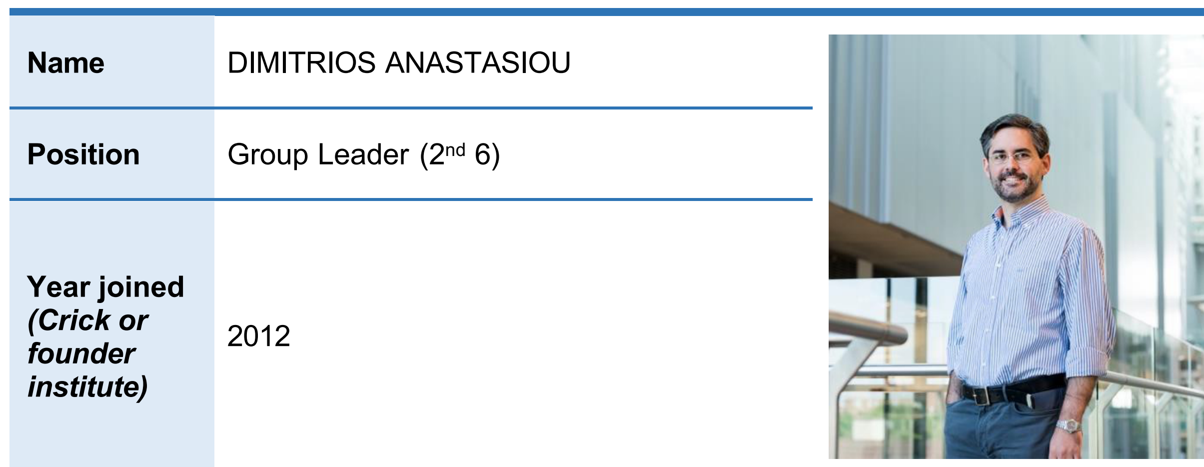
Faculty Overviews 2023

1. Dimitrios Anastasiou
2. David Balchin
3. Patricia Barral
4. David Bauer
5. Rupert Beale
6. Katie Bentley
7. Kate Bishop
8. Michael Blackman
9. Paola Bonfanti
10. Dominique Bonnet
11. Simon Boulton
12. James Briscoe
13. Dinis Calado
14. Margarida Cardoso Moreira
15. Jeremy Carlton
16. Peter Cherepanov
17. Francesca Ciccarelli
18. Alessandro Costa
19. Erika DeBenedictis
20. Mike Devine
21. John Diffley
22. Julian Downward
23. Alberto Elosegui-Artola
24. Radoslav Enchev
25. Gerard Evan
26. Greg Findlay
27. Steve Gamblin
28. Sonia Gandhi
29. Sergi Garcia-Manyes
30. Nathan Goehring
31. Alexander Gould
32. Francois Guillemot
33. Maximiliano Gutierrez
34. Zena Hadjivasiliou
35. Adrian Hayday
36. Jeannine Hess
37. Caroline Hill
38. Florencia Iacaruso
39. Eachan Johnson
40. George Kassiotis
41. Veronica Kinsler
42. Jonny Kohl
43. Jean Langhorne
44. James Lee
45. Leanne Li
46. Vivian Li
47. Robin Lovell-Badge
48. Nicholas Luscombe
49. Ilaria Malanchi
50. Philippa Matthews
51. Neil McDonald
52. Charlie McTernan
53. Maxim Molodstov
54. Mariya Moosajee
55. Naomi Moris
56. Kathy Niakan
57. Paul Nurse
58. Anne O'Garra
59. Snezhana Olifrenko
60. Vassilis Pachnis
61. Venizelos Papayannopoulos
62. Rickie Patani
63. Lucia Prieto Godino
64. Rashmi Priya
65. Peter Ratcliffe
66. Caetano Reis e Sousa
67. Katrin Rittinger
68. Sam Rodriques
69. Peter Rosenthal
70. Erik Sahai
71. Silvia Santos
72. Adam Sateriale
73. Paola Scaffidi
74. Andreas Schaefer
75. Katharina Schmack
76. Anne Schreiber
77. Ben Schumann
78. Andrea Serio
79. Pontus Skoglund
80. Brigitta Stockinger
81. Jonathan Stoye
82. Bart de Strooper
83. Charles Swanton
84. Nicolas Tapon
85. Ian Taylor
86. Saverio Tedesco
87. Pavel Tolar
88. Sharon Tooze
89. Mortiz Treeck
90. Richard Treisman
91. Samra Turajlic
92. James Turner
93. Victor Tybulewicz
94. Frank Uhlmann
95. Sila Ultanir
96. Folkert Van Werven
97. Jean-Paul Vincent
98. Carola Vinuesa
99. Karen Vousden
100. Andreas Wack
101. Louise Walport
102. Michael Way
103. Stephen West
104. Robert Wilkinson
105. Hasan Yardimci
106. Mariia Yuneva
107. Petr Znamenskiy



Name	DIMITRIOS ANASTASIOU
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2012

Lab Name	***Cancer Metabolism Laboratory***

Research programme and achievements

Changes in glucose metabolism have been linked to the pathogenesis of many cancers and are widely used for tumour diagnosis. However, current attempts to target glycolysis for cancer therapy have shown limited success, at least in part due to an incomplete understanding of how glucose metabolism is regulated at the cellular and the subcellular levels. It is also not clear how perturbation of these regulatory mechanisms in tumours affects whole-body glucose homeostasis. The overarching goal of our research is to identify the fundamental principles that underlie the spatiotemporal regulation of glucose metabolism at multiple scales, and then use them to guide improved cancer diagnosis, therapy and prevention.

Since its establishment in 2012, our lab has focused on elucidating cell-autonomous mechanisms that control glycolysis. We generated computational and optogenetics tools that are necessary for our future work to understand how allosteric enzyme control regulates glycolysis. Our work has also revealed new synergies between proteins that provide NAD+ to increase glycolysis, a process that is essential for cancer cell survival during the early stages of hypoxia. Moreover, we have discovered a new mode of action for dimethyloxalylglycine (DMOG), a compound that is widely used to increase glycolysis by stabilising the transcription factor HIF1α. We found that MOG, a major product of DMOG degradation, is selectively cytotoxic to cells that express the pyruvate transporter MCT2, through simultaneous inhibition of multiple key metabolic enzymes. We have synthesised MOG analogues that we are now testing in mice as diagnostic imaging toolsTo study the physiological relevance of our findings in cultured cells and to understand their impact upon whole-body glucose homeostasis, we have established mouse models of hepatocellular carcinoma (HCC) and liver regeneration. To facilitate our research in this area, we have developed new methods, such as 13C-magnetic resonance spectroscopy (MRS), which allow us to measure mouse liver metabolism dynamically in vivo and ex vivo. Using these methods, we discovered a significant increase in the contribution of glycerol to the elevated glucose production in the liver of mice with HCC.

Together, our past work has revealed novel insights into the cell-autonomous mechanisms that regulate glycolysis and has highlighted new molecular mechanisms and target combinations that will help perturb glycolysis specifically in cancer cells, despite its ubiquity throughout the human body. We have also generated mouse models and methods that have highlighted the influence of HCC on host metabolism.

In ongoing and future work, we have been building upon these discoveries

about how glucose metabolism is regulated at the molecular and cellular levels, with an increased emphasis on in vivo interactions between tumour and host metabolism. We have set out to identify the molecular factors from HCC that reprogramme host glucose metabolism and we will genetically and pharmacologically perturb host gluconeogenesis, in part using the tools and insights from our past discoveries. The proposed research programme will allow us to define new ways to target host metabolism, alone or simultaneously with HCC metabolism, in order to prevent and to treat liver cancer.

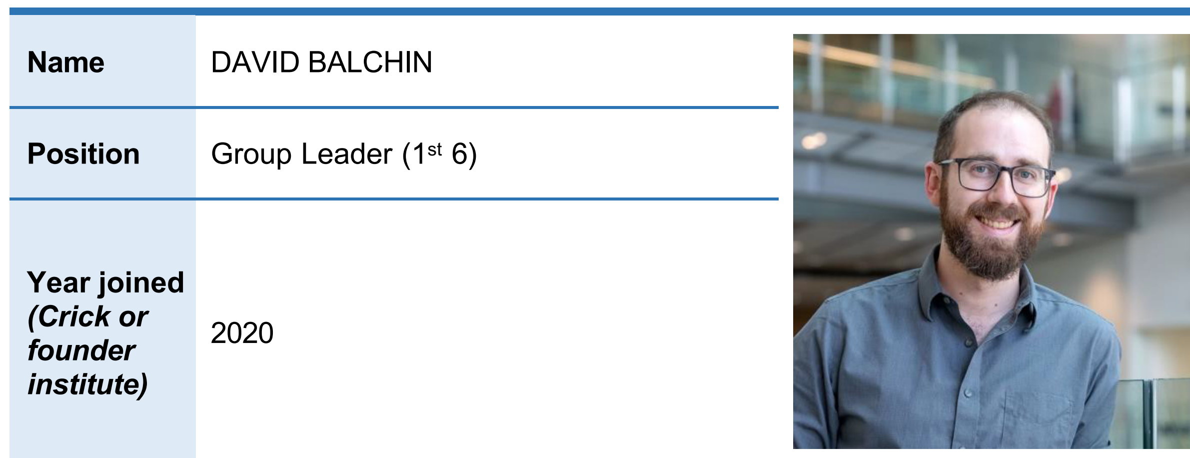
Research outputs

Macpherson, J.A., Theisen, A., Masino, L., Fets, L., Driscoll, P.C., Encheva, V., Snijders, A.P., Martin, S.R., Kleinjung, J., Barran, P.E., Fraternali, F.*, and Anastasiou, D.* (2018). *Functional cross-talk between allosteric effects of activating and inhibiting ligands underlies PKM2 regulation*. e Life 8:e45068 (2019) (* co-corresponding). DOI: 10.7554/eLife.45068
This work reveals that amino acids, rather than FBP, are the relevant cellular regulators of PKM2, a critical node in cancer metabolism. It further elucidates the molecular mechanism of PKM2 regulation by amino acids with a new algorithm that predicts allosteric pathways in proteins, a major and difficult problem in structural biology.

Fets, L., Driscoll, P.C., Grimm, F.*, Jain, A. *, Nunes, P.M. *, Gounis, M., Doglioni, G., Papageorgiou, G., Ragan, T.J., Campos, S., Silva dos Santos, M., MacRae, J.I., O'Reilly, N., Wright, A.J., Benes, C.H., Courtley, K.D., House, D., and Anastasiou, D (* equal contribution). (2018) *MCT2 mediates concentration-dependent inhibition of glutamine metabolism by MOG*. Nat Chem Biol. 14(11):1032-1042. DOI: 10.1038/s41589-018-0136-y
DMOG is a compound that has been widely used to support the involvement of prolyl hydroxylases (PHDs) in disease, thereby leading to multiple, currently active, drug discovery programmes for PHD inhibitors. This paper reveals a new, PHD-independent, mechanism of action for DMOG through targeting of multiple enzymes in glutamine metabolism, thereby leading to cytotoxicity only against some cells.

Gehrig, S., Macpherson, J.A., Driscoll, P.C., Symon, A., Martin, S.R., MacRae, J.I., Kleinjung, J., Fraternali, F., and Anastasiou, D. (2017). *An engineered photoswitchable mammalian pyruvate kinase*. FEBS J 284, 2955-2980. DOI: 10.1111/febs.14175
This paper describes the first light-controllable mammalian metabolic enzyme and demonstrates that glycolysis can be modulated reversibly with light. Optogenetic control of glycolysis opens the door to elucidating the elusive function of glycolytic oscillations in mammalian cells.

Grimm, F., Jain, A., Silva dos Santos, M., Kleinjung, J., Nunes, P.M., Gehrig, S., Fets, L., MacRae, J.I., and Anastasiou, D. *GOT1 primes the cellular response to hypoxia by supporting glycolysis and HIF1α stabilisation*. Cell Reports, under revision
Tumour hypoxia is associated with poor prognosis and resistance to therapy. This manuscript demonstrates that GOT1 and LDHA synergise to support cell survival in early hypoxia; their combined inhibition is cytotoxic only in hypoxia and could therefore be used to selectively kill cancer cells before they adapt to chronic hypoxia.



Name	DAVID BALCHIN
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Protein Biogenesis Laboratory***

Research programme and achievements

The objective of the recently-established Protein Biogenesis laboratory is to understand how cellular factors cooperate to support the folding of nascent proteins. In particular, we focus on the ribosome as the hub that coordinates protein synthesis, folding, chaperone recruitment and quality control.
In my previous role I studied the mechanisms of molecular chaperones. My major achievements in this time were:

1. Determining how the eukaryotic chaperonin complex directs actin folding.
2. Determining how the novel chaperone Hgh1 interacts with elongation factor 2.
3. Demonstrating that the Hsp70 chaperone system is a catalyst of protein folding.
4. Demonstrating that the bacterial chaperonin cavity is a privileged environment that enables rapid folding of endogenous substrate proteins.

Overall, this work provided key support for the new idea that cellular chaperones actively and specifically modulate the folding energy landscape of their client proteins.
Future/current work in my laboratory is focused on two main areas.

1. *In vitro* reconstitution and biophysical analysis of the machineries of protein synthesis and folding in bacteria, with the aim of understanding how the process of translation shapes the folding pathway of nascent proteins on the ribosome.
2. Using mass-spectrometry based approaches to map the pathways of protein biogenesis in bacteria and human cells. From this we hope to discover how protein s are routed through the chaperone network, and how different nodes in the network contribute to overall folding efficiency.

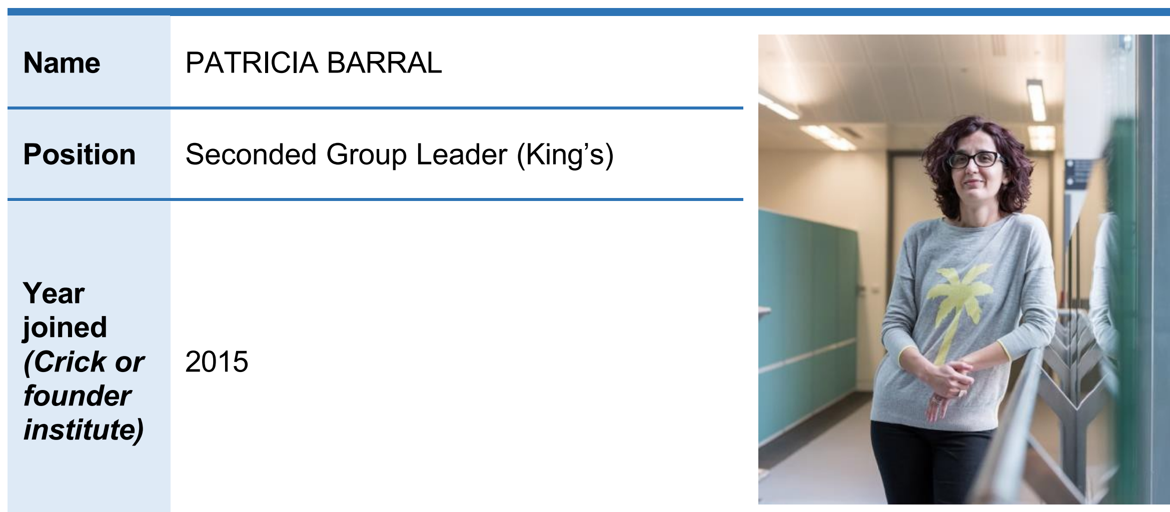
Research outputs

Balchin, D., Miliĉiĉ, G., Strauss, M., Hayer-Hartl, M., and Hartl, F.U. (2018) *Pathway of actin folding directed by the eukaryotic chaperonin TRiC*. Cell 174:1507-1521. DOI: 10.1016/j.cell.2018.07.006
The TRiC chaperonin interacts with ~10% of the nascent cytosolic proteome, and is uniquely able to support the folding of certain essential proteins with complex topologies. Using actin as a model substrate, we applied a range of biophysical methods to establish the protein-folding mechanism of TRiC. This work also put forward the new idea that chaperones can specifically manipulate the folding pathway of some substrates to allow access to the native state.

Moenkemeyer, L., Klaips, C.L., Balchin, D., Koerner, R., Hartl, F.U. and Bracher, A. (2019*) Chaperone function of Hgh1 in the biogenesis of eukaryotic elongation factor*

***2*. Mol Cell 74:88-100. DOI: 10.1016/j.molcel.2019.01.034**
eEF2 is an abundant and essential translation factor with complex biogenesis requirements *in vivo*. We identified Hgh1 as a novel chaperone that cooperates with the TRiC chaperonin to fold EF2 in yeast. These findings allowed us to propose a pathway for EF2 maturation that links multiple chaperone systems with nascent protein folding at the ribosome.

Imamoglu, R., Balchin, D.*, Hayer-Hartl, M.* and Hartl, F.U.* (* Co-corresponding authors). (2020) *Bacterial Hsp70 resolves misfolded states and accelerates productive folding of a multi-domain protein*. Nat Commun 11:1-13. DOI: 10.1038/s41467-019-14245-4
Hsp70 chaperones are the central hub of the protein homeostasis network in bacteria and eukaryotic cells. By reconstituting Hsp70-dependent protein folding under single-molecule conditions, we discovered that Hsp70 can dramatically accelerate folding of a multidomain protein. These findings imply that folding acceleration may be a fundamental feature of chaperone function that underlies both *de novo* protein biogenesis and recovery from proteotoxic stress.



Name	PATRICIA BARRAL
Position	Seconded Group Leader (King's)
Year joined *(Crick or founder institute)*	2015

Lab Name	***Immune Responses to Lipids Laboratory***

Research programme and achievements

My research program aims to understand the cellular mechanisms that initiate and shape immune responses, with a particular emphasis on studying the biology and function of unconventional T cells in health and disease. We specifically focus on immune responses to lipids (both endogenous and from microbes) that are mediated by a family of unconventional T cells called NKT cells. Over recent years, my lab has set-up unique methodologies to dissect NKT cell physiology and to explore the mechanisms underlying NKT cell immunity both in tissue homeostasis as well as during infection and inflammation.

We have established a research programme investigating the mechanisms that regulate unconventional T cell functions. Through this work we have identified a novel cellular crosstalk controlling the activation of NKT cells (Saez de Guinoa*, Jimeno* et al, EMBO Reports 2017), explored the mechanisms by which NKT cells control the intestinal microbial populations and contribute to the establishment of intestinal homeostasis (Saez de Guinoa et al, EMBO J 2018; Jimeno et al, JoVE 2018), and identified the relevance of environmental signals in the regulation of NKT cell functions (Jimeno et al, eLife 2019).

Added to this, I have contributed to the recent discovery of the central role of NKT cells in the control of anti-viral immunity (Gaya, et al, Cell 2018).

<u>Current and future research:</u>
Our current research focuses on exploring the molecular mechanisms regulating the functional specialisation of NKT cells in their anatomical niches and how those in turn contribute to local immunity. Specifically, in collaboration with Gitta Stockinger (Crick), we are exploring the role of Aryl Hydrocarbon Receptor in the development and function of intestinal NKT cells. We are also investigating the role of lipid metabolism in the control of bacterial infection.

Another line of research takes advantage of our expertise in intravital microscopy to further characterise the network of cellular communications that regulate unconventional T cell activation during infection.

Finally, we are expanding the translational potential of our research by analysing the function of unconventional T cells in human patients. We are investigating the mechanisms shaping the functions of lipid-reactive T cells in the skin, and are analysing the effect of alterations in the microbiota associated with liver disease in the phenotype and function of unconventional T cell populations.

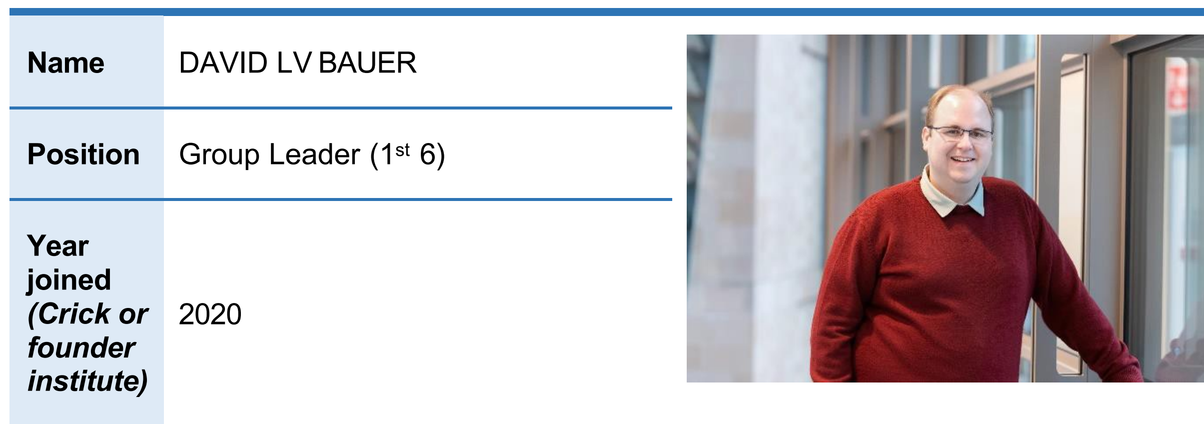
Research outputs

Saez de Guinoa J, Jimeno R, Gaya M, Kipling D, Garzón MJ, Dunn-Walters D, Ubeda C, Barral P. (2018) ***CD1d-mediated lipid presentation by CD11c+ cells regulates intestinal homeostasis.*** **EMBO J 37:e97537. DOI: 10.15252/embj.201797537**
Here we combined various genetic models and deep-sequencing approaches to identify the mechanisms underlying the crosstalk between the intestinal microbiota and NKT cells and their relevance for the regulation of intestinal homeostasis.

Jimeno R, Lebrusant-Fernandez M, Margreitter C, Lucas B, Veerapen N, Kelly G, Besra GS, Fraternali F, Spencer J, Anderson G, Barral P. (2019) ***Tissue-specific shaping of the TCR repertoire and antigen specificity of iNKT cells.*** **eLife e56997. DOI: 10.7554/eLife.51663**
This article explores the mechanisms contributing to the functional specialisation of NKT cells in their anatomical niches and how these control their capacity for lipid recognition.

Saez de Guinoa J*, Jimeno R*, Farhadi N, Jervis P, Cox LB, Besra GS, Barral P. (2017) ***CD1d-mediated activation of group 3 innate lymphoid cells drives IL-22 production*****. EMBO Rep 18:39. DOI: 10.15252/embr.201642412**
This paper defines a novel and completely unexplored mechanism by which innate lymphoid cells regulate CD1d-dependent immune responses.

Gaya M, Barral P, Burbage M, Aggarwal S, Montaner B, Warren Navia A, Aid M, Tsui C, Maldonado P, Nair U, Ghneim K, Fallon PG, Sekaly RP, Barouch DH, Shalek AK, Bruckbauer A, Strid J, Batista FD. (2018) ***Initiation of Antiviral B Cell Immunity Relies on Innate Signals from Spatially Positioned NKT Cells*****. Cell 172:517. DOI: 10.1016/j.cell.2017.11.036**
This paper identifies the major role for NKT cells in the control of antiviral immune responses.



Name	DAVID LV BAUER
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2020

Lab Name	***RNA Virus Replication Laboratory***

Research programme and achievements

We study how RNA viruses replicate in order to understand how they work and to find better ways of treating diseases such as influenza and COVID-19.

Viruses are infectious parasites that can cause disease. Viruses cannot grow on their own — they must infect a 'host' cell in order to reproduce, and often cause disease in the process. All forms of life, from bacteria and fungi to plants and animals, are infected by at least one type of virus.

RNA viruses store their genome as RNA, instead of DNA. They cause a wide range of diseases in humans, from the common cold to more severe illnesses such as gastroenteritis, influenza, Ebola virus disease, measles and COVID-19.

The use of RNA (instead of DNA) by these viruses gives them unique properties. First, they all have a gene for an enzyme to copy the RNA genome, called an 'RNA-directed RNA polymerase' in the case of riboviruses. This enzyme is not found outside of these viruses, which makes it an attractive target for antiviral drugs. Second, unlike DNA, RNA can adopt specific shapes that allow the genome itself to carry out structural and enzymatic functions directly, even though it is not a protein or an enzyme in a conventional sense. All RNA viruses exploit this unique property of RNA in one way or another. At the same time, hosts (including humans) have evolved protein sensors to detect unique features of viral RNA in order to activate the immune system and fight RNA virus infections.

In order to better understand how RNA affects RNA virus replication, our laboratory borrows tools from biochemistry, molecular biology, virology, genomics, and bioinformatics. We also use these tools to design new types of antiviral drugs that can be used to block RNA virus growth, which is especially useful against new or emerging viruses for which conventional drugs or vaccines may not be available.

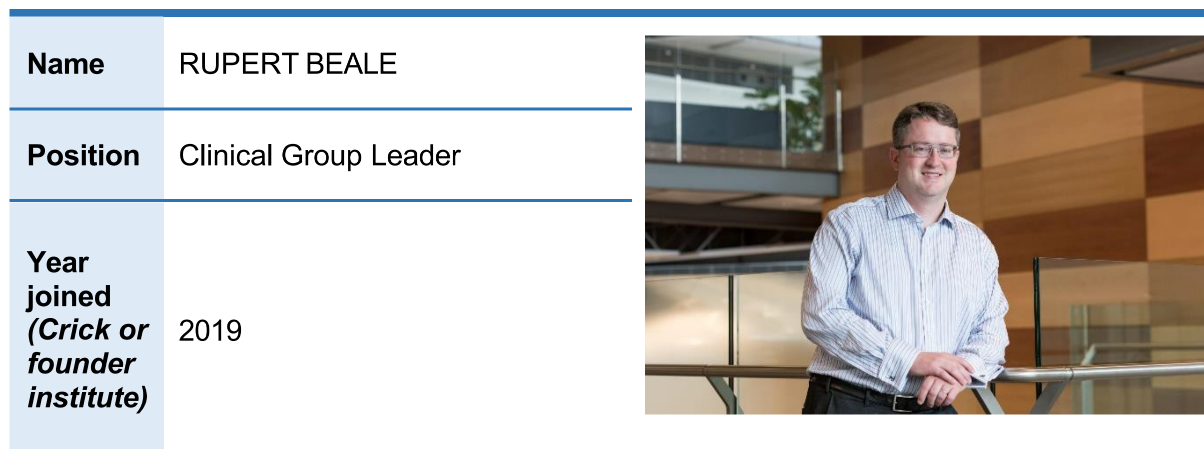
Research outputs

Wall, E. C. et al….Bauer DLV. (2021) ***Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination.*** **Lancet. DOI:10.1016/S0140-6736(21)01290-3.**
Demonstrated reduction in vaccine-induced neutralising antibody titres against emerging SARS-CoV-2 Variants of Concern, as well as age-depending and time-dependent reductions in antibody titres.

Dadonaite B, Gilbertson B, Knight ML, Trifkovic S, Rockman S, Laederach A, Brown LE, Fodor E, and Bauer DLV. (2019) ***The structure of the influenza A virus genome*****. Nature Microbiology 4(11) 1781-1789. DOI: 10.1038/s41564-019-0513-7**
Discovered that the RNA genome of influenza A viruses is much more structured than previously thought, changing the way the field thinks about reassortment (i.e. how a new pandemic could be made) and factors for pathogenicity and cytokine induction.

Bauer DLV*, et al. (2018). ***Influenza Virus Mounts a Two-Pronged Attack on Host RNA Polymerase II Transcription*****. Cell Reports 23, 2119–2129.e3 * corresponding author. DOI: 10.1016/j.celrep.2018.04.047**
Dysregulation of host transcription during influenza virus infection surprisingly can occur independently of the viral NS1 protein's ability to interfere with host mRNA processing (as has been previously assumed), and that this dysregulation caused previously -reported 'downstreamof-gene' transcripts.

Duchi D, Bauer DLV,et al. (2016) ***RNA Polymerase Pausing During Initial Transcription*****. Molecular Cell 63, 939-950. DOI: 10.1016/j.molcel.2016.08.011**
Discovered long-lived pausing during initial transcription in E. coli (rather than rapid abortion of transcription) at position +6 from the TSS and showed it is caused by a clash between nascent transcript and the RNAP σ70 'priming loop' domain.



Name	RUPERT BEALE	
Position	Clinical Group Leader	
Year joined *(Crick or founder institute)*	2019	

Lab Name	***Cell Biology of Infection Laboratory***

Research programme and achievements

Research overview:
Our cells can detect infection by looking for non-self molecular structures, such as bacterial cell walls. Viruses transmit between similar organisms, taking all their structural components from their hosts. They therefore present few targets for host cells to detect. Viruses must hijack the cell to produce progeny, and this results in disturbed homeostasis. Cells therefore also detect perturbed physiology as a potential sign of infection. How this happens, and how the cell responds to these perturbations are the focus of my research.

My lab is particularly interested in what happens to cells when they cannot maintain pH gradients. These gradients are maintained by proton pumps, complicated and energetically expensive molecular machines. Viruses such as influenza and coronaviruses encode channels that allow protons to escape compartments that would normally be acidic. This triggers an inflammatory response, and also a response that looks similar to autophagy. By combining information from genetic screens we discovered that the proton pumps themselves recruit the autophagy machinery under these circumstances. We are trying to work out what this means for the cell and what this means for viruses. The evidence so far points to modulation of antiviral and inflammatory responses – the responses that determine the outcome of severe viral infections such as Covid-19.

Achievements:
Discovered (with the Florey group) the basis for triggering ‘non-canonical’ autophagy in response to disrupted pH gradients during viral infection. This requires the autophagy protein ATG16L1 to interact with the vacuolar ATPase via its C-terminal WD40 domain (Fletcher et al. 2018, Ulferts et al. in press).

Set up simple cell culture models of SARS-CoV-2 infection that are widely used within the Crick, underpinning live virus neutralisation assays and small molecule inhibitor screens. Helped establish the SARS-CoV-2 diagnostic pipeline at the Crick and provided critical data to inform public health responses (Ambrose et al., Houlihan et al., Hellewell et al.,

2020).

Future directions:
The unexpectedly beneficial effect of corticosteroids on survival in oxygen-dependent Covid patients underlines how little we really understand inflammatory responses to infection. Corticosteroids are an exceptionally blunt implement in therapeutic terms, but have proved highly effective. In the case of SARS-CoV-2, this targeting of the host response has proved more useful than targeting the virus itself.
The focus of my research in the next few years will be to try to understand the mechanisms that underly inflammatory and other potentially maladaptive responses to infection, using clinical observations as well as advances in molecular virology to inform the design of experimental models. We intend to discover how viral ion channels (viroporins) such as influenza M2 and SARS-CoV-2 E trigger inflammation, and how this is related to the 'non-canonical' autophagy phenomena that they also trigger and which we have partly characterised. We are also interested in how certain genes implicated by GWAS studies as important for host responses to viral infection (including autophagy genes) might impinge on inflammatory responses at both a cell biological and whole organism level. Understanding these processes better may ultimately enable us to escape the dichotomy of targeting either host response or the virus itself by focussing on the most important host:pathogen interaction.

Research outputs

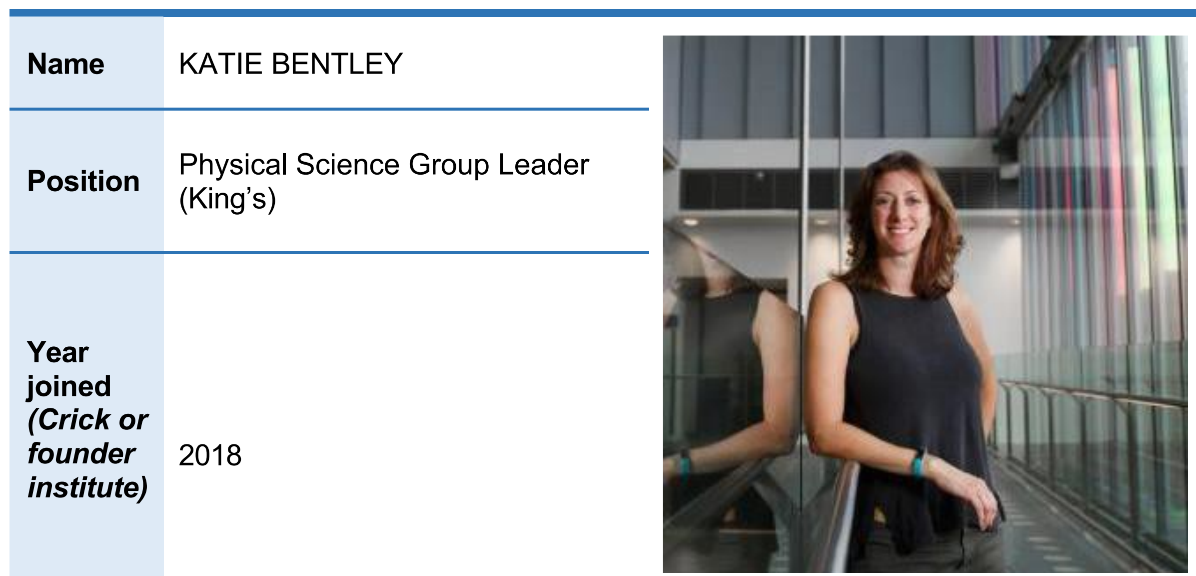
Fletcher K*, Ulferts R*, Jaquin E, Veith T, Gammoh N, Aresteh JM, Mayer U, Carding SR, Wileman T, Beale R†, Florey O†. (2018) ***The WD40 domain of ATG16L1 is required for its non-canonical role in lipidation of LC3 at single membranes*****. EMBO J 37(4):e97840. DOI: 10.15252/embj.201797840**
We showed that multiple forms of so called 'non-canonical' autophagy depend on a different domain of ATG16L1 than canonical autophagy. This important mechanistic advance paved the way for paper 2, and a further manuscript (not yet available as pre-print) shows this depends on an interaction with the vacuolar ATPase.

Durgan J, Lystad A, Sloan K, Carlsson S, Wilson M, Elena Marcassa, Ulferts R, Webster J, Lopez-Clavijo A, Wakelam M, Beale R, Simonsen A, Oxley D, Florey O. (2021) ***Non-canonical autophagy drives alternative ATG8 conjugation to phosphatidylserine.*** **Molecular Cell 81, 9: 2031 – 2040. DOI: 10.1016/j.molcel.2021.03.020**
Oliver Florey's lab went on to show that non-canonical lipidation includes phosphatidylserine rather than just phosphatidylethanolamine. We provided evidence (in collaboration) that ATG4D recycles this conjugate.

Catherine F Houlihan, Nina Vora, Thomas Byrne, Dan Lewer, Gavin Kelly, Judith Heaney, Sonia Gandhi, Moira J Spyer, Rupert Beale, Peter Cherepanov, David Moore, Richard Gilson, Steve Gamblin, George Kassiotis, Laura E McCoy, Charles Swanton, Crick COVID-19 Consortium; Andrew Hayward, Eleni Nastouli, SAFER Investigators. (2020) ***Pandemic peak SARS-CoV-2 infection and seroconversion rates in London frontline health-care workers*****. Lancet 396(10246):e6-e7. DOI: 10.1016/S0140-6736(20)31484-7.**
This important paper showed very high levels of infection amongst healthcare workers in a local hospital. It has influenced government policy – asymptomatic healthcare workers are to be screened as per our recommendation (announced October 12th).

Ambrose K, Beale R and many others in the Crick Covid-19 Consortium. (2020) *Scalable and robust SARS-CoV-2 testing in an academic center*. Nat Biotechnol 38, 927-931. DOI: 10.1038/s41587-020-0588-y
Along with many others I helped design and implement the Crick testing pipeline. My particular responsibility was the initial viral inactivation and RNA preservation step.

Houlihan CF, Beale R. (2020) *The complexities of SARS-CoV-2 serology*. Lancet Infect Dis 20(12), 1350 – 1351. DOI: 10.1016/S1473-3099(20)30699-X
This commentary arose from my work advising both PHE and the Department of Health and Social Care on serological testing.



Name	KATIE BENTLEY
Position	Physical Science Group Leader (King's)
Year joined *(Crick or founder institute)*	2018
Lab Name	***Cellular Adaptive Behaviour Laboratory***

Research programme and achievements

We use simulations integrated with experiments to explore how cells choose their behaviour at a given moment in time, and how their local environment and neighbouring cells influence them to either help the tissue (adaptive behaviour) or help a pathogen/disease condition persist (maladapted behaviour).

We developed a novel, time-based formulation of endothelial cell behaviour during angiogenesis and through our collection of recent integrated in silico/in vivo studies we have demonstrate that various alterations to cell or tissue conditions act to locally adapt the timing of collective cell decisions and behaviours, impacting when certain cells decide to move, and generating a different spacing of vessel branches in the growing network. We are calling these 'temporal regulators' or 'temporal adaptors' of blood vessel branch spacing and they represent an exciting potential to externally modulate vessel branching under different conditions. As such we are now investigating them further and their potential as therapeutic targets to normalize vessel growth in disease.

Our most recent studies in this area have focused on an unexpected role for actin-based filopodia protrusions as an aid to making quick collective decisions on which cell will be the "tip cell" and lead a new blood vessel sprout. We propose they are a basal form of cognition - a form of "active perception" or sensorimotor feedback – as their movement alters sensory input and vice versa leading to enhanced (faster) tip decisions. Our simulations predict they confer positive feedback to sensing which generates a bistable switch property to speed up the otherwise slow process of notch lateral inhibition. We are currently finalising the first proof of concept paper laying out this theory and plan to perform the full investigation, validation and characterisation of the mechanism while at the Crick with integrated in vitro micropatterning and simulation studies together with our in vivo (zebrafish) collaborator Shane Herbert Manchester University.

We also work on understanding pathological vessel growth, in particular how changes to

cell shape and signalling dynamics contribute to vascular abnormalities. Eye diseases affect millions of people worldwide and can have devasting effects on people's lives. Vascular anomalies are central to many retinopathies. To find new treatment s, scientists need to understand more about how these diseases arise and how they progress. This is challenging and progress has been held back by limitations in current techniques for looking at the eye. We recently showed that light-sheet fluorescent microscopy (or LSFM for short) can quickly produce highly detailed, three-dimensional images of mouse retinas, from the smallest parts of cells to the entire eye. The technique also identified new features in a well-studied mouse model of retina damage caused by excessive oxygen exposure in young mice. Previous studies of this model suggested the disease caused blood vessels in the eye to balloon, hinting that drugs that shrink blood vessels would help. But using LSFM, we revealed that these blood vessels actually take on a twisted, knotted and swirled shape. This suggests that treatments that untangle the vessels rather than shrink them may be more effective.

We also recently identified that timing of notch patterning, key in tip cell selection, abnormally oscillates in retinopathy conditions – so we are actively engaged now in both developing new sophisticated computer modelling methods to better capture the complex 3D shapes and cross-talking signalling driving abnormal knotted vessel growth in retinopathy as well as performing cutting edge 3D/4D imaging and analysis of the tufts in retinas from Crick colonies as well as our collaborator Claudio Franco (IMM Lisbon) to better characterise this newly identified knotted morphology. Overall we aim to discover how they form and predict new ways to therapeutically untangle them.

We are also actively collaborating with software engineers to develop a biologist friendly programming language to aid wider adoption of predictive simulations in biology as they have been instrumental for our research in uncovering hidd

counter-intuitive dynamics and could help many other labs understand how non-linear dynamics and unconventional algorithmic cross-scale or cell shape/signalling mechanisms could be contributing to their cell system's behaviour.

Research outputs

Claudia Prahst*, Parham Ashrafzadeh*, Thomas Mead* (co-first), Ana Figueiredo, Karen Chang, Douglas Richardson, Lakshmi Venkatraman, Mark Richards, Ana Martins Russo, Kyle Harrington, Marie Ouarne, Andreia Pena, Dong Feng Chen, Lena Claesson-Welsh, Kin-Sang Cho, Claudio Franco, Katie Bentley. (2020) *Mouse retinal cell behaviour in space and time using light sheet fluorescence microscopy*. ELife 9:e49779. DOI: 10.7554/eLife.49779

We successfully performed the first lightsheet 3D/4D imaging of mouse retinas (focussing on vessels and neurons) and demonstrated that current confocal methodss distorted vessel tissue. This brings a much improved way to observe and quantify the devastati ng changes to vessels and neurons in retinopathy mouse models. The work also solidified our lab as multidisciplinary performing our own mouse experiments and imaging as well as computer modelling.

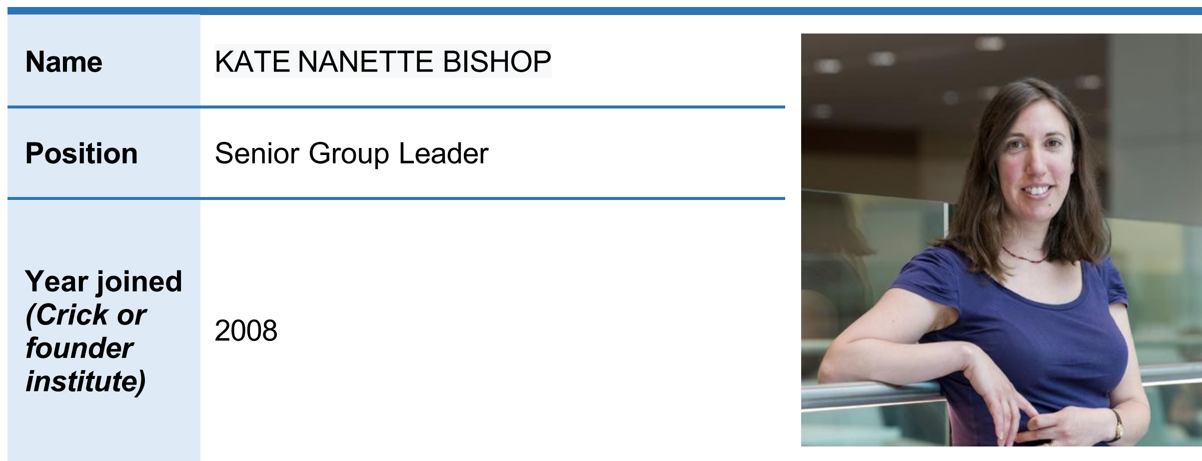
Page, Donna, Thuret, Raphael, Venkatraman, Lakshmi, Takahashi, Tokiharu, Bentley, Katie*, Herbert, Shane P* (co-last). (2019) *Positive feedback defines the timing, magnitude, and robustness of angiogenesis*. Cell Reports 27(11) 3139-3151.e5. DOI: 10.1016/j.celrep.2019.05.052
Collaborative project proving in vivo (zebrafish) validation of our simulation model predictions that positive feedback alters the timing of the important tip cell selection step in blood vessel branching. We also identify a previously unappreciated time window for selection in vivo.

Bentley, Katie, and Shilpa Chakravartula. (2017) *The temporal basis of angiogenesis*. Philosophical Transactions of the Royal Society B: Biological Sciences 372 , 1720. DOI: 10.1098/rstb.2015.0522
Perspective piece detailing my theory that many "temporal regulators" exist to modulate and adapt the timing of tip cell selection during angiogenesis in order to fine tune the vessel branch spacing of new blood vessel networks.

Ubezio, B., Blanco, R.A., Geudens, I., Stanchi, F., Mathivet, T., Jones, M.L., Ragab, A., Bentley, K*. and Gerhardt, H* (co-last). (2016) *Synchronization of endothelial Dll4-Notch dynamics switch blood vessels from branching to expansion*. Elife 5:e12167. DOI: 10.7554/eLife.12167
In vivo validation of simulation predictions, and further simulation studies, sh owing that pathologically high VEGF in tumours and retinopathy cause synchronised Notch oscillations among endothelial cell collectives in growing blood vessels and demonstration that this synchrony contributes to vessel expansion rather than branching in pathological vessels.

The culmination of ten years work when my simulations first predicted patholgical oscillations.

Bahti Zakirov, Georgios Charalambous, Raphael Thuret, Irene M. Aspalter, Kelvin Van-Vuuren, Thomas Mead, Kyle Harrington, Erzsebet Ravasz Regan, Shane Paul Herbert, Katie Bentley. (2021) *Active Perception during Angiogenesis: Filopodia speed up Notch selection of Tip cells in silico and in vivo*. Philosophical Transactions B of the Royal Society 376, 1821. DOI: 10.1098/rstb.2019.0753
Our work addresses Notch mediated tip cell selection preceding angiogenesis. Although the molecular players surrounding this process are well characterized, we have discovered an unexpected role for filopodia (fingerlike, actin-rich cell membrane protrusions) in regulating the timing of Notch patterning. We argue that the filopodia are used by the cells to actively sense morphogens in the environment- thus speeding up patterning decisions. This feeds into the theory that the topology of vascular networks created by angiogenesis is regulated by the timing of Notch patterning, and represents a step towards understanding complex, adaptive morphogenesis in terms of collective cell behaviour and communication.



Name	KATE NANETTE BISHOP	
Position	Senior Group Leader	
Year joined *(Crick or founder institute)*	2008	

Lab Name	***Retroviral Replication Laboratory***

Research programme and achievements

Over 37 million people are infected with HIV and there is no cure, no vaccine and a high economic burden associated with current drugs. Hence, retroviral infection is a major global health issue. My research interest is centred on understanding how retroviruses replicate in cells, which underpins investigations into eradicating HIV reservoirs as well as providing novel targets for HIV therapies. Somewhat surprisingly, there are still many gaps in our knowledge of retroviral replication events and in particular, of the host proteins involved. As obligate intracellular parasites, retroviruses are required to make numerous interactions with cellular proteins in order to complete each replication step. Additionally, they must avoid or overcome cellular proteins that inhibit their replication. As such, retroviruses can give us unique insight into the molecular biology of the cell, and reveal fundamental cellular processes beyond those involved in infection control.

My specific research aims are: (i) to elucidate the function and cellular interactions of viral proteins that are essential for early retroviral replication events and (ii) to identify and characterise the action of cellular proteins that restrict infection during early replication. Within this theme, my research programme is divided into three separate but inter-related projects that collectively will help to decipher the early stages of retroviral replication:

Project 1: Investigating the function of the p12 protein of MLV in early replication
Our studies on p12 have identified two new functions for this retroviral protein during early post-entry replication of MLV. We established that an N-terminal domain of p12 binds the viral capsid protein (CA) and stabilises the viral core. We hypothesise that p12 binds within a pocket on the surface of the CA lattice that is common to all retroviral CA shells, including HIV, and that CA stability and uncoating (breakdown of the viral core) are regulated by binding to this pocket. Additionally, we have demonstrated that a C-terminal motif in p12 directly binds to chromatin to tether the viral pre-integration complex to the host cell DNA, revealing a role for CA in MLV integration. The function of CA and CA-binding factors in HIV-1 integration is currently hotly debated (see below) and p12 provides an excellent model and a novel angle to study this problem. We are currently investigating how p12 binding stabilises CA lattices, and the timing of CA release from pre-integration complexes relative to DNA binding and integration.

Project 2: Investigating the role of capsid in HIV-1 early replication
The relationship between uncoating and other early replication events such as reverse transcription, the trafficking of the core to the nucleus, nuclear entry and integration of HIV is currently unclear. We were the first to demonstrate that HIV-1 uncoating is triggered by a specific step of reverse transcription, and to suggest possible mechanisms that drive the uncoating process. Our current work extends these findings and shows that infections of viruses with hyperstable cores are inhibited at a much later replication step. Therefore, we are collaborating with Crick experts in cryo-electron microscopy to visualise uncoating intermediates that have proved elusive to this point. We are also exploring the localisation of CA during infection and the interactions between CA and cellular factors that are important for replication.

Project 3: Investigating how Vpx and Vpr enhance HIV replication
SAMHD1 is a cellular protein that restricts HIV replication. We have mapped the features of SAMHD1 required for this restriction and established the mechanism of SAMHD1 regulation in cells. This has allowed us to propose a model for SAMHD1 antiviral activity that we have recently refined. Additionally, we have studied the effects of SAMHD1 on nucleoside analogue drugs and have shown that targeting SAMHD1 may enhance particular cancer therapies. We are currently collaborating with GSK in this area. Furthermore, we have uncovered the molecular details of how retroviral Vpx proteins counteract SAMHD1 by directing its degradation. We are now extending our investigations to lentiviral Vpr proteins. Vpr is a paralogue of Vpx, whose function is currently unknown but it is able to cause cell cycle arrest and may interfere with DNA repair.

By combining biochemistry, virology and cell biology, and a successful, long-term collaboration with structural biologists, my lab has been able to reveal novel interactions between retroviruses and their hosts. Furthermore, we have learnt much more about how our own cells work. In the future, we aim to increase our fundamental knowledge of both retroviral replication as well as host cell biology, in order to identify ways to protect ourselves from retroviruses and viral infections in general.

Research outputs

Wanaguru M, Barry DJ, Benton DJ, O'Reilly NJ, Bishop KN. (2018) *Murine leukemia virus p12 tethers the capsid-containing pre-integration complex to chromatin by binding directly to host nucleosomes in mitosis*. PLoS Pathog. 14(6):e1007117. DOI: 10.1371/journal.ppat.1007117
In this paper, we determined that the retroviral p12 protein directly binds to host chromatin to tether the pre-integration complex to DNA ready for integration. This study also revealed that the capsid protein is an important component of this complex and is essential for this stage of viral replication.

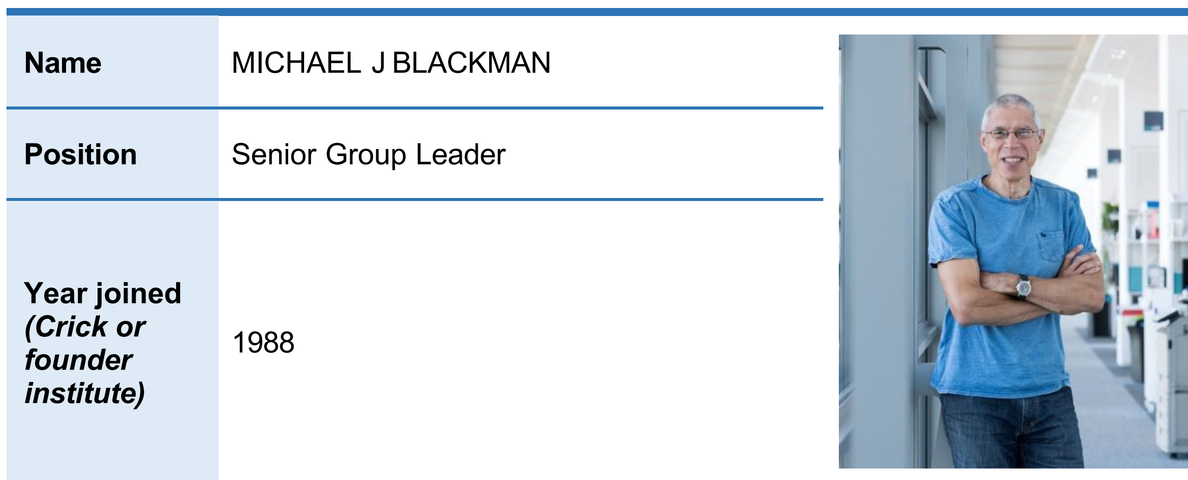
Ordonez P, Kunzelmann S, Groom HC, Yap MW, Weising S, Meier C, Bishop KN, Taylor IA, Stoye JP. (2017) *SAMHD1 enhances nucleoside-analogue efficacy against HIV-1 in myeloid cells*. Sci Rep. Feb 21:7:42824. DOI: 10.1038/srep42824
This study showed that SAMHD1 could influence the activity of several nucleoside analogue drugs used as antiviral or cancer therapies, either by directly degrading them, or through degradation of competing canonical nucleotides and thus raising the drug effective concentration. This suggested that manipulation of SAMHD1 activity may be

useful in combination with existing cancer therapies.

Cosnefroy O, Murray PJ, Bishop KN. (2016) ***HIV-1 capsid uncoating initiates after the first strand transfer of reverse transcription*****. Retrovirology 13(1):58. DOI: 10.1186/s12977-016-0292-7**
In this report, we identified for the first time a stage in reverse transcription that triggers uncoating (breakdown) of HIV-1 cores, a highly controversial area of retrovirology. This in turn suggested possible mechanisms for uncoating and also reopened the long unresolved debate about the location of reverse transcription in the cell.

Arnold LH, Groom HC, Kunzelmann S, Schwefel D, Caswell SJ, OrdonezP, Mann MC, Rueschenbaum S, Goldstone DC, Pennell S, Howell SA, Stoye JP, Webb M, Taylor IA and Bishop KN. (2015) ***Phospho-dependent Regulation of SAMHD1 Oligomerisation Couples Catalysis and Restriction*****. PLoS Pathog 11(10):e1005194. DOI: 10.1371/journal.ppat.1005194**
This study explained the mechanism of SAMHD1 regulation by phosphorylation/ tetramerisation and correlated restriction activity with the capacity of SAMHD1 to form long lived, stable tetramers. These data form the basis of the prevailing model for SAMHD1 restriction of HIV-1 where dNTP-stabilised SAMHD1 tetramers deplete and maintain low levels of dNTPs in the non-permissive cells resistant to HIV-1 infection.

Schwefel D, Boucherit VC, Christodoulou E, Walker PA, Stoye JP, Bishop KN and Taylor IA. (2015) ***Molecular determinants for recognition of divergent SAMHD1 proteins by the lentiviral accessory protein Vpx*****. Cell Host & Microbe 17:489-99. DOI: 10.1016/j.chom.2015.03.004**
This combined virological and structural study revealed how different lineage Vpx proteins are able to target different regions of SAMHD1, whilst still binding to the DCAF1 adaptor to recruit SAMHD1 to the Cullin 4A/E3 ligase complex for degradation. The work provides the first description of how lentiviral accessory proteins employ differing strategies to subvert and inactivate the cell’s viral defence system.



Name	MICHAEL J BLACKMAN
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	1988
Lab Name	***Malaria Biochemistry Laboratory***

Research programme and achievements

Malaria is a devastating disease that impacts the lives of over half of the world's population, causing around 228 million clinical cases and over 400,000 deaths in 2018, mostly in children below the age of five. As a result, malaria is a significant contributor to poverty across much of the developing world and remains a significant threat to travellers. The disease is caused by a mosquito-transmitted protozoan parasite which replicates asexually within circulating red blood cells. Each round of intracellular replication culminates in explosive rupture of the host cell and release (egress) of a new wave of invasive merozoites which rapidly bind to and invade fresh red cells. Increasing parasite levels lead to all the clinical manifestations of malaria, including fever, anaemia, hypoglycaemia, acidosis, respiratory distress, and – in the most severe cases – coma and death. Clinical malaria is a medical emergency and can often be fatal even with access to the best medical intervention. There is no widely available malaria vaccine, and widespread resistance to most antimalarial drugs has led to a pressing need to improve our understanding of parasite biology. Malaria parasites are evolutionarily divergent from model eukaryotes (such as yeast), requiring specialised and often novel approaches to dissecting their cell biology and biochemistry. As an example of this, the parasite replicates by schizogony, in which several rounds of nuclear division first generate a multinucleated syncytium (a schizont), followed by a form of cytokinesis called budding that produces daughter merozoites. The timing of egress must therefore be tightly controlled in order to prevent premature release of non-invasive schizonts.

Our research focuses primarily on the molecular mechanisms by which *Plasmodium falciparum*, the agent of the most dangerous form of malaria, enters and exits its host red blood cell. We have identified and characterised several parasite enzymes with key roles in invasion or egress. These include a cGMP-dependent protein kinase called PKG, which in coordination with a complex cyclic nucleotide signalling pathway triggers egress by activating a parasite subtilisin-like protease called SUB1 which is discharged into the parasitophorous vacuole in which the parasite replicates.

There, SUB1 proteolytically modifies the parasite surface to 'prime' it for egress, and also activates a family of papain-like proteins called the SERA proteins that mediate regulatory roles in egress and that precisely cleave and disrupt the host red cell cytoskeleton. Following egress, a further parasite protease called SUB2 sheds proteins from the merozoite surface to enable invasion of a new red cell and resealing of its membrane. We have led advances in understanding the regulation and molecular functions of these essential enzymes, as well as the role of modification of their various substrates, which include several proteins essential for egress and invasion. We have also developed and applied conditional genetic tools that have revolutionised our capacity to dissect malarial

gene function. We are working to translate the outcomes of our research into health benefits by seeking drug-like inhibitors of the enzymes and other parasite molecules involved in egress and invasion, and promoting their development as antimalarial drugs.

Research outputs

Patel A[+], Perrin AJ[+], Flynn HR, Bisson C, Withers-Martinez C, Treeck M, Flueck C, Nicastro G, Martin SR, Ramos A, Gilberger TW, Snijders AP and Blackman MJ*, Baker DA*. (2019) *Cyclic AMP signalling controls key components of malaria parasite host cell invasion machinery*. PLoS Biology 17(5):e3000264.
DOI: 10.1371/journal.pbio.3000264

This work demonstrated that the cyclic nucleotide cAMP, through its activation of parasite protein kinase A (PKA) controls host cell invasion by malaria merozoites, but plays no role in the regulation of egress, which is regulated by cGMP. Both production of cAMP and activity of PKA are critical for erythrocyte invasion. The study identified and quantified numerous sites, phosphorylation of which are dependent on cAMP signalling, and provided mechanistic insight as to how cAMP-dependent phosphorylation of the cytoplasmic domain of the essential invasion adhesin apical membrane antigen 1 (AMA1) regulates erythrocyte invasion.

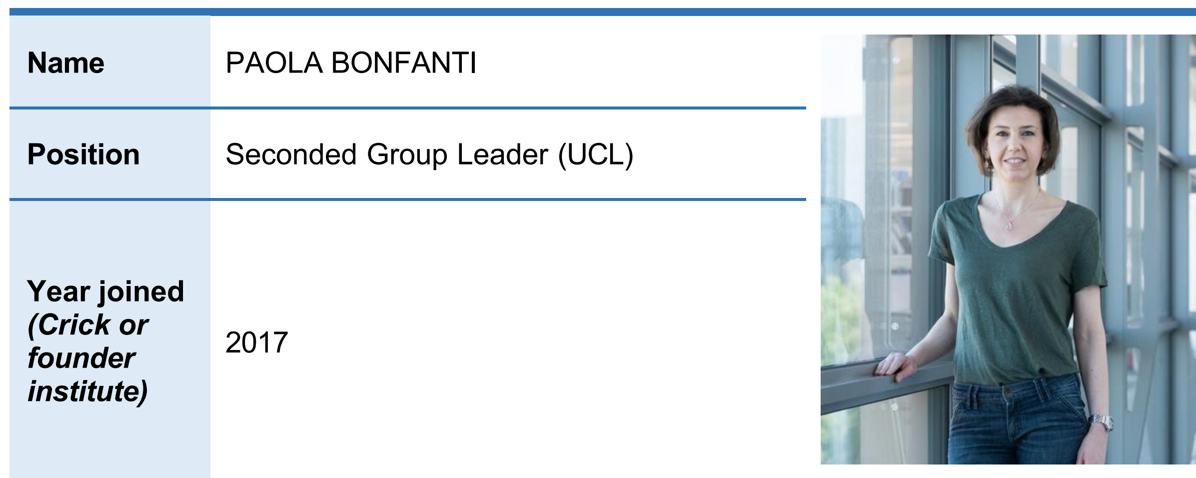
Thomas JA[+], Tan MSY[+], Bisson C, Borg A, Umrekar TR, Hackett F, Hale VL, Vizcay-Barrena G, Fleck RA, Snijders AP, Saibil HR and Blackman MJ. (2018) *A protease cascade regulates release of the human malaria parasite Plasmodium falciparum from host red blood cells*. Nature Microbiology 3:447-455. DOI:10.1038/s41564-018-0111-0
This study showed that egress involves an enzyme cascade in which the serine protease SUB1 activates a second, cysteine protease called SERA6, enabling SERA6 to rapidly and precisely cleave the major red cell cytoskeletal protein β-spectrin and dismantle the cytoskeleton. Provides the first plausible model to explain how the parasite accomplishes timely rupture of its host cell membrane.

Perrin AJ, Collins CR, Russell MRG, Collinson LM, Baker DA and Blackman MJ. (2018) *The Actinomyosin Motor Drives Malaria Parasite Red Blood Cell Invasion but Not Egress*. MBio 9(4):e00905-18. DOI: 10.1016/j.mib.2020.09.003
This study showed that, unlike in the related parasite *Toxoplasma gondii*, egress of *Plasmodium falciparum* merozoites from host erythrocytes does not require actinomyosin-driven motility, placing further emphasis on the protease pathway triggered by cGMP-signalling. In contrast, the *Plasmodium* actinomyosin motor complex is essential for erythrocyte invasion.

Collins CR, Hackett F, Atid J, Tan MSY and Blackman MJ. (2017) *The Plasmodium falciparum pseudoprotease SERA5 regulates the kinetics and efficiency of malaria parasite egress from host erythrocytes*. PLoS Pathogens 13:e1006453. DOI: 10.7554/eLife.61121
We showed that the most abundant protein in the parasite vacuole, SERA5, is a ‘negative regulator’ of egress, controlling the speed of the pathway that leads to malarial egress. Loss of SERA5 leads to accelerated but defective egress and reduced parasite replication rates. This work increased understanding of the molecular mechanisms underlying egress and showed that efficient egress requires precise control of the timing of membrane rupture.

Das S, Hertrich N, Perrin AJ, Withers-Martinez C, Collins CR, Jones ML, Watermeyer JM, Fobes ET, Martin SR, Saibil HR, Wright GJ, Treeck M, Epp C and Blackman MJ. (2015) *Processing of Plasmodium falciparum Merozoite Surface Protein MSP1*

***Activates a Spectrin-Binding Function Enabling Parasite Egress from RBCs*. Cell Host & Microbe 18:433-44. DOI: https://doi.org/10.1016/j.chom.2015.09.007**
We demonstrated that proteolytic processing by SUB1 of the most abundant merozoite surface protein, MSP1, is important for parasite viability and activates its capacity to bind spectrin, a molecular scaffold protein that is the major component of the host erythrocyte cytoskeleton. Merozoites lacking surface-bound MSP1 display a severe egress defect.



Name	PAOLA BONFANTI
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	2017

Lab Name	***Epithelial Stem Cell Biology and Regenerative Medicine Laboratory***

Research programme and achievements

My group aims at understanding how epithelial organs such as thymus, oesophagus and pancreas develop, change with ageing, and become susceptible to cancer. By applying the paradigm of clonal analysis *in vitro*, we dissect long lived epithelial stem cells that regulate homeostasis and regeneration *in vivo*. These properties change during organ morphogenesis, repair and ageing; therefore, we dissect and reassemble the key cellular and molecular players with the ultimate goal of reconstructing functional organs for replacement therapies. The approach is highly interdisciplinary as it combines 2D & 3D *ex vivo* co-cultures, gene editing, tissue engineering, advanced imaging, spatial and single-cell RNA sequencing. We use both small and large animal pre-clinical models and study the cross talk between epithelial cells and the immune system. We have established close collaborations with paediatric surgeons, clinical pathologists and immunologists for developing alternative treatments for oesophageal atresia and congenital athymia.

Thymus biology: We recently identified unique epithelial-mesenchymal hybrid cells, capable of long-term expansion *in vitro*, and able to reconstitute an anatomic phenocopy of the human native thymus, when combined with thymic interstitial cells and a natural decellularised extra cellular matrix (ECM) obtained by a novel method of whole thymus perfusion.

Future plans:

i. engineering a human thymus from organ donors as a tolerising system for immune-suppression free organ transplantation.
ii. a tissue engineered thymus will provide an environment for the maturation of functional T cells in athymic patients. This represents a life-saving treatment which has the potential to be more efficient and safer than thymus transplantation, currently performed by our clinical collaborators at GOSH with cultured organ slices which contain donor lymphocytes often responsible for Graft versus Host Disease (GvHD). A tissue-engineered thymus would cause no tissue damage, and would be free of donor lymphocytes.
iii. By collaborating with the Sanger Institute, we integrate transcriptomic data (Crick) with spatial transcriptomic to identify the niche and exploit the unique properties of thymic clonogenic cells across ages.
iv. Obesity can accelerate thymic involution independently of age, contributing to deficits in adaptive immunity. We engaged with colleagues at the William Harvey Research Institute/Barts NHS Trust for elucidating thymus function in relation to adipose-tissue and systemic inflammation in obese patients.
v. A postdoc is awarded Marie-Curie fellowship (2021-2023) that aims at uncovering how thymus stroma and T-cell subtypes develop in human by dissecting their cellular

and molecular crosstalk.

vi. These projects integrate and complement the Crick collaborations with the Hayday group on investigating how immunosurveillance capabilities are temporally established; with the Bonnet group in developing humanised mouse models with increased T cell output from HSC; it will foster collaboration with the Reis-e-Susa group to study the role of human dendritic cells in thymic selection of human T cells and, in the periphery, in the context of anti-tumour immunity.

<u>Oesophagus biology</u>: Oesophageal replacement remains a major surgical challenge with current options for treatment of long-gap oesophageal atresia that uses substitution with the stomach, colon or jejunum. All these options have important weaknesses with worse reflux and higher risk of cancer. Our current work has proved that oesophageal epithelium can be used for autologous reconstitution of engineered constructs and opens a new avenue for treatment of congenital and acquired disorders:

i. The next step is the validation of such approach in large animal models before phase I/II clinical trials. In collaboration with paediatric surgeons at the GOSH, we have secured funds for a preclinical pig model.

ii. Since our work has contributed to knowledge of epithelial cell biology of the oesophagus that may have important implications in other conditions, we are now proposing to exploit our *ex vivo* systems to overcome the current limitations in cancer modelling. With the Ciccarelli group, expert in large data-sets to predict how abnormal genetic networks in cancer cells drive tumour growth and cancer evolution, we will adapt multiple *ex vivo* assays to monitor metaplasia and transformation of oesophageal cells, genome edited to introduce the most common driver and helper mutations. Thus, we will validate the role of the selected genes in promoting carcinogenesis and, most importantly, will inform future therapeutic strategies of "personalised medicine".

Research outputs

Campinoti S, Gjinovci A, Ragazzini R, Zanieri L, Ariza-McNaughton L, Catucci M, Boeing S, Park JE, Hutchinson JC, Munoz-Ruiz M, Manti PG, Vozza G, Villa E, Phylactopoulos DE, Maurer C, Testa G, Stauss HJ, Teichmann SA, Sebire NJ, Hayday AC, Bonnet D, and Bonfanti P. (2020) *Reconstitution of a functional human thymus by postnatal clonogenic stem/progenitor cells and natural whole organ scaffolds*. Nature Communications 11: 6372. DOI:10.1038/s41467-020-20082-7

In this paper we:

1. Define the heterogeneity and the clonogenic potential of human thymus stroma.
2. Characterise progenitor cells capable of extensive expansion *in vitro*, thereby achieving clinically relevant numbers with resilience to long-term storage.
3. Report an epithelial-mesenchymal hybrid phenotype of thymus epithelial cells *in vivo* and *in vitro* that affects cell behaviour; a unique feature among any epithelia so far reported.
4. Describe a protocol for organs that lack a main vascular access that allowed us to specify the role of natural ECM in supporting organ morphogenesis *ex vivo* and *in vivo*.
5. Reconstitute a functional human thymus long-term *in vivo.*

Meran L, Massie I, Campinoti S, Weston A, Gaifulina R, Faull P, Orford M, Kucharska A, Baulies A, Hirst E, Konig J, Pellegata A, Snijders B, Collinson L, Thapar N, Thomas G, Eaton S, Bonfanti P, De Coppi P, and Li VSW. (2020) *Engineering transplantable mucosal grafts using primary jejunal organoids derived from children with intestinal failure*. Nature Medicine 26:1593-1601. DOI: 10.1038/s41591-020-1024-z

This is a proof-of-principle study demonstrating that autologous jejunal engineered grafts can be obtained with both small intestine and colon scaffolds, thus making them

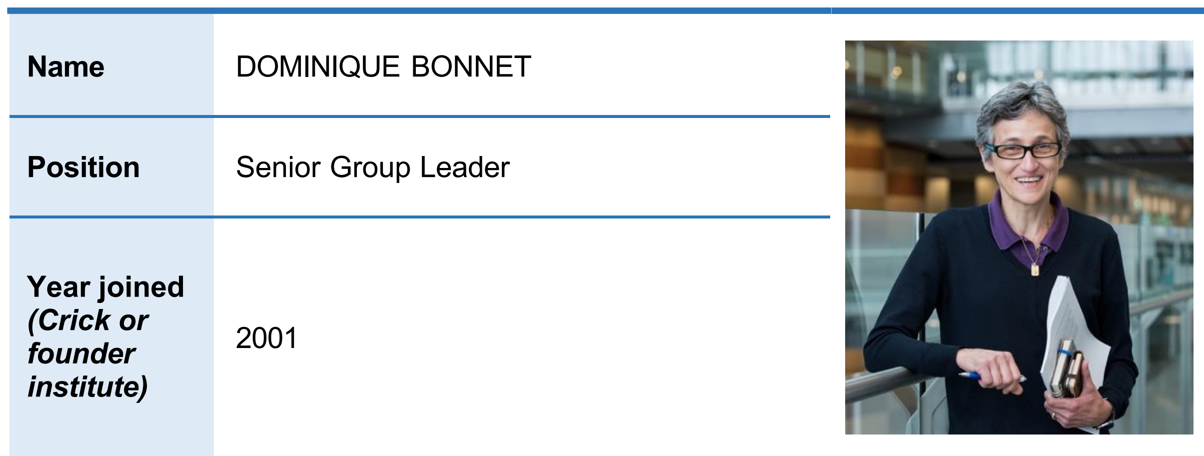
interchangeable for tissue engineering purposes. These constructs survive and form luminal structures after transplantation into the kidney capsule or subcutaneous pockets of mice. This was a Crick collaborative work in which our group developed the *in vivo* assay for intestinal organoids.

Park JE, Botting RA, Conde CD, Popescu DM, Lavaert M, Kunz DJ, Stephenson E, Ragazzini R, Tuck E, Wilbrey-Clark A, Ferdinand JR, Webb S, Maunder D,

Vandamme N, Mahbubani K, Polanski K, Mamanova L, Fuller A, Filby A, Reynolds G, Dixon D, Saeb-Parsy K, Lisgo S, Henderson D, Vento-Tormo R, Meyer KB, Saeys Y, Bonfanti P, Behjati S, Clatworthy MR, Taghon T, Haniffa M, Teichmann SA. (2020) *A cell atlas of human thymic development defines T cell repertoire formation*. Science 21:367(6480) DOI: 10.1126/science.aay3224
In this work a single-cell RNA-sequencing was used to create a cell atlas of the human thymus and to reconstruct T-cell differentiation trajectories across the lifespan with new insights into human thymus. We contributed by validating newly identified markers in isolated and cultured thymus stromal cells.

Giobbe GG, Crowley C, Luni C, Campinoti S, Khedr M, De Santis M, Zambaiti E, Kretzschmar K, Li V, Clevers H, Bonfanti P, Elvassore N, and De Coppi P. (2019) *Extracellular matrix hydrogel derived from decellularized tissues enables endoderm organoids culture.* Nature Communications 11:10(1):5658. DOI: 10.1038/s41467-019-13605-4
The use of extracellular matrix (ECM) hydrogels derived from decellularized tissues can provide an environment capable of directing cell growth. These gels possess the biochemical signature of tissue-specific ECM and have the potential for clinical translation, at variance with commonly used but clinically incompatible matrigel. Gels from decellularized porcine small intestine mucosa/submucosa enable formation and growth of endoderm-derived human organoids, such as gastric, hepatic, and pancreatic. We contributed by establishing the culture conditions for the gastric and pancreatic organoids and develop the transplantation assays to validate long-term function.

Urbani L, Camilli C, Phylactopoulos DE, Crowley C, Natarajan D, Scottoni F, Maghsoudlou P, McCann CJ, Pellegata AF, Urciuolo A, Aruta S, Signorelli MC, Kiely D, Hannon E, Deguchi K, Trevisan M, Wong R, Baradez MO, Moulding D, Khalaf S, Virasami A, Gjinovci A, Loukogeorgakis S, Mantero S, Thapar N, Sebire N, Eaton S, Lowdell M, Cossu G, Bonfanti P and De Coppi P. (2018) *Multi-stage bioengineering of a layered oesophagus with in vitro expanded muscle and epithelial adult progenitors*. Nature Communications 16:9(1):4286. DOI: 10.1038/s41467-020-20082-7
This work shows the reconstruction of a multilayer oesophagus by combining a decellularised oesophagus with *in vitro* expanded epithelial, mesodermal and neural cells, and sets the basis for future use in patients with congenital atresia. The engineered organ was developed in a bioreactor and then transplanted in the omentum of recipient immune deficient mice to allow vascularisation.



Name	DOMINIQUE BONNET
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2001

Lab Name	***Human Haematopoietic Stem Cell Laboratory***

Research programme and achievements

The Bonnet lab works in the fields of human normal and malignant hematopoietic stem cell, focusing on the nature of leukaemic stem cells and their relationship to undiseased human hematopoietic stem cells (HSCs). We were the first to identify and isolate cancer stem cells from human leukaemia.

Our most recent achievements involve:
- Combining next generation sequencing with functional assays, we reported on the evolution history of the genetic mutations present in low--risk myelodysplastic patients and described that in this sub--group of patients, the MDS--propagating cells arise from the haematopoietic stem cell (HSC) compartment (1).
- Using non- invasive intravital imaging techniques to visualise and track human normal and leukaemic hematopoietic development, we showed that AML cells induce vascular leakiness in a response to nitric oxide (NO) production by endothelial cells, which contributes to disease progression and normal HSC dysfunction after chemotherapy, suggesting potential new avenues for therapeutic intervention (3).
- In order to evaluate the role of human BM stroma cells, we developed a new and versatile 3D ossicle model allowing us to grow human AML in a humanized BM niche (2). And also revealed how AML cells impact on normal residual haematopoiesis (4).
- Using proteomic analysis (CYTof) we revealed that RET expression is enriched in human HSC and that human umbilical cord blood HSC treated with the key RET ligand/co-receptor complex, GDNF/GFRa1, show improved progenitor function at primary transplantation and improved long-term HSC function at secondary transplantation (5).

Future plans:

The goal on the lab is to continue evaluating the interactions between normal and leukaemic stem cells and their interactions with the bone marrow microenvironment. We will use spatial transcriptomics, single cell RNAseq, proteomics and imaging of the BM niche to dissect the spatial and temporal organisation of the leukemic subclones in the bone marrow as well as study their response after chemotherapy. We want to provide an atlas of the molecular alterations of the BM microenvironment over time and after chemotherapy intervention. We also aim to validate using Crispr-Cas knock-down or overexpression, the potential micro-environmental “factors” critical for leukaemic stem cell survival and provide approaches to block niche supportive activity. In parallel to this work we aim at further investigating the heterogeneity of the normal HSC compartment and test whether different HSCs reside on distinctive BM niches.

Research outputs

Mian SA, Rouault-Pierre K, Smith AE, Seidl T, Pizzitola I, Kizilors A, Kulasekararaj AG, Bonnet D Mufti GJ. (2015) ***SF3B1 mutant MDS-initiating cells may arise from the haematopoietic stem cell compartment*****. Nat Commun 6:10004. DOI: 10.1038/ncomms10004**
Using next generation sequencing and single cell assays, we report the order of mutations being acquired in human haematopoietic stem cells of patients with MDS which initiate the disease and report on the variegation of the mutations over time and during the transformation from MDS into AML.

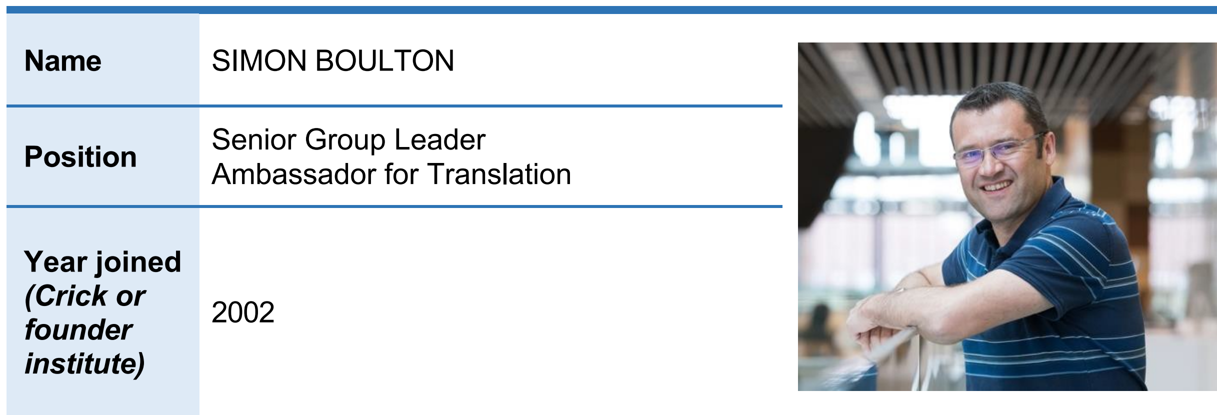
Abarrategi A, Foster K, Hamilton A, Mian SA, Passaro D, Gribben J, Mufti G, Bonnet D. (2017) ***Versatile humanized niche model enables study of normal and malignant human hematopoiesis*****. J Clin Invest. 127(2):543-548. DOI: 10.1172/jci89364**
We developed using a bioengineering scaffold a new versatile humanised bone marrow niche which support the engraftment of both normal and leukaemia stem cells *in vivo*.

Passaro D, Di Tullio A, Abarrategi A, Roualt-Pierre K, Foster K, Ariza-Mc Naughton L, Montaner B, Chakravarty P, Bhaw L, Diana G, Lassailly F, Gribben J, Bonnet D. (2017) ***Increased vascular permeability in the bone marrow microenvironment contributes to disease progression and drug response in acute myeloid leukemia*****. Cancer Cell, 32(3):324-341.e6. DOI: 10.1016/j.ccell.2017.08.001**
Using non-invasive intravital imaging, we reported on the effect of primary AML cells on the bone marrow vasculature increasing the permeability and leakiness of the vessels via increase of nitric oxide synthase (NOS3) and in nitric oxide, demonstrating that AML can directly effect the bone marrow niche components.

Waclawiczek A, Hamilton A, Rouault-Pierre K, Abarrategi A, Albornoz MG, Miraki-Moud F, Bah N, Gribben J, Fitzgibbon J, Taussig D, Bonnet D. (2020) ***Mesenchymal niche remodeling impairs hematopoiesis via stanniocalcin 1 in acute myeloid leukemia*****. J Clin Invest 1,130(6):3038-3050. DOI: 10.1172/JCI133187**
Following on the description by our group in 2013 of the effect of AML on residual normal haematopoietic stem cells, we reported here that AML forces normal HSC into quiescence by inducing the secretion by mesenchymal stroma cells of stanniocalcon.1.

Grey W, Chauhan R, Piganeau M, Huerga Encabo H, Garcia-Albornoz M, McDonald NQ, Bonnet D. (2020) ***Activation of the receptor tyrosine kinase, RET, improves long-term hematopoietic stem cell outgrowth and potency.*** **Blood 136(22):2535-2547. DOI: 10.1182/blood.2020006302**

Recent evidence has implicated the nervous system and glial family ligands (GFLs) as potential drivers of hematopoietic survival and self-renewal in the bone marrow niche, but how to apply this to HSC maintenance and expansion had not been explored. We demonstrated a role for the GFL receptor, RET, at the cell surface of HSCs, in mediating sustained cellular growth, resistance to stress and improved cell survival throughout in vitro expansion. HSCs treated with the key RET ligand/co-receptor complex, GDNF/GFRa1, show improved progenitor function at primary transplantation and improved long-term HSC function at secondary transplantation.



Name	SIMON BOULTON
Position	Senior Group Leader Ambassador for Translation
Year joined *(Crick or founder institute)*	2002

Lab Name	***DSB Repair Metabolism Laboratory***

Research programme and achievements

Since 2015, my lab has provided insights into the regulation of homologous recombination (HR) in normal and pathological situations. We have gained mechanistic insight into several key factors that promote specific steps during the HR reaction and have identified new genes that contribute to the regulation of HR in metazoans and/or function in various DNA repair pathways. Our discovery of RTEL1 as a key regulator of HR and our subsequent insights into its role in meiosis and during DNA replication, laid the foundations for our most important work on the function of RTEL1 in maintaining the integrity of chromosome ends. In a series of studies, we discovered that RTEL1 is co-opted to telomeres to disassemble t-loops, which had been proposed to protect chromosome ends. Unexpectedly, we discovered that telomere dysfunction caused by loss of RTEL1 could be rescued by inactivating telomerase, the reverse transcriptase that normal extends telomeres to solve the end-replication problem. Rather than being unwound by the replisome, we provided evidence that replication forks stall and undergo reversal at persistent t-loops, which creates a pseudo-telomere substrate that is bound and inappropriately stabilised by telomerase, creating a block to telomere replication. This necessitated the excision of the t-loop by SLX1/4 and loss of a substantial part of the telomere. We went on to establish that t-loop unwinding is also compromised by RTEL1 mutations in the telomere dysfunction disorder Hoyeraal-Hreidarsson syndrome and is subject to cell cycle control via a phospho-switch in TRF2. Importantly, we showed that the phospho-switch in TRF2 regulates the transient recruitment and release of RTEL1 from telomeres, which is required to temporarily disassemble t-loops during S-phase to facilitate telomere replication, whilst also preventing promiscuous t-loop unwinding during other cell cycle stages. This work demonstrated beyond any reasonable doubt that the t-loop is a physiologically important structure required to suppress checkpoint activation at telomere ends.

More recently, we discovered that telomere protection is solved by distinct mechanisms in pluripotent and somatic tissues. It was widely acknowledged that TRF2 is essential for t-loop formation and end protection. However, we discovered that this is only true in somatic cells. Loss of TRF2 in stem cells has no overt phenotype, telomeres remain functional, they form t-loops and remain unfused. This work challenges existing dogma, it raises important questions about how and why telomeres in pluripotent cells differ from somatic cells, and how the switch in telomere maintenance mechanism occurs upon differentiation.

In the last few years, we have begun to explore the HR mechanisms that contribute to telomere maintenance in cancers, which acquire unlimited proliferative capacity by either re-expressing telomerase or inducing alternative lengthening of telomeres (ALT) that relies on telomere recombination. Intriguingly, we have recently discovered that infection of cells with Kaposi's Sarcoma Herpes Virus triggers ALT, which we plan to use as a

system to understand the mechanisms responsible for ALT induction and maintenance.

Our ongoing and future work aims to develop our most important discoveries from the last review period in three complementary areas, which share homologous recombination (HR) at their core: 1) telomere maintenance mechanisms; 2) replication-fork conflicts, stabilisation and restart, and; 3) mechanisms of the HR reaction.

Research outputs

Ruis P, Van Ly D, Borel V, Kafer G, McCarthy A, Howell S, Blassberg R, Snijders AP, Howell M, Briscoe J, Niakan K, Marzec P, Cesare AJ & Boulton SJ (2021) *TRF2-independent chromosome end protection during pluripotency*. Nature, 589:103-109 DOI: 10.1038/s41586-020-2960-y
This work revealed that telomere protection is solved by distinct mechanisms in pluripotent and somatic tissues. In somatic cells, TRF2 sequesters the telomere within a t-loop, preventing telomere end-to-end fusions and inviability. In contrast, TRF2 is dispensable for telomere protection in pluripotent cells; ESCs lacking TRF2 grow normally, do not fuse their telomeres and form functional t-loops. Upon differentiation this unique attribute of stem cells is lost and TRF2 assumes its full role in end protection. The retention of end protection in the presence of t-loops, but absence of TRF2, confirmed that t-loops are a key mediator of telomere end protection irrespectively of how they form.

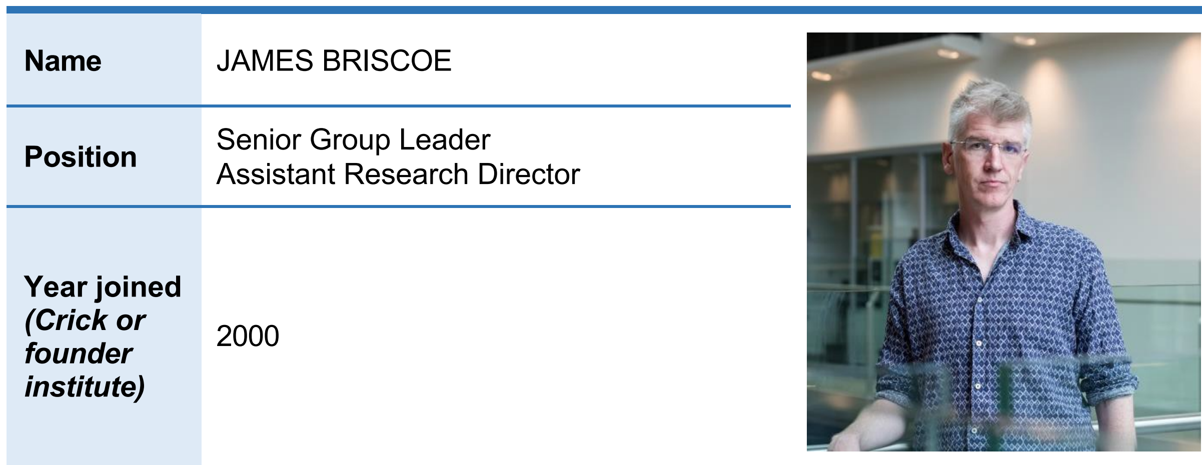
Sarek G, Kotsantis P, Van Ly D, Ruis P, Margalef P, Borel V, Zheng X-F, Flynn H, Snijders B, Choudhury D, Cesare A & Boulton SJ. (2019) *CDK phosphorylation of TRF2 controls t-loop dynamics during the cell cycle*. Nature, 575:523-527. DOI: 10.1038/s41586-019-1744-8
Evidence suggested that the telomere adopts a lasso-like t-loop configuration, which safeguards chromosome ends from being recognised as DNA double strand breaks. However, the regulation and physiological importance of t-loops in end-protection was uncertain. This study uncovered a phospho-switch in TRF2 that coordinates the timely assembly and disassembly of t-loops during the cell cycle, which protects telomeres from replication stress and an unscheduled DNA damage response. These results were the first to definitively establish the t-loop as a physiologically important structure required to suppress checkpoint activation at telomere ends.

Margalef P, Kotsantis P, Borel V, Bellelli R, Panier S & Boulton SJ (2018). *Stabilization of reversed replication forks by telomerase drives telomere catastrophe*. Cell.172: 439-453. DOI: 10.1016/j.cell.2017.11.047
This study defined the mechanism leading to critically short telomeres in the absence of RTEL1 and showed that telomerase, which extends telomeres in normal cells, is pathological when forks encounter an obstacle within the telomere. We showed that replication forks stall and reverse at persistent t-loops, which creates a pseudo-telomere substrate that is inappropriately stabilised by telomerase. Removing telomerase or blocking replication fork reversal rescued telomere dysfunction in *Rtel1* deficient cells. We proposed that when persistent t-loops stall the replisome, telomerase inhibits fork restart, triggering the excision of the t-loop by SLX1/4 and loss of a substantial part of the telomere.

Taylor MR̄G, Špírek M, Chaurasiya KR, Ward JD, Carzaniga R, Yu S, Egelman EH, Collinson LM, Rueda D, Krejci L & Boulton SJ (2015). *Rad51 paralogs remodel pre-synaptic Rad51 filaments to stimulate homologous recombination*. Cell 162, 271-286. DOI: 10.1016/j.cell.2015.06.015
This study was the first to demonstrate that RAD51 paralogues bind to and structurally remodel the pre-synaptic RAD-51-ssDNA filament to a stabilised, “open”, and flexible conformation, which facilitates strand exchange with the template duplex.

We showed that RAD51 paralogues act by binding the end of the presynaptic filament, which induces a conformational change that stabilises RAD-51 bound to ssDNA and primes the filament for strand exchange. These observations established for the first time the underlying mechanism of HR stimulation by Rad51 paralogues and revealed a new paradigm for the action of HR mediator proteins.

Hewitt G, Borel V, Segura-Bayona S, Takaki T, Ruis P, Bellelli R, Lehmann LC, Sommerova L, Vancevska A, Tomas-Loba A, Zhu K, CooperC, Fugger K, Patel H, Goldstone R, Brough R, Lord CJ, West SC, Ahel I, Ahel D, Chapman JR, Deindl S& Boulton SJ (2021). ***Defective ALC1 nucleosome remodelling confers PARPi sensitivity and synthetic lethality with HRD*****. Molecular Cell 81(4): 767-783.e11. DOI: 10.1016/j.molcel.2020.12.006.**
Homologous recombination (HR) is an essential DNA repair mechanism that is frequently inactivated in cancer. Importantly, deficiencies in the HR pathway creates a vulnerability that can be exploited to selectively kill cancer cells by means of synthetic lethality as exemplified by the success of PARP inhibitors in the clinic. This study sought to identify novel synthetic lethal strategies to target cancers and to combat the emerging problem of innate and acquired PARP inhibitor resistance. We discovered that loss of ALC1 confers both PARP inhibitor sensitivity and synthetic lethality with HR deficiency. As such, targeting ALC1 could be employed to augment existing therapeutic strategies for cancer therapy



Name	JAMES BRISCOE
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	2000

Lab Name	***Developmental Dynamics Laboratory***

Research programme and achievements

How are the right types of cells produced in the right place, at the right time, in the right amounts in a developing tissue? In broad terms, initially uncommitted progenitors acquire their fate in response to signals that control transcriptional programmes. These gene regulatory networks (GRNs) determine the spatial and temporal succession of states that progressively define cell identity.

To understand the structure, function and logic of GRNs, we use an experimentally tractable system – the vertebrate spinal cord. We take an interdisciplinary approach combining *in vivo* developmental biology with molecular, genomic and computational methods. Our work has identified mechanisms by which the spatiotemporal pattern in the neural tube is generated, established design principles of the GRN architecture that interpret graded morphogen signalling, and provided insight into the coordination of growth and patterning in the developing neural tube. The work reconciles mechanisms of morphogen activity based on spatial or temporal gradients and provides evidence for how opposing gradients generate and maintain precise patterns in a growing tissue.

Our current directions are driven by three recent advances in the lab. First, we have developed *in vitro* differentiation systems using mouse and human embryonic stem cells that accurately recapitulate developmental processes *ex vivo*. Second, we have embraced new technologies that provide unprecedented ability to manipulate and assay single cells. These include state of the art genomic, genome engineering and imaging methods. Finally, we have embedded collaborations with physicists and computer scientists to develop computational tools and construct data driven mathematical models. Together with our established embryological expertise, this is allowing us to establish a platform for manipulating and analysing the molecular and cellular mechanisms by which cell fate is acquired and tissues organised.

These studies advance beyond qualitative explanations to a dynamic and quantitative understanding of how tissues are patterned and how GRNs operate. We will identify the rules by which cells make decisions and we will define the design logic and network architectures that lead to distinct cell fate choices. The ability to: (i) follow the trajectory of a cell as it transitions to a specific neuronal subtype i*n vivo*; (ii) manipulate the process *in vitro* and *in vivo*; and (iii) model it *in silico*, offers a unique system for understanding organogenesis. By bridging scales - from molecules to cells to tissues – we aim to explain the generation of specific cell types and how a functional, well organised neural tube is assembled. Understanding this will provide insight into the mechanisms that produce and organise cells in complex tissues and establish the foundations for rational, predictive tissue engineering.

Research outputs

Rayon T, Stamataki D, Perez-Carrasco R, Garcia-Perez L, Barrington C, Melchionda M, Exelby E, Tybulewicz V, Fisher EMC, Briscoe J. (2020) *Species-specific pace of development is associated with differences in protein stability*. Science 369(6510) Article number eaba7667. DOI: [10.1126/science.aba7667](https://doi.org/10.1126/science.aba7667)
Despite evolutionarily conservation of molecular mechanisms, the speed of development varies substantially between species. Using *in vitro* directed differentiation of embryonic stem cells to motor neurons, we show that the programme of motor neuron differentiation runs twice as fast in mouse as in human. We provide evidence that a two-fold increase in protein stability and cell cycle duration in human cells compared to mouse can account for the slower pace of human development, indicating that global differences in kinetic parameters play a major role in interspecies differences in developmental tempo. This study establishes a new experimental system in which to address fundamental questions.

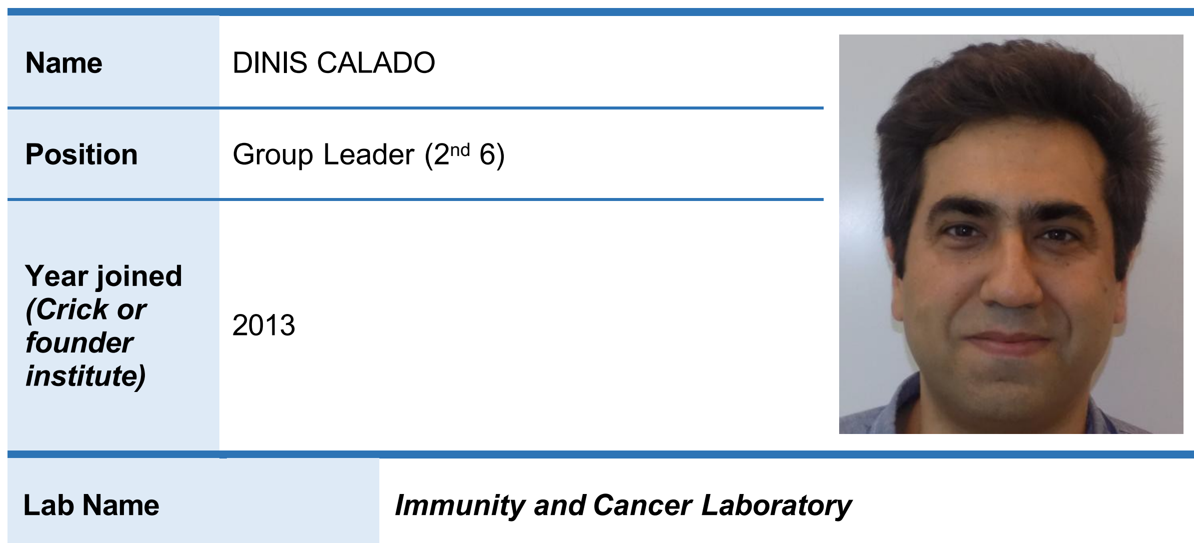
Delile J, Rayon T, Melchionda M, Edwards A, Briscoe J, Sagner A. (2019) *Single cell transcriptomics reveals spatial and temporal dynamics of gene expression in the developing mouse spinal cord*. Development 146 (12): dev173807. DOI: [10.1242/dev.173807](https://doi.org/10.1242/dev.173807)
We used single cell mRNA sequencing to generate a molecular atlas of the mouse neural tube between embryonic days 9.5-13.5. The analysis documented gene expression profiles of developing spinal neurons, but also highlighted a previously underappreciated temporal component to the mechanisms generating neuronal diversity. The data offer insight into the mechanisms responsible for neuronal specification, and provide a compendium of gene expression for classifying spinal cord cell types that will support future studies of neural tube development, function and disease. With ASF, we established 10XGenomics methods at the Crick and also implemented and developed the associated computational methods for data analysis.

Metzis V, Steinhauser S, Pakanavicius E, Gouti M, Stamataki D, Ivanovitch K, Watson T, Rayon T, Mousavy Gharavy SN, Lovell-Badge R, Luscombe NM, Briscoe J. (2018) *Nervous System Regionalization Entails Axial Allocation before Neural Differentiation*. Cell 175(4):1105-1118. DOI: [10.1016/j.cell.2018.09.04](https://doi.org/10.1016/j.cell.2018.09.04)
The prevailing view of neural induction in vertebrate embryos had been that cells are initially induced with anterior (forebrain) identity and then caudalising signals convert a proportion to posterior fates (spinal cord). Using chromatin accessibility, to define how cells adopt region-specific neural fates, combined with genetic and biochemical perturbations, we found that contrary to the established model, cells commit to a regional identity before acquiring neural identity. These findings prompt a revision to textbook models of neural induction. The study illustrates our adoption of new genomic methods (ATACseq) to address long-standing questions, and our capacity to productively collaborate with computational biologists.

Gouti, M; Delile, J; Stamataki, D; Wymeersch, FJ; Huang, Y; Kleinjung, J; Wilson, V and Briscoe, J. (2017) *A gene regulatory network balances neural and mesoderm specification during vertebrate trunk development*. Developmental Cell 41, 243-261. DOI: [10.1016/j.devcel.2017.04.002](https://doi.org/10.1016/j.devcel.2017.04.002)
Here, we reverse-engineered the transcriptional network controlling bipotent neuromesodermal progenitors (NMPs) that fuel embryo elongation by generating spinal cord and trunk mesoderm tissue. We used single-cell transcriptomics to identify the molecular signature of NMPs and together with genetic perturbations, delineated the architecture of a regulatory network architecture that balances the generation of different cell types from bipotential progenitors. The study is an example of our use of stem cell

methods and data driven mathematical modelling to address developmental questions.

Zagorski, M; Tabata, Y; Brandenberg, N; Lutolf, MP; Tkačik, G; Bollenbach, T; Briscoe, J and Kicheva, A. (2017) *Decoding of position in the developing neural tube from antiparallel morphogen gradients*. Science 356, 1379-1383 DOI: 10.1126/science.aam5887
Like many developing tissues, the vertebrate neural tube is patterned by antiparallel morphogen gradients. Using quantitative gene expression and signalling measurements we derived and validated a characteristic decoding map that relates morphogen input to the positional identity of neural progenitors. This revealed a strategy that minimises patterning errors in response to the joint input of noisy opposing gradients. The study illustrates how we integrate quantitative data, developmental and microfluidic experiments with phenological and mechanistic models.

Name	DINIS CALADO	
Position	Group Leader (2nd 6)	
Year joined *(Crick or founder institute)*	2013	

Lab Name	***Immunity and Cancer Laboratory***

Research programme and achievements

The Immunity and Cancer Laboratory studies the germinal center (GC) B-cell reaction, a stage of adaptive immunity critical for long-term protection from infection and underlying vaccination success. The evolutionary trade-off of such a reaction is the occurrence of autoimmunity and haematological cancers.

GCs are formed following the recognition of an infectious or vaccine antigen by the B-cell receptor (BCR) of a mature B-cell. This process gives rise to B-cells carrying BCRs with a wide range of affinities for antigen. The currently favoured model proposes that only those B-cells carrying BCRs with higher antigen affinity are selected to survive.

Once selected, GC B-cells can differentiate into plasma cells (PC) in which the BCR is converted into a secreted form, the antibody, that binds antigen and aids the elimination of the infectious agent. In a far more obscure process, GC B-cells can also form memory B-cells (MBC). These "experienced" B-cells are essential upon re-infection, or exposure to the vaccine antigen.

Clearly, selection of B-cells is critical for GC function, but strict affinity-dependent selection does not explain the retention of a wide range of antigen affinities in the reaction, which may be required for broadly neutralising antibody formation as seen in HIV and Influenza patients. It is also unclear whether MBCs are derived from selected cells. Research in this area is hampered by the exceedingly low number of GC B-cells undergoing selection and the absolute requirement of *in vivo* studies.

Previously, we and others identified the expression of the cell cycle regulator MYC as a marker of GC B-cells undergoing selection. Using this marker, we have characterised GC B-cell selection in unprecedented depth. Single-cell RNA-sequencing revealed unexpected diversity of selected GC B-cells and allowed the identification of surface markers defining sequential clusters of selected cells by flow-cytometry. These studies demonstrated that GC selection is in fact highly permissive and that selected B-cells include those with lower antigen affinity. We also identified clusters of selected B-cells that are candidate PC and MBC precursors. In related work, we showed that the MBC fate within selected B-cells is restricted through the action of a transcriptional repressor complex formed by MYC and MIZ1 (ZBTB17). These works enhance our understanding of GC B-cell selection, and pave the way for interventions that tailor the GC B-cell response to meet specific requirements for infection control and prevention.

In depth analysis of selected GC B-cells may also aid the identification of lymphoma precursors. Activated B-Cell Diffuse Large Cell Lymphomas (ABC-DLBCL) display enforced NF-κB activation downstream of genetic mutations. NF-κB activation plays a crucial role in B-cell to PC differentiation and the gene is transiently expressed, together

with MYC, in PC precursors. MYC expression is then suppressed as PC differentiation ensues, allowing cells to enter a post-mitotic state.

ABC-DLBCLs display the phenotype of a B-cell blocked during PC differentiation, so we wondered whether MYC overexpression could be in part responsible for this. We found instead that NF-κB synergised with MYC to produce a cancer with a PC-like phenotype, demonstrating that MYC overexpression does not interfere with B-cell phenotype loss. The cancer cells had a plasmablast phenotype (i.e. of a PC at an early differentiation stage), and NF-κB and MYC co-activation specifically made these cells addicted to IL-6.

These findings are relevant for cancer therapy, as MYC overexpression is linked to treatment resistance and relapse. We collaborated with Ed Tate's laboratory at Imperial College to show that inhibition of myristoylation was synthetically lethal with high levels of MYC in cancer cells, including in the extremely hard-to-treat double-hit lymphomas that carry concurrent MYC and BCL2 translocations. These studies led to a patent approval, the set-up of the company "Myricx" and to our laboratories being awarded the "2019 Sir David Cooksey Prize in Translation".

Research outputs

Barbosa RR, Xu AQ, D'Andrea D, Copley F, Patel H, Chakravarty P, Clear A, Calaminici M, Janz M, Zhang B, Schmidt-Supprian M, Wang J, Gribben JG, Tooze R, Fitzgibbon J, Franzoso G, Rajewsky K, Calado DP. (2020) ***Co-activation of NF-κB and MYC renders cancer cells addicted to IL6 for survival and phenotypic stability.*** **bioRxiv 2020.04.12.038414. DOI: 10.1101/2020.04.12.038414.**
NF-κB and MYC are found co-deregulated in human B and plasma-cell cancers. Using a mouse system to trace cell lineage and oncogene activation, we found that NF-κB/MYC co-deregulation produced cancers with a plasmablast-like phenotype similar to human plasmablastic lymphoma and also t(8;14)[MYC-IGH] multiple myeloma. Notably, in contrast to NF-κB or MYC activation alone, co-deregulation rendered cells addicted to IL6 for survival and phenotypic stability. We propose that conflicting oncogene-driven differentiation pressures can be accommodated at a cost in poorly-differentiated cancers.

Xu AQ, Barbosa RR, Calado DP. (2020) ***Genetic timestamping of plasma cells in vivo reveals tissue-specific homeostatic population turnover.*** **e Life 2020,9:e59850. DOI: 10.7554/eLife.59850.**
Plasma cells (PCs) are essential for protection from infection, and at the origin of incurable cancers. Current studies do not circumvent the limitations of removing PCs from their microenvironment and confound formation and maintenance. Here we characterize a genetic tool in the mouse that permits first-ever specific genetic manipulation in PCs in vivo, across immunoglobulin isotypes. This tool paves the way for an in-depth mechanistic understanding of PC biology and pathology in vivo, in their microenvironment.

Toboso-Navasa A, Gunawan A, Morlino G, Nakagawa R, Taddei A, Damry D, Patel Y, Chakravarty P, Janz M, Kassiotis G, Brink R, Eilers M, Calado DP. (2020) ***Restriction of memory B cell differentiation at the germinal center B cell positive selection stage.*** **J Exp Med 217(7):e20191933. DOI: 10.1084/jem.20191933.**
Memory B cells (MBCs) are key for protection from pathogen reinfection. However, it is mechanistically unclear how MBCs differentiate. We found that the complex formed by the transcription factors MYC and MIZ1 [ZBTB17] represses the expression of genes associated with MBC differentiation and that mice lacking MYC-MIZ1 complexes increased MBC differentiation. Thus, MYC-MIZ1 complexes restrict MBC differentiation. We propose that interventions that modulate the activity of MYC–MIZ1 complexes may tailor the immune response to meet individual requirements for infection control and prevention.

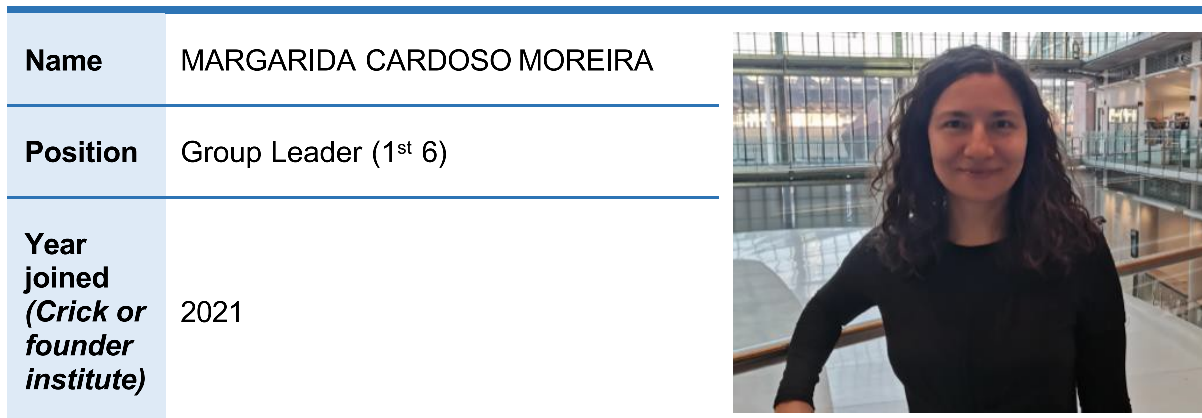
Kallemeijn WW, Lueg GA, Faronato M, Hadavizadeh K, Goya Grocin A, Song OR,

Howell M, Calado DP, Tate EW. (2019) ***Validation and invalidation of chemical probes for the human N-myristoyltransferases*****. Cell Chem Biol. 26(6):892-900.e4. DOI: 10.1016/j.chembiol.2019.03.006.**

On-target, cell-active chemical probes are of fundamental importance in chemical and cell biology, whereas poorly characterized probes often lead to invalid conclusions. Human N- myristoyltransferase (NMT) has attracted increasing interest as target in cancer and infectious diseases. Here we report an in-depth comparison of five compounds widely applied as human NMT inhibitors, using a combination of quantitative whole-proteome N- myristoylation profiling, biochemical enzyme assays, cytotoxicity, in-cell protein synthesis, and cell-cycle assays. We find that N-myristoylation is unaffected by 2-hydroxymyristic acid (100 μM), D-NMAPPD (30 μM), or Tris-DBA palladium (10 μM), with the latter compounds causing cytotoxicity through mechanisms unrelated to NMT. In contrast, drug-like inhibitors IMP-366 (DDD85646) and IMP-1088 delivered complete and specific inhibition of N-myristoylation in a range of cell lines at 1 μM and 100 nM, respectively. This study enables the selection of appropriate on-target probes for future studies and suggests the need for reassessment of previous studies that used off-target compounds.

Nakagawa R, Toboso-Navasa A, Schips M, Young G, Bhaw-Rosun L, Llorian-Sopena M, Chakravarty P, Sesay AK, Kassiotis G, Meyer-Hermann M, Calado DP. (2021) ***Permissive selection followed by affinity-based proliferation of GC light zone B cells dictates cell fate and ensures clonal breadth*****. Proc Natl Acad Sci USA 118(2):e2016425118. DOI: 10.1073/pnas.2016425118.**

Memory B cells (MBCs) and plasma cells (PCs) are formed during the so-called germinal center (GC) B cell reaction. In the GC reaction B cells mutate their B cell receptor (BCR) genes and those that acquire a higher-affinity BCR for a pathogen antigen are presumably selected to survive and differentiate, whereas B cells carrying a lower-affinity BCR die. However, this cannot explain retention of GC B cells with varied BCR affinities and the formation of MBCs that normally carry lower-affinity BCRs. This work re-defines selection of GC B cells as permissive to ensure clonal diversity and broad protection.



Name	MARGARIDA CARDOSO MOREIRA
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2021

Lab Name	***Evolutionary Developmental Biology Laboratory***

Research programme and achievements

We study how new organs originate and how they subsequently change in form and function across species.

A fundamental problem in biology is understanding how new cells, new tissues, or whole new organs are created. Our lab works on this problem by studying an exceptional organ: the placenta.

The placenta controls the physiological exchanges between the mother and her foetus and is essential for pregnancy. Our placenta originated more than 160 million years ago in the ancestors of placental mammals and marsupials. Since then, it has evolved to create an incredible diversity of forms and functions across mammals.

Placentas are thought to have evolved independently as many as 100 times in vertebrates. There are evolutionary young and old placentas in many fishes, lizards, and snakes. This makes the placenta an exceptional organ in which to study how organs originate and how they evolve across species.

In our lab we study the evolution and development of the placenta in fishes and in mammals. In one project we directly address the question of how new organs are created by identifying the genetic, cellular and developmental conditions that have allowed the repeated and independent evolution of placentas in a group of closely related fishes.

In another project we focus on one of the most fascinating aspects of pregnancy - the mother's tolerance to the direct contact between her own cells and those of her foetus. We study how maternal immune systems have evolved different solutions in different mammals (including in humans) to deal with the challenges of the foetus on the mother's immune system.

Research outputs

Wang ZY, et al. (2020) *Transcriptome and translatome co-evolution in mammals.* Nature 588, 642-647. DOI: 10.1038/s41586-020-2899-z
We showed across mammals that the rate of expression divergence is lower at the translational layer than at the transcriptional layer and further demonstrated that this is due to widespread compensatory co-evolution between the two layers.

Cardoso-Moreira M. et al. (2020) ***Developmental gene expression differences between humans and mammalian models.*** **Cell Reports, 33, 108308. DOI: 10.1016/j.celrep.2020.108308**
We systematically compared developmental gene expression profiles between human genes and their counterparts in rhesus macaque, mouse, rat, and rabbit. We found that half of human genes differ from their mouse orthologs in their developmental profiles in at least one major organs. These include more than 200 genes associated with brain, heart, and liver disease, for which mouse models should undergo extra scrutiny.

Cardoso-Moreira M. et al. (2019) ***Gene expression across mammalian organ development*****. Nature, 571, 505-509. DOI: [10.1038/s41586-019-1338-5](10.1038/s41586-019-1338-5)**
We identified general principles underlying the evolution of developmental programs across mammals and identified hundreds of genes likely to be involved in the phenotypic diversification of 7 mammalian organs.

Sarropoulos I., Marin R., Cardoso-Moreira M, Kaessmann H. (2019) ***Developmental dynamics of lncRNAs across mammalian organs and species*****. Nature, 571, 510–514. DOI: [10.1038/s41586-019-1341-x](10.1038/s41586-019-1341-x)**
We showed that developmental gene expression could successfully identify sets of functional long non-coding RNAs and identified key differences in the contribution of lncRNAs to different stages of organ development. (co-senior & co-corresponding author)

Name	JEREMY CARLTON
Position	Seconded Group Leader (King's)
Year joined *(Crick or founder institute)*	2017
Lab Name	***Organelle Dynamics Laboratory***

Research programme and achievements

Achievements from previous role
My lab focus on understanding membrane trafficking and organelle remodelling during dynamic processes such as cell division. As a newly independent group leader, my lab discovered how membranes were sealed around the reforming nuclear envelope during mitotic exit through the employment of a membrane remodelling complex called ESCRT-III. We described roles for this machinery in sealing the nuclear envelope (Olmos et al., Nature, 2015) and discovered a membrane-interacting region of a nuclear envelope-specific ESCRT-III component that was essential for assembling ESCRT-III at the reforming nuclear envelope (Olmos et al., Current Biology 2016).

Current Research Programme

We have used secondment to Crick to expand our research programme, both by developing our analysis of ESCRT-III assembly at the nuclear envelope and initiating new work in a number of areas. For the development of the role of ESCRT-III in nuclear envelope regeneration, we have discovered how the biology of this process is regulated by classical cell cycle control mechanisms, namely direct phosphorylation of CHMP7 by CDK1. This work is currently under revision in eLife. We have also examined the membrane binding element of CHMP7 in a biophysical study where we have benefited from the Crick NMR platform, the protein production STP and lipidomic collaborators at King's. This work is ongoing.

To expand my research programme, I am developing a broader interest in membrane and organellar integrity. Through a Crick/King's PhD student, we are examining the links between nuclear envelope stability and blebbing and the migratory advantage this gives metastatic melanoma cells. This work is nearing completion and has involved collaboration with the Making Lab and the EM core facility and academics at QMUL. Following this membrane integrity theme, I am also branching out into examining the contribution of membrane damage and repair to neurodegenerative diseases through a recently awarded Chan Zuckerburg Initiative grant, and I share supervision of a PhD student examining the role of ESCRT proteins in membrane repair. The major focus of my lab's expansion at Crick has been to analyse the mitotic inheritance of other organelles (notably the ER). This is a largely unresolved question in biology, and we are leveraging support of the EM core to use volume electron microscopical approaches to document how the ER is separated during division. These EM analyses are paired with biochemical and light microscopical examination of the inheritance process in living cells. This is a major project, and I am grateful to Lucy for introducing us to the Zooniverse Citizen Science platform which will be essential for analysing and

reconstructing the large volumes of FIB-SEM data generated.

During the Covid period, we turned to analysing the membrane trafficking pathways exploited by SARS-CoV-2 structural proteins and have discovered an important intracellular trafficking mechanism that we believe will help SARS-CoV-2 assemble.

Future Plans

Future Plans for return to King's involve developing more our analysis of organelle inheritance during division. I would like to look next at the interplay between different organelles, how organelle contacts are remodelled during division and how these contacts (largely with the ER) govern organellar inheritance during this process. We have some interesting data that has come from our FIBSEM analysis which suggests that the ER is remodelled during division by sliding around mitochondria and analysis of the co- regulation of these organelles during division will be an important next step. This will be work that has been initiated and directly stimulated by my interactions with Crick scientists in the EM core. Building upon our discovery of mitotic regulation of ESCRT-III function during nuclear envelope regeneration, I will next look to see how cell signalling programmes can influence organelle inheritance. Lastly, I would like to continue looking at ESCRT-III dependent nuclear envelope regeneration, but in terms of how inner nuclear membrane proteins that are necessary for ESCRT recruitment are released to allow proper structuring of chromatin in the daughter cells. I hope to work these aims into a renewal of my Wellcome Senior Research Fellowship.

Research outputs

Olmos Y, Hodgson L, Mantell J, Verkade P and Carlton JG. (2015) *ESCRT-III controls nuclear envelope reformation*. Nature 522:236-9. DOI: 10.1038/nature14503
This paper showed for the 1st time that the ESCRT-III complex localised to the nuclear envelope and controlled nuclear envelope reformation, opening a new field in ESCRT-biology.

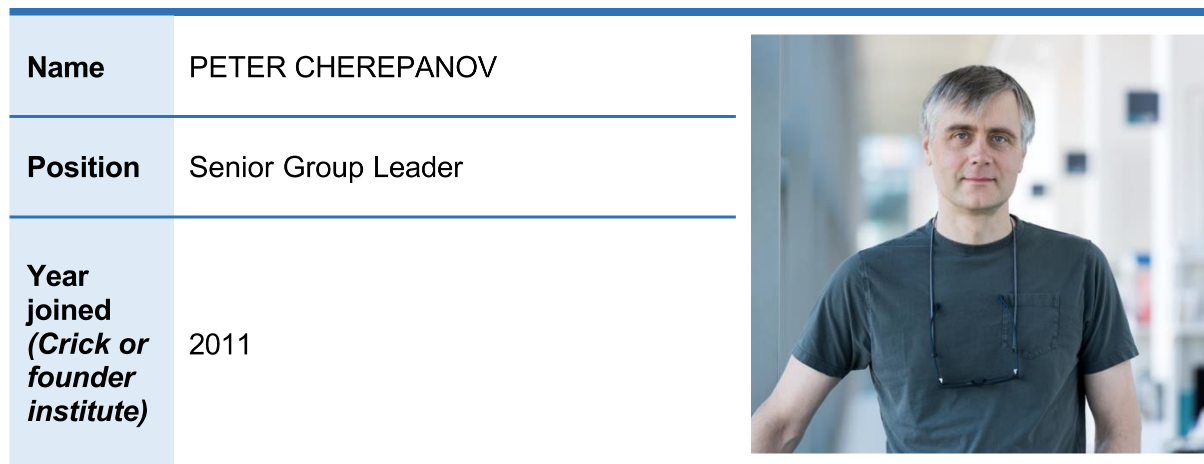
Olmos, Y, Perdrix-Rosell A and Carlton JG. (2016) *Membrane binding CHMP7 directs ESCRT-III-dependent nuclear envelope reformation*. Curr. Biol. 26:2635-41. DOI: 10.1016/j.cub.2016.07.039
This paper showed that CHMP7 is an ER-localised membrane-binding protein. We showed that the ability of CHMP7 to bind ER membranes was essential for assembling ESCRT-III at the reforming NE and for post-mitotic nuclear regeneration.

Monypenny J, Milewicz H, Flores-Borja F, Weitsman G, Cheung A, Chowdhury R, Burgoyne T, Arulappu A, Lawler K, Barber PR, [et al.,] Carlton JG and Ng T. (2018) *ALIX regulates tumour-mediated immunosuppression by controlling EGFR activity and PD-L1 presentation*. Cell Rep. 24:630-41. DOI: 10.1016/j.celrep.2018.06.066
This paper showed that the key immunosuppressive molecule, PD-L1, was secreted from cells on exosomes and that the ESCRT-associated protein, ALIX, was essential for incorporating PD-L1 onto these structures.

Ventimiglia, LN, Cuesta-Geijo MA, Martinelli N, Caballe A, Macheboeuf P, Miguet N, Parnham IM, Olmos Y, Carlton JG, Weissenhorn et al. (2018) *CC2D1B coordinates ESCRT-III activity during the mitotic reformation of the nuclear envelope*. Dev. Cell 47:547-63. DOI: 10.1016/j.devcel.2018.11.012
This paper demonstrated that ESCRT-interacting proteins could control the dynamics of ESCRT-III assembly at the reforming nuclear envelope.

Terry SJ, Dona F, Osenberg P, Carlton JG and Eggert US. (2018) *Capping protein regulates actin dynamics during cytokinetic midbody maturation*. Proc. Natl. Acad.

Sci. U.S.A. 115:2138-43. DOI: 10.1073/pnas.1722281115
This paper showed that retarding actin polymerisation in the midbody was necessary for proper recruitment of ESCRT-III and completion of cytokinesis.



Name	PETER CHEREPANOV
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2011

Lab Name	***Chromatin Structure and Mobile DNA Laboratory***

Research programme and achievements

The focus of our research is retroviral replication and host-pathogen interactions, in particular those involved in HIV entry and DNA integration. Our main techniques are cryo-electron microscopy and X-ray crystallography, which allow us to determine three-dimensional structures of viral components, as well as cellular factors that enable or counteract viral replication.

Integrase is an essential retroviral enzyme that forms the intasome at the termini of linear viral DNA and then inserts them into a host cell chromosome. Replication via a stable proviral form is the unique property that allows HIV and other retroviruses to establish life-long persistent infections. Integrase is essential for retroviral replication and, as such, is an important target for the development of anti-HIV/AIDS therapeutics.

In the past five years we made seminal contributions to understanding the mechanism of retroviral DNA integration. We determined the first structure of the intasome belonging to *Lentivirus,* the retroviral genus that includes HIV. Surprisingly, the structure revealed an extended protein scaffold comprising 16 integrase subunits. We dissected the molecular interactions involving two key viral factors (integrase and the viral structural protein Gag) with the basic repeat unit of chromatin, the nucleosome. For example, we discovered that the intasome locally remodels the nucleosomal structure, literally peeling DNA off the histone octamer surface. More recently, we explained the mode of action of the advanced clinical integrase strand transfer inhibitors Dolutegravir and Bictegravir and the mechanism of HIV resistance to this drug class via mutations in the integrase active site. The interactions with magnesium ions, which are nearly covalent in nature, are partly responsible for the extraordinary tight binding of the strand transfer inhibitors. Our results revealed that the chink in the armor of this drug class, exploited by the virus, is the extreme sensitivity of metal ions for the precise geometry and electronic properties of the ligand chelating cluster. The intasome structures determined in our laboratory are used by pharmaceutical companies to develop this important class of antiretroviral drugs.

Recent research uncovered an array of innate immunity mechanisms employed by host organisms to impede pathogen replication and the specific countermeasures the latter evolve to circumvent host restriction. One such axis of antagonism involves the human transmembrane protein SERINC5, which when incorporated into budding HIV-1 virions, can potently inhibit their subsequent entry into host cells. In addition, SERINC5 strongly enhances the ability of antibodies to neutralize the virus. Using cryo-EM we were able to determine three-dimensional structures of two members of the SERINC protein family, revealing a novel protein fold

comprising ten transmembrane helices. Guided by the structure, we dissected the critical regions of SERINC5 that contribute to its antiviral activities. The mechanism of HIV-1 restriction by the SERINC family of proteins remains one of the hottest topics in the field and is our next goal.

Research outputs

Cook, N.J., Li, W., Berta, D., Badaoui, M., Ballandras-Colas, A., Nans, A., Kotecha, A., Rosta, E., Engelman, A.N. and Cherepanov, P. (2020) *Structural basis of second-generation HIV integrase inhibitor action and virus escape*. Science 367, 806-810. DOI : 10.1126/science.aay4919

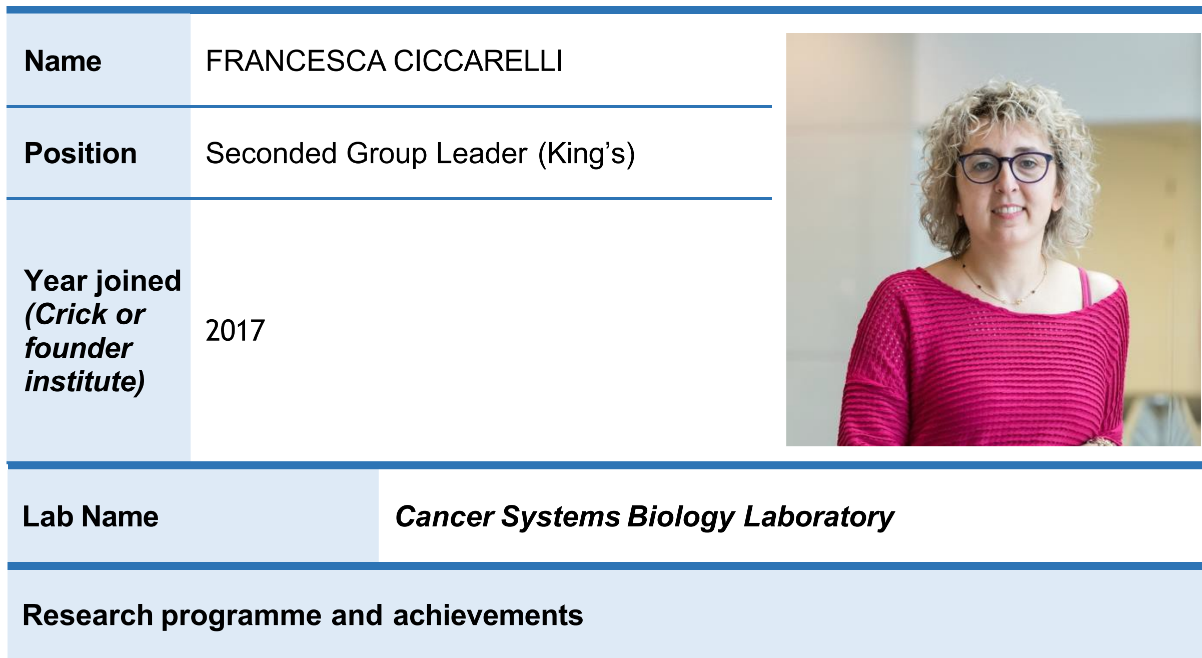
HIV integrase inhibitors represent some of the most impactful antimicrobial inhibitors. The second-generation drugs display improved barriers to the emergence of resistance, which spearheaded their worldwide rollout. Yet not even the most advanced compounds are immune to viral resistance. Our results explained the mechanism of viral resistance associated with the most common drug resistance mutations. Furthermore, we established the key difference between the first and second-generation strand transfer inhibitors, which will inform further development of this drug class.

Pye, V.E., Rosa, A., Bertelli, C., Struwe, W.B., Maslen, S.L., Corey, R., Liko, I., Hassall, M., Mattiuzzo, G., Ballandras-Colas, A., Nans, A., Takeuchi, Y., Stansfeld, P.J., Skehel, J.M., Robinson, C.V., Pizzato, M. and Cherepanov, P. (2020) *A unique bipartite structural organisation defines the SERINC family of HIV-1 restriction factors*. Nat. Struct. Mol. Biol. *27*, 78-83. DOI: 10.1038/s41594-019-0357-0

In this work we determined the structure of SERINC5, a potent HIV-1 restriction factor and discovered a novel transmembrane protein fold. The work has important implications for understanding the antagonistic host-virus interactions and for the development of future antiviral therapies.

Ballandras-Colas, A., Maskell, D.P., Serrao, E., Locke, J., Swuec, P., Jonsson, S.R., Kotecha, A., Cook, N.J., Pye, V.E., Taylor, I.A., Andresdottir, V., Engelman, A.N., Costa, A. and Cherepanov, P. (2017) *A supramolecular assembly mediates lentiviral DNA integration*. Science 355, 93-95. DOI: 10.1126/science.aah7002

Lentiviral IN proteins are notoriously poorly behaved *in vitro*, and the HIV1 intasome has eluded structural biologists for over two decades. Prior research resulted in a collection of partial crystal and NMR structures that did not explain how lentiviral integrase synapses viral DNA ends. This paper described the first structure of the lentiviral intasome, solving



Name	FRANCESCA CICCARELLI
Position	Seconded Group Leader (King's)
Year joined *(Crick or founder institute)*	2017

Lab Name	***Cancer Systems Biology Laboratory***

Research programme and achievements

PAST AND PRESENT

We apply computational and wet-lab approaches to study gastrointestinal cancer genetics, focusing on translational applications.

Representative research lines of my lab include:

(1) identifying cancer driver genes in oesophageal cancer to enhance early detection. We have developed a new approach to identify cancer driver genes based on machine learning to overcome the widespread intra- and inter-patient cancer heterogeneity of oesophageal cancer. This approach builds on our distinctive expertise to characterise the systems-level properties of cancer genes and use them to predict new cancer driver events in individual patients.

(2) targeting cancer vulnerabilities to develop novel intervention. We have optimised a computational and experimental method that led to the successful identification of dependencies involving known tumour suppressors such as SMARCA4, CDH1, DNMT3A, and STAG2. We aim to refine this method to prioritise vulnerabilities with high potential of being therapeutically relevant and test them in patient-derived organoids (PDOs, which we have established in our lab).

(3) profiling immune and genetic heterogeneity in cancer onset and response to therapy. We recently discovered that patients with multiple primary bowel cancers inherit damaging mutations in a variety of immune genes, including cytokines and Toll-like receptors. These patients also present with aberrant immune profiles and high levels of tumour immune infiltrates. The hypothesis is that the altered immune genes modify gut immunity by inducing perturbations in the homeostatic immune network. We therefore set up a high-dimensional, multi-regional and multi-omic platform based on genomic, transcriptomic and single cell immune-phenotyping (solid CyTOF) screening. We are currently applying this platform to unravel the interactions between aberrant immunological functions and cancer presentation and map the dynamic interplay in space and time between immune and genetic intra-tumour heterogeneity in response to immunotherapy.

FUTURE PLANS

I see our future work increasingly intertwined with translational cancer research to exploit the full potential of patient profiling and data integration in directing clinical intervention. In the next years, we will focus mostly on two areas:

(1) apply personalised cancer driver predictions in the clinical setting. We have started to explore the utility of our machine learning approach in the clinic by evaluating our predictions of driver events during the cancer genomic medicine multi-disciplinary team meetings in the oncology department of King's College Hospital. In parallel, together with the CRUK Commercial Partnerships team responsible for the commercialisation of AI and Big Data assets, we are exploring the possibility of patenting and commercialising our

software.
(2) optimise our platform for high-dimensional tumour profiling for patient stratification. We are mapping the dynamic interplay between immune and genetic intra-tumour heterogeneity in tumours from patients showing complete response, primary and acquired resistance to immunotherapy in the context clinical trials. From one side, this will allow us to unravel the roles of innate and adaptive immunity in response to immunotherapy. From the other side, this approach will help patient stratification and inclusion in clinical trials based on biomarkers of response.

Research outputs

Nulsen J, Misetic H, Yau C, Ciccarelli FD. (2021) *Pan-cancer detection of driver genes at the single-patient resolution*. Genome Medicine 13(1):12. DOI: 10.1186/s13073-021-00830-0
Paper describing our machine learning approach that integrates somatic alteration data with systems-level gene properties to predict drivers in individual patients. We demonstrate robust performance and benchmark its performance against other driver detection methods showing a lower false positive rate and superior patient driver coverage.

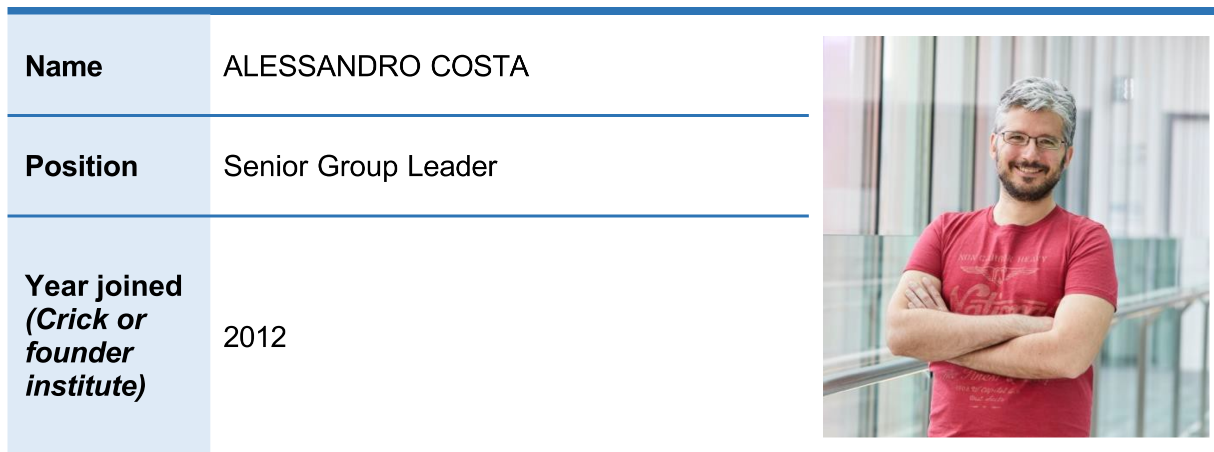
Bortolomeazzi M, Keddar R, Montorsi L, Benedetti L, Temelkovski D, Choi S, Petrov N, Todd K, Ward S, Wilson G, Al Bakir M, Swanton C, John S, Miles J, Banafshe B, Parker PJ, Rodriguez-Justo M, Shiu KK, Spencer J, Ciccarelli FD. (2021) *Immunogenomics of colorectal cancer response to immune checkpoint blockade.* bioRxiv. DOI: 10.1101/2020.12.15.422831
Multi-omic and high dimensional profile of 543 tumour regions and associated tumourmicroenvironment colorectal cancers (CRCs) subsequently treated with Pembrolizumab or Nivolumab. We were able to show that anti-PD1 inhibitors are most effective in highly infiltrated CRCs where they may release the interactions between macrophages and CD8 T cells thus promoting their priming and expansion in intra -tumour niches.

Mourikis, T, Benedetti L, Foxall E, Perner J, Cereda M, Lagergren J, Howell, M, Yau, C, Fitzgerald R, Scaffid P, Ciccarelli FD. (2019) *Patient-specific cancer genes contribute to recurrently perturbed pathways and establish therapeutic vulnerabilities in esophageal adenocarcinoma*. Nature Comms 10:3101. DOI: 10.1038/s41467-019-10898-3
Application of our machine learning method to complete the annotation of driver events in 261 oesophageal cancer patients. We also experimentally validate the potential of predicted drivers to enhance oesophageal cancer cell proliferation capabilities.

Repana D, Nulsen J, Dressler L, Bortolomeazzi M, Kuppili Venkata S, Tourna A, Yakovleva A, Palmieri T and Ciccarelli FD. (2019) *The Network of Cancer Genes (NCG): a comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens*. Genome Biology 20:1. DOI: 10.1186/s13059-018-1612-0
https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1612-0
Latest release of our manually curated repository of 2372 genes whose somatic modifications have known or predicted cancer driver roles. These genes were collected from 275 publications, including two sources of known cancer genes and 273 cancer sequencing screens of more than 100 cancer types from 34,905 cancer donors and multiple primary sites. This represents a more than 1.5-fold content increase compared to the previous version. The resource is widely used as a source of cancer genes.

Cereda M, Gambardella G, Benedetti L, Iannelli F, Guerra R, Mourikis TP, Puccio I, Patel D, Basso G, Sinha S, Laghi L, Spencer J, Rodriguez-Justo M, Ciccarelli FD. (2016) *Patients with genetically heterogeneous synchronous colorectal cancer carry rare damaging germline mutations in immune-related genes.* Nature Communications 7:12072. DOI: 10.1038/ncomms12072
We show that multiple colorectal cancers affecting the same patient have independent

genetic origins, acquire dissimilar somatic alterations, and have different clone composition. These patients show a higher occurrence of inherited damaging mutations in immune-related genes and have a different composition of immune cell populations in tumour and normal mucosa. This suggests an environmental field effect that promotes multiple tumours likely in the background of inflammation.



Name	ALESSANDRO COSTA
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2012

Lab Name	***Macromolecular Machines Laboratory***

Research programme and achievements

In eukaryotic cells, DNA replication is tightly regulated to ensure that the genome is duplicated only once per cell cycle. Errors in the control mechanisms that regulate chromosome ploidy cause genomic instability, which is linked to the development of cellular abnormalities, genetic disease and the onset of cancer.

Initiation of eukaryotic genome duplication requires three distinct steps. First, DNA replication start sites are identified and targeted for the loading of an inactive MCM helicase motor, which encircles the double helix. Second, MCM activators GINS and Cdc45 are recruited, causing duplex-DNA untwisting. Third, upon interaction with additional firing factors, the MCM ring opens to eject one DNA strand, leading to establishment of the replication fork and duplication by dedicated replicative polymerases.

A core interest of our group is studying the molecular mechanism for the activation of an origin of replication. In addition, we seek to understand how sister chromatid cohesion is established at the replication fork, in a process that involves the topological entrapment of two replicated DNA filaments by the ring-shaped cohesin complex. Thirdly, we are interested in the cellular mechanisms to bypass DNA roadblocks that cause fork stalling.

The natural substrate of the eukaryotic replication machinery is not DNA but rather chromatin, formed of nucleosome arrays that compact the genome. Chromatin plays important regulatory roles in all steps of DNA replication, by dictating origin start-site selection and stimulating replication fork progression. Only by studying chromatin replication will we understand the molecular basis of genome propagation. To this end, we are developing new protocols to perform biochemistry experiments under the cryo- electron microscope (cryo-EM), to image chromatin duplication at high resolution, frozen as it is being catalysed. Using these strategies we seek to generate a molecular movie of the entire chromosome replication reaction.

Within the last five years, our group has provided significant contributions to the chromosome biology field. We have employed time-resolved approaches to find that the MCM helicase motor is loaded onto duplex DNA in a concerted and sequential manner. We found that the loaded form of the MCM is a double hexamer that encircles but does not open the DNA duplex. Conversely, our structural studies indicate that the active form of the replicative helicase (Cdc45-MCM-GINS, or CMG) translocates on single-stranded and not duplex DNA. We used two parallel approaches, in collaboration with John Diffley's and Hasan Yardimci's groups at the Crick, to describe a failsafe mechanism whereby bidirectional DNA replication starts only when both MCM helicases have transitioned from interacting with duplex- to

single-stranded DNA, ensuring genome stability maintenance. We found that CMG translocation involves the rotation of DNA inside the central channel of the MCM ring, in a process controlled by ATP binding and hydrolysis. We found that the leading-strand polymerase Pol epsilon is anchored on the posterior side of the advancing CMG helicase, perfectly poised to trap the 3' end of DNA, as it egresses the MCM ring. Working with Frank Uhlmann's group at the Crick we have solved the structure of a loading intermediate of cohesin, explaining how the loader contributes to topologically embracing the double helix, en route to the cohesin ring locking around DNA. We have described the architecture of the Fanconi anemia core complex, providing insights into a key step in the mechanism of interstrand-crosslink repair. This pathway is essential to remove detrimental roadblocks that impair replisome progression. Finally, we have provided our cryo-EM expertise in a collaboration with Peter Cherepanov's group, in three seminal structural studies on the mechanism of retroviral integration.

We have three major goals for the next five years. First, we seek to describe the molecular events that lead to CMG formation. In a manuscript in preparation we explain how only loaded MCM double hexamers and not loading-competent MCM assemblies can be phosphorylated by DDK, in an essential step step for the downstream CMG assembly. We are now structurally characterising the CMG formed at origins to understand how active helicase formation promotes DNA melting. We intend to describe the mechanism of lagging strand ejection from the MCM central channel, in a subsequent step towards replication fork establishment. A second major goal is understanding how the CMG helicase and the three replicative polymerases work together to promote replisome progression, and how nucleosomes are disassembled ahead of the fork during this process, while parental histones are recycled onto duplicated DNA. Finally, we seek to image by cryo-EM *in vitro* reconstituted DNA replication combined with cohesin loading reactions to understand how sister chromatid cohesion is coordinated at the replication fork.

Research outputs

Miller TCR, Locke J, Greiwe JF, Diffley JFX, Costa A. (2019) *MECHANISM OF HEAD-TO-HEAD MCM DOUBLE-HEXAMER FORMATION REVEALED BY CRYO-EM.* Nature 575(7784):704-710. DOI: 10.1038/s41586-019-1768-0
The MCM replicative helicase is loaded onto duplex DNA as a double hexamer. Here we use time-resolved cryo-EM to establish that double hexamer formation is concerted and sequential. Rather than solving one individual macromolecular structure, here we image a whole reaction reconstituted *in vitro*. We employ newly developed *in silico* reconstitution approaches to describe the full context of the helicase loading reaction, studied on a near-native, chromatinised origin of replication. This study radically changes our approach to investigating chromosome replication with cryo-EM.

Goswami P, Abid Ali F, Douglas M, Locke J, Purkiss A, Janska A, Eickhoff P, Early A, Nans A, Cheung A, Diffley JF, Costa A. (2018) *STRUCTURE OF DNA-CMG-POL EPSILON ELUCIDATES THE ROLES OF ESSENTIAL, NON-CATALYTIC POLYMERASE MODULES IN THE EUKARYOTIC REPLISOME.* Nature Commun 9(1):5061. DOI: 10.1038/s41467-018-07417-1
Eukaryotic origin firing depends on the assembly of the CMG helicase. A key step in this process is the recruitment of GINS to MCM, which also requires the leading-strand polymerase Pol epsilon. Here we used cryo-EM to find that the essential function of Pol epsilon in the replisome is to provide the physical link that directly connects the MCM helicase and the GINS activator. Reconstituted DNA replication demonstrates that the

integrity of this physical link is required for origin activation.

Abid Ali F, Douglas ME, Locke J, Pye VE, Nans A, Diffley JF, Costa A. (2017) *CRYO-EM STRUCTURE OF A LICENSED DNA REPLICATION ORIGIN.* Nat. Commun 8(1):2241. DOI: 10.1038/s41467-017-02389-0

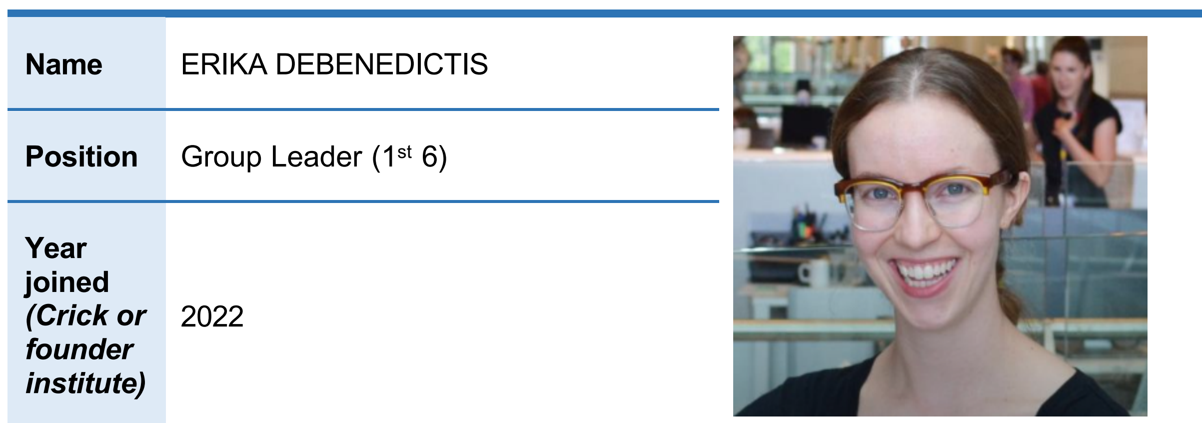
Eukaryotic origin licensing involves the loading of a catalytically inactive double hexamer of MCM around duplex DNA. Whether or not MCM loading is sufficient to melt the DNA double helix has been debated. To resolve this issue we used cryo-EM to demonstrate that helicase loading does not significantly alter the structure of B-form DNA. To perform this study we developed a protocol to discriminate between DNA-bound and DNA-free complexes *in silico*. This tool significantly extends our ability to handle compositional heterogeneity within a single-particle cryo-EM dataset, paving the way to high-resolution imaging of multi-component reactions reconstituted *in vitro*.

Swuec P, Renault L, Borg A, Shah F, Murphy VJ, van Twest S, Snijders AP, Deans AJ, Costa A. (2017) *THE FA CORE COMPLEX CONTAINS A HOMO-DIMERIC CATALYTIC MODULE FOR THE SYMMETRIC MONO-UBIQUITINATION OF FANCI-FANCD2.* Cell Rep 18(3):611-623. DOI: 10.1016/j.celrep.2016.11.013

Activation of the main DNA interstrand crosslink repair pathway in higher eukaryotes requires mono-ubiquitination of FANCI and FANCD2 by FANCL, the E3 ligase subunit of the Fanconi anemia core complex. Here, we found that FANCB, FANCL, and FAAP100 form a dimer of heterotrimers, containing two FANCL molecules that are ideally poised to mono-ubiquitinate both FANCI and FANCD2. This study provides key architectural information on the Fanconi anemia core complex and is our first study combining electron microscopy and crosslinking mass spectrometry. This approach has now become our method of choice for studying the structure of new macromolecular assemblies.

Abid Ali F, Renault L, Gannon J, Gahlon HL, Kotecha A, Zhou JC, Rueda D, Costa A. (2016) *CRYO-EM STRUCTURES OF THE EUKARYOTIC REPLICATIVE HELICASE BOUND TO A TRANSLOCATION SUBSTRATE.* Nat Commun 7:10708. DOI: 10.1038/ncomms10708

The CMG is the active eukaryotic replicative helicase. Whether replication fork progression involves CMG translocation on single- or double-stranded DNA has been long debated. Here we used cryo-EM to describe the structure of CMG bound to a forked-DNA substrate. We found that the MCM ATPase ring encircles and stretches one single DNA filament, indicating that the replicative helicase is a single-stranded DNA translocase. This is the first study where we combined cryo-EM and single-molecule fluorescence approaches, to describe dynamic protein-DNA interaction processes.



Name	ERIKA DEBENEDICTIS
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2022

Lab Name	***Biodesign Lab***

Research programme and achievements

We use evolution as a powerful engineering tool to craft new biological processes. Evolution was one of humanity's first engineering tools. Ancient humans unknowingly used evolution to create useful organisms when they cultivated crops and bred animals. We have used evolution for biological engineering long before it was understood that breeding works through the slow accumulation of DNA changes over many generations.

The Biodesign Laboratory aims to wield evolution as a powerful tool for engineering parts of biology that are still beyond our understanding. Unlike traditional engineering tools that are quite systematic, evolution is highly unpredictable. We use automated robots to help us conduct evolution at scale in a safe, reliable way. This allows us to rapidly work together with nature to create new, useful tools in biology.

At the microscale, we are interested in engineering proteins, which are tiny molecular machines that are responsible for making living organisms work using chemistry. We aim to use systematic evolution to engineer custom proteins so that they can cure diseases or better understand biology. At the larger scale, evolution can help us create new organisms for uses beyond Earth, such as microbes that can grow on Mars and can be used to manufacture useful products like bioplastic and food.

Research outputs

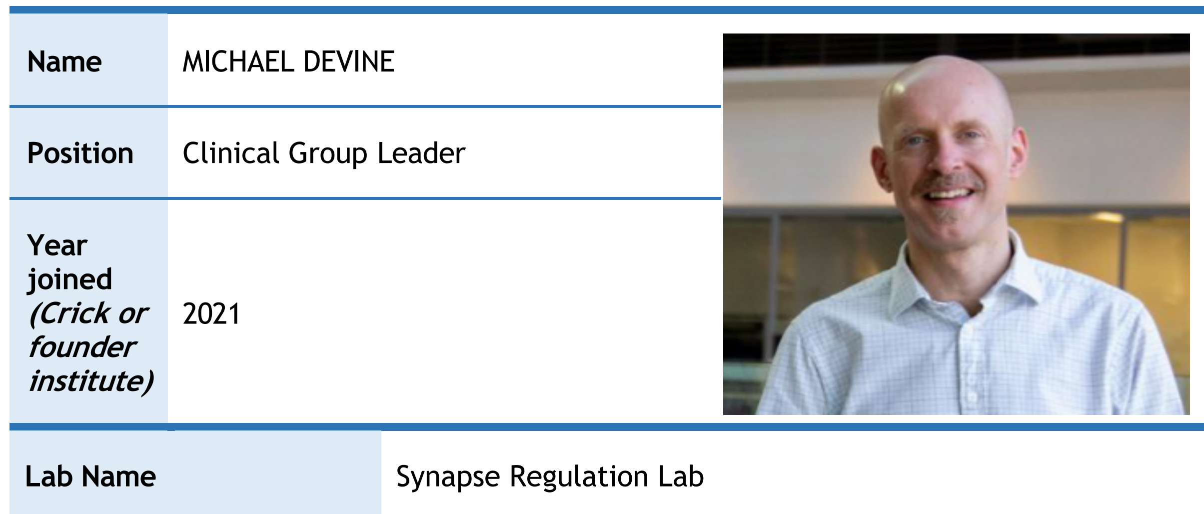
DeBenedictis* (corresponding), Söll, and Esvelt, *Measuring the tolerance of the genetic code to altered codon size*. eLife 2022
Although the canonical genetic code is based on three-DNA base codons, in this paper we show that it is surprisingly tolerant to four-base codons: 9/20 canonical amino acids can be incorporated into proteins in response to four-base codons without any protein engineering.

DeBenedictis*, Chory*, Gretton, Wang, Golas, and Esvelt, *Systematic molecular evolution enables robust biomolecule discovery*. Nature Methods 2021
In this paper, we invented PRANCE, which is a device for high-throughput continuous directed evolution and demonstrate that evolved biomolecules can be produced more robustly using our platform.

DeBenedictis*, Carver*, Chung, Söll, Badran, Multiplex Suppression of Four Quadruplet Codons via tRNA Directed Evolution. Nature Communications 2021
This paper demonstrates how directed evolution can be applied to refactor translation components for use with an expanded codon size. With tRNA refactoring alone, I am able to achieve a substantial amount of quadruplet codon translation: notably, this paper is the first

report of processive translation of adjacent quadruplet codons, an important prerequisite for all-quadruplet translation.

Chory*, Gretton*, DeBenedictis, and Esvelt, "Enabling high-throughput biology with flexible open-source automation", Molecular Systems Biology 2021
In this paper, we show that the standard software that runs liquid handling robots is limiting and show that new types of experiments are possible if they use a more flexible API.



Name	MICHAEL DEVINE
Position	Clinical Group Leader
Year joined *(Crick or founder institute)*	2021

Lab Name	Synapse Regulation Lab

Research programme and achievements

We are working on understanding how neuronal synapses are regulated, and how this regulation goes wrong in diseases that affect the brain. We focus on the range of interactions that take place between mitochondria and synaptic function.

The nervous system is essential, because it enables us to respond to and interact with our environment. However, it is metabolically expensive: the human brain accounts for just 2% of the body's mass, yet demands 20% of the body's energy. Much of this energy is used to power synapses, where neurons connect and communicate with each other. An individual neuron contains many hundreds of synapses, and active synapses use more energy than resting synapses. Therefore, neurons need a way to manage these highly variable energy demands. Mitochondria are small, specialised structures found in large numbers within nearly all cells of the body, and generate most of the energy used to power cellular activity. They are also mobile, and can move around within cells to where they are most needed. As a result, they frequently position themselves at synapses, where they can provide energy to power neuronal communication. Our previous work has shown that, as well as providing energy to support synaptic activity, mitochondria can also move to synapses and reduce their activity by modulating local Ca^{2+} signals at synapses which changes their propensity to release neurotransmitters. This mechanism can be used by neurons as a form of synaptic plasticity. Our subsequent work has suggested that this mechanism is impaired in neurodegenerative diseases such as Parkinson’s.

Our aim is to understand why mitochondria govern synaptic transmission, the molecular mechanisms by which this regulation occurs, and how this regulation changes in neurological and psychiatric disease. Through understanding more about this form of synaptic regulation, we hope to open up new ways of treating these conditions

Research outputs

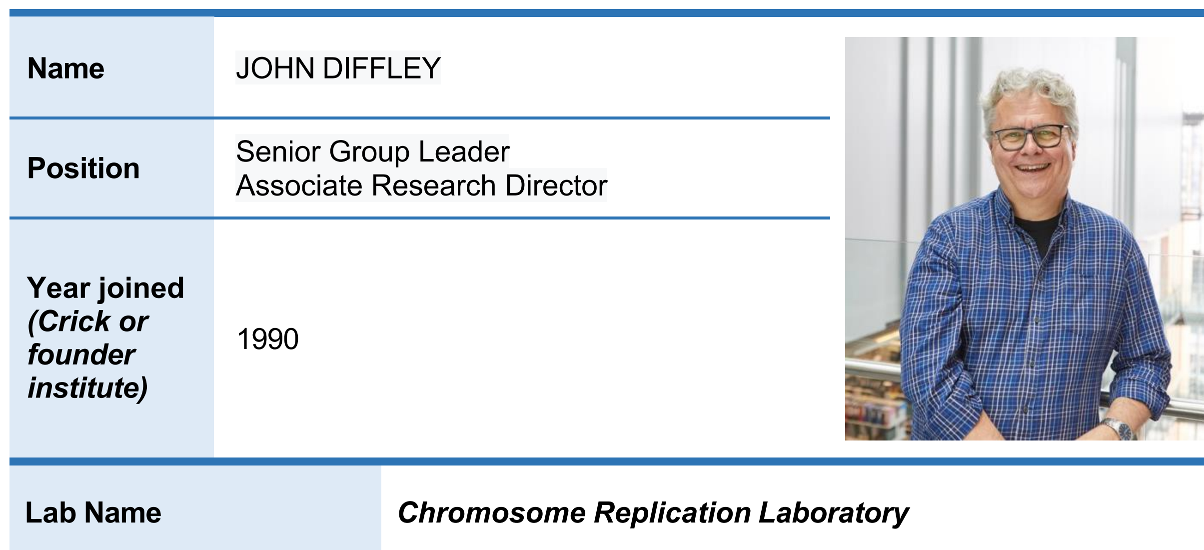
<u>Devine MJ</u>, Szulc BR, Howden JH, López-Doménech G, Ruiz A, Kittler JT (2022) Mitochondrial Ca2+ uniporter haploinsufficiency enhances long-term potentiation at hippocampal mossy fibre synapses. ***Journal of Cell Science*** **135:jcs259823 doi: [10.1242/jcs.259823](https://doi.org/10.1242/jcs.259823)**
Here we used optical and electrophysiological approaches in MCU+/− neurons to reveal a previously unrecognised role for mitochondria in regulating presynaptic plasticity of a major excitatory pathway.

Nadappuram BP, Cadinu P, Barik A, Ainscough AJ, <u>Devine MJ</u>, Kang M, Gonzalez- Garcia J, Kittler JT, Willison KR, Vilar R, Actis P, Wojciak-Stothard B, Oh SH, Ivanov AP, Edel JB (2018) Nanoscale Tweezers for Single Cell Biopsies. ***Nature Nanotechnology*** **14:80 doi: [10.1038/s41565-018-0315-8](https://doi.org/10.1038/s41565-018-0315-8)**
Featured in Nature Medicine (https://www.nature.com/articles/d41591-018-00002-5).
In this report, a new nanobiopsy technique is described for sampling very small numbers of molecules, or individual organelles such as mitochondria, from live cells without affecting cell viability. I provided neuronal cell cultures and worked on the mitochondrial extraction experiments in the paper, with Binoy Nadappuram (1st author).

Little D, Luft C, Mosaku O, Lorvellec M, Yao Z, Paillusson S, Kriston-Vizi J, Gandhi S, Abramov AY, Ketteler R, <u>Devine MJ</u>*, Gissen P* (2018) A single cell high content assay detects mitochondrial dysfunction in iPSC-derived neurons with mutations in SNCA. ***Scientific Reports*** **8(1):9033 doi: [10.1038/s41598-018-27058-0](https://doi.org/10.1038/s41598-018-27058-0)**
*equal contribution
This reports the development of high content assays to distinguish Parkinson’s patient iPSC-derived neurons from control neurons on imaging criteria alone, which could potentially be used in high throughput screens to identify novel therapeutic agents for Parkinson’s disease.

Vaccaro V*, <u>Devine MJ</u>*, Higgs NF, Kittler JT (2017) Miro1-dependent mitochondrial positioning drives the rescaling of presynaptic Ca2+ signals during homeostatic plasticity. ***EMBO Reports*** **18(2):231-240.**
*equal contribution

Here we show that presynaptic mitochondria buffer local Ca^{2+} signals, thereby reducing neurotransmission. This is important because previously presynaptic mitochondria were thought only to sustain or increase synaptic transmission via providing ATP.



Name	JOHN DIFFLEY	
Position	Senior Group Leader Associate Research Director	
Year joined *(Crick or founder institute)*	1990	

Lab Name	***Chromosome Replication Laboratory***

Research programme and achievements

Overview:
My laboratory uses a variety of approaches to understand the mechanism and regulation of eukaryotic DNA replication. We have historically studied this in budding yeast, and we continue to exploit this as a very useful model system, but we are increasingly interested in understanding this process in human cells as well. We are interested in understanding how the MCM helicase is loaded at origins, how it is activated and how it nucleates replisome assembly. We are also interested in how DNA damage checkpoints are activated by stalled replication forks and how active checkpoints regulate replication, especially how they stabilise stalled replication forks. Recently, we have become very interested in understanding how chromatin is replicated, how histones ahead of the fork are re-deposited behind the fork, how this is coordinated with assembly of nucleosomes from newly synthesised histones and how heterochromatin is replicated.

Past quinquennium:
We had previously published the reconstitution of MCM helicase loading at replication origins. In this past quinquennium, we have shown that MCM loading is quasi-symmetrical. ORC loads both MCM hexamers by a similar mechanism (Paper 2) requiring two opposed ORC binding sites. Using time-resolved cryo-EM with our collaborator at the Crick, Alessandro Costa Paper 5), we showed how this works: ORC binds to a high affinity binding site, loads the first hexamer, and then releases from its binding site. Then it or a second ORC molecule binds at a degenerate ORC binding site, stabilised by a novel interaction between ORC and the loaded MCM hexamer, to load the second hexamer by the same mechanism as the first.

In the last month of the previous quinquennium, we published the reconstitution of replication initiation with purified budding yeast proteins. This provided a crucial tool to understand how the MCM double hexamer is converted to two active CMG helicases. We showed (Paper 1) that the critical firing factor Sld3 is a phosphopeptide binding protein which binds specifically to MCM subunits (4 and 6) which have been phosphorylated by Dbf4-dependent kinase. We went on to show that double hexamer separation, CMG formation and initial DNA melting are coordinated with ADP release and ATP rebinding before Mcm10 activates the two CMG helicases to pass each other as they unwind DNA (Paper 4). We showed that, to achieve maximum replication rates, the leading strand must be synthesised by DNA polymerase ε together with PCNA, and the accessory factors Mrc1 and Csm3/Tof1 are required. We showed that a variety of factors are required to replicate chromatinised templates, most important of these being the histone chaperone, FACT (Paper 3).

Future plans:

We continue to dissect the MCM loading and activation mechanisms with purified proteins. We have evidence that different mechanisms are used depending on the distance between the ORC sites. We also continue to try to understand how chromatin is replicated. Using the reconstituted system we have found multiple FACT and histone binding sites in the replisome and we are trying to understand how they work together. We have evidence that the replisome cannot replicate heterochromatin templates; we are trying to identify additional factors required for heterochromatin replication. We also believe we have reconstituted the complete replication checkpoint with purified proteins. We are trying to understand the roles of the two mediators (Mrc1 and Rad9) along with the variety of Mec1 activators in responding to different forms of DNA damage/replication stress. Finally, we are expressing all of the proteins required to reconstitute replication with human proteins.

Research outputs

Deegan, T.D., Yeeles, J.T., and Diffley, J.F.X. (2016) ***Phosphopeptide binding by Sld3 links Dbf4-dependent kinase to MCM replicative helicase activation*****. EMBO J 35, 961-973. DOI: 10.15252/embj.201593552**

Here we showed that Sld3, which we previously identified as being one of two essential cyclin dependent kinase (CDK) substrates in replication, is a phosphopeptide binding protein which binds specifically to Mcm4 and Mcm6 when they have been phosphorylated by Dbf4 dependent kinase (DDK). Sld3 then directly recruits Cdc45 to MCM and, via CDK phosphorylation, recruits the remaining firing factors. We had previously shown that Sld3 is also one of two targets of the DNA damage checkpoint kinase involved in inhibiting origin firing in response to DNA damage. Thus, Sld3 plays key roles with all three kinases that regulate replication (CDK, DDK, Rad53).

Coster, G., and Diffley, J.F.X. (2017) ***Bidirectional eukaryotic DNA replication is established by quasi-symmetrical helicase loading*****. Science** ***357*****, 314-318. DOI: 10.1126/science.aan0063**

This paper shows that loading of the MCM double hexamer is a quasi-symmetrical reaction: two ORC molecules bound at two opposing sites of different affinity each recruit and load a single hexamer. The distance between the ORC binding sites is not critical. Subsequent work has provided further evidence for this from cryo-EM.

Kurat, C.F., Yeeles, J.T., Patel, H., Early, A., and Diffley, J.F.X. (2017) ***Chromatin Controls DNA Replication Origin Selection, Lagging-Strand Synthesis, and Replication Fork Rates*****. Mol Cell** ***65*****, 117-130. DOI: 10.1016/j.molcel.2016.11.016**

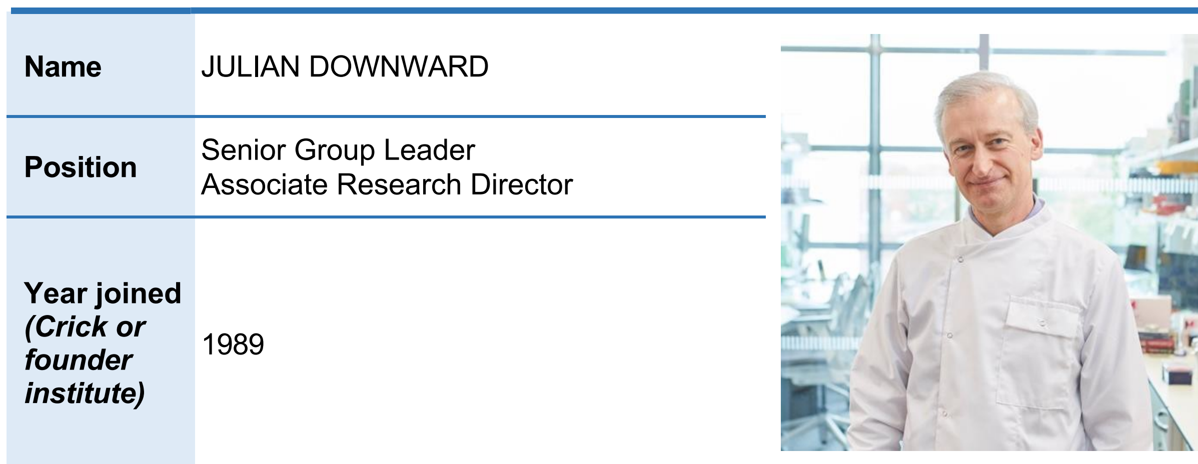
In this and the accompanying paper (Yeeles et al. 2017 Mol Cel 65, 105-116. DOI: 10.1016/j.molcel.2016.11.017) we describe the reconstitution of full chromatin replication. We first identified all of the factors required for complete and rapid replication of naked DNA. Then we identified and characterised factors required to replicate chromatinised templates. We showed FACT is essential for chromatin replication, whilst nucleosome remodellers and histone acetylases help chromatin replication. In addition, chromatin enforces origin specificity and Okazaki fragment processing. Finally, we found that histones are efficiently transferred to nascent DNA.

Douglas, M.E., Ali, F.A., Costa, A., and Diffley, J.F.X. (2018) ***The mechanism of eukaryotic CMG helicase activation*****. Nature** ***555*****, 265-268. DOI: 10.1038/nature25787**

This paper provided the first view of how the inactive MCM double hexamer is converted to two active CMG helicases. We showed MCM remains bound to ADP after loading; firing factors trigger ADP-ATP exchange; ATP rebinding causes double hexamer splitting, initial

DNA melting and CMG formation. Active helicases then translocate N-terminus first.

Miller, T.C.R., Locke, J., Greiwe, J.F., Diffley, J.F.X., and Costa, A. (2019) ***Mechanism of head-to-head MCM double-hexamer formation revealed by cryo-EM*****. Nature** ***575*****, 704-710. DOI: 10.1038/s41586-019-1768-0**
Using time-resolved cryo-EM, we showed that ORC binds to its high affinity binding site to load the first MCM hexamer. ORC then releases this site and it, or another ORC molecule then binds the B2 element, which contains a degenerate ORC binding site. This binding is stabilised by a novel interaction between the Orc6 subunit of ORC and the N-terminus of the MCM hexamer. ORC then recruits and loads the second hexamer by the same mechanism as the first hexamer.



Name	JULIAN DOWNWARD	
Position	Senior Group Leader Associate Research Director	
Year joined *(Crick or founder institute)*	1989	

Lab Name	***Oncogene Biology Laboratory***

Research programme and achievements

The Oncogene Biology Laboratory focuses on characterisation of molecular mechanisms involved in tumorigenesis by RAS and related oncogenes with a view to identifying novel therapeutic strategies for RAS and RAC mutant cancers.

Principal achievements in the past quinquennium have been:
1. Exploration of combinatorial approaches to targeting RAS mutant cancers. This was started before the advent of direct KRAS inhibitors and attempted to find the optimal approach to blocking the actions of mutant RAS through inhibition of druggable signalling nodes both downstream and upstream of RAS. We have now included the use of G12C mutant specific KRAS inhibitors and sought to find ways of potentiating the activity of these agents by interfering with signalling network adaptation to their actions.

We have investigated the role in cancer of RAS signalling through one specific effector enzyme, PI 3-kinase p110α. We have shown this link to be essential in KRAS driven lung tumour maintenance. In addition, we find that wild type RAS signalling to p110α is critical in mutant EGFR driven lung cancer.
2. Our investigation of the interaction of RAS proteins with other isoforms of PI 3-kinase has led us to the conclusion that, unlike p110 α, γ, and δ, the ubiquitously expressed p110β is regulated not by RAS proteins but by related small GTPases from the RAC family. We have further studied the function of an activated oncogenic mutant form of RAC1, P29S, establishing the mechanistic basis for its impact in BRAF mutant melanoma. P29S RAC1 acts through several effectors, in particular the transcriptional regulator SRF/MRTF, to promote in melanoma cells a mesenchymal differentiation state that is resistant to BRAF inhibitors.
3. Due to awareness of the limitations in advanced cancers of even the most effective treatments targeting growth signalling pathways, as evidenced by the common development of resistance to agents targeting mutant BRAF and EGFR oncogenic drivers, we have investigated how RAS oncogenic signalling might be contributing to control of the tumour immune microenvironment. We asked whether there are specific mechanisms by which RAS might promote the ability of tumours to evade the immune system that might provide a rational basis for combining inhibitors of the RAS signalling network with modulators of the immune system. We have shown that in lung cancer cells, RAS, acting principally via the MEK pathway, controls the expression of the immune checkpoint regulatory ligand PD-L1 by modulating the stability of its mRNA through control of the 3' AU rich element binding protein Tristetraprolin.

Future plans for the Oncogene Biology Laboratory will focus on the interplay between the signalling networks in the cancer cells driven by RAS oncoproteins and the patient's immune system, and whether this offers unique therapeutic opportunities. We have

developed a number of mouse models of lung cancer that aim to better replicate the high tumour mutational burden found in the clinic, this being achieved for example by overexpressing genes such as the cytidine deaminase APOBEC3B or deleting genes such as 0'6 methylguanine methyltransferase, resulting in accumulation of clinically relevant mutations. These models are then used to study the response of tumours to therapies targeting RAS signalling, including G12C KRAS inhibitors, focusing on the effects of the interaction between the tumour and the host immune system. We have optimised protocols in imaging mass cytometry and single cell sequencing that allow us to determine the impact of RAS on the tumour immune microenvironment and indicate the optimal approaches to combining RAS pathway targeted therapy with immunotherapy to achieve ultimate complete eradication of tumours.

In addition, we will investigate mechanisms of metastatic spread of RAS mutant cancer cells, in particular the roles of proteins identified as essential for metastasis in a recent functional genomic screen in the lab, including the palmitoyl transferase ZDHHC20. We will also further explore RAF1 signalling downstream of RAS in lung cancer mouse models and investigate the possibility of blocking RAS interaction with effectors in a KRAS isoform selective manner. This latter is the subject of translational drug discovery projects in collaboration with commercial partners such as AstraZeneca.

Research outputs

M.A. Coelho, S. de Carné Trécesson, S. Rana, D. Zecchin, C. Moore, P. East, B. Spencer-Dene, E. Nye, K. Barnouin, A.P. Snijders, W.S. Lai, P.J. Blackshear, J. Downward. (2017) *Oncogenic RAS signalling promotes tumour immunoresistance by stabilising PD-L1 mRNA*. Immunity 47, 1083-1099 DOI: 10.1016/j.immuni.2017.11.016

This work establishes for the first time a link between oncogenic RAS signalling and increased immuno-suppressive expression of the immune checkpoint protein PD-L1. RAS signalling results in phosphorylation and inactivation of TTP, a factor involved in degrading PD-L1 mRNA transcripts. As TTP inactivation causes accumulation of PD-L1 mRNA, interfering with the RAS pathway increases TTP binding to AU-rich elements of the transcripts, decreases PD-L1 protein production, and leads to enhanced antitumor immunity.

M.M. Murillo, S. Rana, B. Spencer-Dene, E. Nye, G. Stamp, J. Downward. (2018) *Disruption of the interaction of RAS with PI 3-kinase induces regression of mutant EGFR-driven lung cancer*. Cell Reports 25, 3545-3553. DOI: 10.1016/j.celrep.2018.12.003

We have investigated the role in cancer of RAS signalling through one specific effector enzyme, PI 3-kinase p110α, creating an inducible mouse model in which we can assess the need for the RAS binding domain (RBD) of p110α in the maintenance of RAS driven lung tumours. As well the interaction of RAS with p110α being required for the maintenance of established KRAS driven lung tumours, we further show here that interaction of endogenous wild type RAS with p110α is also required for lung cancer formation and maintenance driven by upstream signalling components such as mutant EGFR.

D.A. Lionarons, D.C. Hancock, S. Rana, P. East, C. Moore, M.M. Murillo, J. Carvalho, B. Spencer-Dene, E. Herbert, G. Stamp, D. Damry, D.P. Calado, I. Rosewell, R. Fritsch, R.R. Neubig, M. Molina-Arcas, J. Downward. (2019) *RAC1P29S Induces a Mesenchymal Phenotypic Switch via Serum Response Factor to Promote Melanoma Development and Therapy Resistance*. Cancer Cell 36, 68-83. DOI: 10.1016/j.ccell.2019.05.015

Metastatic melanoma is a lethal disease, in part because of rapid acquisition of resistance to therapy. Using genetically engineered mouse models, we demonstrate that the activating RAC1 P29S mutation, present in up to 5% of melanoma patients, cooperates with BRAF as a driver of melanoma initiation and promotes BRAF inhibitor resistance. The critical RAC1 effector pathway in melanoma is shown to be the transcription factor complex SRF/MRTF, which initiates a switch to a mesenchymal-like state characterized by therapy resistance.

Therapeutic targeting of SRF/MRTF may have potential to reverse BRAF inhibitor resistance in melanoma patients bearing the oncogenic RAC1 P29S mutation.

M. Molina-Arcas, C. Moore, S. Rana, F. van Maldegem, E. Mugarza, P. Romero-Clavijo, E. Herbert, S. Horswell, L.S. Li, M.R. Janes, D.C. Hancock, J. Downward. (2019) ***Development of combination therapies to maximize the impact of G12C KRAS inhibitors in lung cancer*****. Science Translational Medicine 11:eaaw7999. DOI: 10.1126/scitranslmed.aaw7999**
KRAS is the most commonly mutated oncogene in human lung cancer, but direct targeting of RAS proteins has proved difficult. A recently developed inhibitor of G12C mutant KRAS protein inhibits lung cancer progression in mouse models but does not provide durable regressions. By studying signalling pathways required for survival of KRAS mutant cells, we demonstrate a strong and selective potentiation of the effects of G12C KRAS inhibitors when mTOR and/or IGF1R are also inhibited. Using mutant specific G12C KRAS inhibitors rather than MEK inhibitors in these combinations is associated with greater specificity and lower toxicity. We propose that adding IGF1R and mTOR inhibitors will increase the impact of G12C KRAS inhibitors in clinical trials.

D. Zecchin, C. Moore, F. Michailidis, S. Horswell, S. Rana, M. Howell, J. Downward (2020). ***Combined targeting of G-protein-coupled receptor and EGF receptor signalling overcomes resistance to PI3K pathway inhibitors in PTEN-null triple negative breast cancer*****. EMBO Molecular Medicine e11987 DOI: 10.15252/emmm.202011987**
As a consequence of loss of PTEN tumor suppressor function, triple-negative breast cancers (TNBCs) show aberrant PI3K pathway activation. However, clinical efficacy of PI3K inhibitory drugs has so far been modest in this setting. We identify here a molecular network that impairs response to PI3K inhibitors in PTEN-null TNBCs. Both the G protein-coupled receptor PAR1 and EGFR were discovered to signal to PI3Kβ in these tumors. Simultaneous inhibition of PI3Kβ and EGFR efficiently blunted the activation of the pathway and produced anti-tumor activity both in vitro and in vivo in different PTEN-null TNBC models.

Name	ALBERTO ELOSEGUI-ARTOLA
Position	Physical Sciences Group Leader
Year joined *(Crick or founder institute)*	2021

Lab Name	Cell and Tissue Mechanobiology Lab

Research programme and achievements

Postdoctoral research: I stayed in Pere Roca-Cusachs' lab at IBEC in Barcelona for over 5 years studying the molecular mechanisms that regulate mechanosensing and rigidity sensing. After several relevant findings, I decided that I wanted to apply this knowledge to more complex, closer to in vivo, three-dimensional environments. Therefore, by the end of 2017 I moved to Boston to start my postdoc in Dave Mooney's lab at Harvard SEAS. Dave's lab is predominantly a biomaterials lab and I wanted to learn from them to create my own niche. The hypothesis that guided my postdoc was that the time-dependent viscoelastic properties of the extracellular matrix regulate tissue spatial and temporal organization, both during development and disease. To be able to address this question and with the help of the Mooney Lab, I developed a hydrogel system with controlled mechanical and chemical properties. This work has just been published and shows that the passive viscoelastic properties of the extracellular matrix regulate tissue growth dynamics (Elosegui-Artola, Gupta et al., Nature Materials 2023). During my first year in Dave's lab, I obtained a Marie Skłodowska-Curie fellowship. Additionally and also during my first year in Boston (2018), I prepared my ERC Starting Grant application. I was awarded with this grant just before COVID-19 started.

Starting my lab at the Crick in 2021: I started the Cell and Tissue Mechanobiology Lab in June 2021. My vision is that the combined influence of chemical and physical cues controls biological functions. Cells in our bodies are in constant communication with other cells and with the extracellular environment. Through this constantly evolving communication, cells receive both physical and chemical signals that dictate their response (reviewed in Elosegui-Artola, COCEBI, 2021). To address the role of these different signals, we need engineered smart systems where we can independently control each cue. Therefore, in the lab we design these systems and apply them to several different projects that have the extracellular matrix mechanics in common. Historically, most research has focused on static conditions and has not considered that tissues are highly dynamic and evolve with time. We are studying the influence of the time-dependent viscoelastic properties of he extracellular matrix in development as well as in cancer progression. During these 20 months, we have been able to develop all protocols that the lab needs, and we have promising results in all our projects. These promising results allow us to have a reasonably clear path as to how to continue our research. Last, I would like to apply for a BBSRC grant this year.

Research outputs

A Elosegui-Artola, A Gupta, A Najibi, BR Seo, R Garry, CM Tringides, I de Lázaro, M Darnell, W Gu, Q Zhou, DA Weitz, L Mahadevan, DJ Mooney. *Matrix viscoelasticity controls spatiotemporal tissue organization*. Nature Materials 22, 117-127 (2023) .
https://doi.org/10.1038/s41563-022-01400-4 .

The most relevant peer-reviewed publication of my research programme. This work demonstrates the importance of the extracellular matrix viscoelasticity in malignant transformation and in morphogenesis.

I Andreu, I Granero-Moya, NR Chahare, K Clein, M Molina Jordàn, AEM Beedle, A Elosegui-Artola, X Trepat, B Raveh, P Roca-Cusachs. Mechanosensitivity of nucleocytoplasmic transport. Nature Cell Biology 24, 896-905. 2022.

This is an important publication that builds on my previous work (Elosegui-Artola et al. Cell 2017) and shows the relevance of nuclear flattening in nucleo-cytoplasmic transport.

Elosegui-Artola A. The extracellular matrix viscoelasticity as a regulator of cell and tissue dynamics. Current Opinion in Cell Biology. https://doi.org/10.1016/j.ceb.2021.04.002

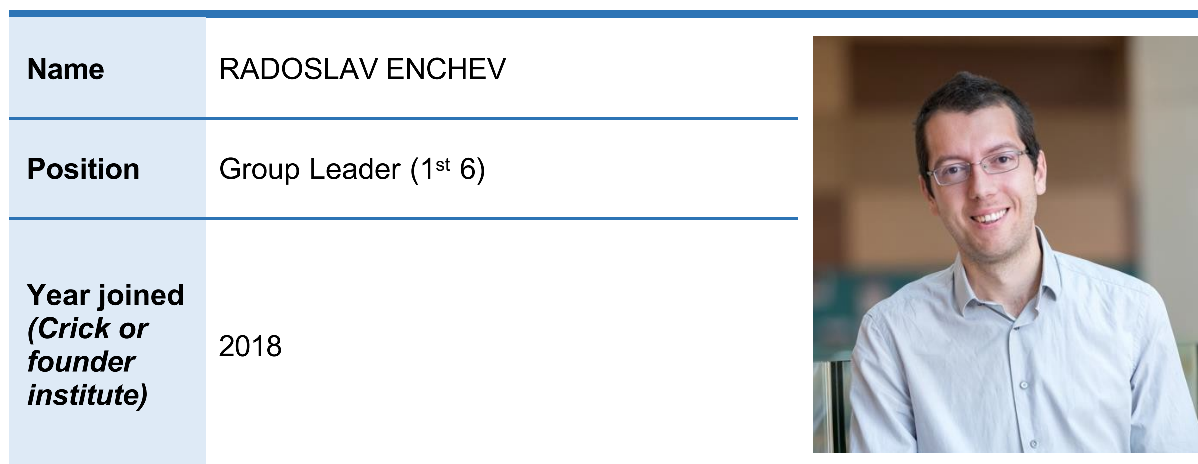
This is a review that explains the importance of time-dependent mechanical properties in biological function.

CM. Tringides, N Vachicouras, I de Lázaro, H Wang, ATrouillet, BR Seo, A Elosegui-Artola, F Fallegger, Y Shin, C Casiraghi, K Kostarelos, SP. Lacour, DJ Mooney. Viscoelastic surface electrode arrays to interface with viscoelastic tissues. Nature Nanotechnology. 16, 1019-1029. 2021.

A relevant and recent publication where we studied the importance of engineering viscoelastic substrates to study electric signals in vivo.

M Uroz, A Garcia-Puig, I Tekeli, A Elosegui-Artola, JF Abenza, A Marín-Llauradó, S Pujals, V Conte, L Albertazzi, P Roca-Cusachs, A Raya, X Trepat. Traction forces at the cytokinetic ring regulate cell division and polyploidy in the migrating zebrafish epicardium. Nature Materials. 18, p1015-1023. 2019.

I have chosen this paper as I developed the computational model. We mainly do wet lab experiments but we can also do computational modelling.



Name	RADOSLAV ENCHEV
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2018

Lab Name	***Visual Biochemistry Laboratory***

Research programme and achievements

Molecular biology research aims to obtain a comprehensive mechanistic description of cellular physiology. In general, the best-case outcome of most biochemical studies is the structural and kinetic characterisation of their underlying chemical processes. Since no single experimental technique can directly deliver such information in a broadly applicable manner, structural studies of usually static macromolecules are complemented by kinetic characterisation and molecular dynamics simulations. In practice progress is often impeded by the typical requirement for large amounts of labelled or crystallised samples. Moreover, the widely-used strategy of using mutants or crosslinking probes to stabilise otherwise short-lived catalytic intermediates may be prone to artefacts and precludes time-resolved tracking of molecular events.

My group is developing and applying a method that largely overcomes these limitations by allowing the direct observation of biochemical processes at atomic spatial- and milliseconds time-resolution by combining microfluidics and electron cryomicroscopy (cryo-EM). We recently published a prototype that reproducibly prepares cryo-EM samples, improves the quality of the sample relative to standard methods and allows monitoring of the progression of a biochemical process over three orders of magnitude of time (tens to thousands of milliseconds)[1]. It consists of a syringe pump driven microfluidic chip that ensures mixing and incubation of two samples, a nozzle which uses gas to spray the sample as a thin plume onto a sample grid and plunging into liquid ethane. The reactants are subjected to subsequent 3D structure determination by cryo-EM and single particle analysis. Iterating the procedure at increasing incubation times after sample mixing allows the visualisation of a biochemical binding and/or enzymatic reaction as a time-lapse "movie".

An engineering subgroup in the lab has substantially improved the published workflow and has nearly completed a next-generation device. It achieves higher quality sample preparation with sub-microlitre sample amounts, owing to complete automation through an on-board computer, elaborate valving and use of additive manufacturing for integration of the microfluidic chip and a modified nozzle. We anticipate that these advances will greatly accelerate the adoption of this new standard in preparing samples for cryo-EM and single particle analysis, enabling off-equilibrium structural studies and facilitating the technology's spread to academic and industrial applications.

Naturally, we are applying our method to fundamental biological questions. The core biological interests of the group are two-fold: elucidating the structural basis for the molecular mechanisms of DNA double strand break repair by homologous

recombination and the regulation of ubiquitin signalling.

Genome integrity is essential for organismal health and reproduction. However, the chemical environment and cellular metabolism frequently cause DNA damage, counteracted by multiple repair pathways. Homologous recombination repair (HRR) is responsible for the nearly error-free restoration of DNA double-strand breaks and is of tremendous importance to human health and gene technology and therapy.

Although many HRR components are amenable to *in vitro* analysis, their functionally relevant interactions are often transient and recalcitrant to conventional trapping techniques. This limits the insights of structural studies and impedes the development of better-targeted diagnostics and therapy. The key questions we are pursuing revolve around uncovering the structural and kinetic basis for specificity during Rad51-mediated homology search. We have already demonstrated the utility of time-resolved cryo-EM to visualise and quantify the ATP-dependent growth of filaments of the bacterial recombinase RecA on ssDNA. We are now focusing on applying the method to study microhomology search intermediates by elucidating structural specificity determinants and directly correlating them to kinetic behaviour. We will extend these studies to human Rad51 and paralogues, and comprehensively describe the defining biochemical step of homologous recombination.

Ubiquitination is a versatile signalling mechanism in eukaryotes, which regulates most aspects of cellular metabolism. Ubiquitination itself is a tightly regulated process and the biochemical pathways regulating its specificity and signalling outcomes present promising drug targets. Cullin-RING E3 ligases (CRLs) comprise nearly half of all cellular ubiquitin ligases. All CRLs are regulated by only a few factors which collectively reshape the cellular CRL pool and enable various rapid adaptive cellular responses to internal and external cues. Despite a wealth of biochemical and cellular data, how a specific CRL is assembled following a signalling event remains poorly understood because the underlying biochemical events are either too short-lived and/or mediated by very low affinity interactions to be easily tractable by conventional structural determination techniques.

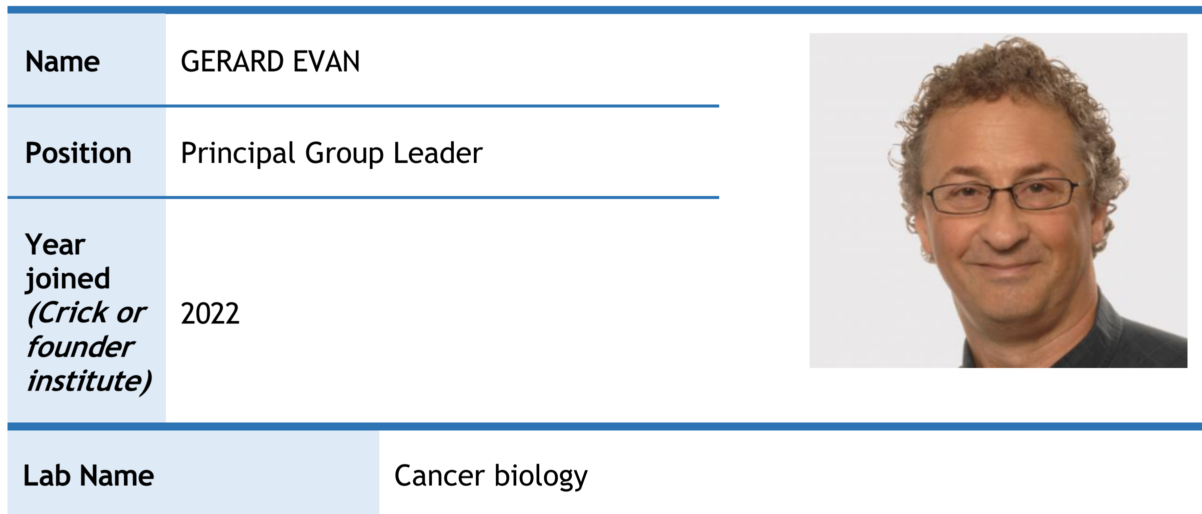
Current projects in the lab are using time-resolved cryo-EM analysis to elucidate the detailed mechanisms of CSN-mediated CRL deneddylation and CAND1-mediated exchange of CRL substrate receptors.

Research outputs

Mäeots M.E., Lee B., Nans A., Jeong, S.G., Esfahani M.M.N., Ding S., Smith D.J., Lee C.S., Lee S., Peter M., Enchev R.I. (2020) *Modular microfluidics enables kinetic insight from time-resolved cryo-EM*. Nat. Commun. 11, 1–14. DOI: 10.1038/s41467-020-17230-4
Cryo-EM has the potential to study any native conformation of a macromolecule. However, the sample preparation time is high, compared to the timescale of most protein interactions and conformational changes. In this paper, we established a robust method of time-resolved cryo-EM sample preparation. We produced high-quality samples for microscopy while speeding up the process of making them by several orders of magnitude. This allowed samples to be collected within 30ms of the initiation of a biochemical reaction, within the timeframe of many critically important and interesting processes. This enables a whole new class of experiments in structural biology research.

Mosadeghi R., Reichermeier K.M., Winkler M., Schreiber A., Reitsma J.M., Zhang Y., Stengel F., Cao J., Kim M., Sweredoski M.J., Hess S., Leitner A., Aebersold R., Peter M., Deshaies R.J and Enchev R. I. (2016) *Structural and kinetic analysis of CSN activation and the cullin–RING ubiquitin ligase deneddylation cycle*. e Life, 10.7554/eLife.12102. DOI: 10.7554/eLife.12102
Cullin–RING ubiquitin E3 ligases (CRL) comprise one of the largest families of eukaryotic

regulatory enzymes and control most cellular processes. They are activated by neddylation, covalent attachment of the ubiquitin-like protein Nedd8. This modification is reversed by a large molecular machine, the COP9 signalosome (CSN). This paper presents a comprehensive kinetic analysis of CSN-CRL binding and de-neddylation and elucidated their structural underpinnings. CSN uses a complex induced-fit mechanism to evolve a very high affinity catalytic intermediate, which rapidly dissociates after catalysis. This mechanism shapes the entire cellular CRL network, enabling an incredibly rapid adaptive response to a changing environment.



Name	GERARD EVAN
Position	Principal Group Leader
Year joined *(Crick or founder institute)*	2022

Lab Name	Cancer biology

Research programme and achievements

Cancers arise through progressive accumulation of mutations and epigenetic errors in somatic cell clones. For this reason, cancers are highly heterogenous, both between cancers in different patients and within the cancer of each patient. This remarkable heterogeneity is beguiling and has fostered the notion that understanding and treating cancers will require a complete description of the mutated landscape of each tumour in each patient – personalized medicine. Moreover, a variety of novel technologies are now available that allow analysis and categorization at the level of individual cells, making possible a finely granular exposition of cancer complexity: Nonetheless, downstream analysis of such data remains largely re-descriptive: cancers are emergent pathologies arising from complex interactions between many cell types and it is not clear that their behaviours can be understood by summing their component parts.

Notwithstanding the evident complexity of human cancers, our laboratory focuses on the profound, but frequently overlooked, commonalities across all cancers. Thus, while many oncogenic mutations reside in functionally degenerate mitogenic signalling networks ,the aggregate outputs of these networks eventually funnel through a common, limited and functionally non-redundant set of YES/NO downstream effectors that then govern the proliferation, survival, migration and stromal interactions of cancer cells. Flux through these common conduits We seek to determine how

Currently, we are specifically addressing two outstanding questions.

First, cancers of a particular provenance (i.e. cell type or organ of origin) closely resemble each other histopathologically, even when driven by different ensembles of oncogenic mutation. Conversely, cancers arising from different tissues but driven by the same oncogenic mutations look quite different from each other. Given that each cancer is unique, where do these signature tissue-specific phenotypes come from and why are they there? Our data reveal that the distinctive phenotypes of different cancers are a property of their host tissue, not their driver oncogenes. In effect, cancers are hacks of tissue-specific regenerative programmes. Normally, activation of regenerative programmes after injury and repair are transient, enforced by the innate attenuation of mitogen-driven mitogenic signalling pathways. However, when hacked by mutations instead of mitogens, such regenerative programmes become persistently engaged and it is this persistent tissue-specific repair phenotype that dictates the tumour phenotype. Our data show that each of these diverse tumour stromal phenotypes is instructed by discrete, tissue-specific ensembles of paracrine instructive signals emanating from the transformed neoplastic epithelium. We are currently determining both the nature and hierarchical relationships of these paracrine, tissue-organizing signals in, specifically, cancers of lung, pancreas and liver.

Second, we are trying to understand how and why cancer therapies that target mitogenic

oncoproteins, such as EGFR, Raf, Ras and Myc, cause tumours to regress (along with all their attendant stroma and inflammatory sequelae) - the expectation is that interference with a mitogenic signals would instead just cause cancer cell arrest but not regress. Our studies have shown that just as active oncogenes hack into physiological tissue-specific regenerative programmes, interruption of Myc, the common downstream obligate effector of all mitogenic oncogenic mutants, hacks into tissue-specific physiological programmes that drive resolution of wound repair after infection and injury, restoring normal tissue architecture and function. When actively imposed on a tumour by Myc inhibition, these same resolution programmes trigger a complex choreography of tumour and stroma that essentially reverses tumour formation and, as with normal injured tissues, restores the neoplastic lesion to its normal mass, architecture and function.

Research outputs

Sodir, N.M., Kortlever, R.M., Barthet, V.J.A., Pellegrinet, L., Campos, T., Kupczak, S., Brown Swigart, L., Soucek, L., Arends, M.J., Littlewood, T.D., Evan, G.I. (2020). Myc instructs and maintains pancreatic adenocarcinoma phenotype. Cancer Discovery. Jan 15. pii: CD-19-0435.

Established a new component of the underlying mechanism by which Ras and Myc cooperate oncogenically and defined new roles for oncogenic signalling in modulating local tumour immune and inflammatory microenvironment. Identified key tumour-stromal signalling that is required for both pancreatic cancer progression when Myc is activated and, remarkably, regression when Myc activity is blocked. Provides a new and coherent mechanism for how and why targeted therapies work in cancer.

Sodir NM, Pellegrinet L, Kortlever RM, Campos T, Kwon YW, Kim S, Garcia D, Perfetto A, Anastasiou P, Swigart LB, Arends MJ, Littlewood TD, Evan GI. Reversible Myc hypomorphism identifies a key Myc-dependency in early cancer evolution. Nat Commun. 2022 Nov 9;13(1):6782. doi: 10.1038/s41467-022-34079-x. PMID: 36351945.

Identifed a critical role for Myc in passage through a hitherto unknown early bottleneck in cancer evolution. Oncogenic mutations in the Ras pathway are insufficient to drive invasion and spread of indolent pre-neoplastic cells because they are unable to modify their adjacent local stroma. Activation of Myc above a critical level kicks such pre-neoplastic cells into cancers by driving release of key chemokines and cytokines that modify local stroma and immunity.

Bywater M, Burkhart D, Sabò A, Straube J, Pendino V, Hudson J, Quaife-Ryan G.A., Porello E.R., Rae J., Parton R.G., Kress T.R., Amati B., Littlewood T.D., *Evan G.I., Wilson C.H. (2020). Reactivation of Myc transcription in the heart unlocks its proliferative capacity. Nature Commun; Apr 14;11(1):1827. doi: 10.1038/s41467-020-15552-x (*corresponding author).

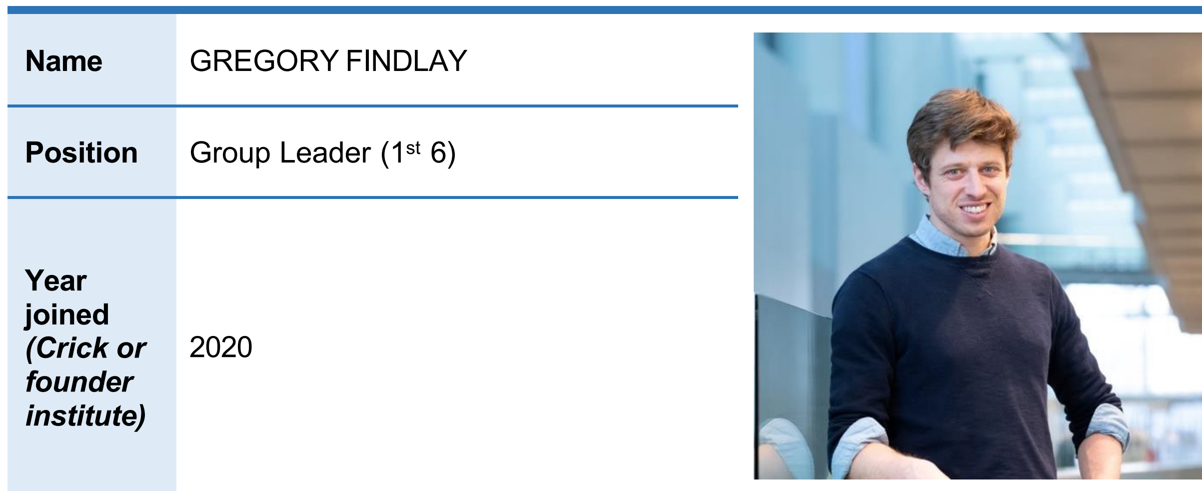
Different tissues show different sensitivities to the mitogenic and oncogenic action of Myc: adult heart is an example of a Myc-refractory tissue. We identified P-TEFb activity as the key limiting determinant of whether tissues are permissive for Myc transcriptional activation and mitogenesis and that Ras signalling induces P-TEFb. Enforced expression of P-TEFb in adult heart allows Myc to drive proliferation in adult carciomyocytes in vivo.

Kreuzaler, P., Clarke, M.A., Brown, E.J., Wilson, C.H., Kortlever, R.M., Piterman, N., Littlewood, T., Evan, G.I., and Fisher, J. (2019). Heterogeneity of Myc expression in breast cancer exposes pharmacological vulnerabilities revealed through executable mechanistic modeling. Proc. Natl. Acad. Sci. 116, 22399-22408. doi: 10.1073/pnas.1903485116.Epub 2019 Oct 14.

Demonstration that breast cancers umors exhibit interclonal mutualism wherein cells with

high-Myc expression facilitate tumor growth by promoting protumorigenic stroma yet concomitantly

suppress Wnt expression, which renders them dependent for survival on paracrine Wnt provided by low-Myc–expressing clones. Such obligate mutualism constitues a potential vulnerability in cancers that we show may be exploited therapeutically.



Name	GREGORY FINDLAY
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Genome Function Laboratory***

Research programme and achievements

The Genome Function Laboratory develops methods to understand genetic differences and the effects they can have on a person's life and health.

The DNA sequence of the human genome is nearly identical between any two people, yet each person also has millions of genetic variants that make their genome unique. With current DNA sequencing technology, we can readily find the set of rare genetic variants present in each individual, yet it remains hugely challenging to predict how each variant will impact health. In the context of certain diseases like cancer and heart disease, recognising a deleterious mutation in a patient early can guide intervention s that prolong life expectancy by many years. This clinical need to understand the effects of variants, coupled with a basic desire to understand the diversity of functions encoded in the human genome, motivates the lab to systematically study genetic variants with a particular focus on tumour suppressor genes.

1. One method we developed called "Saturation Genome Editing" allows us to test the effects of all possible single nucleotide variants across key regions of the human genome. We applied this method to a gene linked to breast and ovarian cancer called *BRCA1*, testing thousands of different variants. Hundreds of these had been previously seen in people, yet their health consequences were largely unknown. Now that we have clear evidence as to whether or not these variants are likely to cause cancer, many patients are getting more accurate diagnoses. We are now applying Saturation Genome Editing to four additional genes in which mutations cause different tumours, with the goal of being better able to predict disease occurrence and treatment response from knowledge of variant effect.

2. A second genome editing technology we previously developed called ScanDel allows us delete thousands of different genomic regions at once to better understand how each one functions and to discover regulatory elements. These high-throughput CRISPR experiments can reveal the logic behind how our genes are regulated and promise to shine light on new therapeutic strategies.

3. We are developing more highly scalable methods that will allow us to engineer and test millions of human variants using the newest genome editing tools, including "prime editing" and "base editing" systems. We hope to apply artificial

intelligence to these large experimental data sets as a means of modelling variants' effects on diverse molecular processes genome-wide.

4. To date, the high-throughput methods we and others use are limited in the depth of information provided about each variant assayed. Future work in the group will couple single-cell genomics approaches to the multiplex genome editing strategies outlined above to reveal in exquisite detail what effects a variant has on processes such as splicing and transcriptional activity genome-wide. We predict this work will allow us to characterise many cancer-relevant mutations whose effects are hard to study through more limited experimental systems.

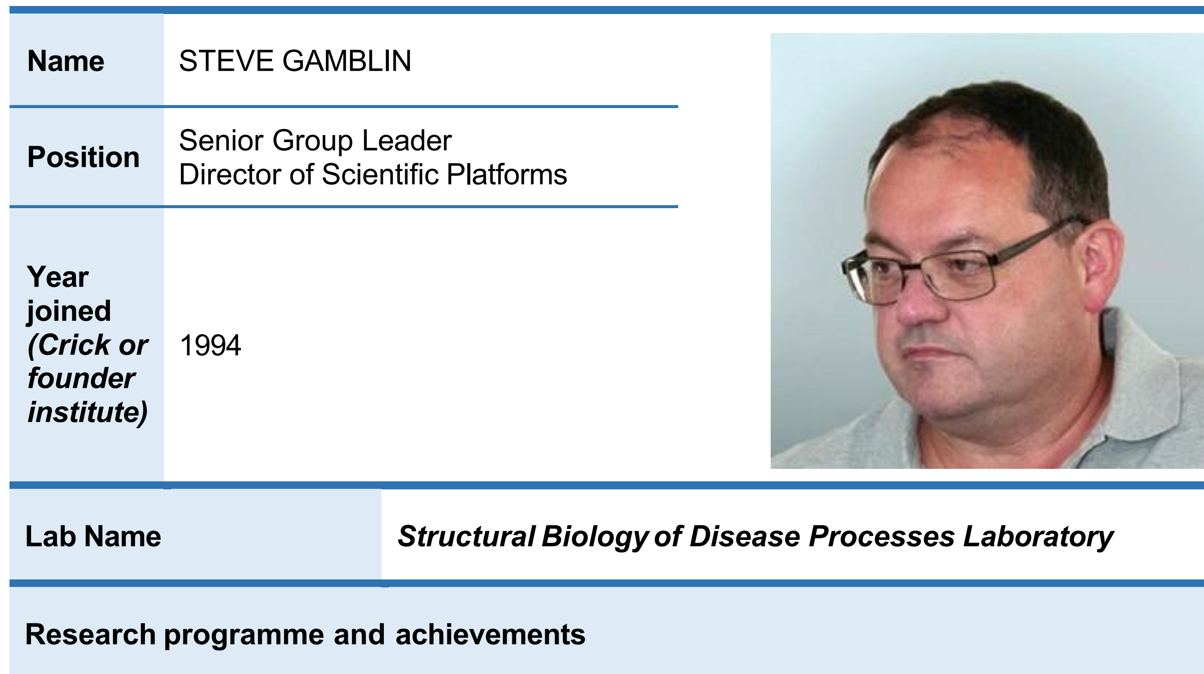
Together, these experiments will help usher in an era of precision medicine in which understanding a patient's genetic differences leads directly to better healthcare. Importantly, our research will help democratise this growing field by providing patients of all genetic backgrounds with high-quality data.

Research outputs

Findlay, G.M., et al. (2018). *Accurate classification of BRCA1 variants with saturation genome editing.* Nature, 562, 217–222. DOI: 10.1038/s41586-018-0461-z
This work was the first to demonstrate how saturation mutagenesis of critical genomic regions can be used to classify variants seen clinically with great accuracy. We engineered thousands of variants in *BRCA1* to ask how each one impacts gene function and RNA expression in human cells. Our "function scores" for each mutation tested achieved near-perfect accuracy (>97%) for predicting pathogenicity, thus establishing an experimental paradigm for adjudicating variant effects applicable to hundreds of genes harbouring thousands of variants likely to be consequential to health and disease.

McKenna, A., Findlay, G.M., et al. (2016). *Whole-organism lineage tracing by combinatorial and cumulative genome editing*. Science 353, aaf7907. DOI: 10.1126/science.aaf7907
This paper marks the first use of CRISPR/Cas9 genome editing as a means of cellular lineage tracing. We deploy the method to tag over a thousand unique embryonic lineages in zebrafish and relate them to one another by patterns of shared mutations. This information allows us to quantitatively study the complex lineage relationships between hundreds of thousands of cells sampled across entire adult organisms.

Findlay, G.M., et al. (2014). *Saturation editing of genomic regions by multiplex homology-directed repair.* Nature 513, 120–123. DOI: 10.1038/nature13695
This paper introduces saturation genome editing (SGE). In this method, a multiplex homology-directed repair step is used to introduce thousands of programmed variants at a single genomic locus of interest. Next-generation sequencing is then used as a means of deciphering the variants' effects on processes such as splicing and protein activity.



Name	STEVE GAMBLIN
Position	Senior Group Leader Director of Scientific Platforms
Year joined *(Crick or founder institute)*	1994

Lab Name	***Structural Biology of Disease Processes Laboratory***

Research programme and achievements

My lab investigates the mechanisms of the cell's protein machinery using structural biology and biophysics guided methodologies. We have three main interests. Firstly, chromatin modification complexes that regulate transcription and have been implicated in cancer. Secondly, viral proteins that facilitate infectivity. In this area historically we have focused on influenza but more recently we have used our experience in these systems to investigate the analogous mechanisms in coronavirus. Finally, we have a longstanding interest in the cellular energy regulator AMP-activated Protein Kinase (AMPK) whose misregulation is linked to diabetes. Our underlying philosophy is that the discovery of the molecular mechanism of fundamental processes will reveal insights into the disease process that will ultimately drive the development of novel therapeutic approaches.

During this quinquennium we completed a long-term project aimed at determining the mechanism that drives allosteric activation of the polycomb repressive complex (PRC2) and leads to spreading of the histone H3K27 methyl silencing mark to generate heterochromatin. Principally this involved the determination of the crystal structure of the catalytic core of the PRC2 complex, bound to both stimulating product and substrate peptides (Justin et al 2016). The structure revealed how the three core subunits interlock to form the catalytic core, and how product binding to a non-catalytic subunit stabilises a series of elements that ultimately promote methylation at the active site. We were then able to exploit the knowledge gained through this initial structure to follow this work directly with a structural investigation of PRC2 blocked with an inhibitor compound, developed by colleagues in pharma (Constellation Pharma ceuticals) (Vaswani et al 2016). This revealed how the inhibitor binds all three subunits locking the complex into an inactive conformation and prevents the binding of cofactor. This structure is being used for further drug development aimed at cancer treatment.

We have had a productive history of using X-Ray crystallography combined with biophysics to investigate the evolution of the major influenza protein, haemagglutinin (HA). This has been used to explain how emerging strains of seasonal influenza h ave modified through mutation of strains in non-human hosts to bind to human receptors. This work is a close collaboration with John Skehel and our colleagues at the World Influenza centre at the Crick. In the current quinquennium we have been able to exploit the recent dramatic advances in resolution of structural Cryo EM technology to further our understanding of the dynamic process of invasion mediated by HA. A high resolution (3.3 Å for the ectodomains) EM structure of full-length HA revealed for the first-time details of how HA is anchored in the virus membrane and the significance of the flexible linker that connects the membrane anchor to the ectodomain (Benton et al 2018). In further work we

showed how binding of a FAB antibody fragment to this region disrupts flexibility and may therefore reduce infectivity. Developing these techniques we have more recently been able to determine structures of intermediates in the endocytosis pathway. As part of the process of cell invasion by the virus the HA proteins undergo a large conformational transformation that ultimately results in fusion of the virus particle to the cell membrane (Benton et al 2020). Using a novel approach, we were able to follow the structural changes to HA induced by incubating the protein at fusion pH, and revealed how the HA1 domain dilates and extends before folding back into the post fusion conformation.

Finally, more recently, in response to the covid crisis we were able to apply our experience in the structural biology of viral infectivity to investigate the changes to the SARS-Cov2 spike protein and its binding to ACE receptors (Wrobel et al 2020). We have shown the importance of the acquired furin cleavage site in enabling the spike protein to adopt a conformation that is favourable to bonding the human receptor.

Research outputs

Justin, N. et al. (2016) *Structural basis of oncogenic histone H3K27M inhibition of human polycomb repressive complex 2*. Nat Commun 7, 11316. DOI: 10.1038/ncomms11316

The PRC2 complex is the major epigenetic driver of programmed transcriptional repression in the cell and its misregulation is associated with development defects and genomic instability. It is a major target for cancer therapeutics. In this paper we described the crystal structure of the multi-protein catalytic core of the PRC2 complex. This analysis provided a description for the molecular basis of allosteric regulation of the EZH2 methyltransferase subunit by its product. Further, the structure contains an oncogenic histone peptide and revealed the mechanism underlying the stalling of the spread of the H3K27 silencing mark in glioma.

Benton DJ, Nans A, Calder LJ, Turner J, Neu U, Lin YP, Ketelaars E, Kallewaard NL, Corti D, Lanzavecchia A, Gamblin SJ, Rosenthal PB, Skehel JJ. (2018) *Influenza hemagglutinin membrane anchor*. Proc Natl Acad Sci USA 115(40):10112-10117. DOI: 10.1073/pnas.1810927115

My lab has a strong record of publications describing the emerging properties of the influenza haemagglutinin (HA) surface protein and its role in infection. In this publication we were able to present a description of the high resolution cryo EM structure of the full-length HA. This structure represented a major development because, for the first time, it includes not just the ectodomain, but also the membrane anchor. This reveals how the HA protein is embedded in the virus membrane and the significance of the flexible linker that connects the membrane anchor to the ectodomain.

Benton DJ, Gamblin SJ, Rosenthal PB, Skehel JJ. (2020) *Structural transitions in influenza haemagglutinin at membrane fusion pH*. Nature 583(7814):150-153. DOI: 10.1038/s41586-020-2333-6.

In order to understand the function of HA in influenza infectivity it is necessary to understand the mechanism of endocytosis. It has previously been established that endocytosis involves a large conformational rearrangement of the HA protein that can be triggered by a change in pH, revealed by structures of initial and final states. In this paper we adapted the cryogenic EM technique in order to trap a series of intermediate HA structures in the pathway. This provided the most complete picture to date of the structural rearrangements that occur in HA during the endocytosis process.

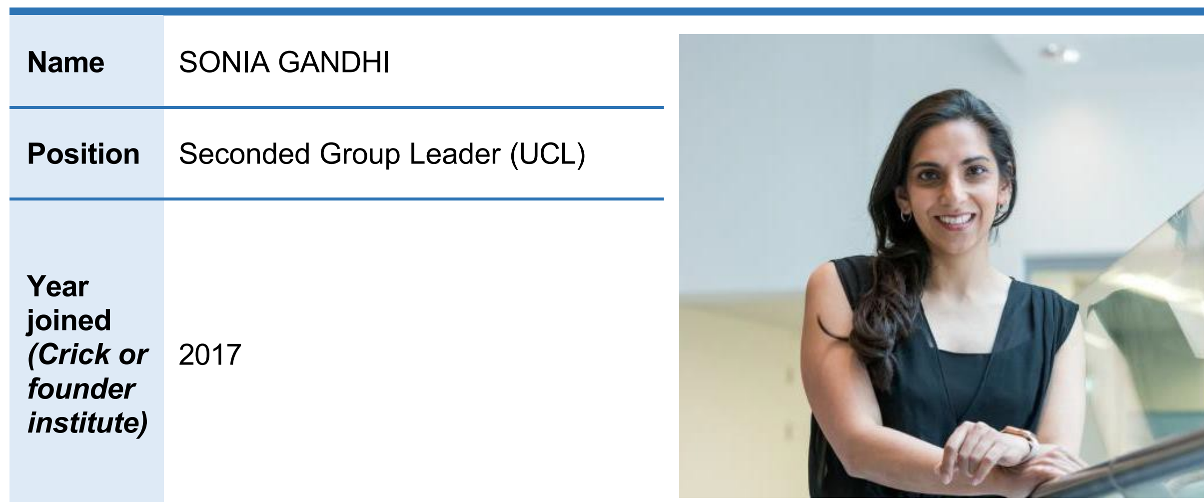
Wrobel AG, Benton DJ, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ. (2020) *SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects*. Nat Struct Mol Biol. 27(8):763-767. DOI: 10.1038/s41594-020-0468-7

We have been able to apply the knowledge we have gained from our work on the infectivity

of the influenza virus to the challenge presented by the recent SARS-CoV-2 virus outbreak. In this paper we present high resolution cryo EM structures of the SARS-CoV-2 and bat RaTG13 spike glycoproteins. We describe from a structural perspective the significant differences between the strains. We draw particular attention to the addition of a furin cleavage site into the human virus spike protein. We discuss its potential role in infectivity and on the evolution of this virulent strain.

Benton, D.J., Wrobel, A.G., Xu, P. Roustan, C., Martin, S.R., Rosenthal, P.B. Skehel, J.J. & Gamblin, S.J. (2020) *Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion*. Nature 588, 327–330. DOI: 10.1038/s41586-020-2772-0

Here we describe the conformational changes that the SARS-Cov2 spike protein undergoes in binding to the human ACE2 receptor. This represents the initial stages of the mechanism of cell invasion by the virus particle during infection. We show a series of ten cryoEM reconstructions of the spike protein binding to ACE2 through its receptor binding domain (RBD), ranging from a closed unbound spike ectodomain trimer to the fully open conformation with each RBD in the trimer bound to an ACE2 receptor. Binding to ACE2 releases the so-called fusion peptide segment and promotes membrane fusion leading to cell invasion.



Name	SONIA GANDHI
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	2017

Lab Name	***Neurodegeneration Biology Laboratory***

Research programme and achievements

Since 2015, my research program has focussed on understanding the molecular and cellular mechanisms that cause neurodegenerative diseases, in particular Parkinson's disease. The accumulation of abnormal insoluble misfolded protein and progressive neuronal death are the key hallmarks of neurodegeneration, although it is not known how protein misfolding and aggregation leads to neuronal dysfunction and death in the huma n brain. To investigate this, my laboratory has:

(i) developed biophysical tools based on highly sensitive single molecule and super resolution approaches that can resolve the process of protein aggregation. Applying these tools to complex biological systems such as human biofluids and human cells, we have shown that during protein aggregation there is a range of intermediates with different structural conformations and sizes. Importantly one of those intermediate species, an oligomer rich in beta sheet structure, is particularly toxic to neurons.

(ii) identified several mechanisms by which the toxic oligomer species of the misfolded protein causes toxicity: we reported an interaction between oligomers and the ATP synthase in mitochondria, that result in bioenergetic impairment, and opening of the mitochondrial permeability transition pore in disease states. We demonstrated how misfolded protein can induce neuronal death through an iron dependent lipid peroxidation pathway termed ferroptosis. We further showed that misfolded protein can activate pro-inflammatory states in astrocytes, inducing TLR4 mediated neuronal injury.

(iii) established a human iPSC-based discovery platform, with in house developed directed differentiation methods to generate region specific CNS cell types affected in disease to test and validate the disease mechanisms.

The future research program of the Neurodegeneration Biology Laboratory incorporates the following themes:

- The interactions between protein misfolding and mitochondrial function; and protein misfolding and lysosomal function; and mitochondrial-lysosomal interactions and signalling in health and disease states in human neurons. This will be explored using the human (patient derived) models based on mutations in the protein misfolding pathway, mitochondrial homeostasis pathway, and lysosomal pathway.
- The dissection of cell autonomous (neuron mediated) vs non cell autonomous processes (astrocyte induced neuronal dysfunction) in disease states. This will be investigated using longitudinal imaging, and transcriptional dynamics of enriched, and co-cultures of different cell types.

- Establishing a transcriptomic and functional cell framework of the inherited forms of Parkinson's disease and determining how the sporadic, or idiopathic forms of disease are related to these familial forms.
- Generating a map of the Parkinson's brain utilising the oligomer as the biomarker for early disease: this will integrate super resolution imaging of oligomers in brain, spatial transcriptomics, and single cell transcriptomic and genomic analyses in postmortem human brain to understand where, how and why protein aggregates form in situ in disease.
- Taking forward the discoveries from MapPD, we will adopt gene editing approaches in our iPSC models to determine how these targets influence protein aggregation, and neuronal pathophysiology.
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Research outputs

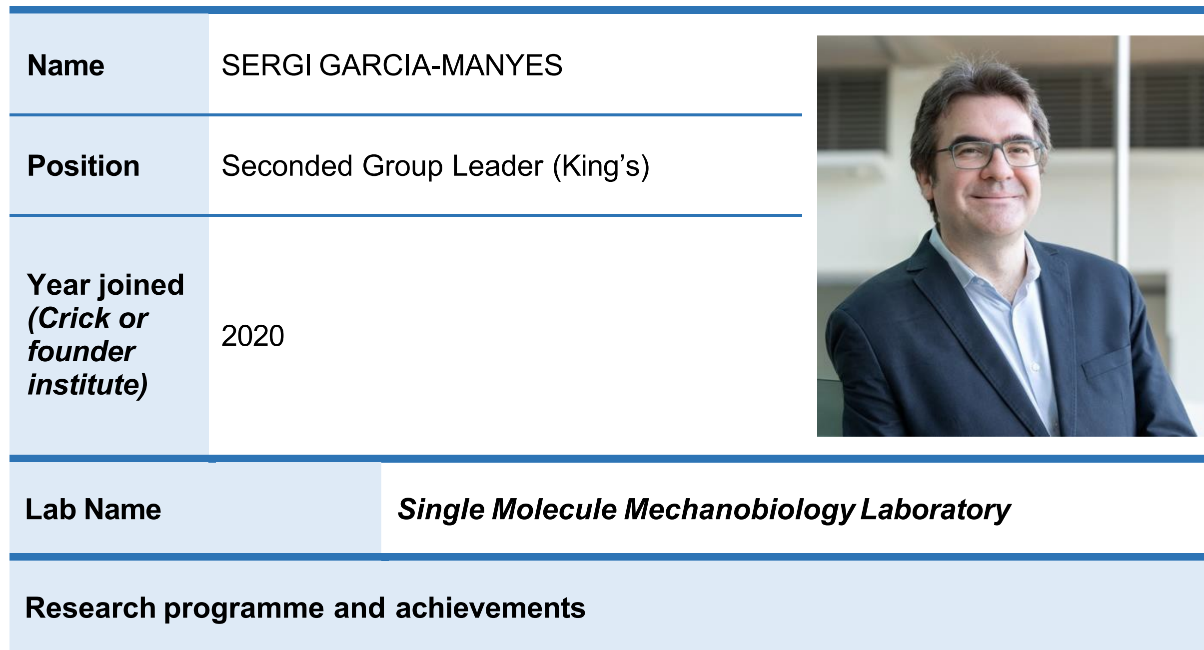
Aitken, J., Ambrose, K., Barrell, S. et al. (2020) *Scalable and robust SARS-CoV-2 testing in an academic center*. Nat Biotechnol 38, 927–931. DOI: 10.1038/s41587-020-0588-y
This paper was driven by the Crick-Covid-19 Consortium in response to the Covid-19 pandemic, and describes how we were able to successfully repurpose the Crick to increase the capacity for Sars-CoV-2 testing in unprecedented times. I have led, and been responsible for many aspects of this work, which has dominated much of the past 6 months, and so I include this output here.

Angelova, P.R., Choi, M.L., Berezhnov, A.V. et al. (2020) *Alpha synuclein aggregation drives ferroptosis: an interplay of iron, calcium and lipid peroxidation*. Cell Death Differ 27, 2781–2796. 10.1038/s41418-020-0542-z
This work demonstrates how lipid peroxidation may a be a critical driver of neuronal toxicity in protein aggregation diseases such as Parkinson's.

Hughes, C.D., Choi, M.L., Ryten, M. et al. (2019) *Picomolar concentrations of oligomeric alpha-synuclein sensitizes TLR4 to play an initiating role in Parkinson's disease pathogenesis*. Acta Neuropathol 137, 103–120. 10.1007/s00401-018-1907-y
This work shows the importance of neuron-glial interactions and inflammatory pathways in Parkinson's.

Ludtmann, M.H.R., Angelova, P.R., Horrocks, M.H. et al. (2018) *α-synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. Nat Commun* 9, 2293 10.1038/s41467-018-04422-2
This work combines single molecule imaging and super resolution methods with mitochondrial imaging and electrophysiology to demonstrate the mechanism by which α-synuclein oligomers alter mitochondrial function.

Hall CE, Yao Z, Choi M, Tyzack GE, Serio A, Luisier R, Harley J, Preza E, Arber C, Crisp SJ, Watson PMD, Kullmann DM, Abramov AY, Wray S, Burley R, Loh SHY, Martins LM, Stevens MM, Luscombe NM, Sibley CR, Lakatos A, Ule J, Gandhi S*, Patani R. (2017) *Progressive Motor Neuron Pathology and the Role of Astrocytes in a Human Stem Cell Model of VCP-Related ALS*. Cell Rep 19(9):1739-1749. DOI: 10.1016/j.celrep.2017.05.024
I led the imaging methodology and functional phenotyping in iPS derived motor neurons and astrocytes.



Name	SERGI GARCIA-MANYES
Position	Seconded Group Leader (King's)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Single Molecule Mechanobiology Laboratory***

Research programme and achievements

Summary of my research programme. My laboratory (joint between King's College London and the Francis Crick Institute) focuses on understanding the fundamental physical principles that govern mechanobiology, from a single molecule perspective.

Scientific rationale. There is now mounting evidence that mechanical stimuli regulate a large number of cellular functions, including growth, motility and differentiation. However, how mechanical forces are channeled through the cytoplasm to eventually reach the nucleus and alter gene expression remains poorly understood. Obtaining a clear molecular picture of nuclear mechanotransduction with traditional cell biology strategies has been challenging. While cell mechanics experiments have provided the most promising approach by measuring the effect of key protein knockdowns on the overall cellular mechanical response, the intrinsic nature of these experiments precludes the establishment of a direct relationship between the force-induced structural changes and the mechanical function of each of the involved molecular players. In contrast, single molecule nanomechanical experiments allow mapping the conformational dynamics of individual proteins under force with great detail. However, the translation of these *in vitro* nanomechanical experiments into the cellular context (with potential knock-on effects at the functional level) is far from trivial. Despite recent promising achievements, we lack global understanding of whether individual molecules inside the cell respond to force according to the same fundamental physical laws established by the single molecule experiments. Closing such a scale gap has become a timely, necessary and unavoidable requirement to enable the natural advancement of the mechanobiology field. **My research programme focuses on employing a combination of state-of-the-art nanomechanical techniques across different force- and length-scales to uncover the molecular details underlying the main force propagation mechanisms of nuclear mechanotransduction.**

Specifically, we have delineated different research lines that we will be exploring at the Crick to dissect the molecular mechanisms of nuclear mechanotransduction from different, complementary angles. (1) We are studying the dynamics under force of each individual protein of the LINC complex, which forms a long molecular tether between the cytoskeleton and the nuclear envelope. We hypothesize that the force-induced conformational changes of the proteins forming the LINC complex are crucial to physically propagate cytoskeletal forces to the nuclear envelope. (2) We aim to investigate the emerging role of the nucleus as a mechanonsensor. In particular, we want to uncover (i) lipidome changes in the nuclear envelop (NE) of cells exposed to mechanical stress and (ii) the mechanical effect of key post-translational modifications of cryptic sites in specific nuclear proteins after mechanical unfolding. Finally, (3) we are using a combined

mechanical/fluorescence optogenetic approach that we have developed to track the dynamics of nuclear shuttling of cytoplasmic transcription factors upon mechanical stimulation. Our main goal is to understand the molecular mechanisms explaining our recent discovery that the mechanical unfolding of translocating proteins across the nuclear pore complex (NPC) accelerates the rate of nuclear import. (4) Finally, we want to understand the downstream effects of extracellular mechanical cues by beginning to interrogate how gene expression is affected by mechanical force, and the knock-on effects on cell function (eg motility, differentiation, etc). Altogether, this multidisciplinary research programme will provide an integrated, mechanistic and quantitative view, from a molecular perspective, on how mechanical forces propagate to the cell nucleus and activate force-induced transcriptional programmes.

The design of this research programme, which we want to uncover in the next 5-7 years, is the result of a natural evolution of my research path. Earlier in my career, I focused on the development of new single molecule AFM instrumentation (*Nature Protocols*, 2013) and experimental approaches to uncover the physical principles underlying (*i*) the mechanical (un)folding of proteins (*PNAS*, 2009 (1); *PNAS*, 2009(2)) (*ii*) the effect of force on chemical reactions with single bond resolution (*Nature Chemistry*, 2009; *JACS*, 2011) and (*iii*) the nanomechanics of membranes (*Biophys. J*, 2005; *JACS*, 2010). We have recently built a single molecule magnetic tweezers technique, complementary to the AFM, which allows to apply low, physiologically relevant forces to individual molecules over extended periods of time of several hours and even days.

In the last few years, and namely within this quinquennium, I became interested in providing our *in vitro* single molecule work with a clear biological context. For example, we uncovered (*iv*) novel non-enzymatic routes to drive oxidative folding (*Nature Communications*, 2016), (*v*) the effect of naturally-occurring post-translational modifications on the elasticity of cardiac titin (*Nature Communications*, 2017) and (*vi*) how mechanical forces modulate chaperone binding (*Science Advances*, 2018).

Motivated by the challenge of integrating data across different scales, specifically aiming to elucidate whether subtle mechanical changes at the single molecule scale have knock-on effects at the cellular level, I have recently expanded the force- and length-range of our experiments to include the cellular scale. Our initial attempts to the field have revealed (*vii*) dynamic changes in membrane stiffness during cell cycle (*Cell*, 2014); that (*viii*) mechanical forces are able to mechanically deform the nuclear pore complex and change its permeability (*Cell*, 2017); and that (*ix*) mechanical unfolding of proteins regulate their translocation rate to the nucleus (*Nature Physics*, 2019)

Research outputs

Infante, E.; Stannard, A.; Board, S.J.; Rico-Lastres, P.; Rostkova, E.; Beedle, A.E.M.; Lezamiz, A.; Wang, Y.J.; Gulaidi Breen, S.; Panagaki, F.; Sundar Rajan, V.; Shanahan, C.; Roca-Cusachs, P.; Garcia-Manyes, S. (2019) *The mechanical stability of proteins regulates their translocation rate into the cell nucleus*. Nature Physics 15, 973-981. DOI: 10.1038/ s41567-019-0551-3

Here we demonstrated that the mechanosensitive MRTF-A transcription factor is imported into the nucleus at a rate that is inversely correlated with its nanomechanical stability, but independent of its thermodynamic stability. Attaching mechanically stable proteins to MRTF-A results in reduced gene expression and the subsequent slowing down of cell migration for three different cancer cell lines. These findings suggest that the modulation of the mechanical stability of transcription factors may represent a general strategy for the control of gene expression.

Beedle, A.E.M.; Mora, M.; Davis, C; Brijnders, B.; Stirnemann, G.; Garcia-Manyes, S. (2018) *Forcing the reversibility of a mechanochemical reaction.* Nature Communications 9(1):3155. DOI: 10.1038/s41467-018-05115-6

Chemical reactions are usually initiated by heat, electric current or light. Here we used single molecule force spectroscopy to show that mechanical force can by-pass thermodynamically locked reactivity in the reduction of thermodynamically stable protein disulfide bonds, having a direct impact on its reversibility.**Perales-Calvo, J.; Giganti, D.; Stirnemann, G.; Garcia-Manyes, S. (2018) *The force- dependent mechanism of DnaK-mediated mechanical folding*. Science Advances 4(2):eaaq0243. DOI: 10.1126/sciadv.aaq0243**
Here we investigated the molecular mechanisms governing chaperone-mediated mechanical protein folding. Using a combination of single molecule force spectroscopy and molecular dynamics simulations, we uncovered the unanticipated role of mechanical force in finely regulating chaperone binding, with direct implications on protein elasticity.

Elosegui-Artola, A.; Andreu, I; Beedle, A.E.M.; Lezamiz, A; Uroz, M.; Kosmalska, A; Oria, R.; Trepat, X.; Navajas, D.; Garcia-Manyes, S.; Roca-Cusachs, P. (2017) *Force triggers YAP nuclear entry by mechanically regulating transport across nucleopores*. Cell 171(6), 1397-1410. DOI: 10.1016/j.cell.2017.10.008
The nuclear translocation of mechanosensitive transcription factors plays a key role in the cells' ability to respond to mechanical signals. In collaboration with the Roca-Cusachs lab, here we demonstrated that extracellular mechanical forces are able to deform the nuclear pore complex, facilitating nuclear import of the YAP transcription factor.

Beedle, A.E.M.; Mora, M.; Lynham, S., Stirnemann, G.; Garcia-Manyes, S. (2017) *Tailoring protein nanomechanics with chemical reactivity*. Nature Communications 8, 15658. DOI: 10.1038/ncomms15658
Post-translational modifications (PTMs) are emerging as key regulators of protein function. Here we discovered a chemical route alternative to enzymes to promote oxidative protein folding using small plasma thiols. Our approach, combining single molecule force clamp spectroscopy, DFT calculations and mass spectrometry, provided a general tool to rationalise the effect of PTMs on modulating protein nanomechanics.

Name	NATHAN GOEHRING
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013
Lab Name	***Polarity and Patterning Networks Laboratory***

Research programme and achievements

<u>Research Programme Summary</u>

Through their ability to link cell architecture and signalling networks, cell polarity networks play fundamental roles in the spatial regulation of intracellular processes and in so doing drive processes as diverse as cell migration, establishment of body axis, cell fate specification, and the development of tissues and organisms.

First identified in *C. elegans*, the *PAR-titioning defective* proteins make up the core of a conserved molecular network involved in polarisation of animal cells, operating from the earliest stages of cell fate specification in species from worms to human. Mutations in polarity-related molecules are linked to cancer and active programmes are in place to develop therapeutics based on manipulating the polarity-related pathways.

At the heart of this network are a set of feedback loops between PAR proteins that enables their self-organisation into discrete membrane-associated domains. Over the past decade, the field has seen a transition from genetic analysis to systems -level approaches as the dynamic and complex nature of PAR polarity has become apparent. Core questions have shifted from identification of molecules and interactions to understanding how this network of molecules generates patterns on the cell membrane.

My research programme has been at the forefront of these efforts, first as a postdoc where I first defined the intracellular mobility of PAR proteins and used this data to construct one of the first generation of mathematical models to understand symmetry-breaking and domain size control. Since starting the lab, we have expanded on this analysis of PAR protein dynamics using the *C. elegans* embryo as a model system and combining genetics, quantitative imaging and mathematical modeling. Our aims are to uncover the design principles of the PAR network that enable intracellular patterning of cells, to determine how these principles emerge from individual and collective properties of the component molecules, and to understand the consequences of these principles as they play out over embryonic development.

Since starting the lab, we have primarily focused on tackling microscale organisation of PAR proteins at the cell membrane, resolving functional complexity in the PAR network, and understanding how features of the PAR network optimise its response to developmental cues (see details in publications below). We have also developed optimised quantitative analysis and genetic techniques to set a foundation for the future.
Our work for the following period will focus on three areas which will leverage our

expertise in quantitative imaging and in vivo network analysis as well as our collaborations at the physics of life interface:

- What is the molecular logic of polarity establishment by the PAR network? We will provide mechanistic insight into key feedback circuits that drive cell polarity and directly link coarse-grained network properties to molecular behaviours.

- How do polarity networks respond to developmental cues? We will explore how PAR proteins respond to developmental cues and how both the cues and the ability of the PAR network to respond to those cues is regulated during development.

- How do dynamic systems cope with energy stress? We will use *C. elegans* embryos as a model to study the nature of suspended animation and explore notions of synchrony and spatial memory as mechanisms for maintaining viability during transitions into and out of suspended states.

Research outputs

Hubatsch, L., Peglion, F., Reich, J.D., Rodrigues, N.T., Hirani, N., Illukkumbura, R., Goehring, N.W. (2019) *A cell size threshold limits cell polarity and asymmetric division potential*. Nat. Phys. 15, 1078-1085. DOI: 10.1038/s41567-019-0601-x
A key requirement for patterning networks is that the scale of pattern be appropriately matched to the size of the system to be patterned. Through a combination of theory and experiment, we show that failure of the PAR network to scale with cell size restricts stable cell polarity to a specific size range and imposes a minimum cell size threshold for polarity. Experimental alteration of cell size indicates that embryos are sensitive to this size threshold. We thus propose a general strategy by which cells can use intrinsic length scales of patterning networks to enable size-dependent decision making.

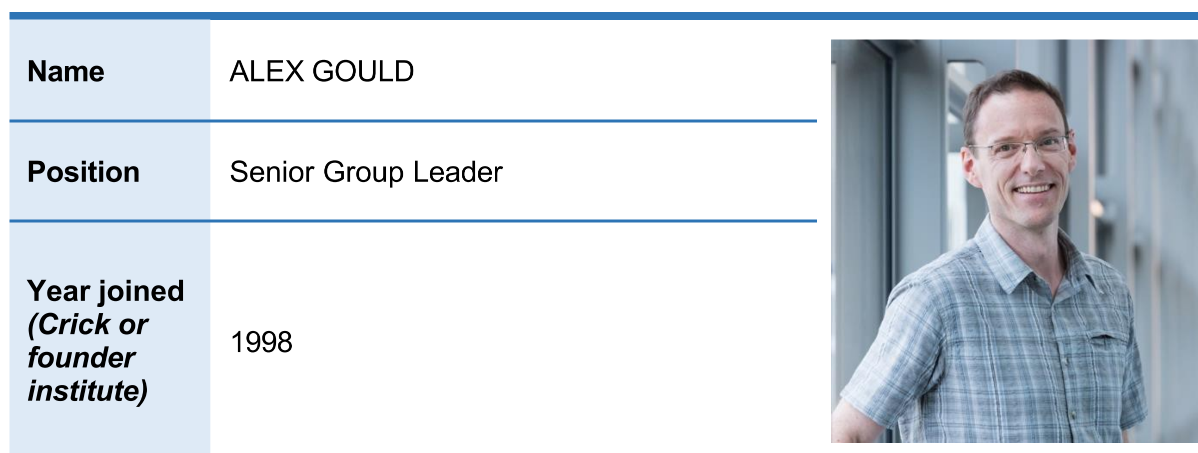
Hirani, N., Illukkumbura, R., Bland, T., Mathonnet, G., Suhner, D., Reymann, A.C., Goehring, N.W. (2019). *Anterior-enriched filopodia create appearance of asymmetric membrane microdomains in polarizing C. elegans zygotes.* J. Cell. Sci. 132, jcs230714. DOI: 10.1242/jcs.230714
This work demonstrates that filopodia-like structures form on the surface of polarising *C. elegans* zygotes. Our analysis forces a re-interpretation of prior reports of asymmetric localisation of membrane-associated molecules such as PIP2 and Rho-family GTPase to membrane microdomains. Our data indicate that these experiments simply report the localisation of filopodia (i.e. excess membrane).

Reich, J.D., Hubatsch, L., Illukkumbura, R., Peglion, F., Bland, T., Hirani, N., Goehring, N.W. (2019) *Regulated activation of the PAR polarity network ensures a timely and specific response to spatial cues*. Curr. Biol. 29, 1911-1923.e5. DOI: 10.1016/j.cub.2019.04.058
In this work, we identify a programme of PAR network activation that is repressed by the cell cycle kinases Aurora A and Polo-like kinase 1. By preventing premature responsiveness of the PAR net- work to potential symmetry-breaking cues, this regulation helps ensure that the embryo polarises along a single axis in response to the centrosome cue provided by sperm upon fertilisation.

Rodriguez, J.*, Peglion, F.*, Martin, J., Hubatsch, L., Reich, J., Hirani, N., Gubieda, A.G., Roffey, J., Fernandes, A.R., St Johnston, D., Ahringer, J., and Goehring, N.W. (2017) *aPKC Cycles between Functionally Distinct PAR Protein Assemblies to Drive Cell Polarity*. Dev. Cell 42, 400-415.e9. DOI: 10.1016/j.devcel.2017.07.007
Through the use of aPKC inhibitors and genetic mutations, we demonstrate that aPKC cycles between distinct PAR-3 and CDC-42 dependent states, which define, respectively, the ability of the aPAR network to respond to spatial cues and to displace pPAR proteins from the membrane. We further show that cue sensing depends

crucially on the oligomeric nature of the PAR-3 state, that the integrity of this cycle is required for coupling of cue-sensing and effector functions of the aPAR network, and that this cycle is enforced by activity of aPKC.

Gross, P., Kumar, K.V., Goehring, N.W., Bois, J.S., Hoege, C., Jülicher, F., and Grill, S.W. (2019) ***Guiding self-organized pattern formation in cell polarity establishment*****. Nat. Phys. 15, 293-300. DOI: 10.1038/s41567-018-0358-7**
This work combines theoretical and experimental approaches to probe the role of guiding cues in polarisation of the PAR polarity network in the *C. elegans* zygote. Notably, this work quantifies a transition point which defines the point beyond which biochemical feedback is sufficient to drive continued polarity in the absence of continued signals from the symmetry-breaking cue, highlighting the subcritical nature of the PAR feedback network and the dominant role of cues in ensuring robust polarisation.



Name	ALEX GOULD
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	1998

Lab Name	***Physiology and Metabolism Laboratory***

Research programme and achievements

Our overarching aim is to understand how developing animals cope with, and sometimes benefit from, exposure to environmental stresses. This is a fundamental problem in developmental biology that is also clinically important, yet mechanistic knowledge in this area remains sparse. Our past work pioneered the study of the role of metabolism in stress adaptation to nutrient restriction during *Drosophila* development.

The main achievements of the laboratory since 2015 are:

1. Establishment of several *Drosophila* and mouse models for the long-term effects of transient developmental stresses on adult physiology.

2. Discovery of a molecular mechanism for selective protection of the CNS (brain sparing) during developmental hypoxia. In response to low oxygen tension, the *Drosophila* neural stem cell niche synthesises lipid droplets with antioxidant functions that are essential to protect neighbouring neural stem cells.

3. Discovery of a mechanism whereby expression of the sex determination pathway in a few identified neurons in the brain regulates the sexual size dimorphism of the entire *Drosophila* body. It overturns long-standing dogma in insects that sexual dimorphism is regulated in a strictly cell-autonomous manner. The morphometric methods developed in this study are now being used to investigate how some developing organs but not others are spared during nutrient restriction.

4. Demonstration that early-life nutrient restriction or mild oxidative stress can significantly extend rather than shorten *Drosophila* lifespan and identification of the underlying mechanisms. Early-life nutrient restriction decreases the concentration of toxic hydrocarbons in the protective lipid barrier coating the adult body, improving its function and thus extending lifespan. Transient exposure to low-dose oxidants permanently changes gut microbiota, eliminating the Acetobacter that trigger age-related hyperimmunity, thus preserving the gut barrier and extending lifespan.

5. The development of an improved chemically defined diet for *Drosophila*, enabling study of the contributions of individual macro- and micro-nutrients to organ growth during development.

6. Multiple technology developments in metabolomics and in mass spectrometry imaging. These have been essential for the laboratory to be able to quantify and to image

metabolism with high precision and single-cell resolution within complex tissues.

Future work of the laboratory will build upon our *Drosophila* findings to provide an in-depth mechanistic understanding of how developing animals cope with environmental stresses. We will embrace new technologies such as mass spectrometry imaging, where we have already invested considerably in method development. We will also embark on a major new research direction - translating key *Drosophila* findings into mice and humans. Recent results indicate exciting and unanticipated parallels between insect and human stress-protective mechanisms. One of these involves a rewiring of lipid metabolism and we will be testing new drugs that target this pathway as a potential therapeutic strategy for glioblastoma.

Research outputs

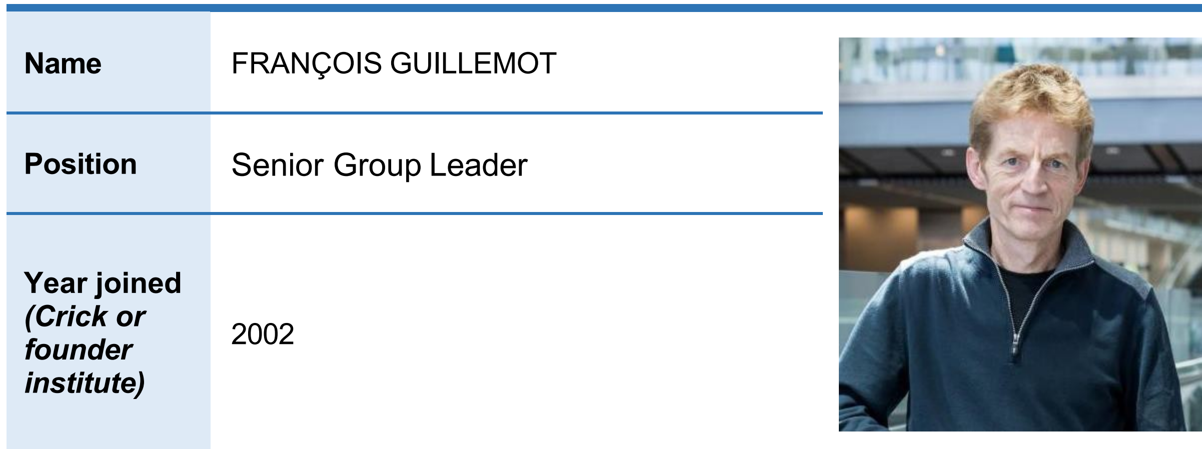
ailey AP, Koster G, Guillermier C, Hirst EM, MacRae JI, Lechene CP, Postle AD and Gould AP (2015). *Antioxidant Role for Lipid Droplets in a Stem Cell Niche of Drosophila*. Cell 163:340-353. DOI: 10.1016/j.cell.2015.09.020
This paper is a continuation of our major research theme on how dividing stem cells in the CNS are able to resist environmental stresses that shut down proliferation in most other developing tissues. It reports the first identification, in any species, of lipid droplets as protectors of stem cells. We discovered that hypoxia induces lipid droplets in the neural stem cell niche and that these protect the neural stem cells themselves from damaging polyunsaturated fatty acid (PUFA) peroxidation reactions. This study laid the foundation for our current mechanistic studies into the antioxidant functions of lipid droplets during development and tumorigenesis.

Stefana MI, Driscoll PC, Obata F, Pengelly AR, Newell CL, MacRae JI and Gould AP (2017). *Developmental diet regulates Drosophila lifespan via lipid autotoxins*. Nature Communications 8:1384. DOI: https://www.nature.com/articles/s41467-017-01740-910.1038/s41467-017-01740-9
This reports the first identification, in any species, of barrier lipids as key mediators of diet induced longevity. We discovered that moderate dietary restriction during development decreases toxic barrier lipids and can more than double lifespan - an effect size comparable or greater than was previously observed with dietary restriction during adulthood. This study has widespread relevance because toxic barrier lipids also influence how longevity is regulated by many other factors, including insulin signalling.

Sawala A and Gould AP (2017). *The sex of specific neurons controls female body growth in Drosophila*. PLoS Biol 15:e2002252. DOI: 10.1371/journal.pbio.2002252
This study identifies a surprising neurohormonal mechanism that links sex to growth and proliferation during development. It overturns long-standing dogma in insects that sexual dimorphism is regulated in a strictly cell-autonomous manner. It also suggests that the principles of sexual differentiation in insects and mammals may be more similar than previously thought.

**Obata F, Fons CO, and Gould AP (2018). *Early-life exposure to low-dose oxidants can increase longevity via microbiome remodelling in Drosophila*.
Nature Communications 9:975. DOI: 10.1038/s41467-018-03070-w**
This reports the first identification, in any species, of the microbiome as a key mediator of developmental stress-induced longevity. We found that mild oxidative stress during development robustly increases lifespan via the selective elimination of Acetob acter from the microbiome. This study also highlights that targeted remodelling of the early-life microbiome can provide an efficient strategy for extending healthspan and lifespan.

**Newell CL, Vorng J-L, MacRae JI, Gilmore, IS and Gould AP (2020). *Cryogenic OrbiSIMS Localizes Semi‐Volatile Molecules in Biological Tissues.*
Angewandte Chemie. DOI: 10.1002/ange.202006881**
This paper reports a technical advance in mass spectrometry imaging. The new cryogenic method decreases molecular fragmentation of lipids and expands the chemical space that is amenable to mass spectrometry imaging with high spatial and mass resolution. For the first time, semi-volatile and non-volatile molecules can now be imaged simultaneously in biological tissues. This recent advance is crucial for our future studies of lipid metabolism at single-cell resolution in complex tissues such as the developing CNS.



Name	FRANÇOIS GUILLEMOT
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2002

Lab Name	***Neural Stem Cell Biology Laboratory***

Research programme and achievements

We are interested in the regulation of stem cell behaviours. We study neural stem cells in the embryonic and adult brain, and we focus on the decisions of neural stem cells to divide or exit the cell cycle and how these decisions are regulated by signalling, transcriptional and epigenetic mechanisms. Our projects can be grouped into two main themes:

1. Regulation of neurogenesis in the embryonic brain.
We study the functions of transcription factors and chromatin remodeling complexes in embryonic neurogenesis, particularly in the human brain, to determine the role of these regulatory factors in the dramatic expansion of neural progenitor populations that occurs during human brain development. We use human iPSC-derived neural cultures and foetal brain tissues to examine the interactions between transcription factors and chromatin regulators, identify the genes they regulate, and determine the steps in neurogenesis they control.

2. Regulation of neurogenesis in the adult brain.
We study stem cells in the hippocampus of adult and ageing mice and address the roles of niche signals and transcriptional and chromatin regulators in the regulation of stem cell behaviour. We also investigate the mechanisms that cause changes in stem cell behaviour at the transition from developmental to adult neurogenesis and during ageing. We are particularly interested in whether the choice of hippocampal stem cells to self-renew or remain quiescent is stochastic or biased by heterogeneity in the niche environment or in the response of stem cells to their environment.

Major Achievements:
We have studied the normal function of genes implicated in neurodevelopmental disorders in the development of the mouse cerebral cortex. We have shown that the centrosome-associated gene Cenpj/CPAP, which causes microcephaly when mutated, regulates the mode of division of cortical progenitors as well as the speed of migration and morphology of newborn neurons. We also found that Nipbl, a subunit of the cohesin complex that has been implicated in syndromic mental retardation, is required for the correct migration of cortical neurons through transcriptional regulation of several neuronal migration genes.

The proliferation of adult hippocampal stem cells is stimulated by the transcription factor Ascl1. We have shown that the choice that these stem cells make between remaining quiescent and becoming active is determined by the posttranscriptional regulation of Ascl1. This involves several mechanisms, including the ubiquitin ligase Huwe1 and the nuclear protein Id4. We have also shown that Ascl1 expression decreases progressively

at the transition from development to adult neurogenesis, due to increasing Huwe1 expression and activity, resulting in hippocampal stem cells returning progressively to quiescence instead of differentiating, and thus ensuring the long-term maintenance of the stem cell pool.

Future plans:
We will further investigate the mechanisms regulating hippocampal stem cell activity, focusing in particular on the niche signals and transcriptional regulators that promote the transition from developmental to adult neurogenesis and that contribute to the decline of neurogenesis during ageing.

We will also analyse the different populations of hippocampal stem cells and the niche signals and transcriptional regulators that control their activity, in other mammalian species than the mouse, including primates and humans, to identify mechanisms responsible for the different dynamics and different extent of adult neurogenesis observed between mammalian species.

We will study the role of proneural transcription factors (ASCL1, MYCN), their regulators (E3 ubiquitin ligase HUWE1) and subunits of the BAF chromatin remodeling complex in human embryonic neurogenesis, to determine their contributions to the extended proliferation of human cortical progenitors. We will also investigate pathological mutations in some of these factors (N-MYC, HUWE1, BAF250A) that cause syndromic or non- syndromic intellectual disability, in order to identify the pathological mechanisms and determine which aspects of human neural development they interfere with.

We will also investigate the mechanism of action of the pioneer transcription factor ASCL1 during neurogenesis by studying how ASCL1 and the BAF chromatin remodeling complex interact when regulating gene expression.

Research outputs

Urban, N., van den Berg, D.L.C., Forget, A., Andersen, J., Demmers, J.A., Hunt, C., Ayrault, O., and Guillemot, F. (2016). *Return to quiescence of mouse neural stem cells by degradation of a pro-activation protein*. Science 353, 292-295. DOI: 10.1126/science.aaf4802
This paper provided the first evidence that stem cells in the adult mouse hippocampus are heterogeneous in their behaviour, with most stem cells differentiating and leaving the niche after they have become active but a small fraction returning to a shallow state of quiescence. These “resting cells” have an essential role in the long-term maintenance of an active stem cell pool.

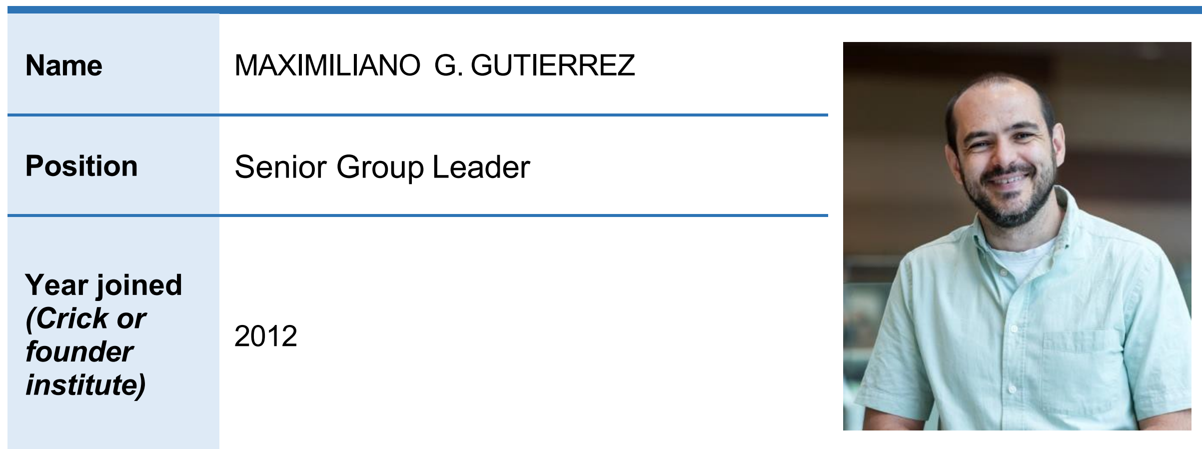
van den Berg, D.L.C., Azzarelli, R., Oishi, K., Martynoga, B., Urbán, N., Dekkers, D.H.W., Demmers, J.A., and Guillemot, F. (2017). *Nipbl interacts with Zfp609 and the Integrator complex to regulate cortical neuron migration*. Neuron 93, 348-361. DOI: 10.1016/j.neuron.2016.11.047
Mutations in the Cohesin subunit Nipbl are the most common cause of a neurodevelopmental disorder, Cornelia de Lange syndrome. In this paper, we investigated the role of Nipbl in normal development of the mouse cerebral cortex. We found that Nipbl regulates transcription during neurogenesis and is in particular required for the expression of genes involved in neuronal migration such as Sema3a and Plxnd1, suggesting that pathological mutations of NIPBL may result in neuronal misplacement in patients’ brains.

Blomfield, I., Rocamonde, B., del Mar Masdeu, M., Mulugeta, E., Vaga, S., van den Berg, D., Huillard, E., Guillemot, F., and Urbán, N. (2019) *Id4 promotes the eliminationof the pro-activation factor Ascl1 to maintain quiescence of adult hippocampal stem cells*. e Life

8:e48561. DOI: **10.7554/eLife.48561**
In this paper, we investigated the mechanisms that regulate Ascl1, a transcription factor that determines the activity state of stem cells in the adult hippocampus. We identified the nuclear protein Id4 as a negative regulator of Ascl1 expression and activity which maintains hippocampal stem cells in quiescence by promoting the degradation of Ascl1 protein.

Harris, L., Rigo, P., Stiehl, T., Gaber, Z., Austin, S.H.L., del Mar Masdeu, M., Edwards, A., Urbán, N., Marciniak-Czochra, A., and Guillemot, F. (2021) *Progressive changes in hippocampal stem cell properties ensure lifelong neurogenesis*. Cell Stem Cell 28, 5. DOI: 10.1016/j.stem.2021.01.003
This paper is a follow up of Urban et al. (2016). We demonstrate that the transition from developmental neurogenesis in the hippocampus, which is marked by a rapid loss of hippocampal stem cells, to adult neurogenesis, when stem cell numbers stabilise, reflects a marked change in stem cell behaviour. During development, stem cells are rapidly eliminated after they have proliferated, while in adults a large fraction of stem cells undergo long-term self-renewal, which involves transient returns to quiescence.

Lattke, M., Goldstone, R., and Guillemot, F. (2020). *Extensive transcriptional and chromatin changes underlie astrocyte maturation in vivo and in culture*. BioRxiv. DOI: **10.1101/2020.04.28.066043**.
Astrocytes are an unusual cell type that remains developmentally plastic for extended times (i.e. they remain capable of proliferating and generating neurons in juvenile mice) and that becomes fully mature only toward adulthood. In this study, we have identified extracellular signals and transcriptional regulators that promote the maturation of astrocytes. These factors might therefore prevent adult astrocytes from acquiring stem cell-like properties upon injury, and thereby limit the capacity of the brain to self-repair.



Name	MAXIMILIANO G. GUTIERREZ
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2012

Lab Name	***Host-Pathogen Interactions in Tuberculosis Laboratory***

Research programme and achievements

My group has developed novel imaging approaches that have allowed us to obtain significant insights into how *M. tuberculosis* interacts with host cells. We have successfully established a research program that combines molecular and cellular biology of macrophages with *in vivo* mouse models and studies in humans. This allowed us to translate our mechanistic studies into more relevant *in vivo* models of TB infection and human disease. Crucially, my group discovered that *M. tuberculosis* can infect human lymphatic endothelial cells (LECs) during extrapulmonary tuberculosis (EPTB). These studies reinforced the idea that cells other than macrophages provide a reservoir for *M. tuberculosis* with important consequences in disease reactivation. Our research also demonstrated that established paradigms can be challenged using new technologies to visualise live virulent *M. tuberculosis*. We found that necrotic cells provide a niche for *M. tuberculosis* replication and also that the proposed mechanism of *M. tuberculosis* phagosome maturation “arrest” is distinct from that proposed in the last 20 years. These studies raise important questions in the field of tuberculosis that my group will address in the future.

The group is now in an unique position to expand the imaging of intracellular *M. tuberculosis* and make major advances in the field of host-pathogen interactions. We will refine imaging technologies to define the molecular mechanisms underpinning *M. tuberculosis* phagosome biology, in particular the factors that contribute to membrane damage and repair and how different populations of *M. tuberculosis* interact with host cell organelles. We will continue our pioneering work on LECs and define the role of these cells in infection and immunity to *M. tuberculosis*. Crucial here is the development of a mouse model of EPTB that will allow us to interrogate important questions at the mechanistic level. Finally, we aim to understand if cellular and tissue environments impact antibiotic efficacy. This question has an enormous relevance for TB treatment and drug-discovery endeavours. In this context, we are pioneering the development of technologies such as correlative nano Secondary Ion Mass Spectrometry (nanoSIMS) to image drugs at the subcellular level.

Research outputs

Herbst, S., Campbell, P., Harvey, J., Bernard, E. M., Papayannopoulos, V., Wood, N. W., . . . Gutierrez, M. G. (2020) *LRRK2 activation controls the repair of damaged endomembranes in macrophages*. The EMBO Journal 39:e104494

DOI:10.15252/embj.2020104494

Mutations in several genes encoding lysosomal proteins are involved in Parkinson's disease (PD). In this work, we show that PD-related leucine-rich repeat kinase 2 (LRRK2) is activated in response to pathogen or membranolytic drug-induced damage of phagolysosomes and lysosomes in macrophages, and
regulates endolysosomal homeostasis by controlling the balance between membrane repair and degradation.

Greenwood, D. J., Dos Santos, M. S., Huang, S., Russell, M. R. G., Collinson, L. M., MacRae, J. I., . . . Gutierrez, M. G. (2019) *Subcellular antibiotic visualization reveals a dynamic drug reservoir in infected macrophages*. Science, 364(6447), 1279-1282. DOI : 10.1126/science.aat9689

Improving chemotherapies against intracellular pathogens requires an understanding of how antibiotic distribution within infected cells affects efficacy. In this work, we developed an approach to visualise antibiotics in human macrophages infected with the tubercle bacillus. We showed that the antitubercular (anti-TB) drug bedaquiline accumulated in host lipid droplets, which seemed to act as an antibiotic reservoir that could be transferred to bacteria during host lipid consumption. Indeed, alterations in host lipid droplet content affected the anti-TB activity of bedaquiline against intracellular bacilli.

Härtlova, A., Herbst, S., Peltier, J., Rodgers, A., Bilkei-Gorzo, O., Fearns, A., . . . Gutierrez, M. G. (2018) *LRRK2 is a negative regulator of Mycobacterium tuberculosis phagosome maturation in macrophages*. EMBO Journal, 37(12). DOI: 10.15252/embj.201798694

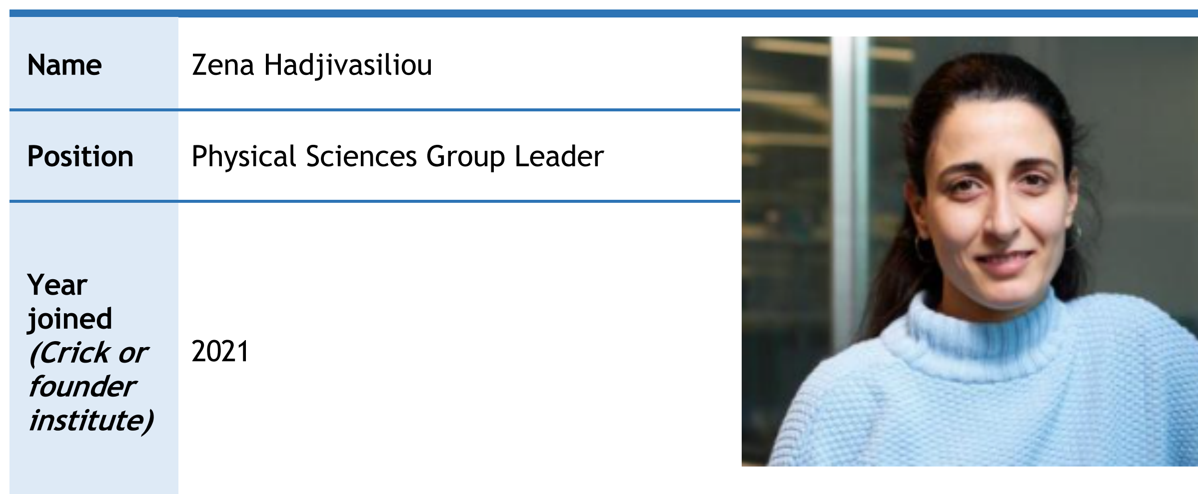
In this study, we found that the Parkinson's disease kinase LRRK2 has an immune function in macrophages. By studying tuberculosis, we have found a possible explanation for why LRRK2 mutations are a genetic risk factor for Parkinson's disease. This study is important because it makes a connection between two important diseases and provide evidence that Parkinson's disease can be associated with defects of the immune function.

Lerner, T. R., Borel, S., Greenwood, D. J., Repnik, U., Russell, M. R. G., Herbst, S., . . . Gutierrez, M. G. (2017) *Mycobacterium tuberculosis replicates within necrotic human macrophages*. Journal of Cell Biology, 216(3), 583-594. DOI: 10.1083/jcb.201603040

This is an example of how live cell imaging is critical to understand host-pathogen interactions in tuberculosis. Using live cell imaging of human primary macrophages infected with *M. tuberculosis* combined with single cell analysis, we showed that in human macrophages, *M. tuberculosis* replicates in pre-necrotic cells. Drugs that inhibit this process impaired bacterial replication.

Schnettger, L., Rodgers, A., Repnik, U., Lai, R. P., Pei, G., Verdoes, M., . . . Gutierrez, M. G. (2017) *A Rab20-dependent membrane trafficking pathway controls M. tuberculosis replication by regulating phagosome spaciousness and integrity*. Cell Host & Microbe, 21(5), 619-628.e5. DOI:10.1016/j.chom.2017.04.004

Here, we examined for the first time the spatiotemporal dynamics of *M. tuberculosis*-containing phagosomes and identified an IFN-γ -stimulated and Rab20-dependent membrane trafficking pathway in macrophages that maintains *M. tuberculosis* in spacious proteolytic phagolysosomes. We uncovered an immune-regulated cellular pathway of defence that promotes maintenance of *M. tuberculosis* within intact membrane-bound compartments for efficient elimination.



Name	Zena Hadjivasiliou
Position	Physical Sciences Group Leader
Year joined *(Crick or founder institute)*	2021

Lab Name	*Mathematical and Physical Biology Lab*

Research programme and achievements

My lab studies how structure and organisation emerge in living systems. We develop mathematical and physical descriptions that help us bridge events that happen at the molecular, cellular, tissue, and whole organism scale. We then use analytical, numerical and computational tools to link our theoretical descriptions to experimental predictions and observations. One of our core interests is the process of development where organisms grow from a single cell to an adult body of well-defined and reproducible size, shape and morphology. We study the developmental machineries that underlie this remarkable transformation in size and organisation. Understanding the mechanisms that control animal growth and patterning can also help explain how the enormous diversity we see across the tree of life has emerged. To do this, our lab examines how developmental machineries mutate and evolve to yield changes and novelty in animal morphology.

My team has recently grown and I am currently supervising a Crick PhD student, one joint HEI-Crick PhD student and a LiDO PhD student. My team also includes an SLRS and two postdocs.

We have recently made progress in developing a theoretical model that allows us to investigate how molecular transport in tissues is coupled to cell and tissue architecture. I am hoping that this work will be written up for publication in a physics journal in 2023 and I am making efforts to set up a collaboration with Alberto Elosegui-Artola's team to explore our theoretical predictions experimentally in an in an in vitro system.

We have also developed an evolution algorithm to explore the design principles that underlie patterning mechanisms that can scale to size during growth. We have found that functional features of morphogen driven patterning, like scalability and robustness, may emerge together in evolution under specific signaling network architectures. I hope that this work will be written up for publication as a theory paper in 2023 and plan to seek collaborations with experimental groups at the Crick to test our predictions going forward.

Two of the PhD students that have joined me this year are co-supervised by Corinne Houart who is at KCL and has a Satellite at the Crick. The students will be performing in vitro and in vivo comparative studies across species and developing mathematical models and quantitative tools to study how brain organization evolves. The projects are at early stages but establish a strong link between my team and that of Corinne Houart and offer excellent experimental systems within which to test our models and predictions about morphogen and Gene Regulatory Network driven diversification.

My last position prior to joining the Crick was as an HFSP Fellow at the University of Geneva and the MPI-PKS. There, I developed statistical and computational tools that allowed us to rigorously quantify the kinetic parameters that dominate morphogen transport. My approach uses a combination of Approximate Bayesian Computation and analytical tools applied to a series of experimental assays. This allowed us, for the first time, to fully quantify the trafficking machinery behind Dpp transport in the fly wing. I used the methodology I developed to understand how the Dpp gradient is formed in WT conditions and in mutants where the Dpp gradient does not scale to size. This work uncovered the machinery via which the Dpp morphogen scales during growth and led to a joint 1st author article published in 2022 and a 2nd author article In Review. I have also used tools from theoretical physics to develop an approach based on Renormalization Group (RG) which shows pattern scaling in biological tissues is a fixed point in RG flow, currently in preparation as a 1st author article. In addition, I initiated a collaboration with physicist Karsten Kruse to explore the evolution of treadmilling in actin and microtubule filaments. This work uses a combination of molecular dynamics simulations and evolution algorithms to explore the selection pressures that could lead to the evolution of complex molecular machines and is currently In Review as a 1st/corresponding author article.

Research outputs

*corresponding author ** equal contribution

Hadjivasiliou Z*, & Karsten Kruse*, Selection for size in molecular self-assembly drives the de novo evolution of a molecular machine. arXiv:2206.06154

In this preprint we use an evolutionary algorithm to explore how molecules can evolve properties that allow them to self-assemble into molecular motors. Our findings could help guide the design of biomolecules that perform directional motion and evolution expeiments in vitro. The evolution algorithm we developed here will form the backbone of some of the work currently developed in my lab.

Romanova-Michaelides M, Hadjivasiliou Z**, Aguilar-Hidalgo D, Basagiannis D, Seum C, Dubois M, Julicher F, & Gonzalez-Gaitan M 2022 Morphogen gradient scaling through recycling of intracelular Dpp.** ***Nature,*** 602, 287-293

This was a key publication from my postdoctoral work where we used a combination of experimental assays, theoretical and computtaional tools to quantify how molecules are transported in tissues and how rates of molecular transport translate into the lengthscale of patterns in tissues. My lab is now building on this work to explore how tissue architecture itself impacts patterning and we are implementing evolution algorithms to explore design principles of morphogen gradient formation.

Mateus R, Holtzer L, Seum C, Hadjivasiliou Z, Dubois M, Julicher F, & Gonzalez-Gaitan M 2020 Scaling of a BMP signalling gradient and growth control in the pectoral fin. ***Cell Reports*****,** 30, 4292- 4302

This collaborative work showed that a scaling BMP gradient controls growth during zebrafish fin development. I contributed a theoretical model that helped drive and interpret experiments to test the central hypotohesis of the article. I hope that my team will continue work in this spirit at the Crick where we work alongside experimental teams to use theoretical models to help guide experiment design and interpratation.

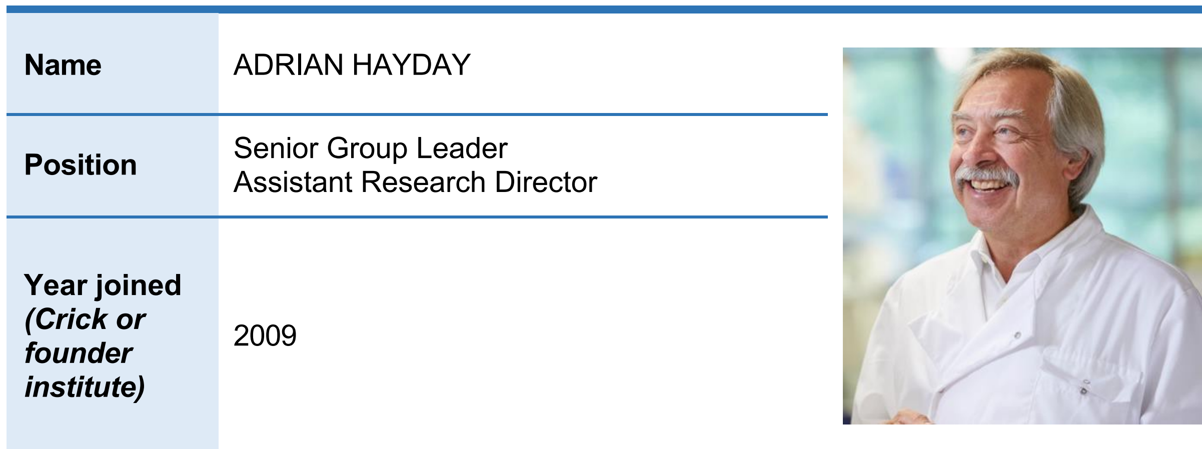
Hadjivasiliou Z*, & Pomiankowski 2019 Evolution of asymmetric gamete signalling and supressed recombination at the mating type locus, ***eLife,*** 8, e48239

In this work I developed an evolution model coupled to the contraints of cell-cell signaling to explore and challenge different hypotheses about the early evolution of sexual

dimorphism. Our findings place cell-cell signaling at the core of the evolution of the sexes and provide a hypothesis for the evolution of reduced recombination and sex chromosomes. My experience in evolution algorithms and using ad-hoc approaches to define fitness as a function of physical constraints is key for the current efforts in my lab to study the evolution of development.

Hadjivasiliou Z**, Moore R**, McIntosh B, Galea G, Clarke J, & Alexandre P 2019 Basal protrusions mediate spatiotemporal patterns of spinal neuron differentiation, *Dev Cell*, 49, 907-919

In this work we show that during neurogenesis developing neurons inhibit other cells in their viccinity from also differentiating into neurons at the same time. This occurs through long, transient protrusions that express Delta and inhibit neurogenesis by activating Notch within their reach. I developed quantitative tools that allowed as to compare experimental data and a theoretical model to test different hypotheses. This is an ongoign collaboration between Paula Alexandre at UCL and myself



Name	ADRIAN HAYDAY
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	2009

Lab Name	***Immunosurveillance Laboratory***

Research programme and achievements

We aim to understand the basis of vertebrate tissue immunosurveillance, focusing on the roles of poorly understood but highly conserved tissue-resident γδ T cells that fill the gap spatially and temporally between innate and adaptive immunity. Within this aim, we seek to understand the inflammatory lesions and increased incidences of cancer that emerge when γδ T cell-mediated surveillance fails. Additionally, we seek to apply our expertise and capabilities to expand the breadth of immunological knowledge and our understanding of the human immune system. We study rodents and humans according to which is most appropriate to the question being asked.

In the quinquennium, we have:

1. Demonstrated that epithelial cells employ hitherto poorly understood, tissue-specific butyrophilin-like (Btnl) molecules to select and maintain signature tissue-resident γδ T cell compartments, and that this system is conserved from rodents through humans.
2. Demonstrated that Btnl molecules work as dimers that directly engage γδ T cell receptors (TCRs), thereby constituting long-sought γδ TCR ligands, and providing an explanation for the association of specific TCRs with specific tissues.
3. Demonstrated that Btnl molecules engage the TCR atypically, binding specifically to Vγ rather than to the γδ TCR CDR3 region that is the conventional site of antigen binding to TCRs or immunoglobulins.
4. Demonstrated that single γδ TCRs can be differentially activated by engaging conventional adaptive ligands and "innate-like" BTNL/Btnl ligands, captured by the term, "adaptate" immunity. This in turn argues that local T cells can actively sense normality as well as stress.
5. Demonstrated that loss-of-function in human BTNL3 and its consequent impact on the colonic γδ T cell compartment is associated with Crohn's Disease severity
6. Identified a tissue-resident Vδ1-expressing compartment in human breast and established its association with positive outcomes in triple negative breast cancer.
7. Developed methods for extraction and large-scale cultivation of human skin-resident Vδ1-expressing T cells that kill solid tumour targets: the basis for the

establishment of Gamma Delta Therapeutics and its off-shoot Adaptate Biotherapeutics in which equity is co-owned by CRUK, King's College and the Crick.

8. Expanded human immune-monitoring capacities, employing them to identify immune signatures of adverse events following swine flu vaccination, and to identify a consensus immune signature of COVID-19.
9. Expanded the immunological knowledge-base by designing and leading a high-throughput, multicentre Infection and Immunity Immunophenotyping (3i) screen of 530 novel single-gene mutant mouse strains that identified 140 immunoregulators of which 80 had never hitherto been implicated in immunology. This study also emphasised that immune systems should be viewed as multidimensional structures rather than as a collection of single parameters.
10. Established an open-access portal wherein we deposit all our raw data pertaining to human and mouse immunophenotyping [www.immunophenotype.org].

Our aims now are to understand:

1. The signal transduction pathway activated by BTNL/Btnl engagement of TCRγδ and how this results in T cell compartment selection and maintenance, by contrast to T cell activation by ligands engaging TCRγδ *via* CDR3;
2. Whether qualitatively differential antigen responsiveness (adaptate immunity) also applies to αβ T cells and B cells.
3. The molecular composition of the BTNL/Btnl "selectosome" unit
4. Molecularly how failures of the BTNL-γδ axis lead to inflammatory hyperactivation of other immune cells, and to increased levels of carcinoma;
5. Practically how this system might be monitored and manipulated in humans as an aid to clinical diagnostics and therapeutics.

Research outputs

Di Marco Barros R, Roberts NA, Dart RJ, Vantourout P, Jandke A, Nussbaumer O, Deban L, Cipolat S, Hart R, Iannitto ML, Laing A, Spencer-Dene B, East P, Gibbons D, Irving PM, Pereira P, Steinhoff U, Hayday AC. (2016) *Epithelia use butyrophilin-like molecules to shape organ-specific γδ T cell compartments* Cell 167(1):203-218. DOI: 10.1016/j.cell.2016.08.030

This paper established that intestinal epithelial cells use BTNL/Btnl molecules to select for and regulate tissue-specific gamma delta T cell compartments. It established a biological mechanism by which epithelial cells communicate with local T cells at steady-state ("normality sensing"). Following on from our prototypic discovery of such a mechanism in mouse skin, the work established conservation of the process across tissues as well as across species. The system is unperturbed by microbial colonisation.

Melandri D, Zlatareva I, Chaleil RAG, Dart RJ, Chancellor A, Nussbaumer O, Polyakova O, Roberts NA, Wesch D, Kabelitz D, Irving PM, John S, Mansour S, Bates PA, Vantourout P, Hayday AC. (2018) *The γδTCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness*. Nature Immunology 19(12):1352-1365. DOI: 10.1038/s41590-018-0253-5

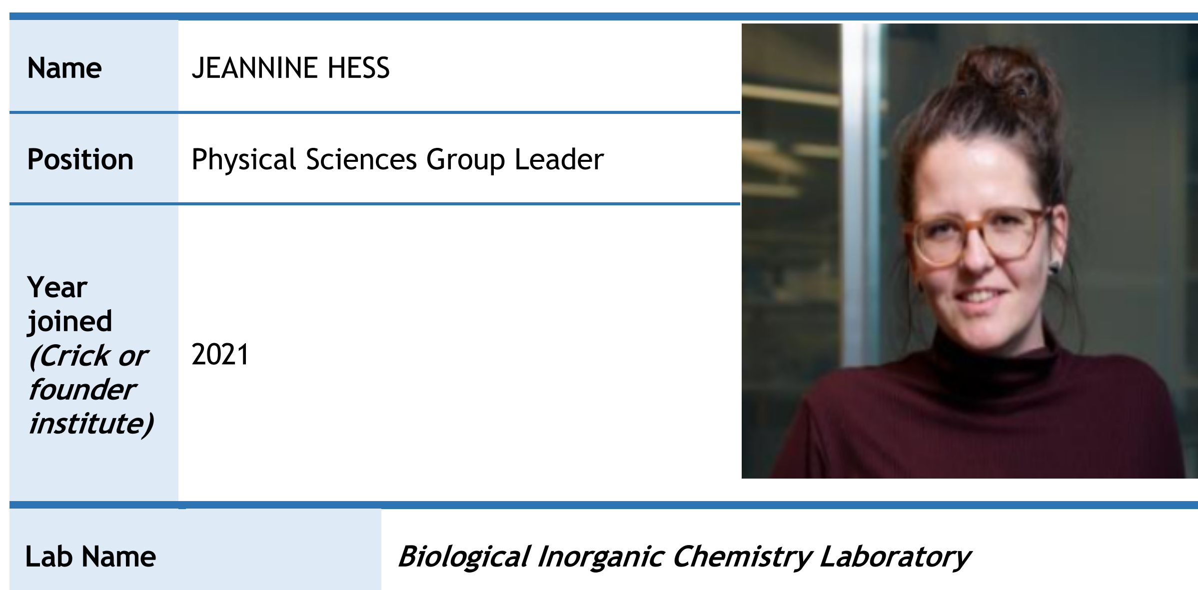
This paper provided molecular genetic data that the selective impacts of BTNL/Btnl molecules on local gamma delta T cell compartments are mediated by the cognate T cell receptors (TCRs) themselves, and it established that the regions responsible were solely within V-gamma, spatially distinct from the regions of the TCR used to engage conventional antigens. As such, BTNL/Btnl molecules emerged as long-sought human/mouse gamma

delta TCR ligands, and evoked the hitherto poorly understood regulation of alpha-beta T cells by V-beta specific "superantigens".

Wu Y, Kyle-Cezar F, Woolf RT, Naceur-Lombardelli C, Owen J, Biswas D, Lorenc A, Vantourout P, Gazinska P, Grigoriadis A, Tutt A, Hayday A. (2019) ***An innate-like Vδ1+ γδ T cell compartment in the human breast is associated with remission in triple-negative breast cancer*****. Science Translational Medicine 11(513) Article number eaax9364. DOI: 10.1126/scitranslmed.aax9364**
This paper provided the first molecular and functional characterisation of a human breast-associated local gamma delta T cell compartment, and linked the activity of that compartment to better outcomes in a breast cancer subtype that remains very challenging to treat. It provided a human validation for several studies associating local gamma delta T cells to cancer protection in mice, and it provided functional and biological validation for several human bio-informatics studies implying host-protective roles for gamma delta T cells in human cancer.

Abeler-Dörner .... Hayday AC. (2020) ***High-throughput phenotyping reveals expansive genetic and structural underpinnings of immune variation***
Nature Immunology 21(1):86-100. DOI: **10.1038/s41590-019-0549-0**
An illustration of the laboratory's breadth and ambition in developing novel approaches and collaborative endeavours to ask horizon-scanning questions in immunology. The results provided the community via an open portal [www/immunophenotype.org] with scores of new immunoregulatory genes and a structural perspective for viewing immune composition.

Laing…… Hayday AC. (2020) A dynamic COVID-19 immune signature includes associations with poor prognosis. Nat Med 26, 1623–1635. DOI: **10.1038/s41591-020-1038-6**
An illustration of how the laboratory's skill-sets and collaborative partnerships could be rapidly deployed to gain insight into the world's most severe infectious disease pandemic in 100 years. To aid those fighting COVID-19, all data were uploaded to an open portal [www/immunophenotype.org].



Name	JEANNINE HESS
Position	Physical Sciences Group Leader
Year joined *(Crick or founder institute)*	2021

Lab Name	*Biological Inorganic Chemistry Laboratory*

Research programme and achievements

Research Programme

Antibiotic resistance is one of the most significant public health challenges of our time and is expected to cause 10 million deaths per year by 2050. In 2019 alone, antimicrobial resistance (AMR) caused more than one million deaths. However, innovative antibacterial development strategies that delivered effective antibiotics are rare.

In the Biological Inorganic Chemistry Laboratory, we investigate the construction of new antibiotics based on novel and original scaffolds, with new mechanisms of action, and that address new targets.

Metal complexes can be the basis of radically novel scaffolds, which in contrast to conventional small molecules, provide several key advantages. *First*, the high three-dimensionality of metal complexes allow targeting larger and more challenging binding sites more efficiently, facilitating the exploration of previously undruggable targets. *Second*, many metal-based scaffolds are new to nature, which can minimise treatment escape through pre-existing resistance mechanisms. *Third*, they can promote metal-specific mechanisms of action based on inherent chemical properties of the metal, such as the production of lethal reactive oxygen species (ROS).

While the development of metal-based drugs has mainly focussed on target-agnostic screening and 'binder-first' approaches, starting from known organic inhibitors that were functionalised with a metal-based moiety, my research groups is streamlining workflows for 'metal-first' approaches allowing the development of metal-based drug candidates.

Major Achievements

Setting up Chemistry at the Crick: Since September 2021, I have successfully recruited a Senior Laboratory Scientist (SLRS), postdoctoral co-workers, PhD students and hosted Master students from King's. We have built a fully functional chemistry lab to safely perform standard chemical reactions (four new fume hoods) and analyse (LC-MS/MS) our newly synthesised metal-based compounds.

Collaborations: As we are building different boundary-crossing approaches to develop new antimicrobials, I collaborate with likeminded national and international groups – Dr Julia Hubbard (Newcastle University, AMR drug discovery, ligand-based NMR screening), Prof. James Collins (UT Southwestern Medical Center, novel *Schistosome* drug targets), Dr Chrysi Sergaki (NIBSC, microbial high-throughput screening of metal-based compounds in health and disease), Prof. Wanhe Wang (Northwestern Polytechnical University Xi'an, iridium complexes as novel antimicrobials), Dr David House (GSK-Links Lab, cysteine-reactive metallo-fragments for novel target identification approaches)

Future Plans

Future projects in the group will focus on the application of activity-based protein profiling

techniques to identify novel drug targets in bacteria. With highly encouraging data in hand on mammalian cells, we will translate this research to Gram-positive and Gram-negative strains with the help of the GSK-Links lab and will draw on their expertise in high-throughput proteomics.
We will expand and screen our in-house metallo-fragment library on other drug targets and are in the process of starting a new collaboration with Dr Ester Morreale (newly recruited Group Leader), to identify metal-based BacPROTACS with multiple mechanisms of action.

Research outputs

J Hess Rational approaches towards inorganic and organometallic antibacterials Biol. Chem., 2022, 403, 363-375. This is my first independent paper and an invited review to Biological Chemistry, where I highlight the past and current approaches in rationally designing metal-based antimicrobials, critically analyse the limitations and future challenges of antibiotic drug discovery and describe the potential for metal-based compounds to considerably impact the field

J. C. Evans, D. Murugesan, J. M. Post, V. Mendes, Z. Wang, N. Nahiyaan, S. L. Lynch, S. Thompson, S. R. Green, P. C. Ray, J. Hess, C. Spry, A. G. Coyne, C. Abell, H. I. M. Boshoff, P. G. Wyatt, K. Y. Rhee, T. L. Blundell, C. E. Barry III, V. Mizrahi, ACS Inf. Dis., 2021, 7, 1666-1679 Targeting Mycobacterium tuberculosis CoaBC through Chemical Inhibition of 4′-Phosphopantothenoyl-l-cysteine Synthetase (CoaB) Activity
Most recent paper from this highly collaborative project that led to the identification of a substrate competitive small-molecule inhibitor of Mtb CoaB via a high-throughput screening approach, which I carried out during my postdoc at the University of Cambridge.

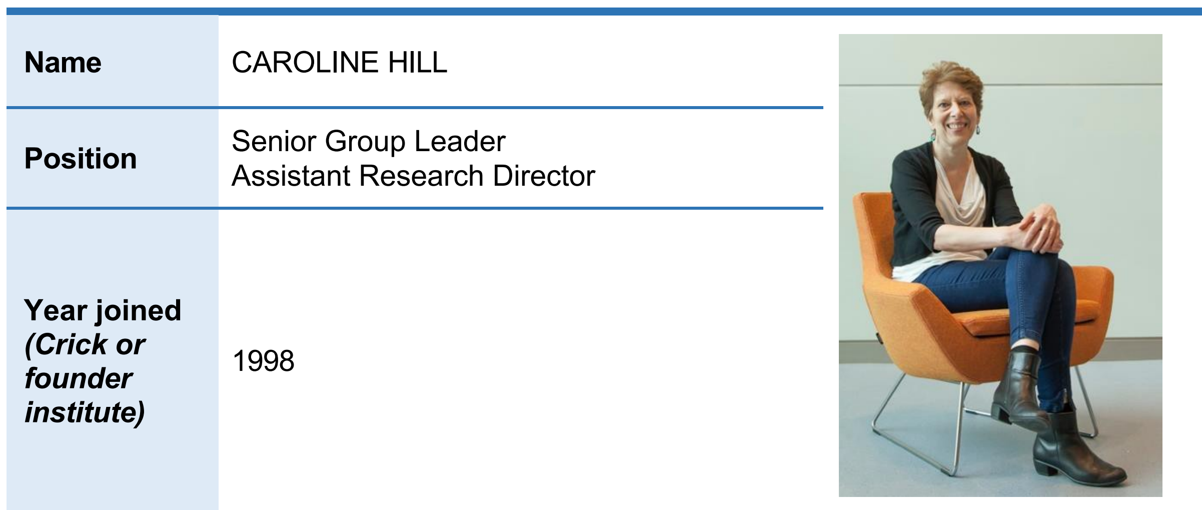
V. Mendes, S. R. Green, J. C. Evans, J. Hess, M. Blaszczyk, C. Spry, O. Bryant, J. Cory-Wright, D. S.-H. Chan, P. HM Torres, Z. Wang, S. O'Neill, S. Damerow, J. Post, T. Bayliss, S. L. Lynch, A. G. Coyne, P. C. Ray, C. Abell, K. Y. Rhee, H. I. M. Boshoff, C. E. Barry, V. Mizrahi, P. G. Wyatt, T. Blundell, Nat. Commun., 2021, 12,143, 1-12. Inhibiting Mycobacterium tuberculosis CoaBC by targeting a new allosteric site
This publication reports the full-length crystal structure of the bifunctional protein Mycobacterium smegmatis CoaBC, a crucial enzyme embedded in the Coenzyme A pathway and a validated drug target. Herein, we report the first allosteric inhibitor of CoaBC identified via a high-throughput screening approach.

D. S.-H. Chan, J. Hess, E. Shaw, C. Spry, R. Starley, C. Dagostin, M. V. B. Diaz, R. Kale, V. Mendes, T. Blundell, A. G. Coyne and C. Abell, Biochem. J., 2019, 476, 3125-3139. Structural insights into Escherichia coli phosphopantothenoylcysteine synthetase by native ion mobility-mass spectrometry
This work describes the applicability and versatility of native ion mobility-mass spectrometry as an innovative technique to gain structural insight into an enzyme target, herein exemplified by studying the CoaB domain of E. coli CoaBC, further highlighting the capability of identifying and assessing a new class of inhibitors of this enzyme.

J. Hess†, G. Panic, M†. Patra, L. Mastrobuoni, O. Blacque, S. Roy, J. Keiser, G. Gasser, Ferrocenyl, Ruthenocenyl, and Benzyl Oxamniquine Derivatives with Cross-Species Activity against Schistosoma mansoni and Schistosoma haematobium ACS Infect. Dis., 2017, 3, 645-652. († equal contributions) Highlighted in ACS Chemical Research in Toxicology.

To develop alternative treatment options against Schistosomiasis, we have designed, synthesised, and evaluated the biological stability and potency of several organometallic derivatives of Oxaminquine (OXA). These first-in-class metal-based compounds demonstrated simultaneous in vitro/in vivo activity against Schistosoma mansoni and were also active against Schistosoma haematobium, thus extending the activity profile of the

parent drug.



Name	CAROLINE HILL	
Position	Senior Group Leader Assistant Research Director	
Year joined *(Crick or founder institute)*	1998	

Lab Name	***Developmental Signalling Laboratory***

Research programme and achievements

The fundamental biological problem I want to solve is how cells use signal transduction pathways to communicate with each other in the context of whole organisms to regulate new programmes of gene expression and induce new behaviours in their neighbours. This is important, as cell communication is fundamental to the decision-making processes that orchestrate embryonic development, thereby determining how tissues and organs of the appropriate size develop in the right place at the right time. Furthermore, mis-regulation of developmental signalling pathways results in severe human diseases, like cancer.

To understand how intercellular signalling functions in embryonic development and disease, my lab focuses on the TGF-β family of ligands, a group of highly evolutionarily-conserved growth and differentiation factors that signal through serine/threonine kinase receptors and the SMAD transcription factors. These ligands play essential roles in the very earliest processes of germ layer specification and patterning, and perturbation of their activity leads not only to cancer, but to a plethora of other disorders, including the Marfan-related syndromes. Our goal is to unravel mechanistically how dynamic spatial and temporal patterns of signals are established, then transduced from receptors to the nucleus, and how this leads to new programmes of gene expression. Furthermore, we want to understand how signalling by TGF-β family ligands functionally cooperates with other signalling pathways to pattern tissues, and to determine how mis-regulation of TGF-β family signalling leads to human pathologies.

A theme that unites the entire programme is the issue of how spatial and temporal information is ‘encoded’ in TGF-β family signalling pathways and interpreted at both the cellular level and more broadly, at the level of tissues and organisms. We take the distinctive approach of studying the pathways in the context of both normal development and disease, which allows us to take fundamental insights from our developmental work and apply them to our studies of deregulated signalling in disease contexts. Working on the diseases in turn uncovers mechanisms relevant for normal physiology.

Our major achievements in the last quinquennium have been to decipher the mechanism underlying how signal duration affects output, and to determine how TGF-β family signals drive dynamic transcriptional programmes, leading to different cell behaviours. In this context, we have uncovered the mechanism whereby domains of BMP and Nodal ligand activity are established in early zebrafish embryos and are functionally interpreted. Furthermore, we have demonstrated how Nodal signalling acts in an incoherent feedforward loop with the FGF pathway to specify mesoderm and endoderm. Importantly, insights from our developmental work on Nodal signalling and the unexpected behaviours

we discovered for TGF-β, prompted us to rethink the role of TGF-β family signalling in tumourigenesis, and has led to a major translational project, developing a potential cancer therapeutic. We have also discovered a new mechanism of receptor activation in TGF-β family signalling that we have now shown to be relevant for aberrant Activin signalling in the diseases Fibrodysplasia Ossificans Progressiva and Diffuse Intrinsic Pontine Glioma.

Our goal in the next five years is to exploit this new knowledge and expertise in a fully integrated project with three main aims:

1. **To dissect the function of TGF-β family signalling in cell fate decision making over time.** We particularly want to solve the mystery of why only a subset of cells in the first two cell tiers of the blastula stage zebrafish embryo are specified to become endoderm progenitors, whilst the adjacent cells, which experience an almost identical environment, become mesodermal progenitors.
2. **To determine the mechanisms by which cells read time to monitor signalling duration and strength in health and disease.** From our previous work we hypothesise that this occurs primarily at the level of receptor trafficking. We are therefore focusing on developing new methods for tracking individual receptors and thus defining trafficking routes and dynamics.
3. **To unravel the consequences of deregulated TGF-β family signalling in disease.** We want to understand the role of aberrant TGF-β family signalling in cancer, in particular in modulating the tumour microenvironment, and are actively developing a potential therapeutic that we hypothesise will act synergistically with immune checkpoint therapies. We are also focused on understanding the molecular mechanisms underlying Marfan-related syndromes, which arise as a result of point mutations in core components of the TGF-β family pathways.

Research outputs

van Boxtel, A. L., Chesebro, J. E., Heliot, C., Ramel, M. C., Stone, R. K., and Hill, C. S. (2015) *A temporal window for signal activation dictates the dimensions of a Nodal signaling domain*. Dev Cell 35, 175-185. DOI: 10.1016/j.devcel.2015.09.014
This paper shows how temporal information in the zebrafish embryo is transformed into a spatial pattern. We demonstrate how the Nodal signalling gradient is formed in the early zebrafish embryo and show that its size and shape are determined by a temporal signal activation window created by a microRNA-mediated delay in the translation of Lefty, a Nodal antagonist. This paper was important as it not only challenged the long-held view in the field that the Nodal gradient was formed by a reaction–diffusion mechanism, but highlighted the importance of signalling duration in gradient formation.

Coda, D. M.*, Gaarenstroom, T.*, East, P., Patel, H., Miller, D. S. J., Lobley, A., Matthews, N., Stewart, A., and Hill, C. S. (2017*) Distinct modes of SMAD2 chromatin binding and remodeling shape the transcriptional response to NODAL/Activin signaling*. Elife 6, e22474. DOI: 10.7554/eLife.22474
This paper explains how SMAD2, the downstream signal transducer of the NODAL/Activin pathway, regulates transcription. We defined the sequence of events that occur from SMAD2 binding to transcriptional activation, and the mechanisms underlying them, thereby establishing new paradigms for signal-dependent transcriptional regulation.

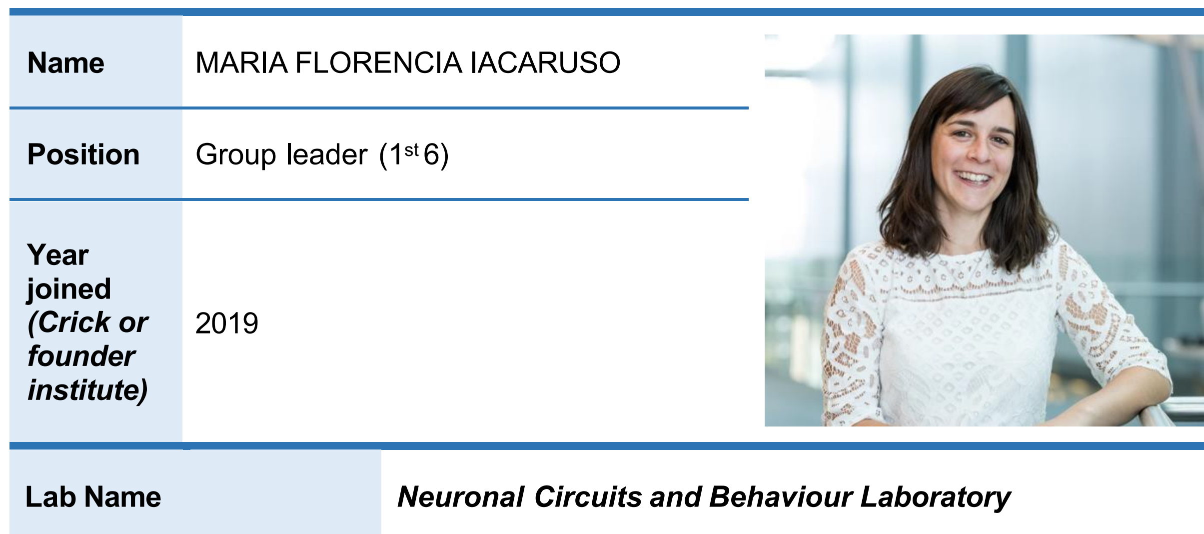
Ramachandran, A., Vizán, P., Das, D., Chakravarty, P., Vogt, J., Rogers, K. W., Müller, P., Hinck, A. P., Sapkota, G. P., and Hill, C. S. (2018) *TGF-β uses a novel mode of receptor activation to phosphorylate SMAD1/5 and induce epithelial-to-mesenchymal transition*. Elife 7, e31756. DOI: 10.7554/eLife.31756

This paper describes a new mechanism for receptor activation, which we believe may be widespread among TGF-β family members. It also demonstrated for the first time the

importance of combinatorial signalling via both SMAD pathways for a functional TGF-β response.

van Boxtel, A. L., Economou, A. D., Heliot, C., and Hill, C. S. (2018) ***Long-range signaling activation and local inhibition separate the mesoderm and endoderm lineages*****. Dev Cell 44, 179-191 DOI: 10.1016/j.devcel.2017.11.021**
This work represents a major step forward in deciphering the organising principles underlying early embryonic patterning. It revises the view that tissues are patterned through a simple long-range morphogen gradient, and instead reveals the importance of feedforward and feedback loops involving multiple signalling pathways.

Miller, D.S.J., Bloxham, R.D., Jiang, M., Gori, I., Saunders, R.E., Das, D., Chakravarty, P., Howell, M., Hill, C.S. (2018) ***The dynamics of TGF-β signaling are dictated by receptor trafficking via the ESCRT machinery*****. Cell Rep 25, 1841-1855.e5. DOI: 10.1016/j.celrep.2018.10.056**
This work revealed for the first time how receptor trafficking shapes signalling dynamics. Using whole genome siRNA screening, we demonstrated that TGF-β receptors are targeted for degradation by the ESCRT machinery. Inhibiting ESCRT components upregulates long-term TGF-β signalling and enhances functional outputs of the pathway to sensitise cells to low levels of ligand in the microenvironment, which we show could be relevant in cancer.



Name	MARIA FLORENCIA IACARUSO
Position	Group leader (1st 6)
Year joined *(Crick or founder institute)*	2019

Lab Name	***Neuronal Circuits and Behaviour Laboratory***

Research programme and achievements

In order to determine behavioural priorities, the brain must integrate sensory information from multiple modalities with motivational and contextual information. Many situations require a fast evaluation of the salience and the relevance of the sensory information, for example during orienting to relevant targets. However, it is not clear how target priority is determined by the brain.

The superior colliculus (SC) is an evolutionary conserved structure, present in all vertebrates, involved in orienting and escape behaviours and it has been implicated in spatial attention and target selection. The SC has access to a wide range of multisensory inputs, as well as non-sensory information from primary and association areas of the neocortex, and the hypothalamus. In turn, the SC projects to the thalamus (mainly to the lateral posterior /pulvinar complex), the dorsolateral periaqueductal gray and to the motor systems in the brainstem and spinal cord. This extensive afferent and efferent connectivity suggests that the SC serves as a hub for linking brain circuits carrying information concerning the location of high-priority targets for an immediate re-orienting response. Thus, the SC provides a unique opportunity to investigate how, within a single brain structure, signals from the different senses are combined with non-sensory information and used to guide motor responses.

At present, it is not clear how functional microcircuits within the SC integrate and assess the relevance of sensory information from different modalities to generate an attentional shift or a motor response. To understand this, we are using two-photon microscopy and extracellular electrophysiological recordings to monitor the activity of hundreds of neurons from the superior colliculus of mice while they perform visual-auditory localisation tasks. The use of genetic markers and optogenetic approaches allows us to identify different neuronal populations and determine their role during behaviour. We are also studying the rules that determine how these different cell types communicate, by performing multiple patch clamp recordings in brain slices to study the strength and probability of their synaptic connections. My long term goal is to elucidate the relationship between the architecture and physiological function of the neuronal circuits underlying the selection of a behaviourally relevant target.

Research outputs

Kim MH, Znamenskiy P, Iacaruso MF, Mrsic-Flogel TD (2018). *Segregated subnetworks of intracortical projection neurons in primary visual cortex*. Neuron 100:1313-1321. DOI : 10.1016/j.neuron.2018.10.023

In the sensory cortex, intermingled neurons encode different attributes of sensory inputs and relay them to different long-range targets. The relationship between synaptic connectivity within an area and long-range projection target remains unclear. We examined the local connectivity and visual responses of primary visual cortex neurons projecting to anterolateral (AL) and posteromedial (PM) higher visual areas in mice. We showed that projection target, in addition to response similarity, constrains local synaptic connectivity of AL and PM projection neurons. We propose that reduced crosstalk between different populations of projection neurons permits independent function of these output channels.

Znamenskiy P, Kim MH, Muir DR, Iacaruso MF, Hofer SB, Mrsic-Flogel TD (2018). *Functional selectivity and specific connectivity of inhibitory neurons in primary visual cortex*. BioRxiv 294835. DOI: 10.1101/294835

In the cerebral cortex, the interaction of excitatory and inhibitory synaptic inputs shapes the responses of neurons to sensory stimuli, stabilises network dynamics and improves the efficiency and robustness of the neural code. Our results indicate that individual parvalbumin-expressing (PV) inhibitory cells in mouse primary visual cortex are preferentially integrated into subnetworks of inter-connected, co-tuned pyramidal cells, stabilising their recurrent dynamics. Conversely, weak but dense inhibitory connectivity between subnetworks is sufficient to support competition between them, de-correlating their output.

Iacaruso MF, Gasler IT, Hofer SB (2017). *Synaptic organization of visual space in primary visual cortex Nature 547:449-452 27*. DOI: 10.1038/nature23019

How a sensory stimulus is processed and perceived depends on the surrounding sensory scene. In the visual cortex, contextual signals can be conveyed by an extensive network of intra- and inter-areal excitatory connections that link neurons representing stimulus features separated in visual space. We show that neurons with displaced receptive fields connect preferentially when their receptive fields are co-oriented and co-axially aligned.

This organisation of synaptic connectivity is ideally suited for the amplification of elongated edges, which are enriched in the visual environment, and thus provides a potential substrate for contour integration and object grouping.

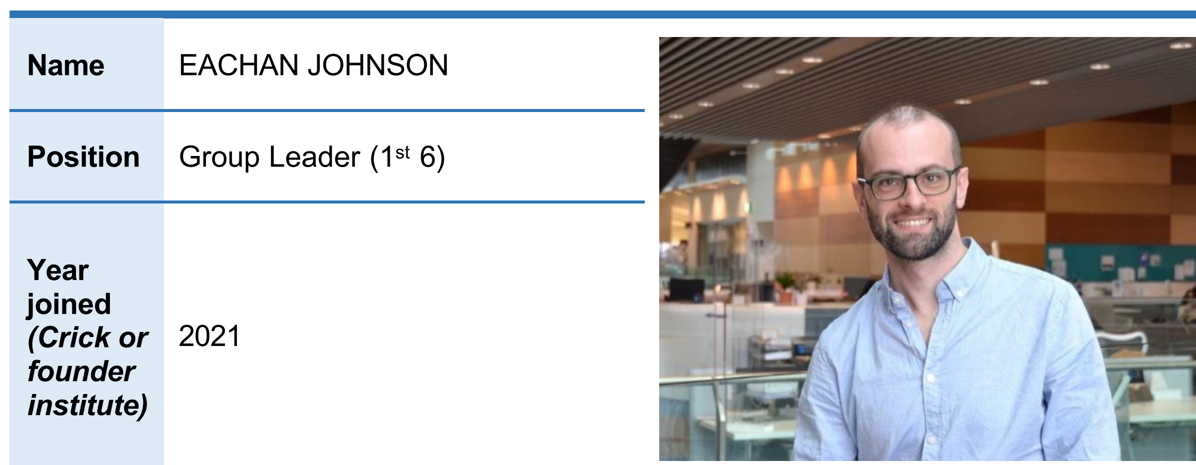
Okun M, Steinmetz NA, Cossell L, Iacaruso MF, Ko H, Barthó P, Moore T, Hofer SB, Mrsic-Flogel TD, Carandini M & Kenneth D. Harris (2015*). Diverse coupling of neurons to populations in sensory cortex*. Nature 521:511-515. DOI: 10.1038/nature14273

A large population of neurons can in principle produce an astronomical number of distinct firing patterns. In the cortex, however, these patterns lie in a space of lower dimension as if individual neurons were “obedient members of a huge orchestra”. We showed that neighbouring neurons can differ in their coupling to the overall firing of the population, ranging from strongly coupled “choristers” to weakly coupled “soloists” and established a measure of population coupling. This measure characterises the relationship of each neuron to a larger population, explaining seemingly complex network firing patterns in terms of basic circuit variables.

Cossell L, Iacaruso MF, Muir DR, Houlton R, Sader EN, Ko H, Hofer SB, Mrsic-Flogel TD (2015). *Functional organization of excitatory synaptic strength in primary visual cortex*. Nature 518:399-403. DOI: 10.1038/nature14182

The strength of synaptic connections fundamentally determines how neurons influence each other's firing. Excitatory connection amplitudes between pairs of cortical neurons vary over two orders of magnitude, comprising only very few strong connections among many weaker ones. Our results showed that the apparently complex organisation of excitatory connection strength reflects the similarity of neuronal responses, and suggest that rare, strong connections mediate stimulus-specific response amplification in cortical microcircuits.

Name	EACHAN JOHNSON
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2021



Lab Name	***Systems Chemical Biology of Infection and Resistance Laboratory***

Research programme and achievements

We are developing precision molecular tools to study how pathogenic bacteria survive and infect, enabling the design of new antimicrobial therapies.

As soon as new antimicrobial drugs are discovered and used in the clinic, pathogenic bacteria inevitably evolve resistance, driving an unsustainable cycle threatening the twentieth century's improvements to public health.

Antibiotics revolutionised modern medicine, but once again millions of lives are threatened by pathogenic bacteria like *M. tuberculosis*, which causes tuberculosis, the deadliest infectious disease and one of the top 10 causes of death worldwide.

Working at the interface of genetics, chemistry, and machine learning, we use chemical 'probes' to systematically and precisely disrupt the cellular machinery of *M. tuberculosis* and study the consequences of this disruption on its ability to survive, infect, and resiliently evolve resistance.

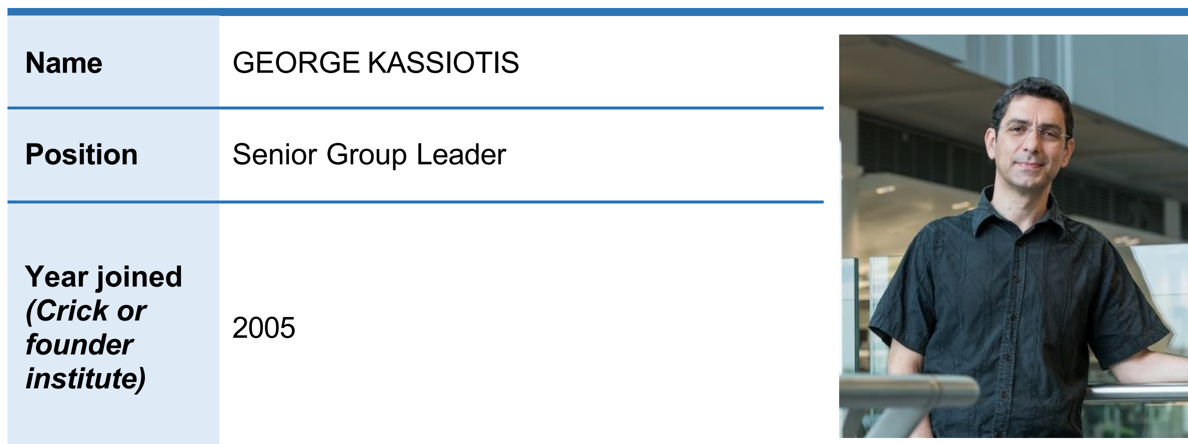
With this approach, we seek to bridge the gap between understanding pathogen biology and designing new therapeutic strategies.

Research outputs

Johnson EO, et al. (2019) *Large-scale chemical-genetics yields new M. tuberculosis inhibitor classes*. Nature, 571, 72–78. DOI: 10.1038/s41586-019-1315-z
Development of a new phenotypic chemical screening strategy in M. tuberculosis that, for the first time, provided mechanism of action information for active compounds in primary phenotypic screening data and sensitively detected bioactive small molecules in new regions of chemical space, enabling compound prioritization based on putative targets instead of simply on potency. Highlighted in Nature Reviews Drug Discovery, Nature Chemical Biology and Biochemistry

Johnson EO, Office E, Kawate T, Orzechowski M, Hung DT. (2019) *A large-scale chemical-genetic strategy to design antimicrobial combination chemotherapy for Mycobacterium tuberculosis*. ACS Infectious Diseases, 6(1), 56–63. 10.1021/acsinfecdis.9b00373

Retrospectively mined the PROSPECT data, for another structurally distinct compound inhibiting EfpA. Resistance conferring mutations of the two compounds are mutually exclusive, and resistance to one compound causes hypersensitivity to the other, in some cases completely suppressing emergence of cross-resistance.



Name	GEORGE KASSIOTIS
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2005

Career History

2000- 2005: Post-doctoral scientist, Molecular Immunology, MRC National Institute for Medical Research, London, UK
2005- 2011: Programme leader-track, Immunoregulation, MRC National Institute for Medical Research, London, UK
2011- 2015: Programme leader, Immunoregulation, MRC National Institute for Medical Research, London, UK
2015- present: date Senior Group Leader, Retroviral Immunology, the Francis Crick Institute, London, UK

Major Awards, Honours and Prizes

1997: Short-term EMBO fellowship, European Molecular Biology Organization (EMBO)
1998: Young Investigator Award, European League Against Rheumatism (EULAR)
2012: Non-Resident Ordinary Member, Medical Research Club, London, UK
2018: Sir David Cooksey Prize in Translation, The Francis Crick Institute, London, UK

Membership of external committees, editorial boards, review panels, SABs etc

2016 – date Supervisor, Wellcome Trust Clinical PhD Programme - Imperial Immunity, Inflammation, Infection and Informatics (4i)
2015 – date Cancer Immunology Expert Review Panel, Cancer Research UK
2016 – date MRC-CRUK Cancer Immunology and Immunotherapy Advisory Group
2020 – date The Children and Young People's Cancer Innovation Award Expert Review Panel, Cancer Research UK
2020 – date Cancer Grand Challenges workshop member, Cancer Research UK
2016 – date Genome Editing Mice for Medicine (GEMM) Review Panel, Harwell, MRC, UK

Lab Name	*Retroviral Immunology Laboratory*

Research programme and achievements

In the last five years, we explored basic aspects of viral disease pathogenesis, the viral and host characteristics that determine the outcome of infection and the quality of the immune response that can afford immune protection to viral infection. At the same time, we initiated work on the role of endogenous retroviruses, particularly in cancer.
We have been focusing on CD4 T helper (Th) cells, the central orchestrators of the immune response, which we study in a mouse model for infection with murine leukaemia virus (MLV), a mouse retrovirus. The immune interplay between MLVs and their natural host involves the numerous MLVs that have invaded the murine germline and are part of the enormous constellation of endogenous retroviruses (ERVs). Consequently, we have begun investigating the impact of ERVs on host physiology and pathology, separately in mice and humans, given the phylogenetic divergence between their ERVs.
We have been able to establish unique experimental systems, which offered novel insights into these key questions. Notable findings include the following:

- The unexpected impact of vaccine vectors on the quality, as well as the magnitude of the CD4 T cell response.
- The rules governing clonal selection of CD4 T cells during the antigen-specific response, particularly the decisive role of B cells.
- The distinctive transcriptional profile, at the single-cell level, of the enigmatic CD4 T cell subset with granzyme-mediated cytotoxic potential.
- The influence of host exposure to unrelated pathogens and commensals on genome-wide ERV transcriptional activity.
- The discovery of transmissible retroviruses resurrected from defective ERVs and their control by host immunity.

We pay particular attention to the interaction between the immune system and chronic retroviral infections, as well as the vast number of ERVs and other retroelements. We first described the complex interplay between immune stimulation by symbiotic or pathogenic microbes, the genome-wide activity of ERVs in the murine host, and immune competence. Immune reactivity to retained viral properties of ERVs ('viral mimicry hypothesis') has now become an area of intense investigation by several groups. We have also generated the bioinformatics tools for probing the transcriptional patterns of ERVs, both in mice and humans.
We also demonstrated the surprising finding that not only is adaptive immunity to ERVs possible to induce, it also critical to prevent ERV 'resurrection'. We uncovered how immunological tolerance to ERVs can be broken, in order to elicit effective anti-ERV immunity. Although autoimmune in nature, we further showed that anti-ERV responses can be used in the protection against tumours, taking advantage of transcriptional ERV induction in transformed cells.
Our most recent work focuses on the transcriptional patterns of human endogenous retroelements, with the recent description of a pan-cancer *de novo* transcript assembly. This laid the foundations for further study of the impact of human endogenous retroelements in molecular and cellular functions in health and disease.
Identification and targeting of ERV-encoded antigens required knowledge of particular ERV proviruses or ERV overlapping transcripts specifically expressed in diverse cancer types. We developed methodology to interrogate RNA-seq data, uncovering characteristic associations between ERVs and all major cancer types. This included investigation of the effect of epigenetic anti-cancer drugs, such as 5-Azacitidine, on ERV expression, with particular emphasis on myelodysplastic syndrome and acute myeloid leukaemia. The discovery of ERV-encoded cancer-specific antigens has led to the creation of a Crick spin-out company, Enara Bio (formerly ERVAXX).
Over the next few years, we will make full use of the pan-cancer *de novo* transcriptome assembly to pinpoint cases of retroelement onco-exaptation, the co-option of endogenous retroelements in the oncogenic process. We have identified over 20 candidate onco-exaptation events in diverse cancer types, which we will validate functionally in *in vitro* and *in vivo* preclinical models. We will further develop and use *in vivo* mouse cancer models expressing human HLA, where human cancer-specific antigens, encoded by

human endogenous retroelements, can be assessed for immunogenicity and anti-tumour immunity.
In the past six months, we have been studying COVID-19, developing research and diagnostic serology assays. We demonstrated protective pre-existing immunity to SARS-CoV-2 from seasonal coronavirus exposure, a finding that attracted considerable attention, and our ERV work identified a novel ACE2 isoform, responsible for the interferon inducibility wrongly attributed to the canonical form, a result which questioned the efficacy of interferon treatment in COVID-19. Although the level of our long-term commitment to COVID-19-related work is currently difficult to decide, we will continue at least part of this work, particularly the research into cross-reactive and cross-protective HCoV antibodies that could be the bases of a pan-coronavirus vaccine.

Research outputs

Merkenschlager, J., Eksmond, U., Danelli, L., Attig, J., Young, G. R., Nowosad, C., Tolar, P. & Kassiotis, G. (2019) ***MHC class II cell-autonomously regulates self-renewal and differentiation of normal and malignant B cells*****. Blood 133, 1108-1118. DOI: 10.1182/blood-2018-11-885467**
A recent example of our interest in basic immunology with ramifications for cancer. We described a role for the best-studied immune molecule, MHC II, that extends beyond its immunological function to cell-intrinsic regulation of stemness and differentiation. This provides an alternative interpretation of the frequent loss of MHC II during tumour evolution.

Attig, J., Young, G. R., Hosie, L., Perkins, D., Encheva-Yokoya, V., Stoye, J. P., Snijders, A. P., Ternette, N. & Kassiotis, G. (2019) ***LTR retroelement expansion of the human cancer transcriptome and immunopeptidome revealed by de novo transcript assembly*****.** ***Genome Res*** **29, 1578-1590. DOI: 10.1101/gr.248922.119**
We assembled and disseminated the most complete, to date, transcriptome with a focus on transcripts initiated by or overlapping with endogenous retroelements. This assembly doubles the number of known transcripts and forms the basis for in-depth analysis of retroelement studies in health and disease, particularly in cancer. It also provided unconventional targets for novel cancer vaccines that are being developed by Enara Bio.

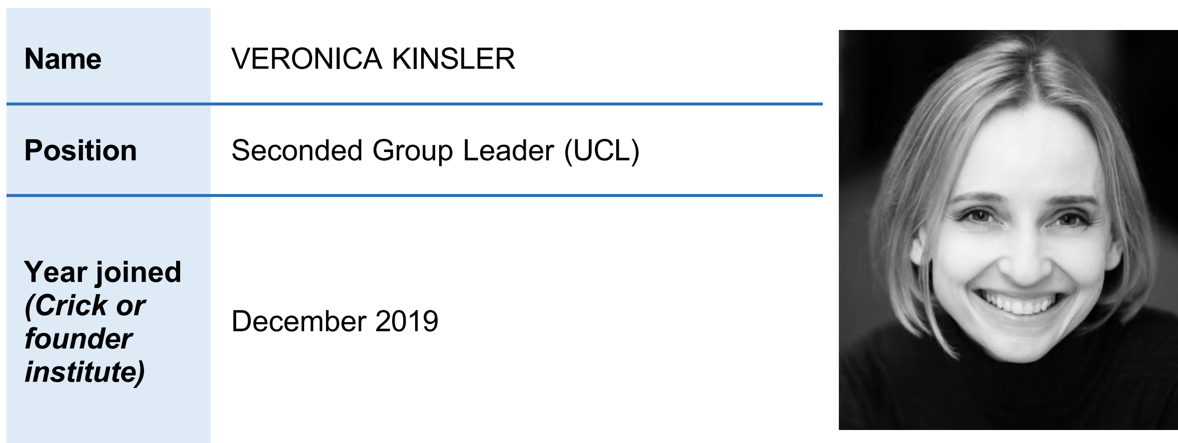
Kazachenka, A., Young, G. R., Attig, J., Kordella, C., Lamprianidou, E., Zoulia, E., Vrachiolias, G., Papoutselis, M., Bernard, E., Papaemmanuil, E., Kotsianidis, I. & Kassiotis, G. (2019) ***Epigenetic therapy of myelodysplastic syndromes connects to cellular differentiation independently of endogenous retroelement derepression*****. Genome Med 11, 86. DOI: 10.1186/s13073-019-0707-x**
We extended our work on endogenous retroelement deregulation in cancer, particularly in bone marrow cancers treated with epigenetic drugs, thought to work through reactivation of these elements (the viral mimicry hypothesis). We described a *de novo* transcript assembly of bone marrow cancers which uncovered alternative splicing as the main determinant of the response to epigenetic drugs.

Ng, K. W., Attig, J., Young, G. R., Ottina, E., Papamichos, S. I., Kotsianidis, I. & Kassiotis, G. (2019) ***Soluble PD-L1 generated by endogenous retroelement exaptation is a receptor antagonist*****. Elife 8. DOI: 10.7554/eLife.50256**
We used our transcript assembly to discover a novel form of PD-L1 with receptor antagonist function, created through exaptation of a normally intronic retroelement, with significant implications for immunity, autoimmunity and cancer immunotherapy.

Ng, K. W., Faulkner, N., Cornish, G. H., Rosa, A., Harvey, R., Hussain, S., Ulferts, R., Earl, C., Wrobel, A., Benton, D., Roustan, C., Bolland, W., Thompson, R., Agua-Doce,

A., Hobson, P., Heaney, J., Rickman, H., Paraskevopoulou, S., Houlihan, C. F., Thomson, K., Sanchez, E., Brealey, D., Shin, G. Y., Spyer, M. J., Joshi, D., O'Reilly, N., Walker, P. A., Kjaer, S., Riddell, A., Moore, C., Jebson, B. R., Wilkinson, M. G. L., Marshall, L. R., Rosser, E. C., Radziszewska, A., Peckham, H., Ciurtin, C., Wedderburn, L. R., Beale, R., Swanton, C., Gandhi, S., Stockinger, B., McCauley, J., Gamblin, S., McCoy, L. E., Cherepanov, P., Nastouli, E. & Kassiotis, G.(2020) ***Pre-existing and de novo humoral immunity to SARS-CoV-2 in humans*****. Science 370:1339-1343 DOI: 10.1126/science.abe1107**

An example of our work on COVID-19 and of the flexible and collaborative nature of the Crick, involving several labs within the Crick and our collaborating universities and university hospitals. In this work, we described the discovery of pre-existing binding and neutralising antibodies against SARS-CoV-2 in uninfected and unexposed individuals. These antibodies, likely induced by exposure to seasonal coronaviruses, are present in a small percent of adults but in the majority of children, consistent with the relative sparing of the latter from the severe form of COVID-19.



Name	VERONICA KINSLER
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	December 2019

Lab Name	***Mosaicism and Precision Medicine Laboratory***

Research programme and achievements

The Kinsler Lab research programme is centred around mosaic disorders, which arise secondary to pathogenic post-zygotic single cell genetic variants during human embryogenesis. The variants are often incompatible with life in the germline, and very frequently are in known oncogenes, resulting in severe congenital malformations and a risk of malignancy. These conditions are universally untreatable currently.

Genetic causes for these conditions have only been discovered in the last decade, due to the technical challenges of finding causal genes in what are sporadic disorders which only affect some tissues and are intermingled with normal cells. In the last 8 years the lab has honed the technical expertise required and have found the causes of 8 of these diseases, with the participation of patients from Great Ormond St Hospital and funding from the Wellcome Trust, and key patient charities Caring Matters Now and Butterfly AVM. This has led to 5 compassionate use trials for repurposed targeted therapies in children (all as yet unpublished). Small molecule therapies however are only effective to some degree and our lab's main thrust has been to understand the basic biology of these diseases with a view to developing novel therapies. Genetic therapies including siRNA and CRISPR base editing have been successfully developed *in vitro* for two diseases thus far, one of which forms the basis of the current NIHR Research Professorship awarded in 2021.

On the basic science front the lab has confirmed their hypothesised principal that these rare diseases of somatic mutagenesis can permit discovery of germline predisposing genetic variants. This has led to the identification of two new loci which predispose to melanocytic neoplasia (naevus and melanoma formation) – the first is a duplication affecting gene *PPP2R3B* (in press) and the second is a cluster of regulatory region variants in a melanoma oncogene (ongoing work). This novel and highly targeted approach is thus capable of identifying cancer predisposition genes which have been thus far undetectable using standard broad screening methods. Furthermore, the unravelling of the mechanism of the predisposition to somatic mutagenesis is not only fascinating but highly likely to be relevant to low penetrance loci in the general population.

Final areas of investigation and achievement in the advancement of knowledge in the scientific field are in embryogenesis. As mosaic disorders are as a results of single cell variants, the offspring of those variants are a visual representation of the process of embryogenesis. Using the patterns of the congenital malformations on the skin we have proposed the existence of a non-neural crest melanocyte precursor, and a large-scale human lineage tracing experiment using NGS is underway to try to confirm its existence. In addition, we have hypothesised the existence of an unsuspected melanocytic- endothelial (pigment-vascular) joint precursor, by demonstrating the same mutation in children in both types of cell in different body areas, and early zebrafish work by our collaborators has confirmed

the presence of these cells.

Research outputs

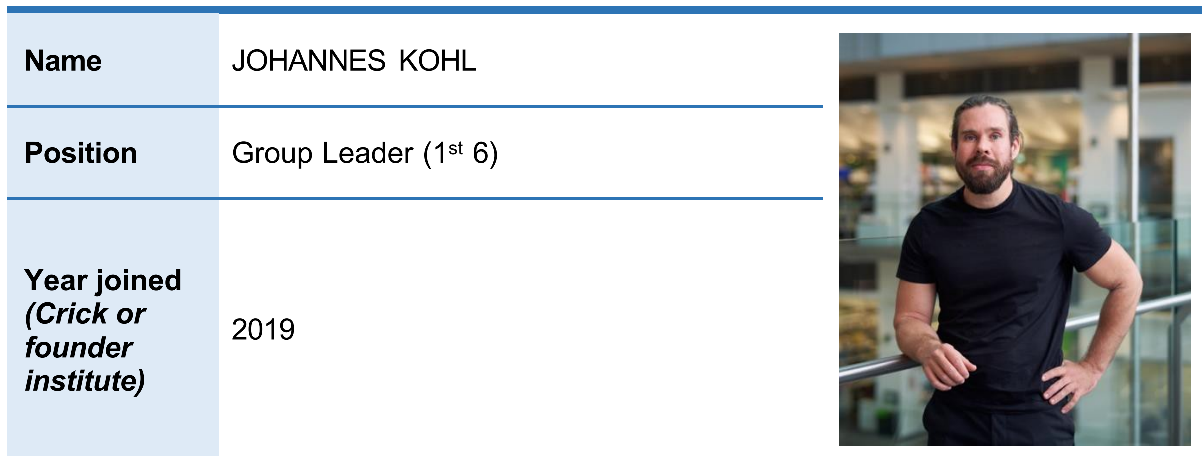
Al-Olabi L, Polubothu S, Dowsett K, Andrews KA, Stadnik P, Joseph AP, Knox R, Pittman A, Clark G, Baird W, Biesecker L, Bulstrode N, Glover M, Hargrave D, Huson SM, Jacques T, James G, Kandolf H, Kangesu L, Keppler-Noreuil K, Khan A, Lindhurst M, Lipson M, O'Hara J, Mahon C, Mosica A, Moss C, Murthy A, Parker V, Rivière J-B, Sapp J, Sebire NJ, Sivakumar B, Thomas A, Virasami A, Waelchli R, Zeng Z, Barnacle A, Topf A, Semple RK, Patton EE, Kinsler VA. (2018) *Mosaic RAS/MAPK variants cause sporadic vascular malformations which respond to targeted therapy*. J Clin Invest 128(4):1496-1508. DOI: 10.1172/JCI98589
This was a seminal publication as we discovered the genetic basis of arteriovenous malformations in children, and demonstrated that targeted therapy in an animal model was able to reverse the phenotype. This paper has led to multiple international clinical trials, currently in progress.

Kinsler VA, Larue L. (2018) *The patterns of birthmarks suggest a novel population of melanocytic precursors*. Pig Cell Mel Res (1):95-109. DOI: 10.1111/pcmr.12645.
This paper is likely to be important in the long term as it proposes a novel population of mesodermal melanocytes. This was based on the study of more than 12,000 human pigmentary patterns and with input from a murine pigmentary expert, and has caused waves in the field of pigment cell research. We are currently undertaking a large scale human lineage tracing study by single cell sequencing from multiple tissues, to attempt to prove the existence of this population, which would have direct implications on the stratification of melanoma.

Kinsler VA, O'Hare P, Bulstrode N, Calonje JE, Chong WK, Hargrave D, Jacques T, Lomas D, Sebire NJ, Slater O. (2017) *Melanoma in congenital melanocytic naevi*. Br J Dermatol 176(5):1131-1143. DOI: 10.1111/bjd.15301
This is a review which also contained new data from our 28 year prospective study of melanoma in 448 patients CMN which is by far the largest study in the world in this condition. It lays out clinical management and has been highly cited so far, and will likely continue to be an important paper in the field.

Thomas AC, Zeng Z, Rivière J-B, O'Shaughnessy R, Al-Olabi L, St-Onge J, Atherton DJ, Aubert H, Bagazgoitia L, Barbarot S, Bourrat E, Chiaverini C, Chong WK, Duffourd Y, Glover M, Groesser L, Hadj-Rabia S, Hamm H, Happle R, Khan I, Jean- Lacour J-P, Waelchli R, Wobser M, Vabres P, Patton EE, Kinsler VA. (2016) *Mosaic activating mutations in GNA11 and GNAQ cause phakomatosis pigmentovascularis*. J Invest Dermatol 136(4):770-8. DOI: 10.1016/j.jid.2015.11.027.
This paper was the discovery of the genetic basis of a neuro-ophthalmo-cutaneous disease known as PPV, a mixture of pigmentary and vascular congenital abnormalities, where there is also a predisposition to melanoma. It has been well cited and will continue to be important as a first description of the genetic cause.

Castillo SD, Tzouanacou E, Zaw-Thin M, Berenjeno IM, Parker VER, Chivite, I, Mila-Guasch M, Pearce W, Solomon E, Dewhurst RE, Knox RG, Scudamore CL, Badar A, Kalber TL, Foster J, Stuckey DJ, David A, Phillips WA, Lythgoe MF, Wilson V, Semple RK, Sebire NJ, Kinsler VA, Graupera M, Vanhaesebroeck B. (2016) ***Somatic Activating Mutations in Pik3ca Cause Sporadic Venous Malformations in Mice and Human*****. Science Trans Med (8(332):332ra43). DOI: 10.1126/scitranslmed.aad9982**
This was a collaborative project where we did the human genetics and the Vanhaesebroeck team did the murine genetics. It has been well cited and the murine models will continue to be important in pre-clinical therapeutic studies.



Name	JOHANNES KOHL	
Position	Group Leader (1st 6)	
Year joined *(Crick or founder institute)*	2019	

Lab Name	***State-dependent Neural Processing Laboratory***

Research programme and achievements

My group studies the mechanisms by which the state of the body controls brain form and function. We are particularly interested in how hormones affect neural information processing.

Over the last years, my scientific interests have evolved around understanding the functional organisation of neural circuits controlling instinctive behaviours. During my postdoctoral research, my goal was to uncover how a small, genetically identified group of neurons in the mouse hypothalamus controls parental behaviour. I found that these neurons are organised in subpopulations, each projecting to a different target region, and each controlling distinct (motor, motivational, hormonal) aspects of parenting. This functional organisation, reminiscent of the control of motor sequences by pools of spinal cord neurons, provides a new model of how discrete elements of a social behaviour can be generated at the circuit level (Kohl et al., 2018).

My own group – which I started in 2019 – now investigates how physiological states shape the formand function of such circuits in models. We use a wide range of behavioural, circuit-level and cellular approaches for this purpose. One current focus is to understand the effects of pregnancy hormones on the neural circuits that control parenting. We have already obtained promising behavioural results suggesting that (1) discrete aspects of parental behaviour are 'switched on' in mid-to-late pregnancy, (2) specific brain areas undergo volumetric remodelling during pregnancy and (3) that several pregnancy hormones are enriched in key nodes of the neural circuit controlling parental behaviour. We are now starting functional experiments which will allow us to understand how pregnancy hormones affect information processing in these circuits. Over the next years we plan to extend such systems neuroscience approaches to obtain a mechanistic, generalisable understanding of how hormones affect brain function at the circuit level ("systems neuroendocrinology"). For this purpose, we are also currently developing viral tools to map and functionally interrogate brain-wide neural circuits.

Research outputs

Monaca F and Kohl J. (2020) ***Neuroscience: Plasticity Matters for Mating*****. Current Biology, 30 (2), R86-R88: DOI: 10.1016/j.cub.2019.11.052**
In this Dispatch article, written jointly with Francesco Monaca, the first PhD student in my lab, we discuss new findings suggesting that brain areas that control 'hard-wired' behaviours are surprisingly malleable.

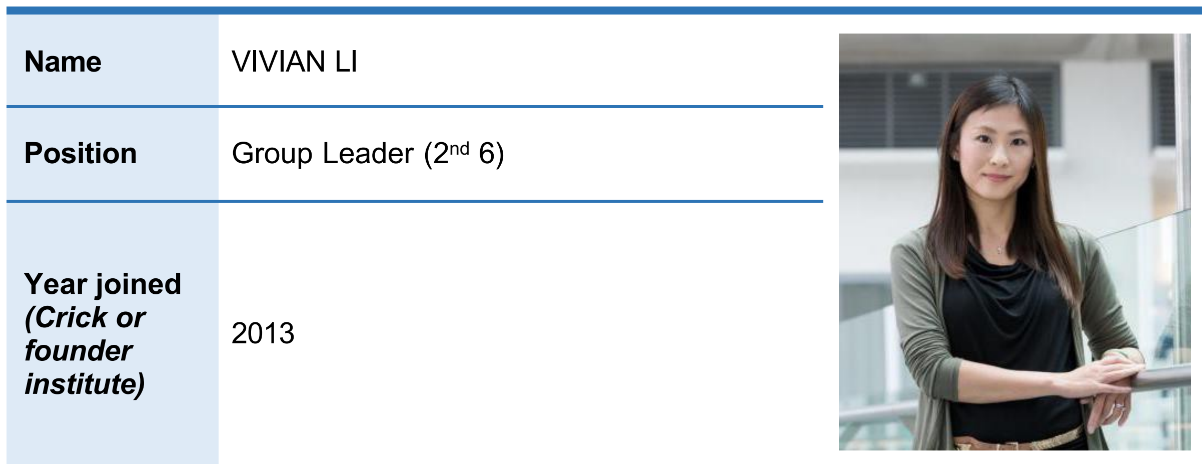
Ammari R and Kohl J. (2020) *Charting a Path Toward Aggression*. Neuron, 106(4):556-558. DOI: **10.1016/j.neuron.2020.04.029**
This review article was a joint effort with the first postdoc in the lab, Rachida Ammari. We discuss recent progress in our understanding of the neural basis of aggression and critically discuss a recent study.

Kohl J. (2020) *Parenting - a paradigm for investigating the neural circuit basis of behaviour*. Curr Op. Neurobiol. 60:84-91 DOI: **10.1016/j.conb.2019.11.011**

In this invited, single-author review, I outline the neural circuit basis of parental behaviour and discuss how it can be used to study/understand how other types of instinctive behaviours are controlled by the brain.

Autry AE, Wu Z, Kohl J, Bambah-Mukku D, Rubinstein ND, Marin-Rodriguez B, Carta I, Sedwick V and Dulac C. (2019) *Perifornical area Urocortin-3 neurons promote infant-directed neglect and aggression*. bioRxiv. DOI: **10.1101/697334**
This study (currently under revision in Nature), finds that infant-directed aggression in mice is orchestrated by specific, molecularly identified neurons, and the circuits in which these neurons function are uncovered. I contributed key functional experiments.

Kohl J, Babayan BM, Rubinstein ND, Autry AE, Marin-Rodriguez B, Kapoor V, Miyamichi K, Zweifel LS, Luo L and Dulac C. (2018) *Functional circuit architecture underlying parental behaviour*. Nature 556(7701):326-331. DOI: 10.1038/s41586-018-0027-0
This postdoctoral project found that a group of neurons crucial for parental behaviour is organised in subpopulations, each projecting to a different target region and each controlling distinct aspects of parenting. Our study provided a new model for how a small population of neurons in the mouse brain can orchestrate a complex social behaviour.



Name	VIVIAN LI
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Lab Name	***Stem Cell and Cancer Biology Laboratory***

Research programme and achievements

The overarching aim of our research is to investigate the signalling regulation in intestinal stem cell (ISC), cancer and damage-induced regeneration, and to exploit their therapeutic potential in intestinal diseases. We focus on investigating Wnt signalling, one of the major pathways contributing to virtually every developmental decision in the lifetime of an organism. We use mouse models and organoids as powerful tools to dissect Wnt signal regulation in intestinal development and cancer. We also explore intestinal tissue engineering strategies using patient-derived organoids (PDOs) for regenerative medicine and disease modelling. The three specific and inter-related research objectives in the lab are:

1. Spatio-temporal regulation of Wnt signalling in ISC homeostasis
Precise control of the Wnt signal strength is crucial to drive stemness and prevent tumorigenesis in the intestine. Our lab investigates how precision Wnt signalling is achieved spatially and temporally in the crypt under ISC homeostasis, and what happens when it goes wrong. We have recently uncovered a number of new Wnt inhibitors that contribute to the fine-tuning of the Wnt signal strength for ISC maintenance and tumorigenesis, including SH3BP4, NEDD4/4L, MTG8 and MTG16. Mtg8 and Mtg16 regulates niche exit and fate decision at the early progenitors via Wnt and Notch regulation, while the underlying expression dynamics of the transcriptional network remains unclear. Given the importance of transcriptional dynamics in regulating other stem cell systems (e.g. embryo and neural progenitors), we will continue to explore the spatio-temporal regulation of the transcriptional network and the related signalling under ISC homeostasis in the next six years.

2. Wnt activating mechanism in colorectal cancer (CRC)
Although Wnt activation is one of the major drivers for CRC, there are significant challenges in targeting the Wnt pathway due to its pivotal role in stem cell function and tissue homeostasis of many body systems. One key objective of our lab is to uncover new druggable targets against Wnt signalling. We have recent discovered the deubiquitinating enzyme USP7 as a tumour-specific drug target for 80% of CRC with *APC*-mutation. Apart from targeting Wnt signalling directly, we are also exploring the link between Wnt activation and immunosuppression in CRC and the therapeutic potential. Our recent results demonstrate that Wnt activation in cancer cells decreases tumour-infiltrating lymphocytes. In the next six years, we will uncover the mechanism underlying Wnt-mediated immune evasion and explore new therapeutic approaches via combination of Wnt inhibition and immunotherapies. Since Wnt activation is the first step for tumour initiation, we will further explore Wnt-induced extracellular biomarkers for non-invasive CRC early detection.

3. Intestinal tissue engineering for regenerative medicine and disease modelling
Our lab is also involved in a EU-funded consortium project of intestinal tissue engineering, aiming to apply organoid technology to reconstruct small intestine to treat intestinal failure, a chronic debilitating disorder without a cure. We have generated proof-of-concept data to engineer patient-specific functional and transplantable jejunal grafts, which could offer a safe and longer-lasting alternative to traditional donor transplants. We will continue and extend the work to engineer CRC with tumour microenvironments to develop improved strategies for disease modelling and drug screening as compared to PDOs.

Research outputs

Meran L, Massie I, Campinoti S, Weston A, Gaifulina R, Tullie L, Faull P, Orford M, Kucharska A, Baulies A, Novellasdemunt L, Angelis N, Hirst E, Konig J, Tedeschi A, Pellegata AF, Eli S, Ambrosius AP, Collison L, Thapar N, Thomas G, Eaton S, Bonfanti P, De Coppi P, Li VS. (2020) *Engineering transplantable jejunal mucosal grafts using patient-derived organoids from children with intestinal failure*. Nature Medicine 26,1593-1601. DOI: 10.1038/s41591-020-1024-z
Children with intestinal failure cannot absorb the nutrients that are essential to be healthy. In the most severe cases, patients may require transplantation. However, there is a shortage of donor organs and complications can arise after surgery. We have shown how intestinal stem cells and intestinal tissues taken from patients can be used to grow functioning intestinal grafts in the laboratory, which could offer a safe and longer-lasting alternative to traditional donor transplants.

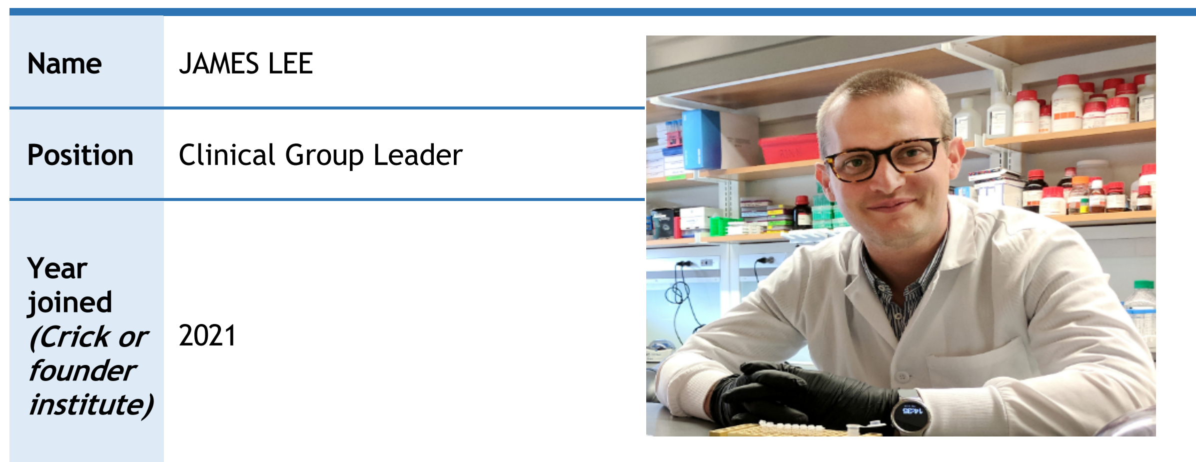
Baulies A, Angelis N, Danielson T, Foglizzo V, Patel H, Kucharska A, Novellasdemunt L, De Coppi P, Li VS. (2020) *The transcriptional co-repressors Mtg8 and Mtg16 regulate exit of intestinal stem cells from their niche and differentiation into enterocyte vs secretory lineages.* Gastroenterology S0016-5085(20)34764-8. DOI: 10.1053/j.gastro.2020.06.012
Despite decades of research, it remains unclear what defines the intestinal stem cell position, and how the binary fate decision between the secretory and absorptive lineages is controlled in the early progenitors. In this study, we uncovered two important transcription regulators, Mtg8 and Mtg16, regulating the intestinal stem cell identities via Notch signalling. The data provide important insights about how intestinal stem cells regenerate, and the role of these genes in tumorigenesis.

Novellasdemunt L, Kucharska A, Jamieson C, Prange-Barczynska M, Baulies A, Antas P, van der Vaart J, Gehart H, Maurice MM, Li VS. (2020) *Nedd4 and Nedd4l regulate Wnt signaling and intestinal stem cell numbers by degrading Lgr5 receptor*. EMBO J. 39(3):e102771. DOI: 10.15252/embj.2019102771
The bona fide intestinal stem cell marker Lgr5 has been extensively studied in the past, mostly focussed on its transcriptional control. However, how the protein level of Lgr5 is regulated remains unknown. In this paper, we showed that the E3 ligase Nedd4 and Nedd4l target the Lgr5 protein for ubiquitination and degradation, and prevent intestinal tumour development and progression. The results may shed light on therapeutic development for CRC.

Antas P, Novellasdemunt L, Kucharska A, Massie I, Carvalho J, Oukrif D, Nye E, Novelli M, Li VS. (2019) *SH3BP4 regulates intestinal stem cells and tumourigenesis by modulating β-catenin nuclear localisation*. Cell Reports 26(9):2266-2273.e4. DOI: 10.1016/j.celrep.2019.01.110
Fine-tuning of the Wnt signal activity along the intestinal crypt to the “just-right” level is crucial to maintain stemness and prevent tumour transformation. In this study, we uncover a novel tumour-suppressive role of SH3BP4 that functions as a negative feedback regulator of Wnt signalling by restricting nuclear localisation of β-catenin. The results provide not only new regulatory mechanism of Wnt signalling, but also the link between Wnt negative feedback mechanism and tumour development.

Novellasdemunt L, Foglizzo V, Cuadrado L, Antas P, Kucharska A, Encheva V, Snijders AP, Li VS. (2017) *USP7 is a tumour-specific WNT activator for APC-mutated colorectal cancer*. Cell Reports 21(3):612-627. DOI: 10.1016/j.celrep.2017.09.072

APC truncating mutation is the hallmark of CRC, leading to hyperactivation of Wnt signalling. Despite of decades of research, targeting Wnt signalling remains challenging due to its essential role in normal stem cell maintenance. There is an urgent unmet need to develop new generation of Wnt inhibitors focusing on tumour-specificity. In this study, we discover that USP7 is responsible for Wnt activation specifically in APC-mutated colon cancer, suggesting that USP7 is a tumour-specific drug target. This is a major discovery in the field of therapeutic intervention of Wnt signalling in cancer.



Name	JAMES LEE
Position	Clinical Group Leader
Year joined *(Crick or founder institute)*	2021

Lab Name	*Genetic Mechanisms of Disease laboratory*

Research programme and achievements

I am a clinician-scientist with a longstanding interest in better understanding the biological mechanisms of immune-mediated disease. I joined the Crick in July 2021, moving my research group from the University of Cambridge.

Over the last 5 years, I have made several contributions to our understanding of immune-mediated disease, which have been recognised by international awards, invited talks and appointments to external panels.

1. Predicting prognosis in inflammatory bowel disease
Crohn's disease and ulcerative colitis are incurable forms of inflammatory bowel disease (IBD) that can have a devastating impact on sufferers' lives. The course of these diseases varies unpredictably between patients, hindering attempts to develop a treatment strategy that is suitable for all patients. Following my previous report of a prognostic CD8 T-cell gene signature in newly-diagnosed IBD patients (JCI 2011), I helped develop and validate a blood-based prognostic biomarker (Gut 2019) that is now available for patients via a spin-out company (PredictImmune Ltd). This is being tested in the PROFILE trial – a UK-wide, biomarker-stratified trial that I designed (BMJ Open) and helped secure funding for (£4.2M; Wellcome Trust Translation Award, co-investigator) and will determine whether personalised therapy can improve outcomes in IBD.

2) Genetics of immune-mediated disease
I have made several contributions to immune-mediated disease genetics, both through the IBD Genetics Consortium (co-author on 5 Nature Genetics papers since 2015, including first author on largest GWAS in IBD) and also in my own work. For example, I was the first to define a genetic contribution to prognosis in immune-mediated disease and show that this is distinct from susceptibility genetics (Nature Genetics 2017). This built on previous work in which I discovered a genetically-regulated pathway that influences prognosis in several diseases by regulating inflammatory responses in monocytes (Cell 2013).

3) Translating genetic discovery to biological insight
My doctoral and post-doctoral work highlighted (1) the need to better understand disease mechanisms, (2) the potential of genetics to do this, and (3) the value of working at the interface of different diseases. Motivated by these points, I began a Wellcome Trust Intermediate Fellowship in 2015, spending 2 years at Harvard before establishing my own group at the University of Cambridge. Here, we adapted a method to simultaneously test the functional effects of hundreds of SNPs in primary immune cells, enabling the identification of putative causal variants via their biological effects and overcoming the limitations of statistical fine-mapping. By combining this approach with methods to

efficiently CRISPR edit primary immune cells without viral transduction, we now have a powerful system that can translate lists of SNPs into disease mechanisms (EMBO Mol Med 2020). These methods, and their extension into new cell-types and diseases, form the basis of our future plans.

Future plans

A fundamental goal of GWAS was to better understand disease biology. As such, despite widespread success in variant discovery, this goal remains unfulfilled – since we have not yet transitioned from lists of associated SNPs to insights into disease mechanisms. During the next quinquennium, we will extend the methods we have developed and apply them to more broadly study inflammatory bowel disease (IBD)-associated loci in a wider range of cellular contexts. This will provide a foundation for experimental studies (both in vitro and in vivo) to resolve the underlying biology – providing mechanistic insights into disease and identifying new therapeutic opportunities. This will include:

1. A comprehensive study of IBD loci that exhibit regulatory activity in CD4 T cells

Using data from ChIP-seq, eQTL, RNA-seq and promoter capture Hi-C experiments, we have identified a subset of IBD-associated loci that are principally biologically active in CD4 T cells, including several with specific T helper lineage activity. We have identified the candidate SNPs at these loci and will perform a CD4 T cell-specific massively-parallel reporter (MPRA) assay to identify putative causal variants for every association.

2. Extension into other disease-relevant primary cell-types, including macrophages and NK cells

Not all IBD loci are biologically active in CD4 T cells. We and others have identified inflammatory macrophages and NK cells as two other cell-types whose regulatory DNA is strongly enriched for IBD SNPs. We have now optimised MPRA for use in macrophages, and shown this can provide insights into disease biology (unpublished). We will similarly develop this method for NK cells to more broadly study IBD-associated loci in the most relevant primary cells.

3. Resolve disease mechanisms and investigate therapeutic opportunities

Using a tiered selection process - based on the MPRA results - we will select loci for downstream studies using both *in vitro* and *in vivo* models. We will particularly focus on loci where candidate genes have unknown functions since these may also provide insights into basic immunology and should provide opportunities for new therapies. These will be explored using small molecule libraries (both novel and re-purposed) to identify modulators of any newly-identified disease pathways.

Research outputs

Bourges C, Groff AF, Burren OS, Gerhardinger C, Mattioli K, Hutchinson A, Hu T, Anand T, Epping MW, Wallace C, Smith KGC, Rinn JL, Lee JC. Resolving mechanisms of immune-mediated disease in primary CD4 T cells. EMBO Mol Med 12:e12112 (2020).
https://doi.org/10.15252/emmm.202012112

The first demonstration that causal genetic variants at GWAS loci can be identified via their functional consequences in primary immune cells, and that this can be used to uncover genetic mechanisms of immune-mediated disease. Contribution: funding acquisition, conceptualisation, study design, methods development, performed experiments, analysis, project oversight, wrote manuscript.

Biasci D*, Lee JC*†, Noor NM, Pombal DR, Hou M, Lewis N, Ahmad T, Hart A, Parkes M, McKinney EF, Lyons PA, Smith KGC†. [* *denotes joint first authors, † denotes co-corresponding authors*]
A blood-based prognostic biomarker in inflammatory bowel disease
Gut. 68(8):1386-1395 (2019). http://dx.doi.org/10.1136/gutjnl-2019-318343

Manuscript describing the development and validation of a whole-blood biomarker that can predict the future disease course in inflammatory bowel disease. This was based on a prognostic CD8 T cell transcriptomic signature that I identified in my doctoral studies and has since formed the basis of a spin-out company (PredictImmune Ltd). Contribution: helped obtain funding, conceptualisation, study design, analysis, wrote manuscript.

Predicting outcomes for Crohn's disease using a molecular biomarker (PROFILE): protocol for a multi-centre, randomised, biomarker-stratified trial.
Parkes M†, Noor NM, Dowling F, Leung H, Bond S, Whitehead L, Upponi S, Kinnon P, Sandham AP, Lyons PA, McKinney EF, Smith KGC, Lee JC†. [† *denotes co-corresponding authors*]
BMJ Open. 5;8(12):e026767 (2018). http://dx.doi.org/10.1136/bmjopen-2018-026767

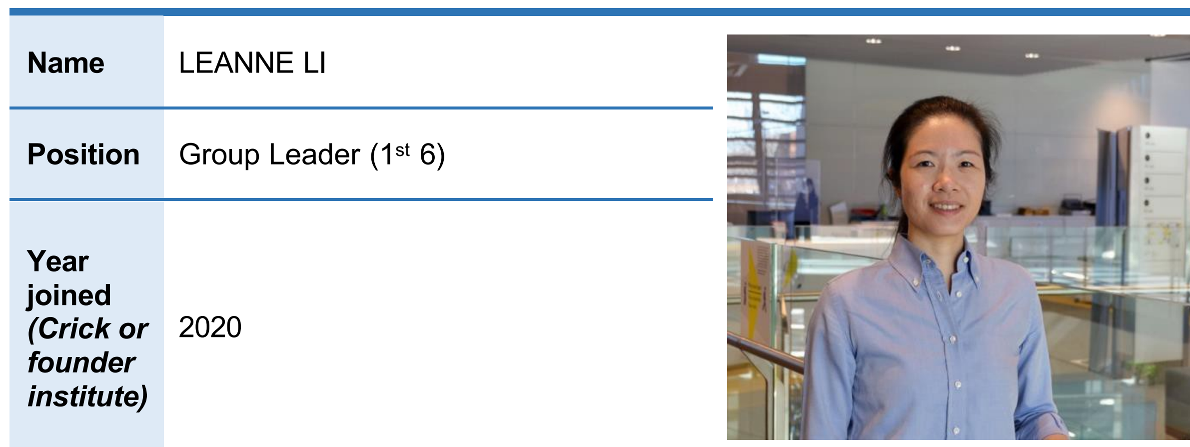
Protocol manuscript describing the PROFILE trial – the first biomarker-stratified trial in any inflammatory disease – to test whether a molecular biomarker can facilitate personalised therapy in Crohn's disease. Contribution: helped obtain funding, study design, trial co-investigator, wrote manuscript.

Genome-wide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn's disease.
Lee JC*‡, Biasci D*, Roberts R, Gearry RB, Mansfield JC, Ahmad T, Prescott NJ, Satsangi J, Wilson DC, Jostins L, Anderson CA; UK IBD Genetics Consortium., Traherne JA, Lyons PA, Parkes M, Smith KGC†. [* *denotes joint first authors, † denotes co-corresponding authors*]
Nature Genetics. 49: 262-268 (2017). https://doi.org/10.1038/ng.3755

The first paper to demonstrate that the genetic architecture of prognosis differs from that of disease susceptibility in inflammatory bowel disease. Contribution: funding acquisition, conceptualisation, study design, project oversight, analysis, wrote manuscript.

de Lange KM*, Moutsianas L*, Lee JC*, Lamb CA, Luo Y, Kennedy NA, Jostins L, Rice DL, Gutierrez-Achury J, Ji SG, Heap G, Nimmo ER, Edwards C, Henderson P, Mowat C, Sanderson J, Satsangi J, Simmons A, Wilson DC, Tremelling M, Hart A, Mathew CG, Newman WG, Parkes M, Lees CW, Uhlig H, Hawkey C, Prescott NJ, Ahmad T, Mansfield JC, Anderson CA, Barrett JC. [* *denotes joint first authors*] Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease.
Nature Genetics. 49: 256-261 (2017). https://doi.org/10.1038/ng.3760

The largest GWAS in IBD to date, increasing the number of associated loci to 242. Contribution: coordinated sample recruitment, preparation and submission, co-ordinated phenotyping collection, analysis, review and editing manuscript.



Name	LEANNE LI
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2020

Lab Name	*Cancer-Neuroscience Laboratory*

Research programme and achievements

Our laboratory combines cancer biology and neuroscience to investigate how tumours communicate with the rest of our body.

Tumours are made up of both cancer cells and host cells that support the tumour's growth. Interactions among the different types of cells in the tumour constantly shape the behaviour of the cancer cells, and affect how the disease will progress.

This means we need to understand how cancer cells communicate with the host or 'stromal' cells within the space directly around the tumour, called the tumour microenvironment. But we also need to know how the tumour communicates with the rest of the body.
The immune system controls part of the conversation, and this piece of the system is relatively extensively-studied at the moment. However, the neuroscience aspects of cancer biology remain mostly unexplored. Our lab uses genetically-engineered mouse models (GEMMs) and GEMM-derived tissue culture systems to answer the following questions: What are the roles of neuronal signalling pathways in cancer? Do cancer cells 'communicate' with our body through the nervous system, and if so, how? Finally, can we interfere with these 'communications' to treat cancer?

One of the major model systems we use is small cell lung cancer (SCLC), a highly aggressive neuroendocrine tumour which metastasizes early and has very limited treatment options. By learning more about the relationship between cancer and neuroscience, we hope to develop potential new treatments for SCLC, and eventually for cancers in general.

Research outputs

Cynthia Hajal, Yoojin Shin, Leanne Li, Jean Carlos Serrano, Tyler Jacks, Roger D. Kamm. (2021) *The CCL2-CCR2 astrocyte-cancer cell axis in tumor extravasation at the brain.* Science Advances (In press).
Extravasation is a rate-limiting factor in brain metastasis, but mechanisms underlying this process have been challenging to investigate, due to the limitations of available technologies. Here we developed a novel in vitro microfluid 3D platform to model the process of cancer cell extravasation in brain vasculatures in real time, and identified novel signalling pathways mediating the brain extravasation of cancer cells.

Rodrigo Romero, Francisco J. Sánchez-Rivera, Peter M. K. Westcott, Kim L. Mercer, Arjun Bhutkar, Alexander Muir, Tania J. González Robles, Swanny Lamboy Rodríguez, Laura Z. Liao, Sheng Rong Ng, Leanne Li, Caterina I. Colón, Santiago Naranjo, Mary Clare Beytagh, Caroline A. Lewis, Peggy P. Hsu, Roderick T. Bronson, Matthew G. Vander Heiden and Tyler Jacks. (2020) *Keap1 mutation renders lung adenocarcinomas dependent on Slc33a1*. Nature Cancer 1: 589–602. DOI: 10.1038/s43018-020-0071-1
Keap1 is frequently co-mutated with Kras in lung adenocarcinoma. Using genetic screens, we identified synthetic lethal targets that could be effective in treating Kras mutant lung adenocarcinomas.

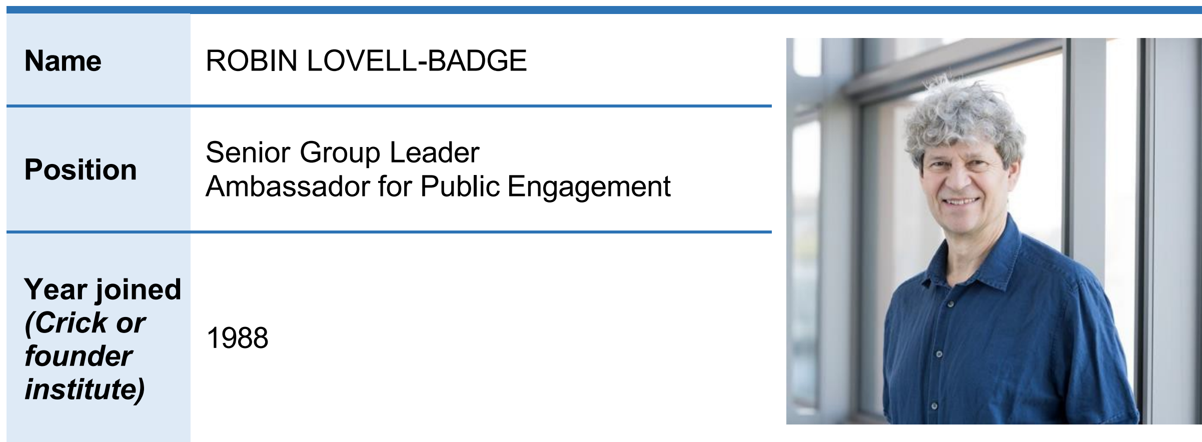
Leanne Li*, Sheng Rong Ng*, et al (*equal contribution). (2019) *Identification of DHODH as a novel therapeutic target in small cell lung cancer*. Science Translational Medicine 11(517):eaaw7852. DOI: 10.1126/scitranslmed.aaw7852
The treatment landscape of small cell lung cancer has remained nearly unchanged over the past 30 years. This study used a cutting-edge CRISPR-mediated genetic screen techique to identify novel therapeutic targets in small cell lung cancer, and validated one of the targets in various pre-clinical models.

Leanne Li*, Qiqun Zeng*, et al. (*Equal contribution). (2018) *GKAP acts as a genetic modulator of NMDAR signaling to govern invasive tumor growth.* Cancer Cell 33: 736-751. DOI: 10.1016/j.ccell.2018.02.011

The finale of a series of proof-of-concept studies, in which a genetically-engineered mouse model of cancer was used to demonstrate the essence of personalized medicine: genetic modifers identified from genome-wise association studies (GWAS) can govern the phenotype of cancer progression through differential activation of signaling pathways, and convey differential susceptibilities to personalized treatments in cancer cells.

Hugh Robinson and Leanne Li. (2017) *Autocrine, paracrine and necrotic NMDA receptor signalling in mouse pancreatic neuroendocrine tumour cells.* Open Biology 7 :170221. DOI: 10.1098/rsob.170221
This is one of the first extensive electrophysiology studies of pancreatic neuroendocrine tumor cells, demonstrating how NMDA receptor signaling functions in these cells.

Name	ROBIN LOVELL-BADGE
Position	Senior Group Leader Ambassador for Public Engagement
Year joined *(Crick or founder institute)*	1988



Lab Name	***Stem Cell Biology and Developmental Genetics Laboratory***

Research programme and achievements

The main goal of the lab is to understand mechanisms underlying cell fate decisions, choosing a number of tractable and clinically relevant systems in the embryo and the adult, including stem cell populations. Many of these involve *Sox* genes, which encode transcription factors that often act as pioneers, and understanding these is also a common theme in our work. Sex determination and differentiation have been a main focus for many years, but CNS and pituitary development, and the biology and role of stem cells in these systems have also been major topics of our research and continue to be so. The work is of clinical relevance, both because it can help reveal the cause of disorders or syndromes, but also because the ability to manipulate cell fate choices is important for regenerative medicine.

Since 2015, our main achievements are:
ï Determined novel genetic causes and provided understanding of the mechanisms underlying several cases of human Disorders of Sex Differentiation (DSDs). This has been achieved through our work in the mouse, often with our clinical collaborators
ï Characterised the regulation of *Sox9* during sex determination, identifying a critical early acting enhancer (amongst a very long and complex regulatory region) that acts at the pivotal point of the decision to become a Sertoli rather than granulosa cell

ï Shown the critical role for *Foxl2* and *Dmrt1* in sex determination in the chick. This is a collaboration with the Roslin Institute and has involved the development of novel methods permitting efficient genome editing and germline transmission in the chick
ï Discovered mechanisms that could be responsible for the 4:1 male:female sex bias seen in Hirschsprung's Disease. This has involved studying mouse models of Hirschsprung's where we have found an unexpected sex bias in survival at birth, and studies on enteric nervous system development in male and female mice, showing precocious development in the latter
ï Through studying the role in the developing CNS of Sox9, which is required for glial cell differentiation, we have identified an astrocytic scaffold required for migration of neuronal progenitors into the dentate gyrus and for normal hippocampus development and function. This work also reveals differences in the gene regulatory network involved in glial cell specification between the brain and spinal cord
ï Characterised the role of several *Sox* genes and their targets (including Notch) in cell fate decisions within the CNS and pituitary
ï Expanded on previous findings showing that the cell cycle inhibitor p27Kip1 can also act as a transcriptional repressor of Sox2, connecting proliferative state and Sox2 function, and understanding how this leads to the development of intermediate lobe tumours in p27

null animals

We are continuing our work on early events in sex determination, in the mouse and chick, but we are also exploring how gonadal sex is maintained. Deletion of *Foxl2* in the adult leads to gonadal sex reversal from ovaries to testis-like organs and understanding this at a molecular and cellular level will be relevant to regenerative medicine. We will also continue to explore *Sox* gene function in the developing and adult CNS, but we have also begun to use them as tools to explore sex differences in the CNS and in neural stem cell populations. We are also continuing work on pituitary stem cells *in vivo*, characterising how these respond to physiological changes, and on how systemic and external factors can influence the hypothalamus and its control of pituitary function. All of these programmes of work are rooted in generating fundamental insights into how decisions of cell fate are established and maintained, but they also have relevance to clinical situations.

Research outputs

Cheung, L., Le Tissier, P., Treier, M., Lovell-Badge, R. and Rizzoti, K. (2018). *NOTCH activity differentially affects alternative cell fate acquisition and maintenance*. e Life 7:e33318. DOI: 10.7554/eLife.33318
Notch signalling tends to maintain an undifferentiated state in progenitor cells, but here we showed that in the embryonic pituitary, while this is the case in one distinct lineage, (POMC cells, where activation can induce a regression towards a progenitor-like state), Notch signalling has no effect on cell fate specification in the POU1F1 lineage. These results have implications for pituitary development, plasticity and regeneration.

Gonen, N., Futtner, C.R., Wood, S., Garcia-Moreno, S.A., Salamone, I.M., Samson, S.C., Sekido, R., Poulat, F., Maatouk, D.M, and Lovell-Badge, R. (2018). *Sex reversal following deletion of a single distal enhancer of Sox9*. Science 360, 1469-1473. DOI: 10.1126/science.aas9408

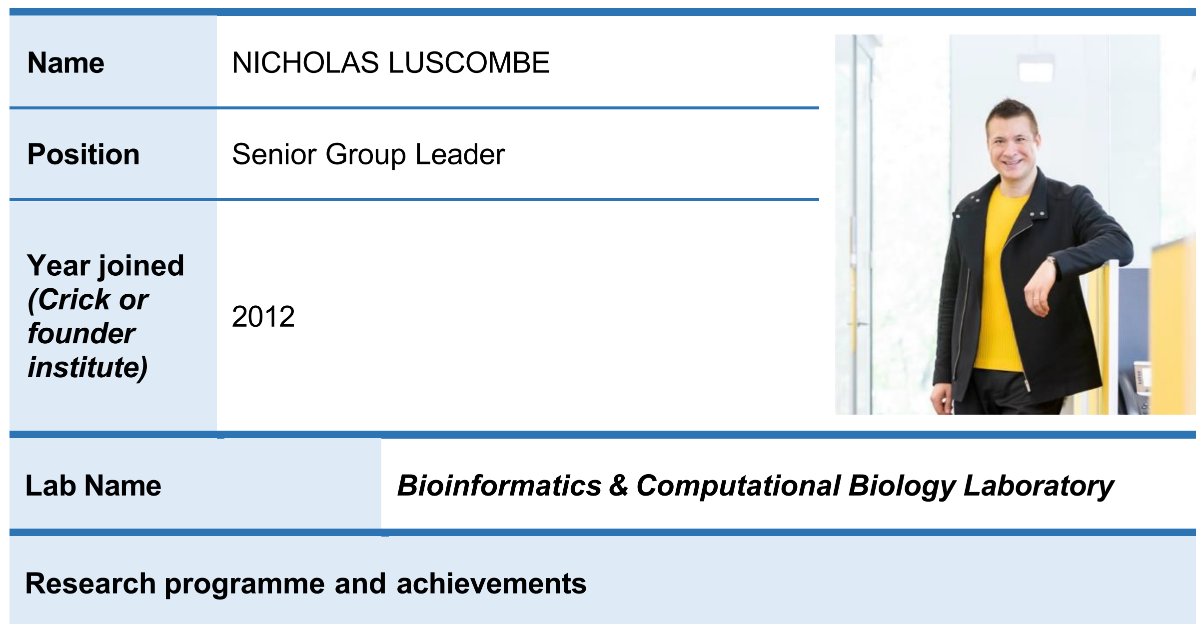
This systematic study revealed the complexity of the *Sox9* regulatory region, but just one enhancer, “Enh13”, was shown by mutation studies to be essential for testis and subsequent male development. *Sox9* expression is at the same very low level in XY Enh13 mutant embryos as in control XX gonads. Enh13 is efficiently bound by Sry *in vivo* and functions to initiate Sertoli cell fate during a short time window. This is in contrast to other redundant enhancers (e.g. TES) that bind Sry, but act later. This study helped explain Disorders of Sex Differentiation (DSDs), due to deletions and duplications mapping far upstream of Sox9, where the human Enh13 equivalent is located, as well as showing that some enhancers can be pioneering rather than redundant.

Guioli, S., Zhao, D., Nandi, S., Clinton, M. and Lovell-Badge, R. (2020) *In the chick embryo, estrogen can induce chromosomally male ZZ left gonad epithelial cells to form an ovarian cortex, which supports oogenesis*. Development 147, 4. DOI: 10.1242/dev.181693
In the chick, many sex-specific characteristics are due to cell autonomous activity of the sex chromosomes, rather than as a response to gonadal hormones. However, we showed here that an ovarian cortex can form independently of the phenotypic sex of the medulla as long as oestrogen, acting via ERalpha, is provided. The presence of a medulla with an “intersex” or male phenotype may compromise germ cell progression into meiosis, causing cortical germ cells to remain in an immature state in the embryo. This may be relevant to human biology, where teratomas develop when germ cells are in an inappropriate environment.

Eozenou, C.*, Gonen, N.*, Touzon, M.S.*, Jorgensen. A., Yatsenko, S.A., Fusee, L.,

Kamel, A., Gellen, B., Guercio, G., Singh, P., Witchel, S., Berman, A.J., Mainpal, R., Totonchi, M., Mohseni Meybodi, A., Askari, M., Merel, T., Bignon-Topalovic, J., Migale, R., Costanzo, M., Marino, R., Ramirez, P., Perez Garrido, N., Berensztein, E., Mekkawy, M.K., Schimenti, J.C., Bertalan, R., Mazen, I., McElreavey, K.*, Belgorosky, A.*, Lovell-Badge, R.*, Rajkovic, A.*, Bashamboo, A.* (2020) ***Testis formation in XX individuals resulting from novel pathogenic variants in Wilms' tumor 1 (WT1) gene*****. Proc. Natl. Acad. Sci. USA. 117, 13680-13699. DOI: 10.1073/pnas.1921676117**
Through analysis of a large collection of clinical cases of Disorders of Sex Differentiation (DSDs), and a mouse model, we showed that unlike previous association of WT1 variants with XY female development, variants of the fourth zinc finger (ZF4) WT1 are a relatively common cause of XX male development, where the gonads are testes or ovotestes. This article is typical of our interaction with clinical geneticists, where our studies on the mouse, including generating models of human disorders, provide valuable insight into conditions affecting patients, as well as revealing novel insights into the underling mechanisms.

Veronica Moncho-Amor, Probir Chakravarty, Christophe Galichet, Ander Matheu, Robin Lovell-Badge*and Karine Rizzoti*. (2021) ***SOX2 is required independently in both stem and differentiated cells for pituitary tumorigenesis in p27 null mice*** **Proceedings of the National Academy of Sciences of USA 118 (7), e2017115118. DOI: 10.1073/pnas.2017115118**
Tumour development depends on cell intrinsic dysfunction, but extrinsic factors can also be important drivers. Deletion of *p27*, which is downregulated in many tumours, predominantly gives pituitary tumours in mice. *Sox2*, which is transcriptionally derepressed in the absence of P27, is also important for tumorigenesis in this and other systems. Using various approaches, we establish the regulatory interaction *in vivo* of SOX2 and p27 and show that SOX2 is required independently, both cell-autonomously in the endocrine cells that form the tumours and non-cell-autonomously, in adjacent pituitary stem cells, to orchestrate tumorigenesis in the absence of P27.



Name	NICHOLAS LUSCOMBE
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2012

Lab Name	***Bioinformatics & Computational Biology Laboratory***

Research programme and achievements

Past work
How do cells recognise and respond appropriately to diverse internal and external stimuli? By ensuring the correct gene expression at the right times and places, regulatory systems control diverse cellular behaviours. Our laboratory undertakes interdisciplinary approaches, using computational and statistical methods to quantify complex molecular and genomic measurements. In this way, we are able to address complex yet far-reaching questions in biology, particularly:
1. How do transcriptional and post-transcriptional regulatory mechanisms control gene expression? We answered such questions by building, for example, extraordinarily accurate and predictive statistical models for transcriptional regulation, which provide insights into molecular mechanisms.
2. How do these mechanisms regulate important biological systems? We explored the use of distal regulatory elements and mRNA splicing during cell-state transitions such as neural development.
3. How do regulatory systems interface with molecular evolutionary mechanisms? Specifically, we examined how mutation rates in genomes are influenced by interactions with components of the gene regulatory system.
In taking a genomic perspective, we uncover general principles that apply across many biological conditions; features unique to individual systems can then be understood within this context. Ultimately, we wish to achieve quantitative explanations of how regulatory systems interpret the information encoded in the genome; in doing so, we seek to predict and modulate the molecular mechanisms by which cellular decisions are made.

Future work
We have spent the last 12 months consolidating our computational work within a background of neural development and degeneration, specifically the development of motor neurones and their untimely destruction in amyotrophic lateral sclerosis (ALS). The availability of representative *in vitro* human and mouse models, combined with sensitive techniques such as single-cell measurements, means that high-resolution -omic investigations are now possible for the neural system and give hope for targeted molecular therapeutic development. We shall address the following questions in the next review period.

1. Can we quantitatively model and modulate transcriptional regulation during neural development?
2. What post-transcriptional systems are involved in neural development?
3. In what way do these mechanisms break down to cause ALS?

The three strands of work are anchored by the computational analysis of genomic datasets - for example, advanced integration of binding site measurements with functional readouts - and development of statistical models that are biologically interpretable. We

will focus on close collaborations with James Briscoe (Crick), Jernej Ule (UCL, seconded to Crick) and Rickie Patani (UCL, seconded to Crick). I will continue with a laboratory of 12 researchers equally divided between the three subject areas; some will be wet/dry scientists co-supervised with one of our collaborators. Proteomics is a new area of particular interest throughout, which will give us orthologous insights into the nature and abundance of interacting molecules.

Research outputs

Ghahramani, A., Watt, F.M., and Luscombe, N.M. (2018) ***Generative adversarial networks uncover epidermal regulators and predict single cell perturbations*****. bioRxiv. DOI: 10.1101/262501**

- A completely novel, orthogonal method for analysing single-cell RNA-seq data, inspired by developments in image-analysis algorithms.
- Trained models simulate realistic single-cell RNA-seq data that covers the full range of cell types.
- Internal parameters of the algorithm can be interpreted in multiple ways to provide biologically meaningful insights into the data.
- Most interesting interpretation is latent space interpolation, a general way to examine trajectories between two or more cells.

Metzis, V., *Steinhauser, S., Pakanavicius, E., Gouti, M., Stamataki, D., Ivanovitch, K., Watson, T., Rayon, T., Mousavy Gharavy, S.N., Lovell-Badge, R., Luscombe, N.M., and Briscoe, J. (2018).** ***Nervous System Regionalization Entails Axial Allocation before Neural Differentiation**. Cell 175, 1105–1118.e17. DOI: 10.1016/j.cell.2018.09.040**

- Re-writes textbook model about the order of events in neural development.
- Examines enhancer accessibility, measured using ATAC-seq, as a sensitive method for defining cell types.
- Advanced statistical analysis and large-scale integration of public datasets identifies differentially accessible enhancers and the features that distinguish them from each other.
- Confirms distinguishing features *in vivo*.

Luisier, R., *Tyzack, G.E., Hall, C.E., Mitchell, J.S., Devine, H., Taha, D.M., Malik, B., Meyer, I., Greensmith, L., Newcombe, J., Ule, J., Luscombe, N.M.+, and Patani, R.+ (2018).** ***Intron retention and nuclear loss of SFPQ are molecular hallmarks of ALS**. Nat Commun 9:2010. DOI: 10.1038/s41467**

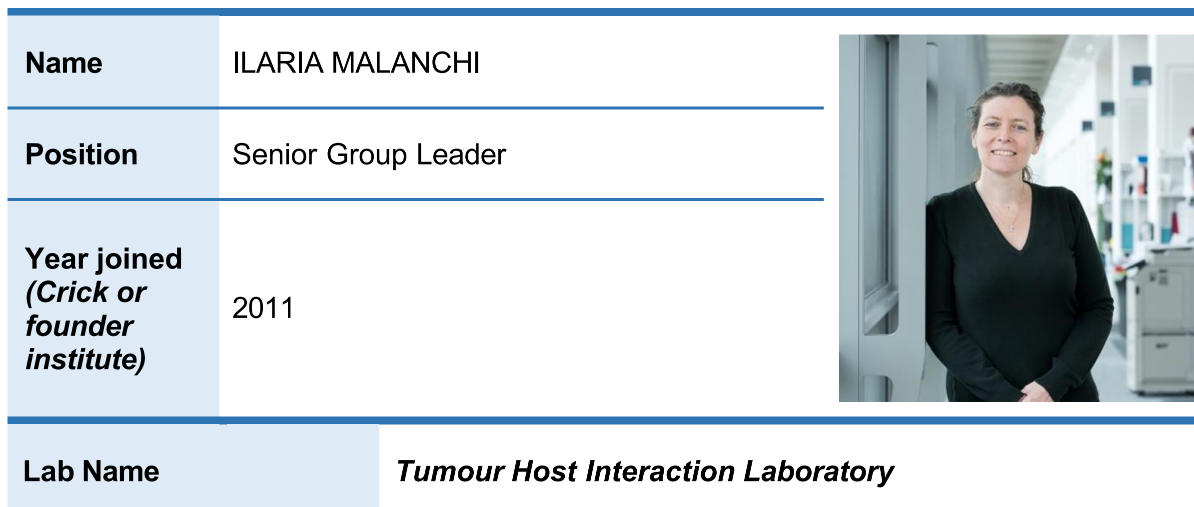
- Amyotrophic lateral sclerosis (ALS) is incurable and invariably fatal, with a lifetime risk of 1 in 400. Avenues for treatment is desperately needed.
- Reveals the earliest molecular events that apply universally to familial and sporadic ALS.
- Detailed statistical analysis of RNA-seq data reveals ~200 transcripts that aberrantly retain long introns at a very early stage of neural development.
- Identifies RNA-binding proteins that regularly bind their own transcripts, mislocalise from the nucleus to the cytoplasm.
- Observations suggest the beginnings of protein aggregation observed at later disease stages, giving insights into possible early stage mechanism.

Mifsud, B., *Tavares-Cadete, F., Young, A.N., Sugar, R., Schoenfelder, S., Ferreira, L., Wingett, S.W., Andrews, S., Grey, W., Ewels, P.A., Herman, B., Happe, S., Higgs, A., Le Proust, E., Follows, G.A., Fraser, P., Luscombe, N.M.+, and Osborne, C.+ (2015).** ***Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C**. Nat. Genet. 47, 598–606. DOI: 10.1038/ng.3286**

- Describes a new high-throughput HiC method that enriches for promoter regions.
- Identifies a regulatory network of physically interacting promoters and non-promoter

regions; many of the latter have hallmarks of gene regulatory activity.
- Opens a new avenue for interpreting the mechanism of action for intergenic disease-associated SNPs.
- Many SNPs overlap with interacting enhancers, thus potentially altering the regulation of the partner gene.

Sugimoto, Y., Vigilante, A., Darbo, E., Zirra, A., Militti, C., D'Ambrogio, A., Luscombe, N.M.+, and Ule, J.+ (2015). ***hiCLIP reveals the in vivo atlas of mRNA secondary structures recognized by Staufen 1*****. Nature** ***519*****, 491–494. DOI: 10.1038/nature14280**
- Introduces a new method to measure RNA-RNA duplexes bound by a double-stranded binding protein.
- Identifies hundreds of previously unseen mRNA secondary structures, with many duplexes spanning thousands of intervening bases.
- Long-range duplex structures occur in mRNAs encoding for specific protein functions and have an effect on translational efficiency and transcript stability.



Name	ILARIA MALANCHI
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2011
Lab Name	***Tumour Host Interaction Laboratory***

Research programme and achievements

The Tumour Host Interaction laboratory studies solid tumours using animal models to focus on the comprehensive analysis of cancer-host cell organisation. We study the cell composition and interactions within the plethora of cell types locally supporting tumour and metastasis, as well as the systemic effects on inflammatory cells caused by the cancer.

RESEARCH ACHIEVEMENTS SINCE 2015:

- My lab has demonstrated that the enhanced ability to trigger a metastatic niche within the foreign organ defines highly metastatic cancer cells. We identified a potential target, Thrombospondin-2 (THSB2) as a novel fibroblast activator produced by cancer cells in an epithelial–mesenchymal transition (EMT) state. Our study challenged the use of drugs targeting the cancer's EMT status by showing its dynamic modulation at the metastatic site derived by the interactions with the host tissue.
- We have significantly contributed to the emerging concept that the cross talk between cancer cells and the host extends beyond local interaction, and that the cancer disease integrates within the whole organism by perturbing the composition of distant organs (pre-metastatic niche). We identified a novel activity of systemically mobilised mature neutrophils within the pre-metastatic niche and highlighted their key role during cancer progression.
- We have also addressed questions about tumour initiation and shown that lung neutrophils are key during the response to carcinogenic agents' exposure and their presence shapes the long-term predisposition to lung cancer.
- The main challenge in studying the early events of niche formation is to directly identify tissue derived cells responding to cancer cells. My laboratory developed a new tool (niche labelling) that can directly identify over time the tissue-derived cells contacting cancer cells in vivo. We hope that this technique will help the broad scientific community to expedite studies on local cellular interaction in vivo.
- This niche labelling strategy allowed us to correlate an early local regenerative response of the tissue to metastatic cells with the presence of a novel type of host cells with tissue stem features.
- We showed that targeting key metabolic requirements in aggressive osteosarcoma cells leads to microenvironment adaptation allowing for cancer persistency. We have suggested a combinatorial adjuvant therapy for tumour eradication by targeting macrophages, the effector of this adaptation.
- Similarly, in the context of breast cancer, we showed that transient inhibition of ROCK-Myosin II driven actomyosin contractility in cancer cells generates an alternative tumour microenvironment, which is unsuitable for long term tumour growth. We described the generation of alternative activated macrophages with anti-cancer function and show that their gene expression signature has a strong prognostic value.
- We showed that healthy tissue exposure to cancer radiotherapy enhances metastasis.

We described a neutrophil pro-inflammatory phenotype in the irradiated healthy lung that, by supporting the integrity of lung alveolar cells, unintentionally creates an environment that enhances metastatic activity of cancer cells. We also identified Notch signalling as the main mediator of this metastatic boost, potentially providing a strategy to combat radiation-enhanced metastases associated with collateral damage of the lung.

THE FUTURE WORK OF THE LAB WILL

- Revolve around following up our previous studies by: 1. investigating the origin and drivers of metastatic niche cells with tissue stem features and the functional implications for metastatic growth; 2. performing a comparative analysis of the metastatic niche environment generated by the same cells in different tissues using our niche labelling tool; 3. continue to investigate the neutrophils' systemic changes in the context of cancer.
- Consolidating or establishing new research questions using two approaches. First, we will study metastatic dormancy in vivo. My laboratory has implemented a protocol to generate mouse-to-mouse dormancy-permissive extramedullary bones and is now using this approach to study the mechanism of cancer cell reactivation upon dormant dissemination. Secondly, we will investigate the relationship between pancreatic cancer neural plasticity (increased nerve density, thickness and fibre switch) and the immune infiltrations during cancer growth and progression.

Research outputs

Wculeck SK, Malanchi I. (2015) ***Neutrophils support lung colonization of metastasis-initiating breast cancer cells.*** **Nature 528(7582):413-7. DOI: 10.1038/nature16140**
In this study we found that via the release of leukotrienes, neutrophils selectively support the more metastatic subset of cancer cells infiltrating the distant tissue and that this activity can be blocked by an inhibitor of leukotriene production. This is one of the most important publications from my laboratory, as it has contributed to the understanding of the crucial responses of neutrophils during metastatic progression.

del Pozo Martin Y, Park D, Ramachandran A, Ombrato L, Calvo F, Chakravarty P, Spencer-Dene B, Derzsi S, Hill CS, Sahai E, and Malanchi I. (2015) ***Mesenchymal Cancer Cell-Stroma Crosstalk Promotes Niche Activation, Epithelial Reversion, and Metastatic Colonization*****. Cell Rep 13(11):2456-69. DOI: 10.1016/j.celrep.2015.11.025**
With this study, my lab highlighted the interdependence between the intrinsic features of cancer cells and the host tissue responses they induce. This highlights the need to study cancer cell functional heterogenicity in the context of cancer-host cell interaction. Importantly, this work showed the challenges in targeting cancer cells' features that might be crucial in a certain moment, but are subsequently modulated over time by interactions with the host tissue.

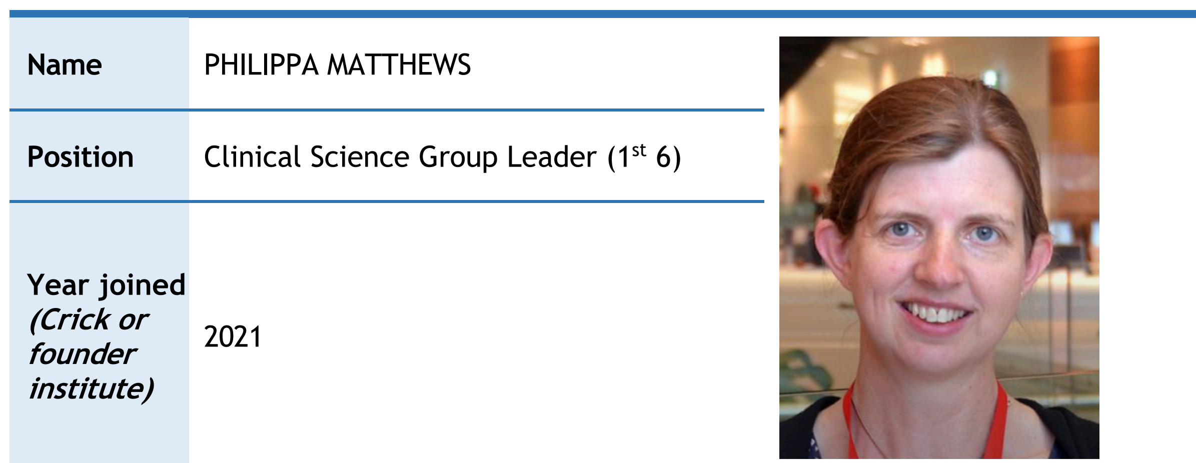
Kurelac I, Iommarini L, Vatrinet R, Amato LB, De Luise M, Leone G, Girolimetti G, Umesh Ganesh N, Bridgeman VL, Ombrato L, Columbaro M, Ragazzi M, Gibellini L, Sollazzo M, Feichtinger RG, Vidali S, Baldassarre M, Foriel S, Vidone M, Cossarizza A, Grifoni D, Kofler B, Malanchi I*, Porcelli AM*, Gasparre G*.(2019) ***Inducing cancer indolence by targeting mitochondrial Complex I is potentiated by blocking macrophage-mediated adaptive responses*****. Nat Commun. 10(1):903. DOI: 10.1038/s41467-019-08839-1**
This work highlights how targeting mitochondrial respiratory complex I in cancer cells leads to microenvironment adaptation involving macrophage recruitment which supports cancer survival. Targeting macrophages in the context of metformin (complex I inhibitor) leads to tumour growth impairment, setting the basis for a potentially efficient combinatorial adjuvant therapy.

Ombrato L, Nolan E, Kurelac I, Mavousian A, Bridgeman VL, Heinze I, Chakravarty P, Horswell S, Gonzalez-Gualda E, Matacchione G, Weston A, Kirkpatrick J, Husain E, Speirs V, Collinson L, Ori A, Lee JH*, Malanchi I*. (2019) *Metastatic-niche labelling reveals parenchymal cells with stem features*. Nature 572(7771):603-608. DOI: [10.1038/s41586-019-1487-6](https://doi.org/10.1038/s41586-019-1487-6)

This work is the result of the long-standing efforts of my lab to directly identify the tissue-derived cells that are early responders to cancer cells.We provided the details of the strategy to the scientific community, and by implementing it in our lung metastatic studies, we described the presence of epithelial cells with tissue stem cell features within the metastatic niche. The presence of these tissue stem cell-like cells offers new insights in the understanding of the origin of cancer's niche.

Wculek SK., Bridgeman VL., Peakman F., & Malanchi I. (2020) *Early Neutrophil Responses to Chemical Carcinogenesis Shape Long-Term Lung Cancer Susceptibility*. Iscience 23(7):101277. DOI: [10.1016/j.isci.2020.101277](https://doi.org/10.1016/j.isci.2020.101277)

Here we show that lung neutrophils are key during the response to carcinogenic agents and that their presence shapes the long-term predisposition to lung cancer. We showed that neutrophils contribute directly to neoplastic transformation by amplifying the effect of a chemical genotoxic agent, but that this activity is uncoupled from the tissue damage activity to which it was previously linked.



Name	PHILIPPA MATTHEWS	
Position	Clinical Science Group Leader (1st 6)	
Year joined *(Crick or founder institute)*	2021	

Lab Name	***HBV Genomics for Elimination Laboratory***

Research programme and achievements

My group works to improve insights into the biology of hepatitis B virus (HBV) infection.

With an estimated 300 million people infected globally, and causing a million deaths every year, this pathogen poses a serious public health threat. International targets have been set for elimination by the year 2030. However, HBV has been neglected by funding, advocacy, education and research, and there are still many gaps in our understanding of transmission, persistence and disease.

Working closely with collaborators in South Africa, as well as with patient cohorts in the UK, we are recruiting adults with chronic HBV to study the different outcomes of infection, which include long term persistence, liver inflammation, cirrhosis and cancer. By sequencing the genetic code of the virus, we can gain insights into interactions between the host and the virus. This provides the opportunity to better understand the role of immune responses in control or clearance of infection, determine the impact of antiviral treatment, and form an evidence base for optimum vaccination strategies.

Overall, our research works towards developing an enhanced picture of the burden of disease caused by HBV in different populations, to inform improved approaches to surveillance and treatment for individual patients, and to enhance public health strategies for prevention, underpinning progress towards global 2030 targets.

Research outputs

Evidence of tenofovir resistance in chronic hepatitis B virus (HBV) infection: An observational case series of South African adults. *Mokaya J, Maponga TG, McNaughton AL, Van Schalkwyk M, Hugo S, Singer JB, Sreenu VB, Bonsall D, de Cesare M, Andersson M, Gabriel S, Taljaard J, Barnes E, Preiser W, Van Rensburg C, Matthews PC. J Clin Virol. 2020;129:104548.*

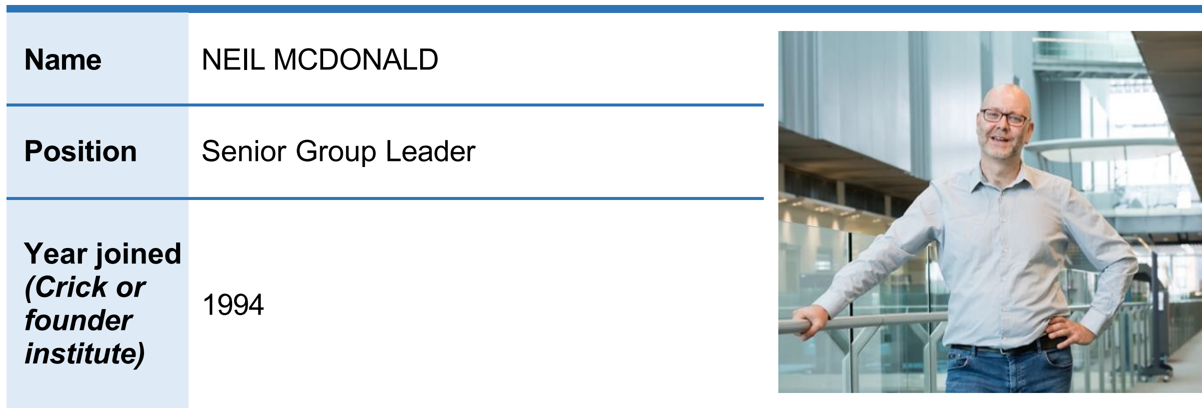
This paper describes a correlation between an HBV drug resistant phenotype (viraemia on treatment) and viral genetic polymorphisms in sequenced virus, showing tenofovir resistance for the first time in an African setting.

Treatment advantage in HBV/HIV coinfection compared to HBV monoinfection in a South African cohort. Maponga TG, McNaughton AL, van Schalkwyk M, Hugo S, Nwankwo C, Taljaard J, Mokaya J, Smith DA, van Vuuren C, Goedhals D, Gabriel S, Andersson MI, Preiser W, van Rensburg C, Matthews PC. J Infect. 2020: S0163-4453(20)30263-2.
This paper is derived from the new South African cohort established with the support of my Wellcome fellowship, demonstrating that adults with HBV infection have better outcomes if they are coinfected with HIV, likely as a result of improved clinical care and antiviral therapy regimens that contain tenofovir.

Hepatitis B virus seroepidemiology data for Africa: Modelling intervention strategies based on a systematic review and meta-analysis. McNaughton AL, Lourenço J, Bester PA, Mokaya J, Lumley SF, Obolski U, Forde D, Maponga TG, Katumba KR, Goedhals D, Gupta S, Seeley J, Newton R, Ocama P, Matthews PC. PLoS Med. 2020;17(4):e1003068.
This paper presents a unique dataset for Africa, in a meta-analysis of HBV serology data to describe the relationship between prevalence of HBV infection (HBsAg) and exposure to infection (anti-HBcore) which we used as a basis to describe different patterns of transmission in different populations, with results that are significant to determining the optimum approaches to intervention.

Electronic Health Informatics Data To Describe Clearance Dynamics of Hepatitis B Surface Antigen (HBsAg) and e Antigen (HBeAg) in Chronic Hepatitis B Virus Infection. Downs LO, Smith DA, Lumley SF, Patel M, McNaughton AL, Mokaya J, Ansari MA, Salih H, Várnai KA, Freeman O, Cripps S, Phillips J, Collier J, Woods K, Channon K, Davies J, Barnes E, Jeffery K, Matthews PC. MBio. 2019;10(3). pii: e00699-19.
Clearance of surface antigen (HBsAg) in chronic HBV infection is an uncommon event; we here used a large unbiased dataset generated through the NIHR Health Informatics Collaborative (HIC) to assess the dynamics of clearance and to characterise the individuals in whom this occurs, setting the scene for the development of mechanistic insights (e.g. based on host or viral genetics).

Illumina and Nanopore methods for whole genome sequencing of hepatitis B virus (HBV). McNaughton AL, Roberts HE, Bonsall D, de Cesare M, Mokaya J, Lumley SF, Golubchik T, Piazza P, Martin JB, de Lara C, Brown A, Ansari MA, Bowden R, Barnes E, Matthews PC. Sci Rep. 2019;9(1):7081.
We present methods using rolling circle amplification to generate full genome HBV sequences, and a bioinformatic approach to correct errors in Nanopore sequence to derive a final sequence data with the same accuracy as the existing ‘gold standard’ short read (Illumina) sequencing.



Name	NEIL MCDONALD
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	1994

Lab Name	***Signalling and Structural Biology Laboratory***

Research programme and achievements

The laboratory studies the molecular mechanisms regulating membrane-linked signalling hubs formed by the neurotrophic receptor tyrosine kinases and the PKC superfamily of serine/threonine kinases. Members of both protein kinase classes act to control fundamental biological processes from cell patterning and polarisation to neuronal physiology, connectivity and survival. Their aberrant activities directly contribute to neurodegeneration and neuropsychiatric disease, heart disease and cancer.

We are addressing two major questions: (1) How do neurotrophic factor receptors assemble and signal to control neuronal function and how are they subverted by oncogenic deregulation in non-neuronal contexts? (2) How do multi-component aPKCi/PKN kinase complexes establish cell membrane polarity and cell shape through a network of activating and inhibitory interactions? Our long-term ambition is to leverage our understanding of these kinase control mechanisms to explain their emergent biological properties and manipulate their functional states using chemical and biological tools.

Our structure-driven programme uses crystallography, cryo-EM and cell biology on reconstituted membrane-linked kinase assemblies, integrated with *in vivo* models through collaborations within the Crick Institute. We have exploited opportunities to develop chemical and biological tools against our molecular targets to contribute to drug-discovery programmes.

The main achievements of the laboratory since 2015 are as follows:

1: We have determined cryo-EM structures of GDNF ligand-bound RET receptor complexes. Insights from these studies have allowed us to pursue a translational project supported by Crick i2i funding with the aim of producing bispecific DARPin anti-RET antagonists. We are probing bispecific DARPin properties on AML cell lines dependent on RET function, both in laboratories at the Crick (Bonnet lab) and in Heidelberg (Scholl lab).

2: We have explored mechanisms of RET tyrosine kinase oncogenic deregulation by missense mutation and identified novel modes of RET chemical inhibition through P-loop engagement. Importantly, this new mode of RET inhibition is largely insensitive to oncogenic gatekeeper mutation.

3: We have reconstituted a key polarity complex containing aPKCi and determined its structure using cryo-electron microscopy. We have combined this analysis with

collaborations within the Crick to examine the impact of targeted disrupting mutations using polarised cells and externally using an *in vivo* model organism.

4. We initiated a successful aPKC iota drug design programme with CRT, together with Peter Parker. This programme directly led to potent, selective inhibitors against aPKC used by Barry Thompson, Kathy Niakan and Nate Goehring for their research programmes and publications. Pre-clinical candidates and a backup series are currently being assessed in a topical formulation to target aberrant Hedgehog signalling in basal cell carcinoma.

4: Structural studies on RPEL motif-containing complexes with G-actin in collaboration with the Treisman Lab have given insights into surprising stoichiometries and mechanisms
of G-actin function. These studies show how G-actin acts to control RPEL motif containing protein function, impacting on either subcellular localisation (MRTFs) or enzymatic activity (Phactrs and RhoGAPs) by steric exclusion mechanisms.

5. Our cryo-EM structures of the DNA repair endonuclease complex XPF-ERCC1 identified an auto-inhibited XPF-ERCC1 complex and a form bound to a ss/dsDNA substrate. We have exploited our structural findings to propose a collaboration with Astra- Zeneca to identify chemical inhibitors in a drug discovery programme.

Future work of the laboratory will focus on the molecular roles of both neuronal tyrosine and serine/threonine kinases in neuronal connectivity, survival and polarisation. This will include: (1) Investigation of how neuronal receptors assemble in membrane and trans- synaptic contexts visualised using a combination of cryo-EM and cryo-ET (2) The development of biological agonists and antagonists against neuronal receptors in relevant disease contexts (3) Defining the interplay between reconstituted polarity complexes that we have solved by cryo-EM and their respective membrane recruitment mechanisms (4) Visualising and manipulating distinct activity states of polarity complexes and their subcellular locations using nanobody tools.

Research outputs

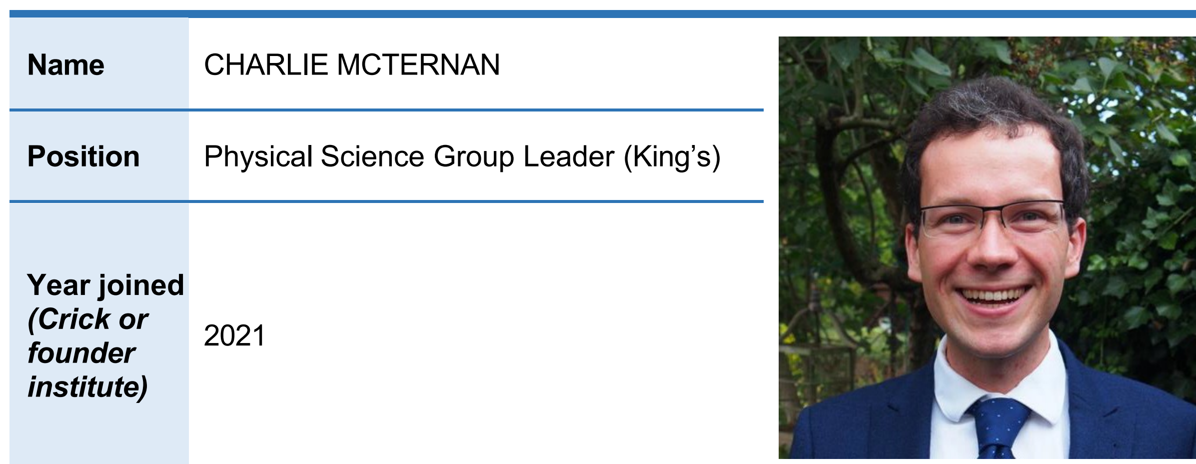
Jones M, Beuron F, Borg A, Nans A, Earl CP, Briggs DC, Snijders AP, Bowles M, Morris EP, Linch M, McDonald NQ. (2020) *Cryo-EM structures of the XPF-ERCC1 endonuclease reveal how DNA-junction engagement disrupts an auto-inhibited conformation*. Nat Commun 11(1):1120. DOI: 10.1038/s41467-020-14856-2
This first cryo-EM structure from the lab determined the first full length XPF family endonuclease structure revealing an auto-inhibited conformer and the initial steps in DNA-junction recognition opening up the endonuclease structure.

Soriano EV, Ivanova ME, Fletcher G, Riou P, Knowles PP, Barnouin K, Purkiss A, Kostelecky B, Saiu P, Linch M, Elbediwy A, Kjær S, O'Reilly N, Snijders AP, Parker PJ, Thompson BJ, McDonald NQ. (2016) *aPKC Inhibition by Par3 CR3 Flanking Regions Controls Substrate Access and Underpins Apical-Junctional Polarization*. Dev Cell 38(4):384-98. DOI: 10.1016/j.devcel.2016.07.018
This three-way collaboration within the Crick combined a molecular and cellular analysis of Par3 interaction with aPKCiota, together with an *in vivo* examination of the polarised follicular epithelium in *Drosophila*. The study suggests a dual role for Par3 as both substrate and competitive inhibitor of aPKCiota function.

Diring J, Mouilleron S, McDonald NQ, Treisman R. (2019) *RPEL-family rhoGAPs link Rac/Cdc42 GTP loading to G-actin availability*. Nat Cell Biol 21(7):845-855. DOI: 10.1038/s41556-019-0337-y
This collaborative publication continued our efforts to apply X-ray crystallography to define the interaction mode and stoichiometry of G-actin binding to three known classes of RPEL motifs: MRTF, Phactrs and RhoGAPs. The study concludes that a subset of RhoGAPs are regulated by G-actin binding a single RPEL-motif, blocking access to the rho-GTPase binding site.

Plaza-Menacho I, Barnouin K, Barry R, Borg A, Orme M, Chauhan R, Mouilleron S, Martínez-Torres RJ, Meier P, McDonald NQ. (2016) *RET Functions as a Dual-Specificity Kinase that Requires Allosteric Inputs from Juxtamembrane Elements*. Cell Reports 17(12):3319-3332. DOI: 10.1016/j.celrep.2016.11.061
This study unexpectedly revealed a serine auto-phosphorylation site within the RET kinase domain required for activation both *in vitro* and *in vivo*. These findings are consistent with a dual-specificity kinase function for this receptor tyrosine kinase.

Adams SE, Purkiss AG, Knowles PP, Nans A, Briggs DC, Borg A, Earl CP, Goodman KM, Narowtek A, Borg A, McIntosh PB, Houghton FM, Kjær S, McDonald NQ. (2021) *A two-site flexible clamp mechanism for RET^{ECD}-GDNF-GFRα1 assembly reveals both conformational adaptation and strict geometric spacing.* Structure 29, 1-15. DOI: 10.1016/j.str.2020.12.012
RET is an unusual receptor tyrosine kinase that recognizes a set of five GDNF family ligand-co-receptors. Our paper reveals the basis for ligand-co-receptor recognition by RET. By comparing crystallographic and cryo-EM structures of unliganded and ligand-co-receptor-bound RET, we explain how RET acts as a two-site molecular clamp. It has a flexible arm that engages different co-receptors through conformational adaptation while a rigid arm recognizes the dimeric nature and molecular dimensions of each GDNF family ligand to drive receptor activation. Our findings have implications for the design of RET receptor modulators relevant to both Parkinson’s disease and RET-driven cancers.



Name	CHARLIE MCTERNAN
Position	Physical Science Group Leader (King's)
Year joined *(Crick or founder institute)*	2021

Lab Name	***Artificial Molecular Machinery Laboratory***

Research programme and achievements

We are a synthetic chemistry group at the Francis Crick Institute and King's College London working to create artificial molecular machines capable of performing complex biological tasks, formed in February 2021.

Supramolecular chemistry is the study of non-covalent interactions between molecules. These non-covalent interactions are critical to biological processes like protein folding, DNA base pairing, and cellular signalling.

Our research looks at how we can combine principles from supramolecular chemistry with biological chemistry and nanotechnology to create new systems capable of carrying out tasks like detecting and responding to chemical signals, or seeking out certain cells in the body.

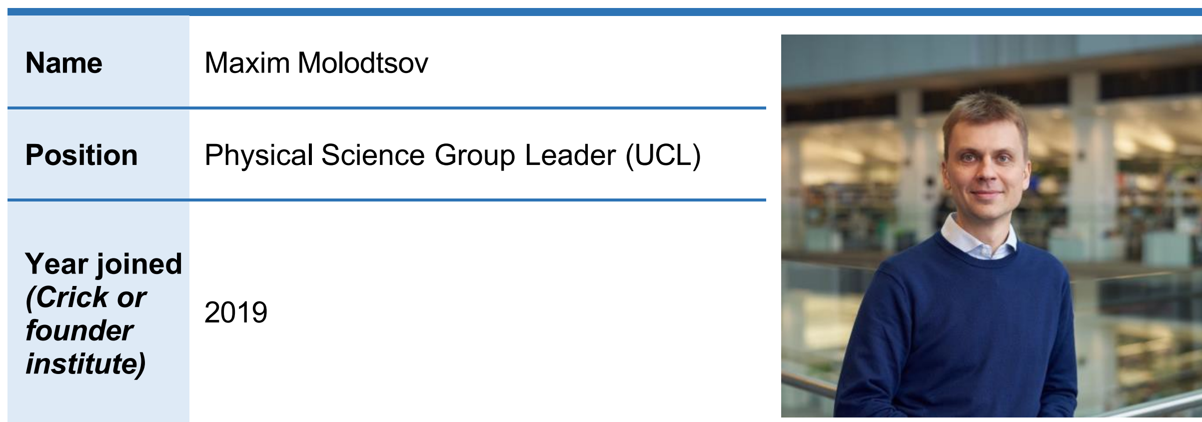
We work with interlocked molecular structures like rotaxanes and catenanes, and molecular capsules with defined internal voids. By tapping into the chemical properties of these structures, we can create a host of useful molecular machines including targeted drug delivery vehicles and artificial cellular receptors.

Research outputs

John P. Carpenter, Charlie T. McTernan, Tanya K. Ronson, Jonathan R. Nitschke. (2019) *Anion Pairs Template a Trigonal Prism with Disilver Vertices*. J. Am. Chem. Soc 141,11409–11413. DOI: 10.1021/jacs.9b05432
This paper showed for the first time that we could use flexible co-ordination to a labile metal to drive the assembly of complex architectures.

Charlie T. McTernan, Tanya K. Ronson, Jonathan R. Nitschke. (2019) *Post-assembly Modification of Phosphine Cages Controls Host-Guest Behavior*. J. Am. Chem. Soc 141, 6837–6842. DOI: 10.1021/jacs.9b02604
This paper showed for the first time that we could control which guest is bound inside a molecular capsule by functionalising the outside of that capsule, and so that post-assembly modifications could control the properties of a molecular capsule.

Stephen D. P. Fielden, David A. Leigh, Charlie T. McTernan, Borja P. Saavedr a. (2018) ***Spontaneous Assembly of Rotaxanes from a Primary Amine, Crown Ether and Electrophile.*** **J. Am. Chem. Soc 140, 6049–6052. DOI: 10.1021/jacs.8b03394**
This paper reported an unexpected and counter-intuitive way to form mechanically interlocked molecules from extremely simple starting materials, providing a general approach to forming rotaxanes.



Name	Maxim Molodtsov
Position	Physical Science Group Leader (UCL)
Year joined *(Crick or founder institute)*	2019

Lab Name	***Mechanobiology and Biophysics Laboratory***

Research programme and achievements

DNA organisation and segregation are inherently mechanical in nature. Understanding how cells organise and segregate genomes requires an understanding of molecular-mechanical forces that drive their movements. The importance of this fundamental problem has been widely recognised, yet there have been very few attempts to address specific molecular mechanisms using single-molecule biophysics. This is mainly due to lack of appropriate physical tools to tackle mechanisms of DNA organisation at the single molecule level. The goal of our laboratory is to develop new physical tools and decipher molecular mechanisms and physical forces that rearrange and organise DNA.

To achieve this, we pursue two lines of research. First, we have generated expertise in developing and using a combination of force spectroscopy and single-molecule microscopy to study molecular mechanisms of recombinant proteins in fully controlled *in vitro* experiments. To this end, we have collaborated with world-leading and pioneering biochemistry groups, which has led to the discovery of a highly conserved mechanism of cytoskeleton rearrangements, and provided novel mechanistic insights into chromatin organisation.

We continue this work in collaboration with groups both inside and outside the Crick. Inside, we collaborate with Frank Uhlmann's group to understand the mechanics of sister chromatid cohesion establishment and maintenance. Outside the Crick we collaborate with Jan-Michael Peters at the Research Institute of Molecular Pathology in Vienna to understand how conformational changes in cohesin molecules generate mechanical work used to bend and shape DNA.

In addition, we initiated a collaboration with Andela Saric's group at the UCL Physics Department to explore quantitative mathematical models of cohesin function, which we believe will be essential to understand how cohesin works. Our physical measurements yield single-molecule rates and stiffnesses that will allow us to develop a coarse-grained mathematical model of cohesin and its interaction with DNA.

The physical approaches that we developed aimed at measuring forces between individual cohesin molecules and DNA and between DNA interactions established by cohesins might be used in future to understand the role of cohesin mutations found in cohesinopathies in maintaining the physical stability of cohesin-DNA interactions. For example, our mechanistic analysis has the potential to uncover the role that regions with high-probability mutations such as those found in Cornelia-de-Lange syndrome or different cancers have in development and progression of the disease.

Our second line of research is to develop new tools for applying and measuring forces generated by specific molecular mechanisms in live cells. Single-molecule approaches revolutionised cell biology due to their ability to quantify and manipulate the interactions of

individual biomolecules. However, most experiments are done on purified molecules, and a paradigm change is required to bring these techniques into live cells. Two main challenges remain unsolved. First, in order to apply forces to specific molecules in cells, precise positioning of multiple force probes is required in live cells. Second, probes need to be specifically attached to the molecules of interest and avoid interaction with the rest of the cellular environment.

To address these long-standing challenges, we are building on recent developments in magnetic micromanipulation, gene editing and crosslinker chemistry that together open up new ways to approach force measurement and manipulation in live cells.

We are developing systems for generating microscale magnetic gradients to independently control positions of magnetic particles with high spatial resolution. We are investigating the use of magnetic tips and microwire matrices to generate micro-fields for manipulating particles at distances of 0.5 – 100 microns with forces going up to 100 pN. In parallel, we are investigating the chemistry that would allow us to bring magnetic probes and attach them in controllable ways to specific molecules inside cells, where microscale magnetic gradients will be used to apply and measure mechanical forces.

The combination of these two approaches will enable us in future to perform highly precise mechanical manipulations of specific intracellular macromolecules and organelles, which we will use to understand the forces that rearrange and pass genomes from one generation to another.

Research outputs

Nöbauer T., Skocek O., Pernía-Andrade A., Weilguny L., Traub F.M., Molodtsov M.I., & Vaziri A (2017). *Video rate volumetric Ca^{2+} imaging across cortex using seeded iterative demixing (SID) microscopy*. Nat. Methods, 14(8):811-818. DOI: 10.1038/nmeth.4341

Obtaining signals from individual cells in tissues is challenging because they are blurred and deteriorated by tissue scatter. This is similar to looking at taillights of a car in front of you during a heavy rain and fog. Here we developed a sophisticated algorithm to capture locations and signals from individual cells in highly scattering tissue and dramatically reduced the time to generate such an image. This technique allowed to capture signals within a 3D mouse brain containing multiple layers of neurons.

Molodtsov M.I., Mieck C., Dobbelaere J., Dammermann A., Westermann S., and Vaziri A. (2016) *A force-induced directional switch of a molecular motor enables parallel microtubule bundle formation*. Cell 167, p. 539-552. DOI: 10.1016/j.cell.2016.09.029

In order to divide, cells completely rearrange their cytoskeleton to form mitotic spindle. Many motor proteins are implicated in cytoskeleton microtubule rearrangements, but how different motors work together and how mechanical forces they generate influence rearrangements are not understood. Here we discovered a mechanism of microtubule organization fundamentally different from all known before. We showed that a two-protein system can use force generated by microtubule tips to guide growth of new microtubules along the pre-existing ones forming parallel microtubule bundles. The mechanism can account for the generation of parallel microtubule networks required for spindle assembly and cell polarization.

Davidson I.F., Götz D.[*], Zaczek M.P.[*], Molodtsov M.I.[*], Huis in 't Veld P.J., Weissmann F., Litos G., Cisneros D., Ocampo-Hafalla M., Ladurner R., Uhlmann F., Vaziri A and Jan-Michael Peters. (2016) *Rapid movement and transcriptional re-localization of human cohesin on DNA*. EMBO J. DOI: 10.15252/embj.201695402

Cohesin is a ring-shaped protein complex required for the spatial organization, expression, repair, and segregation of eukaryotic genomes. Multiple evidence suggests that in cells cohesin is recruited to specific genomic sites and then translocated by unknown mechanism that depends on transcription. It has also been suggested to move along DNA and form DNA loops leading to three-dimensional genomic organization. Here we showed that single molecules of cohesin can move on DNA in a manner consistent with topological entrapment and can pass over some DNA-bound proteins and nucleosomes. The molecules were constrained, however, by transcription and DNA-bound CTCF. These results indicate how transcription can provide directionality to cohesin movements with CTCF being a boundary element.

Tinsley J.N. *, **Molodtsov M.I.***, **Prevedel R., Wartmann D., Espigulé-Pons J., Lauwers M., and Vaziri A (2016).** ***Direct Detection of a Single Photon by Humans*****. Nature Communications 7, 12172. DOI: 10.1038/ncomms12172**
Rod cells in human eye are known to respond to individual photons. Yet whether single photon event can be perceived by a human brain remained a fundamental question. Here we have built a single photon quantum light source and developed mathematical model of human vision to show that humans are capable of distinguishing signals as low as just a single photon.

Mieck C.*, **Molodtsov M.I.***, **Drzewicka K., van der Vaart B., Litos G., Schmauss G., Vaziri A., and Westermann S. (2015)** ***Non-catalytic motor domains enable processive movement and functional diversification of the minus-end directed Kinesin Kar3*****. ELife 4:e04489. DOI: 10.7554/eLife.04489**
Kinesins are molecular motors responsible to transport along microtubules. They can have different structures, but all require two subunits that work together to create a walking-like movement. Here we discovered unconventional mechanism used by kinesin-14 that has only one active subunit. We show that it can nonetheless move along microtubules and an advantage of such mechanism is that the movement of the motor can be easily reversed by external force.

Name	MARIYA MOOSAJEE
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Ocular Genomics and Therapeutics Laboratory***

Research programme and achievements

Our research is focused on dissecting the molecular basis of genetic eye disease using multi-omic approaches such as whole genome sequencing, RNA-Seq, methylomics and metabolomics. In addition to undertaking detailed clinical studies to understand the natural history of these conditions in patients, in the lab we generate zebrafish disease models using CRISPR/Cas9 gene editing and human induced pluripotent stem cell derived retinal cells to further advance our knowledge of disease mechanisms. This permits the identification of potential therapeutic targets for development of treatment strategies and trial outcome measures for a wide range of inherited eye disorders.

At the Crick, we are developing non-viral gene therapy, an alternative to conventional adeno-associated virus (AAV) delivery systems. Non-viral gene therapy has several advantages in that it can accommodate large genes of any size, it lacks viral components and therefore has less chance of evoking an immune response, and it remains episomal, thus reducing the risk of insertional mutagenesis. Together with Crick group leaders, we will use scaffold/matrix attachment region DNA vectors to optimise delivery into photoreceptors and retinal pigment epithelium, whilst exploring the innate intracellular immune response to the introduction of this foreign DNA to assess the safety and efficacy of this approach.

Most significant achievements over the past 5 years have been furthering our understanding of the cellular basis of inherited retinal disease. Using ultrastructure and transcriptomics we have identified disease mechanisms that explain the degeneration of photoreceptors and retinal pigment epithelium. We have used genomics and comparative analysis with mouse and zebrafish models to identify novel genetic causes of disease.
Clinical research using detailed longitudinal phenotyping studies have provided ocular biomarkers for disease prognosis and outcome metrics for future trials.

Research outputs

Toms M, Dubis AM, de Vrieze E, Tracey-White D, Mitsios A, Hayes M, Broekman S, Baxendale S, Utoomprurkporn N, Bamiou D, Bitner-Glindzicz M, Webster AR, Van Wijk E, Moosajee M. (2020) *Clinical and preclinical therapeutic outcome metrics for USH2A-related disease*. Hum Mol Genet 29:11, 1882 -1899. DOI: 10.1093/hmg/ddaa004

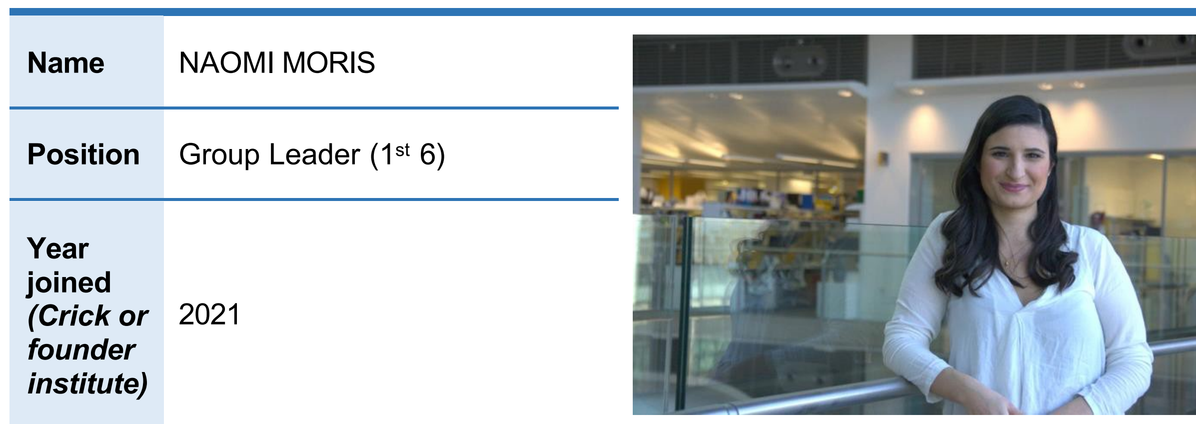
This paper highlights disease mechanisms involved in retinitis pigmentosa and how they lead to the natural history of visual decline seen in humans, hence providing fundamental information on therapeutic targets but also outcome metrics for future clinical trials.

Toms M, Burgoyne T, Tracey-White D, Richardson R, Dubis AM, Webster AR, Futter C, Moosajee M. (2019) *Phagosomal and mitochondrial alterations in RPE may contribute to KCNJ13 retinopathy*. Sci Rep 9, 3793 DOI: 10.1038/s41598-019-40507-8
This paper provides detailed ultrastructural evidence for mechanisms of disease within the retinal pigment epithelial that result in cell death in Leber congenital amaurosis. This provides details on gene function and identifies therapeutic targets.

Richardson R, Tracey-White D, Toms M, Young RM, Owen N, Moosajee M. (2019) *Transcriptomic profiling of zebrafish optic fissure fusion*. Sci Rep 9, 1541. DOI: 10.1038/s41598-018-38379-5
This paper provides detailed longitudinal transcriptomic data over a normal developmental process of optic fissure fusion. This provides insights into the multitude of genes involved, throwing up potential novel candidates for human disease phenotypes and novel mechanisms of eye growth and tissue fusion to pursue.

Sarkar H, Mitsios A, Smart M, Skinner J, Welch A, Kalatzis V, Coffey P, Dubis AM, Webster A, Moosajee M. (2019) *Nonsense-mediated mRNA decay efficiency varies in choroideremia providing a target to boost small molecule therapeutics*. Hum Mol Genet 28, 11. DOI: 10.1093/hmg/ddz028
This paper advances our knowledge of molecular mechanisms that may affect phenotype, and therapeutic targets for treating inherited retinal diseases. It describes how personalised medicine is key for treating conditions with no established genotype-phenotype correlation and phenotypic variability.

Moosajee M, Tracey-White D, Smart M, Weetall M, Torriano S, Kalatzis V, da Cruz L, Coffey P, Webster AR, Welch E. (2016) *Functional rescue of REP1 following treatment with PTC124 and novel derivative PTC-414 in human choroideremia fibroblasts and the nonsense-mediated zebrafish model*. Hum Mol Genet 25(16):3416-3431. DOI: 10.1093/hmg/ddw184
This paper was the basis of our work investigating the utility of small molecule drugs for application to inherited retinal diseases, it provided proof-of-concept for further translational development.

Name	NAOMI MORIS	
Position	Group Leader (1st 6)	
Year joined *(Crick or founder institute)*	2021	

Lab Name	***Stem Cell & Human Development Laboratory***

Research programme and achievements

We study human embryonic development using three-dimensional (3D) stem cell-based models, so that we can better understand both development and disease.

The development of the embryo starts from a few cells that divide and differentiate, eventually forming all the cell types of your body. It is important that the cells do this in a coordinated way, making the right decisions in the right place and at the r ight time.

Because of ethical and technical limitations, we cannot study the human embryo at these stages of development, so we know very little about the dynamics of this process in humans.

Our approach is to use human pluripotent stem cells that we grow under defined conditions to create 3D structures that mirror some of the features of early embryos. Using these model systems, we can examine the emergence of the range of cell types and their spatiotemporal coordination, and the organisation into elements of the mammalian body plan.

Using this model system alongside advanced microscopy, molecular and transcriptomics techniques, we aim to address fundamental questions of developmental biology using systems biology approaches. This will allow us to gain an insight into human-specific aspects of development, as well as enabling the establishment of various disease models that will be used to understand the molecular mechanisms underlying birth defects.

Research outputs

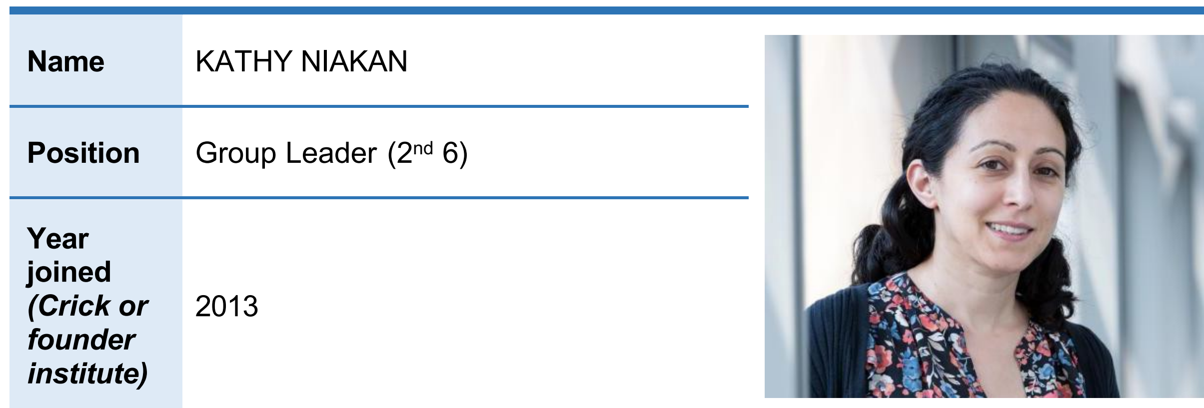
Moris, N., Alev, C., Pera, M. & Martinez Arias, A. (2021) ***Biomedical and societal impacts of in vitro embryo models of mammalian development*****. Stem Cell Reports 16(5):1021-1030. DOI: 10.1016/j.stemcr.2021.03.023**
A discussion about the broader implications of embryo-like models of development, particularly human embryo models. We discuss the challenges, limitations and opportunities for biomedical research and therapeutic advances using stem-cell-based model systems such as the gastruloids.

Moris, N.*, Anlas, K.*, van den Brink, S.*, Alemany, A.* et al. (2020) ***An in vitro model for anteroposterior organisation during human developmen*****t. Nature 582(7812):410-415. DOI: 10.1038/s41586-020-2383-9**
The first demonstration of an axially organised human embryo-like model system, the human gastruloids. We showed that the gastruloids break-symmetry, become polarised in their gene expression and undergo morphological axial elongation. They even have a signature of spatial gene expression organisation indicative of early somitogenesis, that would place them at an equivalent stage as a 20-21 day old human embryo.

van den Brink, S., Alemany A., van Batenburg, V., Moris, N. et al. (2020) ***Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids*****. Nature 582(7812): 405-409. DOI: 10.1038/s41586-020-2024-3**
Using spatial transcriptomics and single cell sequencing, we examined the complexity of the mouse gastruloids, including the diversity of cell types. We also showed that embedding these structures in Matrigel was sufficient to generate morphological 'somite-like' structures and anteroposterior organised somites, for the first time.

Beccari, L.* Moris, N.*, et al. (2018) ***Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids*****. Nature 562, 272–276. DOI: 10.1038/s41586-018-0578-0**
Established the similarities between gastruloids and the developing mouse embryo, using temporal transcriptomics datasets and spatial imaging. It was therefore one of the first to show that self-organisation of ES cells, under defined conditions in suspension, can be used to understand post-implantation embryogenesis including colinearity of Hox genes.

Moris, N., Pina C. & Martinez Arias A. (2016) ***Transition states and cell fate decisions in epigenetic landscapes*****. Nature Reviews Genetics. 17 (11):693-703. DOI: 10.1038/nrg.2016.98**
A strong Perspectives piece that challenged an emerging preconception in the field of single-cell transcriptomics, by suggesting discrete transition states in fate decisions using dynamical systems theory.



Name	KATHY NIAKAN
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013
Lab Name	***Human Embryo and Stem Cell Laboratory***

Research programme and achievements

The aim of research in my laboratory is to provide molecular insights into how early human development is controlled. The mechanisms that regulate early cell lineage decisions in human development remain poorly understood, despite their fundamental biological importance and wide-reaching clinical implications for understanding infertility, miscarriages, developmental disorders and therapeutic applications of stem cells. My laboratory has pioneered approaches to investigate the function of genes that regulate human preimplantation embryo development. During the quinquennium, we have uncovered a mechanism underlying the first lineage decision in human embryogenesis; discovered gene regulatory networks specific to human embryos, which are not found in mouse embryos; and identified mechanisms that are evolutionarily conserved across mammals.

These discoveries validate the need to study human embryos directly. By integrating signalling insights gained from transcriptomic analysis of human blastocysts, we have defined human embryonic stem cell culture conditions that more closely recapitulate the embryonic niche. The foundation of knowledge we have generated will be informative to further improve *ex vivo* models to better understand human biology. Furthermore, by applying the knowledge we gained from dissecting the molecular programmes in the developing embryo, we have identified signalling pathways and transcription factors that mediate a cell fate switch from a pluripotent embryonic stem cell (ESC) to yolk sac or placental progenitor cells. We have demonstrated that these cellular models are tractable systems for molecular genetic analysis and in the future anticipate that they will be informative to understand yolk sac or placental disease.

Our laboratory has contributed to engineering optimised models of early implantation, which has revealed a degree of self-organisation in the absence of maternal tissue. We also generated extensive pre-clinical data that were part of the evidence used to support changes in UK law regulating mitochondrial replacement therapy, a novel reproductive technology to prevent fatal inherited mitochondrial diseases. In all, we have established an international reputation for our expertise in early human development.

Future plans:

Our future plans are to transform our understanding of the molecular mechanisms that control early human development. We seek to uncover when and how human embryonic epiblast cells are established and maintained, and to understand the molecular mechanisms that distinguish these pluripotent cells from extra-embryonic cells during embryogenesis. We will further develop pioneering methods to investigate gene function during human embryogenesis using CRISPR-Cas9-mediated genome editing, TRIM- Away protein depletion, constitutively active and kinase dead variants of proteins and small molecule inhibitors and activators. These approaches will enable us to directly test the function of

genes involved in Hippo and TGFβ signalling, and key transcription factors downstream of these pathways, which we hypothesise are involved in the first and second cell fate decisions, respectively. Altogether, we expect this programme to make significant advances in our understanding of the molecular programmes that shape early human embryogenesis, with the potential to provide fundamental insights and to drive clinical translation.

Research outputs

Gerri C., McCarthy A., Alanis-Lobato G., Demtschenko A., Bruneau A., Loubersac S., Fogarty N.M.E., Hampshire D., Elder K., Snell P., Christie L., David L., Van de Velde H., Fouladi-Nashta A.A. and Niakan K.K. (2020) ***Initiation of a conserved trophectoderm program in human, cow and mouse embryos*****. Nature, 587(7834):443-447. DOI: 10.1038/s41586-020-2759-x**

We discovered that the mechanism underlying the first lineage decision in human embryos is mediated via cell-cell contact, triggering a cascade of broadly evolutionarily conserved molecular events that initiates a switch to a placental progenitor programme. We believe that our study will have clinical impact given that the timing of this decision coincides with the stage when most human embryos arrest.

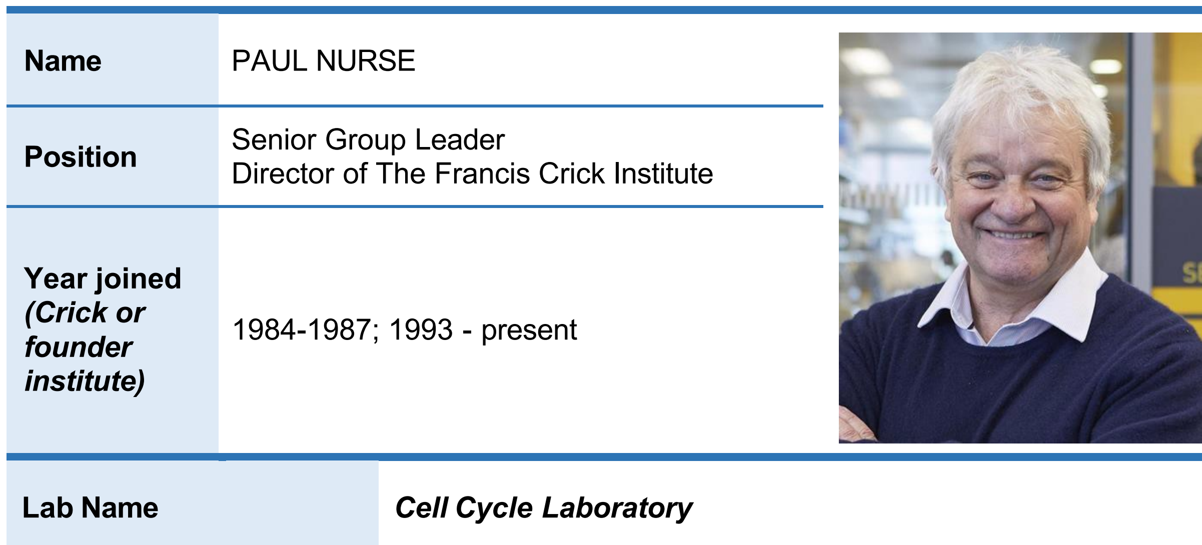
Wamaitha S.E., Grybel K.J., Alanis-Lobato G., Gerri C., Ogushi S., Mahadevaiah S.K., Healy L., Lea R.A., Molina-Arcas M., Elder K., Snell P., Christie L., Downward J., Turner J.M.A. and Niakan K.K. (2020) ***IGF1-mediated human embryonic stem cell self-renewal recapitulates the embryonic niche*****. Nature Communications, 11: 764. DOI: 10.1038/s41467-020-14629-x**
In this work we mined this database to refine hESC culture conditions. These data will be a powerful resource for the community and will lead to changes in how hESCs are cultured in the future. Building on these data, we demonstrated that IGF1-receptor/PI3K/AKT, but not FGF receptor, signalling is required for hESC self-renewal. We built a searchable website that includes a compendium of human embryo gene expression analysis and compiled a list of all possible ligand and receptor interactions.

Fogarty, N.M.E., McCarthy, A., Snijders, K.E., Powell, B.E., Kubikova, N., Blakeley, P., Lea, R., Elder, K., Wamaitha, S.E., Kim, D., Maciulyte, V., Kleinjung, J., Kim, J.-S., Wells, D., Vallier, L., Bertero, A., Turner, J.M.A. and Niakan K.K. (2017) ***Genome editing reveals a role for OCT4 in human embryogenesis*****. Nature, 550(7674): 67-73. DOI: 10.1038/nature24033**
The first demonstration of the utility of CRISPR–Cas9-mediated genome editing for investigating gene function in the context of human embryonic development. We revealed a distinct role for the developmental regulator OCT4 in human versus mouse development.

Hyslop L.A., Blakeley P., Craven L., Richardson J., Fogarty N.M., Fragouli E., Lamb M., Wamaitha S.E., Prathalingam N., Zhang Q., O'Keefe H., Takeda Y., Arizzi L., Alfarawati S., Tuppen H.A., Irving L., Kalleas D., Choudhary M., Wells D., Murdoch A.P., Turnbull D.M., Niakan K.K. and Herbert M. (2016) ***Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease*****. Nature, 534(7607): 383-386. DOI: 10.1038/nature18303**
A preclinical study on the use of pronuclear transplantation for mitochondrial replacement therapy. For this study, my laboratory performed transcriptional comparisons of cells, derived human embryonic stem cells and performed molecular characterisation.

Wamaitha S.E., del Valle I., Cho L.T., Wei Y., Fogarty N.M.E., Blakeley P., Sherwood R.I., Ji H. and Niakan K.K. (2015) ***Gata6 potently initiates reprogramming of pluripotent and differentiated cells to extraembryonic endoderm stem cells*****. Genes and Development, 29(12): 1239-1255. DOI: 10.1101/gad.257071.114**
Using genomics, genome-wide transcriptional and chromatin immunoprecipitation analyses, we discovered that the transcription factor Gata6 is able to rapidly and directly

inhibit core and peripheral genes within the pluripotency regulatory network, as well as directly activate an extraembryonic endoderm program to facilitate cellular reprogramming. The cell lines we engineered and datasets we generated have been used by several international laboratories.



Name	PAUL NURSE
Position	Senior Group Leader Director of The Francis Crick Institute
Year joined *(Crick or founder institute)*	1984-1987; 1993 - present

Lab Name	***Cell Cycle Laboratory***

Research programme and achievements

The objective of the Cell Cycle Laboratory is to better understand the global controls regulating the eukaryotic cell cycle. The organism used for this work is the fission yeast which has many powerful methodologies and resources available for its study, including a 99% genome wide gene deletion collection constructed by a consortium led by the Laboratory.

The main achievements of the Laboratory since 2015 are as follows:

1. Screening of the gene deletion collection which identified 538 cell cycle genes. Diploid haploinsufficiency and haploid mitotic advancement screens of the gene deletions identified 32 genes potentially encoding rate limiting components for cell cycle control and CDK regulation.
2. Establishing that the meiotic and mitotic cell cycles can be driven by a single cyclin-CDK complex, supporting a model proposed by the Laboratory that quantitative increase in total cellular CDK activity is a major principle underlying cell cycle progression.
3. Describing the cell cycle patterns of CDK substrate phosphorylation and identifying substrates phosphorylated early, middle, and late in the cell cycle. Developing an *in vivo* protein kinase assay and using this to reveal that total cellular CDK activity has a wide dynamic range during the cell cycle, rising 50-fold. This results in early substrates involved in S-phase being more easily phosphorylated than late mitotic substrates. Determining the average CDK phosphosite turnover half-life as 2-3 mins and showing it is the same at different cell cycle stages.
4. Demonstrating that CDK and other cell cycle protein kinases at both the microlevel (within mitosis) and macrolevel (throughout the cell cycle) act like rheostats. These can form the basis for maintaining temporal order during the cell cycle. The high CDK phosphosite turnover could create futile cycles to help to ensure there are sharp rises in substrate phosphorylation at cell cycle transitions.
5. Showing that cell size homeostasis at the G2/mitosis transition is maintained in cells that cannot phosphorylate Cdc2Y15, indicating that regulators of Y15 phosphorylation are unlikely to have roles in the primary cell size sensing mechanism as has been proposed, although could co-operate with such a primary mechanism to make it more accurate.
6. Identifying a centrosomal Spindle Pole Body (SPB) targeting motif in the B-type cyclin Cdc13, and showing that this motif is required for cells to enter mitosis. This motif is conserved in human cells targeting cyclin B1 to the centrosome. In the absence of the

Research outputs

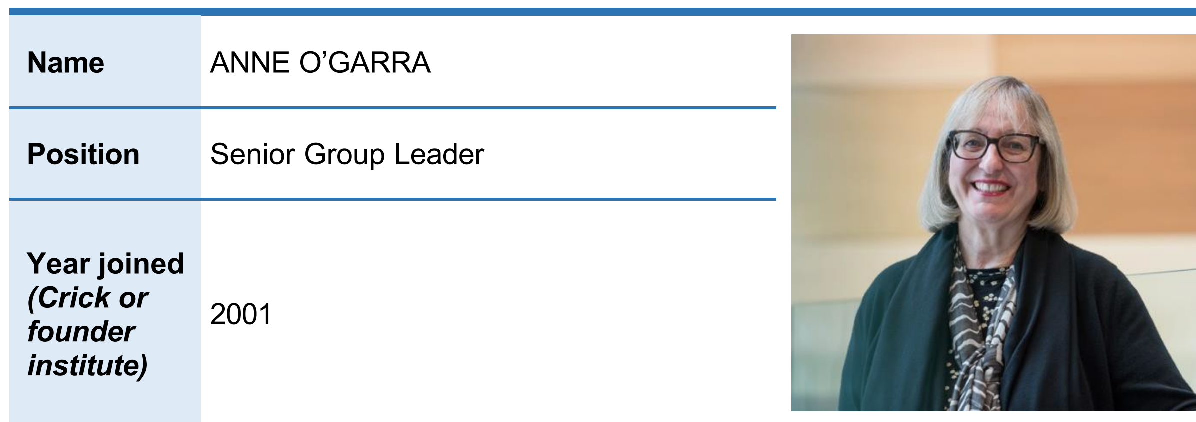
Gutiérrez-Escribano P, and Nurse P. (2015) ***A single cyclin-cdk complex is sufficient for both mitotic and meiotic progression in fission yeast*****. Nat Commun. 6:6871-93. DOI: 10.1038/ncomms7871**
The requirements of the six CDKs expressed during meiosis have been analysed. All six cyclins have been deleted and can be replaced by a single Cdc13-cdc2 fusion protein. We conclude that the meiotic and mitotic cell cycles can be driven by a single CDK complex.

Swaffer MP, Jones AW, Flynn HR, Snijders AP, Nurse P. (2016) ***CDK Substrate Phosphorylation and Ordering the Cell Cycle*****. Cell. 167(7):1750-1761. DOI: 10.1016/j.cell.2016.11.034**
A phosphoproteomics analysis of CDK substrates has shown that the correct cell cycle temporal order of CDK substrate phosphorylation can be established by a single CDK. It is shown that there is a 50-fold increase of *in vivo* CDK activity during the cell cycle. Temporal order is achieved by a combination of this rise with differential sensitivity of substrates to CDK activity. Phosphosite turnover is very rapid which helps ensure sharp cell cycle transitions.

Swaffer MP, Jones AW, Flynn HR, Snijders AP, Nurse P. (2018) ***Quantitative Phosphoproteomics Reveals the Signaling Dynamics of Cell-Cycle Kinases in the Fission Yeast Schizosaccharomyces pombe*****. Cell Rep. 24(2):503-514. DOI: 10.1016/j.celrep.2018.06.036**
A phosphoproteomics analysis of cell cycle protein kinases indicates that different mitotic kinases (CDK, NIMA related, Polo-like and Aurora) are activated sequentially during mitosis. The timing of these waves of activation is determined by the differential sensitivities of the mitotic kinases to the rising level of upstream CDK activity.

Kume K, Cantwell H, Burrell A and Nurse P. (2019) ***Nuclear membrane protein Lem2 regulates nuclear size through membrane flow*****. Nat Commun. 10(1):1871. DOI: 10.1038/s41467-019-09623-x**
Deletion of *lem2* the nuclear membrane protein Lem2 alters the size of the nucleus. Fatty acid synthesis inhibition reduces lipid availability for membranes, and membrane flows rapidly out of the nucleus in cells deleted for *lem2*. It is proposed that membrane flow between organelles is part of the mechanism maintaining nuclear size homeostasis.

Basu S, Roberts EL, Jones AW, Swaffer MP, Snijders AP, Nurse P. (2020) ***The Hydrophobic Patch Directs Cyclin B to Centrosomes to Promote Global CDK Phosphorylation at Mitosis*****. Curr Biol. 30(5):883-892. DOI: 10.1016/j.cub.2019.12.053**
Disruption of a hydrophobic patch in the Cdc13 B-cyclin prevents localisation of CDK at the centrosomal spindle pole body, blocks mitosis, and compromises phosphorylation of the weakest CDK substrates. We propose this mechanism contributes to CDK substrate regulation.



Name	ANNE O'GARRA
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2001

Lab Name	***Immunoregulation and Infection Laboratory***

Research programme and achievements

The Laboratory of Immunoregulation and Infection has a major focus on transcriptional regulation of cytokines, essential for protection against infection, and their regulation by the cytokine IL-10 to prevent collateral damage from an immune response to pathogens and pathobionts. We also continue to study the immune response in tuberculosis (TB), in both mouse models and human disease.

Tuberculosis remains a major cause of death from infectious disease, with 1.5 million deaths in 2018, but diagnosis remains difficult. Moreover, a third of the world is estimated to have been infected by *Mycobacterium tuberculosis*, although most remain asymptomatic or latent, with 10% progressing to active TB, although the host factors determining outcome to infection are unknown. New approaches are needed for TB diagnosis, to determine early which individuals will progress to TB disease, and also to identify the host factors determining the outcome to *M. tuberculosis* infection.

Our identification of a blood neutrophil-driven type I interferon (IFN) -inducible transcriptional signature of active TB, absent in the majority of latently exposed asymptomatic individuals and healthy controls, has now been widely reproduced. We recently demonstrated immunological heterogeneity in the blood transcriptome of a cohort of recent TB contacts. A small proportion expressed a persistent TB signature and subsequently progressed to active TB disease. The composition of this initial blood transcriptional signature in TB progressors suggested a host response evolving towards active disease. Additionally, 50% of contacts who were sensitised to *M. tuberculosis* infection but did not progress to disease exhibited a similar, but not identical, blood signature that was transient and resolved within three months.

Mouse infection models have been used to study the host response to *M. tuberculosis*, but their validity in revealing determinants of human tuberculosis (TB) resistance and disease progression has been heavily debated. We have now shown that the modular transcriptional signature in the blood of TB susceptible C3HeB/FeJ mice infected with a clinical isolate of *M. tuberculosis* resembles that of active human TB disease, with dominance of a type I interferon response and neutrophil activation and recruitment, providing a tractable model to study targets and mechanisms underlying TB pathogenesis. Notably, the blood signature of active disease shared by mice and humans was also evident in latent TB progressors before diagnosis, suggesting that these responses both predict and contribute to the pathogenesis of progressive *M. tuberculosis* infection. Conversely, resistant but not susceptible strains of mice showed increased lung B cell, natural killer and T cell effector responses in the lung upon infection, suggesting that early local immune responses are critical determinants of outcome from infection.

We will identify the early immune events in the airways of human contacts of TB patients

and determine host factors that determine outcome of control of progression to active TB disease. We will in parallel study these early airway events in TB resistant and susceptible mice to identify targets that parallel the host factors determining outcome in human TB, so as to test and identify the early local pathways and mechanisms that determine protection or pathogenesis in TB.

Underpinning our research in TB we continue to define the molecular mechanisms for induction of IL-10 versus inflammatory cytokines in immune cells. We have shown that type I IFN acts as a transcriptional regulator of *Il10* mRNA in *M. tuberculosis* infected or TLR4 stimulated macrophages and continue to identify this in-depth in both *in vitro* and *in vivo* systems. In T cells, we have demonstrated that although the transcription factor c- Maf plays a dominant role in regulation of *Il10* both *in vitro* and *in vivo,* c-Maf exerts context-specific effects in different diseases *in vivo,* through its regulation of other pathways and transcription factors. We continue to study regulation of *Il10* and proinflammatory cytokines *in vivo* by c-Maf and other candidate transcription factors, to determine the mechanisms of their context specific effects on the immune response to intracellular (Th1)-type pathogens and pathobionts, relevant to other intracellular infections such as TB.

Collectively, our studies highlight the complex role of cytokines in protecting or promoting infectious diseases, and we continue to dissect mechanisms underlying their expression and function in this context. We additionally continue to study the diverse roles of type I IFN in the regulation of cytokines in the immune response to *M. tuberculosis,* to provide valuable information for the development of immunomodulatory treatments. We have confirmed the reproducibility of our blood-based transcriptional signature to characterise the immune response determining the outcome of *M. tuberculosis* infection, and to develop tools to support diagnostics and treatment monitoring of TB.

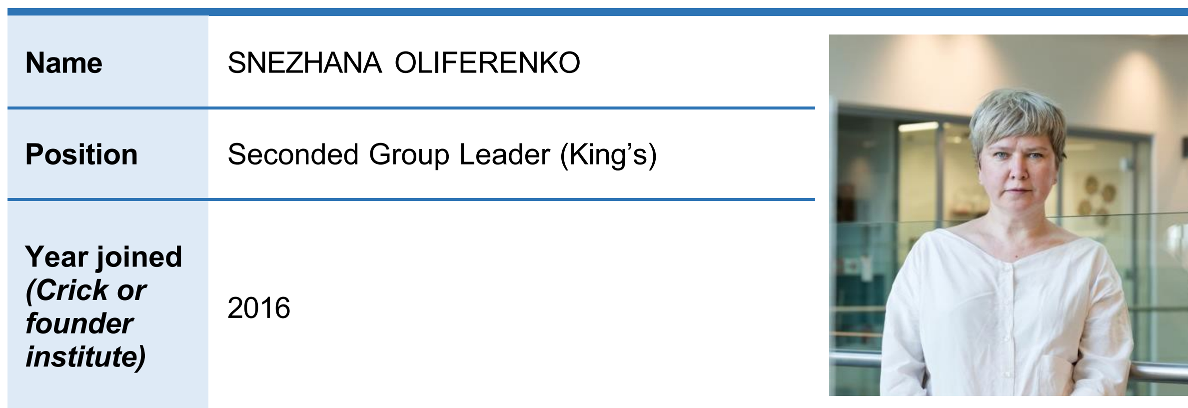
Research outputs

Moreira-Teixeira, L., Redford, P.S., Stavropoulos, E., Ghilardi, N., Maynard, C.L., Weaver, C.T., Freitas do Rosario, A.P., Wu, X., Langhorne, J., and O'Garra, A. (2017) ***T Cell-Derived IL-10 Impairs Host Resistance to Mycobacterium tuberculosis*** **Infection. J Immunol 199, 613-623. DOI: 10.4049/jimmunol.1601340**
This manuscript showed that the T-cell derived suppressive cytokine IL-10 limits the protective immune response to *M. tuberculosis* infection. In the absence of IL-10, decreased bacterial loads correlated with early increased influx of Th1 cells into the site of infection and enhanced production of IFN-γ, indicating that IL-10 contributes to TB pathogenesis.
Gabryšová, L., Alvarez-Martinez, M., Luisier, R., Cox, L.S., Sodenkamp, J., Hosking, C., Pérez-Mazliah, D., Whicher, C., Kannan, Y., Potempa, K., Wu, X., Bhaw, L., Wende, H., Sieweke, M.H., Elgar, G., Wilson, M., Briscoe, J., Metzis, V., Langhorne, J., Luscombe, N. M. and O'Garra, A. (2018) ***C-Maf controls immune responses by regulating disease-specific gene networks and repressing IL-2 in CD4+ T cells.*** **Nature Immunology 9(5):497-507. DOI: 10.1038/s41590-018-0083-5**
We show T cell-specific deletion of c-Maf leads to reduced levels of the suppressive cytokine Il10 gene *in vivo* and and thus increased collateral damage in mouse models of malaria and allergy. Conversely, in an autoimmunity model, T cell-specific deletion of c-Maf, although reducing *Il10* expression, had a protective effect. Analysis of complex gene regulation networks revealed that c-Maf is a central regulator of gene expression in $CD4^{+}$ T cells, explaining its context-specific effects in autoimmunity. Such genomics approaches aid understanding of the complexity of the immune response, and how context-specific activation of transcription factors regulates diverse functions in immunity.

Singhania A, Verma R, Graham CM, Lee J, Tran T, Richardson M, Lecine P, Leissner P, Berry MPR, Wilkinson RJ, Kaiser K, Rodrigue M, Woltmann G, Haldar P, O'Garra A. (2018) ***A modular transcriptional signature identifies phenotypic heterogeneity of***

Singhania A*, Graham CM*, Gabryšová L*, Moreira-Teixeira L, Stavropoulos E, Pitt JM, Chakravarty P, Warnatsch A, Branchett WJ, Conejero L, Lin JW, Davidson S, Wilson MS, Bancroft G, Langhorne J, Frickel E, Sesay AK, Priestnall SL, Herbert E, Ioannou M, Wang Q, Humphreys IR, Dodd J, Openshaw PJM, Mayer-Barber KD, Jankovic D, Sher A, Lloyd CM, Baldwin N, Chaussabel D, Papayannopoulos V, Wack A, Banchereau JF, Pascual VM, O'Garra A. (2019) ***Transcriptional profiling unveils type I and II interferon networks in blood and tissues across diseases*****. Nat Commun 10(1):2887. DOI: 10.1038/s41467-019-10601-6**
Using advanced bioinformatics approaches, we deciphered the global transcriptional response in the lungs of mice infected or challenged with a broad spectrum of infectious pathogens, including parasites, bacteria, viruses, fungi, or allergens; we also determined to what extent each of these responses is preserved in the blood. We demonstrated a unique global transcriptional signature for each of the different diseases in both lung and blood. The lung transcriptional signatures showed a gradation, ranging from IFN-inducible gene clusters, to those associated with granulocyte/neutrophil/IL-17 dominated genes, to responses dominated by expression of genes encoding T_H2 cytokines, mast cells and B cells.

Moreira-Teixeira,. L*., Tabone, *O., Graham, C.M*., Singhania, A, Stavropoulos, E., Redford, P.S., Chakravarty, P., Priestnall, S., Suarez-Bonnet, A., Herbert, E., Mayer-Barber, K.D., Sher, A., Fonseca, K.L., Sousa, J., Cá, B., Verma, R., Haldar, P., Saraiva, M., and O'Garra, A. (2020) ***Mouse transcriptome reveals potential signatures of protection and pathogenesis in human tuberculosis*****. Nature Immunology 21, 464-476. DOI: 10.1038/s41590-020-0610-z**
We demonstrated that TB susceptible mice infected with a clinical isolate of *M. tuberculosis* have a type I IFN-inducible and neutrophil driven transcriptional signature closely resembling the human disease. The signature was also evident in latent tuberculosis progressors before diagnosis. This suggests that these responses both predict and contribute to the pathogenesis of progressive *M. tuberculosis* infection, providing potential prognostic biomarkers for early detection of TB in asymptomatic individuals. Our identification of a TB-susceptible mouse model that recapitulates the human disease provides a tractable system to further dissect the immune mechanisms underlying protection or pathogenesis resulting from *M. tuberculosis* infection.



Name	SNEZHANA OLIFERENKO
Position	Seconded Group Leader (King's)
Year joined *(Crick or founder institute)*	2016

Lab Name	***Comparative biology of mitotic division laboratory***

Research programme and achievements

We have pioneered the use of the fission yeasts *S. pombe* and *S. japonicus* as a composite system for evolutionary cell biology studies. Their similar genetic makeup and experimental amenability allow comparative and reverse engineering analyses, facilitating discovery of the core cellular components and interactions and illuminating the origins of evolutionary innovation. Our comparative approach has come of age in the last five years. We now appreciate its power not only in elucidating the core principles and evolution of specific cell biological mechanisms but also in illuminating unexpected functional links to the rest of cellular and organismal physiology. Several highlights are outlined below.

We discovered that the divergent strategies of mitotic nuclear envelope remodeling in *S. pombe* and *S. japonicus* are necessitated by the distinct phosphoregulation of the phosphatidic acid phosphatase lipin acting as a rheostat to control phospholipid biosynthesis and hence, nuclear membrane expansion [Current Biology 26: 237 (2016)]. We discovered that cellular membranes in these species are made of structurally distinct phospholipids due to the difference in fatty acid synthase activities, and showed that evolutionary changes in lipid metabolism require extensive adaptation of the membrane-associated proteome [Current Biology 30:367-380 (2020)]. We discovered a conceptually novel function and the mechanism for the ESCRT-III/Vps4 machinery in regulating interactions between the inner nuclear membrane proteins and heterochromatin and explained how it impacts nuclear envelope reformation [Developmental Cell, 53:27-41 (2020)]. In the process of working on these projects, we realized that the striking differences in nuclear envelope management between the two species are just the tip of the iceberg. We found that *S. pombe* has evolved an unusual medial division ring assembly mechanism based on neofunctionalization of a recently duplicated anillin paralog but *S. japonicus* regulates ring formation similarly to metazoans [Current Biology 25: 1187 (2015)]. Following this, we made important contributions to illuminating ring organization and function in *S.japonicus* [eLife 210.7554/eLife.21383 (2016); J Cell Biology 216: 2657 (2017)] and discovered the organismal-level function for cellular geometry scaling in maintaining medial division site positioning [Nature Communications 10: 268 (2019)].

We are now wrapping up several projects, including: (1) elucidating the Aurora kinase-dependent mechanism of NE breakage during mitosis; (2) understanding the regulation of the major activator of Lipin, Spo7-Nem1 phosphatase; (3) investigating the divergence of central carbon metabolism between *S. pombe* and S. *japonicus* (an exciting and completely new project started at the Crick with the help of the Crick-King's PhD fellowship)

We are beginning a major effort to define how lipid metabolic capacity controls membrane organization and cellular physiology, by exploiting the natural divergence in membrane

lipid composition between *S. pombe* and *S. japonicus*. This program will be funded by my new Wellcome Trust Investigator Award (awarded in August 2020). Our research will explain how changes in the architecture of glycerophospholipid fatty acyl tails affect membrane properties. It will provide insights into the organization and evolution of genetic networks regulating membrane homeostasis. Finally, it will test if acquisition of new lipid metabolic functionalities engenders diversification of cellular pathways and organismal physiology.

Additionally, we will continue working on elucidating the molecular mechanisms underlying cellular geometry scaling (funded by BBSRC) and probing the functional interactions between the inner nuclear membrane and chromatin organization, including venturing into mammalian cell biology, in collaboration with Jez Carlton at the Crick.

Research outputs

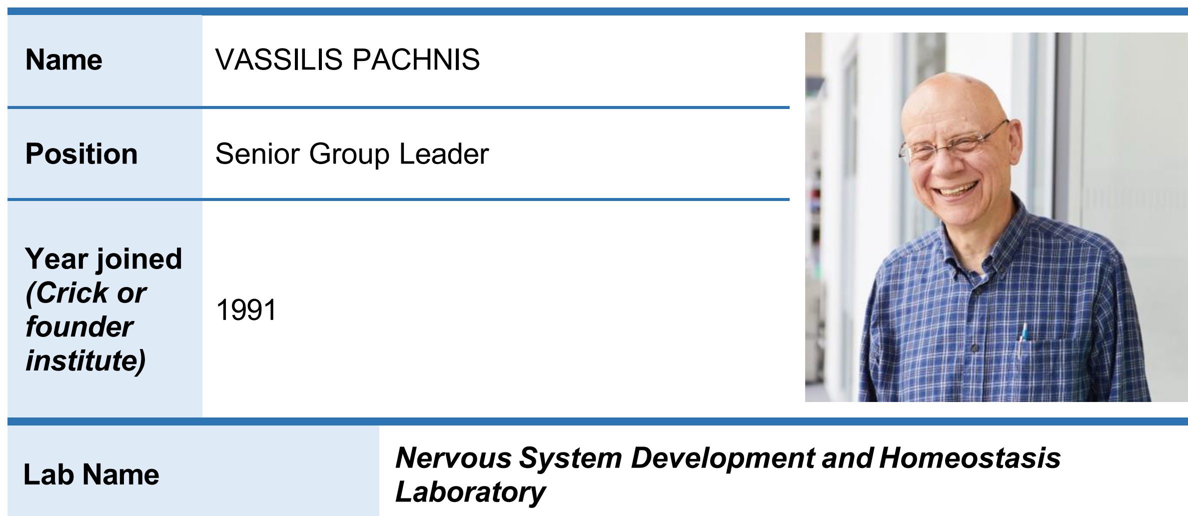
Pieper, G., Sprenger, S., Teis, D. and S. Oliferenko. (2020) *ESCRT-III/Vps4 controls heterochromatin-nuclear envelope attachments*. Developmental Cell 53:27-41. DOI: 10.1016/j.devcel.2020.01.028
Here we show that the inner nuclear membrane Lem2-Nur1 complex serves a substrate for the nuclear ESCRT-III/Vps4 machinery and explain how the dynamic tethering of chromosomes to this complex during interphase is linked to the establishment of nuclear compartmentalization following mitosis.

Makarova, M, Peter, M., Balogh, G., Glatz, A., MacRae, J., Lopez Mora, N., Booth, P., Makeyev, E., Vigh, L. and S. Oliferenko. (2020) *Delineating the rules for structural adaptation of membrane-associated proteins to evolutionary changes in membrane lipidome*. Current Biology 30:367-380. DOI: 10.1016/j.cub.2019.11.043
This work revealing co-evolution of cellular lipidome and transmembrane proteins may lead to a conceptually new understanding of the relationship between the underlying metabolic makeup and the evolution of cellular properties.

Gu, Y. and S. Oliferenko. (2019) *Cellular geometry scaling ensures robust division site positioning*. Nature Communications 10:268. DOI 10.1038/s41467-018-08218-2
Here we describe our discovery that preservation of specific cellular geometry across a range of cell sizes is essential for correct division site positioning and survival, demonstrating the organismal-level function for scaling.

Makarova, M., Gu, Y., Chen, J-S., Beckley, J., Gould, K. and S. Oliferenko. (2016) *Temporal regulation of Lipin activity diverged to account for differences in mitotic programs*. Current Biology. 26: 237-243. DOI 10.1016/j.cub.2015.11.061
Using *S. pombe* and *S. japonicus* we uncovered a molecular basis for variability in nuclear envelope expansion during mitosis. We showed that cells undergoing closed mitosis expand their nuclear envelope prior to division by entraining inactivation of the phosphatidic acid flux regulator lipin to high CDK activity.

Gu, Y., Yam, C. and S. Oliferenko. (2015) *Rewiring of cellular division site selection in evolution of fission yeasts*. Current Biology. 25:1187-1194. DOI: 10.1016/j.cub.2015.02.056
Here we show that placement of the division apparatus is determined by positioning of the actomyosin-plasma membrane linkers and that both identity of the linker and control of its subcellular targeting are subject to evolutionary plasticity.



Name	VASSILIS PACHNIS
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	1991

Lab Name	***Nervous System Development and Homeostasis Laboratory***

Research programme and achievements

Research in my laboratory focuses on understanding the genetic and molecular mechanisms that control the development and homeostasis of the vertebrate nervous system. During the last quinquennium, we addressed these questions in two parts of the nervous system, the forebrain and the enteric nervous system (ENS).

Our work demonstrated that the number of inhibitory neurons in the mouse cortex is regulated cell autonomously by activity-induced apoptosis during a critical postnatal window. This work provided novel insight into the mechanisms that determine the balance between excitation and inhibition in the mammalian forebrain (Denaxa et al. *Cell Rep* 2018).

We also explored the interaction of microbiota and the cell lineages of the ENS. In particular, we demonstrated for the first time that the cellular organisation of the ENS is highly dynamic and regulated by the postnatal colonisation of the gut by microbes (Kabouridis et al. *Neuron* 2015). In addition, we characterised molecular mechanisms underpinning the communication between ENS and the luminal environment of the gut. Collaborative work between my lab and the Stockinger lab demonstrated that central to the ENS-microbiota axis is the transcription factor AhR, which functions as a microbiota and diet-activated biosensor of intestinal neural circuits, thus linking peristaltic activity to the luminal contents of the gut (Obata et al. *Nature* 2020).

We have extended our analysis of the molecular mechanisms that regulate the development of ENS lineages. Using *in vivo* genetic lineage tracing, we examined the developmental potential of mammalian ENS progenitors and characterised their transcriptomic landscape at early stages of ENS ontogenesis. In addition, clonogenic analysis of ENS progenitors led us to propose a new model for the spatial organisation of the mammalian ENS, which links developmental mechanisms employed for the assembly of intestinal neuroglia networks with their functional output (Lasrado et al. *Science*, 2017).

We have also explored the mechanisms that maintain the adult ENS at steady state. Using zebrafish as a model organism, we have demonstrated that enteric glial cells (EGCS), in addition to functioning as canonical glia that provide support and nourishment to enteric neurons and regulates neuronal activity, function as neural stem cells undergoing constitutive neurogenesis. This work reveals previously unappreciated similarities between enteric glia and neural stem cells in the brain and raise hopes that *in vivo* activation of the neurogenic potential of mammalian EGCs could restore the activity of intestinal neural circuits compromised by developmental deficits or disease (McCallum et al. *eLife* 2020).

Our work for the next quinquennium will aim at understanding the developmental basis of the functional units established between the intrinsic neural circuits of the gut and other gut tissues in adult life.

We have now generated a transcriptional landscape of mammalian ENS progenitors, enteric neurons at early stages of differentiation and enteric glia at all stages of development and in the adult. In parallel we have generated cellular and transcriptomic maps of adult zebrafish ENS. These experiments have generated rich datasets which identify a large number of candidate regulators of enteric neuron and glia differentiation and their assembly into functional circuits. We plan to employ hPSC-based *in vitro* models of enteric neurogenesis as well as genetic animal models (mouse and zebrafish) to dissect the molecular cascades that underpin ENS lineage differentiation. In addition to providing fundamental insight into the development of the peripheral nervous system, this work promises to identify molecular pathways implicated in the pathogenesis of neurogenic gut disorders.

Our group was the first to identify signalling queues which originate from the gut mesenchyme and promote the development of the mammalian ENS. We have obtained evidence that, in a reciprocal manner, ENS lineages also regulated the morphogenesis, differentiation and homeostasis of surrounding gut tissues. We plan to characterise such ENS-derived signals and their upstream regulators, and their effect on surrounding tissues at different developmental stages and in adult animals. Of particular interest is our recent discovery that homeostatic immune signals acting on EGCs regulate the inflammatory state of a wide spectrum of non-neuroectodermal cell types of the gut, including fibroblasts, mesothelial cells and macrophages. We will identify the molecular mechanisms underpinning such interactions and their role in tissue maintenance and repair following injury or disease.

Finally, we will examine how changes in the maternal environment, such as dysbiosis, infections, altered diet and stress, influence the development of the ENS in mammalian embryos. We will determine whether adverse maternal environment alters the differentiation and organisation of the ENS during embryogenesis and early postnatal life and how such changes may predispose to gastrointestinal disorders in later life.

Research outputs

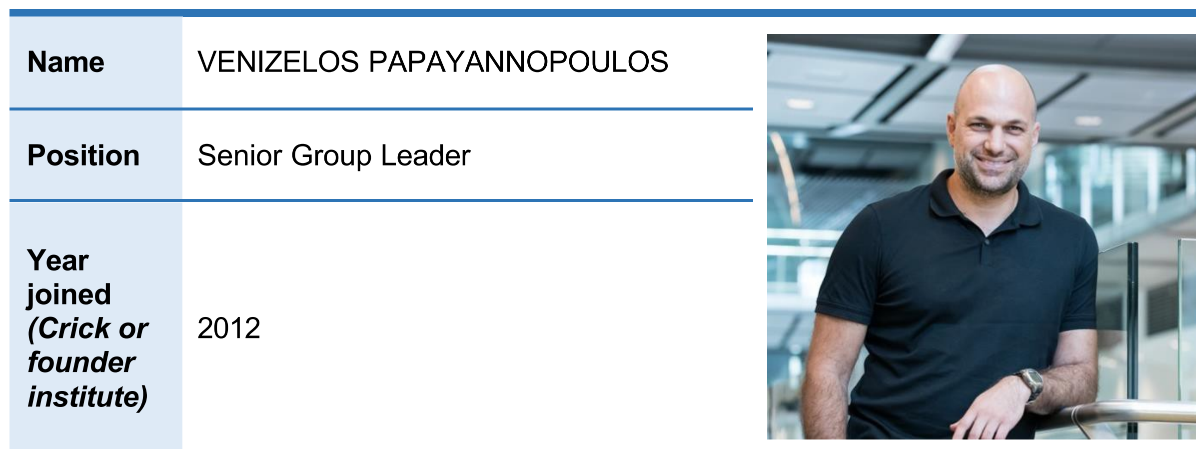
Kabouridis SP, Lasrado R, McCallum S, Chng SH, Snippert H, Clevers H, Pettersson S and Pachnis V. (2015) ***Microbiota controls the homeostasis of glial cells in the gut lamina propria.*** **Neuron 85: 289-295.DOI: 10.1126/science.aam7511**
This paper demonstrates for the first time that the cellular organisation of the mammalian enteric nervous system is highly dynamic and is influenced by the postnatal colonisation of the gut by microbiota.

Lasrado R, Boesmans W, Kleinjung J, Pin C, Bell D, Bhaw L, McCallum S, Zong H, Clevers H, Vanden Berghe P, Pachnis V. (2017) ***Lineage-dependent Spatial and Functional Organization of the Mammalian Enteric Nervous System*****. Science 356:722-726. DOI: 10.1126/science.aaam7511**
In this paper we use genetic lineage tracing and clonal analysis to characterise mammalian enteric nervous system progenitors, define differentiation trajectories for enteric neurons and glia during development and propose a new model for the 3-D organisation of the enteric nervous system.

Denaxa M, Neves G, Burrone J and Pachnis V. (2018) ***Modulation of Apoptosis Controls Inhibitory Interneuron Number in the Cortex*****. Cell Rep 22:1710-1721. DOI: 10.1177/1179069518784277**
Here, we provide evidence that the extent of cortical interneuron apoptosis during the critical early postnatal period is plastic and cell-type specific and can be reduced in a cell-autonomous manner by acute increases in neuronal activity.

Obata Y, Castaño Á, Boeing S, Bon-Frauches AC, Fung C, Fallesen T, Gomez de Agüero M, Yilmaz B, Lopes R, Huseynova A, Horswell S, Rao Maradana M, Boesmans W, Vanden Berghe P, Murray AJ, Stockinger B, Macpherson AJ and Pachnis V. (2020) ***Neuronal programming by microbiota regulates intestinal physiology*****. Nature 578:284-289. DOI: 10.1038/s41586-020-1975-8**
In this paper we explore the molecular mechanisms used by enteric neurons to monitor the luminal environment of the gut. In particular, we demonstrate that the transcription factor AhR functions as a biosensor of intestinal neural circuits, linking their functional output to the microbial environment of the gut lumen.

McCallum S, Obata Y, Fourli E, Boeing S, Peddie CJ, Xu Q, Horsewell S, Kelsh R, Collinson L, Wilkinson D, Pin C, Pachnis V* and Heanue T*. (2020) ***Enteric glia as a source of neural progenitors in zebrafish*****. ELife 9:e56086. DOI: 10.7554/eLife.56086.**
Here we identify the stem cells of the vertebrate enteric nervous system. We demonstrate that enteric glia in the gut of zebrafish have a double character: they function as canonical glial cells, but are also capable of proliferating under physiological conditions giving rise to progeny that differentiate to enteric neurons.

Name	VENIZELOS PAPAYANNOPOULOS	
Position	Senior Group Leader	
Year joined *(Crick or founder institute)*	2012	

Lab Name	***Antimicrobial Defence Laboratory***

Research programme and achievements

Neutrophils are key antimicrobial cells that were once only appreciated for the their antimicrobial properties, which were thought to be implemented via a single antimicrobial programme. The discovery of a defence strategy called NETosis which promotes the release of neutrophil extracellular traps (NETs) raised the possibility that these cells may selectively implement their antimicrobial strategies. Furthermore, it remained unclear whether neutrophils could minimise their destructive effects on tissues. Finally, the role of neutrophils as regulators of inflammation remained unclear.

In 2014 we showed that neutrophils could sense microbe size and release NETs selectively in response to pathogens that are too large to be phagocytised. This discovery inspired subsequent work that highlighted the role of NETs as a strategy to counter large fungi, parasites and bacterial aggregates. It also led to the discovery of a mechanism that allow neutrophils to tune inflammation and swarming according to the size of the microbes they encounter, by sensing the localisation of reactive oxygen species (ROS) generation. This was the first example of ROS localisation being able to influence cellular signalling. This work uncovered mechanisms for intelligent tuning of inflammation.

We also discovered a novel mechanism that allows neutrophils to drive sterile inflammation in atherosclerosis by priming macrophages for the production of pro-inflammatory cytokines via NET-mediated signalling. These studies exemplified the role of neutrophils as regulators of inflammation.

Furthermore, we showed that NET-mediated signalling is driven by NET histones via synergy with NET DNA. This work highlighted a new role for extracellular DNA as a regulator of the cellular localisation of immune receptors and provided the first mechanistic basis for synergy between putative TLR-agonists by showing they trigger sequential distinct functions.

We also contributed to collaborative work that implicated NETs in *Mycobacterium tuberculosis* pathology and allergic airway hypersensitivity as well as gut barrier destruction in Inflammatory bowel disease.

Finally, we uncovered a novel mechanism that promotes neutrophil dysfunction during sepsis and we are investigating how these mechanisms promote severe Covid-19 symptoms. Based on this unpublished work we have established the phase II COVASE clinical trial for Covid-19 patients with the aim of reducing the severity of symptoms by

treating immune pathology using a repurposed Dornase alpha therapy.

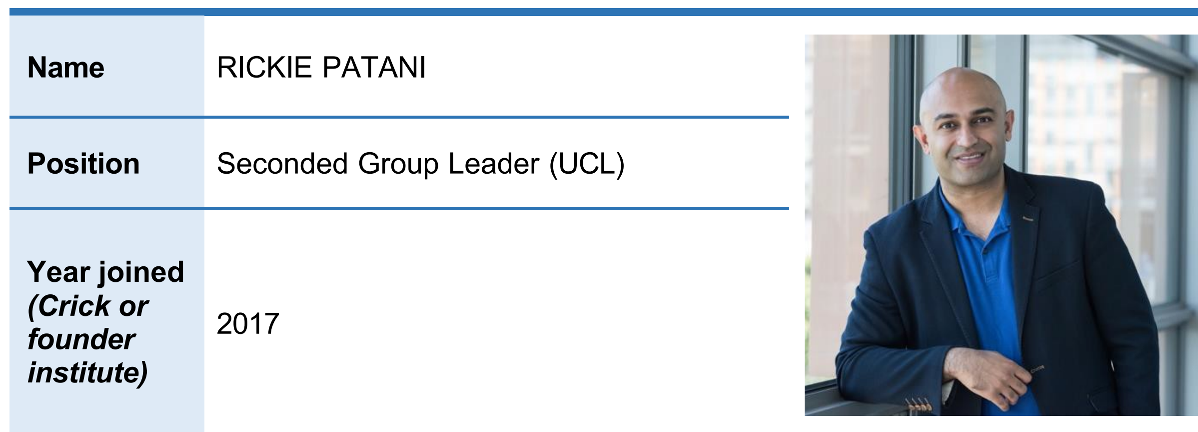
We are currently expanding our studies of the mechanisms regulating the production of neutrophils with alternative properties and understand their contribution to human diseases such as cancer and atherosclerosis particularly at the pre-clinical stage. We are also continuing work on the mechanisms of NET formation and immunomodulatory properties of NETs.

Research outputs

Warnatsch, A., Ioannou, M.,Wang, Q. and Papayannopoulos V. (2015) *Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis*. Science 349 (6245), 316-320. DOI: 10.1126/science.aaa8064
The priming signals in sterile chronic inflammatory diseases remained elusive. Moreover, NETs were mostly thought to serve as an antimicrobial defence mechanism. This work showed that NETs are proinflammatory and provide the priming signals for inflammation in atherosclerosis. It has important implications for our understanding of the mechanisms driving many inflammatory conditions and the important amplification mechanisms involving neutrophil-macrophage crosstalk.

Warnatsch A., Tsourouktsoglou D., Branzk N., Wang Q., Reincke S., Herbst S., Gutierrez M., Papayannopoulos V. (2017) *Reactive oxygen species localization programs inflammation to clear microbes of different size*. Immunity 46(3):421-432. DOI: 10.1016/j.immuni.2017.02.013
How inflammatory programmes are tuned to recruit sufficient numbers of neutrophils to clear microbes of different size remained unknown. Furthermore, neutrophils were not thought to serve as major regulators of inflammation in vivo. We showed that reactive oxygen species localisation allows neutrophils to regulate their own recruitment by setting the appropriate level of cytokine production.

Tsourouktsoglou T.D., Warnatsch A., Ioannou M., Hoving D., Wang Q., Papayannopoulos V. (2020) *Histones, DNA, and Citrullination Promote Neutrophil Extracellular Trap Inflammation by Regulating the Localization and Activation of TLR4*. Cell Rep. 31(5):107602 DOI: 10.1016/j.celrep.2020.107602
This paper uncovered the role of NET histones as major proinflammatory signalling molecules of NET-mediated pathology. It addresses longstanding questions about how cytotoxic chromatin can signal without killing macrophages and changes the way we think about the signaling properties of extracellular DNA. It also answers another longstanding question about the mechanistic basis of synergy between putative TLR agonists. It also offers the first genetic mouse model to interrogate the proinflammatory role of histones *in vivo*. We are building on this story and I think with time, the mechanistic details here will have a great impact on PAMP-induced signaling.



Name	RICKIE PATANI
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	2017

Lab Name	***Human Stem Cells and Neurodegeneration Laboratory***

Research programme and achievements

We study diseases of the nervous system, focusing on motor neuron disease (ALS) and dementia. In ALS, patients lose the ability to move, eat, speak and ultimately breathe. ALS is untreatable because we do not understand the underlying cause(s) of disease. In order to understand disease mechanisms, we use human stem cells generated from real patients. With over a decade of experience using this technology, we can now transform stem cells from patients into human nerve cells and, separately, their support cells (called glia). This approach allows us to determine the sequence of disease-related events within particular cell types. Our overarching goal is to identify precisely what goes wrong, when this begins and in which cell type. We specifically focus on how the following three factors contribute to nerve cell death in ALS: 1) messages called RNAs, which are made from our DNA blueprint; 2) astrocytes, which are star-shaped cells that normally support nerve cells' 3) ageing, which is the biggest risk factor for many neurodegenerative diseases including ALS. The more we understand about human neurological diseases using this approach, the more we will be able to therapeutically target underlying disease mechanisms. We ultimately wish to use this new information to benefit patients with untreatable neurological diseases.

Major Achievements

We have established human induced pluripotent stem cell models of neurodegeneration (Hall et al. 2017 *Cell Reports*). We used this model to described a novel mechanism linking intron retention and nuclear-to-cytoplasmic mislocalisation of RNA binding proteins in iPSC derived neurons, mouse models with ALS causing mutations and further validated in human sporadic ALS postmortem tissue (Luisier et al 2018 *Nature Communications*). This interdisciplinary work combined expertise in stem cell modelling, RNA biology (with Ule lab) and computational analyses (with Luscombe lab), unique to the Crick environment. My group also dissected a new mechanism for the loss of astrocyte-based neuroprotection in ALS (Tyzack et al *2017 Nature Communications*). Two further studies described a new molecular hallmark of ALS (Tyzack et al. 2019 *Brain*) and explore cell type specific differences in the prion-like spread of TDP-43 proteinopathy in ALS (Smethurst et al. 2020 *Brain*).

Future plans

Our vision is to elucidate the role of intron retention in health and neurodegeneration (ALS) using human stem cell models. We will investigate how this post transcriptional phenomenon operates in different cell types (neurons vs astrocytes) and how it is influenced by cellular ageing. Crucially, we also wish to further dissect whether intron retention is mechanistically linked to the nucleocytoplasmic mislocalisation of RNA binding proteins (RBPs). We are developing clear plans to target aberrant intron retention events using ASOs and CRISPR/Cas13 approaches. Likewise, we are also developing strategies to target mislocalised proteins using PROTACs for example. These potentially

therapeutic modalities full rely on a comprehensive understanding of the underlying molecular mechanism e.g. nuclear loss of RBP function and/or altered function in the cytoplasmic compartment, hence our focus on fundamental science (which is driven by a specific clinical need).

Research outputs

Soreq L, UK Brain Expression Consortium, North American Brain Expression Consortium, Rose J, Soreq E, Hardy J, Trabzuni D, Cookson MR, Smith C, Ryten M, Patani R*, Ule J*. (2017) ***Major shifts in glial regional identity are a transcriptional hallmark of human brain aging*****. Cell Reports 18(2):557-570. DOI: 10.1016/j.celrep.2016.12.011**
We examined gene expression changes in post-mortem tissue from 480 individuals (16-106 years old; 10 brain regions). We found that glia are more affected by ageing than neurons, exhibiting major regional identity shifts upon ageing in the substantia nigra and hippocampus, archetypal foci in Parkinson's and Alzheimer's Diseases respectively.

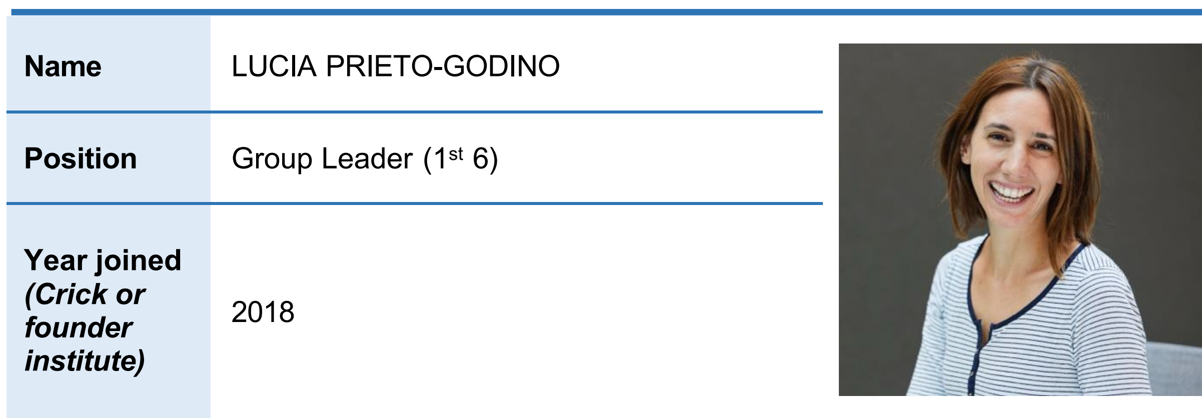
Hall CE, Yao Z, Choi M, Tyzack GE, Serio A, Luisier R, Harley J, Preza E, Arber C, Crisp SJ, Watson PMD, Kullmann DM, Abramov AY, Wray S, Burley R, Loh SHY, Martins LM, Stevens MM, Luscombe NM, Sibley C, Lakatos A, Ule J, Gandhi S*, Patani R*. (2017) ***Progressive motor neuron pathology and the role of astrocytes in a human stem cell model of VCP-related ALS*****. Cell Reports 19(9):1739-1749. DOI: 10.1016/j.celrep.2017.05.024**
We devised some of the most robust, highly enriched and deeply characterised iPSC directed differentiation paradigms into motor neurons and astrocytes. We elucidated the sequence of molecular events in VCP- mutant motor neurons; TDP43 mislocalisation into the cytoplasm and ER stress are primary cytoplasmic events followed by a range of defined secondary phenotypes. We showed that VCP-mutant astrocytes have a survival phenotype and also exert non-cell autonomous effects on motor neurons.

Tyzack GE, Hall CE, Sibley CR, Cymes T, Forostyak S, Carlino G, Meyer I, Schiavo G, Zhang SC, Gibbons GM, Newcombe J, Patani R*, Lakatos A* (2017) ***A neuroprotective astrocyte state is induced by neuronal signal EphB1 but fails in ALS models.*** **Nature Communications 8(1):1164. DOI: 10.1038/s41467-017-01283-z**
We addressed the hypothesis that impairment of neuroprotective astrocytic mechanisms are disrupted in ALS using in vivo models, and patient-specific iPSCs. We found that EphB1, a neuronal signal, can induce a neuroprotective astrocyte phenotype through the EphrinB1 receptor / JAK-STAT pathway and that this response fails in ALS astrocytes.

Luisier R, Tyzack GE, Hall CE, Mitchell JS, Devine H, Taha DM, Malik B, Meyer I, Greensmith L, Newcombe J, Ule J, Luscombe NM*, Patani R*. (2018) ***Intron retention and nuclear loss of SFPQ are molecular hallmarks of ALS*****. Nature Communications 9(1):2010. DOI: 10.1038/s41467-018-04373-8**
We demonstrated aberrant intron retention in ALS-causing mutations. This is the first description of abnormal intron retention in ALS. The most significantly retained intron in is the SFPQ transcript, which 'drags' SFPQ protein out of the nucleus. SFPQ nuclear loss is a new universal molecular hallmark of ALS across iPSC, mouse models and in sporadic ALS post-mortem tissue.

Tyzack GE, Luisier R, Taha DM, Neeves J, Modic M, Mitchell JS, Meyer I, Greensmith L, Newcombe J, Ule J, Luscombe NM*, Patani R*. (2019) ***Widespread FUS mislocalization is a molecular hallmark of amyotrophic lateral sclerosis*****. Brain 142(9):2572-2580. DOI: 10.1093/brain/awz217**
FUS protein was previously thought to only be mislocalised in ALS cases with FUS mutation. This paper demonstrates that FUS nuclear loss is a new molecular

hallmark of ALS across iPSC and mouse models and in sporadic ALS post-mortem tissue. It has evaded detection thus far due to a bias towards studying the molecular constituents of inclusions rather than recognising that RBPs can be mislocalised by not aggregated in the cytoplasm. FUS binds avidly to the retained intron in the SFPQ transcript, which we propose 'drags' FUS protein out of the nucleus



Name	LUCIA PRIETO-GODINO
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2018

Lab Name	***Neural Circuits and Evolution Laboratory***

Research programme and achievements

The goal of the lab is to understand how evolution sculpts nervous systems, giving rise to novel behaviours. Studying the evolutionary forces imposed on neural circuits can provide us with important insights on how brains work and what goes wrong in diseases. We study these questions using a multidisciplinary approach and employing as a model the olfactory systems of several fly species, some of which are pests or contribute to the spread of diseases.

The main achievements since 2015 are the following:

- We discovered that neurons have a mechanism to selectively read through stop codons (1). This finding had two major implications. Firstly, pseudogenes thought to be non-functional can generate fully functional proteins: this shook the olfactory field and made colleagues working on other systems revisit some of their prior findings. Secondly, we uncovered a novel mechanism employed by neurons to generate increased proteomic diversity through the generation of alternative terminal extensions. Currently in the lab we are investigating the molecular mechanisms of neuron-specific readthrough, their role in healthy neuronal function, and its conservation in humans.
- We showed that a single amino acid change is sufficient to evolve the sensitivity of an olfactory receptor towards an ecologically key odour (2). This brought new insights into the molecular mechanisms by which olfactory receptors recognise their ligands. We are following up on these findings by using these receptors as a platform for discovery of the molecular evolutionary mechanisms underlaying the evolution of sensory receptors. Furthermore, this knowledge will be applicable to the rational design of molecules specific to insects' olfactory receptors for pest and vector control.
- We investigated the basis of neuronal number expansions during evolution, demonstrating that both cis- and trans- acting mutations underlie the evolutionary expansion of a sensory population (2). We also identified the regulation of programmed cell death during development as an important mechanism in the evolution of new neural pathways (3).
- To democratise the access to scientific equipment, and promote science reproducibility, we have developed open hardware tools for science, coining the term Open Labware. This includes the co-development of a custom ultra-fast volumetric two-photon microscope at the Crick to perform optogenetically guided circuit tracing. We have carried these activities hand in hand with educational programmes on Open Science in Africa as part of the non-profit organisation I co-founded.

- Our work, together with that of others has shown that olfactory guided behaviours can evolve through changes in the periphery, at the level of olfactory sensory neurons (1,2,3). In addition, behavioural diversity can evolve through modification in how sensory information is processed in the brain, but we know much less about how this happens. A strong current focus of the lab is to investigate how central neural circuits evolve by combining behavioural studies with two photon imaging, electron-microscopy based connectomics, and single cell transcriptomics.
- We have found that evolutionary relatives of the lab model *D. melanogaster* display species-specific behaviours towards odour sources. This is associated with changes their olfactory sensory neuron ensemble, and how these represent the chemical environment. Furthermore, by performing comparative connectomics between *D. melanogaster* and *D. erecta* larvae, we found differences in how their central olfactory processing neurons are interconnected, and by using transcriptomics we are investigating the genetic bases of these changes.

In the future we will build upon our previous discoveries and tool development to address the following main questions. 1) How do central neural circuits evolve? This question will be guided by our recent connectomics results and our unique comparative EM dataset. It will also rely on our establishment of genetic tools across different fly species within the Crick, and our technical capacity to combine fast two-photon volumetric imaging with optogenetic stimulation. 2) What are the functions and mechanisms of neuronal-specific stop codon readthrough? This will build on our previous findings, and novel collaborations that we have made within the Crick. 3) How do insect diseases vectors evolve their olfactory system, and how can we use this knowledge to control them? We have recently established a tsetse fly (vectors of sleeping sickness) facility at the Crick. We have discovered intriguing patterns in the evolution of their olfactory receptor repertoires, and we are investigating how these affect their biology and the diseases vectorial capacity. In addition, we will continue to develop open tools for science, and continue to collaborate with African institutions to make science a truly global endeavour.

Research outputs

Prieto-Godino LL, Rytz R., Bargeton B., Abuin L., Arguello JR, Dal Peraro, M, and Benton, R. (2016) *Olfactory receptor pseudo-pseudogenes*. Nature 539, 93 - 97. DOI: 10.1038/nature19824.
We found that an olfactory receptor pseudogene in *D. sechellia* is functional via readthrough of its premature stop codon. We demonstrated that such functional pseudogenes are common among olfactory receptors in *D. melanogaster*, and that this stop codon readthrough is neuron-specific. This paper had a big impact, making other researchers re-consider whether genes of interest annotated as pseudogenes could be functional.

Prieto-Godino LL, Rytz R, Cruchet S, Bargeton B, Abuin L, Silbering AF, Ruta V, Dal Peraro M, and Benton R. (2017) *Evolution of acid-sensing olfactory circuits in drosophilids*. Neuron 93, 3: 661 - 676. DOI: 10.1016/j.neuron.2016.12.024.
We examined the acid-sensing pathways across two generalists and one specialist *Drosophila* species with divergent odour-guided behaviours. We identified a divergent olfactory pathway in the specialist that responds strongly to an ecologically relevant odour. We discovered that a single amino acid change in a receptor was responsible for the novel odour sensitivity.

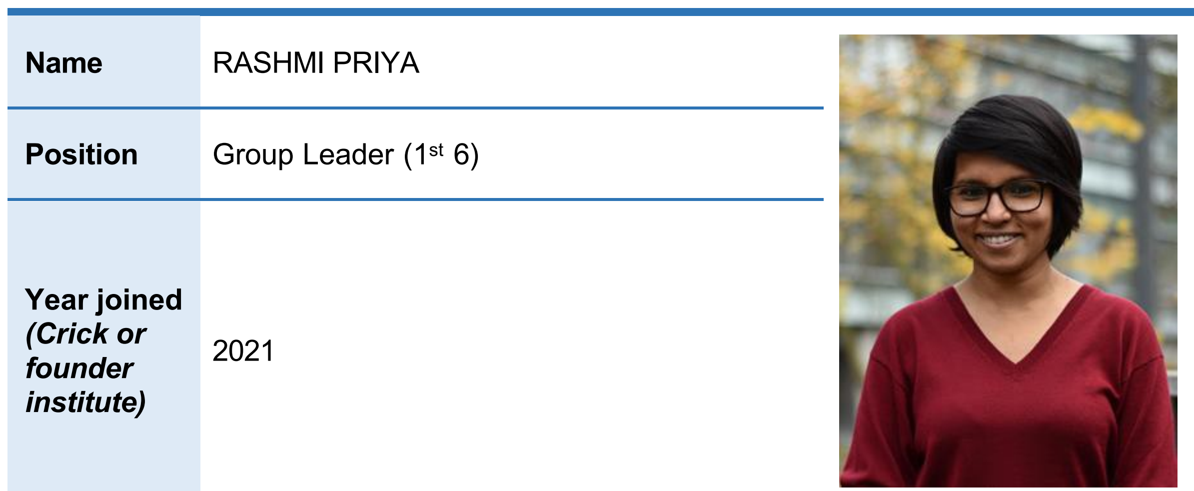
Prieto-Godino LL, Silbering AF, Khallaf MA, Cruchet S, Bojkowska K, Pradervand S, Hansson BS, Knaden M and Benton R. (2020) *Functional integration of "undead" neurons in the olfactory system*. Science Advances 6(11):eaaz7238 DOI:

10.1126/sciadv.aaz7238
Blocking programmed cell death in olfactory sensory neuron precursors in *D. melanogaster* makes new functional neurons. We demonstrated the potential of alterations in programmed cell death to evolve neuronal diversity. This process contributes to evolutionary differences across fly species and between flies and mosquitoes.

Zimmermann MJY, Chagas AM, Bartel P, Pop S, Prieto Godino LL, Baden T. (2020)

***LED Zappelin': An open source LED controller for arbitrary spectrum visual stimulation and optogenetics during 2-photon imaging*. HardwareX 8 E00127. DOI: 10.1016/j.ohx.2020.e00127**
We developed an Open Hardware LED controlled for time-synchronising optogenetic stimulation with two-photon line scans. This helps ameliorate bleed-through of the stimulation light into the recorded data.

Tom Baden, Mahmoud Bukar Maina, Andre Maia Chagas, Yunusa Garba Mohammed, Thomas O. Auer, Ana Silbering, Lukas von Tobel, Marie Pertin, Renee Hartig, Jelena Aleksic, Ibukun Akinrinade, Mosab A. Awadelkareem, Artemis Koumoundourou, Aled Jones, Fabiana Arieti, Andrew Beale, Daniel Munch, Samyra Cury Salek, Sadiq Yusuf and Lucia L. Prieto-Godino. (2020) *TReND in Africa: Toward a Truly Global(Neuro)science Community*. Neuron 107(3), 412 - 416. DOI: 10.1016/j.neuron.2020.06.026
In 2011 I co-founded TReND in Africa, a non-profit organisation to promote science in the African continent. In this article, which was solicited by the Neuron editors, we discussed approaches to address some of the factors that currently stifle Africa's s cientific development and our experience in implementing solutions to them. We also made recommendations to funders, governments and other organisations that were based on other data we had published, consisting of a meta-analysis of neuroscience output from Africa over the last 20 years.



Name	RASHMI PRIYA
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2021

Research programme and achievements

We want to understand how simple structures like groups of cells are moulded into complex organs in a developing embryo.

Organs are composed of thousands of cells that divide, move, differentiate and change their shape to create a complex three-dimensional (3D) architecture. How these key processes are controlled is not well understood. What triggers a particular cell in a population to change its shape or commit to a specific fate? How this information, encoded in mechanical or molecular cues, traverse across biological scales – cells to tissues to organs?

We aim to address these fundamental questions by studying the heart – the first organ to form and to function during embryonic development. To satisfy the demands of a growing embryo, a primitive heart transforms from a hollow tube of a single layer of cells into a highly organized and intricate 3D structure composed of specialized cell types.

Using transparent zebrafish embryos and advanced microscopy, we visualize this remarkable transformation as it unfolds, monitor changes in cell shape and cell fate while the heart is developing and functioning inside the embryo. We combine cross-disciplinary approaches from cell biology, developmental genetics and physics to investigate the mechanical and molecular mechanisms sculpting this vital organ.

The ultimate goal of the lab will be to extend these approaches in other model systems and identify if there are common principles underlying complex organ morphogenesis. We expect that our research will advance our understanding of heart development defects and will have important implications for the fields of regenerative biology and tissue engineering.

Research outputs

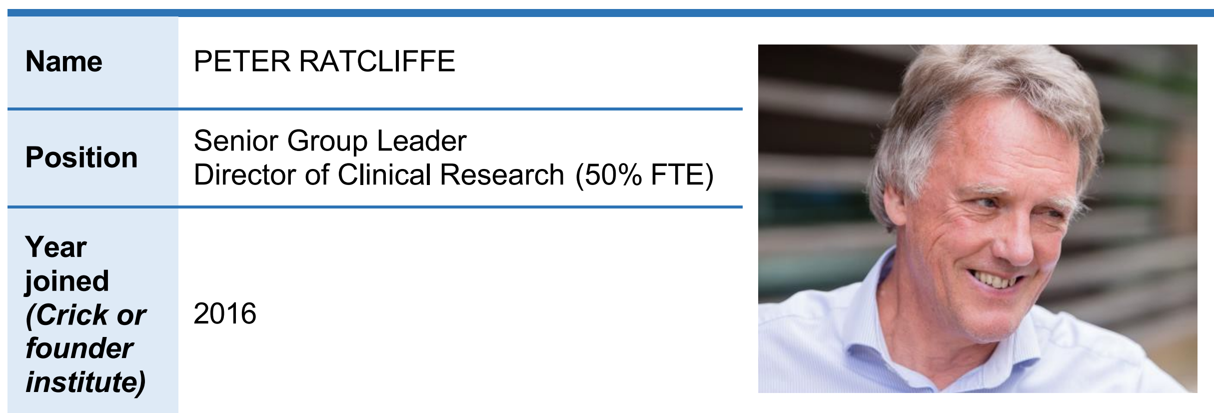
Priya, R.*, Allanki, S., Gentile, A., Mansingh, S., Uribe, V., Maischein, H.-M., and Stainier, D.Y.R. (*corresponding author). (2020) *Tension heterogeneity directs form and fate to pattern the myocardial wall*. Nature 588, 130-134. DOI: 10.1038/s41586-020-2946-9
This work provides the first cogent understanding of how tissue symmetry is broken to specify trabecular cardiomyocytes during embryonic heart development, which is of fundamental importance to the field of cardiovascular and development biology.

Priya, R.*, Liang, X., Teo, J.L., Duszyc, K., Yap, A.S., and Gomez, G.A. (*corresponding author). (2017) ***ROCK1 but not ROCK2 contributes to RhoA signaling and NMIIA-mediated contractility at the epithelial zonula adherens*. Mol Biol Cell 28, 12-20. DOI: 10.1091/mbc.E16-04-0262**
These findings identify ROCK1 as the prominent isoform responsible for contractile properties of epithelial cadherin junctions, and contribute to the growing notion that Rho kinase isoforms do not act redundantly.

Priya, R., Gomez, G.A., Budnar, S., Acharya, B.R., Czirok, A., Yap, A.S., and Neufeld, Z. (2017). ***Bistable front dynamics in a contractile medium: Travelling wave fronts and cortical advection define stable zones of RhoA signaling at epithelial adherens junctions*. PLoS Comput Biol 13, e1005411. DOI: 10.1371/journal.pcbi.1005411**
This paper proposes a new mathematical model for the formation of stable Rho zones, resulting from the interplay between a propagating bistable reaction -diffusion wave and advection by flow due to contractility of actomyosin on the cell cortex.

Priya, R*., Wee, K., Budnar, S., Gomez, G.A., Yap, A.S., and Michael, M. (*corresponding author). (2016) ***Coronin 1B supports RhoA signaling at cell-cell junctions through Myosin II*. Cell Cycle, 1-9. DOI: 10.1080/15384101.2016.1234549**
This paper identifies that Coronin 1B attunes Rho signalling by stabilizing junction Myosin II, and contributes to the growing notion that cytoskeleton regulators can have profound impact on Rho-signalling pathways.

Priya, R., Gomez, G.A., Budnar, S., Verma, S., Cox, H.L., Hamilton, N.A., and Yap, A.S. (2015). ***Feedback regulation through myosin II confers robustness on RhoA signalling at E-cadherin junctions*. Nat Cell Biol 17, 1282-1293. Highlighted in Nature Reviews Molecular Cell Biology 16, 578–579. DOI: 10.1038/ncb3239**
Combining predictive theory and experiments, these findings reveal a novel signalling apparatus (mediated by Myosin II) that ensures the fidelity of Rho zone at epithelial cadherin junctions and buffers it against stochastic biological noise, thus sustaining epithelial homeostasis.



Name	PETER RATCLIFFE	
Position	Senior Group Leader Director of Clinical Research (50% FTE)	
Year joined *(Crick or founder institute)*	2016	

Lab Name	***Hypoxia Biology Laboratory***

Research programme and achievements

The laboratory's principal achievement prior to the quinquennium was the discovery and elucidation of oxygen sensing mechanisms that underpin adaptive responses to low oxygen (hypoxia) in human and animal cells. The process, oxygenase-catalysed post-translational hydroxylation of specific amino acid residues in the hypoxia inducible transcription factor HIF, was unprecedented as a signalling mechanism. Our work has since revealed (i) that oxygenase-catalysed post-translation oxidations, of different types, are not (as had previously been supposed) rare on human intracellular proteins and (ii) that in addition to the HIF system, at least one of these modifications (N-Cysteine di-oxygenation) has a signalling role in oxygen homeostasis alongside the HIF hydroxylase system.

In addition to defining the source(s) of oxygen sensitive signals, we have extensively studied the downstream pathways delivering the physiological outputs of the HIF system. This work includes pan-genomic assays of the direct HIF transcriptional response using chromatin immunoprecipitation and related assays, and characterisation of integrated physiological responses to hypoxic stress in transgenic or recombinant mice. It also includes the testing of the therapeutic potential of pharmacological modulators, both *in vitro*, and *in vivo*.

Future Research
Ongoing and planned research builds on this work in two interrelated ways.

Novel sensors and pathways for oxygen homeostasis
Based on the absolute requirement to maintain oxygen homeostasis over different time-scales, and in tissues operating over widely different ranges of oxygen tensions, we hypothesise that there must be multiple systems in addition to the HIF hydroxylase pathway operating in a co-ordinated fashion to deliver the precision implicit in physiological oxygen homeostasis. We aim to identify novel oxygen-sensitive enzyme-substrate couples and explore their physiological signalling output and therapeutic tractability.

In outline, we are operating two strategies in parallel:
(i) a candidate enzyme-led strategy, focusing on oxygenases that have high candidacy as oxygen sensors, aiming to define their kinetics and substrate repertoire(s) accurately.
(ii) a phenotype-led strategy, exploring pan-genomic and pan-proteomic technologies to display candidate oxidative modifications of proteins and nucleic acids at massive scale. Our intention is to use this output as a "sieve" to define those modifications which are highly sensitive to oxygen levels.

The overall aim is to link the enzyme-led and phenotype-led approach to define key oxygen sensing enzyme-substrate couples.

Since hypoxia can be applied in a quasi-physiological graded manner across different *in vitro*, in *cellulo* and intact organismal systems we also hope to gain insights into a number of conceptual problems in signalling including range-finding, intracellular localisation of signals, and the problems of reconciling interaction with accuracy.

Implications of the oncogenic switching of massive interconnected hypoxia signalling pathway in cancer

Hypoxia signalling pathways are often pathologically activated in cancer, either genetically or by micro-environmental hypoxia. Based on known physiology, and genetic evidence, we have proposed that activation of such pathways entrains multiple anti-tumorigenic as well as pro-tumorigenic actions and that it is the balance of these effects that constrains tumour development.

Under such a model we propose that cancer cells are under selective pressure to modulate or extinguish the anti-tumorigenic properties of hypoxia pathway activation and that this process of “accommodation” drives cancer phenotypes, including those associated with resistance to therapy.

To study these processes it is necessary to track cancer cells accurately from the point of initiating oncogenic mutation. Unfortunately this is not possible in human tumours where history must be inferred from existing cells. Therefore, we have constructed a mouse in which a molecular marker is activated as an obligate consequence of tumour suppressor inactivation. Unlike lineage marking technology, this employs a single allele so that marker gene inactivation can be exactly linked to tumour suppressor inactivation and followed in real-time. We are applying this system to the study of tissue-specific tumour formation, for instance following activation of hypoxia signalling pathways following inactivation of the von Hippel-Lindau tumour suppressor (VHL). We aim to compare and contrast all the events that follow VHL inactivation, in order to better understand how selective pressures are accommodated in cancer-prone as opposed to cancer-resistant tissues. We anticipate that the work should have general relevance to other oncogenic pathways whose “switching” in cancer may likewise alter extensive interconnected pathways, with both anti-tumorigenic as well as pro-tumorigenic potential.

Research outputs

Masson N, Keeley TP, Giuntoli B, White MD, Puerta ML, Perata P, Flashman E, Licausi F, Ratcliffe PJ. (2019) *Conserved N-terminal cysteine dioxygenases transduce responses to hypoxia in animals and plants.* Science 365, 65-69. DOI: 10.1126/science.aaw011

The work identifies cysteamine (2-aminoethanethiol) dioxygenase (ADO) as a high K_mO_2 N-terminal cysteine dioxygenase that transduces cellular response to hypoxia in animals and humans, including the regulation of G-protein signalling by the N-degron pathway. ADO catalyses an identical post-translational modification to the plant cysteine oxidases, defining a mechanism of oxygen sensing that is conserved across multicellular eukaryotes.

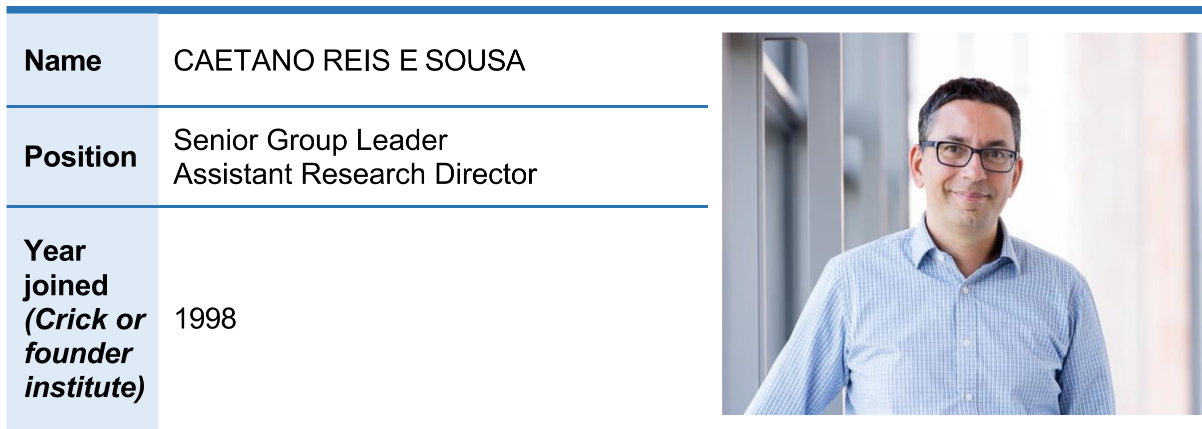
Yamamoto A, Hester J, Macklin PS, Kawai K, Uchiyama M, Biggs D, Bishop T, Bull, K, Cheng X, Cawthorne E, Crockford TL, Davies B, Dow LE, Goldin R, Coleman ML, Kranc K, Kudo H, Lawson H, McAuliffe J, Milward K, Scudamore CL, Soilleux E, Issa F, Ratcliffe PJ, Pugh CW. (2019) *Systemic silencing of PHD2 causes reversible immune regulatory dysfunction*. J Clin Invest 130, pii. 124099. DOI:

10.1172/JCI124099.
The work describes a binary tetracyclin inducible shRNA transgenic system for genetic intervention on the “oxygen sensing” HIF-prolyl hydroxylase enzymes. It demonstrates that systemic silencing of these enzymes has the potential to create an aggressive lymphoproliferative disorder due to abnormal T-cell regulatory function. The work complements cell-specific genetic interventions in the immune system in demonstrating an interface between hypoxia signalling and immune regulation, and highlights the importance of considering this as human therapeutics are developed.

Cockman ME, Lippl K, Tian YM, Pegg HB, Figg WD, Abboud MI, Heilig R, Fischer R, Myllyharju J, Schofield CJ, Ratcliffe PJ. (2019) ***Lack of activity of recombinant HIF prolyl hydroxylases (PHDs) on reported non-HIF substrates*****. eLife 8:e46490. DOI: 10.7554/eLife.46490**
One of the most important current uncertainties in the field of hypoxia biology is whether the “oxygen sensing” prolyl hydroxylases have targets beyond the established Hypoxia Inducible Factor (HIF) system. This paper describes a series of assays using mass spectrometry and radio-chemical detection of hydroxyproline which challenge an accruing body of high-profile literature purporting to describe non-HIF targets of these enzymes. It lays out standards for the assignment of enzyme dependent protein oxidations.

Schmid V, Lafleur VN, Lombardi O, Li R, Salama R, Colli L, Choudhry H, Chanock S, Ratcliffe PJ, Mole DR. (2019) ***Co-incidence of RCC-susceptibility polymorphisms with HIF cis-acting sequences supports a pathway tuning model of cancer*****. Sci Rep 9, 18768. DOI: 10.1038/s41598-019-55098-7**
The work describes highly significant co-incidence of GWAS associated human susceptibility polymorphisms for renal cancer and pan-genomic assays of regulatory elements that transduce the HIF transcriptional cascade. It argues str ongly for a new model of cancer development whereby major switches in oncogenic pathways are initially restrained by the extent and complexity of the downstream pathways and require accommodating modulation to occur for progression to cancer.

Cheng X, Prange-Barczynska M, Fielding JW, Zhang M, Burrell AL, Lima JD, Eckardt L, Argles I, Pugh CW, Buckler KJ, Robbins PA, Hodson EJ, Bruick RK, Collinson LM, Rastinejad F, Bishop T, Ratcliffe PJ. (2020) ***Marked and rapid effects of pharmacological HIF-2α antagonism on hypoxic ventilatory control*****. J Clin Invest 130(5):2237-2251. DOI: 10.1172/JCI133194.**
The paper establishes isoform specificity of the action of Hypoxia Inducible Factors in specific physiological control mechanisms; specifically, the non-redundant role of HIF2 In ventilatory acclimatisation to sustained hypoxia. It also establishes that pharmaceutical antagonism of HIF2 using agents which are undergoing trials in clinical renal cancer have the ability to disrupt normal human ventilatory control.



Name	CAETANO REIS E SOUSA
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	1998

Lab Name	***Immunobiology Laboratory***

Research programme and achievements

The Immunobiology Laboratory studies receptors and signalling pathways that mobilise dendritic cells (DC) and other cell types in response to infection, injury or cancer. In a related programme, the laboratory studies DC heterogeneity and ontogeny.

In the last five years, the Immunobiology Laboratory continued earlier work on cell-intrinsic immunity to RNA viruses to show that antiviral RNA interference, an ancestral form of antiviral defence, is conserved in mammals but masked by the effects of interferons, cytokines elicited by the innate immune response to the virus. In a distinct line of investigation, the lab continued its studies of the Syk-coupled C-type lectin receptor, DNGR-1. This receptor was previously found by the lab to be used by the cDC1 subset of DCs to detect F-actin exposed by cells undergoing necrosis, which suggested that extracellular recognition of cytoskeletal components is an evolutionarily ancient means of detecting tissue damage. Consistent with that notion, the lab found that extracellular -actinin, an actin-binding protein, elicits a response in *Drosophila melanogaster* akin to tissue injury. To further understand the role of extracellular actin recognition, the lab solved the structure of the DNGR-1/F-actin complex and went on to show that the association of F-actin with myosin II greatly potentiates DNGR-1 triggering through ligand cross-linking. The lab then validated the importance of F-actin detection and a pH-induced conformational change in DNGR-1 allowing cDC1s to extract antigens from cell corpses for presentation to $CD8^+$ T cells, a process termed cross-presentation. More recently, the lab discovered the cell biological basis for DNGR-1-dependent cross-presentation by finding that the receptor signals via Syk to induce rupture of phagosomes that contain internalised dead cell debris. This allows wholesale access of antigens associated with cell corpses to the endogenous MHC class I presentation pathway of cDC1s.

Cross-presentation can contribute to immunity to cytopathic viruses and to cancer. In the context of the latter, the lab found that DC activation and immune control can be subverted by prostaglandin E_2 produced by tumour cells. Prostaglandin E_2 also suppresses accumulation of cDC1s in tumours, which led the lab to discover that cDC1 infiltration of cancers is driven by chemokines produced by intratumoural innate lymphocytes such as NK cells.

Independently of its presence and function in differentiated cDC1s, DNGR-1 is also expressed by mouse DC precursors and, in earlier work from the lab, was used to fate-map those cells *in vivo*. In an extension of that work, the lab more recently described the clonal organisation of DCs in tissues and characterised an infection-driven acute recruitment to affected tissues of DC precursors originating from bone marrow. In parallel work, the lab characterised the ontogeny of mouse and human DCs using both *in vitro* cultures and humanised mice.

Overall, the studies of the Immunobiology Laboratory over the last quinquennium have helped build a global picture of the cells, receptors and signalling pathways that regulate immunity with applications in immunotherapy of cancer and infectious diseases. Future plans follow on directly from those of the past quinquennia. Part of the focus will be innate immune recognition of dead cells and its impact on inflammation, infection and cancer. Another focus will be the development and function of DCs, including how DC progenitors seed tissues and to what extent the latter process is regulated by demand.

Research outputs

Canton, J. et al. (2021) *The receptor DNGR-1 signals for phagosomal rupture to promote cross-presentation of dead-cell-associated antigens*. Nat Immunol 264, 1–14. DOI: 10.1038/s41590-020-00824-x
Immune cells such as type 1 conventional dendritic cells (cDC1) can “eat” (phagocytose) dead tumour or virally-infected cells and present associated antigens to CD8+ T cells to elicit a tumour- or virus-specific cytotoxic T cell response. How antigens from the debris get presented on MHC class I (MHC-I) molecules on cDC1 has long been puzzling as MHC-I normally presents antigens found in the cytosol. Canton et al show that cDC1 use the DNGR-1 receptor to induce phagosomal rupture, releasing the debris-associated antigens into the cytosol. These findings have implications for our understanding and manipulation of immunity to infection and cancer.

Cabeza-Cabrerizo, M., J. van Blijswijk, S. Wienert, D. Heim, R.P. Jenkins, P. Chakravarty, N. Rogers, B. Frederico, S. Acton, E. Beerling, J. van Rheenen, H. Clevers, B.U. Schraml, M. Bajénoff, M. Gerner, R.N. Germain, E. Sahai, F. Klauschen and C. Reis e Sousa. (2019) *Tissue clonality of dendritic cell subsets and emergency DCpoiesis revealed by multicolor fate mapping of DC progenitors*. Sci Immunol 4 pii:eaaw1941. DOI: 10.1126/sciimmunol.aaw1941
Conventional dendritic cells (cDCs) originate from a committed precursor in bone marrow (BM) that exits via the blood as a pre-cDC to seed tissues with the cDC1 and cDC2 subsets. We used a multi-colour genetic tracing mouse model to analyse colonisation of tissues by pre-cDC. We found that cDCs in tissues comprise clones mostly composed of a single cDC subset and that ‘flu infection causes an efflux of pre-cDCs from BM and influx into the lungs. The latter finding indicates that cDCpoiesis is responsive to emergency need, which suggests previously undiscovered communication between tissues and cDC progenitors in BM.

Böttcher, J.P., E. Bonavita, P. Chakravarty, H. Blees, M. Cabeza-Cabrerizo, S. Sammicheli, N.C. Rogers, E. Sahai, S. Zelenay and C. Reis e Sousa. (2018) *NK cells*

***stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control*. Cell 172:1022 - 1037. DOI: 10.1016/j.cell.2018.01.004**

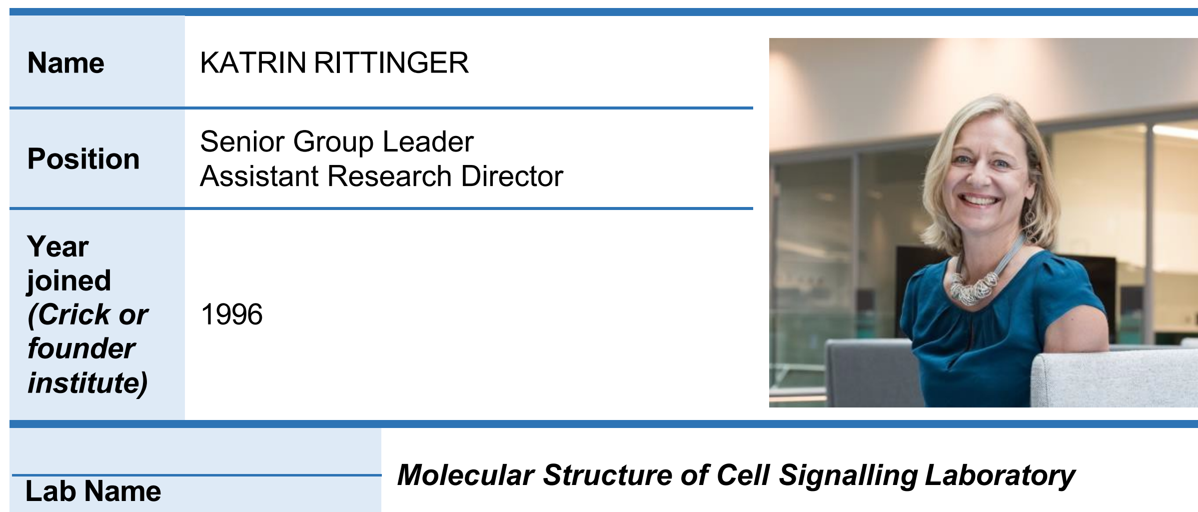
This work follows on from a paper that we published in 2015 (see below). In the present paper, we showed that cDC1 recruitment and infiltration in several mouse tumour models depends on the chemokines CCL5 and XCL1 produced by NK cells. In human cancers, CCL5/XCL chemokine transcripts correlate with gene signatures for NK cells and cDC1 and predict overall survival in melanoma, head and neck cancer, breast cancer and lung adenocarcinoma. Therefore, our data uncovered a mechanism for cDC1 recruitment into tumours that is translatable to humans and cancer patient survival.

Zelenay, S., A.G. van der Veen, J.P. Böttcher, K.J. Snelgrove, N. Rogers, S.E. Acton, P. Chakravarty, M.R. Girotti, R. Marais, S.A. Quezada, E. Sahai, C. Reis e Sousa. (2015) *Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity*. Cell 162:1257-1270. DOI: 10.1016/j.cell.2015.08.015

In this paper, we uncovered a potent mechanism of cancer immune evasion, namely cyclooxygenase (COX)-dependent secretion of prostaglandin E_2 (PGE_2) by tumour cells. We further showed that the growth of PGE_2-secreting tumours in mice can be reversed by a combination of checkpoint blockade immunotherapy and COXinhibitors, suggesting that COX inhibition might be a useful addition to both conventional and immune-based therapy of cancer. This paper led to seven clinical trials worldwide to test combinations of prostaglandin E_2 inhibition with checkpoint blockade cancer therapies and resulted in the attribution of the inaugural Bial Prize in Biomedicine 2019 to the authors.

Hanč, P., Fujii, T., Iborra, S., Yamada, Y., Huotari, J., Schulz, O., Ahrens, S., Kjær, S., Way, M., Sancho, D., Namba, K., C. Reis e Sousa. (2015) *Immune recognition of cell death revealed by the structure of DNGR-1 in complex with F-actin*. Immunity 42:839-849. DOI: 10.1016/j.immuni.2015.04.009

We previously identified DNGR-1 (CLEC9A) as a DC receptor that recognises exposed F-actin and allows for immune responses to dead cells. In this paper, we solved the cryo-EM structure of DNGR-1 bound to F-actin. We identified the key residues involved in binding and show that the latter depends greatly on multimeric interactions that increase receptor avidity. Notably, the binding site was revealed to be a composite of three distinct actin filament subunits explaining why the receptor can only bind to the filamentous form and not to G-actin.



Name	KATRIN RITTINGER
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	1996

Lab Name	***Molecular Structure of Cell Signalling Laboratory***

Research programme and achievements

Protein ubiquitination is a highly versatile post-translational modification that is used in the regulation of virtually all cellular processes, from the maintenance of protein homeostasis to DNA damage responses, cell cycle regulation and immune signalling. E3 ubiquitin ligases, the family of enzymes catalysing the last step of the three-step ubiquitination cascade, select the substrate to be modified, and in some cases also determine the topology of the polyubiquitin chain formed, and thus the physiological outcome. Alterations in the ubiquitin system have been linked to many diseases, including autoimmune and inflammatory disorders, cancer and neurodegenerative diseases. Hence there is immense interest in developing therapeutics that target the ubiquitin system. However, progress towards this endeavour has been slow, and there is a clear need for a more detailed understanding of the structures, molecular mechanisms and regulatory elements of the enzymes involved to enable a more successful drug discovery process.

Research in my group is aimed at providing a molecular understanding of the mechanisms underlying the activity and regulation of enzymes of the ubiquitination cascade, especially E3 ubiquitin ligases, thereby contributing to efforts to explore the ubiquitin system as a drug target. We are employing a broad experimental approach to characterise the function of these E3 ligases on a structural and mechanistic level and collaborate with cell biologists and immunologists to test our working models in a biological context, and with chemists to develop chemical tools to specifically target ubiquitination enzymes.

During this quinquennium we have made important progress towards understanding the mechanism and activity of members of the TRIM family of E3 ligases, many of which regulate innate immune responses. Self-association into higher order assemblies is a key functional property that directs the catalytic activity of this protein family and our work has provided important new insight into the link between oligomeric state and ubiquitination activity. Furthermore, our studies have provided a molecular explanation for the mechanism used by an influenza protein to interfere with TRIM25 function and thereby suppress a host immune response.

In parallel, we have been studying the mechanism of ubiquitin transfer employed by RBR ligases, an E3 subfamily with a catalytic module comprising three subdomains separated by flexible linkers. These subdomains have to work in a coordinated manner to recognise the ubiquitin-loaded E2 and transfer ubiquitin onto a catalytic cysteine within the E3, before its final transfer onto the substrate. Combining structural, biophysical and biochemical techniques we have provided important insight into the mechanism regulating the activity of RBR ligases, especially of the multi—protein E3 LUBAC which is a key regulator of immune and inflammatory signalling, and apoptosis.

We will extend our work on TRIM ligases to cover members from each of the 11 sub-classes of this protein family to identify possible common mechanistic principles that guide the activity of this family. In parallel, we will explore the biological functions of specific subgroups to gain insight into how their E3 ligase activity relates to other biological processes that have been described for specific family members.

At present, no inhibitors or chemical probes specific for TRIM family members exist. We will use chemical biology approaches including fragment-based covalent ligand screening to test if the substrate binding domains of TRIMs could be targeted for chemical probe development. Similarly, in collaboration with GSK/LinkLabs we are extending and improving our fragment-based covalent ligand library to target other E3 ligases containing catalytic cysteine residues, including the RBR ligases discussed above, and bacterial E3 ligases of the NEL family, which are secreted effector proteins. To

develop this technology further and allow screening of covalent libraries in a cellular context without the need to purify individual proteins, we have extended our LinkLabs collaboration to include Bram Snijders from the Proteomics STP to develop fast and high-throughput chemoproteomic pipelines.

In addition to the work on protein ubiquitination, we have established a collaboration with Teresa Thurston from Imperial College London, to complement the cellular work from her group on *Salmonella* effector proteins with structural and mechanistic studies, in order to provide a detailed understanding of how bacterial effector proteins manipulate innate immune signalling.

Research outputs

Tsai YI, Johansson H, Dixon D, Martin S, Chung CW, Clarkson J, House D, Rittinger K. (2020) *Single domain antibodies as crystallization chaperones to enable structure-based inhibitor development for RBR E3 ubiquitin ligases*. Cell Chem Biol. 27(1):83-93.e9. DOI: 10.1016/j.chembiol.2019.11.007
In collaboration with GSK and the Crick-GSK LInkLabs we selected single-domain antibodies (dAbs) based on a human scaffold that recognise the catalytic domain of HOIP, a subunit of the multi-component E3 ligase LUBAC. We used these dAbs to interrogate the ubiquitin transfer mechanism of HOIP, and as crystallisation chaperones to crystallise a HOIP RBR/dAb complex. This complex now serves as a robust platform for soaking of ligands that target the active site cysteine of HOIP, thereby providing easy access to structure-based ligand design for this important class of E3 ligases.

Johansson H, Isabella Tsai YC, Fantom K, Chung CW, Kumper S, Martino L, Thomas DA, Eberl HC, Muelbaier M, House D, Rittinger K (2019) *Fragment-Based Covalent Ligand Screening Enables Rapid Discovery of Inhibitors for the RBR E3 Ubiquitin Ligase HOIP*. J Am Chem Soc 141: 2703-2712. DOI: 10.1021/jacs.8b13193
Protein ubiquitination is a key regulatory mechanism and E3 ubiquitin ligases are the key mediators of ubiquitination providing specificity to the process. In this study we describe the application of fragment-based covalent ligand screening to target the active site of an E3 ubiquitin ligase (HOIP) for which previously no specific inhibitors were known. Combining chemical biology, X-ray crystallography, chemoproteomics and cell biology we were able to identify a covalent binder for HOIP that now forms the basis for further inhibitor development. This study illustrates more generally the power of fragment-based covalent ligand screening to identify lead compounds against challenging targets.

Koliopoulos MG, Lethier M, van der Veen AG, Haubrich K, Hennig J, Kowalinski E, Stevens RV, Martin SR, Reis ESC, Cusack S, Rittinger K (2018) *Molecular mechanism of influenza A NS1-mediated TRIM25 recognition and inhibition*. Nat

Commun 9: 1820. 10.1038/s41467-018-04214-8

Modification with K63-linked poly-ubiquitin chains of RIG-I, a viral RNA sensor that induces type I IFN production in response to viral infection, is crucial for activation of the RIG-I/MAVS signalling pathway. These chains are synthesised by the E3 ubiquitin ligase TRIM25, which is targeted by influenza A virus non-structural protein 1 (NS1) to prevent an efficient host immune response. In this study we provide molecular insight into the mechanism by which NS1 interferes with the correct positioning of the substrate-binding PRYSPRY domain of TRIM25 to suppresses RIG-I ubiquitination and hence downstream signalling.

Esposito D, Gunster RA, Martino L, El Omari K, Wagner A, Thurston TLM, Rittinger K (2018) *Structural basis for the glycosyltransferase activity of the Salmonella effector SseK3*. J Biol Chem 293: 5064-5078. DOI: 10.1074/jbc.RA118.001796
A key part of *Salmonella* pathogenesis is the delivery of virulence proteins (effectors) into the host cell to interfere with host immune responses. SseK3 is a glycosyltransferase that transfers an *N*-acetylglucosamine (GlcNAc) moiety onto a target arginine of host proteins, thereby modulating host cell function. In this study we describe the first detailed structural and functional characterisation of SseK3, which provided important insight into its enzymatic mechanism and substrate selection.

Koliopoulos MG, Esposito D, Christodoulou E, Taylor IA, Rittinger K (2016) *Functional role of TRIM E3 ligase oligomerization and regulation of catalytic activity*. *EMBO J* 35: 1204-18. DOI: 10.15252/embj.201593741
This study presents the first quantitative analysis of the link between the oligomeric state and catalytic activity of TRIM ligases, an important class of E3 ligases that play crucial roles in the regulation of innate immune repsonses, amongst other cellular functions. Our detailed structural and biochemical analysis allowed us to propose models for the architecture of TRIM25 and TRIM32 that differ in the manner in which dimerisation of the catalytic RING domain, which is absolutely required for activity, is achieved. This study now forms the basis for further work interrogating the function of this protein family.

Name	SAMUEL RODRIQUES
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2021

Lab Name	***Applied Biotechnology Laboratory***

Research programme and achievements

Brain disorders affect nearly 15% of the population, and yet the vast majority of new neurotechnologies never make it out of the lab. We develop first-in-class technologies that can be deployed widely to accelerate neuroscience and reduce the number of people suffering from brain disorders. To do so, we combine our core technical competency in neuroengineering with an expertise in biotechnology entrepreneurship that is unique among academic labs, allowing us to identify more important unmet needs and deploy solutions more rapidly.

Our first projects address major unmet needs in drug delivery, diagnostics, and multiomics. We are developing a new generation of viral vectors to allow gene therapies to be delivered to the human brain without brain surgery, to enable a new generation of cures for autism, epilepsy, and neurodegeneration. We are developing a new platform technology for mapping the cell-type and circuit organization of the brain at the single-cell level, so that we can identify and understand subtypes of schizophrenia and depression and develop more targeted therapies. Finally, we are building a medical device that will allow for rapid and minimally invasive sampling of the cerebrospinal fluid, with the goal of enabling everyone to get an annual, risk-free liquid biopsy for Alzheimer's disease.

Research outputs

S.G. Rodriques*, L.M. Chen*, S. Liu, E.D. Zhong, J.R. Scherrer, E.S. Boyden, F. Chen**. (2021) *RNA Timestamps identify the age of single molecules in RNA sequencing*. Nature Biotechnology 39, 320-325. DOI: 10.1038/s41587-020-0704-z**
We invented a method for inferring the age of individual RNAs from a single endpoint sequencing experiment. By aggregating over many RNAs, we were able to infer when promoters were active in the history of a cell, with single-hour time resolution.

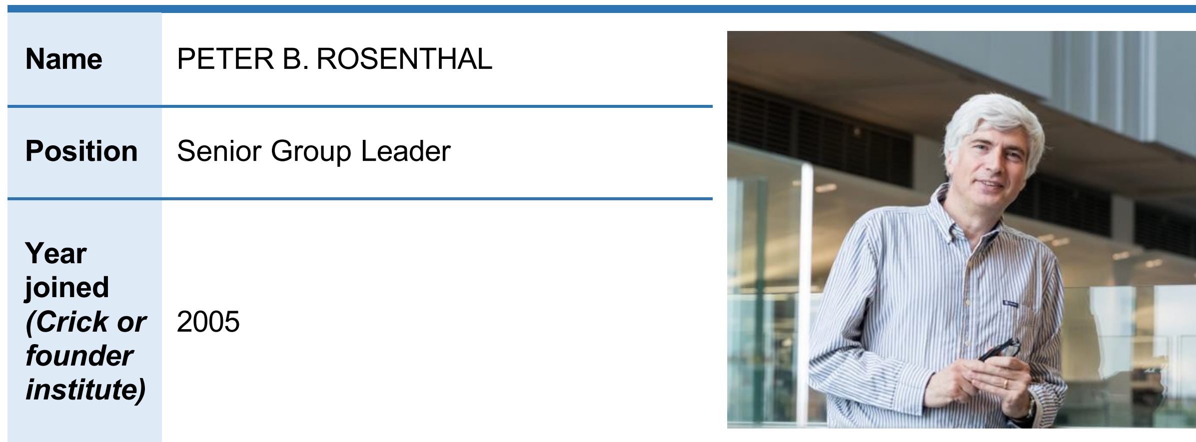
S.G. Rodriques*, R.R. Stickels*, et al. (*Co-first authors). (2019) *Slide-seq: A scalable technology for measuring genome-wide expression with high spatial resolution*. Science, 363(6434): 1463 – 1467. DOI: 10.1126/science.aaw1219

We invented the first spatial transcriptomic technology to provide transcriptome-wide gene expression information with single-cell spatial resolution. This method has now been requested by more than 100 laboratories.

S.G. Rodriques, A.H. Marblestone, E.S. Boyden. (2019) *A theoretical analysis of single molecule protein sequencing via weak binding spectra*. PLoS One, 14(3):e0212868. DOI: 10.1371/journal.pone.0212868
We laid out an approach for inferring the identity of N-terminal amino acids by observing binding and unbinding events of weak and unspecific N-terminal amino acid binders (NAABs). Designing highly specific NAABs is a major bottleneck for protein sequencing which can be overcome using our method, and a company is using our approach.

D. Oran*, S.G. Rodriques*, R. Gao, S. Asano, M. Skylar-Scott, F. Chen, P. Tillberg, A.H. Marblestone, E.S.Boyden** (*Co-first authors, **co-last authors). (2018) *3D nanofabrication by volumetric deposition and controlled shrinkage of patterned scaffolds*. Science, 362(6420): 1281-1285. DOI: 10.1126/science.aau5119**
We invented a new 3D nanofabrication technology that functions by nanofabricating patterns of material inside a hydrogel using diffraction-limited optics, and then shrinking the hydrogel isotropically to obtain 3D nanostructures with feature sizes down to ~30nm. Because the hydrogel serves as a scaffold for the nanostructures, this method can be used to create arbitrary geometries of conductive metals, which is useful for designing antennas that cannot be fabricated with any other process.

S.G. Rodriques*, A.H. Marblestone*, J. Scholvin, J. Dapello, M. Mankin, D. Sarkar, R. Gao, L. Wood, and E. Boyden (*Co-first authors). (2016) *Multiplexed neural recording along a single optical fiber via optical reflectometry*. J.Biomed.Opt. 21(5):57003. DOI: 10.1117/1.jbo.21.5.057003
We proposed a neural recording probe based on detection of the free carrier dispersion effect in an semiconductor waveguide using optical reflectometry. The probe could achieve readout of >1000 independent neural recording sites with a 20um x 20um cross-section. However, the material requirements are currently beyond widely available fabrication capabilities.



Name	PETER B. ROSENTHAL
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2005

Lab Name	***Structural Biology of Cells and Viruses Laboratory***

Research programme and achievements

Our research applies electron cryomicroscopy to study high-resolution biological structure from the molecular to the cellular scale. Our main focus is the structural study of lipid-enveloped viruses in order to understand key steps in virus infection. We study the structural organisation of influenza virus to understand how protein and membrane interactions drive virus assembly. Influenza virus enters cells by binding cell surface receptors and, following receptor-mediated endocytosis, fuses with the endosome membrane. We image dynamic structural changes in the hemagglutinin glycoprotein (HA) and ultrastructural changes in the virus as a whole to understand the mechanism of HA-mediated membrane fusion. We are also interested in related, integrated studies of retrovirus structure. We use electron cryotomography to image high-resolution cell architecture as well as structural information on protein complexes *in situ*. In this area, our main focus is understanding how von Willebrand factor, a glycoprotein with roles in haemostasis, inflammation, and thrombosis, is packaged in storage granules (Weibel-Palade bodies) for acute exocytic release following vascular injury. We also develop experimental and computational methods to improve structure determination by cryomicroscopy in order to extend the range of its application in biology. We lead a UK consortium to develop methods for cryo-EM structure validation.

The main achievements of the laboratory since 2015 are as follows:

- Imaging the fusion of influenza virus with liposome targets at low pH by electron cryotomography revealing structural intermediates of HA and their interaction with membranes.

- Single particle cryomicroscopy resolved changes of the influenza HA glycoprotein at the low pH of membrane fusion, identifying structural intermediates including a dilated arrangement of the membrane distal domains and a 150 Å long extended coiled-coil intermediate at high-resolution.

- Structure of full-length HA solubilised in detergent by single particle cryomicroscopy revealing the structure of the transmembrane region which plays roles in assembly and membrane fusion.

- First structure of a capsid assembly from an endogenous retrovirus (HML2), the first atomic resolution structure of any retroviral shell, showing principles by which a single capsid protein can form closed assemblies consisting of pentamers and hexamers.

- Electron cryotomography of Weibel-Palade bodies, storage granules for von Willebrand factor, in intact endothelial cells identified novel CD63-containing intraluminal vesicles that are released as an exosome during Weibel-Plade body exocytosis.

In our future work, (1) we plan to extend our understanding of the mechanism of HA- mediated membrane fusion by higher resolution studies of influenza HA intermediates to understand their interaction with membranes and to directly image viral membrane fusion in cells (2) we will determine the integrated structure of foamy viruses, a subfamily of *Retroviridae*, which infect a wide range of mammalian hosts. We will perform cryotomography of prototype foamy virus and sub-tomogram averaging of the surface glycoprotein and capsid cores to high-resolution (3) we will extend structural studies of cellular architecture including mammalian filopodia and the structure of von Willebrand factor in Weibel-Palade bodies by cryotomography and sub-tomogram averaging and (4) we will develop new methods for validating cryo-EM maps obtained by single particle analysis and sub-tomogram averaging which are now urgently required following advances in cryomicroscopy.

Research outputs

Benton, DJ, Gamblin, SJ, Rosenthal, PB, and Skehel, JJ. (2020) *Structural transitions in influenza haemagglutinin at membrane fusion pH*. Nature, 583: 150-153. DOI: 10.1038/s41586-020-2333-6

The influenza HA is one of two glycoproteins on the surface of influenza virus and mediates receptor binding and membrane fusion during viral entry. Here, we directly image structural transformations in the HA at the pH of membrane fusion and solve the structure of three structural intermediates including a 150 Å-long triple-helical coiled coil of the HA2 transmembrane subunit. This was a long sought-after result but showed new, surprising concerted conformational rearrangements important to the membrane fusion mechanism. This extended to atomic resolution our application of cryo-EM to study the dynamic process of membrane fusion.

Acton, O, Grant T, Nicastro G, Ball NJ, Goldstone DC, Robertson LE, Sader K, Nans A, Ramos A, Stoye JP, Taylor IA and Rosenthal PB (2019). *Structural basis for Fullerene geometry in a human endogenous retrovirus capsid*. Nature Communications, 10(1). DOI: 10.1038/s41467-019-13786-y

Here we determined the structure by single particle cryo-EM of capsid assembly in an endogenous retrovirus. This is the first atomic resolution structure of a closed capsid shell, which in retroviruses packages and protects the genome. By studying 4 different types of symmetric assemblies, we discovered how the underlying Fullerene geometry is achieved by the CA protein forming both pentamers and hexamers and found structural rules by which invariant pentamers and structurally plastic hexamers associate to form the unique polyhedral structures.

Streetley, J, Fonseca, A-V, Turner, J, Kiskin, NI, Knipe, L, Rosenthal, PB and Carter, T. (2019) *Stimulated release of intraluminal vesicles from Weibel-Palade bodies*. Blood, 133(25), 2707-2717. DOI: 10.1182/blood-2018-09-874552

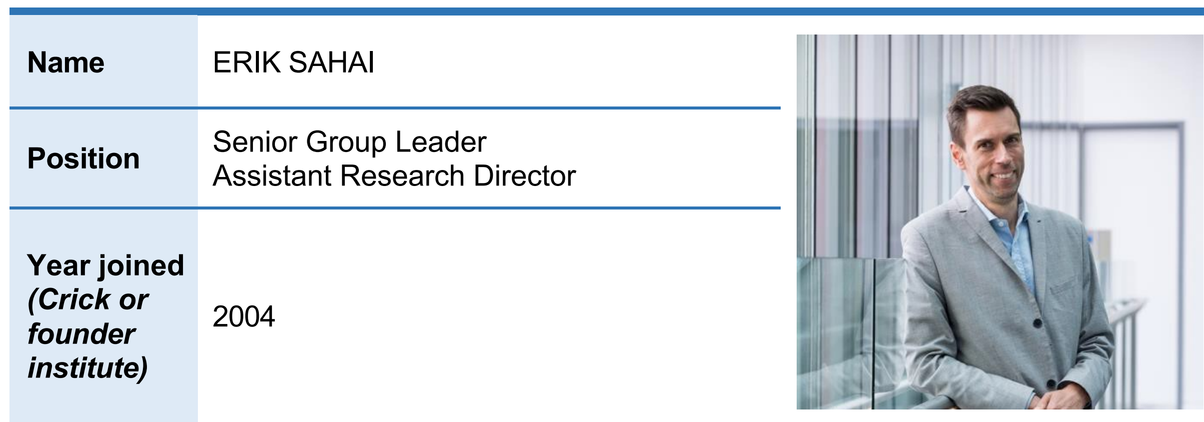
Weibel-Palade bodies (WPBs) are secretory granules that contain von Willebrand factor and P-selectin, molecules that regulate haemostasis and inflammation. We used electron cryo-tomography of frozen-hydrated endothelial cells to image the structure of WPBs and VWF in the context of well-preserved architecture and identified internal vesicles as novel structural features of the WPB lumen. By live-cell fluorescence microscopy, we directly observe the exocytotic release of EGFP-CD63 intralumenal vesicles (ILVs) during WPB exocytosis, describing a novel route for release of ILVs during endothelial cell stimulation. Thematically, this reflects our interest in linking molecular and cellular pictures of cell structure and function.

Benton, DJ, Nans A, Calder LJ, Turner J, Neu U, Lin YP, Ketelaars E, Kallewaard NL, Corti D, Lanzavecchia A, Gamblin SJ, Rosenthal PB and Skehel JJ (2018). ***Influenza hemagglutinin membrane anchor*****. Proceedings of the National Academy of Sciences of the United States of America, 115(40), 10112-10117. DOI: 10.1073/pnas.1810927115**

Here we used single particle cryo-EM to solve the structure of the full-length influenza hemagglutinin (HA) in a detergent micelle showing the central triple-helical structure in the membrane as well as flexible linkers between the ectodomain and the transmembrane domain. We also study the HA in complex with a broadly neutralising monoclonal Fab that binds near the ectodomomain membrane anchor junction and restricts flexibility of the TM region. Flexibility is likely important for the structural re-arrangements that mediate membrane fusion and the Fab may neutralize in part by restricting movement of the glycoprotein.

Calder, LJ and Rosenthal, PB (2016). ***Cryomicroscopy provides structural snapshots of influenza virus membrane fusion*****. Nature Structural & Molecular Biology, 23(9), 853-858. 10.1038/nsmb.3271**

Influenza membrane fusion mediated by the hemagglutinin (HA) glycoprotein has been a paradigm for membrane fusion by enveloped viruses and also by vesicles in intracellular and neuronal contexts. This study advances our understanding of this process by imaging structural re-arrangements of the HA in the context of membrane transformations by electron cryotomography.



Name	ERIK SAHAI
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	2004
Lab Name	***Tumour Cell Biology Laboratory***

Research programme and achievements

At the beginning of the QQR period, the Sahai group was focused on investigating cancer invasion, including regulation of the actomyosin cytoskeleton, and how cancer-associated fibroblasts (CAFs) and the organisation of the extra-cellular matrix (ECM) modulate the behaviour of cancer cells. This work included discovering heterotypic cell-cell contacts between cancer cells and fibroblasts that mechanically couple the two cell types, documenting how cGAMP transfer at this interface promotes the expression of inflammatory modulators by CAFs, and how collisions between fibroblasts lead to the generation of aligned ECM. In the course of this work, we additionally discovered how CAFs modulate responses to targeted therapies, with the relationship between the tumour microenvironment and therapy responses becoming increasingly prominent in our research. We also found out how epithelial cells in the local environment of indolent micro-metastases provide support to cancer cells.

Current and future work in the group is divided into three interconnected areas: 1) Understanding how cell-cell and cell-ECM interactions spatially pattern the tumour microenvironment 2) Studying how non-genetic inter-cellular heterogeneity in cancer cells affects therapy responses and 3) Defining the rules and mechanisms of clonal competition in established tumours and how these are affected by the cell-cell interactions and spatial features of tumours. We employ an inter-disciplinary approach to tackle these problems with mathematical methods of data analysis and computational modelling becoming prominent. These are linked to both experimental models and analysis of patient tissue. We also use various forms of optical imaging to pursue our research, and work closely with the Photonics Satellite group from Imperial College.

Research outputs

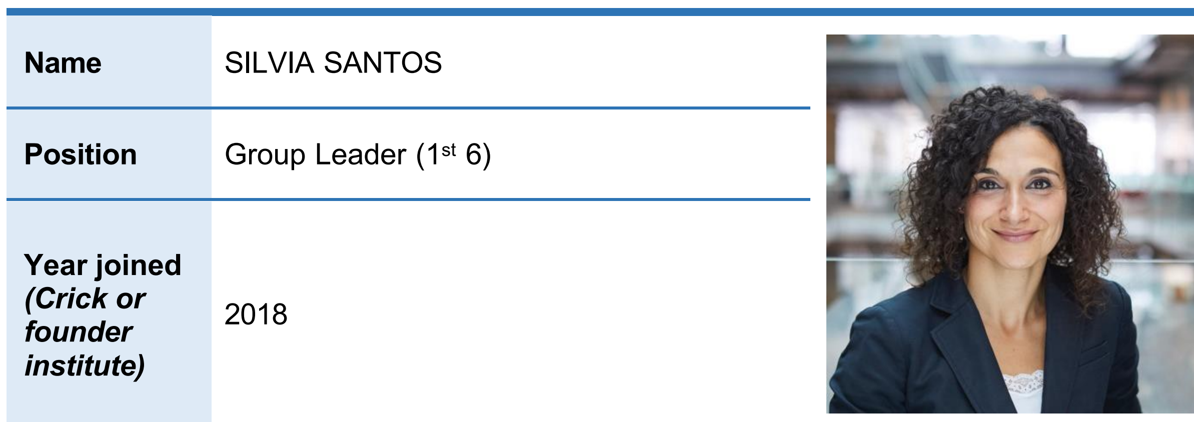
Arwert EN, Milford EL, Rullan A, Derzsi S, Hooper S, Kato T, Mansfield D, Melcher A, Harrington KJ, Sahai E. (2020) *STING and IRF3 in stromal fibroblasts enable sensing of genomic stress in cancer cells to undermine oncolytic viral therapy*. Nature Cell Biology (7):758-766. DOI: 10.1038/s41556-020-0527-7
This work shows why stromal fibroblasts are an important source of inflammatory modulators in tumours. We show fibroblasts can respond to cGAMP produced by cancer cells, but only when the two cell types are in direct contact. This in turn promotes the STING and IRF3 dependent expression of interferon beta and various chemokines. The subsequent up-regulation of interferon-stimulated gene expression undermines the efficacy of oncolytic viruses. We propose the requirement for direct contact represents a ‘tissue level’ mechanism for triggering this response specifically in the context of tissue damage, as in healthy tissue, the basement membrane precludes such interactions.

Montagner M, Bhome R, Hooper S, Chakravarty P, Qin X, Sufi J, Bhargava A, Ratcliffe CDH, Naito Y, Pocaterra A, Tape CJ and Sahai E. (2020) ***Cross-talk with lung epithelial cells regulates Sfrp2 expression enabling disseminated breast cancer cell latency.*** **Nature Cell Biology (3):289-296. DOI: 10.1038/s41556-020-0474-3** We set up a complex model for lung alveoli by co-culturing lung fibroblasts and alveolar epithelial type I and type II cells on a gas permeable support, with the expectation that the fibroblasts would strongly influence the behaviour of cancer cells introduced into the system. However, we discovered that the largest effect came from the alveolar epithelial cells, and we then used a range of approaches to delineate the signalling mechanisms involved. This work, together with a concomitant study from the Malanchi group, established the role of epithelial cells in the tumour microenvironment of indolent and micro-metastases.

Park D, Wershof E, Boeing S, Labernadie A, Jenkins RP, George S, Trepat X, Bates P and Sahai E. (2020) ***Extracellular matrix anisotropy is determined by TFAP2C-dependent regulation of cell collisions.*** **Nature Materials 19, 227-238. DOI: 10.1038/s41563-019-0504-3**
In this study, we used our bank of patient-dervied stromal fibroblasts to ask why some fibroblasts generate highly aligned extra-cellular matrices and other do not. We were able to show how cell migration and cell-cell collisions can dictate the patterns formed by fibroblasts, and that furthermore, the higher order organisation of fibroblasts and matrix is associated with millimetre scale contraction of reconstituted tissues and cancer invasion. The quantitative tool developed during the course of this work and a related study is now being tested for its prognostic value in simple histological stains of breast and prostate cancers.

Ege N, Dowbaj AM, Jiang M, Howell M, Jenkins RP*, Sahai E*. (2018) ***Quantitative Analysis Reveals that Actin and Src-family kinases regulate nuclear YAP1 and its export.*** **Cell Systems 6(6):692-708. DOI: 10.1016/j.cels.2018.05.006**
We previously demonstrated that YAP1 is critically required for the activation of stromal fibroblasts. In this paper, we showed that contrary to received wisdom, YAP1 is not stably sequestered when inactive, and its sub-cellular distribution is largely regulated at the level of nuclear export. Subsequently, Rob Jenkins (SLRS in Sahai group and co-corresponding author) has established a collaboration with the Mathematics department at the University of Nottingham to further develop the quantitative basis of this work, including the derivation of chromatin binding on and off rates from simple photobleaching experiments.

Labernadie A, Kato T, Brugués A, Serra-Picamal X, Derzsi S, Arwert E, Weston A, González-Tarragó V, Elosegui-Artola A, Alcaraz J, Roca-Cusachs P, Sahai E*, Trepat X*. (2017) ***A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion.*** **Nature Cell Biology 19(3):224-237. DOI: 10.1038/ncb3478**
Our previous work showed how stromal fibroblasts lead the collective invasion of cancer cells, and documented how remodelling of the extracellular matrix was important for this behaviour. Following our observation of direct cell-cell contacts between cancer cells and fibroblasts, we hypothesised that the two cells might be mechanically coupled; therefore, we began collaborating with Xavi Trepat (IBEC Barcelona), who is a world leader in the mechanics of multi-cellular systems. By biophysical measurements and a range of conventional cell and molecular biology manipulations, we demonstrated that fibroblasts actively ‘pull’ cancer cells into the surrounding extracellular matrix.



Name	SILVIA SANTOS
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2018

Lab Name	***Quantitative Cell Biology Laboratory***

Research programme and achievements

During cell decision-making, gene and protein networks dynamically change in response to cues in order to trigger different cellular states. How information is decoded and transmitted in order to commit to specific cell fates has been a fundamental question in cell and developmental biology. In this context, my lab aims to understand how signalling molecules are organised into circuits, and how these circuits are spatio-temporally regulated and remodelled in two important cellular decisions: cell division and cellular differentiation.

The decision to divide is a fundamental decision and the conserved networks that trigger cell division adapt and remodel in a variety of biological contexts including developmental transitions and malignancy. We have been exploring spatio-temporal control of cell division in mammalian cells (Santos SDM et al Cell 2012, Araujo et al Mol Cell 2016) and remodelling of cell cycle networks during developmental transitions, using embryonic stem cells as a model system.

Embryonic stem cells have the propensity to differentiate into the three germ layers. The switch between pluripotency and differentiation in these cells has been our paradigm of choice to understand how protein and gene networks decode cellular signals and thereby encode irreversible commitment to different cell fates (Gunne Braden et al Cell Stem Cell 2020).

Both lines of investigation have a profound impact in the understanding of normal human development, reprogramming and de-differentiation and the transition from healthy to disease states, in particular during cancer.
We use quantitative approaches combining experimental methods (based on single cell live cell imaging, genomics, proteomics and chemical biology) with mathematical modelling. Multidisciplinary approaches have revolutionised the way we ask biological questions and have been crucial to uncover regulatory principles in cell decision-making.

Major achievements
During this quinquennium we have made important scientific contributions to two different fields: developmental biology and cell cycle/chromosome biology:

a. We discovered a new class of genes, deemed *early commitment genes* (ECG), which mediate irreversible commitment to cellular differentiation in human early embryonic stem cells (Gunne-Braden et al Cell Stem Cell 2020)
b. We discovered that cells irreversibly commit to undergoing cellular differentiation unexpectedly early during early human embryonic development (Gunne-Braden et al Cell Stem Cell 2020)

c. We discovered a new role for the transcription factor GATA3 in early differentiation (Gunne-Braden et al Cell Stem Cell 2020, Sullivan et al unpublished)

d. We discovered that positive feedback in the networks that regulate the onset of mitosis allow for modularity in cell division cycles (Araujo et al Mol Cell 2016)

e. We discovered that feedback regulation and protein degradation allow for fine tuning of the period of cell division cycles in early embryonic and somatic divisions (Araujo et al under revision)

f. We discovered that a maternal factor, FGF2, is essential during early mouse development for maintaining correct cell number and proportions of specific lineages (Gharibi et al under revision and Gharibi et al unpublished)

g. We discovered that CDK hyperactivation and spatial control are essential for cell cycle remodeling during the embryonic to somatic transition during cellular differentiation (Padgett and Santos Febs Letters 2020, Gharibi et al unpublished and Padgett et al unpublished)

h. We have developed several genome edited human embryonic stem cell lines expressing biosensors, crucial to perform live cell imaging experiments and dynamic perturbations.

i. We have developed artificial intelligence (AI) based image analysis methods for quantification of live cell imaging experiments

j. We have developed and optimised 2D and 3D models to study early development in the lab. These methodologies are going to be crucial to study the interplay between cell cycle regulation and cellular differentiation and for understanding how cells self-organise and pattern during early development.

Summary of future programme

Our future research has three main aims: a) to shed light into embryonic to somatic cell cycle transitions during cellular differentiation in early human development; b) to understand how early embryonic cells decode intracellular signals and signals from the embryo niche and specify specific fates; c) to understand the interplay between cell cycle regulation and cellular differentiation in early development.

We will continue to rely on multi-disciplinary approaches, based on single cell imaging, 2D and 3D models for embryogenesis and genomic approaches and combine these with mathematical models and computations.

Research outputs

Padgett, J., & Santos, S. D. M. (2020). *From clocks to dominoes: lessons on cell cycle remodelling from embryonic stem cells*. FEBS Letters, 594(13), 2031-2045. DOI: 10.1002/1873-3468.13862

This manuscript is the first review we published on cell cycle regulation. It focuses on how the cell cycle remodels during differentiation of human embryonic stem cells. We highlighted that these cells are an incredbily trackable system to understand embryonic-to-somatic transition and highlight the incredible variability in cell cycle regulation in human cells (from early embryonic, pluripotent, somatic to post-mitotic).

Sullivan, A. E., & Santos, S. D. M. (2020). *An optimized protocol for ChIP-Seq from human embryonic stem cell cultures*. STAR Protocols 1, 2. DOI: 10.1016/j.xpro.2020.100062

This manuscript is a methods paper. While at the Crick we have developed methods and optimised protocols to work with human embryonic stem cells, namely ChIPsequencing to perfom high quality and highly reproducible sequencing. While working closely with the Advanced Sequencing facility we were asked to share our protocols with several labs at the Crick, since our data with ASF was of consistently good quality. We decided to submit one of these methods to be useful for the wider community of stem cells/developmental biologists.

Gunne-Braden, A., Sullivan, A., Gharibi, B., Sheriff, RSM., Maity, A., Wang, Y-F., Edwards, A., Jiang, M., Howell, M., Goldstone, R., Wollman, R., East, P. and Santos, SDM*. (2020) *GATA3 mediates a fast, irreversible commitment to BMP4-driven differentiation in human embryonic stem cells*. Cell Stem Cell 26, 693-706. DOI: 10.1016/j.stem.2020.03.005

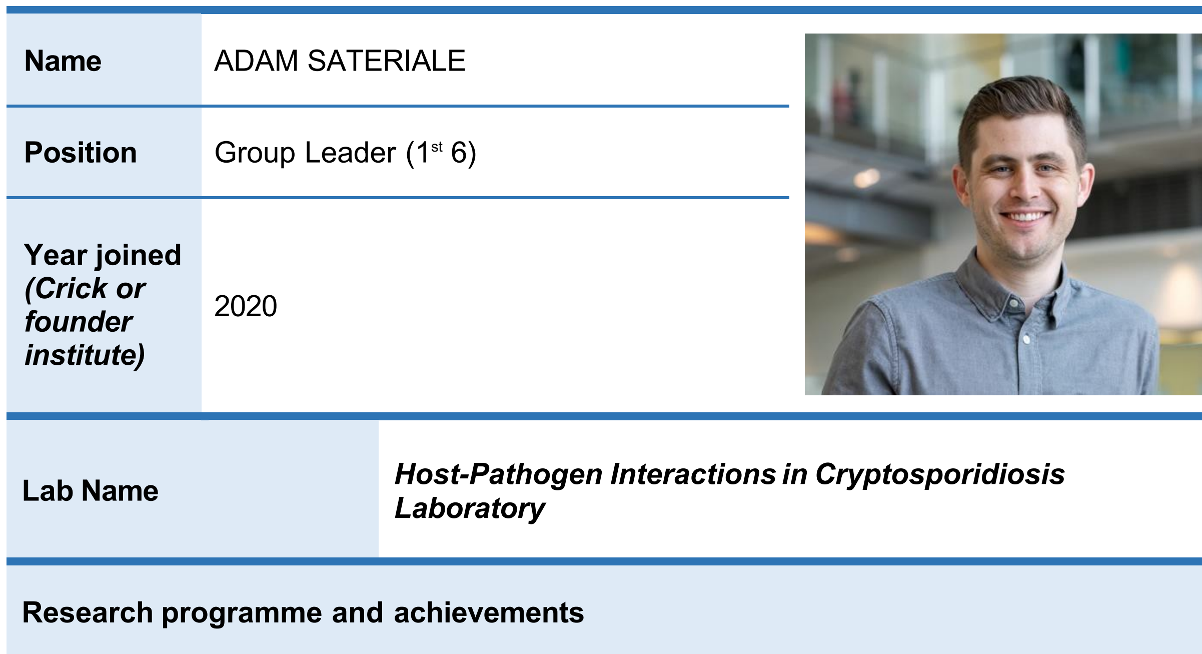
This manuscript was the first demostrations that irreversible commitment to cellular differentiation during early development happens unexpectedly early. The paper reflects our interdisciplinary work, combining single cell imaging, mathematical modelling and - omics approaches. We discovered a new class of genes which we termed early commitment genes (ECG) that are responsible for the pluripotency-to-differentiation transition. It was also our first manuscript in developmental biology, a new field outside of our lab's expertise.

Ochoa, D., Jonikas, M., Lawrence, R. T., El Debs, B., Selkrig, J., Typas, A., Santos, S D M, Beltrao, P. (2016). *An atlas of human kinase regulation*. Molecular Systems Biology, 12(12). DOI: 10.15252/msb.20167295

This manuscript was an interdisciplinary study combining bioinformatics, modelling and experimental approaches to understand regulation of phosphorylation in human cells. The study also developed a computational method to derive activation of kinases from proteomics data. We helped validated our collaborators' methodology with imaging approaches and human embryonic stem cells.

Araujo AR., Gelens L., Sheriff RSM. and Santos SDM* (2016). *Positive feedback keeps duration of mitosis temporally insulated from upstream cell cycle events*. Molecular Cell 64, 362-375. DOI: 10.1016/j.molcel.2016.09.018

This manuscript was our lab's first manuscript. It was an important discovery in the cell cycle field: that despite high variability in cell cycle dynamics in mammalian tissues, duration of mitosis is kept surprisingly constant. We described the molecular basis for this: positive feedback regulation in the networks that regulate the onset of mitosis and mitotic progression. The paper mirors well our interdisciplinary work, combining single cell imaging, mathematical modeling and omics approaches. The study is a collaboration with Dr Gelens, a long term collaborator from the University of Leuven in Belgium.



Name	ADAM SATERIALE
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2020
Lab Name	***Host-Pathogen Interactions in Cryptosporidiosis Laboratory***

Research programme and achievements

Cryptosporidiosis is a diarrhoeal disease caused by the eukaryotic parasite, *Cryptosporidium*. Once thought to be a disease limited to immunocompromised patients, cryptosporidiosis is now recognized as one of the leading causes of morbidity and mortality in children. There is no vaccine for *Cryptosporidium* and the only available treatment, nitazoxanide, is no better than placebo in the immunocompromised and malnourished. Despite this massive impact of cryptosporidiosis on public health, fundamental *Cryptosporidium* research is severely lacking. *I joined the Francis Crick Institute in January 2020 to develop a research program focused on Cryptosporidium host-pathogen interactions, in the hope that a better understanding of disease pathogenesis will lead to new treatments.*

My postdoctoral work with Boris Striepen at the University of Pennsylvania centred on creating and developing the tools to study cryptosporidiosis. In 2015, we adapted CRISPR-driven homologous recombination to genetically manipulate Cryptosporidium parasites. This advancement allowed us to begin to probe parasite biology, yet *in vivo* experiments were limited to a handful of immunocompromised mouse models. To overcome this restriction, in 2017 we isolated a natural mouse pathogen, *Cryptosporidium tyzzeri*. This strain is the first reported lab strain of *C. tyzzeri*, although infections have been reported in both the Old World and New World mice. *Cryptosporidium tyzzeri* is able to infect a wide range of immunocompetent laboratory mouse strains and using CRISPR we can genetically manipulate the parasite. In summary, we now have a powerful mouse model for human cryptosporidiosis where both host and parasite are genetically tractable.

In the short-term, we are leveraging the mouse model to explore howthe host immune system recognises and responds to Cryptosporidium. What pro-inflammatory cytokines impact cryptosporidiosis, and how do they function? What signals or pathogen-associated molecular patterns are being detected and by which host cell receptors? Healthy adult mice infected with *C. tyzzeri* control the infection in roughly a month and demonstrate a robust immunity to re-infection. This is similar to what is seen in human cryptosporidiosis; children growing up areas with widespread cryptosporidiosis show robust immunity by the age of 2 and healthy adults from endemic areas rarely show infection. What cells mediate the protective immunity seen upon rechallenge, and how do they prevent reinfection? How does malnutrition affect this acquired immunity? *In the short term, we are also investigating how Cryptosporidium subverts the immune system and manipulates infected host cells*. After invading a host cell, Cryptosporidium represses apoptosis pathways while the parasite steals nutrients, nucleotides, and amino acids to aid in its own development and replication. Cryptosporidium, like other intracellular parasites, likely secretes hundreds of proteins into the host cell to exert this influence, yet as of this writing there

are no candidate proteins identified. My group is currently characterising the function and secretion mechanism of several gene families that are host cell secreted and will likely prove to be critical for parasite virulence.

In the long term, we will develop forward genetic screens using Cryptosporidium tyzzeri. Unlike most parasites, Cryptosporidium are able to complete their full sexual cycle within a single infected host. Using genetically labelled strains we can easily and rapidly select for recombinant parasite progeny from infected mice. *Cryptosporidium* also have a very small genome, around 9Mb, slightly larger than that of your average bacteria. Put simply: *Cryptosporidium tyzzeri* are perfectly suited for genetic screens. Parasites can be crossed within mice, put through phenotypic selection, then genotyped to identify corresponding loci. We are particularly interested in the genetic loci associated with virulence, however, this approach is applicable to a wide range of research goals including the identification of drug targets.

Research outputs

Sateriale A, Gullicksrud JA, Engiles JB, MacLeoD B, Kugler E, Brodski IE, Shin S, Hunter CA, Striepen B. (2021) *The intestinal parasite Cryptosporidium is controlled by an enterocyte intrinsic inflammasome that depends on NLRP6*. PNAS 118 (2) e2007807118. DOI: 10.1073/pnas.2007807118
The mechanisms of innate recognition are crucial to differentiating between pathogen and commensal organism and then mount an appropriate inflammatory response. In this publication we lay some of the initial groundwork for host cell recognition of a Cryptosporidium infection. In it, we present a working model of innate recognition of Cryptosporidium infection through an NLRP6-dependent and enterocyte intrinsic inflammasome that leads to the release of IL-18 required for parasite control.

Sateriale A, Šlapeta J, Baptista R, Engiles J, Gullicksrud JA, Herbert GT, Brooks C, Kugler E, Kissinger J, Hunter CA, Striepen B. (2019) *A genetically tractable, natural mouse model of cryptosporidiosis offers insight into host protective immunity*. Cell Host Microbe, 26:135-146. DOI: 10.1016/j.chom.2019.05.006
While experimental studies in humans and animals support the development of resistance to cryptosporidium infection, we do not understand the mechanisms that underlie protective immunity. Here, we derive an in vivo model of Cryptosporidium infection in immunocompetent laboratory mice by isolating parasites from naturally infected wild mice. Mice that controlled a live infection were resistant to secondary challenge and vaccination with attenuated parasites provided protection equal to live infection. Importantly, both parasite and host are genetically tractable and this in vivo model, which will facilitate mechanistic investigation and rational vaccine design.

Tandel J, English E, Sateriale A, Gullicksrud J, Beiting D, Sullivan MC, Pinkston B, Striepen B. (2019) *Lifecycle progression and sexual development of the apicomplexan parasite Cryptosporidium parvum*. Nat Microbiol 4, 2226–2236. DOI: 10.1038/s41564-019-0539-x
Parasite transmission occurs through ingestion of oocysts, through either direct contact or consumption of contaminated water or food. Oocysts are meiotic spores and the product of parasite sex. Cryptosporidium has a single-host life cycle in which both asexual and sexual processes occur in the intestine of infected hosts. Here, we genetically engineered strains of Cryptosporidium to make life cycle progression and parasite sex tractable. Our findings suggest obligate developmental progression towards sex in Cryptosporidium, which has important implications for the treatment and prevention of the infection.

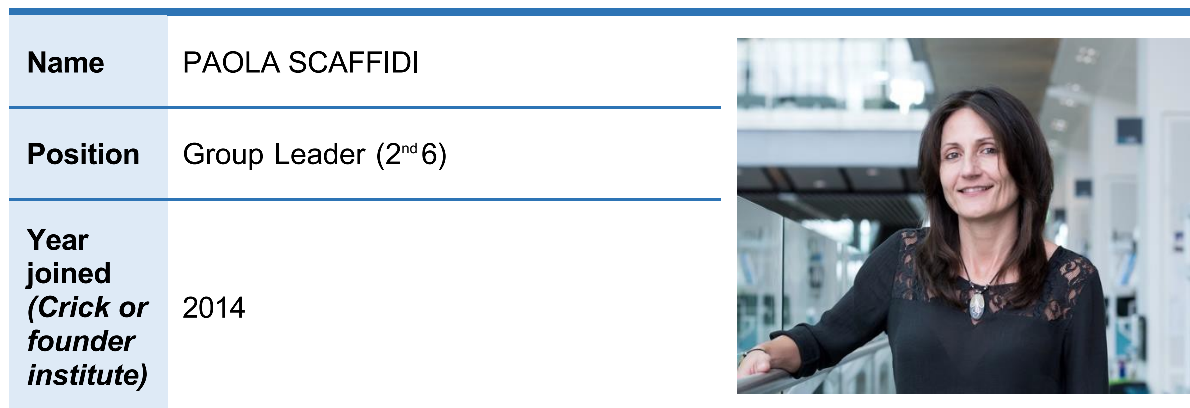
Manjunatha UH, Vinayak S, Zambriski JA, Chao A, Sy T, Noble C, Bonamy G,

Kondreddi R, Zhou B, Gedeck P, Brooks C, Herbert G, Sateriale A, Tandel J, Noh S, Lakshminarayana S, Lim S, Goodman L, Bodenreider C, Feng G, Lijun Z, Blasco F, Wagner J, Leong J, Striepen B, Diagana, T. (2017) ***A Cryptosporidium PI(4)K inhibitor is a drug candidate for cryptosporidiosis*****. Nature 546, 376–380. DOI: 10.1038/nature22337**

For Cryptosporidium, there is neither a vaccine nor an effective treatment. Here we establish a drug discovery process built on scalable phenotypic assays and mouse models that take advantage of transgenic parasites. Screening a library of compounds with anti-parasitic activity, we identify pyrazolopyridines as potent inhibitors of Cryptosporidium infection. Since this publication, the lead compound KDU731 has moved into clinical trials and represents the leading novel treatment candidate for cryptosporidiosis.

Vinayak S*, Pawlowic MC*, Sateriale A*, Brooks CF, Studstill CJ, Bar-Peled Y, Cipriano MJ, Striepen B. (2015) ***Genetic manipulation of the diarrhoeal pathogen Cryptosporidium parvum*****. Nature 523, 477–480. DOI: 10.1038/nature14651**

Here we describe an experimental framework to genetically modify Cryptosporidium parasites using the (CRISPR)/Cas9 system. We derive reporter parasites suitable for in vitro and in vivo drug screening, and then evaluate the basis of drug susceptibility by gene knockout. This paper represents a landmark for the field, laying the foundation for basic research on this previously genetically intractable parasite. This research was highlighted in Nature, Nature Reviews Genetics, Trends in Parasitology, and picked up by several news outlets, including National Public Radio.



Name	PAOLA SCAFFIDI
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2014

Lab Name	***Cancer Epigenetics Laboratory***

Research programme and achievements

The Cancer Epigenetics Laboratory investigates non-genetic alterations important for cancer development, with a particular focus on chromatin-based mechanisms. In physiological conditions, epigenetic regulation mediates acquisition of cell fate and maintains stable cell identity, ensuring tissue homeostasis. These processes rely on modulation of chromatin and DNA modifications in response to environmental conditions, and regulation of gene expression programmess that constrain cellular capabilities. In cancer, epigenetic alterations induced by oncogenic signalling, mutations in epigenetic regulators and pathological environments corrupt cell identity and unleash the inherent plasticity of cells leading to aberrant cell behaviour. We seek to understand how epigenetic mechanisms cooperate with genetic alterations and extracellular signalling in establishing malignant phenotypes at various stages of the disease.

By combining genetic approaches, genome-wide molecular methods and *in vivo* functional assays, we have uncovered both tumour-suppressive and tumour-promoting epigenetic mechanisms that regulate the long-term proliferative capacity of cancer cells, and identified epigenetic determinants of functional diversity within individual tumours. These findings have revealed novel therapeutic opportunities, increased our understanding of how chromatin regulates self-renewing cellular states, and provided insights into the physiological role of the linker histone, one of the most enigmatic components of chromatin. Key achievements during the quinquennium are:

- Discovered epigenetic determinants of intratumour functional heterogeneity relevant for numerous cancer types
- Identified an integral component of chromatin, the linker histone H1.0, as a strong inhibitor of cancer cell self-renewal
- Identified a compound that selectively inhibits cancer cell self-renewal without affecting normal stem cell function
- Dissected how cancer cells corrupt the function of the Polycomb repressive complex 2 to subvert developmental programmes in brain cancer
- Discovered a vulnerability in chromosomally unstable cancers - the MSL acetyltransferase complex.
- Generated and characterised an arrayed CRISPR library that targets human epigenetic regulators
- Revealed the importance of genetic context for precise CRISPR-induced genome editing

In the next six years, we will exploit our accumulated knowledge and technical expertise to dissect how mutations in epigenetic regulators, commonly found across all cancer types, favour disease development, and to uncover how compromised robustness of

epigenetic regulation in malignant cells creates vulnerabilities exploitable for therapeutic purposes.

Research outputs

Torres, C.M., Biran, A., Burney, M.J., Patel, H., Henser-Brownhill, T., Cohen, A.S., Li, Y., Ben-Hamo, R., Nye, E., Spencer-Dene, B., Chakravarty, P., Efroni, S., Matthews, N., Misteli, T., Meshorer, E., and Scaffidi, P. (2016). *The linker histone H1.0 generates epigenetic and functional intratumor heterogeneity*. Science 353. DOI: 10.1126/science.aaf1644
This study showed that epigenetic mechanisms play an important role in generating functional heterogeneity within tumours, and can override genetic alterations that initiate the disease by inhibiting cell proliferative potential during tumour growth. The finding that heterogeneous patterns of H1.0 are broadly observed in cancer and that H1.0 is an independent predictor of patient survival in multiple types of solid tumours makes a strong case for a general role of epigenetic regulators in cancer. Mechanistic characterisation of how H1.0 controls malignant self-renewing states also provided insights into general mechanisms through which the linker histone regulates gene expression.

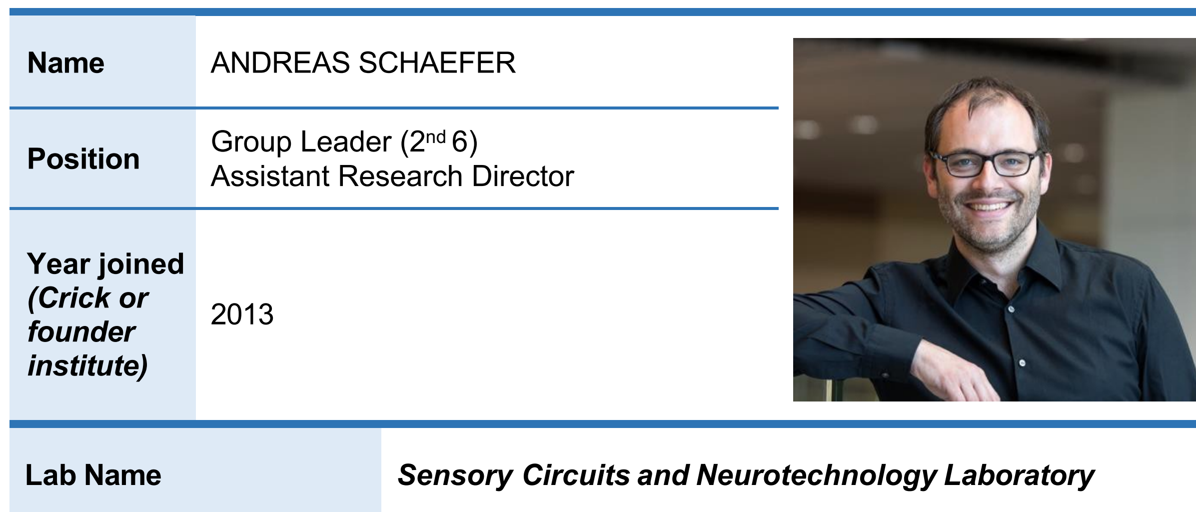
Henser-Brownhill, T., Monserrat, J., and Scaffidi, P. (2017). *Generation of an arrayed CRISPR-Cas9 library targeting epigenetic regulators: from high-content screens to in vivo assays*. Epigenetics 12, 1065-1075. DOI: 10.1080/15592294.2017.1395121
We generated a unique resource that allows systematic inactivation of over 400 epigenetic regulators in human cells, and assessment of functional interactions within the network of proteins that regulate chromatin and DNA methylation. The library is being used in several projects currently ongoing in the laboratory to perform both unbiased screening and hypothesis-driven investigation, and has been shared with multiple laboratories worldwide.

Chakrabarti, A.M. *, Henser-Brownhill, T. *, Monserrat, J.*, Poetsch, A.R., Luscombe, N.M., and Scaffidi, P. (2019). *Target-Specific Precision of CRISPR-Mediated Genome Editing*. Molecular cell 73, 699-713 e696. DOI: 10.1016/j.molcel.2018.11.031
Taking advantage of our sgRNA library, we performed a large-scale characterisation of CRISPR-induced in/del patterns and discovered that Cas9-induced double strand breaks are repaired in a predictable or unpredictable way, depending on the target site. These findings provided the broad scientific community with guidelines for a more effective and safer use of CRISPR technology, with important implications for clinical applications. They also revealed a striking influence of DNA sequence in dictating DSB repair outcomes and laid the foundation for future mechanistic studies that can increase our understanding of end-joining processes in human cells.

Mortimer, T., Wainwright, E.N., Patel, H., Siow, B.M., Jaunmuktane, Z., Brandner, S., and Scaffidi, P. (2019). *Redistribution of EZH2 promotes malignant phenotypes by rewiring developmental programmes*. EMBO Rep 20, e48155. DOI: 10.15252/embr.201948155
Many epigenetic regulators are co-opted by cancer cells to sustain malignant phenotypes, but how cells repurpose key regulators of cell identity as tumour-promoting factors has been unclear. Focusing on the Polycomb component EZH2 and its role in the central nervous system, we found that oncogenic signalling induces a redistribution of EZH2 across the genome. Through this process, cancer cells subvert developmental transcriptional programmes that specify normal cell identity and remove physiological breaks that restrain cell proliferation.

Morales-Torres, C., Wu, M.Y., Hobor, S., Wainwright, E.N., Martin, M., Patel, H., Grey,

W., Grönroos, E., Howell, S., Carvalho, J., Snijders, A.P., Bustin, M., Bonnet, D., Smith, P.D., Swanton, C., Howell, M., and Scaffidi, P. (2020). ***Selective inhibition of cancer cell self-renewal through a Quisinostat-histone H1.0 axis*****. Nature communications 11(1):1792. DOI: 10.1038/s41467-020-15615-z**
As a follow up to our previous study on histone H1.0, we have identified the well-tolerated compound Quisinostat as a strong inducer of H1.0, which halts the maintenance of various cancer types and inhibits disease relapse by trapping resistant cells in a non-diving state. We have also shown that Quisinostat does not affect normal tissue stem cells, providing an attractive means to target malignant self-renewing cells without inducing severe side effects in patients.



Name	ANDREAS SCHAEFER
Position	Group Leader (2nd 6) Assistant Research Director
Year joined *(Crick or founder institute)*	2013

Lab Name	***Sensory Circuits and Neurotechnology Laboratory***

Research programme and achievements

My lab's central ambition is to elucidate how information is processed in the mammalian brain, and to investigate the cellular and circuit mechanisms by which neurons represent and process information. We employ the mouse olfactory system as a tractable, anatomically compact model system of high ethological relevance in a highly genetically accessible mammalian model species. In the past, we have recorded activity from projection neurons in different states – anaesthetised, awake passive and awake actively behaving. We have found that the two projection neuron classes represent odour inputs differently and that these differences are set up by the local interneuron network.

Moreover, we found that using unbiased whole-cell recordings differences between awake and anesthetised state are subtle but that there are learning induced changes that can in part be traced back to changes in sampling behaviour.

In order to in the future further dissect the circuit mechanisms underlying odour processing, we have developed new technical approaches: first, we have developed a home-cage based training system that allows efficient operant conditioning of group-housed mice over periods of months, allowing us now to broadly sample the psychophysical limits of odour representation; second, we have started to dissect the cellular and anatomical basis of local processing in the olfactory bulb combining physiology and anatomy, providing the basis for comprehensive, volume EM based analysis of local information processing; third, to enable sampling information from a significant fraction of all projection neurons in the OB, we have developed scalable electrophysiology that allows recording from 10^2-10^3 of neurons arranged in 3D in a minimally invasive manner.

In the future, we will follow three intertwined lines of research. First, we will assess how the mammalian olfactory system extracts information from temporal structure in odours to gain insight into the spatial structure of the olfactory landscape. This is based on the observation that natural odours are transported by turbulent air flow that imposes rich dynamics on odour concentrations. In ongoing work we have demonstrated that mice can detect concentration fluctuations with frequencies exceeding 40 Hz and that they can use this information to perform a source separation task. In the future we will assess which features of turbulent odour plumes are used to gauge distance or direction of odours, how these are represented in the early olfactory system and how the local interneuron networks enable extraction of this information.

Second, we will explore the topology of neural activity in the OB by combining high-dimensional temporally structured odour stimuli with large-scale electrical recording and local perturbation of the circuitry. By recording from increasing ensembles of neurons in the olfactory bulb and more and more cortical areas in response to high dimensional sensory stimuli and by stimulating large numbers of sites independently we will gain

insight into the complex topology of sensory representation. Technical improvements will further increase the number of sites, recording density and 3D distribution and allow us and others to perform chronic recordings to both increase accessible stimulus space and enable us to assess stability of neural representation.

Third, we will directly link outputs and inputs in the OB and dissect the circuits underlying this transformation by combining *in vivo* imaging with detailed circuit reconstructions using electron microscopy and synchrotron X-ray tomography. Based on ongoing work we will further improve synchrotron X-ray tomography to allow us to capture subcellular detail over mm^3 volumes.

Together, this research programme will provide mechanistic insight into how the mouse olfactory bulb neural circuitry structures neural activity and extracts information about the odour landscape.

Research outputs

Obaid, A.M.*, Hanna, M-E.S.*, Wu Y-E.*, Kollo M.*, Racz R.R., Angle M.R., Muller J., Brackbill N., Wray W., Franke F., Chichilinisky E.J., Hierlemann A., Ding J.B., Schaefer A.T., Melosh N.A., (2020) *Massively Parallel Microwire Arrays Integrated with CMOS chips for Neural Recording*. Science Advances 12:eaay2789 DOI: 10.1126/sciadv.aay2789
Neuroprosthetics and neuroscience research alike are limited by the bandwidth of neural recording. At the same time, ever-more powerful silicon-based technology is ubiquitous in our phones, tablets and computers. Here we showed that progress in neural recording can be coupled to this by fusing bundles of microwires to pixel array chips, lifting silicon technology to the third dimension for deep brain recording.

Erskine A, Bus T, Herb J.T., Schaefer A.T., (2019) *AutonoMouse: High throughput operant conditioning reveals progressive impairment with graded olfactory bulb lesions.* PLoS One e0211571. DOI: 10.1371/journal.pone.0211571
Operant conditioning is a crucial tool in neuroscience research for probing brain function. Here we described a fully automated, high-throughput system for self-initiated conditioning of group-housed mice over periods of several months and >106 trials. We used this "AutonoMouse" system to systematically probe the impact of graded olfactory bulb lesions on olfactory behaviour, demonstrating that while odour discrimination in general is robust to even the most extensive disruptions, small olfactory bulb lesions already impair odour detection. The modular nature and open-source design of AutonoMouse makes it a versatile platform for efficiently and systematically assessing behaviour in mice.

Koldaeva A., Schaefer A.T., Fukunaga I. (2019) *Rapid task-dependent tuning of the mouse olfactory bulb.* e Life e43558. DOI: 10.7554/eLife.43558
Adapting neural representation to rapidly changing behavioural demands is a key challenge for the nervous system. Here, we demonstrated that the output of the primary olfactory area of the mouse, the olfactory bulb, is already a target of dynamic and reproducible modulation. The modulation depends on the stimulus tuning of a given neuron, making olfactory responses more discriminable through selective amplification in a demand-specific way.

Jordan R., Fukunaga I., Kollo M., and Schaefer A.T. (2018) *Active Sampling State Dynamically Enhances Olfactory Bulb Odor Representation.* Neuron 98:1214-1228. DOI: 10.1016/j.neuron.2018.05.016
Animals engage actively with their environment, yet how active sampling strategies impact neural activity was unknown. We showed that mice adapt sniffing during learning

in a way that enhances neuronal representation. Furthermore, this work resolves a long-standing conundrum that seemingly non-olfactory information is prominently represented in the OB: context influences sniffing, which in turn changes neural activity.

Schwarz D., M. Kollo, C. Bosch, C. Feinauer, I. Whiteley, T. W. Margrie, T. Cutforth and Schaefer A.T. (2018) ***Architecture of a mammalian glomerular domain revealed by novel volume electroporation using nanoengineered microelectrodes.*** **Nature Communications 9:183. DOI: 10.1038/s41467-017-02560-7**

Mechanistic understanding of neural circuit function requires a wiring diagram. Electron microscopy is, however, limited to small volumes, such that generally only parts of neurites are captured. Here we developed a new technique that allows comprehensive labelling of cells that extend neurites into a defined volume, adding context to local circuits. We apply this to for the first time register all projection neurons of a genetically identified glomerulus.

Name	KATHARINA SCHMACK
Position	Clinical Group Leader (1st 6)
Year joined ***(Crick or founder institute)***	2021
Lab Name	***Neural Circuits and Immunity in Psychosis Lab***

Research programme and achievements

We study the neural and immune mechanisms underlying psychosis, a state characterised by unfounded perceptions. Our aim is to understand perception in health and disease, and to find new ways to treat brain disorders such as schizophrenia. We use a cross-species approach that combines in-vivo investigations in mice and humans with in-silico computational modelling.

My main achievements that led up to this research programme are as follows:

1. **Demonstrated altered influence of perceptual priors in psychosis.** One major challenge in psychosis research is that the condition is exclusively defined by subjective symptoms that are difficult to objectively measure. When I was a clinician scientist at Charité, my group and I demonstrated that psychotic symptoms are related to alterations in how prior expectations influence perception. In a series of studies in non-clinical and in clinical populations, we showed that psychotic experiences are related to an increased influence of high-level priors and a decreased influence of low-level priors. This body of work stipulated a re-conceptualization of psychotic symptoms as quantifiable perceptual alterations, and thereby provided a foothold for developing objective readouts of psychosis.
2. **Established mouse model of hallucinations to dissect neural circuits relevant to psychosis.** Unsatisfied with the inability to rigorously probe mechanisms in humans, I joined Cold Spring Harbor Laboratory as a research investigator to translate my work to mice. I established and validated a computational-behavioural approach to measure hallucination-like perceptions in humans and mice alike. This allowed me to mechanistically dissect the causal role of dopamine-dependent neural circuits in hallucination-like perception. These findings yielded circuit-level insights into the long-standing dopamine hypothesis of psychosis and opened new avenues for the biological study of psychosis.

In our ongoing work, we use our cross-species approach to investigate how neural and immune processes give rise to psychosis. We currently pursue two main project areas (1) **Neural circuits**: We aim to understand how mechanisms upstream of dopamine contribute to psychosis. To this end, we study how auditory cortex interneurons and striatal cholinergic interneurons modulate dopaminergic signalling underpinning hallucination-like perception. We have implemented our behavioural setups and will get our first neurophysiological recordings in the coming months. We expect our results to connect between currently disparate theories of psychosis, which will deepen our understanding of unfounded perception. Thereby, we hope to pave the way for novel mechanistic antipsychotic drugs that lack the limitations of current anti-dopaminergic drugs. (2) **Immune processes.** We aim to establish whether and how brain-

directed immune processes cause psychosis. To this end, we characterise immune cells and antibodies in the cerebrospinal fluid and blood of psychosis patients, and then probe how these brain-directed immune processes can lead to psychosis-like behaviours in mice. Here, we have started to implement novel assays to detect neuronal autoantibodies with increased specificity and sensitivity, and we have applied for regulatory approval to collect blood and cerebrospinal fluid from psychosis patients. We have also established protocols for passive human-to-mouse brain antibody transfers and for active immunisation against relevant brain antigens. We expect our results to enable us to uncover mechanisms by which the immune system and the brain interact in psychosis. Thereby, we hope to identify novel immunological treatment targets for psychotic disorders.

Research outputs

Schmack K, Ott T, Kepecs A (2022). Computational Psychiatry Across Species to Study the Biology of Hallucinations. JAMA Psychiatry 79(1):75-76. https://doi.org/10.1001/jamapsychiatry.2021.3200

This opinion article lays out a new conceptual framework that allows to bridge between the subjective experience at the core of mental illness and neural circuit dysfunction using computational models. This article has had an impact in the field, as most directly evidenced by a large collaborative grant initiative that brought together mouse systems neuroscientists and human cognitive neuroscientists from different London institutions to apply this computational psychiatry framework to different areas of mental health.

Schmack K, Bosc M, Ott T, Sturgill JF, Kepecs A (2021). Striatal dopamine mediates hallucination-like perception in mice. Science 372(6537):eabf4740. https://doi.org/10.1126/science.abf4740

This work established a new computational-behavioral framework for quantifying hallucination-like perceptions in mice and humans, and dissected dopamine-dependent neural circuits mediating such hallucination-like perceptions. Our findings confirmed and refined the long-standing dopamine hypothesis of psychosis and established the cross-species approach that our laboratory at the Crick is using for the biological study of psychosis.

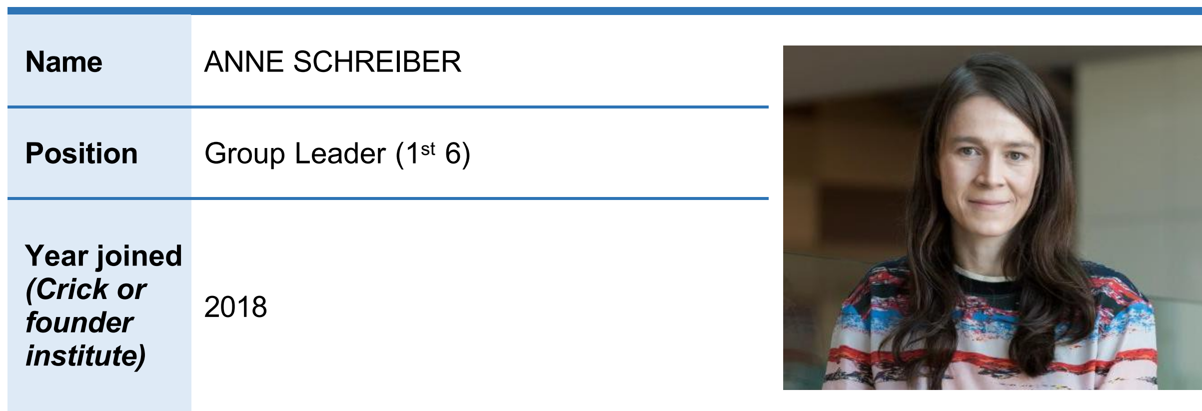
Stuke H, Weilnhammer V, Sterzer P, Schmack K (2019). Delusion Proneness Is Linked to a Reduced Usage of Prior Beliefs in Perceptual Decisions. Schizophrenia Bulletin Jan 1;45(1):80-86. https://doi.org/10.1093/schbul/sbx189

In individuals from the general population, we corroborated our our framework that explain psychotic experiences as altered perceptual inference, which provided an avenue forward for modeling psychosis in mice. Studying psychotic experiences in a non-clinical population allowed us to generate insights that could not be explained by confounding factors such as antipsychotic medication, a common problem in research on psychosis. In our laboratory at the Crick, we will continue to study non-clinical human populations to translationally validate our biological insights into perception from mice.

Weilnhammer V., Stuke H, Sterzer P, Schmack K (2018). The Neural Correlates of Hierarchical Predictions for Perceptual Decisions. Journal of Neuroscience. May 23;38(21):5008-5021. https://doi.org/10.1523/JNEUROSCI.2901-17.2018

In this work, we used computational models to map different types of sensory priors to different levels of the cortical hierarchy, which advanced the understanding of perceptual

inference in the human brain. In our laboratory at the Crick, we continue to use this computational approach to relate behavioral and neural observations in humans and mice, which we expect to generate theory-driven cross-species insights into perception.



Name	ANNE SCHREIBER
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2018

Lab Name	***Cellular Degradation Systems Laboratory***

Research programme and achievements

How does the cell form a customised waste basket when bacteria suddenly invade the cytoplasm, when protein aggregates accumulate, when mitochondria stop functioning and threaten the cell by releasing reactive oxygen species, or when nutrients become so scarce that the cell needs to reclaim a diverse range of cellular building blocks to maintain cellular homeostasis in a changing environment?

Autophagy holds the answer to all these seemingly unrelated challenges. Autophagy initiates a striking cellular response by converting small vesicles into sheet-like structures which, upon localised phospholipid transfer, expand and give rise to double membrane vesicles. The resultant carrier, also known as an autophagosome, fuses with the lysosome/vacuole allowing for the degradation of its cargo and recycling of all contained building blocks. This ensures cellular homeostasis in an ever-changing environment and allows for the rapid neutralisation of potentially cytotoxic threads (such as invading pathogens, damaged or non-functional organelles or protein aggregates), explaining the many beneficial properties attributed to autophagy, and conversely, why its deregulation is associated with many human pathologies such as cancer, neurodegeneration, cardiovascular or infectious diseases.

Autophagy is a captivating but also very complex process which requires a multitude of cellular factors and different layers of regulation. The individual steps involved are difficult to study in the crowded environment of the cell. Therefore, we have developed a cell-free system to rebuild the process of autophagosome formation in the test tube, and to understand when, where and how each member of the core machinery contributes to autophagosome formation. This system can be manipulated at will, allowing us to dissect and visualise the different steps in order to gain insights into the molecular mechanism underlying autophagosome formation. The system also allows us to study the posttranslational regulation of autophagy by protein phosphorylation, acetylation or the covalent conjugation of ubiquitin and ubiquitin-like proteins.

So far, we have successfully used our system to understand how the master regulator of autophagy, the Atg1 kinase (Ulk1/2 in higher eukaryotes), regulates key processes required for autophagy. Having reconstituted the core autophagy machinery we could screen for the direct functional consequences of Atg1 mediated protein phosphorylation. The findings from those *in vitro* studies paved the way for highly targeted *in vivo* studies (using budding yeast as a well established model system) providing a wealth of insights into the complex regulatory principles underlying both bulk and selective autophagy.

Our findings show that Atg1 dependent phosphorylation of its interaction partner Atg13 triggers Atg1 complex disassembly resulting in rapid turnover of Atg1 based complexes at

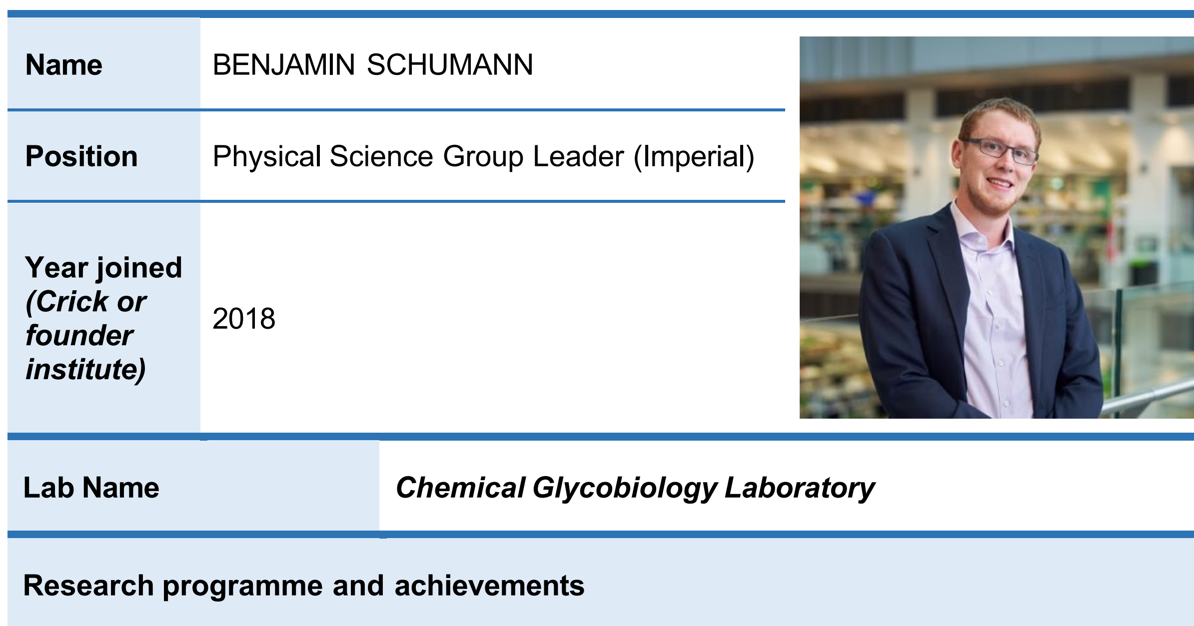
the site of autophagosome formation. As the Atg1 complex is the key factor mediating vesicle tethering our work provides new clues into the mechanism and dynamic nature of membrane formation. Additionally, our system helps us understand the regulation of Atg1 kinase activity by revealing that Atg1 is activated along the growing autophagosomal membrane by lipidated Atg8 (Atg8-PE) locally stimulating substrate phosphorylation. Resultant Atg1 recruitment further self-regulates Atg8-PE levels in the growing autophagosomal membrane by phosphorylating and inhibiting the Atg8 specific lipidation machinery. Taken together, our work uncovers positive and negative feedback imposed by the Atg1 kinase, the underlying molecular mechanism and how opposing phosphorylation and dephosphorylation events underpin the spatiotemporal regulation of autophagy. Our findings significantly change the thinking in the field, establishing Atg1 not only as an activator of autophagy but also a potent inhibitor suppressing some of the key processes required for autophagosome formation. This highlights the importance of protein phosphatases as drivers of autophagy.

To study the process of autophagosomal membrane formation, my lab will use our cell-free system to understand the structural and mechanistic basis of vesicle tethering and fusion – the very first steps during autophagosome formation. We will employ biophysical approaches including single-molecule fluorescence resonance energy transfer (FRET)-based vesicle fusion assays to identify the machinery required for vesicle tethering and fusion. In parallel, we will use biochemical and cell biology approaches to better understand how posttranslational modifications regulate membrane formation. Functional membrane tethering and fusion intermediates will be analysed by cryo-electron microscopy and single particle analysis or by tomography-based approaches. High resolution structures of vesicle tethering and fusion intermediates will significantly advance our knowledge of autophagy, guiding future efforts to identify novel strategies for therapeutic intervention.

Research outputs

Fumagalli F, Noack J, Bergmann TJ, Cebollero E, Pisoni GB, Fasana E, Fregno I, Galli C, Loi M, Soldà T, D'Antuono R, Raimondi A, Jung M, Melnyk A, Schorr S, Schreiber A, Simonelli L, Varani L, Wilson-Zbinden C, Zerbe O, Hofmann K, Peter M, Quadroni M, Zimmermann R and Molinari M. (2016) ***Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery*****. Nature Cell Biology 18:1173-1184. DOI: 10.1038/ncb3423**

As part of my postdoctoral research I also participated in a collaborative study which aimed to shed light on the degradation of parts of the endoplasmic reticulum by selective autophagy (ER-phagy). The work illustrates the applicability of our recombinant autophagy system not only for the study of bulk autophagy but also for the investigation of selective autophagy pathways, providing the basis for our efforts to better understand autophagy initiation during the selective uptake of mitochondria, bacteria and protein aggregates.



Name	BENJAMIN SCHUMANN
Position	Physical Science Group Leader (Imperial)
Year joined *(Crick or founder institute)*	2018

Lab Name	***Chemical Glycobiology Laboratory***

Research programme and achievements

The Chemical Glycobiology Lab uses chemical biology to develop tools that help understand and perturb the roles of glycans in health and disease. Before joining the Crick, my research focused on the development of carbohydrate-based vaccines, with three patents licensed to Vaxxilon AG (Berlin). Being one of the biggest markets in pharma (>>6 billion USD/year), carbohydrate-based vaccines have gained rapid attention to protect against pathogenic bacteria. As opposed to the conventional approach of generating vaccines from isolated bacterial polysaccharides, we chemically synthesised selected and defined antigens on the bacterial cell surface to assess their efficacy in animal models. In addition to their superior accessibility, synthetic antigens offer the possibility to precisely map the epitopes that are important to invoke protective immunity (*4*). Deeper understanding of the relevance of carbohydrate structures in immunity led me to support a Crick African Network fellow working on profiling immunity against *Mycobacterium tuberculosis* in cohorts that are seemingly resistant against infection after being vaccinated with Bacillus Calmette-Guerin (BCG).

During my postdoctoral work at Stanford, I combined my skills in chemistry with cell biology and biochemistry, and spearheaded the first application of a glycosyltransferase bump-and-hole engineering approach in the living cell (*2*). Through structure-informed protein engineering, the active site of a glycosyltransferase is enlarged to accommodate a chemically modified carbohydrate donor that carries a traceable tag. Applying this tactic in the living cell was instrumental to my Lab's work at the Crick, as it allowed us for the first time to dissect the very complex yet disease-relevant products of individual glycosyltransferases. This is no trivial matter, as the approach requires structural data on glycosyltransferases as well as state-of-the-art techniques in mass spectrometry, glycoproteomics and genome engineering. The Crick offers the optimal scientific environment for such an endeavour.

My lab is using the bump-and-hole tactic to develop "precision tools" that will help understand the roles of glycans in biological processes. Since the community currently does not have many useful tools to discriminate between different glycan sub-types or products of different glycosyltransferases, precision tools will be very important reporter reagents especially given the recent advances in quantitative biology. To this end, my group has developed a bioorthogonal reagent that is specific for highly cancer-relevant O-GalNAc glycans (1). We have applied the reagent to image glycosylation with intestinal organoids in collaboration with Vivian Li. Further, we have established and used a chemical glycoproteomics workflow with the Proteomics STP, which makes us one of very few labs worldwide with both the experimental capacity and know-how to site-selectively analyse the products of individual glycosyltransferases. Currently, my group is expanding

these projects to be used in experiments of cell-selective (glyco-)proteomics with Ilaria Malanchi.

Further, we are using structural and chemical biology to assess the impact of glycans on neurotrophic signalling in collaboration with the McDonald lab. Through analysis of glycan binding partners of the Glial cell derived neurotrophic factor (GDNF) and related proteins, we have established an optimal binding partner that we are currently assessing in X-ray crystallography. Our initial findings suggest a rich glycobiology perspective on signalling which is rather unexplored to date.

Research outputs

Debets, M. F., Tastan, O. Y., Wisnovsky, S. P., Malaker, S. A., Angelis, N., Moeckl, L. K. R., Choi, J., Flynn, H., Wagner, L. J. S., Bineva-Todd, G., Antononopulos, A., Cioce, A., Browne, W. M., Li, Z., Briggs, D. C., Douglas, H. L., Hess, G. T., Agbay, A. J., Roustan, C., Kjaer, S., Haslam, S. M., Snijders, A., Bassik, M. C., Moerner, W. E, Li, V. S. W., Bertozzi, C. R., Schumann, B. (2020) ***Metabolic precision labeling enables selective probing of O-linked N-acetylgalactosamine glycosylation*****. Proc. Natl. Acad. Sci. USA 117(41)25293-25301. DOI: 10.1073/pnas.2007297117**
The first publication from our group at the Crick comprises the development of a precision tool to understand O-GalNAc glycosylation, one of the most abundant and disease-relevant types of glycans. We apply the technique to run state-of-the-art methods of biology, including chemical glycoproteomics with the Proteomics STP and a genome-wise CRISPR screen with collaborators from Stanford. We also collaborate in-house with Vivian Li to apply the probe to imaging intestinal organoids.

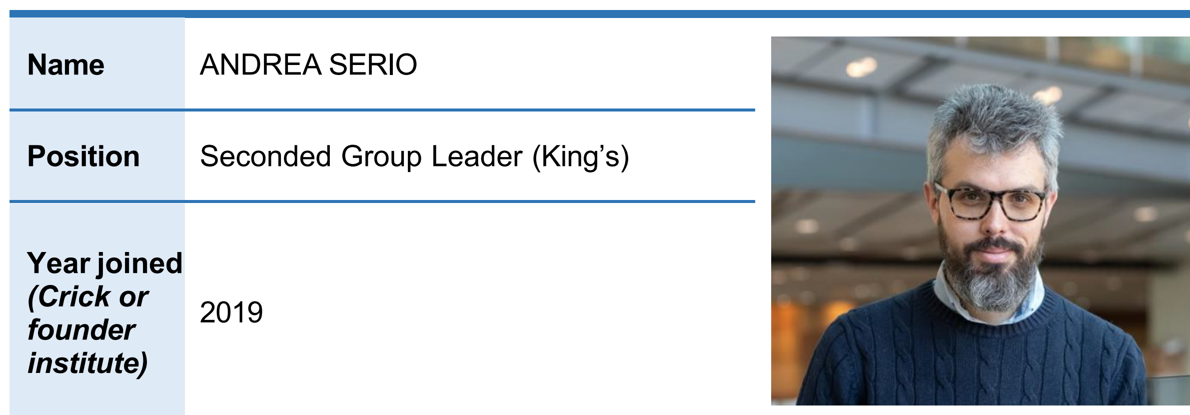
Schumann, B., Malaker, S. A., Wisnovsky, S. P., Debets, M. F., Agbay, A. J., Fernandez, D., Wagner, L.J. S., Lin, L., Choi, J., Fox, D. M., Peh, J., Gray, M. A., Pedram, K., Kayvon Pedram, Kohler, J. J., Mrksich, M., Bertozzi, C. R. (2020) ***Bump-and-hole engineering identifies specific substrates of glycosyltransferases in living cells*****. Mol. Cell 78. DOI: 10.1016/j.molcel.2020.03.030**
This paper represents the first in-cell application of a glycosyltransferase bump-and-hole system which is the basis for our work on chemical precision tools to understand glycobiology.

Choi, J., Wagner, L. J. S., Timmermans, S. B. P. E., Malaker, S. A., Schumann, B., Gray, M. A., Debets, M. F., Takashima, M., Gehring, J., Bertozzi, C. R. (2019) ***Engineering Orthogonal Polypeptide GalNAc-Transferase and UDP-Sugar Pairs*****. J. Am. Chem. Soc. 141. DOI: 10.1021/jacs.9b04695**
This paper is a crucial account of how to engineer glycosyltransferases to transform them into reporter systems to dissect the details of protein glycosylation. The techniques depicted herein, including chemical synthesis, protein engineering and enzymology, are routinely used by our lab at the Crick and an important precedent to our work.

Schumann, B., Hahm, H. S., Parameswarappa, S. G., Reppe, K., Wahlbrink, A., Govindan, S., Kaplonek, P., Pirofski, L., Witzenrath, M., Anish, C., Pereira, C. L., Seeberger, P. H. (2017) ***A Semisynthetic Streptococcus pneumoniae Serotype 8 Glycoconjugate Vaccine*****. Sci. Transl. Med. 9. DOI: 10.1126/scitranslmed.aaf5347**
This paper from my PhD work illustrates the power of synthetic tools to understand, probe and perturb biology. We synthesised glycans normally found on the surface of highly pathogenic streptococci to fashion the next generation of carbohydrate-based vaccines. The work described here has been patented and licensed to a start-up company in Berlin, Germany. We are using some of the same principles and knowledge in collaboration with

the Crick African Network to tackle Mycobacterium tuberculosis.

Name	ANDREA SERIO
Position	Seconded Group Leader (King's)
Year joined *(Crick or founder institute)*	2019



Lab Name	***Neural Circuit Bioengineering and Disease Modelling Laboratory***

Research programme and achievements

Summary of research program: We are creating new models of the nervous system by combining stem cell research with bioengineering. The models allow us to learn more about how our nervous system functions, and see how conditions like motor neurone disease (MND) damage it.

Using pluripotent stem cells to grow neurons in the lab and model diseases of the nervous system transformed the field of neuroscience. However, it is still difficult to create a model that comes close to the complexity seen in our own neural circuits. Our group focuses on finding ways to model the nervous system more accurately using custom-made devices and advanced bioengineering.

We use a range of techniques to support and direct the neurons' growth, including microfabricating new surfaces that resemble human tissue, with channels to 'steer' the growth of axons. Through using new imaging techniques to examine these models in real time, we are able to gain new insights into how these circuits change and develop over time, and identify new options for treatments.

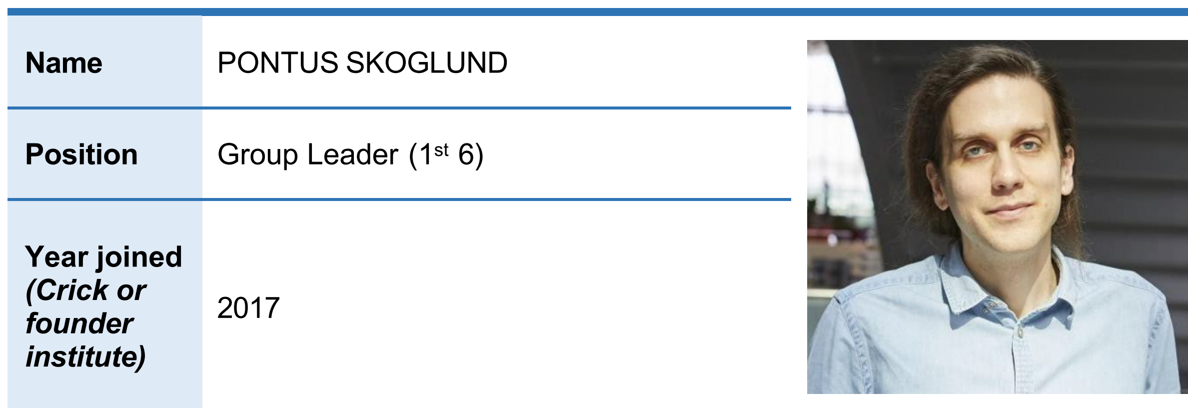
Future Plans: For the bulk of my secondment at the Crick (2020-2025), I plan to grow my research portfolio by developing a novel area of comparative in vitro evolutive neurobiology, where we will use bioengineering and stem cell technology to compare the fine molecular tuning and differences occurring in neural circuits processing across different species, from mouse to primates. This research will take advantage of novel collaborations I am establishing within the Crick (e.g. Tedesco Lab, Rodrigues Lab, etc.).

Research outputs

Hagemann C, Tyzack G, Taha D, Devine H, Greensmith L, Newcombe J, Patani R*, Serio A*, Luisier R* (2021). *Automated and unbiased discrimination of ALS from control tissue at single cell resolution.* Brain Pathol 11;e12937. DOI: 10.1111/bpa.12937
This paper described a novel pipeline based on machine learning and image nalaysis to classify and analyse single cells within patient tissues, to develop a more precise tool of unbiassed phenotyping in ALS pathologies. This publication is one the first outputs from our secondment as well as from my independent laboratory. It is authored by my PhD student Cathleen Hagemann and represent the product of a collaboration with Rickie Patani at the Crick and Raphaelle Luisier (formerly at the Crick) which would not have been possible without my secondment

Harley J*, Hagemann C*, Serio A#, Patani R (2020). ***FUS is lost from nuclei and gained in neurites of motor neurons in a human stem cell model of VCP-related ALS"*** **(2020). Brain 143(12):e103. DOI: 10.1093/brain/awaa339**

One of the first senior author outputs from my lab, produced in the context of my collaboration with the Patani lab, integral for our secondment



Name	PONTUS SKOGLUND
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2017

Lab Name	***Ancient Genomics Laboratory***

Research programme and achievements

All human biology and disease is the outcome of evolution, and many current health challenges have appeared or radically changed their dynamics recently with changes such as agriculture, urbanisation, and industrialisation. But how does human biology adapt to such challenges? The Ancient Genomics Lab uses ancient DNA as a direct source of information from the past to understand the evolutionary processes that shaped human genome variation. The goal is to link evolution and population history to historical factors such as epidemics, environmental and societal changes, and thus uncover new insights about human biology and its mechanisms.

Our three main research directions are currently:

Fine-scale reconstruction of evolution in the ancestry of the UK Biobank
We are sequencing 1,000 ancient British genomes to add a third dimension to the world-leading resource in human medical genomics that is the UK BioBank, and the future NHS 100,000 genomes. We aim to reconstruct evolutionary processes in the genome across the past few thousand years, in response to past epidemics, and societal changes.

Adaptation of human biology and societies during worldwide transitions to agriculture. We are studying human adaptation to one of the key evolutionary transitions in the human past: that to agricultural societies. Questions surround dietary shifts, and infectious disease. We are also studying domestic dogs as a separate organism that went through the same transition.

Co-evolution of human, pathogen, and domestic animal genomes. Zoonoses are a major source of current global health challenges for infectious disease. We are retrieving ancient pathogen genomes from past individuals to directly study their past genomic diversity, and in parallel reconstruct the evolutionary history of the link between humans and domestic animals.

Since the founding of the lab, we have reconstructed the evolutionary history of domestic dog and human populations over the past 11,000 years, revealing an ancient origin of the dog. In addition, we find that adaptation to diet, such as the expansion in amylase family copy numbers also seen in humans, was more recent. We have also provided a synthesis of our understanding of human origins, which is critical of previous simplistic concepts and ideas, and contributed to collaborative projects on present-day human genomic diversity with the Sanger Institute, and early modern humans in Europe with the Max Planck Institute of Evolutionary Anthropology. Other new finds still in preparation include preliminary detection of syphilis and leprosy genomes in ancient skeletons, and finds of previously unknown adaptations to agriculture in ancient Europe.

As a postdoc at Harvard Medical School during the period of the quinquennium that spans April 2015-November 2017, my research revealed complex founding of population structure in the Americas, the origins of population structure in the Pacific, and adaptations and past genomic diversity in Africa. I also studied the origin of dogs, the early history of Easter Island, and ancestry of archaic humans in Eurasia.

Research outputs

A Bergström, C Stringer, M Hajdinjak, E Scerri, P Skoglund. (2021) *Origins of modern human ancestry*. Nature 590(7845): 229-237. DOI: 10.1038/s41586-021-03244-5
This is a review paper that addresses the question on the origins of the ancestry of modern humans, *Homo sapiens.* Together with experts in the fossil and archaeological records, we synthesize recent evidence in those fields as well as genetics to conclude that there is currently no support for often-cited simple narratives of the origin of human ancestry. We currently can't identify a specific time or place of origin with a larger period and the region of Africa.

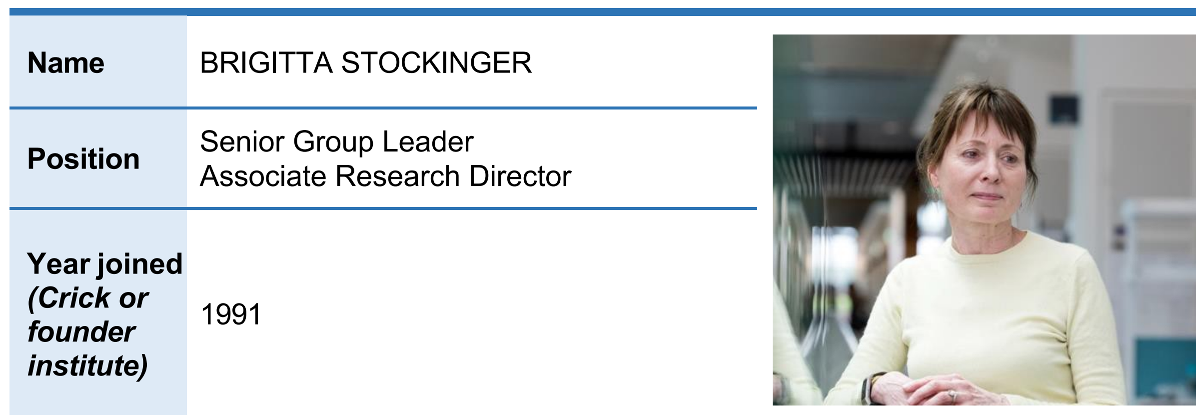
Bergström A et al. (2020) *Origins and genetic legacy of prehistoric dogs*. Science 370 (6516), 557 – 564. DOI: 10.1126/science.aba9572
This first research paper led by our lab presents the first large-scale study of ancient genomes from early domestic dogs. We show that dogs were domesticated prior to the agricultural transition, with a dynamic history that includes collapse of early genetic diversity of dogs in Europe, and a complex evolution of genetic adaptation to starch rich diets.

A Bergström, S A McCarthy, R Hui, MA Almarri, Q Ayub, P Danecek, Y Chen, Sabine Felkel, P Hallast, J Kamm, H Blanché, J-F Deleuze, H Cann, S Mallick, D Reich, MS Sandhu, P Skoglund, A Scally, Y Xue, R Durbin, C Tyler-Smith. (2020) *Insights into human genetic variation and population history from 929 diverse genomes*, Science, 367(6484):eaay5012. DOI: 10.1126/science.aay5012
In this paper, led by the Sanger Institute, we characterised global human genetic diversity in whole-genomes at a greater scale and with broader diversity than before. We documented complex divergence between modern humans and archaic groups such as Neanderthals. Lead author Anders Bergström started work on the project during PhD studies at the Sanger, and continued it with my input as a Crick postdoc from April 2018 until publication in 2020.

P Skoglund, J Thompson, ME Prendergast, A Mittnik*, K Sirak*, M Hajdinjak*, T Salie*, N Rohland, S Mallick, A Peltzer, A Heinze, I Olalde, M Ferry, E Harney, M Michel, K Stewardson, J Cerezo-Roman, C Chiumia, A Crowther, E Gomani-Chindebvu, AO Gidna, KM Grillo, G Hellenthal, R Helm, M Horton, S Lopez, AZP Mabulla, J Parkington, C Shipton, R Tibesasa, M Welling, V Hayes, DJ Kennett, R Ramesar, M Meyer, S Pääbo, N Patterson, A Morris, N Boivin, R Pinhasi, J Krause, D Reich (2017) *Reconstructing prehistoric African population structure.* Cell, 171:59-71. DOI: 10.1016/j.cell.2017.08.049
The first population genomic study of prehistoric Africa. We developed new methods for studying selective sweeps and polygenic selection using ancient genome data, revealing previously unknown diversity and adaptation in Africa during the past 8,000 years.

P Skoglund, C Posth, K Sirak, M Spriggs, F Valentin, S Bedford, GA Clark, C Reepmeyer, D Fernandes, Q Fu, E Harney, M Lipson, S Mallick, M Novak, N Rohland, K Stewardson, S Abdullah, MP Cox, FR Friedlaender, JS Friedlaender, T Kivisild, G Koki, P Kusuma, DA Merriwether, F-X Ricaut, JTS Wee, N Patterson, J

Krause, R Pinhasi*, D Reich*. (2016) ***Genomic insights into the peopling of the Southwest Pacific.*** **Nature, 538:510–513. DOI: 10.1038/nature19844**
In this study of the first tropical ancient DNA, we revealed how the first people in Remote Oceania had an ancestry that contributed to groups across the region today.



Name	BRIGITTA STOCKINGER
Position	Senior Group Leader Associate Research Director
Year joined *(Crick or founder institute)*	1991
Lab Name	***AhRimmunity Laboratory***

Research programme and achievements

Work in my lab has focused until recently on two areas of research: plasticity of effector T cells, in particular Th17 cells, and investigation of environmental influences on intestinal homeostasis and inflammation.

With our identification of the main characteristics of protective Th17 cells vs inflammatory Th17 cells in the gut (see Ref.1) and the departure of the postdoc leading this project, I have terminated our Th17 program. The lab is now concentrating fully on the investigation of environmental triggers via the aryl hydrocarbon receptor (AHR) and their influence in barrier organs, particularly the gut (Ref 2). This work has so far established a critical role for AHR in homeostasis of the intestinal immune system, extended recently by our demonstration that AHR also has cell intrinsic functions in intestinal epithelial cells, notably crypt stem cells (Ref3). AHR regulation of the Wnt pathway is one of the regulatory functions of AHR that prevent overproliferation of stem cells and their malignant transformation. AHR ligands supplied by the diet and generated by the microbiota play critical roles for AHR activation. Most recently a collaboration with Vassilis Pachnis' lab has shown that AHR is also important in the function of enteric neurons so that the interaction between the enteric nervous system, environmental factors and the microbiota maintains the functional output of the gut barrier (Ref 4).

Future work will also look at AHR function in endothelial cells and decipher the molecular mechanisms underlying the complex functions of AHR in the intestine and beyond.

Research outputs

Omenetti, S., Bussi, C., Metidji, A., Iseppon, A., Lee, S., Tolaini, M., Li, Y., Kelly,G. Chakravarty, P., Shoaie, S., Gutierrez, M.G., and Stockinger, B. (2019) *The intestine harbors functionally distinct homeostatic tissue-resident and inflammatory Th17 cells*. Immunity 51, 77-89. DOI: 10.1016/j.immuni.2019.05.004
In this paper, we identified the distinction between inflammatory Th17 cells elicited by pathogens and tissue resident, barrier protective Th17 cells. This will be important in therapeutic interventions targeting Th17 cells as it will allow safeguarding the resident beneficial population.

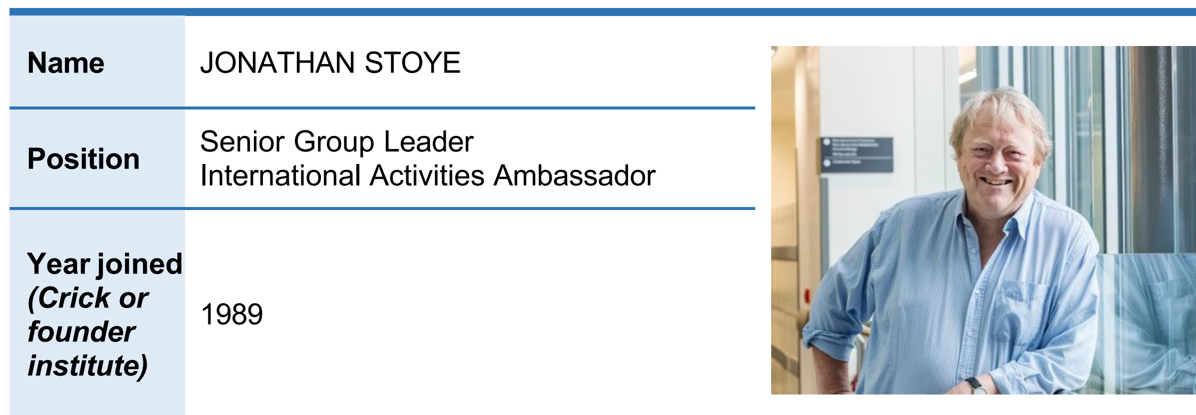
Schiering, C; Wincent, E; Metidji, A; Iseppon, A; Li, Y; Potocnik, AJ; Omenetti, S; Henderson, CJ; Wolf, CR; Nebert, DW and Stockinger, B. (2017) *Feedback control of AHR signalling regulates intestinal immunity*. Nature 542, 242-245. DOI: 10.1038/nature21080

This paper established the AHR induced feedback system of cytochrome P4501 metabolising enzymes as critical regulators of AHR signalling. Excessive ligand degradation via Cytochrome P4501 phenocopies AHR deficiency and has detrimental consequences for intestinal health which can be counterbalanced by increasing the intake of AHR ligands in the diet. The intestinal epithelium acts as gatekeeper for the supply of ligands throughout the body emphasising the importance of the gut barrier for whole body physiology.

Metidji, A., Omenetti, S., Crotta, S., Li, Y., Nye, E., Ross, E., Li, V., Maradana, M., Schiering, C., and Stockinger, B. (2018) ***The environmental sensor AHR protects from inflammatory damage by maintaining intestinal stem cell homeostasis and barrier integrity*****. Immunity, 49, 353-362. DOI: 10.1016/j.immuni.2018.07.010**
This paper demonstrates a cell intrinsic role for AHR in intestinal stem cells. AHR deficiency in intestinal epithelium causes dysregulation of the Wnt pathway,

overproliferation of crypt stem cells and impaired epithelial differentiation following injury, culminating in tumorigenesis.

Obata, Y., Castano, A., Boeing, S. Bon-Frauches, A.C., Fung, C., Fallesen, T., Gomez de Aguero, M., Yilmaz, B., Lopes, R., Huseynova, A., Horswell, S., Maradana, M., Boesmans, W., Vanden Berghe, P., Murray, A.J., Stockinger, B., Macpherson, A., and Pachnis, V. (2020***) Neuronal programming by microbiota regulates intestinal physiology*****. Nature 578, 284-289. DOI: 10.1038/s41586-020-1975-8**
This paper identified AHR signalling in enteric neurons as a regulatory node that integrates the microbiota with the physiological output of intestinal neural circuits to maintain gut homeostasis.



Name	JONATHAN STOYE
Position	Senior Group Leader International Activities Ambassador
Year joined *(Crick or founder institute)*	1989

Lab Name	***Retrovirus-Host Interactions Laboratory***

Research programme and achievements

Throughout evolutionary history, eukaryotes have been subjected to genetic assault by a variety of different retroelements capable of inserting their genomes into those of their hosts. Some of these elements (such as retroviruses like HIV-1) also cause harm by non-integrative mechanisms. As a consequence, a number of intrinsic defence mechanisms have evolved to neutralise these retroelements; in turn, viral countermeasures have developed. My interests span various aspects of the ensuing genetic to and fro. Specifically, we study:

A. **Mechanisms for preventing infection**

Still our prime focus is the restriction gene Fv1, arguably the prototypic restriction factor and first identified in my lab, and other host factors that bind to the capsid core of newly infecting viruses, blocking an early step in the viral life cycle. We would like to understand the long-term survival of these factors and the principles underlying virus recognition. Recently we have shown that maintenance of the Fv1 open reading frame requires continued selection, implying an on-going series of virus infections reaching back millions of years. We have shown the presence of Fv1 in a wide variety of Muridae and identified variants with the ability to restrict five different genera of retroviruses carrying CA proteins with very different primary sequences. This has prompted us, in collaboration with the group of Ian Taylor, to examine the 3D structures of CA from retroelements with increasing divergence from modern day retroviruses. We have demonstrated significant structural conservation in the capsid core of all retroelements, including those of yeast and *Drosophila*, and this may explain why a limited number of sequence changes in Fv1 can allow restriction of viruses showing 90% sequence divergence. We are currently collaborating with a group in the USA to study copy number control of Ty1 elements in yeast which appears to operate by a mechanism closely resembling Fv1 restriction of murine leukemia virus (MLV). We anticipate that these comparative approaches will shed further light on the mechanism of Fv1 action.

We also study the restriction factor SAMHD1 which we have shown to act on retrovirus replication by reducing intracellular dNTP pools with consequent effects on reverse transcription (collaborations with Kate Bishop and Ian Taylor).

Lentiviruses encode a handful of accessory proteins capable of targeting potential restriction factors for destruction by the cellular proteasomes. Probing the interaction between one such protein, Vpx, SAMHD1 and cellular ubiquitinating enzymes has revealed how lentiviral accessory proteins have been retargeted during virus evolution to deal with different host proteins.

Our studies have also highlighted the key role played by SAMHD1 as a regulatory enzyme in cell metabolism. We are currently seeking to identify small molecules targeting SAMHD1 function in high throughput screens.

B. **Mechanisms for silencing insertions.**

Cells possess several mechanisms for silencing inserted retroelements. One of these is the HUSH system which silences gene expression by a mechanism involving the spread of H3K9me3 marks on integrated transgenes. We are examining how this system acts on newly integrated viruses. In addition to its effects on SAMHD1, Vpx can also act to prevent HUSH silencing by a mechanism seemingly involving degradation of one or more HUSH components by proteasomes. We are examining the hypothesis that this represents a further example of accessory protein retargeting to cause degradation of a factor inhibiting virus expression, mediated by hijacking of the cellular pathway for proteolysis.

A second silencing mechanism involves the recruitment of Trim28/Kap1 and Setdb1 to ERVs by cellular Krüppel-associated box domain zinc finger proteins (ZFPs) to mediate their silencing by histone methylation. In collaboration with George Kassiotis, we have recently shown that *Gv1*, a murine gene regulating ERV expression in multiple strains of mice, encodes the ZFP *2410141K09Rik* and are exploring its mode of action in greater detail to shed greater light on the roles of ZFPs in controlling ERV expression. Despite control mechanisms of this kind, ERVs can be expressed with a variety of consequences. We therefore continue to explore patterns of ERV expression and their potential roles in normal physiology and pathology.

Research outputs

Young, G. R., M. W. Yap, J. R. Michaux, S. J. Steppan and J. P. Stoye. (2018). *Evolutionary journey of the retrovirus restriction gene Fv1*. Proc Natl Acad Sci., USA. 115:10130-10135. DOI: 10.1073/pnas.1808516115

This manuscript explores the evolutionary history of Fv1, demonstrating that the gene has its origins 45 million ago and thus is much older than previously thought. Modelling studies indicated that the maintenance of the gene's open reading frame for this period of time can only be explained by repeated selection events from waves of retroviral infection throughout murid evolution.

Yap, M. W., G. R. Young, R. Varnaite, S. Morand and J. P. Stoye. (2020). *Duplication and divergence of the retrovirus restriction gene Fv1 in Mus caroli allows protection from multiple retroviruses*. PLoS Genetics. 16(6):e1008471. DOI: 10.1371/journal.pgen.1008471.

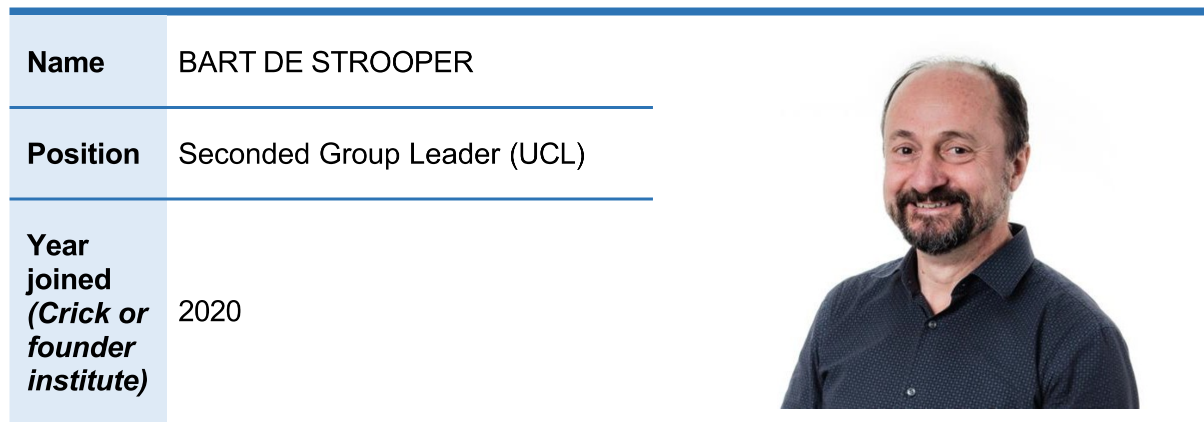
It is thought that retroviruses and their hosts are locked in an evolutionary arms race characterised by development and escape from novel restriction mechanisms. This study examines the status of Fv1 in wild populations of mice from Thailand. It describes a gene duplication event allowing selection of simultaneous restriction of two virus genera. It helps to define limits to the range of restriction possible with a single factor.

Ball, N. J., G. Nicastro, M. Dutta, D. J. Pollard, D. C. Goldstone, M. Sanz-Ramos, A. Ramos, E. Müllers, K. Stiornnagel, N. Stanke, D. Lindemann, J. P. Stoye, W. R. Taylor, P. B. Rosenthal and I. A. Taylor. (2016). *Structure of a spumavirus Gag central domain reveals an ancient retroviral capsid*. PLoS Pathogens. 12(11):e1005981. DOI: 10.1371/journal.ppat.1005981.

In contrast to orthoretroviruses, spumaraviruses contain an unprocessed Gag protein. We had previously shown that (i) Fv1 and Trim5 restriction was dependent on an assembled viral core and (ii) that they could restrict spumaviruses. This prompted us to examine the structure of the central domain of the spumavirus Gag protein, revealing a previously unsuspected structural similarity to orthoviral CA implying a common evolutionary origin.

Cottee, M. A., S. C. Letham, G. R. Young, J. P. Stoye and I. A. Taylor. (2020). ***Structure of the Drosophila melanogaster ARC1 reveals a repurposed molecule with characteristics of retroviral Gag*****. Sci Adv. 6(1):eaay6354. DOI: 10.1126/sciadv.aay6354**
A study of a *Drosophila* protein important for learning and memory, derived from an exapted retrotransposon of the Metaviridae family. Its structure is fully consistent with hypothesised roles for ARC1 in packaging mRNA for transfer across synaptic junctions. However, subtle differences between the *Drosophila* and tetrapod proteins suggest independent exaption events. The overall similarity with the CA protein of the Retriviridae provides further evidence for common evolutionary origins of all retroelements.

Ordonez, P., S. Kunzelmann, H. C. Groom, M. W. Yap, S. Weising, C. Meier, K. N. Bishop, I. A. Taylor and J. P. Stoye. (2017). ***SAMHD1 enhances nucleoside-analogue efficiency against HIV-1 in myeloid cells*****. Sci Rep 7:42824. DOI: 10.1038/srep42824.**
A study examining the effects of dNTP depletion by introduced SAMHD1 on the anti-HIV activity of a number of nucleotide analogues that are not normally considered antiretrovirals. It points to the possibility of manipulating the activities of such drugs, not just allowing redirection towards HIV, but also in potentiating anti-cancer effects, by altering SAMHD1 activity.



Name	BART DE STROOPER
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Cellular Phase of Alzheimer's Disease Laboratory***

Research programme and achievements

Alzheimer's disease (AD) is characterised by the build-up of abnormal proteins and widespread neurodegeneration. In individuals with the familial form of the condition, mutations in the amyloid precursor protein (APP), and proteins involved in processing o f APP, such as gamma & beta secretase, cause the production of longer amyloidogenic amyloid beta (Aβ) peptides – thought to be the primary cause of AD. The amyloid-cascade hypothesis has been the prevailing theory for disease pathogenesis, however, results from clinical trials that target components of this pathway have proved disappointing. However, we have much to learn from their 'failures', and we must also address the heterogeneity and stages of different disease types.

There is now a greater appreciation within the field as to the vast complexity of AD and other neurodegenerative disorders. To decipher disease mechanisms, Prof Bart De Strooper's research has interrogated the genetics of both the familial and sporadic forms of the condition. Genome wide association studies (GWAS) consistently flag microglia and astroglia besides the amyloid pathway and Bart believes that targeting the cellular reaction to the Aβ may prove more effective than the protein itself.

In this new programme at the UK DRI at UCL, Bart and his team will focus on determining the downstream effects of Aβ, from the biochemical to cellular phase, and deciphering both the beneficial and detrimental response of brain cells such as microglia, astrocytes and oligodendrocytes. Recent evidence suggests that there are thousands of risk genes that confer a low risk for the development of AD. Bart is particularly interested in these previously overlooked genes as, when combined with other risk genes, and/or in aggravating conditions e.g. ageing or Aβ deposition, they may have a greater detrimental impact and lower the threshold for disease onset. This may be a critical aspect of sporadic forms of AD, where a strong genetic component, e.g. mutations in APP, is not observed.

Although there are numerous animal models aiming to mimic AD, none of them recapitulate all characteristics and pathologies of the condition fully. Furthermore, while mouse and human immune cells, such as microglia, have similar transcriptional signatures, this partially changes with age. Recent evidence also suggests the signatures from human microglia derived from AD brains, differ dramatically from mouse microglia in disease conditions. In attempts to overcome these issues, Bart's group has recently developed a chimeric model by injecting human microglia derived from embryonic stem cells into brains of young mice. Eight weeks later, the human cells had taken on a seemingly normal, ramified appearance and distributed across the mouse brain in a classic microglial tiling pattern. Crucially, it appears they also keep their human transcriptomic signature. This model, therefore, has the potential to revolutionise microglia studies, yielding results more accurately reflecting the human condition.

The transcriptomic work carried out thus far has highlighted the extensive heterogeneity across microglial cell phenotypes, however, questions still remain as to the functional consequences in a disease context. By building collaborations and drawing on expertise in areas such as proteomics and lipidomics, Bart's group will bring together this knowledge to push toward therapeutic development for the clinic.

Main objectives and research goals:

1. Elucidate the downstream effects of Aβ with respect to cellular response.

2. Utilise the novel humanised chimeric models to study the role of human microglia, astroglia, human neurons and oligodendrocytes in the cellular phase of Alzheimer's Disease.

3. Explore low-risk genetic factors that may reveal cellular components and pathways important in AD mechanism.

4. Use Spatial transcriptomics and single cell technologies to make the map of the molecular and cellular changes in Alzheimer's disease over different Braak and Thal stages.

5. Identify targets for early therapeutic intervention.

Research outputs

Key Publications covering career highlights:

Cellular phase of Alzheimer's Disease

Mancuso R, Van Den Daele J, Fattorelli N, Wolfs L, Balusu S, Burton O, Liston A, Sierksma A, Fourne Y, Poovathingal S, Arranz-Mendiguren A, Sala Frigerio C, Claes C, Serneels L, Theys T, Perry VH, Verfaillie C, Fiers M, De Strooper B. (2019) *Stem-cell-derived human microglia transplanted in mouse brain to study human disease.* Nat Neurosci 22, 2111-2116. DOI: https://doi.org/10.1038/s41593-019-0525-x

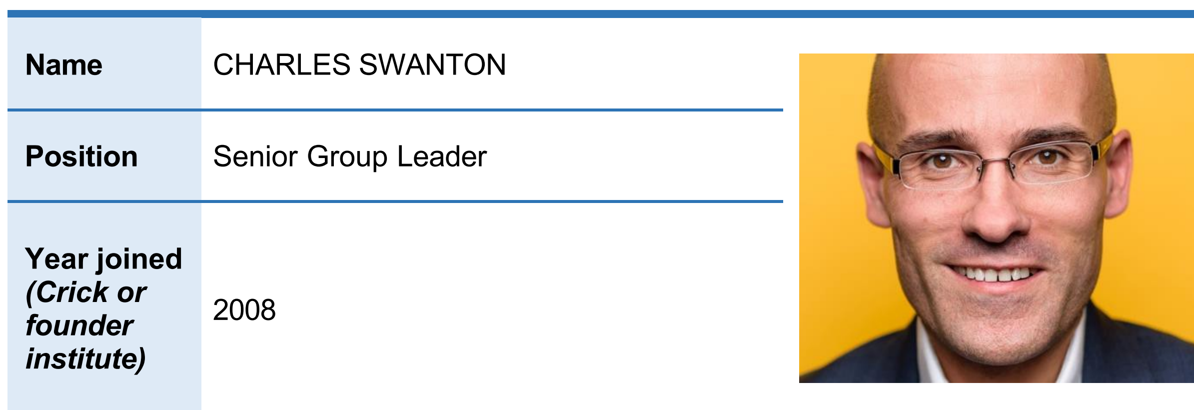
Chen WT, Lu A, Craessaerts K, Pavie B, Sala Frigerio C, Corthout N, Qian X, Laláková J, Kühnemund M, Voytyuk , Wolfs L, Mancuso R, Salta E, Balusu S, Snellinx A, Munck S, Jurek A, ez Navarro JF, Saido TC, Huitinga I, Lundeberg J, Fiers M, De Strooper B. (2020) *Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease.* Cell 182(4):976-991.e.19. DOI: https://doi.org/10.1016/j.cell.2020.06.038

Studies of the genes causing Alzheimer's Disease

Rice HC, de Malmazet D, Schreurs A, Frere S, Van Molle I, Volkov AN, Creemers E, Vertkin I, Nys J, Ranaivoson FM, Comoletti D, Savas JN, Remaut H, Balschun D, Wierda KD, Slutsky I, Farrow K, De Strooper B, de Wit J. (2019) *Secreted amyloid-β precursor protein functions as a $GABA_BR1a$ ligand to modulate synaptic transmission*. Science 363, 6423:eaao4827. DOI: 10.1126/science.aao4827

Habets RA, de Bock CE, Serneels L, Lodewijckx I, Verbeke D, Nittner D, Narlawar R, Demeyer S, Dooley J, Liston A, Taghon T, Cools J, De Strooper B. (2019) *Safe targeting of T cell acute lymphoblastic leukemia by pathology-specific NOTCH inhibition*. Science Transl Med 11, 494:eaau6246. DOI: 10.1126/scitranslmed.aau6246

Szaruga M, Munteanu B, Lismont S, Veugelen S, Horré K, Mercken M, Saido TC, Ryan NS, De Vos T, Savvides SN, Gallardo R, Schymkowitz J, Rousseau F, Fox NC, Hopf C, De Strooper B, Chávez-Gutiérrez L. (2017) *Alzheimer's-Causing Mutations Shift Aβ Length by Destabilizing γ-Secretase-Aβn Interactions.* Cell 170(3):443-456.e14. DOI: 10.1016/j.cell.2017.07.004



Name	CHARLES SWANTON
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2008

Lab Name	***Cancer Evolution and Genome Instability Laboratory***

Research programme and achievements

The laboratory's goals are to address the causes and consequences of tumour evolution in the context of the clinical challenges of cancer drug resistance. The group has focused on deciphering drivers of intercellular heterogeneity precipitating intratumour heterogeneity and its impact upon cancer evolution and drug resistance in the clinical setting.

Through the longitudinal cancer evolution program, TRACERx, the laboratory has contributed to an understanding of tumour branched evolution and selection, the role of DNA replication stress, APOBEC mutagenesis, HLA loss of heterozygosity, chromosomal instability, genome doubling events and cancer cytotoxic drugs as key factors contributing to cancer subclonal evolution. We have found that chromosomal instability, rather than point mutational diversity, is associated with rapid disease progression and widespread dissemination in both non-small cell lung cancer and renal cancer. We have developed new publicly available tools to decipher tumour mutational signatures and HLA Loss of heterozygosity events and their impact upon immune evasion from cancer exome datasets. We have identified mechanisms contributing to the tolerance and initiation of chromosomal instability and genome doubling in cancers, and described how these large- scale genomic events are subject to selection and parallel evolution in solid tumours. We have developed bespoke approaches to detect and monitor tumour branched evolution following surgery through circulating tumour DNA analysis (ctDNA) leading to novel adjuvant clinical trial platforms to target minimal residual disease (MRD) in lung cancer that have been adopted into global clinical trials through a $300m clinical trials programme funded by Astrazeneca.

The laboratory is deriving approaches to limit cancer evolution through the targeting of multiple clonal events; we have found evidence for the importance of CD8+ cytotoxic T cells that recognise clonal neoantigens as mediators of immune surveillance, tumour control and immunotherapy response. This has led to a Crick spin-out company, Achilles Therapeutics (Wellcome Trust/Syncona; $150m series A and B funding), focused on the detection, expansion and therapeutic delivery of adoptive T cell therapies to patients, by targeting multiple clonal neoantigens in lung cancer and melanoma. In 2020, this resulted in the initiation of two first in man Phase 1 clinical trials.

Over the next five years we aim to identify tumour microenvironmental constraints to tumour evolution and mechanisms of immune escape, immune checkpoint inhibitor and drug resistance and subclonal cancer evolution. We plan to investigate the mechanistic basis for the selection of subclones during evolution that precipitate metastatic dissemination in hundreds of patients from TRACERx and the PEACE autopsy programmes. Finally, we are taking our discovery

research findings into clinical trials where we will test these novel diagnostic MRD strategies and therapeutic approaches with neoantigen reactive T cells targeting multiple clonal antigens to attempt to delay resistance and improve survival.

Research outputs

Jamal-Hanjani M et al. (2017) ***Tracking the evolution of non-small-cell lung cancer*****. New England Journal of Medicine 376, 2109 – 2121. DOI: 10.1056/NEJMoa1616288.**
This work evaluates the relationship between intratumour heterogeneity of single nucleotide variants and somatic copy number aberrations and recurrence free survival in non-small cell lung cancer. Diversity of chromosome number or structure rather than single nucleotide variants is associated with poorer recurrence free survival, independent of tumour stage in multivariable analyses. Through subclonal copy number analyses, mirrored subclonal allelic imbalance is found, driving parallel evolution of chromosome copy number gains or losses on either the maternal or paternal chromosome in different regions of the same tumour.

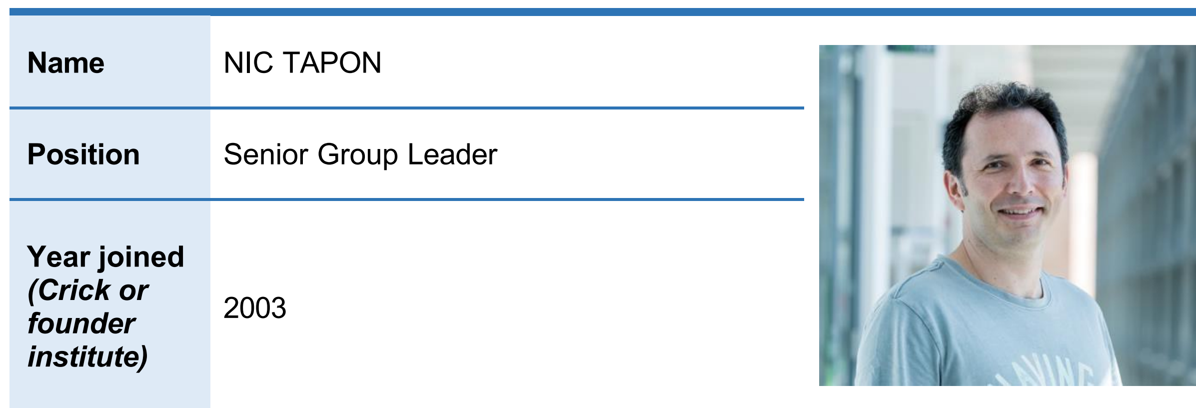
Abbosh C et al. (2017) ***Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution*****. Nature 545, 446 – 451. DOI: 10.1038/nature22364**
This TRACERx work shows that bespoke patient-specific panels to analyse ctDNA can be used to monitor MRD recurrence and tumour branched evolution in the adjuvant setting in the absence of macroscopic disease, and that tumour Ki67 index, necrosis, squamous histology and FDG-PET avidity are closely associated with ctDNA release. We further demonstrate the limitations of ctDNA approaches for early detection as a function of tumour volume and cancer cell number, and show that the subclone identified in ctDNA prior to disease recurrence is identical to the tumour subclone identified at metastatic sites, permitting adjuvant MRD studies to prevent recurrence.

McGranahan N et al. (2016) ***Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade*****. Science 351 (6280), 1463 – 1469. DOI: 10.1126/science.aaf1490**
We investigated how intratumour heterogeneity affects response to checkpoint blockade and how cancers might be treated based on evolutionary principles. Durable clinical benefit to checkpoint blockade in lung cancer and melanoma was associated with a high burden of clonal neoantigens; in contrast, tumours that progressed early on therapy had a higher burden of heterogeneous neoantigens. We could predict clonal neoantigens present in every tumour cell and identify immune cells that recognise them, suggesting that development of adoptive T cell or vaccination strategies targeting such clonal neoantigens might limit therapeutic escape and resistance mechanisms.

McGranahan N et al. (2017) ***Allele-specific HLA loss and immune escape in lung cancer evolution*****. Cell 171 (6), 1259 – 1271. DOI: 10.1016/j.cell.2017.10.00**
Through an analysis of TRACERx, extended from our haplotyping analysis (reference 1), we developed an algorithm called LOHHLA which infers allele specific copy number aberrations in HLA. We find HLA loss occurs in 40% of early stage lung cancers, usually as a subclonal event, and is permissive for branched evolution associated with expansion of mutations predicted to bind the lost HLA allele.

Rosenthal R et al. (2019***) Neoantigen-directed immune escape in lung cancer evolution*****. Nature 567, 479 – 485. DOI: 10.1038/s41586-019-1032-7**
We analysed the first 100 TRACERx patients to unravel how escape from adaptive immunity occurs in non-small cell lung cancer. Immune ‘hot’ tumours, characterised by a

brisk lymphoid infiltrate, had been selected for HLA LOH or deleterious mutations in the antigen presentation machinery. In contrast immune ‘cold’ tumours with an absent lymphoid infiltrate had lost clonal neoantigens through DNA copy number loss events. We found evidence for negative selection of subclones early in tumour evolution harbouring neoantigens in genes essential for non-small cell lung cancer viability. Patient outcome was worse for tumours with evidence of an immune evasion event.



Name	NIC TAPON
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2003

Lab Name	***Apoptosis and proliferation control laboratory***

Research programme and achievements

Lab interests
How do cells in a developing organism stop growing and dividing when the correct body size and shape have been reached? How is tissue size maintained in an adult organism? These fundamental biological questions have clear implications for cancer, where cells lose the ability to respond to tissue size boundaries, and for regenerative medicine, where the proliferative potential of quiescent cells must be unleashed in a controlled manner. Our long-term goal is to unravel how a diverse set of cues acting at the local, tissue autonomous level (e.g. mechanical forces, tissue architecture, developmental signalling pathways) are integrated with systemic signals (e.g. nutrient availability, hormones) to determine final animal size. Many of these signals modulate the activity of the Hippo signalling pathway. Thus, much of our work is focused on studying how the Hippo pathway senses growth-regulatory signals.

We use a multidisciplinary set of approaches, including *Drosophila* and mouse genetics, quantitative *in vivo* imaging, biophysics, mathematical modelling and proteomics, as well as structural biology.

Past work
In the past quinquennium, our major contributions have defined some of the key signals that tune Hippo pathway activity *in vivo*:

(I) We identified the energy sensor AMPK as an upstream input that scales the Hippo pathway growth-regulatory signal with energy availability. This work also demonstrated remarkable differences in the wiring of the Hippo pathway in different neural stem cell populations, suggesting that targeting Hippo signalling for therapeutic benefit will entail a better understanding of context-dependent signalling.

(II) Cell-cell junctions are a key site of Hippo pathway regulation, particularly by mechanical forces. Our work elucidated the mechanism through which the mechanosensitive protein Zyxin modulates junctional Hippo signalling. Zyxin antagonises the function of the key Hippo pathway upstream component Expanded via its ability to promote Enabled-dependent actin assembly. Furthermore, we elucidated a mechanism through which rapid ubiquitin-dependent turnover of Expanded at the apical membrane allows the Hippo pathway to be poised to rapidly respond to upstream signals.

(III) Finally, our interest in Hippo pathway phosphatases led us to structurally define a new PP1 recognition motif and to identify a novel coupling mechanism between different types of cell polarity.

Future work

During the past quinquennium, we have devoted substantial effort to establishing genetic, *in vivo* imaging and quantitative biology tools to exploit the *Drosophila* abdomen as a powerful system to study growth dynamics and growth termination during development.

This includes the creation of machine-learning and mathematical modelling approaches in collaboration with the Salbreux lab at the Crick which are providing us with unprecedented access to the dynamics of a growing epithelium. We will use this system to understand how tissue size is defined in a developing organism. In particular, we will unravel how diverse outputs such as mechanical forces, circulating hormones/growth factor and nutrients are integrated through the Hippo pathway and other signalling networks in order to precisely tune growth rates, growth termination and differentiation to yield highly reproducible organ size. We are also in the process of generating genetic tools in non-*melanogaster* species of different sizes. This cross-species approach will allow us to define which size control mechanisms are conserved across species, and which underpin the wide variety in tissue size and shape that is a striking characteristic of the animal kingdom.

In parallel to our *in vivo* work, we are developing cell-based systems that enable us to precisely manipulate the mechanical environment of cells through cell-cell junctions and cell-extracellular matrix contacts. We will use these tools to understand how the Hippo signalling pathway is regulated by mechanical forces emanating from the cellular microenvironment, and which upstream molecular pathways mediate these responses. This biophysical and molecular approach will enable us to better understand how the Hippo pathway functions in a complex mechanical environment *in vivo*.

Finally, we will elucidate the role of Hippo pathway-regulating PP1 phosphatase complexes in tissue growth and architecture. To approach this question, we will study the function of the RASSF protein family, many members of which are key regulatory subunits in PP1 complexes, using both *in vivo* mouse genetics and *exvivo* organoid culture approaches.

Research outputs

Bertran MT, Mouilleron S, Zhou Y, Bajaj R, Uliana F, Kumar GS, van Drogen A, Lee R, Banerjee JJ, Hauri S, O'Reilly N, Gstaiger M, Page R, Peti W, Tapon N (2019) *ASPP proteins discriminate between PP1 catalytic subunits through their SH3 domain and the PP1 C-tail*. Nature Communications 10(1):771-771 DOI: 10.1038/s41467-019-08686-0
Our past work had indicated that the ASSP proteins are key regulators of junctional dynamics during morphogenesis. Here we used a multidisciplinary approach encompassing X-ray crystallography, NMR, biochemistry and genomic engineering in flies to unravel the function of ASPP as a PP1 phosphatase targeting subunit. We identified a novel PP1 rectuitment mode where ASPP uses both a canonical RVxF motif and its SH3 domain to discriminate between PP1 isoforms based on the divergent PP1 C-tail. This new mode of SH3 domain/PP1 interaction vastly increases the scope to discover new substrates and accessory subunits for PP1 holoenzymes.

Fulford AD*, Holder MV*, Frith D, Snijders AP, Tapon N+, Ribeiro PS+ (2019) *Casein kinase 1 family proteins promote Slimb-dependent Expanded degradation*. eLife 8 e46592. DOI: 10.7554/eLife.46592
The Hippo signalling pathway is a central regulators of tissue size during development and adult homeostasis. Although we have a good static picture of how the Hippo pathway functions, its signalling dynamics remain poorly understood. Here we elucidated how the Hippo pathway upstream component Expanded is constantly turned over at its site of activity at the apical membrane. This occurs through its recruitment by the polarity protein Crumbs, followed by Casein Kinase 1-dependent phosphorylation and recruitment of the

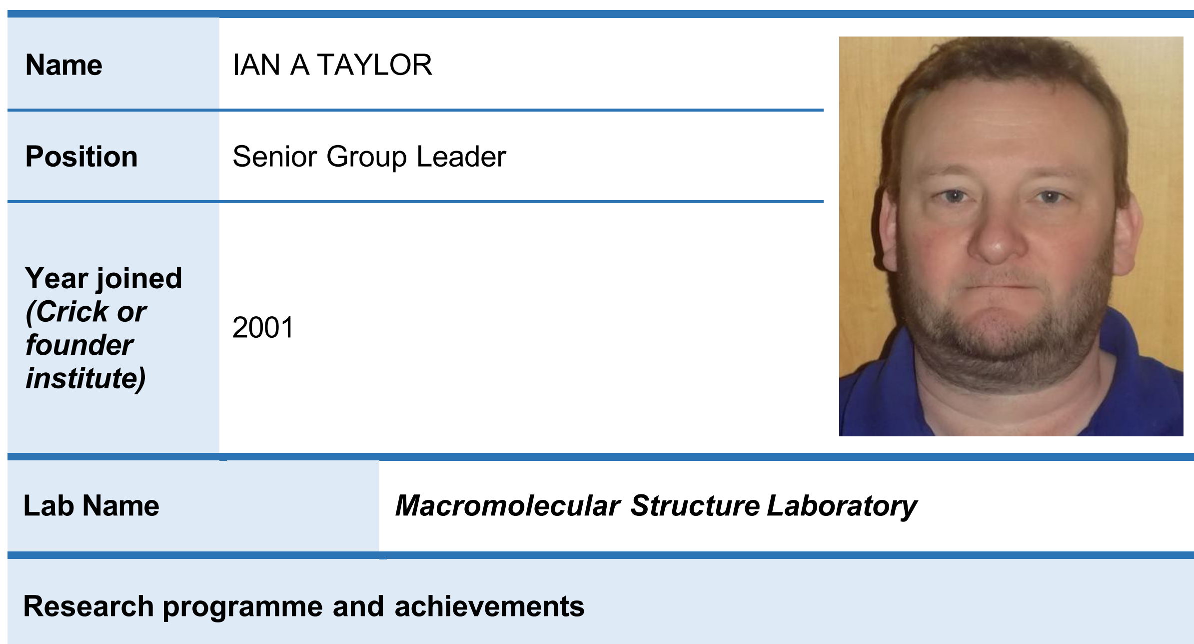
SCF^{Slimb} ubiquitin ligase complex. This ensures that Hippo signalling is poised to respond to external stimuli such as loss of cell polarity.

Banerjee JJ, Aerne BL, Holder MV, Hauri S, Gstaiger M, Tapon N (2017) *Meru couples planar cell polarity with apical-basal polarity during asymmetric cell division*. e Life 6 e25014. DOI: 10.7554/eLife.25014

Polarity is a shared feature of most cells. From a limited set of core building blocks (e.g. the Par complexes and the Frizzled/Dishevelled Planar Cell Polarity complexes), a vastly diverse array of polarised cells and tissues is generated. This suggests the existence of little-studied tissue-specific factors that rewire the core polarity modules to the appropriate conformation for each cellular context. We identified the RASSF family protein Meru as such a factor, and showed that it fulfils a key function in bridging apico-basal and planar cell polarity during asymmetric cell division.

Gailite I, Aerne BL, Tapon N (2015) *Differential control of Yorkie activity by LKB1/AMPK and the Hippo/Warts cascade in the central nervous system*. Proc Nat Acad Sci USA 112(37):E5169-E5178. DOI: 10.1073/pnas.1505512112
The Hippo pathway is a highly conserved tumour suppressor network that restricts developmental tissue growth and regulates stem cell proliferation and differentiation. As a growth regulatory pathway, Hippo signalling needs to integrate information about nutrient and energy levels to ensure that growth proceeds only when sufficient resources are available. In this study, we uncovered a key regulatory input from the energy sensor AMP-activated protein kinase (AMPK) in repressing the activity of Yki, the transcription factor target of the Hippo pathway.

Gaspar P, Holder MV, Aerne BL, Janody F, Tapon N (2015) *Zyxin antagonizes the FERM protein expanded to couple F-actin and Yorkie-dependent organ growth*. Current Biology 25(6):679-689. DOI: 10.1016/j.cub.2015.01.010
Coordinated multicellular growth during development is achieved by the sensing of spatial and nutritional boundaries. The conserved Hippo signalling pathway has been proposed to restrict tissue growth by perceiving mechanical constraints through actin cytoskeleton networks. Here, we elucidated the mechanism through which the mechanosensor Zyxin modulate Hippo pathway activity. We showed that Zyxin antagonises the FERM-domain protein Expanded (Ex) to control tissue growth, eye differentiation, and F-actin accumulation and established a link between actin filament polymerisation and Hipppo pathway activity.



Name	IAN A TAYLOR
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2001

Lab Name	***Macromolecular Structure Laboratory***

Research programme and achievements

Despite many years of progress, HIV infection and AIDS still remain global health concerns. HIV-1 is presently incurable, there is no vaccine and the economic burden of current drug regimens is significant. Therefore, research into HIV-1 and other retroviruses has the goal of furthering our understanding of retrovirus biology with a view to disease eradication but also the development of antiviral compounds and new drug strategies for HIV-1 intervention. To this end, my research is focused on three key areas of retrovirus research; the retroviral capsid and post entry capsid-associating restriction factors, restriction of HIV-1 replication by SAMHD1 and lentiviral accessory proteins, viral countermeasures that evade host-cell defences.

Research into retroviral assembly and post-entry restriction factors has focused on the retroviral core, specifically the capsid protein (CA) and its interaction with host cell restriction factors Trim5α, TrimCyp, Fv1 and Mx2. We have employed structural biology methods - X-ray crystallography, solution and solid-state NMR and cryo-electron microscopy - to determine the structures of restriction factors and individual CA proteins, but also to visualise CA assemblies and analyse their interaction with restriction factors, other viral components and small molecules. These studies aim to provide a full description of the intermolecular interactions required for capsid assembly, and the molecular details of the capsid interaction with restriction factors and drugs. Longer term, we aim to translate this research into development of antiretroviral compounds that act through interaction with capsid, to work alongside existing reverse transcriptase, protease and integrase inhibitors.

Other restriction factor research is focussed on SAMHD1, a dNTP triphosphohydrolase that inhibits HIV-1 replication through depletion of the cellular dNTP pool. SAMHD1 also maintains normal cellular dNTP homeostasis, and controls the efficacy of nucleotide- based antiviral and chemotherapies.Its mutation is a cause of dNTP imbalance, genome instability and human disease. SAMHD1 nucleotide binding, quaternary structure and phosphorylation all contribute to allosteric regulation and catalysis. We have employed structural, enzymological and nucleotide analogue studies to determine the mechanism of allostery and catalysis and how they impact on HIV-1 restriction, normal cellular dNTP homeostasis and drug metabolism together with the dynamics of SAMHD1 quaternary structure. One longer-term goal is to draw on these studies to prepare small molecule activators or inhibitors of SAMHD1 that can be employed in SAMHD1-targeted anti-HIV-1 and anticancer therapies.

To counter the inhibition by restriction factors, lentiviruses express the accessory proteins Vpr and Vpx, which disable cellular defence mechanisms through

reprogramming of the substrate adaptor DCAF1, recruiting restriction factors to the Cul4 E3 ubiquitin ligase to be tagged for degradation. SAMHD1 is the target for Vpx, and some Vpr proteins from Simian Immunodeficiency Viruses (SIVs) also target SAMHD1. However, in other lentiviruses, including HIV-1, the Vpr target is unclear. We have undertaken, virological, cell biological, biophysical and structural studies focused on Vpx(r)-DCAF1-SAMHD1 complexes to determine the structural basis of these host-pathogen interactions at a molecular level. These studies have been expanded into other Vpr proteins and their cellular targets and in the longer-term, these structural data will guide experimentation into a new class of small molecule "therapeutic disrupters" that work through inhibiting Vpx(r)-DCAF1 interactions, preventing the recruitment of host-cell defence proteins for degradation and exposing the virus to the effects of restriction factors.

Research outputs

Morris ER, Caswell SJ, Kunzelmann S, Arnold LH, Purkiss AG, Kelly G, Taylor IA. (2020) ***Crystal structures of SAMHD1 inhibitor complexes reveal the mechanism of water-mediated dNTP hydrolysis*****. Nat Commun 11: 3165. DOI: 10.1038/s41467-020-16983-2**
This ground-breaking structural and biochemical study determined the precise chemical mechanism of metal-water mediated SAMHD1 catalysis and provided the molecular details of SAMHD1 inhibition by nucleotide-based compounds. The study revealed how SAMHD1 both down-regulates cellular dNTP and decreases the efficacy of nucleoside-based anti-cancer and anti-viral therapies, paving the way for rational design of future SAMHD1 inhibitors.

Cottee MA, Letham SC, Young GR, Stoye JP, Taylor IA. (2020) ***Structure of Drosophila melanogaster ARC1 reveals a repurposed molecule with characteristics of retroviral Gag*****. Sci Adv 6: eaay6354. DOI: 10.1126/sciadv.aay6354**

In this structural/evolutionary study, we determined the crystal structure of neuronal protein dARC, which is essential for memory and learning, and examined its relationship with the capsid of retroviruses and retrotransposons. The study revealed that ARC proteins that arose as a result of the exaptation of ancient retrotransposon Gag genes have retained the basic building block structure and can assemble into shells with the overall architecture seen in spuma- and orthoretroviruses. Nevertheless, through adaptation, their original viral genome packaging function has been repurposed to now enable encapsidation and transfer of genetic information across synapses in the brain.

Acton O, Grant T, Nicastro G, Ball NJ, Goldstone DC, Robertson LE, Sader K, Nans A, Ramos A, Stoye JP, Taylor IA*, Rosenthal PB*. (2019) ***Structural basis for Fullerene geometry in a human endogenous retrovirus capsid*****. Nat Commun 10: 5822. DOI: 10.1038/s41467-019-13786-y**
This study used combined structural methods of solution NMR, X-ray crystallography and Cryo-EM to examine the capsid of the human endogenous retrovirus HERV-K. We determined the high-resolution structures of four classes of Fullerene CA-assemblies that demonstrated how invariant CA pentamers combine with plastic CA hexamers to build the polyhedral structures that define the retroviral core. The study also revealed how adaptability and symmetry breaking of intra- and interprotomer CA-CA interactions combine to accommodate the variable curvature of the CA shell.

Arnold LH, Groom HCT, Kunzelmann S, Schwefel D, Caswell SJ, Ordonez P, Mann MC, Rueschenbaum S, Goldstone DC, Pennell S, Howell SA, Stoye JP, Webb M, Taylor IA*, Bishop KN*. (2015) ***Phospho-dependent regulation of SAMHD1 oligomerisation couples catalysis and restriction.*** **PLoS pathogens 11(10): e1005194. DOI: 10.1371/journal.ppat.1005194**
This structural, biochemical and virological study revealed the tetramerisation /phosphorylation dependent mechanism of SAMHD1 regulation. These data form the basis of the prevailing model for SAMHD1 restriction of HIV-1 where dephosphorylation switches housekeeping SAMHD1, found in cycling cells, to a high-activity stable tetrameric form that depletes and maintains low levels of dNTPs in the non-permissive cells resistant to HIV-1 infection.

Schwefel D, Boucherit VC, Christodoulou E, Walker PA, Stoye JP, Bishop KN, Taylor IA. (2015) ***Molecular Determinants for Recognition of Divergent SAMHD1 Proteins by the Lentiviral Accessory Protein Vpx*****. Cell host & microbe 17(4): 489-499. DOI: 10.1016/j.chom.2015.03.004**
This study revealed the crystal structure of a molecular complex containing the lentiviral accessory protein Vpx, a component of the human cellular degradation machinery DCAF1, and a C-terminal region of the anti-HIV-1 restriction factor SAMHD1. The structure combined with our previous 2014 publication provides the explanation of how lentiviral accessory proteins can use different modes of action to subvert the cell’s normal protein degradation pathway to inactivate the viral defence system.

Name	FRANCESCO SAVERIO TEDESCO
Position	Seconded Group Leader (UCL)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Stem Cells and Neuromuscular Regeneration Laboratory***

Research programme and achievements

My laboratory studies skeletal muscle stem cells and regeneration, focusing on the development of novel experimental therapies for incurable neuromuscular disorders. My work pioneered the use of human artificial chromosomes and induced pluripotent stem (iPS) cells for muscle gene and cell therapies (3,4). We have also pioneered the development of the first disease-specific, human iPS cell-derived bioengineered muscle (2). Recent projects investigated the use of small molecules to improve stem cell delivery (1) and the application of iPS cell-derived myogenesis for neuromuscular disease modelling, (Steele-Stallard et al., *Front Physiol* 2018).

My upcoming five years of research programme at the Crick will focus on:

1. Elucidating molecular mechanisms of muscular dystrophies. Questions to be addressed include: how can the same mutation cause distinct muscle diseases, and why are only certain tissues are affected?
2. Resolving molecular determinants of cellular maturation in bioengineered muscles. Questions include: What is the contribution of non-myogenic cells to human muscle maturation and regeneration? Which synthetic stimuli induce myofibre maturation for high-fidelity modelling?
3. Maximising therapeutic potential of human iPS cell-derived myogenic progenitors. Here we will focus on defining human muscle stem cell fate plasticity and on identifying mechanisms and druggable pathways to improve migration of myogenic progenitors.

The overall goal of the Tedesco lab is the clinical translation of these novel regenerative strategies into therapies for severe neuromuscular disorders.

Research outputs

Gerli MFM, Moyle LA, Benedetti S, Ferrari G, Ucuncu E, Ragazzi M, Constantinou C, Louca I, Sakai H, Ala P, De Coppi P, Tajbakhsh P, Cossu G, Tedesco FS. (2019) ***Combined Notch and PDGF Signalling Enhances Migration and Expression of Stem Cell Markers while Inducing Perivascular Cell Features in Muscle Satellite Cells*****. Stem Cell Reports 12(3):461-473. DOI: [10.1016/j.stemcr.2019.01.007]**

- We showed that modulation of pathways involved in developmental specification of pericytes can be used to reversibly convert adult mouse and human myoblasts into pericyte-like cells.
- DLL4 & PDGF-BB-treated cells acquire pericyte properties, markers of stem cell self-renewal and trans-endothelial migration ability while remaining capable of engrafting skeletal muscle.
- These results extend our understanding of muscle stem cell fate plasticity and provide evidence of a novel druggable pathway with clinical relevance for muscle cell therapy.

Maffioletti SM, Sarcar S, Henderson ABH, Mannhardt I, Pinton L, Moyle LA, Steele-Stallard H, Cappellari O, Wells KE, Ferrari G, Mitchell JS, Tyzack GE, Kotiadis VN, Khedr M, Ragazzi M, Wang W, Duchen MR, Patani R, Zammit PS, Wells D, Eschenhagen T, Tedesco FS. (2018) ***Three-dimensional Human iPSC-derived Artificial Skeletal Muscles Model Muscular Dystrophies and Enable Multilineage Tissue Engineering*****. Cell Reports 23(3):899-908. DOI: [10.1016/j.celrep.2018.03.091]**

- In this work we described the development of the first human 3D artificial skeletal muscle entirely derived from pluripotent stem cells of patients with muscle diseases.
- Artificial muscles model severe, incurable forms of muscular dystrophy with high fidelity, providing the foundation for a novel, stem cell-based, drug screening platform.
- Isogenic human vascular-like networks and motor neurons develop within artificial muscles.

Benedetti S, Uno N, Hoshiya H, Ragazzi M, Ferrari G, Kazuki Y, Moyle LA, Tonlorenzi R, Lombardo A, Chaouch A, Mouly V, Moore M, Popplewell L, Kazuki K, Katoh M, Naldini L, Dickson G, Messina G, Oshimura M, Cossu G, Tedesco FS. (2017) ***Reversible Immortalisation Enables Genetic Correction of Human Muscle Progenitors and Engineering of Next-Generation Human Artificial Chromosomes for Duchenne Muscular Dystrophy*****. EMBO Molecular Medicine 10:254-275. DOI: [10.15252/emmm.201607284]**

- This study extended our pioneering work on human artificial chromosomes for ex vivo gene therapy to human muscle stem cells, describing also the largest gene therapy vector developed to date.
- It follows up my PhD paper in which I described the first evidence of pre-clinical gene and cell therapy with a human artificial chromosome in any genetic disease.

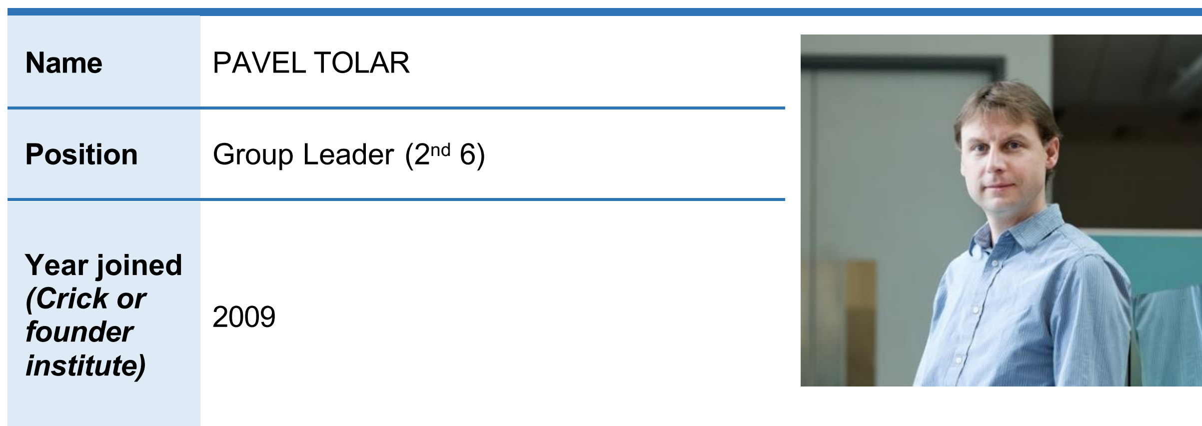
Cossu G, Previtali SC, Napolitano S, Cicalese MP, Tedesco FS, Nicastro F, Noviello M, Roostalu U, Natali Sora MG, Scarlato M, De Pellegrin M, Godi C, Giuliani S, Ciotti F, Tonlorenzi R, Lorenzetti I, Rivellini C, Benedetti S, Gatti R, Marktel S, Mazzi B, Tettamanti A, Ragazzi M, Imro MA, Marano G, Ambrosi A, Fiori R, Sormani MP, Bonini C, Venturini M, Politi LS, Torrente Y, Ciceri F. (2015) ***Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne Muscular Dystrophy*****. EMBO Molecular Medicine 7:1513 -1528. DOI: [10.15252/emmm.201505636]**

- First-in-human, phase I-IIa clinical trial of intra-arterial HLA-matched donor myogenic progenitor cell transplantation in 5 children with Duchenne muscular dystrophy.
- Donor DNA was detected in muscle biopsies of 4/5 patients and donor-derived dystrophin in 1.
- Complex study achieving for the first-time delivery of skeletal myogenic cells through

the intravascular, arterial route for human cell therapy.

Maffioletti SM, Gerli MFM, Ragazzi M, Dastidar S, Benedetti S, Loperfido M, Vandendriessche T, Chuah M, Tedesco FS. (2015) *Efficient Derivation and Inducible Differentiation of Expandable Skeletal Myogenic Cells from Human ES and Patient-Specific iPS Cells*. Nature Protocols 10, 941–958. DOI: 10.1038/nprot.2015.057

- Detailed method to derive transplantable myogenic cells from a wide range of human pluripotent stem cells.
- Derived cells are amenable to genetic correction and to large scale expansion.
- Follows up pioneering work on human iPS cell differentiation and genetic correction in musucalr dystrophy published during my postdoc (Tedesco FS et al., *Sci Transl Med* 2012).



Name	PAVEL TOLAR
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2009

Lab Name	***Immune Receptor Activation Structure Laboratory***

Research programme and achievements

Balanced antibody production underlies healthy immune protection and is the basis of most successful vaccines. This part of our immunity is driven by B lymphocyte responses to foreign antigens. A central focus of my lab is to explain the molecular mechanisms that enable specific and efficient responses of B lymphocytes to antigenic challenge and provide insight into why they sometimes contribute to immune pathology such as immune deficiency, autoimmunity and B cell cancers. In the past five years, my lab has particularly focused on the role of cellular biomechanics in antigen recognition by the B cell antigen receptor (BCR) expressed on B cell surfaces. Using novel imaging methods, nanotechnology, CRISPR-based genetics and mouse models, we showed that BCR mechanosensing, along with specific BCR signalling and endocytic trafficking, plays an important role in T cell-dependent clonal selection of B cells in germinal centres (GCs), a critical process in the development of protective, but also pathogenic antibodies.
Our major findings were:

1. Mouse and human GC B cells bind antigens through immune synapses with unusual BCR patterning and with altered intracellular signalling and cytoskeletal dynamics. This results in enhanced cellular forces on the BCR, which increase the stringency of antigen affinity discrimination during antigen uptake, thus regulating T-cell driven B cell clonal expansion and selection. These results indicate that B cell mechanics of immune synapses with cell-presented antigens significantly contribute to selection of high-affinity somatically mutated BCR variants in the GC and therefore to the affinity maturation of the antibody response.
2. The B cell affinity-dependent uptake of antigens from antigen-presenting cells is regulated by the mechanical properties of the antigen presenting cells' membranes. Increased membrane stiffness promotes affinity discrimination, while low membrane stiffness promotes sensitivity of low-affinity BCRs. We identified follicular dendritic cells as a stiff stromal cell subset mechanically promoting B cell affinity discrimination in the GC.
3. Mechanical B cell extraction of antigens requires formation of dynamic F-actin foci produced by the Arp2/3 complex, and also depends on contractility provided by the non-muscle myosin IIa. Consequently, myosin IIa is important for B cell antigen uptake from follicular dendritic cells in vivo and is essential for B cell responses.
4. Using whole-genome CRISPR screening, we comprehensively characterised endocytic components of B cell antigen uptake. We uncovered a key role of a previously unrecognised, clathrin-independent endocytic pathway mediated by Endophilin A2. We showed that in addition to the BCR, Endophilin A2 regulates endocytosis of a number of key receptors in B cells and is particularly required for GC B cell responses in vivo.
5. Determining one of the first full structures of the IgM antibody and describing its unique dynamics.

In the future, we will focus on the role of follicular dendritic cell-based antigen presentation for the development of the protective antibody responses and on the intracellular wiring of B cell signalling and endocytic pathways in B cell activation and pathology. We aim to:

1. Determine the mechanism regulating antigen and vaccine capture, retention and presentation by follicular dendritic cells in vivo.
2. Determine the role of B cell mechanics in selection of broadly neutralising antibodies against HIV.
3. Identify the wiring of BCR signalling pathways differentially regulating class-switched IgG and IgE B cell differentiation into antibody-secreting cells in allergy.

Determine the role of altered BCR signalling in the growth of IgM- and IgG-switched follicular and diffuse large B cell lymphoma.

Research outputs

Nowosad, C. R., Spillane, K. M., and Tolar, P. (2016) Germinal center B cells recognize antigen through a specialized immune synapse architecture. Nat Immunol 17, 870-77. DOI: 10.1038/ni.3458
Using new high-throughput imaging designed for rare cell subsets, we revealed that germinal centre B cells form uniquely patterned immune synapses to bind antigens. The separation of antigen into small clusters, along with specific cytoskeletal organisation results in enhanced mechanical forces transferred onto the B cell receptor-antigen bonds, increasing the mechanical affinity-discrimination power of germinal centre B cells compared to other B cell subsets. The work implicated germinal centre B cell mechanics in selection of high-affinity B cell clones in antibody responses and introduced novel DNA nanosensors for measuring cellular forces.

Spillane, K. M., Tolar, P. (2017) *B cell antigen extraction is regulated by the physical properties of antigen presenting cells*. J. Cell Biol. 216, 217–230. DOI: 10.1083/jcb.201607064
We demonstrated that mechanical forces and not enzymatic liberation are the physiological mechanism for acquisition of antigens by B cells from live presenting cells. Using DNA-based nanosensors we showed that B cell affinity discrimination is regulated by physical properties of the antigen-presenting cells and identified follicular dendritic cells as a stiff antigen presenting subset that promotes B cell affinity discrimination in germinal centres.

Hoogeboom, R., Natkanski, E.M., Nowosad, C. R., Malinova, D., Menon, R. P., Casal, A. and Tolar, P. (2018) *Myosin IIa promotes antibody responses by regulating B cell activation, acquisition of antigen, and proliferation*. Cell Rep. 216, 2342-2353. DOI: 10.1016/j.celrep.2018.04.087
We showed that the non-muscle motor myosin IIa is important for B cell extraction of antigens from presenting cells in vivo and also for B cell development, proliferation and antibody production. These results established the importance of acto-myosin forces in B cell antigen extraction in vivo.

Kwak, K., Quizon, N., Sohn, H.W., Saniee, A., Manzella-Lapeira, J., Holla, P.,

Brzostowski, J., Lu, J., Xie, H., Xu, C., Spillane, K. M., Tolar, P. and Pierce, S.K. (2018) *Intrinsic properties of human germinal-center B cells set antigen-affinity thresholds*. Sci. Immunol. 3, eaau6598. DOI: 10.1126/sciimmunol.aau6598
We showed that affinity discrimination of human germinal centre B cells is mediated by their altered intracellular signalling, specialised cytoskeletal arrangements and enhanced cell mechanics. Imaging of these cells revealed that antigen extraction occurr ed through actin based pod-like structures at the periphery of immune synapses, which transduce high forces on the B cell receptor. This work implicated mechanical affinity discrimination in the function of human germinal centre B cells.

Roper, S. I., Wasim, L., Malinova, D., Way, M., Cox, S., Tolar, P. (2019) *B cells extract antigens at Arp2/3-generated actin foci interspersed with linear filaments*. eLife 8. DOI: 10.7554/eLife.48093
Super-resolution imaging revealed previously unrecognised actin foci that mediate antigen extraction in B cell synapses. We identified the actin polymerisation pathways that generate these structures and defined the role of their dynamic formation in antigen uptake.

Name	SHARON TOOZE
Position	Senior Group leader
Year joined *(Crick or founder institute)*	1994

Lab Name	***Molecular Cell Biology of Autophagy Laboratory***

Research programme and achievements

Autophagy is a highly conserved intracellular degradation process which acts both as an intracellular recycling pathway and a surveillance mechanism to ensure cell homeostasis and survival. As a highly regulated process, autophagy enables the cell to respond to prevent or negate external and internal damage, proteostasis or metabolic dysfunction, and even pathogen invasion by sequestering the damaging agent within autophagosomes. Formation of the double membrane autophagosome membrane is controlled by both protein and lipid kinase signalling, and these concerted activities are key to the formation of the autophagosome and the selection of cargo incorporated into the autophagosome for delivery to the lysosome and degradation.

My main research interest is to understand at a molecular level how autophagosomes are formed in mammalian cells. My lab has identified and continues to delineate the function of dedicated core autophagy proteins (ATG proteins) and novel regulators, the latter largely those involved in vesicular trafficking. My research programme addresses the role of the ATG proteins required for autophagosome initiation including ATG9A, a multi-spanning membrane protein, ULK1, a serine-threonine protein kinase found in the ULK complex, and WIPI2, the phosphoinositide effector required for lipidation of the ATG8 cargo receptor family. The study of these three proteins is my mainstream activity, as the function and regulation of all three during autophagy remains incompletely understood.

Building upon this core activity my lab has investigated trafficking proteins identified by the lab including TBC1D14, TRAPPIII, and less well understood proteins, WAC, SCOC and FEZ1. We also investigated the selectivity of the LIR (LC3 interacting region) motif in the interaction with autophagy regulators or cargo, in particular PCM1, a centriolar satellite protein. Cargo (ubiquitinated, misfolded or damaged proteins) binds directly to, or through autophagy cargo receptors to, lipidated ATG8 family members (LC3A, B and C, GABARAP, GABARAPL1, and GABARAPL2) via LIR motifs. This allows the recruitment of regulators or delivery of cargo to the autophagosome for degradation in the lysosome.

Using my expertise in molecular cell biology and organelle biogenesis, my work has and will continue to address the fundamental question of how autophagy is initiated. A complete understanding of this complex membrane-mediated process will provide a knowledge base and tools to address the role of autophagy in human disease, infection and ageing. My research is discovery science which by elucidating function and mechanism of proteins and lipids required for autophagy, in both a temporal and spatial context in mammalian cells, underpins the translational work needed to address the treatment of human disease and infection, and mitigate the impact of ageing.

Through a rigorous investigation of the core machinery, the effectors, and trafficking regulators, I aim to obtain a fuller understanding of the autophagosome

formation process. My goal is to reach a near atomic level of understanding of the function of the proteins, and protein complexes involved in the formation process by combining cell and biochemical analyses, high resolution microscopy techniques, temporally and spatially defined analysis and in vitro reconstitution. This will be done using two approaches, a targeted analysis based on understanding i) substrates of the ULK complex, ii), the specific function and regulation of the function of WIPI2 and iii) ATG9A, and secondly a reconstitution approach focusing on i) membrane templates, ii) lipids and iii) cargo recruitment.

Research outputs

Judith, D., Jefferies, H. B. J., Boeing, S., Frith, D., Snijders, A. P., & Tooze, S. A. (2019). *ATG9A shapes the forming autophagosome through Arfaptin 2 and phosphatidylinositol 4-kinase IIIβ*. Journal of Cell Biology, *218*(5), 1634-1652. DOI:10.1083/jcb.201901115
This paper represents an important step forward in our understanding of ATG9, the only multi-spanning autophagy protein and a major focus of my lab's current work. Here we discovered the composition of the ATG9 vesicle and uncovered an important role for a protein which can induce membrane curvature and a lipid kinase. I chose this work as it has provided us with important insights into the function of ATG9A.

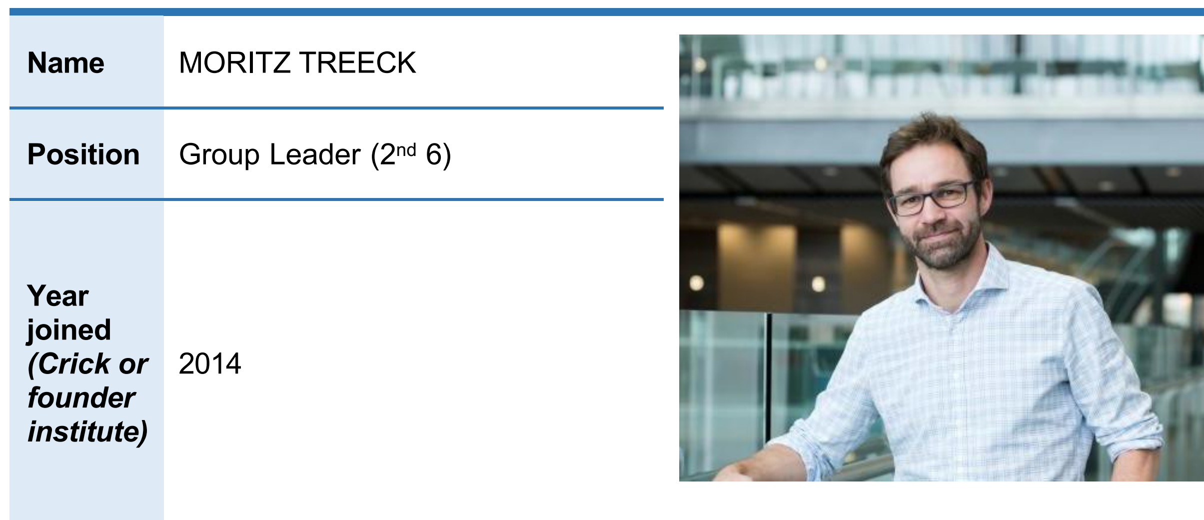
Wirth, M., Zhang, W., Razi, M., Nyoni, L., Joshi, D., O'Reilly, N., Johansen, T., Tooze, S.A., Mouilleron, S. (2019). *Molecular determinants regulating selective binding of autophagy adapters and receptors to ATG8 proteins*. Nature Communications, 10(1). DOI:10.1038/s41467-019-10059-6
This paper follows on from our work on WAC and the role of centrosomes in autophagy. We discovered an important centriolar protein has a specific motif (LIR motif) enabling its binding to a key autophagy protein. In collaborative work, we determined the structure and the important features of the LIR motif, and extended the findings to a group of autophagy proteins to provide an important advance on our understanding of selective autophagy. I chose this work because it is a tour de force of structure and biochemistry and a very substantial collaboration between Structural Biology and Peptide Chemistry STPs.

New, M., Van Acker, T., Sakamaki, J. -I., Jiang, M., Saunders, R. E., Long, J., Wang, V.M.Y., Behrens, A., Cerveira, J., Korcsmaros, T., Jefferies, H.B.J., Ryan, K.M., Howell, M., Tooze, S. A. (2019). *MDH1 and MPP7 regulate autophagy in pancreatic ductal adenocarcinoma*. Cancer Research, 79(8), 1884-1898. DOI:10.1158/0008-5472.Can-18-2553
This work represents findings from a collaborative effort between my lab, Astellas Pharmaceuticals and the Ryan lab at the Beatson Institute. Using a differential siGenome screening pipeline we identified candidates which are important for autophagy-dependent survival of pancreatic ductal adenocarcinoma cells. I chose this paper because the work was funded by and done in a joint collaborations with a pharmaceutical company, and together we identified novel targets which may have a therapeutic benefit in future work.

Lamb, C. A., Nühlen, S., Judith, D., Frith, D., Snijders, A. P., Behrends, C., & Tooze, S. A. (2016). *TBC1D14 regulates autophagy via the TRAPP complex and ATG9 traffic*. EMBO Journal, 35(3), 281-301. DOI:10.15252/embj.201592695
This work uncovered a role for the RabGAP TBC1D14 in vesicle traffic between the endosome and the Golgi complex. Importantly, it highlighted the crucial role of the recycling endosome in formation of the autophagosome. I chose this paper because it identified for the first time the mammalian TRAPPIII complex and showed that, in contrast to yeast, TRAPPIII functions to control ATG9A positioning in the cell, a crucial aspect to allow an immediate response to starvation signals.

Joachim, J., Jefferies, H. B. J., Razi, M., Frith, D., Snijders, A. P., Chakravarty, P.,

Judith, D., Tooze, S. A. (2015). ***Activation of ULK kinase and autophagy by GABARAP trafficking from the centrosome is regulated by WAC and GM130*****.**
Molecular Cell, 60(6), 899-913. DOI:[10.1016/j.molcel.2015.11.018](https://doi.org/10.1016/j.molcel.2015.11.018)
This paper uncovered the function of WAC, which we identified in a siGenome screen, as a positive regulator of autophagy. Furthermore, it provided data to understand how in a non-hierarchical mechanism the ULK1 kinase activation can be maintained during the expansion of the phagophore. Finally, it revealed a role for the centrosome and centriolar satellites in controlling autophagosome formation. I chose this paper because the starting point was the identification of an uncharacterised protein in a siGenome screen which through our study revealed its function and an unexpected link between the Golgi complex, centrosomes, and formation of autophagosomes.



Name	MORITZ TREECK
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2014

Lab Name	***Signalling in Apicomplexan Parasites Laboratory***

Research programme and achievements

My research group focuses on the interaction of two related parasites (Plasmodium falciparum and Toxoplasma gondii) with their human or animal host cell. We study how parasites exit, invade and remodel the host cells they are required to infect. We have developed novel technologies that allow us to investigate these questions in higher throughput.

Using these novel tools, we have been able to overcome major hurdles in the field. We have shown that the species-specific expansion and remodelling of human red blood cells is mediated by parasite-secreted kinases in the most virulent malaria-causing parasite, have identified for the first time that most virulence factors of Toxoplasma in a mouse act on the cell-autonomous immune response, and demonstrated that a key Toxoplasma regulator of host cell interaction is acting in a non-cell autonomous manner, leading to a new appreciation of the control of the systemic immune response by the parasite. We showed that myristoylation of a parasite-secreted protein is required for the entry into host cell; to our knowledge this is the first myristoylated protein entering the secretory pathway in any eukaryote.

In the future we will investigate how Toxoplasma is one of the most successful parasites on earth, using the CRISPR technologies we have developed to identify all virulence factors, and their genetic interactions in various hosts and cell types. We will measure how these factors influence the immune system.

For the malaria-causing parasite, we will investigate in detail how the kinases we identified modulate disease outcome on the molecular level, and investigate their function in host-pathogen interaction in endemic countries through the analysis of field isolate data and studies on infected red blood cells directly from patients.

Research outputs

Broncel, M., Dominicus, C., Vigetti, L., Nofal, S. D., Bartlett, E. J., Touquet, B. et al. & Treeck M. (2020) ***Profiling of myristoylation in Toxoplasma gondii reveals an N-myristoylated protein important for host cell penetration*****. Elife 9:e57861. DOI: 10.7554/eLife.57861**
In this study we describe the Toxoplasma myristoylated proteome, validate inhibitors of P. falciparum N-Myristoyl-transferases to be potent against Toxoplasma and identify N-Myristoylation on a parasite-secreted protein. This is the first description of myristoylation on a secreted protein important for cell-cell interaction.

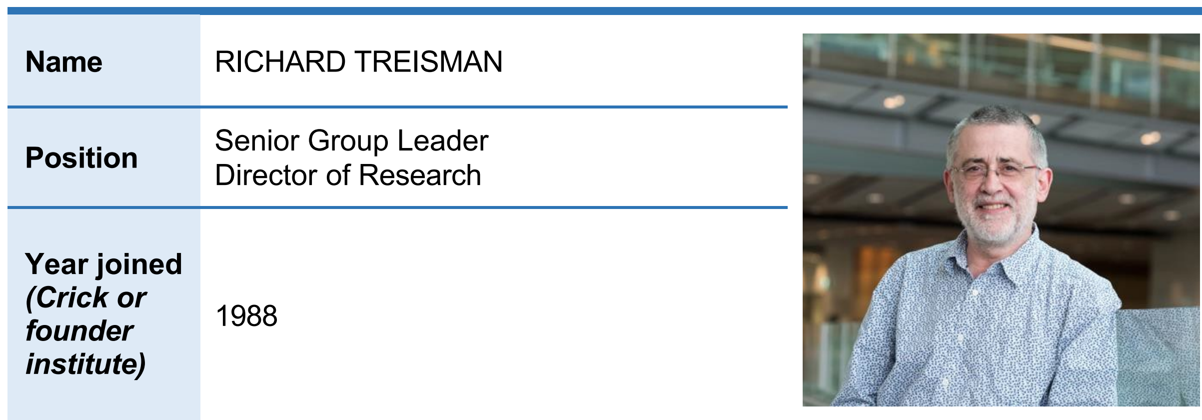
Davies, H., Belda, H., Broncel, M., Ye, X., Bisson, C., Introini, V. et al. & Treeck M. (2020) ***An exported kinase family mediates species-specific erythrocyte remodelling and virulence in human malaria*****. Nat Microbiol 5, 848-863. DOI: 10.1038/s41564-020-0702-4**
We demonstrate species-specific remodelling of red blood cells by the most virulent malaria-causing parasite, mediated by a species-specific expansion of an exported kinase family. Systematic deletion of all 20 members and quantitative phosphoproteomics identifies the kinase targets and supports their role in pathogenesis.

Hunt, A., Russell, M. R. G., Wagener, J., Kent, R., Carmeille, R., Peddie, C. J. et al. & Treeck M. (2019) ***Differential requirements for cyclase-associated protein (CAP) in actin-dependent processes of Toxoplasma gondii*****. Elife 8:e50598. DOI: 10.7554/eLife.50598**
Toxoplasma, as many other related pathogens, has a limited set of actin regulators. Here we demonstrate that complex actin regulation can be achieved by partially overlapping functions of actin regulators. We demonstrate a role for the actin regulator CAP in host cell invasion and maintenance of parasite integrity during division.

Tiburcio, M., Yang, A. S. P., Yahata, K., Suarez-Cortes, P., Belda, H., Baumgarten, S. et al. & Treeck M. (2019) ***A novel tool for the generation of conditional knockouts to study gene function across the Plasmodium falciparum life cycle*****. mBio 10(5):e01170-19. DOI: 10.1128/mBio.01170-19**
We generate the first malaria parasite line that enables conditional gene deletions across the full lifecycle. This is a major advance to probe the "dark boxes" of parasite biology in the mosquito host and human liver stages and paves the way for drug target validation and production of attenuated parasites for vaccination studies.

Young, J., Dominicus, C., Wagener, J., Butterworth, S., Ye, X., Kelly, G. et al. & Treeck M. (2019) ***A CRISPR platform for targeted in vivo screens identifies Toxoplasma gondii virulence factors in mice*****. Nature communications 10, 3963. DOI: 10.1038/s41467-019-11855-w**
We devised a flexible CRISPR platform to generate mutant Toxoplasma pools for tailored genetic screens and used it in vivo for the first time to identify novel effector proteins that ensure parasite survival. We show that known virulence factors are

separated by their function in this screen.



Name	RICHARD TREISMAN
Position	Senior Group Leader Director of Research
Year joined *(Crick or founder institute)*	1988

Lab Name	***Signalling and Transcription Laboratory***

Research programme and achievements

My group has a long-term interest in transcriptional regulation by extracellular signals, with a central focus transcription factor SRF and its partners, the TCFs and MRTFs, which are regulated by ras-ERK and rho-actin signalling respectively. The MRTFs are RPEL proteins, which bind G-actin, and RPEL protein biology isour second focus.

The SRF network

Our work on SRF has centred on genomic analysis of the SRF network and its role *in vivo.* We study proliferative and migratory responses in cancer and immune models. We extended our previous genomic studies of the MRTF-SRF signalling to examine the role of TCF-SRF signalling in the fibroblast response to ERK activation. We found that most ERK-induced chromatin modification is TCF-dependent, as is the majority of ERK-induced transcription, consistent with a transcription cascade model. We also showed that phosphorylation acts both positively and negatively in control of the TCFs and MRTFs. We also established that TCF-MRTF competition is an important determinant of cell contractility in normal fibroblasts.
Our recent studies indicate that MRTF-SRF signalling is essential to prevent senescence in fibroblasts, and we are investigating the relationship of this to cytoskeletal dynamics and the target genes involved. We showed that cancer-associated fibroblasts (CAFs) exhibit heightened sensitivity of MRTF-SRF signalling to mechanical stress, and that in this system MRTF-SRF and YAP-TEAD signalling are mutually dependent. Future work will seek to identify the contribution of MRTF-SRF signalling to CAFs' pro-tumorigenic activity. We are will examine the role of the SRF network in melanoma, in which senescence, Ras-ERK signalling, and Rac signalling are implicated.
We demonstrated that MRTF-SRF signalling is required for the proliferative response of peripheral CD8 T cells to infection. We are currently testing the idea that it is required for the homotypic T-T cell interactions that mediate cytokine signalling. We have also found that SRF-null HSCs cannot colonise bone as a result of MRTF-SRF dependent cell migration defects.

Rho-actin signalling and RPEL proteins

We have explored our understanding of G-actin/RPEL interaction to develop a new class of FRET-based G-actin sensors, which at last allow us to visualise rho-actin signalling directly. We are developing sensors targeted to different subcellular locations, and sensor transgenes, which we will use to evaluate rho-actin signalling dynamics in rgw various biological contexts that under investigation, and make them available to the community of the study of actin dynamics more generally.
We continue to work on the molecular mechanisms of RPEL protein regulation by G-actin. We have found that G-actin negatively regulates recruitment of MRTFs to DNA via SRF,

and have also shown that G-actin/MRTF interaction inhibits productive transcription, apparently promoting recruitment of the RNA exosome to nascent transcripts. Current work aims to understand the phenomena at the molecular level.
We demonstrated that the Phactr1/PP1 complex is a sequence-specific PP1 holoenzyme. Using proteomics, we have identified candidate dephosphorylation targets in fibroblasts and neurons, many of which are cytoskeletal regulators, and can now ask questions about the biology of the Phactr proteins. We are also developing Phactr1/PP1 inhibitory tool compounds.

We used structural and biochemical approaches to demonstrate that the ArhGAP12 and ArhGAP32 families of rhoGAPs are RPEL proteins controlled by rho-actin signalling. RPEL proteins. We are working to establish the role of G-actin binding in control of their functions, particularly in the control of phagocytosis and cell junction dynamics.

Research outputs

Gualdrini, F., *Esnault, C., Horswell, S., Stewart, A., Mathews, N., and Treisman, R. (2016)** ***SRF Co-factors Control the Balance between Cell Proliferation and Contractility**. Mol Cell. 64,1048-1061. DOI: 10.1016/j.molcel.2016.10.016**
The TCFs were the first transcription factors to be identified as targets for ERK signalling, but their contribution to the transcriptional response to ERK signalling has remained unclear. We used MEFs from animals lacking all three TCFs to show that TCF inactivation significantly inhibits over 60% of TPA-inducible gene transcription. Using an integrated ChIPseq/Hi-C approach, we distinguished direct and indirect targets of TCF signalling. The TCFs and MRTFs compete for a common surface on SRF, and this antagonism controls the efficiency of MRTF signalling: cells lacking the TCFs exhibit elevated MRTF/SRF signalling, and enhanced contractility and adhesiveness.

Esnault, C., *Gualdrini, F., Horswell, S., Kelly, G., Stewart, A., Mathews, N., East, P., and Treisman, R. (2017)** ***ERK-induced activation of TCF family of SRF cofactors initiates a chromatin modification cascade required for transcription**. Mol. Cell 65, (6):1081-1095. DOI: 10.1016/j.molcel.2017.02.005**
The relation between signalling to chromatin and transcriptional activation is poorly understood. In parallel with the above study, we investigated the relationship between ERK signalling, histone modifications, and transcription factor activity, focusing on the ERK-regulated ternary complex factor family of SRF partner proteins. We showed much ERK-induced chromatin modification at TSS regions is TCF-dependent. At direct TCF-SRF targets, we used reconstitution of TKO MEFs with Elk-1 mutants to show that signalling induced chromatin modification requires both Elk-1 phosphorylation and recruitment of the transcription machinery. Induction of histone modifications following ERK stimulation is thus directed by transcription factor activation and transcription.

Foster, C., Gualdrini, F., Treisman, R. (2017) ***Mutual dependence of the MRTF-SRF and YAP-TEAD pathways in cancer-associated fibroblasts is indirect and mediated by cytoskeletal dynamics*****. Genes Dev 31, 2361-2375. DOI: 10.1101/gad.304501.117**
The MRTF-SRF and the YAP-TEAD transcriptional regulatory networks both respond to extracellular signals and mechanical stimuli: the MRTFs are controlled directly by G-actin, while YAP activity is somehow potentiated by F-actin. Cancer-associated fibroblasts play an important pro-invasive role in stimulating cancer progression, and previous studies have shown that this involves YAP-TEAD signalling. This paper shows that CAFs also exhibit mechanically-dependent MRTF activation, which is also required for their contractile and pro-invasive activity. The two pathways are mutually dependent, requiring recruitment of MRTF and YAP to DNA via their respective DNA-binding partners, and reflecting their ability to control cytoskeletal gene expression.

Diring, J., Mouilleron, S., McDonald, N.Q. and Treisman, R. (2019) *RPEL family rhoGAPs link Rac/Cdc42 GTP loading to G-actin availability*. Nat Cell Biol. 2019 Jul;21(7):845-855. DOI: 10.1038/s41556-019-0337-y

This paper shows that the ArhGAP9/12/15/27 and ArhGAP32/33 families of rhoGAPs are RPEL proteins whose activity is coupled to G-actin concentration. G-actin forms a 1:1 complex with these ArhGAPs, interacting with an RPEL motif located between the PH and GAP domains, thereby inhibiting their GAP activity. Mutations that block G-actin binding exhibit elevated GAP activity towards their substrate GTPases Rac and Cdc42. Strikingly, treatment of cells with drugs enhancing or inhibiting G-actin/ArhGAP interaction has corresponding effects on Rac GTP loading. These results establish a novel homeostatic feedback loop, in which ArhGAP12-family (and presumably ArhGAP32-family) GAP activity increases when G-actin levels become limiting.

Fedoryshchak*, R.O., Přechová*, M., Butler, A., Lee, R., O'Reilly, N., Flynn, H., Snijders, A.P, Eder, N., Ultanir, S., Mouilleron, S. Treisman, R. (2020) *Molecular basis for substrate specificity of the Phactr1/PP1 phosphatase holoenzyme*. ELife 9:e61509. DOI: 10.7554/eLife.61509

Unlike kinases, PPP-family phosphatases such as PP1 have little intrinsic specificity. PP1 acts in partnership with over 200 different PP1-interacting proteins, but it has remained unclear how they might confer sequence-specificity on PP1. We used proteomics to identify dozens of candidate Phactr1/PP1 substrates, and used structural and biochemical approaches to show that the Phactr1/PP1 holoenzyme is sequence-specific. Phactr1 binding reshapes the PP1 hydrophobic groove, thereby creating a novel composite hydrophobic surface for substrate recognition. This study explains how cofactors can enhance the reactivity of PP1 toward specific substrates, and suggests a way forward for the development of PP1 holoenzyme-specific inhibitors.

Name	SAMRA TURAJLIC	
Position	Clinical Group Leader	
Year joined *(Crick or founder institute)*	2019	

Lab Name	***Cancer Dynamics Laboratory***

Research programme and achievements

Research Programme
In clinical practice, we both over and under-treat patients with cancer. This is in part due to our inability to accurately predict evolutionary potential of individual tumours. The aim of our group is to build a framework for understanding and robustly predicting evolutionary trajectories of primary and metastatic tumours. Initially the approach will involve testing genetic dependencies, evolutionary repeatability and the sources of evolutionary constraint. In the medium-term testable predictions will be examined in wider clinical cohorts across different tumour types. Long-term, the approaches we develop will inform a fundamental understanding of cancer evolution, but also the optimal timing and nature of intervention for individual patients.

Major Achievements
1. Evolutionary understanding of renal cancer. I used my expertise in cancer genetics and genomics to develop tools for evolutionary understanding of cancer, including bespoke sampling and molecular profiling methods (predictive biomarker patent P113326GB). I supervised the development of computational approaches that eventually allowed us to derive evolutionary inferences from >2000 tumour samples. During this time, I set up and led, as the Chief Investigator, a longitudinal prospective study TRACERx Renal (NCT03226886) which has formed the backbone of our discoveries in renal cancer. I brought together a multidisciplinary team of clinicians and researchers who contribute to the work nationally and internationally (TRACERx Renal consortium http://tracerx.co.uk/studies/renal/). By resolving the patterns of driver event ordering, co-occurrence and mutual exclusivity at clone level, we revealed the deterministic nature of clonal evolution and grouped renal cancers into evolutionary subtypes which associate with distinct patterns of cancer progression and clinical outcomes (Turajlic et al. Cell, 2018a). These insights reconciled for the first time the extremely variable clinical behaviour of renal cancer suggesting that evolutionary potential could be used as a biomarker for intervention and surveillance. In a related study, the distinguishing features of metastasis-competent clones showed that they are enriched from high risk somatic copy number alterations (Turajlic et al. Cell, 2018b) which can serve as markers of risk of metastatic disease.
2. Cancer mutations and immunogenicity. In the context of somatic mutations and tumour immunogenicity I have shown that insertion and deletions (indels) are a highly immunogenic mutational class, which can trigger an increased abundance of neoantigens and greater mutant-binding specificity. Extending on these findings in the context of checkpoint inhibitionI have shown that indel burden is a potential biomarker of response to therapy (Turajlic et al. TLO 2017). These findings also support the targeting of frameshift indels by vaccine and cell therapy approaches (patents PCTGB2018/051892 and PCTGB2018/051893).

3. Novel method of tumour profiling. Together with an industry partner (Ventana) I developed an approach to creating a representative tumour sample through homogenisation of left-over surgical tissue (patent filing underway). Analysis of the pilot data revealed robust mutational calling and specifically a more robust determination of the cancer cell fraction avoiding the risk of clonal illusion that is associated with single biopsy molecular assessments. Further methods from these samples include dissociation into single cells that can serve as the input material for flow cytometry analyses of immune and tumour biomarkers for cancer immunotherapy (eg PD-L1 expression, T-lymphocyte cell surface markers), as well as input for single cell sequencing. On the bases of the pilot results I have set up a study at the Royal Marsden Hospital – HoLST-F - Homogenization of Leftover Surgical Tissue, with the aim of assessing the utility of this approach in a pan-cancer setting.

Research outputs

Litchfield K, Stanislaw S, Spain L, Gallegos LL, Rowan A, Schnidrig D, Rosenbaum H, Harle A, Au L, Hill SM, Tippu Z, Thomas J, Thompson L, Xu H, Horswell S, Barhoumi A, Jones C, Leith KF, Burgess DL, Watkins TBK, Lim E, Birkbak NJ, Lamy P, Nordentoft I, Dyrskjøt L, Pickering L, Hazell S, Jamal-Hanjani M; PEACE Consortium, Larkin J, Swanton C, Alexander NR, Turajlic S. (2020) ***Representative Sequencing: Unbiased Sampling of Solid Tumor Tissue*****. Cell Rep 31(5):107550. DOI: [10.1016/j.celrep.2020.107550](https://doi.org/10.1016/j.celrep.2020.107550)**
Representative sequencing offers an improved sampling protocol for tumour profiling, with significant potential for improved clinical utility and more accurate inferences of cancer evolution. Patent arising.

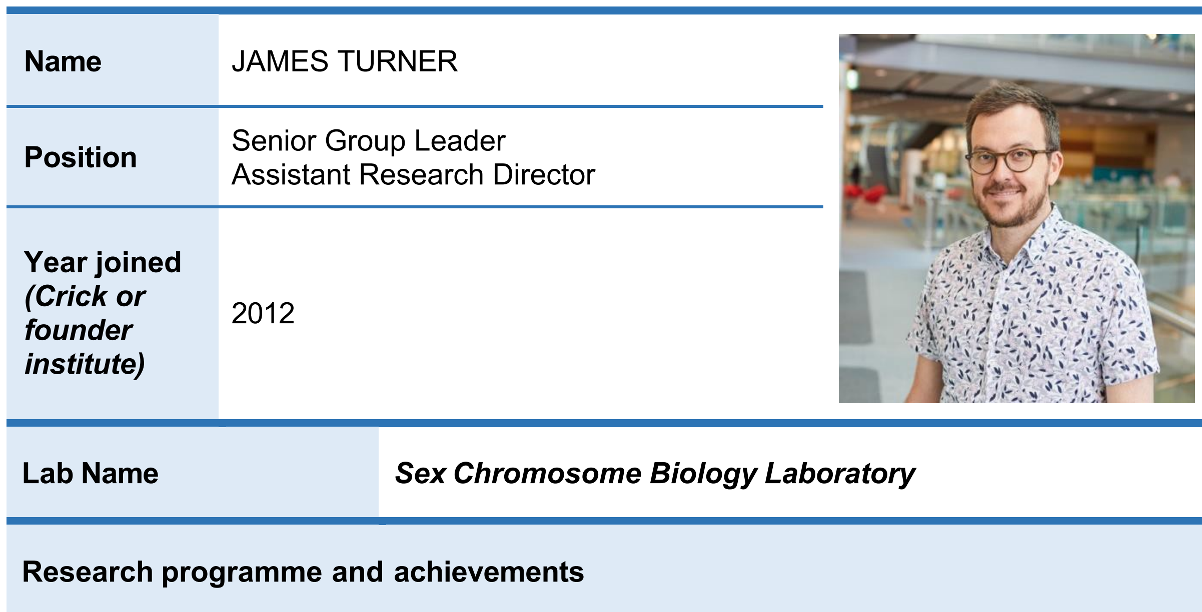
Turajlic S et al. (2018) ***Deterministic evolutionary trajectories influence primary tumour growth: TRACERx Renal*****. Cell 173(3):595-610. DOI: [10.1016/j.cell.2018.03.043](https://doi.org/10.1016/j.cell.2018.03.043)**
This is the largest genomic study ever to be conducted in renal cell cancer and the first to show how evolutionary features of the tumour impact the clinical phenotype. Patent arising.

Turajlic S et al. (2018) ***Tracking cancer evolution reveals constrained routes to metastases: TRACERx Renal*****. Cell 173(3):581-594. DOI: [10.1016/j.cell.2018.03.057](https://doi.org/10.1016/j.cell.2018.03.057)**
This is the first prospective study in any cancer type that resolved the origin of the metastasising clone in the primary tumour characterising its genetic features and uncovering high risk events that confer risk of death.

Turajlic S*, Litchfield K*, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong YNS, Rowan A, Kanu N, Al Bakir M, Chambers T, Salgado R, Savas P, Loi S, Birkbak NJ, Sansregret L, Gore M, Larkin J, Quezada SA, Swanton C. (2017) ***Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis*****. Lancet Oncology 18(8):1009-1021. DOI: [10.1016/s1470-2045(17)30516-8](https://doi.org/10.1016/s1470-2045(17)30516-8)**
The first pan-tumour study to evaluate the contribution of fremashift mutations to generation of immunogenic peptides and anti-tumour immunity. Patent arising.

K Litchfield, E Lim, H Xu, P Liu, M AL-Bakir, S Wong, J Reading, A Rowan, S Funt, T Merghoub, M Lauss, IM Svane, G Jönsson, J Herrero, M Gore, J Larkin, S Quezada, M Hellman, S Turajlic, C Swanton. (2020) ***Escape from nonsense mediated decay associates with anti-tumor immunogenicity.*** **Nature Communications 11(1):3800. DOI: [10.1038/s41467-020-17526-5](https://doi.org/10.1038/s41467-020-17526-5)**

Fs-indels that escape the nonsense-mediated decay (NMD) pathway, can elicit anti-tumor immune responses, especially those the highly elongated neo open reading frames. NMD- escape fs-indels represent an attractive target for biomarker optimisation and immunotherapy design.



Name	JAMES TURNER
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	2012
Lab Name	***Sex Chromosome Biology Laboratory***

Research programme and achievements

Our lab studies how the mammalian sex chromosomes regulate health and disease in women (XX) and men (XY). We achieve our aims using genetic, cell biological, computational, evolutionary and stem cell approaches, and employ eutherian and marsupial mammalian model systems. We have identified mechanisms driving sex chromosome-associated phenotypes and have devised therapeutic approaches to reverse them.

Sex chromosome aneuploidies, including Turner (XO), Klinefelter (XXY) and Jacob (XYY) syndrome, are the most common chromosome abnormalities in humans. Individuals with these conditions are often infertile. We found that early germ cells from Turner and Klinefelter syndrome mice exhibit aberrant X-dosage compensation states, which we proposed contribute to their infertility phenotypes. We devised the first approach to reverse sex chromosome trisomy-associated infertility. Reprogramming of skin cells from sterile XXY and XYY mice caused loss of the extra sex chromosome. The resulting corrected XY stem cells were converted *in vitro* into germ cells, which then produced healthy fertile offspring. Reprogramming also corrected trisomy in human XXY and Down syndrome cells. Our work thus identified a novel role for reprogramming as a trisomy therapy.

In individuals with sex chromosome abnormalities, meiotic synapsis between homologues is defective, leading to germ cell loss via ill-defined mechanisms. In early work, we discovered that unsynapsed chromosomes are transcriptionally inactivated, a process we called meiotic silencing. Epigenetic mechanisms underlying meiotic silencing, and its role in infertility, were unclear. We showed that meiotic silencing is mediated by DNA-damage genes *Atr* and *Topbp1*, and histone methyltransferase *Setdb1*. By ablating meiotic silencing, we rescued oocyte loss in XO female mice. We showed that *Atr* has additional meiotic functions, co-operating with *Atm* during wild-type meiosis to regulate synapsis and recombination. These findings provided insight into the gonadal phenotypes in patients with mutations in *Atr* (Seckel syndrome) and *Atm* (Ataxia Telangiectasia syndrome).

Defective meiotic recombination can cause mutations and aneuploidy in offspring. Germ cells with unresolved recombination intermediates are therefore eliminated by the recombination checkpoint, but how elimination is mediated was not clear. We identified critical functions for BCL-2 pathway members *Puma*, *Noxa* and *Bax* in checkpoint-mediated elimination. Our findings suggest that allelic variants of the BCL-2 pathway may influence the risk of embryonic aneuploidy, and that BCL-2 inhibitors may be of utility in fertility preservation.

Another of our research areas focuses on X-chromosome inactivation, which equalises X-

dosage between females and males. X-inactivation defects have been linked to various diseases, including cancer. Using our *Monodelphis domestica* (opossum) colony, we discovered the marsupial *XIST* equivalent, *RSX* and its antisense partner *XSR*. This work provided a long-sought comparative system with which to understand RNA-mediated chromatin remodelling. We performed the first single-cell RNA-seq analysis of marsupial embryos, identifying lineage-specification and pluripotency factors that are deeply conserved and thus likely to serve critical developmental functions. This study also generated insight into the evolution of imprinted versus random X-inactivation.

Research outputs

Mahadevaiah, S.K., Sangrithi, M.N., Hirota, T. and Turner, J.M.A. (2020) *A single-cell atlas of marsupial embryogenesis and X-inactivation*. Nature 586, 612 -617. DOI: 10.1038/s41586-020-2629-6
Single-cell RNA sequencing of embryos can resolve the transcriptional landscape of development at unprecedented resolution, but such studies of mammalian embryos had focused exclusively on placental species. Analysis of mammalian outgroups might identify deeply-conserved lineage specification and pluripotency factors. In this study, we performed the first single-cell RNA-sequencing in a marsupial, which diverged from eutherians 160 million years ago. We identified many critical developmental regulators pre-dating the placental-marsupial separation which are thus likely to be especially important for embryogenesis. Our study has important implications for understanding the high rates of miscarriage in humans and for developing improved conditions for assisted reproduction.

Ellnati, E., Zielinska, A., Maciulyte, V., Mahadevaiah, S.K., Sangrithi, M.N., Wells, D., Niakan, K.K., Schuh, M. and Turner, J.M.A. (2020) *The BCL-2 pathway maintains genome integrity by elimination recombination-defective oocytes*. Nature Communications 11:2598. DOI: 10.1038/s41467-020-16441-z
Aneuploidy is remarkably common in human embryos, and most often results from defective recombination in the maternal germ line. There is therefore great interest in determining mechanisms that eliminate recombination-defective oocytes, and how defects in these mechanisms cause chromosome abnormalities in offspring. In this study, we showed that recombination-defective oocytes are eliminated via the BCL-2 pathway components Puma, Noxa and Bax. Our findings raised the possibility that allelic variants of the BCL-2 pathway could influence the risk of embryonic aneuploidy.

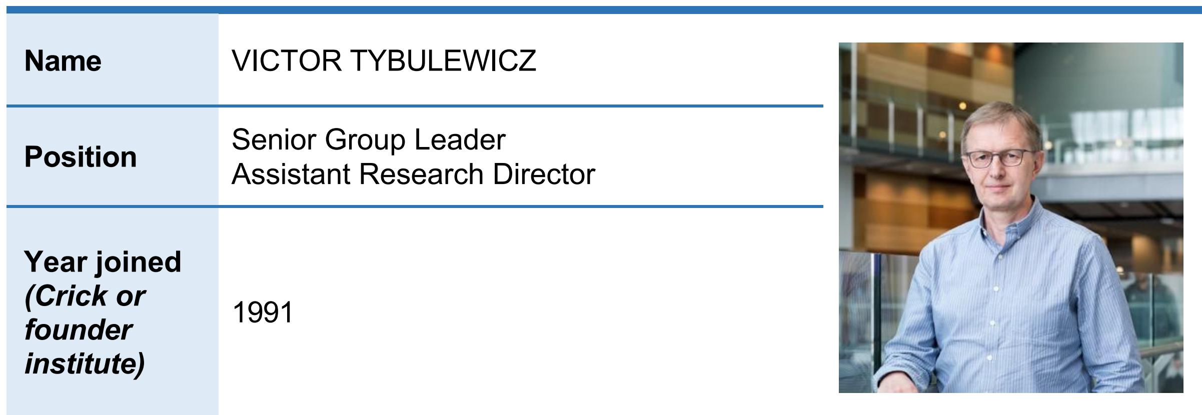
Hirota, T., Blakeley, O., Sangrithi, M.N., Mahadevaiah, S.K., Encheva, V., Snijders, A., Ellnati, E., Ojarikre, O., de Rooij, D.G., Niakan, K.K. and Turner, J.M.A. (2018) *SETDB1 links the meiotic DNA damage response to sex chromosome silencing in mice*. Developmental Cell 47:645. DOI: 10.1016/j.devcel.2018.10.004
Unresolved DNA damage causes chromosome silencing, but how was unclear. We showed that during meiosis, unresolved double-strand breaks induce chromosome silencing via the histone H3-lysine-9 methyltransferase SETDB1. The work provided insight into how cells recognise and repair DNA damage, thereby preserving genome integrity.

Hirota, T., Ohta, H., Powell, B.E., Mahadevaiah, S.K., Ojarikre, O.A., Saitou, M., and Turner, J.M.A. (2017) *Fertile offspring from sterile sex chromosome trisomic mice*. Science 357:932. DOI: 10.1126/science.aam9046
Here, we described a technique for reversing infertility in XXY (Klinefelter) and XYY (Jacob) syndrome mice. We showed that reprogramming of fibroblasts from these mice resulted in elimination of the extra sex chromosome, and that resulting XY cells could be converted by *in vitro* gametogenesis into functional sperm. Reprogramming could also

chromosomally correct cells from Down syndrome mice and patients. The work revealed an unexpected role for reprogramming as a form of chromosome therapy.

Sangrithi, M.N., Royo, H., Mahadevaiah, S.K., Ojarikre, O., Bhaw, L., Sesay, A., Peters, A.H., Stadler, M., and Turner, J.M.A. (2017) *Non-canonical and sexually dimorphic X dosage compensation states in the mouse and human germline*. Developmental Cell 40:289. DOI: 10.1016/j.devcel.2016.12.023
X-dosage compensation was thought to be essential in all mammalian cell types. In this study, we showed that male and female germ cells exhibit unusual X-dosage compensations states, providing an important exception to this rule. We showed for the first time that sex chromosomally-abnormal germ cells exhibit X-dosage compensation states that are unmatched to their somatic gonadal environment, thereby providing a new potential mechanism for sex chromosome-related infertility.

Name	VICTOR TYBULEWICZ
Position	Senior Group Leader Assistant Research Director
Year joined *(Crick or founder institute)*	1991



Lab Name	*Immune Cell Biology and Down Syndrome Laboratory*

Research programme and achievements

Signalling in the Immune System

Major Achievements in the Quinquennium

Our aim is to understand signalling pathways that control the development, survival, migration, activation, and differentiation of lymphocytes. We use a combination of mouse genetics, biochemistry, cell biology and immunology.

We showed that memory B cell (MBC) survival requires the B cell antigen receptor (BCR) and the SYK kinase (Ackermann *et al*, 2015, Müller-Winkler *et al*, 2020). Overturning previous publications, we found that MBC survival is very dependent on BAFF and BAFFR, an important finding because anti-BAFF treatment is a major therapeutic approach for autoimmune diseases.

We previously showed that BAFFR transduces signals in B cells via the BCR and SYK, demonstrating unexpected cooperation between these receptors (Schweighoffer *et al*, 2013). More recently, we extended this to show that the LPS receptor TLR4 also signals via BCR and SYK (Schweighoffer *et al*, 2017).

Using a genetic screen, we discovered that the WNK1 kinase is a negative regulator of CD4+ T cell adhesion and a positive regulator of T cell migration, and acts via the OXSR1 and STK39 kinases and the SLC12A-family of ion co-transporters, implying that movement of ions across the membrane is critical for these processes (Köchl *et al*, 2016).

Future Plans

A major aim of our future work is to understand how BAFFR and BCR transduce survival signals to naïve B cells, and what other receptors may play a role. We will use CRISPR screens, proteomics, transcriptomics, genetics and biochemical analysis. We will investigate how stromal cells provide survival signals to B cells through cell-cell contact.

Secondly, we will investigate how MBC develop, survive and function. Using single cell RNAseq we will establish the differentiation pathways that generate MBC, identifying key transcription factors that control this process.

We will investigate how WNK1 regulates T cell migration and adhesion, focussing on the roles pf the downstream OXSR1 and STK39 kinases and the SLC12A-family of ion co-transporters, aiming to understand how ion and water movement regulate T cell biology.

Genetics of Down Syndrome

Major Achievements in the Quinquennium

Down Syndrome (DS), trisomy of human chromosome 21 (Hsa21), results in learning & memory deficits, congenital heart defects, craniofacial alterations and early-onset Alzheimer's, most likely caused by an extra copy of one or more of the ~230 Hsa21 coding genes. In collaboration with Elizabeth Fisher (UCL), we aim to identify the genes required in three copies to cause DS phenotypes and to establish their pathological mechanisms.

Hsa21 is orthologous to regions on mouse chromosome 10 (Mmu10), Mmu16 and Mmu17. We generated a mouse strain, Dp1Tyb, with increased dosage of the 148-gene Mmu16 region, as well as 8 other strains with shorter duplications, forming a mapping panel to identify the location of DS genes (Lana-Elola *et al* 2016).

We showed that Dp1Tyb embryos develop congenital heart defects very similar to those seen in DS babies and demonstrated that there must be ≥2 causative genes (Lana-Elola *et al* 2016).

We showed that Dp1Tyb mice have locomotor deficits caused by ≥2 genes, one of which is *Dyrk1a*, a kinase (Watson-Scales *et al*, 2018). Furthermore, we found motor neuron loss in these mice and showed a similar loss in humans with DS.

Finally, we showed that while gene expression is dysregulated in DS in clusters, as previously reported, this clustering is unrelated to DS and occurs whenever gene expression changes (Ahlfors *et al*, 2019).

Future Plans

A major future aim is to identify the genes on Hsa21 that are responsible for learning & memory deficits, congenital heart defects and craniofacial changes, and to establish the mechanisms by which they act.

With Trevor Smart (UCL) we have shown that Dp1Tyb mice have increased GABA-mediated inhibition in the dentate gyrus of the hippocampus. We will identify the genes that cause this.

We have found that one of the genes causing congenital heart defects is *Dyrk1a* and mapped a second causative gene to a 6-gene region. We will identify this second gene and establish the mechanisms by which these genes act and the affected cell types.

We have shown that Dp1Tyb mice have craniofacial defects (Toussaint *et al*, bioRxiv). We will map the location of the causative genes and establish their pathological mechanisms.

Finally, we will investigate whether aneuploidy, i.e. an extra chromosome, contributes to DS phenotypes in addition to the effect of increased gene dosage. We will engineer mouse strains carrying Hsa21 either as a separate chromosome or attached to a mouse chromosome; comparison of these will establish whether an additional chromosome contributes to DS pathology.

Research outputs

Lana-Elola, E, Watson-Scales, S, Slender, A, Gibbins, D, Martineau, A, Douglas, C, Mohun, T, Fisher, EMC, Tybulewicz, VLJ (2016). ***Genetic dissection of Down syndrome-associated congenital heart defects using a new mouse mapping panel*****. ELife 5:e11614. DOI:10.7554/eLife.11614.001**

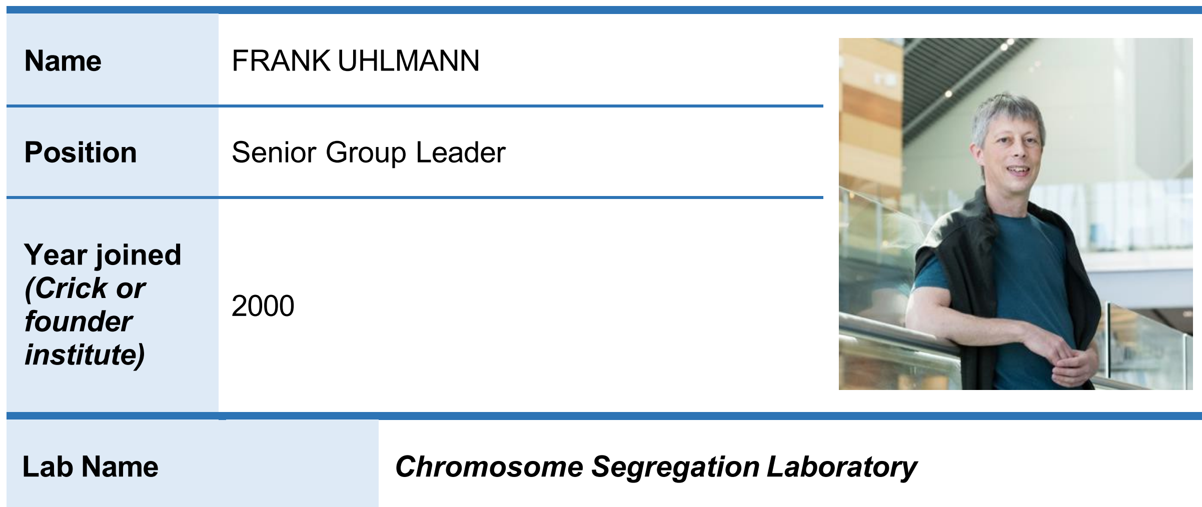
This paper reported the generation of a mouse mapping panel of mouse strains containing duplications of different regions of the mouse genome that are orthologous to human chromosome 21. This panel can be used to study Down syndrome phenotypes and to identify the location of causative genes. Here we showed that the Dp1Tyb mouse strain has congenital heart defects that are very similar to those seen in DS and demonstrated that there must be at least two causative genes mapping their location to a 39-gene region.

Köchl, R, Thelen, F, Vanes, L, Brazão, TF, Fountain, K, Xie, J, Huang, C-L, Lyck, R, Stein, JV, Tybulewicz, VLJ (2016). ***WNK1 kinase balances T cell adhesion and migration in vivo.*** **Nat Immunol, 17, 1075-1083. DOI: 10.1038/ni.3495**
In this study we identified the WNK1 kinase as a negative regulator of CD4+ T cell adhesion and a positive regulator of T cell migration. Furthermore, we showed that WNK1 controls migration through the OXSR1 and STK39 kinases and the SLC12A2 ion co-transporter. This was an unexpected finding since WNK1 had been previously shown to regulate salt homeostasis in the kidney. Our study is the first to have implicated movement of Na+, K+ and Cl- ions in the regulation of T cell migration.

Schweighoffer, E, Nys, J, Vanes, L, Smithers, N, Tybulewicz, VL (2017). ***TLR4 signals in B lymphocytes are transduced via the B cell antigen receptor and SYK*****. J Exp Med, 214, 1269-1280. DOI: 10.1084/jem.20161117**
Here we showed that in B cells, the TLR4 receptor for LPS transduces signals that control B cell activation and proliferation via the BCR and the SYK tyrosine kinase. This was an unexpected finding showing how two distinct receptors signal co-operatively and echoed an earlier finding where we had shown that BAFFR also transduces signals via BCR and SYK.

Watson-Scales, S, Kalmar, B, Lana-Elola, E, Gibbins, D, La Russa, F, Wiseman, F, Williamson, M, Saccon, R, Slender, A, Olerinyova, A, Mahmood, R, Nye, E, Cater, H, Wells, S, Yu, YE, Bennett, DLH, Greensmith, L, Fisher, EMC, Tybulewicz, VLJ (2018). ***Analysis of Motor Dysfunction in Down Syndrome reveals Motor Neuron Degeneration*****. PLoS Genetics, 14:e1007383. DOI: 10.1371/journal.pgen.1007383**
In this study we showed that the Dp1Tyb mouse model of DS has locomotor defects, mapped the causative genes to a 25-gene region and identified that *Dyrk1a* is one of these. Furthermore, we found an unexpected progressive loss of motor neurons in these mice and showed that a similar loss is seen in humans with DS.

Müller-Winkler, J, Mitter, R, Rappe, J, Vanes, L, Schweighoffer, E, Mohammadi, H, Wack, A, Tybulewicz, VLJ (2021). ***Critical requirement for BCR and BAFFR in memory B cell survival*****. J Exp Med 218(2):e20191393. DOI: 10.1084/jem.20191393**
Using conditional genetic ablation, we showed that the survival of memory B cells requires the BCR and its signalling subunit CD79A, as well as BAFF and its receptor BAFFR. Finally, we also showed that memory B cells require IKK2 for their survival. Previous studies had only identified 2 proteins that are required for memory B cell survival – this study identified 5 further proteins, substantially increasing our understanding of how these key memory cells are kept alive.



Name	FRANK UHLMANN
Position	Senior Group Leader
Year joined ***(Crick or founder institute)***	2000

Lab Name	***Chromosome Segregation Laboratory***

Research programme and achievements

All living organisms inherit their genomes in the form of chromosomes. The Chromosome Segregation Laboratory investigates molecular mechanisms that underpin chromosome segregation, in particular the contribution of structural chromosomal proteins to sister chromatid cohesion and mitotic chromosome formation. We also study how the cell cycle control network orchestrates faithful chromosome segregation. Advances over the past five years include:

1. The biochemical reconstitution of topological loading of the cohesin ring onto DNA, as well as uncovering a structure-based mechanism for the DNA entry reaction into the cohesin ring. We also clarified the DNA exit mechanism and its cell cycle regulation by cohesin acetylation and we discovered a mechanism by which cohesin captures a second DNA which is likely important during the establishment sister chromatid cohesion.

2. We described the roles of replication fork-associated protein factors Ctf4, Chl1 and Ctf18-RFC in the establishment of sister chromatid cohesion. Ctf4 recruits Chl1, which in turn contacts cohesin during DNA replication to prevent cohesin loss from chromatin as the replication fork passes. We found that Ctf18-RFC acts by enriching PCNA in the wake of the replication fork as a landing pad for the Eco1 cohesin acetyl transferase.

3. We characterised the cell cycle regulation of the condensin complex and developed a joint computational and experimental approach to analyse how reduced dynamic condensin turnover in mitosis leads to mitotic chromosome formation in budding yeast, as well as how it changes the chromatin interaction landscape in fission yeast.

4. We provided evidence of how Cdk-counteracting phosphatases control the phosphorylation timing of key cell cycle regulators to shape the timely progression of the cell division cycle, including by structural analysis of a new phosphatase substrate targeting motif.

A key remaining puzzle in the chromosome biology field, that we will attempt to solve in the next five years, is the establishment of sister chromatid cohesion during DNA replication. What happens to the cohesin complex when the replication fork passes? How does cohesin ensure that replicated sister chromatids remain connected to each other? We aim to answer these question through the biochemical reconstitution of cohesion establishment during DNA replication. Taking advantage of our unique position at the Crick, next to John Diffley's lab who have reconstituted complete eukaryotic chromatin replication, we will combine cohesin loading onto DNA with DNA replication in the presence of all known cohesion establishment factors. This will allow us to dissect what happens to cohesin during DNA replication so that it embraces both sister DNAs when the fork has passed.

We will also continue to investigate other aspects of cohesin function, including the interplay between the local chromatin landscape and binding of the cohesin and condensin complexes to chromosomes, with important implications for the understanding of Cornelia de Lange syndrome and other human developmental disorders. We have also started to biochemically characterise the condensin complex to begin to evaluate different current models of mitotic chromosome formation. These studies will be conducted in parallel with our continuing work to decipher how phosphorylation timing of cell cycle regulators is established during the cell cycle through the interplay of kinases and phosphatases that act on each substrate.

Research outputs

Y. Murayama and F. Uhlmann. (2015) *DNA entry into and exit out of the cohesin ring by an interlocking gate mechanism*. Cell 163, 1628-1640. DOI: 10.1016/j.cell.2015.11.030

Building on our successful biochemical reconstitution of topological cohesin loading onto DNA, we completed the reconstitution of both dynamic loading as well as unloading. We realised that both loading and unloading follow a very similar trajectory through sequential ATPase and kleisin gates, only one of which can be open at any one time. This formed the basis for our unified DNA passage proposal both into and out of the ring.

Y. Kakui, A. Rabinowitz, D. J. Barry and F. Uhlmann. (2017) *Condensin-mediated remodeling of the mitotic chromatin landscape*. Nat Genet. 49, 1553-1557. DOI: 10.1038/ng.3938

In addition to understanding the molecular mechanism by which SMC complexes work with DNA, we need to know the patterns with which these interactions occur *in vivo*. Here we probed how the condensin complex shapes intra- and intermolecular DNA interactions as chromosomes condense in mitosis. This uncovered a characteristic size-range of condensin-mediated intrachromosomal interactions in the hundreds of kb length scale. This provides a framework against which we can now develop models of how condensin achieves this.

M. Godfrey, S. A. Touati, M. Kataria, A. Jones, A. P. Snijders and F. Uhlmann (2017) *PP2ACdc55 phosphatase imposes ordered cell cycle phosphorylation by opposing threonine phosphorylation*. Mol. Cell *65*, 393-402. DOI: 10.1016/j.molcel.2016.12.018

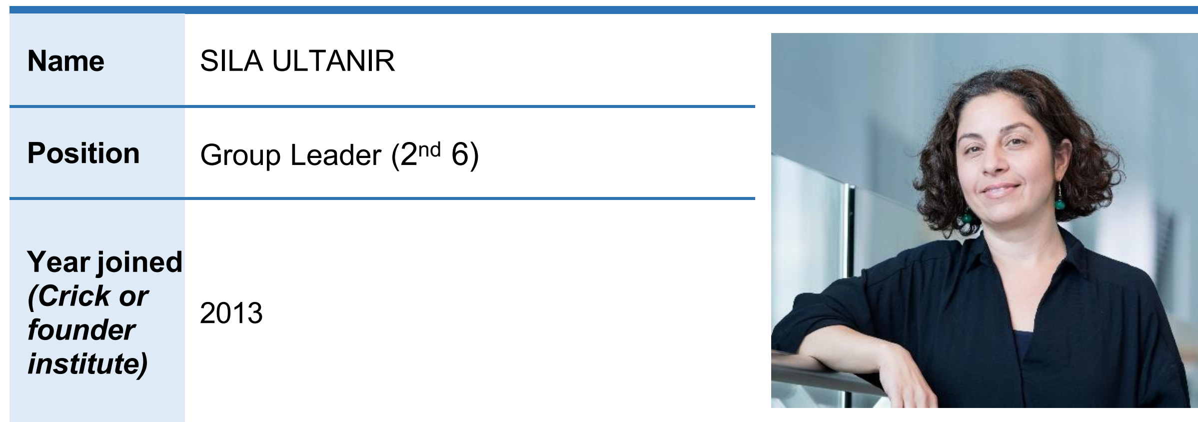
The eukaryotic cell cycle comprises a robustly ordered series of phosphorylation events. In this study, we show that the phosphatase $PP2A^{Cdc55}$ counteracts and thereby delays cell cycle phosphorylation by cyclin-dependent kinase specifically. Strikingly $PP2A^{Cdc55}$ counteracts threonine but less so serine phosphorylation. This reveals an ordering principle based on the phospho-amino acid identity that might be also relevant in other signalling networks.

Y. Murayama, C. P. Samora, Y. Kurokawa, H. Iwasaki and F. Uhlmann (2018). *Establishment of DNA-DNA interactions by the cohesin ring*. Cell 172, 465-477. DOI: 10.1016/j.cell.2017.12.021

SMC complexes not only topologically load onto one DNA, they engage in interactions between more than one DNA. Here we have biochemically reconstituted sequential entrapment of two DNAs by cohesin. Unexpectedly, this is only possible if the second DNA is a single stranded DNA. ds-ssDNA cohesion can then be converted to ds-dsDNA cohesion by DNA synthesis. This is reminiscent of what is found at replication forks where cohesin establishes sister chromatid cohesion.

T. L. Higashi, P. Eickhoff, J. S. Sousa, J. Locke, A. Nans, H. R. Flynn, A. P. Snijders, G. Papageorgiou, N. O'Reilly, Z. A. Chen, F. J. O'Reilly, J. Rappsilber, A. Costa and

F. Uhlmann. (2020) *A Structure-Based Mechanism for DNA Entry into the Cohesin Ring.* Mol. Cell 79 (6), 917–933. DOI: 10.1016/j.molcel.2020.07.013
Cohesin is a ring-shaped protein complex that topologically entraps DNA to fulfil key functions in chromosome architecture. In a collaborative and multidisciplinary approach, we used cryo-EM, biochemical and biophysical techniques to describe how ATP-fuelled structural changes of the cohesin complex drive the DNA entry reaction into the cohesin ring. This solves one of the outstanding riddles in molecular biology.



Name	SILA ULTANIR
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Lab Name	***Kinases and Brain Development Laboratory***

Research programme and achievements

Formation of the neuronal circuitry relies on a large repertoire of signalling mechanisms that play roles in formation of neuronal dendrites, axons and synapses. My lab studies how kinases and protein phosphorylation regulate neuronal development and brain and may contribute to the disease of the nervous system. Numerous kinases are expressed in the nervous system and several are genetically associated with neurological diseases. My lab has a unique combination of expertise in kinase biology and cellular neuroscience. We strive to bridge large gaps in our knowledge of kinase functions with a focus on neurological disease-linked kinases.

In the past five years my lab worked on kinases with known genetic associations to human neurological disorders, and the hippo kinase signalling pathway, which has conserved roles in animal models of neuronal differentiation.

A large part of my lab now studies the kinase called CDKL5. Mutations in CDKL5 cause a severe neurodevelopmental disorder with seizures. We used chemical genetics to identify direct substrates of CDKL5, and via these substrates we revealed a role for CDKL5 in regulating microtubule dynamics. Our findings opened up a new research area that we are actively working on. Reflecting the timeliness of our discovery, the phosphospecific antibodies we generated are widely used in CDKL5 research, particularly for preclinical studies by companies looking to restore CDKL5 function.

We also revealed a novel regulatory mechanism downstream of GAK, a kinase implicated in Parkinson’s Disease. We discovered that GAK phosphorylates the alpha subunit of the Na+/K+ pump and showed that this phosphorylation regulates its trafficking.

In our hippo kinase research strand, we showed that LATS1/LATS2 kinases and their substrate, the transcription factor YAP1, are essential for preventing hyperproliferation and ependymoma-like tumour formation in mouse models. This study also revealed HOPX to be a novel factor present in a YAP1-fusion subtype of ependymoma, indicating that HOPX can be a marker for classifying ependymoma.

We also investigated NDR1/2 kinases, also thought to be downstream of the hippo pathway, using neuronal conditional NDR1/2 knockout mice. We revealed that NDR1/2 is critical for membrane trafficking and efficient autophagy-mediated protein clearance and that loss of NDR1/2 leads to neurodegeneration in the forebrain in mice.

Future Plans:

1. We will determine the function of CDKL5 signalling at molecular, cellular and behavioural levels using a wide array of interdisciplinary approaches. The Crick's many technology platforms are enabling us to conduct this research programme; we use light microscopy, proteomics, advanced sequencing, structural biology, electron microscopy, histopathology, genetic engineering in mouse models and BRF. At the end of a 3 year programme, I hope to have revealed novel kinase signalling mechanisms critical for brain development and plasticity.
2. I aim to expand my lab in the direction of kinase signalling that impacts neurodegeneration/ neuronal protein homeostasis.

Where possible, I would like to have our findings be translated to preclinical research. Antibody reagents that we generated are being used by several USA companies developing gene therapies for CDKL5 Deficiency Disorder, as preclinical markers of CDKL5 activity. While these antibodies are effective, better antibodies can be generated using rabbit monoclonal antibody technology, which is also very costly. It is one of my aims to initiate a pipeline of producing better versions of our antibodies (perhaps in collaboration with Abcam), where we see a clear value for research and therapeutic purposes.

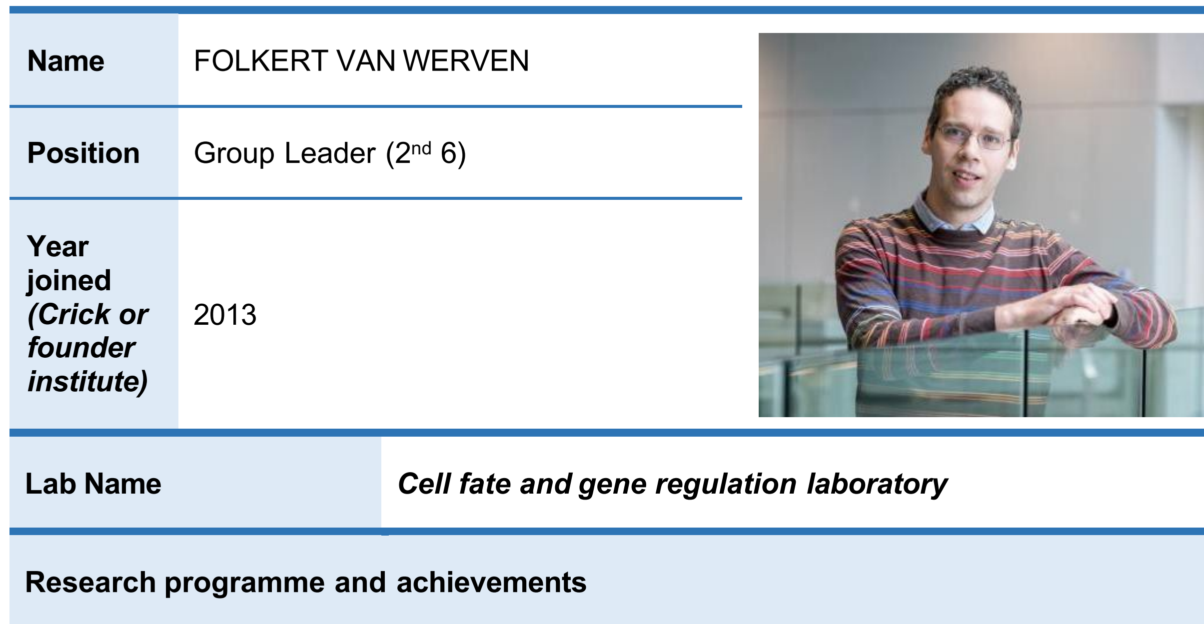
Research outputs

Eder N., Roncaroli F., Domart, MC., Horswell, S., Andreiuolo F., Flynn, H.R., Lopes, A.T., Claxton, S., Kilday, J-P., Collinson, L., Mao, J-H., Pietsch, T.,Thompson, B., Snijders, A.P., Ultanir, S.K. (2020*). YAP1/TAZ drives ependymoma-like tumour formation in mice*. Nature Commun 11, 2380. DOI: 10.1038/s41467-020-16167-y
We showed that active YAP1 in radial glia derived neural precursor cells induces ependymoma-like tumours in mice. We demonstrated that YAP1 is necessary and sufficient using mouse models. We found that transcription coactivator HOPX, a factor consistently suppressed in malignancies, is highly expressed in our mouse models and in YAP1-fusion human ependymoma. HOPX differentiates YAP1-fusion subtype from the highly malignant RELA-fusion human ependymomas. This supports the notion for subtype-specific care for ependymoma.

Lin, A.W., Gill, K.K., Castaneda, M.S., Matucci, I., Eder, N., Claxton, S., Flynn, H., Snijders, A.P., George, R., and Ultanir, S.K. (2018). *Chemical genetic identification of GAK substrates reveals its role in regulating Na(+)/K(+)-ATPase*. Life Sci Alliance 1(6):e201800118. DOI: 10.26508/lsa.201800118
GAK is a serine/threonine kinase implicated in Parkinson's by GWAS. In this paper we identify GAK's direct substrates, and validated ATP1a3 as a substrate in cells. The role of the kinase domain of GAK was unknown, and we show that it enables recycling of Na+/K+ pump from early endosomes back to the plasma membrane. ATP1a3 is also implicated in movement disorders, including rapid onset dystonia. The ATP1a3 T705 phosphomutant is lethal in mice, indicating the significance of this phosphorylation site.

Baltussen, L.L., Negraes, P.D., Silvestre, M., Claxton, S., Moeskops, M ., Christodoulou, E., Flynn, H.R., Snijders, A.P., Muotri, A.R., and Ultanir, S.K. (2018). *Chemical genetic identification of CDKL5 substrates reveals its role in neuronal microtubule dynamics*. EMBO J 37 37(24):e99763. DOI: 10.15252/embj.201899763
We identified novel physiological substrates of CDKL5, a kinase linked to a severe neurodevelopmental disorder in humans. This study is important as it revealed for the first time that CDKL5 was a regulator of neuronal microtubules, thus identifying a new

mechanism by which it regulates neuronal development and function. Our phospho-specific antibodies met the immediate need for a preclinical biomarker for studies in areas such as gene therapy.



Name	FOLKERT VAN WERVEN
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Lab Name	***Cell fate and gene regulation laboratory***

Research programme and achievements

Understanding how a multicellular organism arises from a single cell is a major challenge in biology. Each cell fate or cell differentiation programme is controlled by multiple regulatory signals that drive changes in gene expression. Dissecting the mechanisms of gene regulation is key to the understanding of development, but also to diseases such as cancer. As such, how gene expression is regulated during cell differentiation remains poorly understood. In my laboratory we use budding yeast and the yeast gametogenesis or sporulation programme as a basic model for deciphering principles of regulating gene expression in general and during cell fate changes. The research can be summarised by three key questions: How do signals integrate and control the promoter of a cell fate regulator? How do noncoding RNAs and alternative mRNA isoforms control gene expression? How does a highly conserved RNA modification, *N6*-methyladenosine (m6A), regulate gene expression? The main findings from this research over the past five years can be summarised as follows:

We have deciphered multiple regulatory mechanisms describing how the promoter of a master regulator controls the decision to enter meiosis. Specifically, we identified key regulatory functions for a cluster of transcription factors, a co-repressor complex and two noncoding RNAs. The regulatory principles identified in this work show features of how enhancers in mammalian cells are controlled. For example, many enhancers are primed for activation, integrate various signals, and multiple transcription factors associate with enhancers.

We found new gene regulatory functions for transcription of noncoding RNAs and mRNA isoforms, and a novel regulatory mechanism that ensures repression of aberrant transcription in intergenic regions. We have developed technology to quantify transcript heterogeneity by measuring transcript start and end site usage, and have demonstrated that changes in transcription start site usage form a major regulatory feature of cell fate transitions in yeast. Our results give new and important insights into how gene expression is controlled when cells transition from one state to another and may shed new light on how gene expression is mis-regulated in diseases such as cancer and neurodevelopment disorders, which display pervasive mis-regulation of alternative transcription start sites and alternative promoters.

We investigated the function of the N6-methyladenosine (m6A) modification in mRNAs. This modification is highly abundant, and present in eukaryotes from yeast to mammals, and it alters the fate of transcripts in various ways: it can stimulate turnover of transcripts, promote translation and RNA splicing, alter transcript localisation, and more. The m6A mark also has essential functions in development; for example, the m6A writer and reader machinery is critical for stem cell differentiation and oocyte development. We developed a research programme to dissect the functions and regulation of the m6A modification in

mRNAs in yeast, and have developed various techniques to measure the abundance and regulation of the m6A mark. We have identified a conserved protein that associates with the m6A mark, which we are currently studying further. The work will be the starting point for several new investigations, and the outcome from the research will provide important new insights on the function of the m6A mark and the highly conserved m6A machinery.

My future research programme will centre on two questions:

1) How does transcript heterogeneity shape gene expression? We propose that a primary function for transcript heterogeneity is to regulate and tune the transcription of main protein-coding mRNA isoforms often through transcription coupled chromatin changes. Along term aim of my laboratory is to identify the regulatory principles required for regulation of gene expression via these alternative transcription events. The results fromour studies will reveal how transcript heterogeneity shapes gene expression and how this controls cell state changes in yeast and mammalian cells. Our work may provide important new insights into how mis-regulation of alternative transcription events drives the onset of diseases such as cancer or neural developmental disorders, which often display pervasive mis-regulation of alternative promoters

2) How does the m6A machinery regulate gene expression and cell fate? The long-term aim is to decipher the functions and mechanisms by which the m6A machinery controls gene expression. It is our hope that the work in yeast will provide insights onto the function and regulation of m6A in human cells and help to understand how mis-regulation of m6A contributes to the onset of diseases such as cancer.

Research outputs

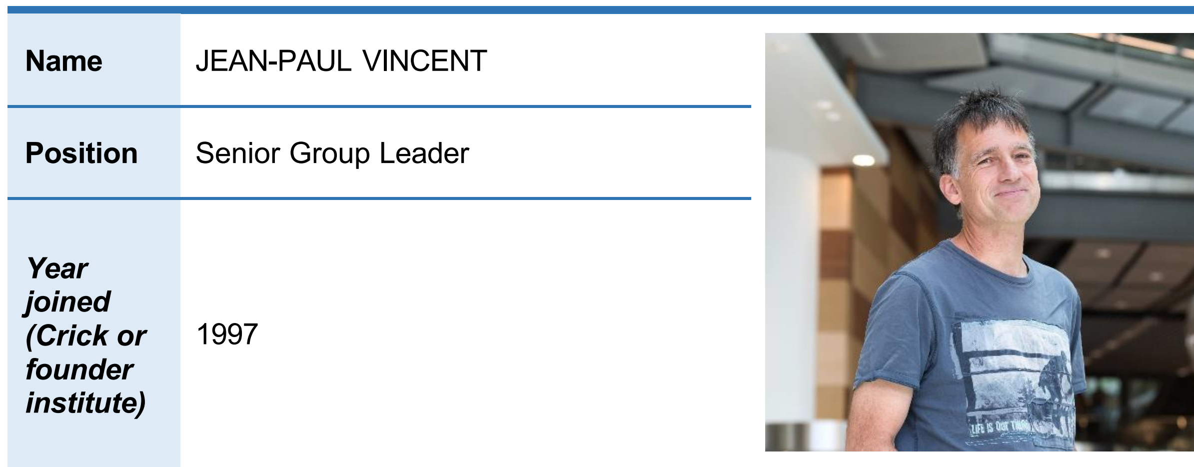
Moretto F., N.E. Wood, M. Chia, C. Li, N.M. Luscombe, F.J. van Werven. (2021) ***Transcription levels of a long noncoding RNA orchestrate opposing regulatory and cell fate outcomes in yeast*****. Cell Reports. 34(3):108643. DOI:** **10.1016/j.celrep.2020.108643**
This work demonstrates how yeast cells have put in place a robust regulatory circuit to ensure that the cell fate decision leading to meiosis is tightly controlled, and that only diploid but not haploid cells enter meiosis. We identified a new mechanism by which transcription of noncoding RNAs can activate gene expression. Our findings have important implications for the gene regulatory potential of long non-coding RNAs (lncRNAs) in general. We propose that transcription level dependent chromatin changes may explain the opposing regulatory activities exerted by lncRNAs.

Chia M.*, C. Li*, S. Marques, V. Pelechano, N.M. Luscombe and F.J. van Werven (*equal contributors). (2021) ***High-resolution analysis of cell-state transitions in yeast suggests widespread transcriptional tuning by alternative starts*****. Genome Biology. 22, 46. DOI: 10.1186/s13059-021-02274-6.**
The start and end sites of messenger RNAs (TSSs and TESs) are highly regulated, often in a cell-type specific manner. Yet the contribution of transcript diversity in regulating gene expression remains largely elusive. We performed an integrative analysis of multiple highly synchronized cell-fate transitions and quantitative genomic techniques in Saccharomyces cerevisiae to identify regulatory functions associated with transcribing alternative isoforms. Increased upstream alternative TSS usage is linked to various effects on canonical TSS levels, which range from co-activation to repression. We identified two key features linked to these outcomes: an interplay between alternative and canonical promoter strengths, and distance between alternative and canonical TSSs. These two regulatory properties give a plausible explanation of how locally transcribed alternative TSSs control gene transcription. Our integrative analysis of multiple cell fate transitions suggests the presence of a regulatory control system of alternative TSSs that is important for dynamic tuning of gene expression.

Tam J., and F.J. van Werven. (2020) ***Regulated repression, and not activation, governs the cell fate promoter controlling yeast meiosis*****. Nature Communications. 11, 2271. DOI: 10.1038/s41467-020-16107-w.**
Our findings provide important new insights into how the decision to enter meiosis and produce gametes is regulated; environmental signals regulate the association and disassociation of transcription factors that recruit Tup1-Cyc8 to the *IME1* promoter. In addition, the work on the *IME1* promoter gives novel insights into how Tup1-Cyc8 regulates gene transcription in general. Transcription factors designated to recruit Tup1-Cyc8 and transcriptional activators prime the *IME1* promoter, but these proteins are likely not the same proteins as have been proposed before. The regulatory principles identified in this work show features of how enhancers in mammalian cells are controlled.

Wu, A.C.K., H. Patel, M. Chia, F. Moretto, D. Frith, A.P. Snijders, F.J. van Werven. (2018) ***Repression of Divergent Noncoding Transcription by a Sequence-Specific Transcription Factor*****. Molecular Cell 72(6):942-954. DOI: 10.1016/j.molcel.2018.10.018.**
Transcription factors typically activate transcription by recruiting cofactors, but our data in this paper illustrate a new function. We show that the sequence-specific transcription factor Rap1 prevents regulatory elements from initiating transcription in the divergent direction. We define a novel mechanism for providing directionality towards productive transcription, as Rap1 promotes directionality, at least in part, by directly interfering with transcription initiation in the divergent direction. Our study reveals a new important layer of regulation, describing how genomes restrict the accumulation of aberrant transcripts and ensure productive coding gene expression.

Chia M., A. Tresenrider, J. Chen, G. Spedale, V. Jorgensen, E. Ünal* and F.J. van Werven* (*co-corresponding authors). (2017) ***Transcription of a 5' extended mRNA isoform directs dynamic chromatin changes and interference of a downstream promoter*****. Elife 6:e27420. DOI: 10.7554/eLife.27420.**
This work demonstrates that 5' extended mRNA isoforms can act as repressors of gene expression. During meiotic prophase in *S. cerevisiae,* the kinetochore complex subunit Ndc80 is downregulated by transcription from a distal *NDC80* promoter directing histone H3K4 and H3K36 methylation to establish a repressive chromatin state on the downstream canonical *NDC80* promoter. This mechanism may be conserved, suggesting that such simple switches may affect meiosis in higher eukaryotes. The findings have potentially wide implications for interpreting gene expression studies, which often report changes in expression levels without taking into account changes in isoform length; genes that appear up-regulated may actually be repressed.



Name	JEAN-PAUL VINCENT
Position	Senior Group Leader
Year joined (Crick or founder institute)	1997

Lab Name	***Epithelial Cell Interactions Laboratory***

Research programme and achievements

My group investigates how, in the context of developing tissues, the range and activity of signalling pathways (signalling landscapes) are generated and then interpreted to control growth, apoptosis, and cell fate decisions.

Establishment of signalling landscapes. During the past five years we have begun to understand how morphogens (lipidated and soluble) form long-range gradients. First, we have tracked the trafficking routes that Wnts take in producing cells. We then found that the Wnt lipid moiety is shielded by a subclass of glypicans defined by the Dally-like protein. We suggest that this interaction allows Wnts to spread in the extracellular space. In another study, we have engineered GFP into a signalling molecule recapitulating the activity of a soluble endogenous morphogen and shown that if one considers the presence of extracellular binding partners, diffusion can account for the formation of a morphogen gradient.
We are planning to continue our multidisciplinary approaches to track the journey of Wnt proteins from secretion to engagement with signalling receptors. This will involve structural and biochemical analysis of Wnts' interactions with their numerous partners (including Wntless, glypicans, and Frizzled receptors) and mathematical modelling to generate a testable theory of Wnt gradient formation in tissues. Additional roles of glypicans in morphogen transport will also be investigated. This programme of work will be in close collaboration with Yvonne Jones and Guillaume Salbreux.

Signalling landscapes that control apoptosis and cell survival. Cell-cell signalling ensures the organised elimination of defective cells. We have shown previously that JNK signaling triggers apoptosis in response to epithelial disruption, and found that this activity is modulated by adenosine signalling, which in turn boosts the production of TNFα, a proapoptotic signal. We also discovered the mechanism underlying the massive apoptosis suffered by many developmental mutants, showing that this follows from disruption of the EGFR signaling landscape in these mutants.
Future work on the regulation of apoptosis will be incorporated in a programme to understand the coordination of compensatory proliferation and apoptosis in a stressed epithelium (see below).

From signalling landscapes to growth, pattern and polarity. In order for well-proportioned structures to form during development, growth and patterning must be coordinated. This is achieved in part because patterning signals are required for growth. We have begun to investigate how these signalling pathways, particularly those mediated by Wingless and Dpp, impinge on growth. We have previously shown that Wingless acts as a permissive

signal for growth (i.e. graded signalling is not required) and that the same is true for Dpp. We also found that graded Wnt signalling is not required for the establishment of planar cell polarity.

How Dpp and Wnt signalling combine with other growth modulating signals at the molecular level remains unknown, and in the next quinquennium, we will follow three lines of research to investigate growth control during development. (1) We will identify the mediators of the pro-growth activities of the two main morphogens that pattern the fly wing. (2) To widen our understanding of growth regulation, we will investigate how changes in systemic ecdysteroids modulate tissue growth. (3) We will also be taking an unbiased approach to identify genes that correlate with growth rate. Overall this programme will address the molecular basis of phenomena such as the progressive slowing down of growth, the termination of growth, the coordination of growth and patterning, and scaling.

Signalling dynamics and plasticity of cell fate decisions. Several signalling pathways interact dynamically to control vein formation in the *Drosophila* wing. We have invested significant effort in designing fast reporters and optogenetic actuators (with Y. Bellaiche) to probe this behaviour in live tissue. We will develop a quantitative understanding of the signalling network and show how it accounts for the formation of reliably sized veins (with G. Salbreux).

Balancing cell removal and replenishment in a stress epithelium. We have found that ribosomal protein (RP) insufficiency (in a *Drosophila* model of a human ribosomopathy) triggers the unfolded protein response. Remarkably, RP-deficient tissues develop into normal adult structures despite massive apoptosis. We will determine why only a subset of cells die even though they all have the same RP-deficient genotype. We also will determine how compensatory proliferation perfectly compensates for cell loss. We know that several signalling pathways are involved and anticipate synergy with our work on signalling dynamics.

A diagnostic tool for colorectal cancer. In collaboration with Svend Kjaer, we are developing reagents to detect Notum and validate them for the diagnostic of colorectal cancer.

Research outputs

Staporwongkul, K., de Gennes,M., Cocconi, L., Salbreux,G.*, and Vincent, J.-P.* (2020) ***Patterning and growth control in vivo by an engineered GFP gradient*. Science, 370, 6514. DOI: 10.1126/science.abb8205**

By merging the power of molecular genetics, basic physical chemistry and mathematical modeling we show quantitatively how an inert protein can be turned into a morphogen that provides positional information in a developing tissue. This is the first time that an active synthetic morphogen gradient is reconstituted *in vivo*.

McGough, I.J., Vecchia, L., Bishop, B., Malinauskas, T., Beckett, K., Joshi, D., O'Reilly, N., Siebold, C., Jones, E.Y and Vincent. J.-P. (2020) ***Glypicans shield the lipid moiety of Wnts to enable signalling at a distance*. Nature 585(7823):85 – 90. DOI: 10.1038/s41586-020-2498-z**

This paper solves the Wnt solubility problem, which has preoccupied the Wnt field for the past 15 years. It explains how the lipid of Wnts can be maintained in the extracellular space. A great example of combining the expertise of the Jones & Vincent groups.

Crossman, S., Streichan, S., and Vincent, J.-P. (2018) ***Loss of EGFR signaling accounts for the pattern of apoptosis in a wide range of developmental mutants*. PLoS biology 16(10): e3000027. DOI: 10.1371/journal.pbio.3000027**

This paper provides a simple explanation for a seemingly mysterious process whereby mis-specified cells seem to recognise that they are in the wrong place and undergo apoptosis as

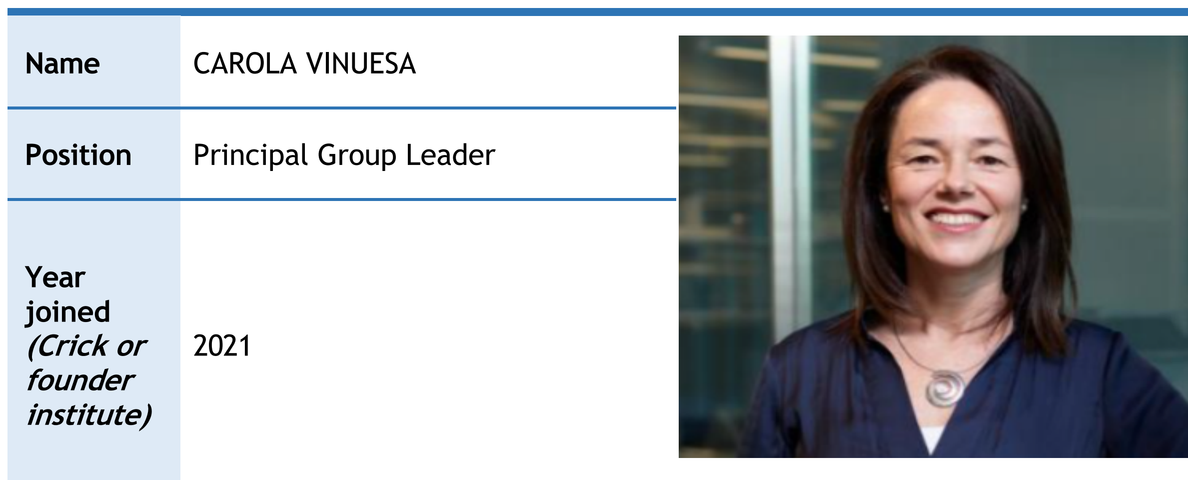
a result. We show that patterning mutants disrupt the segmental pattern of EGFR signalling which is in turn required for cell survival.

Sanchez Bosch, P., Ziukaite, R., Alexandre, C., Basler, K., and Vincent. J.-P. (2017) ***Dpp controls growth and patterning in Drosophila wing precursors through distinct modes of action*. e Life 6:e22546. DOI: 10.7554/eLife.22546.**

This paper settles the question of whether a gradient of Dpp signalling is needed for the growth of wing primordia. It demonstrates that flat, constant Dpp signalling is sufficient to sustain proliferation.

Carles Recasens-Alvarez, Cyrille Alexandre, Joanna Kirkpatrick, Hisashi Nojima, David J. Huels, Ambrosius P. Snijders and Jean-Paul Vincent. (2021)
***Ribosomopathy- associated mutations cause proteotoxic stress that is alleviated by TOR inhibition* .**
Nature Cell Biology 23, 127 - 135. DOI: 10.1038/s41556-020-00626-1

In this paper, we uncover the cell biological basis of a human ribosomopathy. Somewhat unusually, this project was spurred by a member of the public who asked us to create a Drosophila model of his son's condition caused by a ribosomal mutation (RPS23[R67K]. Flies carrying this mutation are viable but display many markers of cellular stress.
Surprisingly, this is not caused by insufficient protein synthesis capacity but instead by a reduced ability to eliminate misfolded proteins, perhaps because of an unusual burden from orphaned ribosomal proteins. We found that pharmacological or
genetic inhibition of TOR significantly rescued cellular stress in these animals, suggesting a therapeutic strategy to alleviate the symptoms of this and other ribosomopathies.

Name	CAROLA VINUESA	
Position	Principal Group Leader	
Year joined *(Crick or founder institute)*	2021	

Lab Name	***Autoimmunity lab***

Research programme and achievements

My lab investigates how the immune system generates protective and lasting understand antibody responses, and how B cells become dysregulated to cause autoimmunity. We do this by unravelling the complex interactions between the cells and molecules that control B cell activation and differentiation, and by identifying rare genetic abnormalities with large effects that interfere with this process in patients with autoimmune diseases.

The major achievements of the lab since 2018 are:

1.Sequencing and analysis of exomes or genomes from kindreds with immune diseases to understand disease mechanisms through the discovery of novel and rare gene variants and their functional validation. The major achievements include:

- Establishing that over 80% of patients with systemic autoimmunity carry rare coding variants in genes implicated in autoimmune diseases by GWAS, and demonstrating that for two of the genes found to harbour such variants in a large frequency of patients – *BLK* and *BANK1*, the rare variants are functional and limit TRAF6/IRF5 signaling to prevent repression of type I IFN.
- Through whole exome sequencing of 73 paediatric lupus trios in Shanghai, identification of a novel role for P2RY8 in human B cell tolerance, and demonstration that rare coding variants in this gene increase PI3K and ERK activity, impair B cell confinement in germinal centres, and negative selection of immature self-reactive B cells.
- Identification of an intronic deletion in *VANGL1* as a kidney intrinsic risk for renal damage in the context of autoantibody-mediated disease (lupus).
- Discovery of a novel monogenic cause of lupus, likely to be the central hub in disease development: gain of function mutations in the RNA viral sensor TLR7. Gain-of-function variants increase affinity for self-nucleic acids and cause TLR7-dependent and B cell intrinsic accumulation of extrafollicular age-associated CD11c+ B cells (ABCs). This discovery offers a new therapeutic avenue for lupus.
- Identification of a de novo variant in PACSIN1 that forms a complex with TRAF4 to regulate TLR7 signaling

Collectively, these studies suggest that increased TLR7 signaling is central to lupus pathogenesis and pays a central role in directing extrafollicular differentiation of self-reactive B cells into pathogenic ABCs.

2. Demonstration that the process of immunoglobulin class switching in B cells, also known as class switch recombination (CSR) does not occur in germinal centers as previously thought, but during cognate interactions of naïve or memory B cells with primed T cells at the T:B border. This is important because it affects the classification of B cell lymphomas and our understanding of memory B cell diversification. It has changed immunology textbooks.

3. Discovery of the long-sought mechanism of autoantibody and IgE repression by regulatory T cells and the mechanism of action of follicular regulatory T cells (Tfr cells): production of the neuropeptide 'neuritin' that enters B cells to limit differentiation of self-reactive cells into plasma cells and IgE switching. This work provides a novel approach to treat allergies and autoimmune disorders, including tissue-specific autoimmunity.

4. Discovery and organising functional validation of a novel variant in *CALM2* that provides an explanation for the sudden unexpected death of the daughters of Australian Kathleen Folbigg, who has been in jail for the past 20 years accused of their murder. I will be presenting this and further related evidence at the second Inquiry into her convictions to be held in Sydney from 13-20 Feb 2023.

Research outputs

Brown GJ, Cañete PF, Wang H, Medhavy A, Bones J, Roco JA, He Y, Qin Y, Cappello J, Ellyard JI, Bassett K, Shen Q, Burgio G, Zhang Y, Turnbull C, Meng X, Wu P, Cho E, Miosge L, Andrews TD, Field MA, Tvorogov D, Lopez AF, Babon JJ, Aparicio López C, Gónzalez-Murillo A, Clemente Garulo D, Pascual V, Levy T, Mallack EJ, Calame DG, Lotze T, Lupski JR, Ding H, Ullah TR, Walters GD, Koina ME, Cook MC, Shen N, de Lucas Collantes C, Corry B, Gantier MP, Athanasopoulos V and Vinuesa CG. *TLR7* gain-of-function genetic variation causes human lupus. *Nature,* 2022. *In press.* Link to preprint.

An important discovery providing definitive genetic evidence that gain-of-function mutations in TLR7, a known viral RNA sensor, cause human lupus. We show that such mutations increase the receptors affinity for self-nucleic acids. It provides an explanation for the increased female risk for systemic autoimmunity and offers a very promising therapeutic target to treat this often very debilitating disease. This work is the result of sequencing the genomes of children with severe systemic autoimmunity and introducing candidate monogenic variants into mice, to obtain functional validation and mechanistic insights. Importantly, we show that germinal centers are beneficial rather than pathogenic in lupus, limiting self-reactivity.

He Y, Gallman AE, Xie C, Shen Q, Ma J, Wolfreys FD, Sandy M, Arsov T, Wu X, Qin Y, Zhang P, Jiang S, Stanley M, Wu P, Tan J, Ding H, Xue H, Chen W, Xu J, Criswell LA, Nititham J, Adamski M, Kitching AR, Cook MC, Cao L, Shen N, Cyster JG and Vinuesa CG. P2RY8 variants in lupus patients uncover a role for the receptor in immunological tolerance. *J Exp Med.* 2022 Jan 3;219(1):e20211004. Link here.

This paper uncovers a novel role for P2RY8 in immunological tolerance through promoting migration to germinal centers and limiting ERK and PI3K activity in B cells. It demonstrates the contribution of rare P2RY8 germline coding variants to human systemic autoimmunity. This is the first paper from Vinuesa's lab in Shanghai and a result of undertaking whole exome sequencing in local patients with childhood-onset SLE.

Gonzalez-Figueroa P, Roco JA, Papa I, Núñez Villacís L, **Stanley M, Linterman MA,** Dent A, **Canete PF** and **Vinuesa CG**. Follicular regulatory T cells produce neuritin to regulate B cells. ***Cell.*** 2021 Apr 1;184(7):1775-1789. Link here.

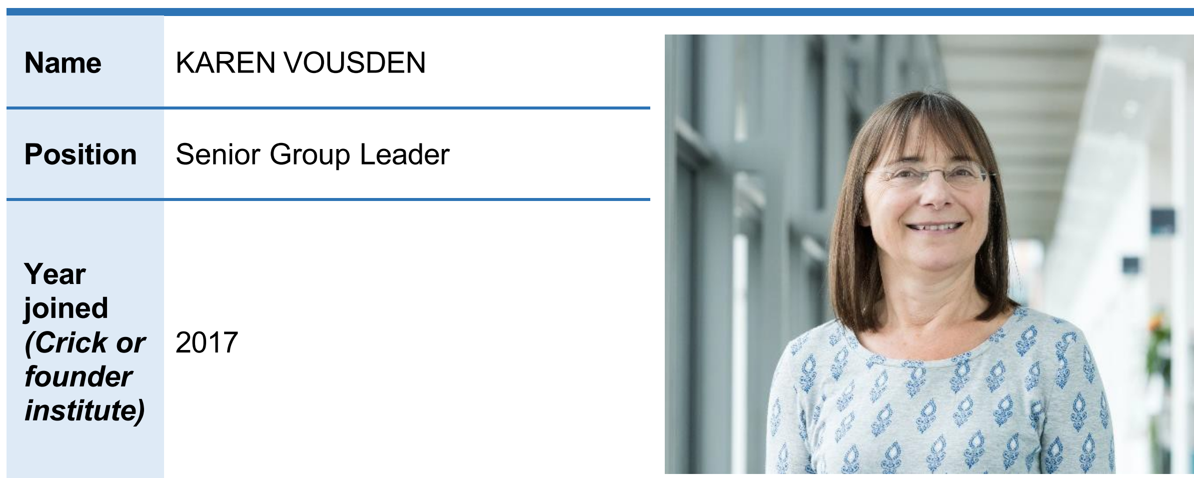
This paper uncovers the long-sought mechanism by which regulatory T cells – and specifically follicular regulatory T cells (Tfr) - regulate pathogenic antibody responses. It identifies a neuropeptide of previously unknown immune function, neuritin, produced by Tfr cells that enters B cells and prevents their differentiation into plasma cells and autoantibody production. It demonstrates that neuritin also prevents switching to IgE and anaphylaxis. This is an attractive new therapeutic strategy to treat antibody-driven diseases.

Roco JA, Mesin L, Binder SC, Nefzger C, Gonzalez-Figueroa P, Canete PF, Ellyard J, Shen Q, Robert PA, Cappello J, Vohra H, Zhang Y, Nowosad CR, Schiepers A, Corcoran LM, Toellner KM, Polo JM, Meyer-Hermann M, Victora GD and Vinuesa CG. Class-Switch Recombination Occurs Infrequently in Germinal Centers. *Immunity.* 2019 Aug 20;51(2):337-350.e7. Link here.

Overturns the prevailing dogma that class switch recombination (CSR) predominantly occurs in germinal centers. We show that CSR ceases in GCs. This work is re-writing the textbooks and has implications for understanding regulation of genome integrity and the origin of B cell lymphomas and their treatment. It was the journal cover's art and it is an ISI highly cited paper.

Jiang SH, Athanasopoulos V, Ellyard JI, Chuah A, Cappello J, Cook A, Prabhu SB, Cardenas J, Gu J, Stanley M, Roco JA, Papa I, Yabas M, Walters GD, Burgio G, McKeon K, Byers JM, Burrin C, Enders A, Miosge LA, Canete PF, Jelusic M, Tasic V, Lungu AC, Alexander SI, Kitching AR, Fulcher DA, Shen N, Arsov T, Gatenby PA, Babon JJ, Mallon DF, de Lucas Collantes C, Stone EA, Wu P, Field MA, Andrews TD, Cho E, Pascual V, Cook MC, Vinuesa CG. Functional rare and low frequency variants in BLK and BANK1 contribute to human lupus. *Nat Commun.* 2019 May 17;10(1):2201.

Demonstration that a large fraction of lupus patients carry functional rare variants, many of them occurring in the BLK and BANK1 genes. We identify novel roles for these genes in repressing TRAF6 and IRF5 signaling downstream of endosomal TLR and Myd88 signals. This was timely because there was resistance in the field of human autoimmunity to accept a role for rare variants to disease pathogenesis



Name	KAREN VOUSDEN
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2017

Lab Name	***P53 and metabolism laboratory***

Research programme and achievements

Over the past five years we have continued to develop our understanding of the regulation and function of p53, a tumour suppressor that is lost or mutated in most cancers. We have made a significant contribution to the understanding of how p53 is regulated and the responses that are driven by p53. These studies led us to investigate the role of metabolism in the control of tumorigenesis, focusing on serine metabolism and the regulation of oxidative stress.

As the principal E3 ligase that targets p53 for degradation, MDM2 is essential for survival. Our structural analysis showing how the RING domain of MDM2 interacts with the E2/ubiquitin complex allowed us to identify MDM2 mutants that cannot target p53 for degradation but retain the ability to control p53 function. Using these mutants, we are validating a new approach to MDM2 inhibition for cancer therapy, based on the selective targeting of MDM2's E3 function.

Our early studies focused on understanding functions of p53 – like the induction of cell death - that drive the elimination of nascent cancer cells. However, more recently we have pursued the unexpected observation that p53 can also function to allow survival and adaptation to non-genotoxic signals such as metabolic stress. Loss of p53 in cancer cells results in an inability to survive serine or glutamine starvation (3), an observation that we developed to show a therapeutic efficacy of dietary serine and glycine depletion in various mouse models (4). We also found that these survival functions can be retained by cancer derived mutant p53s, suggesting that the selective retention of this wild type p53 activity is beneficial during cancer progression. This work led us to examine more generally the role of serine and one carbon metabolism in cancer development, where we showed the importance of *de novo* ATP synthesis in supporting the methionine cycle and DNA methylation (5). At least some of the survival functions of p53 are associated with the ability of p53 to assist in antioxidant defence, mediated through the transcriptional activation of a number of target genes including p21, MDM2 and TIGAR. Regulation of oxidative stress can have opposing effects during tumour development, with evidence for tumour promoting functions of both pro- and antioxidant signals. We found that cancer cells placed into detached conditions undergo metabolic remodelling to remove damaged mitochondria and limit increased ROS (2). While this response allows cancer cell survival during loss of attachment to the extracellular matrix, we also showed that defects in antioxidant defence during pancreas tumour development can enhance metastasis (1). Taken together our work indicates that temporal, dynamic control of ROS underpins full malignant progression and helps to rationalise conflicting reports of pro- and anti-tumor effects of antioxidant treatment.

Finally, we showed that loss of p53 in cancer cells modulates the tumor-immune

landscape to circumvent immune destruction. This response to loss of p53 reflected both an increase in myeloid and Treg recruitment and an attenuation of the CD4+ and CD8+ T- cell response *in vivo*. Interestingly, we were able to show that two key drivers of tumorigenesis, activation of KRAS and deletion of p53, cooperate to promote immune tolerance.

Moving forward we are focusing of a few key areas. We continue to assess the potential of dietary manipulation of amino acid availability to complement cancer treatment, expanding our work to examine the role of amino acids beyond serine and glycine. We have established a small company (Faeth Therapetics) with colleagues from the UK and US to take the idea of precision nutrition into clinical trials. We are testing potential new targets in mitochondrial one carbon metabolism that could affect the balance of metabolite production and antioxidant defence and so provide therapeutic efficacy. Most importantly, we will continue to assess the consequences of modulating nutrient availability on the immune response to cancer. It is clear that the tumour microenvironment is critical in providing support for cancer cells and we are determining the role of adipocytes in this context. The interaction between tumour cells can also impact oncogenic progression and we are investigating how differences in p53 status affect the way cancer cells interact with each other. Finally, we have developed a mouse model to test the therapeutic response to inhibition of the ability of MDM2 to ubiquitinate p53, and in collaboration with Astra Zeneca we will explore the potential of this approach in cancer treatment.

Research outputs

Cheung EC, De Nicola GM, Nixon C, Blyth K, Labuschagne CF, Tuveson DA, Vousden KH. (2020) ***Dynamic ROS control by TIGAR regulates the initiation and progression of pancreatic cancer*****. Cancer Cell 37:168-182. DOI: 10.1016/j.ccell.2019.12.012**
We show that during the development of pancreatic cancer, regulation of reactive oxygen species can both promote or restrain tumorigenesis, depending on the stage of malignant progression. This work highlights the complexity of the response to targeting ROS regulation in cancer therapy.

Labuschagne CF, Cheung EC, Blagih J, Domart M-C, Vousden KH. (2019) ***Cell clustering promotes a metabolic switch that supports metastatic colonisation*****. Cell Metabolism 30:720-734. DOI: 10.1016/j.cmet.2019.07.014**
We show that the clustering of cancer cells following detachment from ECM results in hypoxia, which activates mitophagy to remove damaged mitochondria and reductive metabolism to support glycolysis. These responses limit mitochondrial ROS production, allowing cell survival and metastasis.

Tajan M, Hock AK, Blagih J, Robertson NA, Labuschagne CF, Kruiswijk F, Humpton T, Adams PD and Vousden KH[*]. (2018) ***A role for p53 in the adaptation to glutamine starvation through the expression of SLC1A3*****. Cell Metabolism 28:721-736. DOI: 10.1016/j.cmet.2018.07.005**
In this paper we show that the ability of cells to survive glutamine depletion depends on aspartate metabolism, which is supported by the aspartate/glutamate transporter SLC1A3. The tumor suppressor p53 is shown to induce the expression of SLC1A3, explaining in part how p53 can help cancer cells survive under glutamine starvation.

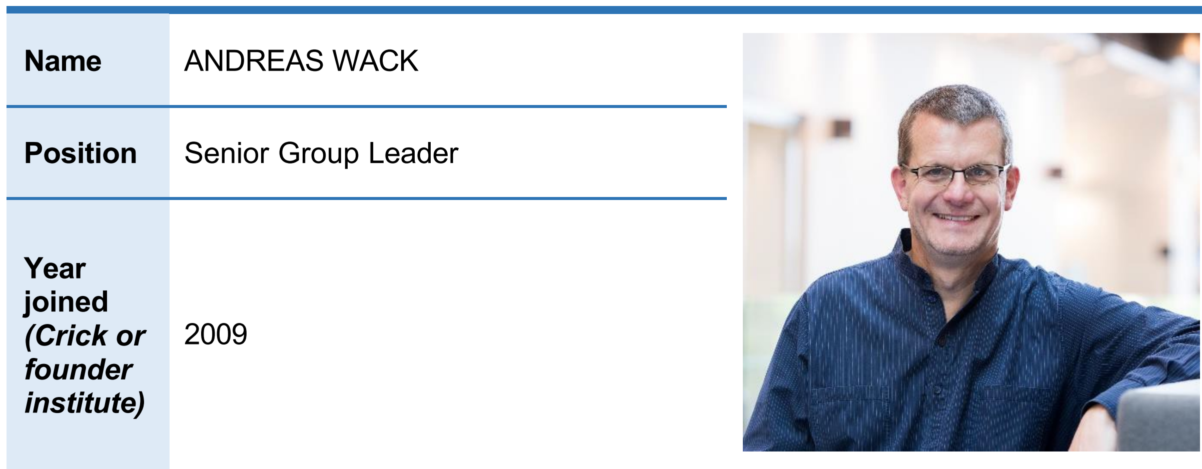
Maddocks ODK, Athineos D, Cheung EC, Lee P, Zhang T, van den Broek NJF, MacKay GM, Labuschagne CF, Gay D, Kruiswijk F, Blagih J, Vincent DF, Campbell KJ, Ceteci F, Sansom OJ, Blyth K and Vousden KH. (2017) ***Modulating the therapeutic response of tumours to serine and glycine starvation*****. Nature 544, 372-376. DOI: 10.1038/nature22056**

This paper expands on our previous work to show the therapeutic benefits of dietary serine and glycine starvation in several genetically engineeredmouse models. We find that activation of KRAS makes tumours less responsive to this approach, reflecting an ability of activated KRAS to increase expression of serine synthesis pathway (SSP) enzymes and *de novo* serine synthesis.

Tajan M, Hennequart M, Cheung E, Zani F, Hock AK, Legrave N, Maddocks ODK, Ridgway RA, Athineos D, Suárez-Bonnet A, Ludwig RL, Novellasdemunt L, Angelis N, Li VSW, Vlachogiannis G, Valeri N, Mainolfi N, Suri V, Friedman F, Manfredi M, Blyth K, Sansom OJ and Vousden KH (2021) ***Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy*****. Nature Communications 12, 366. DOI: 10.1038/s41467-020-20223-y**

Targeting the nutritional requirements of cancers through selective dietary intervention is an emerging therapeutic approach. Dietary limitation of the non-essential amino acids serine and glycine can limit the growth of some, but not all, cancers. This study extends this approach by showing combined treatment with an inhibitor of the intrinsic serine synthesis pathway with a serine/glycine free diet improves the therapeutic response and inhibits the growth of cancers that are not responsive to the diet alone. Extension of this work to human studies may offer an important new avenue for the treatment of a broad range of cancers.

Name	ANDREAS WACK
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2009



Lab Name	***Immunoregulation Laboratory***

Research programme and achievements

Acute infections and chronic inflammation in the respiratory tract remain among the leading causes of morbidity and death worldwide. In a vicious circle, chronic inflammatory lung conditions such as chronic obstructive pulmonary disease (COPD) and asthma facilitate infections, which in turn aggravate inflammation.
Lung-infecting microbes cause tissue damage that requires timely repair. The host response to infection can increase tissue damage, and can impact positively or negatively on repair processes. We study the interplay between infection, immune response, inflammation, tissue damage and repair, to understand pathogenesis and protection in lung infection.

1. Complex effects of interferons (IFNs) in viral infection
IFNs are prototypic antiviral cytokines. However, we showed that due to their pleiotropic functions, IFNs are not always protective but have more complex effects in viral infection. Type I IFNs (IFN-α and IFN-β) drive inflammation, CCL2-dependent monocyte recruitment and airway damage early during influenza, while type III IFNs (IFN-λ) do not have these proinflammatory properties. In contrast, late in infection IFN-λ impairs epithelial proliferation and differentiation, thus delaying tissue repair, an effect that is less evident for IFN–α and □□β. We demonstrated that unregulated IFN responses not only increase influenza severity but also facilitate bacterial superinfection, a common complication of influenza today, and observed in >95% of biopsies tested from victims of the 1918 influenza pandemic which claimed an estimated 50 million deaths. Therefore, tight IFN regulation is crucial, and we test whether drugs can be repurposed to harness IFN-mediated protection and limit IFN-driven pathology. Our studies extend beyond the above areas and have obvious clinical relevance to identify host-directed treatment options for known and novel respiratory viruses.

2. Long-term changes to lung immunity by self-limited influenza infection
Influenza yearly infects 8-15% of the world population, and most people survive infection. We found that influenza leaves a lung imprint beyond recovery, with long-term changes in lung immunity in post-influenza mice. Post-influenza immune reactivity is increased due to newly recruited, monocyte-derived cells that contribute to the pool of alveolar macrophages, while self-renewing tissue-resident macrophages that are hyporesponsive remain unchanged. Newly recruited macrophages slowly change over time of residence in the recovering lung, to become eventually as hyporesponsive as resident alveolar macrophages.

We envisage that origin and residence time in the lung are major determinants of macrophage function, with the (inflamed or uninflamed) lung environment further

impacting on macrophage responsiveness. We plan to investigate the mechanisms underlying the slow change from hyperresponsive to hyporesponsive lung macrophages. Given that macrophage reactivity is important in cancer and chronic inflammation in the human lung, we are using our mouse dataset to identify surface markers that indicate the origins of human macrophages: monocyte-derived recruited versus embryonic-derived tissue-resident alveolar macrophages.

Another candidate for influenza-induced long-term changes are lung epithelia, as epithelial precursors reside locally and can potentially be imprinted. As epigenetic changes to lung epithelia are known to be central to COPD, we have started investigating whether there is also epithelial memory of prior lung infections.

3. Airway epithelial cells (AECs): Conditions for (re-)development and repair
Airway epithelia are targeted by pathogens, trigger early immune responses, and need to be intact to ensure organ function. Correct epithelial repair is an important determinant of recovery from infection, and incorrectly differentiated airways are a hallmark of COPD. In many organs, metabolism co-determines cellular differentiation, and metabolic alterations contribute to pathogenesis. We have followed metabolic changes during lung epithelial differentiation and find that a switch to fatty acid oxidation is required for differentiation *in vitro* and *in vivo*. We have previously described that the aryl hydrocarbon receptor (AhR), known as a sensor of pollutants and toxins, orchestrates development of multiciliated cells in airway epithelia. We now find that AhR has an important role in limiting epithelial damage during viral infection, and we are currently investigating if AhR also affects metabolic rewiring of epithelia, as suggested in other cell types. Fam13a, the most consistent COPD GWAS hit, has been implicated in regulating fatty acid oxidation in epithelia, and we are testing the hypothesis that Fam13a contributes to COPD through epithelial dysfunction due to metabolic alterations.

To summarise, the integrated analysis of lung immune responses and epithelial differentiation will take our understanding of infections and chronic inflammatory conditions to a new level.

Research outputs

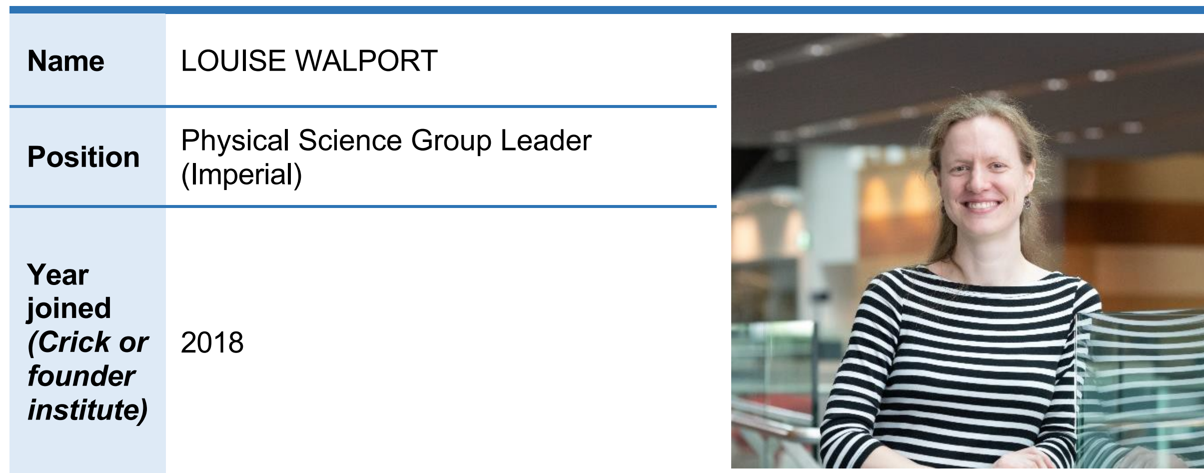
Major, J., Crotta, S., Llorian, M., McCabe, T.M., Gad, H.H., Priestnall, S.L., Hartmann, R. & Wack, A. (2020) *Type I and III interferons disrupt lung epithelial repair during recovery from viral infection*. Science 369(6504):712-717. DOI: 10.1126/science.abc2061.
We showed that interferons (IFNs), known to have antiviral effect, can aggravate respiratory viral infection if present late during infection when epithelial repair sets in. IFN-β and, most potently, IFN-λ reduce airway epithelial proliferation and differentiation in that recovery phase. This is important to understand the complex roles of IFNs in viral infections and has important implications for IFN treatments as presently discussed for COVID-19.

Aegerter, H., Kulikauskaite, J., Crotta, S., Patel, H., Kelly, G., Hessel, E.M., Mack, M., Beinke, S. & Wack, A. (2020) *Influenza-induced monocyte-derived alveolar macrophages confer prolonged antibacterial protection*. Nature Immunology 21(2):145-157. DOI: 10.1038/s41590-019-0568-x
We found long-lasting changes in lung immunity 28 days after influenza infection in alveolar macrophages (AMs), a population that in naïve mice is embryonically derived and self-renewing. Post influenza, cells derived from monocytes recruited early during influenza contribute to the AM pool. They are indistinguishable from embryonically derived AM by surface phenotype, but show a functional, transcriptomic and chromatin profile more similar to blood monocytes. Hence, the changed functionality is due to the changed composition of the pool of AMs. The AM population in humans will likely be a mosaic of cells recruited at different times with different functionalities.

Bradley, K.C., Finsterbusch, K., Schnepf, D., Crotta, S., Davidson, S., Fuchs, S.Y., Staeheli, P. & Wack, A. (2019) ***Microbiota-driven tonic IFNsignals in lung epithelial cells protect from influenza infection*****. Cell Reports 28, 245-256. DOI: 10.1016/j.celrep.2019.05.105**
This study showed that gut microbiota help maintain the IFN signature in lung epithelia and thus contribute to antiviral protection. Antibiotic-treated mice show a reduction in the IFN signature in lung epithelia, the main target of influenza virus infection, leading to increased virus replication in these cells as early as eight hours post infection. The study has important clinical implications and for livestock farming, indicating that antibiotic treatment of healthy organisms likely increases susceptibility to respiratory viral infections.

Villa, M., Crotta, S., Dingwell, K.S., Hirst, E.M.A., Gialitakis, M., Ahlfors, H., Smith, J.C., Stockinger, B. & Wack, A. (2016) ***The aryl hydrocarbon receptor controls cyclin O to promote epithelial multiciliogenesis*****. Nature Communications 7, 12652. DOI: 10.1038/ncomms12652**
The aryl hydrocarbon receptor (AhR) has physiological roles at barriers and in mucosal immunology, but its roles in the lung are less well known. We showed here that AhR directly targets and induces cyclin O, a master regulator of the differentiation of multiciliated cells in airway epithelia. In the absence of AhR, multiciliated cells develop less well, with likely consequences for the mucociliary escalator mediating non-inflammatory removal of particles from the lung. Toxic ligands direct AhR away from inducing a multiciliation programme towards activating a detoxification programme, demonstrating a molecular link between airway pollutants and lung disease.

Davidson, S.*, McCabe, T.*, Crotta, S., Gad, H.H., Hessel, E.M., Beinke, S., Hartmann, R. & Wack, A (*equal contribution). (2016) ***IFNλ is a potent anti-influenza therapeutic without the inflammatory side effects of IFNα treatment*****. EMBO Mol. Med 8(9):1099-1112. DOI: 10.15252/emmm.201606413**
We have previously shown that IFNα can aggravate influenza infection by enhancing pathogenic inflammation. In a direct comparison of IFNα and IFNλ, we showed that IFNλ does not have these proinflammatory effects. IFNλ is therefore the preferred treatment option early in influenza, as the antiviral effects are similer to those of IFNα, but immunopathology is not enhanced by IFNλ. This paper suggests that IFNλ should be used for COVID-19 treatment, but early use is important (see Major et al, above).



Name	LOUISE WALPORT
Position	Physical Science Group Leader (Imperial)
Year joined *(Crick or founder institute)*	2018

Lab Name	***Protein-Protein Interaction Laboratory***

Research programme and achievements

My lab was established in October 2018. We apply a state-of-art cyclic peptide discovery system, known as the RaPID system, to develop chemical probes with which we and others can manipulate a range of biological systems. We have achieved our initial goal of establishing the RaPID screening technology in house. Since then, each lab member has begun working on individual but complementary projects towards our shared lab vision.

Work in the group builds on my background in chemical probe discovery for epigenetic targets, assay development and enzymology. Prior to joining the Crick, I demonstrated that arginine methylation could be removed by a family of non-haem Fe(II) enzymes, settling a long-standing question in the field as to whether this modification was a reversible post-translational modification (PTM). My lab has maintained an interest in arginine PTMs and now focuses on the peptidyl arginine deiminases (PADIs) which convert arginine to citrulline (see below). More recently, in a study profiling the diversity of cyclic peptides found within encoded libraries (in collaboration with the Mackay Lab (University of Sydney)) we have revealed that encoded peptide libraries contain members with many different structural elements that can bind to similar proteins in surprisingly diverse ways. This work provides an experimental explanation for the widely accepted view that peptide library approaches can be used to identify potent and selective protein binders of almost any target of choice.

On the technology development side, my lab now seeks to extend the chemical functionalities that can be included in peptides within DNA-encoded libraries to further expand their applicability. This currently includes incorporating photoswitchable moieties to produce tools that can be switched “on” and “off”, reactive chemical functionalities to produce covalent protein binders and non-canonical amino acids that allow direct visualisation of peptides in cells or enhance their proteolytic stability. We are also exploring the possibility of developing more tailored methods to identify peptide tools with defined functionalities, such as protein activation. We use the RaPID system as a model but aim to develop strategies that could be applied more widely to other DNA encoded drug discovery platforms.

On the biomedical application side, we are using the peptide tools we produce, combined with other more classical biochemical and molecular biology techniques, to rigorously explore the biological significance of protein citrullination in health and disease. The long- term aim of this work is to connect the biochemical effects of protein citrullination with animal physiology, informing on how the chemistry of (methylated)guanidino and citrulline groups relates to biological function; a field in

which there has been relatively little work compared to many other PTMs.

We have produced peptide inhibitors and first-in-class activators of the nuclear member of the PADI family, PADI4. These highly selective peptides provide us with tools to both increase and decrease PADI4 activity in a precise and specific manner. We are currently applying these in cell-based systems to better understand its cellular regulation. We also have a particular interest in the fifth member of the PADI family, PADI6. PADI6 is essential for early embryogenesis, with mutations and deletions resulting in female infertility. However, despite this profound phenotype, the precise molecular mechanisms of PADI6 biological activity remain to be elucidated. We are currently combining our own *in vitro* expertise, with *in/ex vivo* approaches used in the Turner and Niakin labs to dissect the molecular mechanisms behind this phenotype. This approach has been enabled by my location at the Crick. In the longer term I anticipate exploring the use of peptide tools to further manipulate PADI6 activity and understand whether it could be therapeutically targeted.

In addition, we also work with others at the Crick to produce tools to address their research questions. We have established collaborations with several other Crick researchers, including Simon Boulton and Jernej Ule, to produce tools that could be used to explore their biology. Over the remainder of my time at the Crick I hope to be able to extend these and other collaborations. I anticipate maintaining these well beyond my return to Imperial.

Research outputs

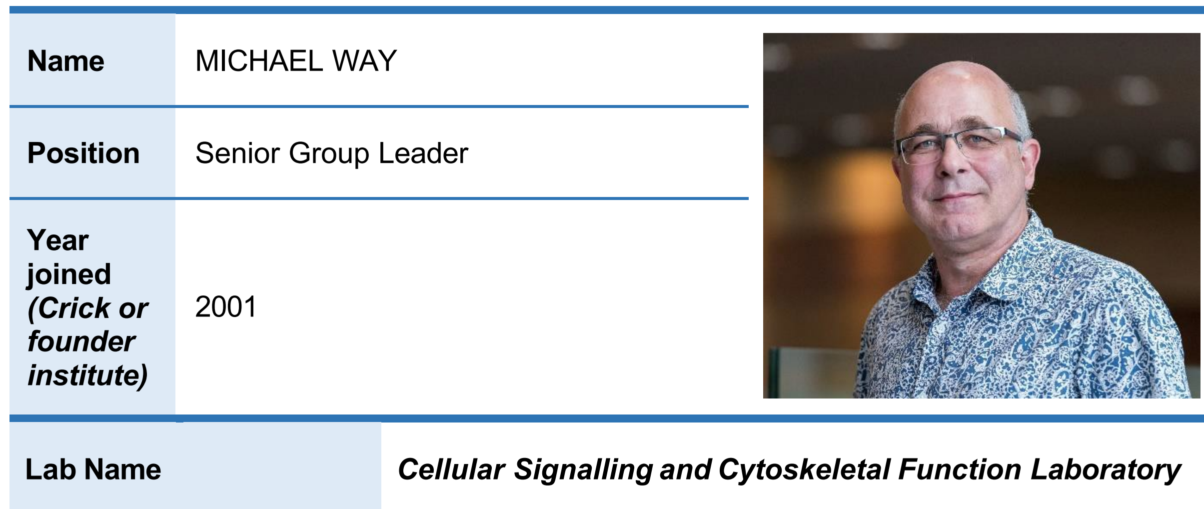
Karishma Patel,† Louise J. Walport,†* James L. Walshe, Paul D. Solomon, Jason K. K. Low, Daniel H. Tran, Kevork S. Mouradian, Ana P. G. Silva, Lorna Wilkinson-White, Alexander Norman, Charlotte Franck, Jacqueline M. Matthews, J. Mitchell Guss, Richard J. Payne, Toby Passioura, Hiroaki Suga,* Joel P. Mackay* (†these authors contributed equally to this publication) (*co-corresponding authors). (2020) *Cyclic peptides can engage a single binding pocket through highly divergent modes.* PNAS 117 (43) 26728 – 26738. DOI: 10.1073/pnas.2003086117
This publication highlights the power of the RaPID system to produce potent and highly selective inhibitors. Despite their widespread application, the inherent structural diversity in encoded cyclic peptide libraries has been little explored. Here, through screening against several highly conserved proteins with a single library, we revealed unprecedented structural and functional diversity in peptide hits. This provides an experimental explanation for the wide applicability of these libraries.

Walport LJ, Hopkinson RJ, Chowdhury R, Schiller R, Ge W, Kawamura A, Schofield CJ. (2016) *Arginine demethylation is catalysed by a subset of JmjC histone lysine demethylases.* Nature Communications 7, 11974. DOI: 10.1038/ncomms11974
In this publication we demonstrate that a family of enzymes that are known to catalyse lysine demethylation of histones can also catalyse the demethylation of arginine residues. This addressed a longstanding question in the field as to whether this PTM was reversible. The publication highlights my use of multiple approaches (here including work with peptides, *in vitro* kinetics, x-ray crystallography and cell-based experiments) to dissect enzymatic function, an approach we continue to take in the lab.

Kawamura A, Münzel M, Kojima T, Yapp C, Bhushan B, Goto Y, Tumber A, Katoh T, King ONF, Passioura T, Walport LJ, Hatch SB, Madden S, Müller S, Brennan PE, Chowdhury R, Hopkinson, RJ, Suga H, Schofield CJ. (2017) *Highly selective inhibition of histone demethylases by de novo macrocyclic peptides.* Nature Communications 8, 14773. DOI: 10.1038/ncomms14773

This publication demonstrates how the RaPID system can be used to develop extremely selective compounds against conserved enzyme families. In particular it highlights how highly specific cyclic peptide tools can be optimised and applied to modulating an epigenetic target in a cellular context. We are applying similar strategies to new peptides identified in my lab.

Hsu, KF, Wilkins SE, Hopkinson RJ, Sekirnik R, Flashman E, Kawamura A, McCullagh JSO, Walport LJ*, Schofield CJ* (*co-corresponding authors). (2021) *Hypoxia and hypoxia mimetics differentially modulate histone post-translational*.
Epigenetics 16(1):14-27. DOI: [10.1080/15592294.2020.1786305](https://doi.org/10.1080/15592294.2020.1786305)
In this paper we profile the effects of hypoxia mimetic drugs on histone post-translational modifications and compare this with hypoxia itself. It highlights our approach to using chemical tools responsibly. Used appropriately synthetic drugs can be powerful tools to modulate biology, but they must always be carefully profiled and used with caution to avoid data misinterpretation.



Name	MICHAEL WAY
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2001
Lab Name	***Cellular Signalling and Cytoskeletal Function Laboratory***

Research programme and achievements

Intracellular pathogens have co-evolved with their hosts to develop multiple mechanisms to hijack the many cellular processes of their hosts to facilitate their entry, replication, survival and cell-to-cell spread. Understanding how pathogens take advantage of their host offers the promise of obtaining fundamental insights into basic cellular processes that are frequently deregulated during pathogenic situations. It also provides important insights into the underlying cause of disease and helps identify potential targets for therapeutic intervention. My lab's research uses a combination of quantitative imaging and biochemical approaches to study Vaccinia virus as a model system to interrogate the regulation and function of Src and Rho GTPase signalling, actin and microtubule-based transport as well as cell migration.

Main lab achievements since 2015

1. We have developed software designed for the automated quantification of cell migration and morphodynamics. Implemented as a plug-in for the open-source platform, ImageJ, ADAPT is capable of rapid, automated analysis of migration and membrane protrusions, together with associated fluorescently labelled proteins, across multiple cells.

2. Using actin-based motility of Vaccinia as a model, we established that the human Arp2/3 complex actually comprises a family of eight complexes with different actin nucleating properties. This finding has far-reaching implications as Arp2/3-driven actin polymerisation is essential for multiple fundamental cellular processes. For example, a collaboration with Edgar Gomes in IMM Lisbon has subsequently demonstrated unique roles for Arp2/3 isoforms in T-tubule organization and nuclei positioning in skeletal muscle, while others have found mutations in one Arp2/3 isoform result in severe inflammation and immunodeficiency.

3. During its egress Vaccinia recruits a signalling network to induce actin polymerisation to enhance its cell-to-cell spread. In our efforts to understand how this complex signalling network is organised and operates we have uncovered the basis for the recruitment of intersectin-1, a RhoGEF that locally activates Cdc42. This activation promotes N- WASP-Arp2/3 driven actin polymerisation and viral spread.

4. In contrast to most DNA viruses, Vaccinia replicates its genome in the cell cytoplasm. For over 30 years, it has been thought that genome replication was mediated by viral proteins and independent of host involvement. We have shown,

in contrast to these long- held beliefs, that Vaccinia recruits components of the eukaryote DNA replication and repair machinery to amplify its genome in the host cytoplasm.

5. The actin cortex regulates the shape and mechanical integrity of cells. It also plays an important role in controlling what gets in and out of a cell. By analysing the basis
of Vaccinia-induced cell blebbing early during infection we uncovered a new RhoGTPase signalling pathway involving RhoD, Pak6 and RhoC that regulates myosin driven cell contraction.

6. We showed that septins, conserved components of the cytoskeleton, suppress the release of Vaccinia from infected cells by acting as “restriction factors” to entrap virions at the plasma membrane. Nck-mediated recruitment of dynamin by the virus as well
as formin-driven actin polymerisation displaces septins to overcome their antiviral effect. This is the first demonstration that septins can inhibit the spread of viral infection.

Ongoing and Future work

1. We continue to examine how different subunit isoforms impact on the properties, interactions and cellular function of Arp2/3 complex family members. We have also generated conditional mice to examine the role of Arp2/3 isoforms in development and tissue homeostasis.

2. We are re-wiring the Vaccinia signalling cascade and building synthetic networks to understand the principals of how signalling networks regulate actin polymerisation.

3. We are analysing microtubule-based motility of Vaccinia including establishing *in vitro* motility assays to understand the mechanistic basis of motor recruitment and regulation.

4. We are performing cryo-electron tomography of infected cells to uncover the ultrastructural organisation of septins and clathrin on Vaccinia.

5. We are analysing how Vaccinia manipulates RhoGTPases and their signalling.

6. We are using Vaccinia to develop novel oncolytic strategies for ovarian cancer.

Research outputs

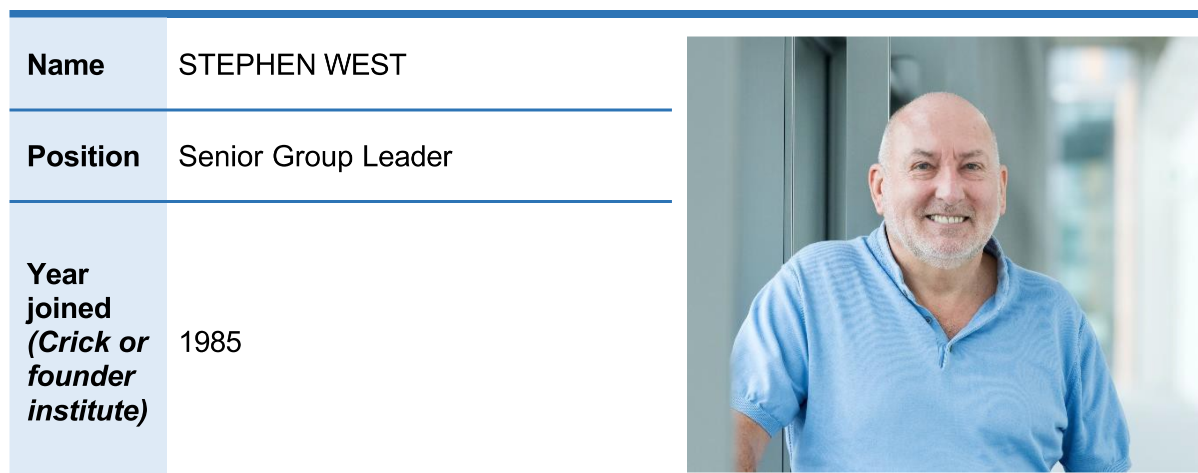
Jasmine V. G. Abella, Chiara Galloni, Julien Pernier, David J Barry, Svend Kjær, Marie-France Carlier and Michael Way. (2016) *Isoform diversity in the Arp2/3 complex determines actin filament dynamics*. Nature Cell Biology 18: 76-86. DOI: 10.1038/ncb3286
The Arp2/3 complex, consisting of seven evolutionarily conserved subunits, generates branched actin networks during many fundamental cellular processes. Taking advantage of actin-based motility of Vaccinia virus as a model system, we demonstrate for the first time that in humans the Arp2/3 complex is actually a family of different complexes with distinct actin-nucleating properties.

Xenia Snetkov, Ina Weisswange, Julia Pfanzelter, Ashley C. Humphries and Michael Way. (2016) ***NPF motifs in the vaccinia virus protein A36 recruit intersectin-1 to promote Cdc42:N-WASP-mediated viral release from infected cells*****. Nature Microbiology 1:16141. DOI: 10.1038/nmicrobiol.2016.141**
Vaccinia virus recruits a signalling network to induce actin polymerisation to enhance its cell-to-cell spread. This study, which described the first viral protein containing NPF motifs, uncovered the molecular basis for the recruitment of intersectin-1, a RhoGEF that activates Cdc42 to increase exit of Vaccinia from infected cells using its actin-based motility.

Charlotte H. Durkin, Flavia Leite, João V. Cordeiro, Yutaka Handa, Yoshiki Arakawa, Ferran Valderrama and Michael Way. (2017) ***RhoD inhibits RhoC-ROCK dependent cell contraction via PAK6*****. Developmental Cell 41:315-329. DOI: 10.1016/j.devcel.2017.04.010**
RhoA-mediated regulation of myosin-II activity in the actin cortex controls the ability of cells to contract and bleb during a variety of cellular processes. Cell contraction and blebbing are also frequently observed as part of the cytopathic effects induced by many different viruses during their replication cycles. By analysing the molecular basis of Vaccinia-induced cell blebbing early during infection we uncovered a new RhoGTPase signalling pathway regulating myosin driven cell contraction.

Julia Pfanzelter, Serge Mostowy and Michael Way. (2018) ***Septins suppress the release of Vaccinia virus from infected cells*****. Journal of Cell Biology 217:2911-2929. DOI: 10.1083/jcb.201708091**
Septins are conserved components of the cytoskeleton that play important roles in many cellular processes including division and migration. They can also suppress bacterial infection by forming cage-like structures around intracellular pathogens such as Shigella. Using a combination of approaches, we demonstrated that septins act as “restriction factors” to entrap virions at the plasma membrane and inhibit the release of Vaccinia virus from infected cells. This study represented the first demonstration that septins can inhibit the spread of viral infection.

Ottilie von Loeffelholz, Andrew Purkiss, Luyan Cao, Svend Kjaer, Naoko Kogata, Guillaume Romet-Lemonne, Michael Way* and Carolyn A. Moores*. (2020) ***Cryo-EM of human Arp2/3 complexes provides structural insights into actin nucleation modulation by ARPC5 isoforms*****. Biology Open 9: bio.054304. DOI: 10.1242/bio.054304**
In 2016 my lab was the first to demonstrate that the Arp2/3 complex in humans and other mammals is actually a family of complexes with different properties (1). However, it is still unclear why these eight complexes are so different and whether they have distinct cellular functions. This study, which was the first cryo-EM analysis of the Arp23 complex when it appeared on BIoRxiv provided structural insights into differences between the Arp2/3 family members.



Name	STEPHEN WEST
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	1985

Lab Name	***DNA Recombination and Repair Laboratory***

Research programme and achievements

Our genetic material is continually subjected to damage, either from endogenous sources such as reactive oxygen species, produced as by-products of oxidative metabolism, from the breakdown of replication forks during cell growth, or by agents in the environment such as ionising radiation or carcinogenic chemicals. To cope with DNA damage, cells employ elaborate and effective repair processes that specifically recognise a wide variety of lesions in DNA. These repair systems are essential for the maintenance of genome integrity. Unfortunately, some individuals are genetically predisposed to crippling diseases or cancers that are the direct result of mutations in genes involved in the DNA damage response.

For several years, our work has been at the forefront of basic biological research in the area of DNA repair, and in particular we have made significant contributions to the understanding of heritable diseases such as breast cancer, Fanconi anaemia, and the neurodegenerative disorder Ataxia with Oculomotor Apraxia (AOA). In particular, our focus has been directed towards: (i) determining the mechanism of action and high-resolution structure of the BRCA2 breast cancer tumour suppressor, and to provide a detailed picture of the interplay between BRCA2, PALB2, and the RAD51 paralogs, in terms of RAD51 filament assembly/disassembly, using biochemical, electron microscopic and cell biological approaches, (ii) to determine how the nucleases that resolve recombination intermediates are regulated in simple (yeast) and complex (human) organisms, to determine the biological role of a unique six-subunit structure-selective tri-nuclease complex (SLX1-SLX4-MUS81-EME1-XPF-ERCC1), and (iii) to understand the actions of Senataxin, which is defective in AOA2, in protecting against genome instability in neuronal cells. These distinct and yet inter-related areas of the research programme will provide an improved understanding of basic mechanisms of DNA repair and thereby underpin future therapeutic developments that will help individuals afflicted with these diseases.

Research outputs

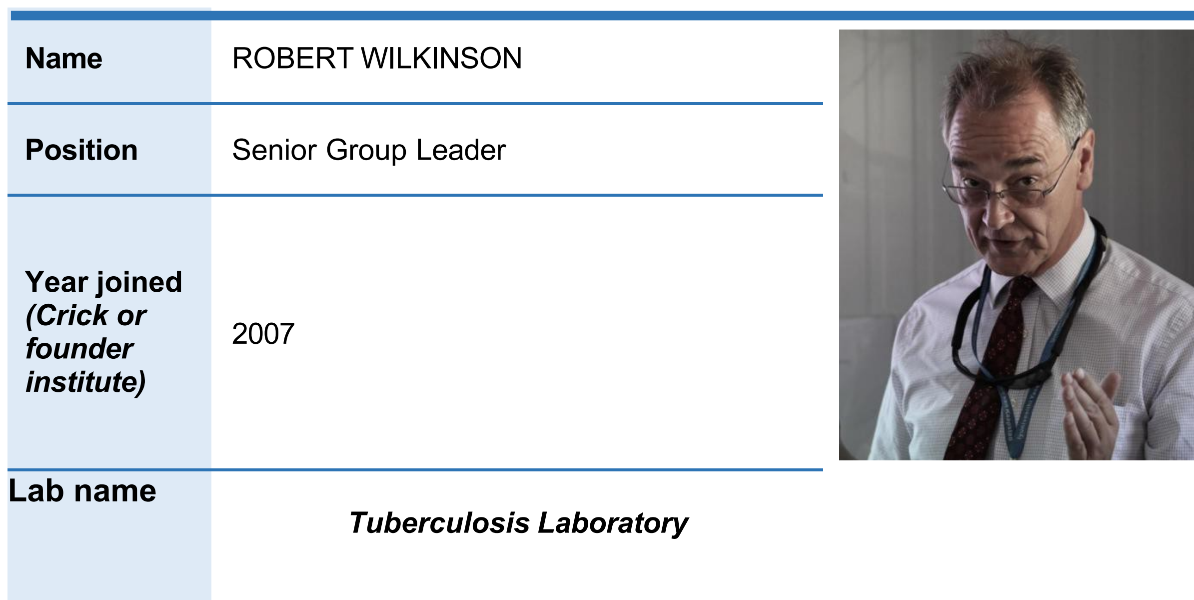
van Wietmarschen N, Sridharan S, Nathan W, Tubbs A, Chan EM, Callen E, Wu W, Belinky F, Tripathi V, Wong N, Foster K, Noorbakhsh J, Garimella K, Cruz-Mignoni A, Sommers JA, Fugger K, Walker RL, Dolzhenko E, Eberle MA, Hayward BE, Usdin K, Freudenreich CH, Brosh RM, West SC, McHugh P, Meltzer PS, Bass AJ and Nussenzweig A (2020) *Werner helicase prevents cell death in cancers with microsatellite instability by resolving large-scale expanded (TA)n repeats*. Nature, in press.

The RecQ DNA helicase WRN is a synthetic lethal target for cancers with microsatellite instability (MSI). WRN depletion induces widespread DNA double strand breaks (DSBs) in MSI cells, leading by an unknown mechanism to cell cycle arrest and/or apoptosis. Here, we show that TA-dinucleotide repeats are highly unstable in MSI cells, exhibiting large-scale expansions. The expanded TA repeats form non-B DNA secondary structures that stall replication forks, activate the ATR checkpoint kinase, and necessitate unwinding by the WRN helicase. In the absence of WRN, the expanded TA-dinucleotide repeats are susceptible to MUS81 nuclease cleavage, resulting in massive chromosome shattering.

Chan YW, Fugger K and West SC (2018). ***Unresolved recombination intermediates lead to a novel class of ultra-fine bridges, chromosome breaks and aberrations*****. Nature Cell Biol 20:92-103. DOI: 10.1038/s41556-017-0011-1**
The generation of CRISPR-Cas9 GEN1 k/o cell lines (supplemented with MUS81 siRNA) allowed us to develop the first model system to analyse the phenotypes of 'resolvase-deficient' human cells. We discovered that recombination intermediates persist until anaphase (despite the presence of the BLM-TopoIII-RMI1-RMI2 dissolvasome) where they form ultra-fine bridges (UFBs). These UFBs represent a new class of ultrafine bridges (we termed them HR-UFBs) distinct from replication stress induced UFBs or centromeric UFBs. HR-UFBs were targeted and processed by PICH/BLM, leading to the formation of ssDNA bridges that were broken at cytokinesis. Loss of GEN1 and MUS81 activity led to synthetic lethality.

Shah Punatar R, Martin MJ, Wyatt HD, Chan YW and West SC (2017) ***Resolution of single and double Holliday junction recombination intermediates by GEN1*****. Proc Natl Acad Sci USA 114:443-450. DOI: 10.1073/pnas.1619790114**
DNA recombination leads to the formation of DNA intermediates that need to be resolved prior to chromosome segregation. These intermediates contain either single- or double Holliday junctions that from a covalent attachment between interaction duplexes. In this work we found that the GEN1 Holliday junction efficiently resolves both single and double junctions. Moreover, we found that GEN1 exhibits a weak sequence preference for incision between two G residues that reside in a T-rich region of DNA.

Wyatt HDM, Laister RC, Martin SR, Arrowsmith CH and West SC. (2017) ***The SMX DNA repair tri-nuclease*****. Molecular Cell 65:848-860. DOI: 10.1016/j.molcel.2017.01.031**
First description of the SMX tri-nuclease that resolves recombination intermediates. Composed of SLX1-SLX4, MUS81-EME1 and XPF-ERCC1, the six-subunit complex was purified following baculovirus expression in insect cells. Characterization of the Holliday junction cleavage reaction revealed that the first incision was introduced by SLX1-SLX4, while the second was mediated by MUS81-EME1. We also found that MUS81-EME1 was activated by interaction with the SLX4 scaffold, ensuring that the second cut occurs in concert with SLX1-SLX4's initial incision. The formation of SMX and activation of MUS81-EME1 provides a mechanistic basis for restriction of SMX activity to the later stages of the cell cycle.



Name	ROBERT WILKINSON
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2007
Lab name	***Tuberculosis Laboratory***

Research programme and achievements

I am a Physician Scientist with significant external appointments at Imperial College London and at the University of Cape Town where I direct that institution's Wellcome Centre for Infectious Diseases Research in Africa. This provides access to substantial numbers of tuberculosis and HIV-tuberculosis co-infected persons in proximity to sophisticated clinical research facilities, giving us a globally-unparalleled ability to phenotype accurately. The role of the allied Crick programme is to understand pathogenesis and thereby improve prevention and treatment, and there is particular emphasis on multi-omic analysis which the Crick is very well-provisioned to provide and interpret.

The programme renewed in 2017 was substantially directed to the role of immunopathological inflammation in tuberculosis, in particular in understanding and managing the HIV-tuberculosis associated immune reconstitution inflammatory syndrome (TB-IRIS), and on the modulatory effects of corticosteroid therapy. Since that time we received results and materials from a placebo-controlled randomised controlled trial in 268 patients at risk of TB-IRIS and found adjunctive steroids reduce the frequency of TB-IRIS by 30% (paper 2). In a reverse translational approach we are using the stored materials arising to dissect the mechanism by which corticosteroids prevented inflammation, and expect this to illuminate pathways that could be targeted by more specific and powerful adjunctive immunotherapies. We were first to definitively show this syndrome is associated with a cytokine release syndrome that arises as a consequence of activation of both canonical and non-canonical inflammasomes (paper 5). The general relevance of this work is increased by the recent finding that corticosteroids also reduce immunopathology and thus death associated with severe COVID-19 infection.

A second major area of activity is in understanding and preventing the progression of early tuberculosis infection as this is key to global elimination. In particular we have pioneered the novel use of high resolution functional and anatomical imaging to benchmark risk of progression (paper 4). Downstream multi-omic analysis has thereby implicated a previously unsuspected role for complement activation in the very earliest stages of progression (paper 5). We are pursuing these findings in much larger clinical studies. In work not directly related to the Crick programme, but nevertheless of potentially very great significance in reducing the 1.6 million annual deaths due to tuberculosis, I also collaborated with industry and thereby contributed as co-senior author to a report of a novel tuberculosis vaccine (GSK biologicals M72AS01E) that reduces the risk of progression of tuberculosis by around 50% (publication 1).

Other very severe immunopathological manifestations of tuberculosis affect the central nervous system (meningitis) and heart (pericarditis). Within the last 18 months we have begun work to advance clinical and pathological studies of these conditions. We will extend multi-omic analysis as above to advance knowledge and potentially improve treatments. In addition we have most recently documented common trilateral association of incident SARS-CoV2 and tuberculosis in HIV-1 infected persons in South Africa. A rapidly established prospective hospital-based study of SARS-CoV2 patients in South Africa has recruited ~140 participants with (unlike many clinical studies of SARS-CoV2 hitherto) adequate and appropriate control groups. We hope to conduct multi-omic analysis of materials collected from this study as well.

Research outputs

Tait, D.R., Hatherill, M., Van Der Meeren, O., Ginsberg, A.M., Van Brakel, E., Salaun, B., Scriba, T.J., Akite, E.J., Ayles, H.M., Bollaerts, A., Demoitié, M-A., Diacon, A., Evans, T.G., Gillard M.D., Hellström, E., Innes, J.C., Lempicki, M., Malahleha, M., Martinson, N., Vela, D.M., Muyoyeta, M., Nduba, V., Pascal, T., Tameris, M., Thienemann, F., Wilkinson, R.J., Roman, F. (2019) *Final Analysis of a Trial of M72/AS01$_E$ Vaccine to Prevent Tuberculosis*. New England Journal of Medicine 381:2429-2439. DOI: 10.1056/NEJMoa1909953

Among adults infected with *M. tuberculosis*, vaccination with M72/AS01$_E$ elicited an immune response and provided protection against progression to pulmonary tuberculosis disease for at least 3 years. This is the first vaccine against tuberculosis to be shown effective in humans since the advent of BCG in 1921. A product development and further testing plan has recently been agreed and we will contribute to the latter.

Meintjes, G., Stek, C., Blumenthal, L., Thienemann, F., Schutz, C., Buyze, J., Ravinetto, R., van Loen, H., Nair, A., Jackson, A., Colebunders, R., Maartens, G., Wilkinson, R.J., Lynen, L. on behalf of the *PredART* trial team. (2018) *Prednisone for prevention of paradoxical tuberculosis-associated IRIS.* New England Journal of Medicine 379:1915-25. DOI: 10.1056/NEJMoa1800762

Prednisone treatment during the first four weeks after the initiation of antiretroviral therapy for HIV-1 infection resulted in a lower incidence of tuberculosis-associated immune reconstitution inflammatory syndrome than placebo, without evidence of an increased risk of severe infections or cancers.

Esmail, H.E., Lai, R. P-J., Lesosky, M., Wilkinson, K.A., Graham, C.M., Horswell, S., Coussens, A.K., Barry III, C.E., O'Garra, A.,Wilkinson, R.J. (2018) *Complement pathway gene activation and rising circulating immune complexes characterize early disease in HIV associated tuberculosis.* Proc Nat Acad Scis USA 115(5):E964-E973 DOI: 10.1073/pnas.1711853115

This *ex vivo* analysis of materials arising from paper 4 (below) showed that transcripts representing the classical complement pathway and Fcγ receptor 1 were differentially expressed before disease presentation. Our results indicate that levels of antibody/antigen complexes increase early in disease, associated with increased gene expression of receptors that bind them.

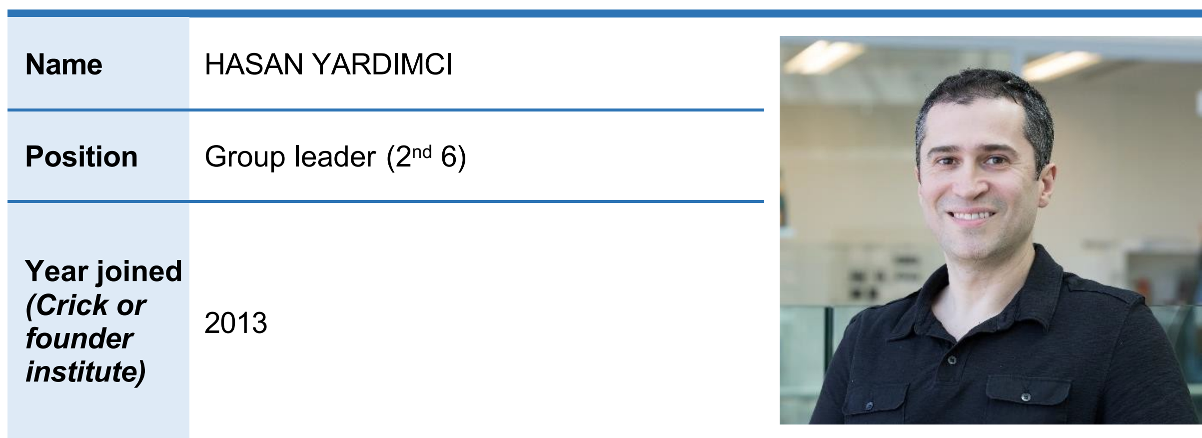
Esmail, H., Lai R.P-J., Lesosky, M., Wilkinson, K.A., Graham, C.M., Coussens, A.K., Oni, T., Warwick, J.M., Said-Hartley, Q., Koegelenberg, C.F., Walzl, G., Flynn, J.L., Young, D.B., Barry 3rd, C.E., O'Garra, A., Wilkinson, R.J. (2016) *[^{18}F]- fluorodeoxyglucose combined positron emission and computed tomographic characterisation of progressive HIV-associated tuberculosis*. Nature Medicine 22(10):1090-1093. DOI: 10.1038/nm.4161

This work used high-resolution PET/CT imaging to establish for the first time in

humans the existence of a high-risk asymptomatic transition state between latent infection and active disease. The technique is thus a phenotypic benchmark for further Experimental Medicine studies of interventions to prevent progression of asymptomatic subclinical tuberculosis.

Lai, R.P-J., Meintjes, G., Wilkinson, K.A., Graham, C.M., Marais, S., Van der Plas, H., Deffur, A., Schutz, C., Bloom, C., Munagala, I., Anguiano, E., Goliath, G., Maartens, G.,

Banchereau, J., Chaussabel, D., O'Garra, A., and Wilkinson, R.J. (2015) ***HIV- Tuberculosis-Associated Immune Reconstitution Inflammatory Syndrome Is Characterized by Toll-Like Receptor And Inflammasome Signalling.*** **Nature Communications 6: 8451. DOI: 10:1038/ncomms9451**
This transcriptomic analysis definitively identified the key immunopathological event in tuberculosis immune reconstitution inflammatory syndrome to be *Mycobacterium tuberculosis* induced activation of the inflammasome attendant on rapid antiretroviral- mediated suppression of HIV-1 replication.



Name	HASAN YARDIMCI
Position	Group leader (2nd 6)
Year joined *(Crick or founder institute)*	2013
Lab Name	***Single Molecule Imaging of Genome Duplication and Maintenance Laboratory***

Research programme and achievements

In eukaryotes, prompt duplication of large genomes requires replication initiation at many sites, called origins. Each origin is 'licensed' for replication through co-ordinated assembly of inactive Mcm2-7 double hexamers onto double-stranded (ds) DNA, forming the pre- replication complex (pre-RC). Mcm2-7 hexamers are activated in S phase after association of Cdc45 and GINS. Cdc45-Mcm2-7-GINS (CMG) complex unwinds duplex DNA at the replication fork and acts as a hub to organise other replication factors around itself, thus assembling the replisome. Despite its importance as the foundation of the eukaryotic replisome, how the CMG helicase interacts with DNA at the replication fork and its dynamics at replication barriers have been controversial. My laboratory showed for the first time that recombinant purified CMG can efficiently bypass protein roadblocks on the lagging-strand template consistent with the steric exclusion model where the active helicase encircles single-stranded (ss) DNA in its central channel. Furthermore, by measuring DNA unwinding by individual CMG complexes with magnetic tweezers, we discovered that CMG unwinds DNA via a random walk, exhibiting both unwinding and backwards motion, with a significant propensity to pause. To elucidate the origin of frequent pausing by isolated CMG, we examined how DNA translocation by the helicase is regulated by its interaction with the replication fork. We discovered that the mechanistic basis for an order of magnitude slower duplex unwinding by CMG compared to its ssDNA translocation rate is engagement of the helicase with the parental duplex. Importantly, we found that association of ssDNA-binding-protein, RPA, with the excluded DNA strand prevents duplex engagement by the helicase and speeds up the helicase at the fork. We further showed that backwards helicase motion is induced by DNA reannealing and can rescue a stalled helicase, serving a significant biological function. This "bottom-up" approach is allowing us to elucidate the dynamics of isolated CMG at the replication fork and to determine how other replisome components can mediate proper DNA engagement by the replicative helicase to achieve efficient fork progression.

The eukaryotic replisome navigates through a protein-rich chromatin environment and must overcome various protein obstacles including nucleosomes. To gain insight into how the replisome complex deals with nucleosomes, we examined DNA replication
in *Xenopus laevis* egg extracts at the single-molecule level. Surprisingly, we found that the majority of parental histones are ejected from DNA upon replication fork arrival, with histone recycling, nucleosome sliding and replication fork stalling also

occurring but at lower frequencies. We showed that expected local histone transfer only became dominant upon removal of free histones from extracts. Our studies provide the first direct evidence that parental histones remain in close proximity to their original loci during recycling and reveal that provision of excess histones results in impaired histone recycling, which has the potential to affect epigenetic memory. We are using the egg extract system as a "top-down" approach to illuminate the dynamics of pre-RCs and cohesin rings upon fork collision.

Research outputs

Burnham DR, Kose HB, Hoyle RB, Yardimci H. (2019) ***The mechanism of DNA unwinding by the eukaryotic replicative helicase.*** **Nature Communications 10, 2159. DOI: 10.1038/s41467-019-09896-2**
In this work, we monitored the movement of single CMG complexes in real time with high spatial and temporal resolution using magnetic tweezers. Our data showed that isolated CMG unwinds DNA one to two orders of magnitude slower compared to *in vivo* fork rates due to frequent helicase stalling at the replication fork. This is the first reported study to interrogate CMG dynamics at this level.

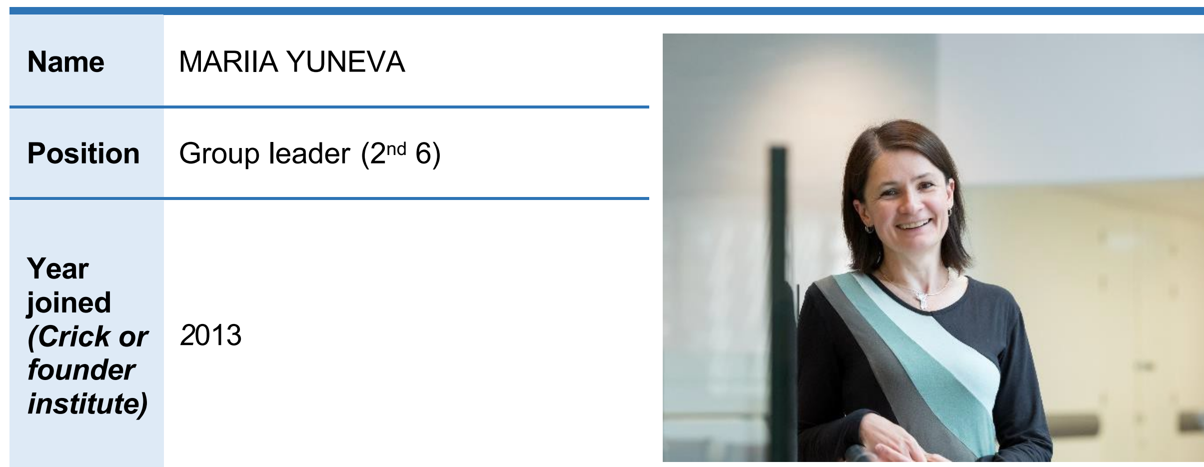
Kose HB, Larsen NB, Duxin JP, Yardimci H. (2019) ***Dynamics of the eukaryotic replicative helicase at lagging-strand protein barriers support the steric exclusion model*****. Cell Reports 26, 2113-2125. DOI: 10.1016/j.celrep.2019.01.086**
Ring-shaped MCM hexamers initially load onto dsDNA at origins. However, whether the active CMG complex encircles dsDNA or ssDNA at the replication fork has been controversial. By analysing the outcome of CMG encountering strand-specific roadblocks, we showed that CMG encircles only one strand in its central channel during unwinding, which has important implications for the architecture of the eukaryotic replisome.

Eickhoff P, Kose HB, Martino F, Petojevic T, Abid Ali F, Locke J, Tamberg N, Nans A, Berger JM, Botchan MR, Yardimci H*, Costa A*. (2019) ***Molecular Basis for ATP-Hydrolysis-Driven DNA Translocation by the CMG Helicase of the Eukaryotic Replisome*****. Cell Reports 28, 2673–2688. *corresponding authors. DOI: 10.1016/j.celrep.2019.07.104**
In this study, we used cryo-EM to image the eukaryotic replicative helicase as it performs ATP-hydrolysis-driven DNA translocation. We describe the molecular basis of fork unwinding and explain why not all sites around the ATPase hexamer are strictly required for translocation. We demonstrate for the first time that vertical movement of ssDNA through the hexameric ATPase pore involves substrate rotation inside the helicase ring with a set of four subunits staircasing around ssDNA.

Gruszka D, Xie S, Kimura H, Yardimci H. (2020) ***Single-molecule imaging reveals control of parental histone recycling by free histones during DNA replication*****. Science Advances 6, 38. DOI: 10.1126/sciadv.abc0330**
Parental histone recycling at the replication fork constitutes the mechanistic basis for epigenetic memory through cell division. The current consensus model suggests that most (if not all) parental histones are locally recycled during DNA replication. With single-molecule imaging, we unravel intricate histone dynamics and discover that, contrary to the prevailing view, not all histones are faithfully transferred onto daughter strands during replication. Importantly, we find that the efficiency of local histone recycling at the replication fork is a function of the free histone concentration.

Kose HB, Xie S, Cameron G, Strycharska MS, Yardimci H. (2020) ***Duplex DNA engagement and RPA oppositely regulate the DNA-unwinding rate of CMG***

***helicase*. Nature Communications 11, 3713. DOI: 10.1038/s41467-020-17443-7**
The origin of relatively slow translocation rates of isolated replicative helicases and the mechanism by which other replication components increase helicase speed remained unclear. Here, we demonstrate that engagement of the eukaryotic CMG helicase with template DNA at the replication fork impairs its helicase activity, which is alleviated by RPA. Our work provides a mechanistic basis for relatively slow DNA unwinding by replicative helicases and explains how replisome components that interact with the excluded DNA strand can increase fork rates.



Name	MARIIA YUNEVA
Position	Group leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Lab Name	***Oncogenes and Tumour Metabolism Laboratory***

Research programme and achievements

The overarching aim of our research is to identify the relationship between genetic profiles, tumour microenvironment and metabolism in *in vivo* settings, and to exploit these relationships to stratify patient groups and develop efficient therapeutic approaches. We focus on investigating the role of one of the major oncogenes in human cancers, MYC, in regulating metabolism of tumour cells and their environment and defining metabolic vulnerabilities of tumours.

The main achievements of the laboratory since 2015 are as follows:
1. Demonstrating differential metabolism between two major subtypes of lung cancer, adenocarcinomas (AdCs) and squamous cell carcinomas (SCCs) both in primary human tumours and tumour slices incubated *ex vivo*. We identified a 24-gene metabolic signature sufficient for the separation of SCCs from AdCs and non-cancerous lung. This suggested potential therapeutic targets specifically in SCCs. We demonstrated an association of metabolic profile of SCCs with Notch signalling, and showed the relationship between Notch1, metabolic signature and cancer type was lost in cancer cell lines, underlining the importance of studying cancer metabolism *in vivo*.

2. Demonstrating that different isoforms of pyruvate kinase can sustain MYC-induced tumorigenesis. Only pyruvate kinase M2 isoforms had been previously considered tumour-specific.
3. Demonstrating the extreme multi-level flexibility of tumour metabolism sustaining tumour progression when specific enzyme/pathways are inhibited: inhibition of enzyme isoforms expressed in a tumour can be compensated by an isoform expressed in the normal tissue from which a tumour originated; inhibition of a metabolic pathway can be compensated by an alternative pathway; inhibiting both glucose and glutamine catabolism, two major pathways feeding the Krebs cycle, is required to significantly decrease the levels of Krebs cycle intermediates and inhibit tumour progression; inhibiting production of metabolites like serine and fatty acids can be compensated by the uptake of these nutrients from the diet/blood stream. The requirement of inhibiting both glutaminase and amidotransferases and combining fatty acid synthase inhibition with low fat diet in order to have a significant effect on tumour progression has direct clinical application as both glutaminase and fatty acid synthase inhibitors are currently in clinical trials.
4. Demonstrating the role of glutamine and serine metabolism in MYC-induced mammary gland tumorigenesis.
5. Demonstrating that increased glutamine catabolism in MYC-induced mammary gland tumours is associated with increased glycosylation of one of the major glutamine

transporters Slc1A5 (ASCT2) and its localisation at the plasma membrane, which promotes its activity. We also demonstrated that ASCT2 glycosylation is glutamine-dependent and that increased expression of ASCT2 regulated by MYC is sufficient to induce its glycosylation and membrane localisation. MYC tumour cells are more sensitive to GPNA, an ASCT2 inhibitor, than ERBB2 tumour derived cells, which do not have glycosylated and membrane-localised ASCT2 and have lower glutamine catabolism than a MYC tumour cell.
6. Demonstrating differential acetate catabolism depending on a tumour-driving lesion. Acetate catabolism through the Krebs cycle is driven by the mitochondrial isoform of Acyl-CoA synthetase 1, ACSS1, specifically in MYC tumours (previous studies demonstrated the importance of nuclear/cytosolic isoform of ACSS2). We also demonstrated the requirement of ACSS1 for tumour development and the role of acetate in supporting tumour cells when glucose catabolism is inhibited.
6. Establishing a pipeline for identifying the activity of metabolic pathways in tumours *in situ*. Demonstrating the heterogenous distribution of metabolic pathways *in situ* defined by genetic backgrounds of tumour cells.
7. Demonstrating an association of high levels of pantothenic acid (vitamin B5) with regions of high Krebs cycle activity and the requirement of pantothenic acid for tumour progression.

In our future work we will focus on:
1. further identifying mechanisms allowing tumour cells to overcome metabolic perturbations. Specifically, we will identify the role of specific amidotransferases and aminotransferases in supporting the proliferation of tumour cells in the absence of glutaminase activity.
2. exploring the role of dietary amino acids in supporting formation and progression of MYC-induced tumours.
3. exploring metabolic interactions between tumour cells and their microenvironment and how these interactions are affected by inhibiting glutamine catabolism.
4. identifying the role of pantothenic acid in supporting tumour progression.
5. establishing the spatial relationship between genetic and metabolic heterogeneity in preclinical tumour models of breast cancer and human tumour samples.
6. evaluating the role of metabolic pathways in supporting lung metastases.

Research outputs

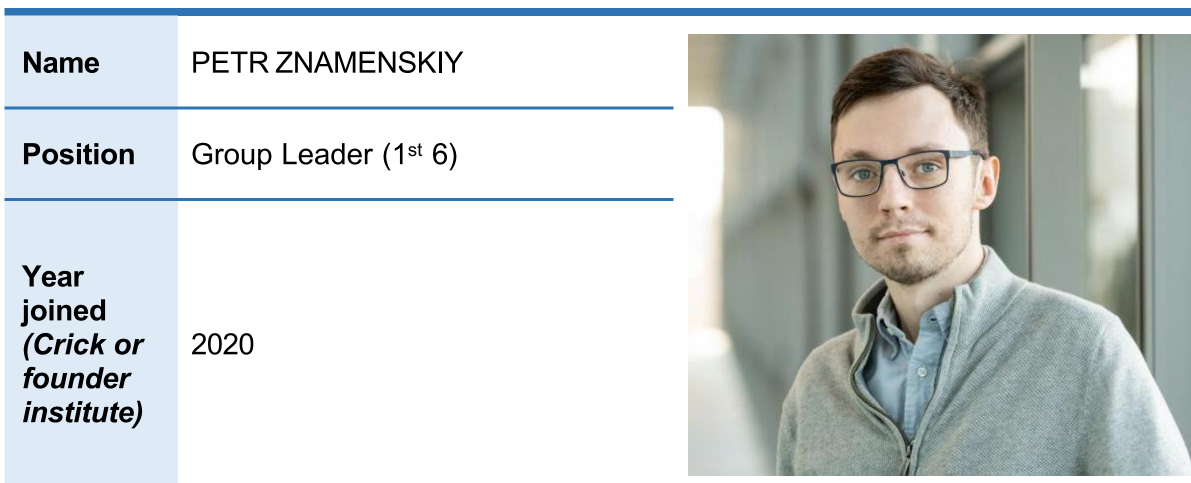
Méndez-Lucas A, Li X, Hu J, Che L, Song X, Jia J, Wang J, Xie C, Driscoll PC, Tschaharganeh DF, Calvisi DF, Yuneva M, Chen X. (2017) *Glucose catabolism in liver tumours induced by c-MYC can be sustained by various PKM1/PKM2 ratios and pyruvate kinase activities*. Cancer Res 77:4355-4364. DOI: 10.1158/0008-5472.can-17-0498
We demonstrated that different isoforms of pyruvate kinase can sustain MYC-induced tumorigenesis. Only pyruvate kinase M2 isoforms had been previously considered tumour-specific.

Tarrado-Castellarnau M, de Atauri P, Tarragó-Celada J, Perarnau J, Yuneva M, Thomson TM, Cascante M. (2017) *De novo MYC addiction as an adaptive response of cancer cells to CDK4/6 inhibition*. Mol Syst Biol 13:940. DOI: 10.15252/msb.20167321
Treatment of colorectal cancer cells with a CDK4/6 inhibitor leads to increased expression of MYC and metabolic reprograming, including increased expression of glutaminase and activation of the mTOR patwhay. As the result, cells resistant to CDK4/6 inhibition become sensitive to inhibitors of MYC, glutaminase and mTOR. This demonstrates how metabolic reprogramming in response to cell cycle inhibitors can lead to new metabolic vulnerabilities.

Sellers K, Allen TD, Bousamra M II, Tan J, Méndez-Lucas A, Lin W, Bah

N, Chernyavskaya Y, MacRae JI, Higashi RM, Lane AN, Fan TW and Yuneva MO. (2019) *Metabolic reprogramming and Notch activity distinguish between non-small cell lung cancer subtypes*. BJC 121, 51-64. DOI: [10.1038/s41416-019-0464-z](https://doi.org/10.1038/s41416-019-0464-z)
Using stable isotope labelling in human lung cancer patients and ex vivo tumour slices we have demonstrated differential metabolism between two major subtypes of lung cancer, adenocarcinomas (AdCs) and squamous cell carcinomas (SCCs). We identified a 24-gene metabolic signature that is sufficient for the separation of SCCs from AdCs and non-cancerous lung. This suggested potential therapeutic targets specifically in SCCs. We identified Notch as a signalling pathway associated with the increased metabolic activities in SCCs.

Méndez-Lucas A, Lin W, Driscoll PC, Legrave N, Novellasdemunt Vilaseca L, Xie C, Charles M, Wilson Z, Jones NP, Rayport S, Rodríguez-Justo M, Li V, MacRae JI, Hay N, Chen X, Yuneva M. (2020) *Identifying strategies to target the metabolic flexibility of tumours.* Nat Metab 2, 335–350. DOI: [10.1038/s42255-020-0195-8](https://doi.org/10.1038/s42255-020-0195-8)
Extreme multi-level flexibility of tumour metabolism is revealed. Demonstrates that 1) inhibition of enzyme isoforms expressed in a tumour can be compensated by an isoform expressed in normal tissue a tumour originated from; 2) inhibition of a metabolic pathway can be compensated by an alternative pathway, 3) inhibiting both glucose and glutamine catabolism, two major pathways feeding the Krebs cycle is required to significantly decrease the levels of Krebs cycle intermediates and inhibit tumour progression; 4) inhibiting production of metabolites like serine and fatty acids can be compensated by the uptake of these nutrients from the diet/blood stream.



Name	PETR ZNAMENSKIY
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2020

Lab Name	***Specification and Function of Neural Circuits Laboratory***

Research programme and achievements

The neocortex plays a key role in perception, cognition, learning and behavioural control. These processes are products of coordinated activity of specialised neuronal cell types, connected into networks according to wiring rules we are only beginning to understand. In my postdoctoral research before joining the Francis Crick Institute, I focused on understanding the patterns of connectivity of different classes of cortical neurons that give rise to computations carried out by cortical circuits, using mouse primary visual cortex (V1) as a model.

We have shown that parvalbumin-positive inhibitory neurons, previously thought to non-specifically provide inhibition to the majority of nearby cells, make the strongest inhibitory connections with pyramidal neurons that have similar visual responses. This fine-tuning of synaptic strength explains how specific inhibition can emerge despite dense synaptic connectivity and has important implications for models of neocortical networks. We have also discovered that V1 neurons projecting to different higher visual areas rarely make connections with each other. Activity-dependent wiring rules appear insufficient to explain their low rate of connectivity, suggesting that hard-wired mechanisms constrain which inputs these cells receive. The functional role of these projection neuron populations and their specific wiring rules remains unknown and will be the subject of my research at the Crick.

At the Crick, I plan to extend this research programme in two complementary directions. First, my lab will aim to understand the molecular mechanisms that implement such wiring rules by guiding different classes of cortical neurons in selecting their input and output connections during development. To accomplish this goal, we are developing methods that will allow us to characterise the effects of disruption of developmental gene expression programmes on cortical wiring at scale. Our approach combines tools relying on molecular barcoding to read out the projection patterns of single neurons with CRISPR gene editing or activation to test the developmental function of dozens of genes in parallel in different cell populations in the same animal.

Second, we will aim to understand the role of the specialised wiring rules of cortical projection neurons in circuit function and behaviour. Simply put, how do the diverse cell types of the visual cortex enable animals to see? To answer this question, we must move beyond simple visual stimuli such as gratings or static images and probe how different V1 neurons are engaged in more complex naturalistic visual scenes. Behavioural studies have shown that rodents have an innate capacity to perceive and discriminate depth that depends on the visual cortex. This suggests that V1 is a part of a hard-wired circuit that enables animals to parse the 3D structure of visual scenes. To understand how different classes of V1 projection neurons contribute to these computations, we plan to measure and manipulate their activity in mice navigating 3D virtual environments.

Finally, we are aiming to develop new approaches for reconstructing the patterns of synaptic connectivity of cortical neurons. Current methods relying on multiple patch clamp recordings or

serial electron microscopy are extremely laborious and are limited to measuring local neuronal connections. We are aiming to use rabies viruses expressing molecular barcodes in order to exploit their ability to transit synaptic connections. This approach will enable us to measure presynaptic connectomes of many single neurons in parallel and can be readily combined with emerging spatial transcriptomics methods to reconstruct local and long-range synaptic connections together with the molecular identity of pre- and post-synaptic neurons.

Research outputs

Xiong, Q.*, Znamenskiy, P.*, Zador, A.M. (2015) *Selective corticostriatal plasticity during acquisition of an auditory discrimination task*. Nature, 521, 348-51. DOI: 10.1038/nature14225
Corticostriatal synaptic plasticity has long been proposed as a key circuit substrate for reinforcement learning. Using a new approach to measure the strength of connections of corticostriatal neurons with their targets in rats, we demonstrate that these connections are potentiated during the acquisition of a perceptual discrimination task. For the first time, we demonstrated that the associations between sensory stimuli and motor responses learned by the animals can be decoded by measuring the the organization of corticostriatal synaptic strength *ex vivo*.

Kim, M.-H.*, Znamenskiy, P.*, Iacaruso, M.F., Mrsic-Flogel, T.D. (2018) *Segregated functional subnetworks of intracortical projection neurons in primary visual cortex*. Neuron, 100 (6), 1313-1321. DOI: 10.1016/j.neuron.2018.10.023
This paper demonstrates a surprising dearth of connections between primary visual cortex neurons projecting to different higher visual areas. Although these neuronal populations have distinct preferences for visual stimuli, simultaneous functional imaging experiments suggest that activity-dependent wiring rules alone are unlikely to explain the lack ofconnections between them. These results support the idea that input connectivity of different subtypes of cortical neurons is constrained by hard-wired molecular mechanisms.

Znamenskiy, P.*, Kim, M.-H.*, Muir, D.R.*, Iacaruso, M.F., Hofer, H.B., Mrsic-Flogel, T.D. (2018) *Functional selectivity and specic connectivity of inhibitory neurons in primary visual cortex*. BioRxiv. DOI:10.1101/294835
By simultaneously characterising visual responses and local connectivity of PV-positive interneurons, one of the major sources of inhibition in the cortex, we demonstrated that these cells preferentially provide inhibition to and receive inhibition from nearby excitatory cells with similar visual responses. This modulation of synaptic strength reconciles the co- tuning excitatory and inhibitory inputs received by individual excitatory cells with the seemingly contradictory observations of dense connectivity of major interneuron subtypes.

Orsolic, I., Rio, M., Mrsic-Flogel, T.D., Znamenskiy, P. (2021) *Mesoscale cortical dynamics reflect the interaction of sensory evidence and temporal expectation during perceptual decision-making*. Neuron (in press) DOI: 10.1016/j.neuron.2021.03.031
This study aimed to understand how visual signals are processed to influence behavioural decision on a cortex-wide scale. To this end, we carried out widefield imaging of the dorsal cortex in mice performing a visual change detection task designed to disambiguate neuronal responses associated with processing of sensory evidence and execution of motor responses. These experiments revealed a localised cascade of activity underlying processing of visual signals in the task and pointed to the secondary motor cortex as a key area modulated both by current sensory evidence and animals' expectations.