A Model of Intracellular Calcium Increase via Light-induced Melanopsin Activation

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Chapter 1

Introduction

The field of synthetic biology is one that has, over the last decade or so, become a more powerful field for increasing our understanding of biological systems and for providing biological systems for a variety of practical applications.

One particular branch of synthetic biology, optogenetics, is concerned with developing biological systems which use light as a stimulus control the behaviour of the system. It allows for spatially restricted excitation or suppression of certain cell function, usually via transcription mediation.

Melanopsin is a photoreceptor that makes certain retinal ganglion cells photosensitive. It shares aspects of its form and function with rhabdomeric rhodopsin, as is found in Drosophila. Melanopsin has already been used in some synthetic biology systems to control, for example, blood-glucose homeostasis.

In order for components in the synthetic biology tool kit to have greater practical application, predictive models that describe how they operate are necessary. Biological systems are inherently noisy due to several factors such as concurrent, interfering reactions occurring. One way to capture the inherent stochasticity in a biological system is to use modelling techniques such as chemical master equations or stochastic differential equations.
Chapter 2

Biological Background

The engineering of biological systems to carry out desirable tasks in a predictable manner is the goal of synthetic biology. The aim of much current research is to develop a tool kit from which one can build up more complex systems for medical (Ruder et al. 2011, Bacchus et al. 2013, Weber & Fussenegger 2011), computational (Auländer et al. 2012, Regot et al. 2010) or other purposes (Khalil & Collins 2010). As such, toggle switches have been developed that respond to several different stimuli (Kramer et al. 2004, Deans et al. 2007). Oscillators have been developed that have tunable frequencies (Tigges et al. 2009).

The medical applications of synthetic biology are only starting to become apparent. Rössger et al. (2013) developed a gene circuit that monitors blood fatty acid level and releases an appetite-suppressing hormone when levels reach a peak level. The system had a built in switch which allowed it to be turned off at any time by application of a skin lotion containing phloretin. An overview of the currently imagined and implemented medical applications of synthetic biology is given by Ruder et al. (2011).

Most of the initial development of synthetic biology systems involved the regulation of gene transcription, as in the example above. The period over which these circuits change state or promote an effect is on the order of several minutes. Some effort has been put into developing components that respond on the second time-scale, such as the translocation of proteins using light (Levskaya et al. 2009).

2.1 Optogenetics

A branch of synthetic biology, optogenetics, focuses on using light as an input for a synthetic system. There are several systems in nature that use light for vision, circadian rhythm synchronisation, growth stimulus etc. (Lucas et al. 2012, Van Volkenburgh & Davies 1983, Bacchus & Fussenegger 2012). Recently scientists have tried to harness these natural photo-receptors and
use them in synthetic circuits [Bernstein & Boyden 2011]. Beyond this, novel photo-receptors have been engineered in order to respond to different wavelengths of light or to utilise different signalling pathways [Gradinaru et al. 2010]. Berndt et al. (2008) modified channelrhodopsins to extend the period for which they occupy the open state. This allows them to respond to light two orders of magnitude lower in intensity. Two new systems developed by [Airan et al. 2009] allow the stimulation of one photoreceptor by short wavelength light, activating a PLC signalling pathway (see section 2.2.3). The other photoreceptor was stimulated by longer wavelength light, activating the adenylyl cyclase pathway eventually leading to opening of cAMP-gated Ca\(^{2+}\) channels.

In addition to the systems engineered from extant vertebrate and invertebrate photo-receptors, several systems utilising microbial and plant photosensitive proteins have been developed. Konermann et al. (2013) developed "light-inducible transcriptional effectors" using light sensitive proteins from Arabidopsis thaliana and a DNA-binding domain from Xanthomonas.

A review of the use of optogenetic tools in neuroscience is undertaken by Bernstein & Boyden (2011). They conclude that spatially specific mechanisms, i.e. the activation of single or a small number of cells rather than populations of cells, are needed. Further, those mechanisms that utilise longer wavelength light allow for "less-invasive, deeper-tissue optical neural control" (Bernstein & Boyden 2011).

Wang et al. (2012) note that coupling photo-receptors to existing signalling pathways can lead to interference with endogenous signalling events.

### 2.2 Melanopsin Signalling Pathway

The ability of certain non-mammalian vertebrates to continue to be able to synchronise their circadian rhythms in the absence of eyes indicated that there exists an extra-ocular photoreceptor [Groos 1982]. Mammals also use light for synchronisation of circadian rhythm, as well as for other non-visual purposes. However, in mammals these non-visual processes are restricted to the eyes [Nelson & Zucker 1981]. It was shown that certain photic processes still occur in the absence of rods and cones and as such there must be another photoreceptor in the eye [Freedman et al. 1999]. Provencio et al. (2000) showed that melanopsin expression in retinal ganglion cells (RGCs) provides a third ocular photoreceptor.

There are three classes of inherently photosensitive retinal ganglion cells (ipRGCs), with differing patterns of activity in response to stimulation by light [Tu et al. 2005]. Along with rods and cones, they account for all photodetection in mammalian eyes [Tu et al. 2005]. Melanopsin stimulation in ipRGCs results in an increase in intracellular calcium [Panda et al. 2005]. The signalling pathway through which this occurs has been the subject of
2.2.1 Melanopsin

Melanopsin is a G-protein-coupled receptor (GPCR) of the opsin family. In M1 ipRGCs the density of melanopsin is on the order of $3 \mu m^{-2}$ (Do et al. 2008), which is $10^4$ fold lower than in rods and cones (Do & Yau 2010). Low melanopsin density means light can easily pass through in order to get to the rods and cones (Do & Yau 2010).

Melanopsin’s roles include activation of the pupillary light reflex (PLR), circadian photo-entrainment, negative masking, sleep regulation and the suppression of pineal melatonin (Do & Yau 2010, Xue et al. 2011). One of the differences between melanopsin signalling and the mechanisms used by rods and cones is the relatively large current produced after its stimulation (Xue et al. 2011). Single photon response of melanopsin is $> 1pA$ at body temperature; larger than mouse rods and $\sim 100$ times that of ground-squirrel cones (Do et al. 2008). Its response time is also far slower than that of rods (20 times that of mouse rods) or cones (> 100 times that of rodent cones) (Do et al. 2008).

Melanopsin is activated by the absorption of a photon of light by a chromophore, 11-cis-retinal, that is bound to the extracellular part of the melanopsin. The photon isomerises the 11-cis-retinal to all-trans-retinal, initiating a conformational change in the opsin (Do & Yau 2010). This conformational change allows Gq heterotrimers to complex with the activated melanopsin (Oldham & Hamm 2008).

Retinal regeneration

In order for the melanopsin to be able to respond to light stimulation the presence of 11-cis-retinal is required (Melyan et al. 2005). This means that there needs to be a mechanism for transforming all-trans-retinal back in to 11-cis-retinal. For rods and cones this is done in retinal pigment epithelial cells and additionally, for cones, in Muller cells (Yau & Hardie 2009). ipRGCs are far removed from the pigment epithelium and, as such, it is believed there must be an alternative method for regenerating 11-cis-retinal.

Limulus, Drosophila and other invertebrate opsins are bistable, meaning that the chromophore does not dissociate from the opsin. Instead light of a different wavelength converts the pigment back to its original state (Do & Yau 2010). Mure et al. (2007) has shown that light with a wavelength of 620nm seems to allow for the regeneration of melanopsin’s sensitivity to its activating wavelength of $\sim 480nm$, indicating bi-stability. Experiments by Panda et al. (2005) showed that co-expression of various arrestins

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1The reduction in locomotion in nocturnal animals when challenged with light.
Figure 2.1: The regeneration of 11-cis-retinal for ciliary photoreceptors involves a complex process undertaken in pigment epithelial cells and Muller cells. The bi-stability of Drosophila rhodopsin is a simpler mechanism in which the all-trans-retinal remains bound to the rhodopsin and is isomerised by a longer wavelength photon than the initial stimuli. \cite{YauHardie2009} with melanopsin results in large and consistent photo-currents in cells incubated with all-trans-retinal. Further, secondary pulses had a reduced response in the absence of arrestin but were potentiated in the presence of arrestin. However, \cite{Qiu2005} failed to attain a termination of persistent post-stimulus response when shining a narrow band stimuli on activated melanopsin. This may indicate a lack of bi-stability, though it could also be the case that they were not using the light of the necessary wavelength.

However, it seems as though there must be light-independent pathway for chromophore regeneration regardless of the possible bi-stability of melanopsin \cite{Walker2008}.

### 2.2.2 G-protein q

Many GPCRs aren’t selective about which G protein family they allow to bind but melanopsin seems to be \cite{Qiu2005}. Experiments involving the competitive inhibition of G-protein q seem to point towards this being the cognate G protein for melanopsin \cite{Qiu2005}. Further evidence comes from the suppression of photo-sensitivity when an antagonist of PLCβ is applied; PLCβ being the effector enzyme for G-protein q.
Gq heterotrimer complex

G proteins form heterotrimers of alpha beta and gamma subunits. The inactive heterotrimer, with GDP bound to the Gα subunit, binds to its corresponding activated GPCR. The activated GPCR catalyses the release of GDP from the Gα subunit which leads to the activation of the G-protein through the binding of GTP. A conformational change occurs in the Gα subunit, on binding with GTP, which allows it dissociation from the heterotrimer and the dissociation of the constituent parts of the heterotrimer from the GPCR [Oldham & Hamm (2008)]. The rate at which this process occurs has not been studied in melanopsin but it has been for some other GPCRs. The rhodopsin is activated within 6 ms and remains in its active state for 6 minutes during which it can activate 200 Gt molecules per second [Heck & Hofmann (2001)]. M1-muscarinic receptors catalyse the release of GDP from the Gq complex at a maximal rate of $\sim 1.8s^{-1}$ [Mukhopadhyay & Ross (1999)].

Gα activation/deactivation

The dissociation of the Gq heterotrimer results in two products, one of which is a Gα subunit with GTP bound to it. The mechanism through which this signalling molecule has an effect is by activating PLCβ [Singer et al. (2001)]. Hydrolysis of the bound GTP in to GDP deactivates the Gα, causing dissociation from the PLCβ and the possibility of reformation of the G protein heterotrimer. Although hydrolysis of Gα bound GTP occurs in the absence of PLCβ, it is 1000 to 2000 fold faster when complexed with it [Mukhopadhyay & Ross (1999)]. The rapidity of the activation-deactivation cycle of the Gα “allows frequent re-interrogation of receptor populations to coordinately control signal amplitude and termination” [Singer et al. (2001)].

The Gβγ complex also plays a role in signalling separate to that of the Gα subunit, though it can also activate some PLCβ isoforms [Oldham & Hamm (2006)].

2.2.3 Phospholipase C

Of the four classes of phospholipase C (PLC) isozymes, only PLCβ directly interacts with GTP-bound Gα subunits [Singer et al. (2001)]. Xue et al. (2011) showed that PLCβ4 is necessary in melanopsin signalling but that in its absence some transient responses to light remain, which may be due to another PLCβ isoform. Gβγ is also a strong activator of PLCβ though not the PLCβ4 isoform that seems to be required in melanopsin signalling [Rebecchi & Pentyala (2000)].

2Though it is unlikely to be PLCβ2 which seems unresponsive to Gq [Rebecchi & Pentyala (2000)].
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PLCβ is intrinsically linked to an increase in intracellular calcium (Putney & Tomita 2012). Activated PLCβ hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2), producing two secondary messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Rebecchi & Pentyala 2000). Both of these products are involved in calcium influx into the cytosol but through different mechanisms.

2.2.4 IP3 and the endoplasmic reticulum

One such mechanism is through the secondary messenger IP3 which binds to IP3 receptors on the membrane of endoplasmic reticulum allowing the flow of stored Ca2+ into the cytosol. There have been suggestions that the depletion of intracellular calcium stores are a factor in the activation of plasma membrane ion channels which allows extracellular Ca2+ influx (Putney & Tomita 2012).

2.2.5 Transient receptor protein channels

Canonical transient receptor protein channels (TRPC) channels are a close "homologue of Drosophila TRP/TRPL channels which mediate rhabdomeric phototransduction" (Xue et al. 2011). The TRPC family has seven members, with TRPC3, 6 and 7 being closely related, sharing amino acid identity and function (Vazquez et al. 2004). TRPC3, 6 and 7 form non-selective cation channels, however TRPC6 is far more Ca2+ selective than the others (Vazquez et al. 2004).

TRPC6 and TRPC7 channels seem to be a likely source of Ca2+ influx in the melanopsin signalling pathway (Xue et al. 2011). Research by Xue et al. (2011) showed that knocking out both channels in mice removed the intrinsic photo-sensitivity of the mice, while having one or the other reduces sensitivity but does not remove the response altogether. Removal of TRPC3 has a minor effect but its presence in the TRPC6TRPC7 knockout didn’t have any effect on the sensitivity reduction. However, Qiu et al. (2005) was able to produce photosensitive human embryonic kidney cells (HEK293) by getting them to express melanopsin. These cells stably express TRPC3 and as such suggests that these could be one of the ion channels used in this pathway.

The mechanism of activation of TRPC3, 6 and 7 channels is still contentious. It is evident that its activation occurs downstream of PLC activation. As such, the main theories focus on three results of PLC activation: increase in IP3 and subsequent depletion of calcium stores, increase of DAG and decrease of PIP2.
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DAG activation

Although it is generally accepted that DAG plays a role in the activation of some TRPC channels, it is contested as to how this actually occurs. Hofmann et al. (1999) showed that applying a DAG analogue (OAG) to excised TRPC3 and TRPC6 channels (and later TRPC7 (Vazquez et al. 2004)), one could activate the channel. This certainly showed that DAG has a role in the activation of the channel but doesn’t illuminate whether DAG binds directly to the channel to activate it or whether it works through a mediator. As the channels were excised from a cell rather than in situ this would seem to limit the candidate mediators. The Hofmann et al. (1999) study is backed up by several other studies in showing that IP$_3$ is not necessary for TRPC channel activity (Vazquez et al. 2004). Trebak et al. (2003) not only reinforced the results of Hofmann et al. (1999) but also showed that protein kinase C (PKC), which is activated by DAG, negatively regulates TRPC3 channels.

IP$_3$, IP$_3$R coupling and calcium store depletion

As mentioned previously, PLC$\beta$-PIP$_2$ interaction leads to the production of IP$_3$ which in turn binds to IP$_3$ receptors on the endoplasmic reticulum membrane, see section 2.2.4. Several studies have been undertaken which show that IP$_3$ is not necessary for the activation of TRPC3 channels (Trebak et al. 2003). However, the cytoplasmic C-terminus of TRP channels includes the calmodulin/IP$_3$ receptor binding (CIRB) region (Vazquez et al. 2004). Kiselyov et al. (1999) experiments indicate that IP$_3$R, in its bound state with IP$_3$, that is not associated with an intracellular store, perhaps through store depletion, is capable of activating TRPC3 channels. Further indication that store depletion may play a role in TRP channel activation is the ability of STIM1, the endoplasmic reticulum calcium sensor, to interact with TRPC channels leading some to propose that it may contribute to their opening (Rohacs 2013).

Activation or inhibition by PIP$_2$

It has also been suggested that PIP$_2$ may play both an activatory (by binding directly to ion channels) and inhibitory (by interacting with a regulatory molecule) role in ion channel activation (Putney & Tomita 2012). PLC’s role in breaking down PIP$_2$ could lead to the removal of its inhibitory complex and in that way promote the activity of TRPC5 channels (Trebak et al. 2009). On the other hand, Lemonnier et al. (2008) claim that PIP$_2$ is necessary for the activation of TRPC7 and that its reduction by PLC, though creating the necessary DAG, can be inhibitory to TRPC activation. Rohacs (2013) sums it up by saying PIP$_2$’s interactions with TRPCs are complex and can act as both activator and inhibitor.
Empirical experimentation has vastly increased our knowledge of how certain systems react under a given stimulus or in a particular environment. However, there are several limitations of empirical experimentation in biology. When considering an intracellular system, the size of the actors in the system do not allow for easy tracking and certainly don’t allow viewing of the intricacies of the interactions. Further, working on real biological systems is costly in terms of time and money.

It is important to remember that the outcome of an experiment on a model is only ever the outcome for that model. Falsification allows for the dismissal of a particular model being an analogue of the system being modelled. However, positive results do not prove anything about the system in question, just that the model reflects the system under the tested circumstances. Although these caveats exist, it is clear that models are useful tools for understanding real systems. It allows for the extensive testing of hypotheses with greater efficiency than most empirical work.

A system of mass action equations was produced to describe the relevant parts of the melanopsin signalling pathway. From this a system of ordinary differential equations was developed. Experimental data from a variety of sources was used to aid with rate estimation.

3.1 Chemical Reactions

Wilkinson (2012) describes chemical reactions as a middle ground between the verbal and diagrammatic approach traditionally favoured by biologists and the systems of differential equations favoured by applied mathematicians. We develop a system of chemical reaction equations to describe the melanopsin signalling pathway between melanopsin signalling and calcium influx via TRPC channels.

The 11-cis-retinal bound to a melanopsin protein (M) absorbs a photon (\(\lambda\)), stimulating its isomerisation to all-trans-retinal. This provokes a con-
formational change in the melanopsin transforming it into its active form ($M^*$).

\[ M + \lambda \xrightarrow{k_1} M^* \]

A $G_q$ heterotrimer ($G_q^*$) binds to an activated melanopsin. The melanopsin catalyses the exchange of GDP for GTP on the $G_\alpha$ subunit. The activated $G_\alpha$ ($G_q^*$) dissociates from the heterotrimer and melanopsin.

\[ G_q + M^* \xrightarrow{k_2} G_q^* + M^* \]

An activated $G_\alpha$ binds to a PLC$\beta$ ($PLC\beta$) molecule creating an active form of PLC$\beta$ ($PLC\beta.G_q^*$) that can stimulate downstream signalling processes.

\[ G_q^* + PLC\beta \xrightarrow{k_3} PLC\beta.G_q^* \]

In the presence of active PLC$\beta$, through mediators such as PIP$_2$ and DAG, TRPC channels ($TRPC$) are activated, creating open TRPC channels ($TRPC^*$).

\[ PLC\beta.G_q^* + TRPC \xrightarrow{k_4} PLC\beta.G_q^* + TRPC^* \]

Open TRPC channels allow the flow of extracellular calcium ($Ca^{2+}_{EC}$) across the cell membrane and into the cell, producing intracellular calcium ($Ca^{2+}_I$).

\[ TRPC^* + Ca^{2+}_{EC} \xrightarrow{k_5} TRPC^* + Ca^{2+}_I \]

The all-trans-retinal in the activated melanopsin is converted back to 11-cis-retinal, enabling the melanopsin to be photo-activated again. As we are only modelling a single photon excitation of a single melanopsin we don’t need to be too concerned about the mechanism through which melanopsin becomes activatable again. We only need to concern ourselves with the rate at which it transforms to a state in which it can no longer activate $G_q$ complexes. It is likely that this either requires an incident photon or that melanopsin changes to an intermediate state in which it is neither photosensitive nor able to activate $G_q$.

\[ M^* \xrightarrow{k_6} M \]

Active $G_\alpha$ hydrolyses the attached GTP into GDP and rebinds to a $G_\beta\gamma$ complex forming a $G_q$ heterotrimer.

\[ G_q^* \xrightarrow{k_7} G_q \]

Active PLC$\beta$ catalyses the hydrolysis of the GTP bound to the complexed $G_\alpha$. The $G_\alpha$ dissociates from the PLC$\beta$ and rebinds to a $G_\beta\gamma$ complex forming a $G_q$ heterotrimer.

\[ PLC\beta.G_q^* \xrightarrow{k_8} PLC\beta + G_q \]
Figure 3.1: A Petri net of the melanopsin model described by the set of mass action equations described in section 3.1.

The open TRPC channel closes.

\[ TRPC^* \xrightarrow{k_0} TRPC \]

3.1.1 Petri nets

As a simple, diagrammatic way of displaying a system of reactions, Petri nets are rather useful. Due to the fact that there is a mathematical formalism for Petri nets and that they can easily displayed in matrix form, they are also a useful tool for calculating, for example, the reachability of a particular state given a starting state.

3.2 Ordinary differential equations

From the mass action model, a system of differential equations was built to describe the change in concentration of the species within the model over time.

\[ \frac{d[\lambda]}{dt} = -k1.[M].[\lambda] \]

\[ \frac{d[M]}{dt} = k6.[M^*] - k1.[M].[\lambda] \]

\[ \frac{d[M^*]}{dt} = k1.[M].[\lambda] - k6.[M^*] \]

\[ \frac{d[G_q]}{dt} = k7.[G^*_q] + k8.[PLC\beta.G_q^*] - k2.[M^*].[G_q] \]
\[
\frac{d[G_q^*]}{dt} = k2.[M^*].[G_q] - k7.[G_q^*] - k3.[PLC\beta].[G_q^*]
\]
\[
\frac{d[PLC\beta]}{dt} = k8.[PLC\beta.G_q^*] - k3.[PLC\beta].[G_q^*]
\]
\[
\frac{d[PLC\beta.G_q^*]}{dt} = k3.[PLC\beta].[G_q^*] - k8.[PLC\beta.G_q^*]
\]
\[
\frac{d[TRPC]}{dt} = k9.[TRPC^*] - k4.[PLC\beta.G_q^*].[TRPC]
\]
\[
\frac{d[TRPC^*]}{dt} = k4.[PLC\beta.G_q^*].[TRPC] - k9.[TRPC^*]
\]
\[
\frac{d[Ca^{2+}_{EC}]}{dt} = -k5.[Ca^{2+}_{EC}].[TRPC^*]
\]
\[
\frac{d[Ca^{2+}_{I}]}{dt} = k5.[Ca^{2+}_{EC}].[TRPC^*]
\]

### 3.3 Parameter Estimation

The rate for melanopsin activation and deactivation had not been measured but we can compare to other GPCRs that have similar form or function. As mentioned previously, rhodopsin is activated within 6\( ms \) and remains in its active state for 6 minutes (Heck & Hofmann 2001). Rhodopsin is similar in both form and function to melanopsin, though it is thought to act faster. However, it has been suggested that this is due to scaffolding proteins that hold the constituent parts of the signalling pathway in a restricted spatial domain (Do et al. 2008) and, as such, the rates for melanopsin activation and deactivation may indeed be similar to rhodopsin’s.

Similarly to the rate of melanopsin activation, no research has been done on the rate at which it catalyses the exchange of GDP for GTP and subsequent dissociation of the activated Go. Again, we can make some assumptions based on other receptors that are similar and have been studied. M1-muscarinic receptors catalyse the release of GDP from the G\(_q\) complex at a maximal rate of \( \sim 1.8s^{-1} \) (Mukhopadhyay & Ross 1999). Rhodopsin activates \( \sim 200 \) G\(_t\) molecules per second (Heck & Hofmann 2001). Finally, A\(_{2A}\)-adenosine and \( \beta_1\)-adrenergic activates G\(_s\) in 500\( ms \) (Hein et al. 2006). Oldham & Hamm (2008) states that the rapidity with which rhodopsin is capable of activating G\(_t\) is still not understood. The limiting factor seems to be the rate at which the receptor can provoke the dissociation of GDP and the dissociation of the activated complex rather than the association
rate of GTP to the Go subunit \cite{Dowal2006}. We therefore assume melanopsin behaves similarly to the other receptors mentioned.

\cite{Dowal2006} suggests that PLCβ can associate with Gq prior to its activation by a GPCR. This allows for speeding up of signalling, but there results show there is a far greater affinity between PLCβ and activated Go rather than GDP bound Go. As such, unless the activated Go is saturated by PLCβ, Gq-PLCβ complexes are unlikely to form. It is difficult to find any data on the rate at which activated Go associates with PLCβ, thus activating it, though \cite{Mukhopadhyay1999} state that the lifetime of activated Go is 25–75ms. If we take the lifetime of activated Go bound to PLCβ to be \( \sim 40ms \) (see below) we can make the assumption that the rate of association is \( > 28s^{-1} \).

The rate at which activated PLCβ opens TRPC channels is harder to garner than the other rates in this model. We are concatenating several processes in to a single process in order to navigate the issues associated with the ambiguity of this part of the signalling pathway. As there is no data on the rate of TRPC channel activation in response to PLCβ activation we use parameter estimation tools in Copasi to calculate a possible rate.

\cite{Mukhopadhyay1999} showed that the hydrolysis of GTP on Go when complexed with PLCβ was \( 9–12s^{-1} \) at 30℃. They did not make measurements at physiological temperatures but estimate the rate to be \( \sim 25s^{-1} \). We make the assumption that PLCβ4, which is the likely PLC isoform in the modelled pathway, behaves similarly to PLCβ1. They also state that this is on the around 2000 times faster than hydrolysis in the absence of PLCβ1.

When developing a stochastic model of single photon response in Drosophila photoreceptors, \cite{Nikolic2010} note that the mean open time of the TRP channels that concern them is \( \sim 1ms \) and \( 1–2ms \) for TRPL channels. We use this as a guideline for estimating the rate for TRPC channels.

\cite{Do2008} experiments showed that the single photon response of an ipRGC causes a current that takes the form of the convolution of two single-exponentials, \( A(e^{-t/\tau_1} - e^{-t/\tau_2}) \). They also calculate that the time constants should take the values \( \tau_1 = 0.4 \pm 0.2s \) and \( \tau_2 = 6.6 \pm 4.4s \). If we take the rate of change of intracellular calcium, \( d[Ca^{2+}_{I}] / dt \), to be proportional to the current produced by calcium entering the cell we have some data to try to fit our output to.

We based initial numbers of particles on those used in model developed by \cite{Nikolic2010} which were in turn based on figures put forward by \cite{Hardie2001}.
3.4 Rate of TRPC channel activation

As the mechanism through which the TRPC channels are activated downstream of PLCβ is contentious, a useful rate to evaluate would be the rate at which activated PLCβ causes TRPC channels to open. We explored this by scanning across a range of values for $k4$.

Doing so makes a flaw in our model immediately apparent. The equation:

$$\text{PLC}\beta.\text{G}_q^* + \text{TRPC} \xrightarrow{k4} \text{PLC}\beta.\text{G}_q^* + \text{TRPC}^*$$

is a flattening of a chain of reactions that includes mediators such as PIP$_2$ and DAG. In our model, the rate of deactivation of activated PLCβ has a direct effect on the number of TRPC channels that will open. As stated previously, this rate is reasonably fast. When we have the TRPC activation rate set to a high value several channels open, allow calcium influx, close quickly and are capable of being reactivated by $\text{PLC}\beta.\text{G}_q^*$ that is still in the system. As the activation rate of TRPC is lessened we lower activation of channels and once the channels deactivate the $\text{PLC}\beta.\text{G}_q^*$ that they need to reactivate is less abundant as it has had more time to hydrolyse the attached GTP. This leads to a lower total calcium influx rather than just a slower onset and decline, as can be seen in figure 3.2. If in the real system activated PLCβ interacts with PIP$_2$ quickly but the products are long lived, our model
can’t reflect the dynamics of the real system.
Chapter 4

Discussion

Synthetic biology requires a set of well understood components from which one can build a system without the need to concern oneself too much with the inner workings of each component. In order for this to happen we need to develop models of viable components from which one can make predictions. Just as with electronics, a designer needs to know that a given input will produce a predictable output.

Melanopsin may be able to provide the basis for a photo-detection component. It has already been used by Ye et al. (2011) in a synthetic system to control the expression of a gene involved in blood-glucose homeostasis in mice. For a system like this to be a viable medical device for humans a greater understanding of the dynamics of the pathways used is necessary.

A lot of research has been carried out on the Drosophila rhodopsin pathway, which it is believed the melanopsin pathway may be similar to. There are however clear differences, at a high level, in terms of speed of function. Many of the probable actors in the melanopsin pathway have been researched, providing a good grounding for our model. However, some parts remain contentious and will require further exploration before a realistic model can be produced.

We developed a model which was shown to be flawed. It showed the problem with trying to simplify parts of a system which are fundamental to the systems operation. A greater understanding of the cascade between PLCβ and the TRPC channels is necessary for a more complete model. Experiments on the kinetics of the melanopsin receptor would allow for better parametrisation of the model.


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