New and Enhanced Precision Spectroscopic Techniques for Förster Resonance Energy Transfer (FRET)

Background

Förster resonance energy transfer (FRET) is the non-radiative transfer of electronic energy from an excited molecule (donor) to a nearby unexcited molecule (acceptor). This process is strongly distance dependent with a transfer rate that only becomes significant once the donor-acceptor separation becomes compatible with their Förster radius, typically a distance in the region of 3 to 5 nanometers. This property has led to FRET being widely applied as a "nanometer ruler" to study molecular interactions in biology, biophysics and nanotechnology.

The award of the 2008 Nobel Prize "for the discovery and development of the green fluorescent protein" was an important milestone in the FRET field. Energy transfer between genetically encodable donors and acceptors permits the study of intermolecular interactions and biomolecular processes in living cells and tissues. These techniques have allowed advances to be made in our understanding of a wide range of diseases. However, recent work in our laboratory led to the significant discovery that the states involved in FRET between a widely-used fluorescent protein (FP) pair (EGFP to mCherry) are a much reduced subset of those accessed by optical excitation, hitherto assumed to be wholly FRET active. This was made possible by combining time- and wavelength-resolved sensitized fluorescence intensity and anisotropy measurements; techniques not employed in routine FRET studies. If not accounted for, these restrictions can lead to order of magnitude errors in the determination of protein-protein interacting fractions. The consequences of this phenomenon for the wide range of existing FRET techniques that are unable to detect these anomalies are profound.

The complexity of fluorescence signals arising from both intrinsic FP heterogeneity (multiple emitting states) and variations in FP environments requires the development of different experimental and analytical approaches. This PhD project will consist of two coherent strands: (A) The investigation of the "true" picture of FP FRET by probing FRET heterogeneity and state restriction in a wide range of FP donor-acceptor (D-A) pairs and systems (e.g. fused D-A pairs and D-A FRET arising from intermolecular association and conformational change) (B) The development of new techniques to probe higher order dipole moment correlation functions in resonance energy transfer (un-measurable by spontaneous emission) based on time-resolved stimulated emission depletion (trSTED) measurements following two-photon donor excitation.

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