

Mapping Distances between Chromophores in Macromolecules using FRET-FLIM Microspectroscopy : Comparison with predicted 3D Structures

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Resonance energy transfer is an elegant tool for studying protein interactions among macromolecules, conformational changes and sensing intracellular microenvironment. Combined with lifetime imaging and molecular modelling, it is now possible to predict the structure of proteins and verify it using FRET-FLIM methods. With the introduction of spectrally separable GFPs it is also now possible to study dynamic interactions inside different subcellular compartments with genetically targeted XFP-fusion proteins. Here with the help of a novel method called Time and Space Correlated Single Photon Counting (TSCSPC), we are able to record the spectrum and lifetime (using DL Detector) and image and lifetime (using Quadrant Anode Detector) from a living cell at minimal invasive conditions without inducing photo damage and photo conversion. It is also possible to study the fluorescence dynamics of the Donor and Acceptor simultaneously using the foresaid detectors.

With the present system, a difference in energy transfer dynamics in constructs which differ in lengths even up to eight amino acids could be detected. These constructs were the size variants of the ratiometric chloride sensor Clomeleon developed by Kuner et al [1]. They consisted of fluorescent proteins CFP and Topaz (a chloride sensitive variant of YFP) separated by different spacers (8aa, 16aa, 24aa respectively). The changes in transfer times were observed by understanding different lifetime components involved and plotting the Decay Associated Spectrum (DAS). The Decay Associated Spectra were plotted for all the constructs by analysing the respective intensity decays by Global analysis. It was also possible to study FRET systems involving Donor and Acceptor which were only few nanometres apart in their fluorescence emission maxima, which in turn gives a hint of the sensitivity of the system.

We were able to experimentally support the structure of the different clomeleon constructs as predicted by molecular modelling. We have studied energy transfer in these constructs in different cell types by quantifying and analysing the sign of the pre-exponential factor of the donor (CFP) and acceptor (YFP) lifetimes. With the mapping of pre-exponential factors along the wavelength axes, we could analyse the contribution of different fluorescent species along the wavelength and thereby finding the component which may undergo energy transfer and hence calculate the distance between chromophores involved. This method of combining molecular modelling with FRET-FLIM studies will enable us to understand better the folding patterns of different proteins in their natural environment and map the distances between interacting domains in complexes involving different macromolecules in living cells.

[1] Thomas Kuner and George J Augustine, "A genetically encoded ratiometric indicator for chloride: Capturing Chloride Transients in cultured Hippocampal Neurons", *Neuron*, **27**, 447-459 (2000).