Investigating protein-protein interaction in living cells using FLIM Microscopy

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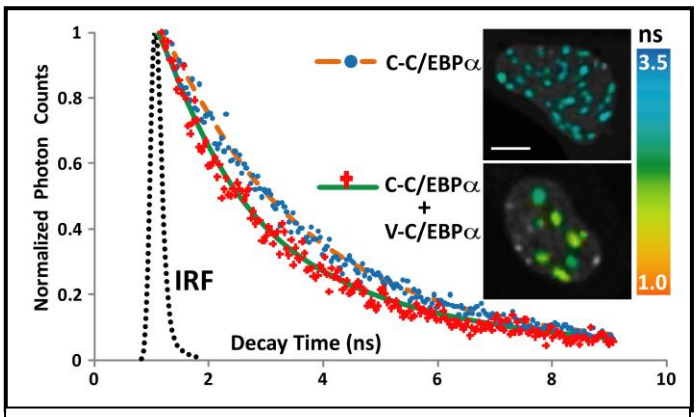
Fluorescence lifetime imaging microscopy (FLIM) is now routinely used for dynamic measurements of many signaling events in living cells, tissues, and live animals including in the measurement of pH, ion concentrations, NADH, FAD, stem cell research and in cancer research[1]. An important application of FLIM is investigation of Forster resonance energy transfer (FRET) between two- or three-protein interactions in living specimens[1-4]. FRET is a photophysical phenomenon which can be applied with any methodology that can probe the fluorescence intensity or lifetime. Intensity based light microcopy FRET system has been widely used to investigate the protein-protein interactions but these investigations provided contaminations in quntitating the FRET signals. There are number methodologies applied to quantify the protein signals collected under the light microscopy systems. These quantifying algorithms do provide reasonable information of protein-protein interactions after applying the mathematical corrections[4]. On the other hand the FLIM technique provides dynamic information of the conformational changes with high temporal and spatial resolution. Protein localization studies using FLIM-FRET techniques can indicate what proteins are expressed, where proteins are expressed, and where they go over time. Tracking these parameters would allow us to gain a greater understanding of these proteins' functions and determine for example, which are likely to be the best drug targets. FRET is a process involving the radiationless transfer of energy from a donor fluorophore to an appropriately positioned (within 1-10 nm) acceptor fluorophore, sufficiently large spectral overlap, and favorable dipole-dipole orientation. Upon energy transfer, donor fluorescence is quenched and acceptor fluorescence is increased (sensitized), resulting in a decrease in donor excitation lifetime.



Dimerized C/EBPα protein in GHFT1 living cell nucleus. TCSPC SPC150 (B&H), Biorad Radiance 2100, Nikon 60X NA1.2 W. Ex 820 nm, Em480/40 nm. Scale 10 micron. Nature Protocol 2011.

In this presentation, we describe the interactions of the transcription factor CAATT/enhancer binding protein alpha (C/EBPα) in living pituitary cells using FLIM-FRET techniques.

[1]Sun, Y., Wallrabe, H., Seo, S.-A. and Periasamy, A. (2011) FRET Microscopy in 2010: The Legacy of Theodor Förster on the 100th Anniversary of his Birth. ChemPhysChem,12:462-474.
[2]Periasamy, A. and Clegg, R.M. 2010 FLIM Microscopy in Biology and Medicine, CRC (Francis & Taylor group) Press.
[3]Chen, Y. J.P. Mauldin, R.N. Day and Periasamy, A. (2007) Characterization of spectral FRET imaging microscopy for monitoring the nuclear protein interactions. J Microscopy, 228:139-152.
[4]Periasamy, A. and Day, R.N. (2005) Moleculalr Imaging: FRET Microscopy and Spectroscopy. Oxford University Press, New York.