Molecular Imaging: FLIM-FRET Microscopy

Ammasi Periasamy and Ye Chen
W.M. Keck Center for Cellular Imaging, Departments of Biology and Biomedical Engineering, Gilmer Hall (064), University of Virginia, Charlottesville, Virginia 22904, USA

Key Words: FLIM, FRET, Two-photon Excitation, C/EBPα protein dimerization.

Molecular interactions in living cells are dynamic, and techniques that rely on chemical fixation or disruption of cell structure can provide only limited information about these interactions. Technological advances in light microscopy imaging, combined with the availability of genetically encoded fluorescent proteins now provide the tools to obtain spatial and temporal distribution of protein associations in living cells [1-4]. The technique of Förster (fluorescence) resonance energy transfer (FRET), for example, can provide information about the interactions between labeled cellular proteins on the spatial scale of angstroms. Energy transfer, however, occurs on a temporal scale of nanoseconds, and steady-state digitized fluorescence microscopy techniques cannot acquire images on this temporal scale. The fluorescence lifetime imaging (FLIM) microscopy technique measures the nanosecond duration of the excited state of fluorophores within the living cell [4]. The fluorescence lifetime of a fluorophore is critically dependent upon the local environment that surrounds the probe. An important advantage of these time-resolved fluorescent lifetime measurements is that they are independent of change in probe concentration, photobleaching, and other factors that limit intensity-based steady-state measurements. When combined with FRET, this approach can provide direct evidence for the physical interactions between proteins with very high temporal resolution, providing the methodology for analyzing dynamic protein interactions in 2- or 3-dimensions. In this study, we used two-photon excitation FLIM-FRET microscopy techniques to visualize the dimerization of the transcription factor CAATT/enhancer binding protein alpha (C/EBPα) in living pituitary cells [2]. Biorad Radiance2100 confocal/multiphoton system was configured to integrate the Becker-Hickl TCSPC (time-correlated single photon counting) lifetime imaging board to monitor the protein associations in living cells and tissues.

References:

[1] Wallrabe, H., Elangovan, M., Burchard, A., Periasamy, A. and Barroso, M. (2003) Confocal FRET microscopy to measure clustering of receptor-ligand complexes in endocytic membranes. Biophysical J. 85:559-571.

[2] Day, R.N., Voss, T.C., Enwright III, J.F., Booker, C.F., Periasamy, A. and Schaufels, F. (2003) Imaging the localized protein interactions between Pit-1 and the CCAAT/enhancer binding protein alpha (C/EBPα) in the living pituitary cell nucleus. Mol. Endo. 17(3), 333-345.

[3] Chen, Y., Elangovan, M. and Periasamy, A., *FRET data analysis:The algorithm*. in Molecular Imaging:FRET Microscopy and Spectroscopy, ed. A. Periasamy and R.N.Day. 2005, New York: Oxford University Press. Chapter 7. In Press.

[4] Wallrabe, H., and Periasamy, A. (2005) FRET-FLIM microscopy and spectroscopy in the biomedical sciences. Current Opinion in Biotechnology. Vol. 16, Issue 1. In Press.