

Protein Localization in Cells and Tissues: 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 FLIM, C/EBP α protein dimerization.

Protein interactions in the living cell 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. The technique of 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 video 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. 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. Importantly, because only one protein partner, the donor, is monitored, it is unnecessary to use spectral bleed-through correction in FRET-FLIM images.

In this study, we used two-photon excitation FRET-FLIM microscopy techniques to visualize the dimerization of the transcription factor CAATT/enhancer binding protein alpha (C/EBP α) in living pituitary cells. Biorad Radiance2100 confocal/multiphoton system was configured to integrate the Becker-Hickl TCSPC lifetime imaging board to monitor the protein localization 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/EBP α) in the living pituitary cell nucleus. *Mol. Endo.* 17(3), 333-345.
3. Sekar, R.B. and Periasamy, A. (2003) Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localization. *J. Cell Biol.*, 160:629-633.
4. Chen, Y. and Periasamy, A. (2004) Characterization of two-photon excitation fluorescence lifetime imaging microscopy for protein localization. *Microscopy Research and Techniques.* 63:72-80.