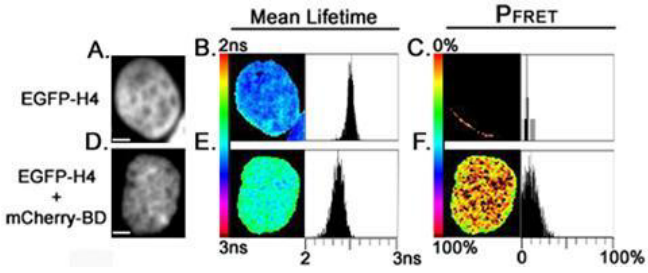
Homo- versus hetero-FRET to probe molecular interactions in living cells: Fluorescence Anisotropy and Lifetime Imaging Microscopy methods.

Marc Tramier, Nicolas Audugé, Sergi Padilla, and Maïté Coppey-Moisan

Institut Jacques Monod, Paris 75251 (France). E-mail : coppey@ijm.jussieu.fr

Such progress has been made in fluorescence microscopy in both the methods and engineering of fluorescent probes that the biology of the cell can now be investigated at macromolecular levels in biological space and time. For example, it is possible to use FRET imaging to monitor protein-protein interactions[1] , biochemical reactions[2] and polymer organization[3] within living cells. The determination and the quantification of FRET are, however, difficult tasks to carry out under the microscope in living cells. Moreover, processes such as photoconversion, the occurrence of a "dark state", photobleaching, or co-presence of other fluorescent species, can produce pitfalls in FRET determination[4] . Through standardized probes and biological examples, we will show how different methods for FRET imaging can bring reliable quantitative FRET determination in living cell.

Fluorescence Lifetime Imaging Microscopy (FLIM) is the most reliable method for hetero-FRET measurement in living cell[5] . The time-correlated single photon counting (TCSPC) method provides the possibility to resolved multiexponential decay functions thanks to their high-time resolution. The single photon counting rate is however the limiting step in image acquisition. By combining multifocal multiphoton excitation and a fast-gated CCD camera we have created a novel confocal FLIM system (TRIM-FLIM), which provides fluorescence decay maps from the time-gated fluorescence intensity images at increasing intervals after excitation. We used this system to show that the nuclear map of the fraction of the acetylated EGFP-Histone H4 can be determined with high spatial resolution from the mean fluorescence lifetime images of EGFP-H4 in presence of mCherry-Bromo domain protein.



Visualization of the fraction of acetylated EGFP-H4 (P_{FRET}) in live cells with the TRIM-FLIM. Two-photon EGFP-H4 images in the absence (A) and in the presence (D) of mCherry BD, and the corresponding mean fluorescence lifetime images (B, E) and the histogram of P_{FRET} (C, F).

Hetero-FRET requires the use of two spectrally different chromophores. In contrast, homo-FRET can occur between like chromophores. This transfer does not change the fluorescence steady-state intensity nor the fluorescence lifetime. This homo-transfer can only be monitored by fluorescence anisotropy. Time-resolved fluorescence anisotropy decay and steady-state fluorescence anisotropy can be performed in microscopy[6] . We will show that two-photon excitation steady-state Fluorescence Anisotropy Imaging Microscopy (TRIM-FAIM) is a powerfull tool for the visualization of homo-dimerization of proteins in living cells and can be used for time-laspe homo-FRET.

References: [1] Y. Yan, G. Marriott, *Curr. Opin. Chem. Biol.* 7 (2003) 635-640. [2] Miyawaki A. *Dev. Cell* 4 (2003) 295. [3] E. Delbarre et al. *Hum. Mol. Genet.* [4] G. Valentin et al. *Nat. Methods* 2 (2005) 801. [5] W. YU, W. Mantulin, E. Gratton, *Emerging Tools for Single Cell Analysis* (2000) New-York: Wiley. [6] M. Tramier et al. *Methods Enzymol.* 360 (2003) 580.