Cell imaging analysis of protein interactions and dynamics in living cells

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Förster (or Fluorescence) Resonance Energy Transfer (FRET) is a physical property of fluorophores that permits the visualisation of inter-molecular interactions, thanks to the development and the use of fusion fluorescent proteins. GFP has been cloned by Chalfie and Tsien in the early 90's (Chalfie, M. et al. Green fluorescent protein as a marker for gene expression. *Science*, 1994 Vol.263(5148), p802, & Heim, R., Prasher, D. C., & Tsien, R. Y. Wavelength mutations and posttranslational autoxidation of green fluorescent protein. *PNAS*, 1994 Vol.91(26), p12501). In less than a decade, fluorescent protein became a major tool in cell imaging and multiple applications of fluorescent proteins have been developed. We propose in the course of this practical to illustrate the interest of FRET to study protein-protein interactions in living cells. Using a combination of photobleaching and imaging analysis, FRET signal can be imaged and further quantified.

The aim of the session is to practice FRET measurements through live-cell imaging and complementary approaches in order to analyse protein interactions in cells.

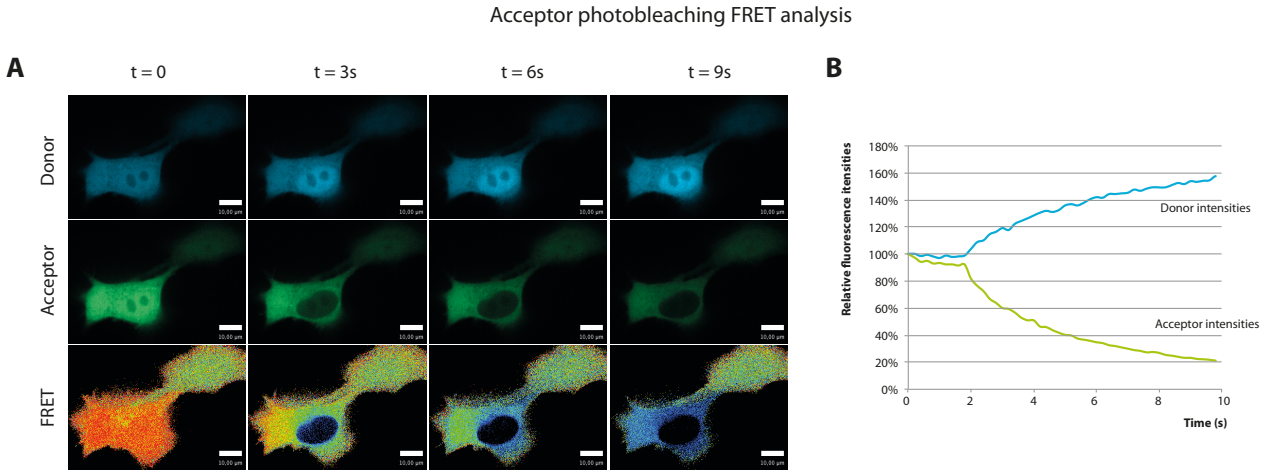

Illustration of a FRET signal (red max., blue min.) visualized on living cells (Panel A, HeLa) expressing mTurquoise (donor) and YFP (acceptor) fluorescent proteins. In this control experiment a maximum FRET signal is detected by its loss while the acceptor fluorophore is photobleached in the nucleus (B, relative intensities curves). Images are acquired through a spinning disk confocal (Andor/Olympus) with a 512x512 16bits/pixel ultrasensitive EMCCD Andor iXon camera (M4D platform, JPK and FL)