PhD Thesis proposal form

Discipline
Biophysical Chemistry

Doctoral School
Ecole Doctorale de Chimie de Paris Sud

Thesis subject title:

**Fluorescence lifetime imaging (FLIM) in the living cell with improved GFP variants**

- Laboratory name and web site: Laboratory of Chemical Physics (LCP, UMR8000)
  http://www.lcp.u-psud.fr

- PhD supervisor (contact person):
  - Name: MEROLA Fabienne
  - Position: Research Director CNRS, leader of Photobiology team and coordinator of Biophysics group at LCP.
  - email: fabienne.merola@u-psud.fr
  - Phone number: +33 1 69 15 30 17

- Thesis proposal (max 1500 words):

  Green fluorescent proteins (GFPs) have given rise to new powerful bioanalytical imaging techniques based on FRET (fluorescence resonant energy transfer), that are increasingly used in pharmacological and clinical research, as well as in environmental sciences and biotechnologies. FRET imaging reports on close molecular proximity (<100Å) between a donor-acceptor pair of fluorophores, and provides a highly versatile optical readout of specific chemical events within complex media such as living cells. Two cyan and yellow GFP variants, CFP and YFP, incorporated into a variety of genetic constructs, are used as the donor-acceptor pair in the vast majority of these FRET studies. Fluorescence lifetime imaging microscopy (FLIM) is a very promising method for quantitative FRET detection, as it precisely measures the CFP fluorescence lifetime, a parameter that is directly related to the FRET efficiency \((J, 2)\). However, quantitative FRET-FLIM studies have remained limited until now by the suboptimal performances of the CFP fluorophore, which is characterized by low brightness and photostability, a complex photophysics including multi-exponential fluorescence decays, and a strong environmental sensitivity \((3)\).

Recently, our laboratory has genetically engineered a new bright, photostable, and pH-insensitive cyan fluorescent protein variant showing a fluorescence quantum yield close to 90% and a near-single exponential fluorescence decay (Pasquier et al, 2011, National patent pending CNRS - Paris Sud). The use of this new fluorophore as a donor in FRET studies will greatly improve the amount
and quality of informations obtained along FRET-FLIM experiments, which opens the way to accurate quantifications of interacting vs non-interacting populations, the separation of specific vs non-specific FRET components, and the analysis of stoechiometry within multi-protein complexes (2, 4).

**Figure 1 (above).** Video microscopy images of new CFP variant in MDCK cells (a) cytosolic CFP and (b) membrane anchored MyrPalmCFP. **Figure 2 (right).** Fluorescence intensity (grey) and TCSPC-FLIM (color) images of CFP-tagged progesterone receptor.

The thesis subject will be to undertake the first applications of this new FRET donor in FRET-FLIM experiments, and to contribute to the development of FLIM methodologies for higher throughput, higher content, live cell bioassays. This will be pursued in the frame of the following pathophysiological studies of clinical interest.

One FLIM-FRET application study, in collaboration with the team of Pr-MD Marc LOMBES (UMR-S INSERM 693, Medical College of Bicêtre, Paris Sud University) is oriented towards the understanding of steroid receptor control of transcriptional activity, through the monitoring of their hormone-dependent interactions with coregulators (5). The functional interactions between steroid receptors with their transcriptional coregulators will be studied by FRET-FLIM using different steroid ligands in breast and uterine cell models.

The second study, in collaboration with the team of Pr Laurent COMBETTES (UMR-S INSERM 757, Paris Sud University) consists in deciphering the molecular mechanism of action of various biliary acids which are impairing communication between hepatocytes. By using a membrane organization probe in FLIM experiments, we will analyse and quantify the hepatocyte cell membrane perturbations caused by biliary acids. Related modifications of the interactions and assembly of GFP-tagged connexins will be explored and quantified by FLIM-FRET analyses.

The candidate's mission will be to ensure proper advancement of these ongoing biomedical collaborations, while improving and optimizing the FLIM methodologies. He will be in charge of cell cultures and transfections, and of microscopy data collection and analyses. The work will be realized in the frame of a larger biophysics program led by the Photobiology team at LCP, aiming at the development and evaluation of new GFPs for bioanalytical imaging, and will benefit from all required supports in molecular biology, biochemistry and fluorescence spectroscopy available in the Biophysics Group.

**Techniques used in this study:** laser-scanning TCSPC FLIM imaging and wide field video-microscopy imaging, cultures of cell lines stably or transiently expressing GFP-tagged proteins of interest, cell transfections (all techniques available at the laboratory, cell lines provided by our collaborators).

**Complementary techniques** in nearby imaging platforms or in the frame of our collaborations: cytometry, FCS, High Throughput Microscopy (Array Scan V11 HCS Reader).
References


- Publications of the laboratory in the field (max 5):


- Specific requirements to apply, if any:

Master students with backgrounds in chemistry, physics or biology are all welcome, and will be trained in the complementary disciplines and techniques required. A good level in english, a strong motivation for interdisciplinary research, and a high sense of rigorous experimental practice are the most important qualities for this program.