

2 PhD positions in biophysics at the Institute of Structural Biology

IBS Grenoble, France

MECHANISTIC INVESTIGATIONS OF PHOTOSWITCHABLE FLUORESCENT PROTEINS BY NMR, X-RAY CRYSTALLOGRAPHY AND SINGLE-MOLECULE IMAGING

The recruited students will investigate the photophysical mechanisms of reversibly photoswitchable fluorescent proteins employing a combination of solution NMR spectroscopy coupled with in-situ laser illumination (PhD 1), and X-ray crystallography /single-molecule fluorescence imaging (PhD 2). The goal will be to contribute fundamental knowledge of these essential fluorescent markers and to engineer improved variants.

Advanced fluorescence imaging is essential to discover the secrets of life, and has largely benefited from the discovery of Fluorescent Proteins (FPs) and other protein-based fluorescent markers. Reversibly Switchable Fluorescent Proteins (RSFPs, [Ref 1](#)) are capable to switch between a fluorescent on-state and a nonfluorescent off-state under specific light illumination, and have fostered many types of imaging applications including some super-resolution methods. Yet, RSFPs are still imperfect: e.g. their brightness is limited, their switching kinetics is perturbed by the involvement of multiple photophysical states, their resistance to irreversible photobleaching is lower than that of typical organic dyes. Moreover, whereas green RSFPs are performing relatively well, red RSFPs do not switch nicely and have been lagging behind. Non FP-based RSFPs also exist, such as FAST which is based on reversible fluorogenic chromophore binding to protein targets ([Ref 2](#)). The switching properties of green and red RSFPs and FAST systems, notably the intertwining with their intrinsic or light-activated protein dynamics can be studied by combining structural biology approaches such as NMR ([Ref 3](#)) and X-ray crystallography with optical spectroscopy and fluorescence imaging ([Ref 4](#)). In the proposed PhD projects, those techniques will be developed and used to better understand a variety of RSFPs and facilitate their rational engineering towards brighter and more photo-resistant variants.

Grenoble is situated in the middle of the beautiful French Alps, and the IBS provides a unique environment for state-of-the-art integrated cellular and structural biology (<http://www.ibs.fr/>).

Candidates should have a strong interest in biophysical approaches with good basic knowledge of biochemistry, molecular biology and/or cell biology. Preliminary experience in NMR spectroscopy, or advanced optical microscopy and protein crystallography is advantageous, but not mandatory.

The project is financed by the French ANR (project: Photoswitch NMR), and the students will be employed by CEA (<https://instn.cea.fr/en/theses-and-phds-at-the-cea/>)

Applications are now open. Please send a CV, a detailed motivation letter, your transcripts, and at least one reference letter to Bernhard Brutscher (bernhard.brutscher@ibs.fr) (PhD 1) or Dominique Bourgeois (dominique.bourgeois@ibs.fr) (PhD 2), **before May 31, 2023.**

