

## Title of the PhD project:

InfraredFP800: Development of infrared fluorescent proteins with emission towards 800 nm

## PhD supervisors:

Antoine Royant [antoine.royant@ibs.fr](mailto:antoine.royant@ibs.fr)

Jérôme Dupuy [jerome.dupuy@ibs.fr](mailto:jerome.dupuy@ibs.fr)

## Host laboratory:

Institut de Biologie Structurale (IBS), GSY group

<https://www.ibs.fr/research/research-groups/synchrotron-group/>

## Project summary:

Fluorescence cell imaging benefited from the Green Fluorescent Protein revolution in the early 1990's, which saw the development of genetically-encoded fluorophores covering the whole visible spectrum. 15 years later, another family of fluorescent proteins (FPs) were developed from the chromophore-binding domain of phytochromes, with emission peaks between 670 and 720 nm. Such infrared FPs (IFPs) can be used for whole-body imaging in the optical window of biological tissues (between 660 and 900 nm), in which light is minimally absorbed by haemoglobin and water. Phytochromes are photoreceptors shuffling between a red-light-absorbing (Pr) and a far-red-light-absorbing state (Pfr), whose absorption maxima differ by 50 to 70 nm. As IFPs were developed by stabilizing the chromophore configuration observed in the Pr state, a lead to generate largely red-shifted IFPs would be to stabilize the chromophore configuration observed in the Pfr state. We will engineer the protein environment in the chromophore-binding region so as to change its affinity towards different configurations, including that observed in the Pfr state. IFPs harbouring these chromophores will exhibit red-shifting of their fluorescence excitation and emission properties, thus extending the possibilities of multicolour imaging in the optical window. To this end, we will use high-pressure crystallography, structure prediction using artificial intelligence and molecular dynamics simulations in order to further our understanding of protein dynamics.

**Preferred skills:** We are looking for a student with a Master in molecular biology, biochemistry or structural biology, who has proven experience in molecular biology and biochemistry and skills in one or more of the following techniques: optical spectroscopy, X-ray crystallography and/or bioinformatics.

**Student role:** The student will perform in silico mutagenesis using the structure prediction software AlphaFold, based on information gained through structural analysis obtained by various techniques, including high-pressure crystallography and molecular dynamics simulations. The most promising mutants will be used as starting material for saturation mutagenesis in bacteria. High-throughput spectroscopic characterization of the resulting colonies will be performed to identify fluorescent proteins of interest, which will be crystallized for structure determination and subject to iterative rounds of optimization. Promising proteins will be used in whole-organism imaging experiments in collaborators' labs.

## Keywords:

Fluorescent proteins, whole-body fluorescence imaging, structure-guided protein evolution

## Relevant publications of the team:

Lambert GG, Depernet H, Gotthard G, Schultz DT, Navizet I, Lambert T, Adams SR, Torreblanca-Zanca A, Chu M, Bindels DS, Levesque V, Nero Moffatt J, Salih A, **Royant A**, Shaner NC (2020) Aequorea's secrets revealed: New fluorescent proteins with unique properties for bioimaging and biosensing. *PLoS Biol.* 18:e3000936

Bindels DS, Haarbosch L, van Weeren L, Postma M, Wiese KE, Mastop M, Aumonier S, Gotthard G, **Royant A**, Hink MA, Gadella (2017) TW Jr. mScarlet: a bright monomeric red fluorescent protein for cellular imaging. *Nat. Methods* 14, 53-56

Feliks M, Lafaye C, Shu X, **Royant A**, Field M (2016) Structural Determinants of Improved Fluorescence in a Family of Bacteriophytochrome-Based Infrared Fluorescent Proteins: Insights from Continuum Electrostatic Calculations and Molecular Dynamics Simulations. *Biochemistry* 55, 4263-4274

Yu D, Gustafson WC, Han C, Lafaye C, Noirclerc-Savoye M, Ge WP, Thayer DA, Huang H, Kornberg TB, **Royant A**, Jan LY, Jan YN, Weiss WA, Shu X (2014) An improved monomeric infrared fluorescent protein for neuronal and tumour brain imaging. *Nat. Commun.* 5, 3626

Shu X, **Royant A**, Lin MZ, Aguilera TA, Lev-Ram V, Steinbach PA, Tsien, RY (2009) Mammalian expression of Infrared fluorescent proteins engineered from a bacterial phytochrome. *Science* 324, 804-807