UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2020-2021

Laboratory/Institute: IBS
Director: Winfried WEISSENHORN
Team: GSY/icOS
Head of the team: Antoine ROYANT (HDR)

Name and status of the scientist in charge of the project: Jérome DUPUY **HDR:** no ■
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Program of the Master's degree in Biology:

■ Immunology, Microbiology, Infectious Diseases ■ Integrative Structural Biology
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology
☐ Planta International

Title of the project: Optimization of the red fluorescent protein mScarlet through mutational and structural analysis

Objectives (up to 3 lines): Fluorescent proteins (FPs) have been developed for all colours of the UV-visible-Near Infrared spectrum, but only optimized for fluorescence efficiency in the cyan, green and yellow parts. FPs in the red, far-red and near-infrared parts still need to be significantly optimized.

Abstract (up to 10 lines): The first fluorescent protein (FP), GFP (Green Fluorescent Protein), was identified in the jellyfish *Aequorea victoria* as early as 1962, but mutational efforts to tune its emission wavelength to other colour had to wait until the cloning of its gene and the discovery of red-shifted homologues in the 1990's. Since then, a large numbers of proteins of various colours have been developed, resulting in the fluorescence microscopy revolution, which it possible to observe many previously invisible biological processes and subcellular structures. The development of the bright red FP mScarlet was based on an innovative approach: the generation of a consensus sequence among all known red FPs and its rational evolution. mScarlet is a bright monomeric FP usable as an acceptor for a green or yellow FP in FRET experiments, destined to probe protein-protein interactions *in cellulo*. However, mScarlet is still amenable to improvements regarding its maturation speed and photostability properties.

Methods (up to 3 lines): Following up on our previous highly successful approach used to develop mScarlet, we will collaborate with the lab of Prof. Gadella in Amsterdam to crystallize and solve the structure of several mutants of mScarlet to understand the influence of key interactions at the vicinity of the fluorescent chromophore.

Up to 3 relevant publications of the team:

Goedhart J, von Stetten D, Noirclerc-Savoye M, Lelimousin M, Joosen L, Hink MA, van Weeren L, Gadella TW Jr, **Royant A** (2012) Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%. *Nat. Commun.* **3**:751.

Clavel D, Gotthard G, von Stetten D, De Sanctis D, Pasquier H, Lambert GG, Shaner NC, **Royant A** (2016) Structural analysis of the bright monomeric yellow-green fluorescent protein mNeonGreen obtained by directed evolution. *Acta Crystallogr D Struct Biol.* **72**:1298-1307.

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

Requested domains of expertise (up to 5 keywords): Molecular biology; Biochemistry; UV-vis absorption and fluorescence spectroscopy; X-ray synchrotron crystallography; Structural analysis