

**Master 2 internship project
Year 2021-2022**

Laboratory/Institute: IBS
Team: icOS (équipe Royant)

Director: W. Weissenhorn
Head of the team: Antoine Royant

Name and status of the scientist in charge of the project: HDR: yes no

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Program of the Master's degree in Biology:

- Immunology, Microbiology, Infectious Diseases Structural Biology of Pathogens
 Physiology, Epigenetics, Differentiation, Cancer Neurosciences and Neurobiology
 Planta International

Title of the project:

Study and optimization of the fluorescent protein mScarlet through the analysis of several mutants.

Objectives (up to 3 lines):

FRET is an extremely powerful tool for studying intra- and intermolecular interactions and is becoming an essential tool in cell biology. Although cyan and yellow fluorescent proteins work very well, their excitation wavelengths remain very harmful to living biological samples, hence the interest in developing new tools in the red.

Abstract (up to 10 lines):

The first fluorescent protein, the "green fluorescent protein" or GFP, was observed in the jellyfish *Aequorea victoria* in 1962, and since then many new colours of fluorescent proteins have been identified and developed, making it possible to observe many previously invisible biological processes and/or structures. Fluorescent proteins, in their many colours and properties, have revolutionized modern cell biology as we know it and continue to be an essential tool for fundamental research.

The mScarlet protein is a monomeric version of a red fluorescent protein (RFP) that has been optimized to have a longer fluorescence lifetime, higher quantum efficiency and optimal brightness. This protein offers significantly improved physical properties compared to previous generations of monomeric RFPs, making its use in techniques such as FRET possible. mScarlet also has low cytotoxicity and no maturation problems at normal physiological temperature for a mammalian cell. These bio-physical properties make mScarlet one of the best candidates to date for making FRET in the red.

Methods (up to 3 lines):

Up to 3 relevant publications of the team:

- Goedhart J, von Stetten D, Noirclerc-Savoye M, Lelimosin M, Joosen L, Hink MA, van Weeren L, Gadella TW Jr, **Royant A**. Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%. *Nat Commun*. 2012 Mar 20;3:751.

- Clavel D, Gotthard G, von Stetten D, De Sanctis D, Pasquier H, Lambert GG, Shaner NC, **Royant A**. Structural analysis of the bright monomeric yellow-green fluorescent protein mNeonGreen obtained by directed evolution. *Acta Crystallogr D Struct Biol*. 2016 Dec 1;72(Pt 12):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. mScarlet: a bright monomeric red fluorescent protein for cellular

imaging. Nat Methods. 2017 Jan;14(1):53-56.

Requested domains of expertise (up to 5 keywords):

- biochemistry
- structure-function relationship analysis
- crystallography interest
- spectroscopy interest
- synchrotron radiation interest