Elucidation of the singlet-oxygen quenching mechanism of the Orange Carotenoid Protein by use of time-resolved serial crystallography.

Host laboratory: Institut de Biologie Structurale, DYNAMOP group
Project "SINGLET", UGA-IRGA, 2024-2027

An international PhD fellowship is available in the DYNAMOP group of Grenoble's Institute of Structural Biology (IBS) to structurally study the singlet-oxygen quenching mechanism of the orange carotenoid protein (OCP) by means of pump-probe time-resolved crystallography. The PhD research will be conducted under the direction of Jacques-Philippe Colletier.

Scientific background: Carotenoids are essential components of the photosynthetic machinery, supporting a variety of key functions such as light-absorption, electron transfer, as well as energy and singlet-oxygen quenching (Frank & Cogdell, 1996). Case in point, the cyanobacterial two-domain **orange carotenoid protein (OCP) is involved in the protection of the photosynthetic apparatus by its ability to quench both singlet oxygen** and the excess of energy absorbed by cyanobacterial light harvesting antennae (*i.e.*, phycobilisomes) (Kirilovsky & Kerfeld, 2016). By combining these two photoprotective mechanisms into a single protein, OCP has the potential to become a central brick in the design of bio-mimetic photosynthetic systems (Stahl & Sies, 2003; Kruk & Szymanska, 2021), where it would function as a dual molecular fuse. The pre-requisite, however, is that the protein must be evolved further so that it can carry out these two functions with higher efficiency than the natural scaffold. A better understanding of the underlying mechanisms is of utmost importance with view to ultimately design OCP variants endowed with higher singlet-oxygen quenching potency.
The proposed PhD project focuses on the singlet oxygen quenching function, and aims at deciphering, controlling and potentially evolving the mechanism(s). Specifically, **we will conduct pump-probe time-resolved serial-crystallography** at the unique ID29 beamline of the ESRF (TR-SSX) and at X-ray Free Electron Laser facilities (TR-SFX) with the objective to capture the structure of the OCP intermediates that form along the process. **We have established the feasibility** of the proposed experiments by collecting diffraction data and optical spectra from OCP crystals soaked in methylene blue (photosensitizer) and maintained under constant illumination at 630 nm – both at cryogenic temperature and room-temperature. The TR-SSX and TR-SFX experiments to be carried out in the framework of the PhD will allow to directly visualize the discrete steps enabling singlet-oxygen quenching by OCP. As a result, we will resolve the *modus operandi*, opening avenues towards the **design of more potent singlet oxygen quenchers**. Indeed, residues crucially important for the singlet oxygen quenching activity need to be identified before attempts can be undertaken to evolve the protein by rational or artificial-intelligence based design. Additionally, our work will allow to better understand (and possibly control) chemical quenching of singlet oxygen by carotenoids. Our current preliminary data indeed support the notion that crystalline OCP could be used as a 'nano-reactor' to produce carotenoid peroxides and determine their high-resolution structures, in a single experiment.

Methods: The PhD student will use state-of-the-art methods to film OCP in the process of quenching singlet oxygen, i.e. time-resolved serial X-ray crystallography at X-ray free electron lasers (XFELs; fs-ms time scale) and synchrotrons (ms-s time scale) (Barends et al., 2022). The DYNAMOP group has extensive experience in these methods. The fast structural changes occurring on the ns-µs timescale will be studied by time-resolved serial-femtosecond-crystallography (TR-SFX) at XFELs, while the late conformational changes occurring on the ms-s timescale will be investigated by time-resolved serial-synchrotron-crystallography (TR-SSX). Specifically, the research will be conducted on the OCP from Planktothrix PCC7805 (Andreeva et al., 2022; Wilson et al., 2022).

Recent key publications of the IBS DYNAMOP group :

Barends TRM et al., 2024 **Nature**, 626, 905-911
Sorigue et al., 2021, **Science,** 372: eabd5687
Nass-Kovacs et al., 2019, **Nature Commun,** 10:3177
Tayeb-Fligelman et al., 2017, **Science,** 355:831-833.
Tetreau et al., 2022, **Nature Commun,** 13:4376
Tetreau et al., 2020, **Nature Commun,** 11:1153
Coquelle et al., 2018, **Nature Chem,** 10, 31-37.
Colletier et al., 2016, **Nature,** 539:43-47.

Selection procedure: Applicants should **apply by sending a CV, an application letter and the name of two references to "colletier@ibs.fr". The selection will be done by June 2024.**