Funded PhD project in Integrated Structural & Cell Biology in Grenoble, France

Title of the PhD project:
CryoACTINOX - Structural characterization of activated NADPH oxidase complex by CryoEM

PhD supervisor:
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Host laboratory: Institut de Biologie Structurale
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Project summary:
The NOX family of NADPH oxidases is an important family of membrane enzyme complexes, which are involved, through the generation of reactive oxygen species (ROS), in innate immunity but also in functions such as vascular tone regulation, hormone synthesis, etc. The 7 human isoforms have a homologous transmembrane component, NOX, containing 2 hemes and a FAD (NOX1 to 5 and 2 DUOX). Beyond that, they differ in their activation mechanism and the subunits associated with the NOX component. NOX1 to 4 require co-expression with a common membrane subunit, p22phox, to form a functional heterodimer. These 4 isoforms then differ in the soluble subunits required for their activation and the nature of the ROS produced. With the development of efficient expression systems for membrane proteins, we are reaching a stage where the structural study of these enzyme complexes become possible and highly competitive. A new expression system validated for NOX2/p22phox heterodimer co-expression in our lab, will be adapted for other membranous heterodimers (p22phox with NOX isoforms). These different NOX/p22phox heterodimer isoforms will be inserted into nanodiscs and assembled with corresponding soluble factors. After stability and activity test, the recombinant activated complex of the isoform identified as the best candidate for structural studies will be used for structure resolution by CryoEM. This project will allow us to characterize at the molecular level the activation process of NADPH oxidases (with potential isoforms specificities) as well as, possibly the mechanisms leading to the production of different ROS. These structural data will be essential to promote drug design strategy on this family of membrane enzyme reluctant up to now to the identification of specific inhibitors.

Preferred skills: Biochemistry (eukaryotic and bacterial cell culture, recombinant protein purification, activity test), an affinity for biophysical studies (sample analysis) and structural biology (electron microscopy).

Student role: The student will be involved in the sample production (cell culture & purification), sample characterization/validation (activity test and complex formation) and structural characterization using Electron Microscopy (CryoEM). *The goal in the end is to train a PhD student to become a Cryo-EM Structural biologist autonomous from the sample production to the structure resolution, but also with some knowledge on the functional analysis of his system (managing enzymatic assays, …).*

Keywords: integral membrane protein, Cryo-Electron Microscopy, assembly and activation of macro-molecular complex, innate immunity, reactive oxygen species, redox biochemistry, microbicidal activity

Relevant publications of the teams:

Structural basis of branch site recognition by the human spliceosome. Tholen J, Razew M, **Weis F**, Galej WP. *Science*. 2022 Jan 7;375(6576):50-57.

NADPH Oxidases (NOX): An Overview from Discovery, Molecular Mechanisms to Physiology and Pathology. Vermot A, Petit-Härtlein I, Smith SME, **Fieschi F**. *Antioxidants* 2021 Jun 1;10(6):890.

Interdomain Flexibility within NADPH Oxidase Suggested by SANS Using LMNG Stealth Carrier. Vermot A, Petit-Härtlein I, Breyton C, Le Roy A, Thépaut M, Vivès C, Moulin M, Härtlein M, Grudinin S, Smith SME, Ebel C, Martel A, **Fieschi F**. *Biophys J.* 2020 Aug 4;119(3):605-618.

Elucidation of the viral disassembly switch of tobacco mosaic virus. **Weis F**, Beckers M, von der Hocht I, Sachse C. *EMBO Rep.* 2019 Nov 5;20(11):e48451. doi: 10.15252/embr.201948451.

The NOX Family of Proteins Is Also Present in Bacteria. Hajjar C, Cherrier MV, Dias Mirandela G, Petit-Hartlein I, Stasia MJ, Fontecilla-Camps JC, **Fieschi F**, Dupuy J. *mBio.* 2017 Nov 7;8(6):e01487-17.