

**Offre de stage M1/M1 Internship offer
in the Membrane and Pathogens Group
(F. Fieschi's team)**

Institut de Biologie Structurale

Biochemical and structural characterization of a bacterial NADPH oxidase

The ROS producing protein NADPH oxydase (NOX) firstly identified in the membrane of phagocytic cells, is involved in crucial physiological processes such as immune defense, cellular signaling, cardiovascular tone regulation and many other mechanisms. Thus, a NOX deficiency can lead to a wide range of severe pathologies (1) giving this membrane protein a strong therapeutic interest. Given the lack of structural data on eukaryotic NOX and the difficulties to produce such a eukaryotic protein, the recent success of the team in finding the first prokaryotic Nox (pNox) homolog represents a huge advance toward understanding NOX enzymology. Extensive characterizations have been performed on purified pNox to demonstrate a strong structural and functional homology with known eukaryotic NOX enzymes. A crystallization assay has been started and crystals have been obtained but the diffraction resolution is too low at this stage for structure determination.

This project aims to use a newly developed NanoDSF (differential scanning fluorimetry) to assess the thermostability of full-length pNox and its transmembrane domain only to enhance the quality of the crystals. This technic allows to screen the stabilizing conditions including pH, salt concentrations, detergent and small molecules while overcoming the membrane protein related issues faced during a classic thermal shift assay. This method will also be used to characterize the stability of pNox variants to select mutants that could stabilize the membrane protein during crystallogenesis. In addition, the student will have the opportunity, depending on the progress made, to take part in the studies reaction mechanisms involving pNox-cofactors interactions, and/or crystallization assay and X-ray diffraction experiments.

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Keywords : Biochemistry, Molecular Biology – Protein Expression, purification – Thermostabiliy assay – Cofactors interaction characterization – Crystallogenesis- FAD - NADPH- Heme – Enzymatic studies

1. Nathan, C., and Cunningham-Bussel, A. (2013) Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat. Rev. Immunol.* 13, 349–361