

M1-Molecular and Cellular Biology (MCB)
Internship Proposal Form
Chemistry-Biology Department

(Deadline Friday 18th December 2020)

Laboratory Address and Affiliation:

Institut de Biologie Structurale
71 avenue des Martyrs
38000 Grenoble

Laboratory/Team Research area (Keyword)

Groupe Membrane & Pathogens (Team F.Fieschi)

Keywords: Immunity, NADPH oxidase, bacterial homologs, crystallisation, site directed mutagenesis, enzyme activity, reactive oxygen species, FAD, NADPH, Heme, membrane protein, detergent.

Summary of the Proposed Internship Project (10 lines)

Title:

Structural characterization and mechanistic studies of a bacterial NADPH oxidase.

DESCRIPTION:

The Membrane and Pathogens team has been working for many years on neutrophilic NADPH oxidase (NOX) which is a key enzyme system of the innate immune response. NADPH oxidase catalyzes the electrons transfer from cytosolic NADPH to oxygen within the phagosomal compartment to lead to the generation of HOCl, a highly toxic reactive oxygen species responsible for the microbicidal activity of neutrophil towards invading pathogens. Due to its membrane protein nature, this eukaryotic membrane protein were not easy to overexpress to performed structural and mechanistic studies. Over the last years, we identified bacterial homologs of the eukaryotic enzyme allowing us to produce such membrane protein in *E.coli* expression system. We have initiated structural studies of this protein and in the timeframe of this internship, efforts will be pursued toward the crystallogenesis of different constructs of the protein alone or in complex with specific blocking antibody (nanobodies). Moreover, in order to understand better, the mechanism of the electron transfer within the enzyme, we have generated several site directed mutation in the enzyme. The student will have to evaluate the impact of these mutations on the activity (their role in cofactors binding, structure integrity and/or electron transfer, ..)

During the internship, the student will have to produce and purify the various mutants of this NOX membrane protein. He(she) will characterize their activity, cofactor binding capacity, evolution of their spectral properties and complex formation with nanobodies. Interaction between NOX and nanobodies might be studied by Surface plasmon resonance. The best NOX/nanobodies complexes will be used for co-crystallization experiment.

Methodologies and/or Techniques to be used potentially

Over-expression of recombinant proteins (*E.coli*),
Protein purification of membrane protein,
Enzymatic activity test, UV-Visible spectroscopy, Biacore
Crystallization

Person to contact:

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Additional information

Upon application please provide your transcripts of records of the last two years and CV.
This internship proposal is, of course, dependent on the evolution of the pandemic and health conditions.