Master 2 internship project
Year 2019-2020

Laboratory/Institute: Institute de Biologie Structurale **Director:** W. Weissenhorn
Team: Membrane & Pathogens **Head of the team:** Franck Fieschi

Name, status of the scientists in charge: F. Fieschi & C. Deniaud **HDR: yes ☒ & no ☒**
Address: 71 Avenue des Martyrs, CS 10090, F-38044 Grenoble Cedex 9
Phone: 33 (0)457428523 **e-mail:** franck.fieschi@ibs.fr

Program of the Master's degree in Biology:

☐ Immunology, Microbiology, Infectious Diseases ☒ Integrative Structural Biology
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology
☐ Planta International

Title of the project:

Resistance to HOCl: characterization of a new enzyme system involved in bacterial resistance

Objectives (up to 3 lines):

Progress toward the structural and mechanistic mechanism of an enzymatic system, composed of membranous and soluble components, that seems to be involved in resistance to inflammation processes, to oxidative stress. It could lead to the development of alternative anti-bacterial therapeutics to face antibiotic multi-resistance.

Abstract (up to 10 lines):

Reactive chlorine species are extremely toxic for bacteria. Indeed, methionine oxidation of the proteins leads to a loss of structure and function and ultimately to the pathogen death. A novel enzymatic system with a methionine sulfoxide reductase (Msr) activity has recently been identified in some bacteria. It comprises a membrane protein (MsrQ1) and a periplasmic protein (MsrP1) encoded by the same operon and is proposed to enable resistance to moderate HOCl doses by reducing altered methionines and restoring periplasmic protein integrity. Interestingly, some intracellular pathogen bacteria present a duplication of this operon (MsrP2-MsrQ2) in pathogenicity islands. This second operon, also observed in some uropathogenic bacteria, contains a small methionine rich periplasmic protein, MrpX, that could act as a sink for HOCl and be subsequently regenerated by the MsrP2-MsrQ2 system. In our laboratory, we have in hands *E.coli* MsrP1/MsrQ1 and Mrpx from a uropathogenic strain. We want to express both independently and as an operon MsrP2/MsrQ2 to undertake a molecular, functional and structural characterization of this system and demonstrate its activity as functional redundant Msr activity and its role in virulence. The long-term objective is the design of inhibitors of these proteins on the basis of the structure function information obtained.

Methods (up to 3 lines):

Production and purification of MsrQ2 and MsrP2 membrane components will be set up. It will be characterized regarding its cofactor composition and activity. They will be compare to MsrQ1and MsrP1. Nanobodies directed against MsrQ1 will be selected and Crystallization fo both membrane component, MsrQ1 and MsrQ2 will be attempted with or without the use of these nanobodies.

Up to 3 relevant publications of the team:

1. Juillan-Binard C, Picciocchi A, Andrieu JP, Dupuy J, Petit-Hartlein I, Caux-Thang C, Vivès C, Nivière V, Fieschi F (2017). A Two component NADPH Oxidase (NOX)-like System in Bacteria Is Involved in the Electron Transfer Chain to the Methionine Sulfoxide Reductase MsrP. J. Biol. Chem. 292(6):2485-2494.

2. Hajjar C, Cherrier MV, Dias Mirandela G, Petit-Hartlein I, Stasia,MJ, Fontecilla-Camps JC, Fieschi F, Dupuy J (2017) The NOX Family of Proteins Is Also Present in Bacteria. mBio 8(6). pii: e01487-17. doi: 10.1128/mBio.01487-17

Requested domains of expertise (up to 5 keywords):

Microbiology (bacterial recombinant expression), protein purification and characterisation, Activity test, UV Visible spectroscopy, Crystallogenesis, X-ray crystallography.