**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** Mechanism of regulation and functional conservation of IMPDH, a key enzyme of the guanine nucleotide metabolism

**Keywords:** Invasive bacteria, nucleotide metabolism, IMPDH, supramolecular assembly, Bateman domain, effector binding site, enzymology, biophysics, structural biology, two-hybrid screening, interactome

**Department:** Structural Biology and Chemistry

**Name of the lab:** Chemistry and Biocatalysis Unit

**Head of the lab:** Sylvie Pochet

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**Web site address of the lab:** http://www.pasteur.fr/ip/easysite/pasteur/en/research/scientific-departments/structural-biology-and-chemistry/units-and-groups/chemistry-and-biocatalysis/home

***Doctoral school affiliation and University*:** Doctoral school MTCI n°563 partnered with Paris Descartes, Paris Diderot and Paris 13 Universities

Presentation of the laboratory and its research topics:

The research projects of the Unit lie at the interface of biology and chemistry, and are mainly focused on the characterization of unexplored proteins involved in the nucleotide metabolism, and the design and synthesis of molecules that interfere with this metabolism. The ongoing research aims to a better understanding of cellular processes as invasive bacteria transits through the various stages of their developmental program focusing on those that are the most promising targets for drug development.

Description of the project:

Nucleotides are absolutely essential to all living cells, and metabolic pathways involved in their biosynthesis are prime targets for therapeutic drugs. Recent findings on enzymes belonging to these pathways have revealed a much more organized and tight regulation than initially thought. In particular, reversible assembly of metabolic enzymes into supramolecular complexes constitutes a novel level of regulation*1-4*.

Thanks to a multi-approach strategy based on complementary expertise, the project aims at deciphering the organization of nucleotide metabolism enzymes in bacteria and at unravelling potential links between these metabolic enzymes and other pathways in prokaryotes. Inosine 5’-monophosphate dehydrogenase (IMPDH) has been selected as a first candidate for several reasons: i) it occupies a key position in the purine nucleotide metabolism*5*; ii) our recent findings6-8 on different bacterial IMPDHs provide indirect experimental evidences at the molecular level that IMPDHs are part of multi-protein complexes. Our working hypothesis is that IMPDH might be involved in other cellular activities (in addition to its classic biosynthetic function: drosophila IMPDH was recently found to be a DNA-binding transcriptional repressor*9*) related to its participation to multi-protein complexes. The interaction with other partners might be fine-tuned through quaternary structure modulations.

The first objective will be to map the interactors of three bacterial IMPDHs using complementary approaches, including bacterial two-hybrid (BACHT)*10, 11*. BACTH chromosomal DNA libraries from different bacteria will be constructed and screened for interacting polypeptides with IMPDHs. Besides the wild-type enzymes, different variants of IMPDHs, which aminoacid substitutions or deletion were shown to impact on the cooperativity, will be considered in order to maximize the coverage of protein-protein interactions and to correlate our structure-function relationships data to the complexes formation. These results will be refined and compiled, and interactome network will be built, taking also into account online databases. Finally, this network will be validated by different biochemical and genetic approaches, and the interacting properties of the validated partners will be further characterized.

Another major aspect of the project will be to correlate structure-function relationships analysis to cellular data. Our previous studies have revealed a complex mechanism of quaternary structure modulation involving one of the two structural IMPDH domains, namely the Bateman domain. A more detailed study of the oligomeric states of different IMPDHs and of the structural variations upon effector or substrates binding will be done using molecular-scale hydrodynamic approaches. The data obtained will help to identify essential features and/or substrate or effector that may influence the stability and the quaternary structure (in particular the overall shape) and relate the results to their activity. In parallel, the physiological importance of the different structural domains of IMPDHs and their conservation among bacteria will be deciphered. Mutant *E. coli* strains will be constructed reexpressing the *guaB* gene coding for IMPDHs from different bacteria (either WT or variants) and the physiology and fitness of these mutant strains will be precisely analysed.

Altogether this project will bring important insights into nucleotide metabolism organization in bacteria and will decipher original links between nucleotide metabolism enzymes, and possibly other pathways, which may represent novel pharmacological targets. This project will thus open the way to original strategies for drug development, urgently needed with the growing public health threat posed by antibiotic resistance.

References:

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Expected profile of the candidate (optional):

The proposed PhD project encompasses knowledge and skills from various scientific fields, ranging from microbiology and biochemistry to biophysics and structural biology. The candidate should have at least some expertise in biochemistry and structural biology, and a strong interest for multidisciplinary approaches.

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