**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:** The actinobacterial exception: structure and regulation of a central metabolic supercomplex

**Keywords:** Actinobacteria; tuberculosis; *Corynebacterium*; integrative structural biology; macromolecular complexes; metabolism; X-ray crystallography; cryo-EM

**Department:** Structural Biology and Chemistry

**Name of the lab:** Structural Microbiology

**Head of the lab:** Pedro M. Alzari

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Presentation of the laboratory and its research topics:

The Structural Microbiology Unit, located at the Institut Pasteur in Paris since 1998 and part of the Structural Biology and Chemistry Department, has a long-lasting interest in bacterial biochemistry and structural biology. Although spanning over several projects, the lab’s activity has been focused on the elucidation of the molecular basis of signal transduction in bacteria, with notable achievements on the structure and function of Ser/Thr kinases 1-3, phosphatases 4,5 and two-component systems 6-8. The lab is now organized in four groups led, respectively, by Pedro M. Alzari (Unit leader), Claudine Mayer, Jean-Christophe Barale and Marco Bellinzoni, in which the PhD student will be enrolled. The group is dedicated to the study of structure and regulation of macromolecular complexes in actinobacteria and has contributed to show, through a wide European collaboration, how mycobacteria can tune their central metabolism according to the available carbon and nitrogen sources 9-11. A strong multidisciplinary approach that includes microbiology, biochemistry and structural biology is the common theme in the Unit and stays at the heart of our research activities. The Unit has several, well-established collaborations both at the national and international level, including groups in Europe (Germany, UK, Italy), South America (Argentina, Brazil, Uruguay) and Asia (Cambodia). Elucidating the molecular basis of key biological processes to develop new therapeutic strategies is our common goal.

Description of the project:

Actinobacteria represent one of the largest eubacterial taxonomic groups and include important human pathogens like *Mycobacterium tuberculosis*, invaluable sources of antibiotics and natural compounds like *Streptomyces*, and major industrial cell factories like *Corynebacterium glutamicum* 12. The need to improve our knowledge of the actinobacterial molecular physiology has been increasing, either for therapeutics (e.g. development of new antibiotics), or for biotechnological purposes, *i.e.* the optimization of strains for the industrial production of chemicals that range from amino acids to terpenes and alcohols. In our lab, we uncovered unique features in the way some of the most conserved reactions in central metabolism are carried out and regulated in *Mycobacterium,* especially the ones situated at two of the most central metabolic nodes, pyruvate and -ketoglutarate 11,13. Despite being considered as universally conserved, we showed that two of the most important complexes, i.e. pyruvate dehydrogenase (PDH) and oxoglutarate dehydrogenase (ODH), are actually merged together in these bacteria to make a mixed ‘supercomplex’. Our recent data suggest this supercomplex to represent a trait of the Actinobacteria phylum. Such findings not only have focused our interest as structural biologists, willing to solve the 3D structure of such a fascinating enzymatic machine, but raise a lot of questions about how this supercomplex is regulated, in a way to coordinate key reactions and metabolic fluxes at two crucial nodes, where carbon metabolism and nitrogen assimilation diverge.

The PhD student will be enrolled in our ongoing work, whose first goal is to isolate this complex and/or reconstitute it *in vitro*, in order to characterize it by an integrative structural biology approach. In practice, daily work will involve different methodologies carried out through high-end equipment, either on the institute’s campus or outside, including X-ray crystallography, SAXS (Small Angle X-ray scattering), mass spectrometry and cryo-electron microscopy, in close collaboration with the cutting-edge platforms at Pasteur. We already determined the crystal structures of the four enzymes forming the supercomplex, and have succeeded in coexpressing them together in *E. coli*, opening the way to the structural characterization of the whole complex. The student will therefore have the opportunity to learn and apply a number of techniques to determine this exciting structure and see how the complex functions, alternating wet-lab with computational work. In addition, through a recently established collaboration with the groups of Profs. Michael Bott and Bernhard Eikmanns, both located in Germany, we wish to go beyond a static structural picture, identifying the regulators that act to coordinate these reactions. *C. glutamicum* will be our working organism, given that this species is a well-established lab model and a major tool for the biotechnological industry 14. The final goal is to clarify the dynamic processes by which the different enzymatic activities may be temporally and spatially coordinated, and to understand, in the end, by which molecular mechanisms (and in response to which stimuli) such a huge machinery could be regulated. In turn, his may open exciting perspectives both for drug development (*e.g.* by targeting essential protein-protein interactions), and for the improvement of industrially relevant strains. Indeed, the metabolic checkpoints we are looking at are hotspots for metabolic engineering 14.

References:

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Expected profile of the candidate:

The ideal PhD candidate for this project is a brilliant and enthusiastic master-level student, interested in microbiology as well as protein biochemistry and structural biology, and willing to learn a portfolio of complementary techniques. Solid communication and presenting skills, as well as a good sense of teamwork are essential. Documented experience in protein biochemistry, biophysics or structural biology would be an asset.

Contact:

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