**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 role of calcium in biogenesis and function of extra-cytoplasmic proteins in Gram-negative bacteria

**Keywords:** protein folding, calcium, disulfide bonds, protein export, type 2 secretion

**Department:** Structural biology and chemistry

**Name of the lab:** Biochemistry of Macromolecular Interactions

**Head of the lab:** Daniel Ladant

**PhD advisor:** Olivera Francetic

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**Web site address of the lab:**

***Doctoral school affiliation and University*:** B3MI, University Paris 7

Presentation of the laboratory and its research topics:

The BIM Unit is interested in understanding the molecular mechanisms that govern protein-protein and protein-membrane interactions in bacterial and eukaryotic cells. The main working model in the unit is the CyaA adenylyl cyclase toxin of *Bordetella pertussis*. Studies of other secreted proteins and protein complexes with roles in virulence and adaptation of *Pseudomonas*, *Klebsiella* and *Salmonella* sp. are studied to unravel their biogenesis, transport, dynamics and molecular function.

Description of the project:

Gram-negative bacterial envelope contains two lipid bilayer membranes that delimit the periplasm, an oxidative compartment with specific properties and protein content. About one third of bacterial proteins is localized in extra-cytoplasmic compartments, that in addition to the periplasm include inner and outer membranes, and bacterial cell surface. The vast majority of these proteins use the Sec export machinery to cross the inner membrane. The narrow SecYEG channel accommodates and transports only unfolded, linear polypeptides. Extensive studies provided an in-depth knowledge of early steps of protein export and chaperons that prevent folding and maintain precursors of Sec dependent proteins in an export competent state. Upon export, rapid and correct folding of these linear polypeptides in the periplasm is critical for their stability and function. Yet, with a notable exception is the DsbABCD machinery that catalyzes disulfide bond formation in the periplasm, little is known about the late steps of protein biogenesis in extra-cytoplasmic compartments.

Calcium is abundant in many bacterial environments and its concentration in the gut, an environment of gut microbiota, is estimated at 2 mM. In addition, early measurements suggest that calcium levels in the periplasm of *Escherichia coli* are high (M range) while cytoplasmic levels are maintained in nM range, suggesting tight regulation.1 Many structural studies reveal the presence of calcium in extracellular proteins and demonstrate the role of calcium in specific adaptive functions2. Our recent study of PulG, a component of the type 2 secretion system, showed that calcium is essential for its folding, stability and function.3 We hypothesize that maintaining high calcium levels in the periplasm is essential for protein folding. Calcium depletion might lead to protein degradation and lead extra-cytoplasmic stress responses4.

The goal of this PhD project is to further our understanding of calcium homeostasis and its cellular and molecular function in bacteria, mainly using *E. coli* as model. Specific goals are the following.

1. Establish and measure *in vivo* calcium levels in different bacterial compartments under a range of calcium concentrations in the growth media, including calcium depletion by EGTA. To this end, calcium specific eukaryotic fluorescent protein probes base on GFP derivatives and fluorescence microscopy will be used to measure cytoplasmic and periplasmic calcium concentrations. Dynamic measurements in live bacteria will be used.
2. Identify proteins whose biogenesis and stability requires calcium, by comparing the proteome of bacteria grown in the presence and absence of extracellular calcium. This part of the work will be done in collaboration with the Mass spectrometry unit and facility of Institut Pasteur.
3. Determine calcium levels in compartments of selected *E. coli* mutants with defects in the *sec* genes, the calcium leak channel *ycc5*, calcium and transporters, as well as putative chaperons induced in conditions of extracytoplasmic stress. Genetic and biochemical approaches will be combined to identify members of calcium regulatory network in *E. coli*.
4. Study the role of calcium on protein folding *in vitro* using model proteins of the type 4 pili family with and without disulfide bonds, with the goal to establish specific roles and interdependence of calcium and Dsb system in protein folding and stability. Protein purification and biophysical techniques including fluorescence measurements will be used. In collaboration with the group of Nadia Izadi-Pruneyre in Institut Pasteur, NMR spectroscopy will be used to establish the effects of calcium on model proteins of known structure at the atomic level.

The results of these studies are expected to provide a better understanding of calcium homeostasis and its function in bacterial adaptation and survival in the environment, its interactions with the host. This fundamental knowledge should also be relevant for bacterial eukaryotic organelles of bacterial origin.

References:

1. Jones et al, (2002) Cell Calcium 32 : 183-92.
2. Dominguez, D.C. Molecular Microbiology (2004)54(2), 291–297
3. Lopez Castilla, A., Thomassin, J-L, Bardiiaux, B *et al*, (2017) Nature Miscrobiology, in press.
4. Grabowicz M, Silhavy TJ. Trends Biochem Sci. 2017 Mar;42(3):232-242.
5. van Stelten et al, (2009) Science 325: 753.

Expected profile of the candidate (optional):

The PhD candidate is expected to have good knowledge of molecular biology and biochemistry. Theoretical and practical knowledge in protein chemistry, protein expression and purification is desirable. Experience with fluorescence microscopy techniques is a plus.

Contact:

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