**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 Full Ph.D. project:** Membrane insertion and translocation of the CyaA toxin

**Keywords:** protein membrane interaction, protein membrane translocation, lipid bilayer, biophysics, fluorescence, FRET, biochemistry

**Department:** Structural Biology and Chemistry

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

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***Doctoral school affiliation and University*:** BioSPC

Presentation of the laboratory and its research topics:

The main objectives of our Research Unit “Biochemistry of Macromolecular Interactions ” is to decipher the molecular basis of action of two bacterial adenylate cyclase toxins that are key virulence factors from *Bordetella pertussis* (CyaA) and *Pseudomonas aeruginosa* (ExoY), two important human pathogens. Fundamental knowledge on the original mechanisms of action of CyaA is exploited in translational science for development of innovative therapeutic vaccines, anti-infective strategies, and novel biological screening techniques, such as the bacterial adenylate cyclase two-hybrid system.

Our Research Unit has previously made some major contributions in the study of the adenylate cyclase (CyaA) toxin from B. pertussis, the causative agent of whooping cough, particularly in the engineering of CyaA into a potent antigen-delivery vehicle that has recently entered into clinical trials. More recently, we developed a method to produce a monomeric, stable and functional CyaA protein that paves new ways to investigate the molecular processes involved in the intoxication mechanism of CyaA, including its calcium-dependent folding and its translocation process across the plasma membrane of target cells. Our projects are build on the established skills of the team in molecular biology, protein engineering, biochemistry and biophysics of proteins and membranes, and rely extensively on collaborations with numerous groups and facilities from Institut Pasteur as well as from national and international Institutions.

The CyaA toxin is a major virulent factor produced by *Bordetella pertussis*, the causative agent of whooping cough. Its translocation process in target cells remains, however, largely unknown. The aim of the PhD project is (i) to decipher the structural mechanism of CyaA membrane insertion and catalytic domain transport across the lipid bilayer (ii) to provide new insights into CyaA-based biotechnological applications developed in the lab, i.e., to improve the use of CyaA as antigen delivery vehicle and to contribute to the development of a new generation of pertussis vaccine. The biochemical, biophysical and functional properties of CyaA will be characterized using a combination of standard and cutting-edge methodologies available in the Unit, in the Institut Pasteur Technological Centers and thanks to national and international collaborations.

Description of the project:

**I. Background**

The adenylate cyclase toxin (CyaA) plays an important role in the early stages of respiratory tract colonization by B. pertussis, the causative agent of whooping cough. CyaA is a 1706-residue long protein organized in a modular fashion: the ATP-cyclizing, CaM-activated, catalytic domain (ACD) is located in the 364 amino-proximal residues. The region spanning residues 400 to 500 is involved in the translocation process of ACD while the C-terminal part of the molecule (from 500 to 1706) is involved in membrane insertion and toxin binding to a specific cellular receptor (CD11b/CD18). The CyaA toxin is synthesized as an inactive precursor, pro-CyaA, that is converted into the active toxin upon specific acylation of two lysine residues (Lys 860 and Lys 983). One of the main originalities of CyaA stems from its unique mechanism of penetration into eukaryotic cells: a direct translocation of the catalytic domain across the plasma membrane. The molecular mechanism by which CyaA enters into target cells remains, however, largely unknown. Once translocated, ACD binds to the endogenous cytosolic calmodulin and produces supraphysiologic levels of cAMP that in turn alters cellular physiology, leading to cell death.

**II- Proposed PhD project**

**II.A. Structure of membrane-inserted CyaA and pro-CyaA toxins**

The conformational changes of CyaA upon membrane interaction will be characterized by a combination of biophysical techniques (CD, FTIR, ATR-FTIR, fluorescence, FRET) in kinetic and steady-state modes available at Institut Pasteur. The low-resolution structure and oligomerization status of CyaA inserted in the membrane will be further investigated by a combination of electron microscopy (EM, IP), neutron specular reflectometry (ILL, Grenoble) and dual polarization interferometry (DPI, IP). Collectively, these data will be crucial to propose a molecular and kinetic description of the membrane insertion process of both CyaA and pro-CyaA. Moreover, the comparison of these two toxins will allow us to decipher the effect of the acylation on the membrane insertion process, which should be different as CyaA does efficiently translocate ACD into the cytosol while ACD is not transported across membrane within pro-CyaA.

**II.B. Structure of CyaA upon ACD translocation across lipid bilayers**

We will describe the impact of the acylation on the successive steps of ACD translocation across lipid bilayers in vitro and, as a future perspective, across the target cell membrane in vivo (erythrocytes, alveolar macrophages and dendritic cells). Two fluorescent assays to monitor the translocation process developed in the lab will be used. Moreover, our in vitro FRET translocation assay should be easily adapted to eukaryotic cells. Altogether, the proposed studies should provide valuable data on the structure and kinetics of the successive steps of the translocation process. Finally, these studies on the intoxication process will be instrumental (i) for the design of improved CyaA-based antigen delivery vectors and (ii) toward the development of a new, safe and efficient pertussis vaccine.

**III. Concluding remarks on the objectives of the PhD project**

The PhD project aims to solve several unanswered key questions regarding the molecular mechanism of CyaA intoxication:

- the successive steps leading to membrane insertion of CyaA,

- the structure and oligomerization status of CyaA inserted into membrane,

- the effects of CyaA acylation on the membrane insertion process, i.e., the differences of membrane insertion mechanisms between non-acylated proCyaA and acylated CyaA toxins,

- the molecular process of ACD translocation across membranes *in vitro* and *in cellula*,

- the impact of lipid properties on the successive steps leading to ACD translocation

References (only those related to this project):

1. *Cannella SE, Ntsogo Enguéné VY, Davi M, Malosse C, Sotomayor Pérez AC, Chamot-Rooke J, Vachette P, Durand D, Ladant D and Chenal A. Stability, structural and functional properties of a monomeric, calcium–loaded adenylate cyclase toxin, CyaA, from Bordetella pertussis; Nature Scientific Reports 2017.*
2. *O'Brien DP, Hernandez B, Durand D, Hourdel V, Sotomayor-Pérez AC, Vachette P, Ghomi M, Chamot-Rooke J, Ladant D, Brier S, Chenal A. Structural models of intrinsically disordered and calcium-bound folded states of a protein adapted for secretion. Sci Rep. 2015 Sep 16;5:14223.*
3. *Karst JC, Ntsogo Enguéné VY, Cannella SE, Subrini O, Hessel A, Debard S, Ladant D, Chenal A. Calcium, acylation, and molecular confinement favor folding of Bordetella pertussis adenylate cyclase CyaA toxin into a monomeric and cytotoxic form. J Biol Chem. 2014 Oct 31;289(44):30702-16.*
4. *Subrini O, Sotomayor-Pérez AC, Hessel A, Spiaczka-Karst J, Selwa E, Sapay N, Veneziano R, Pansieri J, Chopineau J, Ladant D, Chenal A. Characterization of a membrane-active peptide from the Bordetella pertussis CyaA toxin. J Biol Chem. 2013 Nov 8;288(45):32585-98.*
5. *Sotomayor-Pérez AC, Subrini O, Hessel A, Ladant D, Chenal A. Molecular crowding stabilizes both the intrinsically disordered calcium-free state and the folded calcium-bound state of a repeat in toxin (RTX) protein. J Am Chem Soc. 2013 Aug 14;135(32):11929-34.*

*Veneziano R, Rossi C, Chenal A, Devoisselle JM, Ladant D, Chopineau J. Bordetella pertussis adenylate cyclase toxin translocation across a tethered lipid bilayer. Proc Natl Acad Sci U S A. 2013 Dec 17;110(51):20473-8.*

1. *Karst JC, Barker R, Devi U, Swann MJ, Davi M, Roser SJ, Ladant D, Chenal A. Identification of a region that assists membrane insertion and translocation of the catalytic domain of Bordetella pertussis CyaA toxin. J Biol Chem. 2012 Mar 16;287(12):9200-12.*

Expected profile of the candidate:

During this 3-year PhD project on the translocation process of the CyaA toxin, the PhD student will be trained and exposed to various environments and methods in molecular biology, biochemistry and biophysics of proteins and protein / membrane interactions. The project will be mainly performed in the Unit but also involves several collaborations and therefore requires a strong motivation, a team-spirited PhD student, capable of taking self-initiatives for the benefit of his/her doctoral project.

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