**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:** Deciphering theinteractome of bacterial pathogens with mass spectrometry

**Keywords:** Mass Spectrometry, Top-Down Proteomic, Cross-Linking, Native Mass Spectrometry,

**Department: Structural Biology and Chemistry**

**Name of the lab:** Utechs Mass Spectrometry for Biology

**Head of the lab:** Julia Chamot-Rooke

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**Web site address of the lab: https://research.pasteur.fr/fr/team/structural-mass-spectrometry-and-proteomics/**

***Doctoral school affiliation and University*:** Bio Sorbonne Paris Cité

Presentation of the laboratory and its research topics:

The overarching objective of the Unit is to develop new Mass Spectrometry-based methodologies for the analysis of proteins and protein complexes involved in infectious disease. The unit focuses particularly on “top-down” approaches, performed on the entire protein, that are particularly useful for the complete characterization of post-translationally modified proteins. Top-down and high throughput bottom-up approaches are also combined to identify and fully characterize sets of proteins involved in host-pathogen interactions. A second branch of the research team

Description of the project:

*(1 page, Arial font size 11: 600 words in total with at least 50% dedicated specifically to the proposed PhD project(s))*

Protein-protein interactions (PPIs) are involved in most, if not all, cellular functions. The comprehensive mapping of these complex networks of stable and transient associations remains a key goal, both for biological system characterization and for focused biological studies. Despite the significant challenges to achieve such a goal, major advances have been made over the past few years. They include improvements in the computation prediction of PPIs, but also an increase of high-quality data from high-throughput experimental PPI mapping strategies. Quickly, Mass Spectrometry based approaches revealed their great potential taking advantages of their speed and sensitivity. These include approaches like Affinity Purification coupled to Mass Spectrometry analyses (AP-MS), Cross-linking Mass Spectrometry experiments (XL-MS) or Mass Spectrometry Protein Correlation Profilling (PCP).

Pursuing the goal of always better characterizing biological system, our lab acquired expertise in various structural proteomics techniques, including XL-MS [1], native MS and Top-Down Proteomics [2, 3] that we now want combine to profile the interactome of bacterial pathogens. The subject of the proposed PhD project is therefore to optimize these 3 complementary approaches and show that they can provide a new way to address virulence or resistance.

The candidate will first focus on identifying protein-protein interactions. To do so, she/he will adapt the novel XL-MS method developed in the lab for an entire proteome while preserving transient protein associations. This method is based on a new cross-linker, named NNP9, which allows a very efficient one-step enrichment of cross-link species using click-chemistry.

Because subtle post-translational modifications (phosphorylation, alkylation, glycosylation …) can be relevant in terms of pathogenicity or resistance, the candidate will in a second step seek to identify all the possible forms in which a protein is produced, called proteoforms. Unfortunately, classical bottom-up approach used in XL-MS (where the proteins are digested before being analyzed by LC-MS) cannot address peptide inference problem (a peptide can belong to multiple proteins) nor combinatorial issue of the protein decorations. To overcome these problems the student will use Top-Down proteomic approach, the method of choice to characterize the diverse proteoforms extracted from the different bacterial strains. To achieve the more comprehensive analysis, the student will have access to high end mass spectrometer (Orbitrap Fusion Lumos) equipped with a UV laser for UV photodissociation (UVPD) which already proved its efficiency to fragment intact protein in a liquid chromatography time scale [4].

In a third step, the PhD student will extract intact complexes in their native forms and analyze them intact by Native Mass Spectrometry using the PPI identified by XL-MS and the mass of the various proteoforms measured in Top-down analyses as guidelines. Diverse lysis conditions (osmotic pressure, mechanical shredding…) and native separation technics like GelFREE or size exclusion chromatography will be explored in this third step to fractionate and isolate native complexes.

Finally the data obtain will be aggregated to produce an integrated interactome map which will be compared to pin point critical differences between pathogen strains.

Resistant and non-resistant *E. coli* strains will be chosen to start the project but the methods developed will be broadly applicable to other pathogens.

All along its PhD, the student will work within a team of experts in each technique presented above and can count on their supportive collaborations. To tackle the project, she/he will have large time access to state of the art high resolution mass spectrometers (Obitrap Fusion Lumos, Synapt HDMS). The project will also require dedicated informatics tools which will be developed by a computer scientist in the lab.

The student will participate actively in the lab life and will have several occasions to present his/her work in national or international scientific meetings.

References:

1. Nury C, Redeker V, Dautrey S, Romieu A, van der Rest G, Renard PY, Melki R, Chamot-Rooke J: A Novel Bio-Orthogonal Cross-Linker for Improved Protein/Protein Interaction Analysis. *Anal Chem* 2015, **87**(3):1853-1860.

2. Chamot-Rooke J, Mikaty G, Malosse C, Soyer M, Dumont A, Gault J, Imhaus AF, Martin P, Trellet M, Clary G *et al*: Posttranslational modification of pili upon cell contact triggers N. meningitidis dissemination. 2011, **331**(6018):778-782.

3. Vinella D, Fischer F, Vorontsov E, Gallaud J, Malosse C, Michel V, Cavazza C, Robbe-Saule M, Richaud P, Chamot-Rooke J *et al*: Evolution of Helicobacter: Acquisition by Gastric Species of Two Histidine-Rich Proteins Essential for Colonization. 2015, **11**(12):e1005312.

4. Cleland TP, DeHart CJ, Fellers RT, VanNispen AJ, Greer JB, LeDuc RD, Parker WR, Thomas PM, Kelleher NL, Brodbelt JS: High-Throughput Analysis of Intact Human Proteins Using UVPD and HCD on an Orbitrap Mass Spectrometer. 2017, **16**(5):2072-2079.

Expected profile of the candidate (optional):

Chemist, biochemist or biologist with a first experience in mass spectrometry.

The candidate should show a great interest in method development and technology.

Contact:

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