**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:** Synthetic carbohydrate-based conjugates toward a broad coverage vaccine against bacillary dysentery

**Keywords:** Chemical biology,Glycochemistry, Target synthesis, Molecular tools, Glycosylation, Protecting group manipulation, Bioconjugates, Carbohydrate-protein interactions, Antigens, Vaccines, Diarrheal diseases, *Shigella*

**Department:** Structural Biology and Chemistry

**Name of the lab:** Chemistry of Biomolecules

**Head of the lab:** Laurence Mulard

**PhD advisor:** Laurence Mulard

**Email address:** laurence.mulard@pasteur.fr

**Web site address of the lab: *Doctoral school affiliation and University***: Ecole Doctorale MTCI (Médicament, Toxicologie, Chimie, Environnement) Institut de Formation Doctorale Université Paris Descartes 12 rue de l'Ecole de Médecine

75270 PARIS Cedex 06

Presentation of the laboratory and its research topics:

The “Chemistry of Biomolecules” laboratory evolves in the areas of Chemical Biology, Organic and Bioorganic Chemistry. Major interests are in carbohydrates, peptides, proteins and bioconjugates of importance in Health Sciences. Research programs are focused on the design and synthesis of molecular tools and chemical probes aimed at understanding and modulating the interplay between structure and function of those biomolecules, especially when related to human pathologies. For a selection of topics, the gained knowledge serves as strong basis for the development of novel therapeutic agents, vaccines or diagnostic tools.

Central to multidisciplinary collaborative projects, synthetic chemistry addresses a variety of challenges to provide often complex molecules in pure form and sufficient amount to investigate the biological functions of native biomolecules. Research programs aim at (i) validating biological targets of medical interest, (ii) investigating the role that some proteins, polysaccharides, and glycoconjugates play in disease progression and microbial pathogenesis in humans, and (iii) developing strategies for the rational design of novel compounds able to interfere with impaired biological processes and/or diseases as seen from three representative achievements:

* A fully synthetic mCD4-peptide conjugate was proposed as a new bi-substrate HIV-1 entry inhibitor. The designed conjugate displays low nanomolar activity against a number of clinical HIV-1 isolates.
* A fully synthetic glycopeptide, MAG-Tn3, was developed as an immunogen based on the tumor-associated Tn antigen. MAG-Tn3 is a promising therapeutic vaccine against adenocarcinomas. A phase I clinical trial is ongoing.
* A fine-tuned neoglycoprotein immunogen, SF2a-TT15, was developed as the first promising synthetic carbohydrate-based conjugate vaccine candidate against endemic shigellosis. The selected carbohydrate component was designed to act as a functional mimic of the polysaccharide antigen present at the surface of the bacteria causing disease. A phase I clinical trial is ongoing.

Additional examples of topics under study in the unit include the development of, among others, chemo-enzymatic strategies to complex oligosaccharides by use of engineered glycoenzymes, labeled nanobody conjugates for the early diagnosis of Alzheimer’s disease, or the synthesis of site-specifically labeled peptides as biomarkers allowing the exclusive staining of the human colonic mucus.

Description of the project:

Carbohydrate-protein interactions are key players in host/pathogen recognition. Bacterial surface polysaccharides are important virulence factors as well as major targets of the host humoral immune response mounted following natural infection. This observation has led to the development of polysaccharide-protein conjugate vaccines, a major breakthrough in the field of infectious diseases. Yet, there is no polysaccharide conjugate vaccine available for disease caused by bacteria whose surface polysaccharide is the O-antigen moiety of their lipopolysaccharide, as exemplified for *Shigella*. *Shigella* are Gram negative enteroinvasive bacteria, which are the cause of bacillary dysentery, or shigellosis, one of the top four diarrheal diseases circulating in the pediatric population.[1] There is a critical need for developing a vaccine against shigellosis to defeat at least *S. sonnei*and all the *S. flexneri* types and subtypes.[2] The latter are differentiated based on the structure of their O-antigen.[3]

Instead of working with polysaccharides extracted from cell culture, we have addressed this challenging goal by use of synthetic oligosaccharides designed to act as functional mimics of the natural polysaccharide antigens. A first vaccine candidate was developed against *S. flexneri* 2a, the most prevalent serotype.[4] The selected hapten is a synthetic pentadecasaccharide corresponding to an O-antigen segment made of three basic repeating units.[5] A phase I clinical trial is ongoing.[6]

**This project is part of a research program aimed at broadening *Shigella* species and serotype vaccine coverage. It is based on carbohydrate chemistry and developed in combination with structural biology and vaccinology by means of collaborations in and out of campus.**

The project is meant to investigate a novel strategy toward a synthetic carbohydrate-based vaccine candidate against major *S. flexneri* serotypes. With regards to the biological context, it is organized around three objectives.

* (i) to identify potent antigenic mimics of the chosen polysaccharide antigens based on *in vitro* and structural studies involving a broad diversity of synthetic O-antigen fragments in combination with protective monoclonal antibodies.
* (ii) to identify potent immunogenic mimic of the same O-antigens, by means of *in vivo* studies involving a series of glycoconjugates derived from oligosaccharides identified as the most promising antigenic mimics for the investigated serotypes.
* (iii) to identify trivalent vaccine compositions, capable of inducing protective sera targeting each one of the involved serotypes, and possibly cross-reactive with other *Shigella*.

To achieve these objectives, a large number of chemically defined oligosaccharides differing in size and composition are required. In this context, the successful candidate will be in charge of developing efficient syntheses of panels of type-specific O-antigen fragments equipped with a linker for site-selective conjugation. Multiple-step chemical and/or chemo-enzymatic strategies based on optimized protecting group pattern and glycosylation chemistry shall enable the synthesis of oligosaccharides of increasing length and complexity. Developments will go beyond state-of-the-art glycochemistry to tackle some remaining challenging issues such as regio- and stereocontrol during glycosylation, non-stoichiometric substitutions, orthogonal protecting group manipulation, and/or robustness validation on large scale for compatibility with technical transfer. Synthetic targets and intermediates will be characterized by NMR and MS. The latter will be purified by flash chromatography and crystallography, whereas the final compounds will be purified by HPLC. Binding to available antibodies will use the SPR technology, supported by structural analysis and possibly ITC.

The most promising oligosaccharides identified *in vitro* will be converted to glycoconjugates and screened *in vivo* for their immunogenic properties. Following this primary selection, fine tuning of carrier/hapten combinations will be investigated in the search for improved immunogenicity and cross-protective efficacy. This will be supported by extensive biophysical characterization of the conjugates.

References:

1. *K*. L. Kotloff, *et al*, *Lancet* **2016**, *388*, 1291-1301.
2. S. Livio, *et al*, *Clin Infect Dis* **2014**, *59*, 933-941.
3. [A. V. Perepelov, *et al*, *FEMS Immunol Med Microbiol* **2012**, *66*, 201-210.
4. [R. M. F. van der Put, *et al*, *Bioconjugate Chem* **2016**, *27*, 883-892.
5. F. Bélot, *et al*, *Chem Eur J* **2005**, *11*, 1625-1635.
6. [*https://clinicaltrials.gov/ct2/show/NCT02797236*](https://clinicaltrials.gov/ct2/show/NCT02797236)*.*

Expected profile of the candidate:

Strong skills in the theory and experimental aspects of organic synthesis, including some background in the field of multi-step synthesis, purification and analysis of complex molecules, are desirable. Prior experience in oligosaccharide synthesis and interest in methodology would be an asset.

Applicants are expected to be thorough and curious. They should enjoy teamwork and show a strong motivation for multidisciplinary projects in addition to good communication skills. Knowledge of French and/or strong willingness to learn it is recommended.

Contact:

Laurence Mulard

Unité de Chimie des Biomolécules (UMR CNRS 3523)

Institut Pasteur

28 rue du Dr Roux, 75 724 Paris Cedex 15

E-mail: [laurence.mulard@pasteur.fr](mailto:laurence.mulard@pasteur.fr), Phone: +33 (0)1 40 61 38 20