**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:** Unraveling the molecular mechanism of type IIB topoisomerases

**Keywords:** Integrative structural biology, topoisomerase, new DNA topoisomerase, topoisomerase VI, topoisomerase VIII

**Department:** Structural biology and chemistry

**Name of the lab:** Structural microbiology

**Head of the lab:** Pedro Alzari

**PhD advisor:** Claudine MAYER

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**Web site address of the lab:** https://www.pasteur.fr/en/research/structural-biology-chemistry/units-groups/structural-microbiology

***Doctoral school affiliation and University*:** MTCI – Université Paris Diderot

Presentation of the laboratory and its research topics:

The research activities of the unit are oriented towards the biochemical, biophysical and structural studies of proteins involved in microbial physiology and pathogenesis, with a particular focus on bacterial cell signaling mechanisms. They are organized in four main topics: (i) control of glutamate metabolism in mycobacteria, (ii) envelope stress response, (iii) PknA/PknB in mycobacterial cell division and (iv) structural studies of type II topoisomerases.

Description of the project:

Type II topoisomerases are fascinating macromolecular machines essential for solving topological problems arising during DNA metabolic processes and are therefore critical for the preservation of genome stability. They can interconvert different topological forms of DNA during transcription and replication since they catalyse DNA double strand breaks and have gained a high level of importance as targets in antimicrobial and anti-cancer therapy. They are classified into subtypes IIA and IIB, based on sequence and structural similarities. The IIA subclass includes DNA gyrase, found in the three domains of life, bacterial topoisomerase IV and eukaryotic topoisomerase II. Until now, subtype IIB only comprised topoisomerase VI, which was originally discovered in archaea, but has more recently been found in plants and apicomplexa such as plasmodial parasites. These enzymes are thought to play a role in endoreduplication in plants and schizogeny in apicomplexa. However, exactly what they do and how they act is not presently clear.

Archaeal and bacterial types IIA and IIB enzymes are heterotetramers composed of two different subunits (A and B), whereas type IIA enzymes from eukaryotes are homodimers, with the B and A moieties fused into a single polypeptide. The two families share three functional domains, the ATPase domain located in the B subunit, the Toprim domain located either in the B or A subunit, and a winged helix domain in the A subunit, that contain the catalytic tyrosine. Many studies have been published on the mechanism of action of Topo IIA and their interactions with antibiotics and antitumoral drugs. In contrast, very few studies have been performed on Topo IIB. Today, no structural data is available concerning the interaction with DNA. **The first part of the project will focus on the structural characterization of the DNA recognition mode by topoisomerase VI.**

We recently discovered and characterised a new type IIB topoisomerase, topoisomerase VIII, from bacterial, archaeal and plasmid origins (Gadelle 2014). This new enzyme adds to our knowledge of the origins and functions of the type IIB enzymes, and their smaller sizes may facilitate studies of structure and function. The genes encoding these proteins correspond to the fusion of the B and A subunits of type IIB topoisomerases. Intriguingly, their topoisomerase activities are relatively weak and puzzling and it is not yet clear what their biological functions are. We showed that the enzyme encoded by a bacterial plasmid exhibits low ATP-dependent relaxation and decatenation activities, the hallmark of type II topoisomerases, whereas two other enzymes, encoded by integrated elements, exhibit only DNA cleavage activity, producing DNA double-stranded breaks, and/or ATP-independent relaxation activity. **The second part of the project will focus on the biochemical and structural characterization of these new DNA topoisomerases using integrative structural biology methods.**

References:

1. ***J. Piton****,* ***S. Petrella****, M. Delarue, G. André-Leroux, V. Jarlier, A. Aubry and* ***C. Mayer****. (2010). Structural insights into the quinolone resistance mechanism of Mycobacterium tuberculosis DNA gyrase.* ***PLoS ONE,******5 (8)****, e12245.*
2. *Bouige,* ***A. Darmon****,* ***J. Piton****,* ***M. Roué****,* ***S. Petrella****, E. Capton, P. Forterre, A. Aubry, and* ***C. Mayer****. (2013). Mycobacterium tuberculosis DNA gyrase possesses two functional GyrA-boxes.* ***Biochem J.,******455 (3)****, 285-294.*
3. *Agrawal,* ***M. Roué****, C. Spitzfaden,* ***S. Petrella****, A. Aubry, MM. Hann, B. Bax, and* ***C. Mayer****. (2013). Mycobacterium tuberculosis DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion.* ***Biochem J., 456 (2)****, 263-273.*
4. *Gadelle, M. Krupovic, K. Raymann,* ***C. Mayer****, and P. Forterre. (2014). DNA topoisomerase VIII: a novel subfamily of type IIB topoisomerases encoded by free or integrated plasmids in Archaea and Bacteria.* ***Nucleic Acids Res****.****, 42 (13)****, 8578-8591.*
5. *N. Vrielynck, A. Chambon, D. Vezon, L. Pereira, L. Chelysheva, A. De Muyt, C. Mézard,* ***C. Mayer****, M. Grelon. (2016). A DNA topoisomerase VI-like complex initiates meiotic recombination.* ***Science****.* ***351 (6276)****, 939-943.*

Expected profile of the candidate (optional):

Knowledge in biochemistry (activity assays, protein purification), biophysics and structural biology

Contact:

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