**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:** New tool for visualizing and detecting Klebsiella pneumoniae infections

**Keywords:** aptamer, nanobody, Klebsiella pneumoniae, in vivo imaging

**Department:** Center for Innovation and Technological Research & Cell Biology and Infection

**Name of the lab:** Photonic BioImaging (UtechS)

**Head of the lab:** Spencer Shorte

**Ph.D. advisor:** Régis Tournebize

**E-mail address:** regis.tournebize@pasteur.fr

**Web site address of the lab:** https://research.pasteur.fr/en/team/photonic-bioimaging-utechs-pbi/

***Doctoral school affiliation and University*:** ED Bio Sorbonne Paris Cité – Université Paris Diderot

Presentation of the laboratory and its research topics:

The UTechS Photonic BioImaging is a research technology platform (core facility) providing expertise and support in optical imaging methods for researchers at the Institut Pasteur and performing technology-oriented research activities. The R&D is founded upon the need to develop optical imaging methods and tools that bring new understanding of host-pathogen interactions and in situ high-content imaging techniques and their application to infection, cell biology, cellular microbiology, and microbiology.

Platform activities are highly multi-disciplined, and collaborative, with the mission goal focused on the use of quantitative imaging and analysis to understand the processes of cell/tissue-biology, and their usurpation by infection and disease.

Description of the project:

Infectious diseases have a major impact on public health worldwide as we observe the emergence or re-emergence of diseases combined with the appearance of bacterial strains resistant to multiple antibiotics. An early and accurate diagnosis of infectious agents followed by prompt and relevant therapy are major prerequisites for a favorable disease outcome, and more and more infections require precise identification of the pathogen before an efficient therapy is started. However, this task can be sometimes difficult to perform, as, for instance, in clinical setting where the infectious site is often difficult to access and the infectious sample could be contaminated by resident flora from adjacent zones. Altogether, these observations call for new and innovative tools and methodologies that will enable rapid, specific and sensitive identification of disease-causing pathogens that can be applied to the emerging field of non-invasive molecular imaging in biomedical and clinical research (*1*).

Nowadays, a small group of ESKAPE pathogens (for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella. pneumoniae* and *Escherichia coli*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and Enterobacter species) represent a major public health challenge, both in developed and developing countries (*2*). These pathogens are responsible for numerous pathologies in the community, and in hospital, including septicaemia, urinary and lung infections (*3*).

This project proposes to target the *E. coli* and *K. pneumoniae* species and to **develop novel molecular imaging probes** that will allow to **directly recognise and visualise them *in vivo*.**

A recent study identified the adhesin MrKA as a potent antigen for the design of vaccines, indicating that this outer membrane protein is easily accessible. Moreover MrkA is largely conserved among Enterobacteriaceae. These observations thus identify MrkA as good candidate for selecting components targeting this protein. Such components will be able to recognise a large number of Gram negative pathogenic bacteria.

Two different types of targeting moieties will be selected during this PhD project:

* Aptamers, short oligonucleotides sequences that are capable of binding to specific targets with very high efficiency,
* Nanobodies, small single domain antibodies derived from camelids origin.

During this project the PhD candidate will work with the group of Marcel Hollenstein to select aptamers and will interact with the antibody generation team of Pierre Lafaye to select nanobodies. The project will consist of

* producing the MrkA adhesin,
* selecting aptamers and nanobodies,
* biochemical and biophysical characterization of the candidates (highest affinity in vitro, identification of binding site, stability…),
* labelling them with appropriate fluorophores,
* testing them for their capacity to recognise efficiently and selectively the bacteria *in vitro* and *in vivo* in models of infections using small animal optical imaging.

This work will develop further to adapting the aptamers and nanobodies to other modalities of in vivo imaging (PET, MRI…) via external collaboration.

This cross-disciplinary work will make use of a large set of techniques ranging from molecular biology, biochemistry, microbiology, FACS, various microscopy technologies, in vivo imaging. It will be conducted in a team with experience in physiopathology and imaging of infection diseases (*4*-*7*), and will benefit from the input and support of several platforms and units from the campus.

References:

1. S. K. Jain, The Promise of Molecular Imaging in the Study and Treatment of Infectious Diseases. *Mol Imaging Biol*. **19**, 341–347 (2017).

2. L. B. Rice, Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. *J Infect Dis*. **197**, 1079–1081 (2008).

3. J. N. Pendleton, S. P. Gorman, B. F. Gilmore, Clinical relevance of the ESKAPE pathogens. *Expert Rev Anti Infect Ther*. **11**, 297–308 (2013).

4. L. M. S. Lery *et al.*, Comparative analysis of Klebsiella pneumoniae genomes identifies a phospholipase D family protein as a novel virulence factor. *BMC biology*. **12**, 41 (2014).

5. C. Fevre *et al.*, A novel murine model of rhinoscleroma identifies Mikulicz cells, the disease signature, as IL-10 dependent derivatives of inflammatory monocytes. *EMBO Mol Med*. **5**, 516–530 (2013).

6. J. Dragavon *et al.*, In vivo excitation of nanoparticles using luminescent bacteria. *Proc Natl Acad Sci USA*. **109**, 8890–8895 (2012).

7. R. Tournebize *et al.*, Magnetic resonance imaging of Klebsiella pneumoniae-induced pneumonia in mice. *Cell Microbiol*. **8**, 33–43 (2006).

Expected profile of the candidate (optional):

Candidates are expected to have good knowledge and experience in biochemistry, molecular biology, and possibly microbiology. They should be willing to work with animal or have previous experience in animal experimentation and handling.

Contact:

Régis Tournebize

Photonic BioImaging (UTechS PBI)

Center for innovation and Technological Research (Citech)

INSERM 1202

Institut Pasteur

25-28 rue du Dr. Roux

75724 Paris Cedex 15

France

Tel : +33-140613394

email : regis.tournebize@pasteur.fr