**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:** Control of bacterial cell shape

**Keywords:** Regulation of bacterial growth and morphogenesis; Peptidoglycan cell wall; High-resolution fluorescence microscopy; Genetics of cell-shape regulation

**Department:** Microbiology

**Name of the lab:** Lab of Bacterial Morphogenesis and Growth

**Head of the lab:** Sven van Teeffelen

**PhD advisor:** Sven van Teeffelen

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**Web site address of the lab:** sites.google.com/site/vanteeffelenlab/

***Doctoral school affiliation and University*:** ED Physique en Ile de France (ED-PIF, ED-)

Presentation of the laboratory and its research topics:

Our lab is interested in the regulation of bacterial shape and growth. We study how bacteria physically establish their own shape, how they coordinate general physiology with size, and how they decide to grow and divide as a function of extra-cellular cues. For example, how do bacteria 'know' in a certain nutrient environment at what rate they have to expand their cell envelope to make space for intracellular protein, DNA, etc.? How do they know when to divide? How do these behaviors depend on the environment, e.g., in a biofilm? Our interdisciplinary lab addresses these questions with combined techniques from physics and molecular microbiology: high-resolution fluorescence microscopy, quantitative image analysis, genetics, and modeling.

1. *Wong F, Renner LD, Özbaykal G, Paulose J, Weibel DB, van Teeffelen S, Amir A, Mechanical strain-sensing implicated in cell shape recovery in Escherichia coli, Nat. Microbio. 2017; 2:17115.*
2. *Vigouroux A, Oldewurtel ER, Cui L, Bikard D and van Teeffelen S, Engineered CRISPR-Cas9 system enables noiseless, fine-tuned and multiplexed repression of bacterial genes, bioRxiv, doi:10.1101/164384.*
3. *Amir A, van Teeffelen S, Getting into shape: How do rod-like bacteria control their geometry? Syst Synth Biol 2014 Sep;8(3):227-35.*
4. *van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, Gitai Z, The bacterial actin MreB rotates, and rotation depends on cell-wall assembly, Proc. Natl. Acad. Sci. U.S.A. 2011 Sep;108(38):15822-7.*

Description of the project:

Bacteria have evolved the capacity to control their cell shapes with high precision during growth. At the same time, shape is adaptable to changes in growth conditions and the cell cycle. Despite decades of research and a large amount of accumulated knowledge about the individual proteins and enzymes involved, we still do not know how these proteins interact to control, e.g., cell diameter and length. Specific questions within this proposed project are: How does the cell maintain a macroscopic diameter hundreds of times larger than the size of a typical protein? How does the cell know how fast to increase its volume to maintain cell integrity and function? Within this project we aim to use new technologies such as high-resolution fluorescence microscopy and micro-fluidics to gain new insights into the determinants of cell shape and cell-shape regulation. The project will have a strong focus on biophysics, microscopy, and image analysis. A specific project is detailed below. However, the final project will be developed together with the candidate.

The major component responsible for cell shape is the peptidoglycan (PG) cell wall. In Gram-negative bacteria glycan strands are arranged horizontally on the surface encircling the cell as a monolayer [1]. Thus, before new PG can be inserted into the cell wall, old material must be cleaved. Bacterial hydrolysis and synthesis are therefore the major ways to control cell-wall expansion. While the molecular components of the cell wall and some of the PG-modifying enzymes are well characterized [2,3], we are only now starting to understand their spatio-temporal dynamics [5-7], which are ultimately responsible for cell-wall architecture and shape, are less well understood.

Following the dynamics of individual enzymes by single-particle microscopy. Along one possible direction of the PhD project the student will explore the dynamics and activity of major cell-wall synthesis and hydrolysis enzymes using state-of-the art high-resolution fluorescence microscopy. To that end we have built an ultra-stable fluorescence microscope that allows the precise tracking of individual fluorescently labeled proteins in space and time. Once the imaging of single bacterial enzymes is established we will study the change of enzyme dynamics during steady-state growth and upon inhibition of major components of the cell-wall synthesis machinery, thus planning to use enzyme motion as an indirect readout for enzyme activity and cell-wall remodeling.

Regulation of cell division during the cell cycle. During fast growth bacteria double their size and divide about every 20 minutes. At the same time the cells have to replicate and segregate their DNA to make sure that every daughter receives a chromosome. Cell-cycle progression and cell division must therefore be tightly regulated. Different modes of cell-cycle regulation have been proposed recently [8-10], but these proposals need further testing and will likely need to be modified depending on physiological conditions. Furthermore, the molecular factors involved in controlling cell division are not known. In this project we will try to identify the determinants of cell division at the systems and at the molecular level by following cell lineages over multiple generations using time-lapse fluorescence microscopy and different reporters for cell-cycle events and cell division.

References:

1. *Gan L, Chen S, Jensen GJ (2008) PNAS 105:18953-18957*
2. *Typas A, et al. (2011) Nat Rev Microbiol 10(2):123–136.*
3. *Den Blaauwen T, et al. (2003) Mol Microbiol 47(2):539–547.*
4. *Vollmer, W., et al. (2008) FEMS Microbiology Reviews, 32: 259–286.*
5. *Amir A, vanTeeffelen S (2014) Sys Synt Biol 14:9143:9152.*
6. *van Teeffelen S, et al. (2011) PNAS 108:15822*
7. *Ursell TS, et al. (2014) PNAS 111(11):E1025–E1034*
8. *Harris, LK and Theriot JA (2016) Cell 165:1479-92*
9. *Wallden M, et al. (2016) Cell 166:729-39*
10. *Ho PY and Amir A (2015) Front Microbiol 6:662*

Expected profile of the candidate (optional):

As a highly interdisciplinary research team, we are searching for physicists, chemists, and engineers with a strong background in quantitative methods and with a strong interest in biology. Experience in one or several of the following is a plus: microscopy and optics, microfluidics, biophysical modeling, molecular microbiology including genetics and biochemistry. The candidate should bring experience in computer programming.

Contact:

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