Title of the PhD project:

Mechanophysiology of Bacterial Microcolonies

PhD Supervisor

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Title of the team : MécanoMicroBiologie
Team leader (*if different*) *:* -
Doctoral School : ED PIF (demande de rattachement en cours)

Overview of the scientific projects of the team
L'équipe MécanoMicroBiologie s'intéresse au rôle des forces mécaniques en microbiologie, et plus particulièrement chez les bactéries. Pour se faire, nous combinons biologie moléculaire, génétique, microscopie et biophysique expérimentale. Nous sommes particulièrement intéressés par le développement des biofilms, leur impact sur la résistance aux antibiotiques et la compréhension de la physiologie bactérienne en général, ainsi que par la mécanique des pili de type IV, un outil bactérien ubiquitaire.

Main publications since January 1er, 2016

Pönisch W., Eckenrode K.B., Alzurqa K., Nasrollahi H., Weber C., Zaburdaev V., and **N. Biais**. (2018). Pili mediated intercellular forces shape heterogeneous bacterial microcolonies prior to multicellular differentiation. *Scientific Reports*. 8(1): 16567

Ellison C.K., Dalia T.N., Vidal Ceballos A., Wang J.C., **Biais N.,** Brun Y.V., and A.B. Dalia. (2018**)**. Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural transformation in *Vibrio cholerae*. *Nature Microbiology.* 3(7): 773-780.

Ellison C.K., Kan J., Dillard R.S., Kysela D.T., Ducret A., Berne C., Hampton C.M., Ke Z., Wright E.R., **Biais N.**, Dalia A.B., and Y.V. Brun. (2017). Obstruction of pilus retraction stimulates bacterial surface sensing. *Science.* 358(6362): 535-538.

Ng D., Harn T., Altindal S., Marles J.M., Lala R., Spielman I., Gao Y., Hauke C.A., Kovacikova G., Verjee Z., Taylor R.K., **Biais N.**, and L. Craig. (2016). The *Vibrio cholerae* minor pilin TcpB initiates assembly and retraction of toxin-coregulated pilus. *PLoS Pathogens.* 12(12): e1006109.

Higashi D.L., **Biais N.,** Donahue D.L., Mayfield J.A., Tessier C., Rodriguez K., Ashfeld B.L., Luchetti J., Ploplis V.A., Castellino F.J., and S.W. Lee. (2016). Activation of band 3 mediates group A *Streptococcus* streptolysin S-based beta-hemolysis. *Nature Microbiology.* 1: 15004.

PhD Co-Supervisor

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Team leader *(if different) :* -
Doctoral School : ED SMAER - 391

Overview of the scientific projects of the team

Le groupe microrobotique de l'équipe « Interactions Multi-Échelles » s'intéresse à la robotique interactive aux petites échelles pour développer des méthodes d'interaction avec un monde intangible et invisible pour les humains. L'un des défis est la manipulation et la caractérisation physique de micro-objets individuels dans leur environnement naturel. Nous associons l'automatisation à l'interaction directe avec le micro-monde et des micro-objets pour développer des instruments scientifiques, en concevant des outils de précision pour l'application et la captation des interactions mécaniques.

Main publications since January 1er, 2016

1. *Gerena, Edison and Régnier, Stéphane and **Haliyo, Sinan** (2019). High-bandwidth 3D Multi-Trap Actuation Technique for 6-DoF Real-Time Control of Optical Robots.IEEE Robotics and Automation Letters, IEEE, publisher. Vol 4 No 2 Pages 647 – 654*
2. *Gerena, Edison and Legendre, Florent and Molawade, Akshay and Vitry, Youen and Régnier, Stéphane and **Haliyo, Sinan** (2019). Tele–Robotic Platform for Dexterous Optical Single-Cell Manipulation. Micromachines. Vol 10 Pages 677.*
3. *Mohand Ousaid, A. and **Haliyo, S.** and Régnier, S. and Hayward, V. (2020). High Fidelity Force Feedback Facilitates Manual Injection in Biological Samples. IEEE Robotics and Automation Letters. Vol 5 No 2 Pages 1758-1763.*
4. *Bayraktaroglu, Zeki Y. and Argin, Omer F. and **Haliyo, Sinan** (2019). A Modular Bilateral Haptic Control Framework for Teleoperation of Robots. Robotica, Cambridge University Press, publisher. Vol 37 No 2 Pages 338–357.*
5. *Ameline, Olivier and **Haliyo, Sinan** and Huang, Xing Xi and Cognet, Jean A. H. (2017). Classifications of ideal 3D elastica shapes at equilibrium. Journal of Mathematical Physics. Vol 58 No 6 Pages 062902.*

Doctoral Project

Title: Mechanophysiology of Bacterial Microcolonies

Abstract : The role of physical cues in shaping the development of multicellular eukaryotic organisms is now firmly established. Even though it is now also appreciated that bacteria mostly live within dense multicellular communities called biofilms, the understanding of the role of physical cues within these communities is still in its infancy. This doctoral project will aim at studying the role of physical forces in the early biofilm formation of species of the *Neisseria genus*. Focusing on a pair of species, one pathogen and one commensal, the PhD candidate will combine molecular biology, genetics, biophysics and microscopy to tackle the role of physical cues in the physiology of these members of the human microbiota in order to both unravel the fundamental role of mechanical cues in bacterial physiology and understand how to use these cues to control the spread of these bacteria.

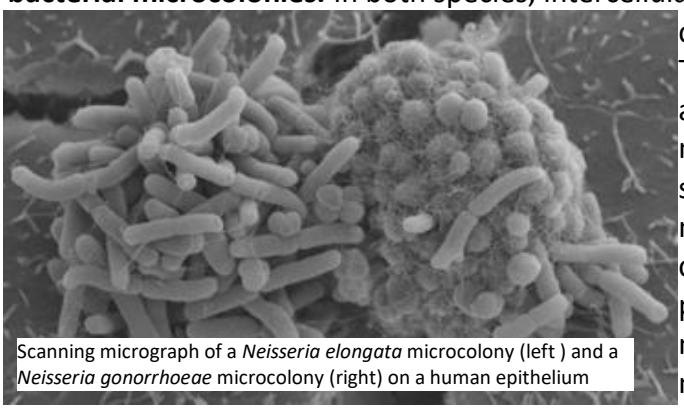
Context and objective :

Most of what is known about bacterial physiology has been figured out for bacteria living a planktonic lifestyle, free-floating in relative low density in liquids. We now understand that the majority of bacteria live a sessile lifestyle attached to surfaces where they form organized physically connected communities called biofilms. One of the main differences in these two lifestyles is the existence of physical forces between bacteria and between bacteria and surfaces in the case of the biofilms. In line with the realization that the development of multicellular eukaryotic organisms is not solely due to the chemical signals within and in between cells but might involve at least a feedback of physical signals, it is time to understand the development of the biofilm superorganism as integrating both chemical and physical signals.

Type IV pili (Tfp) are ubiquitous prokaryotic polymers that have the unusual capacity to be highly dynamical. With a diameter of 6 to 10 nm, Tfp can extend hundreds of microns away from the cellular body. When Tfp retract, they can exert mechanical forces on their surroundings whether an abiotic surface, host cells or other bacterial cells. Tfp are the only outside organelles present in most bacteria from the genus *Neisseria*, hence are the main way to mediate physical interactions between cells. In the case of WT cells, the stochastic cycles of elongation and retractions of Tfp are what enables and structures the early biofilm formation. Within hours, WT bacteria form little balls of cells comprised of a few tens to a few thousands bacteria, called microcolonies. The bacterial microcolonies are, in respect to biofilms, not dissimilar to the embryo in respect to a developed metazoan.

In the case of the causative agent gonorrhea, *Neisseria gonorrhoeae*, we have demonstrated that the dynamics of Tfp and the forces they can generate can induce a heterogeneous motility within microcolonies that leads to spatially differentiated gene expression. We have also demonstrated that dynamics of Tfp and the forces Tfp can generate affect globally the physiology of *Neisseria gonorrhoeae* cells whether in their survival in stationary phase or their sensitivity to antibiotics. The close cousin commensal bacterium, *Neisseria elongata*, shows a similar dependence on Tfp for the early formation of biofilm and its general physiology.

Mechanical interactions generated by Tfp appears to be the main driver in the formation of microcolonies of these two evolutionarily closely related bacterial species, as well as in their physiologies. **The main objective of this PhD project is to investigate the relationship between the mechanical parameters and the biological functions within bacterial microcolonies.** In both species, intercellular forces can be modified by either changing the adhesion forces between Tfp or their ability to retract. The adhesion forces between pili can be modified most efficiently by changing the sequence or post-translational modifications on the monomer constituting the pilus, the major pilin pilE. The retraction forces can be modified most efficiently by removing or mutating the molecular motor pilT. Cells deprived of Tfp stay planktonic, do not interact with each other and don't form microcolonies. We have already characterized biophysically quite a few of these mutants and the **first axis of this PhD project** will be dedicated to fully characterize them in terms of modification of the spatio-temporal expression of genes and the impact of these mutants on the bacteria physiology. For each mutant, we will be able to correlate the biophysical intercellular forces with the pattern of gene expression across microcolonies as exhibited by fluorescent gene reporters and the impact on physiology as exhibited by survival during stationary phase.



Scanning micrograph of a *Neisseria elongata* microcolony (left) and a *Neisseria gonorrhoeae* microcolony (right) on a human epithelium

In the case of *Neisseria gonorrhoae,* knocking out the molecular motor pilT abrogates Tfp retraction. The pili are still extended but they don't retract. The ΔpilT mutant cells still adhere to each other and can form microcolonies but they don't exert retraction forces on each other. ΔpilT cells survive less in stationary phase. It is thus possible to find conditions where WT cells are still thriving while ΔpilT cells are all dead. This is the perfect situation to utilize the power of genetics. We can mutagenize ΔpilT cells and look for compensatory mutations that would enable the cells to survive. As the molecular motor pilT is responsible for retraction, mutations should either restore retraction or point out to the mechanisms linked to mechanosensation in these cells. A saturated screen for compensatory mutations and their characterization will be the **second axis of this PhD project**.

In order to fully understand the role of mechanical stimuli on the *Neisseria* microcolonies, it doesn't suffice to genetically modify the internal forces that are at play in their self-assembly. It will be important to apply external mechanical stimuli and follow their impact on both gene expression and cell physiology. To this end, we will use a microrobotic system to apply very localized forces on single microcolonies. This device is based on a robotic evolution of optical trapping techniques, which offers contactless nanonewton range 3D force generation and sensing with micrometer resolution. We will also design and implement an robotized cell stretcher device compatible with microscopy and long term incubator growth to apply controlled cyclical strain to a surface of 6 well plate well covered with microcolonies. This will be the **third axis of this PhD project**.

The three axes of this PhD project (characterization of Tfp mutants, genetic screen and application of external forces) are largely independent and can be started in parallel. These three axes will all converge in a complementary fashion towards the main objective of the project. The relative advances along the different axes will be monitored in close partnership with the Thesis Advisory Committee, and will guide, in an organic manner, the

advancement of the doctoral student and the emphasis to be given to them as the thesis progresses.

Justification of suitability for *i-Bio*: This project is truly interdisciplinary and even transdisciplinary bringing together physics, biology and engineering. Besides this project is touching on at least two of the major fields of exploration of i-Bio. The very nature of the formation of microcolonies and the comparison of two closely related species aligns itself perfectly with the i-Bio interest on Evolution, biodiversity and super-organisms. The dynamics of Tfp and the robustness of the self assembly of microcolonies is an example of the interaction between stochasticity and robustness in biological processes.

Role of each supervisor / skills provided: Nicolas Biais is a leading expert on the characterization of Tfp biophysical properties. He has also studied Neisseria species for over 15 years and designed genetic tools to easily genetically modify these organisms. He will be the leading supervisor on the first two axes. Sinan Haliyo is a leading expert on microrobotics and micro-manipulation of biological samples. He was at the helm of the conception and realization of many scientific instruments around force-sensing and control at microscales. He will be the leading supervisor on the third axis. Constant communication between the two coordinators will be facilitated by the physical closeness of the two labs. The student will spend times in both labs.

Profile of the desired student: The ideal student will have a background in biology, physics or engineering with previous experience in microbiology, microscopy and experimental biophysics. But more importantly we are interested in students that are ready to embrace the challenge of this truly interdisciplinary project.