Doctoral Project

Title: Studying extracellular matrix assembly in a multi-species model biofilm using micro-Raman spectroscopy

Abstract

Our project aims at studying the assembly of the polymer extracellular matrix of a multispecies bacterial biofilm. This is a central organ of these communities, widespread in human and natural organizations. Our objective is to perform an in situ chemical and functional spatiotemporal imaging of the matrix. To this purpose, we will combine new advances in compressive Raman microspectroscopy which will provide a high speed, label-free, molecular signature of the material — and a microrheology mapping of the matrix which will provide the functional imaging. The experiments will be performed in a recently developed laboratory model of multi-species biofilm growing in a millifluidic device. This project is expected to bring about significant instrumental advances in Raman imaging and provide a unique vision of the mechanisms underpinning the assembly of the biological living material formed by bacteria.

Context and objective

Due to their small size - at most a few microns - and their high speed of division - on the order of the hour - bacteria have long offered an exquisite model to analyze key questions of fundamental biology. Microbiologists have now became aware that the main way of life of bacteria in nature is that of biofilm, this living architecture that bacteria form when they adhere to the surfaces going on dividing and embedding themselves in a self-secreted extracellular polymer matrix. In these organizations where the physical and physicochemical environment is completely reshaped compared to the planktonic situation, bacteria grow in an extremely dense mode where inter-cellular communications are intensified. They thus form complex communities where they acquire specific functions which provide them with greater tolerance to various aggressions and greater persistence in hostile environments (Watnick et al., J Bacteriol 182, 2675-2679, 2000). The extracellular matrix plays a key role in these systems both from a structural and functional point of view (Flemming et al., Nat Rev Microbiol. 14, 563-75 2016).

These systems were first studied based on single-species models but it now increasingly appears that inter-species interactions have a crucial impact on the functioning of all natural communities. As a result, and despite the complexity brought about by the introduction of several species, a trend has started recently to set up multi-species models and start to decipher the main rules underlying their formation and survival. Pioneer researches have thus made the case that these simplified systems could exhibit complex behaviors observed in higher ecosystems involving social interactions such as cooperation, competition or mutualism (I. Parijs et al., ISME J 12, 2061-2075, 2018). In the context of the Anthropocene and the growing threats to the planetary environment, we seek to highlight, on these simplified systems — holding time and size scales allowing both test parallelization and advantageous temporal contraction - the main laws linking the physical and physico-chemical parameters of the system to the behavior of the populations and the evolution of their interactions (Oliveira et al., PLoS Biol 13, e100219, 2015).

At the Jean Perrin Laboratory (LJP), we have been studying for several years, in the group of Biophysics of microorganisms (MOB), the mechanisms of development of mono-species bacterial biofilms. We have been able to highlight the importance of the interactions
and physical properties of these systems on their functioning (refs 1-4). Recently, we set up in the laboratory a 4-species biofilm model (4S) developing under hydrodynamic flow in a millimetric millifluidic channel. Having parallelized and automated this device, we have been able to show that the 4 species (Bacillus thuringiensis, Pseudomonas aeruginosa, Kocuria varians, Rhodocyclus sp.) from a natural biofilm, coexisted to deterministically form a community at equilibrium after 36 hours. We have been able to establish the kinetic properties of development and began to understand the main driving forces (Amaury Monmeyran phd defended on November 4, 2019 and publication in preparation). To do so, we have developed real-time, in situ monitoring of community development in optical and fluorescence microscopy. We have defined several quantitative descriptors such as the growth kinetics of the different tagged species in the adherent community, the heterogeneity of the spatio-temporal distribution, the local dynamics based on reporters expressed by the cells.

In the recent years, Hilton Barbosa de Aguiar has developed a new technique, coined Compressive Raman imaging for high-speed chemical quantification. Indeed, traditional Raman microspectroscopy is intrinsically a slow imaging technique, therefore generally poorly applicable to dynamic living systems. The basic idea in compressive Raman is to perform the chemical analysis during the measurement by combining a non-conventional spectral sampling with efficient reconstruction algorithms. Such a combination of smart sampling with mathematical modelling allows for more sensitive detection (refs 5,6) and the fastest Raman bio-imaging to date (ref. 7). The enhancement in sensitivity and speed brought about by the compressive Raman imaging framework will enable to quantify various chemical species composing bacterial biofilms (protein, nucleic acids, polysaccharides).

Objective: We want now to understand how the multispecies community assembles its extracellular matrix. How the different species cooperate or not to form the tissue of their community is a crucial question to elucidate for a better understanding of the adherent bacterial systems. This is a completely new question requiring to build new tools enabling to identify the individual signature of each species and their respective contribution to the production of the polymer material constituting the matrix. We will have to do it in a non-destructive mode if we are to understand assembly and we propose here to implement compressive Raman microspectroscopy to address this question. Thanks to the high-speed character of the technique, we will achieve separating the extracellular matrix information from the bacterial cells themselves. The analysis of reduced biofilm combinations (1,2 or 3 species), as we have already done in light microscopy, will help to decipher the complete assembly.

Challenges: Challenge number 1 is to achieve the first in situ chemical microanalysis in a growing living community. Challenge number 2 will to take advantage of this data set to reveal the assembly mechanisms of the extracellular matrix in a multi-species bacterial biofilm.

Justification of suitability for i-Bio:

Our strategy in this work is to couple instrumental physics latest innovations - the compressive Raman microspectroscopy- and the control of a model living system, complex enough to provide a relevant model of ecological community - the multispecies bacterial biofilm. The project will create a collaboration between two laboratories SU, LKB and LJP, and is fully aligned with the overarching goal of developing novel optical techniques to address biological questions.
Role of each supervisor / skills provided:

Nelly Henry is a senior researchers having her background in physical chemistry and used to collaborate with scientists from different fields both experimentalists and theoreticians. She will supervise all the steps of the formation and real-time monitoring of the multispecies biofilm in video-microscopy as well as the physical properties characterization.

Hilton B. de Aguiar recently hired as tenured CNRS Associate Junior Researcher at the LKB, has joined the group “Complex Media Optics Lab” to expand its activities in nonlinear microspectroscopy. HBdA is an expert of Computational Microscop, compressive Raman in particular. He will supervise all the micro-Raman developments, data acquisitions and analyses.

The experimental time will be shared between LJP on Campus Jussieu and LKB on Campus ENS-Ulm.

Profile of the desired student:

We are looking for a candidate whose main background is in physics or physico-chemistry with a strong interest in biology - a master 2 student at the interface of these disciplines would be highly appreciated. Yet a strong motivation for biological systems will also be favorably considered for a candidate with no background in biology.

Main references of co-supervisors in relation to the subject:

3. P. Thomen et al., Bacterial biofilm under flow: First a physical struggle to stay, then a matter of breathing. PLoS ONE 12, e0175197 (2017)