Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces 2020

Intitulé du Projet de Recherche Doctoral : Studying extracellular matrix assembly in a multi-species model biofilm using micro-Raman spectroscopy (Bio-Raman)

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

NOM : **HENRY** Prénom : **Nelly**
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e-mail : nelly.henry@upmc.fr
Adresse professionnelle : Campus Jussieu - T 32_33 - P524
(site, adresse, bât., bureau)

Unité de Recherche :
Intitulé : Laboratoire Jean perrin
Code *(ex. UMR xxxx)* : UMR 8237

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED388-ChimiePhysiqueChimieAnalytiqu**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :

-1 doctorant; 2018; 75%

Co-encadrant :

NOM : **BARBOSA DE AGUIAR** Prénom : **Hilton**
Titre : Choisissez un élément : ou Junior Research Chair HDR ☐
e-mail : h.aguiar@phys.ens.fr

Unité de Recherche :
Intitulé : Laboratoire Kastkler-Brossel (LKB)
Code *(ex. UMR xxxx)* : UMR 8552

Ecole Doctorale de rattachement : **Choisissez un élément :** Ou si ED non Alliance SU : **EDPIF**

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences scientifiques apportées. Indiquer les publications/productions des encadrants en lien avec le

projet.
Préciser le profil d'étudiant(e) recherché.

Abstract: We propose to study the assembly of the extracellular matrix of a multispecies bacterial biofilm by Raman micro-spectroscopy. This project relies on researches carried out with Nelly Henry at LJP where a robust experimental model of multi-species biofilm has just been established and characterized and on recent technological innovations introduced in compressive Raman at LKB by Hilton Barbosa de Aguiar. This interdisciplinary collaboration will enable to (i) carry out the first in situ spectroscopic micro-analysis of a living adherent community (ii) to understand the rules of assembly of a controlled model of ecological community - the multispecies bacterial biofilm.

Context: 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 exquiste 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).

We have been studying at the Jean Perrin Laboratory (LJP) 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. We want now to understand how this community assembles its extracellular matrix. This is a completely new question in a multi-species context.

To answer this question, we need to build new tools enabling to identify the individual signature of each species and their respective contributionto 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.

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.

PhD Objective : We aim at performing a Raman microspectroscopy analysis of the extracellular matrix assembling in a multi-species bacterial biofilm.

Strategy: We propose here a PhD co-supervised by Nelly Henry from Laboratoire Jean Perrin (LJP) who will be in charge of multi-species bacterial biofilm elaboration and control and by Hilton Barbosa de Aguiar from Laboratoire Kastler-Brossel (LKB) for the implementation of Raman micro-spectroscopy on these living systems.

The Raman effect provides high chemical selectivity with excellent optical resolution in imaging without labeling. The inherent fingerprint of molecules'vibrational spectrum is used therefore allowing disentangling the proportions of the various chemical species. It is a visible light spectroscopy, therefore providing a resolution of the order of hundreds of nanometers which should allow precise mapping of the extracellular matrix of the bacterial biofilm.

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 intriscally 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 enhacement 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). Thanks to the high-speed character of the technique, we will achieve separating the extracellular matrix information from the bacterial cells themselves. We will also implement isotopic labelings by using appropriate pre-growth media (e.g. containing deuterated glucose) targeting for a given species of the consortium. Thereby, the contribution of the targeted species will appear very distinctly. 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. The experimental time will be shared between LJP on Campus Jussieu and LKB on Campus ENS-Ulm.

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.

Initiative relevance: 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.

Required profile : 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:

1. P. Thomen, J. D. P. Valentin, A. F. Bitbol, N. Henry, Spatiotemporal pattern formation in E.coli biofilms explained by a simple physical energy balance. Soft Matter 16, 494-504 (2020)

2. Monmeyran et al., The inducible chemical-genetic fluorescent marker FAST outperforms classical fluorescent proteins in the quantitative reporting of bacterial biofilm dynamics. Sci Rep 8, 10336 (2018)

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)

4. O. Galy et al., Mapping of bacterial biofilm local mechanics by magnetic microparticle actuation. Biophysical journal 5, 1400-1408 (2012).

5. S. H. Donaldson Jr., H. B. de Aguiar. Molecular Imaging of Cholesterol and Lipid Distributions in Model Membranes. J. Phys. Chem. Lett. 9 1528 (2018)

6. B. Sturm, F. Soldevila, E. Tajahuerce, S. Gigan, H. Rigneault, H. B. de Aguiar. High-sensitivity high-speed compressive spectrometer for Raman imaging. ACS Photonics 6, 1409–1415 (2019)

7. F. Soldevila, J. Dong, E. Tajahuerce, S. Gigan, H. B. de Aguiar. Fast compressive Raman bio-imaging via matrix completion. Optica 6, 341–346 (2019)

Merci de nommer votre fichier pdf :
«ACRONYME de l'institut/initiative_2_NOM Porteur Projet_2020 »

à envoyer simultanément par e-mail à l'ED de rattachement et au programme :
cd_instituts_et_initiatives@listes.upmc.fr avant le 30 mars.