



Mission pour les
Initiatives
Transverses et
Interdisciplinaires

80 | PRIME Appel à projets 2020

Formulaire de candidature

**Ce formulaire doit être libellé « 80PRIME2020_Formulaire_Nomcandidat »
et obligatoirement être déposé par le porteur ou la porteuse du projet
à la fin du questionnaire en ligne en format pdf.**

IDENTIFICATION

Civilité/NOM/Prénom du porteur ou de la porteuse du projet	Eric Clément		
Section du comité national de la recherche scientifique			
Etablissement de rattachement (CNRS, Université de Nantes, CEA, etc.)	ESPCI		
Code Unité (UMR, UPR, EA, etc.)	UMR7636		
Nom du laboratoire et/ou de l'équipe	PMMH		
Rattachement de l'UMR	Institut principal (INSB, INSU, ...)	INSIS	
	Délégation régionale	Paris B	

Projet

Titre long du projet (150 caractères maximum)	BACTERIA IN DROPLET SELF-ASSEMBLIES: FROM HYBRID MATERIALS TO NEW ASSAY STRATEGIES FOR MICROBIAL ECOLOGY
Acronyme du projet	BACTEMUL

Identification des équipes travaillant sur le projet

Code Unité (UMR, UPR, etc.)	Nom du laboratoire	Rattachement*		Civilité/NOM/Prénom des personnes impliquées
		Institut principal (INSB, INSU, ...)	Délégation régionale (DR07, DR12, ...)	
UMR 7636	PMMH	INSIS	ParisB	M. Eric CLEMENT Mme Anke LINDNER
UMR 7083	Gulliver	INC	ParisB	Mme Teresa LOPEZ-LEON
New York – University, USA	Center for Soft Matter Research, Department of Physics			Ms Jasna Brujic

* Pour être éligible le projet doit impliquer *a minima* deux laboratoires issus de différents instituts.

Curriculum vitae

Eric Clement is a member of the UMR 7636 PMMH at the ESPCI and professor of Physics at the UPMC in Paris. He graduated in 1985 from the ESPCI and received in 1989 a PhD from the University of Michigan. He did a Post-doctoral research at the UPMC in 1990 before joining the CNRS as a Research Scientist. He received a Professor position from the UPMC in 2000 and moved to the ESPCI in 2004 with his team. He is working on projects at the interface of mechanics and physics such as granular materials, suspensions hydrodynamics and the statistical physics of bacterial fluids. His

group is specialized in hydrodynamics, including the rheology of complex fluids, studying fundamental properties displayed by fluids laden with bacteria such as transport in a flow, hydrodynamic dispersion or rheology. His team developed various tools to create controllable environments for bacteria and innovative visualization tools to monitor under the microscope, the swimming and spatial exploration of motile bacteria. He recently worked on Magnetotactic bacteria confined in droplets.

See long curriculum in <https://blog.espci.fr/eclement/2018/10/06/curriculum/> and publication list <https://blog.espci.fr/eclement/2018/10/06/recent-publications/>

Teresa Lopez-Leon is a member of the UMR 7083 Gulliver Laboratory at the ESPCI and a Research Scientist at the CNRS. She got her B Sc, M Sc and PhD degrees in Physics from the University of Granada (Spain). She was a postdoctoral researcher at Harvard University (US), Georgia Institute of Technology (US) and University of Montpellier II (France). Her research has focused on the physics of soft materials, including colloidal suspensions, gels, liquid crystals and emulsion droplets. Her current research is on the effect of confinement and curvature on the properties of liquid crystals with the perspective to generate, by self-assembly, novel materials applications such as metamaterials or photonic crystals. She received in 2019 the Bronze medal of the CNRS.

See long curriculum in: <https://www.ec2m.espci.fr/accueil/membres/teresa-lopez-leon.html>

Jasna Brujic is associate professor with tenure in the department of physics at NYU. She received a PhD in Physics from the University of Cambridge (UK) in 2004. She did her post-doc in the Columbia University Biology Department and joined NYU in 2007. Her current research is on Soft matter, jammed matter and biophysics, in particular on protein folding and biomimetic systems. See more extended CV and publications list from <http://www.physics.nyu.edu/~jb2929>

PROJET DE RECHERCHE

1 - Résumé (10 lignes maximum)

Le but du projet est de comprendre comment les bactéries motiles, soit individuellement soit collectivement, explorent, s'auto-organisent et répondent à des stimuli, dans des environnements complexes mais contrôlables tels que des gouttelettes ou à plus grande échelle dans des émulsions possédant des géométries ou des topologies complexes. Nous cherchons à produire par des techniques de micro-fluidique, des états de la matière originaux en combinant les principes d'auto-assemblage de la matière molle avec l'activité inhérente des fluides bactériens. Un des objectifs est aussi de créer des environnements versatiles pour tester des idées fondamentales sur la sélection par la pression environnementale en mettant en œuvre les possibilités offertes par des structures physicochimiques auto-organisées afin d'effectuer des essais micro-biologiques d'un nouveau type.

2 – Mots-clés

collective motion, micro-swimmers, self-organization, geometrical confinement, emulsions, bacteria, active matter, microfluidics

3 - Exposé scientifique du projet explicitant les points suivants (4 pages maximum) :

GENERAL CONTEXT

Motivation: Active matter describes systems in which their elementary constituents consume energy to produce motion. The interaction between these elementary active objects usually leads to fascinating collective behaviors, which are widely observed in nature, from bird flocks to cellular cytoskeletons. How the behavior of the elementary active components determines the macroscopic behavior of the system is still unraveled [Marchetti 2013]. Microswimmers, such as bacteria, are model systems that allow us to address some of these fundamental questions.

In natural environment, bacteria are either dispersed in fluids (planktonic state) or assembled on surfaces as biofilms. These are archetypes of complex societies where internal biochemical cascades govern the cell behavior inducing individual or collective motion. Bacterial motility is involved in survival strategies, optimization of environmental resources and symbiotic interactions with other living species. *Understanding these mechanisms, specifically how environment impacts bacterial motion is of critical interest, non-only in fundamental biology or ecology but also in bio-engineering or medicine.*

Objective: The aim of this proposal is to address the question of how bacteria explore, self-organize and respond to stimuli in complex but controllable environments such as droplets, micro-sized spherical films (shells), or large emulsions with complex geometries/topologies. We seek to develop original states of matter combining the self-assembly principles of soft-matter with the inherent activity of bacterial fluids using microfluidic techniques.

Scientific and social impact: Emulsions offer the possibility of bringing bacteria and other microorganisms to highly constraint conditions, where confinement and curvature are expected to play a crucial role in bacterial behavior. Conversely, bacteria activity, i.e. the micro-organism motion either individual or collective, is expected to influence the space in which they are confined. For more than a decade, droplet bioreactors have been used to monitor bio-activity and to create high throughput assay conditions to test bioenergetics budget or responses to drugs [Boitard2011]. However, the impact of activity on the stability of the different mesoscopic emulsion phases is not well-established. It was recently suggested on theoretical grounds, that the presence of activity in a suspension could be key to design new composite and hybrid materials with a strikingly rich range of morphologies [Bonelli2019]. *In this project, we propose to harness jointly the collective organization capabilities of motile micro-organisms and soft-matter in the perspective of producing original states of matter and novel materials. This project also opens exiting perspectives in terms of microbial ecology, since it will enable to test important ideas on environmental pressure selection using the possibilities offered by such controllable structures to perform new kind of assays. In microbiology, this question is both a fundamental challenge and also major health threat.*

SCIENTIFIC AND TECHNICAL CHALLENGES: BUILDING A ROAD-MAP

Interdisciplinary: This project is inherently interdisciplinary, since it requires combining expertise in: i) biochemistry and biophysics to produce bacterial suspensions with controlled properties (motility, division rate, etc), ii) hydrodynamics and statistical physics to study their collective motion, iii) microfluidics and surface chemistry to produce complex confining environments, iv) topology and geometry to analyze the patterns emerging in these constrained environments.

Complementarity: This expertise is gathered by two groups at ESPCI The first one is the active matter group at **PMMH laboratory** (Eric Clément, Anke Lindner), which has developed an original line of research at the interface between statistical physics, hydrodynamics and biology. In particular, the group is internationally recognized for its works on bacterial fluids and collective motion. The second one is the group of Teresa Lopez Leon at **Gulliver laboratory**, whose expertise lies at the interface between soft matter, topology and microfluidics. In particular, the group is internationally recognized by its works on the self-organization of liquid crystals in confined environments.

The line of research developed in the last decade by the PMMH group aimed at revisiting some fundamental concepts underlying the physics of colloidal suspensions using bacterial suspensions as a model system. Three research axes were developed: (i) the active Brownian motion of colloids in an active bath [Mino2011], (ii) the transport of bacteria in confined environments [Figueroa2015, Creppy2019, Mathijssen2019, Junot2019 Figueroa2020a, Figueroa2020b] and (iii) the emergence of constitutive properties in active suspensions [Gachelin2013, Lopez2015, Martinez2019]. They developed two bacterial models. The first consists in motile E. coli suspended in a minimally energetic environment, which halts the growth of the population and allows

controlled hydrodynamic studies. They also use genetically modified strains to vary motility. The second model consists in a motile magnetotactic strain reactive to both the magnetic field and the oxygen gradients. *The research carried out by the Gulliver group in the last years aimed at investigating the effect of confinement on the self-organization of soft materials. Their achievements can be grouped in four categories.* (i) By using cutting-edge microfluidic techniques, they have been able to encapsulate soft materials, in particular liquid crystals, into complex micro-environments [Lopez-Leon2011a, He2019, Kim2019]. (ii) They have shown that geometrical frustration induced by confinement and curvature can lead to fascinating ways of self-organizing [Lopez-Leon2011b, Darmon2016, Tran2017] and developed a toolbox to control the way in which the system self-organizes [Durey 2019]. (iii) They have exploited these ideas for producing new materials with interesting applications, such as reconfigurable chiral micro-objects or micro-lasers [Lopez-leon2011a, Amine2013, Ansell2019, Kim2019]. Very recently, they have extended their studies to active materials, where the self-assembly units are not passive molecules, but active (motile) nano-objects [Hardoüin2019].

Research plan: To carry out this challenging research project, where we pave the way for a novel generation of hybrid materials and new assay strategies for microbial ecology, we have developed the research plan described below, where we highlight 4 crucial questions to be addressed.

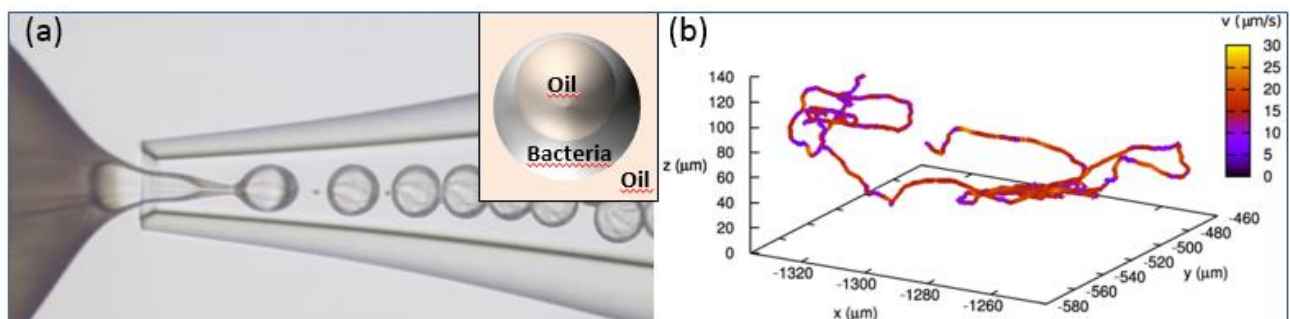


Figure 1. (a) Microfluidic device in the Gulliver laboratory, producing a double emulsion (shell) and encapsulating a suspension of *E. coli*. The outer droplet size is 100 microns. (b) 3D tracking of a wild-type *E. coli* using the PMMH Lagrangian tracking device and showing the sequence of run/tumble processes. Color chart is the swimming velocity.

I – Combined effects of curvature and confinement on the collective organization of bacteria

In the first part of this project, we will explore how confinement and curvature impact the collective motion of bacteria by confining them to micro-droplets or shells (spherical films suspended in water). When a dense population of magnetotactic bacteria (bacteria able to move along the lines of a magnetic field) is confined to a droplet and submitted to a constant magnetic field, it self-assembles into a reversible rotary motor [Vincenti2019]. We have shown that this intriguing behavior stems from a subtle interplay between confinement along a curved interface and swimming activity. We have also shown that the emergence of a “super-fluid” phase (no apparent macroscopic viscosity [Lopez2015]) in a suspension of dense active micro-swimmers is linked to the onset of collective motion [Martinez2019]. Additionally, when a bacterial suspension is confined between two horizontal solid planes, we have seen that the spatial extension of the emerging collective vortices scales linearly with the confinement height (unpublished results). All these properties are a direct consequence of the large scale hydrodynamic interactions existing between swimming bacteria, inducing inherent self-organizing properties. But as in any low Reynolds hydrodynamic problem, the existence of complex boundary conditions such as liquid/liquid interfaces of different geometrical shapes is expected to significantly influence the macroscopic outcome and the organization properties of the micro-swimmers. Such an interplay between confinement and collective organization in complex geometries and topologies is an important fundamental question to be addressed, which requires a thorough experimental and theoretical investigation.

In this study, we will introduce the curvature of the confining space as a control parameter to study and tune the collective organization of bacterial suspensions. The confining geometry will be systematically changed, not only by producing droplets of different curvature radius, but also by producing spherical shells, where the bacteria will be confined between two nested spheres. This original geometry allows us to independently control confinement and curvature, by tuning the thickness and outer radius of the shell,

respectively. To produce droplets and shells with controlled geometries, we will use glass-capillary microfluidics, a technique set by Teresa Lopez-Leon at Gulliver lab, in particular to follow the topological organization of various liquid crystalline phases [Lopez-Leon2011a]. We will assess bacterial motion using confocal microscopy. For increasing concentrations of bacteria, the onset of collective motion as well as its spatial extension will be determined and compared to different theoretical approaches, such as the large-scale lattice Boltzmann simulations performed in the group of A. Morozov in Edinburgh [Stenhammar2017] with whom we currently collaborate.

II – Active confined thermal bath and active mixing

In a bath of active particles, either dilute or concentrated, a passive object, being a molecular species, a nanoscale protein or a particle of microscopic size, will undergo agitation that resembles Brownian motion. This generic situation is currently called an “active thermal bath”. However, the diffusive properties, the collective mixing properties, and the possibility to self-organize and be driven by activity, bestow on the passive objects very novel emergent properties, far beyond the classical behavior resulting from Brownian motion. For example, the standard fluctuation-dissipation theorem does not hold anymore [Burkholdera2019]. It has also been demonstrated the possibility to extract work from fluctuations at a fixed thermal temperature or to concentrate particles starting from an initial random distribution, properties that are essentially different from the standard outcome of equilibrium or close to equilibrium thermodynamics [Fodor2016].

A population of swimming bacteria trapped inside a drop or a shell will constitute an active thermal bath. The agitation properties due the swimming hydrodynamics will determine, for example, the mixing of molecular species, or could accelerate the rate of chemical reactions. Note also that, in some instances, the catalytic properties of the bacteria themselves are part of the chemical kinetics. This is an important mechanism, for instance, in clouds, where the biological activity within the droplets is thought to be a dominant factor for the cloud stability and the rain onset [Delort2010]. This type of active thermal bath can also favor the out-of-equilibrium organization of macroscopic objects trapped inside the droplet. Our objective is to create and study model active thermal baths and contrast the outcome of our measurements with several propositions recently developed to describe active thermal baths in the framework of out-of-equilibrium stochastic thermodynamics (see [Pietzonka2019] and refs inside).

First, we will look at the fluctuating motion of an inner droplet of a shell containing bacteria when systematically changing the outer and inner radii of the shell (see figure 1). Then, we will characterize the motion of solid objects with different shapes trapped inside bulk droplets. We expect to observe interesting self-organization phenomena when several of these objects are encapsulated inside the droplet. For this study, we will use the confocal microscope of the Gulliver lab mounted on a platform that can be tilted with respect to gravity. Also we will use the 3D Lagrangian tracking set-up built at the PMMH [Darnige2017]. Note that solid objects of different complex shapes and micron-size scale can be obtained by the 3D nano-printer of the ESPCI hosted in the PMMH laboratory.

III – Coalescence and necking dynamics of active droplets

The coalescence dynamics of two droplets, or conversely the necking problem leading to droplet separation, are two important problems present in a wide variety of industrial and natural processes. The coalescence dynamics controls natural coarsening mechanisms of all sorts of emulsions. The onset of coalescence is controlled by surface fluctuations driven by thermal agitation before the hydrodynamic regime takes place, leading to the formation of a new droplet (see [Perumanath2019] and refs inside). If these droplets are filled with particles which have some partial affinity for the surrounding medium, one can observe coalescence-arrested Pickering emulsions. However, nothing is known about coalescence between droplets that contain active particles. On the other hand, the detachment dynamics of a viscous droplet through a necking process controlled by viscosity and inertia, is a well-studied process [Eggers1997]. A decade ago, the presence of passive particles in the droplet was investigated at the PMMH by Anke Lindner and Eric Clément, who showed that solid particles even at low concentration do strongly influence the detachment dynamics [Bonnoit2012].

Here we propose to study these dynamical processes, coalescence and necking, in droplets filled with active bacteria. We will use microfluidic techniques and micromanipulation tools to control the conditions of drop coalescence and drop detachment via necking dynamics. The processes will be filmed with a fast camera mounted on the microscope. The objective will be to assess the dynamics as well as the flows inside the drops.

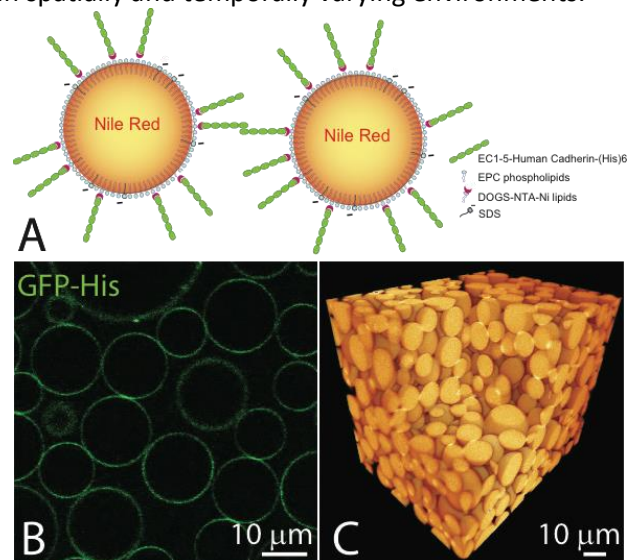
The experimental control parameters will be the droplet size, and mixtures of motile and non-motile bacteria to identify the influence of activity in the dynamical processes.

IV - *Evolution under stress and fluctuations: towards novel complex topologies and bacterial assay strategies*

The final part of the project will include the study of the stability of more complex emulsified phases in the presence of a bacterial active fluid. The ultimate aim would be to establish a new droplet-based system that enables simultaneous spatio-temporal evolution of many bacterial populations. The system set-up will be done in collaboration with the Prof. Jasna Brujic in NYU, whose group recently designed methods to control many dynamic parameters in emulsions [Zhang2017] and obtains a large variety of self-organized droplet geometries [McMullen2018]. We will seek to establish a new droplet-based system that enables simultaneous evolution of many bacterial populations in spatially and temporally varying environments.

For example, the NY group can control the rate of droplet fusion, leading to a distribution of antibiotic levels to which bacteria are exposed, this will give rise to fluctuations in nutrient and antibiotic concentrations, thus mimicking the environmental pressure of some natural situations. Then, we will apply this system to study the rapid evolution of antibiotic resistance in *Escherichia coli* under common antibiotic ciprofloxacin. This part builds on recent work by the Kussell group at NYU that demonstrated the critical importance of gradients and fluctuations in evolutionary dynamics, and which were shown to greatly increase the rate at which resistant bacteria evolve. We hope that our study, which avoids complex microfluidics by using an innovative emulsion-based approach, will have a major impact on experimental evolution.

This final part of the project will combine expertise on emulsion imaging, coarsening, and analysis by the Brujic group, effects of spatial-confinement on the growth and organization of bacteria by Clément and Lopez-Leon, and evolutionary dynamics and adaptation by the NYU Kussell's team (<https://as.nyu.edu/content/nyu-as/as/faculty/edo-kussell.html>).



ROLE OF THE PHD STUDENT

This scientific project is accompanied with a request for a PhD student, who will be co-directed by E. Clément and Teresa Lopez-Leon. We will look for an experimentalist with a background in physics of fluids or a physical-chemist with knowledge in soft matter. During the thesis, the student will essentially follow the four-step roadmap described previously. We are aware that the research program sketched in the proposal may look quite ambitious for a three-year PhD, but at the same time, all the experimental tools to be used in this thesis are already set-up and running in the PMMH lab (bacteria strains, preparations protocols, epifluorescence microscopy, 3D tracking system) and in Gulliver lab (Confocal microscopy, micro-fluidic devices to produce droplets and shells with controlled geometries). The student will also visit for extended periods the group of Jasna Brujic in NYU to get familiar with the controlled emulsification techniques developed there and also to learn new biology techniques to create environmental and evolutionary pressure, in Edo Russell's lab.

BUDGET

1 – Budget détaillé et justifié par poste de dépenses et par équipe pour l'année 2020 (une page maximum). Se référer aux modalités administratives et financières précisées dans le texte de l'appel à projets. Ce budget ne doit pas inclure le contrat doctoral demandé. Le budget peut atteindre 30k€ par an. Seuls quelques projets, sur la base d'une argumentation solide, seront financés à cette hauteur.

Déplacements :

- Sojourns in New York University for the student 1-2 month/year

6000€

Organisation de manifestations : colloques, ateliers, etc. Détail : <ul style="list-style-type: none"> • • 	€
Fonctionnement Détail : Produits et consommables pour culture biologique, <ul style="list-style-type: none"> • Produits chimiques 1 kE • Consommables 3 kE 	3000€
Équipement non-amortissable (montant unitaire inférieur à 800 € HT) Détail : <ul style="list-style-type: none"> • 	€
Équipement amortissable (montant unitaire supérieur à 800 € HT) Détail : i) caméra E.CMOS pour visualisé les bactéries, à monter sur le microscope confocal, qui allie rapidité et sensibilité et (ii) système de manipulation pour organiser les gouttes à l'échelle microscopique, en particulier crucial pour les contrôle des expériences de coalescence. <ul style="list-style-type: none"> • Camera Orca Flash V4 1300 kE (Gulliver) • Micromanipulateur TransferMan 4r (PMMH) 13 kE 	26000€
Prestations de service étroitement liées à la mise en œuvre du projet Détail : <ul style="list-style-type: none"> • • 	€
TOTAL	34 000€

BIBLIOGRAPHIC ADDENDUM (PMMH group articles in red color, Gulliver articles in blue color)

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Modalités administratives et financières

- Les crédits sont alloués au porteur ou à la porteuse et versés à son unité de rattachement CNRS. Ils sont de type subvention d'Etat, ce qui implique qu'ils doivent être entièrement consommés avant le 31 décembre de chaque année du projet et qu'aucun frais de gestion ne pourra être prélevé. Le porteur ou la porteuse les engage pour l'ensemble des partenaires.
- Le projet peut impliquer des partenaires étrangers mais ces derniers ne peuvent pas être financés.

Restitution des résultats

Un bref rapport financier sera demandé aux lauréates et lauréats à la fin de l'année 2020 pour arbitrer le budget 2021. Un rapport scientifique et financier sera demandé fin 2022. Par ailleurs, les lauréats et lauréates s'engagent à déposer la production scientifique issue par ce projet dans l'archive ouverte HAL du CNRS.

Par ailleurs, il est demandé aux lauréates et lauréats de mentionner le financement obtenu (Ce projet a obtenu le soutien financier du CNRS à travers les programmes interdisciplinaires de la MITI ; This project has received financial support from the CNRS through the MITI interdisciplinary programs) dans toute production scientifique et de la déposer systématiquement dans l'archive ouverte HAL.