ECOS-*Sud*

(Argentine - Chili - Uruguay)

Programme de coopération ECOS Sud-ANID (Chili)

Fiche-projet

(doit être adressée à ECOS-Sud, avec les documents annexes au plus tard le **1er juin 2021**, sous forme électronique exclusivement).

1. Titre du projet :

Active emulsions and motors in a droplet

Mots-clés (4 maximum) : Active matter, Bacteria, Physics of fluids, Emulsions

Champ disciplinaire (cocher) : ☐ Sciences Humaines et Sociales ☐ Sciences de la Vie ☐ Sciences de la Santé ☐ Sciences de l'Univers X Sciences Exactes

2. Établissement principal :

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Nom du Directeur Damien Vandembroucq ....

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4. Liste des chercheurs confirmés (grade, HDR, structure de rattachement) et chercheurs en formation (structure de rattachement) participant au projet (distinguer si nécessaire entre chercheurs principaux, bénéficiaires des missions, et chercheurs associés ou occasionnels) : un nombre de 3 à 4 chercheurs principaux hormis les doctorant(e)s par équipe paraît raisonnable

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- Anke Lindner (HDR), Professeur Université de Paris, Paris France, PMMH ESPCI
- Zhenyang Liu , Post Doctorant , PMMH ESPCI
- Renaud Baillou , Doctorant, Sorbonne Université Paris , PMMH ESPCI
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au Chili :

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- Rodrigo Soto, Professeur (encadrant), Physics Department, Universidad de Chile
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5. Résumés non confidentiels du projet (français ET anglais). Les résumés des projets retenus seront communiqués sur le site ECOS-Sud.

In this project, we aim at characterizing and harnessing the fluctuations of bacterial suspensions to build different models of “bacterial motors” using methods of emulsion fabrication and droplet encapsulation. The problem will be decomposed in two. On one hand, we will investigate the possibility to drive the motion of droplets by encapsulating magnetotactic bacteria and applying a magnetic field. Depending on the concentration, the collective bacterial motion inside the drop will be rectified by the external magnetic stimulus and produce a rotation and translation of the drop. We will also look at global self-organization processes induced in such novel activated emulsions. On a second hand, we will look at fluctuations due to bacterial motion (motile E.coli) induced on a passive object hence realizing an “active thermal reservoir” in a drop, with a “thermal probe” being the passive object. In such a bath of active particles, the passive object, being another droplet or a micro-fabricated particle of special design, will undergo agitation, resembling Brownian motion. However, the diffusive properties, the collective mixing behaviour, and the possibility of self-organization bestow on the driven passive objects novel emergent properties, far beyond what can be expected for classical Brownian motion.

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6. Description du projet scientifique (problématique et contexte bibliographique (3 pages max.), objectifs et hypothèses (1 page max.), méthodologie et plan du travail (2 pages max.), implication de chaque équipe, valeur ajoutée de la collaboration, formation des doctorants, incluant plan de travail et stages) **en moins de 10 pages (taille de caractère : 12)**

Scientific context - Active matter is a relatively new field of physics focused on systems composed of particles that, through some kind of internal motor, are able to self-propel [1, 2]. Active matter is an example of out of equilibrium system, with energy injection and dissipation at the scale of the particles that compose the system. Although artificial active systems such as active colloids [3] exist, most examples of active matter are based on living systems such as schools of fishes [4] or cytoskeletal components [5]. Thus, active matter is an inherently multidisciplinary subject, lying at the edge of the physical and life sciences.

A paradigmatic example of an active system is a suspension of motile bacteria [6]. Although the details of the bacterial swimming are system-specific, many bacteria swim thanks to the action of flagellar systems [7, 8], as shown in figure 1(a). Flagella are rotating helicoidal appendages powered by molecular motors embedded in the cellular membrane. It is thanks to the non-reciprocal rotation of these flagella that bacteria can self-propel in the viscous medium where they live at low Reynolds number [9]. Bacterial suspensions show fascinating behaviors. At the individual level, bacteria interact with each other through hydrodynamic and steric forces, which tend to align two colliding bacteria. These bacterial interactions are at the basis of the collective behavior observed when the bacterial concentration increases beyond the dilute limit, such as formation of clusters [10, 11] and the unstable generation of random, fluctuating vortices and jets characteristic of active turbulence [12]. Moreover, bacteria can sense external signals such as chemical gradients. An internal cascade of processes causes a bias in the bacterial swimming direction toward regions of high (low) concentration of chemoattractants (chemorepellents), a behavior known as chemotaxis. Other taxes, ie. movement in response to a stimulus, exist, such as aerotaxis (in response to oxygen gradients), gravitaxis (to gravity), magnetotaxis (to magnetic fields), phototaxis (to light), or thermotaxis (to temperature gradients), to name a few.

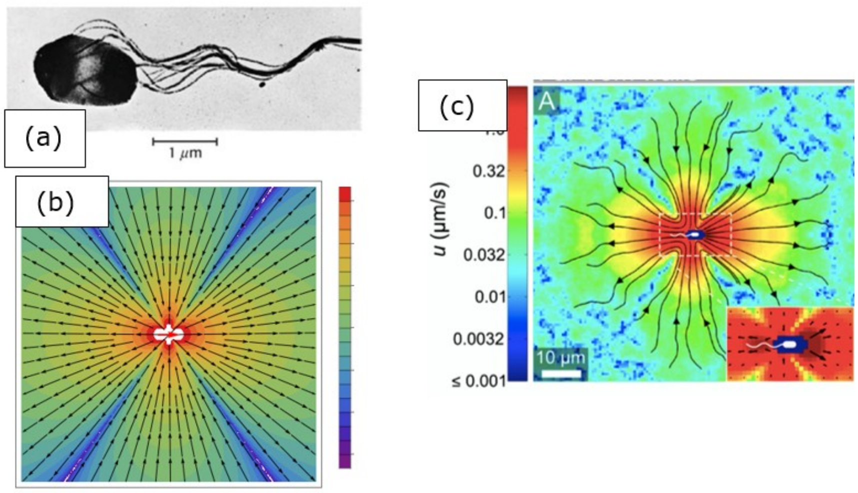

Figure 1 - (a) Transmission electron micrography of bacterium *Salmonella abortus-equi* (from [8]). (b) Theoretical far-field flow around a pusher-type of microswimmer. Lines with arrows represent the streamlines and the color scale represents the velocity magnitude, in arbitrary units. The red arrow in the center represents the microswimmer. (c) Measured flow field around a swimming E. coli far from walls (from [14]).

Harnessing the swimming power - In the far field, microswimmers such as bacteria can be modelled as a force dipole of strength f_0, with the sign of f_0 determining if the microswimmer is a pusher ($f_0>0$) or a puller ($f_0<0$). The velocity field induced by the rotation of the flagella is shown in figures 1(b) and 1(c) [13, 14]. By considering the characteristic size L and speed V_0 of a bacterium swimming in a viscous medium of viscosity η, the thrust force can be estimated as $f_0 \sim \eta L V_0$. Thus, in a suspension with volumetric concentration n of bacteria, there is a rate of energy injection per unit volume $P \sim n\, f_0\, V_0 = n\eta L\, V_0^2$, which could be used to drive microengines. The swimming of microorganisms is subjected to several noise

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sources, including well-known thermal fluctuations from the environment, internal chemical fluctuations involving the flagellar motors, and interactions with other individuals, which cause them to perform a long-term random motion. Although thermodynamic laws prevent the extraction of directed work from Brownian motion in equilibrium, this is not true in out-of-equilibrium conditions, in which case directed work can be extracted if spatial invariance is broken [15, 16]. In bacterial suspensions, this can be achieved for example with an external stimulus, geometrical constraints, or by exploiting bacterial taxes. In 2010, two groups used bacterial suspensions to rotate microscopic gears [17, 18]. The rotating gears of Sokolov and DiLeonardo relied on their asymmetric teeth that rectified the motion of the bacteria, much like the “ratchet and pawl” thought experiment of Feynman [16]. When bacteria got trapped in the corners of the gear’s teeth, they pushed on the gear and led to its directional rotation. Typical length scales for the bacteria and gear radius are 1 µm and 100 µm, respectively, meaning that the bacteria were able to rotate an object hundreds of times larger than them.

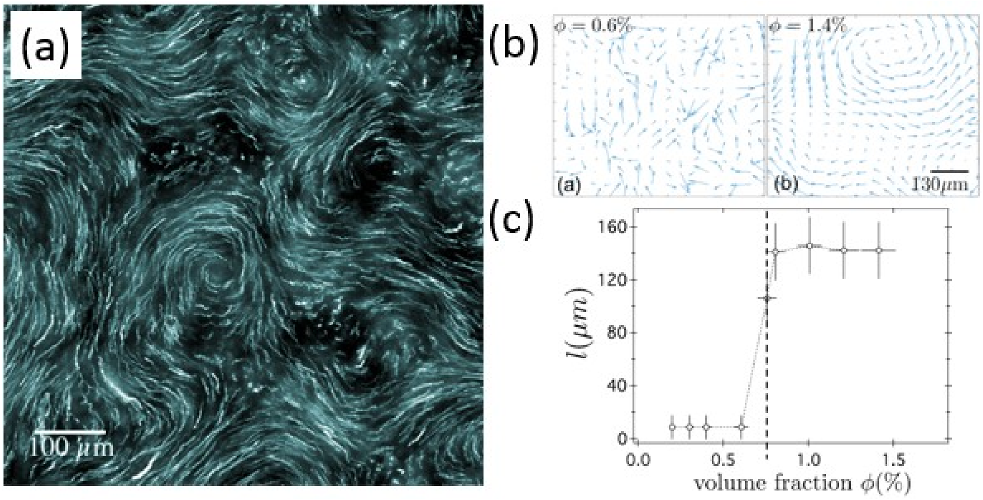

Figure 2 - Emergence of collective motion for a dense suspension of motile E.coli confined between two parallel plates (a) Direct visualization of large-scale collective swirls. (b) Velocity field obtained by PIV, showing a stark change in fluid motion on both sides of the concentration transition at a confinement height H = 220 μm. (c) Velocity correlation length l displayed as a function of the bacteria volume fraction ϕ showing the onset of collective motion **[20].**

This example demonstrates the possibility for using the collective motion of thousands of bacteria to power microscopic engines. To fully exploit this possibility requires a robust rectification protocol that ensures directionality of the collective bacterial movement. Recent works by the Paris group have shown the importance of confinement in the emergence of collective motion, which drives collective motion at a critical concentration concomitantly with the appearance of a « super-fluid » state with no macrocopic viscosity (figure 2) [19, 20]. All these properties are direct consequences of large-scale hydrodynamic interactions existing between swimming bacteria acting in interplay with the nature of the confinement. This suggests that the presence of complex boundary conditions, such as those considered in this study for bacteria confined in emulsions, will significantly influence the macroscopic features of the collective swimming. This is the main object of this proposal.

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Active emulsions turned into a motor - In a recent work, in a collaboration between the French and Chilean team, we showed the resulting dynamics that appeared when bacterial suspensions are encapsulated in drops immersed in oil. We used a type of magnetotactic bacteria (MTB) approximately 1 µm in length that grow magnetic nanoparticles inside their cytoplasm (figure 3(a)). Consequently, these MTB align their swimming direction to magnetic field lines. In nature, this allows MTB to swim following the earth magnetic lines toward the anoxic limit, where they find their ecological niche [21]. We encapsulated suspensions of MTB in aqueous, ~100 µm diameter drops immersed in oil and applied a constant magnetic field [22]. When the bacterial concentration was high enough, they organized in a big, robust vortex whose axis was perpendicular to the magnetic field, as show in figure 3(b). The overall rotation of the drop was demonstrated with tracer particles in the carrier oil, see figure 3(c).

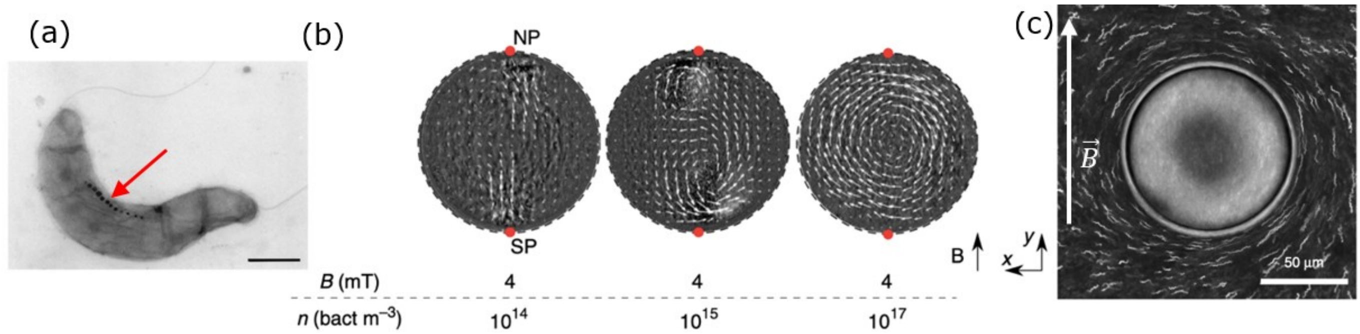

Figure 3 - Previous work on swimming bacteria encapsulated in emulsion drops: (a) Transmission electron micrography of an MTB, from [21]. The red arrow indicates the chain of magnetosomes (magnetic nanoparticles in the cytoplasm of the bacterium). Scale bas is 0.5 µm. (b) Dynamics of MTB encapsulated in a drop with application of a constant magnetic field. Bacterial concentration increases from left to right. At sufficiently high bacterial concentration, a steady vortex is created inside the drop. Drop radius varies between 43 µm and 89 µm. (c) Overall rotation of a drop filled with MTB is evidenced by passive tracers in the carrier oil. (b) and (c) are from [22].

Active thermal baths in a shell - 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, undergoes agitation resembling Brownian motion. This generic situation is currently called an “active thermal bath”. Diffusion, collective mixing and self-organizing possibilities driven by activity, bestow on the passive objects novel emergent properties, far beyond the classical picture stemming from Brownian motion. For example, the standard fluctuation-dissipation theorem does not hold anymore [23]. A population of swimming bacteria agitating a passive colloid indeed constitutes an example already reported and analyzed by the franco-Chilean collaboration in the context of kinetic theory [24, 25].

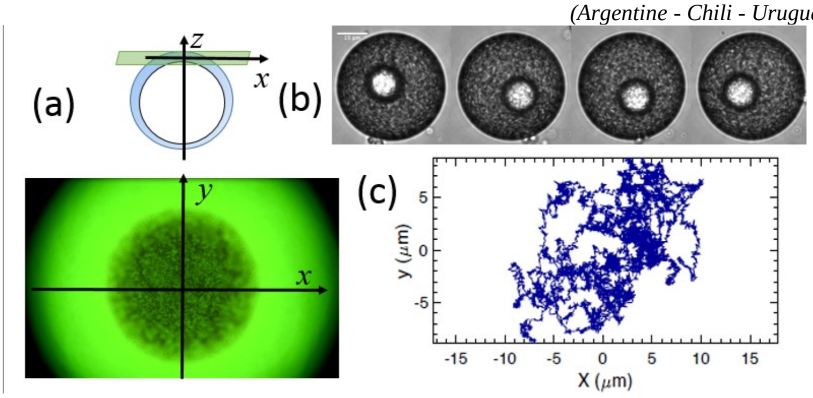

Figure 4 - Preliminary results obtained between Paris and Santiago, showing suspensions of E.coli confined to a shell, visualized by confocal microscopy. (a) Bacterial collective motion at the upper pole of a shell (top view). (b) and (c) Random walk motion of the shell inner droplet (white circle in the 4 top images) resulting from the activity of the bacterial suspension in which the droplet is embedded (top view).

It has also been demonstrated that it is possible to extract work from fluctuations at a fixed thermal temperature or to concentrate particles starting from an initial random distribution. All these properties are strikingly at odd with the standard outcome of equilibrium or close to equilibrium thermodynamics [26, 27].

For populations of swimming bacteria trapped inside a droplet, the agitation properties due the swimming hydrodynamics will determine, for example, the mixing of molecular species, or could accelerate the rate of chemical reactions. Also note 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 [28]. This type of active thermal bath can also favor the out-of-equilibrium organization of macroscopic objects trapped inside the droplet.

At the occasion of an internship at the PMMH in 2019, Cristian Villalobos, PhD student in the group of Profs. Cordero and Soto, performed preliminary experiment on « active shells » i.e. on small droplets confined in a bigger droplet that contains a bacterial suspension (see fig. 4). The inner drop motion was visualized via a confocal microscope that also allows the possibility for a tilt with respect to gravity and thus observe motion in all directions (not only in the XY plane). This work is also done in collaboration with Dr. Teresa Lopez-Leon at the Gulliver laboratory of the ESPCI, who developped the associated techniques for controlling the double emulsion fabrication process. Currently, there is a post-doctorant working in the French group (Zhengyang Liu) on fluctuating motion of objects trapped in droplets and activated by bacteria. Moreover, Cristian Villalobos pursued his PhD activities of activated shells in Chile, both experimentally and using numerical simulations. Recently the Chilean group has demonstrated the inner motion of bacteria in a drop could drive a persistent random walk [33] hence opening the way to the assembly of mesoscopic motors composed of micro swimmers. This proposal would be a unique occasion to join our complementary forces on this subject. These results also have driven several recent theoretical investigation on the fundamental hydrodynamic principles at the origin of this active motion [34,35].

Research plan and objectives

The main objective of this project is to harness bacterial swimming and fluctuations to extract useful, directed work from the motion of encapsulated bacteria. Here, we propose to study the fundamental principles leading to bacteria organization in fluid droplets eventually leading to the building of active emulsions. Also, it is very likely that in the context of soft matter emulsions, bacterial activity could significantly modify the geometry of the boundaries which might lead to novel organizational phases and activation possibilities still unraveled [29]. 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 and the ability to produce macroscopic work. The confining geometry will be systematically changed, not only by producing droplets of different curvature radii, but also by producing spherical shells, where the bacteria will be confined between two nested spheres. This original geometry will allow 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 different techniques such as for example, the glass-capillary microfluidics technique developed by Teresa Lopez-Leon at Gulliver lab, in particular to follow the topological organization of various liquid crystalline phases [30].

The specific goals will be :

- To measure and characterize the fluctuations of the collective motion of bacteria at high densities
- To use external stimuli to rectify the collective motion of bacteria confined in a drop to produce a rotating and translating motor.

The hypotheses of this work are:

- Bacteria in general, and E. coli in particular, are prototype examples of active fluids at low Reynolds number. Hence their behavior can be extrapolated to other microscopic swimmers.
- The collective motion of a dense bacterial suspensions can generate fluid flow at the scale of a droplet, even when the drop is hundreds of times larger than a bacterium, and this flow can be used to move the drop or other microscopic objects.
- Kinetic theory, hydrodynamics and agent-based simulations are appropriate tools to model theoretically these systems.

The research plan is organized two folds :

(i) Active driven droplets

This part will deal with magnetotactic bacteria confined in droplets. We will investigate the role of an external magnetic field and its orientation in time to produce net motion of drops rolling on surfaces. Also, we will investigate to which extend the collective organization properties of the emulsion can be modified in this context. We will also seek to develop techniques to track individual magnetotactic bacteria in 3D inside droplets, along the line of the technique already developed at the PMMH for E.coli [31, 32].

(ii) Active thermal bath in a drop.

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 and also the suspension concentration, ranging from dilute to dense suspension when collective motion can appear. Then, we will characterize the motion of solid objects of different shapes trapped inside the bulk droplets. We expect to observe interesting self-organization phenomena when several of these objects are encapsulated inside the droplet. For this study, in Paris, 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 [31, 32]. 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.

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Résultats escomptés au terme de l'action : NB : toute publication, sur tout support (papier, affiches, film, vidéo…) y compris les résumés (abstracts) produits lors de congrès, devra impérativement mentionner qu'elle s'inscrit dans le cadre du programme ECOS Sud – Chili, soutenu par le ministère de l'Europe des Affaires étrangères (MEAE) et le ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (MESRI) pour la partie française et de la ANID pour la partie chilienne. Le logo du programme sera repris à cet effet

This project is a new endeavor from both teams, towards an emerging field that is the one of active emusions and, more generally, active soft-matter phases. We hope that joining our forces on this new and exciting subject will produce a better understanding of this state of matter.
To build this novel path we rely on the collaboration between experimental and theoretical approaches done in both groups.
As a consequence, the project will result in joint publications related to the project objectives. Also, graduate students and young post-doctoral researchers will benefit from access to different complementary experimental and theoretical approaches to what is currently developed in their respective groups.

7. Antécédents de coopération avec la partie chilienne (joindre en annexe, le cas échéant, les références des publications co-signées, thèses, équipements réalisés, projets conjoints, brevets, etc.). Cette rubrique devra obligatoirement faire mention des actions antérieures ECOS auxquelles a participé *chacun* des membres du projet

The Franco-Chilean consortium has a history of collaboration that dates back more than ten years, on subjects such as granular matter, suspensions and active fluids. Until 2019, they were also a part of a CNRS international laboratory (Laboratoire International Associé « Matière : Structure et Dynamique » (LIA-MSD)), grouping French and Chilean research institutions (ENS Lyon, ESPCI, Univ. Chile, Univ. Santiago, Univ. Católica de Valparaíso). They have a history of multiple exchanges of students and researchers and along those 10 years of active collaboration, they published jointly 10 papers in international journals.

Publications cosignées

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- A. ALVAREZ, R. SOTO, E. CLÉMENT, Confined suspension jet and long-range hydrodynamic interactions: A destabilization scenario, Phys. Fluids 18, 083301 (2006)
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Projets conjoints

- 2012 – 2014: ECOS project C11E04 Transport in active suspensions and dense granular matter, R. SOTO (Chilean responsible), E. CLÉMENT (French responsible)
- 2016 – 2019: ECOS project C16E03 Active fluids in confined environments, R. SOTO (Chilean responsible), E. CLÉMENT (French responsible), M. L. CORDERO (Chilean associate), A. LINDNER (French associate).

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8. Moyens :

Moyens propres se rattachant au projet et provenant d'autres sources de financement (à indiquer <u>OBLIGATOIREMENT,</u> y compris les moyens provenant d'un partenaire industriel, de l'ANR, d'un établissement d'enseignement supérieur, d'un organisme français de recherche et/ou de l'Union européenne) :..

- Crédits récurrents UMR 7536 du CNRS

- ANR 2021-2025 - BACMAG "*Harnessing field-assisted transport and rheology of a bacterial magnetofluid*" with Dr Cécile Cottin-Bizonne (PI) Univ. Lyon I and Dr Damien Faivre CEA, Cadarache.

- Innovative Training Networks H2020-MSCA-ITN-2020 - PHYMOT « *Physics of Microbial Motility* » with Prof. A. Lindner and 13 other European groups. PI Prof. G. Gompper Forschugzentrum, Julich.

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Moyens sollicités dans le cadre ECOS Sud-ANID <u>pour la première année du projet</u> :

1. Nombre de missions pour chercheurs confirmés, avec justification scientifique, en mentionnant <u>obligatoirement</u> leur durée (aucune durée de mission n'est inférieure à 14 jours), la période possible et les bénéficiaires :

a - France -Chili

1 mission Senior Eric CLEMENT 14 jours Juin 2022..

1 mission Senior Anke LINDNER 14 jours Novembre 2022...

....

b - Chili-France :

1 mission Senior María Luisa CORDERO 14 jours Septembre 2022

1 mission Senior Rodrigo SOTO 14 jours Décembre 2022......................................

....

2. Séjours de durée moyenne (1 mois ; aucune durée de séjour n'est inférieure à un mois) pour jeunes chercheurs (doctorant(e)s ou post-doctorant(e)s) en mentionnant le nom des bénéficiaires (fournir un CV et le plan de travail) :

a - Jeunes chercheurs chiliens se rendant en France (nombre : 2) :

1 Cristian VILLALOBOS, doctorand 2nde année, November 2022, fluctuations inside bacterial shells

2 Benjamin OLIVA, masters student, November 2022, magnetotactic bacteria

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b - Jeunes chercheurs français se rendant au Chili (nombre : 2) :

1 Renaud BAILLOU doctorant 2nde année, Septembre 2022 suivi de bactéries confinées dans une goutte

2 Noveau doctorant recruté en septembre 2021 sur ANR BACMAG, bactéries magnétotactiques

3 Zhenyang Liu Post-doctorant PMMH.

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NB : Le programme ECOS-ANID n'accorde pas de financements doctoraux mais encourage la participation de leur bénéficiaires dans les actions.

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9. Informations complémentaires (cocher) :

X Ce projet est partiellement soutenu par le CNRS (Programme MITI-Defi auto organisation-2021), Post-doctorant 2021-2022 plus environnement (14 kE) en 2021.

☐ Ce projet a été retenu pour la période du ……. au….

☐ Ce projet n'a été soumis à aucun organisme, même partiellement.

☐ Ce projet est tripartite avec l'Argentine☐, l'Uruguay ☐ : En cas de réponse positive, le projet doit être déposé également dans le cadre de l'appel à projets correspondant (Uruguay et/ou Argentine.)

10. Liste des experts recommandés (sans conflits d'interêts) avec leurs coordonnées :

1 - Thomas Bickel - **thomas**.**bickel**@u-**bordeaux**.fr university of Bordeaux…………
……………………………………………………………………………………………………

2 - Philippe Peyla – **philippe**.**peyla**@univ-**grenoble**-alpes.fr University of Grenoble
……………………………………………………………………………………………………

3 - Eric Climent - **eric.climent**@imft.fr IMFT Toulouse
……………………………………………………………………………………………………

4 – Harmut Loewen - **hlowen@thphy.uni-duesseldorf.de**, Heinrich-Heine-Universität, Düsseldorf

~~Date :~~

~~Nom et signature du **responsable français du projet**~~

~~Eric Clément~~

~~Avis, nom et signature du **responsable de l'unité de recherche** à laquelle appartient le responsable de projet~~

~~Damien Vandembroucq, directeur du PMMH-ESPCI , Paris~~

~~Avis, nom et signature du **Chef d'Établissement** (les projets provenant d'UMR Université-CNRS pourront transiter indifféremment soit par l'université ou par le Délégué Régional du CNRS compétent ; pour les autres EPST, il s'agira du Directeur des Relations Internationales ou de l'Administrateur Délégué Régional, qui se chargera de recueillir les avis scientifiques éventuellement requis.)~~

~~**ATTENTION :** Les projets ne seront recevables **que sous forme électronique et en un seul fichier** (Word, RTF ou PDF) regroupant : 1) la fiche projet en français avec ses annexes ; 2) un Curriculum Vitae **(2 pages Max)** et la liste des publications sur les cinq dernières années des responsables français et chilien, ainsi que le Curriculum Vitae des participants **(2 pages Max)**~~
~~Par ailleurs un exemplaire **papier** de la fiche projet (sans nécessairement le descriptif intégral du projet scientifique) doit être adressé, dûment signé, **sous couvert du chef d'établissement**.~~
~~**Le fichier électronique doit nous parvenir au plus tard le 1er juin 2021**~~

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