***1.1Quality and pertinence of the project’s research and innovation objectives (and the extent to which they are ambitious, and go beyond the state of the art)***

Original non-equilibrium phenomena arise in active matter in the presence of confinement. This project aims at studying the fundamental principles governing the emergence of collective organization and structured interaction **when active and passive objects interact both** under **confinement and in the presence of curvature**. This project will pave the way towards the elaboration of novel soft-matter exotic phases.

Active matter, denoting collections interacting self-propelled particles, is an emerging scientific concept in statistical physics at the interface between physics, chemistry and biology. Due to the constant energy injection at the single particle scale, active matter is constantly driven **out of equilibrium** and behaves very differently from well-understood equilibrium -and even close to equilibrium- thermal systems. From all the surprising emerging phenomena the presence of boundaries promoting the confinement of active systems is perhaps the most elusive and ill-understood. This feature leads to novel exotic **non-equilibrium phenomena**, such as wall accumulation, motion rectification, ratchet effect and upstream swimming and are particularly present **for boundaries with complex geometries**. Understanding the impact of confining geometries non-only only poses new challenges to fundamental biology and ecology, but also imply world-changing applications in therapeutics and robotics.1,2

The interplay between complex environments and active matter suggests **a possibility to control and engineer active matter** by carefully designing the confinement structures.

**State of the art:** It is now well established that confinement may influence transport3, rheology4–7, pressure8, spatial distribution9–11 and collective motion12,13,22,23,14–21 of active matter.

**However, curved confining walls, which are ubiquitous in biological systems**, show of their own, specific rich and intriguing effects on active matter.

Inspired by the collective motion of confined driven filaments13, Woodhouse and Goldstein constructed a theoretical model, demonstrating that the combination of circular confinement and activity allows for the emergence of stable self-organized rotational streaming.14 Such circular confinement was then realized by emulsions and elastomer chambers, where single vortical flows were observed.17,18,20 Liu et al. recently showed intriguing oscillatory dynamics in a similar geometry.23 Ravnik and Yeomans studied the dynamics of active nematics under cylindrical confinement using simulation based on continuum equations. They showed that the collective vortical flows not only emerge along the cylinder axis (as shown by Woodhouse and others), but also within the plane of the cylinder.16 Fily and co-workers developed a statistical theory for non-aligning, non-interacting active particles to study spatial distributions under strong confinement. They showed that in such confinement, particle concentrations at boundaries were proportional to the local curvature.24 Nikola and co-workers showed, using particle-based theory and simulation, that not only the collective motion, but also the shear stress exerted by active particles on confining walls was wall-dependent.8 These works show that curved confining walls alter the behavior and macroscopic properties of active matter. In particular, **the only key parameter of curved confinement, curvature**, has been shown to play an important role in particle spatial distributions24 and collective motions17. On the other hand, complex-shape passive objects show intriguing persistent and directed motions in active baths, which can be used for the extraction of work.25–28 Angelani and co-workers, using numerical simulations, showed that asymmetric gear-like objects spontaneously rotated in a directed way in active active baths, forming the concept of “micromotor” powered by active matter.27 . This idea was then realized in experiment in experimental25,26 and theoretical grounds.28

Under confinement, the interactions between active bath and passive objects are modified. Experiments have demonstrated interesting behaviors, such as self-organizing into a single vortex and spontaneous oscillatory motions, when dense bacterial suspensions are confined in droplets. Despite the extensive confinement effects illustrated, it remains challenging to predict the behavior under specific geometrical parameters, due to the lack of **well controlled and measurable experiments**. In this project, we propose to build such an experimental system, which will not only allow us to understand active matter better, but also advance the technical frontier for other fields of study.

**Systematic experiments** in a **well-controlled and measurable systems** will deepen our understanding in the confinement effect on active-passive interactions, guiding real-life applications with active matter.

A major step revealing the specific character of active fluids was to study the interaction between a bath of active particle and a passive object immersed in it [WU PRL + Mino PRL]. Through the historical analysis of A. Einstein in 1905 this situation known as the *Brownian particle problem* underlines in thermal matter, the most fundamental and deep features of the concept of temperature and its relation with mesoscopic fluctuations close to equilibrium through the fluctuation/dissipation theorem. Experiments involving bacteria, active algae or active colloids have shown that the fundamental irreversibility of hydrodynamic interactions is at the core of the tracer motion which leads to tracer fluctuations sometimes called “active temperature” but here, essentially controlled by hydrodynamic processes where confinement is an essential piece [MINO J. FLUID MECH].

**The first objective** of this proposal is to build a well-controlled active matter experiment based on the 3D tracking of particles driven by a bath of motile bacteria and confined inside spherical droplets first **using a double emulsion technique.** This novel experimental system stems from two key technological innovations and will enable a systematic investigation of the “active temperature” concept induced by a bath of bacteria confined in a curved environment.

**Well-controlled experimental device for quantitative investigation of active matter under spherical confinement**

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|  | Emulsions offer controllable and high throughput experimental capacities for studying curved confinement effects. In this project, I propose to bring together and adapt two recently developed techniques: concentric capillary microfluidic device (Fig.1a) and 3D Lagrangian tracking microscope.  ✔**Concentric capillary micro-capillary device** allow us to produce **double emulsions** with well controlled compositions and size, as illustrated in Fig.1 b-e. This technique was extensively used to study liquid crystals in Teresa Lopez-Leon’s group at Gulliver, ESPCI (one of the host labs). I will bring together this technique and active matter physics to make a novel and powerful **model experimental system**.  ✔**3D Lagrangian tracking microscope**, an advanced 3D imaging technique, is under development in Eric Clément’s lab at PMMH, ESPCI (one of the host labs). This system already demonstrated its capability of capturing 3D motions of fluorescent bacteria [Darnige RSI, Figueroa PRX, Junot EPJ, Junot PRE, Junot Preprint]. |
| ***Figure 1****: Sketch of the concentric capillary microfluidic device, which generates emulsions with controlled sizes. Inverse double emulsions with bacteria swimming in the aqueous phase. (a, b) Double emulsions in a micro-capillary device, operating at different flow rates. (c, d) Collected double emulsions observed with a higher magnification.* |

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| My objective here is to develop an image processing based feedback control in order to **achieve the 3D tracking of spherical droplets and also complex-shape objects** in a drop**.** In Fig.2 is displayed a preliminary result using the 3D tracking microscope of the PMMH. I obtained by a manual tracking, the trajectory of the inner oil droplet, already showing the combined effects of activity and radial confinement. |  |
| ***Figure 2:*** *3D trajectory obtained by manually keeping the object in focus. Black dashed line indicates the trajectory of the inner droplet. Red dashed frames indicates the surface of the outer droplet.* |

**Active motion of passive spheres confined in droplets**

Combining the microfluidic emulsion device and the 3D imaging technique, I will be able to study systematically how the conjunction of confinement and curvature influence the motion of a spherical droplet in a bath of active bacteria. Bacterial concentration can be a-priori defined in the suspension preparation and the possibility to develop collective motion is also a crucial control parameter of the set-up. Interestingly, in this situation the “creaming effect” on the inner droplet, i.e. the combined influence of weight and Archimedes force, provide a confining potential centered at the top of the outer droplet. I also propose to control this parameter using density matching technique already used in the PMMH team for bacterial fluids [Gachelin PRL, LopezPRL], hence providing a supplementary experimental handle, crucial to compare with theoretical propositions. As shown in Fig.1. a highly controlled oil/water/oil double emulsion can be created, with the active bath of bacteria as the aqueous phase where the sizes of both the outer and inner droplets can be obtained by operating the device at different flow rates. Then, for a large range of geometrical characteristics, the trajectories of the individual spheres can be extracted and several statistical properties analyzed, such as the mean square displacement (MSD) the velocity autocorrelation function (VACF) and the corresponding probability density function (PDF).

**Exploring the validity and consequences of the “active temperature” concept**

The outcome of these results will be compared with different theoretical propositions and in particular, stochastic models developed in the group of Rodrigo Soto at the University of Chile a current collaborator of the PMMH host group. From those models an effective “active temperature” defining the level of fluctuations of the central object can be predicted as well as the VACF and PDF as a function of different geometrical characteristics of the confinement.

**Complex-shape objects in confined active bath: towards new self-assembly principle?**

As discussed previously, active bath can display novel phenomenology subtly dependent of the shape of the driven object. For example, active bath offer the possibility to produce work if the object has a ratchetted shape. This is strongly at odd with thermal equilibrium as, according to the classical Kelvin’s theorem, work cannot be extracted from a single thermal bath. Somehow for active matter, this property can be seen as a hallmark and for this reason deserves more extensive and systematic investigation.

In this second part of the proposal, I will be using a nano-printing device of the ESPCI to fabricate objects of complex form and of several microns size. I will seek to adapt the microfluidic device to insert such objects in the core of an inverted emulsion of different droplet radii.

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| ***Figure 3****: 3D Nanoscribe® nanoprinter at the ESPCI allowing the additive printing of micron-size objects with submicron resolution. (a) Principle of the Nanoscribe nanoprinter using two photon polymerization technique creating at the focal volume an elementary voxel of 300x140 nm resolution. (b) Simple illustration of the variety of complex micro-objects that can be constructed at a micron size. The white bars are 10 mm length.* |

✔ With this system, I will first observe and quantify the motion and the fluctuations of a single complex-shape particles in a confined active bath and in **particular the rotation degrees of freedom.** Note that these solid particle can eventually be rendered fluorescent.

✔ Thereafter, I will try to encapsulate multiple complex-shape particles into droplets. Hence I expect to observe novel self-assembly principles assisted by active matter under confinement.

**Supervision**

The supervisors, Prof. Eric Clement, Prof. Anke Lindner and Prof. Teresa Lopez-Leon are experts in their respective fields, with demonstrated excellence in research project managing, scientific publications and student supervision.

Prof. Eric Clement and his group are world leading experimentalists on the study of granular matter, suspensions and active fluids, with special expertise in the behavior of active particles in complex environments. On the subject of active matter, he was the PI of the ANR project BacFlow (2015-2020): *Hydrodynamic transport and dispersion of bacterial suspensions: from the micro-hydrodynamic scale up to porous media*, the co-PI of a Joint Research Program (PRC) CNRS-Royal Society (2017-2019), focusing on *Macroscopic and Microscopic properties of active matter*. Currently, he is a co-PI of the ANR project BACMAG (2021-2025): *Harnessing field-assisted transport and rheology of a bacterial magnetofluid*. With Anke Lindner he is a part of the **European Training Network PHYMOT** *Physics of microbial motility*. He published more than 115 papers in international journals and has one patent pending on rheometric techniques. Prof. Clement has developed many close collaborations with researchers of international level. Current international collaborators include Profs Rodrigo Soto and Maria-Luisa Cordero (Univ. de Chile), Prof. Wilson Poon (Univ. Edinburgh) and Prof. Jasna Brujic (New York University). He supervised 22 (2 current) PhD students and 10 postdocs. Many former students and post-docs are now working in academia as for ex. at the Universities of Paris, Lyon, Kyushu, Minas-Gerais, Monterey, Colorado, or in French research institutions such a CNRS or CEA.

Prof. Anke Lindner is an internationally acknowledged specialist in the interactions between anisotropic particles and flow. She has been the leader of many projects, including the ongoing PaDyFlow (particle dynamics in flow of complex suspensions) that was selected for a **consolidator ERC grant (2016-2021)**. She is also part of the collaborative **ITN project CALIPER** starting in September 2019. She is a co-PI of the **ETN-PHYMOT** *Physics of microbial motility* starting in 2021. She has been awarded the **“Maurice Couette” award** and **CNRS silver medal** for her study of complex fluids in flow. Former students of hers now work as research scientists at companies such as “Total”, “Arkema” and small start-up companies as “DNA script” and “Aratinga Bio”. Many of them pursue a career in academia and hold positions as assistant professors at Montpellier University, Sorbonne University, Perdue University or University of Dortmund. She has published 63 primary research papers, 1 book chapter and 1 patent which is currently pending. To complement her experimental research Prof. Lindner has developed many close collaborations, acknowledged by common publications, with theoreticians on an international level. Ongoing collaborations comprise for example Mike Shelley, Courant Institute, and Simons Foundation New York, USA, David Saintillan (UCSD, USA), Howard Stone, Princeton University, USA and François Gallaire, EPFL, Switzerland.

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