Part B-1

1. Excellence

1.1 Quality 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)

Exotic non-equilibrium phenomena of active matter arise in the presence of confinement. This project aims to study the fundamental principles governing the **interactions between active and passive objects** under **confinement with curvature**.

Active matter, denoting collections of actively moving particles, is an emerging subject of study at the interface between physics, chemistry and biology. Due to constant energy injection at single particle scale, active matter is constantly driven **out of equilibrium** and behaves very differently from well-understood equilibrium systems. **Exotic nonequilibrium phenomena**, such as wall accumulation, motion rectification, ratchet effect and upstream swimming, are particularly observed **in the presence of boundaries with complex geometries**. These phenomena not only pose 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: Confinement influences transport[3], rheology[4–7], pressure[8], spatial distribution[9–11] and collective motion[12,13,22,23,14–21] of active matter. **Curved confining walls, which are ubiquitous in biological systems**, show rich and intriguing confinement effect on active matter. Inspired by the collective motion of confined driven filaments[13], 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 distributions[24] and collective motions[17]. 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 experimental[25,26] and theoretical grounds.[28]

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

Figure 1: Sketch of concentric capillary microfluidic device, which generates emulsions with controlled sizes.

Under confinement, the interactions between active bath and passive objects are modified. Experiments have demonstrated
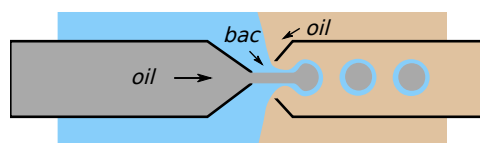
interesting behaviors, such as self-organizing into a single vortex and spontaneous oscillatory motions, when dense bacterial suspensions are confined in droplets. Despite the extensive confinement effects illustrated, it remains challenging to predict the behavior under specific geometrical parameters, 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.

Our first objective is to **build a well controlled and measurable confined active matter experimental system**. This system, involving key technological innovations, will enable systematic investigation into curved confinement.
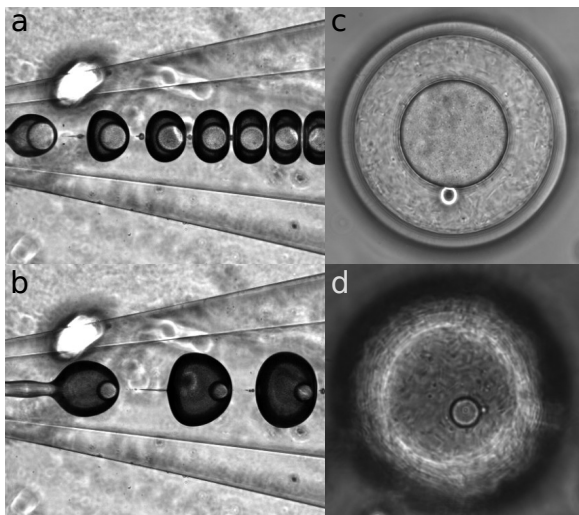

Figure 2: Double emulsions with swimming bacteria in the middle aqueous phase. (a, b) Double emulsions in a micro-capillary device, operating at different flow rates. (c, d) Collected double emulsions observed with a higher magnification.

Building a well controlled and measurable experimental system: Emulsions offer controllable and high throughput experimental systems for studying confinement effect. In this project, we propose to bring together and adapt two recently developed techniques: concentric capillary microfluidic device (Fig.1) and 3D Lagrangian tracking microscope.

✔ **Concentric capillary microcapillary device** allow us to produce **double emulsions** with well controlled compositions and size, as illustrated in Fig.2. This technique has been used extensively 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 to make a novel and powerful **model experimental system**.[29]

✔ **3D Lagrangian tracking microscope**, an advanced 3D imaging technique, is under development in Eric Clement's lab at PMMH, ESPCI (one of the host labs). This system has demonstrated capability of capturing 3D motions of fluorescent bacteria.[30] I will develop the image processing based feedback control to **achieve 3D tracking of spherical droplets and complex-shape objects.** A preliminary result obtained by manual tracking is shown in Fig.3.

Our second objective is to **study the motions of passive objects in confined active bath**. This knowledge will suggests **a possibility to control and engineer active matter** by carefully designing the confinement structures, thus lay the foundation for bioremediation and drug delivery with active matter.

Motions of passive spheres in droplets: Combining the microfluidic and 3D imaging techniques, we will be able to study systematically how confinement geometries influence the motions of spherical particles.

✔ **Create oil/water/oil double emulsions** with bacterial suspensions in its middle aqueous phase as the active bath. The sizes of both outer and inner droplets can be controlled by operating the device at different flow rates, as demonstrated in Fig.2.

Figure 3: 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.

✔ Extract trajectories of individual spheres and analyse their **characteristic statistical properties**, such as mean square displacement (MSD), velocity autocorrelation function (VACF) and probability density function (PDF).

✔ From the properties identified from the experiment, model the trajectories of passive spheres considering geometrical constraints.

Complex-shape objects in confined active bath:

✔ Fabricate complex-shape particles using 3D nano-printer at ESPCI

✔ Study the motions and fluctuations of complex-shape particles in confined active bath

✔ encapsulate multiple complex-shape particles into a droplet comprising active matter. We expect to observe novel self-assembly principles assisted by active matter under confinement.

Expected outcome: Combining the microfluidic techniques and the advanced 3D microscopy, we expect to generate a complete set of fluctuation data (trajectories) of passive oil droplets in confined active bath of various confining curvature and active particle concentration. An automated 3D tracking system, incorporating artificial intelligence technology, will also be demonstrated. Fluctuations of non-spherical passive particles will be compared with spherical particles. Self-assembly of non-spherical particles in active matter will be demonstrated. These experimental data are expected to facilitate further development of active matter models, and eventually close the gap between theory and application.

1.2 *Soundness of the proposed methodology (including interdisciplinary approaches, consideration of the gender dimension and other diversity aspects if relevant for the research project, and the quality of open science practices, including sharing and management of research outputs and engagement of citizens, civil society and end users, where appropriate)*

Overall methodology: Our proposed research aims to systematically study the motions of passive particles in active baths under different confining curvature. The concept of the project is illustrated in Fig.4. Taking the advantage of the **concentric capillary microfluidic device**, experimental system, we can access **a wide range of the key parameter – curvature –** by generating emulsions with various sizes. To overcome the key experimental challenge, **3D imaging**, we have three candidate methods (3D tracking, tilted confocal and holography) ready to be tested. These techniques will enable us to systematically study how curvature influences the fluctuations of an active bath. Furthermore, **3D nano-printer** hosted in ESPCI enables me to produce micro-particles with arbitrary shapes. Taken together these techniques, the objectives of the proposed research can be achieved.

Figure 4: Concept of the project: Understanding the motions of passive objects in active baths by combining 3D imaging and microfluidic techniques.

Integration of disciplines: This project is highly interdisciplinary, requiring expertise in: i) computer image processing to improve the 3D tracking system, ii) biochemistry and biophysics to produce bacterial suspensions with controlled properties, iii) hydrodynamics and statistical physics to study their fluctuations and collective motion, iv) microfluidics and surface chemistry to produce complex confining environments. The host labs have all the equipments that are needed in this project: a clean room, a 3D tracking microscope, a 3D nano-printer and various bacterial
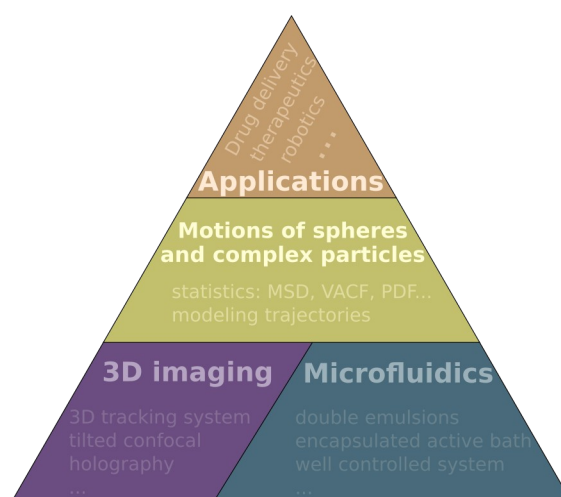
strains. I have extensive experience in studying active matter under confinement, as demonstrated in my CV in part 4. As the researcher, I have a well fitted background and skill set to bring together the expertise from the 3 host labs, as well as the equipments, to achieve the objectives of this project.

Gender dimension and other diversity aspects: Special attention has been brought by the host labs to ensure gender balance.

Open science practices and data management plan: Open Science is an action to promote the benefits of science by giving access to the general public. This helps disseminate our research works and can promote collaborations in a broader community. We will share transferrable skills and technologies generated in this project, such as softwares, algorithms and experiment protocols on GitHub. The data and scientific publications generated in this project will also be deposited in open access repositories. Special attention will be focused on **research data management**. We have an initial data management plan: data will be organized and uploaded to open repositories every month with a report describing the purpose and statistics of them. A separate document will be maintained to account for the status and evolution of the whole data set over time. In addition, all the participants will come together and update the plan regularly to ensure efficient knowledge transferring.

1.3 *Quality of the supervision, training and of the two-way transfer of knowledge between the researcher and the host*

<u>Supervision</u>

The supervisors, Prof. Eric Clement, Prof. Anke Lindner and Prof. Teresa Lopez-Leon are experts and promising scientists 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 active fluids, with special expertise in the behavior of active particles in complex environments. He has been the co-PI of **ANR project BacFlow (2015-2020)**, studying *Hydrodynamic transport and dispersion of bacterial suspensions: from the micro-hydrodynamic scale up to porous media*. He has also been the co-PI of **Joint Research Program (PRC) CNRS-Royal Society (2017-2019)**, focusing on *Macroscopic and Microscopic properties of active matter*. He has published more than 115 papers in international journals. Prof. Clement has developed many close collaborations with researchers with on an international level. Current collaborators include Prof. Rodrigo Soto and Maria-Luisa Cordero (Universitad de Chile) and Prof. Jasna Brujic (New York University). He has supervised XX PhD students and XX postdocs. Former students now work as XX in XX.

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 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.

Prof. Teresa Lopez-Leon is an expert in studying the effect of confinement and curvature on the properties of liquid crystals. She has been the leader of XX project. She has been awarded XX award for her contribution in XX. She has published XX research papers in international journals. She has developed collaborations with XX and XX. She has advised XX PhD students and XX postdocs. She has the technique of producing double emulsions readily available for the proposed research. Her knowledge on confinement effect in general will provide new perspectives to understand the behavior of confined active matter.

Two-way transfer of knowledge between the researcher and the host organisation

Host-to-researcher: the hosts have knowledge on active matter, complex fluids and microfluidics, which forms an ideal combination of knowledge and techniques that are necessary for conducting the proposed research, and will be a great opportunity for me to acquire new knowledge and to carry out an interdisciplinary and impactful study. During the course of pursuing the proposed project, I will be trained by experienced colleagues on various techniques. For example, I will be working with the lab engineer Thierry Darnige on developing the 3D tracking microscope, which will deepen my knowledge in image processing and LabView. I will also be working with our external collaborators Prof. Rodrigo Soto, an expert in modelling and simulating active matter. Such experience will improve my understanding on the subject from theoretical perspective. (maybe we can propose a secondment in Chile?)

Researcher-to-host: My knowledge of studying the diffusion, rheology and collective motions of bacterial suspensions, combined with working experience of microfluidics, advanced microscopy and image analysis together aligns perfectly with the required skill set for carrying out the proposed research. This will allow me to **bring together the expertise from the 3 host labs**, as well as the equipments, to achieve the objectives of this project. Additionally, I can share with the host **a mutant strain of *E. coli*** whose motility can be controlled by light. I genetically engineered the mutant strain during my PhD at the University of Minnesota, and I believe it can lead to a more versatile experimental system and more concrete outcome of this research project. I will also share **holographic imaging technique** with the host lab, which can be a candidate method for 3D imaging.

Planned training activities for the researcher

I plan to set up **regular meetings with the supervisors on a bi-weekly basis** to discuss specific aspects of the project, such as new data and technical issues, as well as my career development plans. In addition, **data management meetings** will take place every 3 months during the course of the project to ensure efficient communication within the group, as well as to the broader community and the general public. **A set of seminars** are planned to communicate new results within the host institute and secondment labs.

1.4 Quality and appropriateness of the researcher's professional experience, competences and skills

I obtained bachelor's degree from Tsinghua University in 2014 studying *Self-assembly of Janus nanoparticles* and PhD degree in 2021 with my thesis entitled *Novel properties and collective phenomena of active fluids*. During these investigations, I have acquired expertise in various experimental techniques, including confocal microscopy, microfluidics, genetic engineering, particle synthesis, image analysis, as well as experience in numerical simulation. These studies have led to 7 publications in international peer reviewed journals, with additional papers under preparation.

The proposed research topic matches well with my experience and skill set. I will be able to quickly adapt my expertise to the new work environment. In the mean time, the supervision from world leading scientists and being able to use the state-of-the-art equipments will benefit my career development enormously.

2. Impact

2.1 Credibility of the measures to enhance the career perspectives and employability of the researcher and contribution to his/her skills development

This fellowship is crucial for me to acquire necessary trainings to become an independent researcher in academia to advance the fundamental understanding of active matter, which has potential to overcome challenges in therapeutics and robotics. By working with the host groups at PMMH and Gulliver in ESPCI on investigating into cutting-edge problems in the interdisciplinary field of active matter, my career perspectives and employability will be enhanced in several aspects:

● Improve my technical skills in specific methods

● Improve my communication skill by presenting my research to audience of different background and expertise

● Learn to define and manage a large research project by setting objectives, planning and making adjustments to the objectives and plans

● Improve mentoring skills by working together with junior students

● Publish high impact research papers to improve the chance I will be employed in academia

2.2 Suitability and quality of the measures to maximise expected outcomes and impacts, as set out in the dissemination and exploitation plan, including communication activities

I will share the results of this project in open access high impact journals. Specifically, I plan to publish a method paper on the improved 3D tracking microscope from WP1, and two research paper on the motions of particles in confined active bath from WP2 and WP3.

The results will also be disseminated by presentations in seminars and international conferences. I plan to give a set of seminars to communicate new data within the host institute and secondment institute. Internationally, I will present in the American Physical Society annual meetings to disseminate the work to a broader scientific community.

The host institute, ESPCI, has created a public scientific venue Espace des Sciences Pierre-Gilles de Gennes (ESPGG), aiming at fostering the science culture in Paris and communicating science with the general public through outreach activities. I will participate in activities organized by ESPGG, such as "lab open days", which opens the lab door to the general public for visit.

The table below presents an overview of the actions to disseminate the results generated in this project:

Type	Action	Date	Target public	Form
Scientific publications	Publications in scientific journals	Month 6, 15, 23 of the project	Scientific community of active matter and fluid mechanics	Publications
Conferences	APS DFD 2022	Nov 2022	International scientific community	Presentation
	APS March 2023	March 2023	International scientific community	Presentation
	APS DFD 2023	Nov 2023	International scientific community	Presentation
	APS March 2024	March 2024	International scientific community	Presentation
Seminars	PMMH	July 2022		Presentation
	Gulliver	Feb 2023		Presentation
	UChile	July 2023		Presentation

2.3. The magnitude and importance of the project's contribution to the expected scientific, societal and economic impacts

We will demonstrate an interdisciplinary approach, bringing together active matter, 3D imaging and microfluidics, to construct a well-controlled and biomimetic confined environment to study the confinement effect on the self-organization and complex interactions of active matter and particles. We will generate data that will be interesting to the **active matter community**, which is **rapidly growing** as evidenced in the number of publications in high impact international journals and the growing number of focused sessions in international conferences. We will also reinforce the 3D Lagrangian tracking system, potentially by incorporating AI technologies, which will provide a crucial technique not only for active matter experiment, but also for imaging tasks in general where the object of interest is moving fast in 3D.

In this project, we will focus on the fundamental aspect of the subject. A complete confinement parameter space will be explored and a data-guided trajectory modelling will be carried out. Nonetheless, the result of this research has the potential of being applied in therapeutics and robotics, which would have broad civil impact.

3.   Quality and Efficiency of the Implementation

3.1   Quality and effectiveness of the work plan, assessment of risks and appropriateness of the effort assigned to work packages

List of major milestones:

Milestones	Description	Month
M1	Improve 3D tracking microscope for droplets	6
M2	Set up protocols of double emulsion generation, 3D imaging and data analysis	8
M3	Complete trajectory data under different confinement	14
M4	Fabrication of anisotropic particles	17
M5	Complete trajectory data of anisotropic particles in active bath	21

The project is divided into 3 work packages according to the objectives presented in section 1.1.

Work package number	1	Start month	1	End month	6
Work package title	Improve 3D tracking				
Objectives	Improve 3D tracking microscope for droplets				
Description	- Collect images where the current tracking system malfunctions - Identify the issues in the image processing of the tracking system - Improve the image processing to handle the tracking in droplets				
Deliverables	Report on specific activities or results, data management plans, ethics or security requirements			Month	
	D1.1	Collect images		2	
	D1.2	Analyze images and improve image processing code		4	
	D1.3	Write towards a method paper		6	

Work package number	2	Start month	7	End month	15
Work package title	Thermometer				
Objectives	Measure the fluctuations of spheres of confined active bath				
Description	- Demonstrate the capabilities of the proposed methods: producing double emulsions with microfluidic device, recording 3D motion of inner droplets with Lagrangian 3D tracking system and confocal microscopy - Set up an experimental protocol				

	- Collect inner droplet trajectory data in various curvatures - Write programs to extract the trajectories from images	
Deliverables	Report on specific activities or results, data management plans, ethics or security requirements	Month
	D2.1 Conference – APS DFD meeting USA, November 2022	8
	D2.2 Make double emulsions with dense bacterial shell	9
	D2.3 APS March meeting USA, March 2023	12
	D2.4 Write towards a manuscript	15

Work package number	3	Start month	16	End month	24
Work package title	Complex-shape object				
Objectives	Measure the dynamics of complex-shape particles in active bath				
Description	- Fabricate complex-shape particles using 3D nano-printer - Measure fluctuations of single complex-shape particles in active emulsion - Investigate self-assembly of complex-shape particles in active emulsion				
Deliverables	Report on specific activities or results, data management plans, ethics or security requirements				Month
	D3.1	Fabrication of complex particles using 3D printer			18
	D3.2	APS DFD Meeting, USA 2023			20
	D3.3	Emulsions of bacterial suspensions with particles			22
	D3.4	Write towards a manuscript			23
	D3.5	APS March Meeting, USA 2024			24

The work plan of the project is outlined in the Gantt chart below (April 2022 – April 2024):

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
WP1																								
WP2																								
WP3																								
Management	G1			G2			G3			G4			G5			G6			G7			G8		
Milestone						M1		M2						M3			M4				M5			
Deliverable		D1.1			D1.2			D1.3	D2.1			D2.2			D2.3			D3.1		D3.2		D3.3	D3.4	D3.5
Secondment																								
Conference								DFD				APS								DFD				APS
Seminar				S1						S2					S3									
Outreach						O1				O2								O3				O4		

Risk management

Milestones	Risk	Intensity	Contingency plan
M1	3D tracking microscope does not work in droplets	High	Tilted confocal microscope with quasi-2D droplets or manual tracking of objects
M2	Initial image analysis does not achieve acceptable accuracy	Middle	Try other available image analysis methods
M3	Complete trajectory data under different confinement	Low	
M4	Fabrication of anisotropic particles	Low	Lindner group have studied particles of various shapes, such as helix and soft fibers.
M5	Complete trajectory data of anisotropic particles in active	Middle	Characterizing motions of complex particles is still challenging. If I fail to automate the

bath		procedure, I will hand-label the most interesting behavior.

3.2 Quality and capacity of the host institutions and participating organisations, including hosting arrangements

The host institution ESPCI in Paris is a prestigious institute of research and higher education in science and engineering, with five Nobel Prize winners associated with the School - Pierre Curie, Marie Curie, Frédéric Joliot-Curie, Pierre-Gilles de Gennes, and Georges Charpak. It hosts 9 high level laboratories with many world class researchers focusing on active matter, the topic I am pursuing. It also has a strong international scientific committee that is composed of eminent, internationally recognized figures from the academic world, research and industry. As a champion for scientific excellence, ESPCI Paris is actively working to take science and the love of science beyond the labs and lecture halls. Inspiring an appetite for knowledge by fostering curiosity and interest in science and experimentation is increasingly vital today, in light of an obvious lack of interest in the western world for scientific studies and careers. A renewed interest in science today will contribute to tomorrow's knowledge-based economy. The seven ESPCI Paris auditoriums are the venue for a variety of lectures, ranging from the Experimental Lectures series with unique lab-bench demonstrations to the "Les Chantiers du Savoir" series which tackles more challenging topics. The School campus also hosts popular events for the general public all year round, including the Sciences on the Seine Festival, Fête de la Science, Researchers' Night, and Science Académie. The host labs have regular activities that can help me integrate in the team and the institution, such as seminars, lab trips, meetings at various levels. The labs are also equipped with top-level infrastructure and instruments that are essential for carrying out the proposed research. The ESPCI Paris also provides its employee with services and benefits from the CNAS (National Social Action Committee), services to improve the material and moral conditions of employees. The ESPCI Paris being a funder member of the PSL University, I will have access to the full scope of PSL services (student and campus services, events, sports, culture, etc). In addition, the geographical location of ESPCI is in the core of Paris, with famous institutions like the École Normale Supérieure, Ecole des Mines and Institut Curie located close-by, providing an exceptional scientific environment for research.

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