Harnessing field-assisted transport and rheology of a bacterial magnetofluid

I. Pre-proposal's context, positioning and objective(s)

The project's objective is to study the fundamental principles governing **the emergence of a new class of fluids laden** with motile **MagneTotactic Bacteria (MTB)**. These suspensions will develop **original field-controlled transport and magneto-rheological properties** through the motility and mesoscopic organization of the bacteria.

- **MTB, a swimming compass with external handles-** In the 70's, Blakemore discovered a novel type of bacteria able to grow a micron-size magnetic compass, consisting in a linear assembly of nano-crystals of ferrite called magnetosomes [Blake75, Faivre2008]. Many other bacterial species were then discovered in freshwater or marine environments [Lefevre2013]. The motility features associated with a magnetism-response has been called **magnetotaxis**. Another property in relation with the ecological niche where these bacteria live i.e. oxic-anoxic interfaces, is their **chemotactic response,** which makes them escape from oxygen-rich regions to gather in places with low oxygen concentration [Popp2014]. Finally, a less well-studied property of MTB is their **ability to respond to light**. Several species were indeed reported to react to such trigger [Chen2011].

Overall, **MTB** are remarkable as their **movement and organization** can be **remotely controlled by external fields**: primary the magnetic field, but also the hydrodynamic, chemical, or light fields. Our project aims at capitalizing on those unique capabilities to obtain new fluids with original properties.

We expect that this **original combination of motility and reaction to external fields** will lead to **novel transport properties** and modifications in the constitutive relations with a possible emergence of **macroscopic flows** or stresses. Furthermore, the nature of the suspending fluid can be varied as it can be a simple viscous liquid but also a complex viscoelastic fluid opening **new perspectives to manipulate the constitutive properties of the fluid** matrix. Remarkably, **all these activable properties can be adjusted to a given situation by a retroactive action via the external fields**. In this sense, from an engineering perspective, such system is a "smart material".

- **Transport of MTB in complex environments-** Soon after MTB were discovered, a vivid line of research was set to understand and genetically engineer the cells in order to create novel strains with prescribed functionalities. By internalization of various metallic compounds dispersed in the environment, they could for instance act as depolluting machines [San19]. Another long-sought application has been to exploit the potential of MTB as controllable vectors for drug delivery. Playing concurrently with other external fields has proven beneficial in this endeavor: The alliance of magnetotaxis and micro-aerotaxis was used as a promising drug vector for cancer therapy since tumor cells produce oxygen-depleted surroundings that attract the MTB [Health2016].

Such studies though, are based solely on mesoscopic approaches. **Microscopic studies on the mechanisms at work in such processes are still missing**. Drug delivery and bioremediation both rely on the ability of MTB to invade the surrounding medium, such as living tissues or sediments. Both are complex environments with interstitial fluid and disordered microstructure and possibly with flows. **How exactly the invasion process happens in those conditions is currently poorly understood**. Several recent clues point at the key role of bacterial motility in the dispersion process. Both experiments with *Escherichia coli* and theory suggest that the motile character of transported species deeply alters the classic, hydrodynamic picture in view of what is currently known for colloids [Creppy2019, Junot2019]. It is thus clear that **understanding at a fundamental and microscopic level, transport properties in confined, geometrically or rheologically complex environments is a crucial step** to be able to consider any reliable applications in the domains of medical therapy or environmental engineering in the future.

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CE30 - Physique de la matière condensée et de la matière diluée		

Our **first objective** will be to understand the magnetic **field-assisted transport of MTB in** confined, geometrically and rheologically **complex environments. We will develop model microfluidic experiments** changing the geometrical features of the flow or the nature of the suspending fluid.

- **Emergence of Tunable Rheological Properties-** Magnetically driven particles suspended in a fluid confer novel constitutive properties to the suspension. These properties are tunable under the action of a magnetic field [Shlo72]. Such magnetorheological fluids have long been studied. A more recent realization is based on the **microscopic motility of the individual entities leading to a paradigmatic shift in emergent constitutive relations** [Lopez2015, Martinez2020]. In this case, the feeding sources of energy being microscopic, a collection of such objects is bound to self-organize at high or moderate concentrations, producing new phases [Theurkauff2012, Bricard2013], as well as novel hierarchical structures [Aubret2018, Vincenti2018], impacting the suspension rheology [Vincenti2019].

Our **second objective** will be **to merge the two lines of research on the effect of motility and magnetic moments,** that have so far mostly been explored independently. Their confluence offers remarkable perspectives for developing fluids with on-demand rheological properties.

The proposal is structured along three main axes and the work program is the following:

Axis 1- Motility of MTB.

An important step in addressing the issues outlined above is to first **characterize** and **model the individual swimming dynamics and properties** for the three species of MTB varying in magnetosome arrangement (chain vs. cluster, *Magnetospirillum* MSR-1 wild-type vs. Δ*mamJ* mutant) and cell morphology (MSR-1 vs *Magnetococcus* MC-1), providing a new look on MTB and crucial new information necessary for the interpretation of many of the following studies of the project.

- ✓ Develop the **3D tracking tools** developed for *E.Coli* (*Fig.1*) to monitor **MTB trajectories** (**PMMH**), transfer the tracking technique to the **BIAM** team and observe bacteria motility at larger scale on their microscopic platform that includes a magnetic system with 3D control via Helmholtz coils.
- ✓ MTB **swimming modes in various environmental conditions** (controlled magnetic field and oxygen concentrations) (**BIAM**). Characterization of their **phototactic** behavior.
- ✓ From the swimming strategies identified in the experiments, **modelling of the MTB swimming** trajectories (**ILM**).

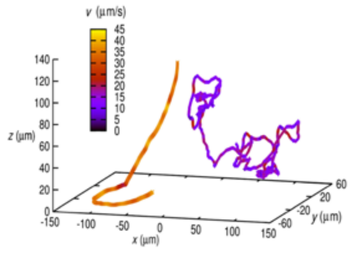

Fig.1 *E. Coli trajectories by Lagrangian tracking [Figueroa2020b]*

Axis 2- Transport of MTB in complex environments.

- ✓ **Transport** in **controlled 2D geometrical environments with obstacles** (**ILM**). Consider pilar network (*Fig.2*) (mimicking porous system), tuning characteristic distances λ between the obstacles (from few to tens of µm). In microfluidics, **individual tracking** of the MTB under the microscope.
- ✓ Transport in **a complex chemical environment** at a **collective level** (**BIAM**). **Collective motion** in varying gradient of oxygen, then in a varying magnetic field and finally in presence of obstacles.
- ✓ **MTB transport and dispersion in the presence of an external flow and external driving** (**PMMH**). Characterize how external handles modifies the swimming kinematics.

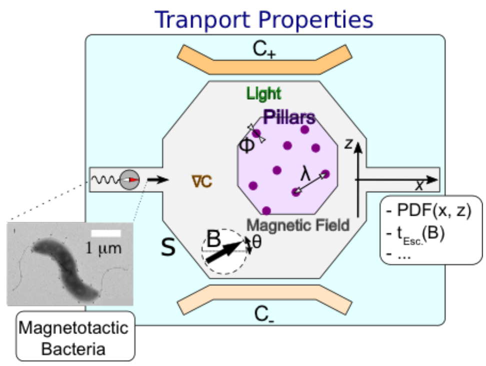

Fig.2. *Schematics of transport properties studies*

The **impact of the magnetic field** is expected to be decisive and we will seek to **identify those optimal protocols that will be most efficient to enhance transport.**

Axis 3- Emergence of Tunable Rheological Properties.

- ✓ Preparation of large bacteria quantities (**BIAM**) to obtain significant active magneto-rheological effects.
- ✓ Active magneto-rheology (**PMMH**) with a Y shape microfluidic rheometer (**Fig.C**). Characterize the viscosity, as a **function of the flow rate** and the direction of **B.**
- ✓ Micro-rheology properties of complex magneto-activable fluids (**ILM**), considering MTB, as an **additive to a non-Newtonian fluid**: Carbopol -a transparent yield-stress fluid.

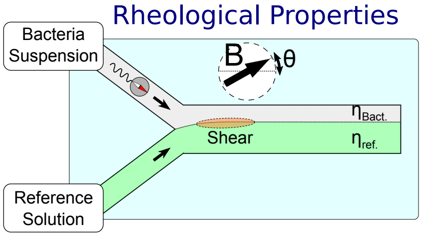

***Fig.3** Rheological properties using Y-Shape channel see [Gachelin2013]*

The **external control of motility orientation via the magnetic field that may be combined with temporal or spatial driving,** this will allow to remotely alter the spontaneous organization and microstructure, and thereby the macroscopic constitutive relations.

II. Consortium

PI: Cécile Cottin-Bizonne (60%) is a CNRS researcher, team leader of the "*Liquid and Interfaces*" group at ILM (~40 members). She has a strong experience in leading collaborative research and a recognized expertise in active matter with abiotic systems or bacteria. She was invited to more than 25 conferences. In 2021, she co-organizes a workshop "Active matter: the next 25 years" in Leiden.

The BACMAG consortium regroups researchers **from interdisciplinary and complementary disciplines** around a challenging project that will answer fundamental questions with future social impact in environmental and medical applications. The ILM team and the PMMH team have **pioneered the issues developed in this proposal in terms of response and self-assembly of MTB in suspension**. The ILM team has shown that the conjunction of flow and magnetic field is able to create coherent moving MTB swarms [Waisbord2016]. The PMMH team has recently demonstrated that MTB dispersed in an emulsion self-assemble in the droplets under a magnetic field to form a rotary motor [Vincenti2019]. Both teams are recognized specialists of active matter with experience in bacterial and abiotic systems. They **have developed complementary approaches** in the understanding of the organization and transport properties i.e. **statistical physics** (ILM) **and hydrodynamics** (PMMH). The third partner (BIAM) consists of **leading specialists in the magnetoaerotaxis and ecology of MTB**. They have a strong and recognized expertise in the genetic and microbiological aspect of such strains associated with its relationship to motility response in various environments. They **have a culture collection of MTB species** with different morphologies and different magnetoaerotactic behavior that would be useful for the two axes. ILM and BIAM have already collaborated together [Waisbord2016].

ILM (Liquid at Interfaces, ILM, Université Lyon 1): C. Cottin-Bizonne (DR) and Christophe Ybert (DR 20%) have developed over the recent years a strong activity on active matter. They designed statistical physics models of self-propelled colloids to study sedimentation, equation of state and clustering effects. They also developed additional studies around biological MTB (strain MC-1) in microfluidic systems. François Detcheverry (20%,CR) who is doing simulations and modeling on active matter will also join the consortium to bring his expertise on the theoretical side.

PMMH (PMMH, ESPCI, Paris) E.Clément (Pr, Co-PI 50%), T.Darnige (IE 20%), X. Benoit-Gonin (IE 20%). The PMMH group developed a model approach on bacterial fluids laden with motile *E.coli* and more recently worked with *Magnetospirillum gryphiswaldense* MSR-1 MTB. They studied transport properties, hydrodynamic dispersion [Creppy2019, Figueroa2020b], rheology and the emergence of collective motion in *E. Coli* suspension using various microfluidic techniques [Lopez2015, Martinez2010]. They also develop a unique tool of 3D Lagrangian tracking to reconstruct the tracks [Darnige2017, Figueroa2020].

BIAM (BIAM, CEA Cadarache) D. Faivre (DR, Co-PI 40%), C. Lefèvre (CR, 15%), and S. Grosse (Tech. 30 %). D. Faivre's research includes biomineralization by MTB and design and properties of synthetic and biological magnetic microswimmers. D. Faivre was the coordinator of a project on the swimming properties of MTB within a DFG priority program. He also received an ERC Starting Grant for a project combining biomineralization and development of bio-inspired microswimmers from which he developed a magnetic microscope that will be used for this project. The team will be complemented by C. Lefèvre, a specialist in the isolation, culture, genomic and evolution of MTB. His knowledge in the culture of different species of bacteria will be critical for the success of the project.

This project is a resubmission (project finally ranked 1st on the waiting list last year). Following recommendations, we clarified the different tasks. Should the project go to step 2 there would be a discussion on the risks for each task.

III. References related to the project

[Blake75] Blakemore R., *Magnetotactic bacteria*, Science, **190**, 377 (1975) DOI
[Bricard2013] Bricard A., *et al*., *Emergence of macroscopic directed motion in populations of motile colloids.* Nature, **503**, 95 (2013) DOI
[Chen2011] Chen C. et al., *Phototaxis in the Magnetotactic Bacterium Magnetospirillum Magneticum Strain AMB-1 is Independent of Magnetic Fields.* Appl. Microbiol. Biotechnol., **90** , 269 (2011) DOI
[Creppy2019] A.Creppy, E.Clement, C.Douarche, M.-V. D'Angelo, H.Auradou, *Effect of motility on the transport of bacteria populations through a porous medium,* Phys. Rev. Fluids, **4**, 013102 (2019) DOI
[Faivre2008] D. Faivre and D. Schüler, *Magnetotactic Bacteria and Magnetosomes*, Chem. Rev., **108**, 4875 (2008) DOI
[Darnige2017] T. Darnige, N. Figueroa-Morales, P. Bohec, A. Lindner, and E. Clément, *Lagrangian 3D tracking of fluorescent microscopic objects under flow*, Review of Scientific Instruments, **88**, 055106 (2017) DOI
[Detchev2017] F.Detcheverry, *Generalized run-and-turn motions: From bacteria to Lévy walks*, PRE **96**, 012415 (2017) DOI
[Figueroa2020a] Figueroa-Morales, N. et al. *E. coli* "super-contaminates" narrow ducts fostered by broad run-time distribution. Sci. Adv. **6**, eaay0155 (2020). DOI
[Figueroa2020b] N. Figueroa-Morales, T. Darnige, C.Douarche, V. Martinez, R. Soto, A. Lindner, E.Clément, 3D spatial exploration by E. coli echoes motor temporal variability Phys.Rev.X, **10**, 021004 (2020). DOI
[Gachelin2013] J.Gachelin, G.Mino, H. Berthet, A.Lindner, A.Rousselet, Clement, *Non-Newtonian active viscosity of E-coli suspensions*, Phys.Rev.Lett. **110**, 268103 (2013). DOI
[Ginot2018] F. Ginot, I. Theurkauff, F. Detcheverry, C. Ybert, C. Cottin-Bizonne*, Aggregation-fragmentation and individual dynamics of active clusters*. Nat. Com. **9** (2018) DOI
[Health2016] O. Felfoul et al., *Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions*, Nat Nanotechnol., **11**, (2016) DOI S. Martel *et al*, *Micromachines,Switching between Magnetotactic and Aerotactic Displacement Controls to Enhance the Efficacy of MC-1 Magneto-Aerotactic Bacteria as Cancer-Fighting* Nanorobots, **7**, 97 (2016) DOI
[Junott2019] G. Junot, N.Figueroa, T.Darnige, A. Lindner, R. Soto, H.Auradou, E. Clément, *Bacterium swimming in Poiseuille flow: the quest for active Bretherton-Jeffery trajectories*, Europhys. Lett, **126**, 44003 (2019) DOI
[Lefevre2013] C.T. Lefèvre, D. A. Bazylinski, Ecology, *Diversity, and Evolution of Magnetotactic Bacteria, Microbiology and Molecular* Biology Reviews, **77**, 497 (2013) DOI
[Lefevre2014] C.T. Lefevre, […], D.A. Bazylinski, R.B. Frankel, S. Klumpp, D. Faivre, *Diversity of magneto-aerotactic behaviors and oxygen sensing mechanisms in cultured magnetotactic bacteria,* Biophys. J., **107**, 527 (2014) DOI
[Li2019] Q. Li, H. Chen, *et al* ,*Nanoparticle-regulated semiartificial magnetotactic bacteria with tunable magnetic moment and magnetic sensitivity,* Small, **15**, 1900427 (2019) DOI
[Lopez2015] H. M. Lopez., J. Gachelin, C. Douarche, H. Auradou, E. Clement, *Turning Bacteria Suspensions into Superfluids*. Phys. Rev. Lett., **115**, 028301 (2015) DOI
[Martinez2020] V. Martinez, E. Clément, J. Arlt, C. Douarche, A. Dawson, J. Schwarz-Linek, A. Creppy, V. Skultéty, A. Morozov, H. Auradou and W. Poon, A combined rheometry and imaging study of viscosity reduction in bacterial suspensions*,* Proceedings of the National Academy of Sciences, **117**, 2326-2331 (2020). DOI
[Mathijssen2019] A.Mathijssen, N. Figueroa-Morales, G. Junot,E. Clement, A.Lindner, A. Zöttl*, Oscillatory surface rheotaxis of swimming E. coli bacteria*, Nature Com. **10**, 3434 (2019) DOI
[Theurkauff2012] I. Theurkauff, C. Cottin-Bizonne, J. Palacci, C. Ybert, L. Bocquet. *Dynamic Clustering in Active Colloidal Suspensions with Chemical Signaling*, Phys. Rev. Lett. **108**, 268303 (2012) DOI
[San19] S. Sannigrahi, *et al, Metal recovery from printed circuit boards by magnetotactic bacteria*, Hydrometal. **187**, 113 (2019) DOI S. Staniland, *et al*, *Controlled cobalt doping of magnetosomes in vivo,* Nature Nanotech., **3**, 158 (2008) DOI
[Shlo72] M. I. Shlomis, *Effective Viscosity of Magnetic Suspensions*, Zh. Eksp. Teor. Fiz. **61**, 2411-2418 (1972).
[Vincenti2018] *B.* Vincenti, C. Douarche and E. Clement*, Actuated rheology of magnetic micro-swimmers suspensions: Emergence of motor and brake states*, Phys. Rev. Fluids **3**, 033302 (2018) DOI
[Vincenti2019] B. Vincenti, G. Ramos, M.-L. Cordero, C. Douarche, R. Soto, E. Clement, *Magnetotactic bacteria in a droplet self-assemble into a rotary motor,* Nat. Comm. 10.1038 (2019) DOI
[Waisbord2016] N. Waisbord, C. Lefevre, L. Bocquet, C. Ybert, C., Cottin-Bizonne, *Destabilization of a flow focused suspension of magnetotactic bacteria.* Phys. Rev. Fluids, **1**, 053203 (2016) DOI