

Active matter and biology at the PMMH

The projects currently developed at the PMMH, in the “*active fluids group*” are oriented towards applying the concepts of “active matter” to the dynamics and hydrodynamics of bacterial populations. Active matter is a subject at the crossing point between statistical physics, hydrodynamics and biology. In this context, the Active fluid group is currently assessing the swimming properties of motile bacteria exploring individually or collectively, different controlled environmental situations. We are using soft-lithography microfluidic techniques to design micro-channels representing different levels of geometrical complexity.

Varying the chemical and rheological properties of the suspending fluid, we currently seek to determine the emergent transport properties of bacterial suspensions and relate individual swimming properties to the large scale transport processes. To this purpose, we developed an original tools such as an automated Lagrangian tracking device (see Fig.1) suited to follow in 3D, fluorescent motile bacteria and eventually visualize in situ their flagellar dynamics.

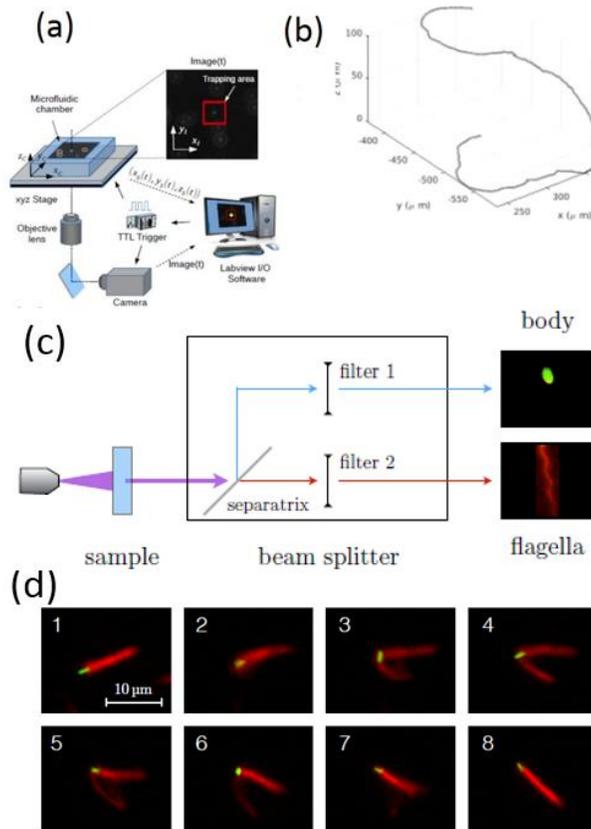


Figure 1 – Lagrangian tracking device allowing a 3D tracking of fluorescent bacteria in microfluidic channels. (a) Schematic presentation of the feedback loop acting on a bacterium body image producing a XYZ motion of 3 mechanical and piezoelectric stages controlled numerically such as to maintain the fluorescent object in the focal plane and in a virtual trapping area (inset). (b) Reconstruction of a swimming trajectory of a wild-type *E. coli*. The bacterium is starting from the upper glass wall and reaches the lower wall. (c) Extension of the tracking device by the adjunction of a dichroic splitter allowing an image reconstruction of the body (in green) and flagella (in red). 3D tracking is done on the green body image. (d) Time lapse of a bacterium undergoing a tumbling event in a flow and leading to a change of swimming direction.

We already obtained important information on the swimming and exploration properties of model *E. coli* bacteria, below and above the gel transition of a model complex fluid (poly-acrylic acid polymer). A salient feature of this study pointed on the crucial importance of mechanical heterogeneities controlling the exploration process even deep in the gel phase (Hector Urrea PhD thesis).

Recent publications (2017-2020) of the PMMH-Active fluid group

- **3D spatial exploration by *E. coli* echoes motor temporal variability.** N. Figueroa-Morales, T. Darnige, C. Douarche, V. Martinez, R. Soto, A. Lindner, E. Clément, to be published Phys. Rev. X (2020) arXiv:1803.01295.
- ***E. coli* « super-contaminates » narrow channels fostered by broad motor switching statistics.** N. Figueroa-Morales, A. Rivera, E. Altshuler, R. Soto, A. Lindner, E. Clement, Science Advances, on line March 13th (2020). arXiv:1904.02801v1
- **A combined rheometry and imaging study of viscosity reduction in bacterial suspensions,** V. Martinez, E. Clément, J. Arlt, C. Douarche, A. Dawson, J. Schwarz-Linek, A. Creppy, V. Skultéty, A. Morozov, H. Auradou and W. Poon, Proceedings of the National Academy of Sciences, **117**, 2326-2331 (2020).
- **Vortex flow generation in magnetotactic bacteria droplets,** B. Vincenti, G. Ramos, M.-L. Cordero, C. Douarche, R. Soto, E. Clement, Nature Comm, **10**, 5082 (2019).
- **Oscillatory surface rheotaxis of swimming *E. coli* bacteria ,** Mathijssen, N. Figueroa-Morales, G. Junot, E. Clément, A. Lindner, A. Zöttl, Nature Comm. **10**, 3434 (2019).

- ***Bacterium swimming in Poiseuille flow: the quest for active Bretherton-Jeffery trajectories***, Junot, N. Figueroa-Morales, T. Darnige, A. Lindner, R. Soto, H. Auradou, E. Clément, *Europhys. Lett*, **126**, 44003 (2019).
- ***Effect of motility on the transport of bacteria populations through a porous medium***, A. Creppy, E. Clément, C. Douarche, M.-V. D'Angelo, H. Auradou, *Phys. Rev. Fluids*, **4**, 013102 (2019).
- ***Actuated rheology of magnetic micro-swimmers suspensions: Emergence of motor and brake states***, B. Vincenti, C. Douarche and E. Clément, *Phys. Rev. Fluids* **3**, 033302 (2018).
- ***E coli Accumulation behind an Obstacle***, G. L. Miño, M. Baabour, R. Chertcoff, G. Gutkind, E. Clément, H. Auradou, I. Ippolito, *Adv. Microbiology*, **8**, 451-464 (2018).
- ***Viscosité d'une suspension de bactéries : des efforts individuels aux efforts collectifs***, H. Auradou, C. Douarche, A. Creppy, H.-M. Lopez et É. Clément, *Reflets phys.* **57**, 20-23 (2018).
- ***Lagrangian 3D tracking of fluorescent microscopic objects under flow***, *Review of Scientific Instruments*, T. Darnige, N. Figueroa-Morales, P. Bohec, A. Lindner and E. Clément, **88**, 055106 (2017).

Subject title - Crossing of the mucus barriers by motile microorganisms: from physical models to new cues on inflammatory-related pathologies.

State-of-the-art - Living species have designed physical defense barriers, such as mucus layers covering the epithelial cells, to avoid tissue colonization by microorganisms. In infectious diseases, the increase in microbial motility is associated with the degree of virulence; serious microbial infections were shown to stem directly from a breach in the barrier [Ottemann, *Mol. Microbiol.* **24**, 1109 (1997)]. However, changes in bacterial motility have been shown not only in the conditions of infection (i.e. by listeria or Salmonella), but also in lower inflammation status such as cancers [Allali, *Gut Microbes* **6**, 161 (2015)] or intestine inflammation. In high fat diet induced rodent obesity, metagenomics analysis identified the importance of metagenomics signatures related to the bacterial motility machinery [Zheng, *J. Diabetes* **11**, 32 (2019)]. However, in spite of the multiple recent evidences pointing on the role of motility in inflammatory related pathologies, there is no clear mechanistic model relating bacteria motility to the inherently complex environment displayed by the mucus barriers.

Collaborative team - This project, is a collaboration with two teams of medical doctors and biologists

(i) Prof. K. Clément (Sorbonne University/Inserm, UMRS1269 "NutriOMics" Pitié-Salpêtrière) specialist of obesity related pathologies and gut microbiota metagenomics. **Prof. K. Clément will be the co-director of the doctorate.** Her team has showed the importance of gut microbiota dysbiosis [Cotillard, *Nature* **500**, 585, 2013] in metabolic diseases in relation with diet [Shoae, *Cell Metabolism*, **22**, 320, 2015]. They demonstrated in obesity, the link between the gut barrier alteration and inflammation [Genser, *J. Pathol.* **246**, 217, 2018].

(ii) Dr B. Chassaing (Institut Cochin-INSERM U1016) an INSERM is a microbiologist who recently joined the Cochin Institute in Paris. With pioneering experiments and seminal papers, he showed that microbiota encroachment is a feature of inflammatory diseases with bacteria penetrating the normally sterile inner mucus layer [Chassaing, *Nature* **519**, 92 (2015)]. He also revealed the importance of flagellar expression in inflammatory processes [Cullender, *Cell Host Microbe* **14**, 571–581 (2013)].

In 2020 the three teams started a collaboration around a common post-doc (Tiphaine Le Roy, January-December 2020, PMMH/Pitié-Salpêtrière) and a PMMH-Sorbonne University doctorate (Hector Urrea) who will finish in December 2020. The present proposal is an extension of this work which has already provided preliminary results on the possibility to track bacteria in mucin gels at different concentrations. The current objective is to prepare directly mucus out of in vitro goblet cells (cells producing mucus) and submit this culture to various bio-chemical strains such as to obtain mucus in which the motility features of different bacteria can be tested.

Working hypothesis - The main hypothesis underlying this doctoral project is that in metabolic diseases (obesity, diabetes) dramatic switches in bacterial motility occur due to changes in the intestine environment (mucus composition unbalance, low-grade inflammation, microbiota dysbiosis), hence promoting alterations in mucus/microbiota links (see Fig.2). Therefore there is a fundamental interest in establishing a direct connection between the swimming properties of various motile bacteria in a mucus gel (eventually partially altered by biochemical conditions stemming from the diet) and the emergence of inflammatory diseases when bacteria are able to cross the mucus protective layer.

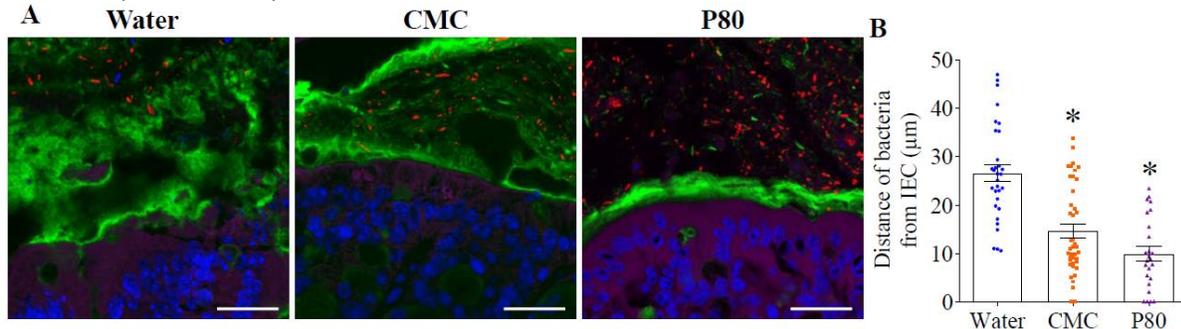


Figure 2 Alterations in mucus/microbiota link - Direct evidence that dietary emulsifiers commonly used to process industrial food (CMC or P80) alter the microbiota localization in an animal model (mice). Microbiota localization for water-, CMC- and P80- treated animals. The mucus localization (*Muc2*) is in green, actin in purple, bacteria in red and epithelial cell nuclei in blue. B: Distances for the closest bacteria to the intestinal epithelial cells [after Chassaing, *Nature* **519**, 92 (2015)].

Doctoral project – The doctoral project is structured in three parts:

- (i) The first objective of the doctorate is to establish reliable physical and mechanical picture of bacterial transport through complex fluids addressing explicitly the role of local heterogeneities either geometrical or mechanical in the exploration process. The project will start with Lagrangian tracking experiments on different standard complex fluids to elaborate hydrodynamic and stochastic theoretical models describing swimming and tumbling in such environments. This knowledge will be used to test directly mucin gels which have been prepared in vitro under different conditions, also using the Lagrangian tracking technique. To assess geometrical heterogeneities in the gels we will use confocal microscopy and low angle diffusion scattering. Complementary macroscopic and microscopic rheology techniques will be performed to identify and characterize the heterogeneities present in the mechanical landscape directly experienced by the swimming bacteria. In connection with the group of B. Chassaing different microorganisms implied in epithelial inflammatory processes will be tested.
- (ii) Then, a direct connection will be made with mucus extracts from animal models submitted to various diets (connection with the group of K. Clément) to develop tools for rheological characterization including geometrical and mechanical heterogeneities, and compared with the complex fluid models developed in part (i).
- (iii) Finally, in collaboration with both teams, we will aim to design novel standardized assays to provide indications on the gut barrier quality by quantifying, for various mucus extracts, the penetration of a model bacterium.

In conclusion, we expect from this research, to be able in conjunction with the medical and microbiologist teams, to obtain news results on the penetration capacities of gut bacteria in mucus protective layers at different stage of its degradation and bring some new cues on the emergence of low grade inflammatory diseases.