Title of the PhD project:

A Biomimetic Approach of Cell-Cell Communication

PhD Supervisor

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Title of the team : Mechanics of artificial and integrated biological systems
Team leader (*if different*) *:*
Doctoral School : ED 564 : Physique en Ile de France

Overview of the scientific projects of the team

L'équipe MEBS s'intéresse aux propriétés mécaniques des systèmes biologiques intégrés et artificiels. Nous étudions d'une part les processus de transduction mécanique depuis l'échelle macroscopique (Collaborations B. Le Reverrend, Nestlé, E. Lauga, Cambridge) jusqu'aux échelles moléculaires et cellulaires (Collaboration V. Noireaux, Univ. Minnesota), en combinant approches biomimétiques et un système biologique simple, la paramécie (Collaboration R. Brette, Inst.de la Vision). Nous étudions d'autre part la mécanique de tissus biomimétiques modèles. Il s'agit ici d'étudier la réponse élasto-plastique d'émulsions soumises à des perturbations mécaniques contrôlées, afin de mieux comprendre les bases physiques qui sous-tendent le remodelage collectif de tissus biologiques (Collaboration T. Bertrand Imperial College, London UK).

Main publications since January 1er, 2016

1. J.-B. Thomazo, E. Lauga, B. Le Révérend, **E. Wandersman** and A. M. Prevost. Collective stiffening of soft hair assemblies. *Phys. Rev. E* **(R)** 102, 010602 (2020).

2. M. Valet, L.-L. Pontani, R. Voituriez, **E. Wandersman** and A. Prevost (2019) Diffusion through Nanopores in Connected Lipid Bilayer Networks, *Phys. Rev. Lett.* **123** 088101.

3. J.-B. Thomazo, J. Contreras Pastenes, C. Pipe, B. Le Révérend, **E. Wandersman** and A. M. Prevost (2019) Probing the in-mouth texture perception with a biomimetic tongue, *J. Roy. Soc. Interface* **16** (159) 20190362.

4. M. Valet, L.-L. Pontani, A. Prevost and **E. Wandersman** (2018) Quasi-static microdroplet production in a capillary trap, *Phys. Rev. Applied* **9** 014002.

5. N. Claverie, Y. Boubenec, G. Debrégeas, A. Prevost and **E. Wandersman** (2017), Whisker contact detection of rodents based on slow/fast mechanical inputs, *Frontiers Behav. Neuroscience,* **10**, 251.

PhD Co-Supervisor

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Team leader *(if different) : NA*
Doctoral School : NA

Overview of the scientific projects of the team

Noireaux Lab has developed a cell-free transcription-translation (TXTL) platform to construct biochemical systems in vitro by executing synthetic gene circuits. Unlike the other cell-free expression systems, our platform is based on an E. coli extract that uses the endogenous TX and TL machineries. The circuits (plasmids or linear DNA) are executed in a cell-free TXTL mix entirely prepared in our lab. Our research is based on this unique system and includes: (I) prototyping regulatory elements and circuits, (II) quantitative biology of self-assembly with phages as models, (III) bottom-up construction of a minimal cell, (IV) application to biotechnologies and medicine. Our work is both fundamental and applied and covers the research areas of synthetic biology and quantitative biology such as biological physics.

Main publications since January 1er, 2016

Noireaux, V., Liu, A.P. (2020). The new age of cell-free biology. *Annu. Rev. Biomed.* **22**, 51-77.

Garenne, D., Libchaber, A.J., **Noireaux, V.** (2020). Membrane molecular crowding enhances MreB polymerization to shape synthetic cells from spheres to rods. *Proc. Nat. Acad. Sci. USA* **117**, 1902-1909.

Agrawal, .K., Marshall, R., **Noireaux, V.**, Sontag, E.D. (2019). In vitro implementation of robust gene regulation in a synthetic biomolecular integral controller. *Nat. Commun.* **10**, 5760.

Marshall, R., **Noireaux, V**. (2019). Quantitative modeling of transcription and translation of an all E. coli cell-free system. *Sci. Rep.* 9(1), 11980.

Garamella, J., Majumder, S., Liu, A.P., **Noireaux, V**. (2019). An adaptive synthetic cell based on mechanosensing, biosensing, and inducible gene circuits. *ACS Synth. Biol.*, **8(8)**, 1913-1920.

Doctoral Project

Title: A Biomimetic Approach of Cell-Cell Communication

Abstract : Cell-cell communication participates in regulating and synchronizing cellular functions. In tissues, the transport of ions and molecules from a cell to another occurs in particular through protein nanometric pores across the cells membrane. Such nanopores can be inert (simple nano-holes) or mechanosensitive with an ionic/molecular permeability that depends on the stress acting on the membrane. This project proposes a biomimetic approach of cell-cell communication in tissues. Tissues will be mimicked with 2D arrays of aqueous droplets connected by lipid membranes decorated with transmembrane proteins, inert or mechanosensitive. Mechanosensitive proteins will be synthetized directly within the droplets using cell free Transcription Translation (TxTl) reactions. For inert networks, we will probe with epifluorescence microscopy how the diffusion of Ca^{2+} ions depends on the network topology and the pores concentration. For mechanosensitive networks, we will probe with a rheo-microscope how controlled deformations of the network affect the transport properties. Our results will be modeled using random walks in nanoporous media, in which the opening gate probability depends on the local stress.

Context and objective :

This project focuses on cell-cell communication, the process by which cells exchange physical or chemical information. Cell-cell communication plays a fundamental role in the regulation and synchronization of cellular functions [1-3]. One particular communication mode, that exists in cell tissues when cells membranes are in direct physical contact, takes place via protein clusters or gap junctions that form channels across the membranes of adjacent cells [2,4,5-7]. These protein channels can be either inert, i.e. simple passive nanometric holes or mechanosensitive. Under mechanical stresses, the ionic and molecular permeability of some gap junction is modified [4,8]. The transport of ions and molecules at the tissue scale can therefore be affected by external mechanical stresses. Moreover, the external stresses can in turn perturb the tissue shape, its remodeling and thus its topology [9]. There are therefore complex couplings between the chemical transport *at the tissue scale* and stresses at *the cell membrane scale* in cellular tissue, that we wish to elucidate.

Fully describing the transport of ions and molecules in cell populations is thus complex as it stems from multiple communication modes that can also be combined [2] and hard to disentangle.

To improve our understanding of cell-cell communication, we will use a simplified biomimetic approach, coupled to a theoretical, statistical physics approach. We will mimic a cellular tissue with a 2D network of Droplet Interface Bilayers (DIBs, [10]). DIBs consist of aqueous droplets as analogues of cells, connected by lipid membranes decorated with a single type of transmembrane pores either inert or mechanosensitive. Such proteins will be expressed directly within the droplets using synthetic biology transcription-translation reactions (TxTL) thanks to our collaboration with Vincent Noireaux (University of Minnesota, USA) who will be co-director of the PhD project [14,15]. Our aim will be to measure the transport properties of calcium ions (since Ca^{2+} is a key player in cell regulation processes) across both inert and mechanosensitive 2D networks. When the communication gates are mechanosensitive, we will study how a mechanical perturbation affects the diffusion properties.

The first objective of the project is technical and will consist in designing biomimetic tissues with a controlled topology. We will use a droplet printer to accurately position aqueous micro-droplets in an oil/lipid bath, as precursors of the DIB network. The printer's mode of operation will rely on a new

droplet formation mechanism that we have identified recently [11-13]. Our aim will be to control precisely the topology of the network while keeping a high rate of droplet production.
Our second objective will be to probe and model how calcium ions diffuse in the DIB networks, when all DIBs are decorated with inert nanopores. This will be done using calcium imaging and fluorescence microscopy techniques. We will investigate how the diffusion laws depend on the coordination of the network (hexagonal versus square arrays) on the concentration of nanopores, on the size of the droplets and on the adhesion between them. Theoretically, we will model the data using Continuous Time Random Walks to describe the diffusion of the ions in the network, with a characteristic time set by the first passage time to the pores.
The third objective of the project will consist in producing DIB networks in which the mechanosensitive protein MscL [16-19] are embedded. The PhD student will spend 3 months in Vincent Noireaux's lab to be trained to the use of synthetic Biology tools. In particular, he will learn how to control the concentration of expressed mechanosensitive proteins (tuning the plasmid concentration and/or the physico-chemical properties of the reaction). He will also work on expressing the mutant MscL protein V23T wich has a lower stress threshold of activation [20], compared to the wild type protein.
Back at LJP, our aim will be to excite mechanically the network and study how such perturbations affect the transport properties of Ca^{2+} ions. A first step will consist here in developing a micro-rheological setup made of a pool equipped with agar piston. Displacement of the piston can be used to compress the droplets and form the DIB network (see a preliminary movie here) and, once formed to apply periodic deformation using a piezo electric transducer (see a preliminary movie here). We will couple this mechanical excitation setup to calcium imaging microscopy, to probe ion transport. We will build the mechano-chemical transfer function of this artificial tissue, by investigating how the characteristic diffusion time of calcium ions depends on the imposed deformation amplitudes and frequencies. We will also measure how a spatial pattern of diffusion can be modulated by the applied stress. Experimental results will be confronted to theoretical models of transport in mechanosensitve network, that will be developed all along the project. Theoretically, we will use random walk models in which the mechanosensitive and inherently stochastic nature of the gates is taken into account. Transport laws in presence of a time fluctuating stress landscape will be predicted.

References

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Justification of suitability for *i-Bio*:
Due to its intrinsic interdisciplinary character at the interface between soft condensed matter Physics and Biology, the PhD proposal falls well within the scope of the doctoral project's call "i-Bio". The project will also help in developing new synthetic Biology tools at LJP and at IBPS.

Role of each supervisor / skills provided:
Elie Wandersman will coordinate the scientific program. On a technical side, he will supervise the PhD student for:
- the microfluidics/microfabrication aspects of the project (droplet formation, surface patterning to trap the droplets.)
- the physico-chemical aspects (DIB stability, adhesion control…)
- the rheological aspects (DIB network deformation)
- the imaging/image analysis aspects (Ca^{2+} imaging, extraction of droplet shape and fluorescence data…)

Vincent Noireaux will supervise the student for the synthetic biology aspects of the project. The student will spend 3 months in his lab to be trained using the TxTl tools.

Profile of the desired student:
Experimental practice in either biophysics, physico-chemistry, mechanics, optics or image analysis will be assets for the candidate. Knowledge in synthetic Biology, Statistical Physics, Hydrodynamics will be appreciated.