Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI 2020

Intitulé du Projet de Recherche Doctoral :
A Biomimetic Approach of Cell-Cell Communication

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

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Unité de Recherche :
Intitulé : Laboratoire Jean Perrin
Code *(ex. UMR xxxx)* : UMR 8237

ED564-Physique en IdF
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant :

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 0

Co-encadrant :

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Unité de Recherche :
Intitulé : Noireaux's Lab
Code *(ex. UMR xxxx)* : University of Minnesota, Minneapolis, USA

Ecole Doctorale de rattachement : x
Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 2
Aset Khakimzhan (2018 – 2022), 100%
Juan Velasco (2019 – 2023), 100%

Cotutelle internationale : Non x Oui

PhD proposal at Laboratoire Jean Perrin – LJP, IBPS, SU/CNRS

A Biomimetic Approach of Cell-Cell Communication

Abstract

Cell-cell communication participates in regulating cellular functions. In tissues, the transport of chemicals across cells membranes can occur through protein nanopores, either inert or mechanosensitive. We will study this specific communication with a biomimetic approach. Tissues will be mimicked with 2D arrays of aqueous droplets connected by lipid membranes decorated with inert or mechanosensitive nanopores. In inert networks, we will probe with fluorescence microscopy how the ion transport depends on the network topology and the pores concentration. In mechanosensitive networks, we will probe how deformations of the network affect the transport. Our results will be modeled using random walks in nanoporous media.

1 – Context and objectives

Cell-cell communication plays a fundamental role in the regulation and synchronization of cellular functions [1]. The exchange of chemical information between cells allows for example, a control of their proliferation, apoptosis and differentiation during morphogenesis [2], or even of their response to chemical stimulations [3]. Such communication occurs via many different modes depending notably on whether cells are connected physically or not. When they are well separated, communication can for instance occur through ion channels, which are transmembrane proteins embedded in the plasma membrane. In this case, ions can be transported through these nanopores to the extra cellular medium in which they can diffuse. Communication between separated cells can also occur via physical links. Tunneling nanotubes have been observed between different cells [2] and shown to coordinate metabolism and signaling. When cells membranes are in direct physical contact (such as in an epithelial tissues), communication can take place via protein clusters that form large channels across membranes of adjacent cells [2,4,5]. In animal cells, these are called “gap junctions” and in vegetable cells, they are referred to as “plasmodesmata”.

Fully describing the transport of ions and molecules in populations of cells is thus complex as it can stem from multiple communication modes that can be combined [2] and hard to disentangle. Another source of complexity arises from the fact that protein channels can be either inert (simple passive nanometric holes) or mechanosensitive. Under mechanical stresses, the ionic and molecular permeability of mechanosensitive ion channels [6,7] or gap junctions [4,8] is modified. The transport of ions and molecules at the tissue scale is therefore affected by external mechanical stresses. Moreover, the external stresses can in turn perturb the tissue shape and its remodeling [9] and thus its topology. There is therefore a non-trivial feedback loop between chemical transport *at the tissue scale* and stresses at *the cell membrane scale* in these systems.

To improve our understanding of cell-cell communication, one possible strategy consists in adopting a simplified biomimetic approach. In this way, we propose in this PhD project to 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 (Fig.1a), either inert or mechanosensitive. Such proteins will be synthetized directly within the droplets using synthetic biology transcription-translation reactions, thanks to an ongoing collaboration with Vincent Noireaux (University of Minnesota, USA) that has been established during a previous PhD thesis in our group [11-13]. Our aim is 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. Theoretically, we will provide a statistical description of the molecular transport in a connected tissue under mechanical stress using random walks in nanoporous media.

This PhD project is intrinsically interdisciplinary since it is at the interface between Soft Matter

Physics and Biology. It thus clearly fits the scope of the i-Bio initiative. The PhD thesis is expected to follow the three successive steps detailed just below.

2 – PhD thesis program

2.1 – A fast droplet printer to produce biomimetic tissues (month 1 to month 4)
The first objective of the PhD will be to design biomimetic tissues with a controlled topology. This will be done by building a printer of micro-droplets (diameter ~100 µm) with a high rate of droplet production. Its functioning will rely on a new droplet formation mechanism that we have identified recently, referred to as the capillary trap method [12]. A pending aqueous droplet is first grown through a glass capillary that bathes in a lipid/oil mixture (Fig. 1b, left image of inset). Once grown, the droplet is detached from the glass capillary by moving up the glass capillary across the oil/air interface (Fig.1b, middle image of inset). By keeping a continuous aqueous flow, the process can be repeated periodically to produce droplets that sink to the bottom of the container and eventually form assemblies.
We have built a preliminary droplet printer by adding an XY automated stage to sequentially move and hold the oil container at prescribed positions. We have also shown that a precise topology of the DIB network can be obtained by trapping the droplets into micro-spherical cavities at the bottom of the oil container. Square and hexagonal arrays can be designed (Fig.1c). The PhD candidate will first work on technical improvements and characterizations of this droplet printer, in order to speed up the network production and systematize the droplet production deposition. Our aim will also be to gain reproducibility on the network topology. This will facilitate the analysis of a large sample of networks to improve the statistical significance.

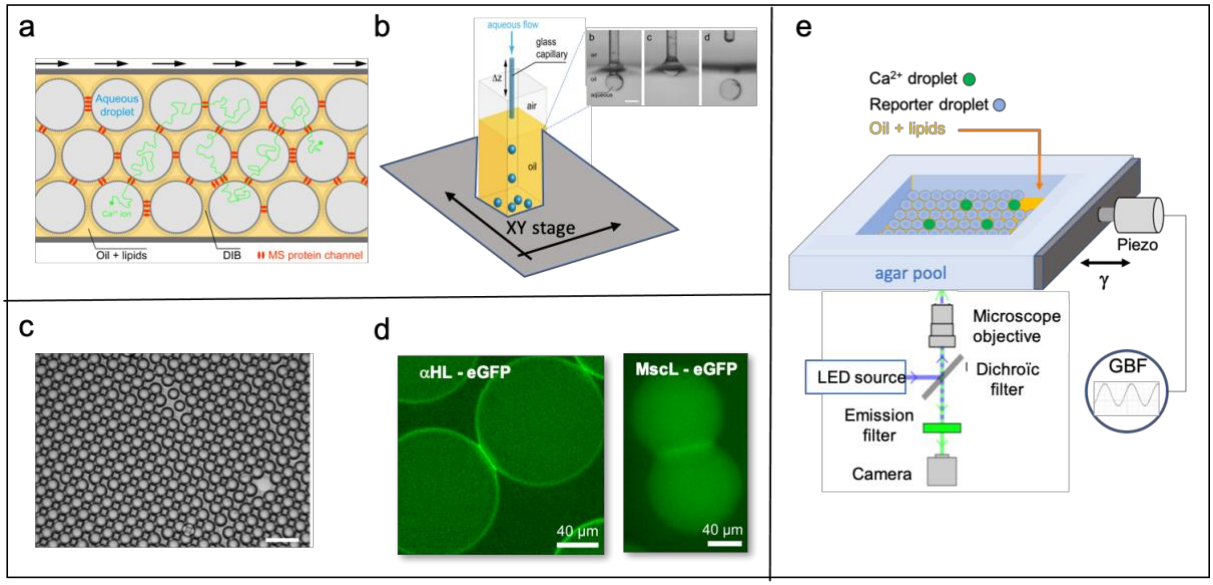

Figure 1. (a) *Principle of this project.* **(b)** *Sketch of the capillary trap droplet printer.* **Inset:** *zoom on the oil/air interface. Snapshots of the detachment process at different times in a cycle, from [12].* **(c)** *A square network of DIBs (the white bar is 1 mm long) made with our preliminary printer.* **(d)** *Stable DIBs encapsulating a TX-TL reaction. The transmembrane proteins αHL (left) and MscL (right) are tagged with an eGFP fluorescent protein.* **(e)** *Sketch of the proposed setup to mechanically excite a mechanosensitive DIB network while imaging molecular transport across the network.*

2.2 – 2D diffusion in artificial tissues of controlled topologies (month 5 to month 12)
The second objective of the PhD program will be to probe and model how calcium ions diffuse in the DIB networks, when all DIBs are decorated with inert αHL nanopores that can be purchased commercially. At t=0, droplets will be seeded with calcic reporters to the exception of some source droplets that will contain calcium ions. This will allow to probe for t>0 how calcium ions diffuse across the network using calcium imaging and an inverted fluorescence microscope (Fig.1e). We will investigate how the diffusion laws depend on the coordination of the network (hexagonal versus square arrays) and on the concentration of nanopores. Theoretically, we will model the data using Continuous Time Random Walks do describe the

diffusion of the ions in the network, with a characteristic time set by the first passage time to the pores [13].

2.3 – Ion transport in mechanosensitive networks under stress (month 13 to month 30)

The third objective of the PhD will be to produce mechanosensitive DIB networks with DIBs decorated with mechanosensitive proteins. These proteins will be expressed directly within the droplets using the synthetic biology tools that V. Noireaux has developed [14,15] and with whom we have been collaborating for several years. His transcription-translation reaction system (TX-TL) allows to express in vitro different types of proteins, in particular the mechanosensitive MscL protein that E. Coli bacteria use to prevent osmotic shock [16-18]. It is a non-selective pore, with an internal diameter of about 2.8 nm in its open state [19]. MscL proteins have been successfully expressed in giant liposomes, both by V. Noireaux and his team and at LJP (Fig. 1b), and we have very recently succeeded in producing stable DIBs which encapsulate the TX-TL reaction [11].
Mastering all production steps of the TX-TL reactions being crucial for this PhD project, the PhD candidate will spend 3 months in V. Noireaux's Lab from month 13 to month 16 of his thesis. In particular, the PhD candidate will develop methods to control the stability of DIBs encapsulating a TX-TL reaction, as well as the kinetics of expression of MscL in order to finely tune its concentration within the membrane. This will be obtained by varying the plasmid concentration in the reaction and/or the physico-chemical conditions. The candidate will also use the TX-TL reaction to produce the MscL mutant V23T which has a lower mechanical threshold of activation [20].

From month 17 to month 30, the candidate will come back at LJP to measure the transport of calcium ions in 2D mechanosensitive DIB networks, upon mechanical excitation. The network will be produced in an agar pool (Fig. 1e), so that the droplets at the boundaries are bound to the pool walls. Mechanical deformations of the agar pool walls will be directly transmitted to the DIB network (see a preliminary test here). Some inner portion of the network will be filled with droplets containing calcium ions. The rest of the network will be made with droplets which contain a calcic reporter. Under minute oscillations (of controlled amplitude and frequencies), we will probe the deformation of the network in bright field and in fluorescence to measure calcium leakage, using fast imaging capabilities.
We will build the mechano-chemical transfer function of this artificial tissue, by investigating for instance, how the characteristic diffusion time of calcium ions, depends on the imposed stress amplitudes and frequencies. We will also measure how the spatial pattern of diffusion can be modulated by the applied stress. Our results will be modelled by 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.

The last 6 months of the PhD will be devoted to writing the manuscript and papers.

3- Profile of the PhD candidate

For this project, we seek a candidate with a very good experimental practice in either biophysics, physico-chemistry, mechanics, optics or image analysis. Additional knowledge in synthetic Biology, Statistical Physics and Hydrodynamics will be appreciated.

4 - Reference

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