Laboratoire Physico-Chimie Curie

Institut Curie,

11 Rue Pierre et Marie Curie-75005 PARIS

PROPOSITIONS DE STAGES DE MASTER 2 ET THESES

MASTER2 and PhD THESIS PROPOSAL

Directeur du laboratoire : Pascal HERSEN

Le laboratoire Physico Chimie Curie :

The goal of the unit is to uncover the role of physical laws in the architecture and functions of cellular systems. To this end, the teams follow cross-disciplinary approaches involving physics, chemistry and biology.

Studies cover a breadth of topics ranging from single molecules (molecular motors, DNA-protein interactions, membrane proteins) to cellular functions (cell adhesion, cell division, cell motility, intracellular transport) and the collective behaviour of cells in tissues and organisms (wound healing, morphogenesis). They include the use of many experimental systems going from isolated molecular assemblies and biomimetic systems to cellular and multicellular systems.

The approaches combine theoretical studies – including statistical physics of non-equilibrium systems – and a variety of experimental techniques such as optical and electron microscopy, as well as microfluidics and micropatterning, optogenetics, or mechanical micromanipulation using optical or magnetic tweezers.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Curved membrane protein-mediated actin network assembly

DIRECTEUR de Stage : Feng-Ching Tsai (P. Bassereau team), feng-ching.tsai@curie.fr

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de la thèse :

The actin cytoskeleton plays an important role in cellular functions, in particular in membrane remodeling. During cell migration, the actin cytoskeleton exerts forces on the plasma membrane to push the membrane forwards to form membrane protrusions such as membrane ruffles and lamellipodia. These membrane protrusions are very thin and sheet-like, and they are filled with actin. Moreover, they facilitate cells to explore the surrounding environment. Another example is during endocytosis where a cell uptakes nutrients or proteins from its surrounding or on the plasma membrane. During endocytosis, the plasma membrane is bent inwards into the cell to form a spherical bud or a thin tube, and this bud or tube is then cut from the plasma membrane to generate a transport vesicle. Here, actin assembly facilitates the deformation of the plasma membrane. For actin to fulfill its cellular functions, it is critical that actin assembly is precisely regulated spatially and temporally. Cell biology studies have provided a never-ending list of proteins that regulate actin dynamics. Understanding how these actin regulatory proteins mediate actin assembly is critical to deepen our knowledge of actin's cellular functions. Here, we focus on one of the actin regulatory proteins, a curved membrane protein called sorting nexin 9 (SNX9). SNX9 has been shown to regulate actin assembly in the formation of membrane ruffling and during endocytosis. Yet, it is poorly understood how SNX9 mediates actin assembly that subsequently results in different membrane shapes: sheet-like for membrane ruffles and tubular shape in endocytosis. To address this question, we will perform experiments using cell-free model systems composed of purified SNX9, actin and artificial model membranes. Our preliminary experiments have provided us promising results to start this project. First, we successfully assembled an actin network in the presence of SNX9 (Fig. 1A). Second, we observed that when placing SNX9 outside giant membrane vesicles, SNX9 binds to the membrane and generate outward membrane tubes (Fig. 1B). In this project, we propose to perform experiments using two types of membrane assays, supported lipid bilayers (SLBs) and giant membrane vesicles. We will examine how SNX9 mediates actin assembly using TIRF microscopy for the SLB assay and confocal microscopy for the vesicle assay (Fig. 1C). Based on our results, we expect to provide biochemical and physical mechanisms underlying SNX9-mediated actin assembly.

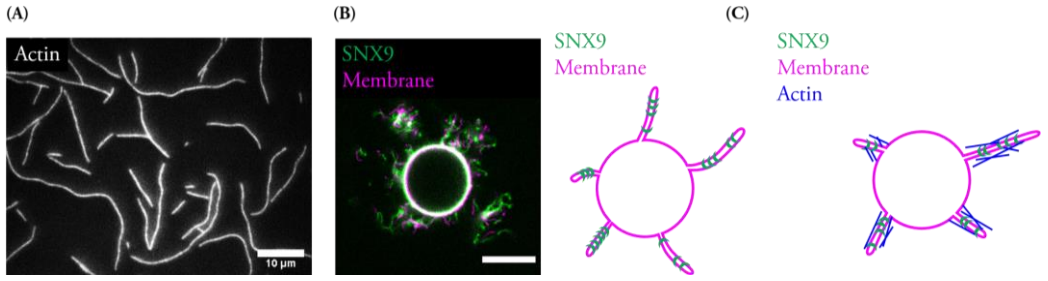


Figure 1. (A) TIRF image of actin networks assembled in the presence of SNX9. Actin is labelled with AX488 phalloidin. Scale bar, 10 µm. (B) Confocal image of a vesicle with tubes generated by SNX9. Scale bar, 5 µm. (C) Schematic of membrane tubes generated by SNX9 and actin.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Physical mechanism of curved protein-mediated membrane remodeling

DIRECTEUR de Stage : Feng-Ching Tsai (P. Bassereau team), feng-ching.tsai@curie.fr

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de la thèse :

It is vital for cells to generate transport vesicles inside the cells. These vesicles allow cells to transport nutrients or proteins to different organelles to fulfill specific cellular functions. To generate these vesicles, mechanistically, a small patch of a membrane is deformed into a spherical bud. This bud is connected to the donor membrane via a narrow tubular neck. To generate a vesicle, the neck is cut to detach the bud from the donor membrane (Fig. 1A). A rich set of cell biology studies have shown that many proteins are involved in the process of generating transport vesicles in cells. Here, we are interested to understand how a curved membrane protein called sorting nexin 9 (SNX9) contributes to vesicle generation. On the plasma membrane, SNX9 has been shown to be recruited to the membrane neck (Fig. 1A). Furthermore, a recent cell biology study suggested that SNX9 can constrict the membrane neck. However, there is no direct evidence supporting this hypothesis. In this project, we aim to reveal physical mechanisms underlying SNX9-mediated membrane remodeling by using biophysical methods and in a quantitative manner. We will establish a cell-free system using purified SNX9 and artificial model membranes. To generate membrane geometry like that of the cellular tubular neck, we will pull membrane nanotubes from giant membrane vesicles using optical tweezers (Fig. 1B). We will measure forces applied by SNX9 on the tubes using optical tweezers and we will correlate tube radius and SNX9 density on the tube using confocal laser scanning microscopy. Eventually, we will provide a comprehensive understanding of the role of SNX9 in membrane remodeling and provide insight into the functions of SNX9 in cells.

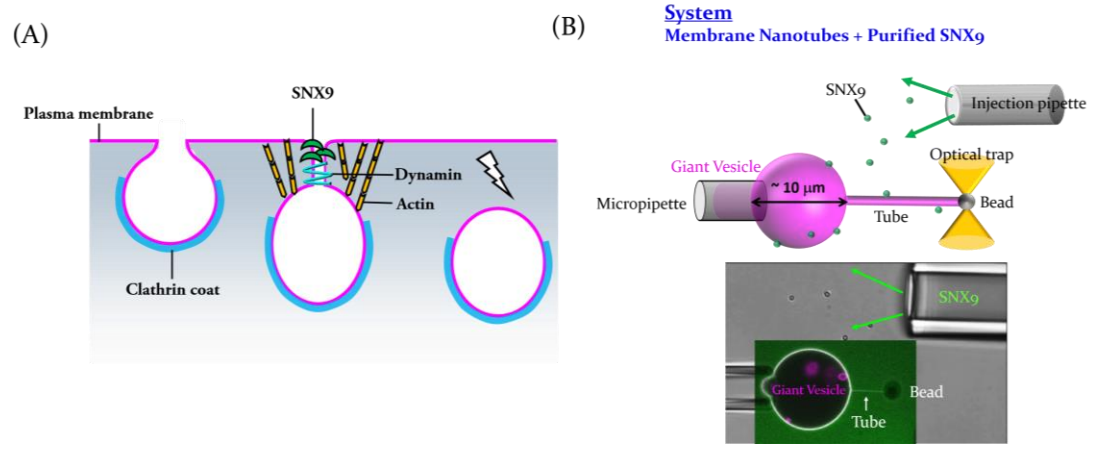


Figure 1. (A) SNX9 is involved in vesicle generation on the plasms membrane in endocytosis. (B) Schematic of the experimental system. The green signal is from SNX9 and the purple signal is from the vesicle.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

Proposal 1: Deciphering communication between cell organelles at subnanometric resolution by cryo-EM

DIRECTEUR de Stage : Manuela Dezi, manuela.dezi@curie.fr

Ce stage peut être poursuivi en thèse : OUI

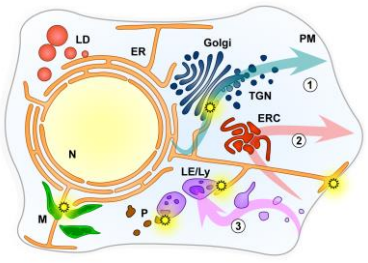
Si oui, la thèse est-elle financée : possible

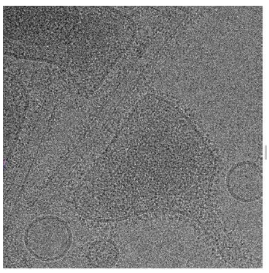
SUJET de la thèse :

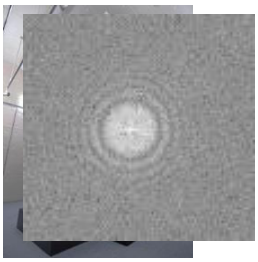
Cryo-electron microscopy (cryo-EM) is an imaging method that provides information at atomic resolutions of purified protein and subnanometric resolutions directly in cellulo. This approach, awarded by the Nobel Prize in Chemistry in 2017, combined with advanced computational analyses and deep learning developments is changing our vision of the cell. In this context, our team is interested in the functions associated with membranes and the 3D architecture of membrane proteins. The Master 2 internship is part of our research on how communication occurs within the cell.

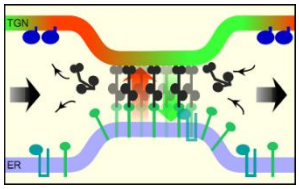
Within the cell, organelles communicate with each other thanks to vesicles that traffic between the compartments. In recent years, another mode of communication has been discovered. It consists of proteins that directly connect two facing membranes of different compartments. Thus, the endoplasmic reticulum unifies the entire cell through these contact site membranes (MCS). Within these contacts, binding proteins also perform major cell functions such as lipid transport, calcium signaling or organelle inheritance. We have been able to understand how a contact is formed between VAP, a transmembrane protein of ER and OSBP a cholesterol transport protein. In particular, we have shown that disorder is essential for dynamics and function within the confined space of the MCS (Dev. Cell 2019). We have also shown that the flexibility and elasticity of VAP-A modulates the geometry of the MCS and the separation between the facing membranes. We believe that thanks to the flexibility of VAP-A, contact areas can include proteins of different sizes and create a localized multi-protein functional area. To support this hypothesis, the student will create in vitro MSCs from those already designed in the lab with VAP with different flexibility and dimensions. Once formed, the MCS will be analyzed by cryo-EM and cryo-tomography. The goal is to decipher the behavior of VAP in a confined space at subnanometric resolution. These results will be compared with in cellulo experiments carried out by our collaborators (B. Antonny, IPBC, Valbonne, Cell 2013, EMBO J. 2017)

The student must have training in biophysics, an interest in cryo-EM, computational analysis and in vitro approaches. The student will be trained in the membrane biochemistry, cryo-electron microscopy and image analysis with the help of biochemists and cryo-electron microscopists in the Molecular Microscopy of Membranes group in Institut Curie, Sorbonne Université (see https://science.institut-curie.org/research/multiscale-physics-biology-chemistry/umr168-physical-chemistry/team-levy.









Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

Proposal 2 :Molecular architecture of endoplasmic reticulum-plasma membrane contact site by cryo-electron microscopy

DIRECTEUR de Stage : D. Lévy, daniel.levy@curie.fr

Ce stage peut être poursuivi en thèse : Possible

Si oui, la thèse est-elle financée : non

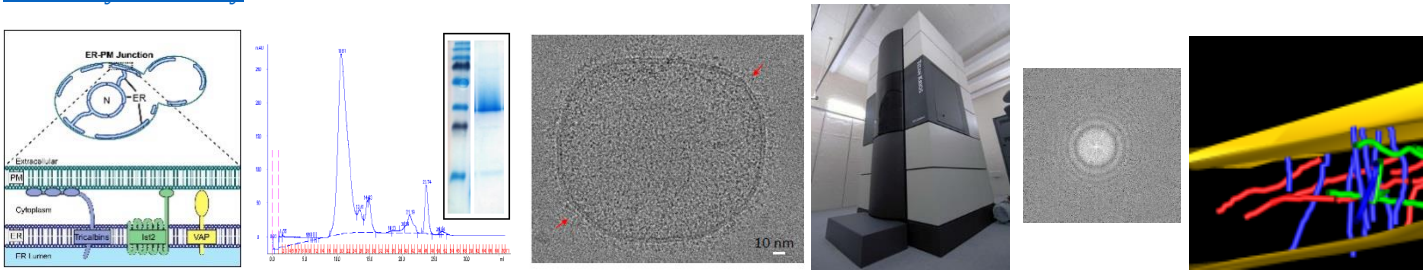
SUJET de la thèse:

Cryo-electron microscopy (cryo-EM) is an imaging method that provides information at atomic resolution of purified protein and at subnanometric resolution in cellulo. This approach, awarded by the Nobel Prize in Chemistry in 2017, combined with advanced computational analyses and deep learning developments is changing our vision of the cell. In this context, our team is interested in the functions associated with membranes and the 3D architecture of membrane proteins. The Master 2 internship is part of our research on how communication occurs within the cell.

Cellular organelles communicate by intravesicular transport and through physical contact zones made of proteins tethering two facing organelle's membranes. These specialized environments are called Membrane contact sites (MCS) and are involved in lipid homeostasis, Ca-signaling or organelle inheritance. MCS dysfunctions are reported in tumor progression and proteins are targets of anti-cancer drugs. They are subject of in-depth studies at the cellular level (Scorrano L, Nat Comm. 2019). However, there is no molecular model of MCS, which limits our understanding of the mechanisms of assembly/disassembly of MCS and associated functions. We reconstituted in vitro MCS between ER and Golgi and analyzed by cryo-electron microscopy (cryo-EM). This revealed the importance of intrinsically disordered regions of the constituent proteins in the 3D function and architecture of MCS (Jacmena D Dev Cell 2019, de la Mora in prep.). In collaboration with W. Kukulski (MRC, UK), we are interested in MCS between ER and plasma membrane formed by Tricalbins and involved in Ca-stimulated lipid transport.

The goal of the internship is to provide the first characterization at the molecular level of Trcb3. Trcb3 will be expressed, purified and reconstituted in model membranes. Important parameters of its organization in membrane will be modulated e.g. lipid composition, protein density, calcium (Reviewed in Lévy, D. BBA 1995). 3D architecture will be derived from cryo-electron microscopy and eventually cryo-tomography and image analysis.

The student must have training in biophysics, an interest in cryo-EM, computational analysis and in vitro approaches. The student will be trained in the membrane biochemistry, cryo-electron microscopy and image analysis with the help of biochemists and cryo-electron microscopists in the Molecular Microscopy of Membranes group in Institut Curie, Sorbonne Université (see https://science.institut-curie.org/research/multiscale-physics-biology-chemistry/umr168-physical-chemistry/team-levy.



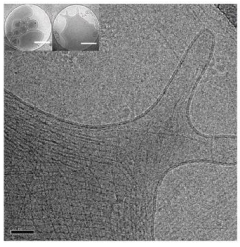
Laboratoire Physico-Chimie Curie
Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS
TITRE DU STAGE :

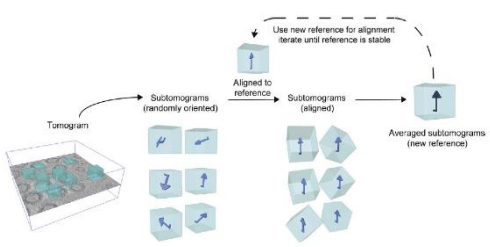
Proposal 3: Reaching sub-nanometric resolution of membrane bound proteins by sub-tomogram averaging and cryo-electron tomography

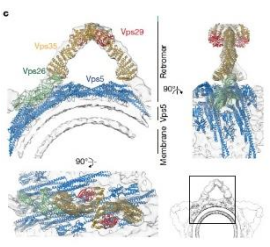
DIRECTEUR de Stage : D. Lévy, daniel.levy@curie.fr
Ce stage peut être poursuivi en thèse : OUI
Si oui, la thèse est-elle financée : possible
SUJET du stage de M2 :

Electron cryo-microscopy is revolutionizing structural biology and protein structure determination. It allows to determine the structure of proteins purified and homogeneous at atomic resolutions. Since the award of the Nobel Prize in Chemistry in 2017, computational instrumental and analytical developments have been carried out and opened up new fields of application. One of these development axes is the analysis of complex, multicomponent and heterogeneous systems in terms of conformation and flexibility. The aim is to determine the structure of proteins in situ within cells or of multicomponent machinery reconstituted in vitro. For this purpose, computational analysis developments are in progress for 3D reconstruction that take into account the heterogeneity and flexibility of biological objects.
In this context, our team is interested in the 3D architecture of machinery composed of proteins associated with membranes and involved in major cellular functions (see https://science.institut-curie.org/research/multiscale-physics-biology-chemistry/umr168-physical-chemistry/team-levy.). To do this, we designed in vitro systems and obtained cryo-tomography data sets of two types of samples on state of the art microscopes. The first, made up of protein filaments assembled on membranes, must allow sub-nanometric resolutions to be achieved. The challenge is to sort out the variability of filament organization. The second is made of proteins that bridge two membranes. The challenge is to sort out the flexibility of the assemblies. In both cases, this requires image analysis approaches called subtomogram averaging. Briefly, sub-volumes extracted from cryo-tomograms are compared and aligned with a template by applying a basic algorithm of iterative refinement, consisting in a refinement loop in which geometrical transformations are applied to every particle, and a selection process followed by averaging. The most common procedure is iterative refinement based on cross correlation optimization, specifically the Roseman's fast scheme (Roseman, 2003) to restrict the computation to a selected region of interest when comparing particles with the template. Structural heterogeneity analysis is derived from 3D Classification based in principal component analysis (PCA) and multireference alignement (MRA).

The student must have training in computational analysis, bioinformatics or physics and an interest in cryo-electron microscopy and structural biology. Knowledge of (*or at least familiar with*) Matlab or Python will be a plus.







Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

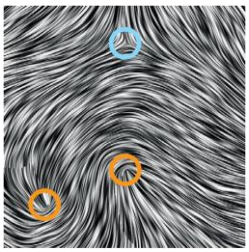
TITRE DU STAGE : ARCHITECTURES AND DYNAMICS OF ACTIVE NEMATIC TISSUES

DIRECTEUR de Stage : Pascal Silberzan

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de la thèse :



The behavior of a biological tissue results from the coordinated actions of the cells that constitute it. *In vivo*, these cell populations cope with their microenvironment, including physical and biochemical cues exerted by the extracellular matrix but also by neighboring cells. Therefore, experiments aiming at mimicking these situations *in vitro* must include such passive or active boundaries and confinement.

In the last years, we have addressed different aspects of collective behaviors of cells with a physics approach. In particular, we have recently shown that spindle-shaped cells tend to adopt a common orientation amounting to a so-called "nematic" order. When confined in well-defined domains of various shapes, monolayers adapt their architecture to accommodate both the long-range orientation order favored by the cells, and the geometry of the confining domain. These model systems mimic the *in vivo* architectures found in some physiological tissues such as muscles or in the vicinity of tumors. They highlight the emergence of organizations and dynamics characteristic of populations of active cells, and the critical importance of defects in these architectures.

In particular, when plated on a stripe much wider than a cell size, these cells spontaneously orient with a well-defined angle with respect to the stripe direction and exhibit a shear flow close to its edges. We understand these behaviors at the light of a continuous theory of active matter but there remain several observations addressing important biological questions that deserve a better understanding. This is the case for instance for the formation of well-defined tridimensional cell cords resulting from a combination of directed flows and cell proliferation and that plays a crucial role in the subsequent differentiation steps.

To address these questions practically, we combine microfabrication, micropatterning and innovative microscopy techniques with cell biology techniques. To realistically model these situations, physical quantities such as the flows of cells within the monolayer or the mechanical forces developed on the substrate are quantitatively measured at all scales in parallel with cellular biological activity.

This interdisciplinary project is developed in close collaboration with groups of biologists and theoreticians at Institut Curie and ENS.

Recent references (selection)

- Duclos G., Blanch-Mercader C., Yashunsky V., Salbreux G., Joanny J.-F., Prost J., Silberzan P.: *Spontaneous shear flow in confined cellular nematics*, Nat. Phys. **14**, (2018), 728.
- Duclos G., Erlenkämper C., Joanny J.-F., Silberzan P.: *Topological defects in confined populations of spindle-shaped cells.* Nat. Phys. **13**, (2017), 58.
- Hakim V., Silberzan P.: *Collective cell migration: a physics perspective.* Rep. Prog. Phys. **80**, (2017), 076601

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Self-organized flagellar-like beating of actin bundles in vitro.

DIRECTEUR de Stage : Pascal MARTIN

Ce stage peut être poursuivi en thèse : OUI – ~~NON~~

Si oui, la thèse est-elle financée : OUI - ~~NON~~

SUJET de la thèse :

The emergent active behaviors of systems comprising large numbers of molecular motors and cytoskeletal filaments remain poorly understood, even though individual molecules have been extensively characterized. We have very recently shown in vitro that flagellar-like beating can be produced naturally and robustly in polar bundles of filaments. Using surface micro-patterns of a nucleation-promoting factor, we control the geometry of actin polymerization to produce thin networks of parallel actin filaments in vitro. In the presence of either myosin Va or heavy-mero myosin II motors added in bulk, we have recently shown that growing actin filaments self-organized into bundles that display periodic wave-like beating resembling those observed in eukaryotic cilia and flagella. The aim of the traineeship will be to study the role of fluid viscosity on the wave properties (frequency, wavelength, waveform) as well as the conditions that allows neighboring bundles to synchronize their oscillations. In the longer term, these artificial cilia may be used to mediate particle transport or as micro-swimmers. Our results will guide theoretical descriptions of wave-like beating in polar bundles of filaments in collaboration with Jean-François Joanny (UMR168 Institut Curie / Collège de France) and Frank Jülicher (MPIPKS Dresden).

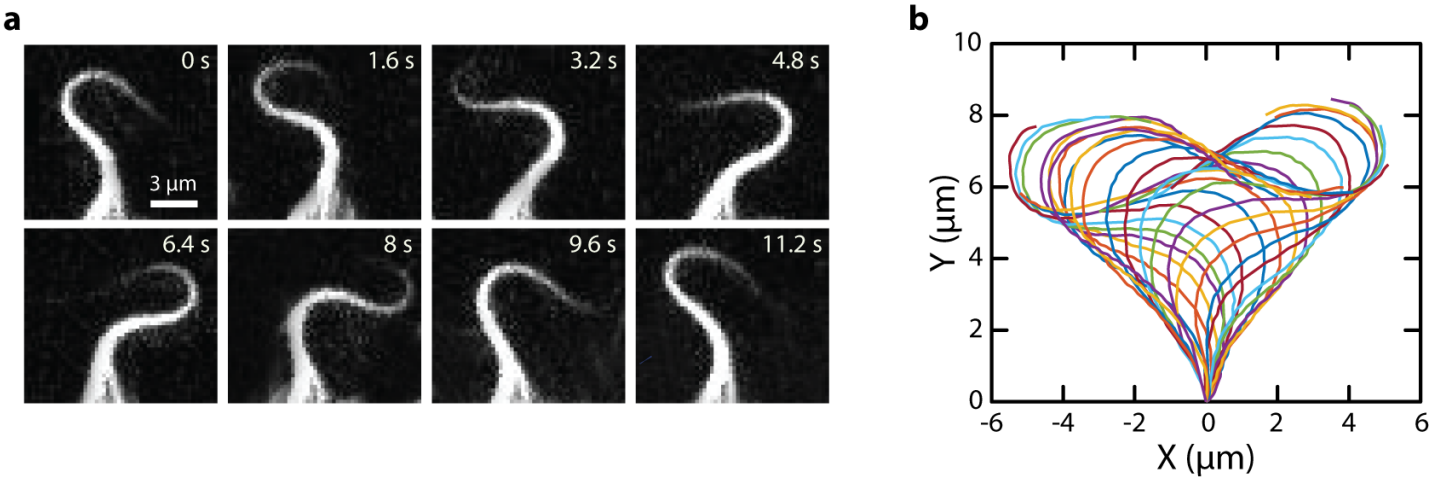


Figure: **a.** Wave-like beating of an actin bundle driven by the mechanical activity of myosin motors in vitro. **b.** automatic tracking of the bundle's shape results in the beating pattern of the actin bundle shown in **a**.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Out-of-equilibrium statistical mechanics of a polymer undergoing loop extrusion

DIRECTEURS de Stage : Vittore Scolari (vittore.scolari@curie.fr) & Antoine Coulon

Ce stage peut être poursuivi en thèse : à discuter.

SUJET du stage :

The secrets of life are encoded in DNA, as a sequence of 4 letters (the sugars A, T, C and G) that contains essential information required for our cells to thrive. Nevertheless, having access to those sequences, collected by the community and stored in the genetic databases, is not enough to understand their meaning. Different cells of the same organism, such as a stomach and a brain cell, share the same genetic code, but look different and perform a totally different duty. This leads us to an extra layer of more volatile information, that is not encoded in the sequence but rather is written on top of that, which is called epigenetics, and consists of a large quantity of molecules and biochemical processes associated to DNA in chromatin, making up the chromosomes. Those molecules are always associated to the physical position of DNA in the 3D space, and often have an important effect on the way the chromosomes fold inside the nucleus. Our overarching objective is to understand the connections between the function of the molecular elements which constitute epigenetics and the spatial organization and dynamics of chromosomes.

Specifically to this project, we are interested in genomic elements called ‘enhancers’: because these elements can switch the transcriptional activity of genes located hundreds of kilobases away from their position in the sequence. How enhancers and genes communicate in space and time is a great mystery and is a major subject of current research. The current paradigm of understanding, is based on “loop extrusion”, as it has been hypothesized that this governs the local conformation of mammalian chromosomes (Fudenberg *et al.*, 2017). It posits that molecular motors create and grow local DNA loops (typically < 1 Mb) until it encounters a roadblock. This model can account for many experimental observations, creating local conformational structures likely to influence the communication between genes and enhancers (Valton and Dekker, 2016).

The goal of this internship is (i) to develop an analytical theory for the dynamics of polymers undergoing loop extrusion, using the tools of out-of-equilibrium statistical mechanics, and (ii) to be able to identify "loop extrusion" in experimental data obtained in our lab and others. To study local genome conformation and enhancer-gene communication in the lab, we measure the 3D trajectories of genomic elements and the activity and position of individuals genes that share the same enhancer(s). The problem arises by the fact that the position of such objects, at mesoscopic scales, is dominated by chaos. And chaos can be the result of purely Brownian diffusion (the Rouse model of free polymers), as well as the outcome of averaging over space

and time (Egolf, 2000). In order to highlight the effect of activity above the background of equilibrium chromatin, the intern will develop the following:

1. Null model theory: parametrization of the Rouse model using a statistical micro-ensemble, analytical calculation of the probability fluxes as a function of time.
2. Adaptation of available numerical simulations of loop extrusion, to test the predictions obtained in point 1, in presence and absence of loop extrusion. Quantification of the effects of polymer relaxation
3. Comparison of the theory to data from our lab and from the literature (e.g. Bintu et al. 2018): contact probability maps of chromosomes (Hi-C), distance distributions and conformation ensembles (Oligopaint DNA FISH), gene activity (RNA FISH), 3D trajectories of genomic loci.
4. explore the effect of different hypotheses in the extrusion model (Ganji *et al.*, 2018; Vian *et al.*, 2018)

Candidates should have substantial programming skills (Python preferred), knowledge in statistical physics, and a genuine interest in approaches combining theory and experiments for solving questions at the physics-biology interface.

More information on the lab: www.coulonlab.org

Related literature:

Bintu, B. et al. (2018). Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science*, **362**(6413), eaau1783–10.

Egolf,D.A. (2000) Equilibrium Regained: From Nonequilibrium Chaos to Statistical Mechanics. *Science*, **287**, 101–104.

Fudenberg,G. *et al.* (2017) Emerging Evidence of Chromosome Folding by Loop Extrusion. *Cold Spring Harb Symp Quant Biol*, **82**, 45–55.

Ganji,M. *et al.* (2018) Real-time imaging of DNA loop extrusion by condensin. *Science*, **360**, 102–105.

Valton,A.-L. and Dekker,J. (2016) TAD disruption as oncogenic driver. *Current Opinion in Genetics & Development*, **36**, 34–40.

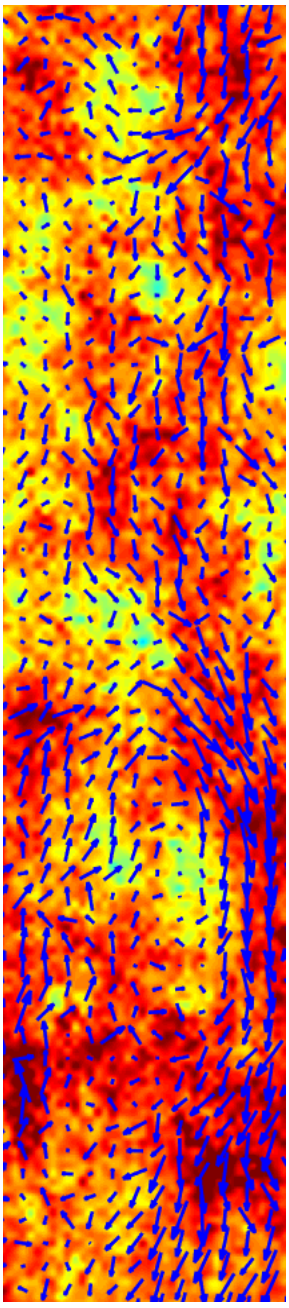
Vian,L. *et al.* (2018) The Energetics and Physiological Impact of Cohesin Extrusion. *Cell*, **173**, 1165-1178.e20.

Laboratoire Physico-Chimie Curie, Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

DIRECTEUR de Stage : **Isabelle BONNET, Maître de Conférences SU,** isabelle.bonnet@curie.fr

Ce stage peut être poursuivi en thèse : **OUI**

Si oui, la thèse est-elle financée : **NON (concours Ecole Doctorale)**



Effect of an oncogenic activation on confined cellular flows

Summary

Collective behaviors are essential for regulating many biological processes. A cell population is not simply a collection of single cells and a coordination among individuals is required to create patterns of collective migration. But, what happens if we biomechanically disrupt this coordination by disturbing cell-cell couplings? To what extent the mechanical properties of individuals impact on the modes of collective behavior?

Our approach is based on optogenetics: we use cells that over-activate the Src oncoprotein (known to be overexpressed in many cancers) when they are illuminated with blue light. Otherwise, they keep a normal phenotype. We thus trigger the oncogenic activity of the cells by illuminating them in blue. These activated cells present biomechanical characteristics of malignant cells (decreased cell-cell adhesion and increased focal adhesions): how such oncogenic activation impact on the collective dynamics?

Our specific aim is to establish the relationships between dynamics (cell flow), genetic (oncogenic state), tissue organization (nematic order) and the environment (confinement). Given that collective cell dynamics is important for cancer as well, such a study should help to unravel the mechanisms of collective cell migration in cancer.

Background

Training at the interface physics / biology would be a plus, but we are mostly looking for highly motivated students.

Competences that will be acquired during the internship

Cell culture, microfabrication, optogenetics, optical microscopy, image analysis.

1. https://www.nature.com/articles/s42005-019-0198-5
2. https://www.nature.com/articles/s41567-018-0099-7

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Single molecule super-resolution imaging in reversibly cryo-arrested cells

DIRECTEUR de Stage : Bassam HAJJ

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de stage :

Single molecule super-resolution techniques such as photo-activated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) provide a detailed view of the molecular organization in biological specimens [1, 2]. However, super-resolution technics face many challenges. Among the most pressing issue is still to acquire high-density super-resolution images in single cells at specific time points.

The purpose of this internship is to setup a reversible cryo-arrest technique for super-resolution imaging at low temperature [3]. On a widefield optical microscope, living cells will be reversibly arrested to low temperature ($-45°C$) by controlled steps of cooling and medium exchange to avoid lethal solid ice formation. At low temperature, the cells will be imaged using PALM/STORM approaches. The same cell will be revived to physiological temperature ($+37°C$) to pursue its different functions. A second step of cryo-arrest will follow to image in super-resolution the evolution of the molecular organization of the same cell in a following time point.

Additionally, low temperature arrest will yield a reduced molecular vibration. The signature of the dipole emission will be revealed by polarization measurements or defocused imaging [4]. The molecule orientation signature will play a role in discriminating adjusting molecules appearing in consecutive acquisition frames. We hypothesis that, thanks to the improved photo-physics of single molecule emissions at low temperatures and the determination of the dipole orientation, a clear resolution improvement should be achievable.

This method will be applied in the context of super-resolution imaging of nuclear organization at specific time points, a very challenging task in super-resolution.

Interested candidates can send a CV and motivation letter to: bassam.hajj@curie.fr

References:

1. Betzig, E., et al., *Imaging intracellular fluorescent proteins at nanometer resolution.* Science, 2006. **313**(5793): p. 1642-5.
2. Rust, M.J., M. Bates, and X. Zhuang, *Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM).* Nat Methods, 2006. **3**(10): p. 793-5.
3. Masip, M.E., et al., *Reversible cryo-arrest for imaging molecules in living cells at high spatial resolution.* Nat Methods, 2016. **13**(8): p. 665-672.
4. Valades Cruz, C.A., et al., *Quantitative nanoscale imaging of orientational order in biological filaments by polarized superresolution microscopy.* Proc Natl Acad Sci U S A, 2016. **113**(7): p. E820-8.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Mapping nuclear environment by 3D single particle tracking

DIRECTEUR de Stage : Bassam HAJJ

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET:

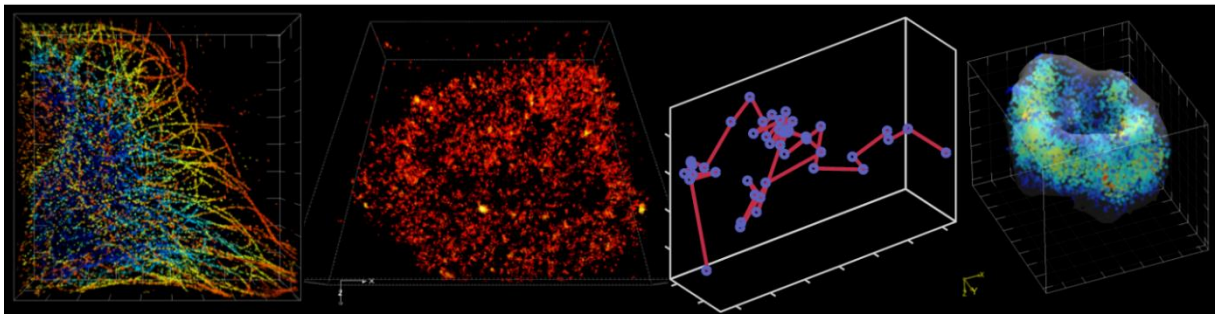
We are seeking a motivated candidate to join our lab for an internship on single particle tracking inside the nucleus of living cells.

The nucleus of a living cell is a crowded environment where nuclear factors such as transcription factors are in constant search for specific DNA sequences. DNA is organized at different levels, from topologically associated domains (TADs) to chromosomes territories. It is known that DNA organization and compaction plays a role in orchestrating the different nuclear functions and gene expression by restricting the accessibility of nuclear players to specific genes. In cancerous cells, it was shown that DNA compaction and chromosome territories are massively altered. However, a single cell characterization of the nuclear environment is still missing.

In order to map the 3D environment and study different properties of the nucleus, we will perform single particle imaging and tracking. Efficient 3D tracking of single particles in the nucleus requires 3D imaging with fast frame rates. Multifocus microscopy (MFM) allows simultaneous acquisition of 9 different focal planes on the same camera, thus covering the whole volume of a nucleus in a single image. With MFM it is possible to acquire volumetric images with hundreds of frames per second.

In this project, the student will benefit from the latest technological advancements in volumetric microscopy to image efficiently the nuclear environment. He or she will inject different particles of different sizes in the nucleus, image them in 3D and follow their dynamics. New approaches for inferring nature and properties of 3D random walks of the particles at high density will be explored using the TRamWAy platform (https://goo.gl/McgJXR) in collaboration with Jean-Baptiste Masson at Pasteur Institute. Moreover, a combination of advanced statistical approaches implemented within a virtual reality software platform (https://goo.gl/dnNueu) will be accessible to analyze and visualize complex 3D pattern of random walk properties. These 3D maps of nucleus will be compared between different cell types.

We are looking for a motivated candidate with the will to learn single particle data processing, optical microscopy, and cell culture. Interested candidates can send a CV and motivation letter to: bassam.hajj@curie.fr



Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Mechanics of somite formation in chicken embryos

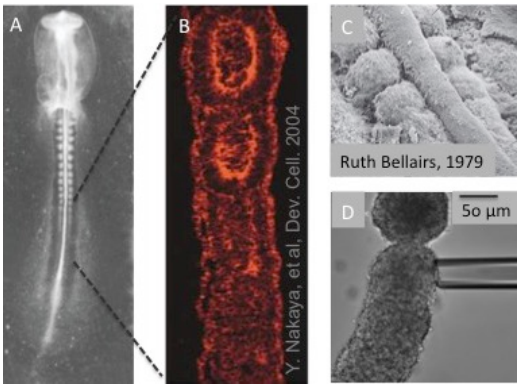
DIRECTEUR de Stage : Karine Guevorkian

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de thèse :

One of the challenges in modern developmental biology is to understand how mechanical cues shape tissue patterns from which organs emerge. A good example of pattern formation is the generation of somites in vertebrate embryos. Somites are compact segments of thousands of cells that pinch-off periodically from the tail tissue of vertebrates, reminiscent of droplets formed from a water jet. Our skeleton, muscles and the skin emerge from these somites. We are interested in understanding the origin of the mechanical instabilities that lead to the separation of somites in the chicken embryo. These instabilities arise from the internal stresses produced by cells inside the tissue, as well as the rheology of the tissue. Therefore, we aim to assess the role of cell and tissue mechanics in the process of somite formation, by borrowing concepts used in soft-matter physics.



(A) Formation of somites in 2-day chicken embryo. (B) Actin reorganization. (C) Electron micrograph showing the detachment of the somites appearing as cellular balls. (D) Micropipette aspiration to assess the rheology of the somites.

In this master's project, we will develop new ex-vivo approaches to explore the role of actin polymerization, and myosin activity during somite formation and characterize the rheological properties of the tissue, using micromanipulation techniques. The continuation of this project as a PhD thesis involves studying the role of cell-cell and cell-matrix interactions, and force generation during somitogenesis by combining in-vivo and ex-vivo approaches.

Our team benefits from the highly interdisciplinary environment at PCC, hosting experimental and theoretical physicist, biologists and biochemists, as well as the vicinity of Cell Biology and Developmental Biology departments of Curie Institute. Please contact Karine Guevorkian (karine.guevorkian@curie.fr) for complementary information.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Nucleus activity triggered by cytoskeletal forces

DIRECTEUR de Stage : Cécile Sykes

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de thèse :

The cytoskeleton is the structure that ensures cell motility and cell shape changes, and also transmits forces throughout the cell. The cytoskeleton contains actin and microtubules, two types of filaments with different mechanical and dynamical properties. Those filaments are highly involved in various aspects of cell fate such as cell division and motility. The Sykes lab has a know-how in the reconstitution, with a minimal number of purified proteins, of actin dynamics and membrane shape changes. This thesis project aims at applying this knowledge to forces transmitted by the actin cytoskeleton to the nucleus.

Alternative to the whole cell approach, a reconstitution approach, using a minimal number of constituents, provides a controlled system to thoroughly study forces and nuclear shape changes. Purified nuclei will be placed in actin networks from purified proteins. Nuclear shape and content will be analyzed when actin networks are put under tension, either by spontaneous tension buildup of the growing actin network, or by the addition of myosin motors.

Whereas the physics of actin networks are now well characterized, the mechanism of force transmission to the nucleus is not known. The use of reconstituted systems is a powerful method that has been developed by the host lab. Mechanical tensions within acto-myosin networks are sufficient to drive deformations of nuclear-like artificial objects, such as oil droplets and liposomes. By replacing these objects with real nuclei, and with the use of optically-tweezed actin coated beads, actin-patterned surfaces and contractile actin shells, we will assess the mechanics of nucleus-actin network contacts in a controlled way.

Tools available in the lab: optical imaging, microfluidics, optical tweezers, biochemistry, cell biology.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Cell motility: quantification of cytoskeleton-nucleus mechanotransduction

DIRECTEUR de Stage : Cécile Sykes

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de thèse :

The cytoskeleton is the structure that ensures cell motility and cell shape changes, and also transmits forces throughout the cell. The cytoskeleton contains actin and microtubules, two types of filaments with different mechanical and dynamical properties. Those filaments are highly involved in various aspects of cell fate such as cell division and motility. The Sykes lab has a know-how in the reconstitution, with a minimal number of purified proteins, of actin dynamics and membrane shape changes. This thesis project aims at applying this knowledge to forces transmitted by the actin cytoskeleton to the nucleus.

A limiting factor for 3-D cell migration is the stiffness of the large and rigid nucleus. Considerable force needs to be applied by the cytoskeleton to the nucleus, yet many aspects of mechanical linkage and force transmission from the cytoskeleton to the nucleus are still poorly understood. Many nuclear envelope proteins, including nesprins, which mechanically link the actin cytoskeleton to the nuclear membrane have been implicated in cancer. A thorough study of the ability of metastatic cells to migrate through narrow pores, their nuclear stiffness and their nucleo-cytosketal connections is increasingly becoming crucial. Microfluidic devices have been developed in the lab to measure the capacity of cells to translocate through narrow spaces of micrometer size. Cells that have more deformable nuclei (due to low lamin A/C levels) can pass through constrictions smaller than 3 µm more easily than wild-type cells. Using these tools, we want to assess the mechanism of nucleus deformation through forces transmitted by the cytoskeleton, and in particular through nesprins. This project will lead to a comprehensive molecular portrait of nuclear and cytoskeleton proteins that are involved in cell translocation. We will dissect the role of the stiffness of the nucleus and its cytoskeletal connections in cell migration. We will develop a high-throughput micropipette aspiration device to measure the mechanical properties of cells and their nuclei, in the presence or absence of the proteins linking the nucleus to the cytoskeleton. A CRISPR labelling of nesprins and lamins is already available in the lab.

Tools available in the lab: optical imaging, microfluidics, optical tweezers, biochemistry, cell biology.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

MUTUAL ROLE OF ESCRT-III AND SEPTINS DURING THE CYTOKINESIS.

DIRECTEURS de Stage : Aurélie BERTIN et Stéphanie MANGENOT

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON
SUJET de la thèse :

Cytokinesis is the last step of cell division, when a mother cell is spitted in two daughter cells. Several proteins are involved in this complex process and their mutual and cooperative role is still under debate.

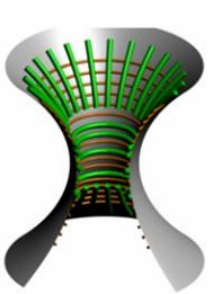
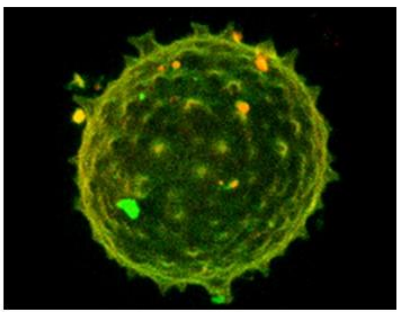
In this proposal, we propose to focus our work on the role of ESCRT-III and septins. Septins are multitasking proteins. During cytokinesis, they form a collar shape around the constriction site. ESCRT-III proteins are then recruited to decrease the neck diameter and to allow membrane scission. ESCRT and septins are involved in several cancer and deseases.

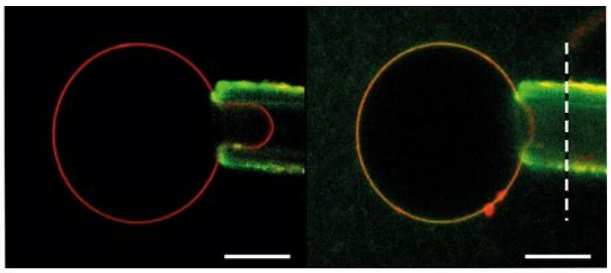
In our teams, we have previously shown that septins are sentive to positive micrometric curvature, whereas ESCRTs are sensitive to negative curvature.

We would like to elucidate the mutual interaction of these two proteins. We propose to use an in-vitro bottom approach, where we will:

1- Elucidate the supramolecular organization of ESCRT-III and septins on flat and curve susbstrate. We propose to perform cryo-electron microscopy on model membrane.
2- Study how ESCRT-III and septins are able to deform model membrane consisting of Giant Unilamellar Vesicle.
3- Reconstitute in vitro a constriction site. We will use micro fabricated approach to built in an in vitro system mimicking the geometry of the constriction site, where we can sequentially added the proteins.

Tools available in the teams: Cryo-electron microscopy, Confocal microscopy, Optical tweezer, micro-pipette manipulation, micro fabrication.



Intership Master 2

Institut Curie UMR 168
Institut Pierre Gilles de Gennes (IPGG)
Group QDevBio (eq. Keil)
6 rue Jean Calvin 75005 Paris

Supervisor: Wolfgang Keil
Tel: +33 (0)1 40 79 59 12
E-mail: Wolfgang.Keil@curie.fr
Start Date: January 2020

Development of a microfluidic system for controlled perturbations of *C. elegans* development during live-imaging

The ability to observe and perturb animal development *in-vivo* at cellular represents a prerequisite for quantitative studies of morphogenesis. However, long-term high-resolution live imaging has largely been restricted to the embryonic stage, leaving a large part of animal development inaccessible. Recently, we developed a microfluidics methodology that for the first time allows high-resolution time-lapse imaging of the small roundworm *C. elegans* for up to 60 hours [1-3].

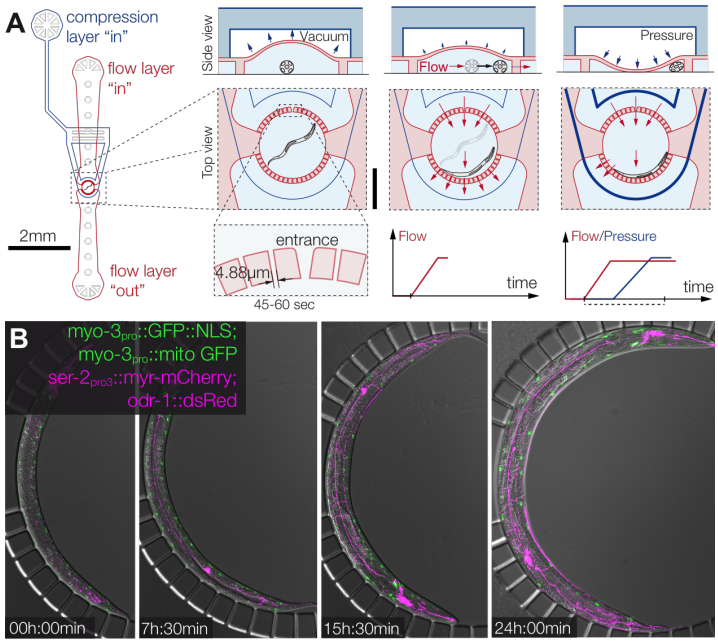


Figure 1. Long-term high-resolution imaging of developing C. elegans larvae with microfluidics. (A) Device layout and operating principle for worm immobilization. Left: Schematic of a worm chamber with inlet and outlet ports for the flow layer (red) and the inlet port for the pressure layer (blue). Right: Side view (top) and top view (bottom) of a worm chamber at subsequent stages of the worm immobilization procedure. (B) Merged Nomarski and fluorescence micrographs of immobilized animals showing the outgrowth of a touch-sensitive neuron (magenta) and body wall muscle cells (green) over 24h.

Up to 10 animals are kept in micro-chambers of 400um diameter (Figure 1A). In these chambers, reliable, reversible, repeatable, and non-damaging immobilization of worms for multi-channel imaging (Figure 1B) is achieved through precise orchestration of flow and pressure in the microfluidic device.

Based on the above system, **the goal of this internship is to develop and test a microfluidics system that**, in addition to confinement and repeated immobilization, **allows for precisely controlled perturbations of *C. elegans* development during live imaging.** This will be achieved by two means: (1) Fast and reversible exchange of the growth medium through on-chip microfluidics valves. (2) Fast and reversible changes in cultivating temperature. Fast changes in growth medium allow conditional and reversible inactivation of proteins with the auxin-inducible degradation (AID) system for C. elegans [4]. Fast temperature changes will enable rapid induction of gene expression or reversible disruption of protein function either through temperature-sensitive mutations or cell-specific heat-shock factor-driven gene expression [5].

This project is highly interdisciplinary. Students are not expected to have experience in all fields, and will learn during the project what is complementary to their own training. The project will be suitable for instance for students following courses in microfluidics & microsystems, physics with strong interest in biology, as well as biology students with strong quantitative interests.

References

[1] **W. Keil**, L. M. Kutscher, S. Shaham, and E. D. Siggia, Dev. Cell 40, 1 (2017).
[2] L. M. Kutscher, **W. Keil**, and S. Shaham, Dev. Cell 47, 222 (2018).
[3] M. A. Attner, **W. Keil**, J. M. Benavidez, and I. Greenwald, Curr. Biol. 29, 1 (2019).
[4] L. Zhang, J. D. Ward, Z. Cheng, and A. F. Dernburg, Development 142, 4374 (2015).
[5] T. Bacaj and S. Shaham, Genetics 176, 2651 (2007).

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Development of microvascularized microfluidic devices based on hybrid textile/polymer technologies for organs-on-chip and regenerative medicine

DIRECTEUR de Stage : Jean-Louis Viovy ; Co-direction avec Catherine Villard.

Ce stage peut être poursuivi en thèse : A voir en fonction des résultats et partenariats

SUJET :

Microfluidics an microfabrication have recently endergone an explosive development in biology. They allow to position, address and study cells with an unprecedented accuracy and resolution. One particular promising application is the development of "organs-on-chip" (OOC), reproducing the structure, physiology and functions of tissues and organs. The constitute an ideal partner to stem cell technologies, adding to this field or research the possibility to pre-position cells, and to stimulate them with physical or biochemical cues with a high spatial and temporal resolution. This has enormous potential for research and for drug and toxicity testing, as an intermediate between conventional in vitro culture and animal models, but also as a way to avoid problems in translation from animals to humans in drug development, thanks to the use of OOC based on human primary cells or IPSCs. Last but not least, these systems will also allow for the preparation of implants for regenerative medicine, based on human cells, and with a pre-structuration improving their function, biocompatibility, and post-implantation survival (e.g. thanks to pre-vascularization). Currently, however, OOC are based on microelectronics clean-room technologies, they are complex and expensive to fabricate, and transposition to biomaterials is not straightforward. Microfluidics an microfabrication have recently endergone an explosive development in biology. They allow to position, address and study cells with an unprecedented accuracy and resolution. One particular promising application is the development of "organs-on-chip" (OOC), reproducing the structure, physiology and functions of tissues and organs. The constitute an ideal partner to stem cell technologies, adding to this field or research the possibility to pre-position cells, and to stimulate them with physical or biochemical cues with a high spatial and temporal resolution. This has enormous potential for research and for drug and toxicity testing, as an intermediate between conventional in vitro culture and animal models, but also as a way to avoid problems in translation from animals to humans in drug development, thanks to the use of OOC based on human primary cells or IPSCs. Last but not least, these systems will also allow for the preparation of implants for regenerative medicine, based on human cells, and with a pre-structuration improving their function, biocompatibility, and post-implantation survival (e.g. thanks to pre-vascularization). Currently, however, OOC are based on microelectronics clean-room technologies, they are complex and expensive to fabricate, and transposition to biomaterials is not straightforward. This very interdisciplinary project will be developed in the team "Macromolecules and Microsystems in Biology and Medicine, a multidisciplinary team of about 20 persons working at the interface between physics, chemistry and biology[1]. The team is located in IPGG the first French Institute entirely dedicated to microfluidics. The project will benefit from the whole technological platform and support of the engineers of IPGG (clean room, microfabrication facility, culture rooms, microscopy), and if needed of the technological platforms and engineers of Curie Institute.

[1] https://science.curie.fr/recherche/physique-chimie-biologie-multi-echelle-et-cancer/physico-chimie/equipe-viovy/

It will also benefit from collaboration with ENSAIT in Roubaix, the National Superior Engineering school and research center, fully dedicated to the development and teaching of textile technologies at the highest level. The project will require knowledge in biomaterials and cell culture, curiosity for new fields and ability to work in team.

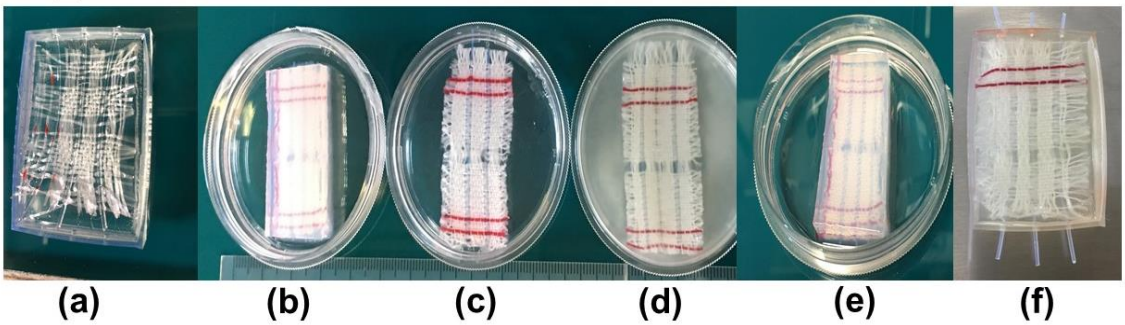


Figure 1: Examples of Hydrogel/Textile chip generated using different biomaterials. (a) PDMS (1:10) (b) agarose (2% w/v) (c) alginate 3% (w/v) (d) agarose/gelatin (10% w/v) (e) alginate/gelatin (f) GelMA (10% w/v)/ Gelatin.

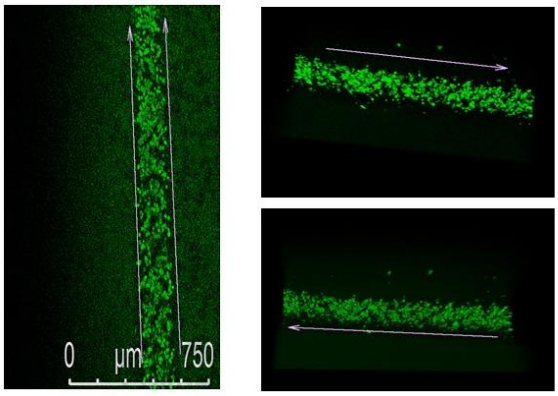


Figure 2: Images of endothelial cells (HUVEC) in hybrid textile/hydrogels chips after 2 days in culture.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Towards a new generation of microfluidic soft robots

DIRECTEUR de Stage : Jean-Louis Viovy ; Co-direction avec Catherine Villard.

Ce stage peut être poursuivi en thèse : On prévoit plutôt une évolution vers un transfert industriel/startup

SUJET :

1. Présentation et description du sujet

Robotics is currently a fast developing field, with huge promises in fields as varied as industry, transport or help to dependent people. Their development however face strong challenges. A first one is the cost: anthropo-mimetic robots with amazing possibilities are now developed (ref), but they cost hundreds of thousands €. Another issue, not always recognized in the public but well known in research and industry, is the danger associated with their power and the hard material they are made with: This make them poorly adapted to the manipulation of fragile objects, and raise a lot of challenges to avoid hurting surrounding people during their operation. To solve these two problems, "soft robots", based on polymers, are becoming popular, and indeed companies start to commercialize them (1). Currently, they are mainly used to manipulate fragile objects, but very exciting systems for helping humans (e.g. handicap control) are also developed (2). In the micro-world, soft robots based on microfluidics were popularized by the group of G. Whitesides (3). They however rely on microfabrication technologies, and their fabrication can rapidly become complex and expensive, notably when willing to integrate to the robot sensing abilities in addition to the actuation potential.

2. Techniques/méthodes utilisées

In this project, we shall explore the potential of a new approach, based on 3D printing and other technologies invented in the lab during ERCadg Cello (4), to overcome the above difficulties: it allows in particular to prepare microfluidic systems with asymmetric mechanical properties and easy integration of actuating and sensing elements, with a potential for mass production at low cost. The fabrication technology does not require clean room, and yields "naturally 3D" micro-robots, without the need of multilayer fabrication, which makes conventional technologies cumbersome and expensive. Transfer to Industry through a startup should be a natural outcome of this project.

3. Résultats attendus

Using the above approach, the student, in collaboration with the other team's members, will prepare and characterize various prototypes, and compare them with the conventional microfabricated systems. This will involve research on microfabrication, microfluidic control, and on specific materials required to achieve the optimal thermos-mechanical properties. If the project advances fast enough, we shall also develop biomimetic and biocompatible systems, which cannot be prepared by the conventional methods, as a route towards a new generation of mechano-active organs on chips.

This is an exploratory, interdisciplinary project, requiring curiosity, interest in real world applications and a taste for challenge. The required background involves a lot of practical sense, ability to work in team and share various competences and experimental skills, and a good background in physics, soft matter and microfluidics. Experience in microfabrication, and/or programming (notably embarked systems such as Arduino or Raspberry pi) will be a plus, but not mandatory.

4. Références

1 : see e.g. https://en.wikipedia.org/wiki/Soft_robotics, or https://www.softroboticsinc.com/ 2 : see e.g Polygerinos et al., **DOI:** 10.1109/ICRA.2015.7139597, or Connolly et al., PNAS , https://doi.org/10.1073/pnas.1615140114

3: G.M. Whitesides, Angew. Chem., https://doi.org/10.1002/anie.201800907

4: Venzac et al., *Microsystems and Nanoengineering*, in press

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Recruitment of Actin in Artificial Cellular Protrusions

DIRECTEUR de Stage : Patricia Bassereau patricia.bassereau@curie.fr
Web: https://science.institut-curie.org/team-bassereau

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de la thèse: Mechanisms of cellular protrusions' generation

Many dynamical protrusions called **filopodia** extend and retract at the edge of the cells that allow cells to sense their environment but also the entry of pathogens. They are thin tubular membrane structures that contain parallel actin filaments. Different types of membrane-actin linkers and proteins involved in actin dynamics are present in these structures [1]. Another type of membrane protrusion has been more recently discovered, **the tunneling membrane nanotubes, Tunneling nanotubes (TNTs**), that are able to connect cells and mediate the transfer of cytoplasmic molecules, organelles, membrane components, RNA, pathogens between cells for remote intercellular interactions [2]. Although filopodia and TNTs apparently share many common morphological features and are sustained by actin bundles, they also exhibit strong differences on their length, dynamics and also on the opposite effect of regulatory proteins interacting with actin [3].
It has been proposed that the **initiation of filopodia growth is mediated by clustering of IRSp53, an I-BAR domain protein** [4] that **next recruits actin nucleators for actin polymerization and the generation of pushing forces by the filaments.** In TNTs, another closely related I-BAR protein, IRKTS, is probably involved. The global objective of the project is to uncover the mechanism of filopodia and TNT generation. we will form an artificial protrusion out of cell membranes using optical tweezers or micromanipulation [5] and we will observe the sequence of recruitment of the proteins in this structure, as well as the condition for actin growth using confocal microscope or SIM. Different cortical actin organizations will be obtained by growing cells on micropatterns, and the respective effect of IRSp53 and IRKTS will be studied. A better distinction on the mechanisms leading to TNTs or filopodia, and more generally on cellular protrusions should be achieved with this study.
This project will be developed in close collaboration with C. Zurzolo (Inst. Pasteur) and P. Lappalainen for the biological aspects.

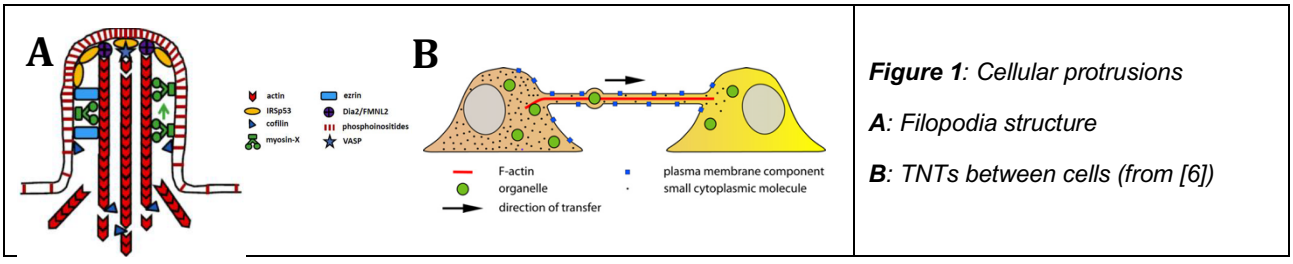


***Figure 1**: Cellular protrusions*

***A**: Filopodia structure*

***B**: TNTs between cells (from [6])*

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[5] B. Pontes *et al.*, Biophys. J. **101**, 43 (2011).
[6] H. H. Gerdes *et al.*, FEBS Lett. **581**, 2194 (2007).

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Characterization of a sphingomyelin sensor with *in vitro* model membrane systems

DIRECTEUR de Stage : Patricia Bassereau patricia.bassereau@curie.fr
Web: https://science.institut-curie.org/team-bassereau

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de la thèse : Mechanism of Caveolin-assisted transport and enrichment of sphingomyelin in the plasma membrane

Lipid and protein sorting are crucial processes that maintain unique biophysical and biological properties of different organelles. Nevertheless, the mechanism by which sphingomyelin (SM) is enriched at the PM is unknown. SM is synthesized at the trans-Golgi network (TGN) and transported to the PM via an uncharacterized pathway. SM enrichment in transport carriers cannot simply be explained by a curvature-based mechanism of lipid sorting, since SM form stiff membranes. A few years ago, we proposed that a mechanism of protein-assisted lipid sorting could explain the enrichment of SM in transport carriers. Caveolin is the main structural protein of caveolae, small PM invaginations present in many cells. Since caveolin has a strong affinity for SM and cholesterol-rich environments, and is transported from TGN to PM to assemble caveolae, it is a good candidate for this protein-assisted mechanism (Fig. 1A). Our preliminary data *in cellulo* support indeed this new hypothesis. The **objective of the PhD** will to **develop *in vitro* reconstituted systems based on Giant Unilamellar Vesicles (GUV), membrane nanotubes of controlled curvature, and purified caveolin as well as quantitative fluorescence analysis to investigate the mechanism of protein-assisted lipid sorting** (Fig. 1C). The project will be developed in **collaboration with C. Lamaze (I. Curie) for the biology part**. These reconstituted systems will also have important potential interest for groups working on caveolae, recently involved in mechanosensing and associated with several pathologies.

Internship: To quantify sphingolipid concentration, we need a **sphingolipid reporter**. C. Bird's group has developed a novel sphingomyelin (SM) reporter: **Equinatoxin** (Eqt). The objective of the internship to **characterize the ability of Equinatoxin to bind SM depending on the organization of the membrane and on the SM content** and to further address its possible applications for in vitro studies requiring SM reporter (Tube pulling assays). First, Giant Unilamellar Vesicles (GUVs) will be used as a model membrane system (Fig. 1B) with fluorescent Eqt. Quantitative measurements will be performed on GUVs with different lipid compositions, corresponding to monophasic or diphasic organization. Protein binding will be analyzed by confocal microscopy imaging. In a second part, giant liposomes formed (GPMVs) from cell membranes will be used and similarly Eqt binding will be quantified. This project has high potential impact since there is a strong need for SM detection/labelling in a wide range of in vitro experiments.

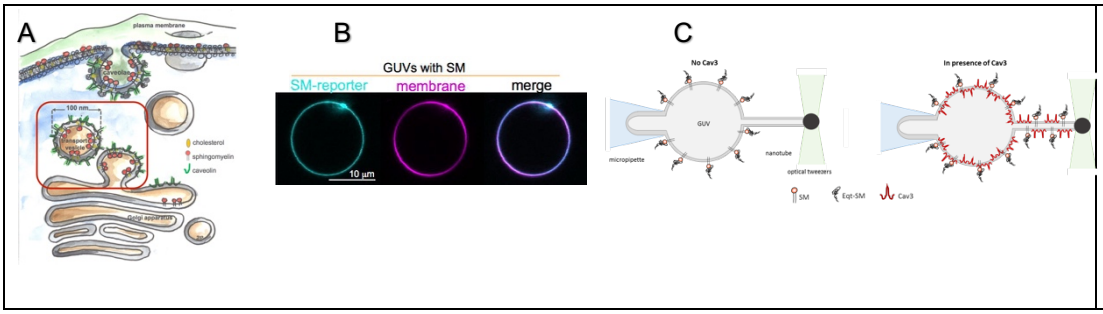

Fig. 1: (A) Caveolin and traffic of sphingomyelin from the Golgi to the plasma membrane. (B) Binding of the SM reporter (Eqt) to GUVs containing SM; confocal microscopy image. (C) Nanotube pulled from GUV for quantifying caveolin-assisted SM enrichement in curved membranes.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Recruitment of curvature-sensitive proteins in biomimetic bud necks

DIRECTEUR de Stage : Patricia Bassereau patricia.bassereau@curie.fr
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Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : OUI (probablement)

SUJET de la thèse : "Reconstitution of membrane scission by ESCRT-III complexes"

ESCRTs are evolutionary conserved protein complexes that mediate membrane remodeling and scission in many cellular events such as cytokinesis (cell division), internal endosomal vesicle (MVB) formation, HIV virus release (Fig. 1A) and nuclear membrane repair [1, 2]. ESCRT-III complexes and VPS4 have been proposed to constitute the membrane fission machinery. The proteins of these complexes tend to assemble into polymer filaments. However, in spite of a fast growing number of studies on the structure of these protein polymeric assemblies *in vitro* and *in cellulo,* **little is currently known about the minimal composition of ESCRT-III actually required for fission and how the mechanics of the different polymers couples to their dynamical organization to produce membrane constriction and cleavage**. *In vivo*, ESCRT-III-induced membrane constriction and cleavage occur at membrane sites with a specific morphology: for instance, at the neck of buds or at the cytokinetic bridge, where membrane has a dumb-bell shape characterized by a negative Gaussian curvature. Whether the shape of the membrane is important in the ESCRT-III proper assembly and function is still an open question. Following on our previous work on the reconstitution of ESCRT-III at the neck of membrane nanotubes [3], we propose to develop different model membranes systems or artificial budding viruses to mimic the membrane shape corresponding to these processes and to find out conditions where a minimal ESCRT-III and VPS4 fission machinery is operant.

In a first step (M2 internship), we will **set-up an *in vitro* system**, using the wrapping of colloidal particles by the membrane of giant vesicle (Fig. 1B) [4] **to form necks that mimic the neck of viral particles**. Using purified ESCRT-III proteins and ATP and by tuning neck shape by adjusting membrane mechanics and particle-membrane adhesion [5] (Fig. 1C), we will find the geometrical and biochemical conditions that lead to neck scission.

This project will be continued by using artificial viral particles provided by our collaborator and by comparing the protein assemblies at the neck of membrane nanotubes. This project will be **developed in close collaboration with W. Weissenhorn (Grenoble**) **for the biological and structural aspects** and **with P. Sens (Inst. Curie)** for the **modeling**. The final aim is to obtain a physical model for membrane scission by ESCTS.

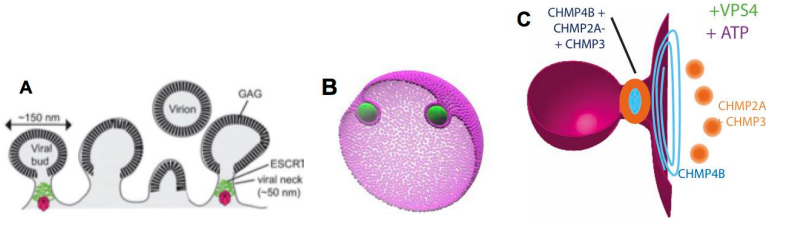

***Figure 1**: **A**:ESCRT-III are recruited at the neck of the HIV virus to induce scission (from[6].) **B**: Forming a membrane neck by wrapping colloidal particles by the membrane of giant unilamellar vesicles (from[4]). **C:** Reconstitution of the assembly of the ESCT-III polymers at the neck of an artificial virus (colloid or synthetic viral capsid)*

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[6] A. Y. Adell *et al.*, The FEBS journal **283**, 3288 (2016).

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE : Inference analysis of timely and spatially coupled datasets : application to modeling ex vivo tumor-immune response ecosystems

DIRECTEUR de Stage : Hervé Isambert http://kinefold.curie.fr/isambertlab

Ce stage peut être poursuivi en thèse : OUI

Si oui, la thèse est-elle financée : NON

SUJET de la thèse :

Inference methods based on Maximum Likelihood approaches assume that the available data includes *independent* samples. While this is indeed the case for various biological datasets, such as single cell gene expression data, or clinical datasets from medical records of patients, many other datasets of interest include in fact *non-independent* samples, such as datasets including time series or phylogenetically related sequences.

Our lab recently developed a novel inference method to learn causal relationships in a broad variety of biological or clinical datasets from single cell transcriptomic data (qRT-PCR or RNA-seq) and genomic alterations in tumors (Verny et al 2017, Sella et al 2018) to medical records of patients (Cabeli et al 2019). The method can learn a large class of graphical models including undirected, directed and possibly bidirected edges originating from latent common causes unobserved in the available data. This machine learning approach combines the analysis of multivariate information with consistent constraint-based graphical models (Li et al 2019). In brief, it starts from a complete graph and iteratively removes dispensable edges, by uncovering significant information contributions from indirect paths, while guaranteeing their consistency with the final graph (Li et al 2019). The remaining edges are then filtered based on their confidence assessment or oriented based on the signature of causality in observational data. The resulting method (miic) outperforms concurrent methods on a broad range of benchmark networks, achieving better results with only ten to hundred times fewer samples and running ten to hundred times faster than the state-of-the-art methods (Verny et al 2017, Cabeli et al 2019).

The present project aims at extending this network inference method to analyze datasets including timely and spatially coupled features extracted from video images of complex tumor ecosystems. We have already extended and benchmarked these inference methods to analyze time series (Verny et al 2017) or phylogenetically related datasets (Sella et al 2018, Singh et al 2019), but a more general approach, not assuming prior knowledge about the type of dependency between samples, will be implemented in the course of this project.

The method, which can have many potential applications, will be applied to analyze the timely and spatially coupled cellular features of tumor-immune response ecosystems. In particular, we will analyze causal effects of chemotherapy and immunotherapy on various tumor ecosystems reconstituted ex vivo by our collaborators at Institut Curie (MC Parrini), see (Nguyen et al 2018).

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Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

TITRE DU STAGE :

Development and validation of a fully automated microfluidic magnetic fluidized bed for exosomes circulating biomarkers

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Lieu du stage :

Institut Curie UMR 168
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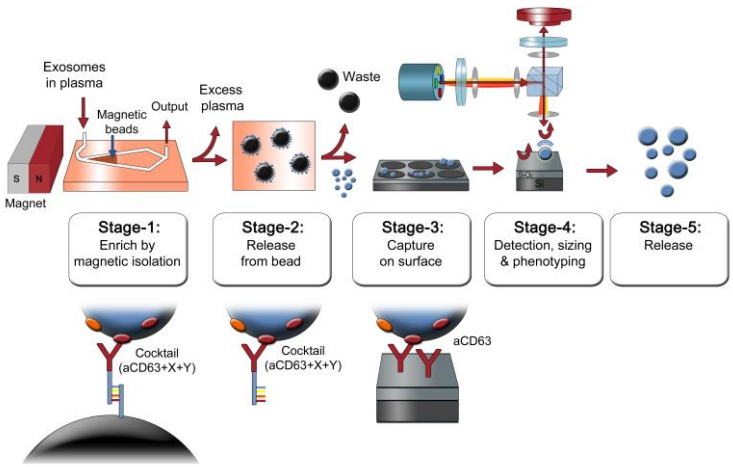
Development and validation of a fully automated microfluidic magnetic fluidized bed for exosomes circulating biomarkers

The Long-term vision is to develop new tools for translational medicine towards utilization of promising biomarkers of the future for molecular diagnostics of cancer, i.e. exosome-based diagnostics. Exosomes are nanoparticles known to mediate communication between cells and facilitate antigen presentation as well as signaling between neighboring cells, and received attention for their potential as diagnostic biomarkers. However, the clinical breakthrough exosomes may present in healthcare could not be realized to date due to their small size (30-100nm).
Our project is aiming to generate the breakthroughs in nano-scale technologies to overcome this challenge, and accelerate the transition of exosome-based research and scientific discoveries to real clinical utility. To achieve this goal, the project will focus on the first step of exosomes immuno-extraction using an original microfluidic approach: microfluidic fluidized bed.



Fluidization is a steady-state dynamic regime in which solid particles show a fluid-like behavior under two directionally opposite but equal forces. Fluidized-beds are commonly used to enhance surface interactions in a solid/liquid mixture, with high stirring and low backpressure. Our team developed a completely new concept of microfluidized-beds [1] based on magnetic micro particles in equilibrium between flow-induced drag forces and

magnetic forces. This system already showed unprecedented efficiency for solid phase extraction and was successfully applied for the detection of bacteria and protein biomarker preconcentration [2].

The main goal of this internship will be to demonstrate how this fluidized bed technology can be used for the extraction and preconcentration of circulating exosomes in collaboration with our partner at CNR in Italy. This work will be part of the European project (INDEX). The main challenge will be to extract specifically exosomes present at in a complex samples, different aspects will be investigated (the capture ligands, temperatures, beads configuration….) and also to setup the release of exosomes, a crucial step of the bioanalytical workflow. Microfluidics will bring to this project its efficiency of mixing and ability of target-capturing during sample injection. This work will be performed in collaboration with the INDEX consortium.

The project is interdisciplinary and translational: students are not expected to have experience in all fields, and will learn during the project what is complementary to his/her own training. The project will be suitable for instance for students following courses in soft matter and complex fluids, nanoscience, microfluidics, microsystems or analytical sciences.

Laboratoire Physico-Chimie Curie

Institut Curie, 11 Rue Pierre et Marie Curie-75005 PARIS

How intestinal epithelial cells are affected by peristaltic motion on 3D scaffold ?

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Our team has recently launched an on organ on chip activity. Organ-on-a-chip devices aim at recapitulating multicellular architectures, physicochemical, biochemical and geometrical features of a specific organ or tissue on a microfluidic chip. These approaches mimic organ functionality and complexity much better than conventional 2D or even 3D culture systems. In close collaboration with the team of Danijela Vignjevic (Institut Curie, UMR 144), we have recently developed an in vitro model of gut on chip in order to decipher the mechanisms underlying gut homeostasis (figure 1). The current gut on chip model we developed captures the complexity of the intestinal mucosa as it reproduces its microarchitecture and the spatial distribution of its molecular and cellular constituents.

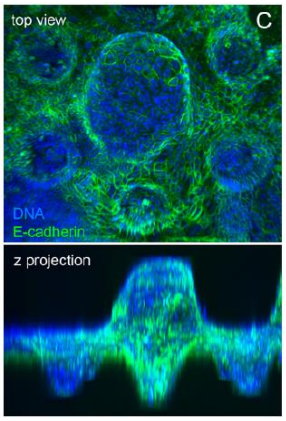

Figure: 1 top view and side view of the 3D gut on chip

Objective :

Based on this existing model, this project aims at determining the minimal number of components necessary to recapitulate gut physiology. In particular, we will investigate how intestinal epithelial cells are affected by peristaltic motion when cultured on a 3D scaffold. To do so a microfluidic device containing different crypts geometries will be used as cell scaffold to compare cells behavior when cyclically stretched or not. In particular, will investigate the influence of cells stretching on intestinal cells shape and orientation as well as on their proliferation and migration. Starting with intestinal cell lines, further experiments will be performed with organoids.

The project is interdisciplinary and translational: students are not expected to have experience in all fields, and will learn during the project what is complementary to his/her own training. The project will be suitable for instance for students following courses in biophysics, microfluidics or microsystems.

Laboratoire Physico-Chimie Curie

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Development and validation of a magnetic hydrogel for Single Cell analysis in droplet microfluidics

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Cancer is the leading cause of death worldwide. It is now accepted that cancer is a highly heterogeneous disease and that subpopulations of cells within a single tumor can exhibit distinct genomic and phenotypic profiles. Tumor heterogeneity arises from clonal evolution, microenvironment interaction and phenotypic plasticity[i]. There are still open questions about tumor heterogeneity and original experimental approaches are required to distinguish the relative contributions of the different sources of heterogeneity to disease progression.

In the last decade Next-Generation Sequencing (NGS) has emerged as relevant tool to tackle such challenges both in clinics and life sciences. Some groups such as Macosko et al.[ii] developed disruptive technologies based on droplets microfluidics barcoding and NGS to study gene expression at single-cell level at high-throughput. Although these methods have been applied mainly on transcriptomics, droplet microfluidics technologies are also used to analyze genomics[iii], proteomics[iv] or epigenetics[v]. Despite the efficiency and the disruptive character of these microfluidic-based technologies, they are limited to one analyte, and studying several "omics" from the same single cell remains a challenge.

To carry out these "multi-omics" studies, a physical separation of the different analytes present in the cell is often needed, making it difficult to apply in droplet microfluidics. Our group is developing an original solid support extraction approach based on magnetic tweezers that allows the transfer of functionalized magnetic microparticles between sub-nanoliter droplets (Fig. 1). The aim of the internship is to develop and characterize a new class of solid support based on hydrogel microbeads, that would increase both efficiency and throughput of the system. This will open the second part of the internship, which will focus on biological application of the developed technology.

This project is multidisciplinary at the interface between physico-chemistry, microfluidics and biology. Thanks to the position of the host laboratory inside Institut Curie and Institut Pierre-Gilles de Gennes (IPGG) for Microfluidics, the intern will benefit from an environment of experienced biologists and renowned physicists and microfluidicians. Furthermore, for all the microfabrication and experimental work, the IPGG is hosting the technological platform (UMS 3750) where facilities such as clean and grey rooms, cell culture, electronic and photonic fluorescent microscopy are available.

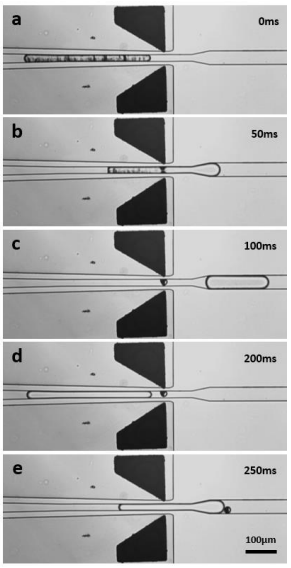

Figure 1: (a,b,c) Capture of around 10ng of magnetic particles (from a 400pL droplet. (d,e) The cluster is pushed out of the tweezers by the following droplet.

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