physico chimie curie
internship
opportunities
2021

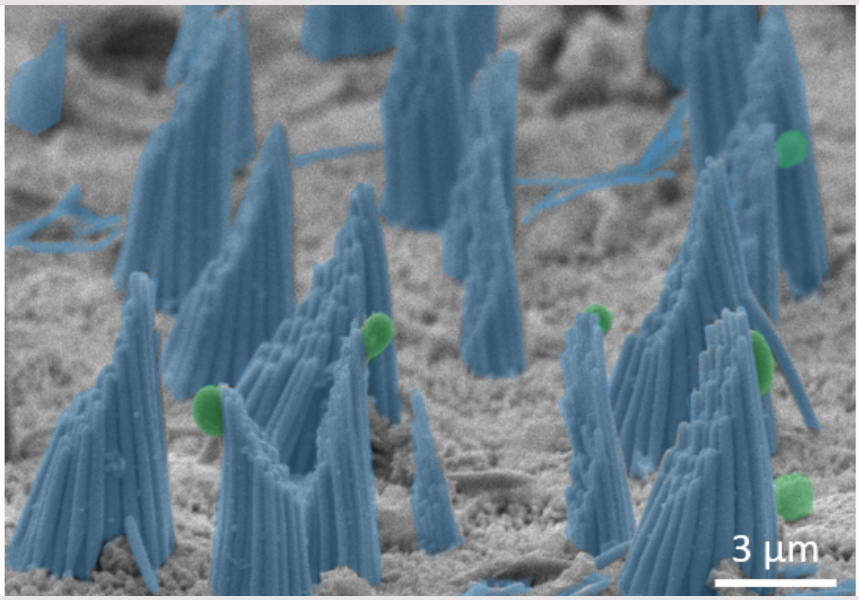
institut Curie

cnrs

SORBONNE UNIVERSITÉ

PSL RESEARCH UNIVERSITY PARIS

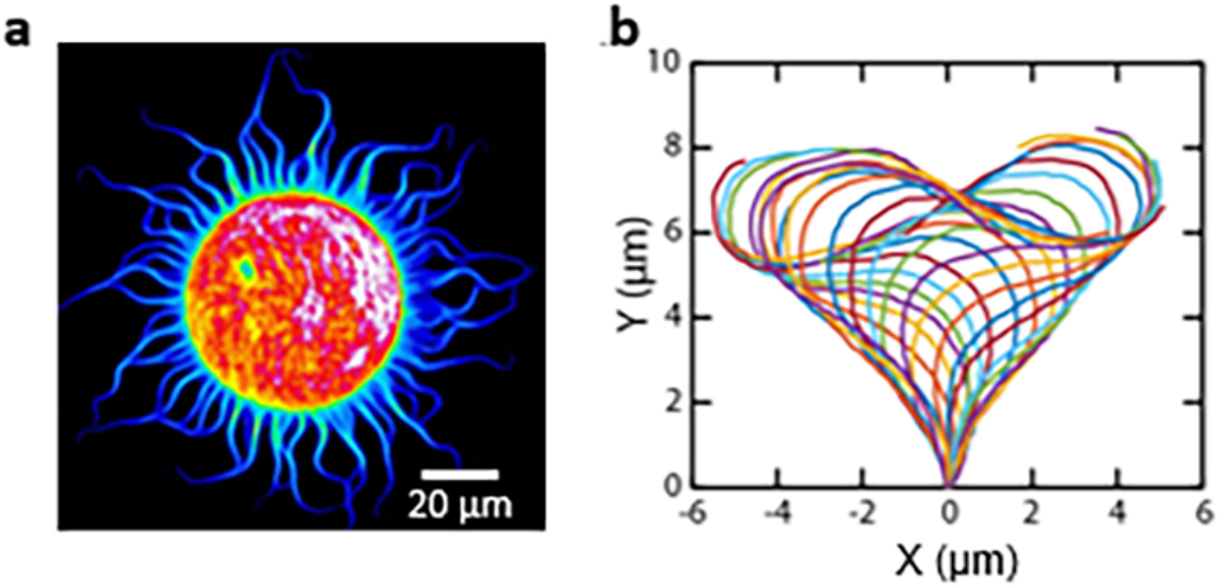
Table of Contents



Active mechanosensitivity by hair cells of the inner ear

Over the past twenty years, Martin's team has developed micromechanical tools (flexible micro-fibers, fluid jet, optical tweezers) to study the active and passive mechanical properties of the hair bundle, the mechanosensory antenna of the hair cells in the inner ear. In close collaboration with theorists, physical models were developed to explain the various regimes of active hair-bundle motility as well as the effects of intrinsic noise, friction, and elastic coupling on hair-bundle mechanosensitivity. In parallel, the team has designed minimal molecular systems comprising purified myosin molecular motors and actin filaments, demonstrating that spontaneous mechanical oscillations resembling those observed with the hair-cell bundle, in muscle fibers, or sperm flagella can arise from a dynamic instability in a motor group under elastic loading. In addition, a similar biomimetic approach was developed to study active transport and positioning of colloidal particles by myosin motors in antiparallel networks of actin filaments.

Team leader : Pascal Martin
Team size : 5
Number of PhDs and Post-docs : 4



Self-Organized Flagellar-Like Beating of Actin Bundles In Vitro

Biological systems are endowed with the remarkable ability to self-organize at large scales by orchestrating the local activity of many small constituents that each consume energy from the environment. This project focuses on the spontaneous emergence of wave-like mechanical oscillations in polar bundles of filaments, as observed in cilia and flagella of eukaryotic cells. We have recently developed a bottom-up approach based on a minimal active molecular system demonstrating in vitro that wave-like beating emerges robustly in polar filament bundles.

Using surface micro-patterns to control the geometry of actin polymerization, we produce a thin network of parallel filaments. In the presence of myosin-motor dimers added in bulk, growing actin filaments self-organize into beating bundles resembling those observed in eukaryotic cilia and flagella (see Figure). A decisive asset of our assay is that the motors can be visualized using fluorescence microscopy while the actin bundle is beating, revealing that actin-bending waves are associated with myosin-density waves.

The trainee will contribute to the following tasks: (i) Study the effect on beating properties of varying the actin-bundle architecture and motor physical properties, (ii) probing the interplay between myosin-motor localization and actin-filament shape, (iii) Study the effect on beating properties of the surrounding-fluid viscosity and of an external localized force (optical tweezers), (iv) Study how a beating actin bundle interacts with its environment to generate flow, synchronize with its neighbors, and apply forces that may lead to swimming.

Our Laboratory is located at the Institut Curie (Paris), which provides an international, supportive, and inspiring environment for scientific research in a broad range of disciplines. Our project has recently been funded for 4 years by the ANR and involves a collaboration between two teams of complementary experimental expertise. Our team aims at studying beating of filament bundles at mesoscales (many filaments and motors), while the group of Guillaume Romet-Lemonne (Institut Jacques Monod, Paris) will work primarily at the molecular scale on single filaments and bundles with only a few filaments. In addition, we collaborate with theorists (Frank Jülicher, MPIKS, Dresden; Jean-François Joanny, Collège de France) to develop a physical description of our observations.

We seek a highly motivated physicist or biologist interested in developing experiments at the interface between physics and biology.

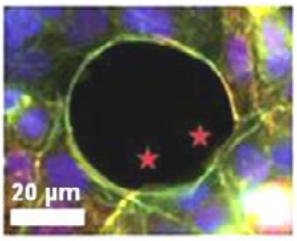
Contact

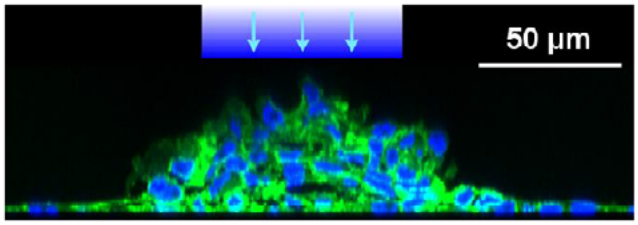
Pascal Martin
pascal.martin@curie.fr

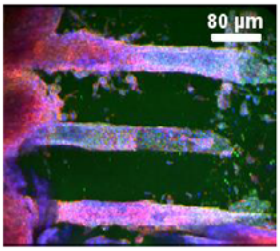
Information

This internship can be followed by a thesis.

The thesis will be funded.







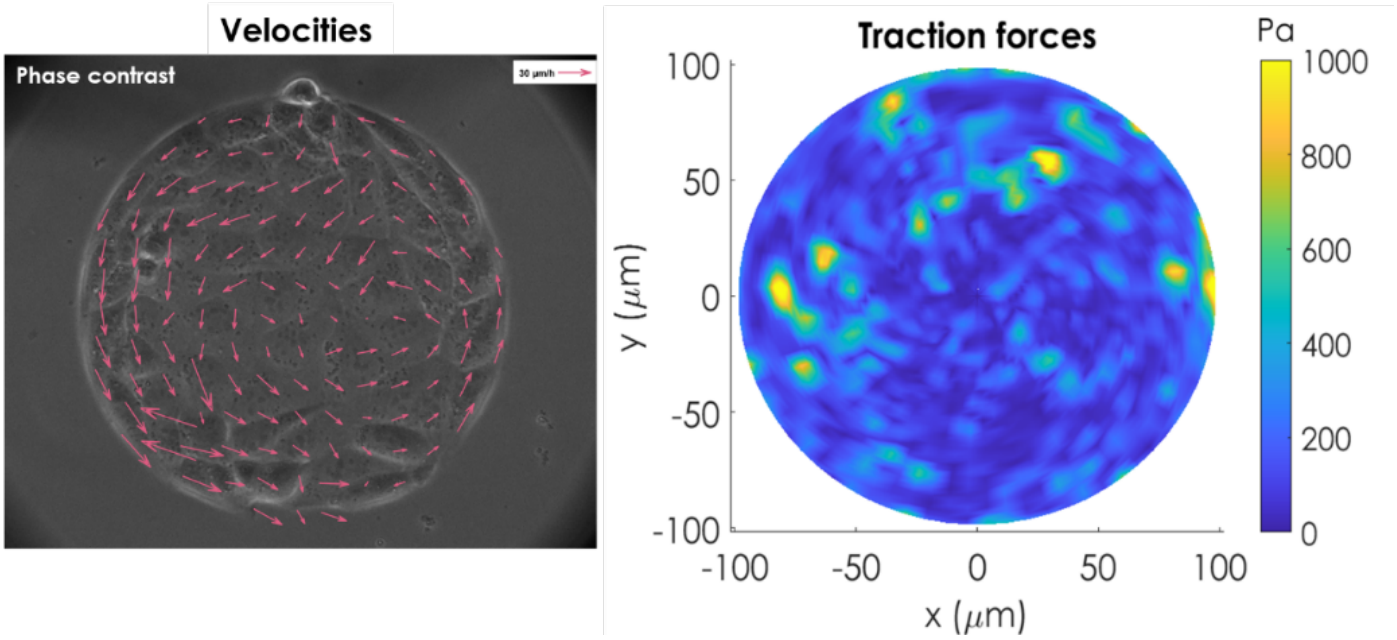
Biology-inspired Physics at MesoScales

Our research focuses primarily on the study of populations of interacting cells using physics concepts and techniques.

We conduct experimental projects addressing different aspects of communication between cells in different systems (including bacteria and mammalian cells). To achieve a good control in time and space on the local microenvironment of the cells, we extensively use the possibilities offered by microfabrication/micropatterning techniques.

We also develop tools to quantitatively analyze our experimental data, which allows interpreting behaviors of populations on the basis of single cells'. The results obtained with this approach have led to several collaborations with theory groups and biology groups in France and abroad.

Team leader : Pascal Silberzan & Axel Buguin
Team size : 10
Number of PhDs and Post-docs : 6



Collective behavior of a confined population of transformed cells

Biological tissues are active cellular materials whose mechanical characterization is a timely subject. How forces and motions drive collective behaviors in population of normal cells tends to be well characterized. Several works have reported propagation of waves in monolayers of normal epithelial cells under confinement. Such physical waves could be responsible for carrying information across the tissue. Much less is known about pathological situations for which collective behaviors are still relevant, like in cancer invasion. In that context, we wonder how mutated cells behave under confinement? More specifically we want to study the impact of an oncogenic transformation on collective flows and forces.

To address this question, we will study the mechanical behavior of confined colonies of cells, which are genetically modified to be light-sensitive. In dark, the cells keep a normal phenotype, while they over-activate the Src oncoprotein (known to be overexpressed in many cancers) if they are exposed to blue light. We will analyze forces and motions of the cells in relation with the oncogenic stress, directly controlled with the blue-light illumination.

To confine cells, we use microfabrication. Image analysis allows us to measure velocity field, the deformation rate and the cell polarity pattern. Traction Force Microscopy gives access to cellular tractions. We will look for correlations between dynamics (cellular flows), mechanics (traction forces) and genetics (oncogenic state).

Understanding the effect of an oncogenic activation on collective behaviors should help to unravel the mechanisms of collective cell migration in cancer.

Skills: cell culture, microfabrication, optical microscopy, optogenetics, image analysis, traction force microscopy.

Contact

Isabelle Bonnet
isabelle.bonnet@curie.fr

Information

This internship can be followed by a thesis.

The thesis may be funded.

Directing cell populations with multiscale environments

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. Importantly, these environments are complex and include different lengthscales from molecular sizes (the biopolymers of the Extra Cellular Matrix (ECM)) to multicellular guiding cues (such as the large scale heterogeneities in the ECM gel). Therefore, experiments aiming at mimicking these situations in vitro should include this complexity in the form of guiding cues, physical boundaries, and confinement.

In the last years, our group has addressed different aspects of collective behaviors of cells with a physics approach. In particular, we have studied various aspects of collective migration in confluent cell sheets in different situations including for instance spontaneous laminar flows, global oscillations or migration over a free surface (wound-healing) under confinement.

In the present project, we propose to increase the complexity of these experiments by designing multiscale structures and studying the impact of the different lengthscales that can act independently or synergistically on high-density cell populations. From these experiments and their theoretical interpretation, we expect to be able to quantitatively extract several relevant parameters such as the cell-substrate effective friction. In a second time, acting on the relevant signaling pathways will allow relating these physical global parameters with the cell molecular players.

To address these questions practically, we combine microfabrication, micropatterning, innovative microscopy techniques and image analysis, with cell biology techniques. Physical quantities such as the cell flows or the mechanical traction forces exerted on the substrate are quantitatively measured at all scales in parallel with biological activity.

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

Contact

Pascal Silberzan
pascal.silberzan@curie.fr

Information

This internship can be followed by a thesis.

The thesis may be funded.

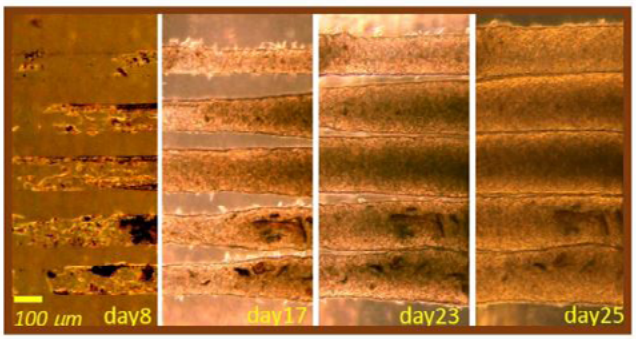
Recent references (selection)

- 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.

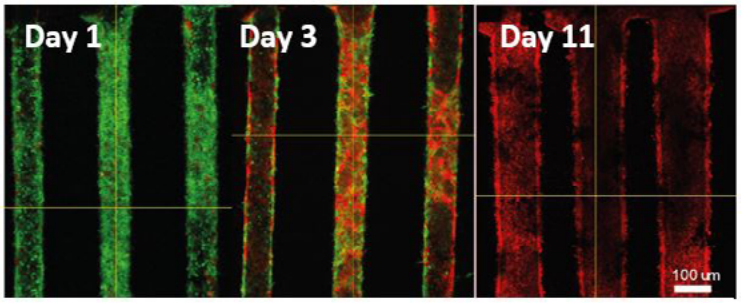
- Duclos G., Erlenkämper C., Joanny J.-F., Silberzan P.: Topological defects in confined populations of spindle-shaped cells. Nat. Phys. 13, (2017), 58.

- Hakim V., Silberzan P.: Collective cell migration: a physics perspective. Rep. Prog. Phys. 80, (2017), 076601

- Reffay M., Parrini M. C., Cochet-Escartin O., Ladoux B., Buguin A., Coscoy S., Amblard F., Camonis J., Silberzan P.: Interplay of RhoA and mechanical forces in collective cell migration driven by leader cells. Nat. Cell. Biol. 16, (2014), 217.



Dilation of tubes lined with ADPKD cells



Kinetics of dilation of tubes lined with a mix of ADPKD healthy (green) and cystic cells (red)

Renal cyst formation: cell competition and mechanical determinants studied in 3D biomimetic environments

Genetic renal kidney diseases lead to the development of numerous cysts in the renal tubules, ultimately leading to kidney failure. Despite extensive work on the genetics and cell biology of these diseases, the precise mechanism of cyst formation, resulting from localized dilatation of the renal tubules, remains imperfectly understood. Renal tubules have an extremely controlled geometry and are permanently subjected to mechanical constraints. Our hypothesis is that these mechanical determinants play a key role in cyst formation. The most common genetic kidney disease, Autosomal Dominant Polycystic Kidney Disease (ADPKD), leads to the alteration of proteins, polycystins, involved in mechanotransduction through their presence in the primary cilia, a flow-sensitive mechanosensitive organelle. Cysts arise initially from somatic mutations inactivating further polycystins in a few cells. This leads presumably to cell competition with the neighbor cells, and to localized tube dilation and cyst formation upon the mechanical constraints experienced by the tubule. In a second time, these mechanical constraints are reinforced due to compression by expanding cysts. Mechanical stimuli are sensed by the primary cilia; paradoxically, removal of primary cilia inhibits cyst growth in animal models of the disease. The aim of the internship is to investigate competition events and the role of primary cilia in tube dilation upon mechanical perturbations.

In order to finely study the mechanical contributions in ADPKD, we have developed several original tube systems with versatile geometries, stiffness and adhesive properties, using microfabrication and microfluidic techniques. These systems can be lined with cell lines either model of healthy cells and/or of the disease, which we have previously shown to induce tube dilation. The purpose of this internship will be to study in our device the tubular deformation induced by model ADPKD cells whose primary cilia length can be modulated (collab. F. Bienaimé, Necker hospital), in the presence or absence of flow or mechanical compression. We will also try to investigate the role of those constraints on tubular deformations on competition assays, in a mix of healthy and ADPKD cells.

This internship will include a microfabrication and microfluidics part for induction of mechanical stresses in the microfluidic devices previously developed in the team, and a part of cell culture, immunofluorescence, and imaging by confocal or video microscopy.

Contacts

Sylvie Coscoy
sylvie.coscoy@curie.fr
Stéphanie Descroix
stephanie.descroix@curie.fr
Brice Lapin
brice.lapin@curie.fr

Information

This internship can be followed by a thesis.

Dynamics of endothelial engagement in complex 3D microstructures built by two-photon photopolymerization

Contacts

Sylvie Coscoy
sylvie.coscoy@curie.fr

Vincent Semetey
vincent.semetey@chimieparistech.psl.eu

Pierre Ucla
pierre.ucla@curie.fr

Information

This internship can be followed by a thesis.

The fundamental understanding of endothelium-extracellular matrix interactions during the formation of new blood vessels meets considerable medical needs. The 3D geometrical organization of matrix fibers is complex, and modified in many pathophysiological conditions, like in tumor microenvironments which favor the formation of tortuous and disorganized vessels. Our group focuses on early steps of angiogenesis, where “leader” tip cells invade extracellular matrix upon growth factors gradients, leading trailing endothelial cells (stalk cells). Tip/stalk transient specification is governed by Dll4/Notch signalling, with tip cells emitting exploratory filopodia important for angiogenesis, while stalk cells are devoid of filopodia.

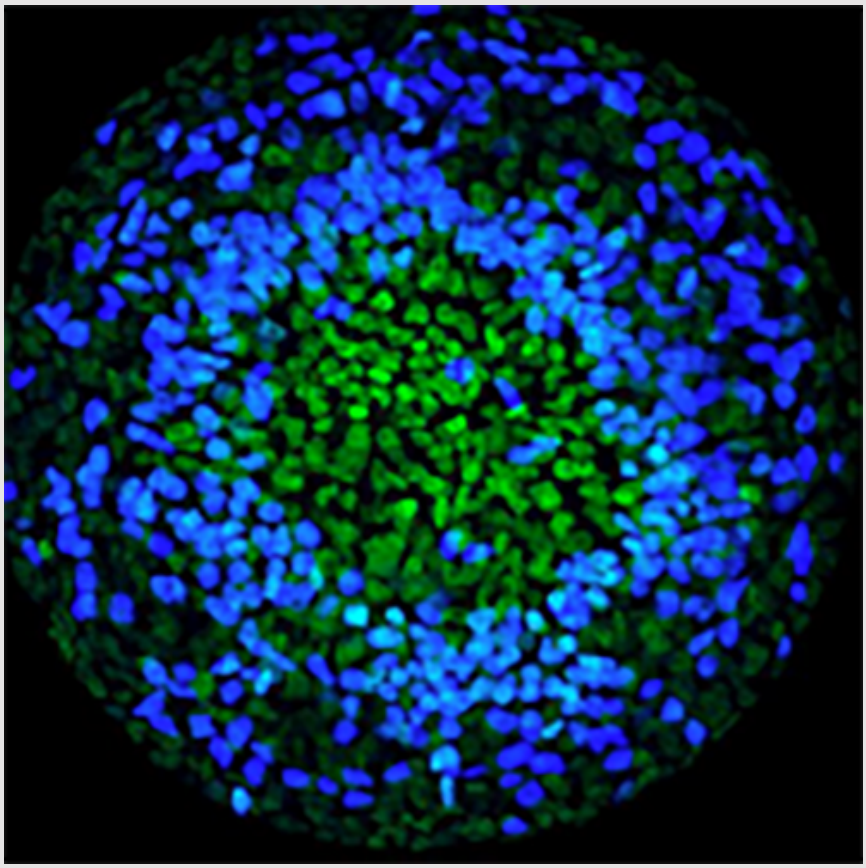
We have previously identified 3D microstructures triggering the formation of endothelial filopodia evocative of a tip cell phenotype (Figure 1). Filopodia were formed with or without Vascular Endothelial Growth Factor, suggesting that geometry alone might lead to tip cell phenotype. Our 3D microstructures are realized by two-photon photopolymerization, which allows to build structures at a subcellular scale thanks to the confinement of the excitation to the focal volume (µm3). We are now building more complex 3D structures in order to study early steps of endothelial multicellular engagement, and filopodia dynamics coupled to collective migration. Our system based on two-photon photopolymerization provides an ideal tool for modifying at will the 3D geometries and stiffness characteristics of the tunnels, and to understand finely the dynamic adaptation of vessel formation to the microenvironment.

This internship aims to optimize the creation of tunnel-like 3D microstructures promoting multicellular engagement and collective migration guided by patterns with varied properties (like stiffness, spatial organization or protein coating). The cell migration mode as well as the formation of filopodia in response to the local geometrical and mechanical constraints will be characterized from a biology perspective (stainings, pharmacology) and from a physics perspective (migration speed, dynamics of filopodia formation, exerted forces).

The intern should have basic knowledge of cell culture techniques. During the period of the internship, he/she will perform cell culture (HUVECs), live imaging (spinning disk, two-photon, lattice light sheet microscopes), stainings (nuclei, cytoskeleton, E-Cadherin, focal adhesion proteins, ion channels, actin regulators…) (collaboration C. Monnot, Collège de France). The intern should demonstrate some interest in microfabrication as part of the work will be dedicated to the fabrication and functionalization of microstructures by two-photon photopolymerization.

Depending on the intern’s profile and interests, the project may also include an aspect of :

- Microfabrication : participation in the design of new architectures (use of CAD software like Sketchup, optimization of slicing and laser parameters for fabrication) and characterization of the resulting structures (SEM and AFM).
- Chemical functionalization : optimization of the spatial patterning of chemical coating in the structures. Coatings include PEG (anti-adhesive), extracellular matrix proteins (collagen, fibronectin, laminin…), angiogenic signalling pathways molecules (VEGF, Dll4…).
- Image analysis : use of Python/Keras for automated segmentation of cellular components via CNN (Convolutional Neural Networks), participation in the annotation of 3D images, in the optimization of the architecture and parameters of the neural network, in the evaluation of performances and comparison with other existing tools.
- Modeling : use of Comsol Multiphysics to model the forces exerted by the cells in the microstructures based on the local deformations.



Dynamic Control of signaling and gene expression

We are an interdisciplinary team working on systems and synthetic biology. We use microfabrication, microfluidics, genome editing, fluorescence and opto-genetics to produce quantitative measurement of cells' dynamical processes. We also use advanced modeling and theoretical tools to extract single cell parameters. Our long term goal is to improve our ability to interact and control cellular processes in vivo, with a specific focus on gene expression and cell-computer interfacing.

Current projects include computer-assisted control of gene expression, optimization of biomolecule production by yeast and control of the spatial organization of developing tissues.

Team leader : Pascal Hersen
Team size : 11
Number of PhDs and Post-docs : 5

Microfluidic gradient chip to understand early human embryonic patterning

One striking property of embryonic tissues is their ability to self-organize in order to form well-defined patterns that prefigure the body plan. One such early morphogenetic event is gastrulation, during which the cells of the pluripotent epiblast are allocated into three germ layers that derive from it: the ectoderm, the mesoderm and the endoderm.

Assessing the relative contribution of parameters controlling this process (morphogen gradients, mechanics…) in a quantitative manner requires at the same time to record fates and movement with single cell resolution but also to be able to apply well defined perturbations to the system, which is usually not possible in live embryos, especially for species developing in utero, like human. Thus, to address these issues in a quantitative manner, we are developing tool that allow in vitro recapitulation of early embryonic patterning and morphogenetic processes (Warmflash et al. Nat Methods 2014).

Recently, we have designed a microfluidic chip to cultivate human Embryonic Stem Cells (hESC) for several days under stimulation of fully controllable gradients of morphogens. Using this unique device, we have studied how the tissue patterning depend on the maximum concentration, and the shape (steepness, linear vs parabolic) of a BMP gradient. Cell identity can be recorded live thanks to fluorescent reporters (Fig1A-C).

Our preliminary results show that in a parabolic gradient of BMP4, some cell types (amnion-like cells, mesoderm) are generated in a concentration dependent manner, in perfect agreement with the classical “French flag model” (Wolpert, 1969). However, endoderm cells are not generated in those parabolic BMP gradients. They were observed however in steeper profiles of BMP concentration, such as step function. These observations suggest a model for patterning of the embryo in which the tradeoff between the spatial profile of BMP and the diffusion properties of mesendoderm inducing molecules (WNT, NODAL) plays a crucial role in the tissue patterning (fig1D). The goal of this internship is to investigate how the patterning evolves when other morphogens are added in order to test and constraint this model that defines the rules for the spatial organization of cell identities present in the gastrula.

During this internship, you will learn: making and operating advanced hybrid 2-layer microfluidic chips with porous membranes, culture and characterization of human embryonic stem cells, confocal microscopy and image analysis.

Contact

Benoit Sorre
benoit.sorre@curie.fr

Information

This internship can be followed by a thesis.

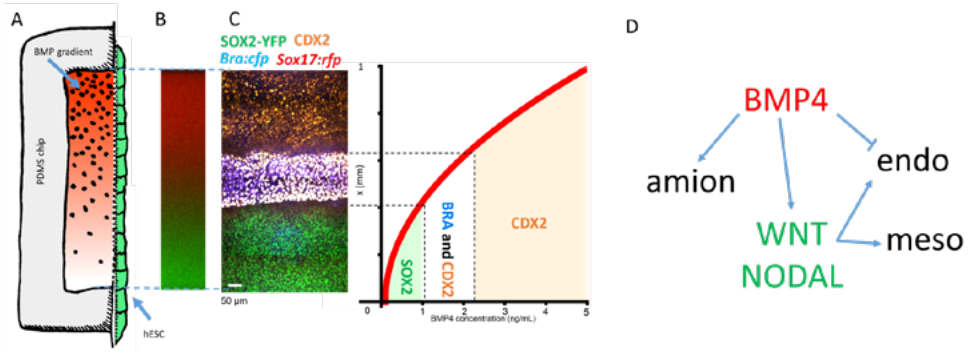


*Fig1: **Antiparallel gradient chip**. A: sketch showing the principle of the chip B: Proof of principle of hESC differentiation in the gradient chip. after 48h of differentiation in a parabolic gradient of BMP. Cells get an extra-embryonic identity at the high end of the BMP gradient (CDX+2, amnion) at intermediate dose cells adopt a mesodermal fate (BRACHYURY+) and stay pluripotent under a certain threshold (SOX2+). (D) Model for the patterning to be tested. BMP4 induces the amnion identity directly and also induces the production of secreted diffusible factors (WNT, NODAL) necessary for formation of endoderm and mesoderm. BMP4 prevents formation of endo*

Bioelectricity and embryonic patterning

All cell membranes present an electric potential. This is called the Resting Membrane Potential (RMP) and is the trace of the difference of ionic charges between the extracellular and the intracellular spaces. This is very well known in the so-called excitable cells (e.g. neurons, muscles) which can change their electric potential thanks to ion channels regulation. In the past years however there has been a regain of interest on the role of the membrane potential in participating to biological regulation at the scale of the cell and the tissue in non-neural cells. There is now a clear set of evidence that membrane voltage is involved in / correlates with non-neural cell regulation in a variety of situations from embryonic development to cancer[1].

Among several striking facts, stable gradients of RMP at the scale of the embryo of model organisms have been observed. These gradients seem to provide positional information much like classical morphogen gradients and appeared to be crucial to establish the body plan[2, 3]. The fact that Embryonic Stem cells are depolarized and become hyperpolarized as they differentiate and that RMP modulates developmentally relevant signaling pathways suggest that it could also play a role in Human development [1, 4]. To test this hypothesis, we propose to use Human Embryonic Stem Cells (hESC) cultured as a monolayer on adhesive circular micro-patterns. This in vitro system recapitulates faithfully what is known of the dynamics of gene expression and key morphogenetic features of the gastrula and is fully accessible to manipulation and observation

The goal of this internship is to use recently developed Genetically Encoded Voltage Indicators (GEVI) to observe RMP in live micropatterned hESC colonies in order to assess if the spatio-temporal variation of RMP (From depolarized stem cells to hyperpolarized differentiated cells) correlates with that of the expression of genetic markers of cell differentiation in hESC colonies. We will also perturb RMP chemical inhibitors and/or optogenetic tools to ask if it plays a causal role in cell fate commitment.

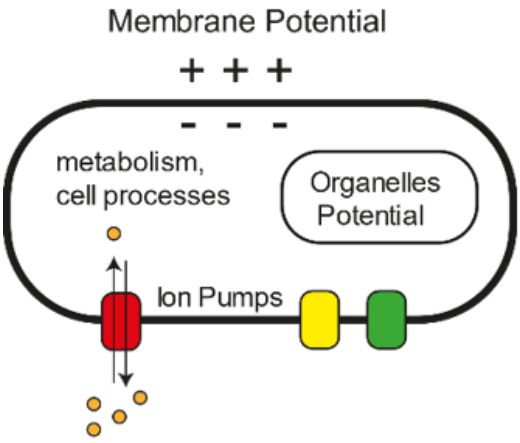
During this internship, you will receive training on: Early mammalian embryology, Electrobiology, Culture and differentiation of human embryonic stem cells, micropatterning, Characterization of cell identity, molecular biology and cloning. Confocal microscopy and quantitative image analysis.

1. Bates E (2015) Ion Channels in Development and Cancer. Annu Rev Cell Dev Biol 31:231–247 . https://doi.org/10.1146/annurev-cellbio-100814-125338

2. Levin M (2021) Bioelectric signaling: Reprogrammable circuits underlying embryogenesis, regeneration, and cancer. Cell 184:1971–1989 . https://doi.org/10.1016/j.cell.2021.02.034

3. Adams DS, Levin M (2013) Endogenous voltage gradients as mediators of cell-cell communication: strategies for investigating bioelectrical signals during pattern formation. Cell Tissue Res 352:95–122 . https://doi.org/10.1007/s00441-012-1329-4

4. Binggeli R, Weinstein RC (1986) Membrane potentials and sodium channels: Hypotheses for growth regulation and cancer formation based on changes in sodium channels and gap junctions. Journal of Theoretical Biology 123:377–401 . https://doi.org/10.1016/S0022-5193(86)80209-0



Self-organized hESC differentiation on micropatterns(SOX2:ectoderm,BRA:mesoderm, CDX2: Amnion). Bar 100µm

Contacts

Benoit Sorre
benoit.sorre@curie.fr

Pascal Hersen
pascal.hersen@curie.fr

Information

This internship can be followed by a thesis.

Mechanical instabilities in axial patterning of vertebrate embryos

A Masters internship position is available at the interface of physics and biology to study the mechanics of tissue patterning and shape emergence in vertebrate embryos. We are interested in understanding how mechanical cues and the feedback between mechanics and biochemical pathways result in the formation of structures during the axial morphogenesis of vertebrate embryos. More specifically, we focus on somite generation process, where packets of epithelial cells emerge from the posterior mesoderm, as the cells undergo mesenchymal to epithelial transition under the action of morphogens. We develop experimental biophysical techniques and approaches borrowed from soft matter physics to quantitatively study the interplay between mechanical forces, signaling, and physico-chemical properties of the environment during this process.

In this project, we will study the mechanical properties of the mesoderm as it differentiates along the axis, in ex vivo controlled conditions. By developing spreading assays, we will evaluate how the tissue flow evolves as the mesoderm differentiates. Our preliminary results show that the mechanics of PSM differentiation can be captured from dynamic profiles of spreading assays. We will quantify these observations and assess how they depends on morphogen concentrations and substrate rigidity. Micromanipulation techniques will be used to evaluate the rheology of the tissue, which combined with the spreading assay, will allow us to describe our observations in a theoretical framework. The findings of this project will help us better understand the origin of the mechanical instability that we observe at the time of the separation of the somite.

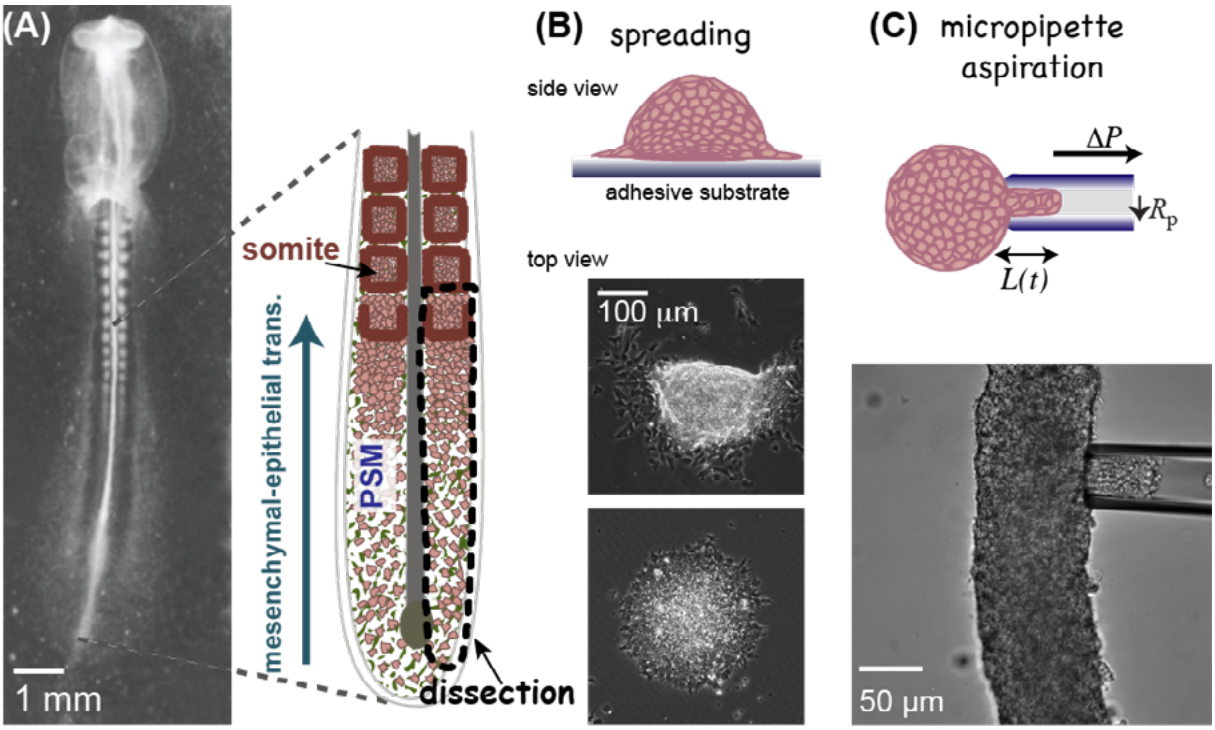
Techniques used: Microdissection and tissue culture, spreading assays, videomicroscopy, image analysis, immunolabeling, micropipette aspiration.

Contact

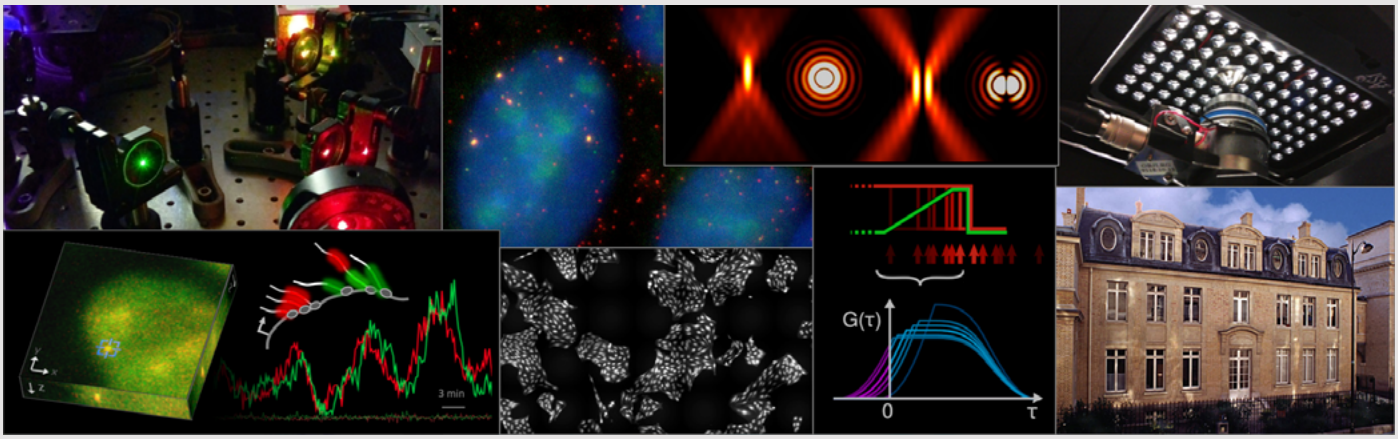
Karine Guevorkian
karine.guevorkian@curie.fr

Information

This internship can be followed by a thesis.



(A) Somitogenesis in chicken embryo. Schematic of the PSM and the somites. (B) Spreading dynamics reveal variations in tissue mechanics along the axis.(C) Rheological measurements using the micropipette aspiration technique.



Genome Functions in Space and Time

Our team studies the physical organization of the genome –in space and in time– in the mammalian nucleus and its relationship with transcriptional regulation or other functional genomic processes. We take an interdisciplinary approach at the physics-biology interface, which relies on quantitative approaches, combining advanced microscopy, mechanical micro-manipulation and physical modeling.

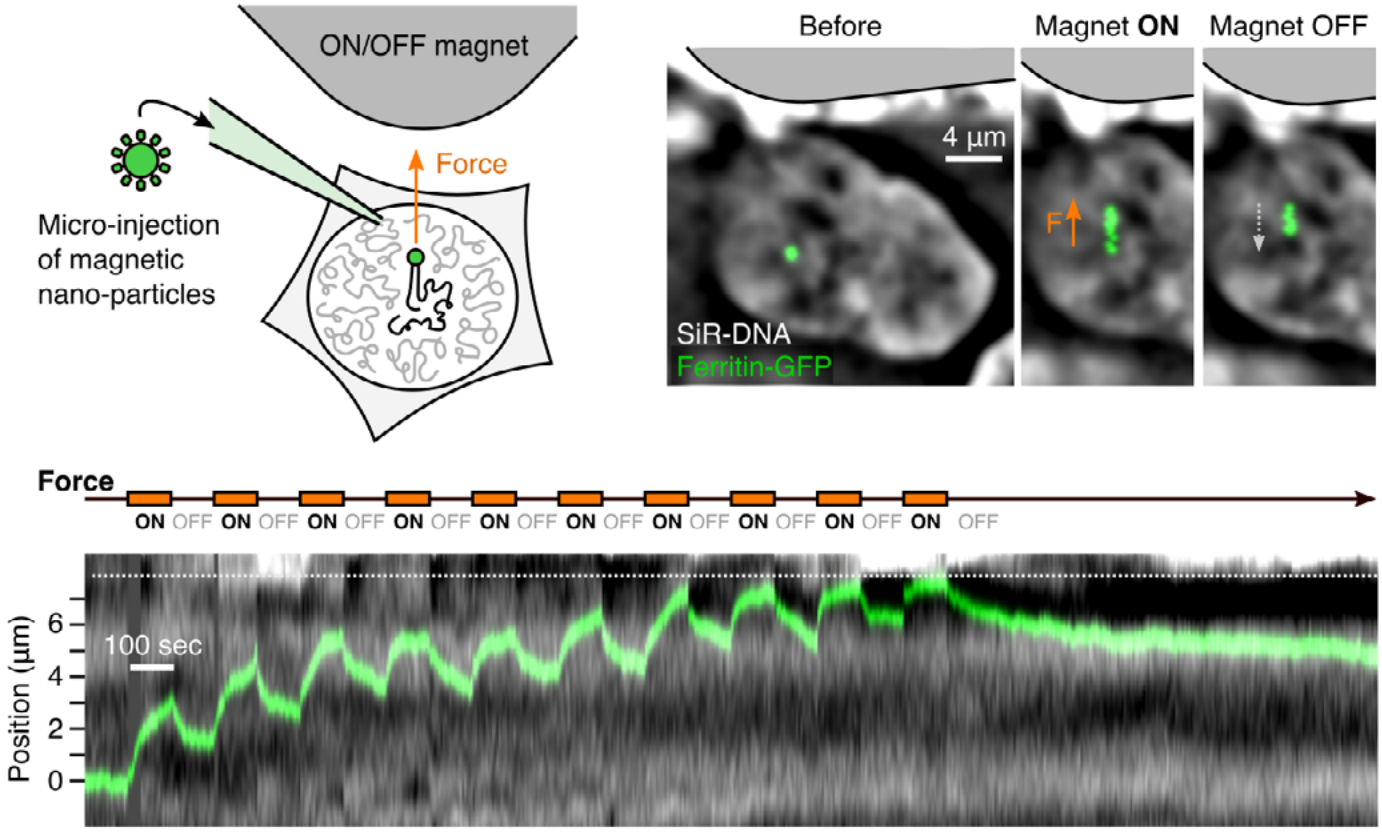
More info about the team: http://www.coulonlab.org/

Our team is part of two research unit at Institut Curie:

- the 'Physical Chemistry' unit (UMR168), with physicists working on diverse biological topics

- the 'Nuclear Dynamics' unit (UMR3664), with biologists studying different aspects of the cell nucleus.

Team leader : Antoine Coulon
Team size : 7
Number of PhDs and Post-docs : 3



Probing chromosome mechanics inside living cells by magnetic micro-manipulation

The physical principles governing the organization and dynamics of chromosomes in 4D inside the nucleus are broadly studied and central for understanding how the genome functions [1,2]. However, fundamental questions about the physical state of chromosomes and chromatin remain open, largely due to the inability to make direct mechanical perturbations and measurements on interphase chromosomes inside living cells.

To address this limitation, our team and collaborators recently developed a new technology to mechanically micro-manipulate chromosomes inside living cells by exerting a point force onto a targeted genomic locus using magnetic nanoparticles [3]. This new technique gives unprecedented access to physical parameters and insight into chromatin mechanics.

More info on this technique: https://doi.org/10.1101/2021.04.20.439763
Example movie: http://minilien.curie.fr/z8wnpm (play speed is 100x)

In this context, we propose internship(s) for 1 or 2 students on 3 possible topics.

I. TECHNOLOGICAL DEVELOPMENTS FOR MAGNETIC MICRO-MANIPULATION

Project – The student will engineer, calibrate and test a new way to apply a magnetic field onto the cells. If time permits, she/he will also interface this system with the microscope acquisition software for on-the-fly live feedback.

Outcome – A more flexible and tunable approach than in our original study [3] to apply

continued on next page

Contact

Antoine Coulon
antoine.coulon@curie.fr

Information

This internship can be followed by a thesis.

The thesis will be funded.

and modulate the force, which will permit more quantitative measurement and a range of downstream applications for the lab.

Expected knowledge and skills:

• • • Magnetism, electroplating/microfabrication, micromanipulation
• • • Arduino programming, electronics
• Basic mammalian cell culture
• Fluorescence microscopy
• • Good general level of programming (python, imagej)

II. BIOLOGICAL DETERMINANTS OF CHROMOSOME MECHANICS

Project – The student will perform chromosome micro-manipulation experiments under different biological perturbations and/or will observe specific biological responses to the mechanical perturbations.

Outcome – An understanding of how the physical nature of chromosomes relates to the biological state of chromatin (e.g. histone marks) and different genomic processes (e.g. transcription, replication), and how the cell responds to such mechanical stresses (DNA damage…).

Expected knowledge and skills:

• • Mammalian cell culture
• • • Immunofluorescence
• • • Transfection, siRNA, drug treatments
• • Fluorescence microscopy and image analysis
• Good general level of programming

III. IMAGING FORCE-INDUCED DEFORMATION & REARRANGEMENT OF CHROMOSOMES

Project – The student will perform chromosome micro-manipulation experiments after fluorescent labeling of genomic elements and chromatin domains using different strategies (nucleotide incorporation, CRISPR/dCas9 based labeling).

Outcome – Following the displacement and deformation of chromosomes and genomic domains will shed light onto the physical principles organizing the genome in space and the material nature of nuclear domains.

Expected knowledge and skills:

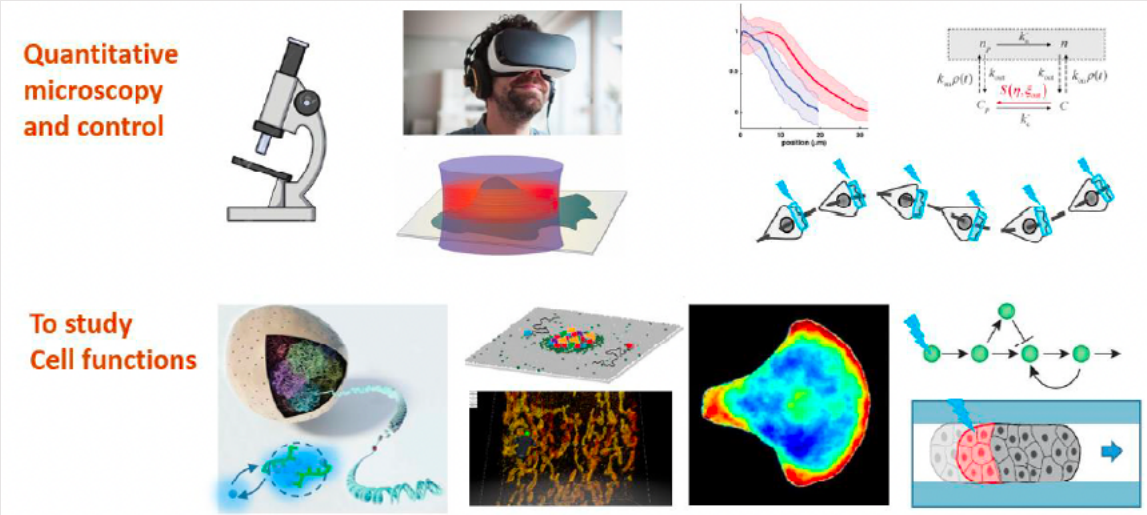
• • • Mammalian cell culture
• • • Fluorescence microscopy and image analysis
• • Good general level of programming
• Protein biochemistry and molecular biology

[1] Misteli, T. (2020) The Self-Organizing Genome: Principles of Genome Architecture and Function. Cell. 183, 28-45.

[2] Agbleke, A. A. et al. (2020) Advances in Chromatin and Chromosome Research: Perspectives from Multiple Fields. Molecular Cell. 79, 881–901.

[3] Keizer V. I. P. e al. (2021) Live-cell micromanipulation of a genomic locus reveals interphase chromatin mechanics. bioRxiv. https://doi.org/10.1101/2021.04.20.439763

• • • necessary – • • recommended – • considered a plus



Light-Based Observation and Control of Cell Organization

Our goal is to discover and study the physical principles underlying biological functions such as cell polarity and migration, signaling, nuclear organization or gene expression. Our approach consists of quantifying and perturbing the organization and dynamics of cellular constituents from molecular to cellular scales using advanced light microscopy.

In recent decades, a large number of interactions between molecules in the cell have been mapped, revealing a gigantic molecular circuitry. However, such a mapping is not sufficient to understand the emergence of cellular functions. Indeed, all functions result from the coordination in time and space of multiple processes such as the transport of molecules by diffusion, transient assemblies, molecular organization or maintenance of inhomogeneous spatial distributions

In order to identify the minimal physicochemical processes involved in cellular functions, we are using and developing quantitative imaging and light-based perturbation tools on living cells. Combining state of the art 3D super-resolution and advanced image processing algorithms with innovative visualization, e.g. use of virtual reality to explore single-molecule localization data, our quantitative approach allows us to discover new cellular structures as well as characterize their dynamics. For example, using single molecule imaging, we were able to discover that the transmission of biochemical signals in the cell occurs collectively via nano-aggregates of a few dozen proteins. We combine this approach with physical modeling and optogenetic tools that induce biochemical reactions with light at a specific location in the cell. We were able to show, for example, how the spatial extension of molecular gradients controls the migration of a cell. This causal approach allows us to directly see the effect of a physicochemical process on the behavior of the cell.

Team leader : Mathieu Coppey
Team size : 12
Number of PhDs and Post-docs : 5

Emergence of collective cell migration

Cancer is a leading cause of death worldwide, mainly due to metastases which are responsible for 90% of the deaths. Metastases form when some cancer cells escape from the primary tumor to migrate in the organism, and create new colonies in other parts of the body. Recently, using explants from patients, Fanny Jaulin's team from Gustave Roussy Institute showed that these metastases could form collectively: instead of migrating individually, groups of around fifty cells collaborate to go across tissues(1).

Combining our expertise with Matthieu Piel and Raphael Voituriez's lab, we demonstrated that these groups of tumor cells use a second mode of collective migration that was never described before, which we named collective amoeboid migration. In non-adherent microfluidic channels, groups of cells propel themselves thanks to a global gradient of contractility and random cell fluctuations(2).

We now want to understand how cells organize themselves to initiate migration, create and maintain a collective polarity. Who is deciding? How? With chemical, electrical or mechanical signals?

To tackle these questions, the student will study this collective migration in a minimal system, with clusters containing 2 to 10 cells where migratory properties already emerge. He will question the way polarity is initiated in microfluidic microchannels, and look at the possible gain of functions of this collective system. He will use fluorescent markers of the cytoskelet, optogenetic techniques(3), fluorescent microscopy as well as different types of microfluidic chips to confine cells at different heights.

Techniques: Cell culture, microfluidic, microscopy, optogenetics, image analysis (Python or Matlab)

Skills required: motivation to learn and make experiments. Basic notions in biology and programming.

(1) Zajac O, Raingeaud J, Libanje F, Lefebvre C, Sabino D, Martins I, Roy P, Benatar C, Canet-Jourdan C, Azorin P, Polrot M, Gonin P, Benbarche S, Souquere S, Pierron G, Nowak D, Bigot L, Ducreux M, Malka D, Lobry C, Scoazec JY, Eveno C, Pocard M, Perfettini JL, Elias D, Dartigues P, Goéré D, Jaulin F. Tumour spheres with inverted polarity drive the formation of peritoneal metastases in patients with hypermethylated colorectal carcinomas. Nat Cell Biol. 2018

(2) Pagès D.-L., Dornier E., De Seze J., Wang L., Luan R., Cartry J., Canet-Jourdan C., Raingeaud J., Voituriez R., Coppey M., Piel M., Jaulin F.. Cell clusters adopt a collective amoeboid mode of migration in confined non-adhesive environments. bioRxiv 2020

(3) Valon L., Etoc F., Remorino A., Pietro F., Morin X., Dahan M., Coppey M.. Predictive Spatiotemporal Manipulation of Signaling Perturbations Using Optogenetics. Biophysical Journal 2015.

Contact

Jean de Seze
jean.de-seze@curie.fr

Information

This internship can be followed by a thesis.
The thesis may be funded.

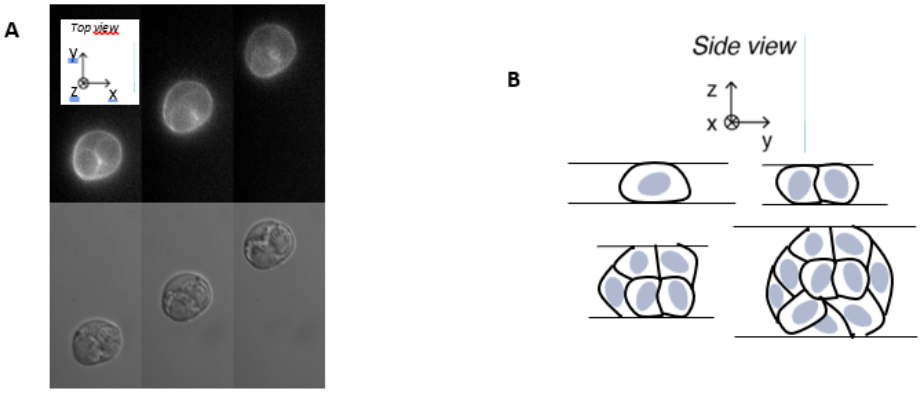
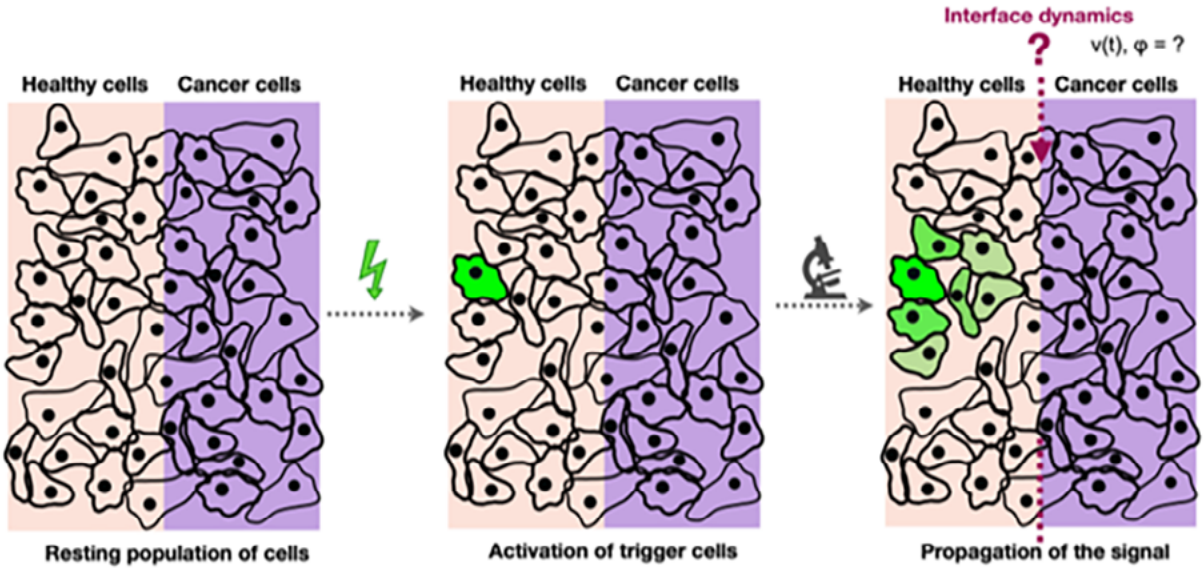


Figure : (A) Migration of a group of 5 cells in a confined environment (labeled with actin in the upper image, and in transmission on the bottom) (B) Sketch of different confinements for one, two, 5 or 10 cells.



Exploring unique bio-electric signatures of cancer cells

While the rapid exchange of electrical impulses is a well-known hallmark of communication between neurons, electrical phenomena in non-neuronal cells remain largely unexplored. A particularly intriguing hypothesis is the existence of 'bioelectric signatures' in cancer — patterns of electrical activity that distinguish cancer cells from their healthy counterparts.

With the advancement of optogenetics, we are freed from the constraints of rigid experimental setups and have the unique opportunity to probe the bioelectric activity in intact epithelia. In this internship, the student will actively participate in fleshing out a novel approach to studying cancer.

Using established mammalian cell lines, the student will use light input to perturb the cells' membrane potentials and analyze the spatio-temporal propagation of signals that arise in cell population after such stimulation. Once the groundwork is laid out, the student will be free to creatively explore the effect of different geometries and arrangements of cancer cells embedded in a population of healthy cells.

By working on this interdisciplinary project, the student will acquire a range of experimental and data analysis skills. In turn, this inherently novel project has the potential of opening new venues into cancer research and will benefit from the student's creativity and curiosity.

Experimental techniques: Live cell microscopy, cell culture, optogenetics, optics, programming (Matlab), image analysis, quantitative biology

Contact

Mathieu Coppey
mathieu.coppey@curie.fr

Information

This internship can be followed by a thesis.

The thesis may be funded.

Spatial mapping of molecular crowding in living cells via phase separation induced by optogenetics

The interior of cells is a medium heavily encumbered by the high density of macromolecules. The space accessible to biological molecules is a crucial parameter because it controls their diffusion as well as their local concentrations, and therefore it controls the reactivity of biological processes. This molecular hindrance is probably inhomogeneous at the subcellular scale, and it is possible that the biochemical activity of the cell is spatially regulated by local hindrance. It is currently very difficult to measure molecular crowding with spatial resolution. The subject of this internship will be to develop a new method, based on the optogenetic control of liquid-liquid phase separation, to map spatially the molecular crowding in a living cell.

Liquid-liquid phase separation is a non-linear process: above a critical concentration, a molecule separates from its solvent and forms droplets. Recently, work has shown that genetically encoded proteins, initially soluble in the cytosol, can be activated by light to induce phase separation. We will use such proteins, and by finely adjusting the amount of light we will reach the critical threshold of concentration. We will then be able to see where in the cells the droplets form first - and therefore identify the most crowded places of the cell interior. By gradually increasing the amount of light, we will then classify cell regions according to their local crowding.

Experimental techniques : The intern will learn the basics of cell culture, transfection, live cell fluorescence microscopy as well as optogenetics.

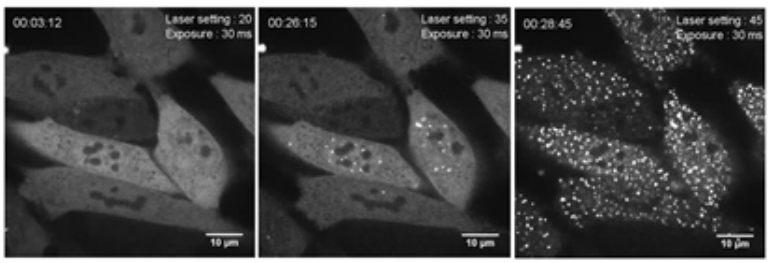
Contact

Mathieu Coppey
mathieu.coppey@curie.fr

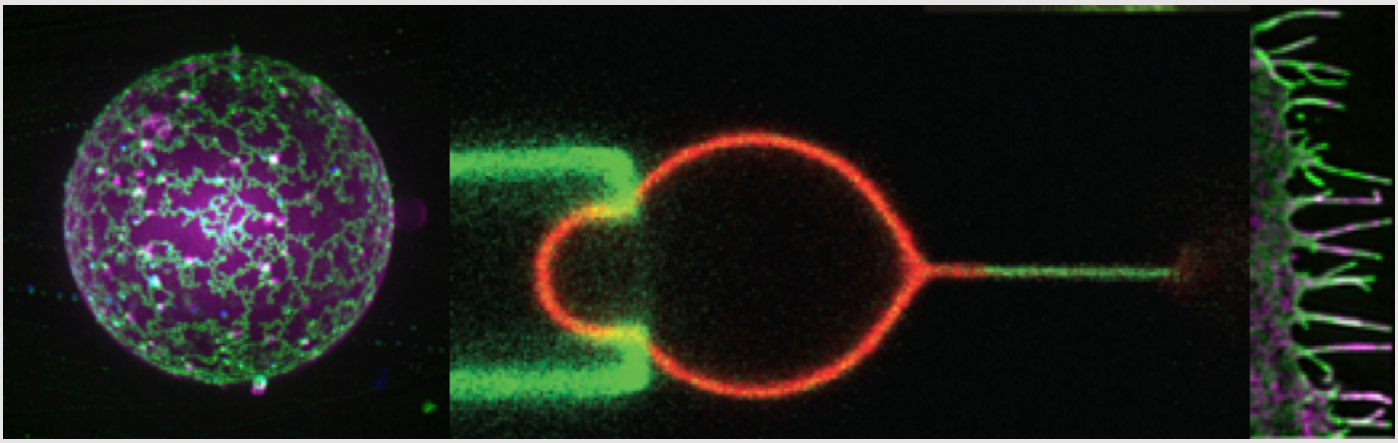
Information

This internship can be followed by a thesis.

The thesis will be funded.



Example of phase separation induced by optogenetics (after Shin et al, Cell 2017): initially the protein is soluble in the cytosol, then at the threshold of phase separation droplets are formed - in this example preferably in the nucleus - and finally beyond the threshold the demixing is complete.



Membranes and Cellular Functions

We develop multidisciplinary approaches, largely based on synthetic biology and biomimetic systems, as well as on quantitative methods of mechanics (optical tweezers, micropipette aspiration, microfluidics) and optical microscopy (confocal microscopy, super-resolution) to understand the role of biological membranes and of their organization in cellular functions such as intracellular trafficking, endo/exocytosis, cytokinesis or adhesion or the formation of cellular protrusions (Filopodia, TNTs). In parallel, we continue to develop advanced physical models of biomembranes by studying the consequences of the non-equilibrium activity of membrane proteins on their diffusion or clustering as well as the effect of the membrane mechanical properties on the activity of these proteins.

Team leader : Patricia Bassereau
Team size : 13
Number of PhDs and Post-docs : 11

Actin assembly mediated by curved membrane proteins

The actin cytoskeleton plays an important role in many cellular functions, for example in endocytosis where cells uptake nutrients from its surrounding environment. In 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 endocytosis. Yet, it is poorly understood how SNX9 mediates actin assembly.

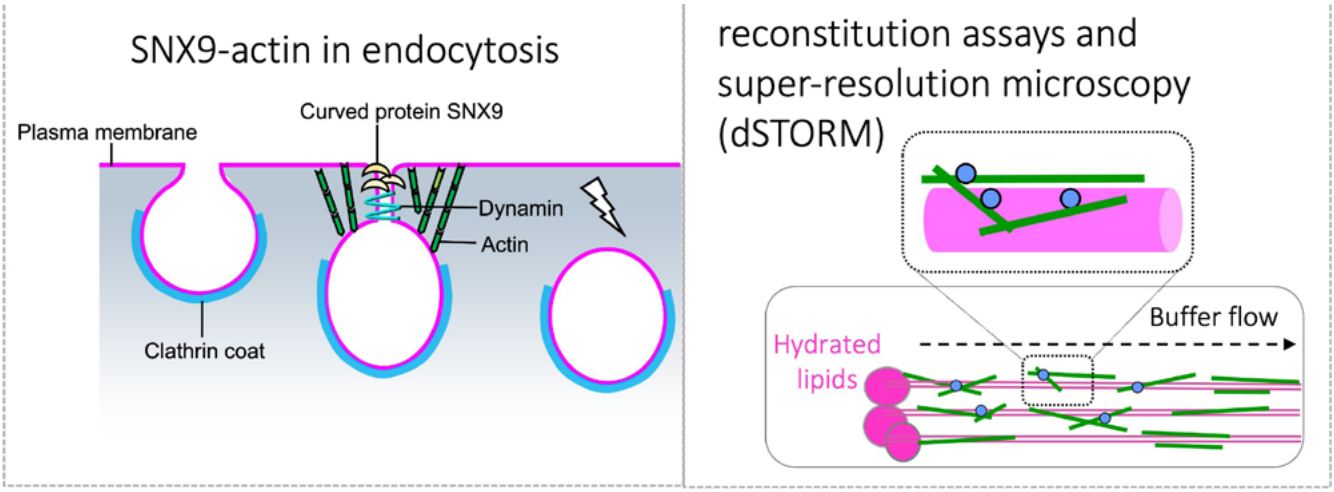
To address this question, we will perform experiments using cell-free model systems composed of purified SNX9, actin and artificial model membranes. We will generate cylindrical membrane tubes using microfluidic devices. We will examine how SNX9 self-assembles on the tubes and mediates actin assembly using super-resolution microscopy, such as dSTORM and single-particle tracking.

Contact

Feng-Ching Tsai
feng-ching.tsai@curie.fr

Information

This internship can be followed by a thesis.



Actin Dynamics in a Confined Tubular Environment

Actin remodeling is at the heart of the response of cells to external or internal stimuli, allowing a variety of thin, tubular shaped membrane protrusions to form. For example, short (less than 10 µm), dynamic filopodia are involved in migration and environmental sensing, while tunneling nanotubes (TNTs) connect distant cells (up to 100 µm) for sustained periods of time such that direct cell-to-cell communication and organelle transfer is achieved. Although filopodia and TNTs appear morphologically similar and are sustained by actin bundles, they exhibit clear differences in regards to their obtainable length scales, dynamics and the involvement of actin regulatory proteins. Different protein types that link the plasma membrane to actin filaments (e.g., inverted Bin/Amphiphysin/Rvs (I-BAR) proteins), nucleate and/or enhance actin polymerization (e.g., Ena/Mena/VASP or formins), or bundle actin filaments (e.g., fascin) have been shown to play key roles in the regulation of protrusion growth in filopodia. However, the detailed mechanisms governing protrusion generation and regulation between filopodia and TNTs are unknown.

Thus, the objective of this project is to uncover governing principles that describe how actin polymerization takes place in confined and thin tubular geometries such as those found in cell protrusions. We will form artificial protrusions (nanotubes) of defined lengths out of cell membranes using optical tweezers and we will observe the sequence of recruitment of actin-related proteins (those known to influence actin dynamics and interface with the plasma membrane) using confocal microscopy. Simultaneously, we will study how these proteins facilitate actin growth into the nanotube using time-lapse imaging or FRAP, and how the growth depends on the initial organization of the actin cortex (manipulated using micropatterns or specific drug inhibitors such as CK666). We will measure the resulting forces that are ultimately generated. The degree of tube stiffness, measured for example through buckling measurements, will be correlated to the structural organization of actin (number of filaments, cross-linking density, etc.) within the pulled nanotube using super resolution imaging methods such as STORM/TIRF available in the team and cryoEM (with A. Bertin).

This project represents a new way to study the mechanisms leading to actin polymerization in a confined environment with a geometry relevant for cellular protrusions (inside a tube) such as filopodia or TNTs.

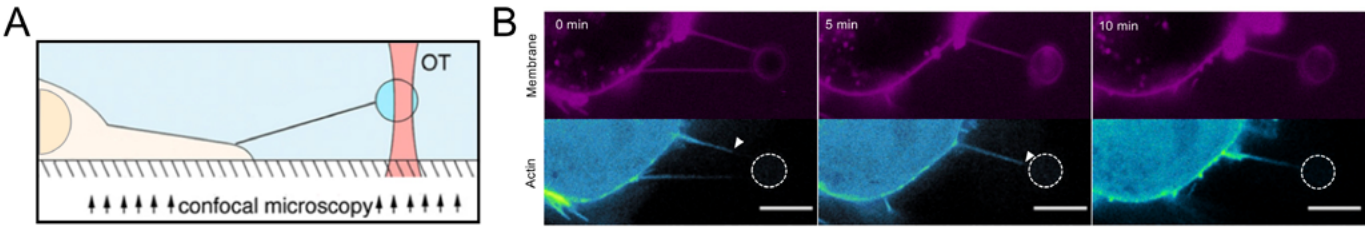
It will be developped in collaboration with the cell biology teams of Chiara Zurzolo (Institut Pasteur) who studies the formation of TNTs between cells and Pekka Lappalainen (Helsinki, Finland) who is an expert on actin polymerization in cells.

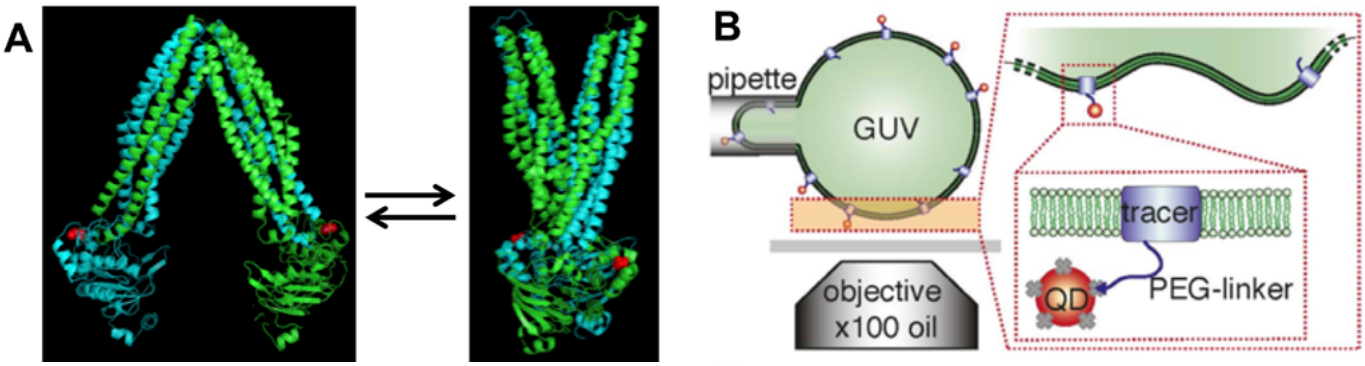
Contact

Patricia Bassereau
patricia.bassereau@curie.fr

Information

This internship can be followed by a thesis.





Effect of non-equilibrium activity on single membrane proteins diffusion and clustering

Trans-membrane proteins are tightly embedded in fluid lipid bilayers where they diffuse laterally. Many membrane proteins are involved in the transport of ions or molecules through the membranes using different sources of energy (ATP hydrolysis, voltage, light, stretching etc…) that allow for conformational changes, thus shape changes. Our group showed that protein shape (conical versus cylindrical, for instance) has an effect on its lateral diffusion. Membrane deformations produced by the inclusion of non-cylindrical proteins can also lead to membrane-mediated attractive interactions and to protein clustering that have not been studied experimentally yet.. Our general objective is to investigate the effect of the functional conformational dynamics of membrane proteins on their diffusion and on their clustering.

We will study BmrA from the ABC transporters family that transport drugs across membranes upon ATP hydrolysis upon changes of conformation (Fig. 1A). During the internship, we will use FRAP (Fluorescence Recovery After Photobleaching) to measure the collective diffusion of BmrA reconstituted in Giant Unilamellar Vesicles, either in its open or its closed conformation. In the longer-term, the objective will be to study the diffusion of active BmrA molecules (with ATP) by FRAP and by single molecule tracking in giant liposomes of controlled tension (Fig. 1B) and in nanotubes of controlled curvature in order to decipher the effect of membrane mechanics on its diffusion. From the diffusion analysis and with super-resolution microscopy, we will study the effect of the protein activity on membrane-mediated clustering.

This project will be developed in close collaboration with D. Lévy (membrane protein expert) in the Physico-Chimie Curie lab.

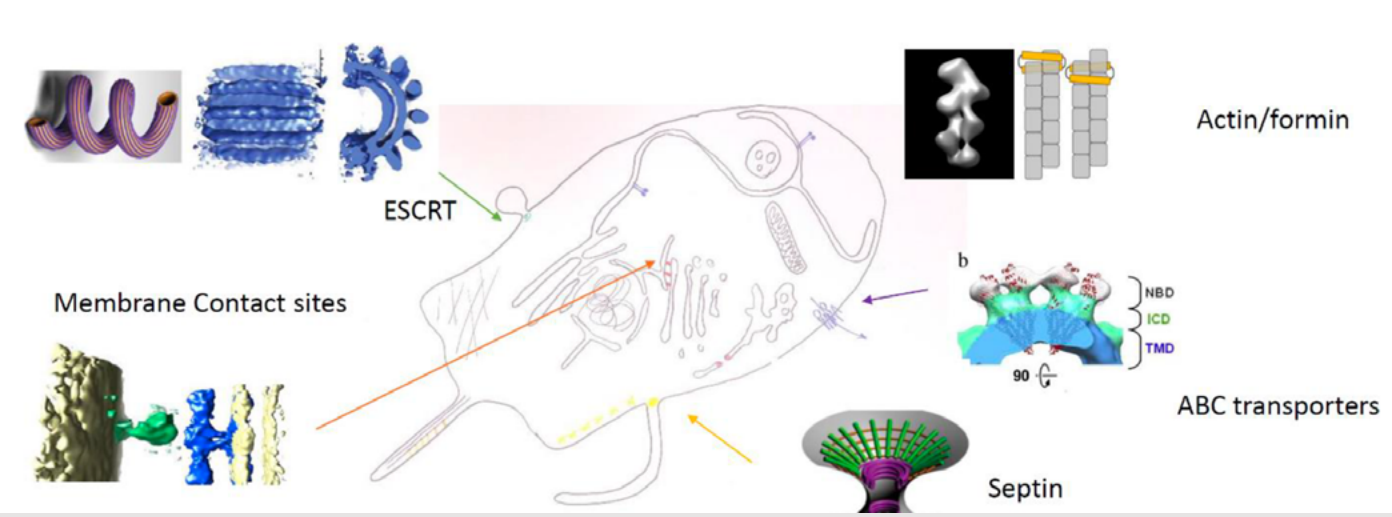
Contact

Patricia Bassereau
patricia.bassereau@curie.fr

Information

This internship can be followed by a thesis.

The thesis will be funded.



Molecular Microscopy of Membranes

How the components of cell membranes, lipids and membrane proteins are organized and how the interplay between their molecular 3D architecture and the surrounding membrane affects their function? Our Team addresses these questions in the context of cell division, cell detoxification and inter-organelles communication. We combine cryo-electron microscopy and in vitro membrane systems to decipher the architectures at subnanometric resolution of proteins and membrane. We integrate our results with data derived from functional analysis, physics of membranes and cell biology to unravel mechanisms at different spatial and temporal scales.

Team leader : Daniel Levy
Team size : 5
Number of PhDs and Post-docs : 4

Interplay between septins and ESCRT by bottom up in vitro assays

Septins are ubiquitous in eukaryotes, essential for numerous cellular processes (cytokinesis, neuro-morphogenesis, …) and are involved in membrane remodeling. Septins are filamentous proteins interacting with the inner plasma membrane. They localize at the cleavage furrow during cell division.

Like Septins, ESCRT proteins assemble into intracellular filamentous structures and are involved in a variety of membrane remodeling processes (like virus budding, cell division and MVB formation). ESCRT proteins are made of five different complexes: ESCRT-0; I; II; III and the ATPase VPS4. ESCRT-0, I and II serve as a platform to recruit ESCRT-III which further assemble into an active filament perform abcission of the membrane during cytokinesis.

Both septins and ESCRTs are essential for cytokinesis, the last step of cell division that physically splits cells into two. The final scission (abscission) requires the concentration of ESCRT-III filaments at the abscission site (Figure 1)

Electron microscopy analysis at the ICB revealed that ESCRT-dependent 17 nm-thick filaments form a conical structure from the midbody to the abscission site (Guizetti et al; Science (2011). However, the nature of these filaments, remains poorly understood, since individual ESCRT-III filaments are much thinner in vitro. We can thus assume that these filaments include a set of proteins including ESCRT-III proteins and septins. Recent data have shown that the depletion of septin leads to anarchic polymerization of ESCRT-III within the midbody and ESCRT-III cannot reach the abscission site.

In the present work, we propose to understand the mutual interplay between the septin cytoskeleton and the ESCRT-proteins.

We will first characterize the interaction of septin filament with ESCRT-III proteins in solution using classical biochemical strategies. We will then characterize the interaction of these proteins on lipidic membrane of various sizes and geometries (Supported lipid bilayer, small and large unilamellar vesicles and Giant vesicles). The ultra-structure of proteins will be visualized using fluorescent microscopy (confocal and super resolution microscopy) and cryo-electron microscopy.

S. Mangenot is an expert of in vitro lipidic systems. In her lab she has all the equipments to produce the in vitro system. The optical imaging will be performed either directly in the lab or at the platform located rue des St Pères. A. Bertin is an expert of septin proteins with a long knowledge of electron microscopy imaging. Electron microscopy imaging will be performed either in Curie or at a Platform at Institut Pasteur. Both supervisors already published articles on the structural organization of septins or ESCRT-III alone (Septins: Beber et al Nature com 2019; ESCRT-III: Bertin et al Nature com 2021).

This work could be follow by a PhD. S. Mangenot and A. Bertin have been granted a 3 years PhD ANR funding to carry out this project.

Francois Iv, Carla Silva Martins, Gerard Castro-Linares, Cyntia Taveneau, Pascale Barbier, Pascal Verdier-Pinard, Luc Camoin, Stéphane Audebert, Feng-Ching Tsai, Laurie Ramond, Alex Llewellyn, Mayssa Belhabib, Koyomi Nakazawa, Aurélie Di Cicco, Renaud Vincentelli, Jerome Wenger, Stéphanie Cabantous, Gijsje H. Koenderink*, Aurélie Bertin*, Manos Mavrakis*. Insights into animal septin assembly and function using recombinant human septin hetero-octamers, J Cell Sci. 2021 Aug 1;134(15):jcs258484. doi: 10.1242/jcs.258484. Epub 2021 Aug 5.

Anthony Vial*, Cyntia Taveneau*, Luca Costa, Brieuc Chauvin, Hussein Nasrallah, Cédric Godefroy, Stéphanie Mangenot, Daniel Lévy, Aurélie Bertin*, Pierre-Emmanuel Milhiet*, Correlative AFM and fluorescence imaging demonstrates a nanoscale membrane remodeling and spontaneou

Contacts

Aurélie Bertin
aurelie.bertin@curie.fr

Stéphanie Mangenot
stephanie.mangenot@parisdescartes.fr

Information

This internship can be followed by a thesis.

The thesis will be funded.

Ultrastructure of the cytokinetic intercellular bridge by cryo-electron tomography

An intercellular bridge (ICB) connects dividing mammalian cells, in late cytokinesis, before they eventually split in two. At the ICB different sets of proteins are recruited. Mis regulations at late cytokinesis can lead to multinucleated cells and thus diseases. Among the regulating factors, septins are recruited by anillin and self-assemble into two ring-like structure on both sides of the central midbody (Figure 1, left). Septins are filamentous proteins interacting with the inner plasma membrane, are ubiquitous in eukaryotes and involved in membrane remodeling. ESCRT-III protein complexes are then recruited in between those septin rings before being relocalized to the future abscission site.

Electron microscopy analysis at the ICB revealed that ESCRT-dependent 17 nm-thick filaments form a conical structure from the midbody to the abscission site (Guizetti et al; Science (2011). However, the nature of these filaments, remains poorly understood, since individual ESCRT-III filaments are much thinner in vitro. We can thus assume that these filaments include a set of proteins including ESCRT-III proteins and septins. Recent data have shown that the depletion of septin leads to anarchic polymerization of ESCRT-III within the midbody and ESCRT-III cannot reach the abscission site.

In the present work we propose to get more insights into the ultrastructure of the midbody using the latest available methods. We want to determine at different time points during cytokinesis how septin, ESCRTs and their partners interact and decipher their fine localization. We want to determine the nature of the filaments present in the 17 nm in diameter filament highlighted a few years ago by electron tomography (Guizetti et al; Science (2011)).

We will develop a methodology to perform correlative microscopy of resin-embedded samples obtained by preservative procedures: high pressure freezing and freeze substitution. On one hand, super resolution multi-focus microscopy (MFM) will be used on resin sections and on another hand the same sections will be transferred to an electron microscope to perform 2D imaging as well as electron tomography. Super resolution MFM will be carried out using the setup designed by Bassam Hajj at PCC (Institut Curie) and electron microscopy will under the supervision of A. Bertin. Ultimately, the global organization and architecture of the ICB we will attempt to describe its ultrastructure at the best possible resolution. To this end, cryo-electron tomography will be performed on vitrified samples followed by sub-tomogram averaging.

This project will be carried out in collaboration with Stéphanie Mangenot (Unv. Paris) and Bassam Hajj (PCC, I. Curie).

Contact

Aurélie Bertin
aurelie.bertin@curie.fr

Information

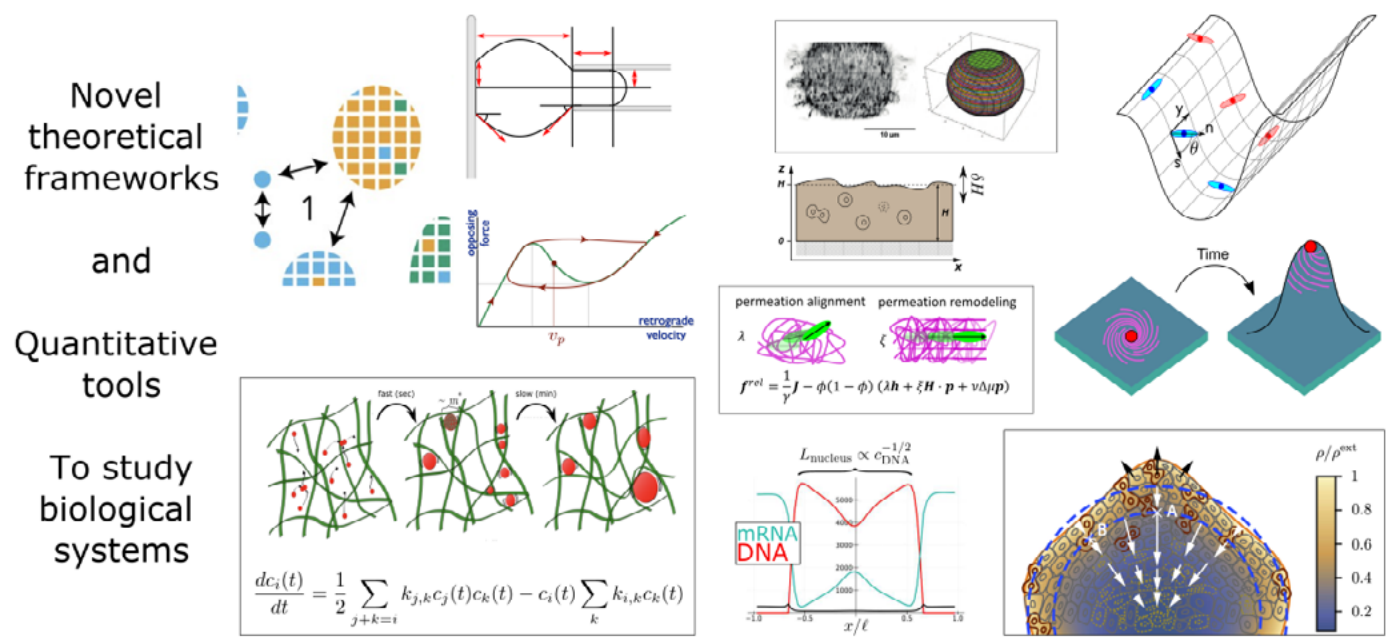
This internship can be followed by a thesis.

Francois Iv, Carla Silva Martins, Gerard Castro-Linares, Cyntia Taveneau, Pascale Barbier, Pascal Verdier-Pinard, Luc Camoin, Stéphane Audebert, Feng-Ching Tsai, Laurie Ramond, Alex Llewellyn, Mayssa Belhabib, Koyomi Nakazawa, Aurélie Di Cicco, Renaud Vincentelli, Jerome Wenger, Stéphanie Cabantous, Gijsje H. Koenderink*, Aurélie Bertin*, Manos Mavrakis*. Insights into animal septin assembly and function using recombinant human septin hetero-octamers, J Cell Sci. 2021 Aug 1;134(15):jcs258484. doi: 10.1242/jcs.258484. Epub 2021 Aug 5.

Anthony Vial*, Cyntia Taveneau*, Luca Costa, Brieuc Chauvin, Hussein Nasrallah, Cédric Godefroy, Stéphanie Mangenot, Daniel Lévy, Aurélie Bertin*, Pierre-Emmanuel Milhiet*, Correlative AFM and fluorescence imaging demonstrates a nanoscale membrane remodeling and spontaneous ring-like and tubular structures formation by Septin, Nanoscale. 2021 Aug 7;13(29):12484-12493. doi: 10.1039/d1nr01978c. Epub 2021 Jul 6.

Agata Szuba, Fouzia Bano, François Iv, Manos Mavrakis, Ralf P. Richter, Aurelie Bertin, Gijsje H. Koenderink, Membrane binding controls ordered self-assembly of animal septins, Elife. 2021 Apr 13;10:e63349. doi: 10.7554/eLife.63349.

Taveneau C., Di Cicco A., Levy D., Pehau-Arnaudet G., Bertin A.,



Physical approaches to biological problems

Cell biology and soft matter physics both share similar orders of magnitude with two important differences: biological systems are out of equilibrium and molecular specificity is highly relevant. We are using tools of soft matter physics to provide a quantitative description of cellular systems. In collaboration with biologists, we are raising interesting new and challenging physical questions related to the non-equilibrium physics of cell morphology and dynamics and of the mechanics and growth of tissues.

Team leader : Pierre Sens
Team size : 12
Number of PhDs and Post-docs : 5

Active membrane dynamics in the secretory pathway

Contact

Pierre Sens
pierre.sens@curie.fr

Information

This internship can be followed by a thesis.

The secretory pathway is a foundational system used by all eukaryotic cells to distribute membrane and secretory proteins. Proteins synthesized in the endoplasmic reticulum (ER) are sorted and exported at ER exit sites (ERES) toward the Golgi apparatus, where additional sorting and protein processing occurs. This dynamics is orchestrated by coat protein complexes (COPII and COPI) able to polymerise into flexible protein scaffold to support membrane deformation and protein sorting. The classical view of ER to Golgi transport is that ER export membranes and proteins inside COPII coated spherical vesicles, while retrograde transport from Golgi to ER is occurs in COPI coated spherical vesicles. This view has recently been challenged by high resolution optical and electron microscopy studies [1,2] (see figure). These showed that ERES are highly intertwined and dynamical tubular networks continuous with the ER by a constricted neck where COPII proteins localise. Transport intermediates between the ERES and the Golgi are dynamical and pearled tubular membranes containing COPI. Proper ERES functioning requires the dynamical turnover of COPII component between the ER membrane and the cytosol, showing the importance of non-equilibrium processes.

- Building on these new observations, and on the recent interest of the cell biology and biophysics community on out-of-equilibrium phase separation with turnover [3], we will construct and analyse physical models of the ERES as resulting from active membrane flow driven by tread-milling of COPII proteins with the constricted connection between ER and ERES. This will involve a description of the out-of-equilibrium hydrodynamics of protein aggregation and a mechanical description of soft, deformable interfaces.

- In a second stage, we will study the dynamics of the tubular network emerging from the ERES, as resulting from the interplay between COPII driven influx of membrane and COPI driven budding off of ERES material [2]. During these two stages we will evaluate the ability of our model structures to perform protein sorting through the differential affinity of cargoes to either COPII or COPI components.

- In a third phase of the project, we will explore more general questions related to active tubular networks. Many cellular organelles, from the ER to mitochondria, form highly dynamical tubular networks maintained by active processes, which control the nucleation, elongation and branching of tubules [4]. We will aim at determining the global statistical properties of the tubular network, and in particular the possibility of coexistence between regions with high and low density of 3-way junctions in this network depend on cellular activity. In equilibrium systems, the usual way to approach such question is to derive a free energy and minimize it with respect to appropriate fields. This approach is inadequate for active systems, in which phase equilibrium can be derived by analyzing the entropy production in the system. We will follow this approach to obtain a phase diagram describing the range of parameters for which the clustering of 3-ways junction can be expected, and compare our results to experimental observations regarding the structural

[1] Uncoating of COPII from ER exit site membranes precedes cargo accumulation and membrane fission. O. Shomron et al. preprint. BioRxiv https://doi.org/10.1101/727107.

[2] ER-to-Golgi protein delivery through an interwoven tubular network extending from the ER. A.V. Weigel et al. Cell 184 (2021) 1

[3] Physics of active emulsions. C. A. Weber, D. Zwicker, F. Jülicher and C. F. Lee. Rep. Prog. Phys. 82 (2019) 064601

[4] Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER. J. Nixon-Abell et al. Science 354 (2016) 433

Reversible aggregation of optogenetic proteins in cells

Aggregation of molecules into clusters can be substantially simplified if the process is diffusion limited: in this scenario, molecule binding processes are irreversible, and cluster sizes monotonically grow in time. In a recent study with our experimental collaborators [A. M. Miangolarra, et al., Biophys. J. 120, 2394 (2021)], we studied the diffusion-limited aggregation of an optogenetic protein, and proposed a physical model for the processes underlying such a cluster-formation process.

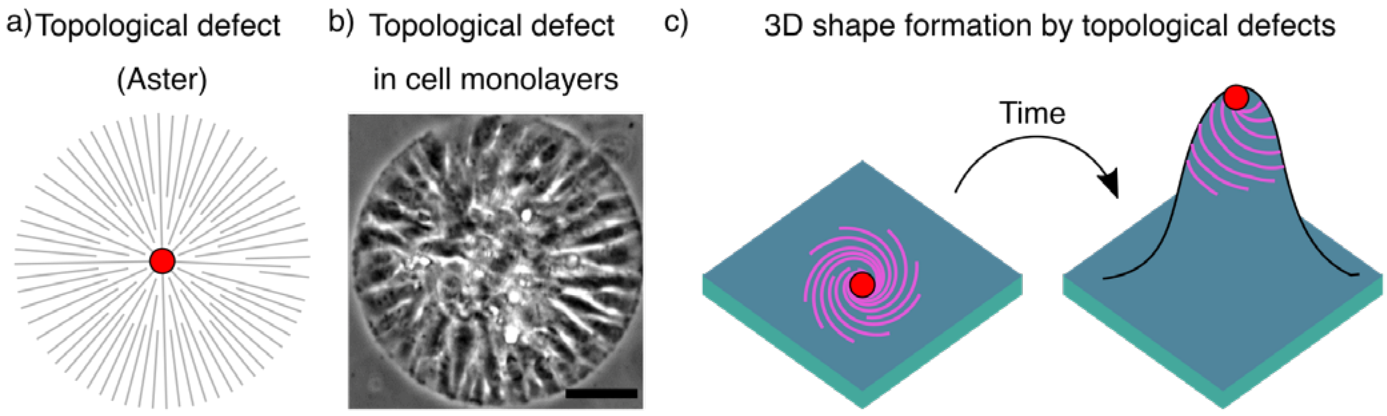
By manually tuning the intrinsic binding affinity of the optogenetic proteins, it is possible to make this aggregation processe reversible, and design a new experiment where bound molecules may unbind from clusters.

Although mathematically challenging, the physics of such novel, non-diffusion-limited process can be described theoretically at different levels of accuracy. This theoretical analysis will be the main topic of this internship: its level of complexity will be tailored to the capabilities, background and expertise of the student, and can range from analytical to numerical approaches.

Overall, a complete understanding of this aggregation mechanism may allow one to control it, and therefore tailor the physiological functions which are associated with it in a living cell, with multiple potential biological implications.

Contact

Michele Castellana
michele .castellana@curie.fr



Shape formation in active liquid crystals by topological defects

Monolayers of anisotropic cells exhibit characteristics of liquid crystals, such as long-range orientational order and topological defects [1]. The latter corresponds to domains in space where the orientation is ill-defined, see Fig. 1a. During development, topological defects are at the core of morphogenetic events and biological processes, such as protrusion formation or cell extrusion [2-4]. However, the interplay between morphogenesis and topological defects remains to be elucidated.

During this M2 internship, the student will aim to understand the linkage between active processes, topological defects and shape formation, Fig 1c. The student will apply an ample set of mathematical tools, such as the active gel theory [5] or differential geometry, to study the dynamics of active liquid crystal surfaces, which is a minimal description of some tissues. This work will help to better understand morphogenetic mechanisms in multicellular systems, as well as open new ways to design shape-morphing materials.

1. To start, we will focus on how order alone can generate morphologies, and consider a minimal system composed of a deformable membrane with a single topological defect. The student will determine the equilibrium morphologies induced by the relaxation of the topological-defect energy. Unlike previous studies [6], we will consider both intrinsic and extrinsic order-geometry couplings in the total free-energy.

2. Tissues typically are out-of-equilibrium systems. Therefore, in the next step, the student will aim to better understand the role of activity in generating shape through order-geometry couplings. Specifically, the student will study the dynamics of active liquid crystal surfaces for configurations with a single topological defect.

[1] G. Duclos, etal, Soft Matter, 10, 2346-2353, (2014), [2] T. B. Saw, etal, Nature, 544, 212-216, (2017), [3] K. Kawaguchi, etal, Nature, 545, 327-331, (2017), [4] Y. Maroudas-Sacks, etal, bioRxiv, (2020), [5] K. Kruse, etal, Eur. Phys. J. E, 16, 5-16, (2020), [6] J.R. Frank and M. Kardar, Phys. Rev. E ,041705, 77 (2008).

Contact

Carles Blanch-Mercader
carles.blanch-mercader@curie.fr

Information

This internship can be followed by a thesis.

Instabilities and geometry of growing tissues

Cilia and flagella are the cellular appendices that drive flows around cells. The flows can provoke cell motion. A typical example is sperm cells, which are propelled by the beating of long flagellas. The actuation of the flagellum by molecular motors creates a bending wave propagating outward from the sperm cell, which induces its motion. Cilia beating in synchrony can also create macroscopic flows around surface

Classical theories of cilia beating consider the cilium as a flexible filament coupled it to the surrounding fluid by an a priori distribution of point-like forces along the filament. The theory of Camalet and Julicher calculates this force distribution based on a theoretical description for the collective behavior of the molecular motors interacting with microtubules. Recently, stimulated by the experiments of the group of P. Martin at Institut Curie, we have proposed a very generic theory of cilia or flagella beating that considers the cilium as an active flexible filament. The active torque dipoles are due to molecular motors and we have supposed that they are curvature controled. This theory allows for the description of both the mechanical beating wave and the distribution of the molecular motors along the beating filament. In addition, it takes into account the hydrodynamic drag but also two types of internal friction associated to the shear inside the filament and the filament curvature changes.

The experiments are done on artificial cilia formed by the assembly of actin filaments and myosin molecular motors. They measure explicitly the shape of the cilium and the wave of molecular motors propagating along the cilium. We have interpreted the experiments using the simplest version of the model, which considers that the dissipation is dominated by the internal shear friction and that the beating is two-dimensional. These are extreme approximations that must be relaxed to obtain a more complete quantitative description of cilia beating. For example, the experimental beating pattern has a clear 3-dimensional character and the hydrodynamic drag is not entirely negligible.

The aim of the internship and PhD thesis is to establish along these theoretical lines, a general 3-dimensional study of cilia beating. A particular attention will be given to the relative importance of internal and external (hydrodynamic) frictions and to all possible active torques allowed by the symmetries of the problem. The thesis will be made under the joined supervision of F. Jülicher at the Max Planck Institute of Complex systems in Dresden and J.F. Joanny at Collège de France and Institut Curie in Paris and could lead to a cotutelle degree between these two institutions. The theoretical work will be done in close collaboration with the experimental work of P. Martin. It will have both theoretical and numerical aspects and is based on non-equilibrium statistical physics and fluid mechanics. Part of the work will be done in Paris and part in Dresden.

Contact

Jean-François Joanny
jean-francois.joanny
@curie.fr

Information

This internship can be followed by a thesis.

The thesis will be funded.

Dynamics of biomimetic active gels and cortex/membrane interaction

The cell cytoskeleton is made up of polar protein filaments that interact with each other via various proteins, either fixed or motile. The polar aspect of its structure gives it properties that resemble those of polymer solutions and liquid crystals. The cytoskeleton however is active: thanks to a direct energy consumption or its coupling to motor proteins, it produces local deformations and stresses that allow the cell to deform, move, exert forces, and divide. A major component of the cell cytoskeleton is actin: biopolymer filaments that can polymerize and depolymerize thanks to the hydrolyzis of ATP to create stresses or deformations.

The group of Cécile Sykes, lately part of Physico Chimie Curie, has a long-standing expertise in developing in-vitro reconstituted systems called biomimetic systems, which attempt at reproducing biological phenomena in controlled conditions and using minimal ingredients. In the recent years, the team has focused on the interaction between the actin cortex—a layer of crosslinked actin filaments that form a gel—and a biological membrane, notably using reconstituted liposomes. The experimental team has observed different shape instabilities of the liposome, even in the absence of actin-associated myosin motors [1]. We propose to study the phenomenon of stress buildup and deformations under the sole action of actin polymerization/depolymerization in this geometry. We shall rely on a previous expertise on the instability of cellular spheroids driven by inhomogeneous cell-renewal distributions [2].

[1] Carvalho et al., Phil. Trans. R. Soc. B 368, 20130005 (2013).

[2] Martin M. and Risler T., New J. Phys. 23, 033032 (2021).

Contact

Thomas Risler
thomas.risler@curie.fr

Information

This internship can be followed by a thesis.

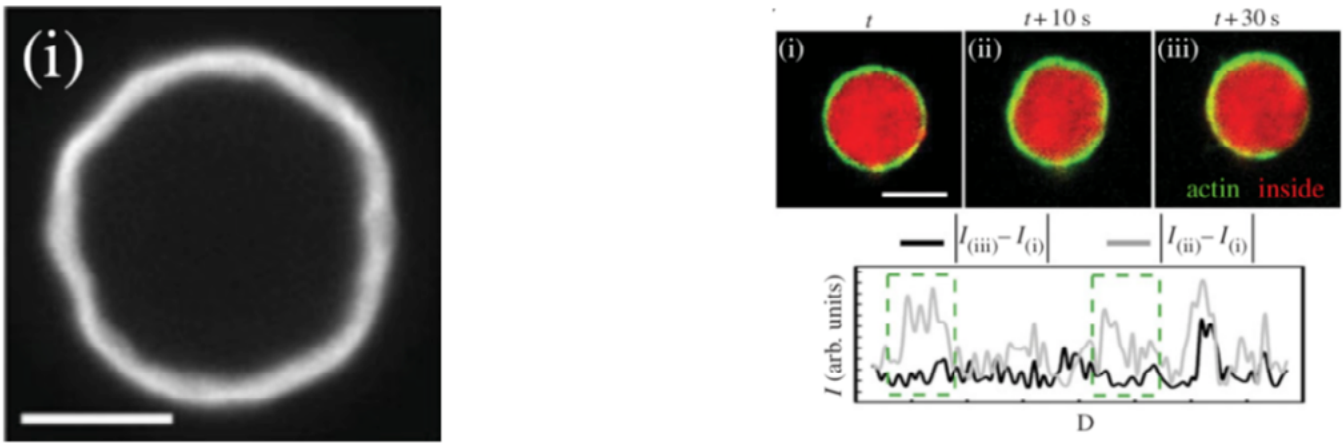


Figure 1: Left – Spinning-disk confocal microscopy image of the deformation of a liposome under polymerization of a branched actin network. Scale bar, 10 microns. Right – Time lapse of a spinning disk confocal slice at the equatorial plane a liposome with actin (green) and inside solution (red), in the presence of myosin motors: (i) before contraction, (ii) just before cracking of the gel, (iii) when the actin network ruptures. Below: absolute value of the intensity difference over the distance D along the contour of the liposome: black curve, difference between (iii) and (i); grey curve, difference between (ii) and (i). Scale bar: 5 microns. Illustrations reproduced from [1].

Anomalous fluctuation spectra of a sensory auditory cell

Contact

Thomas Risler
thomas.risler@curie.fr

Information

This internship can be followed by a thesis.

Hearing relies on mechanoelectrical transduction (MET), the fundamental process that transforms auditory stimuli into electrical signals. In vertebrates, mechanical stimuli carried by sound waves deflect the sensory organelle of specialized receptor cells of the inner ear – the hair bundle – opening mechanosensitive ion channels. Channel gating (opening and closing) and the hair-bundle motion are directly coupled by extracellular filaments called tip links, which tense or relax in response to the hair-bundle deflections and transmit force onto the MET channels, changing their opening probability [1]. Recently, we proposed that hearing relies on the cooperative gating of neighbouring MET channels connected to a common tip link, mobile in the membrane, and coupled by elastic forces generated within the lipid bilayer [2]. This model quantitatively explains, using only realistic parameters, the main characteristics of hair-cell mechanotransduction.

Measured spectra of spontaneous fluctuations of the hair-bundle position reveal unusual Brownian motion, in which the mean-square displacement increases as a fractional power of time [3]. Such anomalous scaling contradicts the canonical model of MET. The goal of the internship will be to extend the study of our two-channel model beyond static quantities, and simulate its dynamics thanks to stochastic simulations to generate in-silico Brownian motion of the modelled hair bundle. Obtained fluctuation spectra will be compared with the measurements. For that project, we shall benefit from an already established collaboration with Dr. A. Kozlov at Imperial College London.

[1] Howard J. and Hudspeth A.J., Neuron 1, 189 (1988).

[2] Gianoli F., Risler T.*, and Kozlov A.S.*, PNAS 114, E11010 (2017).

[3] Kozlov A.S., Andor-Ardó D., and Hudspeth A.J., PNAS 109, 2896 (2012).

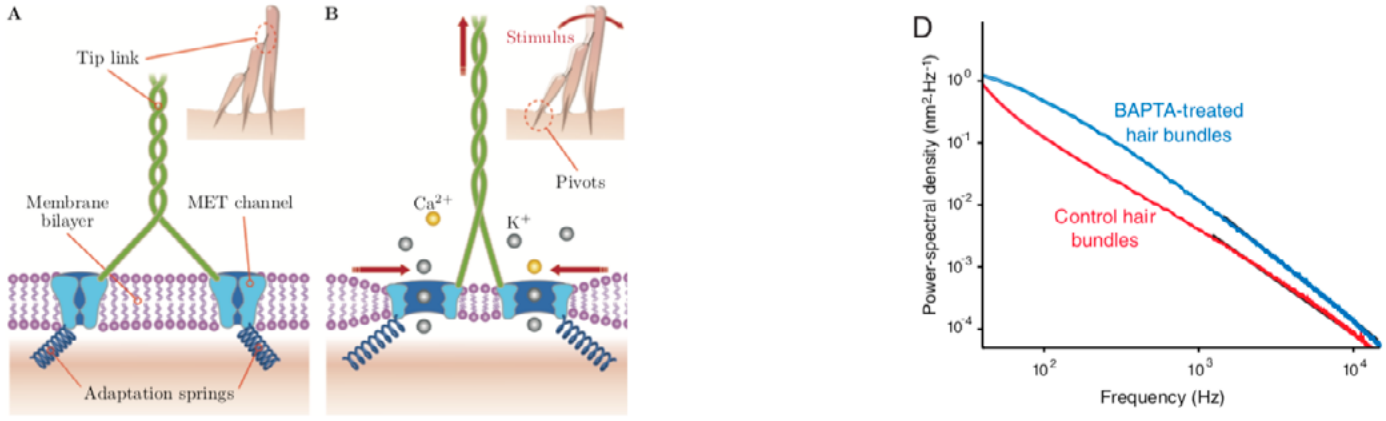


Figure 1: Left - Illustration of the principle of the two-channel model proposed in [2]. A) Two MET channels are connected to the insertion to two transduction channels. The scale bar represents 100 nm. B) As the tip link is tensed, the two channels move closer to each other. (Reproduced from [2]). Right – Average power spectra of intact hair bundles (red) and of hair bundles treated with BAPTA (blue), a drug that cuts the tip links (Reproduced from [3]). In the red curve, the slope at high frequencies is close to -1.75, whereas the blue curve has the standard Brownian slope of -2 (underlying black lines).