Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces **2020**

Intitulé du Projet de Recherche Doctoral : High spatiotemporal resolution microscopy for the study of the multi-scale dynamics of healthy and Huntington's neurons

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

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Unité de Recherche :
Intitulé : Laboratoire Jean Perrin
Code *(ex. UMR xxxx)* : UMR8237

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

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

Co-encadrant :

NOM : **Betuing** Prénom : **Sandrine**
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Unité de Recherche :
Intitulé : Neuroscience Paris Seine
Code *(ex. UMR xxxx)* : UMR8246

Ecole Doctorale de rattachement : ED158-Cerveau, cognition, comportement
Ou si ED non Alliance SU :

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

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences scientifiques apportées. Indiquer les publications/productions des encadrants en lien avec le projet.
Préciser le profil d'étudiant(e) recherché.

High spatio-temporal resolution microscopy for the study of the multi-scale dynamics of healthy and Huntington's neurons

Abstract

The aim of the PhD is to study the influence of the spatio-temporal dynamics of the mesoscale organization of cells on the biological activity of cellular membranes, focusing on mitochondria and plasma membrane that respectively ensure energy production and cellular exchanges. In a systems approach, our efforts to understand these dynamics and their consequences question the multi-scale relationship between structures and functions. The PhD candidate will be in charge of the experimental part of the project, in particular super-resolution, and of image and data analysis. She/he will work in the Lab. Jean Perrin, under the supervision of Prof. S. Bonneau, in collaboration with the Neuroscience Lab of IBPS (sup. Prof S. Betuing). She/he will be interested in both the biological and physical aspects of the project, and is likely to have a good feel for imaging and statistical analysis.

Scientific background

Living cells can be seen as active matter, where transport phenomena are both amplified by the energy produced by the cell itself and restricted by important diffusion limitations. They exhibit significant out-of-equilibrium activity, mainly due to the forces generated by the *cytoskeleton* and molecular motors. The activity of these proteins contributes to the violation of the fluctuation-dissipation theorem in living systems and defines the rheological properties of cells [1,2]. The necessary energy is produced by oxidizing carbon: in aerobic organisms, a major step in this oxidation is carried out by the respiratory chain in the *mitochondria*. This energy production therefore involves the oxidation and ageing of cellular materials. The control of this oxidative activity allows the cells to remain sufficiently far from the thermodynamic equilibrium and the balance between respiration and ageing is thus a major regulatory parameter of the fate of the cells.

The cellular environment is highly structured in space at all levels: from molecular small assemblies to subcellular nano- and micro- compartments and to global micrometer scales at cells level. This approach has led, in recent years, to a profound renewal of our vision of cellular behaviour, in particular thanks to evidence that membrane architecture and shape modulate the regulatory network and cellular functions [3,4]. The *systems biology of cell membranes* thus converges with biophysics, and the multi-scale spatio-temporal organization of cells now appears to organize and coordinate signalling networks and cell behavior.

Such an approach implies the integration of various theoretical and experimental tools. In addition, a major challenge is to link the molecular level at the nanoscale with the classical description of cells at the micron scale, in terms of dynamics, mechanisms and remodeling [5]. In this context, we have developed - on cell lines - an efficient method. We are now extending it to more realistic systems, on primary cells (cortical neurons, obtained from healthy mouse models or models of *Huntington's disease*, HD).

Scientific aims and objectives

The leading question of our project is *how the particular topology of a cell membrane can modulate its biological functions*. The term topology is used here to consider the configuration, dynamics and modulation of the membrane shape. We will focus on two key cell membranes, whose state and activities are related to key points in the physiological and thermodynamic state of the cell:

- The *plasma membrane*, which is directly coupled to the activity of the cytoskeleton, and which controls cellular exchanges, including endo- and exocytosis,
- The *mitochondrial membranes*, whose spatial and temporal organization is linked to the production of cellular energy.

In our pathological model (HD), these two membranes are affected: the plasma membrane, where mesoscale modulations are linked to a planar patern (1D), consisting of elements called lipid rafts [6], and the mitochondrial inner membrane, where the nanoscale organization has 3D membrane invaginations called cristae [7]. These membrane arrangements involve physical and chemical characteristics (such as cholesterol or cardiolopin enrichment, respectively). This induces specific architectures of the membrane surface, resulting in particular

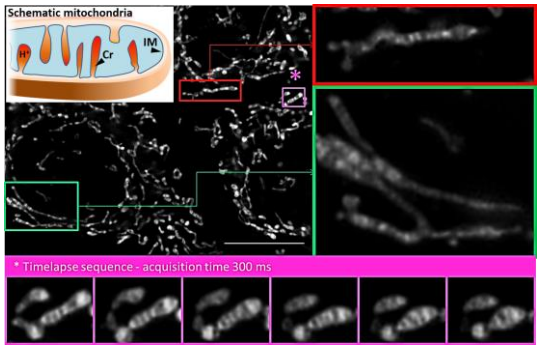


Fig. 1: *Schematic drawing of mitochondria and Fast-SIM image within living cells. Scale bar 10 µm*

modulations of the diffusion processes of molecules and protein complexes (in-plane and 3D confinement effects), capable of modulating their interactions and biological activity. Our approach is based on the combination of the study of the diversity and function of membrane components with the study of the topological and mechanical aspects of the membrane. These studies are made possible from a dynamic point of view by the latest advances in fluorescence imaging microscopy.

High-resolution microscopy is very useful for understanding both the structure and function of cell structures. On the one hand, lipid domains are accessible to super-resolution techniques [8], as are associated proteins [9-11]. For example, structured illumination microscopy (SIM) has made it possible to visualize the reorganization of the membrane during activation of immune cells [12]. On the other hand, because of their small size, intracellular organelles are perfect targets for super-resolution imaging. Until recently, optical microscopy has not been able to study the structures of mitochondria (1 µm), lysosomes (500 nm), endosomes (100 nm) or synaptic vesicles (50 nm). Today, super-resolution microscopy techniques provide access to the organization of mitochondrial membranes within cells [13,14]. However, in addition to this *spatial heterogeneity*, cell membranes also exhibit *temporal heterogeneity*. For studies of living cell dynamics, however, super-resolution techniques have certain limitations, particularly because of the acquisition time (usually tens of seconds to the minute) [15]. Finally, a specific limitation for monitoring membrane dynamics is the limited repertoire of probes.

In order to overcome these limitations and conduct studies on living cells, we have built home-made SIM set-up, whose innovative approach improves the acquisition rate [16]. Experiments on living HeLa cells have given promising results (Fig. 1): we have demonstrated a method, called Fast-SIM, offering a lateral resolution of ~100 nm at a raw data acquisition rate of 15 frames per second for a wide field image of 85µmx85µm ([17] and submitted paper).

Working program and approaches

Applied mathematics and optics for advanced microscopy on living cells. In SIM microscopy, high-frequency characteristics are encoded in the wide-field image. Several images of the same sample are acquired and digitally combined to reconstruct a super-resolved image of the object. This method is of particular interest for biological studies because of its speed, flexibility and the low level of irradiation required. In addition, images are obtained with conventional fluorescent probes which offer a wide range of applications. Spatial resolution is improved by a factor of two. While seven images are generally required, our reconstruction method is based on only four images, thus improving the acquisition speed [16].

Cell models. The experiments are conducted on two cell models. The *HEK cell line* is a labile model and will be used for preliminary experiments, development and experimental set-up. The level of cellular stress will be controlled using a photoactivatable molecule [2,17]. *Primary cell cultures of cortical neurons* constitute a more realistic model well adapted to our problem. Indeed, we have access to models of HD, a neurodegenerative disease. The plasma membrane of neurons with this disease shows increased cholesterol levels, altered lipid rafts [18] and disruption of ionotropic glutamate NMDA (N-methyl-D-aspartate) (GluN2B) receptors. The properties and function of mitochondria are also severely impaired [19]. We have developed biochemical tools for fine control of these membrane compositions (methyl-β-cyclodextrin, a cholesterol chelator, the CYP46A1 enzyme, which breaks down cholesterol into 24S-OH cholesterol [20]). Our strategy is to follow, under controlled conditions:

(1) the cholesterol-rich lipid rafts (Cholera-toxin B Alexa-488) and the GluN2B-Cherry subunit, as well as the cytoskeleton,

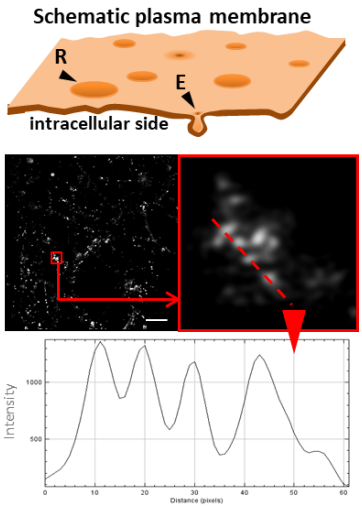


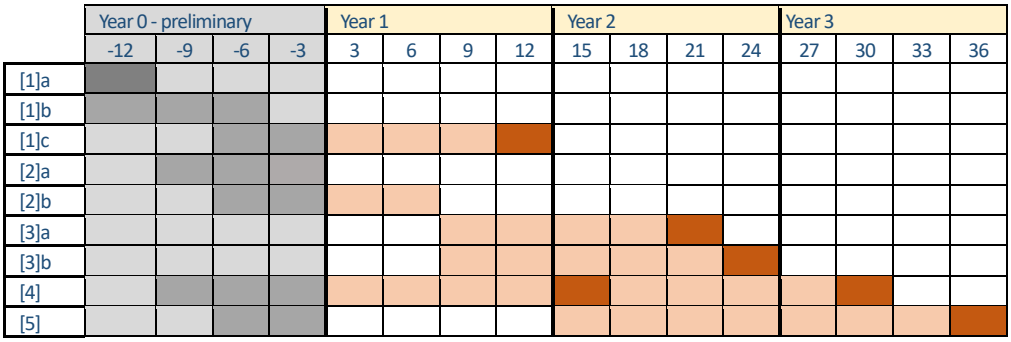
Fig. 2 *Schematic drawing of lipid rafts and Fast-SIM image within HD primary neurons. Scale bar 10µm.*

(2) the intramitochondrial dynamics (MitoTracker Green) and activity (JC-1) and
(3) the local dynamic viscosity of cell compartments to obtain nanoscale mapping of viscoelastic properties (rotor-based fluorescent probes will be obtained from M. Kuimova, Imperial College, London) [21].

We have obtained the first super-resolved images of lipid rafts and mitochondria in primary neurons using the Fast-SIM set-up under development at the LJP (Fig. 2).

Statistical analysis and correlations. The cells will be characterized by classical biochemical approaches (lipidomics, Western Blot, etc.) as well as imaging (both functional and Fast-SIM). Quantitative data will be derived from image analysis (number of rafts and size distribution, number and aspect-ratio of mitochondria, as well as mean square displacements and fission/fusion events) to analyze topology and dynamics. These data are characteristic of the out-of-equilibrium state of the cell. They will be correlated with data obtained from biochemical analysis and functional imaging, which provide access to the pathophysiological state. To interpret these correlations, theoretical models will help to understand how membrane composition controls its structuring at the nanoscale, and how a specific topology influences the interactions of protein complexes and biological function. We develop models of both structure-function coupling and dynamic aspects of the observed structures by integrating coupling effects dependent on smaller scale physical phenomena such as diffusion of ionic species, membrane mechanics and electrostatic effects.

PhD program:



WORKPAKAGES		
	[1] High resolution imaging	b. Calibrate the speed acquisition
	a. Improvement of the acquisition speed	**[3] Data acquisition**
	b. Calibration of the illumination of the sample	a. Tracking experiments (Dynamics of the cells membranes)
	c. Cell line (HeLa, HEK) cells experiments	b. Functional imaging (JC-1, Calcein-Co2+ experiments)
	[2] Primary neuron imaging	**[4] Data analysis and identification of correlations**
	a. Find of setup use condition that preserve cells from stress	**[5] Model computing and correlations analysis**

Conclusions

The strong coupling of biochemical reactions with spatial coordination means that biological signaling is subject to a plethora of physical constraints. Our efforts to understand their mesoscale dynamics and their consequences in a systems approach is then challenging and key to furthering our knowledge of system biophysics of cells, questioning the multi-scale structure/function relationship. Moreover, aging and neurodegenerative diseases are real public health problems. The development of models, knowledge and approaches may open up new diagnostic techniques and, more ambitiously and in the longer term, constitute a first step in proposing bioinspired devices such as biomimetic mitochondria capable to balance pathological energy deregulation.

Bibliography

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