Title of the PhD project:

Early stages of Huntington disease: Spatiotemporal co-ordination of cell reprogramming

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Overview of the scientific projects of the team

Physique et biophysique des membranes. Imagerie super-résolue. Activité mitochondriale

Main publications since January 1er, 2016

1. Mitochondrial cristae modeled as an out-of-equilibrium membrane driven by a proton field - Phys. Rev. E. Nirbhay Patil, **Stéphanie Bonneau**, Fréderic Joubert, Anne-Florence Bitbol, and Hélène Berthoumieux
2. Bour A., Kruglik S.G., Chabanon M., Rangamani P., Puff N., **Bonneau S.** (2019). Lipid Unsaturation Properties Govern the Sensitivity of Membranes to Photoinduced Oxidative Stress. *Biophysical Journal*, **116**, 910-920.
3. Almendro-Vedia V.G., Natale P., Mell M., **Bonneau S.**, Monroy F., Joubert F., López-Montero I. (2017). Nonequilibrium fluctuations of lipid membranes by the rotating motor protein F1F0-ATP synthase. *PNAS*, DOI 10.1073/pnas.1701207114.
4. Andreazza N.L., Vevert-Bizet C., Bourg-Heckly G., Sureau F., Salvador M.J**., Bonneau S.** (2016). Berberine as a Photosensitizing Agent for Antitumoral Photodynamic Therapy: Insights into its Association to Low Density Lipoproteins. *International Journal of pharmaceutics*, **510**, 240-9.

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Overview of the scientific projects of the team

Main publications since January 1ᵉʳ, 2016

1. Kacher R, Lamazière A, Heck N, Kappes V, Mounier C, Despres G, Dembitskaya Y, Perrin E, Christaller W, Sasidharan Nair S, Messent V, Cartier N, Vanhoutte P, Venance L, Saudou F, Néri C, Caboche J, **Betuing S.** (2019). CYP46A1 gene therapy deciphers the role of brain cholesterol metabolism in Huntington's disease. Brain. **142**, 2432-2450.
2. Boussicault L, Kacher R, Lamazière A, Vanhoutte P, Caboche J, **Betuing S**, Potier MC. (2018). CYP46A1 protects against NMDA-mediated excitotoxicity in Huntington's disease: Analysis of lipid raft content. Biochimie. **153**, 70-79.
3. Dos Santos M, Salery M, Forget B, Garcia Perez MA, **Betuing S**, Boudier T, Vanhoutte P, Caboche J, Heck N. (2017). Rapid Synaptogenesis in the Nucleus Accumbens Is Induced by a Single Cocaine Administration and Stabilized by Mitogen-Activated Protein Kinase Interacting Kinase-1 Activity. Biol Psychiatry. **82**, 806-818.
4. Galan-Rodriguez B, Martin E, Brouillet E, Déglon N, **Betuing S**, Caboche J. (2017). Coupling of D2R Short but not D2R Long receptor isoform to the Rho/ROCK signaling pathway renders striatal neurons vulnerable to mutant huntingtin. Eur J Neurosci. **45**, 198-206.
5. Boussicault L, Alves S, Lamazière A, Planques A, Heck N, Moumné L, Despres G, Bolte S, Hu A, Pagès C, Galvan L, Piguet F, Aubourg P, Cartier N, Caboche J, **Betuing S**. (2016). CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease. Brain. 139, 953-70.

Doctoral Project

Title: Early stages of Huntington disease: Spatiotemporal co-ordination of cell reprogramming

Abstract :
To understand the mechanisms of cellular reprogramming in Huntington's disease, we are studying the influence of multi-scale spatio-temporal dynamics of cells on biological activity. We focus on mitochondrial and plasma membranes, which are impacted from the early stages of the disease. We overcome two key problems: the experimental limitations for mesoscale observation of living cells on the one hand, and on the other hand the validation of theoretical models to understand these complex problems. Thus, in a systems approach, our efforts to question the multi-scale relationship between structures and functions constitute a challenge for systems biophysics of cells. Moreover, aging and neurodegenerative diseases are real public health problems. The development of our methodological approaches may open new diagnostic perspectives and, in the long term, may pave the way to mitochondrion-like bioinspired devices to balance energy deregulation.

Context and objective :

Huntington's disease (HD) is a progressive neurodegenerative disorder with a typical phenotype including chorea and dystonia, incoordination, behavioral difficulties and cognitive decline[1]. At the cellular level, mutant huntingtin results in neuronal dysfunction and death through a number of mechanisms, including direct toxicity of the mutant protein but also disruption of mitochondrial functions, cellular trafficking, axonal transport, synaptic assembly and neurotransmission[2]. Early changes appear in the striatum, typically 15 years before the first symptoms onset. Despite this large window of potential intervention, there are currently no disease modifying treatments[3,4]; therefore, **understanding of the cellular mechanisms underlying the pathophysiological state, in particular the early events of the reprogramming of the cells,** is developing rapidly.

Here, we will focus on two major early events, hallmarks of the early stage of the pathology: the mitochondrial dysfunction and the impaired cholesterol metabolism that strongly affects the plasma membrane[5]. This induces important changes in transport and communication within the cells and the multiscale spatiotemporal organization emerges as modulating and coordinating the cellular functions[6–8]. Thus, we address the issue of the links between the different levels: **how changes in membrane components affect its nano-scale ultrastructure, influencing the cell micron-scale shapes and dynamics and thus modulating its functions**. Two key problems have to be overcome[9]: the challenge of imaging such highly dynamic and unstable structures, in particular at the 50-500 nm scale where insight are lacking due to experimental limitations, and the need of an interdisciplinary work to build, implement and validate quantitative models needed to describe and understand these complex interactions.

The plasma membrane is compartmentalized into distinct **domains**, with a high diversity of size, life-time and dynamics[10,11]. The functional role of this lipid and protein clustering is not yet fully understood, but it undoubtedly modulates the protein transport and interactions on the membrane surface[12]. The segregation of lipids and membrane proteins is thought to be driven by the lipid composition itself (justifying the well-known name of "**cholesterol-rich lipid rafts**"), as well as by lipid–protein interactions and diffusional barriers. In the plasma

membrane, lateral diffusivities of membrane proteins ranges from 6 to 10 $\mu m^2.s^{-1}$ and the membrane geometry itself fundamentally impacts the diffusivity of the embedded proteins, in a manner dependent on its thickness and curvature[13]. The **mechanical coupling between the proteins and the membrane** can strongly affect protein mobility. Moreover, due to the purely 2D-nature of the membrane, hydrodynamic correlations extend over the entire domain, leading to the Stokes paradox[1]. Hence, the size of the domains can be an important length-scale for determining the diffusivity of the proteins. Finally, the dynamics of the lipid rafts can themselves involve modulations in mobility of the embedded proteins, in particular due to the possibility of redistribution and exchanges between domains during fusion or fission events. Then, the real problem is not to visualize isolated structures but rather to evidence their complex, dynamic and multi-scale behavior, as it exists within living cell. Here, we will follow the plasma membrane dynamics both at the **nano-scale (rafts)** and by **single-molecule tracking (proteins, receptors)**.

From the early stage of the cellular reprogramming, HD cells presents an increase of cholesterol content within the lipid rafts[14] and a dysregulation of the NMDA (N-methyl-D-aspartate) ionotropic glutamate receptors (GluN2B). These two modifications, evolving and interrelated, sign a strongly impaired membrane dynamics, which could contribute to the neuronal and synaptic defects. The **comparison of plasma membrane dynamics between healthy and HD neurons** will evidence relations between the ultrastructure and the function, as well as physical feature that support the cellular reprogramming of the neurons, giving insight of the modulation of the regulatory network by the organization of the membrane.

In mitochondria, the inner membrane consist of the boundary membrane and 3D-invaginations[15] called **cristae**. This nano-scale organization supports the coordination of the respiratory protein complexes to produce energy: cristae have a higher membrane potential than the intervening boundary membranes. The molecular diffusion is limited by confinement inside the tubular or sheet-like cristae, involving confined proton loops and individual functioning of each cristae within a same mitochondrion[16]. Interestingly, the proton loops in turn influence the shape of the membrane, in which the presence of charged lipids (the cardiolipin) involves a strong **coupling between the membrane shape and the charged proton diffusion**[17]. In this project, we will follow the cristae shape and dynamics, and the membrane potential (at the nano-scale single cristae level). We will compare healthy and HD neurons, where the defects in mitochondrial membranes lead to both a decrease of ATP production and an increase of the oxidative stress[18]. Interestingly, in HD neurons, these alterations of the mitochondrial functions are not due to defects in cardiolipin metabolisms[19], and the mechanisms supporting this coupling remain unknown. This obviously justifies
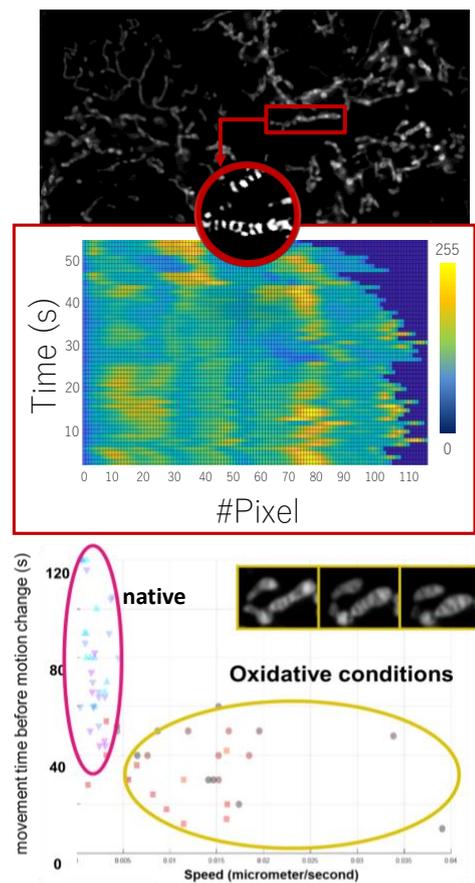

Fig.1 - *Mitochondria Fast-SIM image in living cells (MitoTracker Green) and example of the dynamic changes analysis. Bar 10 µm. (unpublished data).*

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[1] In the 3D case, the Stokes method provides a solution to the problem of flow, for example around a sphere. However, there is no non-trivial steady-state solution to these equations around an infinite cylinder, i.e. there can be no creeping flow in a 2D-system, what constitute the "Stockes paradox".

making them part of the core problem of our study. Moreover, both studied membranes, both early impaired during HD development, are characterized by extremely dynamic and highly structured topologies. Their comparison is very promising regarding the biophysics side of our project, as these specific architectures, induced by very particular compositions (cholesterol and cardiolipin), are able to modulate the transport processes at the vicinity of the membrane, but present very different shapes, dimensions (2D or 3D confinement) and underlying physical processes.

From the early stage of the cellular reprogramming, HD cells presents an increase of cholesterol content within the lipid rafts[14] and a dysregulation of the NMDA (N-methyl-D-aspartate) ionotropic glutamate recep-tors (GluN2B). These two modifications, evolving and interrelated, sign a strongly impaired membrane dynamics, which could contribute to the neuronal and synaptic defects. The **comparison of plasma membrane dynamics between healthy and HD neurons** will evidence relations between the ultrastructure and the function, as well as physical feature that support the cellular reprogramming of the neurons, giving insight of the modulation of the regulatory network by the organization of the membrane.

For the mitochondria, we will implement a similar approach. The mitochondrial inner membrane consist of the boundary membrane and 3D-invaginations[15] called cristae. This nano-scale organization supports the coordination of the respiratory proteins to produce energy: cristae have a higher membrane potential than the intervening boundary membranes. The molecular diffusion is limited by confinement inside the tubular or sheet-like cristae, involving confined proton loops and individual functioning of each cristae within a same mitochondrion[16]. Interestingly, the proton loops in turn influence the shape of the membrane, in which the presence of charged lipids (the cardiolipin) involves a strong **coupling between the membrane shape and the charged proton diffusion**[17]. To investigate these relations, we will use the same optical microscopy techniques than for plasma membrane, to follow the cristae shape and dynamics, and the membrane potential (at the nans-scaled single cristae level). We will compare healthy and HD neurons, where the defects in mitochondrial membranes lead to both a decrease of ATP production and an increase of the oxidative stress[18]. Interestingly, in HD neurons, these alterations of the mitochondrial functions are not due to defects in cardiolipin metabolisms[19], and the mechanisms supporting this coupling remain unknown. This obviously justifies making them the core of our study. Moreover, the two studied membranes are both characterized by extremely dynamic and highly structured topologies. Their comparison is very promising regarding the biophysics side of our project, as these specific architectures, induced by very particular compositions (cholesterol and cardiolipin), are able to modulate the transport processes at the vicinity of the membrane, but present very different shapes, dimensions (2D or 3D confinement) and underlying physical processes.

A first key step for the project achievement is to have well designed biological models. We will work on primary cells cultures of cortical neurons of mouse, which express the human HD gene and are able of generating glutamatergic excitatory synapses. Thanks to Partner 4, we have developed unique biochemical tools enabling a fine control (methyl-β-cyclodextrin, over-expression of the CXP4AI)[20].

A second key step is to **develop experimental approaches enabling to image the spatiotemporal structures and the activity** of the mitochondrial and plasma membranes of healthy and HD models. Enhanced-resolution microscopies have been very helpful to apprehend both structure and function of the cells. In particular, they played a critical role to evidence their **spatial heterogeneity**[21]. For example, 3D-SIM has been used to elucidate the membrane reorganization in immune cells[22]. In addition, due to their small size ($<\mu m$), intracellular organelles are perfect targets for super-resolution imaging[23]. Moreover, cell membranes also exhibit **temporal heterogeneity**, and the leading-edge super-resolution

techniques present some limitations, especially due to the time of acquisition (typically tens of second to minute). Moreover, a specific limitation for tracking the membrane dynamics is the limited repertoire of probes. We wish to overcome these limitations **by taking advantage of** a **homemade** Fast-**SIM** set-up, of which innovative approach increases the acquisition rate[24]. It will enable fast, large field and non-aggressive (low light excitation) imaging with a lateral resolution of ~100 nm at raw data acquisition rate of 15 frames per second for a wide field of 85µmx85µm (Fig.1)[25].

Fig.2 - Rafts Fast-SIM image in living primary neurons and example of the dynamic analysis. Scale bar 10 µm. (unpublished data)

Our work program **will be implement in both involved laboratories.**

- [NSL]: Sample preparation and characterization : The HD human gene will be over-expressed in mice (mutated or WT-*htt*). Primary cell cultures of WT- or HD-cortical neurons will be prepared. The membrane cholesterol content will be modulated by using chelation (treatment with methyl-β-cyclodextrin), and over-expression of CYP46A1. The biochemical cell analysis will be performed (level of expression, membrane composition and activity) providing information on the cellular components and pathophysiological state. For imaging, cells will be co-transfected with GluN2B-m-Cherry subunit.
- [LJP]: Whole-cell Fast-SIM imaging: Our strategy is to work with the samples prepared and characterized by NSL and to track the remodelling of the cristae (MitoTracker) and the lipid raft dynamics (Cholera-toxin B Alexa-488, see preliminary results Fig.1 and 2), in order to :
  - (1) Extract quantitative data (rafts number and size, mitochondrial aspect ratio) and
  - (2) Analyse their motility (velocity, total displacement, MSD), count the fusion and fission events.

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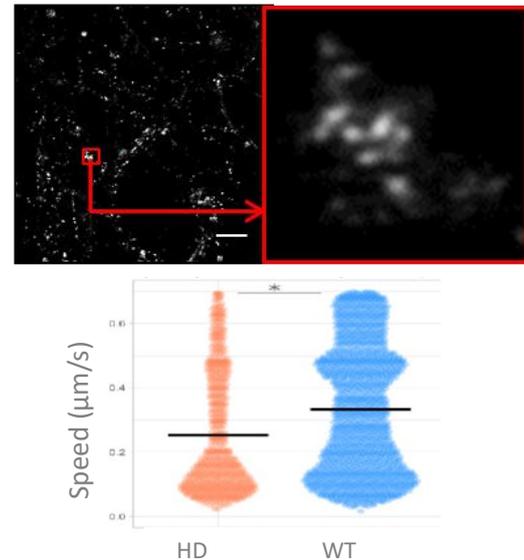
Justification of suitability for *i-Bio*:

Our interdisciplinary project requires different skills, ranging from physics to cell biology, all represent in the two teams. The tools, technical skills and expertise required to achieve our objectives are already implemented in the partner teams, ensuring feasibility of this proposal. Each aim of this project is supported by strong preliminary data obtained through collaboration between the two groups, with direct access to state-of-art core facilities required for successful completion of this project.

Role of each supervisor / skills provided:

[LJP - UMR 8237- IBPS, Sorbonne University/CNRS]: S. Bonneau (PR SU) has an interdisciplinary background covers membrane physics and cell biophysics. She always mixed different techniques (cell biology, physical chemistry, microscopy) with biophysics and coordinate several collaborative projects. For example, she has established the proof of concept of the use of Fast-SIM microscopy for mitochondria cristae imaging. In the team, F. Joubert (CR CNRS) brings his expertise in cellular energetics. F. Sureau (CR CNRS) and S. Kruglik (IR SU) have an important experimental background in optics for biology.

Profile of the desired student:

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.