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 :
Using engineered oil droplets to map compression and traction forces in a developing neuronal circuit

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

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Unité de Recherche :
Intitulé : Laboratoire de Biologie du Développement
Code *(ex. UMR xxxx)* : UMR7622

ED515-Complexité du Vivant
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant :

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

2 doctorants, chacun en co-encadrement (soit 1 au total)

- Pauline Monnot, co-encadrement avec Isabelle Bonnet, Institut Curie (inscription en décembre 2017)

- Pénélope Tignard, co-encadrement avec Alain Trembleau, NPS, IBPS (inscription en octobre 2019)

Marie Breau est actuellement titulaire d'une autorisation à encadrer des thèses sans HDR en tant que jeune cheffe d'équipe (ED CDV) et passera son HDR au cours de l'année universitaire 2020-2021

Co-encadrant :

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

ED564-Physique en IdF
Ecole Doctorale de rattachement : 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) :

2 doctorants, chacun en co-encadrement

- Iaroslava Golovkova, co-encadrement (50%) avec Alexis Prevost, LJP (inscription en Octobre 2017)

- Nicolas Escoubet, co-encadrement (30%) avec Alexis Prevost, LJP, et Romain Brette, Institut de la vision, (inscripton en Octobre 2019)

Lea-Laetitia Pontani s'engage à passer son HDR au cours de l'année universitaire 2020-2021.

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.

Merci de nommer votre fichier pdf :
«ACRONYME de l'institut/initiative_2_NOM Porteur Projet_2020 »

à envoyer simultanément par e-mail à l'ED de rattachement et au programme :
cd_instituts_et_initiatives@listes.upmc.fr avant le 30 mars.

Using engineered oil droplets to map compression and traction forces in a developing neuronal circuit

Summary. This project, lying at the interface between soft matter and biology, proposes to use engineered oil droplets to map and assess the role of mechanical forces in the development of a neuronal circuit. Neuronal migration and axon growth are believed to be primarily guided by chemical signals. However, the role of mechanical cues, such as compression and traction forces, remains poorly understood. To tackle this question we use the zebrafish olfactory circuit, amenable to live imaging and mechanical manipulation. To map forces in a real time, non invasive manner, we develop a biophysical approach based on the injection of oil droplets to use as force sensors. The PhD student will inject and image naked droplets and engineered functionalized droplets to respectively map out compression and tractions forces in the developing neuronal circuit.

Scientific background and preliminary results. Neuronal circuits assemble through a series of developmental steps including neuronal migration and axon growth, which are believed to be primarily guided by chemical cues. However, neurons are surrounded by a complex and dynamic environment exposing them to a variety of mechanical signals, including compression (pushing) or traction (pulling) forces exerted by neighboring cells or tissues. **The role of mechanical forces in neuronal development remains largely unexplored *in vivo*[1]. To address this question, we develop an interdisciplinary strategy combining live imaging, physical approaches to measure and perturb forces *in vivo,* and molecular functional studies.** We use the **zebrafish olfactory circuit** as a model: its superficial location makes it amenable to live imaging and mechanical perturbation, and our previous work suggests an important role for mechanical forces in olfactory axon elongation[2]. We showed that two types of movements are involved in the construction of the circuit, which assembles during olfactory placode (OP) morphogenesis (Fig.1A): neurons from the OP extremities actively migrate along the brain towards the placode center (**active convergence**), whereas central cell bodies are passively displaced from the brain surface while extending their anchored axons (**passive lateral movements/retrograde axon extension**)[2].

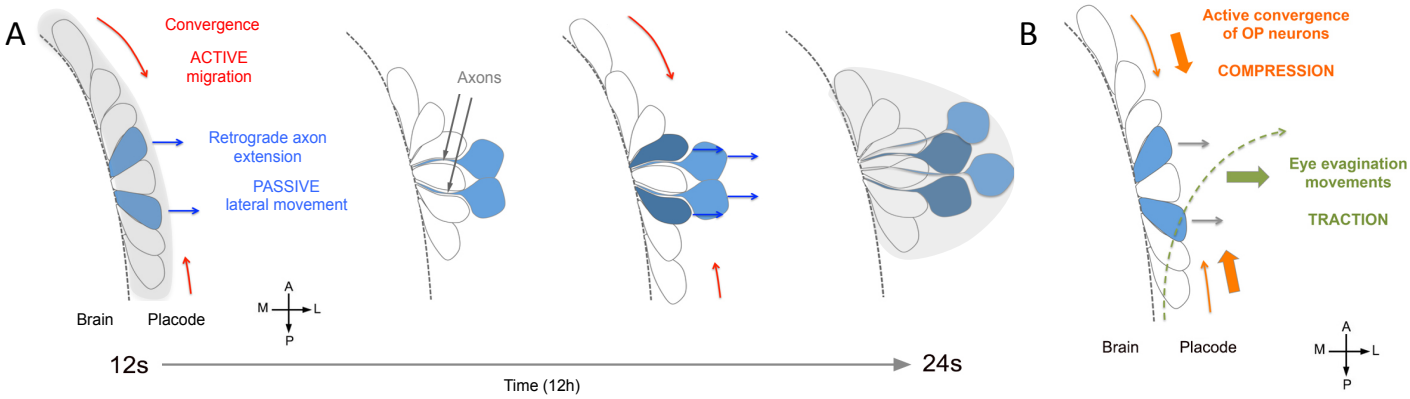

Figure 1. OP morphogenesis and working hypotheses. *(A) Neurons from OP extremities converge towards the center through active migration (red), while cell bodies of central cells passively move away from the brain (blue). As they move laterally, central neurons keep contact with the brain surface through long cytoplasmic protrusions, thereby elongating of their axons (blue, retrograde axon extension). (B) Possible sources of extrinsic mechanical forces driving retrograde axon extension: compression exerted by actively converging cells from placode extremities or traction exerted by the adjacent eye morphogenetic movements. Grey = placode. s=somites.*

The passive nature of the lateral movements suggests that axon extension is driven by extrinsic forces exerted on the central OP cell bodies, pushing or pulling them away from their anchored axon tip. **To decipher the role of mechanical forces, it is essential to map the forces exerted on OP neurons in space and time, in wild type and perturbed conditions.** Our cell movements/imaging data[2] are compatible with two, non exclusive possible sources of extrinsic forces driving axon extension (our two **working hypotheses**, Fig.1B): either anteroposterior (AP) compression from actively converging OP cells squeezes central cell bodies away from their axon tip (**compression hypothesis**, Fig.1B) or ML traction exerted by the morphogenetic movements in the underlying eye tissue pulls cell bodies away from the brain (**traction hypothesis**, Fig.1B). To **map forces in a real time, non invasive manner**, we develop a biophysical approach based on the **injection of oil droplets than can deform upon applied cellular forces, in collaboration with L.-L. Pontani, a specialist in the physics of biomimetic emulsions**[3,4] (Laboratoire Jean Perrin). Proofs of concept for the feasibility of droplet injection in live tissues has already been published[5], but to our knowledge this would be the **first application of this**

technique to map and dissect the contribution of compression and traction forces in an *in vivo* organism. We have been collaborating for 1 year and have already succeeded in injecting and imaging naked oil droplets in the OP, analyzed their deformation and obtained a preliminary map of compressive forces at 16s (Fig.2). OP morphogenesis is not affected by the injection, neither by the presence of the droplet.

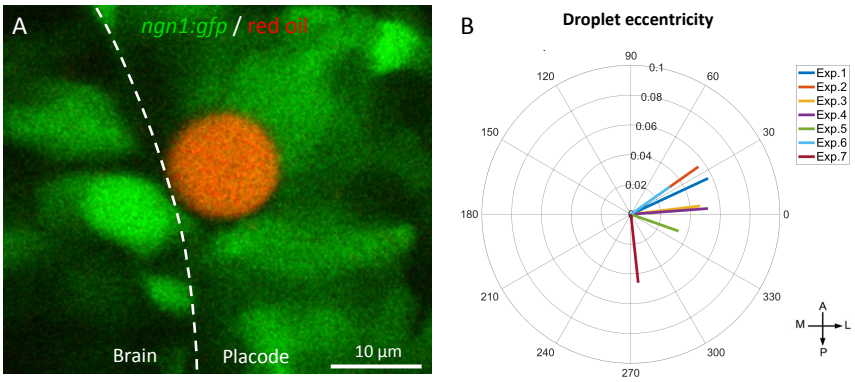

Figure 2. Preliminary compression map in the OP. *(A) 10-20 µm red fluorescent oil droplets were injected in the OP centre of ngn1:gfp embryos, in which OP neurons express cytoplasmic GFP. Confocal live imaging of the droplets was performed between 14s and 18s. (B) Their deformation was quantified using surface segmentation, ellipsoid fitting and eccentricity calculation. Most of the droplets (n = 6/7) showed ML-oriented eccentricity, suggesting the presence of AP compression forces, which is compatible with our compression hypothesis (Fig.1B). Control experiments were conducted, including injection of the droplets in water and in agarose, and in the pre-somitic mesoderm, where ML compression forces have been previously mapped[7] (we reproduced the results, not shown). Exp = experiment.*

PhD project and expected results

The goal of the PhD project is to use oil droplets to map compression and traction forces in the OP and to identify the sources of these forces, i.e. the cells or tissues that exert compression and/or traction on OP neurons. We will use oil droplets that can be deformed only by compressive forces (naked passive droplets) and engineer new droplets that deform upon both compression and traction (adhesive droplets). We will inject these droplets in wild type (wt) OPs and in experimental situations where the potential sources of forces (converging placode cells or eye cells) are affected.

Aim 1. Use naked oil droplets to map compressive forces. Our preliminary map of compression using naked oil droplets in the OP centre (Fig.2) suggests the presence of AP compressive forces in the developing OP, which is consistent with our compression hypothesis (Figs.1,2). To consolidate these results, we will analyze more embryos in the wt situation. We will reuse our established protocol: injection of 10-20 µm oil droplets (bulk injection of soybean oil containing phospholipids, neutral surfactants and nile red) in the OP centre, confocal live imaging and analysis of their deformation using custom scripts. The deformation analysis gives information about the anisotropy of compression. To get the absolute value of the forces, we will measure the surface tension of the oil/water interface through rising drop experiments that will be implemented at LJP. To identify which cells exert compression in the OP, we will inject similar droplets in embryos where the potential sources of extrinsic forces are perturbed: embryos in which converging placode cells have been killed with a biphoton laser, and *rx3* mutant embryos[6] which lack eye morphogenesis. Both conditions are established in the Breau team. The effect of these perturbations on axon extension is currently being analyzed and is not included in this project, which focuses on force distribution in wt and perturbed situations.

Aim 2. Engineer and use functionalized droplets to map traction forces in the placode. In order to measure pulling (and not only pushing) forces, the droplets have to adhere to the surrounding cells. Indeed, if the cells around the droplets are under extension, those pulling forces will be transmitted through the cell/droplet adhesions and measured through droplet deformation. We will thus functionalize the droplets with adhesion proteins such as cadherins or with antibodies against cadherins. In previous studies we successfully functionalized droplets with the extracellular domains of E-cadherin[4]. However, our attempts to inject emulsions, i.e. dispersed droplets, in the zebrafish OP were unsuccessful so far, forcing us to inject the oil in bulk instead, similarly to other groups[7]. In order to combine the bulk injection method with droplet functionalization, we will develop new oil formulations. For instance, we will use the destabilization of an inverted emulsion to our advantage. The first step will consist in making an inverted emulsion (water droplets in oil): the aqueous phase will contain the binders that will be grafted onto lipids dissolved in the oil (Fig. 3A). When a droplet made out of this inverted emulsion is formed, the inner water droplets then fuse with the outer surface of the oil droplet (Fig.3A, middle panel). Upon fusion, the molecules that decorated the inner water droplets will redistribute over the surface of the oil droplet and make it adhere to the cells (Fig. 3A, right panel). We already obtained promising preliminary results showing fluorescence redistribution on the surface

(Fig.3B). We will quantify the amount of protein on droplets surface over time by immunostaining and confocal microscopy. We will also test the functionality of cadherins with aggregation assays performed on droplets formed with this technique[3]. Once fully characterized, these droplets will be injected in zebrafish embryos and analyzed as previously described.

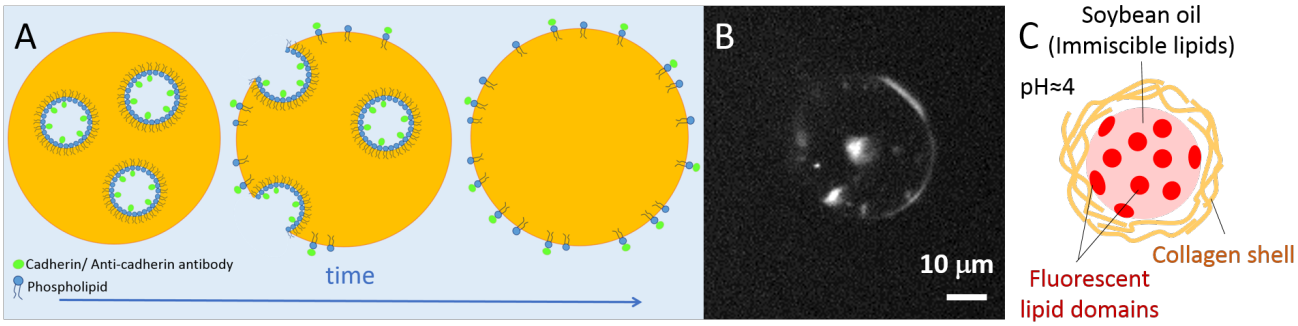

Figure 3. Functionalized droplets. *A) Functionalization upon double emulsion destabilization. Cadherins or cadherin antibodies (green discs) are grafted onto lipids (in blue) inside the water droplets and relocate to the surface upon droplet fusion with the outer surface. B) Fluorescence imaging of a double emulsion droplet injected in agarose. After 1h the fluorescent streptavidin that was grafted on the surface of the inner water droplets redistributes to the outer surface. C) Core-shell structure of collagen-coated droplets. The positively charged surface of the droplets will attract negatively charged collagen at basic pH, while immiscible lipids form fluorescent patches on the surface for imaging.*

Aim 3. Engineer and use functionalized droplets to map traction forces at the placode/eye interface. In addition to mapping of compression and traction in the OP we want to analyze the traction forces exerted at the interface between by the OP and the eye (Fig.1B). We found that OP lateral movements are perturbed in the *rx3* eyeless mutant, suggesting the eye exerts ML traction forces on OP neurons (P.Monnot, unpublished). The interface between the OP and the eye is enriched in extracellular matrix (ECM, P.Monnot, unpublished), including collagen[8]. We hypothesize that this ECM transmits the traction forces from the eye to OP cell bodies. To try and measure these forces we will synthetize a new class of droplets that will be surrounded by a collagen shell, thus ensuring their interaction with the ECM. To do so we can stabilize the droplets with phosphatidic acid lipids that are negatively charged at a pH where collagen is still positive, thus leading to electrostatic interactions. Moreover, we will add immiscible mixtures of lipids inside the droplets in order to form fluorescent domains on their surface[9] (Fig.3C). 3D confocal imaging of such droplets will thus allow us to track the displacement of these surface domains over time, in order to distinguish between compression and shear forces exerted at the interface between the two tissues. The surface coverage of collagen around the droplets will first be characterized *in vitro* through fluorescent labelling and confocal imaging. Mechanical assays will also be developed at LJP to characterize the effective surface tension of these composite objects.

References. **(1)** Gangatharan G et al., Role of mechanical cues in shaping neuronal morphology and connectivity (2018) Biol Cell **(2)** Breau MA et al., Extrinsic mechanical forces mediate retrograde axon extension in a developing neuronal circuit (2017) Nat Comm **(3)** Pontani LL et al., Cis and Trans cooperativity of E-cadherin mediates adhesion in biomimetic lipid droplets (2016) Biophys. J. **(4)** Pontani LL et al., Biomimetic emulsions reveal the effect of mechanical forces on cell-cell adhesion (2012) PNAS **(5)** Campas et al., Quantifying cell-generated mechanical forces within living embryonic tissues (2014) Nat. Methods **(6)** Loosli F, et al. Loss of eyes in zebrafish caused by mutation of chokh/rx3 (2003) EMBO Rep **(7)** Mongera et al., A fluid-to-solid jamming transition underlies vertebrate body axis elongation (2018) Nature. **(8)** Bader et al., Zebrafish collagen XIV is transiently expressed in epithelia and is required for proper function of certain basement membranes (2013) J Biol Chem **(9)** Pontani et al., Immiscible lipids control the morphology of patchy emulsions (2013) Soft Matter.

Adequation to the call "Instituts et Initiatives". The i-Bio PhD funding will serve to **reinforce a recently established (1 year ago) collaboration between two young PIs with complementary expertise** (M.Breau, biologist at LBD, and L.Pontani, physicist at LJP). To initiate their collaboration, they obtained the IBPS Actions Incitatives funding (20 k€ to be used in 2019/2020, covering imaging platform costs and the setting-up of a tensiometer to measure droplet surface tension), and funding from the CNRS MITI (Défi Nouveaux Matériaux, 22 k€ in 2020, to be renewed in 2021), which secures running costs for the next 2 years. However, **no team member is currently fully affiliated to this collaborative work. It is thus absolutely essential to recruit a PhD student to make significant progress in this innovative and interdisciplinary project.**

Expected applicant background. The applicant will be involved at all stages of the project, from the design and characterization of the biocompatible droplets at the LJP, to their injection in the live embryos at LBD and interpretation of the collected data. Due to the strong interdisciplinary aspect of the project he/she is expected to have a background in soft matter as well as in biophysics.