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 : EV-Sens: Label-free BioSensing and profiling of Extracellular Vesicles

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

NOM : **NERI** Prénom : **Christian**
Titre : Directeur de Recherche ou
e-mail : christian.neri@inserm.fr
Adresse professionnelle : IBPS, Bt B6, Sorbonne Université, 9 quai Saint Bernard, 7005 Paris
(site, adresse, bât., bureau)

Unité de Recherche :
Intitulé : Biology of Adaptation and Aging (B2A)
Code *(ex. UMR xxxx)* : UMR 8256

ED158-Cerveau, cognition, comporteme

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) : 1, 2019 (100%)

Co-encadrant :

NOM : **BOUJDAY** Prénom : **Souhir**
Titre : Professeur des Universités ou HDR ☒

e-mail : souhir.boujday@sorbonne-universite.fr

Unité de Recherche :
Intitulé : LRS Laboratoire de Réactivité de Surface
Code *(ex. UMR xxxx)* : 7197

ED397-Physique Chimie des Matériaux

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) : doctorant en 3e année (50%, 2017); 1 doctorant en 2e année (60 %, 2018)

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

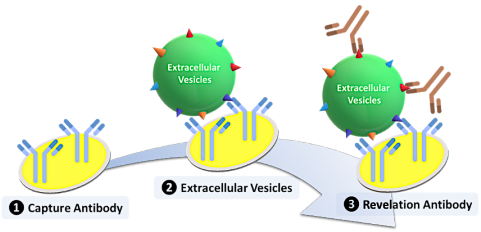
Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences scientifiques apportées. Indiquer les publications/productions des encadrants en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

PhD project EV-Sens: Label-free biosensing and profiling of neuronal extracellular vesicles

Summary: Extracellular Vesicles (EVs) are key mediators of intercellular communication in development, maintenance and human disease. However, interrogating EVs in a precise manner is challenging due to their context-dependent diversity (size, density, trafficking route, cargo content). The EV-Sens PhD project relies on state-of-art technologies in surface chemistry and cellular neurosciences to study the features of EV signaling in the development and maintenance of the normal and neurodegenerative brain. This multidisciplinary project provides high-level training at Sorbonne Université (Jussieu campus) for a candidate with background in physico-chemistry or neurobiology. More detailed information on the project will be communicated to candidates.

Rationale: Extracellular Vesicles (EVs) are key mediators of intercellular communication that are associated with several basic processes (*e.g.* development, maintenance) and human diseases (*e.g.* cancer, neurodegenerative disease). EVs have diagnostic potential as they may reflect the health/disease status of secreting cells and as they can be interrogated in biological fluids (*e.g.* blood). However, interrogating EVs in an easy, precise and reproducible manner is challenging due to their diversity (size, density, origin in the cell, cargo content) and to confounding factors, particularly non-vesicular particles with similar physical features. Depending on the cellular context, EVs may promote or counter-act cellular homeostasis and a pressing question in neurociences is to characterize EV signaling in the context of brain development and maintenance to understand the role of specific EV subtypes in modulating neuronal activity and survival, which has important implications in basic and translational research.

Aim: The aim of the PhD studies is to design a direct, label-free biosensor for the precise detection of EV sub-types based on coupling quartz crystal microbalance with dissipation (QCM-D) and nanoplasmonic spectroscopy (NPS). This approach enables simultaneous *real-time measurements* to be performed while generating *enriched information* on biomolecular recognition under strictly identical conditions thanks to using a gold nanostructured plasmonic_QCM-D sensor chip coated with silica that is mounted in a QCM-D sapphire window module on top of which illumination in reflection mode is carried out by an optical fiber. Using this technology, our **preliminary data** suggest that EV diversity can be finely resolved by information on the amount of transmembrane proteins that are expressed by specific EV subpopulations (see insert below). Moreover, pilot studies in cellular models of neurodegenerative diseases (Huntington's disease: HD, which primarily affects the striatum) suggest that our approach can distinguish EVs that are secreted by wild-type cells from those secreted by diseased cells. The PhD student will further investigate the differences between EV subtypes in normal and disease conditions using relevant cellular models among which panels of human iPSC-derived neural-stem-cells and neurons. In addition, we will design other biosensing methods relying on plasmonic nanoparticles with the ambition of a greater in-depth exploration of the possible wealth of information these EVs can provide.



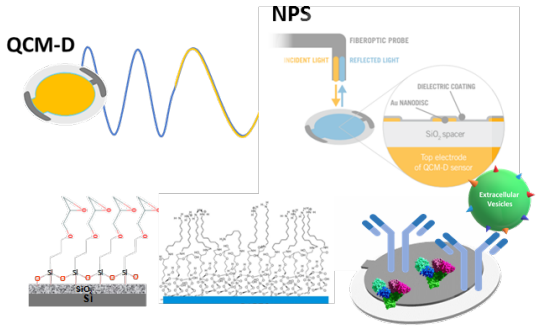
Relevance to the i-Bio initiative: This project cannot be achieved by what each EV-Sens team is capable to achieve on its own. It is directly relevant to the i-Bio initiative as it is multidisciplinary and as it is focused on developing modern research in cellular neurosciences and brain health, bringing together precision chemistry and biology through 4 interconnected workpackages (WP) that are equally distributed between the Neri team at IBPS, expert in aging and neurodegeneration (see References, see also https://www.ibps.upmc.fr/en/research/biological-adaptation-and-ageing/brainc) and the Boujday team at LRS, expert in surface chemistry and biosensors design (see References; see also http://lrs.sorbonne-universite.fr/fr/index.html).

Work Plan:

WP1: Production and characterization of EVs from murine cells @ Brain-C lab (C. Neri) in the Institut de Biologie Paris-Seine (IBPS). The aim of this WP is to produce and characterize the EV samples that will be tested in WP2 using cells that produce high amounts of EVs, here mouse striatal cells derived WT and HD knock-in mice. To this end, we will use state of art techniques that enable different types of EVs to be isolated at high homogeneity from different sources (culture media, tissular extracts, plasma), *e.g.* differential ultracentrifugation (DUC) and size-exclusion chromatography (qEV). This WP will also make use of the most recent techniques for qualifying (electronic microcopy, EM), quantifying and characterizing (immunoprofiling using western blots, functional assays) EV subtypes. Methods: molecular biology, cell biology and quantitative biology (cell imaging, particle quantification, EM).

WP2: Planar immunosensor design @ NanoBioSurf (S. Boujday) in the Laboratoire de Réactivité de Surface (LRS)

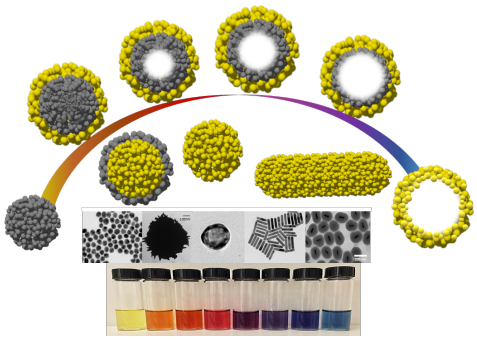
The two techniques utilized in this project are quartz crystal microbalance with dissipation (QCM-D) and nanoplasmonic spectroscopy (NPS), both have limitations regarding the nature of substrates. To remain within the specified constraints of both techniques the biosensing platform will be designed on gold coated-Quartz covered by a thin layer of silica. The objectives of this WP are first mastering surface functionalization to achieve the highest efficiency in terms of sensitivity and specificity. This will be achieved starting through an oxidation step to promote silanol groups and allow for the chemisorption of functional silanes, then, subsequent controlled immobilization of antibodies or their Fab fragments on the surfaces. Considering the very small amounts of biological samples, a major challenge will be reducing the volumes required for the analysis through different means, including the design of microfluidic devices. Once the responsive surfaces mastered, QCM-D and NPS will be combined to enhance the precision and reproducibility of EV-based diagnosis from WP1 in a first stage then, once the volumes are reduced, from WP3. Methods: Surface functionalization and characterization by IR and/or XPS.



WP3: Comparison of the EV subtypes secreted by mouse and human cells @ Brain-C lab (C. Neri, IBPS). The aim of this WP is to investigate the similarities and differences between EVs produced by mouse neural cells and those produced by human neural cells. To this end, we will use protocols that are validated in the Team Brain-C for expanding and

differentiating human normal and HD iPSCs into neural stem cells, pre-patterned cells and neurons. All experiments will be performed using low-passage, quality-controlled lines and clones under highly-controlled cell culture conditions. The challenge will be to optimize the production (at IBPS/Brain-C) and analysis (at LRS) of human EV preparations so that a comprehensive comparison (amount, size, density, mass, function) of EV subtypes can be achieved. Methods: molecular biology, cellular biology, high resolution biology (cell imaging, particle quantification) and stem cell culture.

WP4: Plasmonic nanoparticles @ NanoBioSurf (S. Boujday) in the LRS.
We have developed at LRS an expertise in engineering stable and reliable Bioconjugates combining antibodies and plasmonics nanoparticles. In this project we will synthetize and use different bioconjugates with defined shape and composition leading to identifiable responses in terms of optical properties (LSPR band and color) as well as different electronic properties allowing for their discrimination by Transition Electron Microscopy (TEM). This will be achieved starting from gold and/or silver nanoparticles used bare or coated by silica. Firstly, we will amplify the responses of the techniques mentioned in WP1, by increasing the mass for QCM and coupling the plasmonic properties for LSPR. Secondly, we will, use Cryo-TEM to investigate the external composition of the EVs by engineering different bioconjugates targeting various markers at the EVs surfaces. Methods: colloidal synthesis and respective characterization techniques (UV-Visible, DLS, zêta potential).



References

Neri lab:
https://www.ncbi.nlm.nih.gov/pubmed/31113759
https://www.ncbi.nlm.nih.gov/pubmed/28178240
https://www.ncbi.nlm.nih.gov/pubmed/26681807
https://www.ncbi.nlm.nih.gov/pubmed/24960609
See also:
https://www.ibps.upmc.fr/en/research/biological-adaptation-and-ageing/brainc

Boujday lab:
https://doi.org/10.1021/acsami.9b14980
https://doi/10.1021/acs.jpcc.8b11864
See also:
http://www.lrs.upmc.fr/en/nanosurf-nanomaterials-surfaces-biointerfaces/publications.html