Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral :

Directeur de Thèse porteur du projet (titulaire d'une HDR) :
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Unité de Recherche :
Intitulé : **Laboratoire des biomolecules**
Code : **UMR7203**
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Peptide glycoconjugués et métaux en biologie**
Thématique de recherche : Chimie bioorganique
Responsable d'équipe :
NOM : Grimaud Prénom : Laurence
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED406**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :
En dernière année, soutenant en fin 2021 : 3
En première année, 1 (encadrement 50%)

CO-DIRECTION *(obligatoire)*
Co-Directeur de Thèse (titulaire d'une HDR) :
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Titre : **CRCN INSERM** HDR ☒ oui
e-mail : vincent.vialou@inserm.fr
Unité de Recherche :
Intitulé : **Neurosciences Paris Seine**
Code: **UMR 8246**
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Neurobiologie des maladies psychiatriques**
Thématique de recherche : Neurosciences
Responsable d'équipe :
NOM : **Betancur** Prénom : **Catalina**
Ecole Doctorale de rattachement : **ED158**

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

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

Précisez ici les éventuels co-encadrants (non HDR)

Co-encadrant :
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Titre : **CRCN CNRS** HDR ☐
e-mail : blaise.dumat@ens.psl.eu
Unité de Recherche :

Intitulé : Laboratoire des Biomolécules
Code : UMR7203
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Peptide glycoconjugués et métaux en biologie**
Thématique de recherche : Chimie Bioorganique
Responsable d'équipe :
NOM : **Grimaud** Prénom : **Laurence**
Ecole Doctorale de rattachement : ED406
Ou si ED non SU :

Résumé (2 000 caractères maximum) :

L'objectif de ce projet de thèse est de caractériser le trafic subcellulaire, la sécrétion et la régulation de la protéine hevin - Hevin est une protéine sécrétée exprimée par les astrocytes et les neurones qui module la migration neuronale et la synaptogenèse dans le cerveau en développement. Ainsi, nous pensons que hevin module profondément la réponse comportementale au stress et à l'abus de drogues. Les outils que nous souhaitons développer sont des sondes fluorescentes organiques qui s'activent en se liant à une protéine de marquage (HaloTag). En modulant les propriétés des sondes et en les rendant sensibles aux facteurs environnementaux (pH), le marquage fluorescent d'une protéine de fusion hevin-HaloTag en cellules vivantes nous permettra de suivre la synthèse, la sécrétion et la dégradation de la protéine hevin. Le travail de thèse comportera deux composantes : la synthèse et une première évaluation des sondes (ED 406), et l'utilisation sur culture de cellules primaires (neurones et astrocytes) en imagerie de fluorescence (ED 158).

Joindre en annexe un descriptif du PRD avec références au format pdf
(« NOM_2_IPV_2021 » / 3 pages maximum, taille police 11)

AVIS et VALIDATION de l'ECOLE DOCTORALE :

Avis favorable

Dr. Cyril Ollivier

Dr Cyril OLLIVIER
Directeur de l'Ecole Doctorale
de Chimie Moléculaire

à envoyer simultanément par e-mail à l'ED de rattachement et au programme : interfaces_pour_le_vivant@listes.upmc.fr avant le lundi 15 février minuit.

PhD research project

Hybrid chemogenetic fluorescent toolkit to study protein trafficking, secretion and regulation: application to the synaptic glycoprotein Hevin

Direction: Dr. Jean-Maurice Mallet
UMR7203 Laboratoire des Biomolecules, SU-ENS-CNRS – ED406
Co-direction : Dr. Vincent Vialou
UMR8246-U1130 Neurosciences Paris-Seine, SU-CNRS-Inserm – ED158

Background

The project is a collaboration between a team of chemist led by Jean-maurice Mallet (UMR7203, Sorbonne Université, ENS department of chemistry) working on the development of fluorescent probes for proteins and biological analytes and a team of biologists led by Vincent Vialou (UMR8246-U1130, Sorbonne Université, IBPS) whose research focuses on the elucidation of the molecular mechanisms underlying experience-dependent synaptic plasticity. In particular, the neurobiologist team is studying the role of hevin, a matricellular protein that was identified as a synaptic junction glycoprotein. Hevin is a secreted protein expressed by astrocytes and neurons [1] that modulates neuronal migration and synaptogenesis in the developing brain. Hevin was found to profoundly modulate the behavioral response to stress and drugs of abuse. It is induced by chronic social stress in the nucleus accumbens, a key brain reward region, only in resilient individuals [2]. Using RNA interference to downregulate the expression of hevin in accumbens astrocytes *in vivo* in mice, the team of Vincent vialou observed a decrease in the rewarding properties of cocaine. Neuroanatomical studies show that hevin is ideally localized to modulate adult synaptic plasticity. Indeed, electron microscopy and confocal imaging have localized hevin in perisynaptic astrocytic processes and excitatory post-synaptic density of adult brain. Based on these observations, we hypothesize that hevin participates in adaptive behaviors and coping mechanisms to drugs of abuse and stress by dynamically modulating synaptic plasticity.

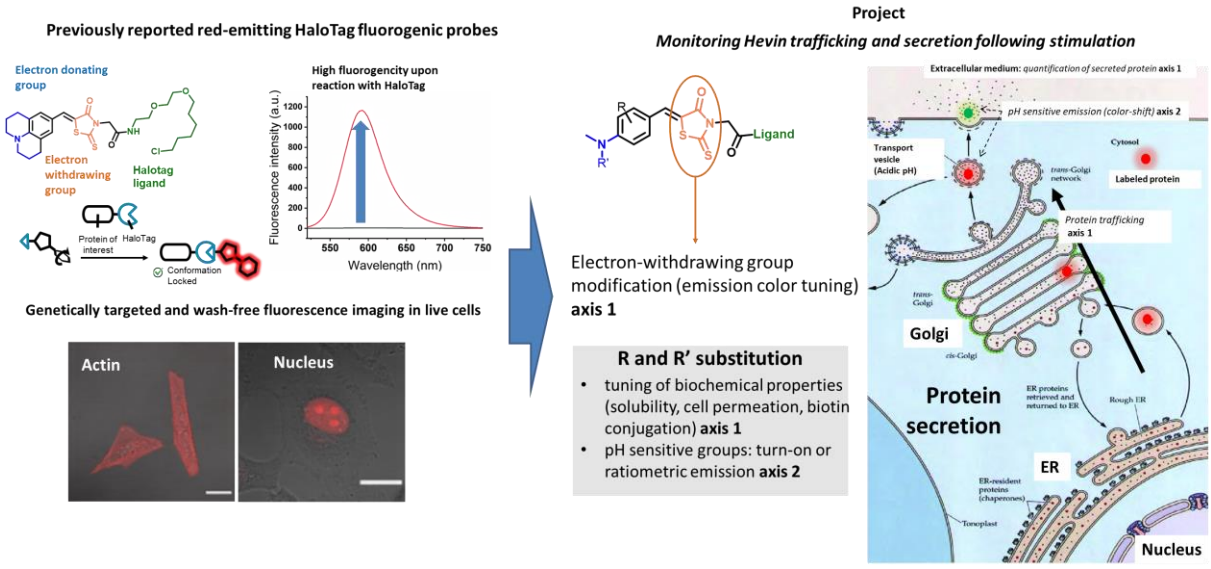
As in most biomedical studies, fluorescence imaging is a key technique used to visualize Hevin in biological samples. It resorts mostly on the use of recombinant hevin fused to fluorescent proteins (FPs). FPs are indeed the tool of choice for biologists to selectively label proteins. They benefit from the unparalleled targeting selectivity of controlled genetic expression. Yet, FPs are not without drawbacks, especially to monitor protein secretion and trafficking since they do not allow any temporal control over the fluorescent labeling. Hybrid chemigenetic systems associating a protein self-labeling tag and a small molecular fluorescent probe have emerged as a valuable alternative [3]. The diversity of available organic fluorophores provides a larger palette of photophysical properties compared to fluorescent proteins and the control of the labeling reaction enables “pulse-chase” experiments useful in dynamic protein imaging. A generation of protein can be labeled at an intant *t* and followed overtime without interference from newly secreted proteins that remain unlabeled. Using multicolor imaging, several generations of proteins can even be labeled at different times with different fluorophores and followed independently.

The team of chemists has recently developed a series of far red-emitting probes targeting a genetically encoded protein self-labeling tag HaloTag for the selective imaging of proteins in live cells

[4]. The fluorescence of the so-called “fluorogenic probes” is activated by the binding to the protein thus enabling high contrast imaging in wash-free conditions.
In this PhD project, the two teams will associate their respective expertise to develop a set of hybrid chemogenetic fluorescent probes based on the HaloTag technology in order to study the **subcellular trafficking**, **secretion** and **regulation** of hevin using live cell imaging. The project will combine organic synthesis and biophysics for the development and characterization of small molecular protein probes (team Mallet) with biochemistry for advanced molecular manipulations of hevin on primary cultures of cortical astrocytes and neurons and fluorescence imaging (team Vialou).

Research project

The proposed project is articulated around two axes.



1. Monitor hevin trafficking using Fluorogenic HaloTag probes

First, we will study the real-time **trafficking of hevin** from endoplasmic reticulum to the site of exocytosis using live imaging of neurons and astrocytes transfected with hevin fused to a Halotag probe. To that end, we will design and synthesize a series of fluorogenic HaloTag probes with photophysical and biochemical properties fine-tuned for this project. The probes are based on push-pull molecular rotor structures. By changing the electron donating or acceptor group, we will tune the absorption and emission wavelength to develop a palette of probes suitable for multicolor imaging. We will also strive to optimize the photophysical properties such as brightness and photostablity that are essential for live imaging. The collaboration between chemists and biologists will also help to adapt the biochemical properties (cell permeation, toxicity, minimization of off-target binding) in true experimental conditions. Cell impermeant probes will also be developed to selectively label extracellular hevin and biotinylated analogues will be designed to enable the simultaneous detection and fishing of hevin protein for further quantification.

2. Monitor Hevin secretion using Dual-input pH/HaloTag probes

The secretion of hevin from acidic transport vesicles into the physiological pH environment will be monitored by the development of a pH-sensitive HaloTag probes. The team of Jean-Maurice Mallet has been working for a decade on functional fluorescent probes for pH and calcium ions [5] and recently, it has focused on the development of dual-input HaloTag/Ca^{2+} probes where the

fluorescence is activated by reaction with HaloTag and detection of Calcium (unpublished results). Using the same principle, we will append a pH sensitive moiety to our newly designed HaloTag probes. We will tune the pKa as well as the photophysical properties to develop turn-on probes that will light-up upon secretion or ratiometric probes able to visualize both acidic and neutral conditions in two different colors.
Probes will be tested in cell culture transfected with the hevin-Halotag protein. We will monitor **the secretion of hevin** in basal and stimulated conditions by time-lapse imaging. Since exocytosis is regulated by cytosolic free calcium, we will test the effects of increased intracellular calcium levels on hevin-Halotag secretion.

This project will enable the development of new chemogenetic fluorescent probes in order to image and study protein trafficking and secretion. The probes will be applied to the study of hevin protein and we expect this collaboration to provide a thorough understanding of the regulation of hevin protein secretion in both neurons and astrocytes, and new insights into the molecular mechanisms of activity-dependent synaptic plasticity. The projet relies on the use of a protein self-labeling tag which makes this fluorescent toolkit virtually applicable to the study of any protein of interest.

Project organization

The PhD will be conducted in two laboratories of Sorbonne Université located close to each other in the center of Paris. Organic synthesis and photophysical characterization of the probes will be carried out at the Laboratoire des Biomolecules located at the Ecole Normale Supérieure de Paris. With its newly refurbished facilities, the lab is fully equiped for these experiments and a has a longstanding experience in the development of functional fluorescent probes [4-5].
Cloning of Halotag into the C terminus of hevin, primary astrocytic and neuronal cultures will be performed in the Neuroscience Paris Seine laboratory located on the Jussieu campus. Confocal TIRF live imaging will be performed in the imaging platform of Institut de Biologie Paris Seine. These techniques have already been used and validated in the laboratory.

[1] R. Mongredien, A. Erdozain, S. Dumas,…, V. Vialou, *Brain. Struct. Funct.* **2019**, 224, 1219-1244.
[2] V. Vialou *et al. Nat. Neurosci*. **2010**, *13*, 745-752.
[3] C. A. Hoelzel, X. Zhang, *ChemBioChem* **2020**, *21*, 1935–1946.
[4] S. P. J. T. Bachollet, C. Addi, N. Pietrancosta, J.-M. Mallet, B. Dumat, *Chem. Eur. J.* **2020**, *26*, 14467–14473.
[5] (a) M. Collot, C. Loukou, A. V. Yakovlev, …, J. M. Mallet, *J. Am. Chem. Soc.* **2012**, *134*, 14923–14931. (b) G. Despras, A. I. Zamaleeva, L. Dardevet, …,J.-M. Mallet, M. Collot, *Chem. Sci.* **2015**, *6*, 5928–5937.