Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Fiche à nommer selon le format Nom_Prénom_ProjetED2021, à enregistrer en format PDF et à renvoyer à l'adresse : edcdv@sorbonne-universite.fr

Nom et prénom du directeur de thèse (et si besoin du co-directeur) :
Le directeur de thèse et le co-directeur doivent impérativement avoir l'HDR ou équivalent
Coordonnées   Tel :   01 44 27 45 85       e-mail : catherine.venien-bryan@upmc.fr

Nom et prénom du co-encadrant (non Hdr) (s’il y a lieu) :
Coordonnées   Tel :

Y-a-t-il un candidat déjà identifié pour le projet: OUI NON

Nom et prénom du responsable de l’équipe : Catherine Vénien-Bryan

Intitulé de l’équipe : « Structure et dynamique des protéines »   groupe BIBIP Bioinformatique et Biophysique
Nombre de chercheurs et enseignants-chercheurs statutaires de l’équipe titulaires d’une HDR (ou équivalent) : 1

Nom et prénom du responsable d'UMR ou de département: Guillaume Fiquet

Intitulé et N° d’UMR ou de département : IMPMC Institut de Minéralogie, Physique des Matériaux et Cosmochimie
UMR 7590

Titre du projet de thèse : *Etudes structurales et fonctionnelles d'un canal à potassium humain, Kir. Effet de modulateurs (Pip2 et miRNA) sur son activité*  

Signature du directeur d'UMR ou de département (vaut avis favorable pour le dépôt du projet) :

Guillaume FIGUET 

Directeur

Spécialité : Biochimie, Biologie Structurale

Résumé du projet de thèse (1 page maximum, en anglais)
Pour les thèses avec 2 co-directeurs, ou en partenariat entre 2 laboratoires ou structures, indiquer la participation de chaque co-directeur et structure dans la gestion du projet
This project aims at understanding the effects of modulators such as PiP2 and microRNA on the structure, the dynamics and the function of Kir2.1, a human potassium channels. One of the most fundamental biological processes is the ability of a cell to facilitate the selective movement of ions and small molecules across the plasma membrane. The rapid movement of inorganic ions such as Na+, K+, Ca2+ and Cl- across the membrane along their electrochemical gradients is achieved by ion channels: integral membrane proteins that provide ion-selective pathways across the otherwise impermeable cell membrane. Of the 80 different K+ channel genes identified in the human genome, 15 belong to the inward rectifier potassium (Kir) channel family composed of 7 members (Kir1.x to Kir7.x) encoded by 16 genes. These channels selectively control the permeation of K+ ions at the cell membranes of a variety of tissues and regulate the membrane electrical excitability and K+ transport in many cell types. Kir channels are found in almost every cell in the body and control such diverse processes as heart rate, vascular tone, insulin secretion and salt/fluid balance. The gating of Kir channels is modulated by various intracellular ligands that bind directly to the channel and enable the electrical activity of these channels to be linked to a wide range of cellular and metabolic pathways. How the channel gates in response to effectors is beginning to be understood. In particular, we recently provided structural insights into the molecular mechanisms of gating by solving the structure of a functionally active mutant of KirBac3.1 S129R\(^1\). We have also identified in situ the change of conformation during the gating using a new method called MDeNM which combine normal modes and molecular dynamics\(^2\). The physiological importance of the Kir channels is highlighted by the fact that genetically-inherited defects in Kir channels are responsible for a number of human diseases (channelopathies) such as in Andersen’s syndrome (Kir2.1). These diseases originate often from missense mutations that alter the gating mechanisms of the channel and/or its response to cellular effectors.

The gating of Kir channels is modulated by various intracellular ligands; it depends critically on phosphatidylinositol 4,5-biphosphate (PIP2). PIP2 activates the channel. MicroRNAs (miRs) play critical roles in regulation of numerous biological events, including cardiac electrophysiology and arrhythmia, through canonical RNA interference (RNAi). However, recently, in collaboration with colleagues, we shed light on a new role of these miRNA. We found that endogenous miR1 could physically bind with cardiac membrane proteins, including an inward-rectifier potassium channel Kir2.1\(^3\). The miR1-Kir2.1 physical interaction was observed in mouse, guinea pig, canine and human cardiomyocytes. miR1 quickly and significantly modulate the physiological homeostasis of the heart through non canonical mechanisms.

The PhD student will focus primarily on the interaction between these two modulators and the human potassium channel Ki2.1. He will study

- The structure at high resolution of the complex Kir2.1 /PiP2 using cryo-EM and image analysis in order to identify the interaction critical for ligand-induced activation.
- The interaction at high resolution of the complex Kir2.2/miRNA using cryo-EM and image analysis with the same objective as above.

The student will investigate the structural and biochemical effects of a particular mutant, the Kir2.1 R312H. R312 is a putative PiP2 binding site. Any mutation on this position will cause the Andersen’s syndrome.

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2 Fagnen et al. (2020) Scientific reports 10:1 (1-14)
3 Yang D. et al. (2021) Circulation. doi: 10.1161/CIRCULATIONAHA.120.050098. PMID: 33590773
The aim of this PhD program is to investigate the interactions critical for ligand-induced activation, of the human kir2.1. For this, the knowledge of the structure at high resolution of this protein in complex with PIP2 or MiRNA is crucial. The PhD student will be trained to express the human Kir2.1 (in Pichia Pastoris) and to purify this protein. The quality of the protein will be checked in term of purity, solubility, homogeneity. All the necessary biochemical equipment is available at IMPMC. The team of C. Vénien-Bryan has recognized expertise in the structural studies of proteins and particularly membrane proteins using cryo-EM combined with image analysis. IMPMC possesses a JEOL 2100F EM, equipped with minimal dose system and all cryo equipment for preliminary cryo-work. C. V-B is a co-investigator in the Equipex project CACSICE; in this context, the laboratory has access to a state of the art microscope (Krios Titan 300KV, FEG, at Pasteur Institute), equipped with the new up-to-date camera crucial for the biological studies at high resolution. C V-B has its own computer cluster with graphic cards for intensive computations for the calculation of 3D maps at high resolution. A robust protocol for the expression and purification of the human Kir2.1 is in place. The protein is usually solubilized in the detergent DDM, and recently we have used successfully the amphipoles. We are developing a protocol for reconstitution of the Kir2.1 into nanodiscs. The composition of the nanodiscs will include PIP2. This will allow to image the complex Ki2.1/PIP2. Kir2.1 in nanodisc in complex with PIP2 or miRNA will be be first imaged in negative staining at IMPMC with our routine microscope in order to assess the best conditions for observation under cryo-conditions. Cryo-EM images will be collected on the sate of the art microscopes at the nanoimaging core facility at Institut Pasteur. Image analysis will take place using software such as (Relion, cryoYOLO, cryoSPARC, Eman2.3...). All the necessary GPU’s computers and the expertise for image analysis at high resolution are available at IMPMC. In order to interpret the 3D map calculated from cryoimages; we will use our recently published software developed in Venien-Bryan’s team which which allow interpreting the 3D map using flexible fitting.

Electro physiology and single channel recording will be performed with our collaborator Dr Said Bendahhou in Nice. Using these advanced methods in structural biology combined with functional tests, the student will also investigate the effects of a representative mutation (R312H) in this Kir2.1 channels that have been linked to severe pathologies such as Anderson syndrome. This PhD proposal is therefore integrated in a larger project in the hosted team which aims at developing a systematic approach for the design of small molecules able to correct the underlying molecular defect and restore normal function.
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Thèses actuellement en cours dans l’équipe
Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d’une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu’IPV, FDV...)

<table>
<thead>
<tr>
<th>Nom et Prénom du doctorant</th>
<th>Directeur(s) de thèse</th>
<th>Année de 1ère inscription</th>
<th>ED</th>
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<tr>
<td>ZUNIGA Dania</td>
<td>Catherine Vénien-Bryan</td>
<td>Octobre 2018</td>
<td>CDV</td>
<td>Concours Ecole Doctorale CDV</td>
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Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s’il y a lieu). Mettre en gras le nom du directeur de thèse.


Docteurs encadrés par le directeur de thèse ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.

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<tr>
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<tbody>
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<td>Date de obtention : 28 Mai 2020</td>
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<td>Durée de thèse (en mois) : 44 mois</td>
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Publications :


Physiology via Directly Binding to Ion Channel Circulatin (impact Factor: 24)  Feb 16. Doi : 10.1161/circulation 120.050098 Online ahead of print PMID : 33590773

4) **Fagnen C**, Bannwarth L, Oubella I, Haouz A, Forest E, Bendahhou S, De Zorzi R, Perahia D, **Vénien-Bryan C**  Gain of function mutation associated with DEND syndrome revealed in high-resolution structure of the Kir channel *Cell Mol Life Science* 2021 in revision

5) **Fagnen C**, Bannwarth L, Dania Zuniga Oubella I, Forest E, Bendahhou S, De Zorzi R, Perahia D, **Vénien-Bryan C**  Unexpected gating of an engineered open state of a potassium channel Submitted to Frontiers in Molecular Biosciences.