PhD offer :

Title: **Study of the role of ion channels and of their molecular environment in endothelial mechanotransduction in the context of Cerebral Cavernous Malformations using an in vitro endothelial model under shear stress.**

Starting date: July 2023
Funding: ANR grant

Scientific context:
The endothelial cells lining the blood vessels are permanently subjected to extracellular mechanical signals generated at their apical side by the blood flow and at their basal side by the stiffness of the extracellular matrix. These mechanical signals are integrated into mechanotransduction pathways to generate appropriate cellular responses.
Our group is focused on better understanding the molecular bases of endothelial mechanotransduction and has identified a complex of proteins called CCM (Cerebral Cavernous Malformations) as a major orchestrator of these pathways. In humans, loss-of-function mutations on the CCM genes lead to the formation of cerebrovascular lesions in which the mutant endothelial cells invade the surrounding neuronal tissue to form stacks of dilated and hemorrhagic vessels. CCMs are present in 0.5% of the worldwide population. Mysteriously, these malformations form only in low blood flow vessels (venous-capillaries), but not in high shear stress which keeps the defects silent. The mechanisms responsible for this hypersensitivity to low flow are still to be discovered. Ion channels are the first molecules activated, within a few milliseconds, when endothelial cells are subjected to flow. We have shown that endothelial cells deficient in the CCM complex overexpress a set of ion channels which are otherwise known to control angiogenesis and vascular permeability. Our first results indicate a functional cross-talk between these channels and suggest that they could lie upstream of the mechanotransduction pathways controlled by the CCM complex.

Objectives and experimental approaches:
The goal of this PhD work will be to develop in vitro culture of CCM mutated endothelial cells under flow for the identification by proximity labeling using BioID of molecular actors involved in the response to flow and responsible for the mutant phenotype.
BioID is a rapidly developing technique. It allows to identify directly in the living cell the proximate molecular environment of a protein of interest thanks to its labeling by biotinylation. The tagged proteins are then extracted and analyzed by mass spectrometry.
We will couple this proximity labeling technique to cell culture under laminar flow. To our knowledge, such an experimental setup has never been published. It should allow us to identify the cellular processes involved in the appearance of defects in mutated endothelial cells when the flow is lowered.

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Keywords:
Mechanotransduction, endothelial cells, ion channels, microfluidics, bioID, proteomic analyses.

Laboratory:
This PhD will be performed in IAB, Grenoble in the group led by Eva Faurobert in DYSAD team. This work will lie in the context of an international collaboration with Prs Hans Van Oosterwyck and Susana Rocha laboratories of the Department of Mechanical Engineering in KU Leuven in Belgium. The student will benefit from our collaboration with the EDyP proteomic facility in CEA Grenoble and from the microscopy facility at IAB (MicroCell) which is equipped with confocal microscopes for live and quantitative microscopy.

Requested knowledge and skills:
The project requires that the candidate has very good knowledge in biochemistry, cell biology, cell biomechanics and biomaterials. Some skills acquired through internships in either of these domains will be appreciated.
The successful applicant must also have the willingness and enthusiasm to work independently while being able to communicate with the various scientists involved in this project whether they are biologists, engineers, or biomechanicians. The ideal candidate should be curious, and should enjoy solving problems and developing new technologies with personal creativity and innovation.

Publications from the lab:
- Vannier DR, Shapeti A, Chuffart F, Planus E, Manet S, Rivier P, Destaing O, Albiges-Rizo C, Van Oosterwyck H, **Faurobert E*.** CCM2 deficient endothelial cells undergo a ROCK dependent reprogramming into senescence associated secretory phenotype. Angiogenesis, 2021, Nov;24(4):843-860. doi: 10.1007/s10456-021-09809-2
- Manet S, Vannier D, Bouin AP, Lisowska J, Albiges-Rizo C and **Faurobert E**. Morphological study by immunofluorescence of cell-cell and cell-extracellular matrix adhesive defects in *in vitro* endothelial CCM model. Juxtacrine role of mutant extracellular matrix on wild-type endothelial cells. **Methods in Mol Biol** 2020;2152:401-416
- Lisowska J, Rödel CJ, Manet S, Miroshnikova YA, Boyault C, Planus E, De Mets R, Lee HH, Destaing O, Mertani H, Boulday G, Tournier−Lasserve E, Balland M, Abdelilah−Seyfried S, Albiges−Rizo C, **Faurobert E***. The CCM1−CCM2 complex controls complementary functions of ROCK1 and ROCK2 that are required for endothelial integrity. **J Cell Science** 2018 Aug 13;131(15).

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