***IFJPAN – JCET***

From the structure of fenestrations in live Liver Sinusoidal Endothelial Cells (LSECs) to real-time in vitro pharmacology

## *1. Research hypothesis*

Under physiological conditions, the unique phenotype of liver sinusoidal endothelial cells (LSECs) is characterized by the presence of fenestrae (or fenestrations). Those are cellular transmembrane pores of size in the range of 50-300 nm. It is known that fenestrae are dynamic structures that actively regulate the transport of solutes and macromolecules, especially lipoproteins, from the vascular system to the interior of the liver. Accordingly, liver disorders are often characterized by the decreased porosity of the endothelium. Due to the methodological limitations the knowledge about the dynamics of fenestration structure, and the mechanisms responsible for their regulation remained rudimentary. With the advent of the novel AFM imaging methodology, enabling studies of fenestrae dynamics in live cells, new possibilities of testing the properties and functionality of fenestrae became possible.

In this project, we will evaluate the link between the changes in nanomechanical properties and the number of fenestrations regulated via vasoprotective mediators during the defenestration process. We hypothesize, that stimulating spectrin-actin cytoskeleton, we could restore fenestrated morphology during liver pathology. We claim that the porosity can be controlled pharmacologically via modulation of the vasoprotective endothelial mediators, offering new therapeutic perspectives for the fenestrae restoration in liver dysfunctional diseases.

## 2. Research methodology

For achieving the project objectives, we will apply a unique methodology developed by our team, based on combination of the atomic force microscopy (AFM) and the atomic force spectroscopy (AFS). Up to date, our methodology is the only one allowing for tracking nearly in real-time and under high resolution, dynamic alterations of the fenestrae nanostructure in the physiologically relevant periods. Taking advantage of the technique, the structural changes in LSEC cytoskeleton will be tracked, both by means of cell morphomechanics - morphology (fenestrae), and nanomechanics (elasticity/stiffness). Using this novel AFM-based methodology, with the support of other techniques (fluorescence microscopy, Western blot), we will verify our hypothesis. We selected three types of defenestration simulating liver pathology. Firstly, we will culture LSECs for 3 days until the loss of fenestrae. Secondly, we will stimulate isolated LSECs using drugs to induce defenestration. Finally, we will isolate LSECs from mice in which defenestration occurs in vivo and investigate them ex vivo. We will try to restore fenestrations and maintain fenestrated morphology for a prolonged time in each of the three models of defenestration. To achieve this, we will focus on the investigation of the organization of fenestrations using: soft substrate, cytoskeletal-altering drugs, vasoprotective mediators. The real-time changes of the cell morphomechanics will be investigated for the first time under different physiologically relevant stimuli of pharmacological agents (i.e. analogs of NO, PGI2, or VEGF).

## 3. References

1. Zapotoczny, B. et al.Sci. Rep.7, 7994 (2017)
2. Zapotoczny, B. et al.Hepatology (2018)
3. B. Zapotoczny et al.,Traffic, 1–11 (2019)