

Development of a robust protocol for multimodal imaging of peptides in cell spheroids

Supervision: Emmanuelle Sachon, emmanuelle.sachon@sorbonne-universite.fr
Team : Chémobiologie des peptides et des protéines

Projet scientifique

1. Description du projet / Description of the project

Homeoproteins (HPs) are transcription factors having the remarkable capacity to transfer between cells. This process, associated to their paracrine activity, is mediated by a cell-penetrating domain located in their sequence.¹ Studies on HPs have led to the discovery of one of the first cell-penetrating peptide (CPP), now widely used as a vector for drug delivery.^{1,2} At the Chimie Physique et Chimie du Vivant (CPCV) laboratory, we are currently designing HP-derived CPPs incorporating non canonical residues with improved cell-penetration properties. To select the best candidates, we have developed quantitative methods of peptide internalization in cells based on mass spectrometry (MS),^{3,4} or fluorescence.⁵ The following step is the transposition of these methods on spheroids, 3D cell culture tumoral models. Such systems have been previously studied by fluorescence microscopy;⁶ which only gives information on the position of the probe. Mass Spectrometry Imaging (MSI), in which mass over charge (m/z) ratio of the molecules is measured, can overcome this downside. **The proposed M2 project focuses on the development of a robust protocol to explore CPP integration in spheroids using fluorescence imaging and Matrix-Assisted Laser Desorption/Ionization Mass spectrometry imaging (MALDI) MSI with a focus on the MALDI-MSI.** In order to do so, CPP sequences labeled with different functionalities adapted for fluorescence imaging or MALDI MSI, have been first synthesized. These CPP sequences will be first internalized into 2D cells and spheroids to determine their internalization yields. Then, MALDI-MSI will be realized on the spheroids sections. Producing thin sections (10 to 15 μm) of spheroids (with diameters of less than 1 μm) using a microtome requires embedding the spheroids in a gel that should be resistant enough to be easily sectioned. Moreover, for a multimodal imaging purpose, the polymer used needs to be transparent to be compatible with fluorescence microscopy, and with no risk of production of interferent ions during the MS analysis. Concerning the MALDI-TOF analysis, different steps will be optimized: 1) MALDI analysis requires the deposition of a uniform film of an organic matrix onto the tissue section; 2) Detecting and quantifying high-molecular-weight peptides by MALDI-MSI within tissue sections can be difficult due to the concomitant presence of lipids from the cell membranes, that are easily ionized and detected by MALDI-TOF.

2. Techniques ou méthodes utilisées / Specific techniques or methods

This highly multidisciplinary and exploratory internship, set within the CNRS-funded project *ImagiCS*, aims at the development of MALDI MSI at CPCV in collaboration with the MS facility of Sorbonne University (MS³U). Optimizing this matrix film, sample preparation steps involved in producing the sections, and quantification strategy to be able to detect and quantify peptides with high signal-to-noise ratio, on the section will form the core of this work.

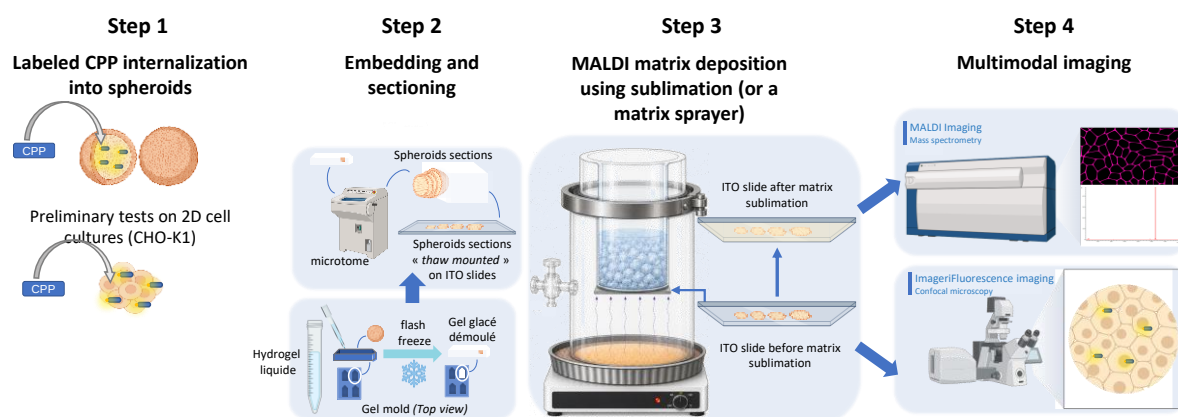


Figure 1 Graphical representation of the M2 internship project

Keywords: multimodal imaging, cell-penetrating peptides (CPPs), quantification methods, spheroids, MALDI-TOF.

3. Références / References

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