

Synthesis and photophysical characterization of multimodal tags to explore peptide penetration in cell spheroids

Homeoproteins (HPs) are transcription factors having the remarkable capacity to transfer between cells. This process, associated to their paracrine activity, is mediated by their cell-penetrating domain located in their homeodomain (HD).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-derivative peptides 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 transposal of these methods on spheroids, 3D cell culture tumoral models. Such systems have been precedentially 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 **multimodal imaging tag** to explore **CPP integration in spheroids** using **fluorescence imaging** and Matrix-Assisted Laser Desorption/Ionization Mass spectrometry imaging (**MALDI**) **MSI**. In order to do so, synthetic HP-derivative peptides obtained by Solid Phase Peptide Synthesis (SPPS) must be produced and modified by a label with different functionalities adapted for both fluorescence imaging and MALDI MSI. The multimodal synthetic probe is based on a core fluorophore (coumarin) bearing a link that upon irradiation produces a cation detectable by MSI. Photoreactivity of the probe will be studied and its structure optimized by modification of substituents or stabilization of the produced cation. To

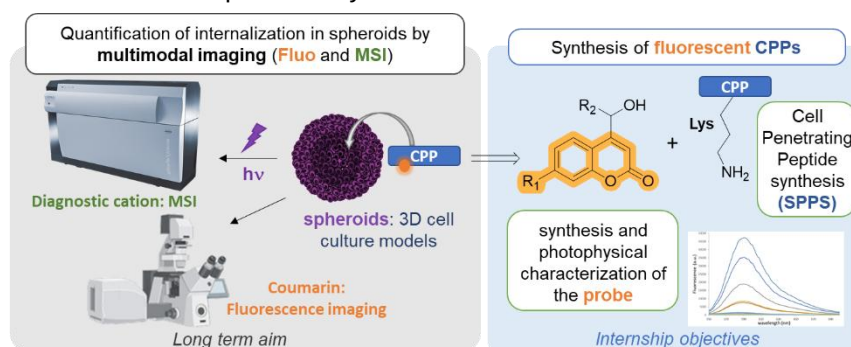


Figure 1 Graphical representation of the M2 internship project

properly quantify peptide internalization into spheroids by qMSI (quantitative MSI), the synthesis of isotopically labeled versions of the probe will be envisaged. This highly multidisciplinary internship, inscribed within the CNRS-funded project *ImagiCS*, combines chemical biology, organic and peptide synthesis with

analytical aspects such as MALDI-TOF mass spectrometry and spectrochemical characterization (absorption and emission wavelengths, fluorescence and photocleavage quantum yields).

Keywords: coumarin, multimodal imaging, homeodomain (HD), solid phase peptide synthesis (SPPS), quantification methods, spheroids.

Highly motivated candidates with a strong background in organic chemistry and a keen interest in working at the interface between chemistry and biology should send their CV, undergraduate (L3) and first-year Master's (M1) transcripts to carolina.chieffo@sorbonne-universite.fr.

References:

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