Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
Elisabetta Boeri Erba, elisabetta.boeri-erba@ibs.fr

Host laboratory:
IBS, https://www.ibs.fr/

Host group/team:
Epigen: Epigenetics & molecular pathways

Title of the M2 research internship:
Sequencing soluble and membrane proteins using mass spectrometry

Project summary:
The primary sequence and post-translational modifications (PTMs) of proteins influence their structure and function, tuning their actions in key cellular processes. Using mass spectrometry (MS), the IBS MS laboratory characterises soluble and membrane proteins and their PTMs. MS can assess the mass of biomolecules with high accuracy, sensitivity and rapidity. Our mass spectrometers allow us to sequence intact proteins. Sequencing shed light on identity, sequences [e.g., loss of first amino acid (often a Met), annotation errors, mutations], identity, truncations, PTMs, and key structural knowledge (such as position of disulfide bridges to distinguish conformational isomers of a protein). To our knowledge, many purified proteins do not have the theoretical mass calculated from the amino acid sequences.
The student will analyse interesting samples (both soluble and membrane proteins) purified in collaboration with our colleagues at the IBS, LPCV (Laboratoire de physiologie cellulaire végétale) and Grenoble-Institut des neurosciences (GIN). Using a MALDI time-of-flight (TOF)/TOF, the student will optimise sample preparation conditions to sequence these proteins. She/he will assess different types of experiments matrices, sample deposition and matrix crystallisation. For instance, she/he tests T3 experiments using distinct laser powers to further sequence fragments. Using a novel instrument, electrospray quadrupole-TOF, the student will set up a middle-up approach to characterise investigated proteins and to maximise mass resolution, accuracy, sensitivity and sequence coverage. Middle-up approach requires short enzymatic step to generate large fragment of proteins, which can be sequenced.
Overall, this project aims to enhance our MS-based ability of sequencing soluble and membrane proteins. It should appeal to students with a background in biology, nanoscience or physics, who are interested in innovative approaches.

Keywords:
membrane proteins, mass spectrometry, sequencing

Relevant publications of the team:
Boeri Erba E, Klein PA, Signor L. Combining a NHS ester and glutaraldehyde improves crosslinking prior to MALDI MS analysis of intact protein complexes. J Mass Spectrom. 2015, 50(10):1114-9. doi: 10.1002/jms.3626

Boeri Erba E. et al. Exploring the structure and dynamics of macromolecular complexes by native mass spectrometry. J Proteomics. 2020. 222:103799. doi: 10.1016/j.jprot.2020.103799

Signor L, Boeri Erba E. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometric analysis of intact proteins larger than 100 kDa. J Vis Exp. 2013, (79). doi: 10.3791/50635