PhD proposal at CBM CNRS, Orléans, France.

Native MALDI Mass Spectrometry with liquid deposits: Application to therapeutic proteins and their complexes

Context:
Native Mass Spectrometry (MS) native consists in analyzing noncovalent biomolecular assemblies directly in an instrument. To this end, biomolecules are exposed to nondenaturing conditions, in order to keep their 3D structure intact from the sample solution to the gas phase. By its speed and sensitivity, this approach has become essential for complex characterization. It can be used to determine binding stoichiometries in specific complexes, to precisely measure their affinity, and to perform ligands screening.

Membrane proteins constitute two thirds of therapeutic targets, yet only 10% of these proteins have been targeted so far. The characterization of membrane proteins by native MS can unlock essential knowledge for biology and pre-clinical research.

To this day, the go-to method for native MS is based on electrospray ionization (ESI). However, membrane protein analysis by native ESI-MS is still a challenge. This is because these proteins necessitate salts and detergents for solubilization in MS-compatible buffers. Detergents in turn can give rise to ion suppression, adduction, and degraded instrument performance.

MALDI (Matrix-Assisted Laser Desorption-Ionization) presents numerous advantages in this context. It is highly tolerant to contaminants and consumes low quantities of sample, making it *de facto* attractive for the native MS analysis of membrane proteins. Recently, we developed a native MALDI-TOF MS method for the analysis of macromolecular complexes. The success of this method can be attributed to the use of liquid deposits, allowing for the smooth transfer of molecular complexes from the sample to the instrument [1]. The proof of concept was established for a protein-protein and protein-ligand systems.

Our group has a lot of experience and knowledge in the analysis of membrane proteins in denaturing conditions [2,3]. More recently, we have developed quantitative methods for the fine characterization of proteins and their interactions with ligands or other proteins. Thus, we can precisely determine the equilibrium dissociation constant K_D by native ESI even when affinities are low [4], or quantify the degree of modification of every single residue of a target protein by a covalent ligand [5]. Finally, our demonstration for native liquid MALDI MS is a worldwide premiere [1].

Project:
Thesis work will be dedicated to extending applications of native liquid MALDI MS methods to the observation of membrane proteins by themselves or in a complex with one or more ligands.
Our group currently investigates ion channels or transporters types of transmembrane proteins. We plan to manipulate complexes directly in the instrument in native MALDI MS. First, we will show the intact protein oligomer complex that forms the ion channel or transporter. Then we will measure the affinity and stoichiometry of the complexes they form with ligands of therapeutic interest and with membrane lipids.

In cases when the ligand binding site is unknown, complementary experiments will be set up to localize the site.

These experiments will require that both sample preparation and instrumental parameters be optimized. More generally, this work will open the way to an easy, fast and sensitive native analysis of hydrophobic proteins or any protein that needs additives with low compatibility with electrospray.
On the basis of existing or emerging collaborations in our group, researches will be extended to the fine characterization of ion channels, chloride transporters, and small ligand transporters of therapeutic interest, so as to contribute to the design of new anticancer treatments.

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[2] Cadene M, Chait BT (2000) A robust, detergent-friendly method for mass spectrometric analysis of integral membrane proteins. *Anal. Chem.* 72, 5655–5658.
[3] Gabant G, Cadene M (2008) Mass spectrometry of full-length integral membrane proteins to define functionally relevant structural features. *Methods* 46, 54-61.
[4] Jaquillard L, Saab F, Schoentgen F, Cadene M (2012) Improved accuracy of low affinity protein–ligand equilibrium dissociation constants directly determined by Electrospray Ionization Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* 23, 908–922.
[5] Gabant G, Boyer A, Cadene M (2016) SSPaQ: A subtractive segmentation approach for the exhaustive parallel quantification of the extent of protein modification at every possible site. *J. Am. Soc. Mass Spectrom.* 27, 1328-44.
[6] Rivera-Torres IO, Jin TB, Cadene M, Chait BT, Poget SF (2016) Discovery and characterization of a novel toxin from *Dendroaspis angusticeps*, named Tx7335, that activates the potassium channel KcsA. *Scientific Reports* 6, 1-10. doi: 10.1038/srep23904.

Financing:

A PhD fellowship has been granted by the University of Orleans with funds from the French Ministry for Research and Higher Education.

Candidates profile:

Candidates with a Masters or an equivalent Engineering School diploma in the fields of Biology or Biochemistry, with a deep interest in therapeutic proteins & protein targets, and the will to invest themselves in Mass Spectrometry are welcome to apply.

To apply:

Please send a CV, motivation letter and the address of 3 referees to:
Martine Cadene, martine.cadene@cnrs-orleans.fr
Functional Mass Spectrometry in Metastasis
Centre de Biophysique Moléculaire (CBM-CNRS, UPR 4301)
Rue Charles Sadron - CS 80054 - 45071 Orléans Cedex 2, France.
Phone: +33 (0)2 38 25 56 24.
http://cbm.cnrs-orleans.fr/spip.php?article100