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Structural biology of membrane proteins and their complexes using Native Liquid MALDI (NALIM) Mass Spectrometry (MS).

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Introduction. Membrane proteins (MPs) represent two-thirds of potential therapeutic targets, yet only 10% have been targeted so far, mainly owing to several analytical constraints such as low sample quantities (linked to difficult overexpression and purification) and a hydrophobic environment. Structural biology requires tools to integrate information at different scales, from individual molecules of all sizes to large molecular assemblies. Mass spectrometry (MS) enables structural characterization of MPs in support of high-resolution structure determination methods such as X-Ray Crystallography, NMR, and Cryo-EM.

Native MS methods have reached the status of powerful tools for the analysis of biomolecular complexes directly in an instrument. To this end, samples are analyzed in carefully controlled non-denaturing conditions which preserve their 3D structure. So far, the go-to method for native MS is based on electrospray ionization (ESI). Nevertheless, analysis by native ESI-MS can still be challenging due to the use of additives and salts for the stabilization of proteins in aqueous buffers, which can give rise to ion suppression, adduction, and degraded instrument performance. By contrast, MALDI is more tolerant to contaminants and consumes small quantities of sample. This is particularly the case for MPs, which require detergents for solubilization, making MALDI-based methods *de facto* attractive for the development of native MS analysis. We previously leveraged MALDI's unique properties to create a new native MS method based on a liquid spot [1], which we have renamed Native Liquid MALDI (NALIM). Here we show how we develop NALIM to make MS-based structural biology studies of MPs more accessible.

Materials and methods. NALIM relies on an ionic liquid matrix solution (HCCA/3-Aq/Glycerol) in native conditions. Analyses were performed on an Ultraflex extreme MALDI-TOF/TOF mass spectrometer from Bruker Daltonics equipped with PANTM technology and a 2 kHz smartbeam-II laser. A 1 μ L aliquot of analyte-matrix solution was spotted onto a MTX stainless steel sample stage. In order to achieve high compatibility of NALIM with MPs at micromolar to submicromolar concentrations, both the sample/matrix preparation and the instrumental parameters setup had to be optimized.

Results and discussion. NALIM was successfully adapted and applied to address challenges related to the analysis of a multimeric ABC transporter which is an archetype for multi-drug resistance transporters. The transporter can be stabilized through binding to its cognate ligands thanks to inorganic ion trapping. Tx7335 is an original actitoxin, meaning that, contrary to all other known potassium channels toxins, this toxin has an activating effect on the channel. Preliminary data from competition experiments gives us insight into the localization of the actitoxin onto a potassium channel. NALIM thus provides easy access to the stoichiometry of membrane protein assemblies, a monitoring method to follow complex stabilization through the binding of a specific ligand, and a tool for localization by competition.

Conclusion. While not as mature as ESI-based methods for native MS, NALIM is proving to be a promising alternative, particularly for large molecular assemblies.

[1] Beaufour, M., Ginguené, D., Le Meur, R., Castaing, B., and Cadene, M. (2018). Liquid Native MALDI Mass Spectrometry for the Detection of Protein-Protein Complexes. *J. Am. Soc. Mass Spectrom.* 29, 1981–1994.

Keywords:

Native mass spectrometry, NALIM, structural biology, membrane protein, ABC transporter, potassium channel, snake toxin.