MOSBRI Molecular-Scale Biophysics Research Infrastructure Basic Level School

Mass spectrometry by MALDI

A. Introduction - Guidelines:

1. Reminder

Mass spectrometry (MS) has become an indispensable technique for protein QC (see Note 1). Many quality-relevant aspects of a protein affect molecular mass, for example primary sequence, processing, proteolytic degradation, and post-translational modifications (PTMs). The high mass accuracy that can be achieved by MS techniques (in the range of one Da) allows sensitive detection of PTMs, such as phosphorylation ($\approx +80$ Da), or proteolytic processing that would evade detection in gel electrophoresis.

Matrix-assisted laser desorption / ionization (MALDI) is a soft ionization processes due to its capacity to transfer a moderate excess of energy to the analyte, which is crucial for fragile and non-volatile compounds such as proteins. MALDI is an ionization process from a solid phase: the protein of interest is dispersed in a saturated solution of a small aromatic molecule called matrix and the whole is co-crystallized by evaporation of the solvent. The solid phase obtained is irradiated, resulting in desorption of the ions. In MALDI, proteins often result in only a singly-charged ion species. One of the main advantages is that the sample droplets can be prepared rapidly on the metal target, allowing the analysis of a large number of soluble or membrane proteins in a very short time.

For protein QC, an **intact mass MS** analysis (top-down strategy) is highly recommended to check both integrity and identity of the protein of interest. This QC parameter is crucial to ensure that the target protein is highly pure without any degradation at the N- or C-terminus. Buffer components such as detergents or glycerol can easily interfere with the measurement and make the analysis of membrane proteins or proteins in glycerol more challenging. Nowadays, most biological, biochemical, biophysical or structural biology institutes have access to MS technology (either in-house or as commercial service) so that this essential test can be used routinely in the QC workflow.

2. Protein Intact mass by MALDI

During sample preparation for a MALDI experiment, a droplet of the target protein-matrix solution is deposited onto a MALDI plate and the solvent is evaporated to allow co-crystallization. There are several ways to prepare deposits, varying in matrix concentration, solvent, method of crystallization, etc. The “dried droplet” method is the most commonly used technique because it is extremely fast and simple to implement. Either the sample can be mixed with the matrix directly on the MALDI plate or the mixture can be prepared beforehand in a tube and a droplet is then deposited on the target plate. Typically, 1 µL of protein is applied to the target plate and 1 µL of matrix is immediately added. The sample and the matrix can be

mixed by repeated pipetting before the deposit, which is still in the form of a droplet, is allowed to dry.

B. Protocol:

1. Sample preparation for intact mass without zip tipping

- Freshly prepare the matrix of interest. HCCA, DHB and sDHB are used for proteins below 20 kDa, whereas HCCA is usually employed for proteins above 20 kDa. A good practice is to prepare these matrices at 25 $mg.mL^{-1}$ in 50 % (v/v) acetonitrile and 0.1 % (v/v) TFA in HPLC water.
- Mix 1 µL of the target protein with 1 µL of the matrix on the MALDI plate and let it dry for 15 min. For a typical 15 kDa protein 1 µL at 0.1 $mg.mL^{-1}$ is sufficient, for a 150 kDa protein around 1 mg.mL-1 are recommended. These concentrations are acceptable for protein samples in phosphate-bufferd saline (PBS)-type buffers. If your buffer is supplemented with more than 1 % (v/v) glycerol or detergent, the sample should be concentrated and then diluted with water to reach the required concentration. This will minimize the effect of the detergent or glycerol. Unfortunately, a few samples are capricious. In these cases, ZipTip pipette tips (C18 for proteins < 20 kDa and C4 for proteins > 20 kDa) may be used to concentrate and desalt the protein of interest.

2. Sample preparation for intact mass with zip tipping

i. Solution to prepare

- 50 % aq ACN containing 0.1 % TFA
- 0.1 % aq TFA
- Matrix solution → HCCA
- Sample to be prepared if possible in 0.1 % aq TFA

ii. Sample preparation

Use a 20 µL pipetman. Make sure there is no contact with air during the whole process. Keep the pipetman on the first notch between uses.

- Activate the ZipTip C4 with 20 µL 50 % aq ACN containing 0.1 % aq TFA. Repeat three times.
- Rinse the ZipTip C4 20 µL with 0.1 % aq TFA. Repeat three times.
- Pipette with the same ZipTip C4 15 µL sample and transfer to another tube.
- Pipette / release the material to ensure proper mixing and homogeneity of the solution Repeat 10 times.
- Desalt and wash the sample attached to the ZipTip C4 with 20 µL 0.1 % aq TFA three times.
- Pipette approximately 2 µL of the matrix solution taking advantage of capillarity and transfer on the MTP 384 ground steel target plate (press up to the second stop position of the pipette to ensure full release (air bubble)).

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3. Configuration of the measurement

Perform the analysis and acquire data on the MALDI time-of-flight (TOF) mass spectrometer. It is recommended to use the minimum laser power required to get a nice spectrum as a high laser power leads to a loss of resolution and precision on the m/z.

Calibrate the experiment with a standard mixture as recommended by the manufacturer, previously prepared.

Notes:

Note 1: The European consensus on protein QC can be found on the websites of the European networks P4EU (https://p4eu.org/protein-quality-standard-pqs) and ARBRE-MOBIEU (https://arbre-mobieu.eu/guidelines-on-protein-quality-control/)