MALDI-TOF Mass Spectrometry

Sample processing:

Perform dialysis if sample buffer in which proteins or peptide is present contains salt. Samples should not contain any salt, organic/inorganic buffer or solvent. **NOTE:** for peptides, use ZipTip to remove salt.

Dialysis

1. Pipet 500µL of protein sample into a dialysis tubing with a desired molecular-weight cutoff (MWCO). Close the tubing with clips.
2. Place the dialysis tubing containing the sample in a 2L beaker filled with Milli-Q. Add a magnet into the beaker and place on a magnetic stirrer at +4°C for overnight incubation.
3. Next day collect sample (~500µL) from the dialysis tubing into an eppendorf tube.

ZipTip:

Zip-Tips are pipette tips that contain immobilized C18, C4 or some other resin attached at their very tip occupying about 0.5µl volume. We used C18 resin. Our usual protocol is:

1. Use a P20 pipetter set to 10µl for Zip-Tips
2. Wash the Zip-Tip with 0.1% trifluroacetic acid (TFA) in acetonitrile
3. Wash the Zip-Tip with 0.1% TFA in 1:1 acetonitrile:water
4. quilibrate the Zip-Tip twice with 0.1% TFA in water
5. The sample, typically dissolved in 10 µl of 0.1% TFA, is passed through the Zip-Tips repeatedly by pipeting in and out to bind the sample to the resin.
6. Wash the Zip-Tip three times with 0.1% TFA, 5% methanol in water
7. Elute the sample from the Zip-Tip in 1.8µl of matrix, typically alpha-cyano-4-hydroxycinnamic acid in 0.1% TFA 50% acetonitrile, directly on the MALDI-TOF sample plate.

Sample preparation:

A. For proteins bigger than 10kD

1. Preparation of protein sample: 1µl of 2mg/ml of recombinant protein solutions in 10% acetic acid.
2. Preparation of the matrix: dissolved sinapinic acid in 50%:50% Milli-Q water : Acetonitrile (ACN) with final concentration 0.1% Trifluoroacetic acid (TFA).
3. Mixed 1µl of prepared sample and 1µl of the matrix.
4. Then place it on MALDI target plates and dried for minimum of 60 min before conducting the experiment.

B. For protein or peptides lower than 10kD

1. Preparation of peptide sample: 1µl of 2mg/ml of peptide solutions in 10% acetic acid.
2. Preparation of the matrix: dissolved a-cyano-4-hydroxycinnamic acid in 70%:30% Milli-Q water: Acetonitrile (ACN) with final concentration 0.05% Trifluoroacetic acid (TFA).
3. Mixed 1µl of prepared sample and 1µl of the matrix.
4. Then place it on MALDI target plates and dried for minimum of 60 min before conducting the experiment.

We used matrix-assisted laser desorption/ionization-time of flight/time of flight (MALDI-TOF-TOF) mass spectrometer (UltrafleXtream™ Bruker, Aalto department of biotechnology and chemical technology facilities, Espoo, Finland) equipped with a 200-Hz smart-beam 1 laser (337 nm, 4 ns pulse) to identify masses of proteins/peptides.

Data collection

Data collection was carried out by operating the instrument in positive ion mode controlled by the flex software packaged (FlexControl, FlexAnalysis). 5,000 laser shots were accumulated per each spectrum in MS modes. Protein Calibration Standard mixture I,II and peptide calibration standard II (Bruker Daltonics) were used to calibrated the MS spectra.

Note: Remember that you also need **calibration standards**. These are just protein and peptides with know molecular weights, which will be used for calibrating the equipment before collecting spectra for the real samples. Side by side with preparation of the samples you need to also follow exactly the same steps for the preparation of the standard whether for case **A** or **B**.