Protocol for Sample Preparation for Native Mass Spectrometry

Native mass spectrometry interrogates gas-phase proteins in their 'native', *i.e.* non-denatured state using electrospray ionisation. This MS approach can be used for answering questions on the stoichiometry of protein complexes and protein binding to small molecules and peptides. Binding affinities can also be calculated from native mass spectra.

As most MS techniques, native MS is quite sensitive and usually requires protein concentration in the range of 1-25 μM. Final volumes of the sample solution can be as low as 15 μl, but usually have to be larger prior to buffer exchange that is required for MS analysis. Protein samples have to be prepared in non-denaturing buffer for electrospraying, for most proteins it means buffer pH around 7. Aqueous *ammonium acetate* is the most common buffer used as it is volatile and provides the required pH. Buffer concentration is usually in the range of 50-200 mM. Samples can tolerate presence of other solvents in the buffer, but usually not above 5 % (depends on the solvent and also protein stability). *Glycerol should be avoided at protein purification steps as it leads to spectral peak broadening even at much smaller concentrations.* Molar concentration of salts can only be in the range of that of the protein, a larger presence of salts has to be reduced during the buffer exchange procedure. If these requirements can lead to protein collapsing in the ammonium acetate buffer within a short while, the buffer exchange can be carried out immediately prior to the mass analysis for each sample.

There are different ways of buffer exchange available. Most common are:

- Micro Bio-Spin 6 chromatography columns, MW exclusion limit 6 kDa (Tris buffer, Bio-Rad, cat. no. 732-6221);
- NanoSep centrifugal devices (various MWCO, Pall Life Sciences) and Vivaspin 500 ml concentrators (various MWCO, Sartorius)

For some proteins that are stable in ammonium acetate the original buffer can be removed more efficiently using dialysis (e.g., Dialysis Micro and Ultra-Micro DispoDialyser (Harvard Apparatus)).

The fastest way of buffer exchange is that offered by Bio-Spin 6 microcolumns (takes 25-30 mins), and it can be carried out right before spraying the sample. It is, therefore, advisable to try it first using the protocol below. *Please note that we do not provide the Bio-Spin columns, you have to purchase them from the vendor.*

Protocol for buffer exchange using Bio-Spin 6 microcolumns

Materials: ammonium acetate solution 50-200 mM, Bio-Spin 6 microcolumns with included waste vials, clean 2 ml Eppendorf vials, 100 and 1000 μl pipettes with tips, centrifuge for Eppendorf-type vials. Initial sample amount 25-80 μl required per single column. As the columns are centrifuged, a common practice is to make buffer exchange on two columns simultaneously counter-balancing them on the centrifuge. Protein concentration can be higher than the final 1-25 μM (please refer to Bio-Spin 6 instruction leaflet), and consequently diluted in ammonium acetate after the buffer exchange.

Procedure:

1. Invert the column sharply several times to resuspend the settled gel and remove any bubbles.
2. Snap off the tip and place column in a 2.0 ml microcentrifuge waste tube (included). Remove the cap. Allow the excess packing buffer to drain by gravity to the top of gel bed. (If the

column does not begin to flow, push cap back into the column and remove). Discard the drained buffer, then place the column back into 2 ml waste tube.

3. Centrifuge for 2 min in a swinging bucket centrifuge at 1,000 x g (to remove the packing buffer). Make sure you balance the column in the centrifuge, better by the same microcolumn/waste tube. Discard the buffer inn the waste tube.
4. Put 500 µl of ammonium acetate buffer in the column, centrifuge 1 min at 1000xg, discard the buffer in the waste tube.
5. Repeat step 4 three-five times discarding the buffer each time. The last time you can use 450 µl of ammonium acetate instead of 500 µl to avoid dilution of the sample in the step 6.
6. Place the column in *a clean 2.0 ml Eppendorf tube*. Carefully apply the sample (25–80 µl) directly to the centre of the column. Application of more or less than the recommended sample volume may decrease column performance.
7. After loading sample, centrifuge the column for 4 min at 1,000 x g. The sample in ammonium acetate will be at the bottom of the Eppendorf tube now.

Contact Dr Victor Mikhalov (victor.mikhailov@chem.ox.ac.uk) if you have any questions related to this protocol or to organise a collaborative Native MS project.