General Tips and Tricks

Native MS

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Keep in mind the following tips and tricks from the Wysocki group when performing native mass spectrometry experiments.

#1: Tip Size Matters

- Different samples will require different kinds of tips for optimal nanospray.
- Larger tips which use higher spray voltage (1-1.4 kV) often work better for larger systems.
- Small (<150 kDa) soluble globular proteins tend to spray best from small tips at low voltage (0.5-0.9 kV).
- Tip size and spray voltage will drastically affect signal and apparent resolution or desolvation
- Don't be afraid to have multiple tip profiles.

#2: Use Fresh Solution of Cesium Iodide

- Cesium iodide for high mass calibration does not keep in solution. Prepare fresh for each calibration in 50/50 water/isopropanol at 2-8 mg/mL dependent on the instrument
- We recommend dissolving ~100 mg of CsI in a small amount of water (~1 mL), aliquoting (20 uL for 2 mg) into 1.5 - 2 mL tubes and then drying for storage. When needed you can resuspend an aliquot.

#3: Blunt Tipped Syringe for Filling Nanospray Capillaries

- If filling Nanospray capillaries using a syringe a blunt tipped syringe is recommended (Hamilton 80085 or similar).

#4: Consider Gel-Loading Tips

- Gel-loading tips (Genesse # 14-352, Eppendorf cat # 022351656) are useful for samples that may contaminate a syringe (ligands, detergent, salt, etc) and to eliminate the possibility of carryover.

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#5: Recovering Limited Sample

- Sample can be recovered from a used tip, dispensed into a small tube, and reloaded into a new tip if sample is extremely limited.
- Samples that are sensitive to air or have been sprayed at high voltage (>2 kV) should not be transferred due to potential oxidation or other degradation.
- Attempting to directly transfer solution tip to tip often results in air bubbles that disrupt spray.

#6: Frequent Source Cleaning

- Simple source cleaning (not requiring venting) should be done daily for best results-particularly when additives such as magnesium acetate or detergents are being used. Frequent source cleaning can reduce internal contamination.
- Some samples are best run using a separate cone or ion transfer tube if available e.g. membrane proteins in detergent.
- Clean Pt wire often, or if contamination is suspected.

#7: Preferred Electrolyte Solutions

- Native mass spec electrolyte solutions are commonly ammonium acetate (Sigma 431311), ethylene diamine diacetate (EDDA) (Sigma 420352) [*Note: Not Ethylenediamine-N,N'-diacetic acid*], and triethyl ammonium acetate (TEAA) (Sigma 90358)
- Commonly used at 100 and 200 mM concentrations
- Complex formation may be affected by ionic strength and pH
    - Screening conditions may be required for unknown samples
- TEAA is added to 20% ionic strength in ammonium acetate ex 160 mM AmAc + 40 mM TEAA to achieve charge reduction
- Recommended to dilute a small amount of 1 M TEAA stock with water to typical ammonium acetate concentration for easy addition of 20% V/V to achieve charge reduction

#8: Using Ammonium acetate

- Ammonium acetate is hydroscopic and quickly absorbs water from the air making accurate ionic strength determination difficult. We recommend dissolving the entire container when received to 5 M in ultrapure water, storing at -20 C, and diluting immediately before use
- Ammonium acetate is volatile and concentration will change over time if left at room temperature or uncapped for extended periods of time
- It is recommended to dilute fresh ammonium acetate every 2 weeks for best results

#9: Dilute, Dilute, Dilute!

- Protein or protein complex concentrations should generally be 500 nM – 10 uM for analysis. Poor spray stability can be an indication of too high concentration, diluting 2-20x is recommended for testing. Excessive concentration can also result in the formation of nonspecific oligomers that are not present in solution