Mass Photometry Notes

Buffer Solvent Recommendations (From Refeyn)

In order to optimize detection, accuracy and resolution of lower molecular weight species and minimize background noise, particularly in the lower molecular weight range, the following steps are recommended.

Using the freshest water and buffer and procedure below yields the best results.

Materials

VWR: VWRL0201-0500 Water, Molecular Biology Grade 0.2um filtered
VWR: 82020-066 HyClone Dulbecco's Phosphate Buffered Saline Solution

Aliquot stock solutions into 15 ml sterile conical tubes by pouring (do not use pipets)

Freeze stock aliquots until use.

On day of use, thaw the required number of stock tubes and aliquot into 1.5 ml microcentrifuge tubes.

Using sterile tips and storing tubes on ice, may minimize bacterial growth. Discard unused solutions after 1 day.

Mass Photometry Notes

Preparation of Calibrants (from Refeyn)

Below are recommendations for the most commonly used proteins calibration standards. Other protein and nucleic acid calibration standard options can be found at the BIF website (bif.mit.edu)

In general, it is best to store calibration solutions at the higher stock concentrations and to prepare working solutions immediately before use. Storage of lower concentration solutions will cause absorption of proteins from the solution onto tube surfaces, reducing their effective concentration, resulting in much lower levels of detection.

Materials:

BSA Bovine Serum Albumin (ThermoFisher 23209): main species is monomer with MW 66kDa

Beta-amylase (Sigma A8781): main species are monomer, dimer and tetramer with MW 56, 112, 224kDa

Thyroglobulin, bovine (Sigma T9145): main species is dimer with MW 670

Native Mark (ThermoFisher LC0725): main species have MW 66, 146, 480, 1048 (not recommended; very low stability standard mix)

Bovine Serum Albumin (BSA)
Preparation and storage of stocks:
1. Vials come at a 2mg/ml concentration in liquid form
2. MW BSA is 66kDa
3. 2mg/ml BSA = 30uM.
4. Crack open the vial and prepare 50ul aliquots and freeze.
5. Stability: 2mg/ml RT for months in intact vials; 0.5-1uM 4C >100ul for months, <100ul for weeks; 50-300nM 4C small volumes <1day. Avoid freeze/thaw cycles.

Preparation of 1uM stocks from 30uM:
1. Thaw 50ul BSA 30uM on ice
2. Add 1450ul PBS 5% glycerol for 1uM and aliquot for medium-term storage (months 4C)
3. To use for measurements, dilute further to 50-300nM in buffer without glycerol for short-term use (<day RT)
4. Measure at a final concentration of 5-10nM in the well

Notes: BSA from ThermoFisher shows very little dimer, but the presence of dimer and larger species will increase over time, thus reducing the effective concentration of monomer. Other sources of BSA usually show higher prevalence of multimers, which might be more/less useful as calibrants.

Beta-amylase (BAM) from sweet potato
Preparation and storage of stocks:
1. Vials contain ~20mg in powder form (check lot details)
2. MW BAM monomer is 56kDa (in solution it exists as a monomer, dimer and tetramer of 56, 112, and 224kDa, respectively)
3. Resuspend at 5.6mg/ml with PBS 5% glycerol
4. 5.6mg/ml BAM = 100uM of monomer.

5. Prepare 100ul aliquots and freeze.
6. Stability: 100uM frozen for >1yr; 10uM -20C for months; 1uM 4C for weeks; 50-300nM <1day RT. Avoid freeze/thaw cycles.

Preparation of 10uM and 1uM stocks from 100uM:
1. Thaw 100ul BAM 100uM on ice
2. Add 900ul PBS 5% glycerol for 10uM, aliquot 10x100ul and freeze -20C.
3. Thaw 100ul BAM 10uM on ice and add 900ul PBS 5% glycerol for 1uM; aliquot 10x100ul and freeze -20C.
4. To use for measurements, dilute further to 50-300nM in buffer without glycerol for short-term use (<1d RT)
5. Measure at a final concentration of 5-10nM in the well

Notes: BAM shows quite a bit of unbinding at neutral pH. This means that you sometimes have to load less than normal so as to not have too many "events" per frame. As you lower the pH you see less unbinding but then the tetramer disappears until you only get monomer at low pH. It is important to be aware of pH when using BAM as a calibrant.
For low mass proteins (i.e. <100kDa), it is best to just use BAM for calibration, the reason being that it is most sensitive to what the y-intercept should be.

Thyroglobulin (TG) from Bovine
Preparation and storage of stocks:
1. Vials contain 35mg in powder form (check lot details)
2. Resuspend at 10mg/ml PBS 5% glycerol
3. MW TG monomer is 335kDa but in solution it mainly exists as a dimer of 670kDa)
4. 10mg/ml TG = 15uM of dimer.
5. Prepare 100ul aliquots and store -20 or -80C.
6. Stability: 15uM -20 or -80C for >year; 0.3uM for months frozen or 4C >100ul; 0.3uM for <week RT. Avoid freeze/thaw cycles.

Preparation of 0.3uM stocks from 15uM:
1. Thaw 100ul 15uM TG on ice; keep at 4C <6mo
2. Take 10ul 15uM and add 490ul PBS glycerol for 0.3uM (stable for weeks 4C; <day RT)
3. Dilute to 30-50nM for RT working stock
4. Measure at a final concentration of 3-5nM in the well

Notes: TG is sensitive to pH (at low pH it falls apart into monomers) and to reducing conditions (dissolves into monomers).

Native Mark (NM)
Preparation and storage of stocks:
1. Vials contain 5x50ul and come frozen.
2. Keep frozen
3. Stability: Unthawed and undiluted -20 or -80C for >year; 4C undiluted for weeks; -20C frozen 1:10 dilution for weeks; thawed 1:10 dilution for hours 4C; 1:100 dilution discard after use
4. NOTE: VERY UNRELIABLE BECAUSE OF INSTABILITY

Notes: After the first thaw, the inherent instability of this marker results in: 1048kDa species disassembles into smaller species increasing background noise; apoferritin forms 48-mers at 960kDa that get confused with 1048kDa species; ADH at 146kDa disassembles into dimers and monomers that overlap with BSA population, making its center peak inaccurate; BSA starts forming more dimer, which is confounded with what remains of 146kDa species. All in all, this standard is very unreliable unless very fresh.
Preparation of 1:10 dilution from stock

1. Thaw 50ul stock aliquot and add 450ul PBS
2. Aliquot 10x50ul and freeze -20C
3. Measure at a final dilution of 1:300 in well

Calibrant Mixes

Mass photometry measures and counts single molecules, and therefore is sensitive to the concentration of the sample analyzed. When measuring a calibration mix it is best not to have too many species nor very high concentrations, specially of the larger mass species, as best measurements are those where molecules do not land next to each other. Whenever deciding to create a calibration mix, make sure you measure each sample individually and then in combination to see if you get equally good calibration curves.

A useful guide: less than 3500 total counts in 1-minute movie in regular image size of a TwoMP; enough counts of each population to fit a gaussian (i.e. >200-300 counts); and have greater than full-width at half-maximum (i.e. resolved) populations. Aim to have less of larger species than smaller MW species. In general, you do not want particles landing right next to each other.
Below are some examples of what has worked for us, but represent only a recommended starting point that needs to be tweaked due to lot-to-lot and user-dependent variability.

Preparation of BAM-TG premix calibration standard (100ul; preferred mix):
10ul BAM 1uM + 10ul TG 0.3uM + 80ul PBS
Stability: weeks at 4C; <day RT
Use 1:10-1:20 final in the well Proprietary & confidential

Notes: in regular image size of a TwoMP and if not too concentrated you should detect 4 main species: 56, 112, 224, and 670kDa species. Consider not including 56kDa species if your max error and r^2 is problematic.