

Sample preparation for AUC sedimentation velocity experiments

General information:

- The optimal sample volume is 400 μ L.
- Between 1 and 3 samples can be run at any one time. Typically these would be either (i) 3 dilutions of a single protein, or (ii) free A, free B and “complex” AB, all at the same concentration.
- The same volume of buffer (preferably dialysate or flow-through from a concentrator) is required for each sample. (It's helpful to have several extra ml of buffer to hand for dilutions/washing *etc.*)
- Spin samples down hard or filter to remove aggregates.

For detection by UV absorbance:

Ideally, aim for between 0.5 and 1.5 OD at a wavelength at which the sample absorbs more significantly than the other components (e.g. 280 nm for proteins, although any wavelength from 190-800 nm is theoretically possible; let us know in advance if this presents a problem). We usually collect interference data too, but it's useful to have good UV absorbance data.

For detection by interference optics:

The minimum loading concentration is c. 0.05 mg/ml. Concentrations around 1 mg/ml are typical, but up to and above 10 mg/ml may be detected as the optics have unlimited linearity, but solution non-ideality usually becomes an issue at very high concentrations.

Buffer requirements:

- The sample and reference buffers must be exactly matched, ideally by dialysis, but flow-through from a concentrator can work well after exhaustive dilution/re-concentration.
- If working in the far-UV (e.g. 230 nm), avoid large amounts of DTT, β -mercaptoethanol, TRIS, HEPES and other absorbing components (e.g. use TCEP).
- No glycerol.

Other requirements:

- Bring/email accurate masses and the protein sequence including any tags/extra residues after cleavage *etc* in **text-only format**, and a PDB file if it exists.
- Bring/email the exact and complete composition of your buffer, as we will calculate the density and viscosity accurately in order to analyse the data.

You may like to view the cell assembly video

<http://www.jove.com/details.stp?ID=1530>

beforehand, just so that what we do looks a bit more familiar on the day.

Katherine Stott

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