ITC sample preparation guideline

All samples and buffers should be filtered by 0.2 µm filter and degassed

Buffers

-Buffers for macromolecule (M) and ligand (L) must be identical to minimize ΔH from dilution.

-If both M and L are large, dialyze simultaneously into the same buffer using separate dialysis vessels.

-If L is too small to be dialyzed, use the "dialysate" to dissolve solid ligand.

-Reducing agents: DTT is not recommended; substitute with β-mercapoethanol or TCEP (not compatible with phosphate buffer) if possible.

-To minimize artifactual heats, the buffer should have a low enthalpy of ionization (e.g.phosphate, citrate, acetate).

- If you are working with synthetic peptides or oligonucleotides, be sure they are desalted prior to suspension in ITC buffer. Residual chemicals from synthesis (e.g., TFA and salts) will cause a buffer mismatch and high heats of dilution.

- A difference in [DMSO] between M and L will yield large ΔH from dilution and obscure binding data. If you use DMSO to solubilize a ligand, you will need to add DMSO to the macromolecule solution to match the concentration of the ligand solution. Many proteins are stable in the short term in up to 2-5% DMSO.

-Add the DMSO to the protein solution immediately before running the ITC experiment

SAMPLES

- Sample volume (Auto-iTC200)

    - Sample cell (Macromolecule) → 430 µL per run;
    - Syringe (Ligand) → 150 µL per run

-Sample concentrations

Typical starting concentrations are 30-50 µM for the sample cell. Generally, the syringe concentration is 10x higher than the concentration in the sample cell; 7x higher for very tight binding and 10-15x (or more) for very weak binding.