Planning your isothermal titration calorimetry (ITC) experiment

Preparing the samples

- 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 same buffer using separate dialysis vessels
  - If L is too small to be dialyzed, use the "dialysate" (post-dialysis buffer) to dissolve solid ligand
    - The pH of both solutions should be checked in this case
- Reducing agents: DTT is not recommended;substitute with β-mercapoethanol or TCEP if possible
- If DMSO is required, either include it in buffer during dialysis *or* add it to M, L and buffer post-dialysis
  - A difference in [DMSO] between M and L will yield large ΔH from dilution and obscure binding data
- Measure concentration post-dialysis and pre-dilution when preparing sample for experiment
- For accurate dilutions, use analytical scale to measure mass of added volume instead of using a pipet
  - Tip: keep a large stock of concentrated M and L from which many ITC samples can be prepared via dilution with matched buffer (keep these stocks pure → only *take*, do not *add*)

Sample requirement guidelines

- (M) Macromolecule (in cell)
  - VP-ITC → 2.5 mL per run (cell holds 1.8 mL); iTC200 → 400 µL per run (cell holds 280 µL)
    - Extra volume facilitates loading and can be used later (concentration should be re-assessed)
  - [M] = 1-10×K_d
    - If K_d is unknown, aim for 10-100 µM
- (L) Ligand (in syringe)
  - VP-ITC → 700 µL per run (syringe holds 250 µL); iTC200 → 70 µL per run (syringe holds 40 µL)
  - [L] = 10-15×[M]
    - Use greater [L] as [M] is reduced closer to K_d (maximum [L] ≈ 15×[M])
- (B) Buffer matched to buffer in M and L via dialysis
  - 20 mL per run
  - Tip: save 1L of filtered buffer post-dialysis

Equipment required

- Solutions: M, L and Buffer
- Tube to hold M for loading: VP-ITC → 2.5+ mL tube; iTC200 → 1.5 mL tube
- Tube to hold L for loading: VP-ITC → 1.5 mL tube; iTC200 → 0.2 mL PCR tube
- Tube to hold M+L post-experiment: VP-ITC → 4 mL ITC tube; iTC200 → 1.5 mL tube
- Pipet and tips for sample transfer: 1000 µL and 200 µL
- Data can be transported via USB (Internet connection is not available)
- Lab notebook

Time required

- 30-60 min to set up each run (extra 60 min for training)
- 3-6 hours per run (it is automated, so this can be done in the absence of experimenter / overnight)

Multiple experiments required for maximum information content

- The first experiment will likely not be maximally informative – plan to repeat it
- Different temperatures (VP-ITC and iTC200 ranges 2-80º C)
  - Temperature can affect binding affinity ΔG, enthalpy ΔH, entropy ΔS and experimental *S/N* ratio
  - Change in heat capacity ΔC_p can be obtained via temperature-dependence of ΔH
  - Example: try 25ºC, then 15ºC then 35ºC
- Different c-values at each temperature
  - $c = n[M] / K_d$ where n is the number of binding sites per macromolecule M
  - This affects shape the shape of the thermogram
  - Varying the c-value is important to probe for multiple binding modes
  - Example (if K_d = 5 µM and n = 1)
    - c = 5 → [M] = $5K_d$ / n = (5)(5 µM) / 1 = 25 µM and [L] = 10[M] = 250 µM
    - c = 50 → [M] = $50K_d$ / n = (50)(5 µM) / 1 = 250 µM and [L] = 10[M] = 2500 µM