ITC Background

ITC – measures the heat of binding of the titrant (ligand in syringe) to the macromolecule in the sample cell. Determine n, K_D, ΔH, ΔS

K_D – Affinity
N – Stoichiometry
ΔH – Enthalpy (Heat of Reaction)
ΔS - Entropy

Y-axis – Power (ucal/sec) needed to keep the sample cell at the same temperature as the reference cell

Exothermic Rxn – spikes descend from baseline, (heat given off in sample cell), so less power is required to compensate the temperature differences

Endothermic Rxn – spikes rise from baseline, (heat absorbed in sampler cell) more power is required to compensate for temperature differences between sample and reference cells

Large initial peak due to ~100% binding, peaks decrease as less binding occurs

Plot kcal/mole of injectant (ligand) versus Molar Ratio

Minimizing Control Heat

Heat of mixing and heat of dilution
Buffer Mismatch – buffer of titrant and macromolecule must be as similar as possible
pH with +/- 0.05
Same ionic strength (salt concentration)

***Know concentrations** of ligand and macromolecules accurately

(Correct calculations (pay attention to units) and accuracy in using micropipettors to make solutions. Neena recommends not using smaller than 10uL pipetting since using smaller amounts requires more experience pipetting accurately) Do Serial Dilutions

Errors in **cell concentration**: affect n-value (stoichiometry)
Little affect on enthalpy
Mild affect on affinity

Errors in titrant concentration: affect n-value (stoichiometry)
Affects enthalpy
Mild affect on affinity

If get high K_D with CaEDTA, indicates some of the EDTA used up by residual metals complexing to it.
Cell may be dirty: Rinse with high concentration of EDTA to complex metals

ITC Helpful Instrument Info

CAUTION: Be very careful to never touch or bend the ITC pipettor/syringe. Even a slight bend will make it wobble and generate heat, making it unusable. Replacements cost $900.

Use: MICROCAL PEAQ-ITC SYSTEM Getting Started Booklet

After training do

Exercise 2: $CaCl_2$/EDTA titration experiment p. 10
Exercise 3: Control experiment p. 17
Exercise 4: Evaluation of Results p. 20
Exercise 5: Experimental Design p. 24

Use Software's step by step Load and Clean guide

Sample cell loading – use 500µL glass syringe with blunt plastic coated needle, pull up about 325µL sample into the syringe and remove bubbles, fill cell halfway then pulse few times and pull up on syringe while dispensing

ITC Syringe loading - Use micropipettor to load PCR tube with at least 75µL of titrant, use loading guide (syringe holds ~38uL MAX but need extra)

-For CaEDTA set reference power to 10.

System must stabilize to within +/-1 of reference power, if it doesn't, likely bubble in sample cell, stop run before any injections, empty and refill sample cell, restart

-For DNA and RNA samples, set reference power to 5 or what Neena says

***Run a Control**

File name should end in _ctrl
Ligand titrated into the buffer/all components except binding molecule
Or if expensive ligand, do a buffer/buffer titration as your control

For Control the y-axis very small changes, keep this in mind that it's okay to see baseline slant

After each run clean cell and syringe

If highly water soluble – cell and syringe Rinse is only needed in between runs
At end of day do cell and syringe wash
1x per month do a soak – Tanya does

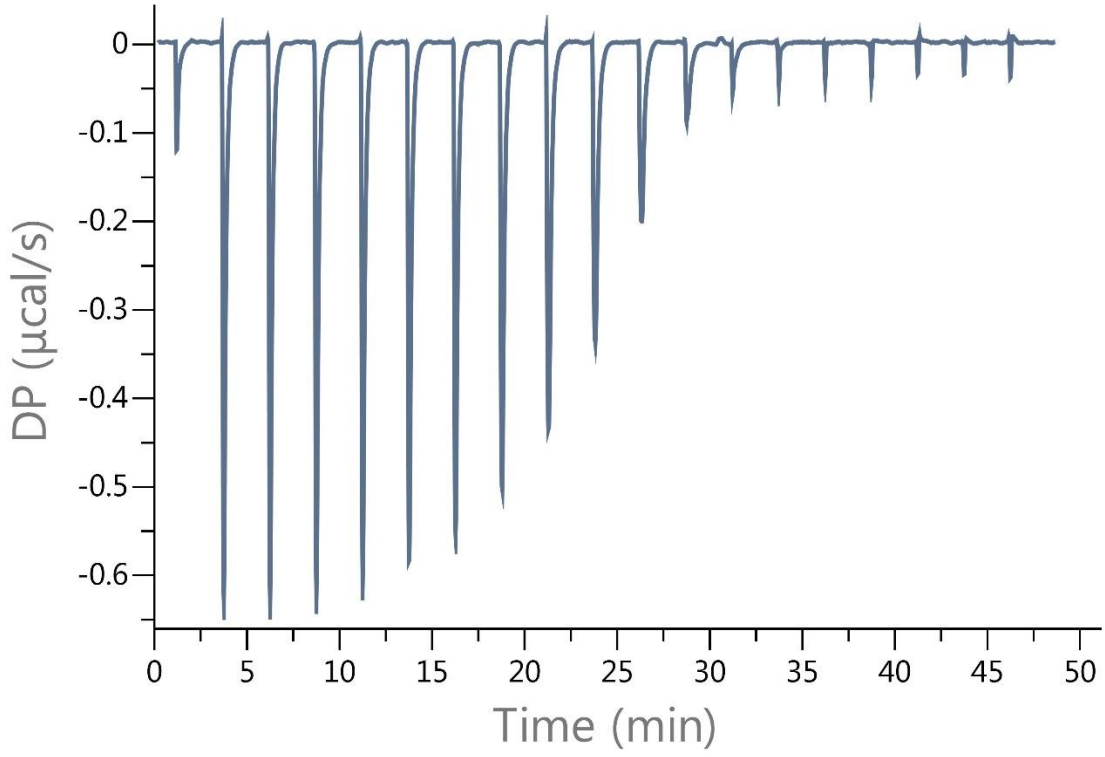
Check cleaning station bottle solution levels:

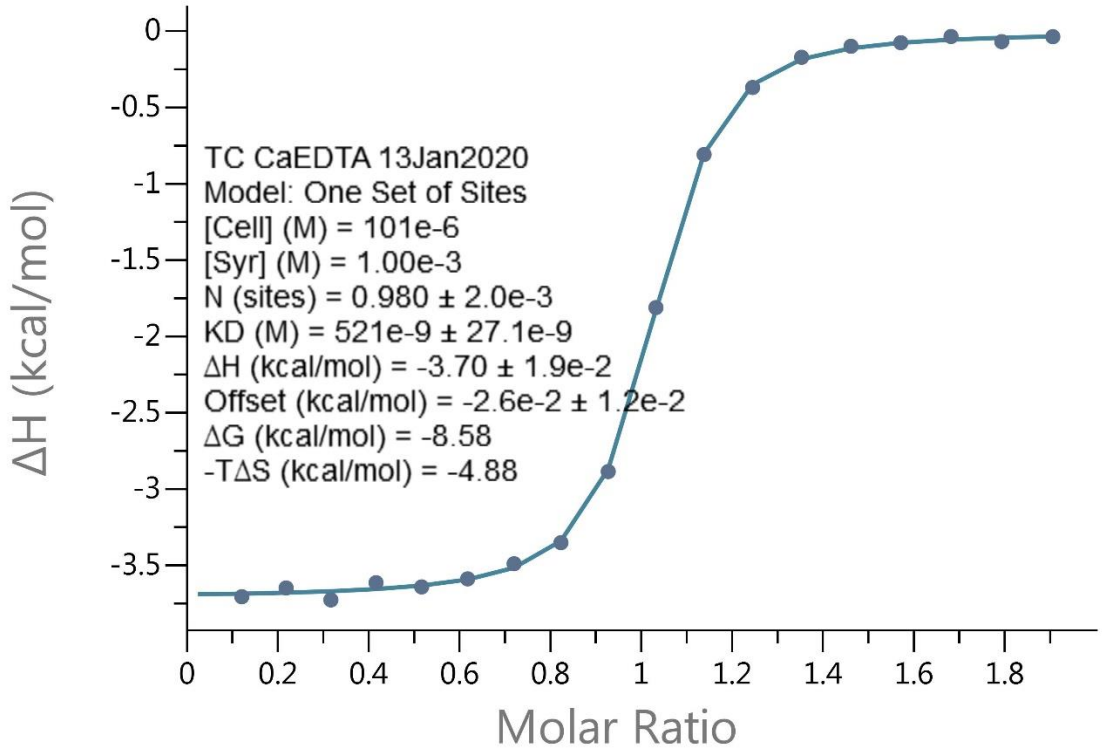
Methanol: Can absorb water from air over time. Use fresh weekly
Detergent: 20% Contrad 70 (make sure no crystals or floating solids)
Water: use MilliQ, change weekly
Waste Bottle: empty to labeled waste bottle

Always leave system with MilliQ water in Sample Cell – For CH383 the Ref Cell has been filled and should not be changed.

Microcal PEAQ-ITC User Manual for sample prep, determining concentrations to use, Troubleshooting p. 97 shows examples

Example ITC - CaEDTA ITC – No control





CaEDTA ITC – with Ca-MES Buffer Control substracted