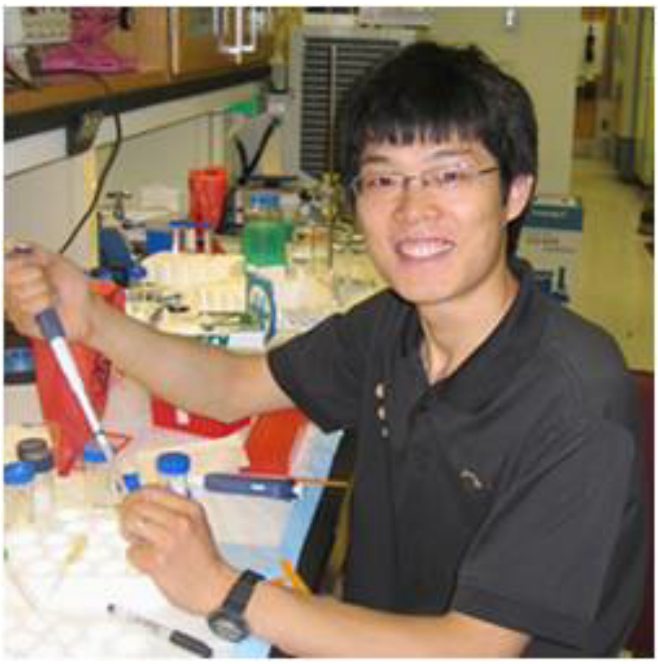
ITC: An Empirical Approach

By Michael Clark

Dedicated to the Most Fearless, Methodical, Legendary ITC Practitioner Ever – Dr. Tao Xie



Guest Editor: Magic Zmyslowski

1. Determine the ITC cleaning schedule. For example, previously the instrument was cleaned every Monday morning. If at all possible plan to do your experiments immediately after Keck staff clean the instrument.
2. Sample Preparation:
    a. Aim to generate two samples. Your first protein should go in the cell, a reasonable concentration is 30-50 μM. Your second protein (usually the smaller, more soluble protein or peptide) will be your titrant, and should be 10 fold higher in concentration.
    b. Match all sample buffers. Dialysis is best (For proteins that are larger than the MW cutoff values). Use Slide-a-lyzers to dialyze volumes too small for snakeskin dialysis tubing (<5 mL). Typical dialysis protocol consists of two steps into 4 L 50 mM sodium phosphate buffer. Add salt to dialysis buffer only if necessary for protein stability. Select a buffer with a low enthalpy of ionization to minimize the effect of any pH mismatches. Phosphate works well. Others have even gotten data with Tris buffers (although this is not recommended).
    c. If you have an HPLC-purified peptide that is too small for dialysis, resuspend lyophilized powder in HPLC buffer C (50/50 H_2O/ACN), freeze and lyophilize.

This will remove residual TFA. Repeat this process again once (repeat twice if paranoid).

d. Measure sample concentrations to high accuracy post-dialysis.
e. Twice sterile filter and degas 50 mL of your buffer (from the 2nd 4 L dialysis). Use this buffer to make the appropriate sample dilutions. If the samples are too dilute, you can concentrate them with a fully equilibrated table top centrifuge concentration unit. This however will create slight buffer mismatches.

3. Log into NUCORE and turn on the instrument.
4. Begin cleaning protocol. This is the single most important part of ITC. If in doubt, CLEAN AGAIN. If not in doubt/ you are confident that you sufficiently cleaned the instrument, CLEAN AGAIN.
    a. Set jacket temp to your run temp. (You should give this parameter careful thought. Most people use 25 °C. If worried about protein stability, use 20 °C. If you want maximum entropy contribution (gains), use 37 °C.
    b. Make sure all wash reservoirs are filled before beginning.
    c. Use **Cell and Syringe Wash**
        i. Place wash tool in cell
        ii. Put syringe in dry port. Ensure plastic tube is connected to the side of syringe. NEVER OVER-TIGHTEN tubing
    d. Wash 500 µL manual syringe with one pull of contrad detergent, 20 pulls of water.
    e. Remove wash tool from cell and use manual syringe to remove residual water ~40 µL, from the cell. Cell should be completely dry
    f. Equilibrate manual syringe with your buffer (10 pulls)
    g. Fill cell with you buffer using manual syringe. Remove and repeat. Incubate with buffer for 5 min.
    h. Inspect automatic syringe. It should be completely dry. If any liquid is seen, use **Syringe Dry**.
    i.
5. Fill the automatic syringe with your titratant:
    a. Put 100 µL of titrant in PCR tube and place in tube holder.
    b. Use **Syringe Fill**
        i. Put syringe in rest position (not in sample!) – syringe will expel air
        ii. Place syringe in titrant. Ensure that syringe fills completely.
            1. If not, use **down** command to expel 40 µl from autosyringe
            2. Try tightening syringe tubing (although not too tight)
    c. Once syringe is filled successfully, remove from fill port, detach the tubing and wash outside of syringe with 300 µl of buffer. Then blot dry with kimwipe.
6. Fill cell with your protein. DO NOT MAKE BUBBLES
    a. Remove buffer that you put in cell for equilibration purposes with manual syringe

b. Fill manual syringe with 350 µl of sample.
c. Put manual syringe in cell and inject sample until you start to see sample coming out of the top. At this point, slowly pull out syringe while still injecting slowly.

7. Suck out extra protein around the rim of the cell. There should not be more than 100 µl left in the manual syringe. If there is extra volume left, there may be a bubble in the cell (Which will require you to refill the cell).
8. Place autosyringe into sample cell.
9. Set Run Parameters
    a. If you have no idea of the binding affinity, do a scouting run (~10 points). If K_D is high, you may have to increase sample concentration. Ideal cell sample concentration is 50-100 times greater than K_D value. Mortals will attempt cell concentrations 10 times the K_D.
10. Reasonable run parameters are below, and are initialized from the **Advanced Experimental Design** tab
    a. 24 total injections
    b. Cell temperature (same as you set jacket temperature)
    c. Reference power = 8
    d. Initial delay = 300 sec (ensuring the flattest of baselines)
    e. Set syringe and cell concentrations
        i. This is important! The concentrations listed will be irreversibly saved in the data file. They cannot be edited, and will have to be manually changed each time in Origin which is annoying.
    f. Stirring speed = 1000 RPM
    g. Feedback mode = high
    h. Set Injection profiles:
        i. First injection is usually bad, set the volume of this to be 0.6 µl, and check **unique** box
        ii. The rest of the injections should be good. Set:
            1. Volume = 1.6 µl
            2. Duration 3.2 sec
            3. Spacing 150 sec
            4. Filter period = 4 sec
    i. Set file name (on **Experimental Design** tab)
    j. Set data storage path (on right-most tab) to your folder
11. Start Run. After this point, just sit there.
    a. Temp should stabilize before baseline equilibration period. If it doesn't, stop run and ask staff for help.
    b. Baseline equilibration should bring the dP value within 10% of your set reference power. For a reference power of 8, a dP value of less than 7.2 is unacceptable.

c. OK data can be collected with aberrant dP values but it is unlikely. Best practice is to refill the cell