Biophysical
Instrumentation
Facility

MICROCAL ITC notes

Instrument settings:

For quicker injections, sharper peaks, and less diffusion, set the injection duration to be half what the injection volume number is. You can override the automatic setting.

For exothermic reactions, set the reference power to > 15. (Keep it at 5 for water/water baseline work.)

If binding is very weak, make the ligand concentration >>> than the protein concentration to drive the equilibrium toward binding.

To force a flatter baseline, stop the run then start it again quickly. This thermally shocks the cells. For flatter baselines in general, start thermostatting the instrument the night before.

Phosphate and Tris buffers take longer to passivate (oxidize) the inside of the chambers. If using these buffers, put some buffer in the sample cell and let it sit overnight.

When setting the Spacing parameter (between injections), the number set for injection 1 is the spacing between it and inj. 2. Spacing set for inj. 2 is for between inj. 2 and 3 (or the end of the experiment if you want the ITC to hold a constant temp. for a long time as in an enzyme kinetics study).

If the tips of peaks are getting cut off, increase the Reference Power.

The reference power range varies with different feedback modes. "None" for feedback mode gives a narrower range than "High". For most work, use “High”. If “Low” is used, the range is only 1-11 and the ITC won’t do 10 well since it’s at the end of the range.

DP should be somewhere near the Reference Power value while things are equilibrating. See page 22 of the ITC manual for an explanation of what the Reference Power is.

If no Equilibration Mode is chosen, you’ll have to move things along manually. When the DP value is green, double click on those numbers to move the ITC along.

Temperature issues:

The temperature range is 2 - 80° but you want to cool things down below 50° before emptying the cells.

Some users like to start with the reference and sample cells much cooler than the run temp. (set at 10° for a 25° run) Chill your solutions to maybe 6.5° before loading since they'll warm up during loading.

If the ITC asks if you want it to passively cool the cells, be aware that saying Yes will send it to 10° below your set point before it reheats. This takes ages, so keep your eye on it and when it's 0.1º below the desired set temp., click on the Set Jacket Temp. button again. Once you've begun a titration, you can't interrupt passive cooling so get the temperature correct before you begin.

If during a titration the ITC gets near the set temperature and keeps flipping from cooling to heating and back again, just leave it alone and it will eventually progress by itself. Or you can stop the run, go back to the Thermostat page, re-enter the same temperature and click the Set Jacket Temp. button.

To check for the heat of dilution in your system, do the controls of injecting buffer into protein and ligand into buffer.

Cell preparation/sensitivity:

Before a run, rinse the whole system with the buffer from the protein and ligand solutions so there will not be any spurious heat of dilution. Ideally, the protein and ligand will have been dialyzed against their buffer overnight.

The safe pH range for the ITC cells is 2 – 12. Outside that range, damage will occur.

Data:

Even if your largest peak is only 0.2 uCal/min, the software will fit the curve as long as the peaks get smaller over time.

A protein concentration at least 10X the Kd is needed to get a sigmoidal binding curve.

If the K_b makes sense but the N doesn't, check the concentration of the actual solutions you used. A small change in concentration will have a large effect on the N value.

Cleaning:

Blowing N_2 through the injector to dry the methanol is NOT okay. Positive pressure will ruin the injector needle. Draw air through it either by hand or with the vacuum drying setup to dry it out.

SDS isn't good for routine cleaning because it's hard to rinse out. If you need to use SDS, 20% ethanol is good to use for rinsing.

If there are metal ions in your buffer, clean the cells with EDTA at the end of your run. See Debby for the protocol.

Printing the experiment parameters window and graphs:
You can print the experiment parameters window by pressing CTRL, Print Screen and putting that into Word. Then enlarge it and print it on the nearby Samsung printer.

Exporting graphs as .TIFF files gives great resolution.

Enzyme kinetics work:
To get your pre-injection baseline, uncheck Auto Equilibration and let the instrument run for 30 minutes (or whatever you've previously determined is right for your system) then tell the software when to start the injections.

Use Origin 7.0 for analyzing data. When you Read Data, load the data as an enzyme assay, still using a .itc filename. Use Method 1 (single injection) to get ΔH then do Method 2 (multi-injections) and plug in that ΔH.

Miscellany:
The ThermoVac pulls 28" Hg vacuum.

The units for ITC parameters are listed on page 14 of the Origin 7 manual.

Water/water injections give tiny peaks because the temperature in the syringe is a little different from the temperature in the cell.

The lowerst Kd the ITC can accurately report is 10 nM. (tight binding)

Troubleshooting:
Final Baseline Equilibration taking more than overnight is caused by either a bent syringe or a bubble in the sample chamber.

If the temperature is holding 1° higher than the set temperature, you probably put the syringe in after starting the run. This can be prevented by putting the syringe in first.

If the injection trace swings back above the baseline, check that the reference cell is completely full of water. If the trace doesn't go back up to the baseline, change the Feedback Mode to High.

If the DP = 0.000, you probably aren't connected right. Check the constants window. If these are okay, check DP response by putting hot water in the sample cell.

If the automatic washer gives a lot of bubbles, change both its inner and outer O-rings.

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