

## MICROCAL Analyzing ITC Data

### Routine Data Analysis

1. Click on the MicroCal Analysis Launcher icon. Double click on VP-ITC and this will open Origin to the RawITC window.
2. Click the Read Data button and choose your file. This opens the DeltaH window. The software will automatically set the baselines and integration ranges for you. If you have very small injection peaks and need more accurately set integration details, return to the RawITC window under Window and click on the Adjust Integrations button. Then follow the instructions in Lesson 2 (pp. 21-25) in the Origin 7.0 manual.
3. If there are any obviously bad points, you can remove them by clicking on the Remove Bad Data button. Move the cross-hairs over the bad point, click on it and hit Enter. (You can also just double-click on the bad point.) To recover a removed point, click on the Concentration button and then OK on that window.
4. Save the DeltaH data by pulling down from Window to DeltaH and Save Area Data. Choose a folder to put the .DH file in.
5. Click on the Concentration button and edit the info there if necessary.
6. Choose a model from the Model Fitting set of buttons. Clicking on One Set of Sites will give you a first fit line in red and N, K, and H values. Click on the 1 iter. or 100 iter. button until the  $\text{Chi}^2$  value stops decreasing, then click Done.
7. Move the text box manually to include it on the delta H graph. To edit its text, right click anywhere in it and select Properties. You can print this graph out, saving it as a .OPJ file.
8. To create a final figure with both the raw data and the delta H curve, pull down from the main ITC menu on the toolbar and choose Final Figure. The raw data will have had its baseline subtracted. (To get your original raw data again, you have to start over from the .ITC file.) To remove the line at  $Y=0$  from the raw data graph, see page 18 of the Origin 7.0 manual. You can copy and paste the fitting parameters text box from the original delta H plot into this version and change its size by right clicking on it.
9. Save the final figure by choosing Save File As from the File menu. You can make a PDF of it using the Cute PDF Writer option under Print.

10. To see your raw data plotted again, go to Window, Raw ITC.

### **Subtracting a Reference Run**

You can do this in one of two ways: 1) with the .DH files (pp. 29-36) or 2) with the raw .ITC files (pp. 36-42).

1) For straightforward data:

- Open both .NDH files (p. 29)
- Put them on the same graph through the Layer 1 icon (axes will rescale) (p. 31)
- Adjust molar ratio so both lines will plot in register (p. 33)
- Subtract them using the Subtract Reference Data button in the DeltaH window (p. 34) (to undo that, add the buffer data set back to the sample data set with the Simple Math function)
- Save it (p. 35)

2) If sample and reference data don't match precisely (no. of injections or spacing):

- Open both .ITC files (p. 37)
- Put both on the same graph through the Layer 1 icon (p. 37)
- Make the ligand concentration the same for both (pp. 37-38)
- There are two ways to deal with having a different number of points:
  - Subtract a constant (p. 38)
    - Use the Data Reader tool to read the Y values for the buffer line and estimate an average
    - Use Simple Math from the Math menu and subtract the average value from the sample data set using the math box (pp. 38-39)
  - Subtract a straight line from the sample data (p. 39)
    - Choose the buffer data set
    - Do Linear Regression on it from the Math menu
    - Use Simple Math and subtract the linear fit from the sample data set using the math box (pp. 39-41)
    - Plot them on the same graph (pp. 41-42)

### **Plotting Multiple Data Sets**

You can plot multiple runs on top of each other by displacing them. See p. 36 of manual.

### **Handling Worksheet Data**

**Open**

- Select File, New, Project: the RawITC window opens
- Click on Read Data: the Open dialog window opens
- Find your file wherever it is, then click OK.
- Go to Format and choose Plot...
- Click on the Worksheet button at the bottom: the worksheet opens
- Note: if there are ###'s in a cell, the column is not wide enough for the resident number, so just widen the column at the column's header.

### **Copy/Paste**

- You can copy cells, rows, columns or fields from the worksheet through the clipboard to a new worksheet or another Windows application using standard copy/paste commands.
- If you get ###'s in a cell, widen the column as above.

### **Export to ASCII file**

- From the RawITC window, choose Data and click the appropriate filename at the bottom.
- Go to File, Export to ASCII.
- Select the filename, then click Save.
- You'll have formatting options, then click OK.

### **Import ASCII file**

- To import an ASCII file to a worksheet or plot window, see p. 48 of the Origin 7.0 manual.

### **Modifying Templates**

- You can modify the DeltaH or RawITC window templates to your own specifications. Options are labeling the right Y axis or changing the scale of the units on either axis (to time in minutes or cal/mole of injectant, for instance). (pp. 49-54)
- If you make changes to the template and save them, those changes will be permanent. You can easily revert to the original template by reversing the steps you took to change it. (p. 52)

### **Advanced Curve Fitting**

In the Origin 7.0 manual, there are detailed instructions for:

**Fitting with the Two Sets of Sites Model** (pp. 55-58)

**NonLinear Least Squares Curve-Fitting Session** (pp. 58-60)

**Deconvolution with Ligand in Cell and Macromolecule in the Syringe** (pp. 60-62)

**Deconvolution with the Sequential Binding Sites Model** (pp. 63-66)

### **Enzyme/substrate/inhibitor Assays**

## **METHOD 1**

### Substrate only

With enzyme (or substrate) in the cell, a single large injection of substrate (or enzyme) is carried out and the resulting decay curve is analyzed. The Michaelis parameters  $K_m$ ,  $k_{cat}$ , and  $\Delta H$  can be determined.

### Substrate plus inhibitor

This method can also be used a second time with a competitive inhibitor in the enzyme so that the Michaelis inhibitor constant,  $K_i$ , can be determined.

## **METHOD 2 (depends on a $\Delta H$ previously determined by a Method 1 single injection)**

### Substrate only

Enzyme solution is in the cell but at a lower concentration than in Method 1. A number of injections are carried out with time allowed for the baseline to equilibrate after each one. A plot of reaction rate vs. total substrate concentration is constructed and the Michaelis parameters  $K_m$ ,  $k_{cat}$ , and  $\Delta H$  can be determined.

### Substrate plus inhibitor

This method can also be used a second time with a competitive inhibitor in the enzyme so that the Michaelis inhibitor constant,  $K_i$ , can be determined.

## **Dimer Dissociation Model**

There is a procedure for analyzing the heat of dilution of a compound (in the syringe) which has a tendency to form dimers. Multiple injections are made from the syringe into buffer and the resulting heats are analyzed to determine the dissociation constant  $K$  and the heat of dissociation  $\Delta H$ . See pp. 75-77 of the Origin 7.0 manual.

## **Competitive Ligand Binding**

To do a competitive binding experiment injecting a strong-binding ligand A into a solution which contains both the macromolecule and the competing ligand B, an initial non-competitive experiment must first be carried out in the conventional way to determine the binding parameters for ligand B itself:  $N_B$ ,  $K_B$ , and  $\Delta H_B$ . With those in hand, the competitive experiment can be designed optimally (p. 77) and analyzed to determine  $N_A$ ,  $K_A$ , and  $\Delta H_A$  (pp. 78-79).

## **Simulating Curves**

There are instructions (pp. 80-81) for simulating fit data even from unrelated raw data.

## **Using Macromolecule Concentration, rather than $n$ , as a Fitting Parameter**

If you know the stoichiometric parameter  $n$  from other studies but can't come up with an accurate estimate of macromolecular concentration, there's a way to calculate that concentration. See p. 82 of the manual.

### **Other Useful Details**

There are more detailed explanations of the following:

**Chi-square ( $\chi^2$ ) Minimization** (p. 100)

**Line Types for Fit Curves** (pp. 101-102)

**Inserting an Origin Graph into Microsoft Word** (pp. 102-103)

**Calculating a Mean Value for Reference Data** (p. 102)

### **Appendix: Equations Used for Fitting ITC Data**

In the Appendix, a general explanation of how cell volume and concentrations are changed with each injection is given, as well as all the equations used to fit the various models of binding.

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