

Using Regression to Extract Thermodynamic Information from ITC Data

- **Conceptual goal:** Understand how nonlinear regression works and can be used to extract biophysical parameters from experiments.
- **Skill goal:** Improved spreadsheet skills; ability to analyze data by regression.

Mechanics

1. Open the *itc-fitting.xlsx* spreadsheet in Excel.
2. Make sure you have “Solver” installed in Excel. (It should be at the bottom of the “Tools” menu). If it is not there, instructions for installing are here <https://tinyurl.com/ydehj42m>.
3. Create a plot of “injection” versus “observed heat.”
4. Fill in the $[MX]/[M]_T$ column with calculated values for $[MX]/[M]_T$ given $[X]_T$, $[M]_T$, and K_D . Have the formula point to the K_D cell in the spreadsheet (indicated in red). Remember:

$$[MX] = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

where

$$a = 1$$

$$b = -(K_D + [M]_T + [X]_T)$$

$$c = [X]_T[M]_T$$

5. Fill in the **calculated heat** column. The heat evolved at injection i depends on the change in the fractional saturation of the reaction and the enthalpy of the reaction. For the enthalpy, point to the ΔH cell (indicated in blue).

$$Q_i = \Delta H \left[\left(\frac{[MX]}{[M]_T} \right)_i - \left(\frac{[MX]}{[M]_T} \right)_{i-1} \right]$$

6. Plot the values in calculated heat versus injection on the same plot as the experimental plot. Change the values in the K_D and ΔH columns to try to match to the data. What values do you find?
7. Fill in **square residuals** column. For each injection, calculate:

$$(Q_{calc} - Q_{obs})^2$$

8. Create a cell holding the sum of the square residuals column.

9. Go to *Tools* → *Solver*.
 - (a) Point the **Set Objective** cell to point to the sum of square residuals that you calculated.
 - (b) Point the **By Changing Variable Cells** cell to point to the K_D and ΔH cells.
 - (c) Make sure that **Make Unconstrained Variables Non-Negative** is unchecked. (If it's not, Excel will helpfully never allow a negative value in the solver.)
 - (d) Select “GRG Nonlinear” for the **Solving Method**
 - (e) Hit “Solve”
10. What cells changed? What is the estimate of K_D and ΔH from this analysis?

Thinking

1. How precise are these estimates? How might you estimate this?
2. How accurate are these estimates? How might you estimate this?
3. What if your protein has two sites and binds two ligands with slight different affinities? Would you be able to tell from these data?
4. The “Replicates” tab has three more columns of experimentally observed heats. Do these provide similar estimates for K_D and ΔH ? (And just how do you define similar?)