

Biocalorimetry

One important task of biophysics is to study interactions between different biomolecules. These interactions can be approached from different sides: structural, thermodynamic, kinetic.

Calorimetry is a method of choice when one wants to determine thermodynamic parameters (ΔG , ΔH , ΔS , etc.) as well as binding constants (K_b) and stoichiometry of the interacting biomolecules. There are two different modes of calorimetry: differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC). The first one can be used to study order–disorder transitions, e.g. protein unfolding or nucleic acid melting. The latter can be used to study ligand–macromolecule or macromolecule–macromolecule binding interactions.

ITC allows directly and without the need of a predetermined model measures the enthalpy change for a bimolecular binding interaction at a constant temperature. This methodology relies upon a differential cell system within the calorimeter assembly. For a qualitative description, refer to the video behind the QR code.



The reference cell contains only water or buffer, while the sample cell contains e.g. a macromolecule receptor. Injection of a ligand into the sample cell results in interactions between the receptor and ligand. Heat (q_i) corresponding to the binding energy ($\Delta G = \Delta H - T\Delta S$) is liberated into the solution.

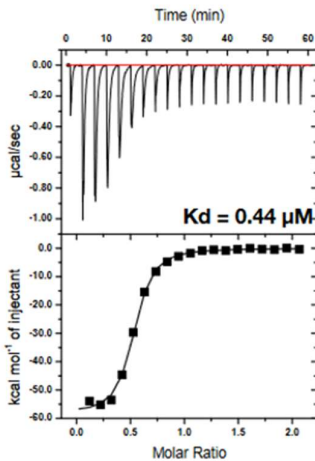


Figure 1

The amount of power that must be applied to actively compensate for the heat produced in the sample cell, after an injection i of ligand, is measured directly and corresponds to:

$$q_i = \Delta H_{app} \cdot V_C ([RL]_{b,i} - [RL]_{b,i-1})$$

with V_C being the volume of the cell, and $[RL]_{b,i}$ being the concentration of ligand-receptor complex after injection i .

This gives a downward peak in Figure 1, top panel. The individual peaks are further integrated and plotted as a function of molar ratio between the ligand and receptor (Figure 1, bottom panel) and fitted to a binding isotherm, e.g. for multisite binding:

$$f = \frac{n[L]}{[L] + K_d} = \frac{[RL]}{[R_{tot}]}$$

The amount heat released after each injection is then given by:

$$q_i = \Delta H_{app} \cdot V_C (f_i [R_{tot}] - f_{i-1} [R_{tot}])$$

with $[R_{tot}]$ being the total receptor concentration. The total heat is then given by:

$$Q = \sum_{i=1}^N q_i = \Delta H_{app} \cdot V_C \cdot [RL] = \Delta H_{app} \cdot V_C \cdot [R_{tot}] \cdot f$$

Note, that this is a model dependent analysis, relying on the type of binding isotherm which is chosen. The equation can vary depending on the ligand–macromolecule interaction stoichiometry and binding mode.

Exercises:

- 1) Consider the following ITC experiment: poly(U)₃₀ oligonucleotide was titrated into a solution containing a protein complex Nmd4/Upf1 (Upf1 is a RNA helicase protein). The resulting ITC curve is shown in Fig. 1.
 - a) From the figure, determine the binding stoichiometry between poly(U)₃₀ and Nmd4/Upf1?
 - b) Is this interaction exo- or endothermic? Estimate ΔH .
 - c) Write down a chemical equilibrium scheme for the interacting molecules and the equation for K_d .
- 2) For a binding reaction with stoichiometry 1:1, it can be shown that

$$\frac{1}{V_C} \frac{dQ}{d[L_{tot}]} = \Delta H_{app} \left(\frac{1}{2} + \frac{1 - \frac{1+r}{2} - \frac{X_r}{2}}{\sqrt{X_r^2 - 2X_r(1-r) + (1+r)^2}} \right) \quad (1)$$

where L_{tot} is the total ligand concentration, free plus bound, in the reaction cell of volume V_C , Q is the heat absorbed or evolved, and ΔH_{app} is the apparent molar enthalpy of binding. X_r and r are two unitless parameters that depend on the binding constant K_b , total ligand concentration (L_{tot}) and the total receptor concentration (R_{tot}):

$$1/r = c = R_{tot}K_b \quad (2)$$

$$X_r = L_{tot}/R_{tot} \quad (3)$$

Show that equation 1 holds true following the steps:

- a) For the equilibrium $L + R = RL$, write the equations for binding constant K_b and total concentrations of the macromolecule and ligand, $[R_{tot}]$, and $[L_{tot}]$ (*in terms of $[R]$, $[L]$ and $[RL]$, NOT X_r and $1/r$*).
- b) Combine the three equations from a) into one, containing a single unknown variable— $[RL]$. You should get a quadratic equation.
- c) Find the real root for this equation. Now you have an expression for $[RL]$.
- 3) The change in RL concentration after each injection can be related to the heat change as $dQ = d[RL] \cdot \Delta H_{app} \cdot V_C$, where ΔH_{app} is the molar enthalpy of binding and V_C is the cell volume. Now, what is the missing step to obtain eq. 1?

- 4) In another experiment, Nmd4 protein was titrated into the cell containing solution of Upf1 protein. Raw data output for the titration is shown in Figure 2. The concentration of Upf1 in the cell was 35 μM . The concentration of Nmd4 in the syringe was 260 μM . After the first small injection of 0.33 μL (to remove a potential air bubble from the syringe), 19 injections of 2 μL of Nmd4 were added to Upf1 at intervals of 180 s. The cell volume is $V_0 = 150 \mu\text{L}$. Assume that the titration was done at 25 $^{\circ}\text{C}$.

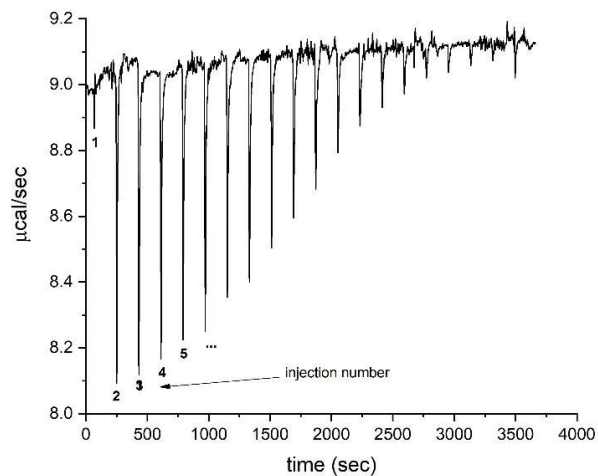


Figure 2

- a) Calculate the molar ratio between Nmd4 and Upf1 after each injection without considering the dilution. *Ignore the dilution factor.* For this and following questions you can use Excel Sheets (Google sheets).

The cell in which titration takes place has the shape of a lollipop with a cylindric communication tube attached to it (see Figure 3). At the beginning of an experiment, both the cell and the communication tube are filled with macromolecule solution, but only volume of the lollipop V_0 is sensed calorimetrically. Because of the total-fill nature of the cell, each injection acts to drive liquid out of the working volume and up into the inactive communication tube as shown by the darkened portion representing ΔV in Fig. 3. Thus, with each injection of the titrant, small dilution of the molecules happens.

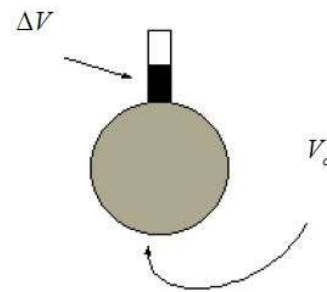


Figure 3

- b) Derive equations to calculate new concentrations of the proteins after each injection to take the dilution into consideration. Recalculate the molar ratio between Nmd4 and Upf1 after each injection. *Hint: consider that dilution happens linearly and the molecule concentration in the excluded volume can be calculated as $0.5 \cdot (C_{i-1} + C_i)$, where C_{i-1} is the known molecule concentration in the cell before the injection, and C_i — new and unknown molecule concentration after the injection.*

After integrating the raw data from Figure 2, normalizing it by the cell volume and subtracting the heat of dilution of the titrant (which was determined from the peaks measured after full saturation of the target by the titrant), the following dataset was obtained:

Injection #	$V_0 \cdot \Delta Q$, kcal/mol	Injection #	$V_0 \cdot \Delta Q$, kcal/mol
1	—	11	-7.24
2	-13.84	12	-5.18
3	-13.63	13	-4.27
4	-13.15	14	-3.11
5	-12.60	15	-2.44

Injection #	$V_0 \cdot \Delta Q$, kcal/mol	Injection #	$V_0 \cdot \Delta Q$, kcal/mol
6	-12.34	16	-1.65
7	-11.90	17	-1.40
8	-10.76	18	-1.09
9	-9.97	19	-1.06
10	-8.70	20	—

- c) Plot these data as a function of Nmd4 and Upf1 ratio obtained from b (or a). Using equation (1) and Excel Solver add-on, generate a curve to fit the data points. What are the calculated values for the binding constant, K_b , and enthalpy ΔH° ?

Hint: how to use Excel Solver add-on can be learned from this short video (scan the QR-code):



- d) Also, calculate ΔG° and ΔS° of the interaction between Nmd4 and Upf1.
- 5) As you saw, from a single ITC experiment one can extract a lot of thermodynamic parameters. Using these energetic signatures, it is often possible to determine the energetic source of the interaction, e.g. whether the binding is largely due to hydrogen bonds or hydrophobic interactions or whether conformational changes take place upon binding. These data can be, for example, useful in the drug discovery process in quantitative SAR (QSAR) to characterize potential drugs interactions with their targets and optimize their structure. Figure 4 shows a schematic representation of a range of thermodynamically distinct interactions all with a binding free energy, ΔG , of about 40 kJ/mol.

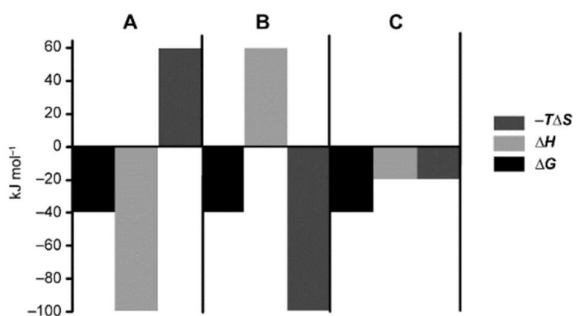


Figure 4

Comment on each of the energy profiles A–C, regarding the contribution of hydrogen bonds/hydrophobic interactions to the binding.

- 6) Another distinct mode of calorimetry is differential scanning calorimetry (DSC). Based on literature search, how DSC is different from ITC? In a few sentences, describe how DSC experiments are performed, what is the read-out of the measurements, and what thermodynamic parameters can be obtained by DSC.
- 7) Elaborate on possible disadvantages of ITC for studying interactions between biomolecules. What are other alternative/supplementary techniques to study biomolecular Interaction?