



Thermodynamics and Calorimetry

Lecture 2 Methods in Molecular Biophysics C1-C3 Intermolecular and Surface Forces 2

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Outline

We start with a brief summary of some important concepts in thermodynamics, with emphasis on biomolecular systems.

Calorimetry techniques are then explained. The two most important ones are differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC).

Everything is based on equilibrium thermodynamics here. Kinetics of biomolecular interactions come in the next lecture!

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Basic Thermodynamics

The first law (conservation of energy) says the internal energy U of a system changes as:

$$dU = dq - dw$$

Here q is the heat supplied to the system and w the work performed by the system (note signs). Usually $dw = PdV$ (mechanical work) but it can also be related to other things like addition or removal of matter (chemical potential)!

Enthalpy definition:

$$H = U + PV$$

Entropy (thermodynamics) for reversible process:

$$dS = \frac{dq}{T}$$

The second law (in the most common formulation) says that the total entropy change (a system and its surroundings) always increases:

$$dS_{\text{tot}} = dS_{\text{sys}} + dS_{\text{sur}} \geq 0$$

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Gibbs Free Energy

The definition of *Gibbs* free energy is:

$$G = U + PV - TS = H - TS$$

The differential becomes:

$$dG = dU + d(PV) - d(TS) = dU + PdV + VdP - TdS - SdT = dq - dw + PdV + VdP - TdS - SdT = TdS - PdV + PdV + VdP - TdS - SdT = \cancel{VdP} - SdT$$

We see that if P is constant V does not influence G .

Gibbs free energy ignores mechanical work which is good for chemistry. It is especially useful in biological systems!

Stating Gibbs free energy means experiments were performed at constant pressure!

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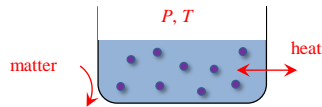
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Free Energy Minimization

“The first law says something about how things must happen while the second law explains why things do happen.”

We work under the assumption that the free energy of an *isothermal* and *closed* system (exchanges heat perfectly but never matter with the environment) will strive towards a minimum.



A system at constant T and P with strive towards a minimum in G .

Why???

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Gibbs Energy Minimization

Using the first law we can see that:

$$dU = dq - dw = TdS - PdV$$

$$dU + d(PV) = dq - PdV + d(PV)$$

$$d(U + PV) = dH = dq - PdV + VdP + PdV = dq + VdP$$

First we see that if the system is kept at constant P the enthalpy change is equal to the heat transferred to the surroundings:

$$dS_{\text{sur}} = -\frac{dH}{T}$$

The second law then gives:

$$dS_{\text{tot}} = dS_{\text{sys}} - \frac{dH}{T} = -\frac{dG}{T} \geq 0 \Rightarrow dG = dH - TdS_{\text{sys}} \leq 0$$

So a process with negative ΔG is thermodynamically favorable!

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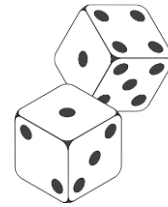


Entropy in Statistical Mechanics

Entropy is about probabilities and the number of *microstates* associated with a certain *macrostate*. The microstates are not observable! Entropy is lack of information.

Two dice have 36 microstates with equal probability. Entropy can be observed in the macrostate represented by the sum. Example: Probability of getting macrostate 7 with two dice is 1/6 (6 out of 36 microstates). The probabilities for getting 2 or 12 are only 1/36 each.

	1	2	3	4	5	6
1	2	3	4	5	6	7
2	3	4	5	6	7	8
3	4	5	6	7	8	9
4	5	6	7	8	9	10
5	6	7	8	9	10	11
6	7	8	9	10	11	12



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Boltzmann's Entropy Formula

Most general entropy formula:

$$S = -k_B \sum_{i=1}^n p_i \log(p_i)$$

The probability of microstate i is p_i . Boltzmann's constant $k_B = 1.3806 \times 10^{-23} \text{ JK}^{-1}$ relates entropy to free energy via temperature.

If all W microstates are equally probable $p = 1/W$ for all i and $n = W$. We can get the simpler formula:

$$S = -k_B \sum_{i=1}^W \frac{1}{W} \log\left(\frac{1}{W}\right) = k_B \sum_{i=1}^W \frac{1}{W} \log(W) = k_B \log(W)$$



Wikipedia: Ludwig Boltzmann

The logarithmic dependence essentially comes from combinatorics: If there are W_A states in system A and W_B states in system B the total number of states is $W_A W_B$, but entropy becomes additive: $S_A + S_B = k_B \log(W_A) + k_B \log(W_B) = k_B \log(W_A W_B)$

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Test: Entropy of Gender

Alice and Bob have two kids...

I: One is a boy.
p(the other is also a boy)?

I: The older is a boy.
p(the younger is also a boy)?

I: Nothing more
p(both are boys)?

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Boltzmann Statistics

N_i is number of entities occupying state i with energy G_i and N_i/N the probability that a given entity is at energy level i at any point in time. (The parameter g is *degeneracy* and we can set $g = 1$ here.) The ratio of the probabilities of occupying one state (B) compared to another (A) is then:

$$\frac{N_i}{N} = \frac{g_i \exp\left(-\frac{G_i}{k_B T}\right)}{\sum_i g_i \exp\left(-\frac{G_i}{k_B T}\right)}$$

$$\frac{N_B}{N_A} = \frac{\frac{\exp\left(-\frac{G_B}{k_B T}\right)}{\sum_i \exp\left(-\frac{G_i}{k_B T}\right)}}{\frac{\exp\left(-\frac{G_A}{k_B T}\right)}{\sum_i \exp\left(-\frac{G_i}{k_B T}\right)}} = \frac{\exp\left(-\frac{G_B}{k_B T}\right)}{\exp\left(-\frac{G_A}{k_B T}\right)} = \exp\left(-\frac{G_B}{k_B T} + \frac{G_A}{k_B T}\right) = \exp\left(-\frac{\Delta G}{k_B T}\right)$$

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Chemical Potential

The chemical potential μ is per definition the free energy required to introduce molecules into the system:

$$\mu = \left. \frac{\partial F}{\partial N} \right|_{T,V} = \left. \frac{\partial G}{\partial N} \right|_{T,P}$$

N is some measure of number of molecules. Boltzmann statistics makes it possible to relate concentration to chemical potential by thermodynamic *activity* a :

$$a = \gamma \frac{C}{C^\circ} = \exp\left(\frac{\mu - \mu^\circ}{k_B T}\right)$$

Here γ is the *activity coefficient* which depends on environmental factors (ideally $\gamma = 1$).

We have also introduced a standard chemical potential μ° which is the chemical potential at a standard state. (Typically $T = 25^\circ\text{C}$ and $P = 1$ bar.) Note that we must work with a dimensionless concentration (volume fraction, mole fraction) or by normalizing to a standard concentration C° .

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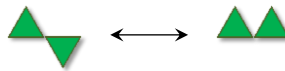
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Simple Equilibrium

Consider the very simple system where a molecule switches between two states:

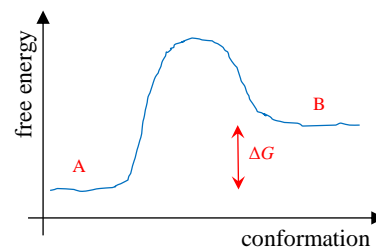


Boltzmann statistics gives the ratio between the probabilities of occupying these states. This must be identical to the concentration ratios. So we have an equilibrium constant:

$$K = \left. \frac{C_B}{C_A} \right|_{T \rightarrow \infty} = \exp\left(-\frac{\Delta G}{k_B T}\right)$$

Here K becomes dimensionless. We can solve for ΔG :

$$\Delta G = -k_B T \log(K)$$



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Simple Interaction

What about an interaction where we have:



The *law of mass action* says that the association reaction rate is $k_{\text{on}}C_A C_B$ and the dissociation rate is $k_{\text{off}}C_{AB}$ where k_{on} and k_{off} are constants.

At equilibrium the rates are equal (not zero). We can define an equilibrium constant by:

$$K = \frac{k_{\text{on}}}{k_{\text{off}}} = \frac{C_{AB}}{C_A C_B}$$

Model is not valid if there is more than one transition state (usually not a problem).

But k_{on} and k_{off} have different units and K is not dimensionless!

How can we now relate K to ΔG ?

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Arbitrary Reactions

Consider an arbitrary reversible reaction:



Here R means reactant (M different types) and P product ($N - M$ different types).

The stoichiometric coefficient (positive integer) is A_i for molecule of type i .

Example: $N_2 + 3H_2 \rightarrow 2NH_3$ has $M = 2$, $N = 3$, $A_1 = 1$, $A_2 = 3$ and $A_3 = 2$.

We now introduce the chemical potential to write the free energy change of one (forward) reaction step.

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Free Energy Change for One Reaction

$$\begin{aligned}
 \Delta G &= -\sum_{i=1}^M A_i \mu_i + \sum_{i=M+1}^N A_i \mu_i = -\sum_{i=1}^M A_i \left[\mu_i^\circ + k_B T \log \left(\gamma_i \frac{C_i}{C^\circ} \right) \right] + \sum_{i=M+1}^N A_i \left[\mu_i^\circ + k_B T \log \left(\gamma_i \frac{C_i}{C^\circ} \right) \right] \\
 &= -\sum_{i=1}^M A_i \mu_i^\circ + \sum_{i=M+1}^N A_i \mu_i^\circ - \sum_{i=1}^M A_i k_B T \log \left(\gamma_i \frac{C_i}{C^\circ} \right) + \sum_{i=M+1}^N A_i k_B T \log \left(\gamma_i \frac{C_i}{C^\circ} \right) \\
 &= \sum_{i=M+1}^N A_i \mu_i^\circ - \sum_{i=1}^M A_i \mu_i^\circ + k_B T \left[\sum_{i=M+1}^N A_i \log \left(\gamma_i \frac{C_i}{C^\circ} \right) - \sum_{i=1}^M A_i \log \left(\gamma_i \frac{C_i}{C^\circ} \right) \right] \\
 &= \sum_{i=M+1}^N A_i \mu_i^\circ - \sum_{i=1}^M A_i \mu_i^\circ + k_B T \left[\sum_{i=M+1}^N \log \left(\left[\gamma_i \frac{C_i}{C^\circ} \right]^{A_i} \right) - \sum_{i=1}^M \log \left(\left[\gamma_i \frac{C_i}{C^\circ} \right]^{A_i} \right) \right] \\
 &= \sum_{i=M+1}^N A_i \mu_i^\circ - \sum_{i=1}^M A_i \mu_i^\circ + k_B T \left[\log \left(\prod_{i=M+1}^N \left[\gamma_i \frac{C_i}{C^\circ} \right]^{A_i} \right) - \log \left(\prod_{i=1}^M \left[\gamma_i \frac{C_i}{C^\circ} \right]^{A_i} \right) \right] \\
 &= \sum_{i=M+1}^N A_i \mu_i^\circ - \sum_{i=1}^M A_i \mu_i^\circ + k_B T \log \left(\frac{\prod_{i=M+1}^N \left[\gamma_i \frac{C_i}{C^\circ} \right]^{A_i}}{\prod_{i=1}^M \left[\gamma_i \frac{C_i}{C^\circ} \right]^{A_i}} \right) = \Delta G^\circ + k_B T \log(Q) \text{ becomes } K \text{ at equilibrium } (\Delta G = 0)
 \end{aligned}$$

$\rightarrow \Delta G^\circ$

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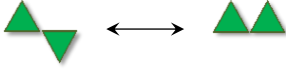
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Applying the Generic Model

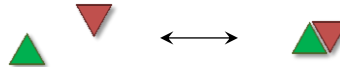
Consider again $A \leftrightarrow B$. If we set the activity coefficients to unity:

$$\Delta G = \Delta G^\circ + k_B N_A T \log \left(\frac{\gamma_A \frac{C_B}{C^\circ}}{\gamma_B \frac{C_A}{C^\circ}} \right) = \Delta G^\circ + k_B N_A T \log \left(\frac{C_B}{C_A} \right)$$


Consider again $A + B \leftrightarrow AB$. One can set $C^\circ = 1 \text{ M}$ but then all concentrations must be inserted in M and the standard state is determined in terms of concentration!

$$\Delta G = \Delta G^\circ + k_B N_A T \log \left(\frac{\gamma_A \frac{C_A}{C^\circ} \gamma_B \frac{C_B}{C^\circ}}{\gamma_{AB} \frac{C_{AB}}{C^\circ}} \right) = \Delta G^\circ + k_B N_A T \log \left(\frac{\frac{C_{AB}}{1}}{\frac{C_A}{1} \frac{C_B}{1}} \right)$$

We make K dimensionless by dividing with one...



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Standard Free Energy

At equilibrium $\Delta G = 0$ so we can write the standard free energy of a reaction per mole:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -k_B N_A T \log(K)$$

The physical meaning of ΔG° is the free energy change when the system starts at the standard state and goes to equilibrium.

Since ΔH° and ΔS° should be independent on T one can use an *van 't Hoff plot* to relate them to K for different T :

$$\log(K) = \frac{\Delta S^\circ}{k_B N_A} - \frac{\Delta H^\circ}{k_B N_A} \times \frac{1}{T}$$

So $\log(K)$ should be linear against $1/T$. Slope gives ΔH° and intersect ΔS° . The *van 't Hoff equation* is equivalent:

$$\Delta H^\circ = k_B N_A T^2 \frac{\partial \log(K)}{\partial T}$$

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Reflections & Questions



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Calorimetry

The principle of calorimetry is to measure the heat transferred to or from a sample.

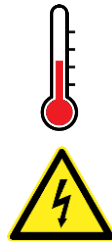
Joseph Black 18th century measured ice melting.

Not long after, first biological experiment by Lavoisier (guinea pig in snow cage).

Calorimetry basically measures heat exchange in a temperature controlled container where something interesting is going on...

Measure heat q (J) that goes in or out! For instance by an electric current and a resistor inside.

Also measure and control the temperature T !



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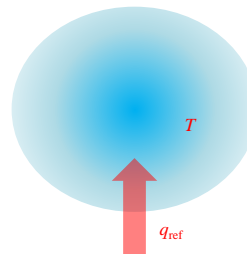
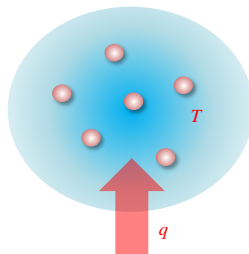
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Reference Chamber

Usually one chamber contains the molecule of interest in solution and the reference chamber contains only the solvent in the same amount.

The instrument makes sure that both chambers have the same temperature, but the amount of heat transfer needed to achieve this will be different because of the content.



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Heat Capacity

The quantity determined in a calorimetry experiment on a macromolecular solution is simply q for a defined T . This is equal to ΔH when P is constant. From the data one can define the heat capacity at constant pressure c_p :

$$c_p = \left. \frac{\partial H}{\partial T} \right|_P = \frac{\frac{\partial q}{\partial t} \text{ measured}}{\frac{\partial T}{\partial t} \text{ user defined and measured}}$$

For biomolecular analysis, the solvent is of course water. Temperature range -10 to 130 °C. (How?) Typical concentrations 0.1-1 g/L, chamber volume 1 mL (μg region).

From the definition of c_p , when changing temperature from T_0 to T :

$$\Delta H = H(T_0) + \int_{T_0}^T c_p dT$$

And the formula for entropy:

$$\Delta S = S(T_0) + \int_{T_0}^T \frac{c_p}{T} dT$$

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Differential Scanning Calorimetry

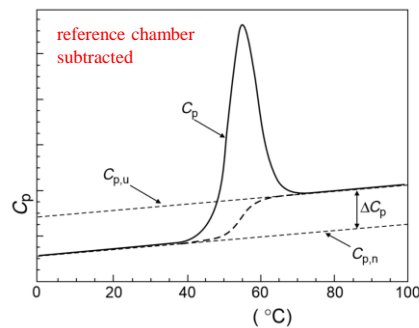
In *differential scanning calorimetry* (DSC) the temperature is scanned (~ 10 K/min) and one measures the heat required to reach the set temperature.

Temperature induced transitions such as protein denaturation are typically studied

Note that the baseline is not flat and not necessarily the same before and after the transition.

One can define $\Delta c_p(T)$ as the difference between measured c_p and the baseline. The “melting” enthalpy is then:

$$\Delta H_m = \int_0^{\infty} \Delta c_p dT$$



I. Serdyuk, N.R. Zaccai, J. Zaccai
Methods in Molecular Biophysics Cambridge University Press 2007

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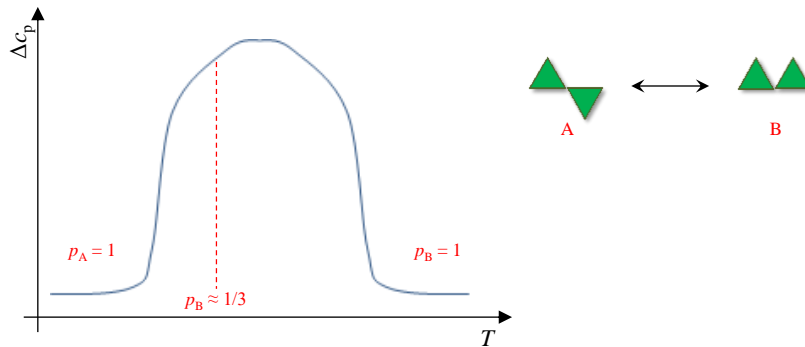
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Notes on DSC

The area under the baseline corrected peak provides information about the fraction of molecules in either state, for each temperature! The integral of $\Delta c_p(T)$ follows the progression of the transition.



Things will be more complicated if there are multiple states that one can reach from the reference state...

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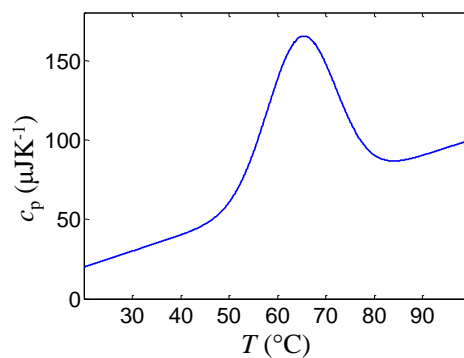
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DSC Exercise

A DSC chamber of 1 mL contains a protein with molecular weight 50 kDa at a concentration of 1 g/L. Estimate the molar enthalpy of denaturation for the protein from the data.



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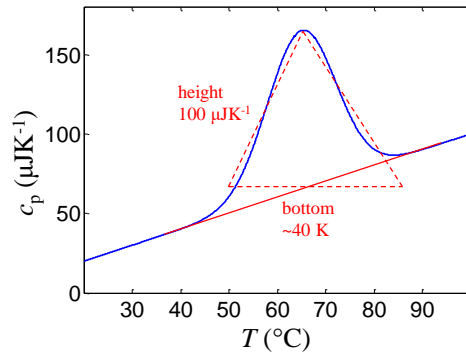
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DSC Exercise Solution

The area under the peak after baseline subtraction is approximately $100 \mu\text{JK}^{-1} \times 40 \text{ K} \times 1/2 = 2 \text{ mJ}$. The amount of protein in the chamber is $10^{-3} \text{ L} \times 1 \text{ gL}^{-1} / [5 \times 10^4 \text{ g mol}^{-1}] = 2 \times 10^{-8} \text{ mol}$. The denaturation enthalpy is then 100 kJ mol^{-1} .



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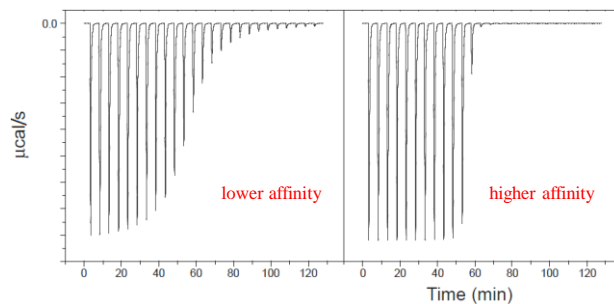
Isothermal Titration Calorimetry

In *isothermal titration calorimetry* (ITC) the calorimeter measures the energy needed/released when maintaining a constant temperature of the chamber (and the reference).

Typically used to acquire the enthalpy associated with biochemical reactions.

One molecule is in the chamber from start and the other is introduced by repeated injections.

Each injection gives a “spike” in the heat transfer rate.



I. Serdyuk, N.R. Zaccai, J. Zaccai
Methods in Molecular Biophysics Cambridge University Press 2007

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ITC Data

ITC data shows reaction events directly in a $\partial q/\partial t$ plot in time. Again, by subtracting the reference the enthalpy of the association or “on” reaction is easily calculated:

$$\Delta H_{\text{on}} \Delta N_{\text{AB}} = \int_0^{\infty} \frac{\partial q}{\partial t} dt$$

A (present)

B (injected)

↔

AB

$K = \frac{C_{\text{AB}}}{C_{\text{A}} C_{\text{B}}}$

Here ΔN_{AB} is the number of AB complexes formed due to the injection.

The amount of A, which may be in the form of AB, is constant and known ($N_{\text{A}(\text{tot})}$).

The amount of B (possibly present as AB) increases but is always known ($N_{\text{B}(\text{tot})}$).

$$\frac{N_{\text{A}(\text{tot})}}{V} = C_{\text{A}} + C_{\text{AB}}$$

$$\frac{N_{\text{B}(\text{tot})}}{V} = C_{\text{B}} + C_{\text{AB}}$$

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ITC Analysis

For injection j :

$$q_j = \Delta H_{\text{on}} N_{\text{A}(\text{tot})} \left[\frac{KC_{\text{B}j}}{1 + KC_{\text{B}j}} - \frac{KC_{\text{B}j-1}}{1 + KC_{\text{B}j-1}} \right]$$

S. Leavitt, E. Freire
Current Opinion in Structural Biology 2001

C_{B} can always be calculated based on $V(j)$, $N_{\text{A}(\text{tot})}$ and $N_{\text{B}(\text{tot})}(j)$ but the formula becomes quite complicated.

Reaction enthalpy is measured in a very direct way as it is simply equal to q (constant pressure) but more information can also be obtained such as entropy of the reaction.

The equilibrium constant can also be determined since one can see to what extent the reactions occur by each injection (the area of each “spike”).

ITC is usually not used to measure *kinetics* of biomolecular interactions (next lecture) although in principle some information is in the timetrace...

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ITC Exercise

In an ITC experiment a 1 mL chamber contains 10^{-8} mol of a protein. A ligand is introduced with injections of 10 μL with concentration 100 μM . It binds to the protein with an equilibrium constant of 10^9 M^{-1} and a reaction enthalpy of 10 kJmol^{-1} .

How much heat evolves during the first injection if one assumes all the introduced molecules bind?

How much heat evolves during the first injection if one does not assume that all ligands bind?

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ITC Exercise Solution

Each injection introduces $10 \times 10^{-6} \text{ L} \times 100 \times 10^{-6} \text{ molL}^{-1} = 10^{-9}$ mol. If everything binds the heat evolved is $10^{-9} \text{ mol} \times 10^4 \text{ Jmol}^{-1} = \underline{10 \mu\text{J}}$.

We can write:

$$C_{\text{A tot}} = C_{\text{A}} + C_{\text{AB}} \quad C_{\text{B tot}} = C_{\text{B}} + C_{\text{AB}}$$

We know that the volume is 1.01 mL after the injection. The total amount of A is 10^{-8} mol and of B 10^{-9} mol. The total concentrations are thus:

$$C_{\text{A tot}} = 10^{-8} / (1.01 \times 10^{-3}) \approx 9.9 \times 10^{-6} \text{ M}$$

$$C_{\text{B tot}} = 10^{-9} / (1.01 \times 10^{-3}) \approx 9.9 \times 10^{-7} \text{ M}$$

Use equilibrium constant to make C_{B} the only unknown:

$$K = \frac{C_{\text{AB}}}{C_{\text{A}} C_{\text{B}}} = \frac{C_{\text{B tot}} - C_{\text{B}}}{C_{\text{B}} [C_{\text{A tot}} - C_{\text{AB}}]} = \frac{C_{\text{B tot}} - C_{\text{B}}}{C_{\text{B}} [C_{\text{A tot}} - C_{\text{B tot}} + C_{\text{B}}]}$$

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ITC Exercise Solution

This is a second degree equation for C_B which can be written as:

$$C_B^2 + \frac{K[C_{A_{tot}} - C_{B_{tot}}] + 1}{K} C_B - \frac{C_{B_{tot}}}{K} = 0$$

The solution is:

$$C_B = -\frac{K[C_{A_{tot}} - C_{B_{tot}}] + 1}{2K} \pm \left[\left[\frac{K[C_{A_{tot}} - C_{B_{tot}}] + 1}{2K} \right]^2 + \frac{C_{B_{tot}}}{K} \right]^{1/2}$$

If $K = 10^9 \text{ M}^{-1}$ we get $C_B = 1.11 \dots \times 10^{-10} \text{ M}$ ignoring the negative solution. (This seems reasonable because it is much smaller than $C_{B_{tot}}$, i.e. most molecules bind.)

Now we can use the formula for the heat generated with $C_B = 0$ previously:

$$q = \Delta H_{on} N_{A_{tot}} \frac{KC_B}{1 + KC_B}$$

This gives $q = 9.9991 \mu\text{J}$ (almost $10 \mu\text{J}$ as expected).

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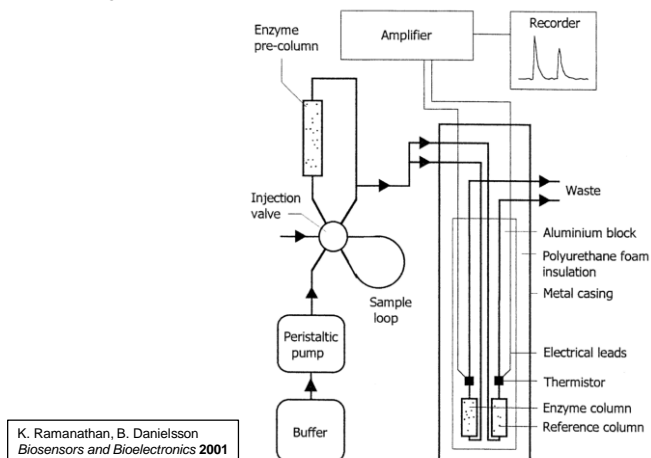
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Calorimetry Biosensors

Note that the calorimeter is a thermal biosensor in the sense that the molecular interaction is detected through the heat transfer!



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Reflections & Questions

