Thermodynamic of proteins

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What we study in the biological system

- We study proteins-protein interactions.
- Proteins are the biomolecules is a unit which the body is built of.
- Proteins as molecules have dynamics.
- So, therefore, we study the dynamic of the proteins by measuring the thermodynamic parameters during the interactions.
- What are the thermodynamic parameters? What are based on?

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What are the thermodynamic parameters? ΔH

- Enthalpy ΔH, a property of a thermodynamic system, is equal to the system's internal energy plus the product of its pressure and volume.
- **(1st law of thermodynamic)**
- In a system enclosed, the heat absorbed or released equals the change in enthalpy

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1- ΔH

- Any biological system underly to Exothermic and/or endothermic process.
- To understand that:
- The dissolving of NaOH is an exothermic reaction with $\Delta H < 0$
- So, the solution becomes warm and heat is transferred to the surroundings.
- In contrast, the dissolution of NaNO3 is an endothermic process,
- with $\Delta H > 0$.
- As a consequence, the solution becomes cold, as heat is drawn from the surroundings.

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2- ΔS

- Entropy ΔS: it is a property of thermodynamic system. it tells how order or disorder the system is.
- ΔS> 0 (+) more disorder.
- ΔS< 0 (-) increase order.
- **2nd law: Entropy of any isolated system always increases.**

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Endergonic and exergonic processes

- Processes with $\Delta G < 0$ are termed *exergonic*, processes with $\Delta G > 0$ are *endergonic*.
- With ΔG, the *free energy G*. The general definition of the free energy is
- $G = H - TS$
- *G* is also called the *Gibbs free energy*. Complete differentiation of *G* gives:
- $\Delta G = \Delta H - T\Delta S$
- For reactions: $\Delta Gr = G$ products $- G$ reactants
- The equilibrium is affected by change on these paprmters.

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Units of the parameters

$$\Delta G = \Delta H - T\Delta S$$
$$\Delta G = RT\ln K_d$$

ΔG- *Gibbs Free Energy, or "available energy"*
ΔH- *Enthalpy change*
T- *Temperature in Kelvin*
ΔS- *Entropy change*
R- *Gas constant, 8.314JK^{-1}mol^{-1}*
K_d- *Dissociation rate*

Standard Free Energy ($\Delta G°$) and Temperature (T)

(consists of 2 terms) $\Delta G° = \Delta H° - T\Delta S°$ *(on equation sheet)*

free energy (kJ/mol)	enthalpy term (kJ/mol)	entropy term (J/mol·K)
max energy used for ***work***	energy transferred as ***heat***	energy ***dispersed*** as disorder

units convert to **kJ**!!!

The **temperature dependence** of free energy comes **from** the **entropy term** ($-T\Delta S°$).

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Solutions ! ΔH -92.22 kJ & ΔS= -198.75 J/K what is ΔG?

$$T_K = 25^\circ C + 273.15 = 298.15\ K$$

$$\Delta S^\circ = -198.75\ J/K \times \frac{1\ kJ}{1000\ J} = -0.19875\ kJ/K$$

$$\Delta H^\circ = -92.22\ kJ$$

$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ Plug in ΔH°, ΔS°, and T

$$\Delta G^\circ = -92.22\ kJ - (298.15\ K)(-0.19875\ kJ/K)$$

$$\Delta G^\circ = -92.22\ kJ + 59.257\ kJ$$

$$\boxed{\Delta G^\circ = -32.96\ kJ}$$

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Thermodynamic and Biomolecules

- Protein-protein interaction are a type of reaction.
- Meaning: thermodynamic parameters of the reactants will differ from the product.
- How can we calculate all of the thermodynamic parameters for protein-protein interactions?
- In the labs, we use ITC isothermal calorimetry techniques to calculate the parameters and also to study the affinity binding Ka and decoration Kd and also the stoichiometry (molar ratio).

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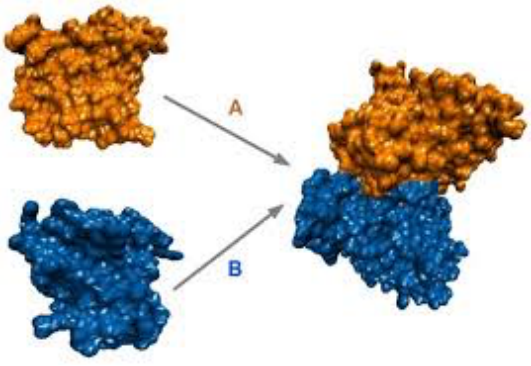
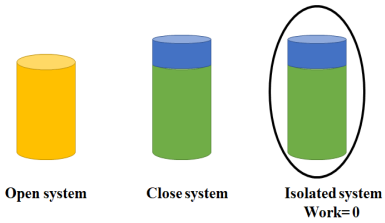
Protein-protein binding

- So, the biomolecules behave as same other non-biomolecules but they are in a closed system.
- Their parameters are changed during the reaction.





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How does ITC work?

- Measurement principle of ITC
- We titrate a protein with another protein and then we measure the enthalpy ΔH of each point.
- By collecting different point, we can calculate the difference in (ΔH)
- When binding occurs, heat is either observed or released (comparing to the reference cell).
- As first injection is made, the micro-calorimeter measures all the heat releases till binding reaction has reached equilibrium.

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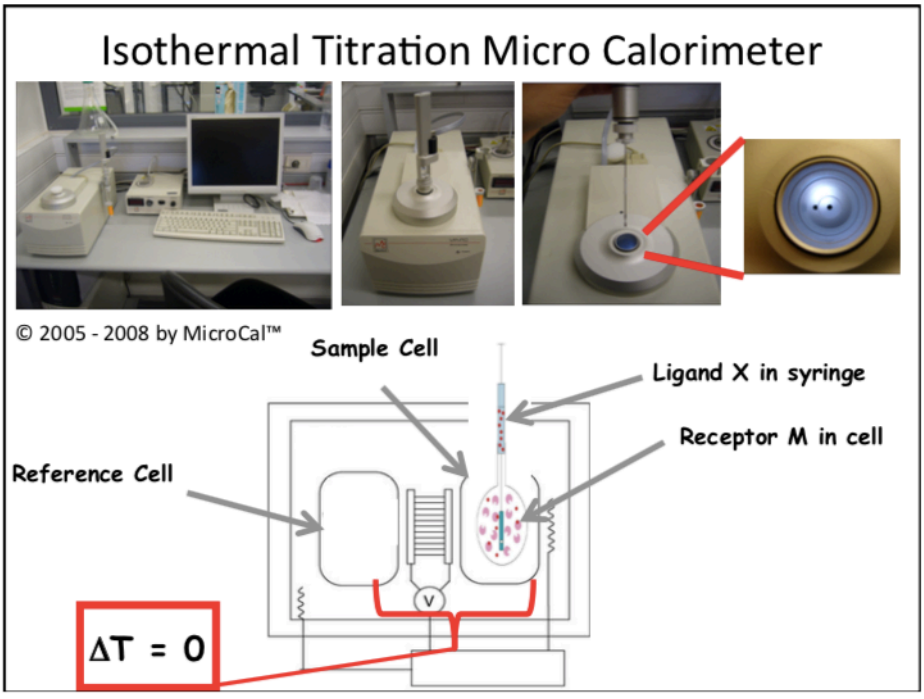

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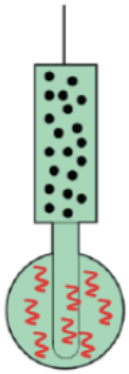
ITC – Before titration



- Ligand – in syringe
- Macromolecule in ITC cell

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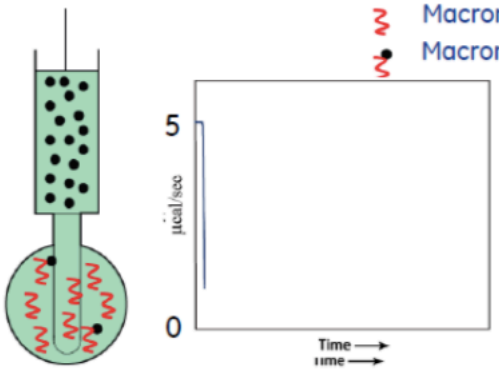
Titration begins: First injection



- Ligand in syringe
- Macromolecule in cell
- Macromolecule-ligand complex

As the first injection is made, all injected ligand is bound to target macromolecule.

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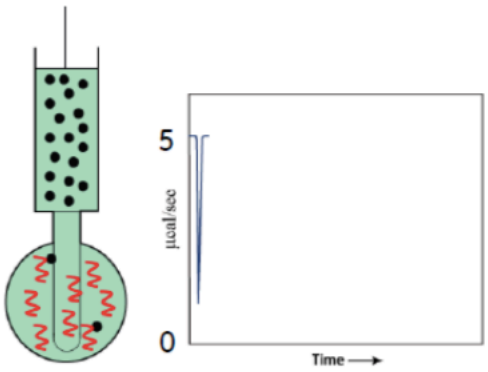
Return to baseline



The signal returns to baseline before the next injection.

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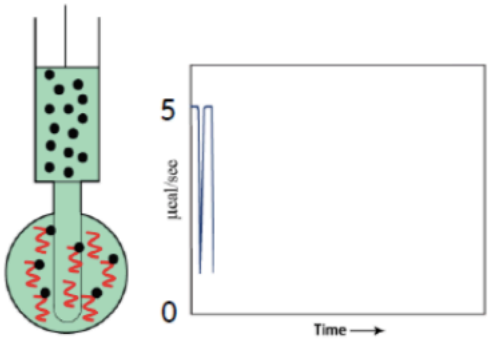
Second injection



As a second injection is made, again all injected ligand becomes bound to the target.

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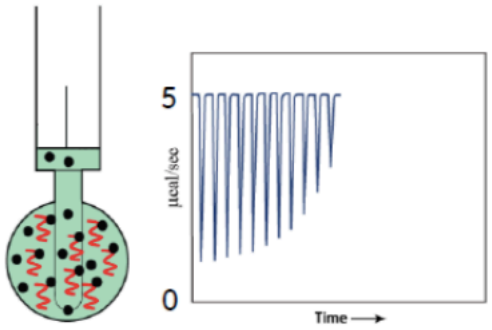
Injections continue



As the injections continue, the target becomes saturated with ligand so less binding occurs and the heat change starts to decrease.

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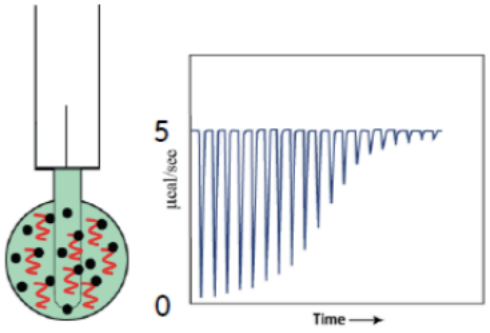
End of titration



When the macromolecule is saturated with ligand, no more binding occurs, and only heat of dilution is observed.

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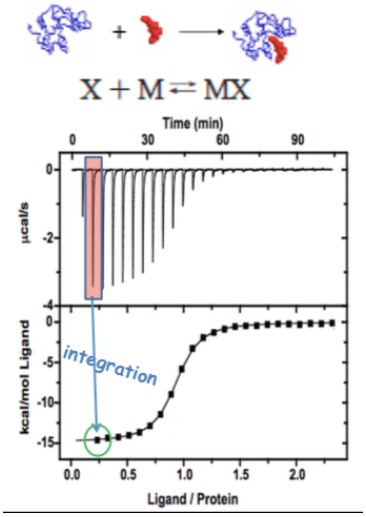
How do we fit the point to calculate the parameters ?

- We fit the collected points of ΔH to a curve equation to calculate the ka (affinity or association constant).
- Kd (dissociation constant)= 1/ka.
- When the binding is weak? Ka and kd
- When the binding is strong? Ka and kd



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Other paramters?

- We can calculate ΔG form:
- ΔG = –RTlnΔKa = RTlnΔKd (Gibbs free energy equation), where: (R: gas constant and T is the absolute temperature).
- Or form ΔG = ΔH – TΔS (Van't Hoff equation).
- What does each tell?
- If ΔG= - or +
- If ΔH= - or +
- If ΔS= - or +
- What is ΔCp:

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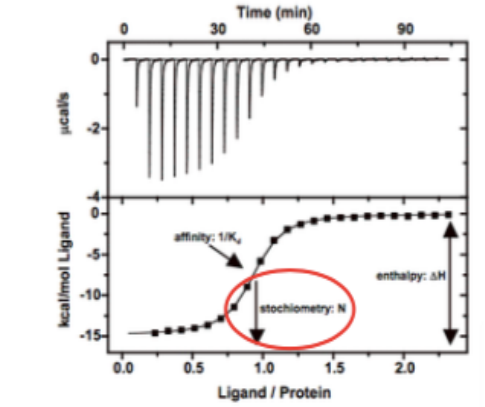

$$\Delta G_{obs} = -RT \ln K_{B,obs}$$

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$$\Delta S_{obs} = \frac{(\Delta H_{obs} - \Delta G_{obs})}{T}$$

$$\Delta C_{p,obs} = d\Delta H_{obs}/dT$$

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Example of determining of thermodynamic parameters

Table 4.9 Determination of Kd of tropomyosin-troponin binding using ITC technique.

TPM	Stoichiometry n.	Kd (μM)	ΔS (cal/mol)	ΔH (kcal/mol)	ΔG (kcal/mol)
TPM1WTAS	1.66 ± 0.033	0.93 ± 0.47	5	-6.56 ± 2.6	-8.22
TPM1WTGA	1.27 ± 0.022	0.62 ± 0.04	3.9	-7.2 ± 0.2	-8.45
K6K7E	0.16 ± 0.15	4.9 ± 1.23*	-1500	-4546 ± 450	-7.23
K48K49E	2.04 ± 0.04	1.58 ± 0.03	11.8	-4.4 ± 0.2	-7.90
R90R91E	0.67 ± 0.01	0.25 ± 0.21	0.68	-8.7 ± 0.2	-8.98
S132R133E	1.48 ± 0.02	0.86 ± 0.43	13	-4.11 ± 0.11	-8.35
R167K168E	0.66 ± 0.02	0.79 ± 0.62	-39.1	-19.9 ± 0.8	-8.34
N202N203	0.68 ± 0.01	0.76 ± 0.90	-13.4	-12.3 ± 0.43	-7.61
R244S245E	0.87 ± 0.01	2.60 ±1.55	-8.77	-10.2 ± 0.9	-7.23

Note: the ΔG is shown in (kcal/mol) unit as it was calculated using the gas constants value (1.94 kcal $K^{-1}mol^{-1}$). For each parameter * indicates significant difference from TPM1WTAS ($p<0.025$). ANOVA oneway Post-Hoc method was used to analysis the data.

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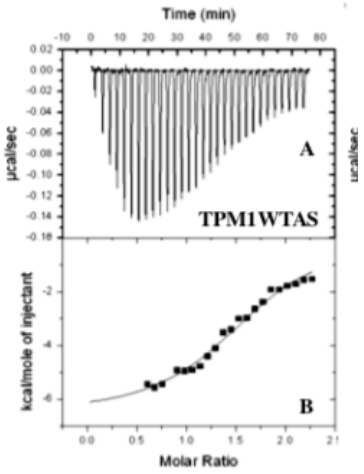
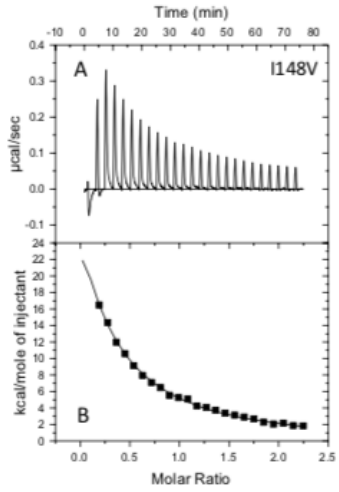
Endothermic and exothermic ITC

- Which one is Endothermic or exothermic reaction?





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What do we get from ITC/ why?

- The thermodynamic parameters tells:
- How much heat the biological system produces.
- Order or disorder? And how order the system is?
- How much energy does the biological system gives or take?
- How many biomolecules per each could bind? Stoichiometry.
- What is the affinity of the reaction? Too speed? Too tight? Or low affinity binding?

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