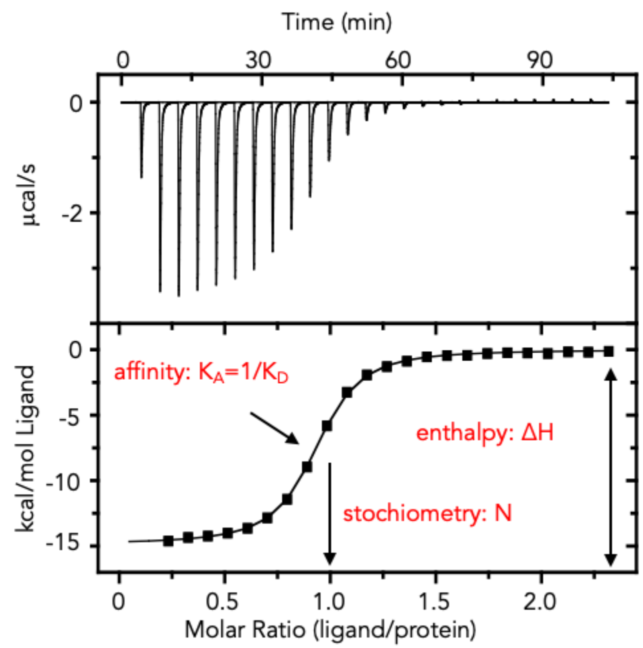
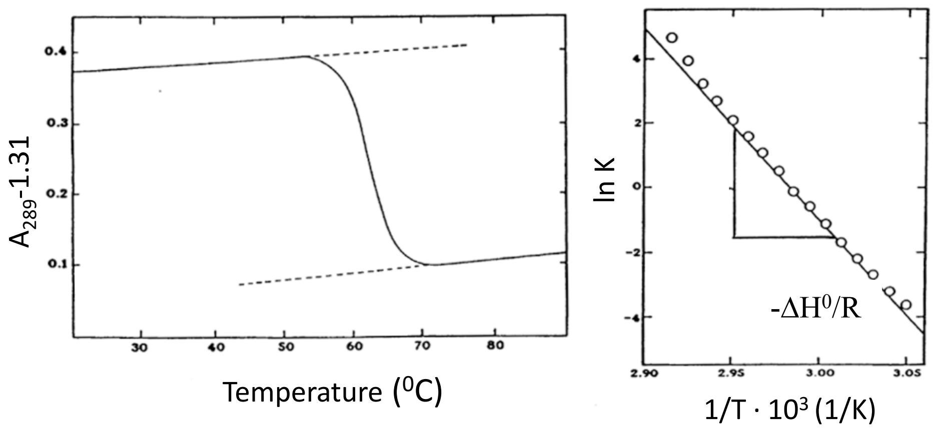
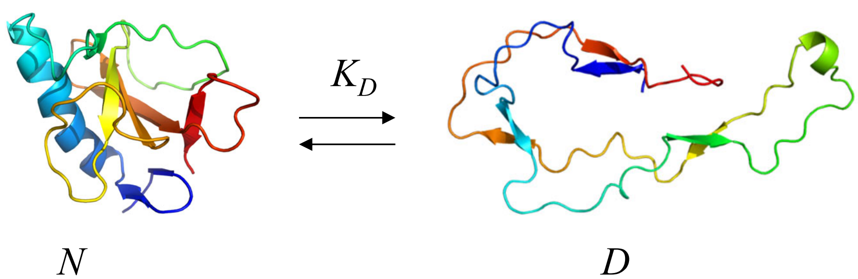
Studying protein structure and binding using calorimetry



Thermal denaturation

Ribonuclease T1





the T-dependence of K is given by:

$$\ln K(T) = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}$$

derivative for T:

$$\boxed{\begin{aligned} \frac{d\ln K}{dT} &= \frac{\Delta H^0_{vH}}{RT^2} \\ \frac{d\ln K}{d1/T} &= -\frac{\Delta H^0_{vH}}{R} \end{aligned}}$$

van't Hoff equation

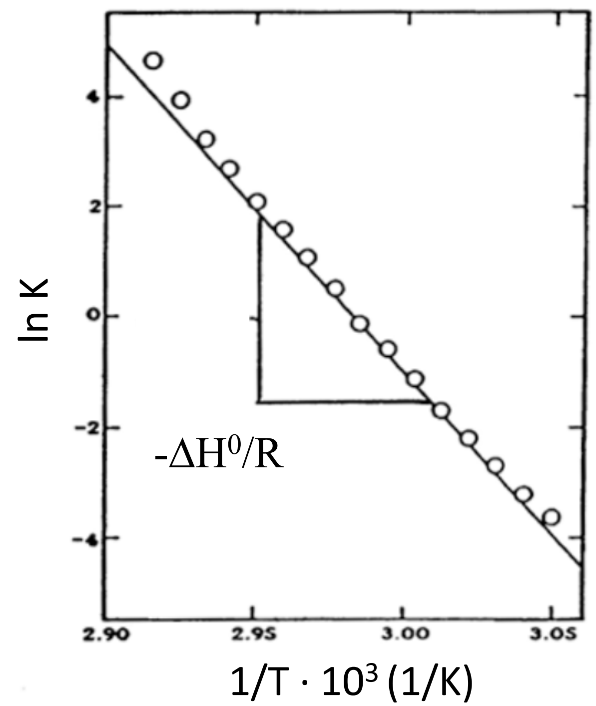
Thermodynamic parameters of protein denaturation

Enthalpy:

Determined from slope of Van't Hoff plot

$$\frac{d\ln K}{d1/T} = -\frac{\Delta H^0_{vH}}{R}$$

within window of linear relationship



Entropy:

At T_m, the temperature of the mid-point of the transition ($\Delta G^0 = 0$)

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

$$\Delta S^0 = \Delta H^0 / T_m \qquad \text{(for } \Delta G^0 = 0\text{)}$$

Quiz 2: Thermal transitions

- Another small protein is 99% folded at 328K and 1 % folded at 340 K
- what is the standard enthalpy (ΔH^0) of its folding transition?
- also estimate the entropy of the transition (ΔS^0)!

Heat capacity changes in protein denaturation

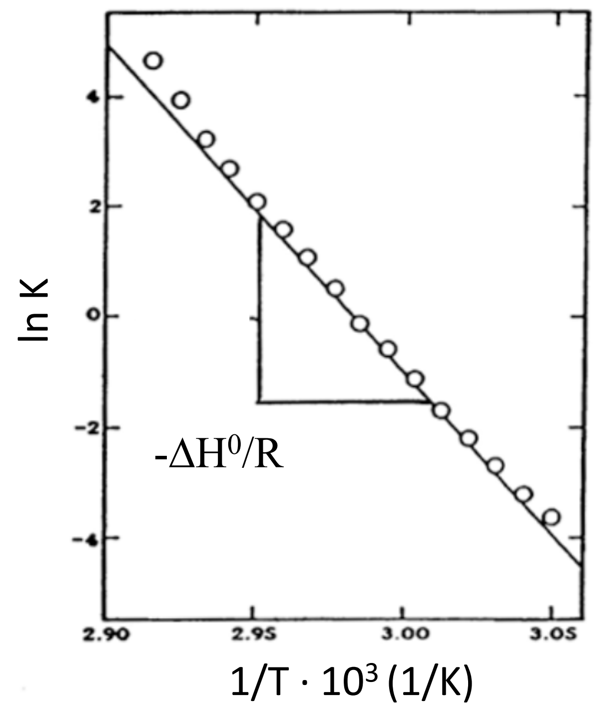
Heat capacity: $$C_P = \left(\frac{\partial H}{\partial T}\right)_P = \left(\frac{\partial Q}{\partial T}\right)_P$$

Change of internal energy upon heating, property of each global state

$$\Delta C_P^0 = C_{P,unfolded}^0 - C_{P,folded}^0$$

Curvature in Van't Hoff relations: The native state and the denatured state have different heat capacities.

Protein unfolding heat capacity is large and positive



$$\boxed{\Delta C_p^{\ 0} > 0}$$

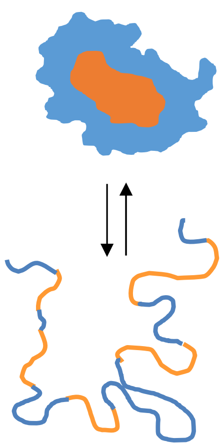
Heat capacity of protein denaturation

Definition of heat capacity:

$$\boxed{\Delta C_p{}^0 = \frac{d\Delta H^0}{dT}}$$

$$\Delta C_p{}^0 / T = \frac{d\Delta S^0}{dT}$$

both, ΔH^0 and ΔS^0 are temperature dependent



The molecular origin of $\Delta C_p{}^0 > 0$:

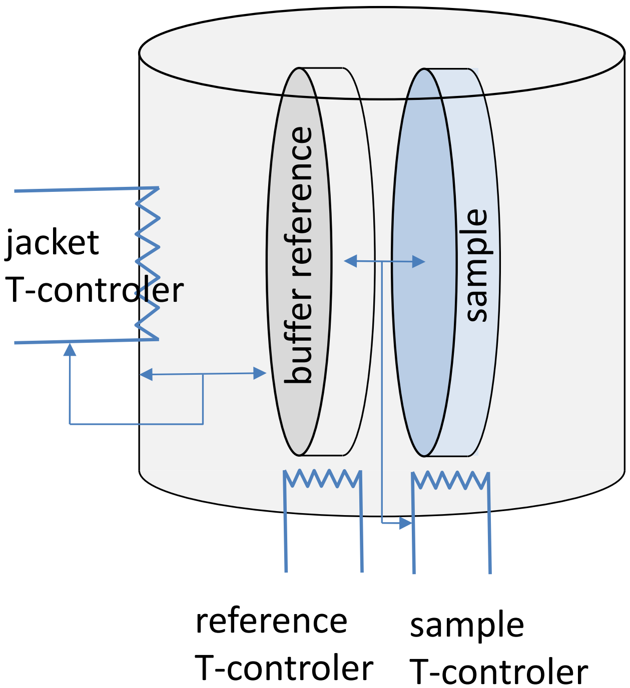
The native and the denatured state exhibit differences in **solvation**

in D, hydrophobic residues are exposed. The **water structure** leads to high heat capacity (compare to Lecture 1)

$\Delta C_p{}^0$ is usually 40-80 J mol^{-1} K^{-1} per residue

related to m-value (also dependent on ASA)

Measuring thermodynamic parameters: Differential Scanning Calorimetry (DSC)



Measures **amount of heat** required to change the **temperature** in the sample vs. the reference.

For an adiabatic isolated microcalorimeter, no heat exchange takes place with the environment

$$\delta Q = 0$$

according to first law

$$dU = dW$$

changes in internal energy solely depend on work within instrument

The partial molar heat capacity Cp

The heat capacity is defined as the **amount of heat** (Q) required to **change the temperature** by dT with constant pressure.

$$C_P = \left(\frac{\partial Q}{\partial T}\right)_P = \left(\frac{\partial H}{\partial T}\right)_P = \frac{dH}{dT}$$

Heat capacity of a protein solution $C_{P,sol}$ is a composite of **partial molar heat capacity** terms:

- Heat capacity of the solvent $C_{P,1}$
- Heat capacity of the protein (e.g. non-covalent interactions) $C_{P,2}$

From $C_{P,2}$ we can determine the enthalpy of protein folding/unfolding (n denotes the molar amounts)

$$C_{P2} = \left(\frac{\partial C_{PSol}}{\partial n_2}\right)_{T,p,n_i \neq 2}$$

Using calorimetry to determine protein stability

Problem: $C_{P,2}$ cannot be directly measured

From calorimetry, the **apparent molar heat capacity** ($C_{P,app}$) is obtained

this is the **difference** between the heat capacity of the solution ($C_{P,sol}$) and of the solvent ($C_{P,1}$)

$$C_{Papp} = \frac{C_{PSol} - n_1 C_{P1}}{n_2}$$

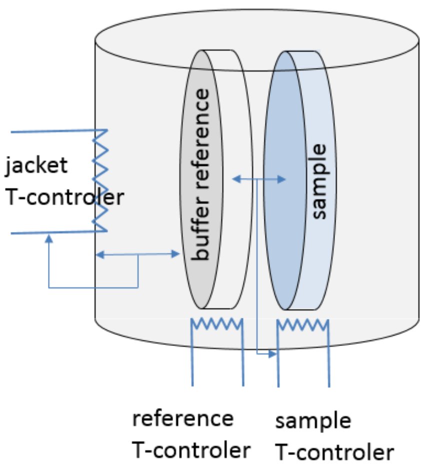
with: $$C_{P2} = \left(\frac{\partial C_{PSol}}{\partial n_2} \right)_{T,p,n_i \neq 2}$$

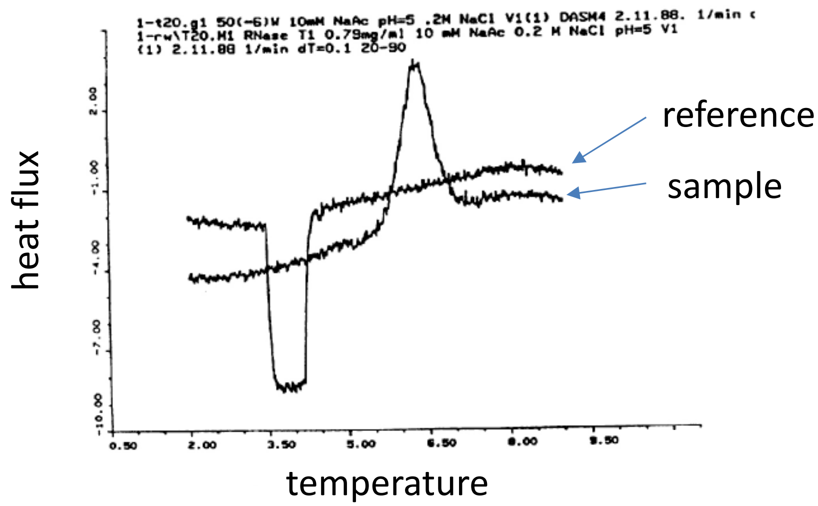
$$C_{P2} = C_{Papp} + \underbrace{n_2 \left(\frac{\partial C_{Papp}}{\partial n_2} \right)_{T,p,n_i \neq 2}}$$

this term can usually be neglected for employed protein concentrations

$$\boxed{C_{P2} = C_{P1} - C_{Papp}}$$

Determining heat capacity from calorimetry





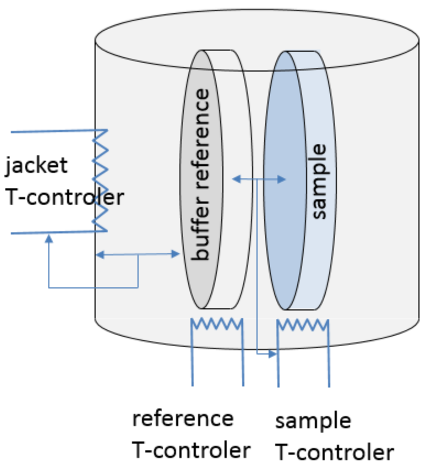
offset correction between sample and reference

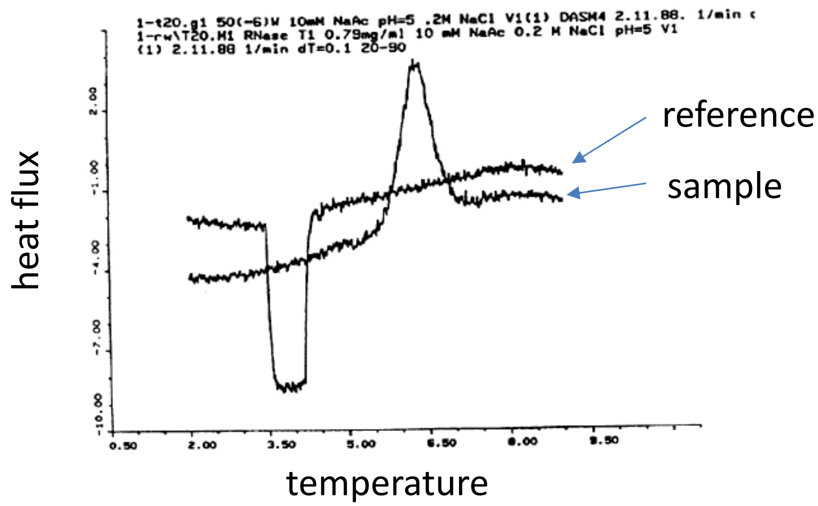
from normalized and calibrated difference follows the **heat capacity change of protein unfolding**

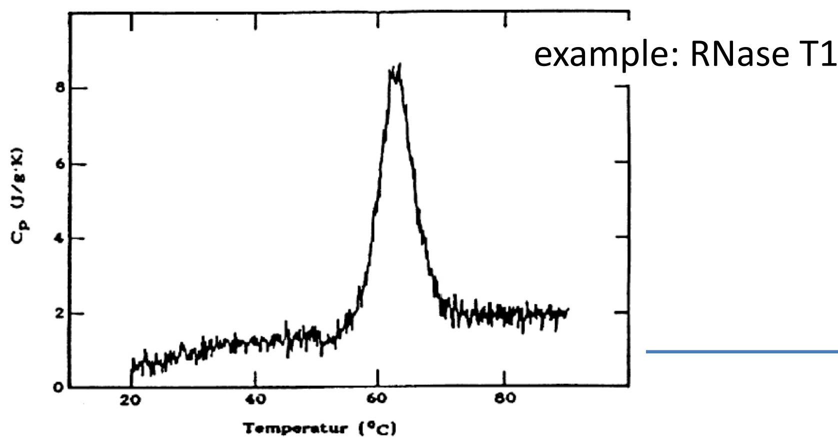
$$C_{P2} = C_{P1}\frac{v_P}{v_1} - \frac{Ck}{m_p}h$$

v_P : partial specific volume of protein
v_1 : partial specific volume of solvent
h: normalization
k: calibration constant

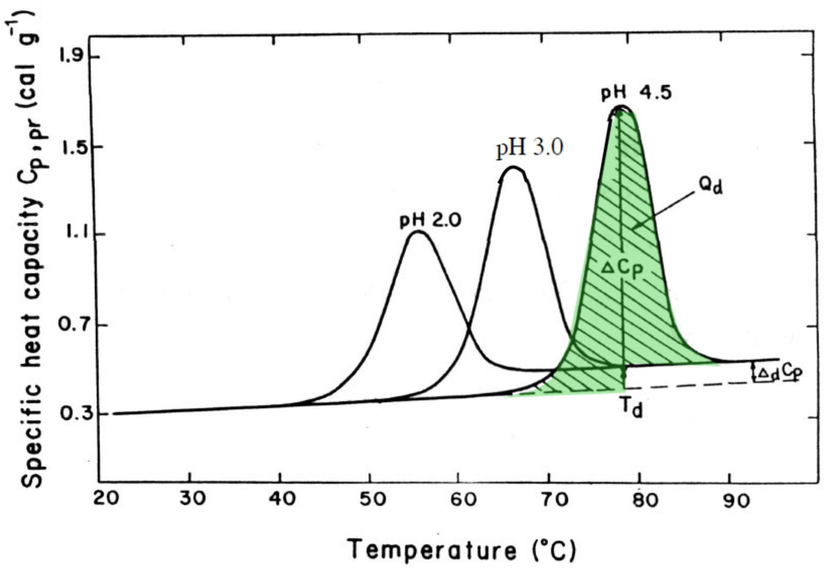
Determining heat capacity from calorimetry







Enthalpy of conversion in protein denaturation



$\Delta H_m(cal)$ of lysozyme denaturation as a function of pH

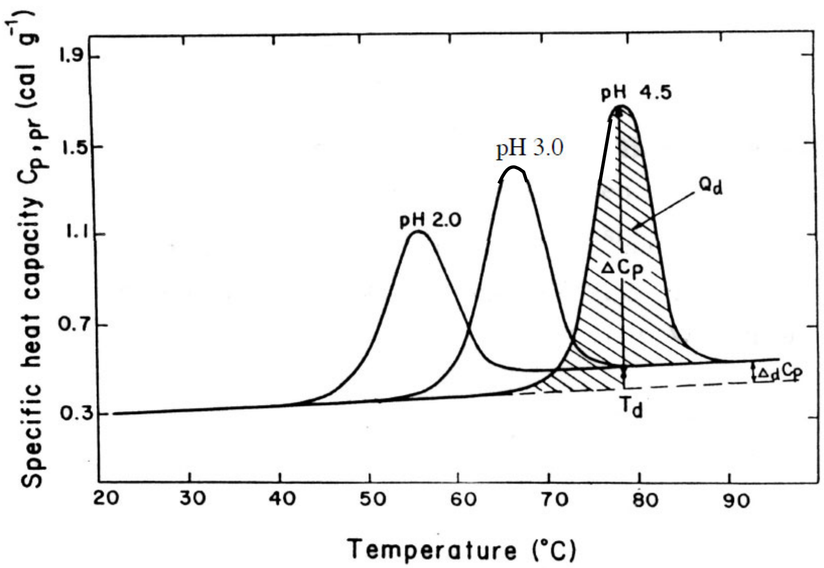
From **integration of the curve** the enthalpy of conversion (folded to unfolded) $\Delta H_m(cal)$ is determined

$$C_P = \frac{dH}{dT}$$

$$\boxed{Q_d = \Delta H_m(cal) = \int C_p dT}$$

model-free determination of enthalpy of state transition

Two-state folding or multistate transition?



$\Delta H_m(cal)$ of lysozyme denaturation as a function of pH

comparing the areas under the curve, an equilibrium constant can be determined.

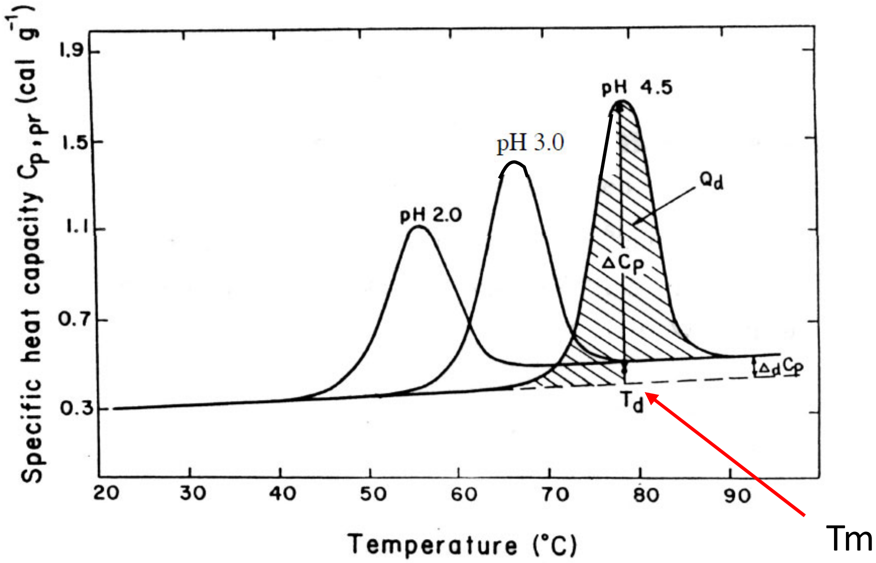
From K, Van't Hoff Enthalpy can be determined immediately, using:

$$\left(\frac{d \ln K}{dT}\right)_p = \frac{\Delta H^0_{v.H.}}{RT^2}$$

This assumes a **two-state transition**

Only in this case, $\Delta H_m(cal) = \Delta H^0_{vH}$

Entropy of conversion



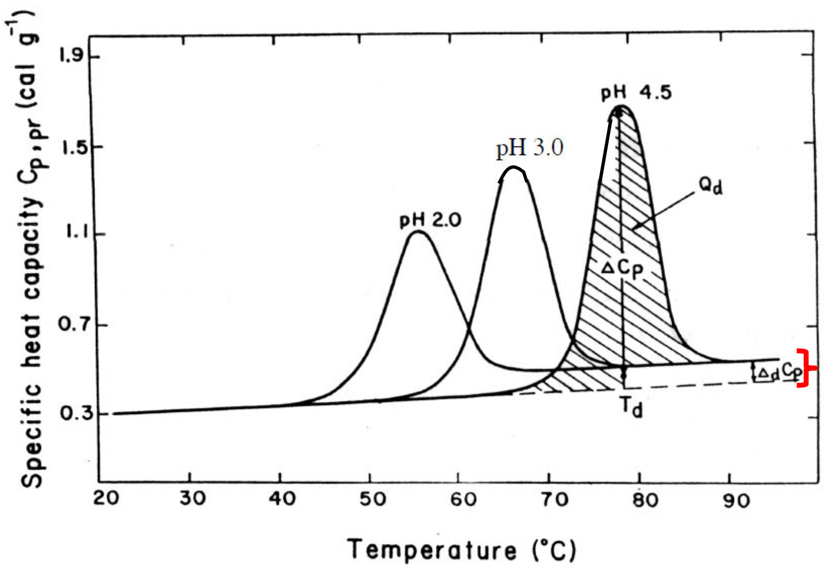
$\Delta H_m(cal)$ of lysozyme denaturation as a function of pH

At the midpoint of the transition (T_m), the entropy can be determined

$$\Delta G = \Delta H - T\Delta S = 0$$

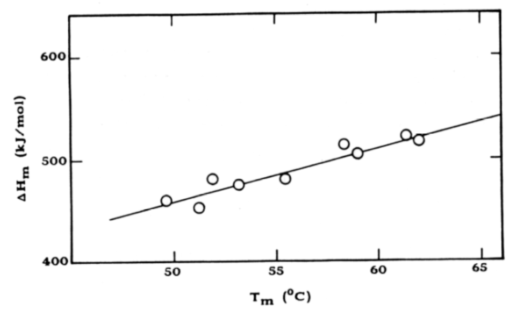
$$\Delta S_m = \frac{\Delta H_m}{T_m}$$

Heat capacity difference of unfolding



ΔC_p can directly be obtained from the measured curve

Can also be determined from the temperature dependence of ΔH_m, e.g. measured under different pH



Rnase T1 denaturation data

The protein stability curve

With heat capacity:

$$\frac{d\Delta H}{dT} = \Delta C_P \qquad \Delta H(T) = \Delta H(T_m) + \Delta C_P (T - T_m)$$

$$\frac{d\Delta S}{dT} T = \Delta C_P \qquad \Delta S(T) = \Delta S(T_m) + \Delta C_P \ln\left(\frac{T}{T_m}\right)$$

With heat capacity, the T-dependence of ΔG^0 becomes:

$$\Delta G^0(T) = \Delta H^0(T_m) - \mathrm{T}\,\Delta S^0(T_m) + \Delta C_p{}^0\left(T - T_m - T\ln\frac{T}{T_m}\right)$$

The protein stability curve

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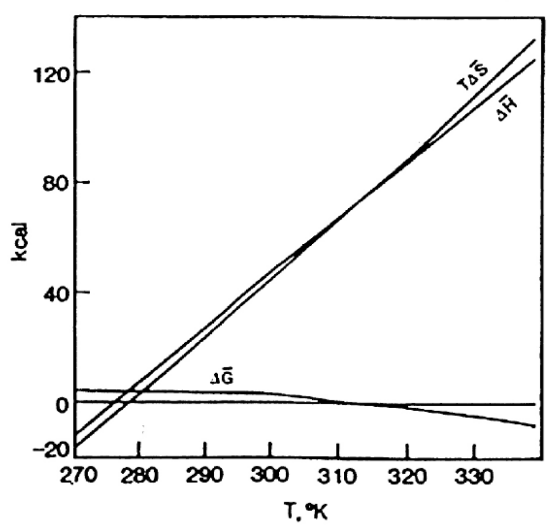
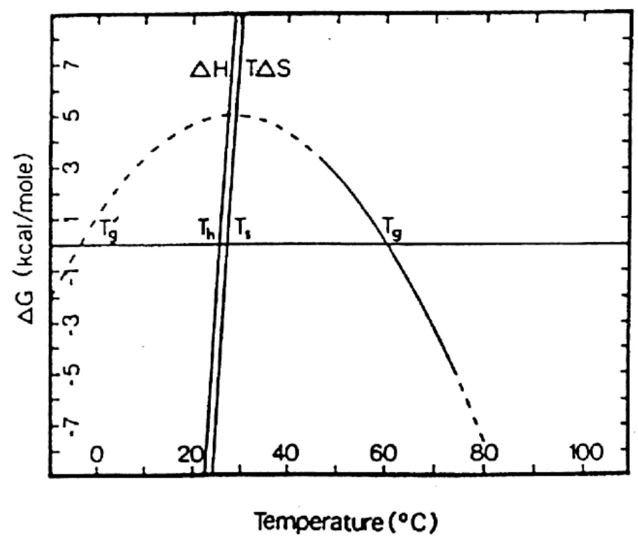
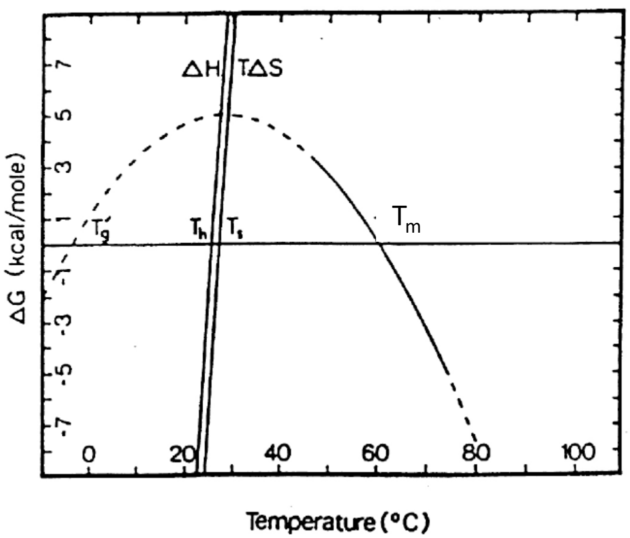
$$\Delta G^0(T) = \Delta H^0(T_m) - \mathrm{T}\,\Delta S^0(T_m) + \Delta C_p{}^0\left(T - T_m - T\ln\frac{T}{T_m}\right)$$

Example





The protein stability curve



Protein stability is maximal at $\Delta S^0 = 0\ (T_S)$

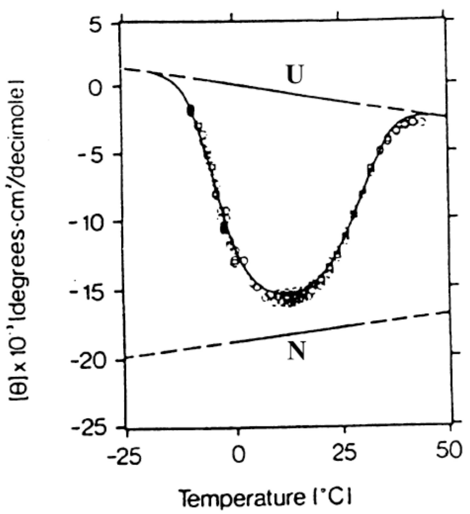
K_D (equilibrium constant) is maximal at $\Delta H^0 = 0\ (T_H)$

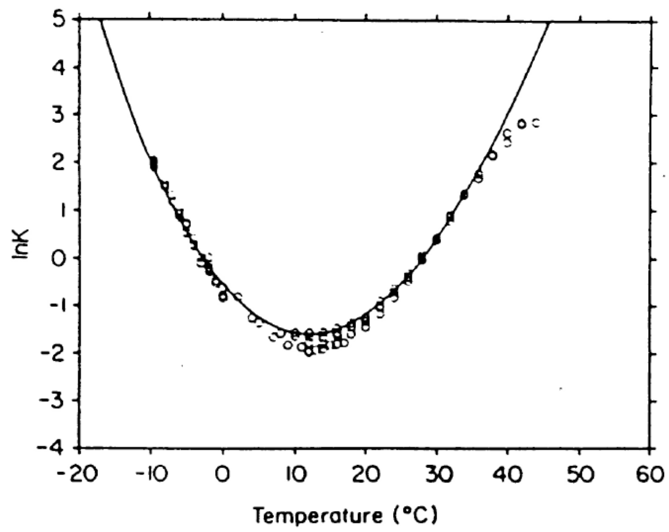
The transition mid-point (T_m) is at $\Delta G^0{=}0$ and $f_N = f_U = 0.5$

Protein cold denaturation

Protein stability curves show second Tm' at low temperature! → cold denaturation

Can be observed for some proteins / mutants:
Cold denaturation of lysozyme (destabilized mutant)

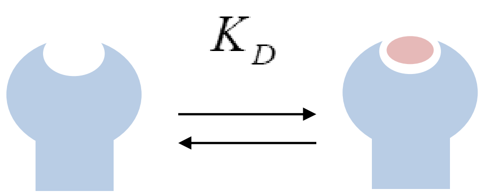




Using calorimetry to determine binding interactions

The energy of a ligand-receptor interaction is determined by ΔG :

$$\boxed{\Delta G = RT \ln K_D}$$



degrees of freedom fixed!

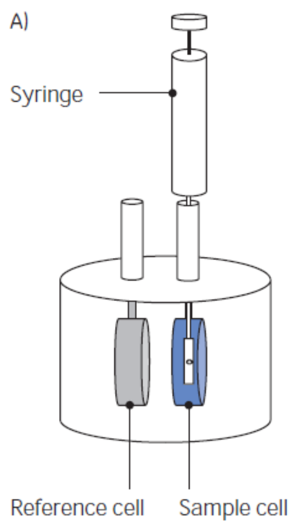
... whereas ΔG itself can be separated into enthalpy and entropy

$$\Delta G = \Delta H - T\Delta S$$

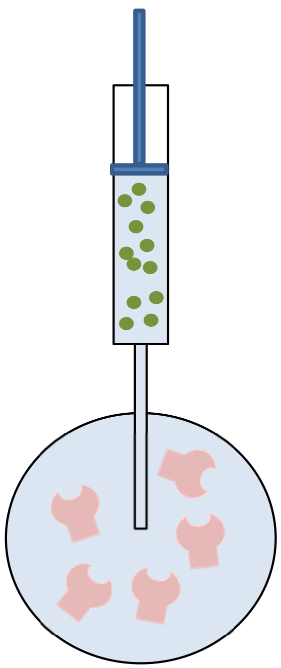
Calorimetry directly informs on thermodynamic parameters ΔH and ΔS

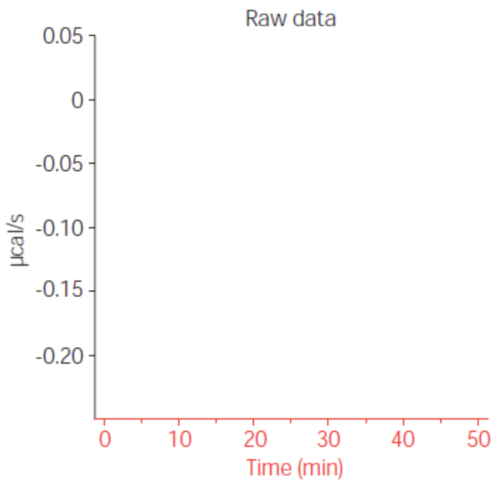
Using calorimetry to determine binding interactions: Isothermal titration calorimetry ITC

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

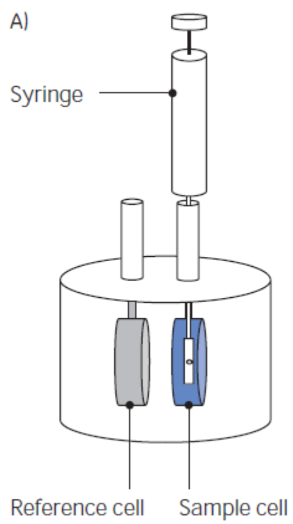


injection of ligand solution into receptor solution



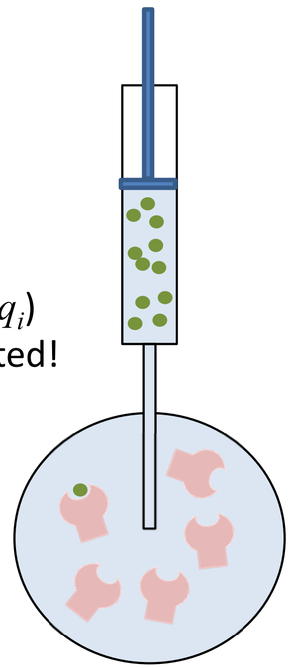


Isothermal titration calorimetry



injection of ligand solution into receptor solution

heat (q_i) detected!



$$\Delta G = RT \ln K_D$$
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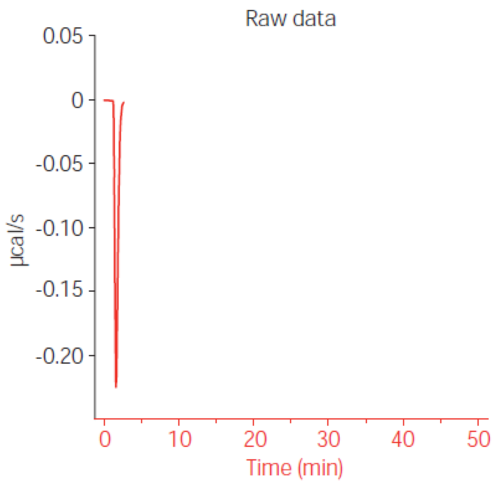
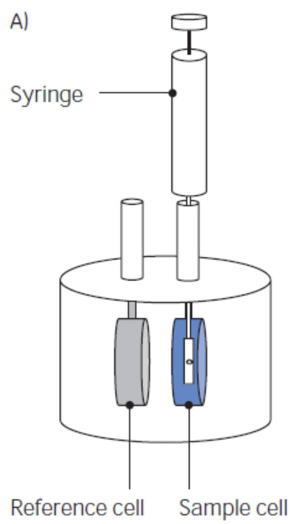


all injected ligand is bound

binding energy is released and is measured as heat

Isothermal titration calorimetry



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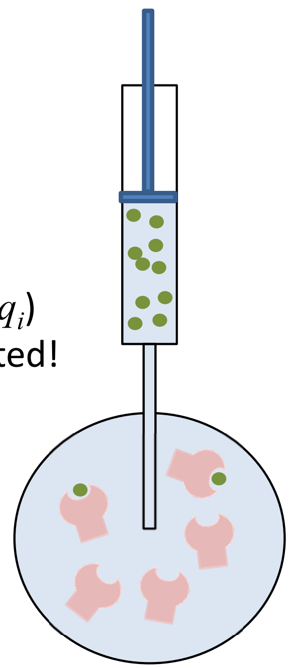

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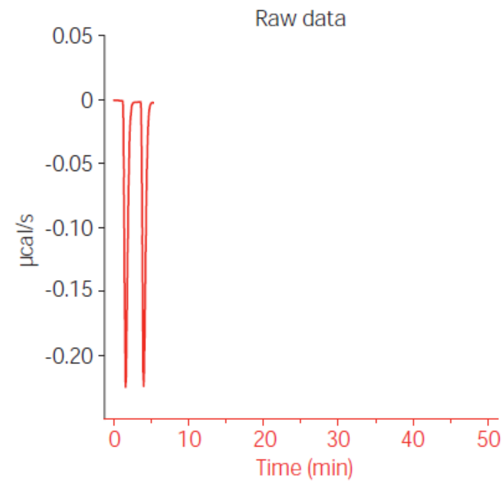

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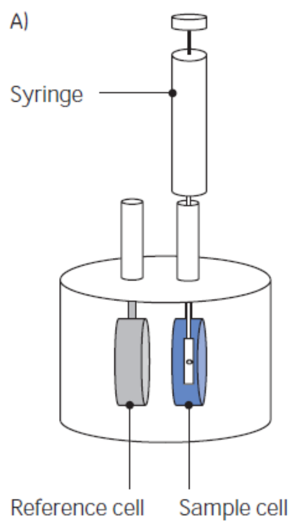

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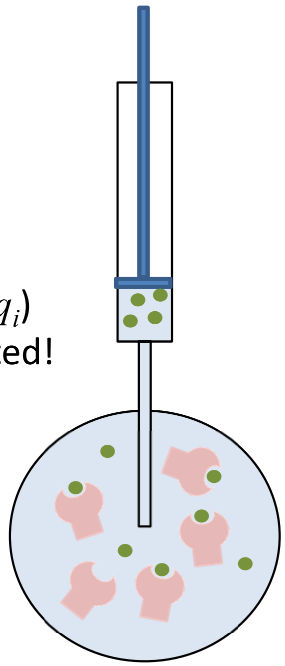

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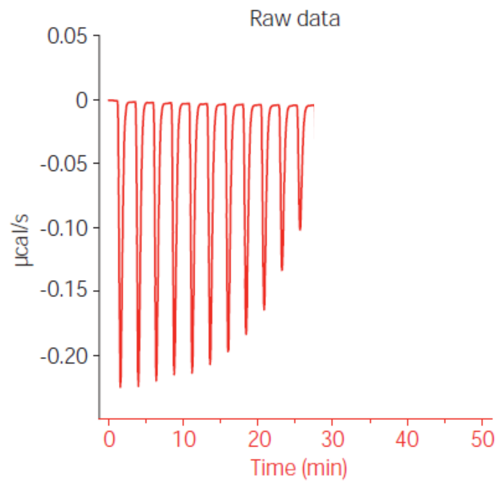
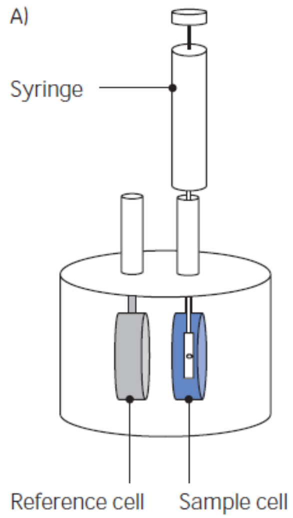


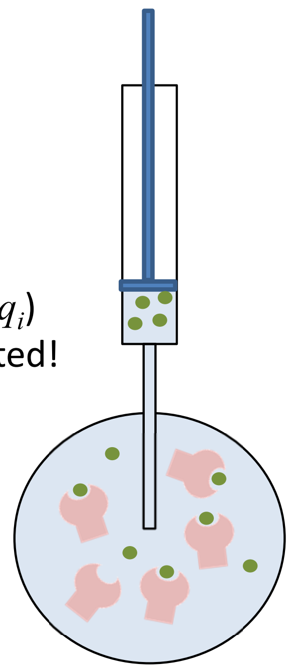
around the Kd, no longer all ligand is bound, less heat is released

Isothermal titration calorimetry



injection of ligand solution into receptor solution

heat (q_i) detected!



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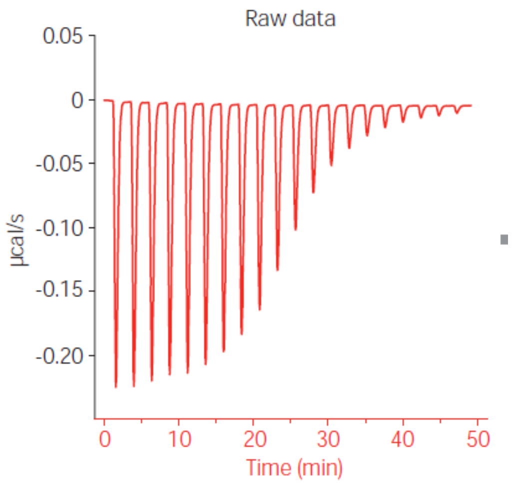

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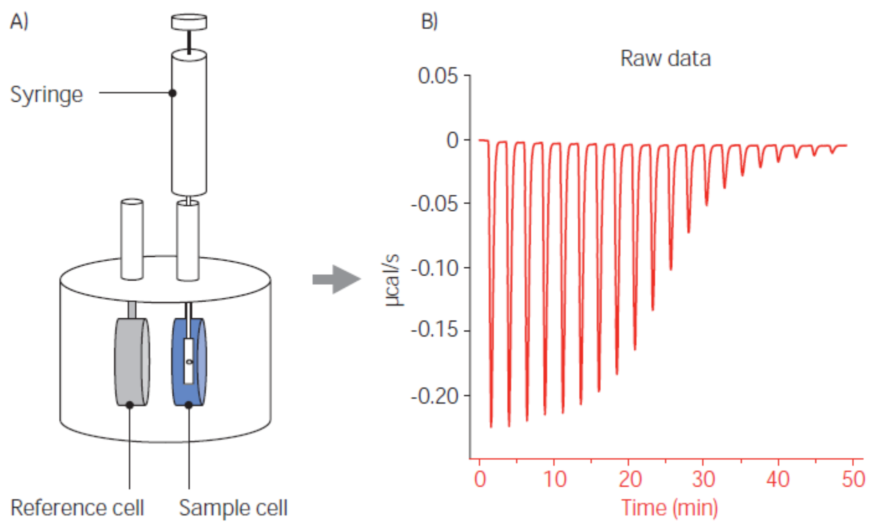
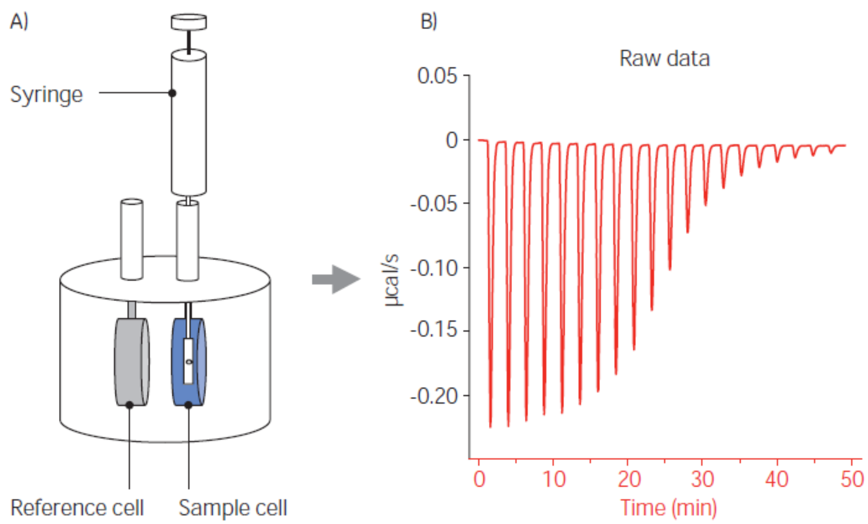


injection of ligand solution into receptor solution

measurement of heat of binding

Isothermal titration calorimetry



injection of ligand solution into receptor solution

measurement of heat of binding

Analysis:

heat released (absorbed) for each injection

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

Vc : volume of the cell

using binding isotherm for multisite binding:

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

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

ITC: Binding to n independent, equal sites

total heat released
(complete integral of curve):

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

using the general solution, as shown before:

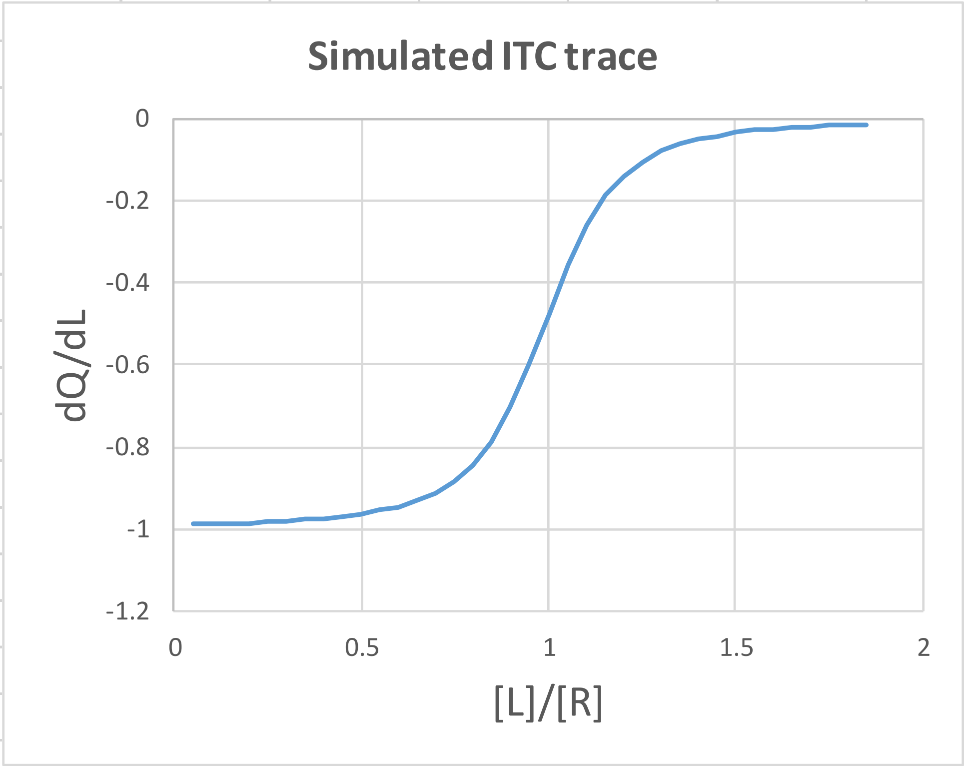
$$Q = \frac{\Delta H_{app} \cdot V_C}{2} \left([L_{tot}] + n[R_{tot}] + K_D - \sqrt{([L_{tot}] + n[R_{tot}] + K_D)^2 - 4n[R_{tot}][L_{tot}]} \right)$$

differentiate for $[L_{tot}]$:

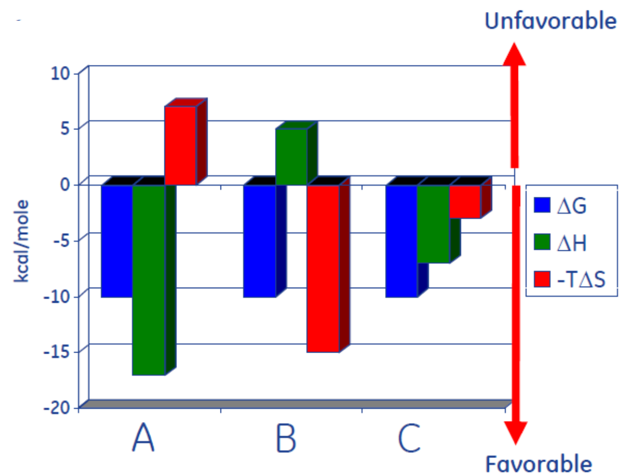
$$\boxed{\frac{dQ}{d[L_{tot}]} = \frac{\Delta H_{app} \cdot V_C}{2} \left(1 - \frac{[L_{tot}] + K_D - n[R_{tot}]}{\sqrt{([L_{tot}] + n[R_{tot}] + K_D)^2 - 4n[R_{tot}][L_{tot}]}} \right)}$$

Simulated ITC traces

Kd	1.00E-09
L (start)	5.00E-09
R	1.00E-07
n	1



ITC can give informations about binding mechanisms



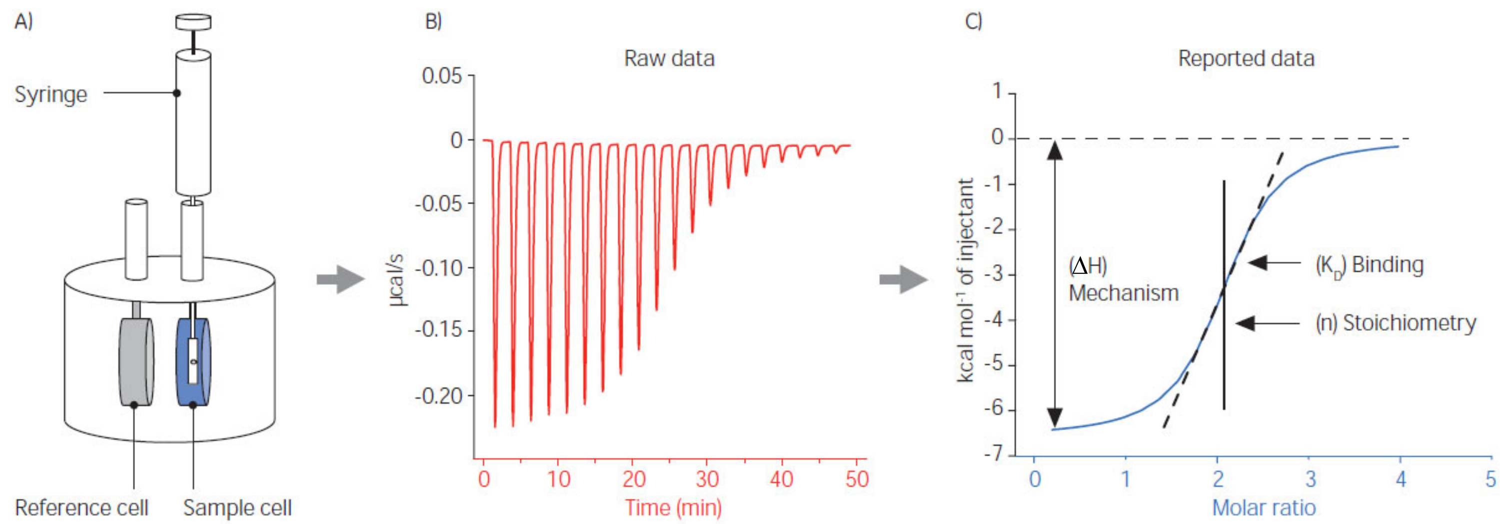
…all three binding reactions have the same ΔG.

A) good hydrogen bonding (enthalpy) and unfavorable conformational changes.

B) Hydrophobic interactions drive binding

C) both favorable enthalpic interactions and hydrophobic interactions

Isothermal titration calorimetry



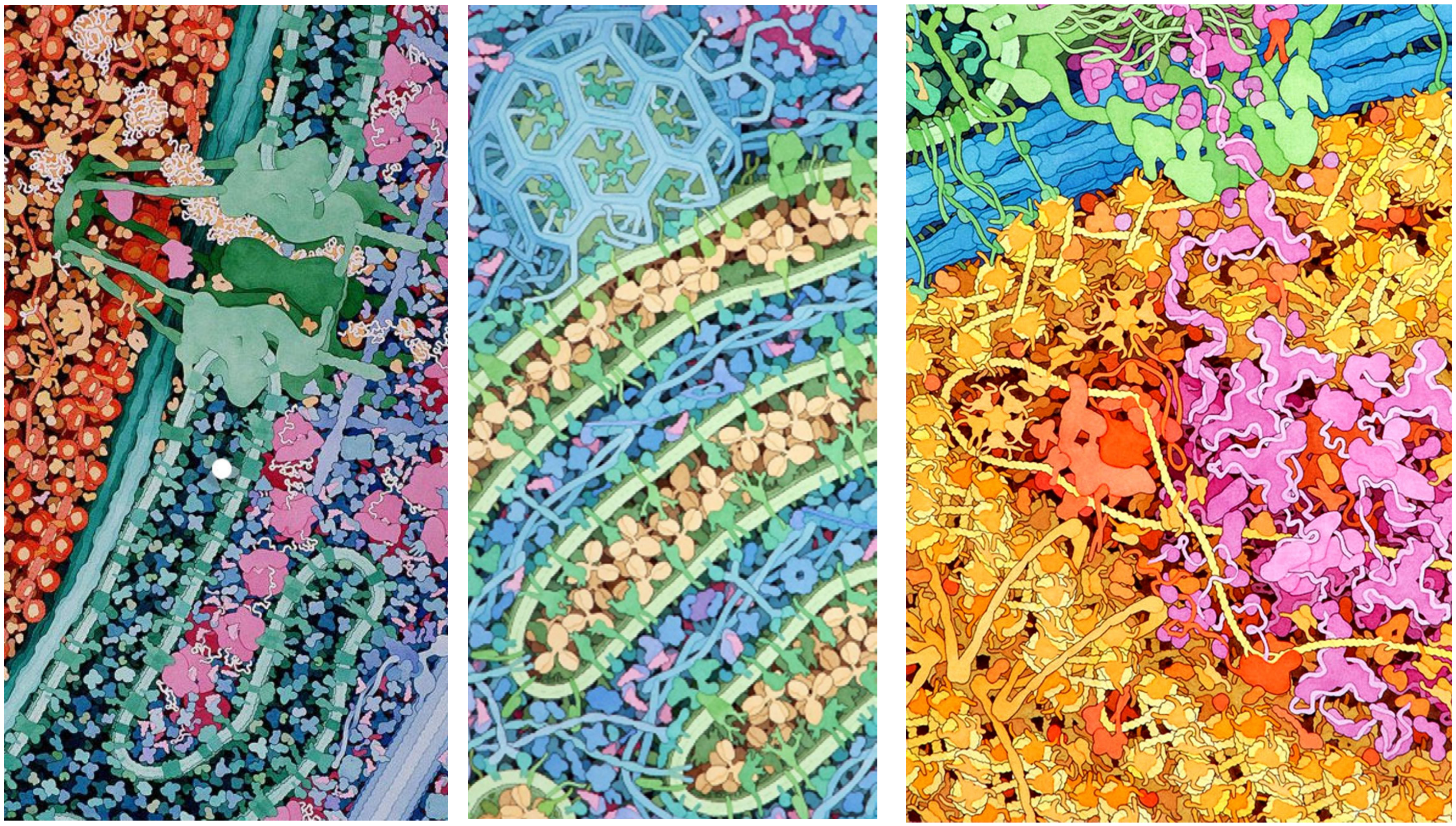
injection of ligand solution into receptor solution

measurement of heat of binding

Fitting to **appropriate model:** determination of all parameters of a binding reaction

for useful traces, $[R_{tot}]/K_D * n$ = 5 to 500 (10 to 100 ideal)

In cells, interactions are managed by compartmentalization



David Goodsell