

- In differential scanning calorimetry, two solutions are heated
- Due to chamber A containing proteins, $C_p(A) > C_p(B)$
- From this, get C_p of protein as a function of T , and the "Melting Curve"

$$\Delta G_{\text{unfolding}} = \Delta H^{\circ}_{\text{unfolding}} - T \Delta S^{\circ}_{\text{unfolding}}$$

general formula

$$H(T_2) - H(T_1) = \int_{T_1}^{T_2} C_p(T) dT$$

$T_m = \text{melting temp} \Rightarrow \Delta G^{\circ}_{\text{unfolding}} = 0 \text{ at } T_m$ equilibrium of unfolding (50/50)

$$\Rightarrow \Delta S^{\circ}_{\text{unfolding}}(T_m) = \frac{\Delta H^{\circ}_{\text{unfolding}}}{T_m}$$

unfolding ← folding

Observe that $C_p^U > C_p^F$

Why? • exposure of hydrophobic residues to water
• increased surface area
• greater conformational freedom

$$\text{From } \Delta H_1 = \int_{T_1}^{T_2} C_p(T) dT; \Delta S = \int_{T_1}^{T_2} \frac{C_p}{T} dT$$

$$\text{get } \Delta H^{\circ}_u(T) = \Delta H^{\circ}_u(T_m) + \Delta C_p (T - T_m)$$

$$\Delta S^{\circ}_u(T) = \Delta S^{\circ}_u(T_m) + \Delta C_p \ln\left(\frac{T}{T_m}\right)$$

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

$$\Delta G = \Delta H_m \left(1 - \frac{T}{T_m}\right) + \Delta C_p \left\{ \left(T - T_m\right) - T \ln\left(\frac{T}{T_m}\right) \right\}$$

* expected to derive this formula. Expect on test next week

To graph, you need $\Delta H_m, T_m, \Delta C_p$. You get this from experimental data

Plot this for a protein

G-81 protein (56 residues)

$$\Delta C_p = 53 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$$

per residue

$\Delta H_{\text{unfolding}}(T_m) =$

4.58 kJ/mol per residue

$$T_m = 85^\circ \text{C}$$

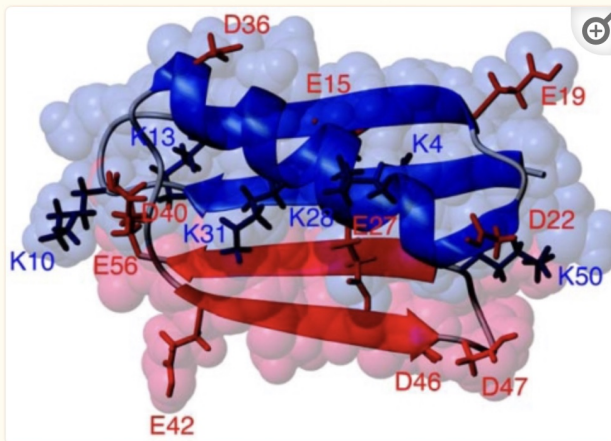
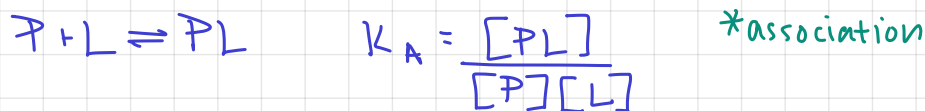


Figure 1.

The structure of PGB1 with residues 1 - 40 in blue and 41 - 56 in red with the backbone shown as a ribbon diagram on top of the space-filling model. Charged sidechains are shown as sticks with Asp and Glu side chains in red and Lys side-chains in dark blue. The figure was prepared from PDB file 2GB1 using MolMol software [35].

Observation: the unfolded protein is stable at high Temp; $T > T_m$
and at low T (but water freezes)
But needs to be above water freezing point

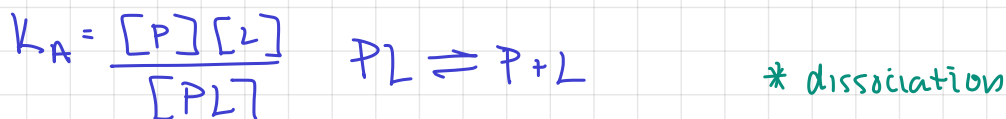
Thermo of ligand binding (during binding)



$$\Delta G_{\text{binding}}^\circ = -RT \ln K_A$$

initial conditions
* 1 mol/L

↳ one molar solution of each species



$$\Delta G_{\text{binding}}^\circ = +RT \ln K_A$$

K_p is how we report the binding affinity

"nanometer" affinity $K_p \sim 10^{-8} \text{ M}$