Lecture 8: Thermodynamics & Protein Stability

Assigned reading in Campbell: Chapter 4.4-4.6

Key Terms:

- $\Delta G = \Delta RT \ln K_{eq} = \Delta H \Delta T \Delta S$
- Transition Curve, Melting Curve, Tm
- ΔH calculation
- ΔS calculation
- van der Waals forces
- Electrostatic interactions
- Hydrogen bonds
- Hydrophobic effect
- Determining ΔH (van't Hoff Plot)
- Determining ΔS

Molecular Interaction	Enthalpy or Entropy?	Stabilizes Folded State (yes/no)	Relationship to structure of folded state
Van der Waals		Yes	Tight packing of residues in non-polar core
Hydrogen Bond			
Configurational Entropy S=RlnW	Entropy		
Hydrophobic Effect			

8.1 Comparison of Calculated and Predicted Thermodynamics for Protein G

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

Enthalpic Term: Hydrogen Bonds

Assume that a hydrogen bond to water has an enthalpy of -20 kJ/mol, while in secondary structure the enthalpy is -22 kJ/mol. Both relative to no hydrogen bond at all. The enthalpy change/H-bond is then:

$$\Delta H = H_{water} \Delta H_{protein} = \Delta 20 \Delta (\Delta 22) = +2 kJ/mol$$

Assuming 60 residues, the total enthalpy change is +120 kJ/mol. In other words, 120 kJ/mole would have to be put into the system to break all of the main-chain hydrogen bonds.

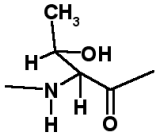
Entropic Term: Configurational Entropy

The entropy is given by S = R lnW.

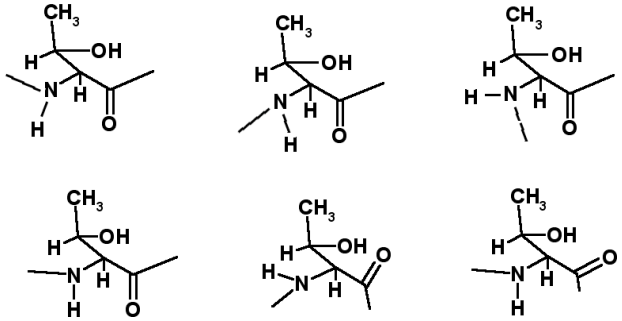
In the *folded* state, W = 1, therefore S = 0
In the *unfolded state*, each residue can assume 9 different combinations of ∏ (3) and ∏ (3) torsional angles. Therefore, the entropy of a single residue in the unfolded state is 9.

For a polypeptide of length N, the number of conformations is $W = 9^N$, giving an entropy of S = NR ln9. In the case of protein G, with its 60 residues:

Folded Protein (Native)



Unfolded Protein



A comparison between the calculated and measured thermodynamic properties:

Thermodynamic parameter	Calculated	Measured
ΔH	+120 kJ/mol (H-bonds only, 2 kJ/mol per H-bond)	+210 kJ/mol
ΔS	+1096 J/mol-deg (Main-chain entropy)	+616 J/mol-deg

The measured ΔH is larger than the calculated ΔH. Where does the excess enthalpy come from?

i. **van der Waals Forces**. Forces between atoms are attractive and occur between any pair of atoms at distances of 4-6 A° (polar or non-polar). Typical ΔG_s are 0.5-1.0 kJ/mol per pair of atoms. The interior of folded proteins is tightly packed. Proteins have very few cavities on the order of the size of a water molecule. Rather, each core side chain fits into a complementary space created by several (5-8) of the other core side chains.

ii. **Hydrogen bonds** are due primarily to partial electrostatic charges. Typical ΔG_s are 1-2 kJ/mol per H-bond. In addition to the main chain H-bonds that are found in regular secondary structure, there are also side chain-side chain as well as side chain-main chain H-bonds in proteins. These additional H-bonds contribute another ~0.5 kJ/residue.

iii. **Electrostatic Interactions.** Although most charged residues are on the surface of a folded protein and contribute little to the stability of the folded state (this is due to the high dielectric constant of water D=80), a charged residue is infrequently seen in the

core of a protein. In this case, there is a large energetic advantage in burying a group of opposite charge nearby (or a corresponding penalty for leaving it unpaired). This is due to the small dielectric constant of the hydrophobic protein core (D=2-4).

$$F = \frac{1}{4\pi\varepsilon_0}\frac{q_1 q_2}{Dr^2}$$

The measured ΔS is smaller than the calculated ΔS. Why? The unfolding of protein G results in a smaller change in entropy than that predicted from a consideration of conformational entropy. Apparently, the large increase in entropy of the protein that arises from releasing the restriction of ϕ and ψ angles is compensated by a decrease in entropy of something else in the system.

This additional decrease in entropy is due to the *hydrophobic effect* – "the driving force for protein folding". The hydrophobic effect is due to the entropy of the *water* in the system. When a non-polar side chain is exposed to water it orders, or decreases the entropy, of the water molecules. However, when the non-polar residue becomes buried in the non-polar center of the protein it releases all of the water that coated it. The released water can now freely diffuse in the solvent.

Energetic Grand Total:
A very rough estimate of the contribution of the above factors to ΔG for unfolding of an ~60 residue protein are (in units of free energy at T = 300K):

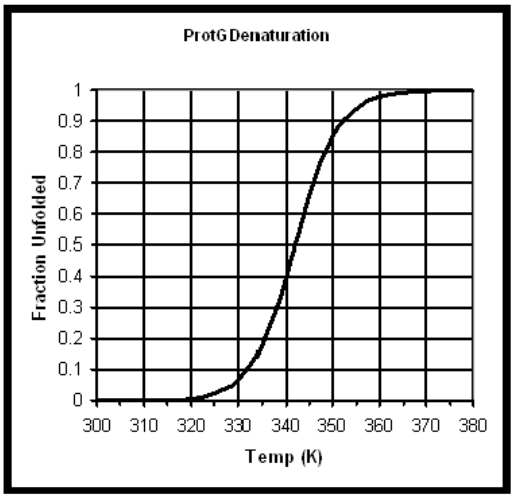
Energetic Term	**Contribution**	**Physical Description**
Conformational Entropy	-1,500 kJ/mol	Disorder of main and side chains
Hydrophobic Effect	+1,255 kJ/mol	Ordering of water molecules
Van der Waals	+170 kJ/mol	Breakage of van der Waals contacts in the protein core
Hydrogen Bonds	+45 kJ/mol	Breakage of main chain and side chain H-bonds
Electrostatic Interactions	+5 kJ/mol	Loss of favorable charge-charge interactions
Net Sum	**+25 kJ/mol**	

The bottom line: Proteins are only marginally stable under physiological conditions!

8.2 Determining ΔH and ΔS from Protein Melting Curves:

In the case of protein *denaturation*, where $N \rightleftharpoons U$

Melting Temperature, T_m: Since ΔG depends on temperature, a temperature exists at which $\Delta G = 0$. At this point native (N, folded) and unfolded (U, denatured) states are equal in energy and therefore equal in concentration. At $\Delta G = 0,\ K_{eq} = \frac{[U]}{[N]} = 1.$



At this temperature:

$$\Delta H = T_M \Delta S$$

Below this temperature, the native state is more stable: ($\Delta G > 0$), $K_{eq} < 1$

Above this temperature, the native state is less stable: ($\Delta G < 0$), $K_{eq} > 1$

Extracting ΔH and ΔS from a melting curve:

The temperature dependence of the equilibrium constant can be used to determine ΔH.

Equating the two expressions for ΔG:

$$\Delta H - T\Delta S = -RT \ln K_{eq}$$

$$\ln K_{eq} = -\frac{\Delta H^\circ}{R}\frac{1}{T} + \frac{\Delta S^\circ}{R} \qquad K_{eq} = \frac{[U]}{[N]}$$

This is the equation of a straight line. If ln K_{eq} is plotted versus 1/T, then:

Slope = $-\Delta H / R$ or ΔH = -R x slope

This plot is referred to as the **van't Hoff Plot**.

Example calculation:

Use the data from protein G for an example. The equilibrium constant at various temperatures is obtained from the melting curve. In practice, reliable values can only be obtained in the range of f_N = 0.95 to 0.05.
Selecting a couple of values from the melting curve:

T (K)	1/T	F_N	$K_{eq} = f_U/f_N$	ln(K_{eq})
330	.00303	0.93	.07/.93 = 0.08	- 2.70
340	.00294	0.60	.40/.60 = 0.66	- 0.41

(Remember $f_U + f_N = 1$)

Calculating the slope:

$$\Delta y/\Delta x = (-0.41-(-2.70))/(.00294-.00303) = 2.29/9\times 10^{-5} = 25,444$$

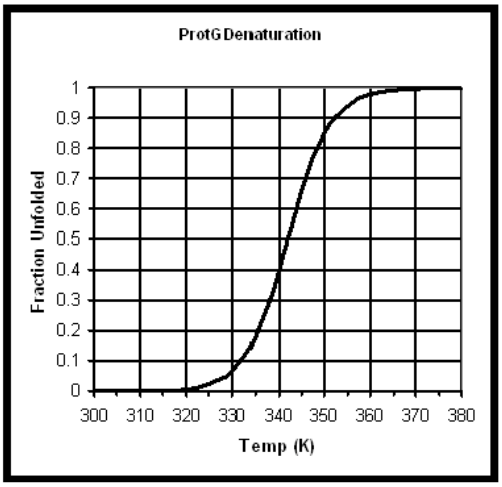
Therefore the ΔH is: **211 kJ/mol (25444 x 8.3 J/mol-K)**, where **R = 8.3 J/mol-degK**.

ΔS calculation:

Once ΔH is found, ΔS can be easily found:

$$\Delta S = \frac{\Delta H}{T_m}$$

Tm = 342K,
so ΔS **= 211,000 J/mol / 342 K = 616 J/mol-degK**



Common Errors:

- Forgetting to work in Kelvin
- Mixing kJ (enthalpy) and J (entropy).