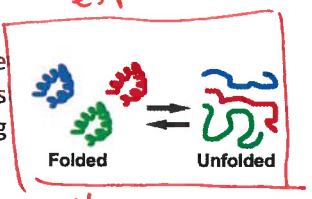
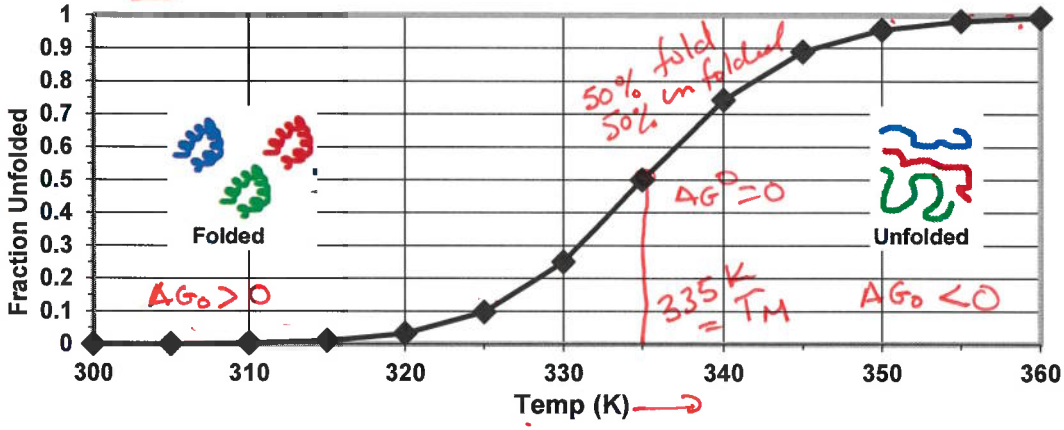
Lecture 9: Measurement of Protein Stability, Amphipathic Structures

- Identify helices and sheets based on location of non-polar residues.
- Determine the ΔH° and ΔS° from thermal denaturation curves.
- Predict amount of folded protein, given ΔH° and ΔS°.
- Interpret changes in ΔH° and ΔS° due to mutations.
- Use of thermal stability in drug discovery.

Thermal Denaturation of Proteins: The relative energy of the native and unfolded state can be changed with temperature. Unfolding occurs at high temperature due to the positive ΔS° during unfolding making ΔG°<0, due to the -T ΔS° contribution the standard energy.





$$f_u = \frac{[U]}{[N]+[U]} \qquad f_N = \frac{[N]}{[N]+[U]} \qquad K_{eq} = \frac{f_u}{f_N}$$

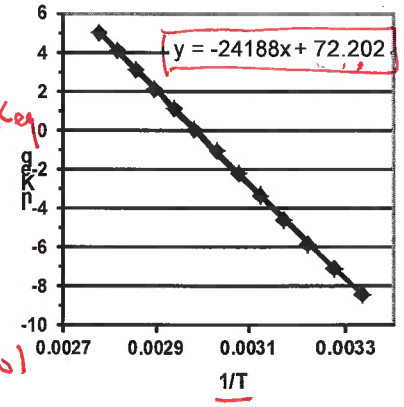
Obtaining ΔH°: The temperature dependence of the equilibrium constant can be used to determine ΔH°. Equating the two expressions for ΔG°:

$$\Delta G^o = \Delta H^o - T\Delta S^o = -RT\, lnK_{EQ}$$

$$lnK_{EQ} = \frac{-\Delta H^o}{R}\frac{1}{T} + \frac{\Delta S^o}{R}$$

$y = mx + b$

$-24188 = -\frac{\Delta H^o}{R} \Rightarrow \Delta H^o = 200\ kJ/mol$



This is the equation of a straight line, if lnK_{EQ} is plotted versus 1/T. This plot is referred to as a **van't Hoff** Plot.

Slope = -ΔH° /R **ΔH° = -slope × R**

Obtaining ΔS°: Once the enthalpy is known, the change in entropy can be calculated from the T_M and from ΔH°. At the melting temperature, T_M, the energy difference between the native and unfolded states is zero.

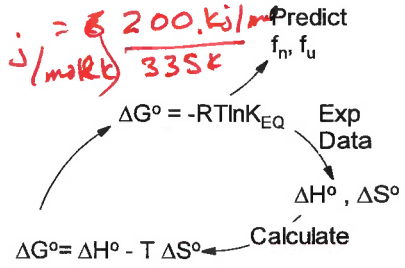
$$\Delta G^o = 0 = \Delta H^o - T_M \Delta S^o$$

$$\Delta S^o = \frac{\Delta H^o}{T_M} = \frac{200\ kJ/mol}{335\ K} = 600\ J/mol\,K$$

Applications of Protein Thermodynamics.

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

- ΔH° and ΔS° are obtained from experimental data
- ΔG° is calculated from ΔH° and ΔS°
- K_{EQ} is calculated from ΔG°
- f_N and f_U are predicted from K_{EQ}.



$\Delta S_{obs} = 600\ J/mol\text{-}K$

$$f_F = \frac{[F]}{[F]+[U]} = \frac{\frac{[F]}{[F]}}{\frac{[F]}{[F]}+\frac{[U]}{[F]}} = \frac{1}{1+K_{EQ}} \qquad f_U = \frac{[U]}{[F]+[U]} = \frac{\frac{[U]}{[F]}}{\frac{[F]}{[F]}+\frac{[U]}{[F]}} = \frac{K_{EQ}}{1+K_{EQ}}$$

Example 1: Given ΔH° and ΔS°, predict the fraction unfolded at any Temp.

You work for a company that uses an enzyme to make the amino acid Lysine, an important amino acid in dinosaur food at Jurassic park. Your supervisor tells you to increase the production of lysine. The reaction is normally run at 290 K (~ room temperature). You know that the rate of the reaction, and therefore the Lysine production, will increase at higher temperatures. Consequently, you increase the reaction temperature to 310K (37° C). Given the following thermodynamic properties of the unfolding of the enzyme used in the reaction: ΔH°= +300 kJ/mol, ΔS°= +1000 J/mol-K, have you just lost your job?

i. Calculate ΔG° at the required temperature, $\Delta G°=\Delta H°-T\Delta S°$

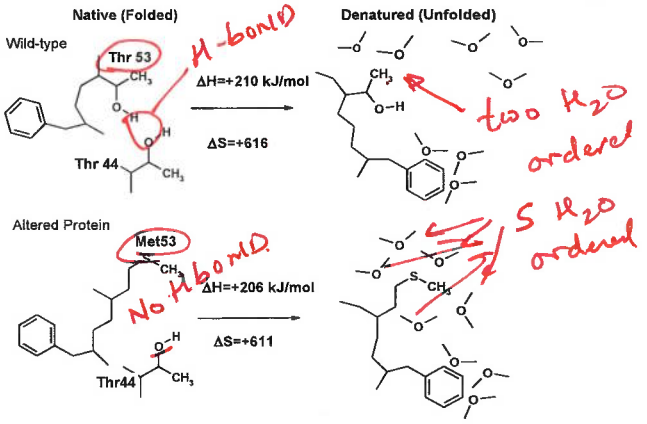
$\Delta G° = 300 - (310)x(1.0) = -10$ kJ/mol

ii. Calculate K_{EQ} at the required temperature: $K_{EQ} = e^{-\Delta G°/RT}$ **$= e^{+10/2.57} = e^4 = 48.9$**

iii. Calculate f_U using K_{EQ}: **$f_U = 48.9/(1+48.9) = 0.98$.**

Example 2: The denaturation curves for both wild-type and mutants of a protein were measured to obtain ΔH° and ΔS°. *Explain the effects on both ΔH° and ΔS°.*

	Wild-Type	Mutant (Thr → Met)
ΔH°	210.0 kJ/mol	206 kJ/mol
ΔS°	616 J/mol-deg	611 J/mol-deg
Tm	341 K	337 K



Analysis of Enthalpy Changes:

$\Delta H°_{OBSERVED} = \Delta H°_{VdW} + \Delta H°_{H\text{-}bond} + \Delta H°_{Electrostatic}$

Enthalpy to unfold the mutant is lower, indicating fewer interactions have to be broken to unfold. *What interaction is important here?*

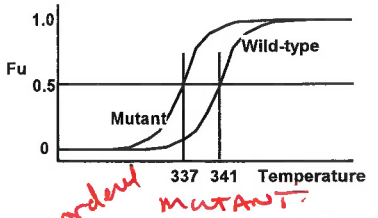
Analysis of Entropy Changes: $\Delta S°_{OBSERVED} = \Delta S°_{CONFORM} + \Delta S°_{H2O}$

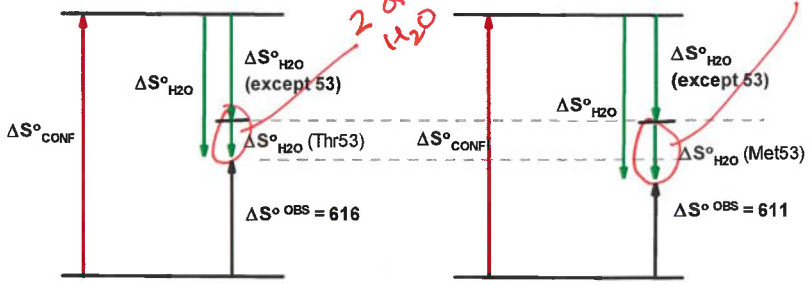
- Each ΔS° is a vector with sign and magnitude:
  - $\Delta S°_{CONFORMATIONAL}$ is large and positive for N→ U
  - $\Delta S°_{H2O}$ is large and negative for N→ U
- Consider each buried non-polar group to contribute to the overall hydrophobic effect:

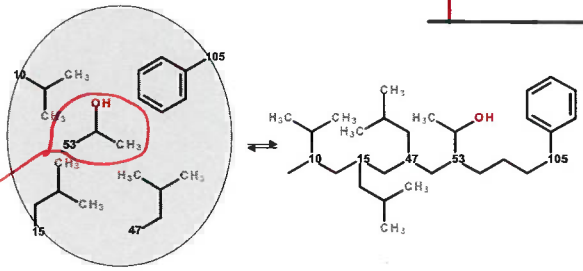
$\Delta S°_{H2O} = \sum \Delta S°_{1\text{-}H2O} + \Delta S°_{2\text{-}H2O} + \ldots + \Delta S°_{n\text{-}H2O}$

$= \Delta S°_{Not\ 53\ H2O} + \Delta S°_{53\ H2O}$

- The observed ΔS° for unfolding the mutant is smaller because $|\Delta S°_{H2O}|$ is larger for Met.

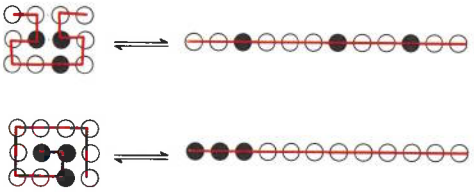


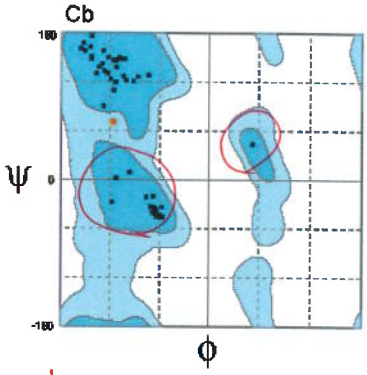




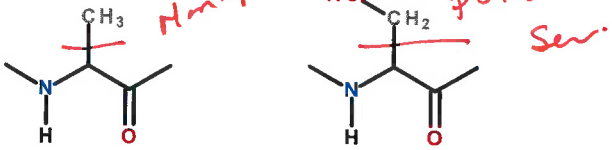
Interplay between 1°, 2° and 3° structure.

Both α-helices and β-sheets are equally stable from the perspective of vdw (both in low energy regions of the Rama. Plot) and H-bonds between mainchain atoms, why is one favored over another when a protein folds?





1. Name the following amino acids and indicate whether they are polar or non-polar.



2. H-bond the C=O of i to the NH of i+4 on the paper strip Where do you find the non-polar Ala sidechains on your helix?

NP NP NP NP
Ala-Ser-Ser-**Ala**-**Ala**-Ser-Ser-**Ala**-Ser-Ser
1 2 3 4 5 6 7 8 9 10

3 or 4th residue

3. What pattern is present in the distribution of polar and non-polar residues in this two stranded β-sheet?

NP NP NP NP
Ala-Ser-**Ala**-Ser-**Ala**-Ser-**Ala**-Ser

Ala-Ser-**Ala**-Ser-**Ala**-Ser-**Ala**-Ser

Many protein structural elements are amphipathic – meaning that one face is polar and the other face is non-polar.