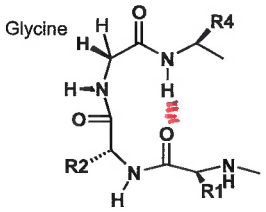
**Lecture 8: Non-Regular Secondary Structure. Energetics of Tertiary Structure**

**Goals:**

- Understand stabilization of regular turns.
- Relate molecular interactions to stability of proteins.
- Categorize enthalpic and entropic effects.
- Understand marginal stability of folded proteins.

**Non-regular secondary structures:** Sharp turns in proteins, particularly at the ends of beta-strands (beta hairpins) have a characteristic geometry and sequence. These turns are also stabilized by hydrogen bonding. These turns often contain Glycine at position 3, because of its unique conformational properties.




**Type II b-turn**

**Features of the Folded State of Globular Proteins:**

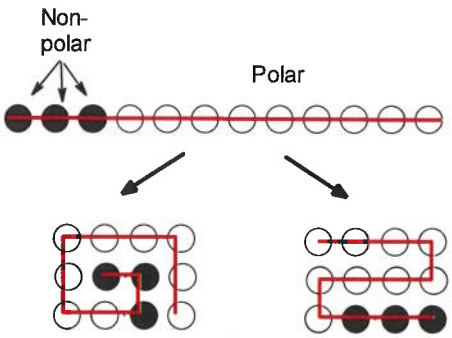
| Amino Acid Distribution | Location | |
|---|---|---|
| | Inside | Surface |
| Charged | | ✓ |
| Polar | | ✓ |
| Non-polar | ✓ | ✓ |

| Folded (Native) State (Tertiary Structure) | Denatured or Unfolded State |
|---|---|
| • Extensive 2° Structure | N |
| • Compact, well packed core | N |
| • Single Conformation | Large # |
| • Low exposure to solvent | Solvent exposure |

~100% Non-polar core.

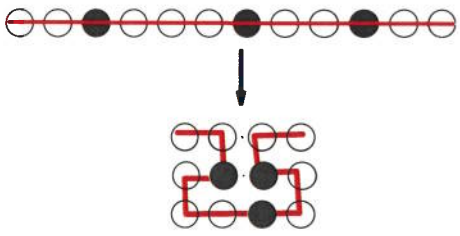
**The tertiary (folded) form of a protein depends (mostly) on the location of non-polar residues.**

A. Which is more stable? Why?




more stable (buried Non-polar)

B. How will this protein fold?



**Thermodynamic Factors that affect the Stability of the Native State:**

**ΔG°:** Change in the energy of the system when one mole of reactant(s) are converted to one mole of product(s).

$$\Delta G^\circ = G^\circ_{\text{Products}} - G^\circ_{\text{Reactants}}$$

ΔG° defines the equilibrium position of a reaction through the relationship: $\Delta G^\circ = -RT \ln K_{EQ}$.

We will consider the direction of the reaction to be: Folded (native) ↔ Unfolded

Both the enthalpy and entropy contribute to the overall Gibbs energy of the system as follows: $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$

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$N \rightleftharpoons U$ $\quad \Delta G^\circ > 0$

E: N ↓ U

- H-bonds
- electro.
- vdW
- hydrophobic effect
- chain entropy

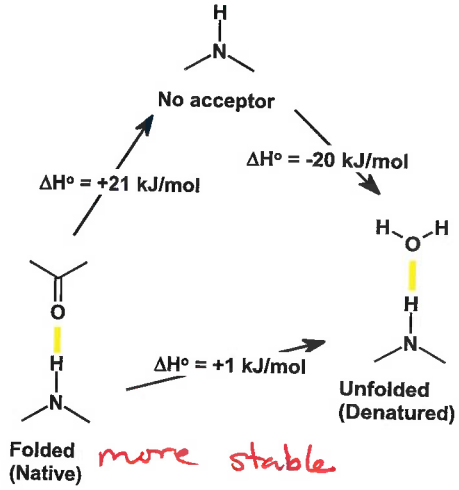
**Enthalpy ($\Delta H°$)** is the amount of heat generated/consumed by the reaction when 1 mole of reactants are converted to one mole of products.

$$\Delta H° = H°_{Products} - H°_{Reactants}$$

The enthalpy is related to changes in molecular interactions/electronic structure (i.e. hydrogen bonds, van der Waals, etc.). In biochemistry, these interactions usually involve **non-covalent** changes.

$\Delta H° > 0$ - unfavorable, the system absorbs heat during the reaction.

$\Delta H° < 0$ – favorable, the system releases heat during the reaction.




**1. $\Delta H°$ - Hydrogen bonds:**

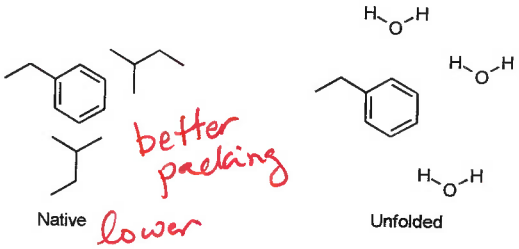
- $\Delta H°$ associated with hydrogen bonding is *favorable* for folding. Hydrogen bonds are more stable in the native form of the protein by about 1 kJ/mol.
- Hydrogen bonds that are broken during folding and then **not** reformed in the folded state cost about 20 kJ/mol.

***Reflection:*** *What is the cost if a protein folds and doesn't reform an internal hydrogen bond?*

destabilized by ≈ 20 kJ/mol

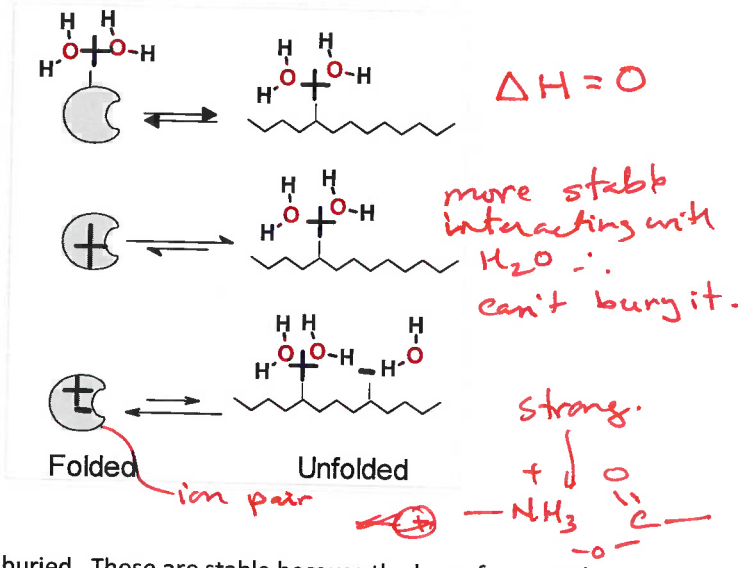
**2. $\Delta H°$ - Van der Waals forces.** This is unfavorable for unfolding. Van der Waals interactions are more stable in the native form of the protein.

***Reflection:*** *What enhances the stability of van der Waals interactions?*

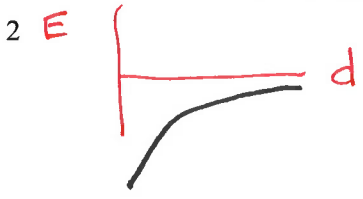



**3. $\Delta H°$ - Electrostatic forces:**

- **Surface charges:** Although these forces can contribute to $\Delta H°$ changes in many biochemical interactions, they are generally not important for protein folding because the charged residues remain on the surface and therefore interact with water equally well in both the native and the denatured state.
- **Buried charges:** The energetic cost of burying a single charge in the core of a protein is extremely high, largely due to desolvation of the ion during the folding process.




- In some proteins *charge-pairs* are buried. These are stable because the loss of energy due to desolvation is regained by favorable electrostatic interactions in a low dielectric media ($E \propto$



1/D).

**ΔS°** : Change in entropy when 1 mole of reactant(s) are converted to one mole of product(s).

$$\Delta S^\circ = S^\circ_{\text{Products}} - S^\circ_{\text{Reactants}}$$

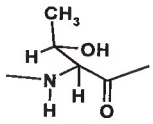
A positive entropy change is favorable since the disorder in the system is increased. The entropy is related to the change in the number of possible configurations (W) of the system when the reaction occurs. The entropy can be calculated from Boltzmann's equation:

**S = R *ln* W** *W is the number of conformations, and R is the gas constant.*

**1. ΔS° -Conformational Entropy of the Protein:** When a protein unfolds the entropy of the molecule increases dramatically due to a change in the conformational freedom of the Φ and Ψ angles of the mainchain, as well as disordering of the sidechain. We can estimate the number of conformations as follows:

Mainchain – since there are three stable secondary structures, the unfolded protein can assume one of three possible structures at any given time. It will inter-convert between these structures while unfolded. Therefore $W_{MC}^{\text{Unfolded}} = 3$.

Sidechain – We will assume at each sidechain can also exist in three possible conformations, $W_{SC}^{\text{Unfolded}}=3$

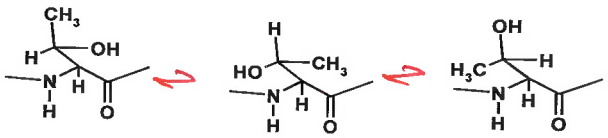


**Folded Protein**

$W = 1$

$S = R \ln W$

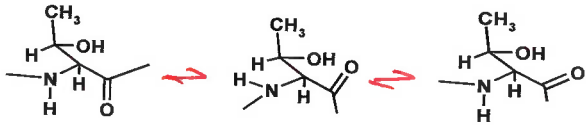
$S_N = 0$



Conformations of sidechain

$W_{SC} = 3$

X



right-handed helix | left-handed helix | beta

Conformations of mainchain

$W_{MC} = 3$

**Unfolded Protein**

$W = 9^{\text{residue}}$

The number of conformations in a 50 residue **folded** protein is: $W = 1$

Giving an entropy of : $S = 0$

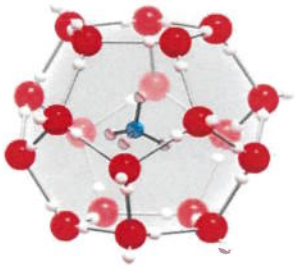
The number of conformations of a 50 residue unfolded protein is: $W = 9^{50}$

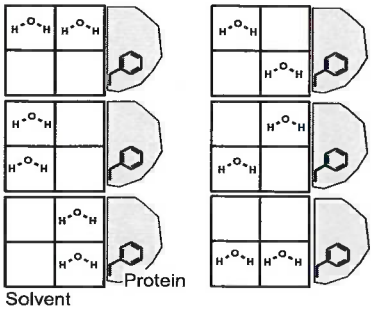
Giving an entropy of: $S = R \ln 9^{50}$

**The net change in entropy**, due to unfolding of a 50 res. protein: $\Delta S = S_u - S_N = R \ln 9^{50}$

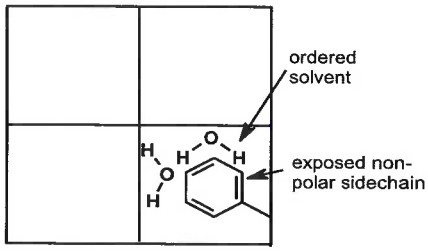
large & Pos ∴ stab. unfolded state

**2. ΔS° - Hydrophobic effect - Entropy Changes of the Solvent:** 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 which coated it. The released water can now freely diffuse in the solvent, resulting in an increase in entropy of the water, thus non-polar groups are "forced" into the non-polar core of the protein.






**Folded State**




**Unfolded State**

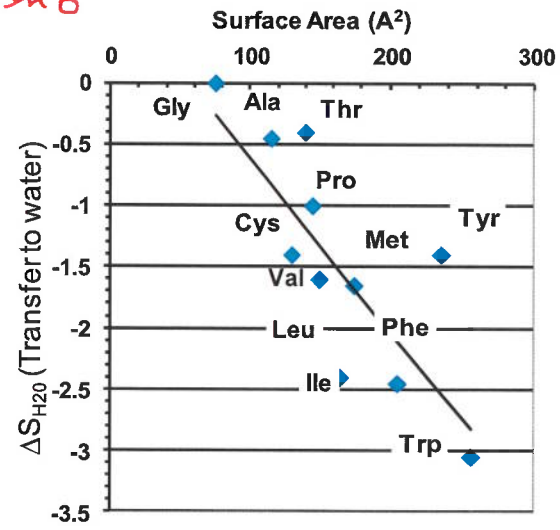
*The white squares represent solvent boxes. The gray area represents the folded protein.*
*How many ways can the two water molecules arrange themselves in the folded state?* $W = 6$ ($H_2O$)

*How many in the unfolded state?* $W = 1 = S_U = 0$ $S_N = R \ln 6$

*What is ΔS (=RlnW) for the* ***water*** *molecules in the folded state and in the unfolded state?*

$\Delta S_{H_2O} = S_U - S_N = -R \ln 6$

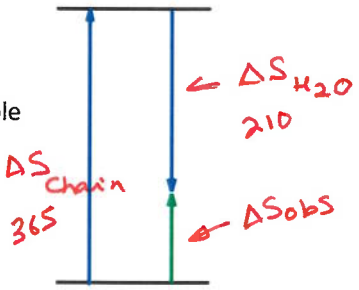
The **hydrophobicity** depends on the non-polar surface area. Glycine is set to zero on this plot to account for the contribution of the mainchain. Note that amino acids with larger non-polar sidechains cause a larger decrease in the entropy of the water when they become exposed. *Why?*




**Overall entropy change:**

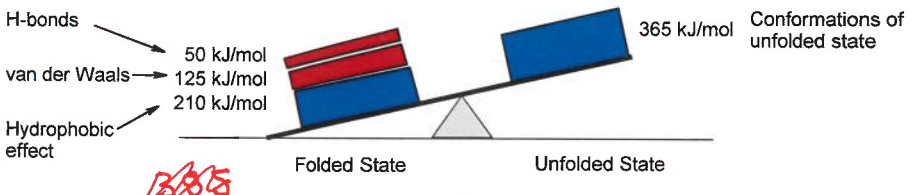
$$\Delta S°_{OBS} = \Delta S°_{Chain} + \Delta S°_{Solvent}$$

Note that these are opposite in sign, for N→U, $\Delta S°_{Chain}$ is large and positive (favorable), while $\Delta S°_{Solvent}$ is large and negative (unfavorable for unfolding), overall the entropy of unfolding is positive, favoring the unfolded form of proteins. The entropy changes can be represented as a vector diagram:




$$\overrightarrow{\Delta S_{OBS}} = \overrightarrow{\Delta S_{Chain}} + \overrightarrow{\Delta S_{Solvent}}$$

**Energy Balance:** An *estimate* of the contribution of the entropy ($-T\Delta S°$) and enthalpy to the overall energy of the folded and unfolded state for a 50 residue protein is illustrated below. Although the energies associated with each term are large, the overall difference in energy between the folded and unfolded state are quite small, about 20 kJ/mol in this case.






0 ← −385 → 365 (20 kJ)