Class 5

(Ch. 4, pp. 143-151)

Protein folding: Thermodynamics & kinetics

(how do unfolded polypeptides acquire their native, biologically active conformations?

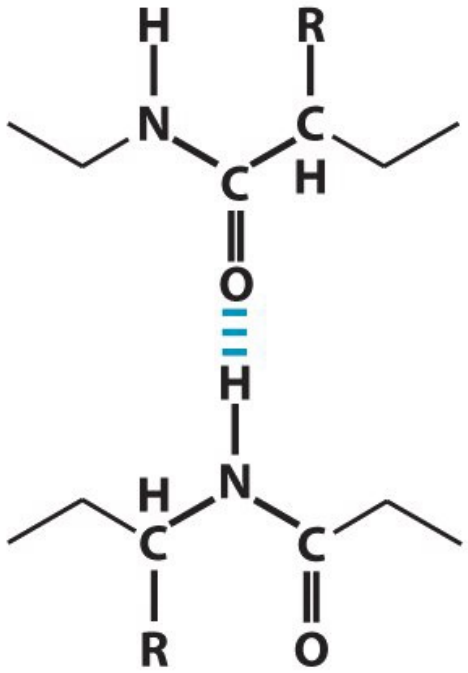
(Portions of this class will be taught using the blackboard)

Interactions that stabilize tertiary structure

	Bond energy (kcal/mol)
1. H bonds	2-5
2. Ionic interactions	10-20
3. Hydrophobic interactions	1-3
4. Van der Waals interactions	1
5. Disulfide bonds (only covalent one)	80

H-bonds

- H bond donor and acceptor within 3 Å of each other.
- Involves backbone and R-groups (in tertiary structure).
- Involves polar residues (if on surface of protein, can H bond with water).
- Individually weak, but additive effect – so significantly stabilizing.

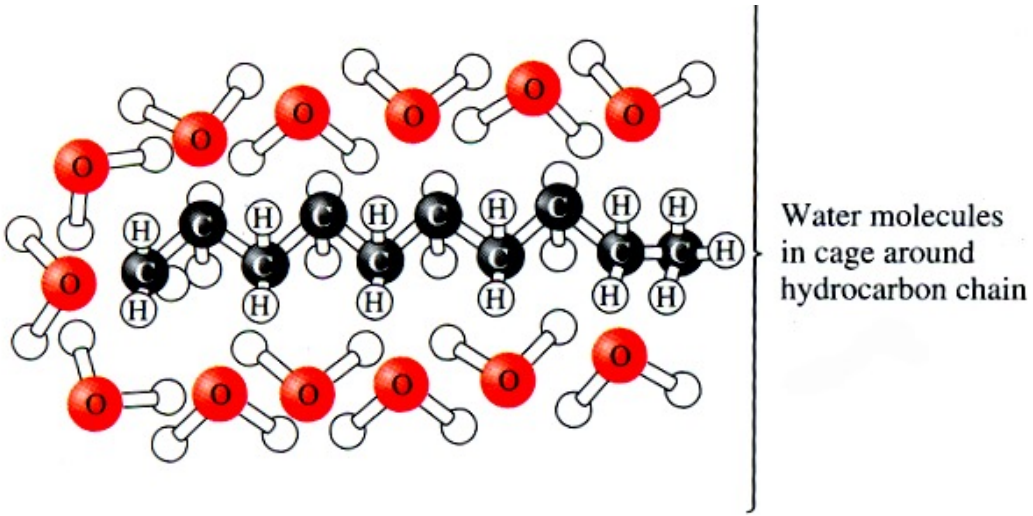


Ionic interactions

- Charge-charge interactions (salt bridge formation)
- Strongest of non-covalent bonds.
- Note that charged amino acids usually found on surface of protein and thus interact with ions in solution.

Hydrophobic interactions

- Very important; individually weak, but additive.
- Involve non polar R-groups – tendency is to hide non polar residues from water.
- Driven by entropy – maximize disorder in universe!



Transferring non polar R group from exterior (solvent-exposed) to hydrophobic interior of protein is thermodynamically favorable

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

Useful energy (ΔG) = total energy (ΔH) - useless energy ($T\Delta S$)

G = Gibb's free energy (useful energy can be used to do work)
H = enthalpy (heat content/bond energy)
S = entropy (disorder)

ΔG predicts if a reaction will be favorable or unfavorable

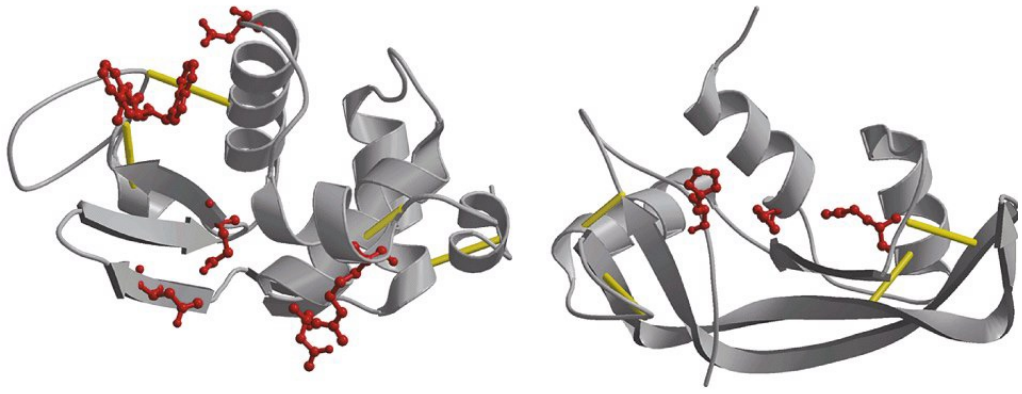
CH_4 in H_2O	**ΔH**	**$T\Delta S$**	**ΔG (kJ/mol)**
transferred to C_6H_6	11.7	22.6	-10.9

Van der Waal's interactions

- Weak attractive forces between electrically neutral molecules which have permanent or induced dipoles (additive).

Disulfide bonds (strong covalent)

- Usually few Cys in proteins, so not too many disulfide bonds (but small proteins utilize disulfide bonds to confer stability (ex. lysozyme, ribonuclease A).



Lysozyme

Ribonuclease

Interactions that stabilize tertiary structure

	Bond energy (kcal/mol)
1. H bonds	2-5
2. Ionic interactions	10-20
3. Hydrophobic interactions	1-3
4. Van der Waals interactions	1
5. Disulfide bonds (only covalent one)	80

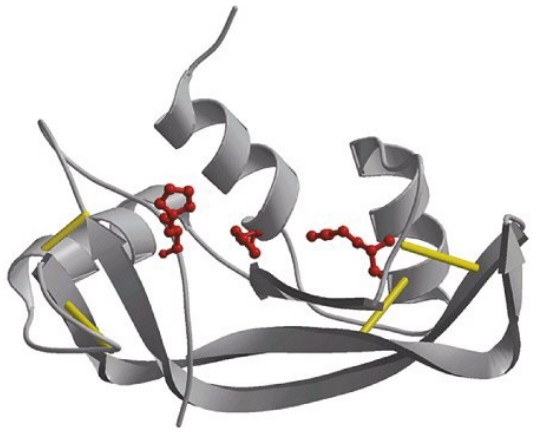
Quaternary structure stabilized by all of the above EXCEPT disulfide bonds!

How do unfolded polypeptides acquire their native, biologically active conformations?

- ***Observation:*** Most proteins fold spontaneously into their native conformations under appropriate physiological conditions.

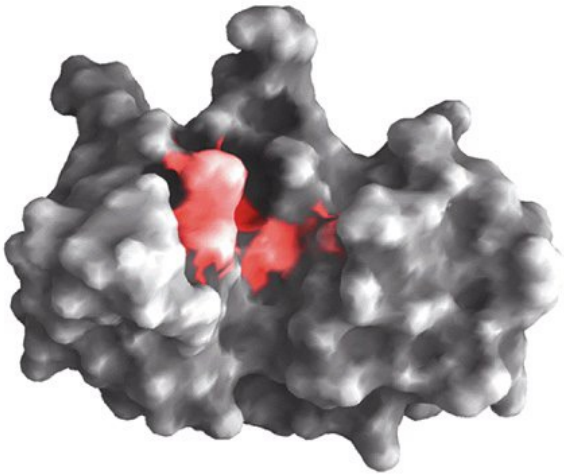
- ***Hypothesis:*** A protein's primary structure alone is sufficient to direct its folding.

- ***Proof:*** True (for the most part!! Read Kimchi-Sarfaty, 2007 paper) and was first demonstrated by Christian Anfinsen in 1957.

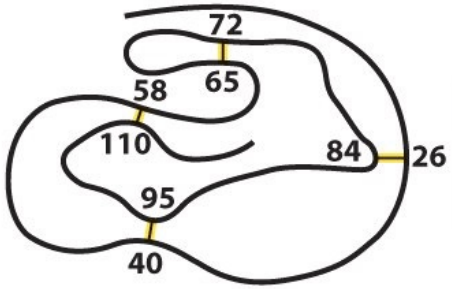


Ribonuclease

Denaturation/renaturation of Ribonuclease A



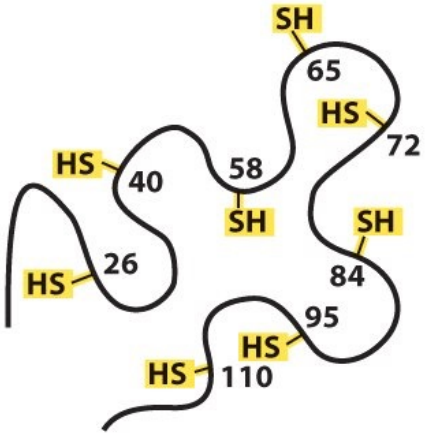
100% active



Native state; catalytically active.

addition of urea and mercapto-ethanol

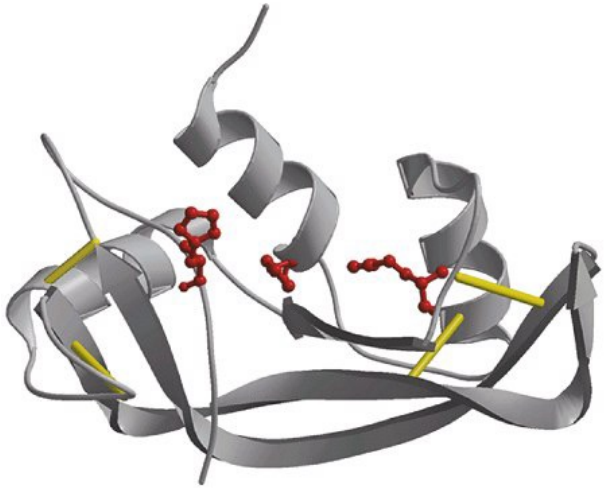
inactive



Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

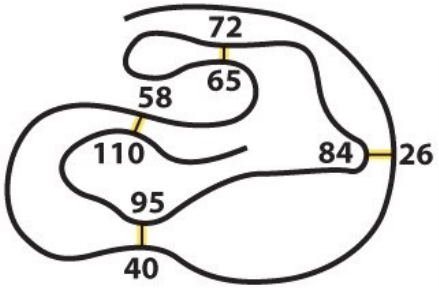
removal of urea and mercapto-ethanol

Slowly dialyze out



Ribonuclease

100% active



Native, catalytically active state. Disulfide cross-links correctly re-formed.

Figure 4-27

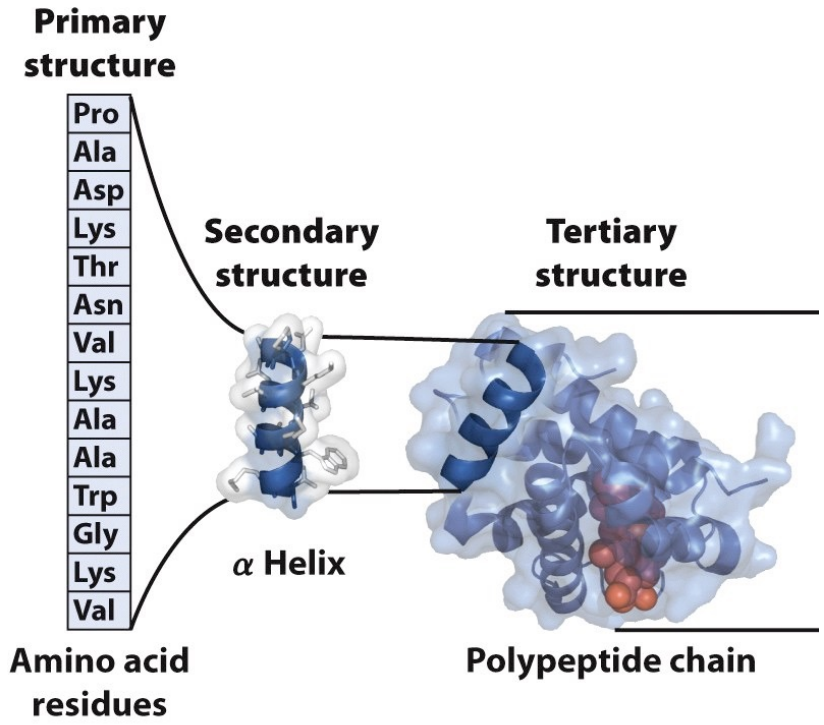
105 possible combinations of forming 4 disulfide bonds between 8 Cys residues

Moral of Anfinsen's story

- **Protein folding is not a random process – it is directed.**

The 3D structure of the native protein is ultimately determined by its primary structure (sequence).

- **The native state of RNaseA is probably its <u>thermodynamically</u> most stable conformation under physiological conditions.**

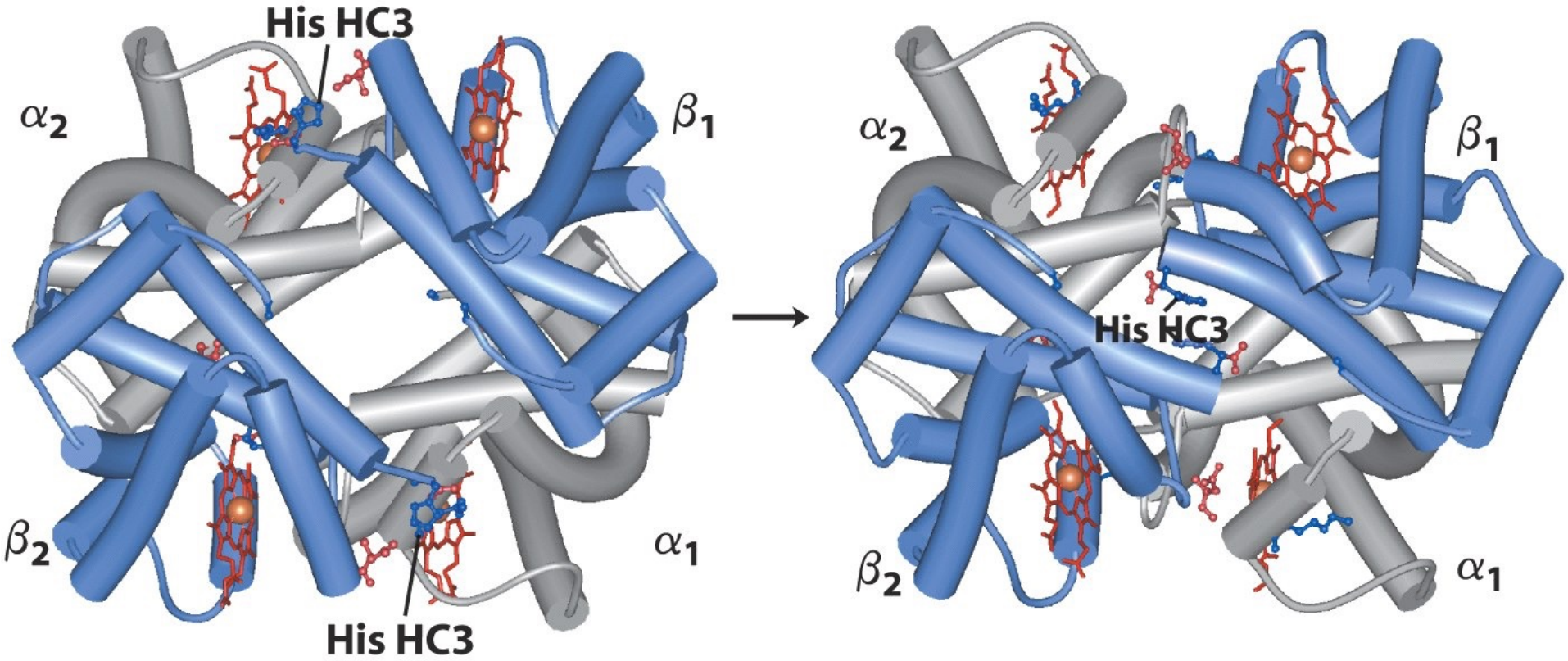


Thermodynamics of protein folding
on blackboard

Proteins are stable, yet flexible and fragile!

	Bond energy (kcal/mol)
1. H bonds	2-5
2. Ionic interactions	10-20
3. Hydrophobic interactions	1-3
4. Van der Waals interactions	1
5. Disulfide bonds (only covalent one)	80

Hb undergoes conformational changes to function as an oxygen-transporter

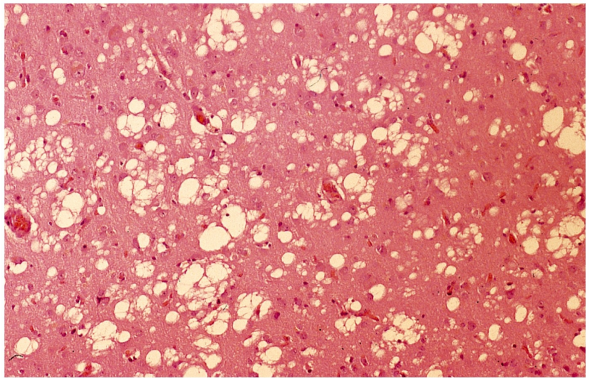


Deoxy-Hb

Oxy-Hb

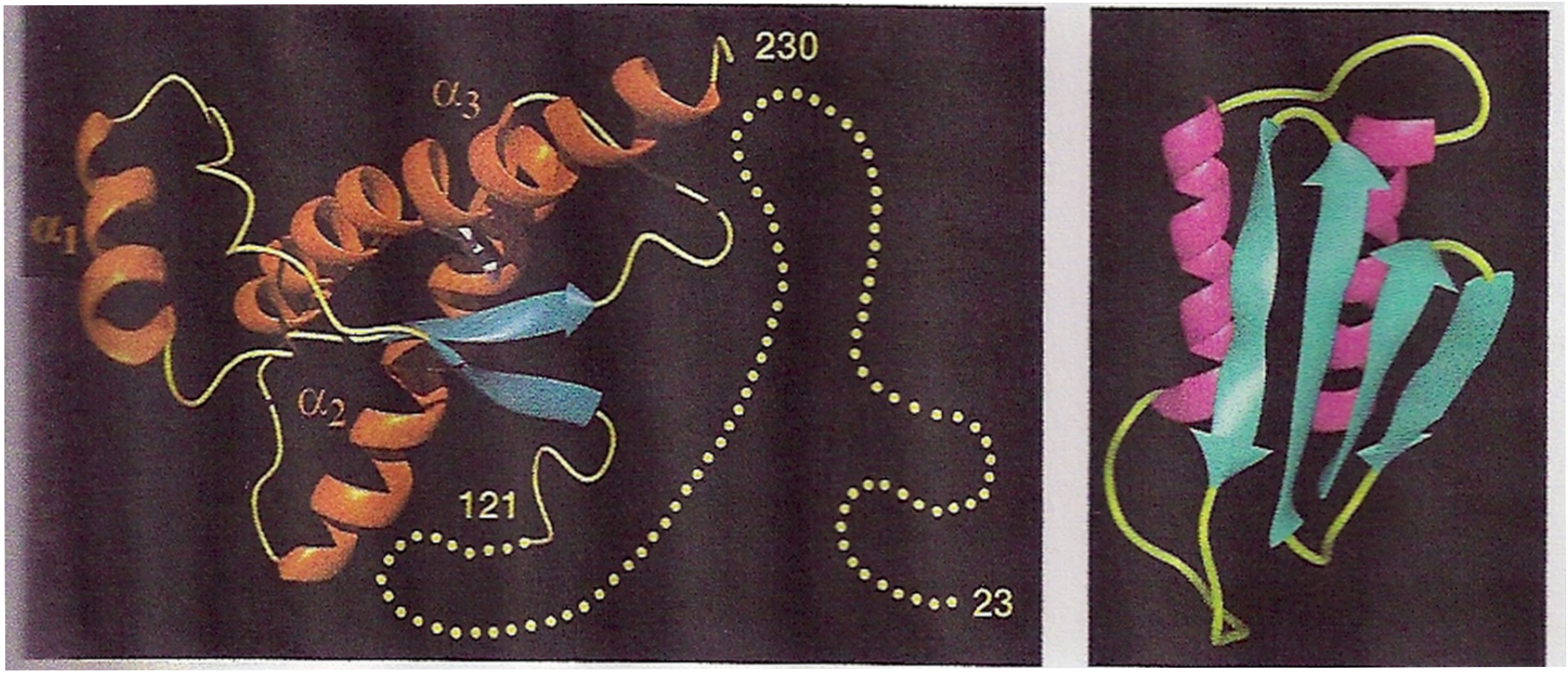
Kinetics (rate) of protein folding

Protein folding diseases -- Prions



PrP^{Sc}: refolded N-terminal domain of prion form

PrP^{c} : normal cellular protein



Voet & Voet, Biochemistry, 3rd ed.