

Protein folding & stability

The Levinthal Paradox

- There are vastly too many different possible conformations for a protein to fold by a random search.
- Consider just for the peptide backbone, there are 3 conformations per amino acid in the unfolded state, For a 100 a.a. protein we have 3^{100} conformations.
- If the chain can sample 10^{12} conformations/sec, it takes 5×10^{35} sec (2×10^{28} year)
- Conclusion: Protein folding is not random, must have pathways.

Proteins folding follow a distinct path

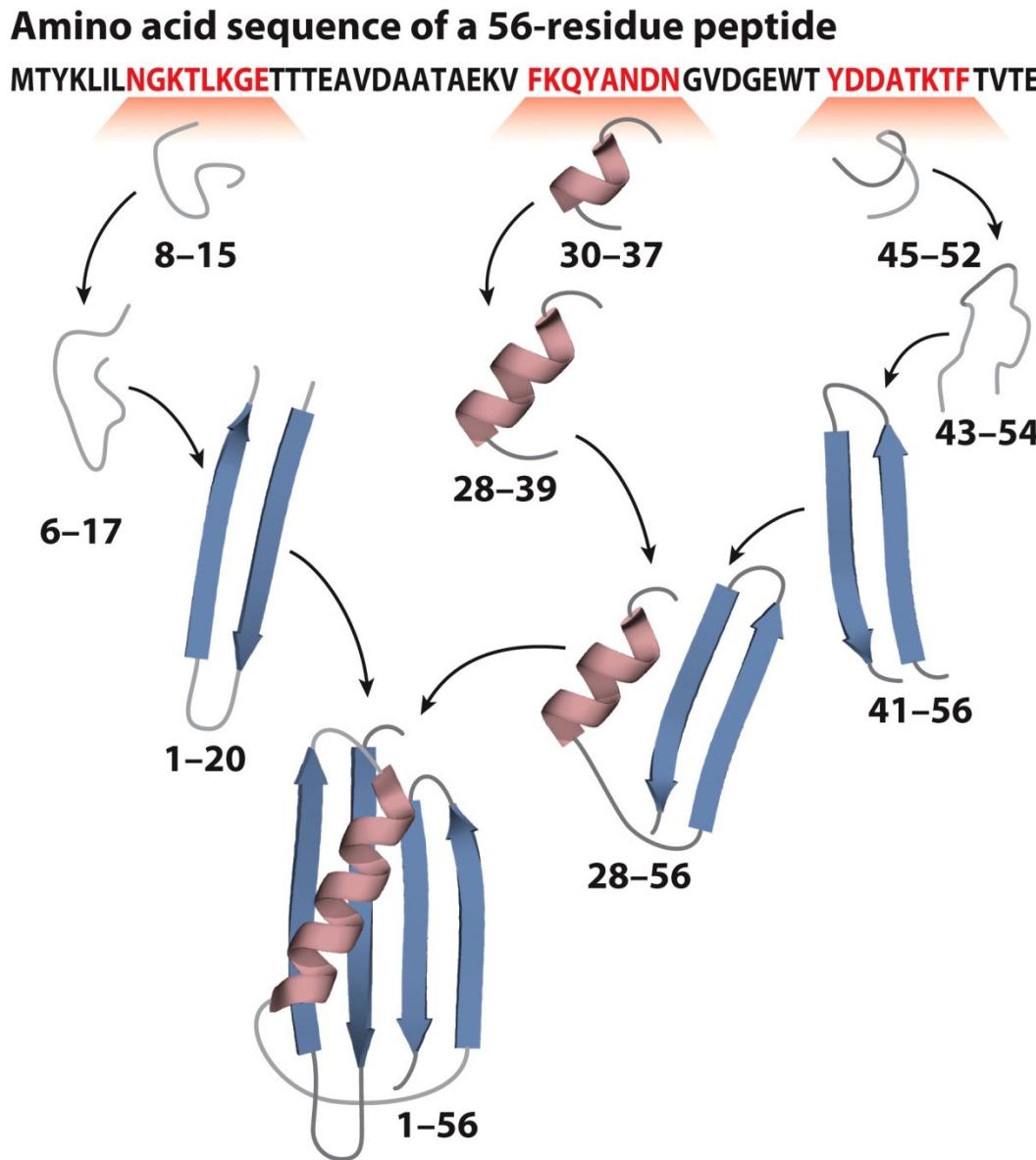
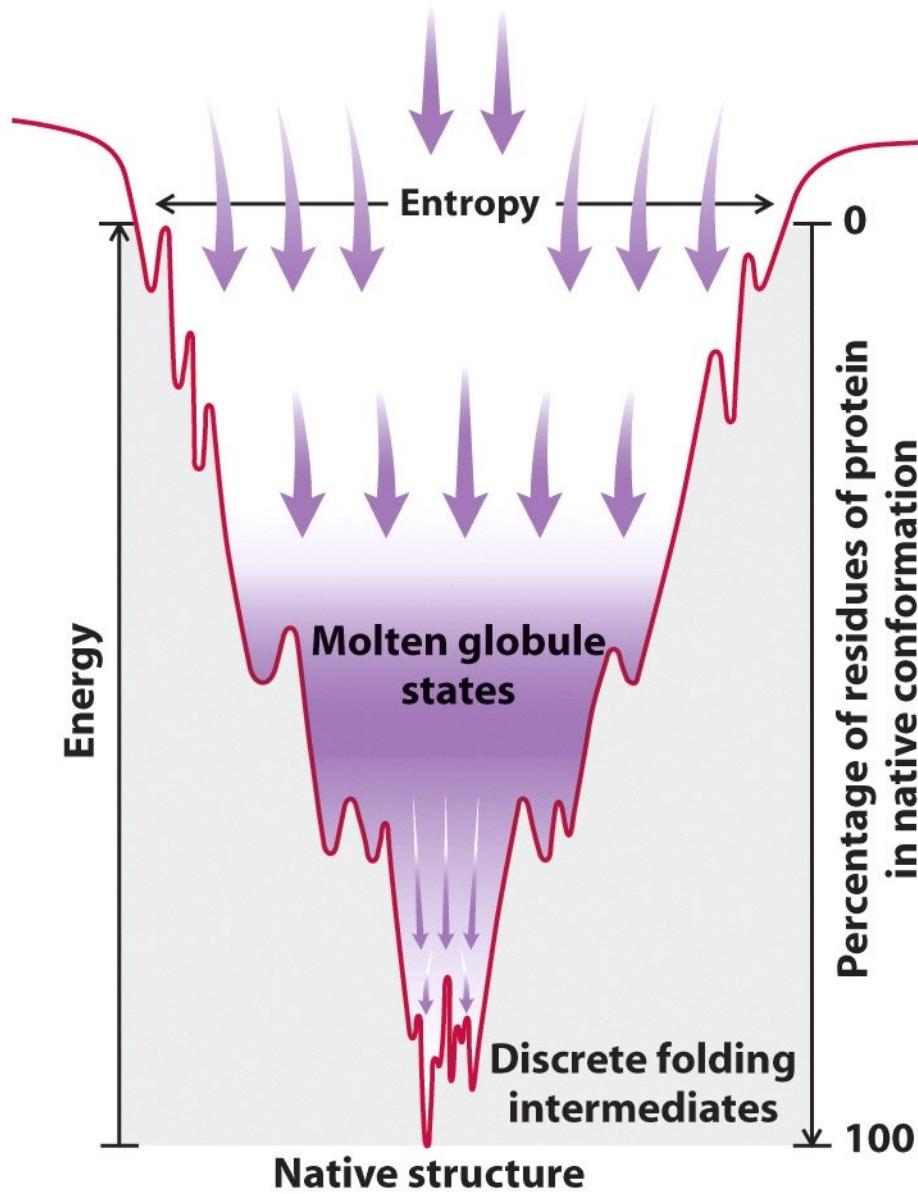
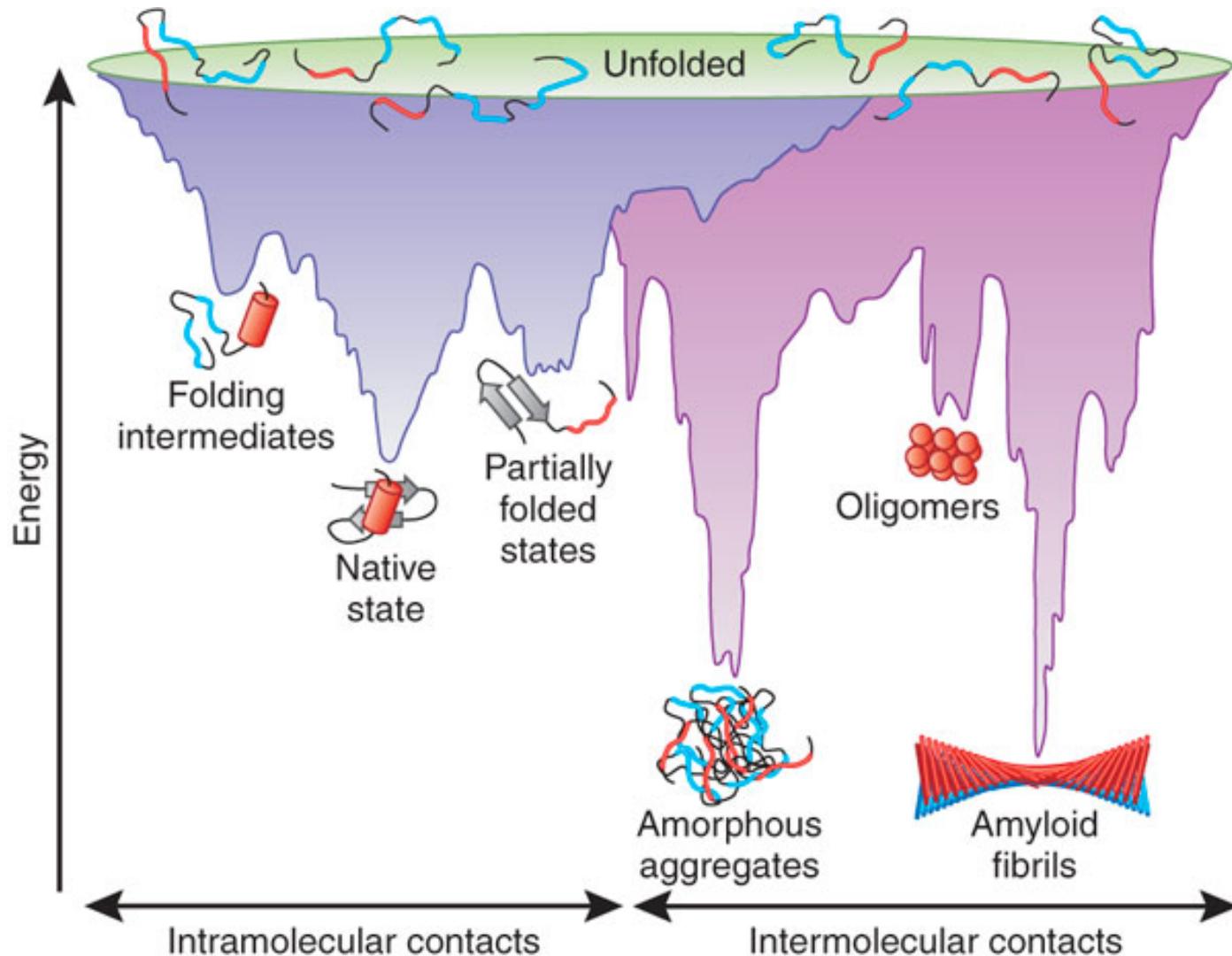


Figure 4-28

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Beginning of helix formation and collapse





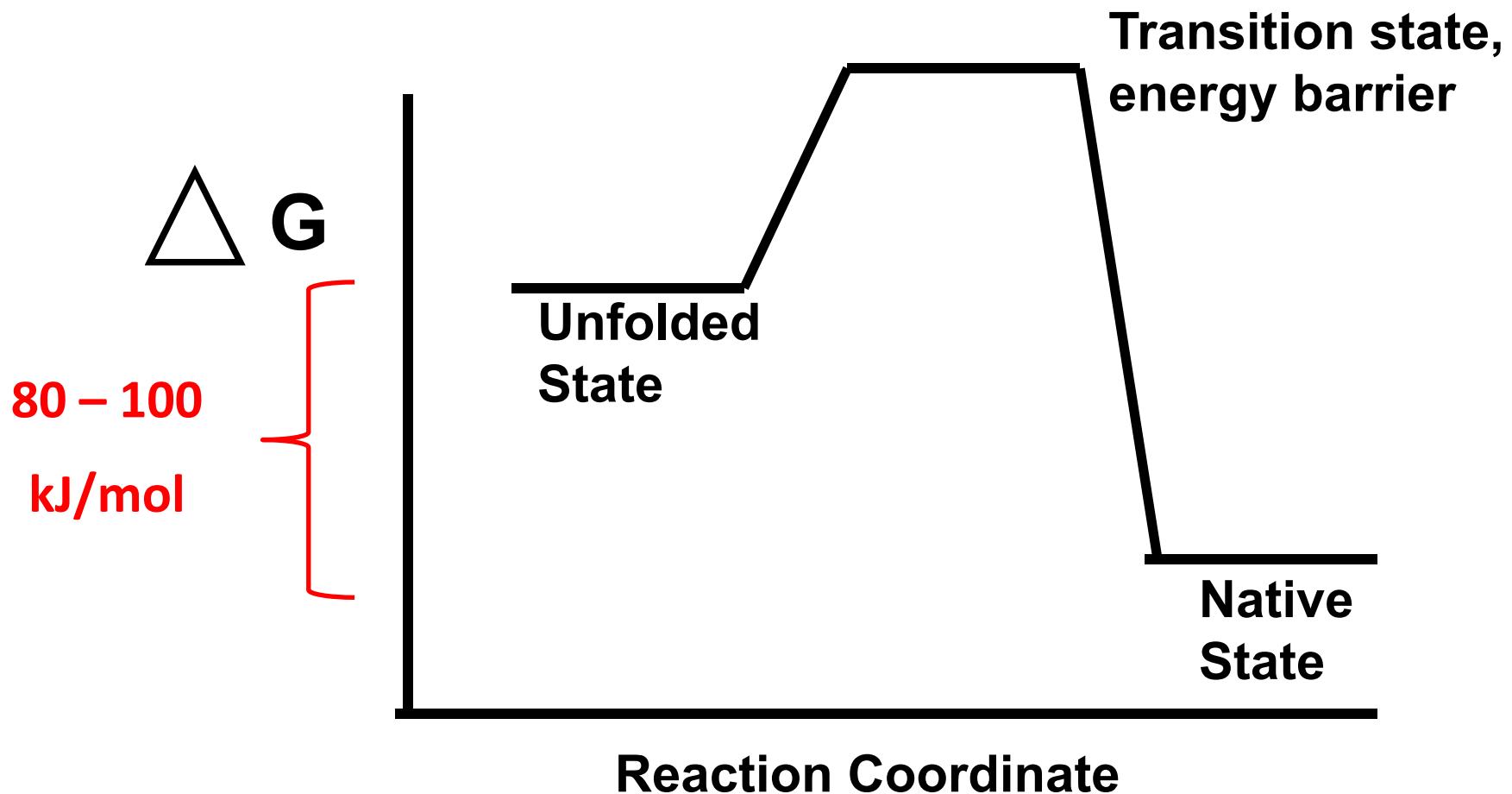
Stability of Proteins

- Stability of the folded structure of a globular protein depends on the interplay of three factors.
 1. The unfavorable conformational entropy change, which favors random chains instead.
 2. The favorable enthalpy contribution arising from intramolecular side group interactions.
 3. The favorable entropy change arising from the burying of hydrophobic groups within the molecule.
- Factor 1 works against folding, whereas 2 and 3 help stabilize folding.

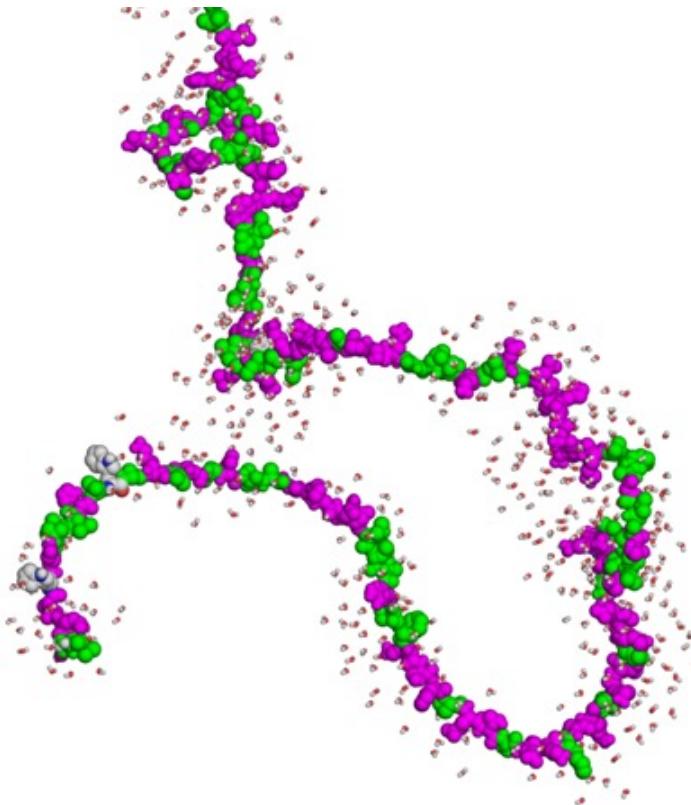
Protein are marginally stable

- The free energy difference between a typical 200-residue polypeptide in its folded versus denatured form is only of the order of 80–100 kJ/mol.
- In the unfolded state, intrachain non-covalent (stabilizing) interactions are not maximized, but extensive hydrogen bonding between appropriate amino acid groups and surrounding water molecules can ‘stabilize’ the denatured state.
- The second law of thermodynamics states that it is more energetically favorable for a molecule to exist in a random order (the concept of entropy).
- Marginal stability renders proteins somewhat flexible, enabling them to more readily undergo various conformational changes central to their biological activity.

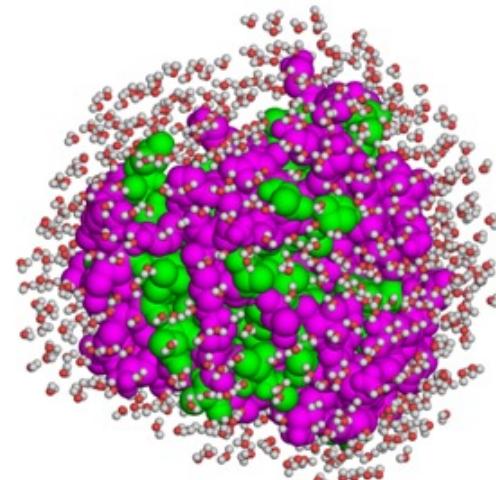
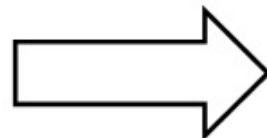
Protein folding thermodynamics



Protein folding thermodynamics



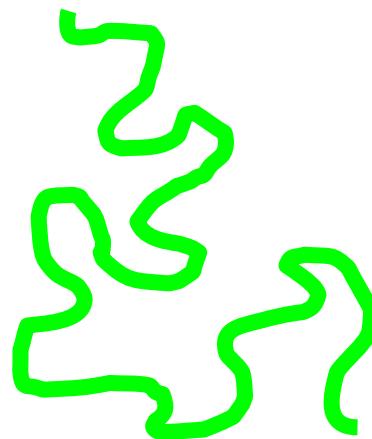
Unfolded



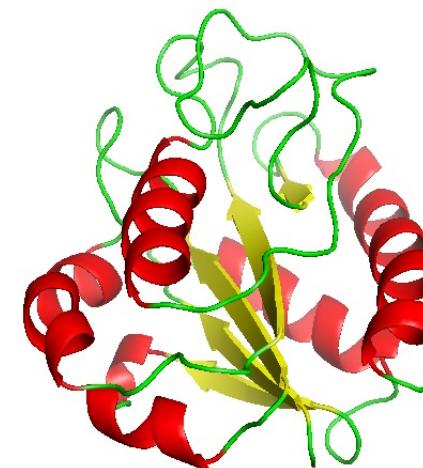
Folded

Native and denatured states

denatured ensemble
unfolded ensemble



native state
folded state



*many different structures fluctuating;
not usually very compact;
disordered but not a “random coil”*

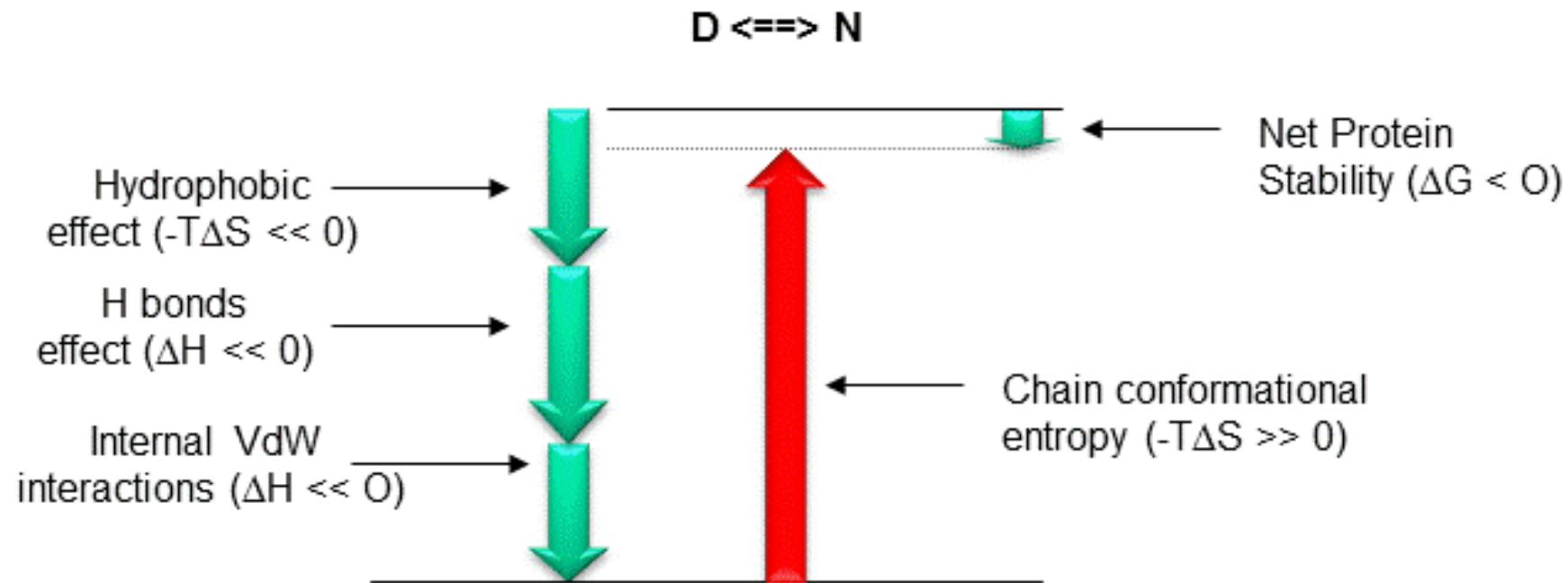


*single structure or ensemble
of very similar structures;
compact*

For some proteins, **but not all**, this process is readily reversible and occurs without populated intermediate forms--> “two-state” folding

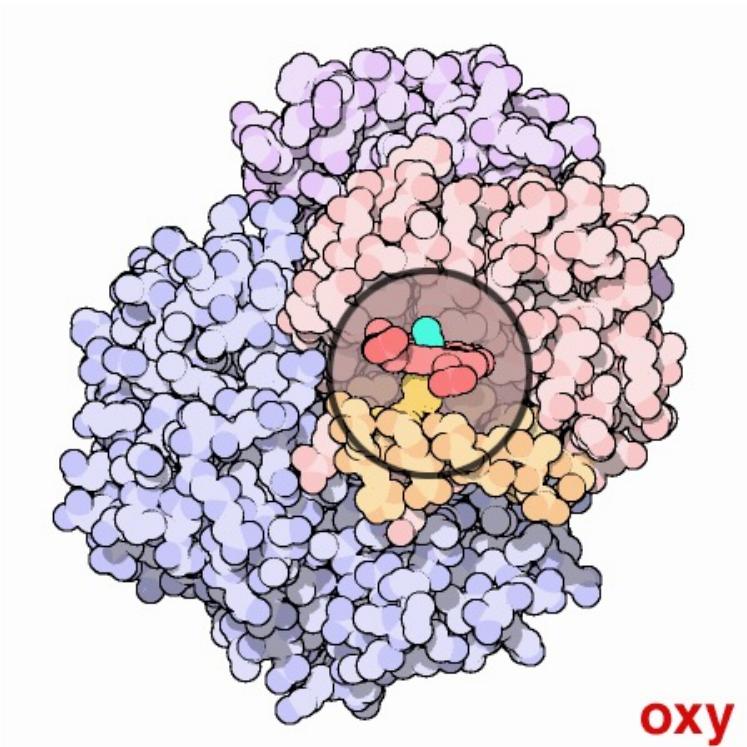
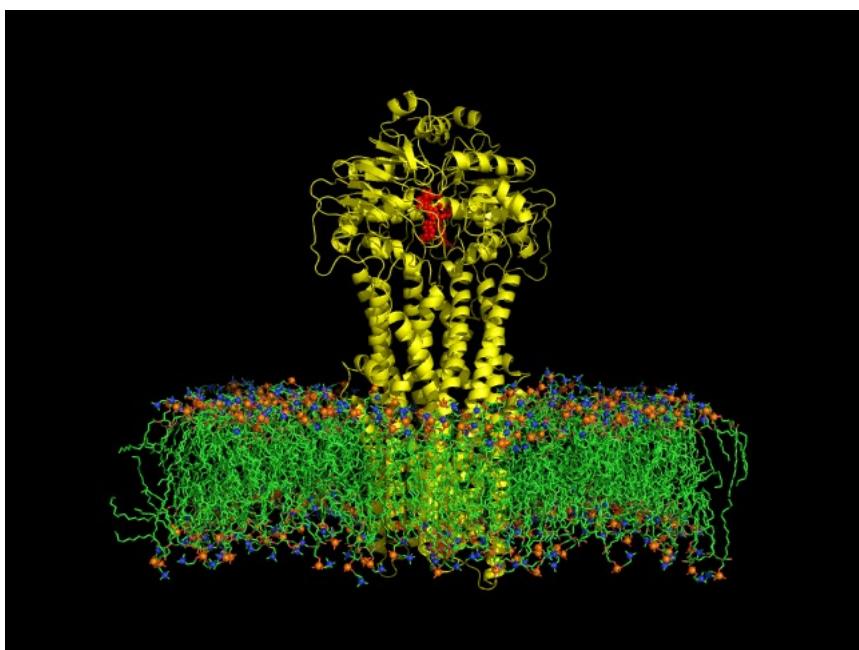
Contributions to free energy of protein folding

Thermodynamics of Protein Folding



Protein are marginally stable

- A protein's atoms are constantly in motion and groups ranging from individual amino acid side chains to entire domains can be displaced via random motion by up to about 0.2 nm.
- A protein's conformation displays a limited degree of flexibility and such movement is termed 'breathing'.
- Breathing can sometimes be functionally significant by, for example allowing small molecules to diffuse in or out of the protein's interior.
- Some proteins undergo conformational changes that are functionally significant. Most often they are induced by biospecific ligand interactions (e.g. binding of a substrate to an enzyme or antigen binding to an antibody).



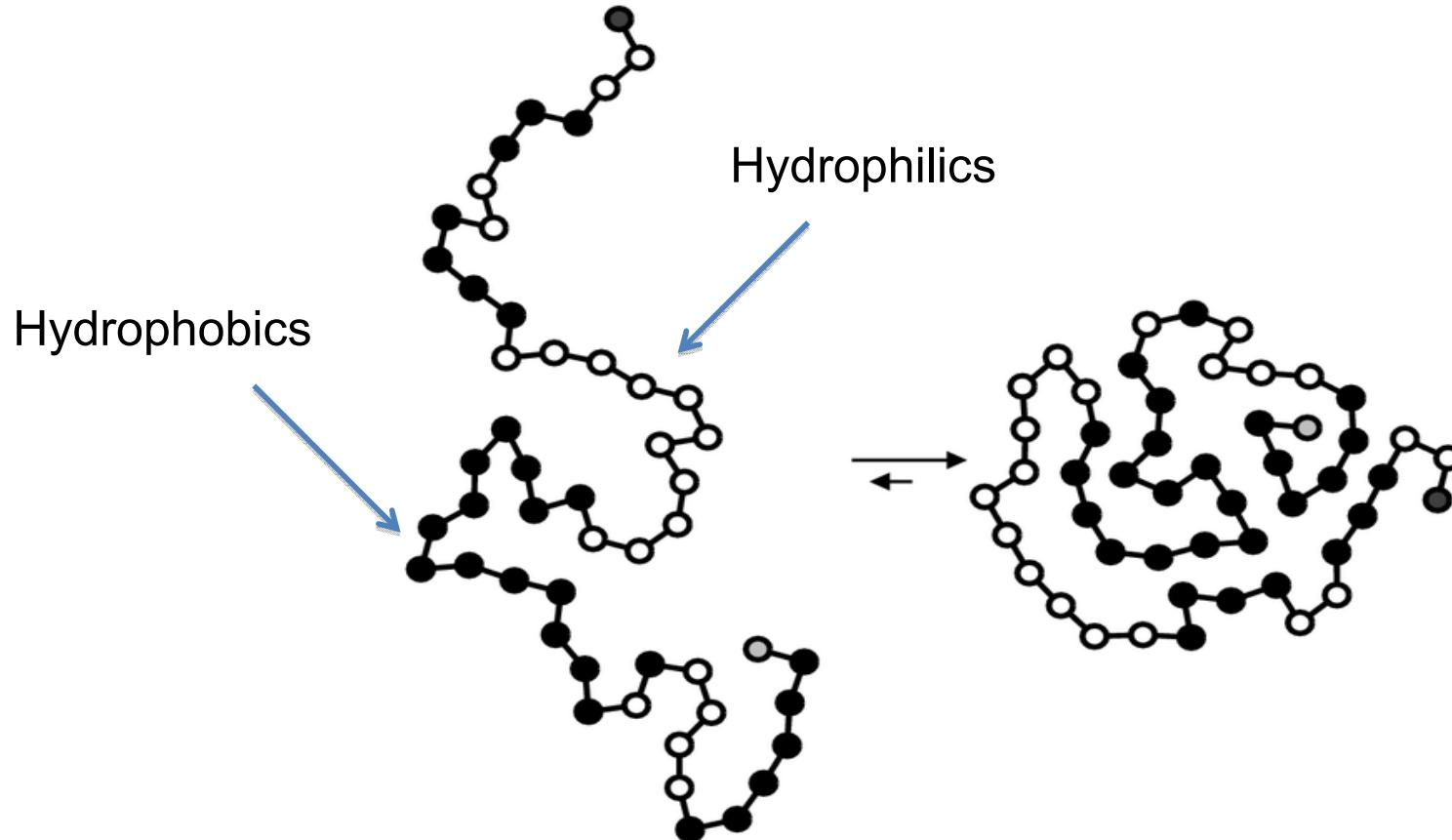
Protein folding

- This process is usually a rapid one, often lasting from under 1 second to several seconds
- The folding pathway proceeds via the initial rapid formation of the more compact, partially folded ‘molten globule’ followed by completion of the folding pathway at a slower pace
- The molten globule exhibits most secondary structural elements of the native protein, but only a limited degree of ultimate tertiary structure.
- Its formation, which requires only several milliseconds, is termed a **hydrophobic collapse**, which is driven by the favorable energy change achieved by bringing hydrophobic amino acid residues into contact with one another and away from surrounding water molecules.

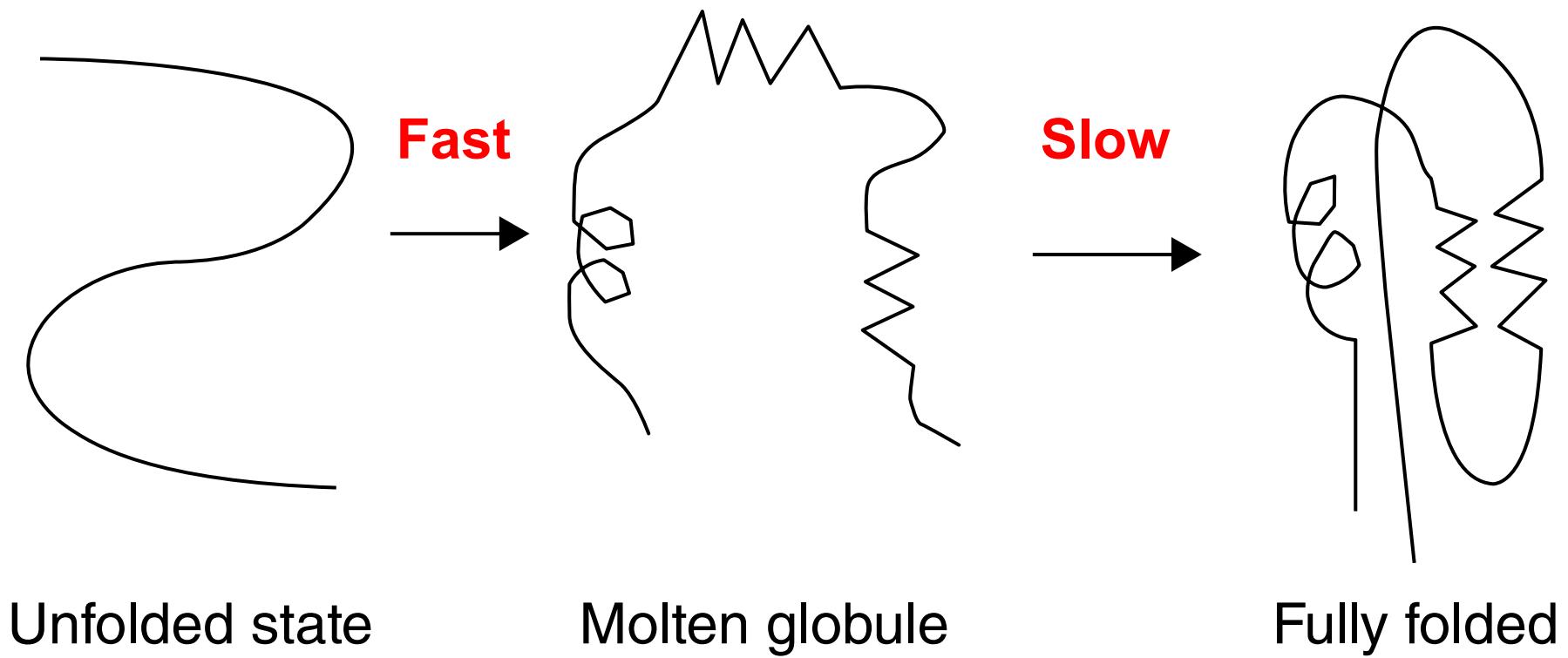
Hydrophobic Collapse

- Hydrophobic collapse drives secondary structure formation to prevent NH and C=O groups in non-polar amino acids from forming stabilizing hydrogen bonds with surrounding water molecules.
- In final phase of protein folding, elements of tertiary structure form. Initially, these are most likely motifs/subdomains, which in turn lead to complete folding.
- In the case of large multidomain proteins, domains near the protein's N-terminus, being synthesized first, may be folded in part or in full before the entire protein is synthesized.

Hydrophobic Collapse



The folding pathway proceeds via the initial rapid formation of the more compact, partially folded ‘molten globule’



Fast folding step

Molten globule formation

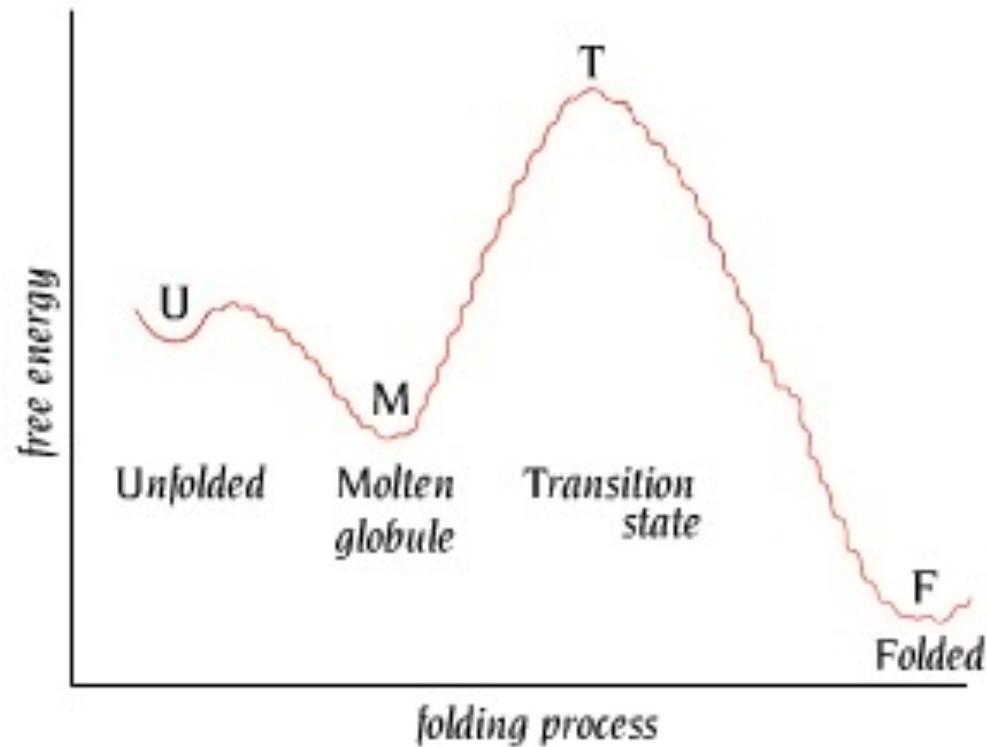
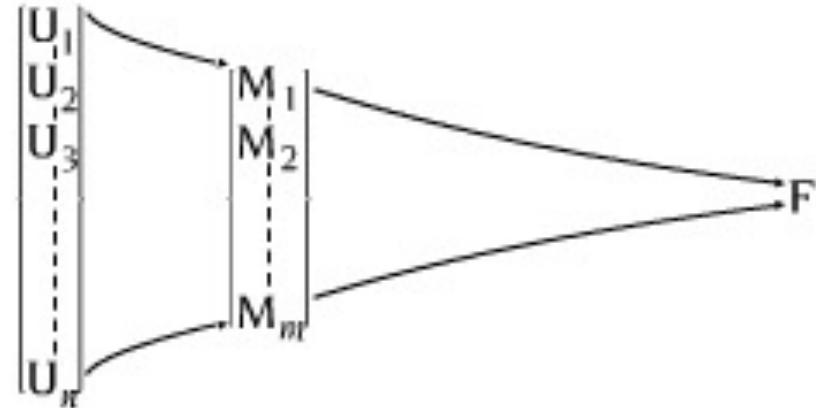
Slow folding steps

Formation of disulfide bond

Protein Disulfide Isomerase

Pro isomerization

Peptidyl Prolyl Isomerase (PPI)



Chaperones prevent misfolding

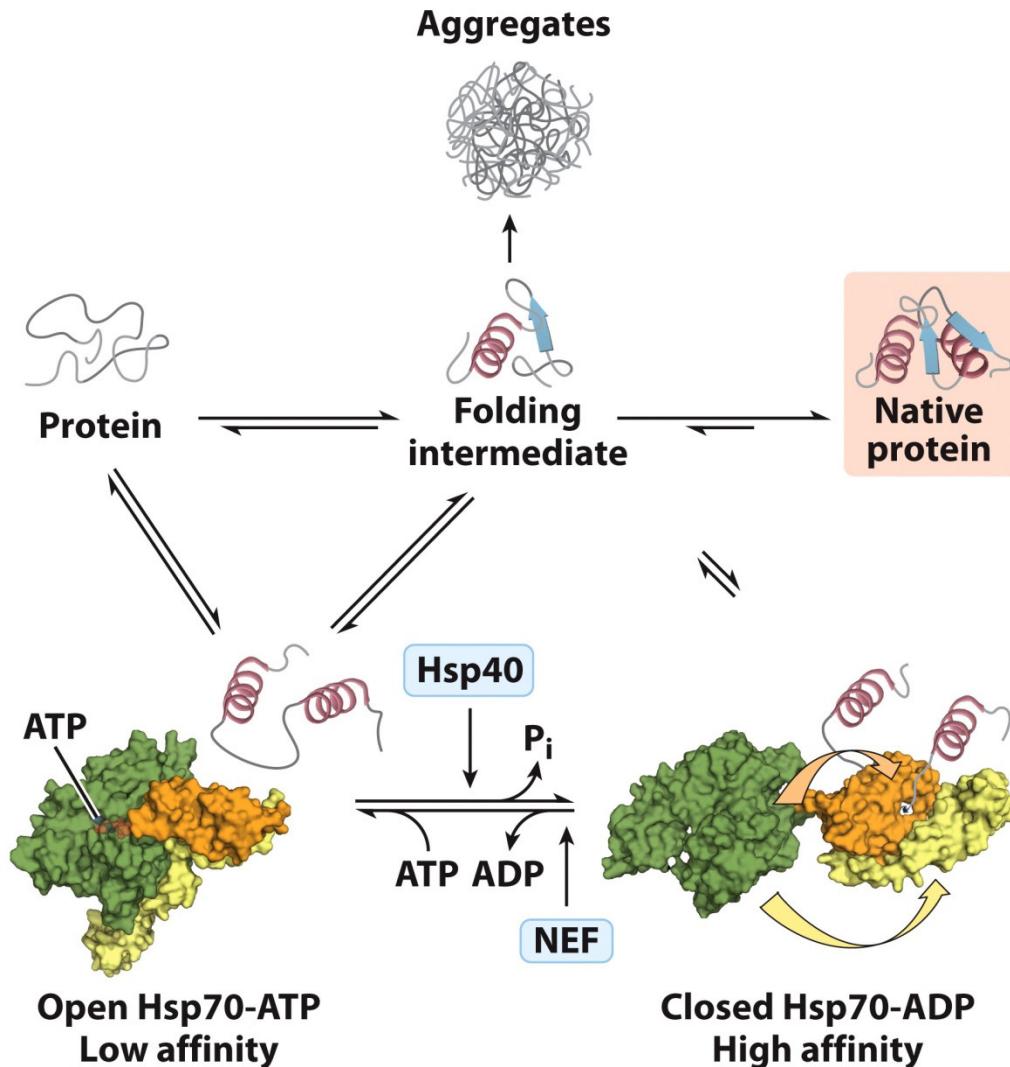
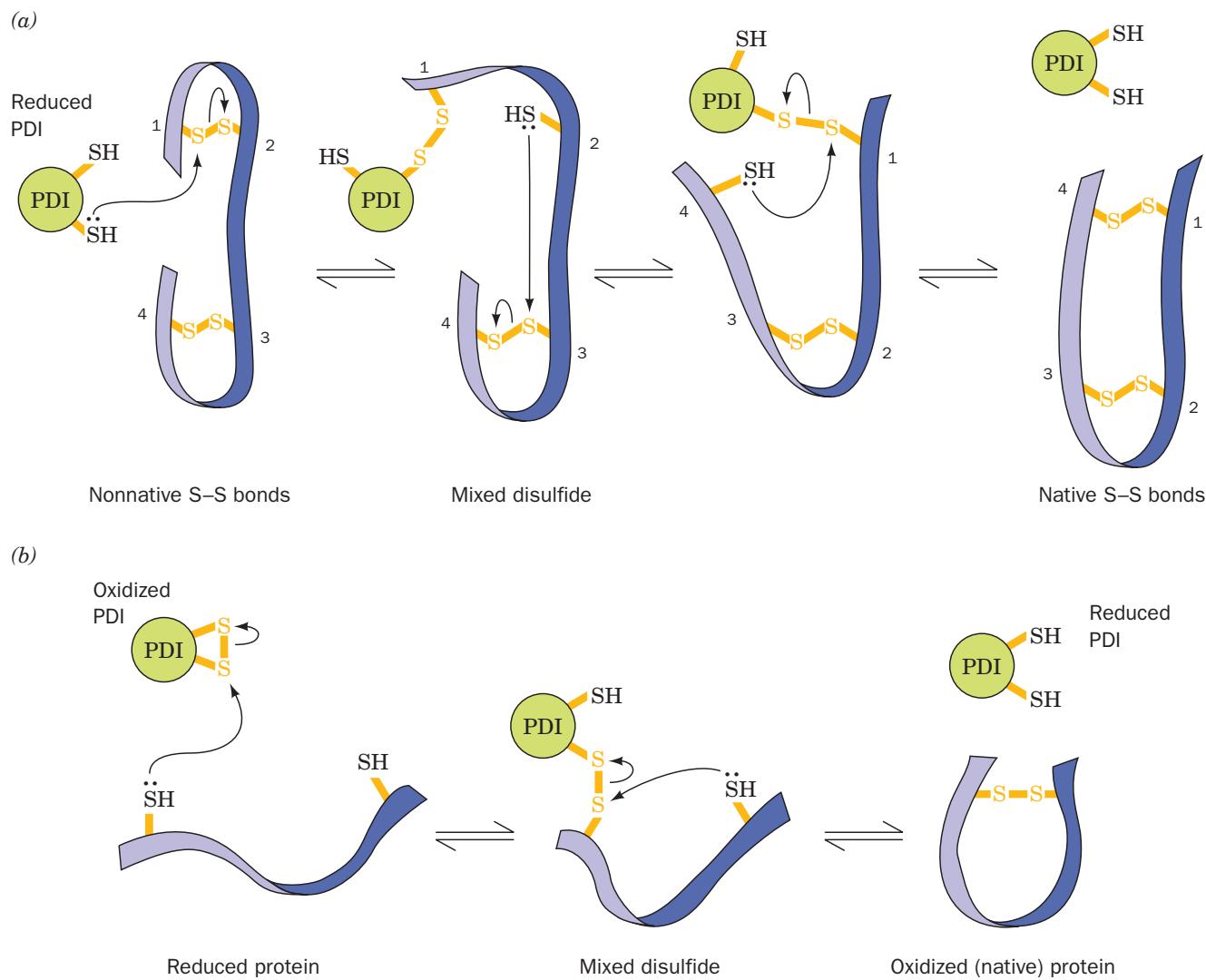


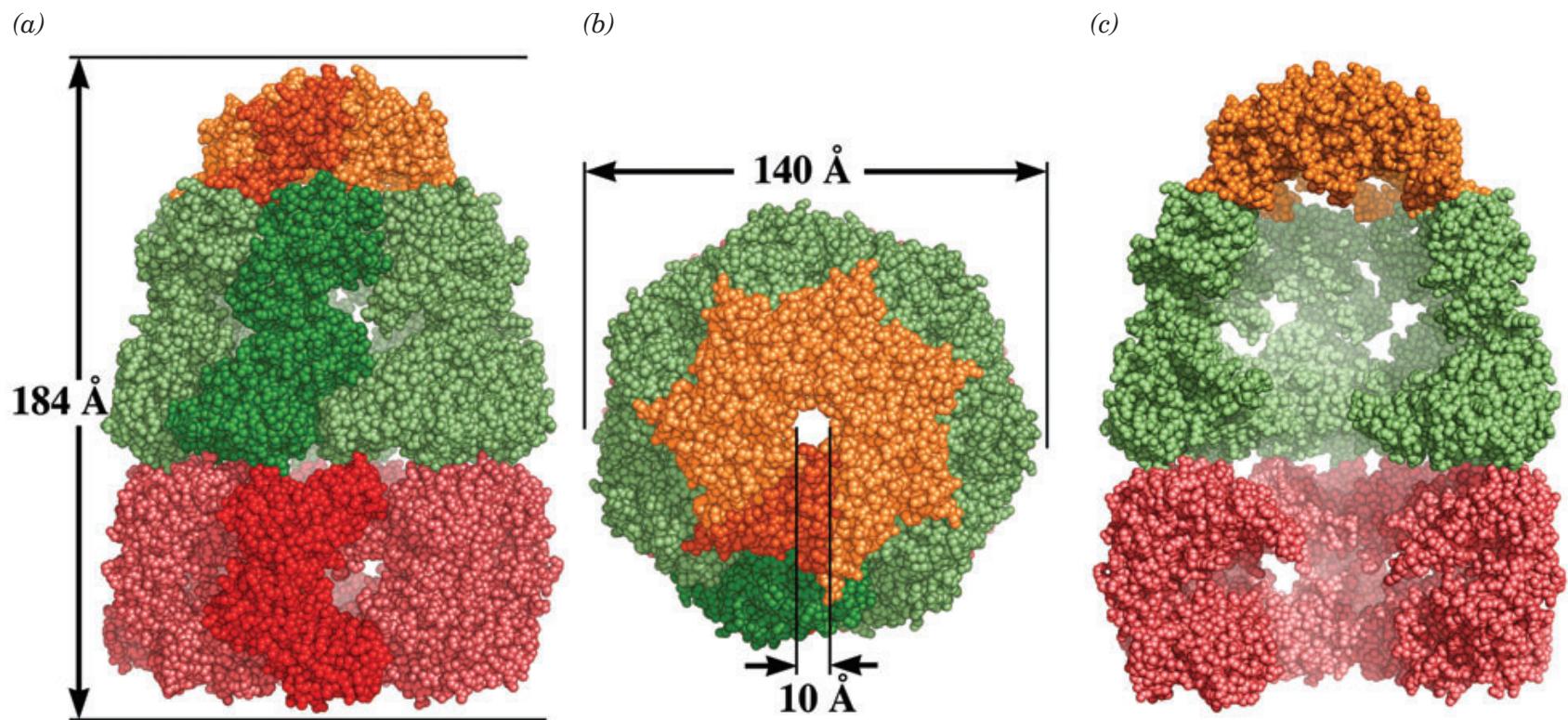
Figure 4-30

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Protein Disulfide Isomerase



GroEL and GroES



Chaperonins facilitate folding

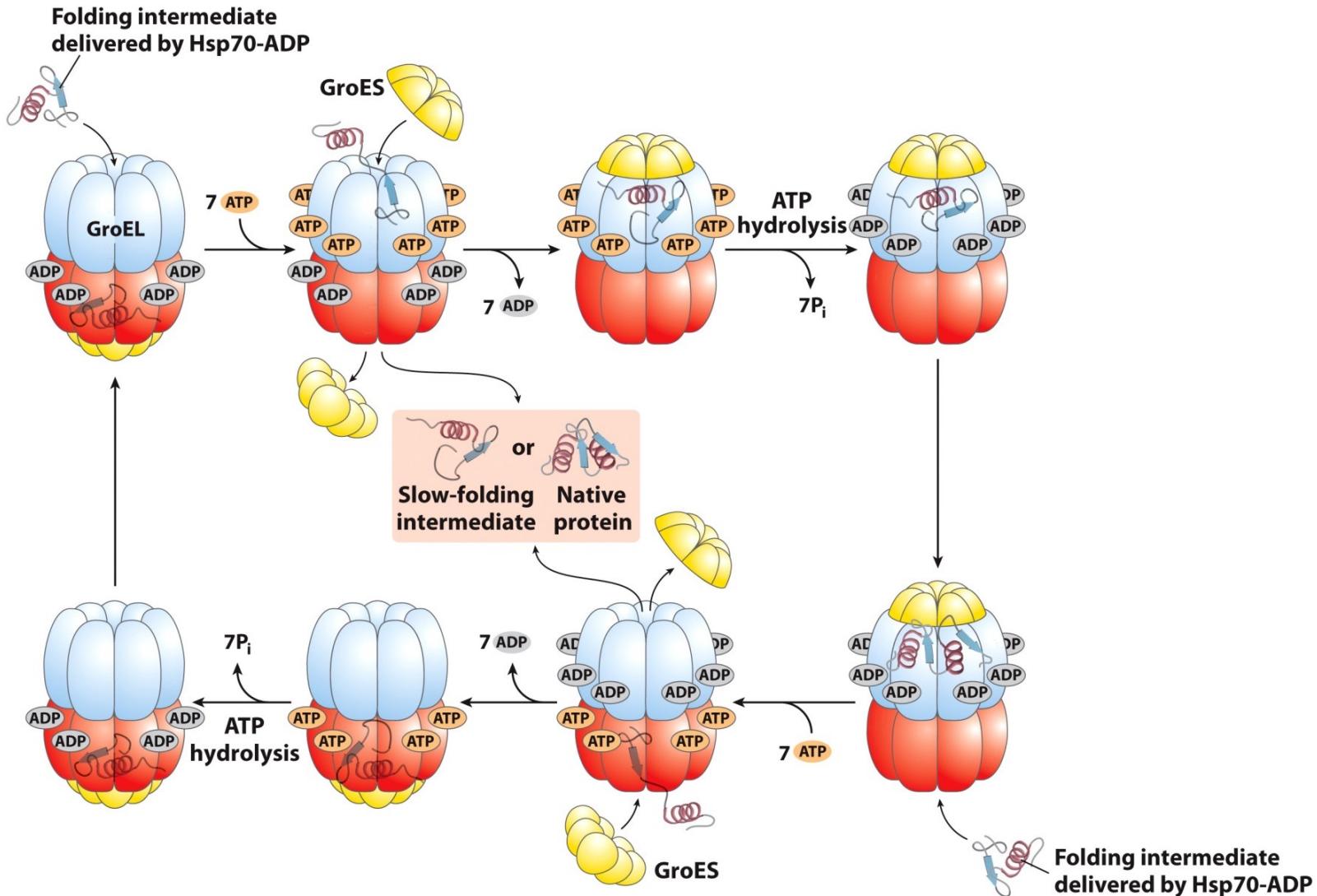
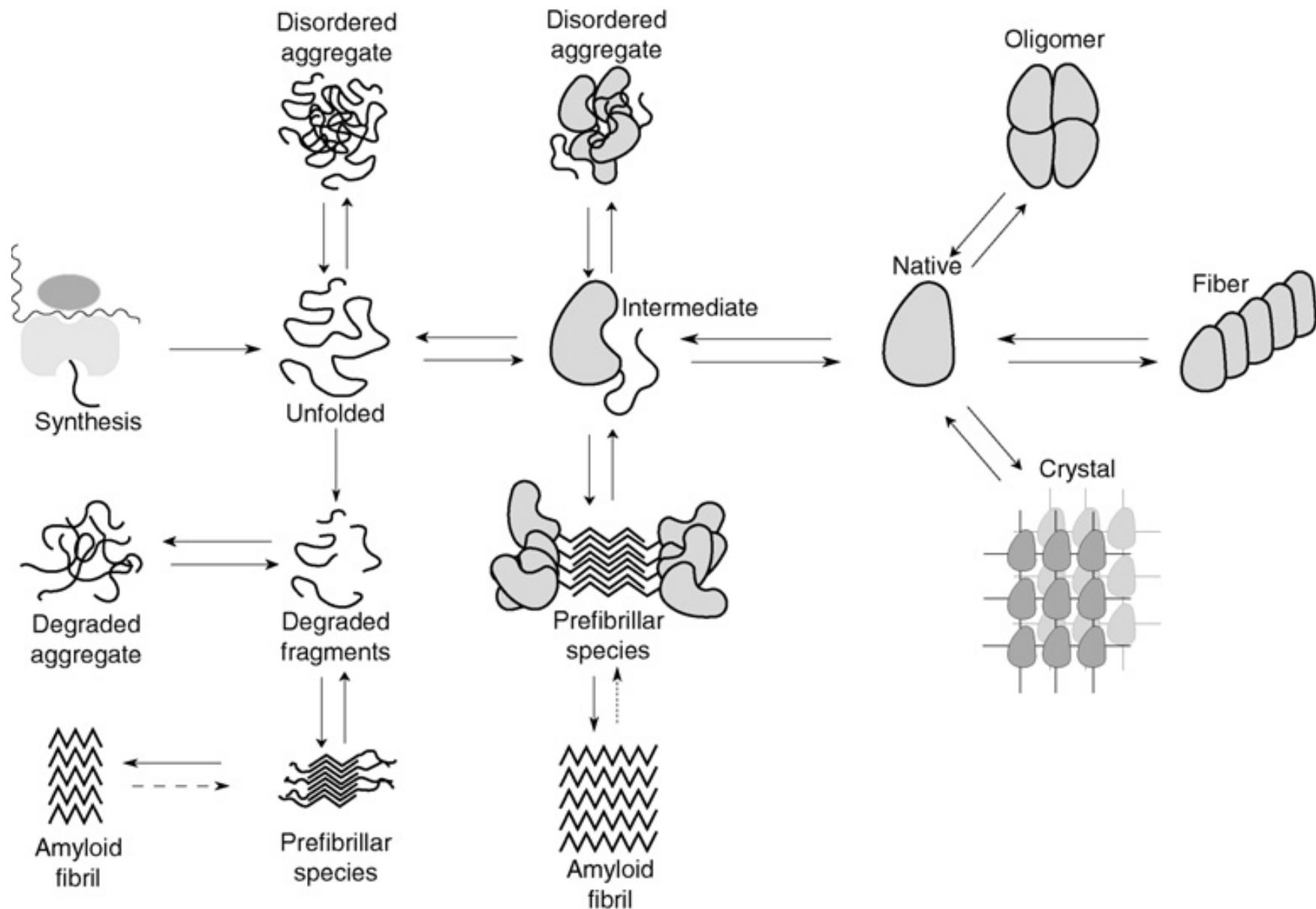


Figure 4-31a

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Major types of structure that can be formed by polypeptide chains



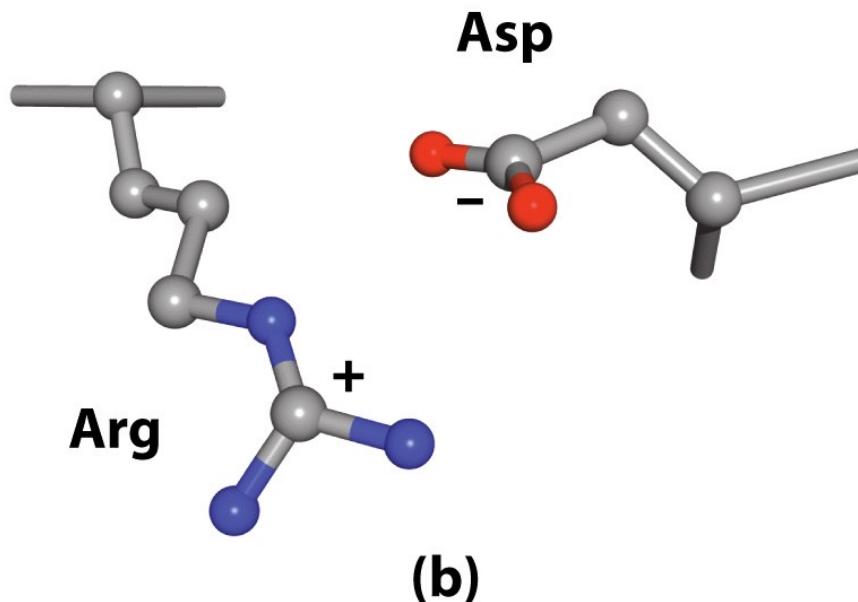
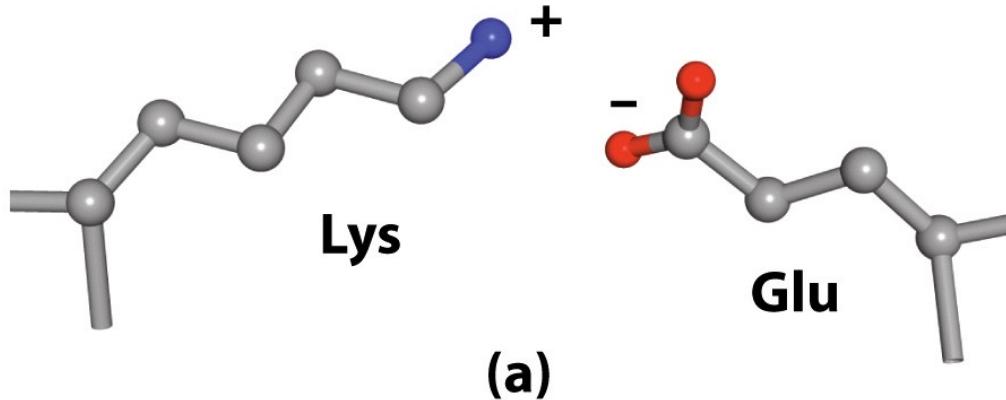
Protein structural stability

The major stabilizing forces of a polypeptide's overall conformation are:

1. Hydrophobic interactions
2. Electrostatic attractions
3. Covalent linkages

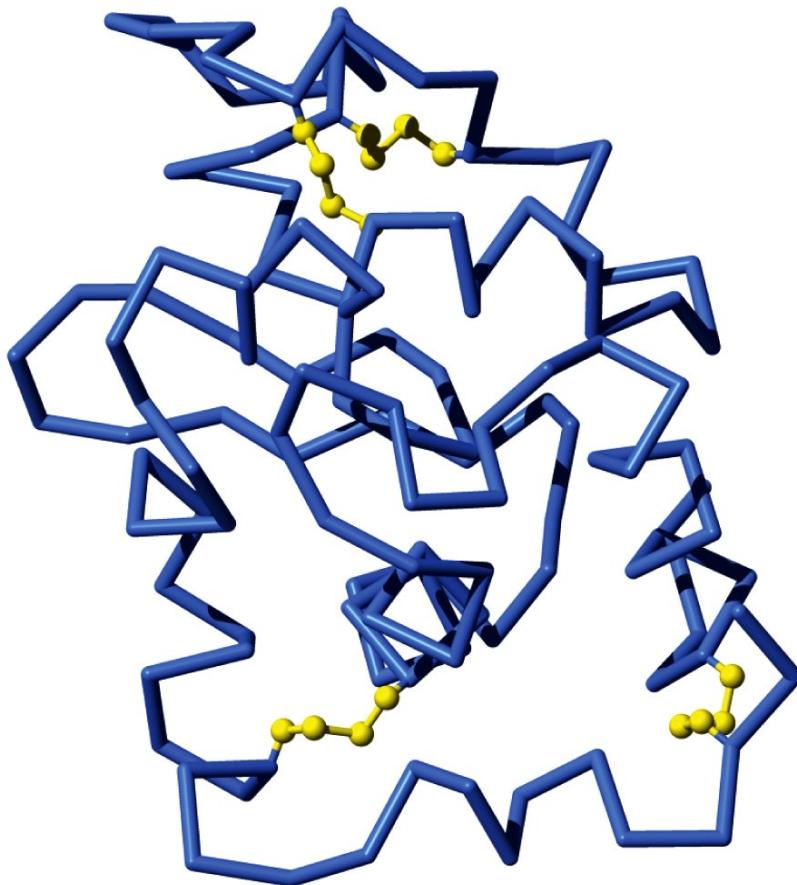
Cross-links stabilize proteins

Electrostatic Interactions



Cross-links stabilize proteins

Disulfide Bridges

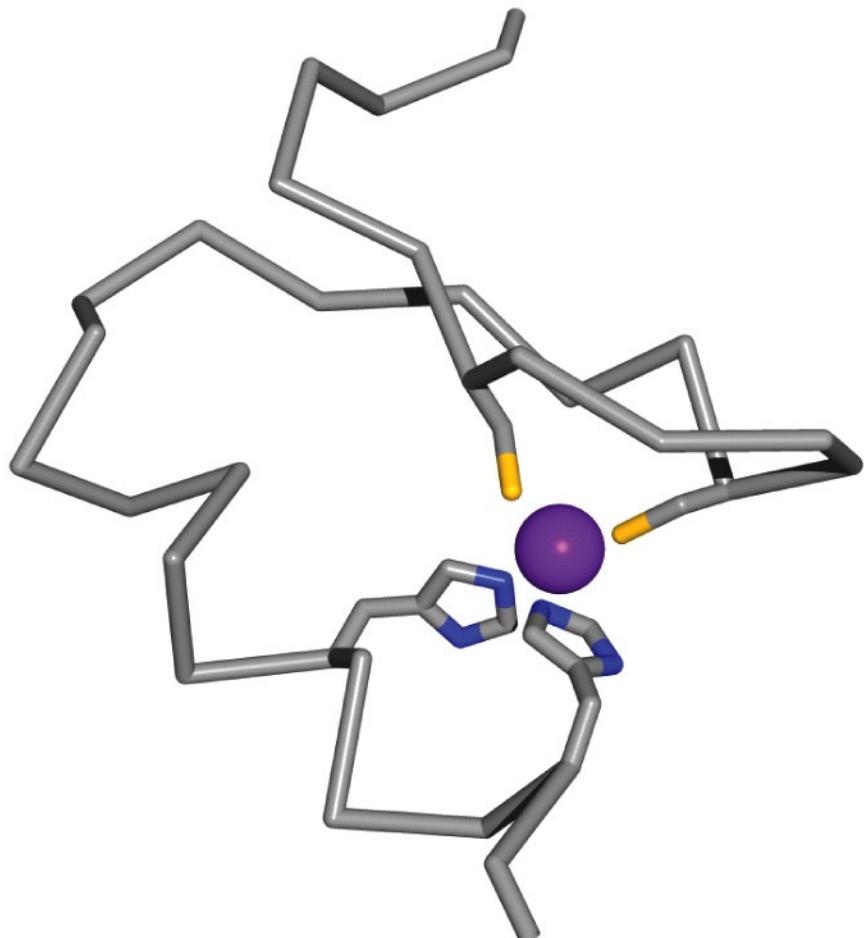


Disulfide linkages

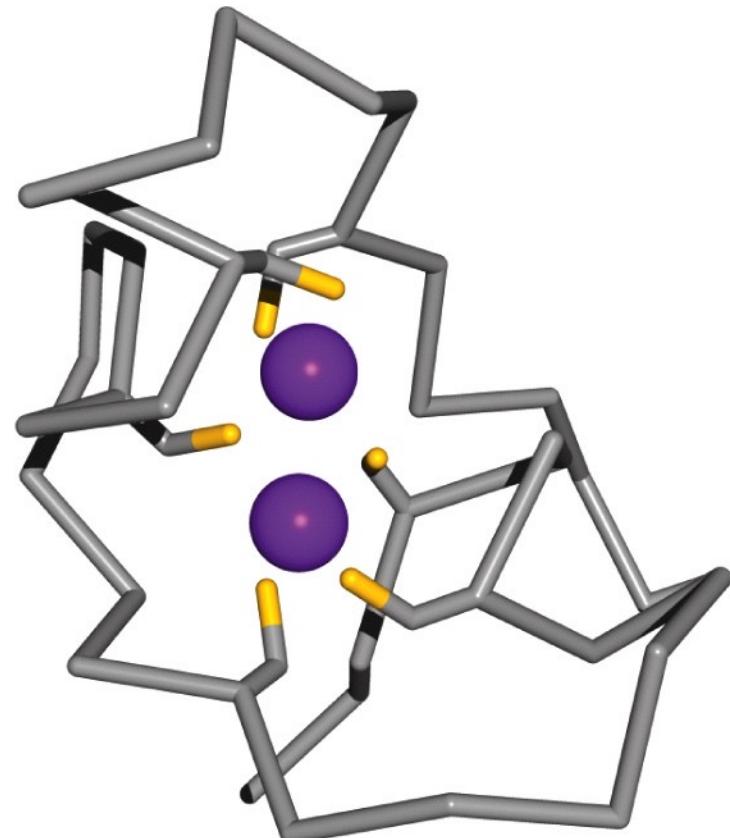
- Intracellular proteins rarely form disulfide linkages due to the reducing environment which prevails within the cell.
- Extracellular proteins in contrast are exposed to a more oxidizing environment, conducive to disulfide bond formation.
- In many proteins the reduction (i.e. breaking) of disulfide linkages has little effect on the native conformation of polypeptides.
- In some proteins, (particularly disulfide-rich proteins) disruption of this covalent linkage does render the protein less conformationally stable. In these cases the disulfide linkages likely serve to 'lock' functional/structurally important elements of domain/tertiary structure in place.

Cross-links stabilize proteins

Metals



(a)



(b)

Electrostatic interactions

- Hydrogen bonds do not contribute very significantly to overall conformational stability.
- This is because atoms that hydrogen bond with each other in a folded polypeptide can form energetically equivalent hydrogen bonds with water molecules if the polypeptide is in the unfolded state.
- Ionic attractions (Salt bridges) between oppositely charged amino acid side chains also contribute modestly to overall protein conformational stability.

Bond type	Bond strength (kJ/mol)
Van der Waals' forces	10
Hydrogen bond	20
Ionic interactions	86
Carbon–carbon bond	350

What does the stability of proteins in solution depend on?

1. Amino acid sequence
 2. Environmental factors (pH, temperature, ionic strength ligands)
- Physiological conditions: The equilibrium between the unfolded and folded state lies very far on the side of the native state.
 - As a consequence, the equilibrium constant cannot be measured, because no method would be sensitive enough to measure the small amount of unfolded protein molecules accurately enough.
 - How then can we measure the stability of proteins?

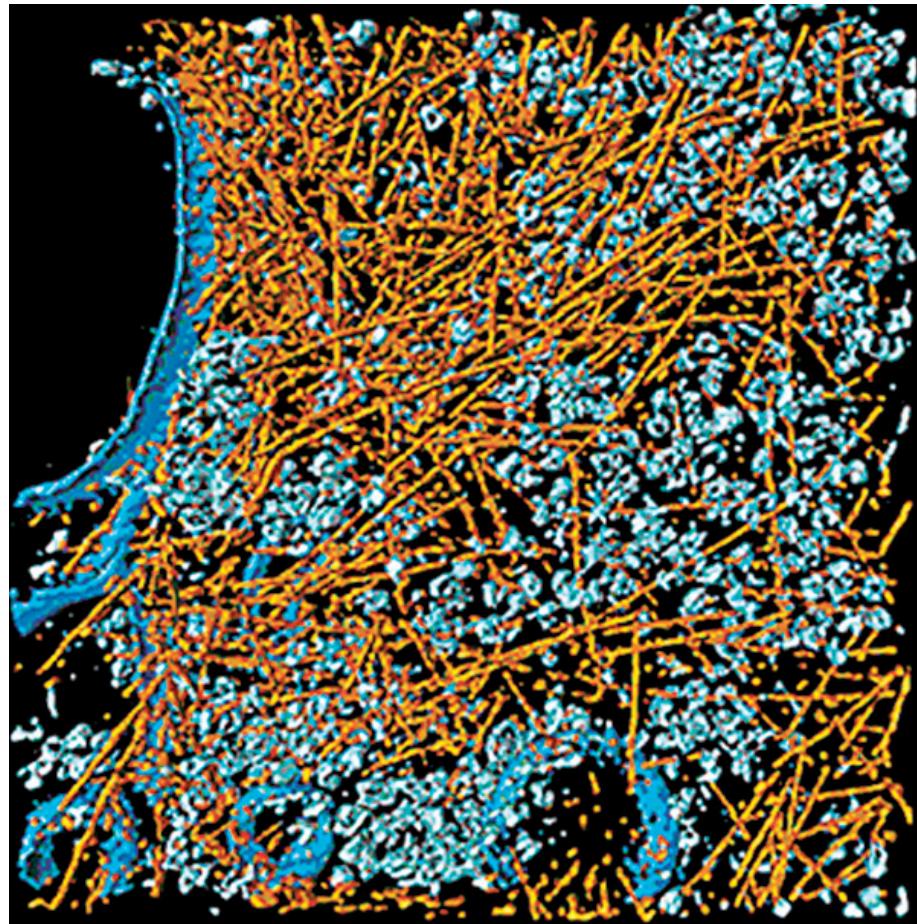
Proteins in cells are densely packed

Proteins in cells are densely packed.

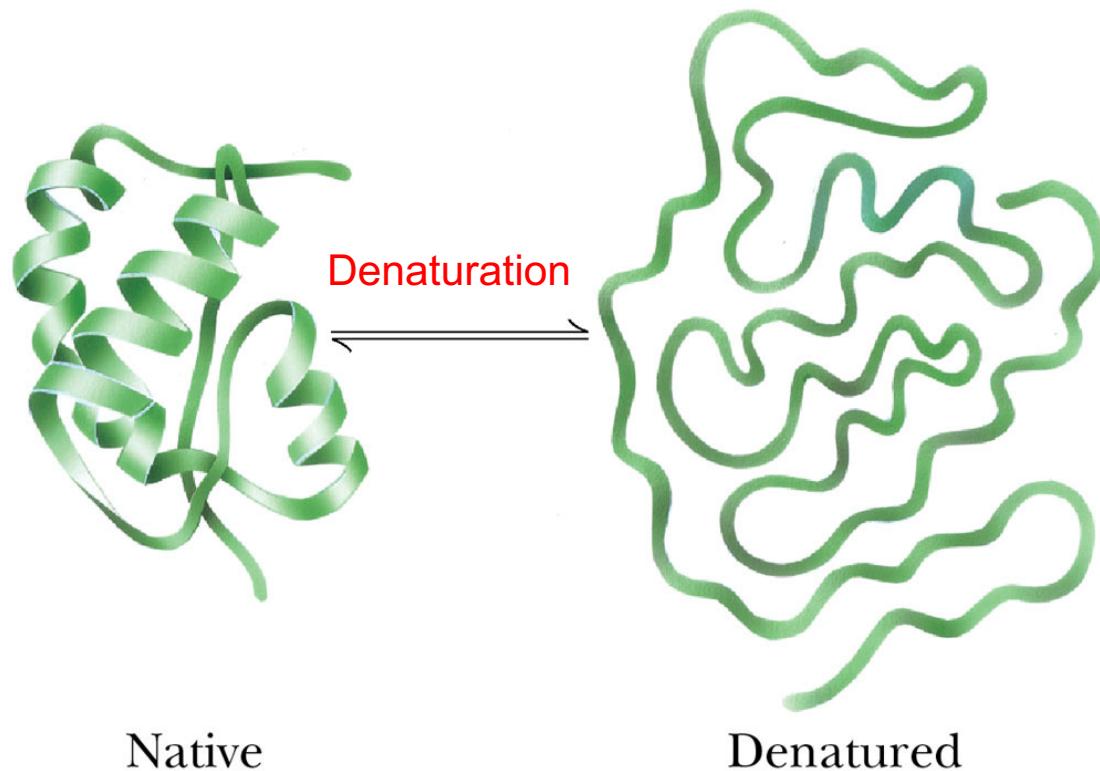
Concentration of total protein inside cells = 200-300g/L

Cellular interiors are 20-30% volume occupied by macromolecules

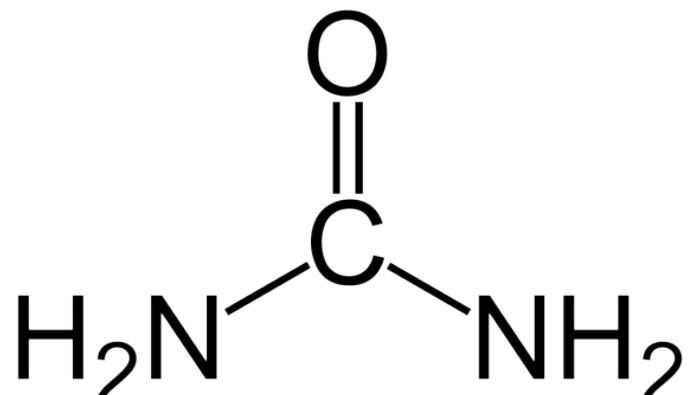
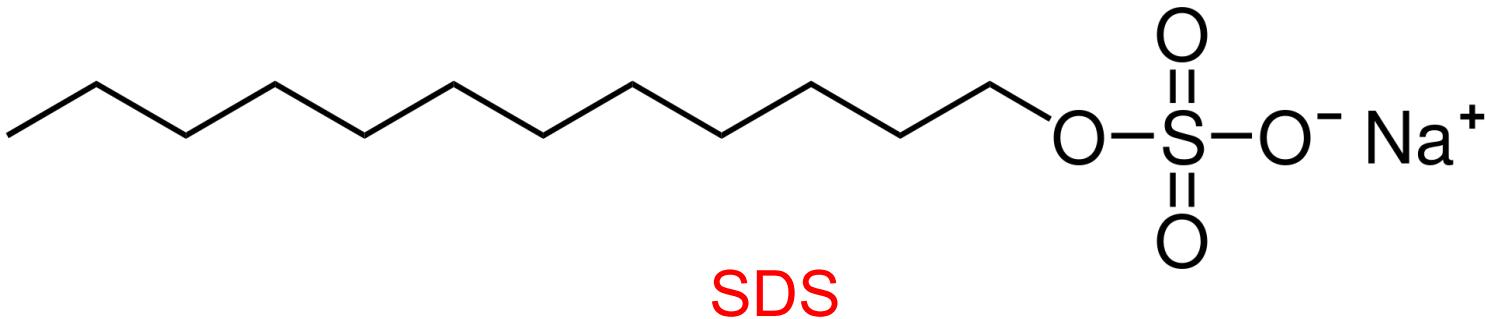
(Red blood cells contain 350g/L of hemoglobin alone)



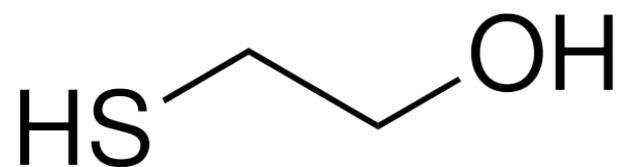
Protein Denaturation



Protein Denaturation

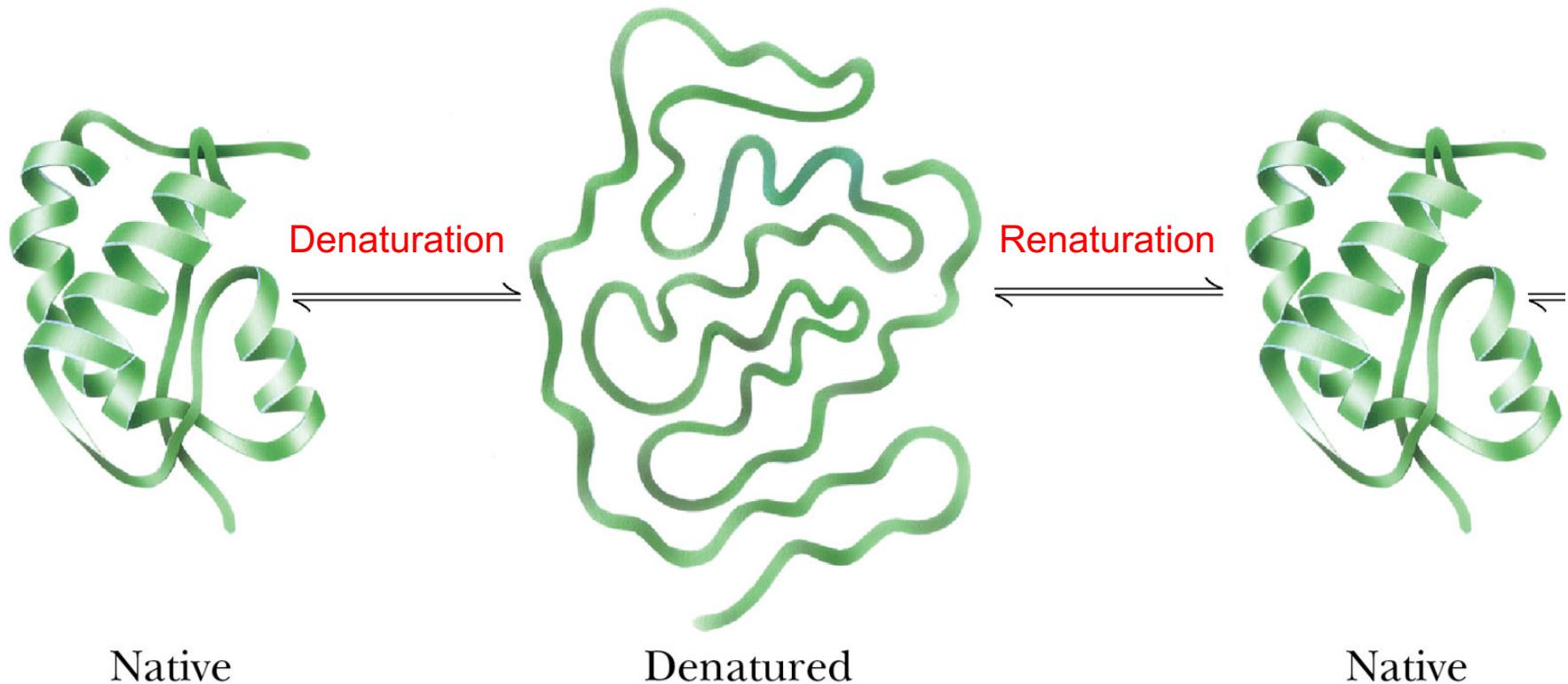


Urea

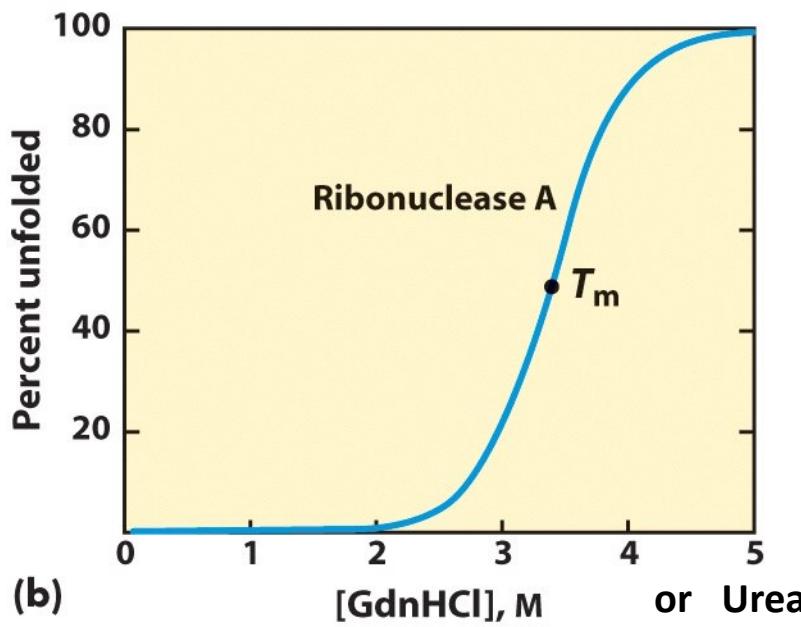
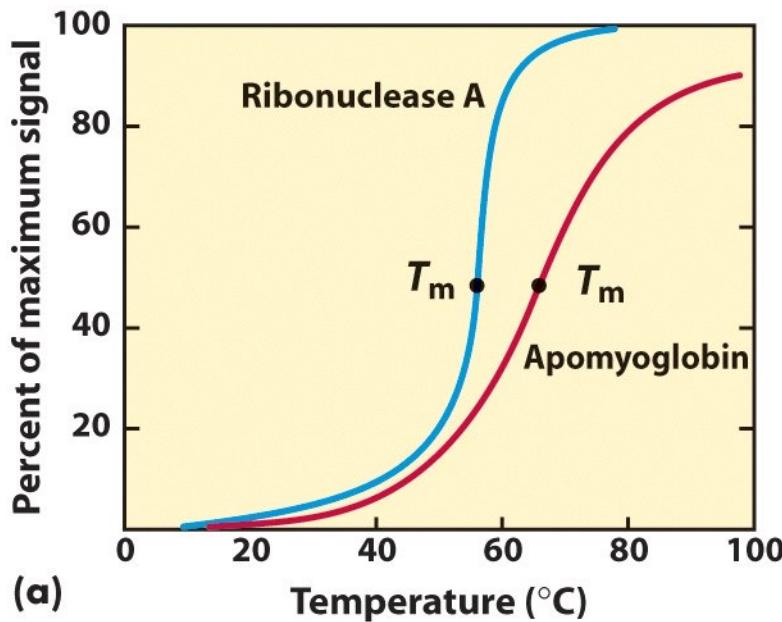


β - Mercaptoethanol

Protein Denaturation



Thermal and Chemical Protein Denaturation



Thermal and Chemical Protein Denaturation



$$\Delta G^\circ = -RT\ln K_{eq}$$

$$K_{eq} = \frac{[F]}{[U]}$$

G = Gibbs Free Energy
Δ = Change

Recall 3 Cases:

$K_{eq} > 1$ rxn favors folded state

$K_{eq} < 1$ rxn favors unfolded state

$K_{eq} = 1$ equal mixture of U and F

3 Cases:

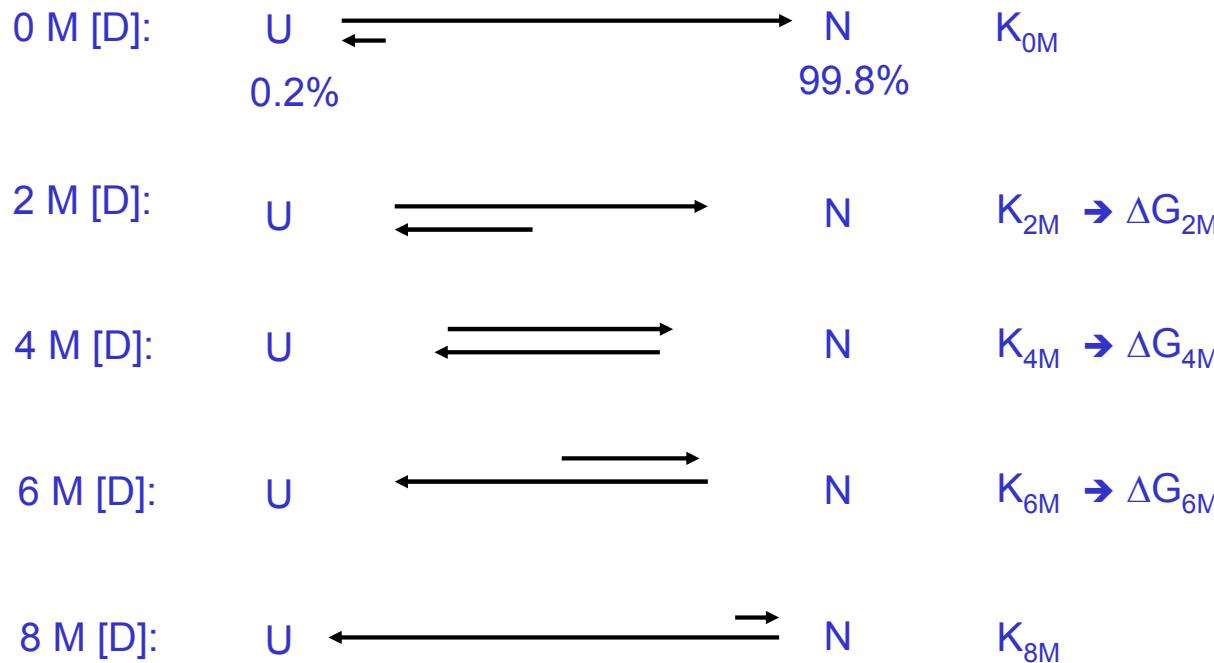
$\Delta G^\circ < 0$ rxn favors folded state

$\Delta G^\circ > 0$ rxn favors unfolded state

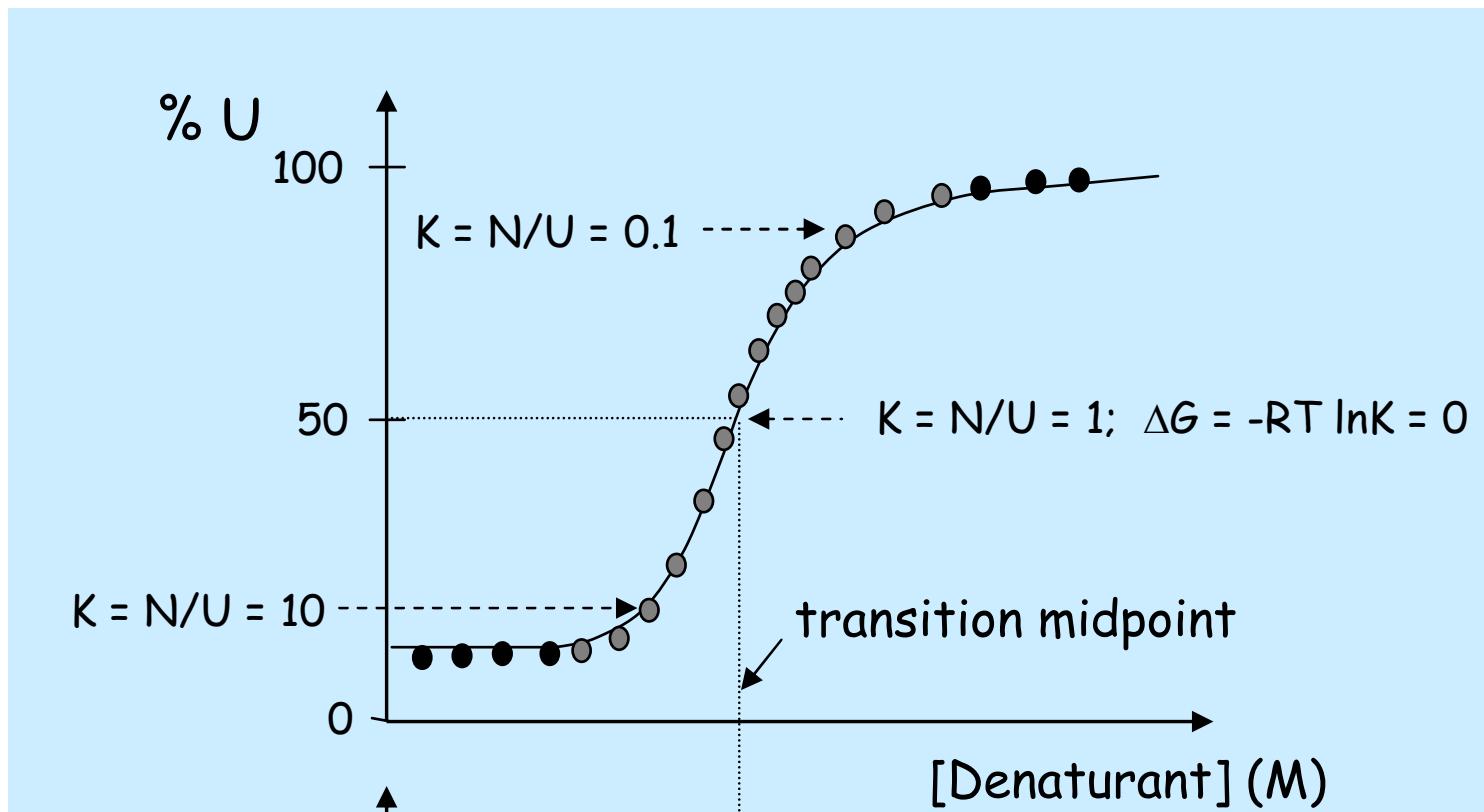
$\Delta G^\circ = 0$ equal mixture of U and F

Thermal and Chemical Protein Denaturation

Shift of the Equilibrium between U and N with Denaturant



Determination of ΔG_F from denaturant-induced equilibrium transitions



Determination of ΔG_F from denaturant-induced equilibrium transitions

