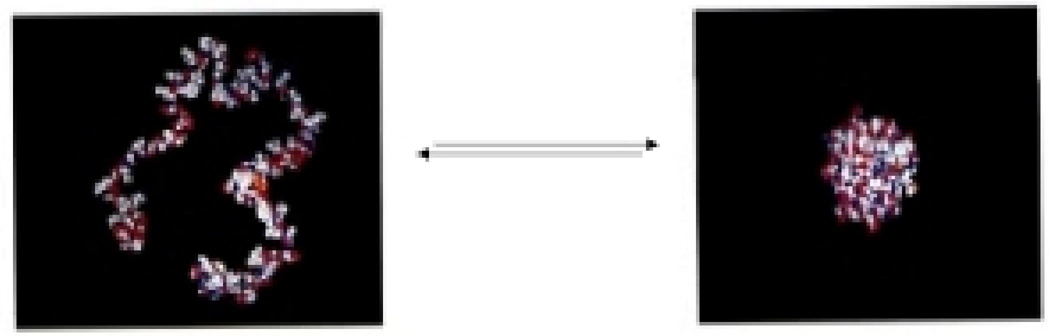
Lecture 11: Protein Folding & Stability

Margaret A. Daugherty
Fall 2004

How do we go from an unfolded polypeptide chain to a compact folded protein?



(Folding of thioredoxin, F. Richards)

Protein Folding: What we know

Structure - Function

1). Amino acid sequence dictates structure.
2). The native structure represents the lowest energy state for a protein (physiological conditions).
3). Proteins are densely packed as small organic crystals.

Protein Folding

4). A protein cannot sample all possible conformations in finding its native structure (Levinthal's paradox).
5). Protein folding *in vitro* is a good model for *in vivo* folding.
6). Protein folding is a cooperative process, usually between N <--> U states.
7). Intermediates with non-native structure can exist is some protein folding pathways.
8). The molten globule is likely to be an intermediate on protein folding pathways.

The Future of Folding

9). The protein folding problem will be solved within 5 years (Walter Gilbert, 1988)
10). Designed proteins usually turn out to be molten globules.
11). We will eventually be able to predict protein structure from sequence.

Anfinsen's protein folding experiment

1916-1995

Ribonuclease A
124 aa, pancreatic enzyme

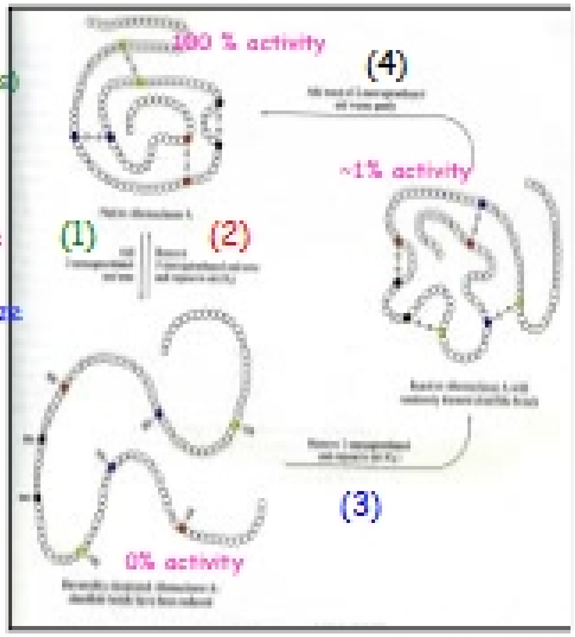
Anfinsen's experiment: sequence dictates structure `57:
Nobel Prize in 1972

(1) a. Reduce protein (β-ME destroys disulfides)
b. Unfold protein in urea

(2) Remove urea - allow protein to refold
Remove β-ME- allow disulfide to reoxidize

(3) Remove β-ME - allow disulfides to reoxidize
Remove urea - allow protein to refold

(4) Add trace β-ME, warm
~10 hrs ==> 100% ACTIVITY
Add trace β-ME + cytosolic* fraction
~2 minutes ==>100% ACTIVITY



Protein folding

Thermodynamic component: *The native structure represents the lowest energy state for a protein*

Kinetic component: *A protein cannot sample all possible conformations in finding its native structure*

PROTEIN FOLDING IS ENERGETICALLY FAVORABLE

Unfavorable (more positive) ← **Free Energy** → Favorable (more negative)

Unfolded	Nucleation of secondary structural elements	Interaction of secondary structural elements "molten globule?"	Native

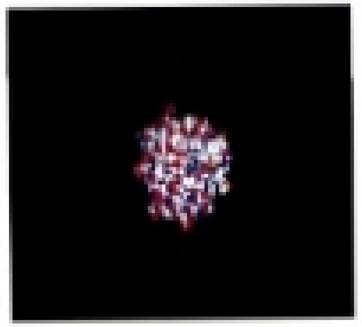
Energetic contributions to protein folding

- Conformational entropy
- Hydrogen bonds
- Electrostatic Forces
- *The hydrophobic effect*
- van der Waals Forces
- Other forces

CONFORMATIONAL ENTROPY

Boltzmann's Equation: $S = k \ln W$

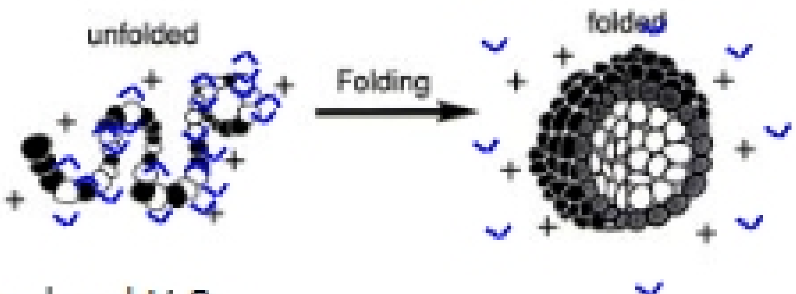
VS.



The polypeptide chain in the unfolded state has more *conformational freedom* than the folded state. Thus, the conformational entropy is a destabilizing component of the native state free energy

HYDROPHOBIC EFFECT

Hydrophobic effect: *Thermodynamic consequence* due to avoidance of H_2O by the apolar side chains of a protein. Predicted in 1959 by Kauzmann (before 3D structures).
Major contributor to stabilization of native state: proportional to apolar surface area buried.



ordered H_2O about hydrophobes result in *clathrate* structures Entropically unfavorable

Sequestering of hydrophobes to interior of protein molecule => Release of H_2O is ENTROPICALLY FAVORABLE

HYDROGEN BONDS IN PROTEINS

Type of hydrogen bond		Typical distance between donor and acceptor atom (nm)
Hydroxyl-hydroxyl	—O—H····O(H)—	0.28
Hydroxyl-carbonyl	—O—H····O=C<	0.28
Amide-carbonyl	>N—H····O=C<	0.29
Amide-hydroxyl	>N—H····O(H)—	0.30
Amide-imidazole nitrogen	>N—H····N (imidazole) NH	0.31

"Almost all groups capable of hydrogen bonding are, in fact, hydrogen bonded"

Baker & Hubbard, 1984