Refolding

When a protein is unfolded under gentle conditions it can refold.

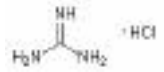
This suggests that the primary structure determines the tertiary structure through short range interactions

RNAase

Native molecule contains 4 disulfide bonds.

Molecule can be reduced, then unfolded in denaturants (6M Guanidine HCl).

When oxidized about 1% of disulfide bonds are in the native conformation.



RNAase

With 8 sulfhydryls, 4 disulfide bonds are possible.

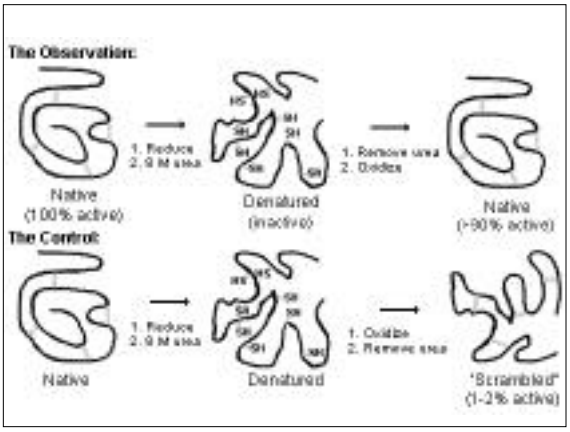
- First bond has 7 possibilities
- Second has 5 possibilities
- Third has 3 possibilities
- Fourth has 1 possibility

Random = 7x5x3x1 = 105 possible combinations.

Native

If the denaturant is slowly removed by dialysis before oxidation, the protein will become native from 70 to 80% of the time.

Doesn't work for activated zyymogens or multi-chain proteins Why not?



Energy of Folding

With a fragment of staph nuclease the energy to go from partially to completely folded is not great.

Segment that is native about 0.02% of the time requires a gain of 0.37 Kcal/residue to become 100% native.

Protein Folding

Protein with 300 amino acids

Molecular weight approximately 33,000

If each amino acid has only 3 possible configurations there are $3^{200} = 2.66 \times 10^{95}$ conformations

Time of Folding

For a polypeptide with 100 amino acids and 10 conformations per amino acid, there are 10^{100} possible conformations.

If it were possible to try 10^{13} conformations per second, it would require 10^{85} seconds or 10^{77} years to samples them all.

Proteins fold in from 10^{-1} to 10^{3} seconds.

Conformational Entropy

Assume a protein with 100 amino acids, each having 10 possible conformations.

Entropy $S = RT\ln W$

At 25°C S = -136 Kcal/mole

To get half of the molecules into a single conformation would require an input of energy of 136 Kcal/mole.

Folding

Not all Φ and Ψ angles are allowed.

For each amino acid we find that some angles are a lower energy and increase the likelihood of that amino acid being in a particular structure.

This does not totally depend upon the neighboring amino acid.

Time

How can a protein fold in seconds or minutes to the conformation of lowest free energy when there are so many to try (10^{100}) requiring 10^{85} seconds or 10^{77} years when the age of the universe is about 10^{10} years?

Do they really achieve the conformation of lowest free energy?

Hydrophobic Collapse

Hydrophobic groups are quickly removed from water

Entropy increases

Volume of protein only slightly larger than completely folded

Secondary structure very similar to final structure

Described as a molten globule

Limits number of conformations to try

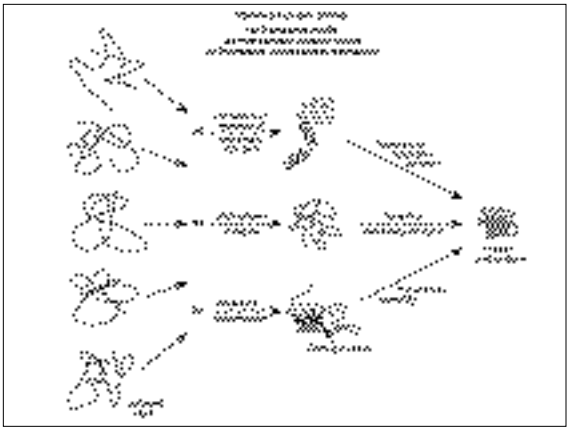
Nucleation Theory

Specific sites tend to fold first

Close together with many hydrogen bonds

Often α-helix or reverse turns

Direct other residues into proximity and limit number of conformations that must be tried.



Energy Landscape Model

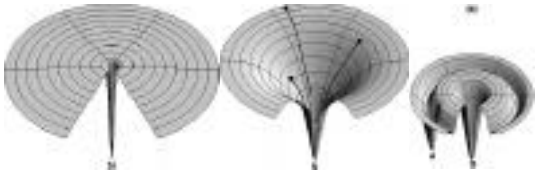
The protein folds to lower its energy.

Many steps are cooperative and once a certain shape is reached, the next follows logically.

The protein may not try all energy states.

The landscape may have areas that trap some proteins in the wrong conformation.

Other Landscapes

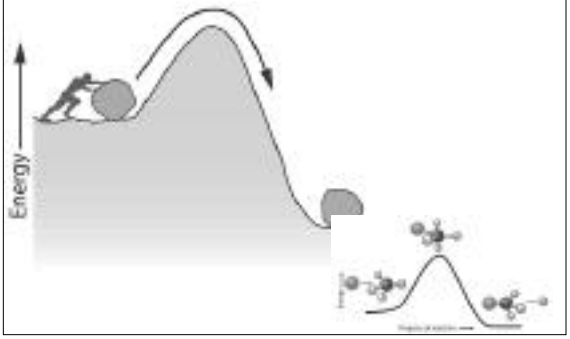


Random structures with no interactions that eventually lead to an energy minimum.

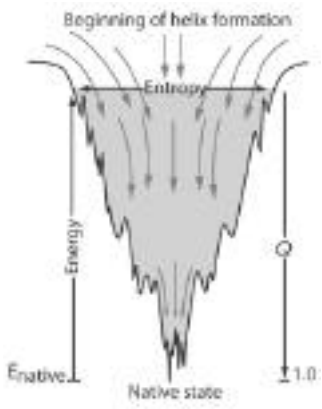
No energy barrier leads to a rapid minimum

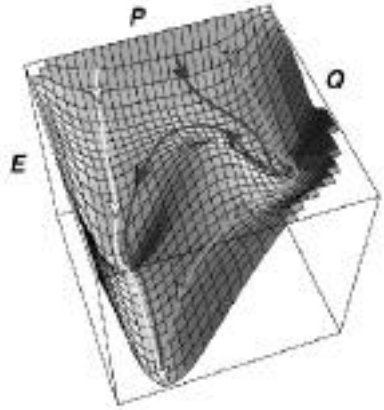
A protein could reach structure A, not the true minimum and never reach B

Energy Barriers



Another Landscape





Amino Acid Tendencies

Helix Breaker	Indifferent	Helix Former
Gly	Lys	Val
Ser	Tyr	Gln
Pro	Asp	Ile
Asn	Thr	His
	Arg	Ala
	Cys	Trp
	Phe	Met
		Leu
		Glu

Help Fold

If a protein gets to an energy minimum that is not global there may be a kinetic barrier to ever achieving its native structure.

This happens during protein synthesis.

Protein needs help to overcome this problem.

Chaperones

Chaperonin 10 (cpn10, hsp10, co-chaperonin, early pregnancy factor, GroES) Co-chaperonin' to chaperonin 60; promotes folding of substrates that are bound to chaperonin 60

Small heat-shock proteins (hsps) Diverse class of proteins; chaperone function is independent of adenosine triphosphate (ATP); bind non-native proteins

Hsp40 (DnaJ related) Co-chaperones that regulate the activity of hsp70 proteins; some can bind non-native proteins themselves

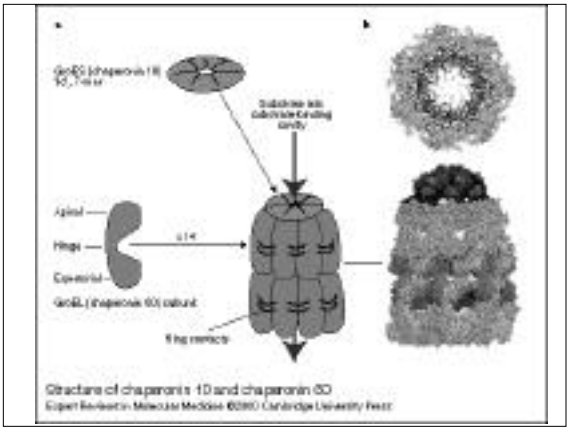
Chaperones

Hsp70 Prevent the aggregation of unfolded polypeptides; disassemble multimeric protein complexes; involved in protein trafficking; regulate the heat-shock response

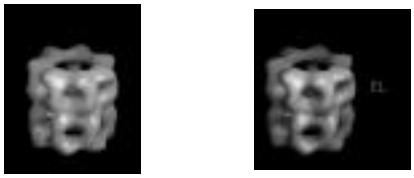
Hsp90 Specific functions in regulating signal transduction pathways, through their actions 'general' chaperone activity

Hsp100 (Clp) Disassemble protein oligomers and aggregates

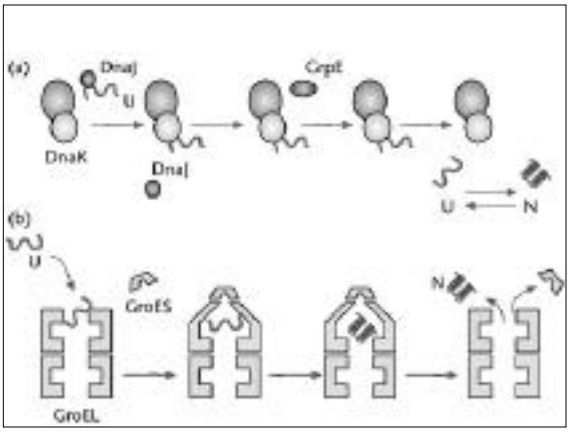
Hsp110 High degree of homology with the hsp70 family; little known about Functions



Chaperone Motion



Molten Globules



SH Groups

The formation of SS groups can limit the number of structures a protein can assume.

May occasionally form in areas that are not appropriate.

Enzymes reduces SS bonds and then reoxidizes the SH bonds formed.

If initial bond was correct - no change, if not correct - the protein has a chance to assume new conformation.