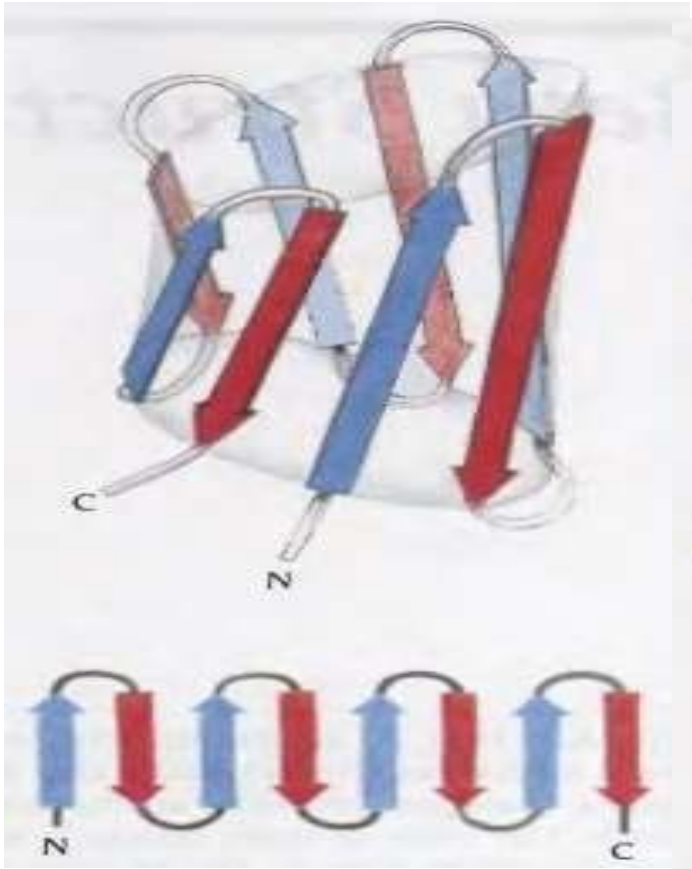
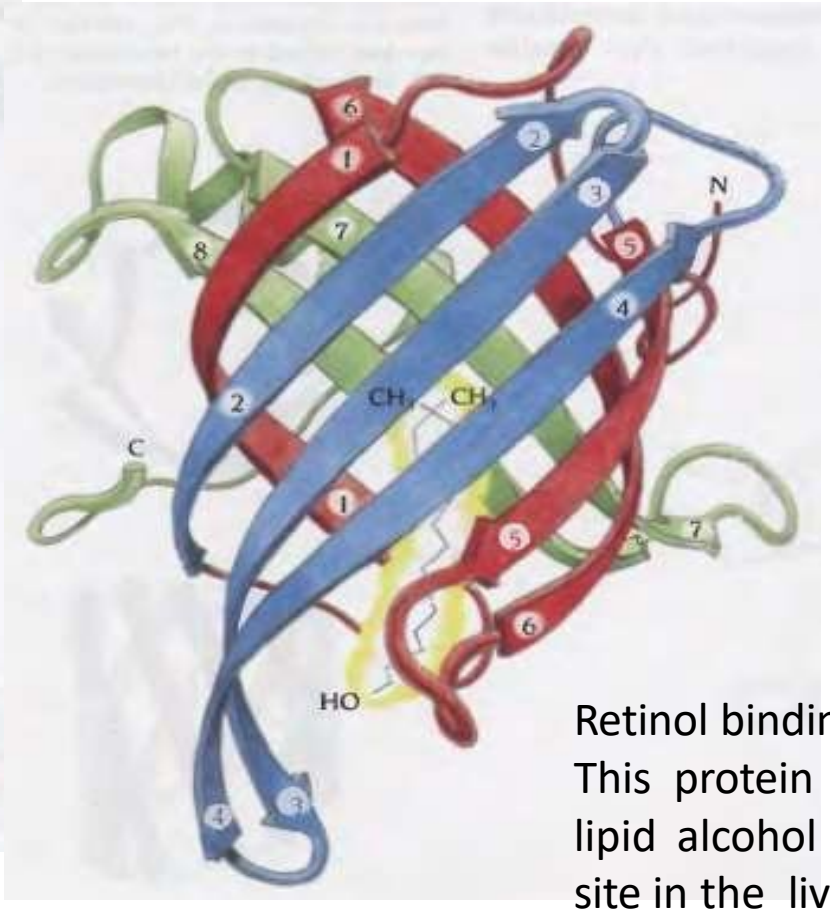
β structure: the core of the domain is built up by β strands that vary in number from 4 to over 10

- β strands are arranged in a predominantly antiparallel fashion –

1) Up and down barrrel - each successive β strand is added adjacent to the previous strand until the last strand is joined by hydrogen bond to the first strand and the barrel is closed

- the arrangement of β strands is similar to that in α/β barrel structure except that strands are antiparallel and all conections are hairpins



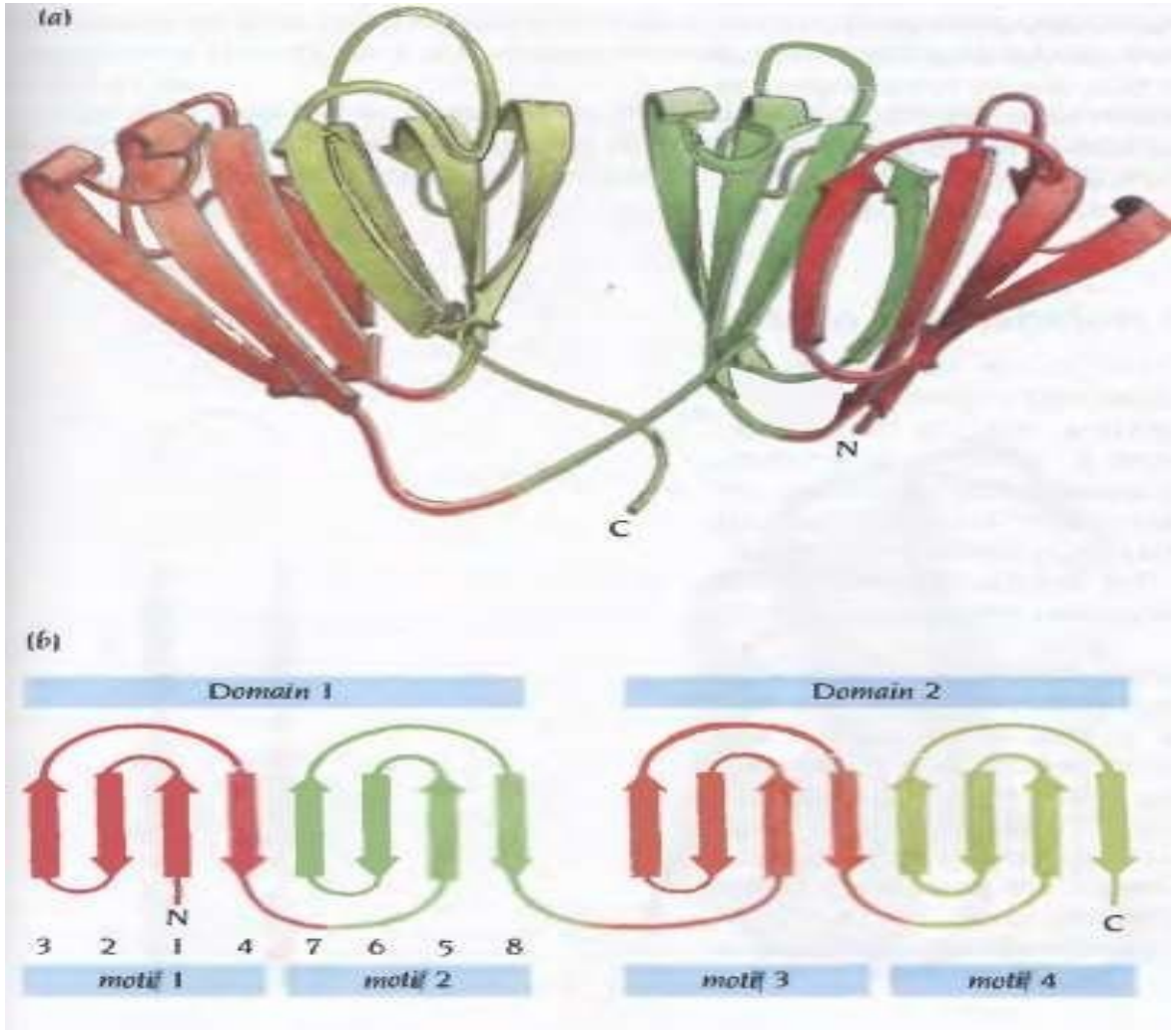


Retinol binding protein:

This protein is responsible for transporting the lipid alcohol vitamin A (retinol) from its storage site in the liver to the tissues

2) Two Greek key motifs form the domain:

γ-crystallin: The transparency and refractive power of the lenses of our eyes depend on a smooth gradient of refractive index for visible light. This is achieved partly by a regular packing arrangement of the cells in the lens and partly by smoothly changing concentration gradient of lens-specific proteins, the crystallins. There are three different classes of crystallins: α, β and γ



Schematic diagram of γ crystallin:

- each domain structure is built up from two β sheets of four antiparallel β strands

Topology diagram of γ crystallin :

Both the domains have the same topology

- each domain is composed of two Greek key motifs that are joined by a short loop region

Protein folding:

The process by which a polypeptide chain acquires its correct three dimensional structure to achieve the biologically active native state is called protein folding

- Polypeptide chain folds i) spontaneously
  ii) may require the assistance of enzymes to catalyse the formation and exchange disulfide bonds
  iii) may require assistance of a class of proteins called chaperone

How to predict the three dimensional structure of a protein from one dimensional structure?

Proteins in its native state is not static: α helices, β sheets, domains undergo small movements

- In general biologically active protein molecules in solution phase is not highly stable

$$\text{Native state} \xrightarrow[\Delta G \approx 5 - 15 \text{ kcal/mol}]{\text{change in pH; temperature}} \text{Denatured state}$$

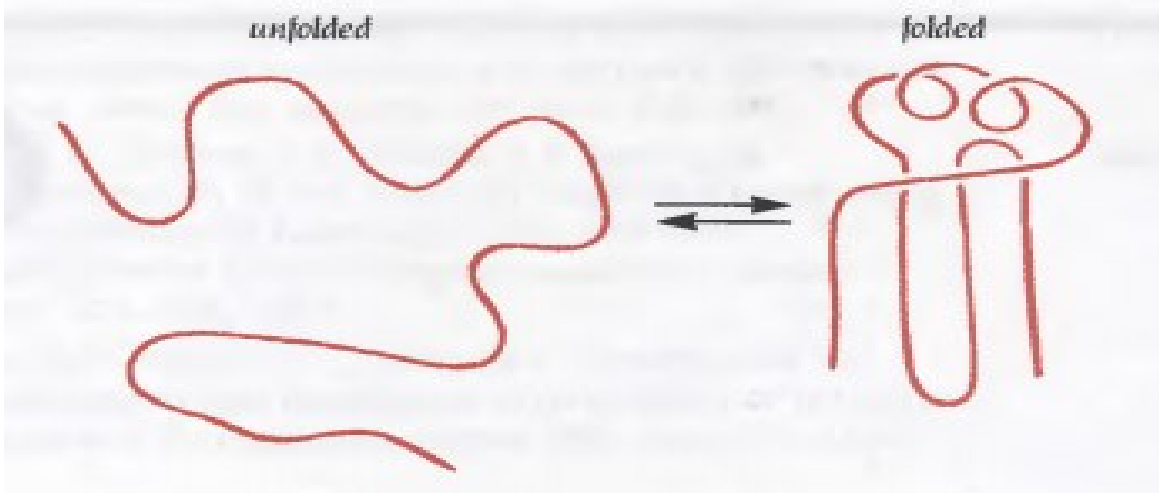
(H-bond ≈ 2 to 5 kcal/mol)

⇒ compared to denatured state, native state of a protein is marginally stable

$\Delta G = \Delta H - T\Delta S$ ⇒ Two major contributors to the energy difference: ΔH; ΔS

ΔH : Noncovalent interactions within the polypeptide chain – i) Hydrophobic interactions; ii) Hydrogen bonds; iii) Ionic bonds

Covalent interactions – Bonds between amino acid residues; disulfide bonds



Native state vs Denatured state

- Covalent interactions between native and denatured states are same with the exception of disulfide bonds between cysteine residues of protein
- Noncovalent interactions differs significantly –
    - Interactions are maximized to produce a compact globular molecule with a tightly packed hydrophobic core in the native state
    - Denatured state is more open and side chains are loosely packed

ΔH : ~ several hundred kcal/mol

ΔS :

✓ Proteins in the native state are highly ordered in one main conformation whereas the denatured state is highly disordered with protein molecules in many different conformations

⇒ Denatured state is entropically favourable comapred to the native state of proteins

ΔS : ~ several hundred cal/mol

⇒ ΔG : small difference (~ 5 to 15 kcal/mol) between native and denatured state

Native state is marginally stable over the denatured state !!!

- It is biologically important to have native proteins in correct quantities in appropriate time
- It is important for living cells to be able to easily degrade proteins as it is able to synthesize them

⇒ Therefore native state of proteins evolved to be marginally stable than denatured state

Kinetic factors for folding:

✓ The specific sequence of a polypeptide chain of a protein appears to yield only a single, compact, biologically active fold in the native state

✓How is this folded state reached **?**

Probable pathway : Protein molecule search through all possible conformations in a random fashion until and unless they are frozen at the lowest energy in the conformation of native state

Levinthal's paradox: (Cyrus Levinthal – 1968)

Each amino acid has only three possible conformations, the allowed regions α, β and L and it converts one conformation into another in a very short time domain, one pico second (10^{-12} seconds)

⇒ A polypeptide chain of 150 residues would then have $3^{150} \approx 10^{68}$ possible conformations

⋙ To search all these conformations would require $10^{68} \times 10^{-12} = 10^{56}$ seconds

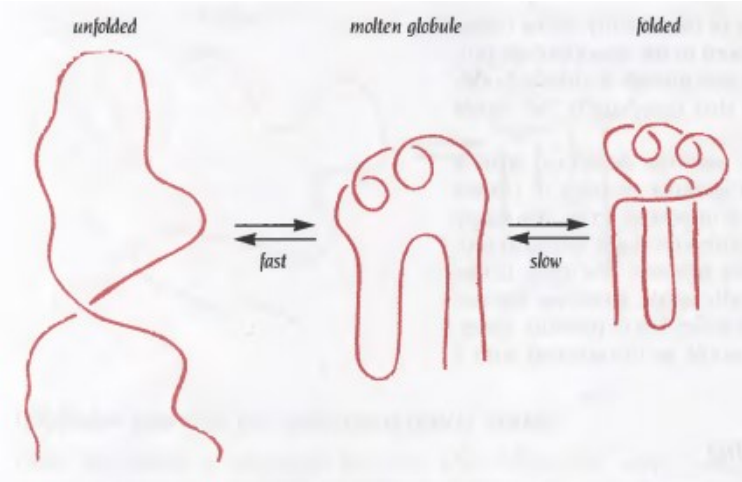
$= 10^{48}$ hours

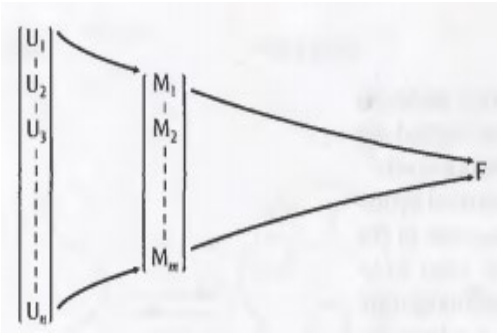
$\approx 10^{44}$ years

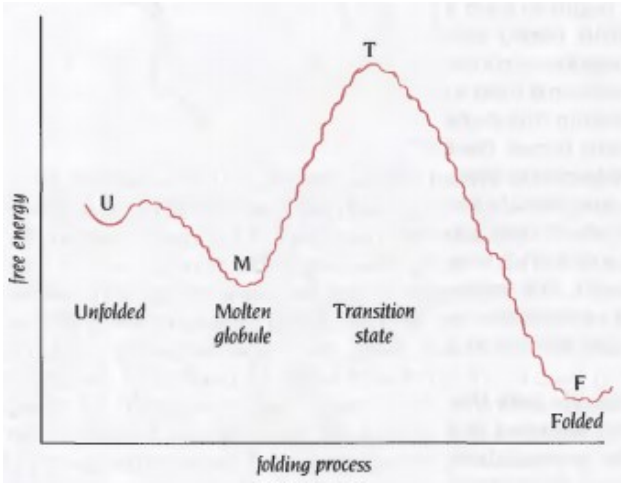
(astronomical number)

Impossible !!

In living cells (*in vivo*) and *in vitro*, the actual folding time is 0.1 to 1000 seconds







The first observable event in the folding pathway of proteins is a collapse of the flexible disordered unfolded polypeptide chain into partly organised globular structure, **Molten Globule**

- Formation of molten globule is a fast event in the folding pathway, usually within the deadtime of experimental observation (few milliseconds)
- The molten globule has most of the secondary structure of the native state and in some cases even native like positions of the α helices and β strands
- It is less compact than the native structure and proper packing interactions in the interior of the protein have not been formed
- The interior side chains may be mobile, more closely resembling a liquid than solid like interior of native state
- Loops and other elements of surface structure remain largely unfolded, with different conformations. Thus molten globule is not single structural entity but as an ensemble of related structures that are rapidly interconverting
- In the second step (~1 second) a single native structure is formed through the formation of native interactions including hydrophobic packing in the interior, fixation of surface loops

What is the Driving force for the collapse of randomly oriented unfolded polypeptide chain to molten globule ?

✓ A little change in ΔG by forming H-bonds in α helices and β sheets

- as unfolded state has equally stable H-bonds with water

➢secondary structure formation can not be thermodynamic driving force for protein folding!

✓ Large ΔG by bringing hydrophobic side chains out of contact with water to the interior

Likely scenario ⇒ The polypeptide chain begins to form a compact shape with the hydrophobic side chains at least partially buried early in the folding process

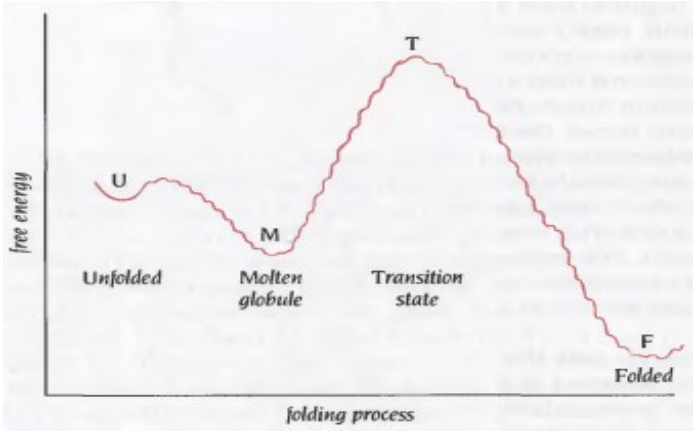
Important consequences :

- It vastly reduces the number of possible conformations that need to be searched because only these that are sterically allowed can be sampled
- When some of the side chains are partially buried, –NH and –CO groups of their backbone are also buried in hydrophobic environment and are unable to form H-bonds with water

➢This is energetically unfavourable until they form H-bonds to each other

⇒ formation of α helices and β sheets

❖ The formation of secondary structure (α helices and β sheets) early in folding process can therefore be regarded as a consequence of burying hydrophobic side chains and not as a driving force for the formation of molten globule



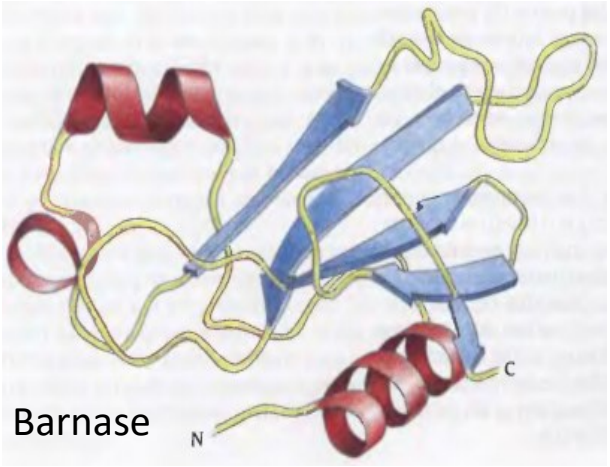
Folding: Protein molecule proceeds from high energy unfolded state to a low energy native state through metastable intermediate with local energy minima separated by unstable transition state of high energy

⇒ important to characterise these intermediate states

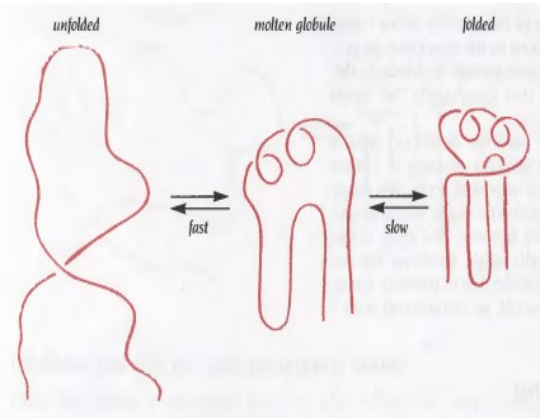
To investigate the folding pathway Alan Fersht developed an unique technique:

- Effect on the energetics of folding upon single site mutation in protein of known structure

Example: Let, Ala to Gly mutation in the solvent exposed face of an α helix results in destabilisation of both intermediate and native states then α helix has already formed in the molten globule state. If the mutation destabilises the native state but not the intermediate state then α helix has not formed in the molten globule state.



Barnase



The intermediate molten globule state has not only most of the native secondary structural elements but also the native like relative positions in the α helix and β sheet as well as the relative positions of the β strands within the β sheet

Folding of Barnase proceeds through single major transition state and consequently through one major pathway