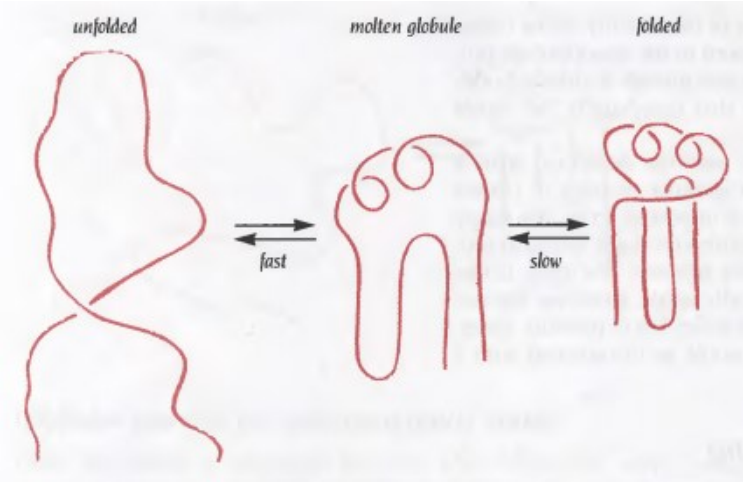
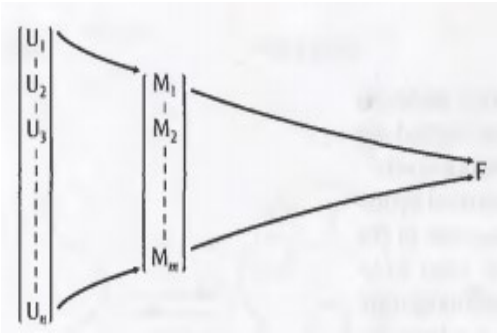
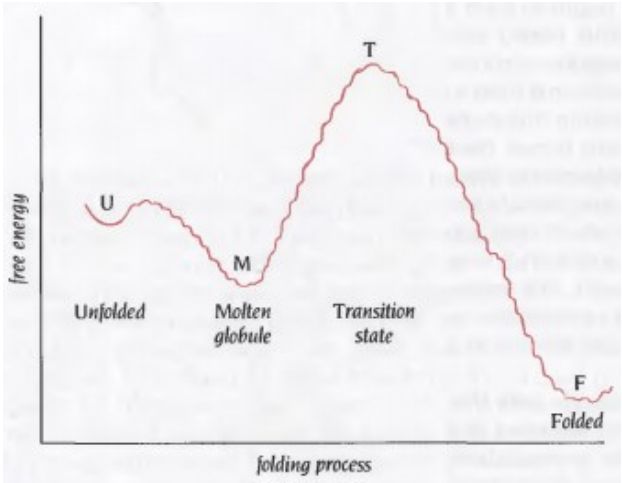






The first observable event in the folding pathway of proteins is a collapse of the flexible disordered unfolded polypeptide chain into partly organised globular structure, **Molten Globule**

- 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

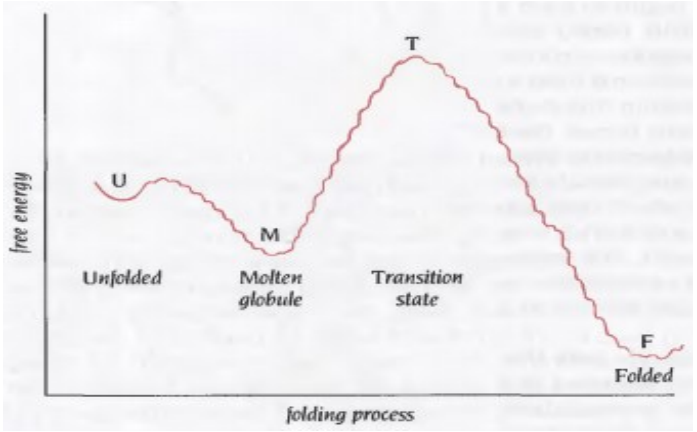
Important consequences :

- 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



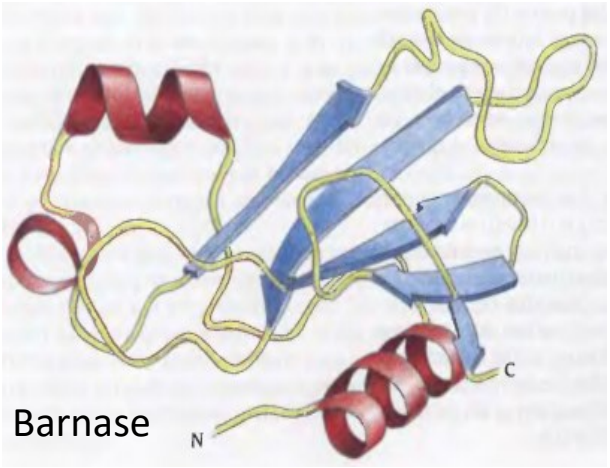
Folding: Protein molecule proceeds from high energy unfolded state to a low energy native state through metastable intermediate with local energy minima separated by unstable transition state of high energy

⇒ important to characterise these intermediate states

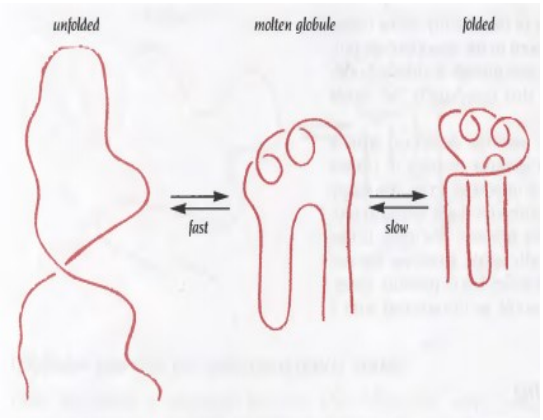
To investigate the folding pathway Alan Fersht developed an unique technique:

- 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.

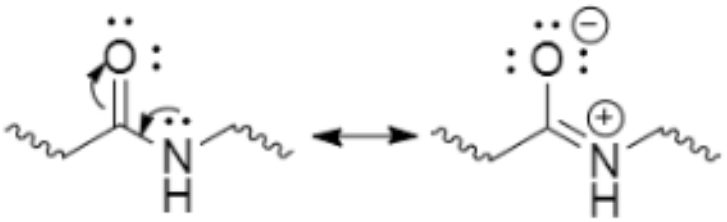


Barnase



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



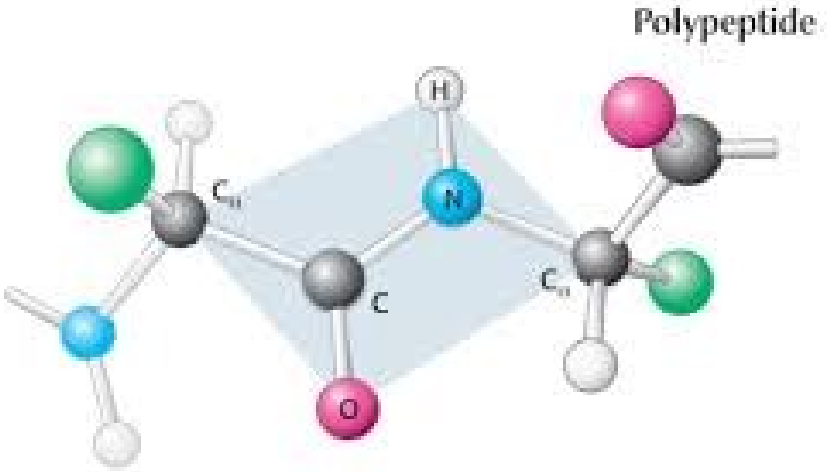
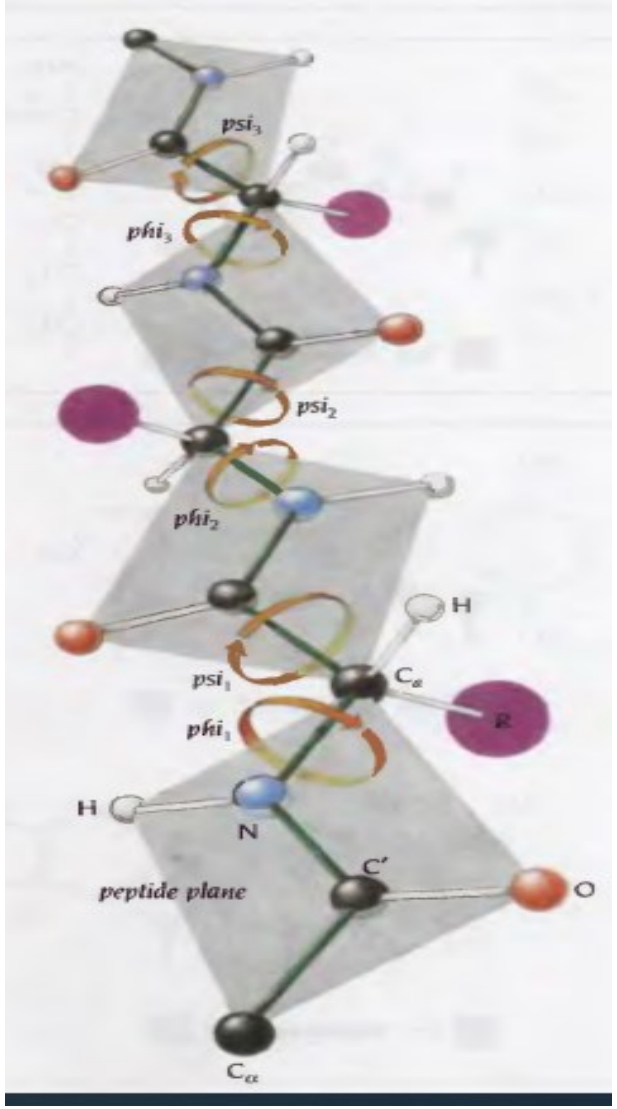


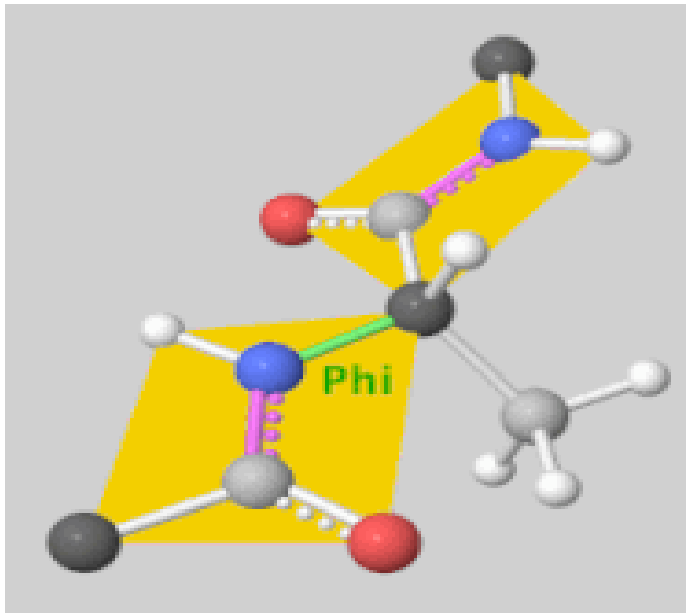
Figure 4.6 Peptide bonds are planar. In a pair of linked amino acids, six atoms (C_α, C, O, N, H, and C_α) lie in a plane. Side chains are shown as green balls.

peptide bond
tures: the elec

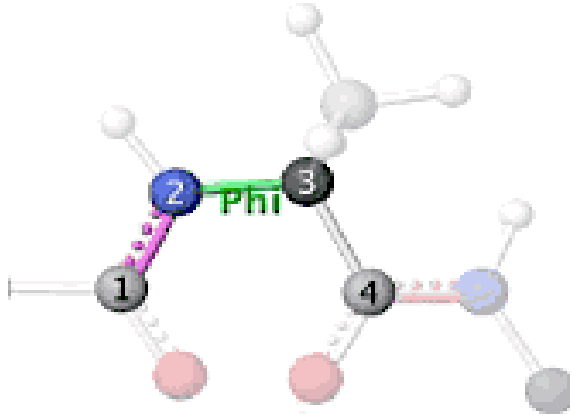
- Different way to visualise the polypeptide chain:
  - Peptide units do not have the side chains
  - Peptide units are rigid
  - Each unit can rotate around C_α–C' and N–C_α bond
  - Angle of rotation around N – C_α : phi (ϕ)
  - Angle of rotation around C_α– C' : psi (ψ)
  - Each amino acid residue is associated with two conformational angle: ϕ and ψ
  - These are the only degrees of freedom that define the conformation of whole main chain

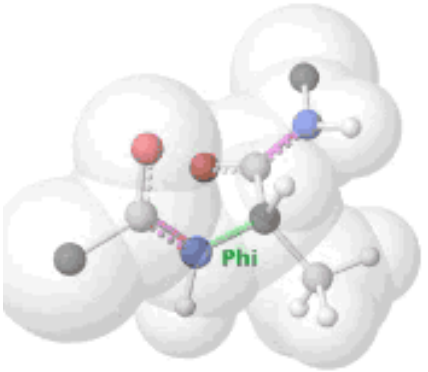


ϕ and ψ are examples of dihedral angles which are the angles between two intersecting planes



Phi = 0°

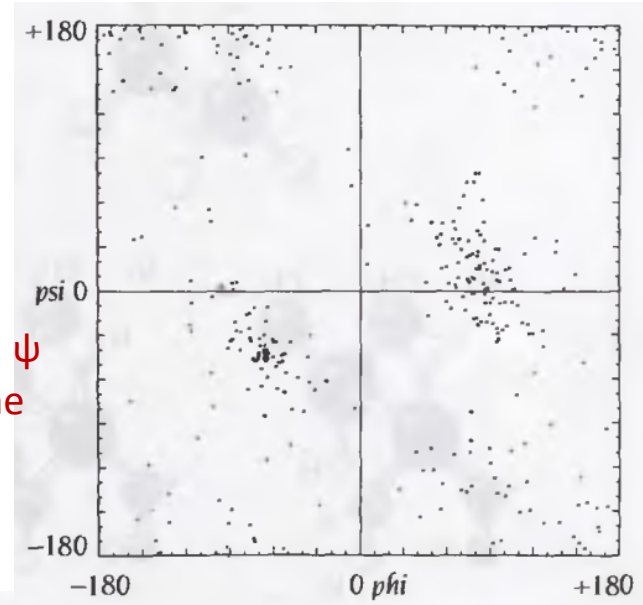




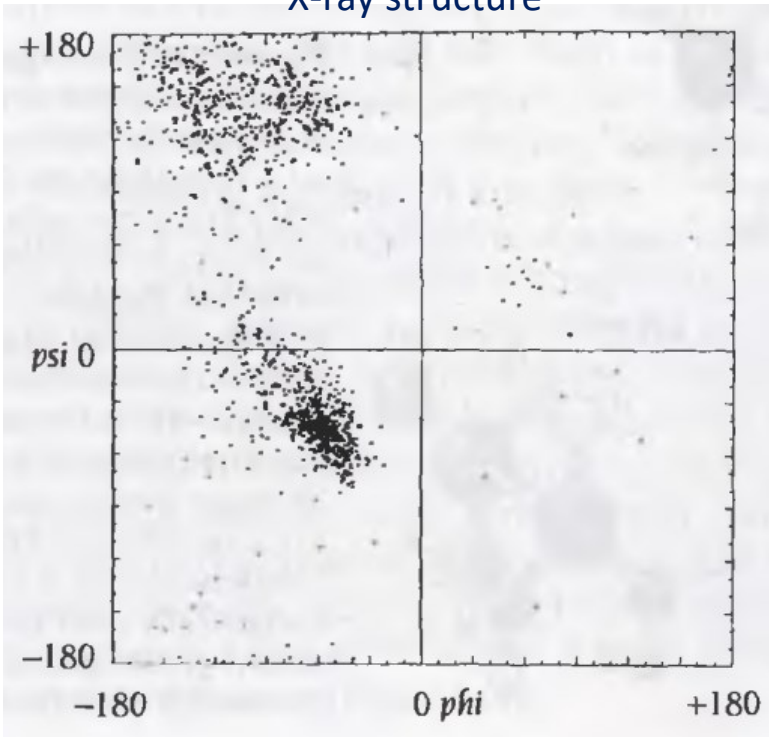
Cα C H N O

- Most combinations of ϕ and ψ angles for an amino acid are not allowed due to steric collisions between side chains and main chain
- D and L forms of an amino acid, which are mirror image of each other, have different allowed ϕ and ψ angles
- HIV-1 protease – D-enzyme, made of D-amino acids, is exactly the mirror image of L-enzyme, made of L-amino acids

Observed ϕ and ψ angles for glycine

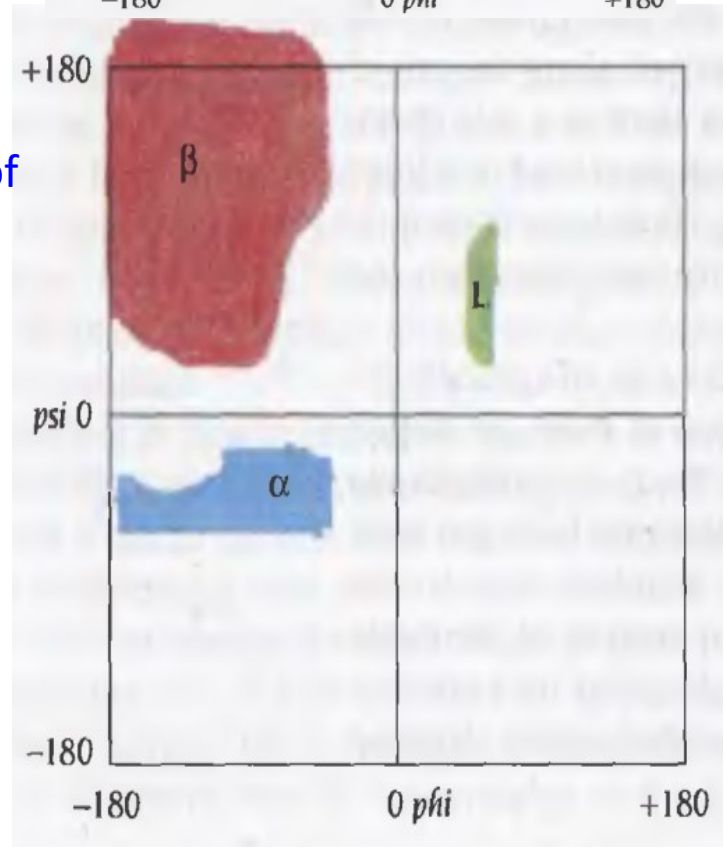


Observed ϕ and ψ angles for all amino acid residues except glycine in a well defined X-ray structure



Ramachandran plot=> calculations of sterically allowed regions

α: right handed α helices
β: β strand
L: left handed α helices



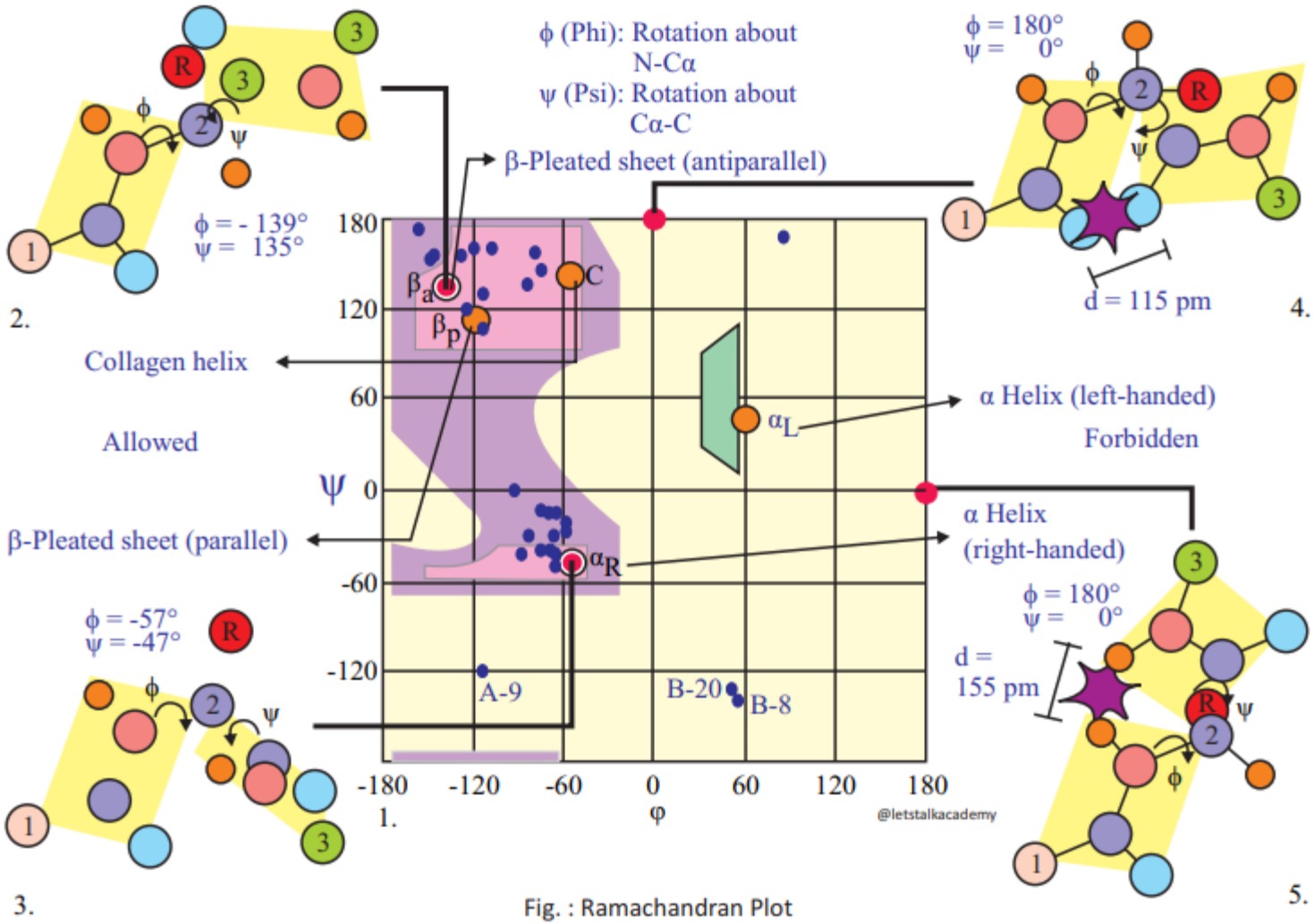


Fig. : Ramachandran Plot