

The background features a dark blue gradient with several circular elements. On the left, there are concentric circles with tick marks and numerical labels (40, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260) arranged in a spiral pattern. Some of these circles have arrows pointing in various directions, suggesting rotation or movement. The overall aesthetic is technical and scientific.

PROTEIN BACKBONE
FLEXIBILITY,
PHI-PSI ANGLE,
THE RAMACHANDRAN PLOT

Proteins And Backbone Flexibility

Proteins are most abundant organic molecules of living system.

They constitute 70% of total dry weight.

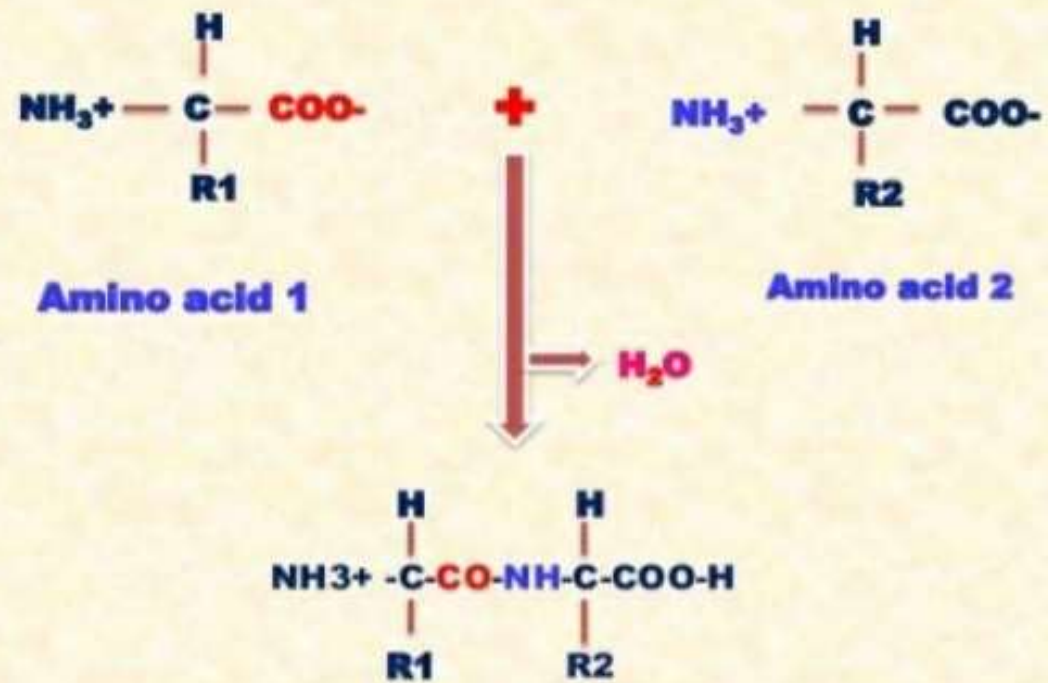
They form fundamental basis of structure & function of life.

Proteins are nitrogenous macromolecules composed of many amino acids.

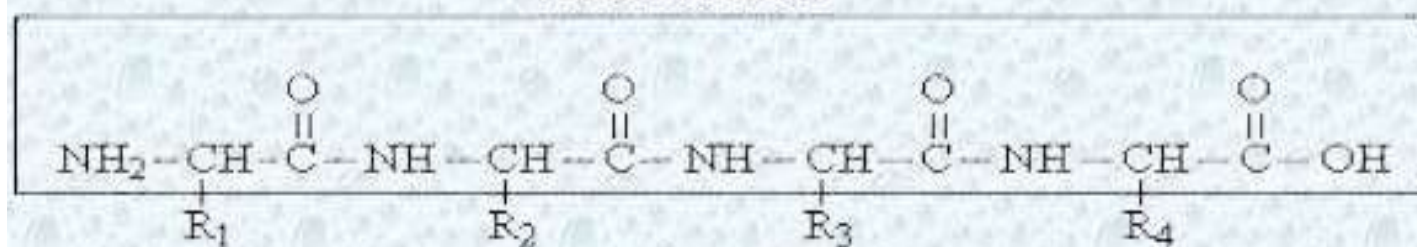
Peptide bond formation

- Alpha carboxyl group of amino acid with forms a covalent peptide bond with alpha amino group of other amino acid by removal of a molecule of water.
- This results in di-peptide , repetition of this process generates polypeptide or protein of specific amino acid sequence.
- Polypeptide backbone is repeating sequence of N-C-C-N-C-C.... In peptide bond, side chain or R-group isn't a part of backbone or peptide bond.

Formation of peptide bond



backbone



sidechains

Overview of protein structure

- Configuration-Geometric relationship between a given set of atoms.
- Conformation-Spatial arrangement of atoms in a protein.
- Thermodynamically most stable conformation exist . Stabilized largely by weak interactions.
- Stability-Tendency to maintain a particular conformation.
- Conformation is stabilized by disulfide bonds and non covalent forces.

Structures of proteins

- Primary structure(determined by covalent bonds)
- Secondary structure
- Tertiary structure
- Quaternary structure
- Secondary ,tertiary, quaternary structures determined by weak forces.

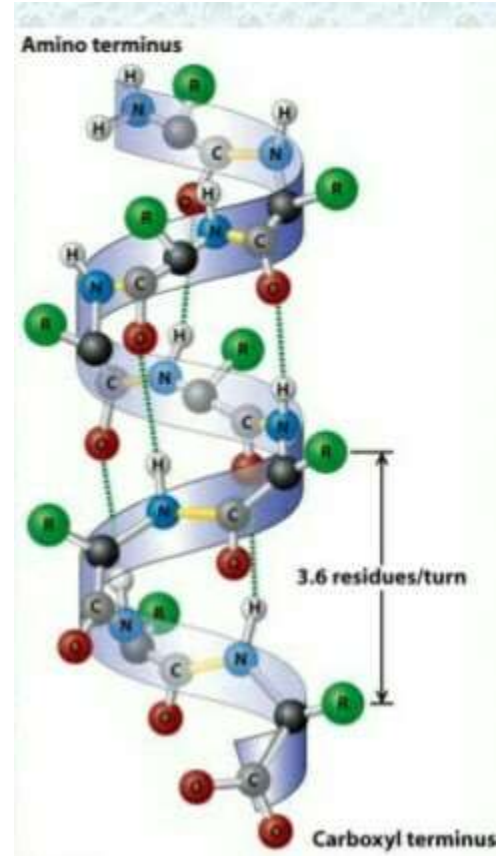
Primary structure

- Refers-Sequence of amino acids present in polypeptide chain . These are formed by assembly joined with help of covalent bonds or peptide bonds.
 - Secondary Structure-
- Local regularly occurring structure in proteins formed due to hydrogen bonds between backbone atoms.
- Examples-1)Alpha helix
2)Beta sheets

Alpha helix

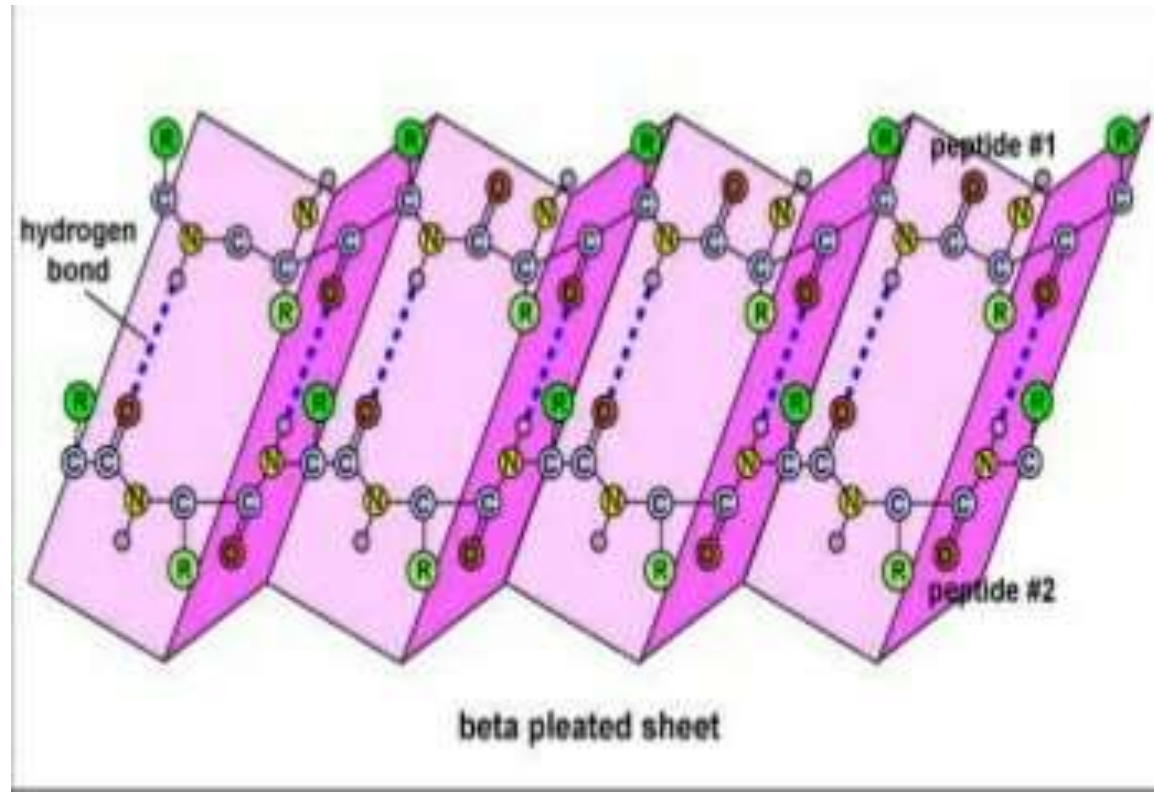
- Right handed spiral structure.
- Side chain extends outwards.
- Pitch-5.4 Å; length-12 residues (approx); helical turns-3 (approx).
- Stabilized by hydrogen bonding that are arranged such that peptide carbonyl oxygen and amide hydrogen.

Fig-Alpha helix



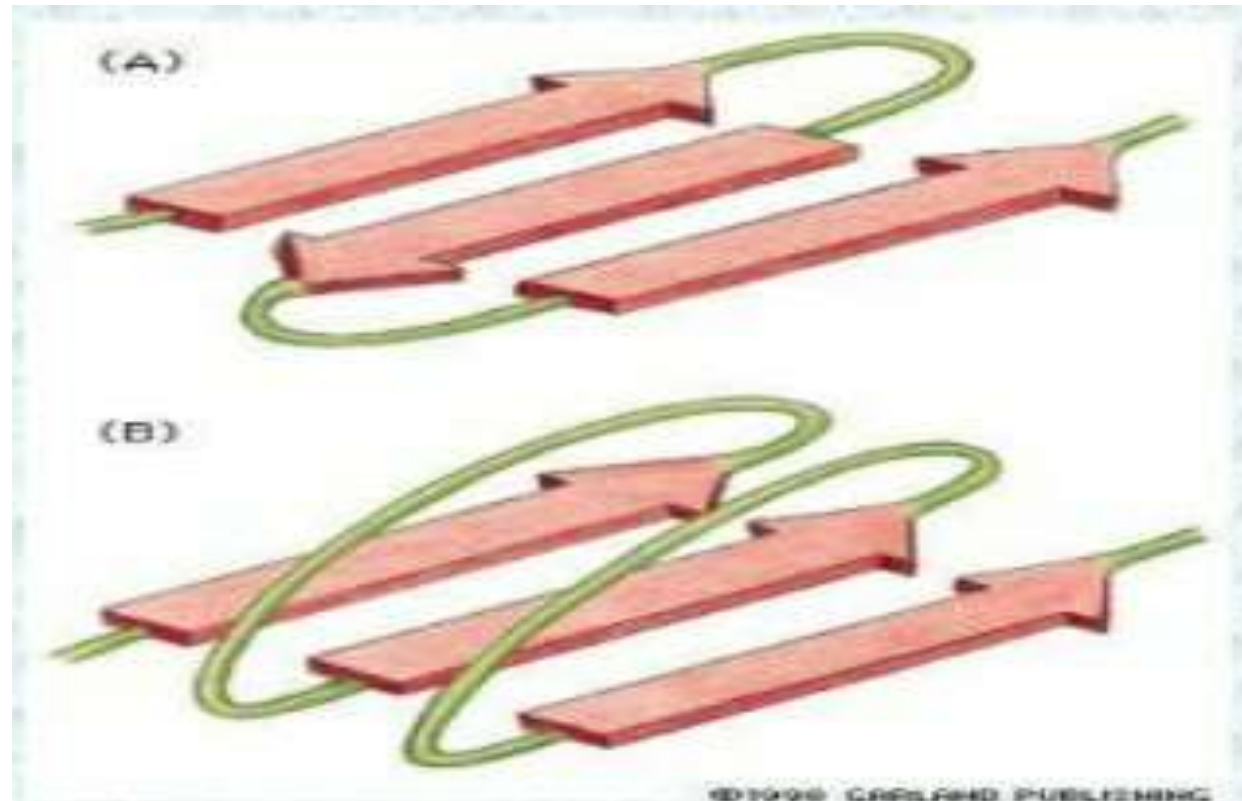
Beta pleated sheet

- Formed when 2 or more polypeptides line up side by side.
- Individual polypeptide-beta strand.
- Each beta strand is fully extended.
- They are stabilized by hydrogen bond between N-H and carbonyl groups of adjacent cells.



- Beta pleated sheets are of two types-
- A)Anti-parallel beta sheet- Neighbouring hydrogen bonded polypeptide chain run in opposite direction.
- B)Parallel beta sheet-Hydrogen bonded chains extended in same direction.

a) Anti parallel & b) parallel



- Two major sorts of connection between beta strands-
- 1) Hairpin or same end connection
- 2) Cross over or opposite end connection.

(a)



hairpin connection
common

(b)



right-handed crossover
common

(c)



left-handed crossover
uncommon

Tertiary Structures

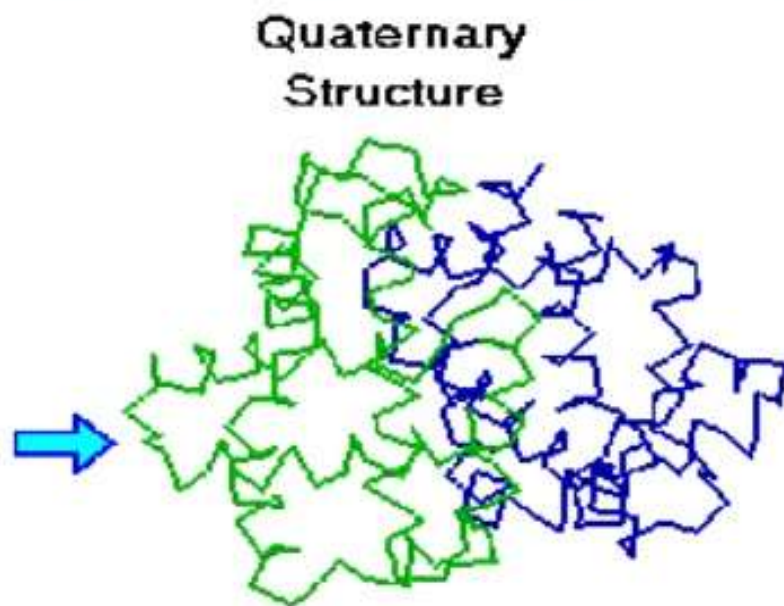
- Defines overall 3-D shape of protein.
- Tertiary structure is based on various types of interactions between side chains of polypeptide chain.
- Example-1) Globular proteins
2) Fibrous proteins

Quaternary structures

Involves clustering of several individual peptide or protein chains into specific shape.

Two types-1) Homodimer -Association between identical polypeptide chains.

2) Heterodimer-Interaction between subunits of very different structures.

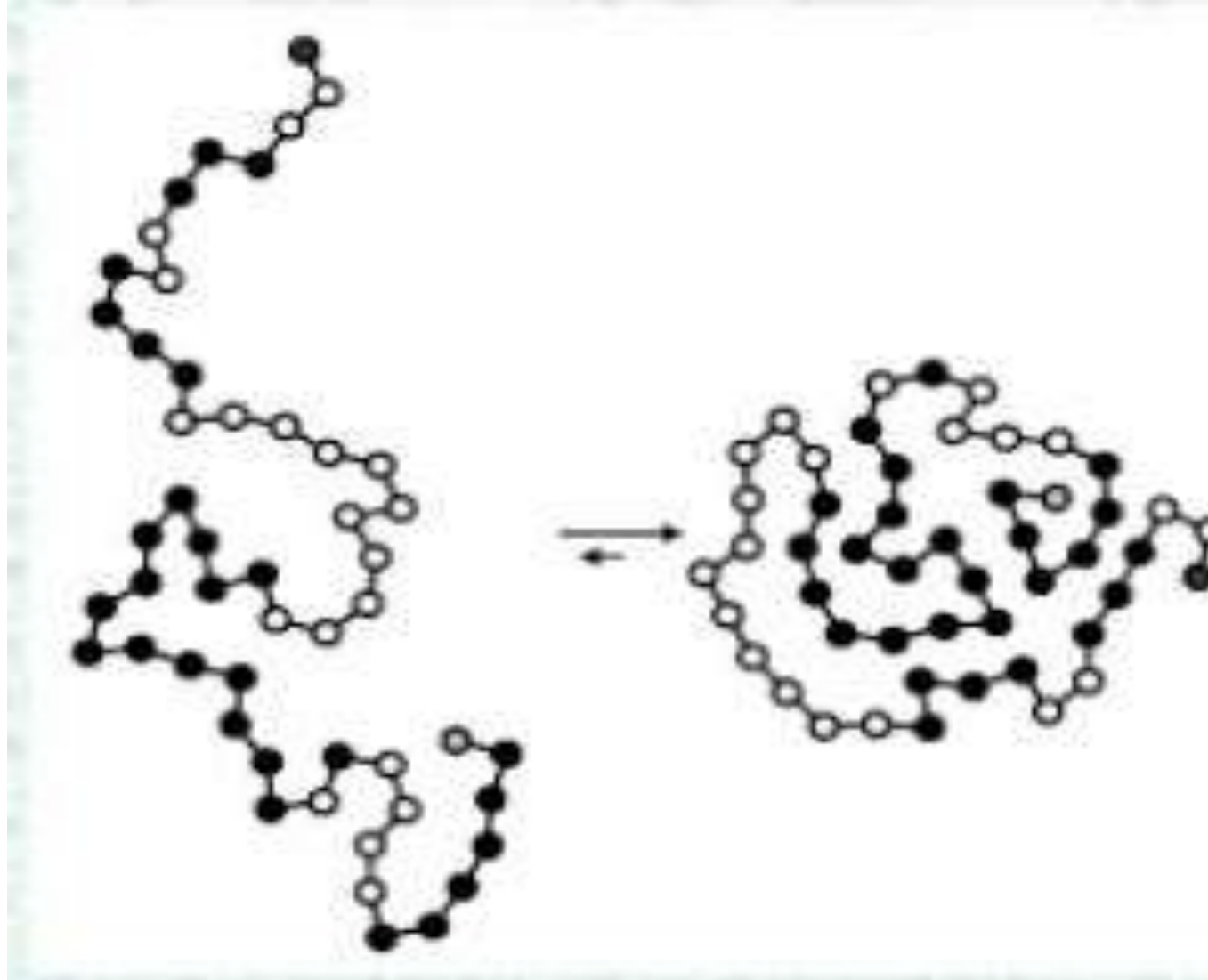


**Structure maintained by
interchain interactions**

Protein Folding

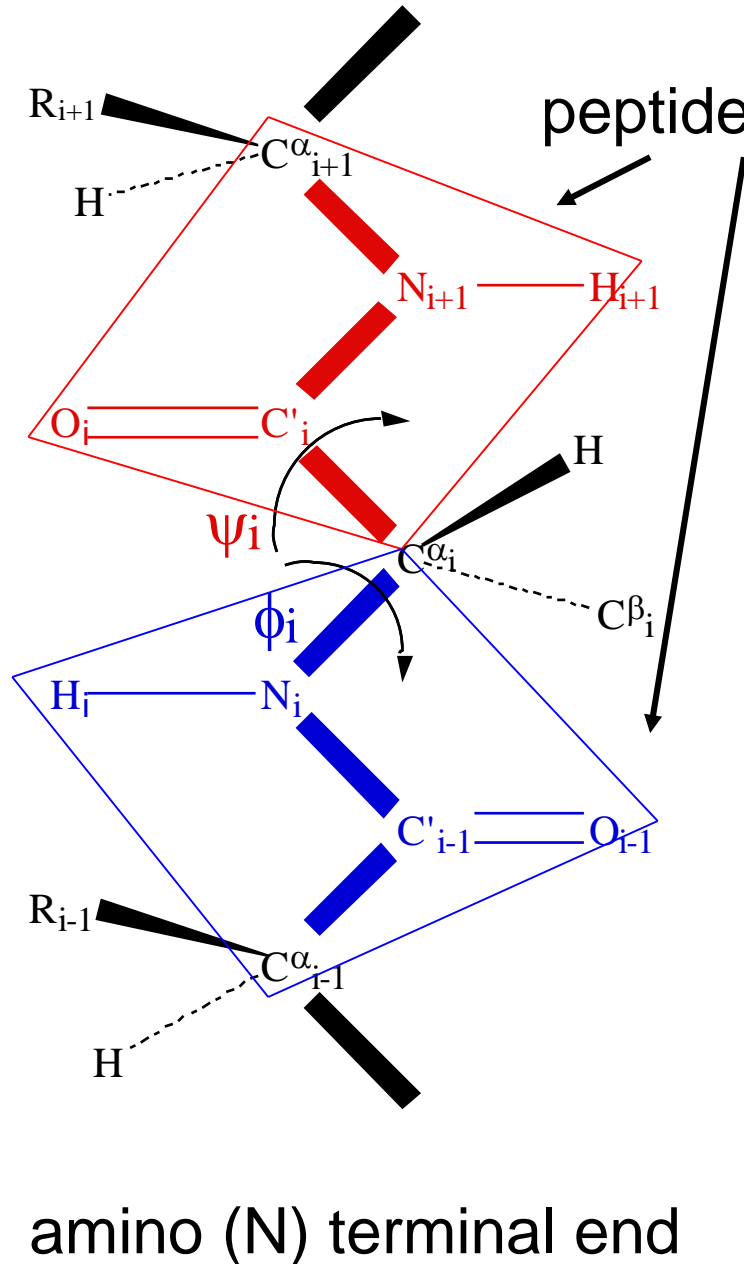
- Peptide bond allows for rotation around it & therefore protein can fold and orient R groups in favourable positions.
 - Turns and loops
- Loops and turns connect alpha and beta helices.
- Loops have only 4-5 amino acid residues called turns when have internal hydrogen bonds.

Protein folding



carboxy (C) terminal end

ϕ and ψ angles



Torsional angles are defined by four atoms:

$$\psi \rightarrow N_i - C^{\alpha} - C'_i - N_{i+1}$$

$$\phi \rightarrow C'_{i-1} - N_i - C^{\alpha} - C'_i$$

Notice that these are defined from N to C terminus, using main chain atoms only.

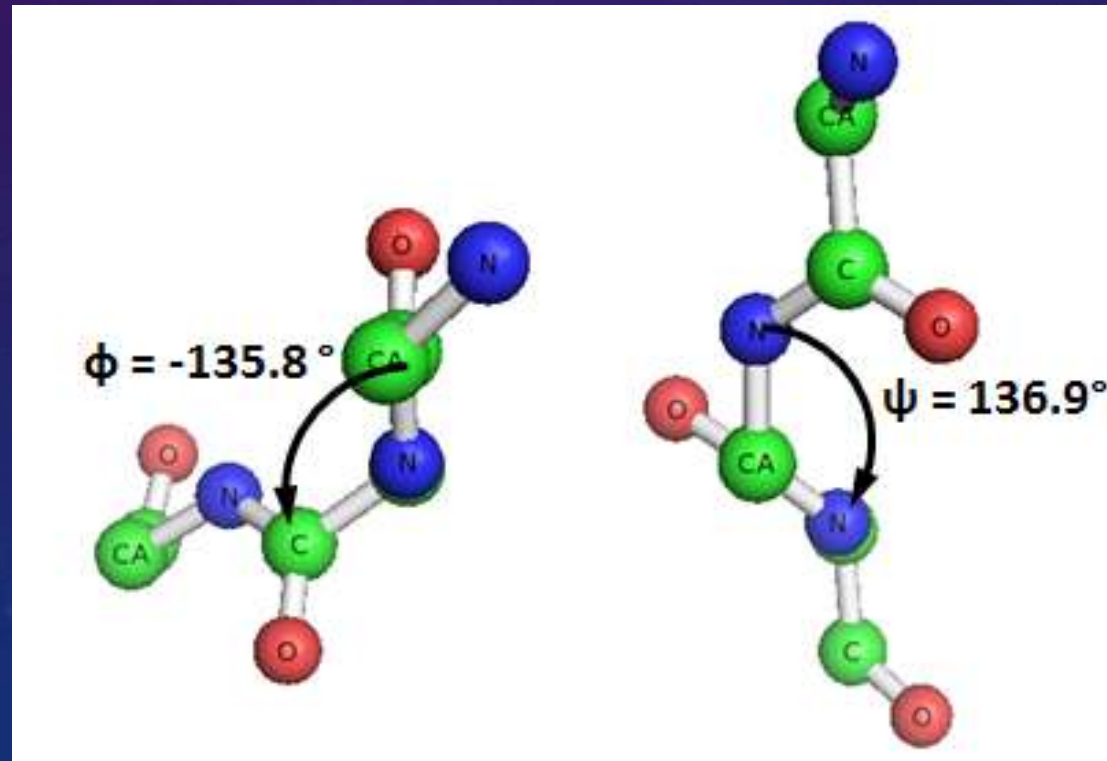
A residue's conformation is usually listed as (ϕ, ψ) , since ω is close to 180 for almost all residues.

ϕ and ψ angles

- The two torsion angles of the polypeptide chain, also called Ramachandran angles, describe the rotations of the polypeptide backbone around the bonds between – N-C α (called Phi, ϕ) and – C α -C (called Psi, ψ).
- The Ramachandran plot provides an easy way to view the distribution of torsion angles of a protein structure

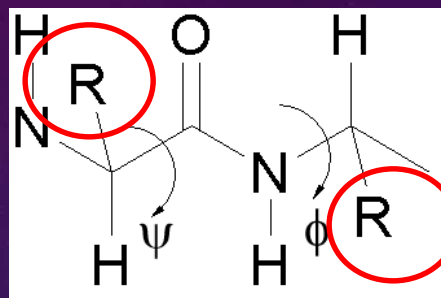
ϕ and ψ angles

- It also provides an overview of allowed and disallowed regions of torsion angle values, serving as an important indicator of the quality of protein three-dimensional structures.

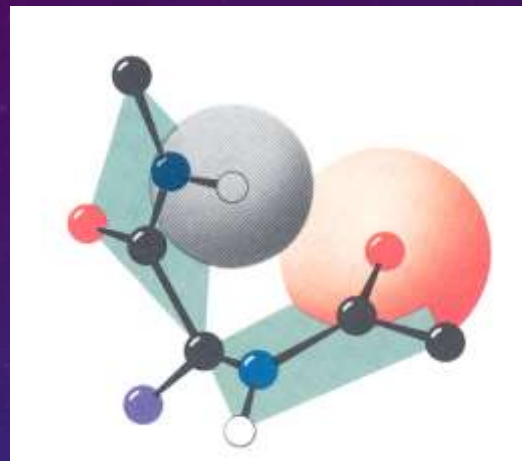


ϕ and ψ angles

- Torsion angles are among the most important local structural parameters that control protein folding
- Essentially, if we would have a way to predict the Ramachandran angles for a particular protein, we would be able to predict its 3D folding.
- The reason is that these angles provide the flexibility required for the polypeptide backbone to adopt a certain fold,
- Since the third possible torsion angle within the protein backbone (called omega, ω) is essentially flat and fixed to 180 degrees

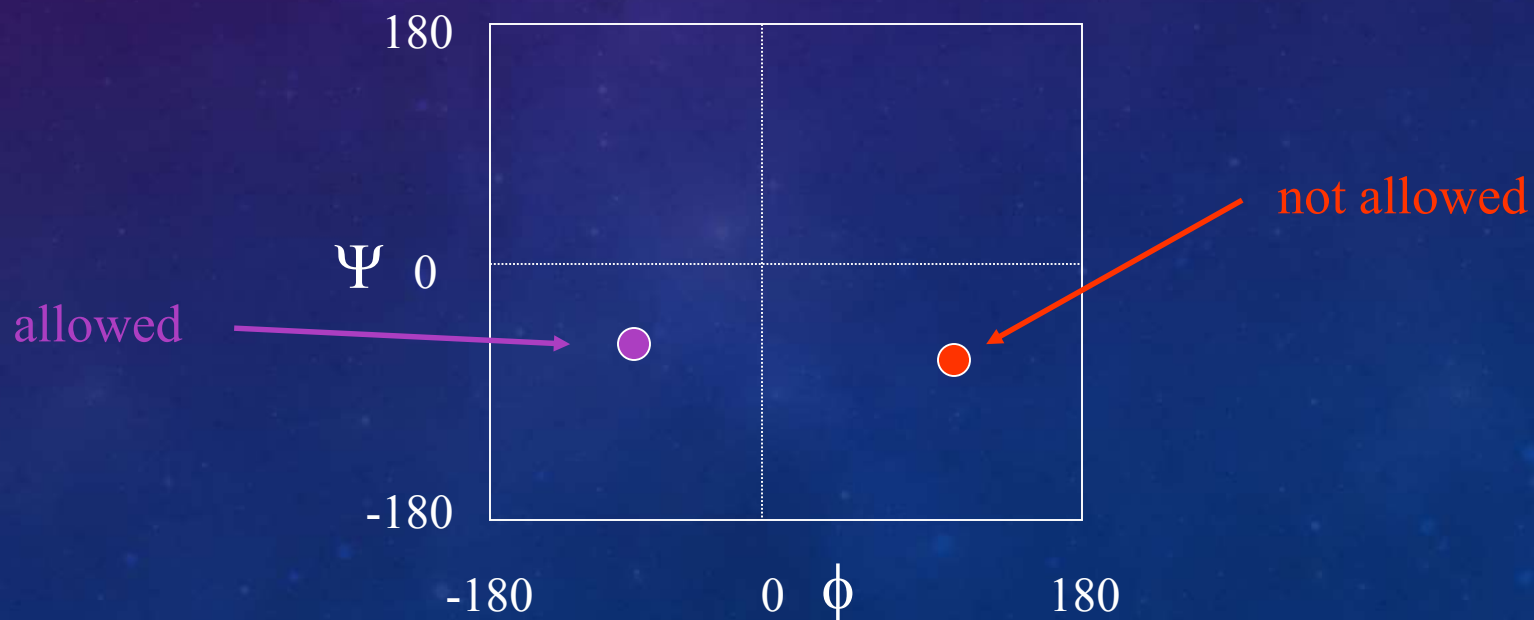


between R groups

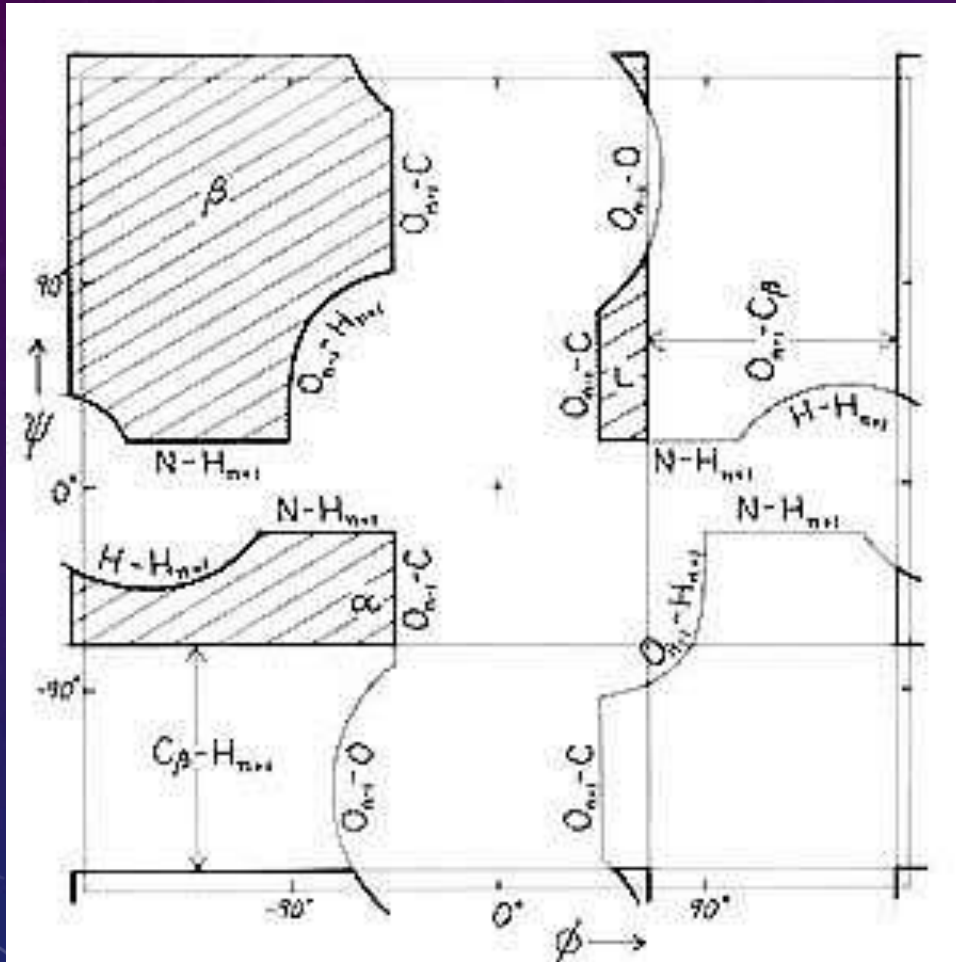


between carbonyls

or combinations of an R group, carbonyl or amide group.



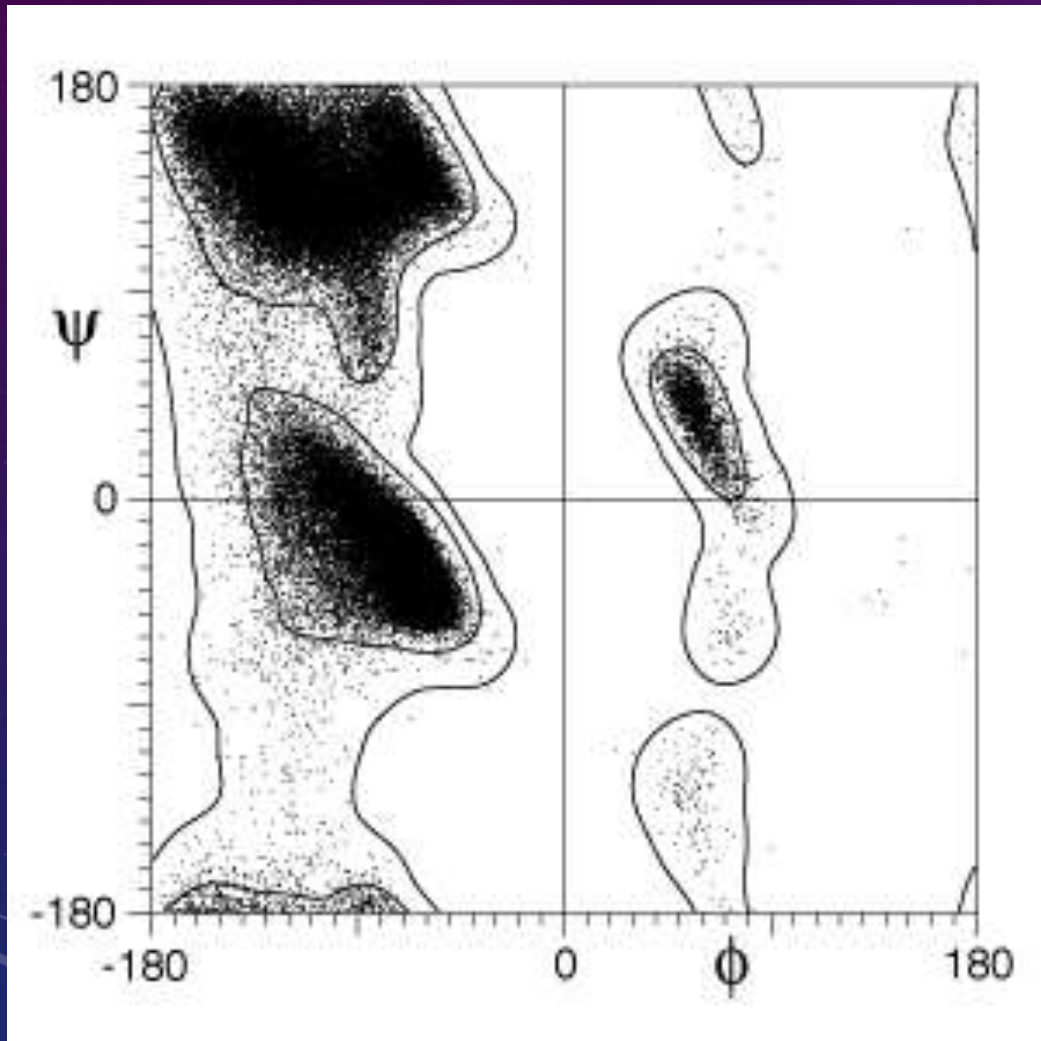
STERIC CLASHES DISALLOW SOME Φ AND Ψ COMBINATIONS



- Theoretical calculations using hard sphere approximations suggest which phi and psi combinations cause clashes, and between which atoms.

- Cross-hatched regions are “allowed” for all residue types. The larger regions in the four corners are allowed for glycine because it lacks a side chain, so that no steric clashes involving the beta carbon are possible.

OBSERVED Φ AND Ψ COMBINATIONS IN PROTEINS



Phi-psi combinations actually observed in proteins with known high-quality structures.

Gly residues are excluded from this plot, as are Pro residues and residues which precede Pro (more on this later).

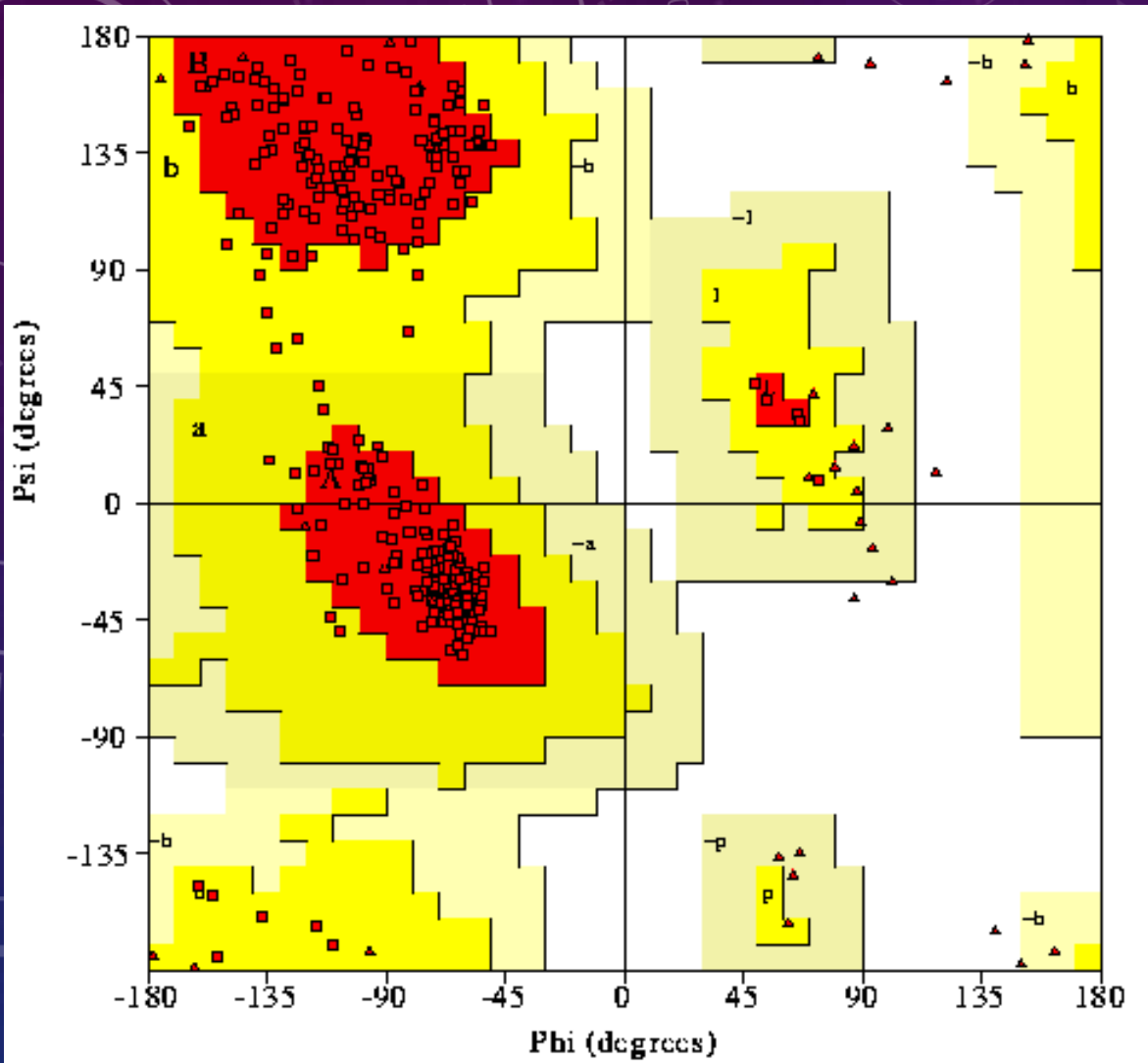
Contours enclose 98% and 99.95% of the data respectively.

Notice that the observed conformations do not exactly coincide with the theoretically allowed/disallowed confs based on steric clashes.

from Lovell SC *et al.*

Proteins **50**, 437 (2003)

SAMPLE “RAMACHANDRAN PLOT” FOR A PROTEIN



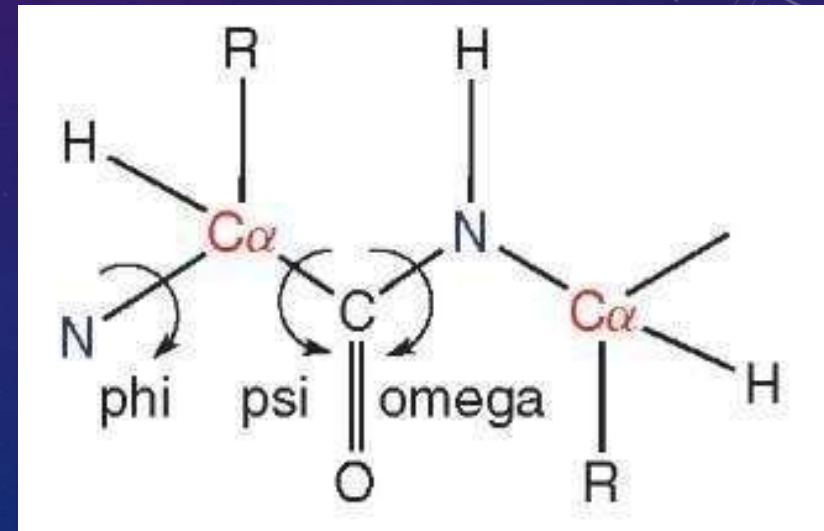
red= allowed
yellow= additionally allowed
pale yellow= generously allowed
white= disallowed

- The squares denote non-glycine residues, while the triangles are glycines. Glycines have no side chain and are not as restricted because of the lack of side chain steric clashes.

- This protein has 219 residues, 90% of which are in the “allowed” region and 10% of which are in “additionally allowed” regions. None are in the other regions (except glycines, which don’t count)

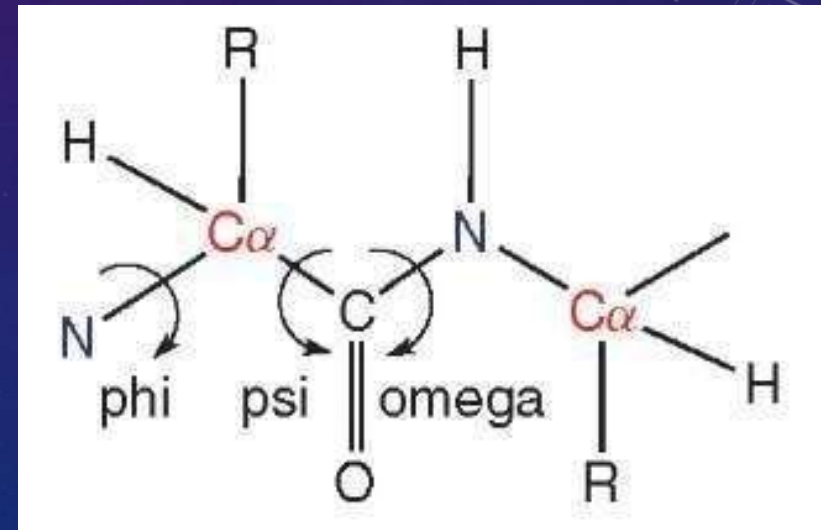
THE RAMACHANDRAN PLOT

- The two torsion angles of the polypeptide chain, describe the rotations of the polypeptide backbone around the bonds between N-C α (called Phi, ϕ) and C α -C (called Psi, ψ)
- It provides an easy way to view the distribution of torsion angles of a protein structure



THE RAMACHANDRAN PLOT

- It also provides an overview of allowed and disallowed regions of torsion angle values
 - serve as an important indicator of the quality of protein three-dimensional structures
- Torsion angles are among the most important local structural parameters that control protein folding
- Third possible torsion angle within the protein backbone (called omega, ω)

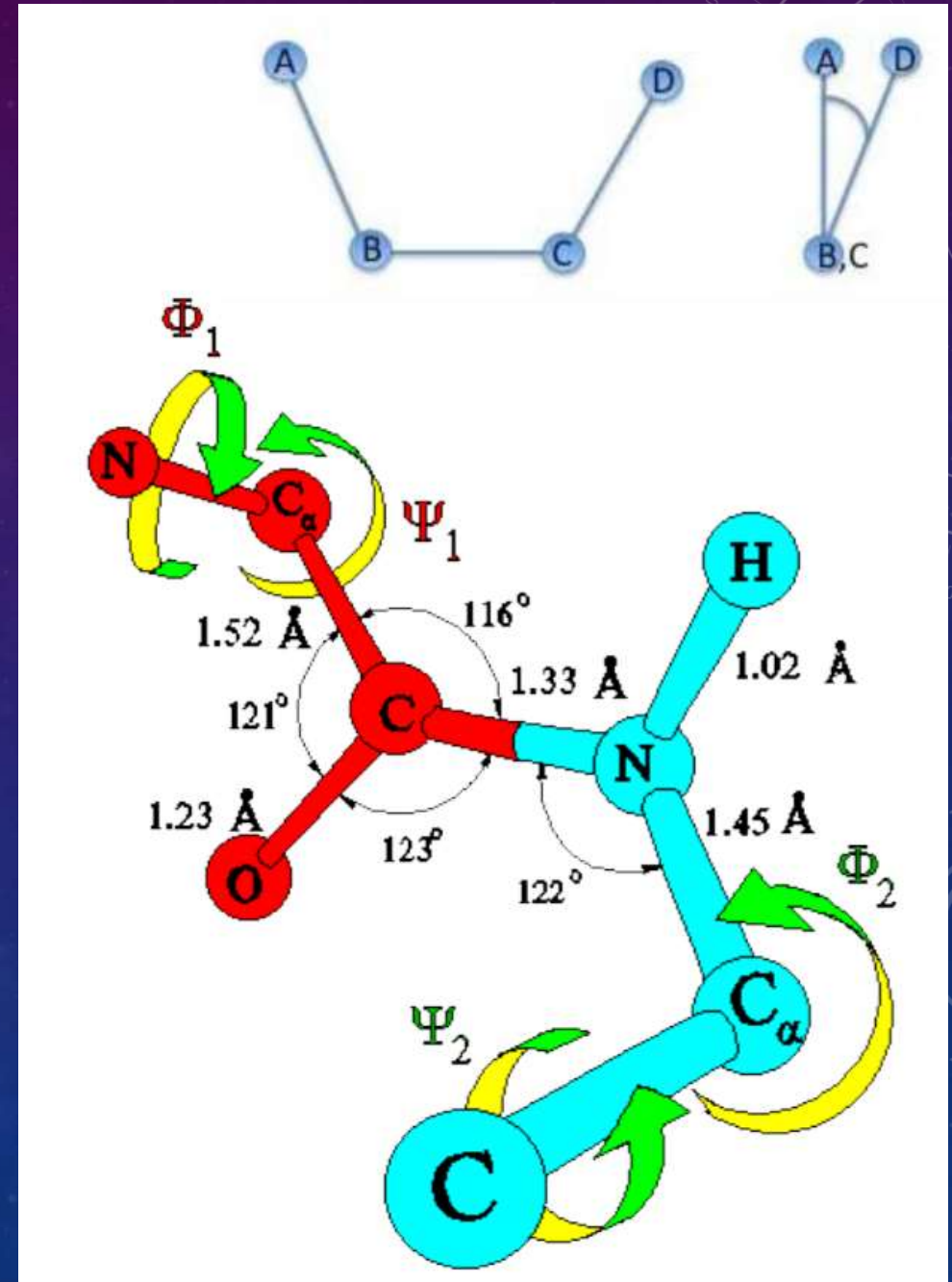


THE RAMACHANDRAN PLOT

- If we would have a way to predict the Ramachandran angles for a particular protein, we would be able to predict its 3D folding
- The reason is that these angles provide the flexibility required for the polypeptide backbone to adopt a certain fold, since ω is essentially flat and fixed to 180 degrees
 - This is due to the partial double-bond character of the peptide bond, which
 - restricts rotation around the C-N bond, placing two successive alpha-carbons and C, O, N and H between them in one plane
 - Thus, rotation of the protein chain can be described as rotation of the peptide bond planes relative to each other

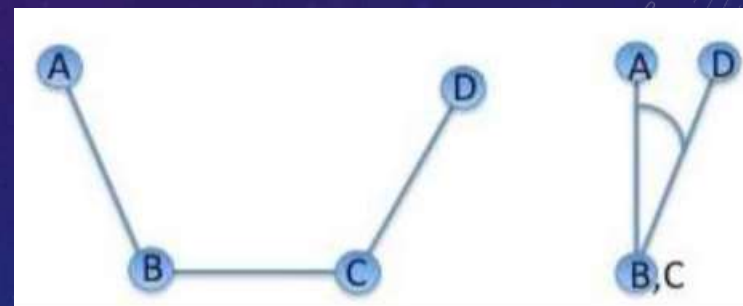
TORSION ANGLES

- Torsion angles are dihedral angles, which are defined by 4 points in space
- In proteins the two torsion angles phi and psi describe the rotation of the polypeptide chain around the two bonds on both sides of the C alpha atom



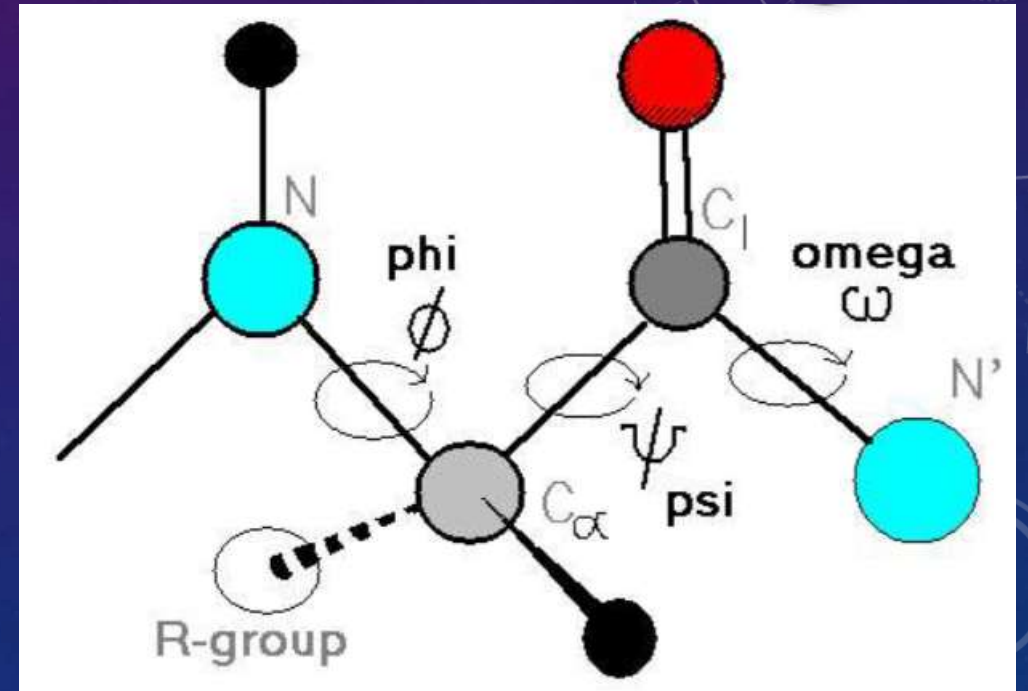
DIHEDRAL ANGLE

- The standard IUPAC definition of a dihedral angle is illustrated in the figure below
- A, B, C and D illustrate the position of the 4 atoms used to define the dihedral angle
- The rotation takes place around the central B-C bond
- The view on the right is along the B-C bond with atom A placed at 12 o'clock
- The rotation around the B-C bond is described by the A-B-D angle shown of the right figure: Positive angles correspond to clockwise rotation



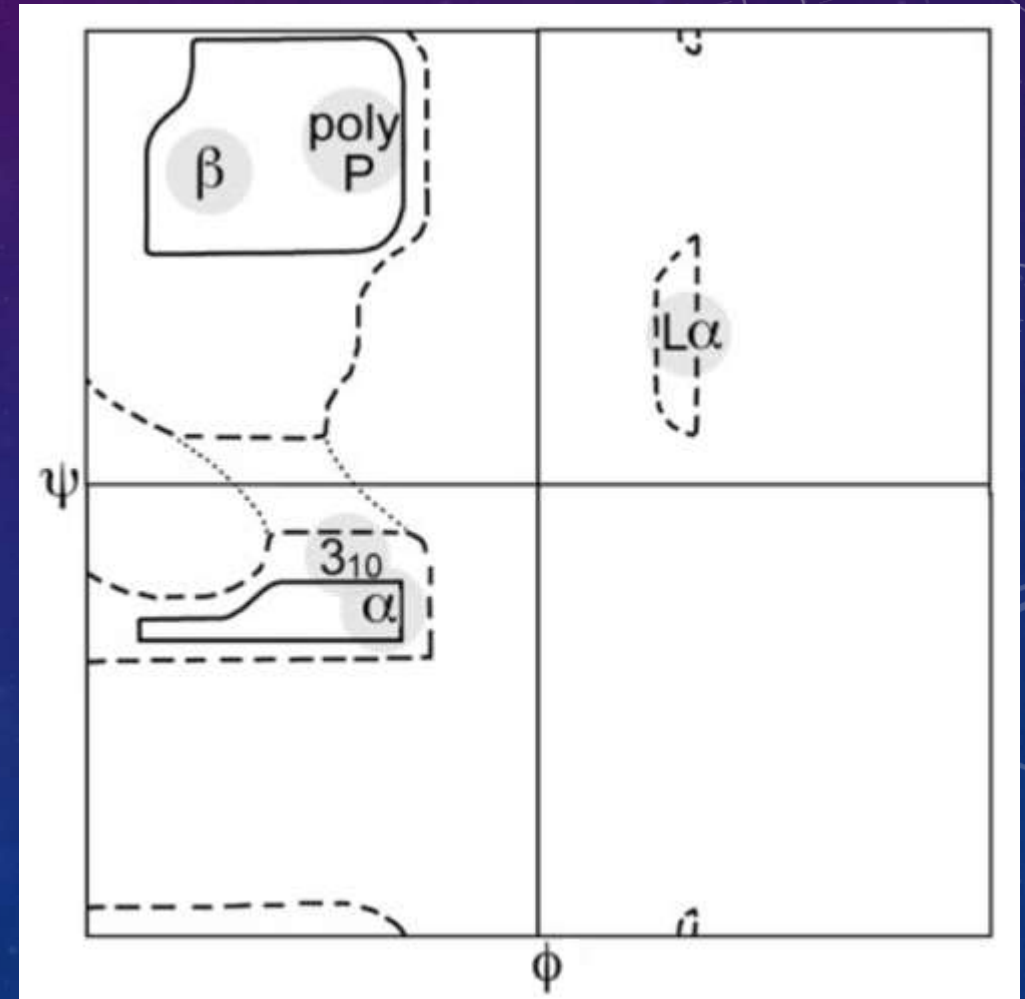
PROTEIN BACKBONE

- The protein backbone can be described in terms of the phi, psi and omega torsion angles of the bonds: The phi angle is the angle around the -N-CA- bond (where 'CA' is the alpha-carbon)
- The psi angle is the angle around the -CA-C- bond.
- The omega angle is the angle around the -C-Nbond (i.e. the peptide bond)



THE RAMACHANDRAN PLOT

- In a polypeptide the main chain N-Calpha and Calpha-C bonds relatively are free to rotate. These rotations are represented by the torsion angles phi and psi, respectively
- G N Ramachandran used computer models of small polypeptides to systematically vary phi and psi with the objective of finding stable conformations
- For each conformation, the structure was examined for close contacts between atoms
- Atoms were treated as hard spheres with dimensions corresponding to their van der Waals radii
- Therefore, phi and psi angles which cause spheres to collide correspond to sterically disallowed conformations of the polypeptide backbone



THE RAMACHANDRAN PLOT

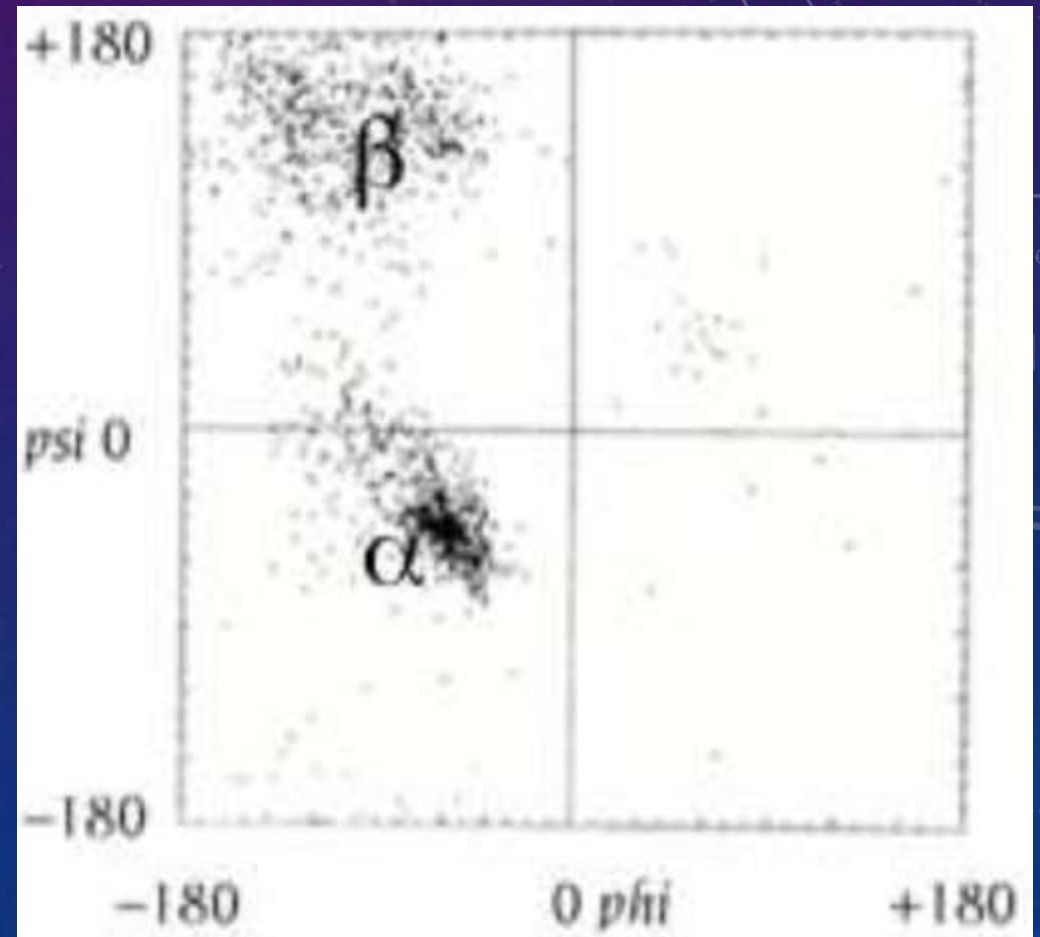
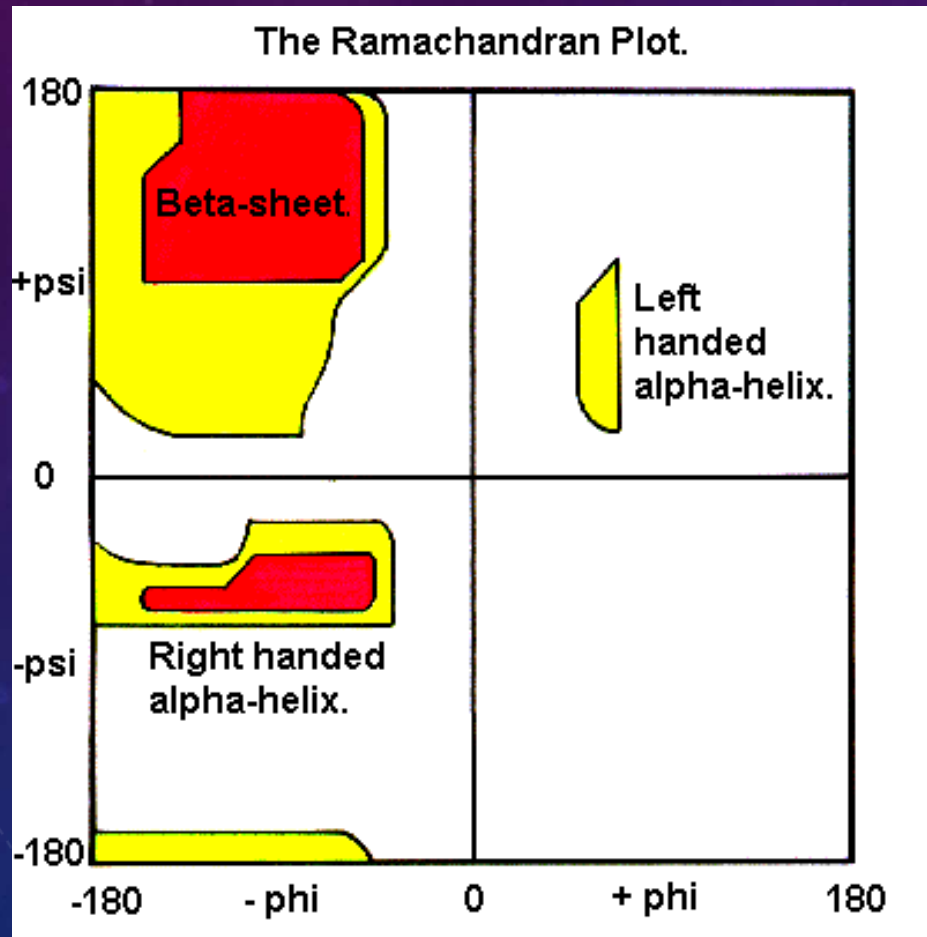
- In the diagram the white areas correspond to conformations where atoms in the polypeptide come closer than the sum of their van der Waals radii.
- These regions are sterically disallowed for all amino acids except glycine which is unique in that it lacks a side chain

THE RAMACHANDRAN PLOT

The red regions correspond to conformations where there are no steric clashes, ie these are the allowed regions namely the alpha-helical and beta-sheet conformations

- The yellow areas show the allowed regions if slightly shorter van der Waals radii are used in the calculation, ie the atoms are allowed to come a little closer together
- This brings out an additional region which corresponds to the left-handed alpha-helix

RAMACHANDRAN PLOT



THE RAMACHANDRAN PLOT

- L-amino acids cannot form extended regions of lefthanded helix – but occasionally individual residues adopt this conformation
 - These residues are usually glycine but can also be asparagine or aspartate where the side chain forms a hydrogen bond with the main chain and therefore stabilises this otherwise unfavourable conformation
 - The 3(10) helix occurs close to the upper right of the alpha-helical region and is on the edge of allowed region indicating lower stability

3₁₀ HELIX

- A 3₁₀ helix is a type of secondary structure
- found (often) in proteins and polypeptides
- Top view of the same helix shown to the right
- Three carbonyl groups are pointing upwards towards the viewer
 - spaced roughly 120° apart on the circle
 - corresponding to 3.0 amino-acid residues per turn of the helix



THE RAMACHANDRAN PLOT

- Disallowed regions generally involve steric hindrance between the side chain C-beta methylene group and main chain atoms
- Glycine has no side chain and therefore can adopt phi and psi angles in all four quadrants of the Ramachandran plot
- Hence it frequently occurs in turn regions of proteins where any other residue would be sterically hindered

SIGNIFICANCE OF RAMACHANDRAN PLOT

- Ramachandran plots show the relationship between the phi and psi angles of a protein referring to dihedral angles between the N and the C-alpha and the C-alpha and the C-beta. As an aside, the omega angle between the C-beta and the N tends to be fixed due to pi-pi interactions.
- There are limits to possible distributions of phi and psi angles due to steric clashes between the side chains. Furthermore other limitations from higher order structure will result in the adoption of defined phi-psi angles. Using data from solved crystal structures, it can be seen that the dihedral angles will adopt specific conformations in a protein.

- Furthermore, it can be noted that some of these conformations relate to specific secondary structures. As seen above, peptides in alpha-helices and beta-sheets adopt a even more limited set of phi-psi angles. Certain amino acids like glycine and proline, which differ from from canonical amino acids have an unique Ramachandran plot.
- The angles from a Ramachandran plot are useful not only for determining a amino acids' role in secondary structure but can also be used to verify the solution to a crystal structure. Furthermore, it assists with constraining structure prediction simulations and helps with defining energy functions