Ramachandran Plot

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Ramachandran Plot: A simplified approach

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Introduction

Ramachandran plot also known as a Ramachandran diagram or [ϕ, ψ] plot was originally developed in 1963 by G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan. Ramachandran plot provides a simple two-dimensional graphic representation of all possible protein structures in terms of torsion angles. Although the plot was developed using theoretical methods, mathematical calculations and models building, once the protein structure began to discover, the importance of plot was realized. Even after 55 years of its discovery, as more and more protein structures are being discovered, it remains an important tool to confirm the accuracy of the structures. In order to know more about Ramachandran plot, let us first understand the basic nature of peptide bond and torsion angles.

Planar nature of peptide bond

Polypeptides or proteins are most commonly, linear and unbranched polymers composed of amino acids linked together by peptide bonds. Peptide bonds are amide linkages formed between α-amino group of one amino acid and the α-carboxyl group of adjacent amino acid. This reaction is a condensation reaction, in which a water molecule is released and the linked amino acids are referred to as *amino acid residues*. Thermodynamically, peptide bond formation is an endergonic process, with $\Delta G^0 \sim +21$ kJ/mol.

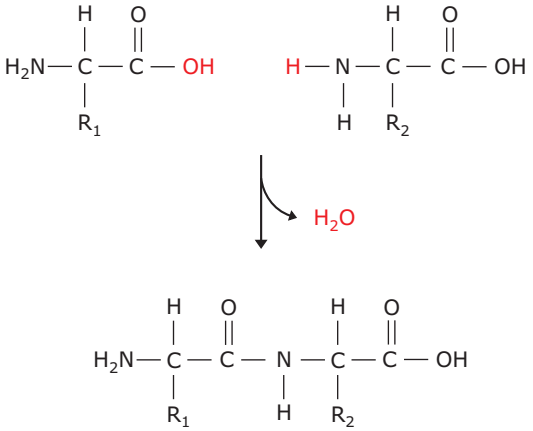
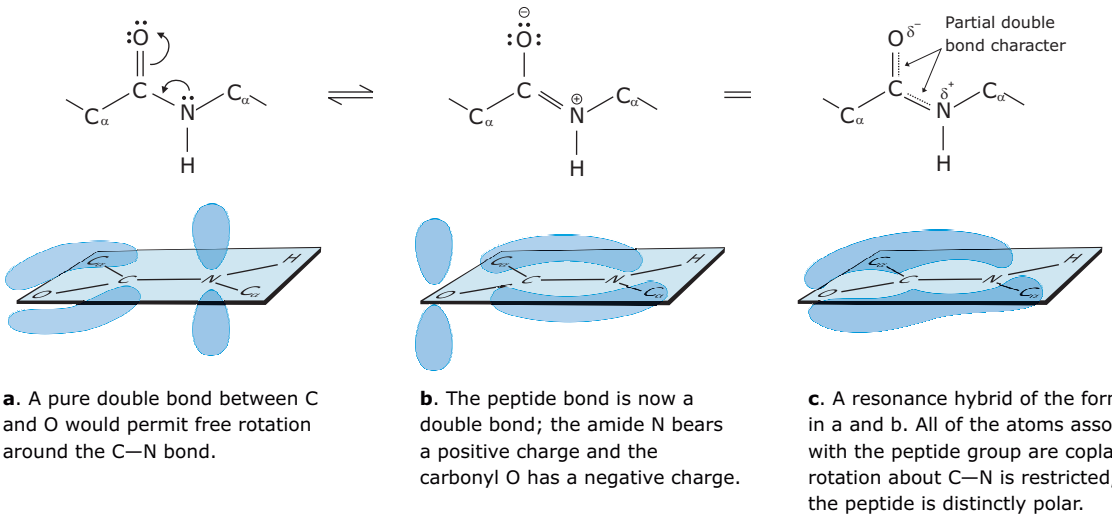


Figure 1 The formation of a peptide bond (also called an *amide bond*) between the α-carboxyl group of one amino acid and the α-amino group of another amino acid is accompanied by the loss of a water molecule.

The peptide C—N bond has a partial double bond character. Consequently, the peptide bond length is only 1.33 Å, which is between the values expected for a C—N single bond (1.49 Å) and a C = N double bond (1.27 Å). The peptide bond appears to have approximately 40 percent double-bonded character due to resonance. The oxygen has a partial

negative charge and the nitrogen has a partial positive charge, setting up a small electric dipole. The partial double bond character keeps the peptide bond in a rigid planar configuration. Hence, for a pair of amino acids linked by a peptide bond, six atoms (C_α, C, O, N, H and C_α) lie in the same plane.



a. A pure double bond between C and O would permit free rotation around the C—N bond.

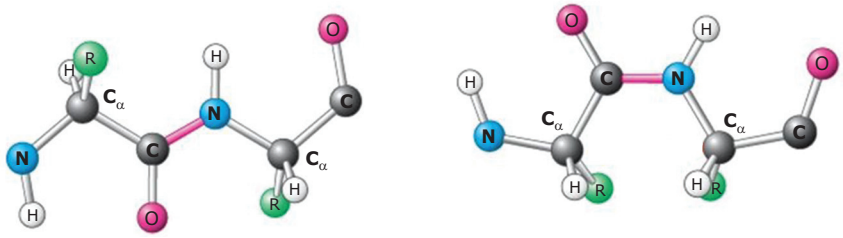
b. The peptide bond is now a double bond; the amide N bears a positive charge and the carbonyl O has a negative charge.

c. A resonance hybrid of the forms in a and b. All of the atoms associated with the peptide group are coplanar, rotation about C—N is restricted, and the peptide is distinctly polar.

Figure 2 Peptide-bond resonance structures. Each peptide bond has some double-bond character due to resonance. It can be written as a resonance hybrid of two structures. The peptide bond is essentially planar. For a pair of amino acids linked by a peptide bond, the six atoms of the peptide group lie in a single plane: the α-carbon atom and CO group of the first amino acid and the NH group and α-carbon atom of the second amino acid.

Torsion angles and peptide bond

Torsion angles (or *dihedral angles*) are defined by four points in space. If a system of four atoms A – B – C – D is projected onto a plane normal to B – C, the angle between the projection of A – B and the projection of C – D is described as the torsion angle about bond B – C; this angle may also be described as the angle between the plane containing atoms A, B and C and the plane containing atoms B, C and D.



trans configuration

cis configuration

Figure 3 A torsion angle is the angle at the intersection of two planes. A, B, C and D illustrate the position of the four atoms used to define the torsion angle. The rotation takes place around the central B-C bond. (Adapted from Biochemistry by Stryer, W. H. Freeman and Company).

As we learnt above, a peptide linkage has partial double-bond character, rotation about this bond is restricted. Two possible configurations, *cis* and *trans*, are observed for a planar peptide bond in polypeptides. In the *cis* configuration, successive α-carbon atoms are on the same side of the peptide bond. In the *trans* configuration, the two successive α-carbon atoms are on opposite sides of the peptide bond (figure 3). The restricted rotation about C—N bond can be specified by torsion angle ω (**omega**). In the *trans* configuration, ω = 180° and in the *cis* configuration, ω = 0°.

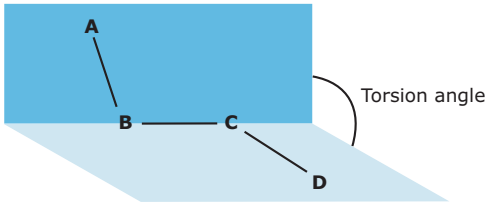


Figure 4 *Cis* and *trans* configuration of peptide bond. A *trans* configuration is more stable than a *cis* configuration, because a steric clash between the side chains. Virtually all peptide bonds in proteins occur in *trans* configuration

Trans configuration is favored by a ratio of approximately 1000:1 over the *cis* configuration. This preference for *trans* over *cis* can be explained by the fact that steric clashes between groups attached to the α-carbon atoms hinder the formation of the *cis* configuration but do not arise in the *trans* configuration. However, this steric interference is reduced in peptide bonds to proline residues, therefore, some peptides containing successive proline residues are known to prefer *cis* configuration.

The bonds between the nitrogen of amino group and the α-carbon atom (i.e. N—C_α bond) and between the α-carbon atom and the carbon of carbonyl group (i.e. C_α—C bond) are pure single bonds. The two adjacent rigid peptide units can rotate about these bonds, acquiring various orientations. The rotations about these bonds can be specified by torsion angles ϕ (**phi**) and ψ (**psi**). The torsion angle about the bond between the amino nitrogen and the α-carbon atoms is called ϕ whereas, the torsion angle about the bond between the α-carbon and the carbonyl carbon atoms is called ψ (figure 5).

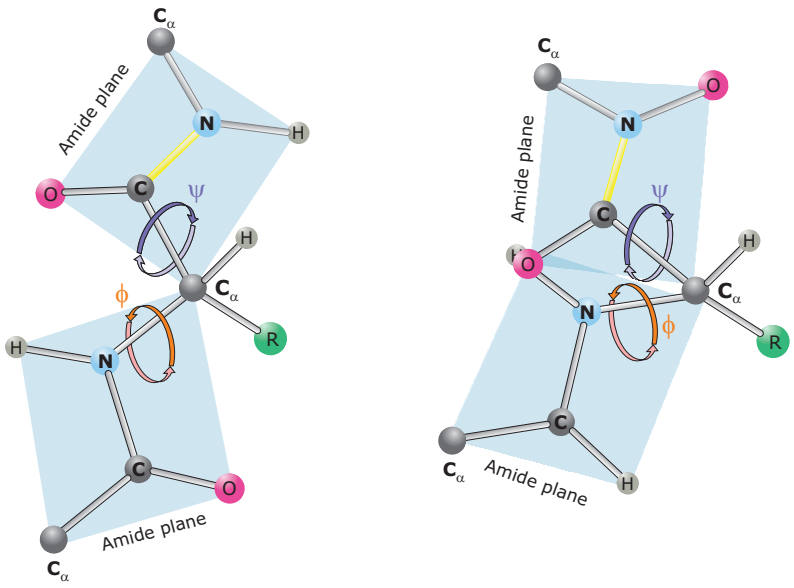


Figure 5 The bonds between the amino nitrogen and the α-carbon atom (i.e. N—C_α bond) and between the α-carbon atom and the carbonyl carbon (i.e. C_α—C bond) are pure single bonds. Hence, rotation can occur about these bonds. The rotations about these bonds can be specified by torsion angles ϕ (phi) and ψ (psi). The torsion angle about the N—C_α bond is called ϕ and that about the C_α—C bond is ψ. The rotations about torsion angles ϕ and ψ angles can vary over range of 0 to 360°.

Understanding the Ramachandran plot

Theoretically, ϕ and ψ can have any value between +180° and −180°, (i.e. 360° of rotation for each). However, not all combinations are possible in reality due to the physical clashes of atoms in 3-dimensional space. Atoms take up space and two atoms cannot occupy the same space at the same time. These physical clashes are called *steric interference*. Most values of ϕ and ψ are therefore not allowed due to steric interference between non-bonded atoms. The permitted values for ϕ and ψ were first determined by G. N. Ramachandran. These permitted values can be visualized on a two-dimensional plot called a **Ramachandran plot** plotted between ϕ and ψ on x-axis and y-axis, respectively.

Polypeptide conformations are defined by the values of ϕ and ψ. Most values of ϕ and ψ are not allowed due to steric interference between non-bonded atoms. Hence, most areas of the Ramachandran plot (i.e. most combinations of ϕ and ψ) represent sterically disallowed conformations of a polypeptide chain because of steric collisions between side chains and main chain. In the figure 6, the white areas correspond to sterically disallowed conformations in which any non-bonding interatomic distance is less than its corresponding van der Waals radii. These regions are sterically disallowed for all amino acids except glycine which is unique as it lacks a side chain. The dark blue regions called *allowed regions* correspond to conformations where there are no steric interferences. Most of ϕ and ψ values of a polypeptide chain fall within these allowed regions of the Ramachandran plot. There are, however, some notable exceptions. Glycine, the amino acid with a smallest side chain, is much less sterically restricted than the other amino acid residues. Hence, its allowed range of ϕ and ψ covers a larger area of the Ramachandran plot. At glycine residues, polypeptide chains often assume conformations that are sterically disallowed to other residues. The cyclic side chain of proline limits its range of ϕ values to angles of around −60°, making it most conformationally restricted amino acid residue. Light blue regions correspond to conformation having outer limit van der Waals distances i.e. the atoms are allowed to come a little closer together.

Since the D- and L-form of the amino acids have their side chain oriented differently with respect to the CO group, hence, they have different allowed ϕ and ψ angles. If polypeptides built from D-amino acids, they have different ϕ and ψ angles than those that are exclusively made up of L-amino acids. For example, a β-sheet made up of L-amino acids occupies upper left quadrant whereas, β-sheet made up of same amino acids of D-enantiomeric form would occupy lower right quadrant.

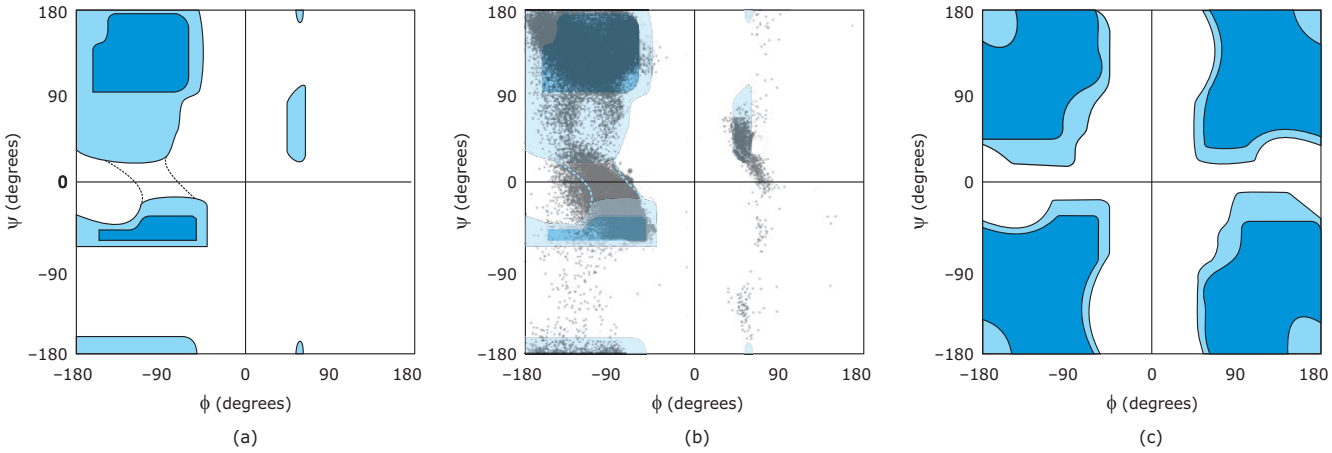


Figure 6 (a) Ramachandran plots showing allowed combinations of the torsion angles ϕ and ψ. Most values of ϕ and ψ are not allowed due to steric interference between nonbonded atoms. The areas shaded dark blue represent the sterically allowed ϕ and ψ values. The plots for L-amino acid residues with unbranched side chains are nearly identical. The allowed ranges for branched residues such as threonine are somewhat smaller than amino acids with unbranched side chains. The glycine residue, which is less sterically hindered, has a much broader allowed range. The cyclic side chain of proline limits its range of ϕ values to angles of around −60°, making it most conformationally restricted amino acid residue. (b) Ramachandran plots showing observed values of torsional angles for most proteins. Each point represents ϕ and ψ values for an amino acid residue obtained using structure determination methods. (c) A typical Ramachandran plot for glycine occupying much larger area due to least steric interference (Adapted from Hollingsworth and Karplus, Biomol Concepts).

Predicting secondary structure from Ramachandran plot

Every amino acid residue in a polypeptide can have specific set of ϕ and ψ angles, therefore, each residue can be represented as a point on Ramachandran plot with corresponding ϕ and ψ angles as x and y coordinates, respectively. Polypeptides, when adopting secondary structures, rotate at specific torsional angles each time so as to form regular repetitive structures such as α-helix and β-sheet. Therefore, on plotting these torsional angles to Ramachandran plot, we obtain, a very restricted area on the plot which can be used to identify and check the secondary structure in a given polypeptide. The backbone torsion angles for right-handed α-helix are approximately $\phi = -57°$ and $\psi = -47°$ and therefore, occupies small area on lower-left quadrant. β-sheet is made up of almost fully extended strands, with ϕ and ψ angles falling in the upper-left quadrant of the Ramachandran plot (figure 7).

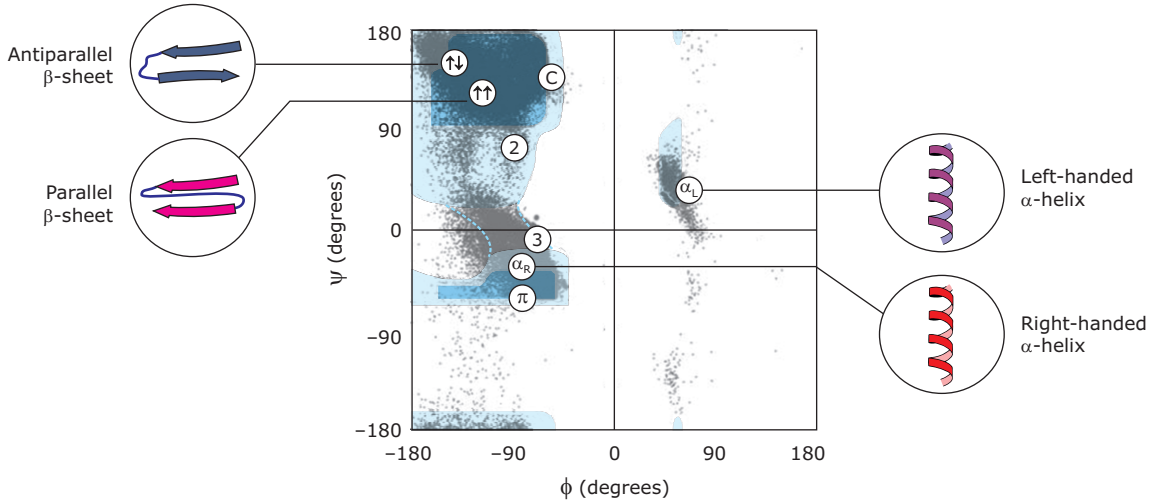


Figure 7 Ramachandran plots showing a variety of secondary structures. The values of ϕ and ψ for various allowed secondary structures are overlaid on the plot. The white circles represent torsion angles of several secondary structures: α-helix (α_R), 3_{10}-helix (3_{10}), π-helix (π), left-handed α-helix (α_L), 2.2_7 helix (2), collagen (C), parallel β-sheet ($\uparrow\uparrow$) and anti-parallel β-sheet ($\uparrow\downarrow$).

Table 1 enlists dihedral angles of some commonly known secondary structures. Several non-repetitive secondary structures such as random coil can be adopted various torsional angles, therefore, can occupy variable regions in the allowed area of Ramachandran plot.

Table 1 Idealized ϕ and ψ angles for common secondary structures in proteins

Secondary structure	Torsion angles (in degree)	
	ϕ	ψ
Antiparallel β-sheet	−139	+135
Parallel β-sheet	−119	+113
Collagen	−51	+153
Right-handed 2.2_7-helix	−78	+59
Right-handed 3_{10}-helix	−49	−26
Right-handed α-helix (3.6_{13}-helix)	−57	−47
Left-handed α-helix	+57	+47
Right-handed π-helix (4.4_{16}-helix)	−57	−70

Note: In real proteins, the torsion angles often vary slightly from these idealized values.

Predicting quality of protein structure using Ramachandran plot

The most important application of Ramachandran plot is the prediction of the quality of various protein structure determined using experimental methods (X-ray crystallography, NMR and Cryo-EM). A good quality structure contains all the set of torsional angles in the allowed area whereas, a bad quality (low resolution) protein structure is reflected as a number of torsional angles falling in the forbidden region. Besides experimental methods, protein structure obtained using homology modeling or *ab-initio* methods are also routinely checked by plotting Ramachandran plot.

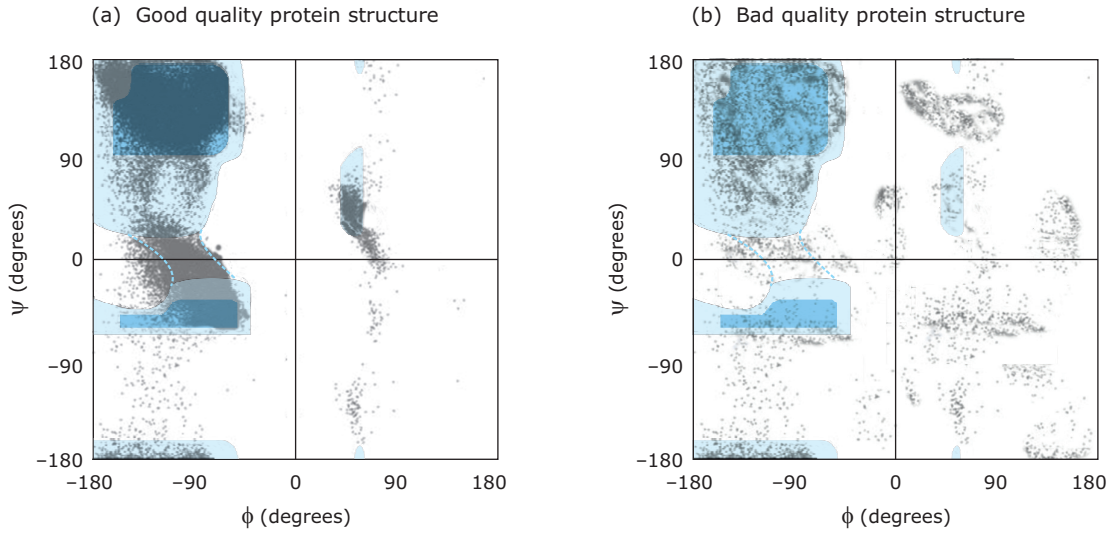


Figure 8 Use of Ramachandran plot to predict the quality of protein structure. (a) A good quality Ramachandran plot contains most torsional angles in allowed region. (b) Bad quality or low-resolution protein structure shows a large number of torsional angles in the forbidden region.

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