Introduction to Protein Structure

Proteins are large heteropolymers usually comprised of 50 – 2500 monomer units, although larger proteins are observed[8]. The monomer units of proteins are amino acids. Proteins are responsible for implementing many of the properties of living systems, including mediating the chemical processes and controlling many of the structural features of cells.

The **primary structure** is the sequence of monomer units in the protein. **Secondary structure** is made up from regular structures within proteins. The **tertiary structure** is the overall three-dimensional structure of the protein. **Quaternary structure** is the organization of multi-subunit proteins (proteins comprised of single polypeptide chains do not have quaternary structure).

A **peptide** is a molecule containing two or more amino acid residues linked by secondary amide bonds. The term peptide typically refers to molecules comprised of relatively small numbers of amino acid residues. This is sometimes made explicit in the term **oligopeptide**: "oligo-" means "short", and thus an oligo peptide is a short polymer of amino acids.

A **polypeptide** is a longer polymer of amino acids. The term "**protein**" is often used interchangeably with "polypeptide". In some cases, however, the term "polypeptide" is used to refer to a single chain peptide part of a molecule that either also contains non-amino acid groups as part of the overall "protein" or which is not folded into a defined structure.

You may have noticed the term amino acid **residue** in the preceding paragraphs. In a strict sense, proteins do not contain actual amino acids; as will be seen, the amino and carboxylic acid functional groups of amino acids incorporated into proteins are transformed into amide linkages, which lack the ionizable groups. Therefore, the monomer units within proteins are properly referred to as "amino acid residues" or simply "residues".

The **primary structure** of a protein is the sequence of amino acids that comprises the protein. The three-dimensional structure of a protein (and therefore, all of its functions) is determined by interaction between the primary structure and the environment in which the protein resides, and by the presence of any incorporated covalent modifications or prosthetic groups.

The primary structure is always written from the amino-terminus (the residue with the free α-amino group) to the carboxy-terminus. This convention has several reasons, including the fact that the protein biosynthetic machinery produces proteins from N-terminus to C-terminus. Thus, anyone seeing a peptide written as GRSKKNS (or the equivalent sequence: Gly-Arg-Ser-Lys-Lys-Asn-Ser) immediately knows that this peptide has an N-terminal glycine and a C-terminal serine.

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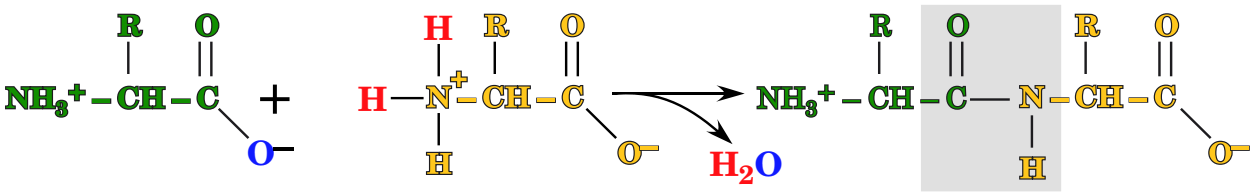
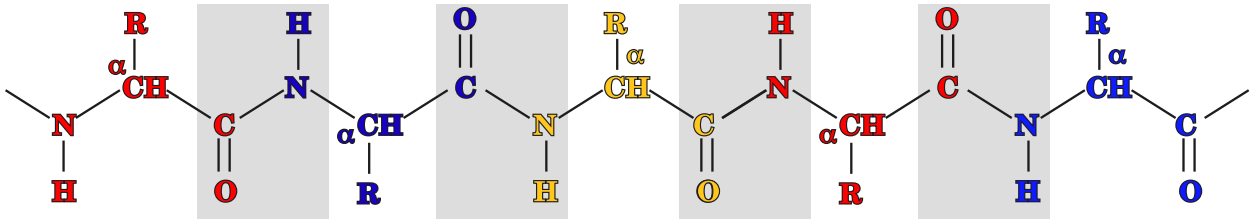
[8] The largest known biological polypeptide, the full length version of the mouse muscle protein called titin, has 35,213 amino acid residues (the human version of titin is smaller, with "only" 34,350 residues in the full length protein).

The peptide bond

Peptides and proteins are **linear** polymers of amino acid residues. The amino acids are connected by a secondary amide linkage frequently referred to as a **peptide bond**. In principle, the peptide bond is formed by removing an H_2O molecule while linking the α-amino group of one amino acid to the α-carboxylic acid of another.

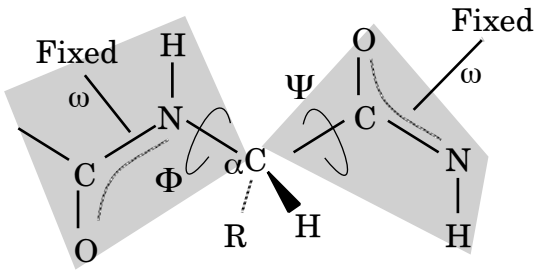


Proteins are polymers of amino acids linked by these peptide bonds, with the properties contingent upon the properties of the residue side-chains.

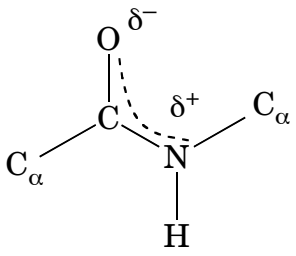


Structural Features of the Peptide Bond

In the peptide bond, the π-electrons from the carbonyl are delocalized between the oxygen and the nitrogen. This means that the peptide bond has ~40% double bond character. This partial double bond character is evident in the shortened bond length of the C–N bond. The length of a normal C–N single bond is 1.45 Å and a C=N double bond is 1.25 Å, while the **peptide C–N bond length is 1.33 Å.**



Because of its partial double bond character, **rotation around the N–C bond is severely restricted**. The peptide bond allows rotation about the bonds from the α-carbon, but not the amide C–N bond. Only the Φ and Ψ dihedral angles (see below) can vary reasonably freely. In addition, the six atoms in the peptide bond (the two α-carbons, the amide O, and the amide N and H) are **coplanar**.



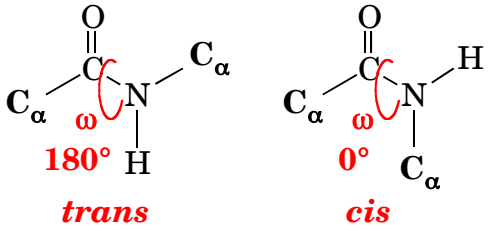
Finally, the peptide bond has a dipole, with the O having a partial negative charge, and the N_{amide} having a partial positive charge. This allows the peptide bond to

participate in electrostatic interactions, and contributes to the hydrogen bond strength between the backbone carbonyl and the N_{amide} proton.

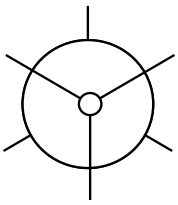
Peptide bond and protein structure

The peptide bond contains three sets of **torsion angles** (also known as **dihedral angles**). The least variable of these torsion angles is the ω angle, which is the dihedral angle around the amide bond. As discussed above, this angle is fixed by the requirement for orbital overlap between the carbonyl double bond and the N_{amide} lone pair orbital. Steric considerations strongly favor the *trans* configuration (*i.e.* an ω angle of 180°), because of steric hindrance between the alpha carbons of adjacent amino acid residues.[9] This means that nearly all peptide bonds in a protein will have an ω angle of 180°.

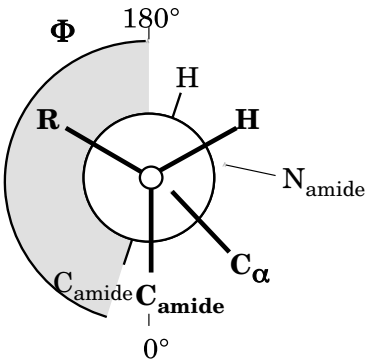
Observed ω angles for the peptide bond

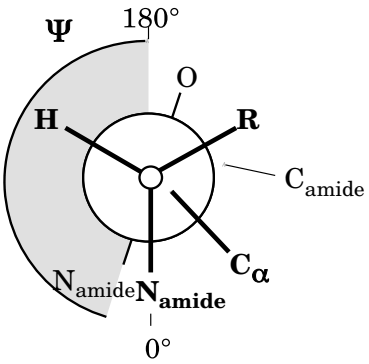


In considering peptide structures, it is usually much more important to look at the backbone dihedral angles that can vary more widely. These angles are the **Φ** (= phi, C_α–N_{amide}) and **Ψ** (= psi, C_α–C_{amide}) angles. By definition, the fully extended conformation corresponds to 180° for both **Φ** and **Ψ**. (Note that 180° = –180°). Numeric values of angles increase in the clockwise direction when looking away from the α-carbon.



Tetrahedral Carbon–Carbon bond





By definition, **Φ** = 0° when the C_{amide}-N_{amide} and C_{amide}-C_α bonds are in the same plane, and **Ψ** = 0° when the N_{amide}-C_{amide} and N_{amide}-C_α bonds are in the same plane. The (+) direction is clockwise while looking away from the C_α.

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[9] Proline is an exception; the tertiary amide formed by an X-Pro peptide bond does not have strong energetic preference for *cis* or *trans* configurations. As a result, both are observed in proteins. Interconversion between *cis* and *trans* X-Pro peptide bonds requires an enzyme (*proline isomerase*), which plays a role in folding of many proteins.

The torsion angles that the atoms of the peptide bond can assume are limited by steric constraints. Some **Φ / Ψ** pairs will result in atoms being closer than allowed by the van der Waals radii of the atoms, and are therefore extremely unlikely to be observed because of steric clashes (for example: 0°:0°, 180°:0°, and 0°:180° are almost never observed because of backbone atom clashes)[10]. For tetrahedral carbons, the substituents are typically found in staggered conformations (see figure, above). Peptide bonds are more complicated, because while the α-carbon is tetrahedral, the two other backbone atom types have planar arrangements. However, while a true staggered conformation is is not possible, the same principle applies: the preferred conformations for peptide bond atoms have the substituent atoms at maximal distances from one another.

A **Ψ** angle of 180° results in an alignment of the N_{amide} with the carbonyl oxygen from the same residue. This is allowed, although not especially favored. A **Ψ** angle of 0° places the N_{amide} from one residue very close to the N_{amide} from the previous residue; this results in a steric clash (as well as an unfavorable electrostatic interaction, because both N_{amide} have partial positive charges).

The residue side-chains also impose steric constraints. Glycine, because of its small side chain, has a much large ranger of possible **Φ / Ψ** pairs than any other residue. Proline has a very limited range of **Φ** angles because its side-chain is covalently bonded to its N_{amide}., while its accessible **Ψ** angles are similar to those of most other residues. Most other residues are limited to relatively few **Φ / Ψ** pairs (although more than proline). This is especially true for the β-branched residues threonine, valine, and isoleucine, which are the most restricted, because these residue side-chains have more steric bulk due to the presence of two groups attached to their β-carbons.

Secondary Structure (α-helix and β-sheet)

Secondary structural elements are repeated regular structures within proteins. In these repeated structures, several successive residues exhibit the same **Φ / Ψ** pairs. The two most common types of secondary structural elements are α-helices and β-sheets.

The α-helix

Examining an α-helix is easiest with a molecular model of a short polypeptide. With a model: start at top with amino terminus, and rotate the chain clockwise as you move downward. The carbonyl oxygens point downward. The carbonyl oxygen from α-carbon #1 forms an H-bond to the amide H–N that follows three intervening carbonyl oxygens; this is the amide proton from the 5th residue. More generally, the **carbonyl$_i$ accepts an H-bond from H–N$_{i+4}$.**

In looking at a physical model, removing the side chains after the β-carbon (*i.e.* constructing polyalanine) makes the demonstration easier, because the extra atoms

[10] Dihedral angles of 0°:0° and the others listed constitute eclipsed conformations, which are energy maxima, and therefore unfavorable.

may be heavy enough to distort chain in a gravity field (which is not a problem with real proteins). As it happens, alanine is frequently found in α-helices.

An α-helix is a **right-handed** helix: looking end on, the rotation is clockwise while moving away from observer. This is true looking at the helix from either N-terminal or C-terminal end.

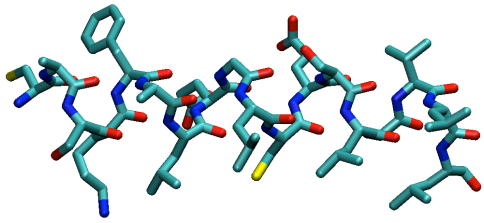
Note that the first 4 nitrogens (counting the one from the initial residue that contains the i=1 carbonyl) cannot form H-bonds within the helix.

Properties of an α-helix

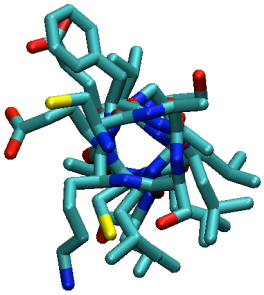
1) The **α-helix allows all of the polar backbone atoms to hydrogen bond** (except for the first 4 N_{amide}, and last 4 carbonyl groups).

2) There is no "hole" in the middle of the helix: instead, the van der Waals surfaces of the backbone atoms are in contact down the center of the helix axis, which is a stabilizing feature of the helix. If you look down the axis of a space-filling model of a helix, you will not see any empty space. The figures below show the same α-helix.[11]

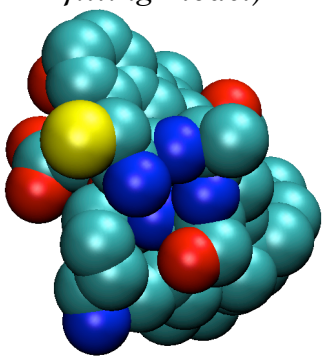
N-terminus → C-terminus



Looking down helix axis from N-terminus (*licorice model*)



Looking down helix axis from N-terminus (*space-filling model*)



3) The **side chains extend outwards** and angled slightly (~30°) toward the N-terminus of the helix.

4) An α-helix has **3.6 residues/turn**. Since a circle contains 360°, each residue is rotated by 360/3.6 = 100°.

5) An α-helix has a **5.4Å pitch/turn**. This means that as the chain moves along the axis of the helix, the helix becomes 5.4 Å longer for each full turn. This corresponds to **1.5 Å/residue.** (A C–C single bond length is ~1.5 Å; since each

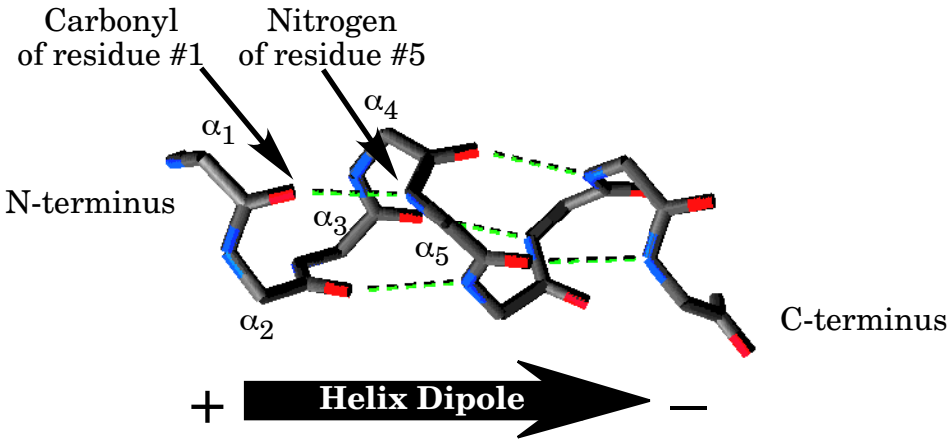
[11] These, and most other figures depicting three dimensional structures of proteins in these pages were created using VMD (http://www.ks.uiuc.edu/Research/vmd/). Another program for viewing an manipulating protein structures in three dimensions is SwissPDBViewer (http://spdbv.vital-it.ch/). Both are powerful programs, although SwissPDBViewer is probably easier to use due to its more heavily graphical user interface.

residue represents two C–C bonds and a C–N bond, 1.5 Å per residue is a very compact structure.)

6) **The backbone of the helix is ~6 Å in diameter** (ignoring side chains).

7) 8) The backbone bond angles are $\Phi = -57°$ and $\Psi = -47°$. Note that the Φ and Ψ angles in real helices are not exact, and may vary by up to 10° from these ideal values and occasionally vary by even larger amounts. As with most features of actual protein structures, the variation in the helical Φ and Ψ angles is due to other factors in the protein structure that require a more or less tightly wound helix for optimal stability.

8) An α-helix has a **dipole**, with the partial positive charge toward N-terminus. This is true because all of the partial charges of the peptide bonds are in alignment.



Two-dimensional representations of α-helices

Drawing a three-dimensional helix on paper is difficult. Two types of two-dimensional representations (helical wheel and helical net diagrams) are commonly used to simplify the analysis of helical segments of proteins. The two-dimensional representations are somewhat stylized, but show the major features more clearly than attempting to draw a three-dimensional structure accurately in two-dimensions.

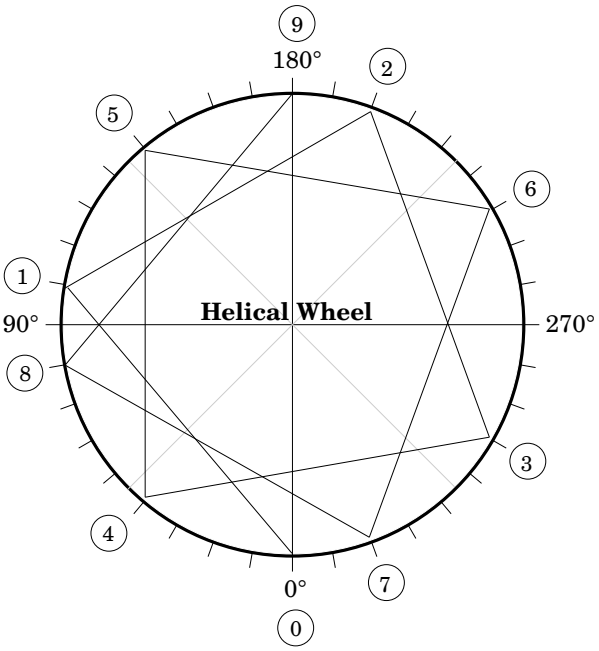
The first type of representation is a **Helical Wheel** diagram. In this diagram, the representation involves looking down the helix axis, and plotting the rotational angle around the helix for each residue. This representation is conceptually easily grasped, but tends to obscure the distance along the helix; residues 0 and 18 are exactly aligned on this diagram, but are actually separated in space by 27 Å. [12]

[12] Numbering the helix beginning with residue #0 simplifies some calculations. Note that distances along helices are measured from the first residue, but, in effect, do not count the first residue. Thus, the length of a four-residue helix is 4.5 Å, not 6 Å.

Helical Wheel

Residue #0 = 0° (by definition)
#1 = 100°
#2 = 200°
#3 = 300°
#4 = 400° = 40°
#5 = 140°
#6 = 240°
#7 = 340°
#8 = 440° = 80°
9# = 900° (from first) = 180°

These angles can be plotted on a circle. Doing so results in a representation that corresponds to the view looking down the long axis of the helix. (**Note that the rotation is clockwise as the residue number increases**.)



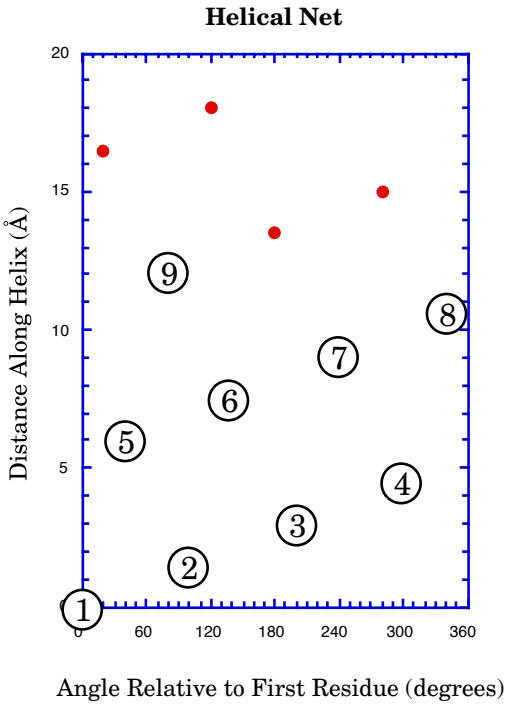
The diagram makes it possible to more easily look for patterns in amino acid properties around the helix.

Helical Net

A separate representation involves a hypothetical razor cut lengthwise along the helix axis, followed by unpeeling the surface of the helix. This results in a plot of distance along the helix *versus* distance around the helix called a **Helical Net**.

While it is sometimes more difficult to see the helix "faces" in a helical net (where vertical bands within net are on same face of helix), the helical net does emphasize the distance **along** the helix, which is ignored in the helical wheel. Compare the two representations, and note the relative positions of different residues.

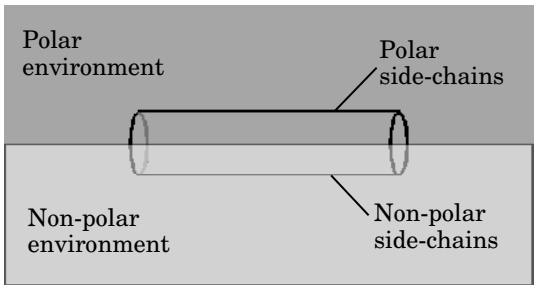
In the helical wheel, residues 1 and 8 are quite close together; the helical net shows that these residues exhibit a 10.5 Å vertical separation (although they are still present on the same side of the helix).



Both helical wheel and helical net analysis types are useful for analyzing the helix for patterns in residue properties around the circumference of the helix. The helical

wheel is somewhat easier to interpret, but leaves out the sometimes-crucial distance information.

A common reason for using a helical wheel or helical net diagram is to look for helical regions that exist at protein surfaces. The interior of a protein is typically non-polar, while the surrounding solvent is polar. A helix that has its axis along the border of this region would be expected to have a corresponding, amphipathic, distribution of polar and non-polar residues. (Amphipathic, meaning "hating both" refers to the presence of both polar and non-polar groups in the helix.) Note, however, that residues 1 and 8 are much closer in position around the helix than are residues 1 and 2. A simple examination of the linear sequence does not reveal amphipathic tendencies; in contrast, helical net, and especially helical wheel diagrams, show amphipathic helical character clearly.



The 3-10 helix

In some proteins, the backbone forms a variant on the α-helix instead. The 3-10 helix (for "3 residues/turn, 10 backbone atoms/turn") has a pitch of 6 Å (2 Å/residue). The residues in a 3-10 helix have slightly different **Φ** and **Ψ** angles to yield a helix that is less tightly wound and more extended than an α-helix.

Feature	α-helix	3-10 Helix
Φ	–57°	–49°
Ψ	–47°	–26°
Rise per residue	1.5 Å	2Å

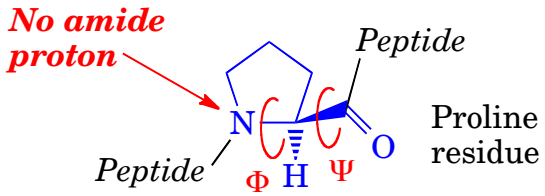
Amino acids in helices

Most amino acids are observed to be present in helices within proteins. The side-chains of helical residues can interact, at least to some degree, but with rare exceptions (see below) the side-chains do not participate in the hydrogen-bonding pattern of the helical backbone.

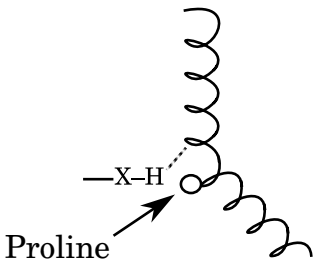
There are a few amino acids that are *relatively* rare in helical structures.

1) While proline has limited conformational flexibility, it is capable of forming **Φ** angles close to -57°. However, **proline lacks an amide proton**, and therefore cannot participate in helix hydrogen bonding pattern. Proline is often found at end of a helix (especially at the N-terminus, when the lack of

the amide proton is not a problem, but also at the C-terminus, where it helps terminate the helix.



2) **Glycine** has a very small side-chain, and therefore has a **large conformational flexibility**. This means that incorporating glycine into the ordered helical structure has a significant **entropic cost**. Therefore, while glycine is capable of readily forming the appropriate **Φ / Ψ** angles for an α-helix, glycine tends to allow too much flexibility to contributed to the stability of the helical structure.



Proline and glycine are sometimes referred to as "helix breakers" because they tend to have destabilizing effects on helices. Proline and glycine also allow "kinks" in a helix, where the direction of the helix axis changes. Note the hydrogen bond donor in the drawing. This hydrogen bond donor from outside the helix is necessary to satisfy the hydrogen bonding requirements of the P_{i-4} residue (*i.e.* the position four residues closer to the N-terminus than the proline). The P_{i-4} residue has a backbone carbonyl that cannot hydrogen bond to the proline (because, as shown above, proline lacks an amide proton). The kink in the helix is a consequence of the missing H-bond from the proline and the likely distortion of the helix to allow room for a hydrogen bond donor from outside the helix.

3) Charged residues can either stabilize or destabilize a helical structure. For example, glutamate residues usually favor helix formation. However, too many glutamates in the absence of positively charged residues can result in sufficient electrostatic repulsion to disrupt helix formation.

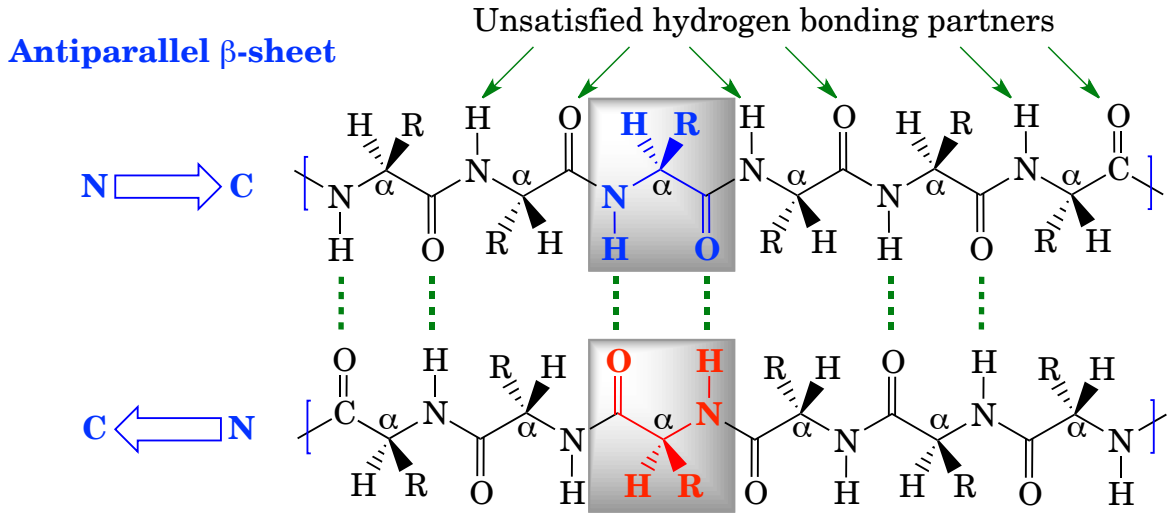
The β-sheet

A second widely observed method for satisfying backbone atom hydrogen bonding requirements is called a β-sheet. A β-sheet is a much more extended form of secondary structure than an α-helix. A β-sheet has the **backbone atoms in an essentially planar structure**, with the **side chains *alternately* pointing above and below** the sheet plane.

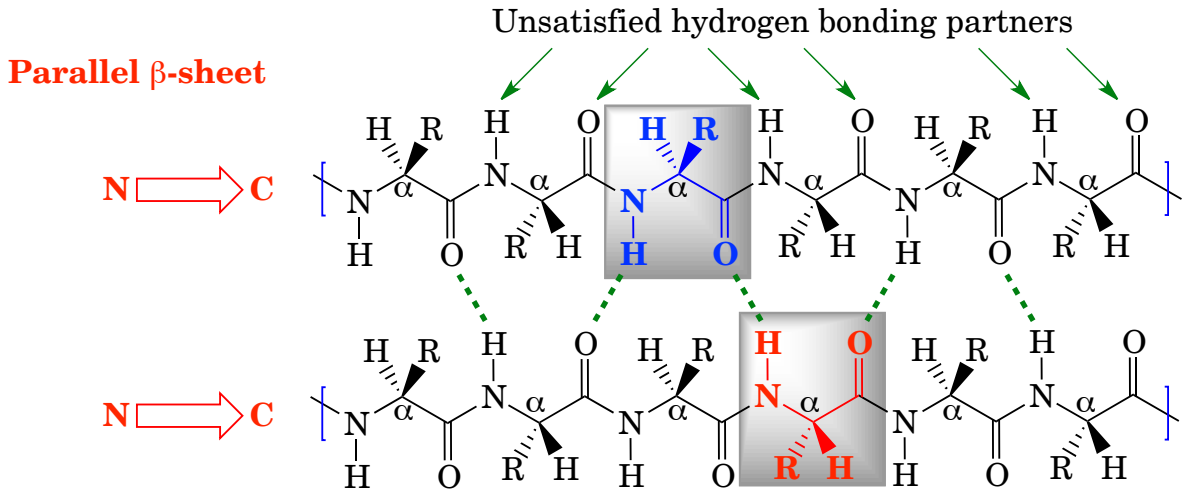
An **antiparallel β-sheet** is a secondary structural element comprised of two (or more) strands leading in **opposite** directions. In an antiparallel β-sheet, the

backbone amide proton and carbonyl from one residue form hydrogen bonds with the corresponding groups from a single residue in another strand.

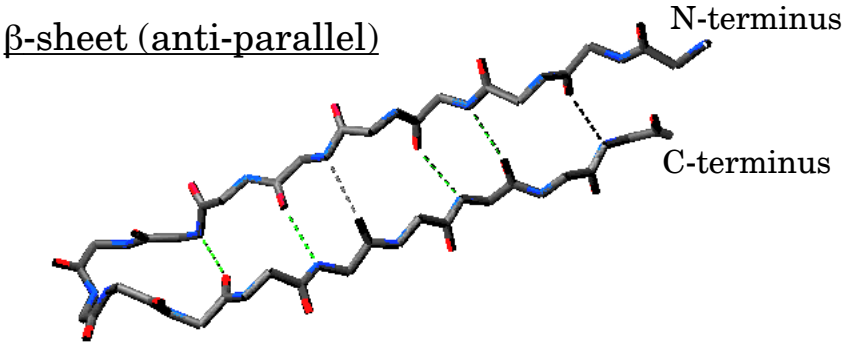
Note the unsatisfied hydrogen bonds on each edge of the two-stranded β-sheet shown in the figure below. These could be satisfied by the presence of additional β-sheet strands (which allows β-sheets to form rather large structures). However, half of the backbone hydrogen bonds at the edge of any planar β-sheet must be formed using groups from outside the secondary structure.



A **parallel β-sheet** is comprised of two (or more) strands leading in the **same** direction. Note that a residue from one strand forms hydrogen bonds to groups from two residues of the other strand. Note also that the hydrogen bonds are somewhat distorted (this distortion is somewhat exaggerated in the diagram). Parallel β-sheets tend to be less stable than their antiparallel counterparts, and are less commonly observed. Most parallel β-sheets are formed from multiple strands.



Note also that forming a parallel β-sheet requires strands from different parts of the protein (an antiparallel sheet can form from a single sequence assuming a turn in the middle (as shown in the diagram below).



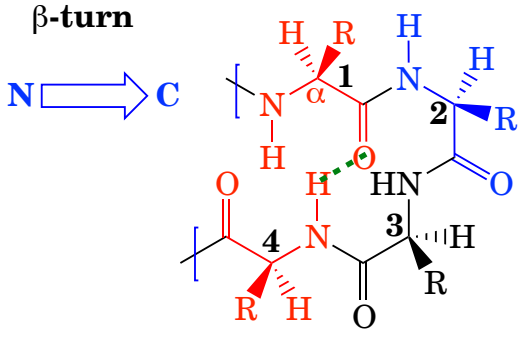
The Φ and Ψ angles required to form a β-sheet are quite different from those used for forming an α-helix. These bond angles result in a much more extended structure (the rise/residue for a β-sheet is about 3.5Å).

Angle	Antiparallel	Parallel
Φ	–139°	–119°
Ψ	135°	113°

Both parallel and antiparallel β-sheets put the residue side-chains on alternating sides of H-bond/backbone plane. Generating a β-sheet with specific properties requires taking this alternating side-chain pattern into account.

β-turn

A third type of secondary structural element observed frequently in proteins is a β-turn (also called a β-bend, or a tight turn, or merely a turn). A β-turn is a motif that allows a rapid reversal of the peptide strand direction.



In a β-turn, the carbonyl oxygen of residue #1 forms a hydrogen to the amide proton of residue #4. In these structures, residue #2 is often glycine (because the glycine

side-chain is small enough to avoid significant steric hindrance at the angles required for the structure). Residue #3 is often proline (which readily assumes the required Φ and Ψ angles, and which lacks an amide proton that may create a steric clash with residue #1).

Other types of secondary structure

Some other regular, repeating sets of Φ and Ψ angles are observed in a few types of proteins. Some fibrous proteins such as α-keratin have α-helices wrapped about one another in a structure called a coiled-coil.

An even more prevalent motif is found in a structural protein called collagen; collagen is comprised of three intertwined chains arranged in a helix. The collagen triple helix residues exhibit Φ and Ψ angles of –51° and 153°, respectively. Most animals have large amounts of a few types of collagen.

A summary of the Φ and Ψ angles in common secondary structural elements is given in the table below. The table also shows the distance spanned by each type of structural element; clearly, the α-helix is a very compact structure, while β-sheet structures are much more extended.

Secondary structure	Φ	Ψ	Rise per residue
α-helix	–57°	–47°	1.5 Å (3.6 residues per turn)
Coiled-coil	–64°	–42°	1.5 Å
β-sheet (antiparallel)	–139°	135°	3.5 Å
β-sheet (parallel)	–119°	113°	3.5 Å
Collagen	–51°	153°	3 Å

A few proteins have a left-handed helix. This is a much less stable structure than the standard α helix, and is not included in the table to avoid confusion.

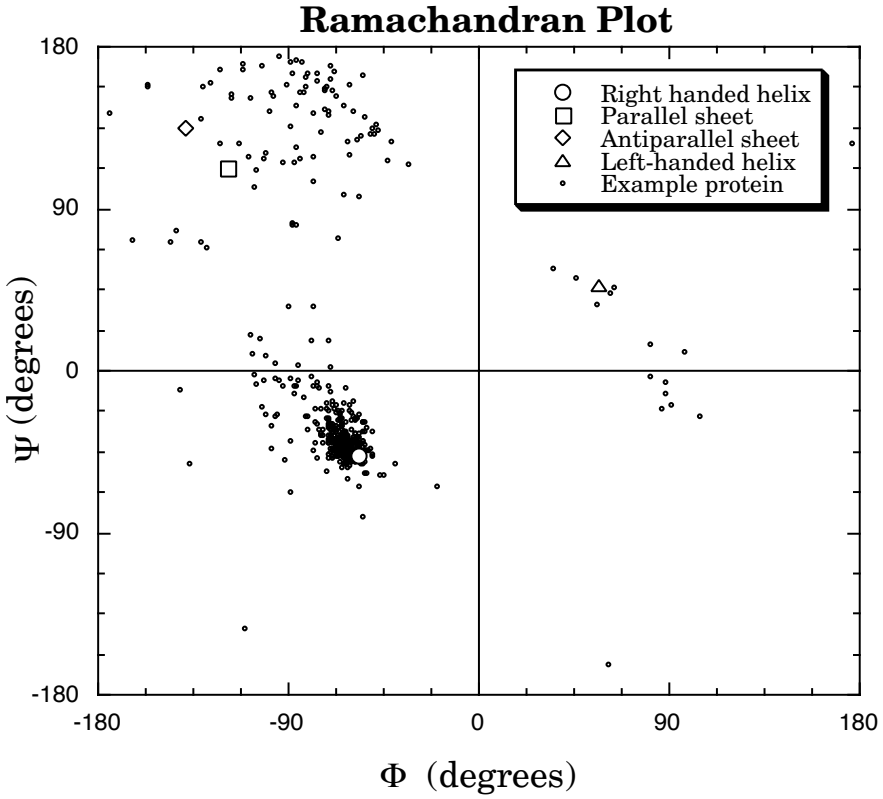
Finally, most proteins have regions in which the Φ and Ψ angles are not repeating. These regions are sometimes referred to as "random coil" although their structures are not actually "random". The non-repeating structures are considered by some protein chemists to comprise types of "secondary structure", in spite of their irregular nature.

Ramachandran diagram

A Ramachandran diagram is a plot of Ψ *versus* Φ angles for each residue in a protein (see example below). The diagram reveals several features about proteins. One obvious feature is the limited Φ / Ψ angle pairs present; many of the pairs are sterically impossible, or so strained as to be extremely rare.

Another obvious feature is the presence of many points in small regions of the diagram. These correspond to many residues with very similar Φ / Ψ angle pairs, and are usually indications that secondary structural elements are present. The example protein shown contains large amounts of α-helix; the region near Φ = –57°; Ψ = –47° is therefore heavily populated. Note, however, that all of the residues do not have identical angles (hence the scatter in this region). This is a general

phenomenon: few residues have the exact angles of a perfect secondary structural element.



The collagen triple helix, the α-helix, β-sheets, and less regular structures all have specific locations on the diagram.

A Ramachandran diagram can be generated for any protein for which structural data are available. (For example, in SwissPDBViewer select **Ramachandran Plot** in the **Wind** menu; note that SwissPDBViewer, will only plot residues selected in the Control Panel.)

Summary

The amide linkage of the peptide bond has a partial double bond character that greatly restricts free rotation for the ω angle (the dihedral angle for the C_{amide}-N_{amide} bond). This means that, in general, only the **Φ** (C_α–N_{amide}) and **Ψ** (C_α–C_{amide}) angles can vary.

Steric constraints limit possible **Φ** / **Ψ** pairs. This is most true for β-branched residues, and least true for glycine, but all residues experience some degree of constraint.

The steric constraints favor certain types of **Φ** / **Ψ** pairs. Regions consisting of the same set of **Φ** / **Ψ** pairs exhibit regular structures known as secondary structure.

The two most common types of secondary structure are α-helices and β-sheets.

α-Helices are compact structures, with each additional residue contributing 1.5 Å to the length of the structure. The backbone rotates 100° clockwise for each additional residue. Side-chains in an α-helix point outwards from the structure.

β-Sheets are extended structures; each additional residue contributes 3.5 Å to the structure. The two types of β-sheet differ by the direction of subsequent strands.

Both α-helices and β-sheets satisfy the backbone atom hydrogen bonding requirements; this hydrogen-bonding pattern is a major driving force for secondary structure formation, particularly in regions of a protein in which other hydrogen bonding would not otherwise be possible.

Ramachandran diagrams are commonly used methods for examining the patterns of **Φ** / **Ψ** pairs present within proteins.