

Protein structure and folding

BIOS 1006

20 June 2025

Objectives

- Know all definitions.
- Describe the types of reactions involving amino acids.
 - Ionic reactions: negative + positive (acidic + basic)
 - Hydrogen bonding: polar + polar (hydrophilic)
 - Disulfide bonds: cysteine + cysteine
 - Peptide bonds: amino acid + amino acid, partial double bond character, rigid (not rotatable)
- Describe properties of the bonds in the polypeptide backbone.
 - Dihedral angles: ϕ (phi) angle/bond (N-C $_{\alpha}$), ψ (psi) angle/bond (C $_{\alpha}$ -C), and ω (omega) angle/bond (C-N)
 - Dihedral angles are rotatable, except for the peptide bond (ω)
- Describe and identify the different classes of protein structure: primary, secondary, tertiary and quaternary.
 - Primary structure: unique sequence of amino acids in a polypeptide
 - Secondary structure: local folding patterns (e.g., α -helices, β -strands)
 - Tertiary structure: overall 3D arrangement of all residues in a polypeptide
 - Quaternary structure: arrangement of multiple polypeptide subunits in a protein complex
- Describe the importance of primary structure in protein folding and the relationships between proteins.
- Describe the properties of features, such as secondary structure elements and motifs, that are found in proteins.
- Describe the forces that stabilize tertiary and quaternary structures of proteins.

- Describe the properties of different protein types, classifications and architectures.
- Understand the role of free energy, dynamics, the forces involved and the factors that influence, aid or impede protein folding, shape and function.

Protein structure

Peptide/amide bonds

Dehydration synthesis or condensation reaction or nucleophilic substitution

Nitrogen with a lone pair attacks the carbonyl carbon of another amino acid, forming a covalent bond and releasing water. This requires energy and a **ribozyme** (enzyme made out of nucleotides, RNA) called a **ribosome** to catalyze the reaction.

Resonance structures

Electrons (usually in double or triple bonds, or lone pairs) can move around and be “shared” or “delocalized” over two or more atoms. This is called **resonance**.

Resonance hybrids result in the...

- C–N bond having **partial double bond character**.
- peptide bond being shorter and essentially planar. (sp^2 hybridized, trigonal planar)

Resonance structures have characteristics of both arrangements.

Resonance structures of the peptide bond

C_α on opposite sides of the amide bond = **trans** conformation (preferred form in proteins)

C_α on the same side of the amide bond = **cis** conformation (less common, leads to steric clashes)

Dihedral angles

- ϕ (**phi**) **angle/bond**: nitrogen - α carbon, C–N– C_α –C
- ψ (**psi**) **angle/bond**: α carbon - carbonyl carbon N– C_α –C–N
- ω (**omega**) **angle/bond**: carbonyl carbon - nitrogen C_α –C–N– C_α
- Rotatable

The only bond that is not rotatable is the peptide bond (amide bond) because it has partial double bond character due to resonance. This means that the C–N bond is rigid and planar, which restricts the rotation of the peptide bond.

Key terms for peptides

residue an amino acid within a peptide

peptide molecule containing amino acids linked together

oligopeptide molecule containing less than 10 amino acids

polypeptide molecule containing more than 10 amino acids

proteins functional molecules consisting of one or more polypeptides

Levels of protein structure

Primary structure

The unique sequence of amino acids that defines a peptide or polypeptide; shows all covalent bonds.

Secondary structure

α -helices (cylinders/arrows) and β -strands (arrows, N- to C-terminus) are connected by random coils

The α helix Looks like a spiral staircase, stabilized by hydrogen bonds between amide groups in the protein backbone. The hydrogen bonding pattern unique to alpha helix is that it requires hydrogen bonds between i and $i + 4$ (where i is the amino acid of interest) and are stronger than the hydrogen bond between a regular amine and carboxyl group.

- 3.6 residues per turn
- Pitch (distance between 2 identical points on adjacent turns): 5.4 Å (0.1 nm)
- Rise (distance between 2 identical points on adjacent residues, pitch/residues per turn): 1.5 Å (0.15 nm)
- Torsion angles: $\phi = \sim -60^\circ$, $\psi = \sim -45^\circ$
- H-bonds parallel to helical axis and point in the same direction. The α helix has a dipole
- R-groups point outwards
- On average, ~ 10 residues per helix
- Directionality: left-handed and right-handed helices. α helix is a right-handed helix
- “Helical wheel” representation of an α -helix (a view down the helical axis)

The β strand Different from β -sheets. Link together to form β -sheets. Strands can have parallel (pointing in the same direction) or antiparallel (pointing in opposite directions) orientations. β strands are stabilized by hydrogen bonds. The hydrogen bonding pattern unique to beta strands is that it requires hydrogen bonds between i and $i + 2$ (where i is the amino acid of interest) and are weaker than the hydrogen bond between a regular amine and carboxyl group.

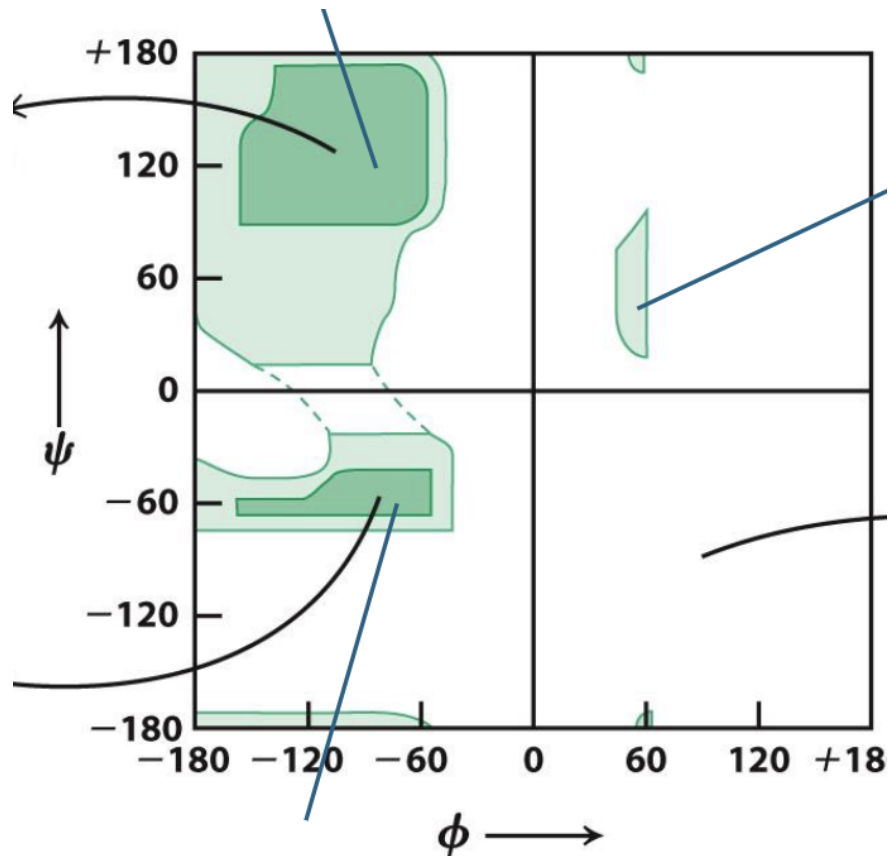
- Amino acids that interact can be distant in primary structure
- Parallel: N \rightarrow C and N \rightarrow C, less ordered hydrogen bonds, angled, not collinear (weaker)

- Anti-parallel: $N \rightarrow C$ and $C \rightarrow N$, very ordered hydrogen bonds, perpendicular, collinear (stronger)
- β -strands form β -pleated sheets
- R groups on opposite sides of sheet
- Amphipathic pattern: 1 polar (hydrophilic) - 1 nonpolar (hydrophobic)
- $\psi = \sim -120^\circ$, $\phi = \sim 120^\circ$

Less common secondary structures The β -turn and left-handed helix are less common. The β turn is a 4 amino acid motif with beta sheet-like characteristics, and left-handed helices are present in collagen.

Secondary structures on Ramachandran plot

- Plots ϕ and ψ values
- 4 quadrants
- Top left: β sheets
- Top right: left-handed α -helix
- Bottom left: right-handed α -helix
- Bottom right: disfavored



Random coil region

A flexible amino acid sequence that does not adopt a uniform secondary structure fold and can play essential roles in the structure and function of the protein.

Tertiary structures

The three-dimensional arrangement of *all* residues that comprise a polypeptide.

- Results from **protein folding**, a process driven by primary structure, hydrophobic effect, and R group interactions
- Tertiary structure is predominantly stabilized by electrostatic interactions (H bonds, salt bridges, van der Waals) and covalent bonds (disulfides)

Folded proteins

- All-helical
- All β sheet
- Mixed α and β

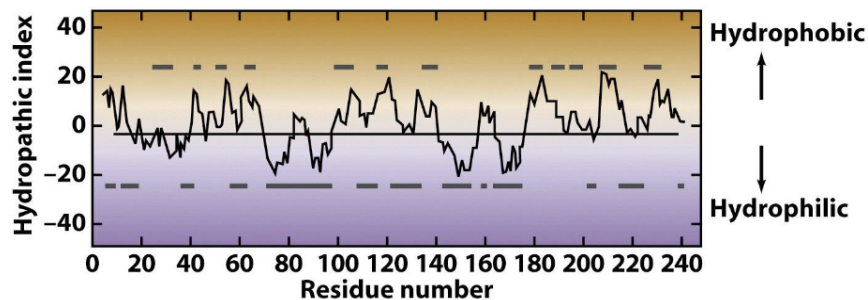
Anfinsen's hypothesis

Primary structure drives protein folding

- Added 8M urea to destroy hydrogen bonding in proteins and mercaptoethanol (reducing agent) to destroy disulfide bonds
- Protein denatured
- Hydrogen bonds and disulfide bonds began to re-form
- Protein refolded

Hydrophobic effect drives protein folding

Hydropathy plot:



Hydrophobic, higher values = in the core of the protein, embedded in the membrane

Hydrophilic, lower values = surface exposed

Quaternary structure

The three-dimensional arrangement of polypeptide subunits in a protein complex containing two or more polypeptides

Nomenclature:

If all subunits are identical, **homo-**

If subunits are not identical, **hetero-**

2 subunits = dimer

3 subunits = trimer

4 subunits = tetramer

5 subunits = pentamer

6 subunits = hexamer

(Example: homodimer, heterotetramer, etc.)

Oligomers = 2 or more subunits are identical

homo-oligomer = all subunits are the same
hetero-oligomer = two or more different subunits
protomer = repeating structural unit

Association of subunits are often driven by hydrophobic effect. Quaternary structure is also stabilized by covalent bonds, salt bridges, hydrogen bonds, and van der Waals interactions.

Shape and stability

- Non-polar amino acids side chains are forced into the interior of the protein by the hydrophobic effect
- Van der Waals interactions organize and stabilize the core structure
- Hydrophilic on the outside, hydrophobic on the inside
- Ionic interactions form between proteins with different charges
- Hydrogen bonds between R groups, R group – water, and R group – backbone

Globular proteins

- Spherical or elliptical
- Play enzymatic or regulatory roles
- Water-soluble

Fibrous proteins

- Rod-shaped
- Play structural and protective roles
- Water-insoluble (so your hair doesn't dissolve...)

Simple proteins

Only composed of amino acids

Conjugated proteins

Proteins that have a bound **prosthetic group** (not peptide-based) that is required for function. Covalently attached or IMF interaction

apo-protein prosthetic group is not present

holo-protein prosthetic group is present

Thermodynamics of protein folding

- Driven by hydrophobic effect and primary structure (R group interactions)
- Allows for proteins to be dynamic
- Folded proteins are in equilibrium with less folded states
- Lowest energy states are most populated
- Dynamics are essential for proper folding and functioning
- Proteins are not rocks!
- Dynamic motions can do work

Intrinsically unstructured proteins

- Few hydrophobic amino acids
- High percentage of hydrophilic amino acids (glycine, proline)
- May become structured upon post-translational modification or binding other biomolecules

Factors that influence protein folding

- Primary structure!
- Amino acid modifications
- Temperature
- pH
- Size
- Binding partners and prosthetic groups
- Presence of **chaperones** (protein complexes that help in the folding of new proteins and old denatured proteins)

Denaturants

- Acid or base - pH should be within 1 pH unit of the isoelectric point
- Organic solvents (ethanol) - disrupt H-bonds in 2°
- Detergents - disrupt protein core in 3° and 4°
- Reducing agents - break disulfide bonds
- Heavy metal ions - disrupt salt bridges and bind cysteine
- Temperature - disrupts all bonds, increases kinetic energy and hurts all IMFs

- Salt concentration - a little helps increase protein stability, but increases hydrophobic effect too much, neutralizes charges on surface
- Mechanical stress - disturbs intramolecular forces