

# Protein structure and folding

BIOS 1006

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## Objectives

- Know all definitions.
- Describe the reactions involving amino acids.
- Describe properties of the bonds in the polypeptide backbone.
- Describe and identify the different classes of protein structure: primary, secondary, tertiary and quaternary.
- 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

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

$C_\alpha$  on opposite sides of the amide bond = **trans** conformation (preferred form in proteins)

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

## Dihedral angles

- $\phi$  (**phi**) **angle**: nitrogen -  $\alpha$  carbon, C–N– $C_\alpha$ –C
- $\psi$  (**psi**) **angle**:  $\alpha$  carbon - carbonyl carbon N– $C_\alpha$ –C–N
- $\omega$  (**omega**) **angle**: carbonyl carbon - nitrogen  $C_\alpha$ –C–N– $C_\alpha$
- Both rotatable

## 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

$\alpha$ -helices (cylinders/arrows) and  $\beta$ -strands (arrows, N- to C-terminus) are connected by random coils

**The  $\alpha$  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  $\alpha$  helix has a dipole
- R-groups point outwards
- On average,  $\sim 10$  residues per helix
- Directionality: left-handed and right-handed helices.  $\alpha$  helix is a right-handed helix
- “Helical wheel” representation of an  $\alpha$ -helix (a view down the helical axis)

**The  $\beta$  strand Different from  $\beta$ -sheets.** Link together to form  $\beta$ -sheets. Strands can have parallel (pointing in the same direction) or antiparallel (pointing in opposite directions) orientations.  $\beta$  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.

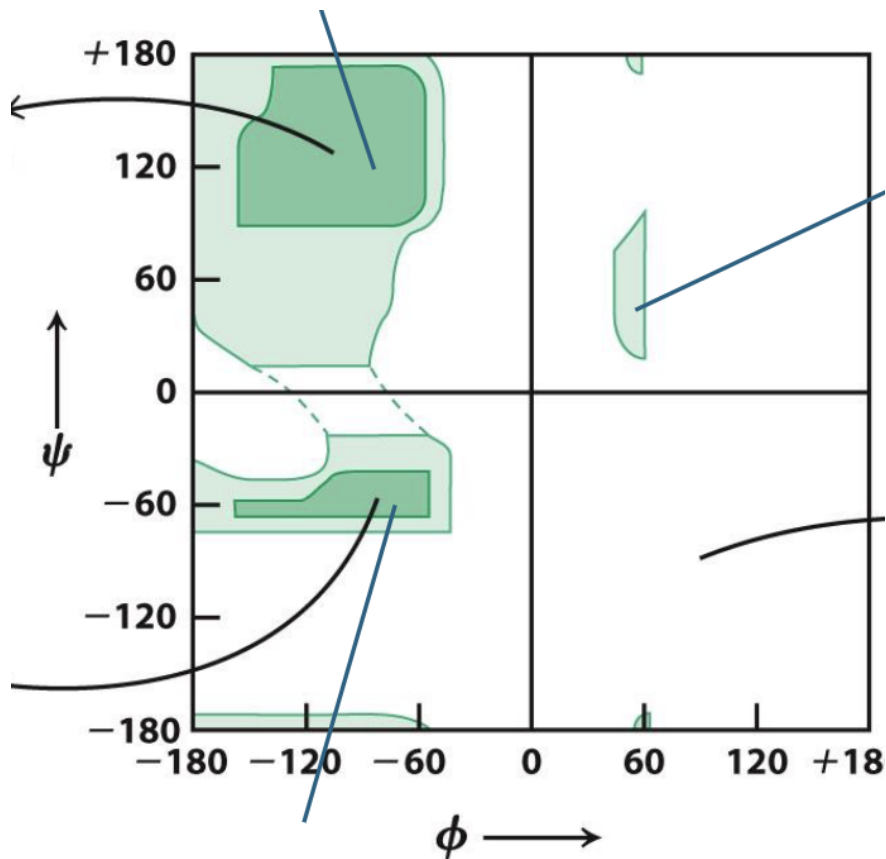
- Amino acids that interact can be distant in primary structure
- Parallel:  $N \rightarrow C$  and  $N \rightarrow C$ , less ordered hydrogen bonds, angled, not collinear (weaker)
- Anti-parallel:  $N \rightarrow C$  and  $C \rightarrow N$ , very ordered hydrogen bonds, perpendicular, collinear (stronger)
- $\beta$ -strands form  $\beta$ -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  $\beta$ -turn and left-handed helix are less common. The  $\beta$  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  $\phi$  and  $\psi$  values
- 4 quadrants
- Top left:  $\beta$  sheets
- Top right: left-handed  $\alpha$ -helix
- Bottom left: right-handed  $\alpha$ -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  $\beta$  sheet
- Mixed  $\alpha$  and  $\beta$

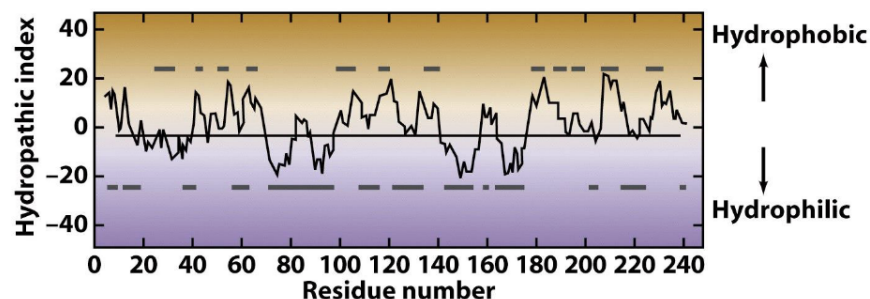
## Anfinsen's hypothesis

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