

CHEM 153A Week 3

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Protein Folding Continued

More Questions - Levinthal's Paradox

- Other experiments like Anfinsen's raised more questions
 - Denatured proteins refold in 0.1-1000 seconds
 - Take a hypothetical protein with 100 amino acids
 - Due to allowed rotations, amino acids can have 3 conformations
 - That's roughly 3^{100} possibilities ($\approx 5 \times 10^{47}$)
- If the protein can visit one conformation every picosecond (10^{-12} s), searching every possibility would take $5 \times 10^{47} \times 10^{-12}$ seconds, or 1.6×10^{28} years.
- This is the left diagram

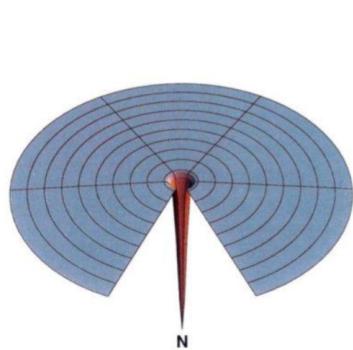


Fig. 1 The Levinthal 'golf-course' landscape. N is the native conformation. The chain searches for N randomly, that is, on a level playing field of energies.

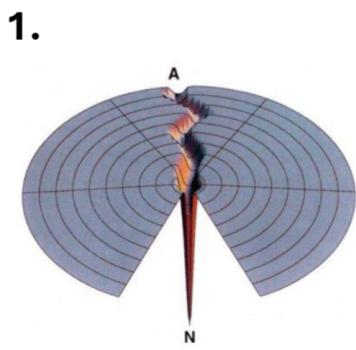


Fig. 2 The 'pathway' solution to the random search problem of Fig. 1. A pathway is assumed to lead from a denatured conformation A to the native conformation N, so conformational searching is more directed and folding is faster than for random searching.

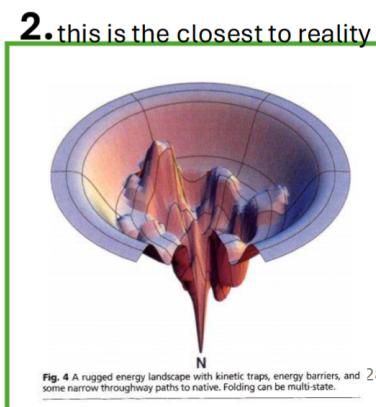


Fig. 4 A rugged energy landscape with kinetic traps, energy barriers, and some narrow throughway paths to native. Folding can be multi-state. 28

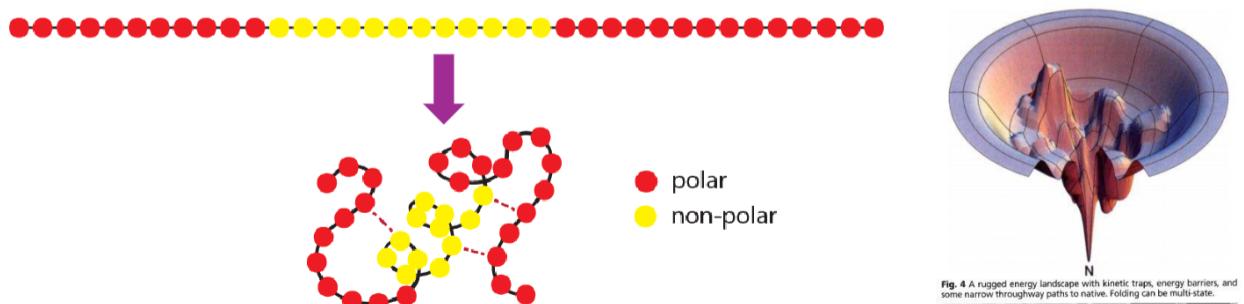
The Thermodynamics of Protein Folding

- Free Energy Funnel:
 - Unfolded states have high degree of conformational entropy, thus there is high free energy.
 - The free energy funnel shows that the closer the protein is to its **native state**, the ideal, lowest energy, folded form, the lower energy it has.
 - * The middle diagram suggests that there is a pathway guiding the protein folding to the lowest energy state. This is on the right track, but is not correct.
 - * The right diagram states there are multiple stable intermediates leading to the final folded protein. This is the most accurate diagram.

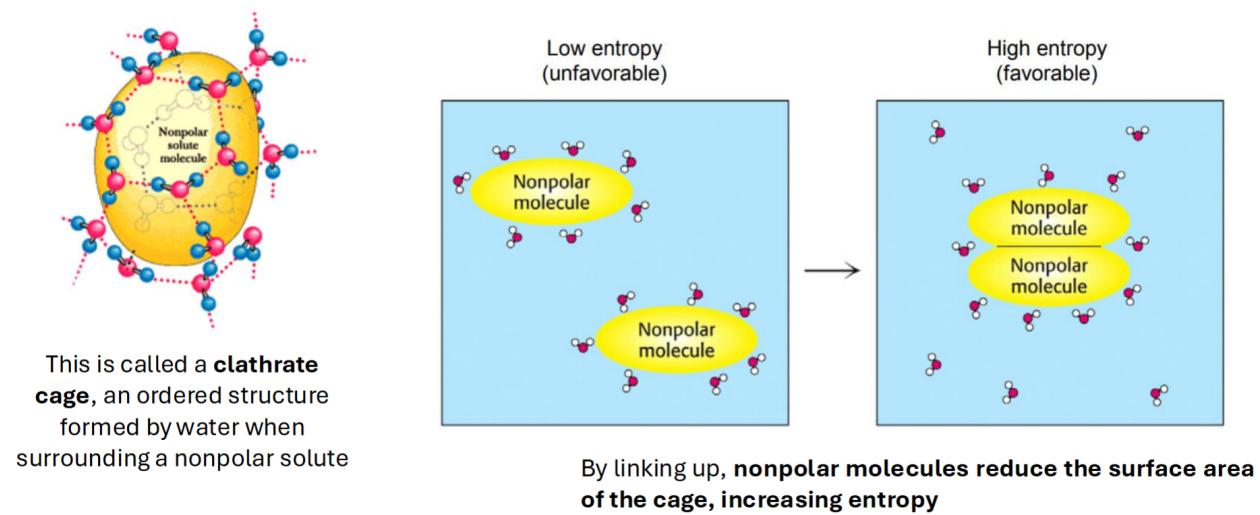
Hydrophobic Collapse → Molten Globule

Hydrophobic collapse is the rapid burying of hydrophobic residues in the center of the protein - they want to escape their watery environment.

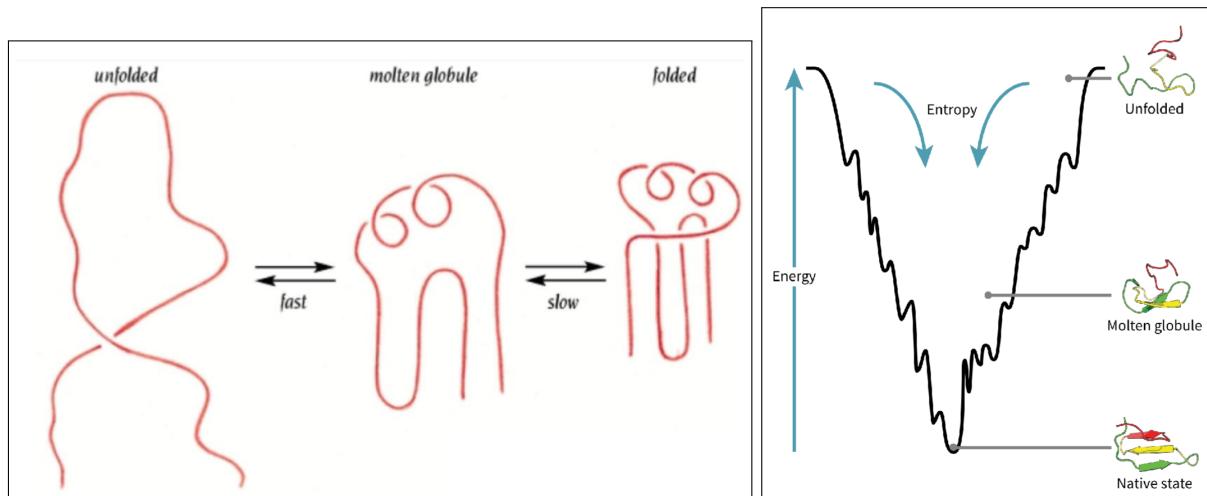
- e.g., Val, Leu, Ile, etc.



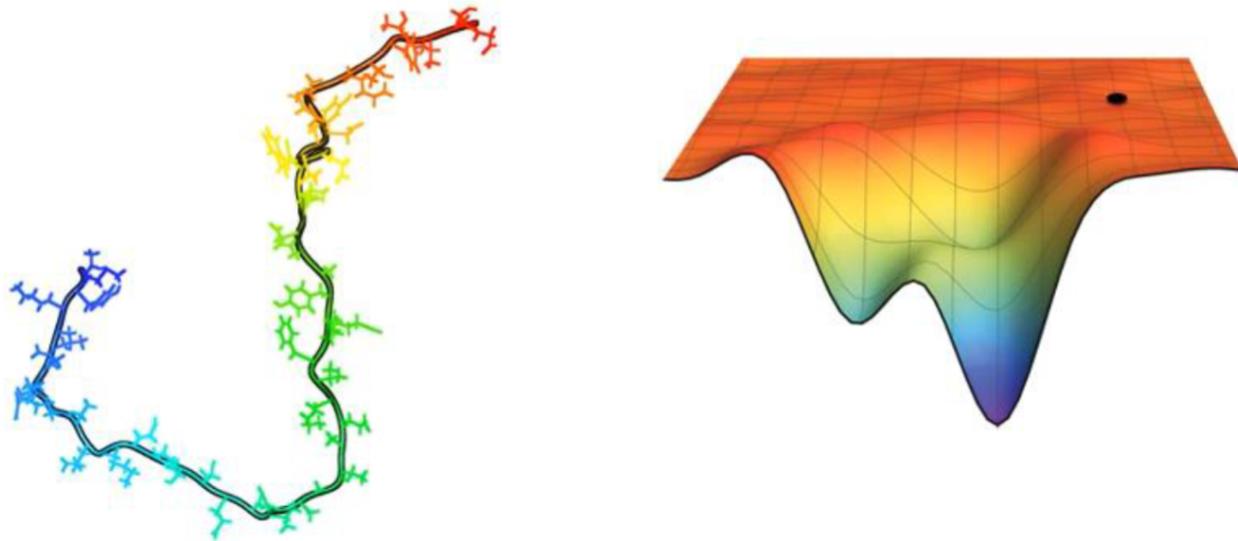
- Hydrophobic collapse is entropy-driven: water molecules become more ordered around hydrophobic residues, and the collapse releases that ordered water, increasing entropy.



- This collapse forms the **molten globule**, an intermediate transitioning to the final form of the protein



Protein Folding and Energy Landscape



Other Significant Factors in Protein Folding

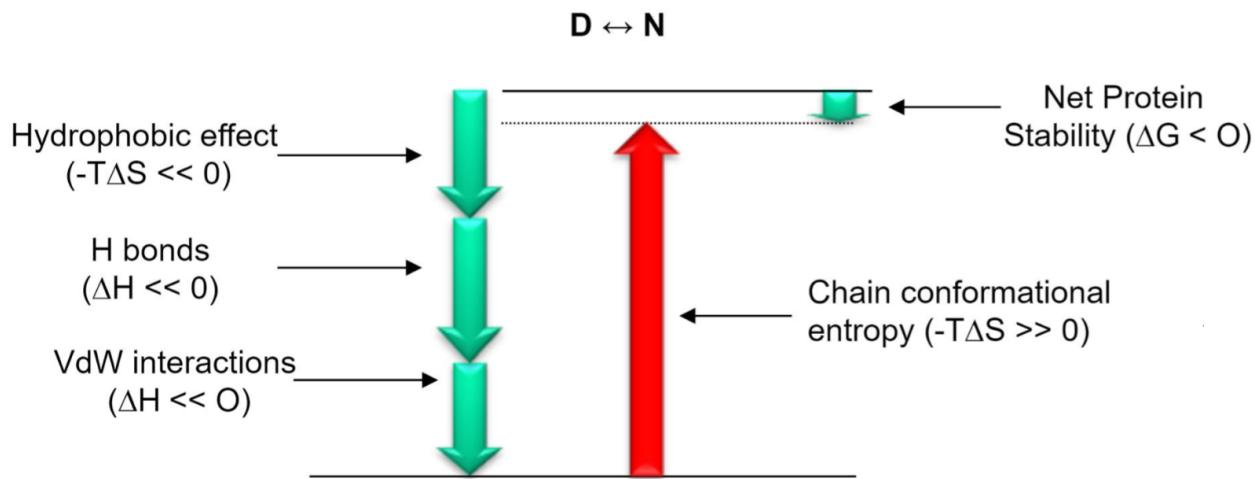
- Chain conformational entropy is the entropy *decrease* due to the formation of an ordered polypeptide
- Hydrogen bonding serves an important role, mediating interactions with the surrounding water as well as connecting the outer surface of the protein with the hydrophobic core
 - They also stabilize interactions between peptide chains (secondary structure)
 - Enthalpy decrease
- London dispersion forces hold together the hydrophobic core (enthalpy decrease)
- Electrostatic forces can form between charged R groups (enthalpy decrease)

Protein folding is enthalpically driven

Although folding a protein reduces its entropy, the process is driven by the **enthalpy gain** from forming stabilizing interactions. These forces - hydrogen bonding, van der Waals interactions, and electrostatic forces - make folding energetically favorable, leading to a stable, functional protein.

- **Conclusion:** Protein folding is **enthalpically favorable** ($\Delta H < 0$) but **entropically unfavorable** ($\Delta S < 0$)

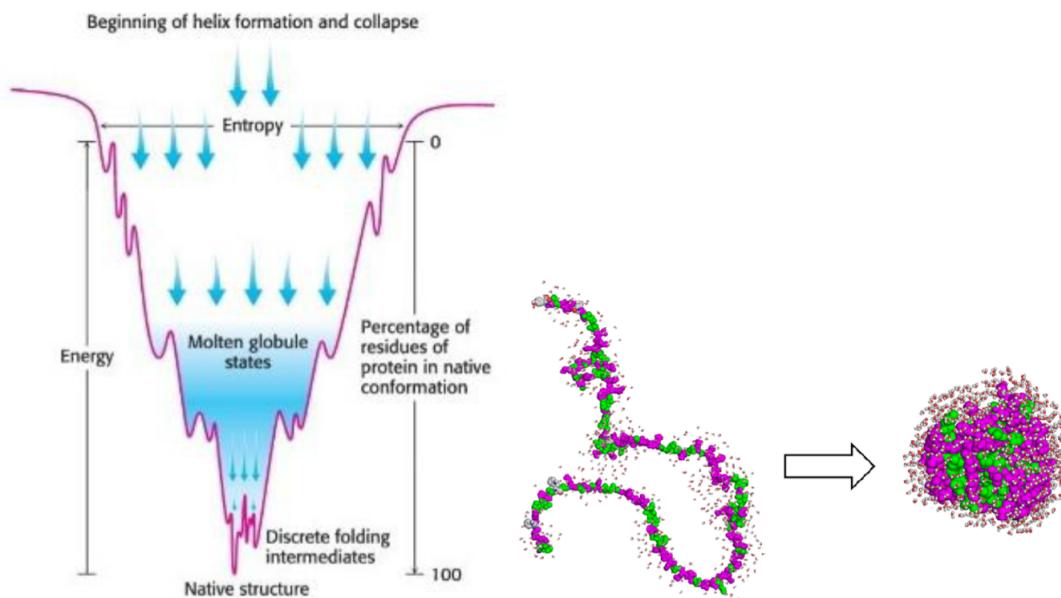
Thermodynamics of Protein Folding



Relative contributions to ΔG for protein folding

Summary of protein folding

- Unfolded protein rapidly collapses (hydrophobic collapse) to increase entropy of surrounding water
- Molten globule state(s) form, with some early secondary structure
 - Serves as pre-folded state, serving to constrain energetic possibilities
 - "Cooperative" effect emerges, once one interaction forms, the next interaction is easier to form
- Molten globule state then slowly works its way to the final native structure.

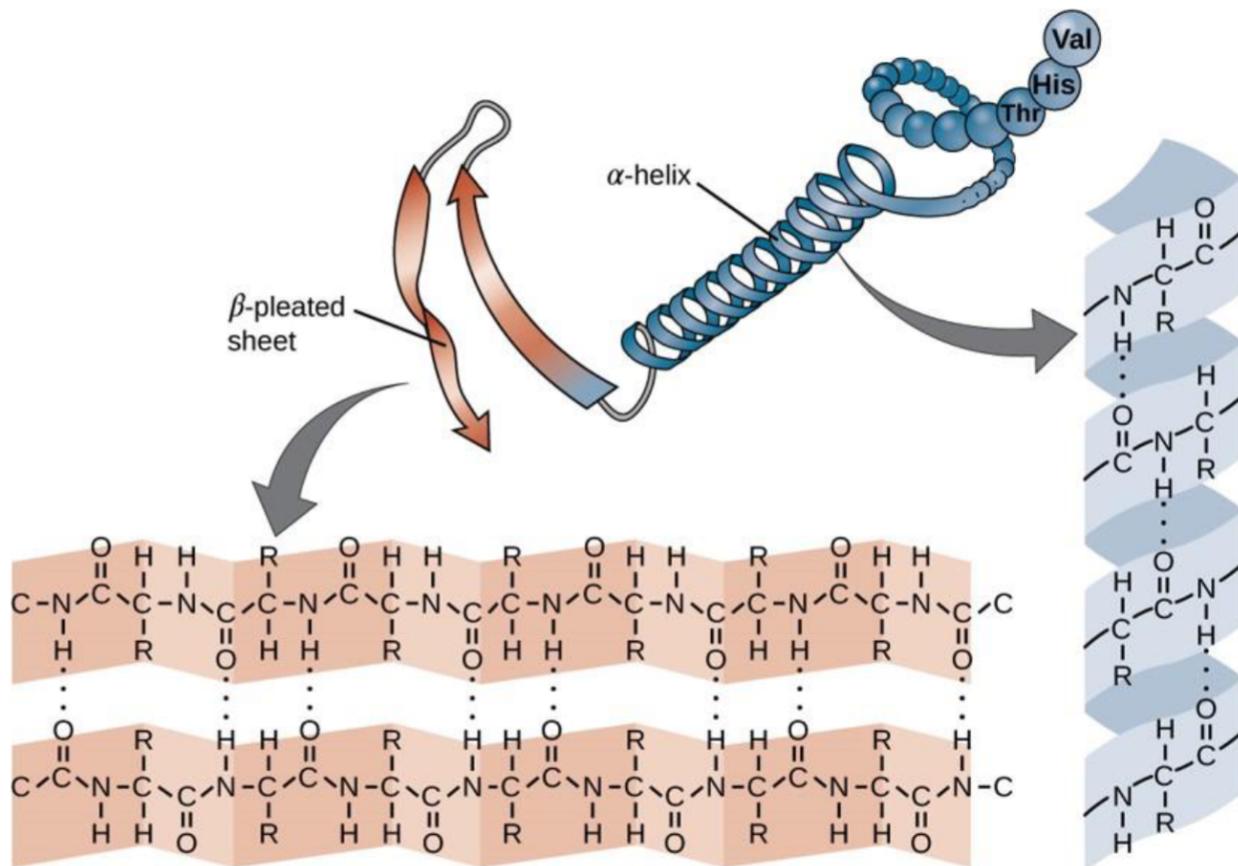


Protein Structures

- Protein segments can adopt regular secondary structures such as the α helix and the β conformation.
- These structures are defined by particular values of ϕ and ψ and their formation is impacted by the amino acid composition on their segment.
- All of the ϕ and ψ values for a given protein structure can be visualized using a Ramachandran plot.

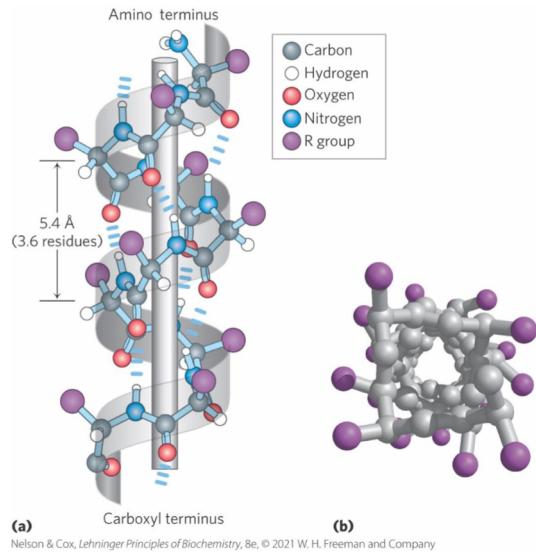
Protein Secondary Structure

- **secondary structure** = describes the spatial arrangement of the main-chain atoms in a segment of a polypeptide chain
 - *regular* secondary structure = ϕ and ψ remain the same throughout the segment
 - common types = α helix, β conformation, β turn, random coils



The α Helix is a Common Protein Secondary Structure

- α helix = simplest arrangement, maximum number of hydrogen bonds
 - backbone wound around an imaginary longitudinal axis
 - R groups protrude out from the backbone
 - Each helical turn = 3.5 residues, $\sim 5.4 \text{ \AA}$



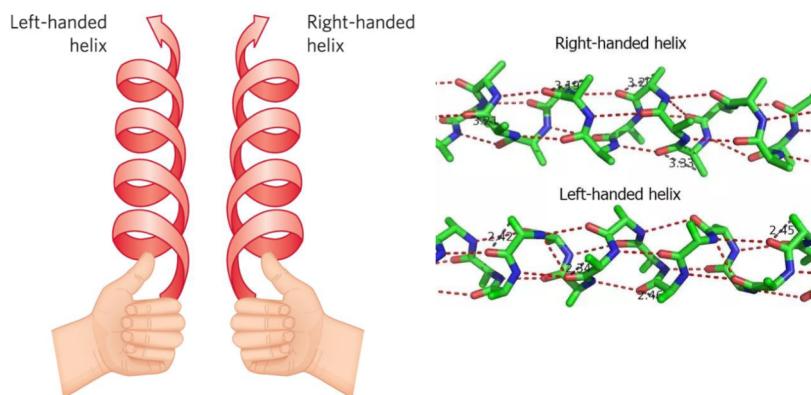
Dihedral Angles Define Protein Conformations

The following table shows idealized ϕ and ψ angles for common secondary structures in proteins

Structure	ϕ	ψ
α Helix	-57°	-47°
Conformation: Antiparallel	-139°	$+135^\circ$
β Conformation: Parallel	-119°	$+113^\circ$
Collagen triple helix	-51°	$+153^\circ$
β Turn type I: $i + 1$	-60°	-30°
β Turn type I: $i + 2$	-90°	0°
β Turn type II: $i + 1$	-60°	$+120^\circ$
β Turn type II: $i + 2$	$+80^\circ$	0°

Handedness of the α Helix

- Right-handed:
 - R groups protruding away from the helical backbone
 - most common
 - extended left-handed: theoretically less stable, not observed in proteins

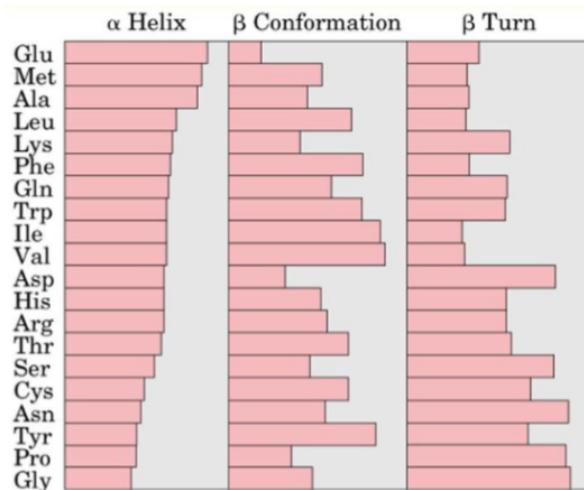


Intrahelical Hydrogen Bonds

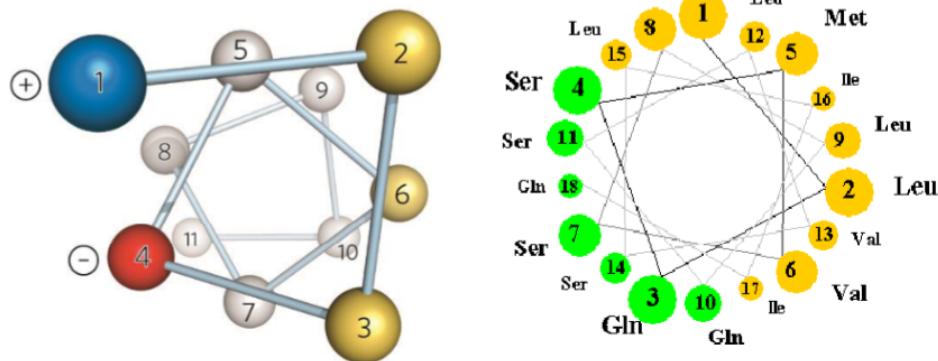
- Between hydrogen atom attached to the electronegative nitrogen atom of residue n and the electronegative carbonyl oxygen atom of residue $n + 4$
- Confers significant stability

Amino Acid Sequence Affects Stability of the α Helix

- amino acid residues have an intrinsic propensity to form an α helix
- α helix = simplest arrangement, maximum number of hydrogen bonds
 - backbone wound around an imaginary longitudinal axis
 - R groups protrude out from the backbone
 - each helical turn = 3.6 residues, 5.4 Å
- Interactions between R chains spaced 3-4 residues apart can stabilize or destabilize α helix
 - charge, size, and shape of R chains can destabilize
 - formation of ion pairs and hydrophobic effect can stabilize



Helical wheel



Amphipathic α -helices

Can be found at the surface of a water-soluble globular protein, whereas hydrophobic helices are on the inside.

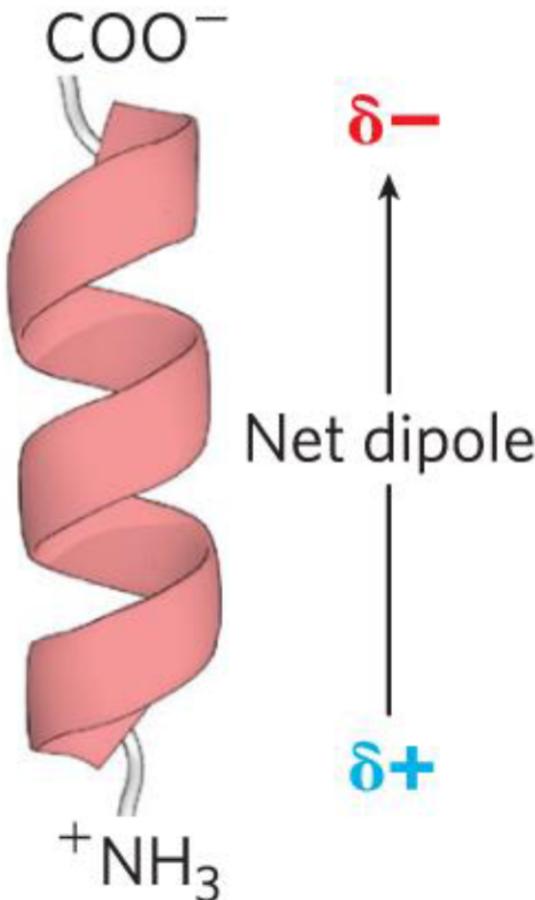
- As the name implies, an **amphipathic** (or **amphiphilic**) **helix** is an α -helix with both **hydrophobic and hydrophilic amino acid residues** arranged in such a way as to create two faces on opposite sides of the helix, one face being hydrophobic.
- An example is the bottom left image above (yellow is nonpolar, green is polar)

Proline and Glycine Occur Infrequently in an α Helix

- Proline = introduces destabilizing kink in helix
 - Nitrogen atom is part of rigid ring
 - rotation about N-C $_{\alpha}$ bond not possible
- Glycine = high conformational flexibility, takes up coiled structures.

Amino Acid Residues Near the End of a α Helix Segment Affect Stability

- small electric dipoles in each peptide bond align through hydrogen bonds
- negatively charged amino acids often found near the NH $_3^+$ terminus
- positively charged amino acid often found near the COO $^-$ terminus



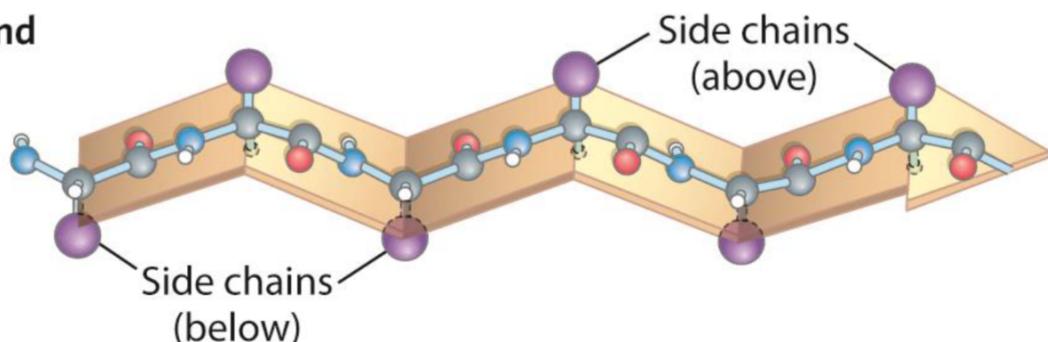
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The β Conformation Organizes Polypeptide Chains into Sheets

- β conformation = backbone extends into a zigzag
 - β strand = single protein segment
 - β sheet = several strands in β conformation side by side

(a) β Strand

Side view



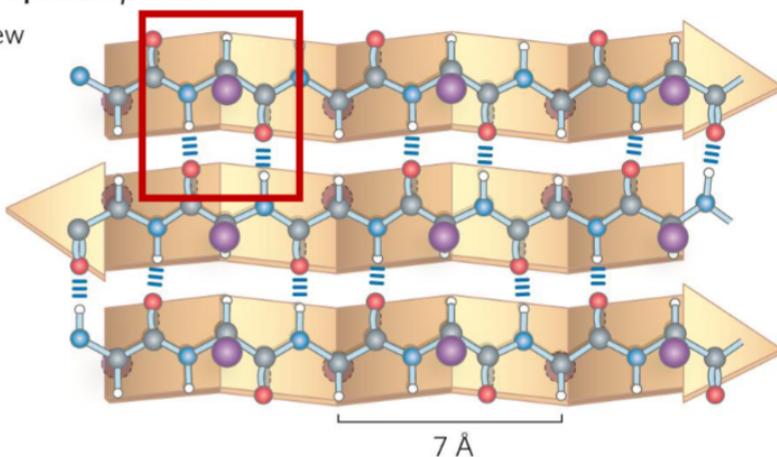
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Adjacent Polypeptide Chains in a β Sheet Can Be Antiparallel or Parallel

- antiparallel = opposite orientation (occur more frequently)
- parallel = same orientation
- H bonds form between backbone atoms of adjacent segments

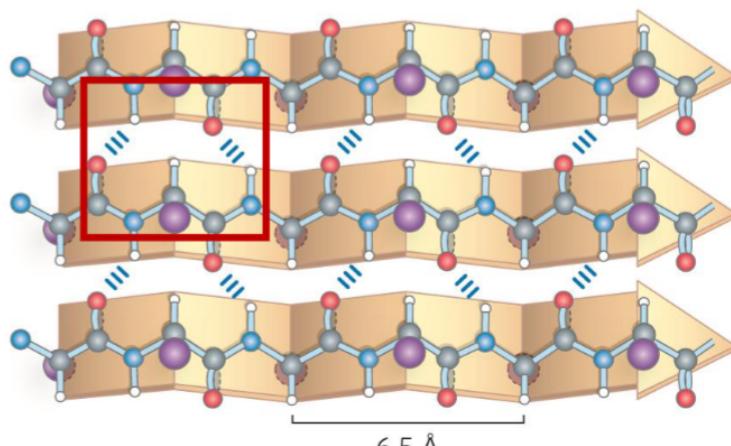
(b) Antiparallel β sheet

Top view



(c) Parallel β sheet

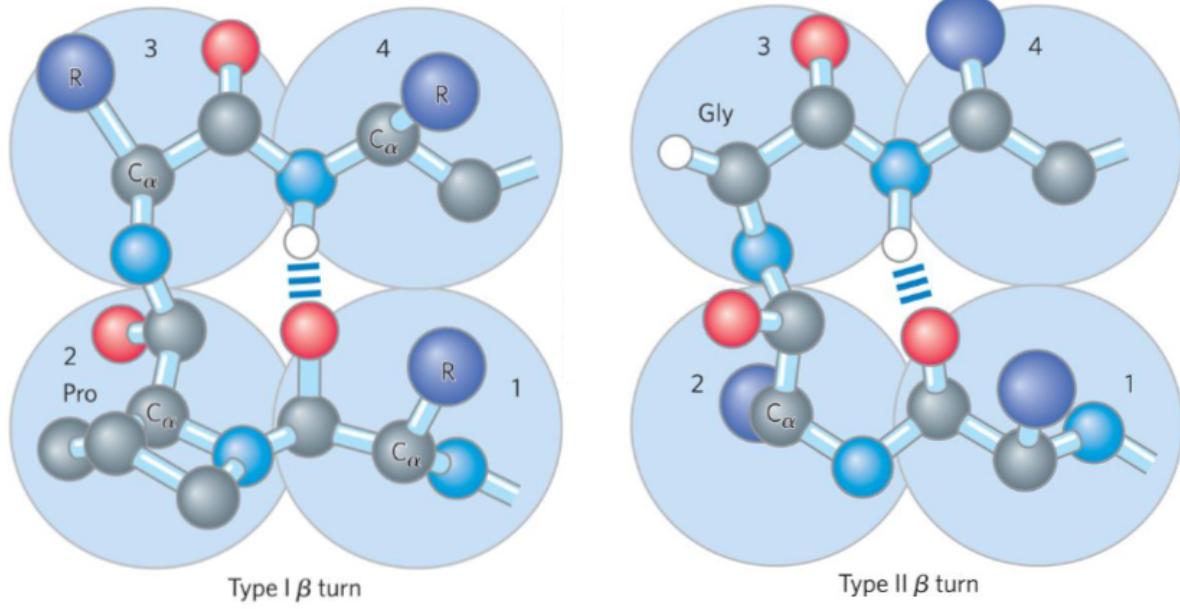
Top view



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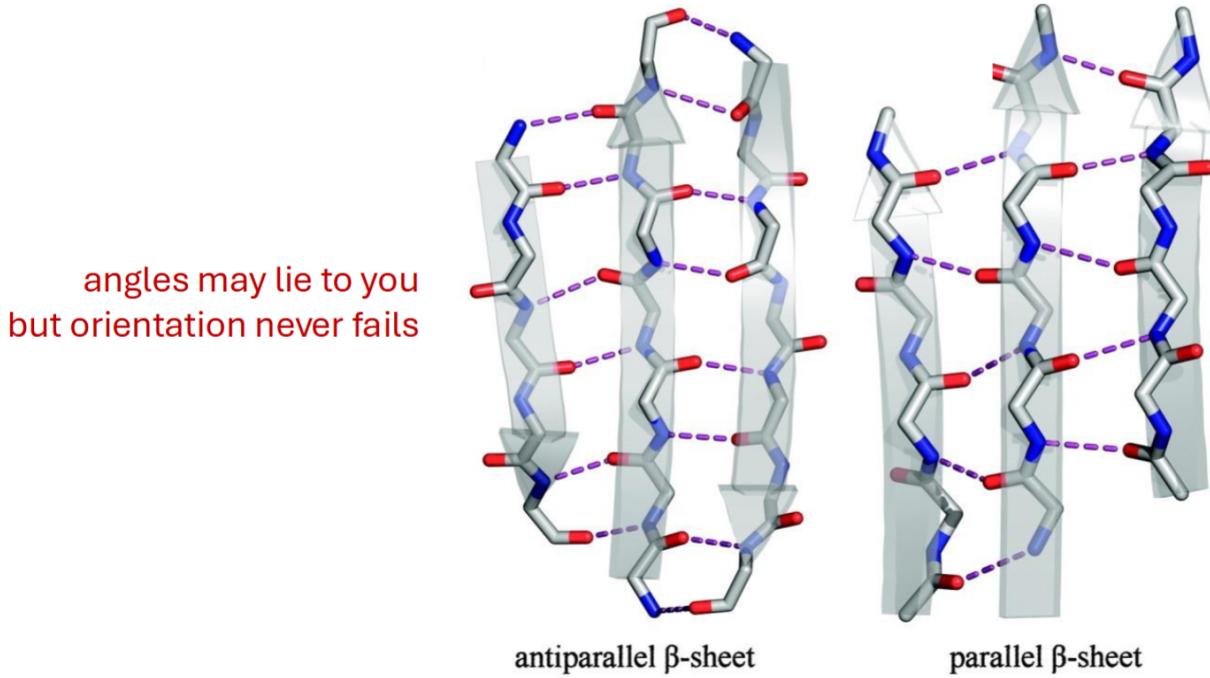
β Turns are Common in Proteins

- β turns = connect ends of two adjacent segments of an antiparallel β sheet
 - 180° turn
 - involves 4 residues
 - hydrogen bond forms between first and fourth residue
 - Gly (residue 2) and Pro (residue 3) often occur in β turns.



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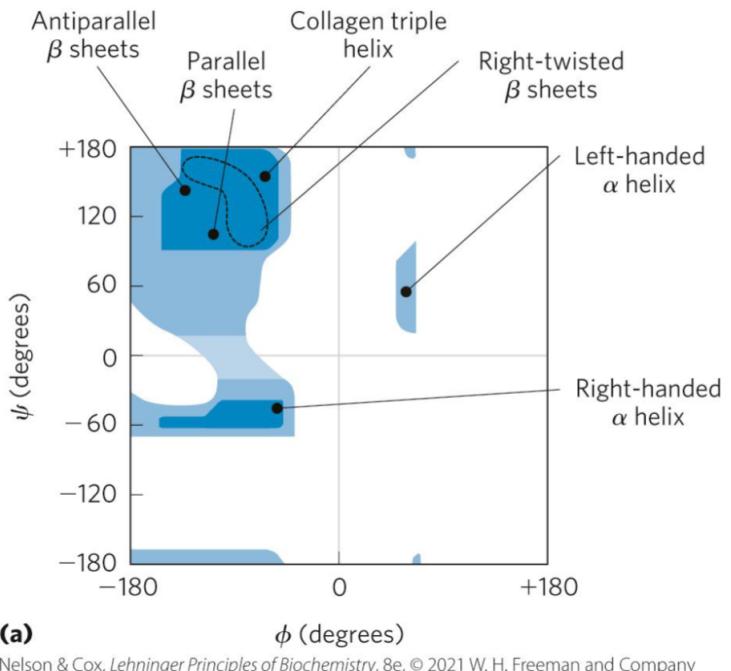
Notes on determining parallel vs antiparallel β sheets



Red labels carbonyl oxygens while blue labels the amine nitrogens

Common Secondary Structures Have Characteristic Dihedral Angles

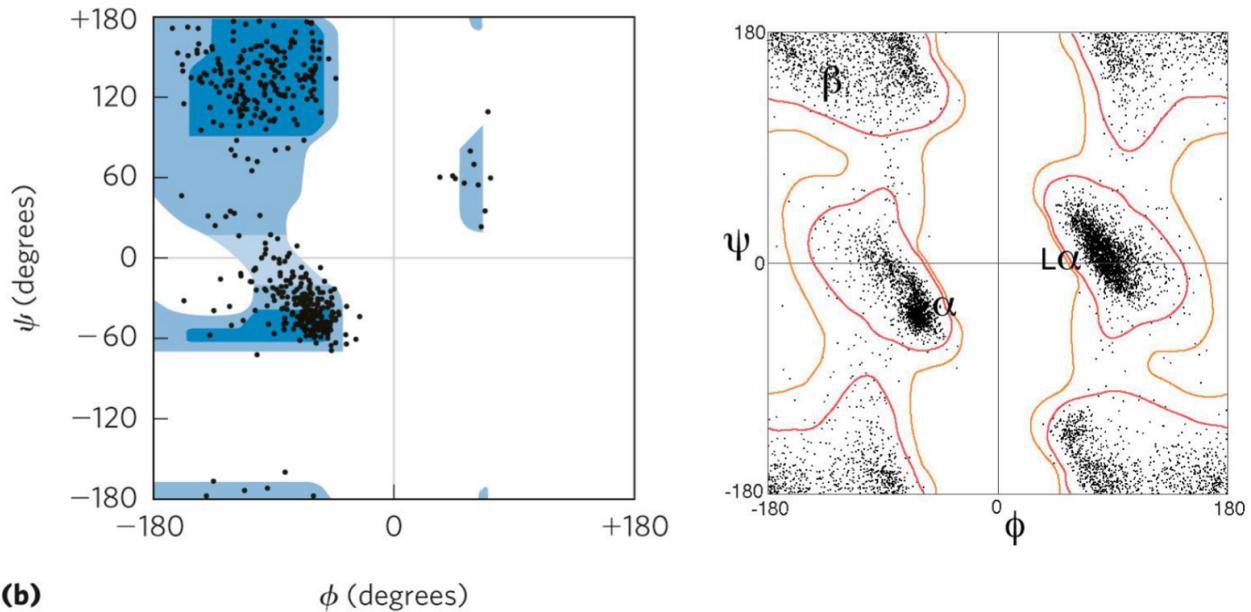
- dihedral angles φ (phi) and ψ (psi) associated with each residue completely describe secondary structure
- **Ramachandran plots:**
 - visualize all φ and ψ angles
 - test quality of three-dimensional protein structures



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Ramachandran Plot - Glycine

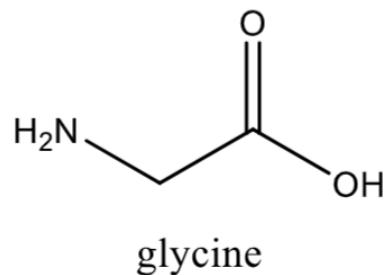
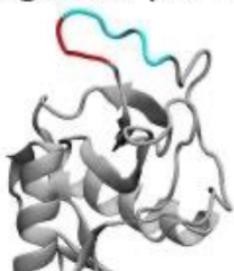
- Glycine falls frequently outside the expected ranges.



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332	331	330	329	328	327	326	325	324	323
GLU	ALA	GLY	SER	SER	GLY	GLY	ASN	ASN	ASN

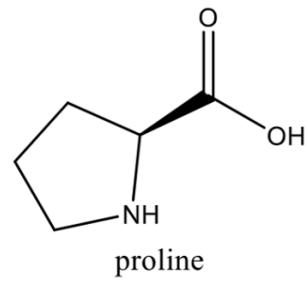
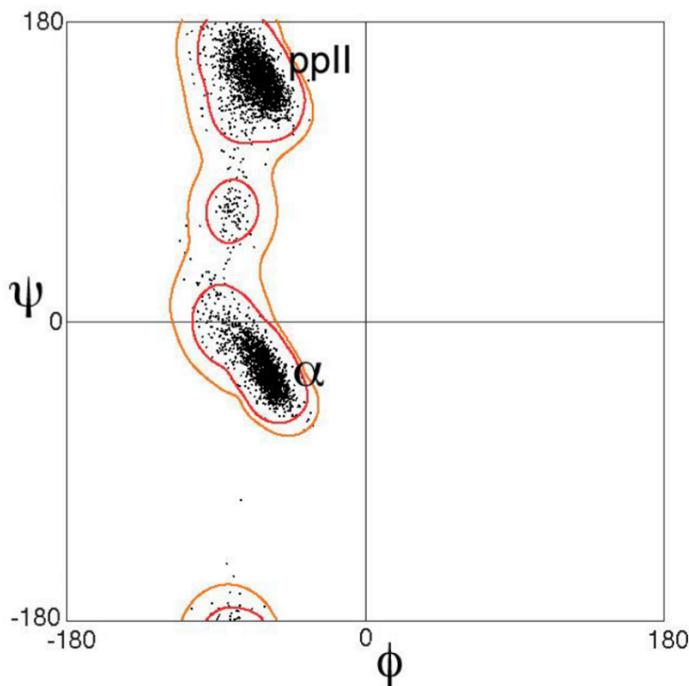
Loop region of pSP-D



- Due to the minimal bulk, glycine residues have far more conformational flexibility than other amino acid residues
 - Actually works against it, destabilizes α and β
- This is why its Ramachandran is so well populated
- This is also why glycine is often found in **loop regions** of the protein structure (polypeptide taking a turn)

Ramachandron Plot - Proline

- Due to its R-group ring structure, proline is highly constrained and can only adopt specific angles
- Not likely to show up in either β sheets or α helices (destabilizes both, creating kinks)



Protein Tertiary and Quaternary Structures

Tertiary structure describes the well-defined, three-dimensional fold adopted by a protein.

Shape → Function

- **tertiary structure** = overall three-dimensional arrangement of all the atoms in a protein
 - weak interactions and covalent bonds hold interacting segments in position
- **quaternary structure** = arrangement of 2+ separate polypeptide chains in three-dimensional complexes