Protein secondary structure

All of the information required for polypeptides to assume their biologically active conformation is inherent in their linear amino acid sequence (and hence, encoded in the DNA)

- Linear sequence = "primary structure"
- Higher order structures:
  - Secondary
  - Tertiary
    - "motifs"
    - "domains"
  - Quaternary

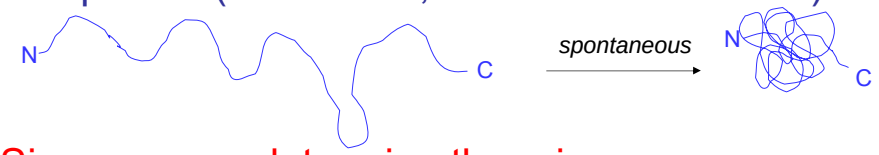
Amino acid sequences of >50,000 proteins are known

- Compare to other known sequences- similarities, families (BLAST)
- Same proteins, different families- evolutionary information
- Genetic defects
- Internal repeats (gene duplication)
- Signal sequences-targeting, processing
- Antibody design
- DNA probes- DNA → protein

Degrees of structural complexity

- Primary (1°): all covalent bonds. The linear sequence of AAs and disulfide bonds
- Secondary (2°): regular, recurring arrangements in space of adjacent AA residues in a polypeptide chain
- Tertiary (3°): spatial relationship between all AAs in polypeptide; the 3-D structure of the polypeptide
- Quaternary (4°): spatial relationship of polypeptides in multisubunit proteins

Dogma: all of the information required for polypeptides to assume their biologically active conformation is inherent in their primary sequence (and hence, encoded in the DNA)

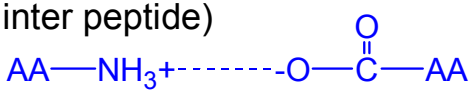


Since we can determine the primary sequence, can we predict higher order structures?

Yes and No

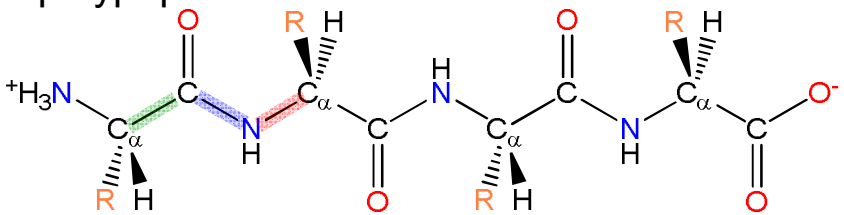
Constraints on possible conformations

Thermodynamics, folded vs. unfolded states in H_2O

- Unfolded protein:
  - High conformational entropy (∞conformations)
  - Favorable AA – H2O interactions
- Folded (native) protein
  - Disulfide bonds (sometimes)
  - H-bonds (intra and inter peptide)
  - Ionic interactions 
  - Van der waals
  - Hydrophobic effect: 8 AA side chains are hydrophobic

Constraints on possible conformations

- About which main chain bonds in a polypeptide can rotation occur?
- Begin by defining the three types of bonds in a polypeptide:



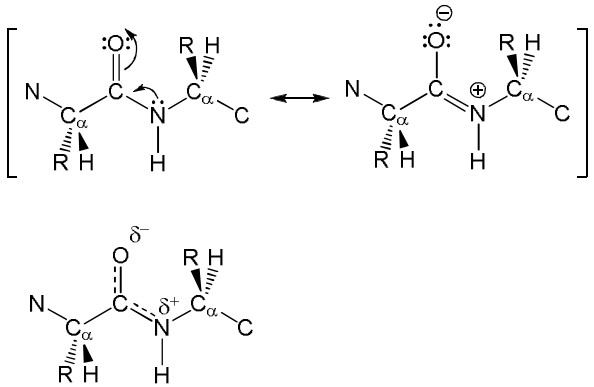
Peptide bond (ω bond): between a $C_{carbonyl}$ and N

Psi (ψ) bond: between a C_α and $C_{carbonyl}$

Phi (ϕ) bond: between a N and C_α

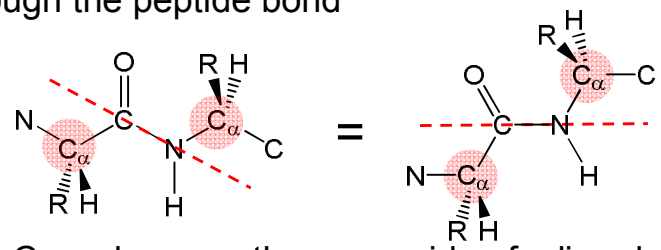
Constraints on possible conformations

- The peptide bond is rigid due to resonance overlap of p orbitals between N and C, so no rotation occurs

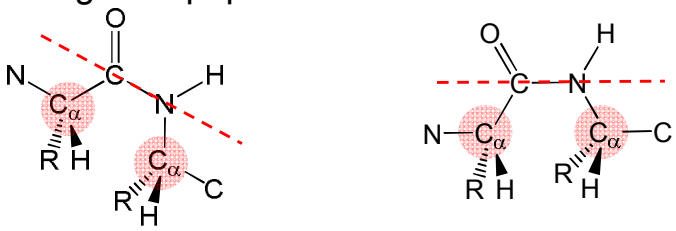


Peptide bond conformations

- Trans: C_α carbons on opposite sides of a line drawn through the peptide bond

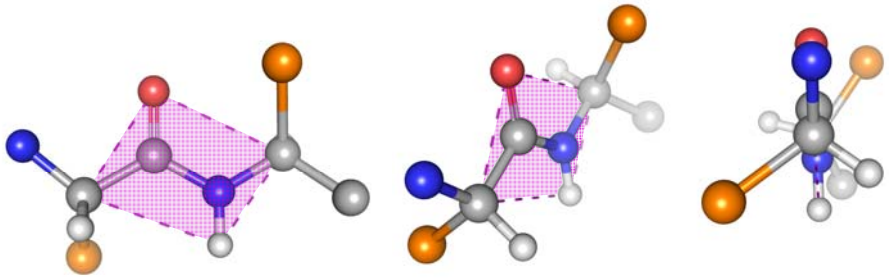


- Cis: C_α carbons on the same side of a line drawn through the peptide bond



Peptide bond conformations

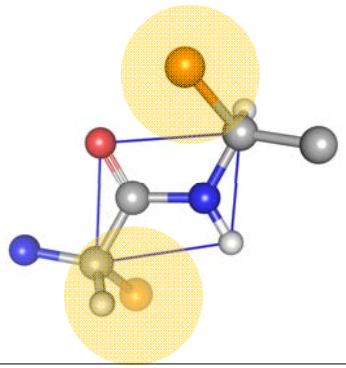
- Note how the atoms directly bonded to the peptide bond form a planar unit with the atoms of the peptide bond



C_α

peptide bond trans sae1.pse

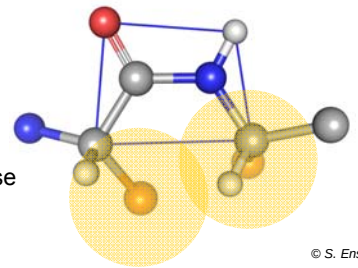
The trans peptide bond is favored based on steric considerations



Trans peptide bond:

common

peptide bond trans sae1.pse

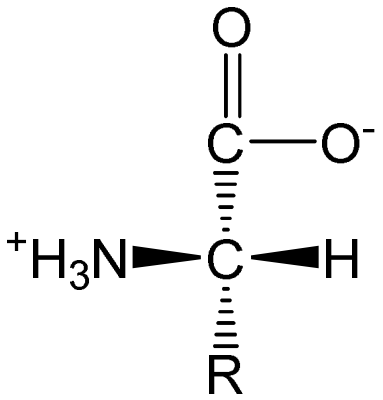


Cis peptide bond

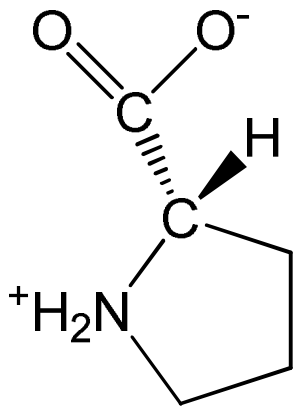
unusual

peptide bond cis sae1.pse

Proline is the only AA that is routinely found in proteins in the cis-configuration

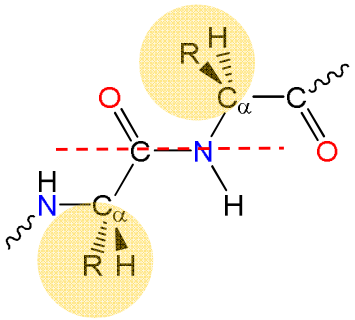


"normal" AA

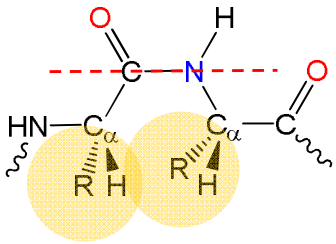


Proline

Steric hindrance of R groups for normal amino acid side chains in cis conformation

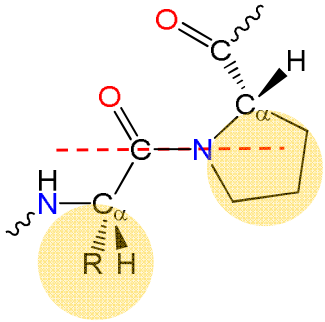


trans

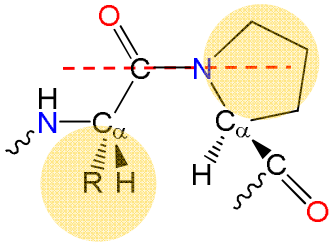


cis

Note how the symmetric cyclic structure of Pro is accommodated in both the trans and cis conformations



trans



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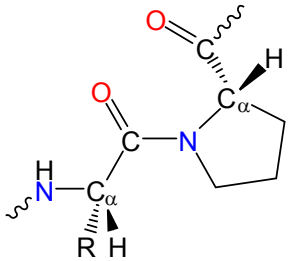
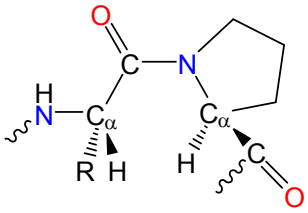


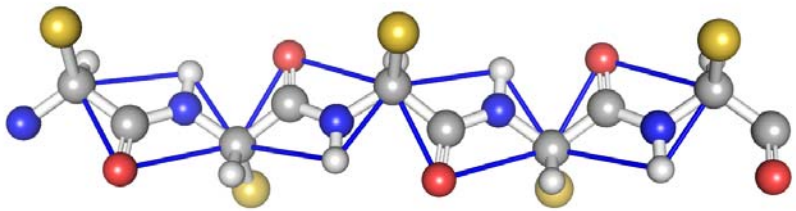
ANIMATION

trans



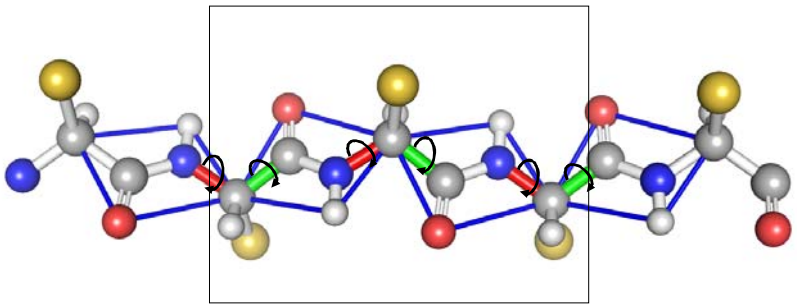
cis

A polypeptide consists of planar peptide units connected by C_α carbons



bsheet generic 5mer sae1.pse Scene 001

Protein conformation is determined by rotation about the N-C_α (ϕ) and C_α-C (ψ) bonds



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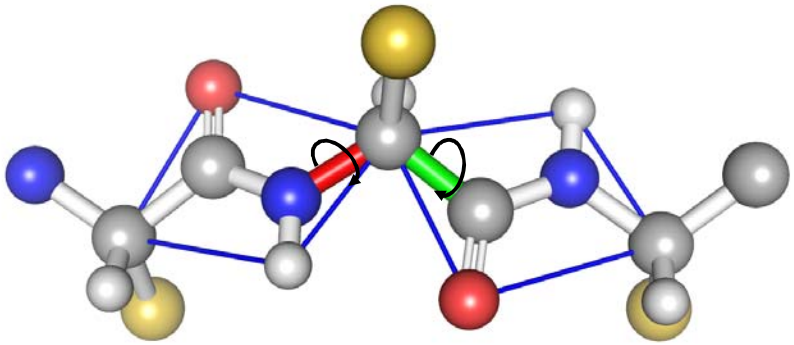
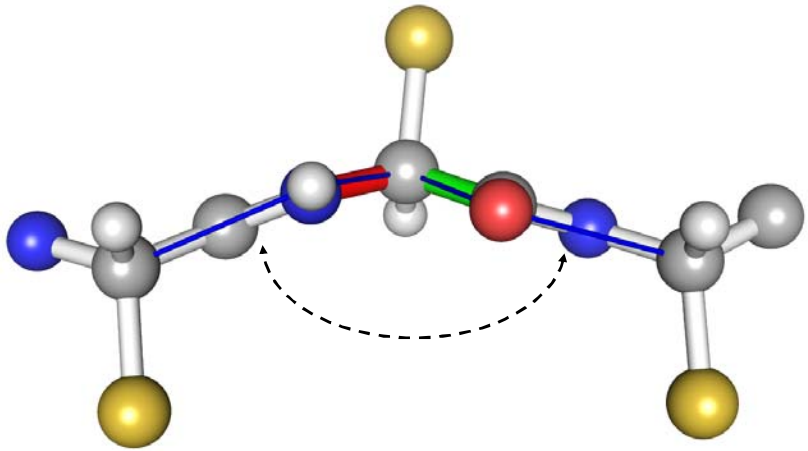


Change view so both peptide planes are in same plane….

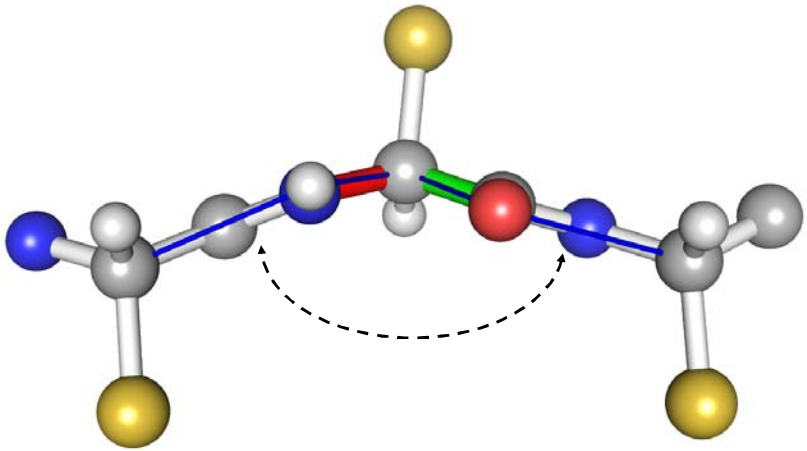
bsheet generic 5mer sae1.pse Scene 003

Protein conformation is determined by rotation about the N-C_α (ϕ) and C_α-C (ψ) bonds



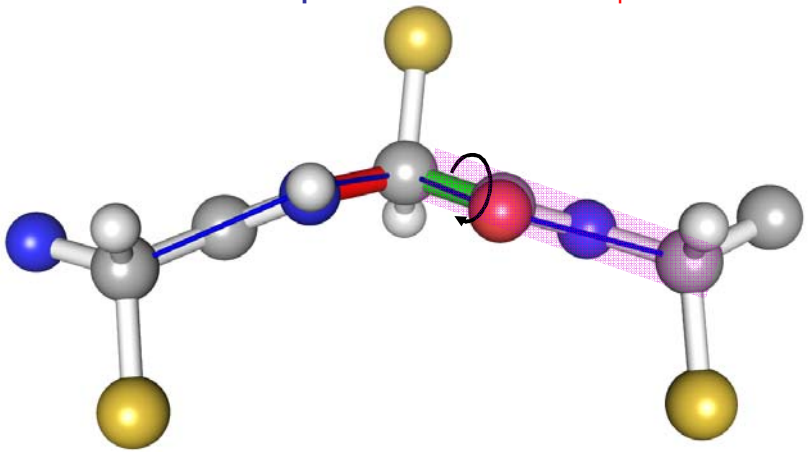
bsheet generic 5mer sae1.pse Scene 003

Rotating about either the ϕ or ψ bond changes the orientation of the two peptide units



bsheet generic 5mer sae1.pse Scene 004

Example: rotate about ϕ …



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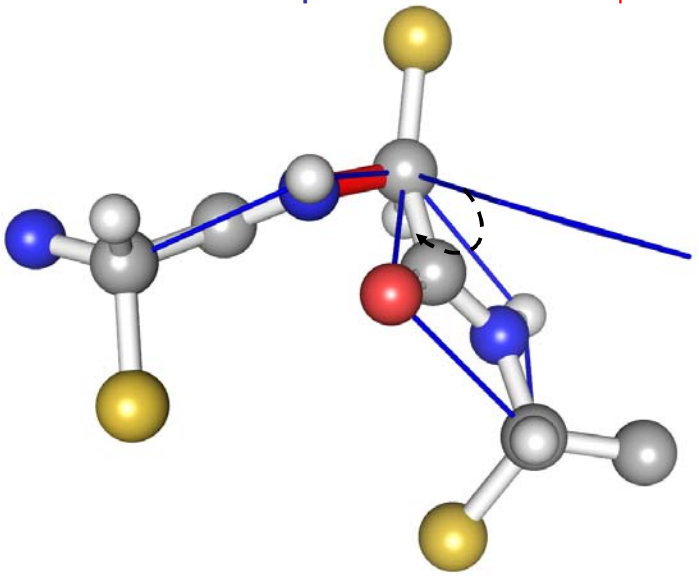
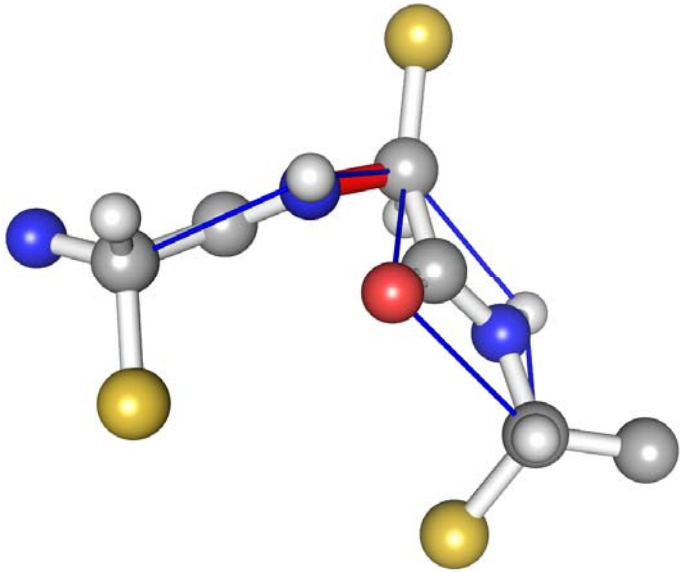
Example: rotate about ϕ …

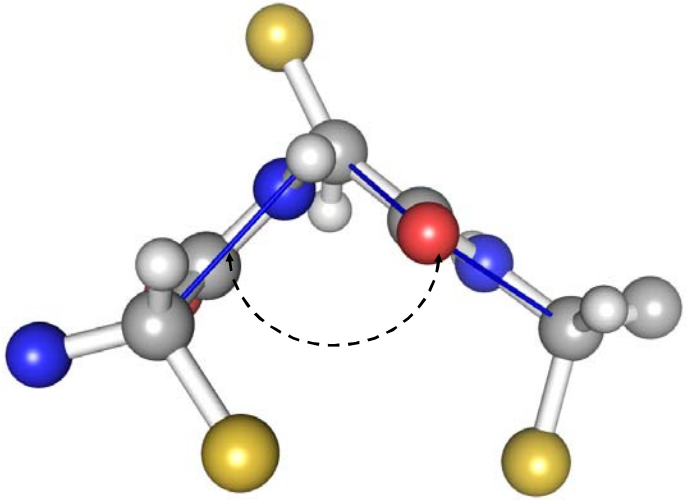


Example: rotate about ϕ …



Change view so both peptide planes are in same plane….

Note how the rotation about ϕ changed the orientation of the two peptide units, bringing them closer together



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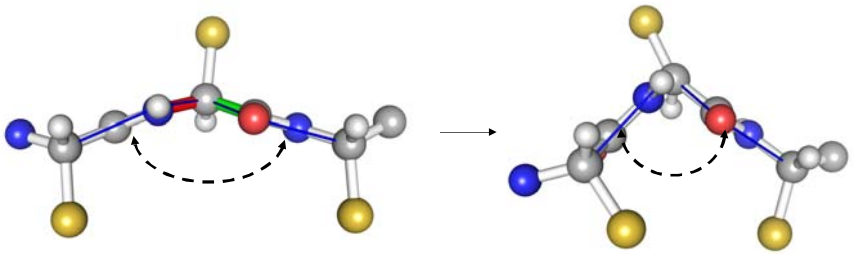
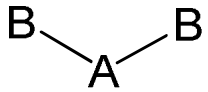
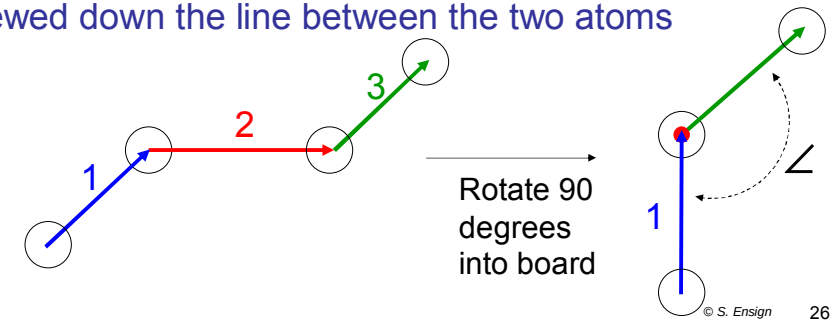


Bond angle vs. dihedral angle

- A bond angle is the angle between three bonded atoms:



- A dihedral (torsion) angle is the angle defined by three vectors, or as applied to molecules, the angle formed between groups attached to two adjacent atoms when viewed down the line between the two atoms



Dihedral angles and ϕ and ψ bonds

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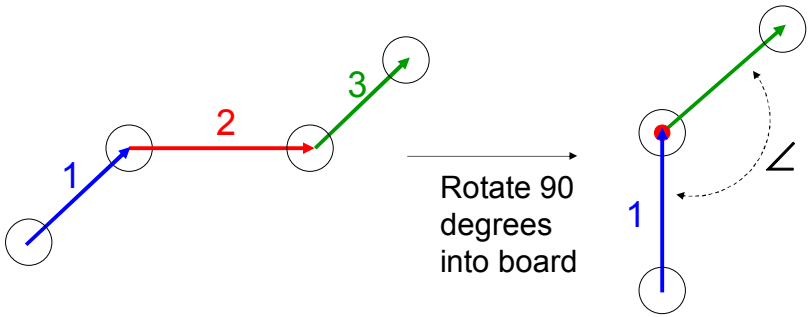
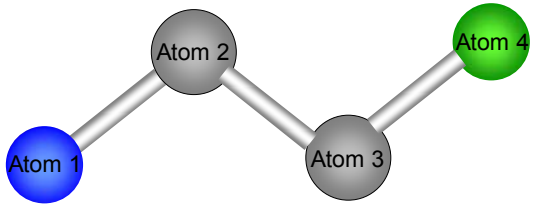
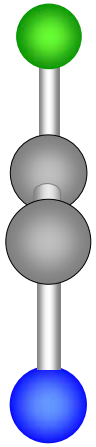


- The two adjacent atoms form the bond associated with the dihedral angle (bond 2)

Animation of dihedral angle changes

- Align four bonded atoms, sighting down the dihedral bond (atoms 2 and 3)
- Begin with two terminal atoms (atoms 1 and 4) 180° apart (DH ∠ = ±180°)
- Rotate about dihedral bond so rear atom rotates clockwise: angles increase from -180 ° to +180 ° after a full turn





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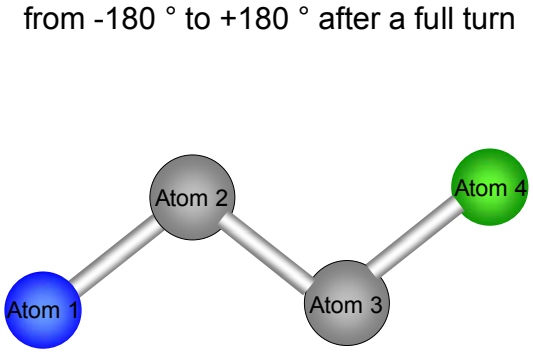

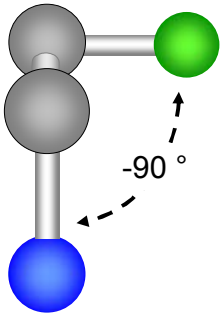

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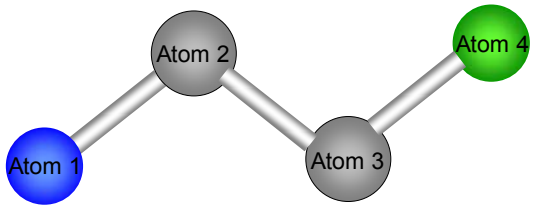

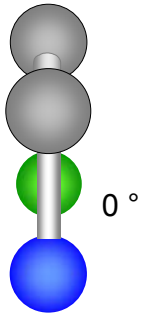

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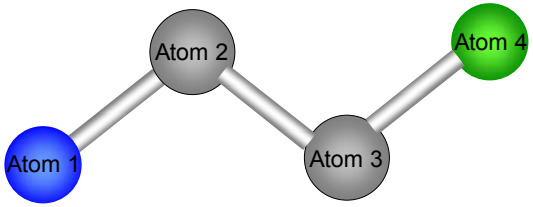

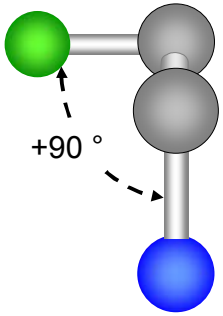

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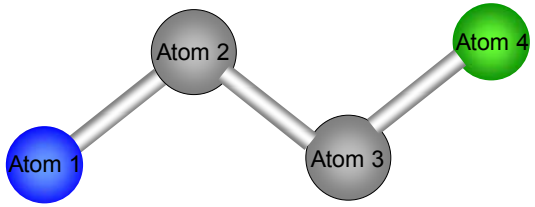

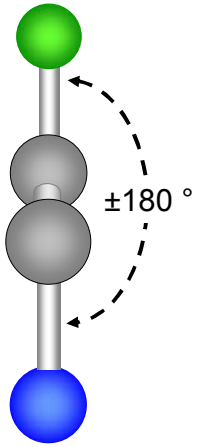

Animation of dihedral angle changes

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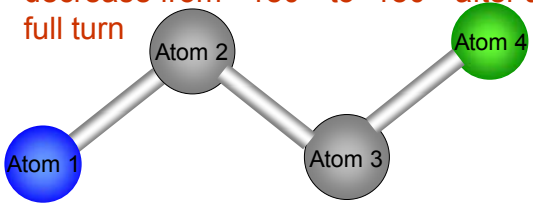
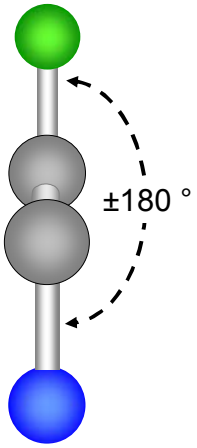
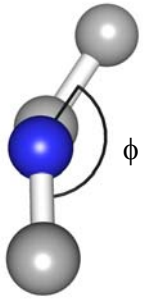
- Rotate about dihedral bond so rear atom rotates clockwise: angles increase from -180 ° to +180 ° after a full turn
- Rotate about dihedral bond so rear atom rotates counterclockwise: angles decrease from +180 ° to -180 ° after a full turn





Measuring phi and psi angles in a polypeptide chain

- First of four bonded atoms is nearest the N-terminus
- phi (ϕ) bond: defined by the dihedral angle C-N-C_α-C
- psi (ψ) bond: defined by the dihedral angle N-C_α-C-N



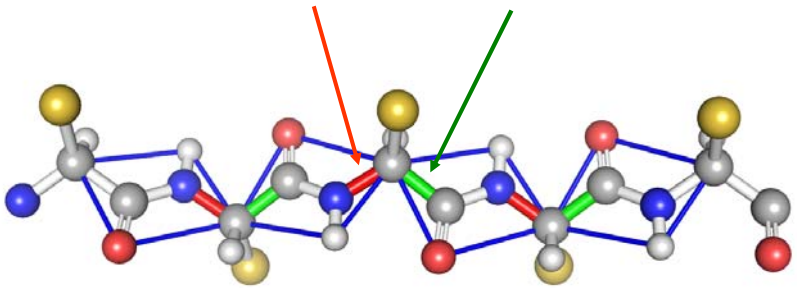
ϕ = -143.3°

ψ = +155.4°

phi (ϕ) bond: C-N-C_α-C

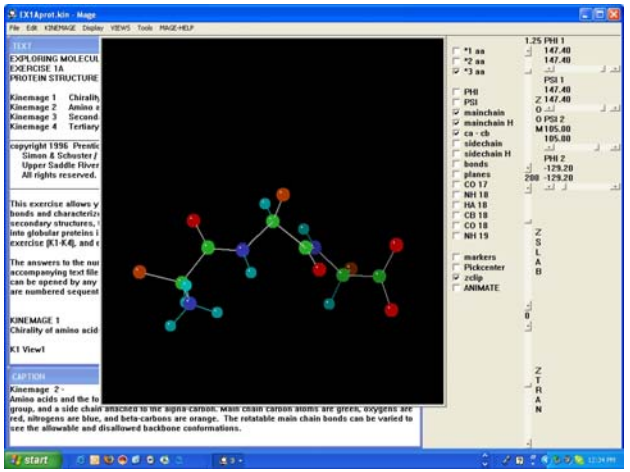
psi (ψ) bond: N-C_α-C-N

Pymol animation showing the values of ϕ and ψ for the indicated bonds in a polypeptide chain



bsheet generic 5mer sae1.pse Scene 001

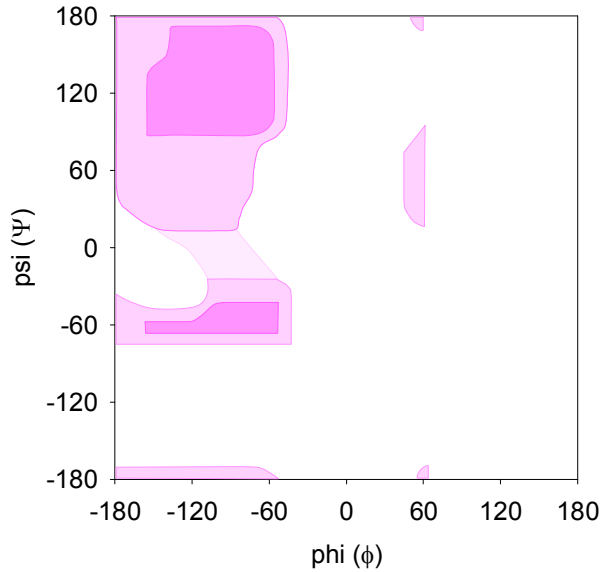
See kinemage exercise EX1Aprot.kin, kinemage 2



Polypeptides adjust ψ and ϕ to:

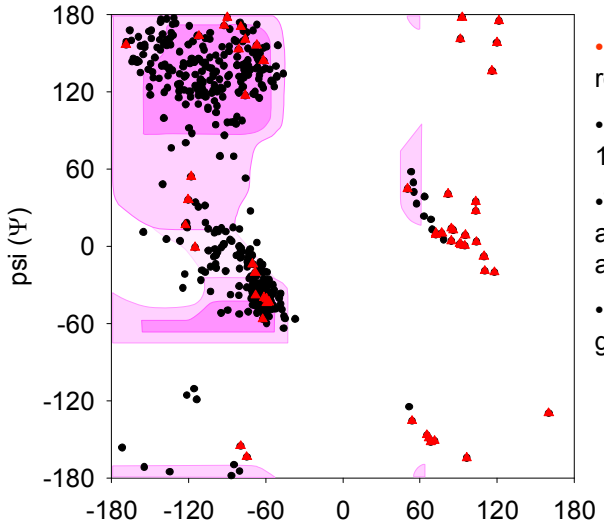
- Decrease steric interactions between $C_{\alpha n-1}$, $C_{\alpha n}$, and $C_{\alpha n+1}$
- Decrease the interactions of nonpolar moieties with water
- Maximize interactions of polar residues with polar "solvents" (membranes, lipids)

Ramachandran plot: A plot of allowed phi and psi angles for a polypeptide



The plot shown is for poly-A. Combinations of phi and psi with darker shading are more sterically favored. Regions with no shading are "not allowed"

The dots represent the phi and psi angles for amino acids present in a "real" protein.



- Red triangles are glycine residues
- Black dots are the other 19 amino acids
- The majority of amino acids have "allowed" phi and psi angles
- Most of the "outliers" are glycine residues

Protein PDB 1MO9

Experimental data generated using: http://dicsoft1.physics.iisc.ernet.in/rp/index.html

Approaches used to determine structure

1. Computer fits, energy minimization- rotations about ψ and ϕ are varied in an attempt to minimize unfavorable interactions

   Usually poor fits to known structures

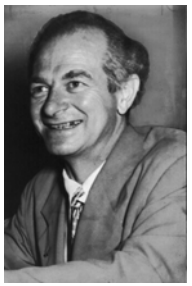
2. Computer fits based on empirical rules generalized from known structures
3. Actual structure determination: ←
   - X-ray crystallography
   - NMR

Common 2° structural elements

- Coiled helices: α helix
- Extended sheets: β strands

The α helix

- Polypeptide backbone is wound around long axis of molecule
- R groups protrude outward
- Repeating unit: a single turn of the helix
- Ψ = -45 to -50˚ ϕ = -60˚
- R-handed helix
- Stabilized by internal H-bonds
- All amino acids are L



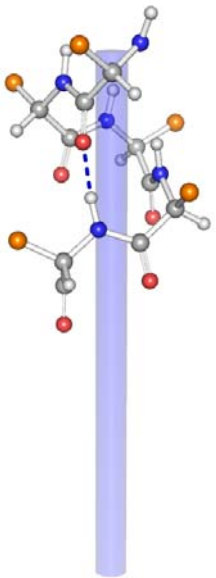
Linus Pauling

The α helix

- Polypeptide backbone is wound around long axis of molecule

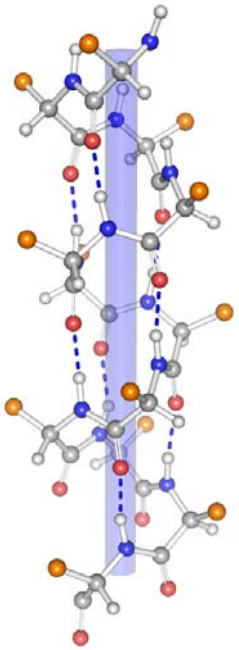
The α helix

- Five residues
- A hydrogen bond is formed between the carbonyl O of residue 1 and amide H of residue 5
- Each successive amino acid results in formation of a new H bond (carbonly O of residue “n” and amide H of residue “n+4”



The α helix

- Connecting the alpha carbons of the polypeptide backbone by straight lines results in a "C_α trace"



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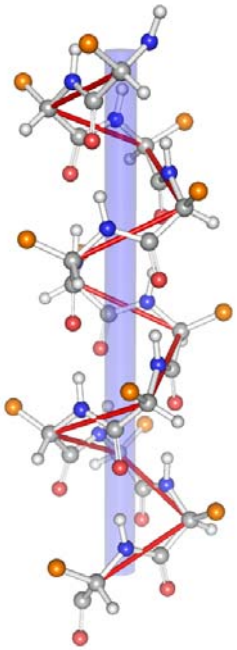

The α helix

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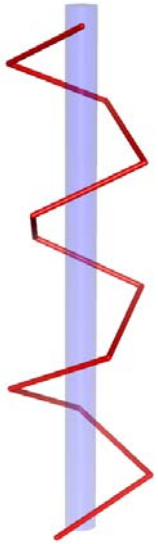
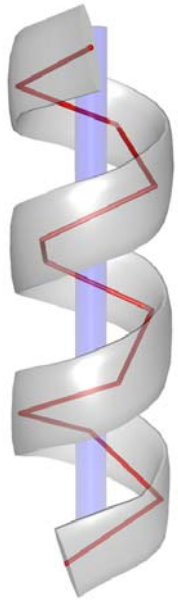


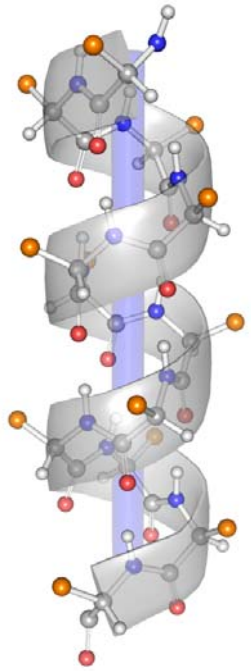
The α helix

- A smooth interpolated line connecting the alpha carbons results in a ribbon diagram:



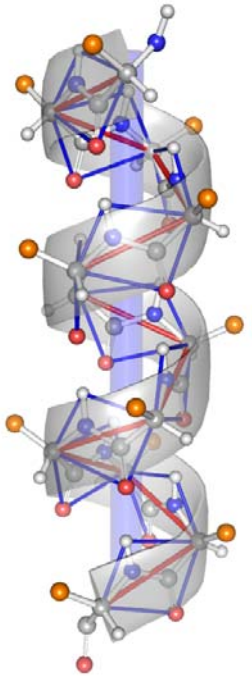
The α helix

- A smooth interpolated line connecting the alpha carbons results in a ribbon “cartoon” diagram:



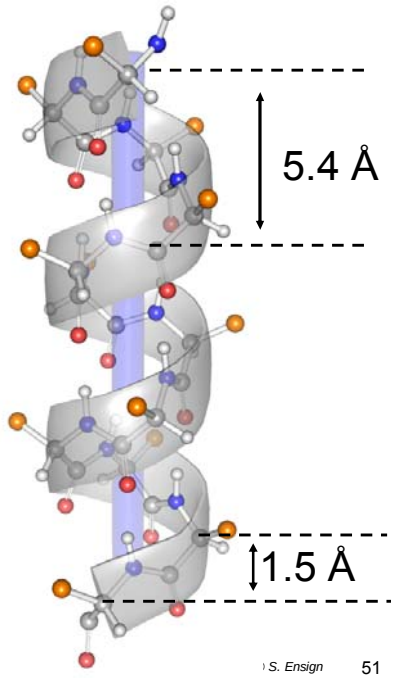
The α helix

- Peptide planes added…



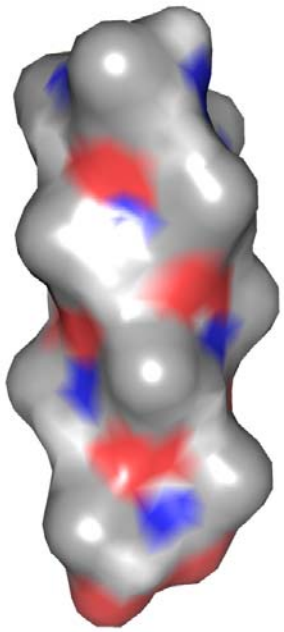
The α helix

- One turn of the helix occurs every 3.6 residues
- Helical pitch (distance per one turn) = 5.4 Å
- Helical rise (distance per residue) = 1.5 Å

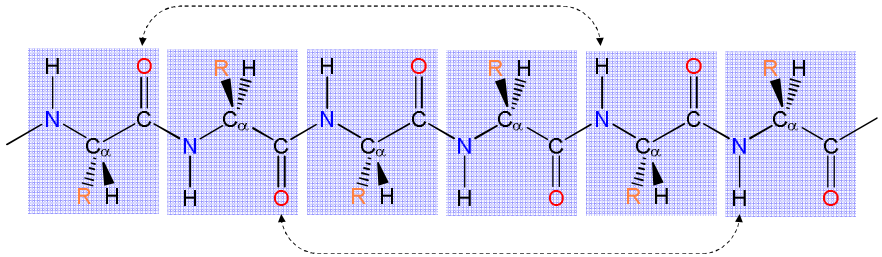


The α helix

- Helical surface (space filling)

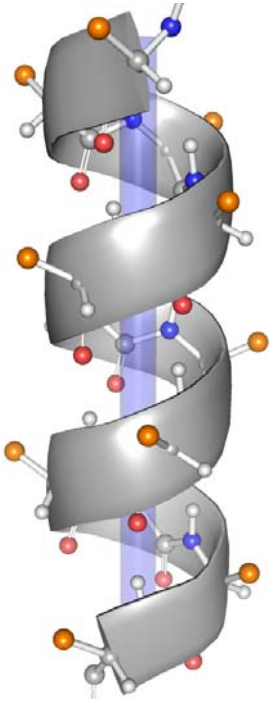


Repeating unit: a single turn of the helix (3.6 residues)

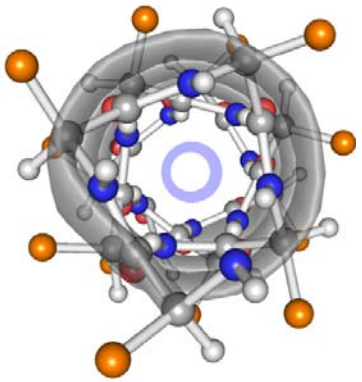


H-bonds are formed between carbonyl “O” and amide “H” four residues away (n → n+4)

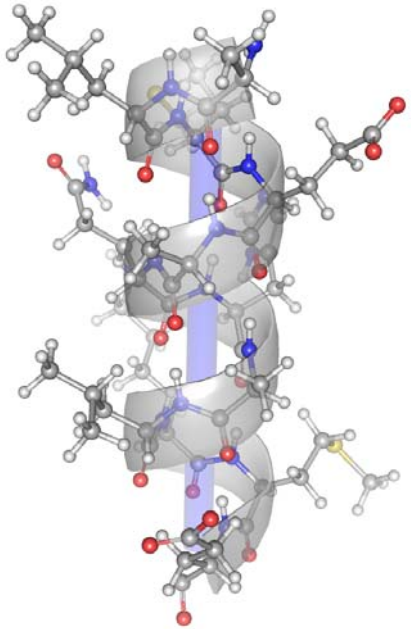
R groups protrude outward

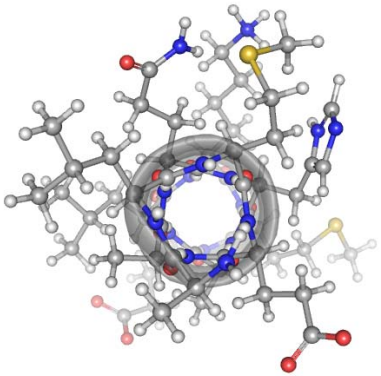


Look down the helical axis



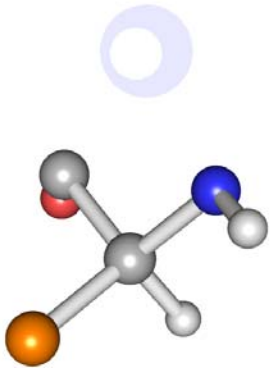
R groups protrude outward





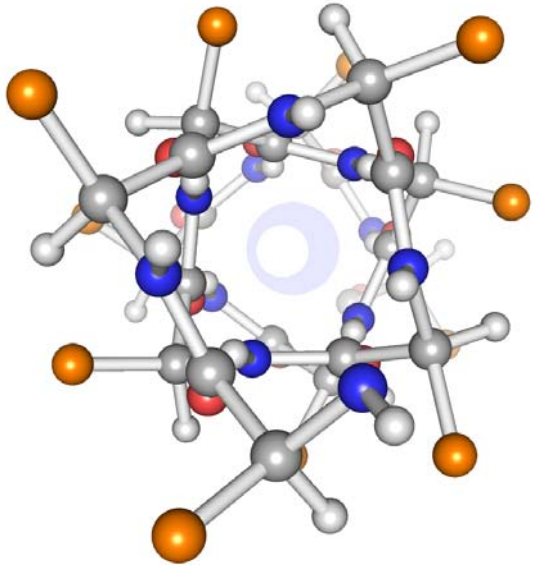
Building the alpha the helix by adding residues from the N-terminal end, looking down the helix axis

- One residues



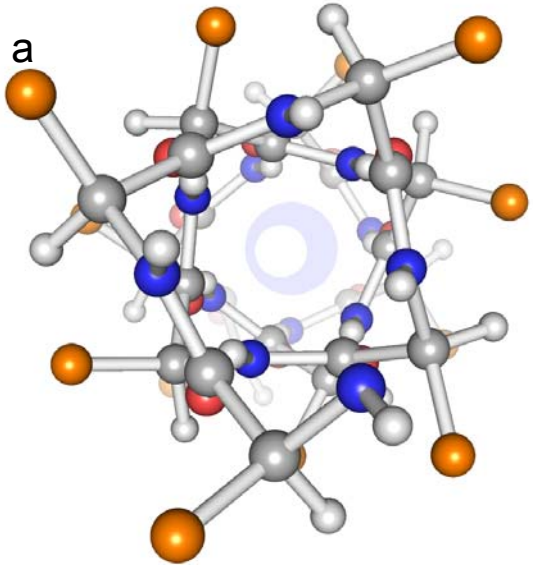
Building the alpha the helix by adding residues from the N-terminal end, looking down the helix axis

- eleven residues



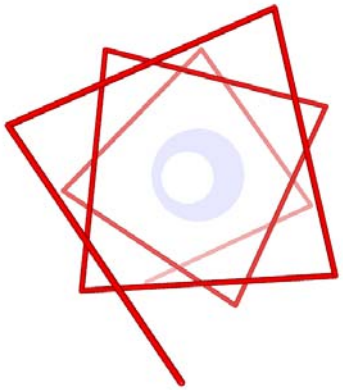
Building the alpha the helix by adding residues from the N-terminal end, looking down the helix axis

- This view is called a "helical wheel"

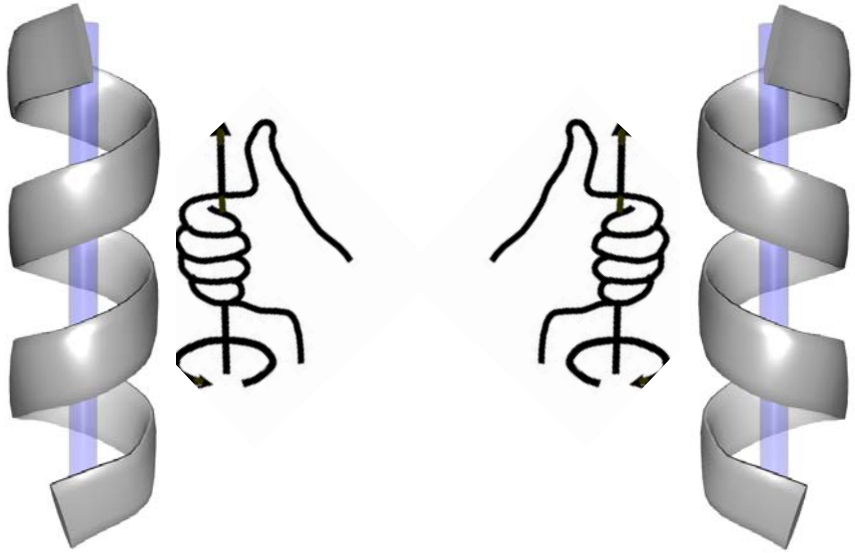


Building the alpha the helix by adding residues from the N-terminal end, looking down the helix axis

- Alpha C trace looking down the helix axis



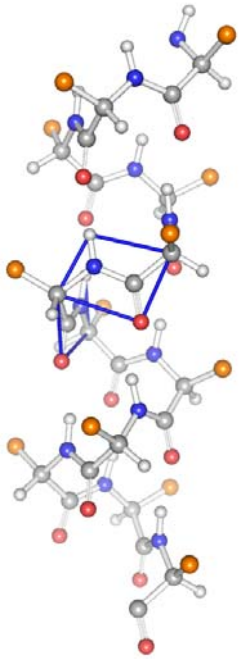
The α helix is a right-handed helix



R-handed helix

L-handed helix

The α helix torsion angles
ϕ = -60˚ , Ψ = -45 to -50˚



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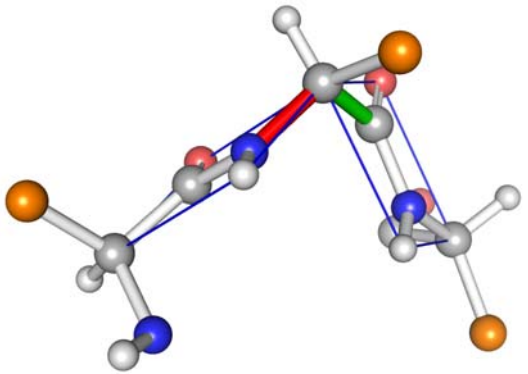
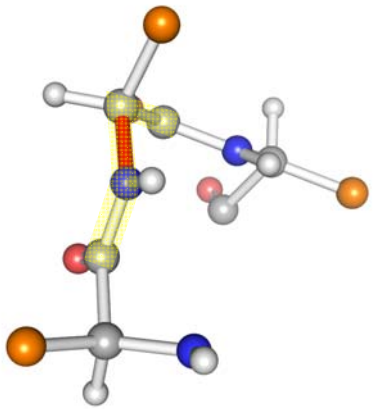


The α helix torsion angles:
ϕ = -60˚ , Ψ = -45 to -50˚

•phi (ϕ) bond: defined by the dihedral angle C-N-C_α-C

•psi (ψ) bond: defined by the dihedral angle N-C_α-C-N



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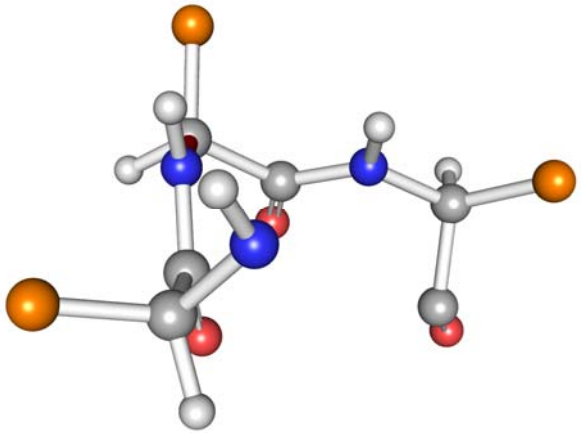

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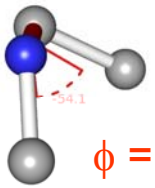
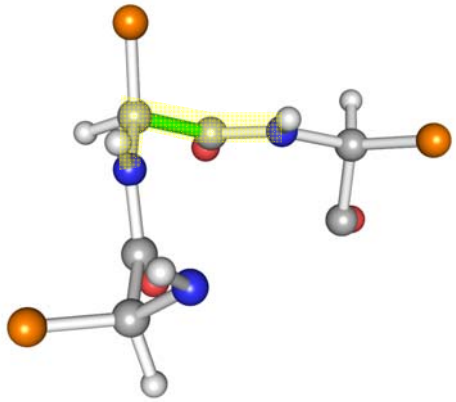


ϕ = -54.1˚

The α helix torsion angles:
ϕ = -60˚ , Ψ = -45 to -50˚

•phi (ϕ) bond: defined by the dihedral angle C-N-C_α-C

•psi (ψ) bond: defined by the dihedral angle N-C_α-C-N



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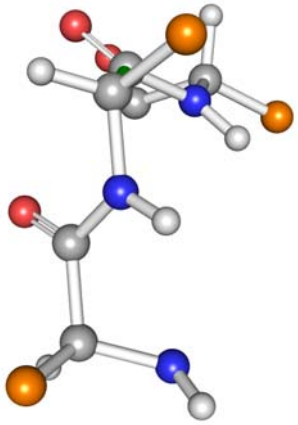

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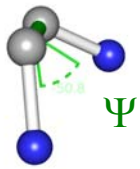
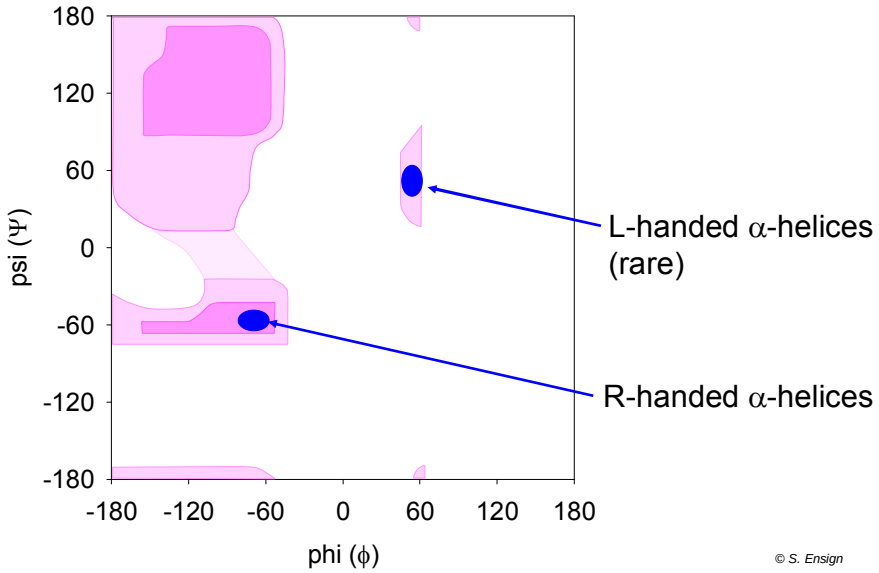


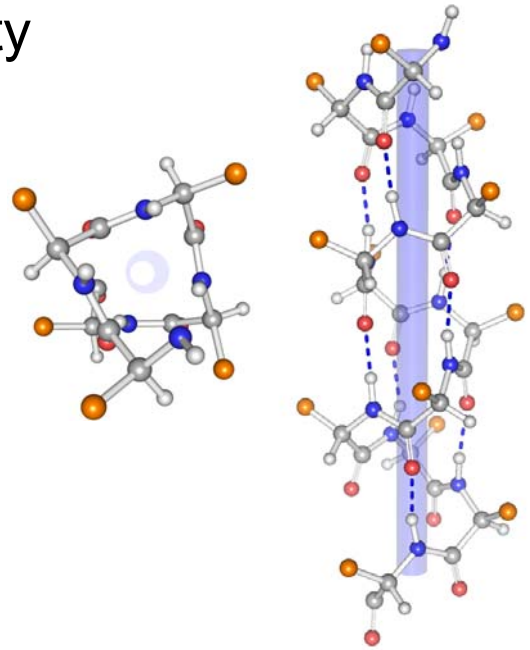
Ψ = -51.8˚

Ramachandran plot: A plot of allowed phi and psi angles for a polypeptide



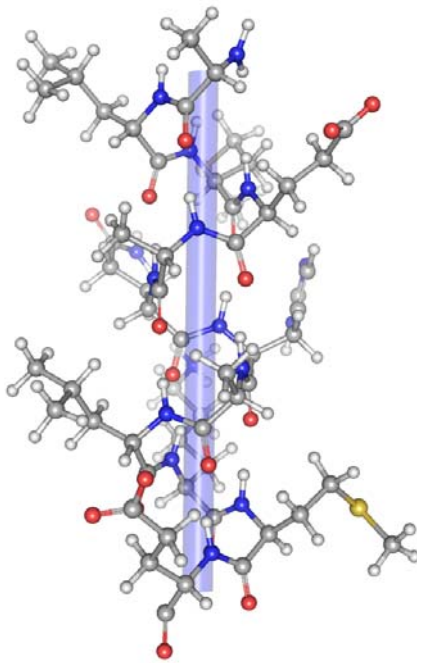
α helix stability

- Destabilized by:
  - Stetches of similar charged polar AAs (electrostatic repulsion)
  - Branched residues (recall residues oriented outward looking down axis)
  - Pro residues- cause the helix to kink
- Stabilized by:
  - Linear (extended) residues (recall residues oriented outward looking down axis)
  - Favorable interactions between amino acid side groups 3-4 residues apart
  - Negative AAs at N-terminal end, positive AAs at C-terminal end



α helix stability

•This helix sequence is ALMEAQHALKME
•Note how many extended side chains are present



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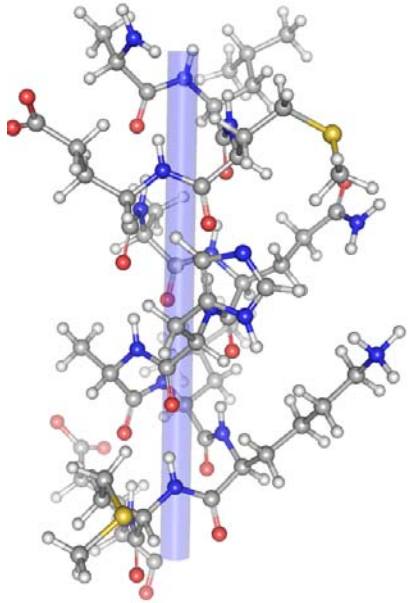
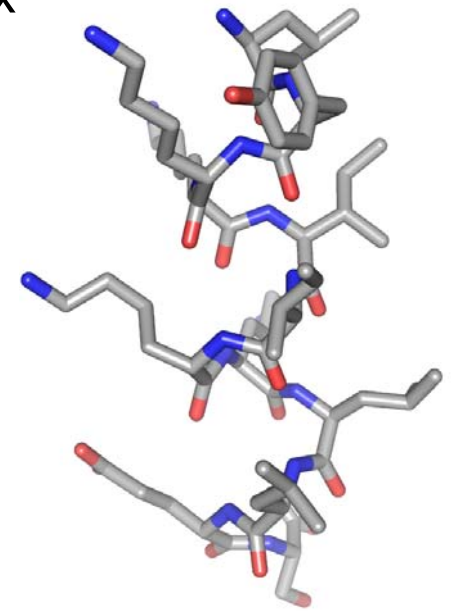


Amphipathic α helices

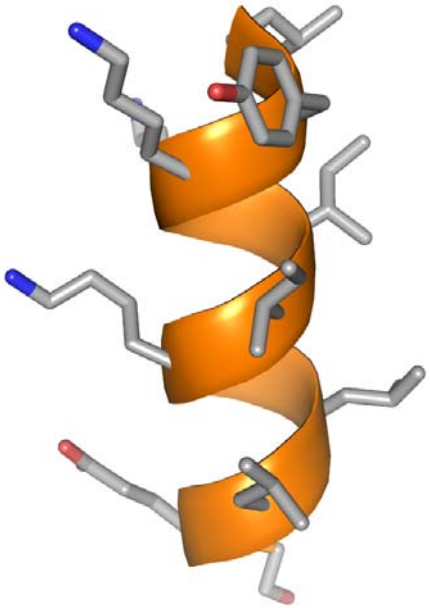
•Amphipathic: having both nonpolar and polar components (a molecule that is both hydrophobic and hydrophilic)
•An amphipathic alpha helix contains polar residues on one side and nonpolar residues on the other side
•The polar side will be exposed to water, and the nonpolar side will be buried within a protein so it interacts with other nonpolar portions

Amphipathic α helix LYKKIIKKLLES



Residues 59 to 70 from human platelet factor 4, pdb 1F9Q

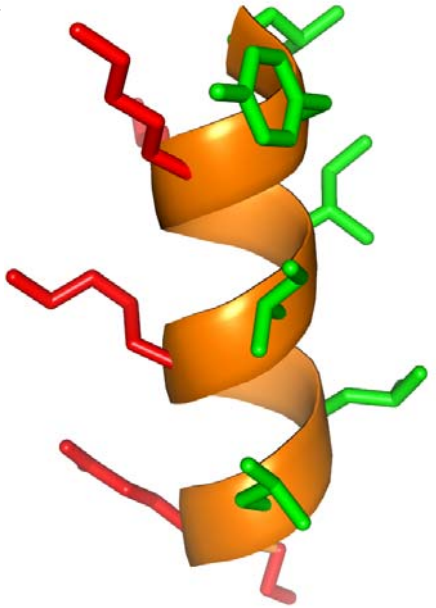
Amphipathic α helix LYKKIIKKLLES



Residues 59 to 70 from human platelet factor 4, pdb 1F9Q

Amphipathic α helix LYKKIIKKLLES

- Hydrophobic side chains in green
- Hydrophilic side chains in red



Residues 59 to 70 from human platelet factor 4, pdb 1F9Q

Amphipathic α helix LYKKIIKKLLES

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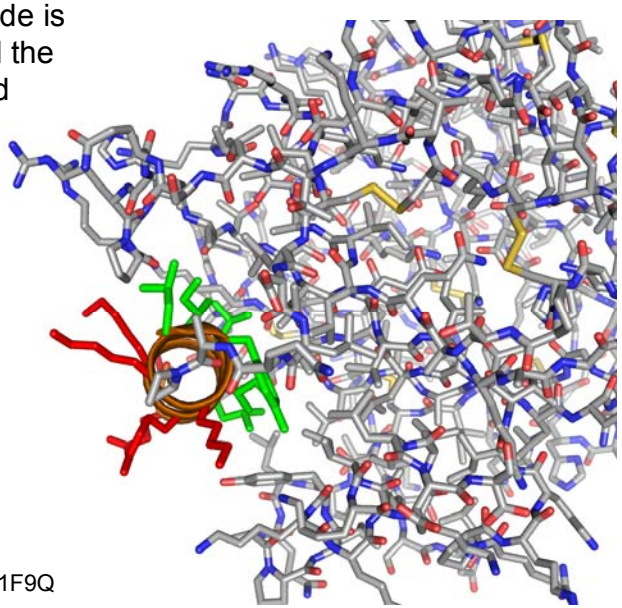
Residues 59 to 70 from human platelet factor 4, pdb 1F9Q

Amphipathic α helix LYKKIIKKLLES

•Note how the polar side is exposed to water, and the nonpolar side is buried within the protein so it interacts with other nonpolar portions

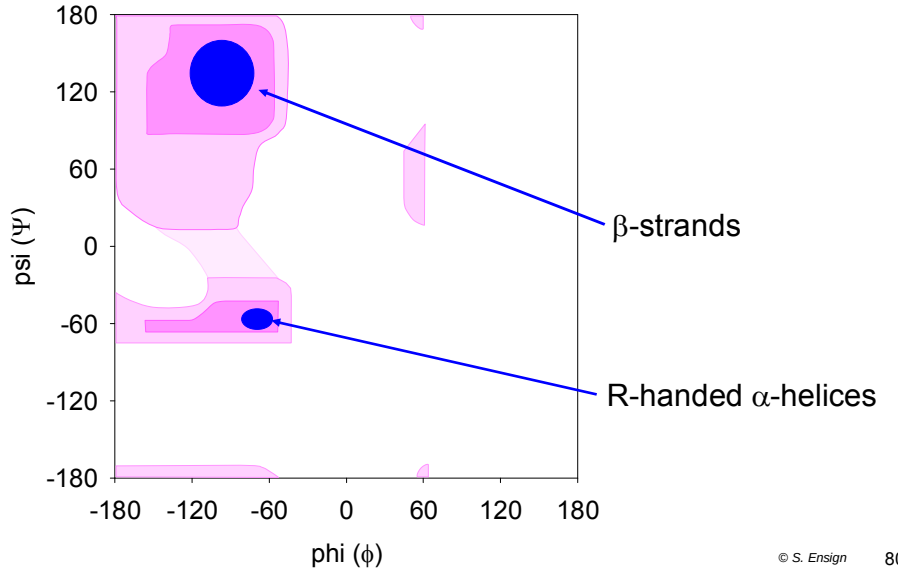


human platelet factor 4, pdb 1F9Q

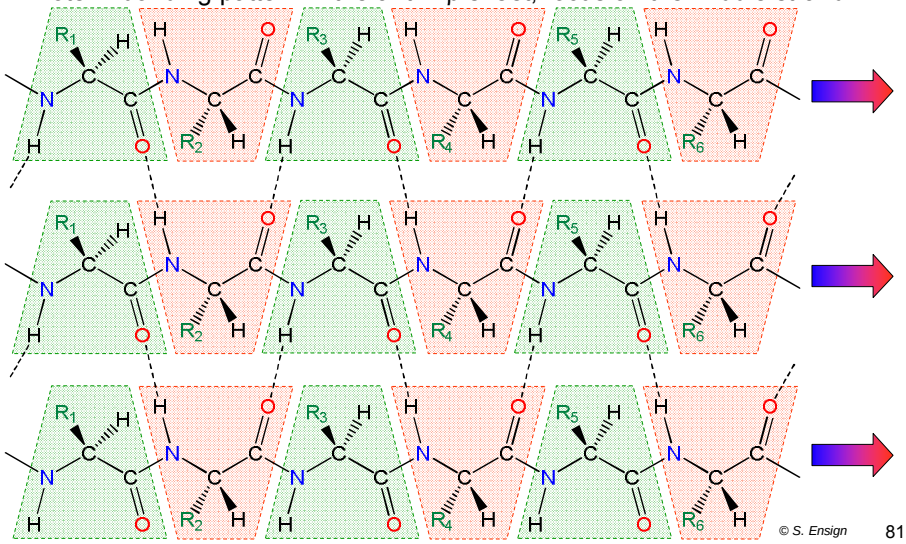
The β sheet

- More extended polypeptide conformation
- Ψ ~ +140˚ ϕ ~ -135˚
- Polypeptide backbone: zig-zag
- H-bonds: intra or interchain
  - Carbonyl O ---- amino H
- R groups of adjacent AAs: protrude outward in opposite directions
- "pleated"
- Antiparallel or parallel strands
- R groups at contact surfaces must be fairly small
- 5-10 AA residues per β strand
- Multiple "strands" associate to form the "sheet"

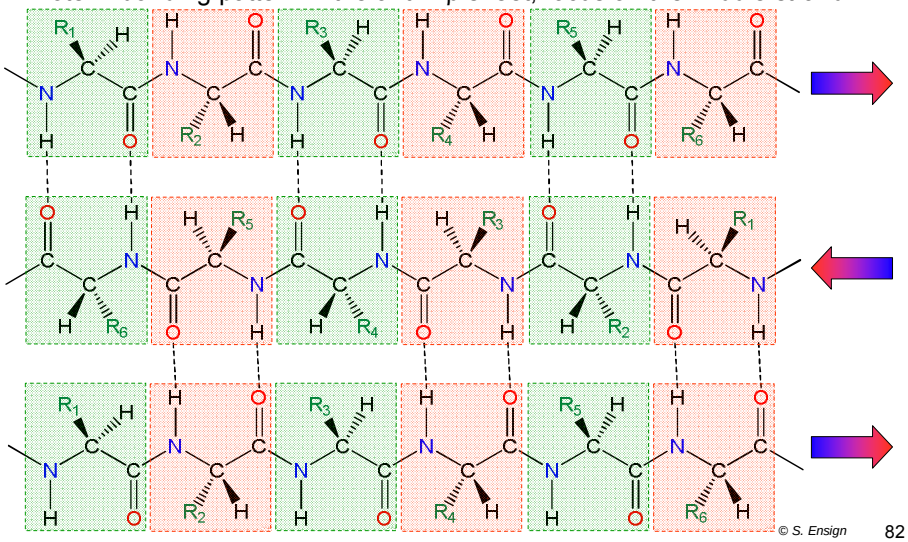
Ramachandran plot: A plot of allowed phi and psi angles for a polypeptide



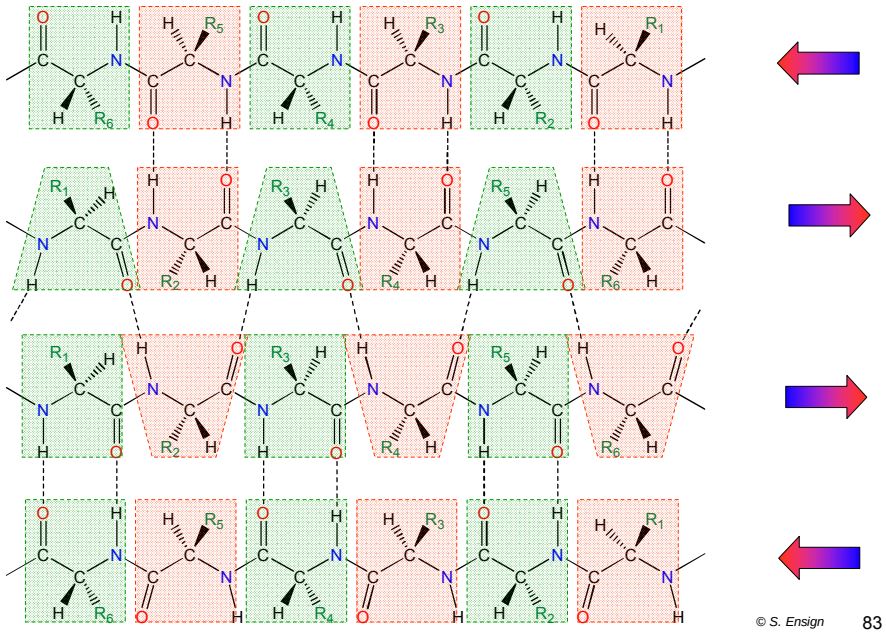
- Parallel β sheet: Strands run in the same direction (N→C and N→ C)
- Strands can be on same or different polypeptide chains (subunits)
- Note “wedge shaped” units created by H-bonds between individual strands
- Note H-bonding pattern in the anti II β sheet, focus on the middle strand



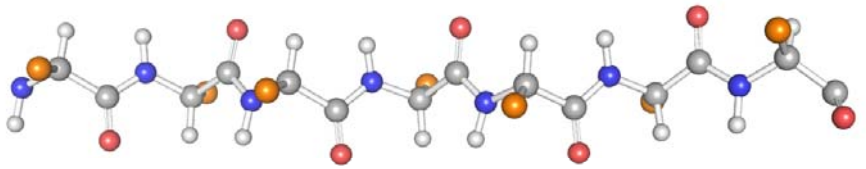
- Antiparallel β sheet: Strands run in opposite directions (N→C and C→ C)
- Strands can be on same or different polypeptide chains (subunits)
- Note “square shaped” units created by H-bonds between individual strands
- Note H-bonding pattern in the anti II β sheet, focus on the middle strand



A β sheet formed from a combination of antiparallel and parallel strands



Build an “ideal” anti II β sheet from 2 strands



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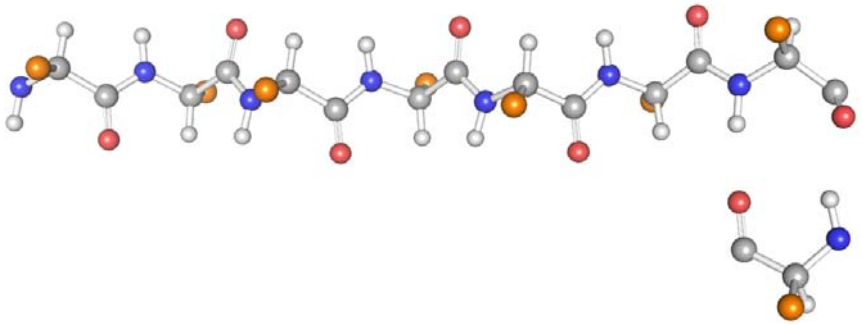
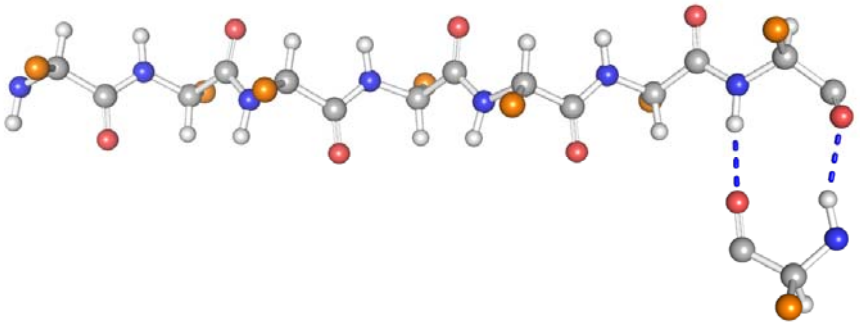
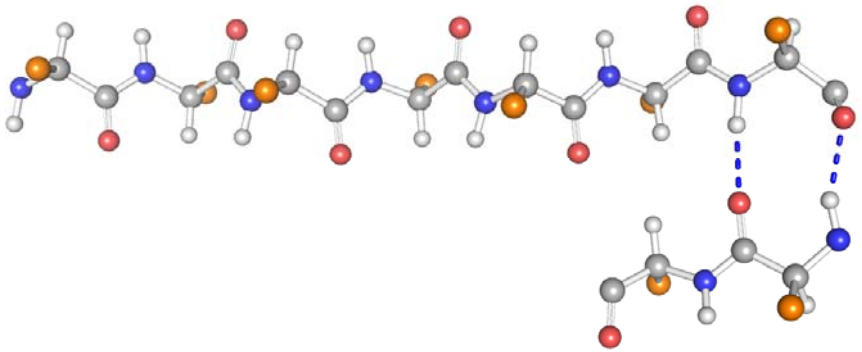


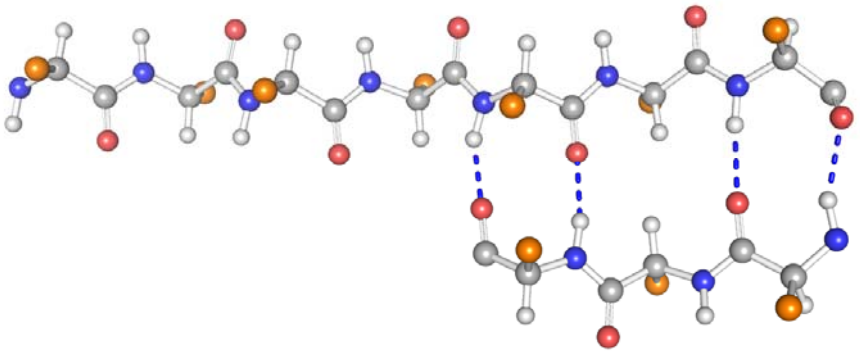
Build an “ideal” anti II β sheet from 2 strands



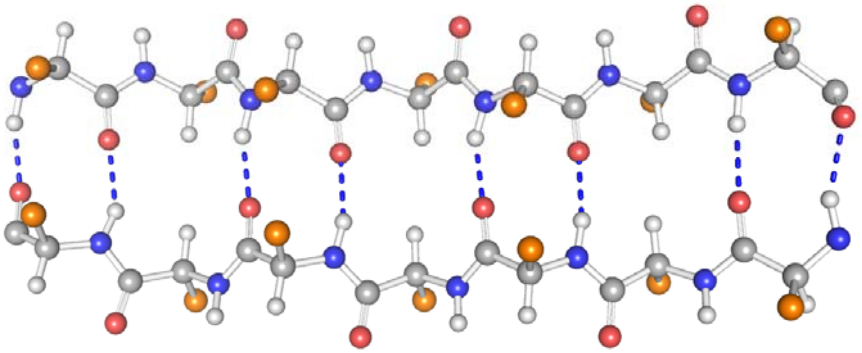
Build an “ideal” anti II β sheet from 2 strands



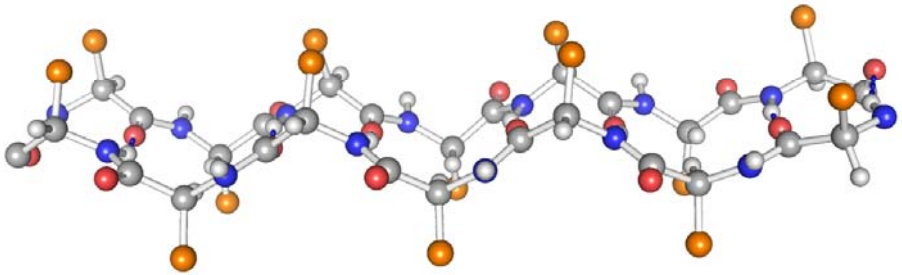
Build an “ideal” anti II β sheet from 2 strands



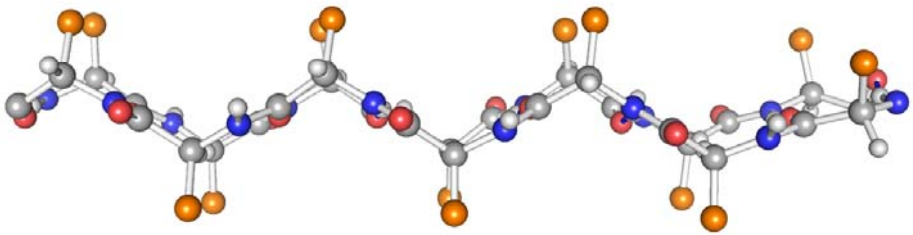
Build an “ideal” anti II β sheet from 2 strands



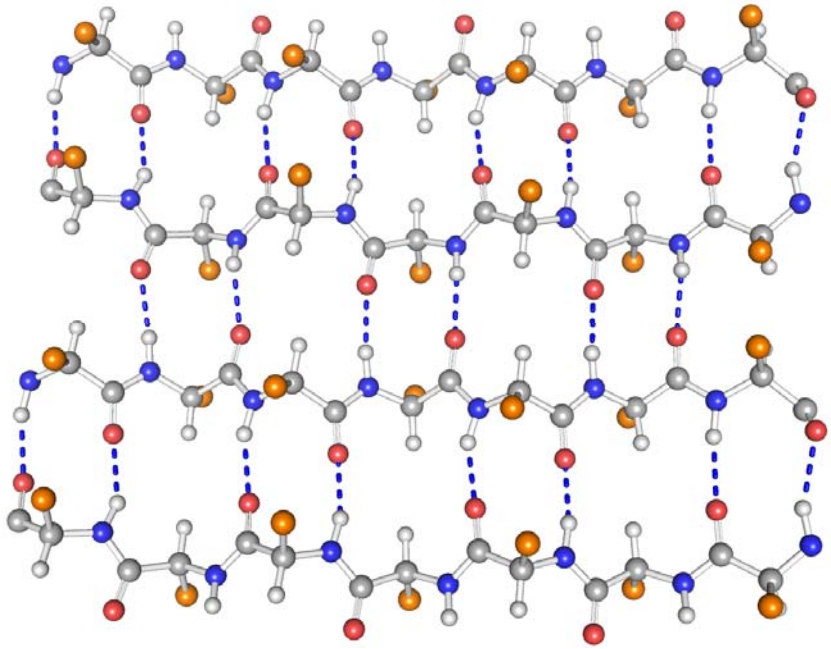
Build an “ideal” anti II β sheet from 2 strands



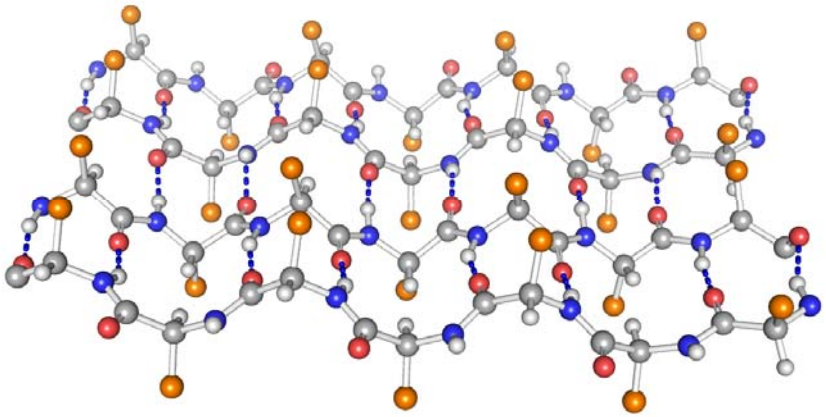
Build an “ideal” anti II β sheet from 2 strands



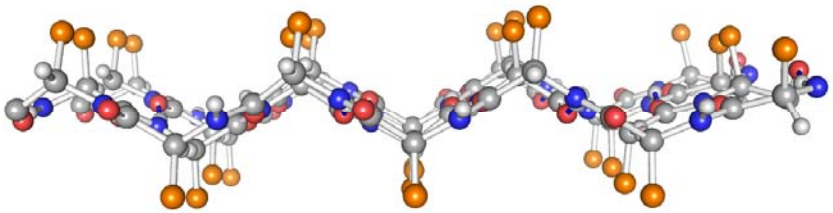
Add a fourth anti II strand……..



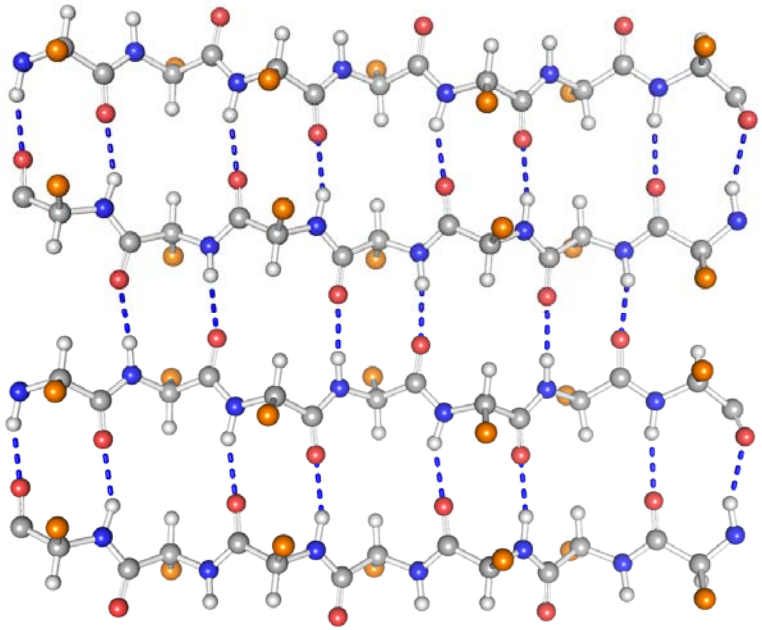
A four strand anti II β sheet



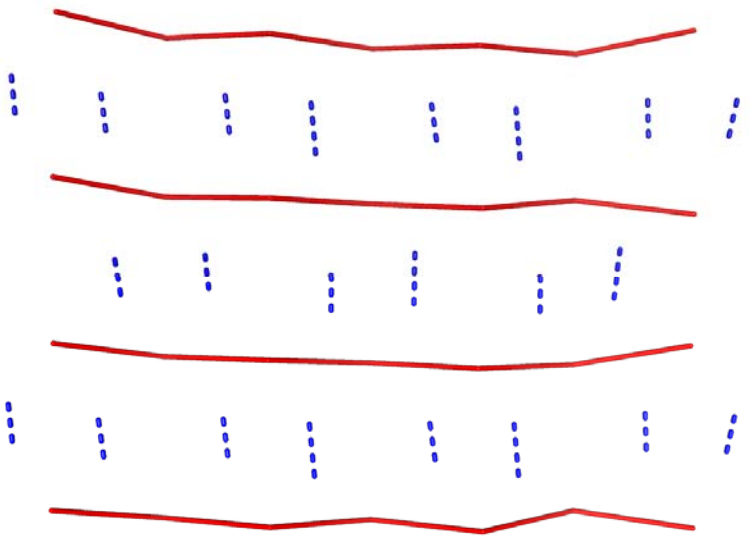
A four strand anti II β sheet



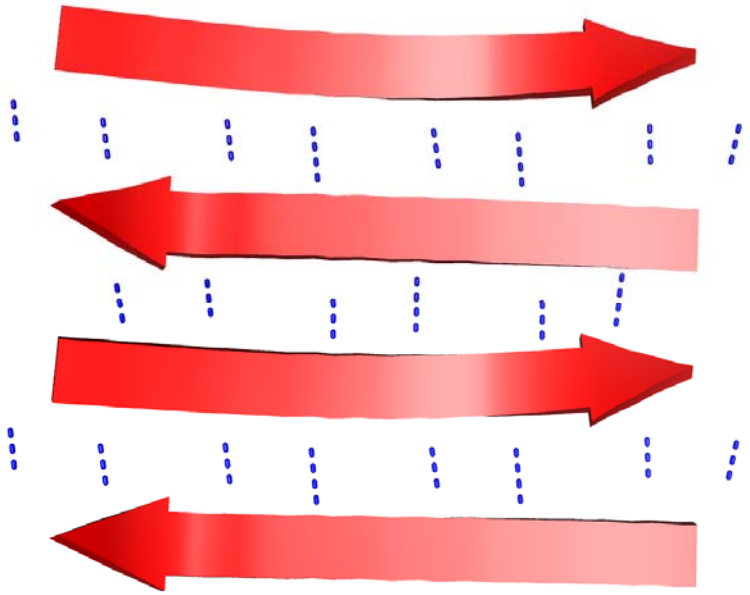
A four strand anti II β sheet

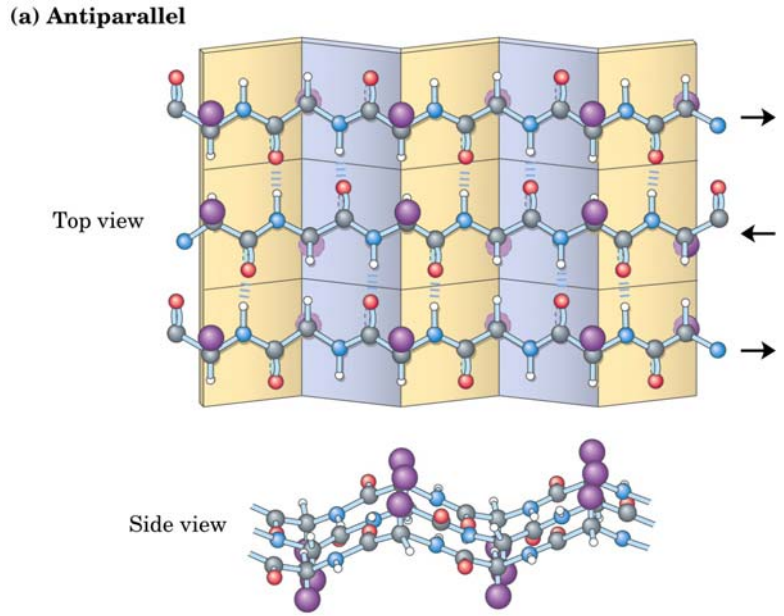


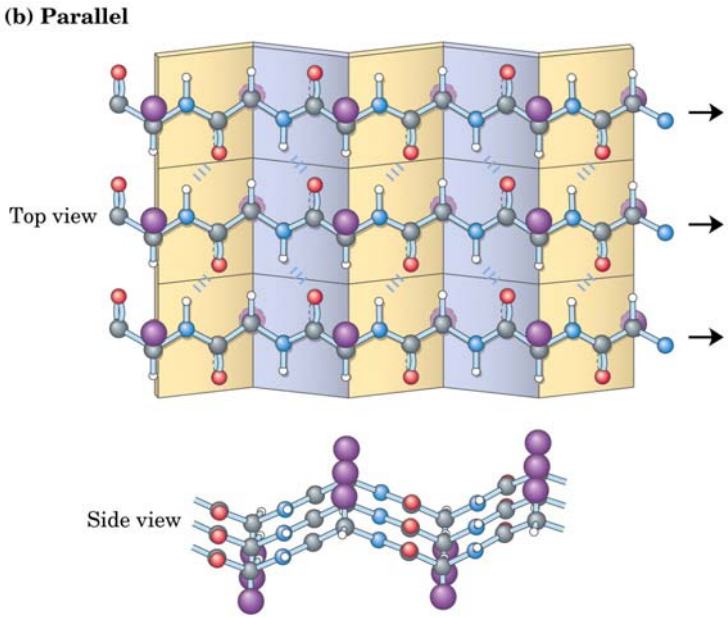
Alpha carbon trace of strands in the anti II β sheet



"Cartoon" representation of strands in the anti II β sheet



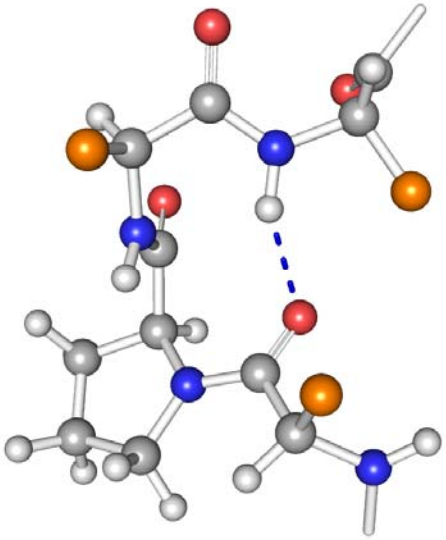




Secondary structural elements

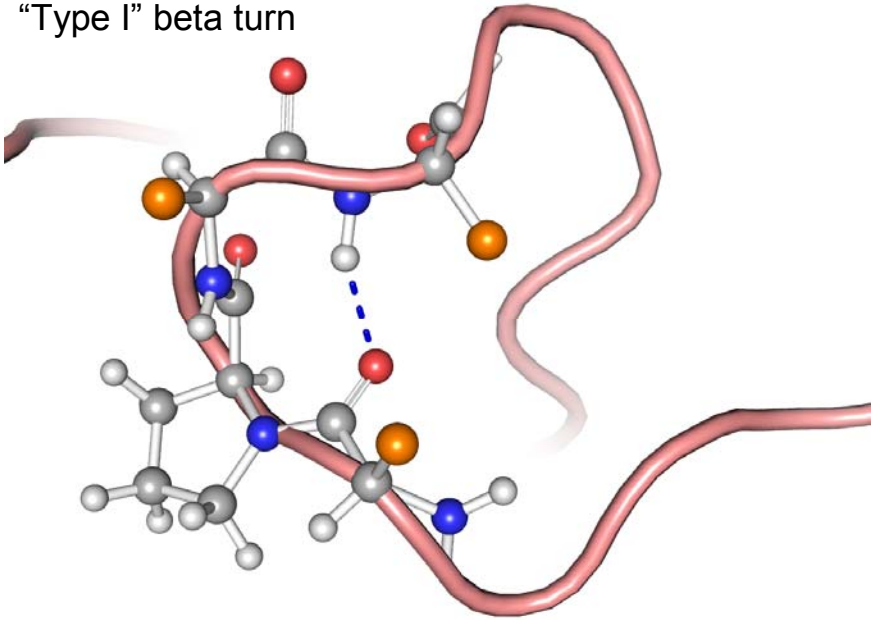
1. α-helix
2. β-pleated sheet
3. β-bend: polypeptide reverses direction
   Tight turn, 4AAs
   Carbonyl O of residue R_1 H-bonds NH of residue R_4
   Gly and Pro common here (often cis-Pro)
4. Collagen helix
5. "random coil": structures that are neither random nor coiled, but cannot be classified in one of the 4 defined elements

"Type I" beta turn



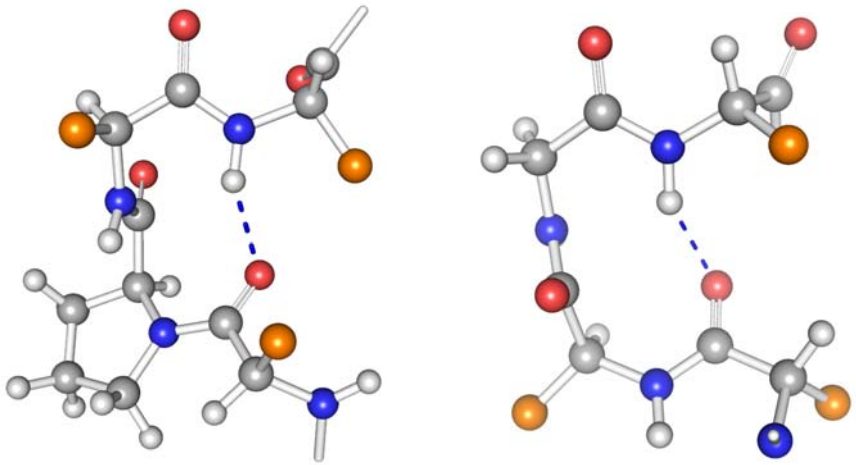
Residues 159-162 (SPCS) of carboxypeptidase A, pdb 5CPA

"Type I" beta turn



Residues 159-162 (SPCS) of carboxypeptidase A, pdb 5CPA

Examples of "Type I" and "Type II" beta turns



Residues 159-162 and 89-92 of carboxypeptidase A, pdb 5CPA

Secondary structural elements

1. α-helix
2. β-pleated sheet
3. β-bend: polypeptide reverses direction

   Tight turn, 4AAs

   Carbonyl O of residue R_1 H-bonds NH of residue R_4

   Gly and Pro common here (cis-Pro)
4. Collagen helix
5. "random coil": structures that are neither random nor coiled, but cannot be classified in one of the 4 defined elements

Proteins vary in secondary structure

Protein	% α-helix	% β-sheet
Myoglobin	78	None
Cytochrome C	39	None
Lysozyme	40	12
Ribonuclease	26	35
Chymotrypsin	14	45

Major protein classes

Globular: almost all soluble and membrane-associated proteins and enzymes

-e.g. myoglobin, hemoglobin

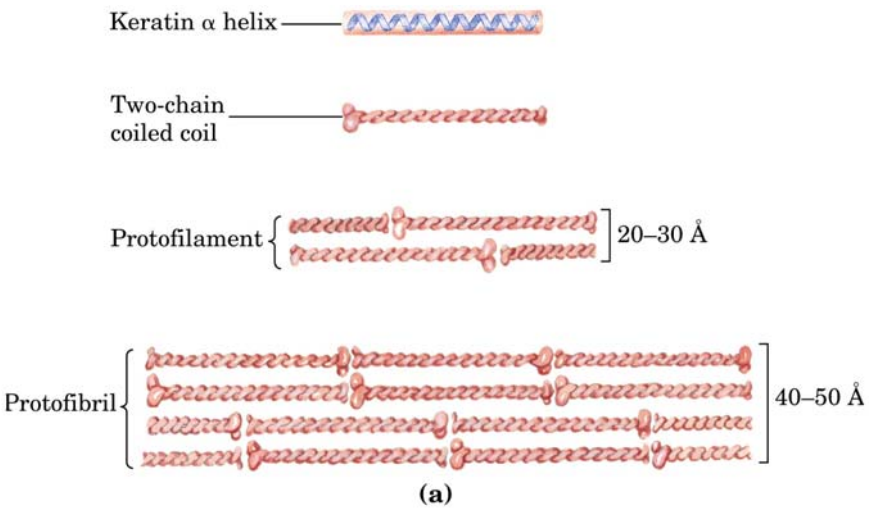
-several 2° structural elements

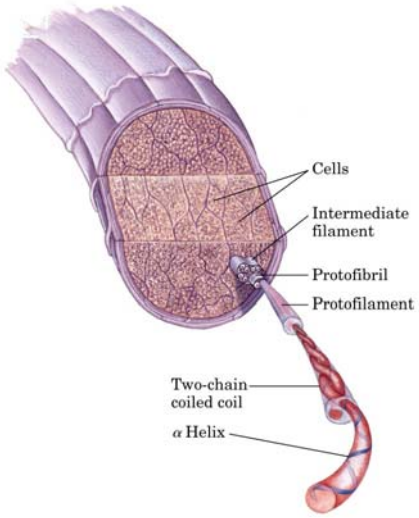
Fibrous: arrays of polypeptides associated in long strands or sheets (1 type of element)

-α-keratin: α-helices

-silk: β-pleated sheets

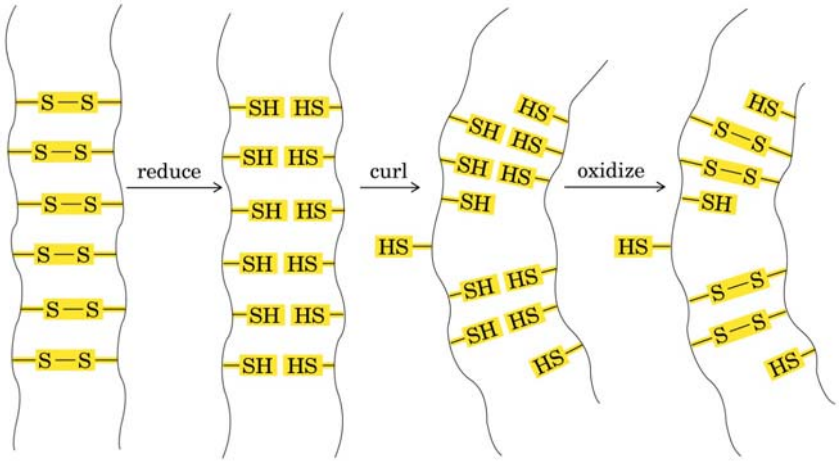
-collagen: triple helix





Cross section of a hair
(b)

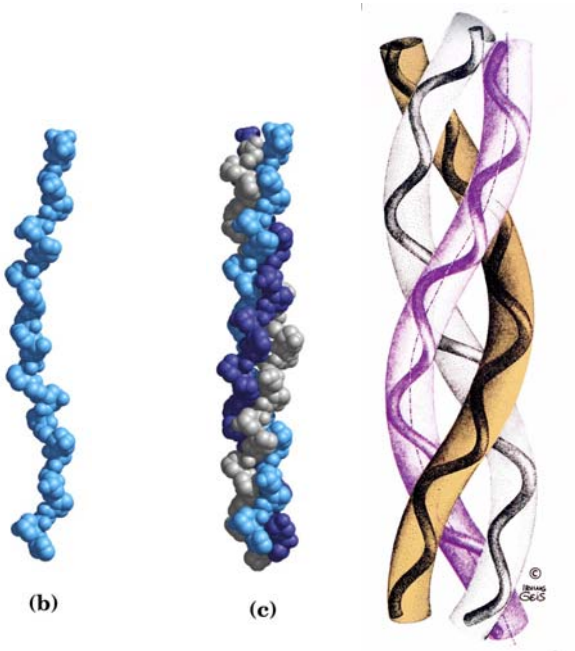
α-keratin is strengthened by interstrand disulfide bonds

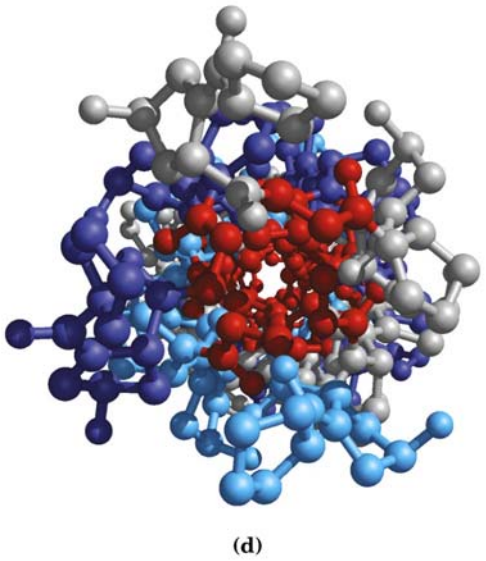


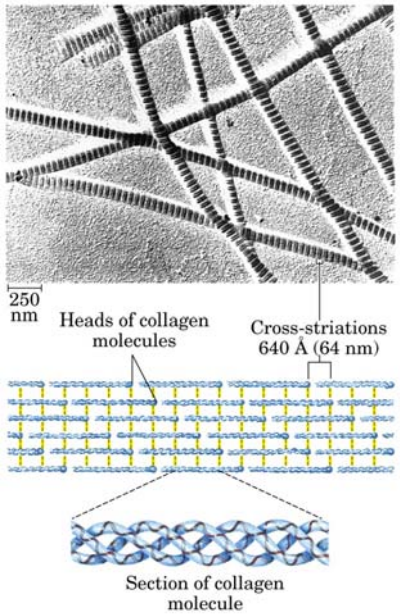
What is the biochemical basis for a hair perm?

Collagen

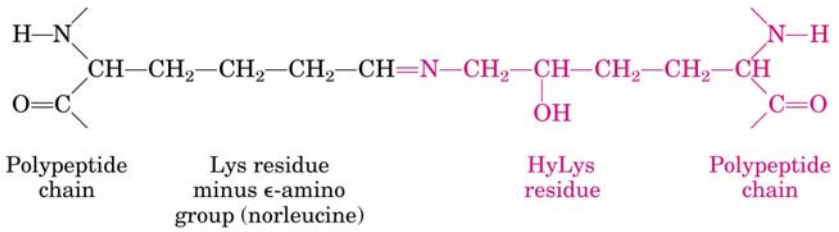
- The most abundant protein in mammals (skin, tendons, bone, cartilage)
- Water insoluble
- Extracellular
- L-handed helix, 3 AA residues/turn
- Contains ~33% glycine, modified AAs (4-hydroxyProline, 5-hydroxyLysine)
- Tropocollagen: triple-stranded collagen fiber
- Pro, OH-Pro, OH-Lys stick out. Gly sticks into core
- Interstrand covalent crosslinks







Crosslinking in collagen



Dehydrohydroxylysinonorleucine