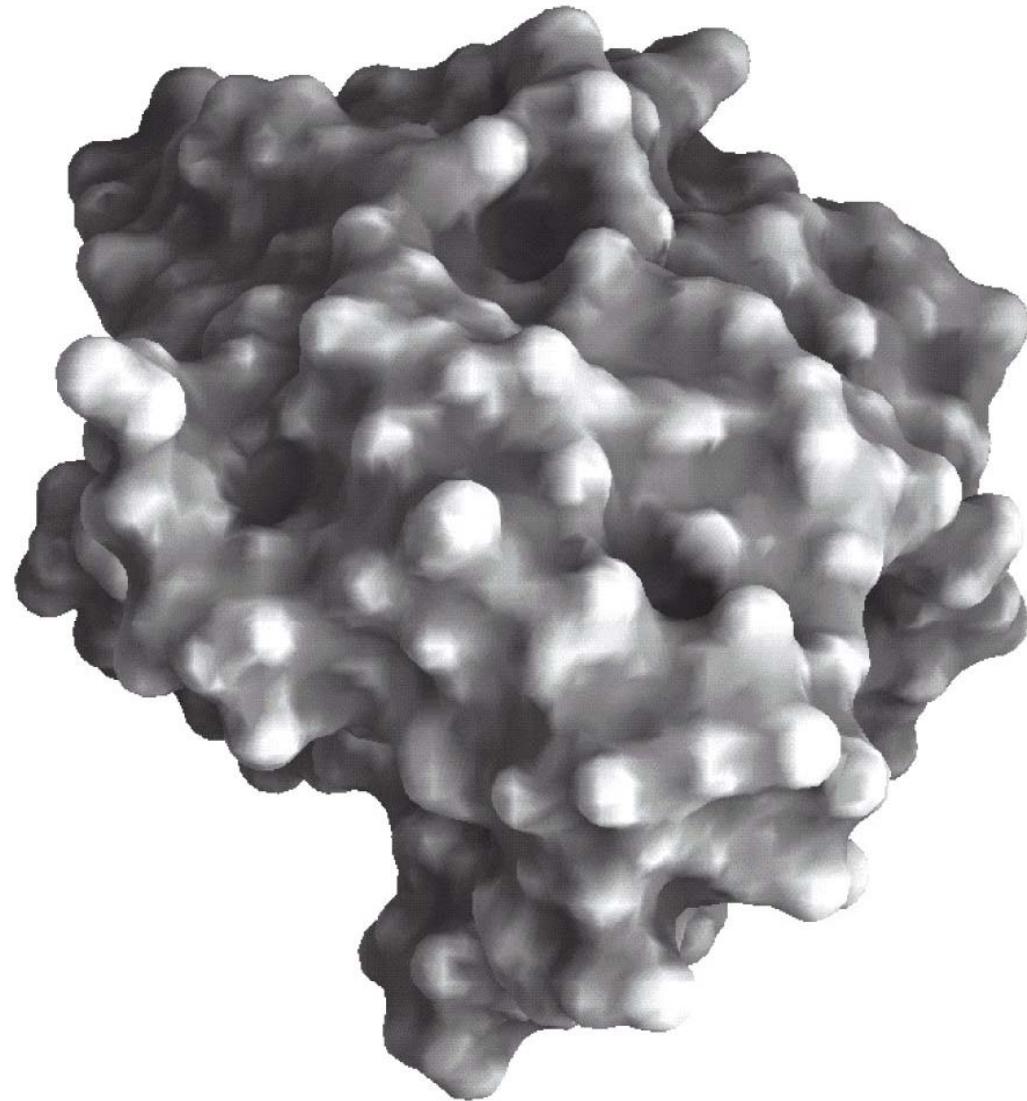


CHAPTER 4

THE THREE- DIMENSIONAL STRUCTURE OF PROTEINS

Chymotrypsin



Glycine

Four Levels of Protein Structure

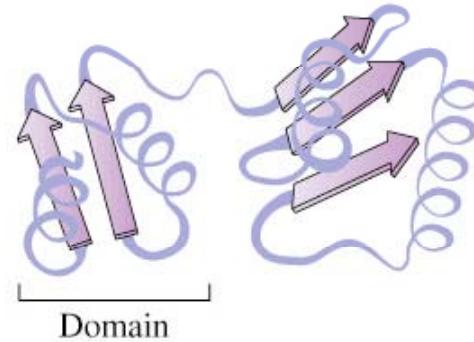
- **Primary structure** - amino acid linear sequence
- **Secondary structure** - regions of regularly repeating conformations of the peptide chain, such as α -helices and β -sheets
- **Tertiary structure** - describes the overall three-dimensional arrangement of all atoms in a protein and the shape of the fully folded polypeptide chain
- **Quaternary structure** - arrangement of two or more polypeptide chains, which may be identical or different, into multisubunit molecule

Four Levels of Protein Structure

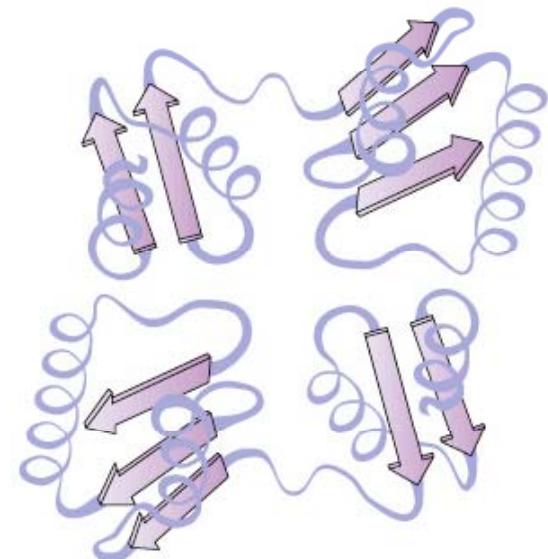
(a) Primary structure

- Ala - Glu - Val - Thr - Asp - Pro - Gly -

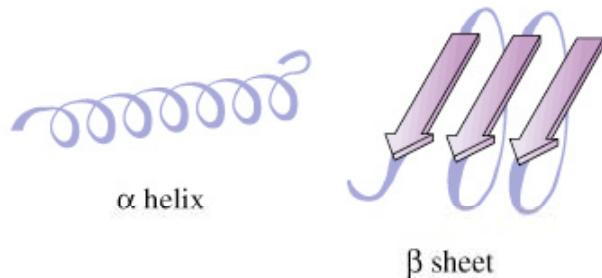
(c) Tertiary structure



(d) Quaternary structure



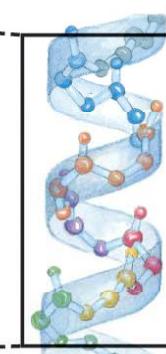
(b) Secondary structure



Primary structure

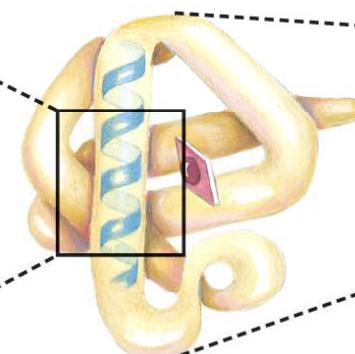
Lys
Lys
Gly
Gly
Leu
Val
Ala
His

Secondary structure



Amino acid residues

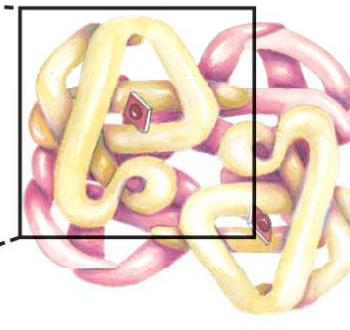
Tertiary structure



α Helix

Polypeptide chain

Quaternary structure



Assembled subunits

Protein: Three dimensional structure and function

- **Conformation** - three dimensional shape
- **Native conformation** - each protein folds into a single stable shape (physiological conditions)
- **Biological function** of a protein depends completely on its **native conformation**
- Proteins in any of their **functional, folded conformations** are called **native proteins**
- A protein may be a single polypeptide chain or composed of several chains

Stabilizing protein native conformations

- **Disulfide bonds and the weak (noncovalent) interactions**
including hydrogen bonds and hydrophobic and ionic interactions stabilize the protein conformations
- **The environment within most cells is highly reducing and thus precludes the formation of disulfide bonds.** In eukaryotes, disulfide bonds are found primarily in secreted, extracellular proteins. Exception: thermophilic bacteria, archaea
- Among those weak interactions, **hydrophobic interactions** generally predominate on the contribution to protein stability; **the interior of a protein is generally a densely packed core of hydrophobic amino acid side chains**; hydrophobic residues are largely buried in the protein interior, away from water; the **number of hydrogen bonds and ionic interactions within the protein is maximized.**

Protein secondary structure

- Describing the local spatial arrangement of its main-chain atoms, without regard to the conformation of its side chains (In fact, R group may affect stability of the α helix)
- A regular secondary structure occurs when each dihedral angle, ϕ and ψ , remains the same or nearly the same throughout the segment
 - α -helix
 - β -sheet (β -conformation)
 - β -turn (β -bend)
 - Random coil (undefined)

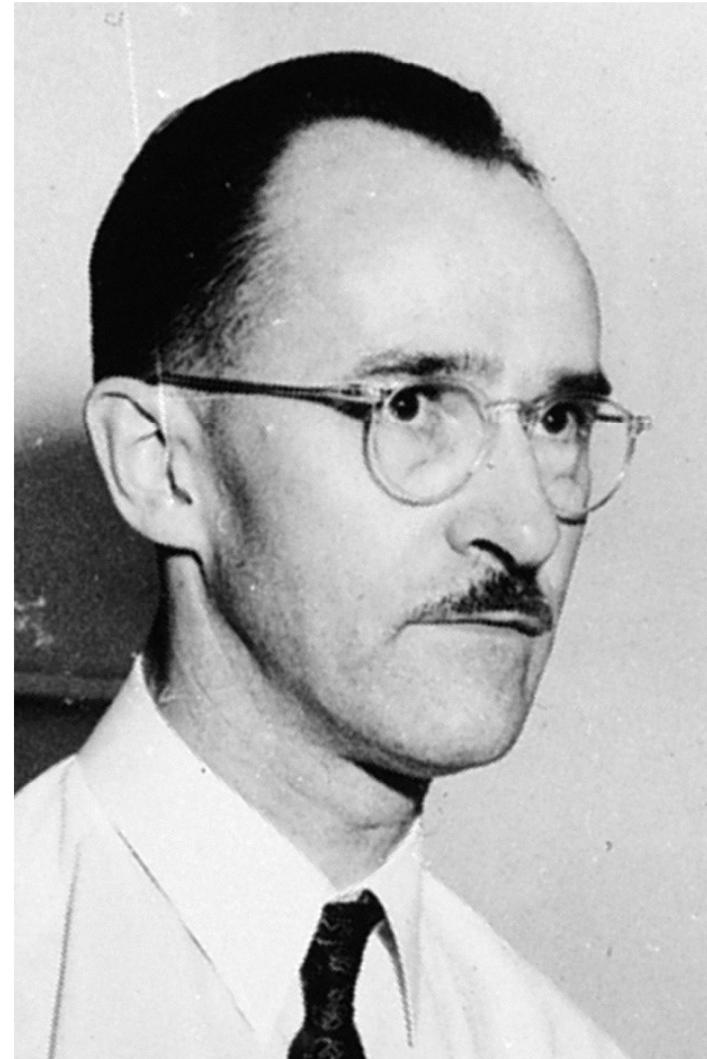
Study on the architecture of the rigid and planar peptide group



Linus Pauling, 1901–1994



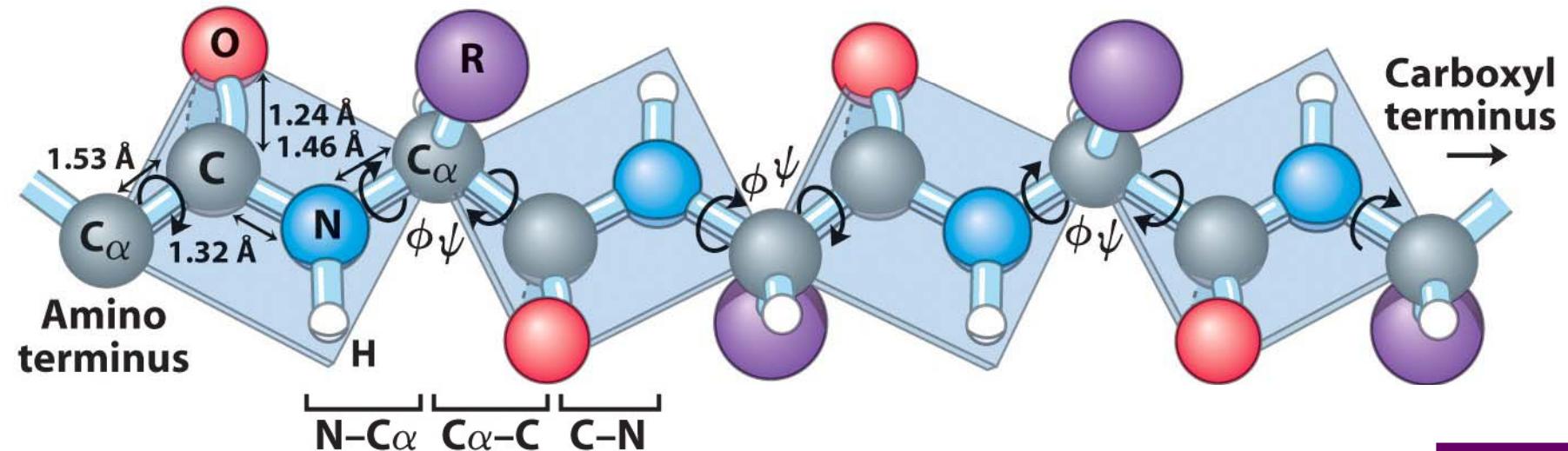
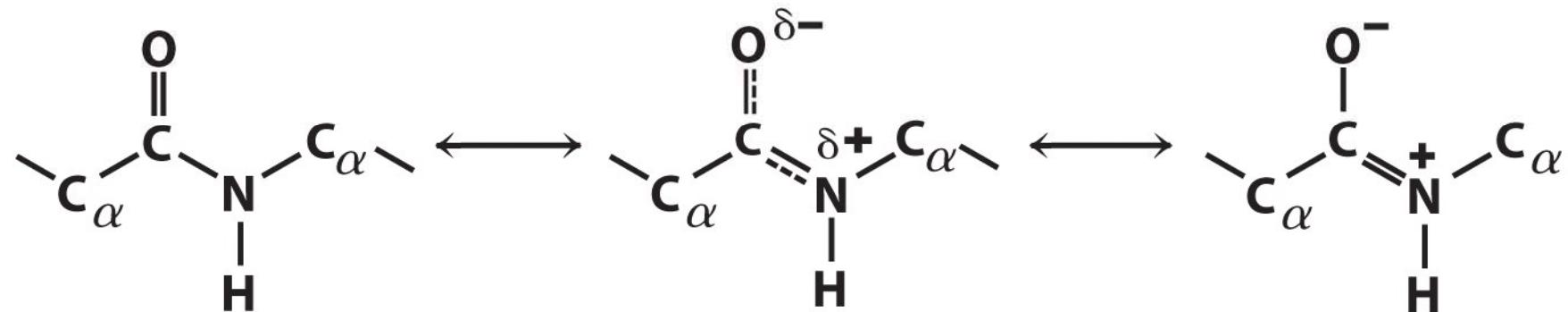
1954 & 1962



Robert Corey, 1897–1971

The planar peptide group

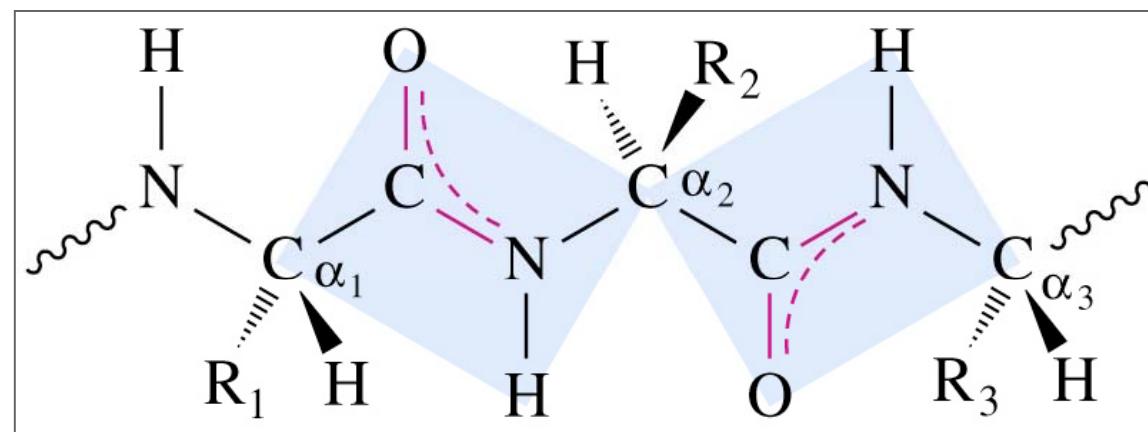
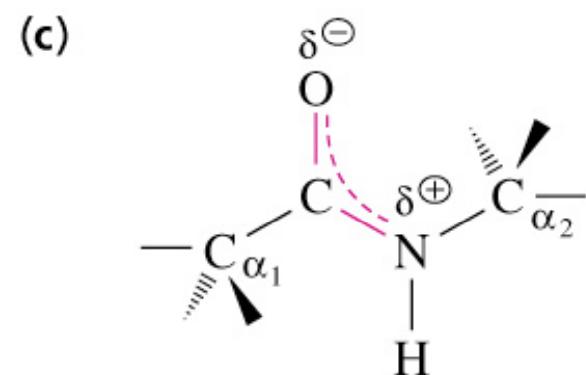
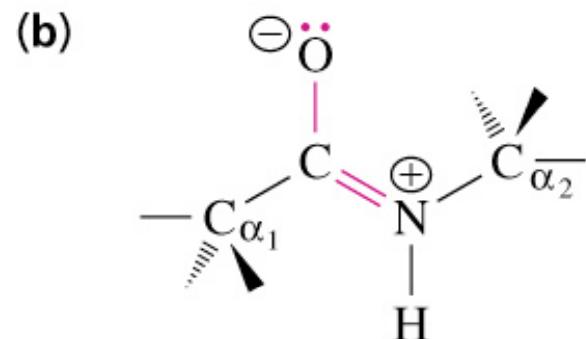
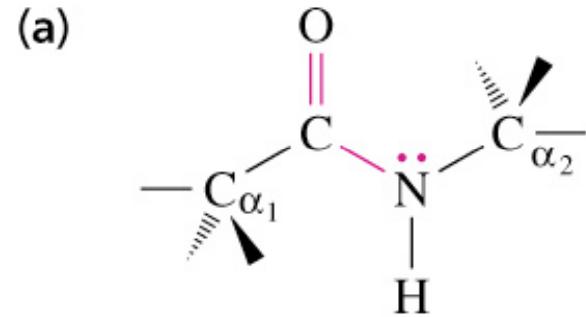
The carbonyl oxygen has a partial negative charge and the amide nitrogen a partial positive charge, setting up a small electric dipole.



ϕ : phi ψ : psi

Resonance structure of the peptide bond

- (a) Peptide bond shown as a C-N single bond
- (b) Peptide bond shown as a double bond
- (c) **Actual structure is a hybrid of the two resonance forms.**
Electrons are delocalized over three atoms: O, C, N



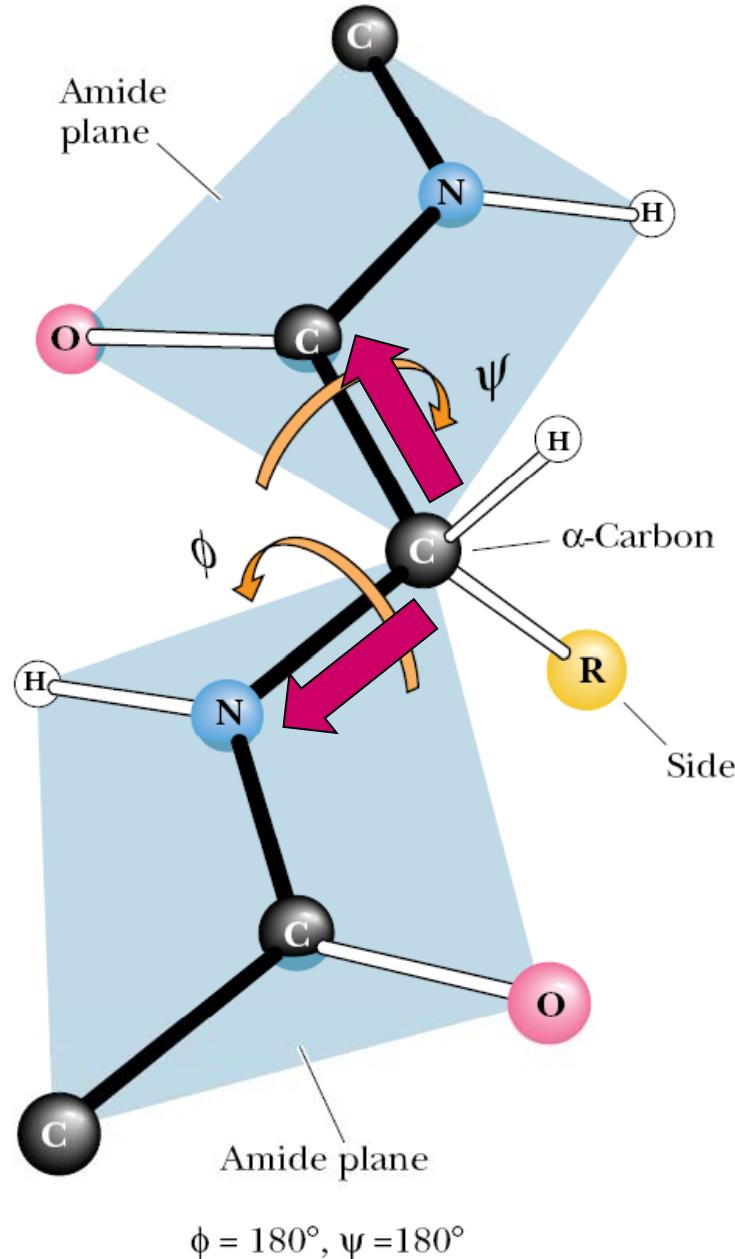


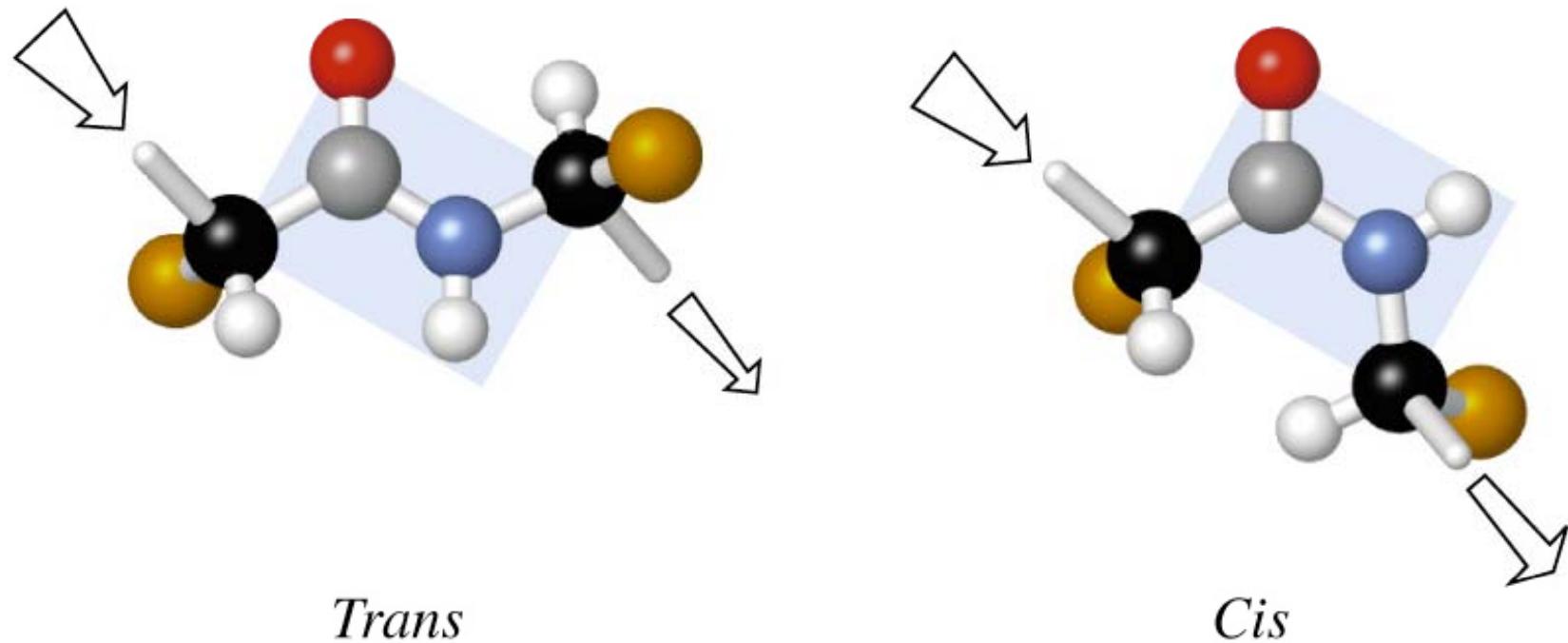
FIGURE 6.2 • The amide or peptide bond planes are joined by the tetrahedral bonds of the α -carbon. The rotation parameters are ϕ and ψ . The conformation shown corresponds to $\phi = 180^\circ$ and $\psi = 180^\circ$. Note that positive values of ϕ and ψ correspond to clockwise rotation as viewed from C_α . Starting from 0° , a rotation of 180° in the clockwise direction ($+180^\circ$) is equivalent to a rotation of 180° in the counterclockwise direction (-180°). (Irving Geis)

The Conformation of the Peptide Group

- The peptide group consists of 6 atoms
- Peptide bonds have some double bond properties so that their conformation is restricted to either *trans* or *cis*
- *Cis* conformation is less favorable than *trans* due to steric interference of α -carbon side chains
- *Cis-trans* isomerases can catalyze the interconversion of *cis* and *trans* conformations

Trans and *cis* conformations of a peptide group

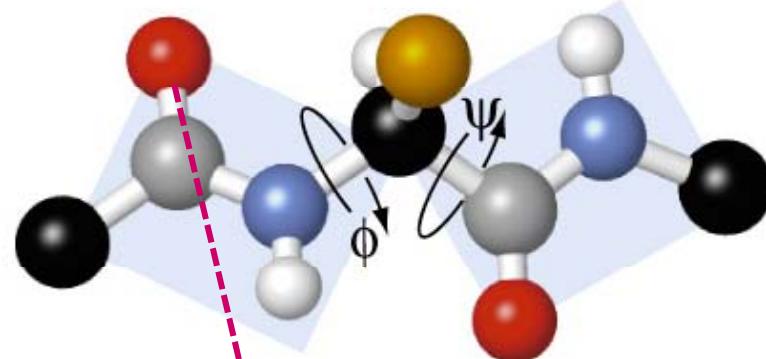
- Nearly all peptide groups in proteins are in the ***trans*** conformation



● α -carbon	○ Hydrogen	● Oxygen
● Carbonyl carbon	● Nitrogen	● Side chain

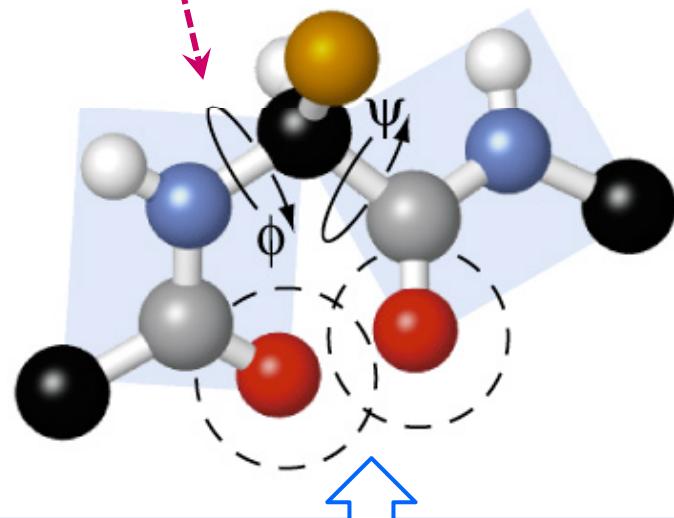
Rotation around the N-C_α and C_α-C bonds that link peptide groups

Trans



(b)

Cis



- α-carbon
- Carbonyl carbon
- Hydrogen
- Nitrogen
- Oxygen
- Side chain

Some conformations are prohibited by steric overlap of atoms

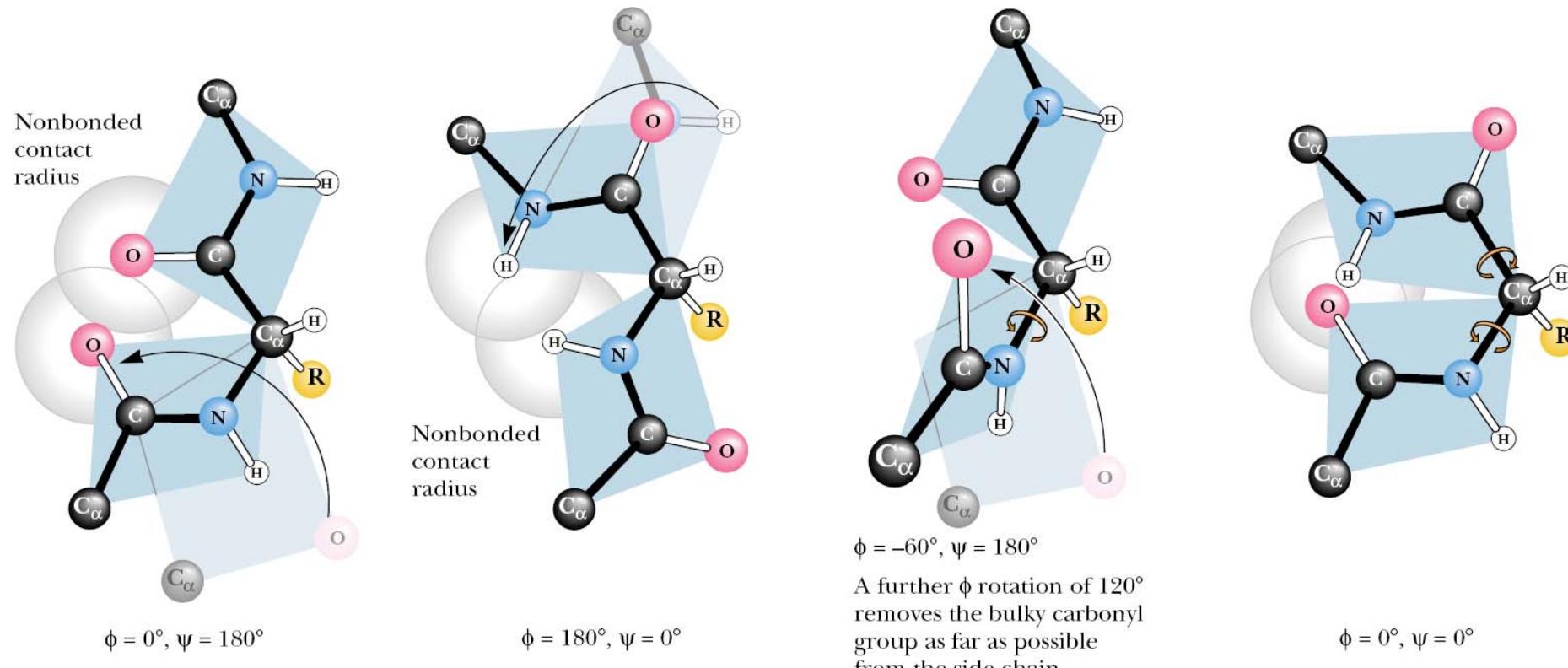


FIGURE 6.3 • Many of the possible conformations about an α -carbon between two peptide planes are forbidden because of steric crowding. Several noteworthy examples are shown here.

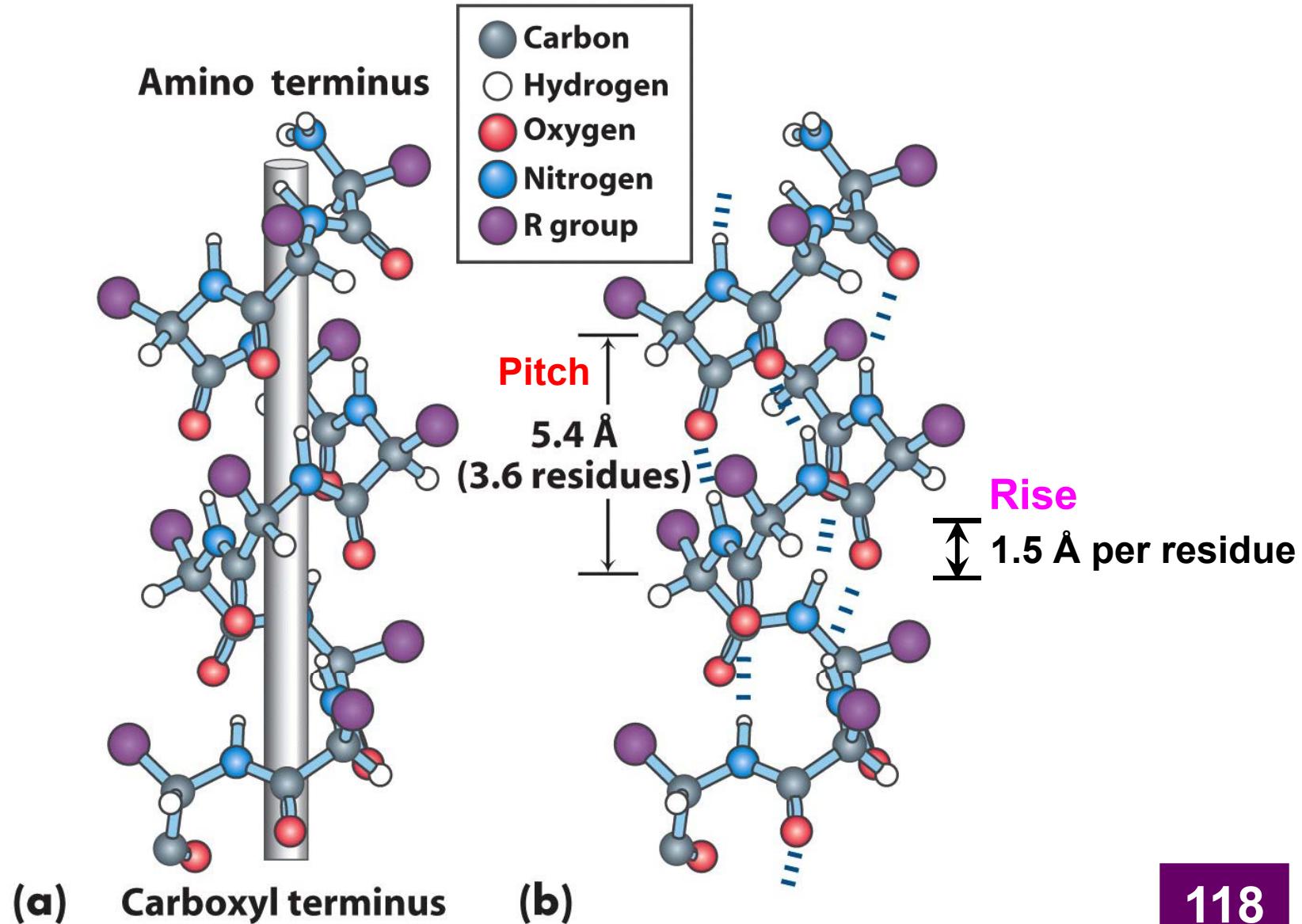
Note: The formal IUPAC-IUB Commission on Biochemical Nomenclature convention for the definition of the torsion angles ϕ and ψ in a polypeptide chain (*Biochemistry* 9:3471–3479, 1970) is different from that used here, where the C_α atom serves as the point of reference for both rotations, but the result is the same. (Irving Geis)

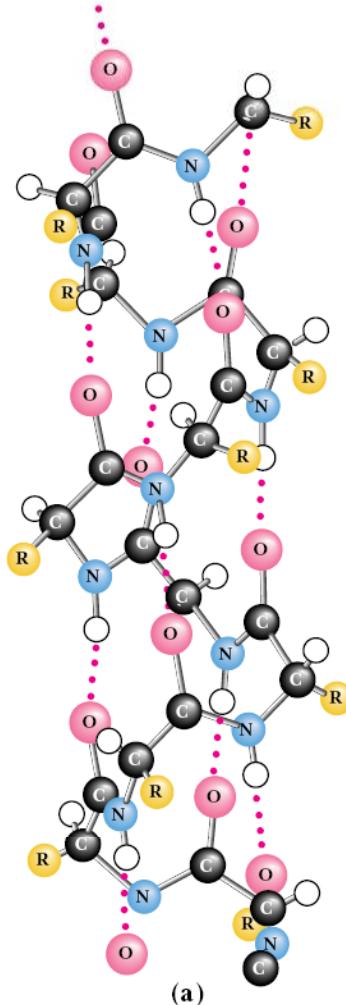
Rotation of atoms in a peptide group

- Peptide group has a repeating $\text{C}_{\alpha 1}\text{-C-N-C}_{\alpha 2}$ backbone.
- Rotation around C-N bond is restricted due to the double-bond nature of the resonance hybrid form.
Peptide groups are therefore planar.
- Rotation about both the $\text{N-C}_{\alpha} (\phi)$ and $\text{C}_{\alpha}\text{-C} (\psi)$ bonds is possible.
- Rotation is restricted by steric interference between main-chain and side-chain atoms.
- **Rotation of the N-C_{α} bond in proline is restricted** because of the pyrrolidine ring structure.

Model of the α helix

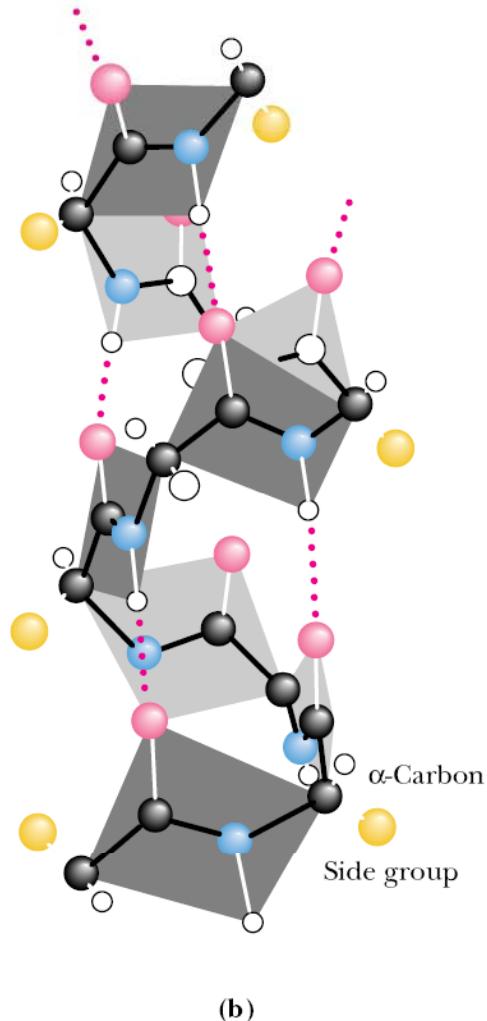
Ball-and-stick model





Hydrogen bonds stabilize the helix structure.

C



The helix can be viewed as a stacked array of peptide planes hinged at the α -carbons and approximately parallel to the helix.

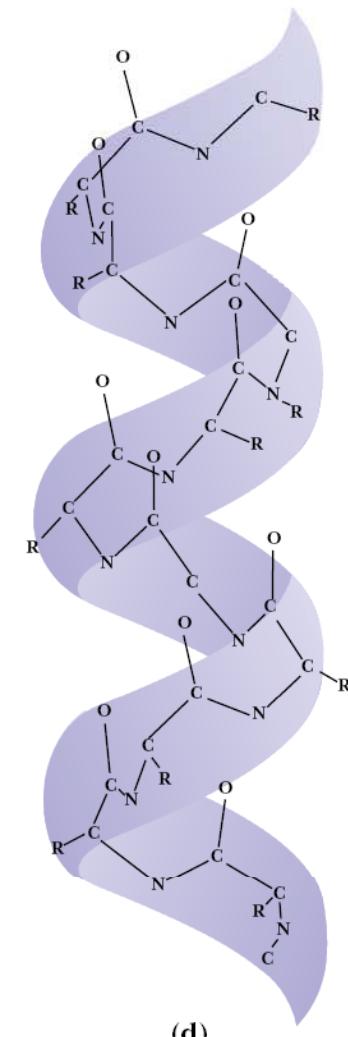
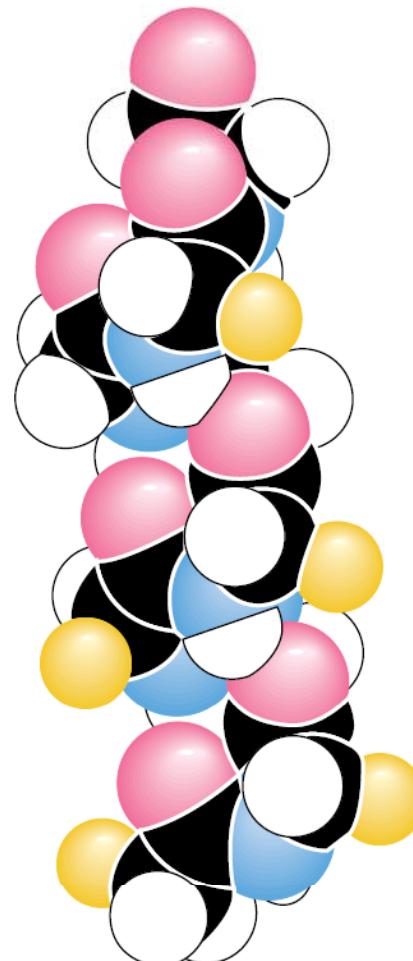
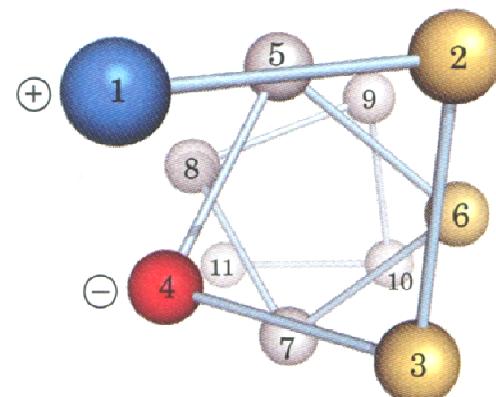
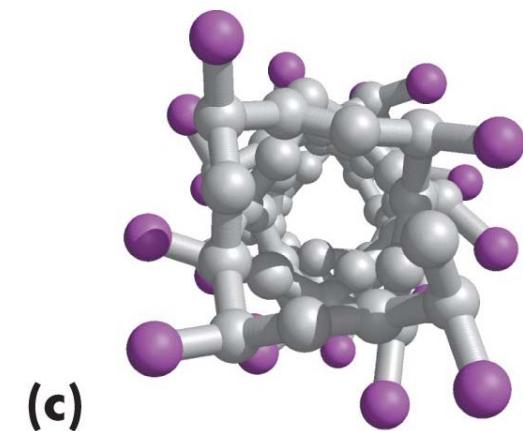


FIGURE 6.6 • Four different graphic representations of the α -helix. (a) As it originally appeared in Pauling's 1960 *The Nature of the Chemical Bond*. (b) Showing the arrangement of peptide planes in the helix. (c) A space-filling computer graphic presentation. (d) A "ribbon structure" with an inlaid stick figure, showing how the ribbon indicates the path of the polypeptide backbone. (Irving Geis)

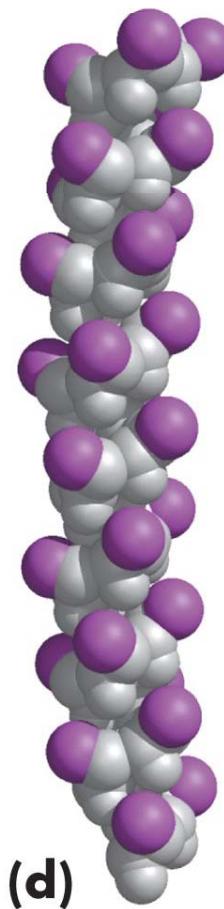
Helical wheel projection



Ball-and-stick model (Top view)

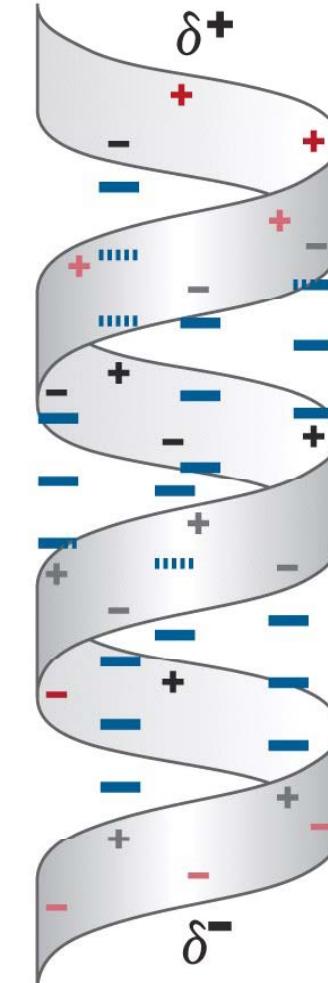


Space -filling model



Helix dipole

Amino terminus



Carboxyl terminus

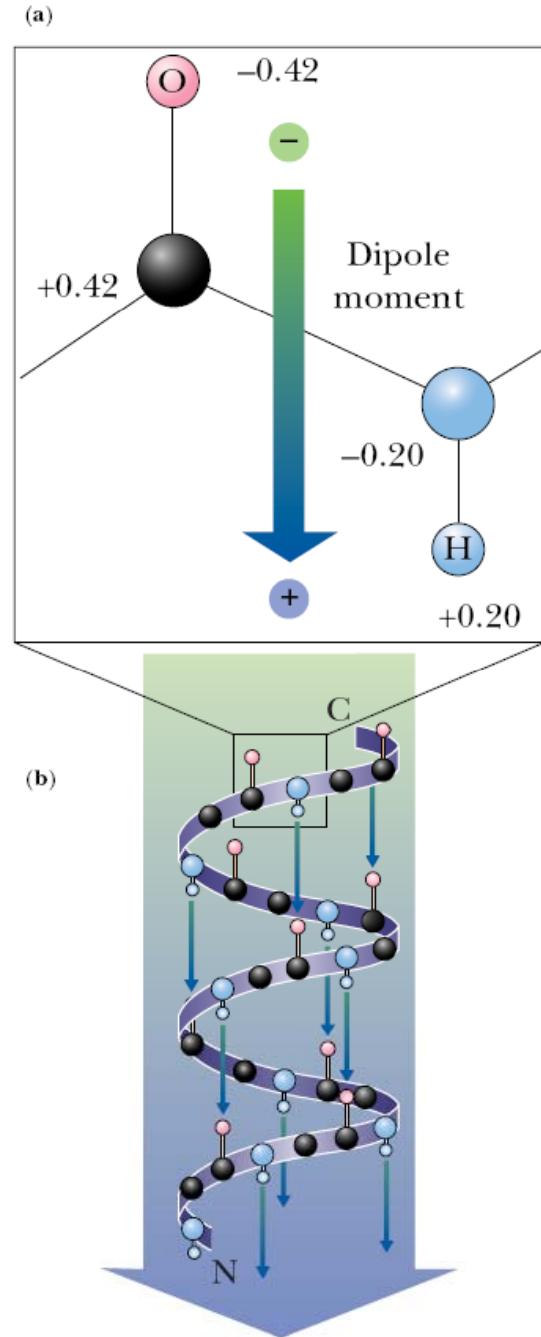


FIGURE 6.8 • The arrangement of N—H and C=O groups (each with an individual dipole moment) along the helix axis creates a large net dipole for the helix. Numbers indicate fractional charges on respective atoms.

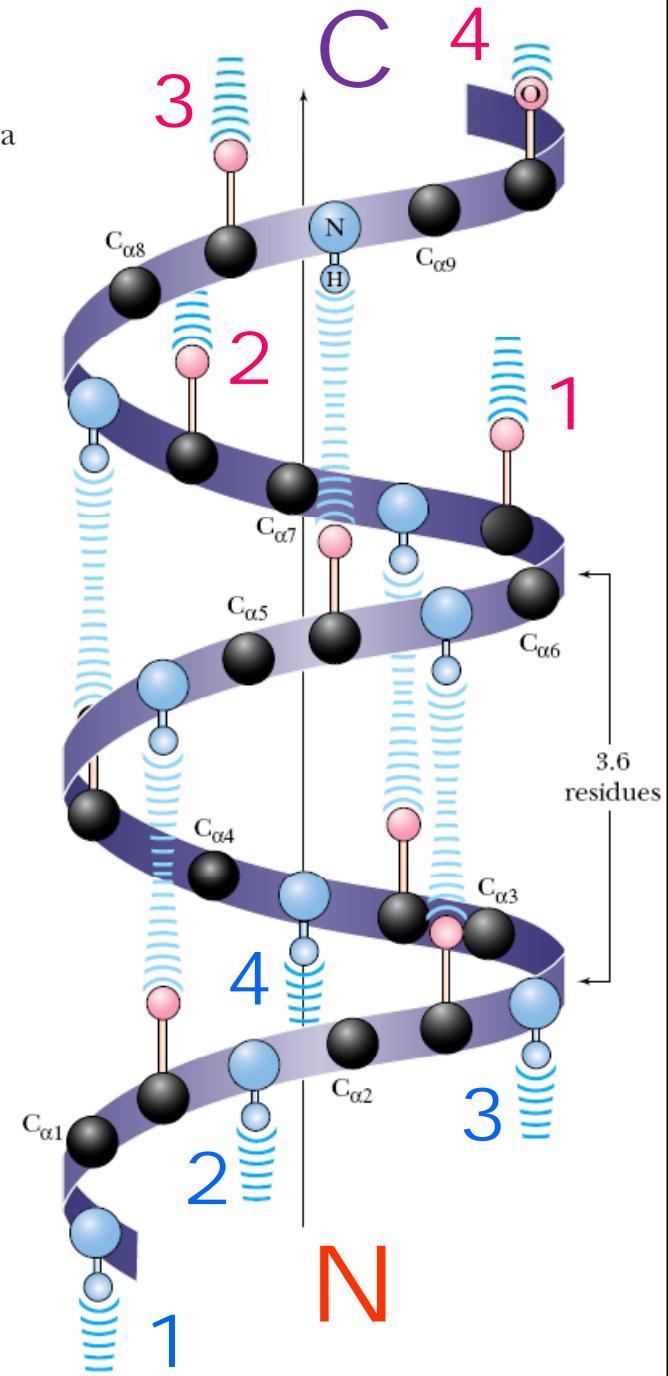
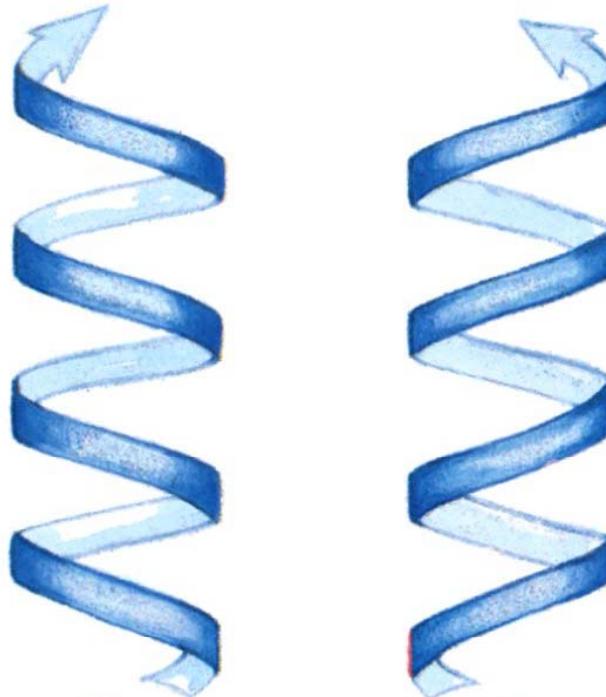


FIGURE 6.9 • Four N—H groups at the N-terminal end of an α -helix and four C=O groups at the C-terminal end cannot participate in hydrogen bonding. The formation of H-bonds with other nearby donor and acceptor groups is referred to as **helix capping**. Capping may also involve appropriate hydrophobic interactions that accommodate non-polar side chains at the ends of helical segments.

The α helix

- Each C=O (residue n) forms a hydrogen bond with the amide hydrogen of residue $n+4$
- Helix is stabilized by many hydrogen bonds (which are nearly parallel to long axis of the helix)
- All C=O groups point toward the C-terminus. (entire helix is a dipole with (+)N, (-) C-termini)
- All side chains project outward from helix axis
- The ϕ and ψ angles of each residue are similar: near -57° (ϕ) and near -47° (ψ)
- Most α helices in proteins are right handed (backbone turns clockwise when viewed along the axis from the N terminus)

Knowing the right hand from the left



In all proteins,
the helical
twist of the α
helix is right-
handed.



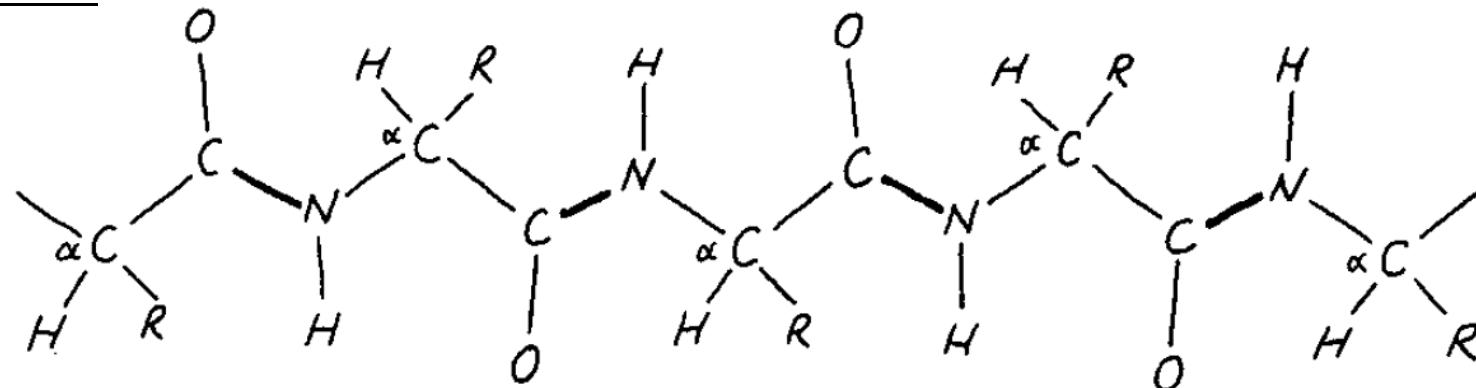
Propensity of amino acids to take up an α -helical conformation

Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)	Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)
Ala	0	Leu	0.79
Arg	0.3	Lys	0.63
Asn	3	Met	0.88
Asp	2.5	Phe	2.0
Cys	3	Pro	>4
Gln	1.3	Ser	2.2
Glu	1.4	Thr	2.4
Gly	4.6	Tyr	2.0
His	2.6	Trp	2.0
Ile	1.4	Val	2.1

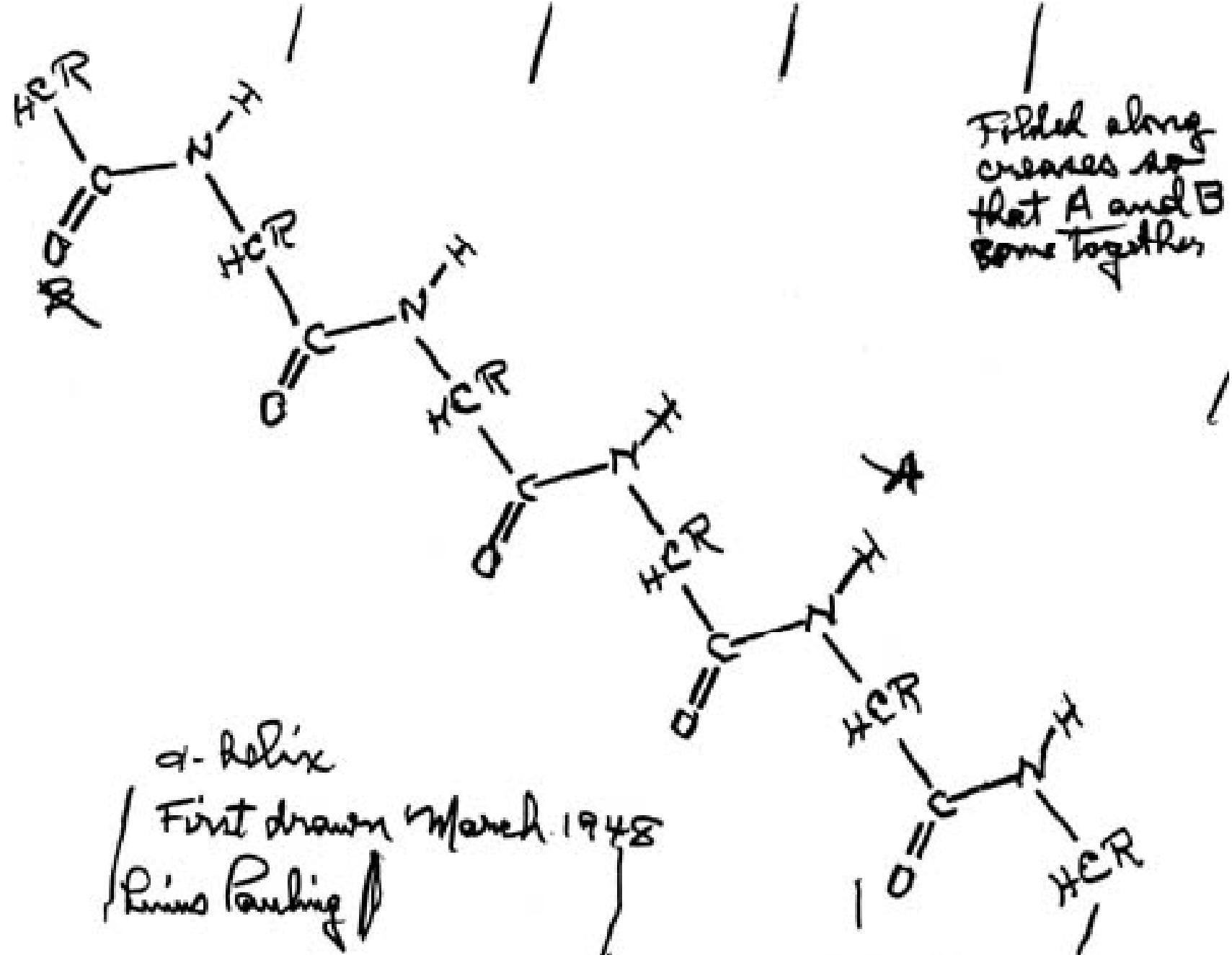
119

$\Delta\Delta G^\circ$ is the difference in free-energy change, relative to alanine, required for the amino acid residue to take up the α -helical conformation. Larger number reflect greater difficulty

In January **1948**, Pauling went to Oxford as a visiting professor... “In Oxford, it was April, I believe, I caught cold. I went to bed, and read detective stories for a day, and got bored, and thought why don’t I have a crack at that problem of alpha keratin.” Pauling called for paper, pencil, and straightedge... “I creased the paper in parallel creases through the alpha carbon atoms, so that I could bend it and make the bonds to the alpha carbons, along the chain, have tetrahedral value. And then I looked to see if I could form hydrogen bonds from one part of the chain to the next.”... After several tries, changing the angle of the parallel creases in order to adjust the pitch of the helix, he found one where the hydrogen bonds would drop into place, connecting the turns, as straight lines of the right length. He had a model.



He put in lengths and bond angles from memory and knew that the peptide bond, at the carbon-to-nitrogen link, was always rigid.



*THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED
HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN*

By LINUS PAULING, ROBERT B. COREY, AND H. R. BRANSON*

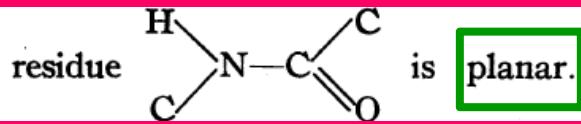
GATES AND CRELLIN LABORATORIES OF CHEMISTRY,
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA†

Communicated February 28, 1951

During the past fifteen years we have been attacking the problem of the structure of proteins in several ways. One of these ways is the complete and accurate determination of the crystal structure of amino acids, peptides, and other simple substances related to proteins, in order that information about interatomic distances, bond angles, and other configurational parameters might be obtained that would permit the reliable prediction of reasonable configurations for the polypeptide chain. We have now used this information to construct two reasonable hydrogen-bonded helical con-

α helix and β helix

We assume that, because of the resonance of the double bond between the carbon-oxygen and carbon-nitrogen positions, the configuration of each



$3.6 \times 2 = 7.2$

7.2 Å

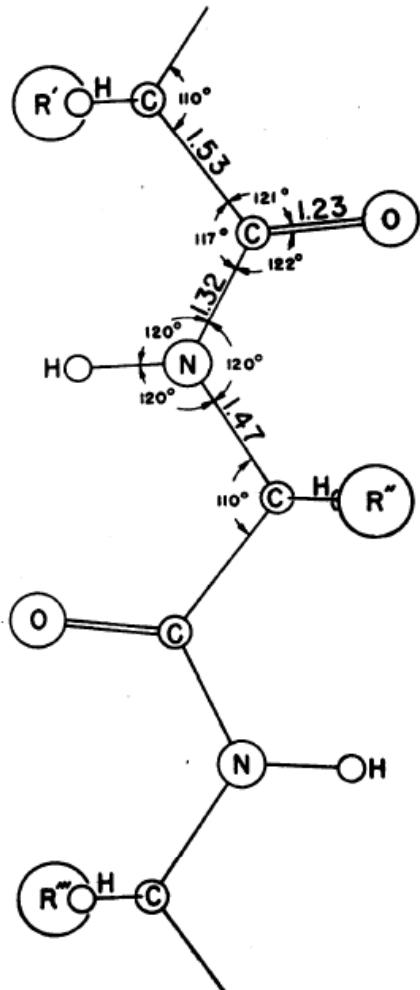


FIGURE 1

Dimensions of the polypeptide chain.

This structural feature has been verified for each of the amides that we have studied. Moreover, the resonance theory is now so well grounded and its experimental substantiation so extensive that there can be no doubt whatever about its application to the amide group. The observed C—N distance, 1.32 Å, corresponds to nearly 50 per cent double-bond character, and we may conclude that rotation by as much as 10° from the planar configuration would result in instability by about 1 kcal. mole⁻¹. The interatomic distances and bond angles within the residue are assumed to have the values shown in figure 1. These values have been formulated² by consideration of the experimental values found in the crystal structure studies of DL-alanine,³ L-threonine,⁴ N-acetylglycine⁵, and β -glycylglycine⁶ that have been made in our

THE PLEATED SHEET, A NEW LAYER CONFIGURATION OF POLYPEPTIDE CHAINS

By LINUS PAULING AND ROBERT B. COREY

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Communicated March 31, 1951

For many years it has been assumed that in silk fibroin, stretched hair and muscle, and other proteins with the β -keratin structure the polypeptide chains are extended to nearly their maximum length, about 3.6 Å per residue, and during the last decade it has been assumed also that the chains form lateral hydrogen bonds with adjacent chains, which have the opposite orientation. A hydrogen-bonded layer of this sort is represented diagrammatically in figure 1.¹⁻⁴

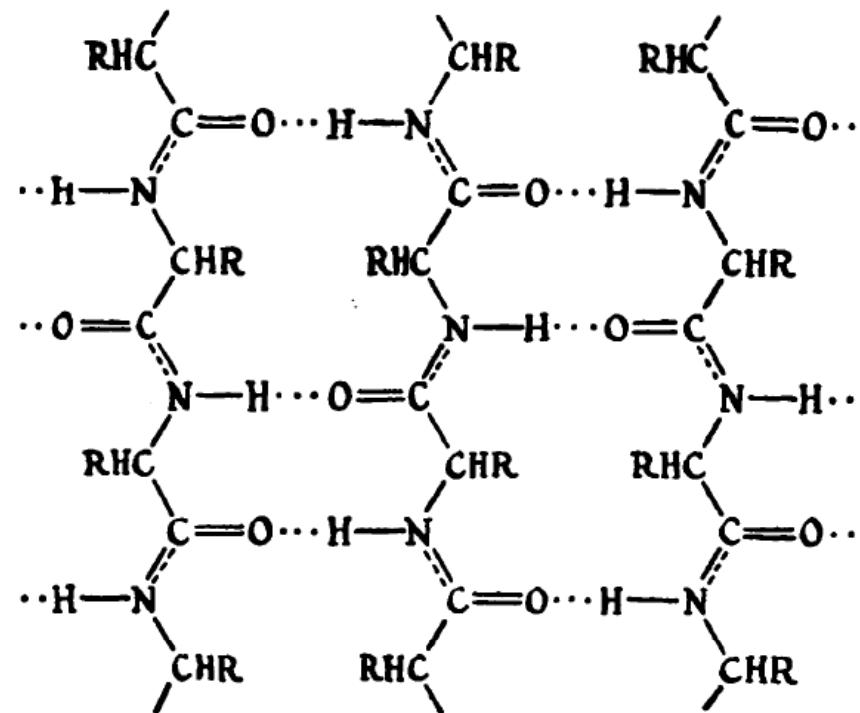


FIGURE 1

Diagrammatic representation of a hydrogen-bonded layer structure of polypeptide chains with alternate chains oppositely oriented.

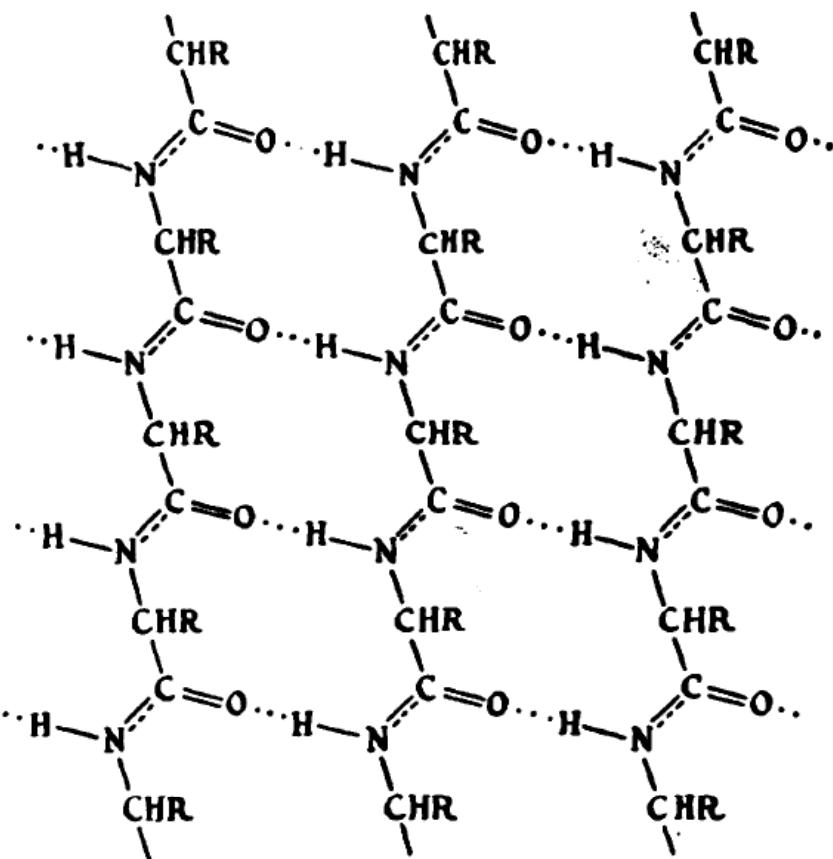
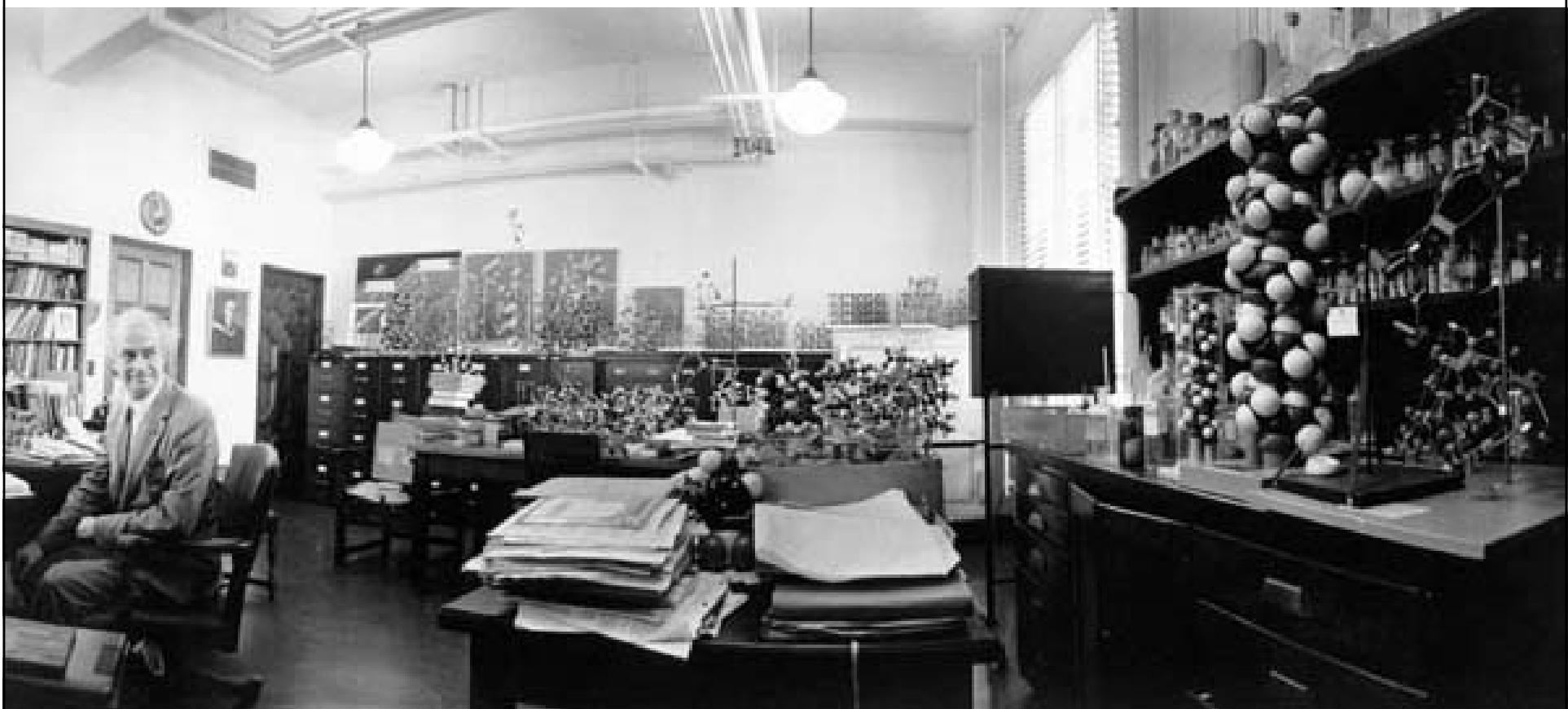


FIGURE 2

Diagrammatic representation of a hydrogen-bonded layer structure of polypeptide chains with all chains similarly oriented (the pleated sheet).

Pauling's Office at Caltech (1957)

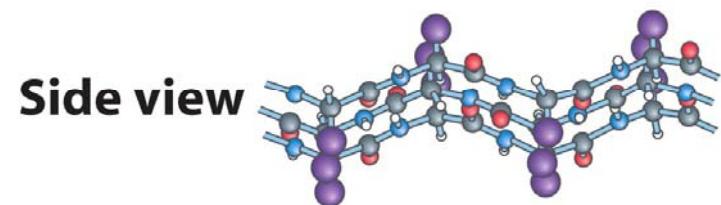
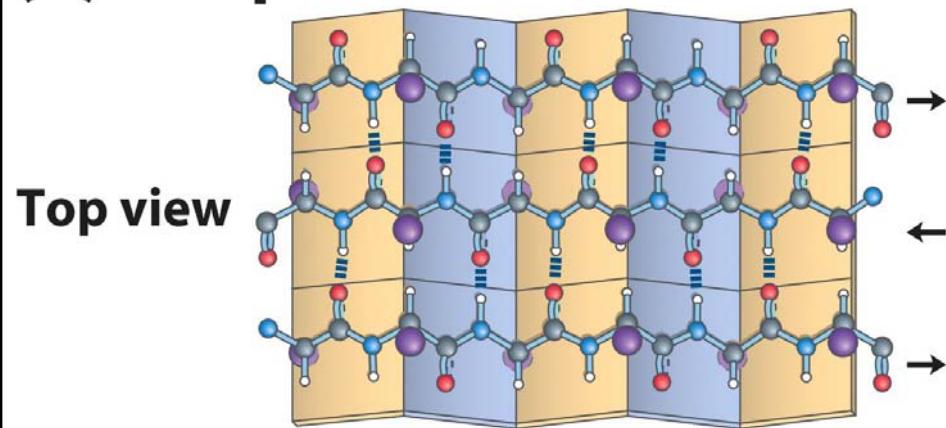
Pauling surrounded by molecular models at the California Institute of Technology, where he established his career.



The β conformation of polypeptide chains

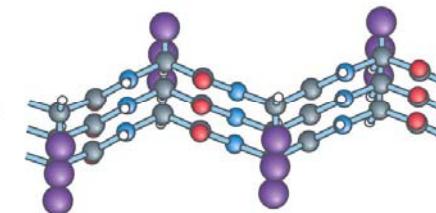
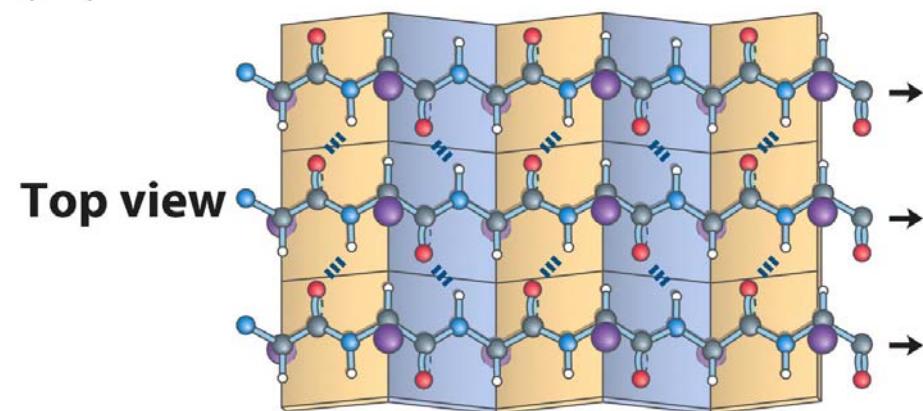
Hydrogen bonds form between adjacent polypeptide chains, which are usually nearby, but can also be quite distant each other in the linear sequence of the polypeptide; they may even be in different polypeptide chains.

(a) Antiparallel



β -pleated sheets

(b) Parallel



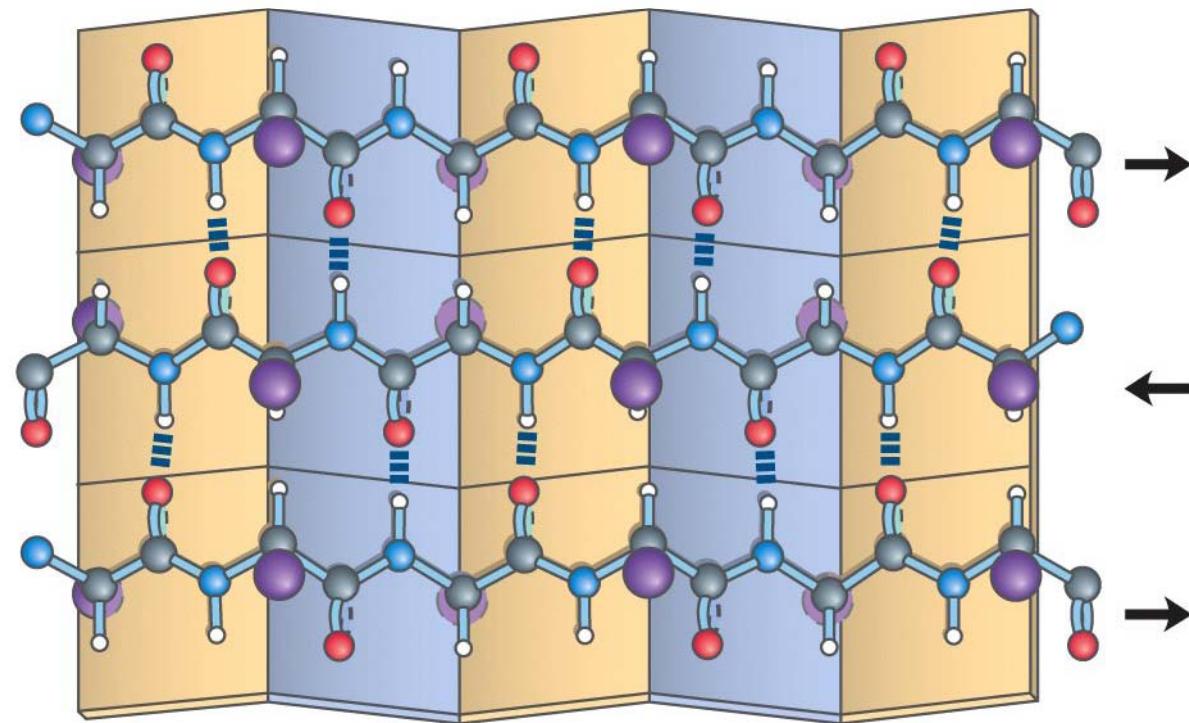
(a) Antiparallel

Top view

β -strand

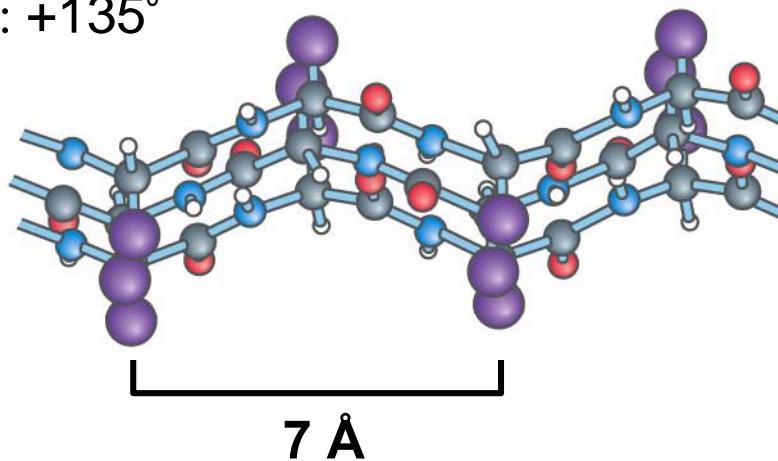
β -strand

β -strand



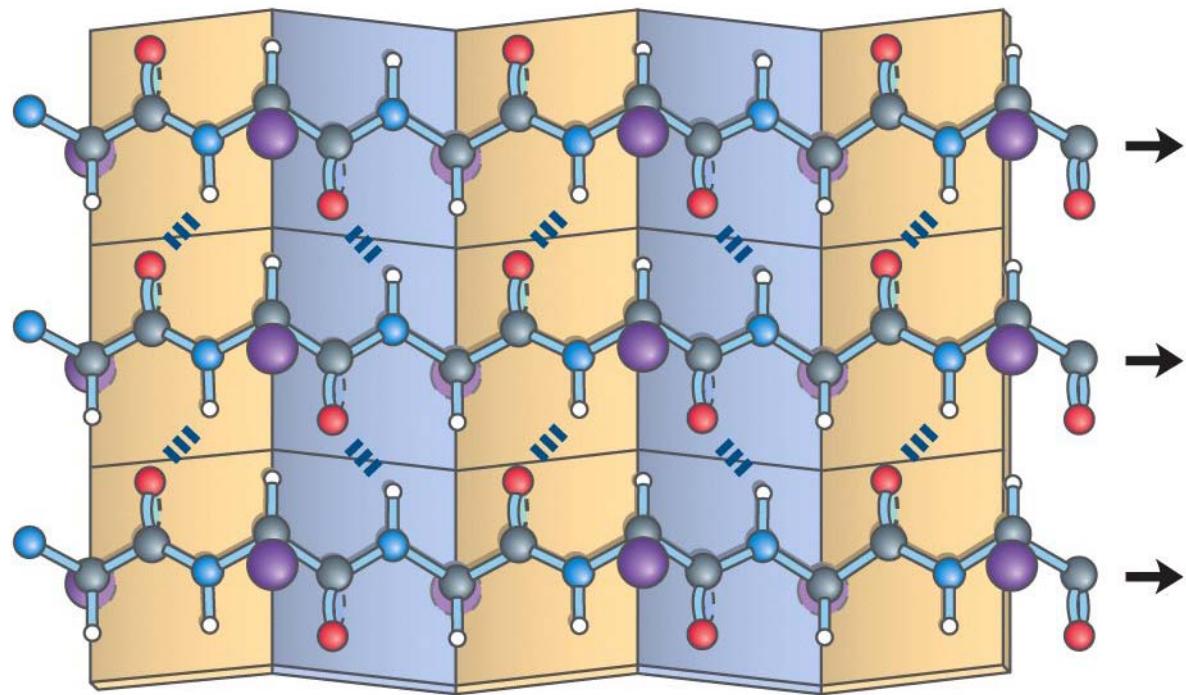
approximate ϕ : -139° and ψ : $+135^\circ$

Side view



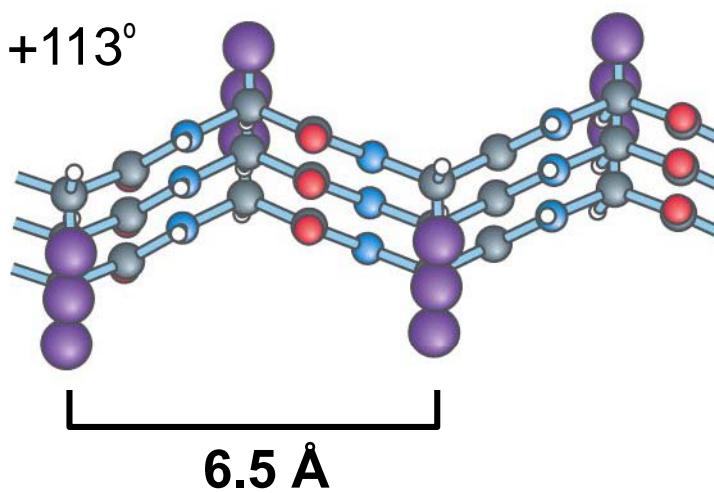
(b) Parallel

Top view



approximate ϕ : -119° and ψ : $+113^\circ$

Side view



This structure was first postulated by Pauling and Corey in 1951

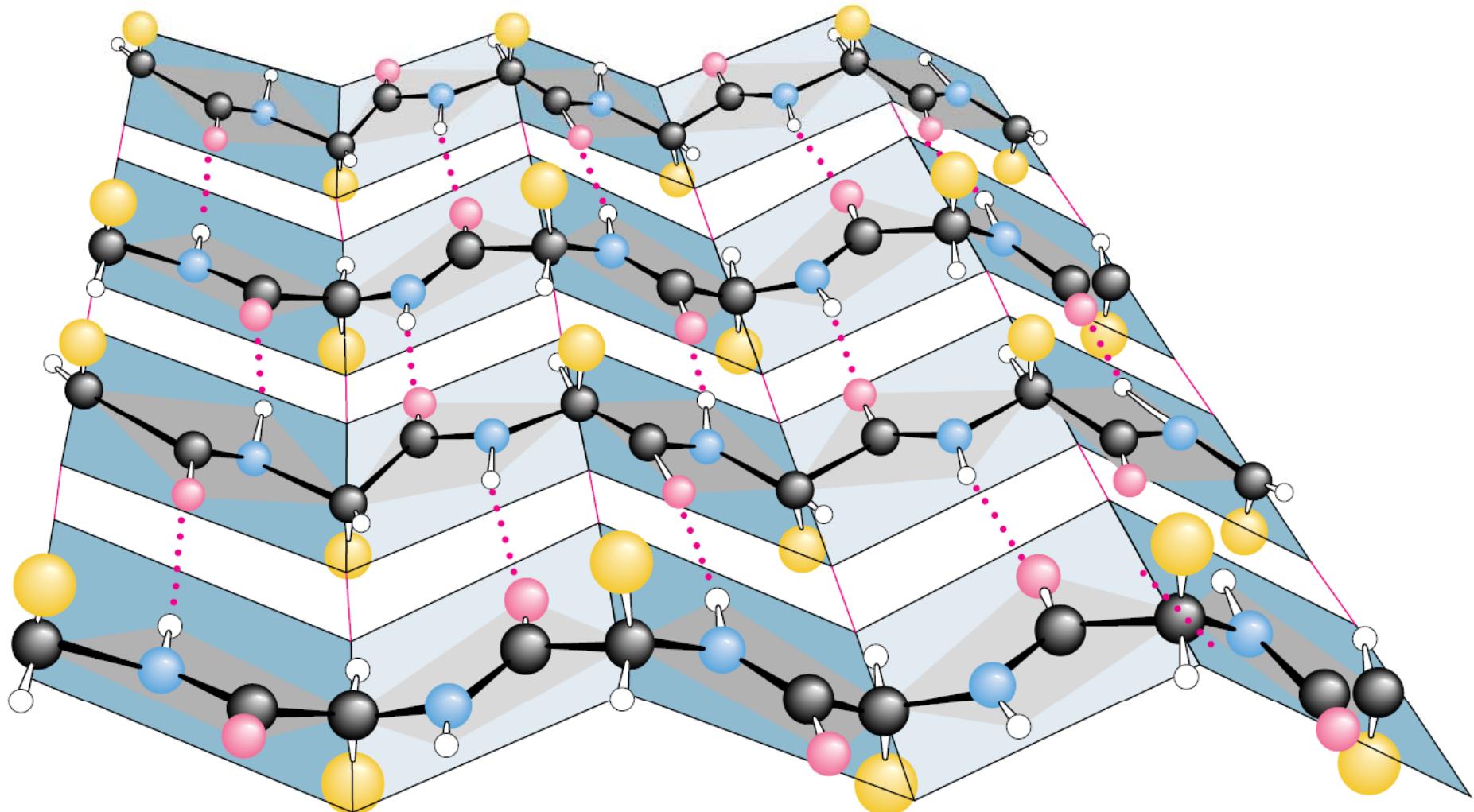
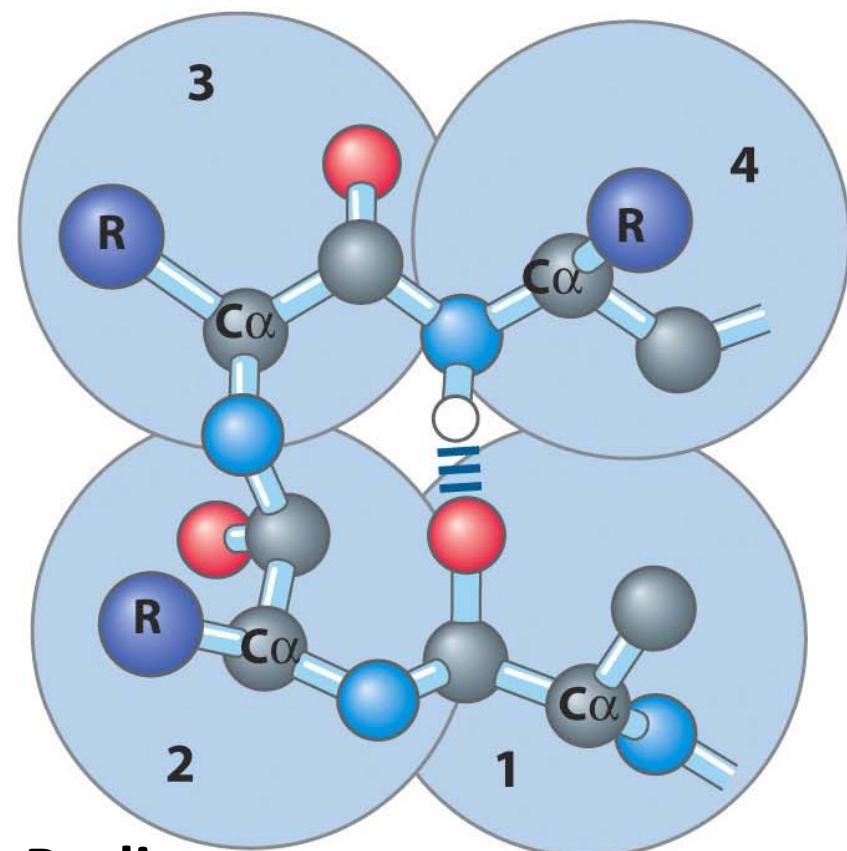


FIGURE 6.10 • A “pleated sheet” of paper with an antiparallel β -sheet drawn on it.
(Irving Geis)

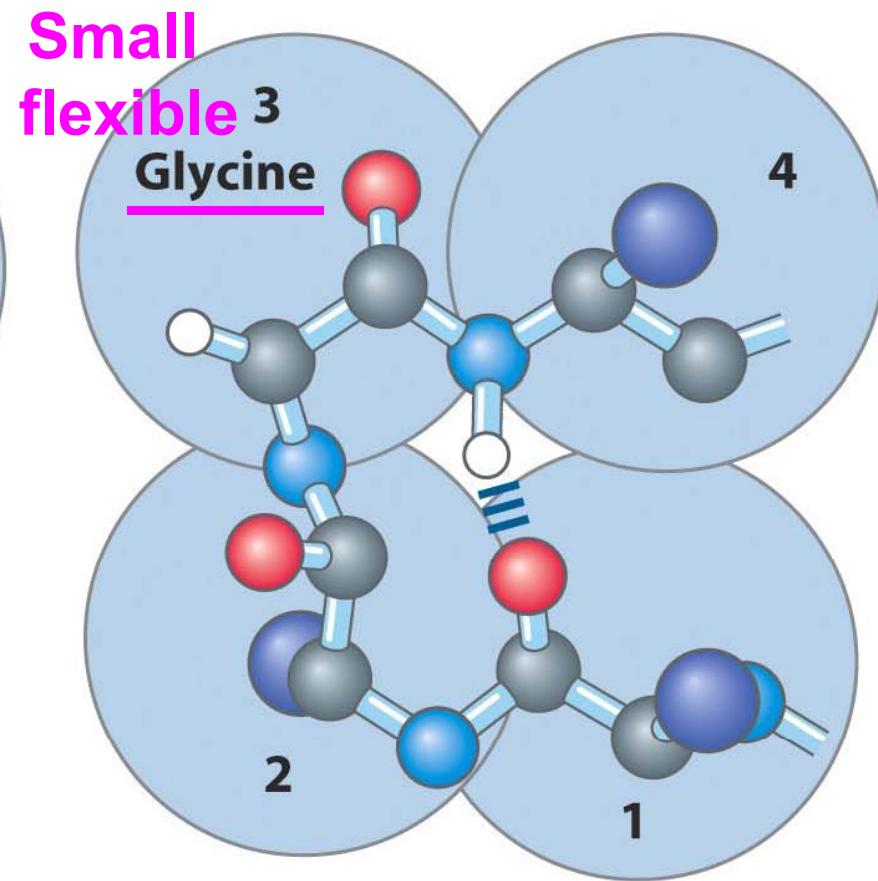
Structures of β turns

(a) β Turns involving 4 amino acids



Proline
Cis configuration

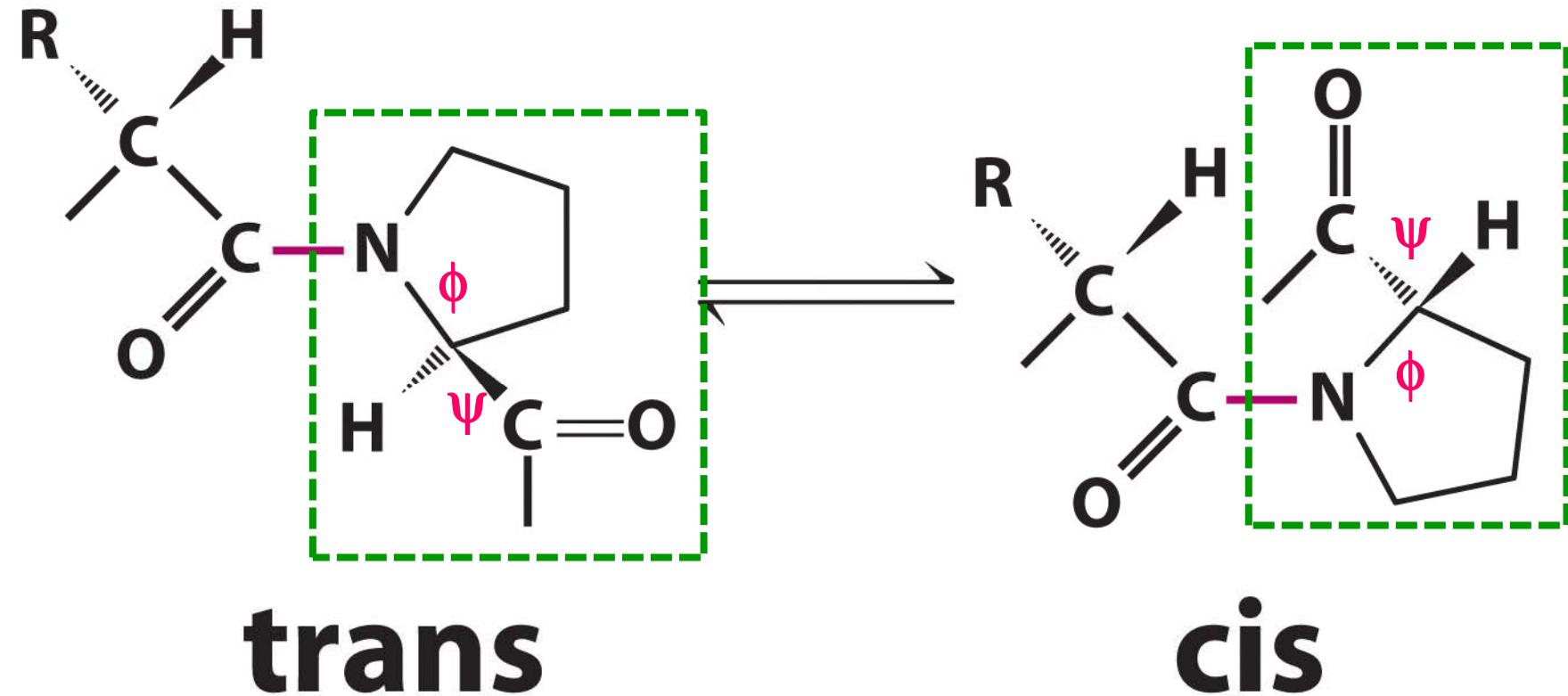
Type I



Type II

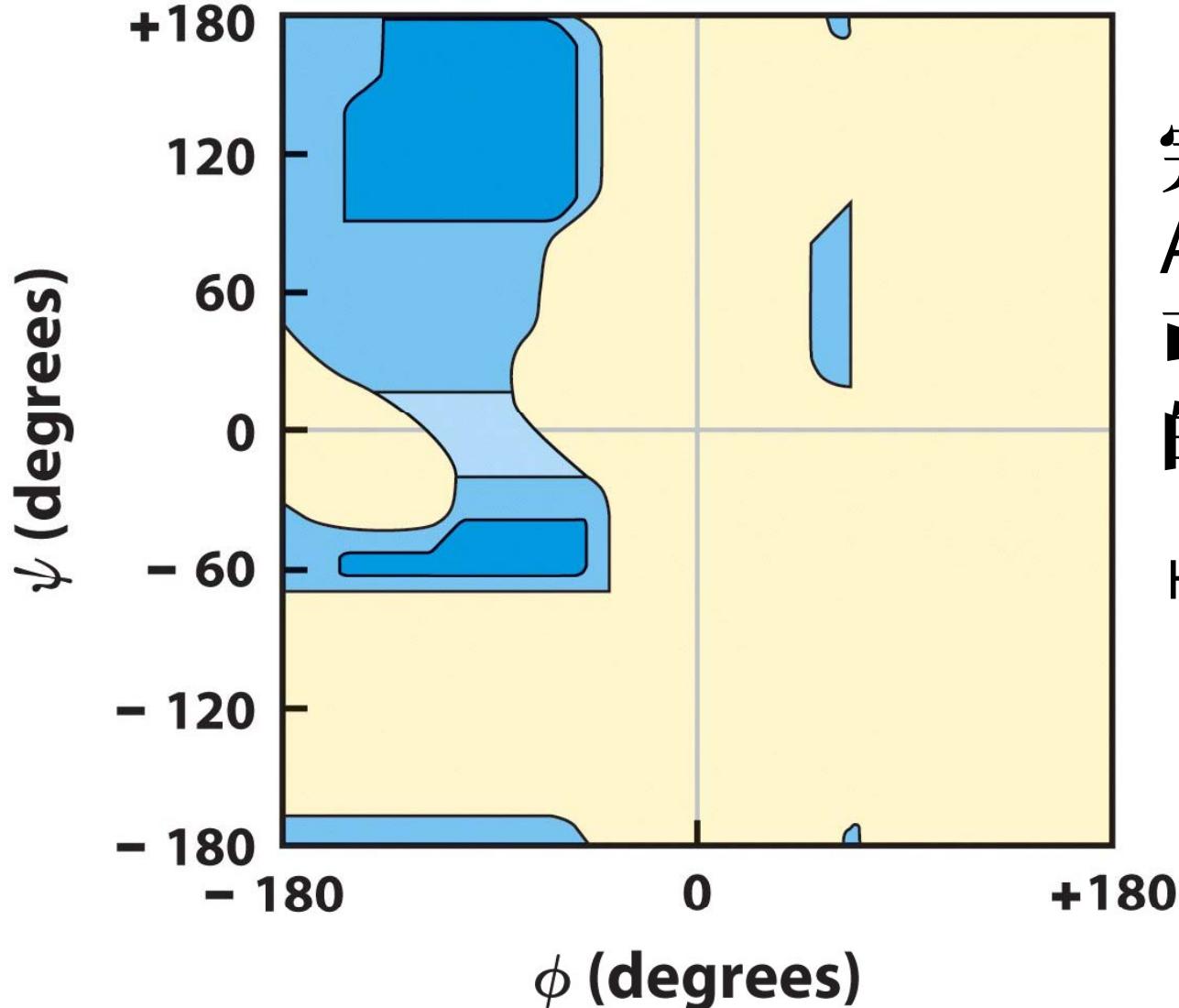
Small
flexible 3
Glycine

Proline isomers



The range for **Pro** residues is greatly restricted because ϕ is limited by the cyclic side chain to the range of -35° to -85°

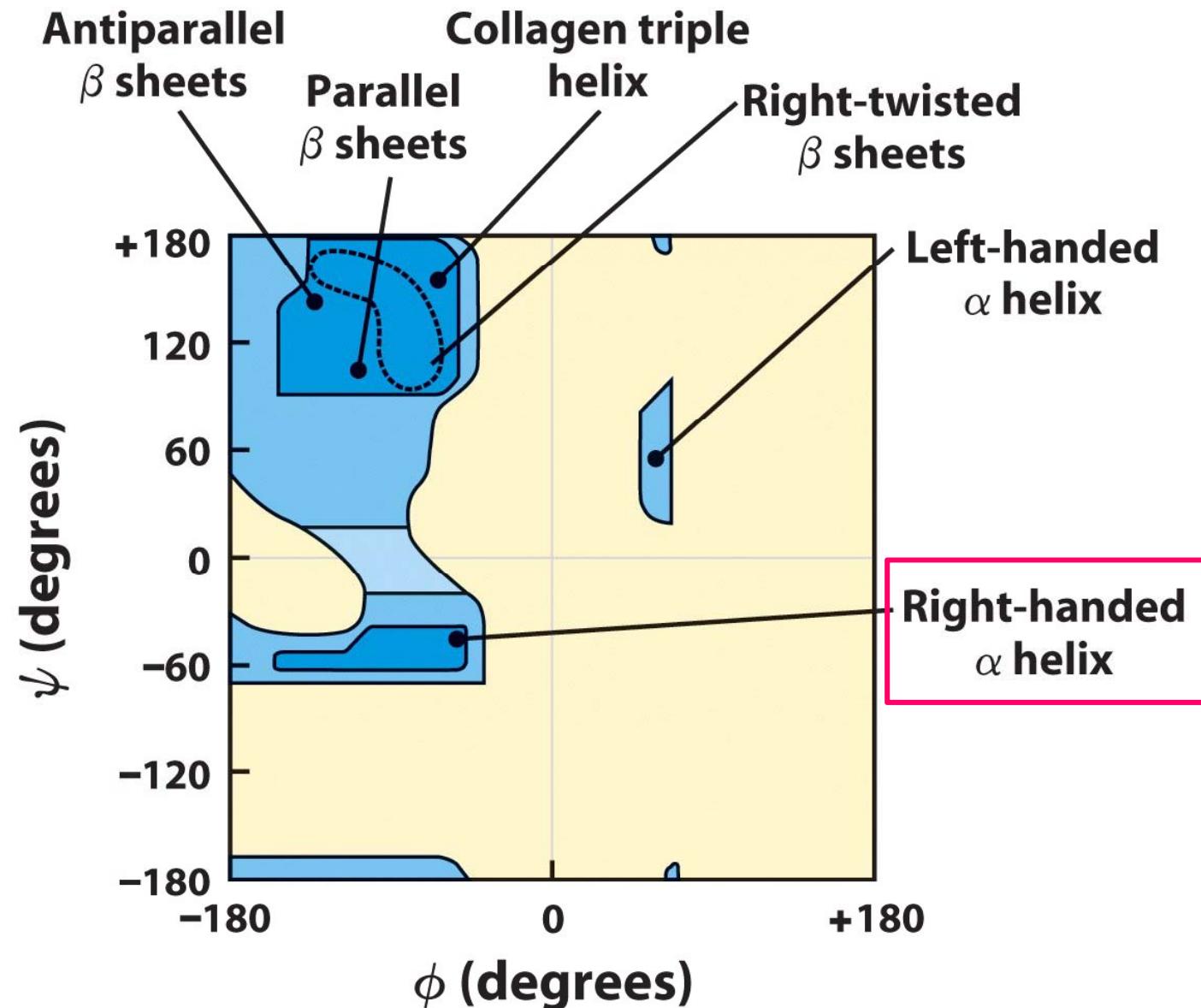
Ramachandran plot for L-Ala residues



完全用Ala-Ala-
Ala-Ala-Ala.....
可以組合起來
的角度

Here Ala means L-Ala

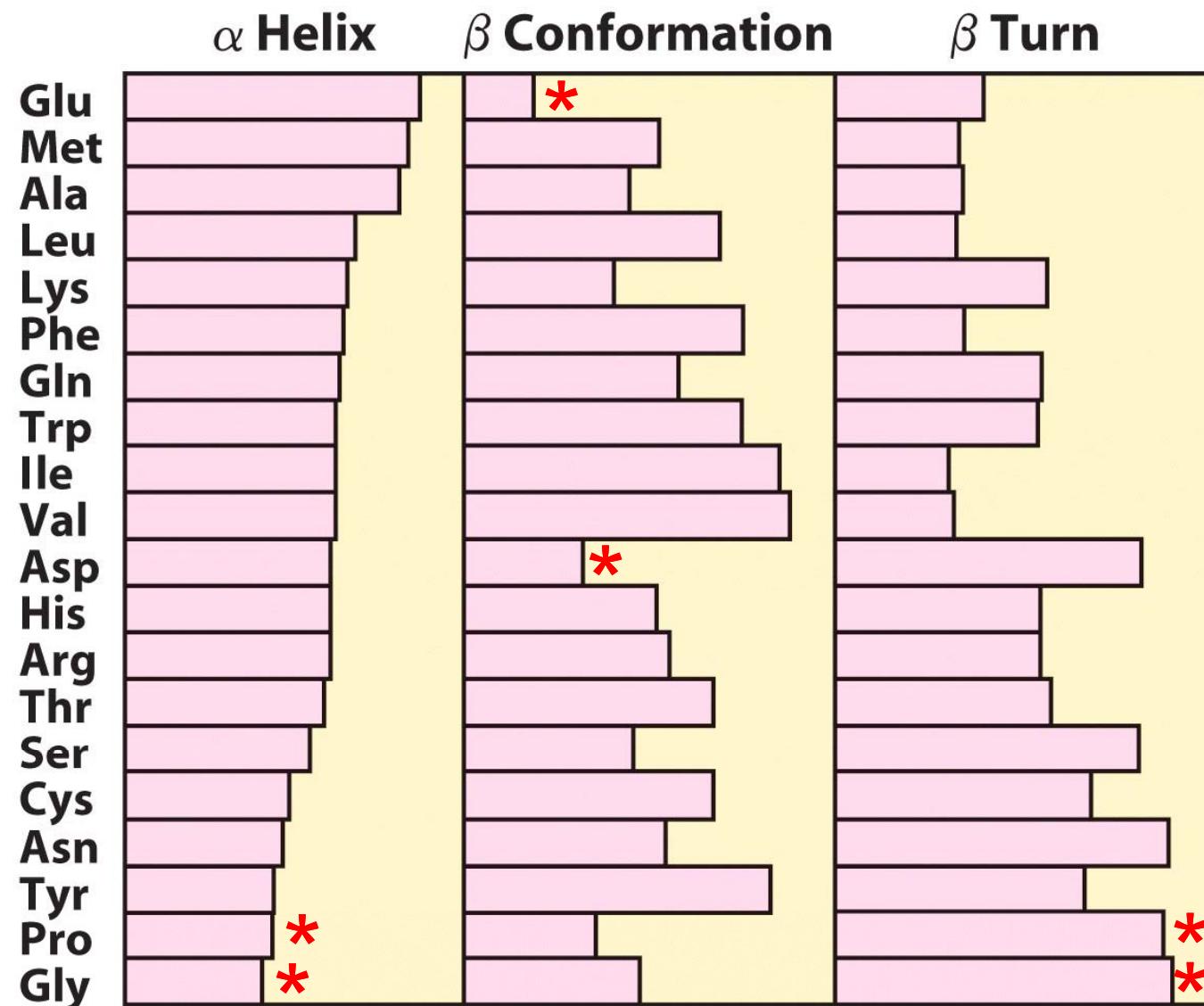
Several types of secondary structures fall within restricted area of Ramachandran plot



Permissible values of ϕ and ψ

- Conformation of a polypeptide chain can be solely described by ϕ and ψ angles
- Ramachandran plots of ϕ and ψ show permissible angles for polypeptide chains
- Some ϕ and ψ angles are not allowed because of steric hindrance
- Conformations of several types of secondary structures fall within permissible area of Ramachandran plot

Relative probabilities that a given amino acid will occur in the three types of secondary structure



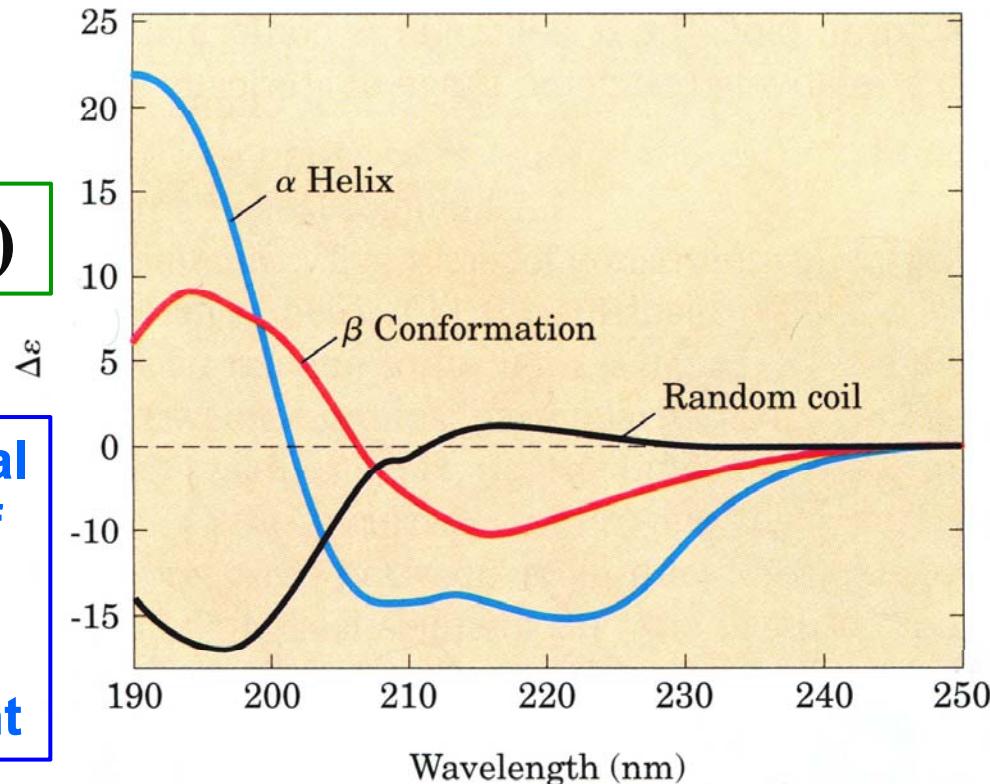
Circular dichroism (CD) spectroscopy

These spectra show **polylysine** entirely as α helix, as β conformation, or as a denatured, random coil

$\Delta\epsilon$: Molar extinction coefficient (分子消光係數)

$$\Delta\epsilon = (\epsilon_L - \epsilon_R)$$

the differential absorption of left and right circularly polarized light



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- CD spectroscopy is a method for assessing common secondary structure and monitoring folding in protein