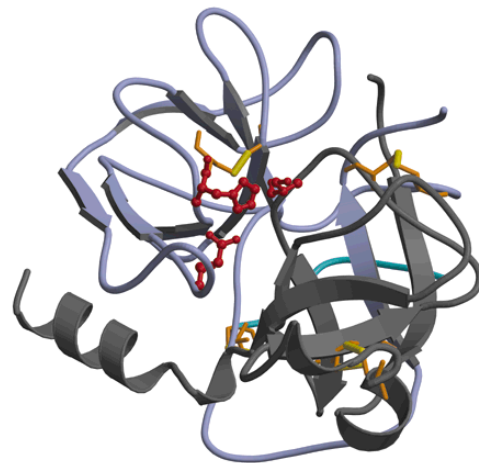
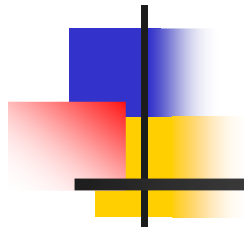


Chapter 4

The 3-D structure of proteins



Ribbon diagram

Chymotrypsin

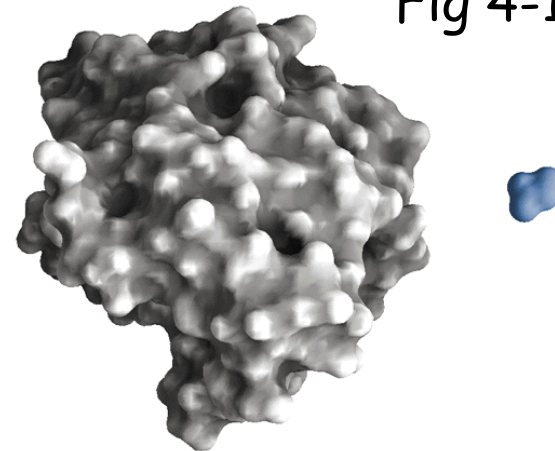


Fig 4-1

Surface contour image



學習目標

1. 瞭解胜肽鍵的特性及限制。
2. 瞭解維持蛋白質各級結構的作用力。
3. 瞭解二級結構中螺旋(α -helix)及摺板(β -sheet)的結構特徵及相似、相異處。
4. 瞭解蛋白質折疊與變性的可能過程。



“Conformation”

p. 113-114

- The spatial arrangement of atoms in a protein (without breaking or forming covalent bonds ζ)
- Thermodynamically stable
= Lowest Gibbs free energy (G)

Native protein

- Protein in its functional, folded conformation.
- Stabilized by disulfide bond and “weak (non-covalent) interactions”
 - ✓ H-bond
 - ✓ Hydrophobic interaction
 - ✓ Ionic interaction
 - ✓ Van der Waals interaction



"Weak"

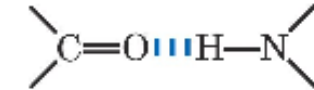
TABLE 2-5 Four Types of Noncovalent ("Weak") Interactions among Biomolecules in Aqueous Solvent

Hydrogen bonds

Between neutral groups

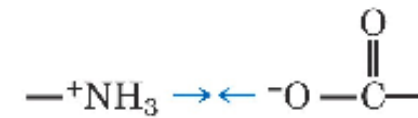


Between peptide bonds



Ionic interactions

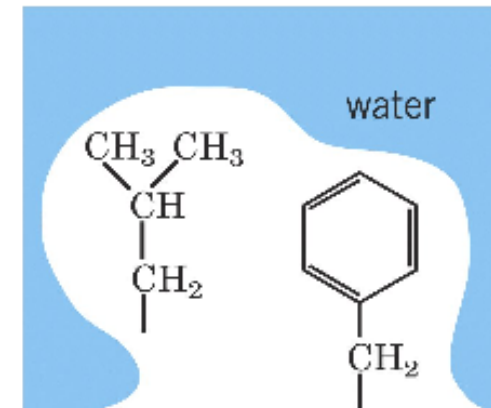
Attraction



Repulsion



Hydrophobic interactions



p. 50

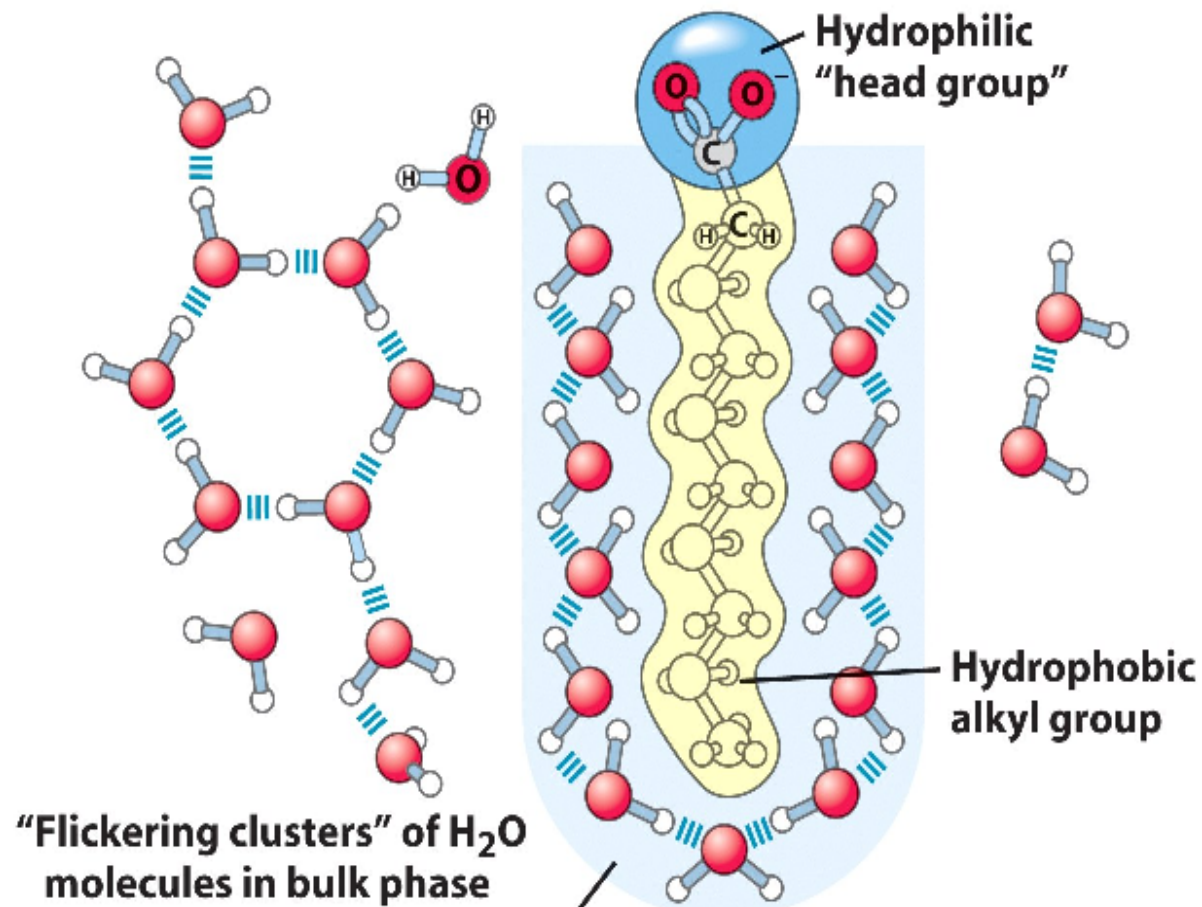
van der Waals interactions

Any two atoms in close proximity

Solvation layer

- Ordered water molecules form H_2O cages around the hydrophobic portion.

Fig 2-7a, p. 48



Hydrophobic interaction

- The forces (**driven by entropy**) that hold the non-polar regions of the molecules together.

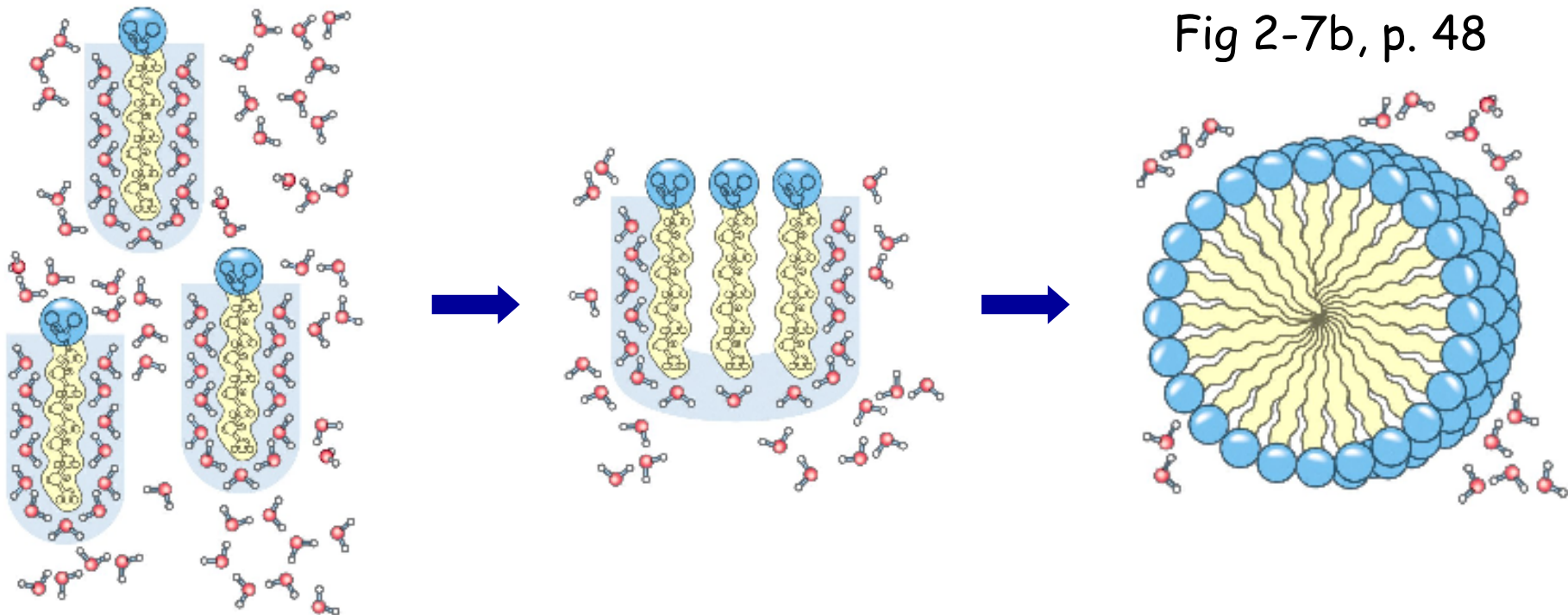


Fig 2-7b, p. 48

Order of H₂O

Entropy, (火商)
= randomness



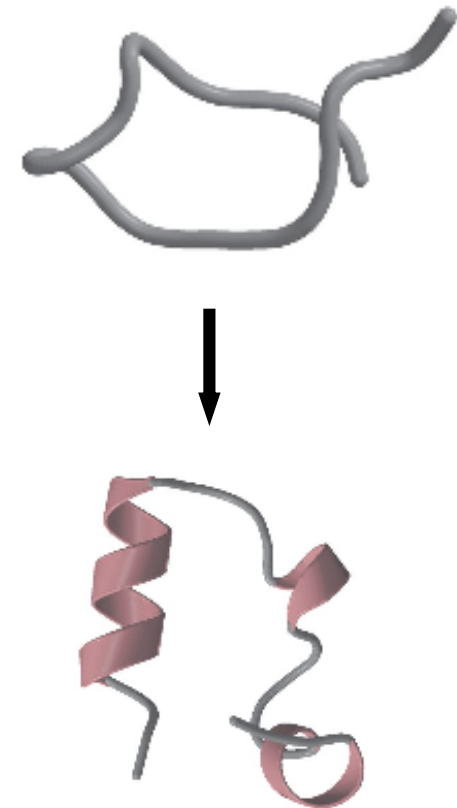
Protein stability (p. 116-117)

Unfolded

- High degree of conformational entropy
- H-bond of polypeptide with solvent (H_2O)

Folded

- Disulfide bond
- Weak (non-covalent) interactions:
 - ✓ H-bond
 - ✓ Hydrophobic
 - ✓ Ionic
 - ✓ Van der Waals interaction

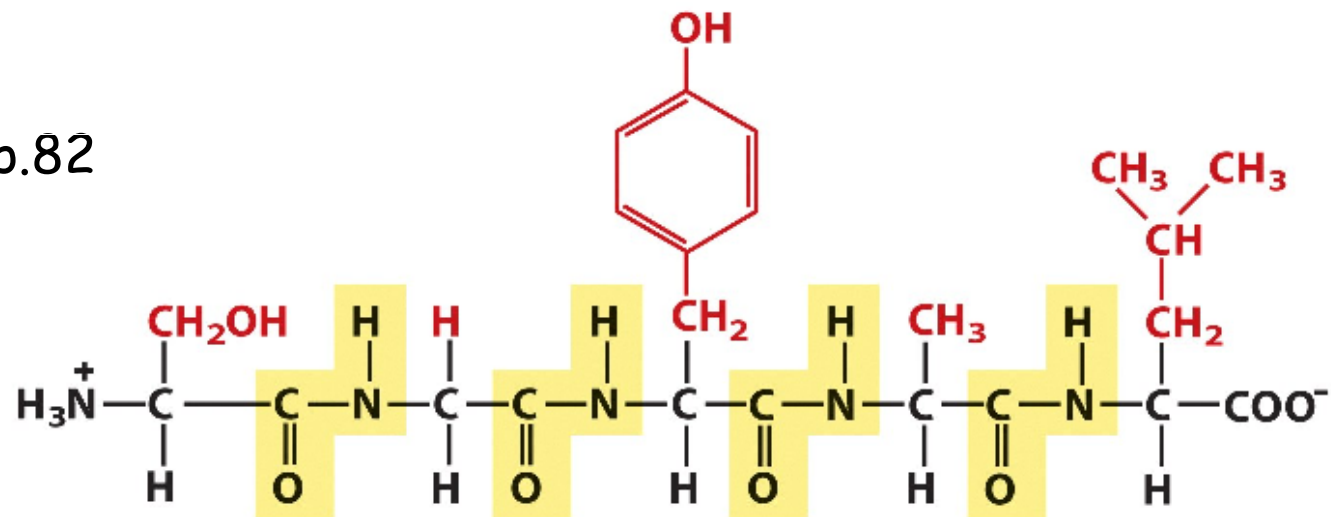


In general, the protein conformation with lowest free energy is the one with the max. no. of weak interactions.

Primary (covalent) structure

- The **composition** and the **order** of the a.a. of a polypeptide chain
- Amino acid sequence of a polypeptide chain
 - ✓ Amino acids are joined by peptide (covalent) bond

Fig 3-14, p.82



- Backbone (N-C_α-C-N-C_α-C-...)
- Side chain (R-groups)

Peptide bond

p.115-116

1. OC-NH is shorter
2. Coplanar peptide group
3. Trans configuration (O vs. H, $C_{1\alpha}$ vs. $C_{2\alpha}$)

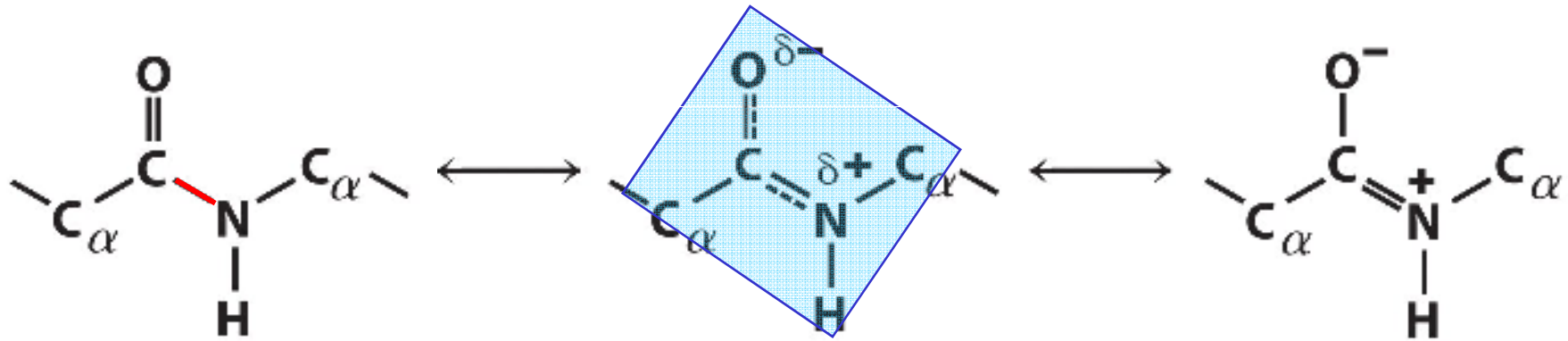


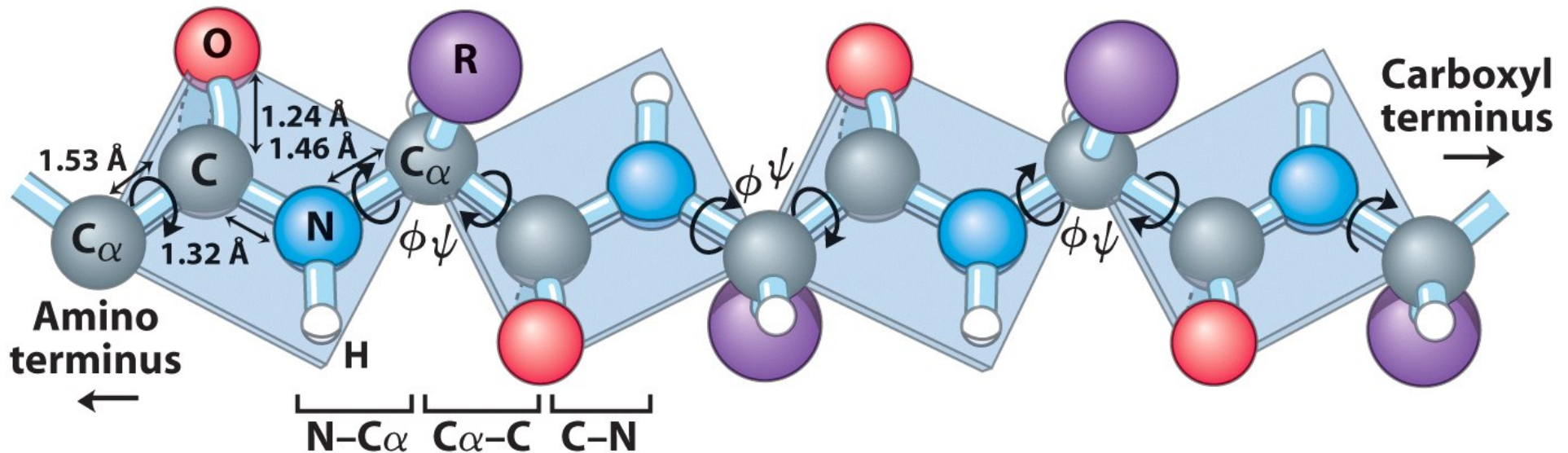
Fig 4-2a, p.116

- Electrons resonance (partial sharing) between the **carbonyl O** and the **amide N** (electric dipole).
- Peptide bond can not rotate.

Planar and rigid peptide bond

1. OC-NH can not rotate
2. C_{α} -C: ψ (psi), $-180^{\circ} \sim 180^{\circ}$
3. N- C_{α} : ϕ (phi), $-180^{\circ} \sim 180^{\circ}$

Fig 4-2b, p.116



Limited ψ and ϕ angles

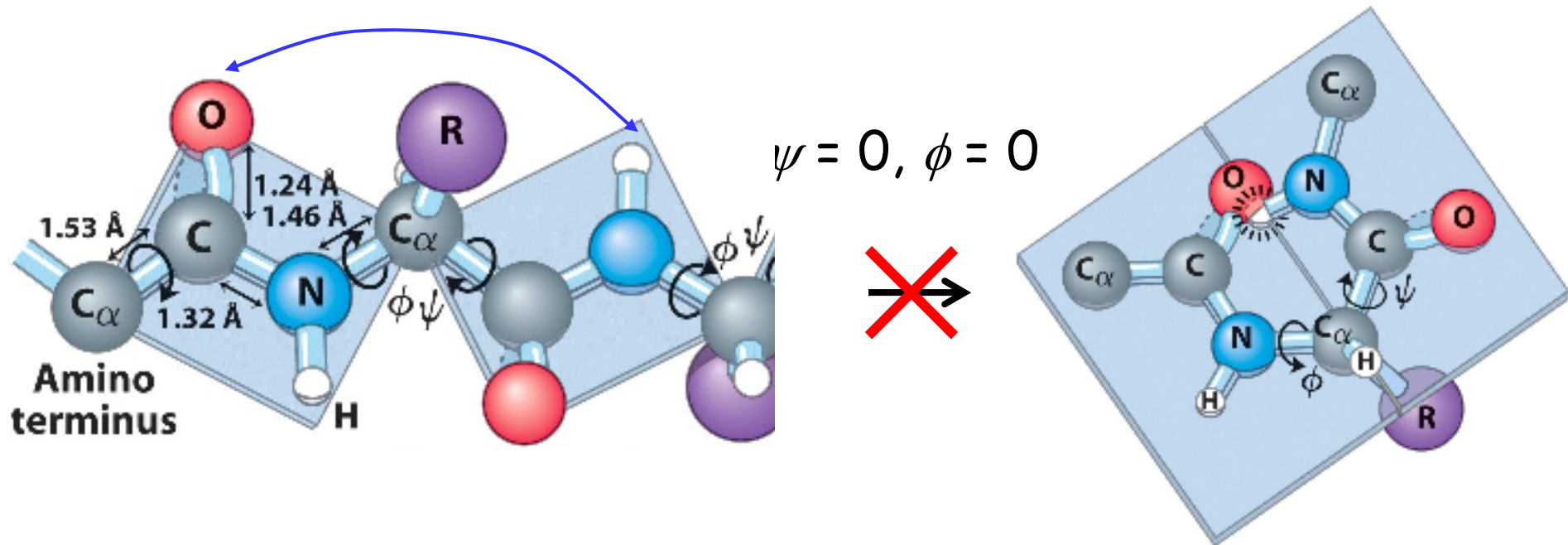


Fig 4-2, from 4th ed.

- Steric interference between atoms in the backbone and side chains

Ramachandran plot

Plot ψ and ϕ for possible conformations

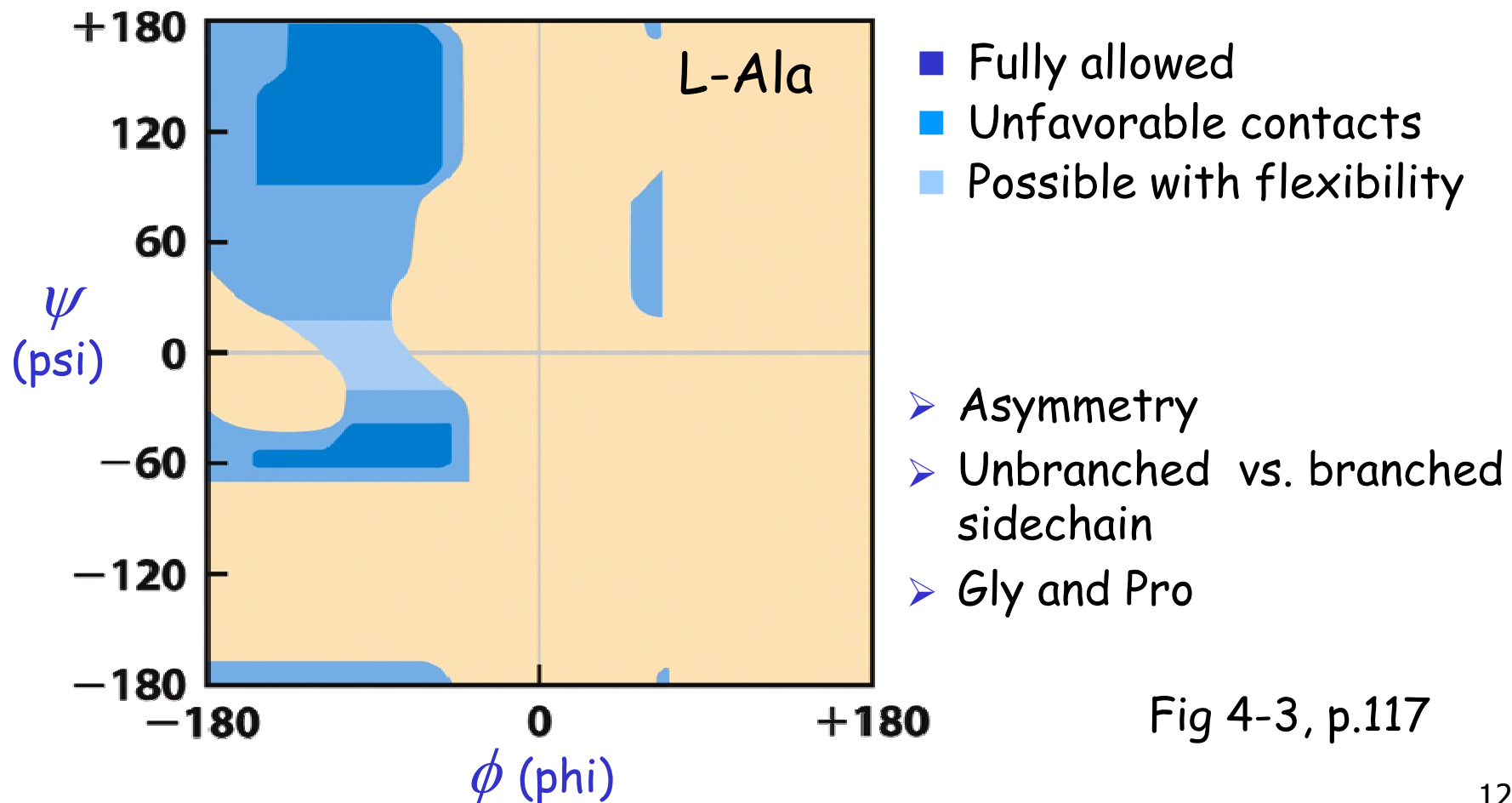
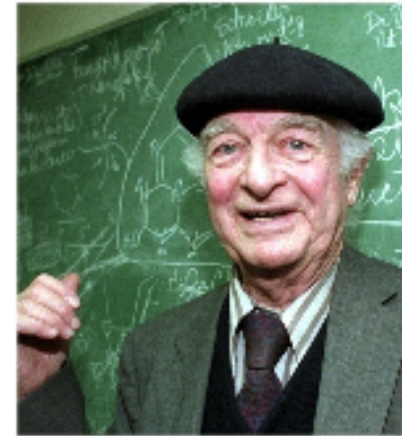


Fig 4-3, p.117

Protein secondary structure

p. 117

- Secondary structure
 - ✓ Local arrange of main-chain atoms
 - ✓ Nearly the same ψ and ϕ
 - ◆ α -helix
 - ◆ β -sheet
 - ◆ β -turn
- Determined by primary structure
 - ✓ Planar peptide bond
 - ✓ Limited ψ and ϕ
 - ✓ Charge and size of the R-groups
- Stabilized by H-bond



Linus Pauling



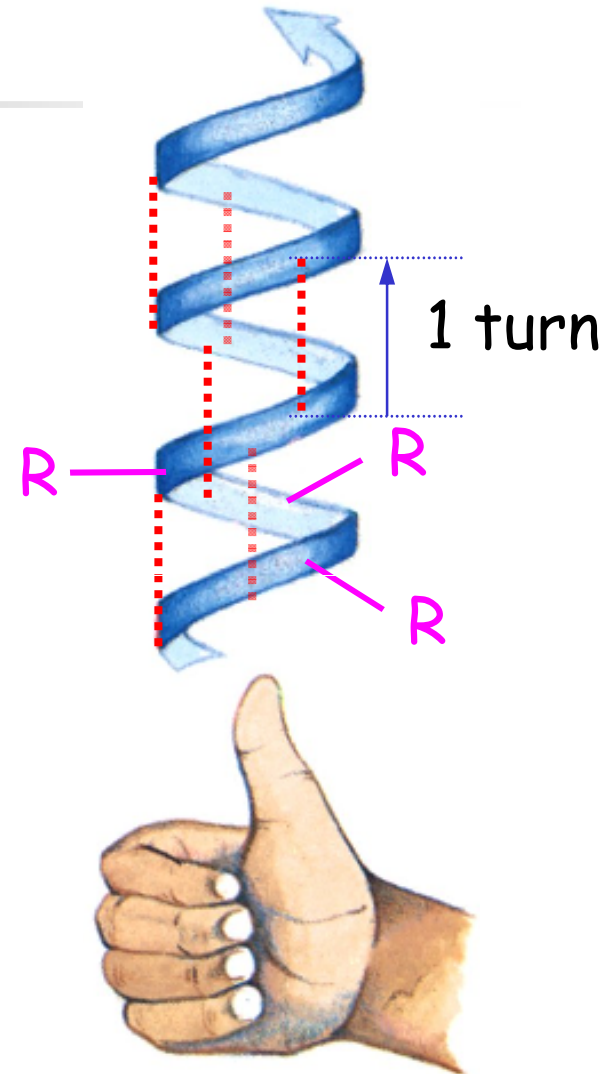
Robert Corey

α -helix

p. 117-118

A right-handed α -helix:

- *3.6 a.a.* per turn
 - $\psi = -45$ to -50° and $\phi = -60^\circ$
- *5.4 Å* (1 Å = 0.1 nm) per turn
- R groups extended *outward perpendicular* to the helical axis
- *H-bonding* between adjacent turns



Box 4-1, p.118

H-bond in α -helix

p.118-119

- H-bond between the -C=O of residue (i) and the -NH of residue (i+3).
 - 13 atoms between a H-bond
- 2 H-bonds per residue
- 3 or 4 H-bonds per turn
- Provide stability

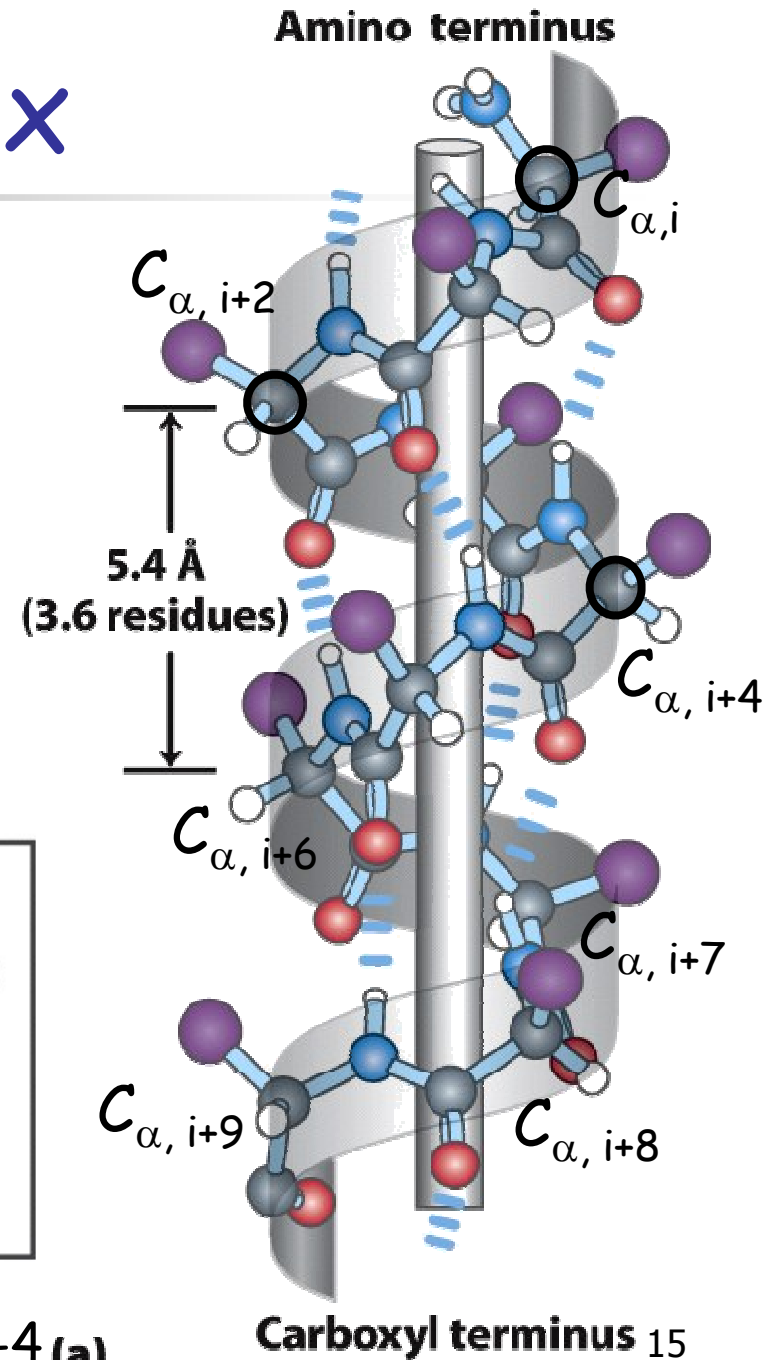
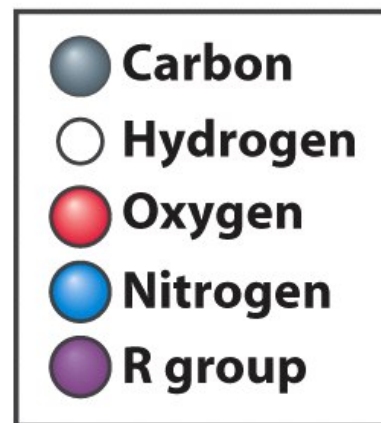


Fig 4-4 (a)

α -helix constraints

1. Helix-forming propensity of each a.a.
 - See Table 4-1 (p.119)
2. Size of the adjacent R group
3. Interactions between R_i and R_{i+3} or R_{i+4}
4. Pro and Gly
 - See Table 4-1 (p.119)
5. End residues (electric dipole)
 - See Fig 4-5 (p.120)

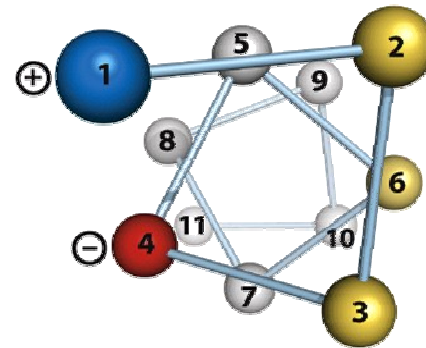
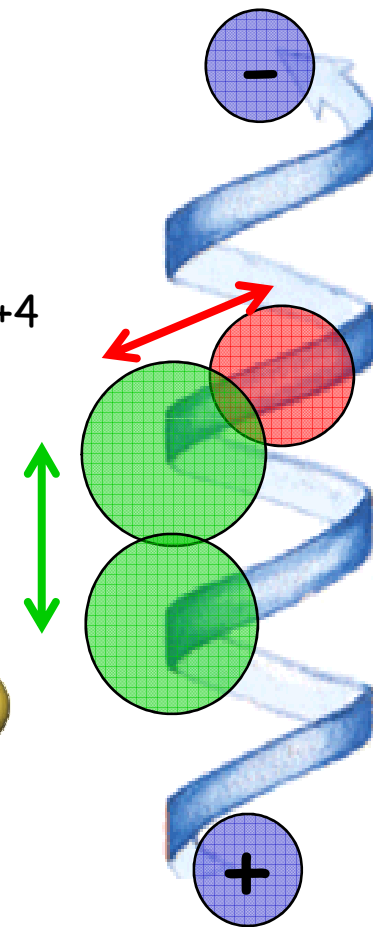


Fig 4-4d

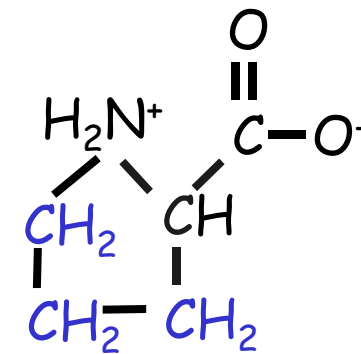
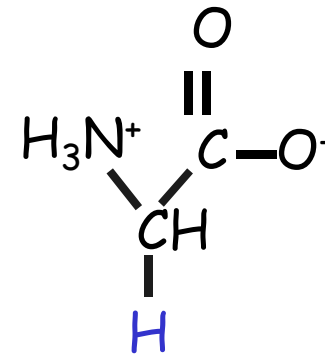


Propensity to form α -helix

TABLE 4-1

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



p. 119

- $\Delta\Delta G^\circ$ relative to Ala. Larger numbers reflect greater difficulty taking up the α -helical structure

Electric dipole of an α -helix

- Peptide bond dipole
- Helix dipole
- End residues and helix stability

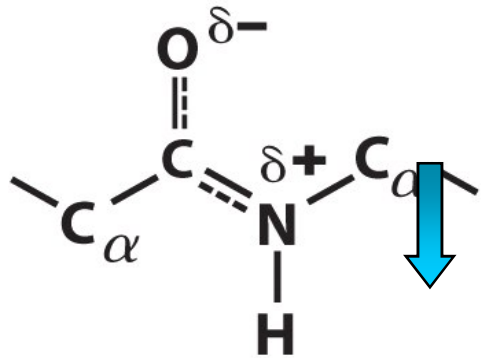
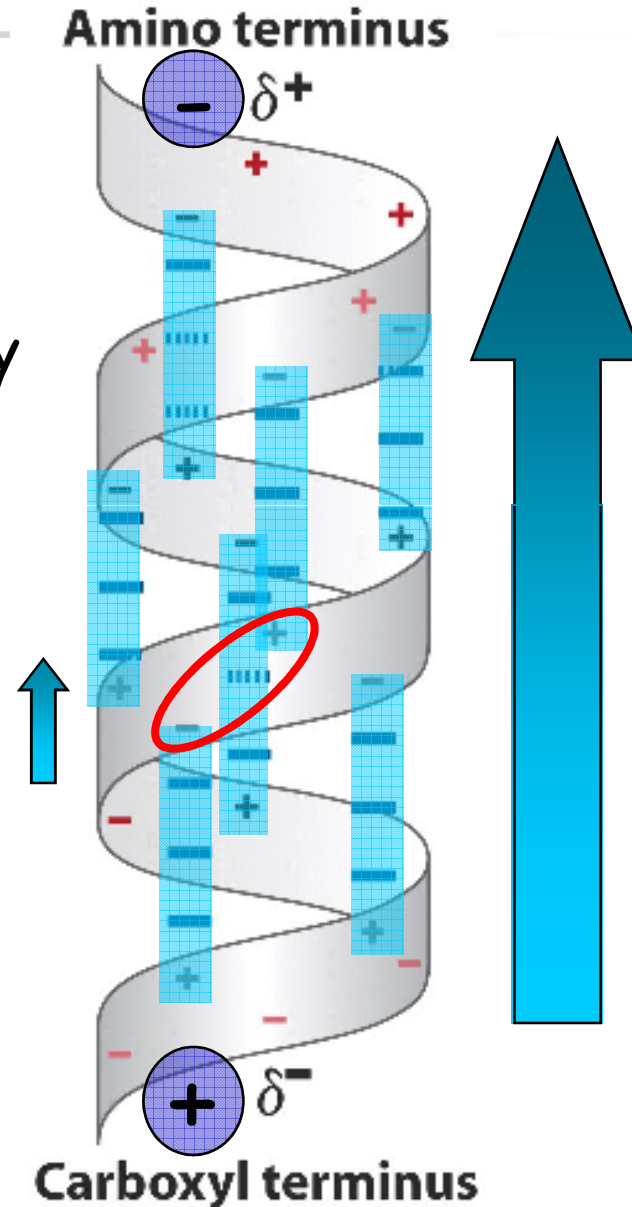


Fig 4-2a

Fig 4-5, p.120





α -helix model

- Ball-and-stick
- Space-filling (with van der Waals)
- Example: α -keratin in hair

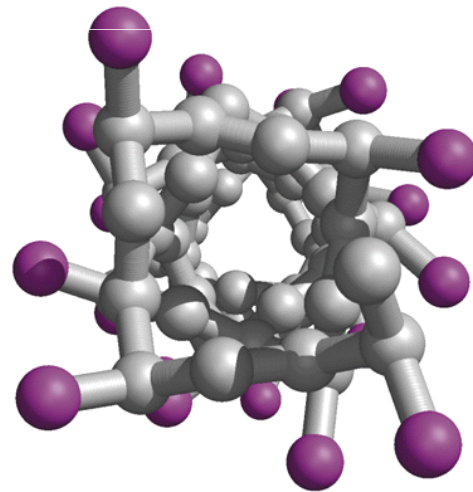
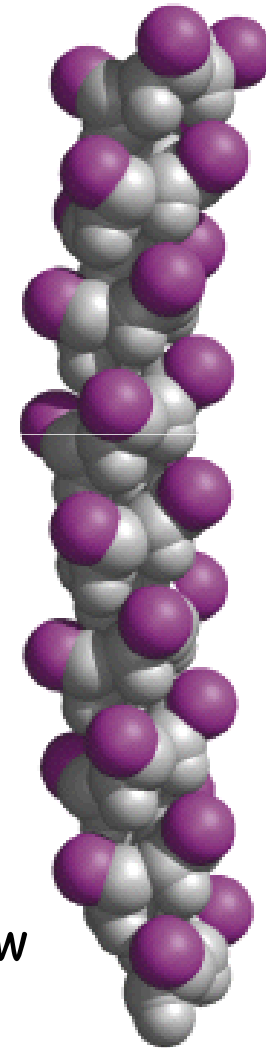


Fig 4-4b,c

Top view

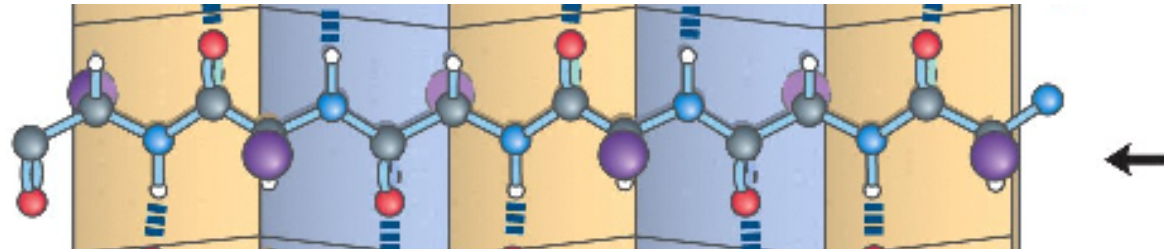


Side view

β -conformation

- Zigzag
- Extended protein chain, with the R groups **alternating above and below the backbone.**

Top view



Side view

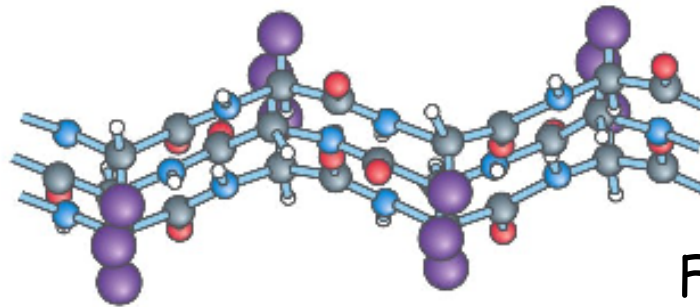
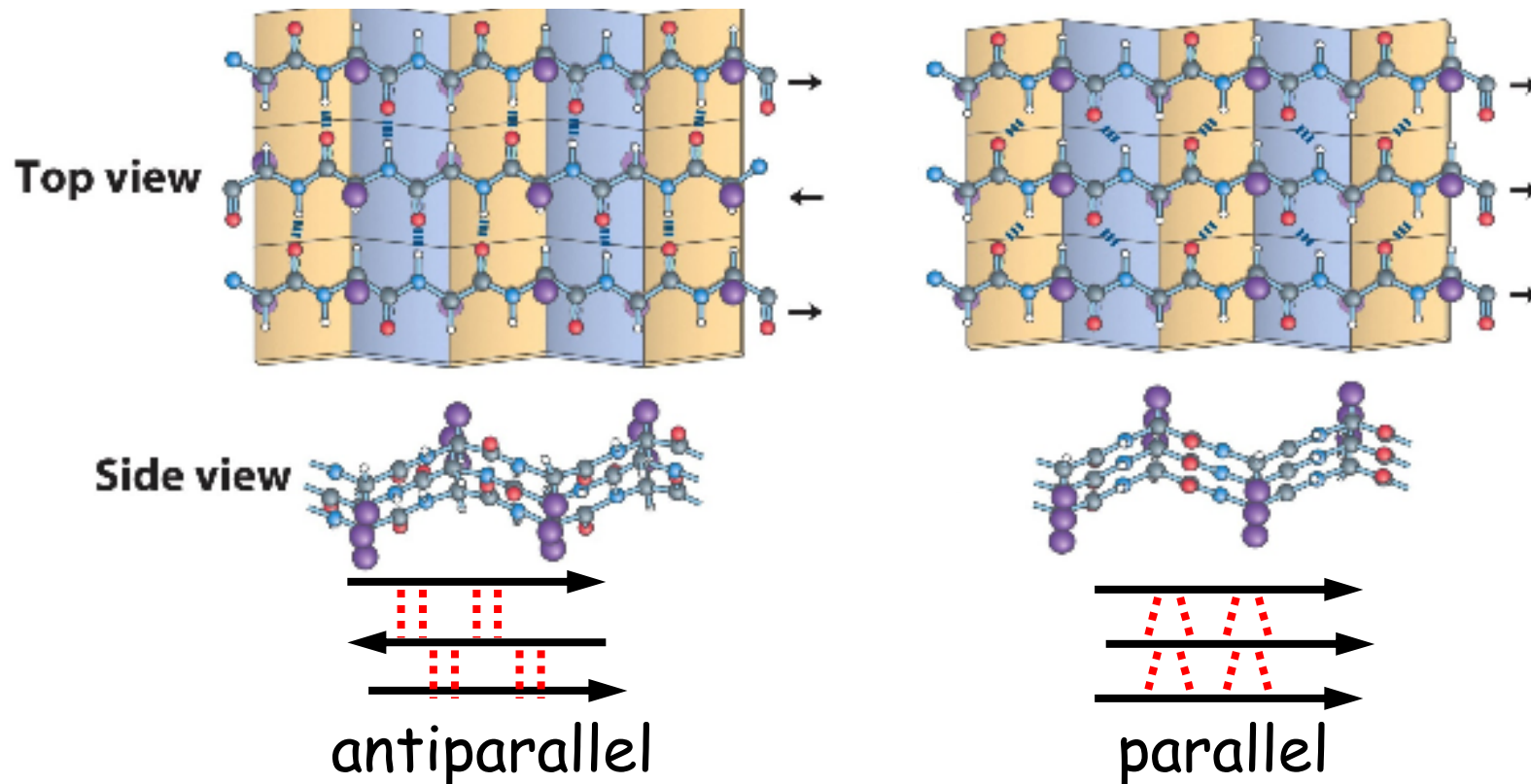


Fig 4-6a, p.120

β -sheet

- Inter- or intra-molecular H-bond
- Parallel or antiparallel orientations
 - ✓ Silk fibroin - layers of β -sheets (Fig 4-13)

Fig 4-6, p.120



β -turn

- A 180° turn involving 4 a.a.
- H-bond between -C=O of the 1st a.a. and the -NH of the 4th a.a.
- Common a.a.
 - ✓ Gly (small and flexible, type II β -turn)
 - ✓ Pro (peptide bonds involving the imino N in *cis* configuration)

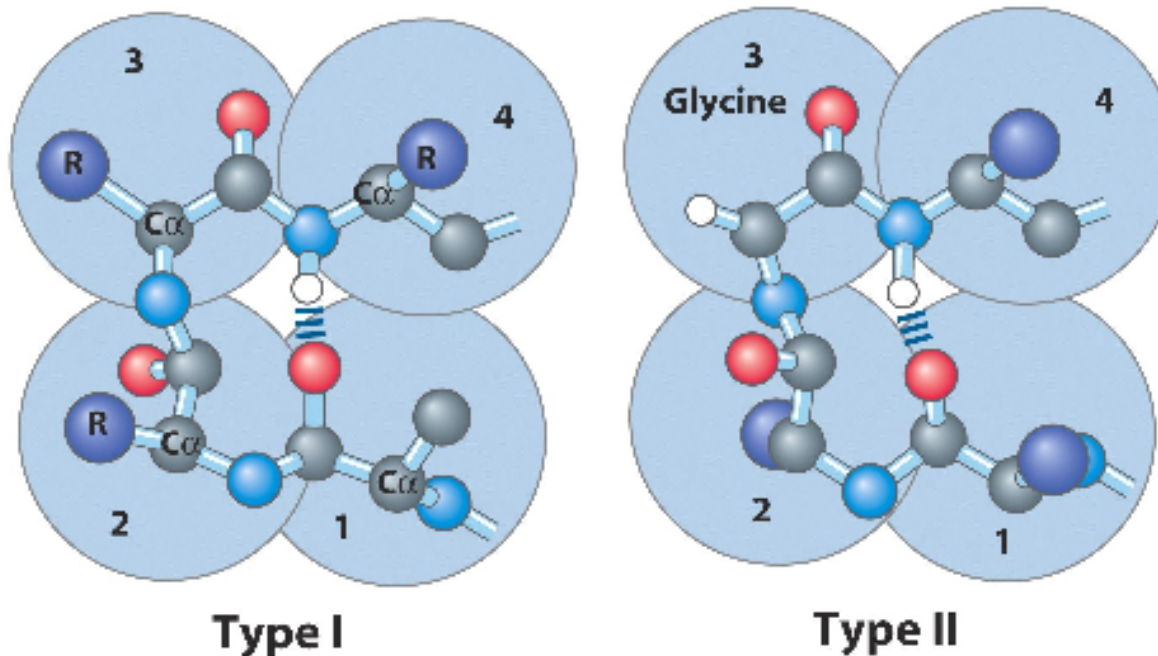


Fig 4-7a,
p.121

ψ and ϕ in 2° structures

- Restricted range in α -helix and β -sheets

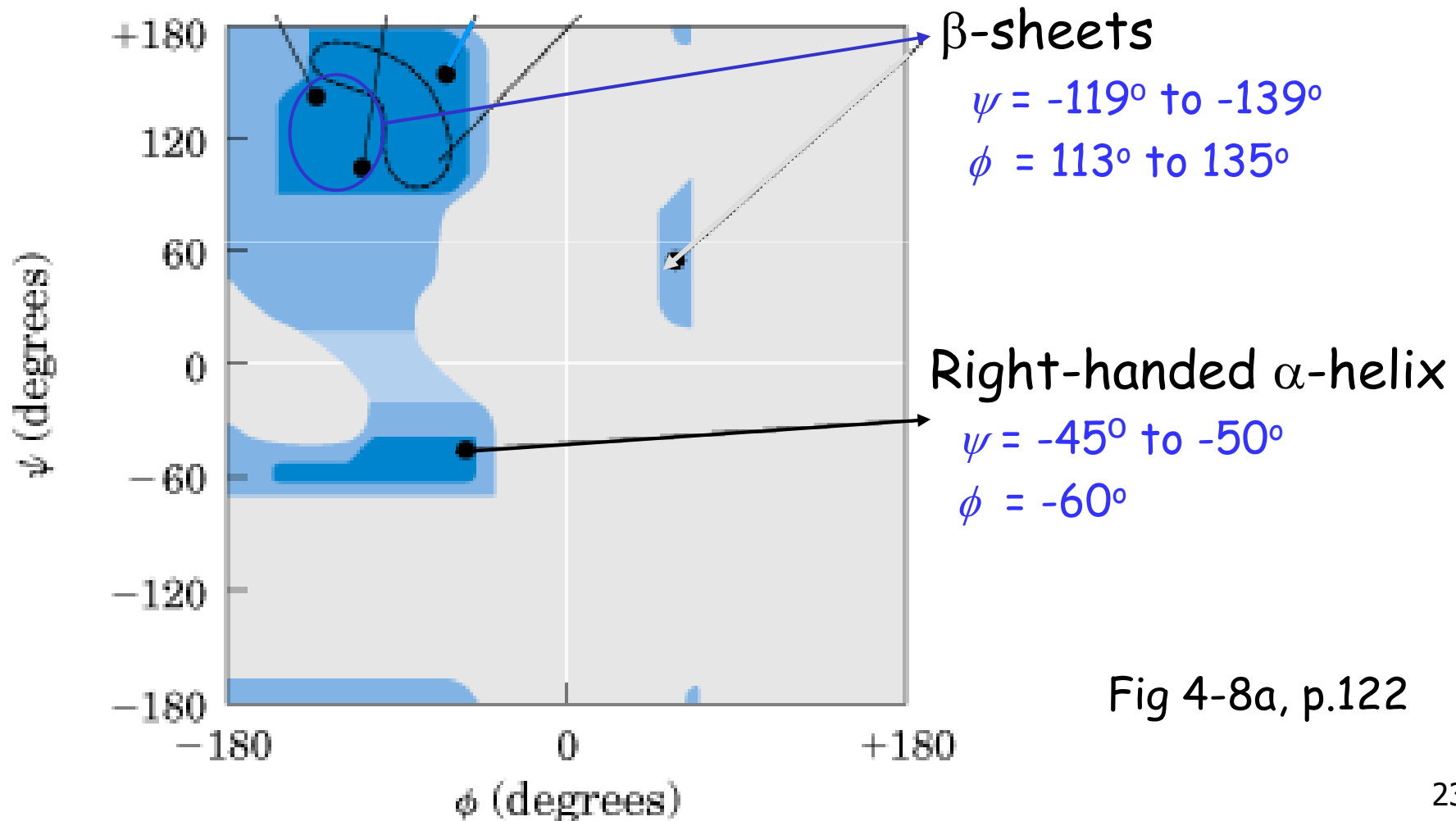


Fig 4-8a, p.122

Circular Dichroism Spectroscopy

- CD, 圓二色極化光譜儀
 - ✓ Structural asymmetry gives rise to differences in absorption of left-handed vs. right-handed plane-polarized light.
 - ✓ Determine the content of 2° structure of a protein

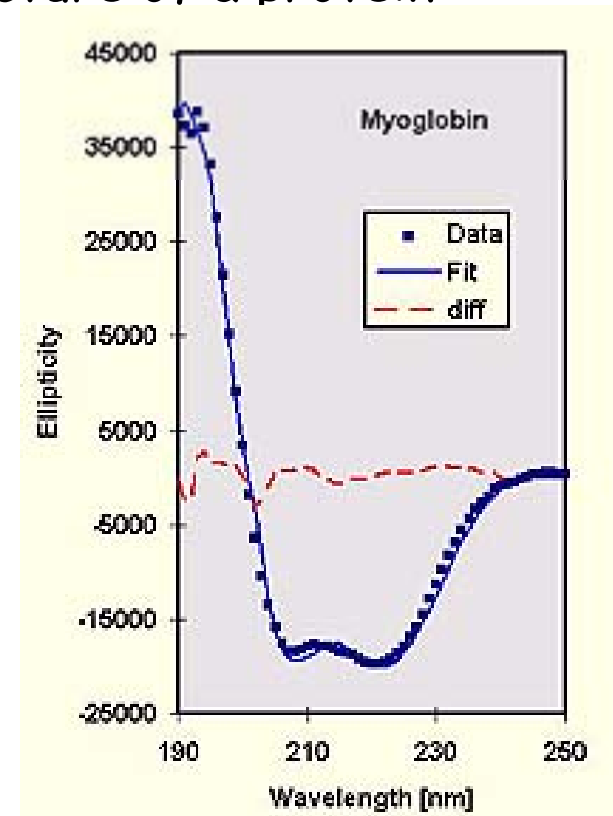
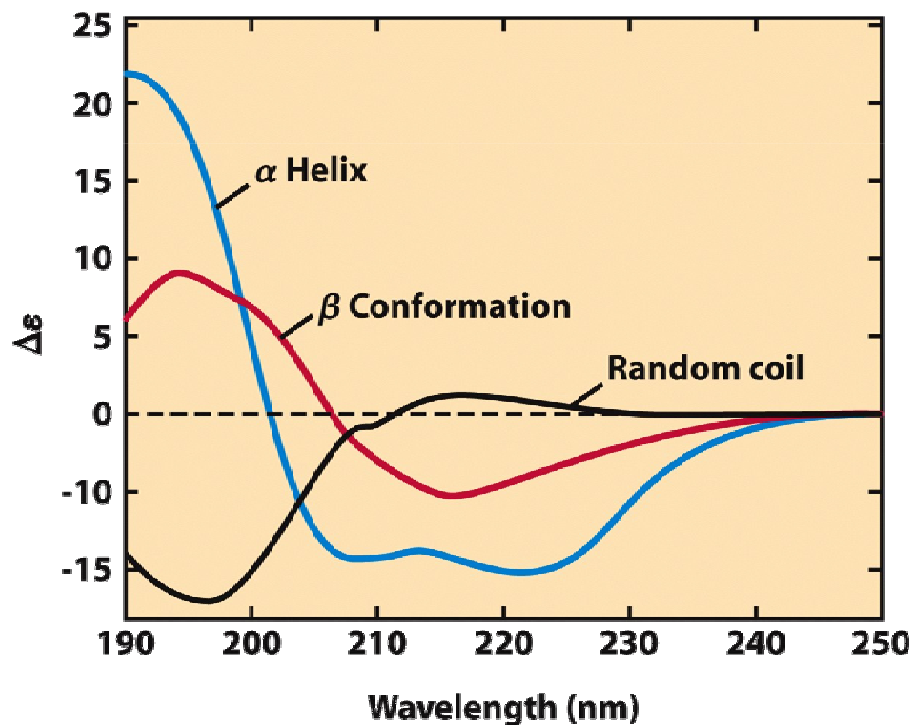
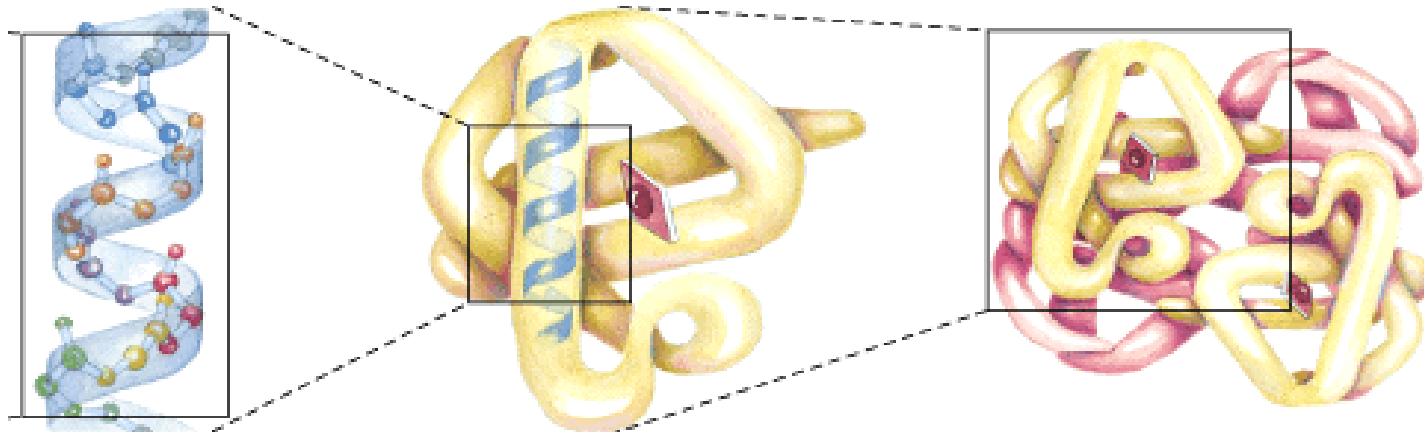


Fig 4-9, p.122

3° and 4° structure

- 3-D structure
- Protein *conformation* in space
- Including *long-range* interactions
- Determined by:
 - ✓ Primary (and secondary) structures
 - ✓ Interactions among R groups
 - ✓ Disulfide bond and weak interactions





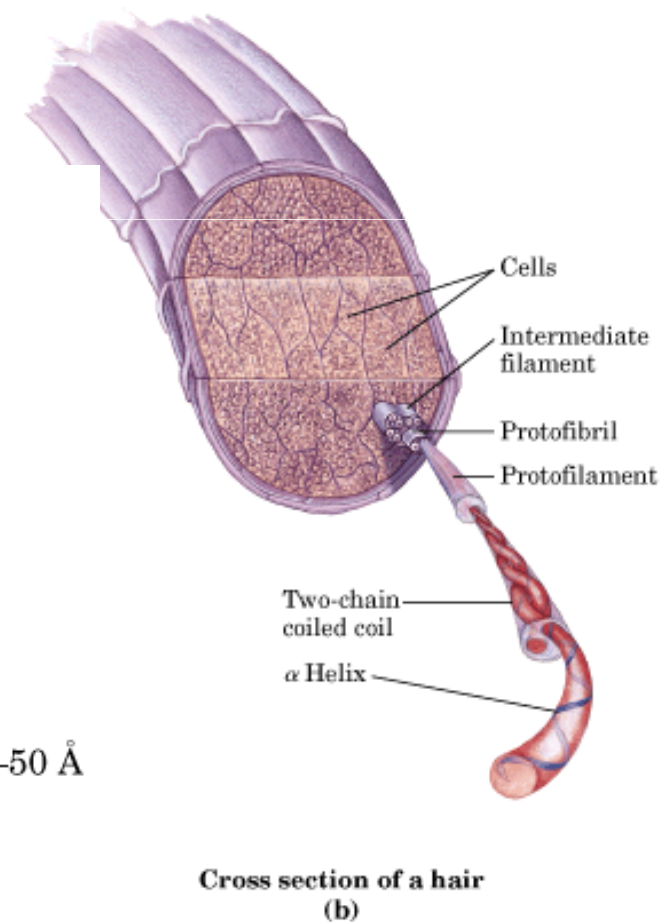
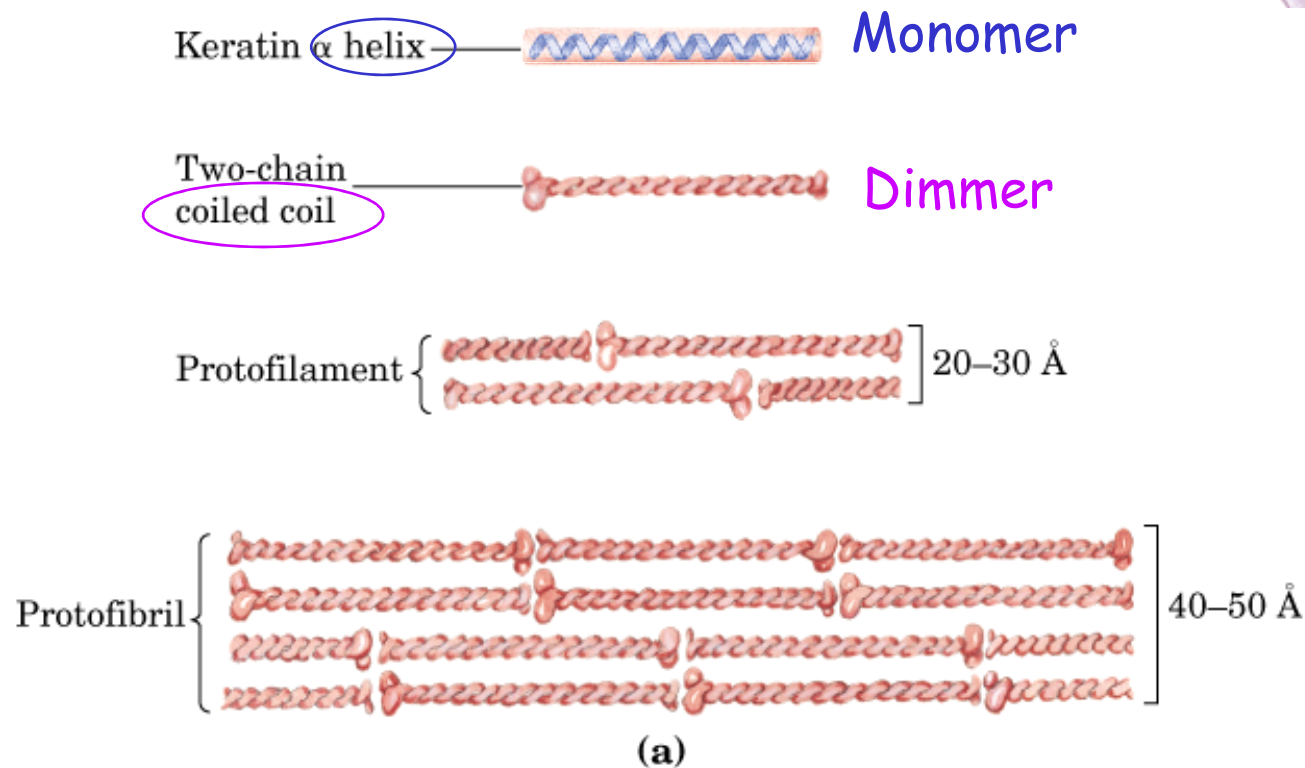
Structural classification

- Fibrous proteins (p. 123-128, e.g. Table 4-2)
 - ✓ Long strands or sheets
 - ✓ Consist of a single type of 2° structure
 - ✓ Function in structure, support, protection
 - ✓ α -keratin, collagen
- Globular proteins (p. 129-131, e.g. Table 4-3)
 - ✓ Spherical or globular shape
 - ✓ Contain several types of 2° structure
 - ✓ Function in regulation
 - ✓ Myoglobin, hemoglobin

Structure of hair

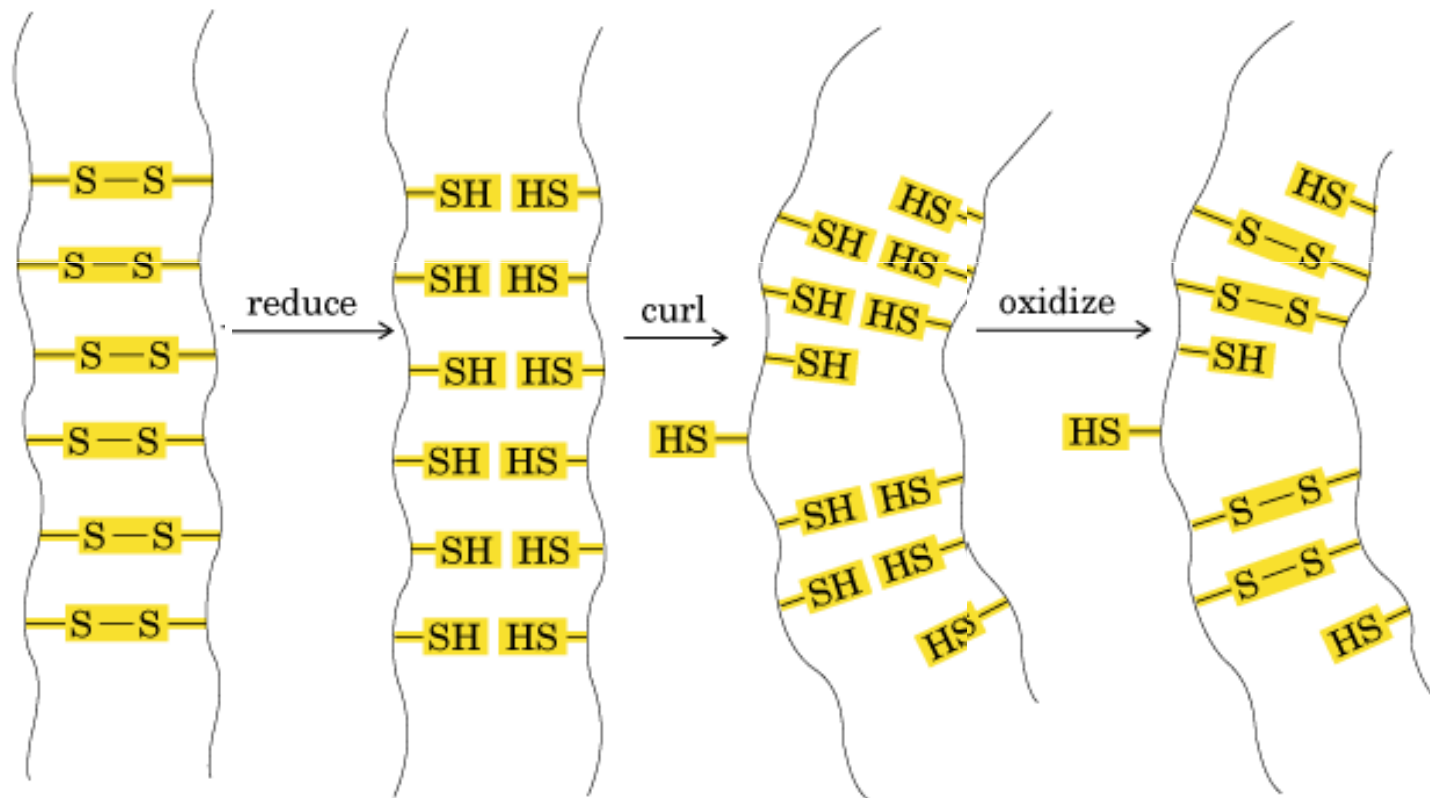
α -keratin: hair, wool, nails, claws, quills, horns, hooves, and the outer layer of skin

Fig 4-10, p. 124



Disulfide bond and Perm

- Permanent waving and biochemical engineering

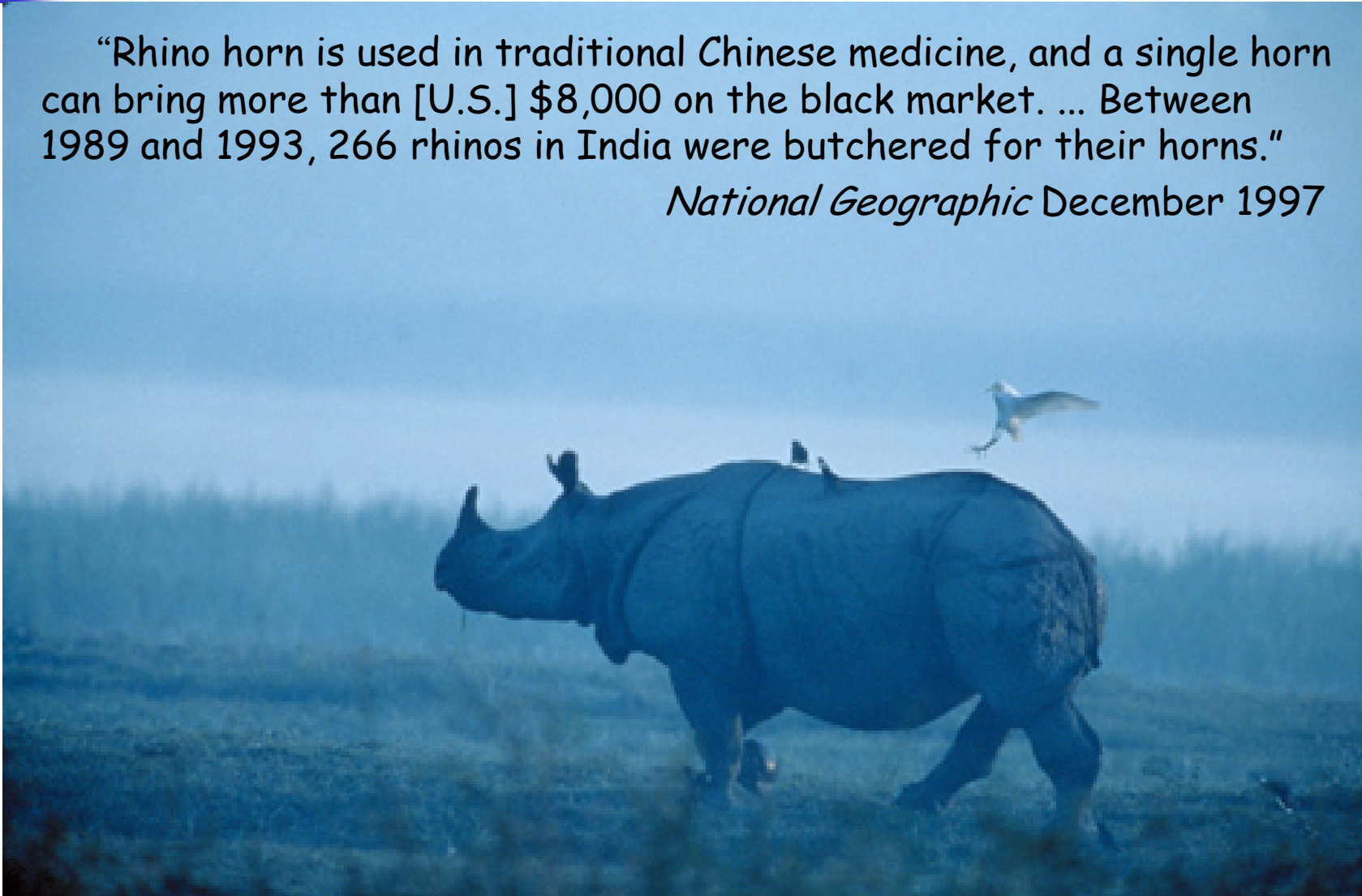


Box 4-2, p. 125

打破犀牛角的迷思 - 角質素(keratin)

“Rhino horn is used in traditional Chinese medicine, and a single horn can bring more than [U.S.] \$8,000 on the black market. ... Between 1989 and 1993, 266 rhinos in India were butchered for their horns.”

National Geographic December 1997

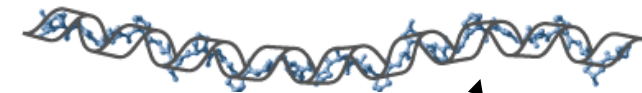


Collagen

p. 124-128

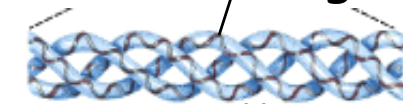
- Tendons, bone, cartilage, skin, and cornea
- Primary sequence:
 - ✓ Gly-X-Pro (HyPro)
 - ✓ Repeating tripeptide unit
- Structure
 - ✓ Monomer (α chain)
 - ✓ Left-handed helix, 3 a.a. per turn
 - ✓ Trimer: coiled-coil (tensile strength).
 - ✓ Stabilized by H-bond
 - ✓ Crosslink between triple helixes (disulfide bond)
- Genetic defect: (p.128)
 - ✓ Osteogenesis imperfecta
 - ✓ Abnormal bone formation in babies
 - ✓ Ehlers-Danlos syndrome
 - ✓ Loose joint

Fig 4-11a

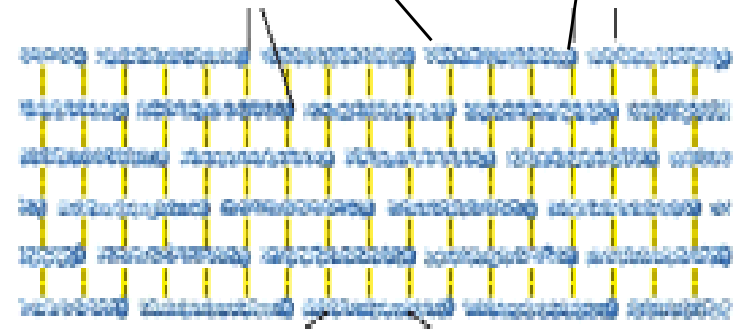


collagen

Fig 4-12

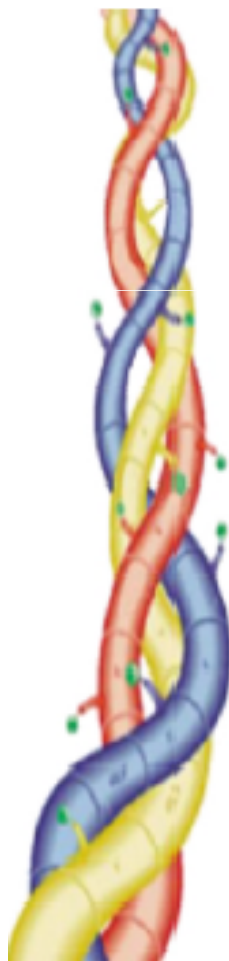


tropocollagen

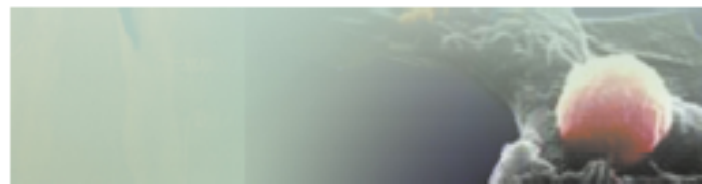


Collagen (膠原蛋白)

- 科學發展月刊九十三年八月號380期 專題報導



專題報導 膠原蛋白 / 陳怡瑜 劉中行 —— 4



避免變性的生醫材料 — 膠原蛋白 / 呂博文 —— 6



膠原蛋白讓你水嫩嫩 / 陳世輝 —— 12



組織工程中的膠原蛋白 / 湯淑娟 —— 18



生醫敷料及人工皮膚 / 黃頌雯 —— 24



膠原蛋白產品的功效 / 洪雅潔 —— 30



More on Collagen ...

Harper's 26th, p. 38-39.

- Procollagen (a larger precursor polypeptide)
 - ✓ Post-translational modification
 - ✓ Pro, Lys → Hydroxyl Pro, Lys (cofactor = ascorbic acid)
 - ✓ Provide H-bond that stabilizes the mature protein
 - ✓ Scurvy: a dietary deficiency of Vit C (Lehninger, Box4-3)
 - ✓ Central portion → triple helix (procollagen → collagen)
 - ✓ The N-, and C-terminal portions are removed
 - ✓ Certain Lys are modified by lysyl oxidase (a copper-containing protein)
 - ✓ Crosslink between polypeptides → increased strength and rigidity.
 - ✓ Menke's syndrome: a dietary deficiency of the copper

3D structure determination

- 1st protein structure solved
 - ✓ Myoglobin at 6Å resolution in 1957
 - ✓ By X-ray diffraction
 - ✓ John Kendrew and Max F. Perutz
 - ✓ 1962 Nobel prize in chemistry
- Box 4-5, p.132-135
 - ✓ X-ray diffraction and crystallography
 - ✓ Nuclear Magnetic Resonance (NMR)

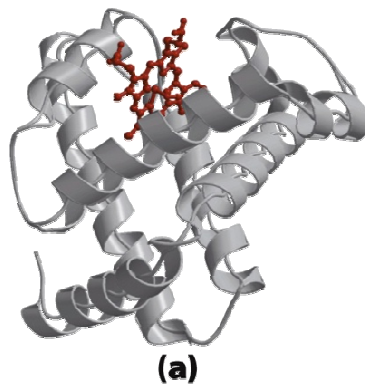


Myoglobin structure

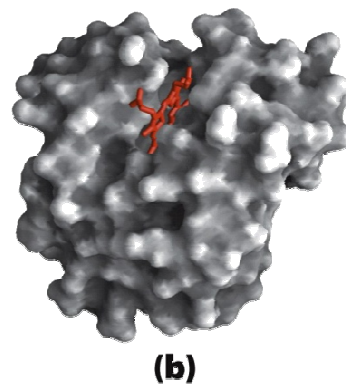
- Sperm whale (抹香鯨) myoglobin



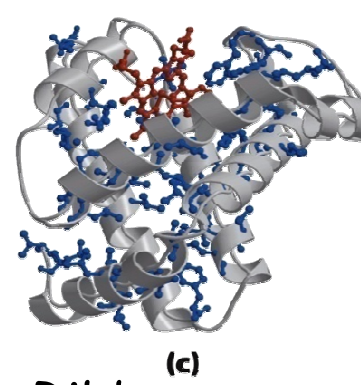
Fig 4-15, p. 130



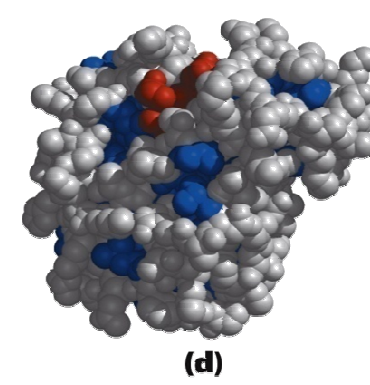
(a)
Ribbon



(b)
Surface contour



(c)
Ribbon + some
side chains



(d)
Space filling



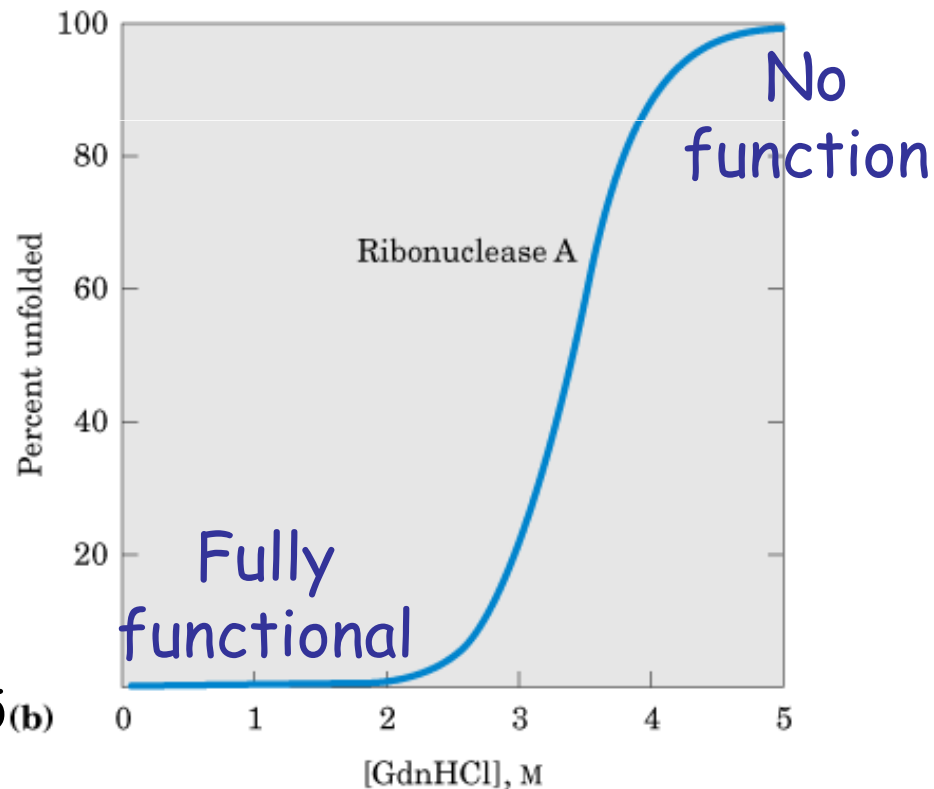
Protein folding

The polypeptide must *fold* during and following synthesis on a ribosome to take up its *native conformation*.

(p. 140)

Denaturation and unfolding

- Loss of function due the structural disruption
- Cooperative process
- Denatured conformation: random but partially folded



p. 141

Fig 4-25(b)

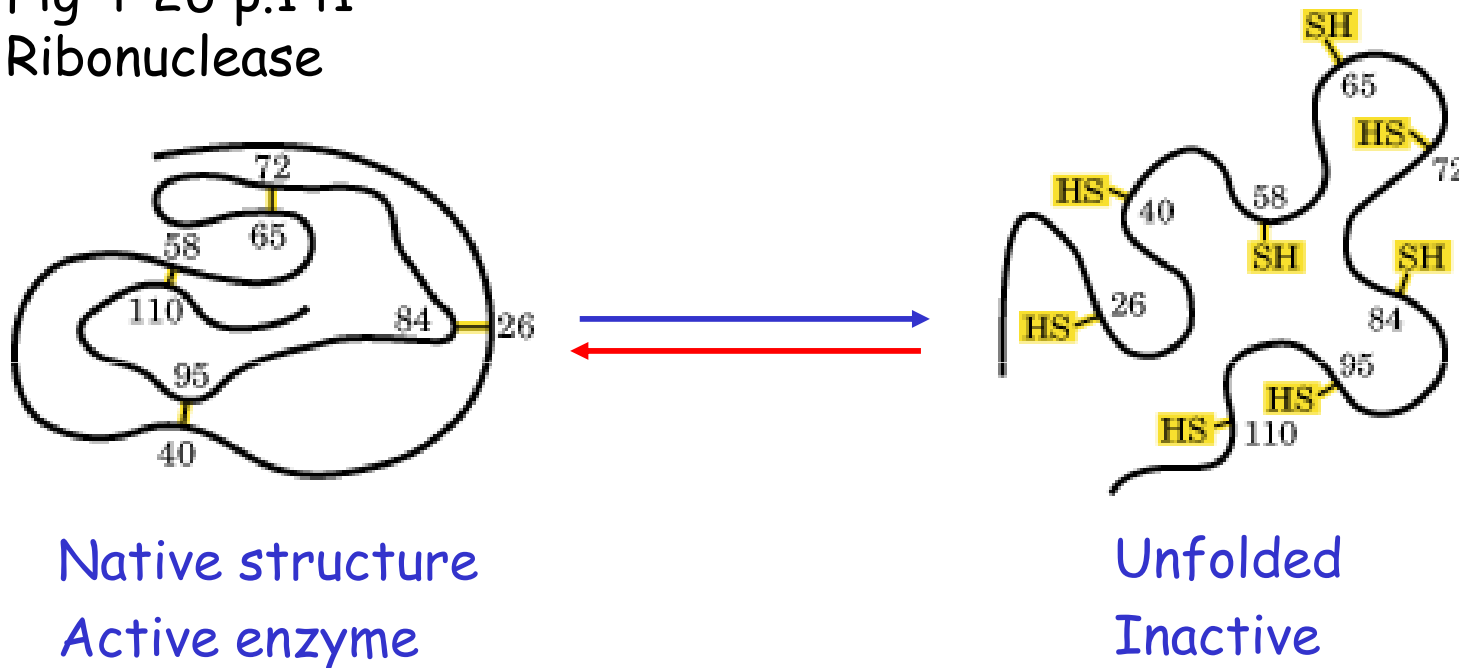


Denaturing agent (p. 141)

- Heat
 - ✓ H-bond
- Extreme pH
 - ✓ Alter net charge
- Miscible organic solvent
 - ✓ Alcohol, acetone
 - ✓ Hydrophobic interactions
- Certain solutes
 - ✓ Urea, guanidino hydrochloride (Gdn HCl), detergent
 - ✓ Hydrophobic interactions
- No covalent bonds in the polypeptide are broken !!

Folding and unfolding

Fig 4-26 p.141
Ribonuclease



- A.A. sequence contains required information for native protein folding



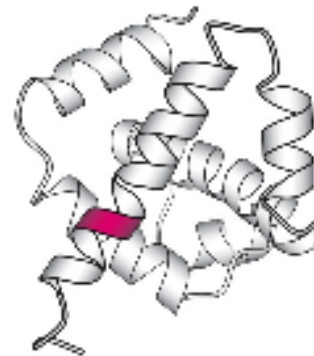
Assisted protein folding

p. 143-145

- Molecular chaperones
 - ✓ Proteins that interact with partially folded or improperly folded polypeptides
 - ✓ Facilitating correct folding pathways or providing microenvironments for folding.
- Hsp70
 - ✓ Heat shock protein, mw = 70,000 in eukaryotics
 - ✓ DnaK/DnaJ in *E. coli*
- Chaperonins
 - ✓ Protein complex required for proper folding
 - ✓ GroEL/GroES in *E. coli*

The prion disease (Box 4-6)

- Spongiform encephalopathies
 - ✓ Disease caused by a protein (prion)
 - ✓ Proteinaceous infectious particle
- Related diseases:
 - ✓ Mad cow disease
 - ✓ Kuru
 - ✓ Creutzfeldt-Jakob disease (human CJD)
 - ✓ Scrapie (sheep)
- Misfolded prion



Pr^{PC}
(normal)



Pr^{PSC}
(infectious)



Ch 4 Summary

- 3-D structure of protein
 - ✓ Planar peptide bond
 - ✓ Level of organization
 - Primary (1°)
 - Secondary (2°): α -helix, β -sheet
 - Tertiary (3°): motif, fold, domain
 - Quaternary (4°)
 - ✓ Protein folding vs. unfolding
 - ✓ Assisted protein folding
- Problems: 3, 7, 9