Tertiary structure

- Folding of secondary structure (hiding polar backbones)
- Positions of side chains

Protein structure determination by

- X-ray crystallography
- nuclear magnetic resonance (NMR)

Protein crystals

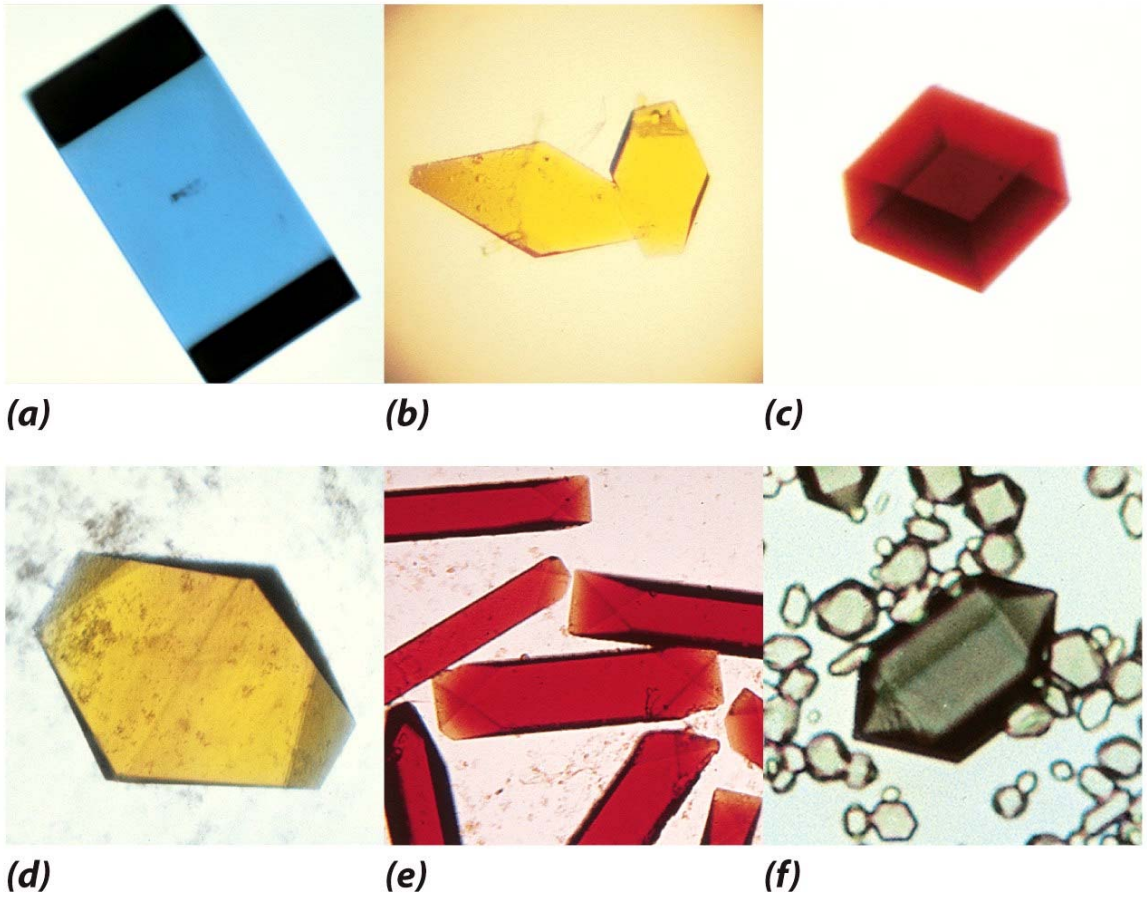
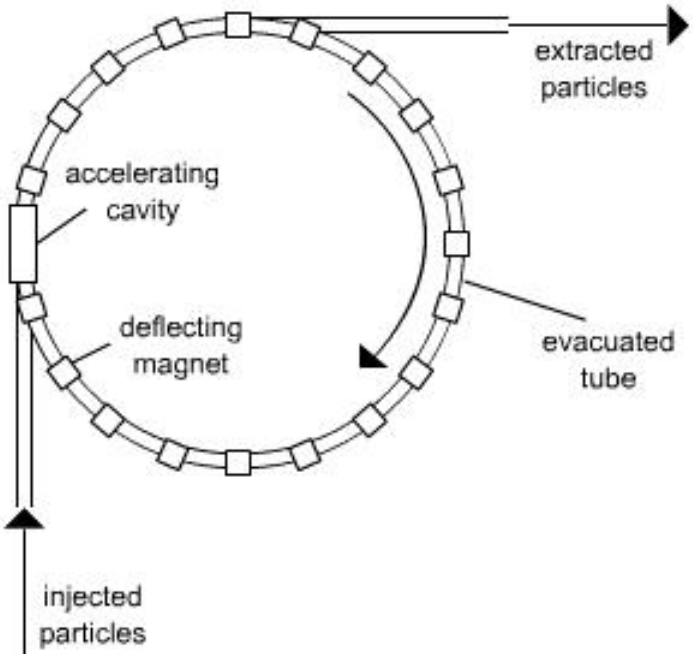
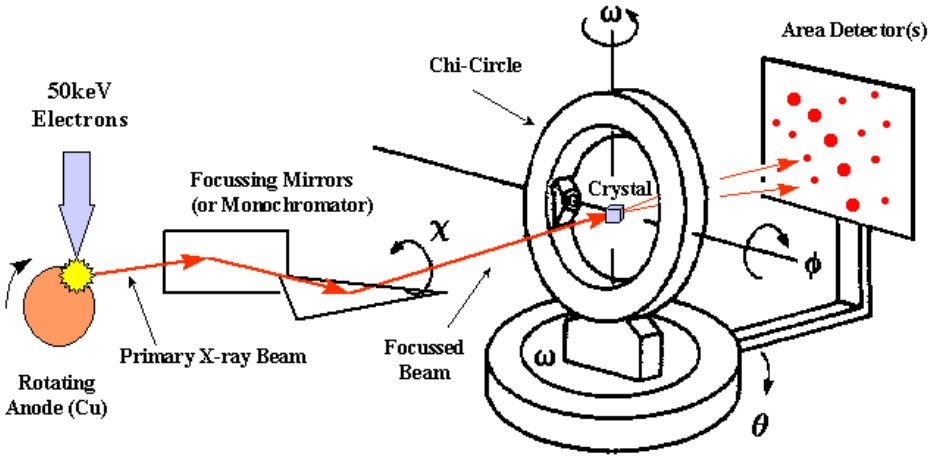



Figure 6-21 Fundamentals of Biochemistry, 2/e




4-Circle Goniometer ( Eulerian or Kappa Geometry)

X-ray crystallography

X-ray generator

Synchrotron

X-rays interact with the electrons in the protein: diffraction pattern

Converted into electron density maps except hydrogen (having only one electron)

A series of diffraction patterns are taken from several angles: 3D structure

Mathematical refinement based on primary structure to position atoms to as little as 0.1 Å

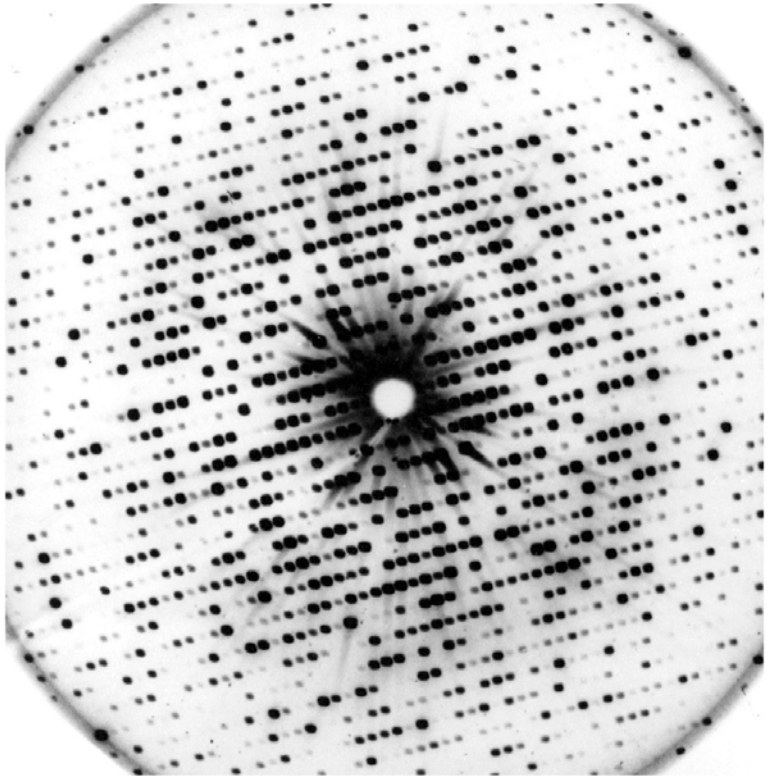
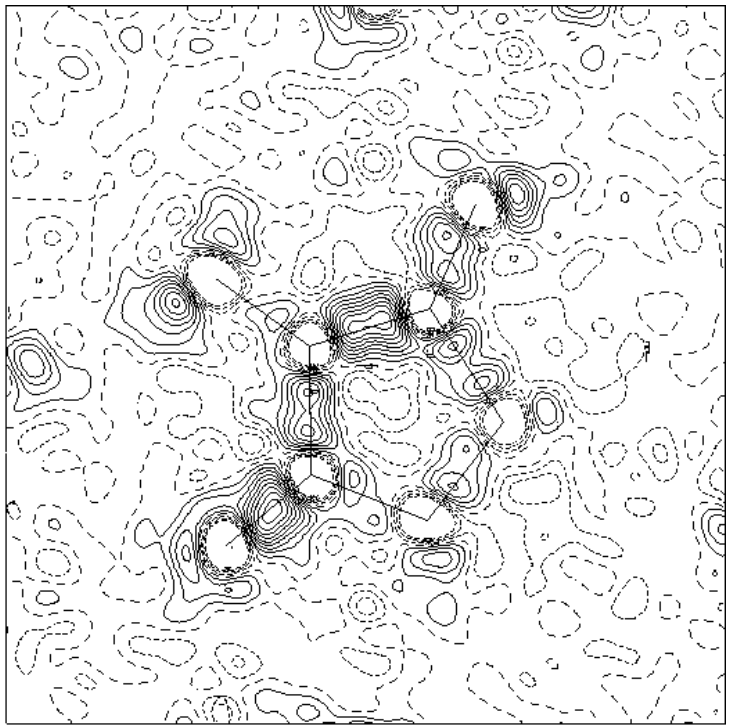


Figure 6-22 Fundamentals of Biochemistry, 2/e

X-ray diffraction photograph of
A crystal of sperm whale myoglobin



Electron density map contoured in 3 dimensions

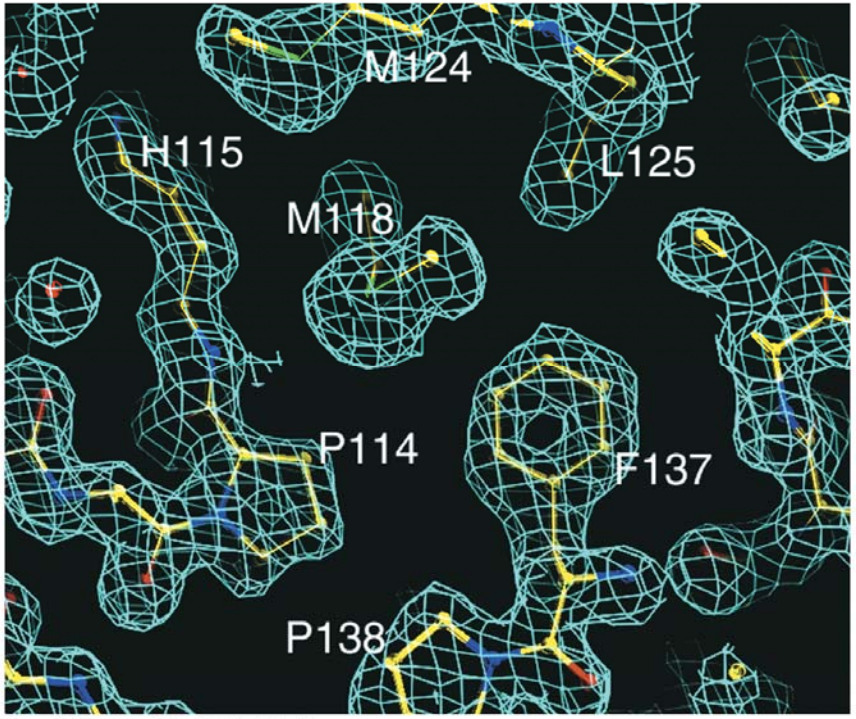



Figure 6-23 Fundamentals of Biochemistry, 2/e

Section through the electron density map calculated at the indicated resolution levels

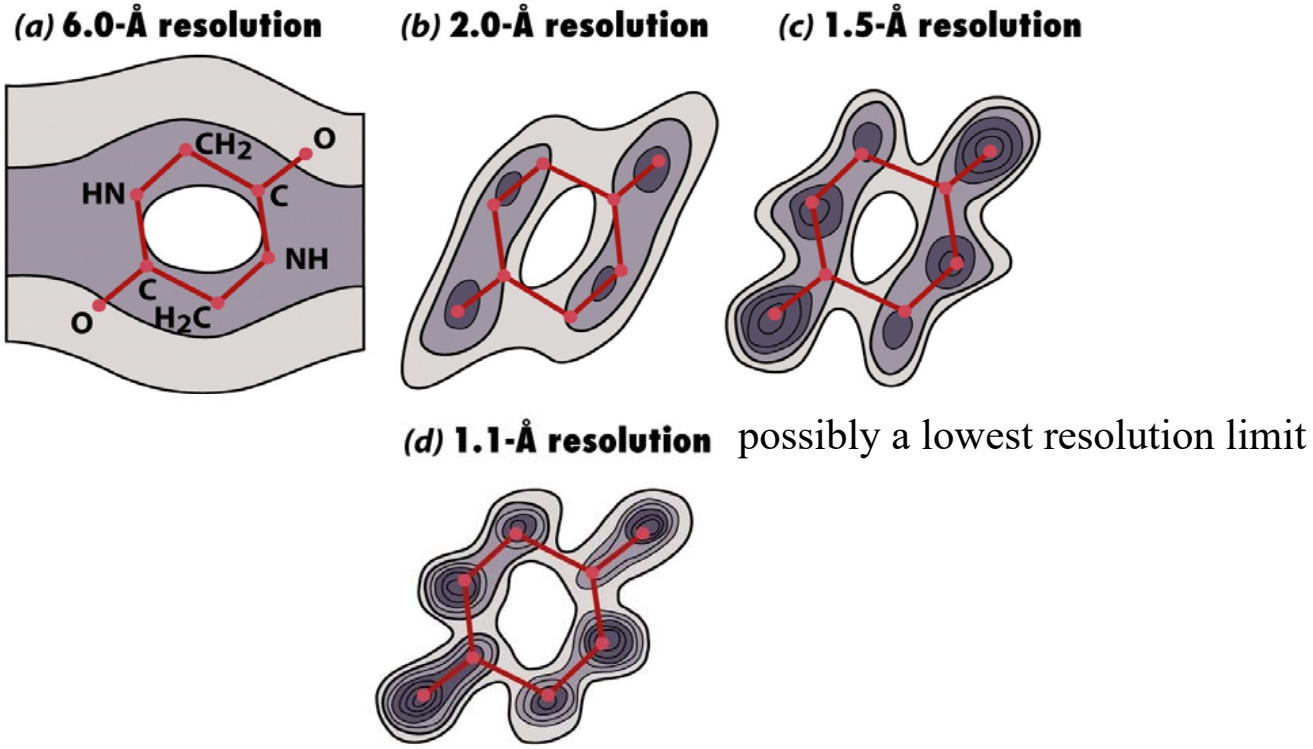



Figure 6-24 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

possibly a lowest resolution limit

<u>Crystalline proteins assume very nearly the same structures that they have in solution</u>

Crystalline protein has 40-60% water content, which is similar to that of many cells

Crystalline protein prepared in different conditions have identical conformations

NMR structure coincides with crystalline protein structure

Many enzymes are catalytically active in the crystalline state

Protein structure determination by NMR

- In aqueous solution
- 2D NMR spectroscopy
- Smaller proteins: less than ~40 kD
- Solution conformation of surface structure
- Motions over time: useful for the study of protein folding and dynamics

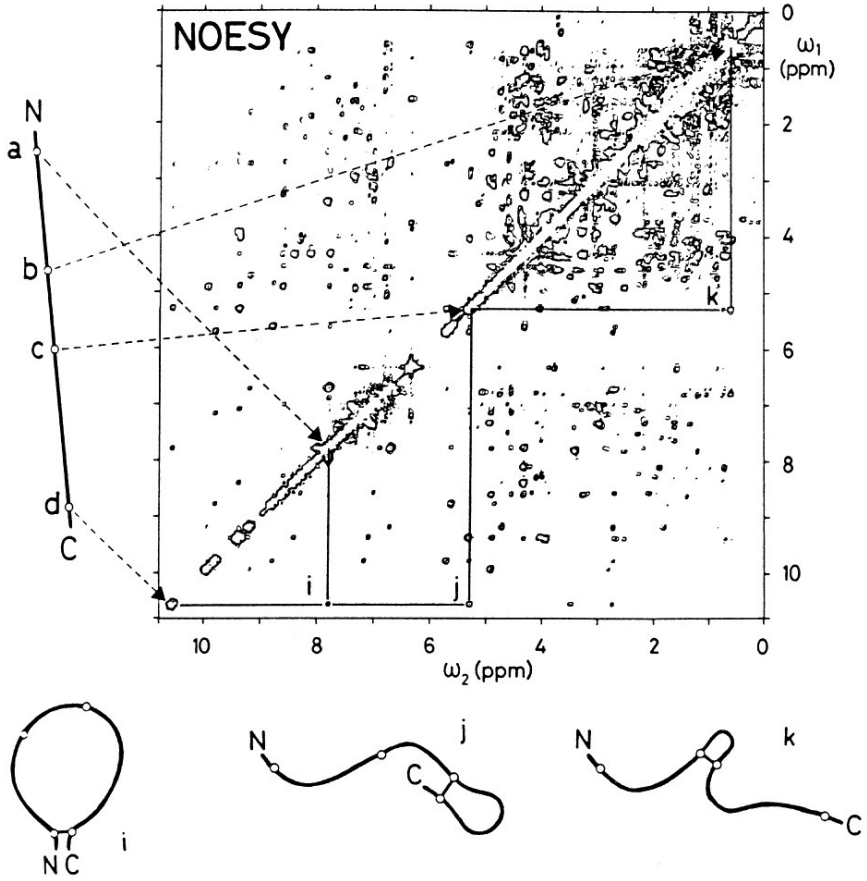



Figure 6-25a Fundamentals of Biochemistry, 2/e

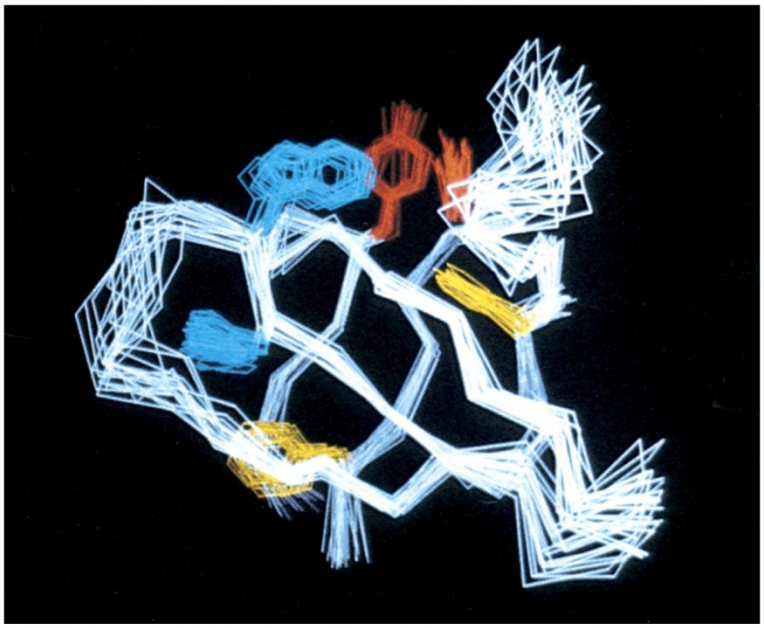


Figure 6-25b Fundamentals of Biochemistry, 2/e

# Side chain location and polarity

General principles obtained from protein structures, which are currently more than (?)
http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.html
The primary structures of globular proteins lack the repeating sequences
Side chain location varies with polarity
- nonpolar residues: interior
- charged polar: surface
- uncharged polar: surface and interior

Most proteins are quite compact, generally excluding water inside

White: backbone
Yellow & brown: nonpolar
Purple: polar

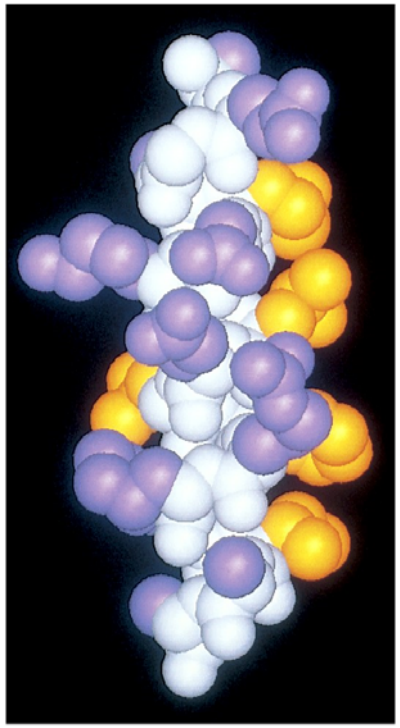


Figure 6-26a Fundamentals of Biochemistry, 2/e

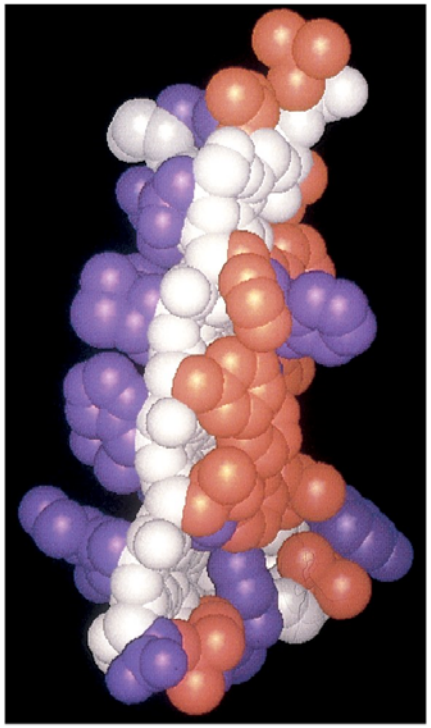


Figure 6-26b Fundamentals of Biochemistry, 2/e

Side chain distribution in horse heart cytochrome c

Green: hydrophilic
Orange: hydrophobic
Yellow: heme group

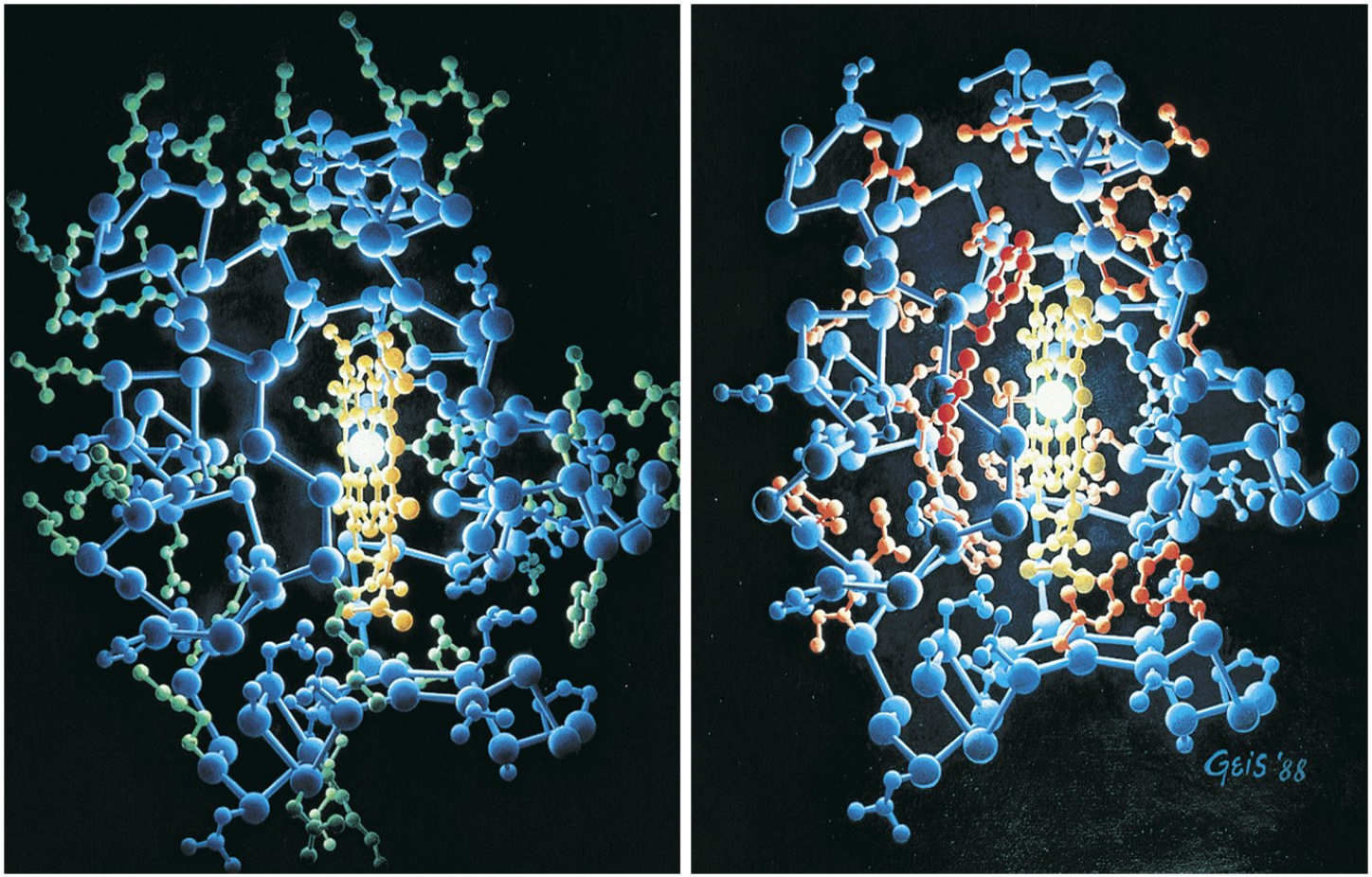



Figure 6-27 Fundamentals of Biochemistry, 2/e

A selection of protein structures

*E. coli* cytochrome c (256B): α-helix

Human Ig Fab (7FAB): β sheet

Dogfish lactate dehydrogenase (6LDH): α-helix and β sheet

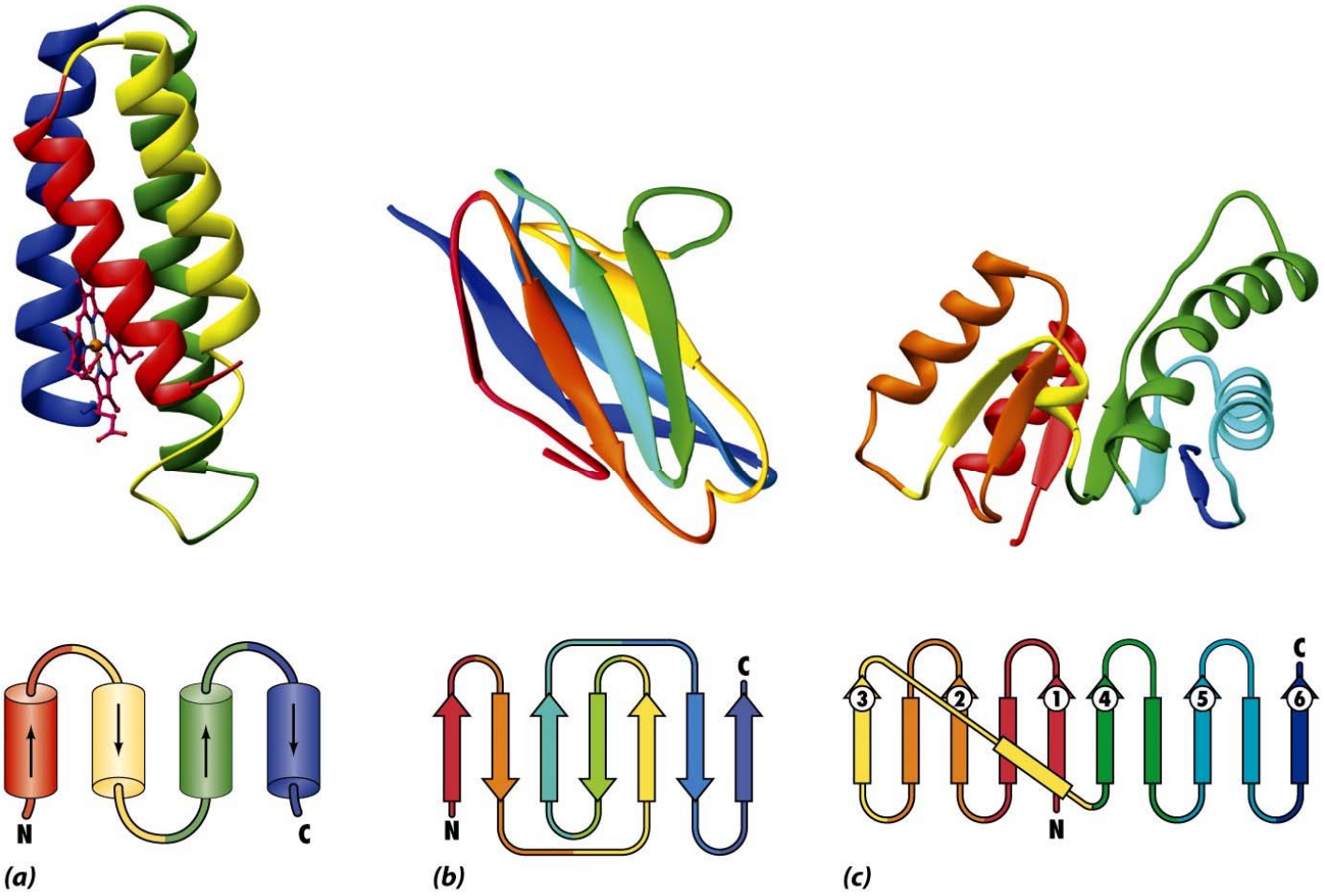



Figure 6-28 Fundamentals of Biochemistry, 2/e

# Supersecondary structures: motif

βαβ motif: two parallel strands of β sheet connecting an α helix

β hairpin motif: tight reverse turn

αα motif: two successive antiparallel helices

Greek key motif: 4-stranded antiparallel β sheet (cf. jelly roll)

β barrels: rolling of extended β sheets

Functional as well as structural significance:

two βαβαβ units combined to form dinucleotide-binding fold

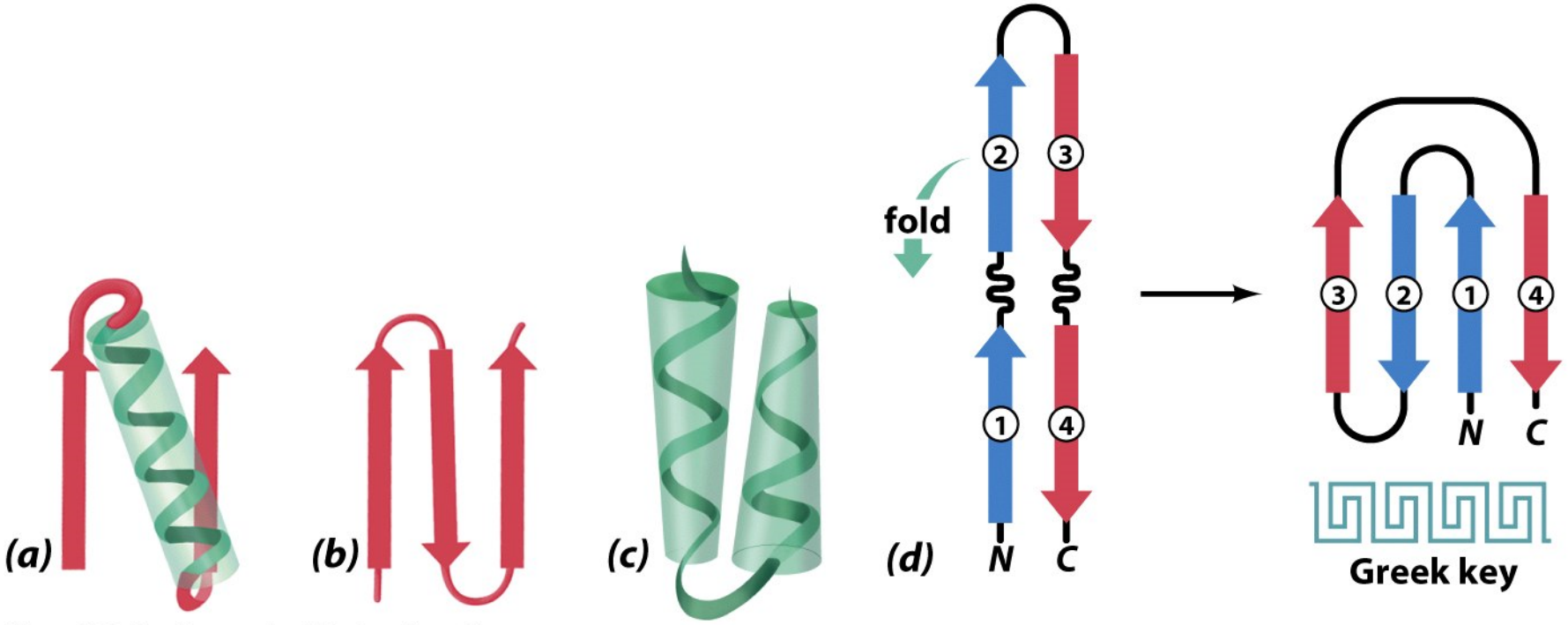



Figure 6-29 Fundamentals of Biochemistry, 2/e

# β barrels

retinol binding protein (1RBP)

peptide-N4-(N-acetyl- β-D-glucosaminyl)asparagine amidase F (1PNG): jelly roll barrel

triose phosphate isomerase (1TIM): α/β barrel

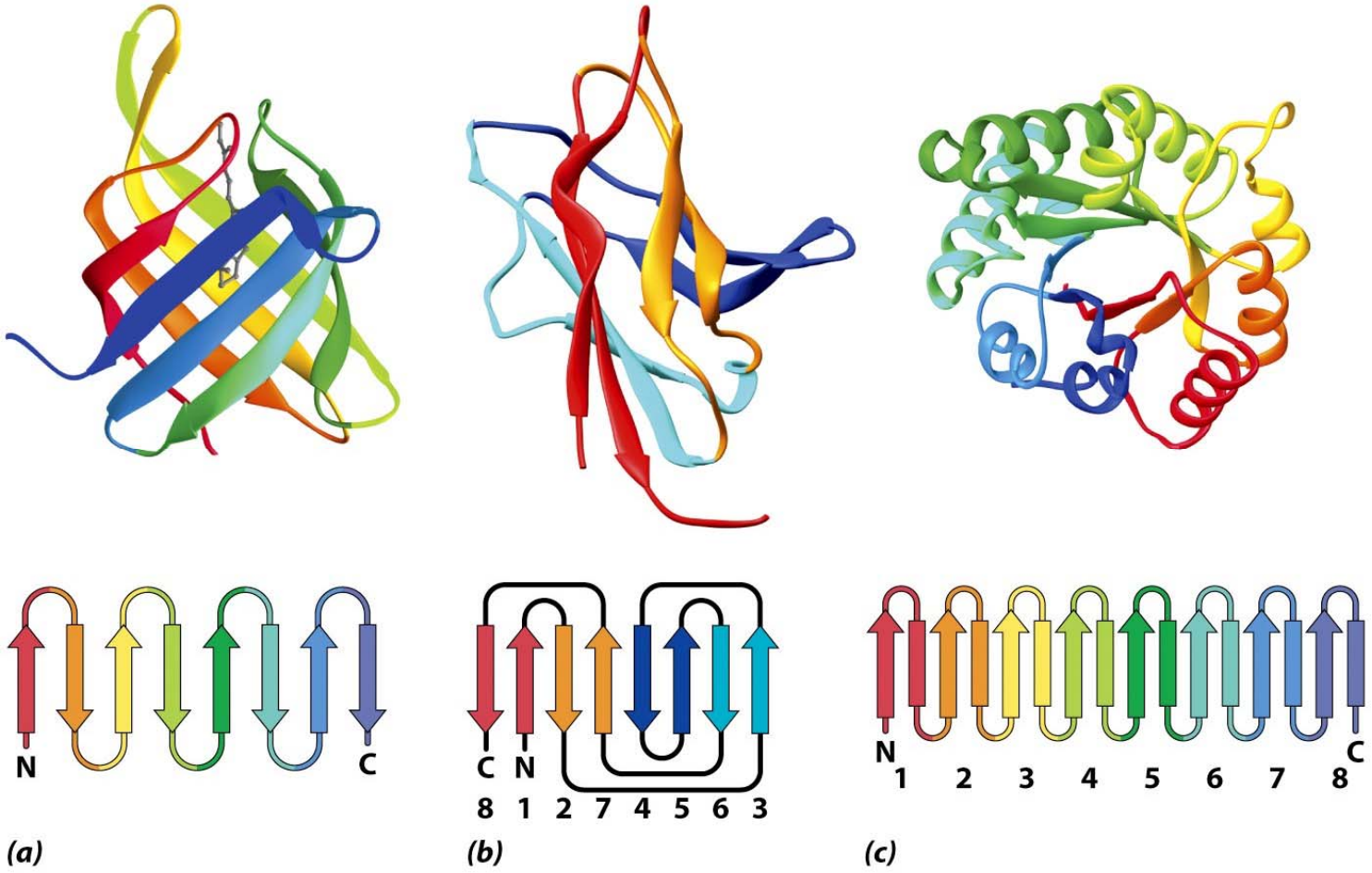



Figure 6-30 Fundamentals of Biochemistry, 2/e

# Domain structure

Large polypeptide (>~200 residues) form domains
Structurally independent units: functionally independent?
Consists of two or more layers of secondary structural elements
Connected by a pliable covalent connection
Binding sites between the domains

Glyceraldehyde-3-phosphate dehydrogenase
Bilobal appearance: two domain structure
Dinucleotide-binding fold

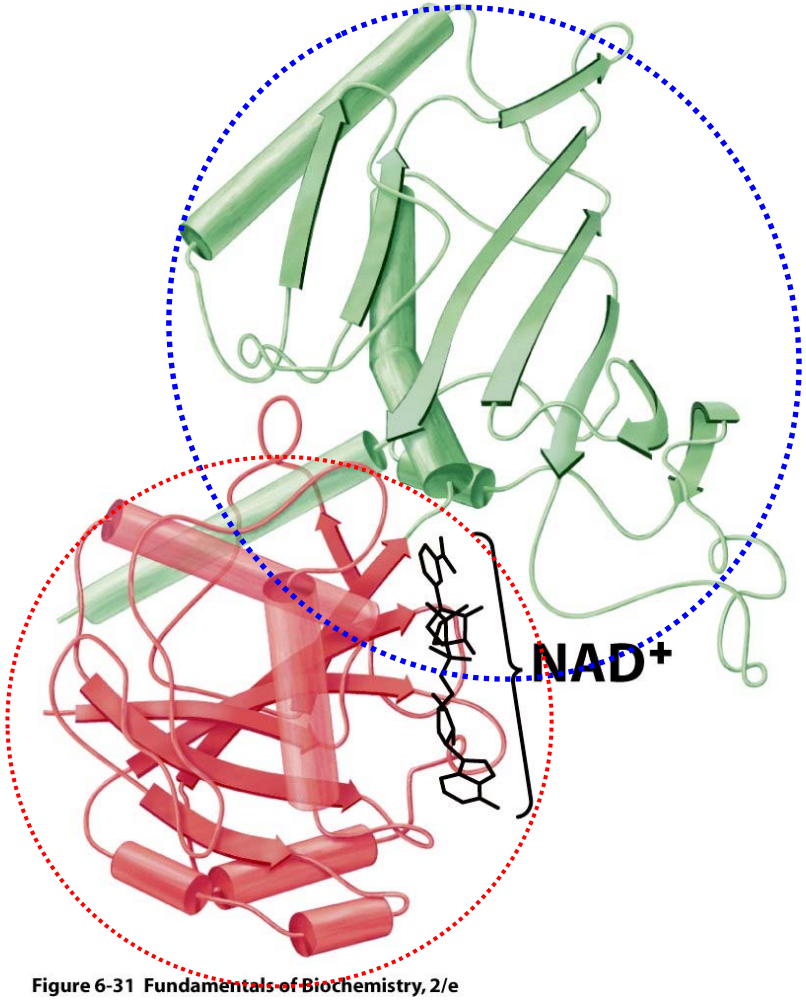



Figure 6-31 Fundamentals of Biochemistry, 2/e

# Protein families

SCOP http://scop.mrc-lmb.cam.ac.uk/scop/count.html#scop-1.69

Unique structural domains: ~1000
A few dozen folding patterns

Primary structure determines tertiary structure

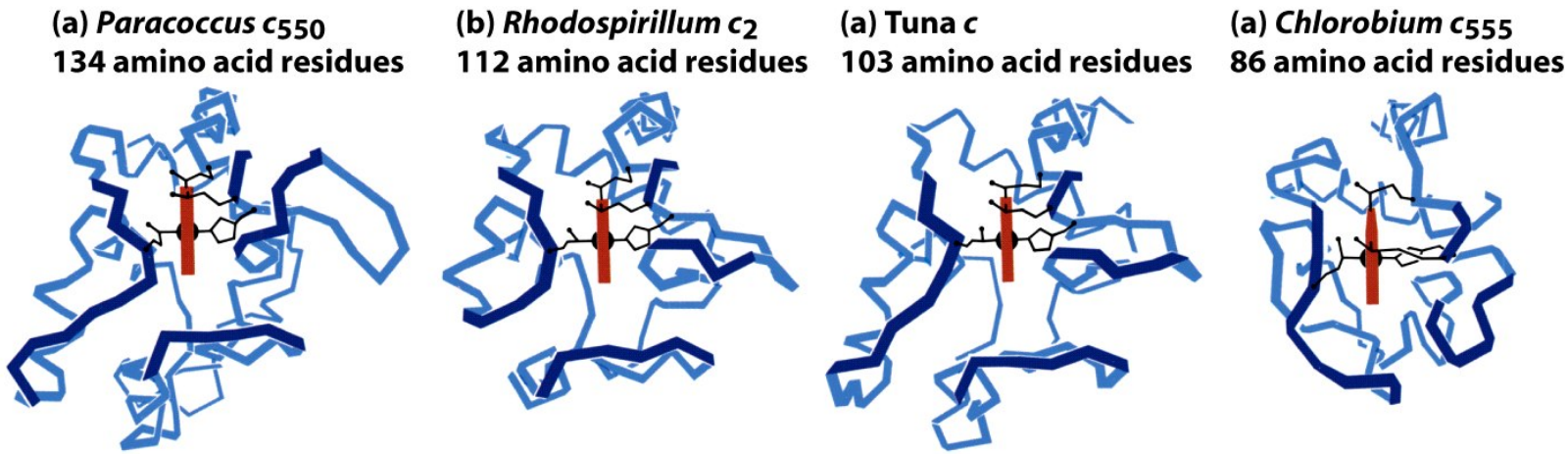
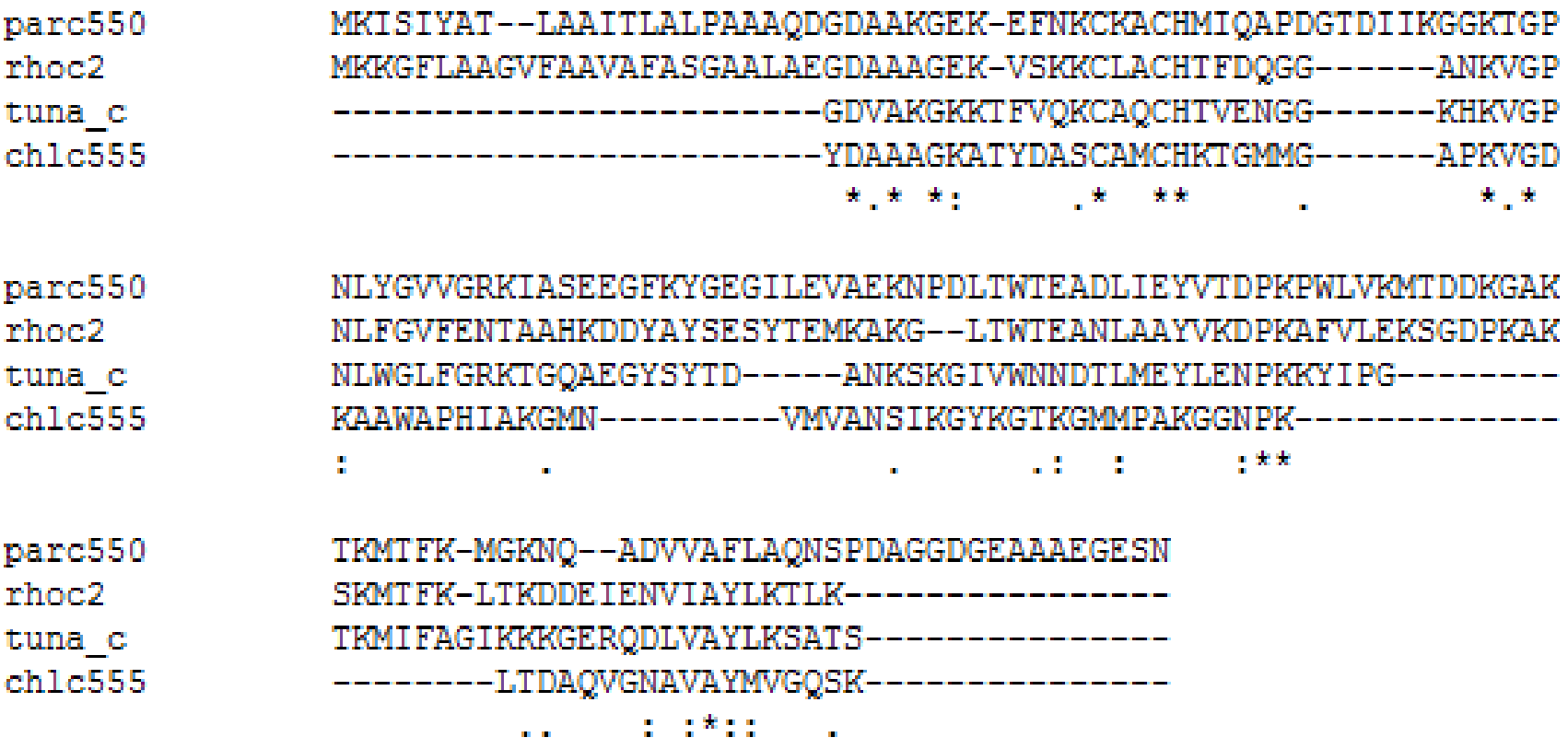



Figure 6-32 Fundamentals of Biochemistry, 2/e

3D-structure of c-type cytochromes
Low degrees of sequence similarity to each other: diverged so far not to be aligned properly
Similar folding and side chain packing in interior
Various polypeptide loops on the surface

Structural & functional elements are conserved, rather than amino acid residues


parc550 MKISIYAT--LAAITLALPAAAQDGDAAKGEK-EFNKCKACHMIQAPDGTDIIKGGKTGP
rhoc2 MKKGFLAAGVFAAVAFASGAALAEGDAAAGEK-VSKKCLACHTFDQGG------ANKVGP
tuna_c ----------GDVAKGKKTFVQKCAQCHTVENGG------KHKVGP
chlc555 ----------YDAAAGKATYDASCAMCHKTGMMG------APKVGD
parc550 NLYGVVGRKIASEEGFKYGEGILEVAEKNPDLIWTEADLIEYVTDPKPWLVKMTDDKGAK
rhoc2 NLFGVFENTAAHKDDYAYSESYTEMKAKG--LIWTEANLAAYVKDPKAFVLEKSGDPKAK
tuna_c NLWGLFGRKTGQAEGYSYTD-----ANKSKGIVWNNDTLMEYLENPKKYIPG--------
chlc555 KAAWAPHIAKGMN---------VMVANSIKGYKGTKGMMPAKGGNPK----------
parc550 TKMTFK-MGKNQ--ADVVAFLAQNSPDAGGDGEAAAEGESN
rhoc2 SKMTFK-LTKDDEIENVIAYLKTLK----------
tuna_c TKMIFAGIKKKGERQDLVAYLKSATS----------
chlc555 --------LTDAQVGNAVAYMVGQSK----------

# Quaternary structure and symmetry

Proteins of more than one polypeptide chain
The spatial arrangement of these subunits: quaternary structure
Homo or hetero polymers (protomers, oligomers)
Why multisubunits are so common? regulation of activities
Usually noncovalent association (some interchain disulfide bonds)

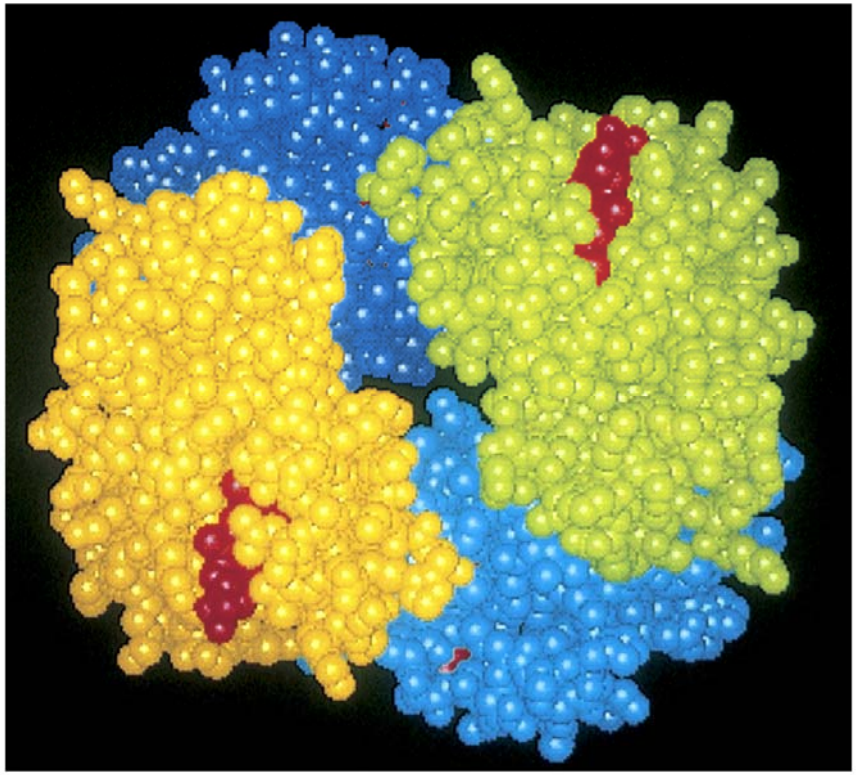
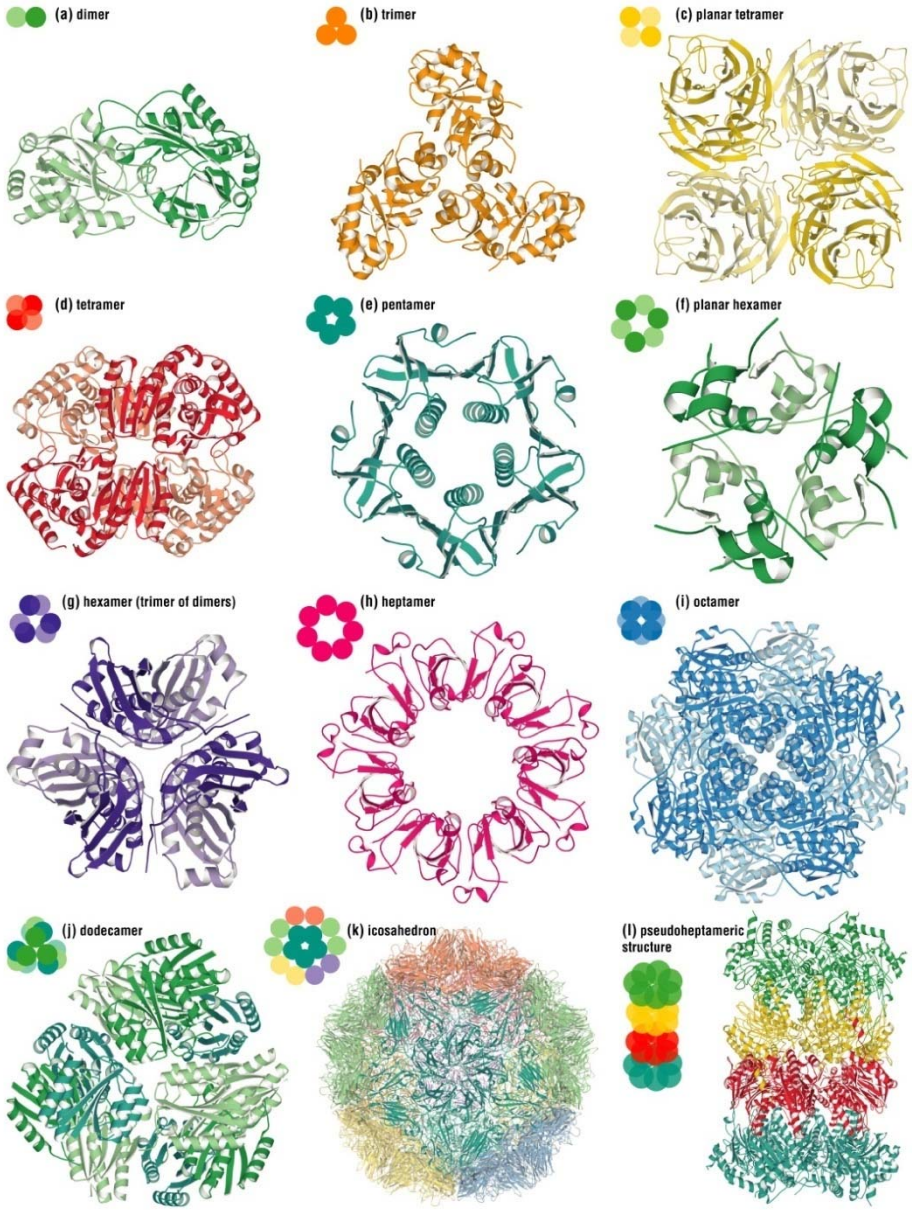


Figure 6-33 Fundamentals of Biochemistry, 2/e

From **Protein Structure and Function** by Gregory A Petsko and Dagmar Ringe






http://www.new-science-press.com/browse/protein/illustrations/1/

Subunits are symmetrically arranged: only rotational symmetry

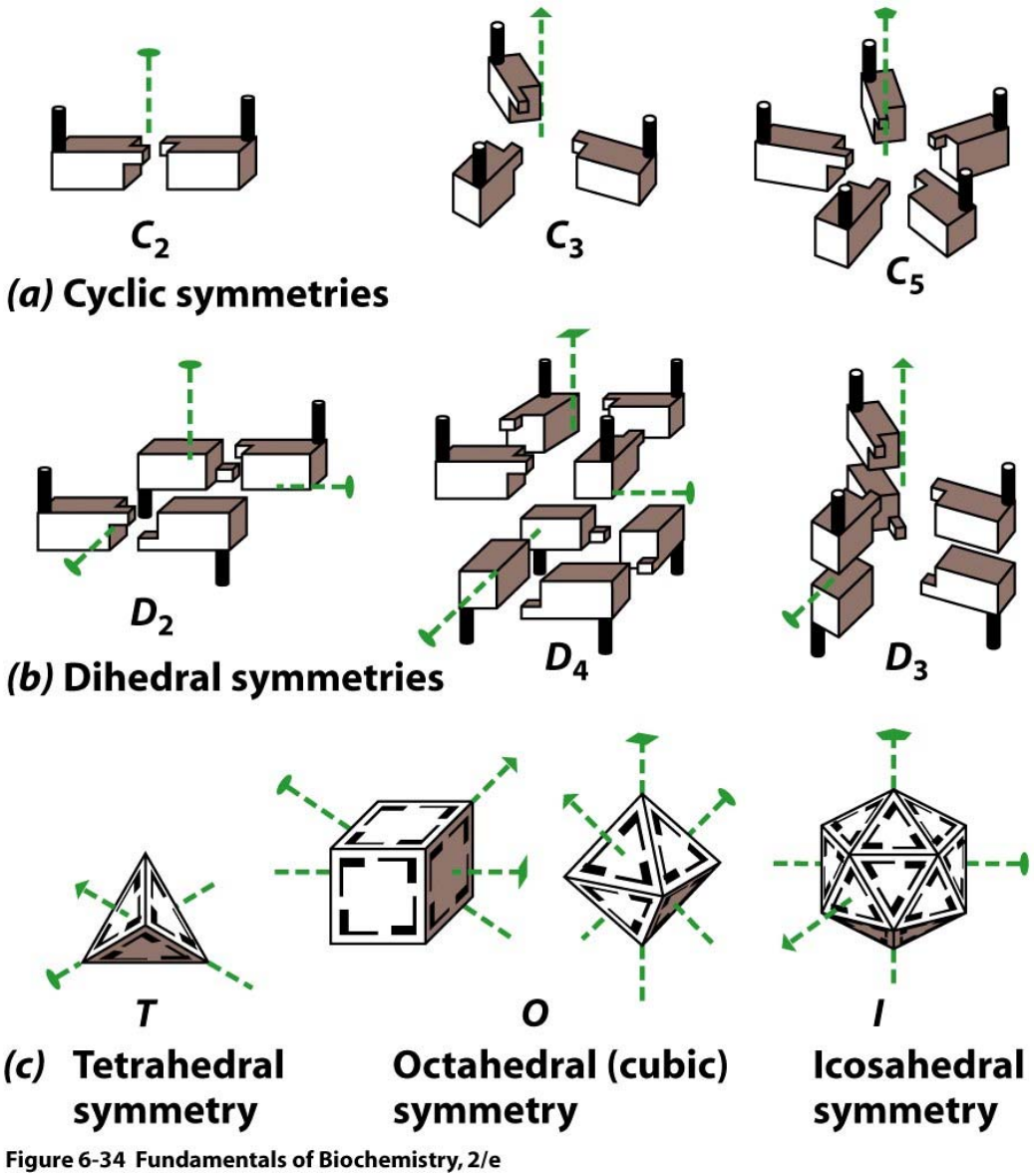
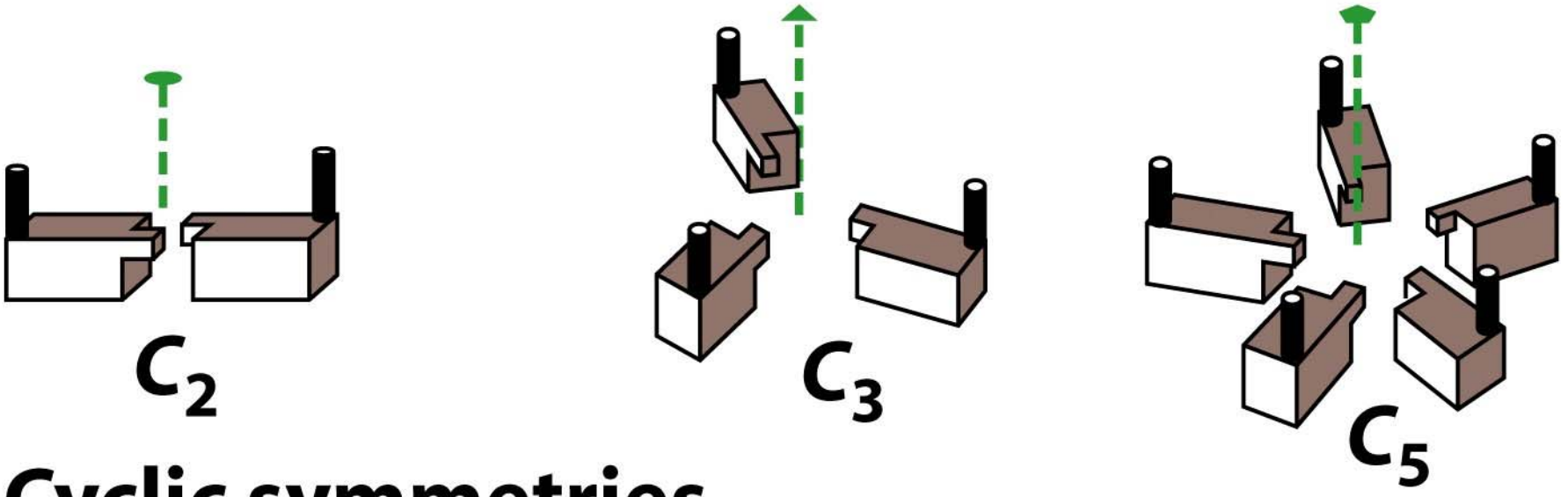


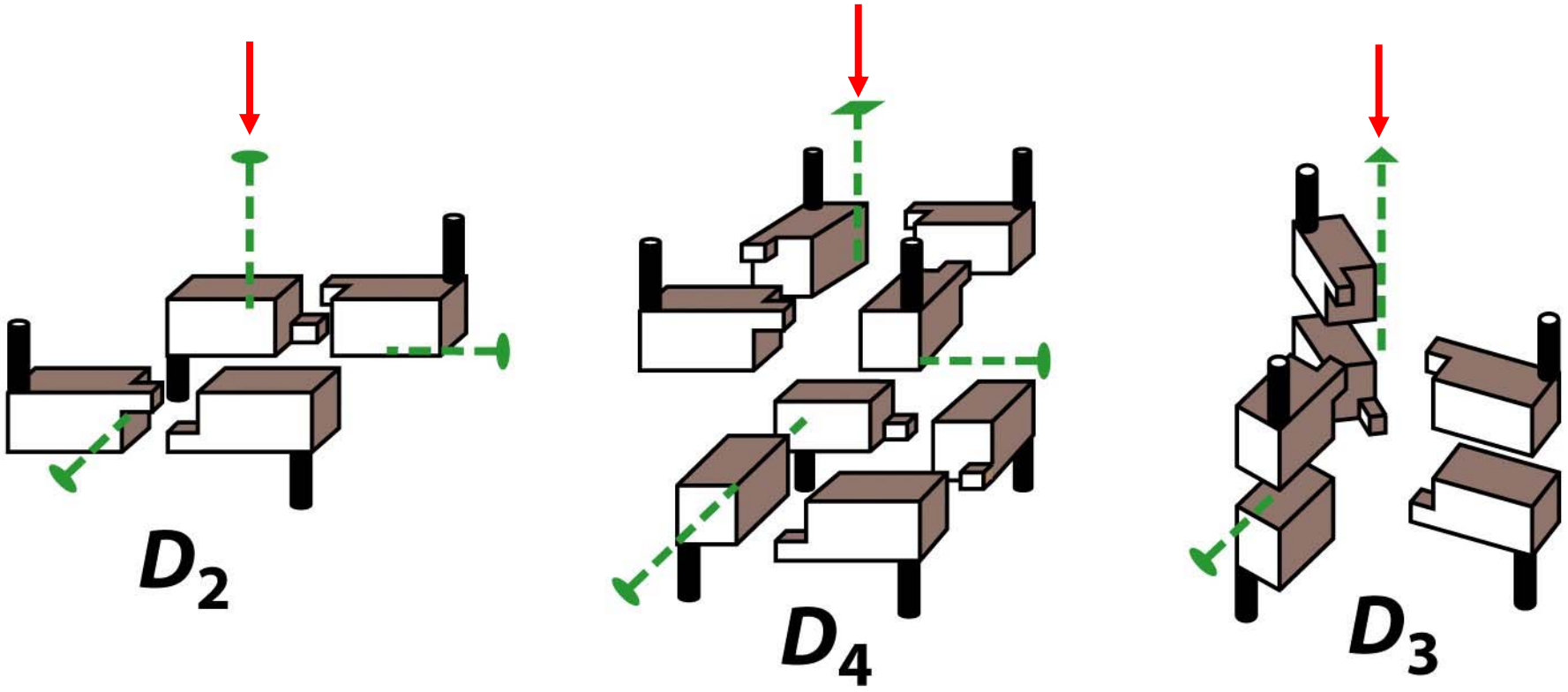

Figure 6-34 Fundamentals of Biochemistry, 2/e




# Cyclic symmetries

Figure 6-34a Fundamentals of Biochemistry, 2/e

Single axis of rotation
C2 are the most common




# Dihedral symmetries

Figure 6-34b Fundamentals of Biochemistry, 2/e

N-fold rotation axis intersects a twofold rotation axis at right angles
An oligomer with *Dn* symmetry consists of *2n* protomers

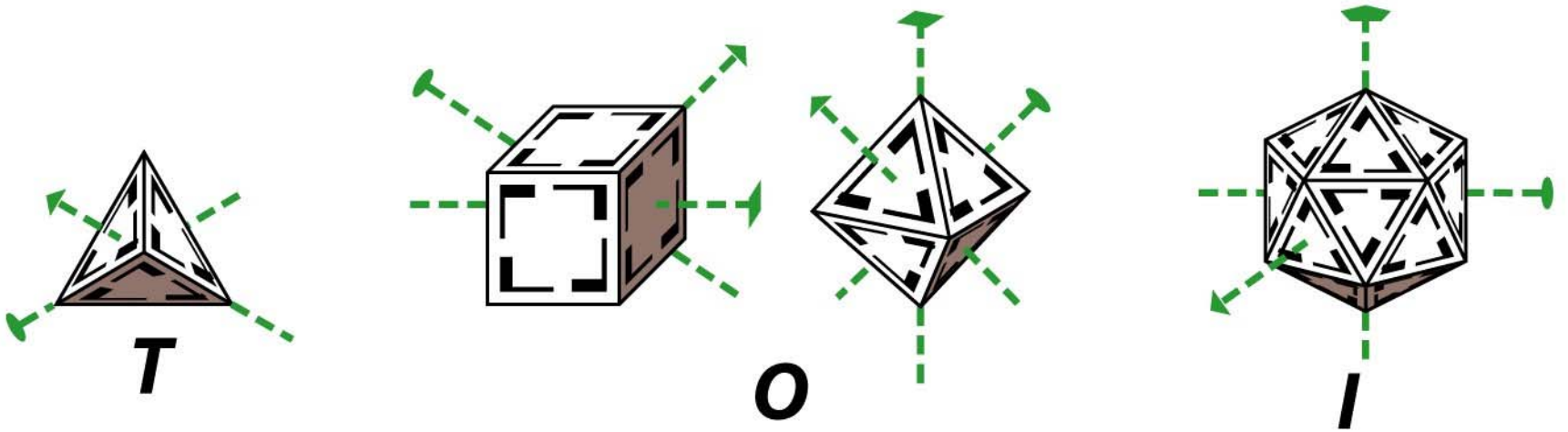



Figure 6-34c Fundamentals of Biochemistry, 2/e

Some multienzyme complexes
Spherical viruses

# Protein stability

Native proteins are only marginally stable under physiological conditions

(the free-energy difference between the folded and the unfolded states of a typical 1000-residue protein is 42 kJ mol−1 and thus each residue contributes on average only 0.42 kJ mol−1 of energy to maintain the folded state.)

Protein structure is the result of a delicate balance among powerful countervailing forces

## Forces that stabilize protein structure

- The hydrophobic effect
  - the major determinant of native protein structure
  - hydropathy
- Electrostatic interactions
- Chemical cross-links

***stability factors suggested by site-directed mutagenesis

- hydrophobicity
- steric compatibility
- volume of the side chain

**Table 6-2 Hydropathy Scale for Amino Acid Side Chains**

| Side Chain | Hydropathy |
|---|---|
| Ile | 4.5 |
| Val | 4.2 |
| Leu | 3.8 |
| Phe | 2.8 |
| Cys | 2.5 |
| Met | 1.9 |
| Ala | 1.8 |
| Gly | −0.4 |
| Thr | −0.7 |
| Ser | −0.8 |
| Trp | −0.9 |
| Tyr | −1.3 |
| Pro | −1.6 |
| His | −3.2 |
| Glu | −3.5 |
| Gln | −3.5 |
| Asp | −3.5 |
| Asn | −3.5 |
| Lys | −3.9 |
| Arg | −4.5 |

*Source:* Kyte, J. and Doolittle, R.F., *J. Mol. Biol.* **157,** 110 (1982).

**Table 6-2 Fundamentals of Biochemistry, 2/e**

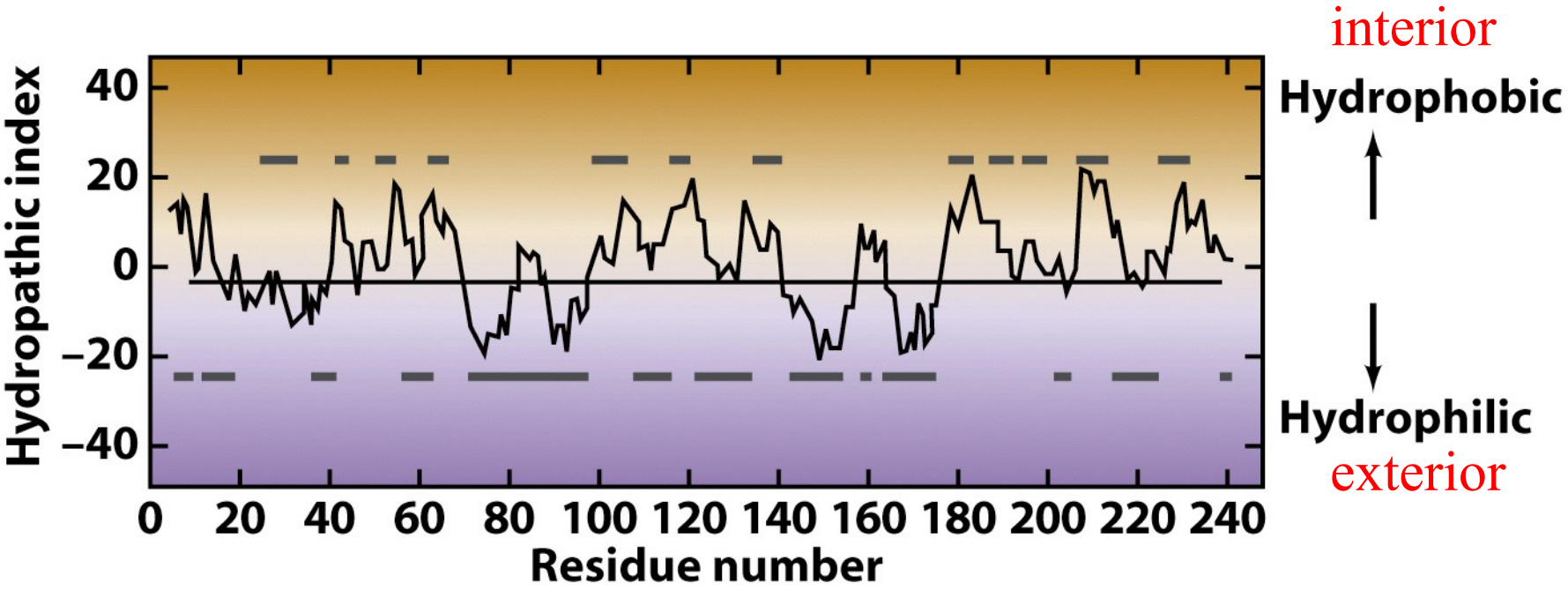



Figure 6-35 Fundamentals of Biochemistry, 2/e


A hydrophobic index plot for bovine chymotrypsinogen

the sum of the hydropathies of nine consecutive residues

**Table 2-1 Bond Energies in Biomolecules**

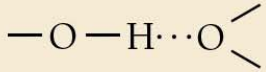
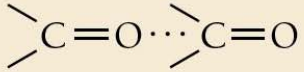
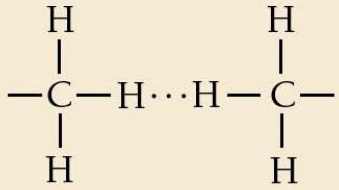
| Type of Bond | Example | Bond Strength ($kJ \cdot mol^{-1}$) |
|---|---|---|
| Covalent | O—H | 460 |
| | C—H | 414 |
| | C—C | 348 |
| Noncovalent | | |
| Ionic interaction | $—COO^- \cdots {}^+H_3N—$ | 86 |
| van der Waals forces | | |
| Hydrogen bond |  | 20 |
| Dipole–dipole interaction |  | 9.3 |
| London dispersion forces |  | 0.3 |

Table 2-1 Fundamentals of Biochemistry, 2/e


Ionic (fully charged): NaCl
Nonionic: covalent (most organic molecules)
polar (partially charged): oxygen
nonpolar: carbon

# Dipole-dipole interactions

permanent

induced

momentary

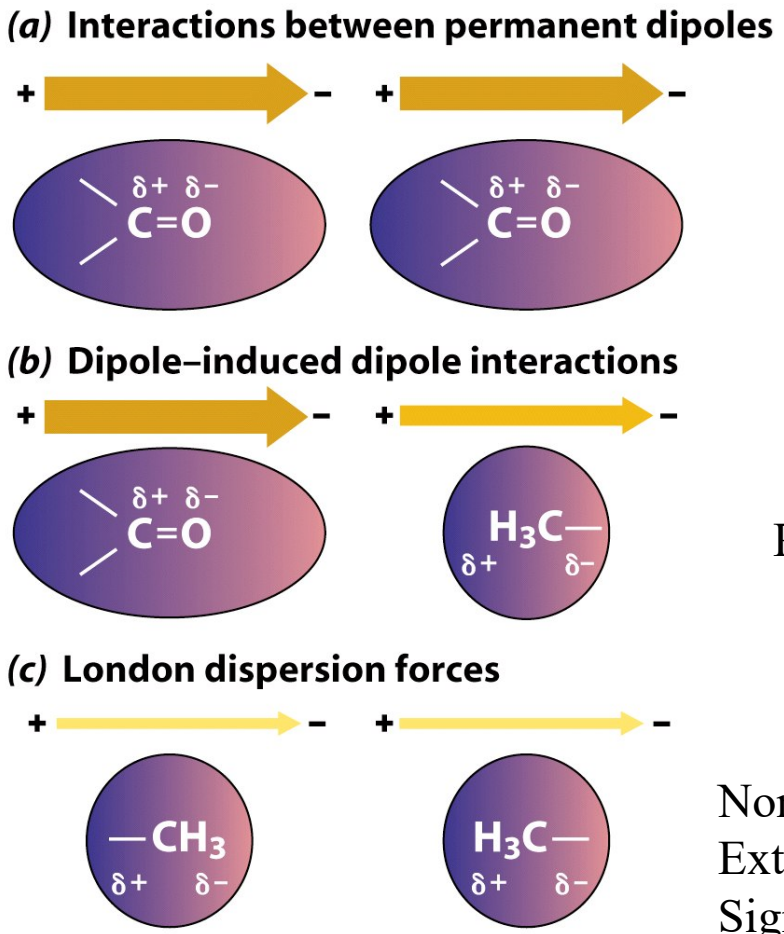



Figure 2-5 Fundamentals of Biochemistry, 2/e


Polar & polar interaction

Polar & nonpolar interaction

Nonpolar & nonpolar interaction
Extremely weak
Significant only in close contact
Extremely important for structure

# Electrostatic interactions

van der Waals forces: an important stabilizing influence

H-bonds: minor contribution to protein stability

because of H-bonds with water molecules in the unfolded state

but important determinants of native protein structures: fine-tuning of tertiary structure

Ion-pair (salt bridge)

about 75% of the charged residues are members of ion pairs located on the protein surface

contribute little to protein stability

ion-pairs are poorly conserved among homologous proteins

Examples of ion-pairs in myoglobin

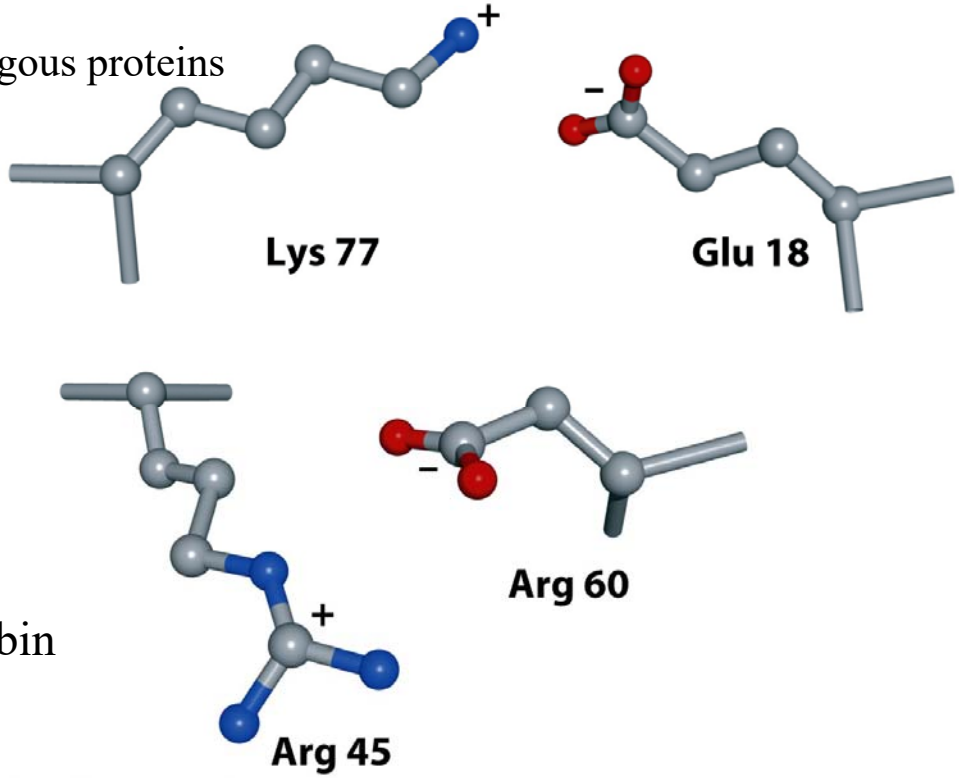



Figure 6-36 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

# Chemical cross-links

Disulfide bonds: not essential stabilizing forces

may be important for “locking in” a particular backbone folding patterns

rare in intracellular proteins

Metal ions

internally cross-link proteins

zinc fingers: tetrahedrally coordinated by His and Cys

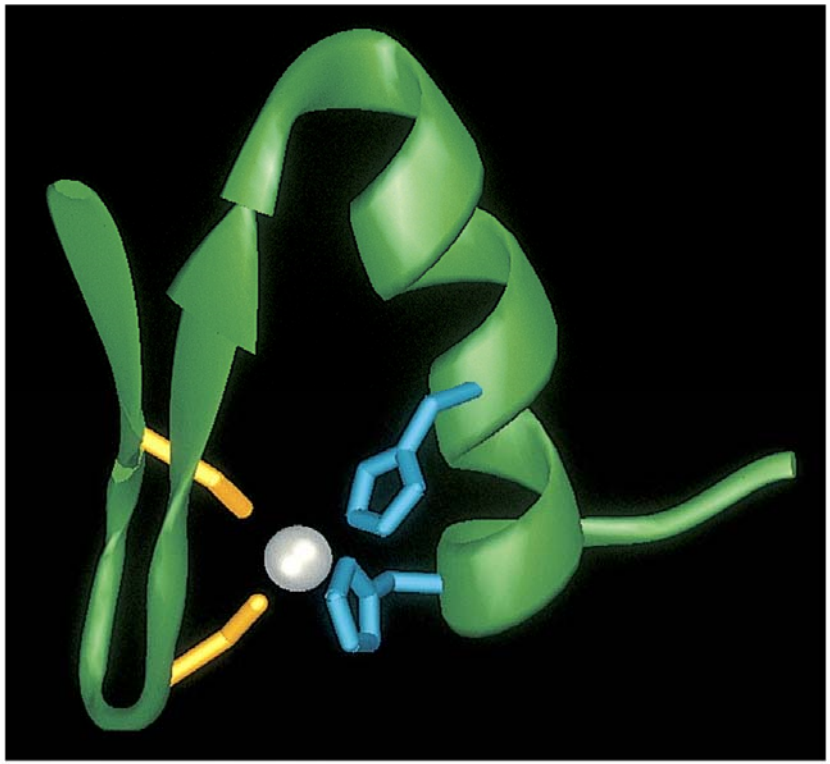


Figure 6-37 Fundamentals of Biochemistry, 2/e

# Protein dynamics

Proteins are flexible and rapidly fluctuating molecules
whose structural mobilities are functionally significant

Conformational flexibility (or breathing) up to ~2Å
Extended side chains (Lys) and the N- & C-termini are especially prone to wave around in solution

## Molecular dynamics of myoglobin

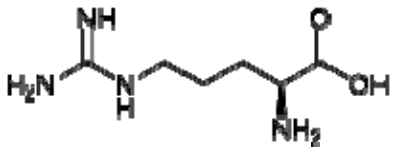
Several snapshots of the protein calculated at intervals of $5 \times 10^{-12}$ seconds are superimposed

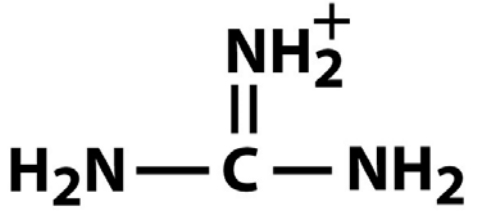
Figure 6-38 Fundamentals of Biochemistry, 2/e

# Protein denaturation and renaturation

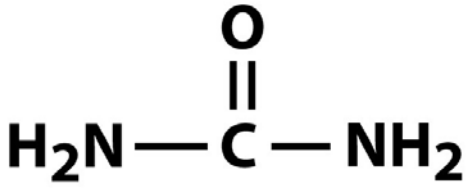
Proteins are susceptible to denaturation
Variety of conditions and substances

Heating
pH: changes charge distribution and H-bonding requirements
Detergents: interfere with the hydrophobic interactions
Chaotropic agents (guanidinium ion and urea): disrupt hydrophobic interactions




Guanidinium ion




Urea

Unnumbered figure pg 159 Fundamentals of Biochemistry, 2/e

# Denatured proteins can be renatured

1957 Christian Anfinsen, RNase A

Demonstration of spontaneous protein folding

A protein's primary structure dictates its 3D structure

Denaturation and renaturation of RNase A

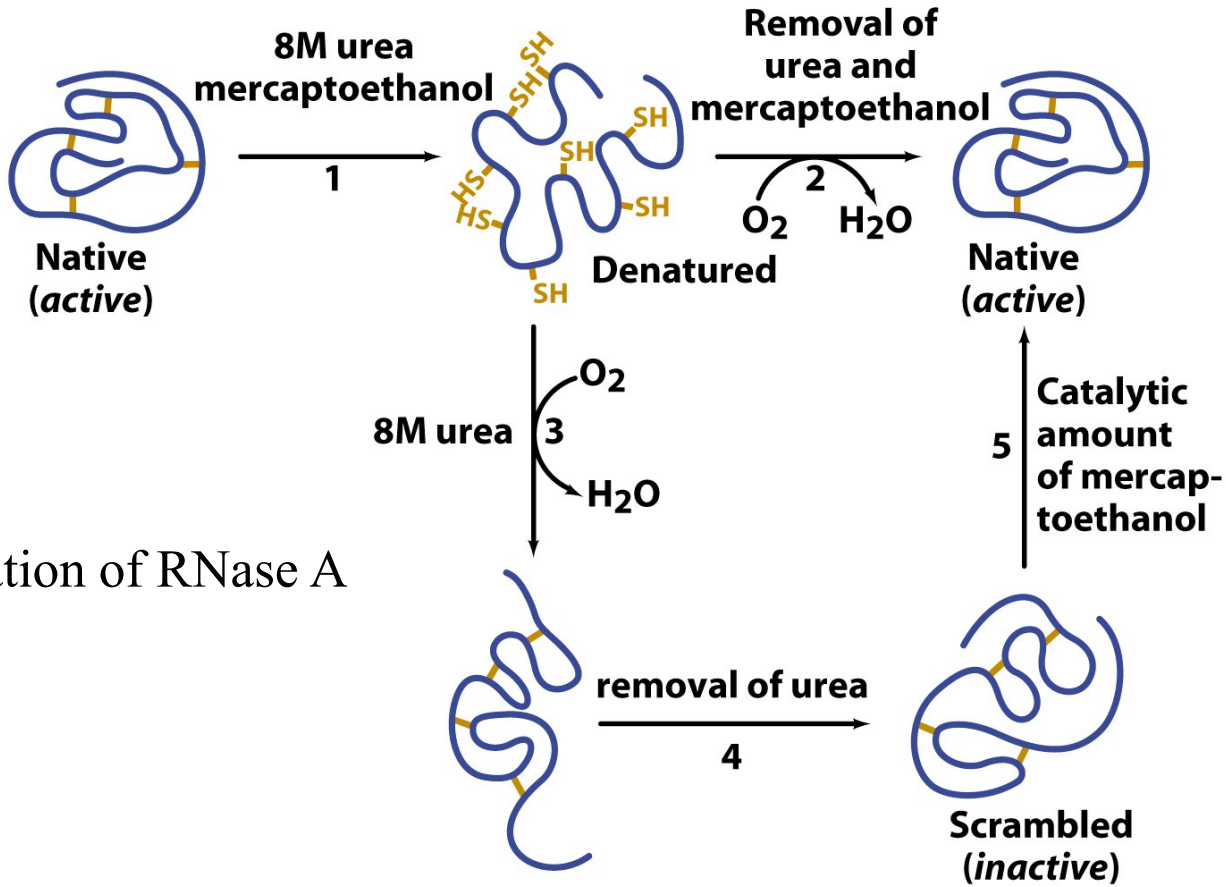



Figure 6-39 Fundamentals of Biochemistry, 2/e

However, there is growing awareness that intrinsically unstructured proteins are quite prevalent in eukaryotic genomes,[Nat Rev Mol Cell Biol. 2005 Mar;6(3):197-208. Intrinsically unstructured proteins and their functions] casting further doubt on the simplest interpretation of Anfinsen's dogma: "sequence determines structure (singular)". In effect, the new paradigm is characterized by the addition of two caveats: "sequence and cellular environment determine structural ensemble".