

Proteins : Structure and Function

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Γ. 208

Books

- Introduction to Protein structure

Carl Branden & John Tooze

Garland Science

- How proteins work

Mike Williamson

Garland Science / Taylor & Francis

- Molecular biology of assemblies and machines

Alasdair Steven, Wolfgang Baumeister, Louise N. Johnson, & Richard N. Perham

Garland Science

A working machine



Figure 0.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Flow of genetic information

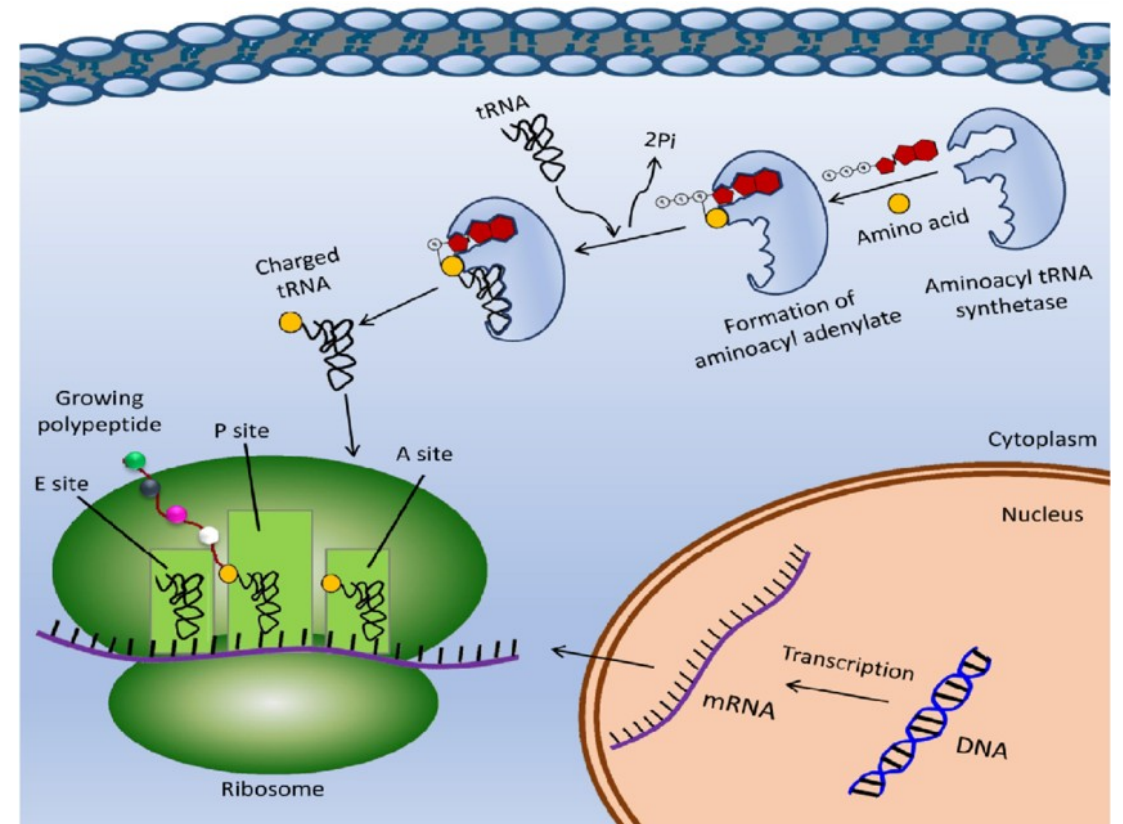
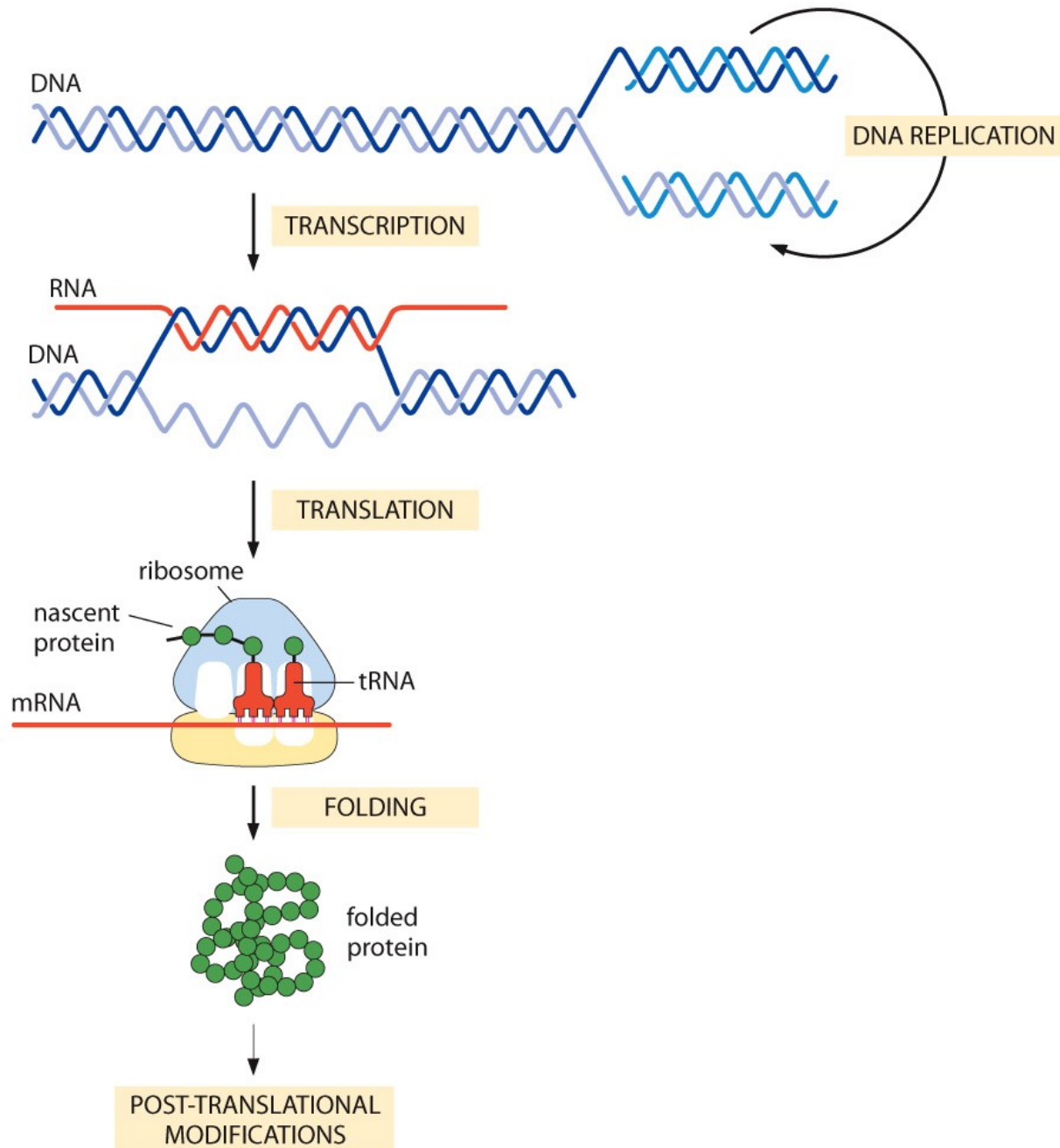
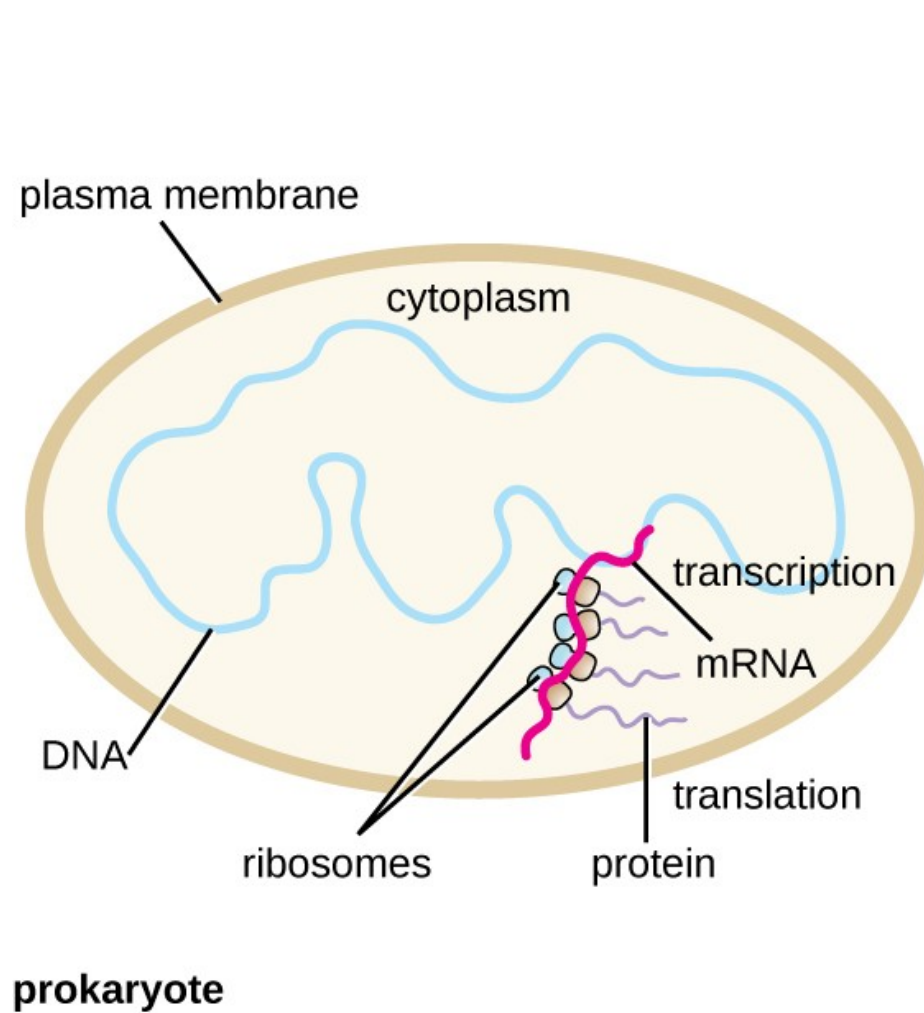
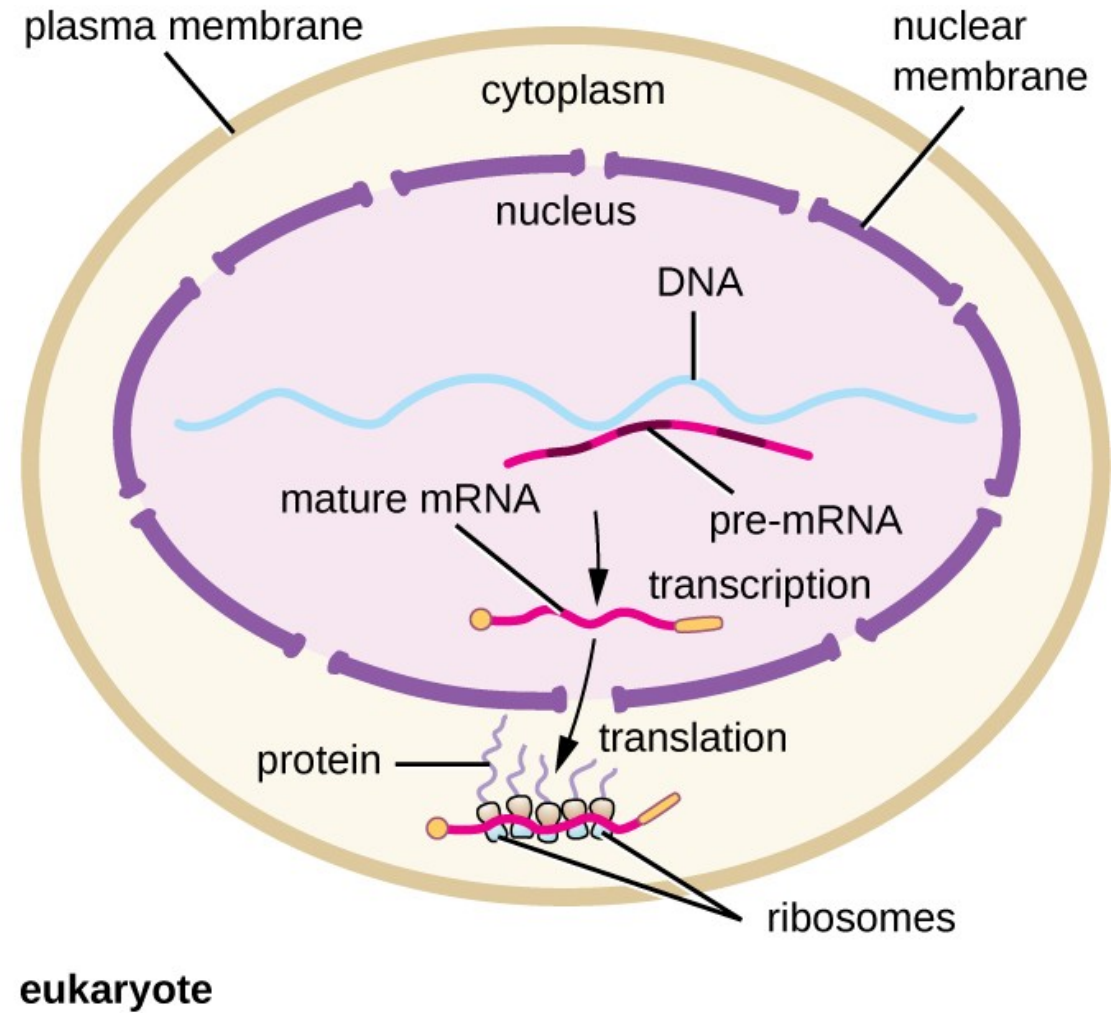


Figure 1.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Transcription and translation



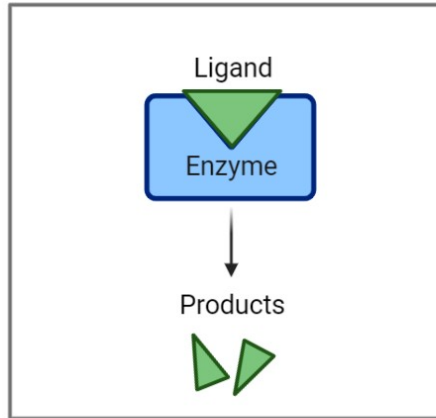
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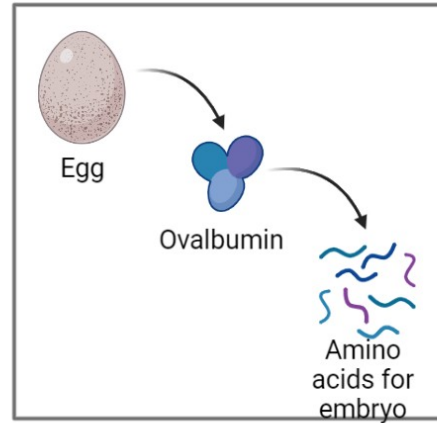
(b)

An Overview of Protein Functions

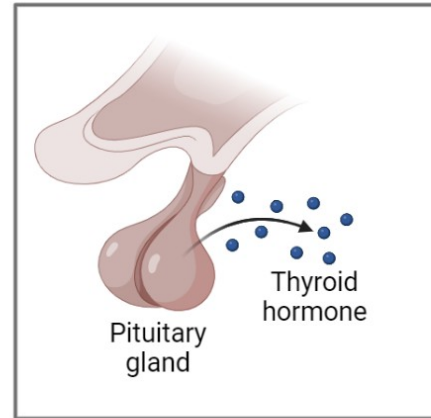
Enzymatic proteins



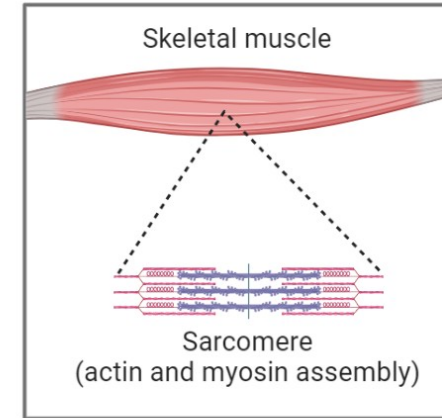
Storage proteins



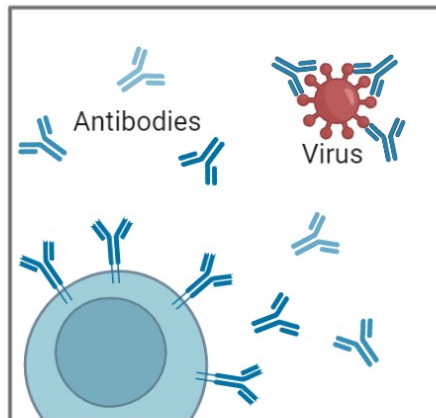
Hormonal proteins



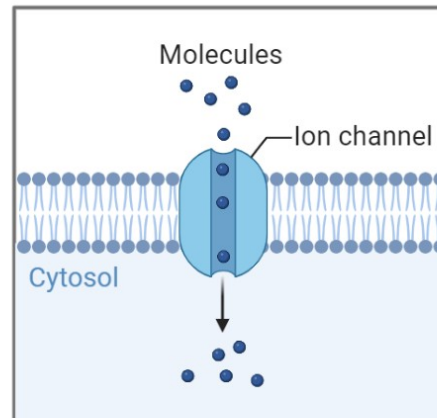
Motor proteins



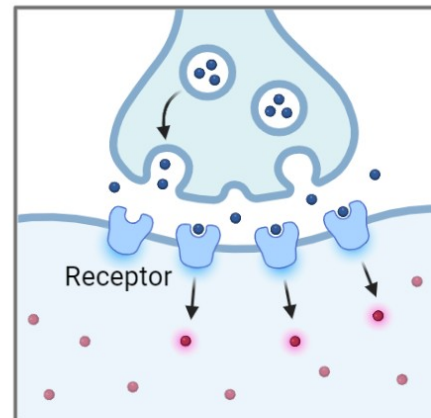
Defensive proteins



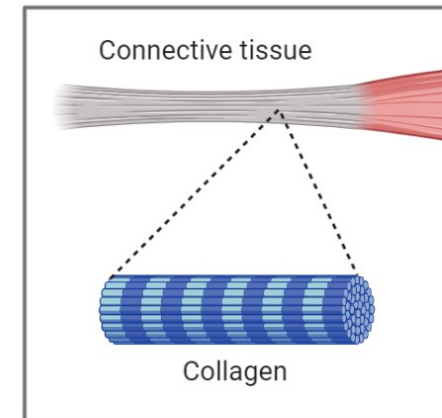
Transport proteins



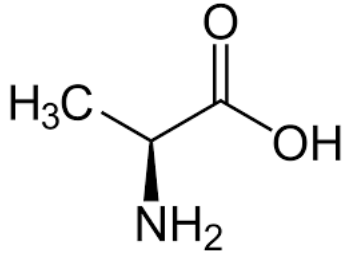
Receptor proteins



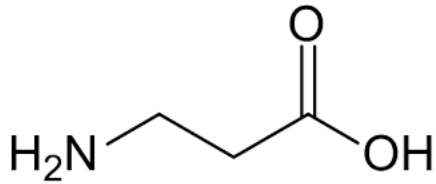
Structural proteins



α -amino acids-building blocks of protein



α -alanine



β -alanine

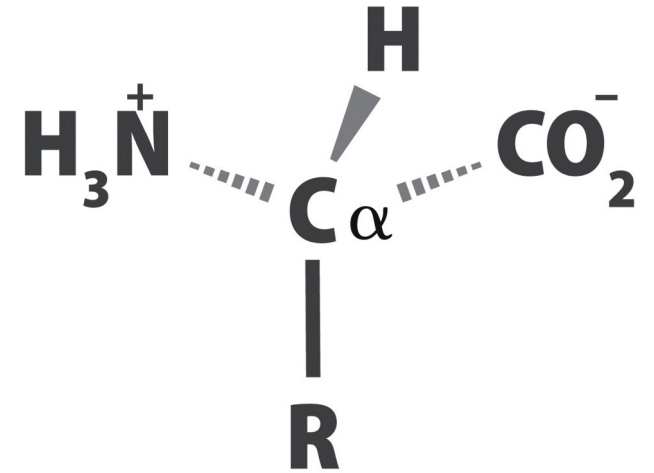


Figure 1.1.1 How Proteins Work (©2012 Garland Science)

L-amino acids

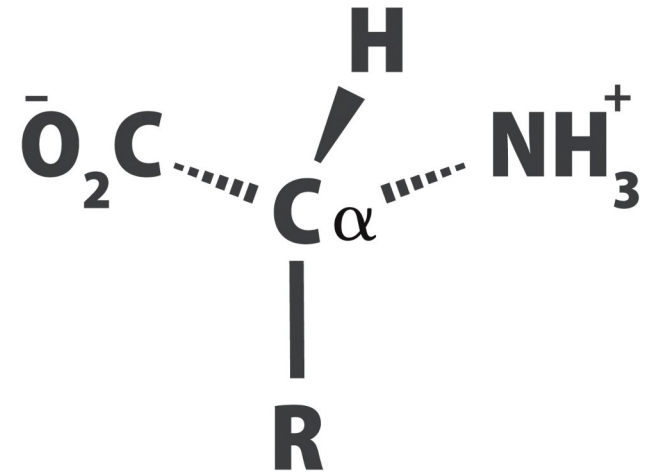


Figure 1.1.2 How Proteins Work (©2012 Garland Science)

D-amino acids

α -amino acid side chain

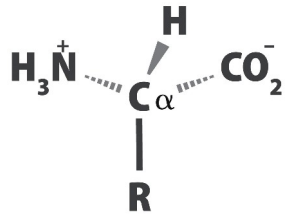
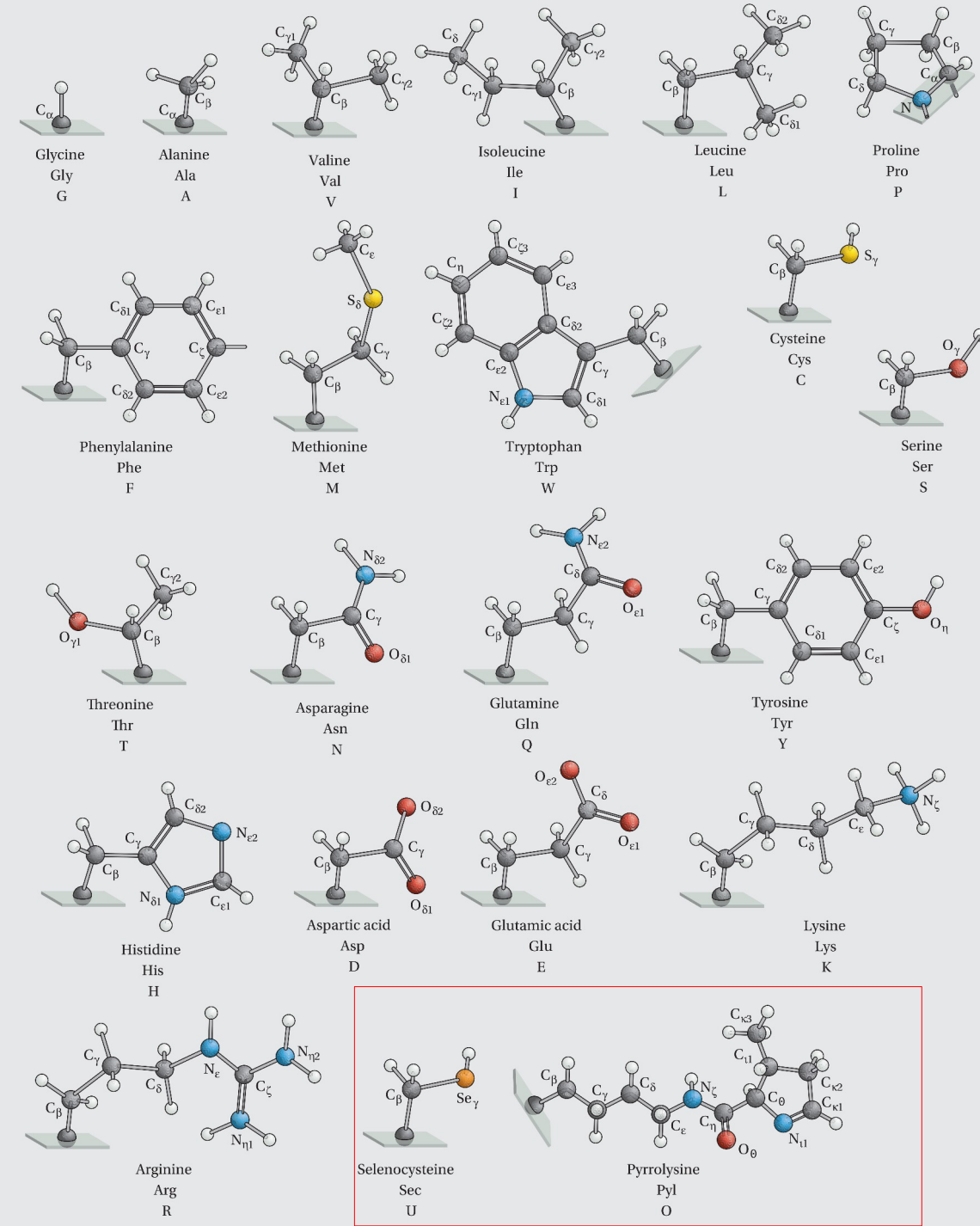


Figure 1.1.1. From Proteins: A Molecular Approach, 2nd Edition, © 2005 Garland Science



α -amino acid side chain

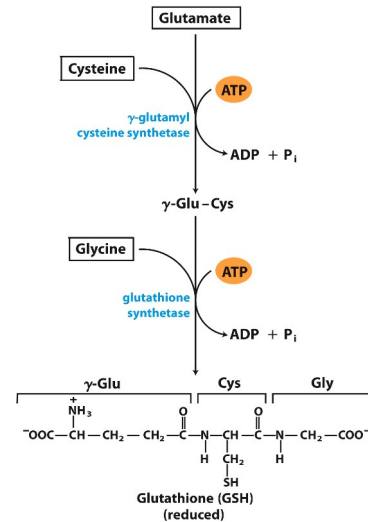
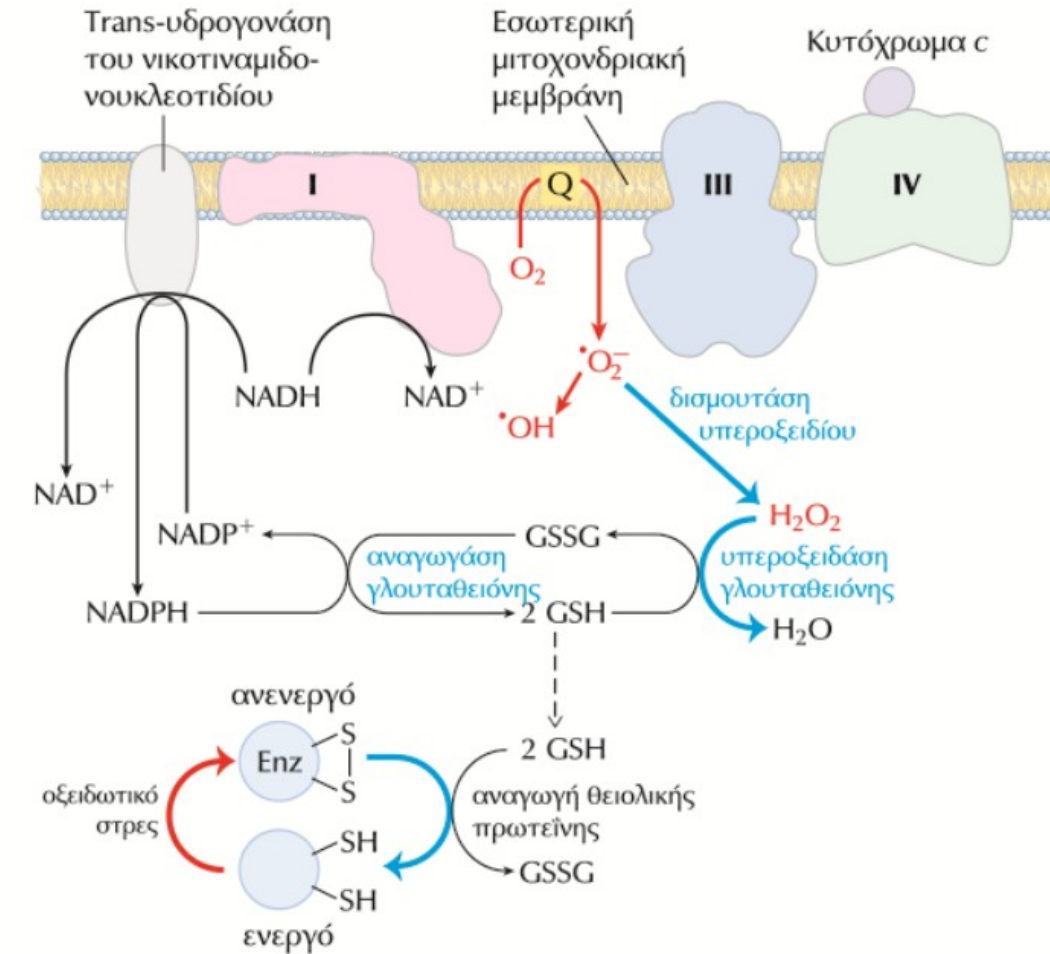
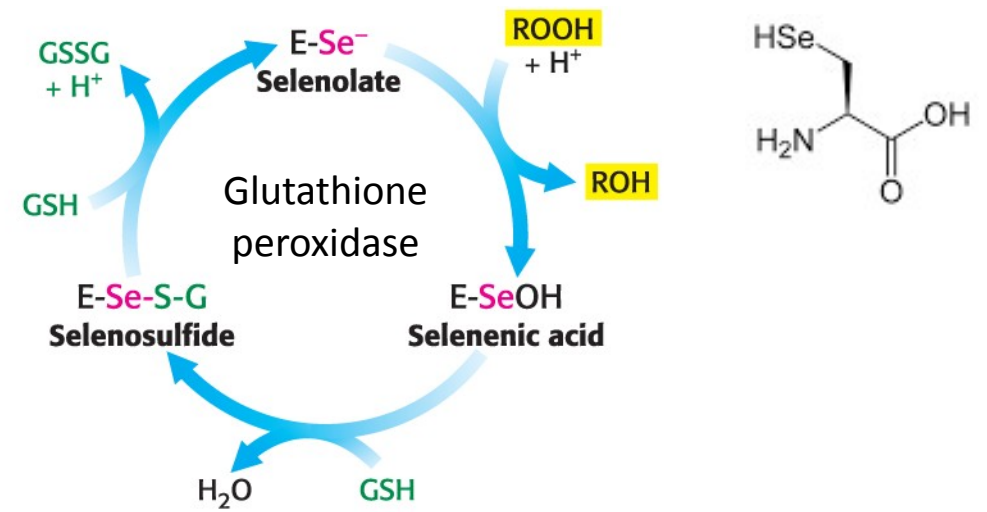
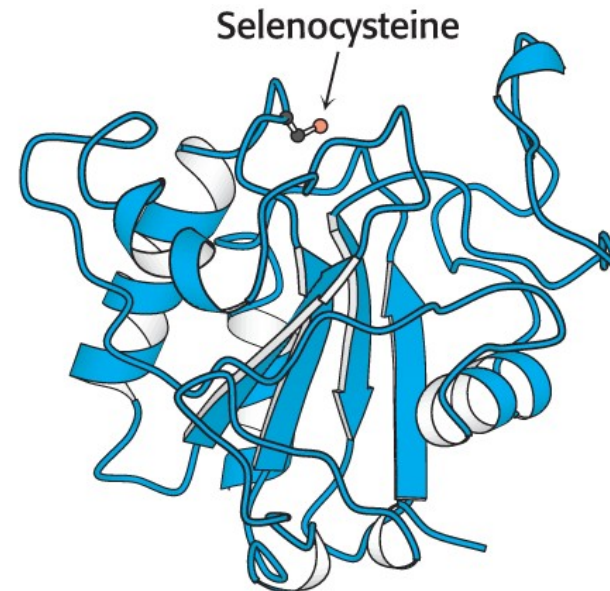


Figure 22-29a
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company



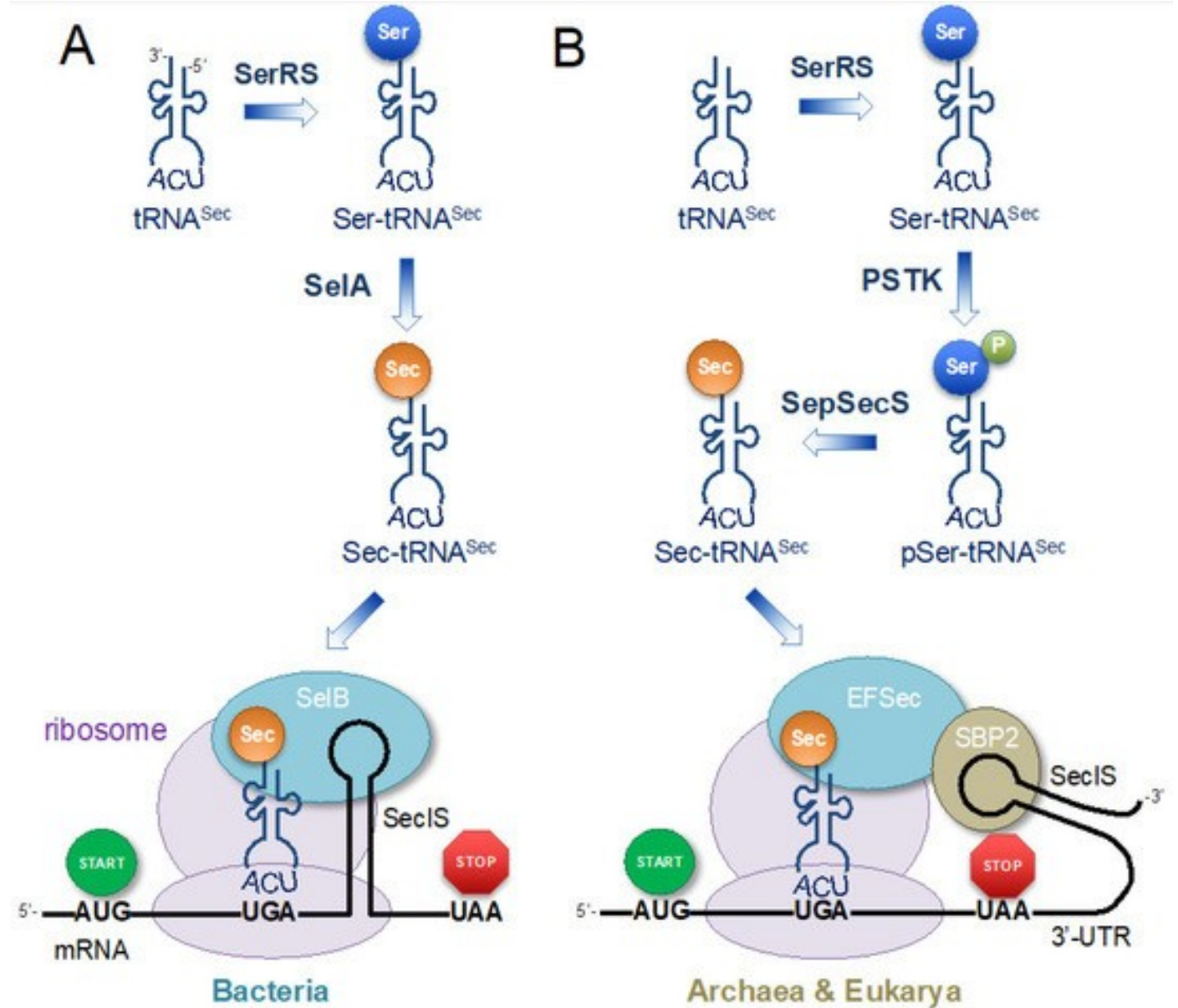
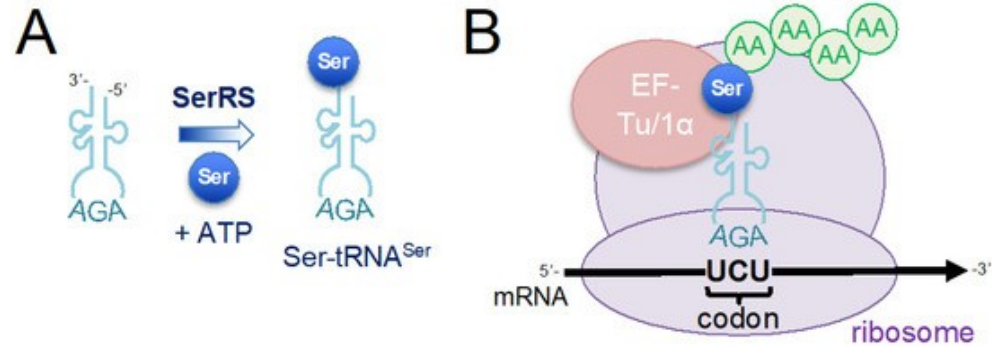
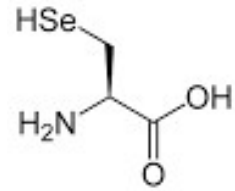
Berg et al., *Biochemistry*, 9e, © 2019
W. H. Freeman and Company



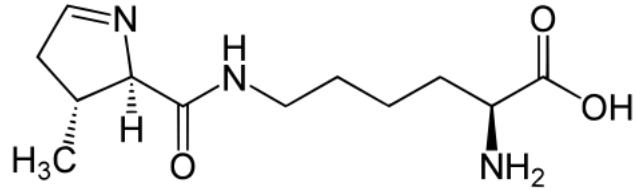
Berg et al., *Biochemistry*, 9e, © 2019
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GSH είναι παρών στα περισσότερα κύτταρα σε υψηλά ποσά
Αναγωγικό / αντιοξειδωτικό
Διατηρεί πρωτεΐνες, μεταλλικά κατιόντα ανηγμένα
Διατηρεί τα ένζυμα οξειδοαναγωγής σε ανηγμένη κατάσταση
Αφαιρεί τοξικά υπεροξειδία Οξειδωνεται σε διμερές (GSSG)

α -amino acid side chain

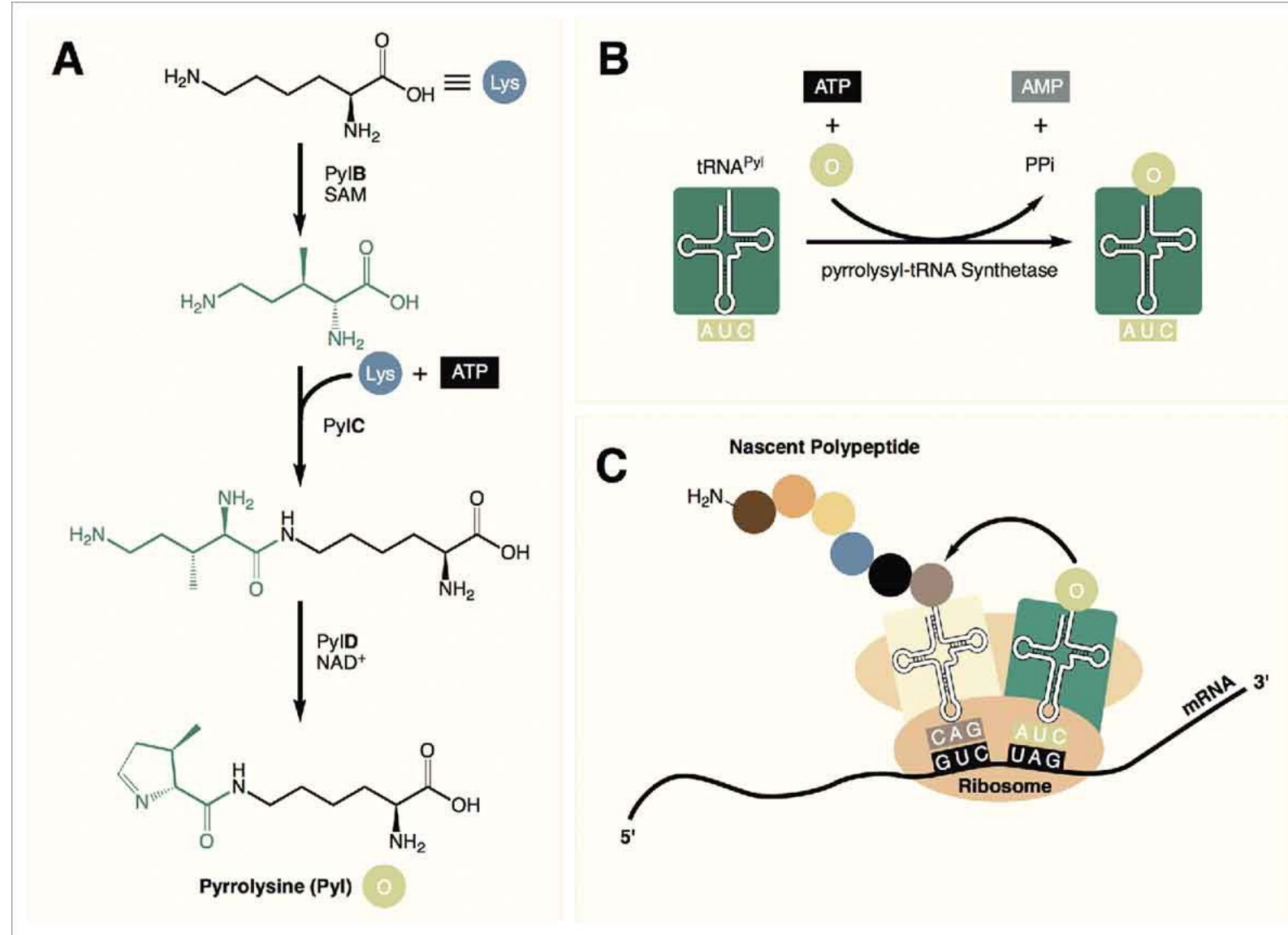


α -amino acid side chain

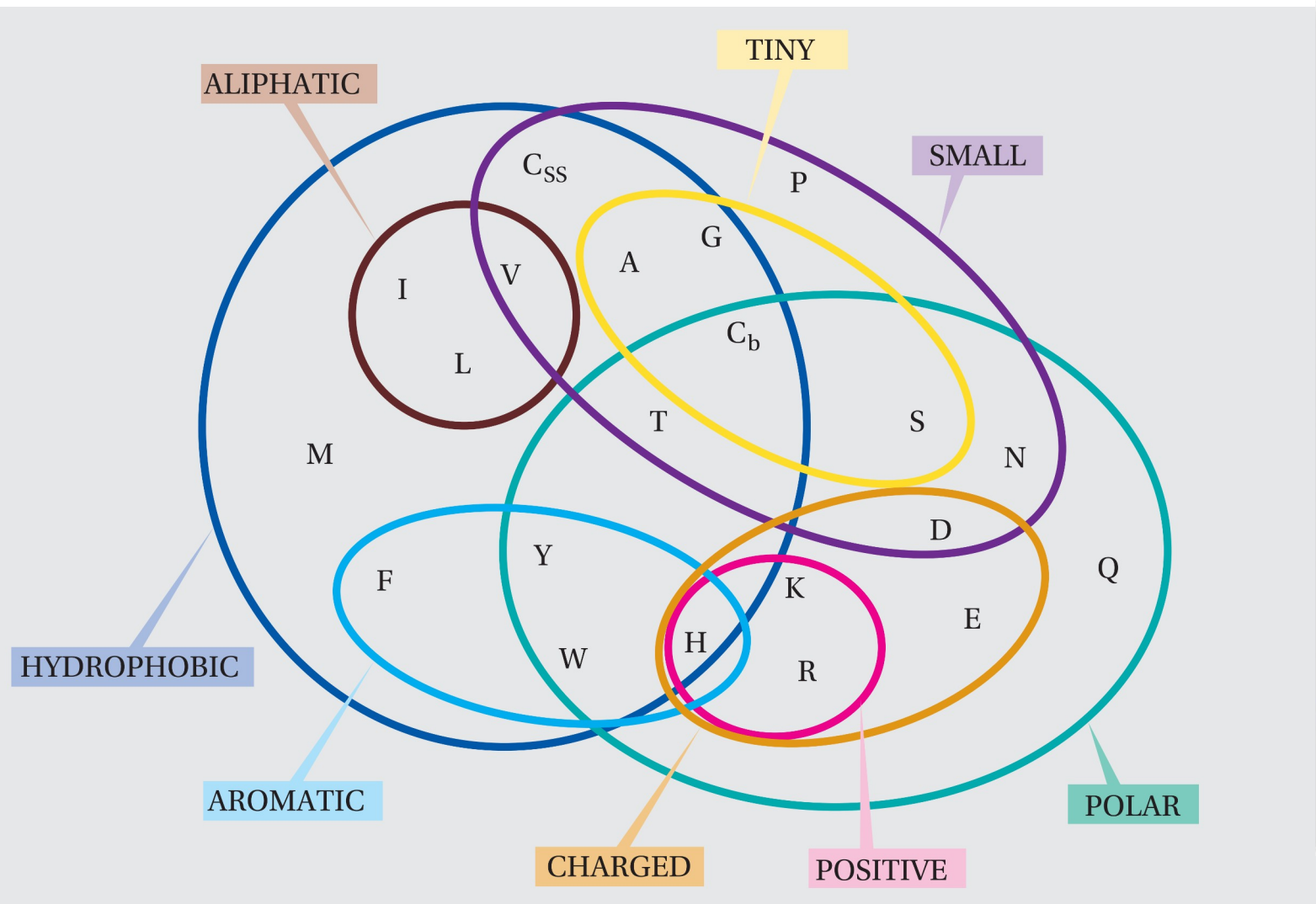


Pyrrolysine functions as a catalytic amino acid in specialized enzymes, particularly those involved in **methylamine metabolism**.

Methylamine → methyltransferase enzyme containing pyrrolysine → transfer of methyl group → energy/carbon metabolism



Physicochemical classifications of the 20 common aa



Amino acid	Short	Abbrev.	Avg. mass (Da)	pI	pK ₁ (α-COOH)	pK ₂ (α-NH ₃)
Alanine	A	Ala	89.09404	6.01	2.35	9.87
Cysteine	C	Cys	121.15404	5.05	1.92	10.70
Aspartic acid	D	Asp	133.10384	2.85	1.99	9.90
Glutamic acid	E	Glu	147.13074	3.15	2.10	9.47
Phenylalanine	F	Phe	165.19184	5.49	2.20	9.31
Glycine	G	Gly	75.06714	6.06	2.35	9.78
Histidine	H	His	155.15634	7.60	1.80	9.33
Isoleucine	I	Ile	131.17464	6.05	2.32	9.76
Lysine	K	Lys	146.18934	9.60	2.16	9.06
Leucine	L	Leu	131.17464	6.01	2.33	9.74
Methionine	M	Met	149.20784	5.74	2.13	9.28
Asparagine	N	Asn	132.11904	5.41	2.14	8.72
Pyrrolysine	O	Pyl	255.31			
Proline	P	Pro	115.13194	6.30	1.95	10.64
Glutamine	Q	Gln	146.14594	5.65	2.17	9.13
Arginine	R	Arg	174.20274	10.76	1.82	8.99
Serine	S	Ser	105.09344	5.68	2.19	9.21
Threonine	T	Thr	119.12034	5.60	2.09	9.10
Selenocysteine	U	Sec	168.053	5.47		
Valine	V	Val	117.14784	6.00	2.39	9.74
Tryptophan	W	Trp	204.22844	5.89	2.46	9.41
Tyrosine	Y	Tyr	181.19124	5.64	2.20	9.21

α -amino acid in proteins

TABLE 1.2 Frequency of occurrence of amino acids in proteins			
Amino acid	Frequency in intracellular proteins (%)	Frequency in membrane proteins (%)	Number of codons
Ala	7.9	8.1	4
Arg	4.9	4.6	6
Asp	5.5	3.8	2
Asn	4.0	3.7	2
Cys	1.9	2.0	2
Glu	7.1	4.6	2
Gln	4.4	3.1	2
Gly	7.1	7.0	4
His	2.1	2.0	2
Ile	5.2	6.7	3
Leu	8.6	11.0	6
Lys	6.7	4.4	2
Met	2.4	2.8	1
Phe	3.9	5.6	2
Pro	5.3	4.7	4
Ser	6.6	7.3	6
Thr	5.3	5.6	4
Trp	1.2	1.8	1
Tyr	3.1	3.3	2
Val	6.8	7.7	4
(Data taken from J. Cedano et al., <i>J. Mol. Biol.</i> 266:594–600, 1997.)			

Table 1.2 How Proteins Work (©2012 Garland Science)

Peptide bond

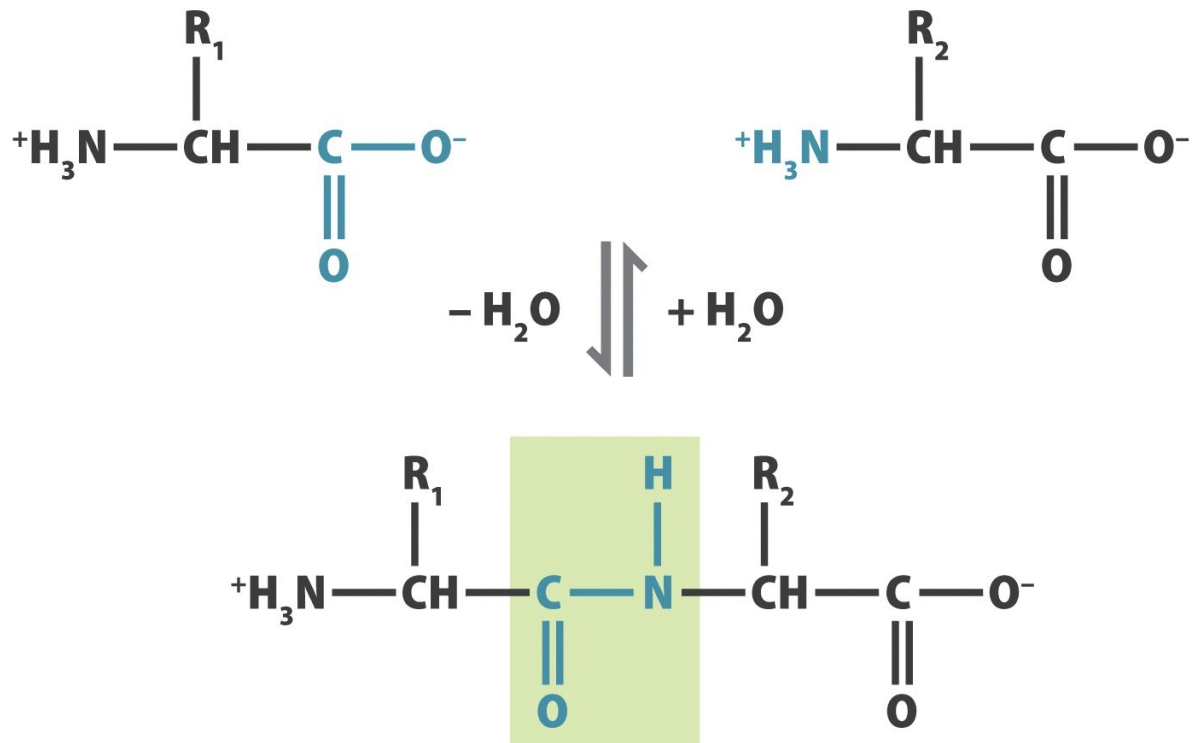


Figure 1.2 How Proteins Work (©2012 Garland Science)

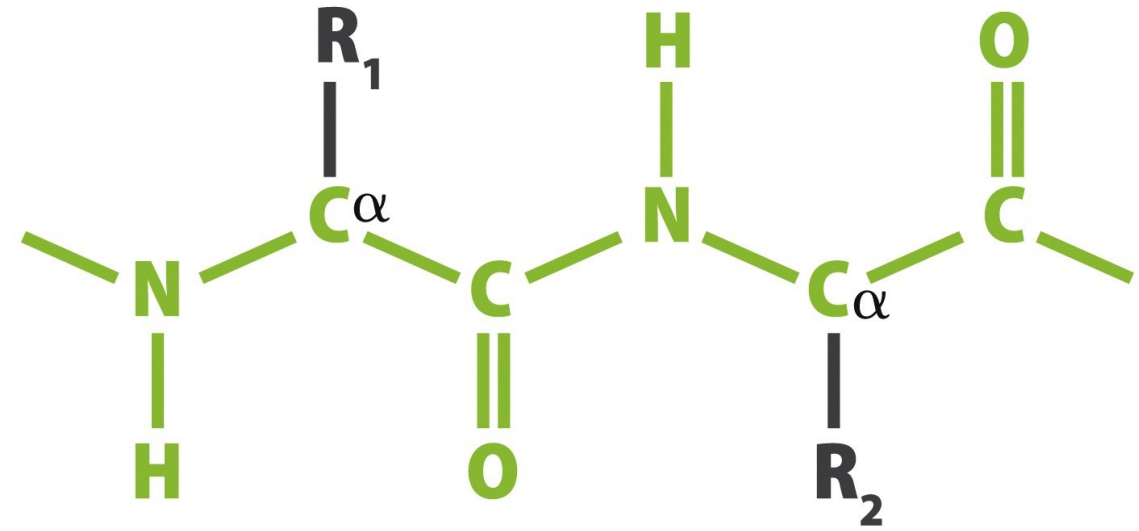


Figure 1.3.1 How Proteins Work (©2012 Garland Science)

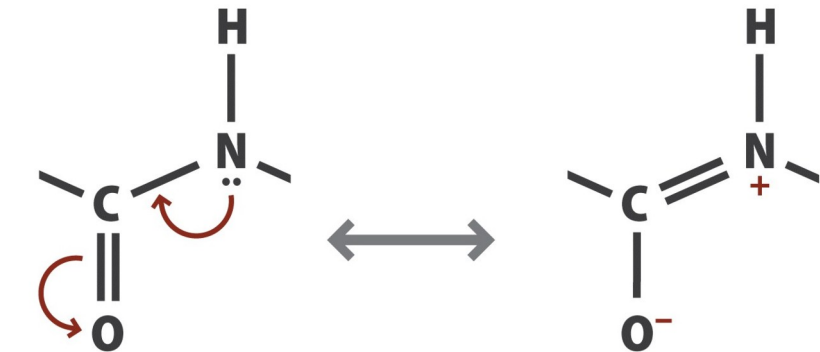


Figure 1.3 How Proteins Work (©2012 Garland Science)

Partial double bond character

Dihedral angle

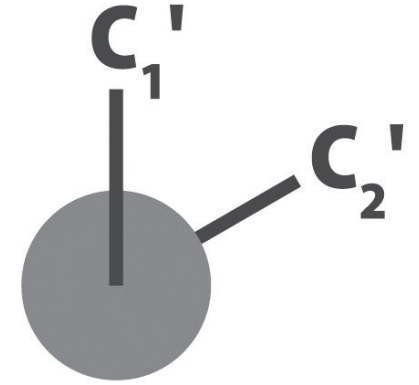


Figure 1.5.1 How Proteins Work (©2012 Garland Science)

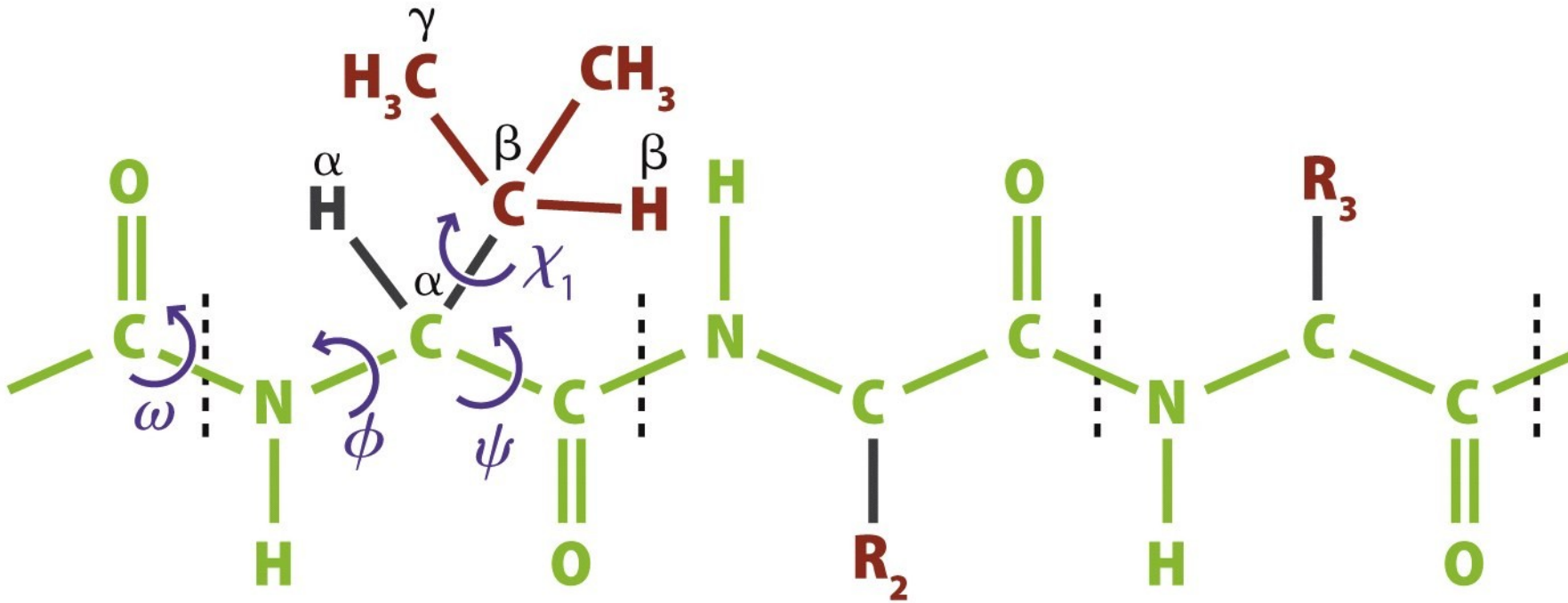
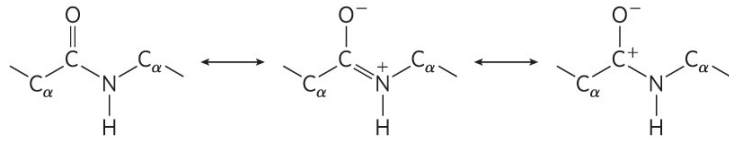
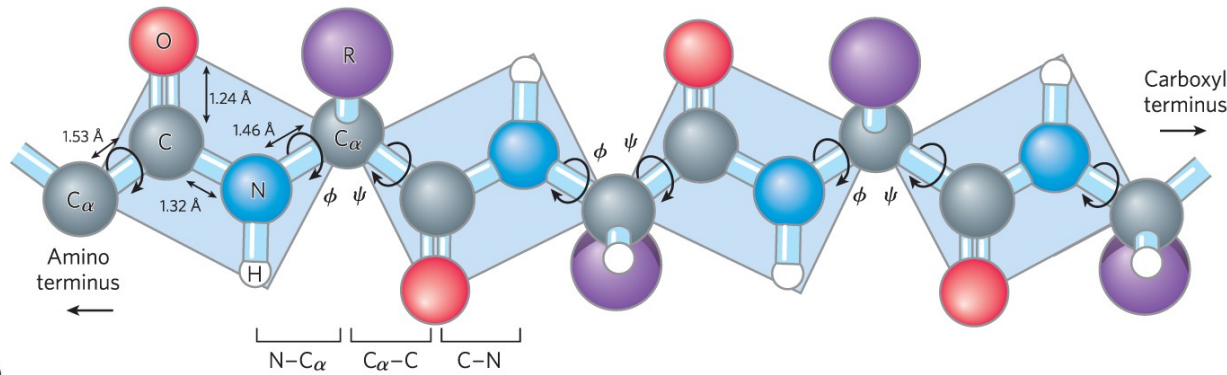


Figure 1.4 How Proteins Work (©2012 Garland Science)

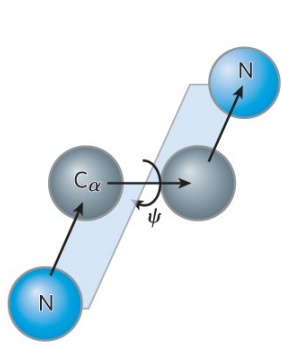
Prohibited conformations



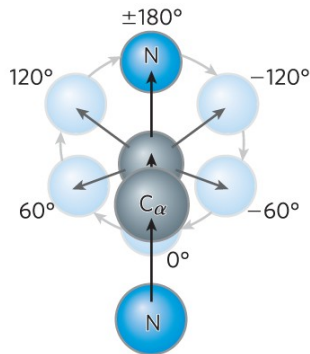
(a)



(b)



(c)



(d)

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

- many ϕ (phi) and ψ (psi) values are prohibited by steric interference
 - ϕ and ψ cannot both = 0 degrees

Ramachandran Plot

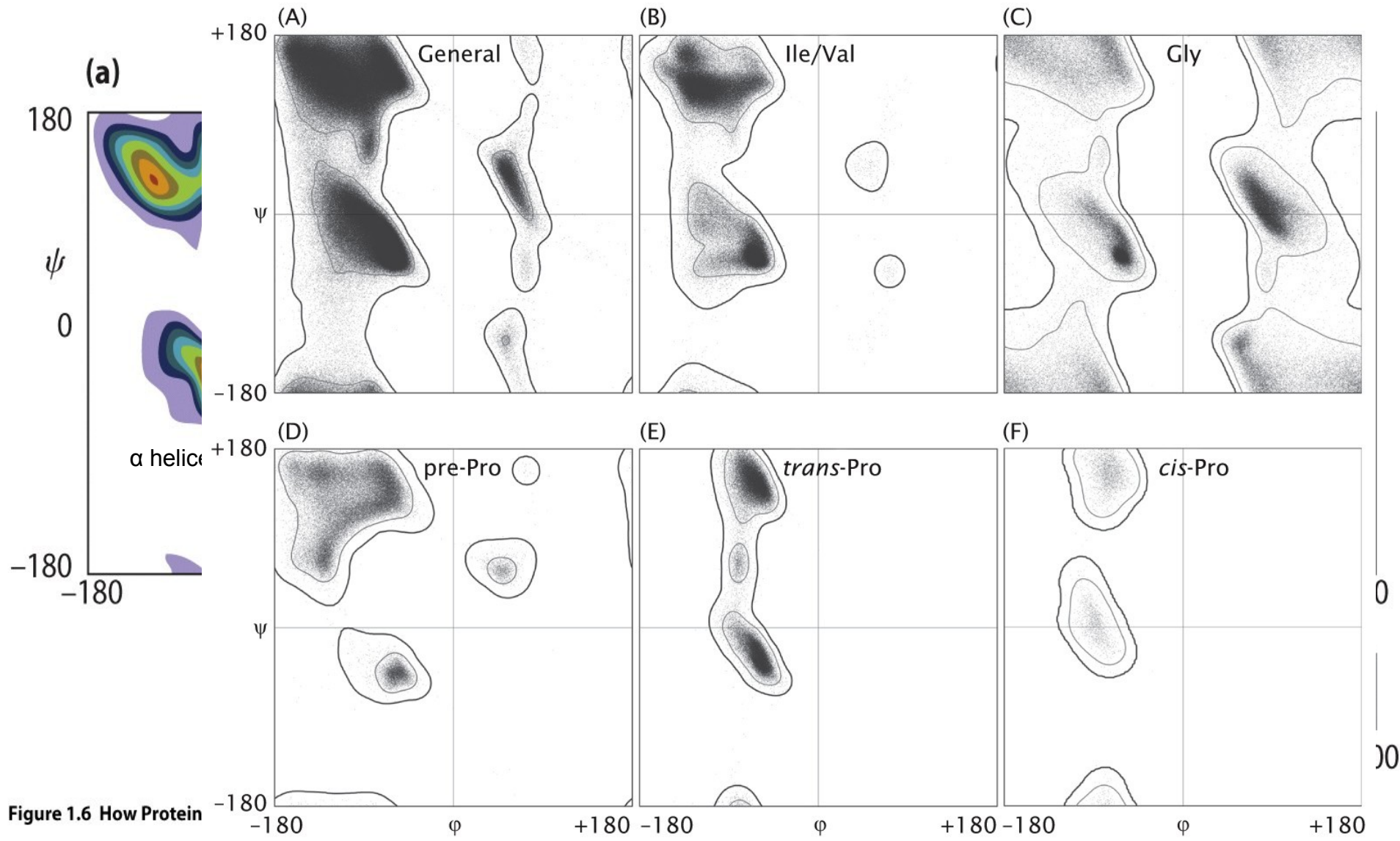


Figure 1.6 How Protein

Table 2.5 Parameters of secondary structure^a

Secondary structure	ϕ (deg)	ψ (deg)	Residues/turn	Rise per residue (Å)
Right-handed α -helix	-57	-47	3.6	1.5
Antiparallel β -sheet	-139	+135	2.0	3.5
Parallel β -sheet	-119	+113	2.0	3.2
3_{10} -Helix	-49	-26	3.0	2.0
Poly(Pro) II, poly(Gly) II	-79	+150	3.0	3.1
Collagen	-51	+153	3.0	3.1

^aData from Ramachandran GN & Sasisekharan V [1968] *Adv Protein Chem* 23:283–437, and IUPAC-IUB Commission on Biochemical Nomenclature [1970] *Biochemistry* 9:3471–3479.

Peptide bond in proline

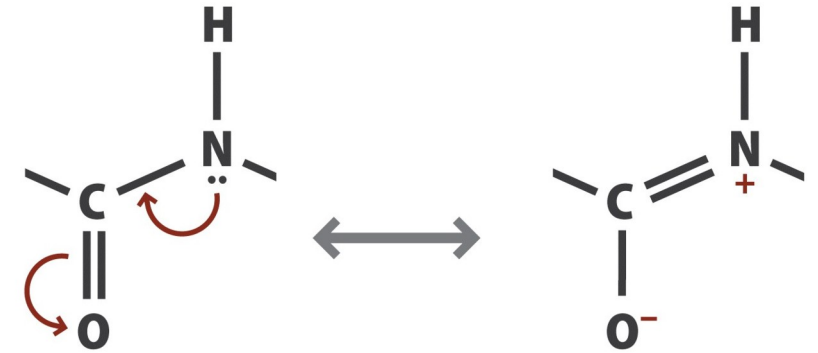


Figure 1.3 How Proteins Work (©2012 Garland Science)

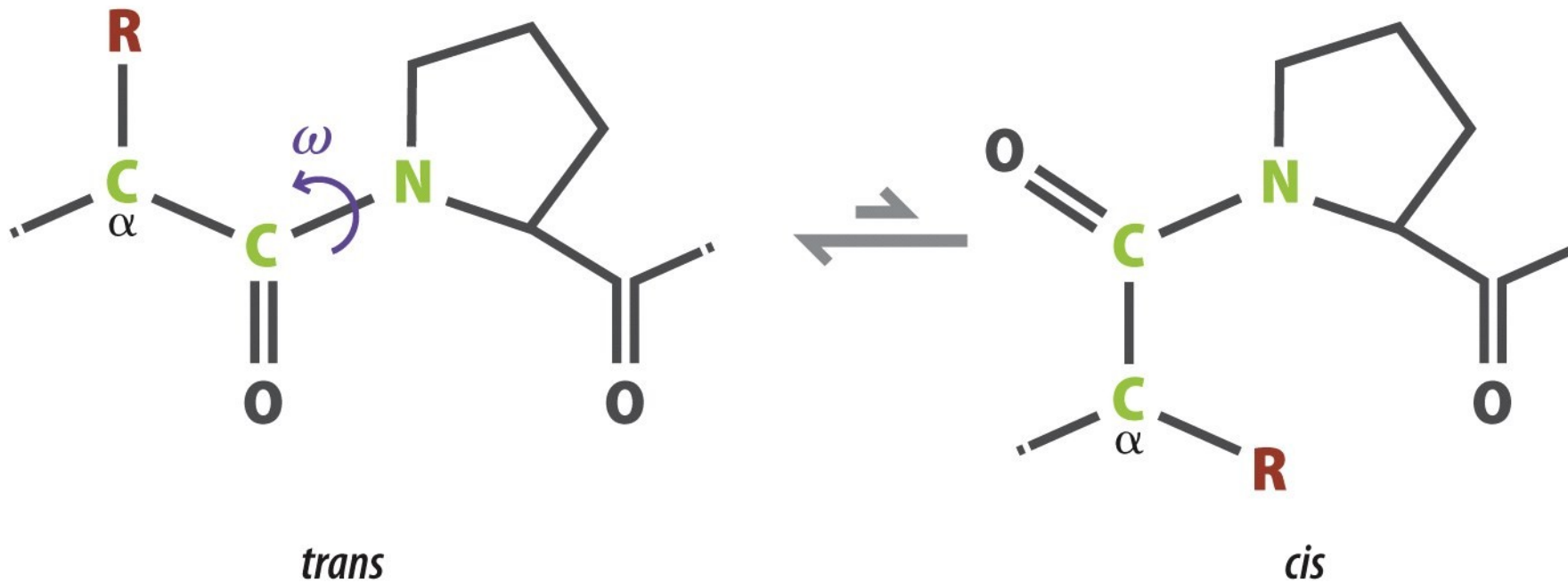
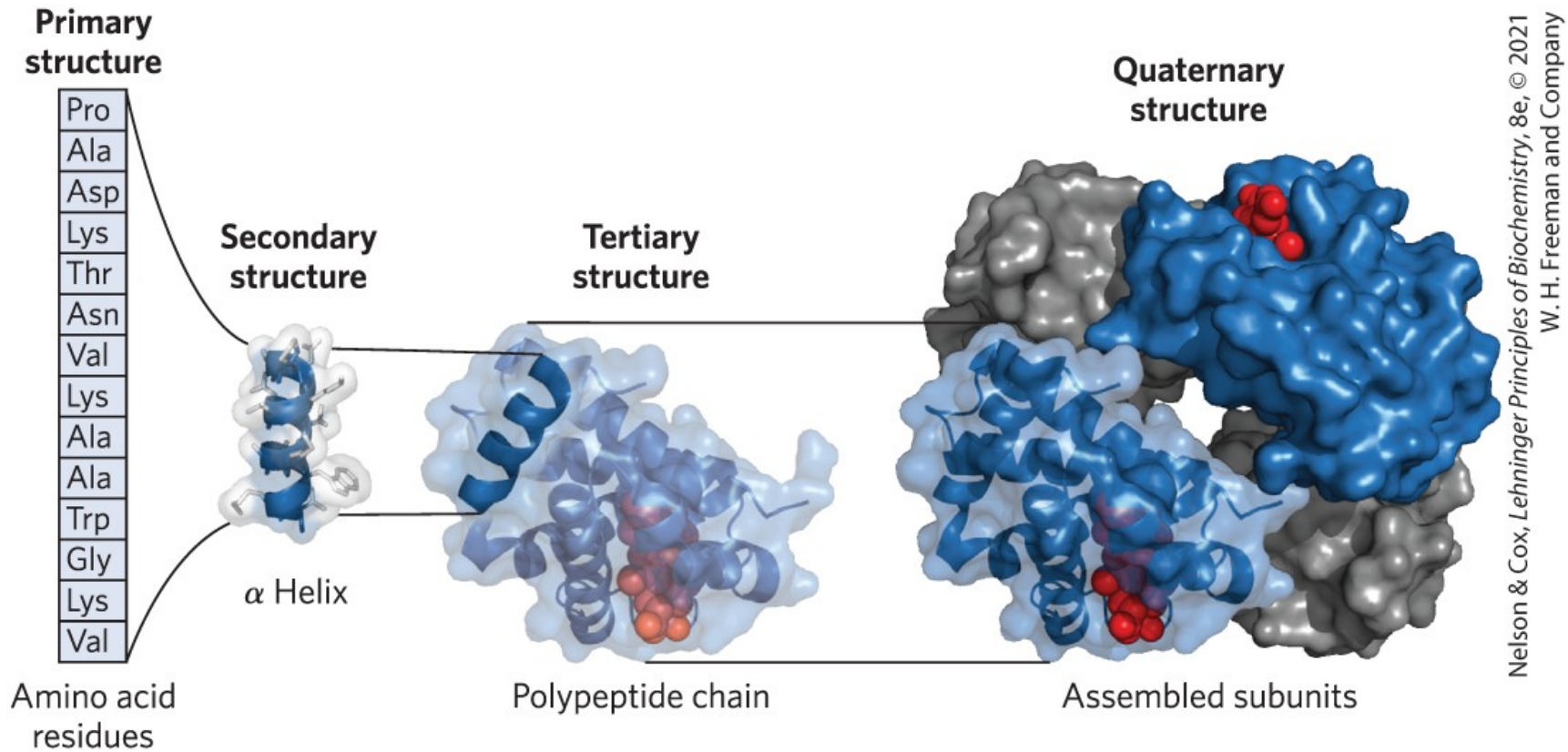


Figure 1.5 How Proteins Work (©2012 Garland Science)

Levels of structure in proteins



- four levels:
 - **primary structure** = covalent bonds linking amino acid residues in a polypeptide chain
 - **secondary structure** = recurring structural patterns
 - **tertiary structure** = 3D folding of polypeptide
 - **quaternary structure** = 2+ polypeptide subunits

Protein sequences help elucidate the history of life on earth

- **bioinformatics:**
 - identifies functional segments in new proteins
 - establishes sequence and structural relationships to known proteins
- essential amino acid residues = conserved over evolutionary time
- less important amino acid residues = vary over evolutionary time

Structure of proteins

secondary structure prediction

[illegible]

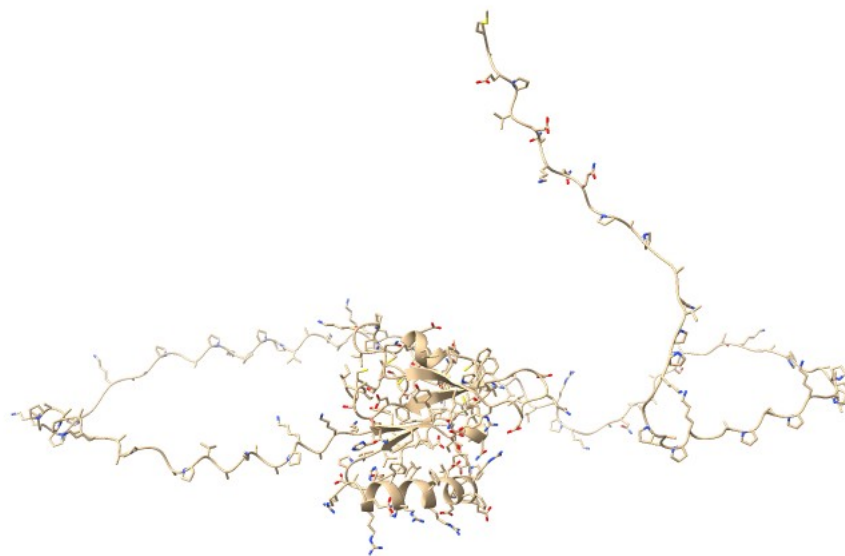
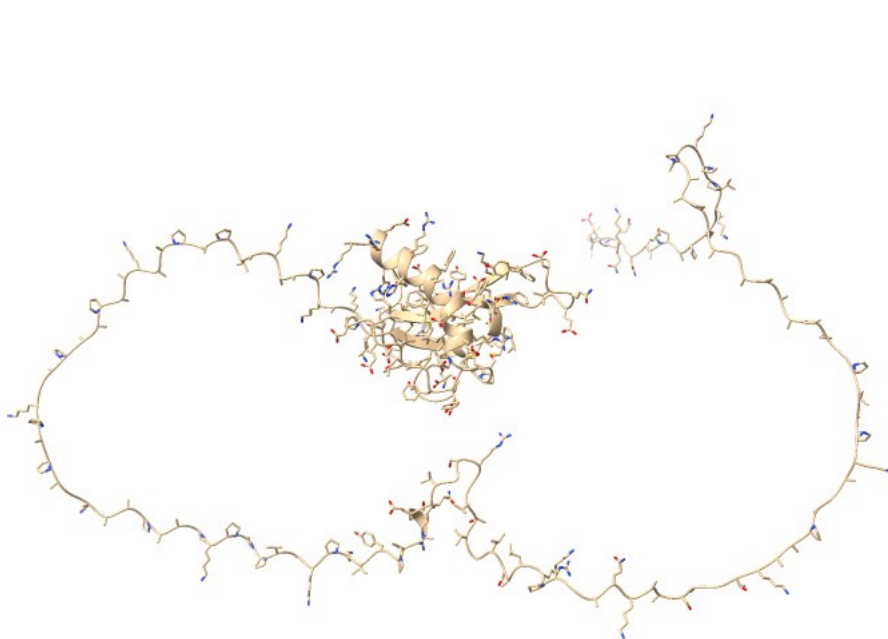
Blue: hydrophobic
Brown: aromatic
Green: polar
Olive: sulfur contain

disorder prediction

index

diso_prob

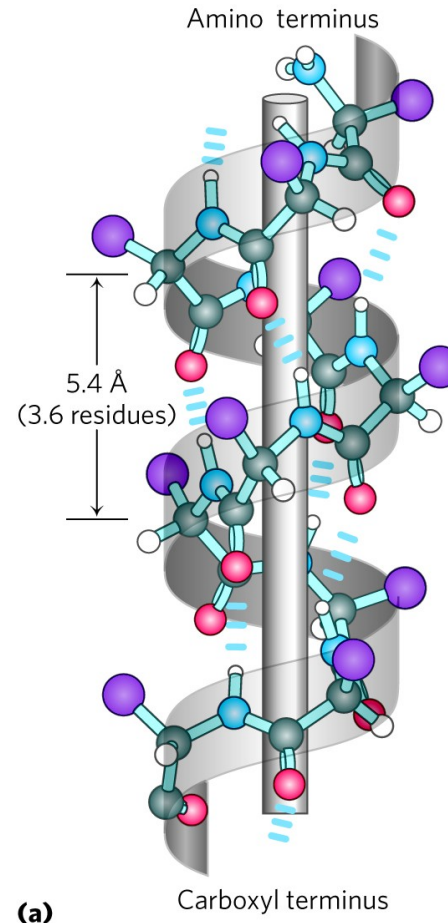
diso_prob



PscB CT2019
AlphaFold

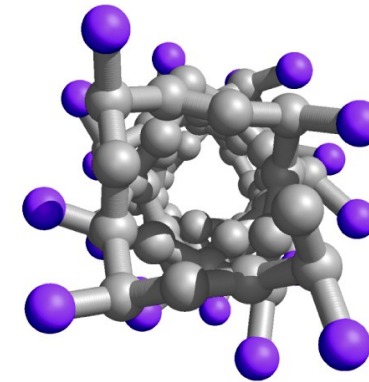
The α Helix Is a common protein secondary structure

- **α helix** = simplest arrangement, maximum number of hydrogen bonds
 - backbone wound around an imaginary longitudinal axis
 - R groups protrude out from the backbone
 - each helical turn = 3.6 residues, ~ 5.4 Å
- between hydrogen atom attached to the electronegative nitrogen atom of residue n and the electronegative carbonyl oxygen atom of residue $n + 4$
- confers significant stability

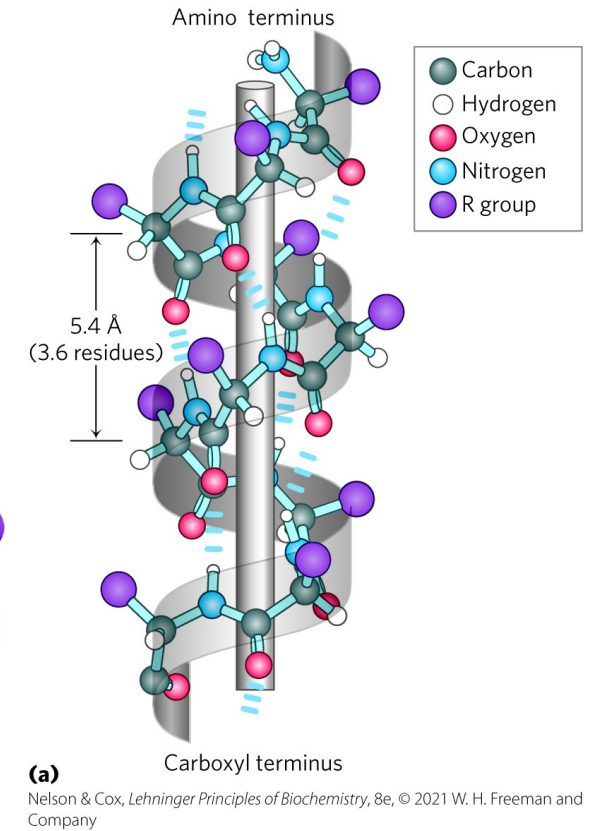


(a)

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company



(b)

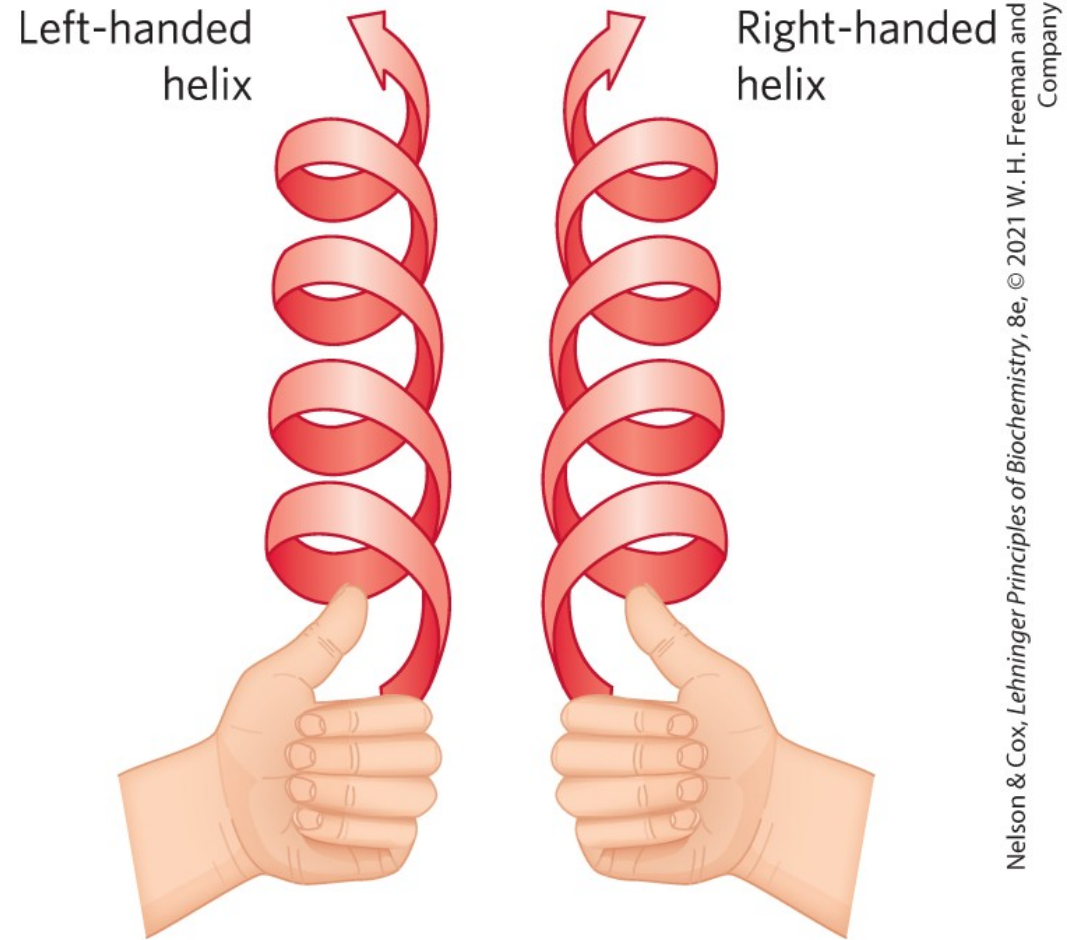


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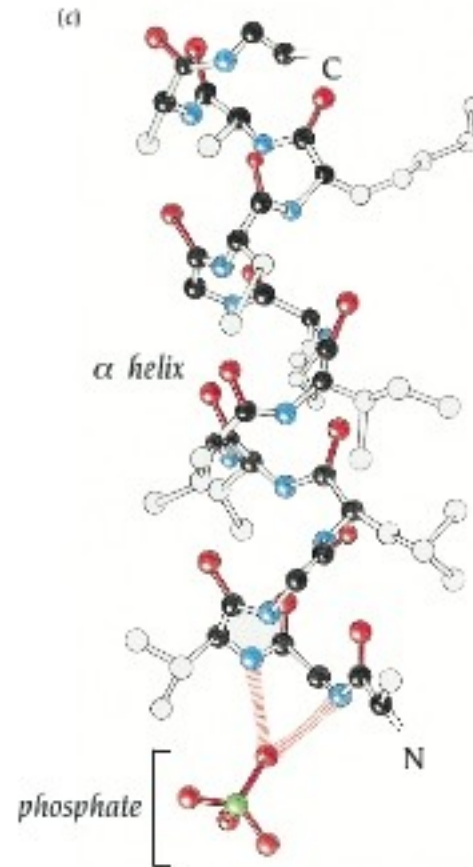
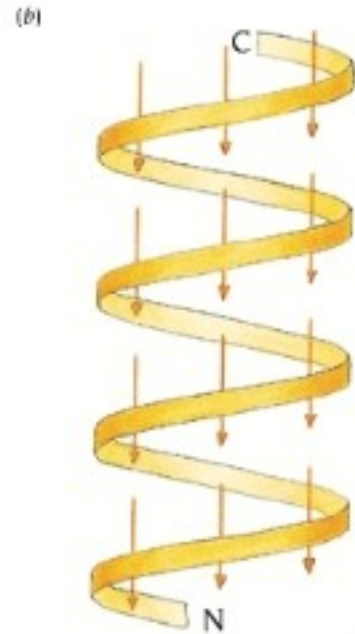
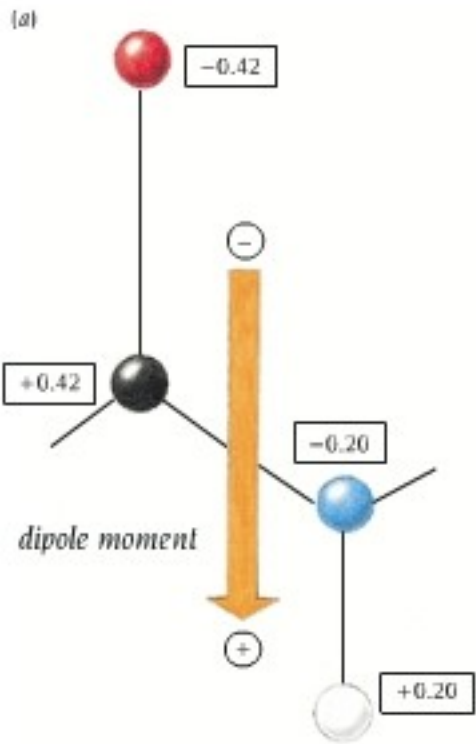
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Handedness of the α Helix

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



α helix

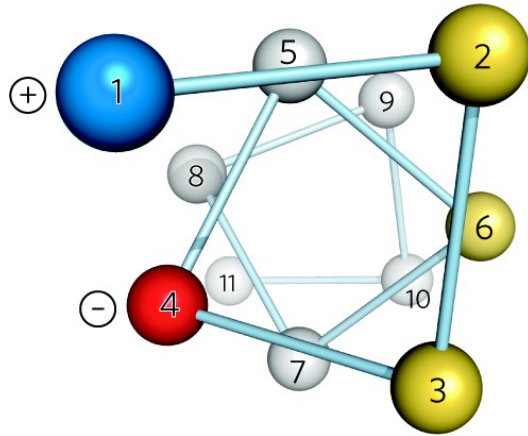


small electric dipoles in each peptide bond align through hydrogen bonds

negatively charged amino acids often found near the NH_3^+ terminus

positively charged amino acids often found near the COO^- terminus

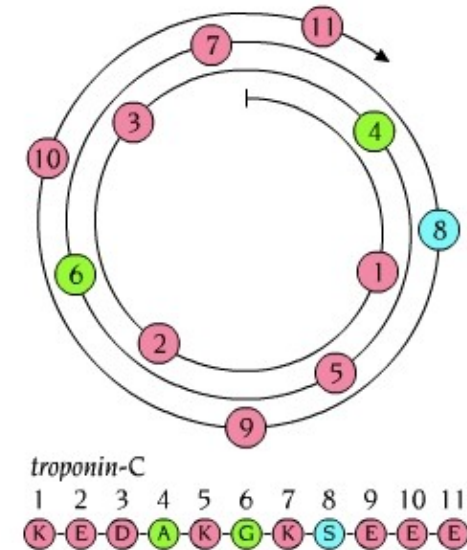
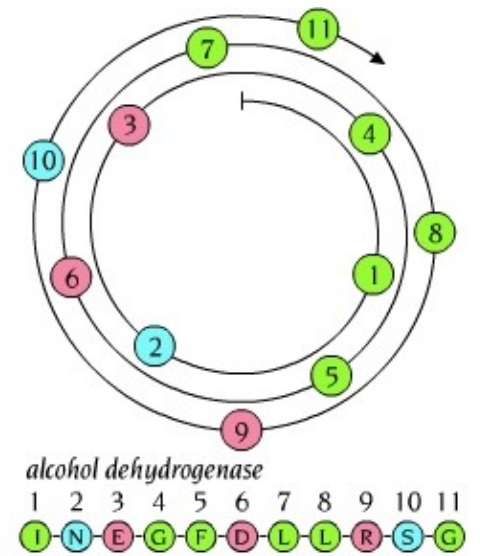
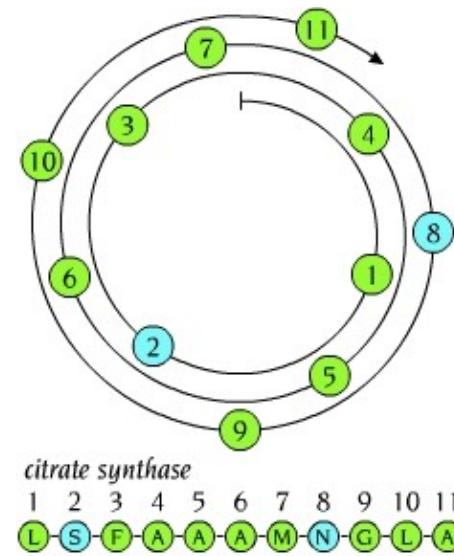
α helix-Helical wheel



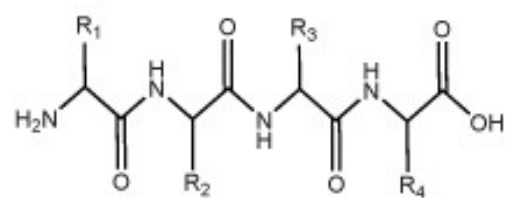
amino acid residues have an intrinsic propensity to form an α helix

interactions between R chains spaced 3–4 residues apart can stabilize or destabilize α helix

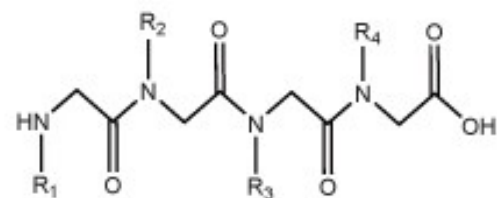
charge, size, and shape of R chains can destabilize formation of ion pairs and hydrophobic effect can stabilize



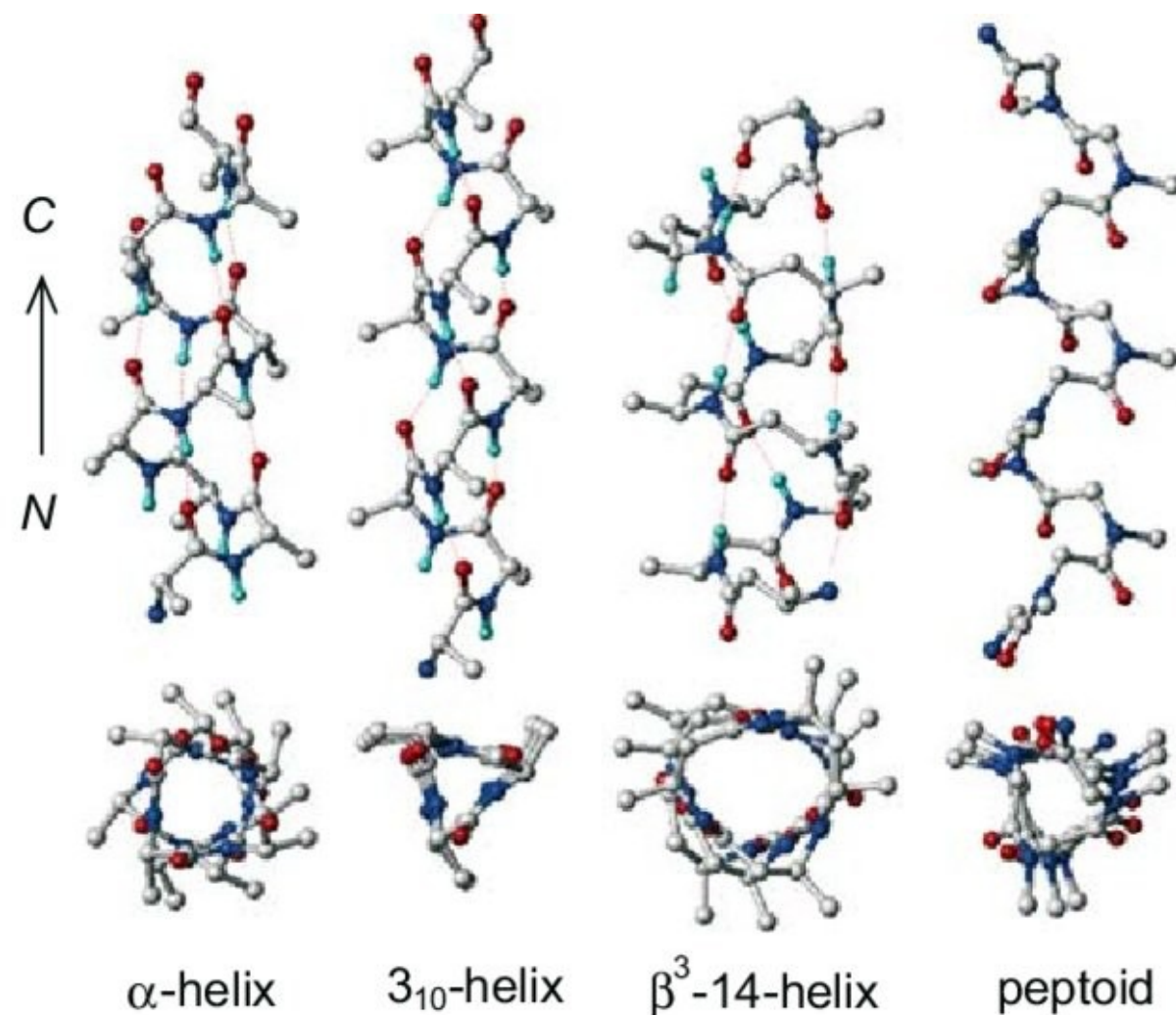
	Backbone torsional angles		Residues per turn	Translation per residue (Å)
	Φ (deg)	Ψ (deg)		
α -Helix	-57	-47	3.6	1.5
3_{10} -Helix	-49	-26	3.0	2.0
Antiparallel β -sheet	-139	+135	2.0	3.4
Parallel β -sheet	-119	+113	2.0	3.2
Polyproline I	-83	+158	3.3	1.9
Polyproline II	-78	+149	3.0	3.1



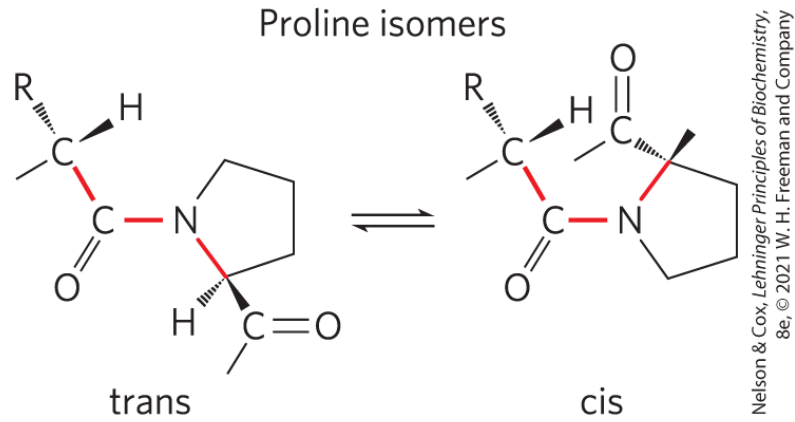
Peptide



Peptoid



Poly-proline



- proline = introduces destabilizing kink in helix
- nitrogen atom is part of rigid ring
- rotation about $\text{N}-\text{C}_\alpha$ bond not possible

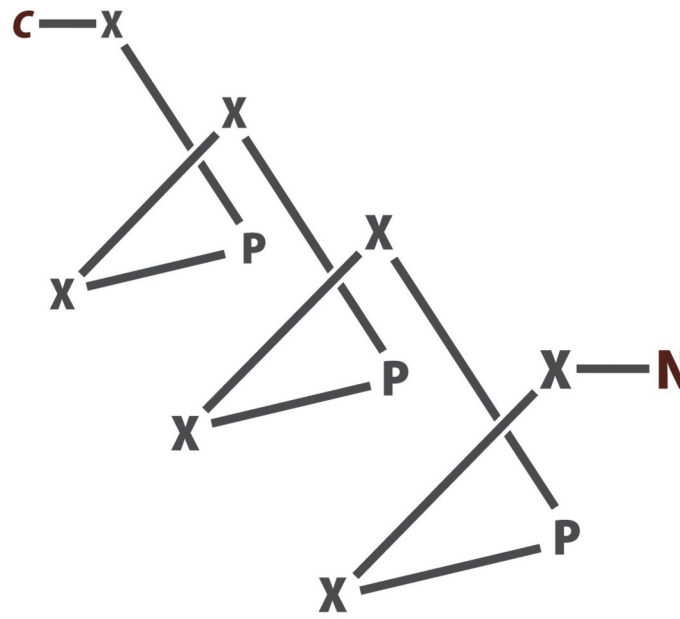
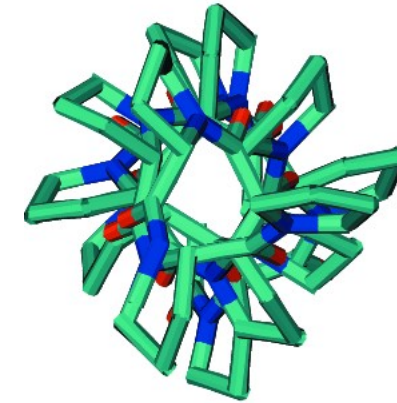
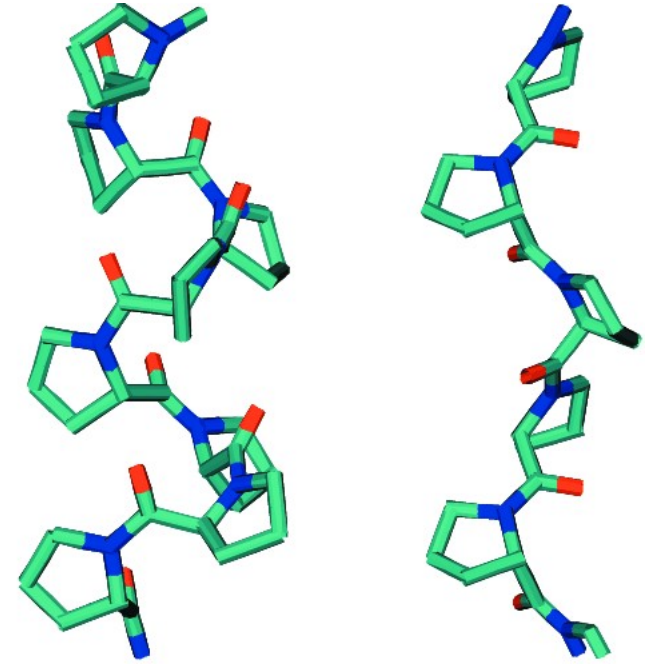
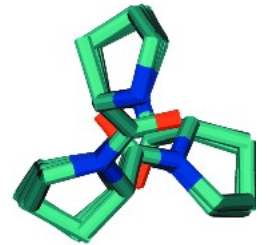


Figure 1.13 How Proteins Work (©2012 Garland Science)



(a)



(b)

β sheet-two types

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

Antiparallel β -sheet
Parallel β -sheet

-139
-119

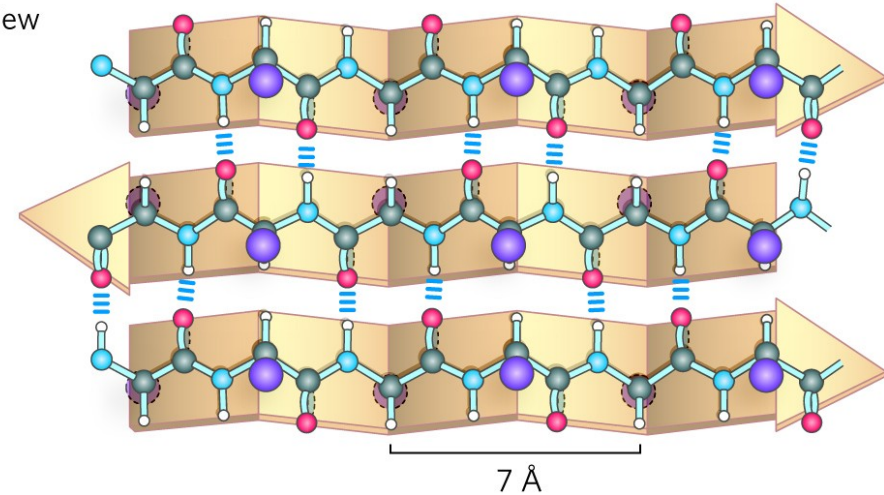
+135
+113

2.0
2.0

3.4
3.2

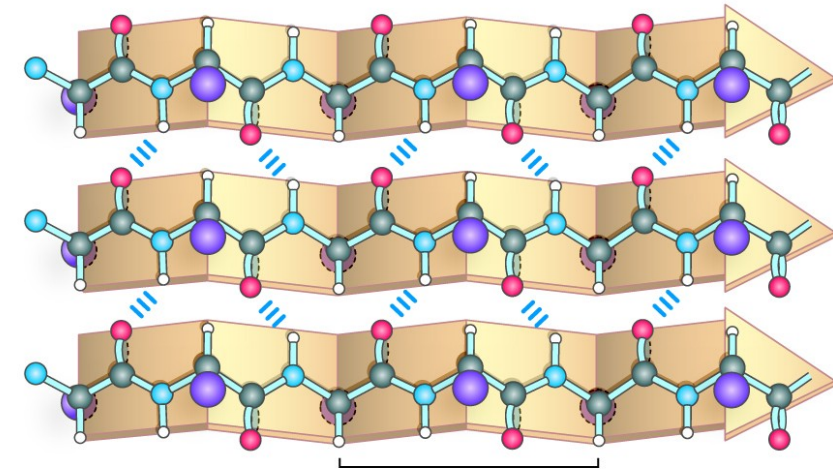
(b) Antiparallel β sheet

Top view

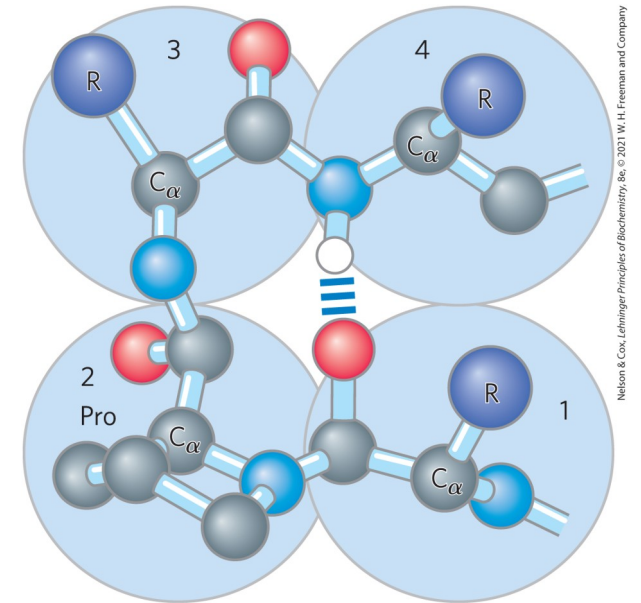
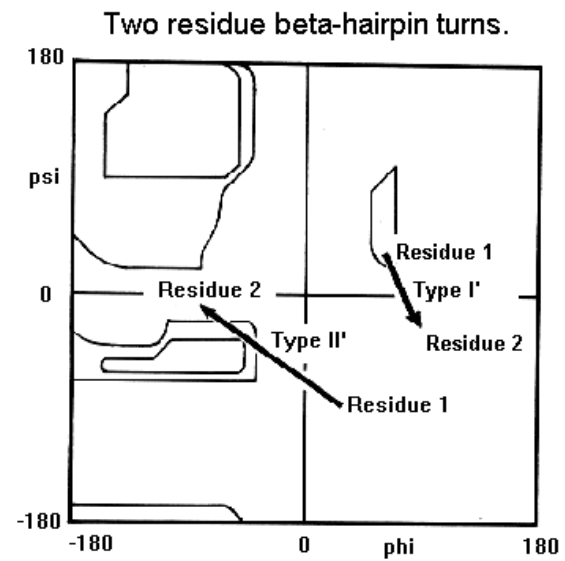
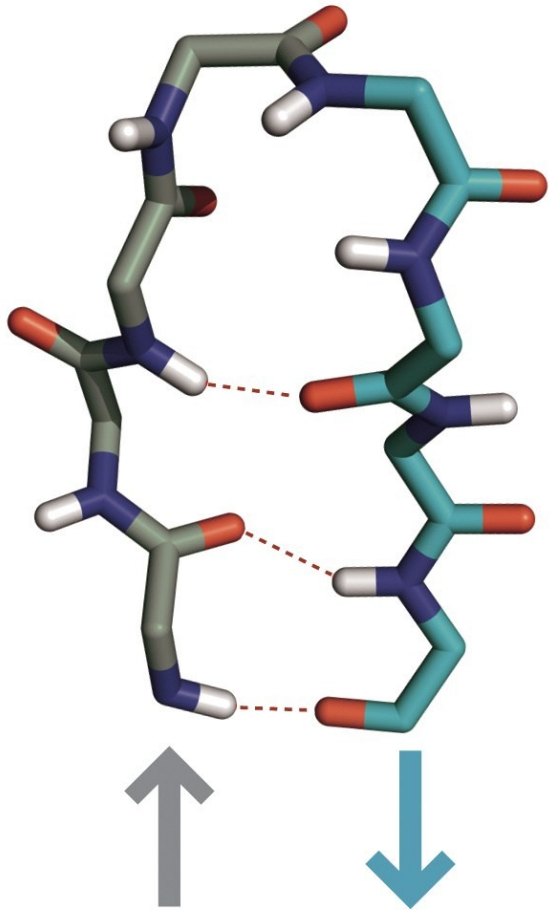


(c) Parallel β sheet

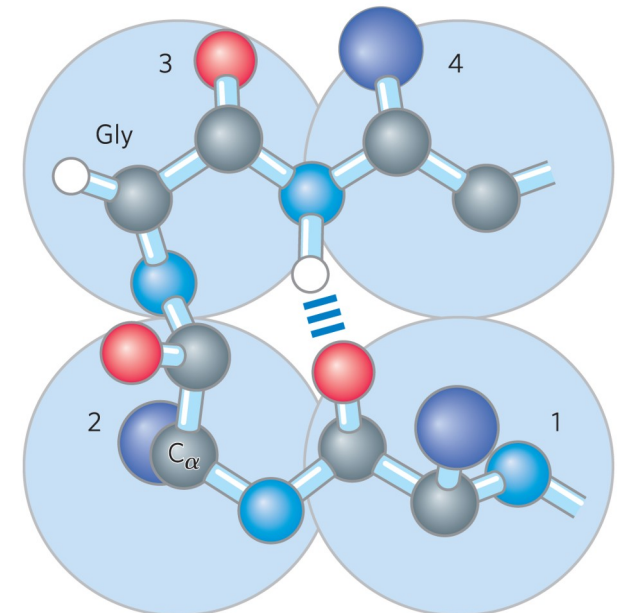
Top view



b-hairpin



Type I β turn



Type II β turn

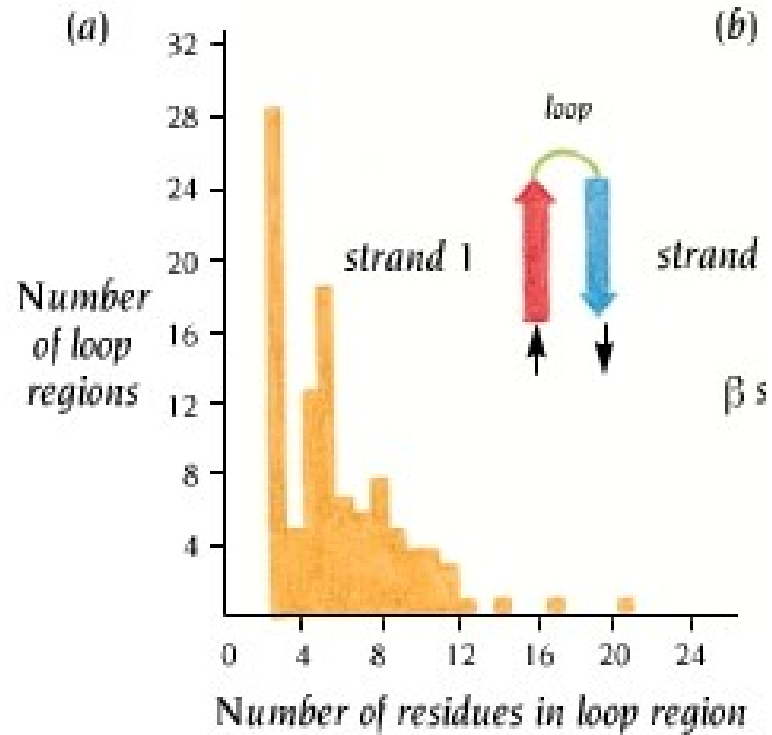
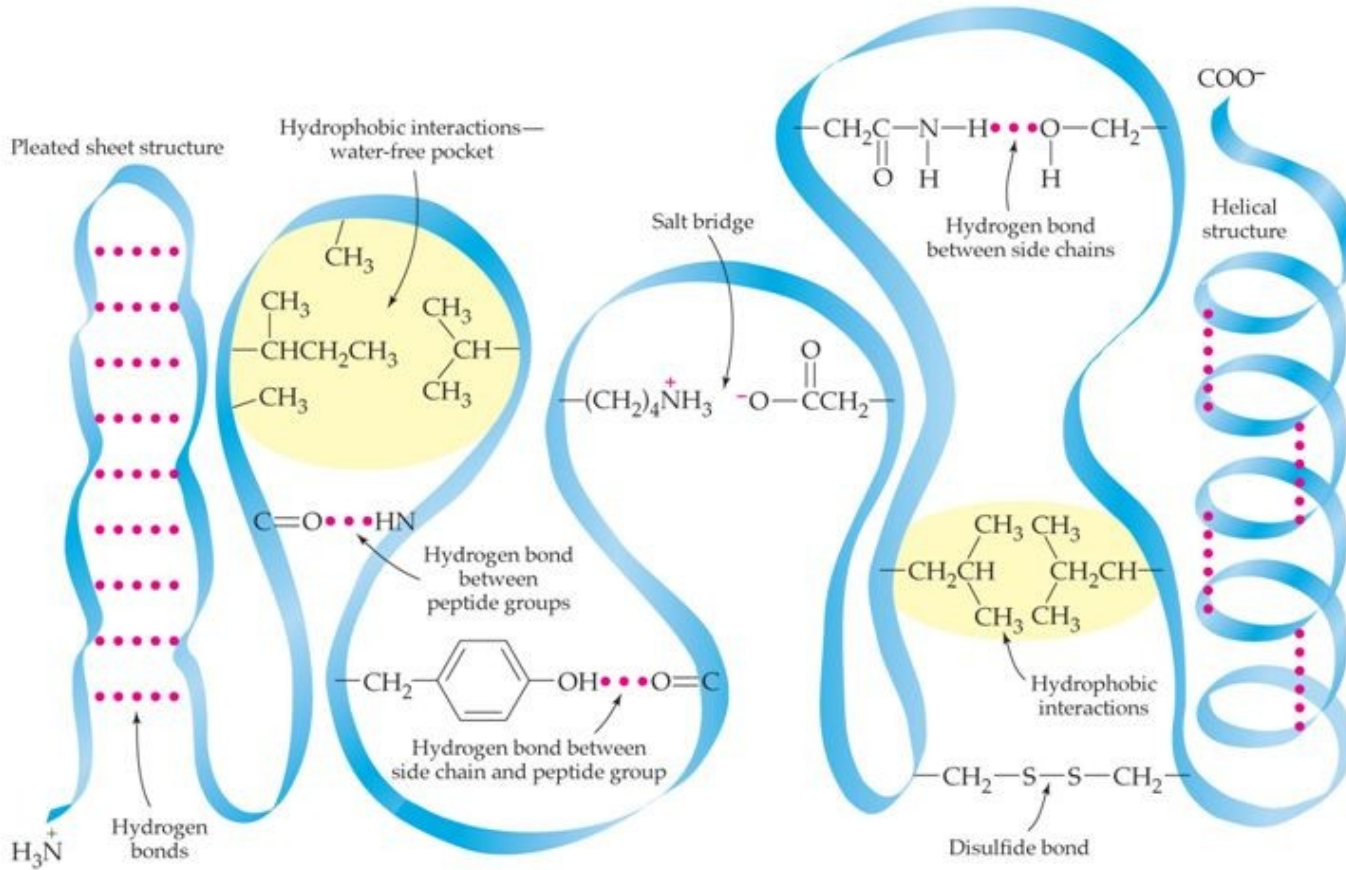


Figure 1.11 How Proteins Work (©2012 Garland Science)

forces holding proteins together

1. Disulfide linkages
2. Hydrogen Bonding
3. Electrostatic interactions
4. Hydrophobic interactions



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Interactions	Typical energy (kJ mol ⁻¹)
Van der Waals	1
Hydrogen bonds	8–29
Electrostatic	17–50
Non-polar	60–80
Disulphide bond (S–S)	250
Single covalent bond	200–500

Water as solvent

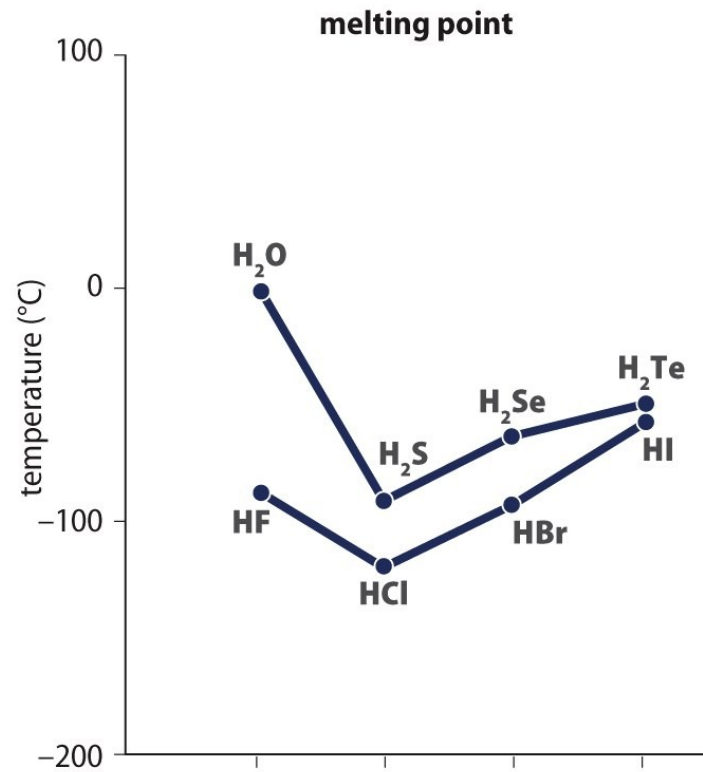
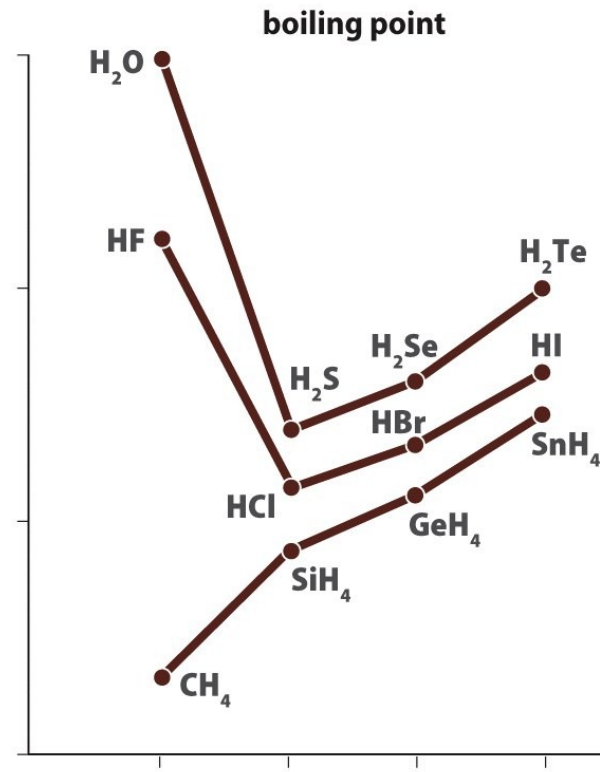
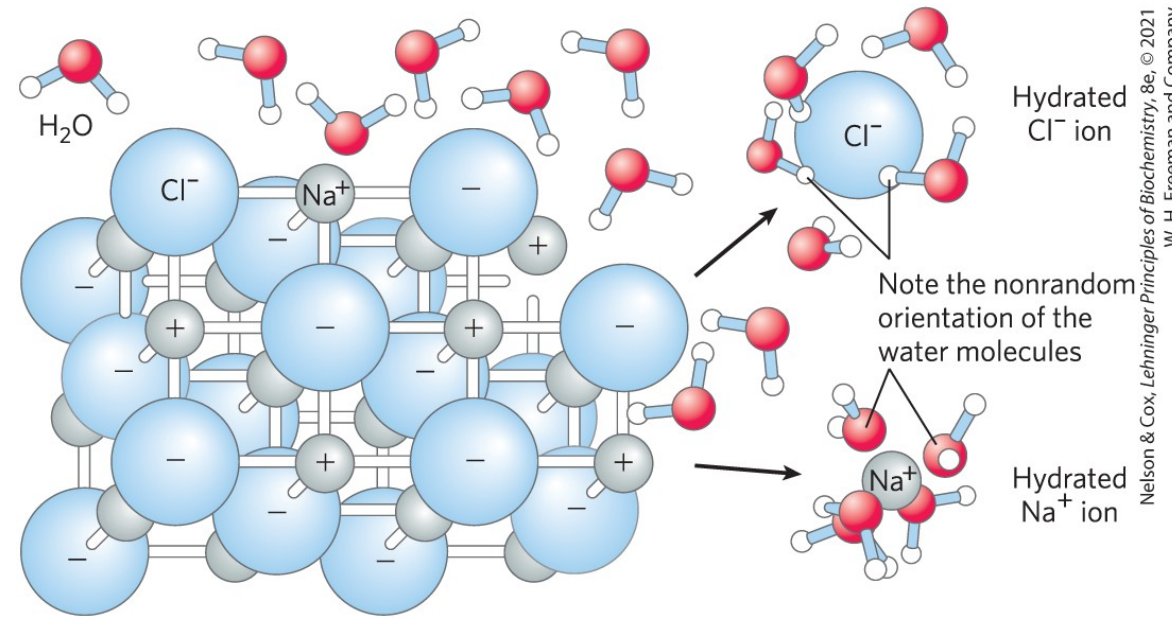


Figure 1.9.1 How Proteins Work (©2012 Garland Science)



Water as solvent



- H₂O dissolves salts and charged biomolecules by screening electrostatic interactions
- the increase in entropy of the system is largely responsible for the ease of dissolving salts in water

Forces holding proteins together electrostatic

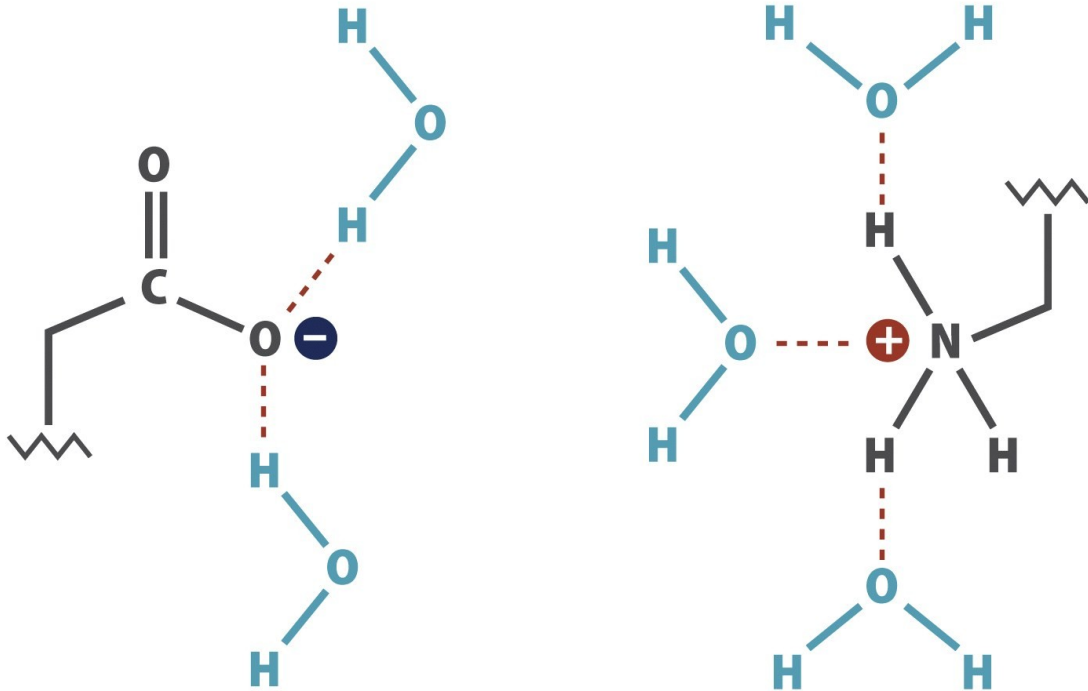
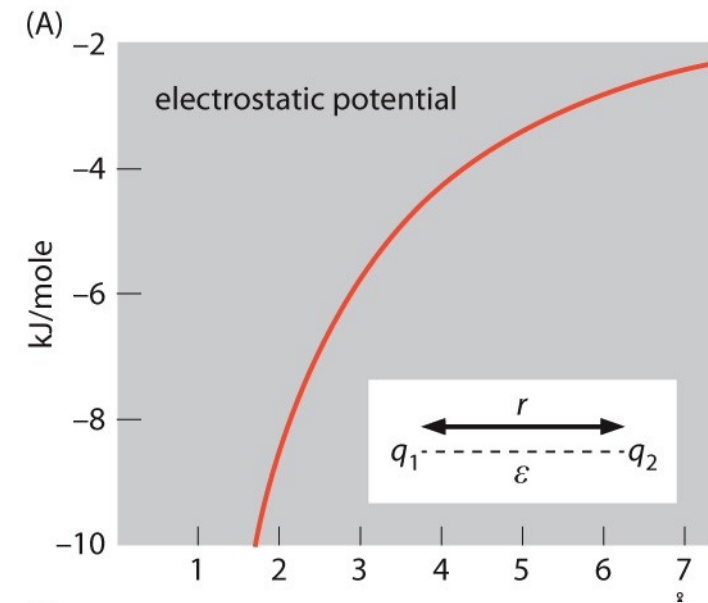
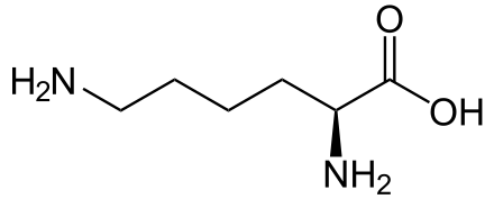


Figure 1.17 How Proteins Work (©2012 Garland Science)

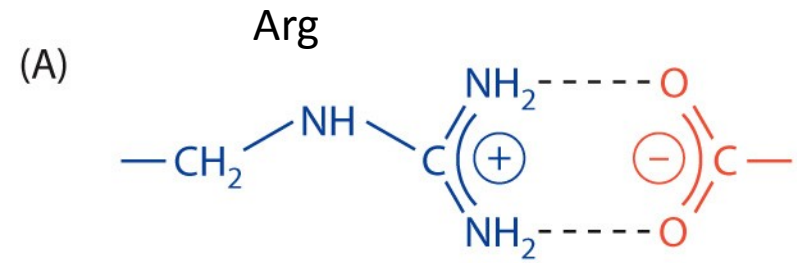


electrostatic interactions

Electrostatic field



Cu-Zn superoxide dismutase



(B)

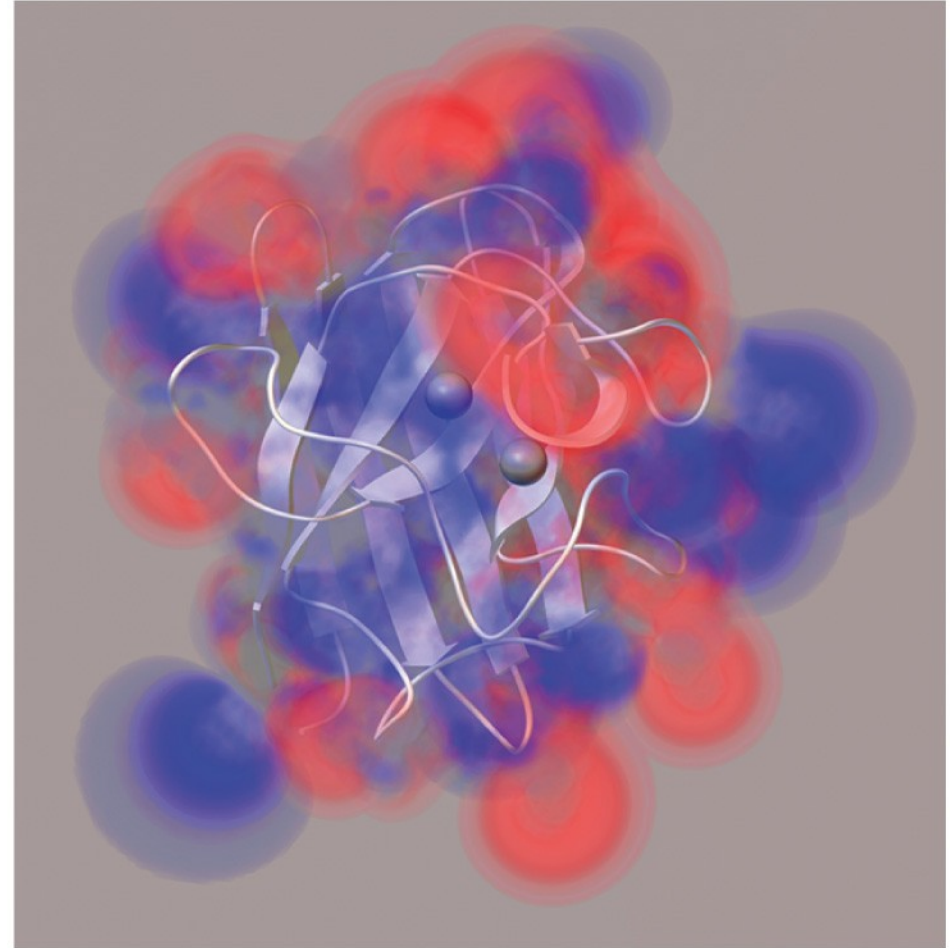


Figure 1.4 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

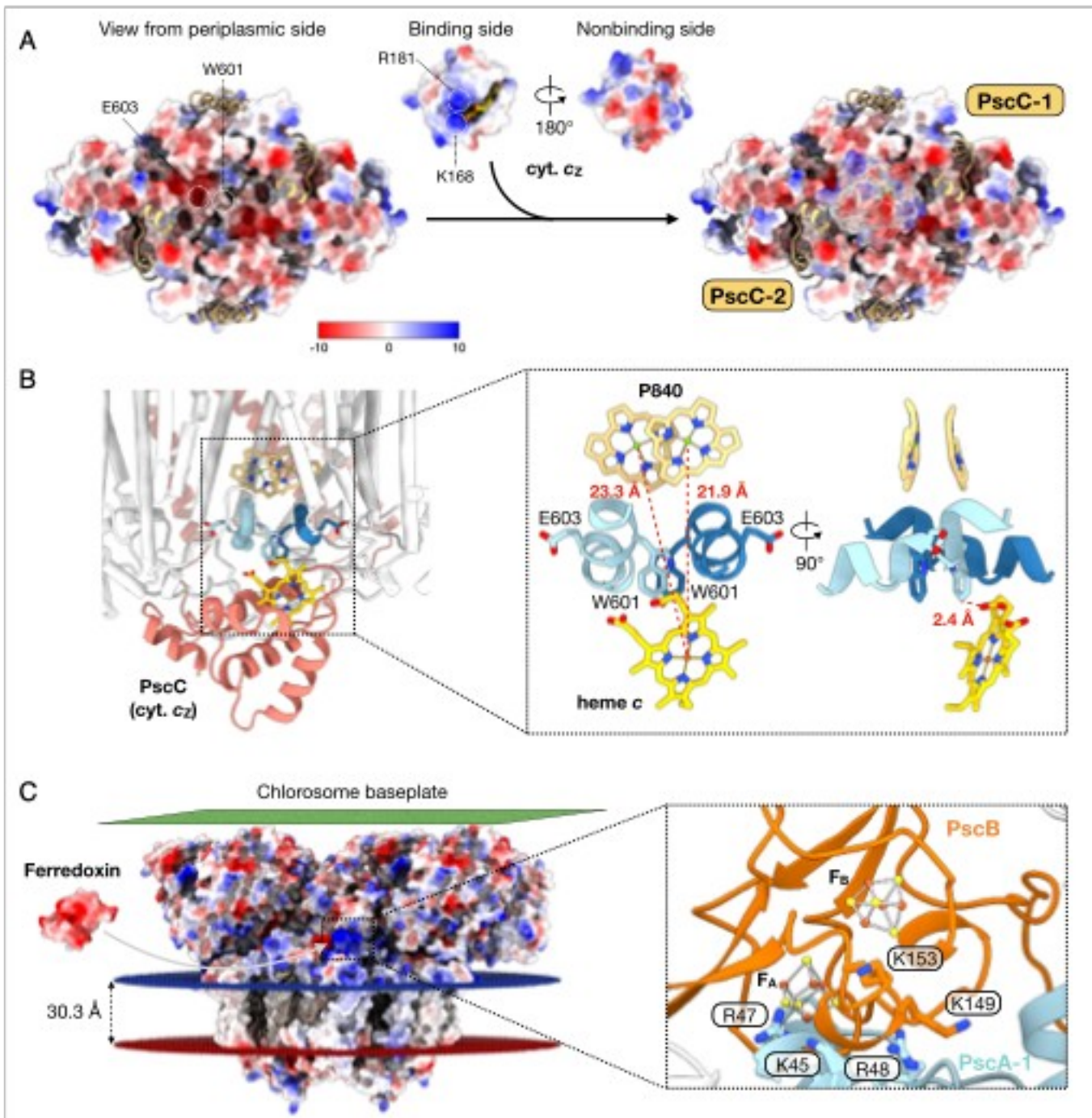
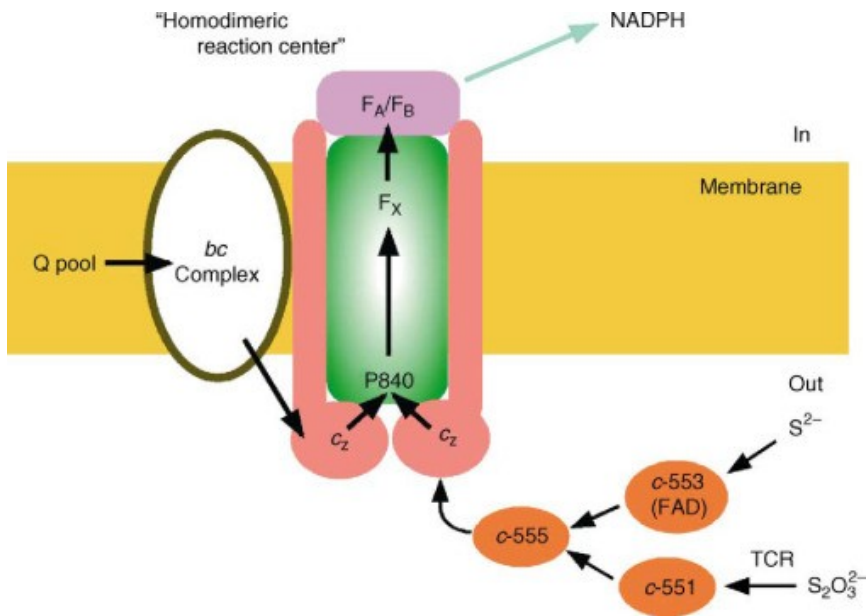


Cryo-EM structure of the whole photosynthetic reaction center apparatus from the green sulfur bacterium *Chlorobaculum tepidum*

Hao Xie^{a,1}, Alexandros Lytratzakis^b, Radhika Khara^a, Myrto Koutantou^b, Sonja Welsch^c, Hartmut Michel^{b,1}, and Georgios Tsiotis^{b,1}

Contributed by Hartmut Michel; received September 30, 2022; accepted December 27, 2022; reviewed by Alexey Arnouts and C. N. Hunter

PNAS 2023 Vol. 120 No. 5 e2216734120



H-bound interactions

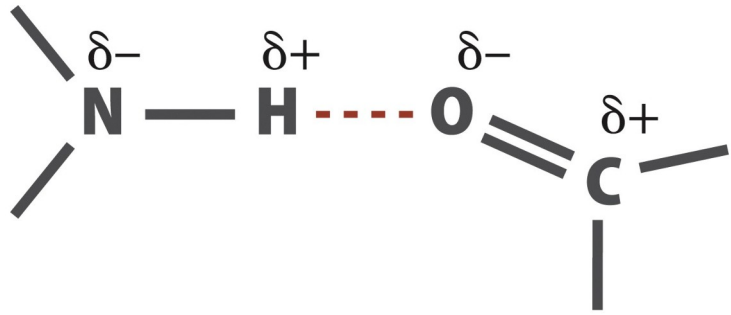


Figure 1.18 How Proteins Work (©2012 Garland Science)

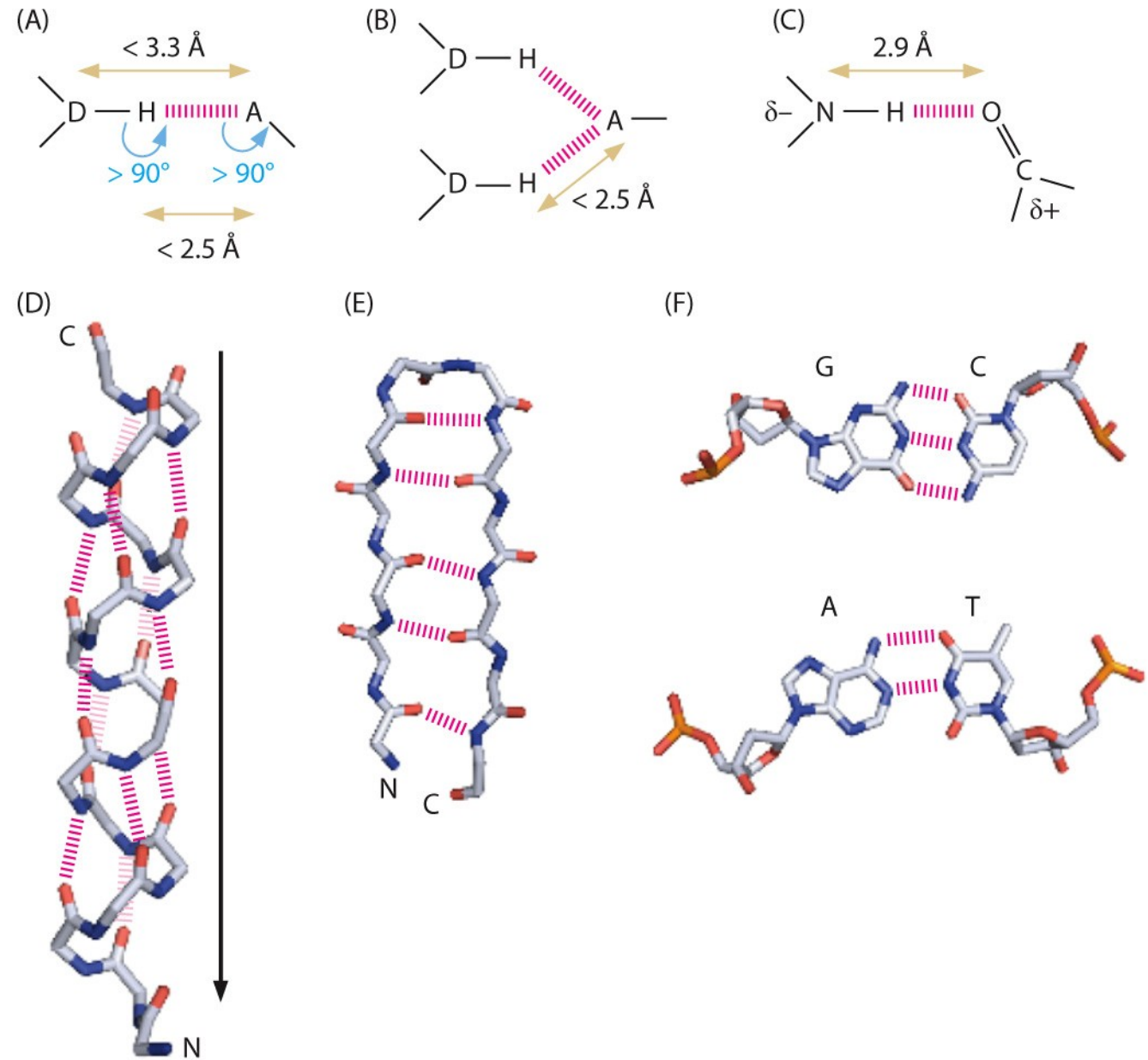


Figure 1.5 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

formation of β -hairpin
is cooperative

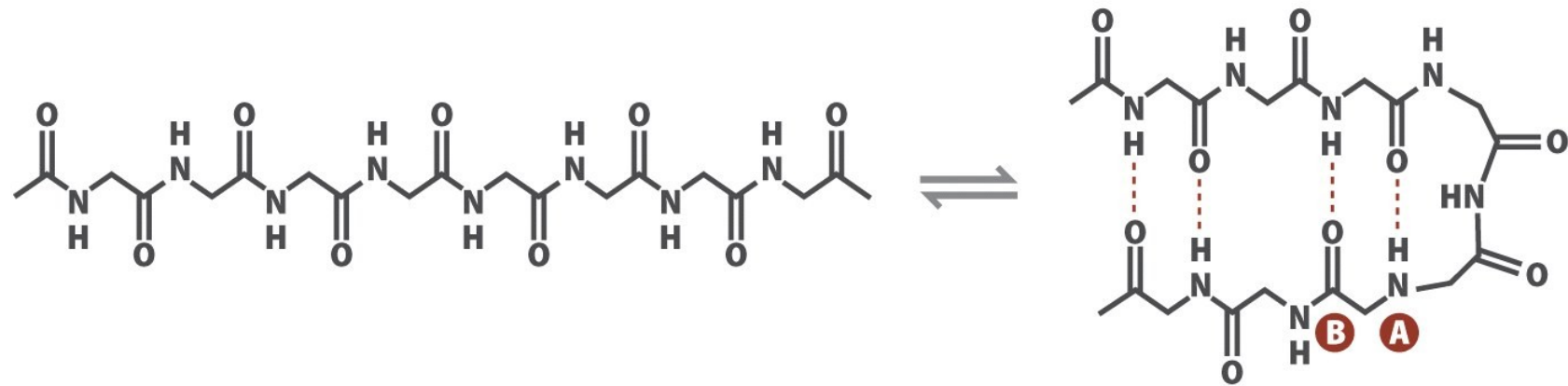


Figure 1.25 How Proteins Work (©2012 Garland Science)

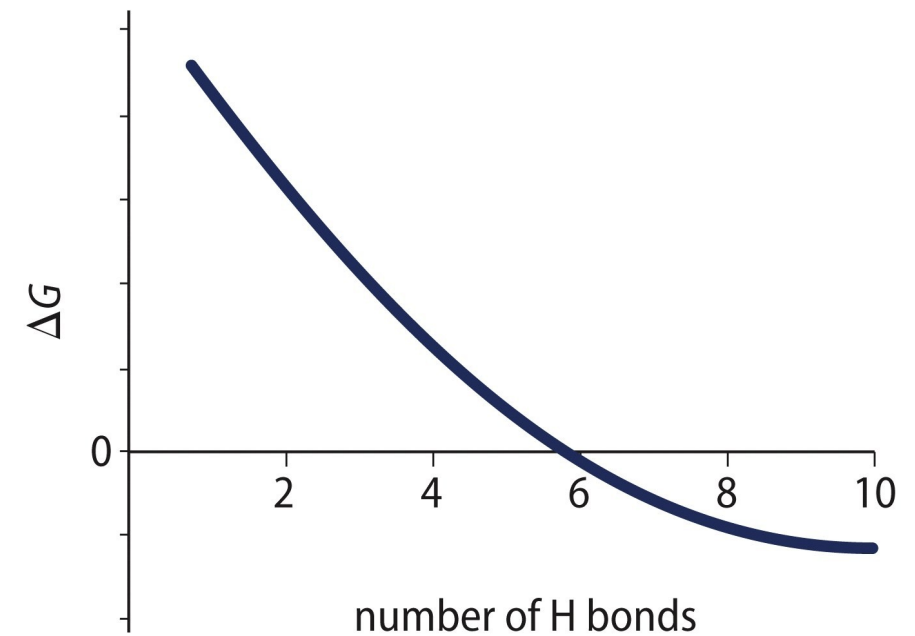


Figure 1.26 How Proteins Work (©2012 Garland Science)

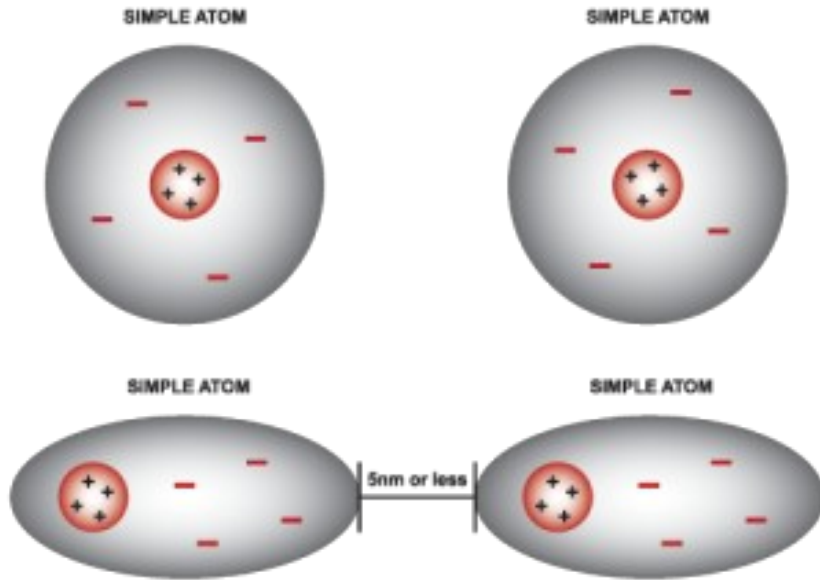
Van der Waals interactions

VAN DER WAALS' FORCES (VDW)
DIAGRAM

KEY

+ POSITIVE NUCLEUS

- NEGATIVE CHARGED ELECTRON CLOUD



When two atoms come within 5 nanometers of each other, there will be a slight interaction between them, thus causing polarity and a slight attraction.

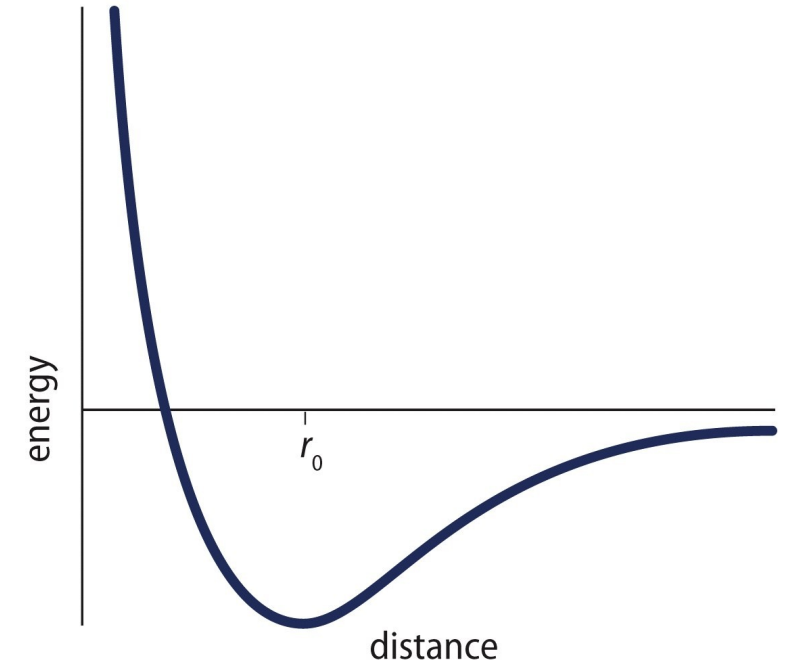


Figure 1.20 How Proteins Work (©2012 Garland Science)



Figure 1.22 How Proteins Work (©2012 Garland Science)

hydrophobic interactions

$$\Delta G = \Delta H - T \Delta S$$

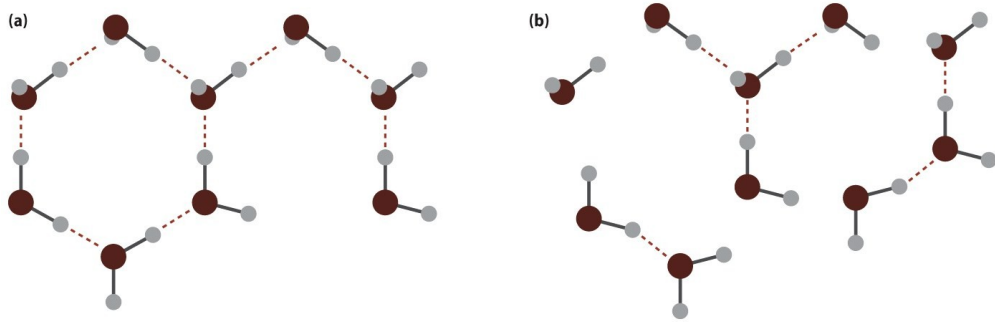


Figure 1.9.2 How Proteins Work (©2012 Garland Science)

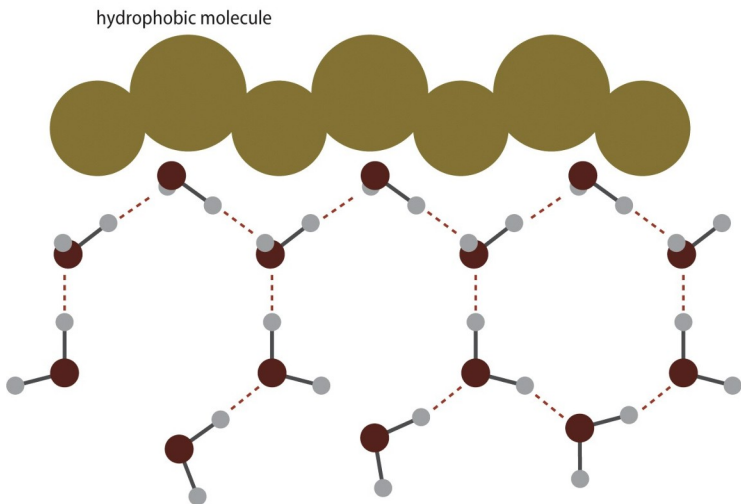
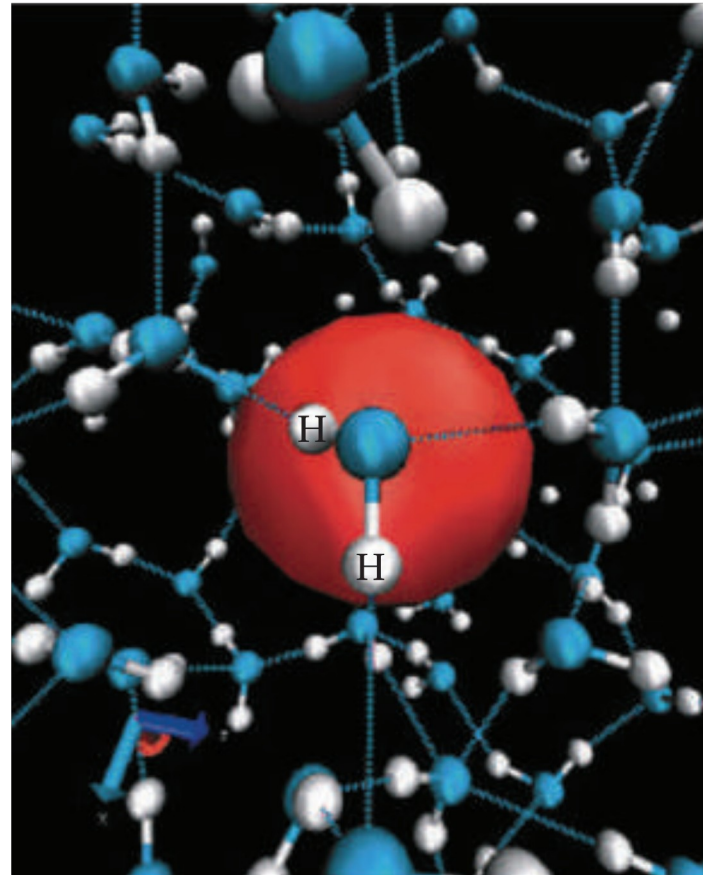
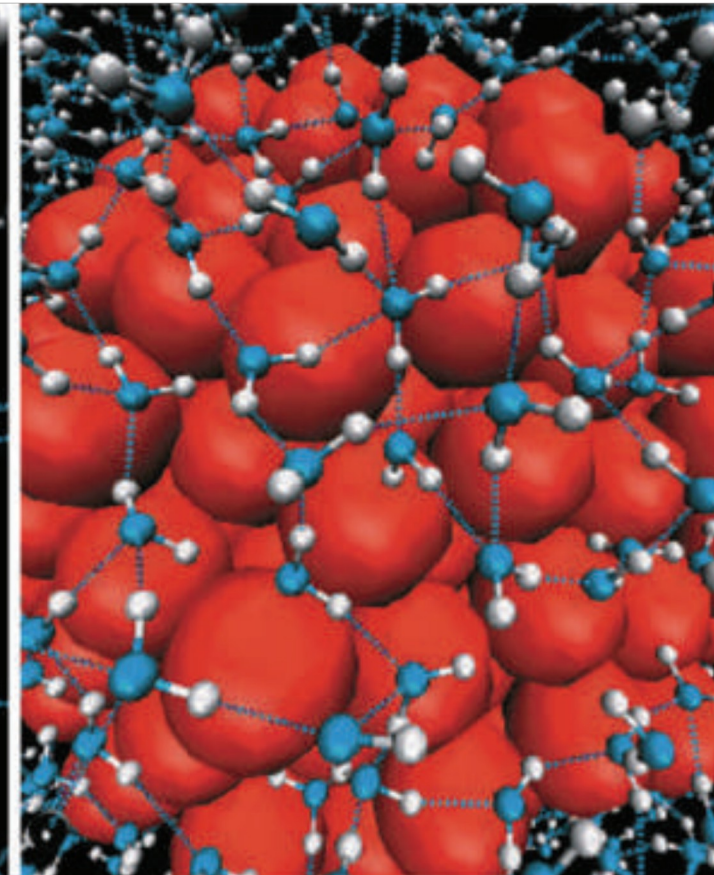


Figure 1.21 How Proteins Work (©2012 Garland Science)

(A)

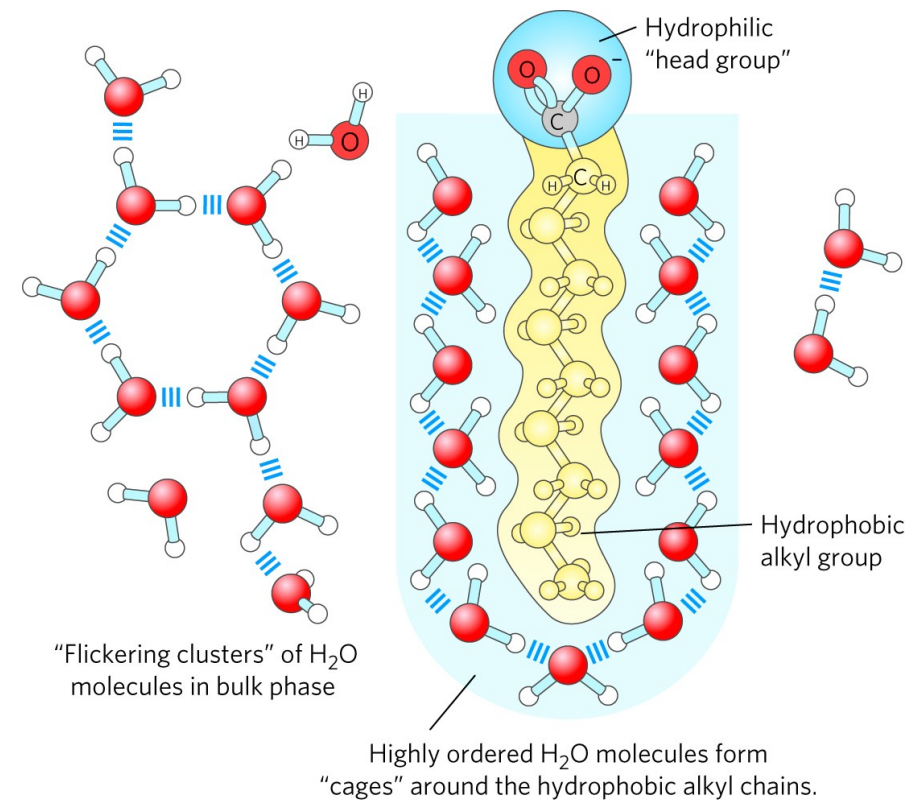


(B)



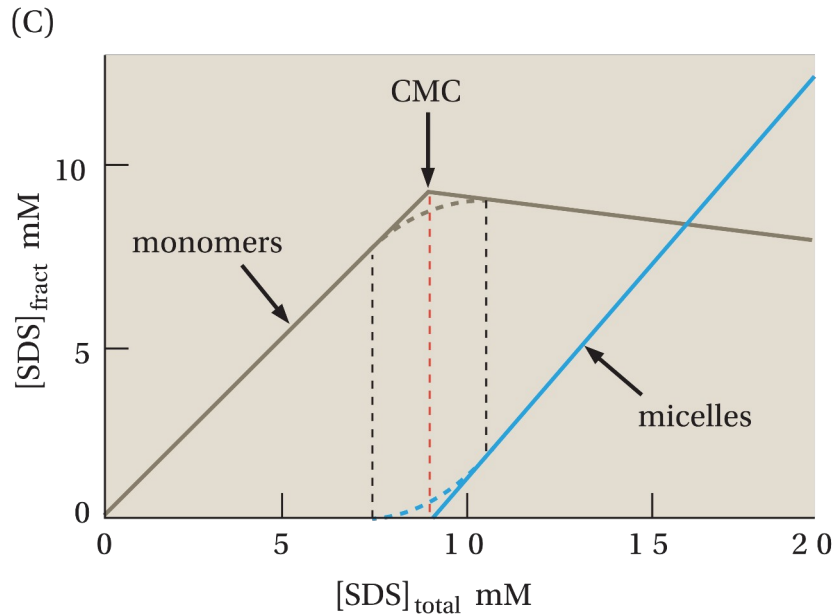
Amphipathic Compounds in Aqueous Solutions

- polar, hydrophilic region interacts favorably with H_2O and tends to dissolve
- nonpolar, hydrophobic region tends to avoid contact with H_2O and cluster together

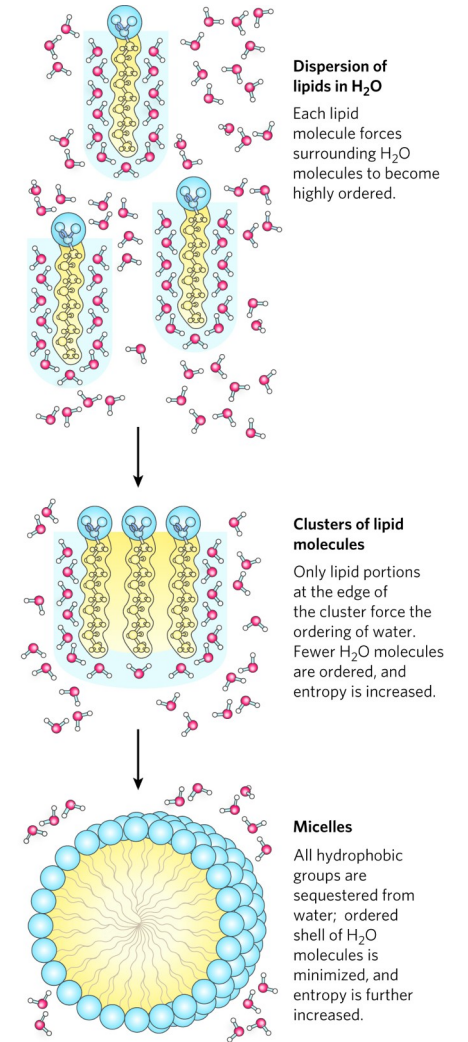
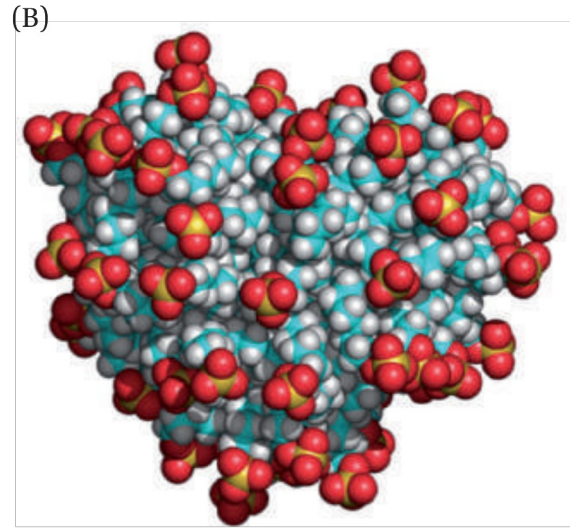


(a)

The Hydrophobic Effect

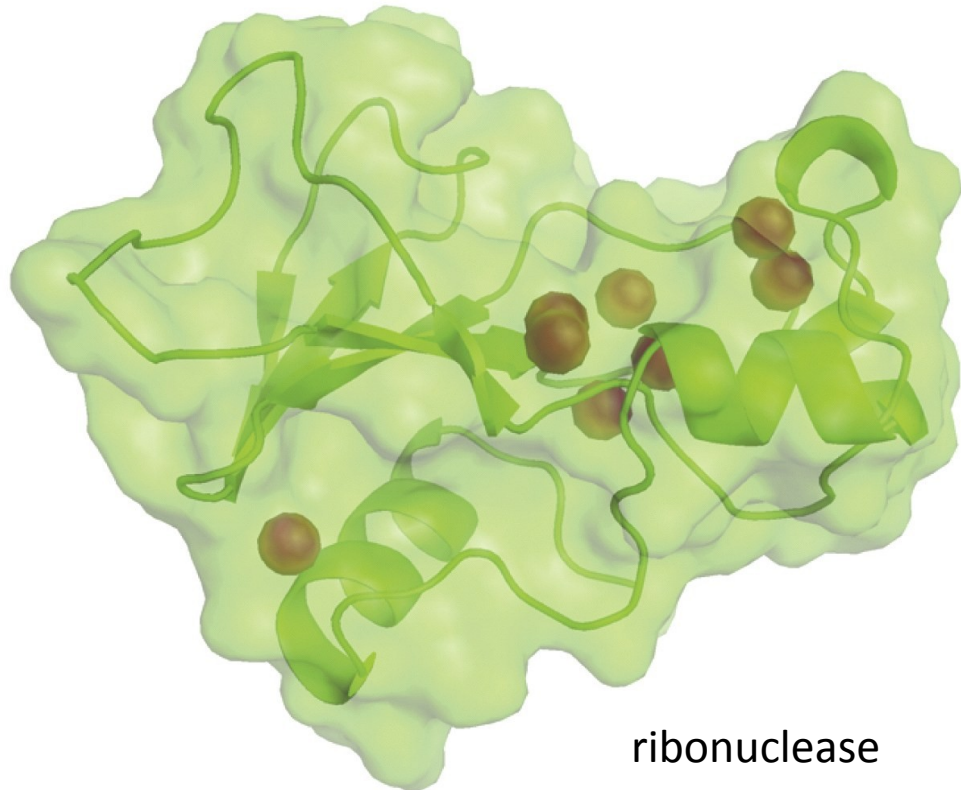


- hydrophobic effect =
 - nonpolar regions cluster together
 - polar regions arrange to maximize interactions with each other and with the solvent
- micelles = thermodynamically stable structures of amphipathic compounds in water



(b)

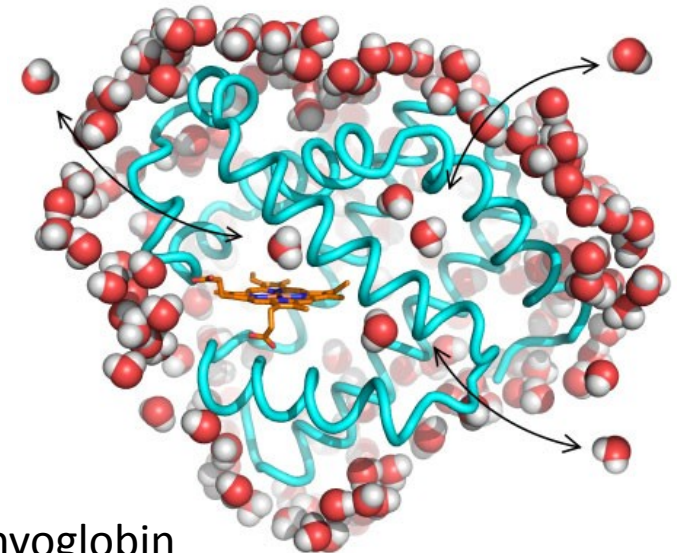
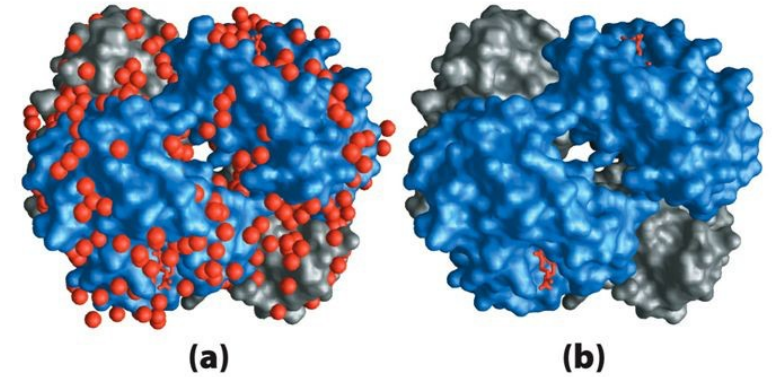
water layer



ribonuclease

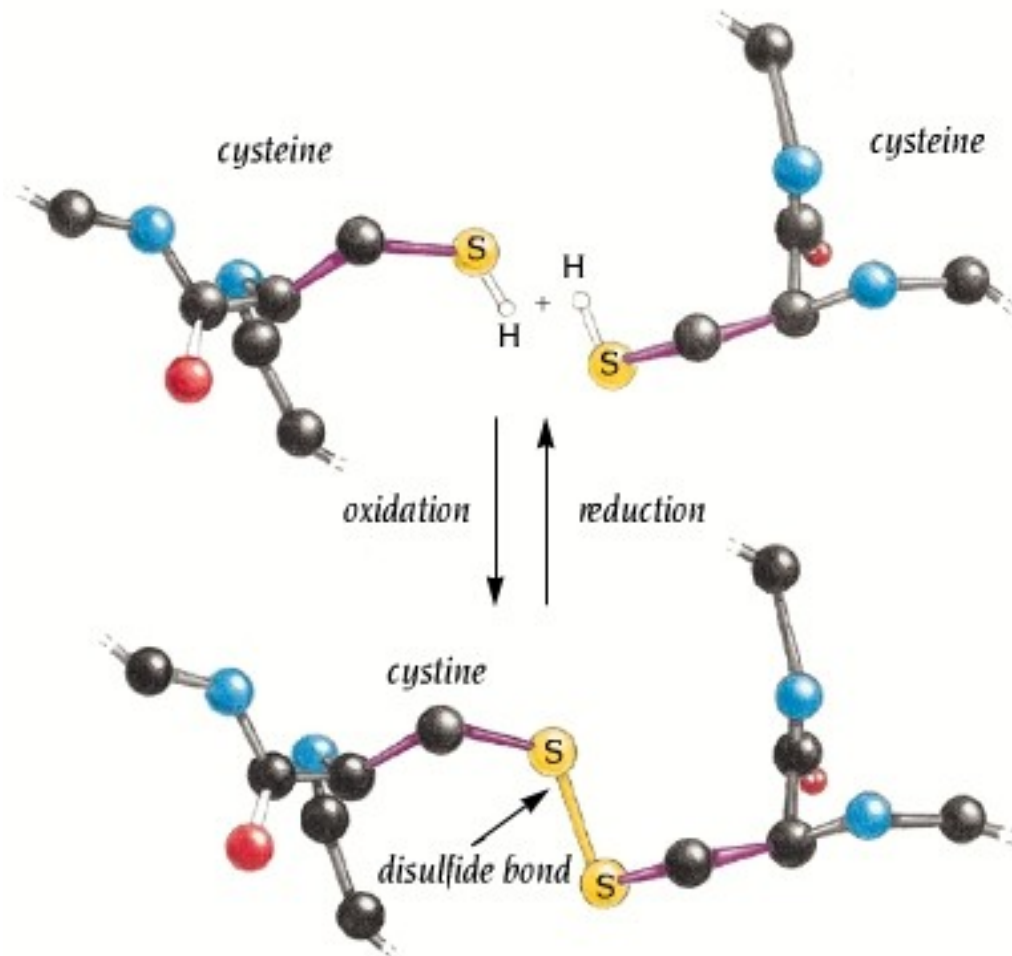
Binding of Water Molecules to Proteins

Structural analysis indicates that some water molecules are tightly bound to proteins. This is illustrated for hemoglobin below in Fig. 2-9, *left*. These water molecules have different properties from those of the bulk water of the solvent. In certain proteins, tightly bound water molecules play roles in catalysis and roles in ligand binding.



Hemoglobin/myoglobin

cysteine and disulphides



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It holds two portions of the protein together, biasing the protein towards the folded topology.

The disulfide bond may form the nucleus of a hydrophobic core of the folded protein

The disulfide bond increases the effective local concentration of protein residues and lowers the effective local concentration of water molecules.

Since water molecules attack amide-amide hydrogen bonds and break up secondary structure, a disulfide bond stabilizes secondary structure in its vicinity.

entropy/enthalpy compensation

$$\Delta G = \Delta H - T \Delta S$$

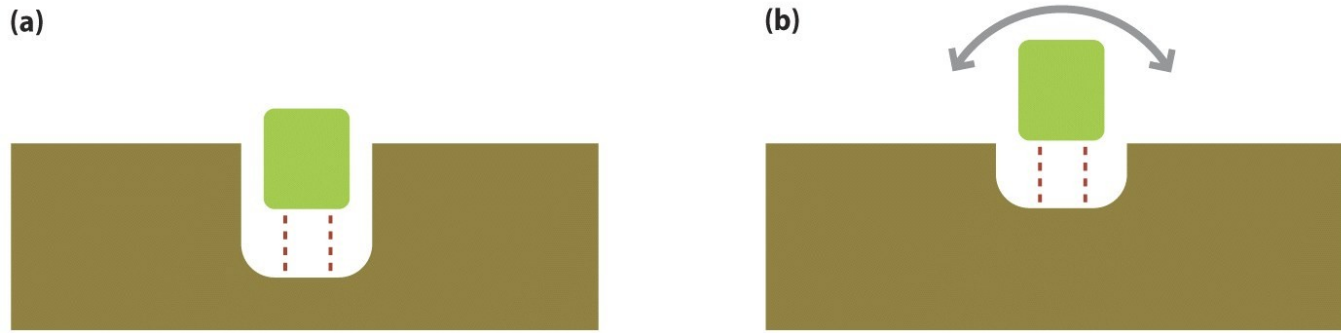


Figure 1.29 How Proteins Work (©2012 Garland Science)

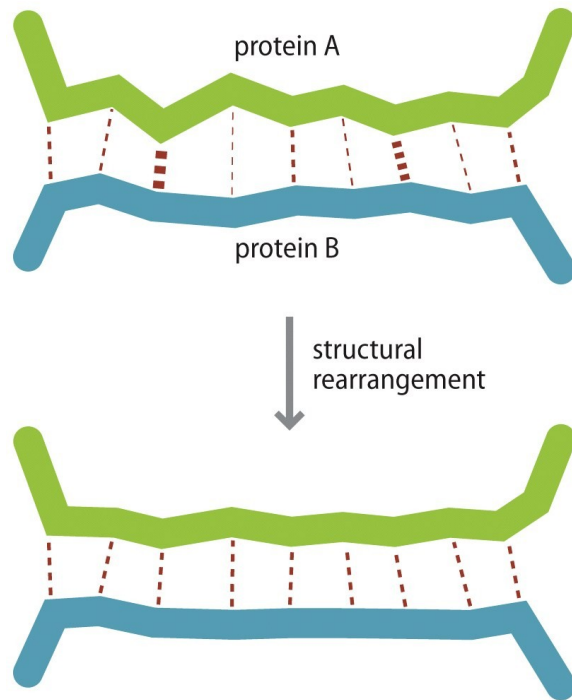


Figure 1.30 How Proteins Work (©2012 Garland Science)

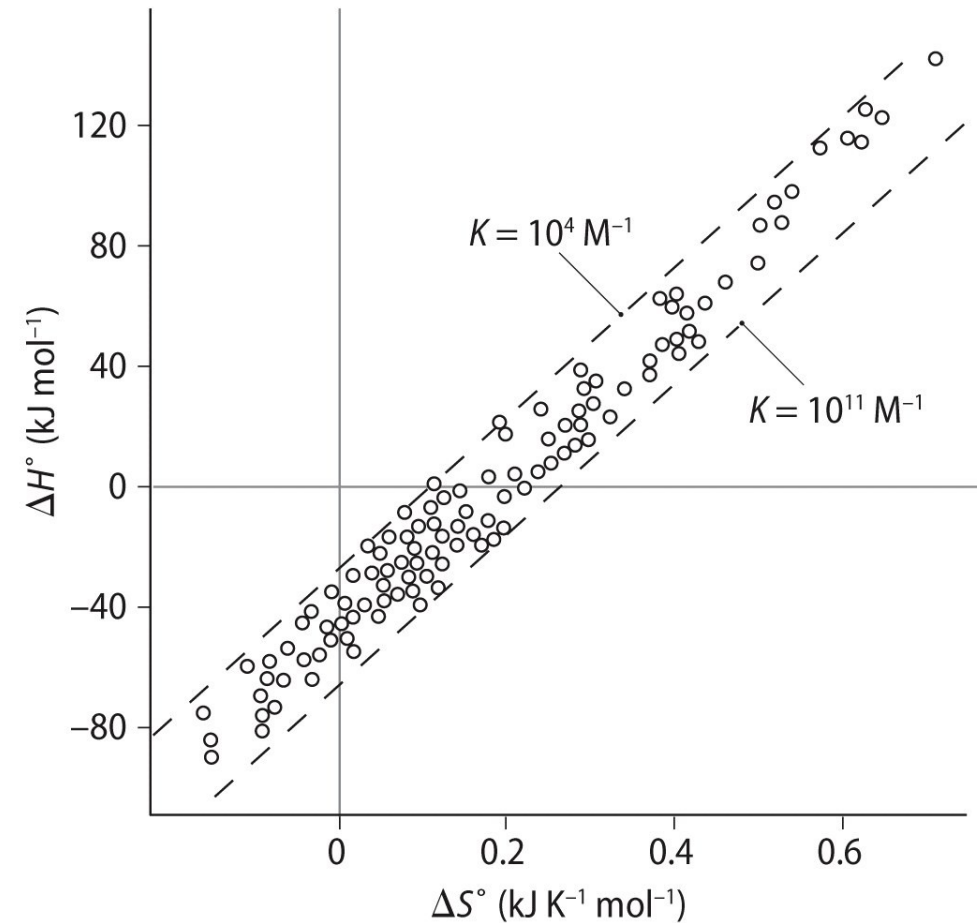
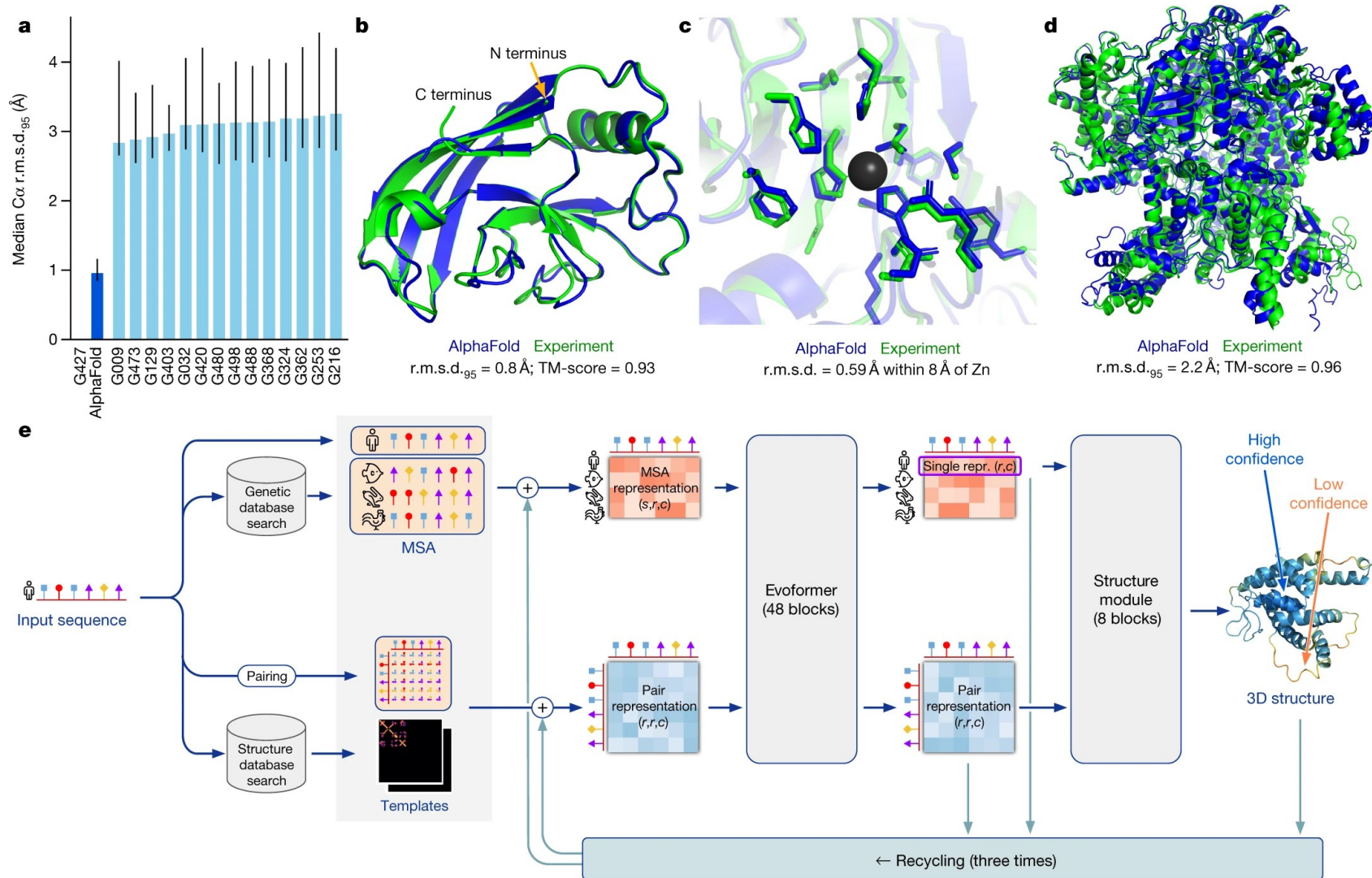


Figure 1.28 How Proteins Work (©2012 Garland Science)

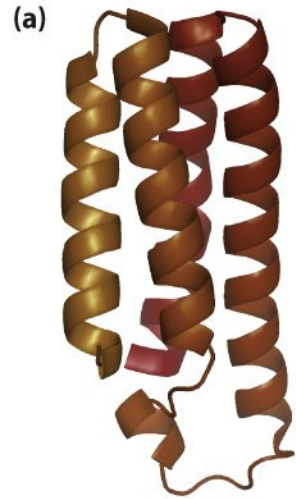
structure prediction

Highly accurate protein structure prediction with AlphaFold

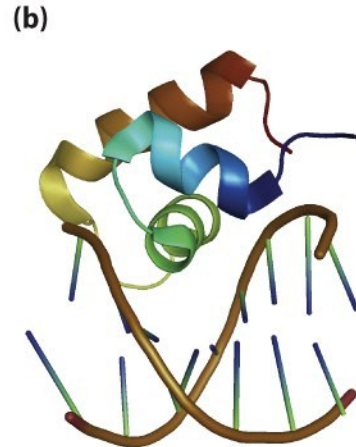


structure motifs

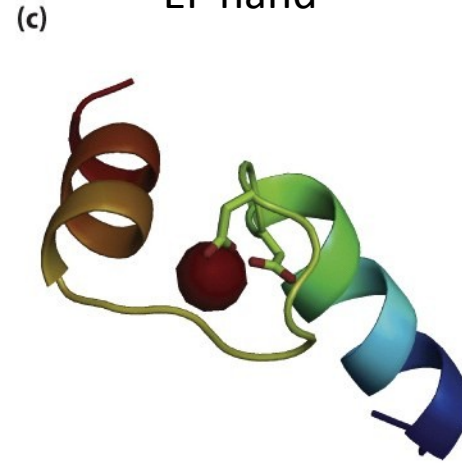
four-helix bundle



helix–turn–helix



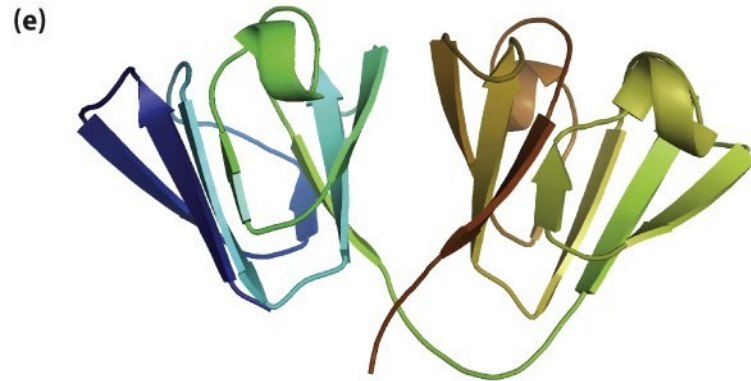
EF hand



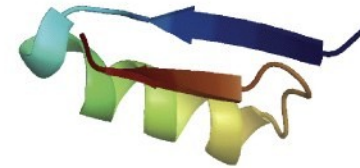
coiled coil



Four Greek keys



(f)



β - α - β motif

(g) β hairpin



β-barrel

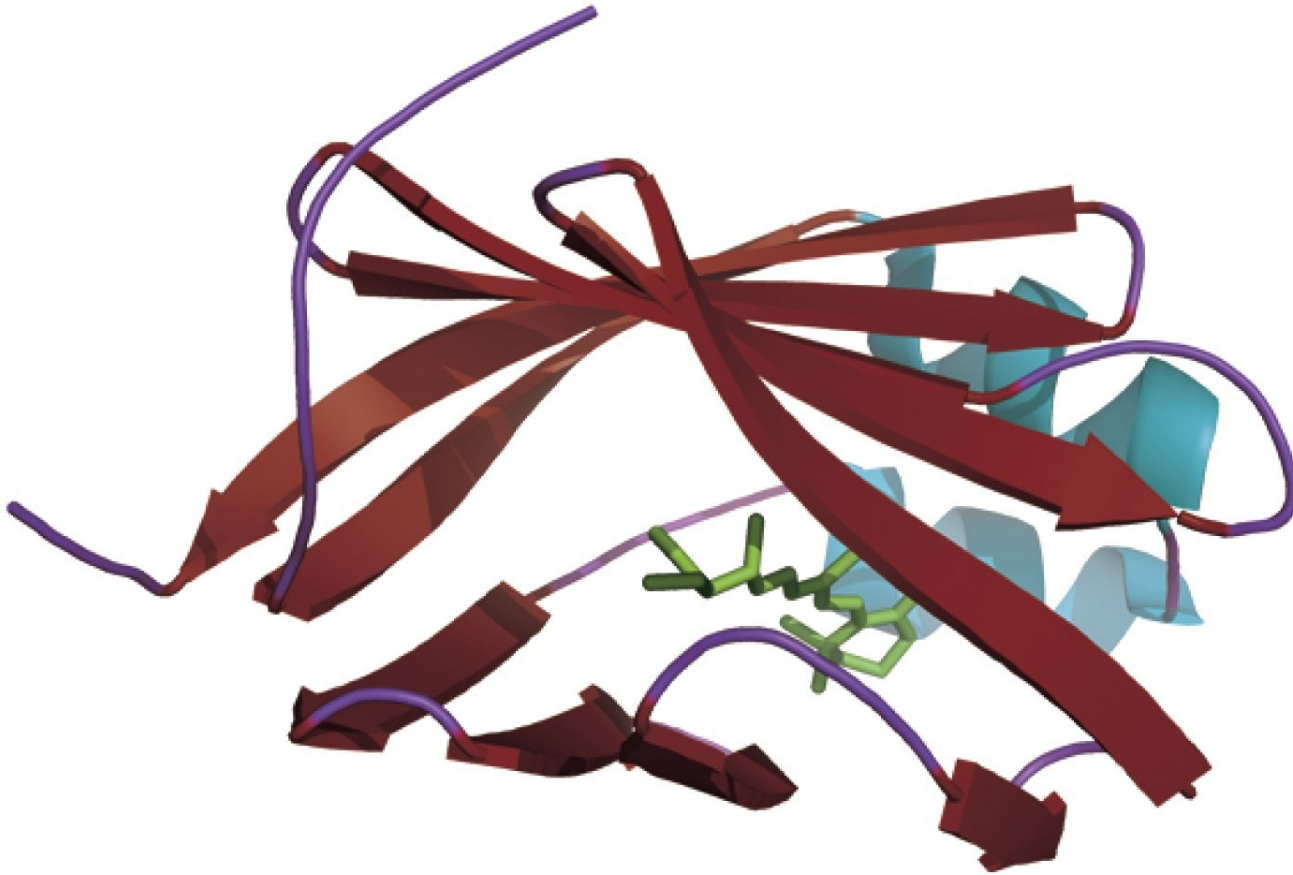


Figure 1.12.1 How Proteins Work (©2012 Garland Science)

Retinol binding protein

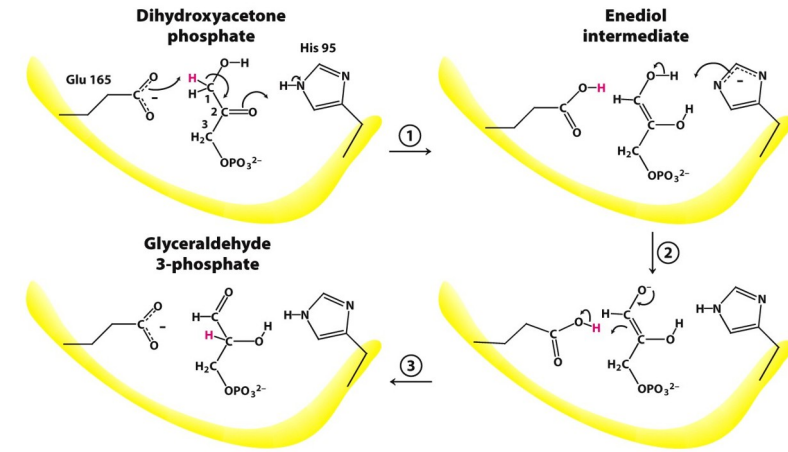
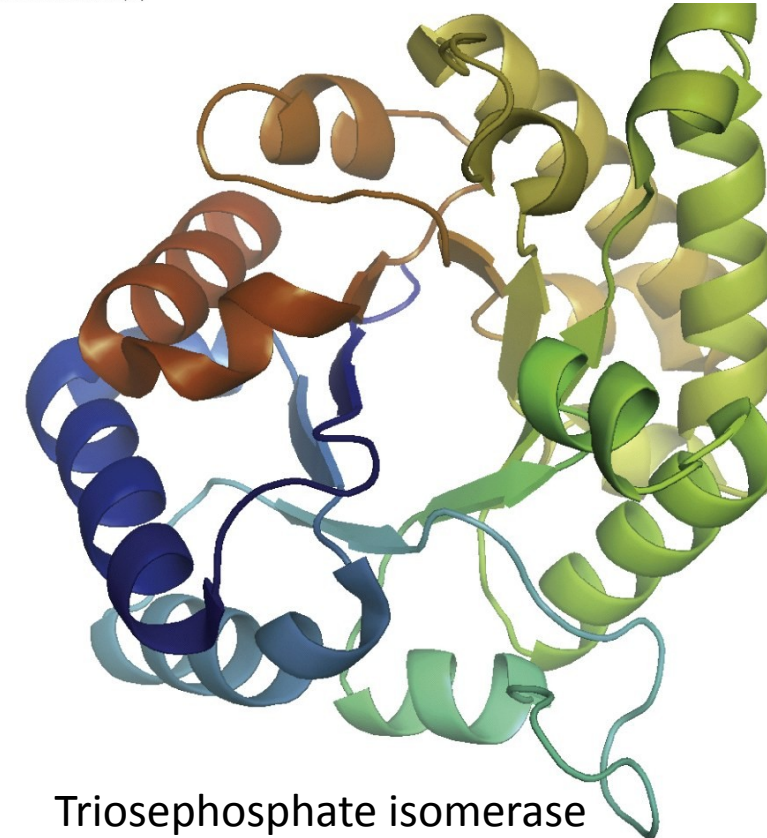


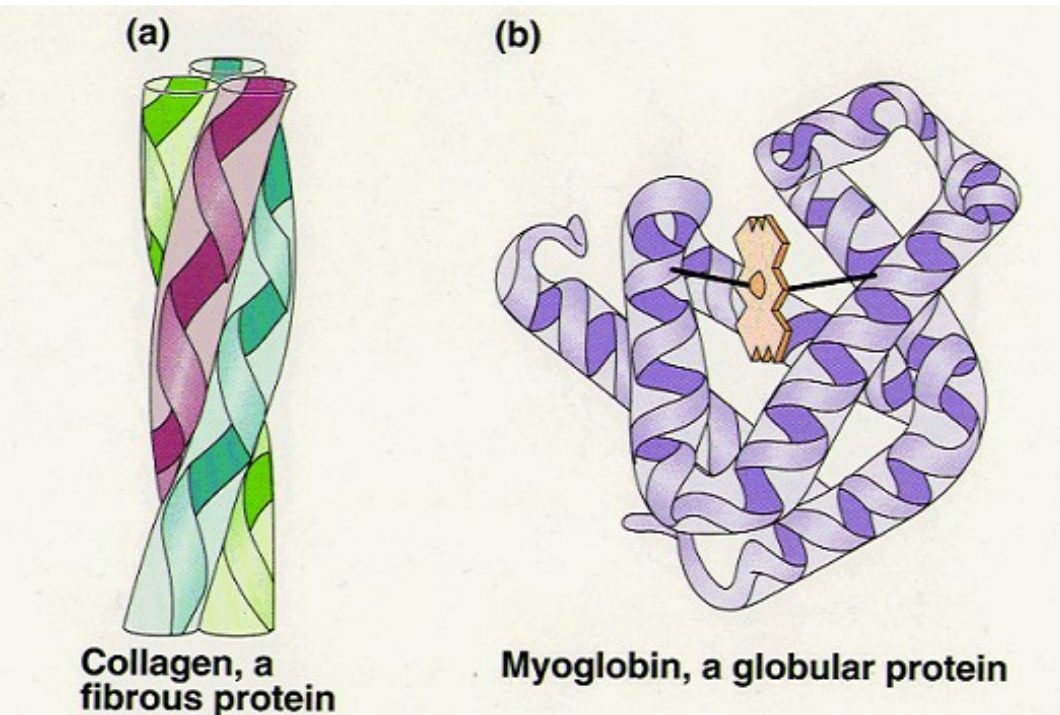
Figure 16.5
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company



Triosephosphate isomerase

Figure 1.34 How Proteins Work (©2012 Garland Science)

Proteins with a helix



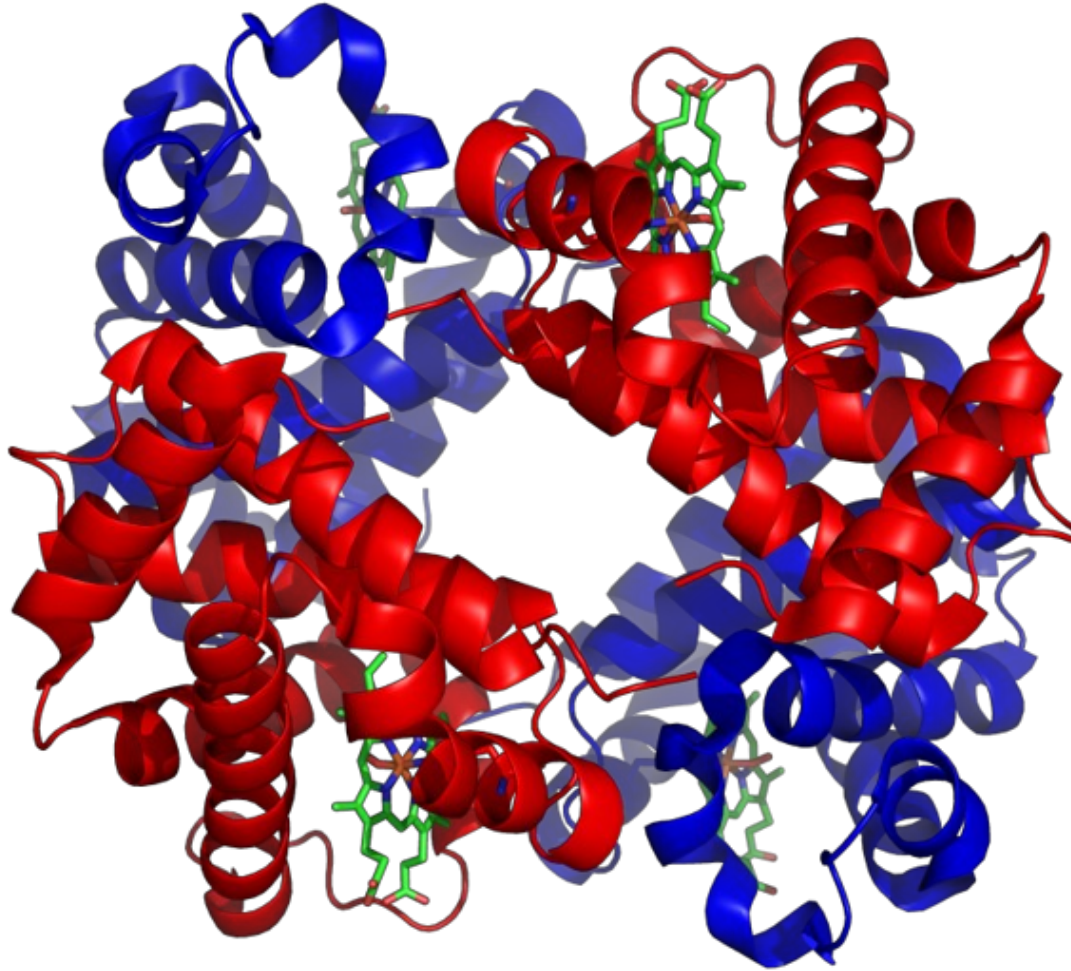
Characteristics	Globular Proteins	Fibrous Proteins
Shape	These are compact and spherical and folded in nature	These are elongated and have helical or sheet-like structures
Structure	Most of their hydrophobic residues are buried inside, and charged residues are at the surface	Each site of these proteins is exposed to interact with other particles
Functions	Mainly involved in the metabolic functions	Mainly constitute structural element
Solubility	Mostly soluble in water	Mostly insoluble in water
Sensitivity	Highly sensitive to changes in pH, temperature, etc.	Less sensitive to changes in pH, temperature, etc.
Example	Lys, BSA, HSA, haemoglobin	Actin, myosin, collagen, keratin

Proteins with a helix: The Globin Fold

A



B



Ridges in grooves model

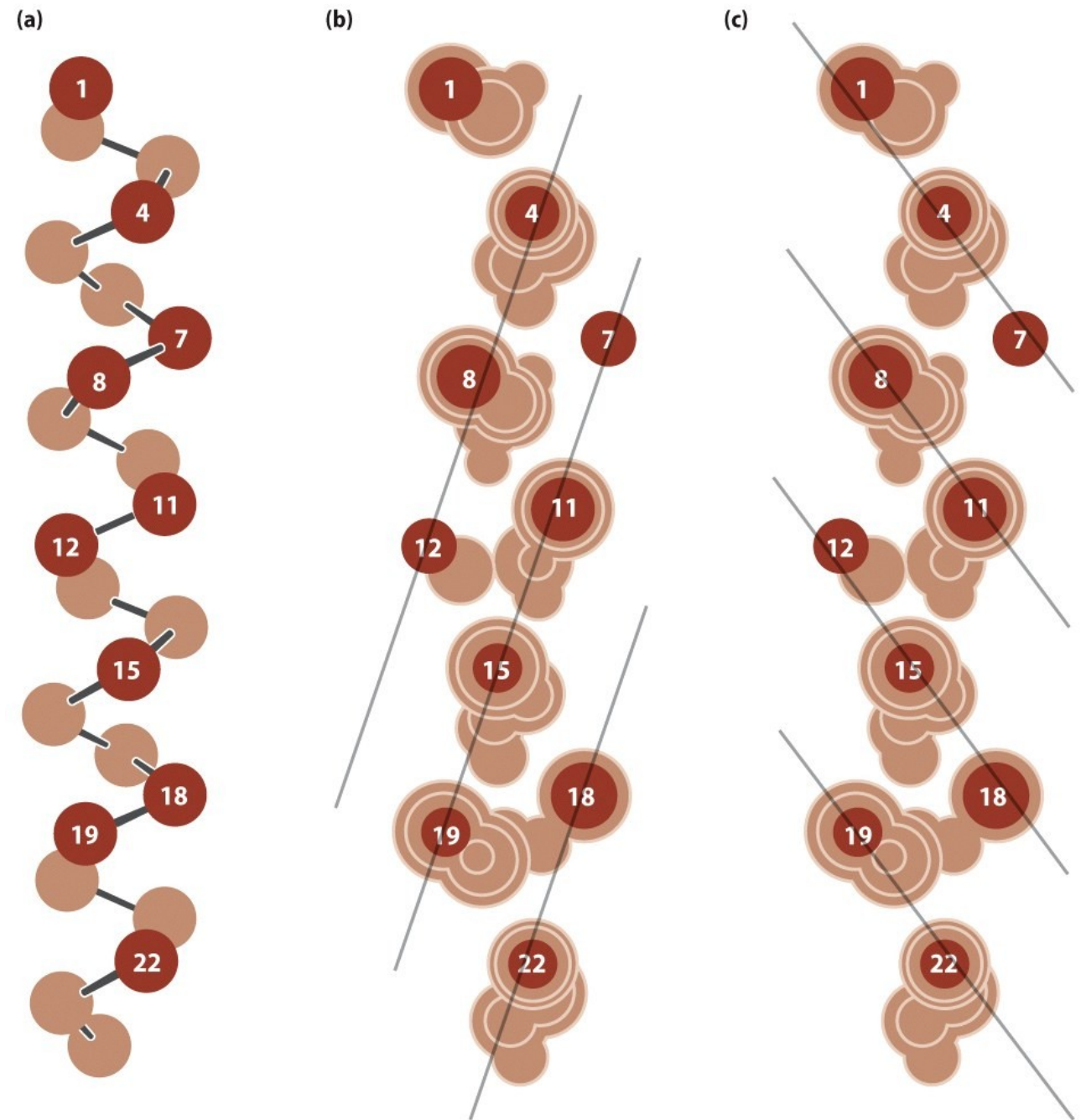


Figure 1.35 How Proteins Work (©2012 Garland Science)

ridges in grooves model

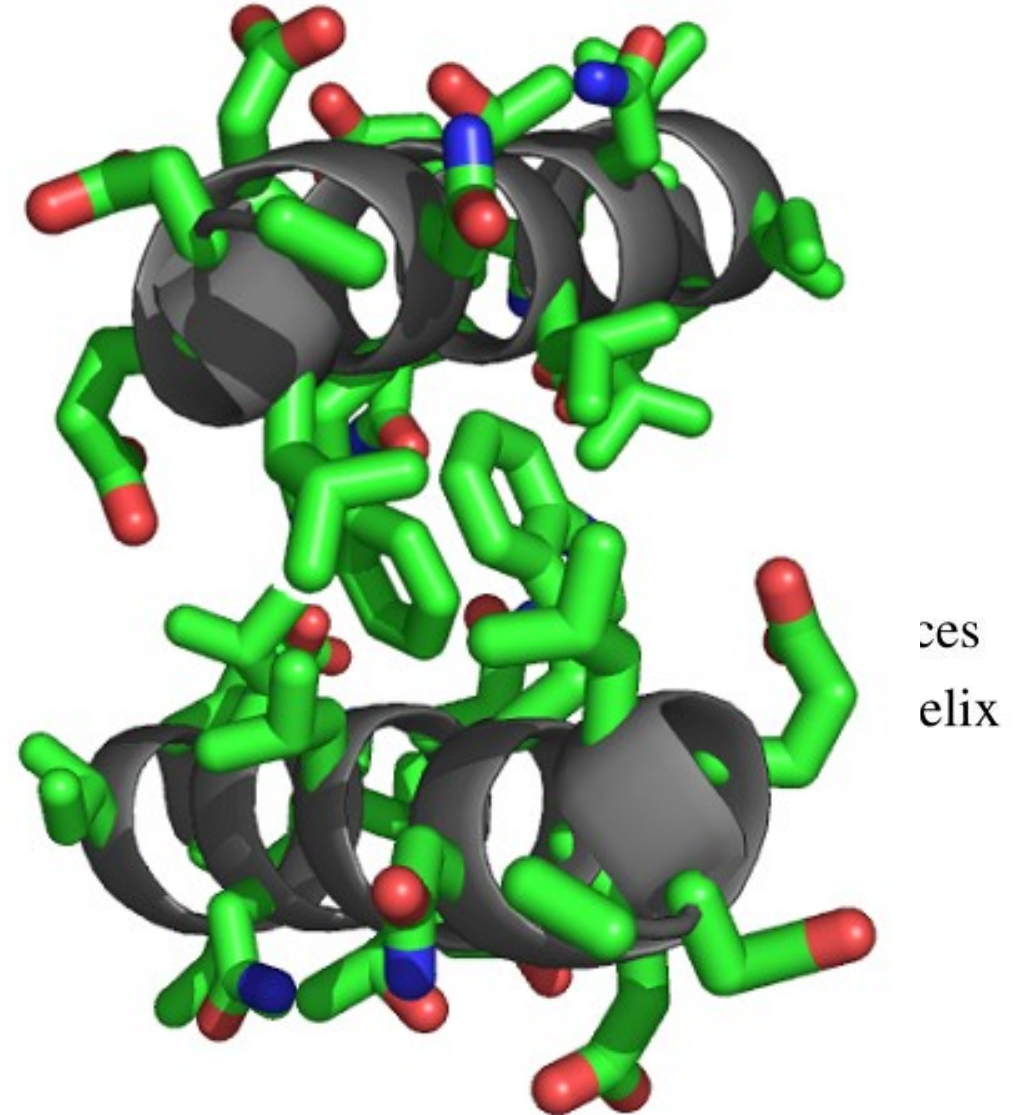
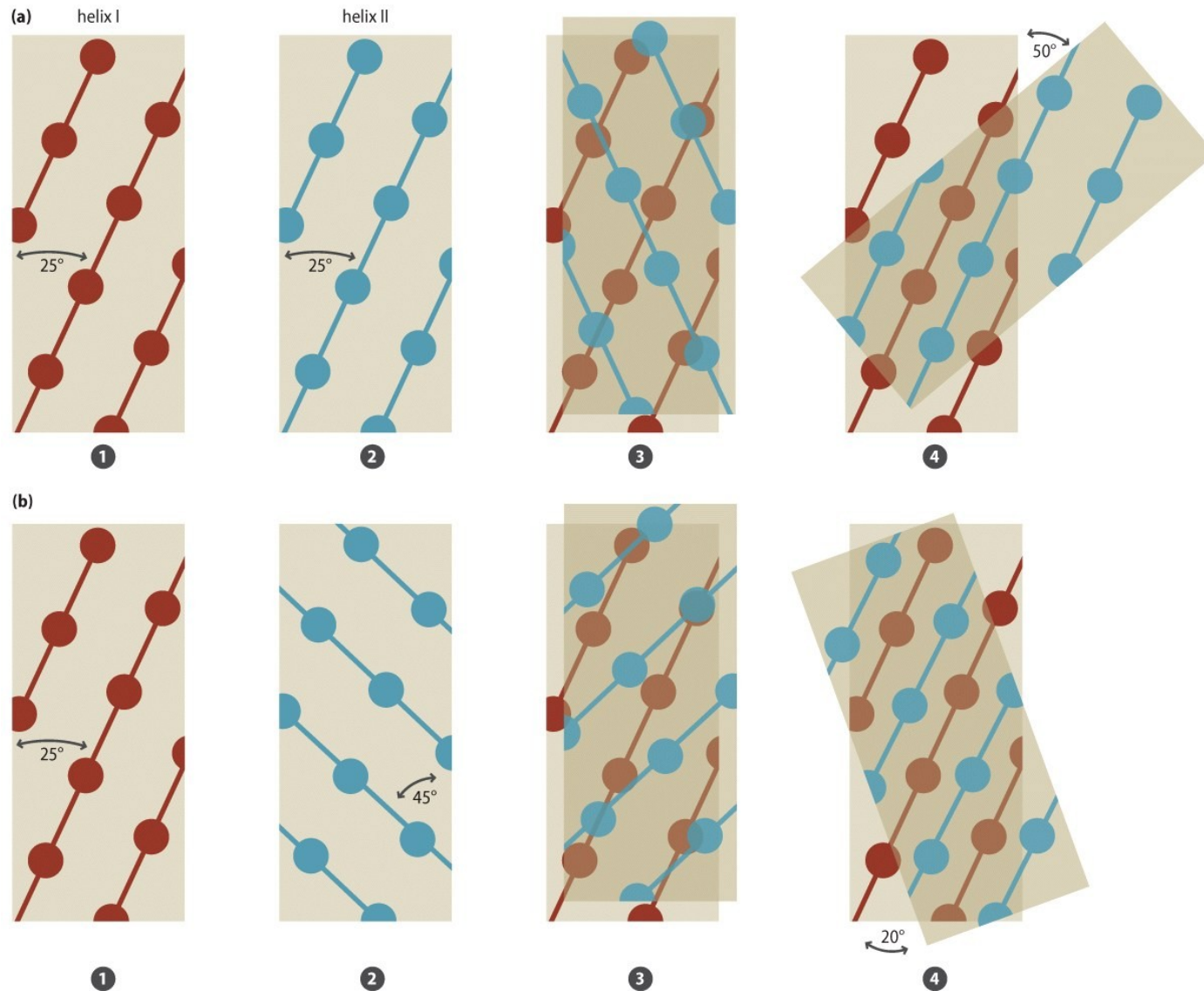
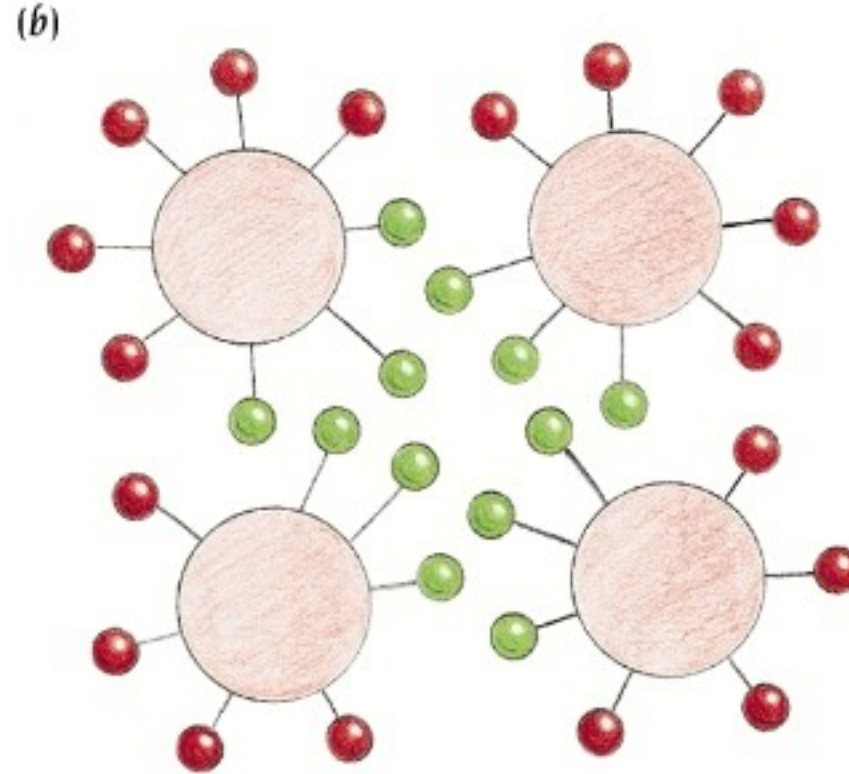


Figure 1.36 How Proteins Work (©2012 Garland Science)

Four helix bundle



Proteins with a four-helix bundle structure include

- growth hormones
- Cytokines
- Ferritins

The four helices may splay apart to generate a binding pocket for cofactors and metal ions

amphipathic

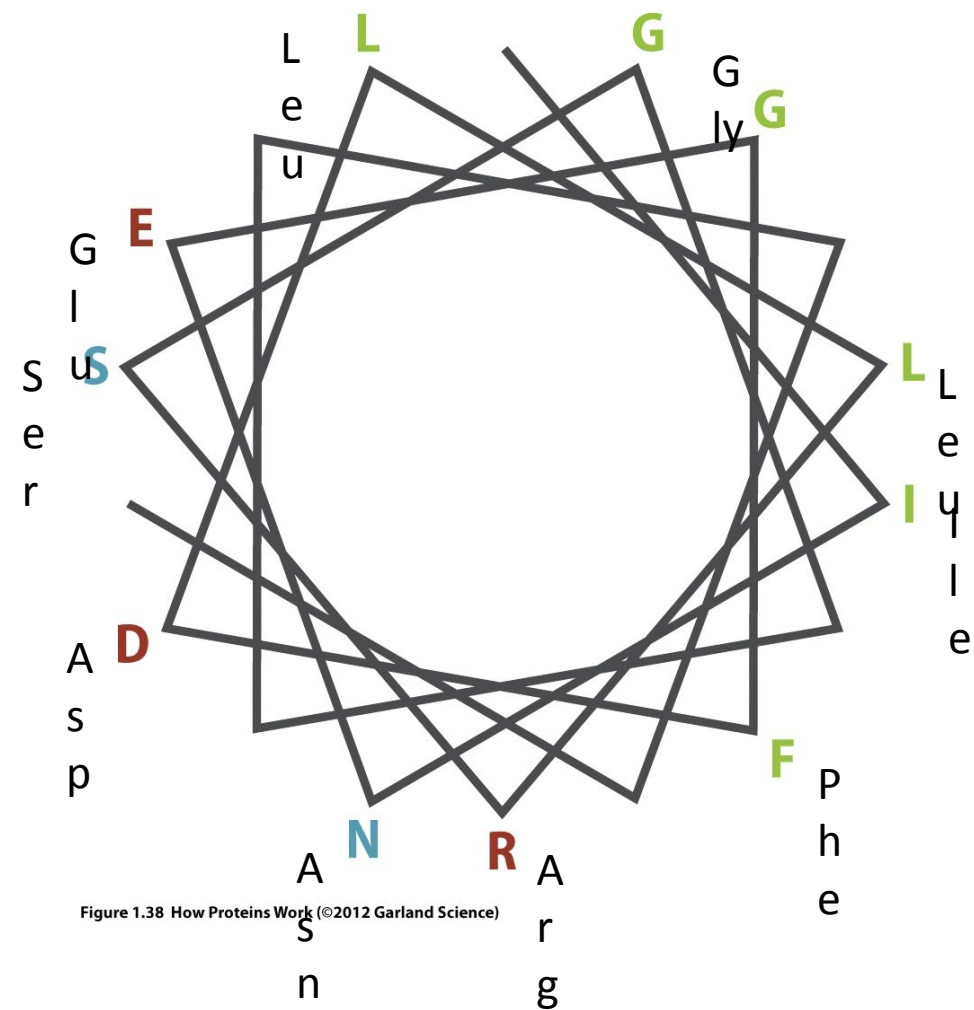
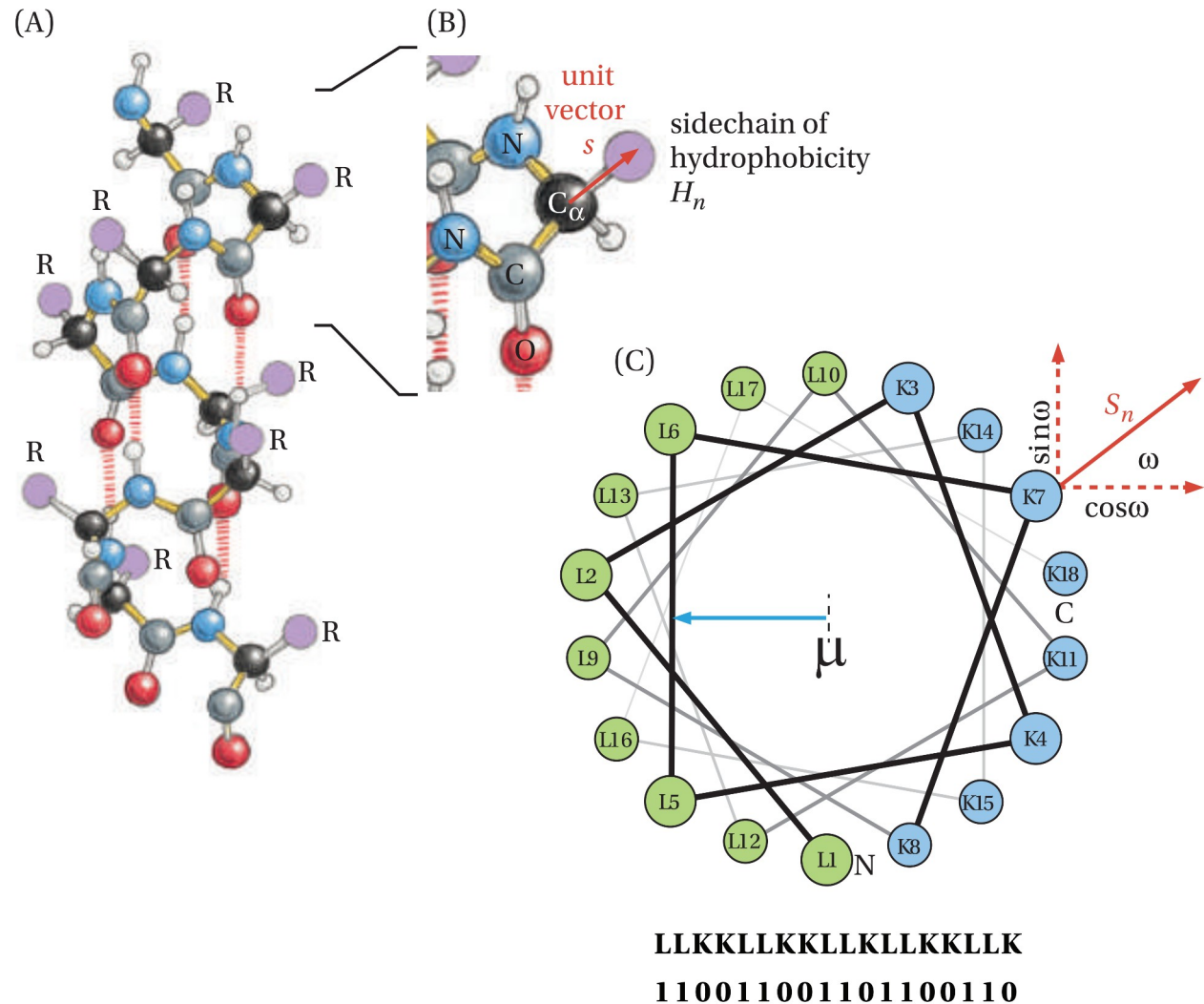
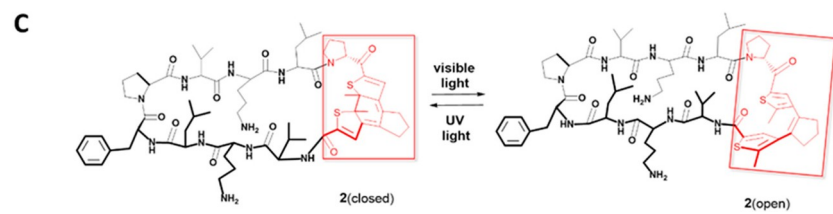
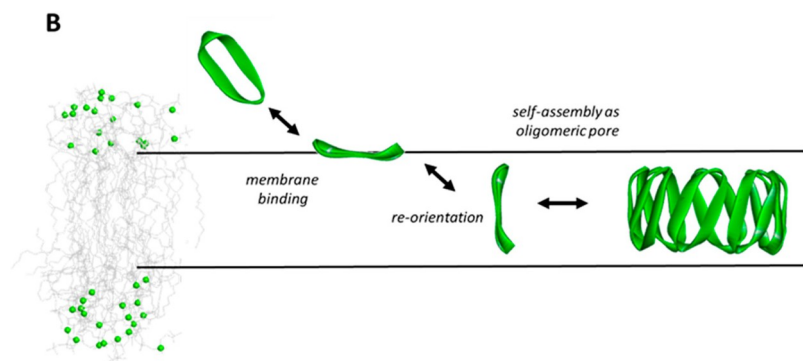
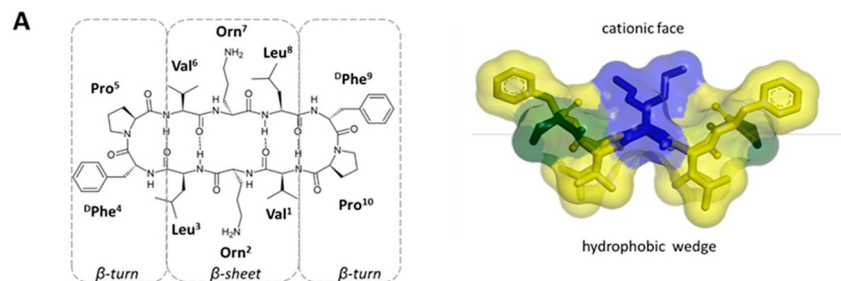
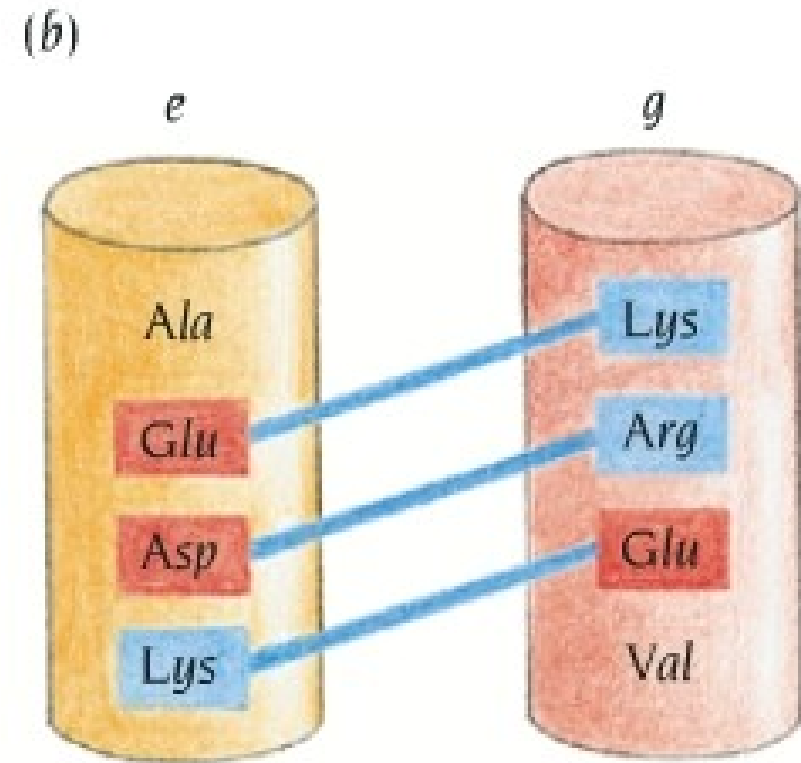
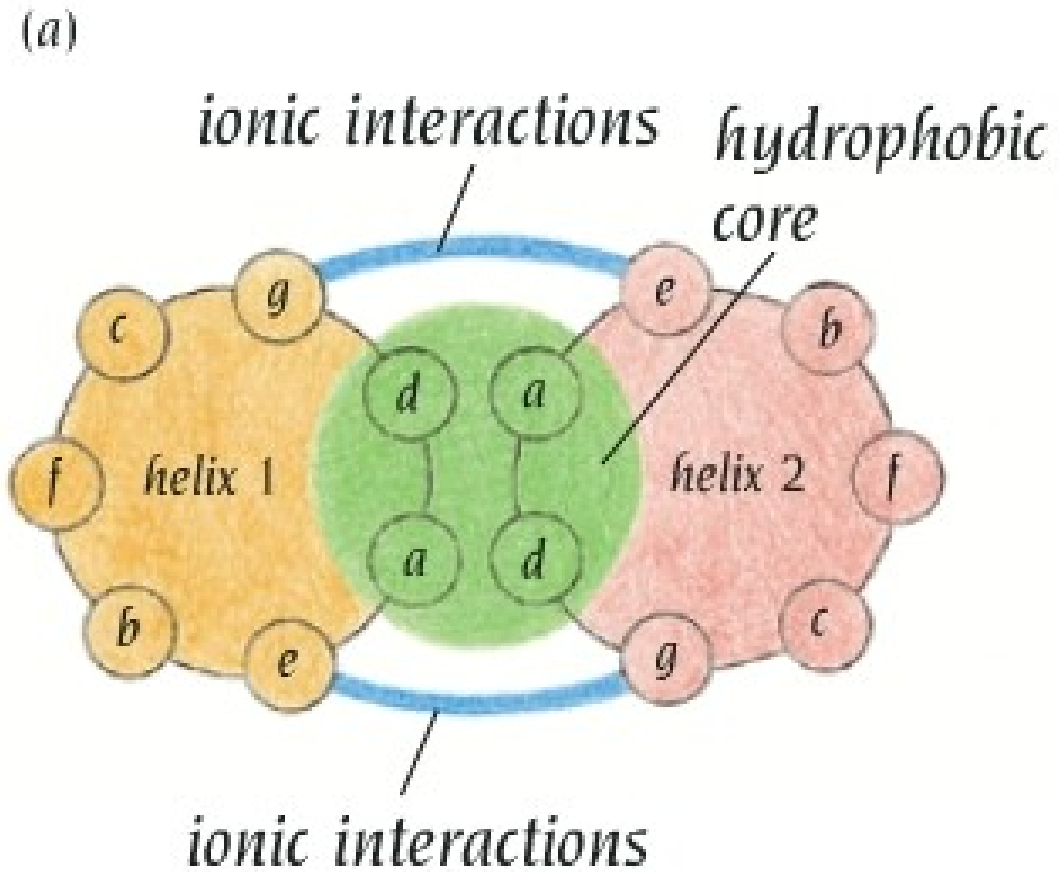


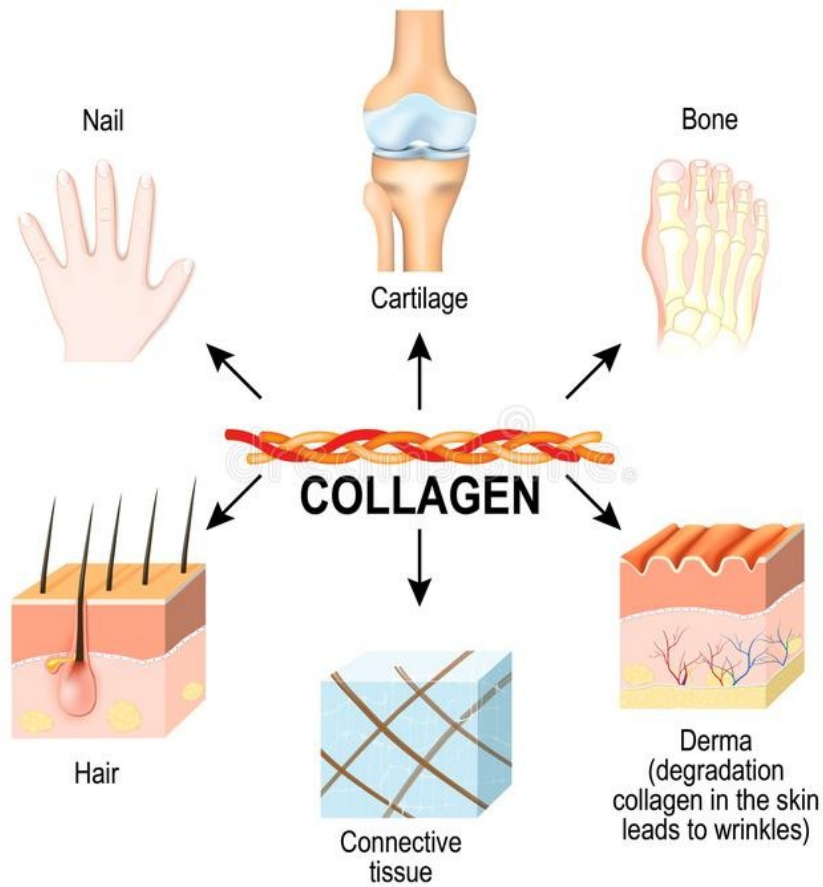
Figure 1.38 How Proteins Work (©2012 Garland Science)



Coiled-coil



coiled coil *fibrous proteins*

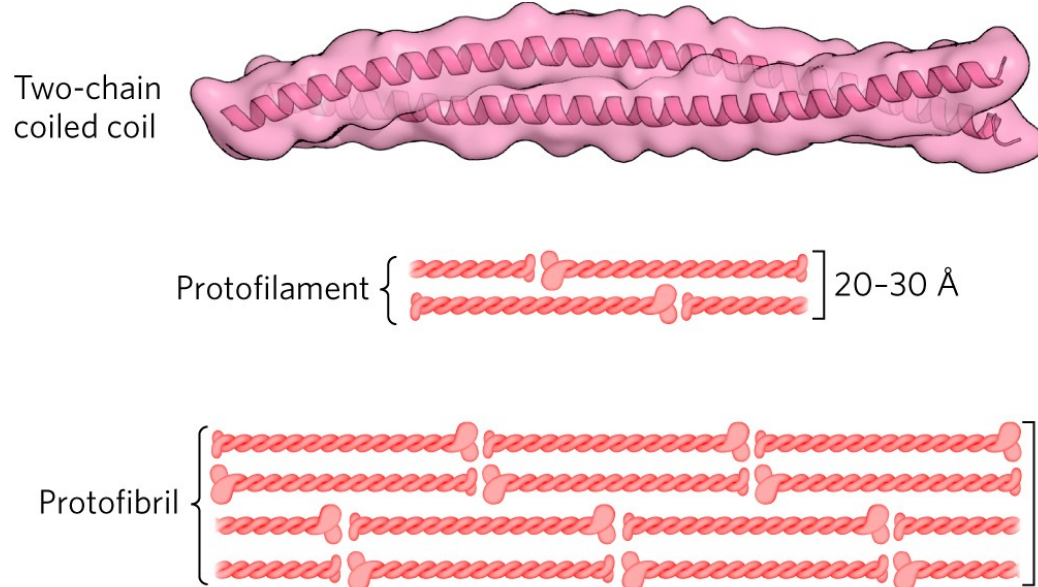


Structure	Characteristics	Examples of occurrence
α Helix, cross-linked by disulfide bonds	Tough, insoluble protective structures of varying hardness and flexibility	α -Keratin of hair, feathers, nails
β Conformation	Soft, flexible filaments	Silk fibron
Collagen triple helix	High tensile strength, without stretch	Collagen of tendons, bone matrix

- give strength and/or flexibility to structures
- simple repeating element of secondary structure
- H₂O insoluble due to high concentrations of hydrophobic residues

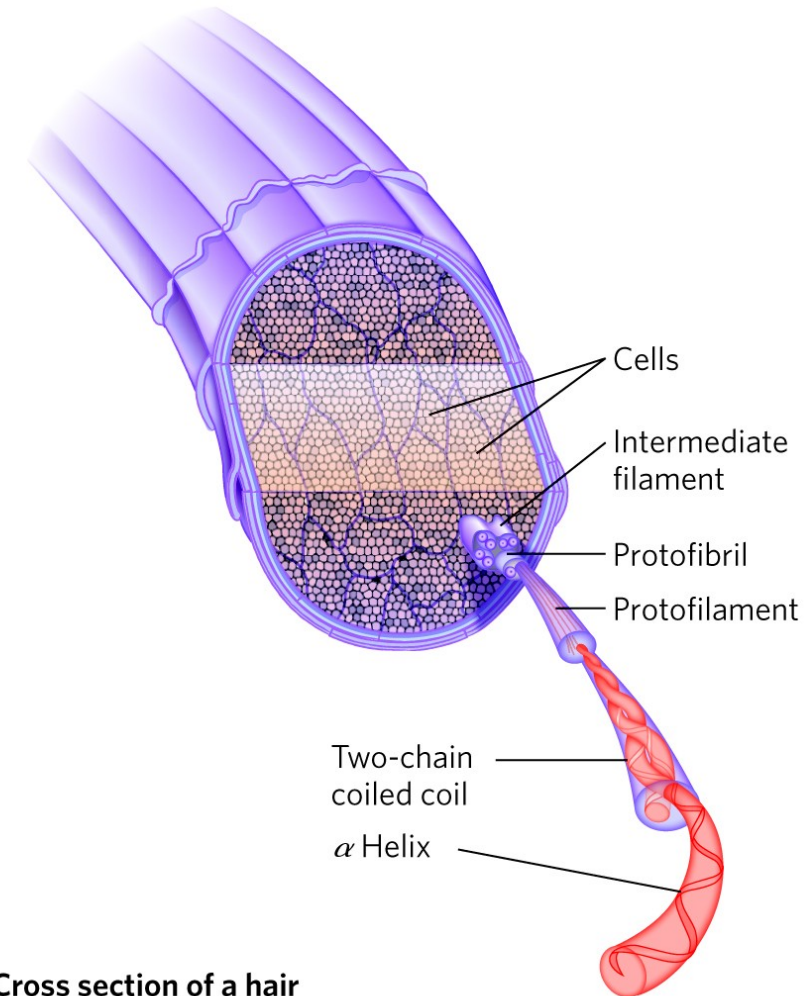
The Structure of α -Keratin in Hair

- α -keratin helix is a right-handed α helix
- two strands of α -keratin, oriented in parallel, wrap about each other to form a supertwisted coiled coil
 - supertwisted helical path is left-handed
- rich in hydrophobic residues: Ala, Val, Leu, Ile, Met, Phe
- cross-links stabilized by disulfide bonds



(a)

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company



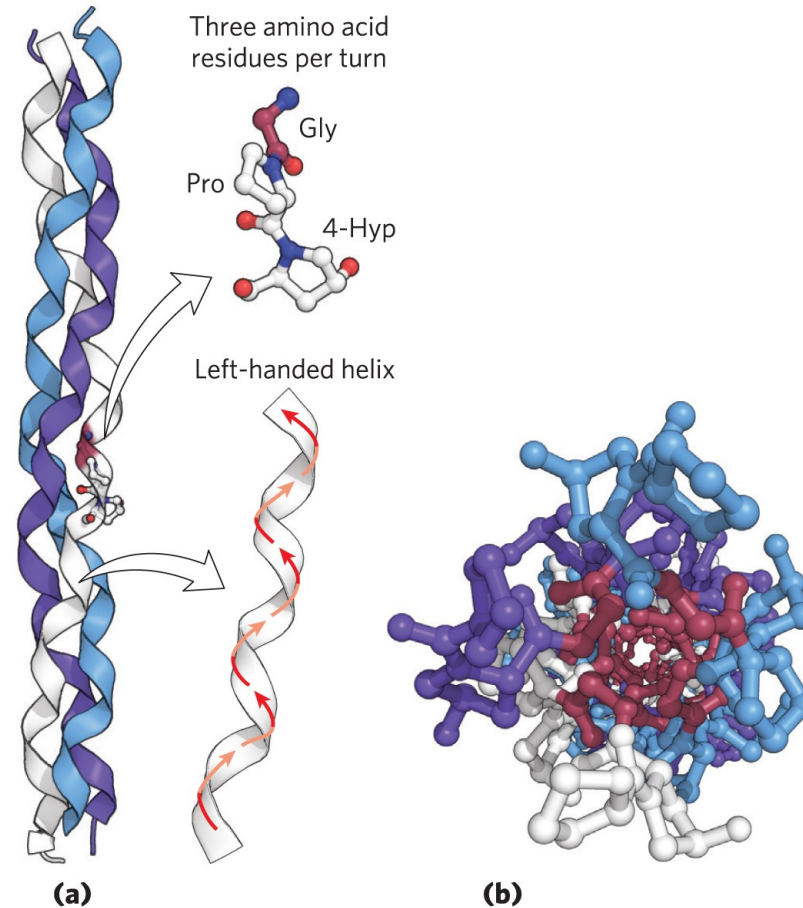
(b) Cross section of a hair

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

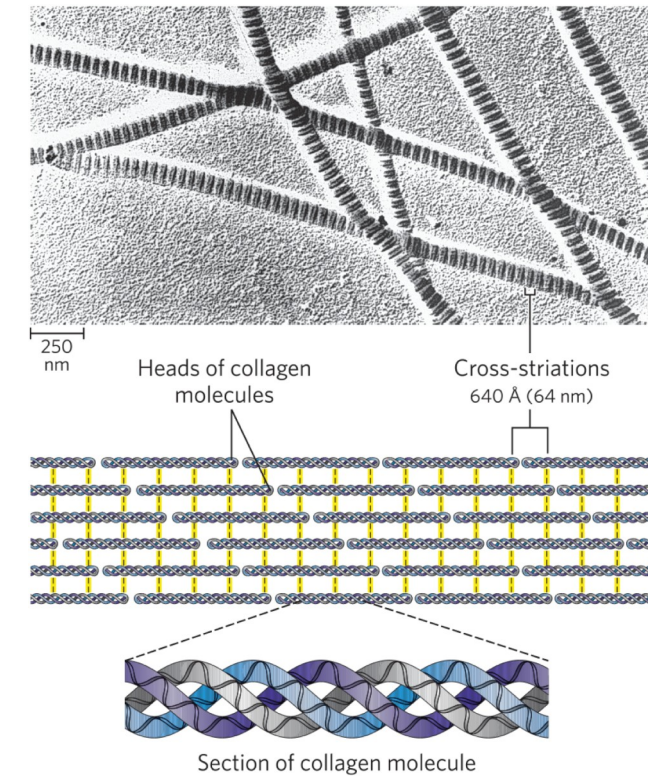
The Structure of Collagen

collagen = found in connective tissue

- secondary structure = left-handed, repeating tripeptide unit Gly–X–Y, where X is often Pro and Y is often 4-Hyp
- tertiary and quaternary structure = right-handed twisting of 3 separate polypeptides



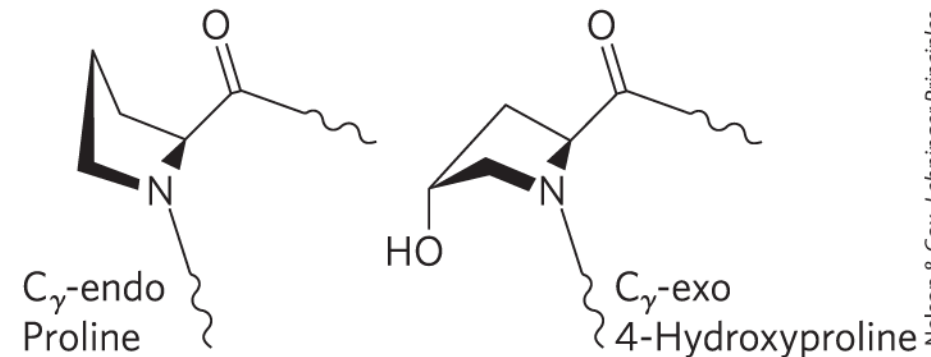
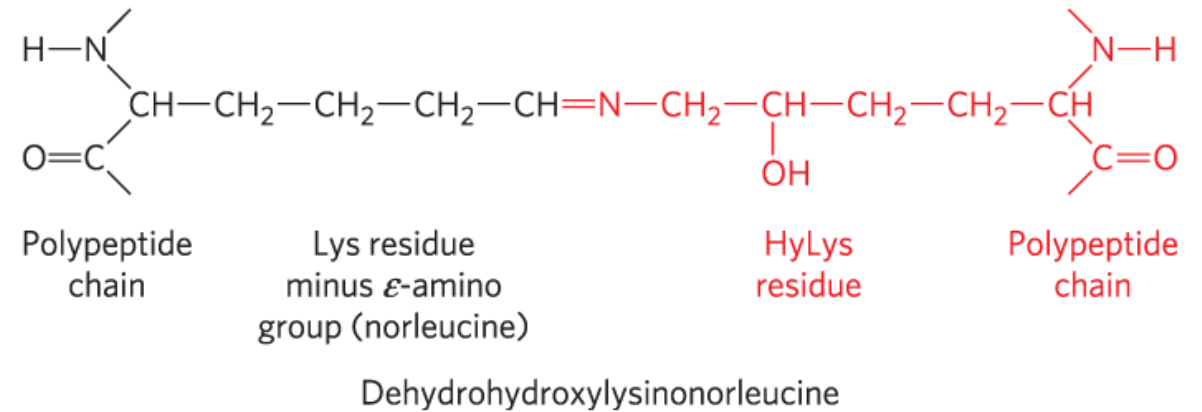
Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company



Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company
J. Gross/Biozentrum, University of Basel/Science Source

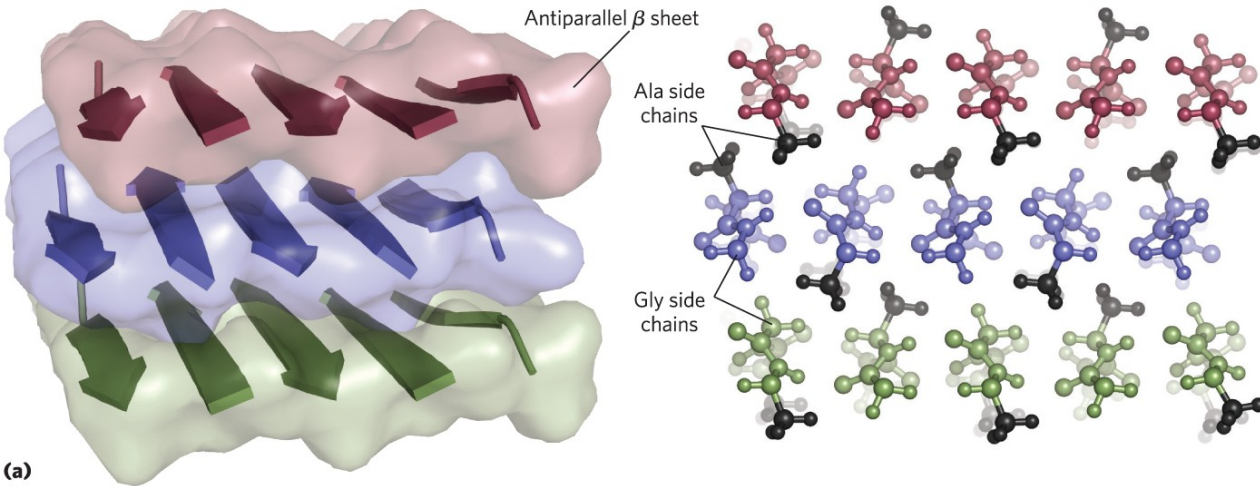
Covalent Cross-Links in Collagen Fibrils

- cross-linked by covalent bonds involving Lys, HyLys (5-hydroxylysine), or His
 - links create uncommon amino acid residues
- scurvy is caused by a lack of vitamin C
 - characterized by general degeneration of connective tissue
- vitamin C is required for the hydroxylation of proline and lysine in collagen



β -sheet fibrous proteins

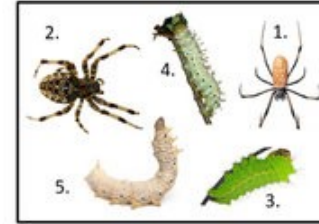
(-Gly-Ser-Gly-Ala-Gly-Ala-)n



- main protein in silk
- predominantly β conformation
- rich in Ala and Gly
- stabilized by hydrogen bonding and van der Waals interactions

Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2011 W. H. Freeman and Company
(b) Tina Weatherby Carvalho/MicroAngela.

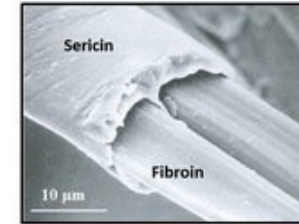
A. Popular silk sources



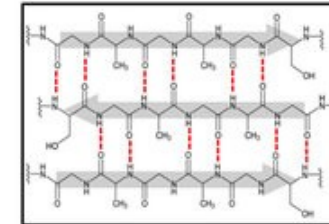
B. *B. mori* silkworm and cocoons



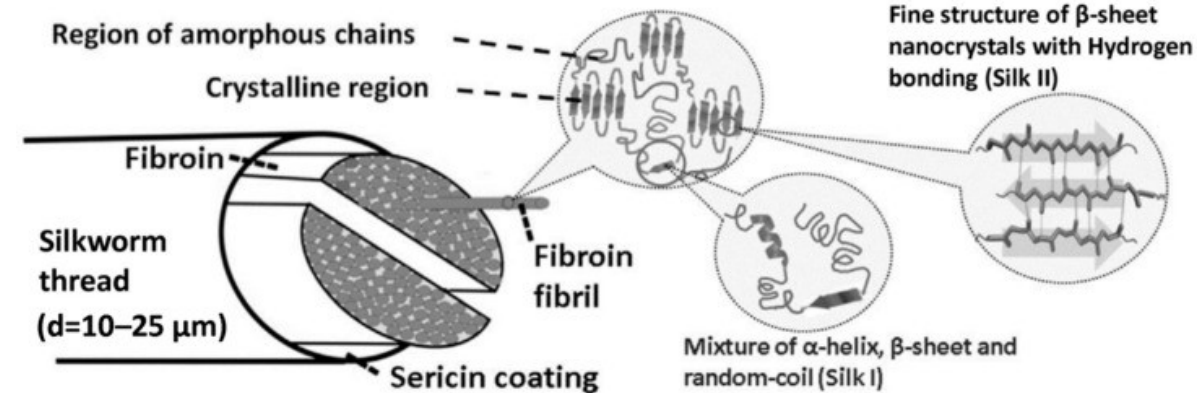
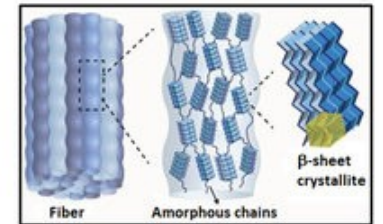
C. Composition of silk protein



D. Amino acid sequence

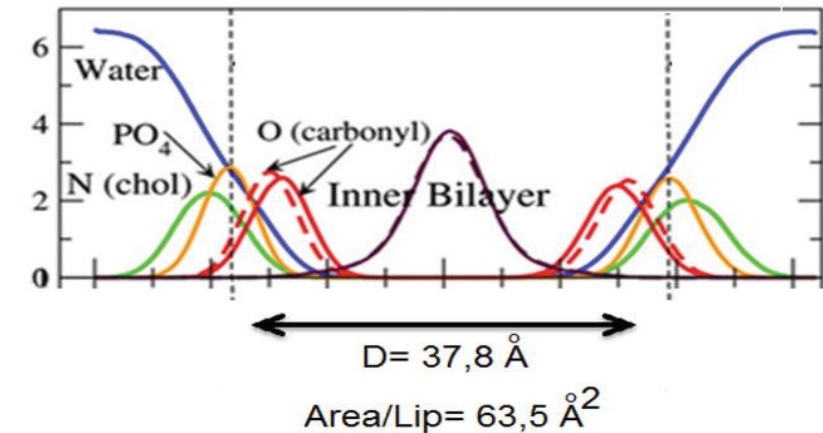
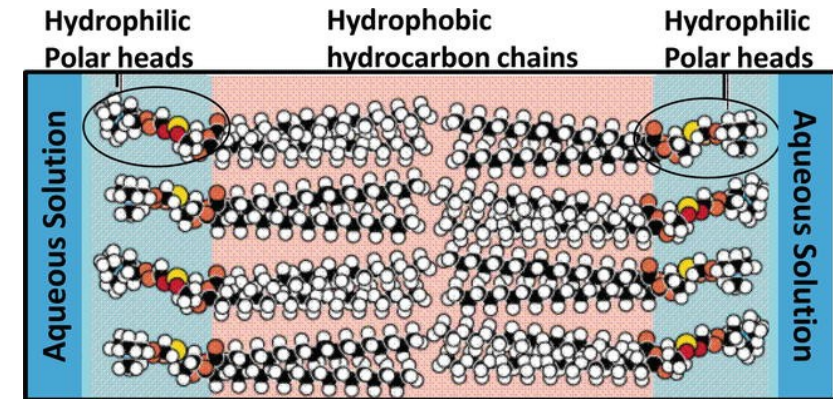
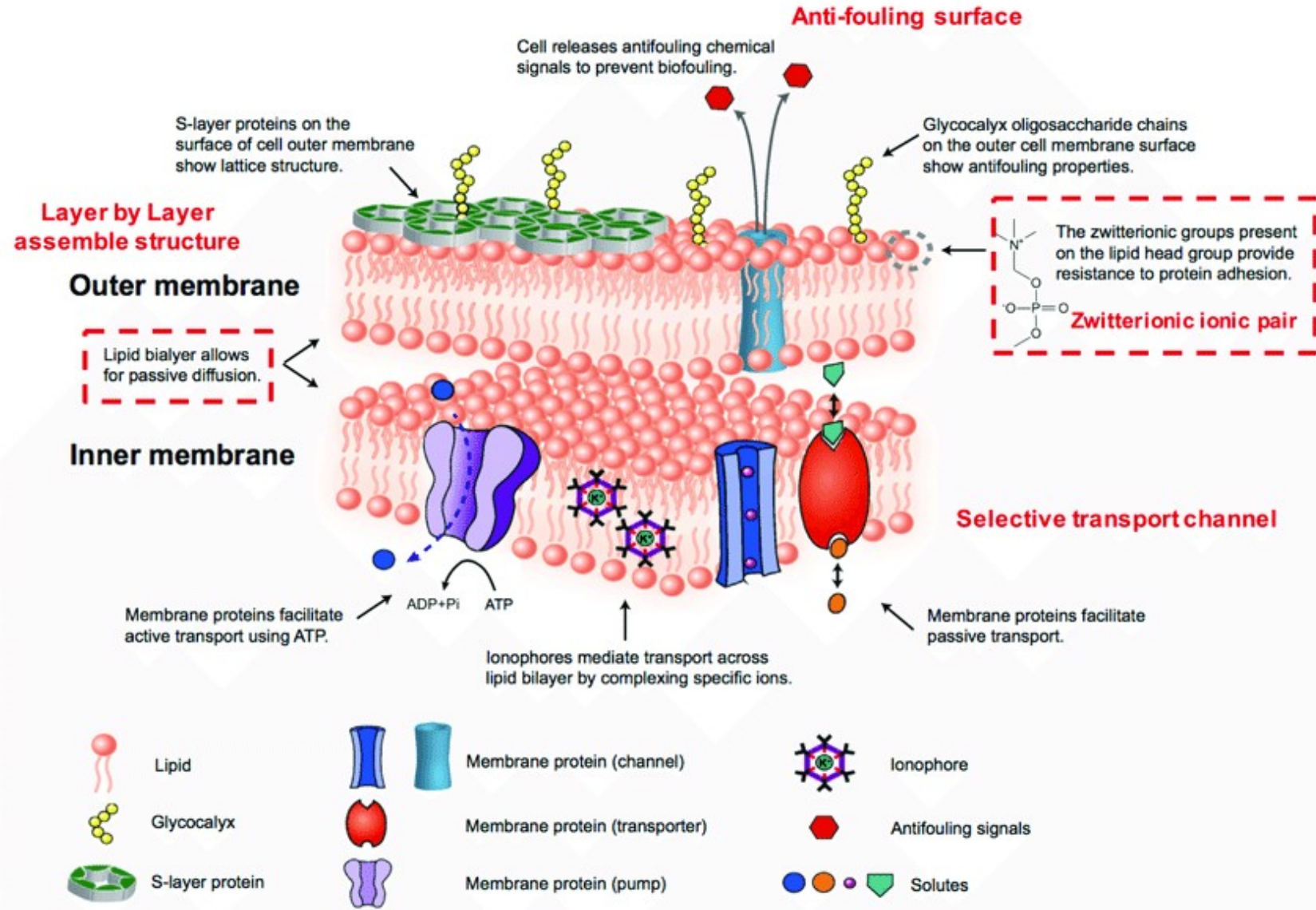


E. Semi-crystalline network



Silk Fibroin is strong, inextensible, insoluble and chemically inert

Proteins in biological membranes



Membrane protein α Helix

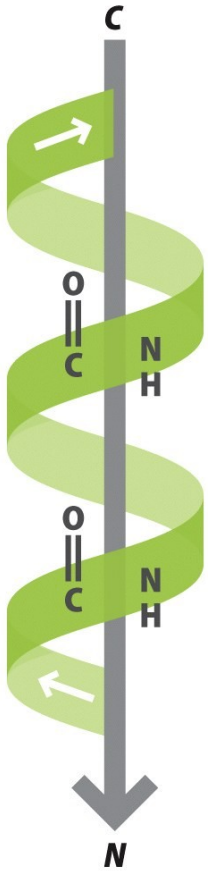


Figure 1.39 How Proteins Work (©2012 Garland Science)

rhodopsin

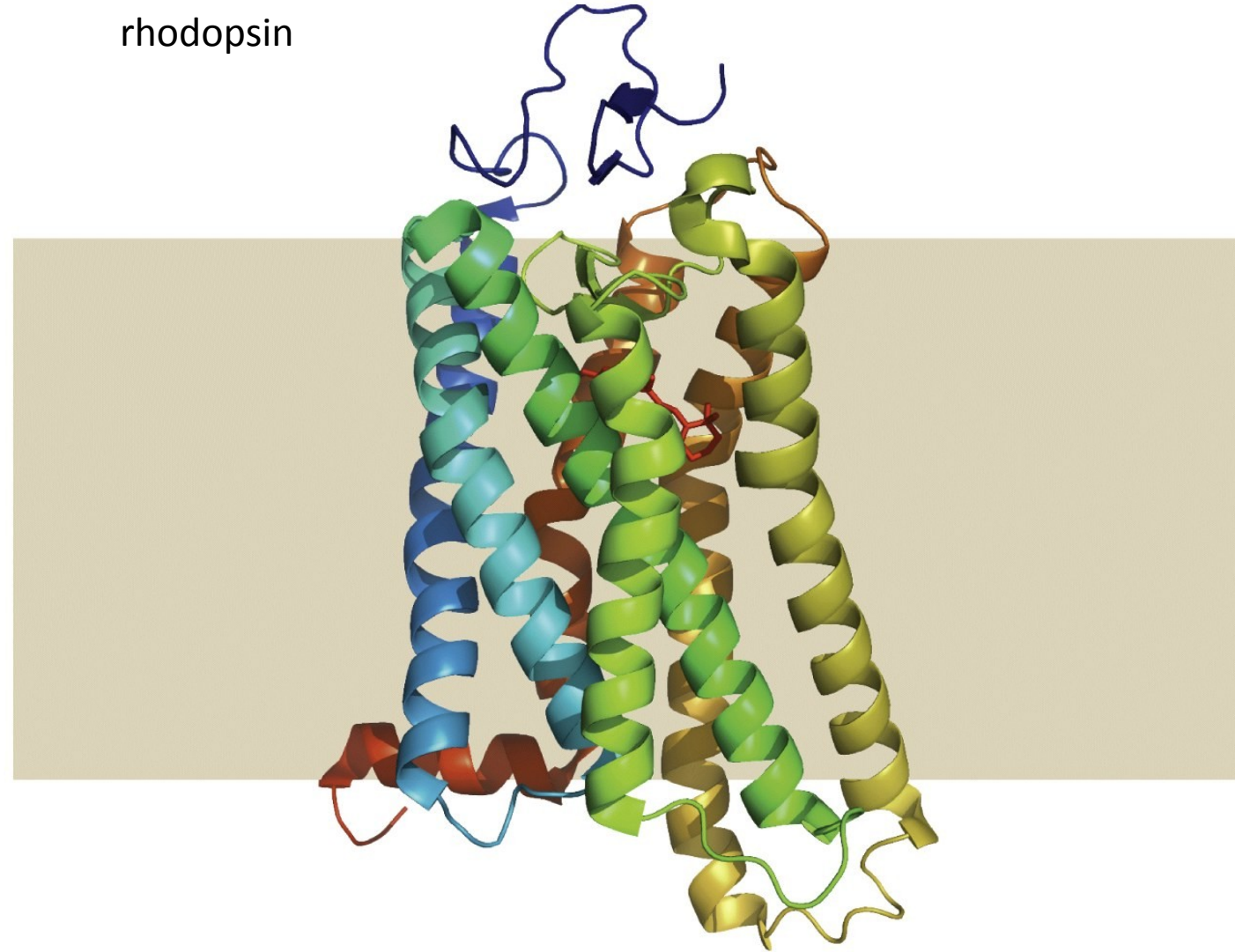


Figure 1.40 How Proteins Work (©2012 Garland Science)

hydrophobic/hydrophilic

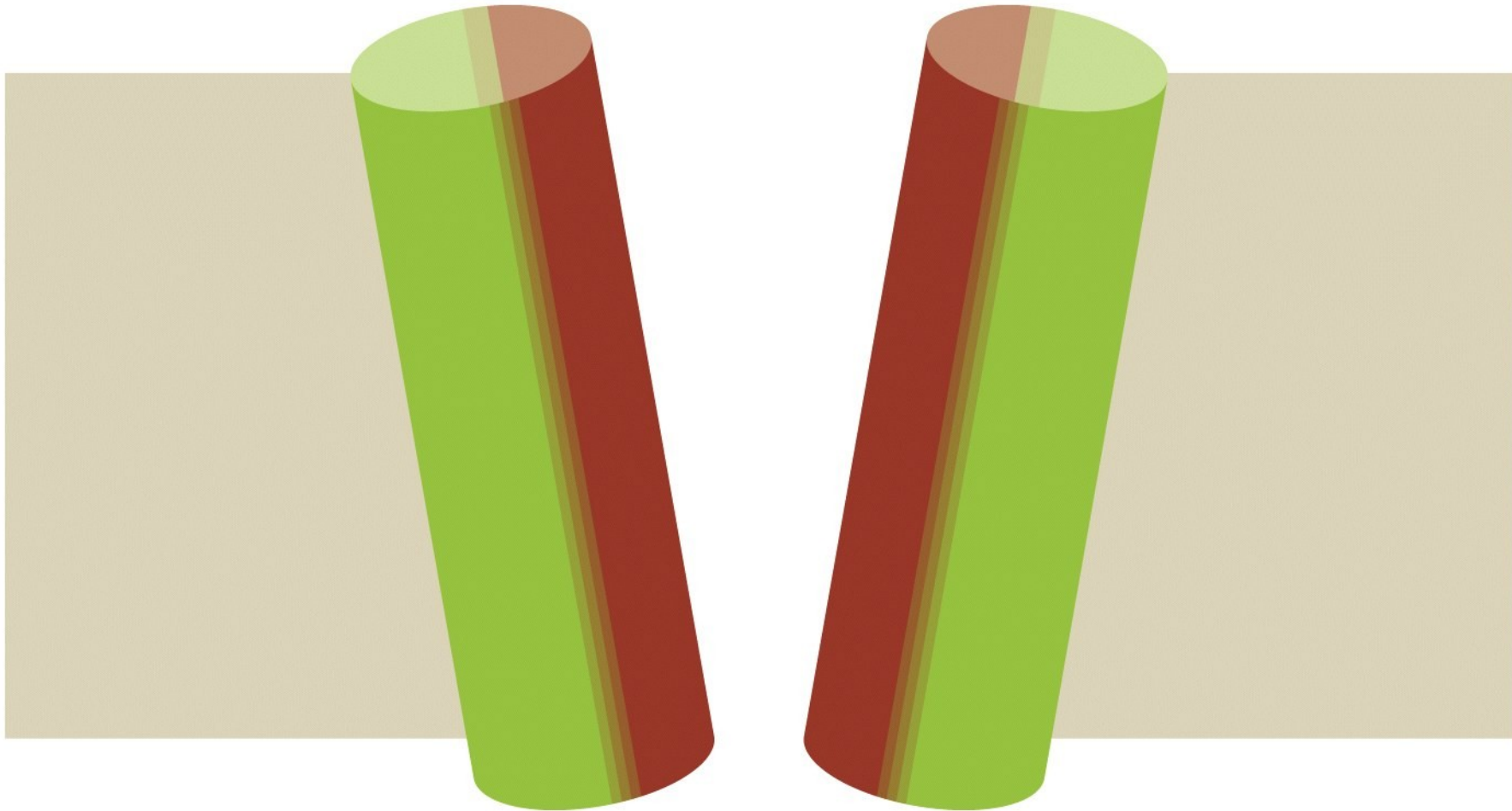


Figure 1.41 How Proteins Work (©2012 Garland Science)

potassium channel

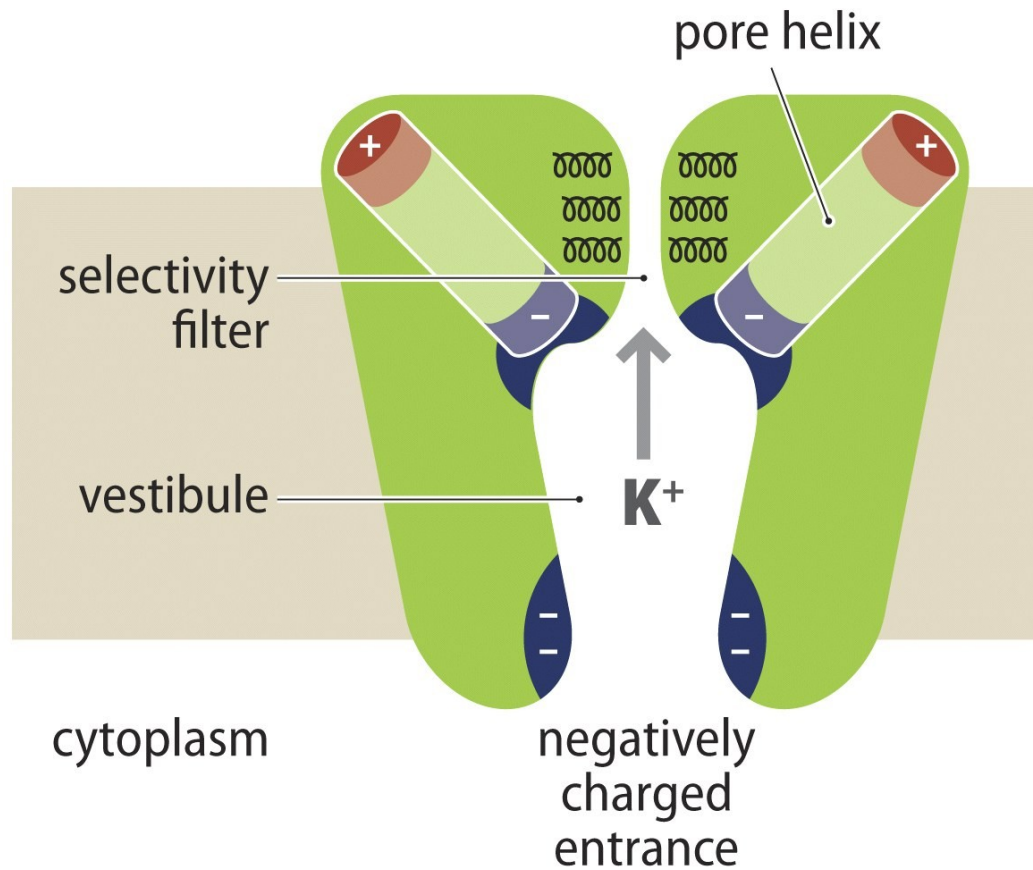


Figure 1.43 How Proteins Work (©2012 Garland Science)

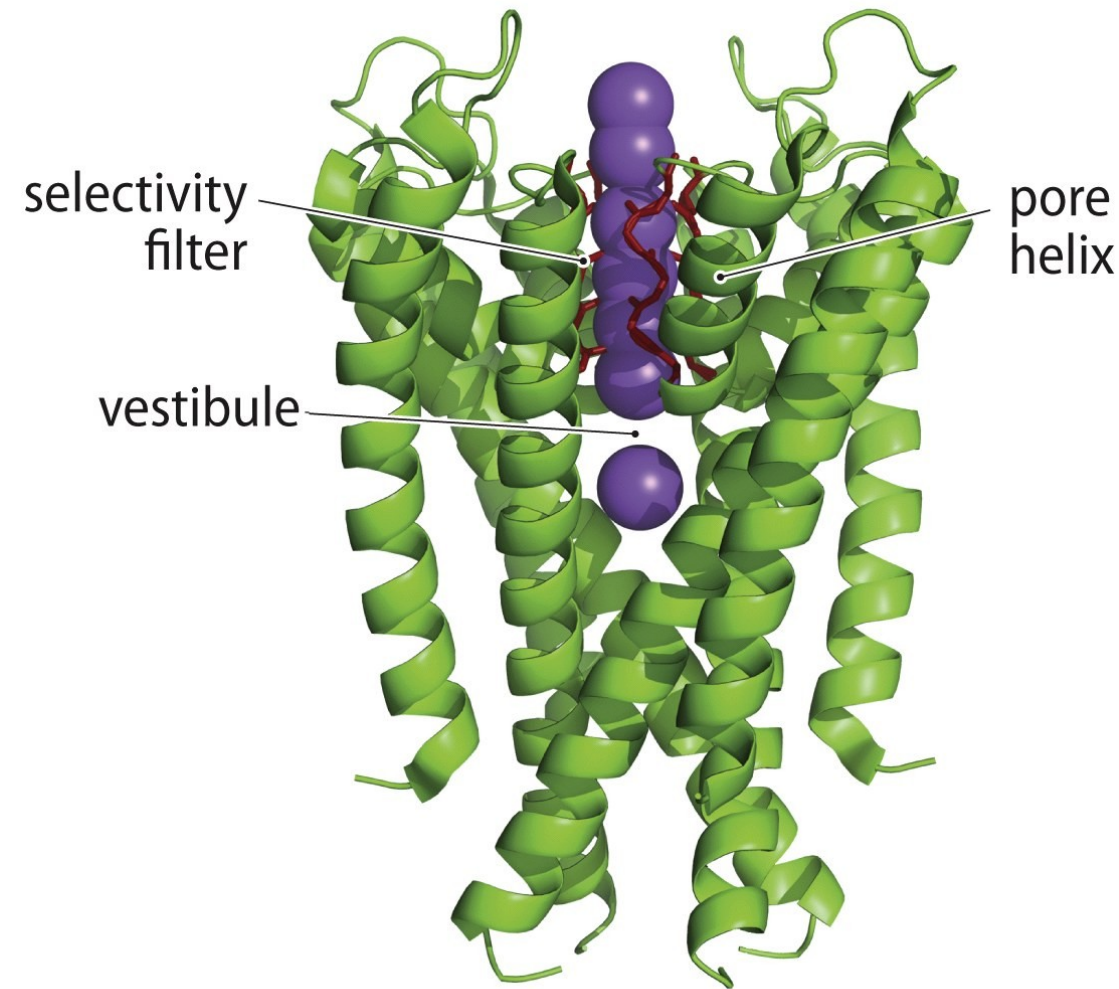


Figure 1.42 How Proteins Work (©2012 Garland Science)

OmpX b-sheet

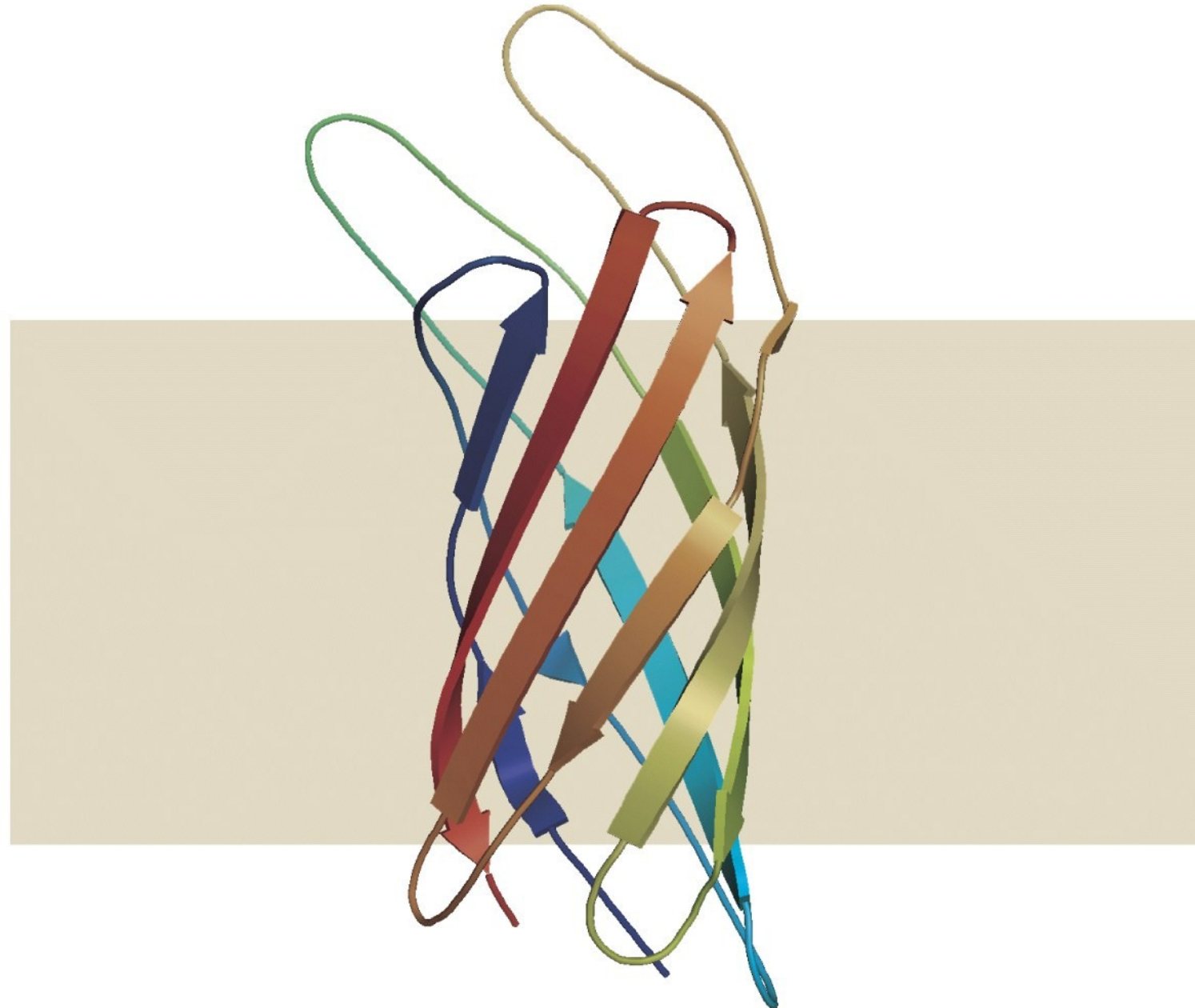


Figure 1.44 How Proteins Work (©2012 Garland Science)