Protein Interactions *in vivo*

$$mv^2/2 = kT/2,$$

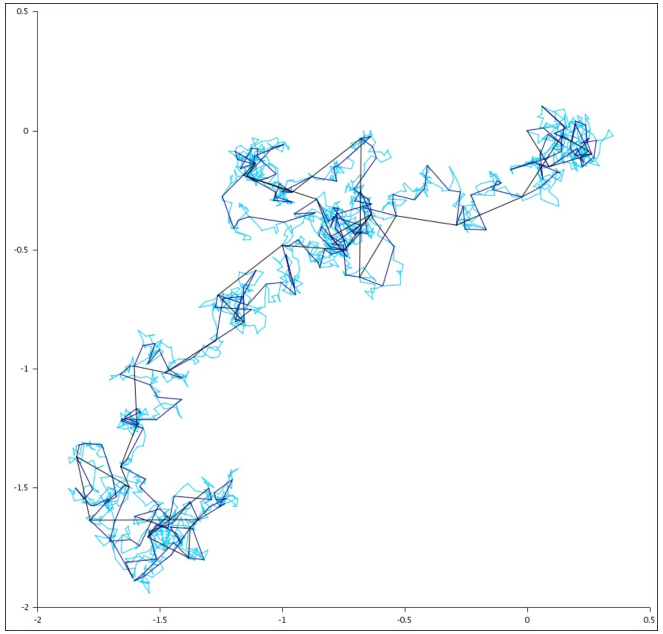
$$v = (kT/m)^{1/2}.$$

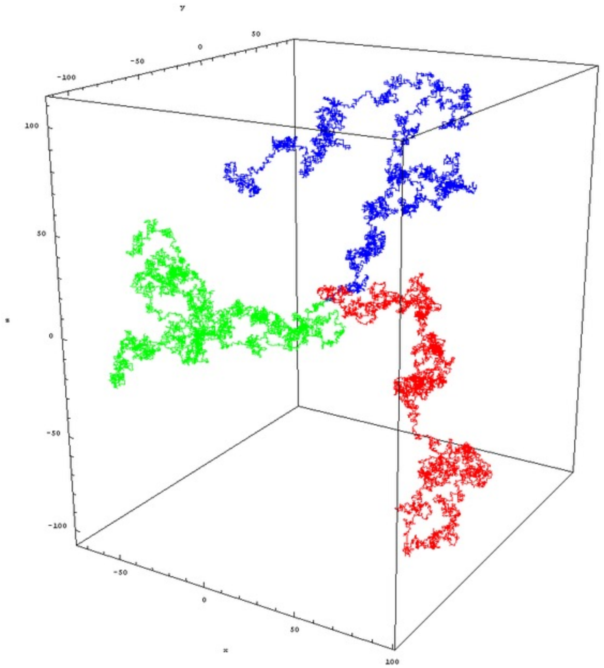
In one direction

In a chemical reaction which is fast enough

An enzyme rate depends on
how fast substrate can diffuse into the active site, and how fast the product can diffuse out.

This rate is proportional to the diffusional collision rate, which in turn depends on the size of the molecules and on the viscosity of the medium





collision rate

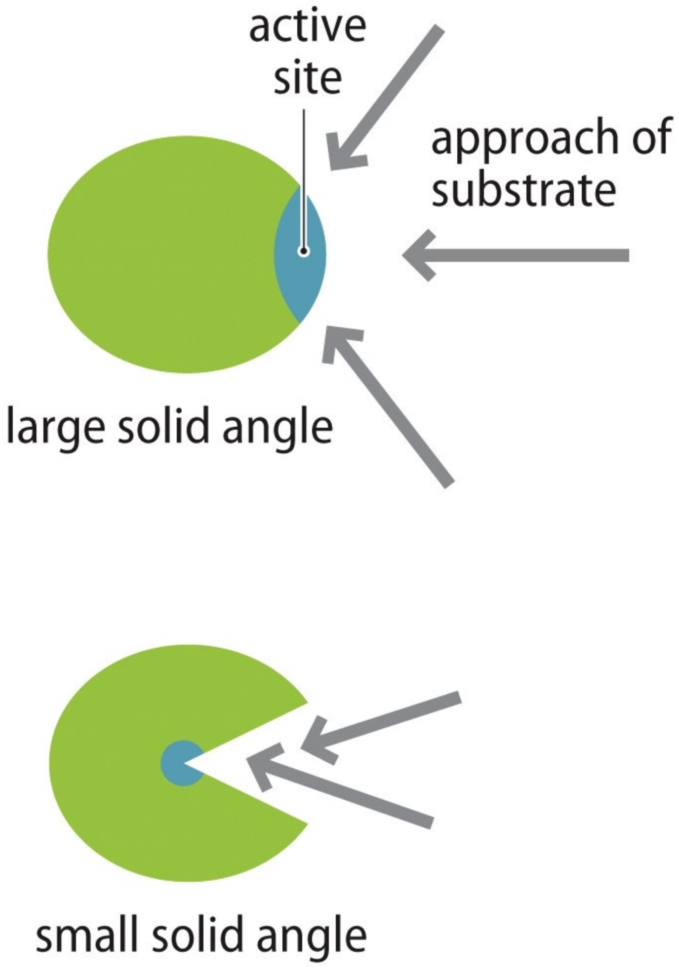


Figure 4.1 How Proteins Work (©2012 Garland Science)

- k_{cat}/K_M is the catalytic efficiency. It is used to rank enzymes. A big k_{cat}/K_M means that an enzyme binds tightly to a substrate (small K_M), with a fast reaction of the ES complex.

- k_{cat}/K_M is an apparent second order rate constant

$$v=k_{cat}/K_M[E]_0[S]$$

*K*cat is the catalytic constant for the conversion of substrate into product

Km is the Michaelis constant

- k_{cat}/K_M is the specificity constant. It is used to distinguish and describe various substrates.

TABLE 4.1 Values of k_{cat}/K_m for some enzymes		
Enzyme	**Substrate**	**k_{cat}/K_m ($M^{-1}\,s^{-1}$)**
Acetylcholinesterase	Acetylcholine	1.5×10^8
Carbonic anhydrase	Carbon dioxide	8.3×10^7
Catalase	Hydrogen peroxide	4.0×10^8
Fumarase	Fumarate	1.6×10^8
Fumarase	Malate	3.6×10^7
Superoxide dismutase	Superoxide	2.8×10^9
Triosephosphate isomerase	Dihydroxyacetone phosphate	7.5×10^5
Triosephosphate isomerase	Glyceraldehyde 3-phosphate	2.4×10^8
Lysozyme	$(NAG\text{-}NAM)_3$	83
Glucose isomerase	Glucose	7.4

Abbreviation: NAG-NAM, *N*-acetylglucosamine–*N*-acetylmuramic acid disaccharide.

Table 4.1 How Proteins Work (©2012 Garland Science)

rate of diffusion

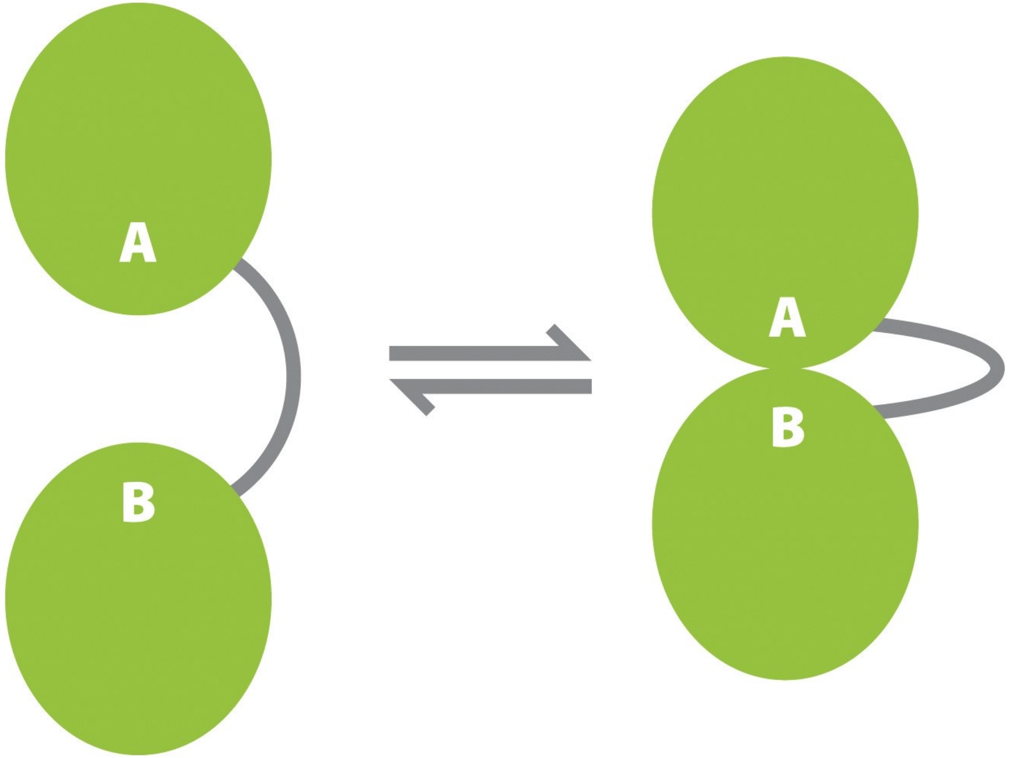


Figure 4.2 How Proteins Work (©2012 Garland Science)

Dielectric constant

$$F = q_1 q_2 / 4\pi\epsilon r^2,$$

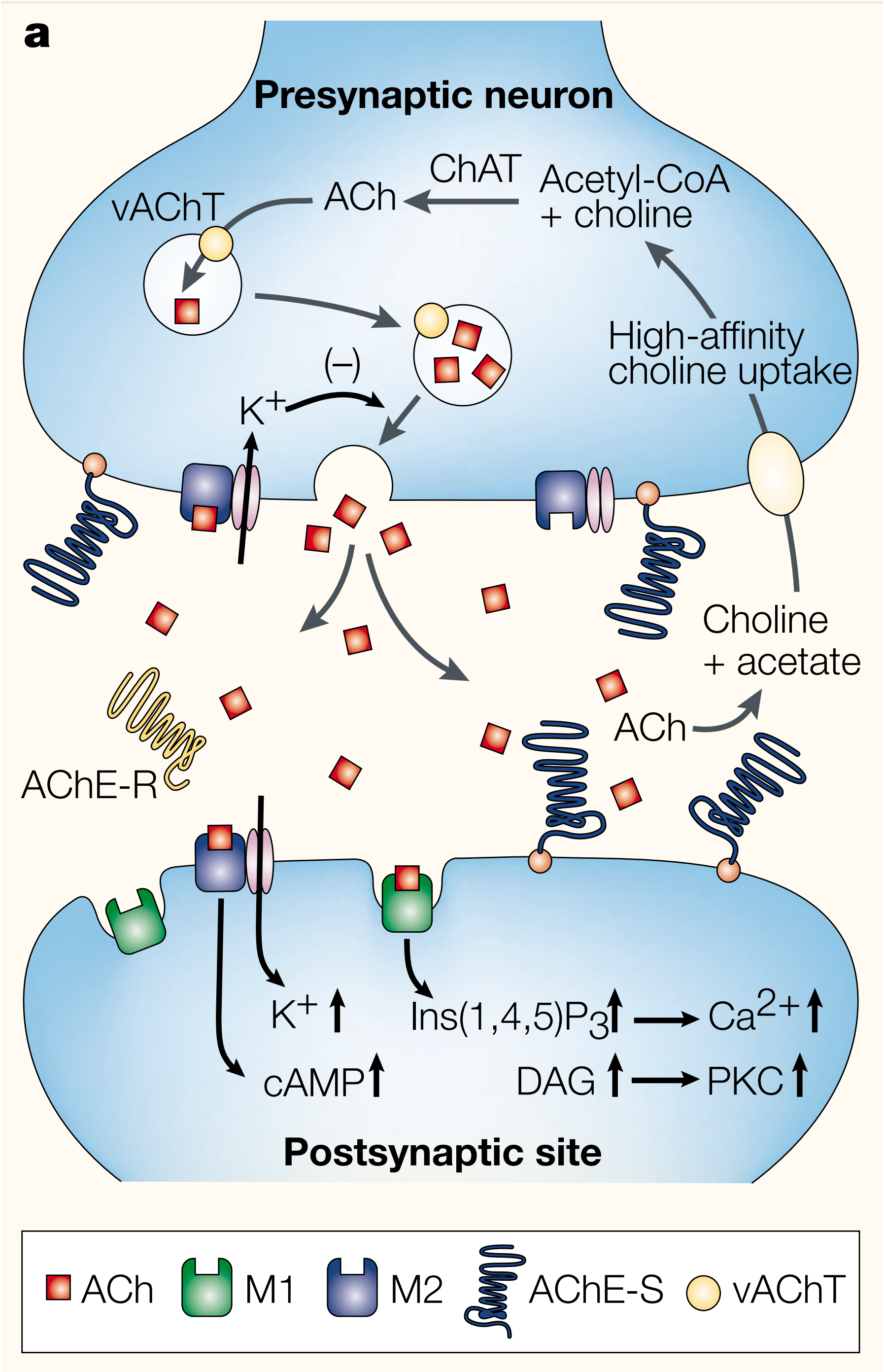
Ionic strength

$$\mu = \tfrac{1}{2} \sum z_i^2 C_i$$

Electrostatic screening

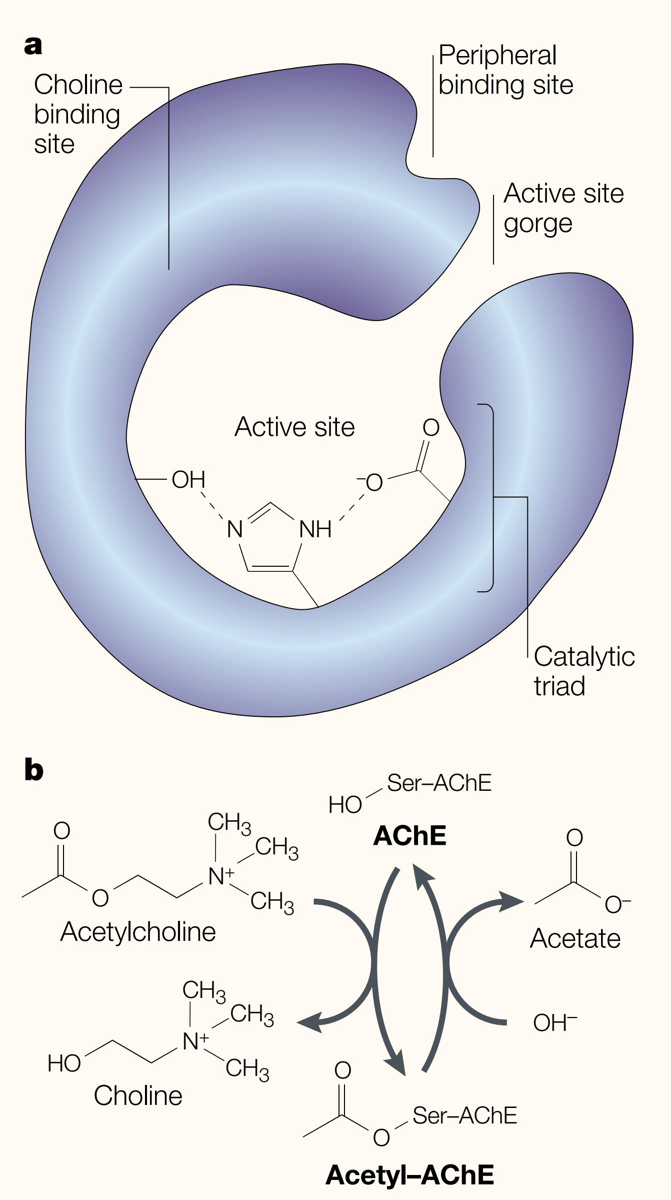
$$r = \left(\frac{\epsilon KT}{2N_0 e^2 \mu}\right)^{1/2}$$

Acetylcholinesterase



Collision rates

electrostatic orientational steering



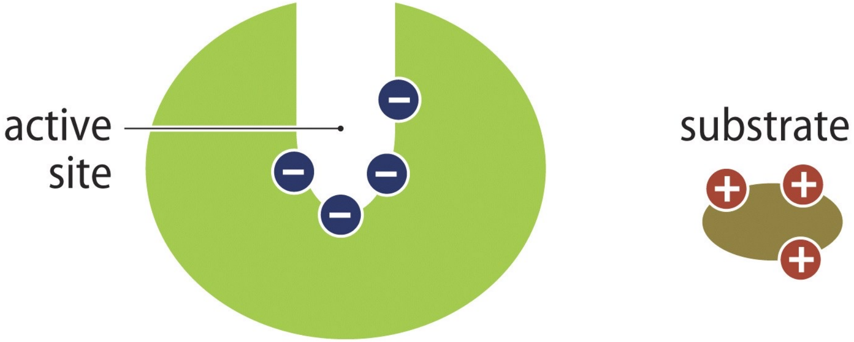


Figure 4.3 How Proteins Work (©2012 Garland Science)

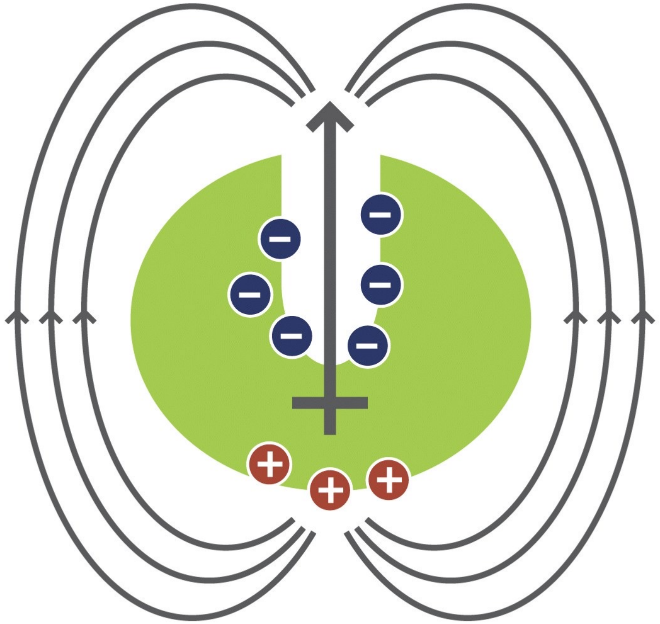


Figure 4.4 How Proteins Work (©2012 Garland Science)

Cytochrome c

Electrostatic steering

Red basic
Green acidic

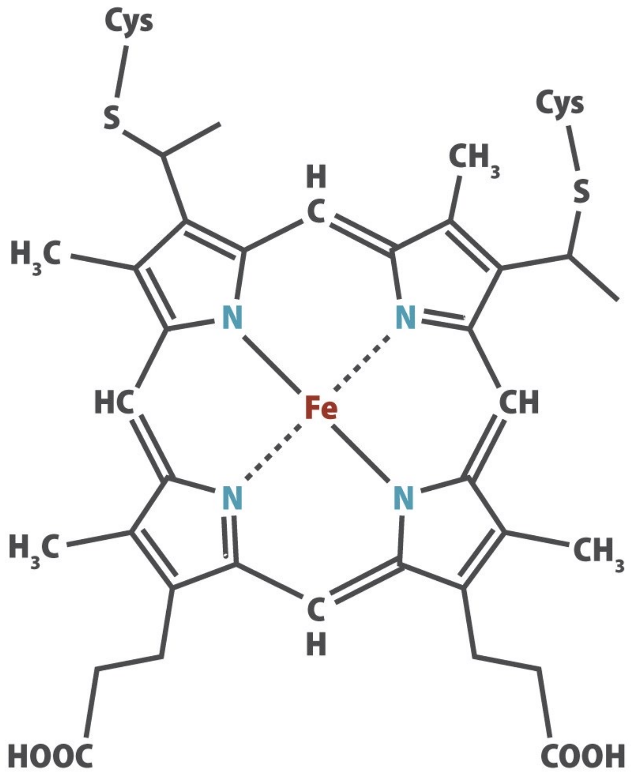


Figure 4.5.1 How Proteins Work (©2012 Garland Science)

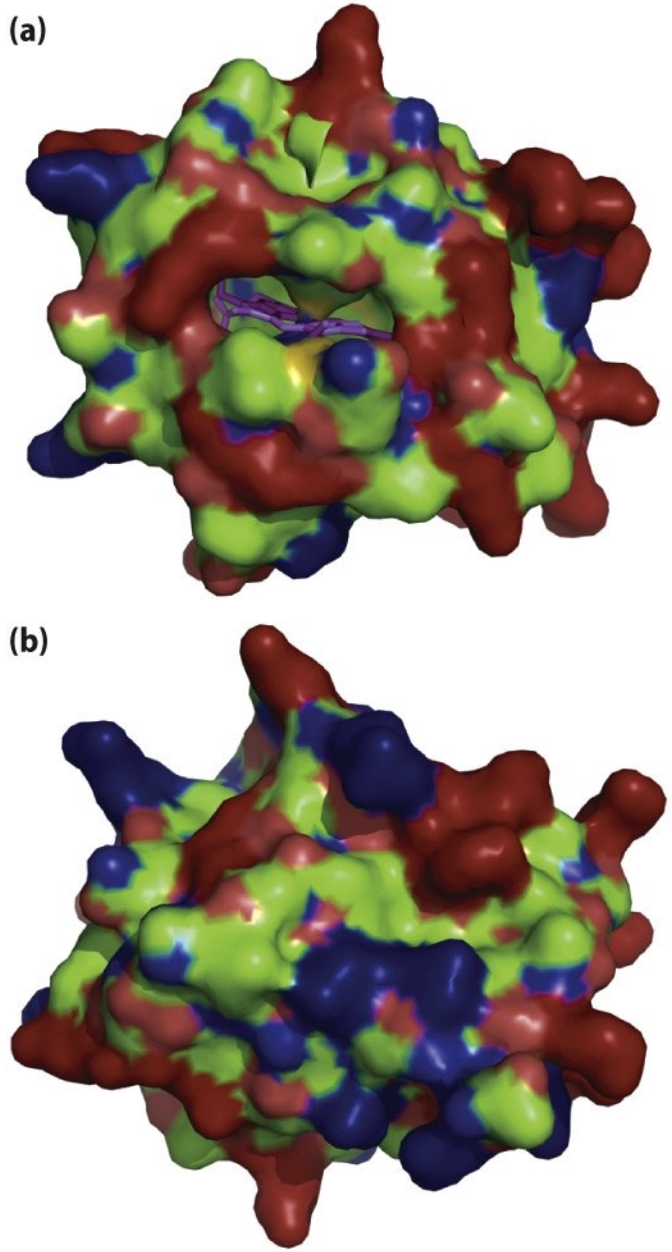


Figure 4.5 How Proteins Work (©2012 Garland Science)

Protein pIs

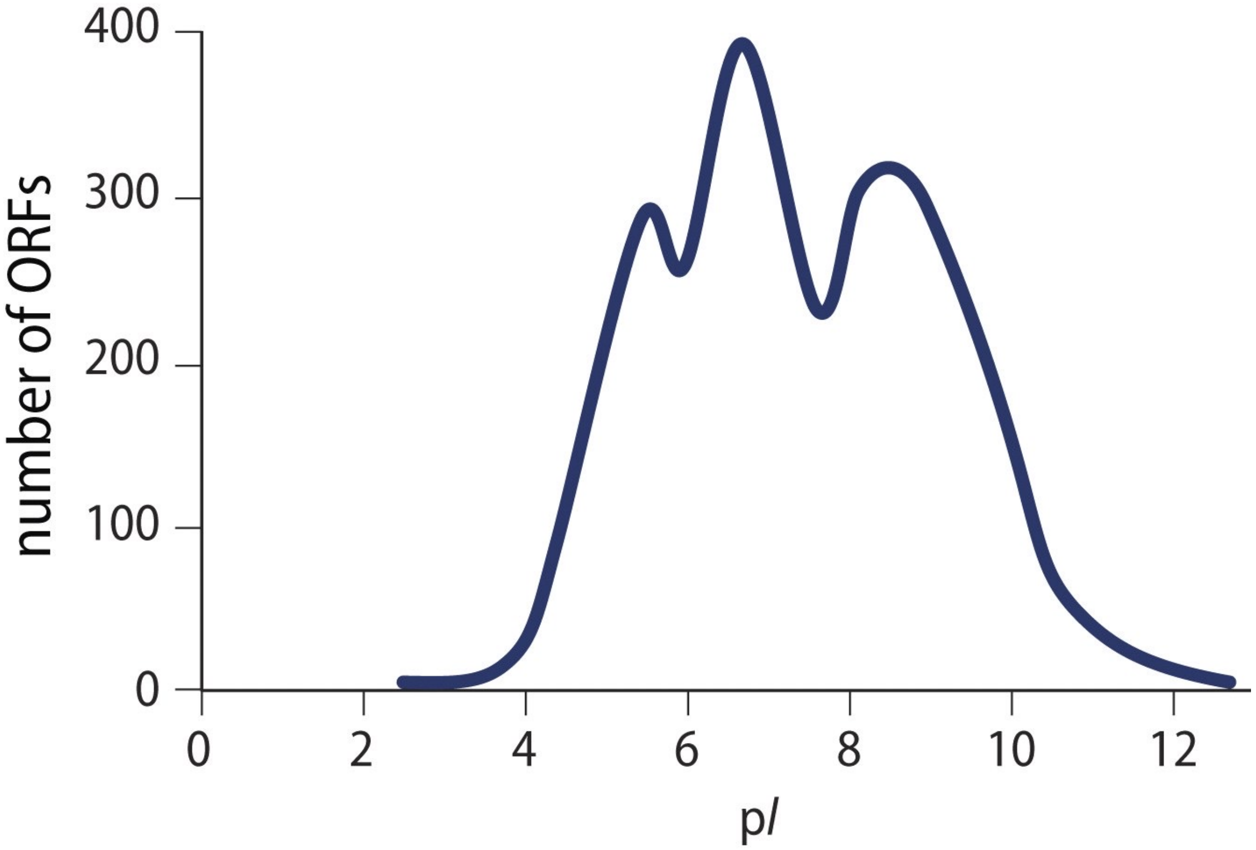


Figure 4.7 How Proteins Work (©2012 Garland Science)

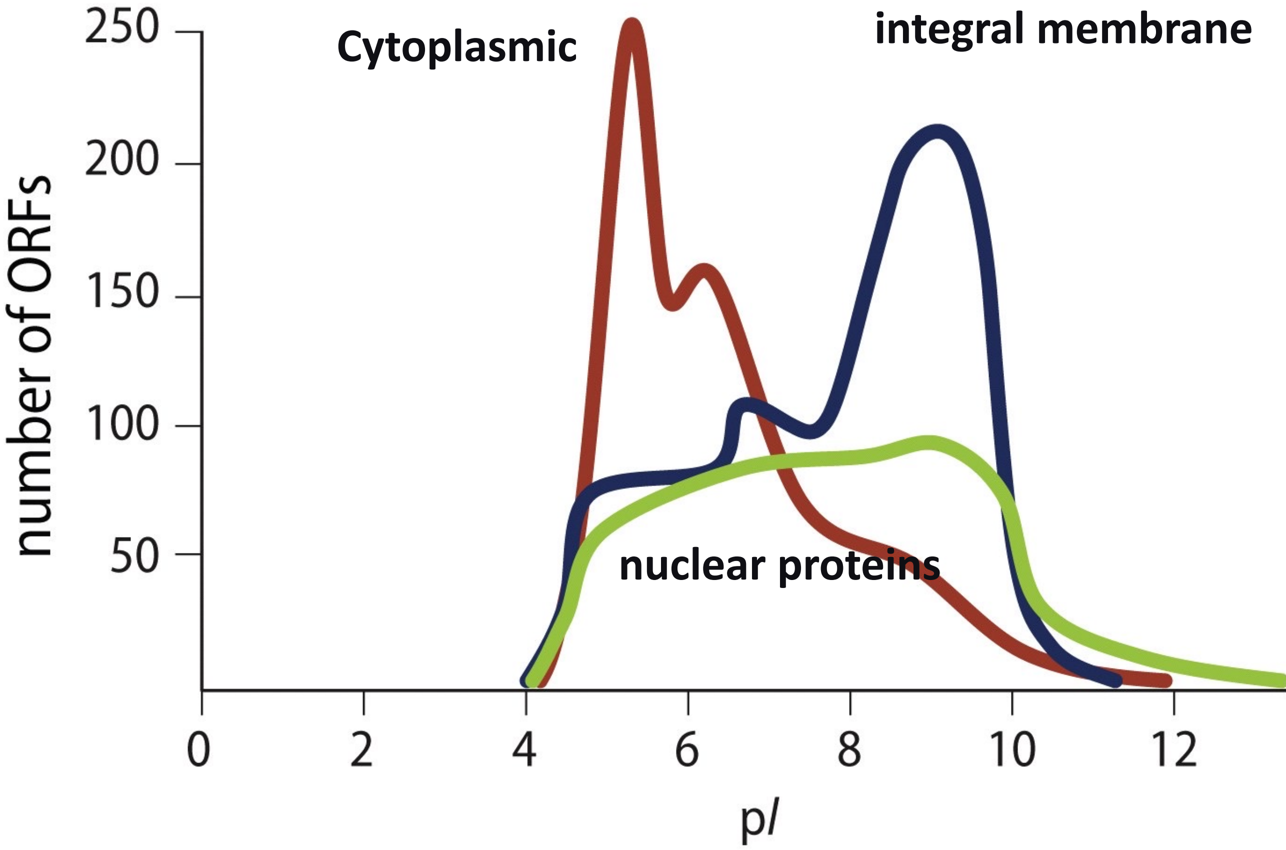


Figure 4.8 How Proteins Work (©2012 Garland Science)

σύμπλεγμα συνάντησης

encounter complex

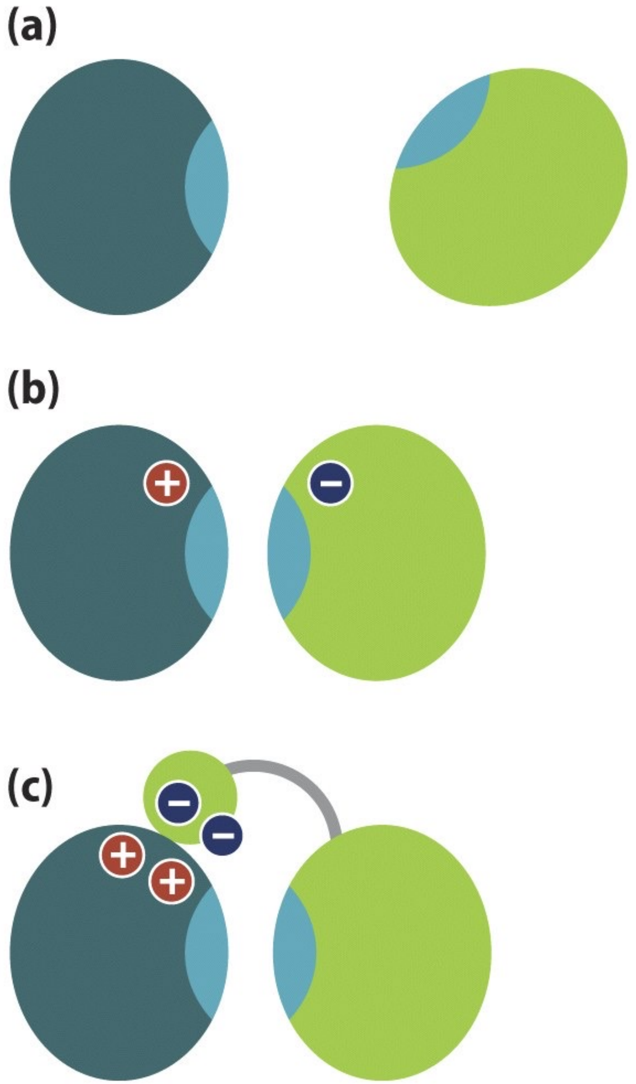
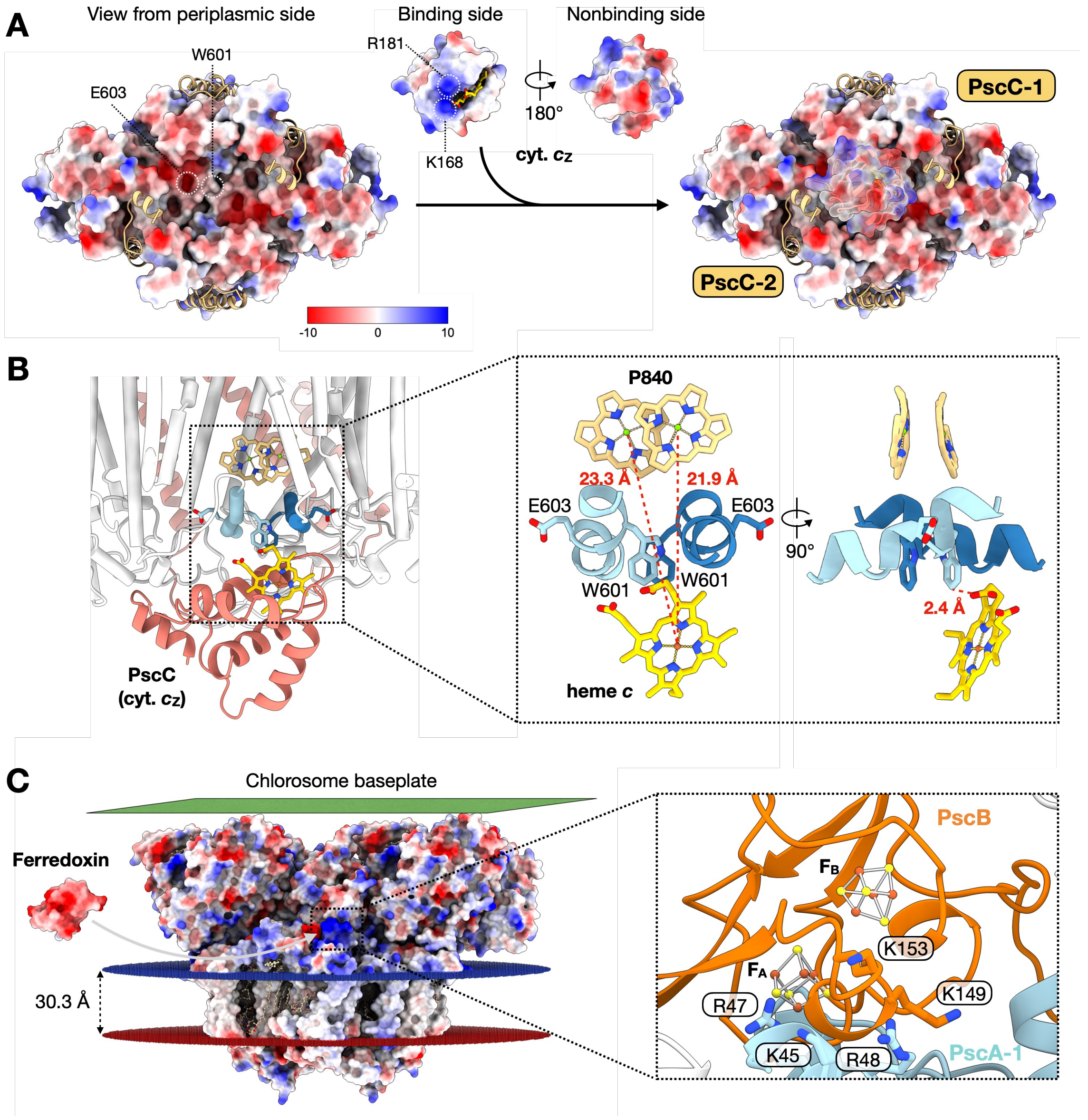
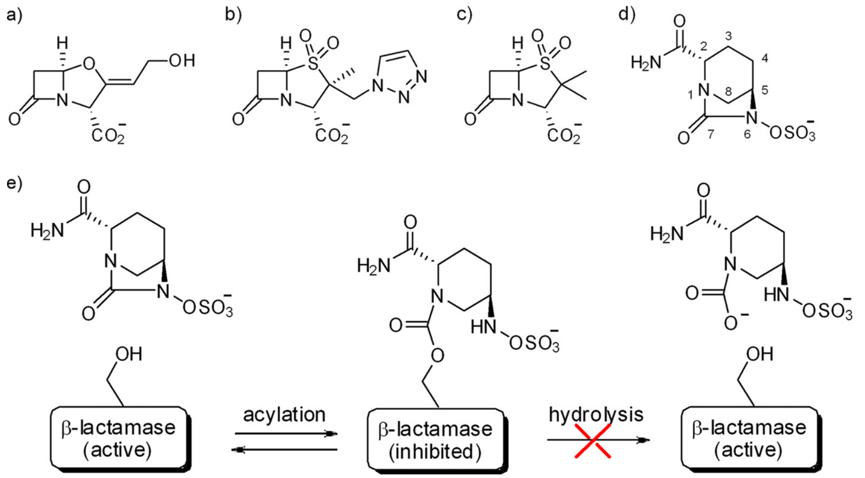


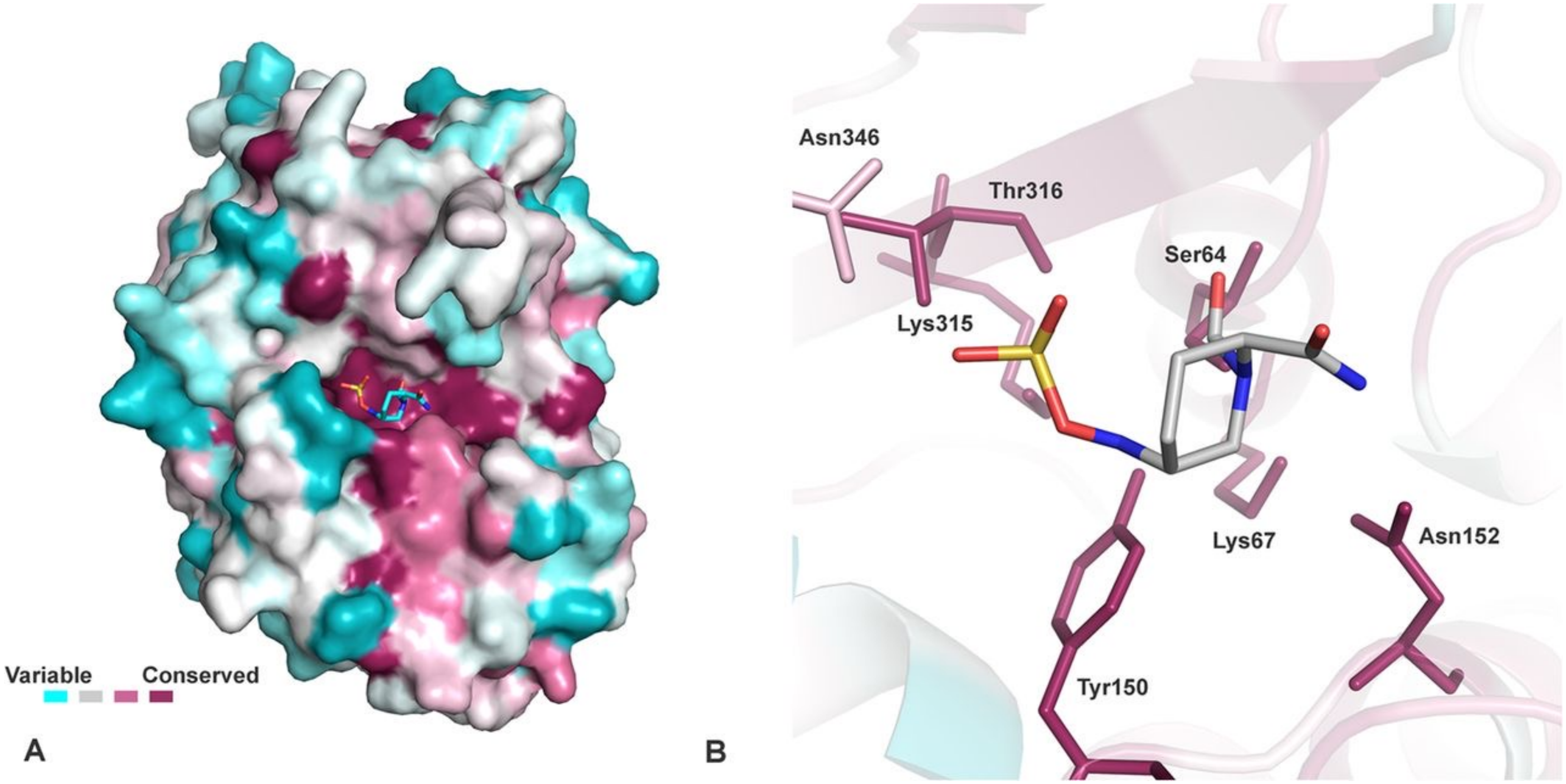
Figure 4.6 How Proteins Work (©2012 Garland Science)



Lyratzakis et al. PNAS 2023

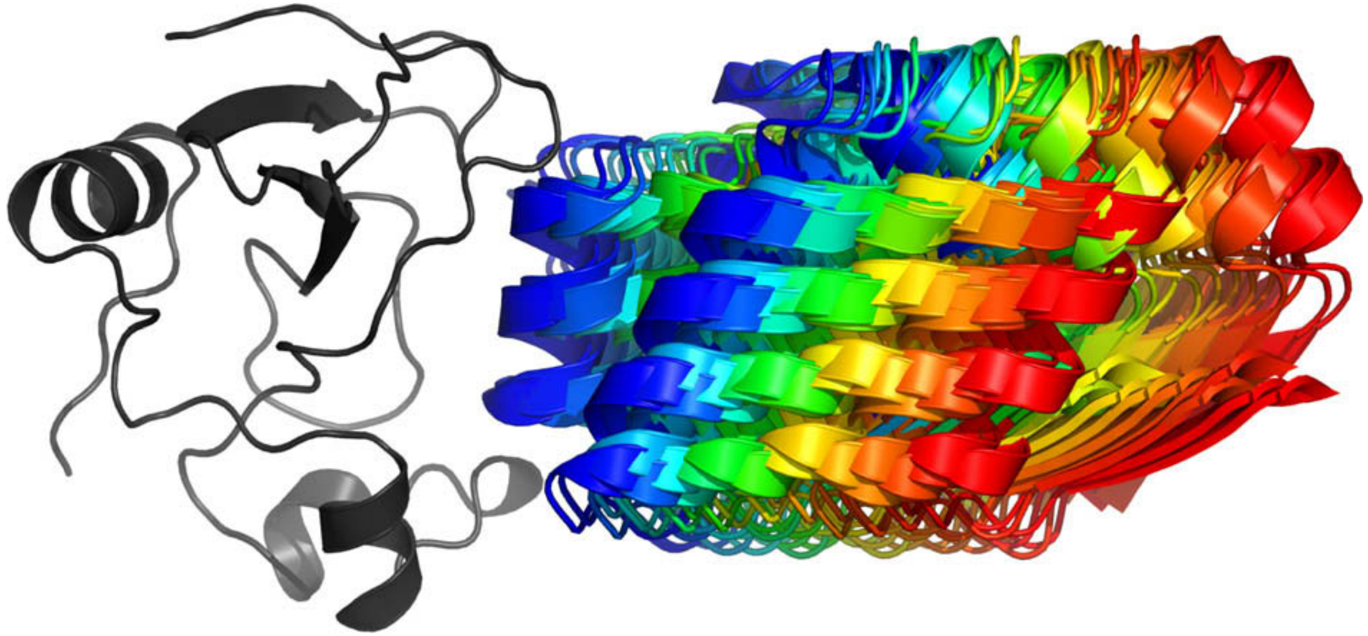
Conservation of the avibactam binding pocket mapped on the AmpC crystal structure.

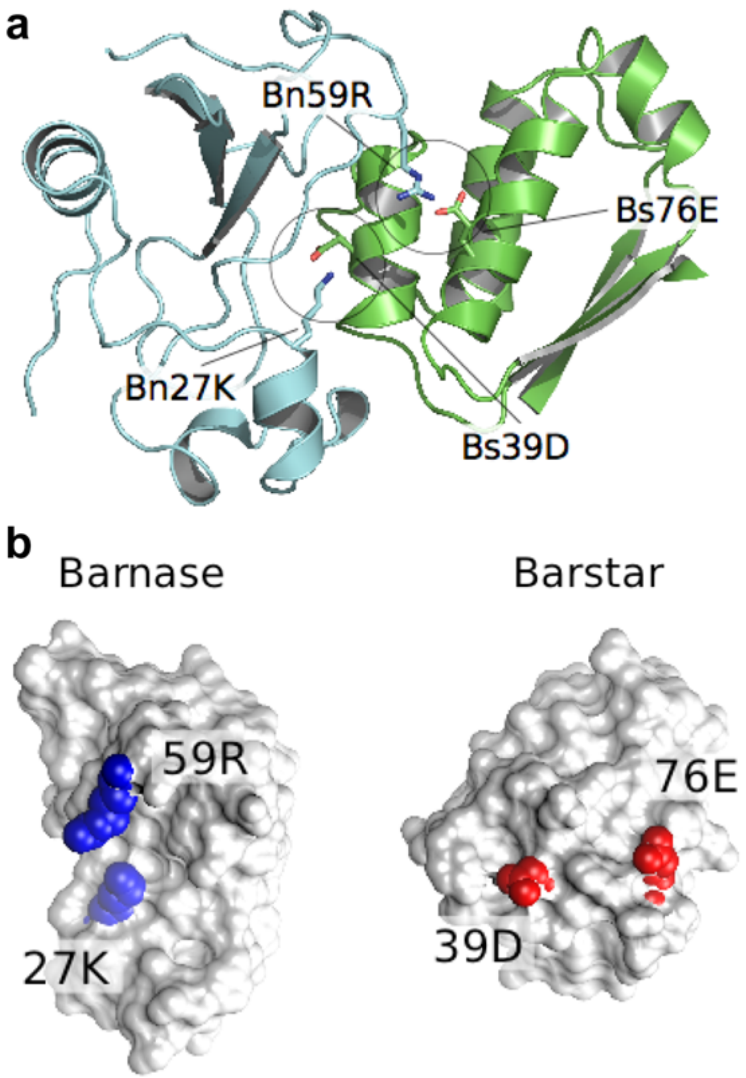




S. D. Lahiri et al. Antimicrob. Agents Chemother. 2014;
doi:10.1128/AAC.03057-14

barnase with inhibitor barstar





M. Hoefling, K.E. Gottschalk /Journal of Structural Biology 171 (2010) 52–63

inside of a cell

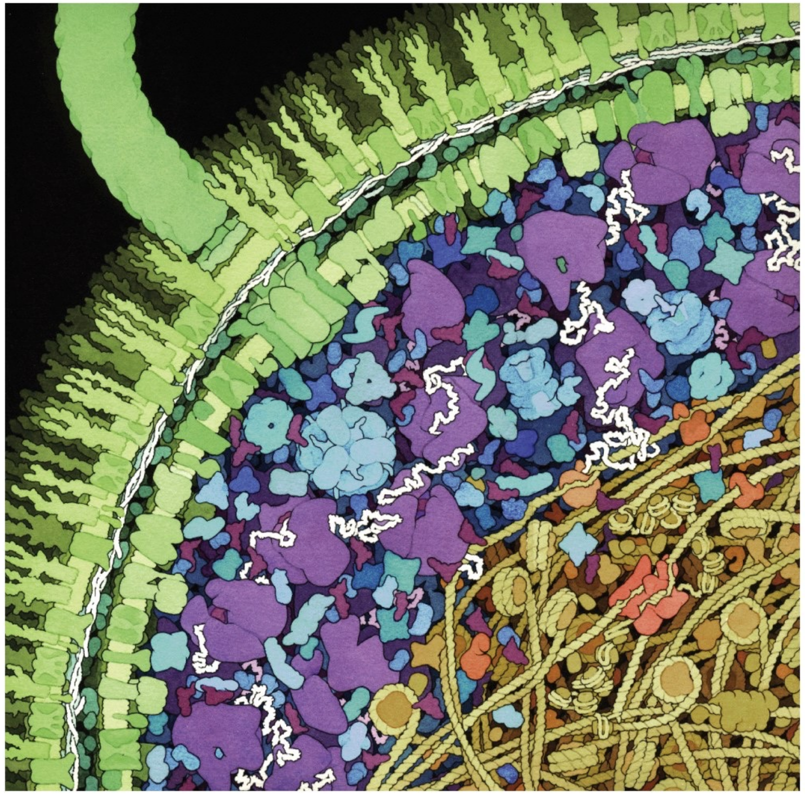
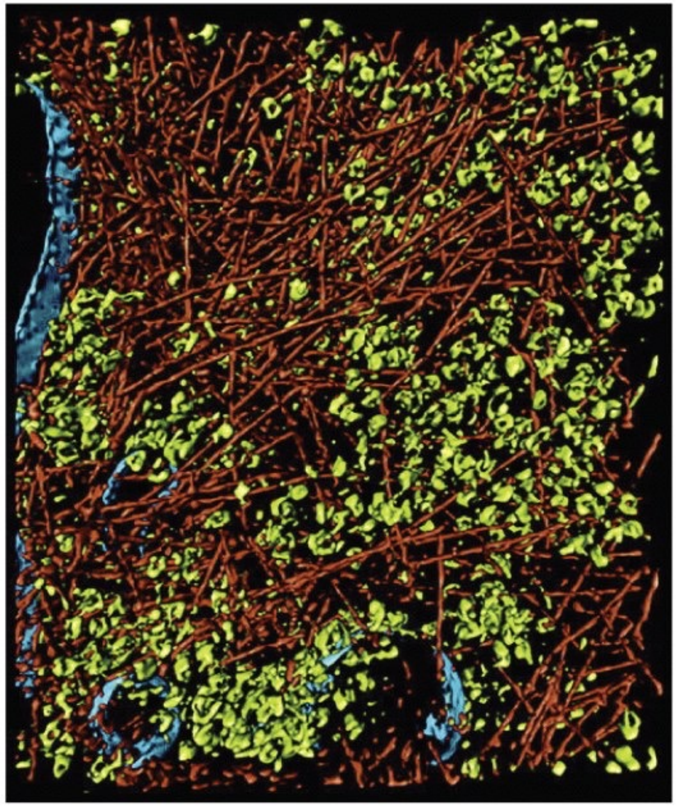


Figure 4.9 How Proteins Work (©2012 Garland Science)

(a)



(b)

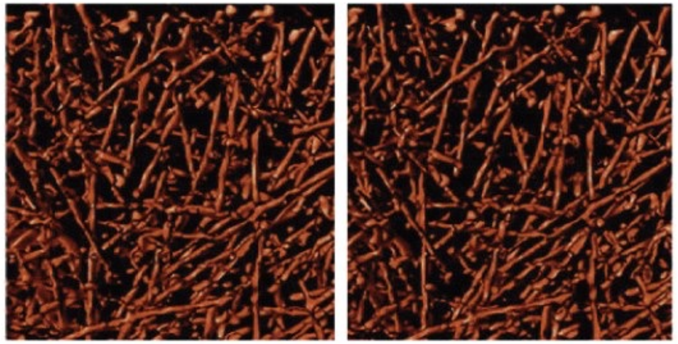


Figure 4.10 How Proteins Work (©2012 Garland Science)

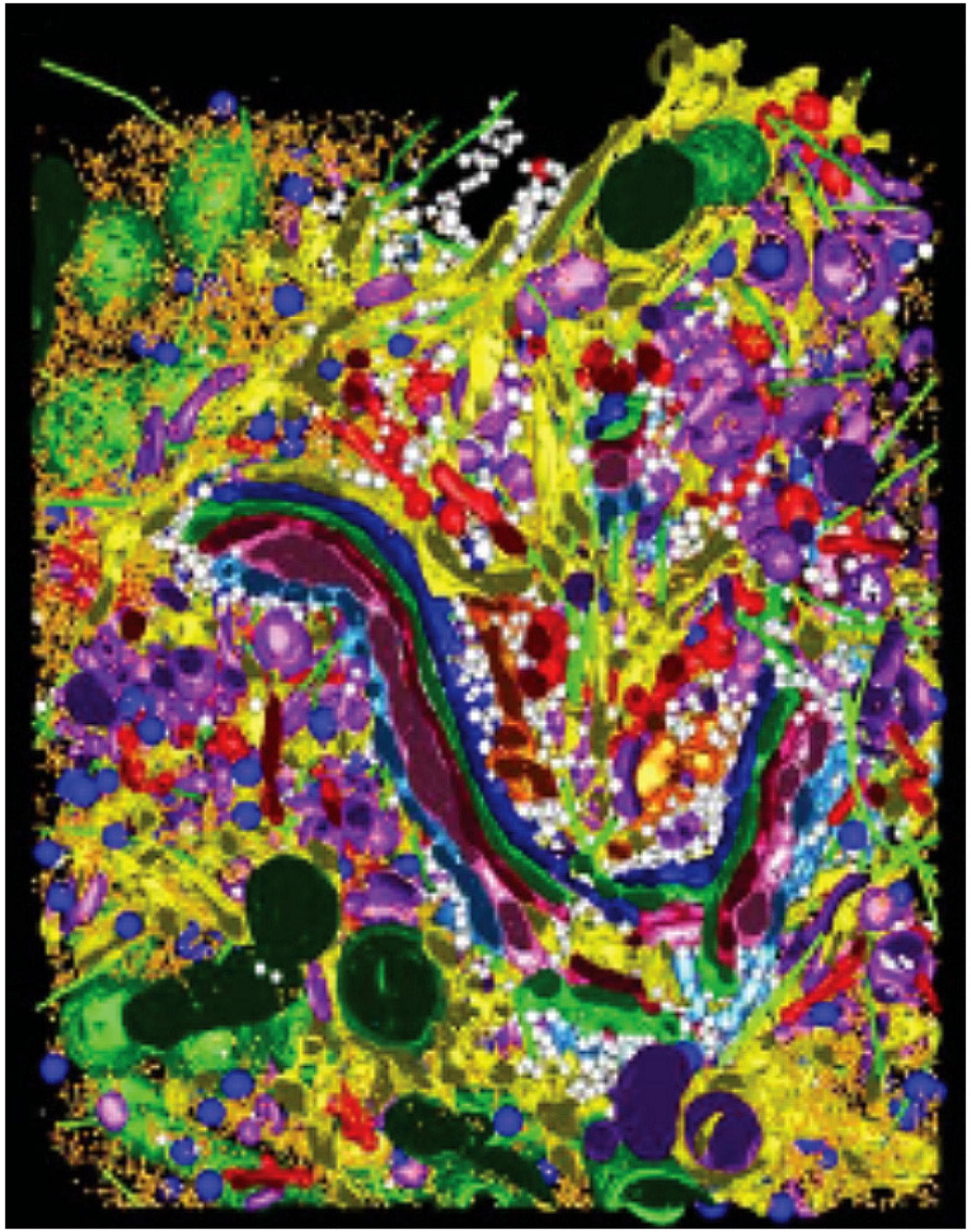
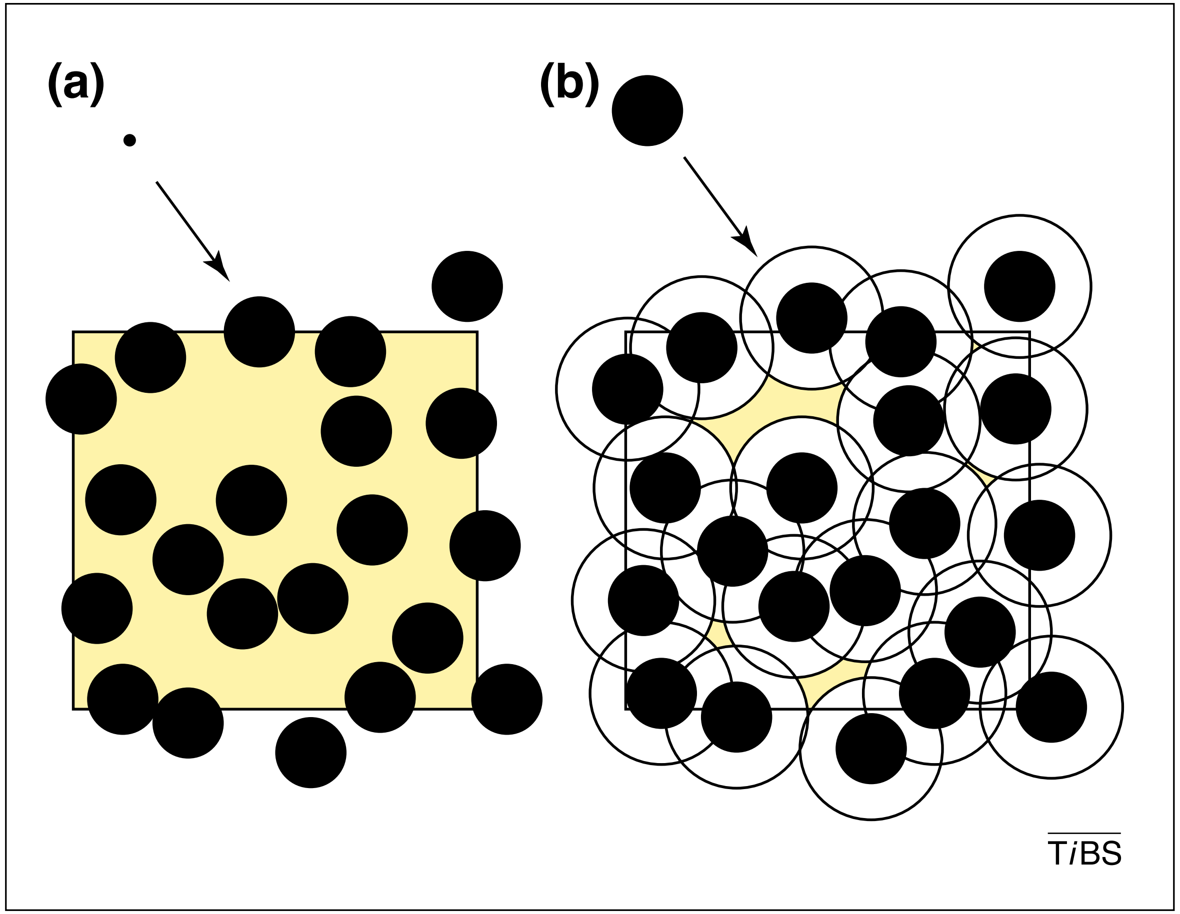


Figure 1.35 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

volume exclusion



TRENDS in Biochemical Sciences Vol.26 No.10 October 2001

relative sizes

The collision rate thus depends most critically on the size r_A of the diffusing molecule:

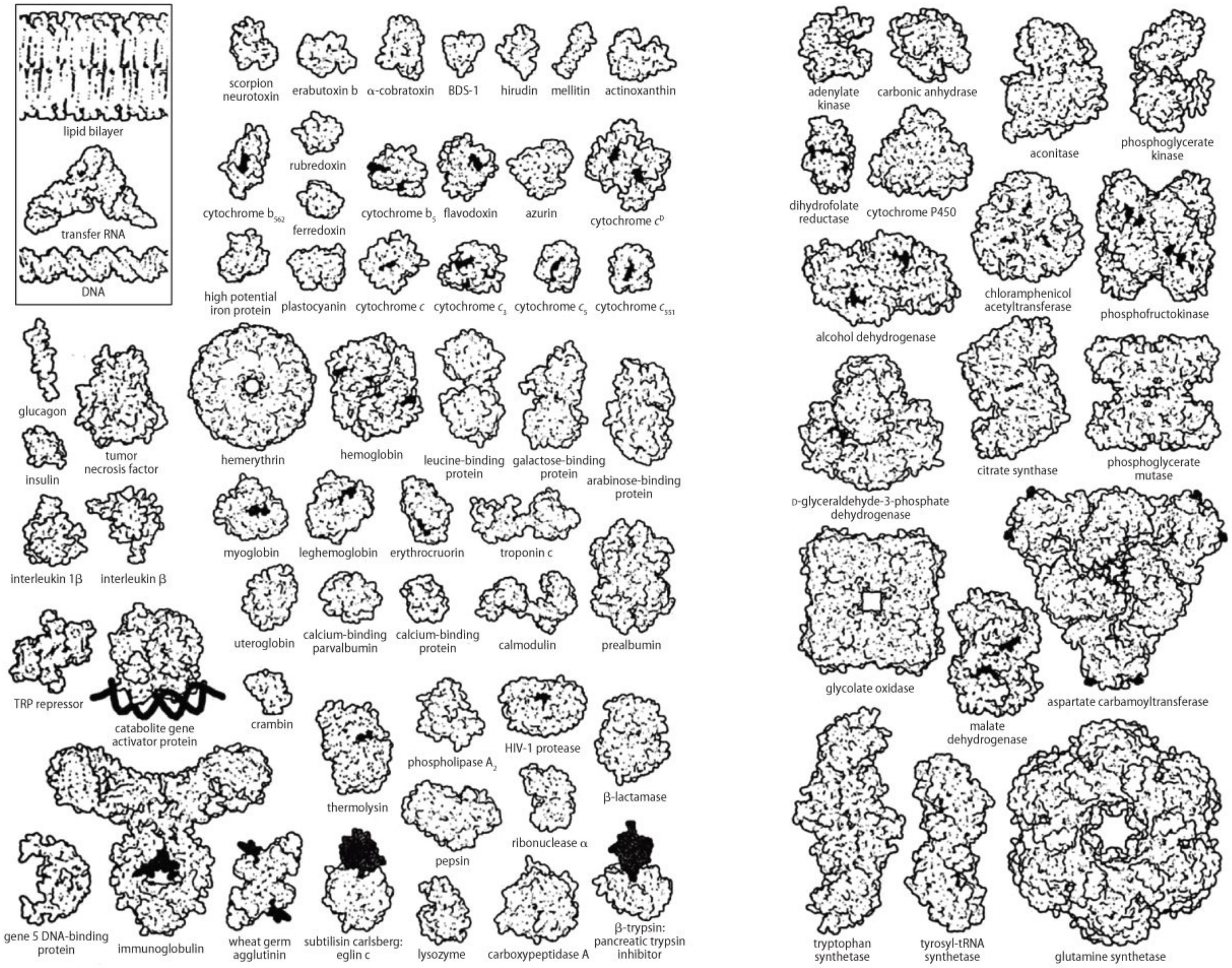


Figure 4.15 How Proteins Work (©2012 Garland Science)

Find the partner

Processivity decreases the off-rate from polymeric substrates

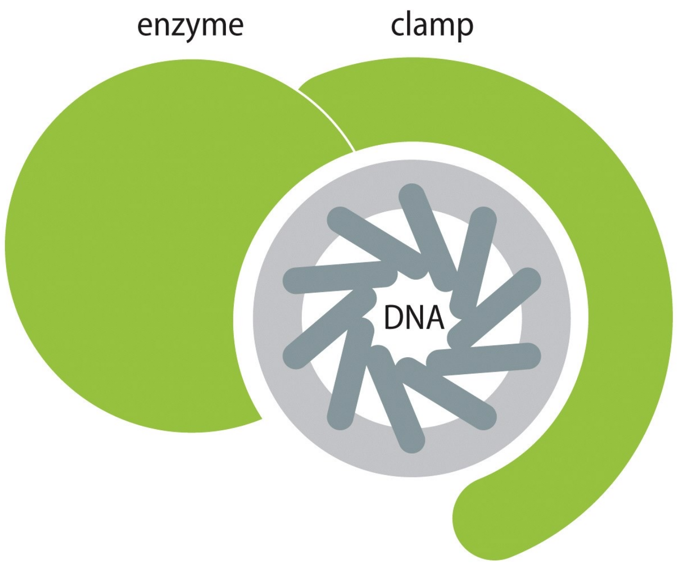
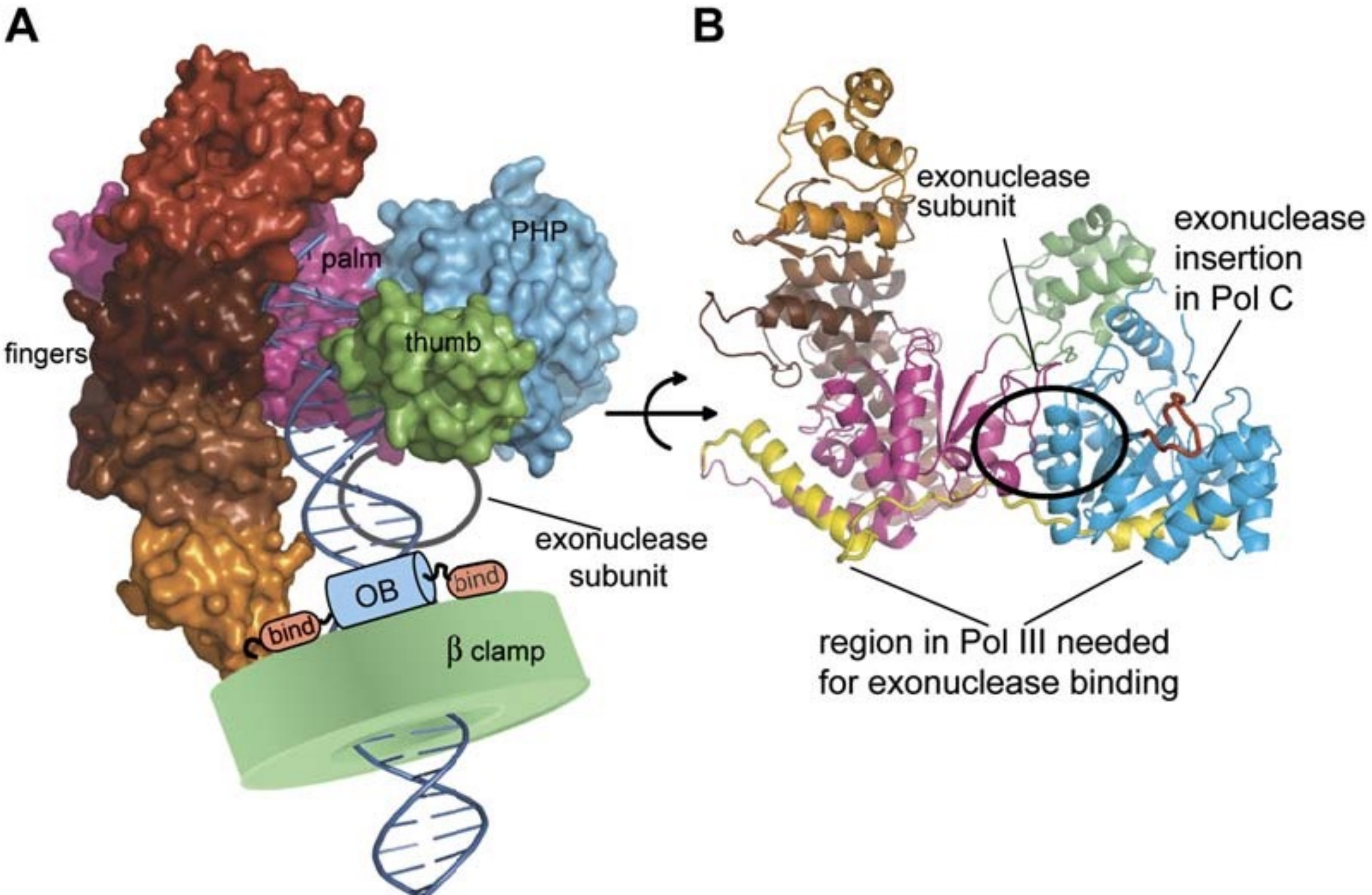
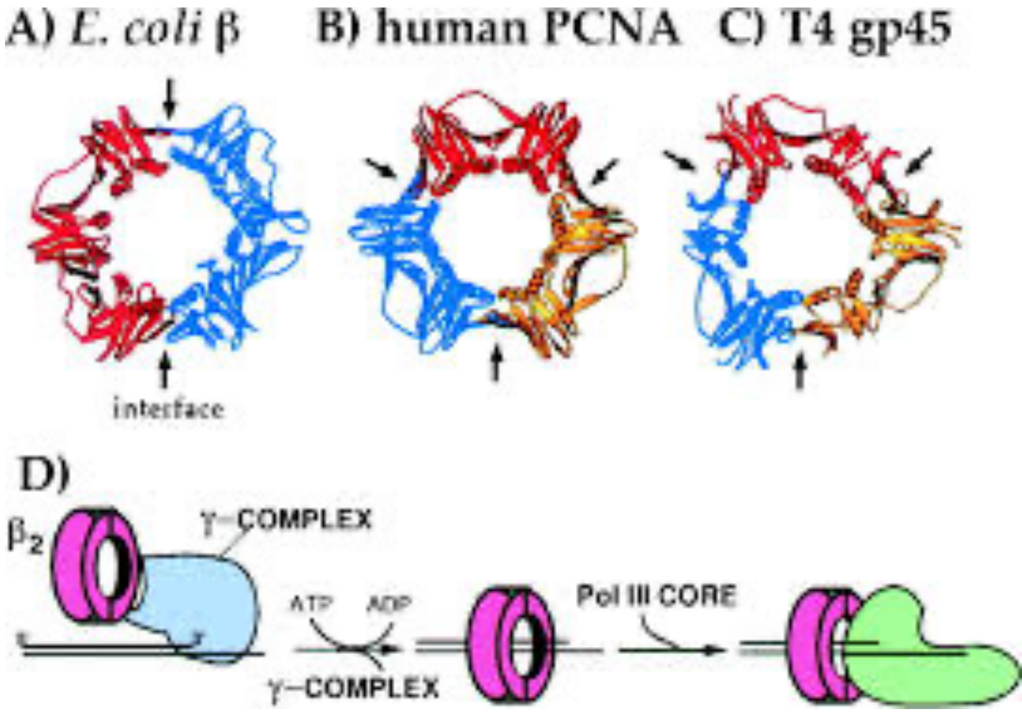
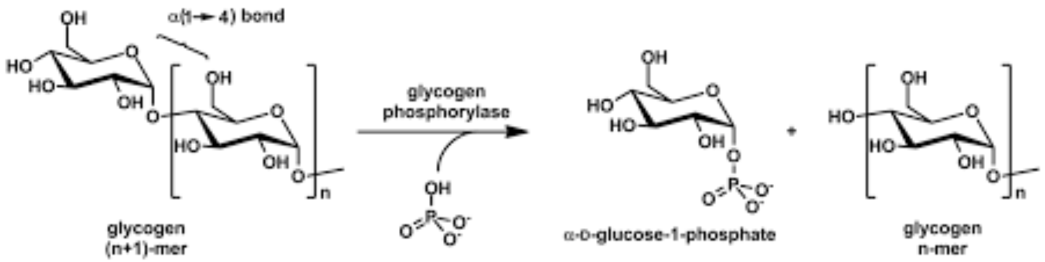


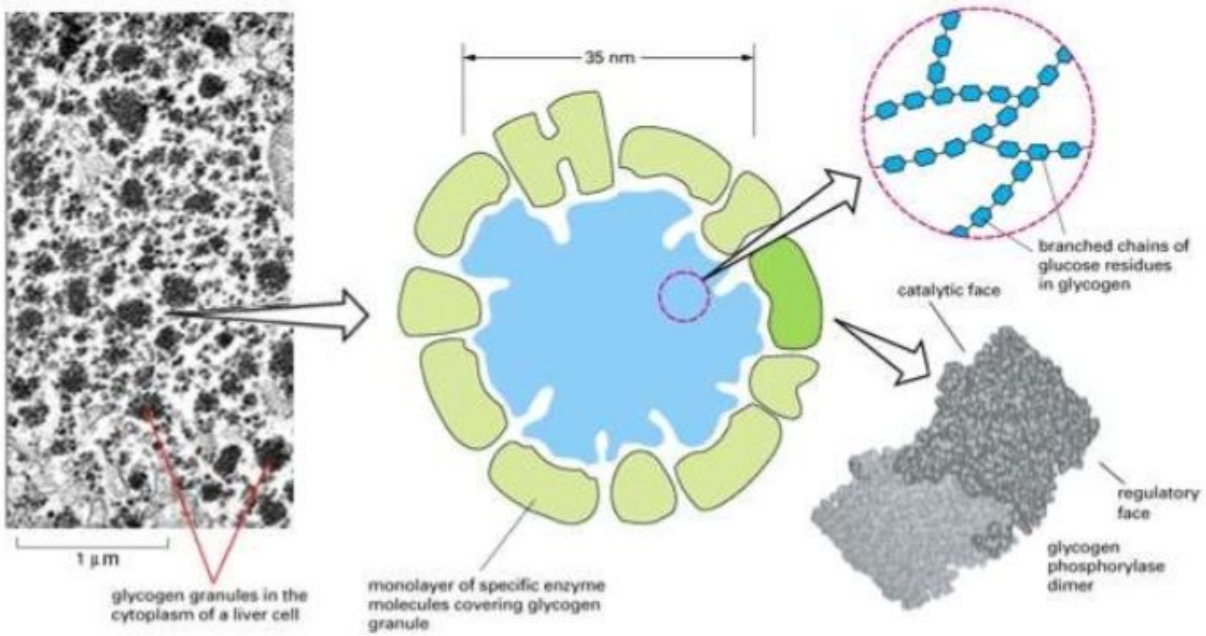
Figure 4.16 How Proteins Work (©2012 Garland Science)



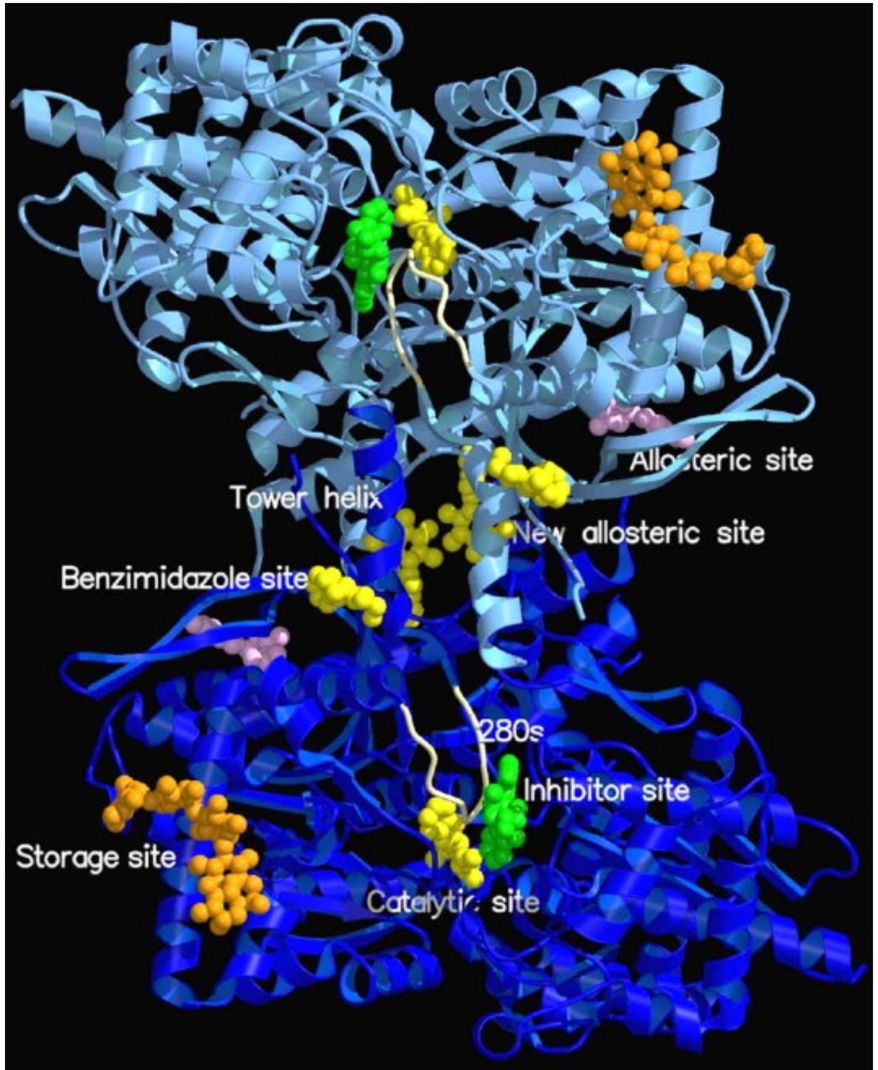


Find the partner

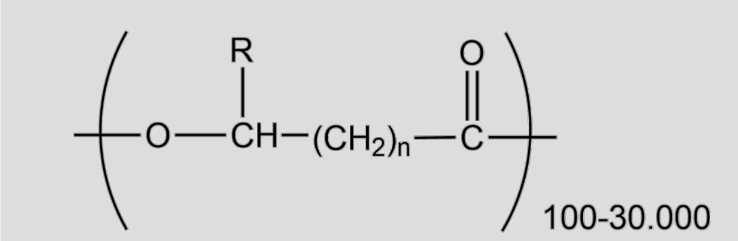


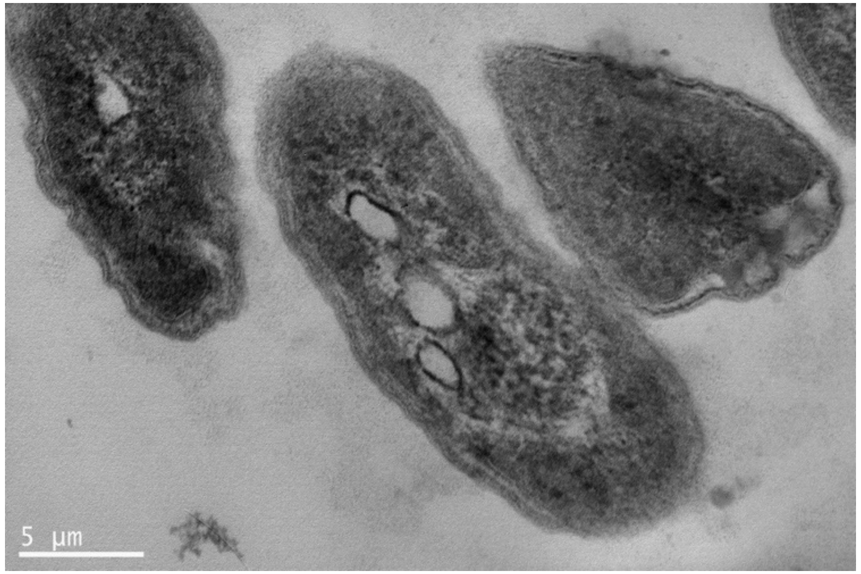


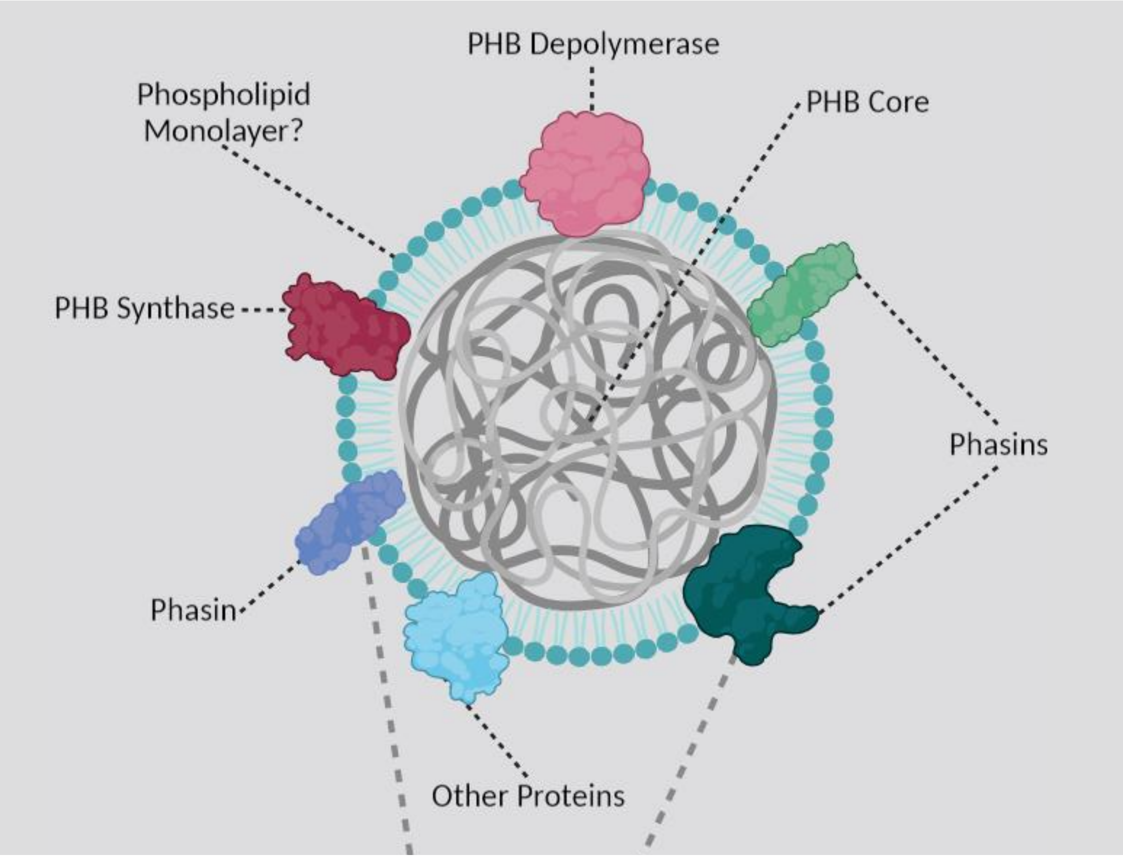
Regulatory proteins, enzymes needed for synthesis and degradation



Find the partner





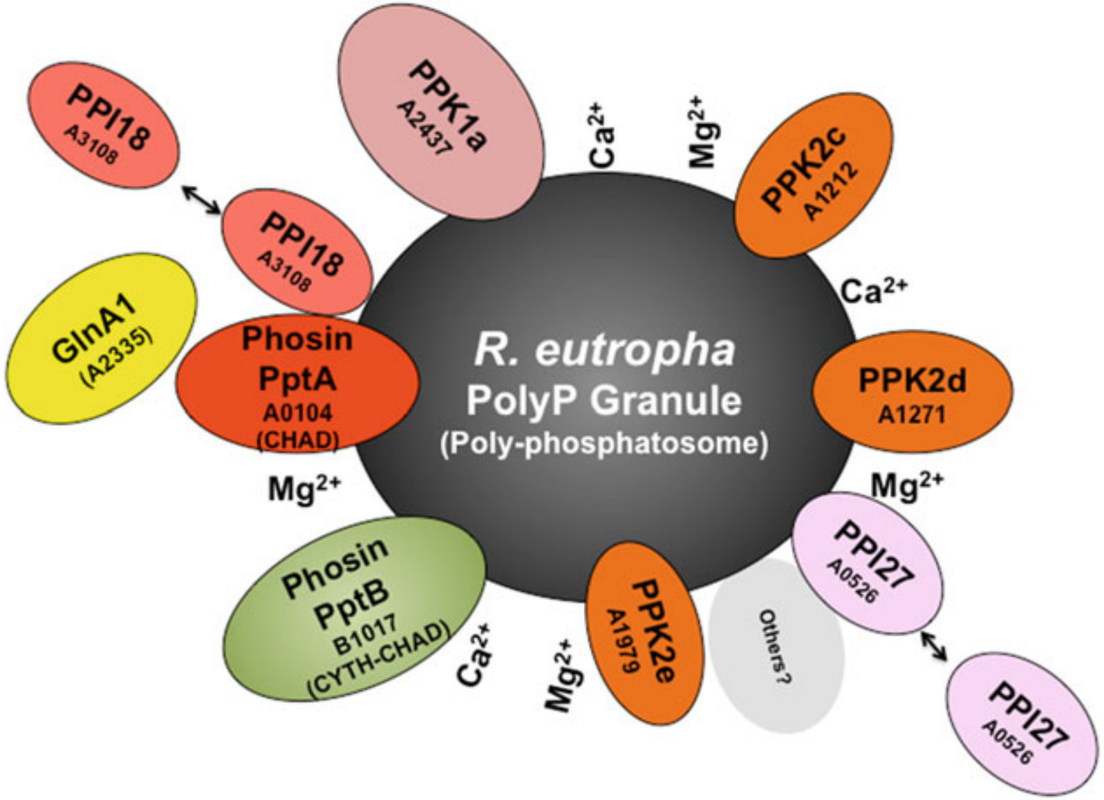


Similar properties to conventional plastics

- Accumulated in the form of granules in microorganisms, as a type of carbon storage
- PHB is the most studied PHA

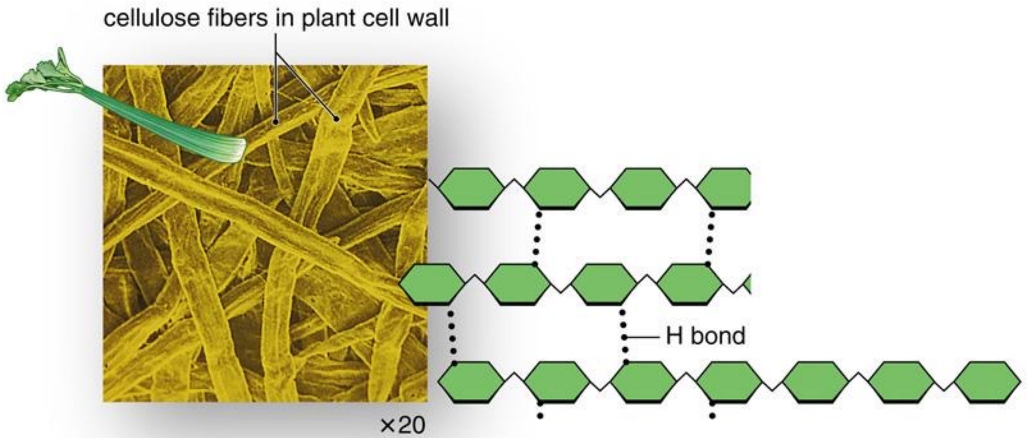
I. Kanavaki, A. Drakonaki, E.D. Geladas, A. Spyros, H. Xie, G. Tsiotis, Polyhydroxyalkanoate (PHA) Production in Pseudomonas sp. phDV1 Strain Grown on Phenol as Carbon Sources., Microorganisms. 9 (2021).

Find the partner

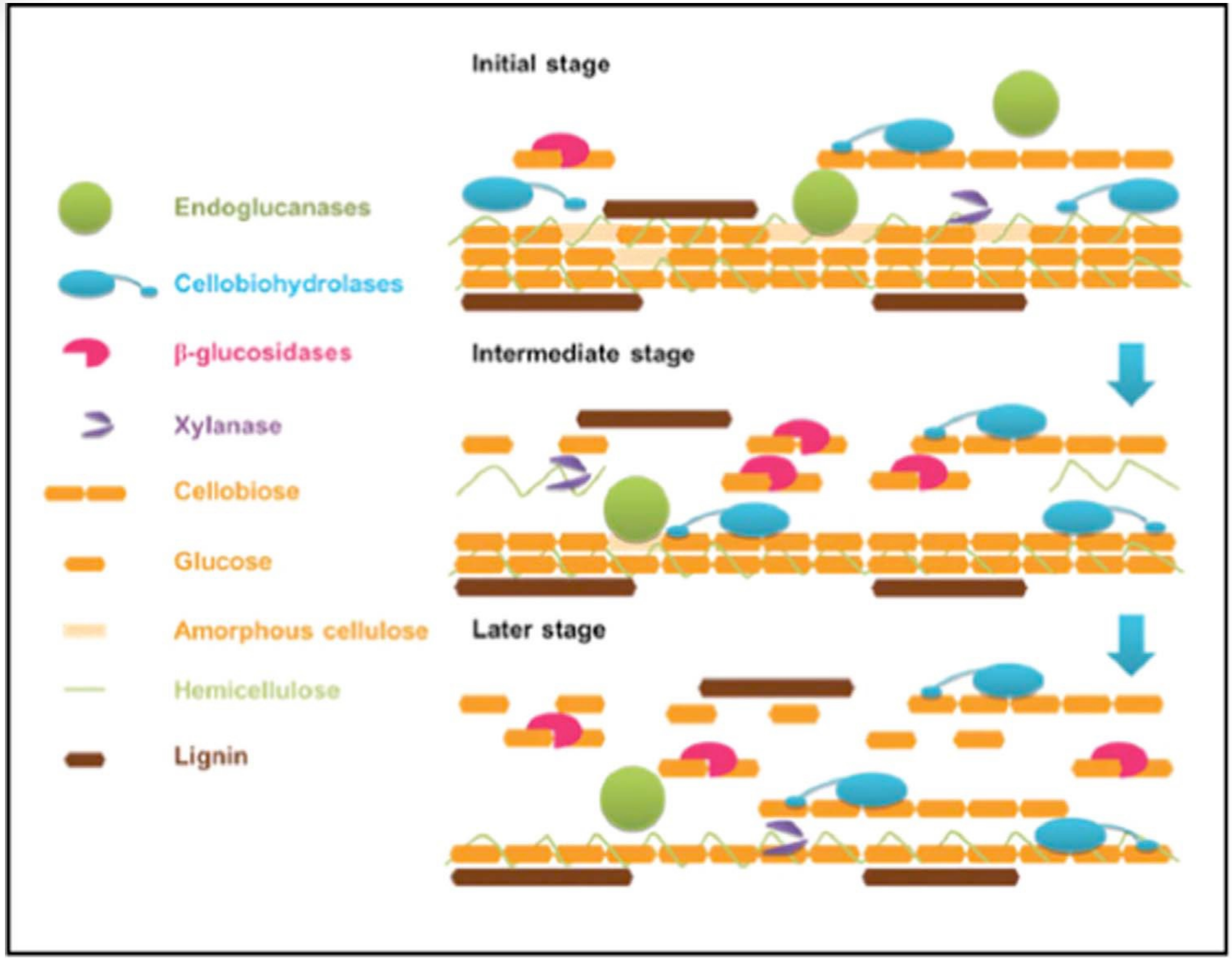


Polyphosphate (polyP) is a linear polymer of phosphate residues linked by energy-rich phospho-anhydride bonds.

cellulose



Cellulose structure



cellulase

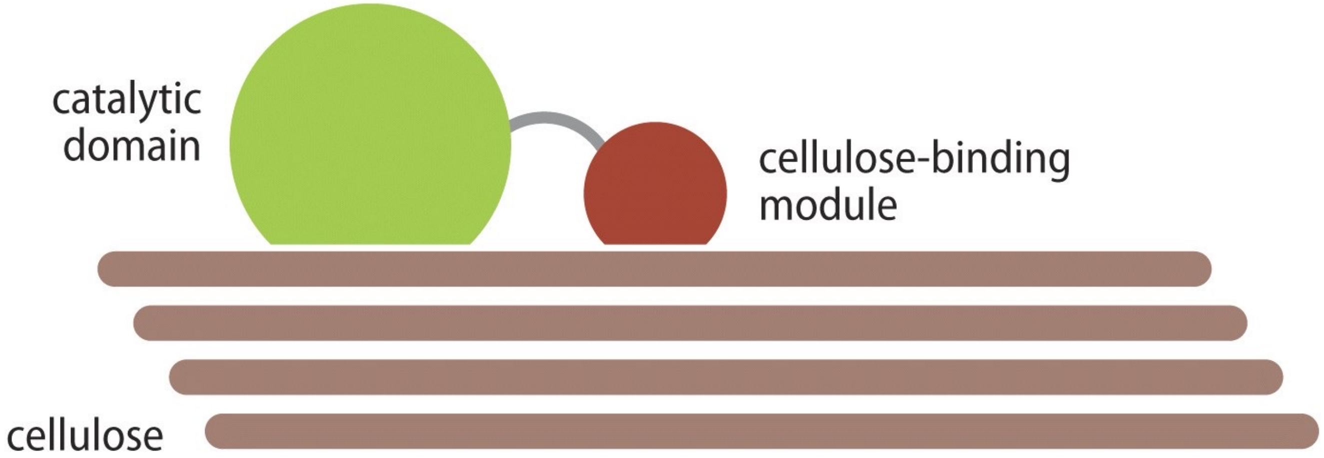
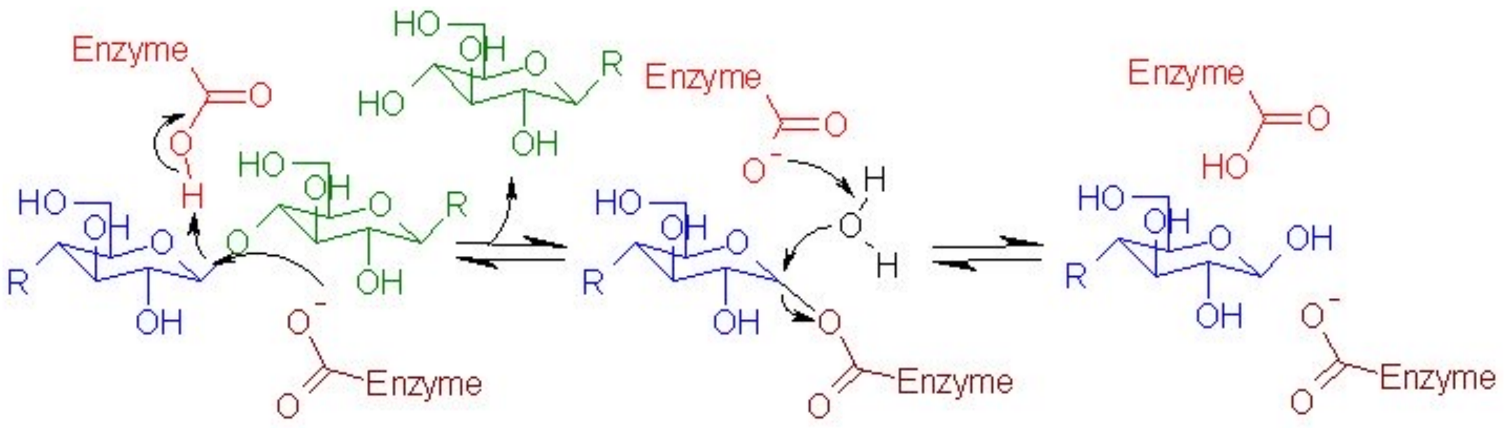
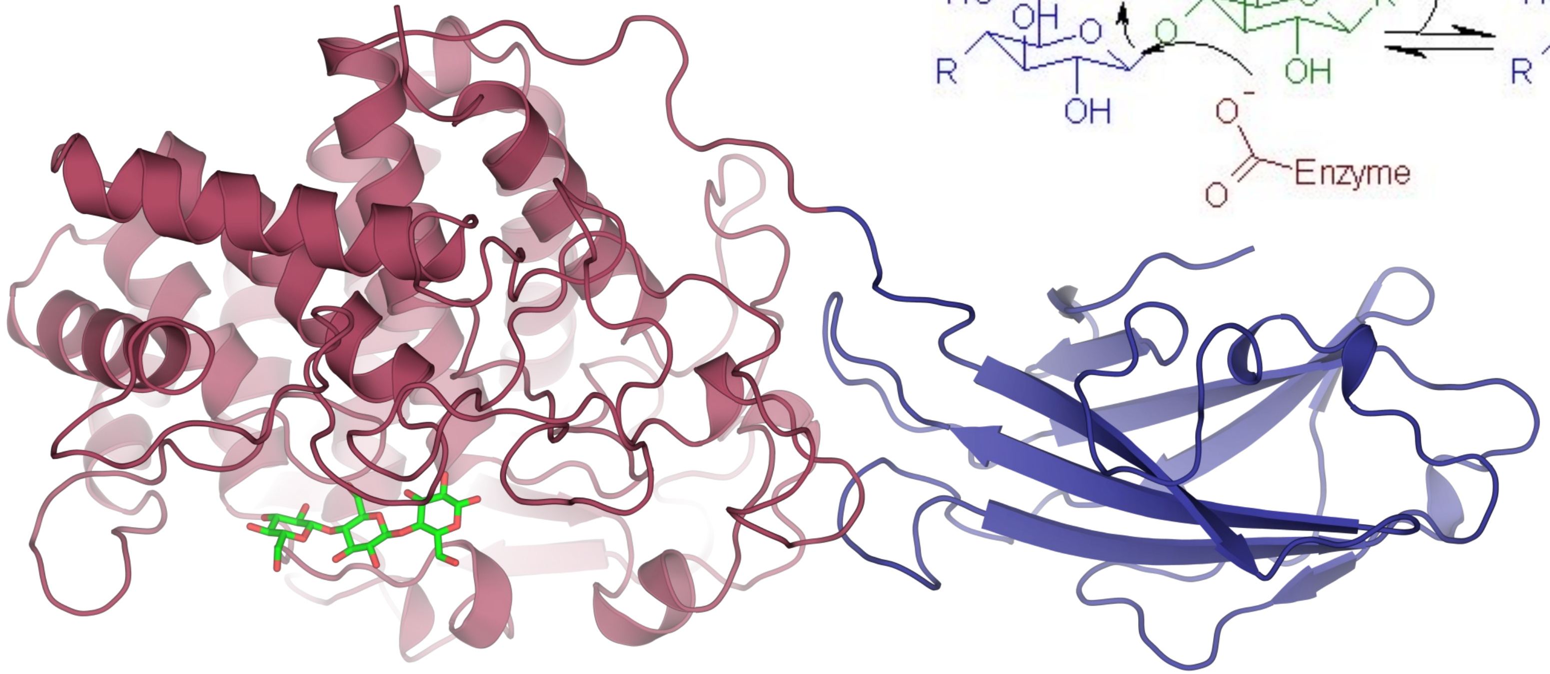


Figure 4.17 How Proteins Work (©2012 Garland Science)





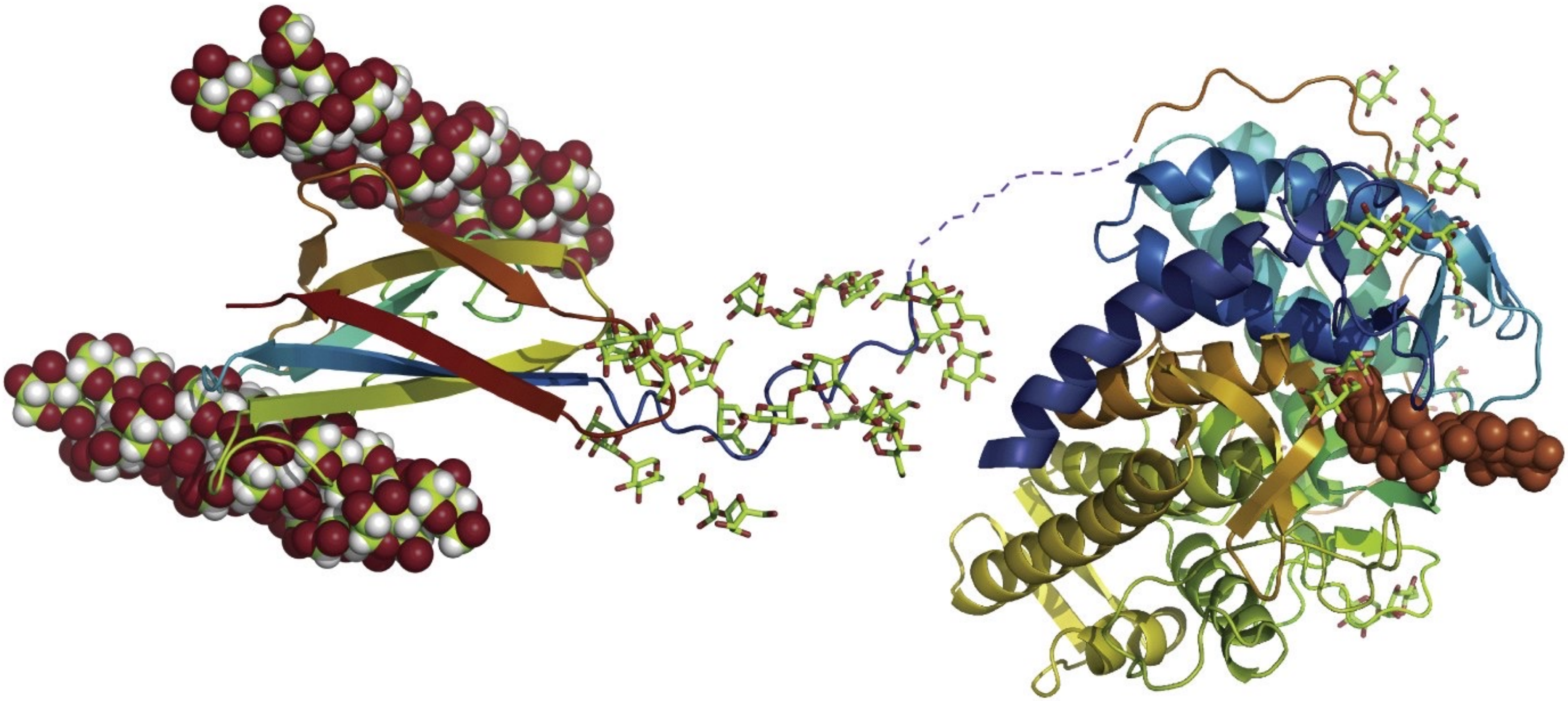


Figure 4.18 How Proteins Work (©2012 Garland Science)

Searching is faster in two dimensions

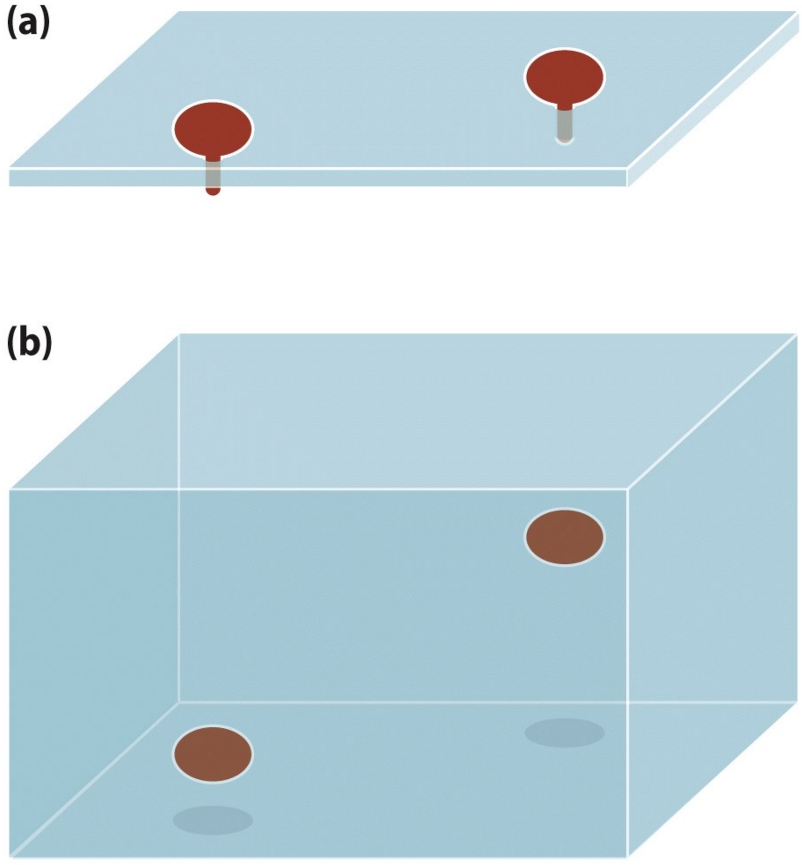
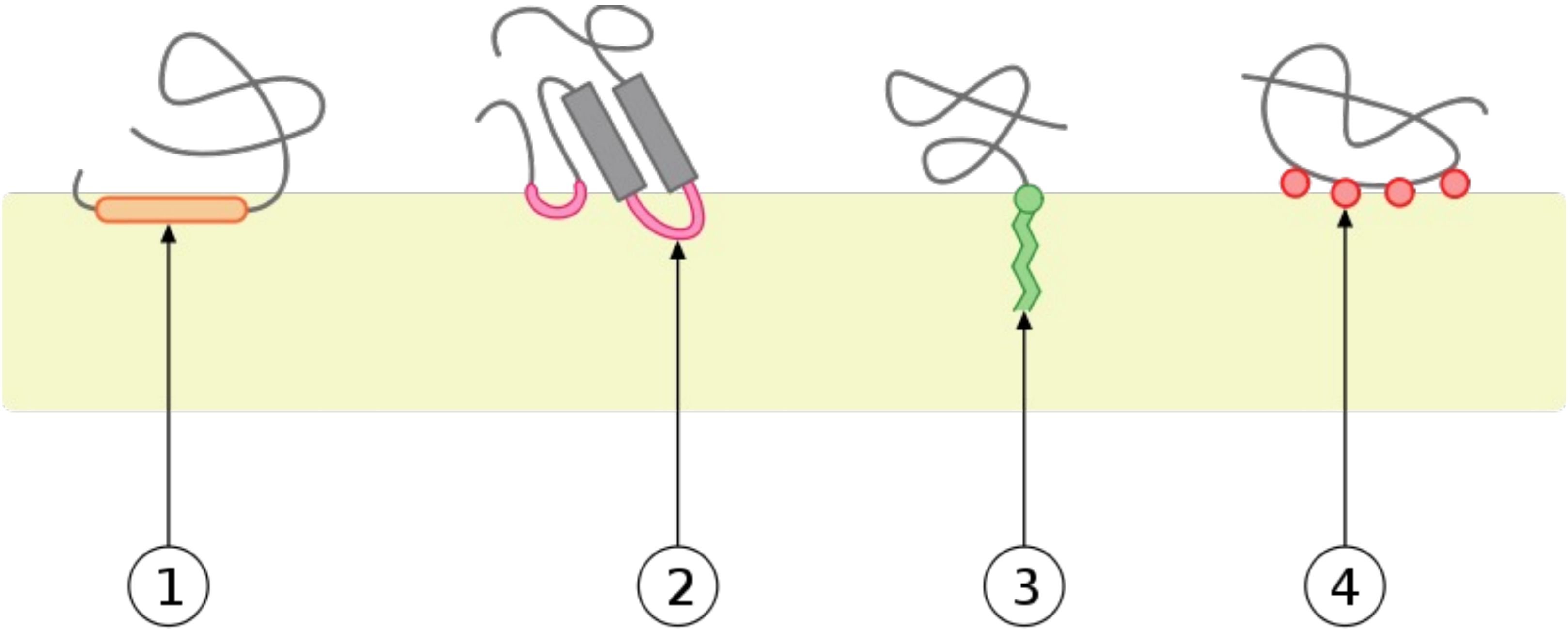
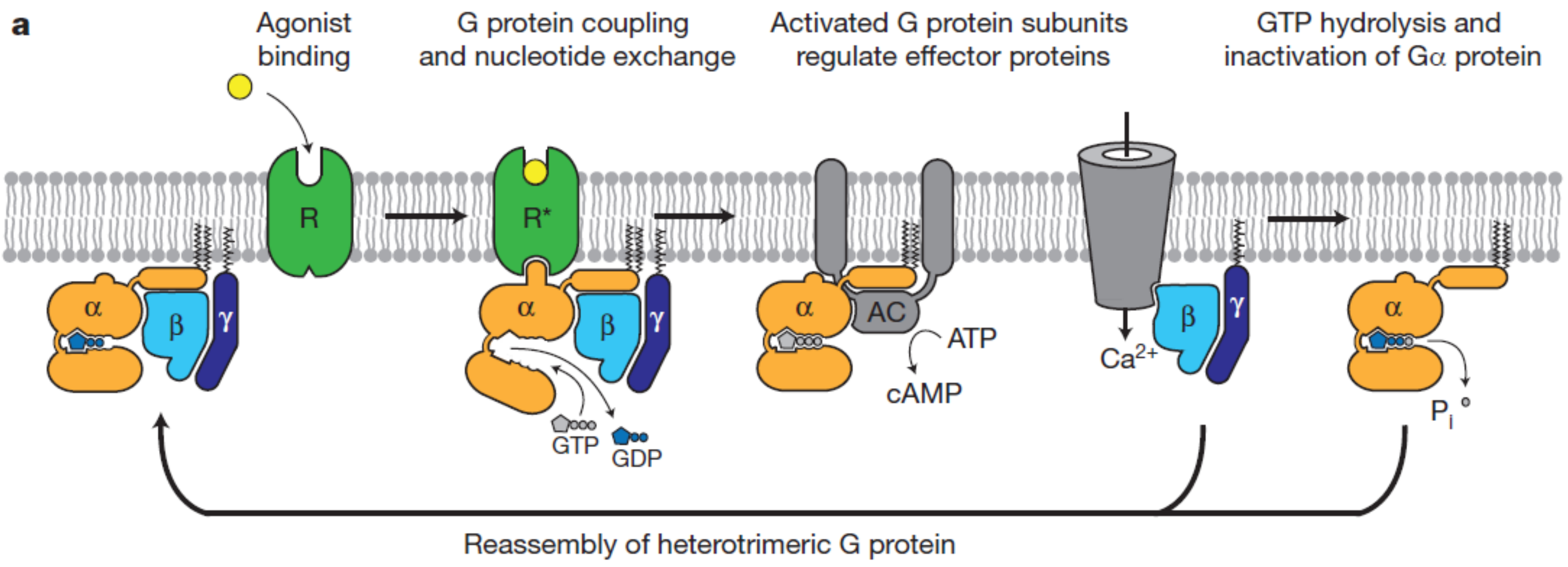


Figure 4.20 How Proteins Work (©2012 Garland Science)



Searching is faster in two dimensions



Searching is slightly faster again in one dimension

Oct-1 has two DNA-binding domains

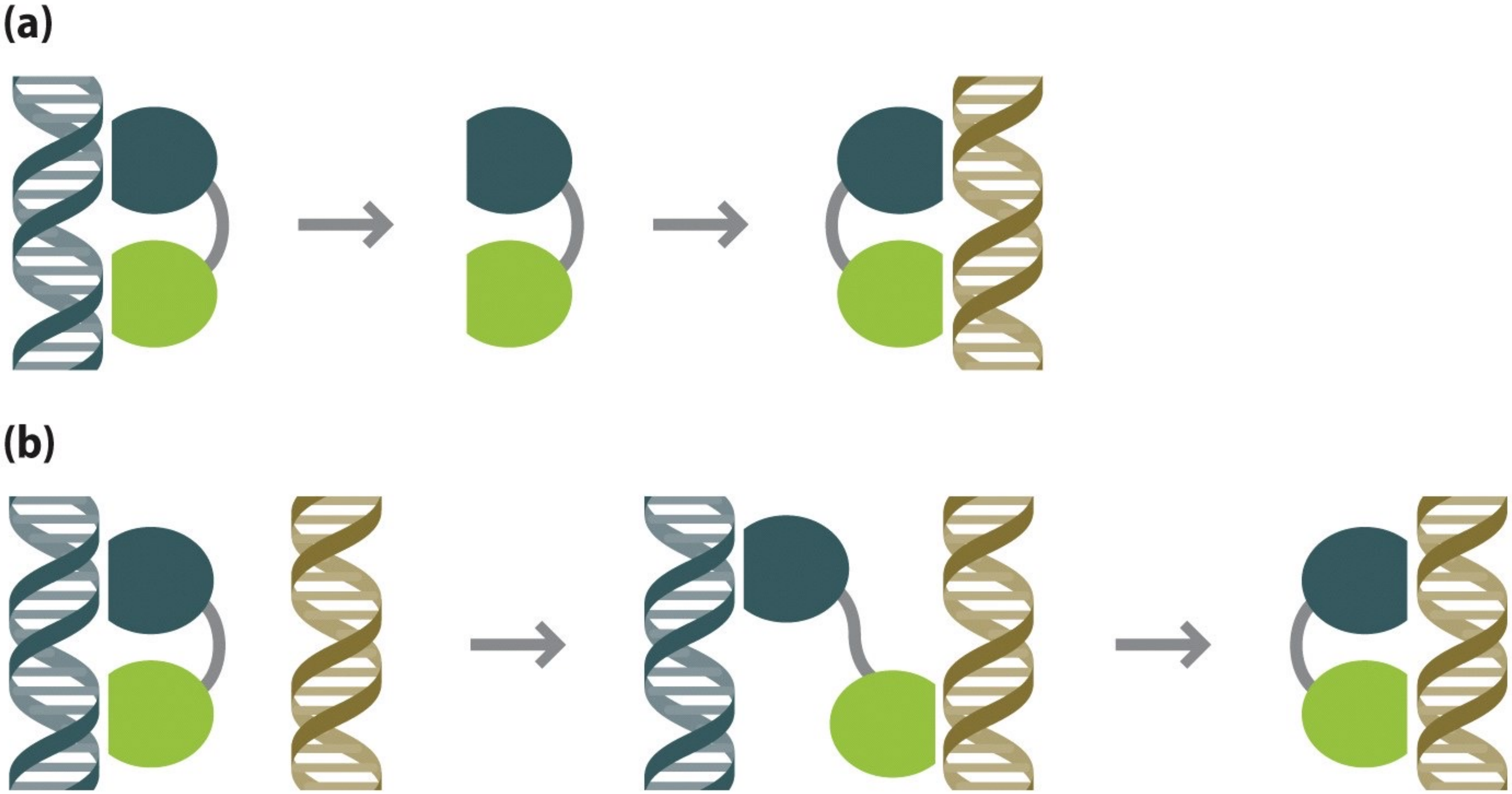
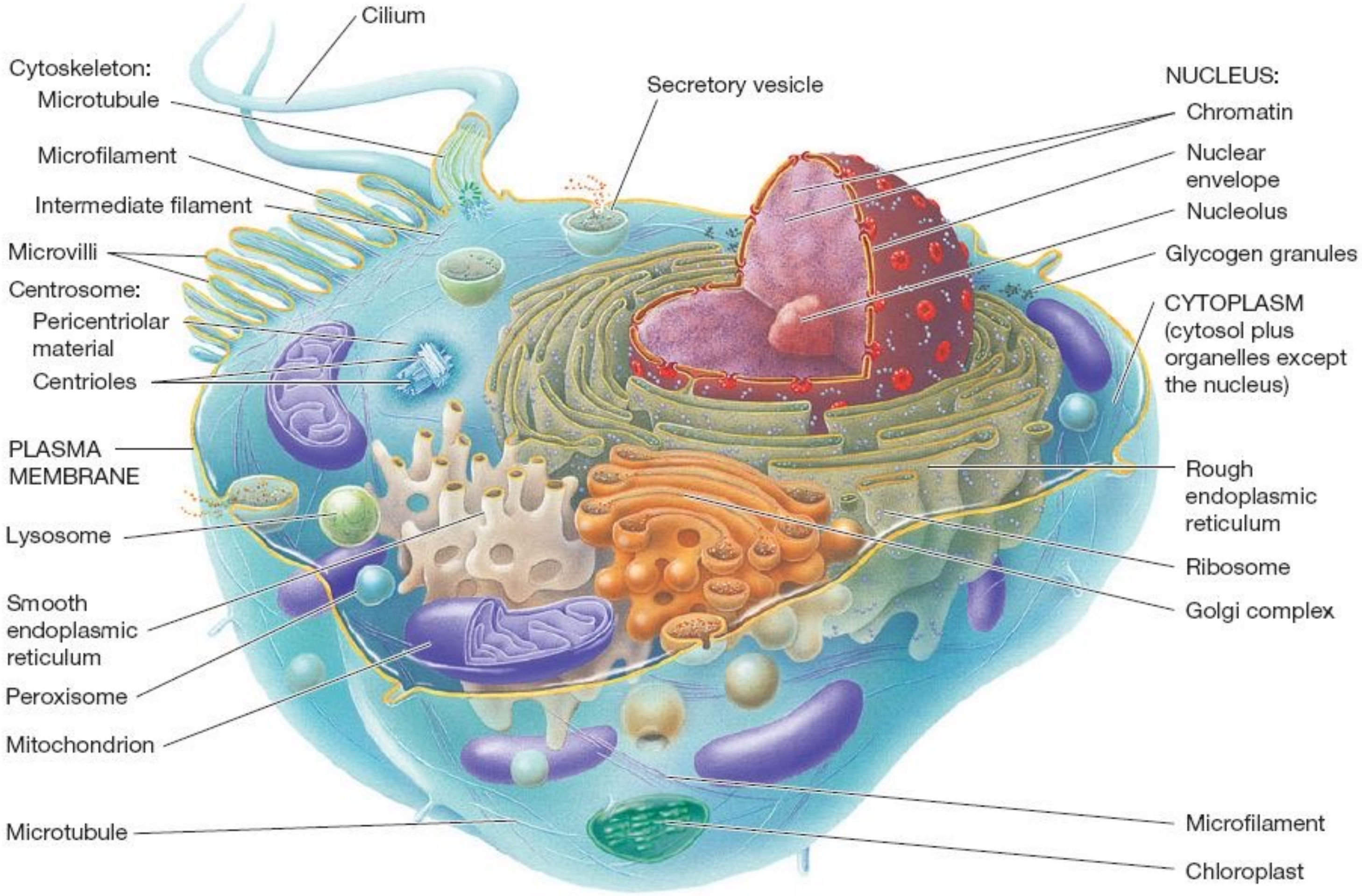


Figure 4.22 How Proteins Work (©2012 Garland Science)

Searching is faster in smaller compartments



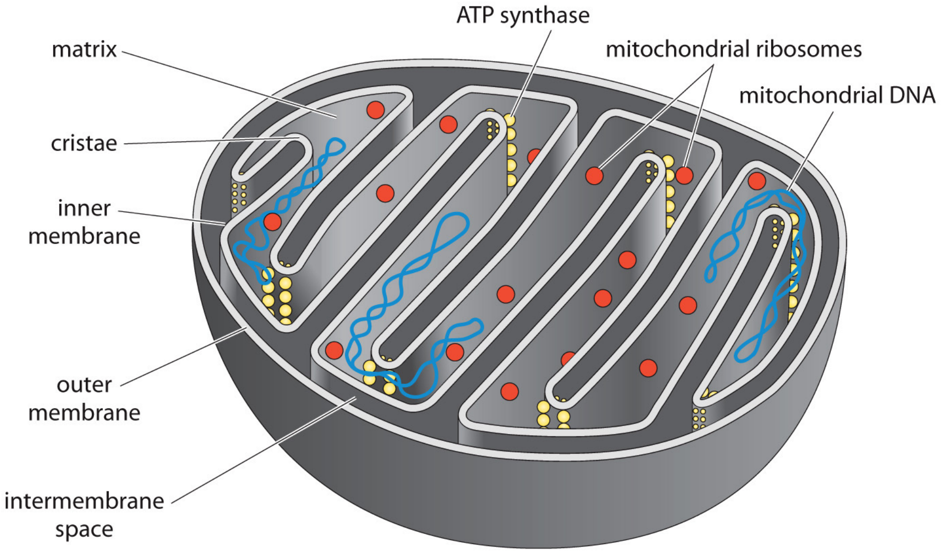


Figure 1.5.1b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Sticky arms

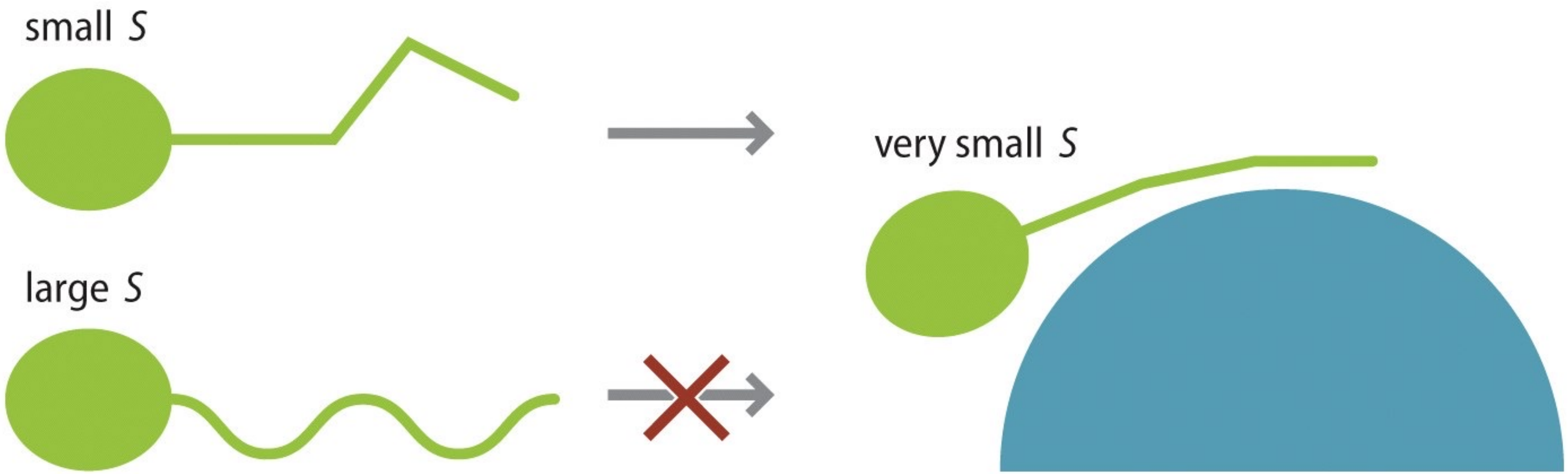
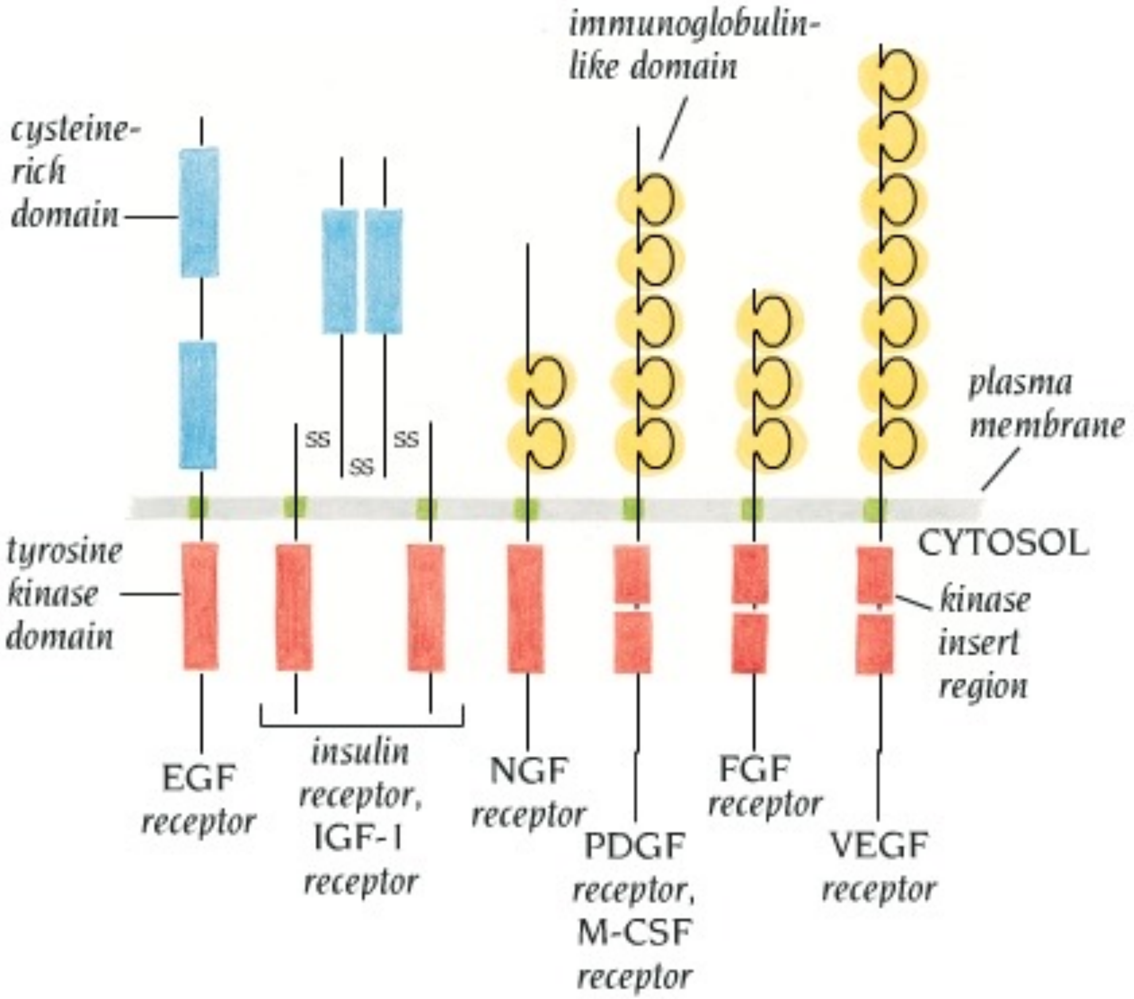
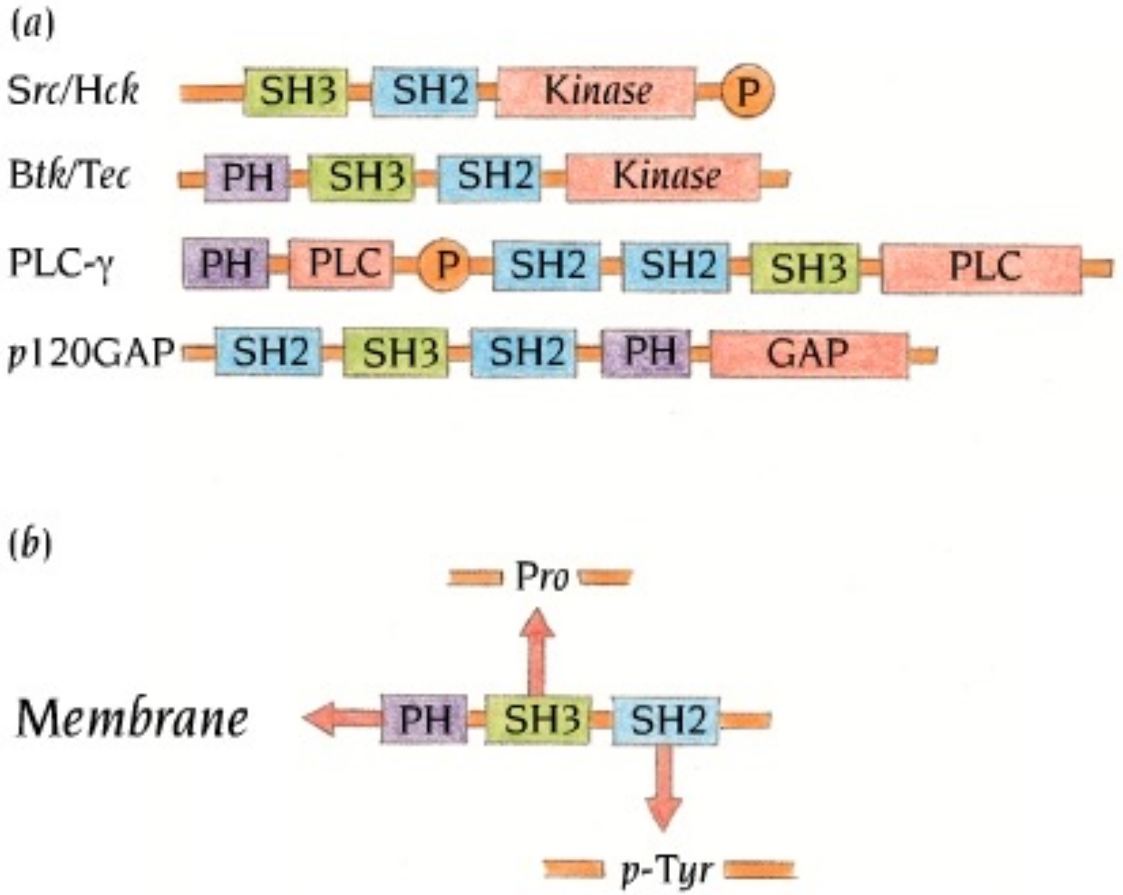


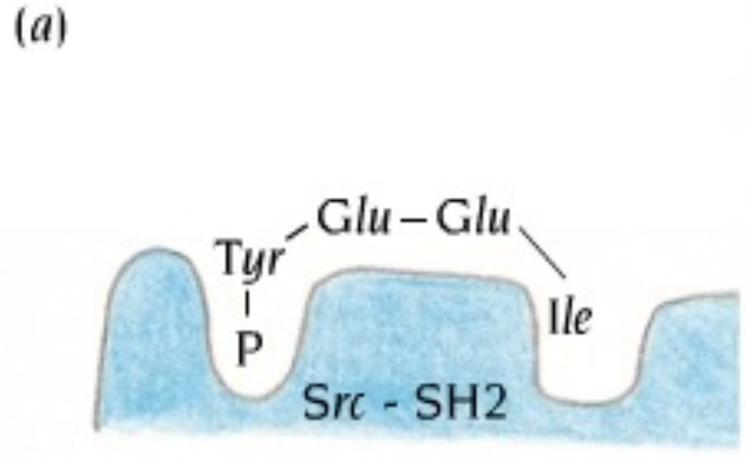
Figure 4.23 How Proteins Work (©2012 Garland Science)

tyrosine kinases receptor

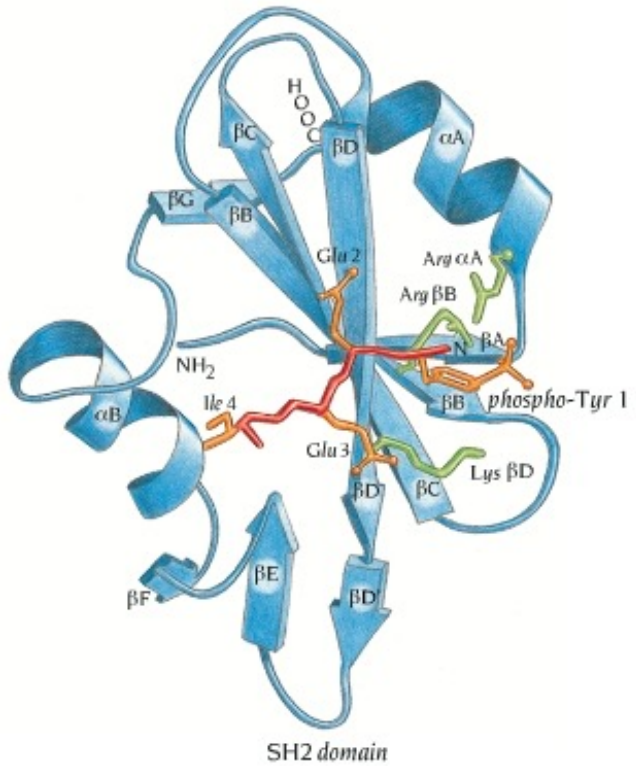




SH2 domains



(a)
Glu – Glu
Tyr
P
Ile
Src - SH2



HOOC
βC
βD
αA
βG
βB
Glu 2
Arg αA
Arg βB
βA
N
NH2
phospho-Tyr 1
αB
Ile 4
βB
Glu 3
Lys βD
βD'
βC
βE
βF
βD'
SH2 domain

SH3 domains

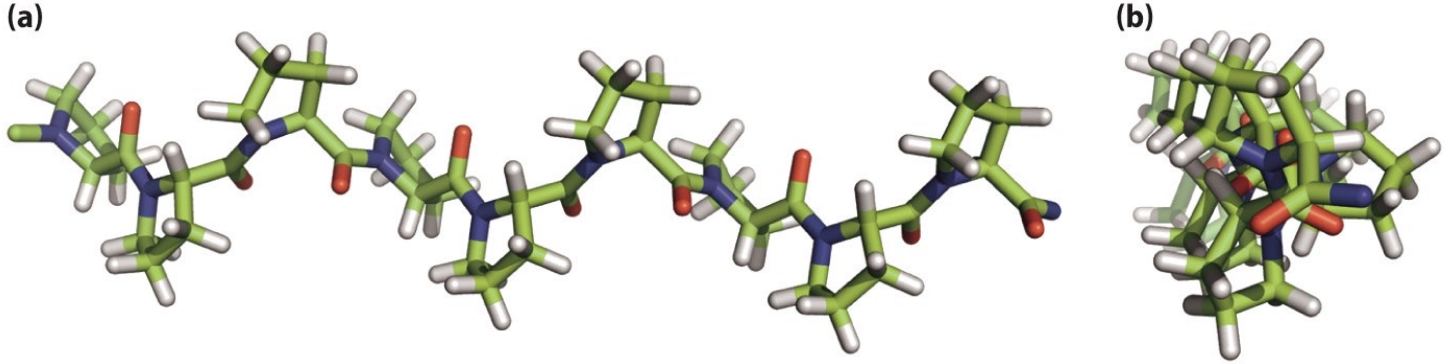
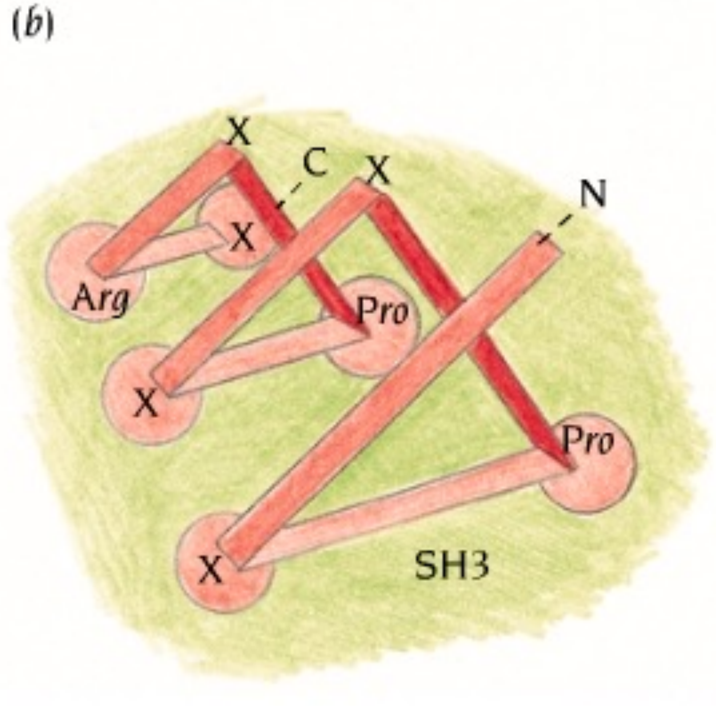


Figure 4.24 How Proteins Work (©2012 Garland Science)



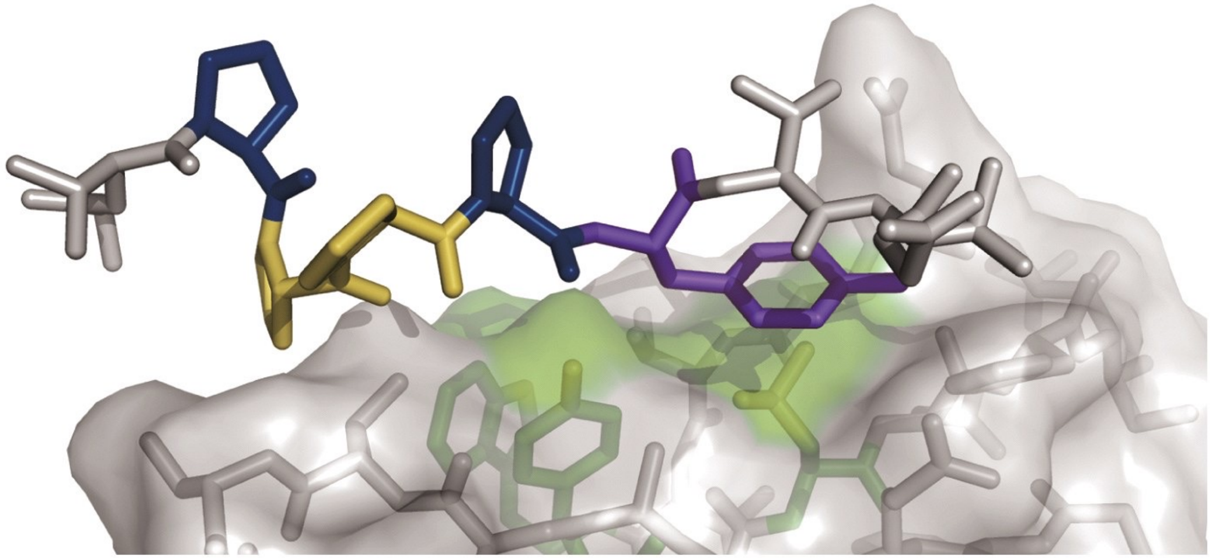


Figure 4.26 How Proteins Work (©2012 Garland Science)

SH3 domain

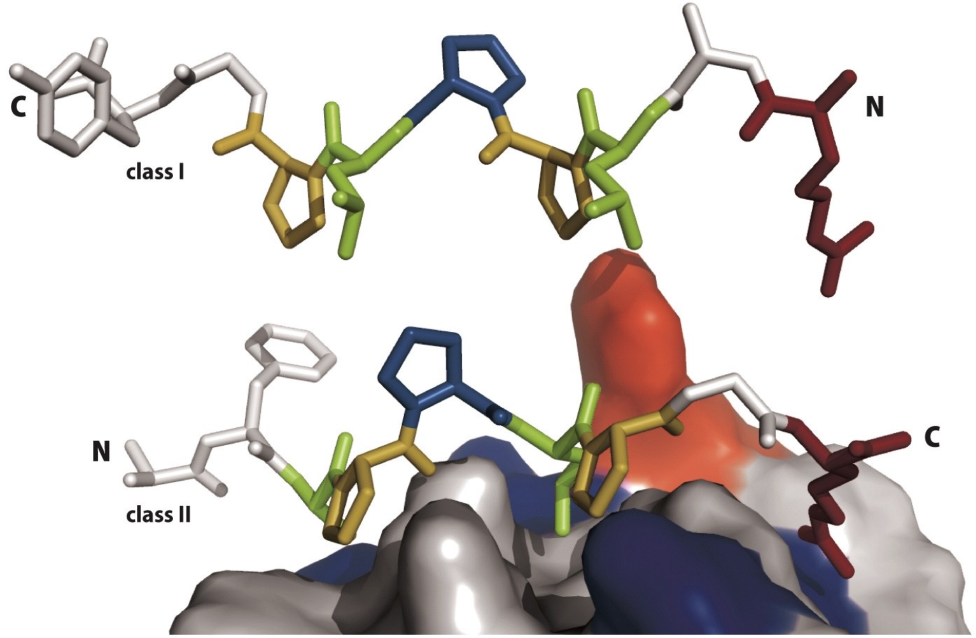
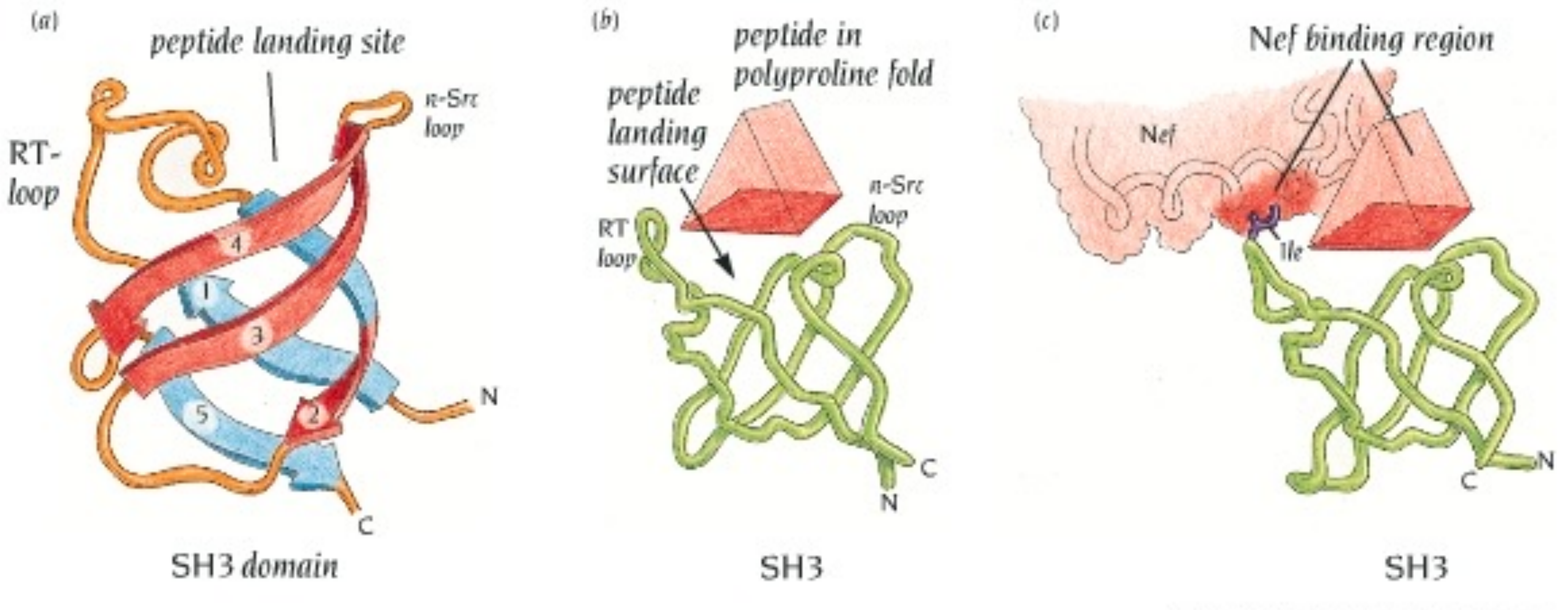
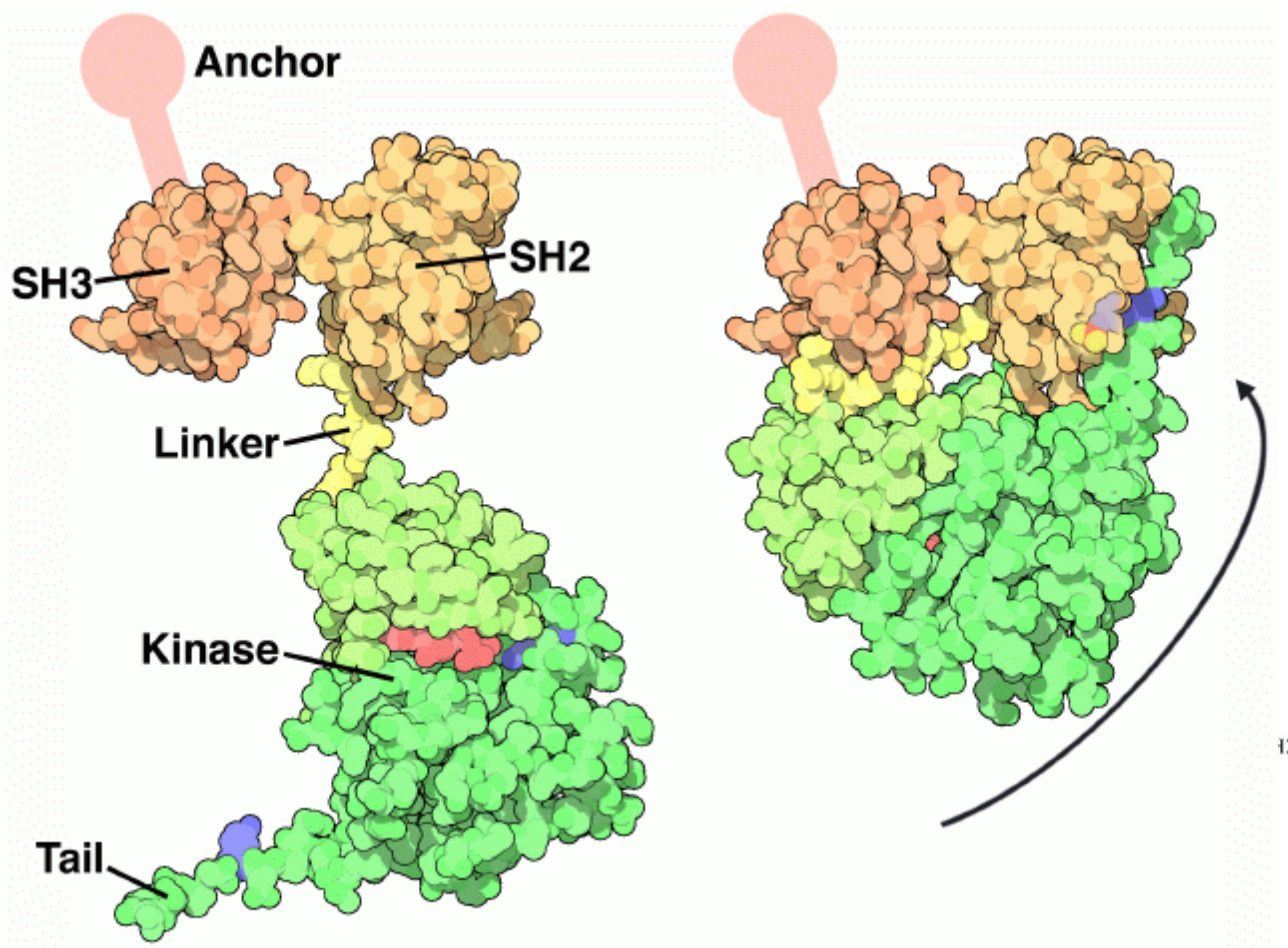
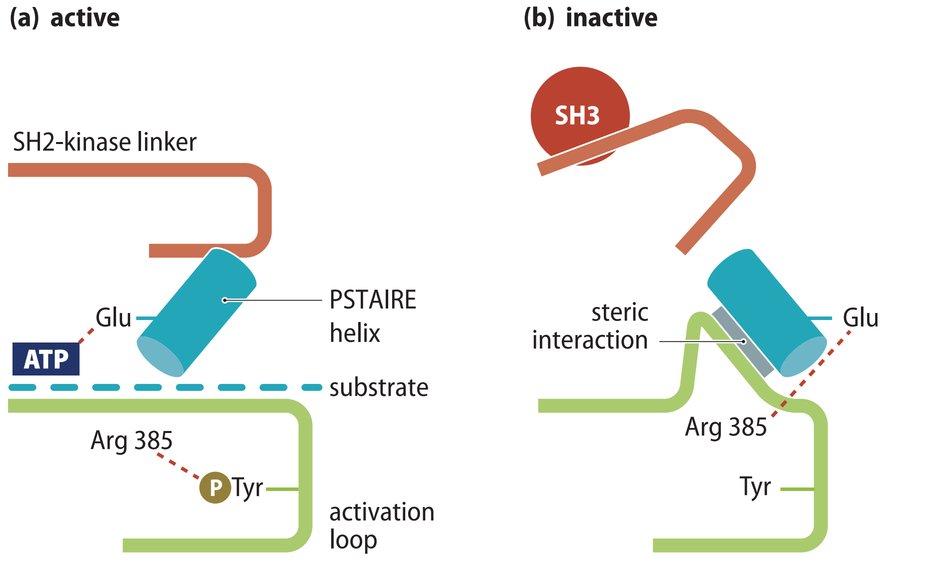


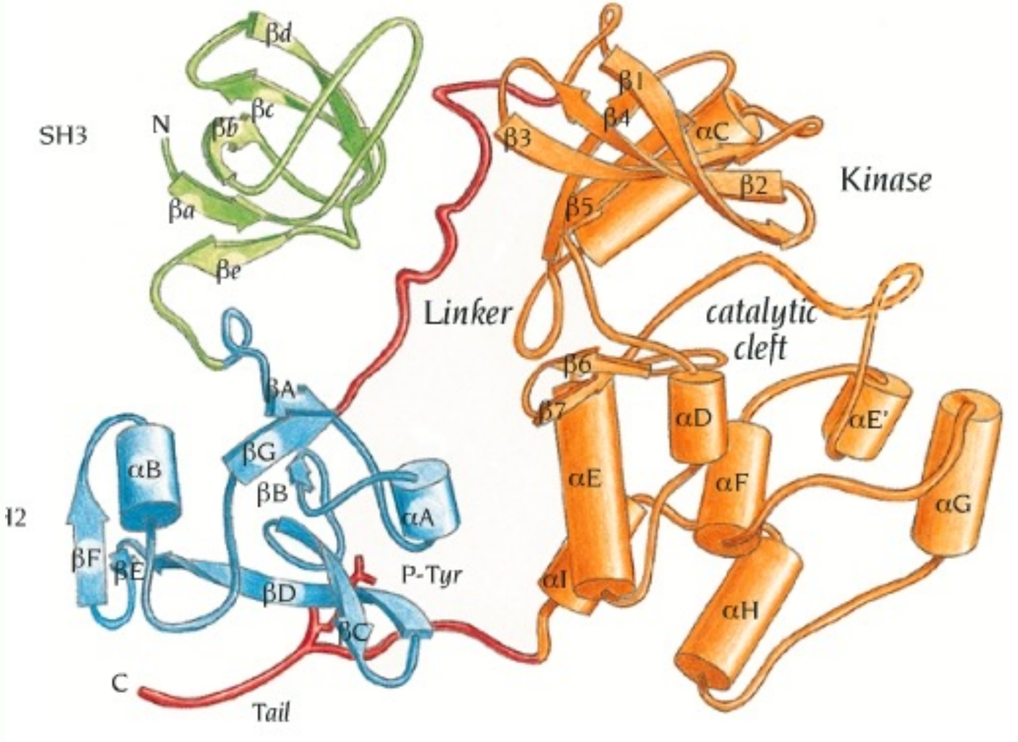
Figure 4.27 How Proteins Work (©2012 Garland Science)



Src tyrosine kinase







Post-translational modifications of proteins

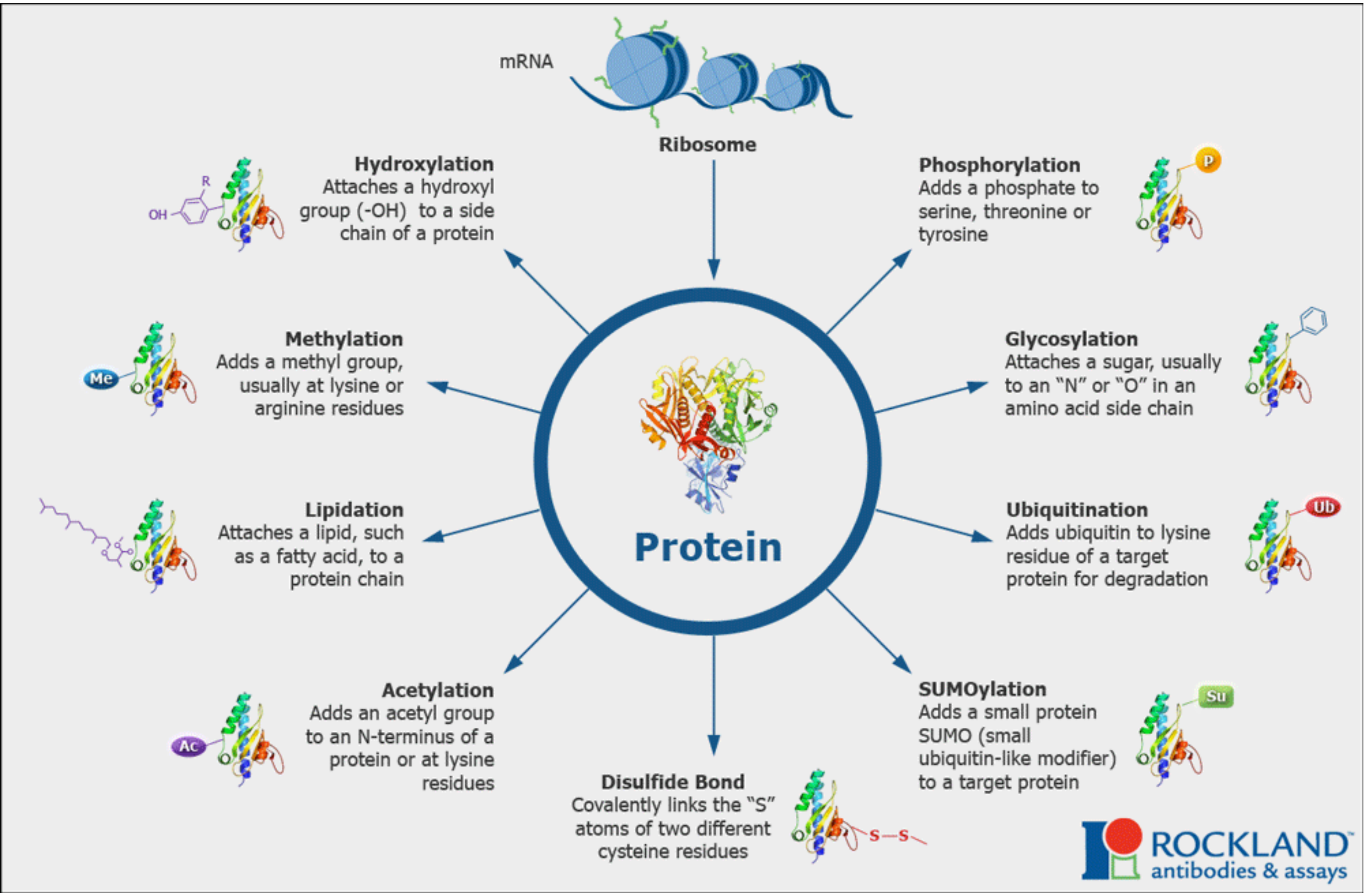


TABLE 4.3 A few covalent modifications of proteins

Modification	Site	Comments
Phosphorylation	Ser, Thr, Tyr	Regulates activity. Regulates assembly
Acetylation	Lys	Creates part of histone code in chromatin
Methylation	Lys	Creates part of histone code in chromatin
Methylation	Arg	
Lipid attachment	Cys, C terminus	Attaches protein to membrane
SUMOylation	Lys	Role in transport, transcriptional regulation, apoptosis
Ubiquitylation	Lys	Regulates transport and degradation, plus histone readout
Limited proteolysis		Activates proteases (zymogens) in extracellular location (e.g. chymotrypsin); activates hormones (e.g. insulin)
Attachment of *N*-acetylglucosamine	Ser, Thr	Regulates activity in enzymes involved in glucose metabolism
Glycosylation	Asn, Ser/Thr	Eukaryotes. Recognition, membrane protein folding
Hydroxylation	Pro	Collagen: to facilitate triple helix formation. Irreversible
ADP ribosylation	Arg, Glu, Asp	As part of signaling, DNA repair and apoptosis
Sulfation	Tyr	Irreversible and probably required for activity
Carboxylation	Glu	Creates γ-carboxyglutamate (Gla), a calcium ligand

Table 4.3 How Proteins Work (©2012 Garland Science)

Phosphorylation

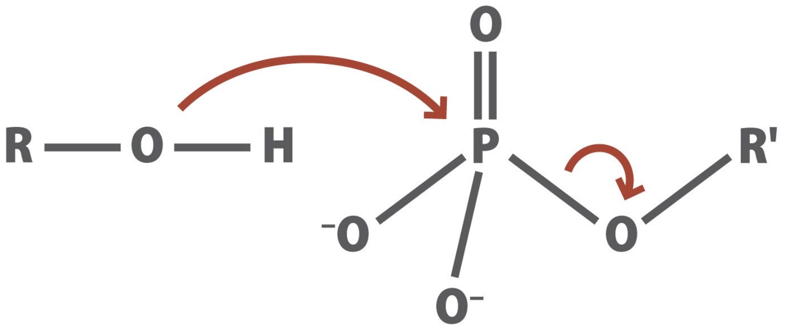


Figure 4.33 How Proteins Work (©2012 Garland Science)

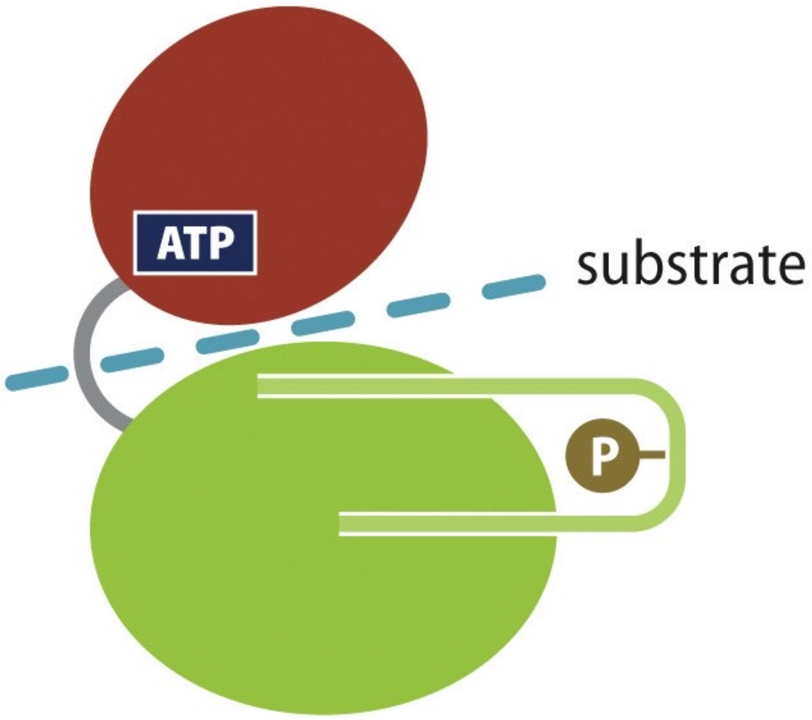


Figure 4.34 How Proteins Work (©2012 Garland Science)

deactivation and activation of kinases

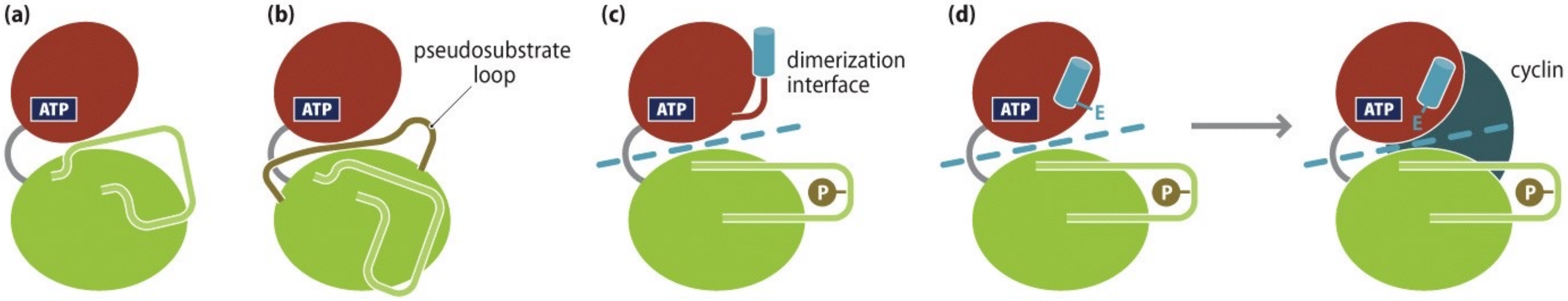
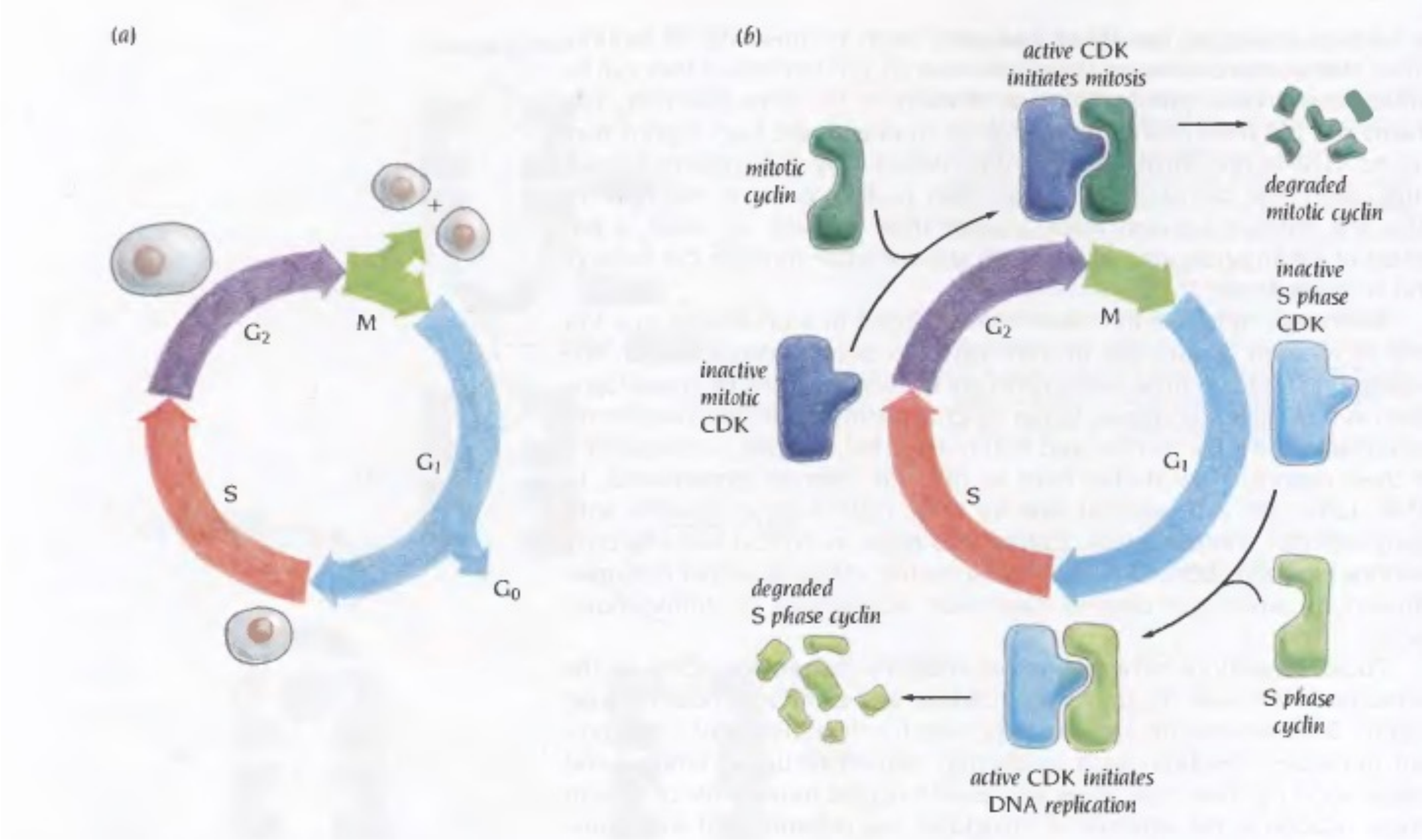


Figure 4.35 How Proteins Work (©2012 Garland Science)

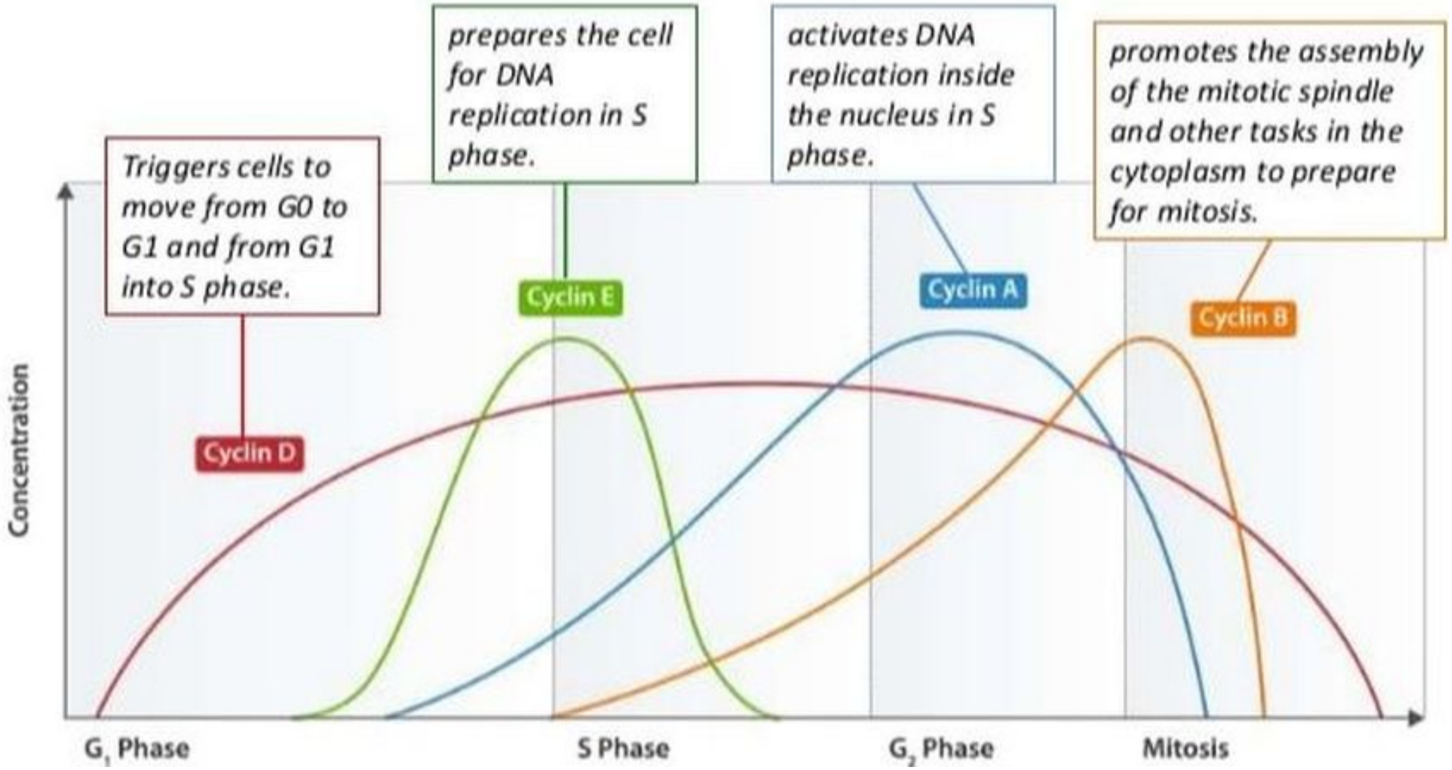
proline-serine-threonine-alanine-isoleucine-arginine-glutamate (PSTAIRE)

cell cycle



(a)
G2
M
G1
S
G0
(b)
active CDK
initiates mitosis
mitotic
cyclin
degraded
mitotic cyclin
inactive
S phase
CDK
inactive
mitotic
CDK
M
G2
G1
S
degraded
S phase cyclin
S phase
cyclin
active CDK initiates
DNA replication

cell cycle



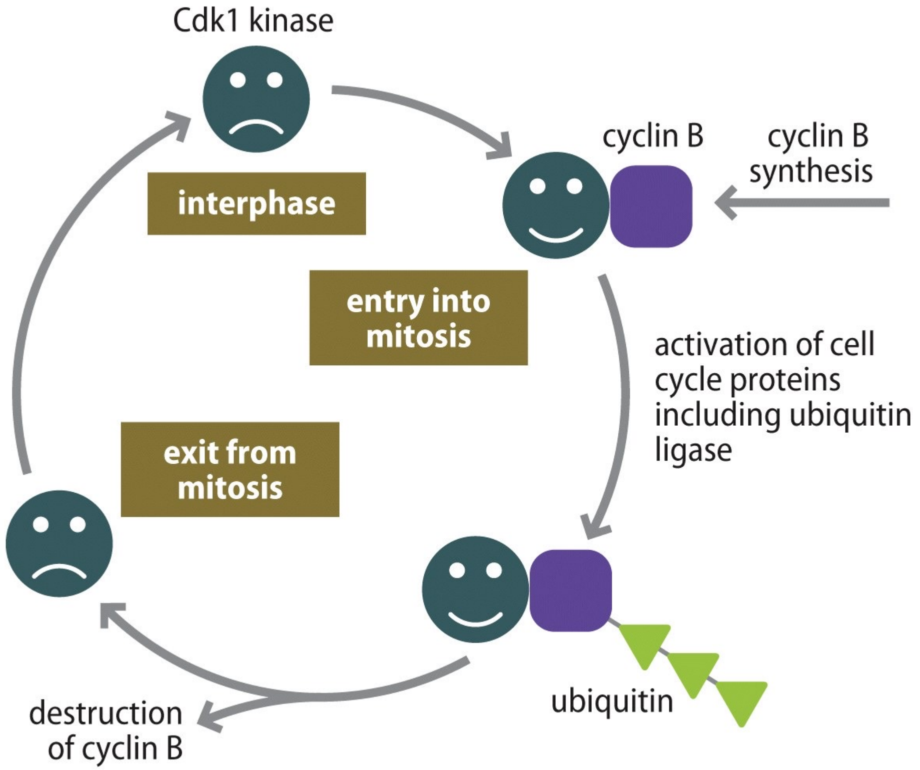
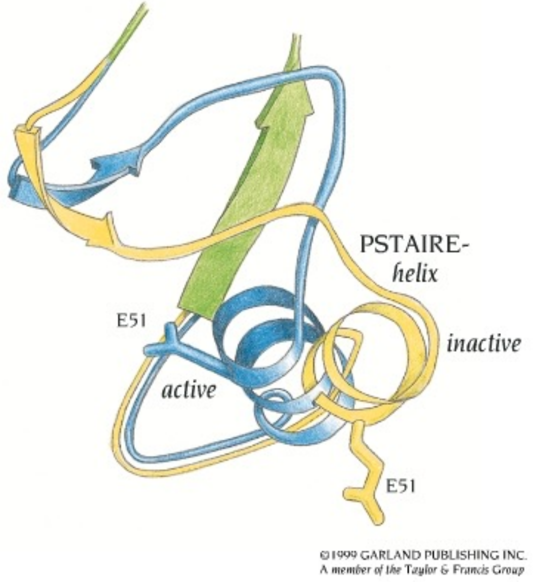
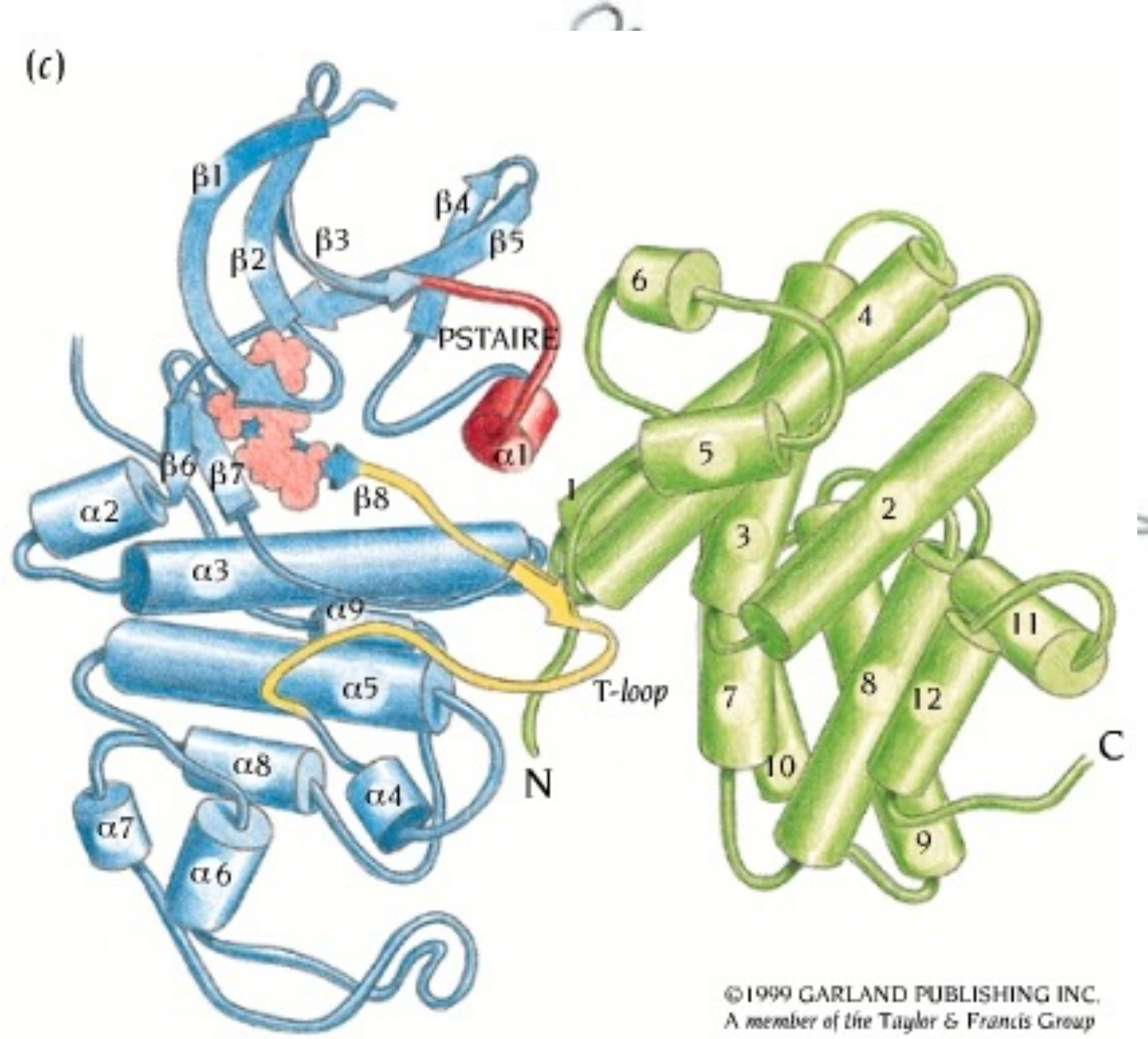


Figure 4.9.3 How Proteins Work (©2012 Garland Science)

CD kinase


PSTAIRE-
helix
E51
inactive
active
E51
©1999 GARLAND PUBLISHING INC.
A member of the Taylor & Francis Group


(c)
β1
β2
β3
β4
β5
PSTAIRE
α1
β6
β7
β8
α2
α3
α9
α5
α8
α4
α7
α6
T-loop
N
C
6
4
5
1
3
2
11
7
8
12
10
9
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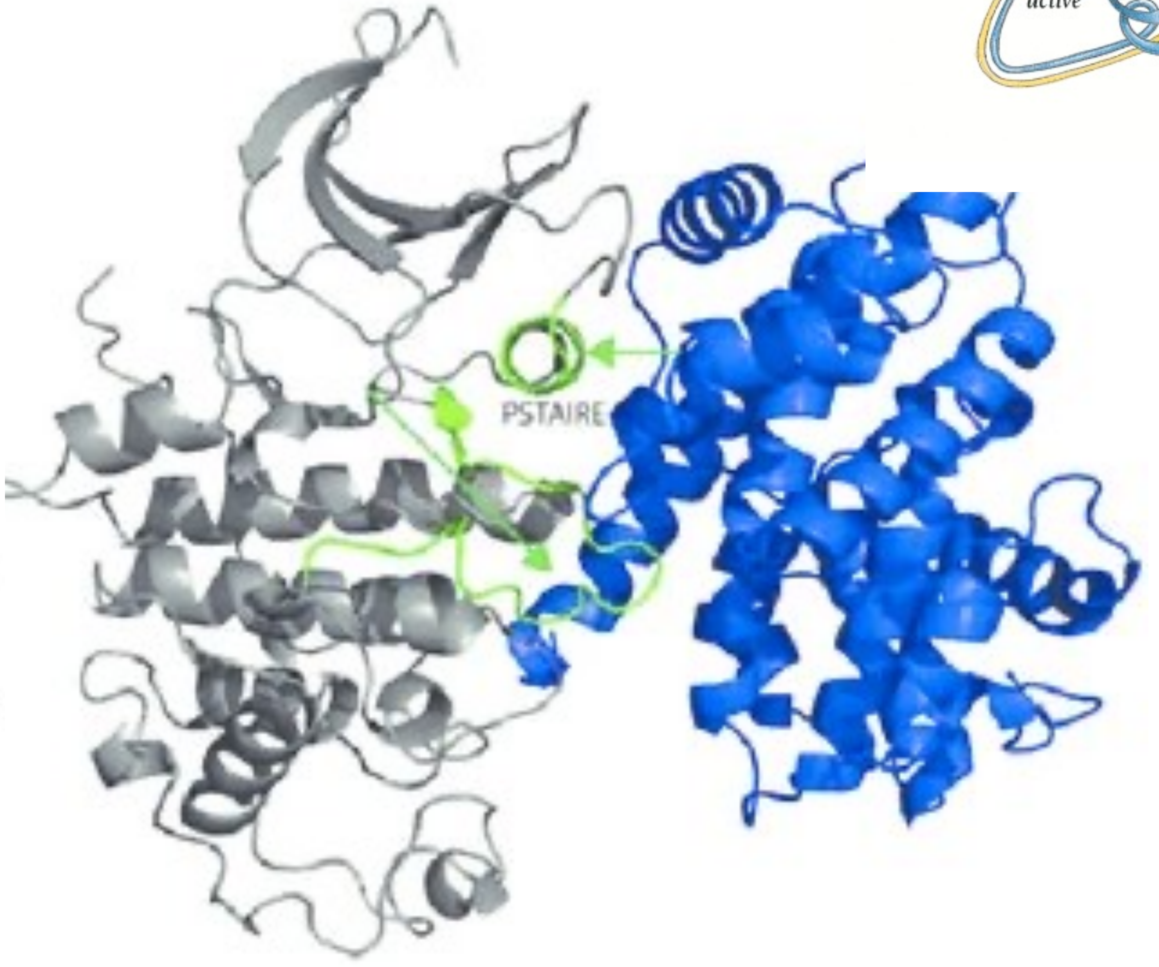
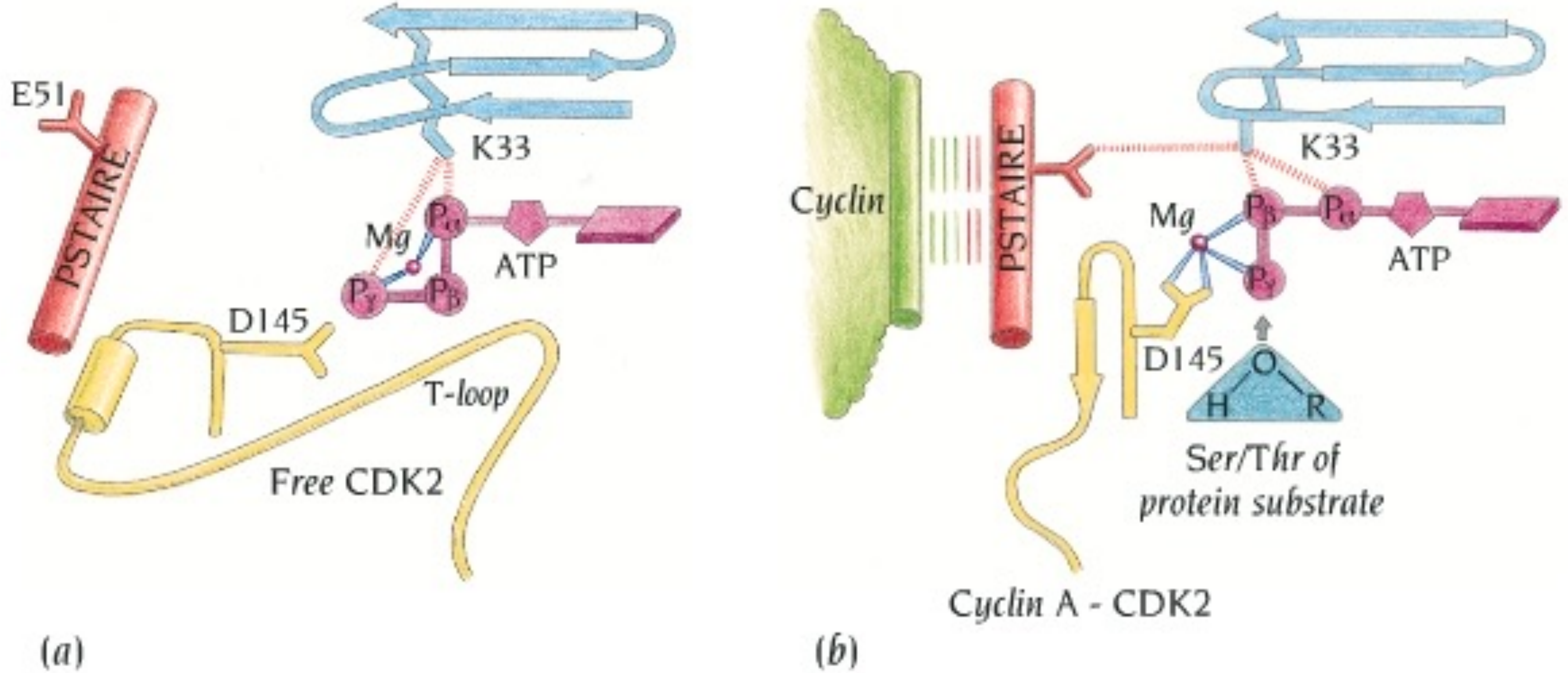
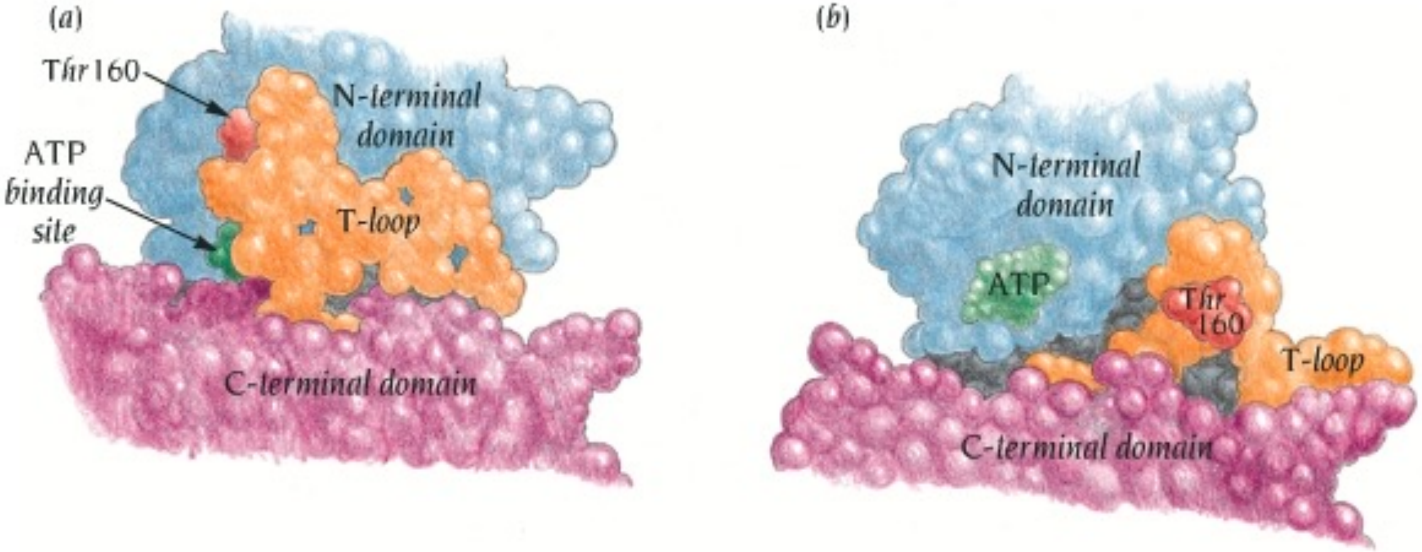

PSTAIRE

CD kinase





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ubiquitylation

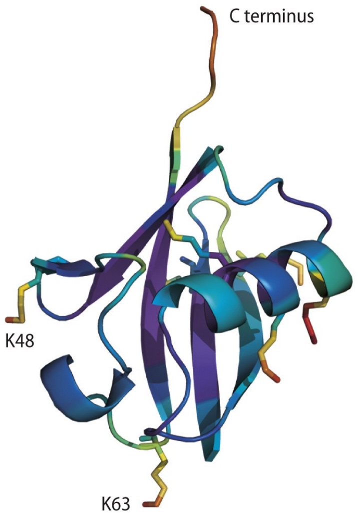


TABLE 4.4 Dependence of half-life of cytoplasmic proteins on their N-terminal residue	
N-terminal residue	Half-life
Met, Gly, Ala, Ser, Thr, Val	> 20 h
Ile, Glu	30 min
Tyr, Gln	10 min
Pro	7 min
Leu, Phe, Asp, Lys	3 min
Arg	2 min

Table 4.4 How Proteins Work (©2012 Garland Science)

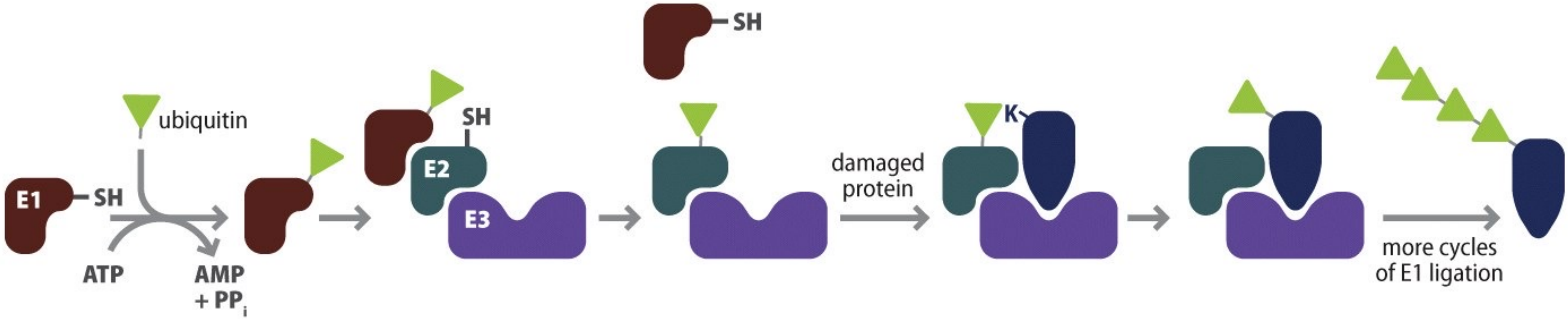
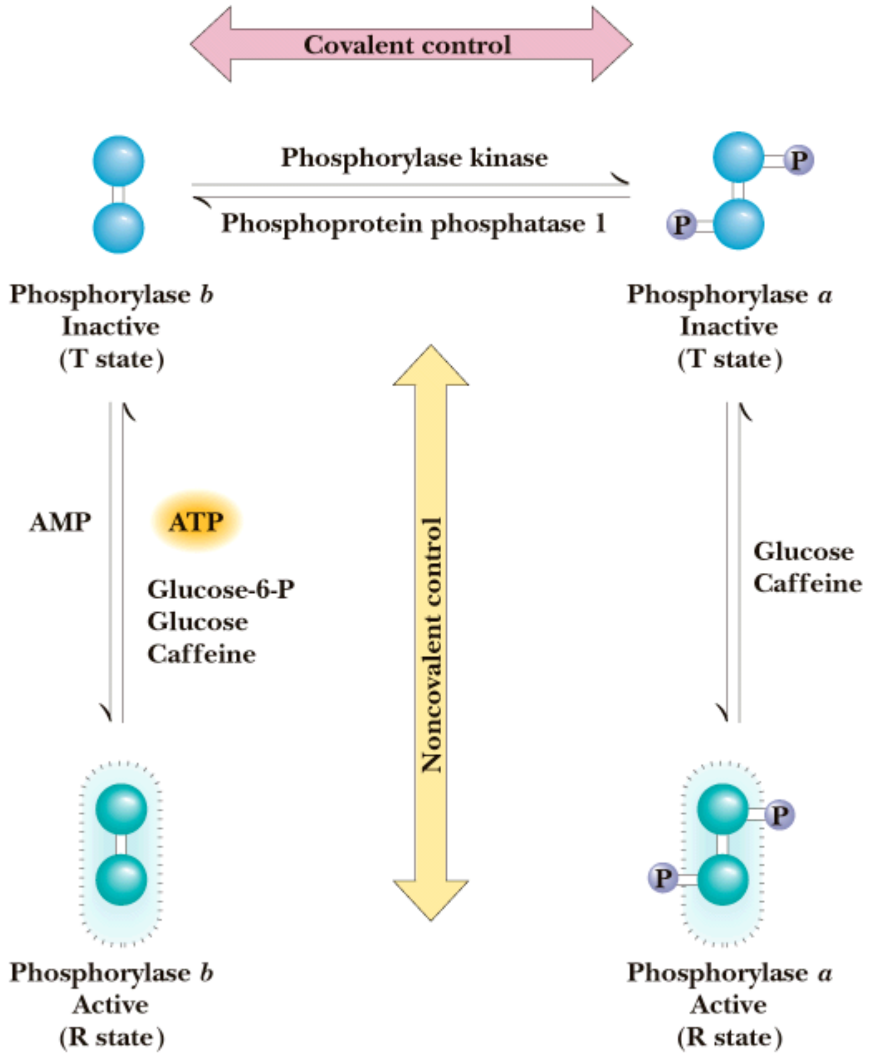
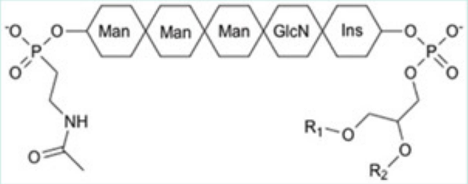
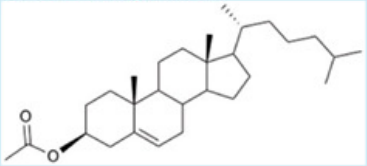
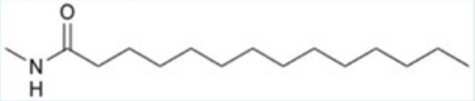
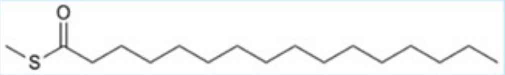
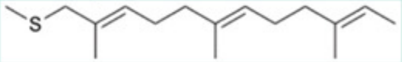
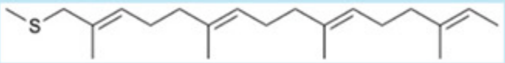


Figure 4.9.2 How Proteins Work (©2012 Garland Science)

Phosphatases

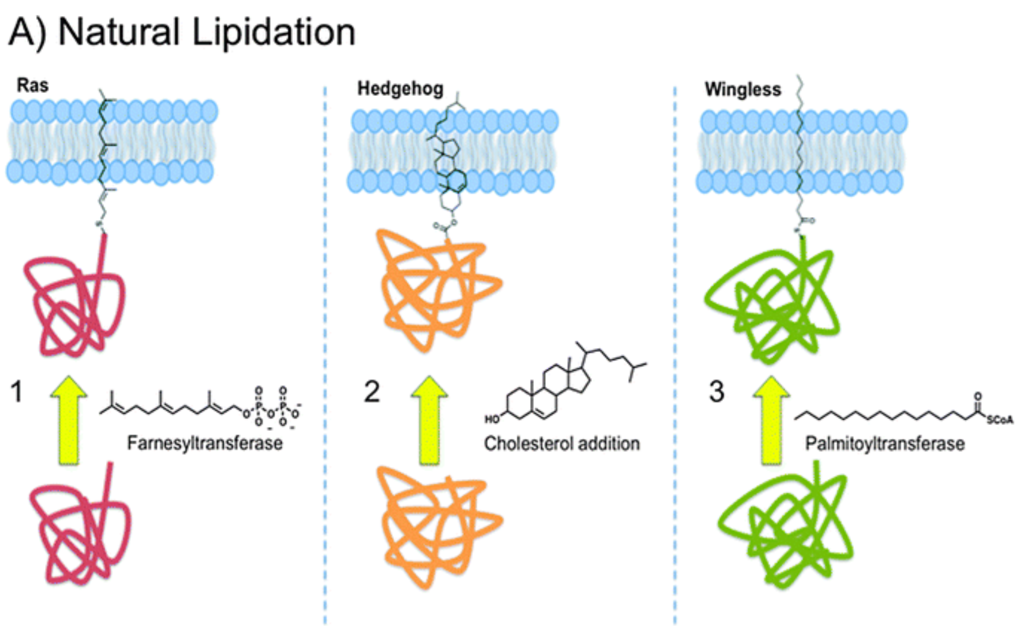


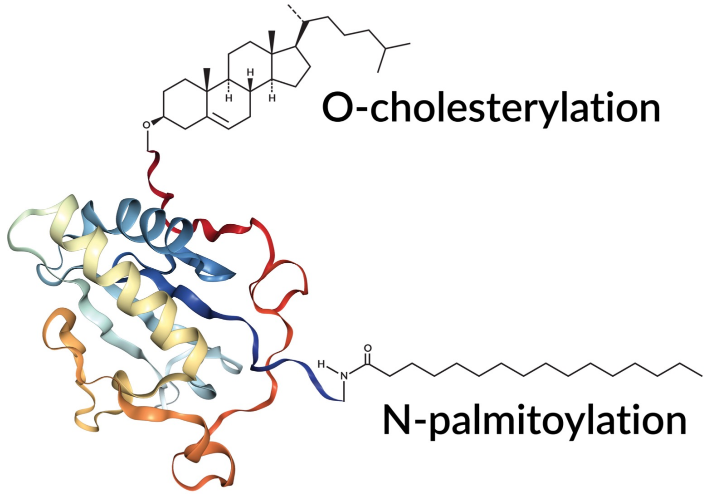
Lipidation

Lipid Structure	Effects on proteins	Effects on downstream signaling in cancer
GPI anchor 	Plasma membrane tethering Incorporation into specific membrane domains Protein-protein interaction	Increase cell division in bladder[197], breast[198], colon[199], and other cancers
Cholesterylation 	Hedgehog signaling activation	Facilitate tumorigenesis and cancer growth in prostate[200], breast[201], bladder[202], and other cancers
Myristoylation 	Membrane localization Autoinhibition	Carcinogenesis in breast [203], lung[204], and other cancers
Palmitoylation 	Plasma membrane localization Partitioning into lipid rafts Protein maturation/quality control	Promote proliferation and invasion in melanoma[170], intestinal[205], and other cancers
Farnesylation 	Membrane localization Conformational change Protein-protein interaction	Promote cell growth, survival and metastasis in lung[206], myeloid leukemia[207], pancreatic[208], and other cancers
Geranylgeranylation 	Membrane localization Protein-protein interaction	Facilitate cell proliferation and migration in lymphoma[177], leukemia[209], and other cancers

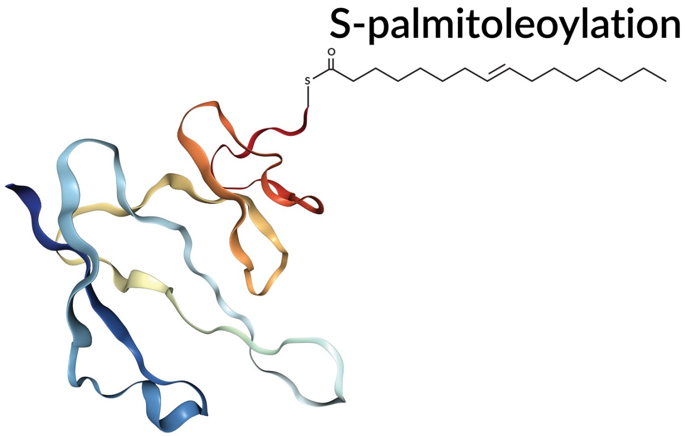
Front. Oncol., 29 June 2017

Lipidation

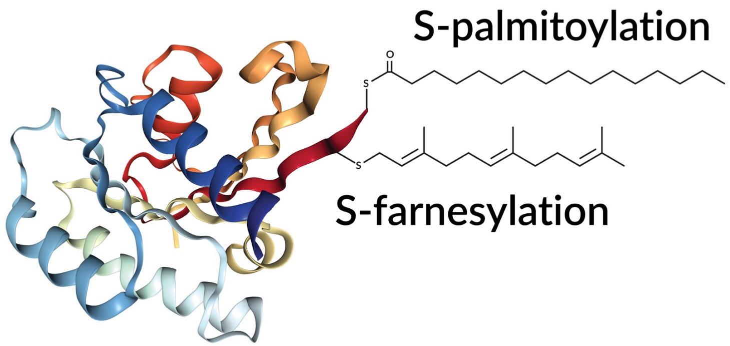




Shh

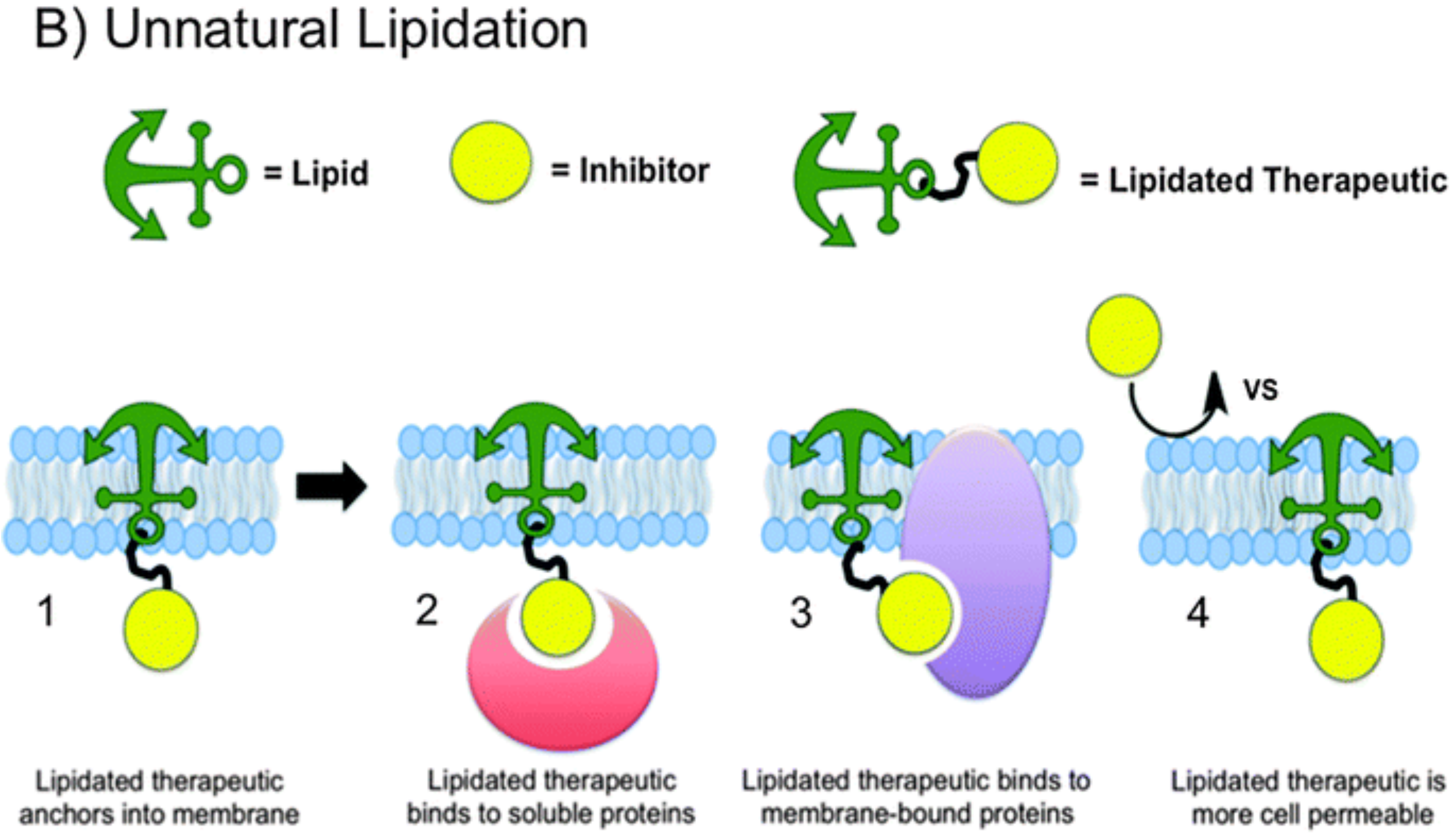


Wnt



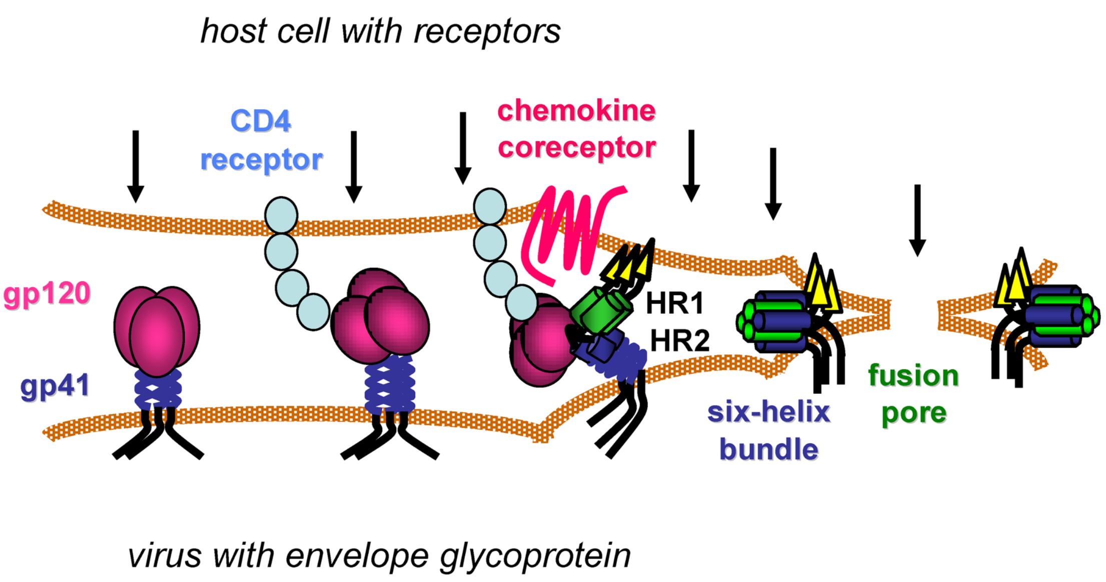
H-Ras

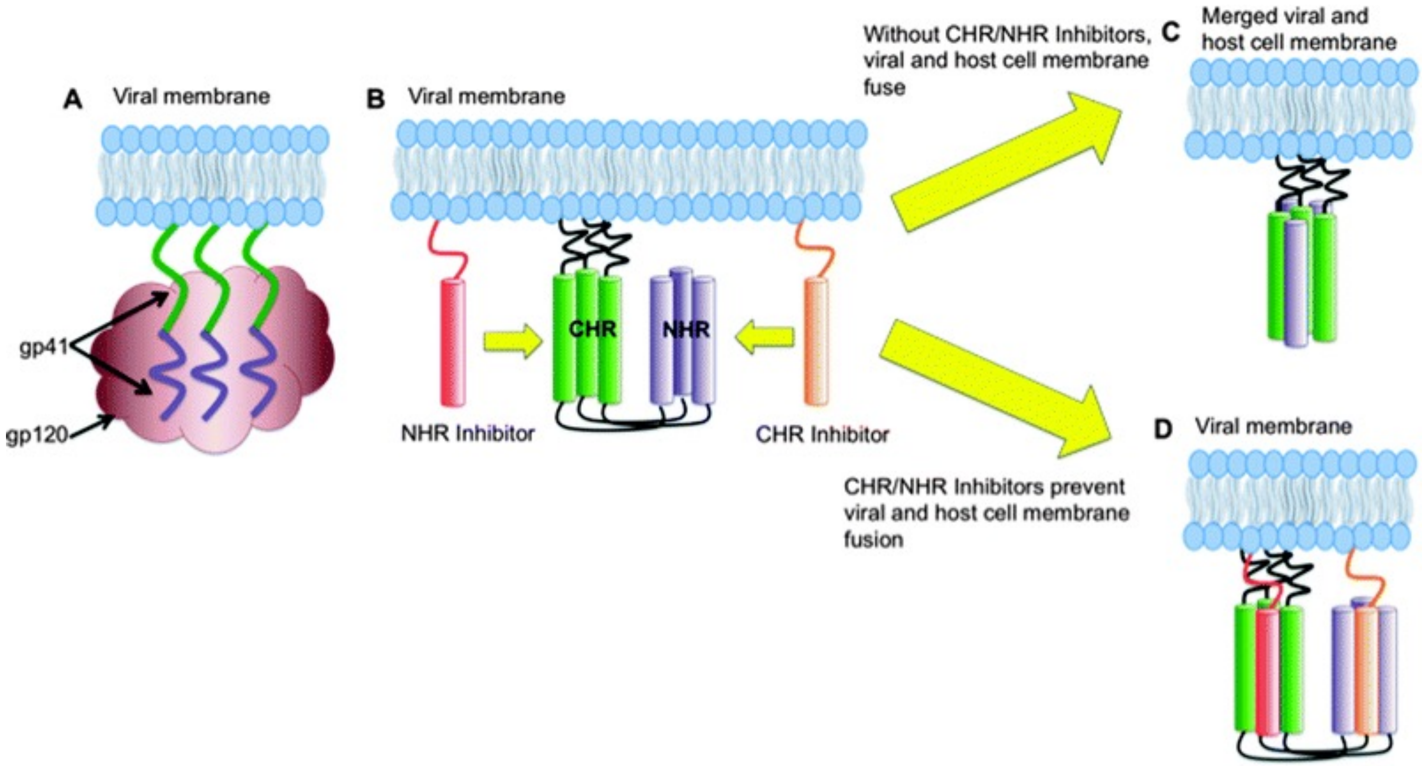
Lipidated therapeutics



Lipidated therapeutics

Escape from Human Immunodeficiency Virus Type 1 (HIV-1)





Viruses **2012**, *4*(12), 3859-3911

chromosome

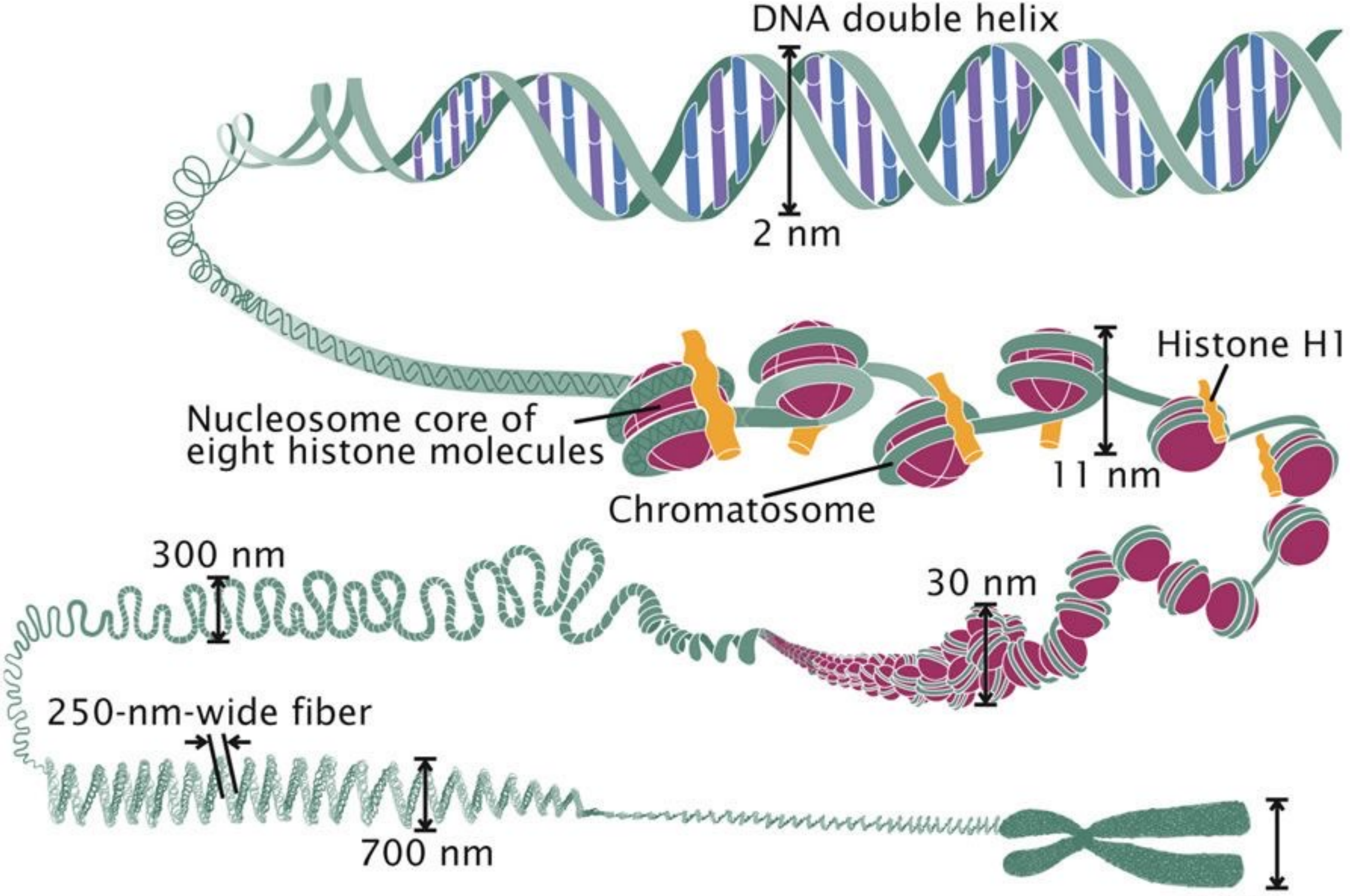


Fig_11-05 *Genetics, Second Edition* © 2005 W.H. Freeman and Company

Drosophila nucleosome core

H2A, H2B, H3, and H4

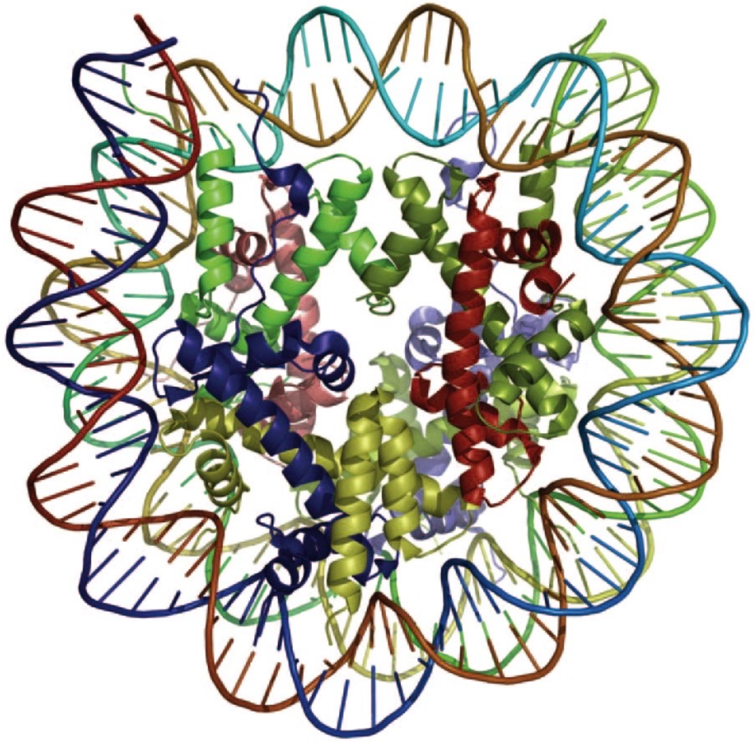
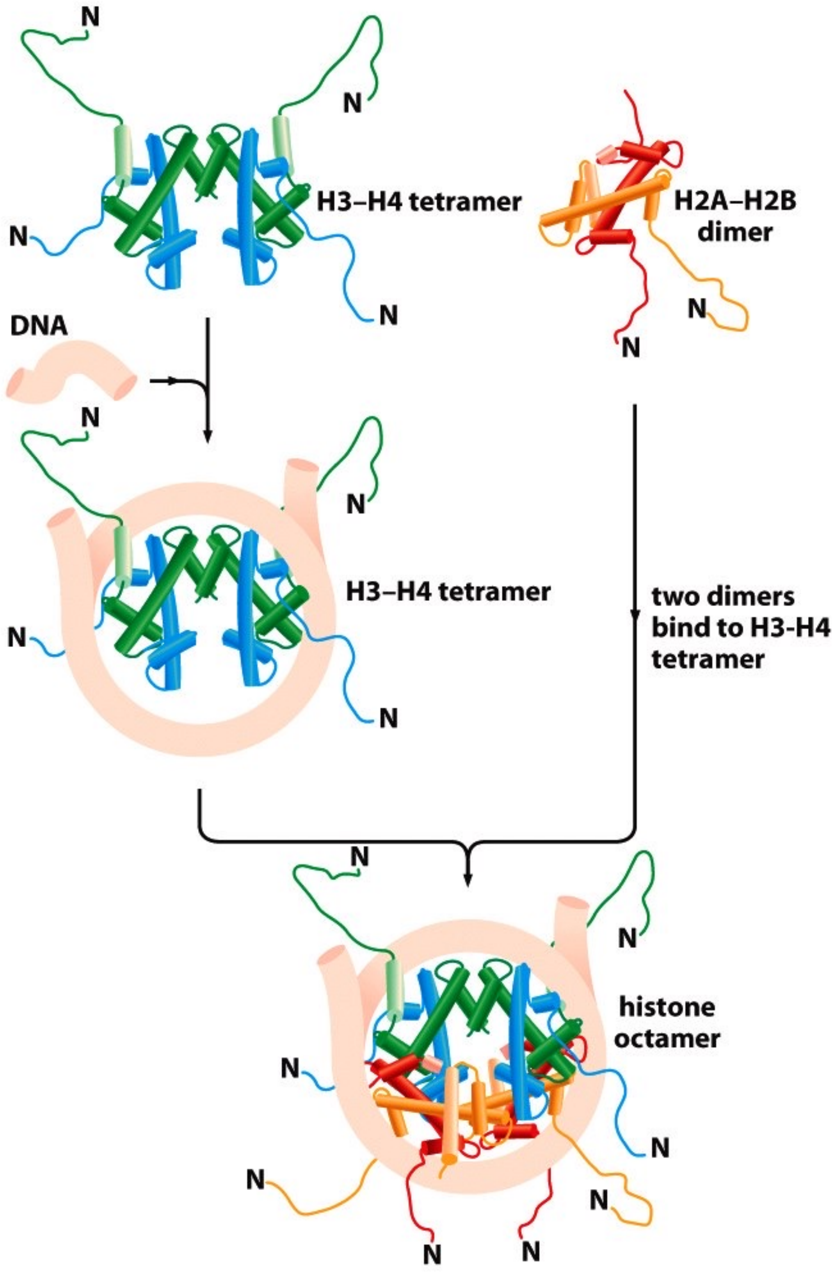
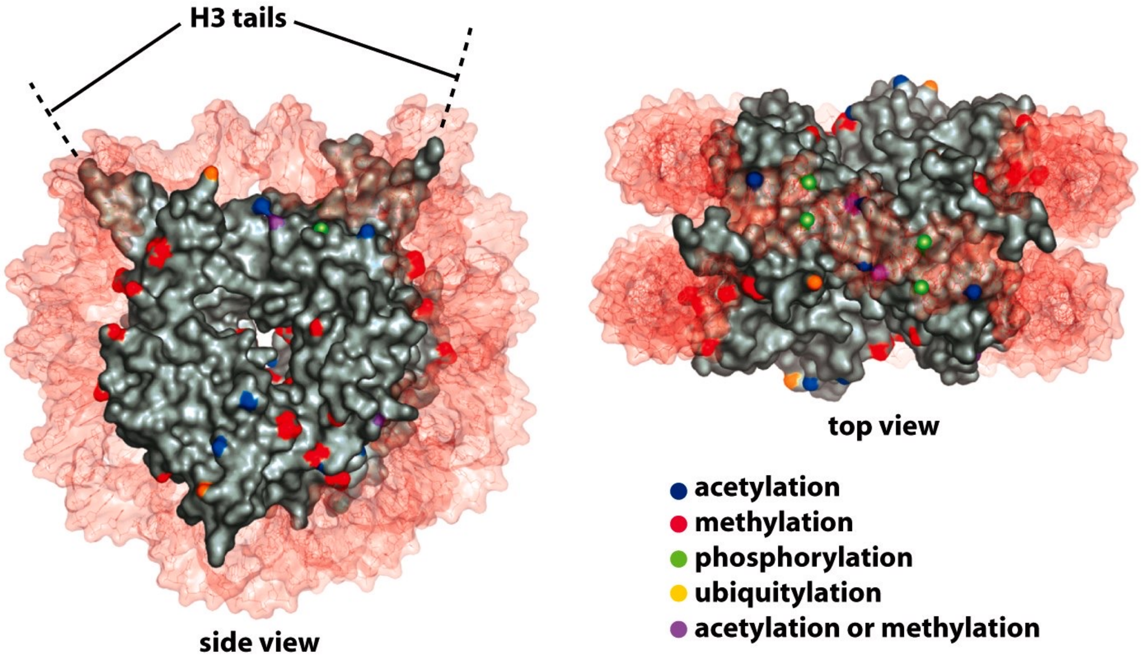
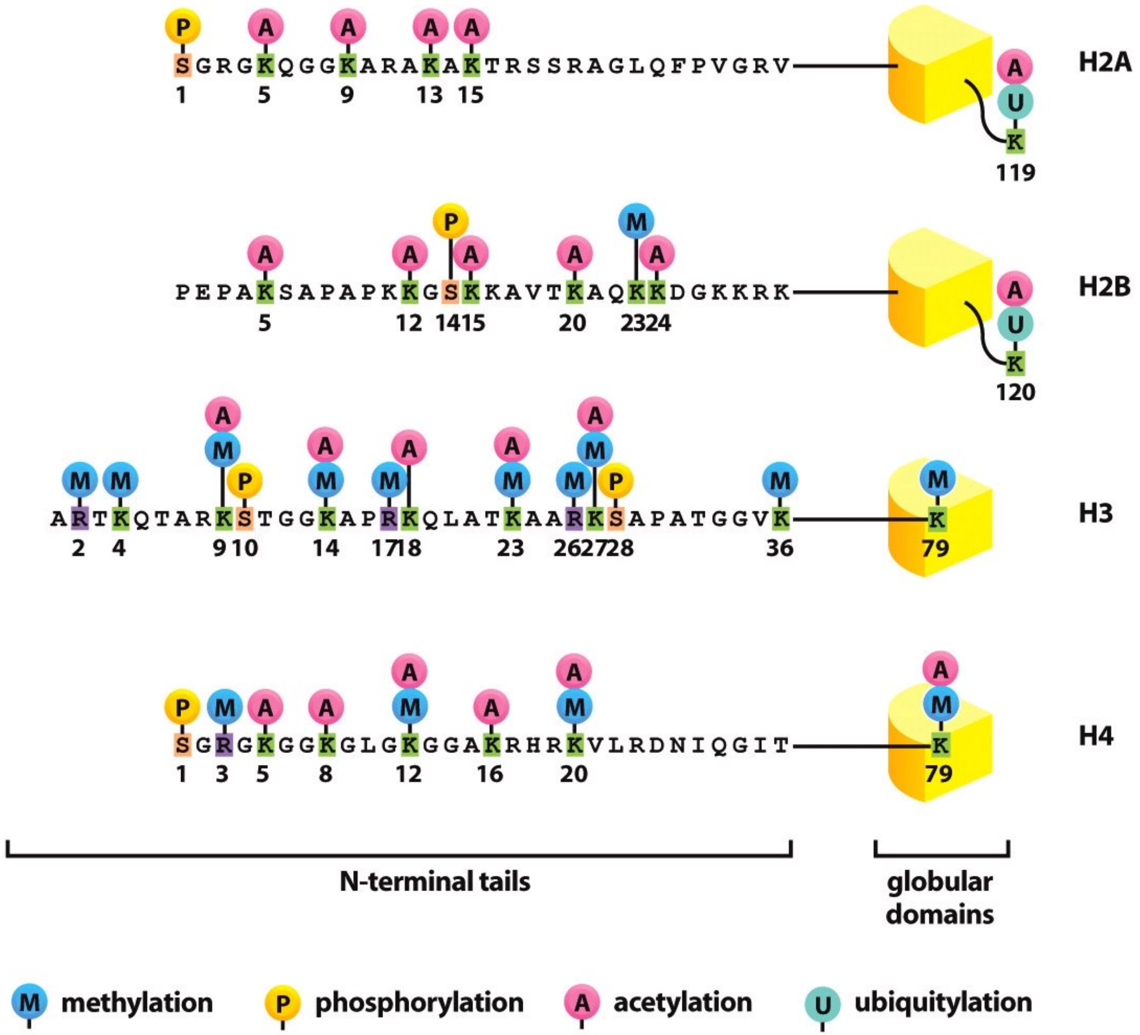


Figure 4.38 How Proteins Work (©2012 Garland Science)

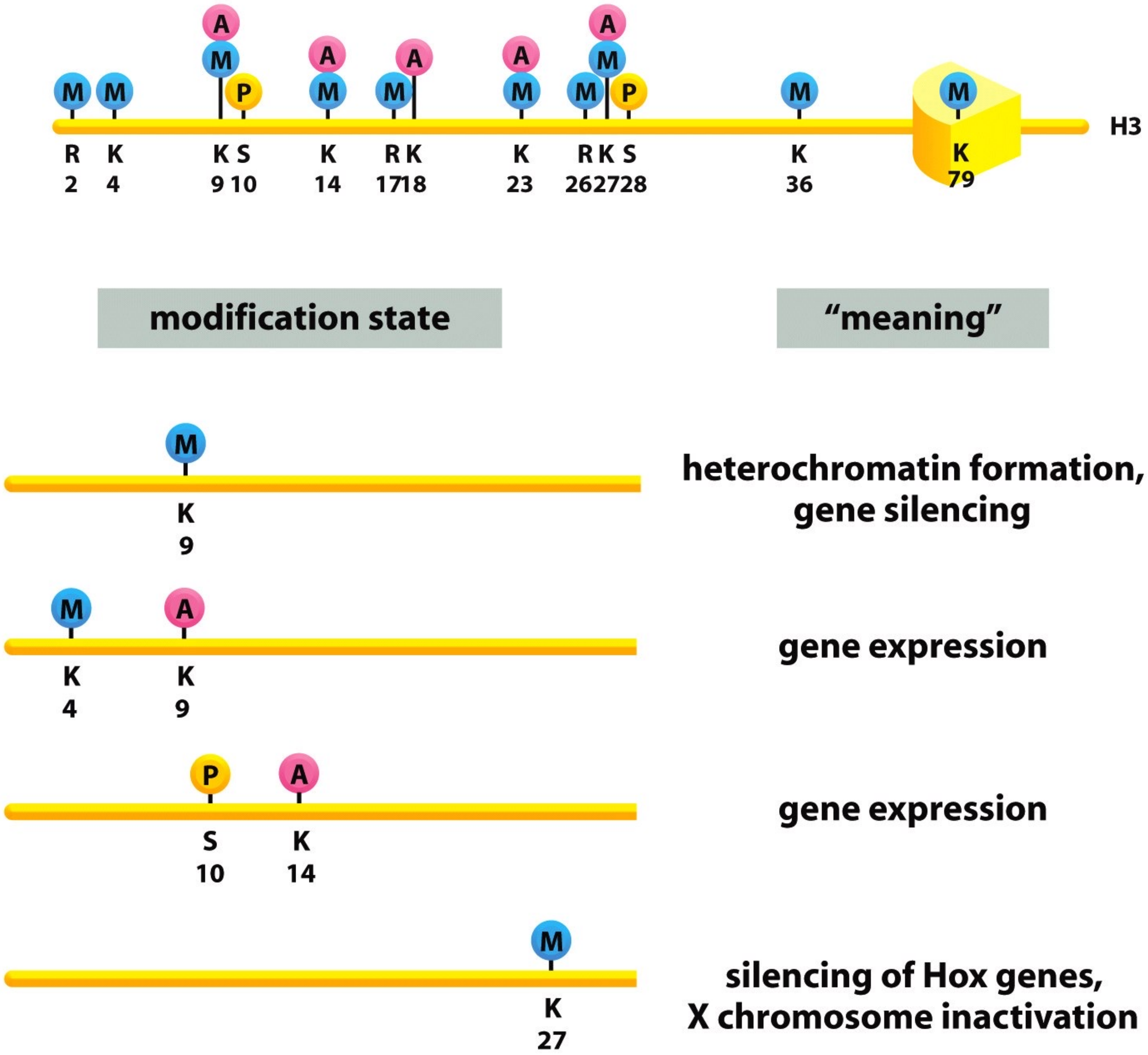


Modification





Effects



M M A M P A M M A A M A M M P M M
R 2
K 4
K 9
S 10
K 14
R 17
K 18
K 23
R 26
K 27
S 28
K 36
K 79
H3
modification state
“meaning”
M K 9
heterochromatin formation, gene silencing
M A K 4 K 9
gene expression
P A S 10 K 14
gene expression
M K 27
silencing of Hox genes, X chromosome inactivation

effects

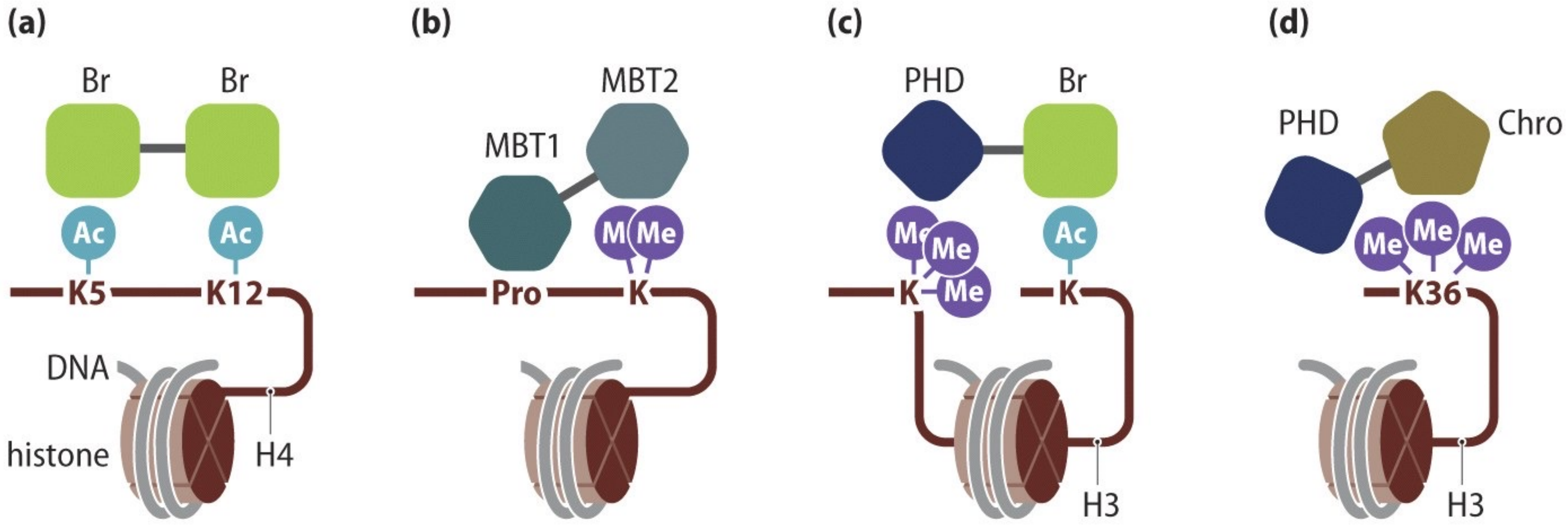
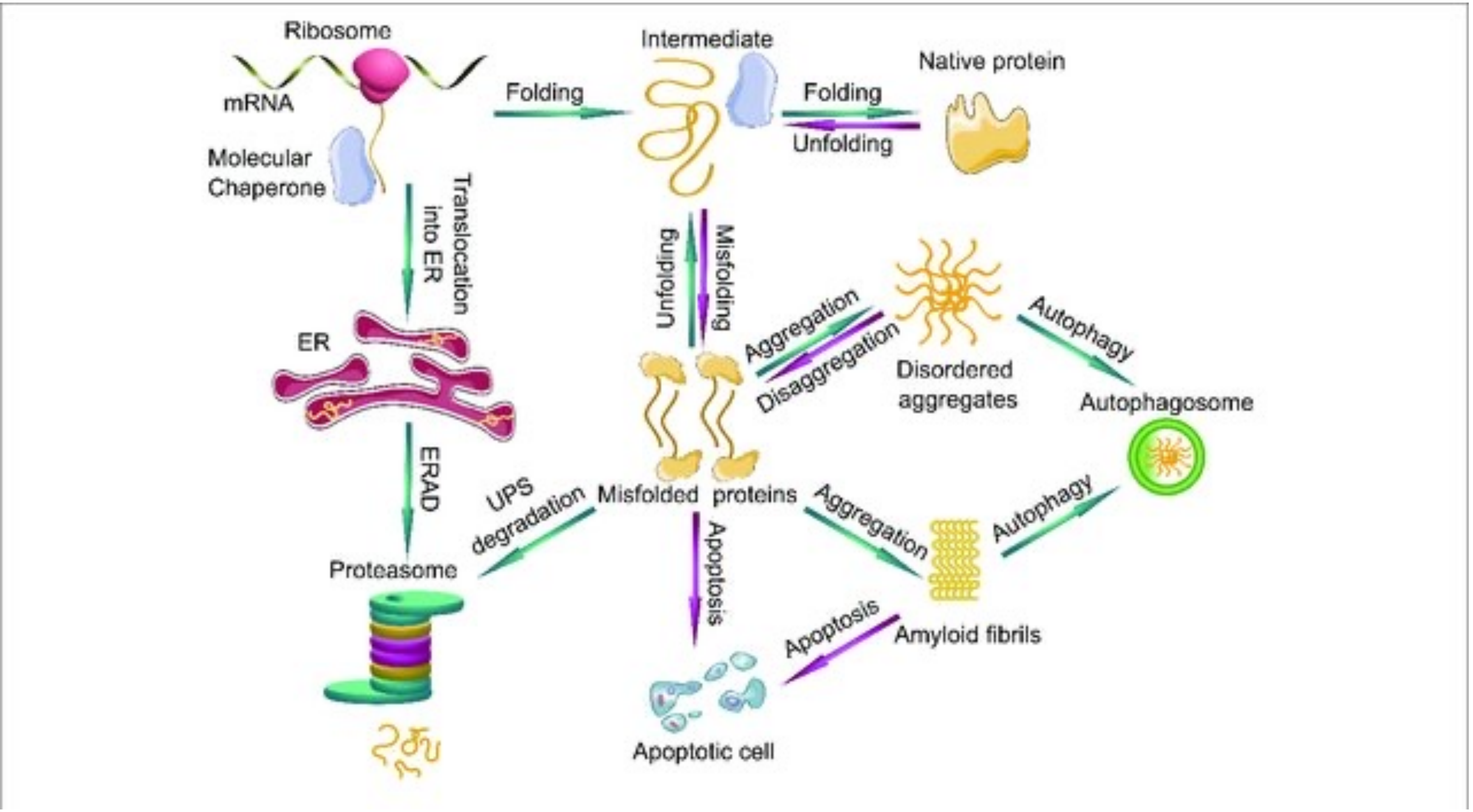


Figure 4.39 How Proteins Work (©2012 Garland Science)

Protein quality control



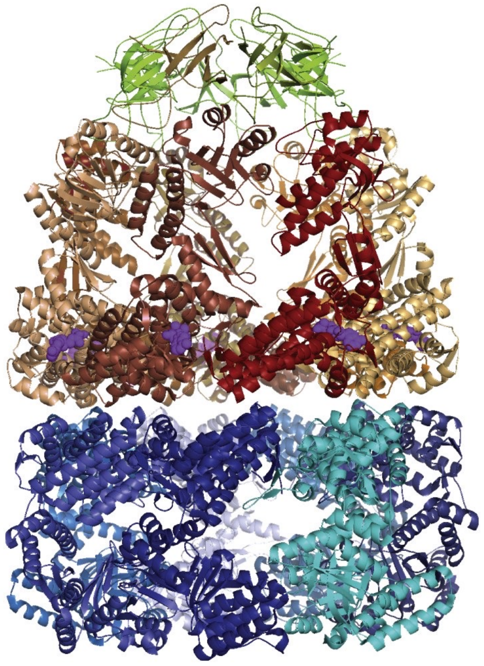


Figure 4.12.1 How Proteins Work (©2012 Garland Science)

Frontiers in Cell and Developmental Biology 8:425

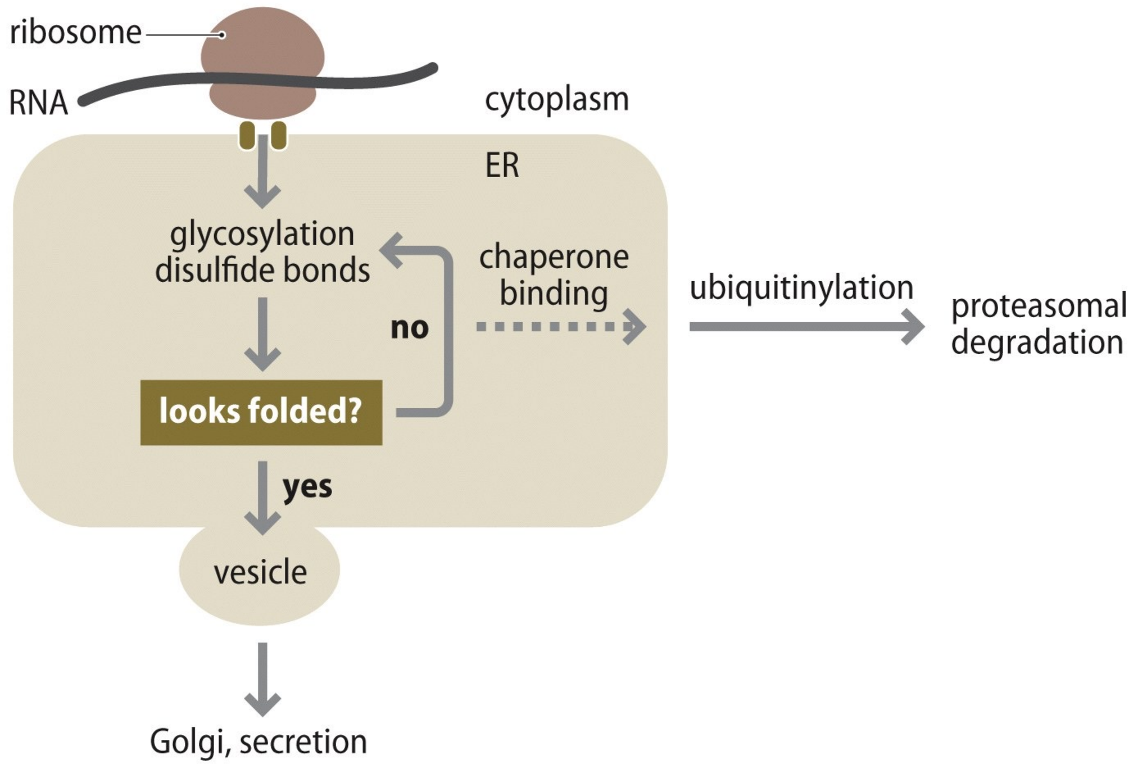


Figure 4.41 How Proteins Work (©2012 Garland Science)

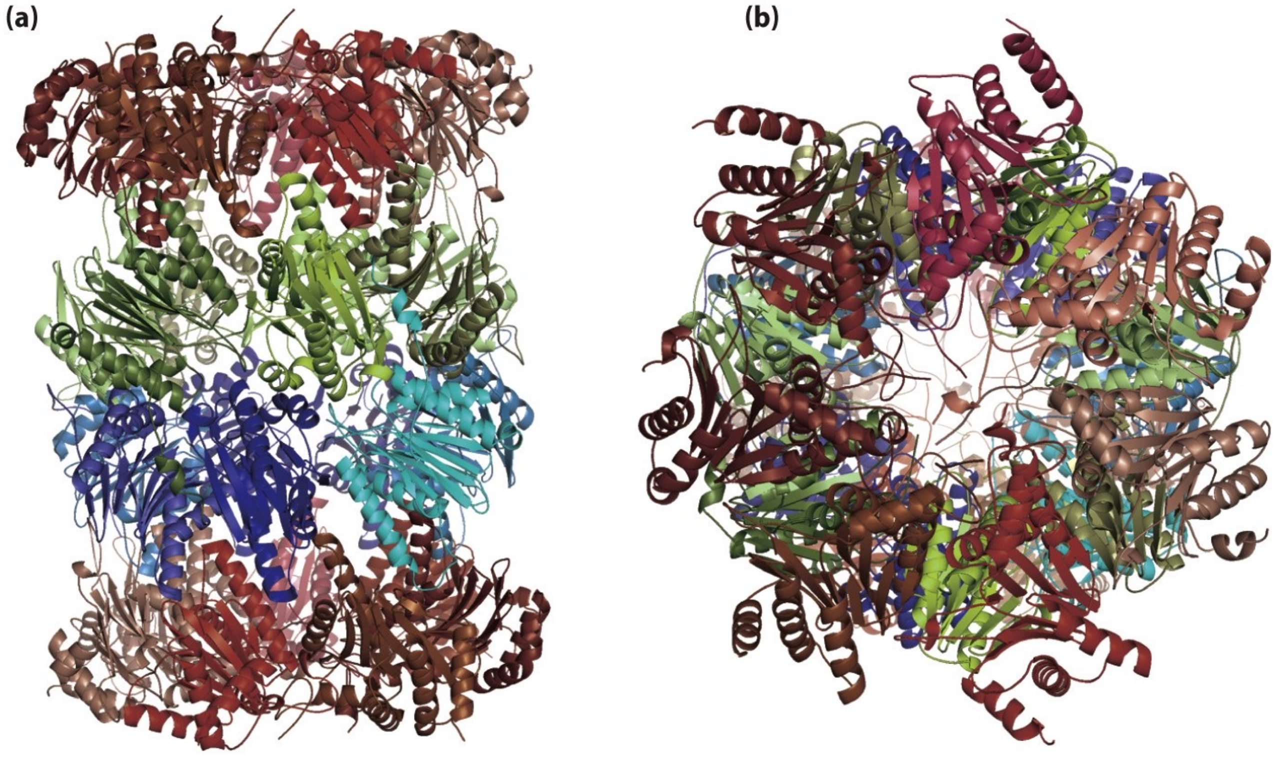
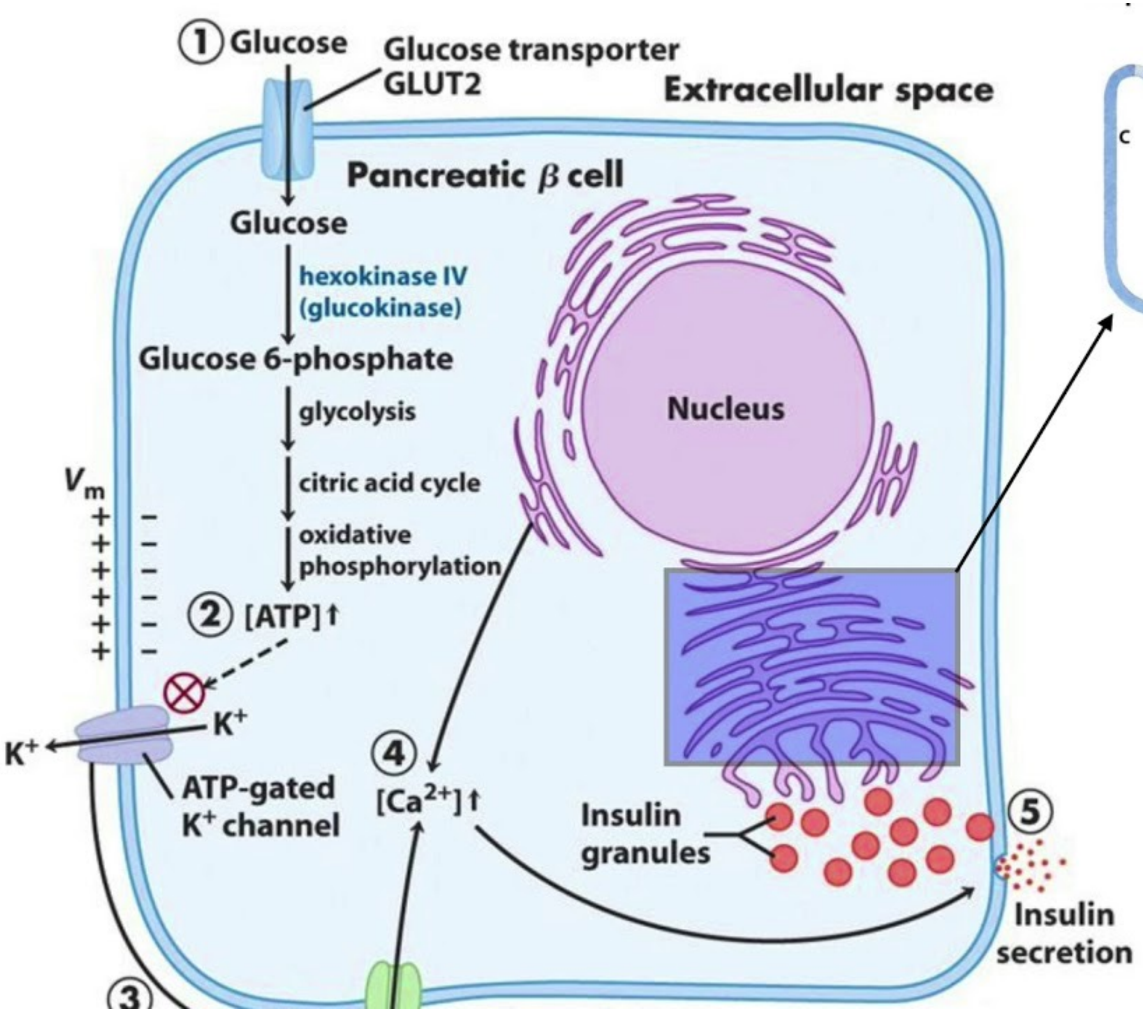
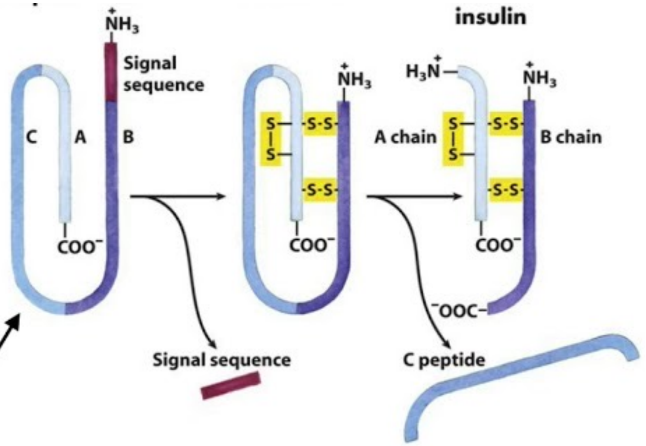


Figure 4.13.1 How Proteins Work (©2012 Garland Science)

Pancreatic β cells





- In pancreatic **β-cells**, glucose release requires **Ca^{2+} influx**.
- **Low** blood glucose:
  - ↓ATP → ↑K^+ efflux → ↑hyperpolarization → ↓Ca^{2+} influx → ↓insulin secretion
- **Elevated** blood glucose
  - **↑ATP** → ↓K^+ efflux →

self-splicing polypeptides

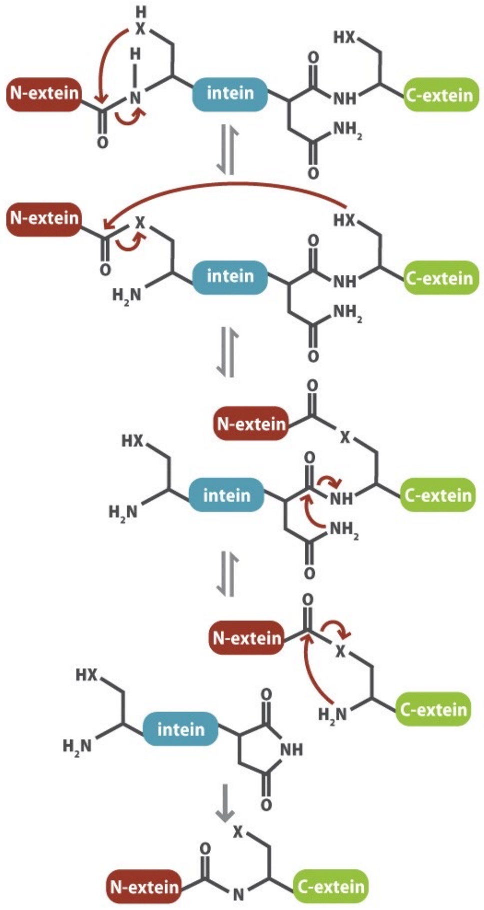


Figure 4.10.1 How Proteins Work (©2012 Garland Science)

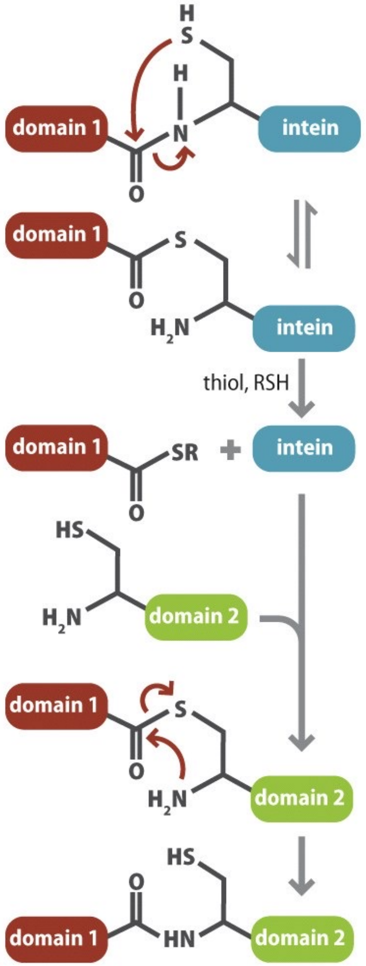
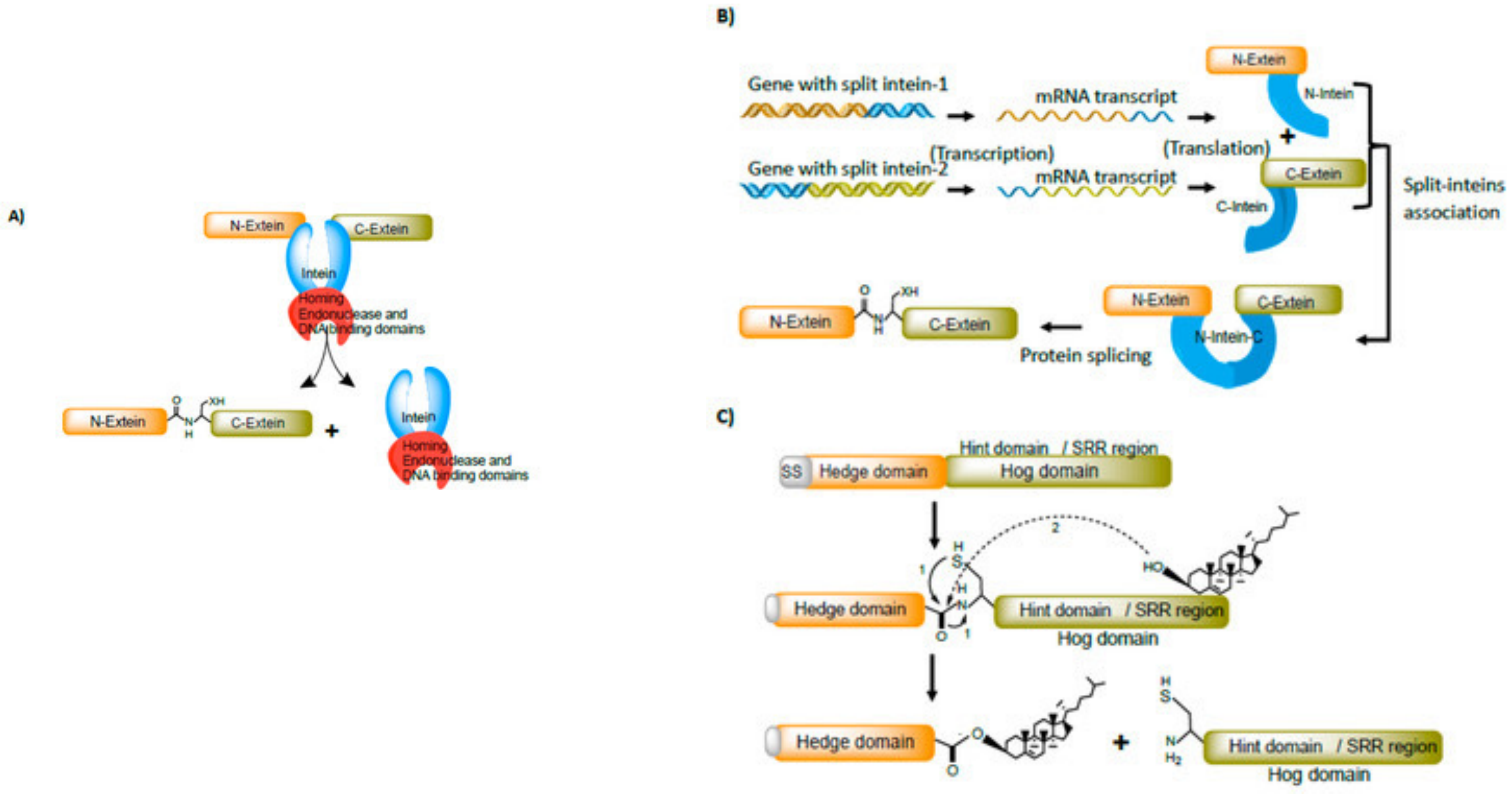
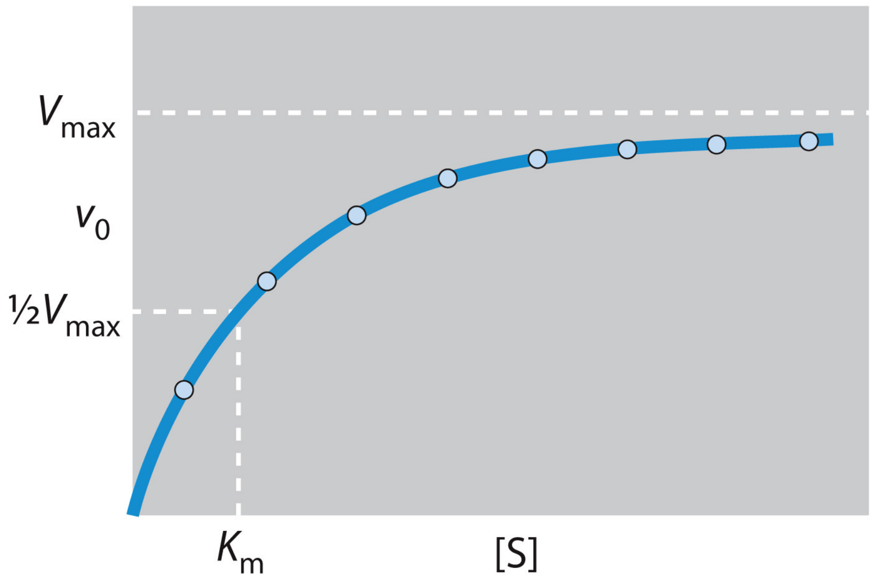


Figure 4.10.2 How Proteins Work (©2012 Garland Science)


A)
N-Extein
C-Extein
Intein
Homing
Endonuclease and
DNA binding domains
O
XH
N-Extein
N
H
C-Extein
+
Intein
Homing
Endonuclease and
DNA binding domains
B)
Gene with split intein-1
mRNA transcript
N-Extein
N-Intein
+
(Transcription)
(Translation)
Gene with split intein-2
mRNA transcript
C-Extein
C-Intein
Split-inteins
association
O
XH
N-Extein
N
H
C-Extein
N-Extein
C-Extein
N-Intein-C
Protein splicing
C)
Hint domain / SRR region
SS
Hedge domain
Hog domain
2
H
S
1
HO
H
N
Hedge domain
O
1
Hint domain / SRR region
Hog domain
Hedge domain
O
O
+
H
S
N
H2
Hint domain / SRR region
Hog domain

Catalysis



free energy profiles of uncatalyzed and enzyme-catalyzed reactions

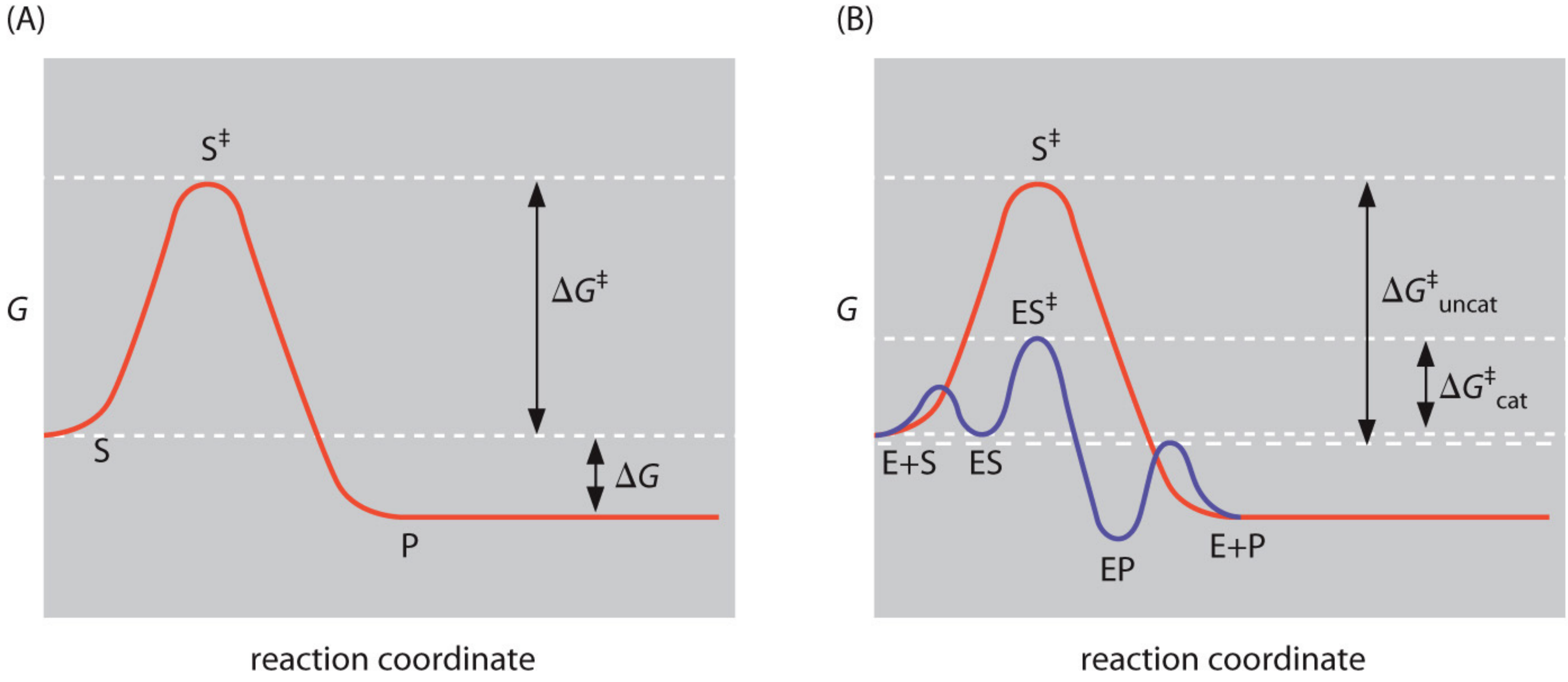
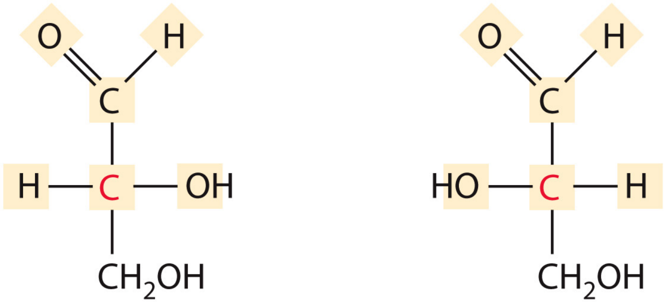


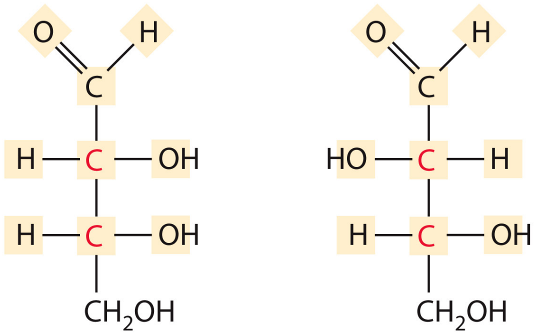
Figure 1.28 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

isomer



D-(+)-glyceraldehyde

L-(–)-glyceraldehyde

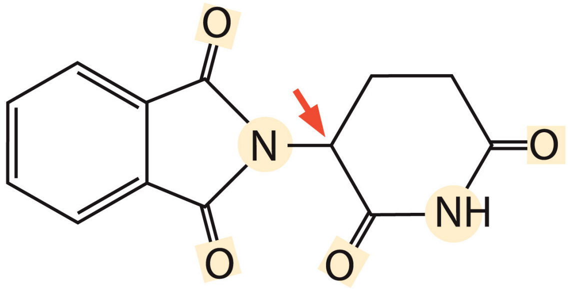


D-erythrose

D-threose

enantiomer

diastereoisomer



thalidomide

catalytic mechanism of the pancreatic protease chymotrypsin

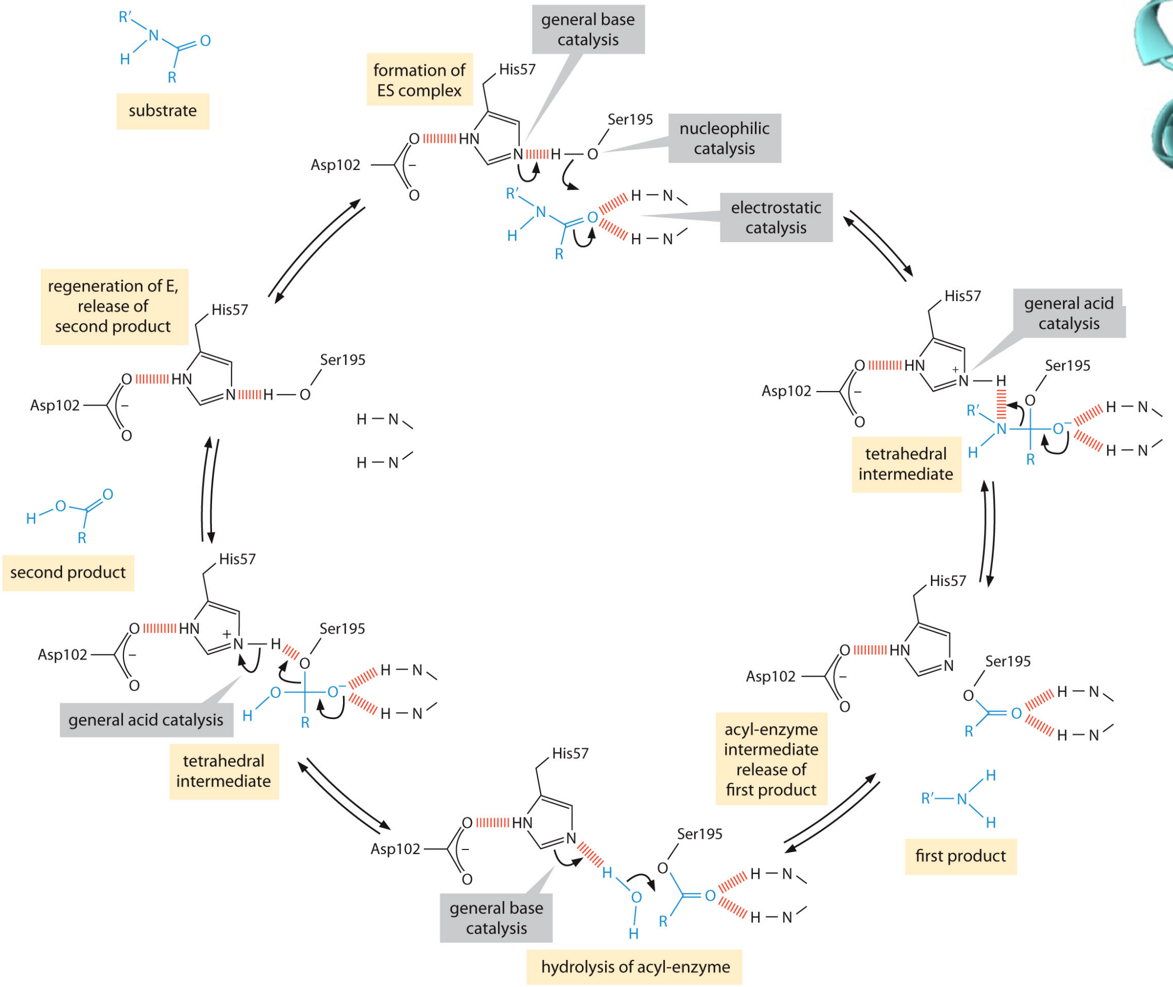
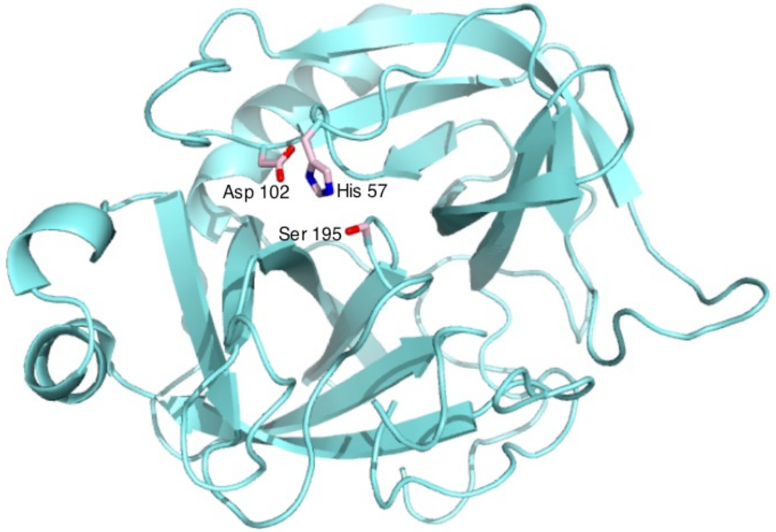


Figure 1.29 Molecular Biology of Assemblies and Machines (© Garland Science 2016)



Substrate specificity

selectivity

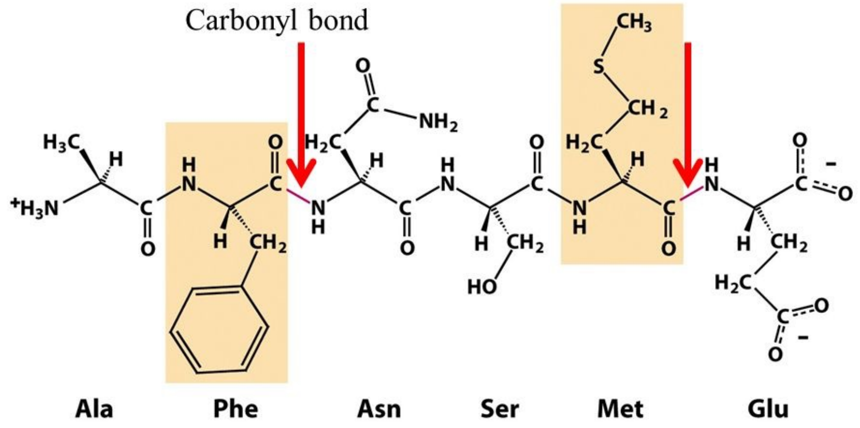
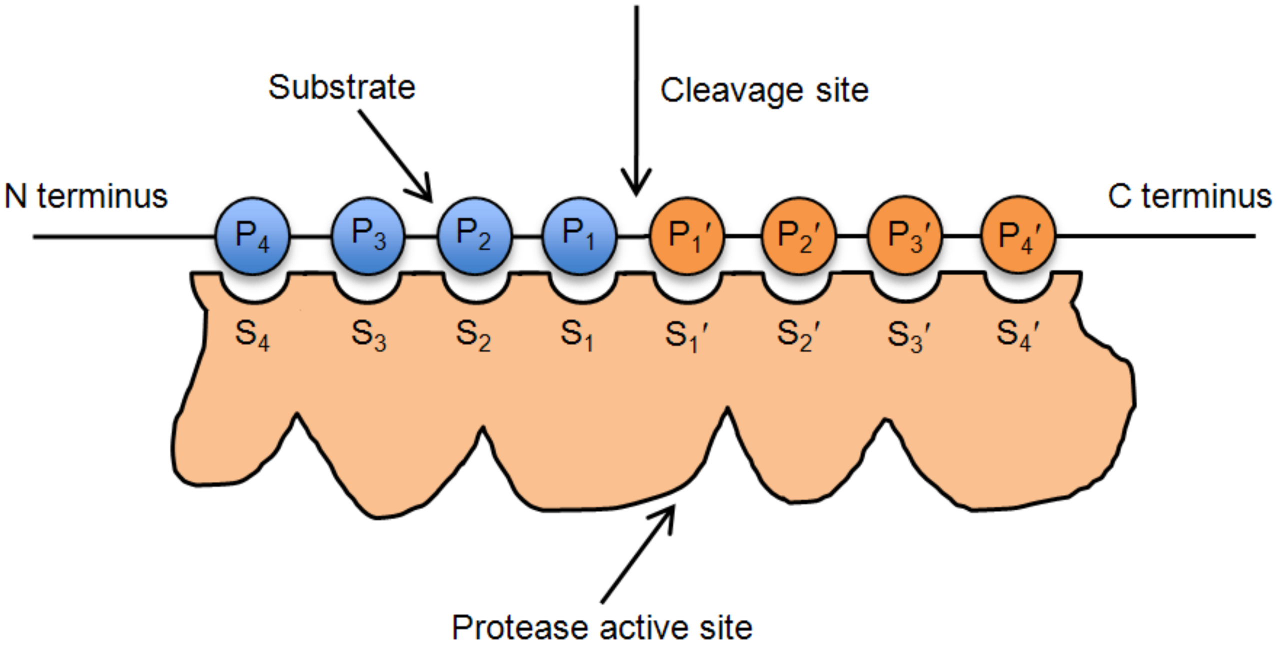
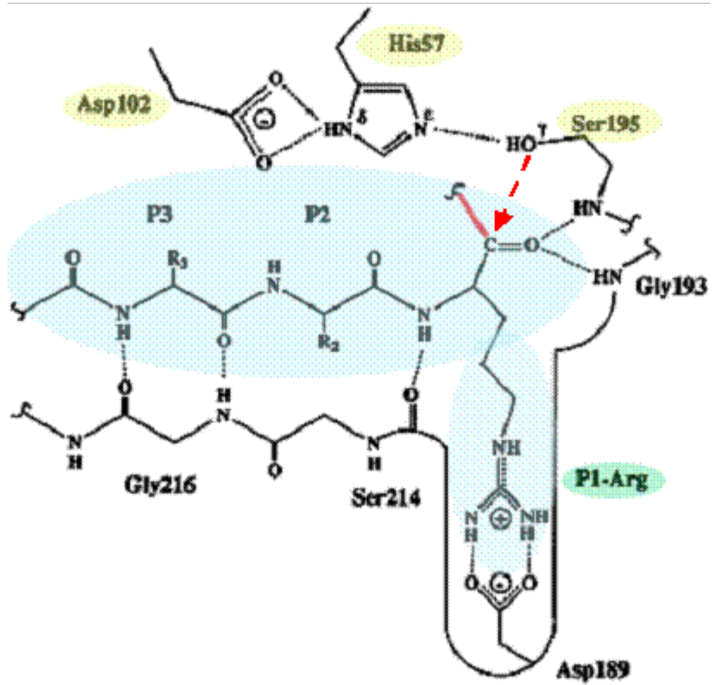


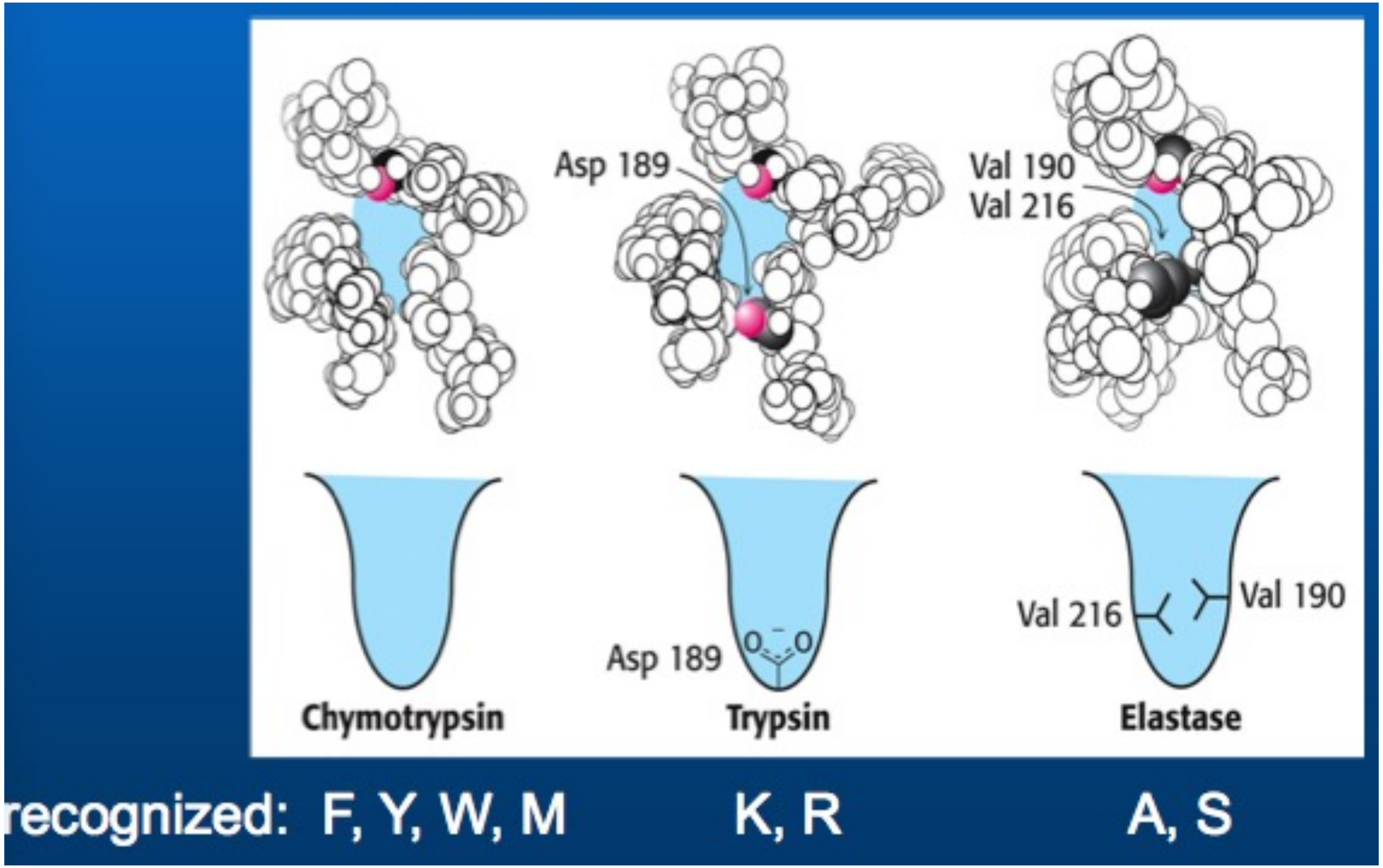
Figure 9-1
Biochemistry, Sixth Edition

Hydrophobic amino acids





Pocket


Asp 189
Val 190
Val 216
Chymotrypsin
Asp 189
Trypsin
Val 216
Val 190
Elastase
recognized: F, Y, W, M
K, R
A, S

related serine peptidases