Oligomers

In Escherichia coli, the average oligomerization state of proteins is 4. Thus, a protein that exists as a monomer is a relatively uncommon event.

A survey of human enzymes shows that approximately two-thirds are oligomers.

An oligomeric protein creates some problems in assembly, in that at least two molecules of protein need to be present in the same place at the same time.

It also makes the protein larger, and therefore less mobile, so there must begood reasons why proteins have evolved to exist as oligomers

Oligomers

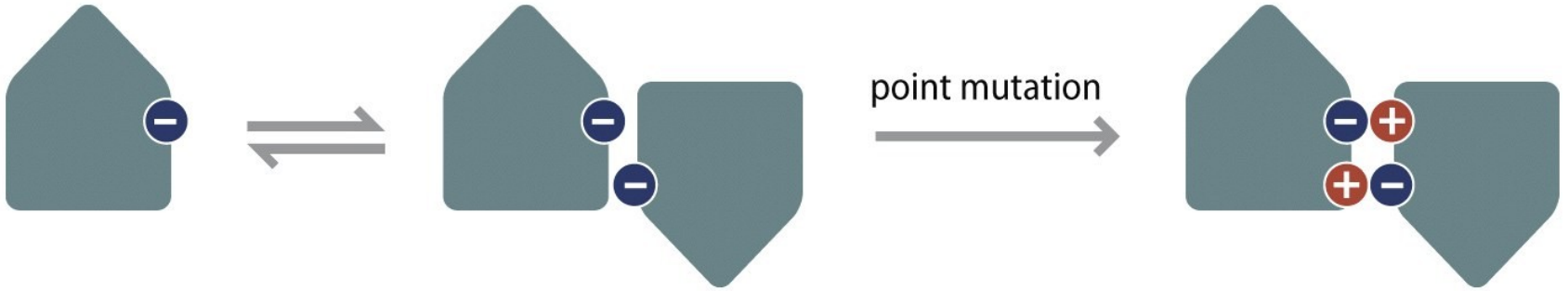


Figure 3.1 How Proteins Work (©2012 Garland Science)

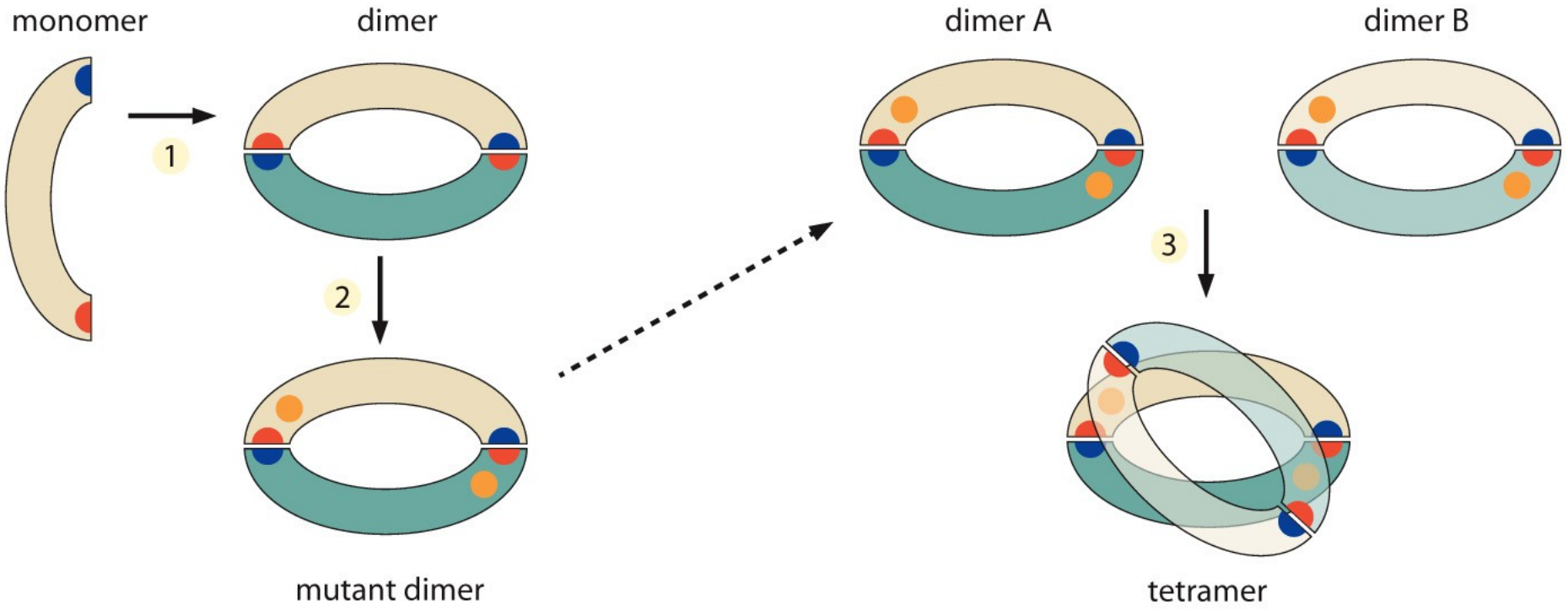
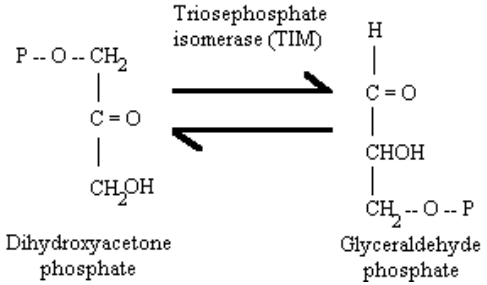


Figure 1.14a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

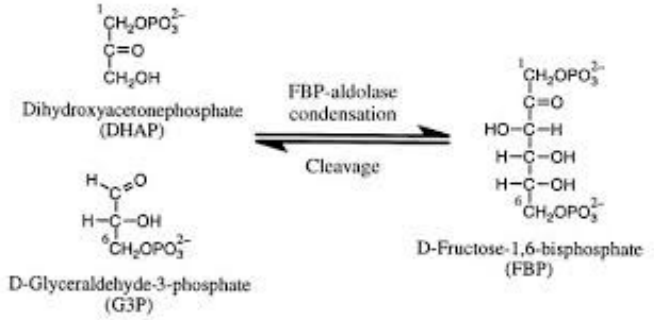
Oligomers

- homo-oligomers by self-association
- hetero-oligomers by binding to a different protein.

triose phosphate isomerase



rabbit muscle fructose-1,6- bisphosphate



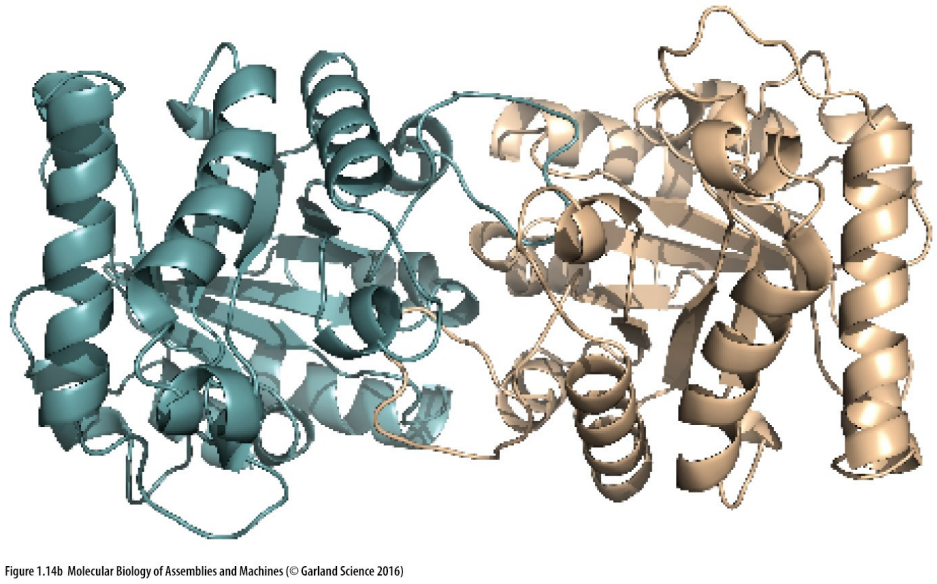


Figure 1.14b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

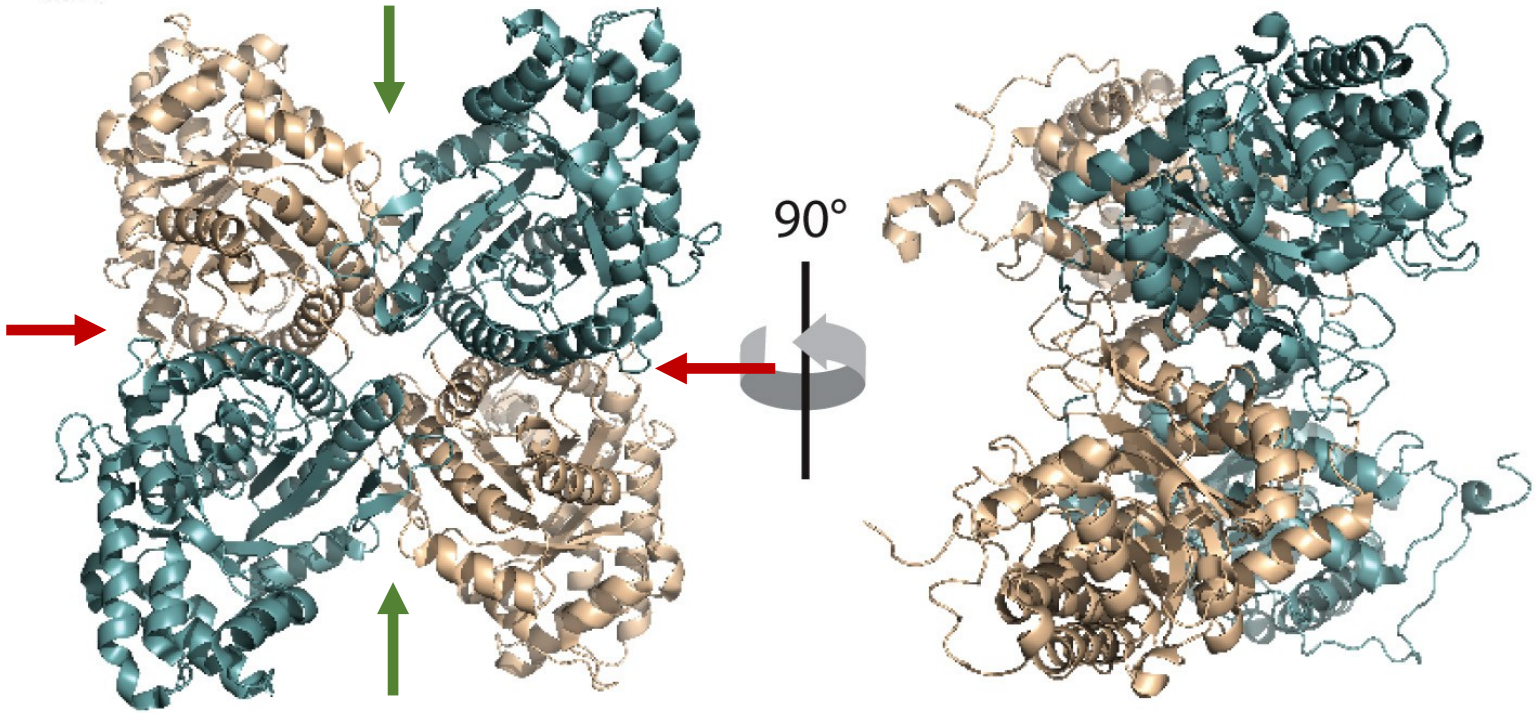


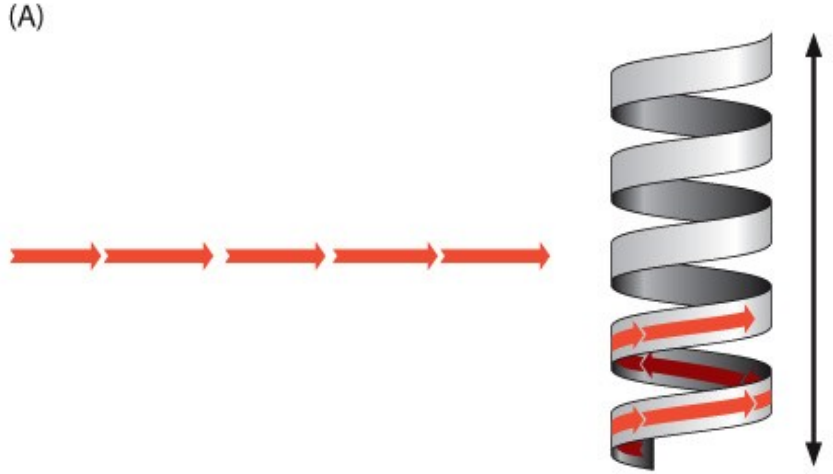
Figure 1.14c Molecular Biology of Assemblies and Machines (© Garland Science 2016)

homo-dimer

homo-tetramerr

symmetry

In line symm



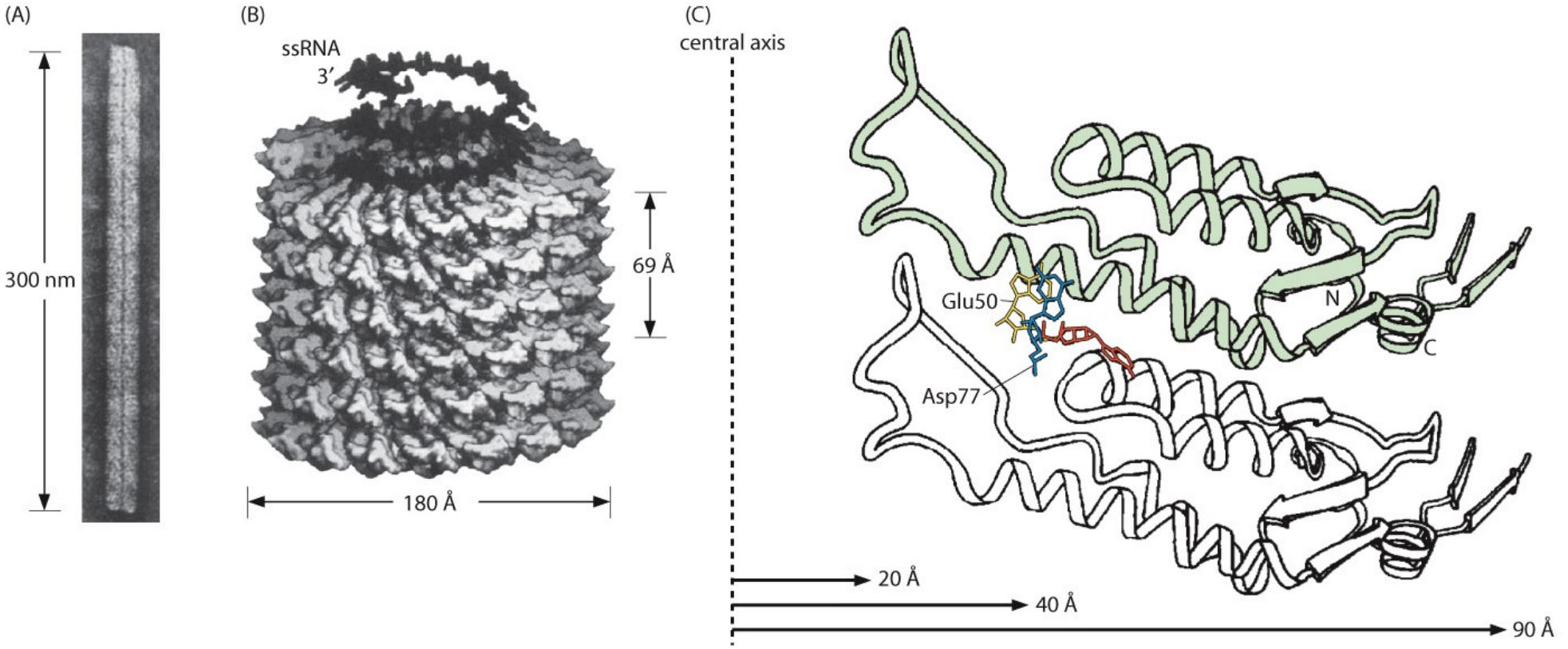


Figure 8.15 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

symmetry

In cyclic symmetry, the subunits form closed rings.

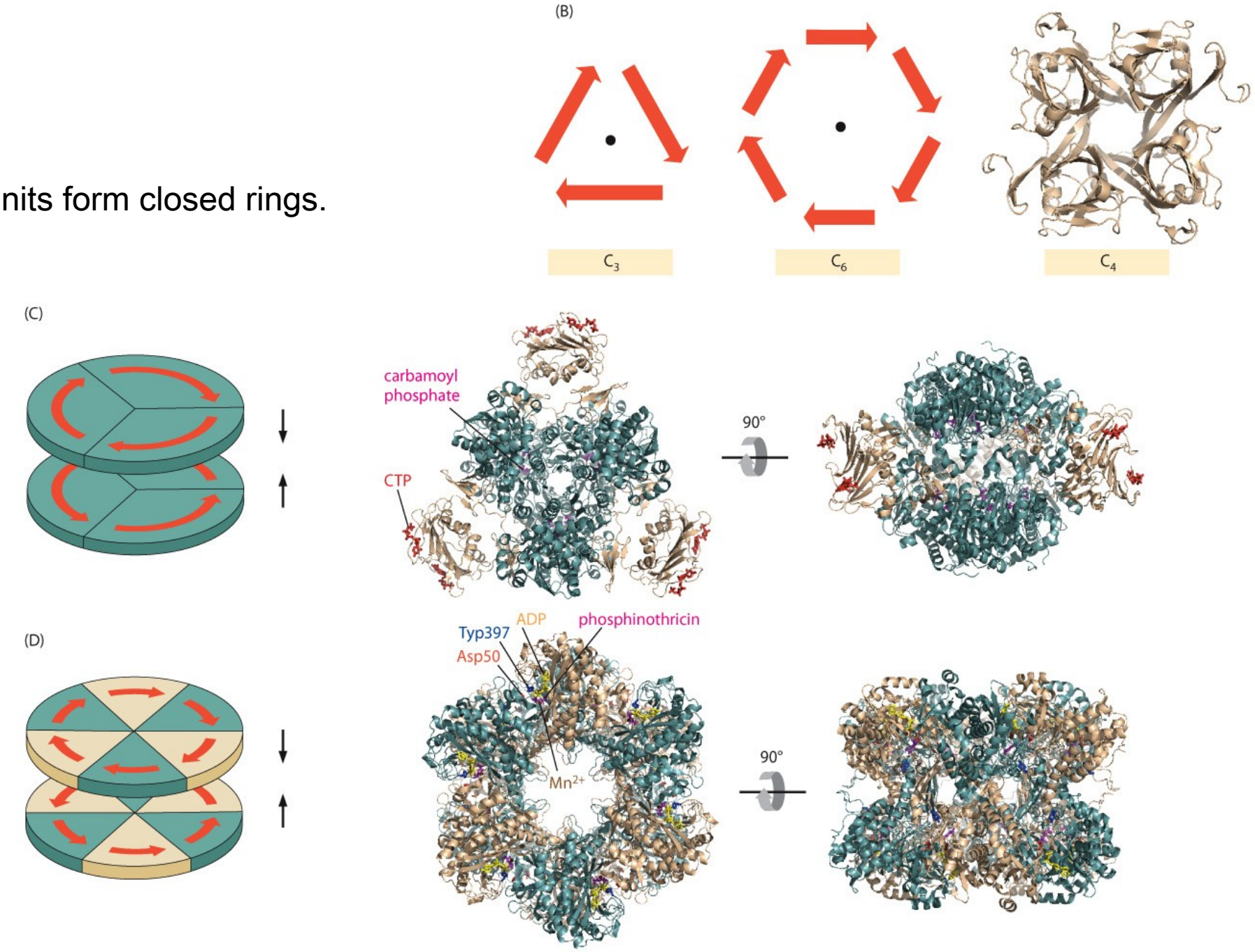
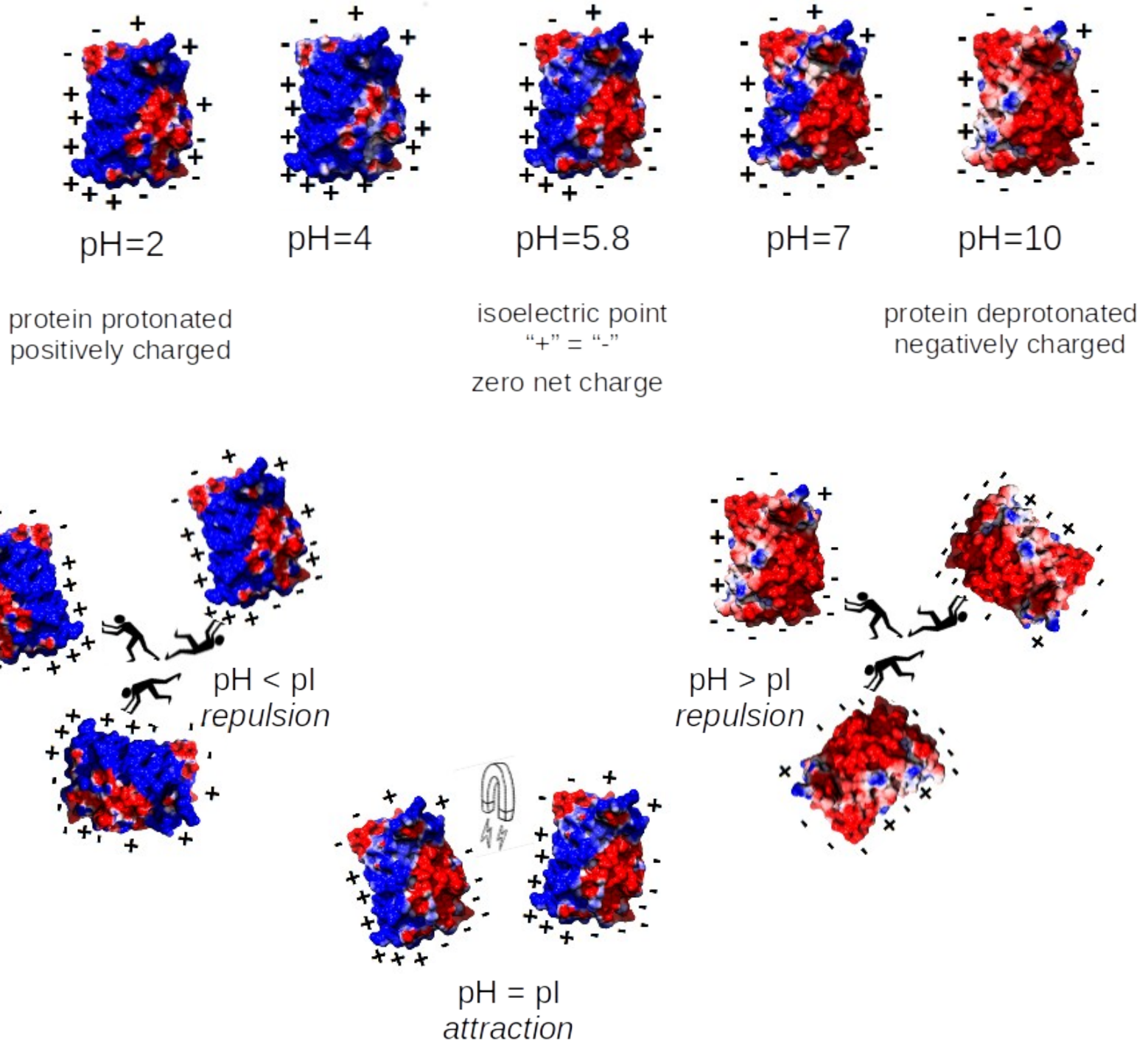


Figure 1.15 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

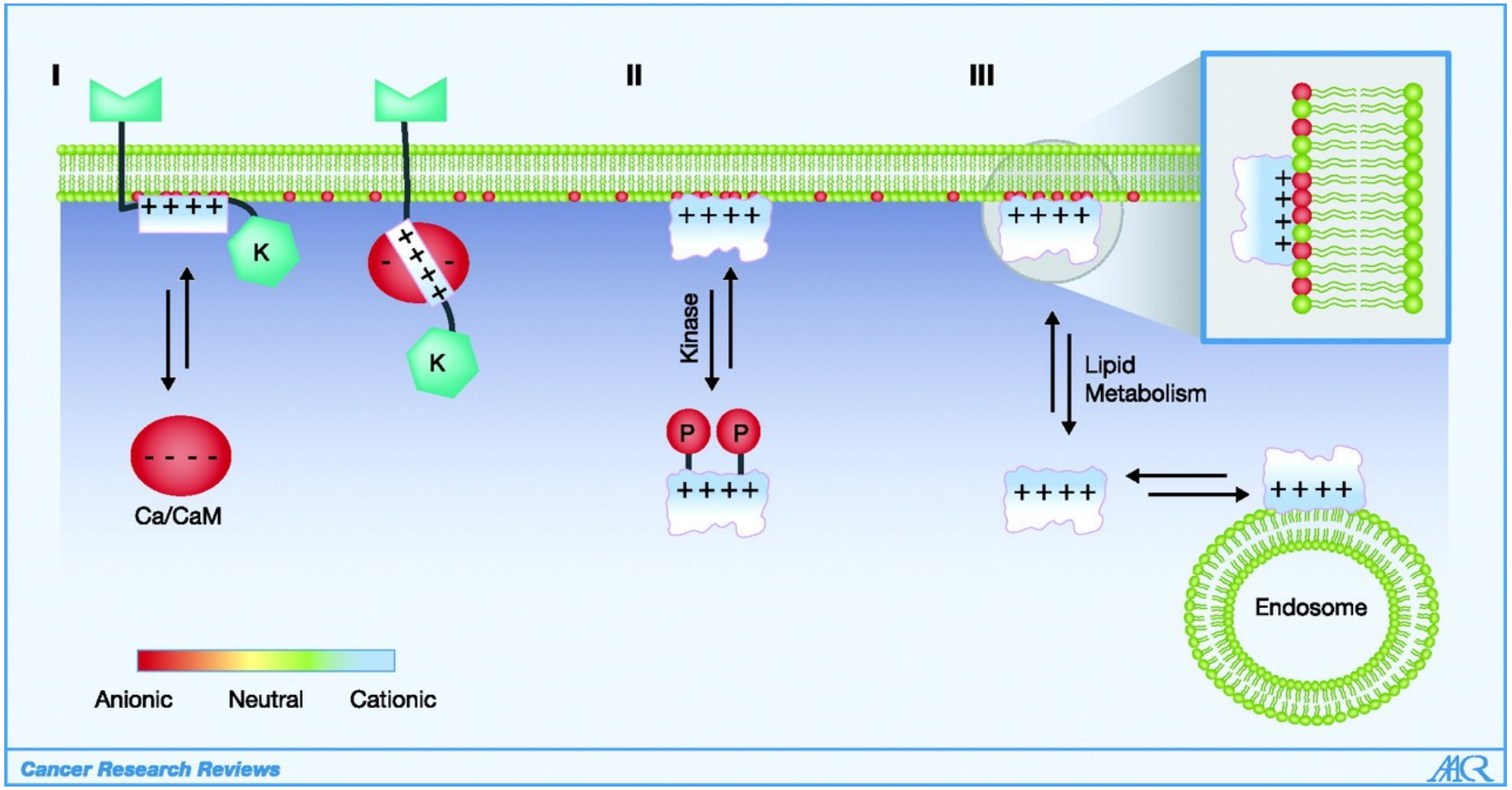
Isoelectric point

Green Fluorescent Protein
(*Aequorea victoria*)
molecular weight of 26.9 kDa (238 aa)
and isoelectric point of ≈5.8.



From: **Surface Charge: A Key Determinant of Protein Localization and Function**

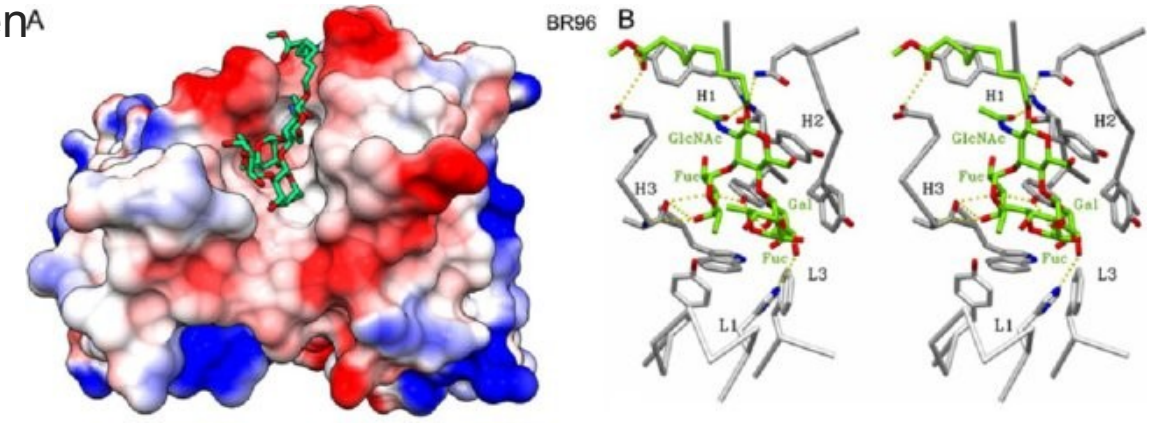
Cancer Res. 2010;70(4):1277-1280. doi:10.1158/0008-5472.CAN-09-2905



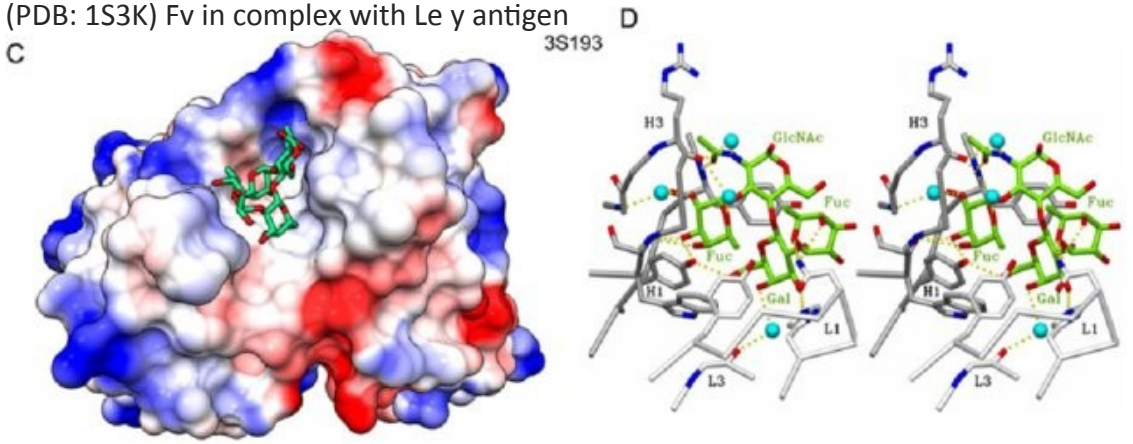
Protein surface

carbohydrate antigen

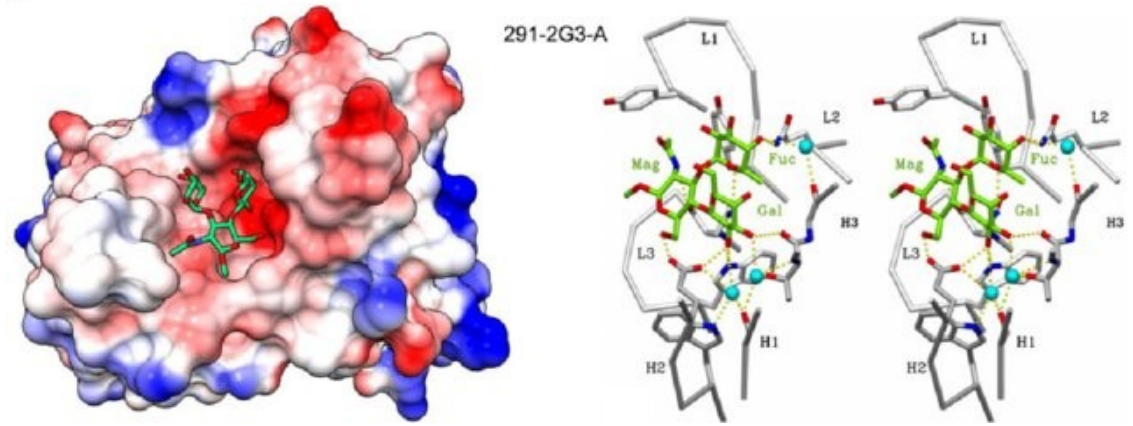
Electrostatic surface potential of chimeric BR96 (PDB: 1CLY) variable domains in complex with nonoate methyl ester derivative of Lewis Y antigen



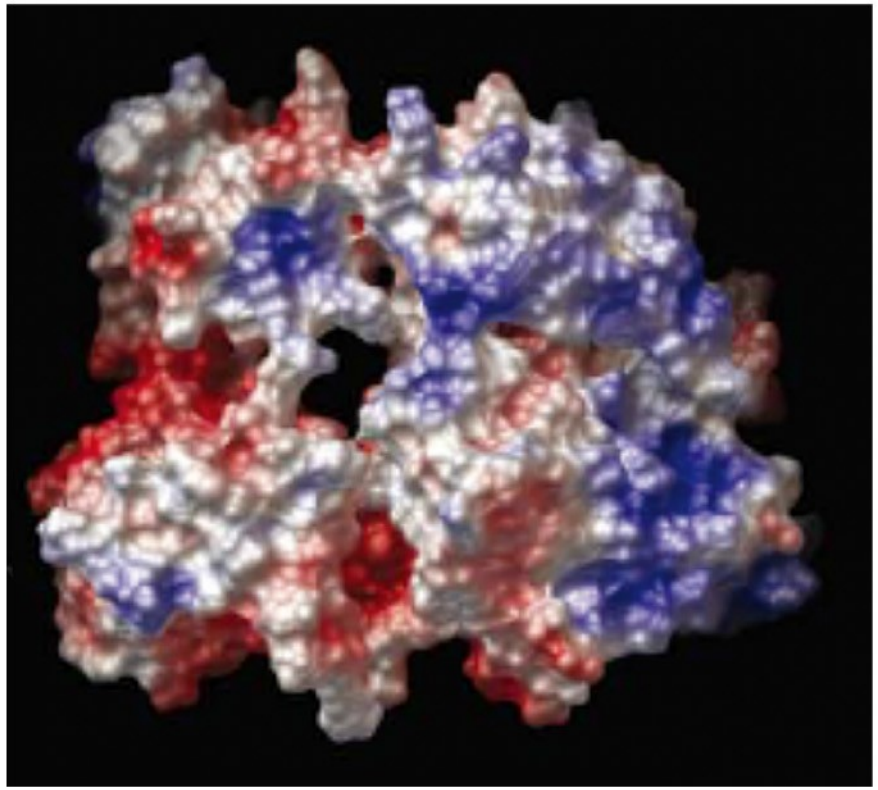
humanized 3S193 (PDB: 1S3K) Fv in complex with Le y antigen



291-2G3-A (PDB: 1UZ8) Fv in complex with the Le x trisaccharide Galβ1,4Fucα1,3GlcNAcβ antigen.



(A)

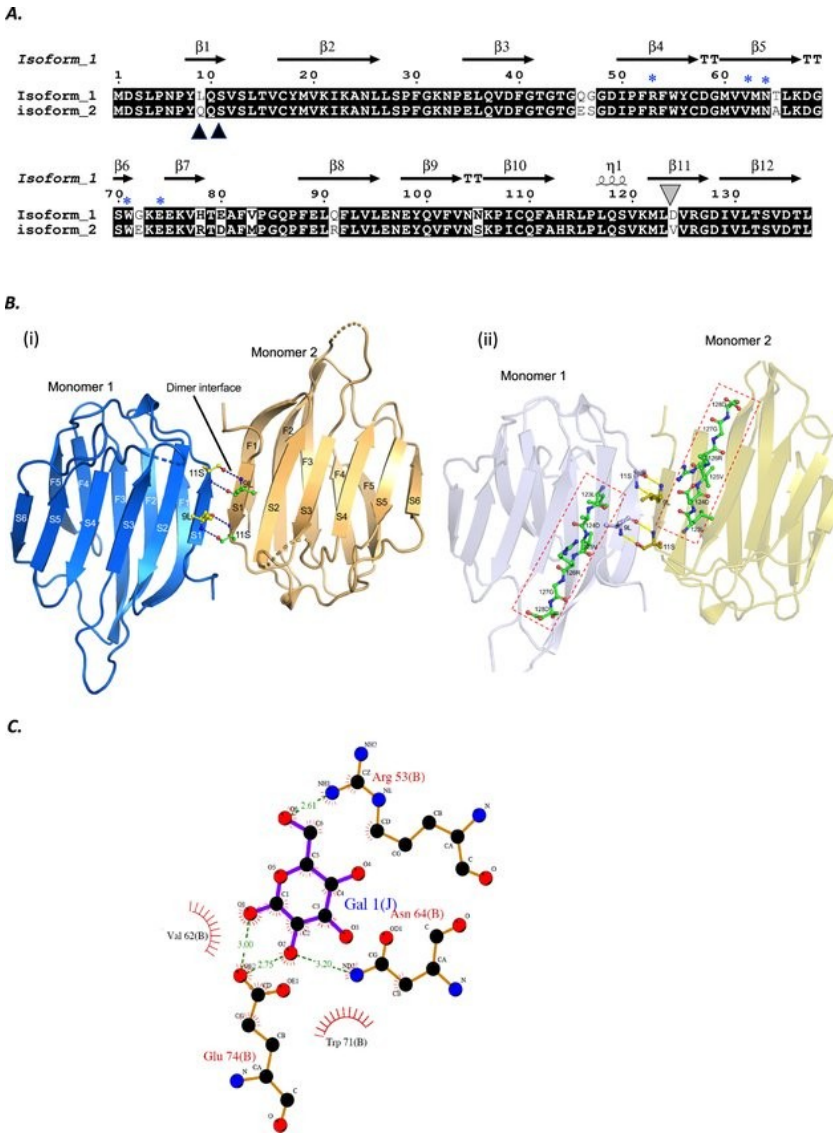


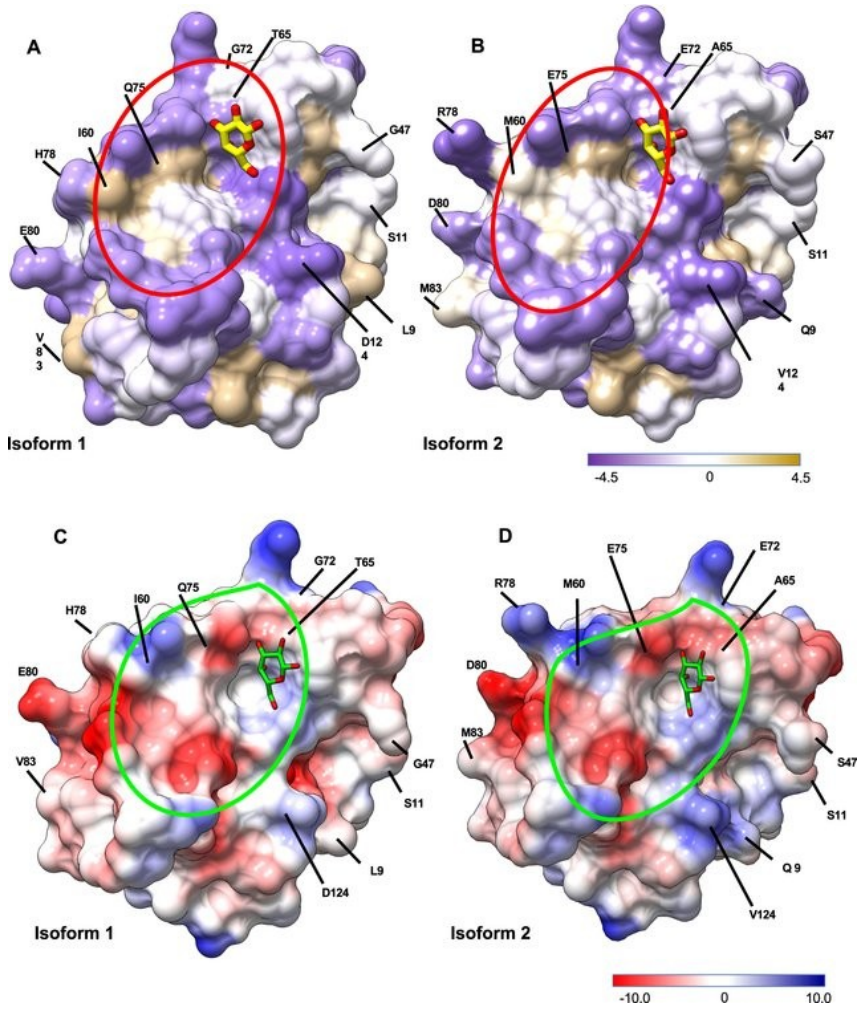
electrostatic potential

Figure 1.7 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

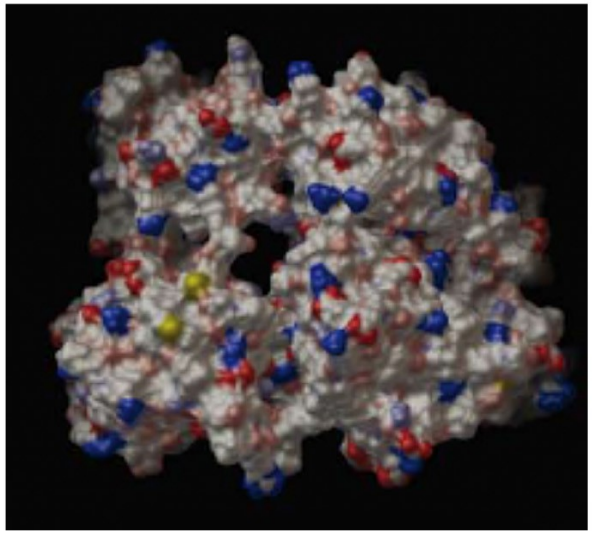
red and blue for negative and positive charges

Protein surface



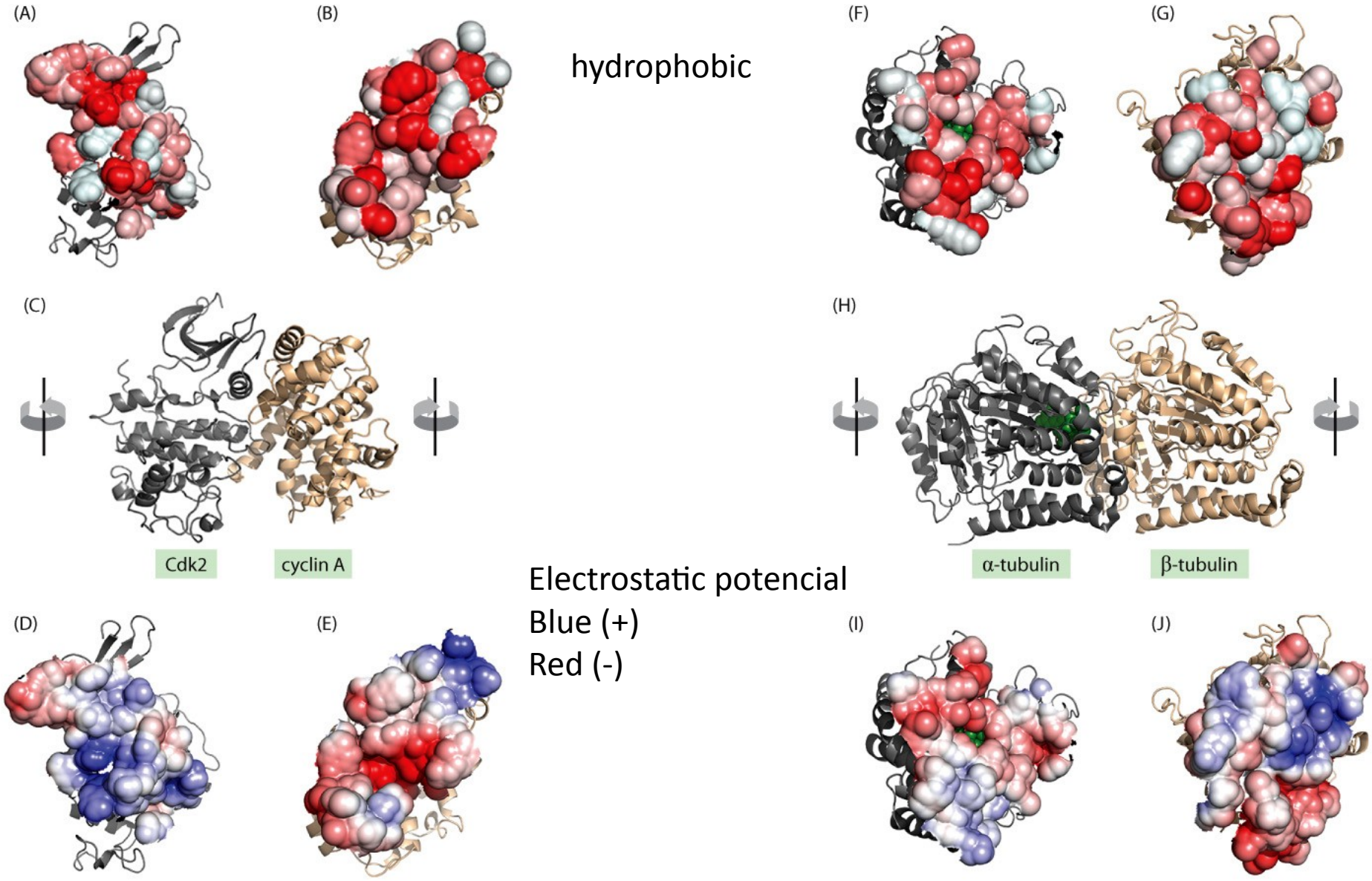


(B)



hydrophobic potential

Interactions



hydrophobic

Electrostatic potencial
Blue (+)
Red (-)

Figure 1.10 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Amino acid in oligomeric interfaces

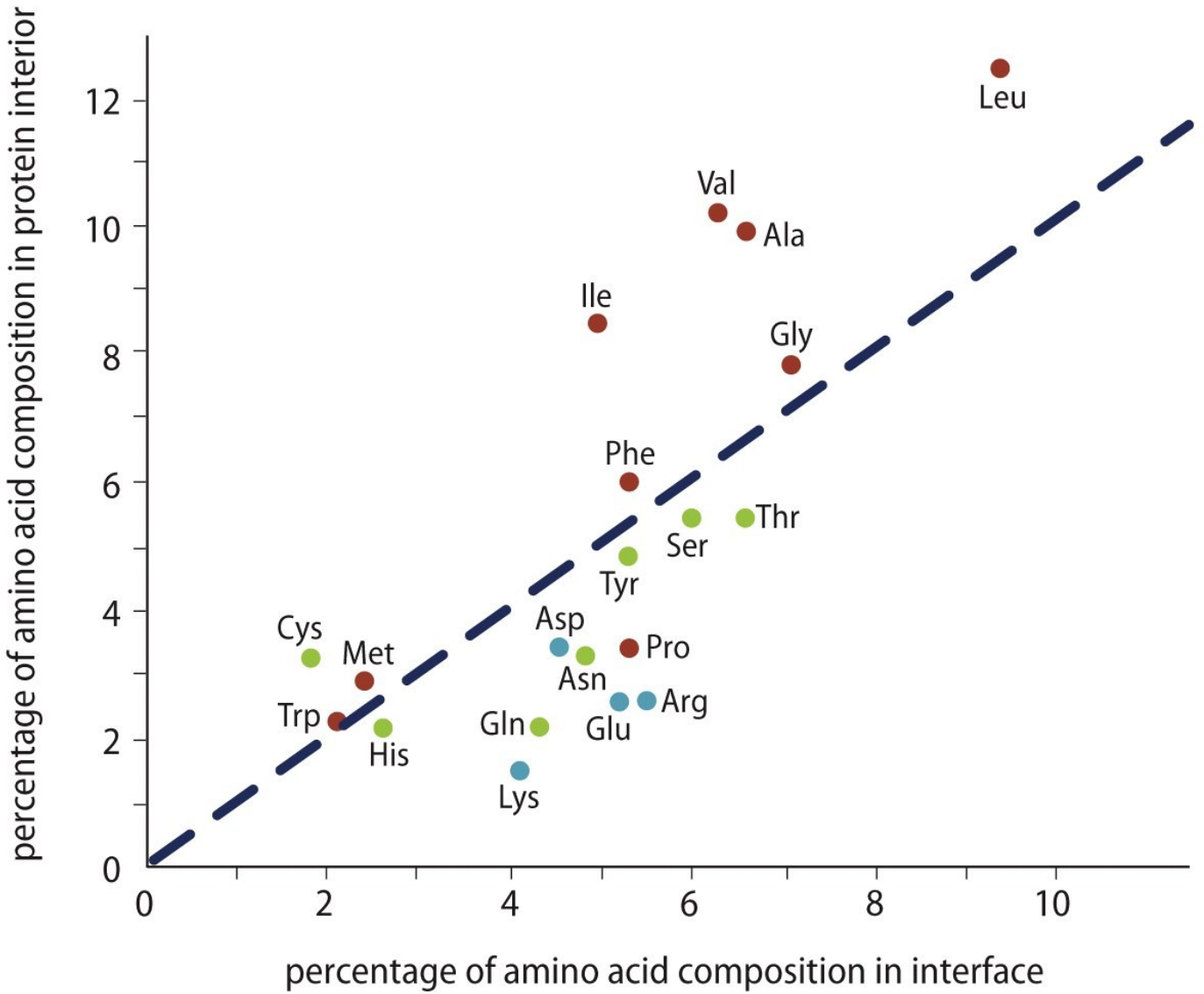


Figure 3.2 How Proteins Work (©2012 Garland Science)

regulation the accessibility of the active site

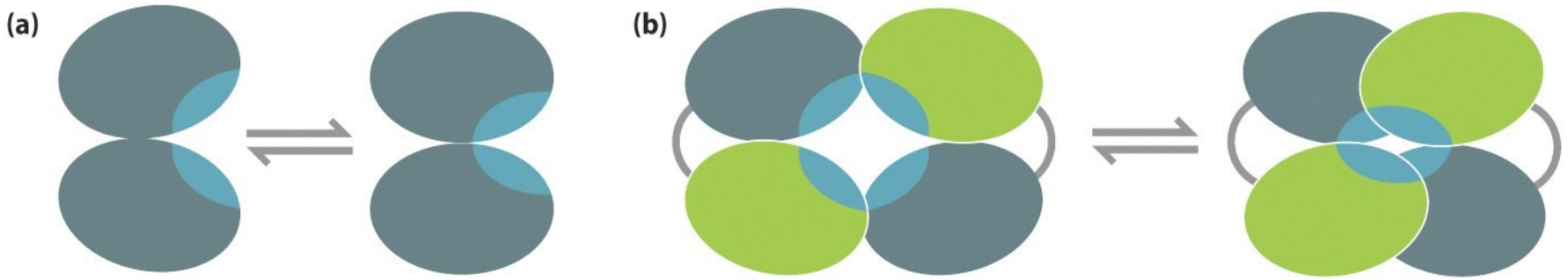
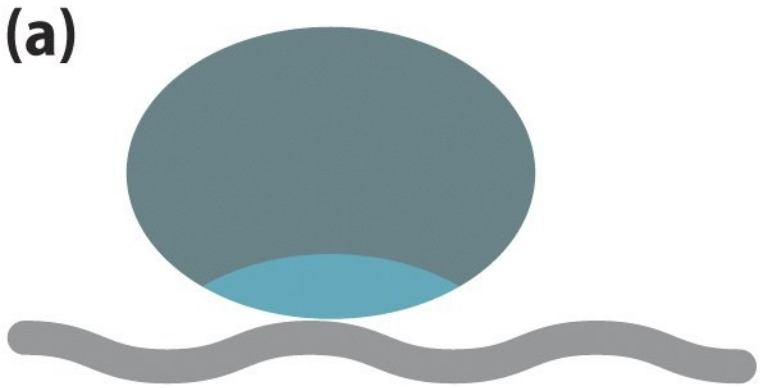


Figure 3.3 How Proteins Work (©2012 Garland Science)

selectivity

Exolytic and endolytic



Exolytic

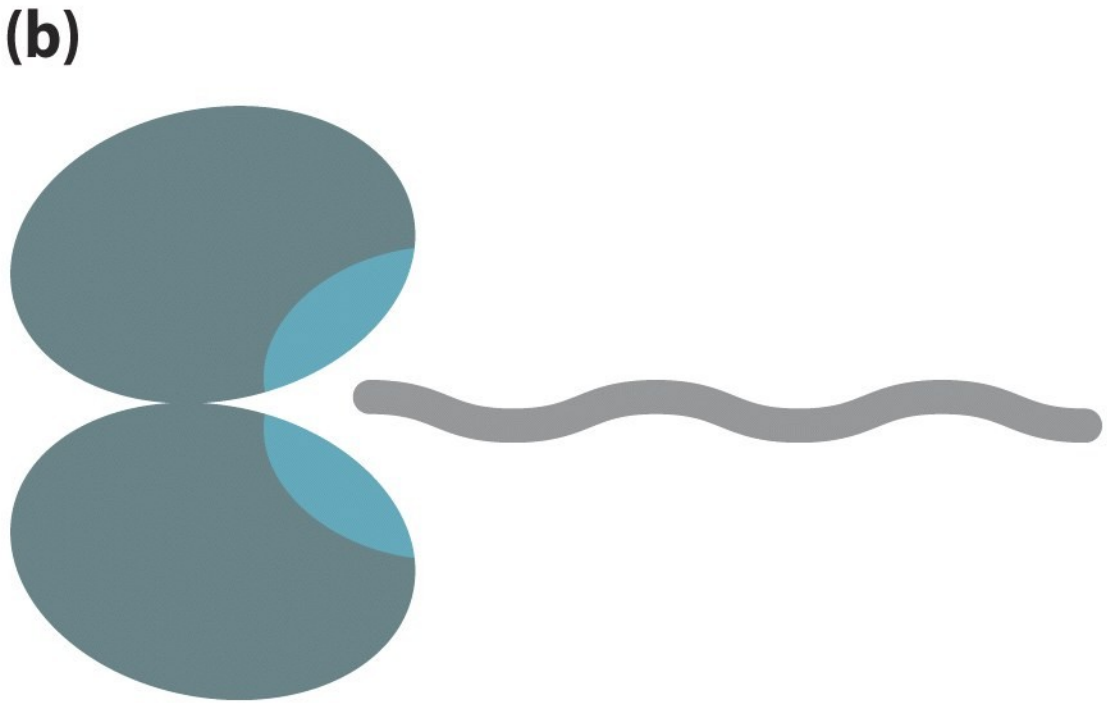


Figure 3.4 How Proteins Work (©2012 Garland Science)

evolution of allosteric effectors

The interface of two domains also creates the possibility of **allostery**

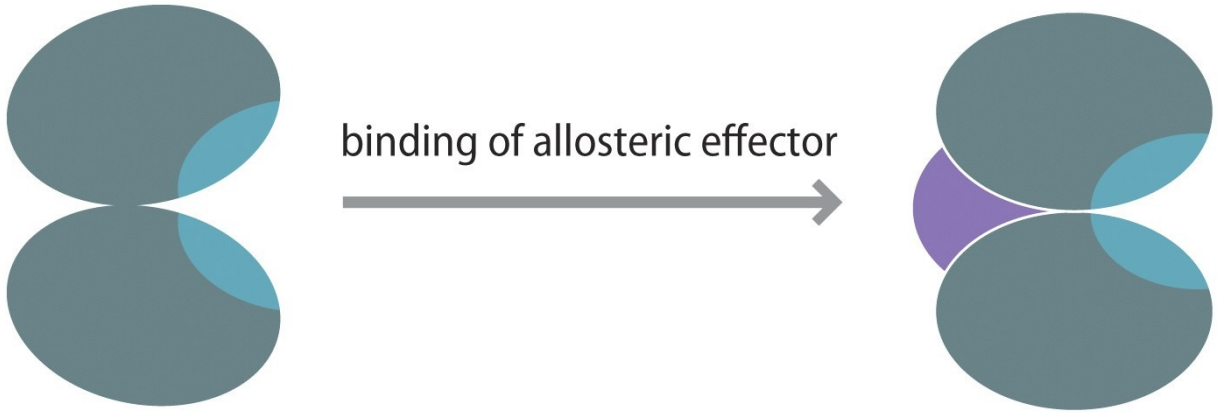


Figure 3.5 How Proteins Work (©2012 Garland Science)

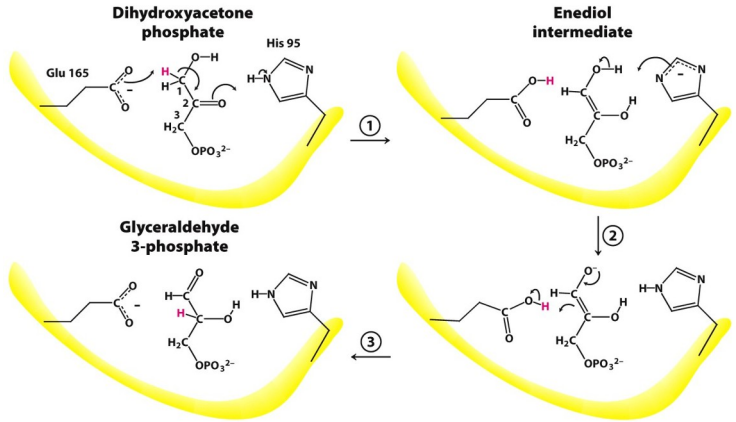


Figure 16.5
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

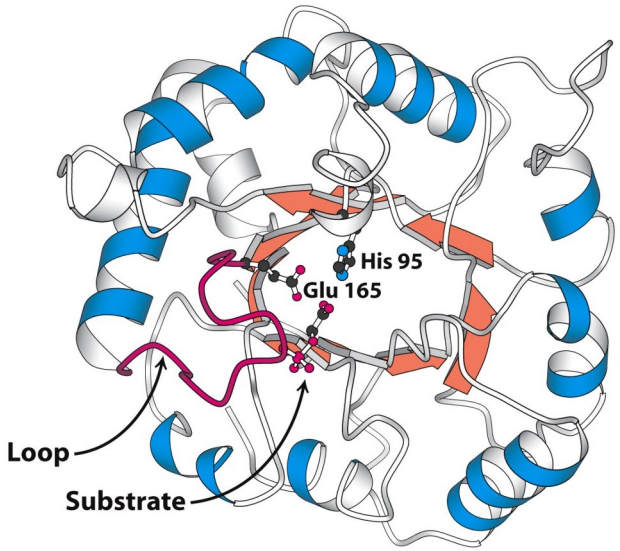
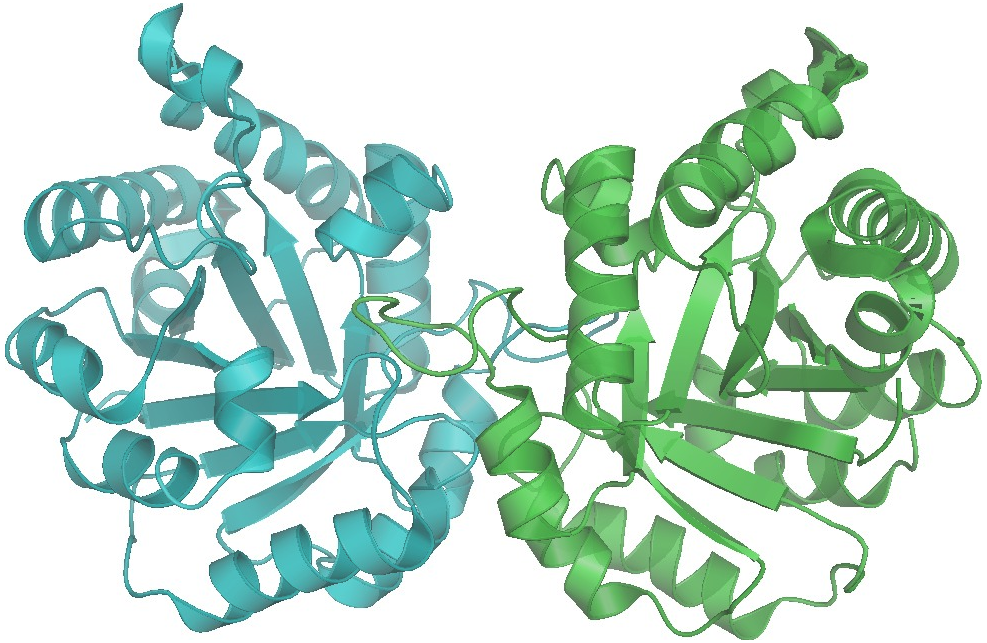
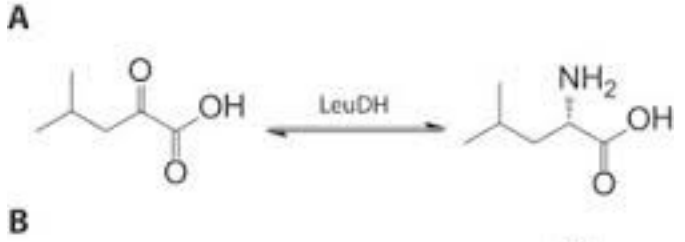


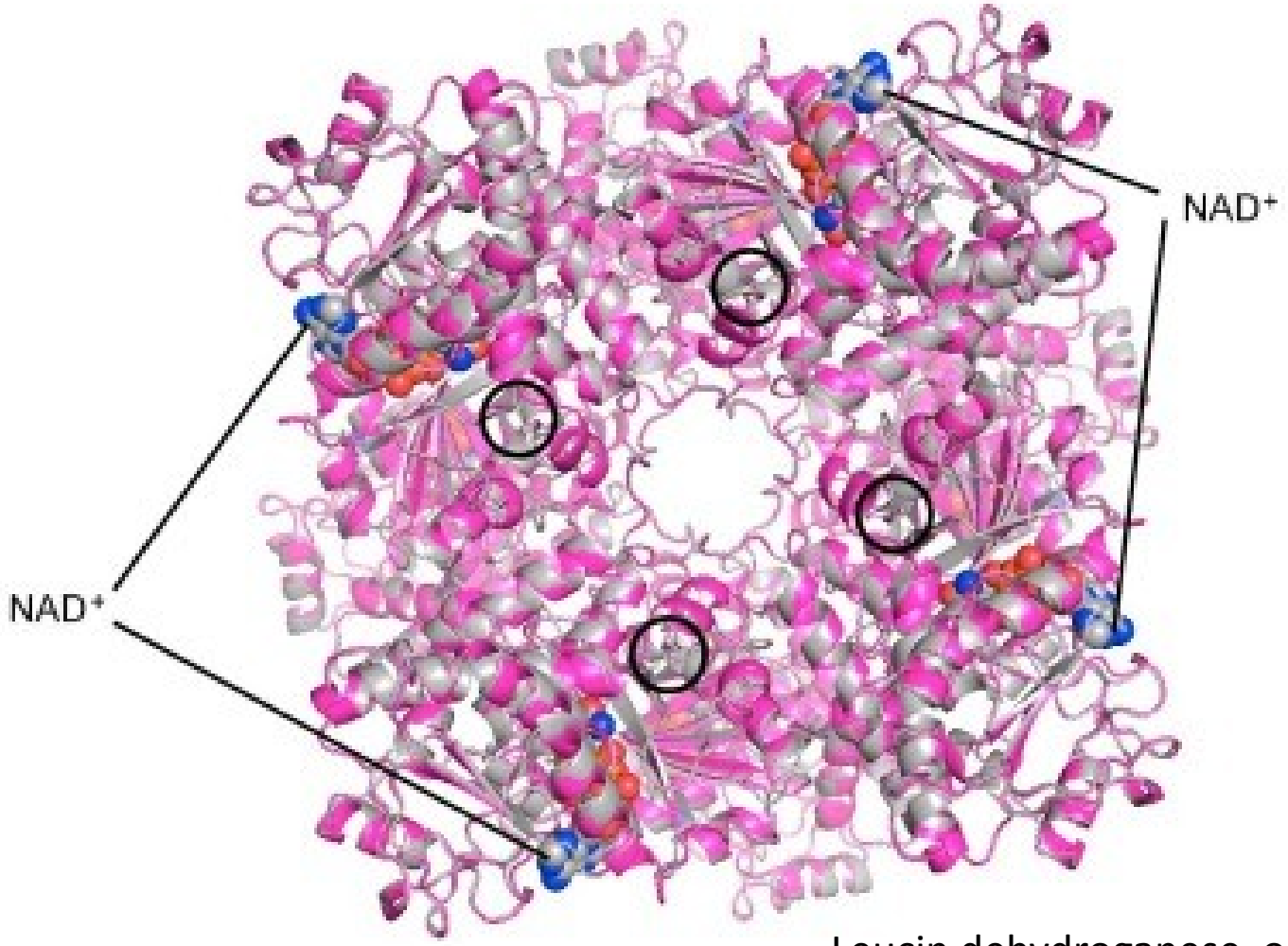
Figure 16.4
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

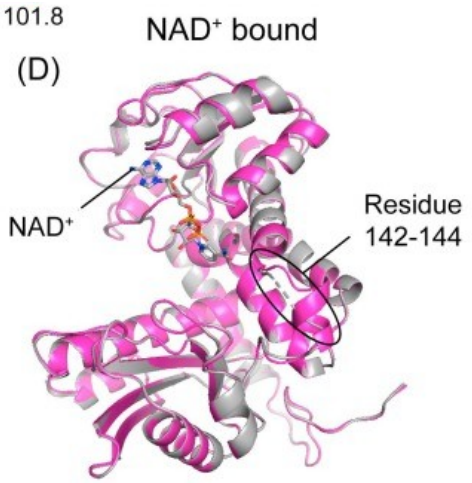


improve of enzyme functionality

Enzymes
low K_m high k_{cat}

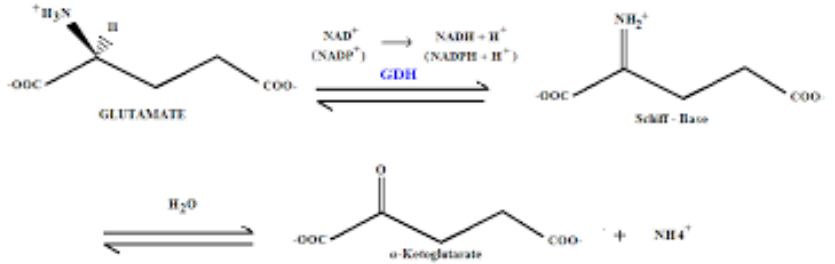


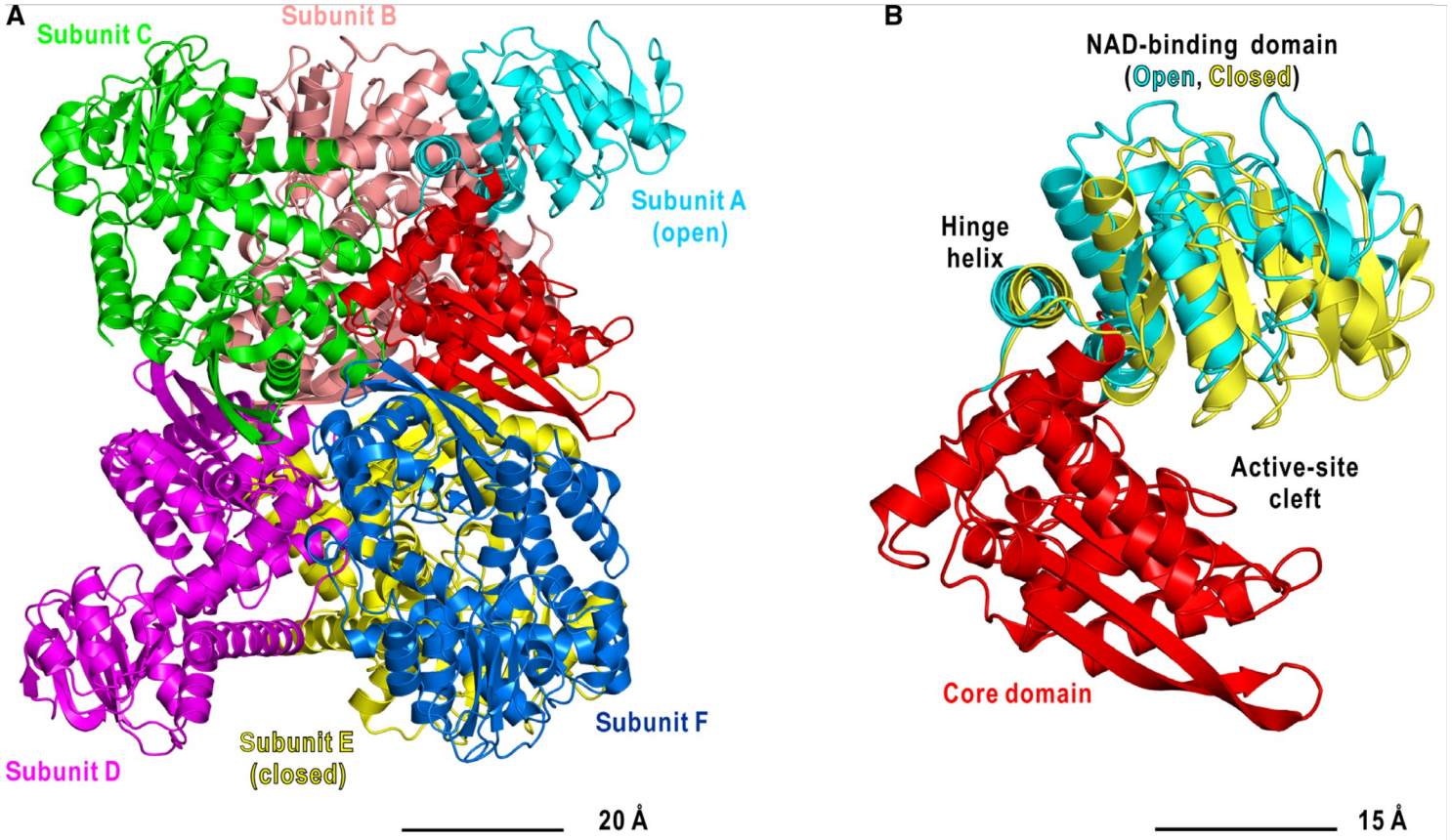




Leucin dehydroganese octamer

improve of enzyme functionality

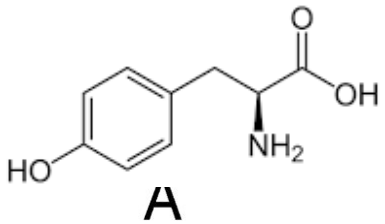




Glutamate dehydrogenase

improve of enzyme functionality

tyrosyl-tRNA and tryptophanyl-tRNA synthetases



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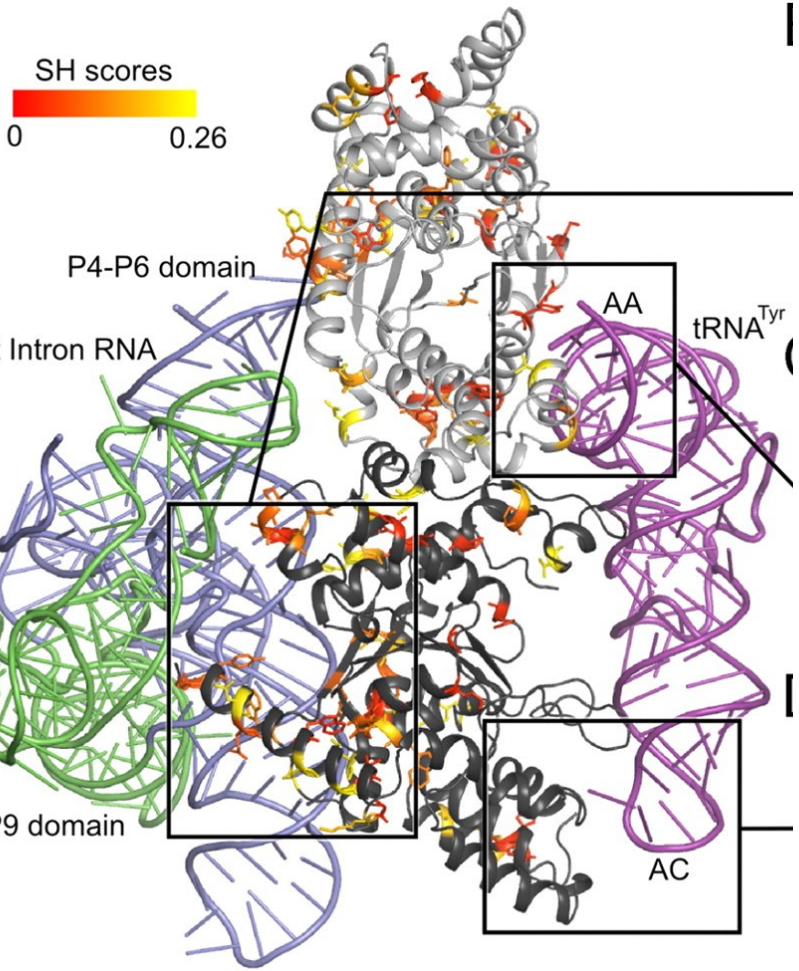
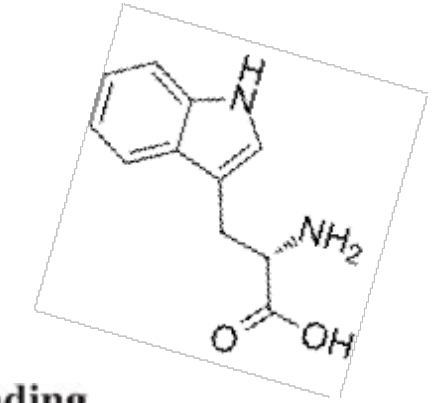
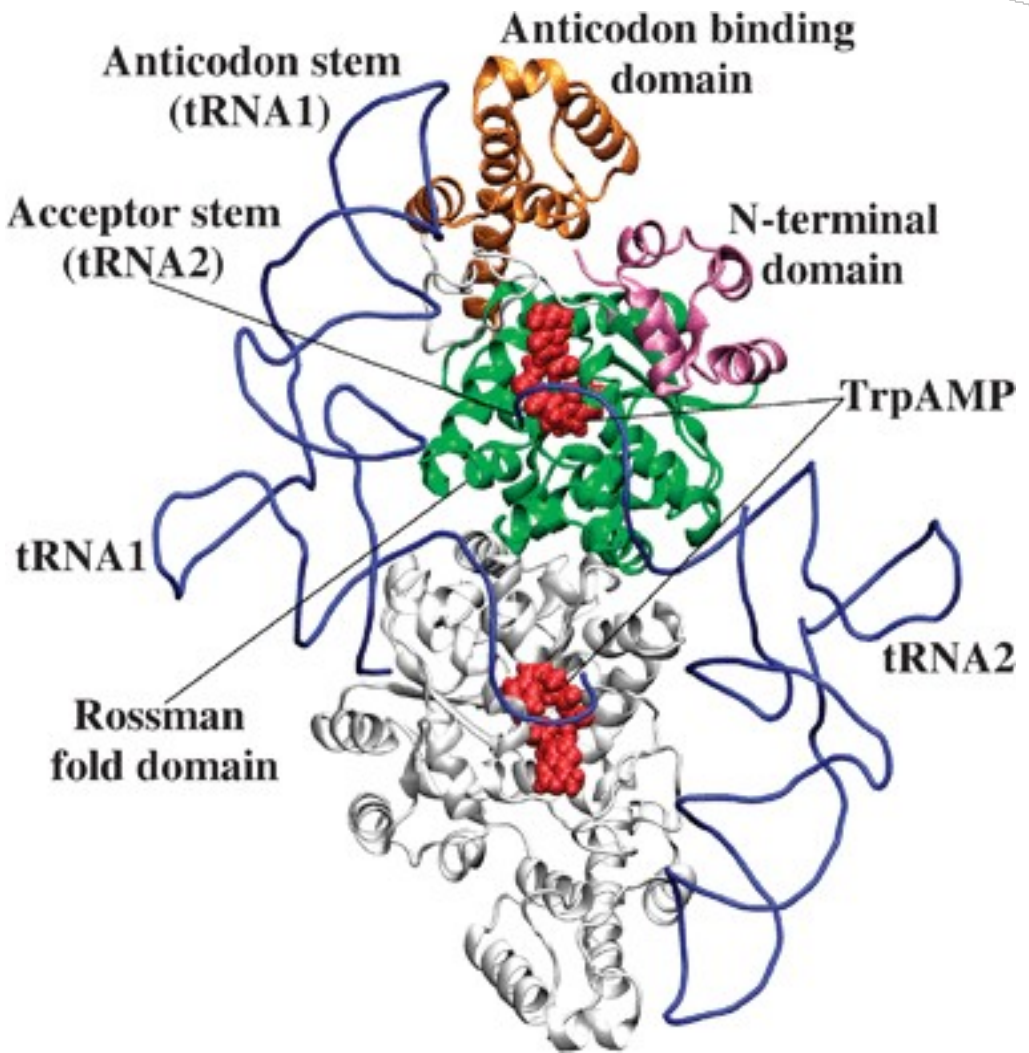






monomer/oligomer

activation of caspase-7 by proteolytic cleavage

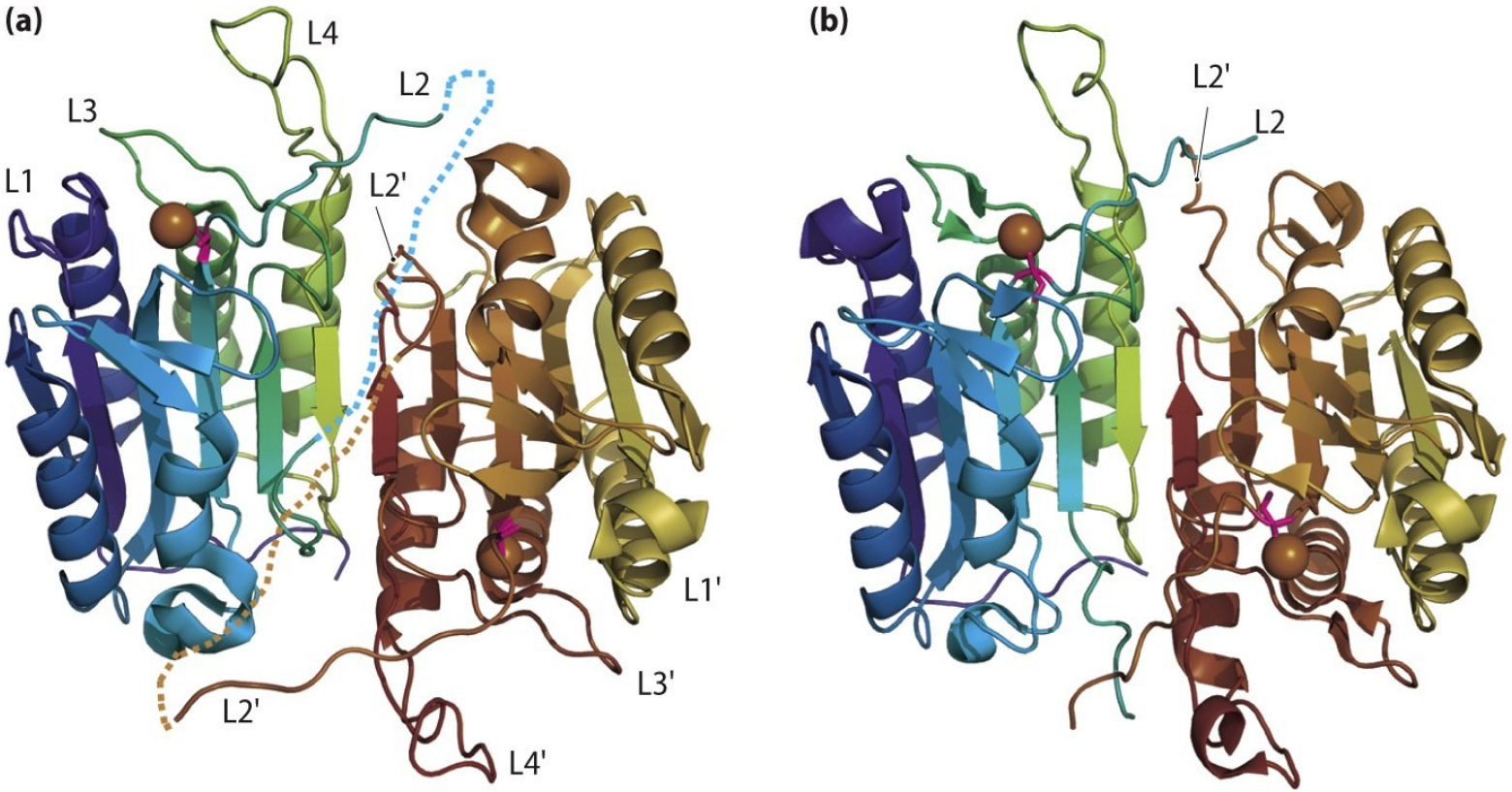
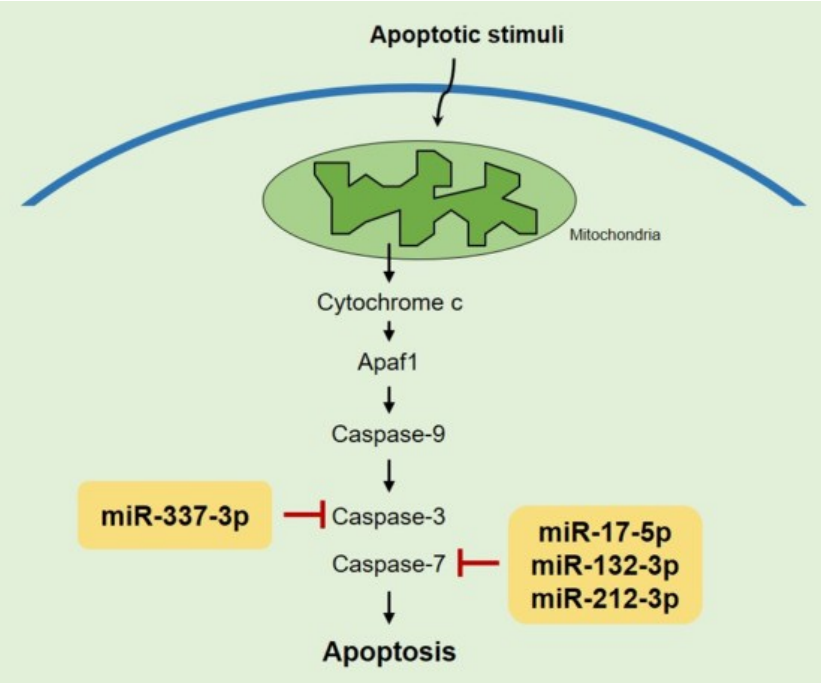
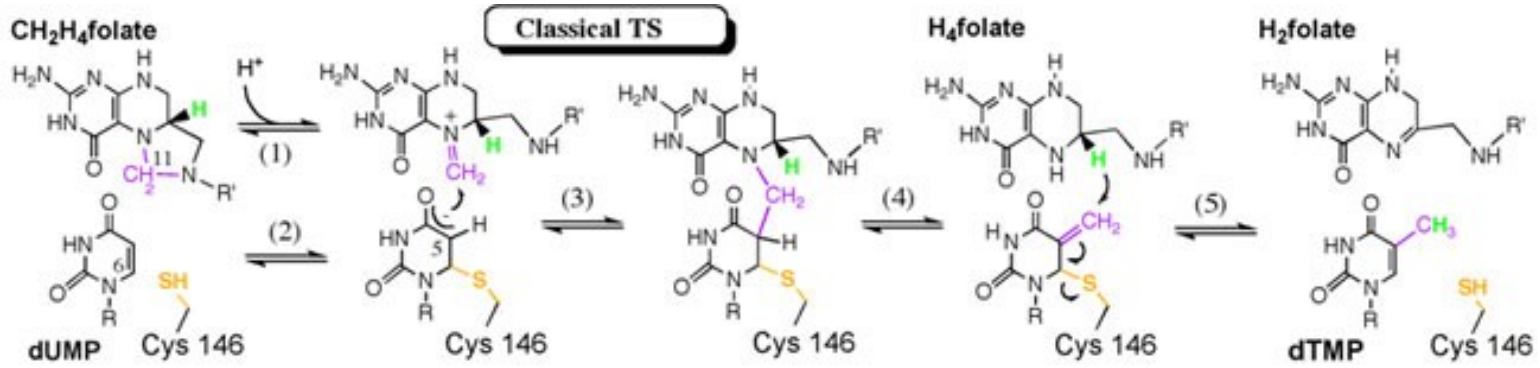
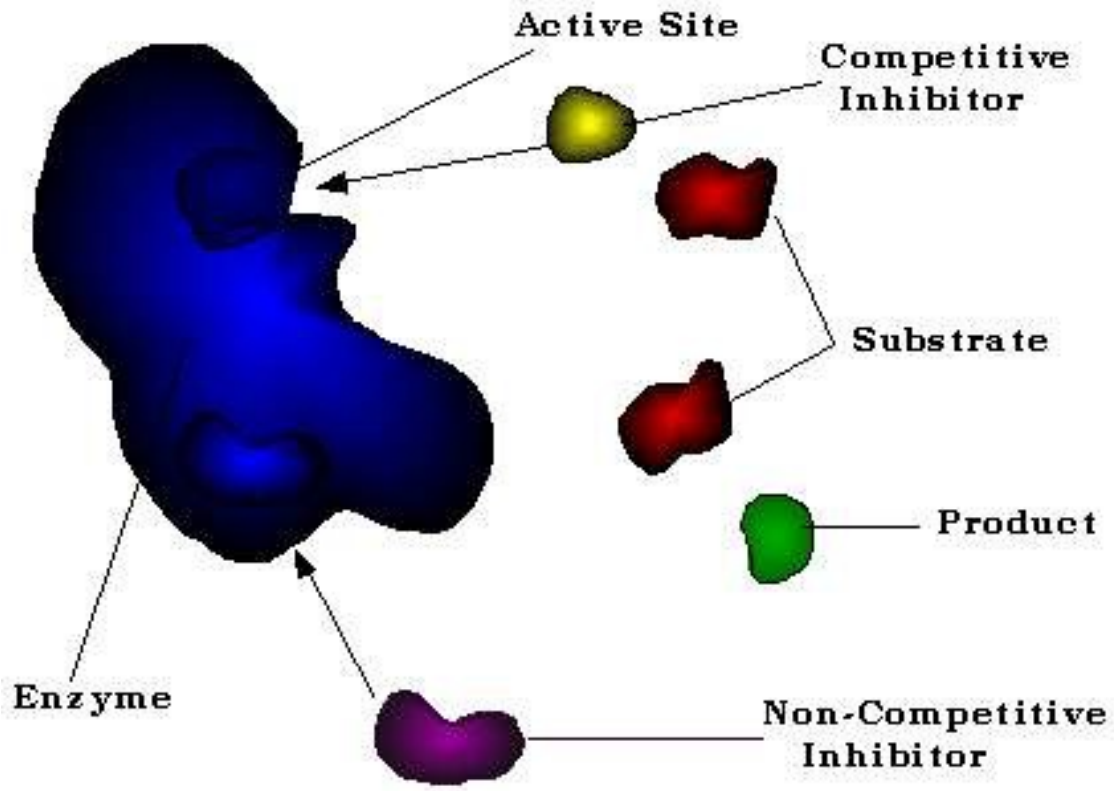


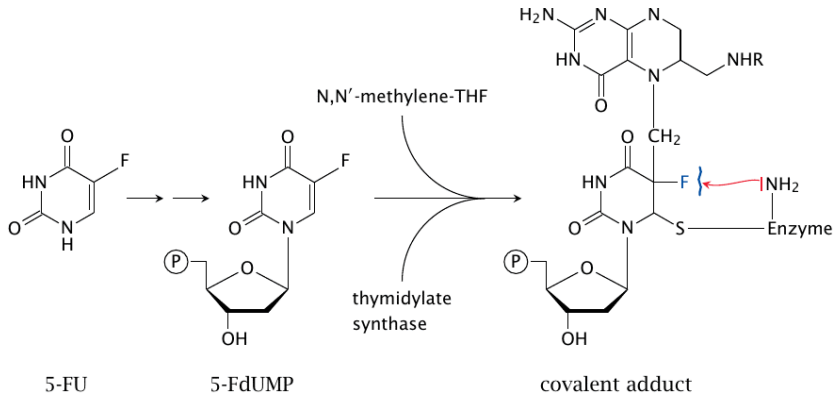
Figure 3.7 How Proteins Work (©2012 Garland Science)



Enzyme regulation







Irreversible
Active site-directed inhibitors

ligand-induced conformational change

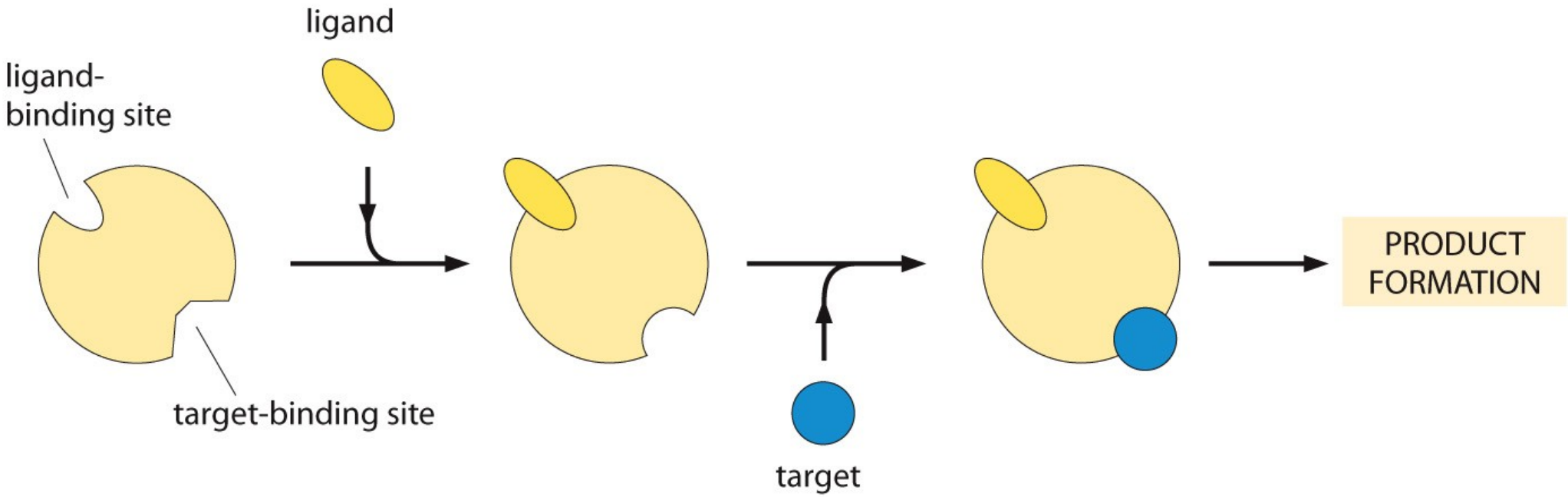
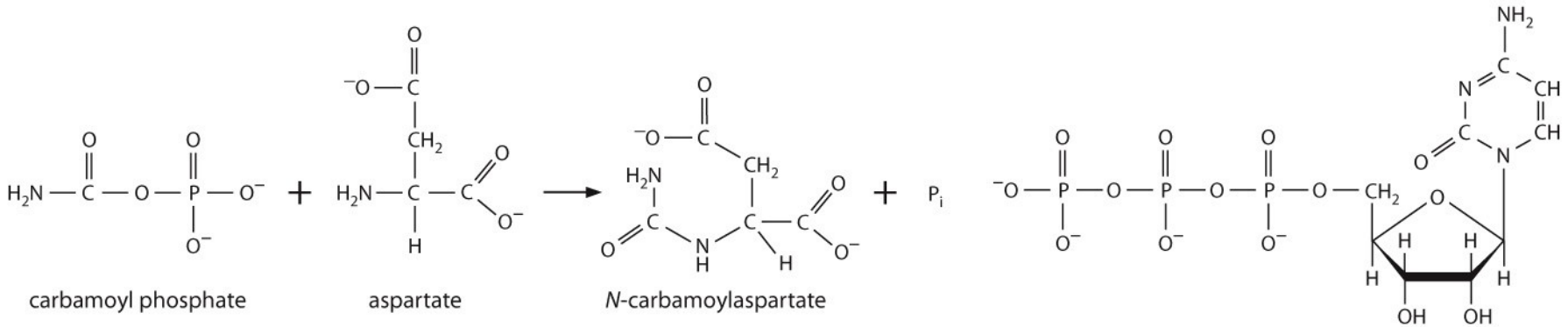


Figure 1.30 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

allosteric regulation of a multi-subunit enzyme

A → B → C→ D → E



aspartate transcarbamoylase

Figure 1.31 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

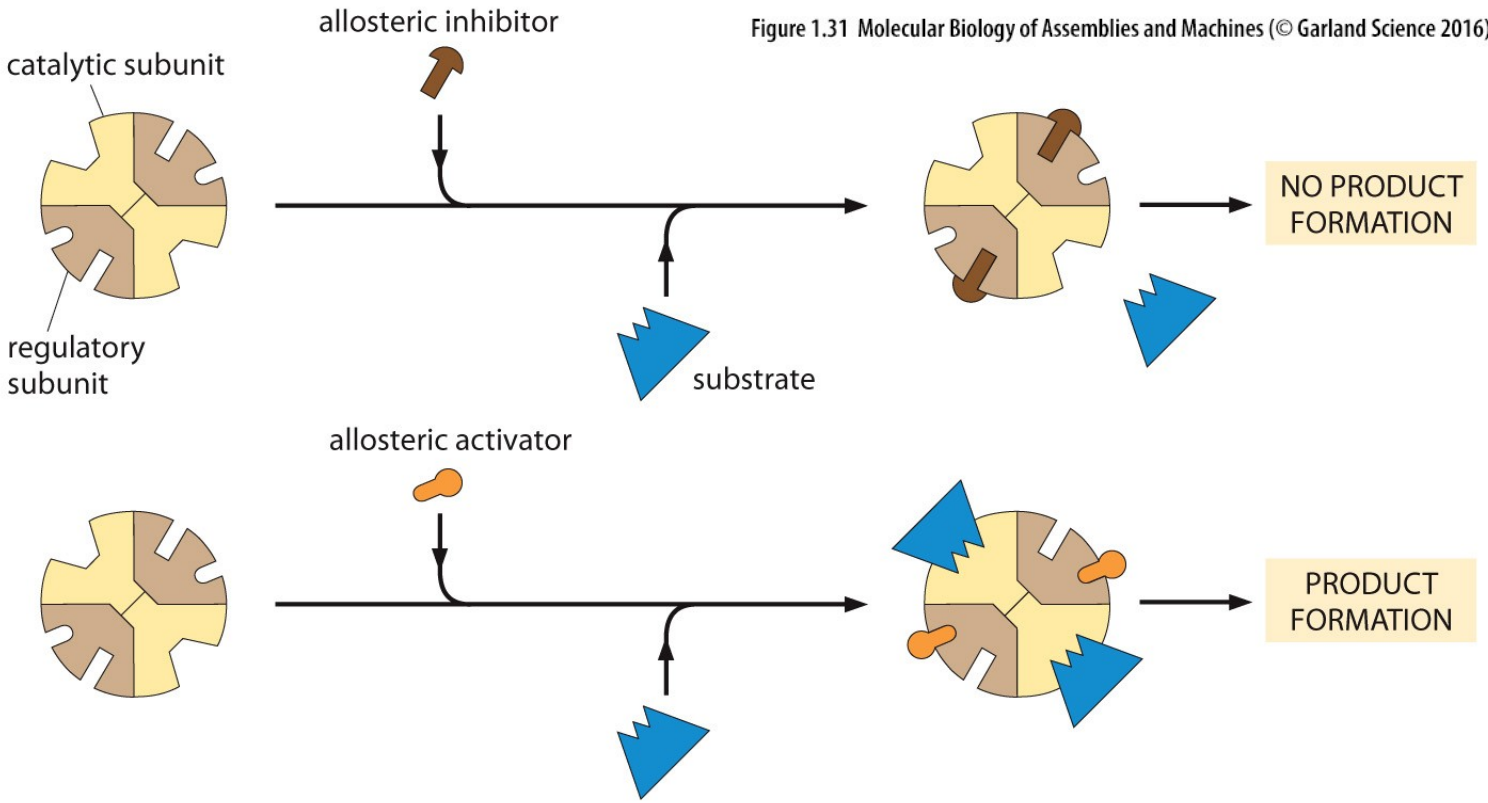


Figure 1.32 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

kinetics of allosteric enzymes

$L_0=[T]/[R]$

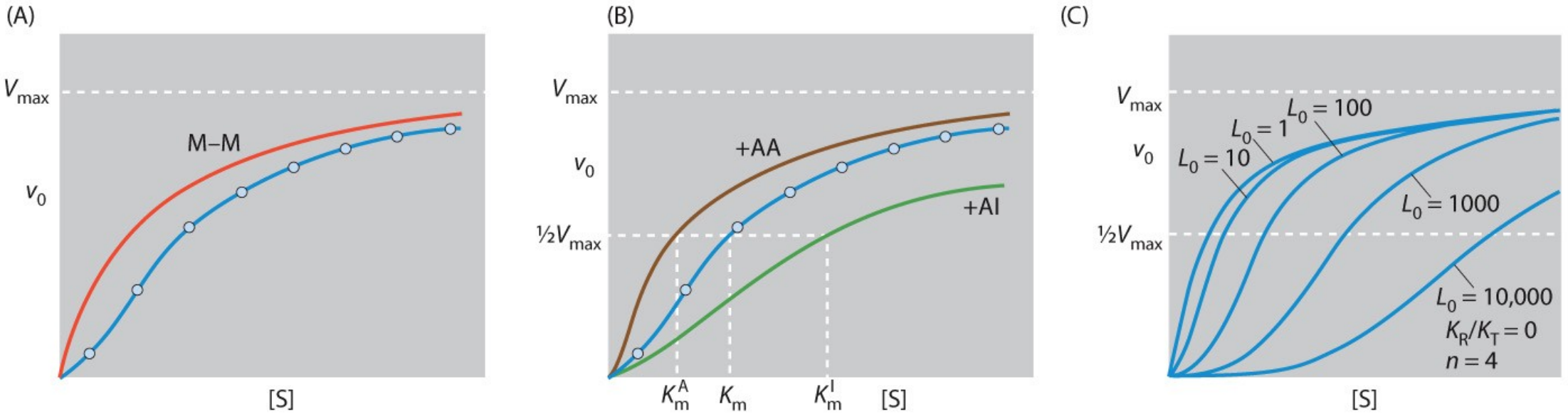


Figure 1.33 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

allostery

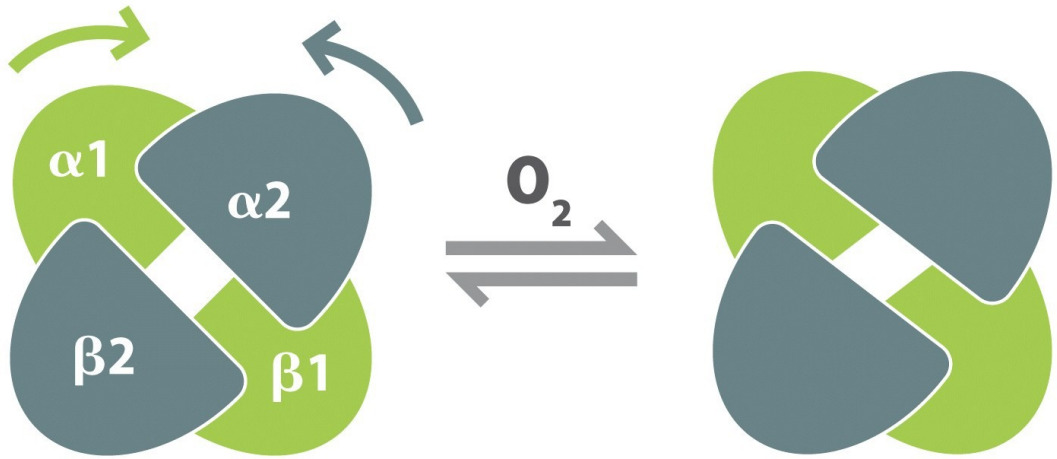
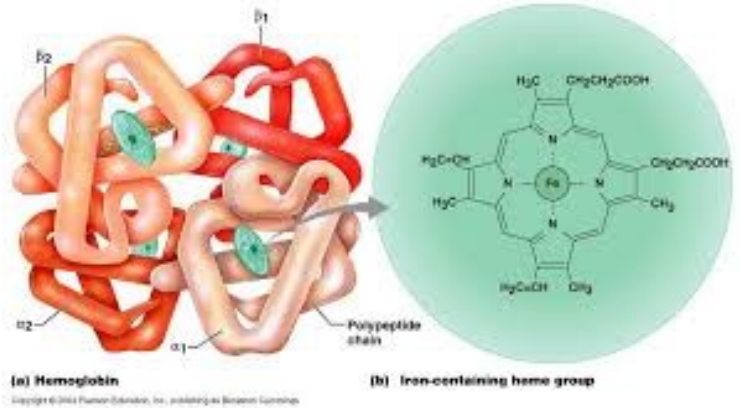
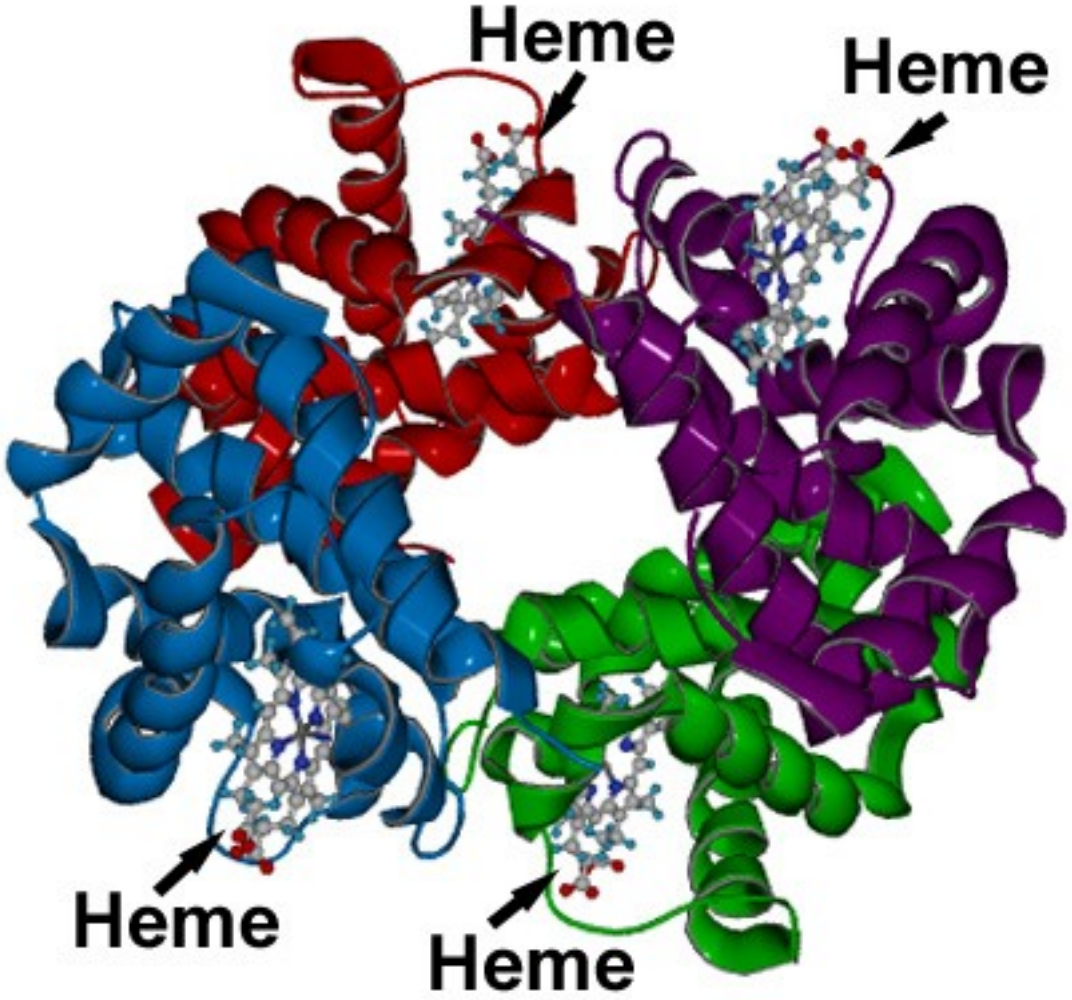


Figure 3.10 How Proteins Work (©2012 Garland Science)





Hemoglobin

effects of oxygen binding

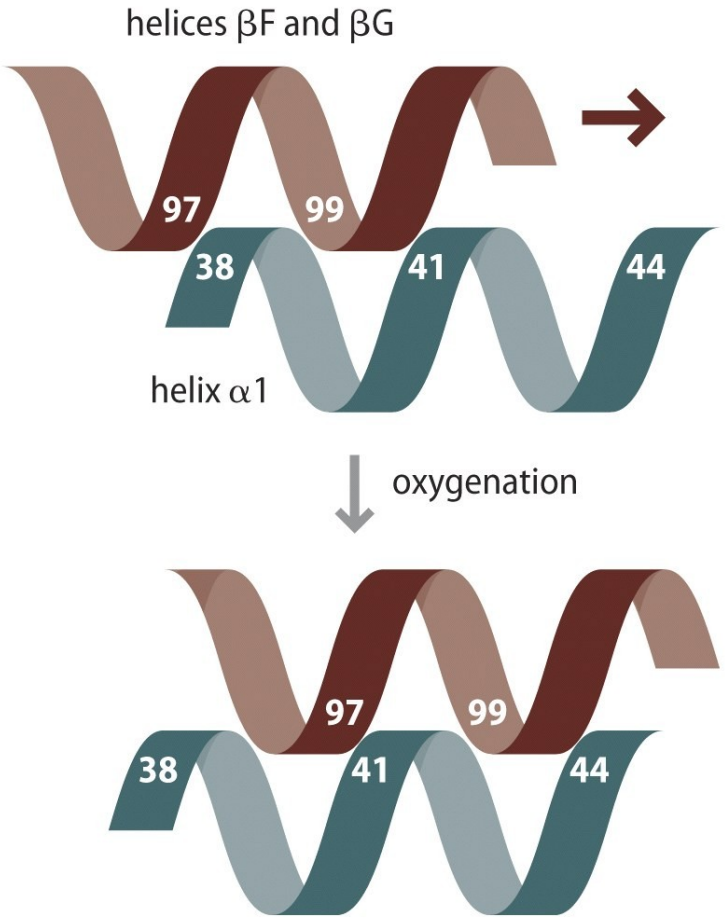


Figure 3.12 How Proteins Work (©2012 Garland Science)

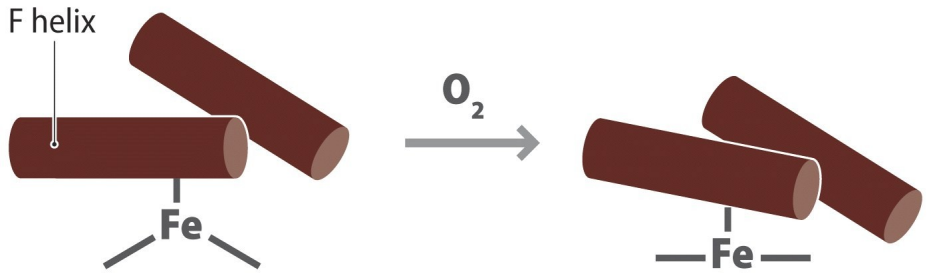
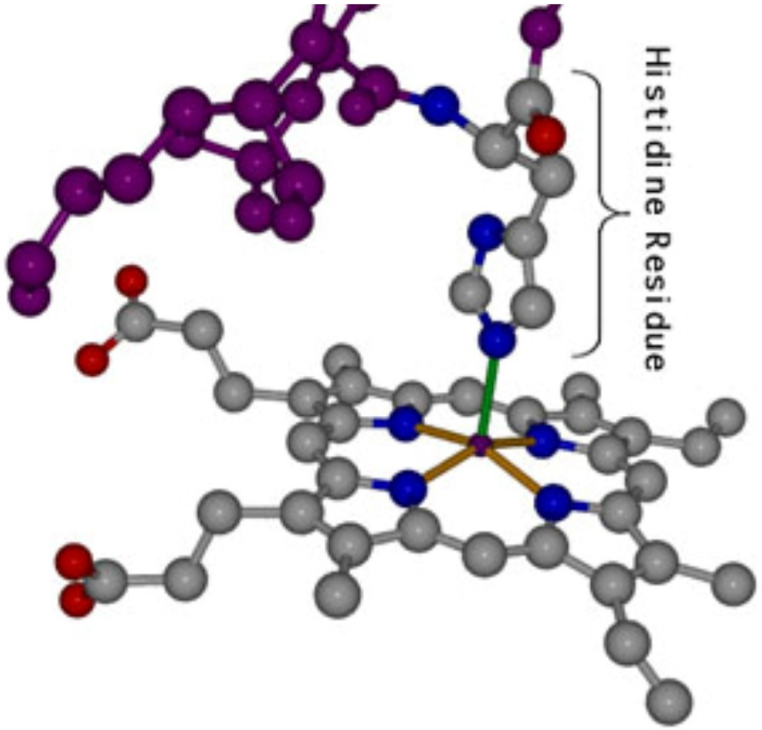
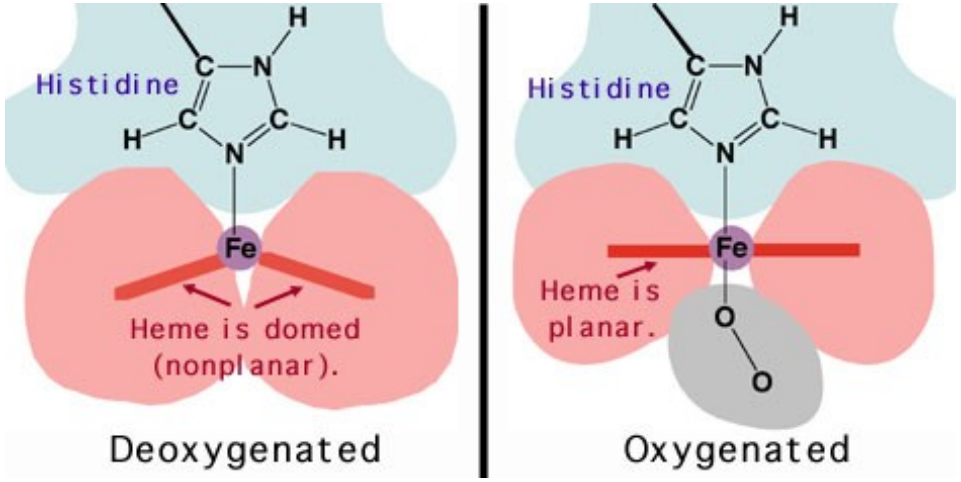


Figure 3.11 How Proteins Work (©2012 Garland Science)





oxygen saturation-cooperative binding

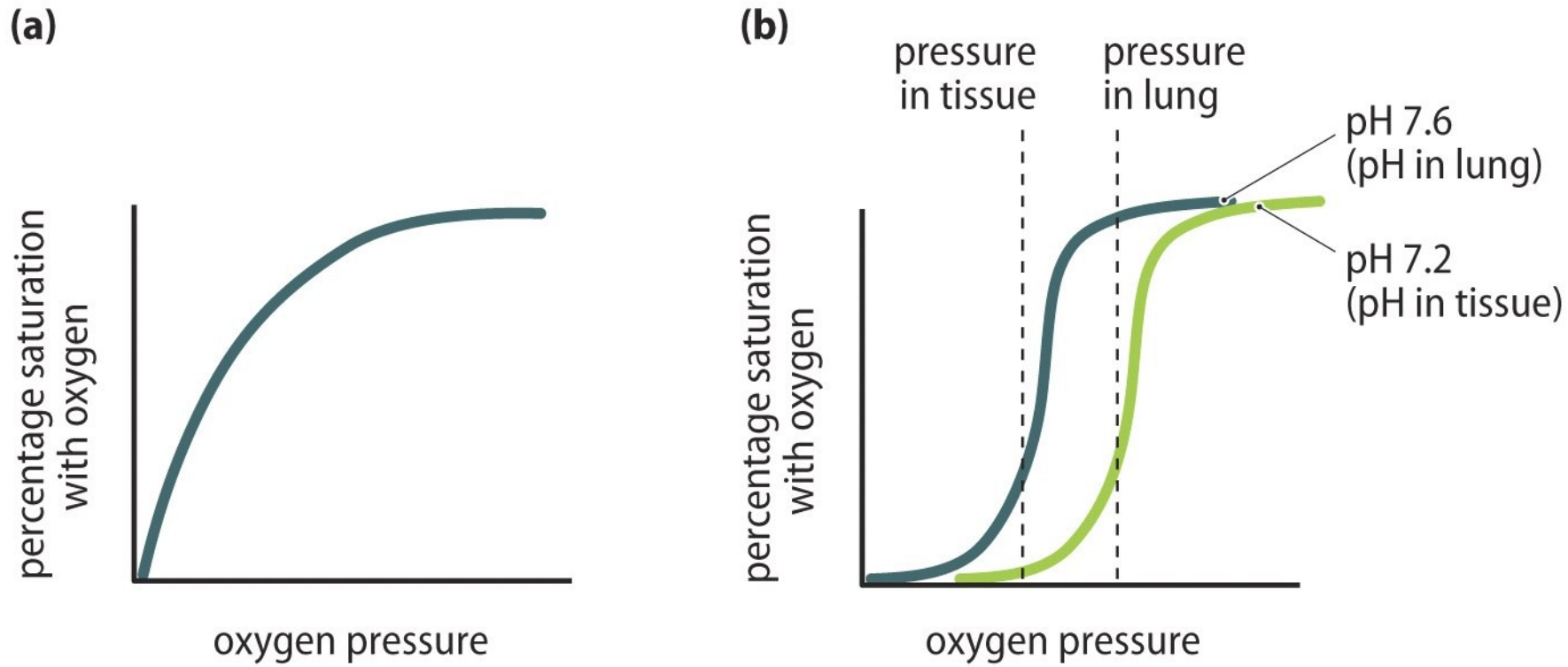
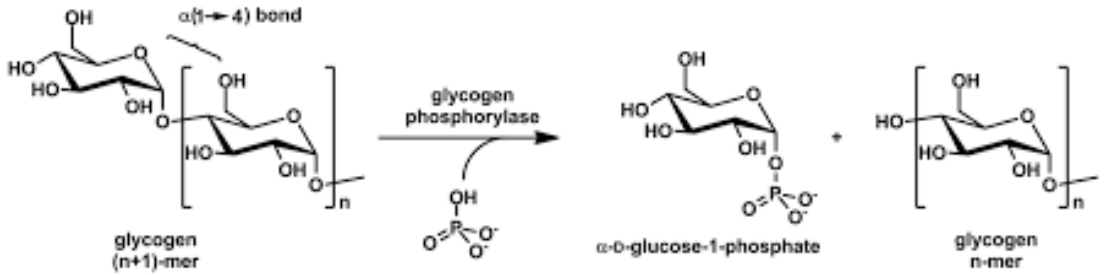


Figure 3.14 How Proteins Work (©2012 Garland Science)

$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$

glycogen phosphorylase



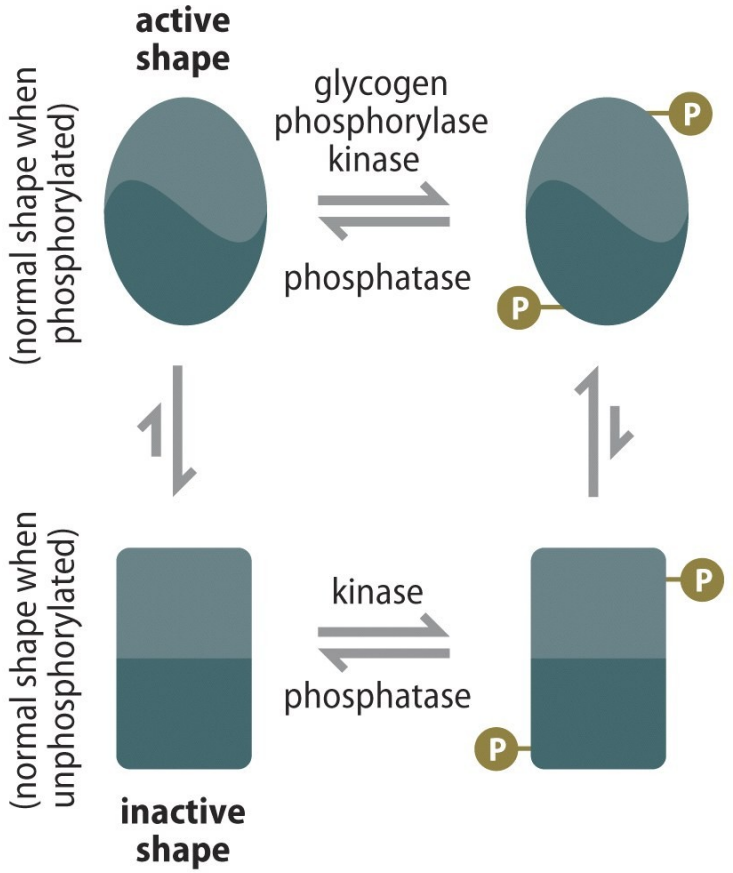
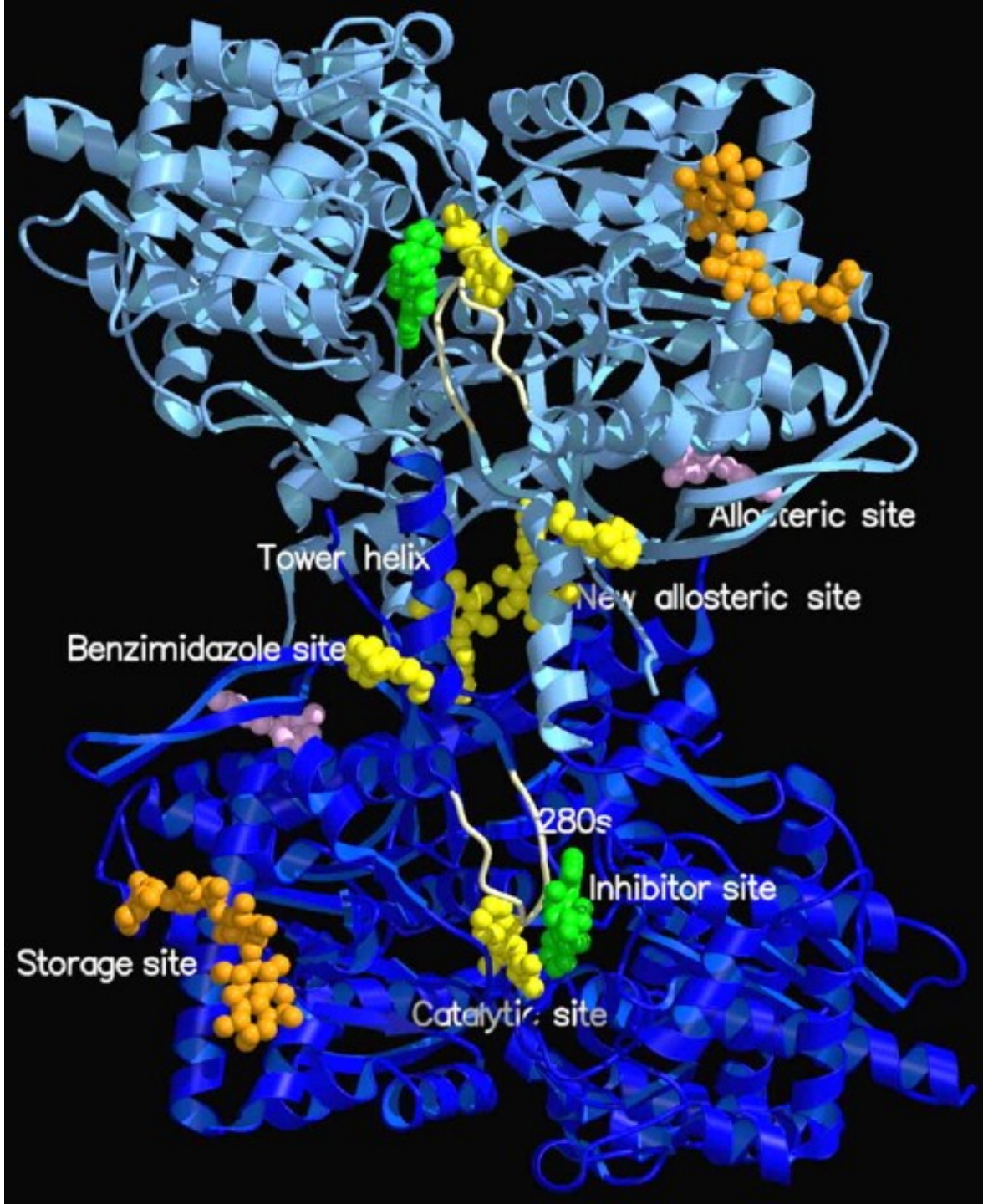


Figure 3.19 How Proteins Work (©2012 Garland Science)



glycogen phosphorylase

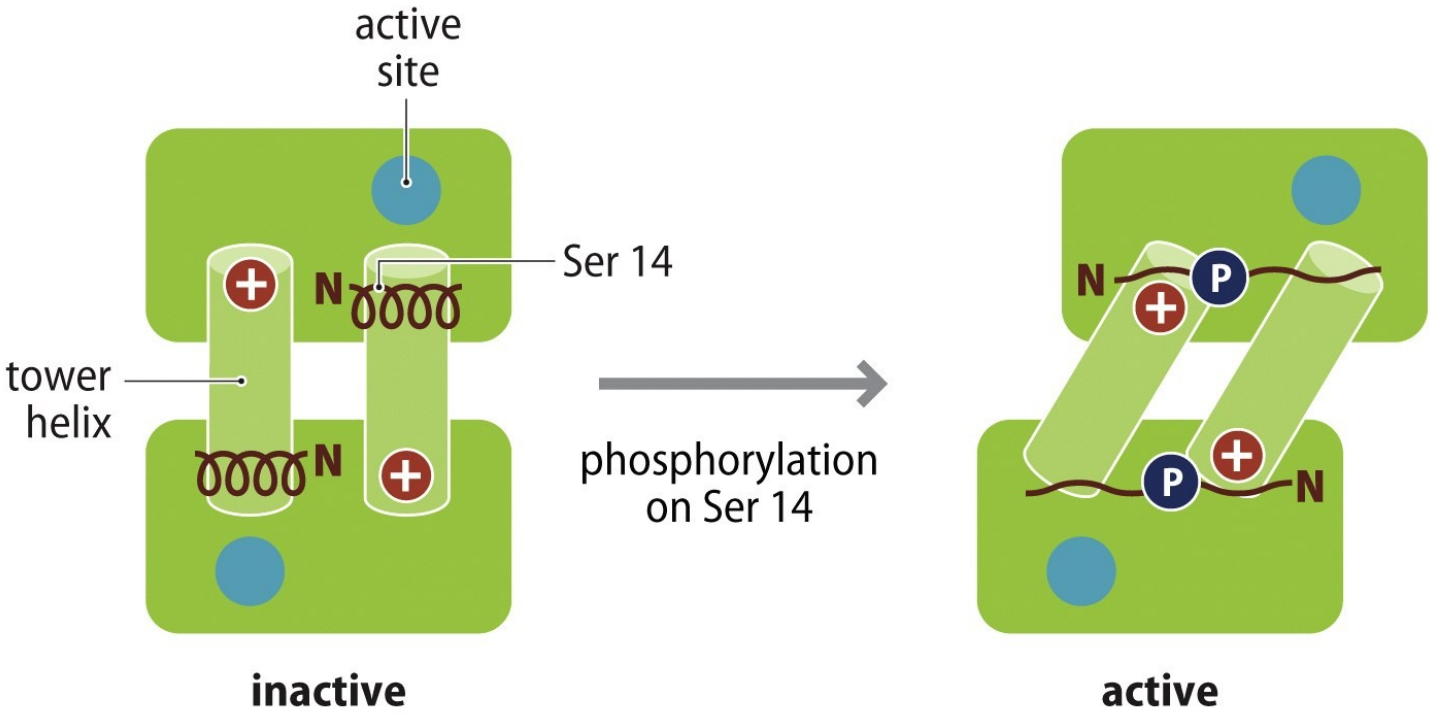
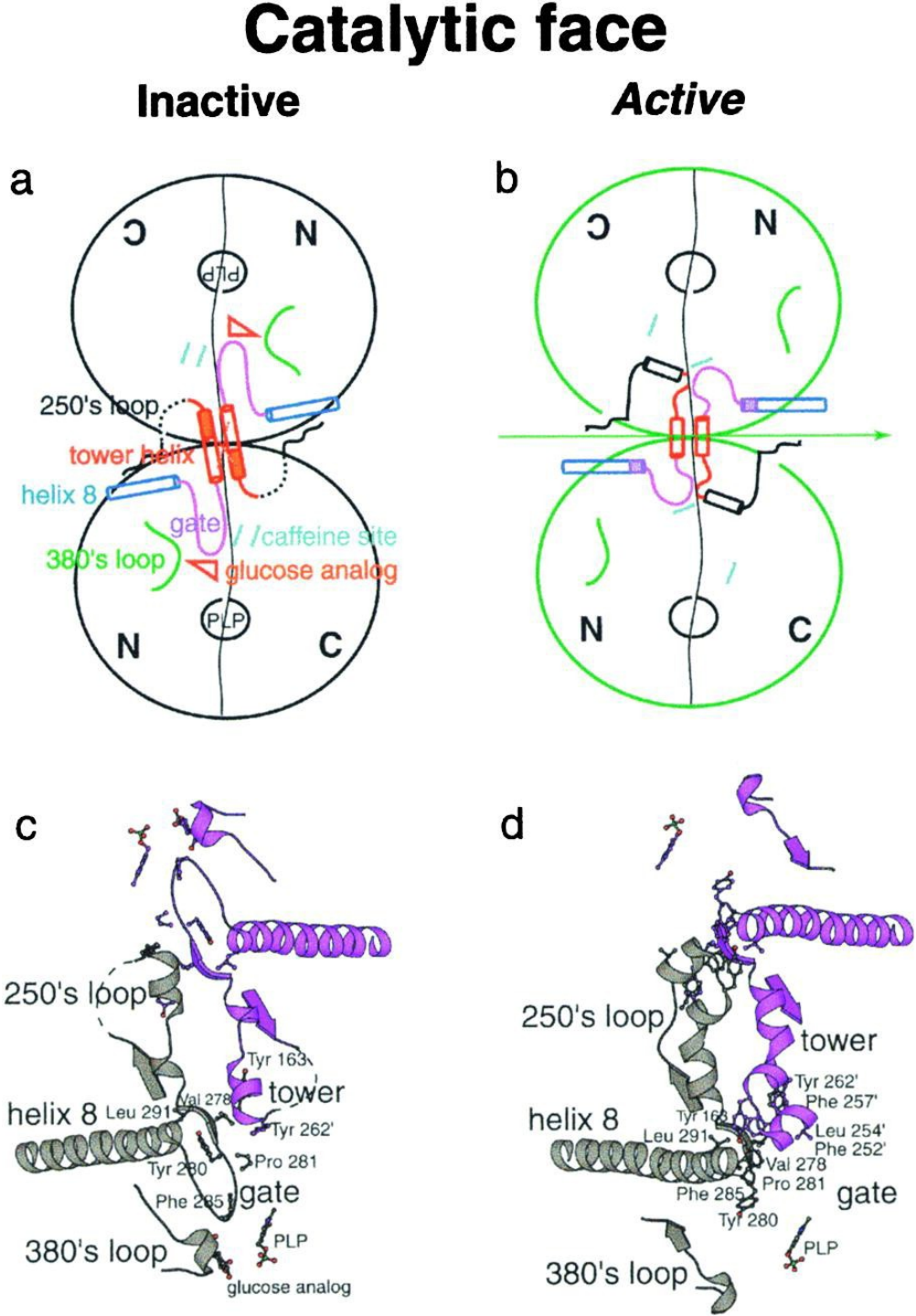
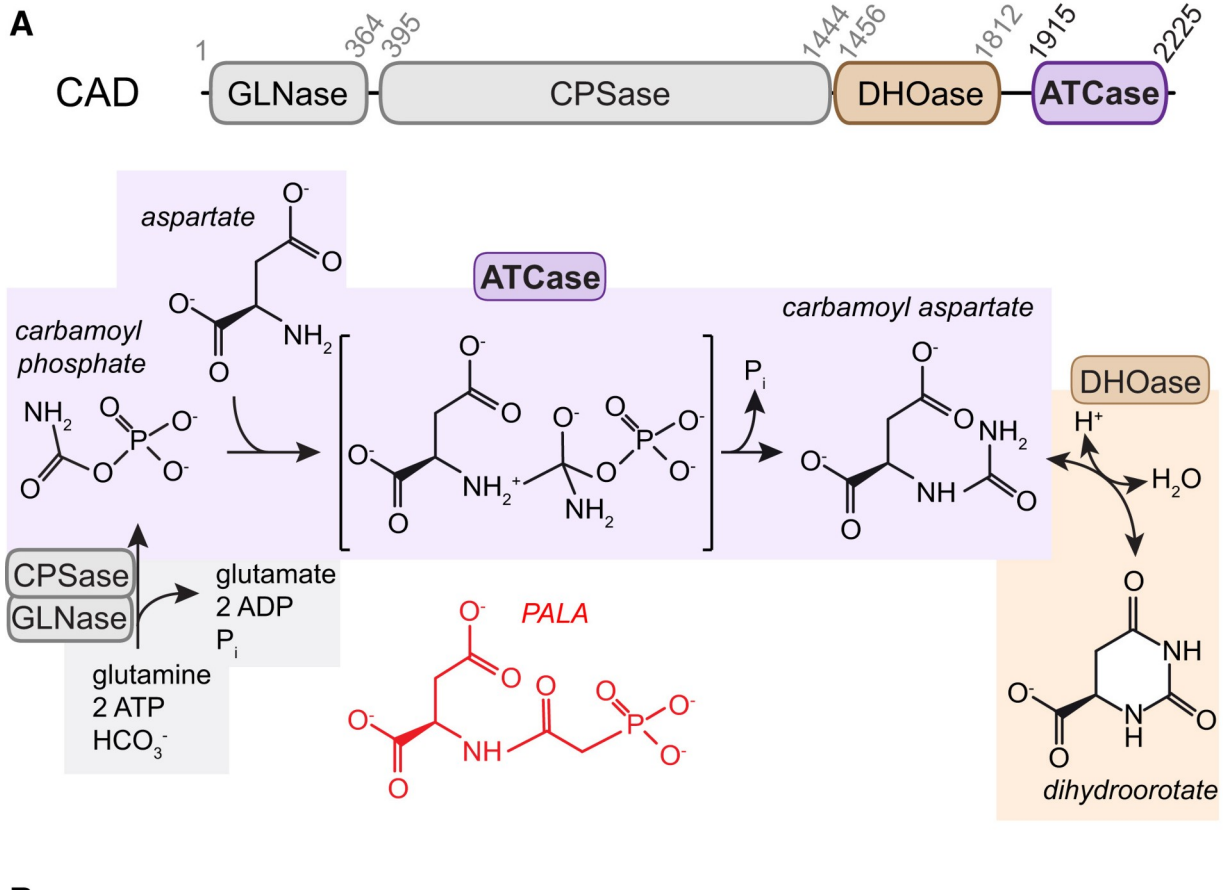
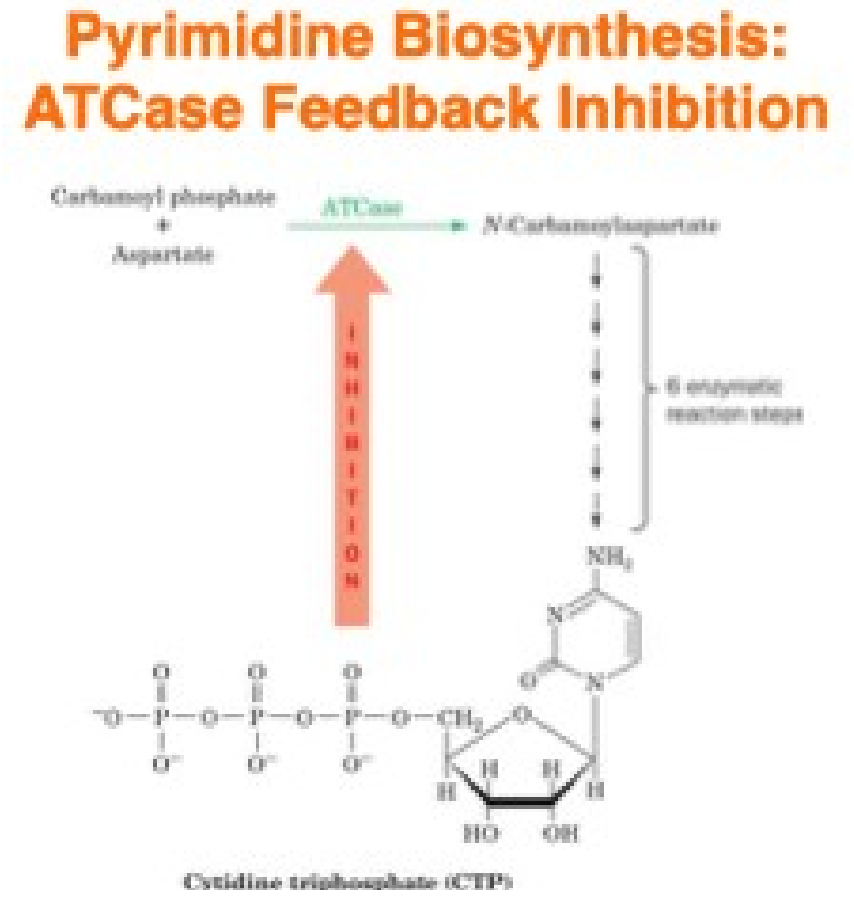


Figure 3.20 How Proteins Work (©2012 Garland Science)



aspartate transcarbamoylase

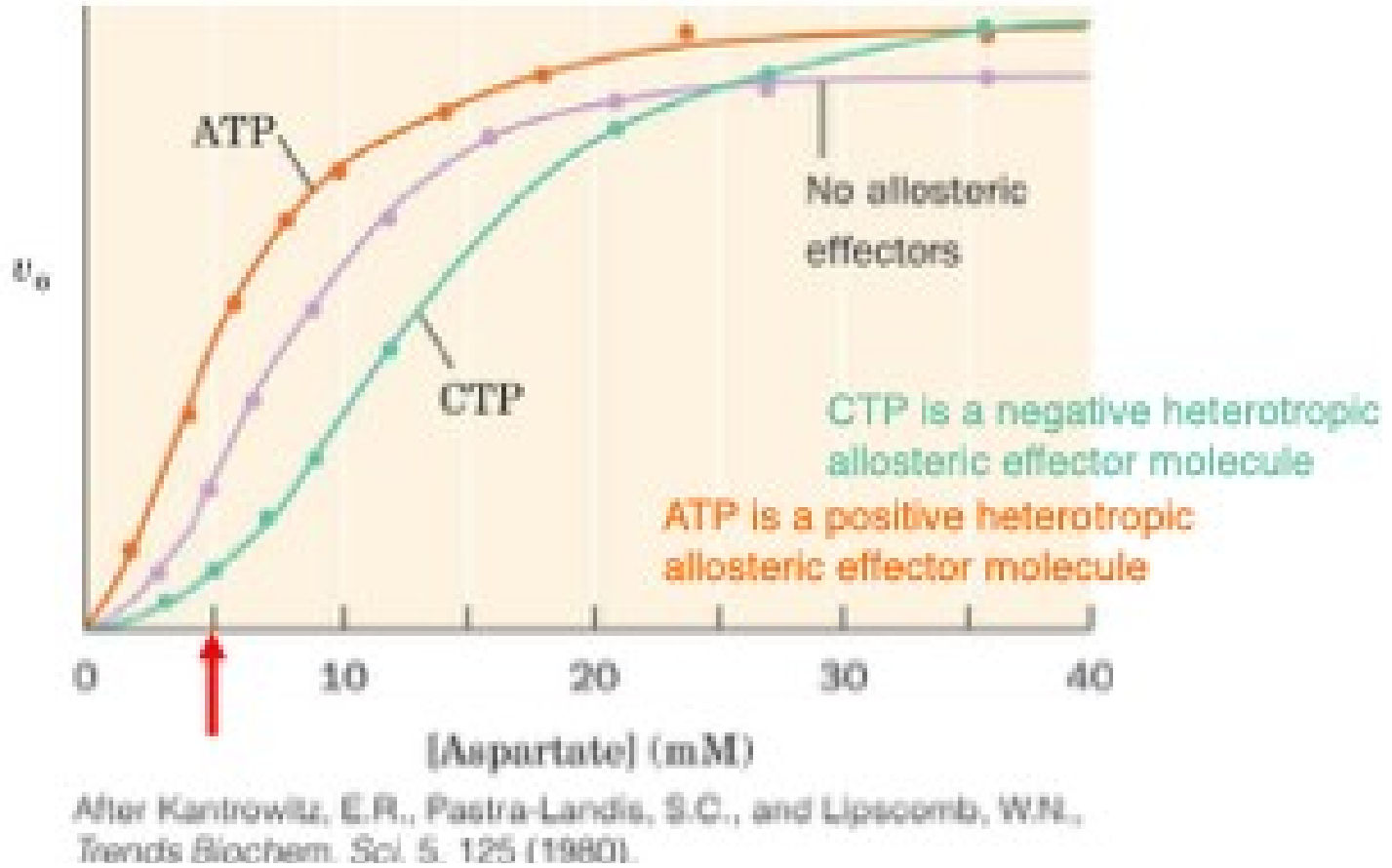




Anti-tumoral Drug PALA

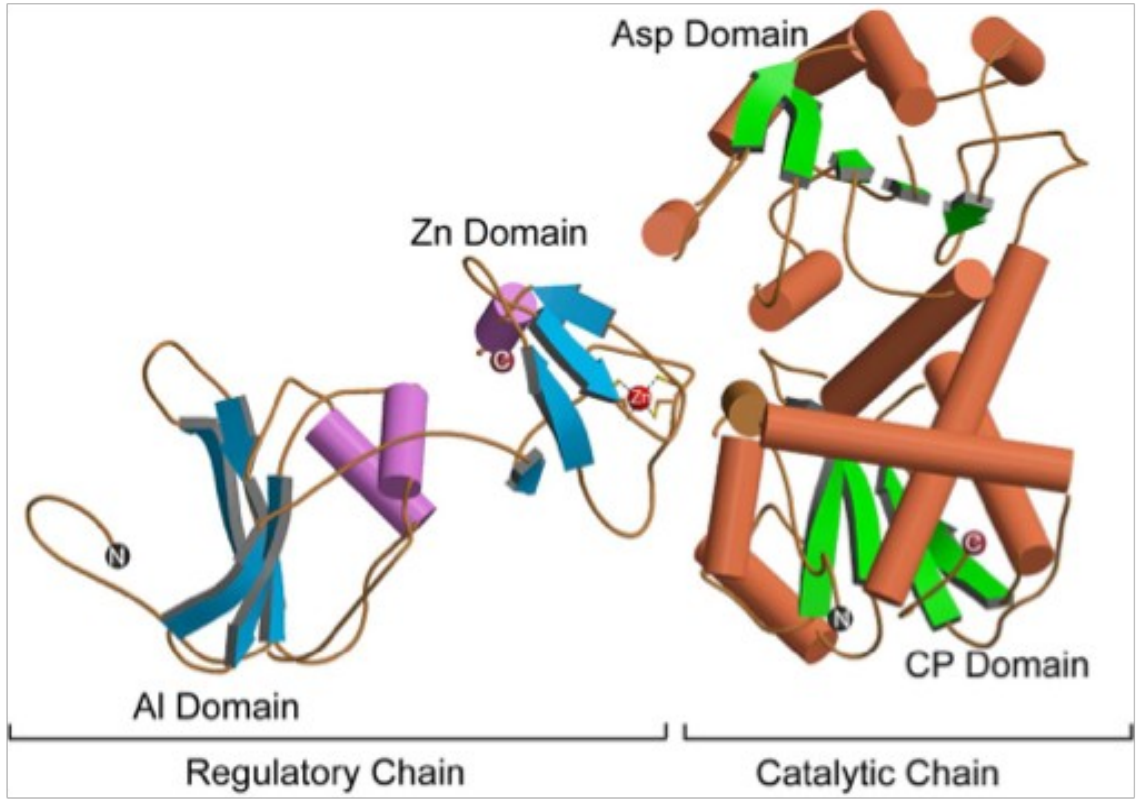
Control by Allostery

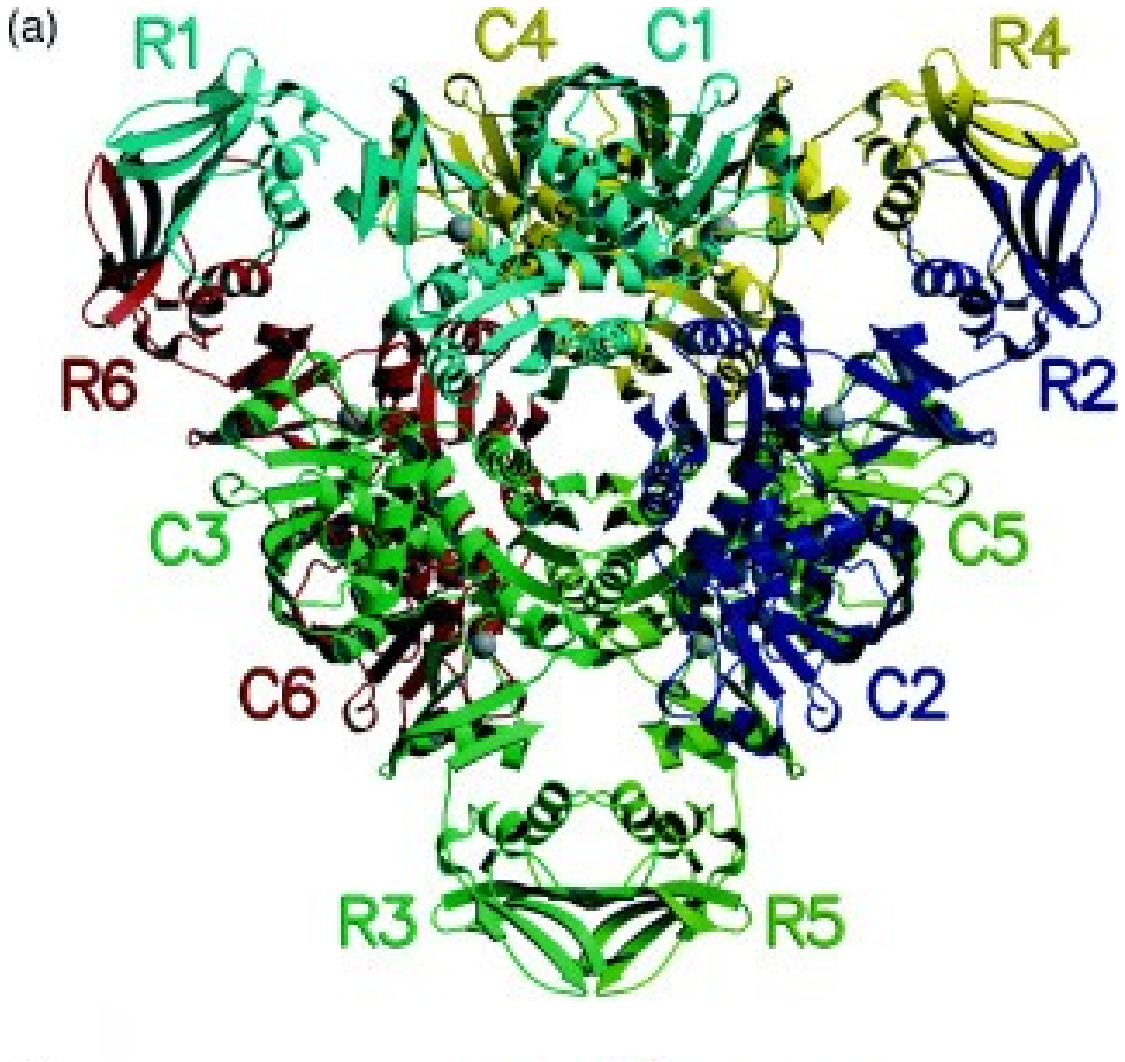
Allosteric Effectors: ATCase Reaction



After Kantrowitz, E.R., Pastra-Landis, S.C., and Lipscomb, W.N., *Trends Biochem. Sci.* 5, 125 (1980).

aspartate transcarbamoylase

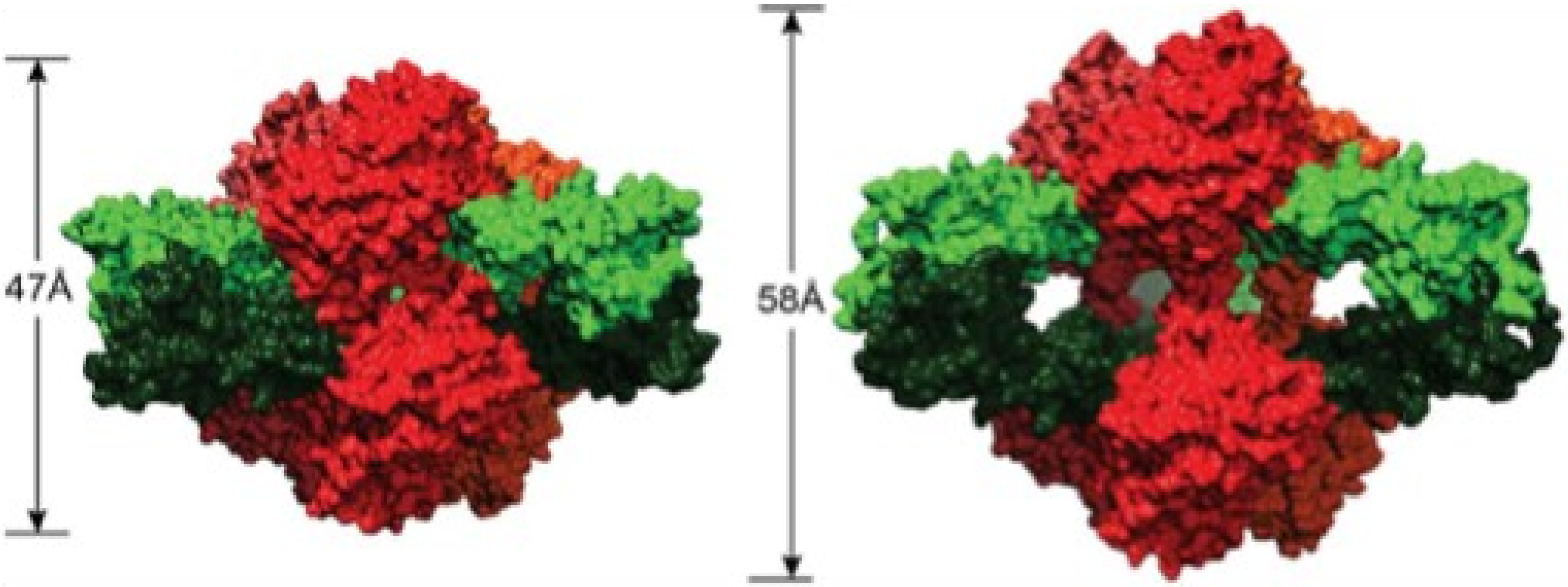




aspartate transcarbamoylase

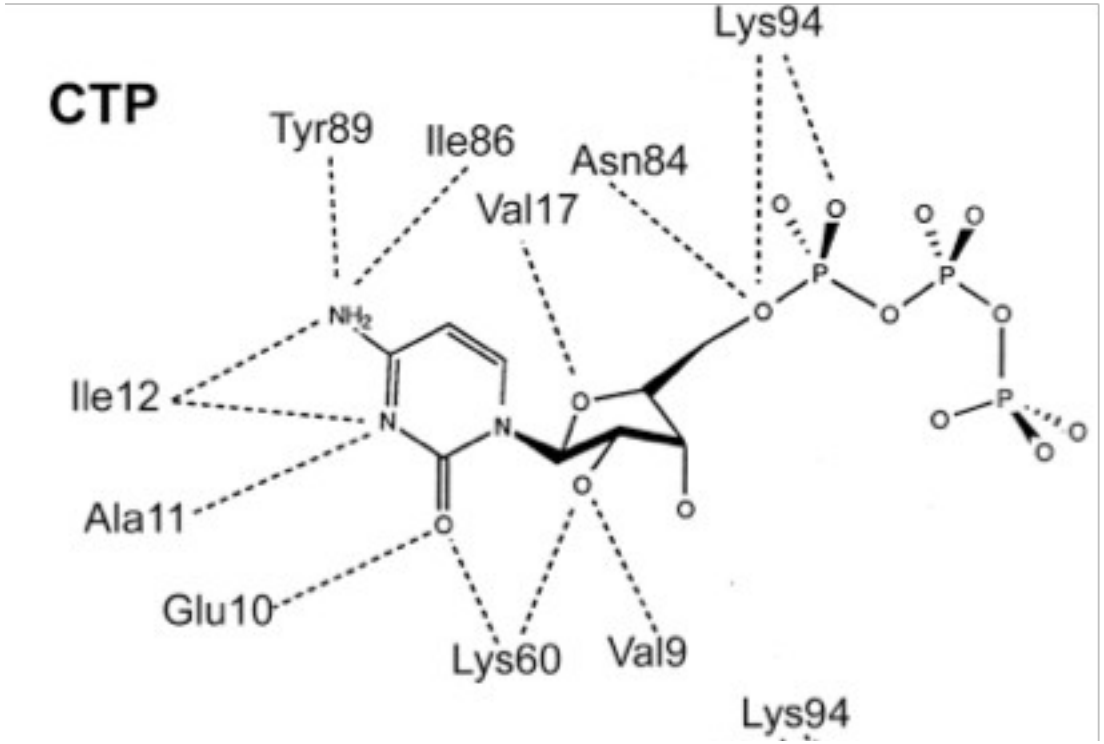
T

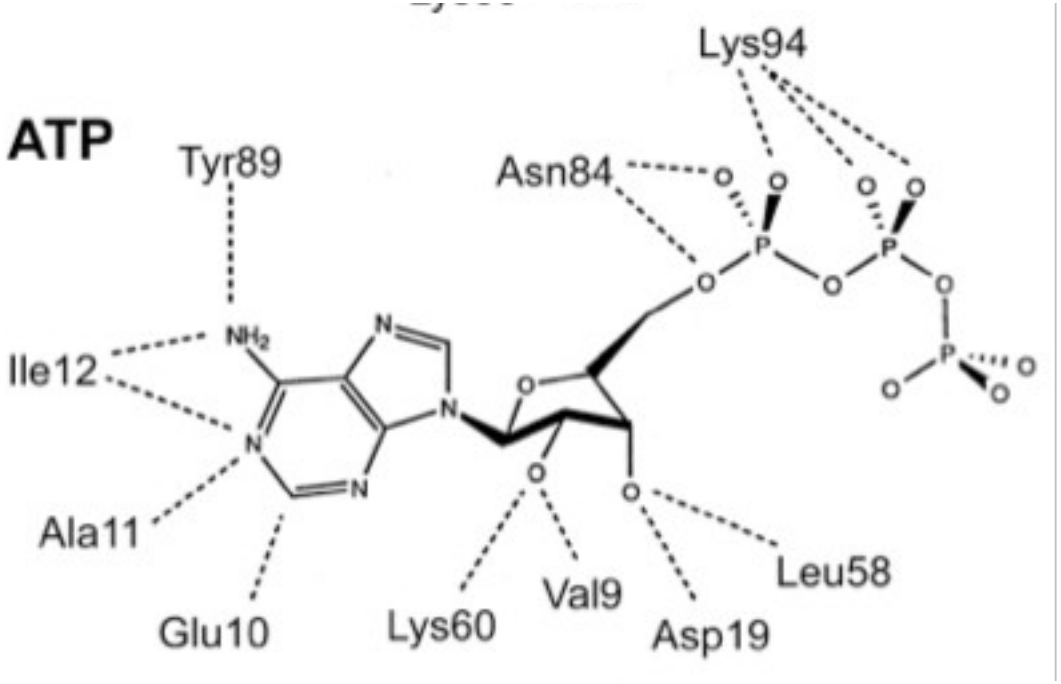
R

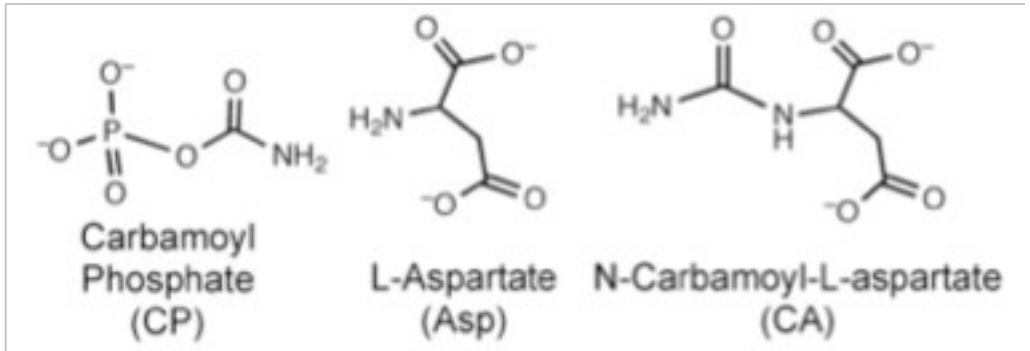


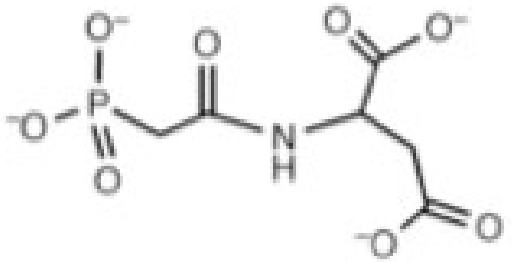
6 CTP

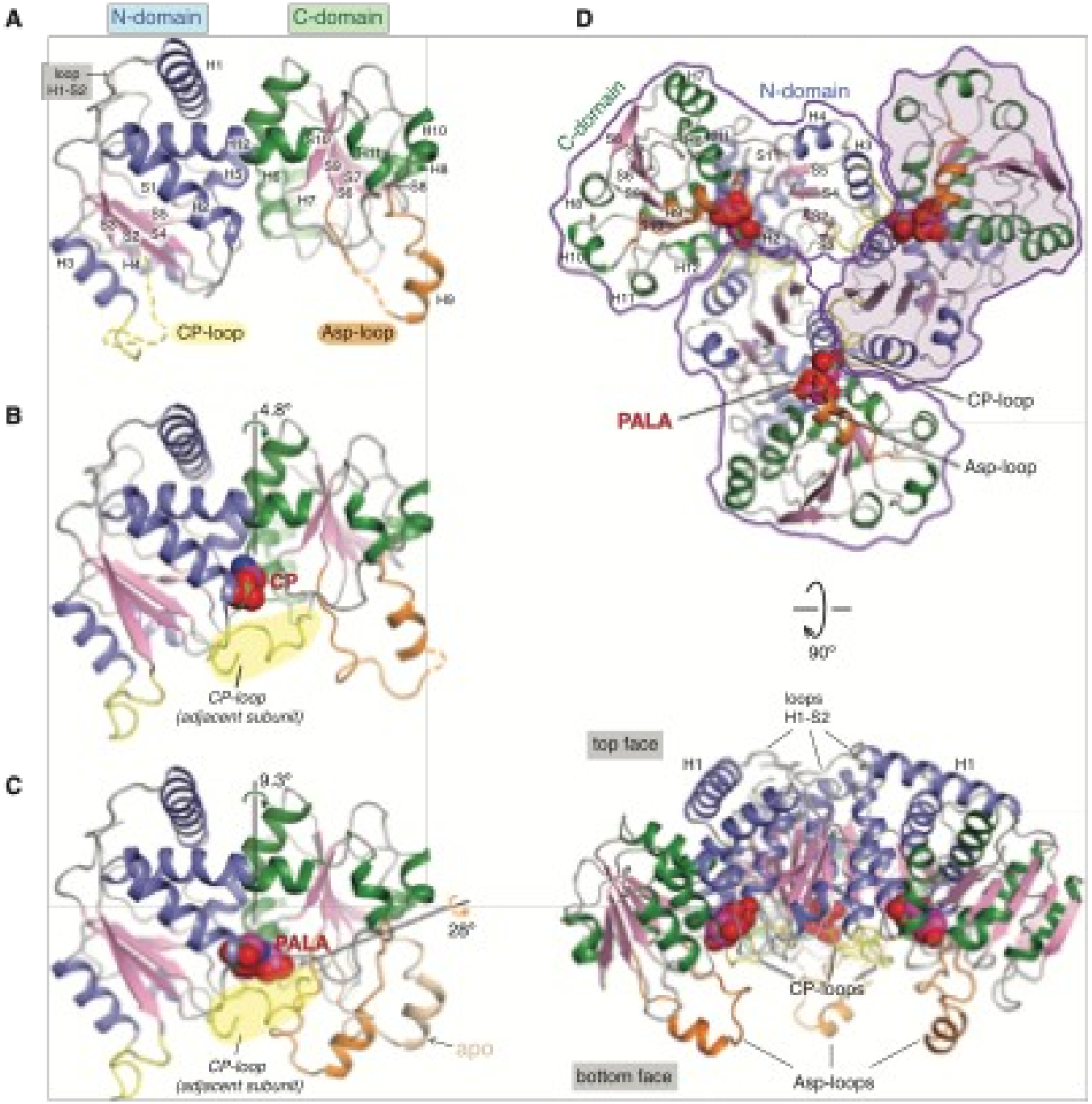
Effectors binding

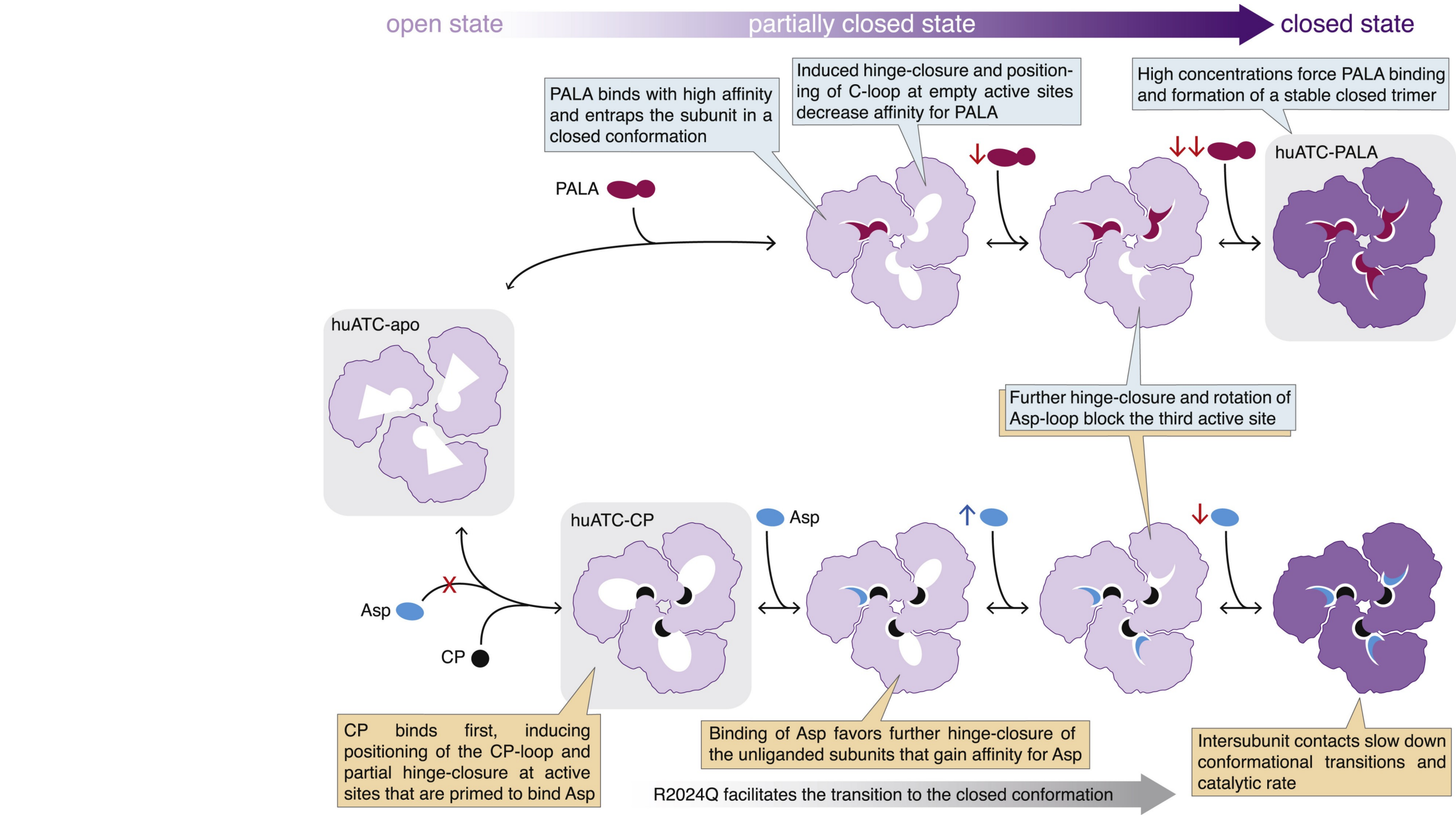





Carbamoyl Phosphate (CP)
L-Aspartate (Asp)
N-Carbamoyl-L-aspartate (CA)


N-phosphonacetyl-L-aspartate (PALA)


A
N-domain
C-domain
loop H1-S2
H1
CP-loop
Asp-loop
B
4.8°
CP
CP-loop (adjacent subunit)
C
9.3°
28°
PALA
apo
CP-loop (adjacent subunit)
D
N-domain
C-domain
PALA
CP-loop
Asp-loop
90°
loops H1-S2
top face
H1
H1
CP-loops
bottom face
Asp-loops


open state
partially closed state
closed state
PALA binds with high affinity and entraps the subunit in a closed conformation
Induced hinge-closure and positioning of C-loop at empty active sites decrease affinity for PALA
High concentrations force PALA binding and formation of a stable closed trimer
PALA
huATC-PALA
huATC-apo
Further hinge-closure and rotation of Asp-loop block the third active site
huATC-CP
Asp
Asp
CP
CP binds first, inducing positioning of the CP-loop and partial hinge-closure at active sites that are primed to bind Asp
Binding of Asp favors further hinge-closure of the unliganded subunits that gain affinity for Asp
Intersubunit contacts slow down conformational transitions and catalytic rate
R2024Q facilitates the transition to the closed conformation

activation

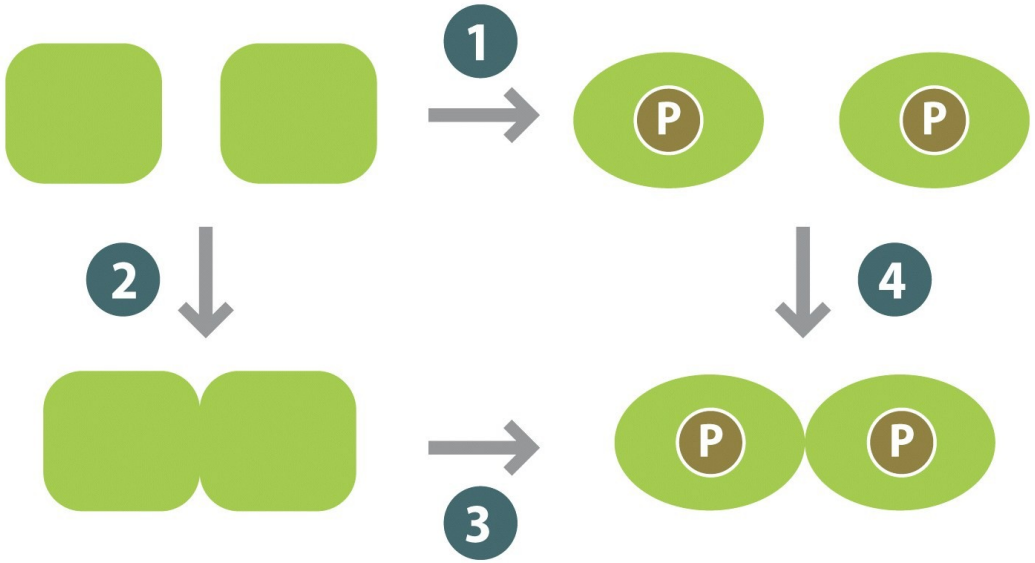


Figure 3.23 How Proteins Work (©2012 Garland Science)

$$\Delta G_1 + \Delta G_4 = \Delta G_2 + \Delta G_3$$

Translation and rotation entropy

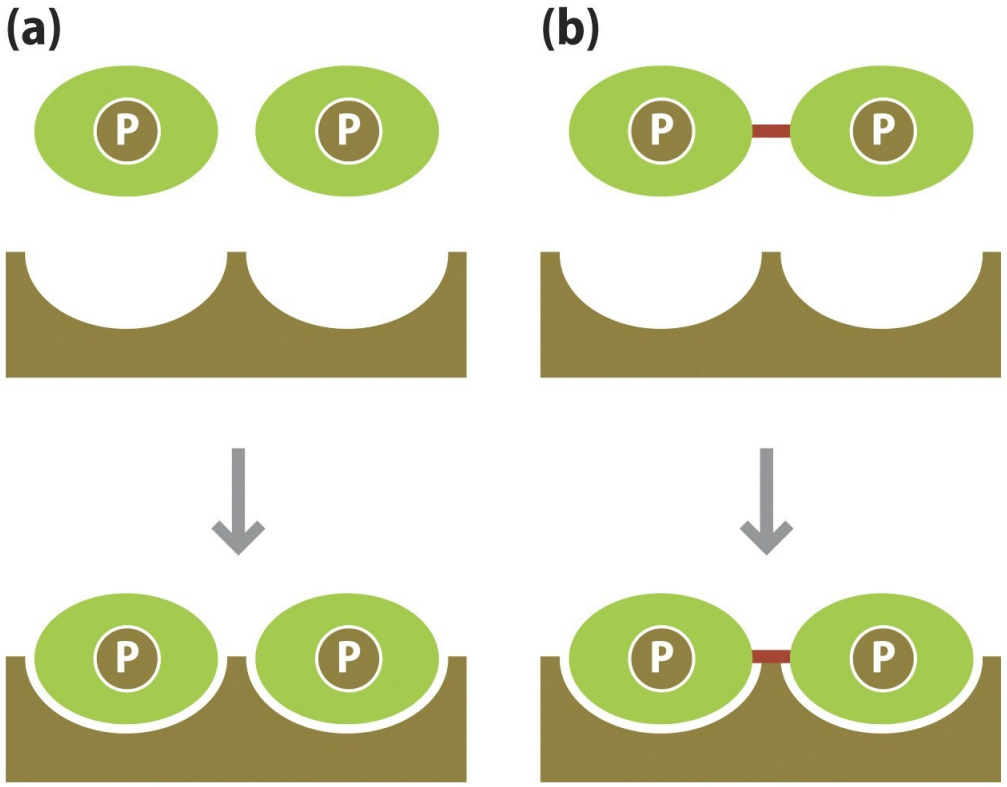


Figure 3.24 How Proteins Work (©2012 Garland Science)

Sequence-specific binding to DNA

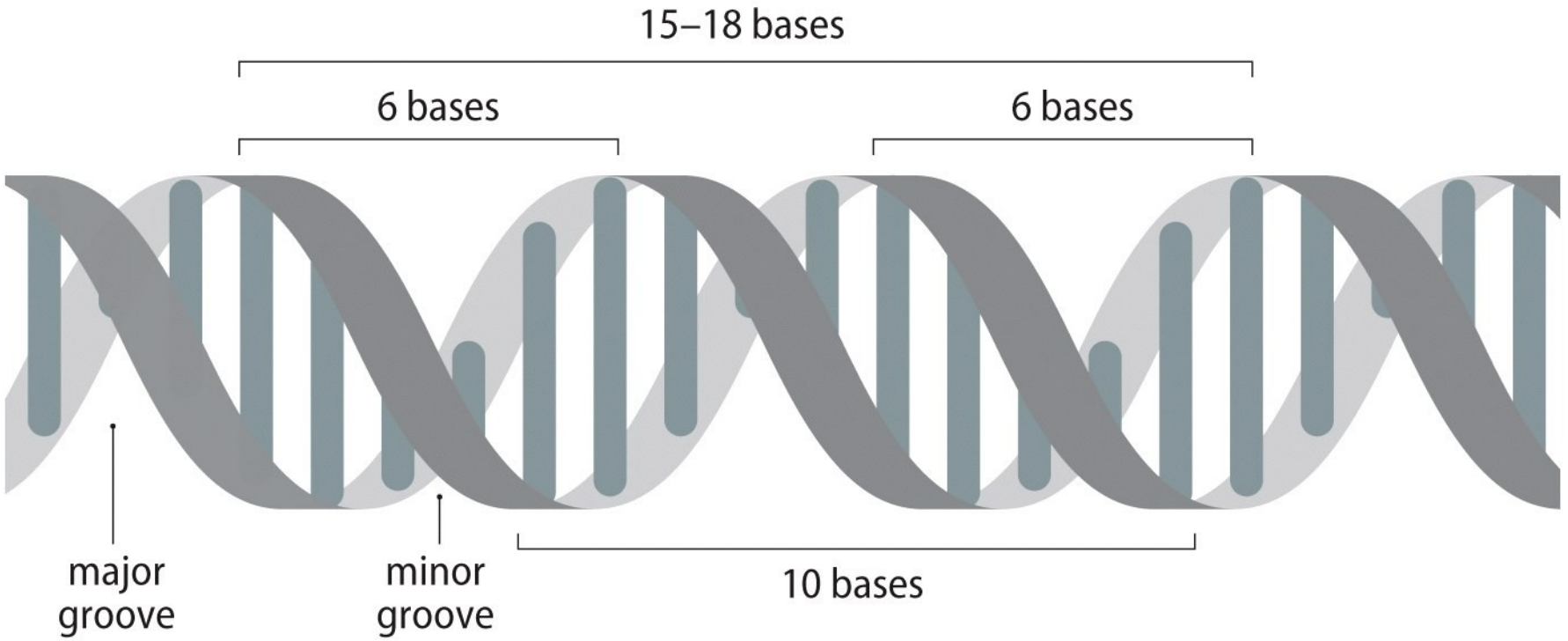


Figure 3.25 How Proteins Work (©2012 Garland Science)

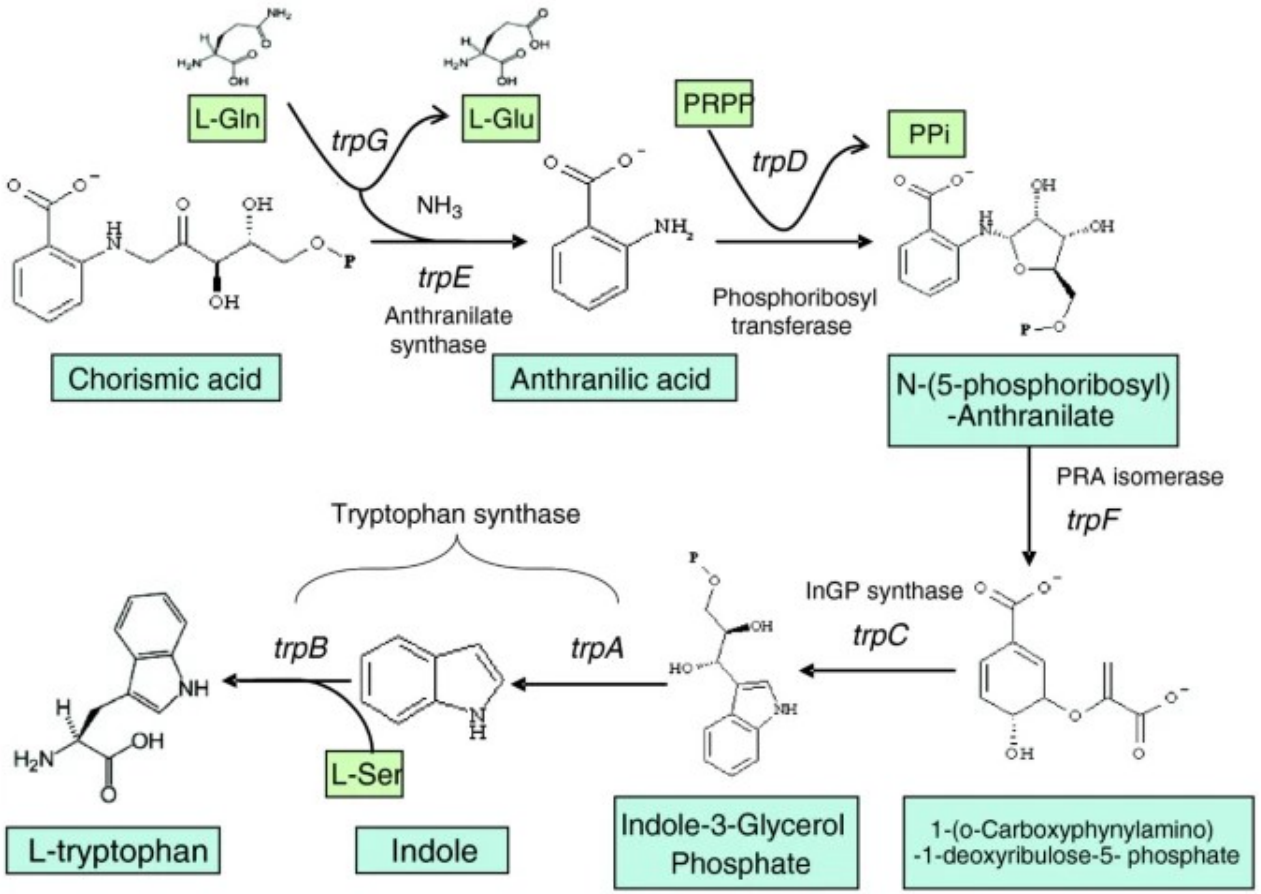
10^7 base pairs long
4^{-n}

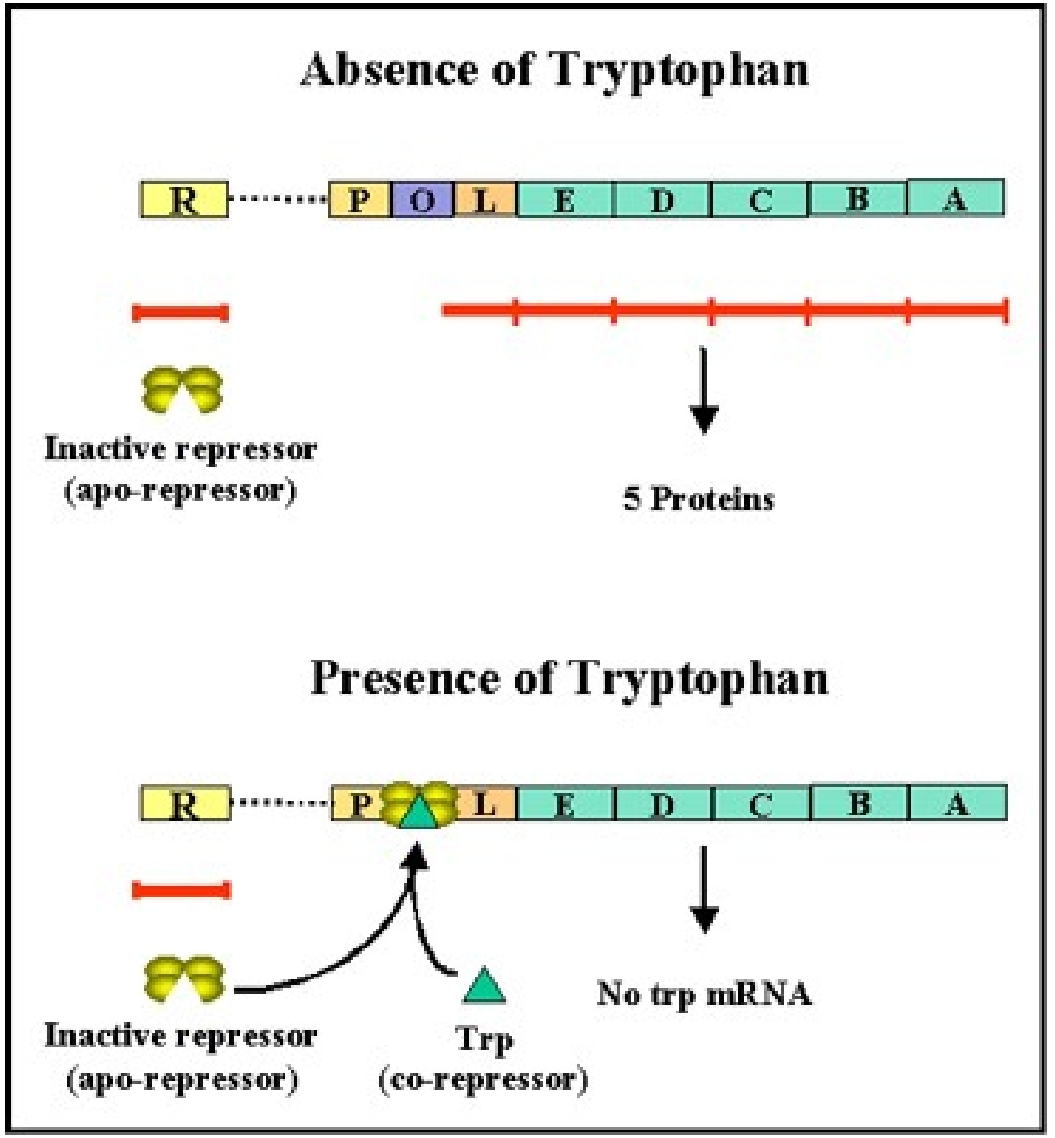
3-mer $p = 0.0156$ or $1.6*10^5$
4-mer with $p = 0.0039$ or $4*10^4$
6-mer 2500
8-mer 150 times
10-mer 10 times
12-mer less than once.

a protein that binds to DNA as a dimer

Figure 3.26 How Proteins Work (©2012 Garland Science)

trp repressor





a helix–turn–helix repressor

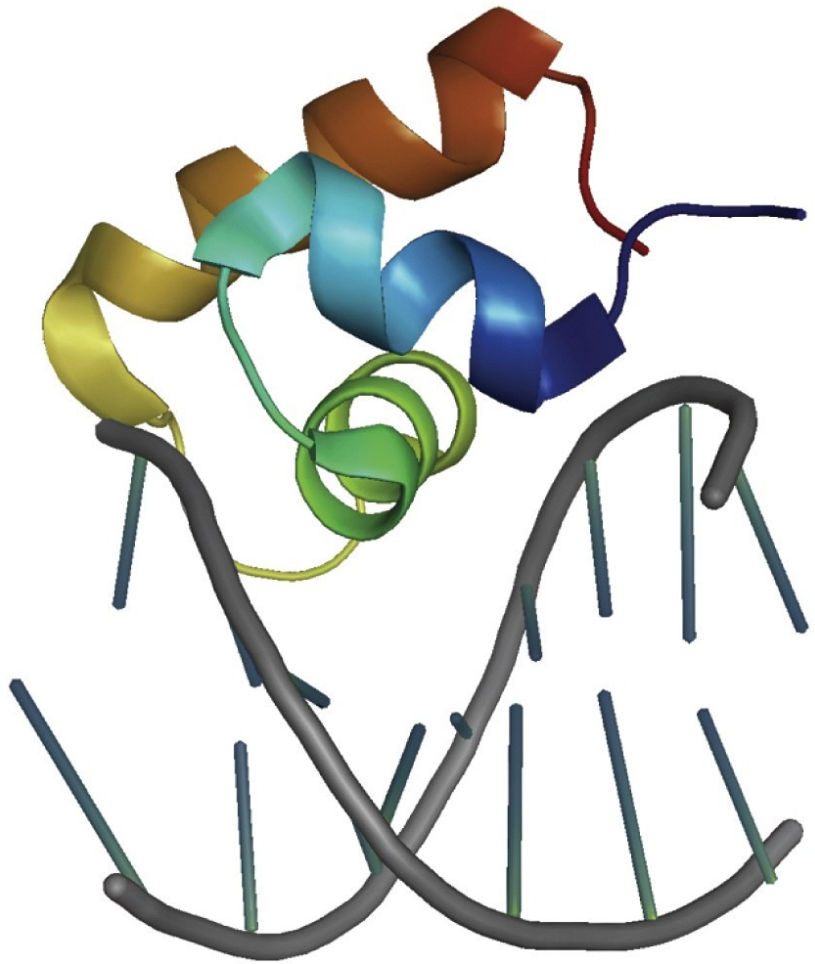
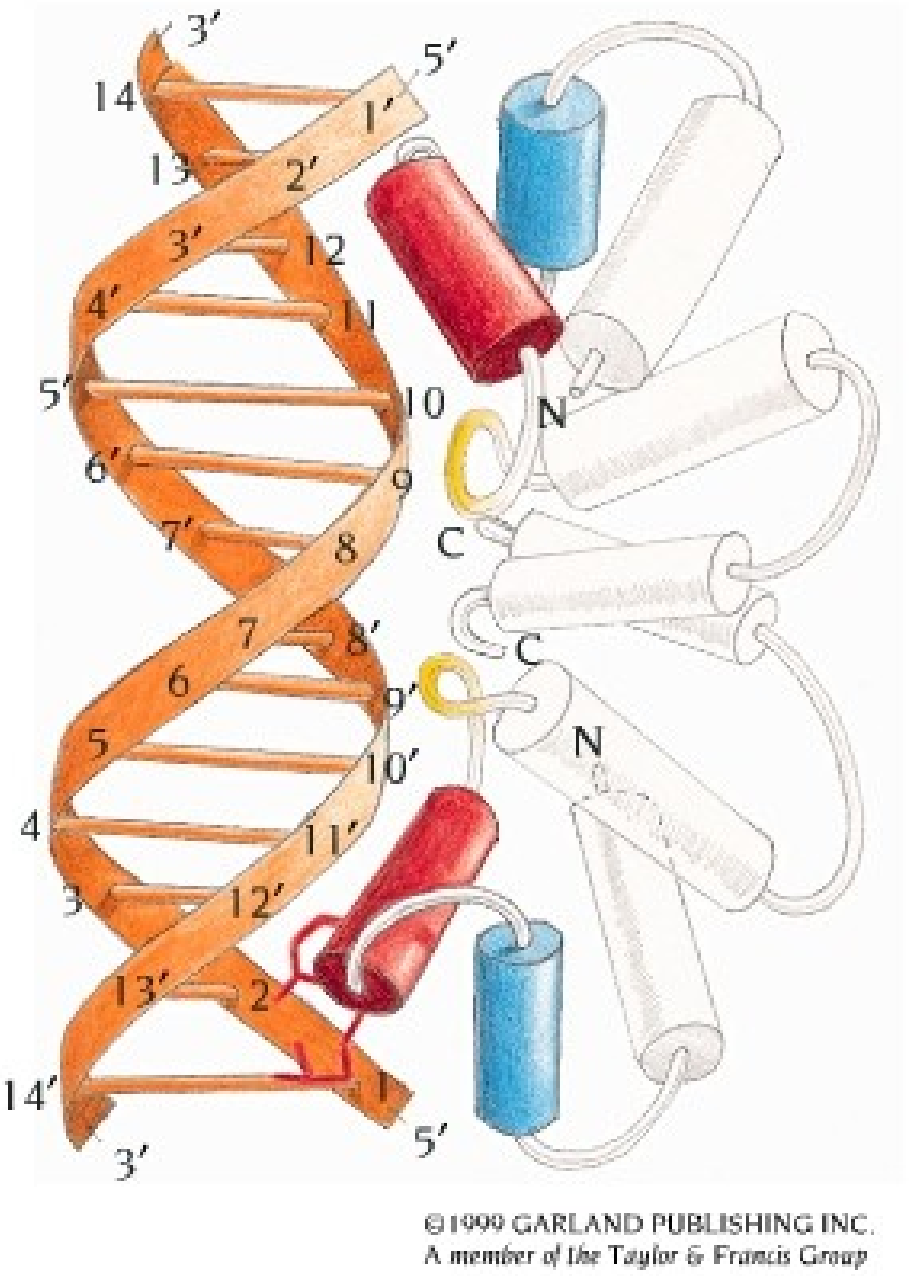
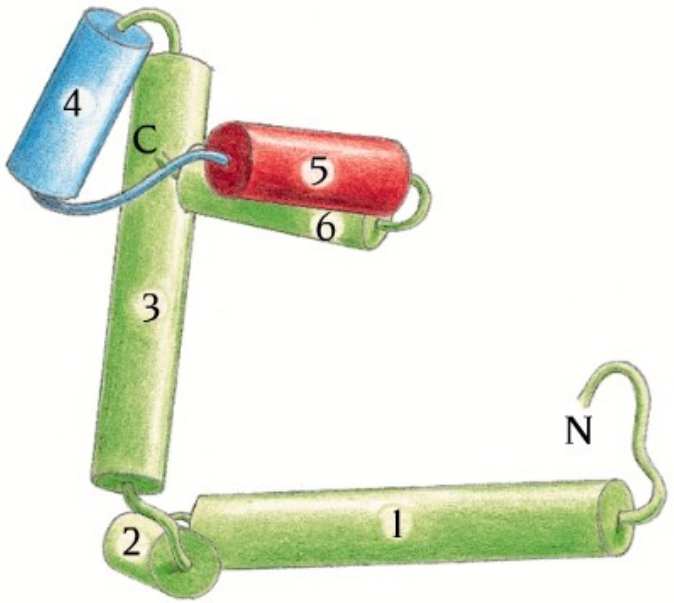
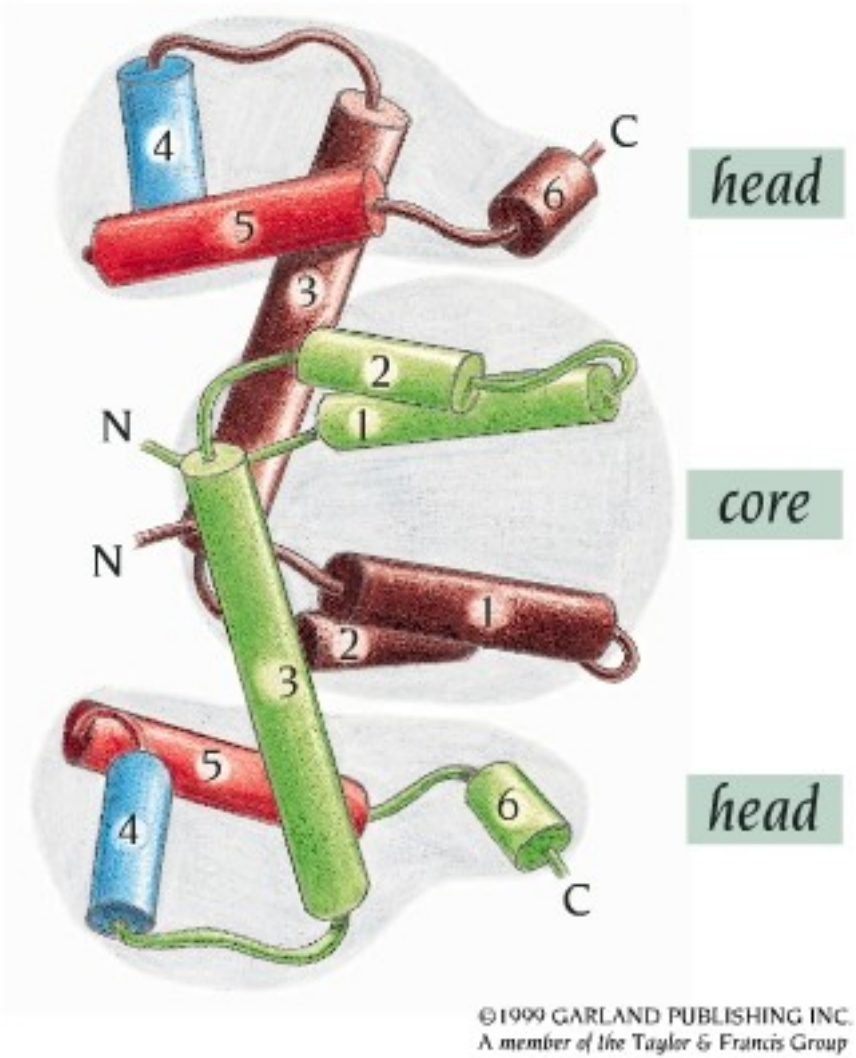


Figure 3.29 How Proteins Work (©2012 Garland Science)



trp repressor





the DNA-binding domain of *trp* repressor

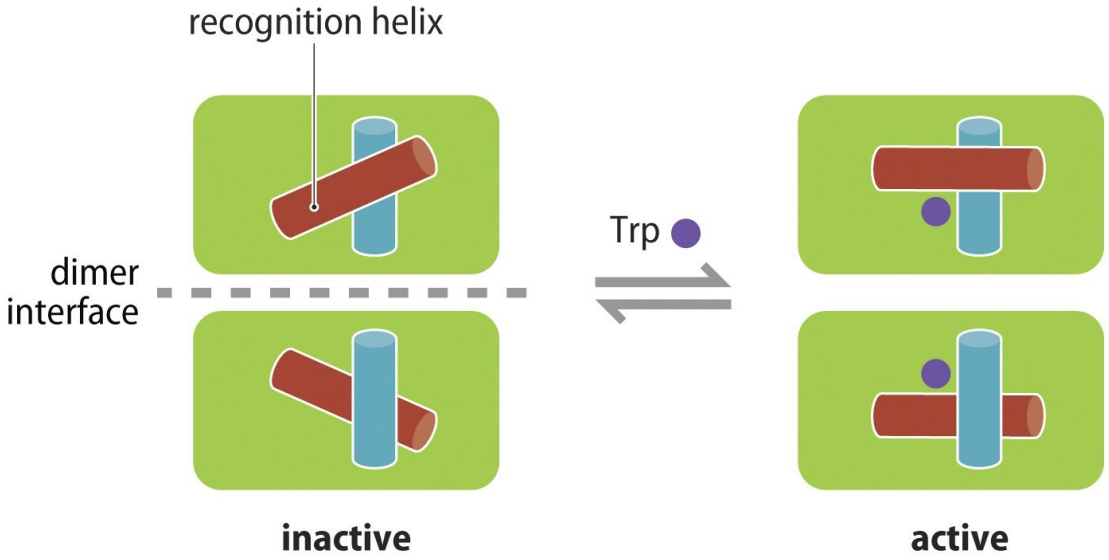
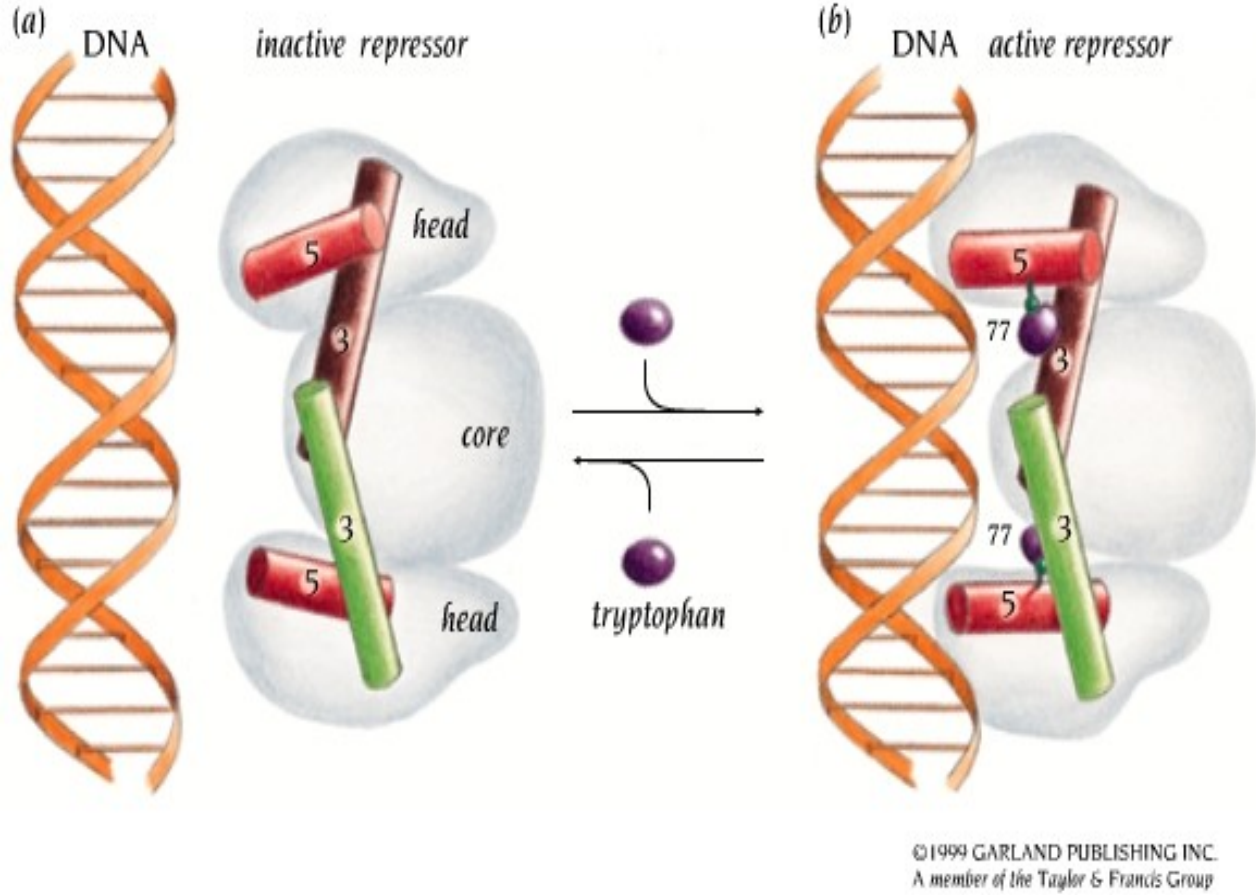
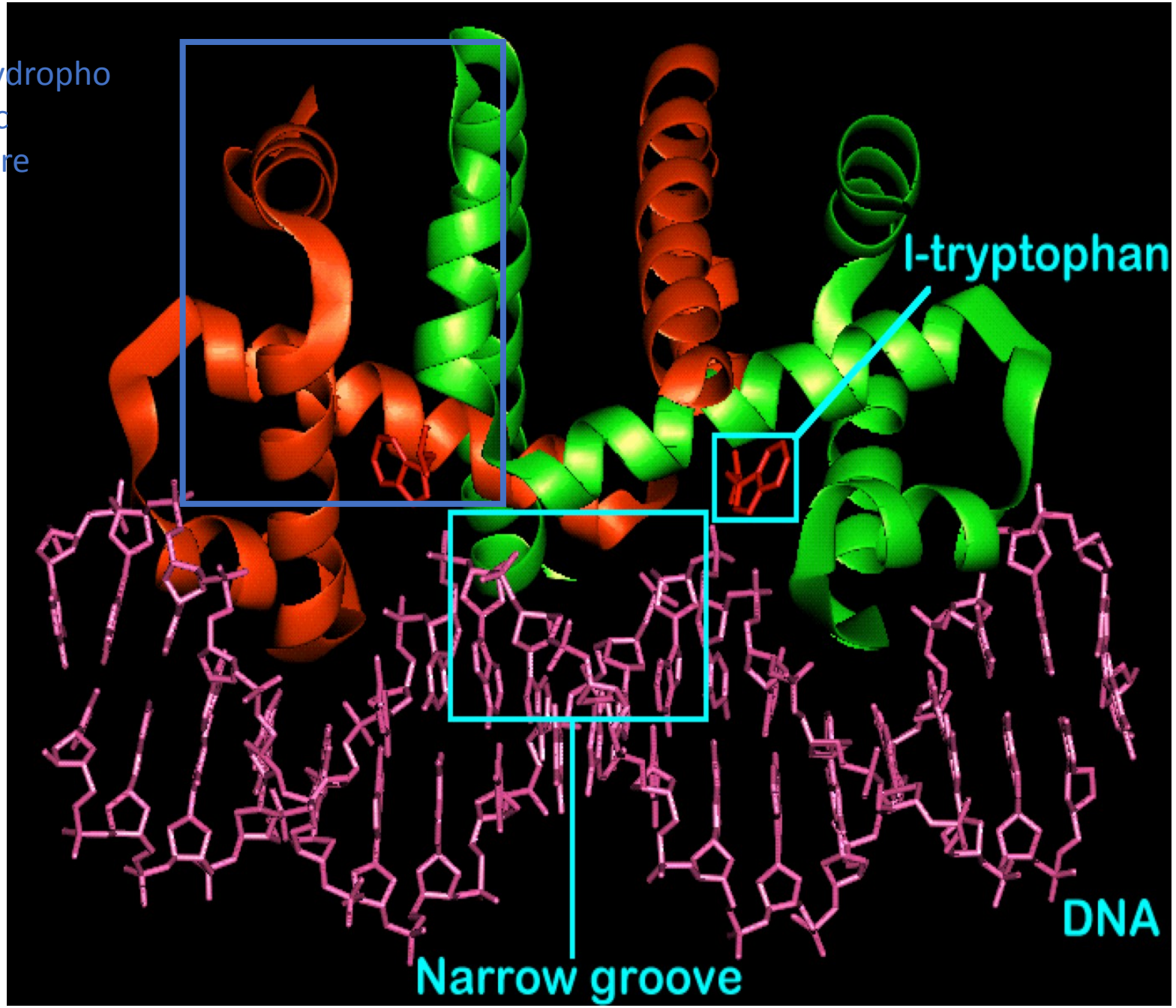


Figure 3.30 How Proteins Work (©2012 Garland Science)

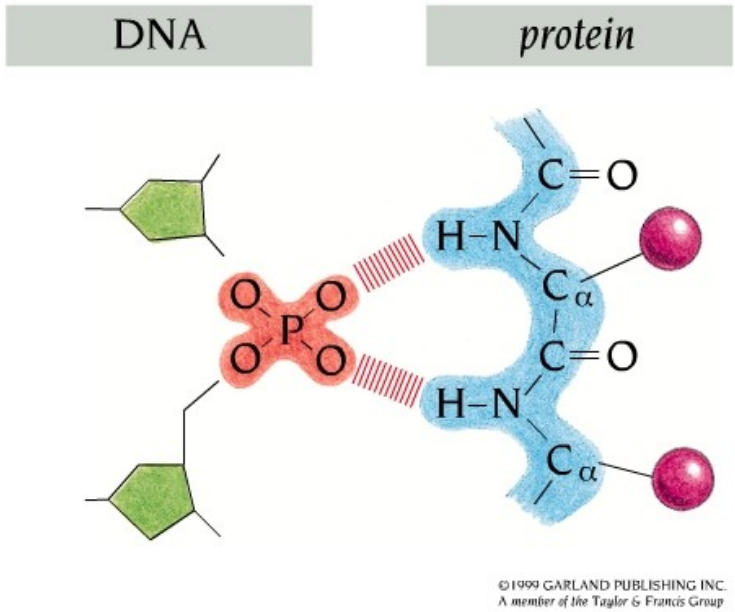


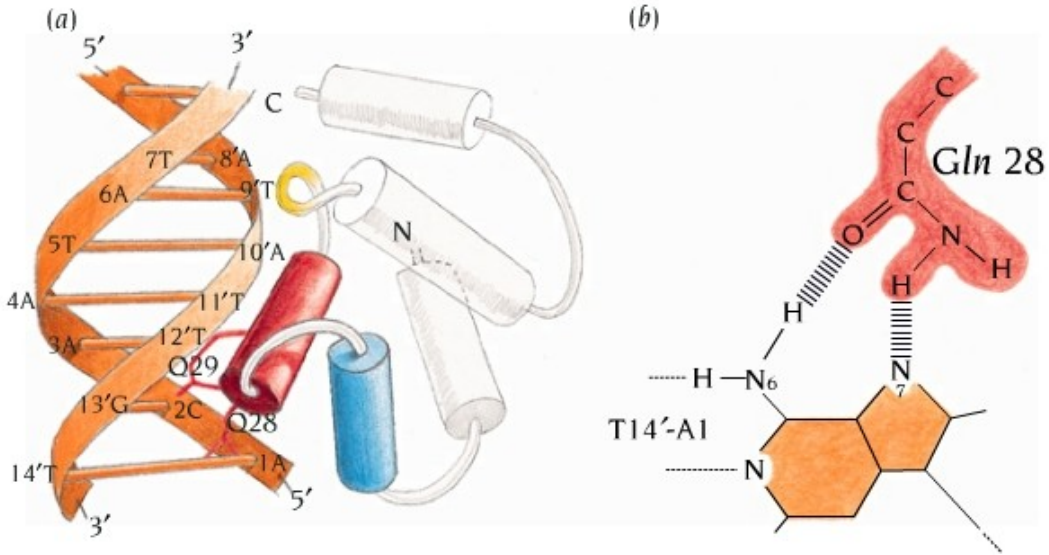
Ala 77--->
Val

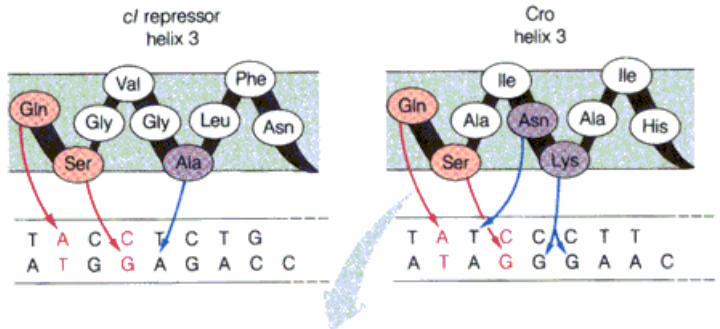
DNA-binding

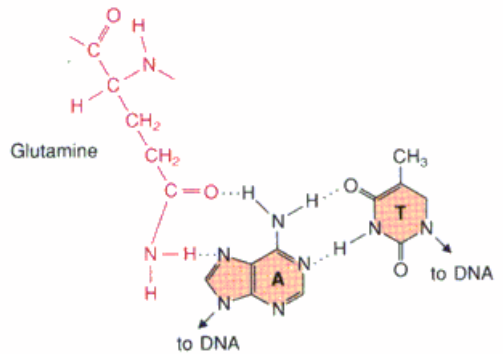


interactions
specific/non specific









CAP protein

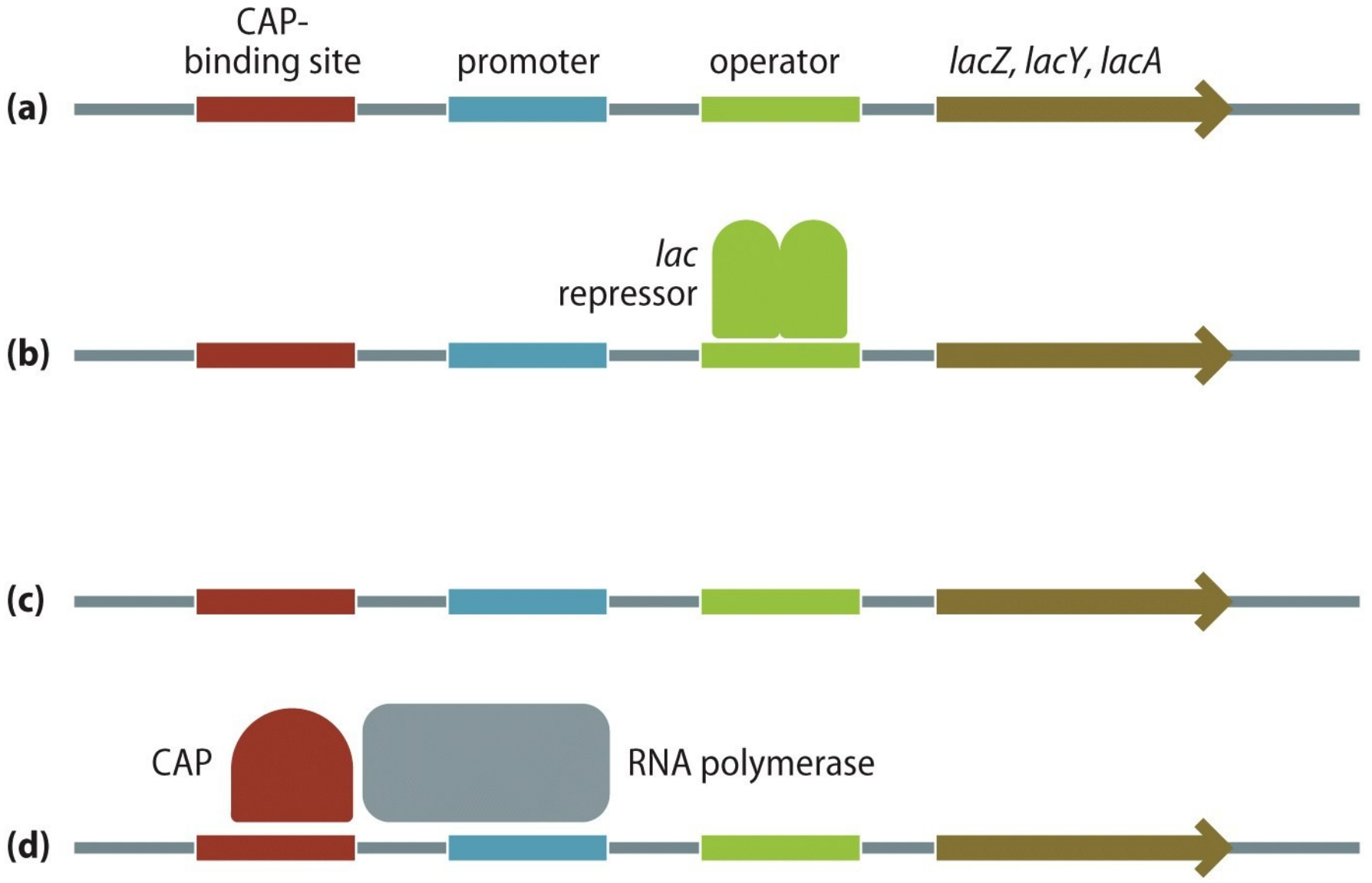


Figure 3.31 How Proteins Work (©2012 Garland Science)

inactive and active form of CAP

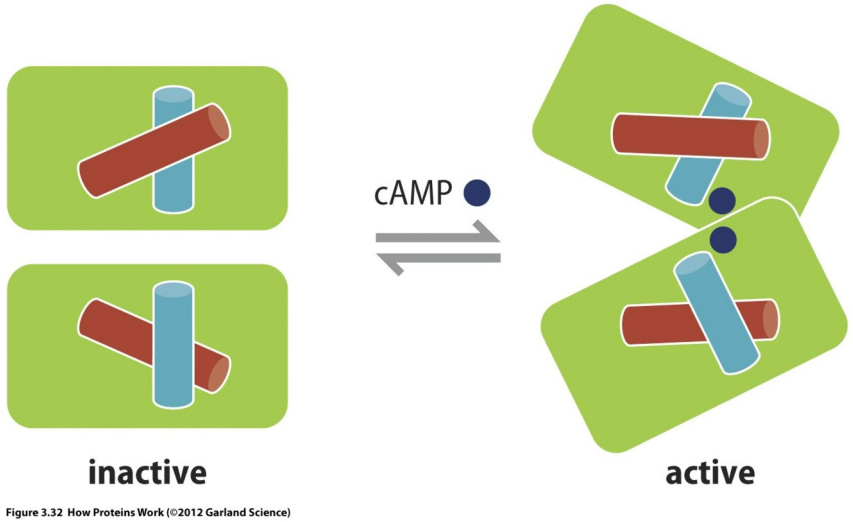
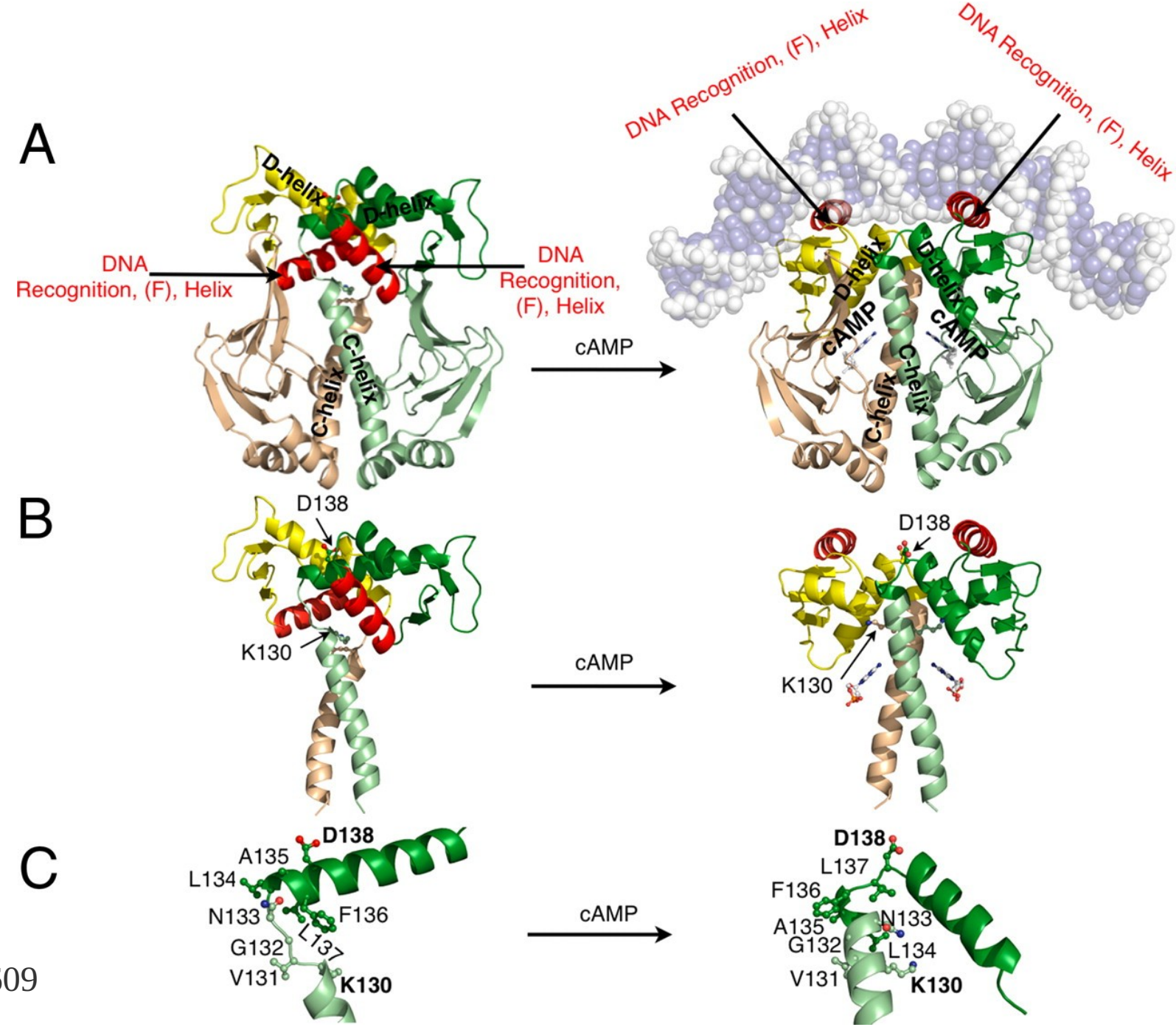
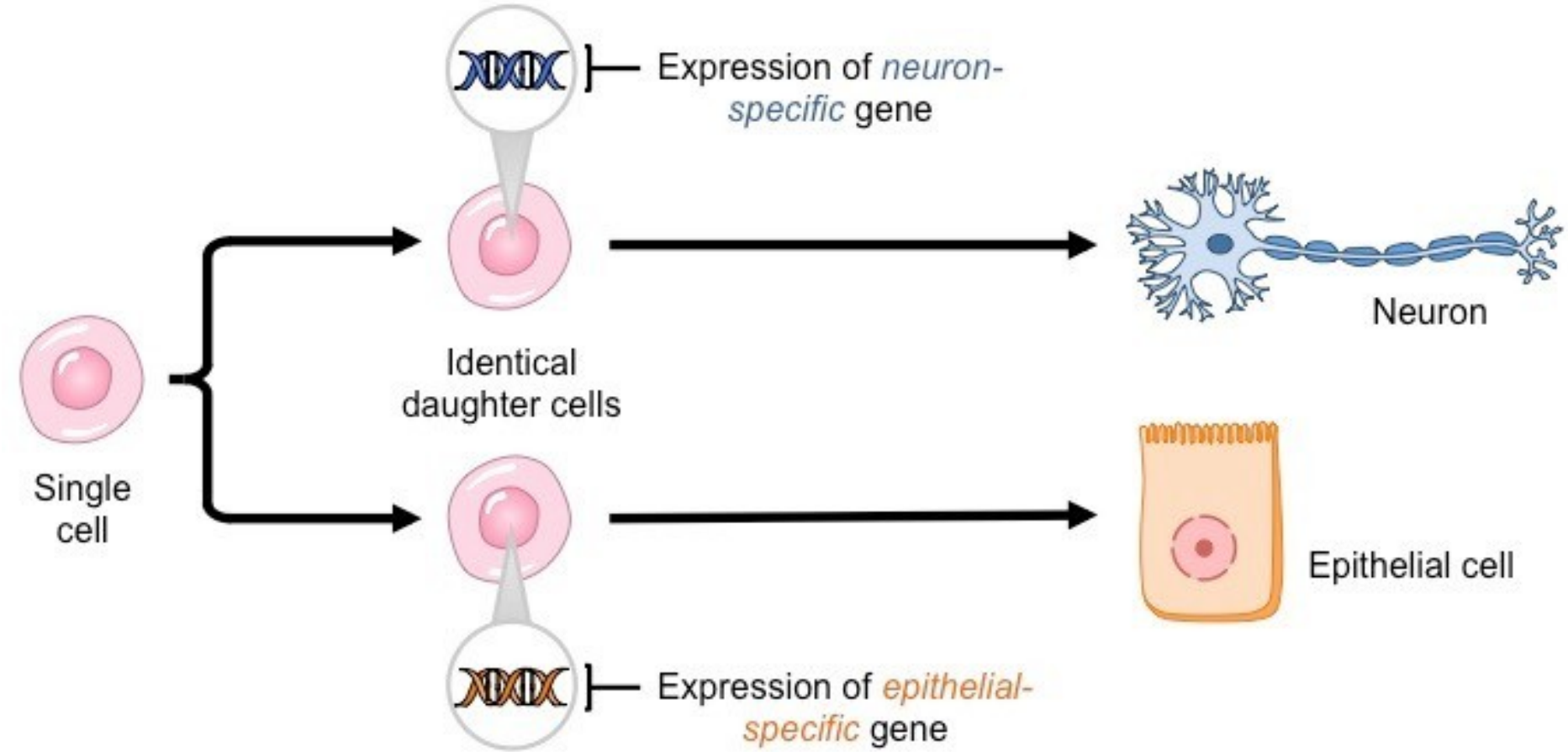


Figure 3.32 How Proteins Work (©2012 Garland Science)



PNAS , 2009 106 (39) 16604-16609

Cell Specialisation via Differential Gene Expression

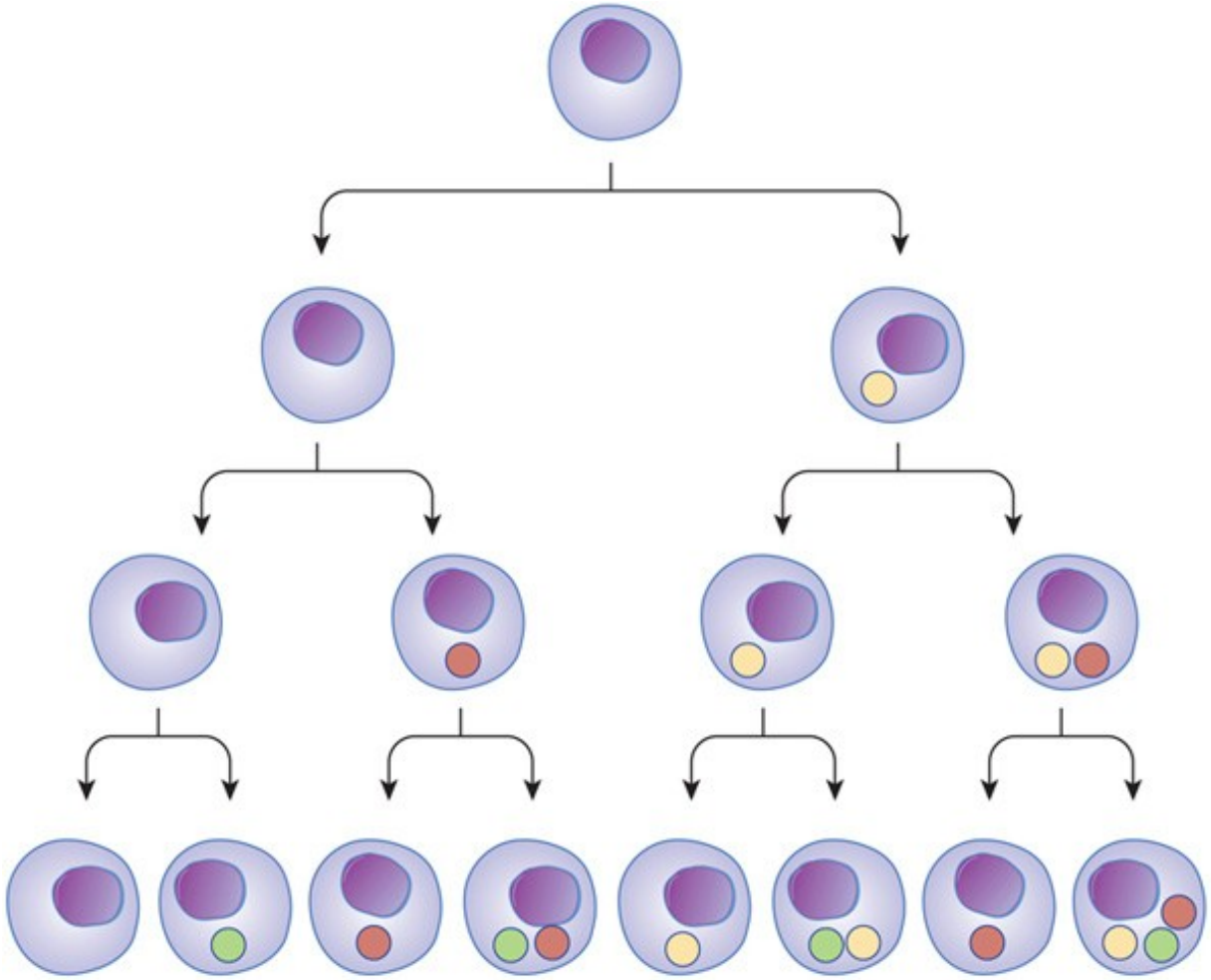


homeodomain proteins

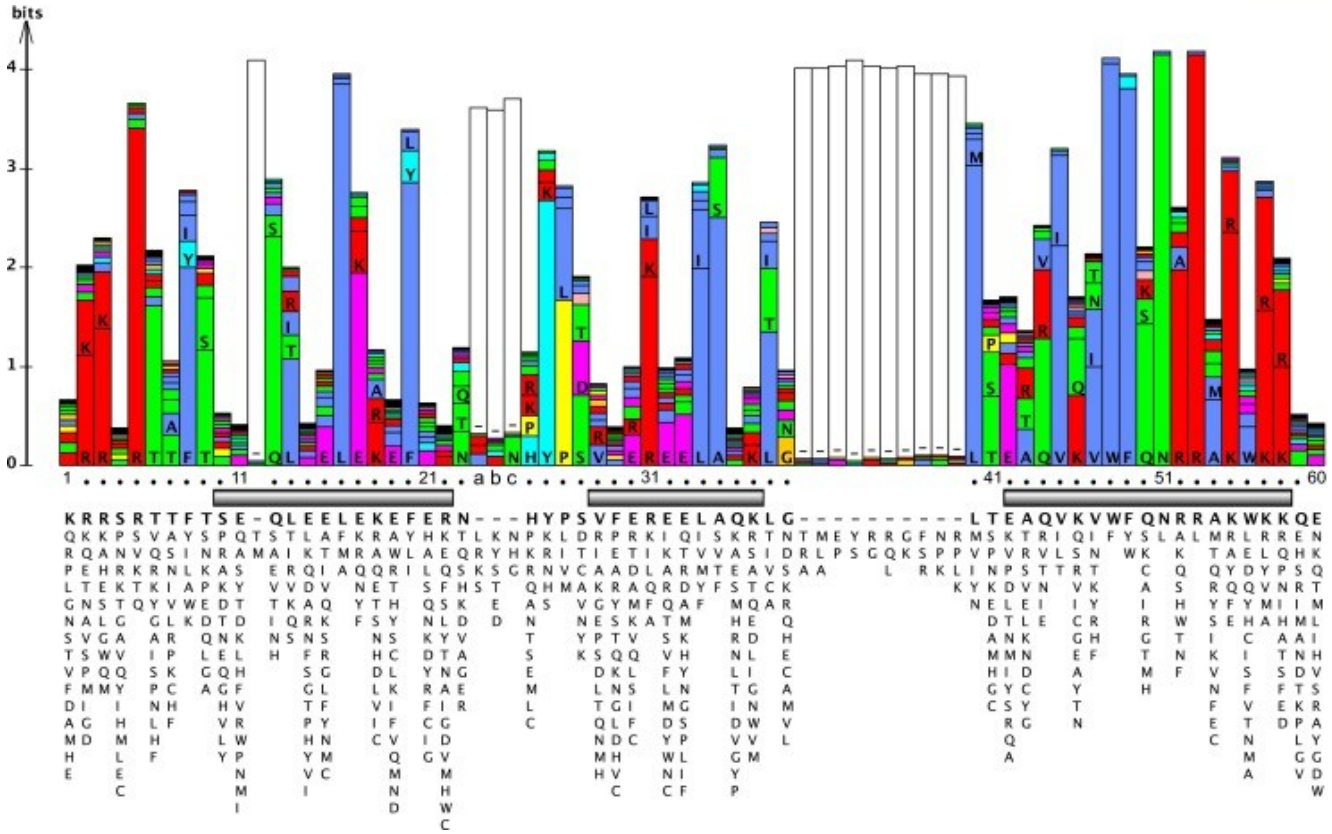
Οι homeodomain proteins κατευθύνουν το σχηματισμό των αξόνων και των δομών του σώματος κατά τη διάρκεια της πρώιμης εμβρυϊκής ανάπτυξης. Πολλές homeodomain proteins προκαλούν κυτταρική διαφοροποίηση, ενεργοποιώντας τις αλυσιδωτές αντιδράσεις των συνρυθμιζόμενων γονιδίων που απαιτούνται για την παραγωγή μεμονωμένων ιστών και οργάνων

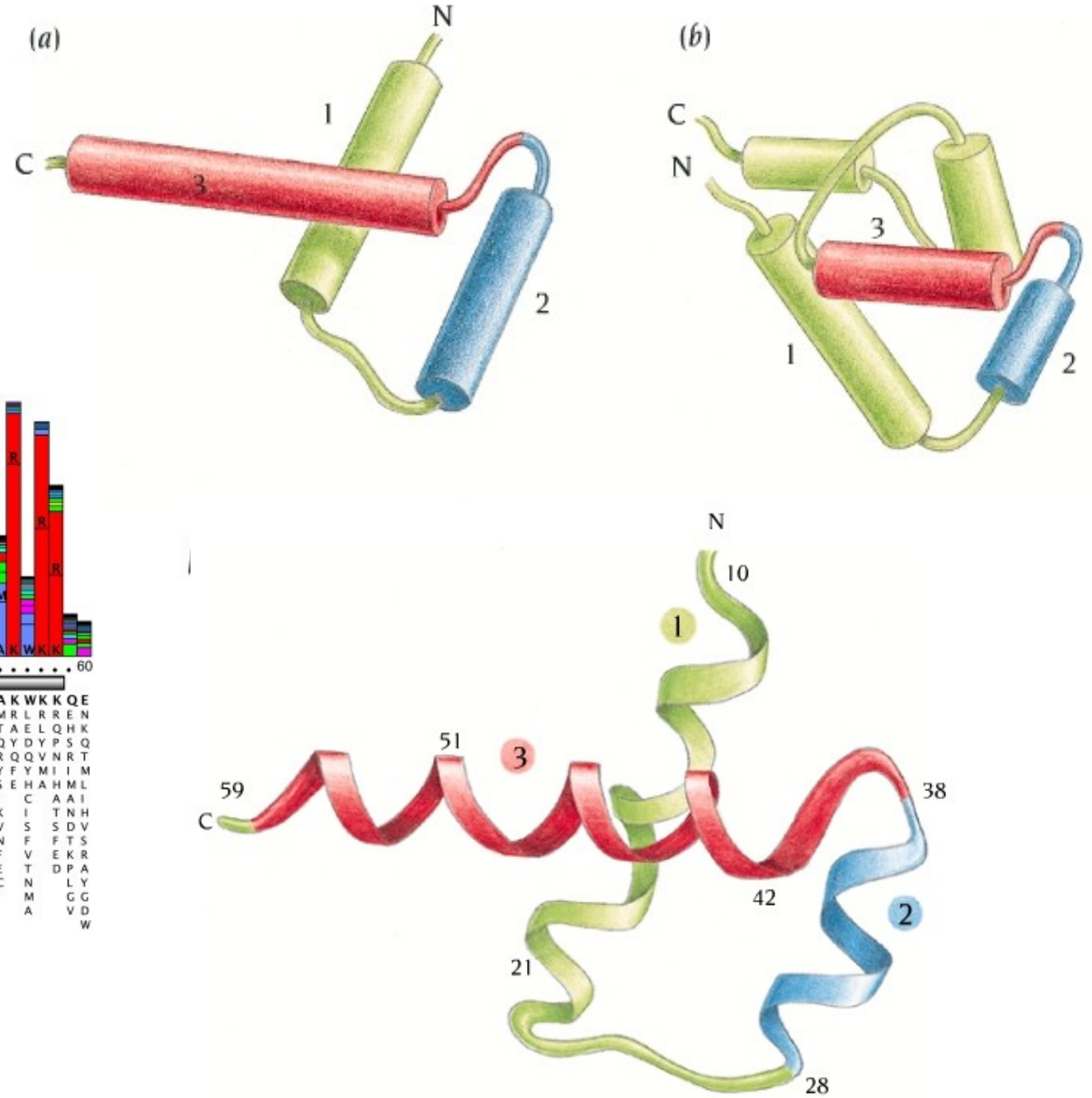
Η αλληλουχία homeobox κωδικοποιεί το HD, έναν σφαιρικό τομέα περίπου 60 αμινοξέων που λειτουργεί κανονικά ως τομέας σύνδεσης με το DNA. Σήμερα γνωρίζουμε ότι στα ζώα υπάρχουν συνήθως περίπου 100 γονίδια homeobox

Συνολικά, περίπου το 15-30 % όλων των παραγόντων μεταγραφής στα ζώα είναι πρωτεΐνες HD, οι οποίες αντιπροσωπεύουν περίπου το 0,5-1,25 % όλων των πρωτεϊνών σε ένα δεδομένο είδος.

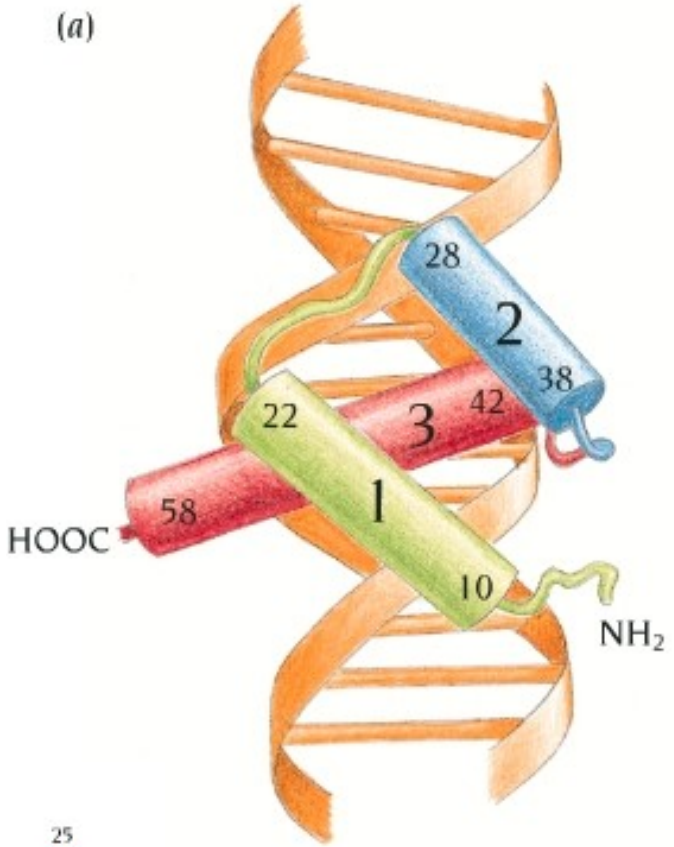


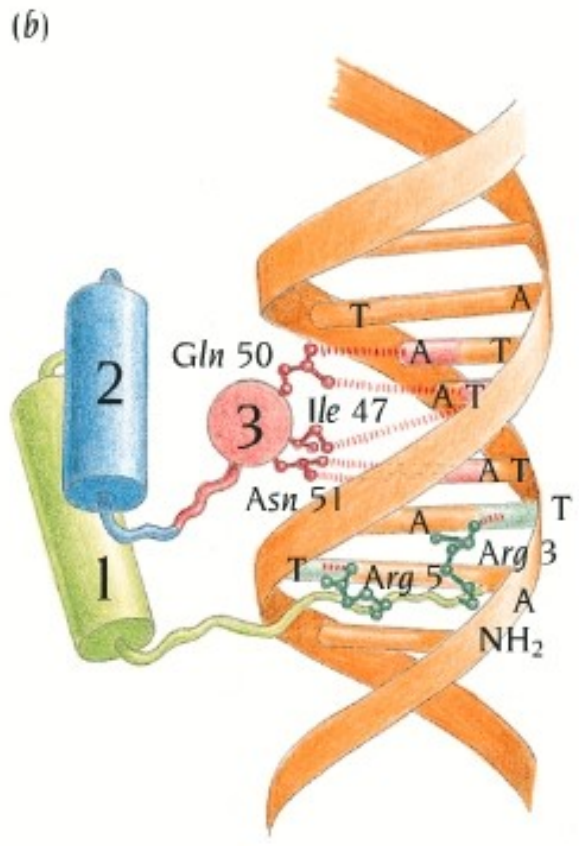
homeodomain proteins

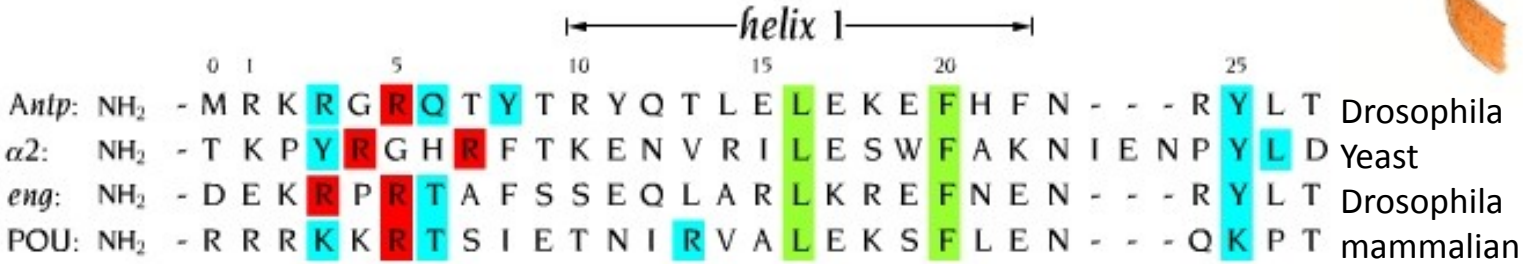




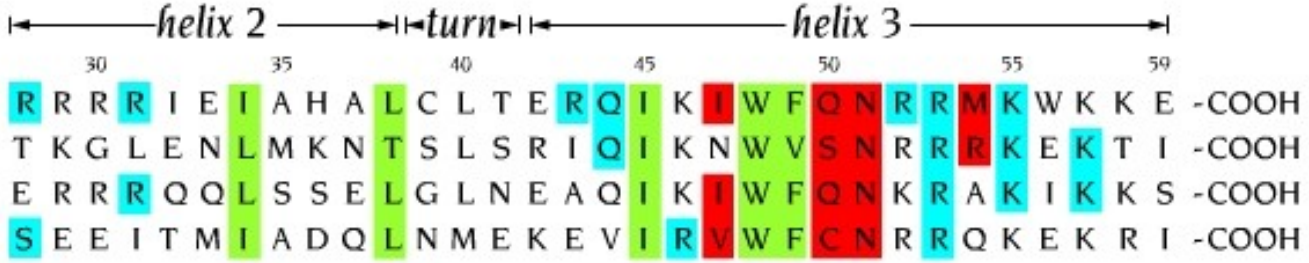
Interactions

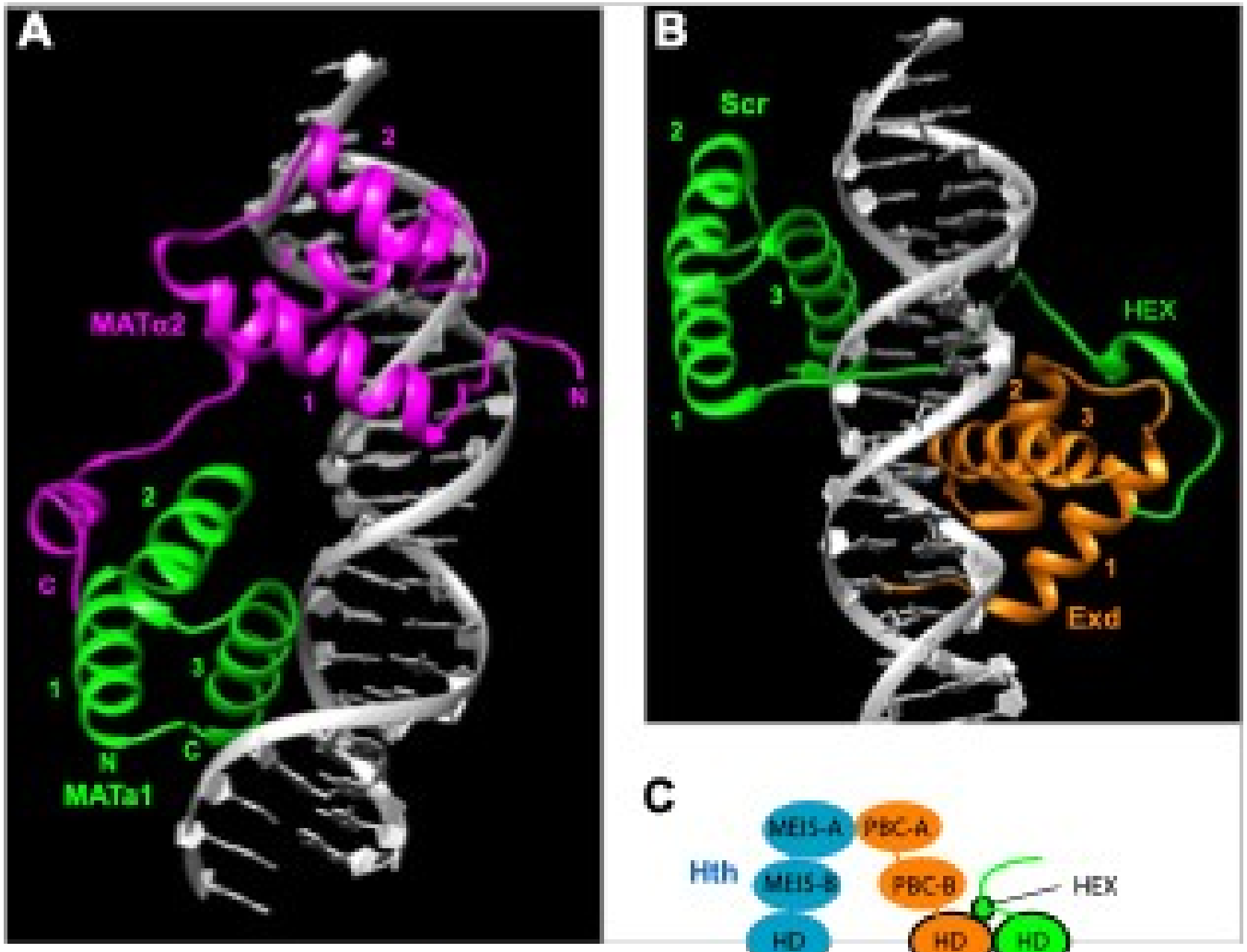




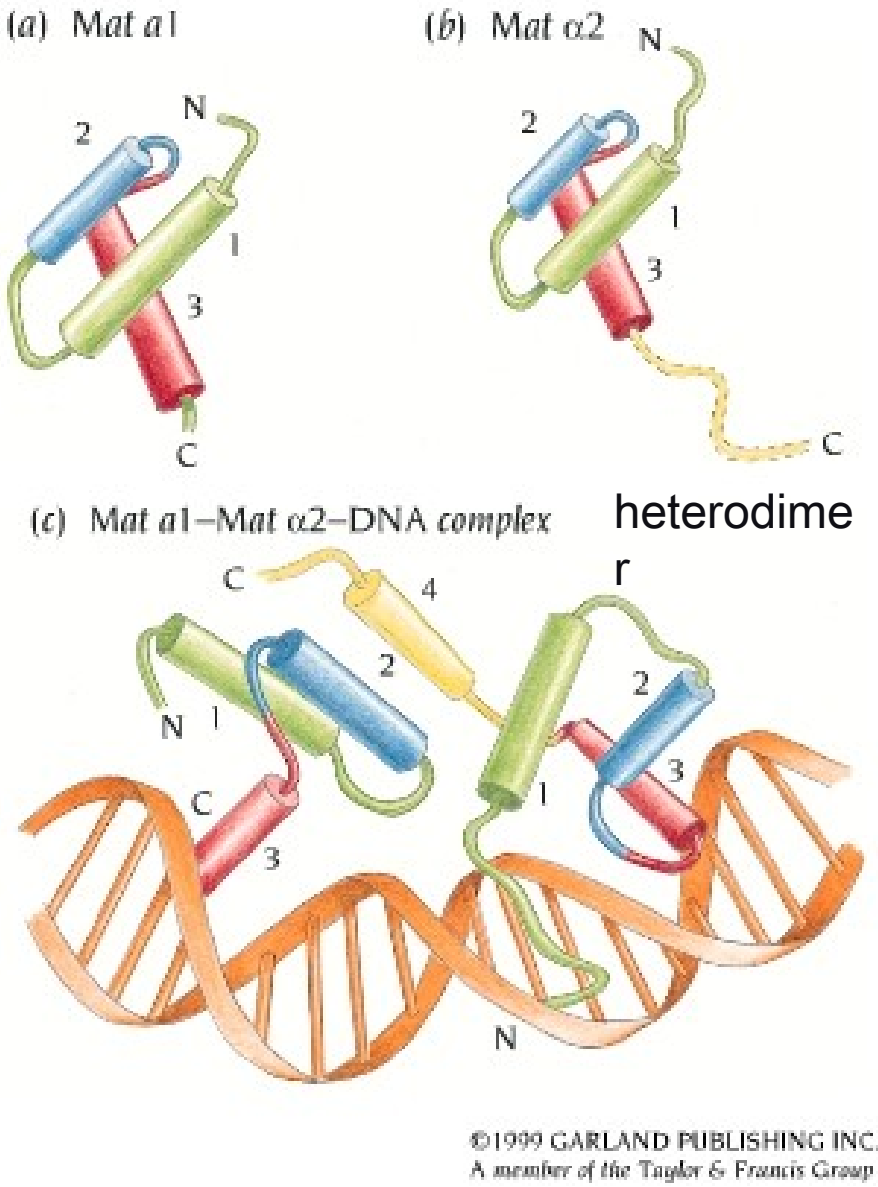


Drosophila
Yeast
Drosophila
mammalian




A
MATα2
MATa1
N
C
B
Scr
HEX
Exd
C
Hth
MEIS-A
PBC-A
MEIS-B
PBC-B
HEX
HD
HD
HD
5'TACATGTAATTTATTACATCA
GTACATTAAATAATGTAGTAT5'
5'ACTCTAAGATTAATCGGCTG
GAGATTCTAATTAGCCGACT5'

interactions with other proteins



heterodimer

MAT a2 heterotetramer with MCM1

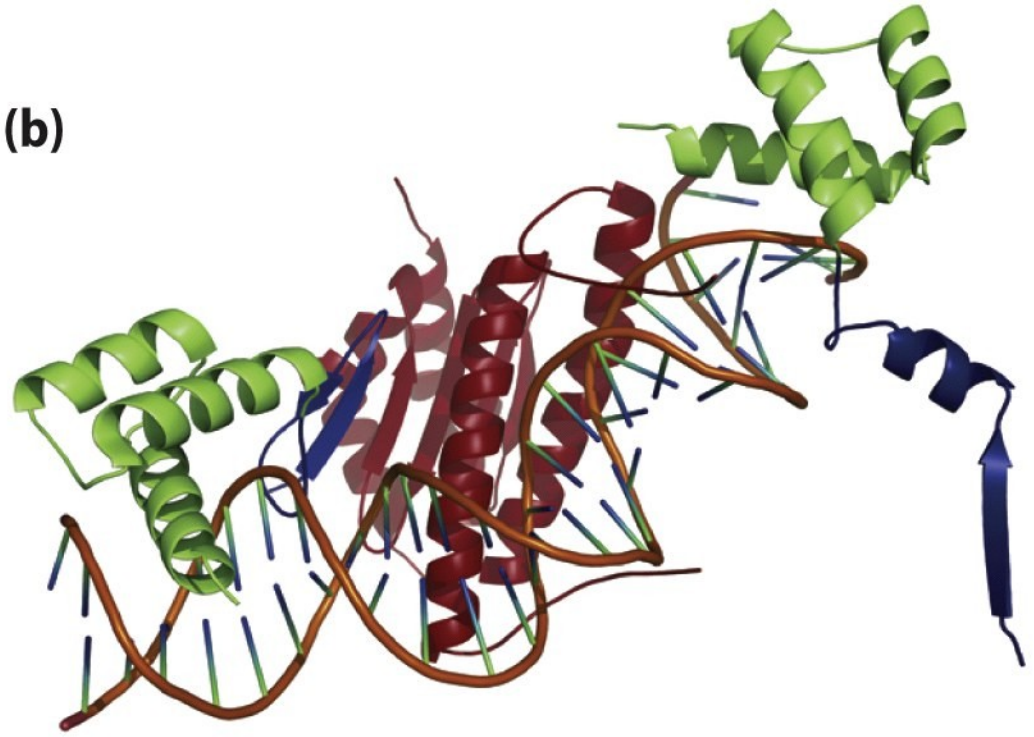
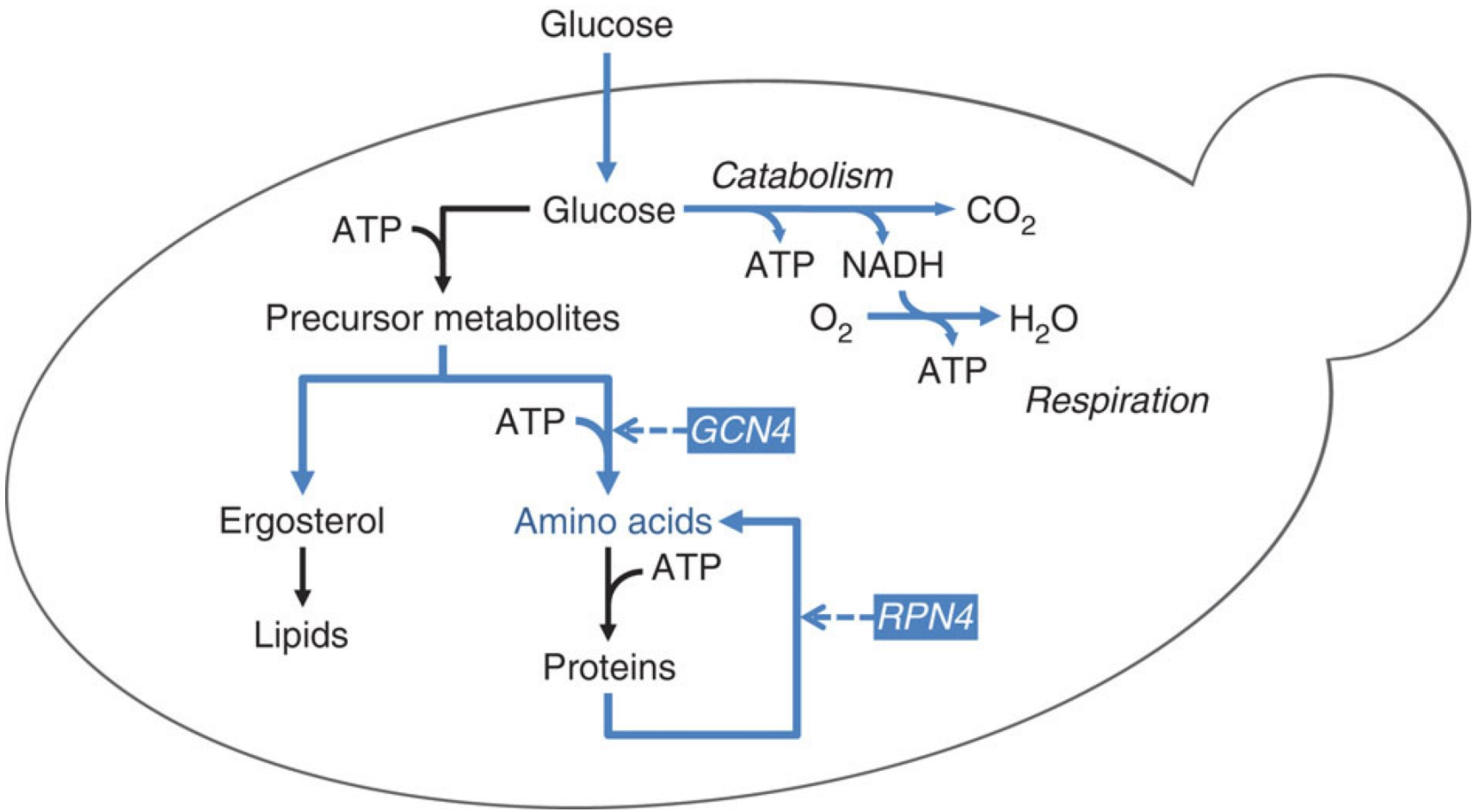


Figure 3.27 How Proteins Work (©2012 Garland Science)

Transcription factor

GCN4 protein

General control transcription factor

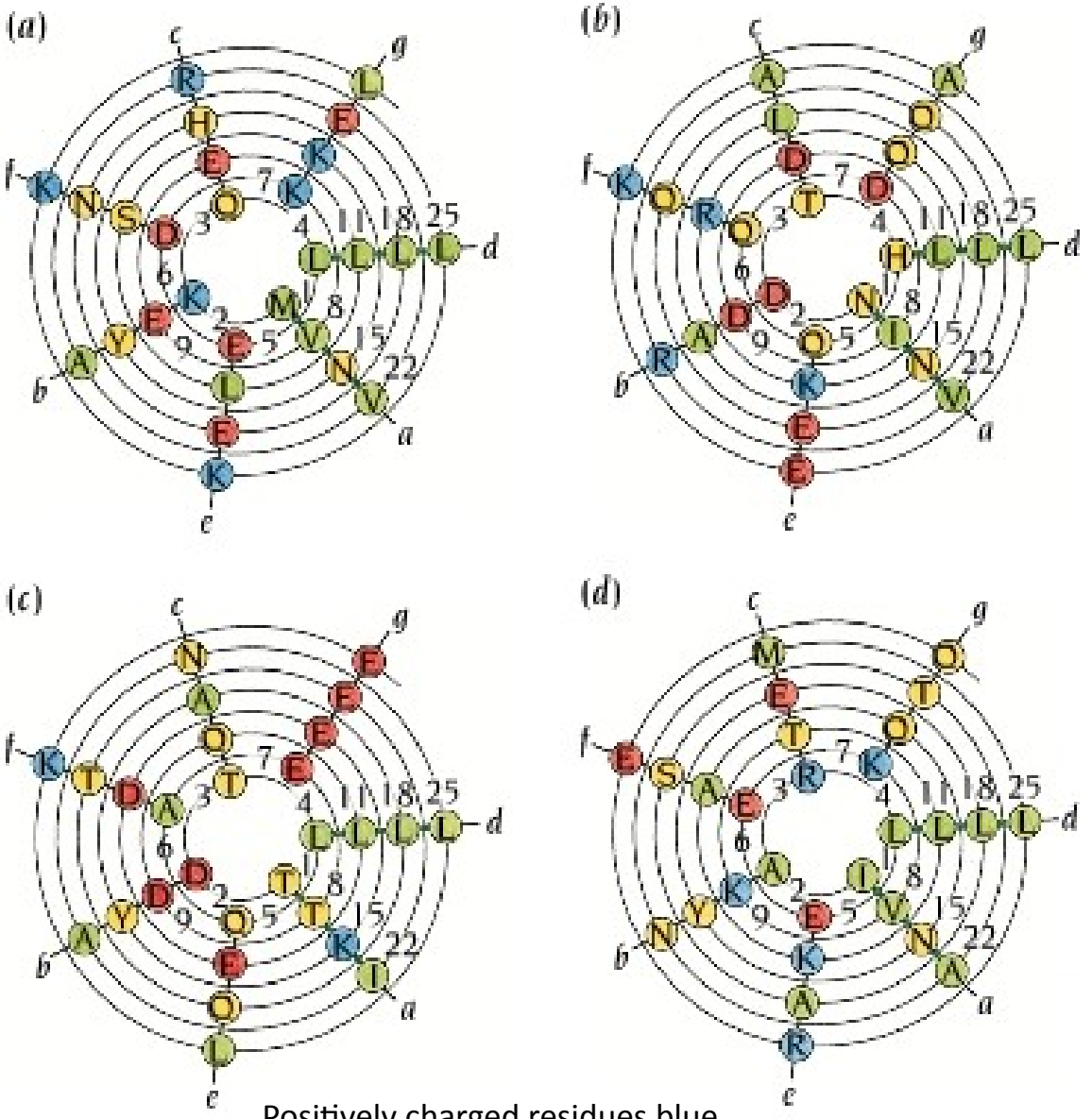


symmetric leucine zipper

Transcriptions factors

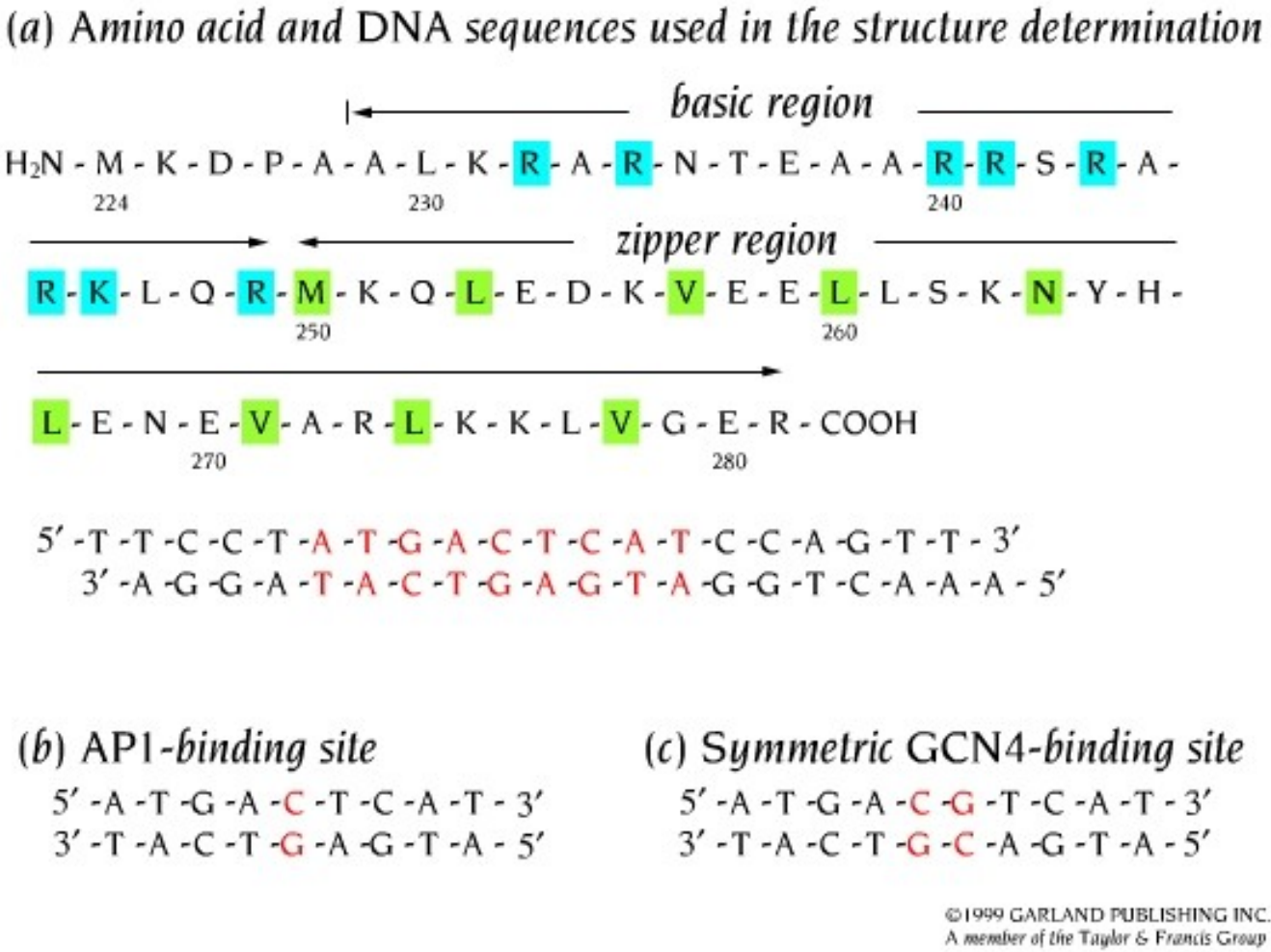
a) GCN4
b) Max
c) Fos (v-Fos)
d) Jun (v-Jun)

proto-oncogene



Positively charged residues blue
negatively charged red
polar residues yellow
Hydrophobic green

Regions


(a) Amino acid and DNA sequences used in the structure determination
basic region
H2N - M - K - D - P - A - A - L - K - R - A - R - N - T - E - A - A - R - R - S - R - A -
224
230
240
zipper region
R - K - L - Q - R - M - K - Q - L - E - D - K - V - E - E - L - L - S - K - N - Y - H -
250
260
L - E - N - E - V - A - R - L - K - K - L - V - G - E - R - COOH
270
280
5′ - T - T - C - C - T - A - T - G - A - C - T - C - A - T - C - C - A - G - T - T - 3′
3′ - A - G - G - A - T - A - C - T - G - A - G - T - A - G - G - T - C - A - A - A - 5′
(b) AP1-binding site
5′ - A - T - G - A - C - T - C - A - T - 3′
3′ - T - A - C - T - G - A - G - T - A - 5′
(c) Symmetric GCN4-binding site
5′ - A - T - G - A - C - G - T - C - A - T - 3′
3′ - T - A - C - T - G - C - A - G - T - A - 5′
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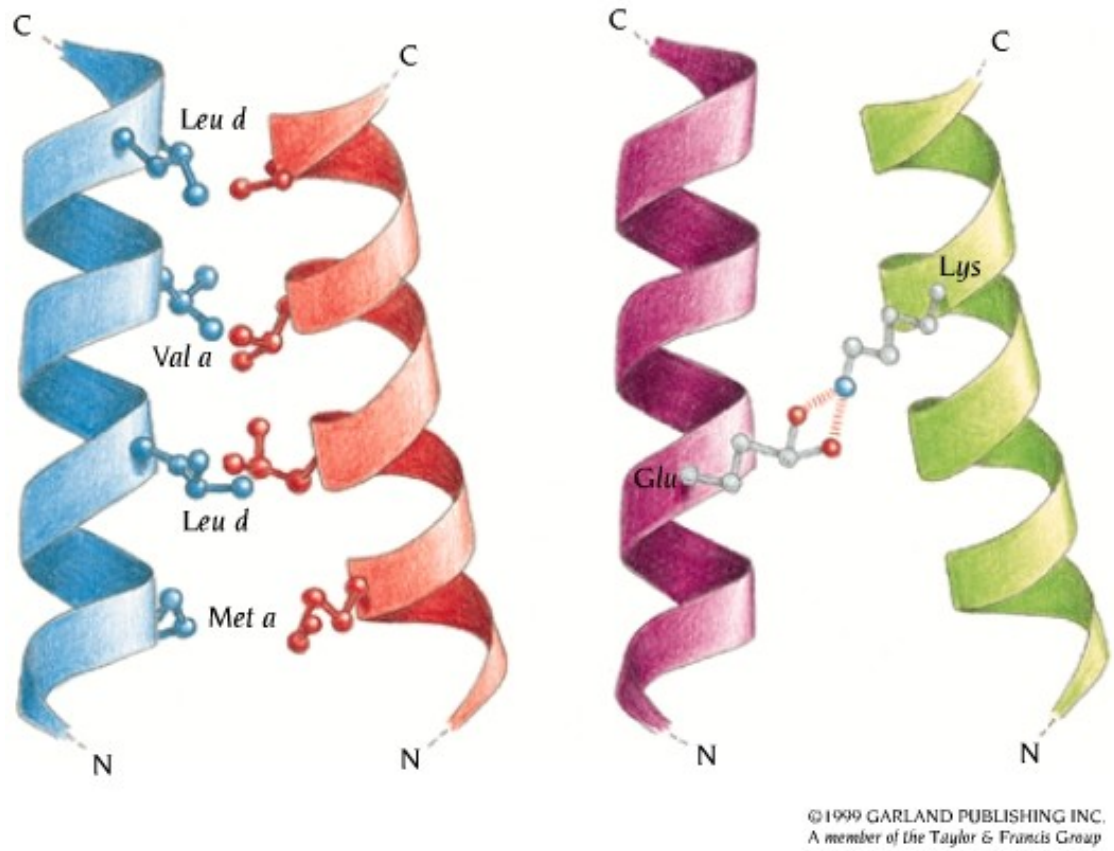
C
C
Leu d
Val a
Leu d
Met a
N
N
C
C
Lys
Glu
N
N
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interactions

(a) Amino acid and DNA sequences used in the structure determination

basic region

H_2N - M - K - D - P - A - A - L - K - R - A - R - N - T - E - A - A - R - R - S - R - A -
224 230 240

zipper region

R - K - L - Q - R - M - K - Q - L - E - D - K - V - E - E - L - L - S - K - N - Y - H -
250 260

L - E - N - E - V - A - R - L - K - K - L - V - G - E - R - COOH
270 280

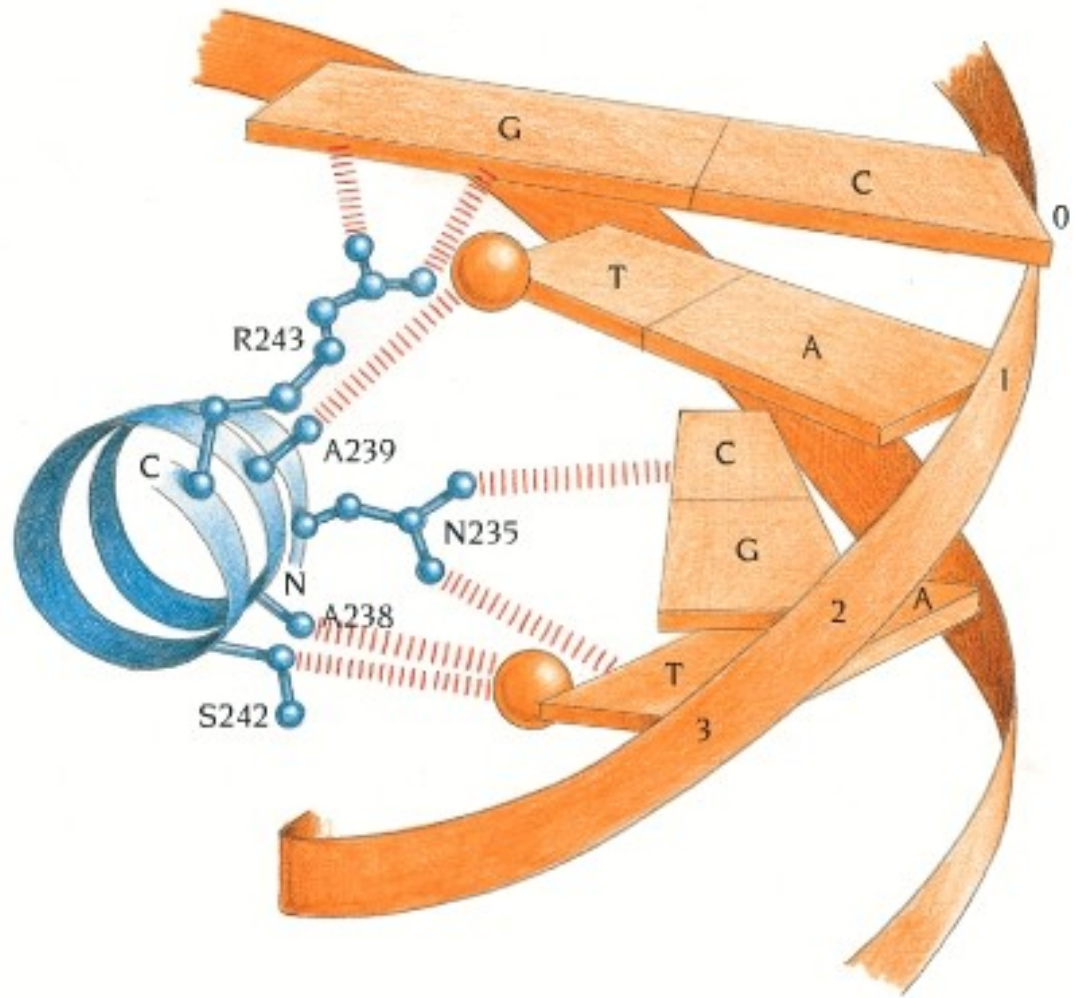
5′ -T -T -C -C -T -A -T -G -A -C -T -C -A -T -C -C -A -G -T -T - 3′
3′ -A -G -G -A -T -A -C -T -G -A -G -T -A -G -G -T -C -A -A -A - 5′

(b) AP1-binding site

5′ -A -T -G -A -C -T -C -A -T - 3′
3′ -T -A -C -T -G -A -G -T -A - 5′

(c) Symmetric GCN4-binding site

5′ -A -T -G -A -C -G -T -C -A -T - 3′
3′ -T -A -C -T -G -C -A -G -T -A - 5′



Fos-Jun

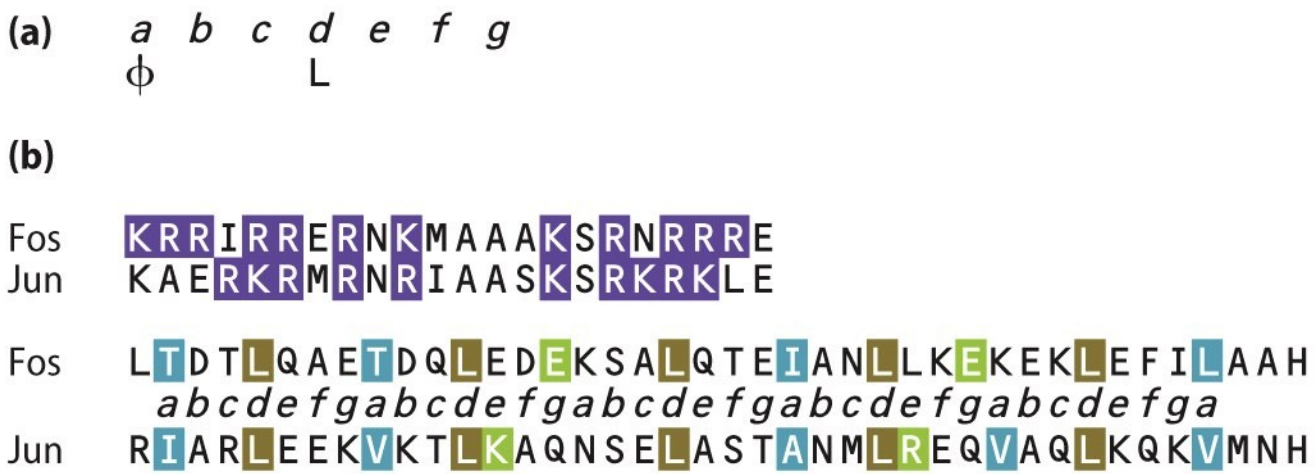


Figure 3.35 How Proteins Work (©2012 Garland Science)

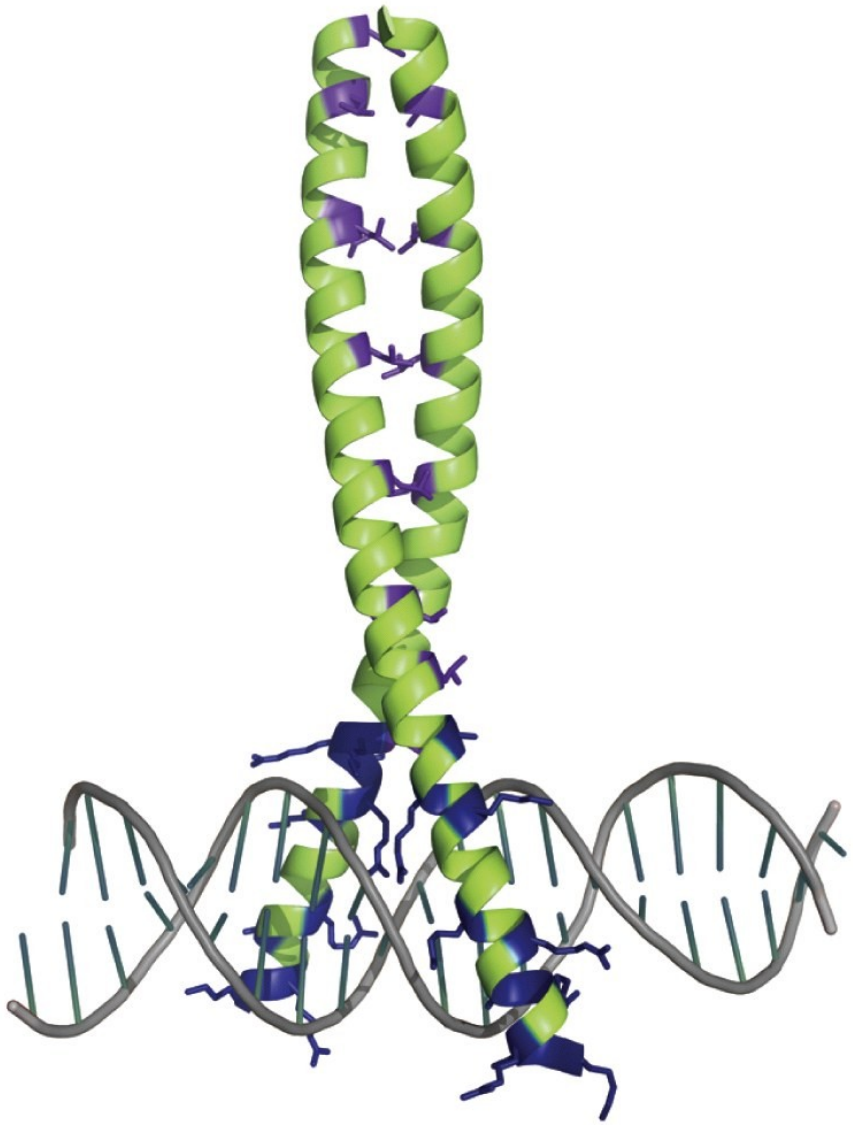


Figure 3.34 How Proteins Work (©2012 Garland Science)

Heterodimerization of leucine zipper proteins

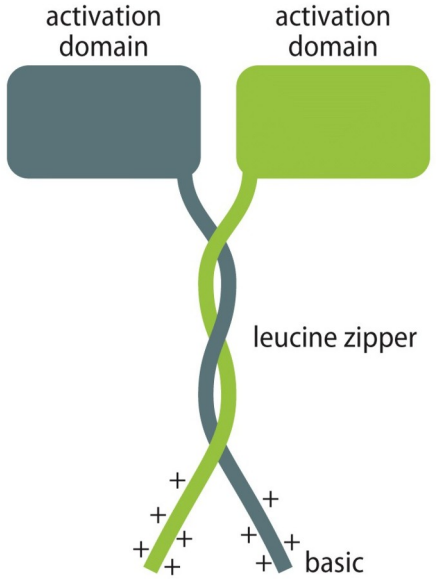
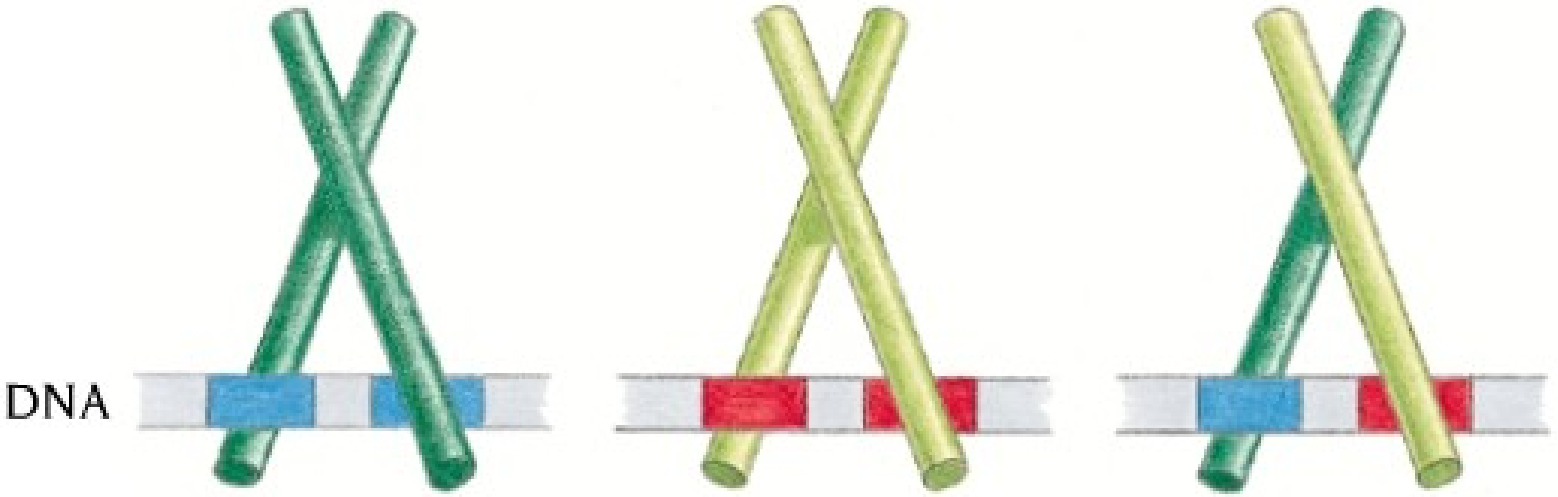


Figure 3.36 How Proteins Work (©2012 Garland Science)



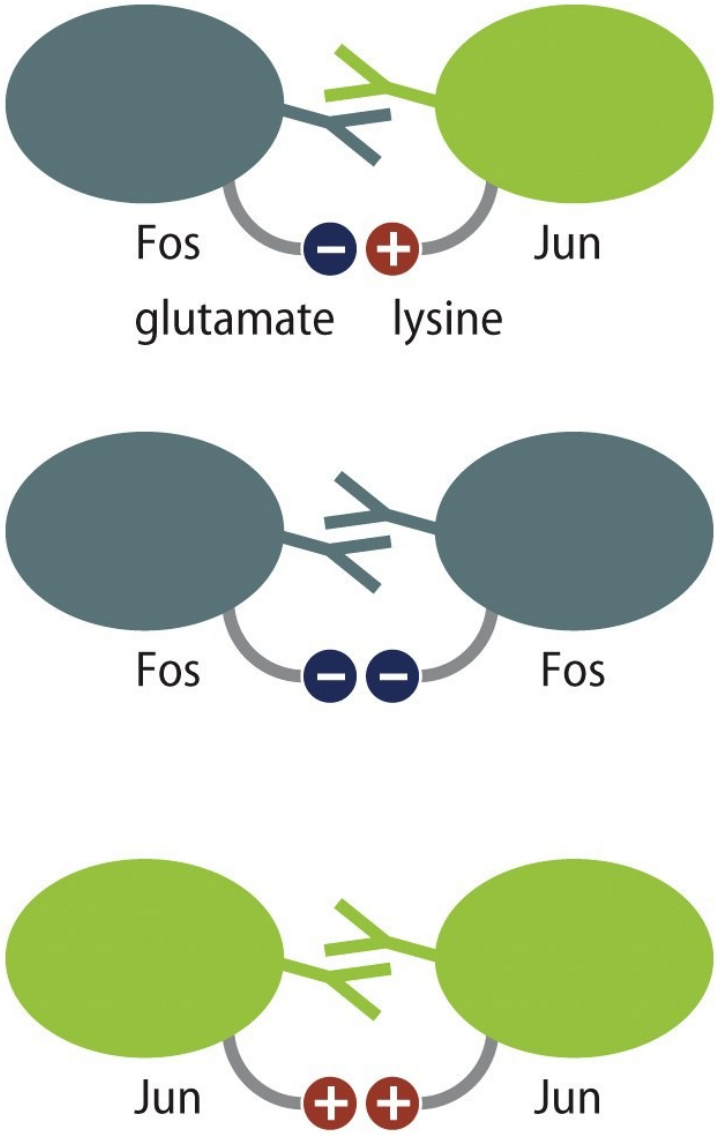
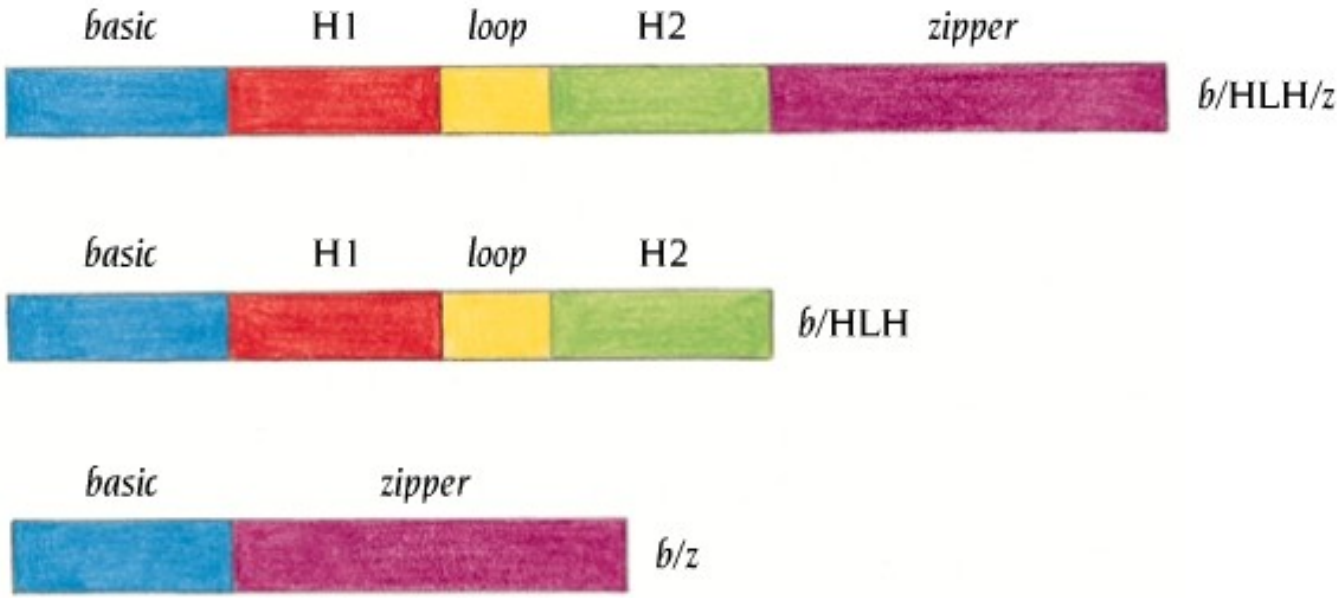
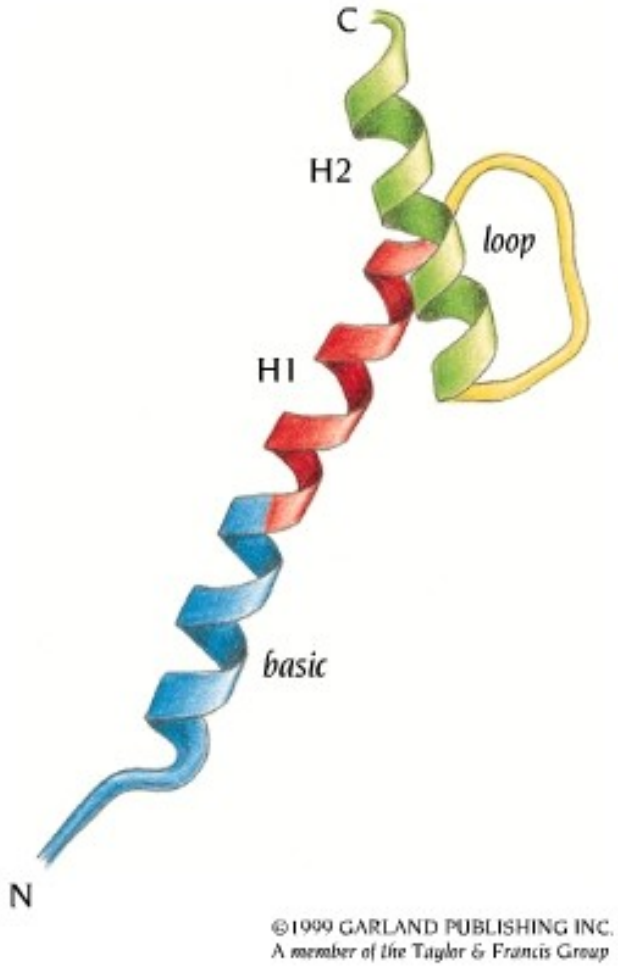


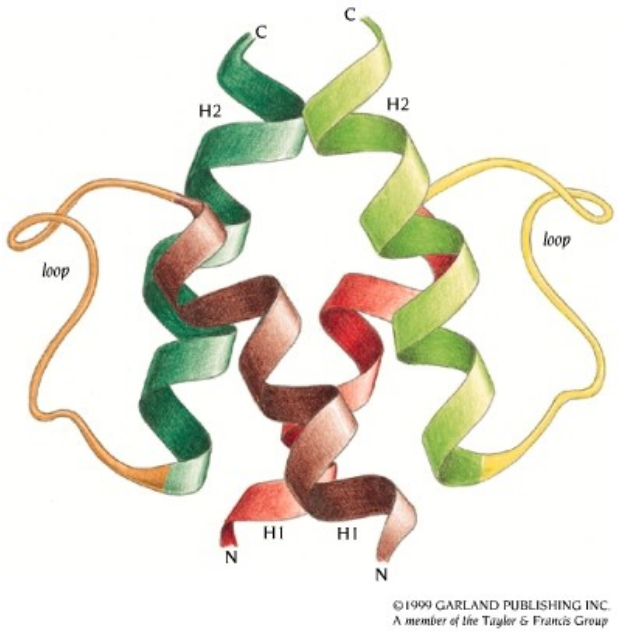
Figure 3.37 How Proteins Work (©2012 Garland Science)

HLH motif

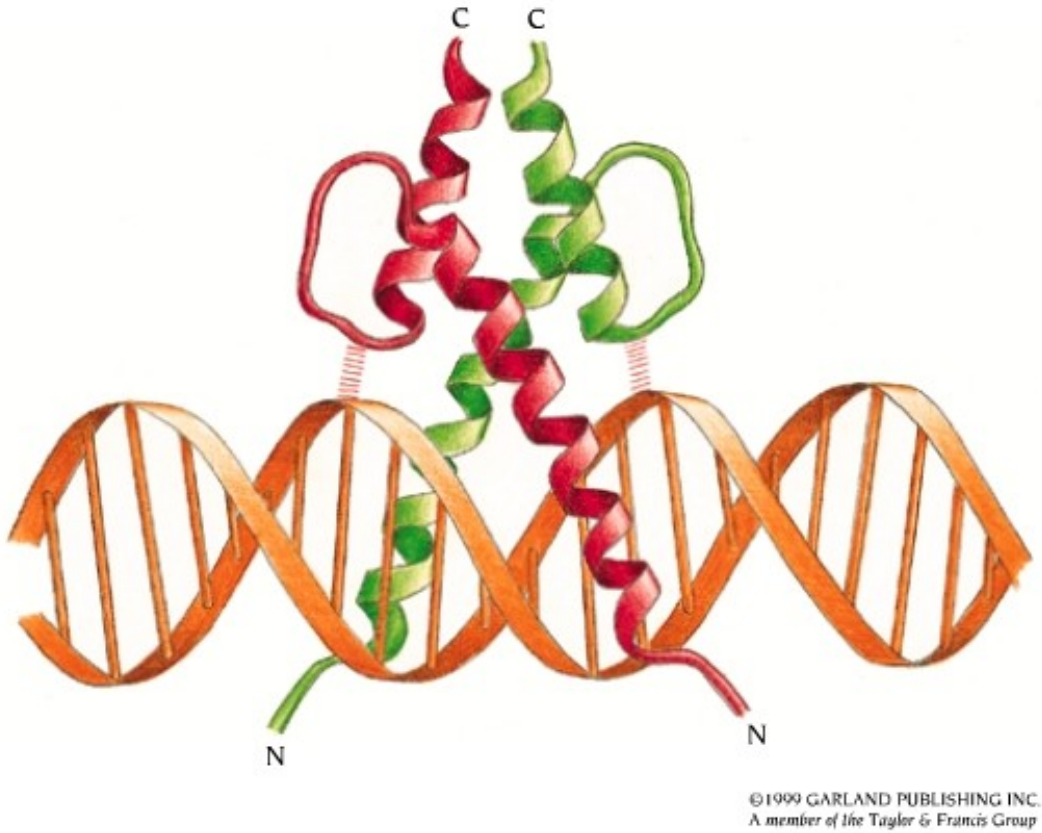


HLH motif

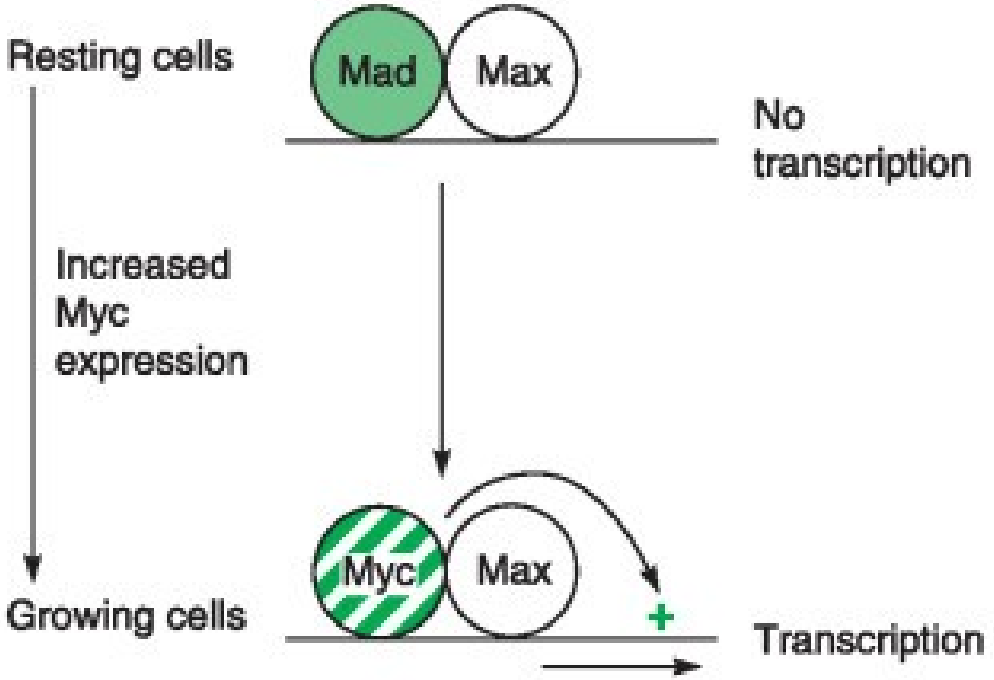




MyoD

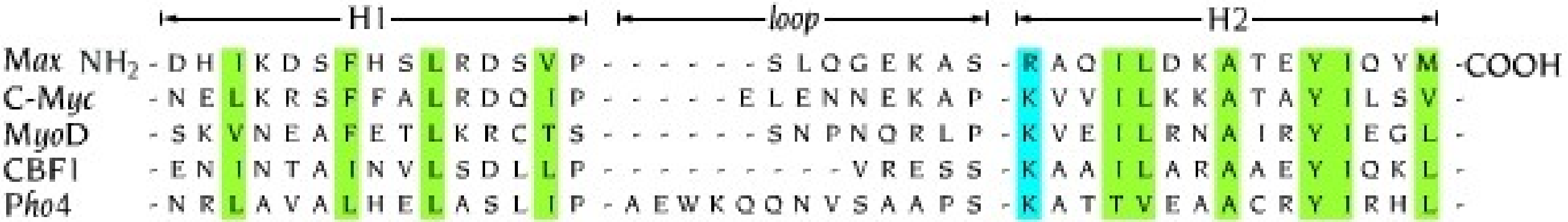


HLH motif

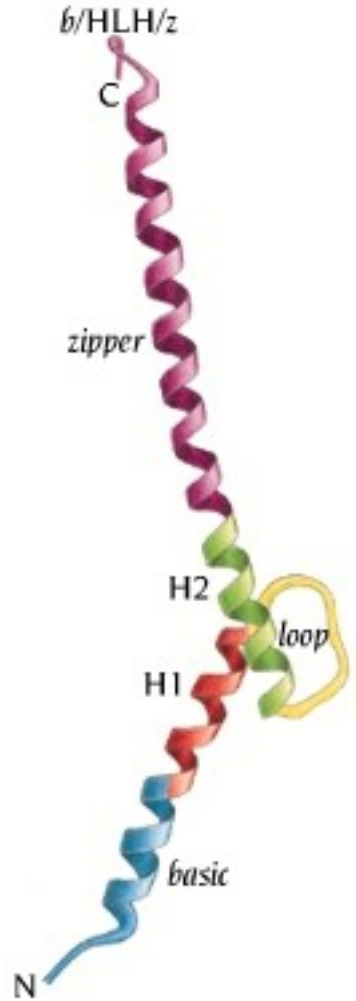


- **Myc-Max:**
- This heterodimer promotes cell growth, proliferation, and differentiation by binding to E-box sequences and upregulating genes necessary for these processes.
- **Mad-Max:**
- This heterodimer, which replaces Myc-Max during differentiation, represses transcription and promotes cell quiescence and differentiation.

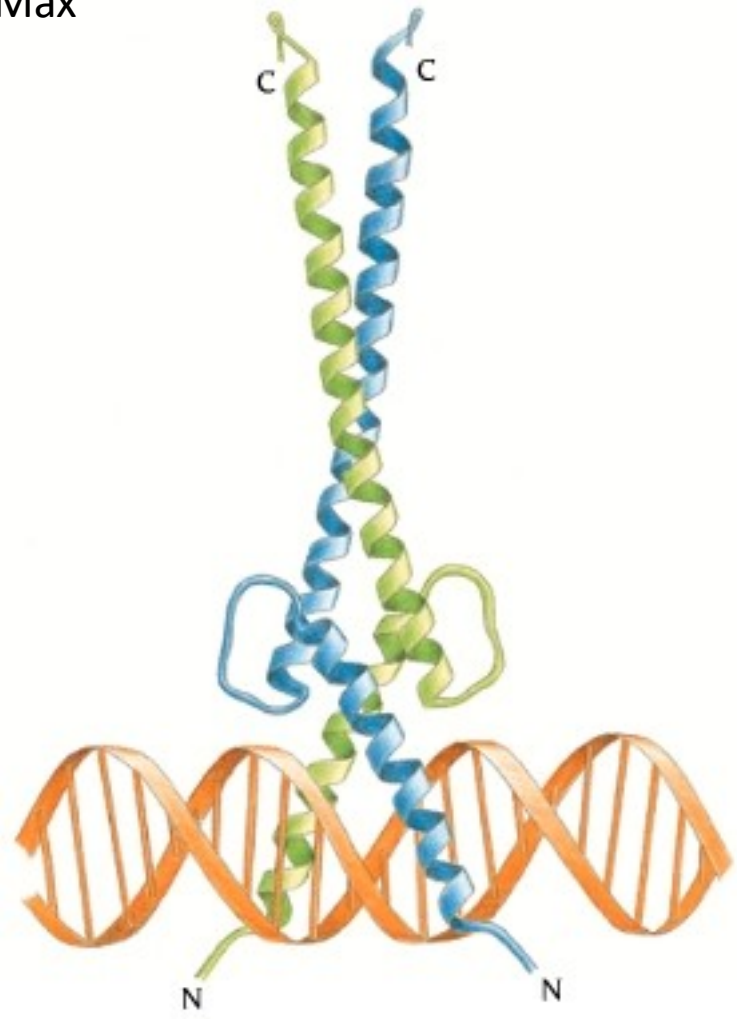
HLH motif

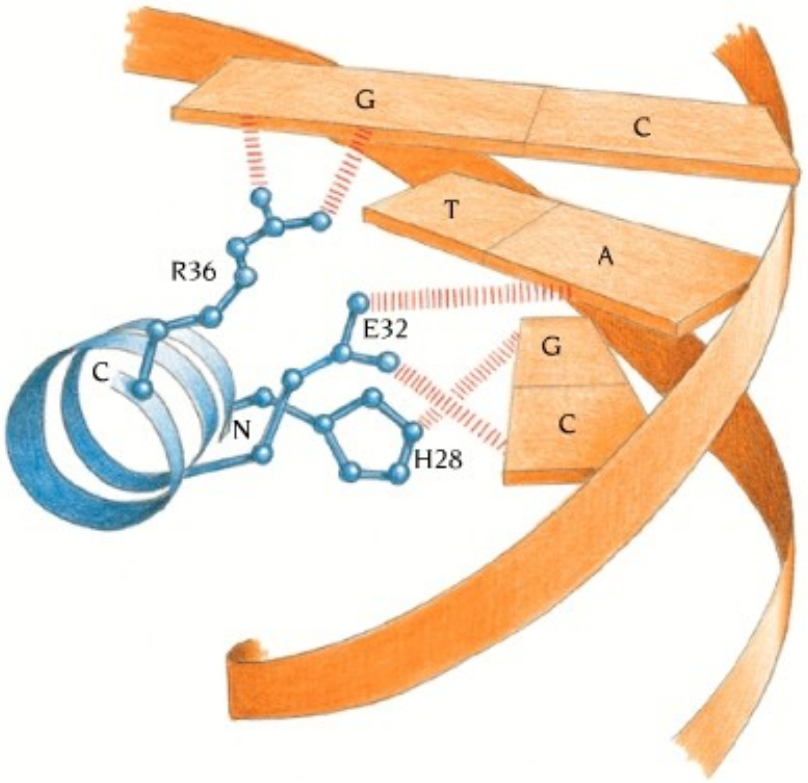


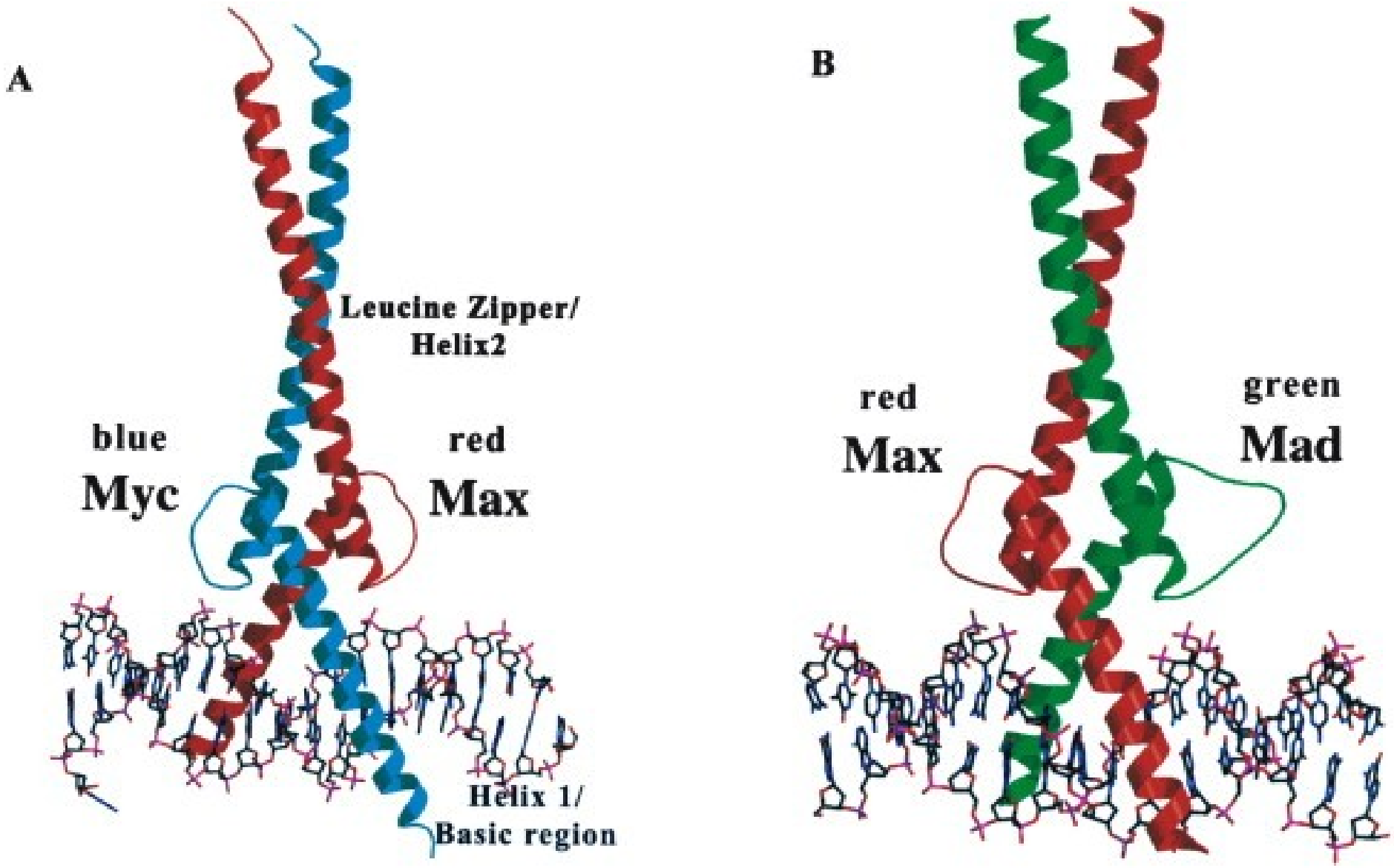
HLH motif



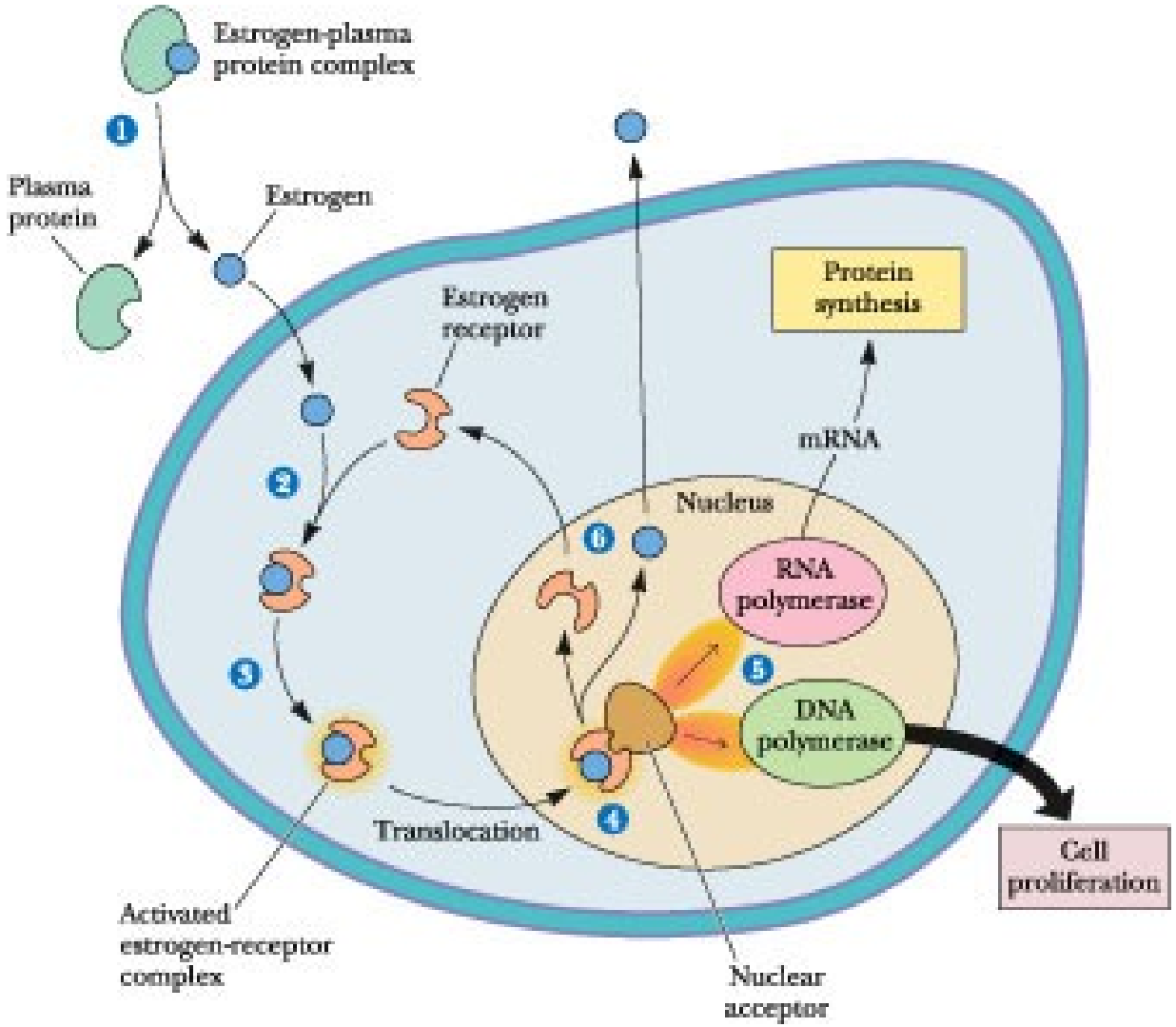
Max



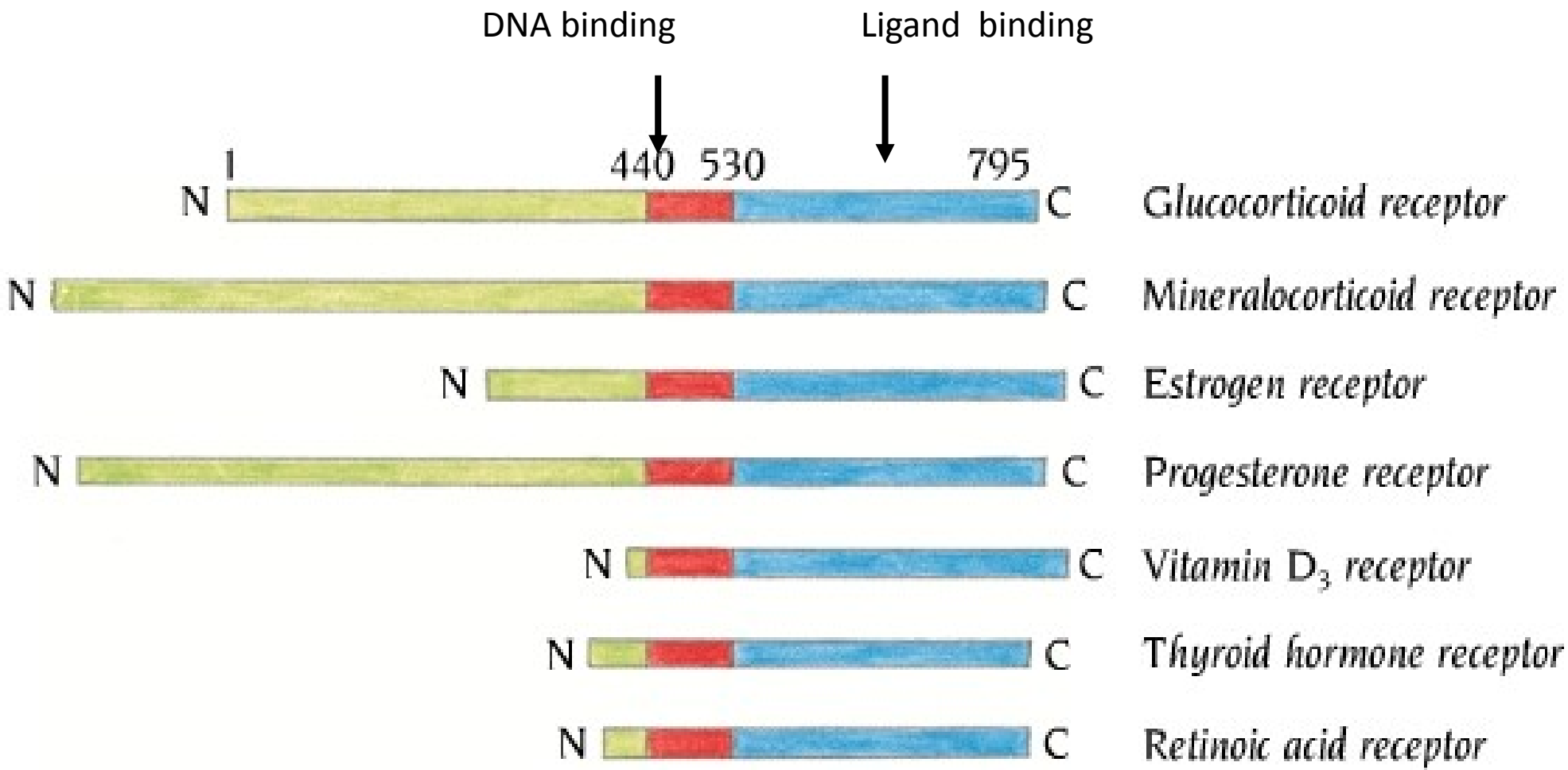



A
Leucine Zipper/
Helix2
blue
Myc
red
Max
Helix 1/
Basic region
B
red
Max
green
Mad

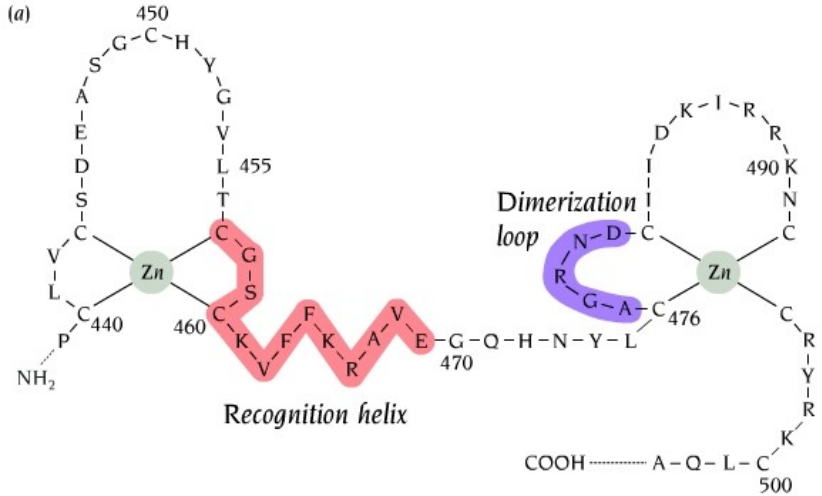
Steroid hormone action



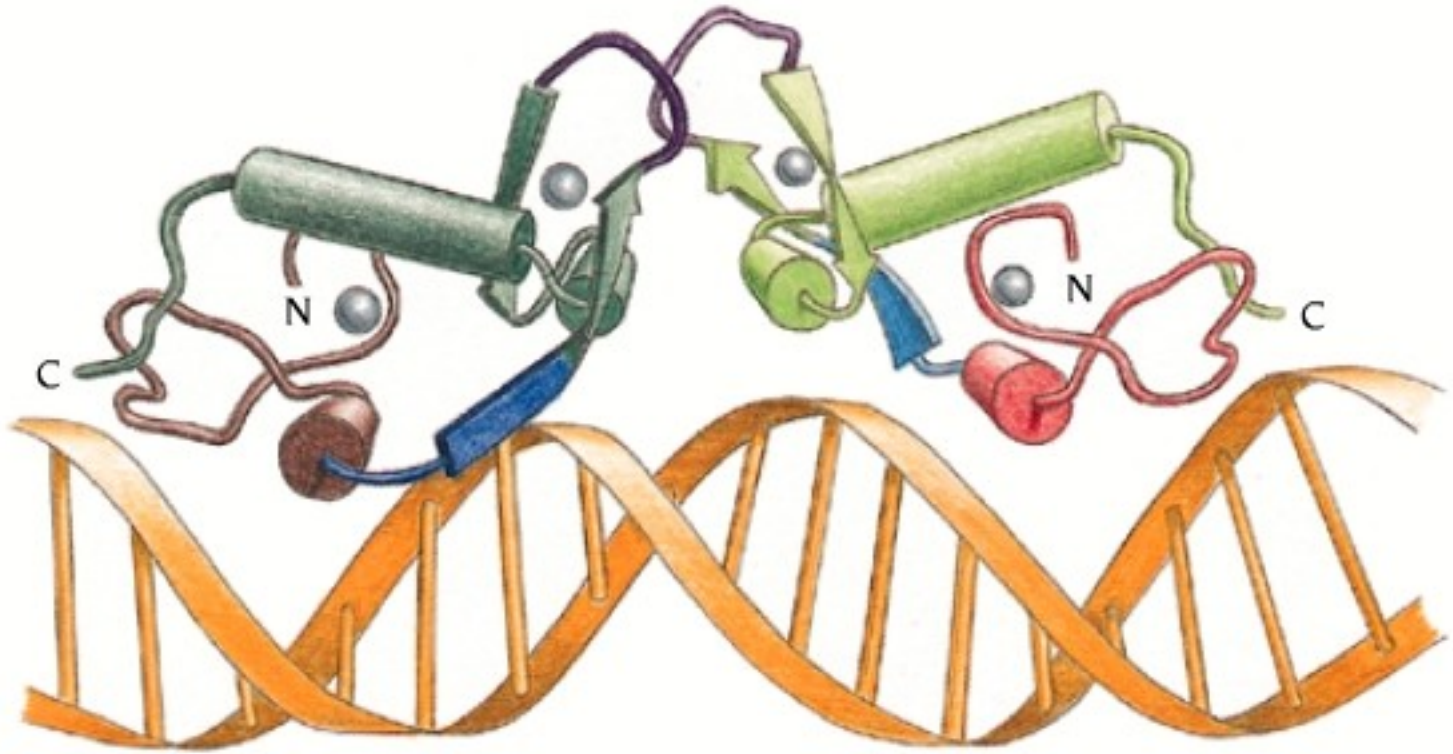
Steroid hormone receptors



Zinc containing motifs



Glycocorticoid receptor



Tandem dimer

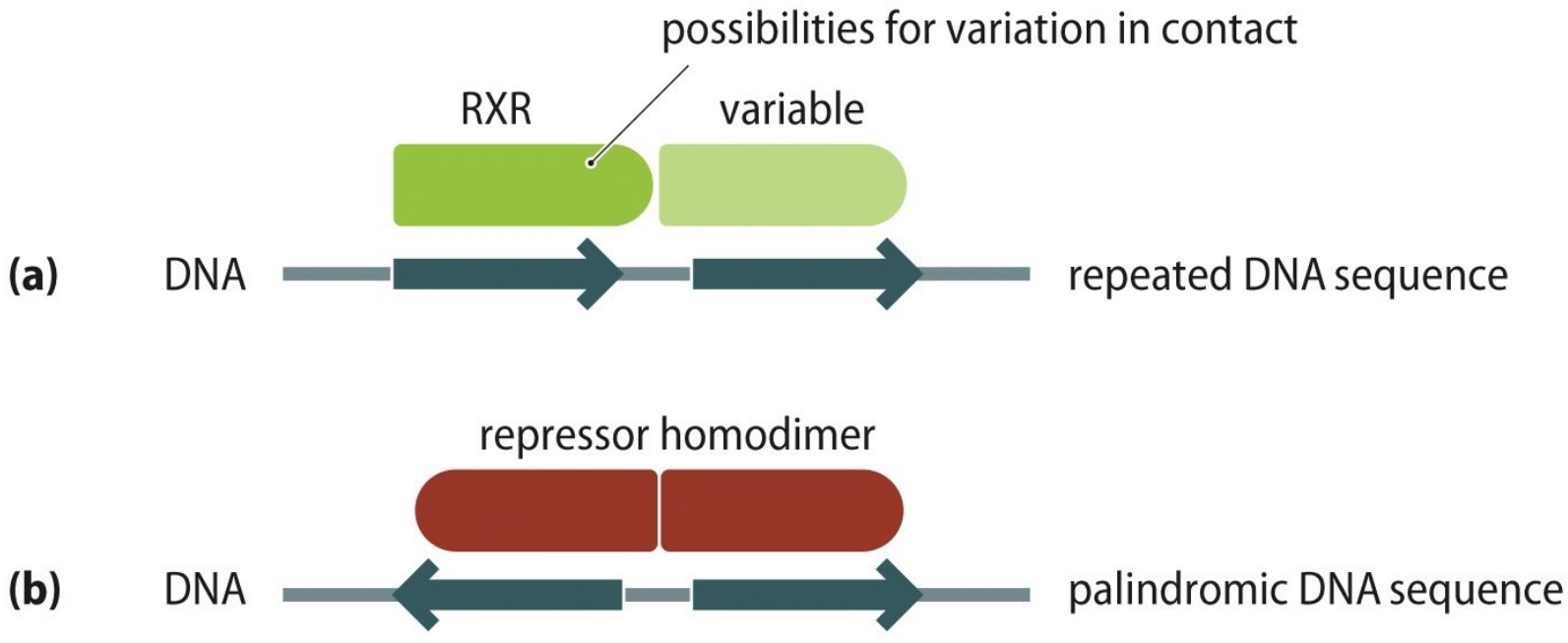


Figure 3.39 How Proteins Work (©2012 Garland Science)

VDR vitamin D
RXR cis retonic acid
TR thyroid hormone
RAR trans retonic acid

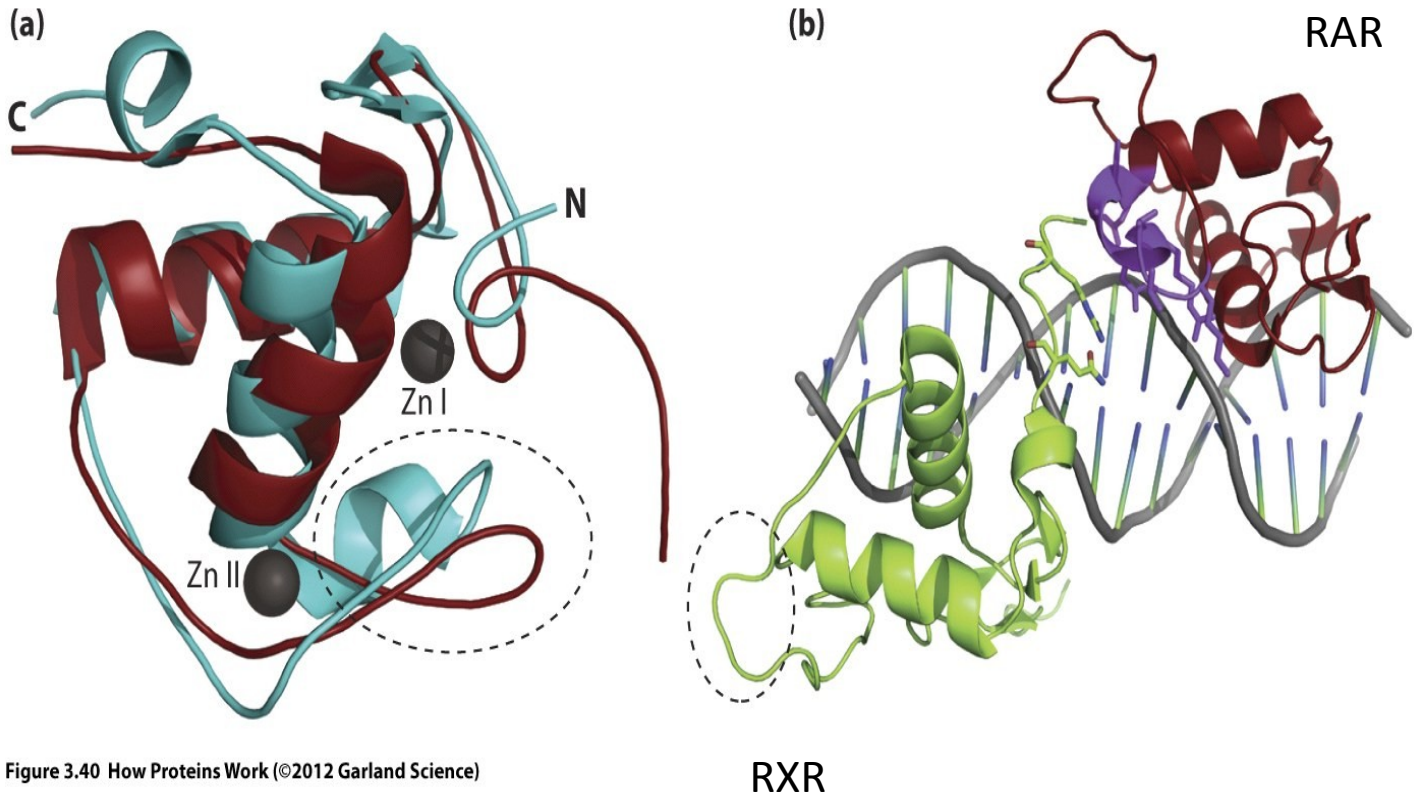


Figure 3.40 How Proteins Work (©2012 Garland Science)

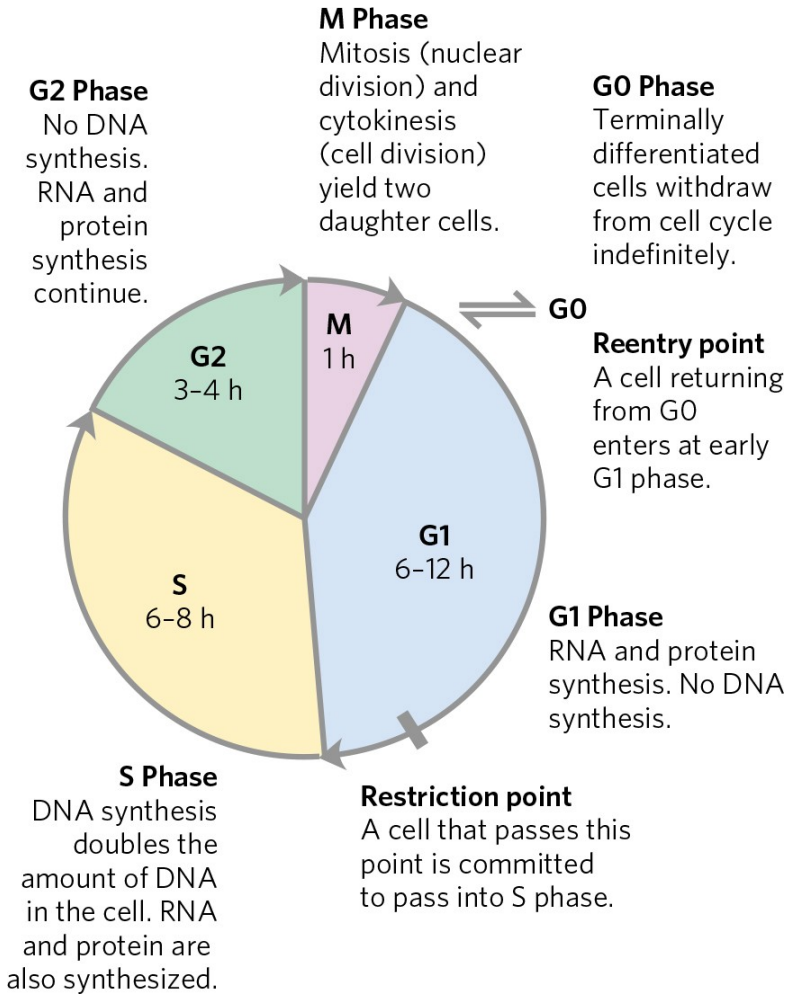
TABLE 3.1 Lipophilic hormone receptor targets

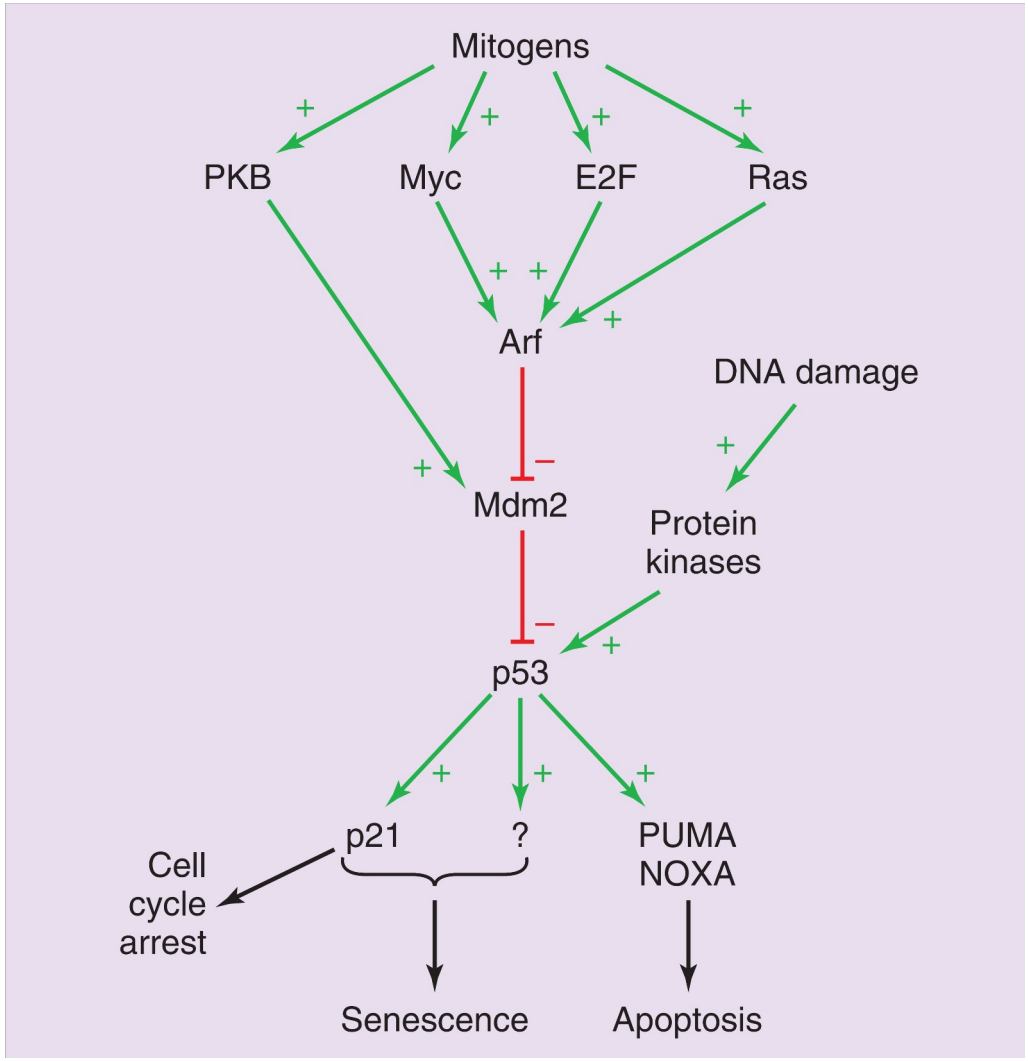
RXR.RXR	AGGTCA*n*AGGTCA
RXR.RAR	AGGTCA*nn*AGGTCA
RXR.VDR	AGGTCA*nnn*AGGTCA
RXR.TR	AGGTCA*nnnn*AGGTCA
RXR.RAR	AGGTCA*nnnnn*AGGTCA

RXR = 9-*cis* retinoic acid receptor; RAR = all-*trans* retinoic acid receptor; VDR = vitamin D1 receptor; TR = thyroid hormone receptor. (Based on a Table in F. Rastinejad, *Curr. Opin. Struct. Biol.* 11:33–38, 2001. With permission from Elsevier.)

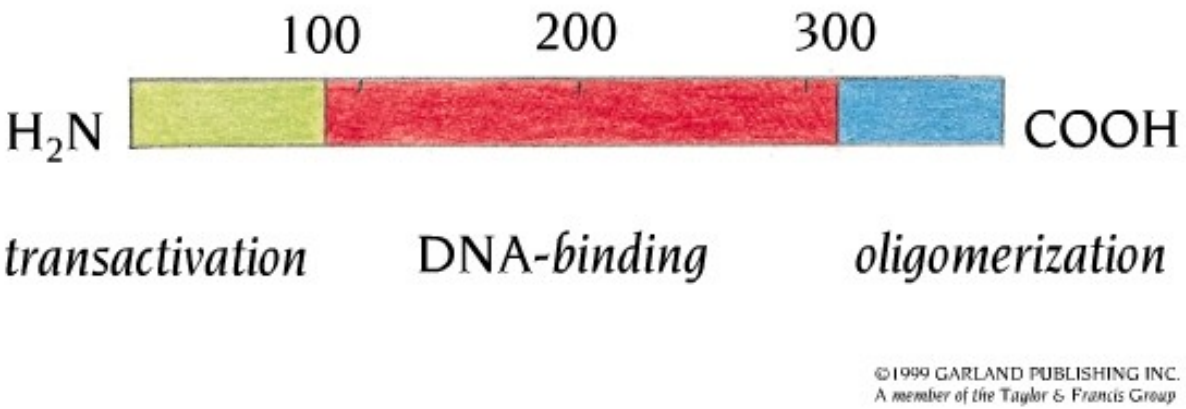
Table 3.1 How Proteins Work (©2012 Garland Science)

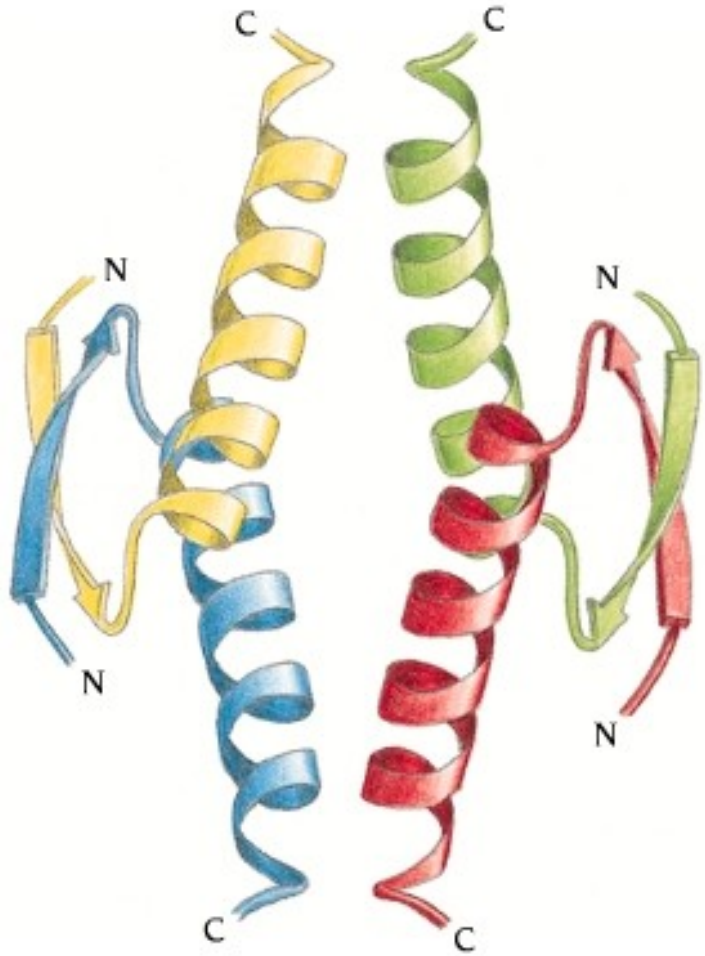
Understanding tumorigenic mutations



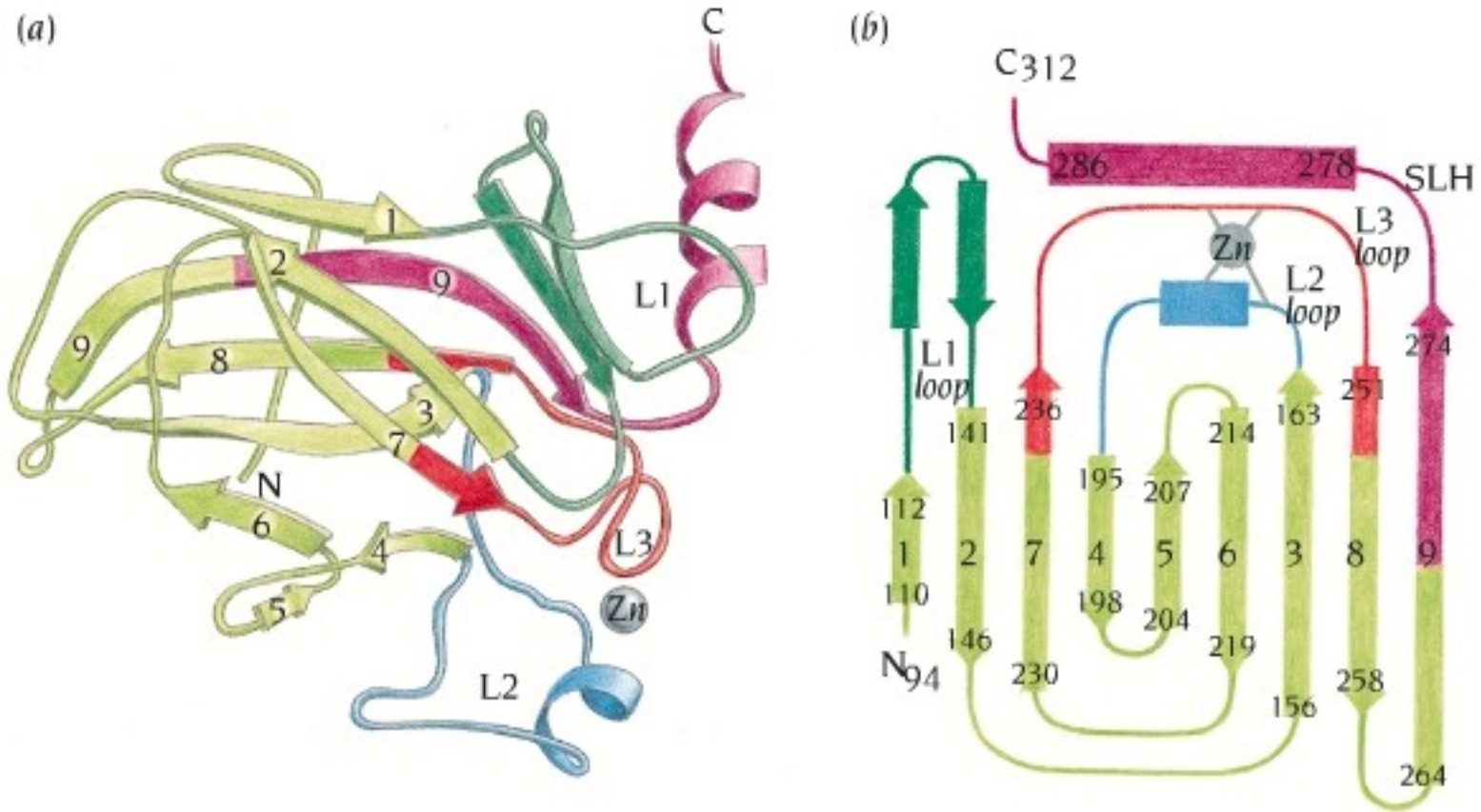


Understanding tumorigenic mutations

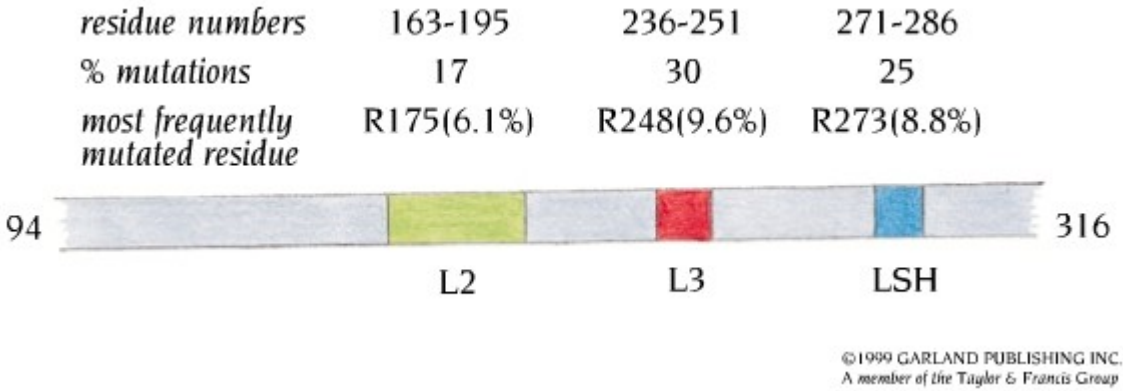


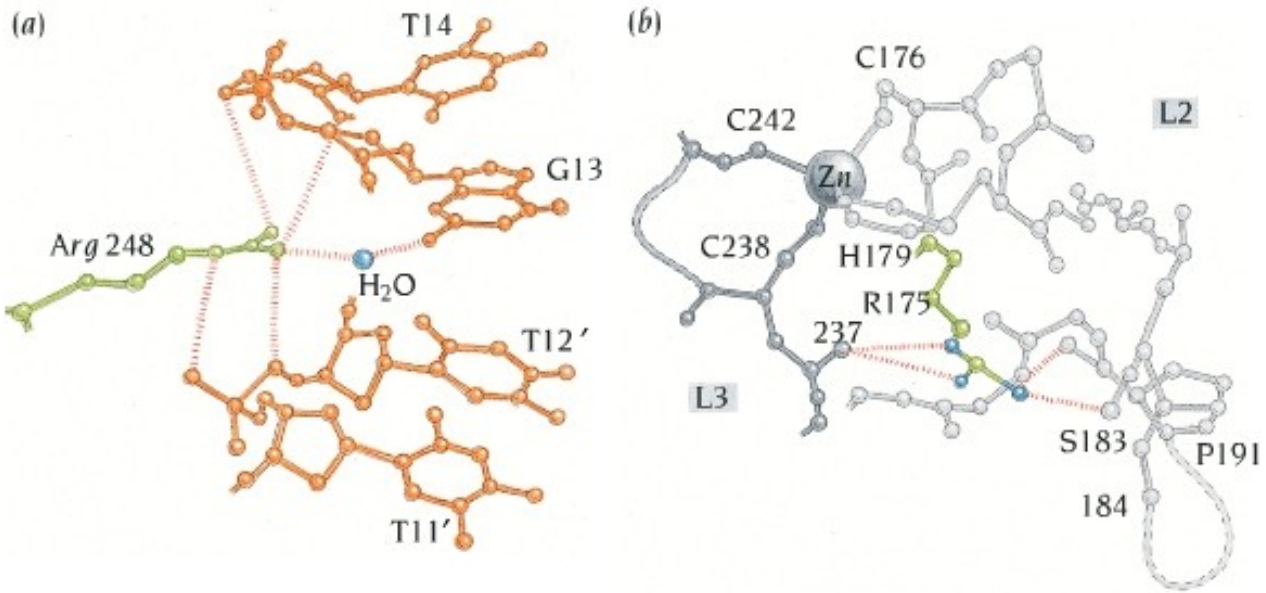


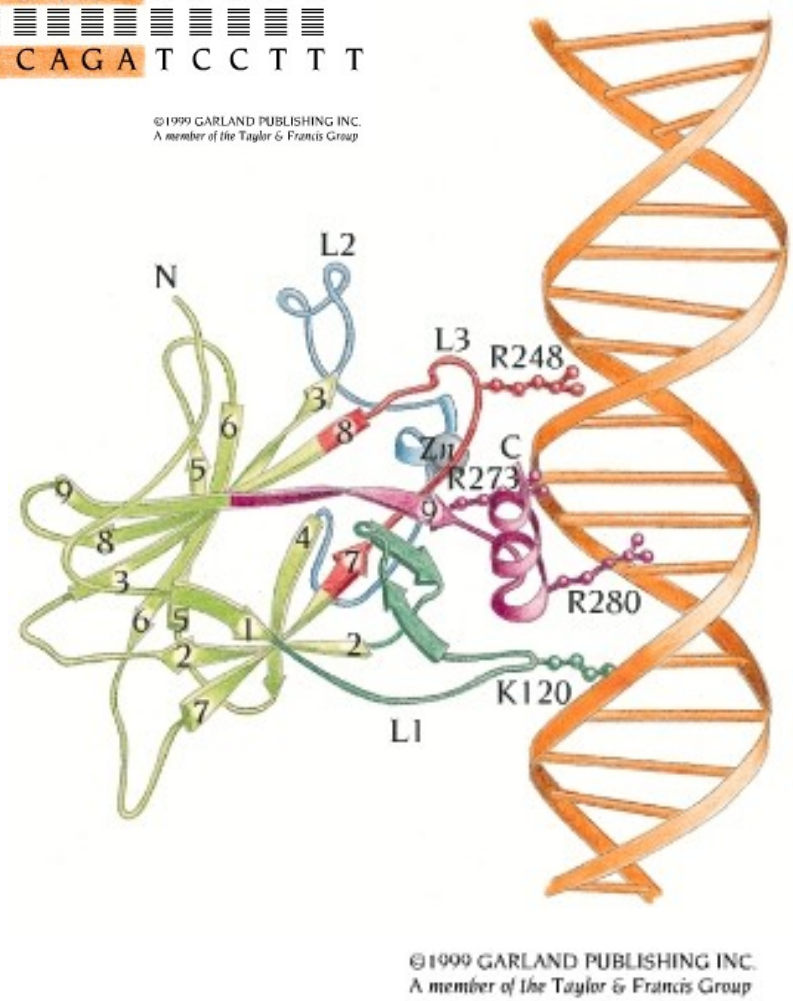
Understanding tumorigenic mutations



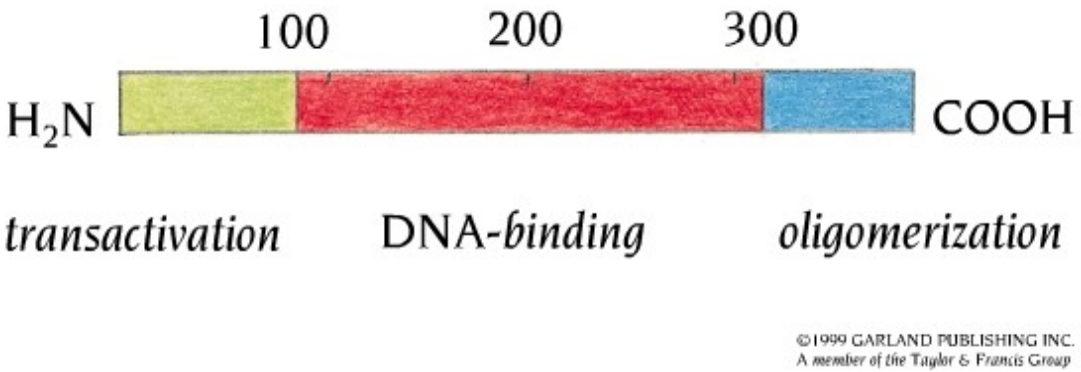
Understanding tumorigenic mutations

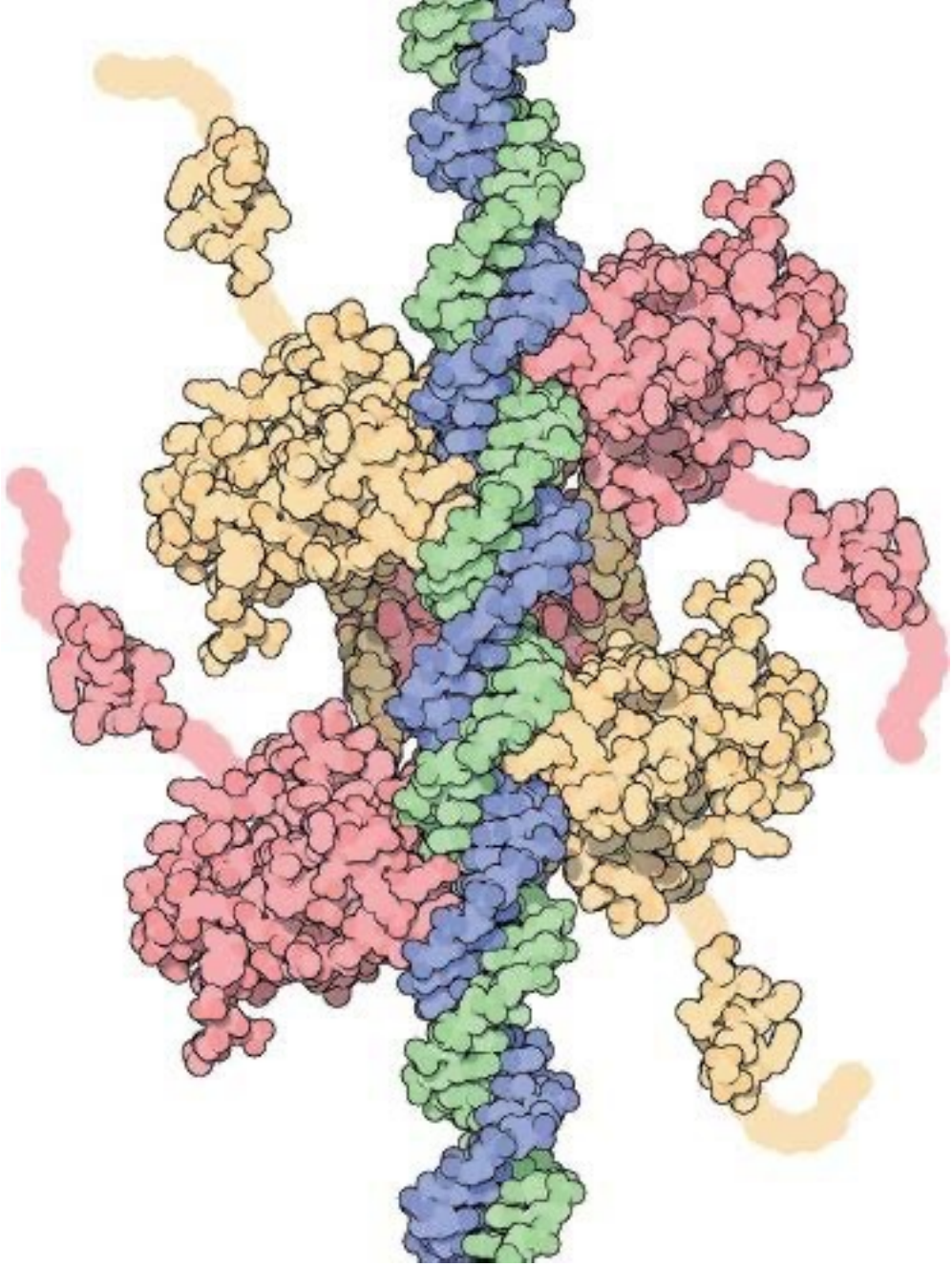






Understanding tumorigenic m





Isozymes

Isozymes or Isoenzymes are proteins with different structure which catalyze the same reaction.

Frequently they are oligomers made with different polypeptide chains, so they usually differ in regulatory mechanisms and in kinetic characteristics.

From the physiological point of view, isozymes allow the existence of similar enzymes with different characteristics, “customized” to specific tissue requirements or metabolic conditions.

The existence of isozymes permits the fine-tuning of metabolism to meet the needs of a given tissue or developmental stage.

Lactate Dehydrogenase

Muscle heart

$$\text{pyruvate} + \text{NADH} \rightleftharpoons \text{lactate} + \text{NAD}^+$$

M_4

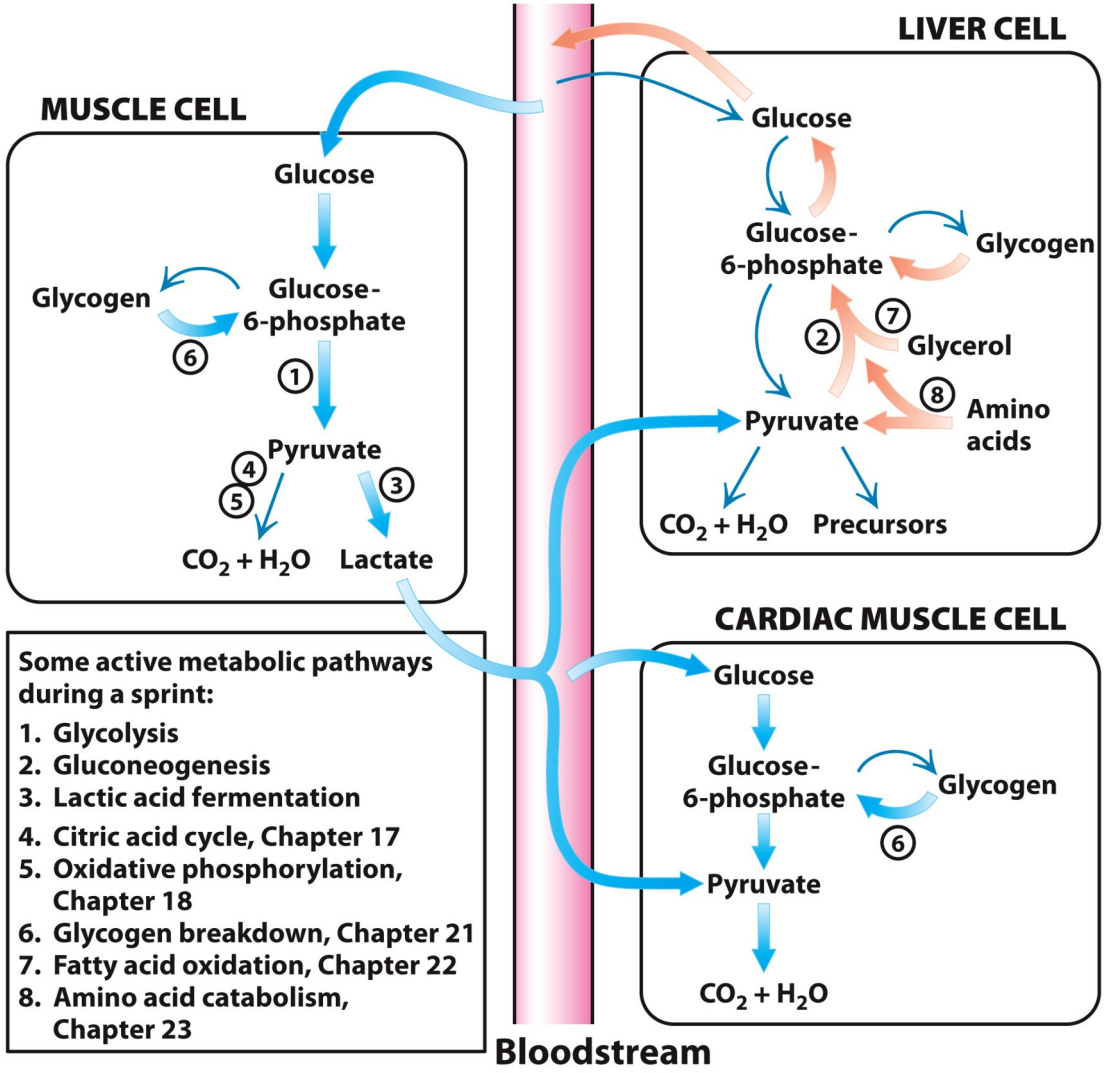


Figure 16.34
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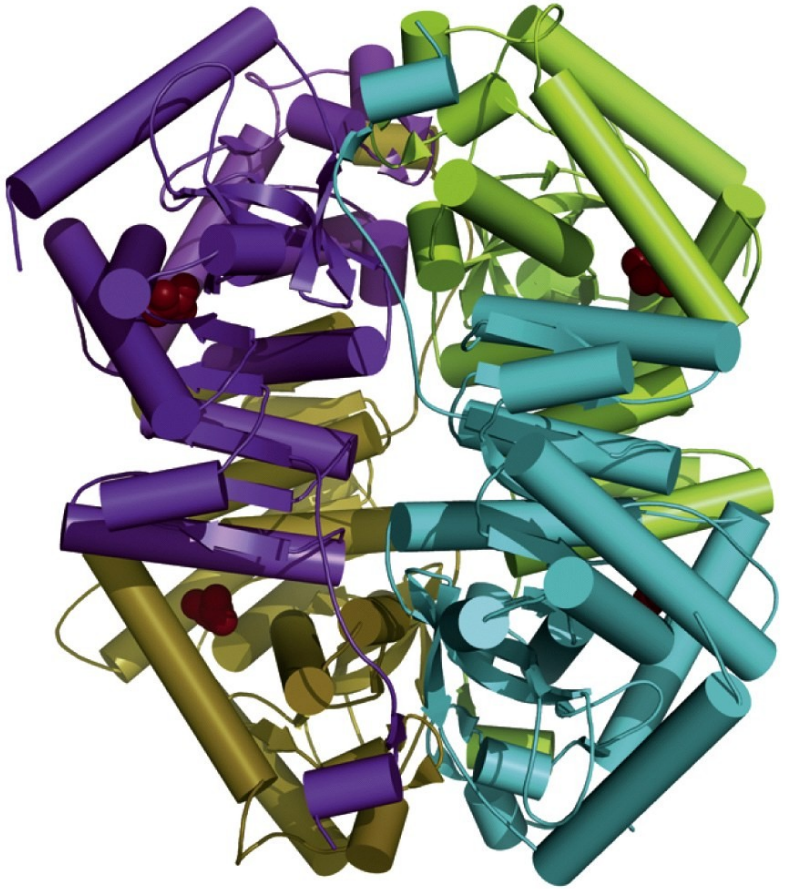
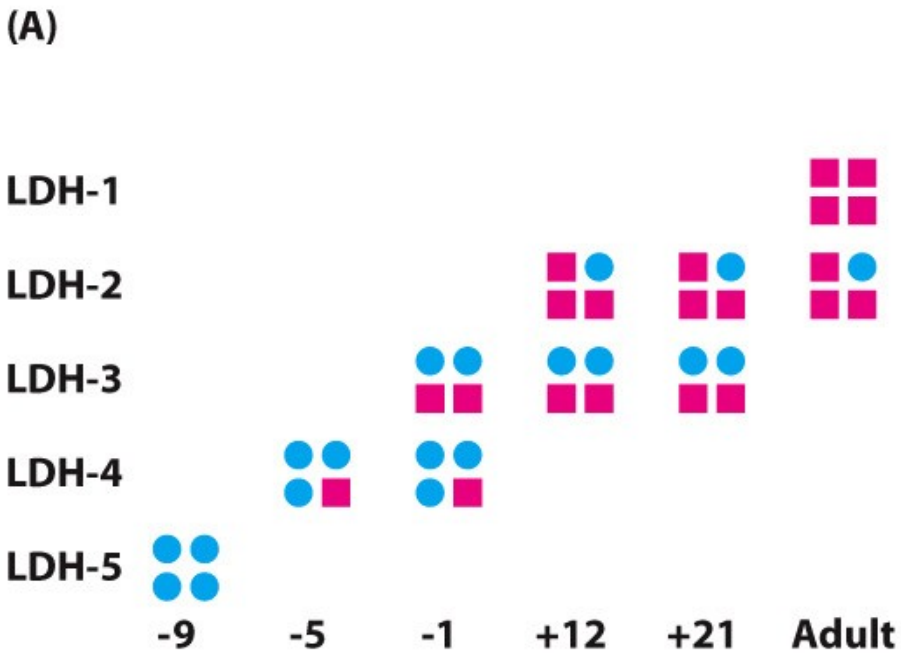


Figure 3.41 How Proteins Work (©2012 Garland Science)

Lactate Dehydrogenase

H isozyme by squares
M isozyme by circles

$$\text{pyruvate} + \text{NADH} \rightleftharpoons \text{lactate} + \text{NAD}^+$$



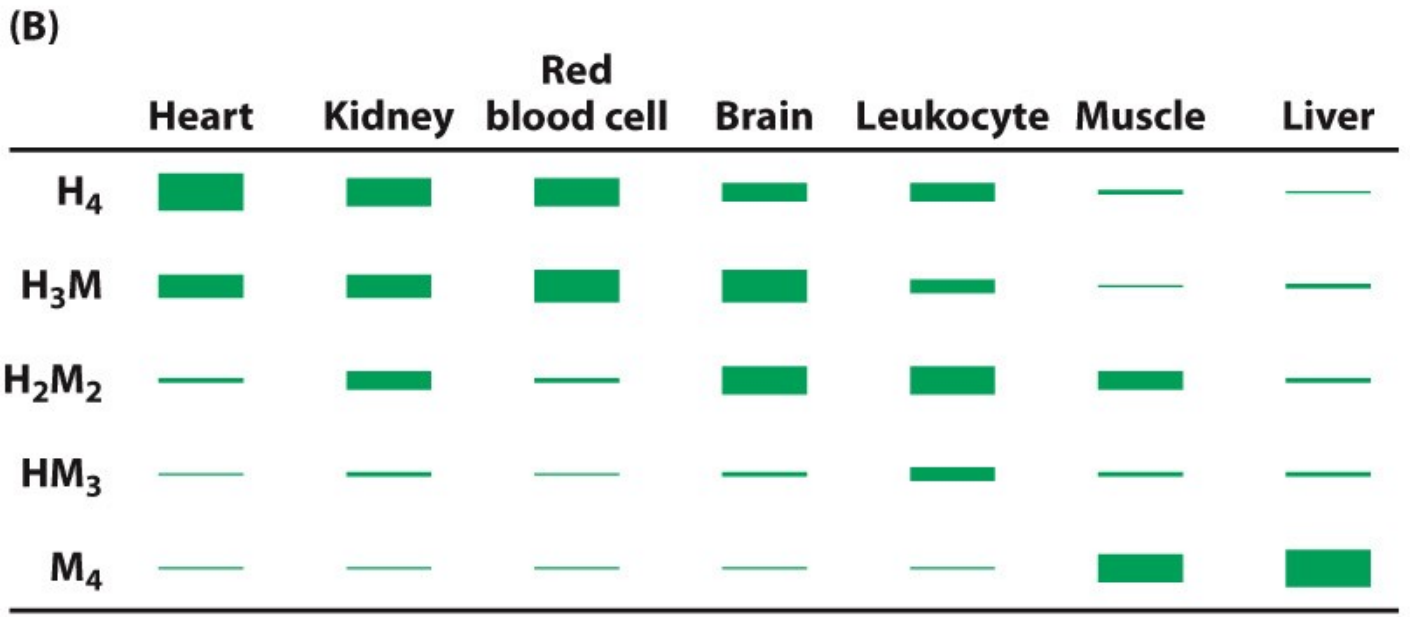


Figure 10.15
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