Multienzyme Complexes: Catalytic Nanomachines

Beyond the catalytic face, enzymes have two additional faces: regulatory and social.
The regulatory site binds a ligand that modifies the rate and specificity of the enzymes.
The social face associates the enzyme with other components, such as a membrane or a scaffold, or complexes with other enzymes.

The sociology of complexes

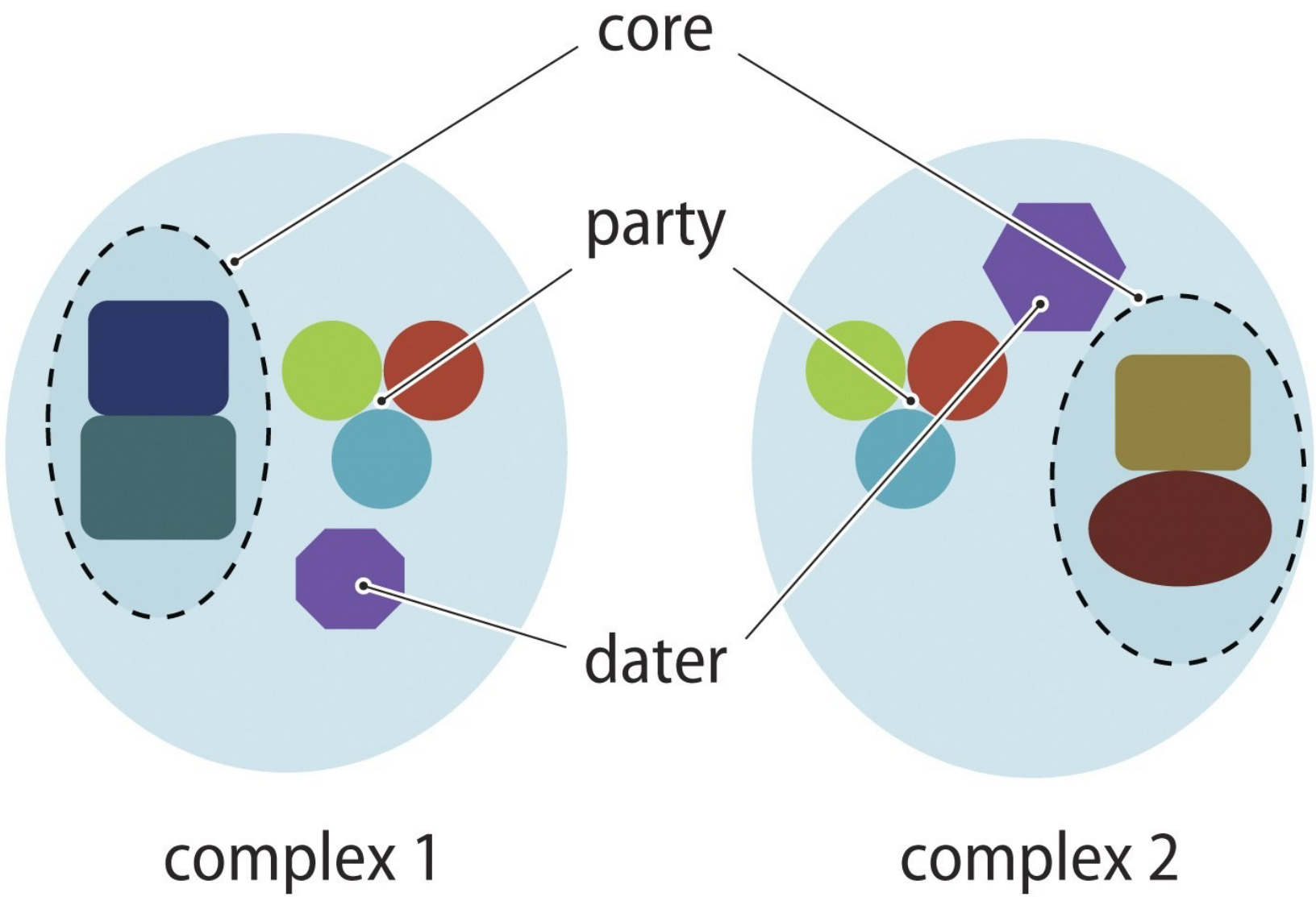
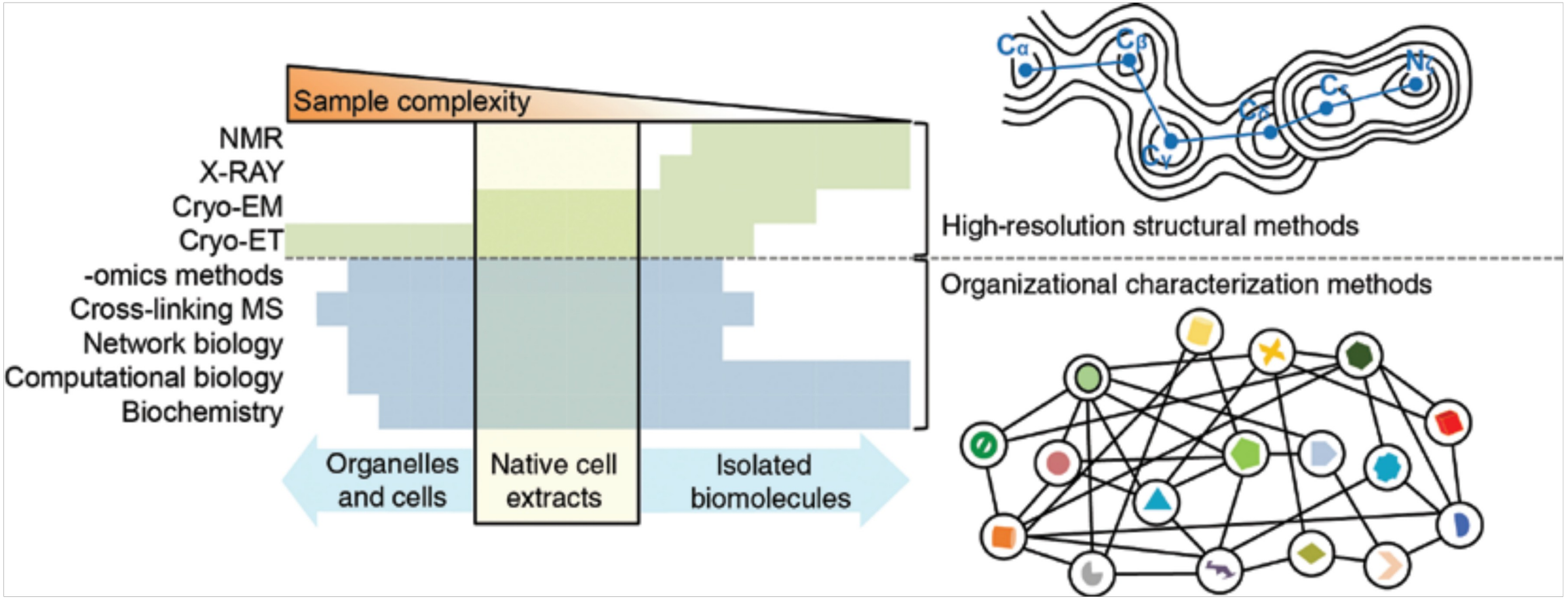


Figure 9.2 How Proteins Work (©2012 Garland Science)

molecular identity gap



Biologica Chemistry 2019

number of components per complex

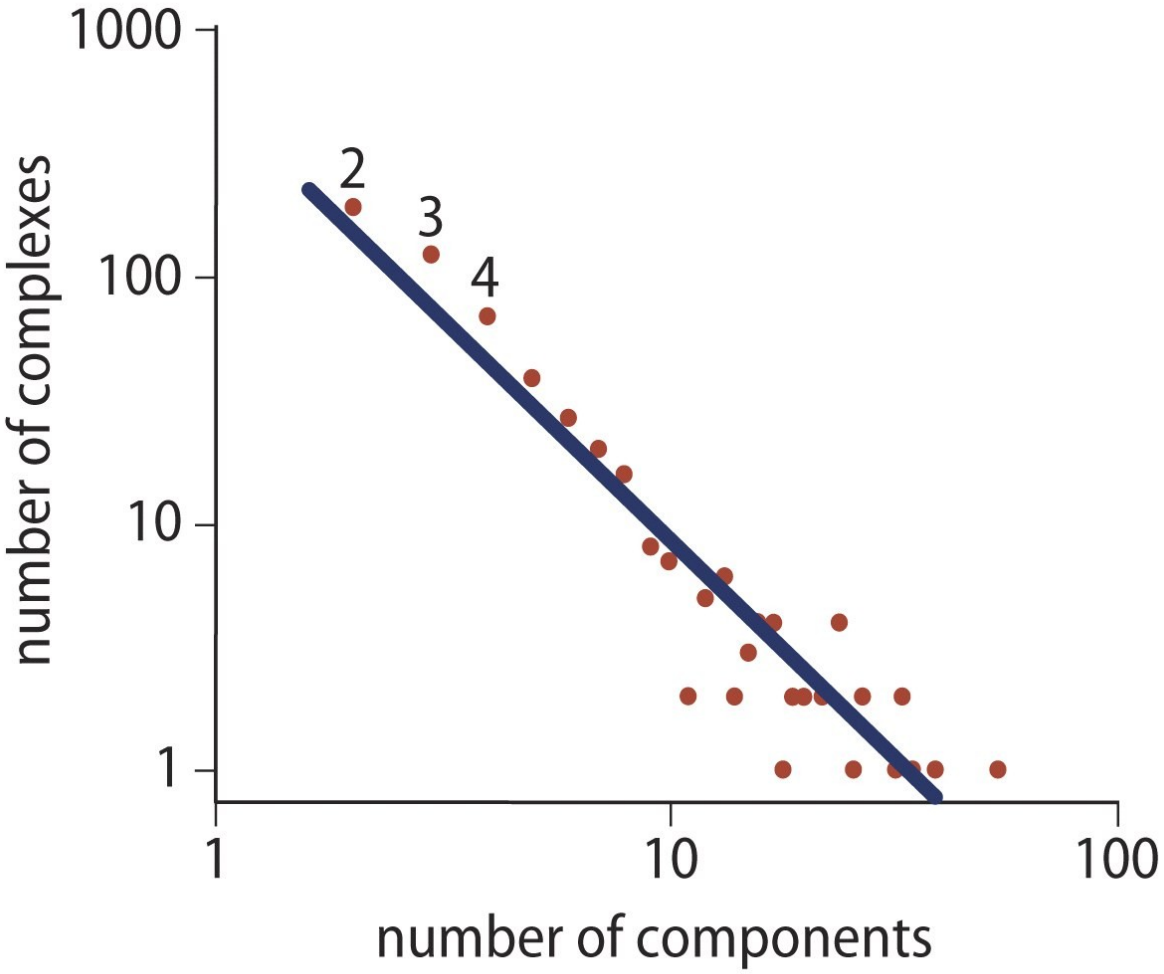
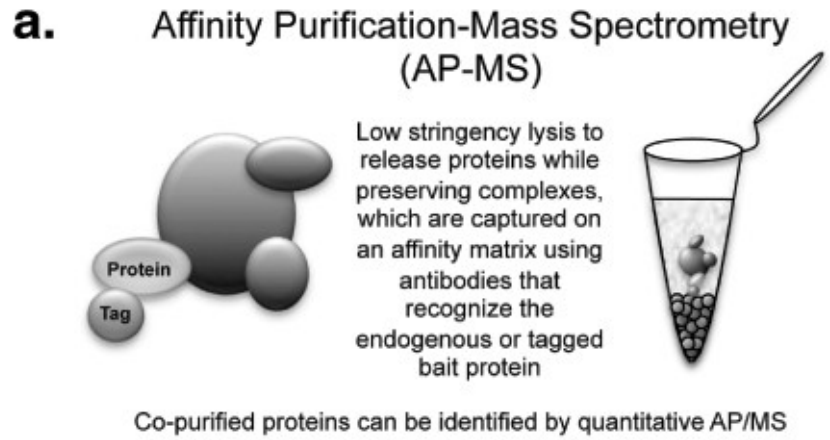
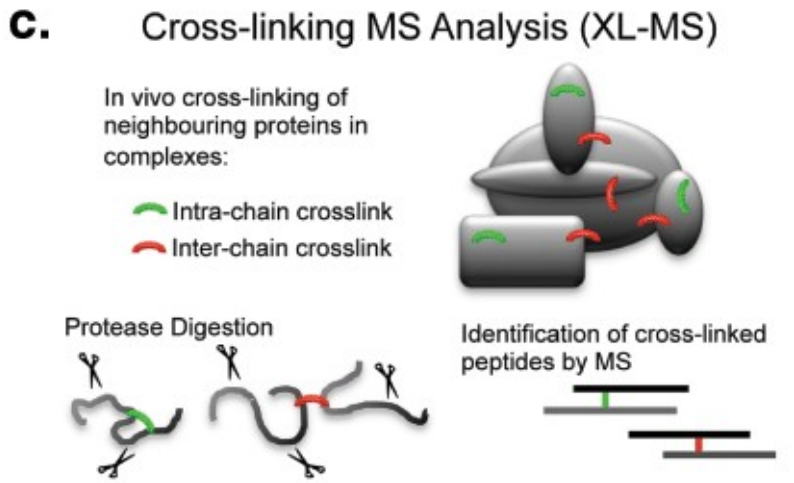
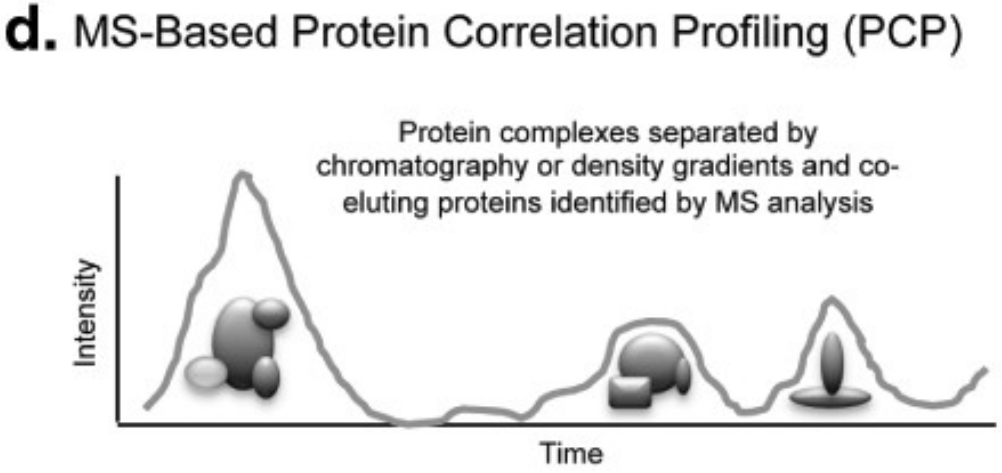


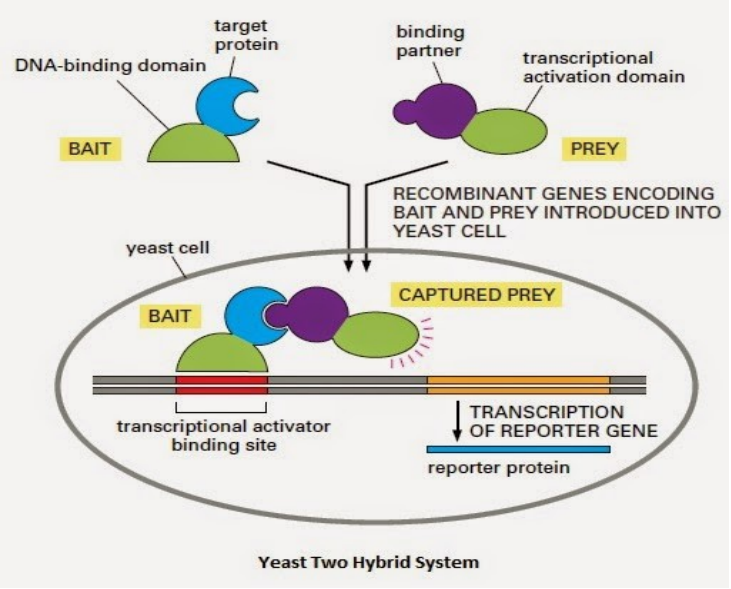
Figure 9.1 How Proteins Work (©2012 Garland Science)

Experimental approaches





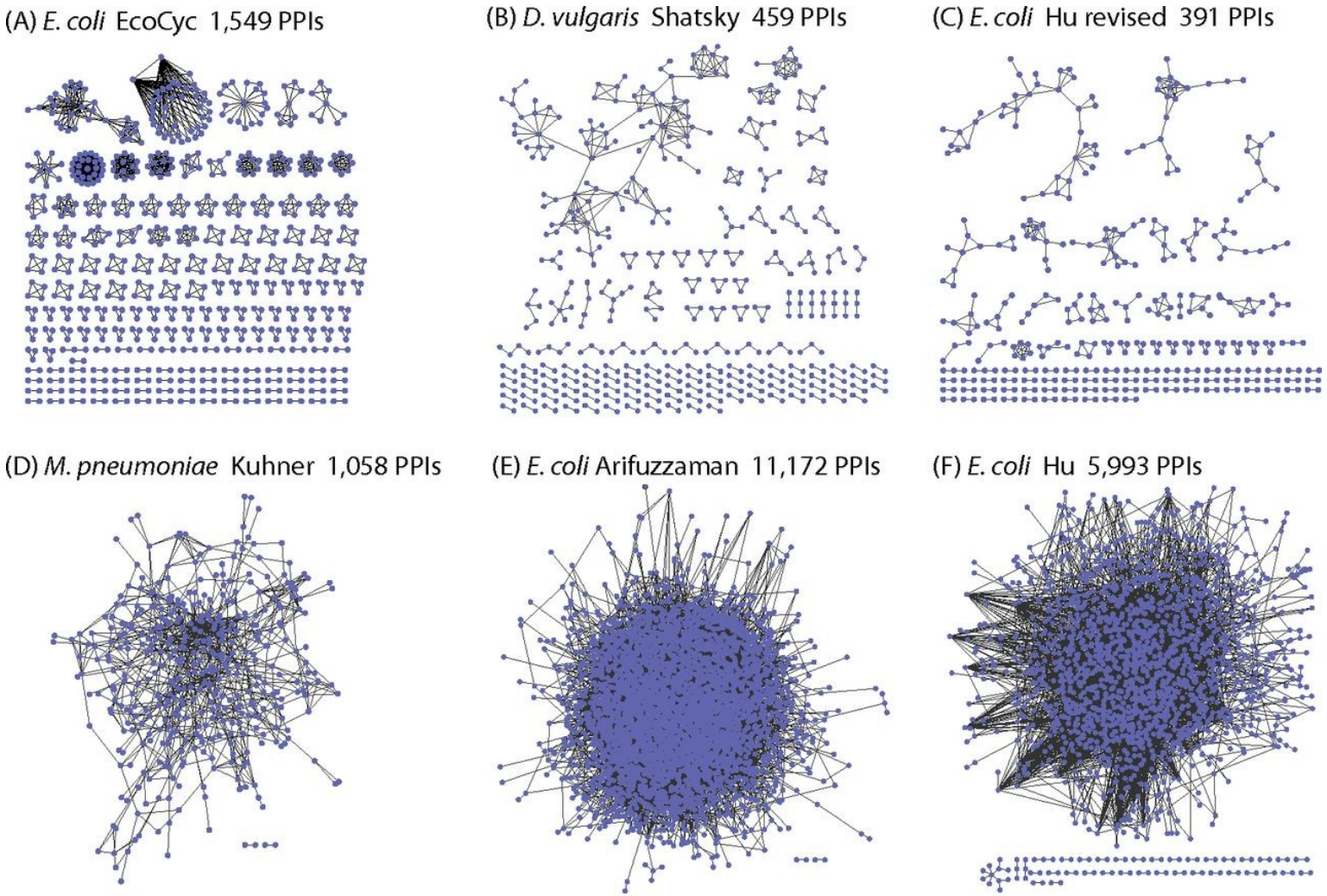




Mehta V and Trinkle-Mulcahy L. Recent advances in large-scale protein interactome mapping [version 1]. F1000Research 2016, 5:782 (doi: 10.12688/f1000research.7629.1)

F1000Research

Interactome connectivity of EcoCyc PPIs and proposed bacterial AP-MS interactomes.

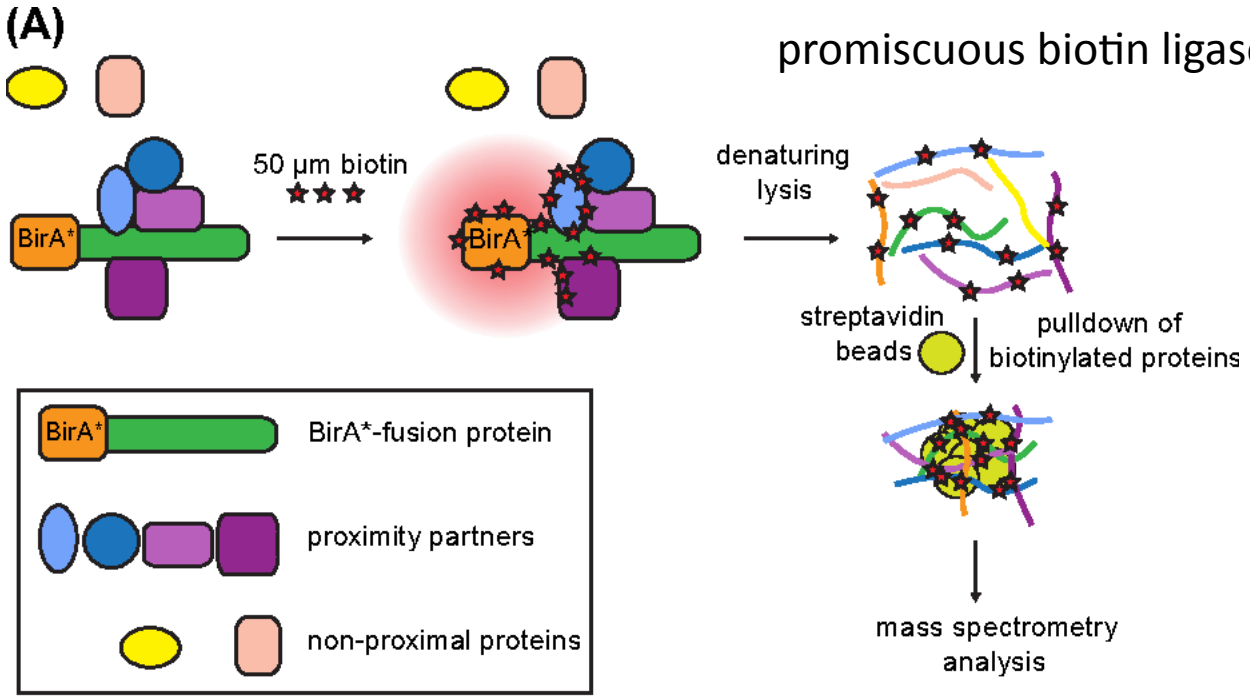


Maxim Shatsky et al. Mol Cell Proteomics 2016;15:1539-1555

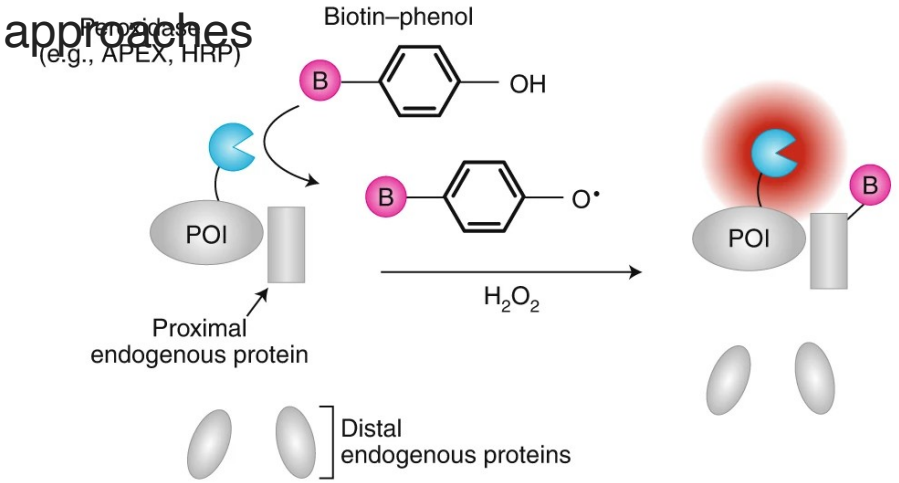
MCP MOLECULAR & CELLULAR PROTEOMICS

BioID & APEX

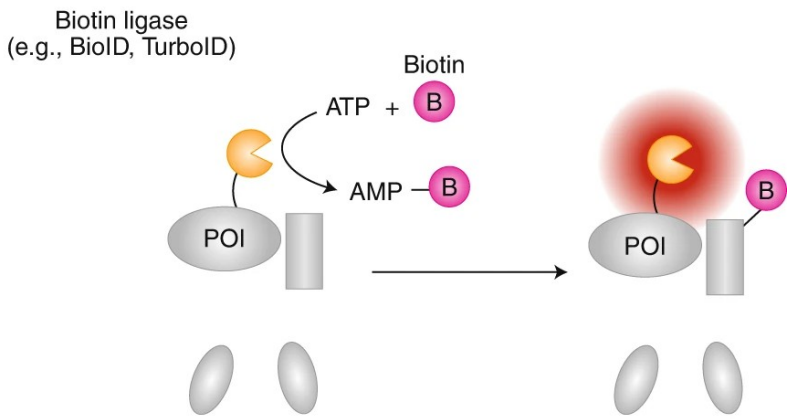
"near neighbor labeling" approaches
that utilize enzymatic reactions to tag proteins



a Peroxidase-based approaches



b



Cell extracts for the structural characterization and identification of molecular species

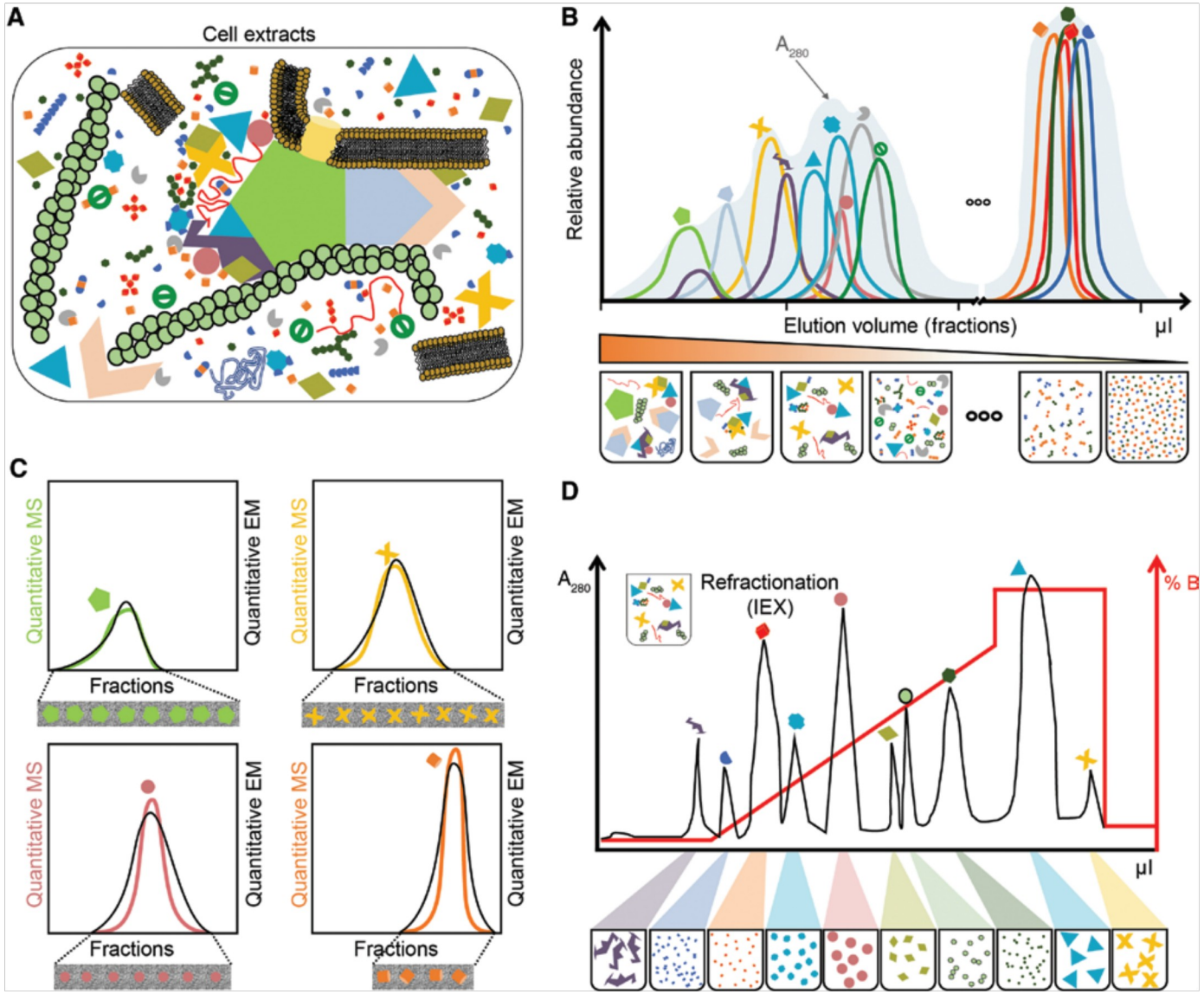
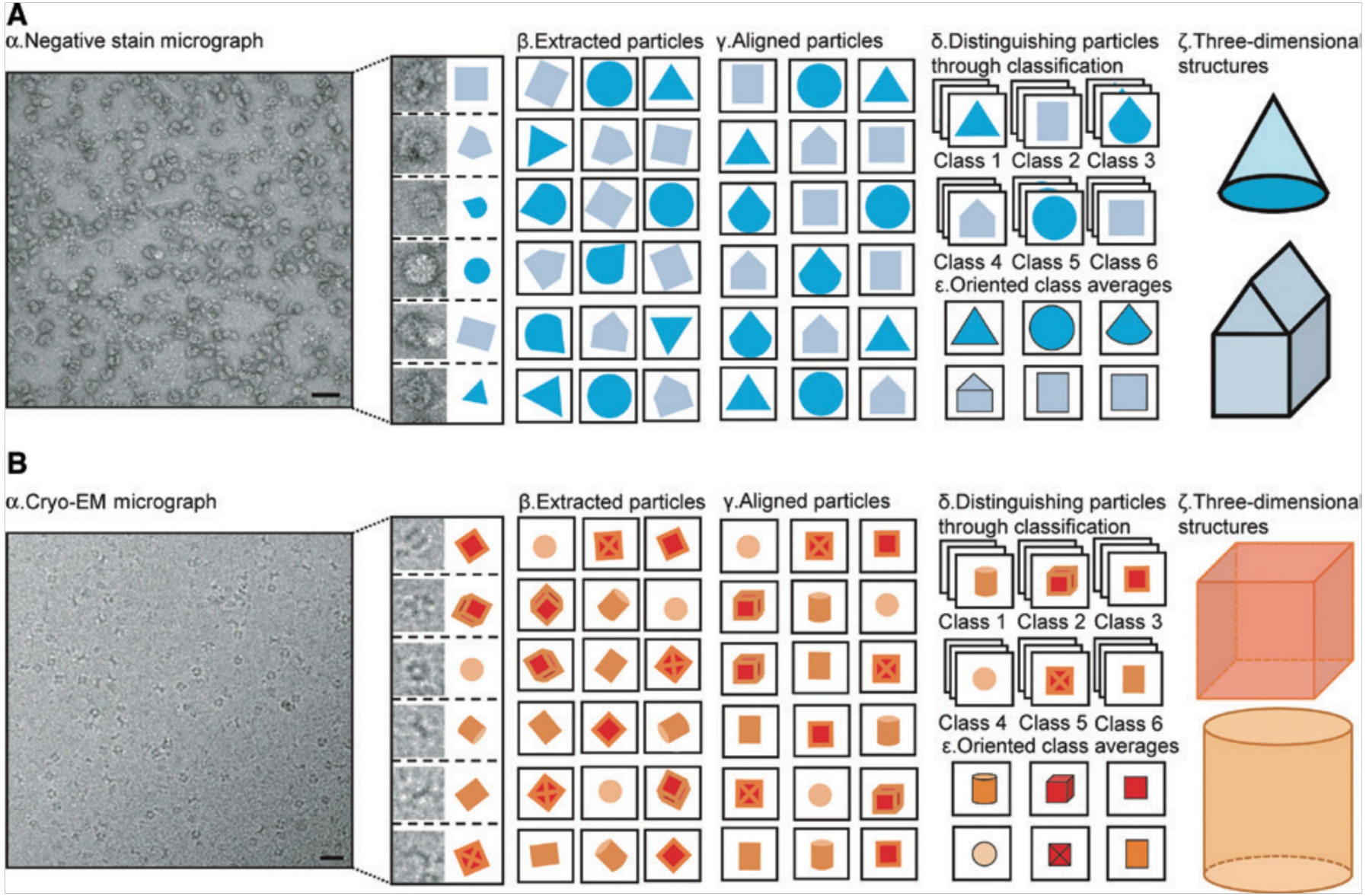
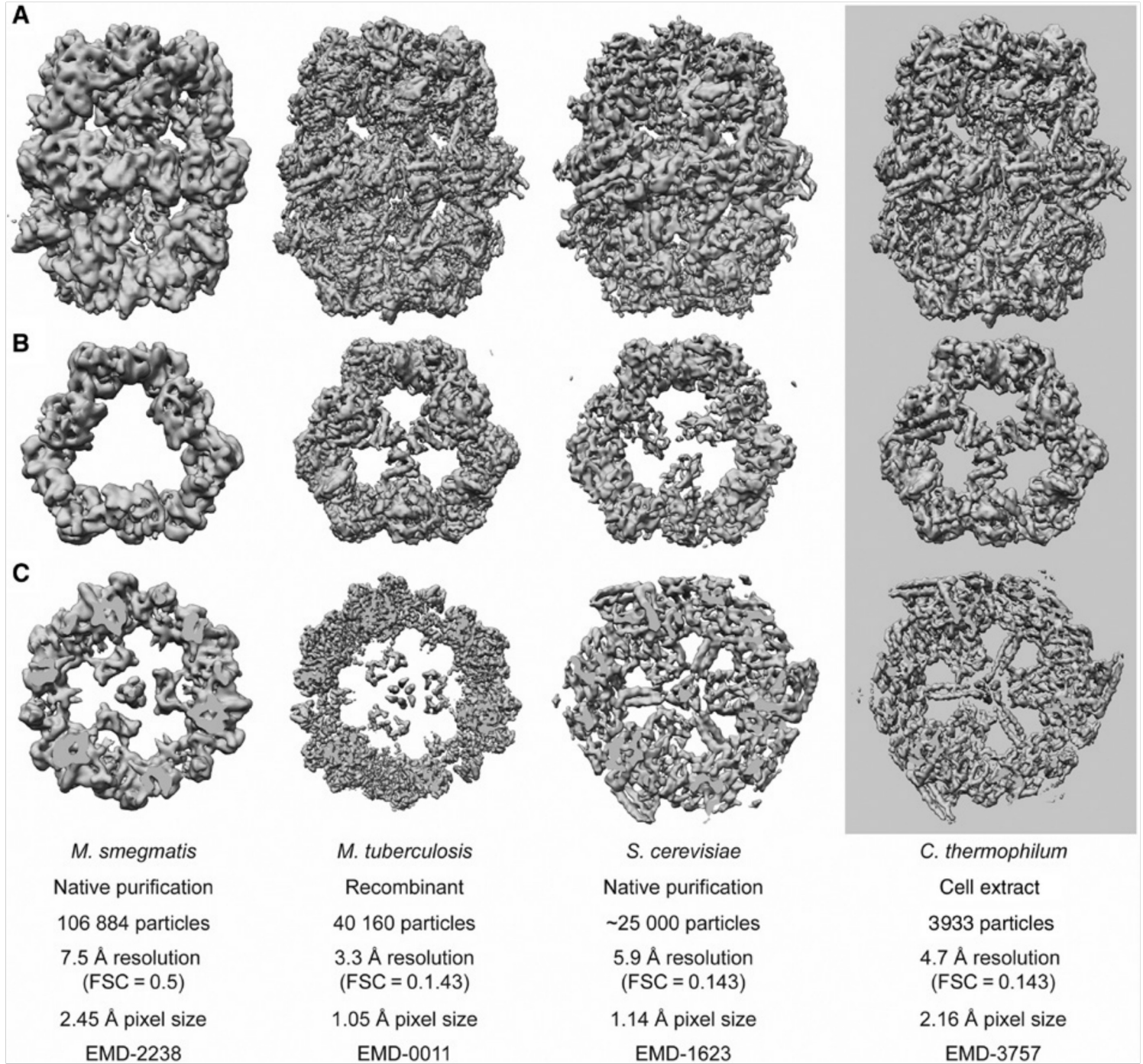


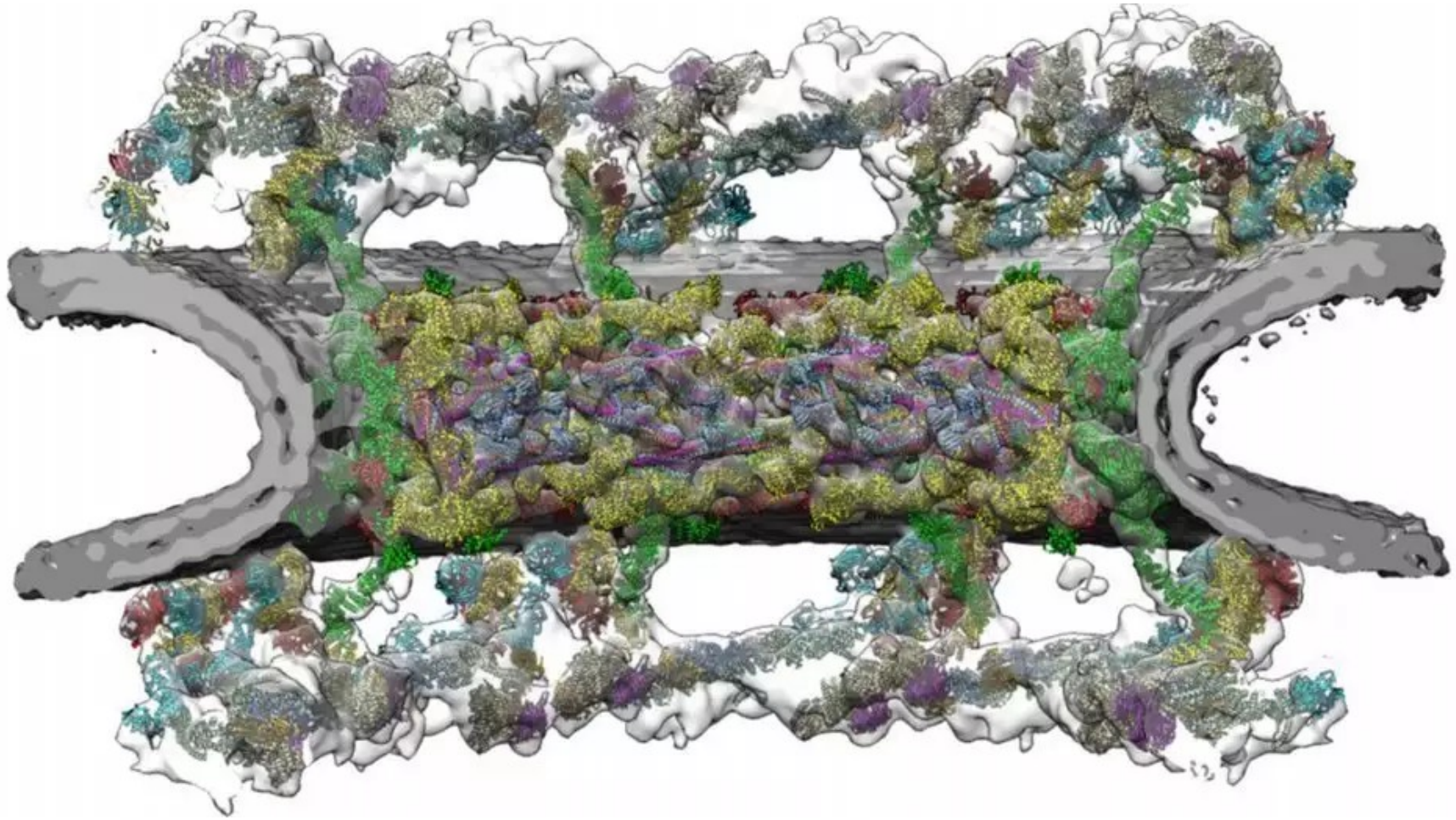
Image processing steps to reconstruct electron optical densities from native cell extracts.

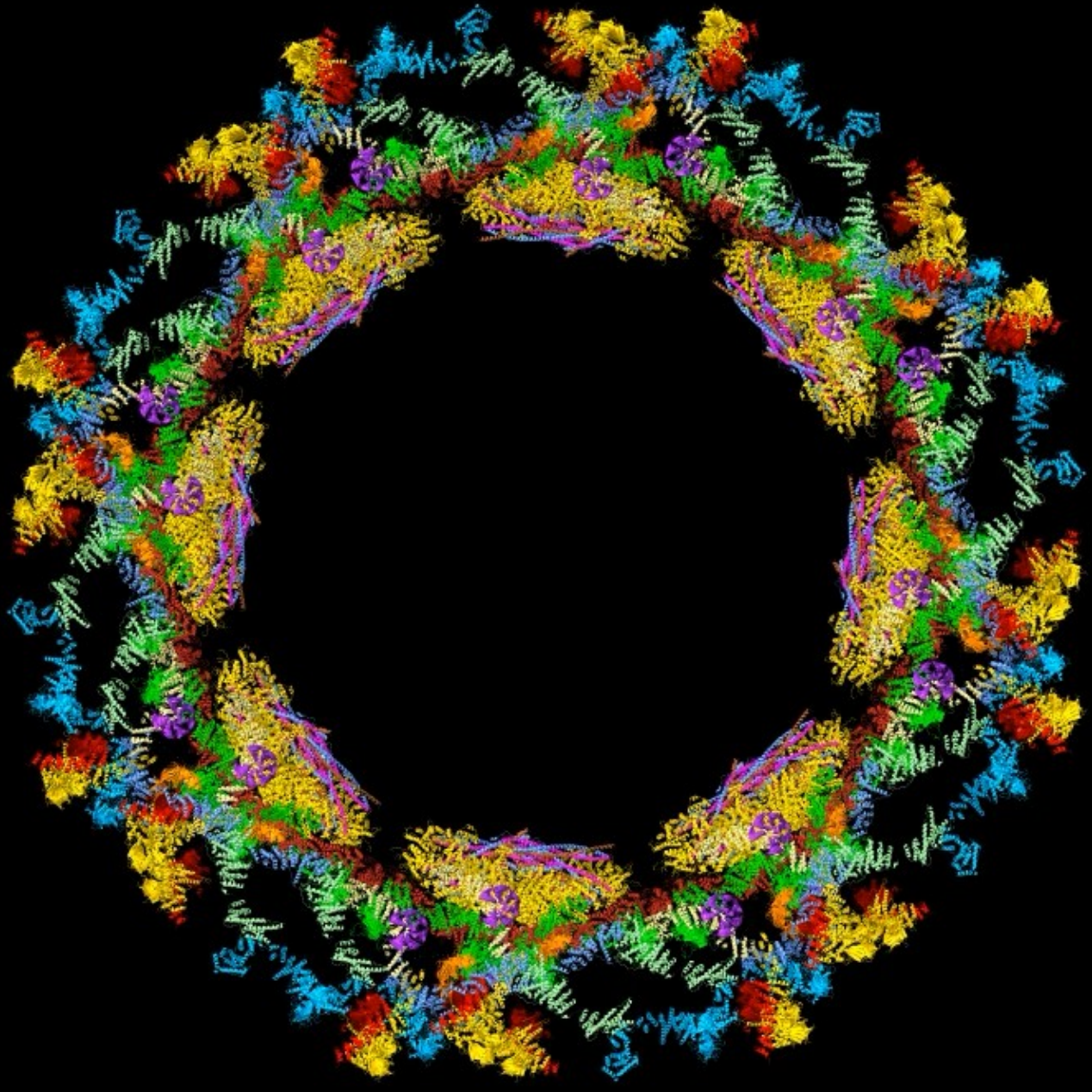



A
B
C
M. smegmatis
Native purification
106 884 particles
7.5 Å resolution
(FSC = 0.5)
2.45 Å pixel size
EMD-2238
M. tuberculosis
Recombinant
40 160 particles
3.3 Å resolution
(FSC = 0.1.43)
1.05 Å pixel size
EMD-0011
S. cerevisiae
Native purification
~25 000 particles
5.9 Å resolution
(FSC = 0.143)
1.14 Å pixel size
EMD-1623
C. thermophilum
Cell extract
3933 particles
4.7 Å resolution
(FSC = 0.143)
2.16 Å pixel size
EMD-3757

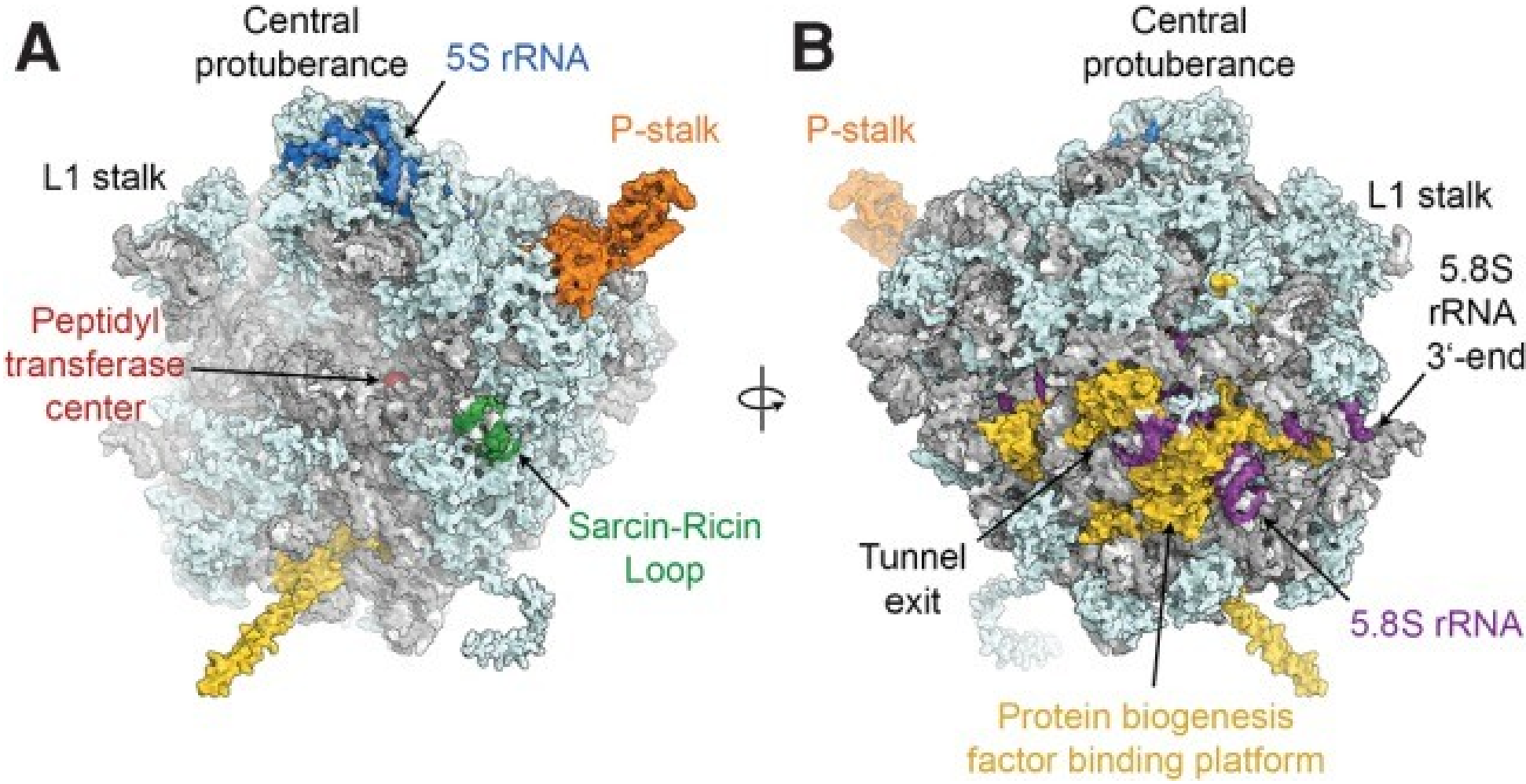
Martin Beck – Molecular Sociology
MPI of Biophysics Frankfurt am Main

How do molecular modules act in concert to generate complex cellular functions?





Structure of the yeast 60S ribosomal subunit



The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath "for studies of the structure and function of the ribosome."

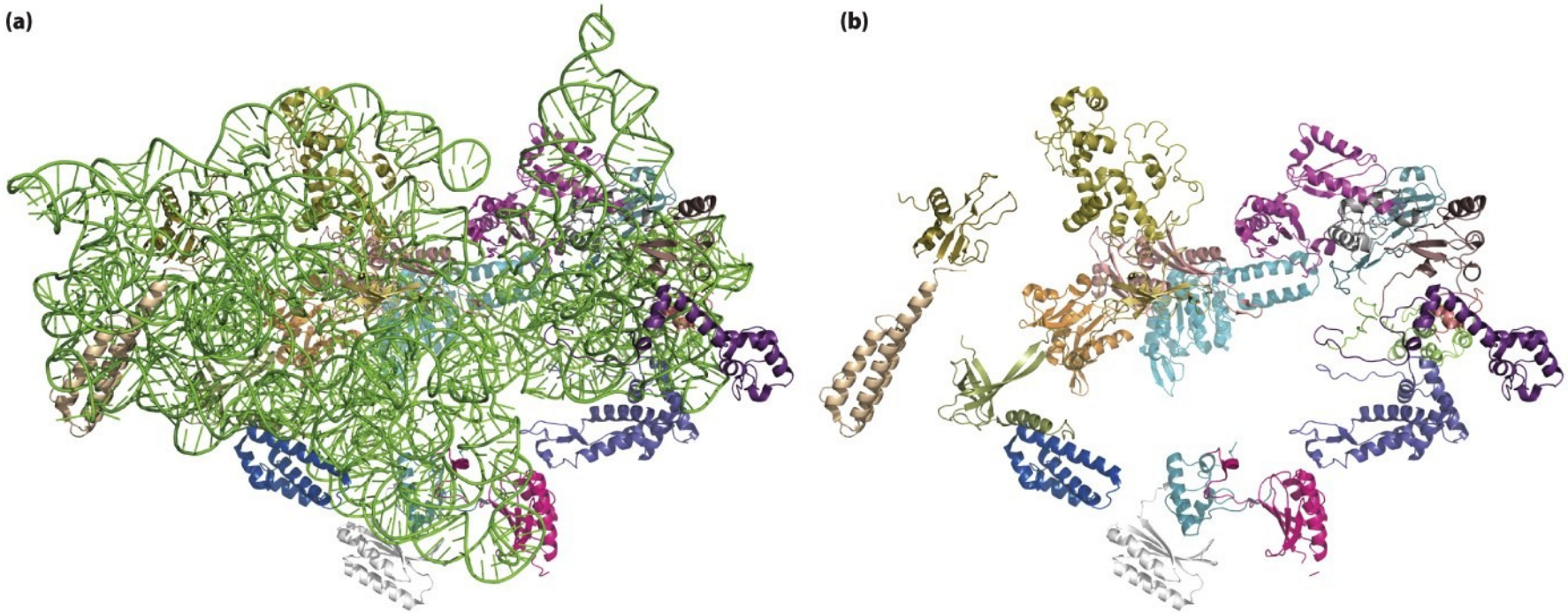


Figure 9.3 How Proteins Work (©2012 Garland Science)

ribosome biogenesis

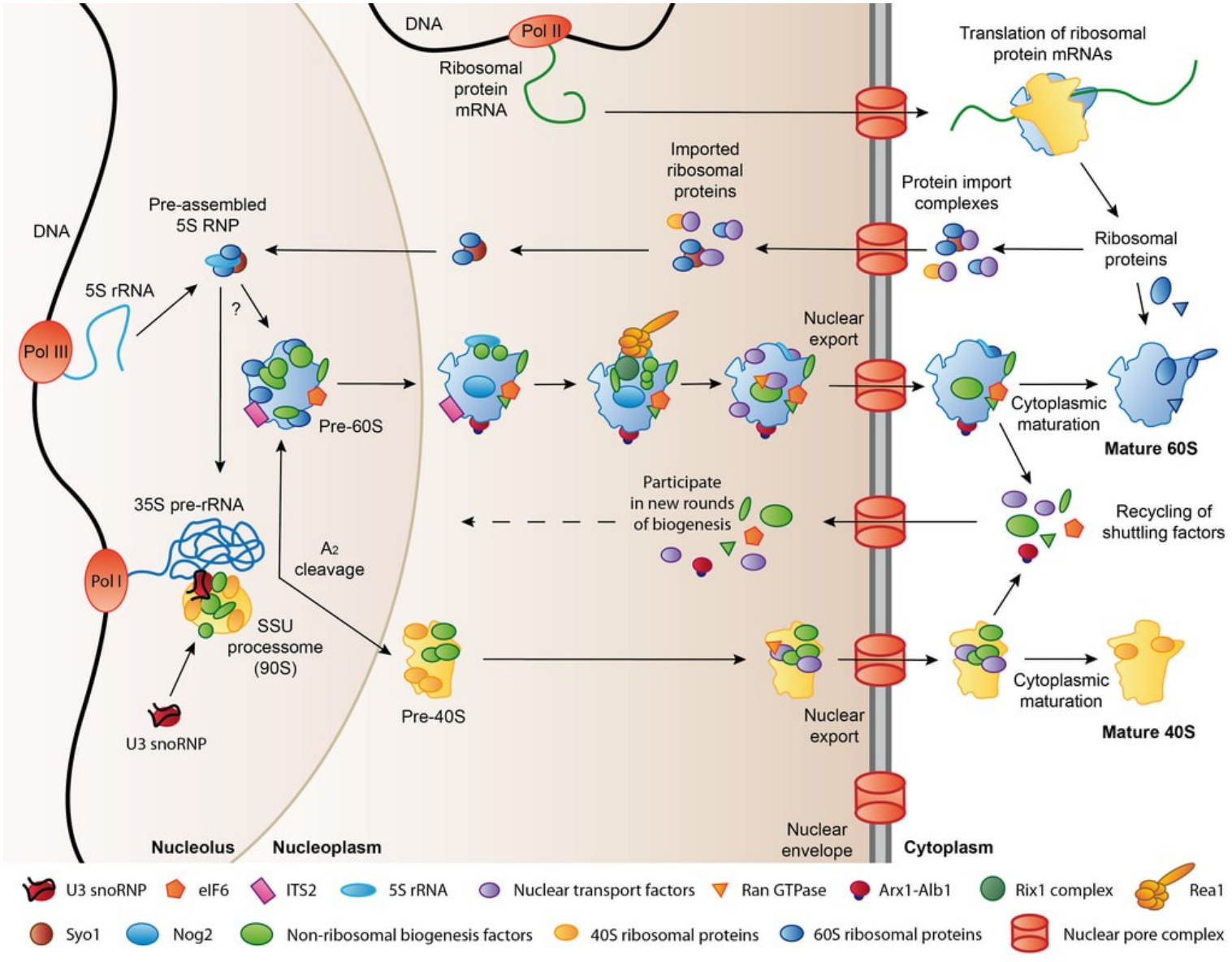
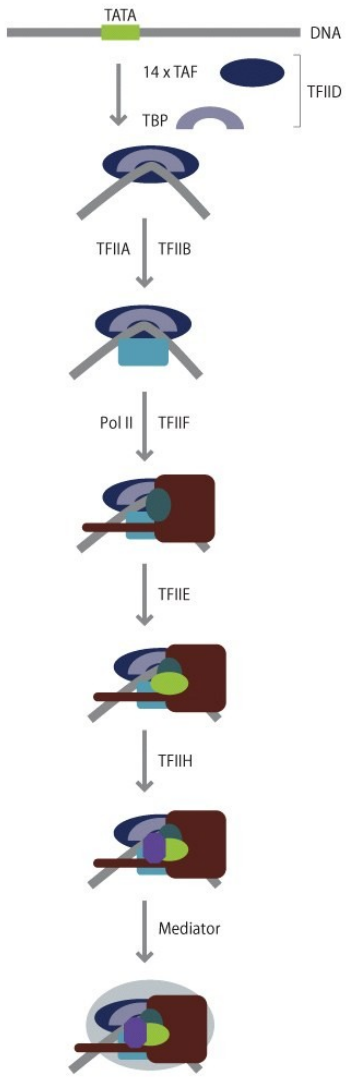


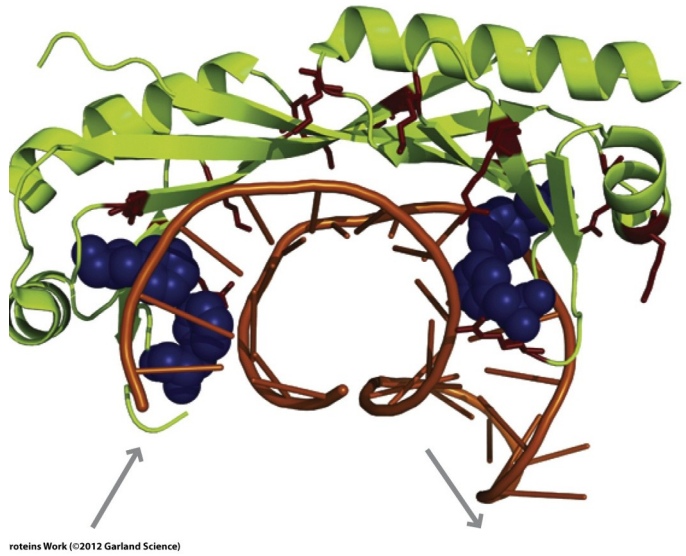
TABLE 9.2 The constituents of yeast RNA polymerase II

Protein	Number of components	Role
Pol II	12	Polymerase
TFIIA	2	Stabilizes TBP and TFIID binding. Blocks transcription inhibitors. Positive and negative gene regulation
TFIIB	1	Binds TBP, Pol II, and DNA. Helps determine start site
TFIID TBP	1	Binds TATA element and bends DNA. Platform for assembly of TFIIB, TFIIA, and TAFs
TFIID TAFs	14	Binds INR and DPE promoters. Target of regulatory factors
Mediator	24	Binds cooperatively with Pol II. Kinase and acetyltransferase activity. Stimulate basal and activated transcription
TFIIF	3	Binds Pol II and is involved in Pol II recruitment to PIC and in open complex formation
TFIIE	2	Binds promoter near transcription start. May help open or stabilize the transcription bubble in the open complex
TFIIH	10	Transcription and DNA repair. Kinase and two helicase activities. Essential for open complex formation
SAGA TAFs	5	Unknown
SAGA Spts, Adas, Sgfs	9	Structural. Interact with TBP, TFIIA, and Gcn5
SAGA Gcn5	1	Histone acetyltransferase
SAGA Tra1	1	Large activator protein. Part of the NuA4 HAT complex
SAGA Ubp8	1	Ubiquitin protease

Table 9.2 How Proteins Work (©2012 Garland Science)

Figure 9.7 How Proteins Work (©2012 Garland Science)





roteins Work (©2012 Garland Science)

Figure 9.9 How Proteins Work (©2012 Garland Science)

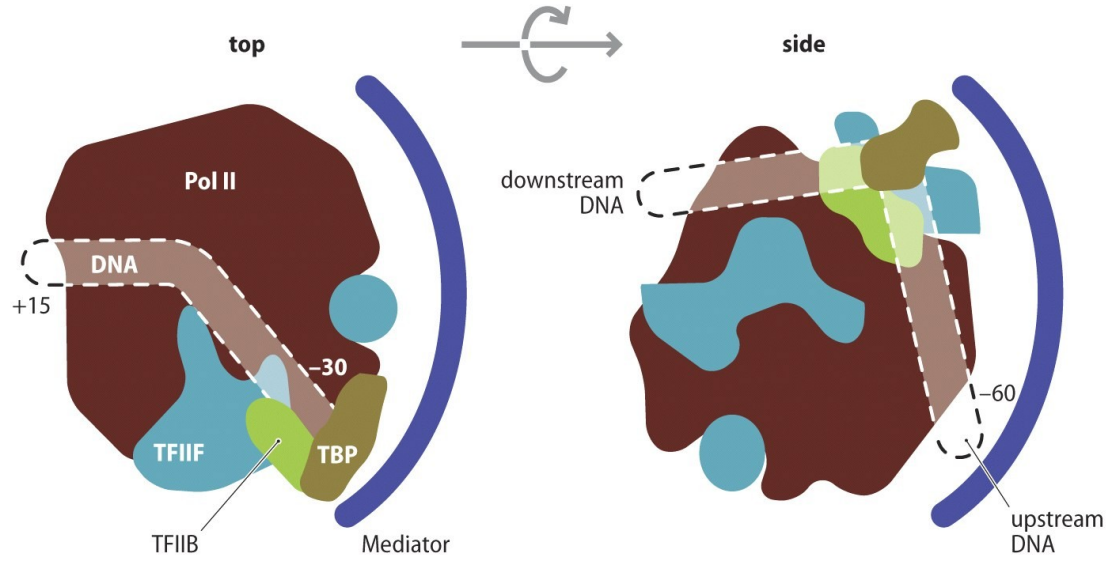


Figure 9.10 How Proteins Work (©2012 Garland Science)

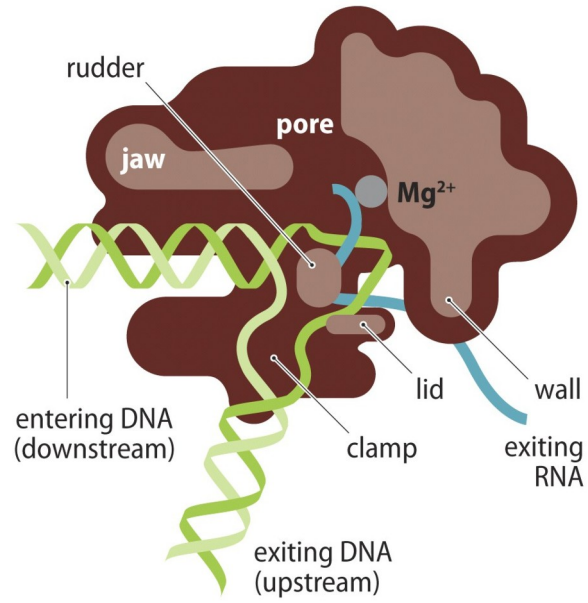
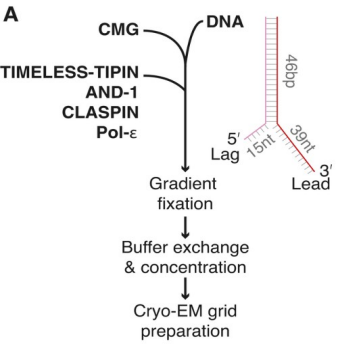
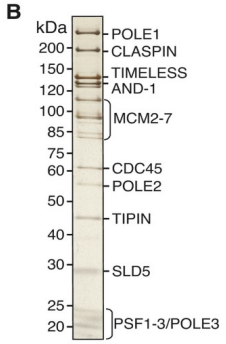
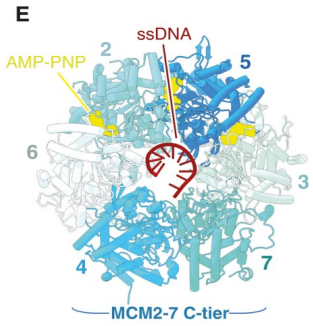


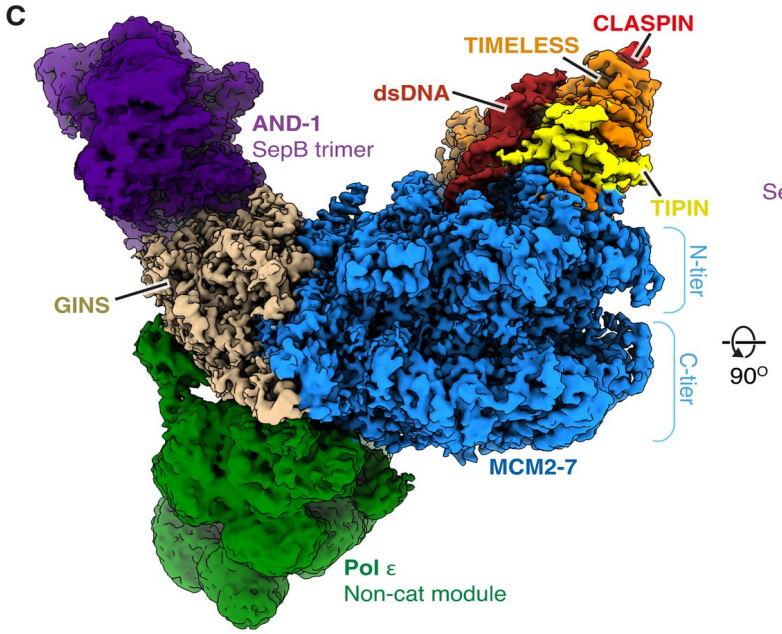
Figure 9.11 How Proteins Work (©2012 Garland Science)

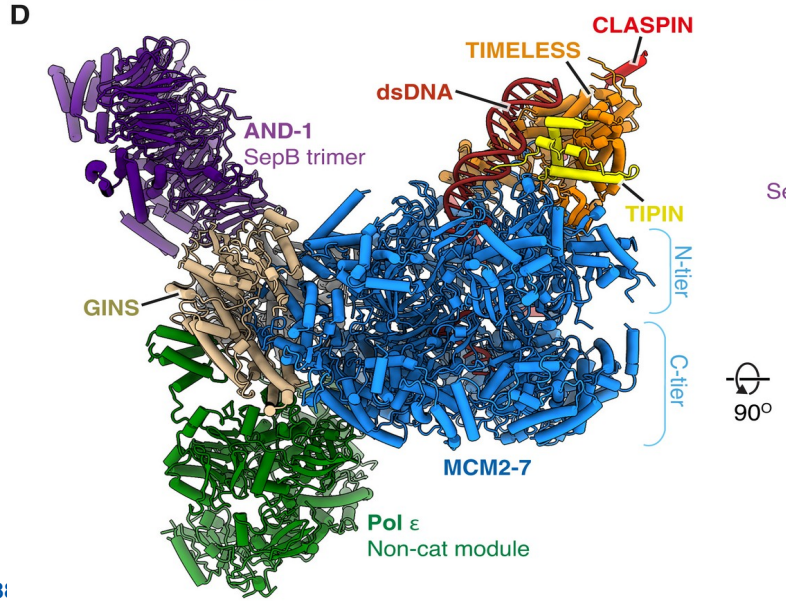
Structure of a human replisome shows the organisation and interactions of a DNA replication machine





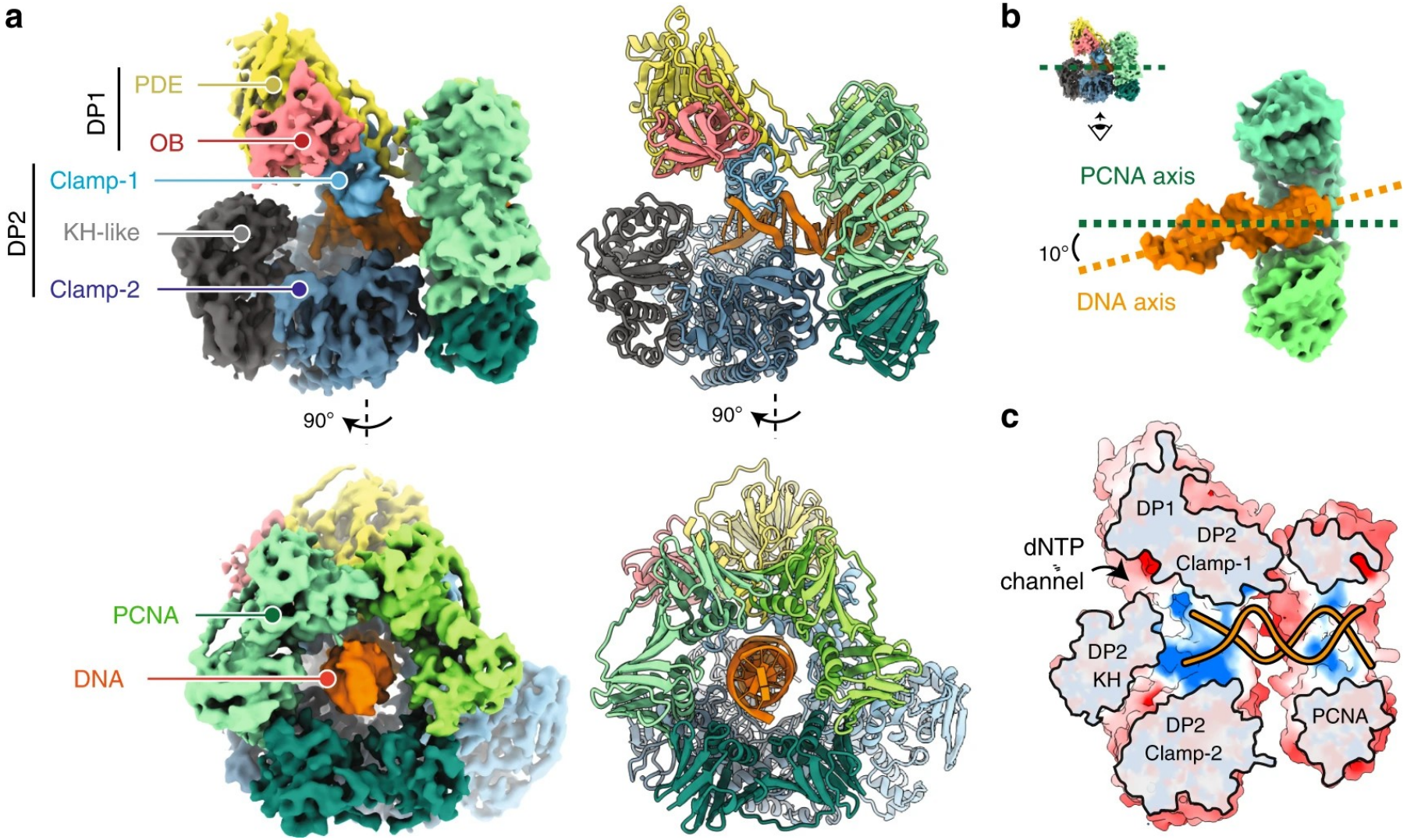




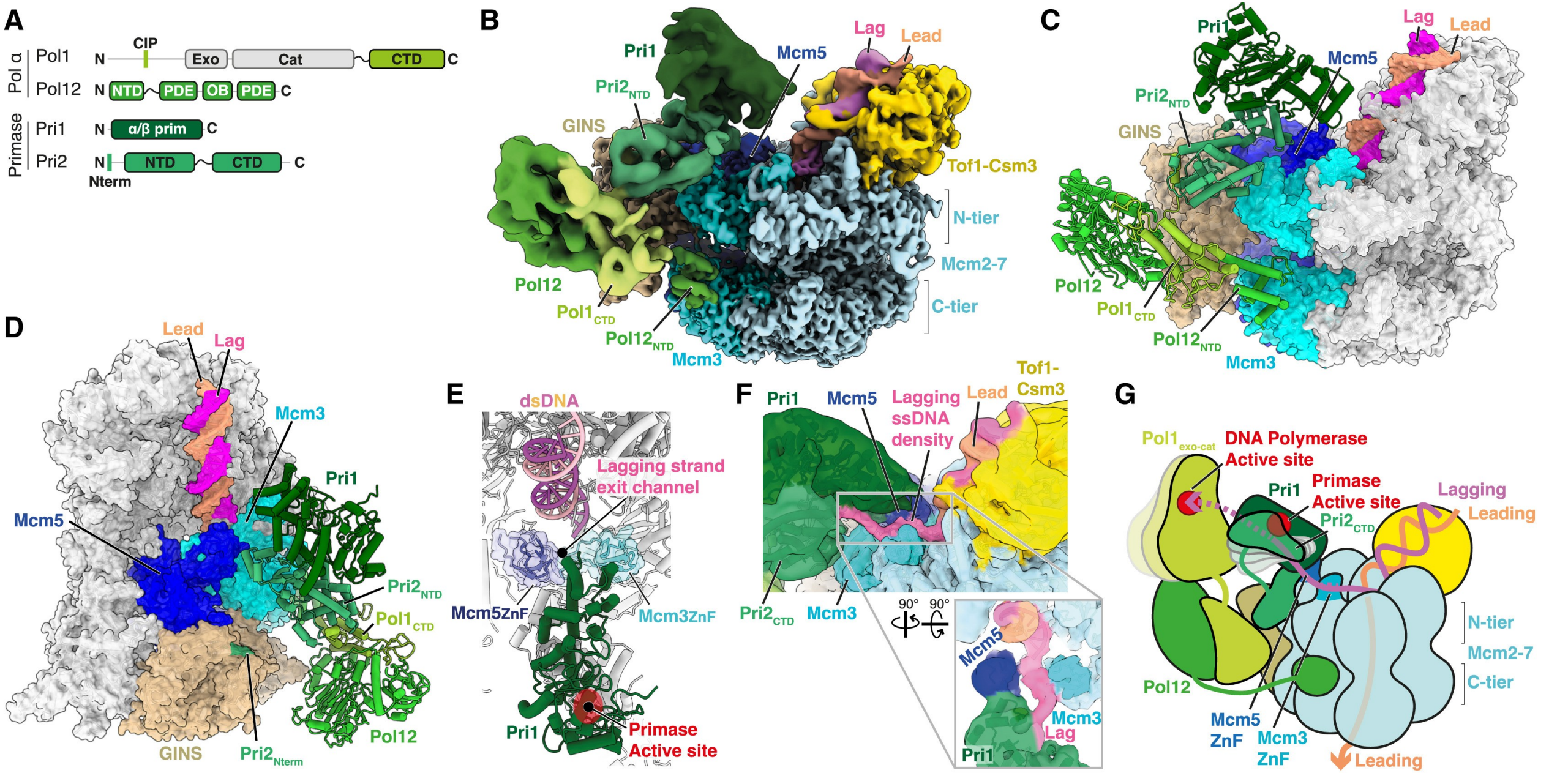


The EMBO Journal, Volume: 40, Issue: 23, First published: 25 October 2021, DOI: (10.15252/embj.2021108

DNA-bound PolD–PCNA processive complex



Nature Communications **volume 11**, Article number: 1109 (2020)


A
Pol α
Pol1
N
CIP
Exo
Cat
CTD
C
Pol12
N
NTD
PDE
OB
PDE
C
Primase
Pri1
N
α/β prim
C
Pri2
N
NTD
CTD
C
Nterm
B
Pri1
Mcm5
Lag
Lead
Pri2NTD
GINS
Tof1-Csm3
N-tier
Mcm2-7
C-tier
Pol12
Pol1CTD
Pol12NTD
Mcm3
C
Pri1
Lag
Lead
Mcm5
Pri2NTD
GINS
Pol12
Pol1CTD
Pol12NTD
Mcm3
D
Lead
Lag
Mcm3
Pri1
Mcm5
Pri2NTD
Pol1CTD
Pol12
GINS
Pri2Nterm
E
dsDNA
Lagging strand
exit channel
Mcm5ZnF
Mcm3ZnF
Pri1
Primase
Active site
F
Pri1
Mcm5
Lagging
ssDNA
density
Lead
Tof1-
Csm3
Pri2CTD
Mcm3
90°
90°
Mcm5
Mcm3
Lag
Pri1
G
Pol1exo-cat
DNA Polymerase
Active site
Pri1
Primase
Active site
Pri2CTD
Lagging
Leading
N-tier
Mcm2-7
C-tier
Pol12
Mcm5
ZnF
Mcm3
ZnF
Leading

human metabolic pathways

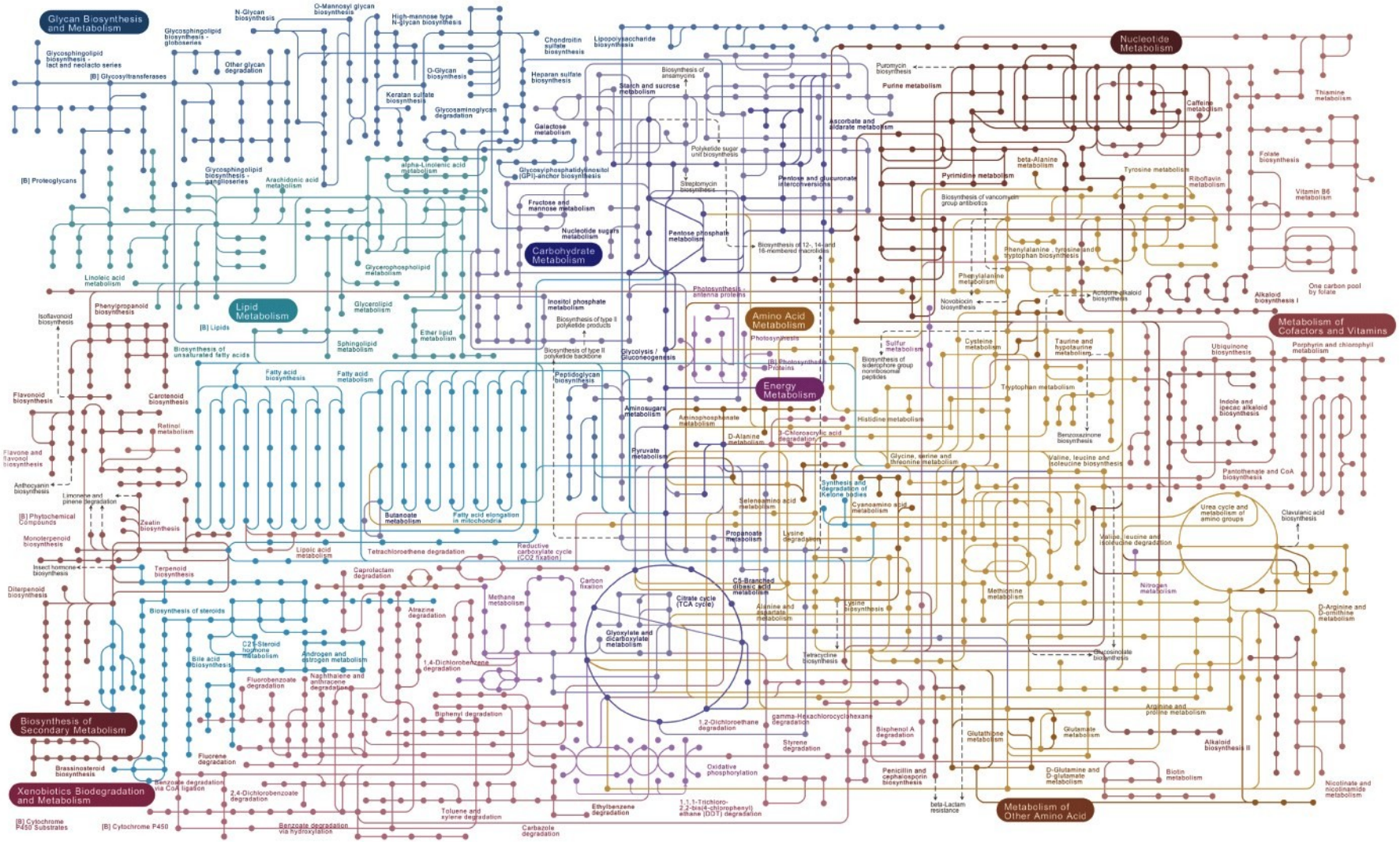


Figure 9.13 How Proteins Work (©2012 Garland Science)

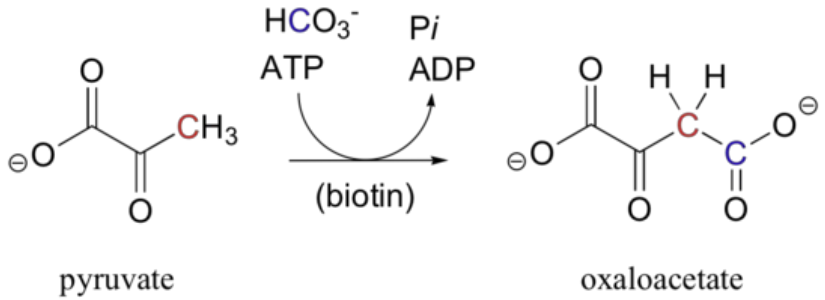
coupling of enzymes

$X + Y \rightarrow XY \qquad \Delta G_1 > 0$

$ATP \rightarrow ADP + P_i \qquad \Delta G_2 < 0$

$X + ATP \rightarrow X\text{-}P + ADP$
$X\text{-}P + Y \rightarrow X\text{-}Y + P_i$

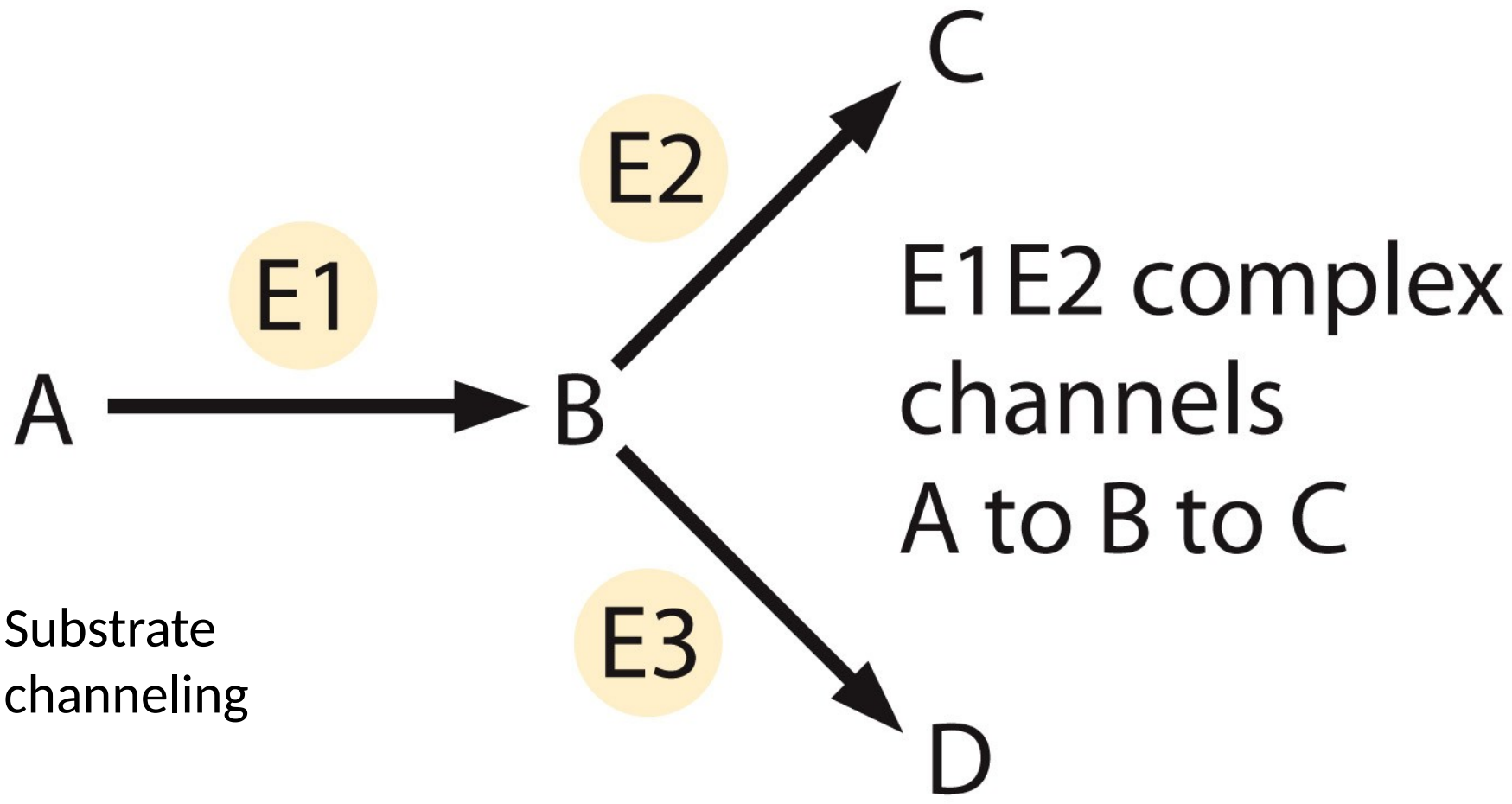
$X + Y + ATP \rightarrow XY + ADP + P_i \qquad \Delta G < 0$ if $\Delta G_2 > \Delta G_1$



DNA Synthesis

Peptide bond by ribosomes

Multienzyme Complexes: Catalytic Nanomachines



Substrate
channeling

Unnumbered 9 p358 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

substrate channeling

D isomer

Molecular channeling

Swinging arm

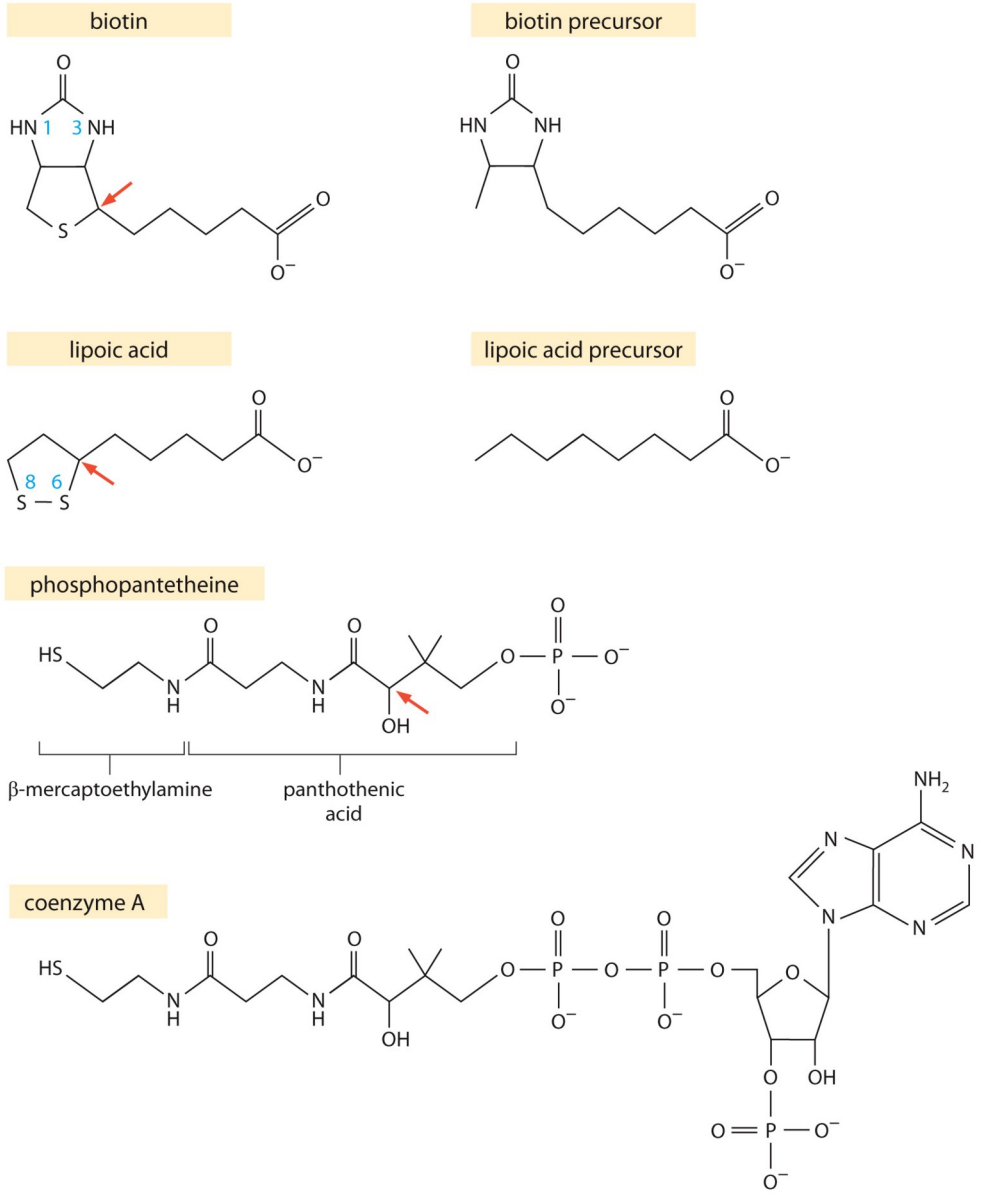


Figure 9.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Channeling

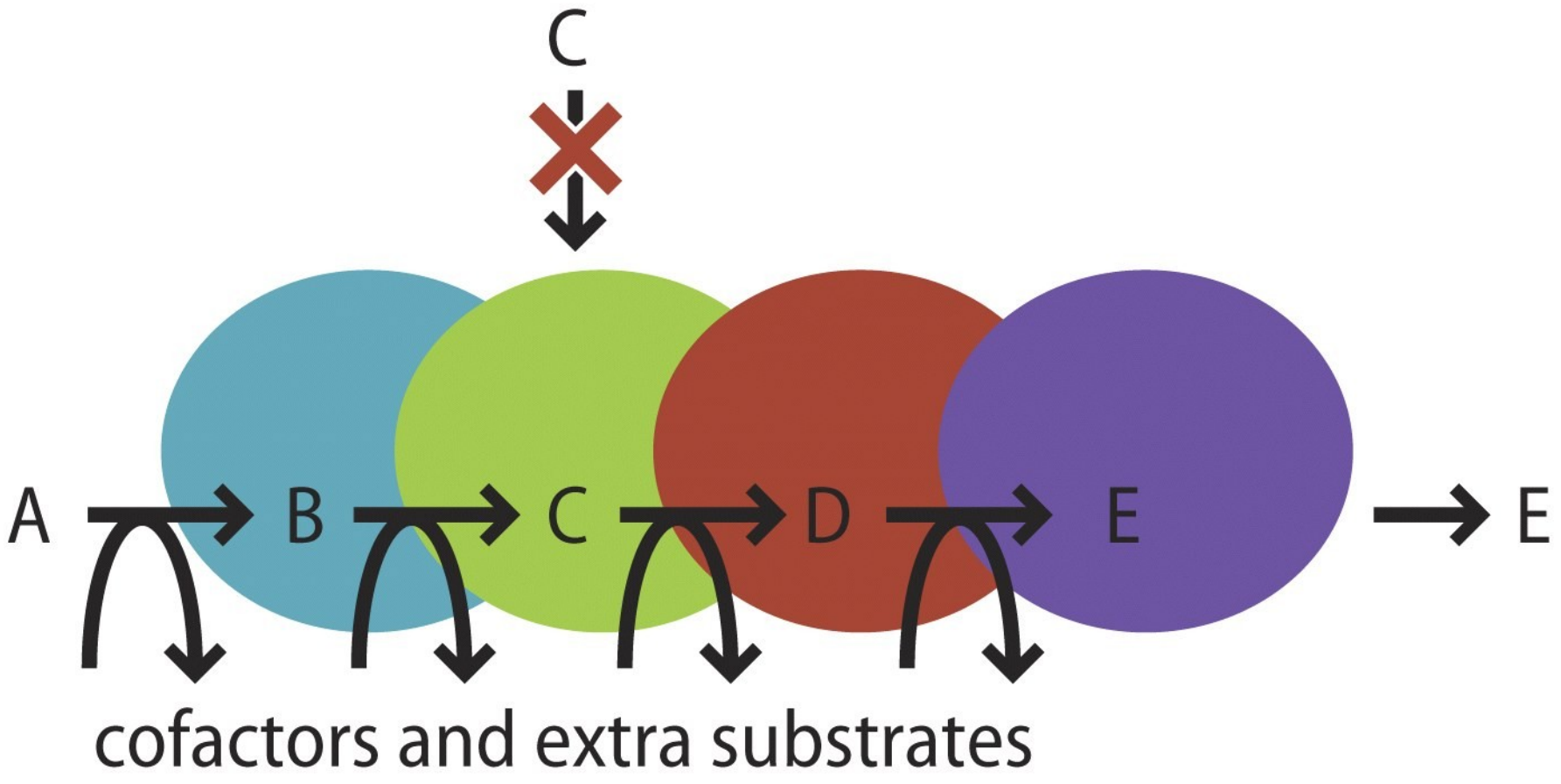


Figure 9.14 How Proteins Work (©2012 Garland Science)

enzyme complexes with tunnels

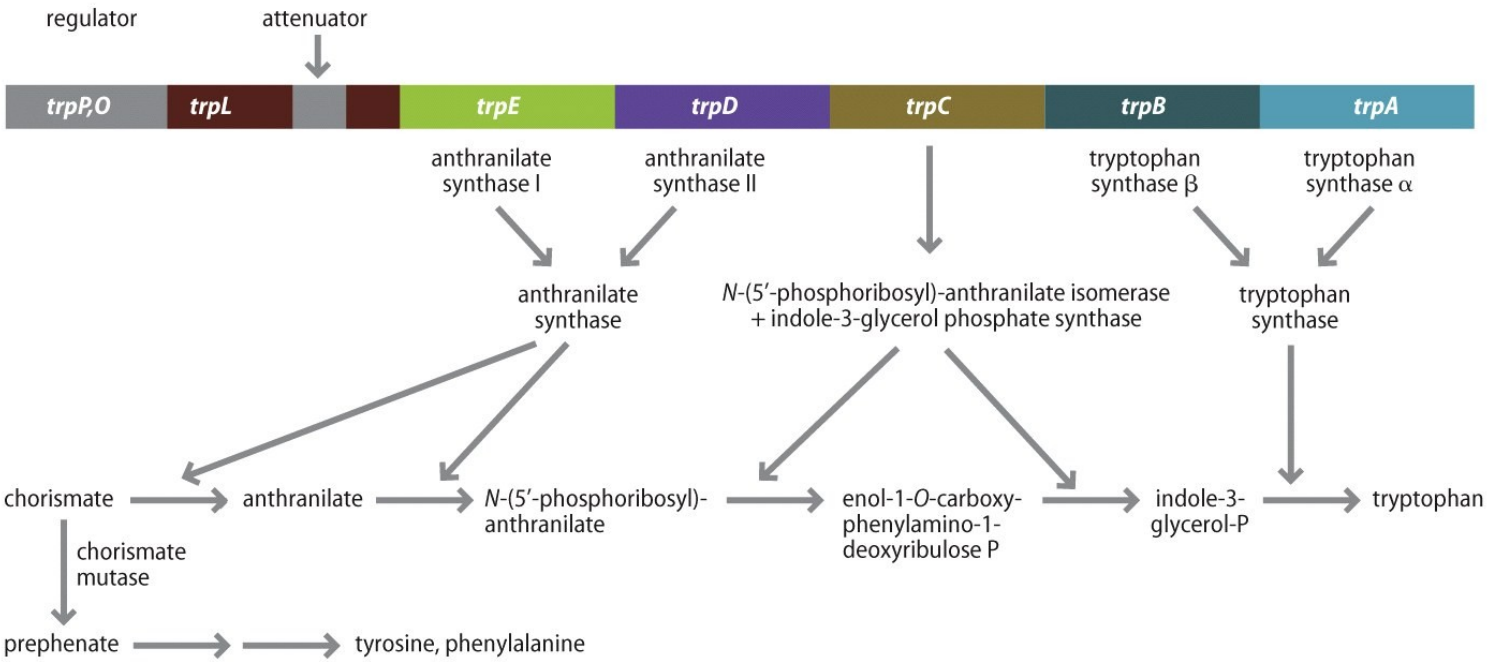
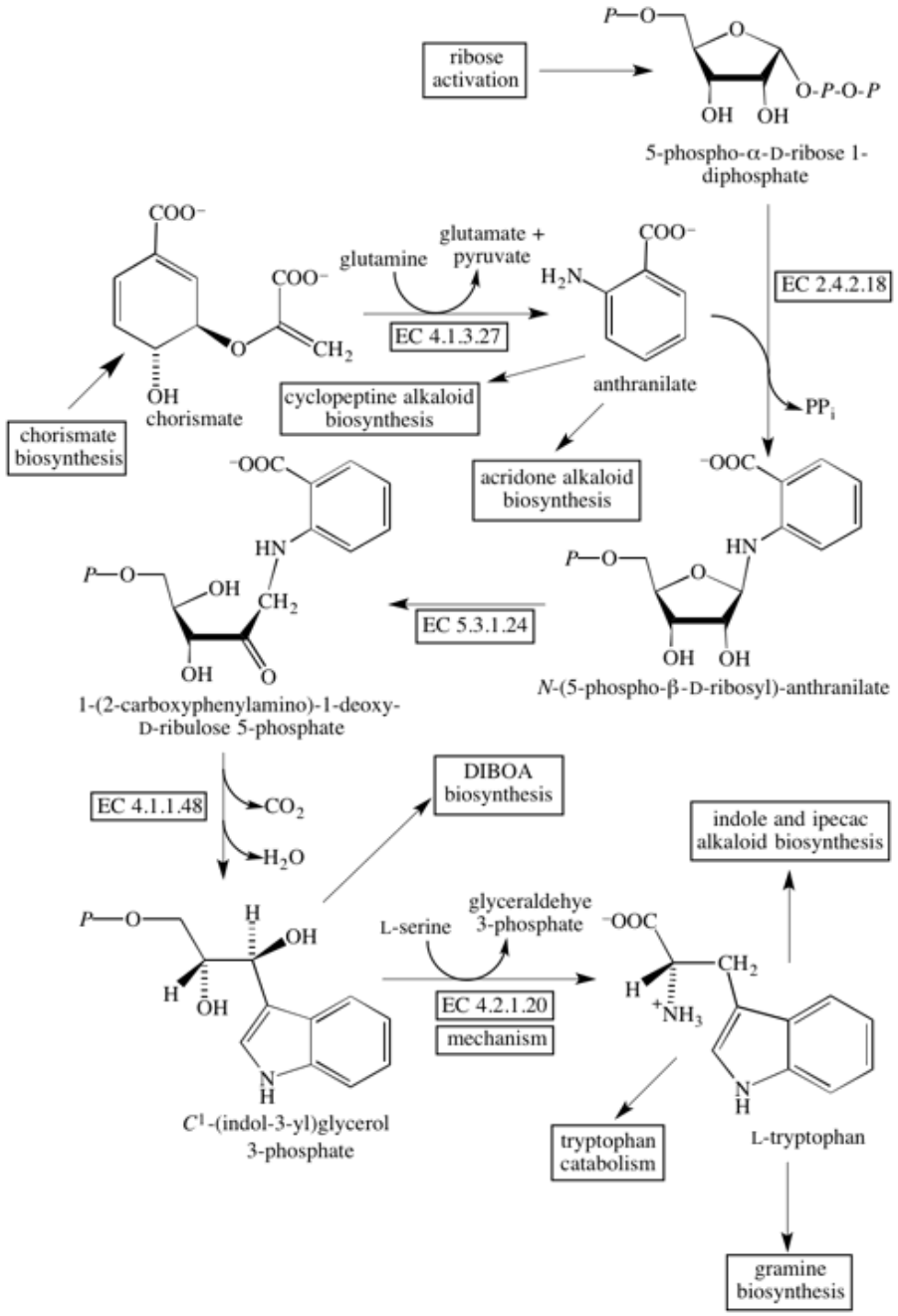


Figure 10.1 How Proteins Work (©2012 Garland Science)



enzyme complexes with tunnels

$\alpha_2\beta_2$ heterotetramer.

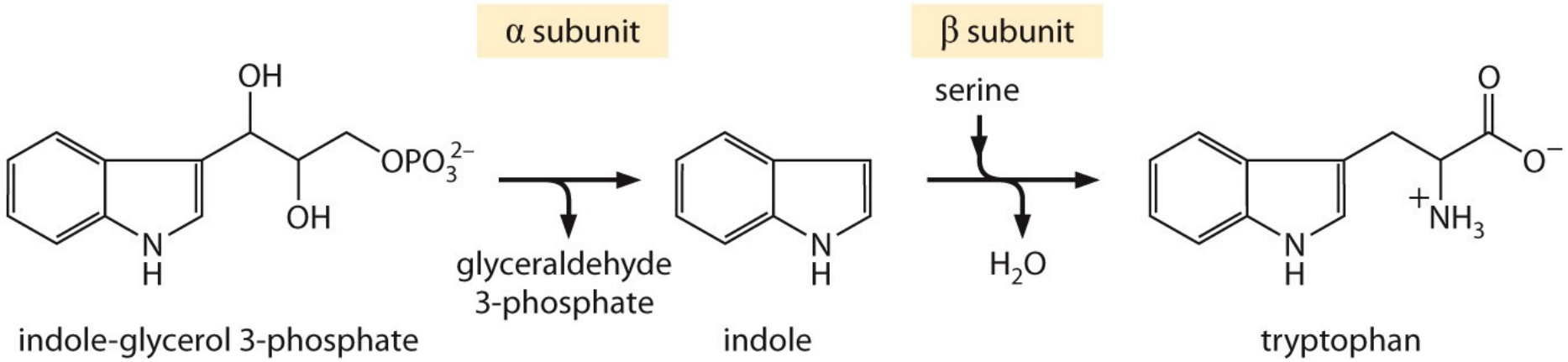
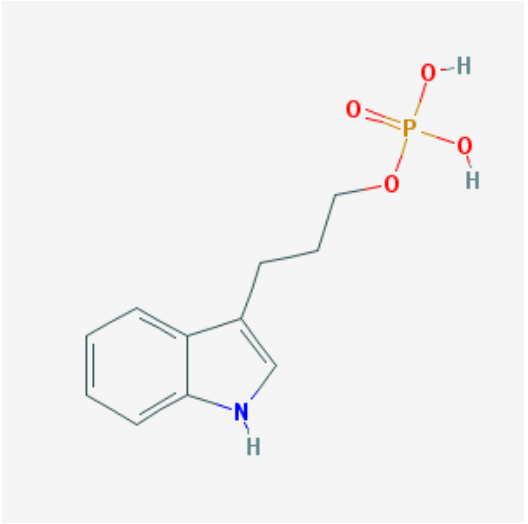


Figure 9.2a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Structure and mechanism of tryptophan synthase

competitive inhibitor



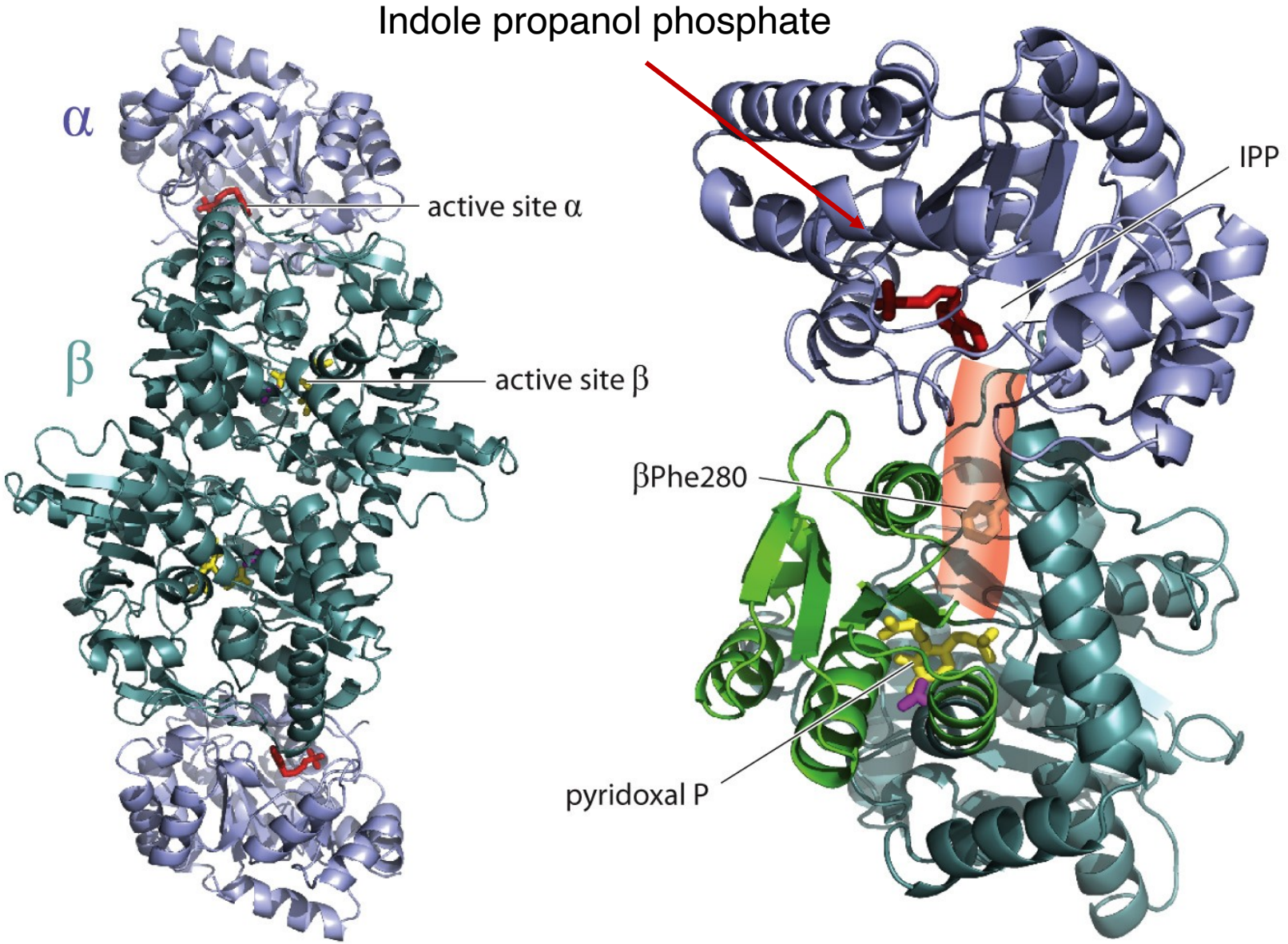
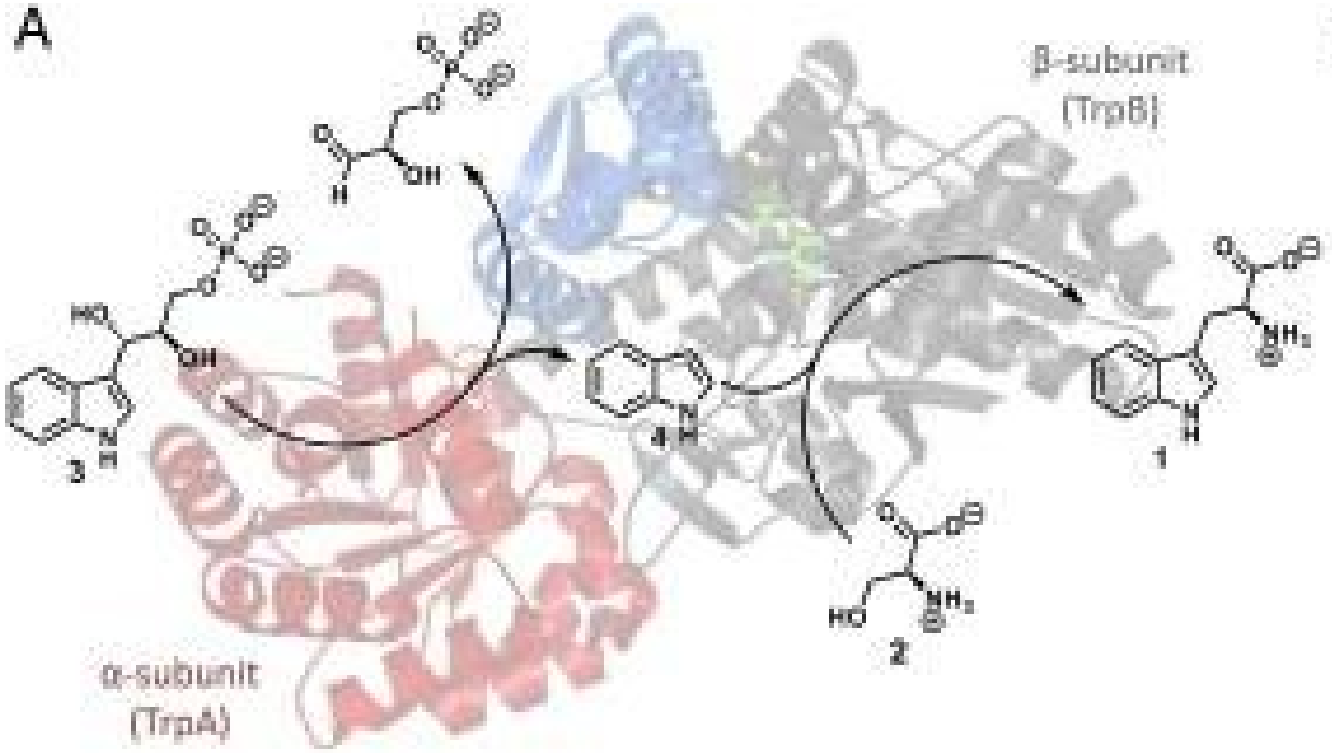
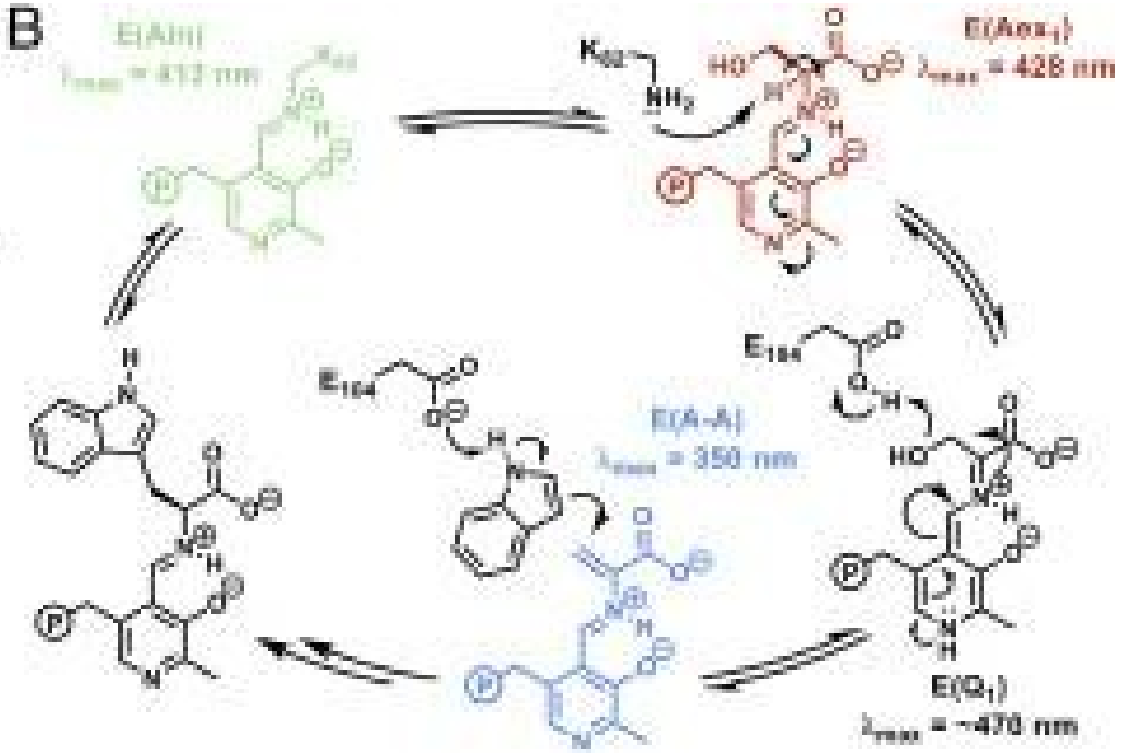
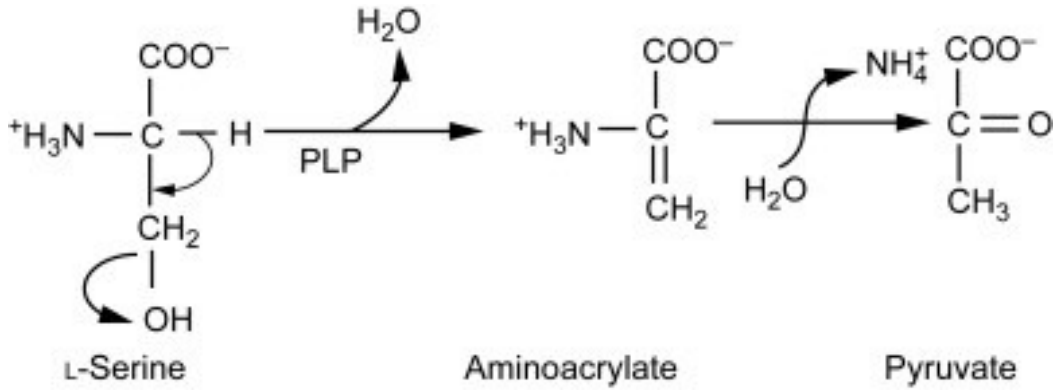


Figure 9.2b Molecular Biology of Assemblies and Machines (© Garland Science 2016)







β subunit as β2 catalyse the conversion of serin to puruvate and NH_3

ammonia as intermediate

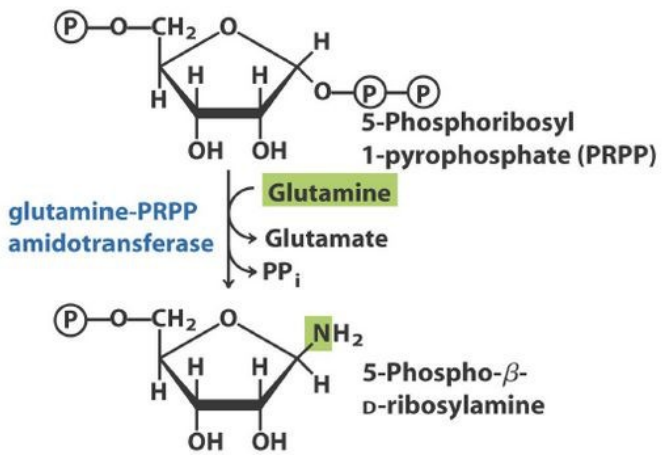
Asparagin synthase

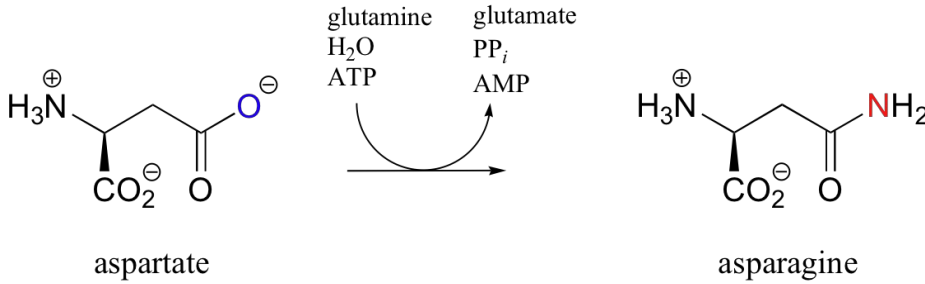
Carbomoyl phosphate synthase

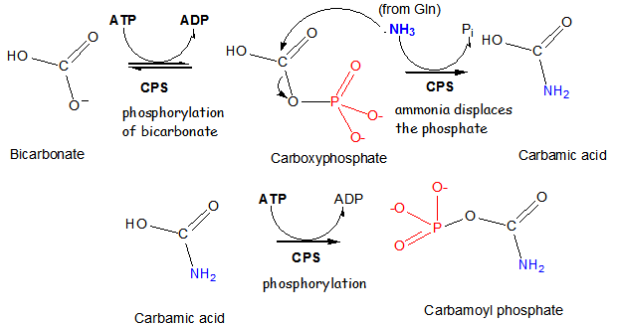
Glutamate synthase

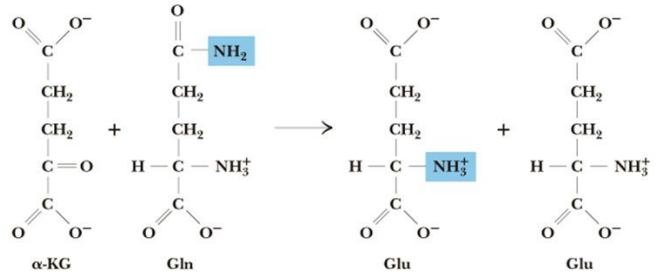
Imidazol glycerol phosphate synthase

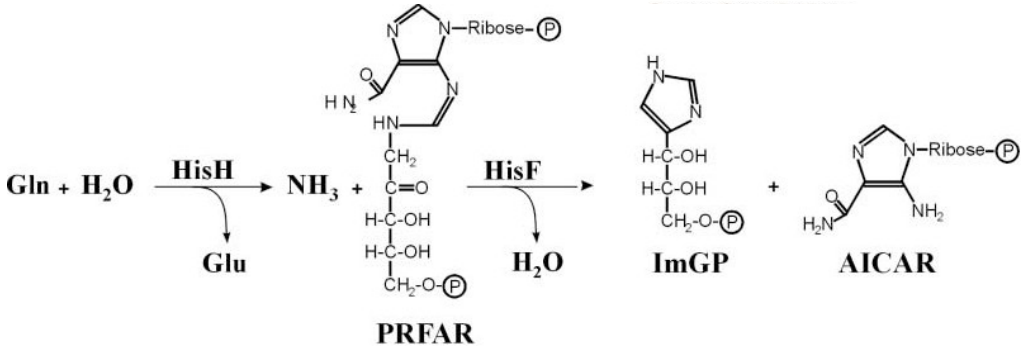
GPAT











structure and mechanism of CPS

Carbomoyl phosphate synthase

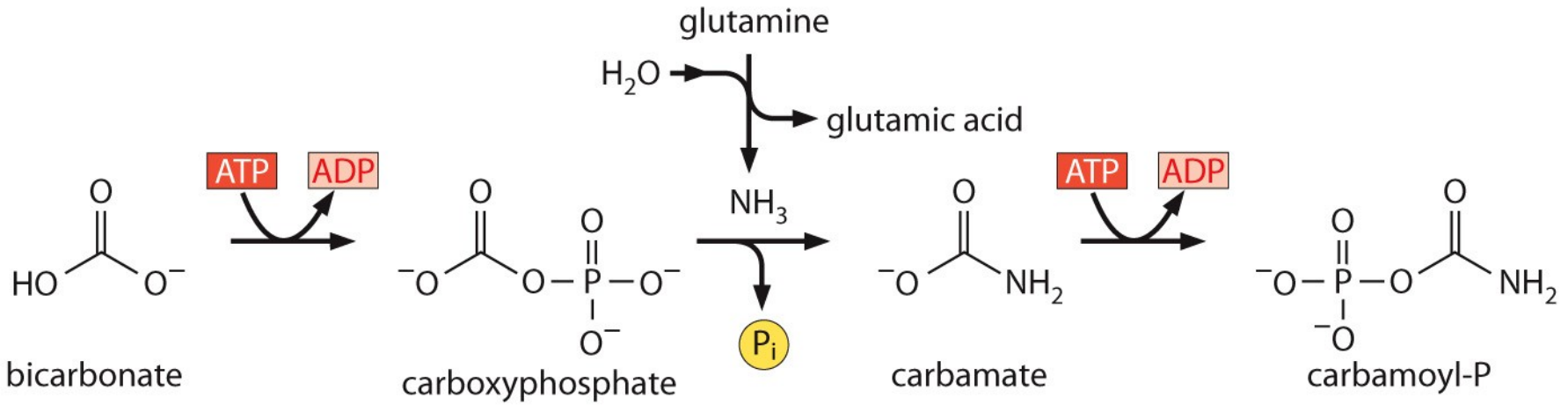


Figure 9.3a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

structure and mechanism of CPS

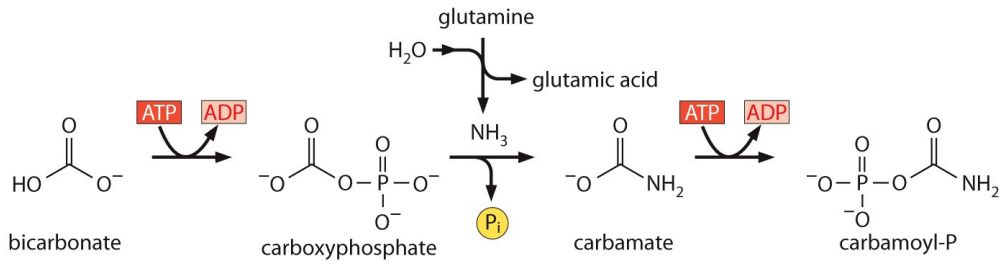


Figure 9.3a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Hetero-octamer

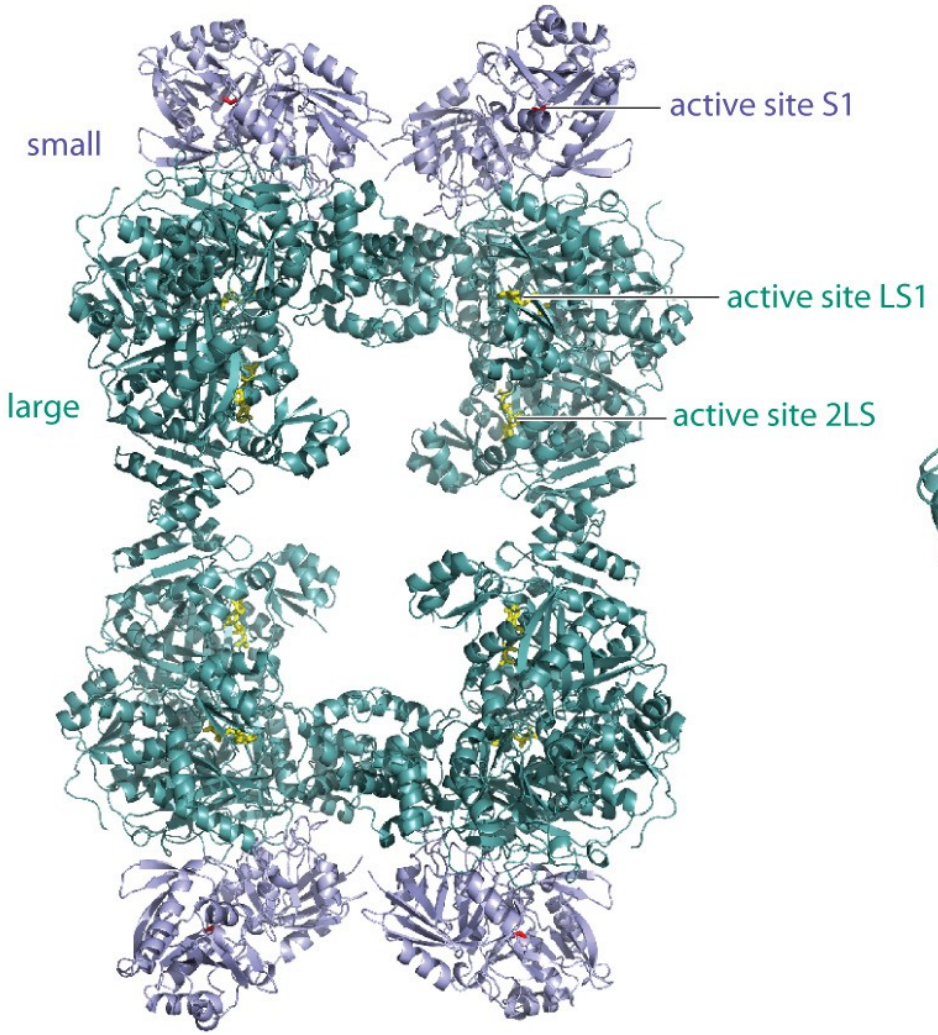
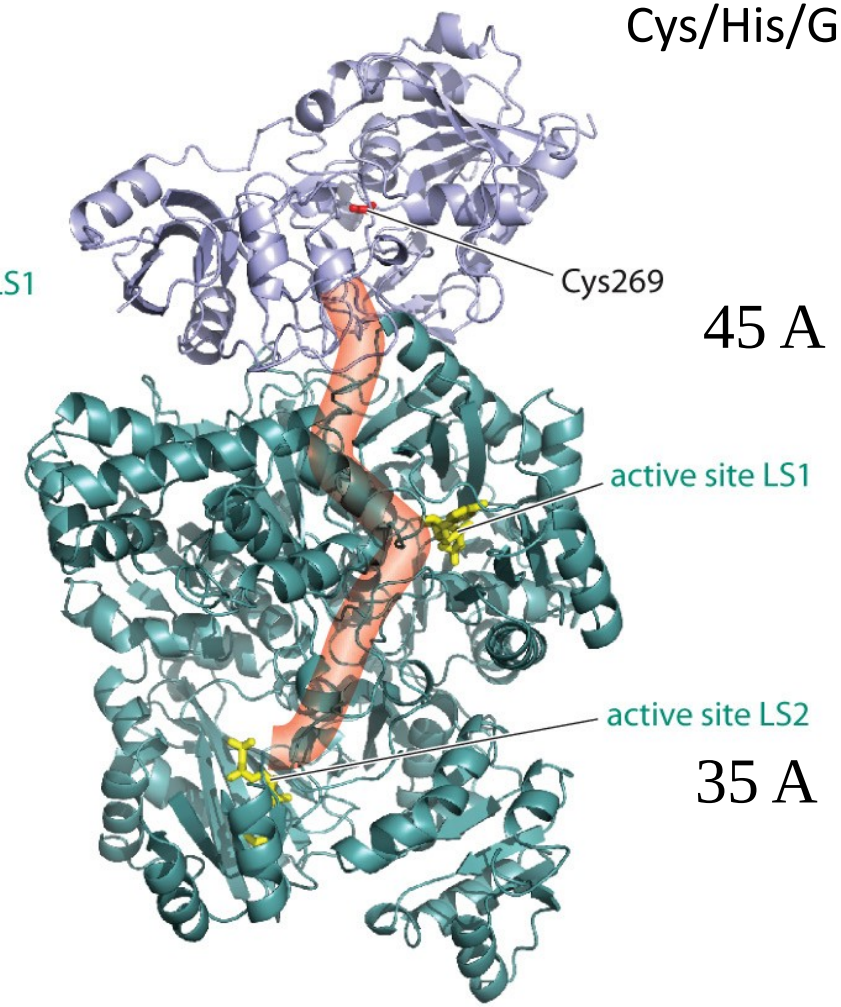
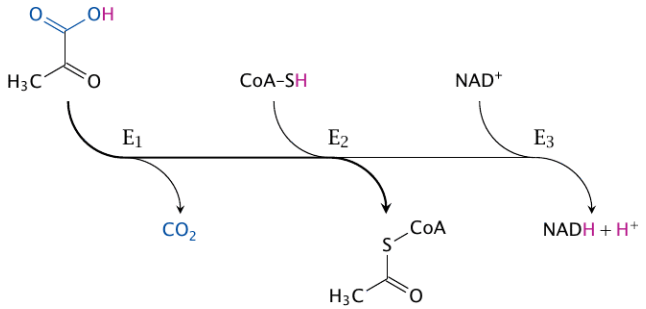


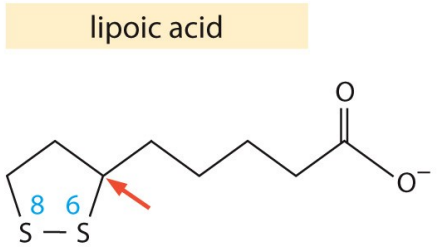
Figure 9.3b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

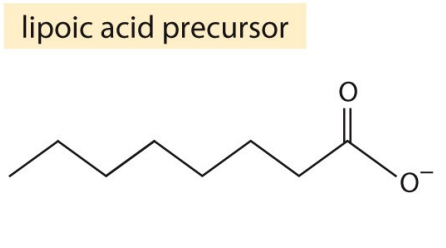
Cys/His/Glu

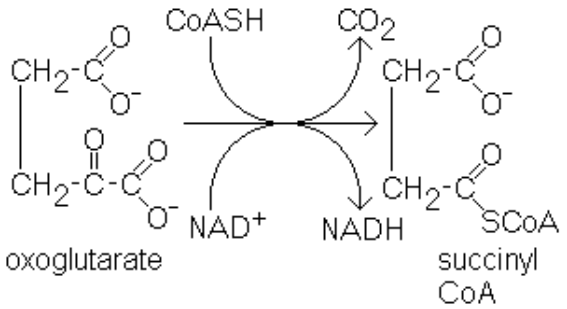


Multienzyme Complexes

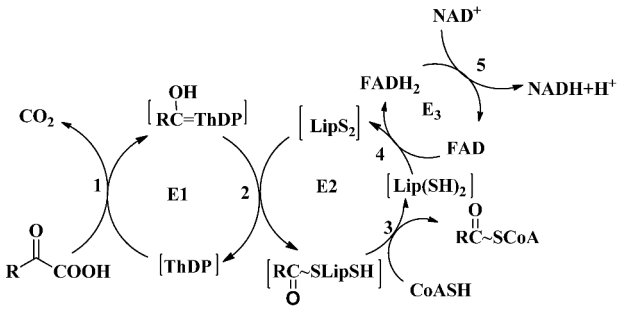




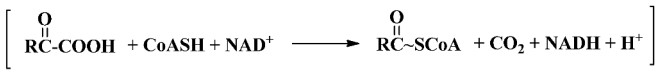




Lipoic acid dependent 2-oxo acid dehydrogenase



5-10 MDa

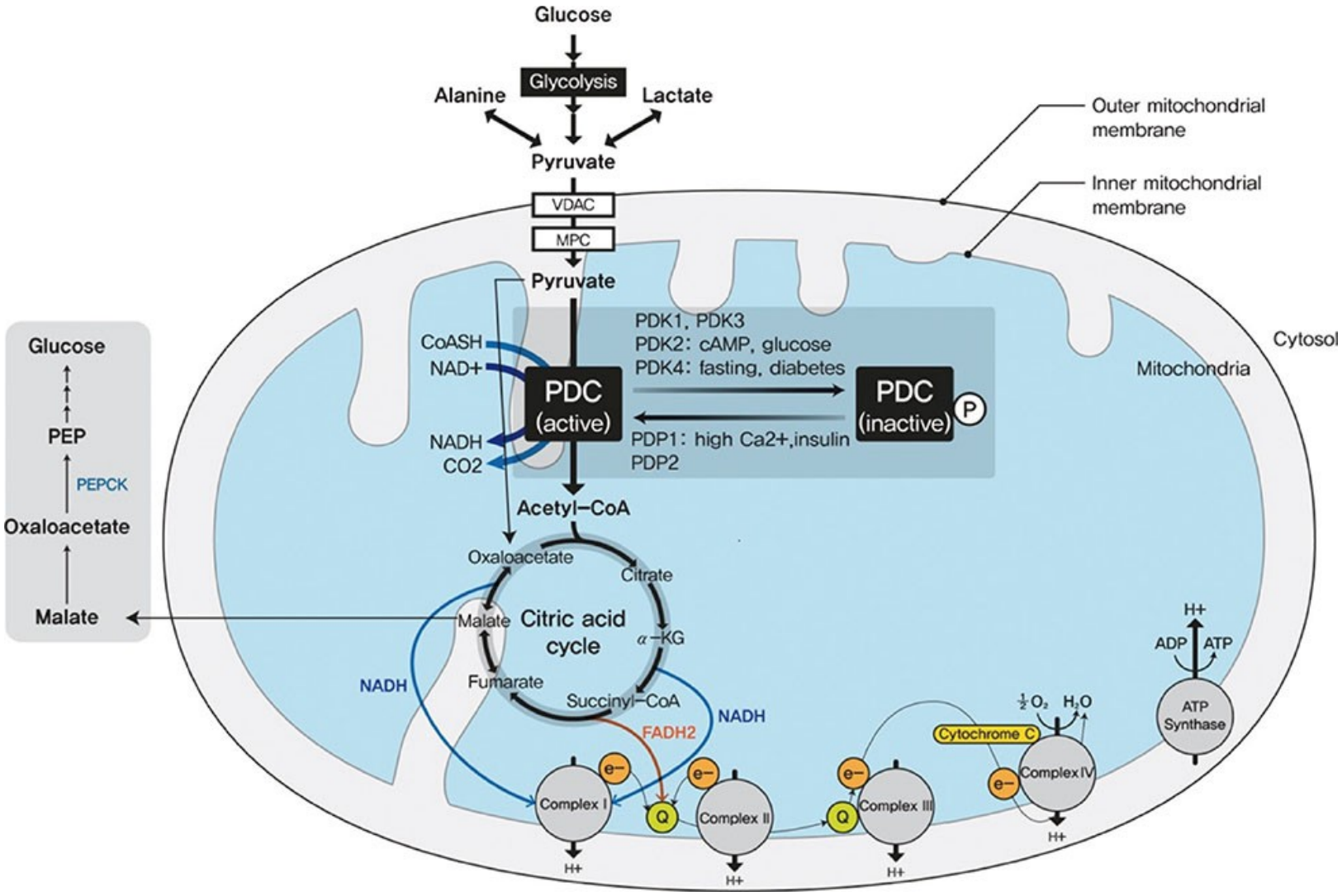


pyruvate dehydrogenase complex

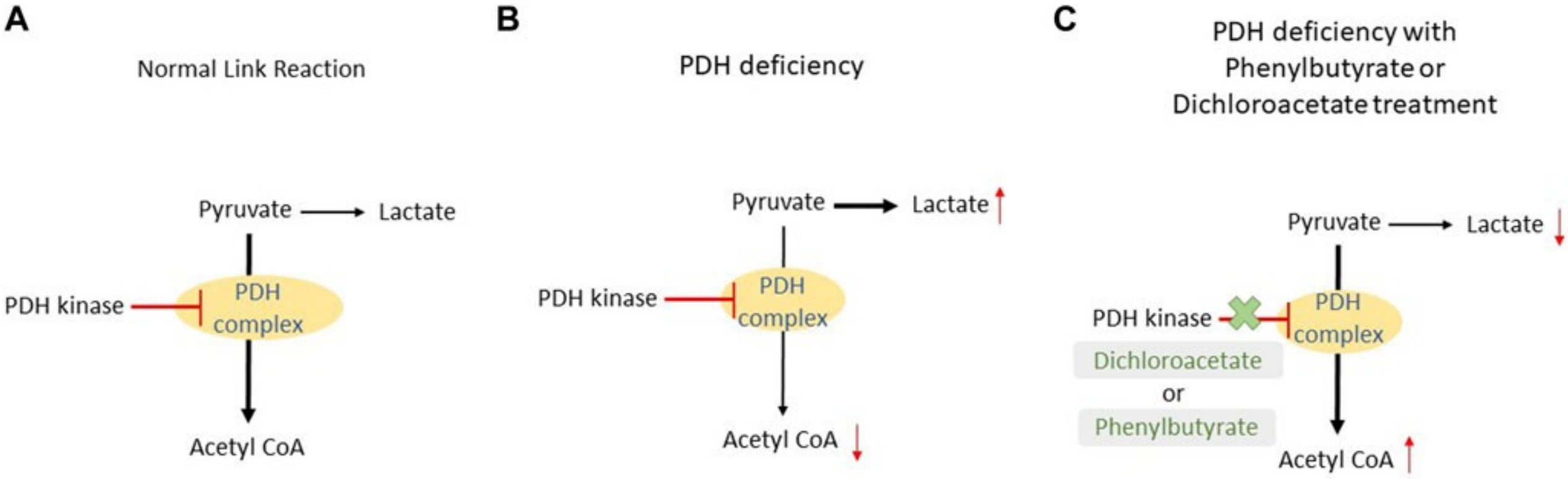
A huge molecular complex links three sequential reactions for energy production

Pyruvate dehydrogenase complex (PDC) deficiency, is an inborn error of mitochondrial energy metabolism.

The pyruvate oxidation route, bridges the cytosolic glycolytic pathway and the mitochondrial tricarboxylic acid cycle



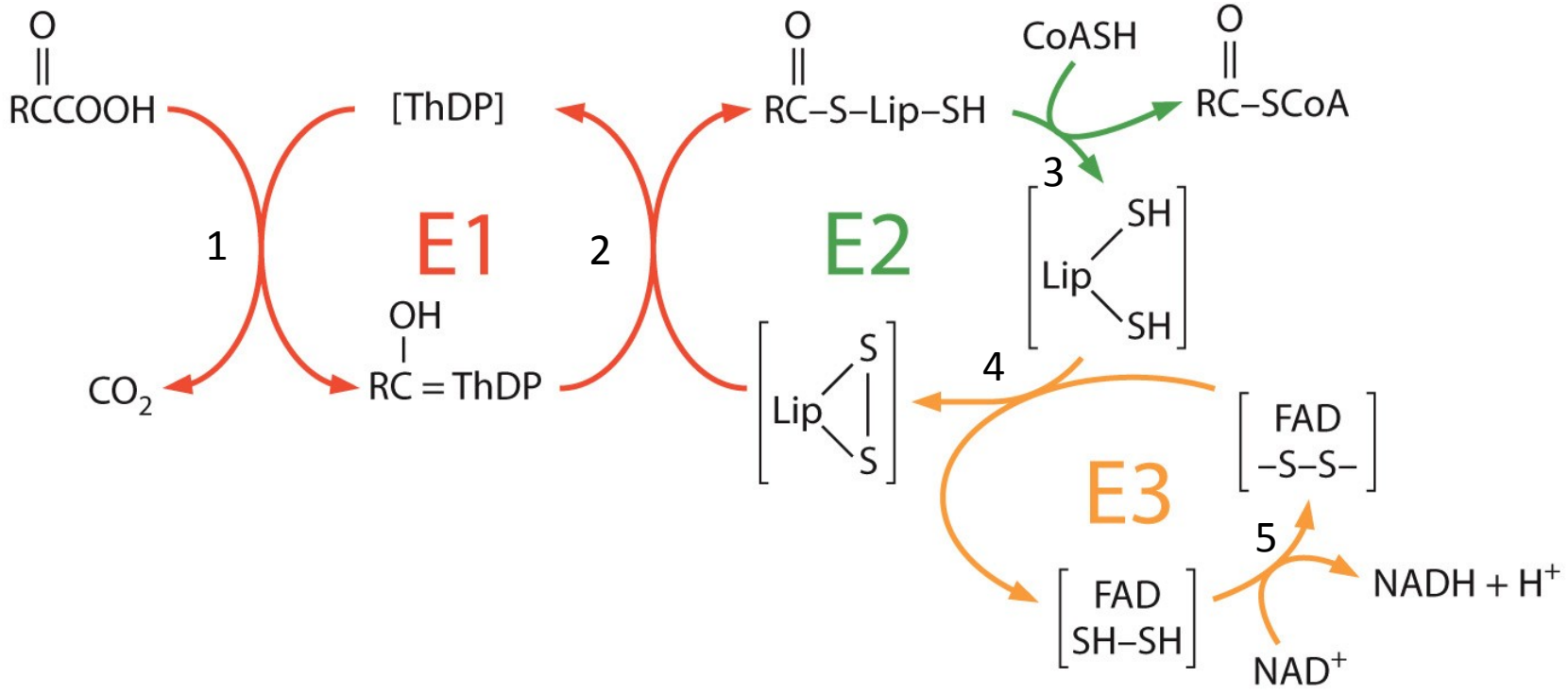
Pyruvate Dehydrogenase Deficiency

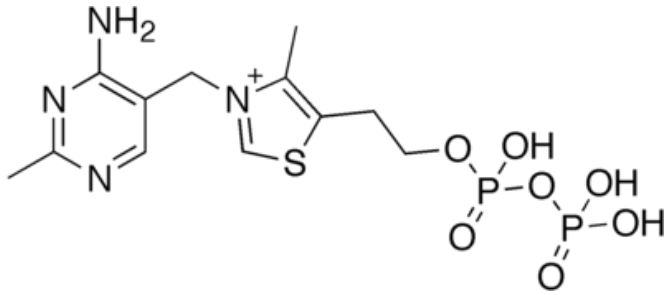


In PDH deficiency patients, a pathogenic variant in the PDH complex causes a slower conversion of pyruvate to acetyl CoA.

Br J Biomed Sci, 19 May 2022
https://doi.org/10.3389/bjbs.2022.10382

2 oxo acid dehydrogenase complexes





ThDP, thiamin diphosphate;

LipS-S, lipoyl group covalently attached to a lysine residue in E2 in oxidized form

R-CO-S-Lip-SH, reductively acylated lipoyl group

$Lip(SH)_2$, dihydrolipoyl group

NET REACTION

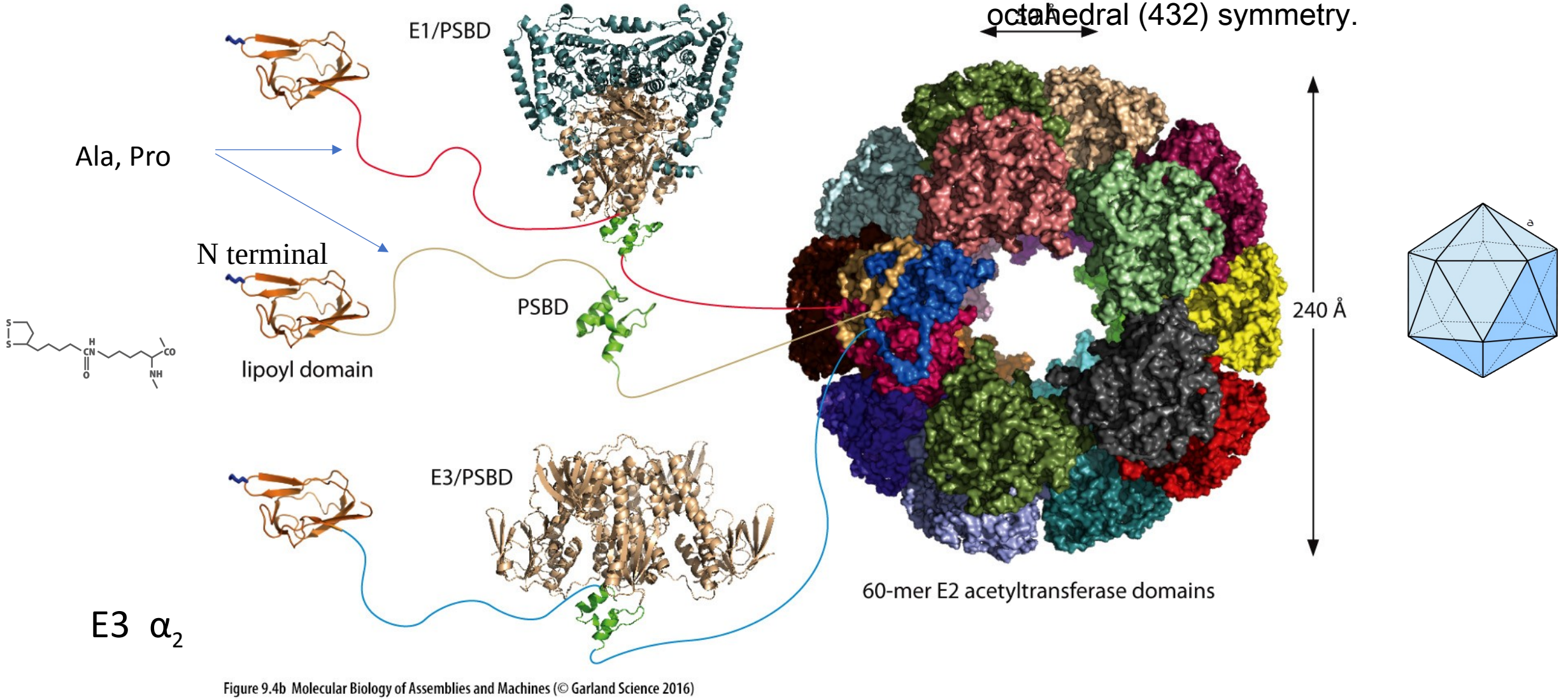
Figure 9.4a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Structure

peripheral subunit binding domain (PSBD).

60 polypeptide chains (each 46 kDa) 2.8 MDa
icosahedral (532) symmetry
E2 cores (24mers)
octahedral (432) symmetry.

E1 $\alpha_2 \beta_2$



E3 α_2

Figure 9.4b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

lipoyl arm

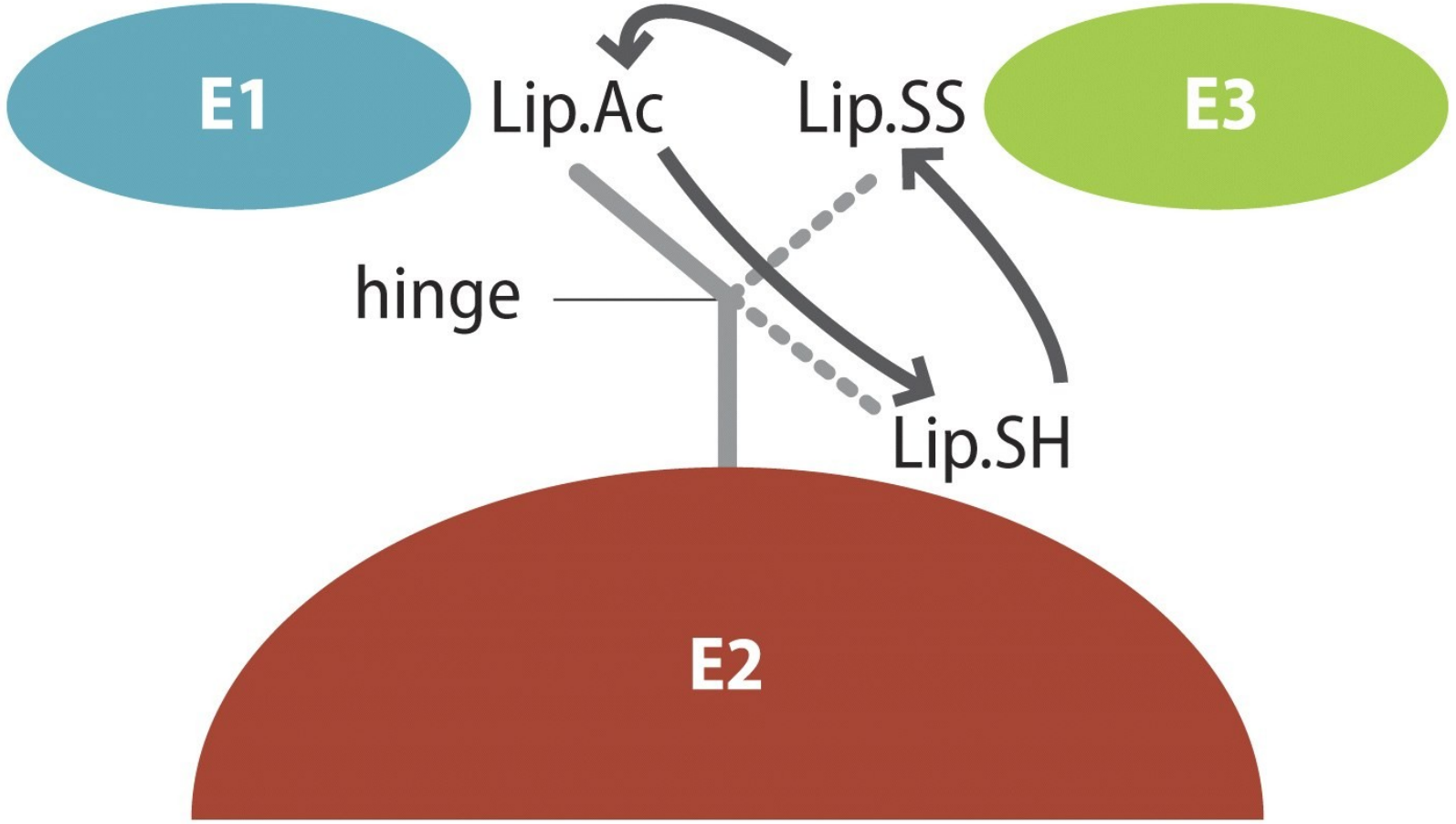


Figure 10.14 How Proteins Work (©2012 Garland Science)

polyproline II helix, hinged sticky arm

Figure 4.25 How Proteins Work (©2012 Garland Science)

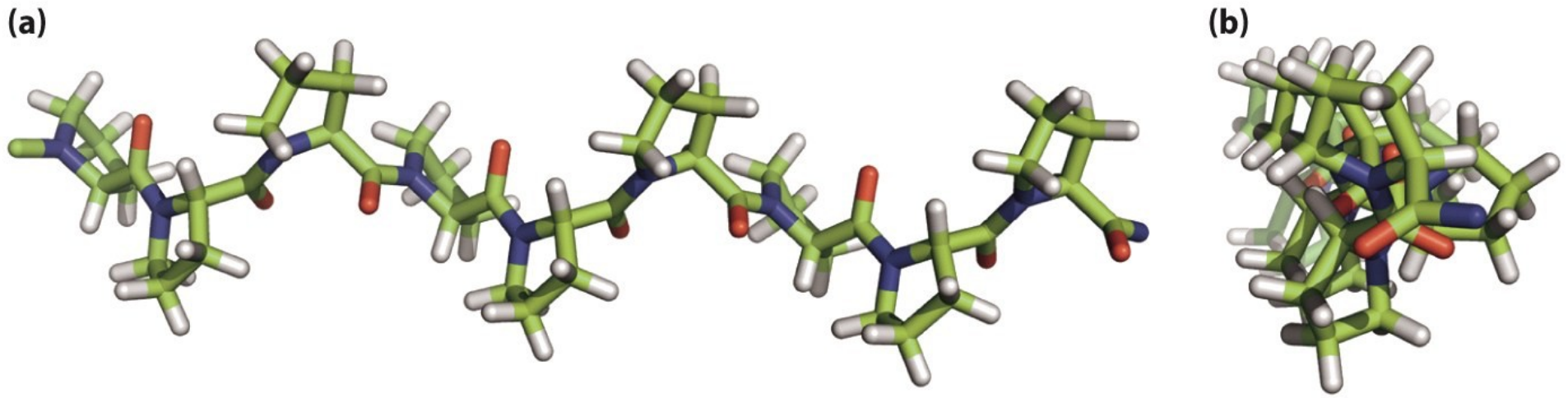


Figure 4.24 How Proteins Work (©2012 Garland Science)

E2 polypeptide chain in *E. coli*

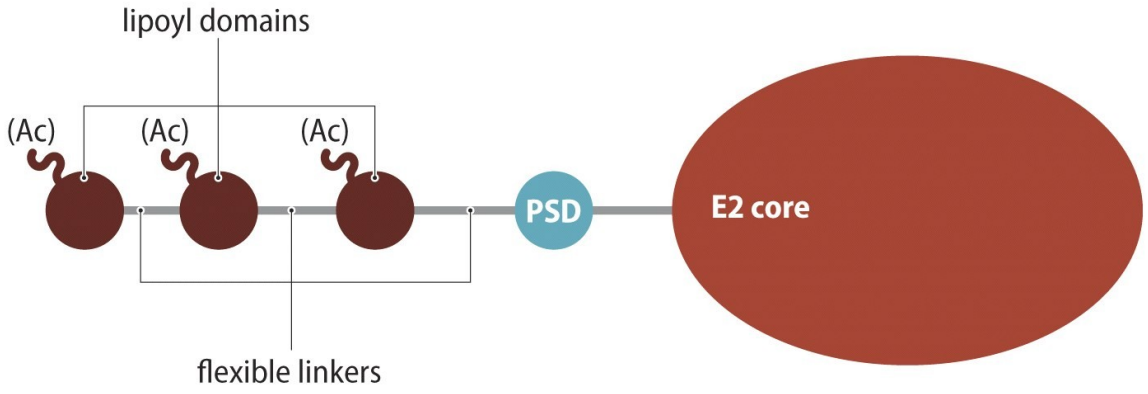


Figure 10.13 How Proteins Work (©2012 Garland Science)

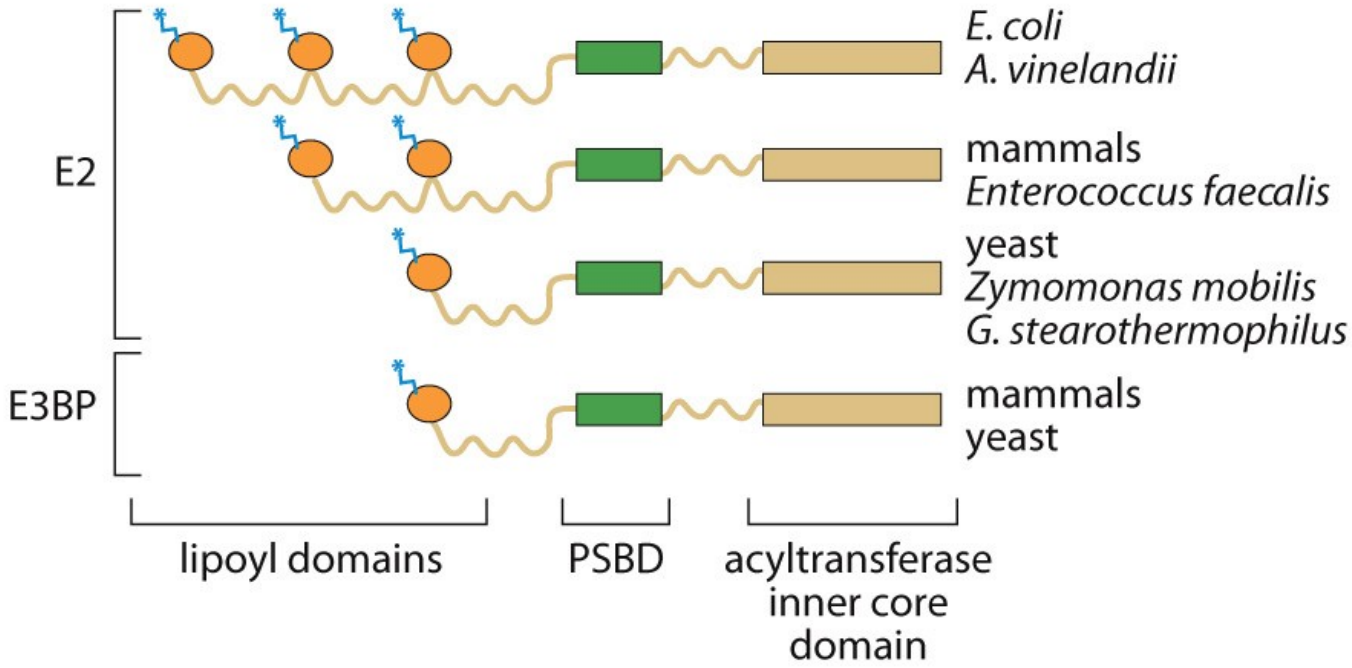


Figure 9.8 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Active-site coupling

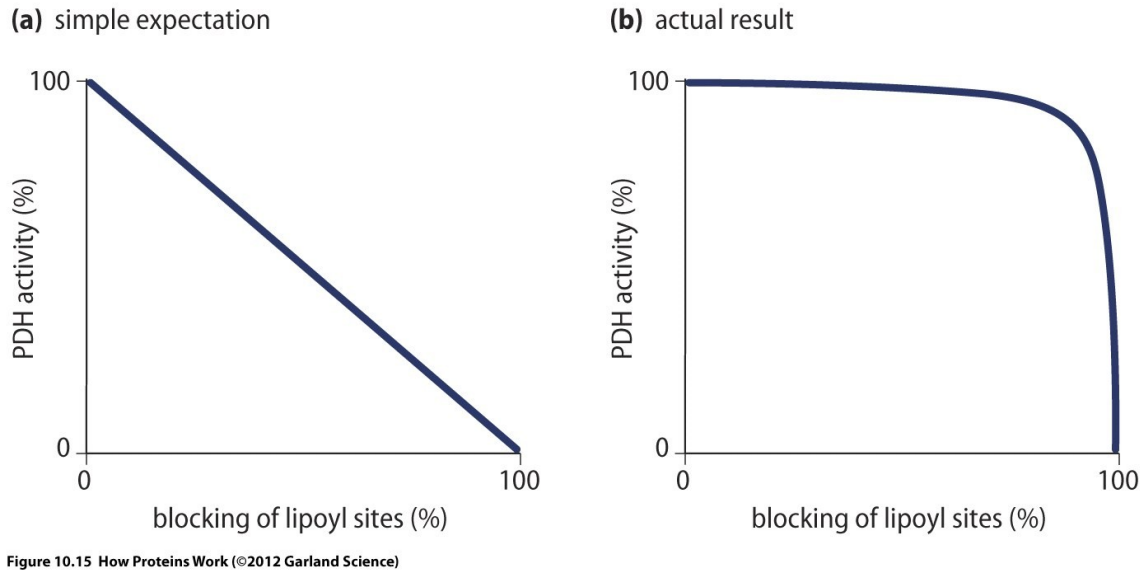
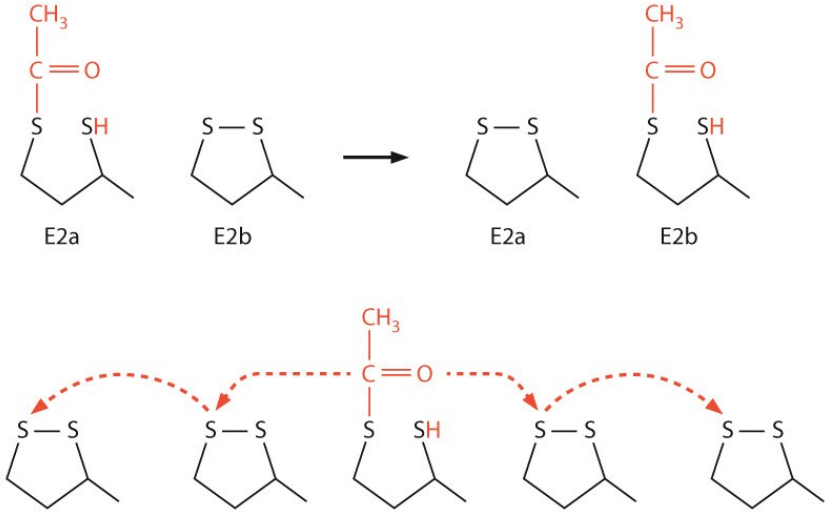


Figure 10.15 How Proteins Work (©2012 Garland Science)



active-site coupling

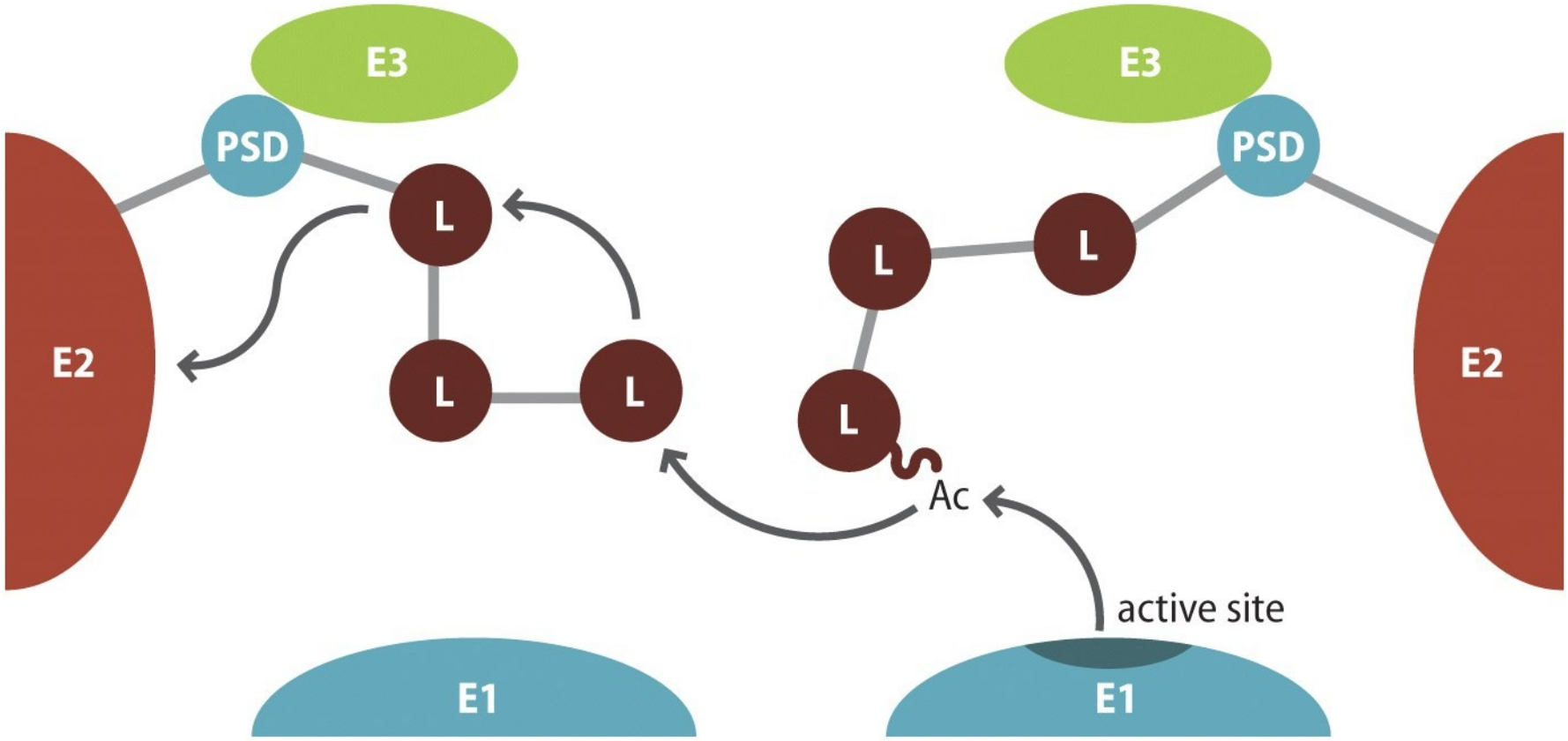
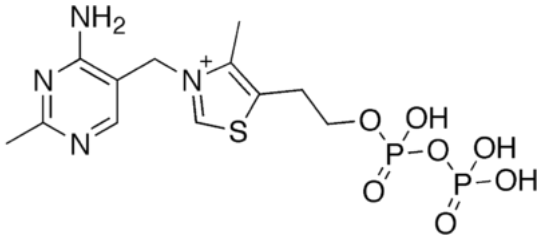
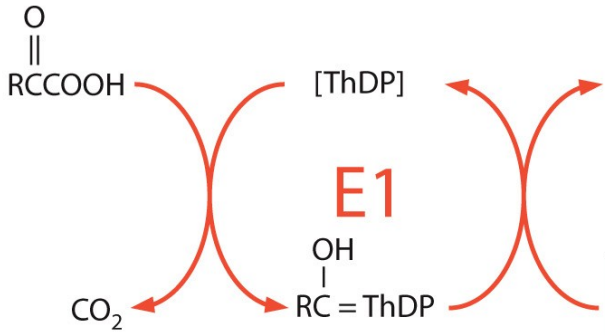


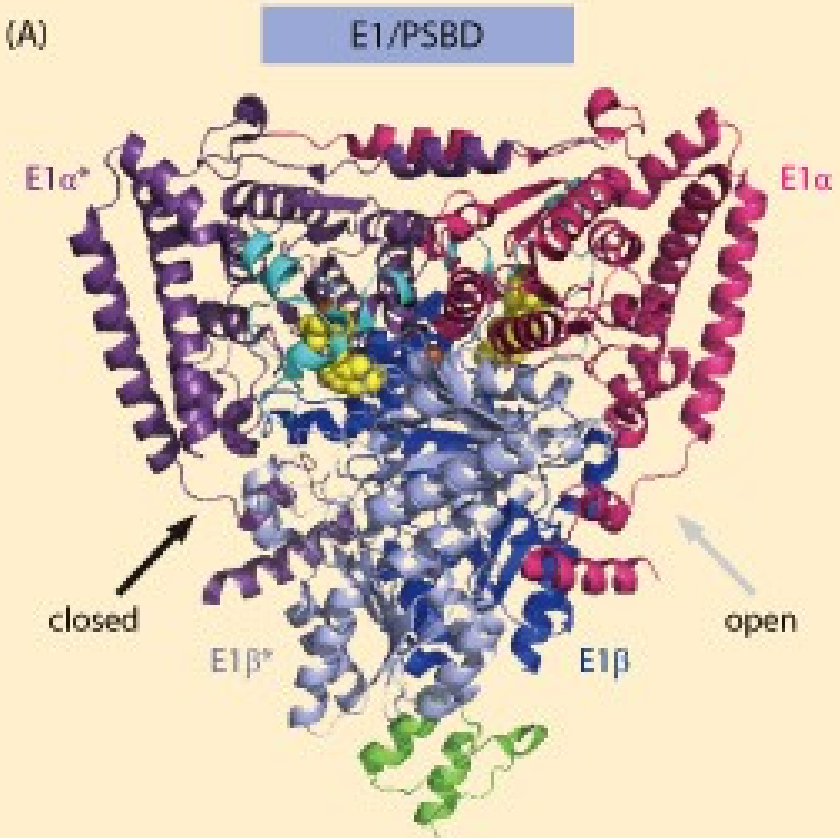
Figure 10.16 How Proteins Work (©2012 Garland Science)

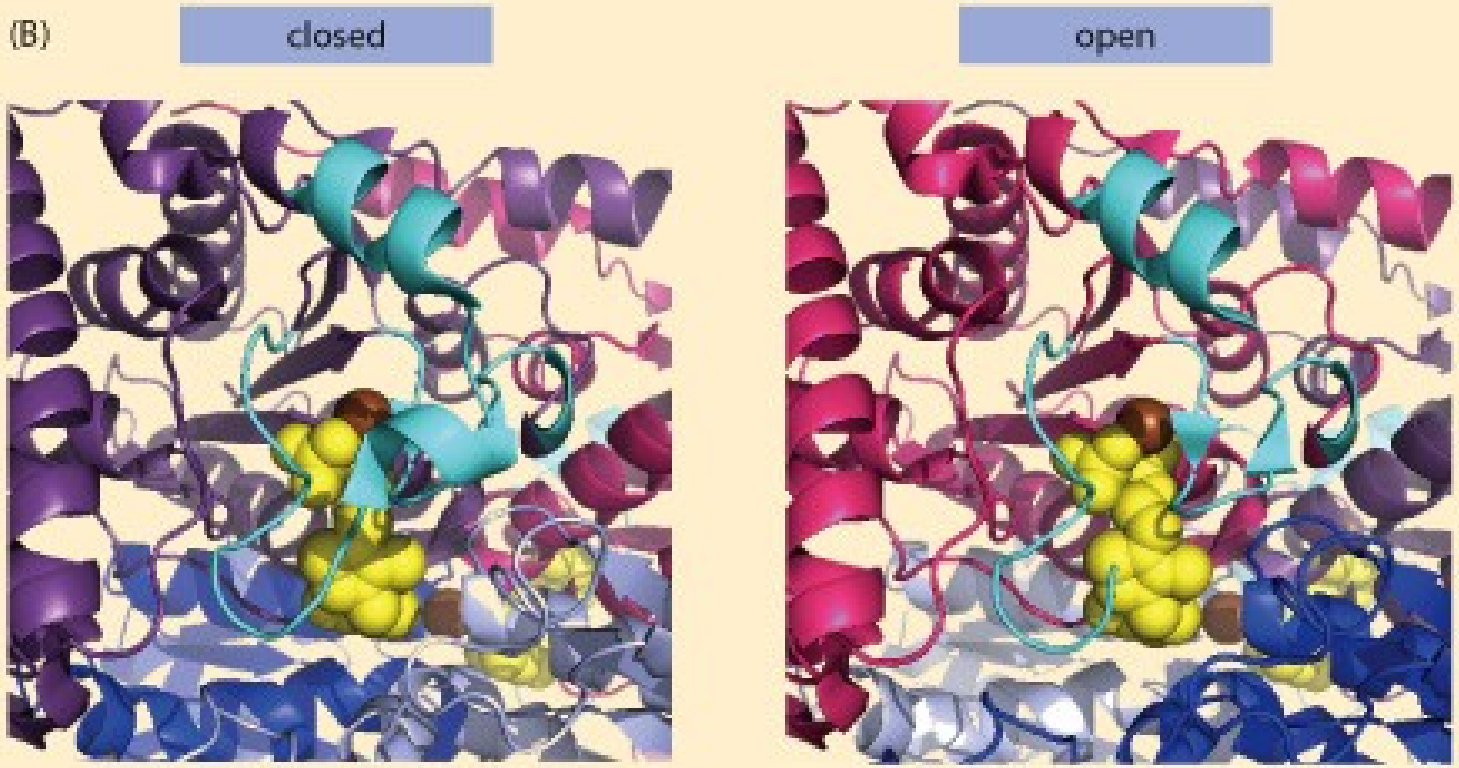
E1 subunit

Thiamin diphosphate

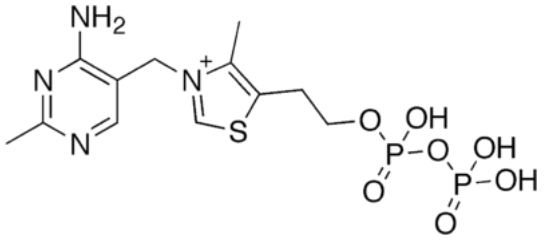


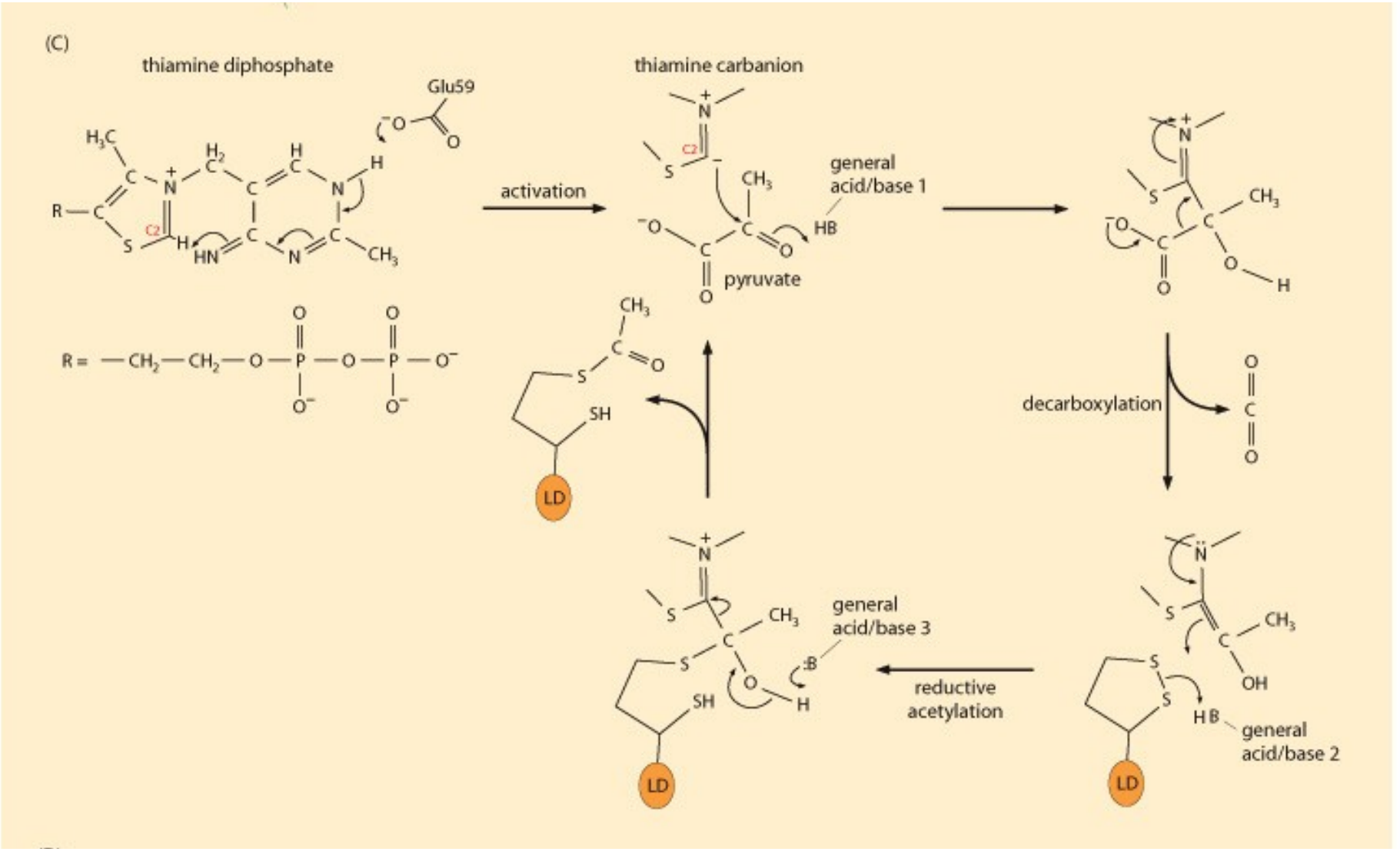




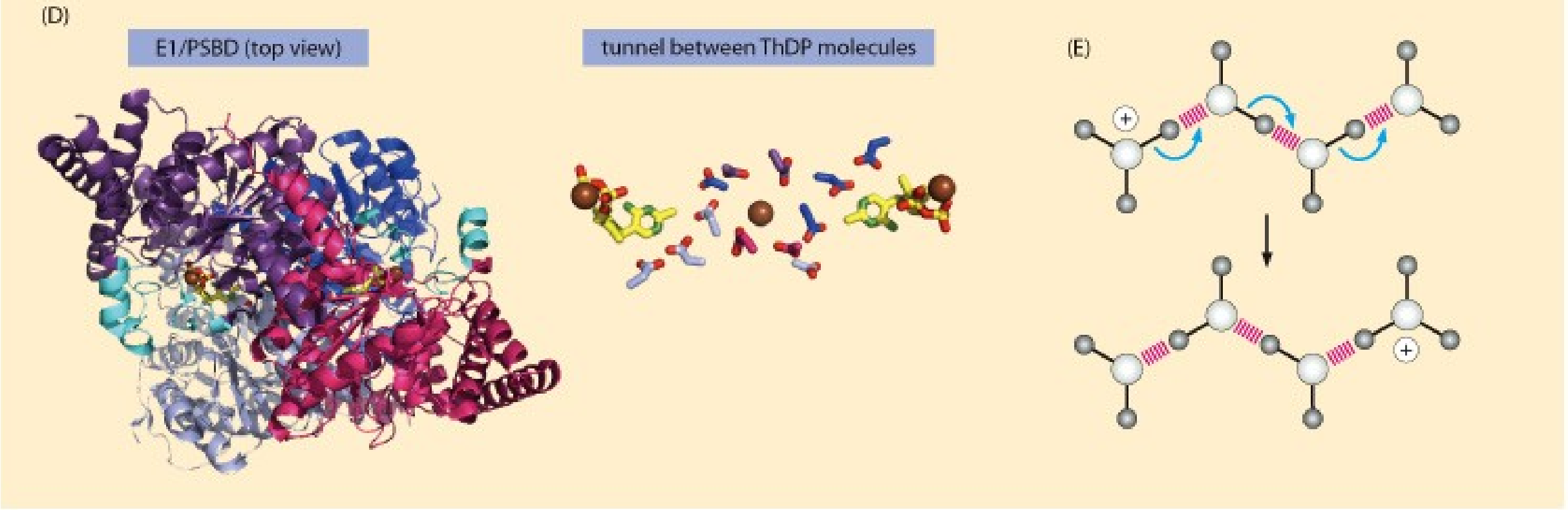


E1 mechanism

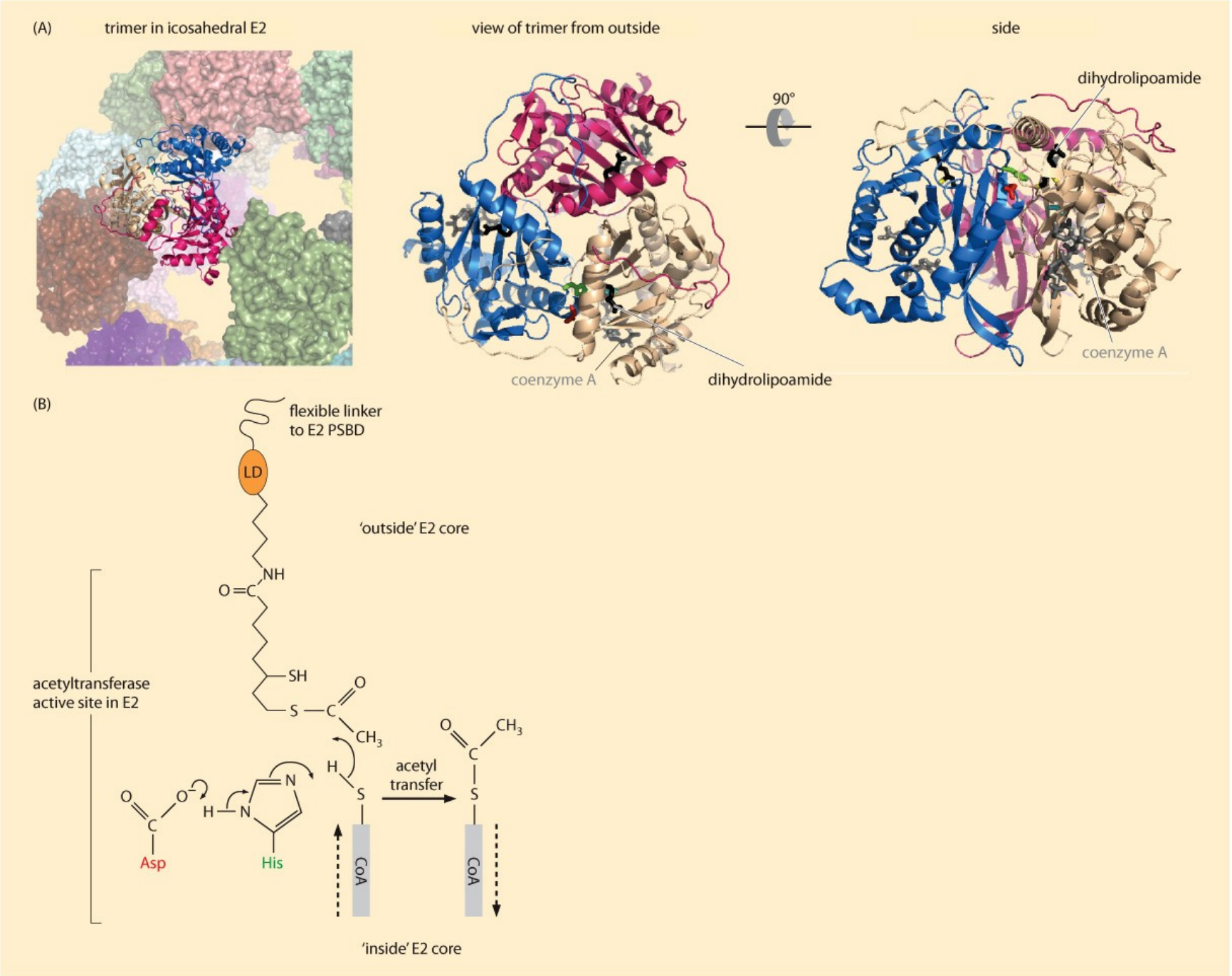




E1 communication

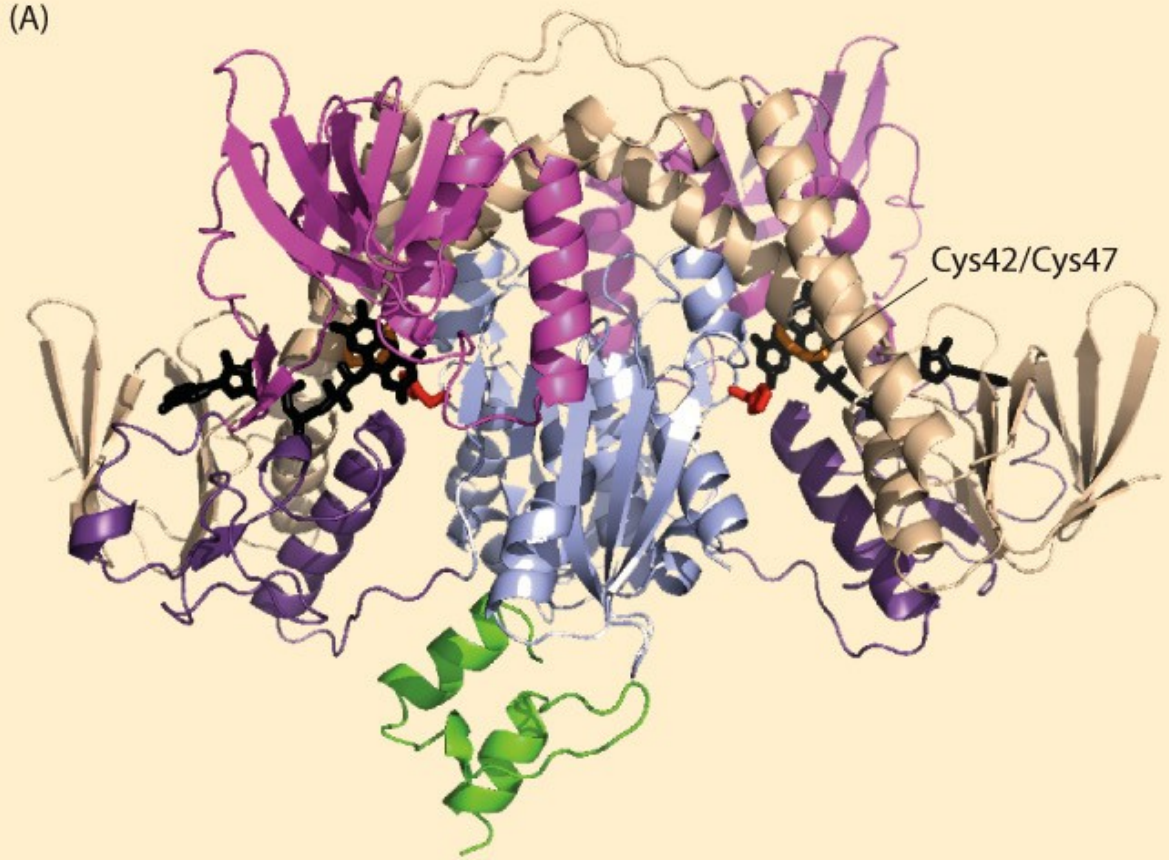


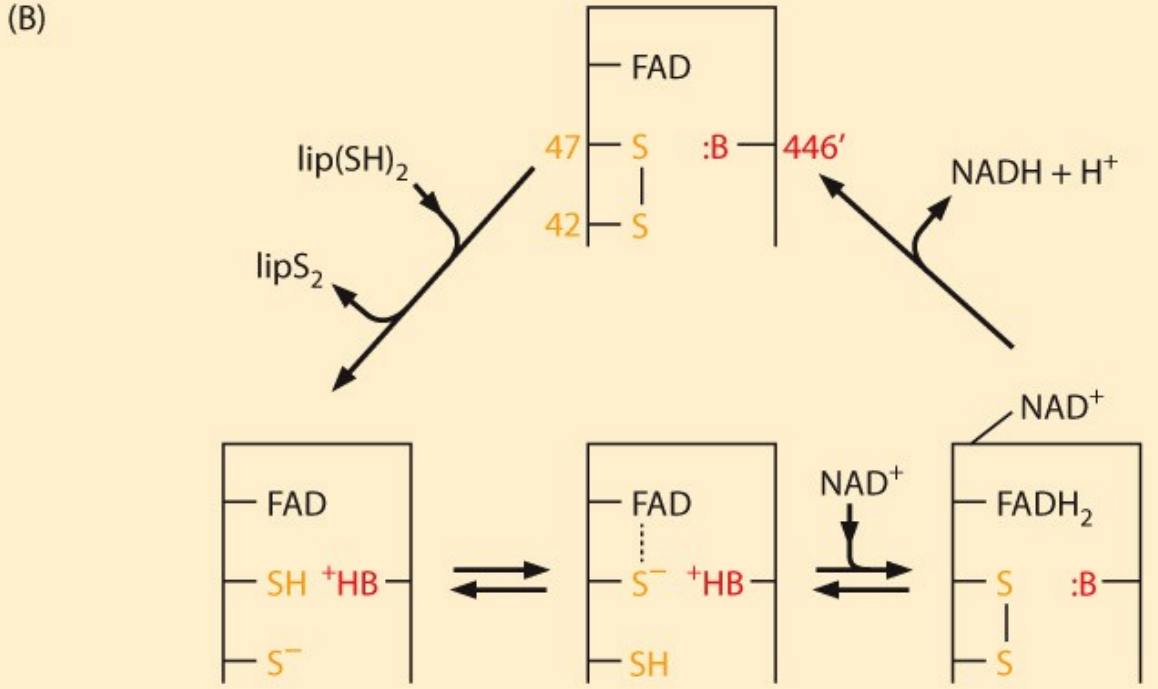
E2 Mechanism



Box 9.1 Figure 9.1.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Mechanism on E3





Box 9.3 Figure 9.3.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Cryo EM of the E2/E3

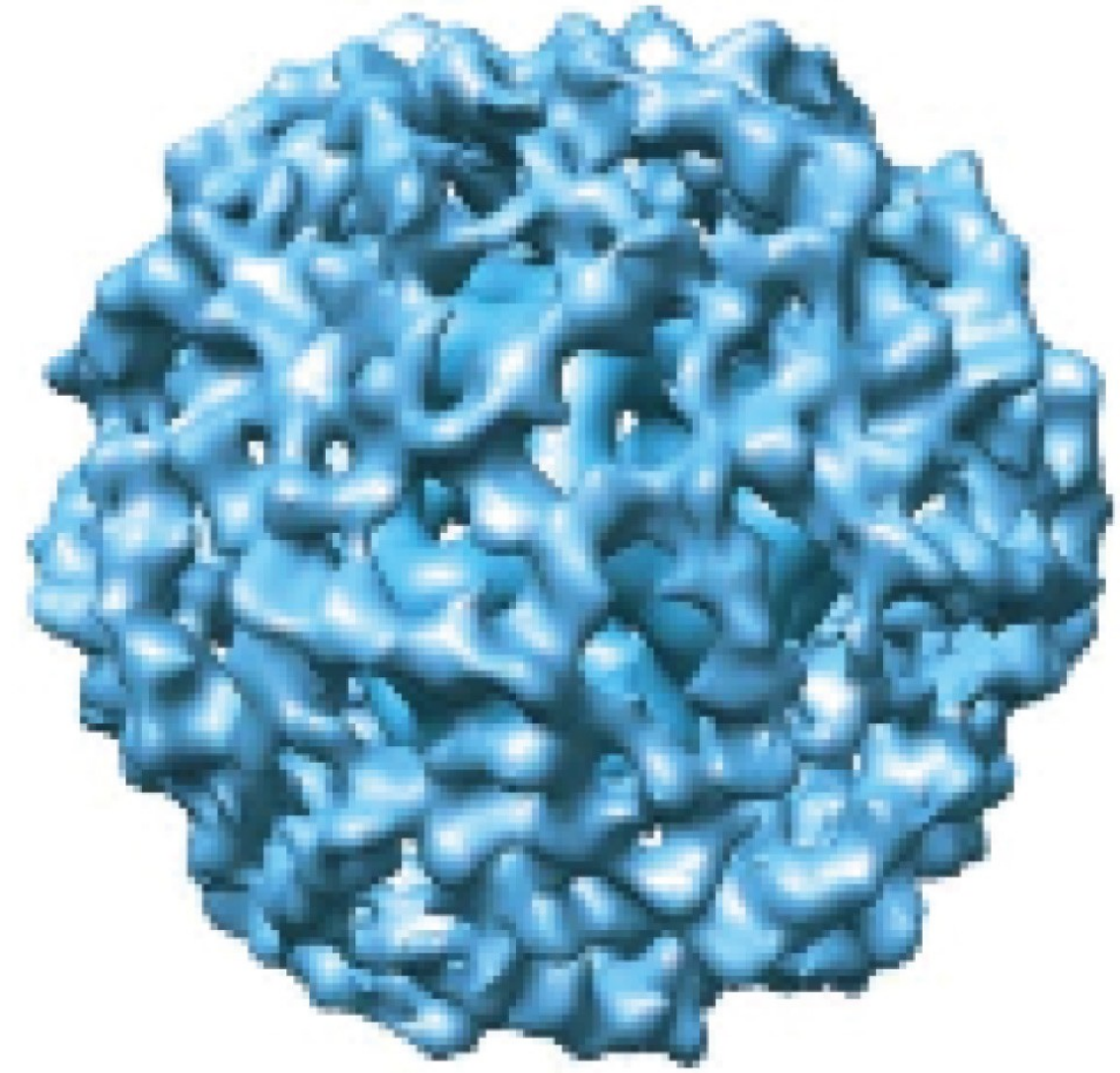
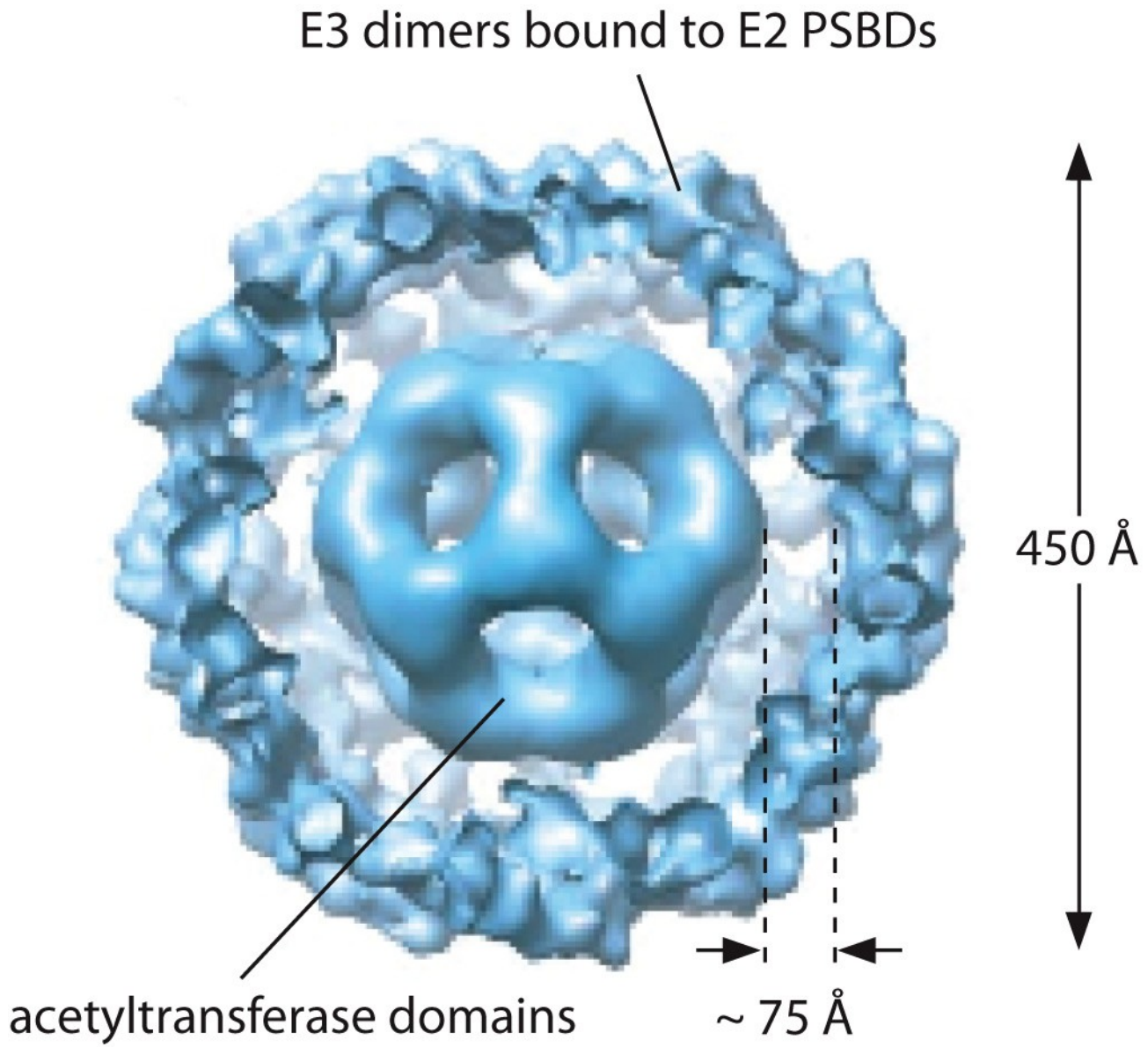
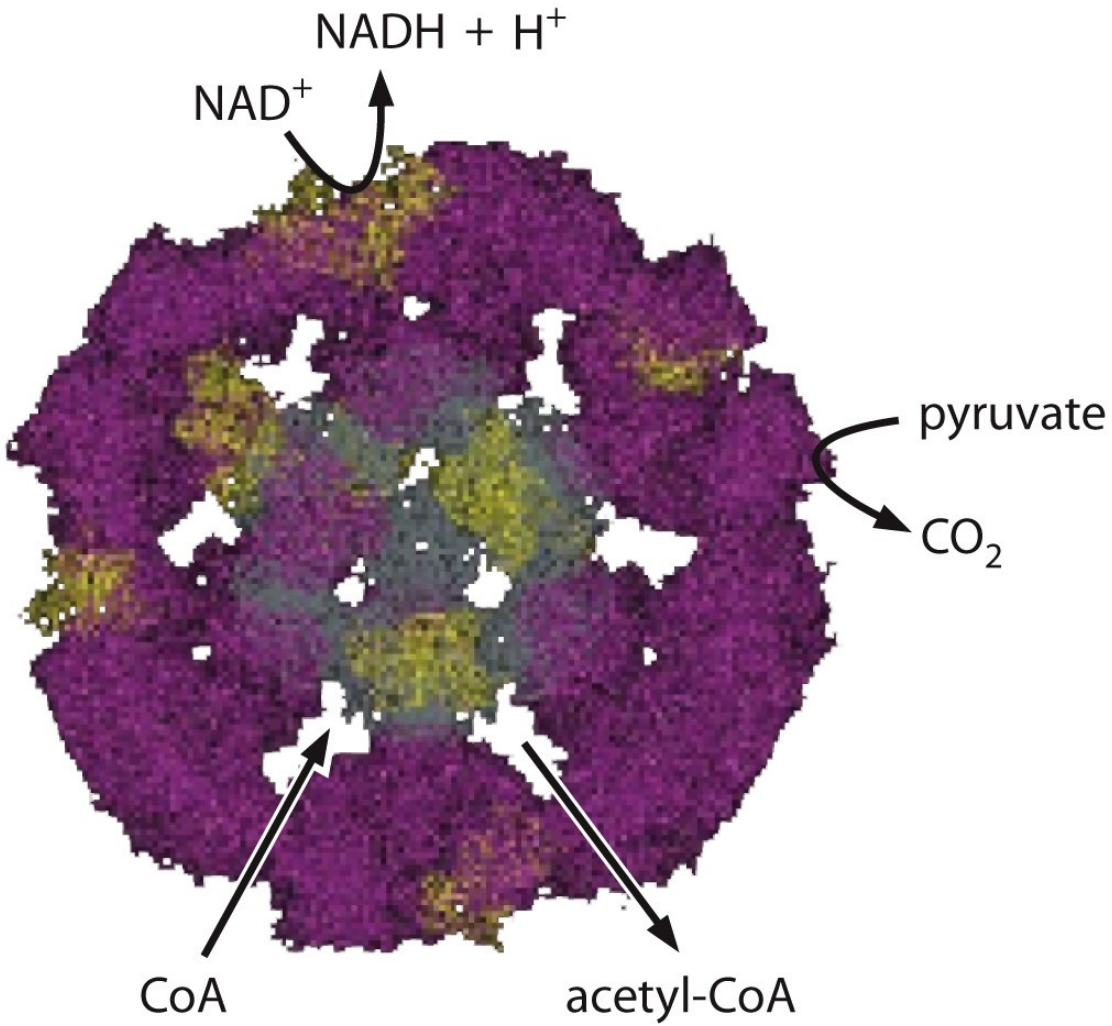


Figure 9.7a Molecular Biology of Assemblies and Machines (© Garland Science 2016)



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

Model of PDH



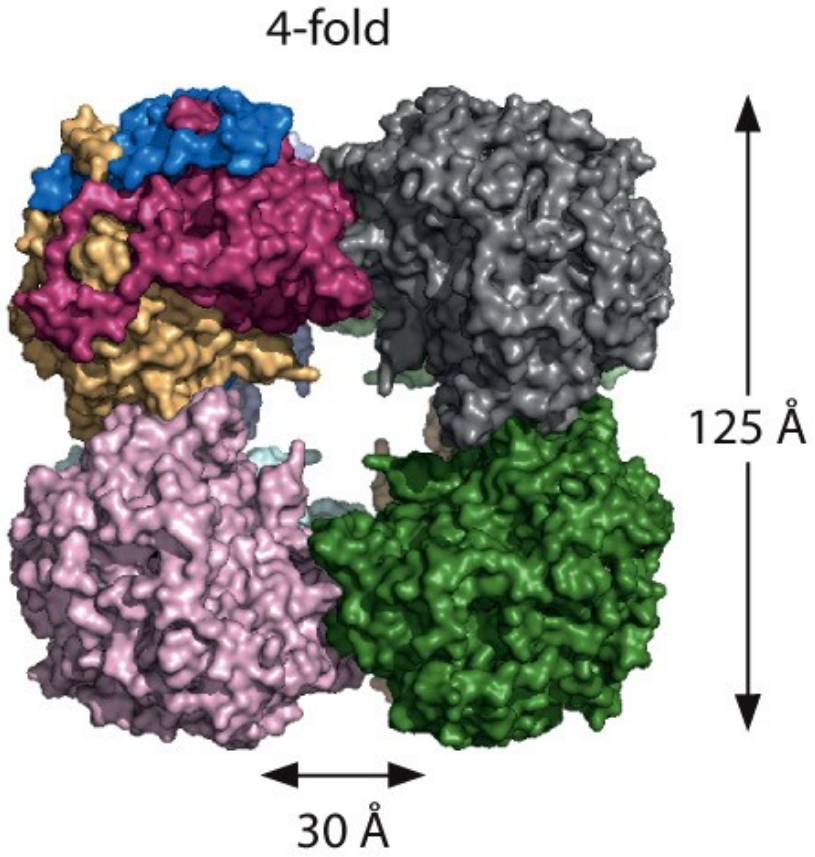
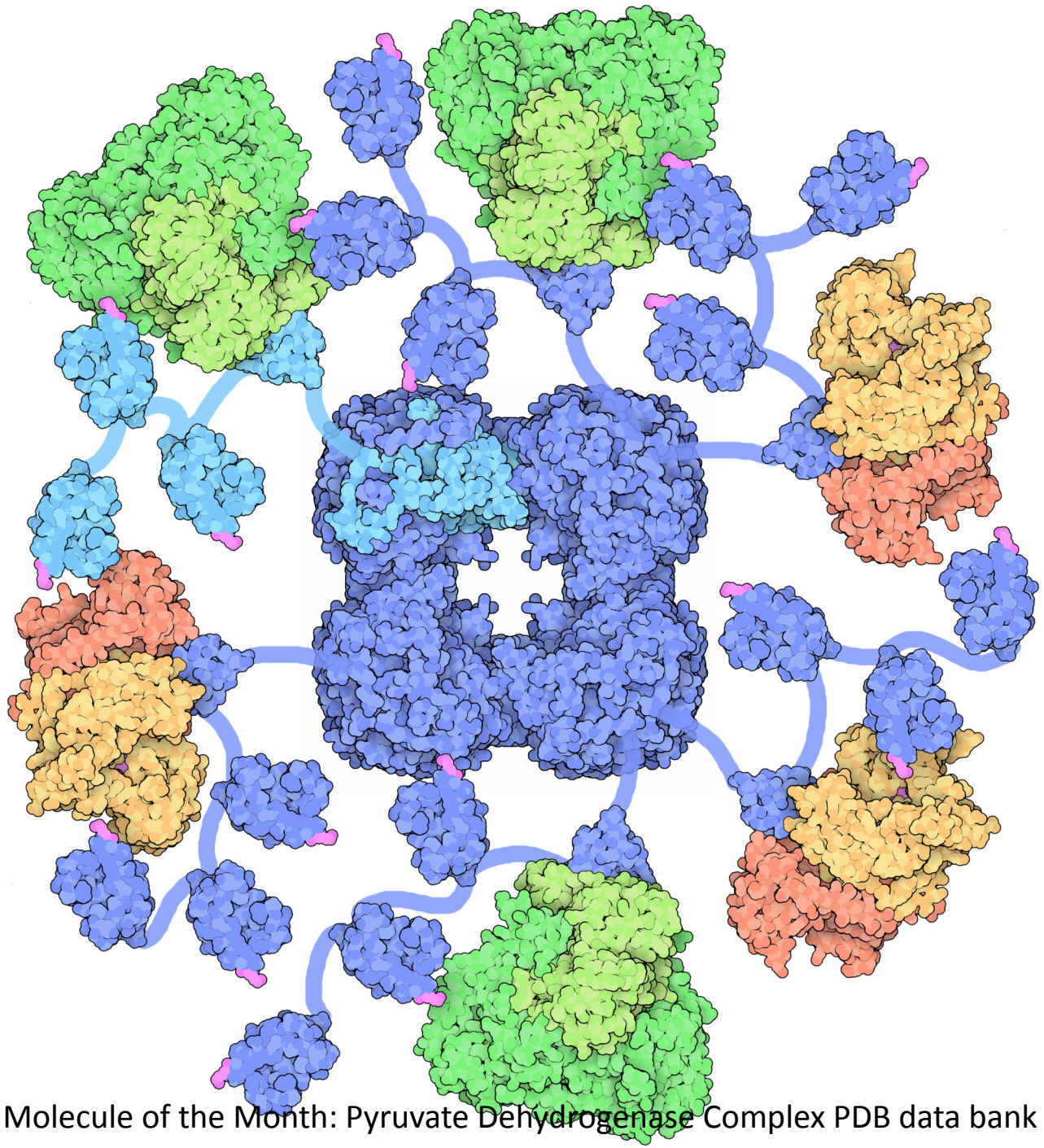
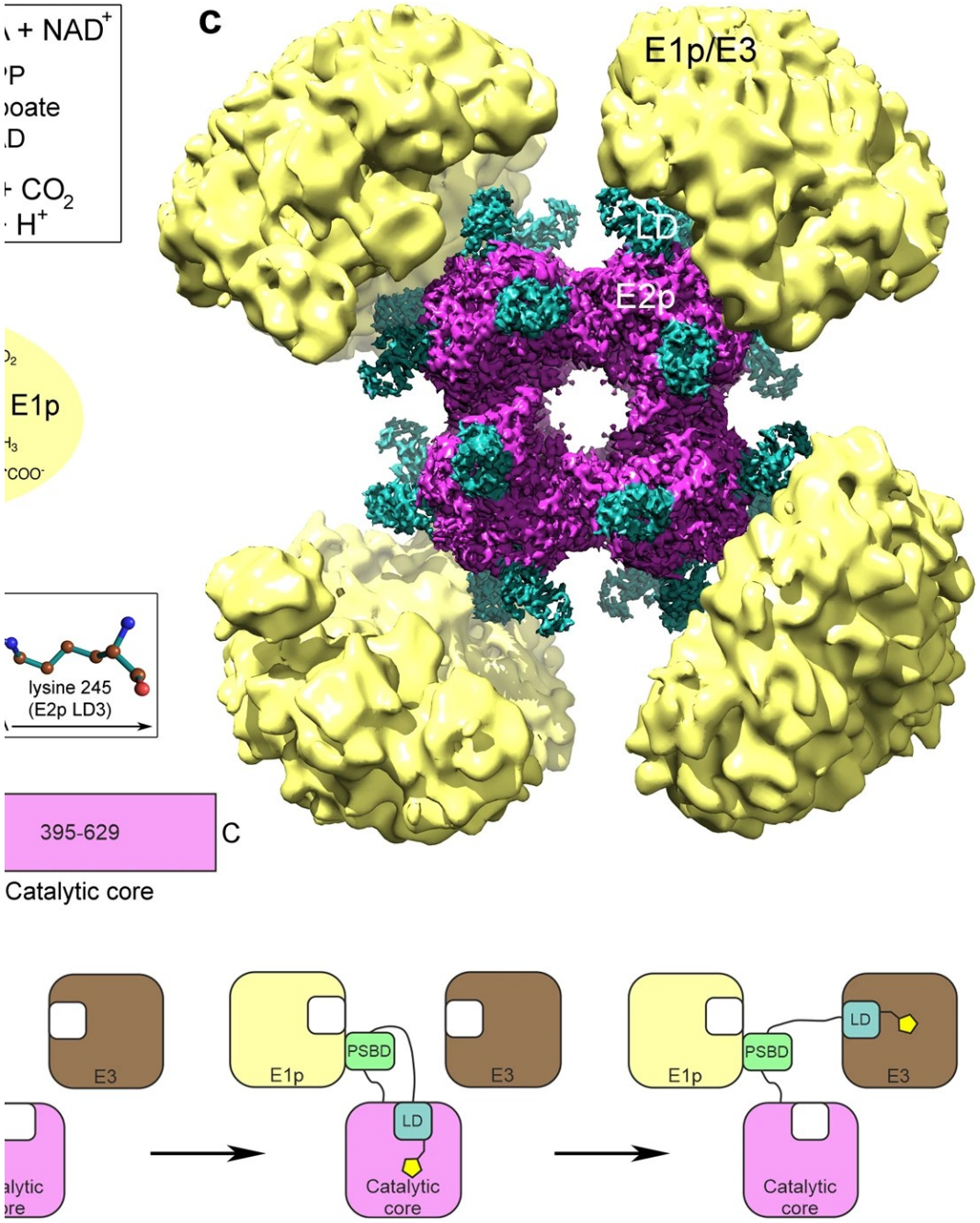


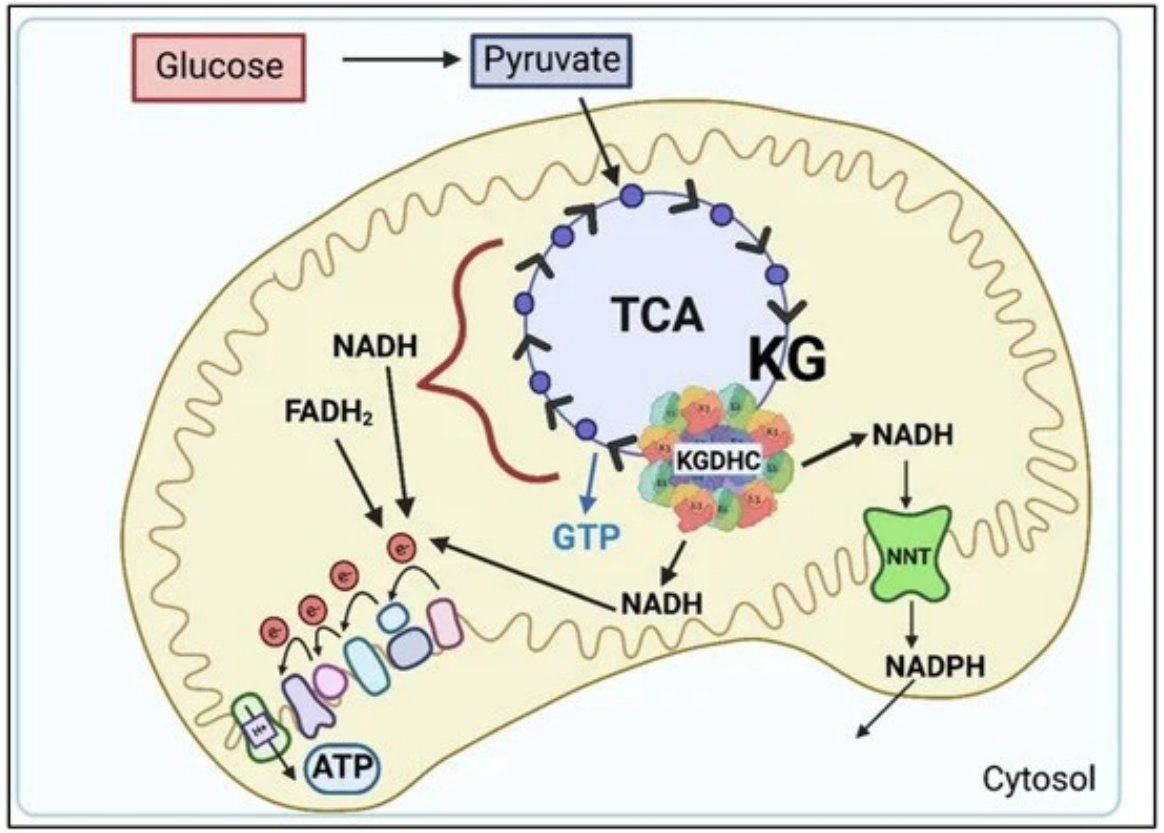
Figure 9.7c Molecular Biology of Assemblies and Machines (© Garland Science 2016)

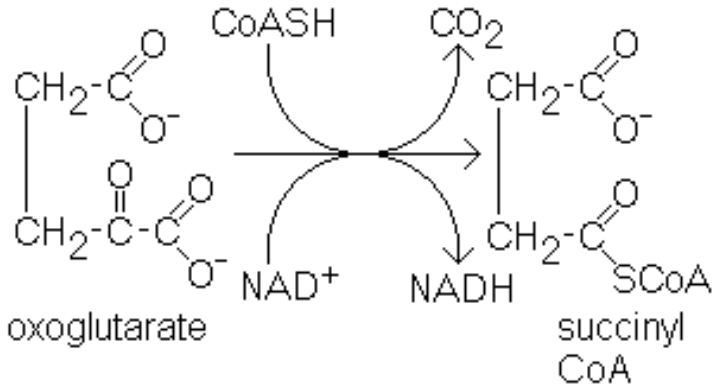




Molecule of the Month: Pyruvate Dehydrogenase Complex PDB data bank

a-ketoglutarate dehydrogenase

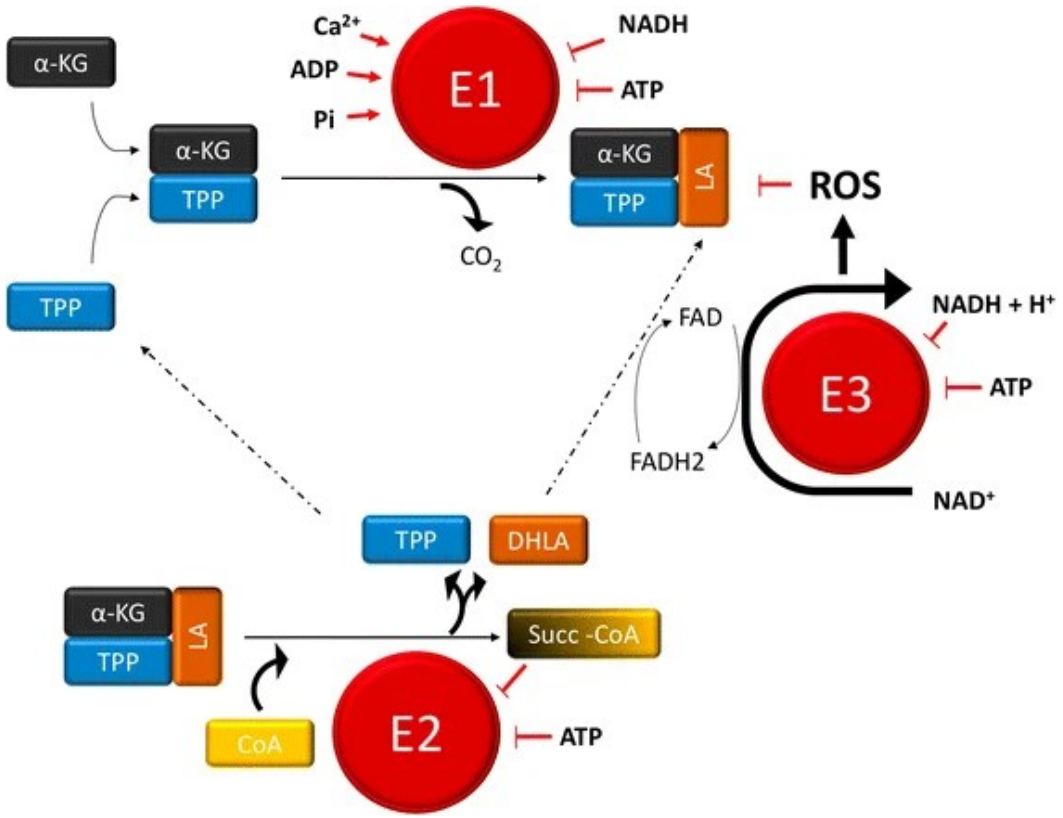




ketoglutarate (KG), serve as a signaling hub that regulates multiple cellular processes:

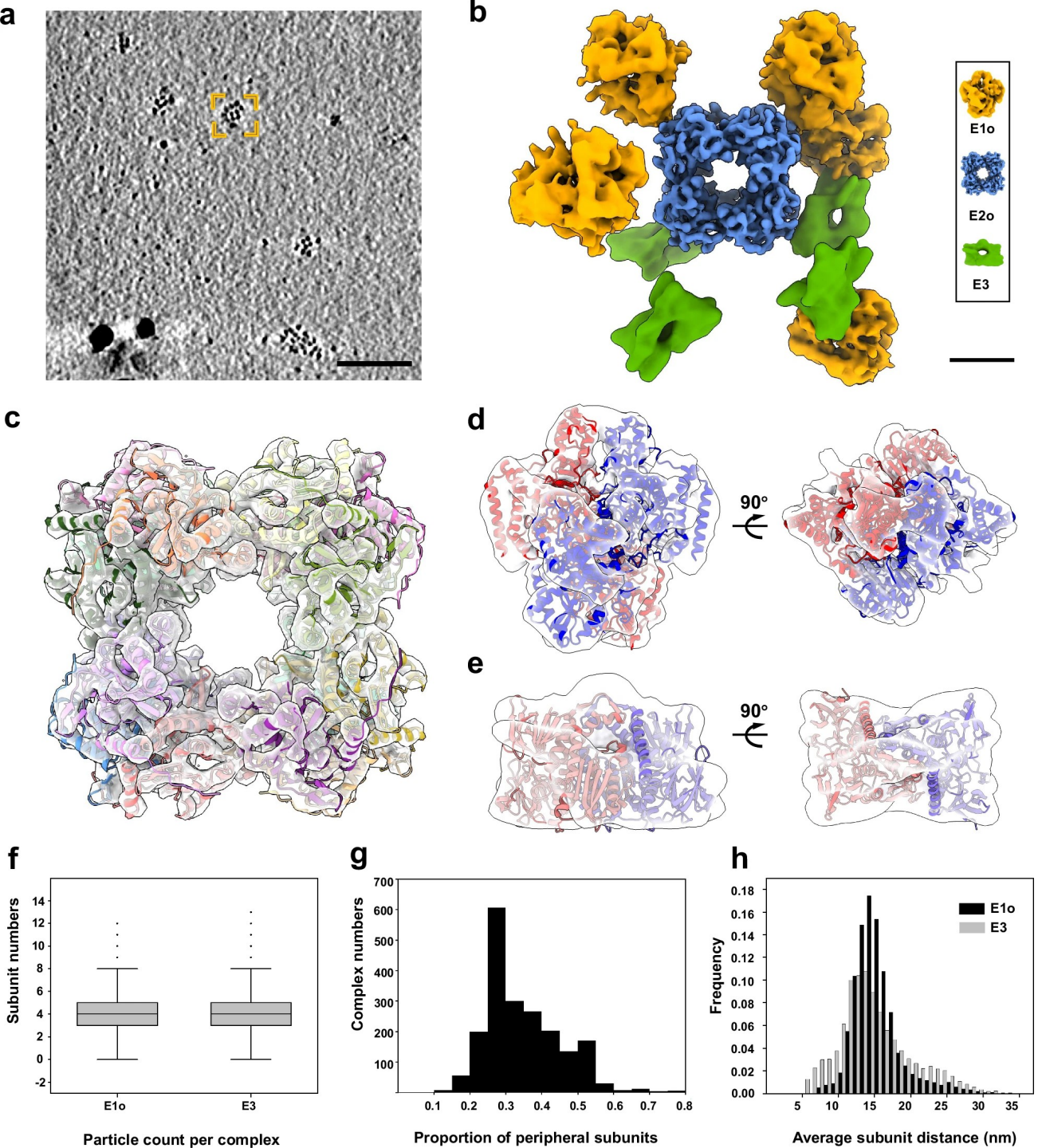
1) is the rate-limiting step of the TCA cycle,
2) is sensitive to reactive oxygen species (ROS) and produces ROS
3) determines whether KG is used for energy or synthesis of compounds to support growth
4) regulates the cellular responses to hypoxia
5) controls the post-translational modification of hundreds of cell proteins in the mitochondria, cytosol, and nucleus through succinylation
6) controls critical aspects of transcription
7) modulates protein signaling within cells
8) modulates cellular calcium.

a-ketoglutarate dehydrogenase

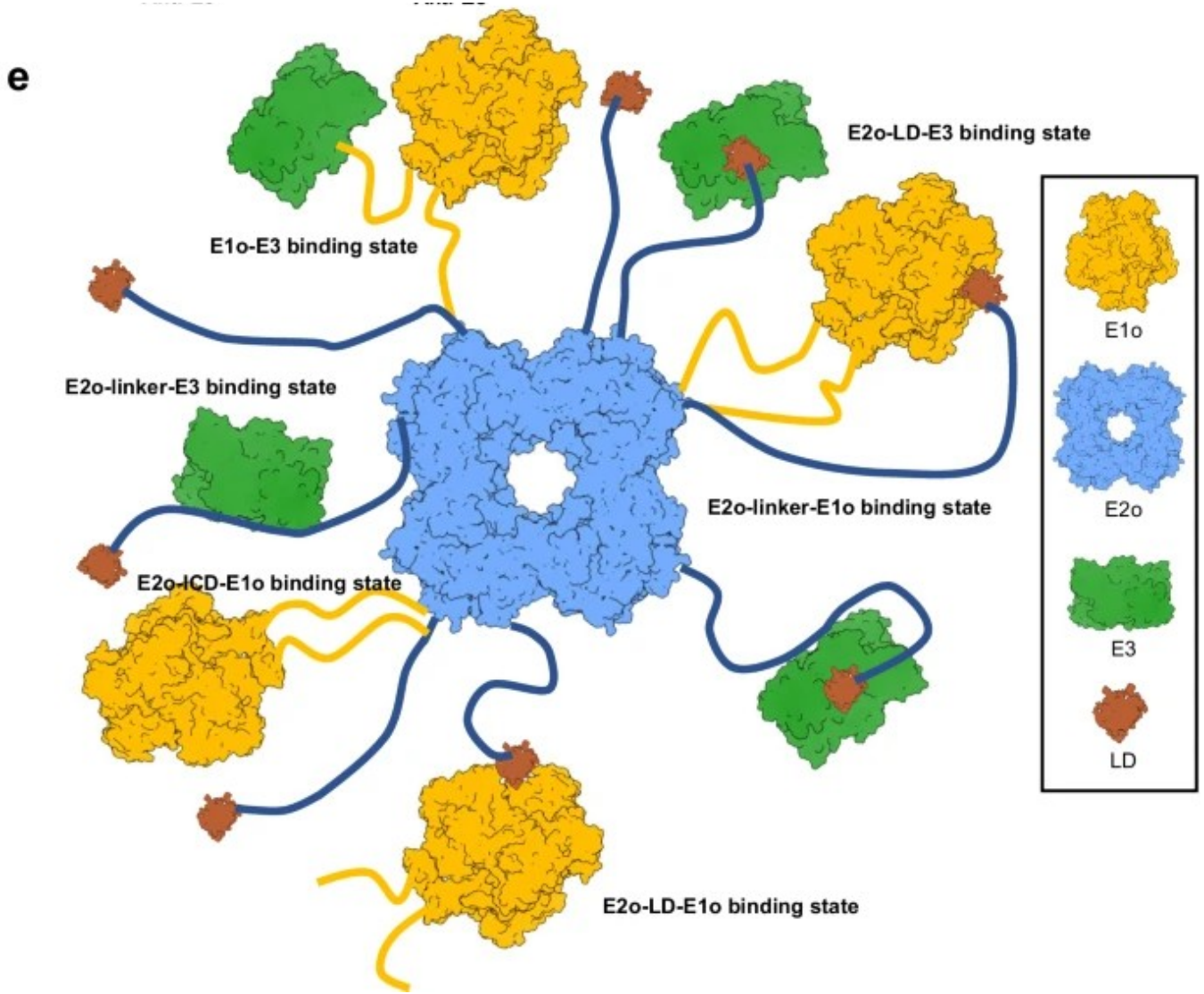


Its E1 and E2 domains are homologous with those of PDH,
E3 domain (which regenerates E2 and therefore does not interact directly with the ketoacid) is identical;
the structure of each OGDHc subunit has been solved, the architecture of the intact complex and inter-subunit interactions still remain unknown

a-ketoglutarate dehydrogenase



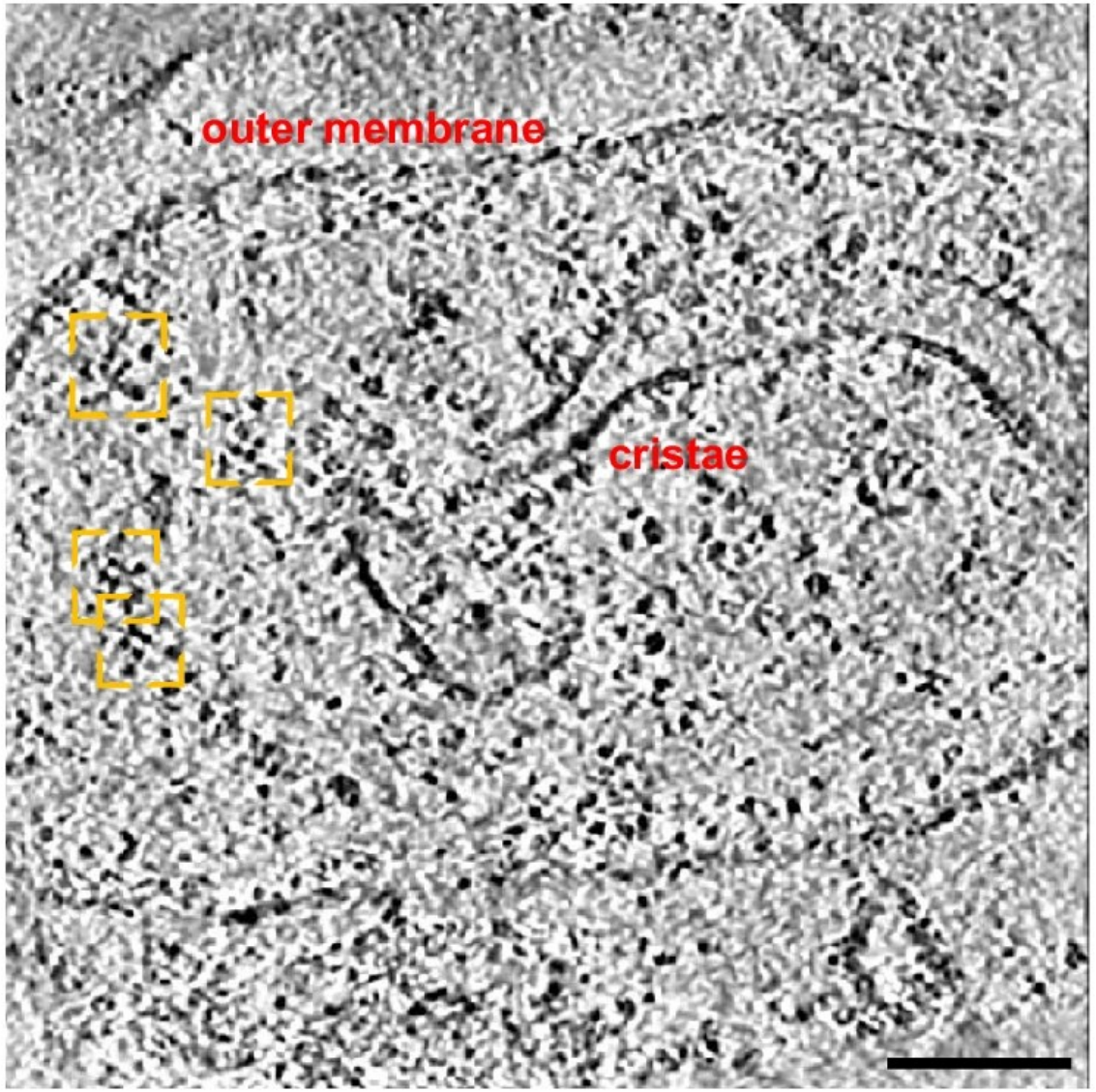
Nature Communications
volume 15
Article number: 8407 (2024)



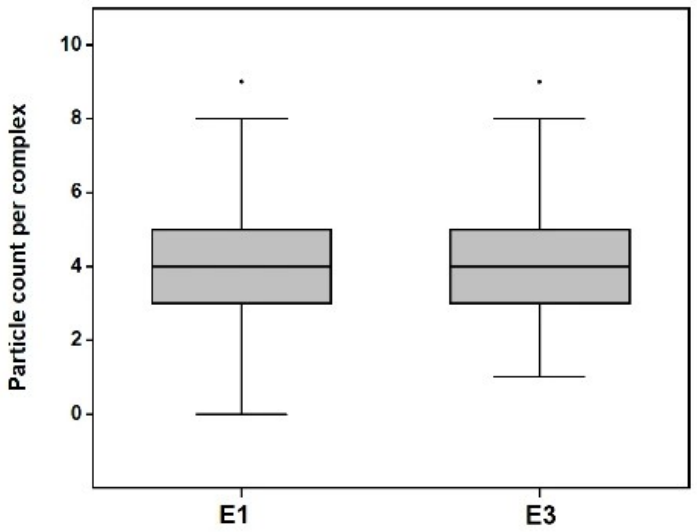
(i) E1o-E2o_linker binding state
(ii) E1o-E2o_ICD binding state
(iii) E1o-E2o_LD binding state
(iv) E3-E2o_liker binding state
(v) E3-E2o_LD binding state
(vi) E1o-E3 binding state.

Nature Communications
volume 15
Article number: 8407 (2024)

a-ketoglutarate dehydrogenase



b



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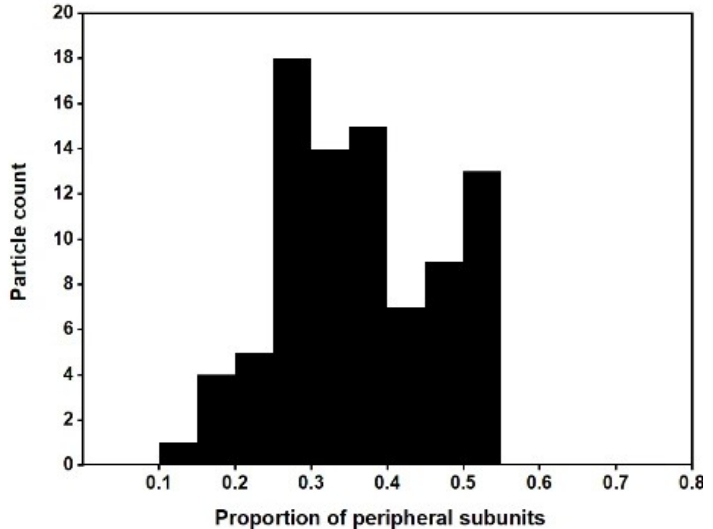
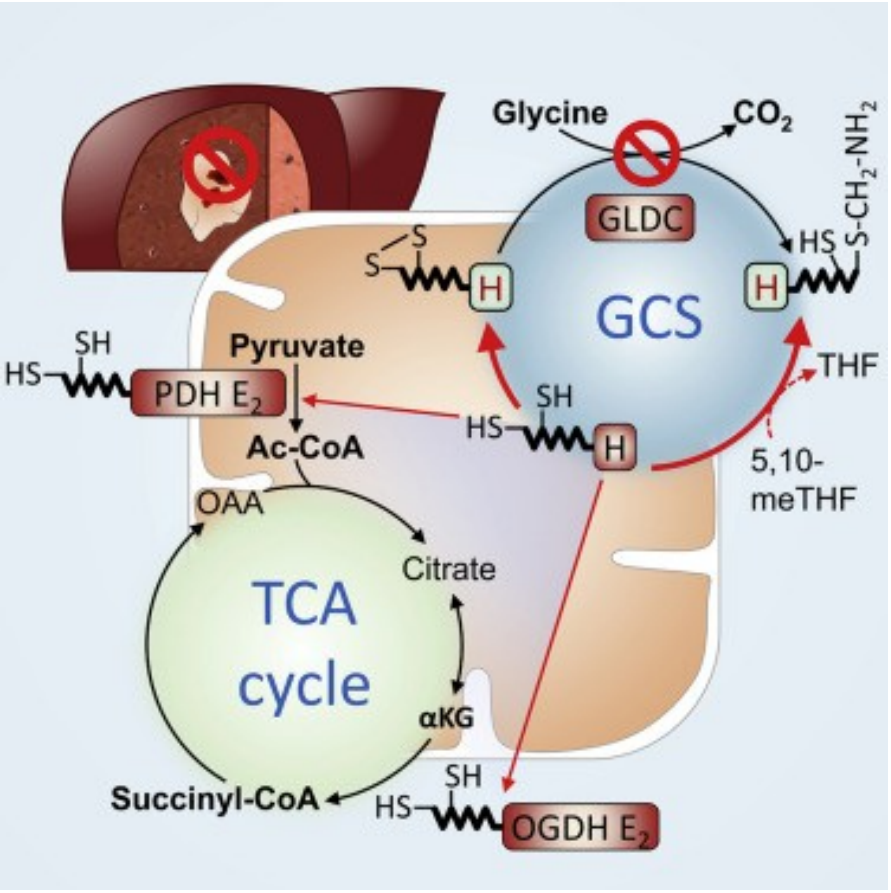


glycine decarboxylase



H, lipoylated H-protein
P, PLP-dependent glycine decarboxylase;
T, a tetrahydrofolate-dependent transferase;
SHMT, a PLP-dependent serine hydroxymethyl transferase
H4FGlu*n*, 5,6,7,8-tetrahydrofolate

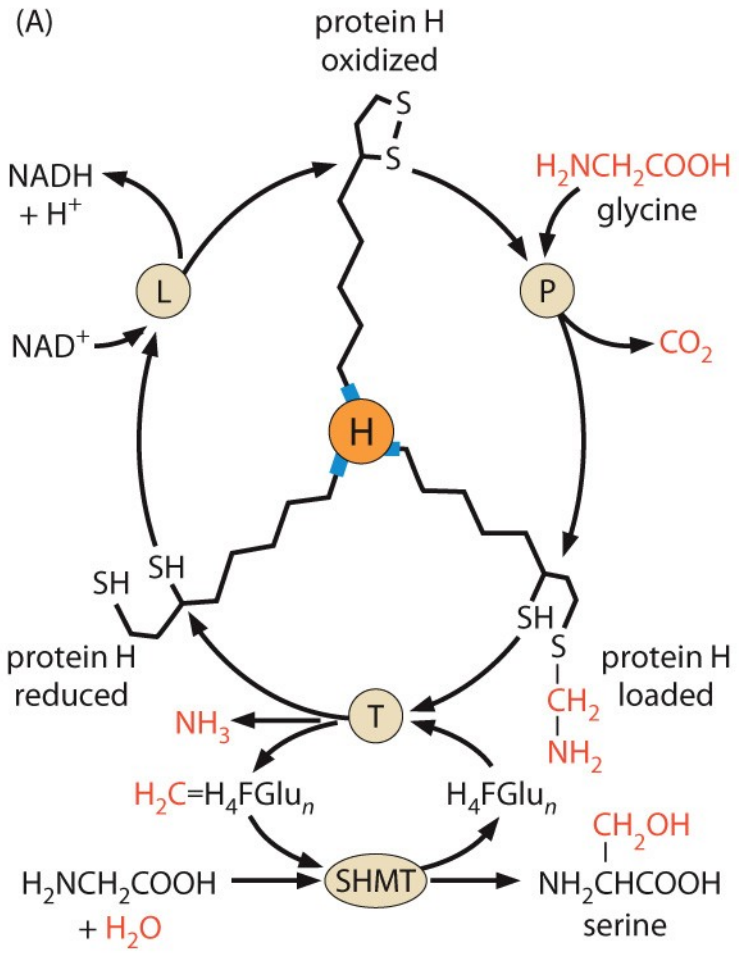
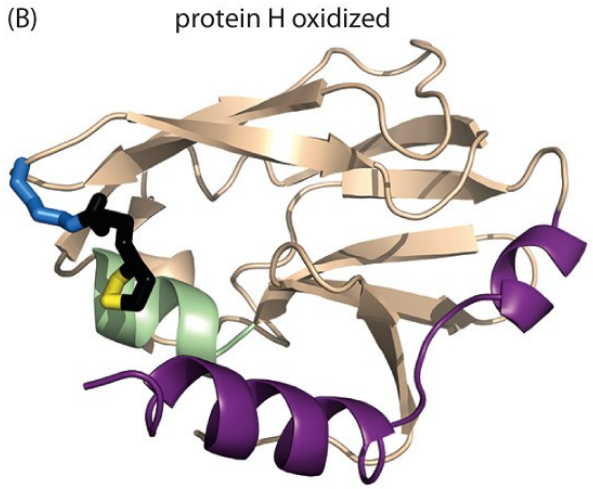
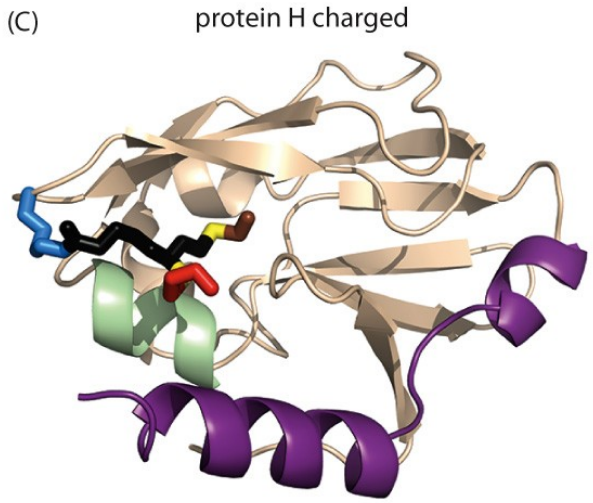
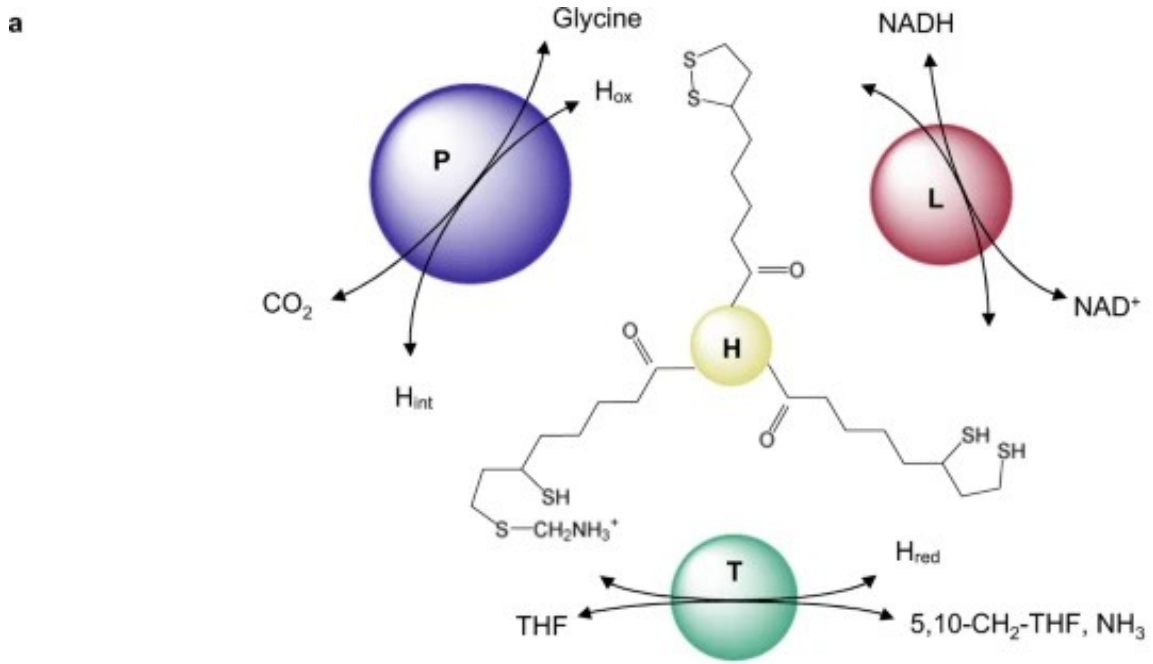


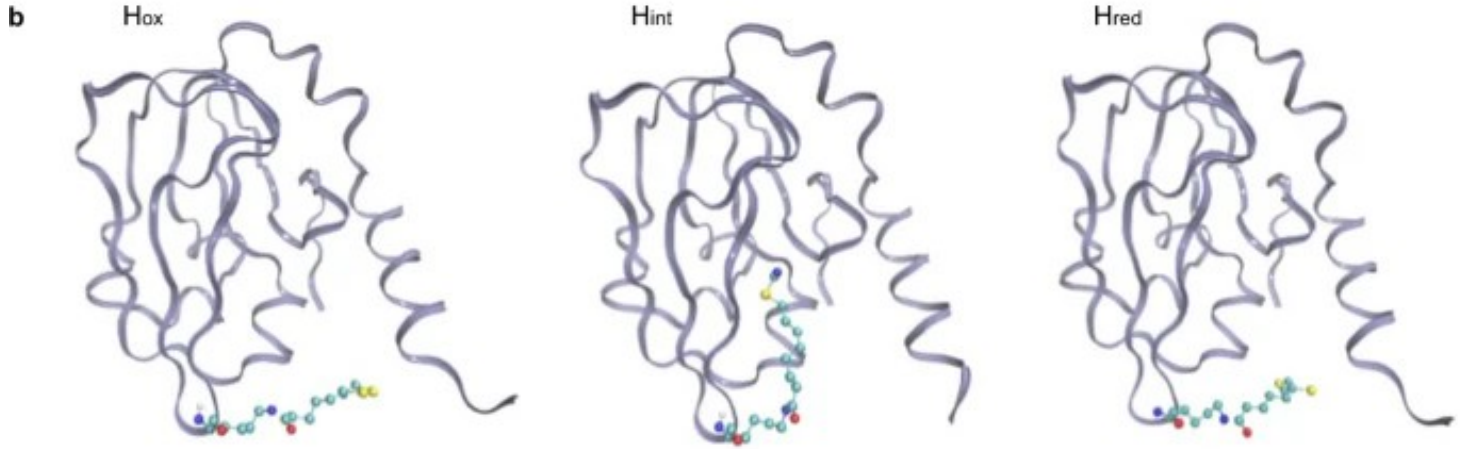
Figure 9.9 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

glycine decarboxylase

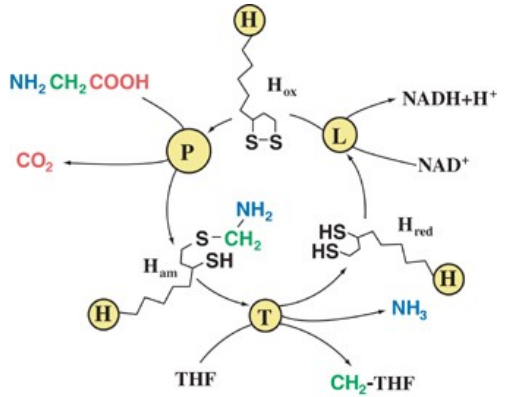


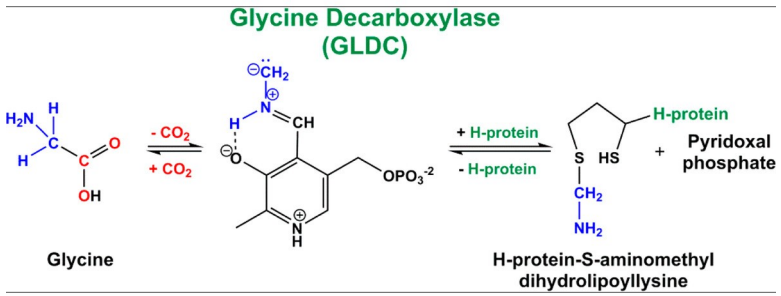


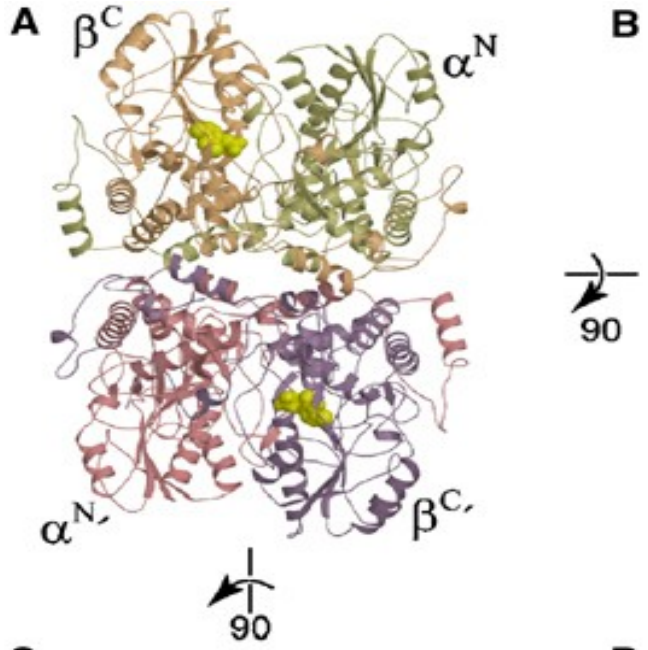


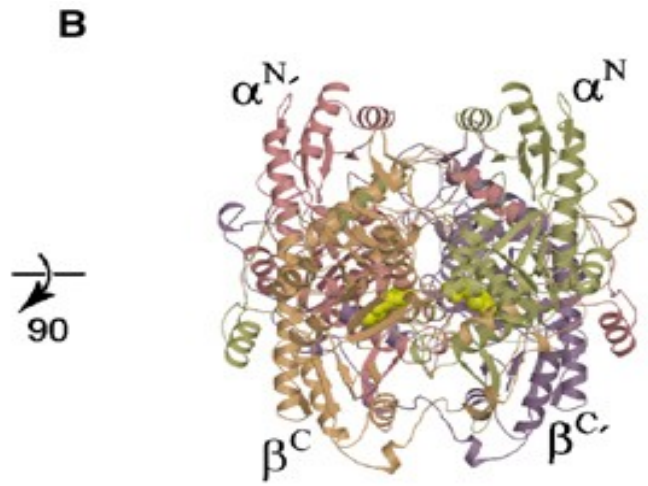


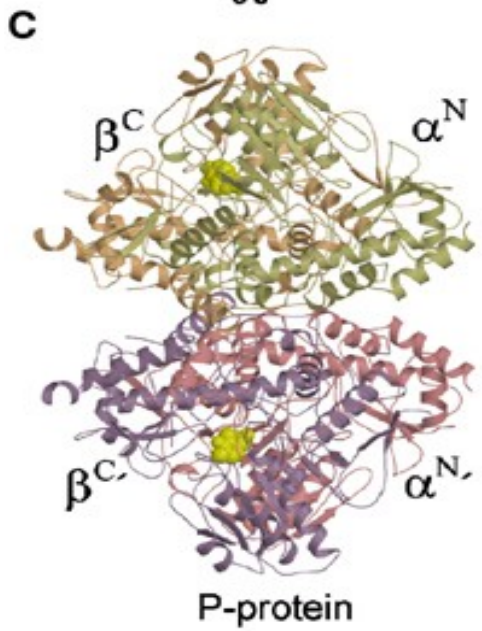
COMMUNICATIONS BIOLOGY | https://doi.org/10.1038/s42003-020-01401-6

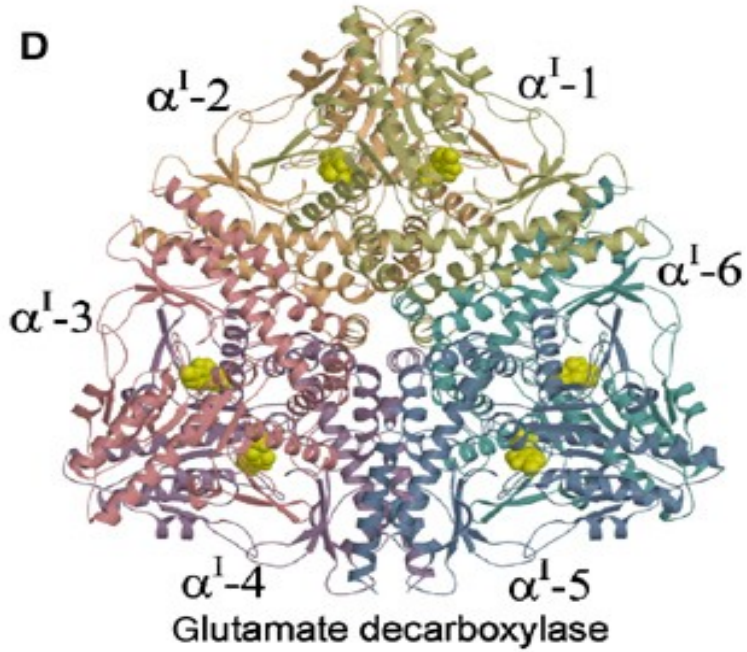






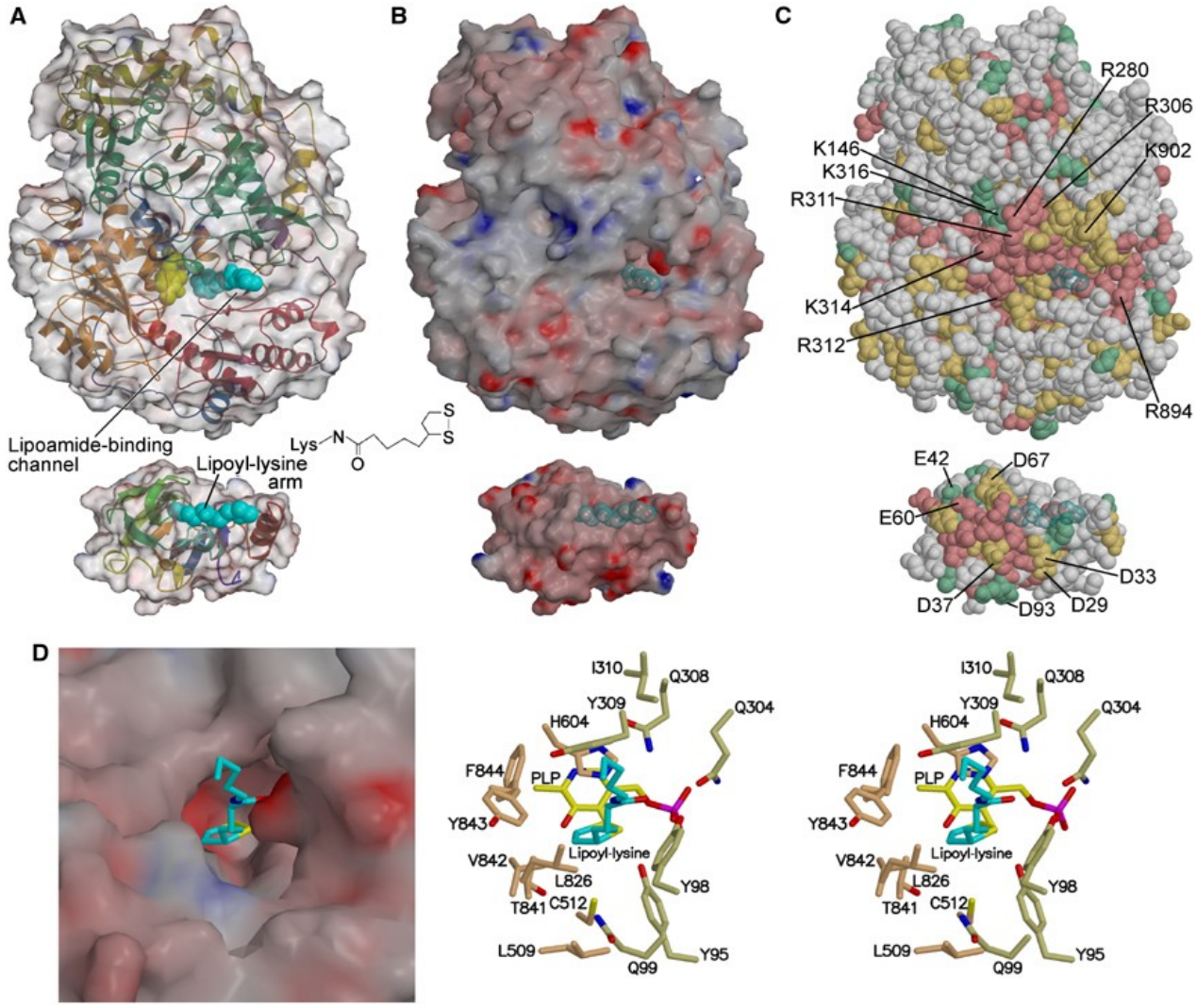






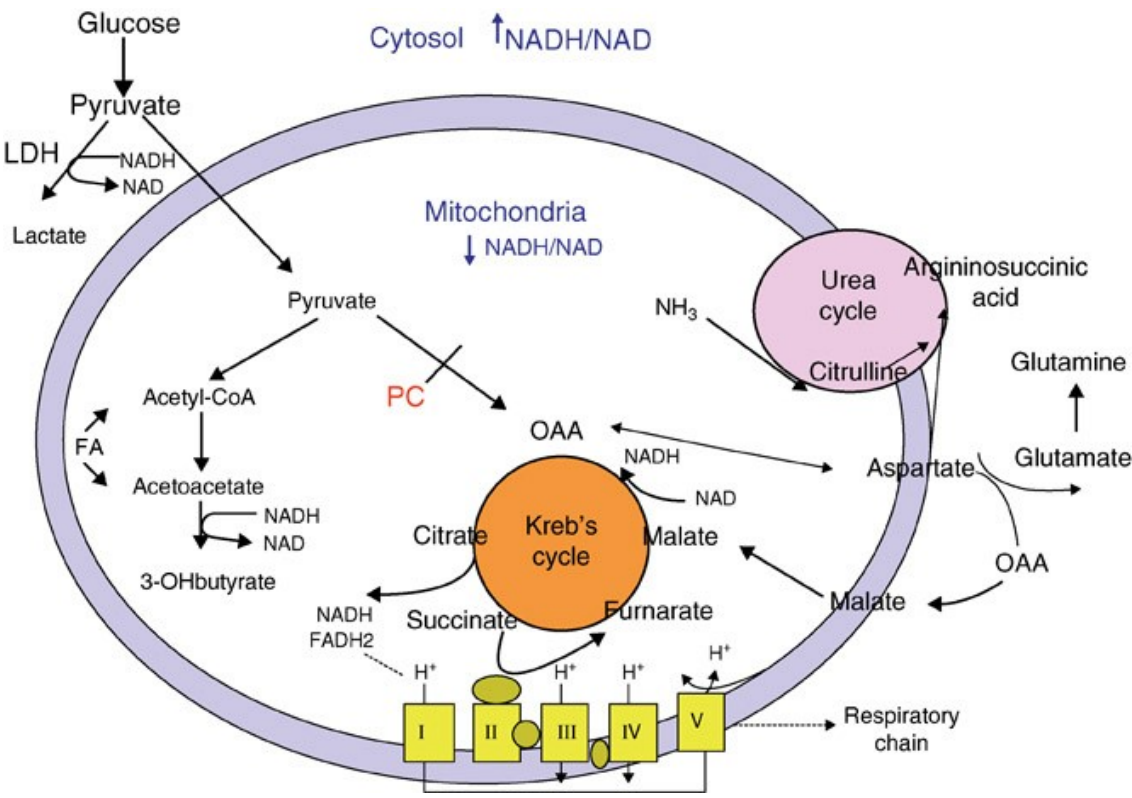
The EMBO Journal, Volume: 24, Issue: 8, Pages: 1523-1536, First published: 24 March 2005, DOI: (10.1038/sj.emboj.7600632)

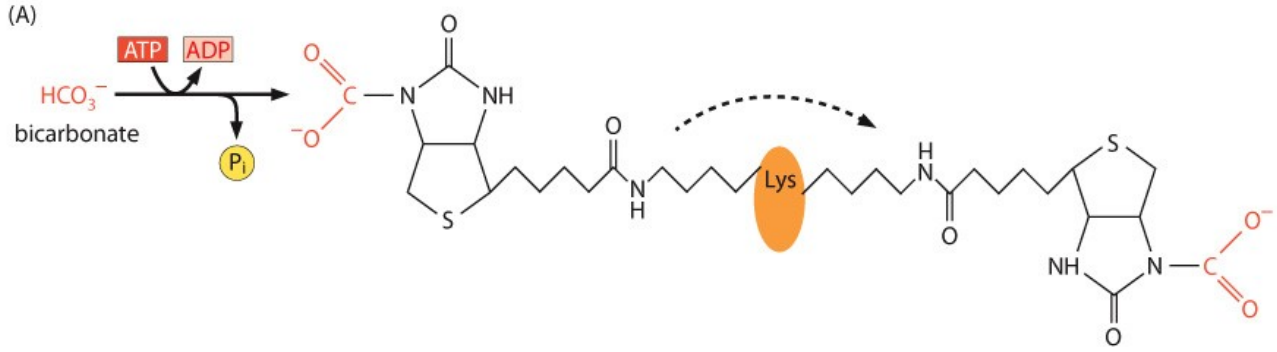
Structure of P□protein of the glycine cleavage system: implications for nonketotic hyperglycinemia

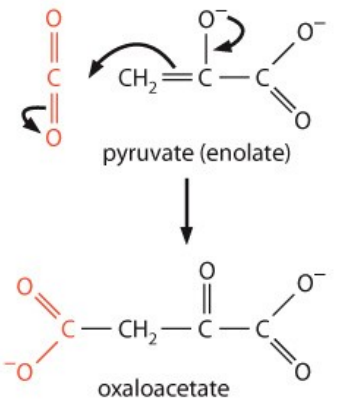


The EMBO Journal, Volume: 24, Issue: 8, Pages: 1523-1536, First published: 24 March 2005, DOI: (10.1038/sj.emboj.7600632)

pyruvate carboxylase



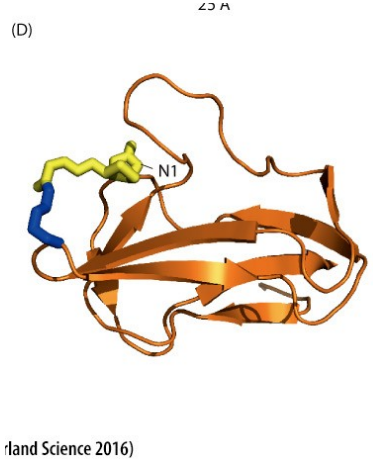




carboxylase domain — BCCP domain, potential arm span of ~30 Å — carboxyltransferase domain

pyruvate carboxylase

BC, biotin carboxylase; BCCP, biotin carboxy carrier protein; AD, allosteric domain; CT, carboxyltransferase domain



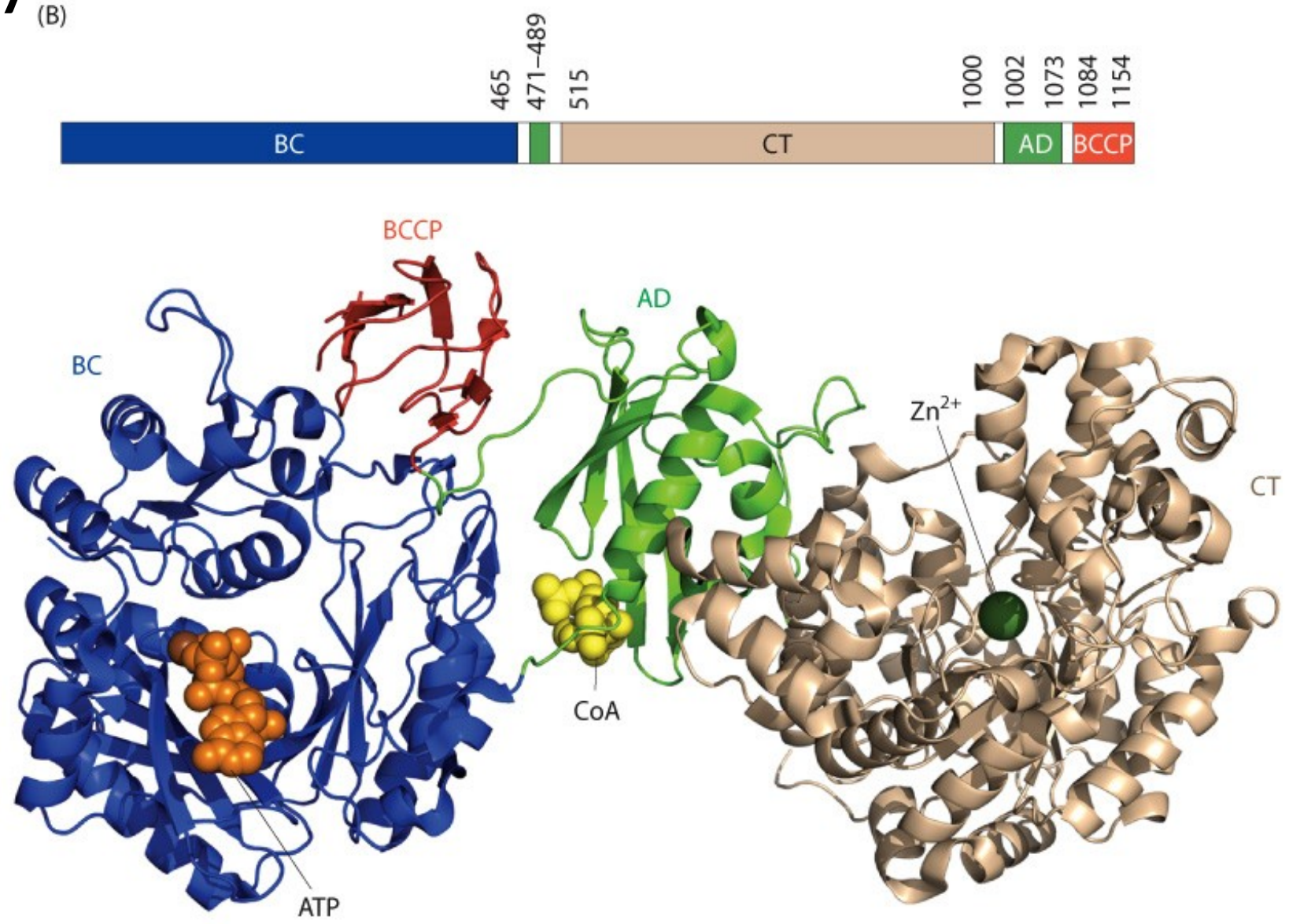


Figure 9.10 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

pyruvate carboxylase

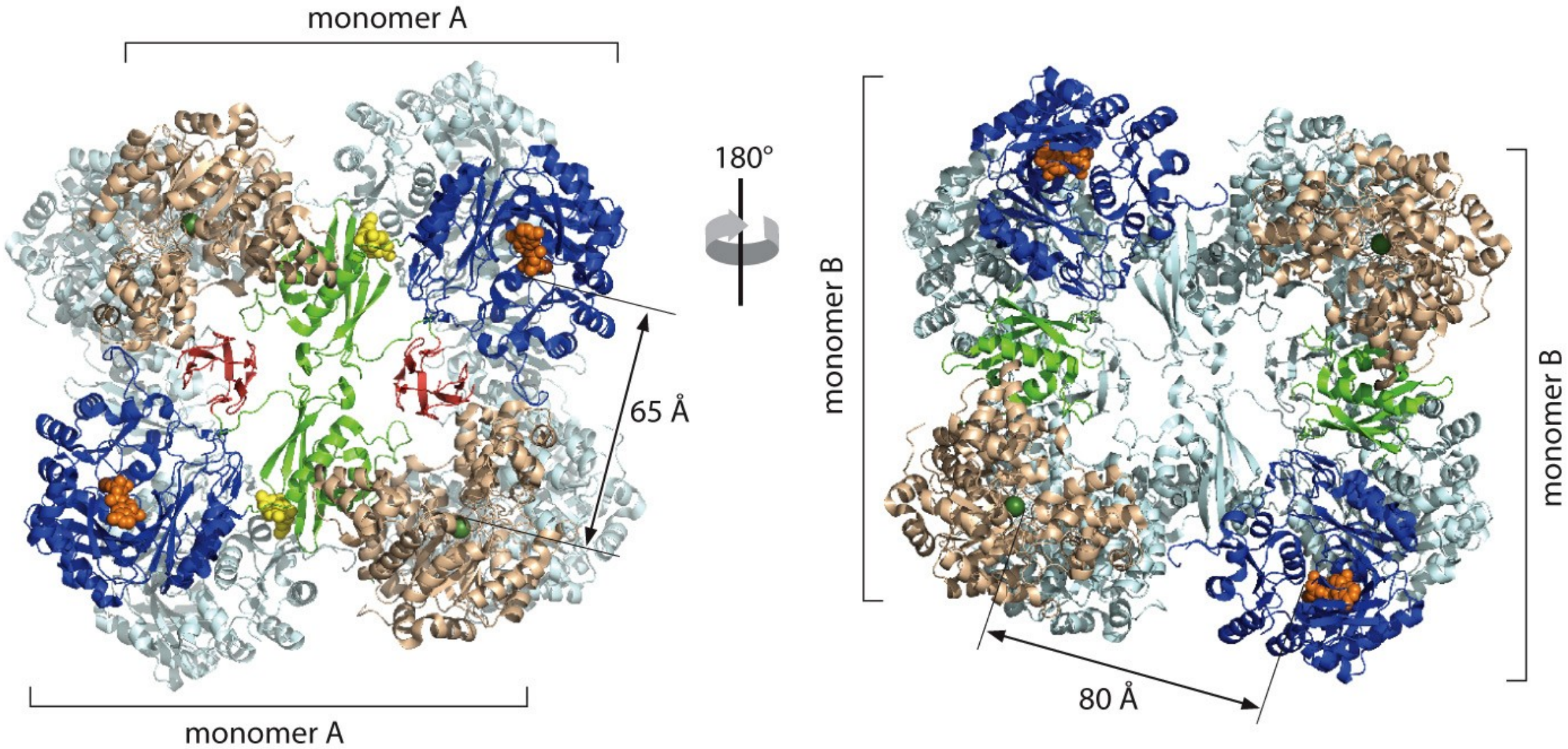
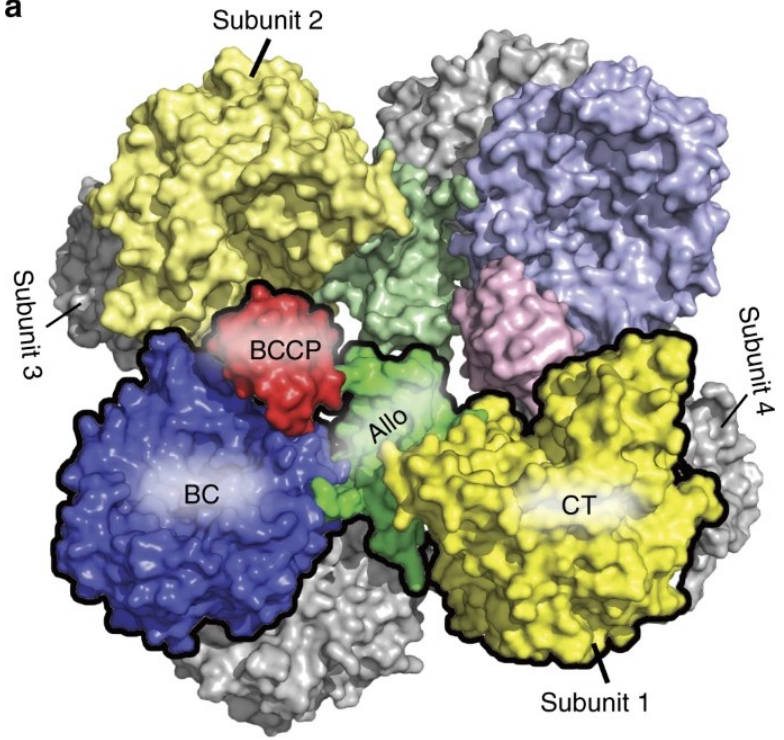
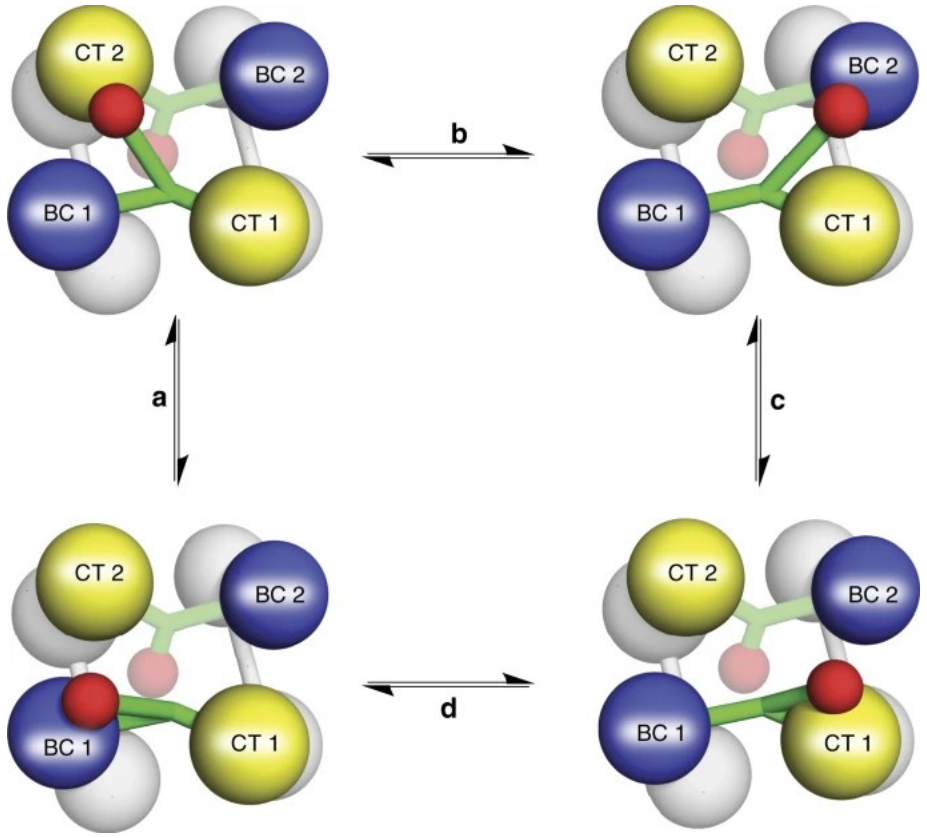


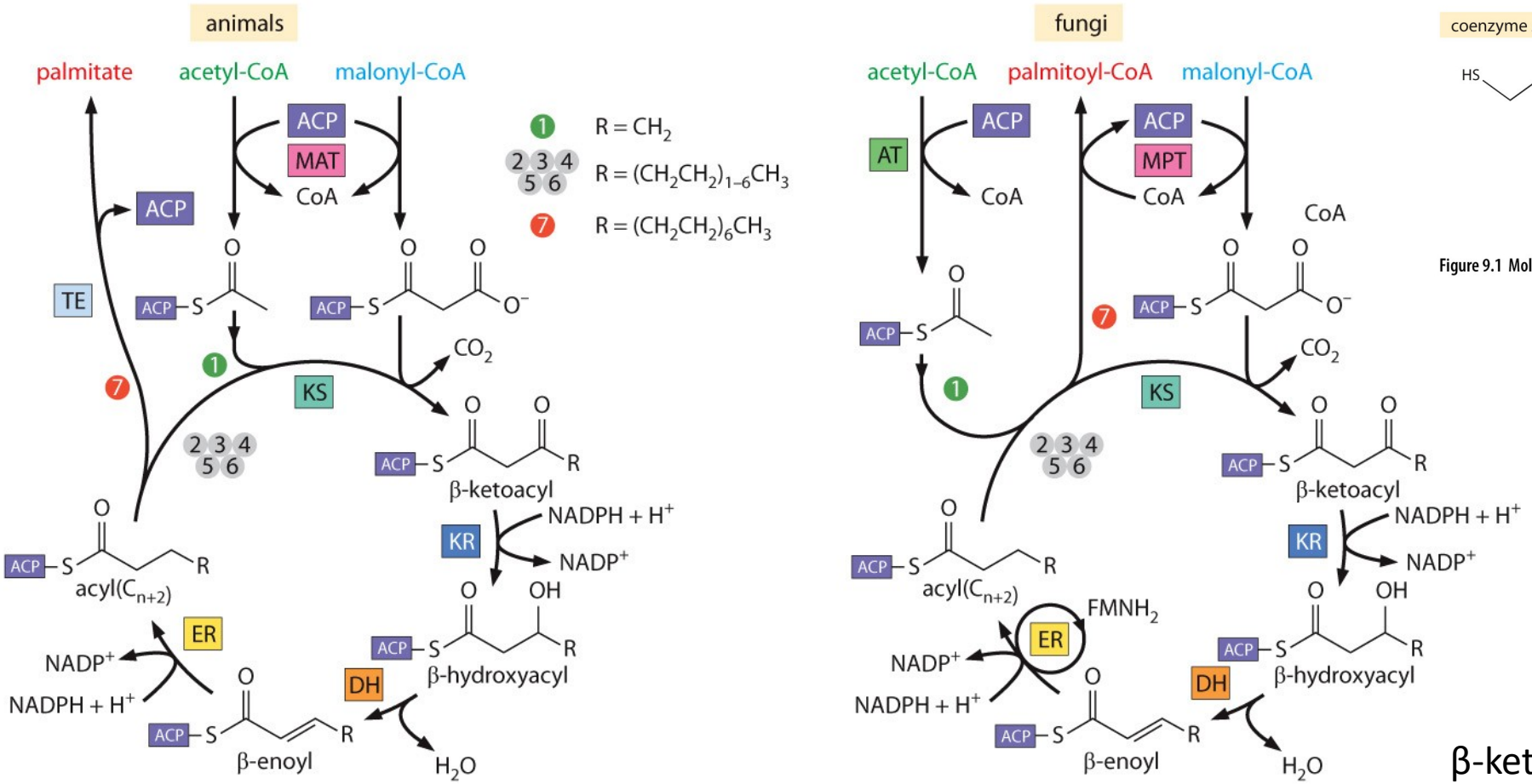
Figure 9.11 Molecular Biology of Assemblies and Machines (© Garland Science 2016)





Nature Communications **volume 9**,
Article number: 1384 (2018

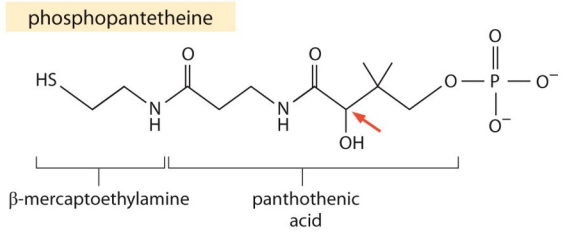
fatty acid synthases



$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO^-$

palmitate

Figure 9.13 Molecular Biology of Assemblies and Machines (© Garland Science 2016)



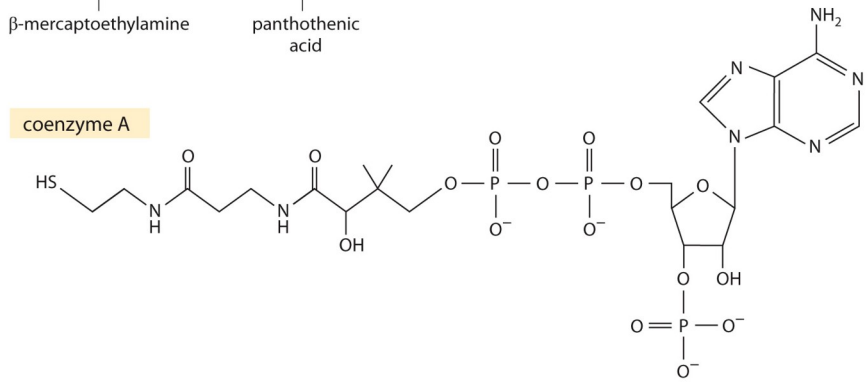


Figure 9.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

β-ketoacyl-ACP synthase (KS)
β-ketoacyl-ACP reductase (KR)
β-hydroxyacyl-ACP dehydratase (DH)
β-enoyl-ACP reductase (ER)

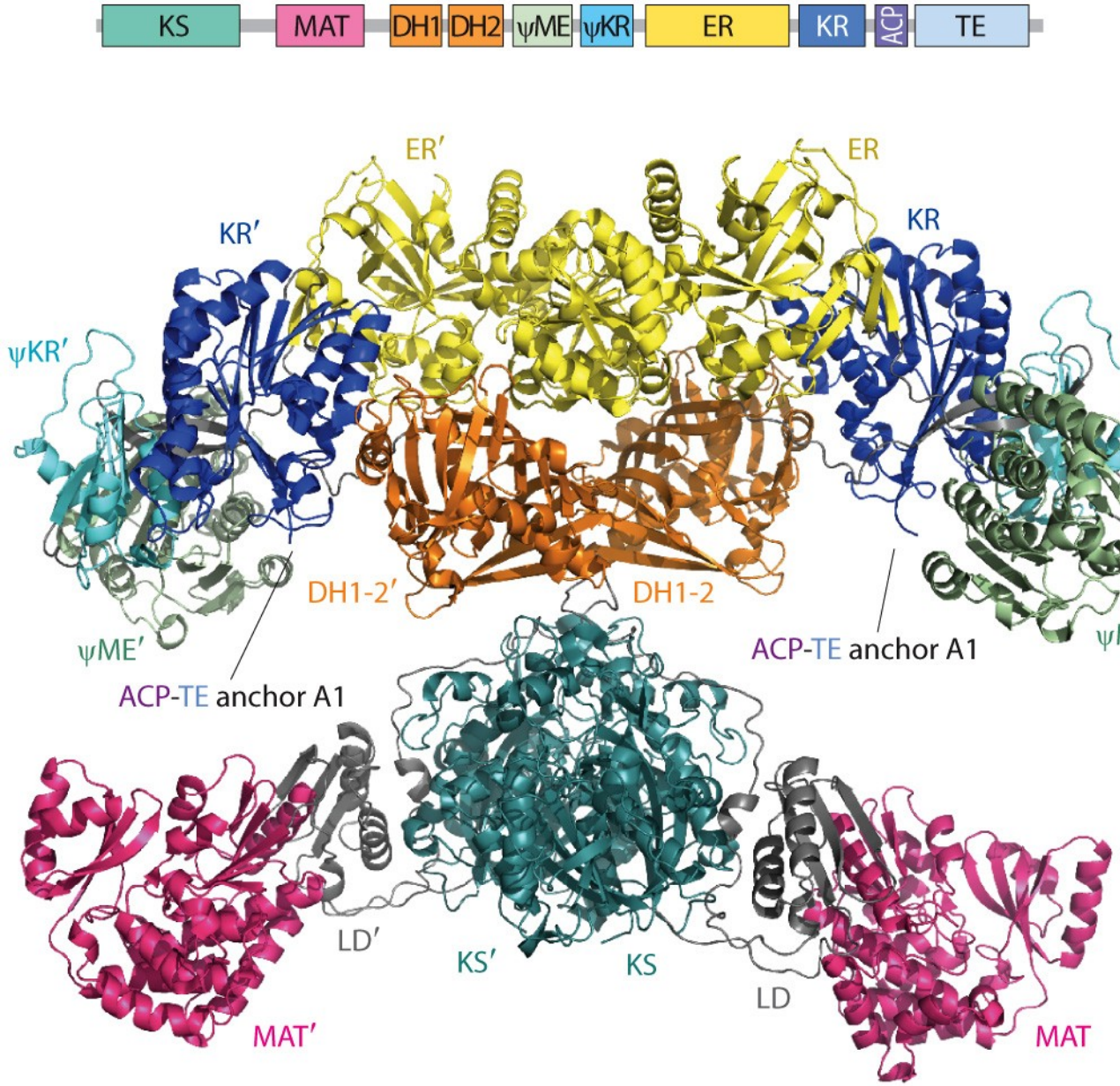


Figure 9.14 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

animal FAS

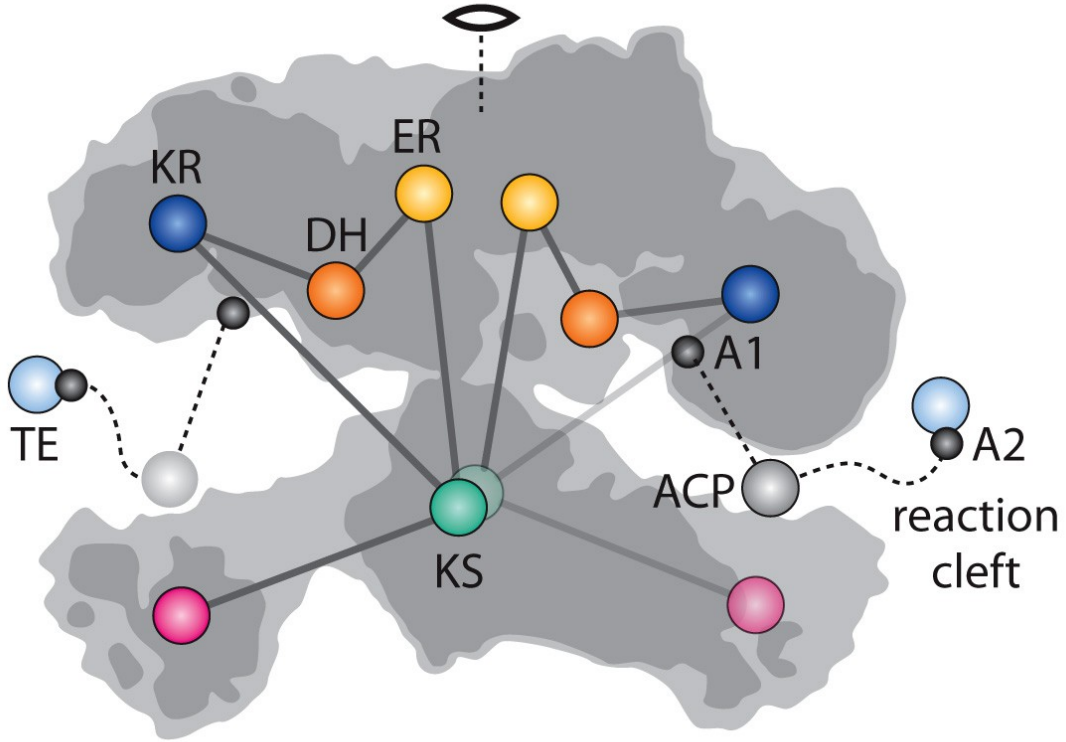


Figure 9.17a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Structure of ACP and the interaction with KS

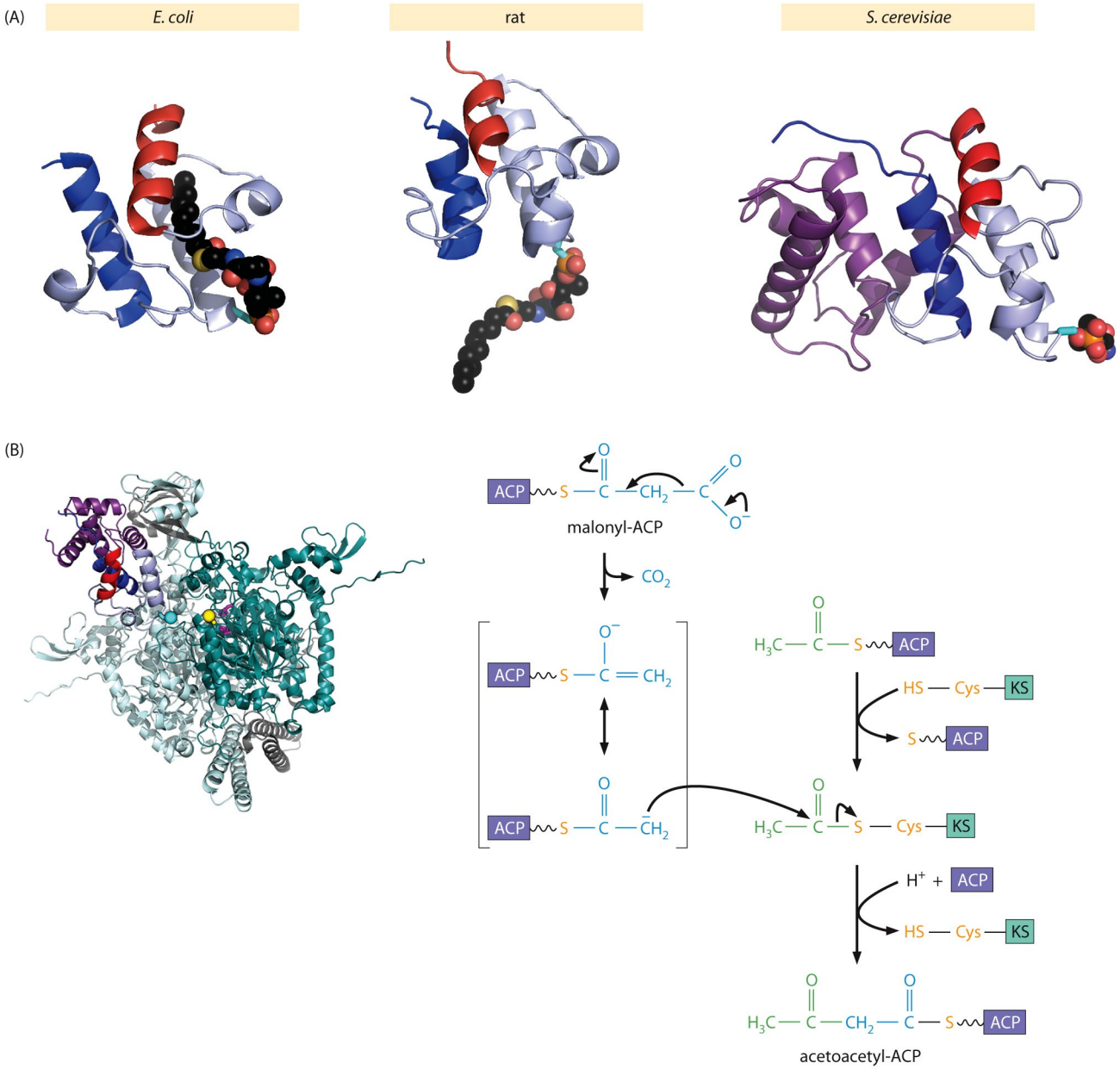
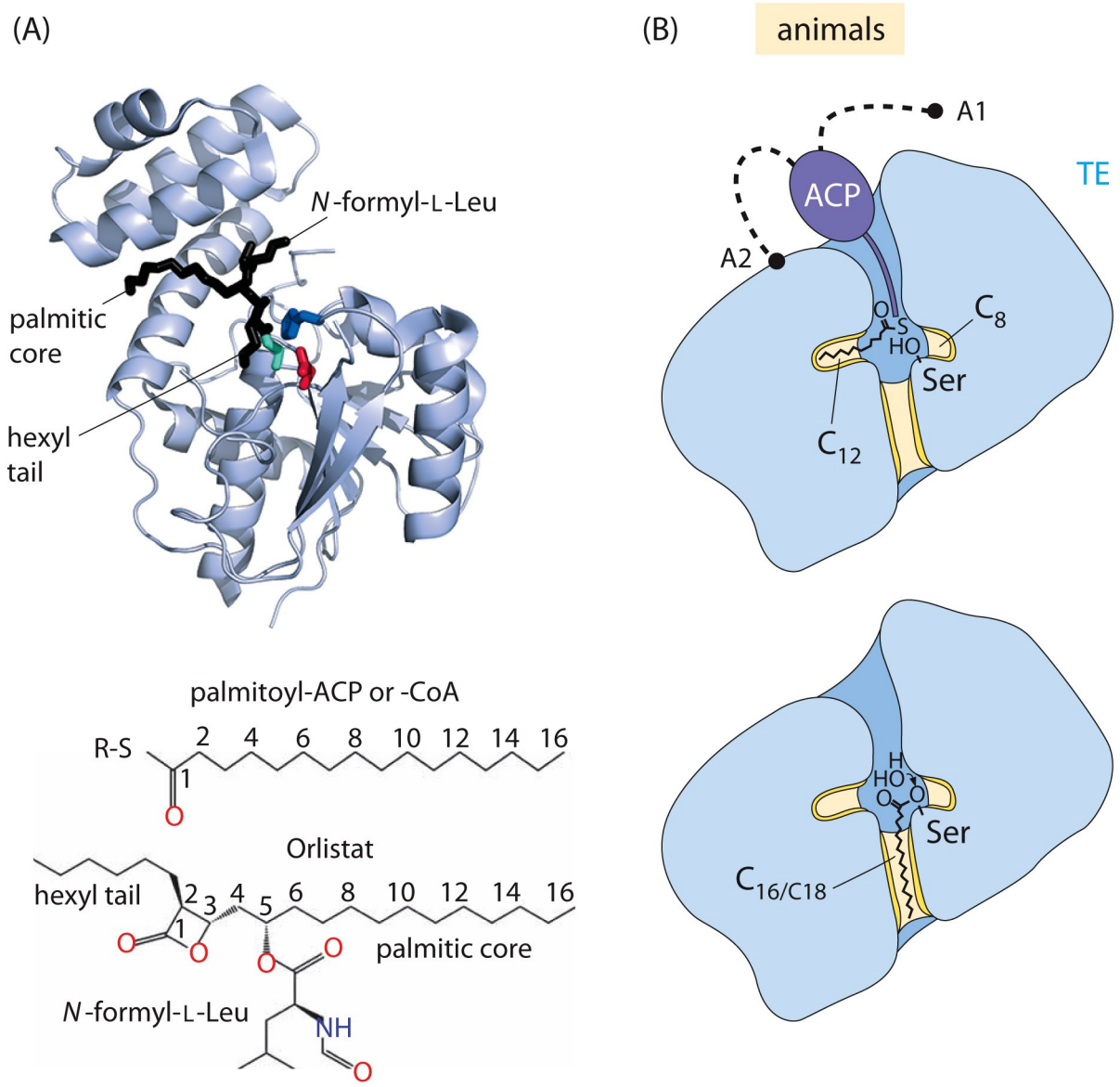


Figure 9.18 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Determination of fatty acyl chain length



Thioesterase domain

Figure 9.19 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Fatty acid degradation

There are four ACDs that differ in their specificity for the length of the acyl group

ACD, FAD-dependent fatty acyl-CoA dehydrogenase
ECH, enoyl-CoA hydratase
HACD, NAD_+-dependent hydroxyacyl-CoA dehydrogenase
KACT, ketoacyl-CoA thiolase.

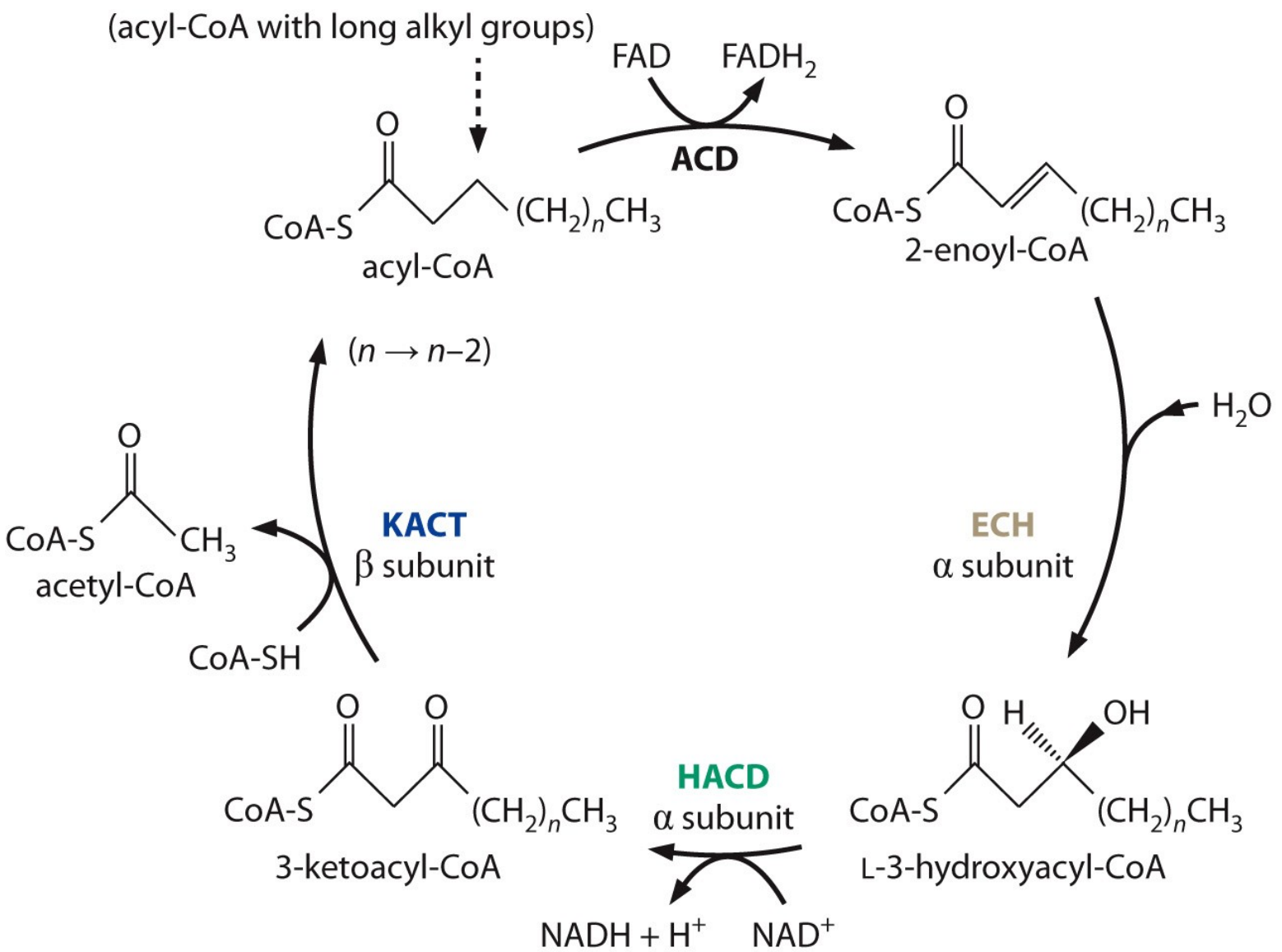
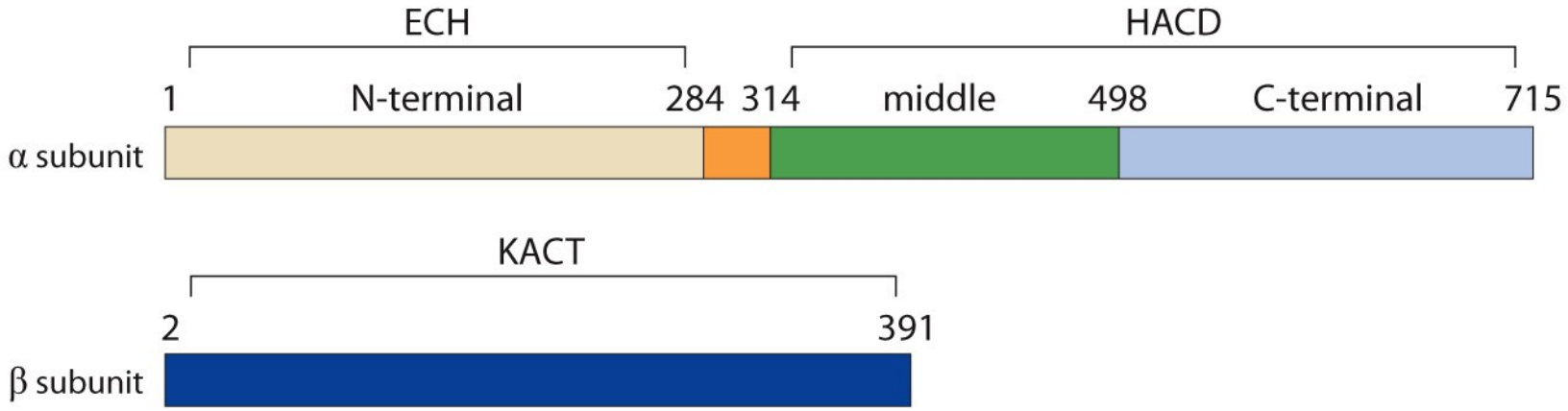


Figure 9.20a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

FAO complex

ECH, enoyl-CoA hydratase;
HACD, NAD_+-dependent hydroxyacyl-CoA dehydrogenase;
KACT, ketoacyl-CoA thiolase.



In animals, the FAO complex is an $\alpha_4\beta_4$ heterooctamer,
in bacteria it is an $\alpha_2\beta_2$ heterotetramer.

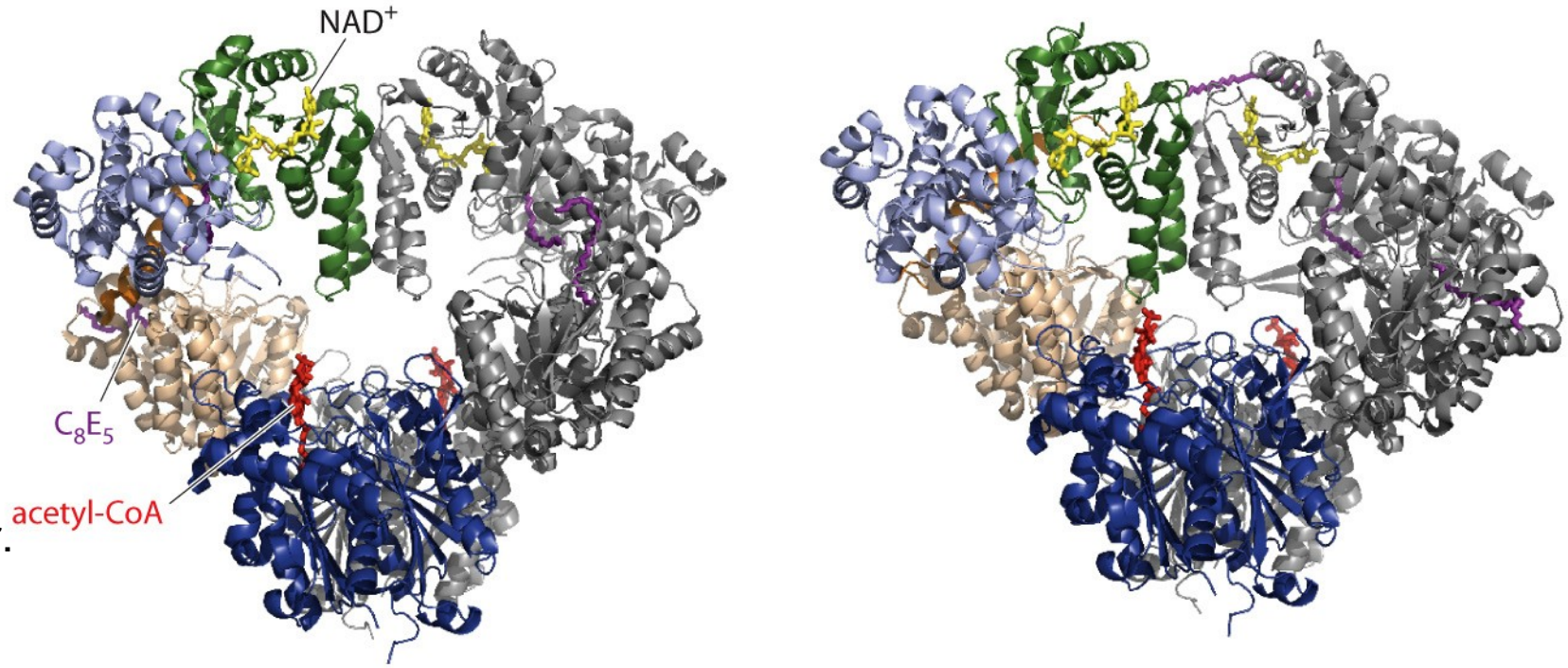
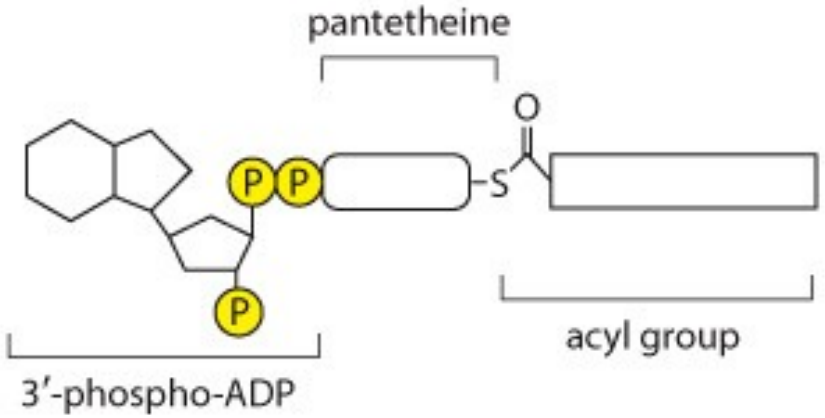


Figure 9.20b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Substrate channeling in FAO complex



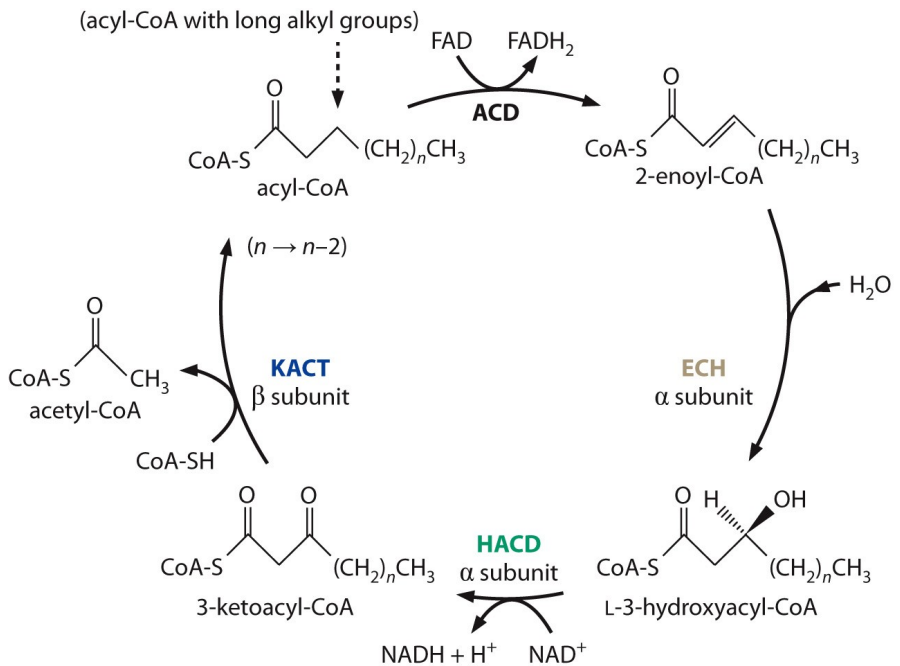


Figure 9.20a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

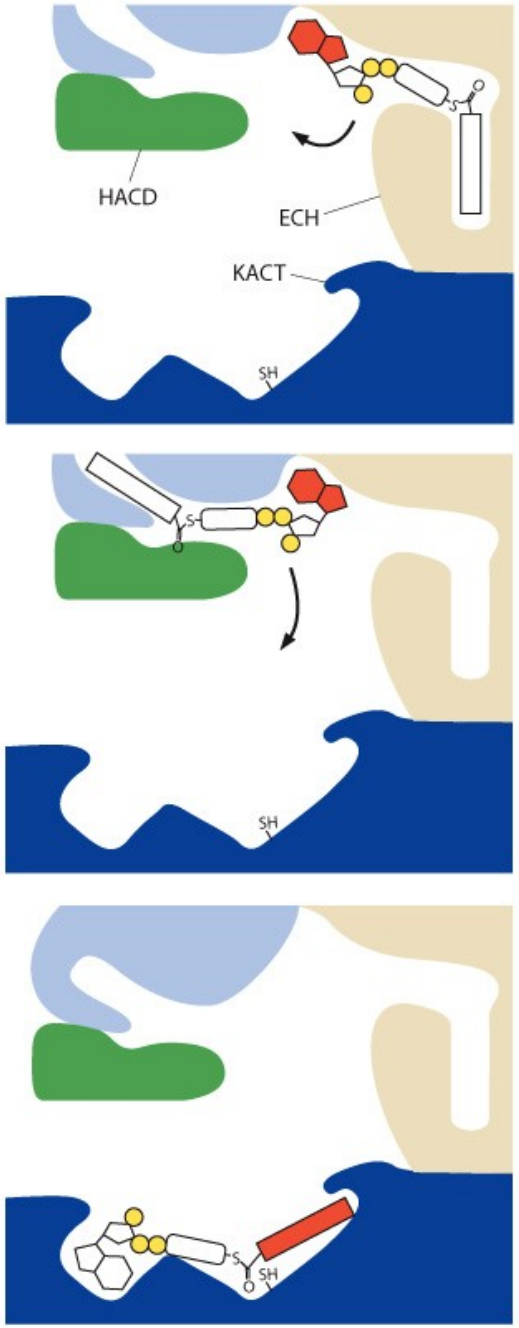
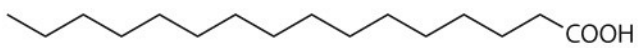
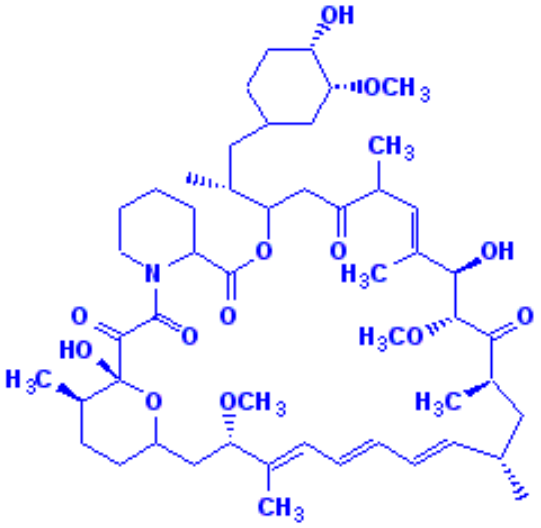


Figure 9.21 Molecular Biology of Assemblies and

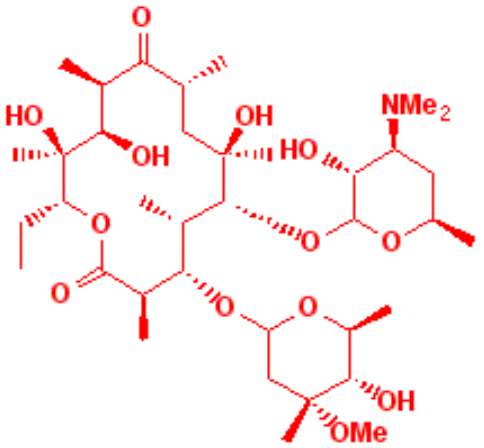
polyketide synthases

palmitic acid

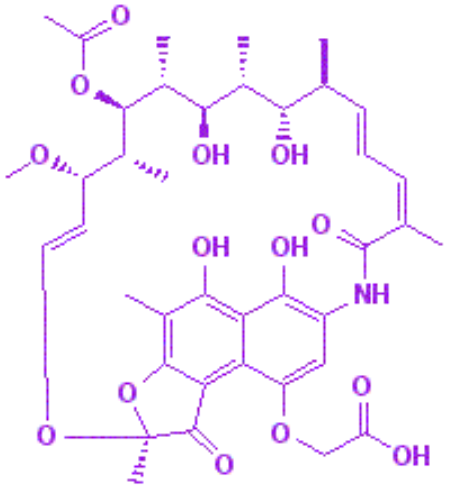




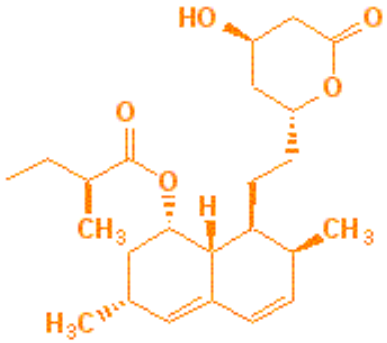
rapamycin
(immunosuppressant)



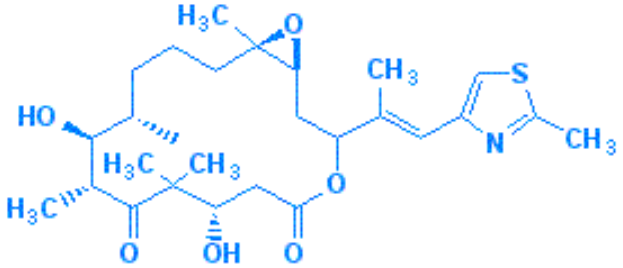
erythromycin A
(antibacterial)



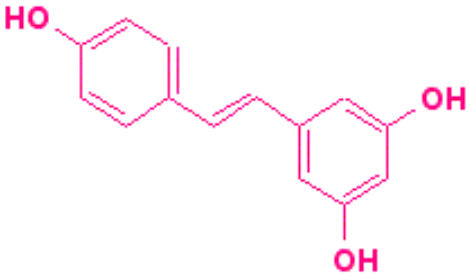
rifamycin B
(antituberculosis)



lovastatin
(anticholesterol)



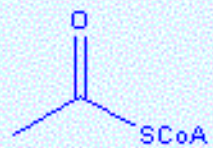
epothilone B
(anticancer)



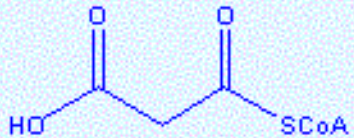
(E)-resveratrol
(chemopreventive)

STARTER AND EXTENDER UNITS

FAS

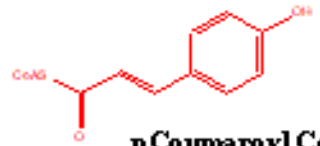


Acetyl CoA



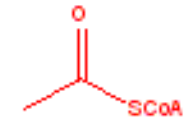
Malonyl CoA

PKS

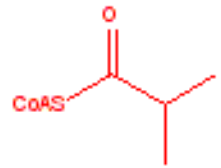


p Coumaroyl CoA

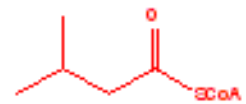
Octanoyl CoA



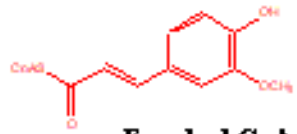
Acetyl CoA



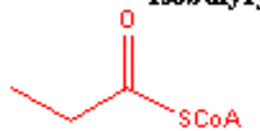
Isobutyryl CoA



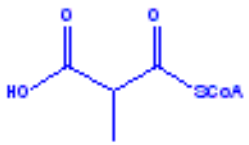
Isovaleryl CoA



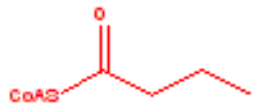
Feruloyl CoA



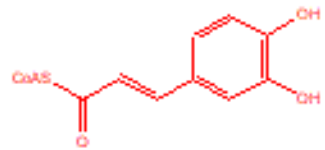
Propionyl CoA



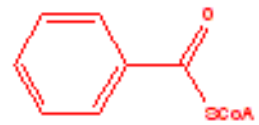
Methylmalonyl CoA



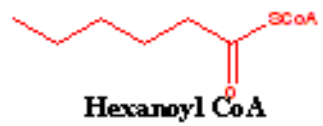
Butyryl CoA



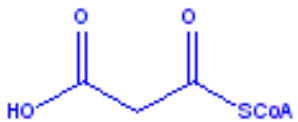
Caffeoyl CoA



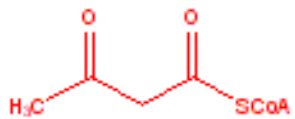
Benzoyl CoA



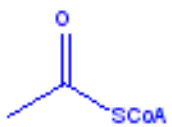
Hexanoyl CoA



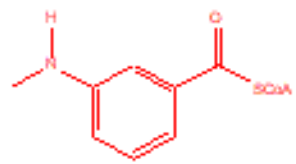
Malonyl CoA



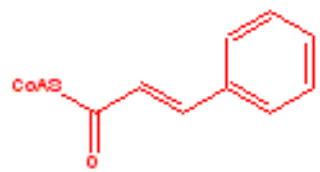
Acetoacetyl CoA



Acetyl CoA



N methy anthraniloyl CoA



Cinnamoyl CoA

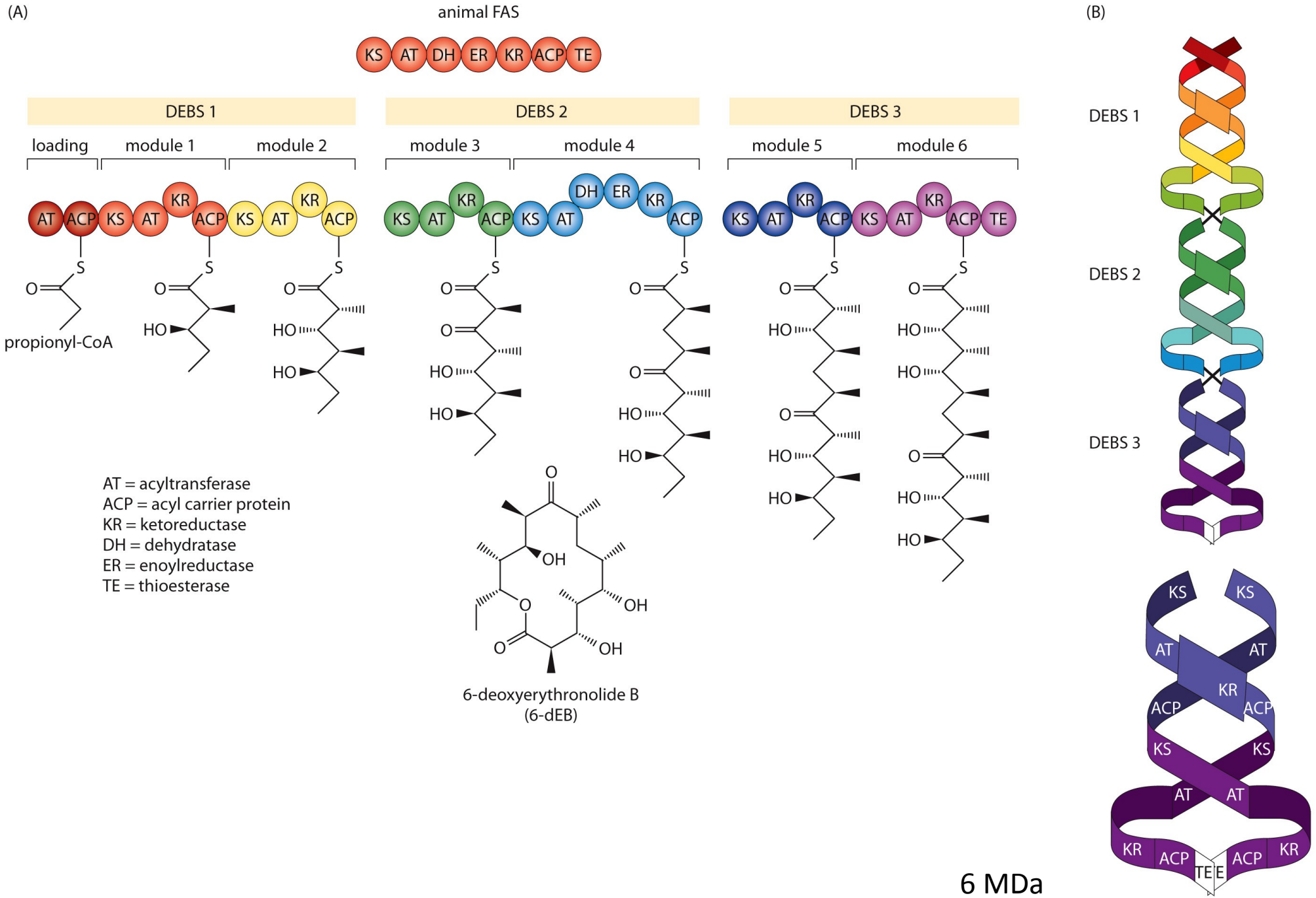


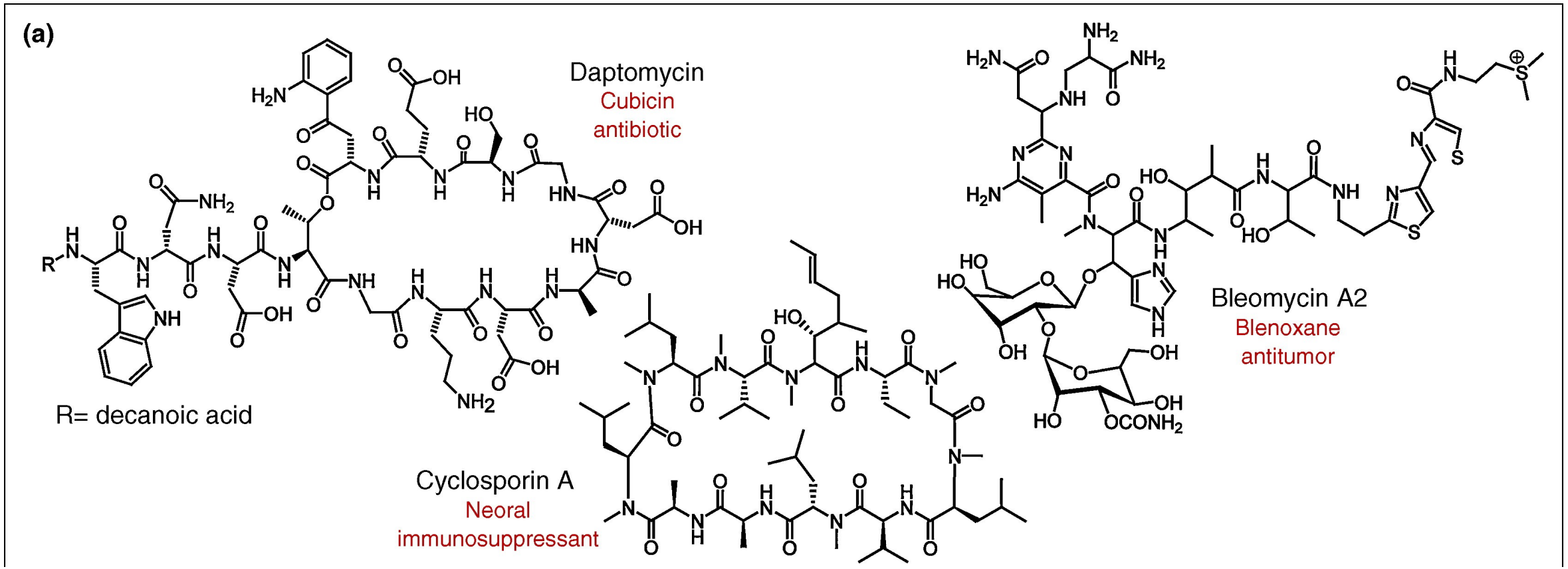
Figure 9.23 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

non-ribosomal peptide synthases

Nonribosomal peptide synthetases (NRPSs) are large multimodular biocatalysts that utilize complex regiospecific and stereospecific reactions to assemble structurally and functionally diverse peptides that have important medicinal applications.

During this ribosome-independent peptide synthesis, catalytic domains of NRPS select, activate or modify the covalently tethered reaction intermediates to control the iterative chain elongation process and product release.

non-ribosomal peptide synthases



https://doi.org/10.1016/j.sbi.2010.01.009

non-ribosomal peptide synthases