# 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

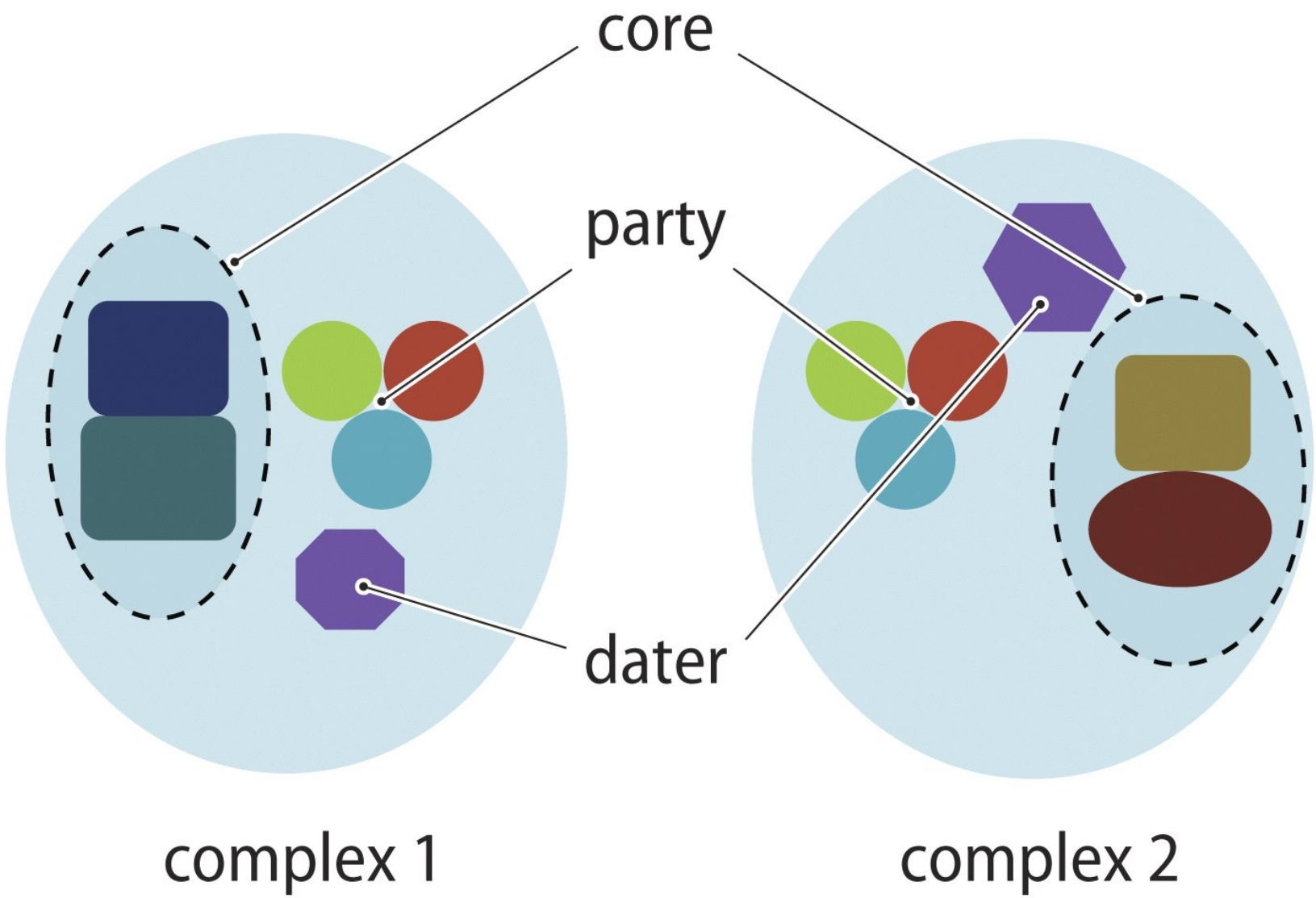
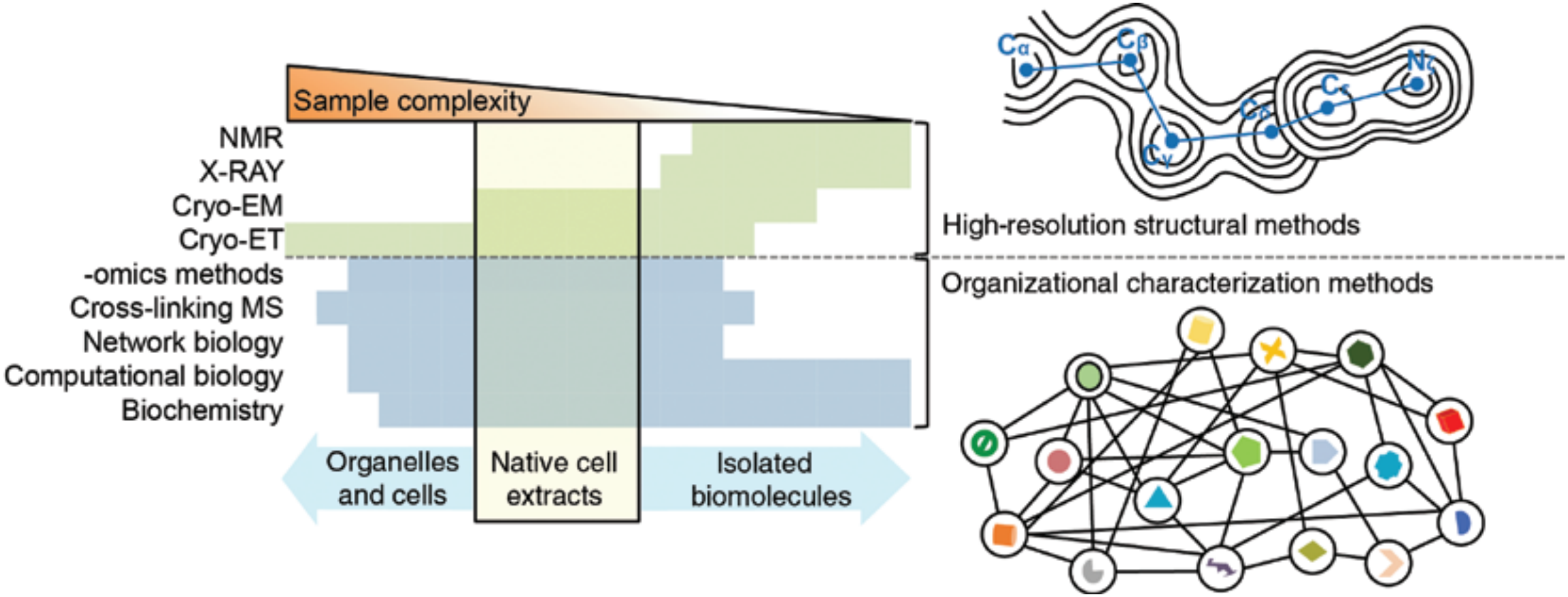



Figure 9.2 How Proteins Work (©2012 Garland Science)

# molecular identity gap




Biologica Chemistry 2019

# number of components per complex

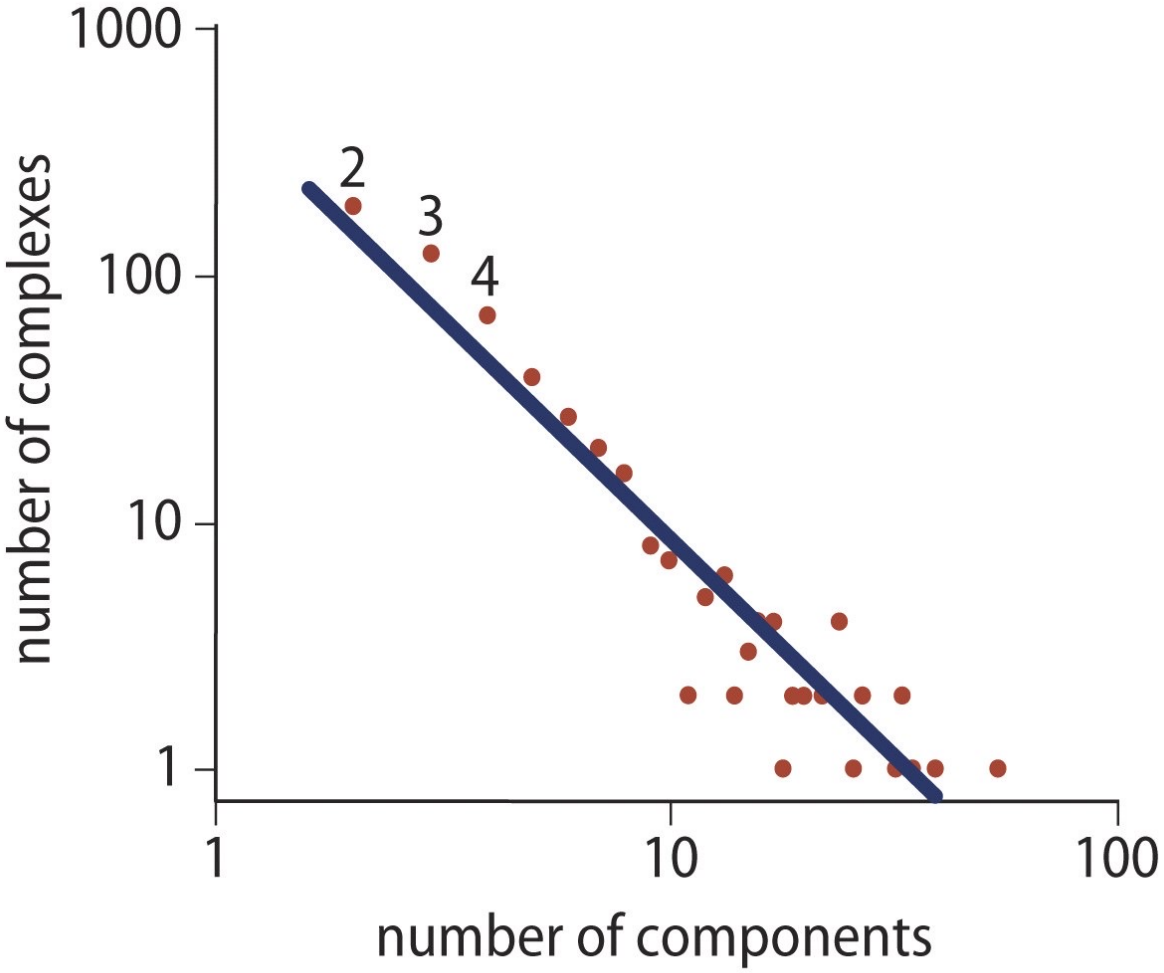
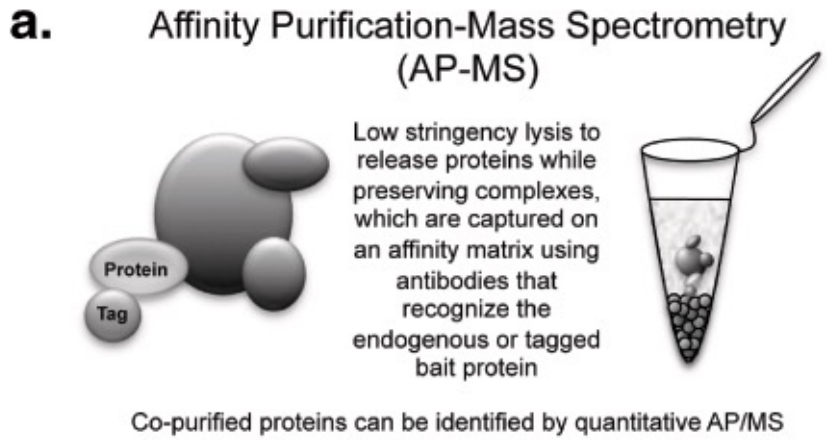
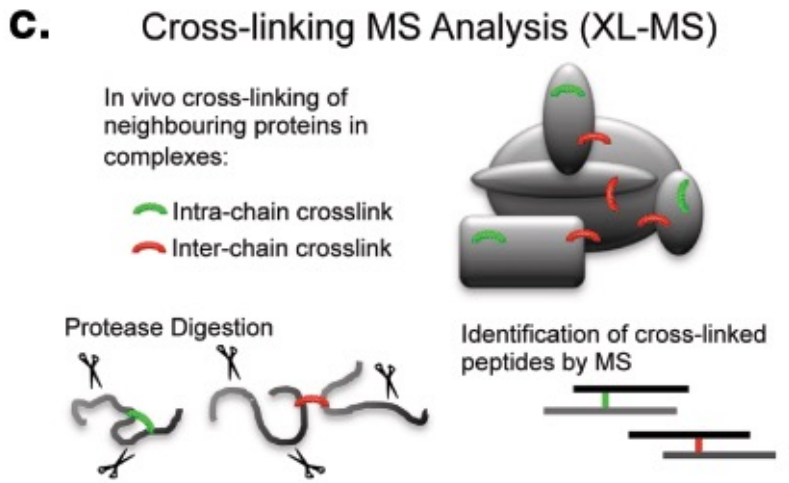
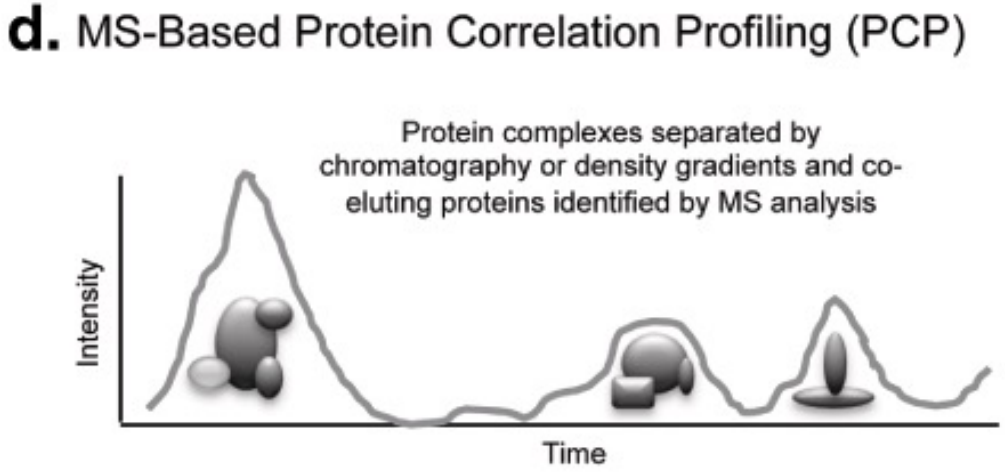


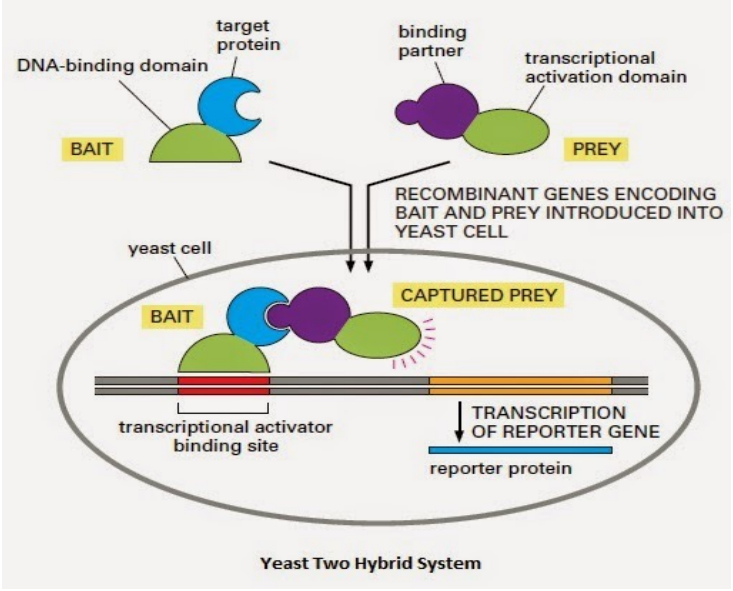

Figure 9.1 How Proteins Work (©2012 Garland Science)

# Experimental approaches



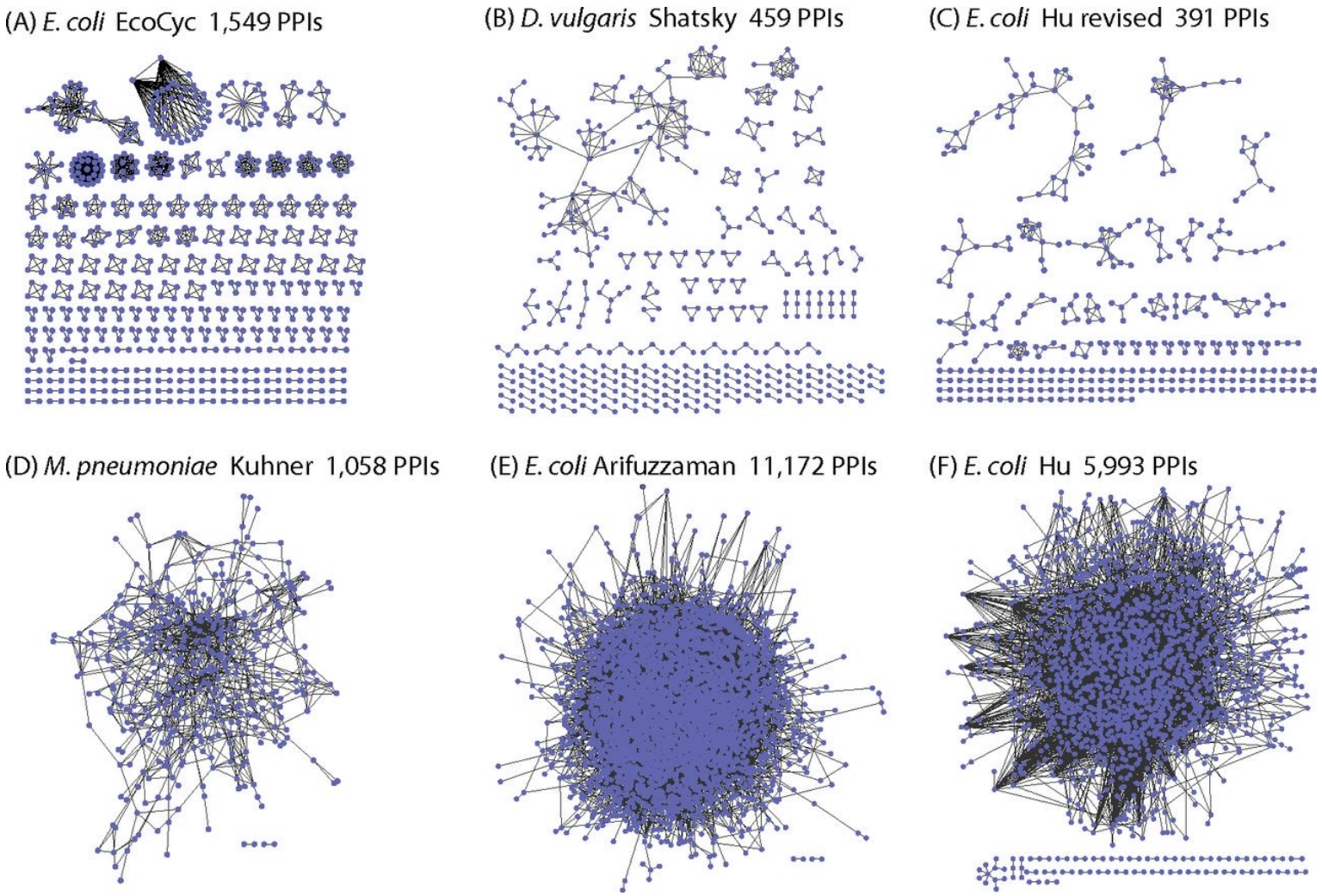




Mehta V and Trinkle-Mulcahy L. Recent advances in large-scale protein interactome mapping [version 1]. F1000Research 2016, 5:782 (doi: 10.12688/f1000research.7629.1)

F1000Research

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



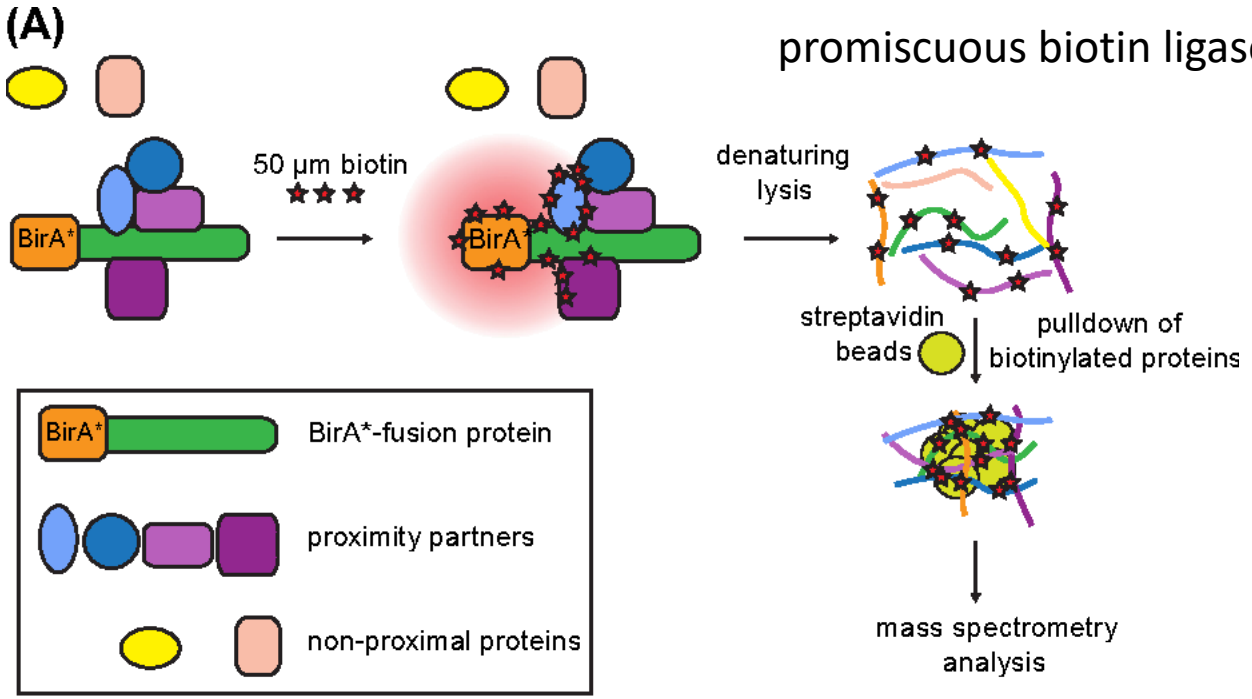

**Maxim Shatsky et al. Mol Cell Proteomics 2016;15:1539-1555**



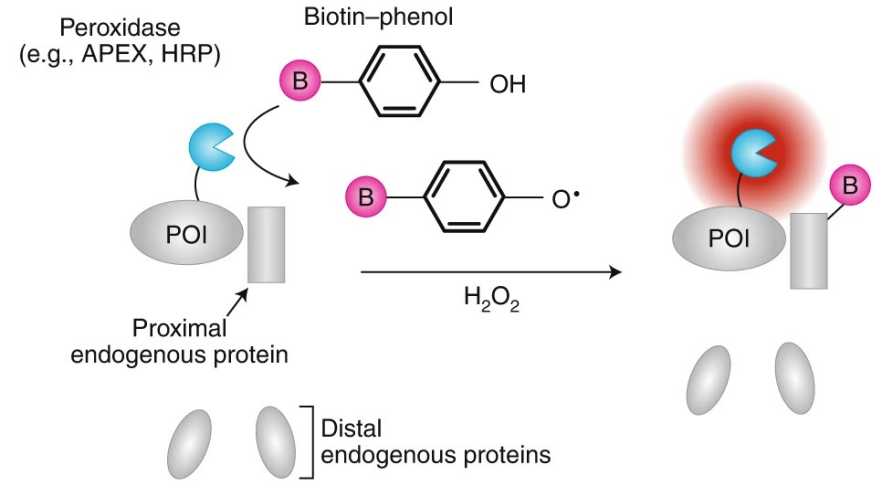
MCP MOLECULAR & CELLULAR PROTEOMICS

# BioID & APEX

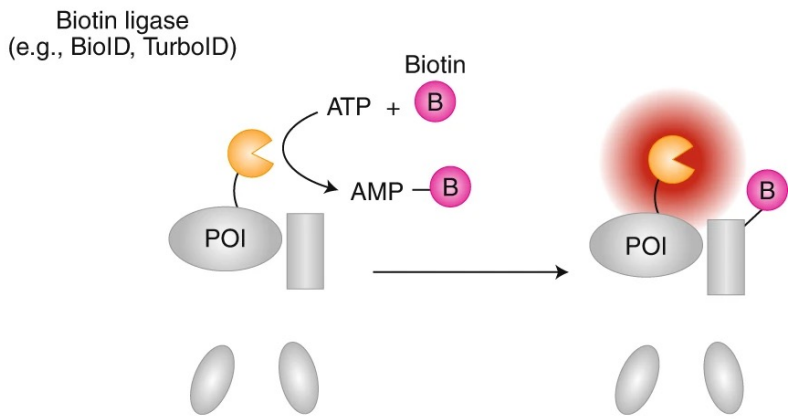
"near neighbor labeling" approaches
that utilize enzymatic reactions to tag proteins



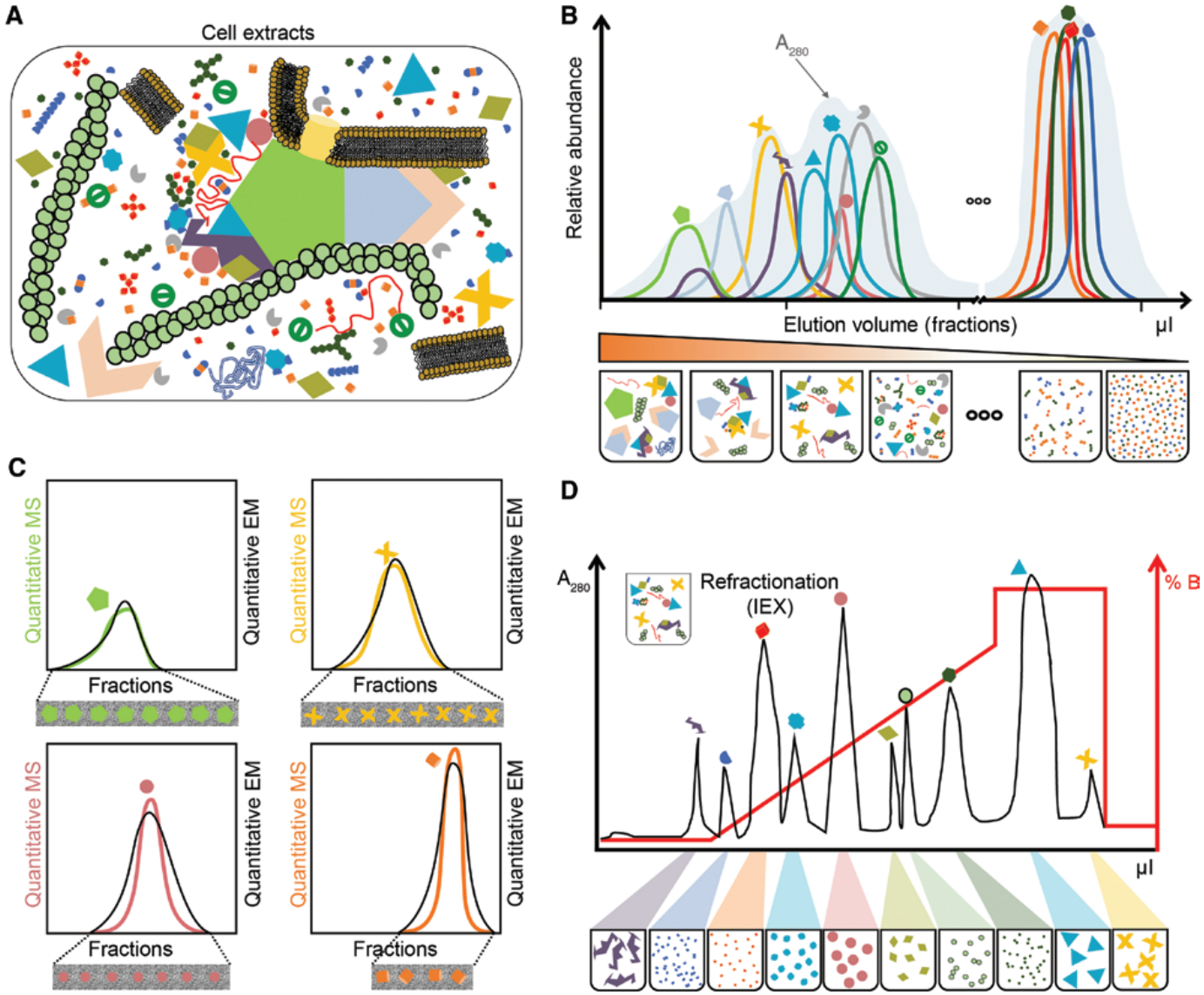

a Peroxidase-based approaches



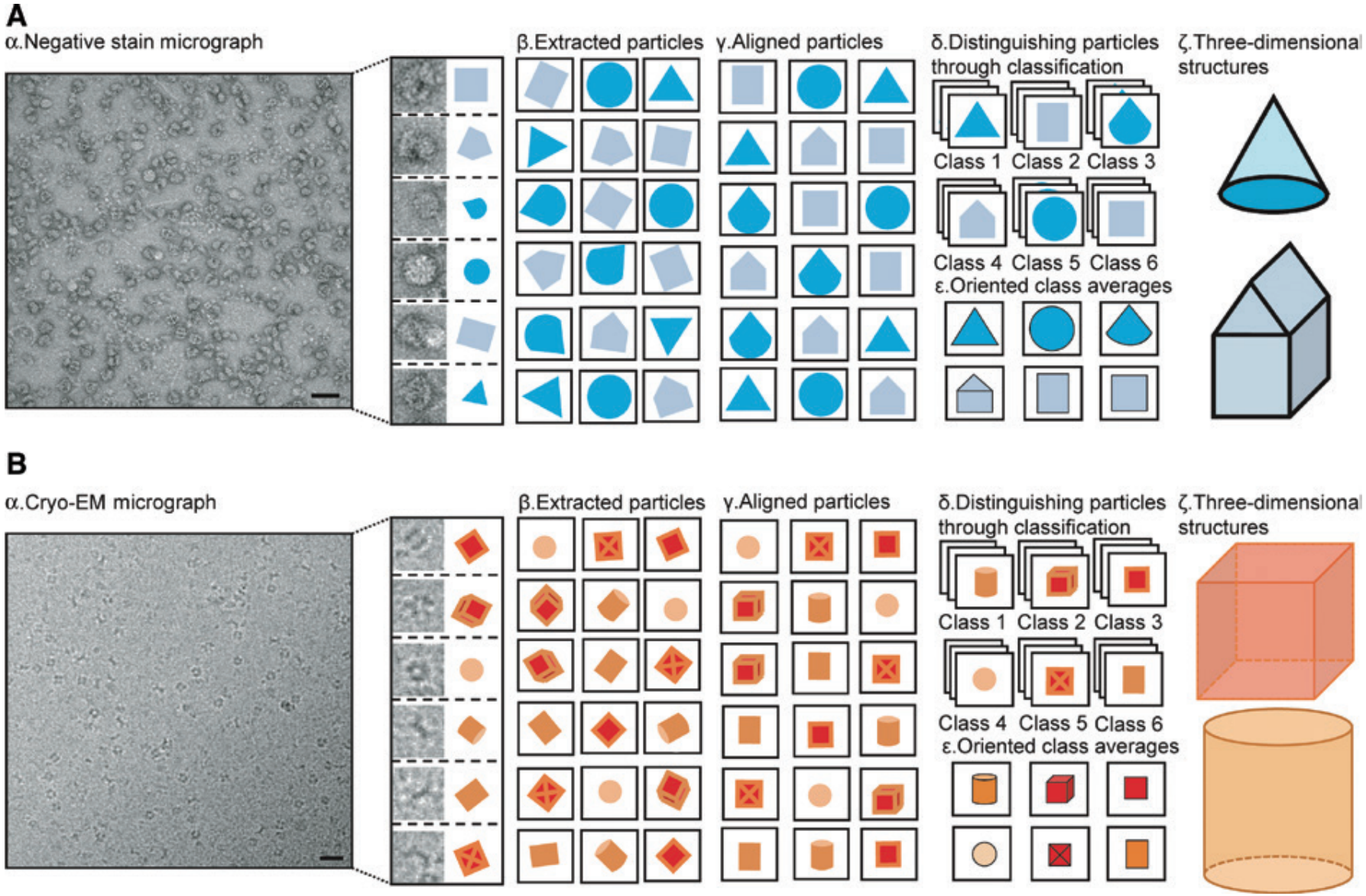

b

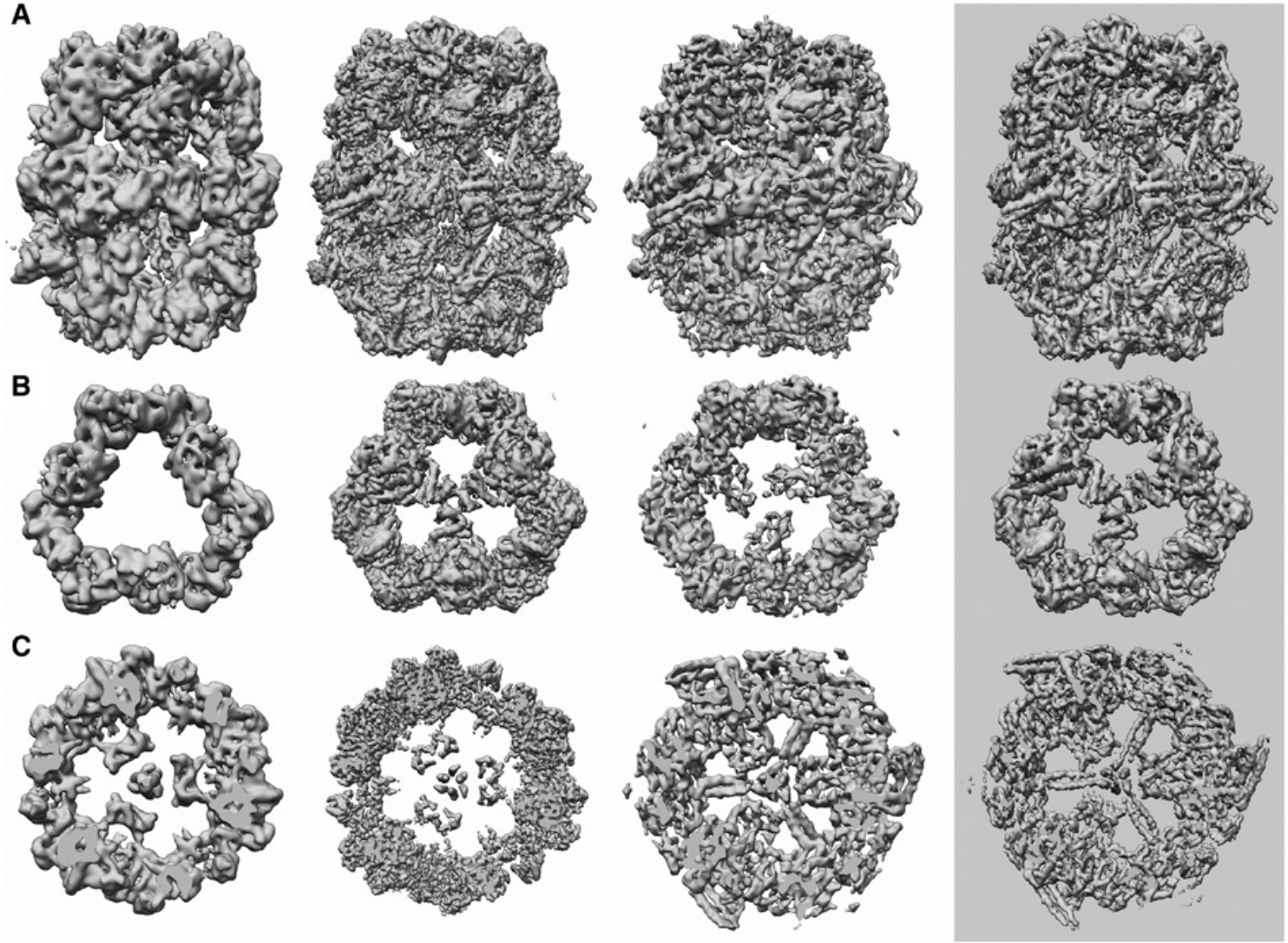


# Cell extracts for the structural characterization and identification of molecular species



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

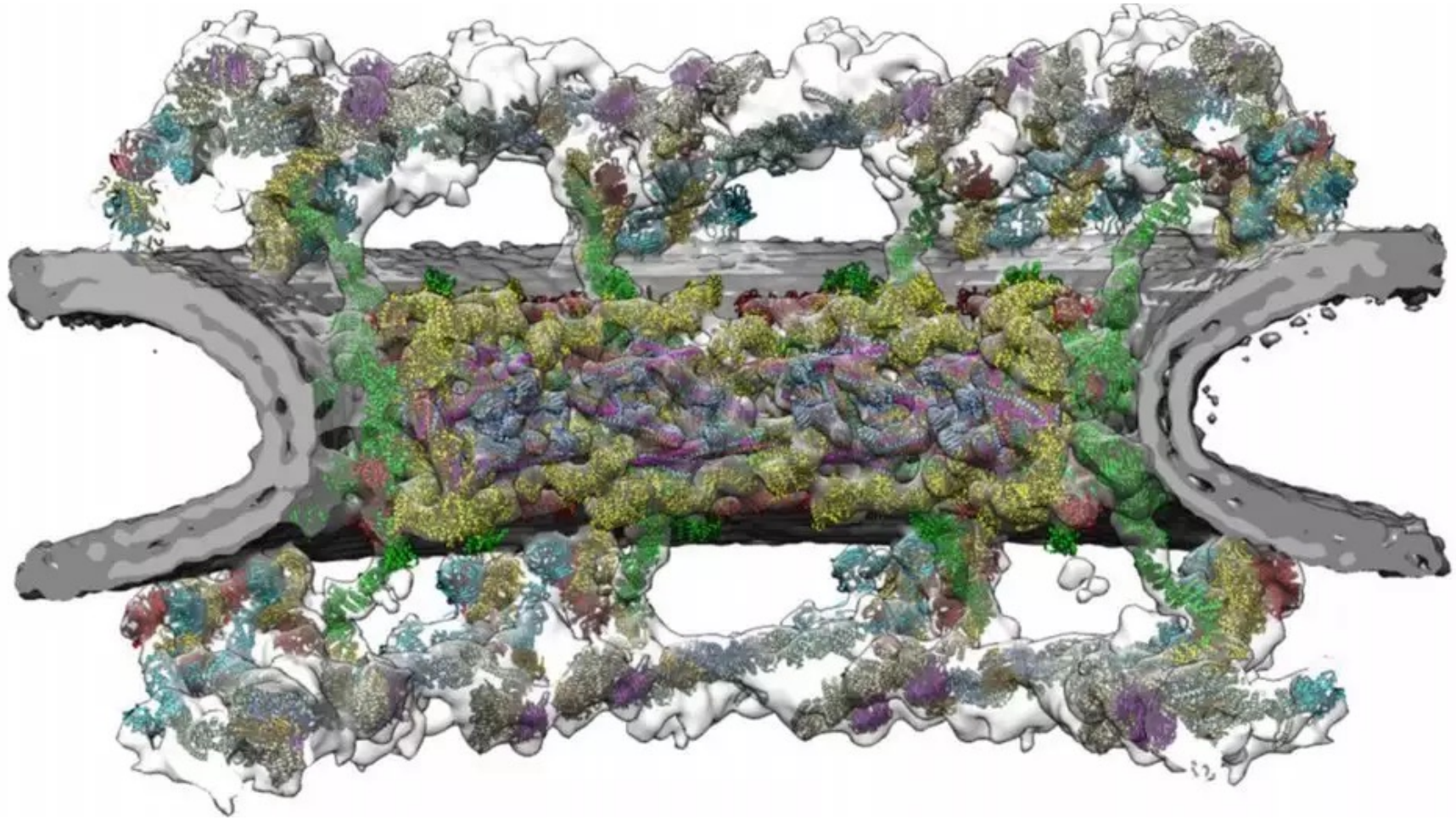


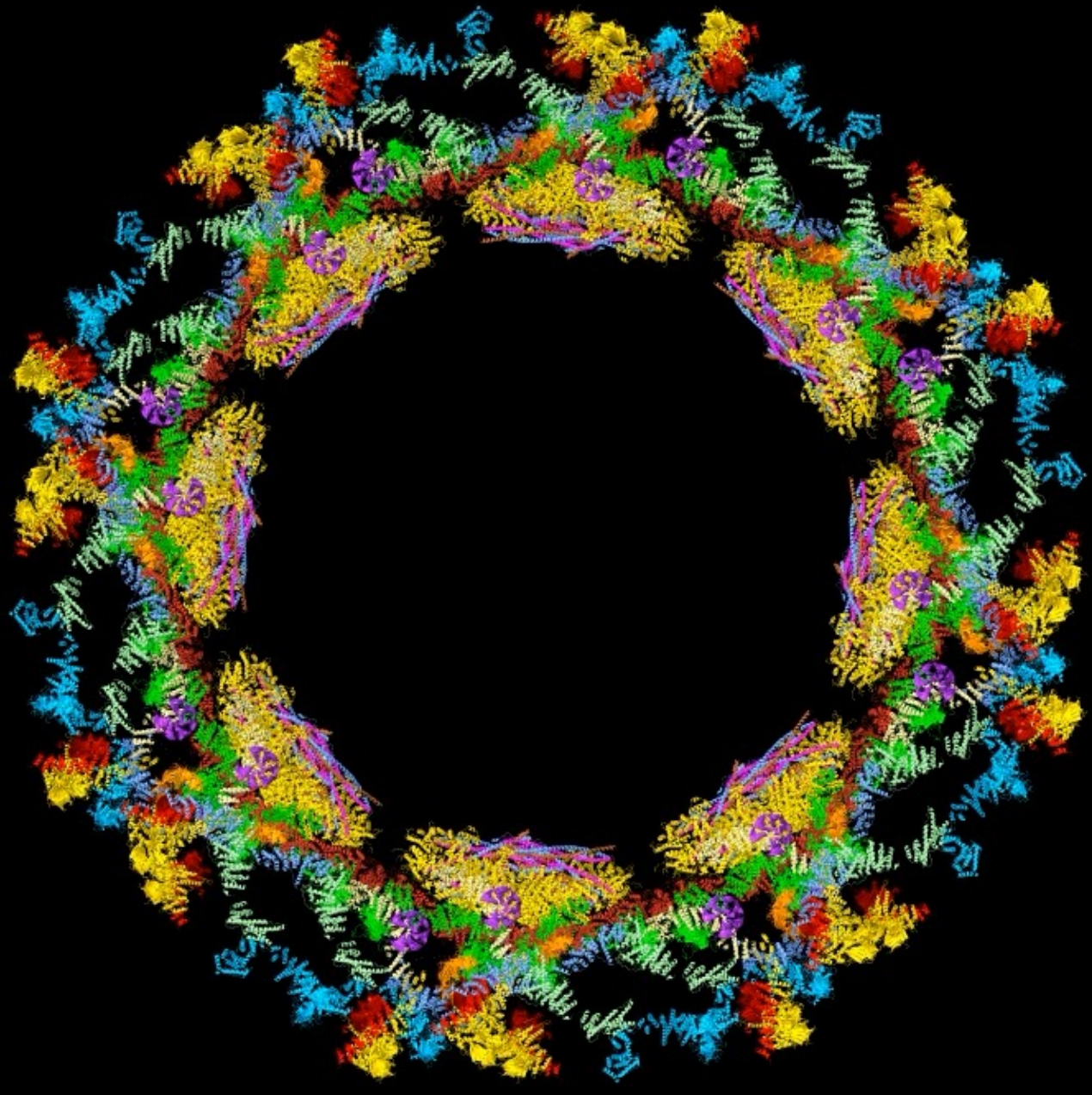



| *M. smegmatis* | *M. tuberculosis* | *S. cerevisiae* | *C. thermophilum* |
|---|---|---|---|
| Native purification | Recombinant | Native purification | Cell extract |
| 106 884 particles | 40 160 particles | ~25 000 particles | 3933 particles |
| 7.5 Å resolution (FSC = 0.5) | 3.3 Å resolution (FSC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

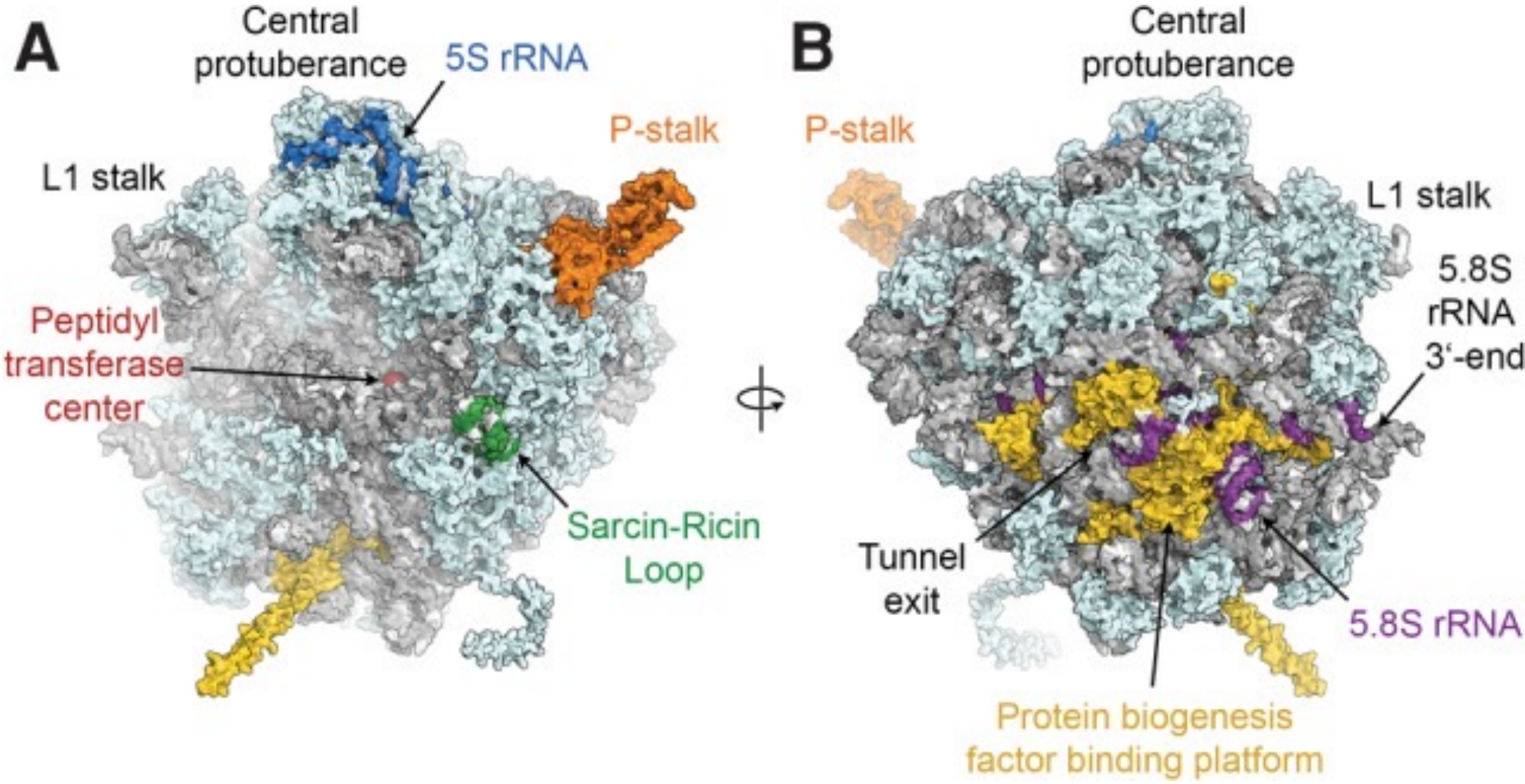
Martin Beck – Molecular Sociology
MPI of Biophysics Frankfurt am Main

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."

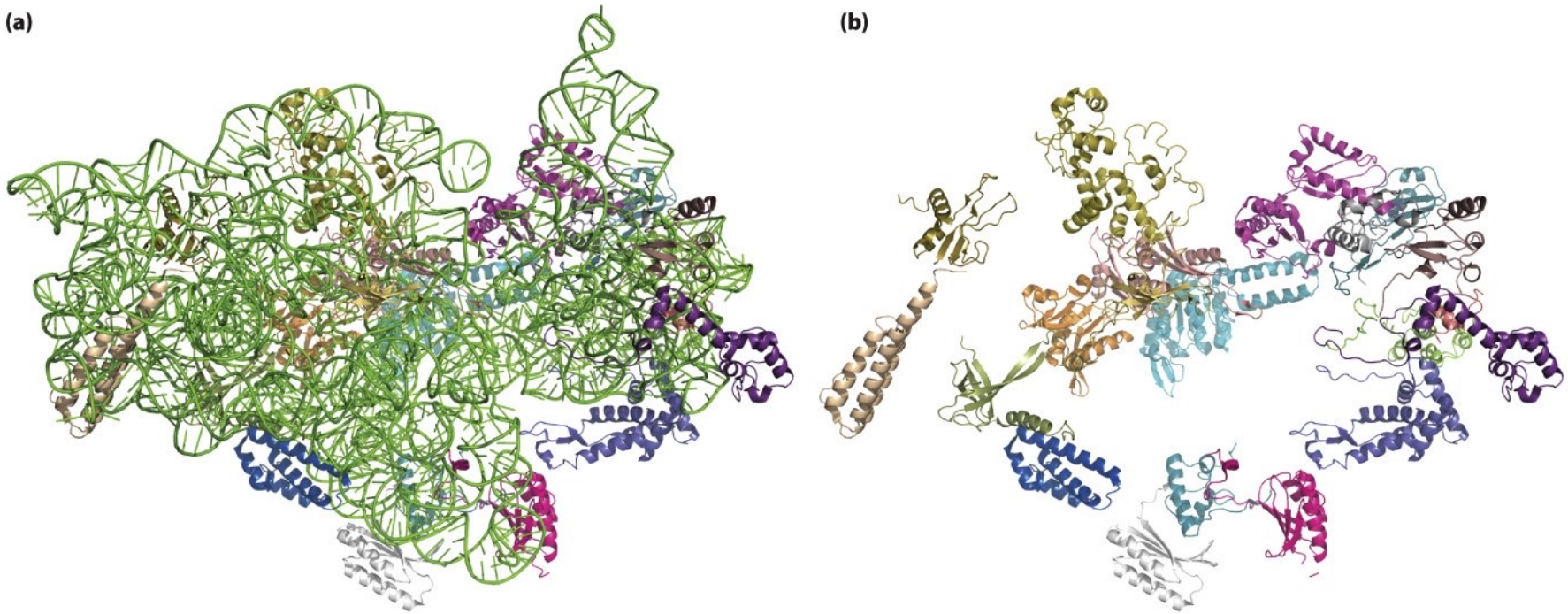



Figure 9.3 How Proteins Work (©2012 Garland Science)

# ribosome biogenesis

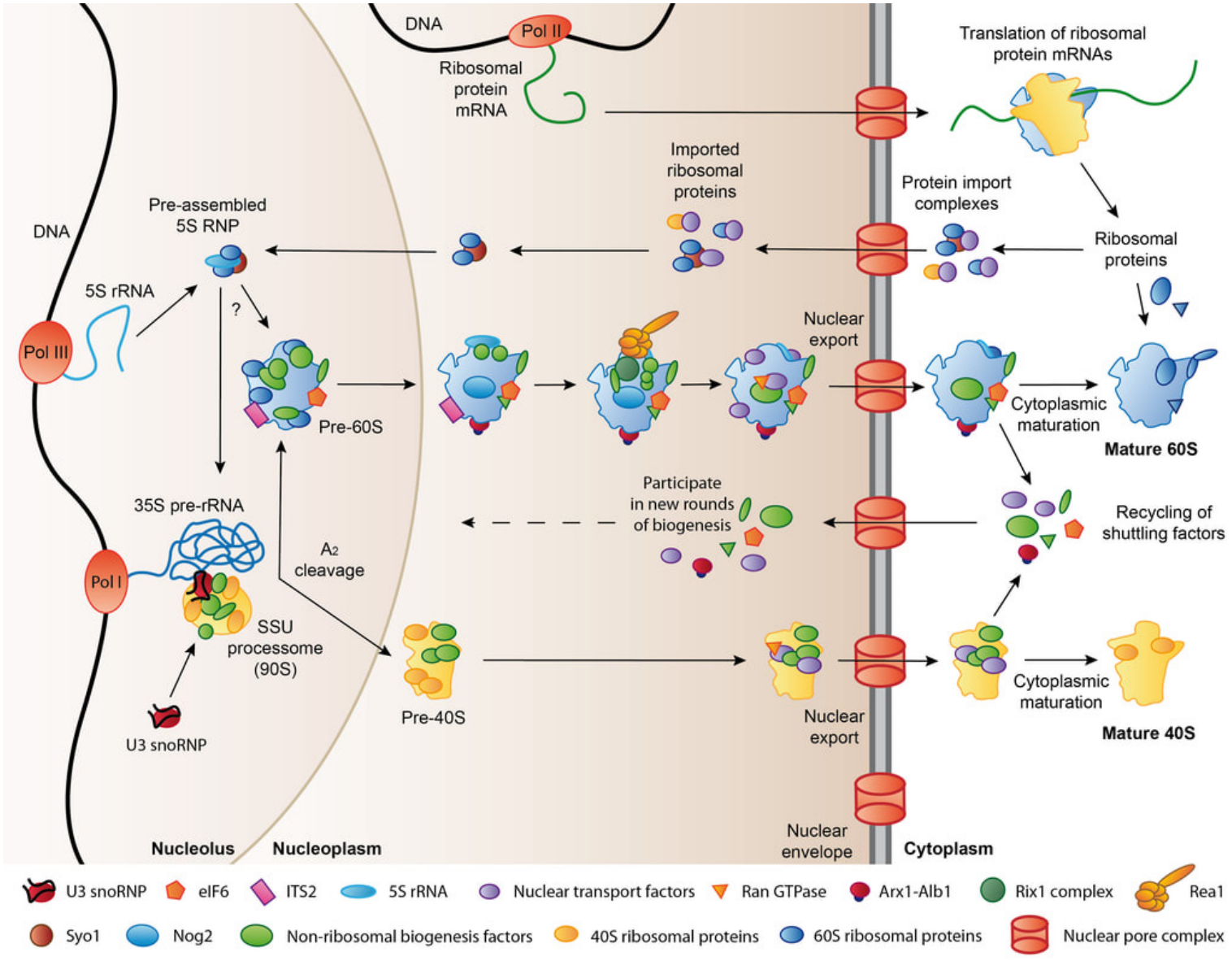
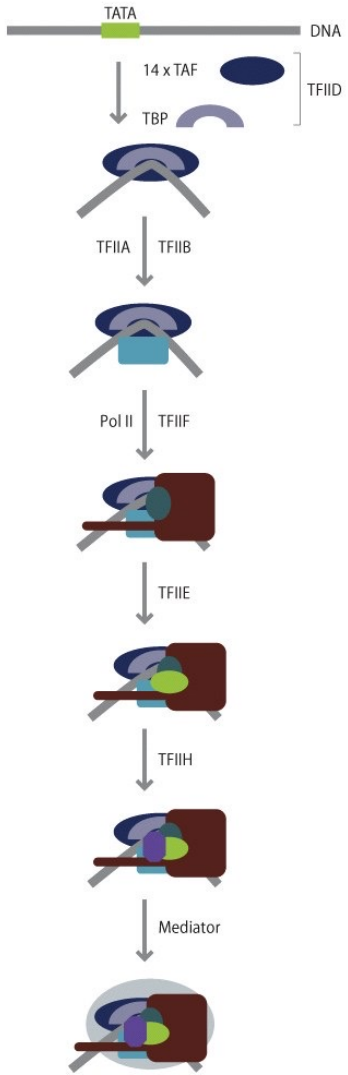


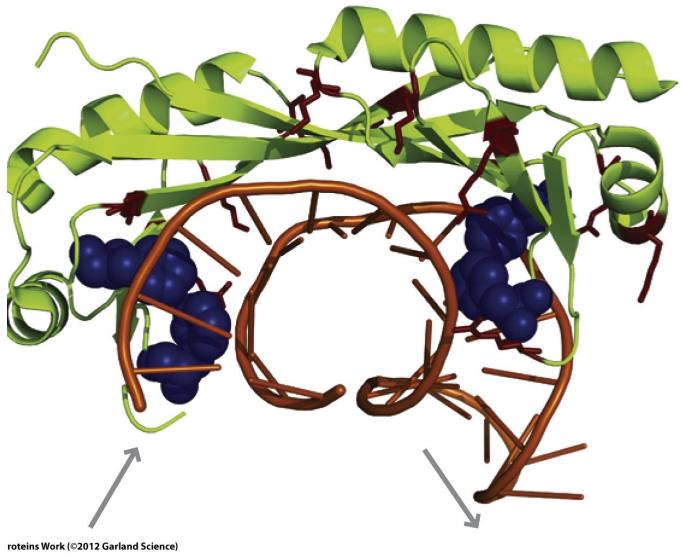
**TABLE 9.2 The constituents of yeast RNA polymerase II**

| 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)

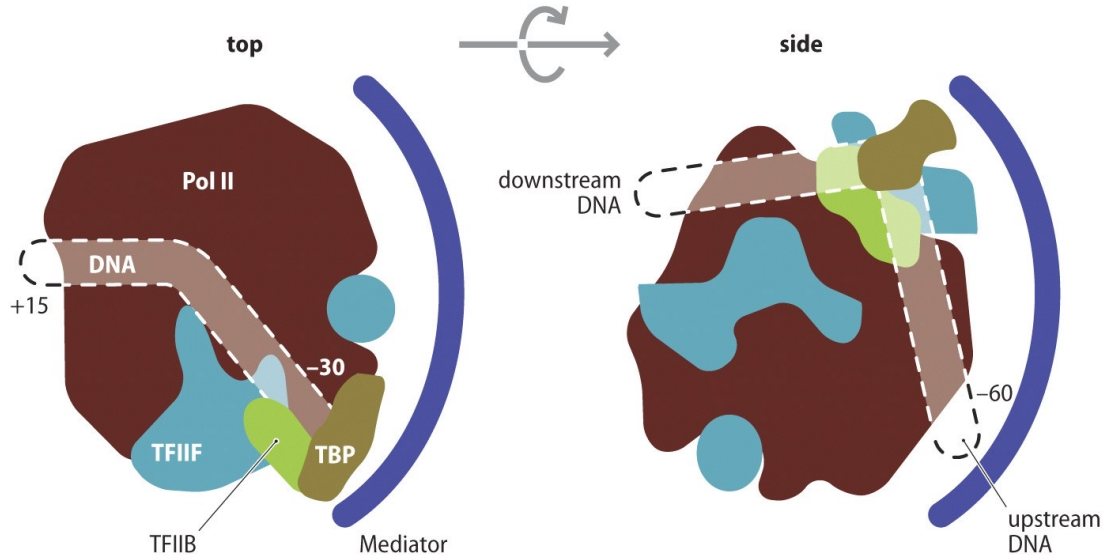



Figure 9.10 How Proteins Work (©2012 Garland Science)

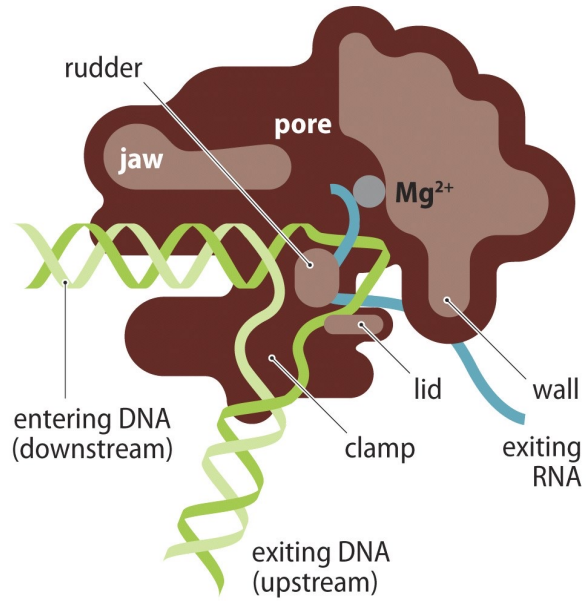



Figure 9.11 How Proteins Work (©2012 Garland Science)

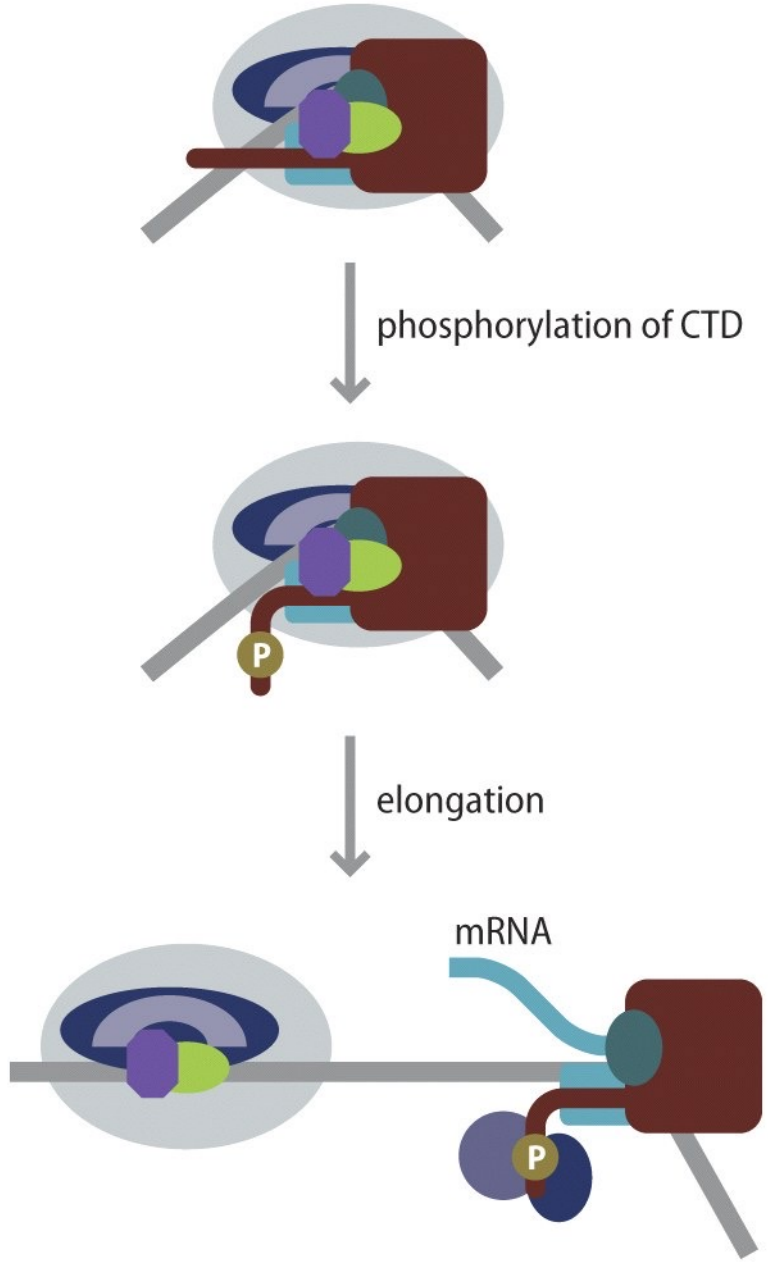



**Figure 9.12 How Proteins Work (©2012 Garland Science)**

# human metabolic pathways

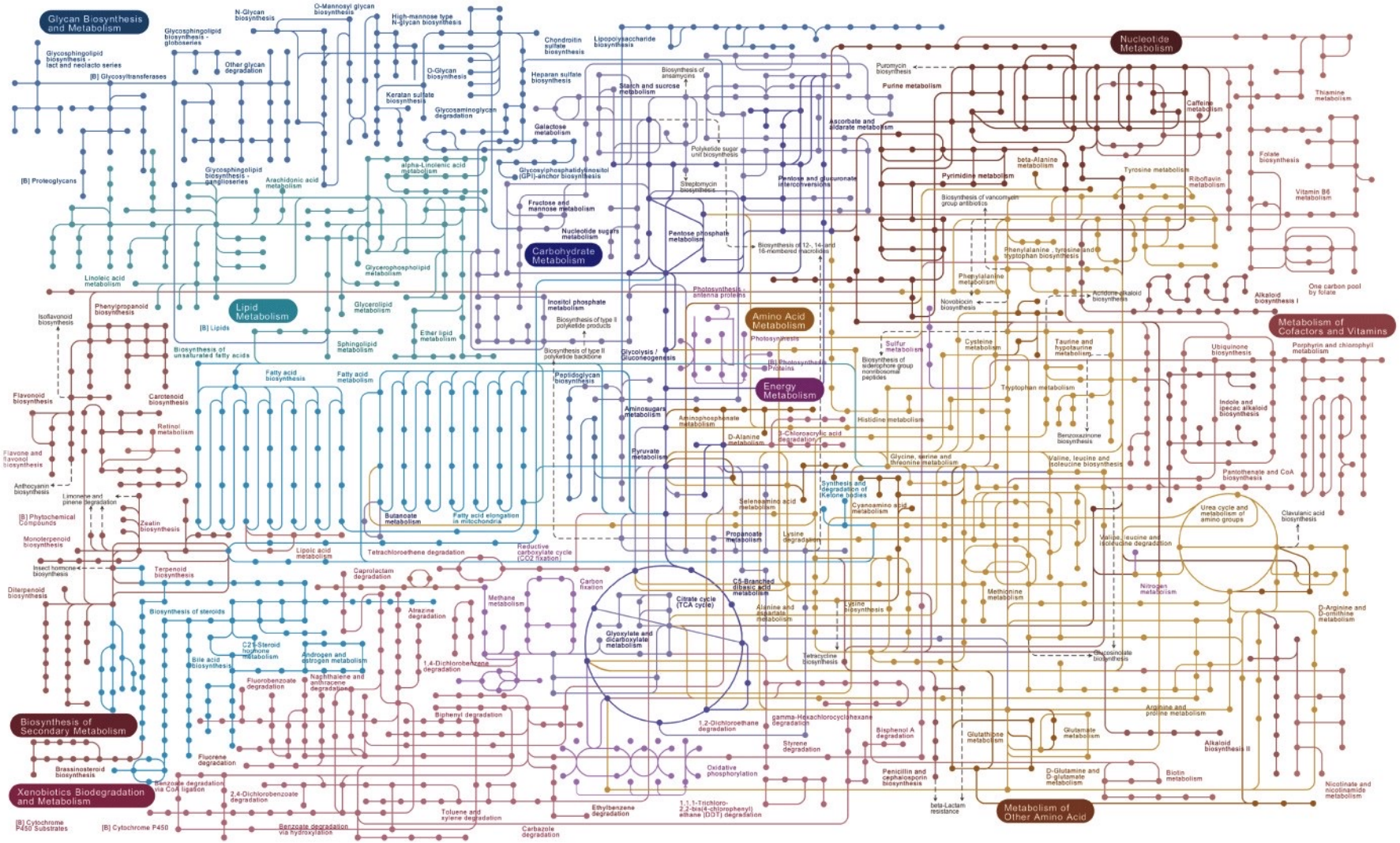



**Figure 9.13 How Proteins Work (©2012 Garland Science)**

# coupling of enzymes

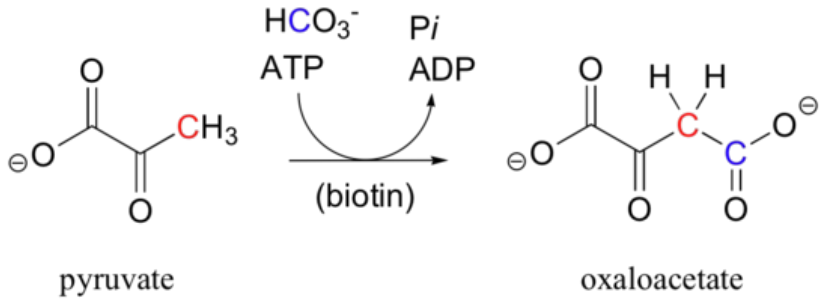
$X + Y \rightarrow XY \qquad \Delta G_1 > 0$

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

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

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

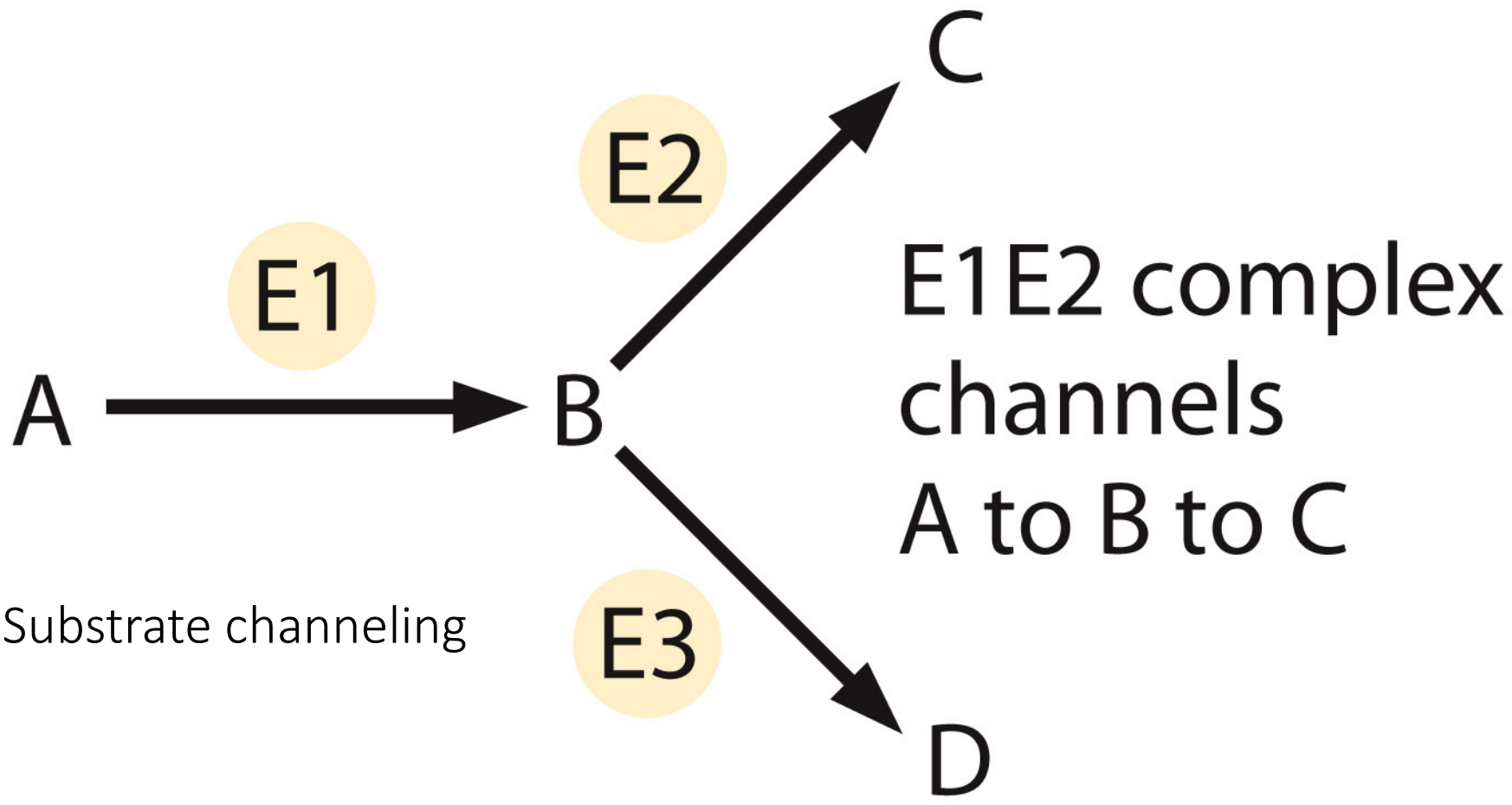
$X + Y + ATP \rightarrow XY + ADP + P_i \qquad \Delta G < 0 \text{ if } \Delta G_2 > \Delta G_1$




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

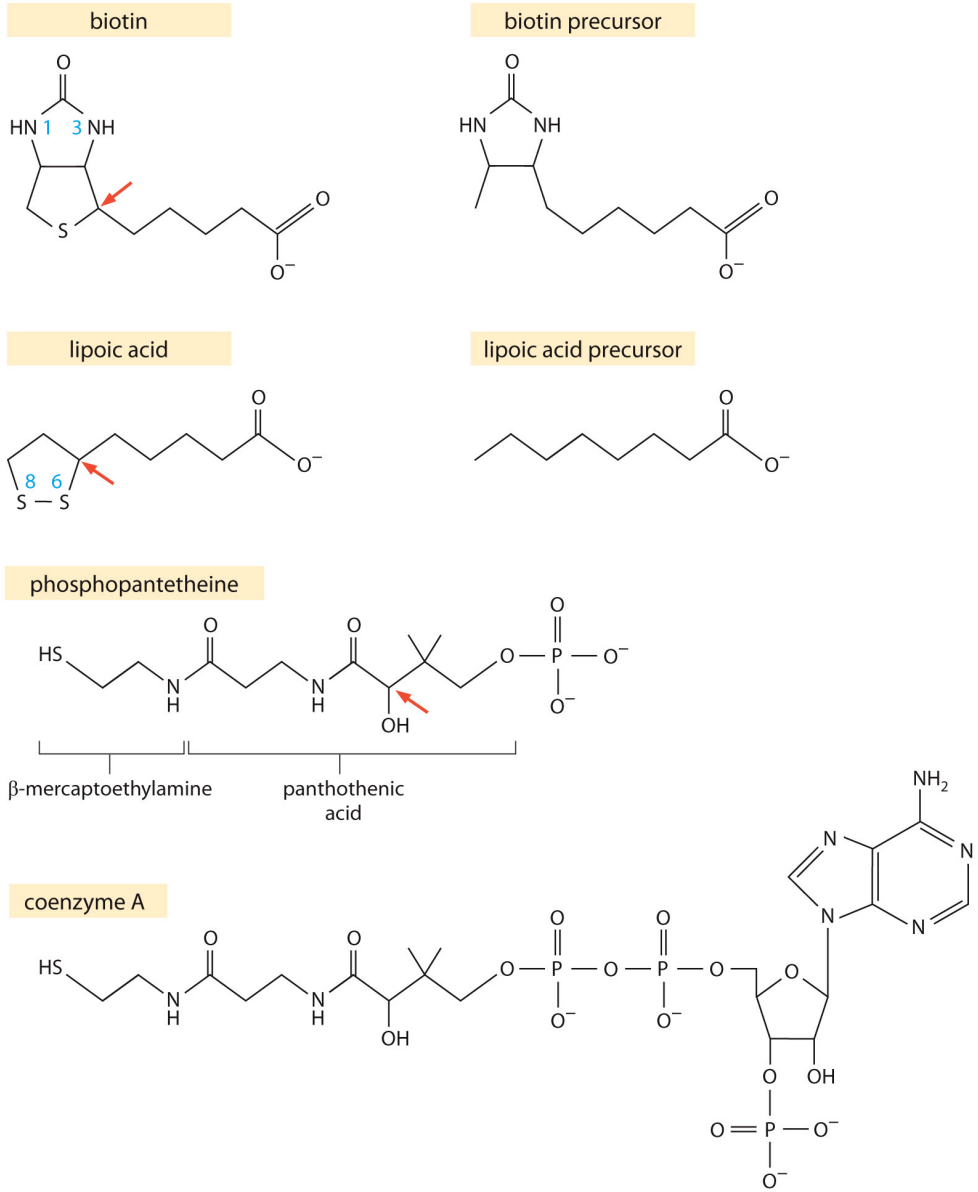



Figure 9.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# Channeling

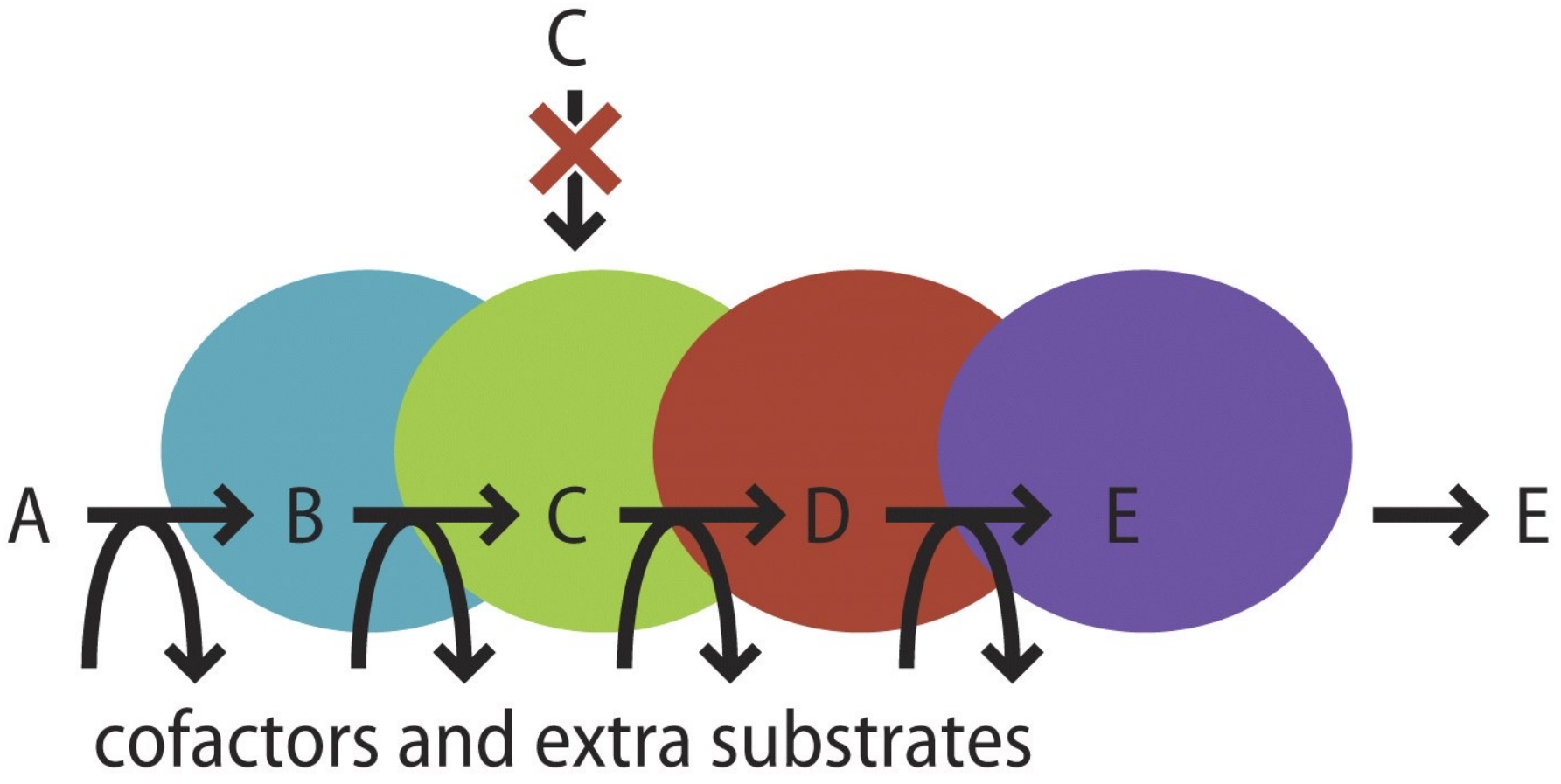



Figure 9.14 How Proteins Work (©2012 Garland Science)

# enzyme complexes with tunnels

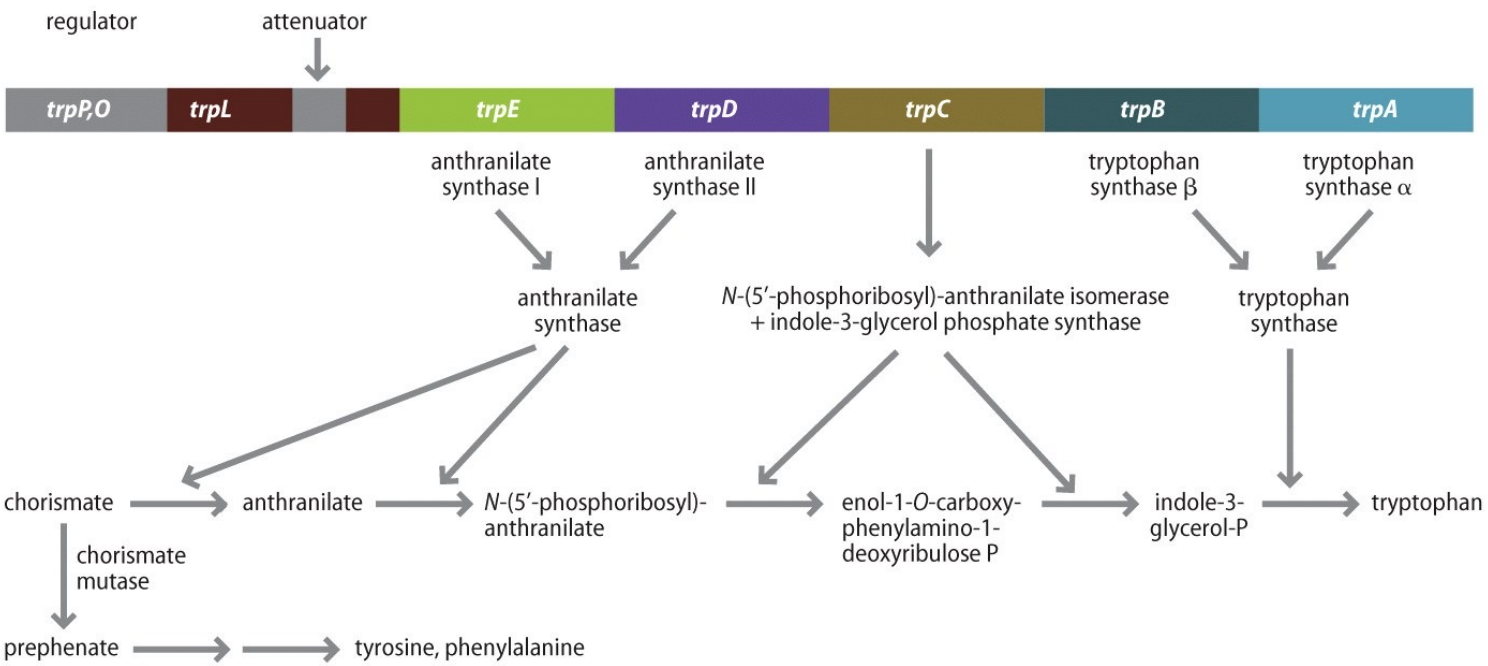
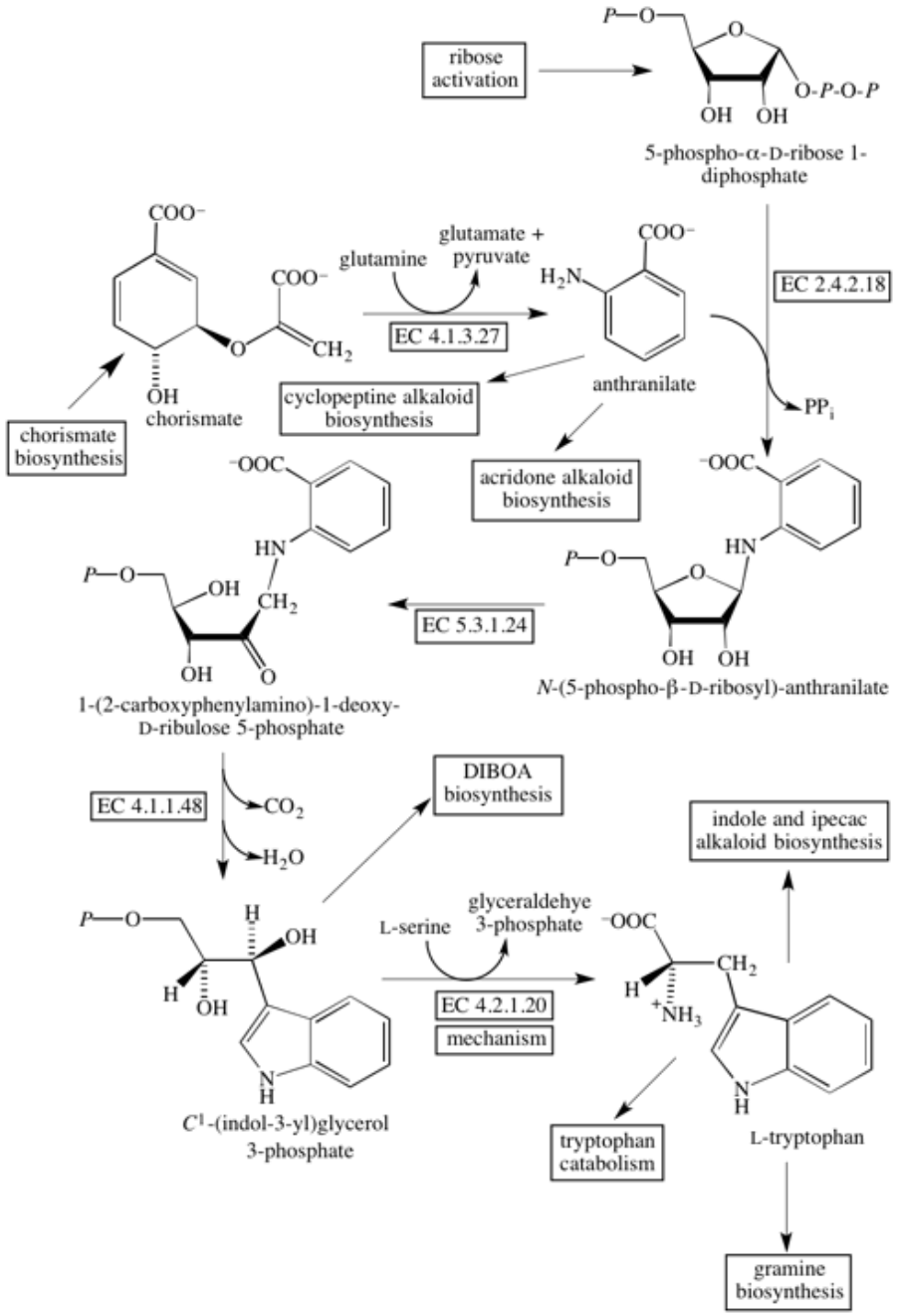



Figure 10.1 How Proteins Work (©2012 Garland Science)



# enzyme complexes with tunnels

$\alpha_2\beta_2$ heterotetramer.

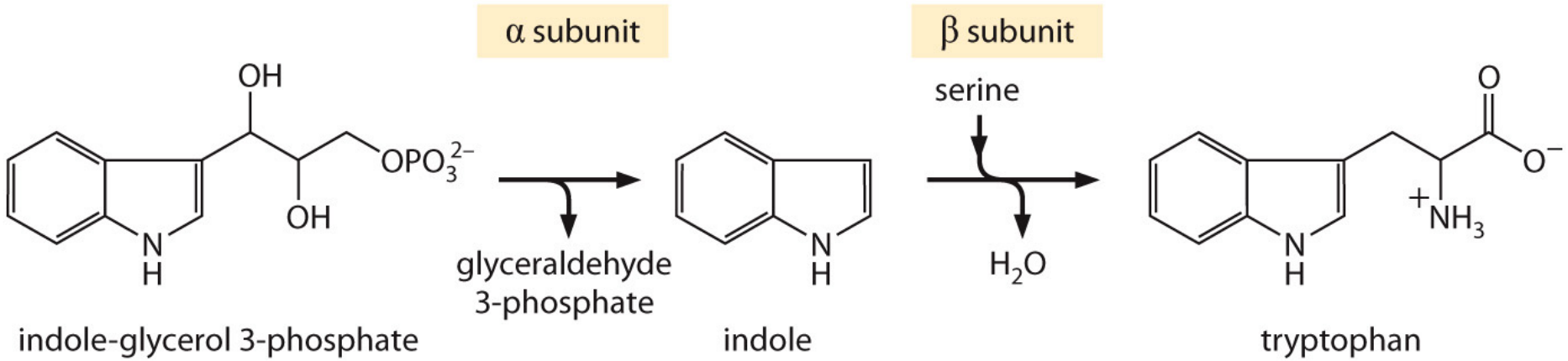
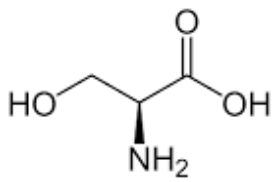
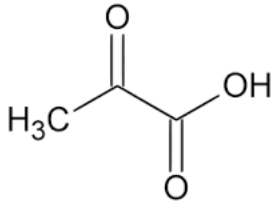



Figure 9.2a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

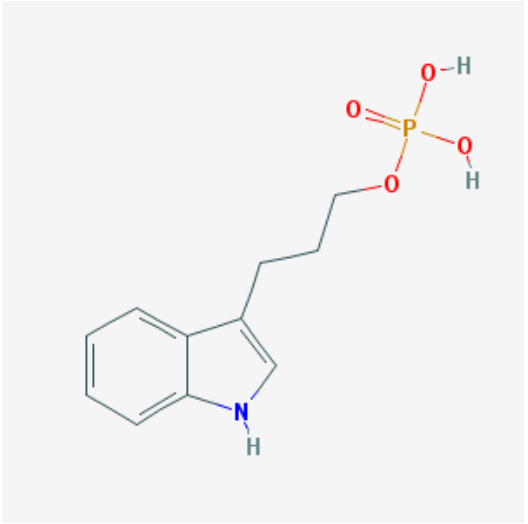



β subunit as β2 catalyse the conversion of serin to puruvate and $NH_3$

# Structure and mechanism of tryptophan synthase

competitive inhibitor



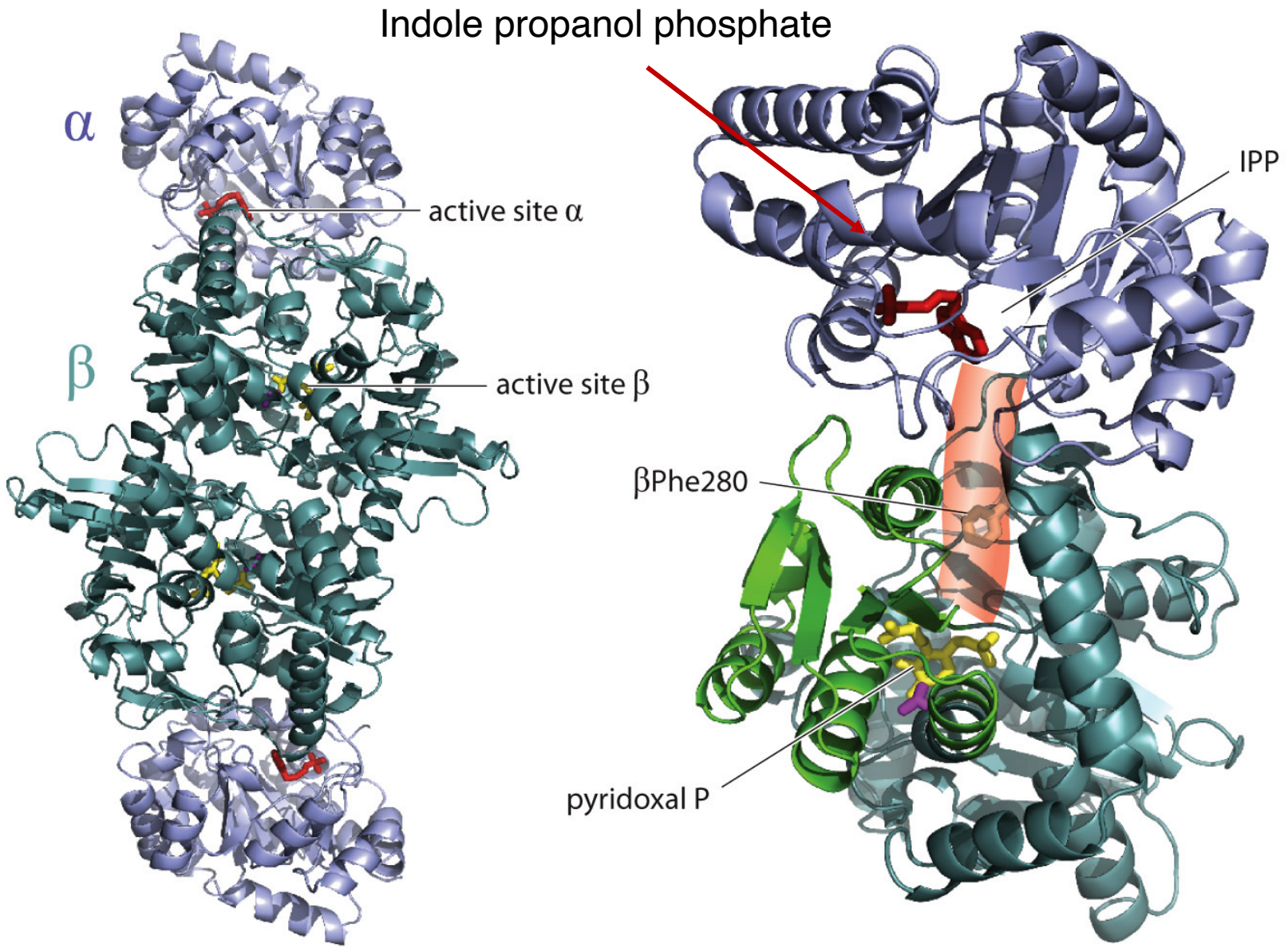




Figure 9.2b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# ammonia as intermediate

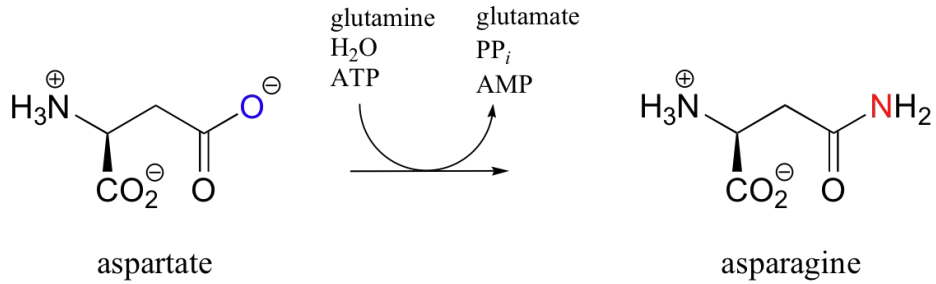
Asparagin synthase

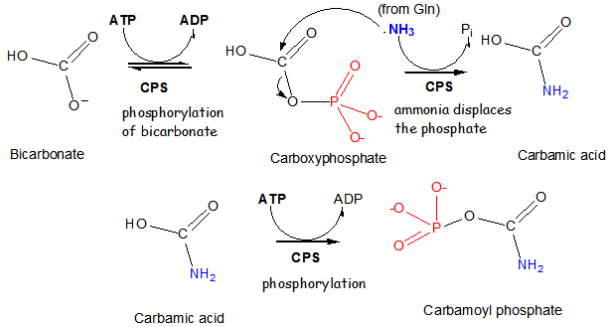
Carbomoyl phosphate synthase

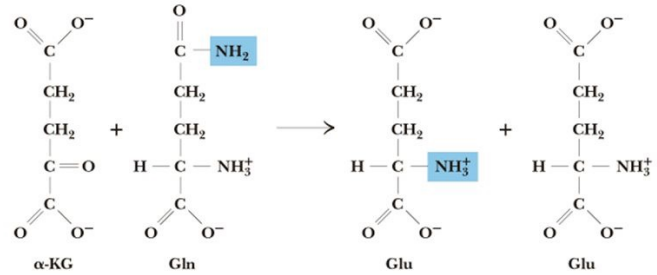
Glutamate synthase

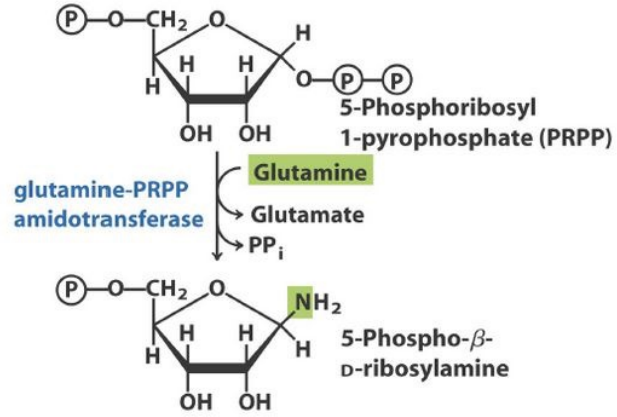
Imidazol glycerol phosphate synthase

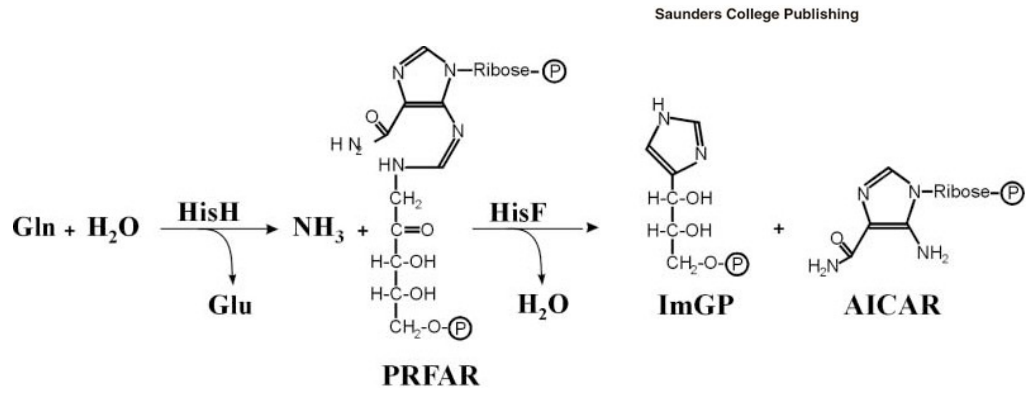
GPAT



# structure and mechanism of CPS

Carbomoyl phosphate synthase

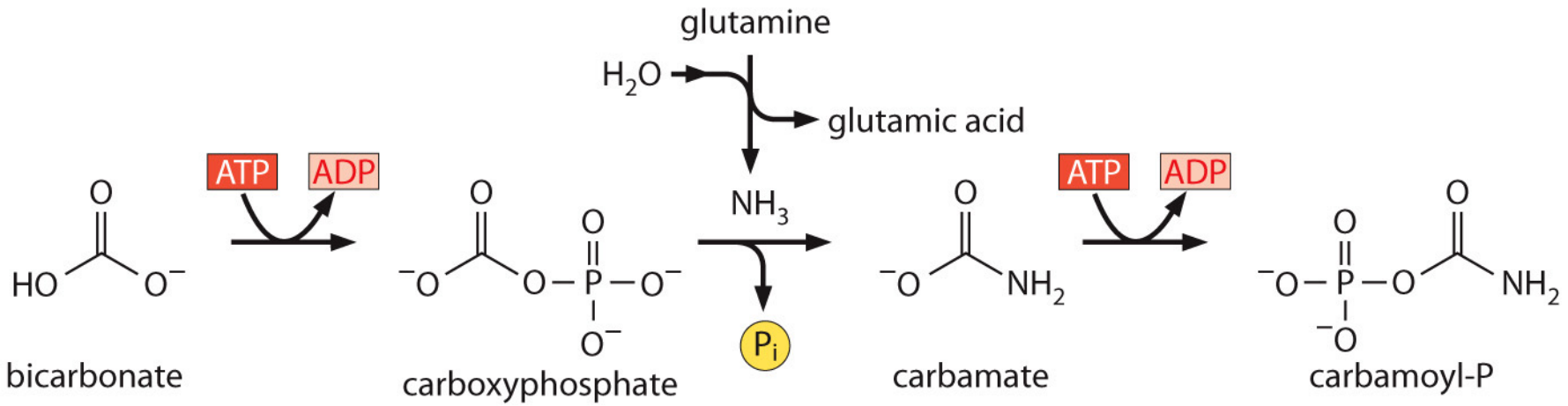



Figure 9.3a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# structure and mechanism of CPS

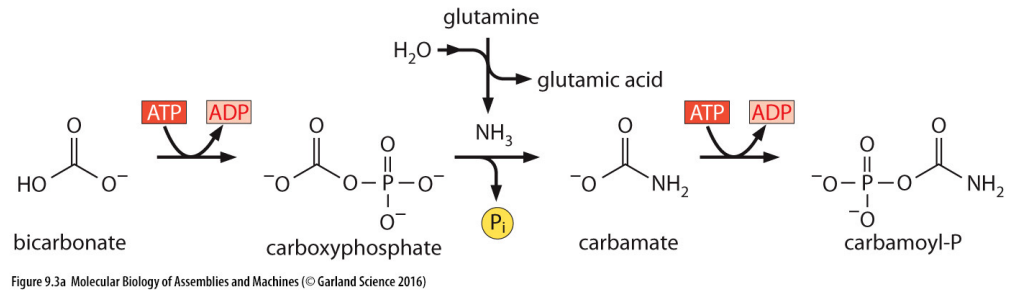



Figure 9.3a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

Hetero-octamer

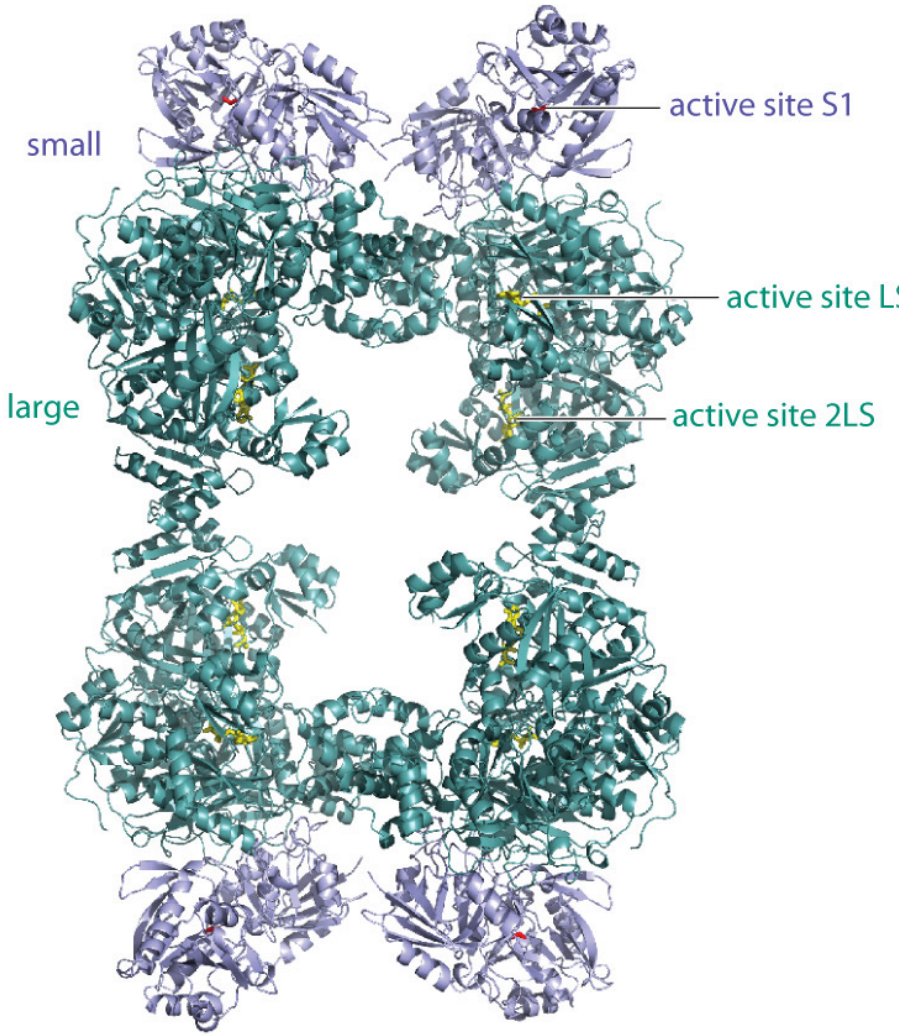
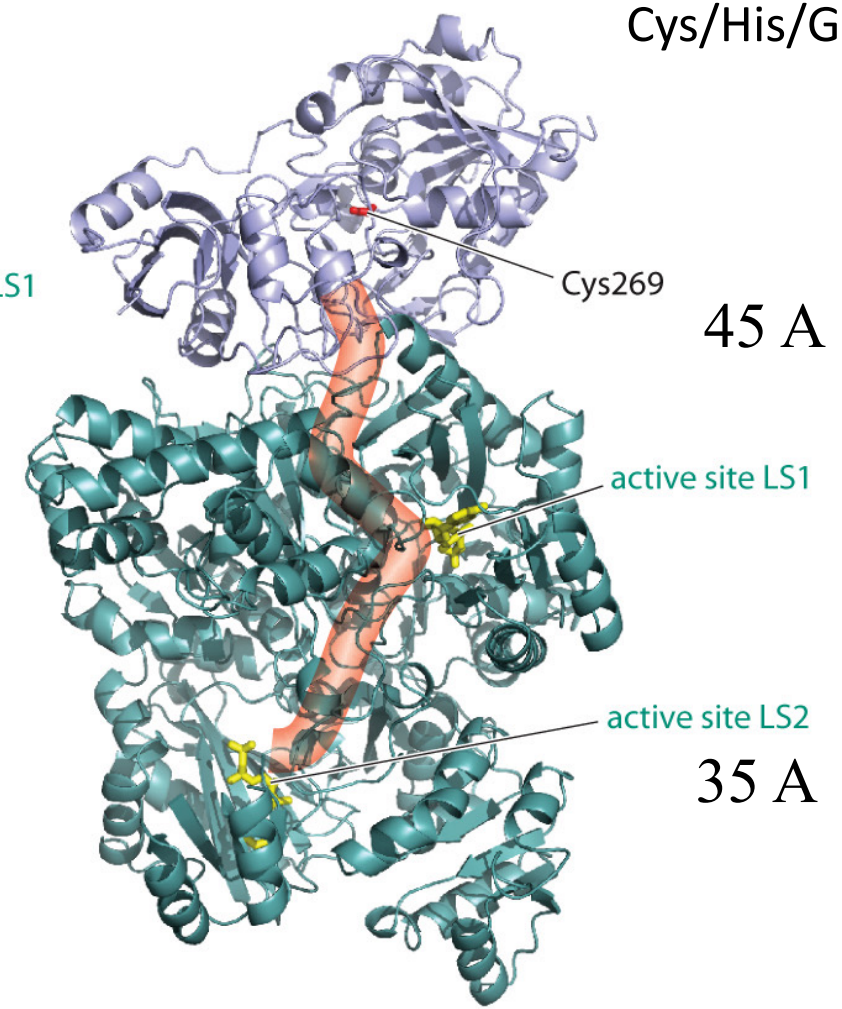
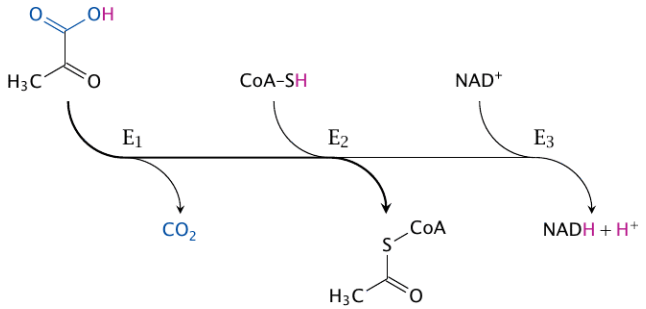



Figure 9.3b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

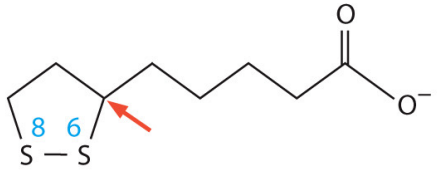
Cys/His/Glu



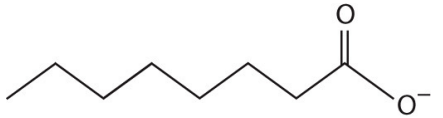
# Multienzyme Complexes

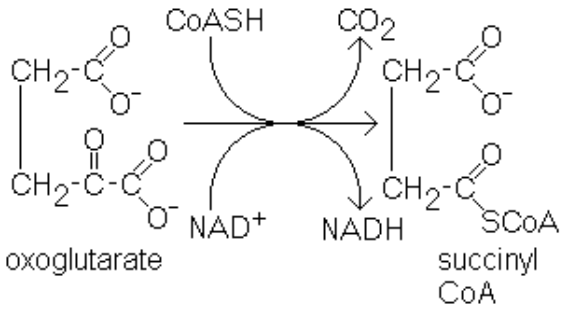



lipoic acid

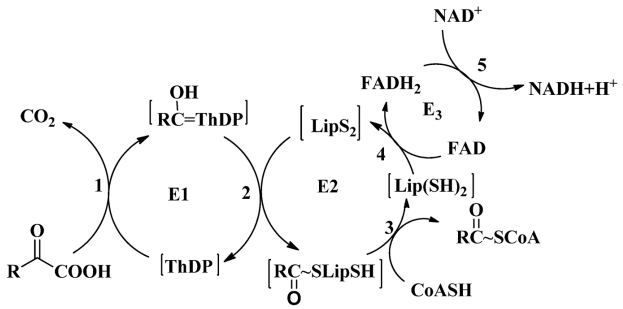



lipoic acid precursor

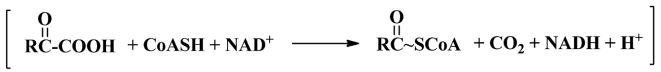



Lipoic acid dependent 2-oxo acid dehydrogenase




5-10 MDa

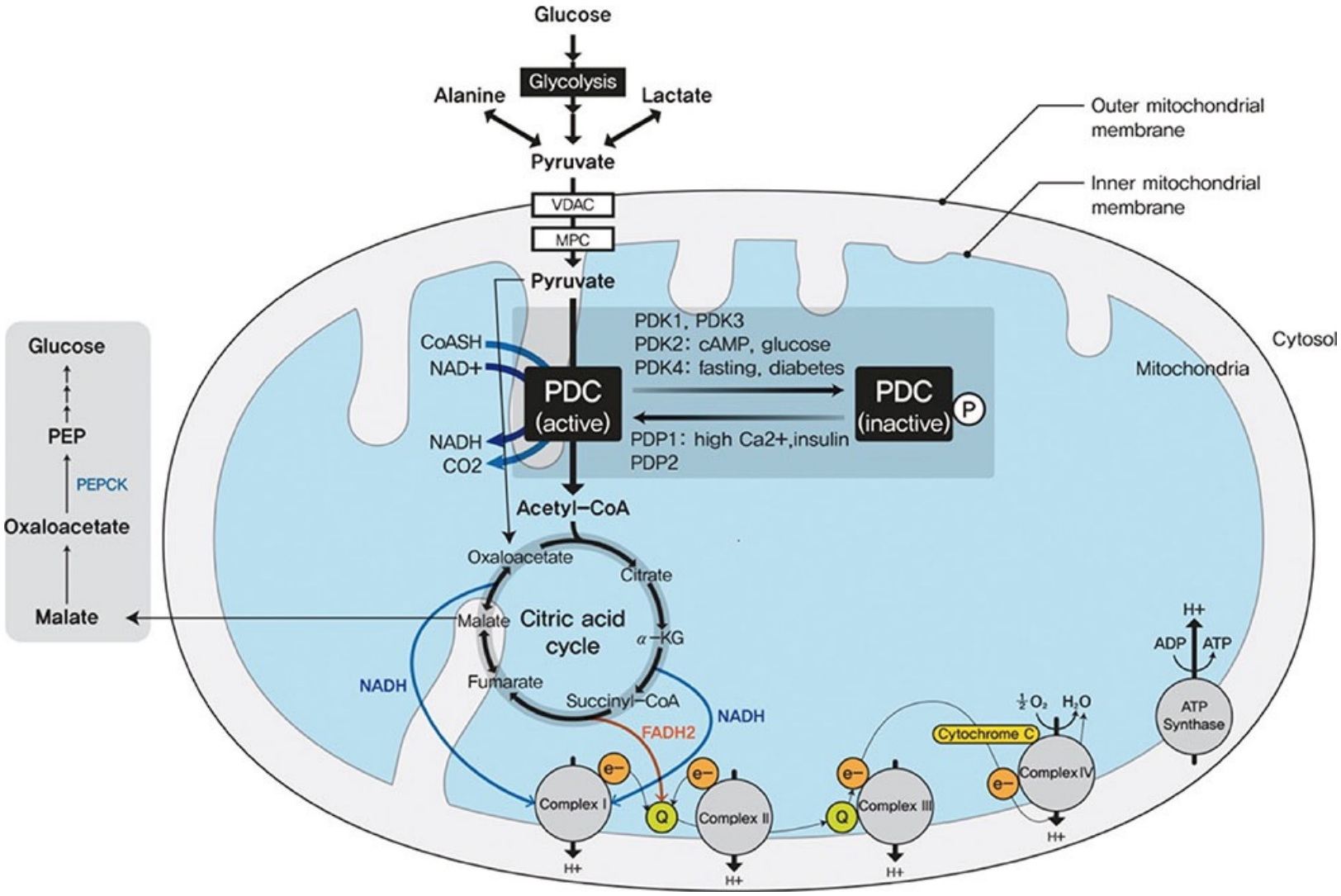


# pyruvate dehydrogenase complex

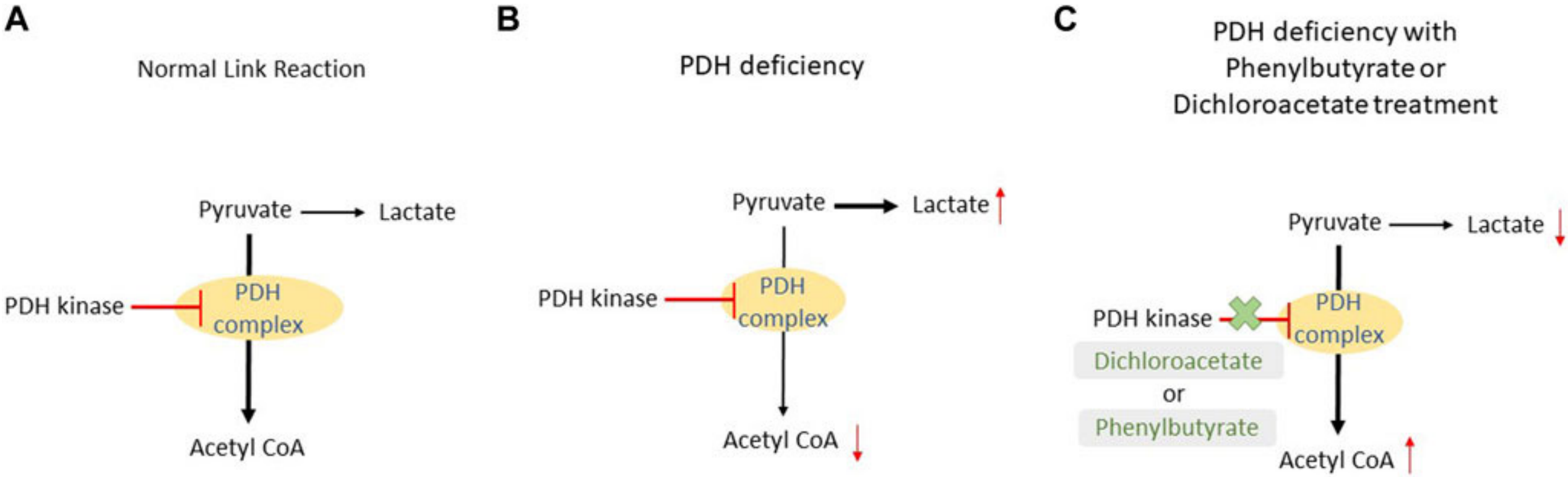
*A huge molecular complex links three sequential reactions for energy production*

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

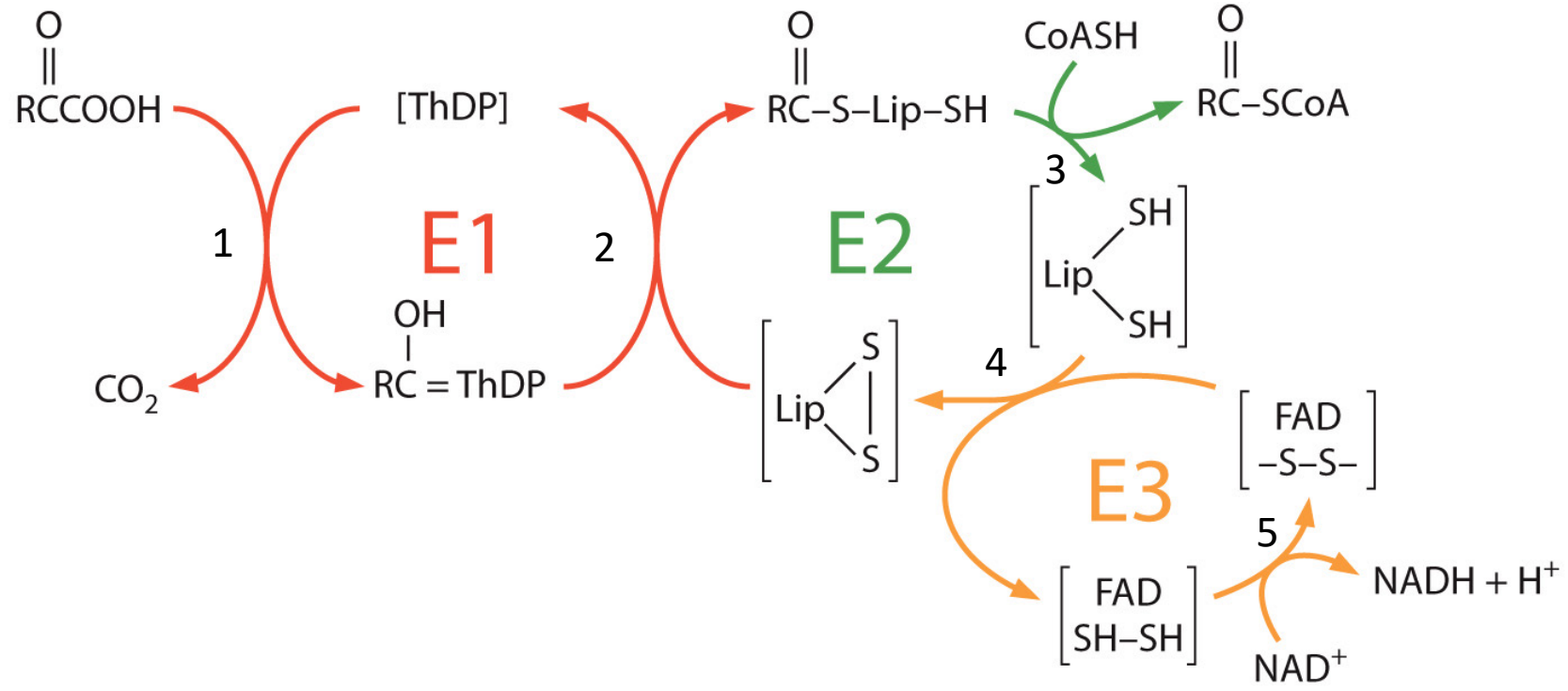


# Pyruvate Dehydrogenase Deficiency




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

# 2 oxo acid dehydrogenase complexes




NET REACTION


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

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

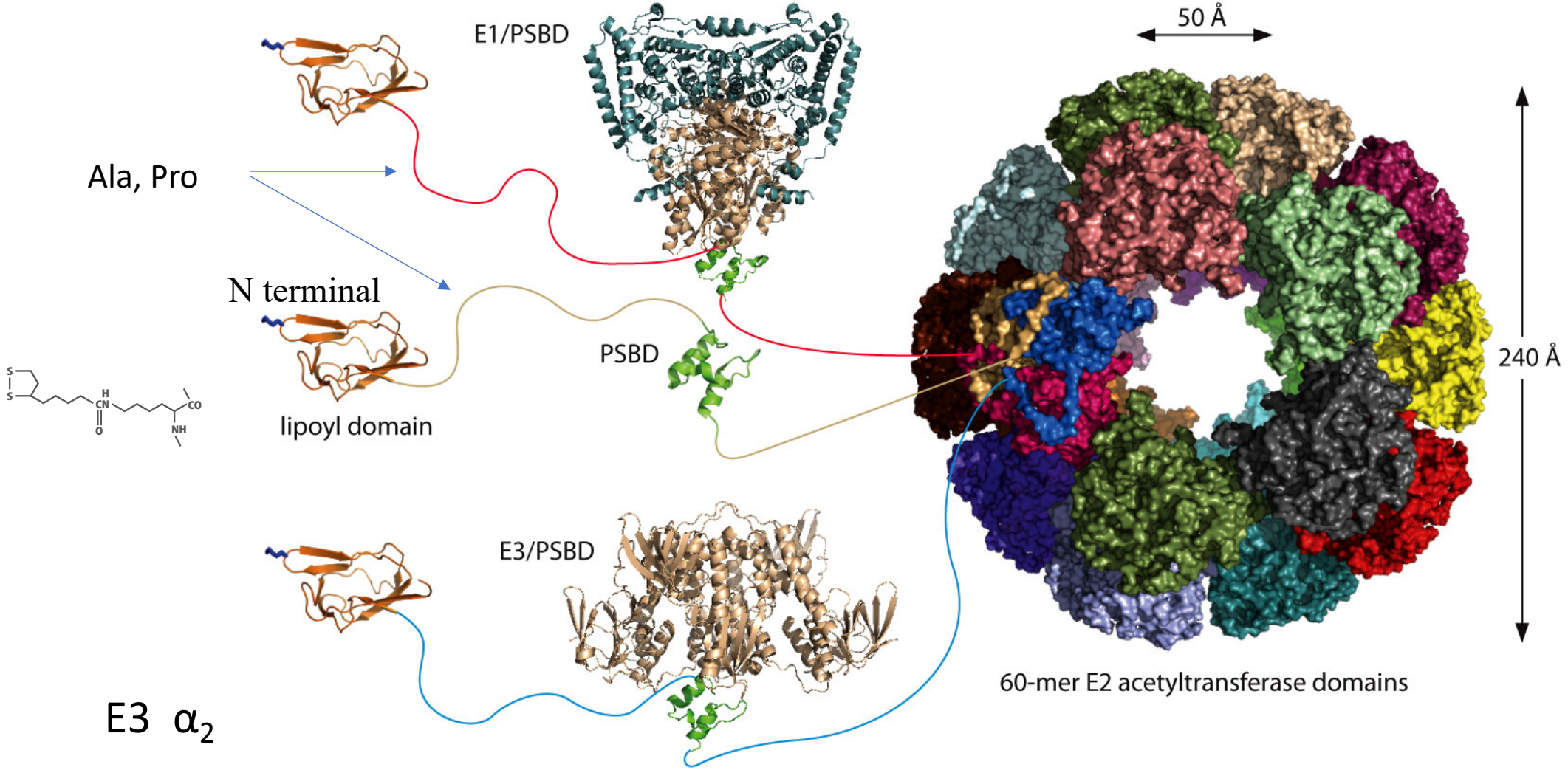
$Lip(SH)_2$, dihydrolipoyl group

# 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 $\alpha_2$

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

# lipoyl arm

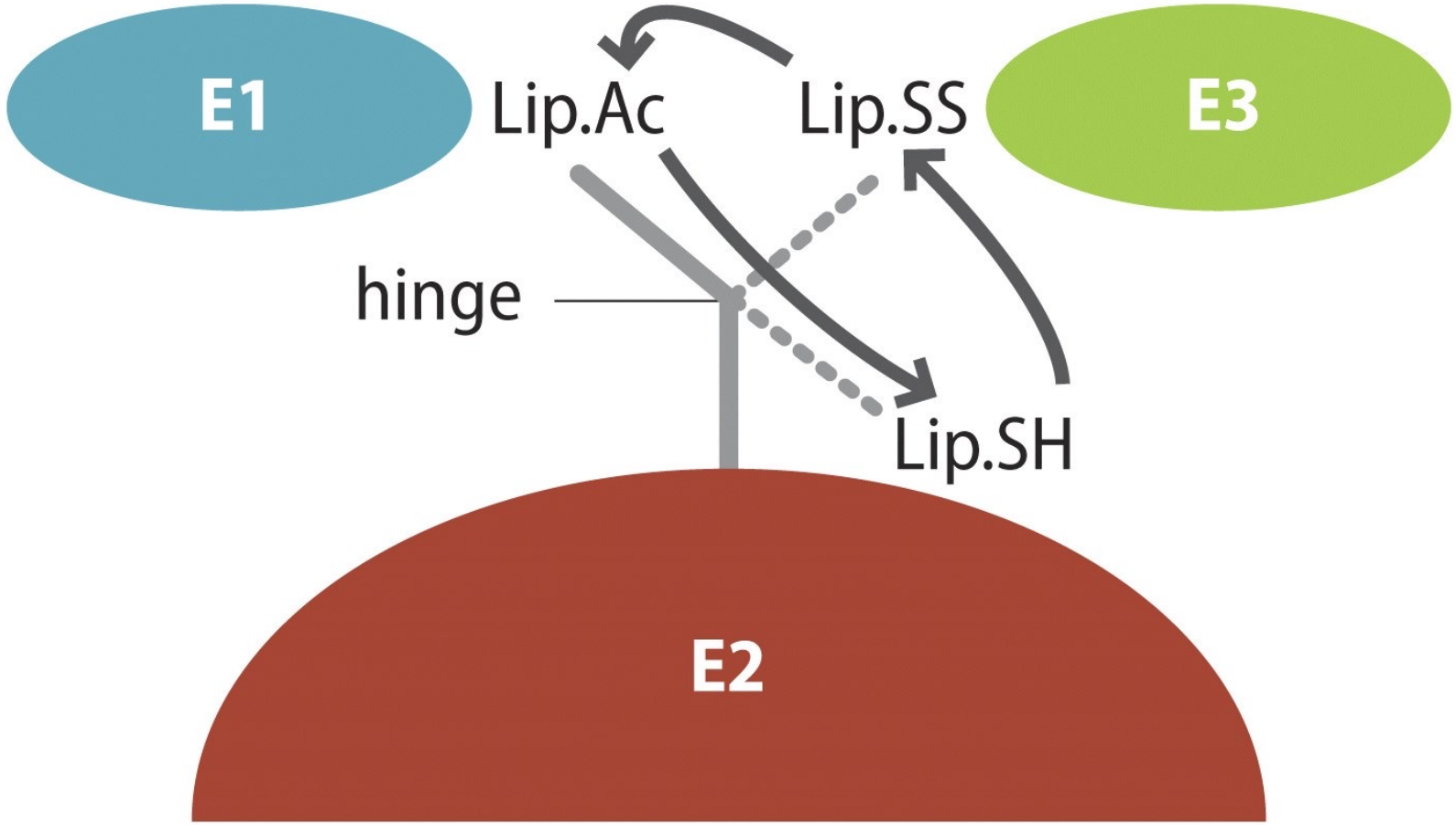



Figure 10.14 How Proteins Work (©2012 Garland Science)

# polyproline II helix, hinged sticky arm

**Pro/Ala**


Figure 4.25 How Proteins Work (©2012 Garland Science)

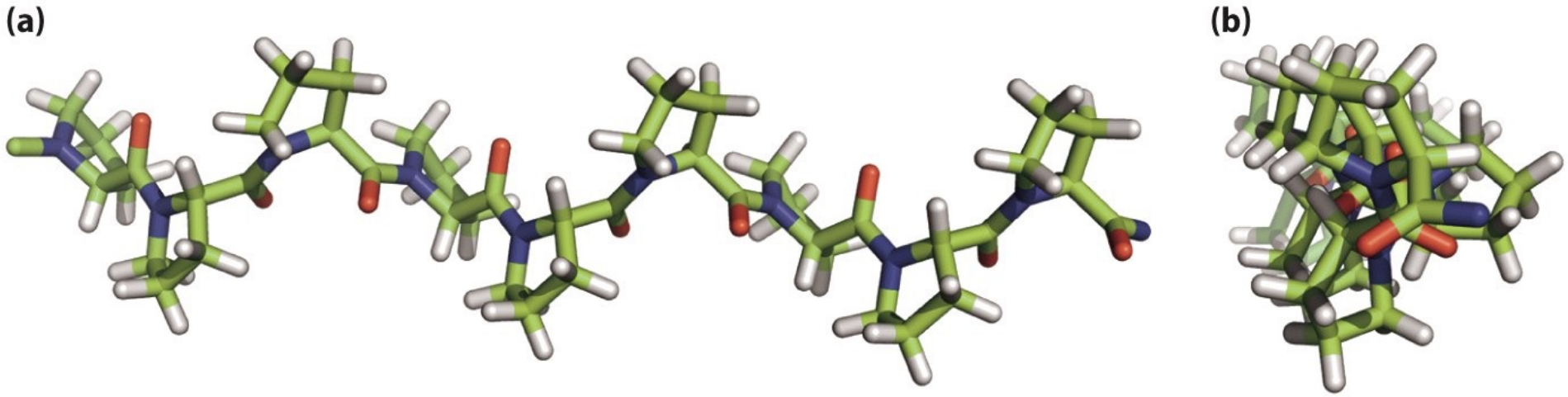



Figure 4.24 How Proteins Work (©2012 Garland Science)

# E2 polypeptide chain in *E. coli*

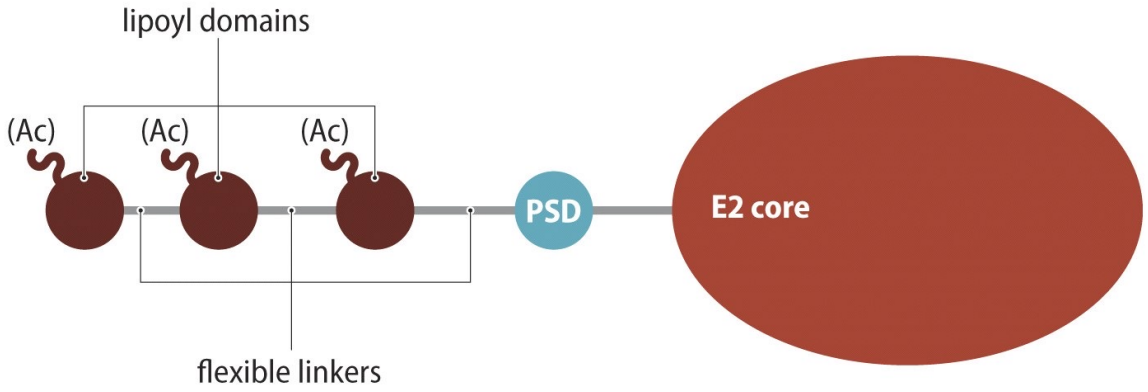



Figure 10.13 How Proteins Work (©2012 Garland Science)

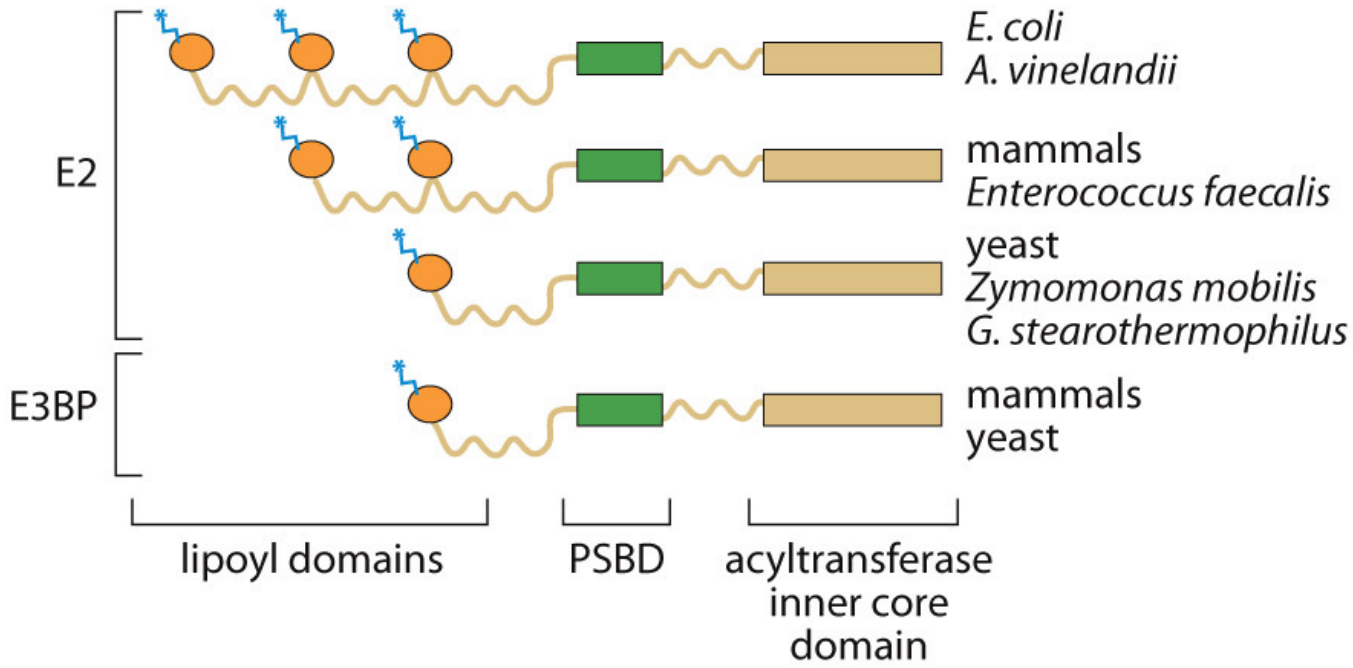



Figure 9.8 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# Active-site coupling

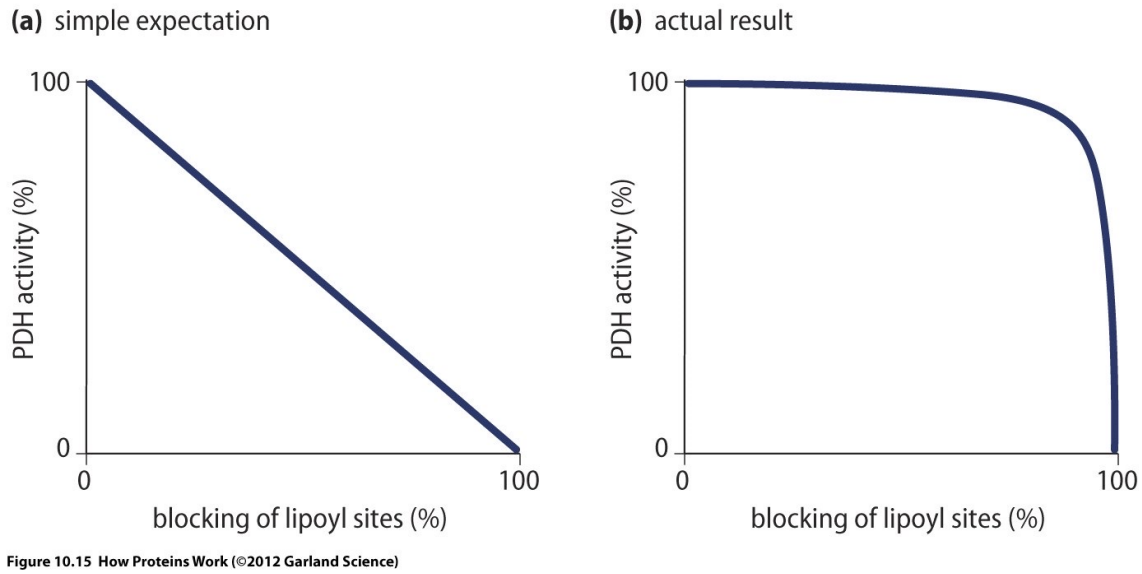
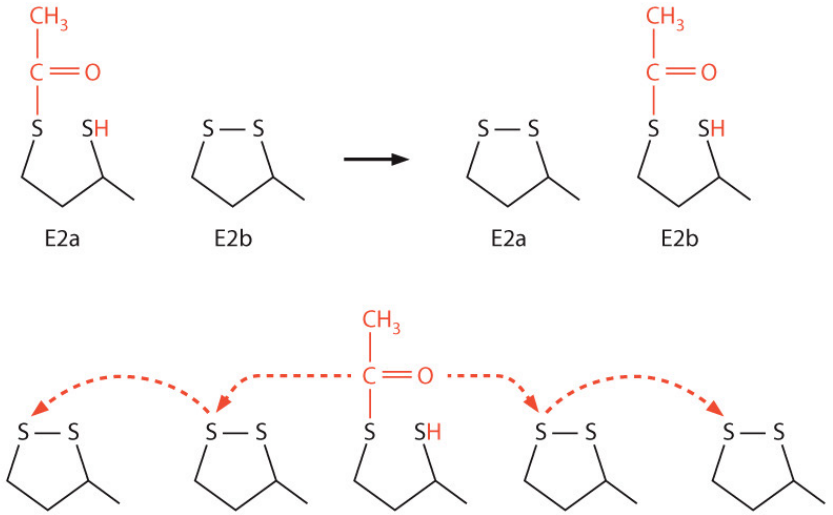



Figure 10.15 How Proteins Work (©2012 Garland Science)



# active-site coupling

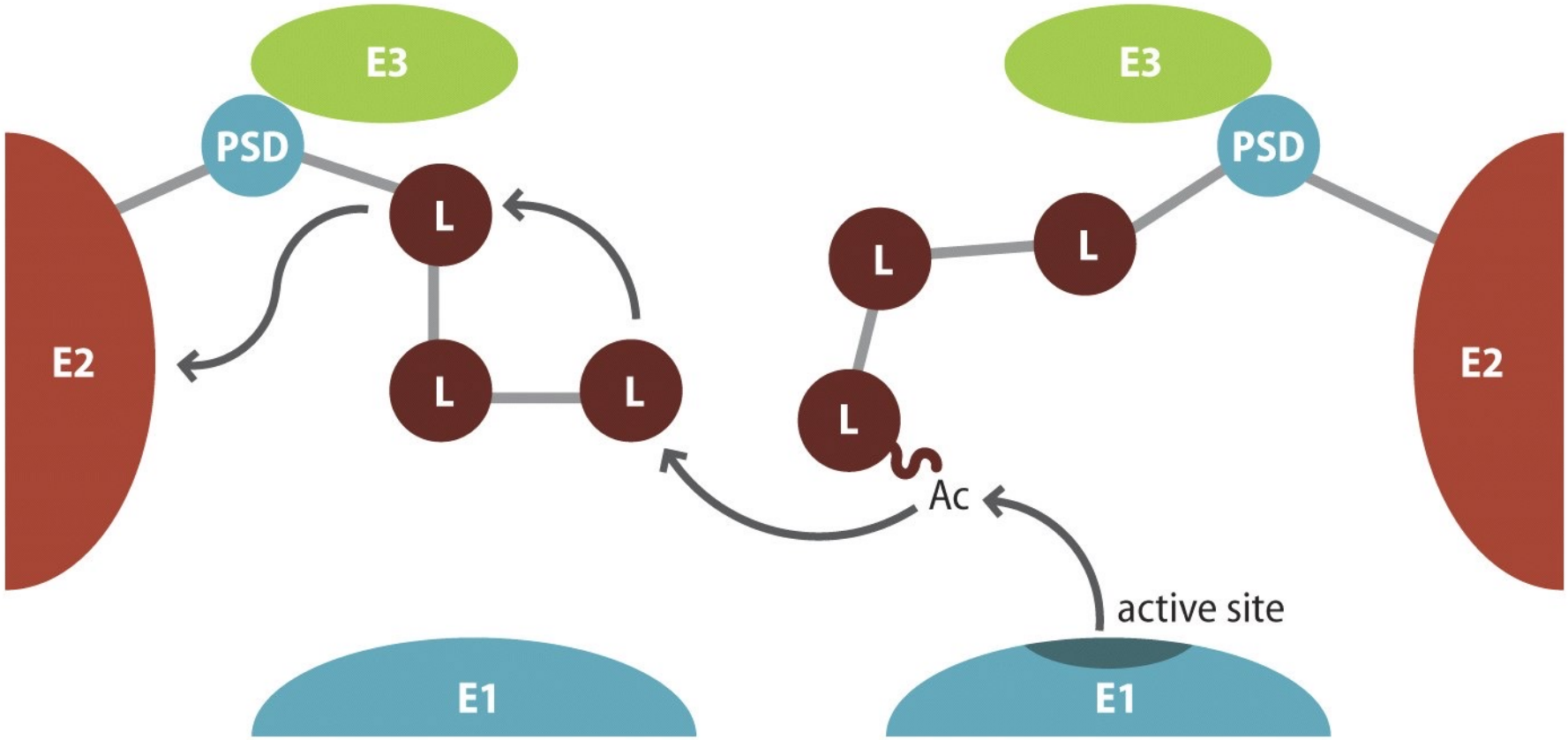
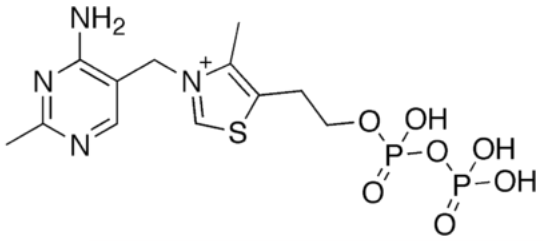
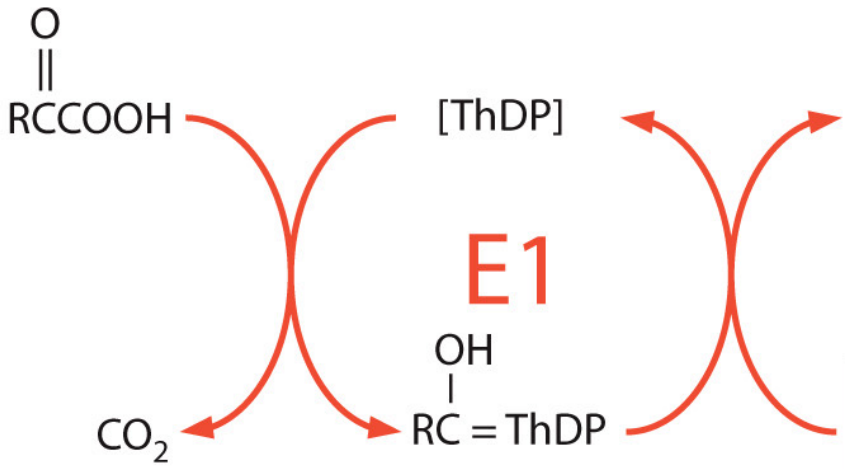


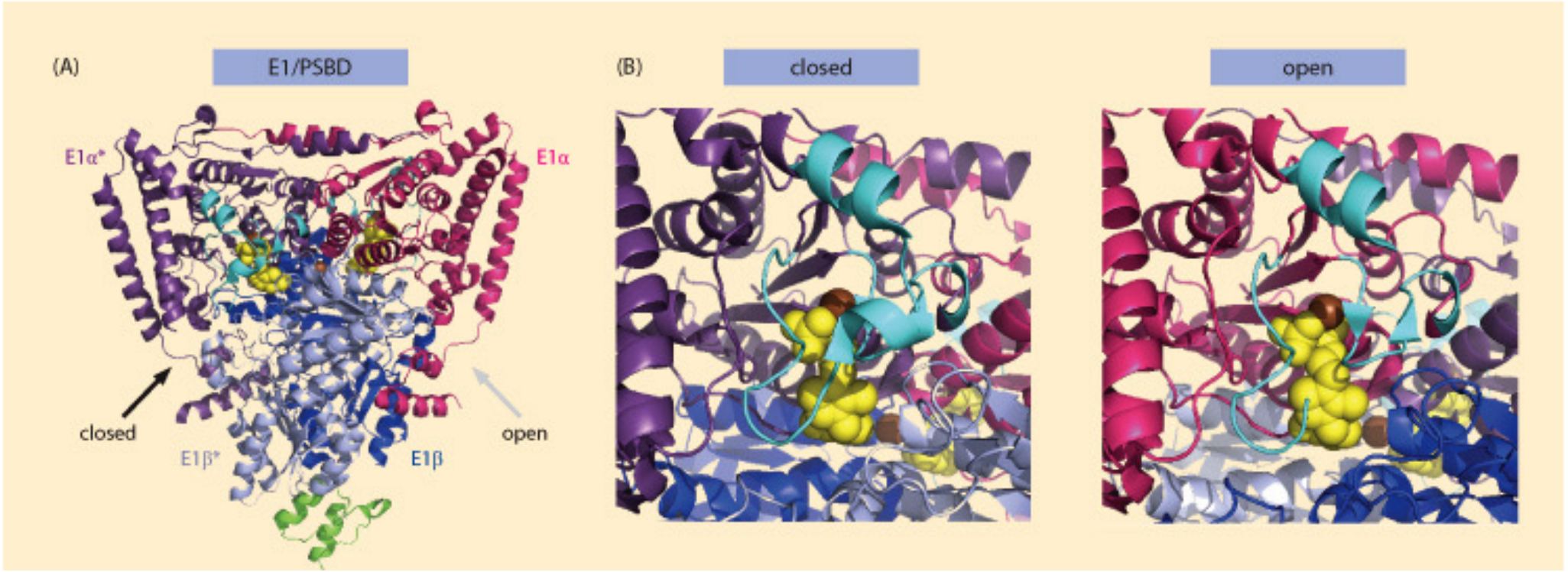

Figure 10.16 How Proteins Work (©2012 Garland Science)

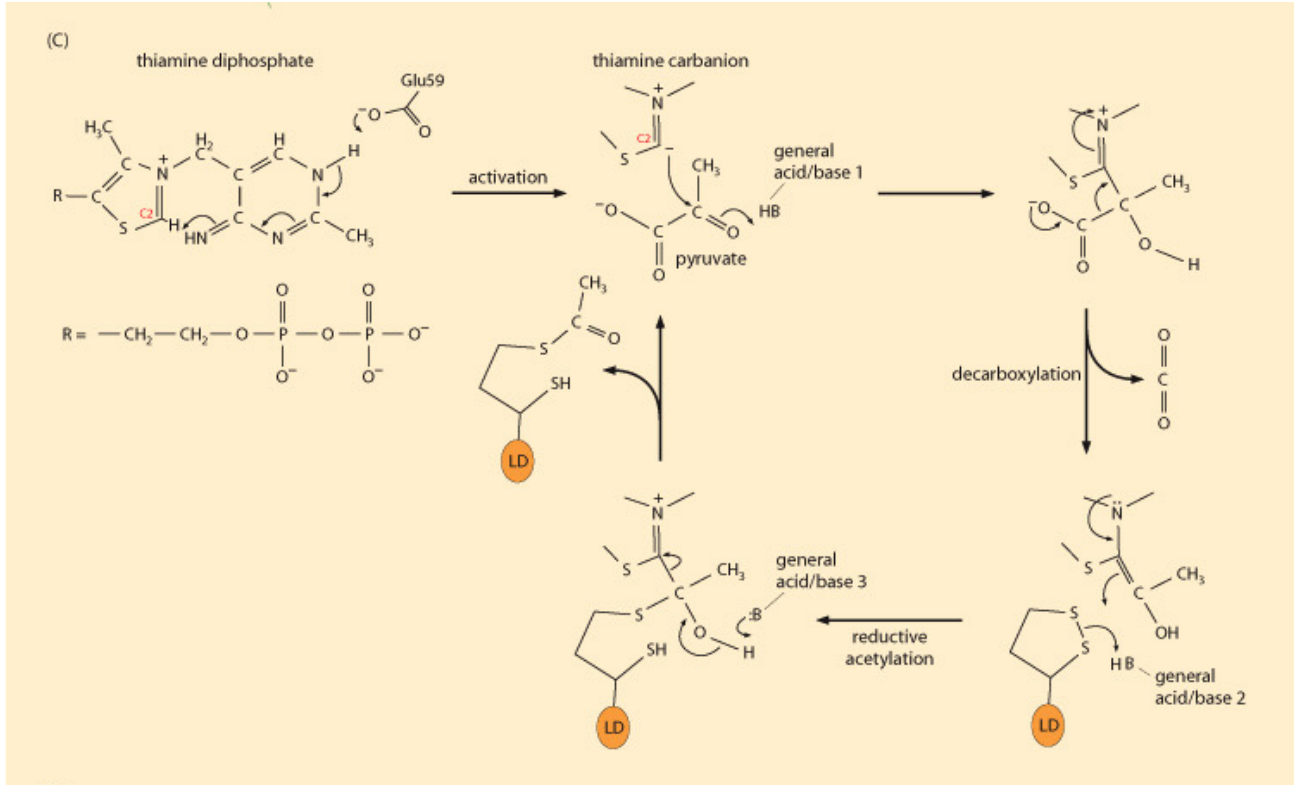
# E1 subunit

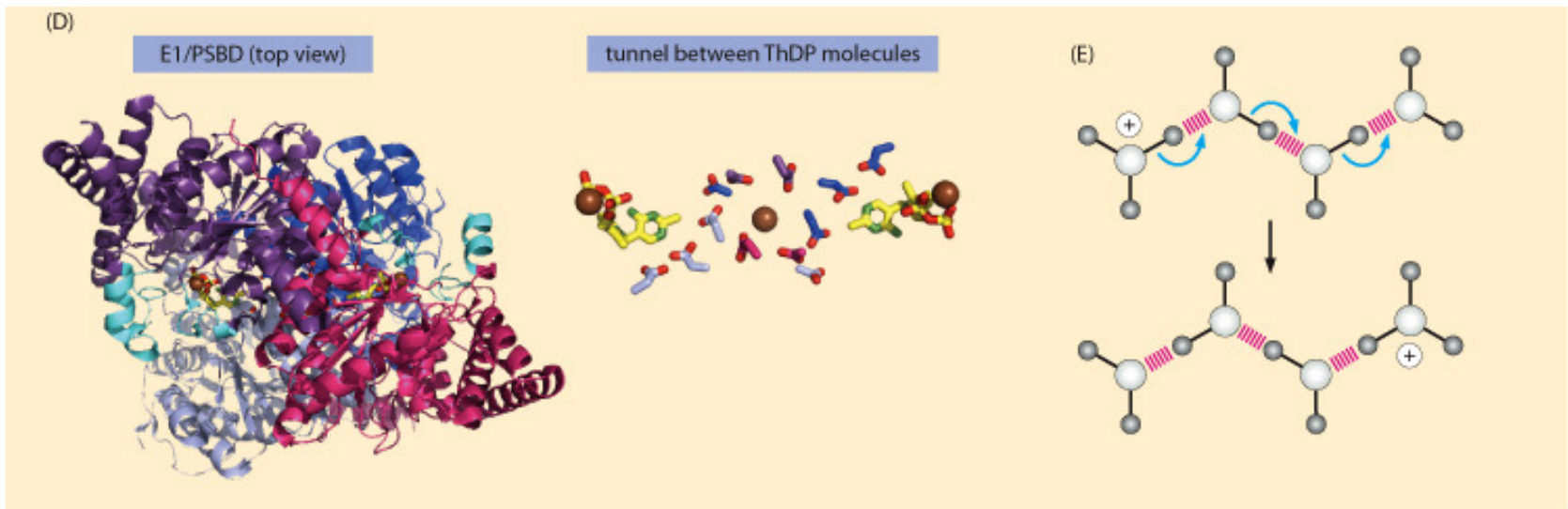
Thiamin diphosphate



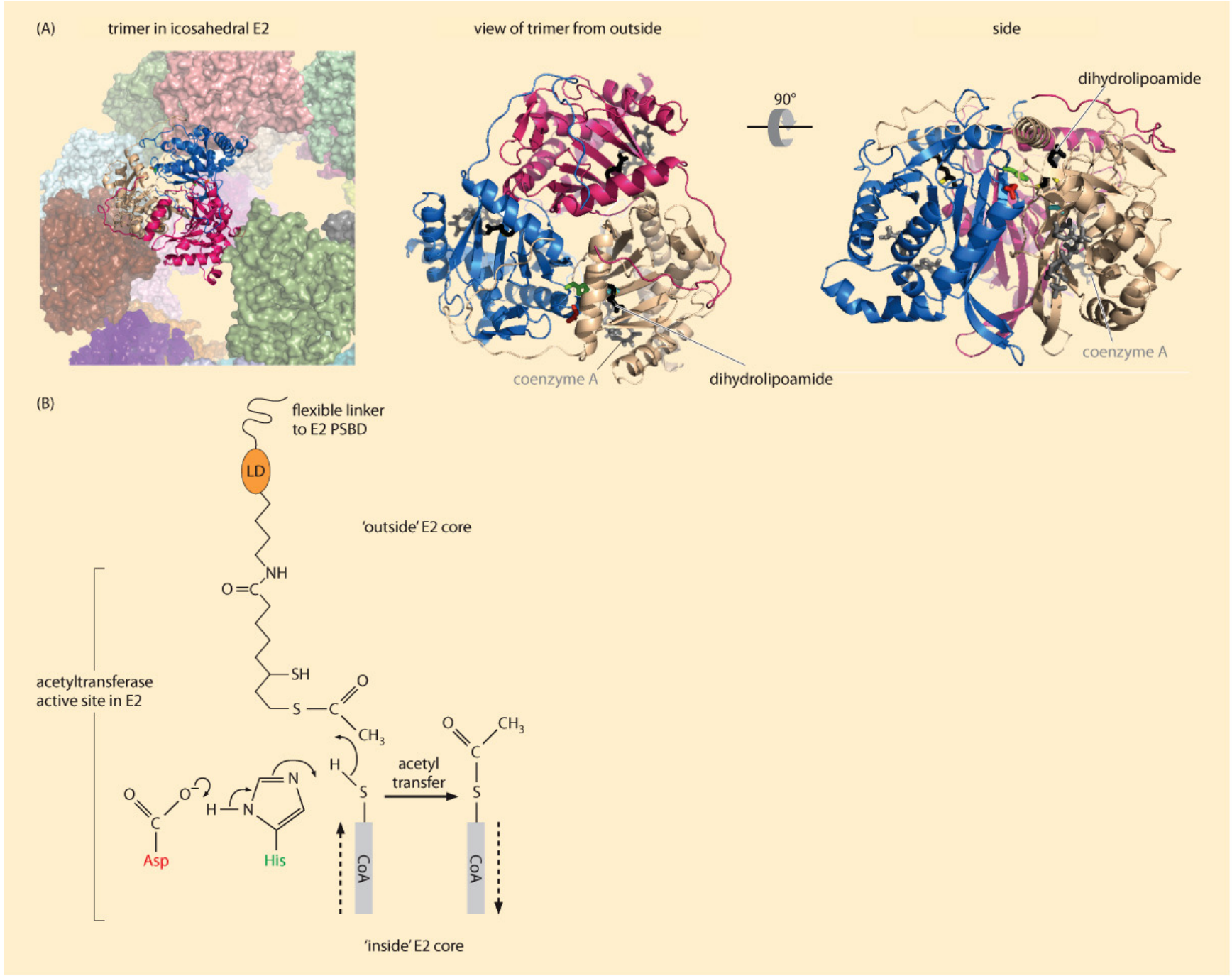





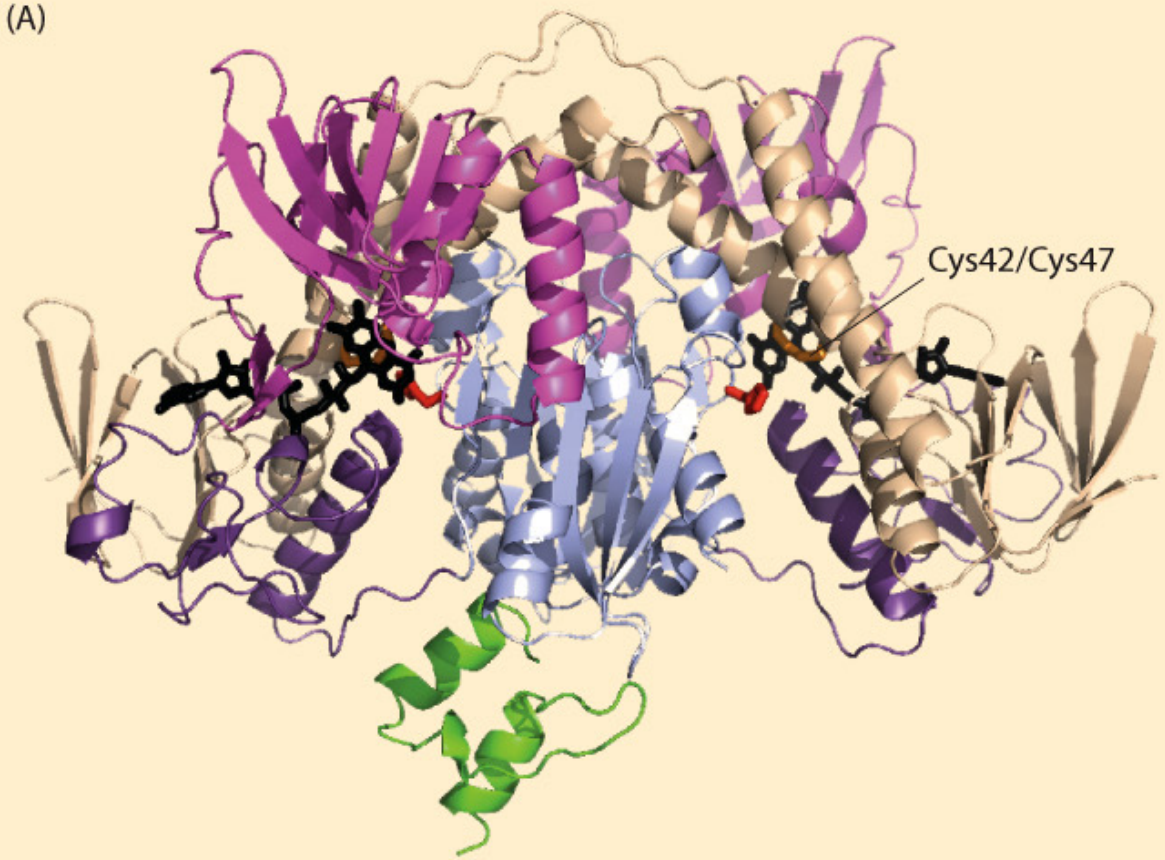

Box 9.2 Figure 9.2.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

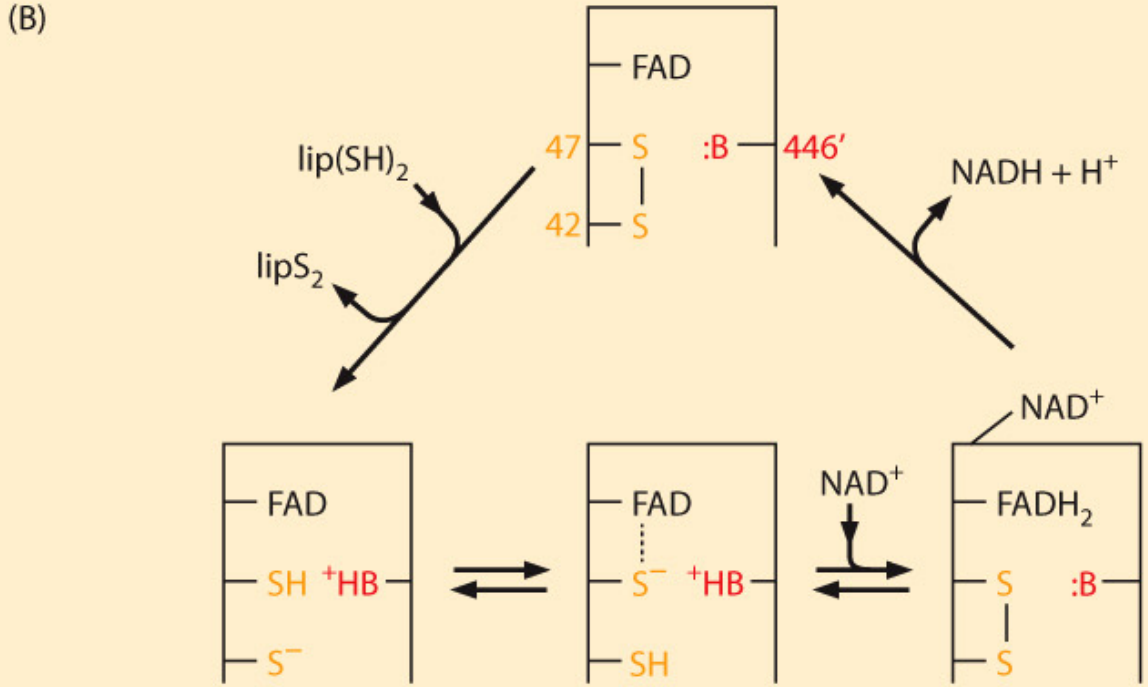
# Mechanism on E2




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

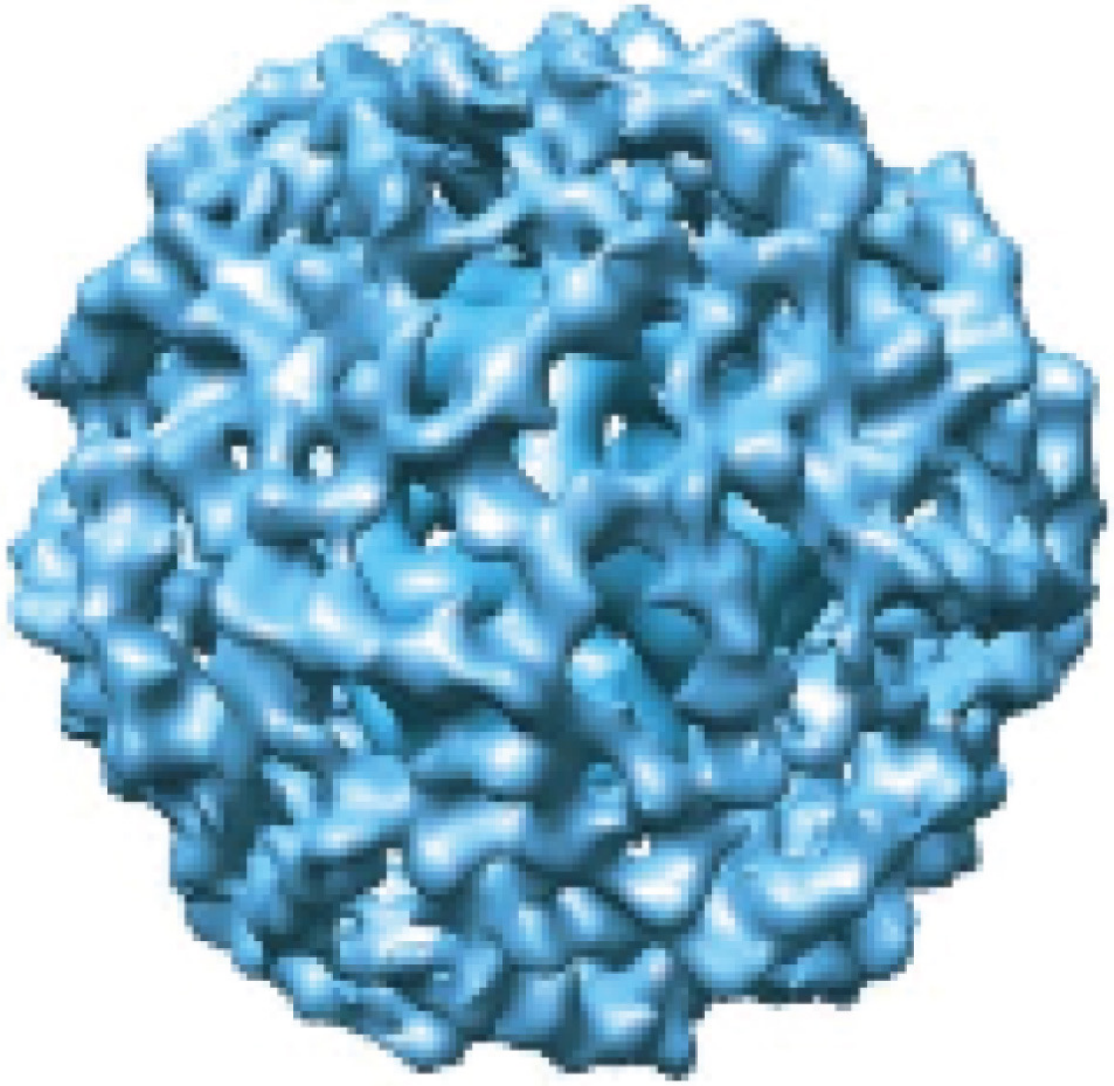
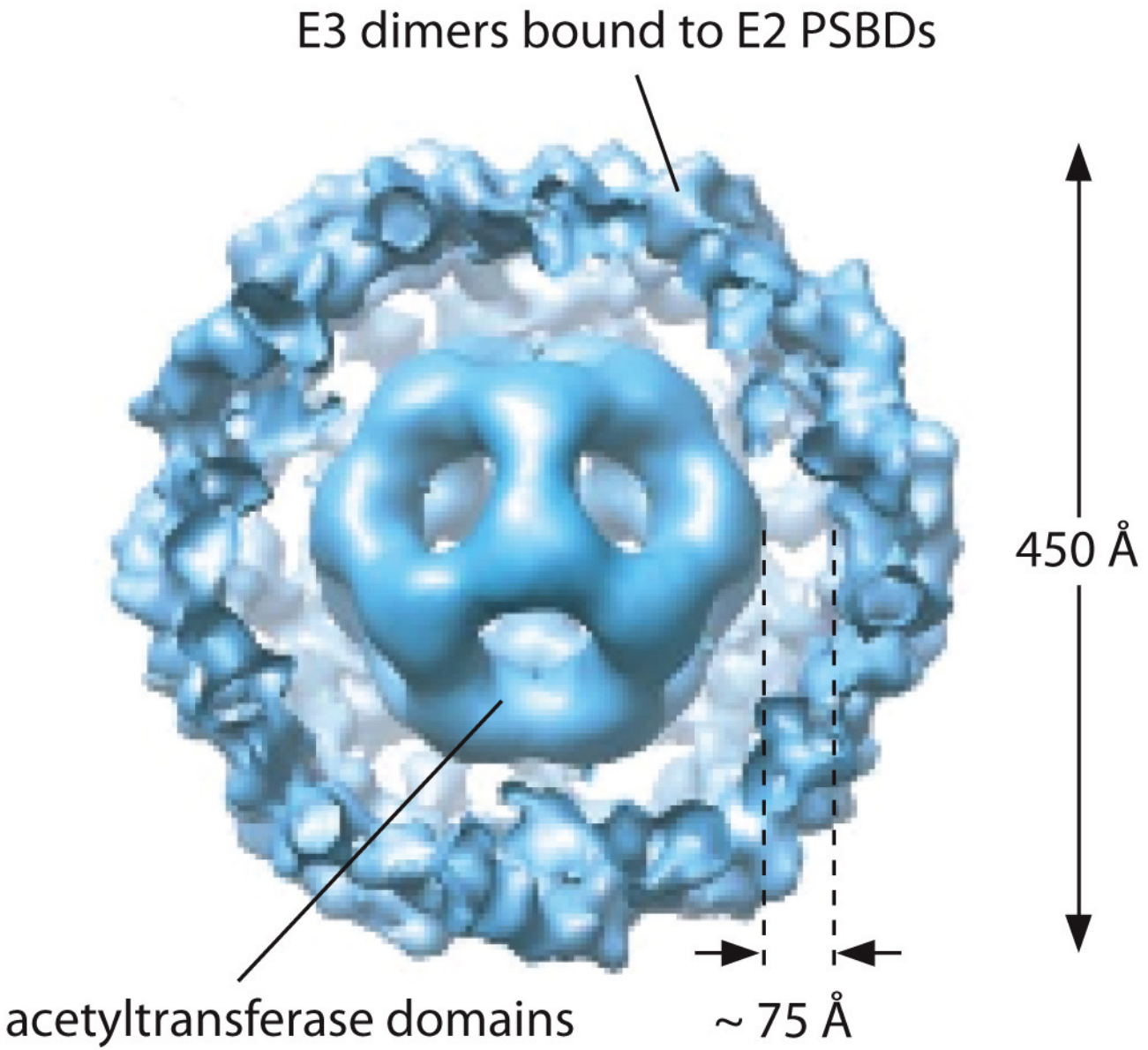
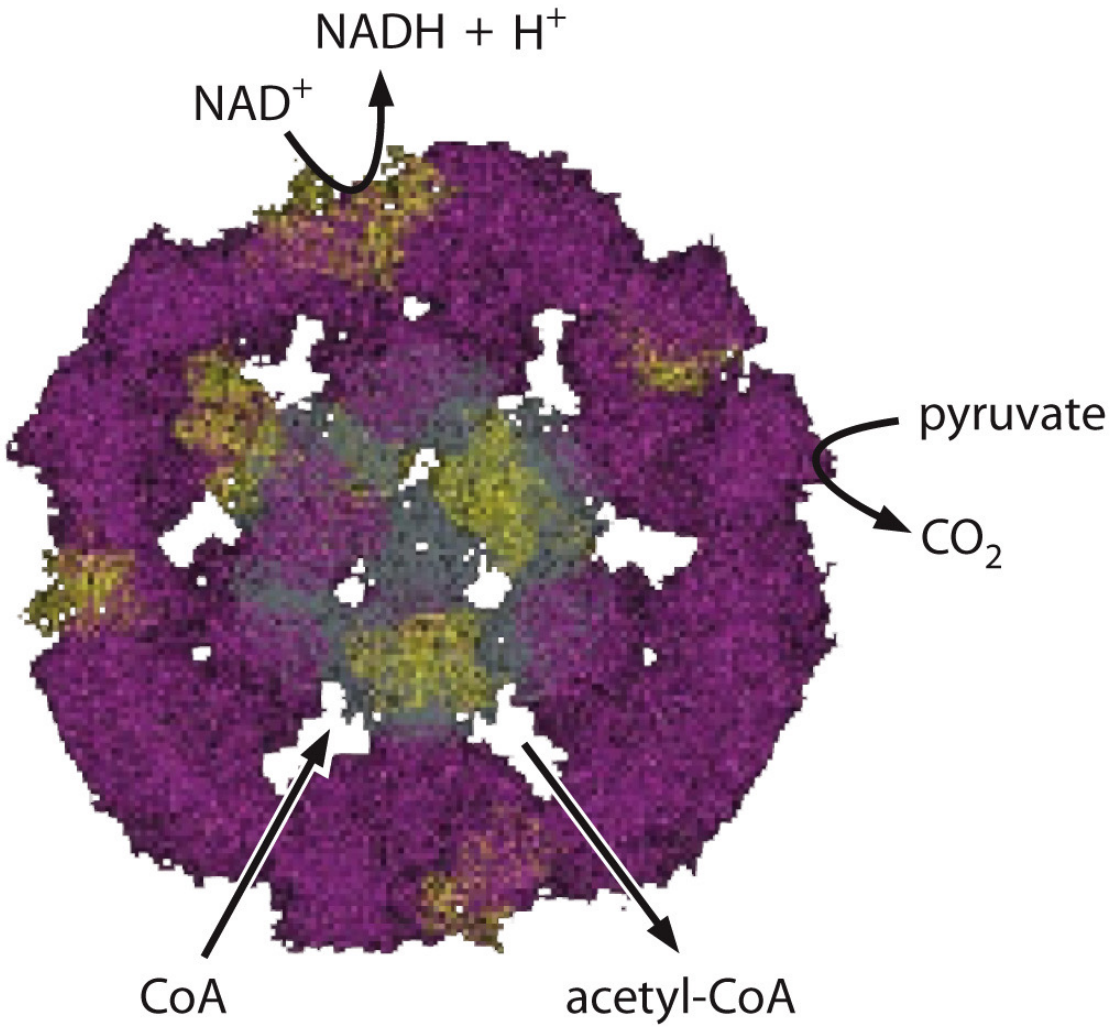


Figure 9.7a Molecular Biology of Assemblies and Machines (© Garland Science 2016)




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

# Model of PDH



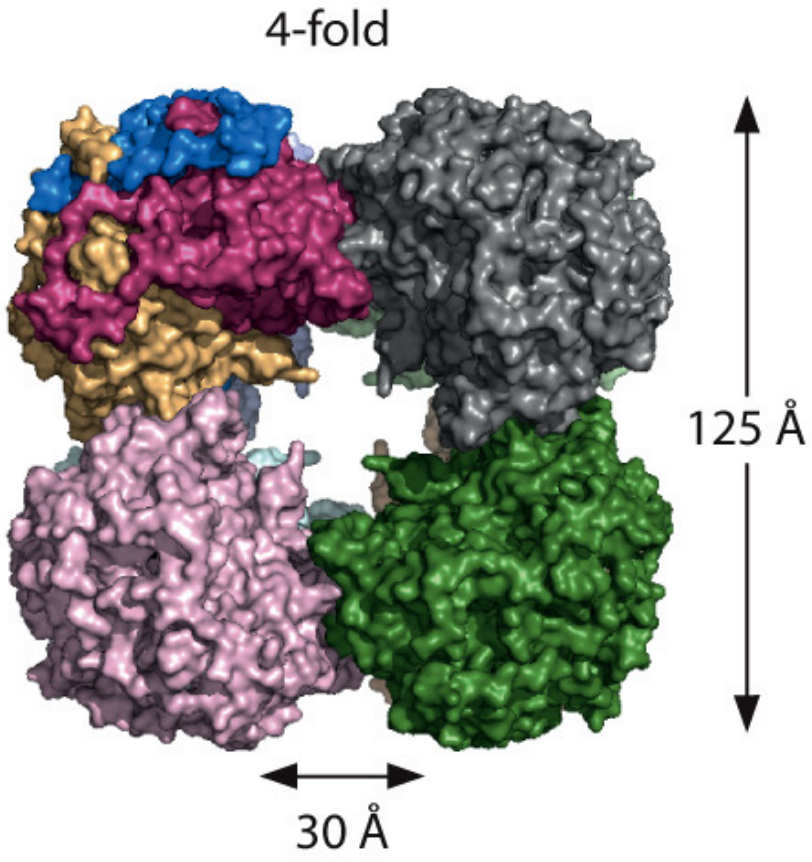
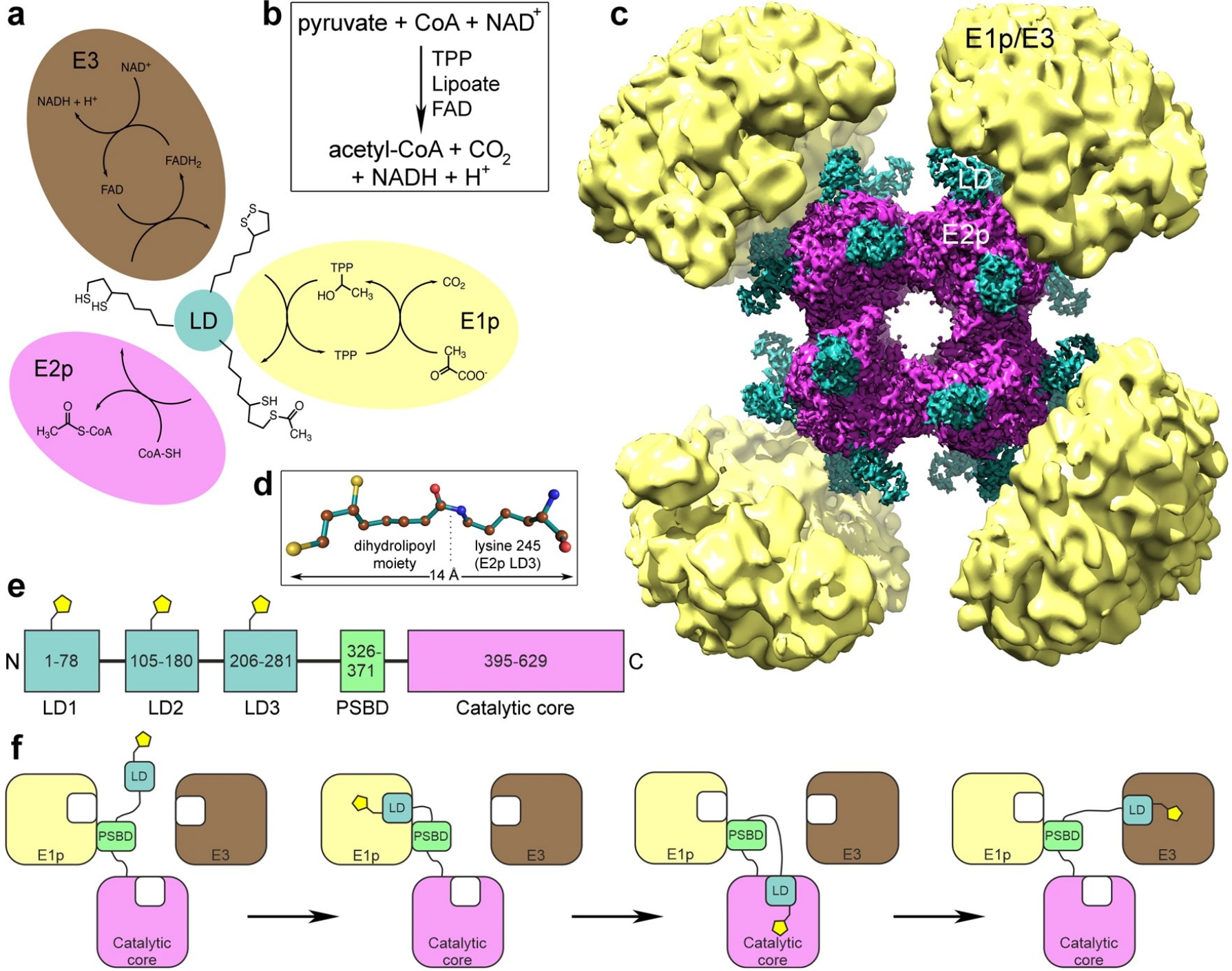
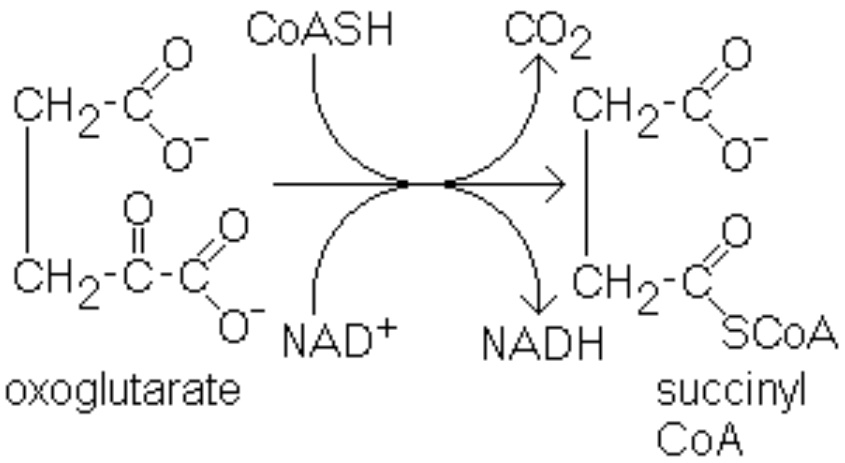



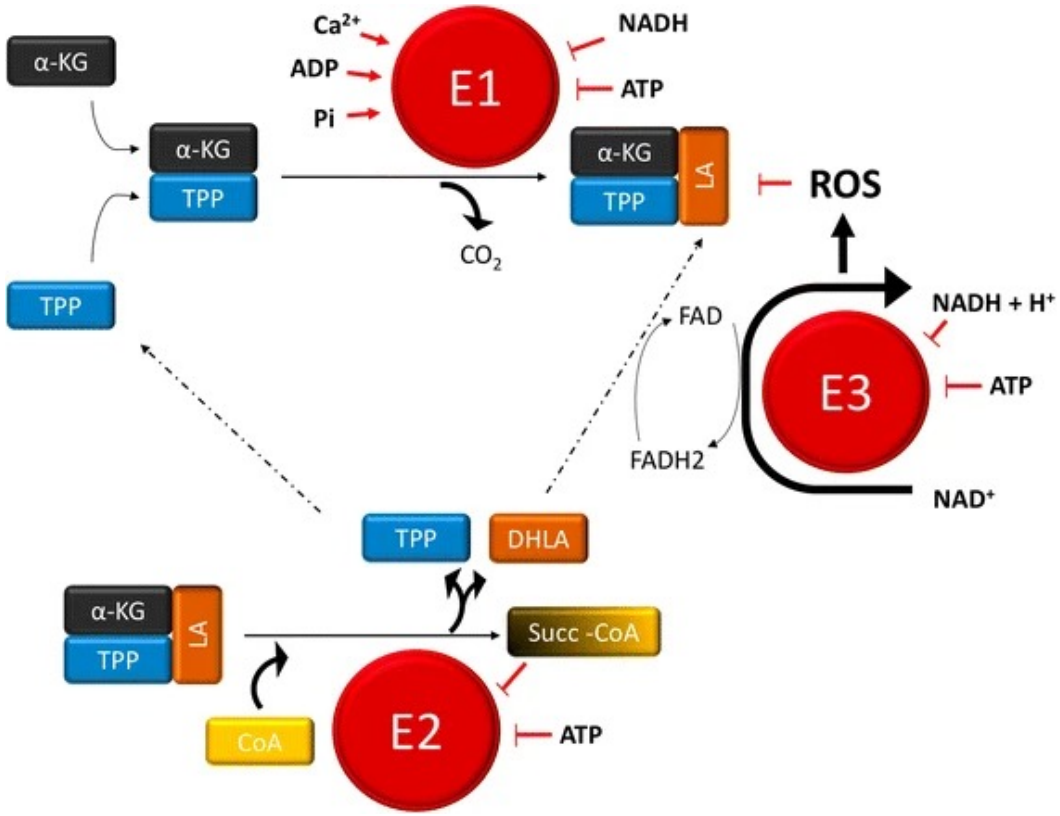

Figure 9.7c Molecular Biology of Assemblies and Machines (© Garland Science 2016)




*Nat Commun* **12**, 5277 (2021).

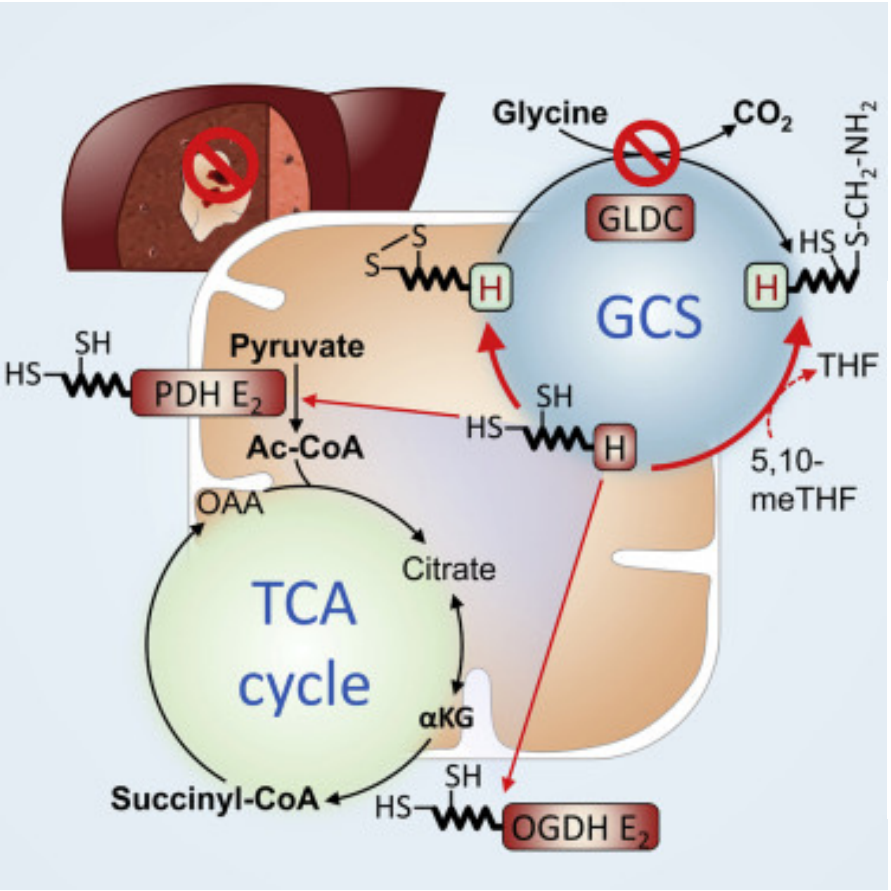
# a-ketoglutarate dehydrogenase

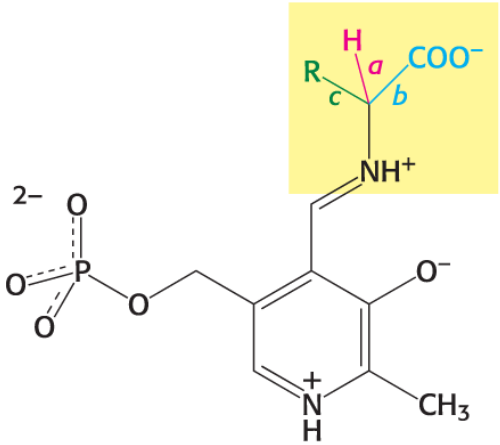


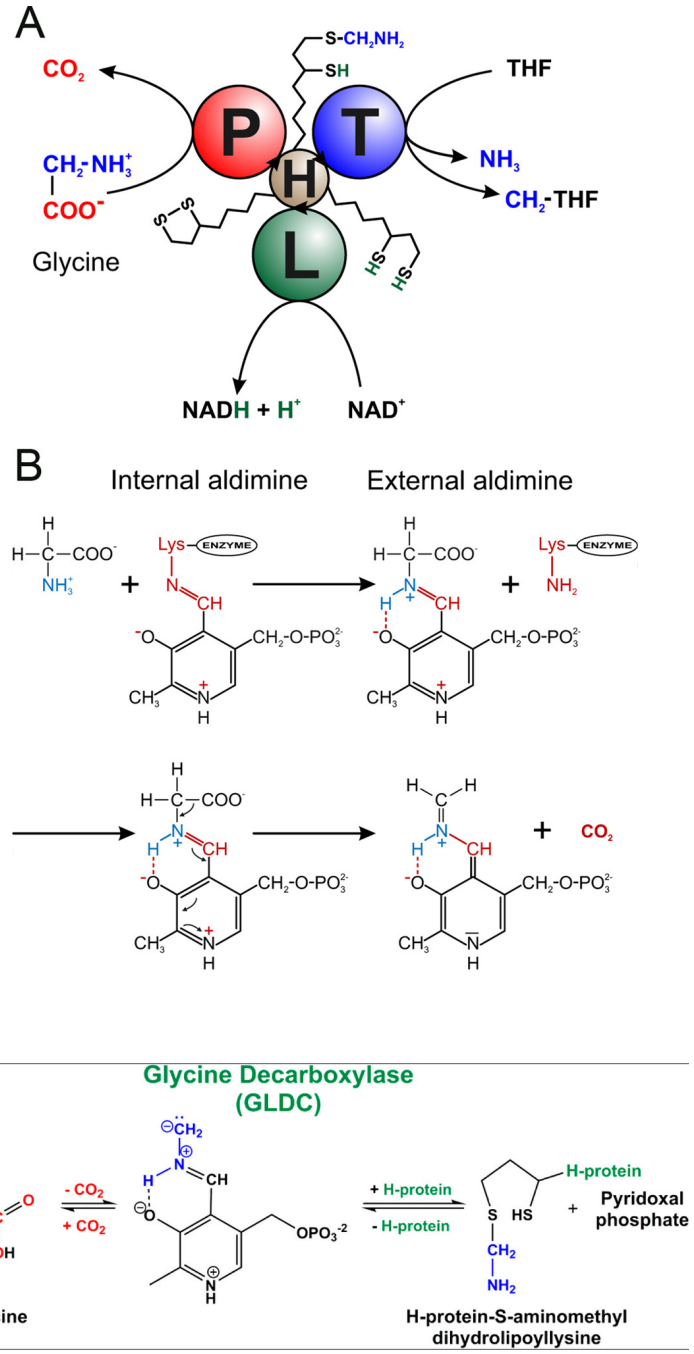

Its E1 and E2 domains are homologous with those of PDH,
E3 domain (which regenerates E2 and therefore does not interact directly with the ketoacid) is identical;

# glycine decarboxylase



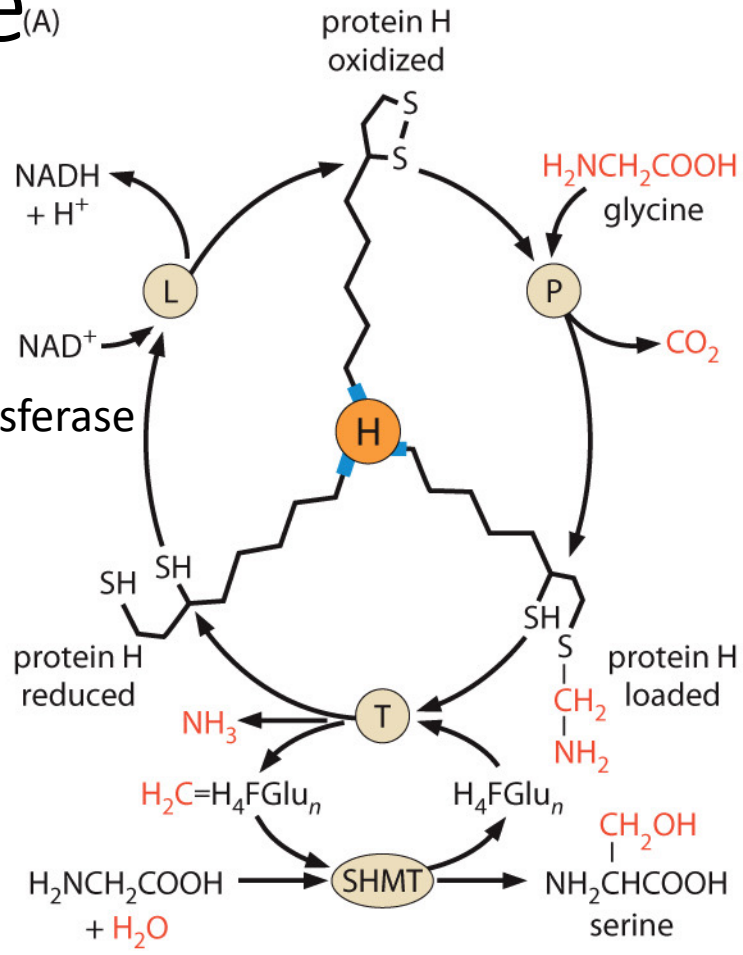



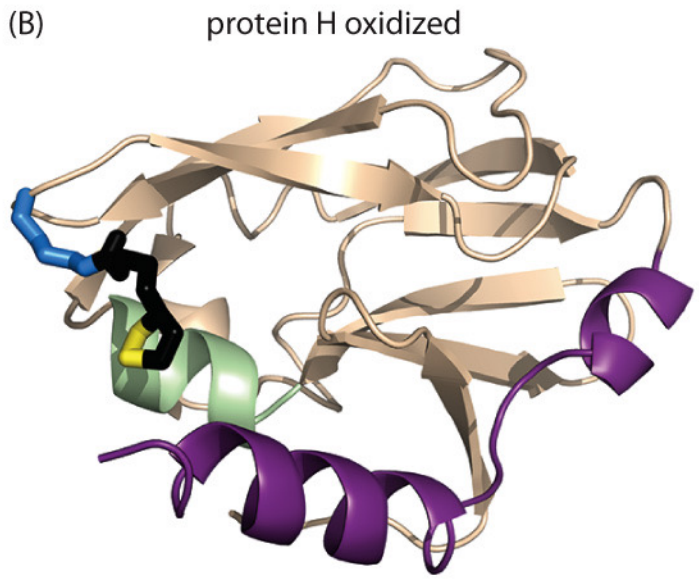
Berg et al., *Biochemistry*, 9e, © 2019
W. H. Freeman and Company



# 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



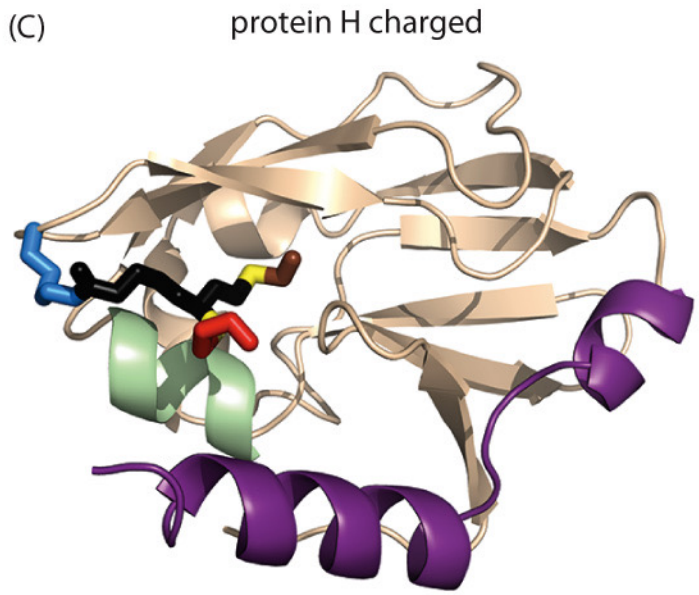
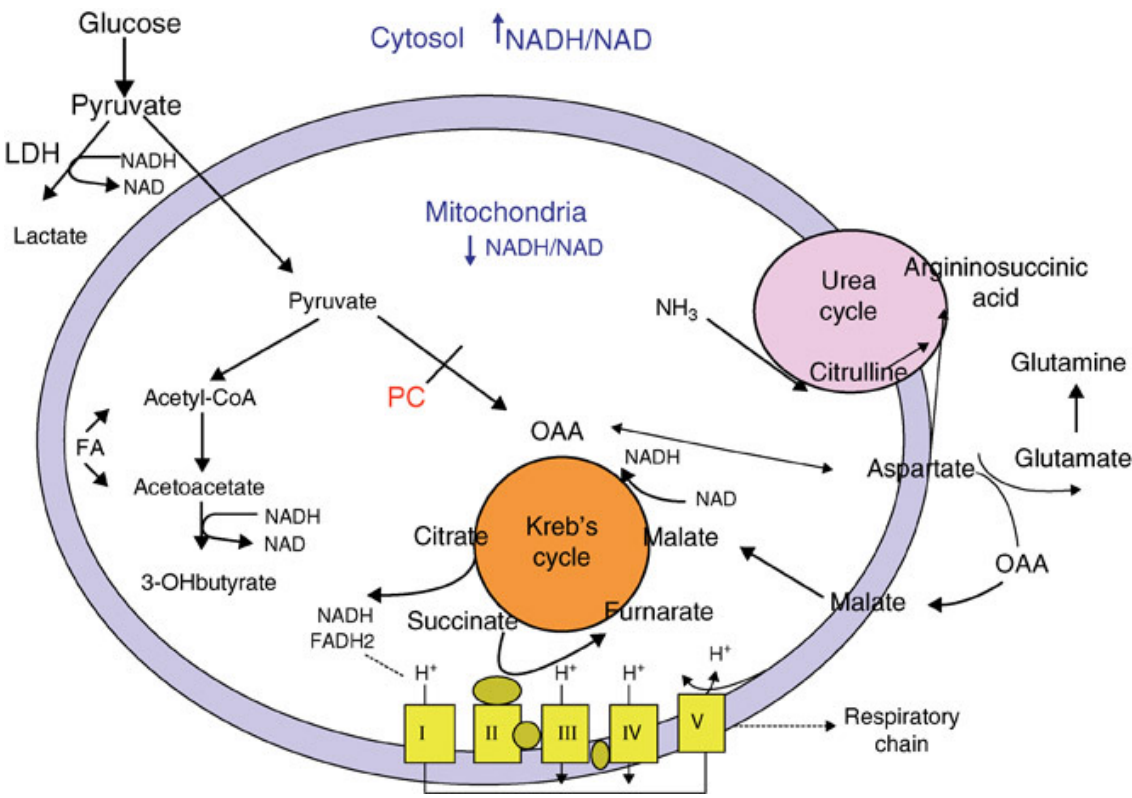
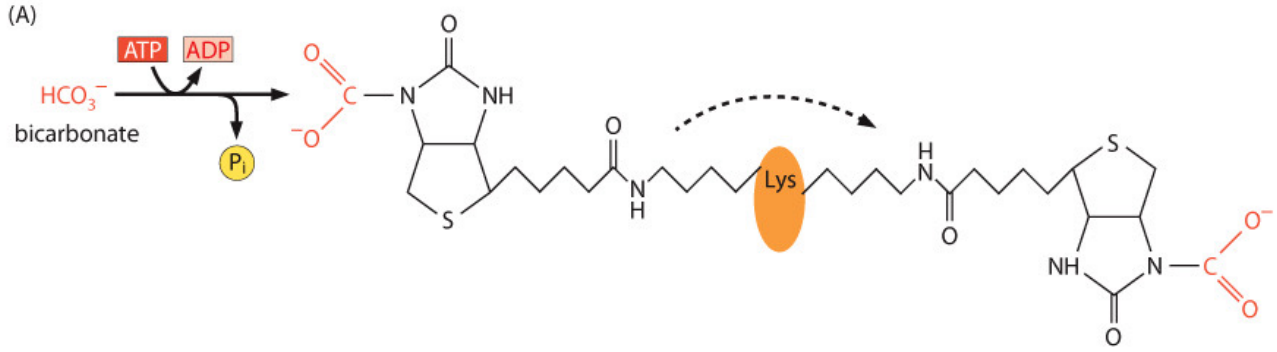
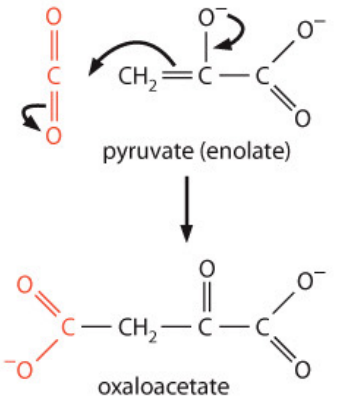




Figure 9.9 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# pyruvate carboxylase



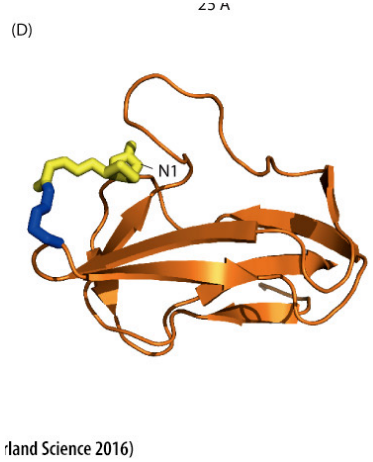




carboxylase domain — BCCP domain, potential arm span of ~30 Å — carboxyltransferase domain

# pyruvate carboxylase

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



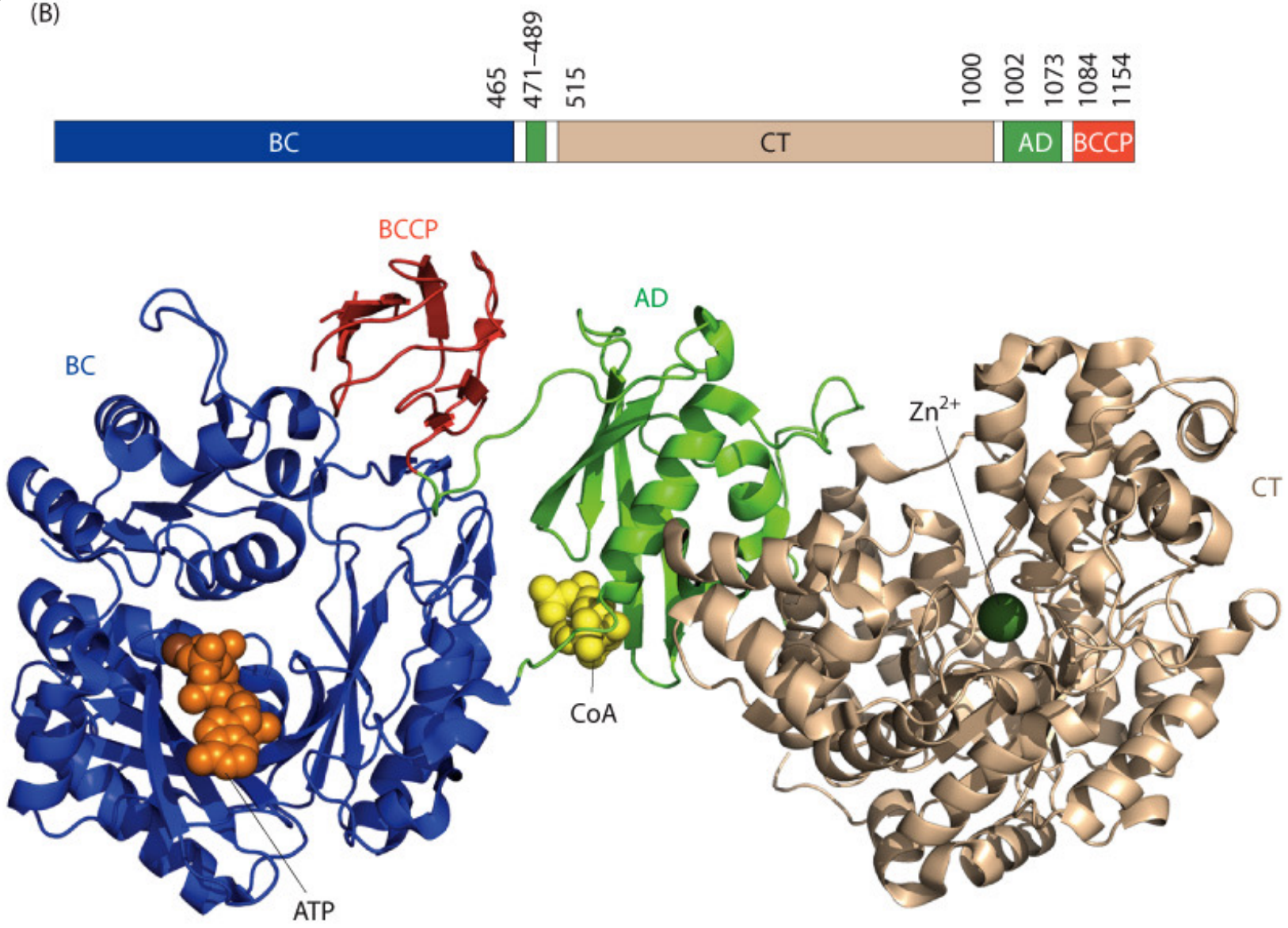




Figure 9.10 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# pyruvate carboxylase

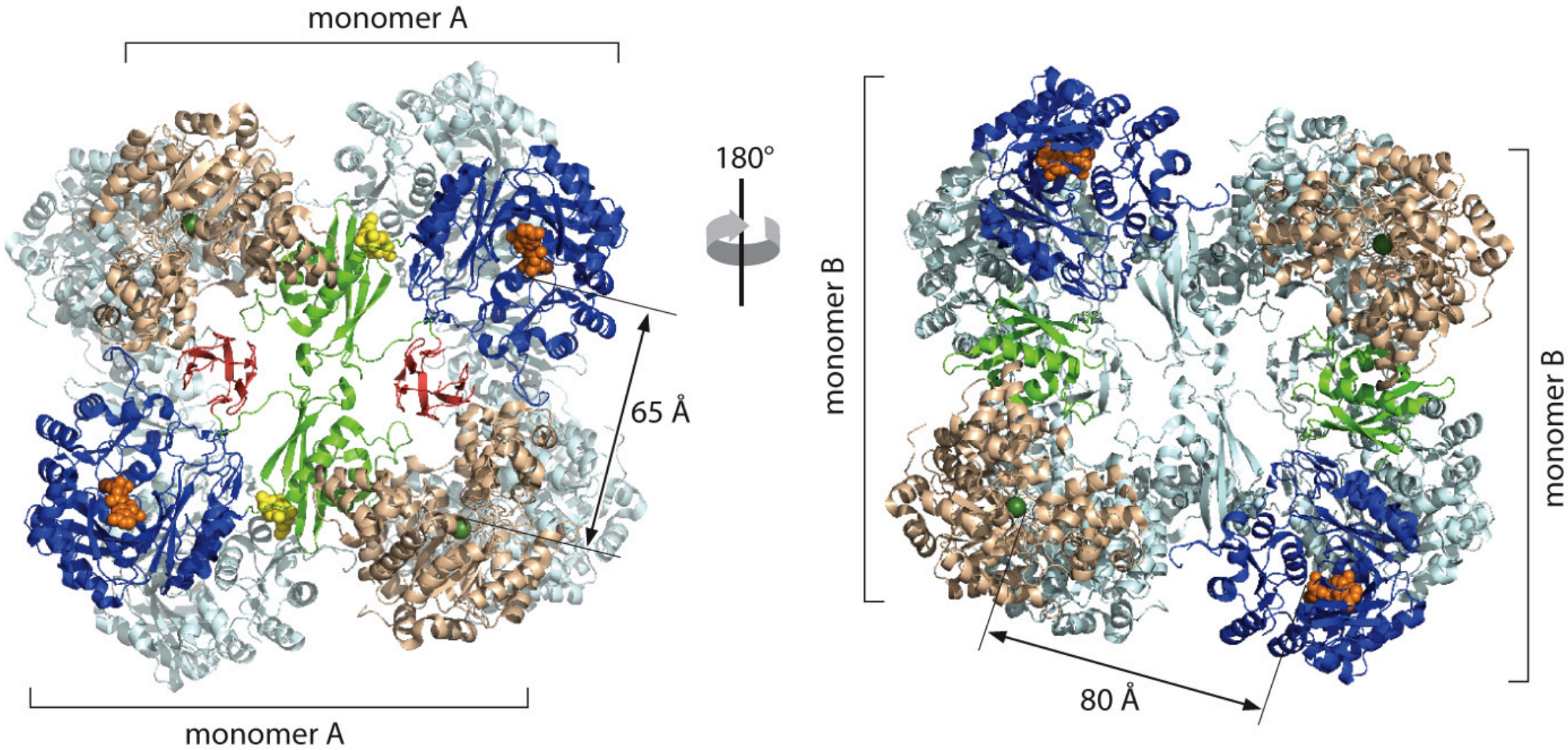
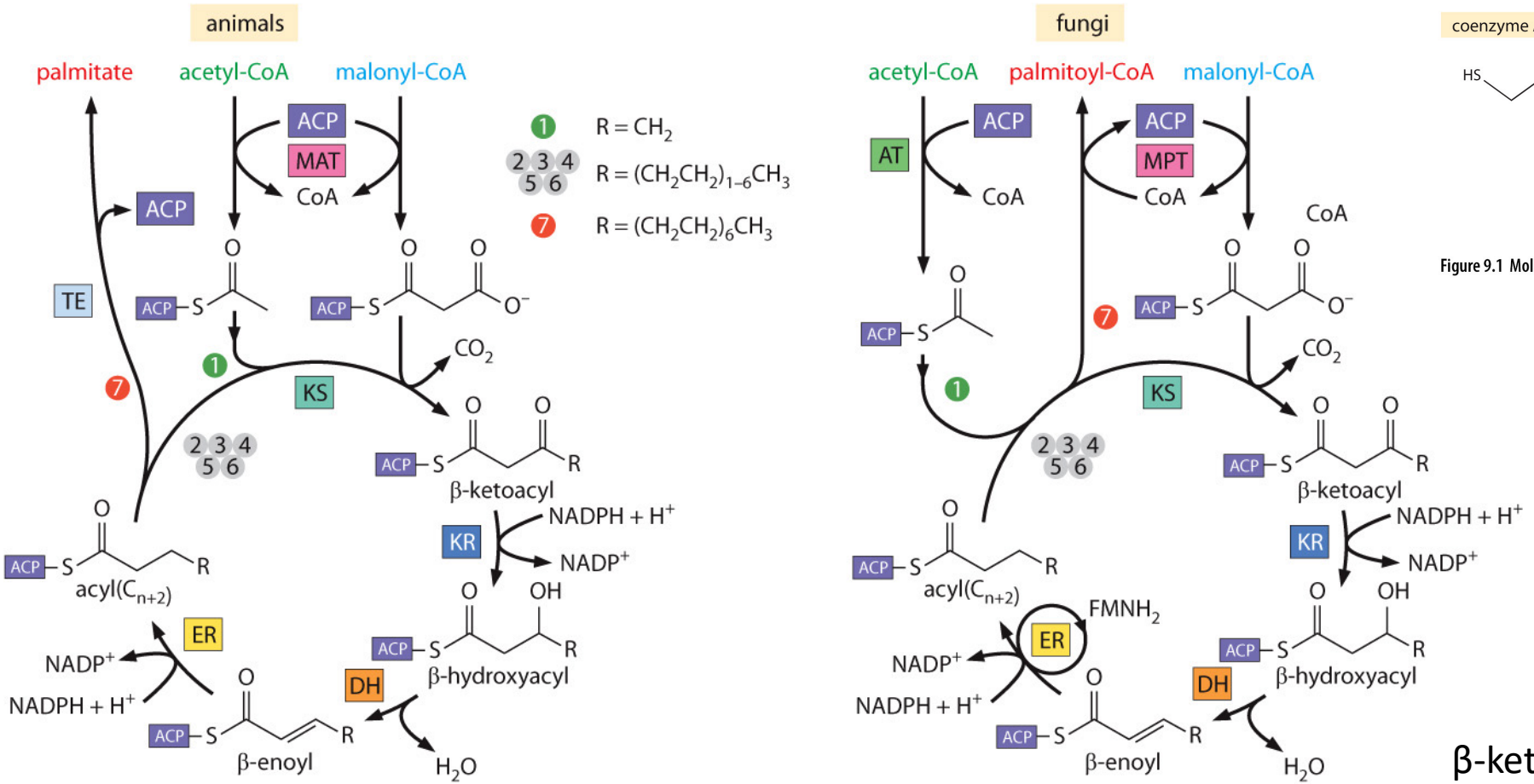



Figure 9.11 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# fatty acid synthases



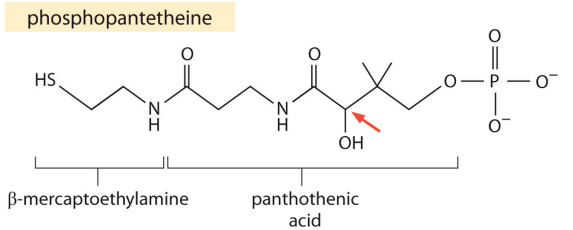




$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO^-$

palmitate

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



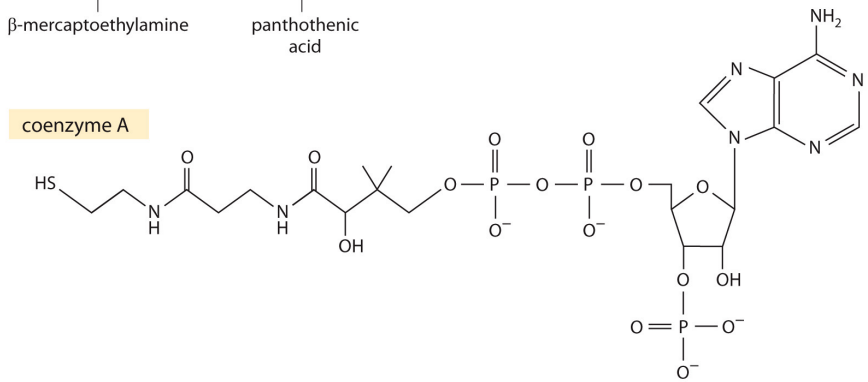




Figure 9.1 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

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

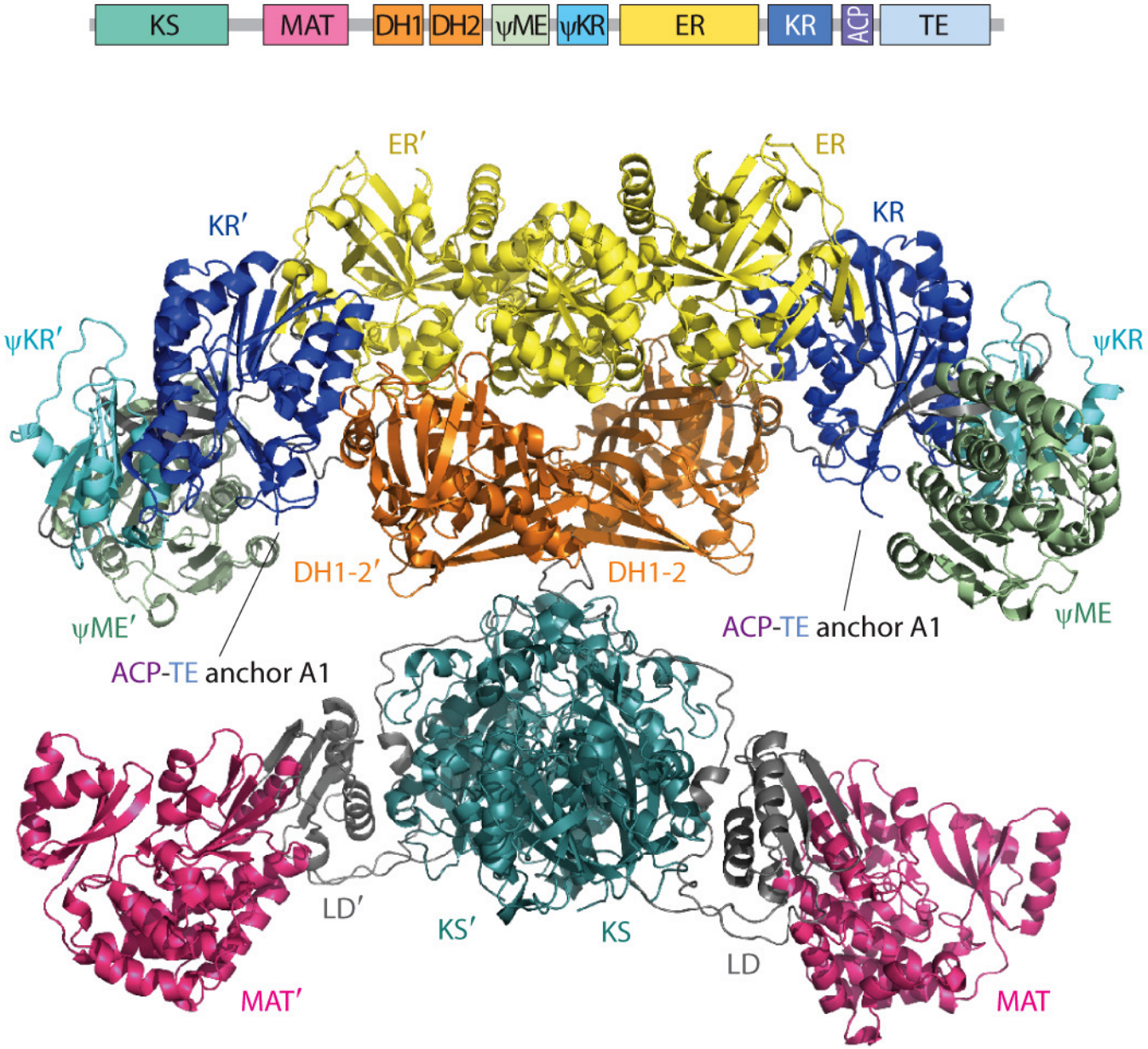



Figure 9.14 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

## animal FAS

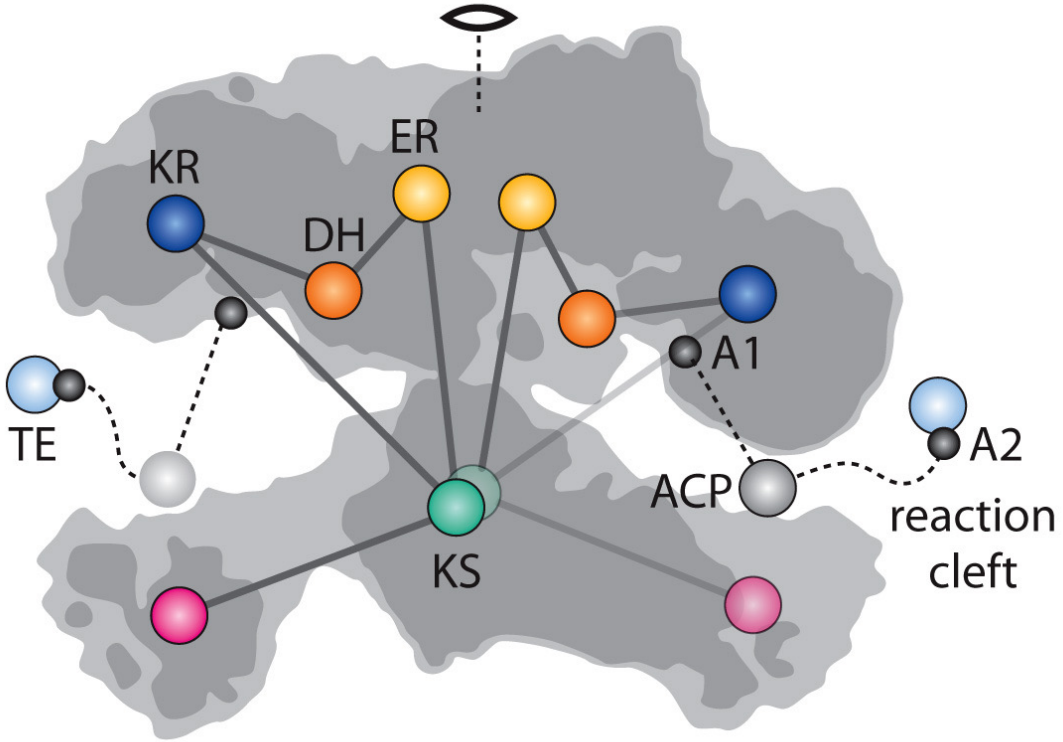



Figure 9.17a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# Structure of ACP and the interaction with KS

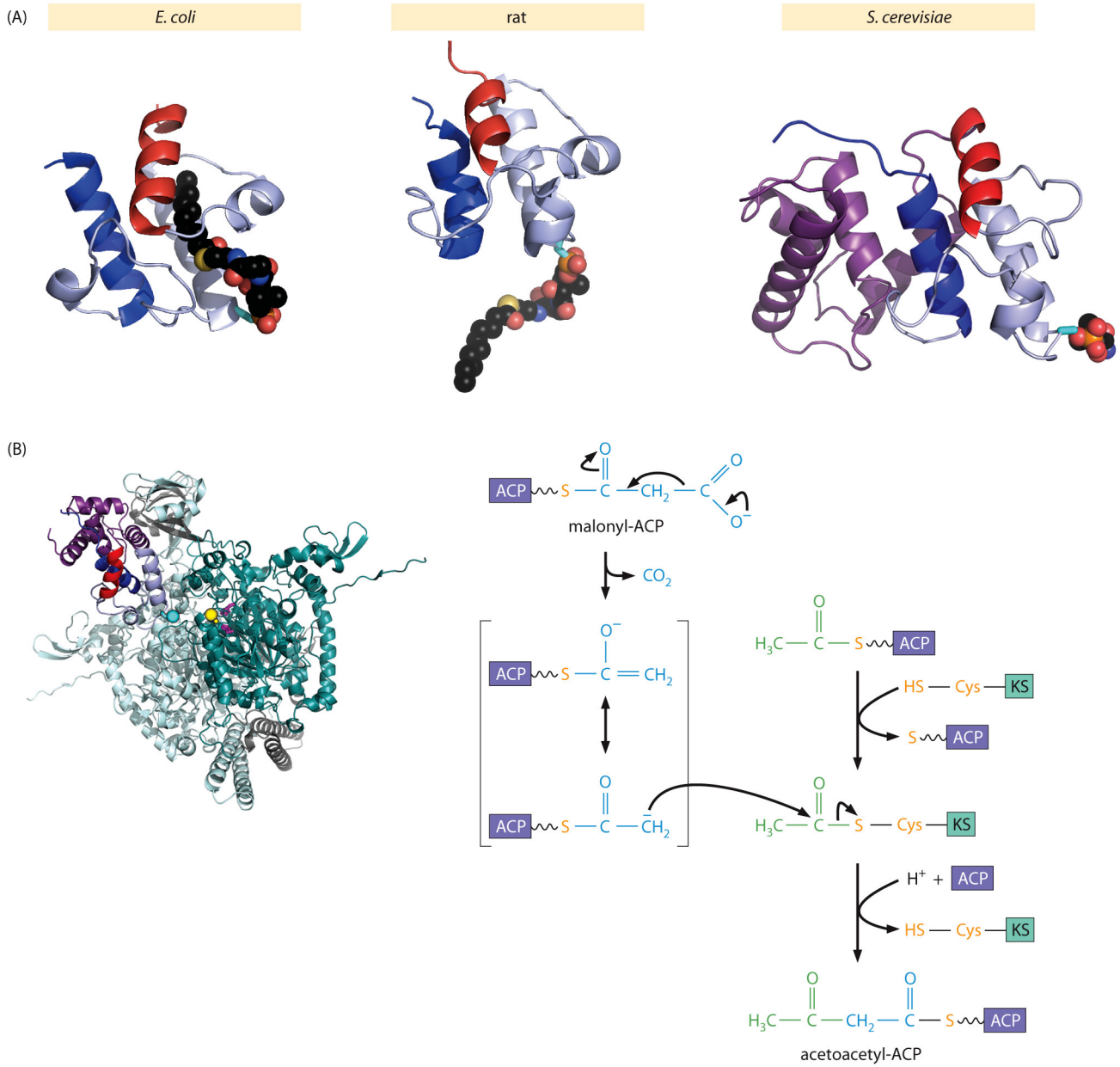
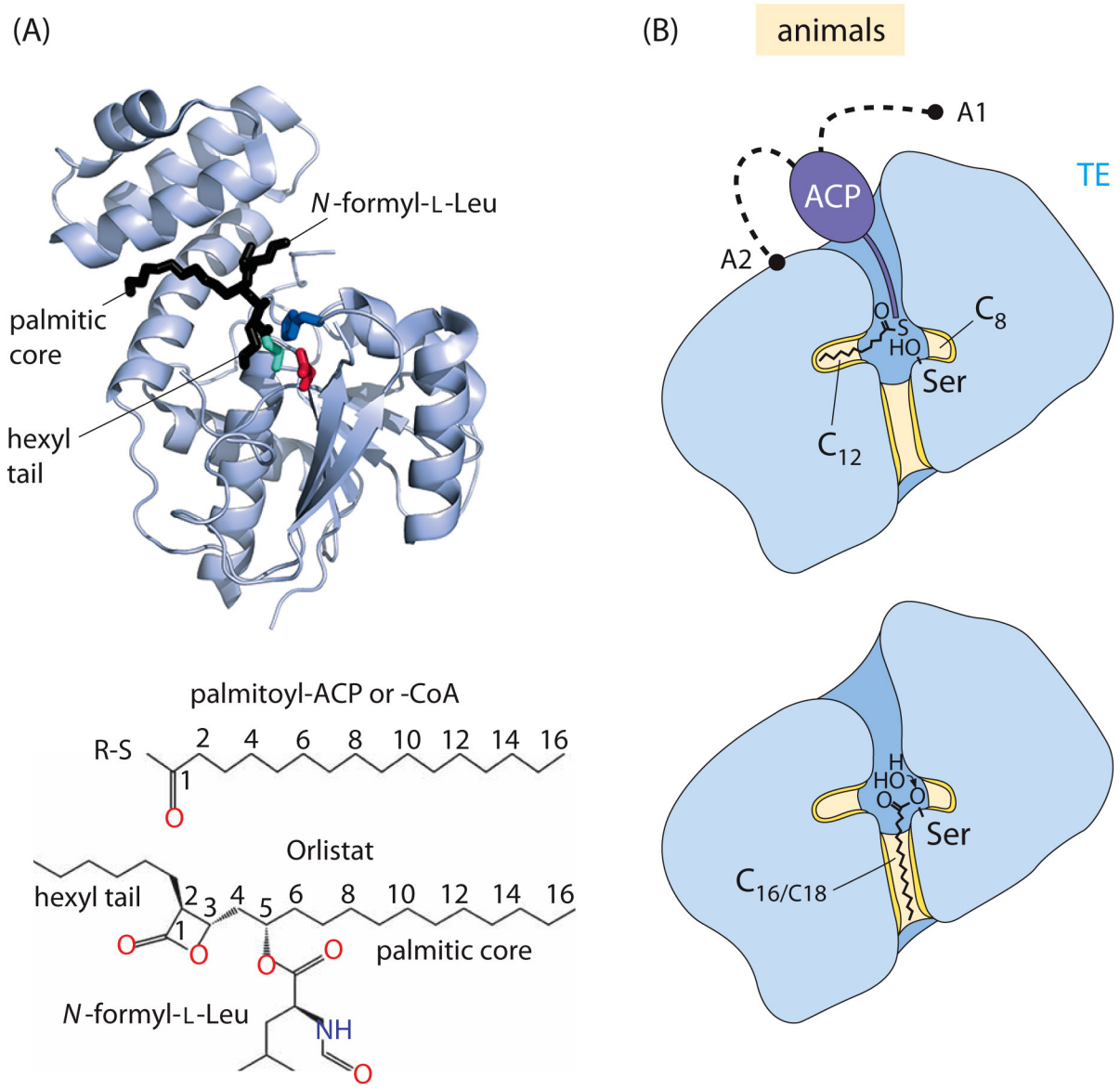



Figure 9.18 Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# Determination of fatty acyl chain length




Thioesterase domain

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

# Fatty acid degradation

$$\text{R-COOH} + \text{ATP} + \text{CoA-SH} \longrightarrow \text{R-CO-S-CoA} + \text{AMP} + \text{PP}_i$$

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.

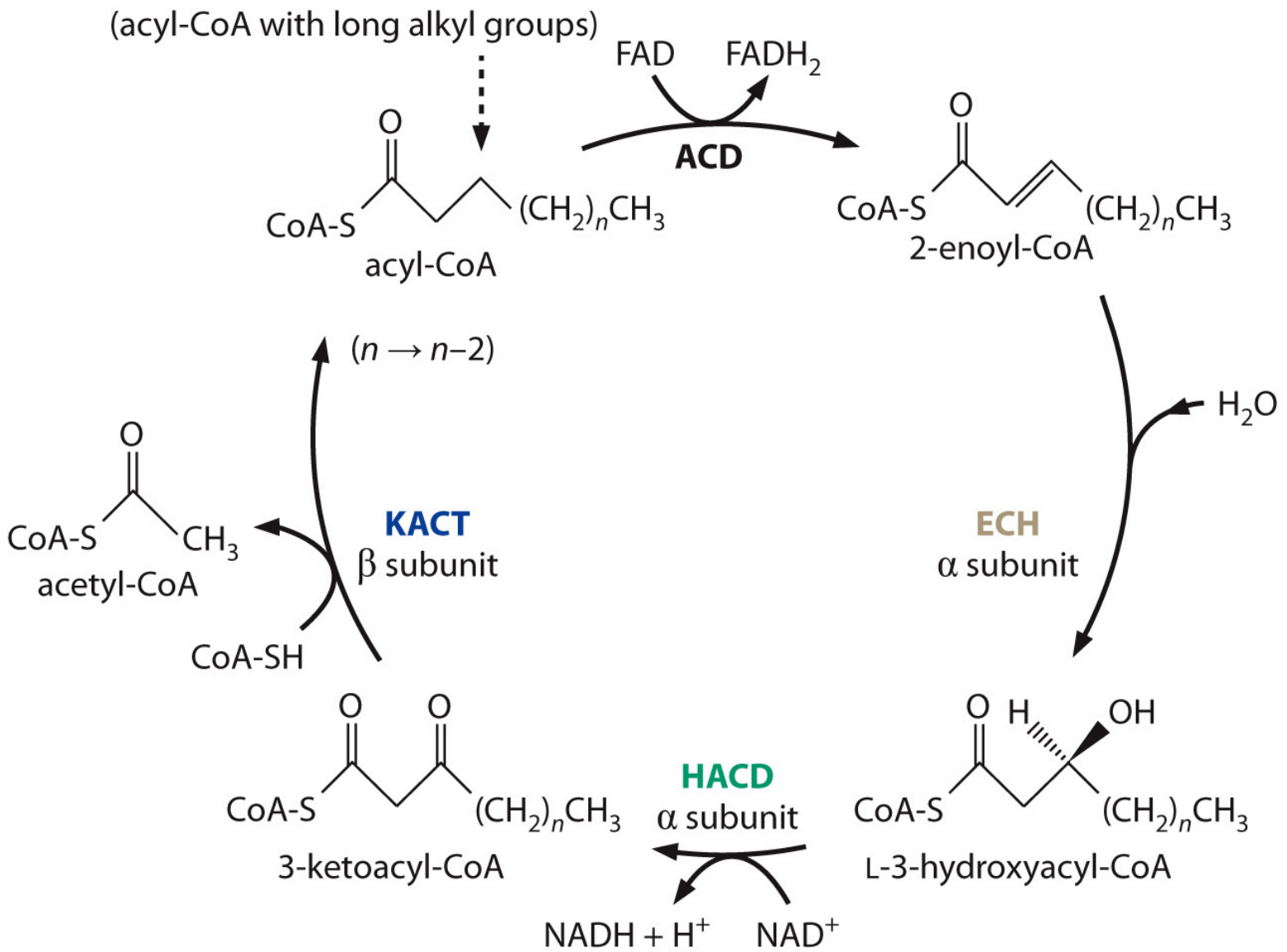
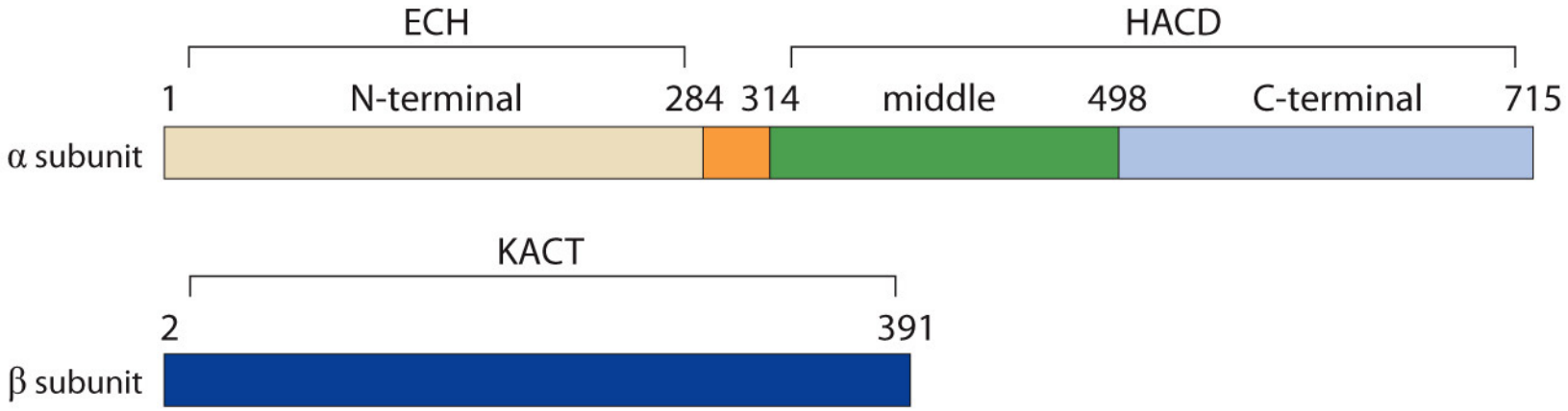



Figure 9.20a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# 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.

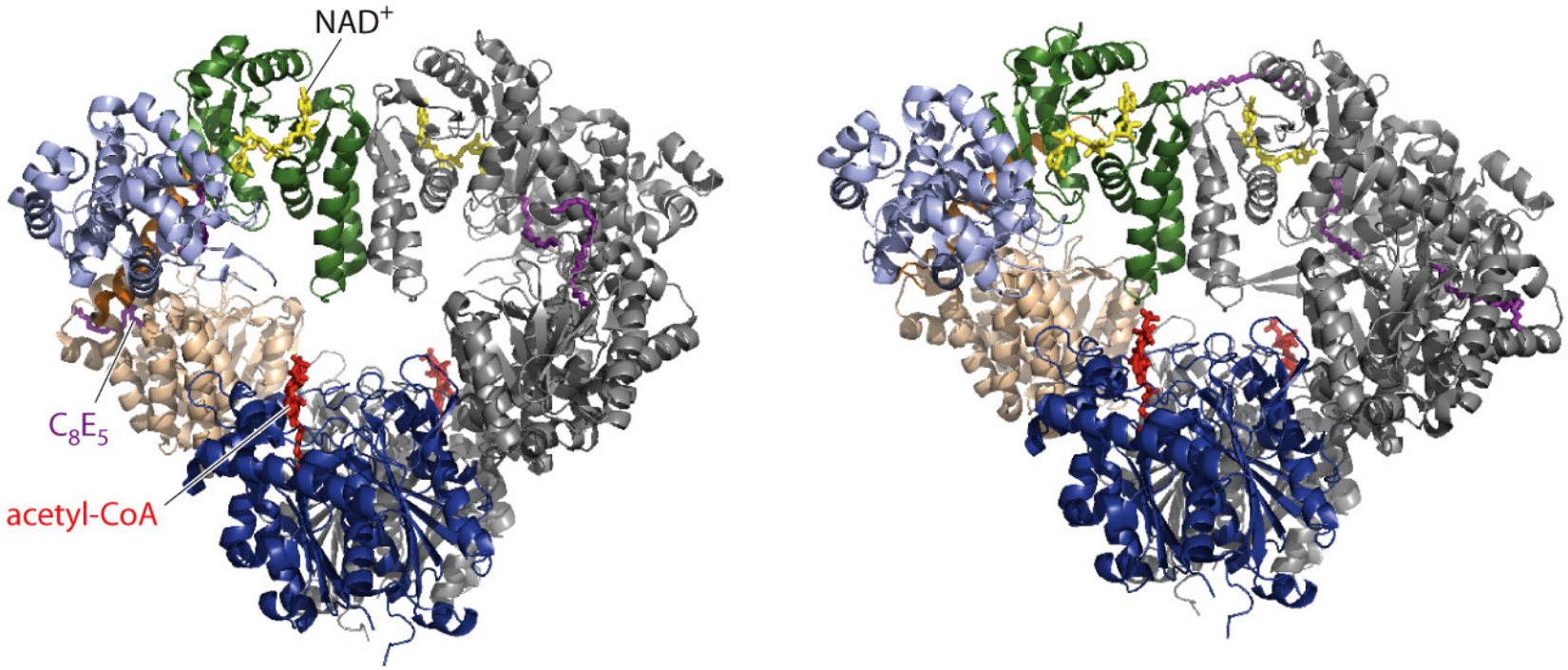
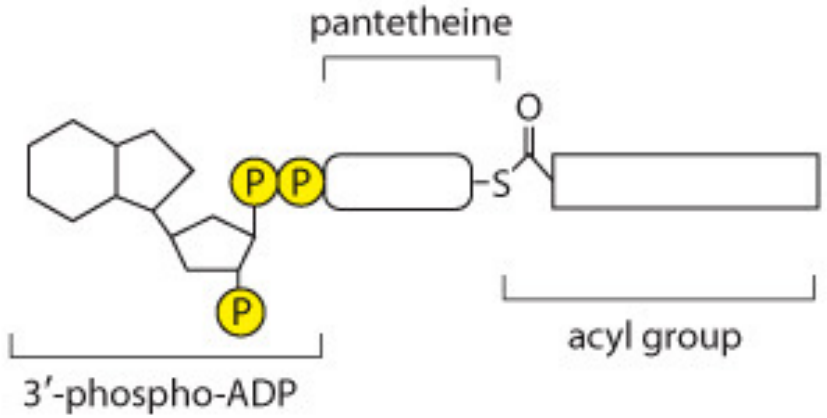



Figure 9.20b Molecular Biology of Assemblies and Machines (© Garland Science 2016)

# Substrate channeling in FAO complex



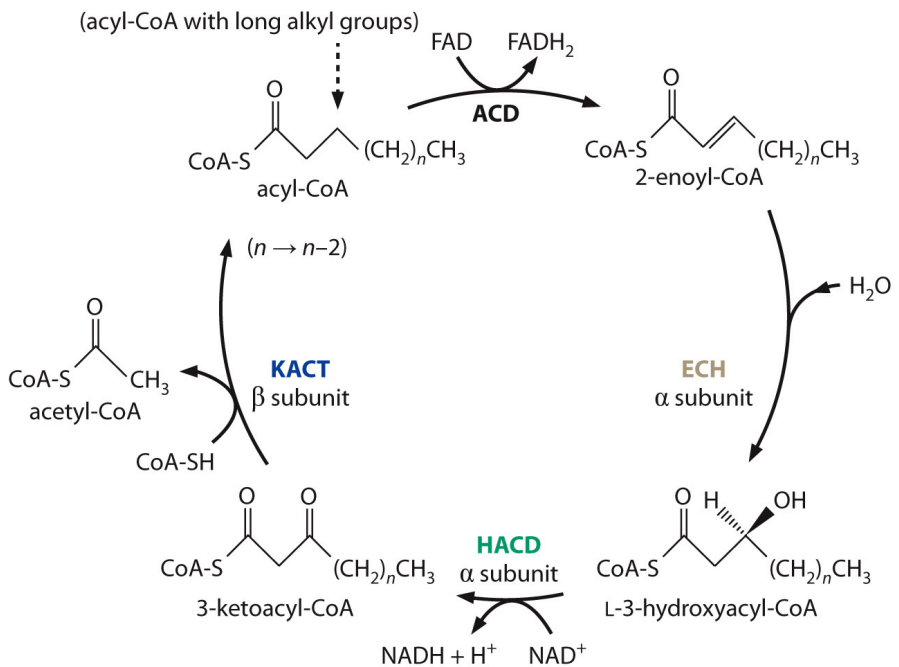




Figure 9.20a Molecular Biology of Assemblies and Machines (© Garland Science 2016)

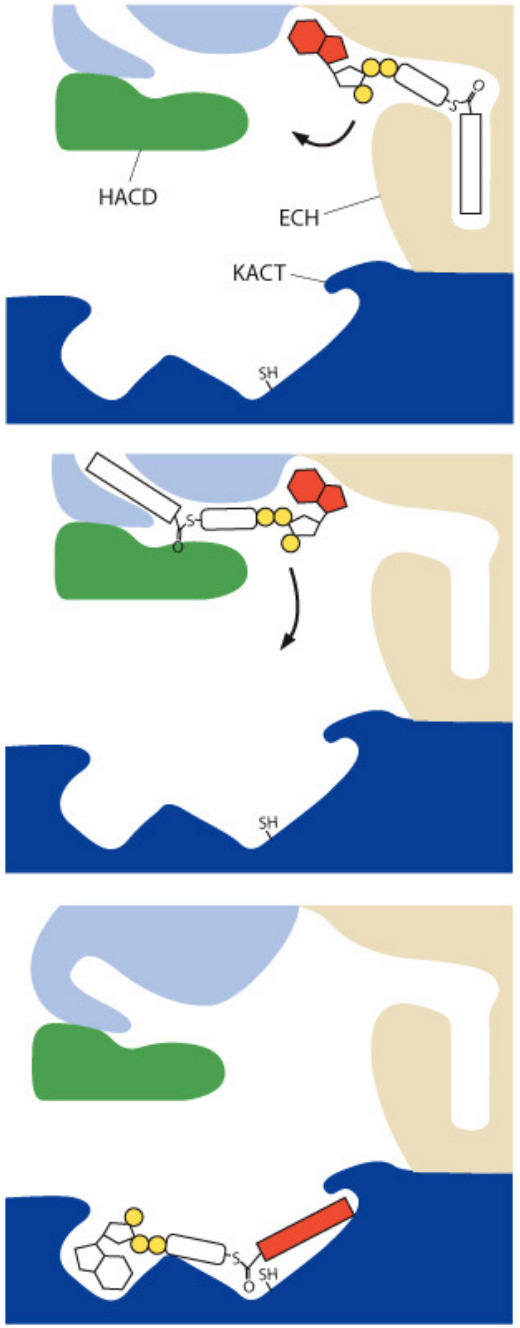
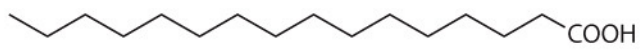
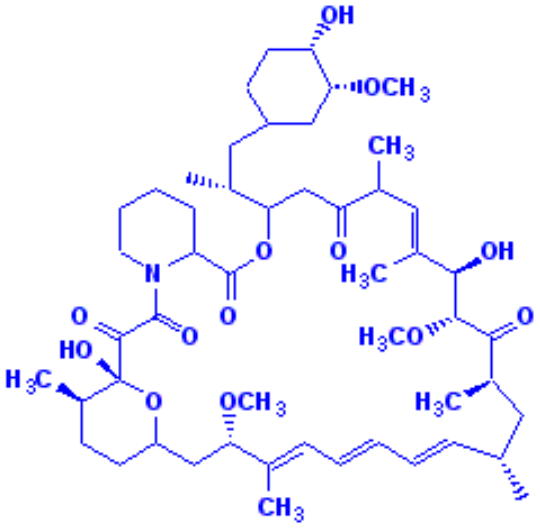



Figure 9.21 Molecular Biology of Assemblies and

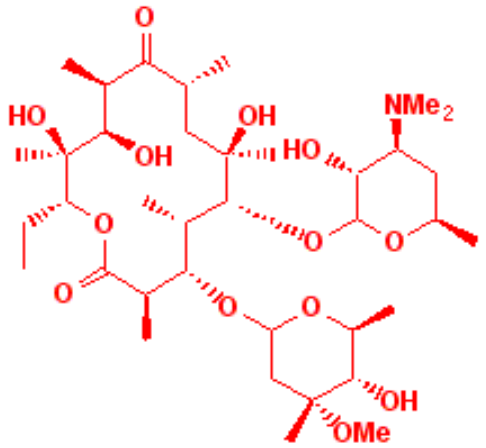
# polyketide synthases

palmitic acid

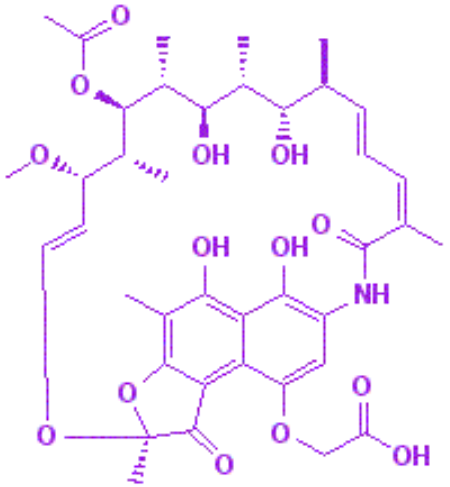


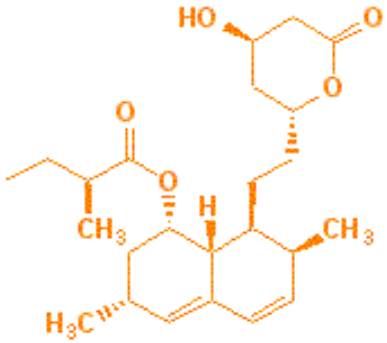
***rapamycin***
***(immunosuppressant)***



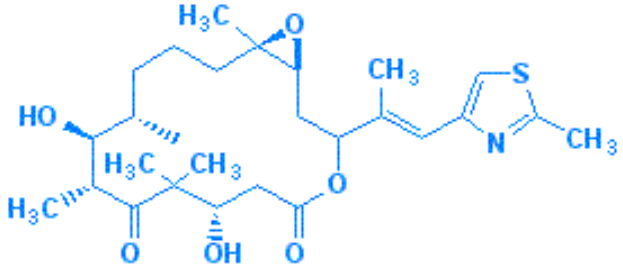
***erythromycin A***
***(antibacterial)***



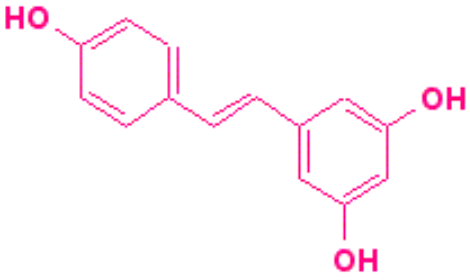
***rifamycin B***
***(antituberculosis)***



***lovastatin***
***(anticholesterol)***



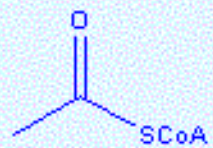
***epothilone B***
***(anticancer)***



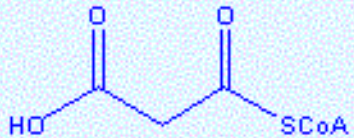
***(E)-resveratrol***
***(chemopreventive)***

# STARTER AND EXTENDER UNITS

## FAS

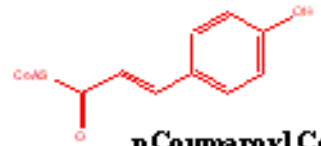



Acetyl CoA



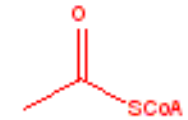

Malonyl CoA

## PKS

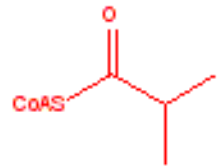



p Coumaroyl CoA

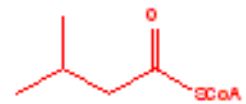

Octanoyl CoA



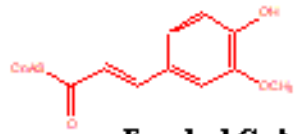

Acetyl CoA



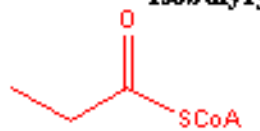

Isobutyryl CoA



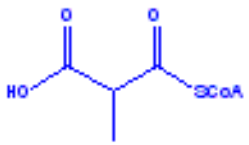

Isovaleryl CoA



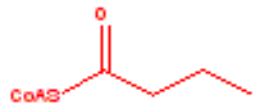

Feruloyl CoA



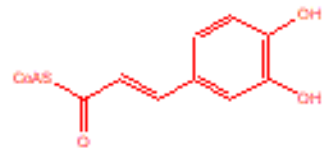

Propionyl CoA



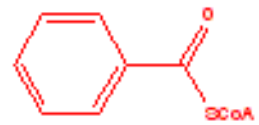

Methylmalonyl CoA



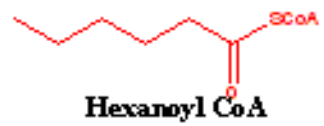

Butyryl CoA



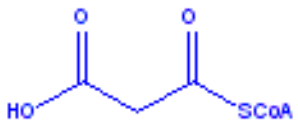

Caffeoyl CoA



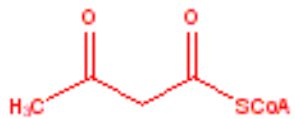

Benzoyl CoA



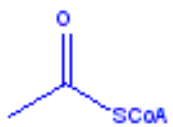

Hexanoyl CoA



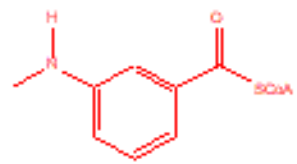

Malonyl CoA



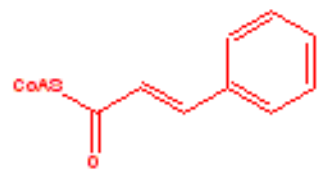

Acetoacetyl CoA




Acetyl CoA




N methy anthraniloyl CoA




Cinnamoyl CoA

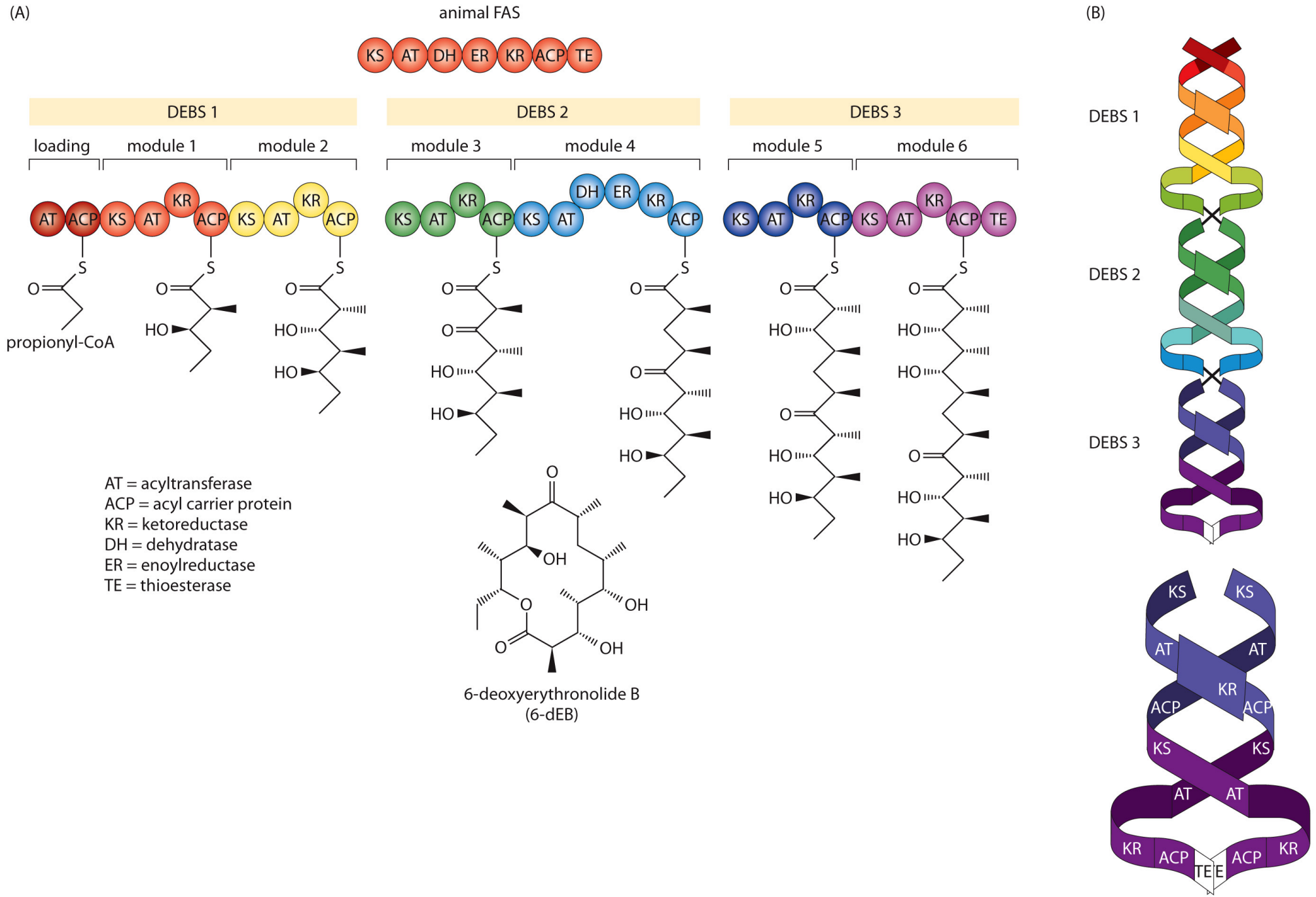


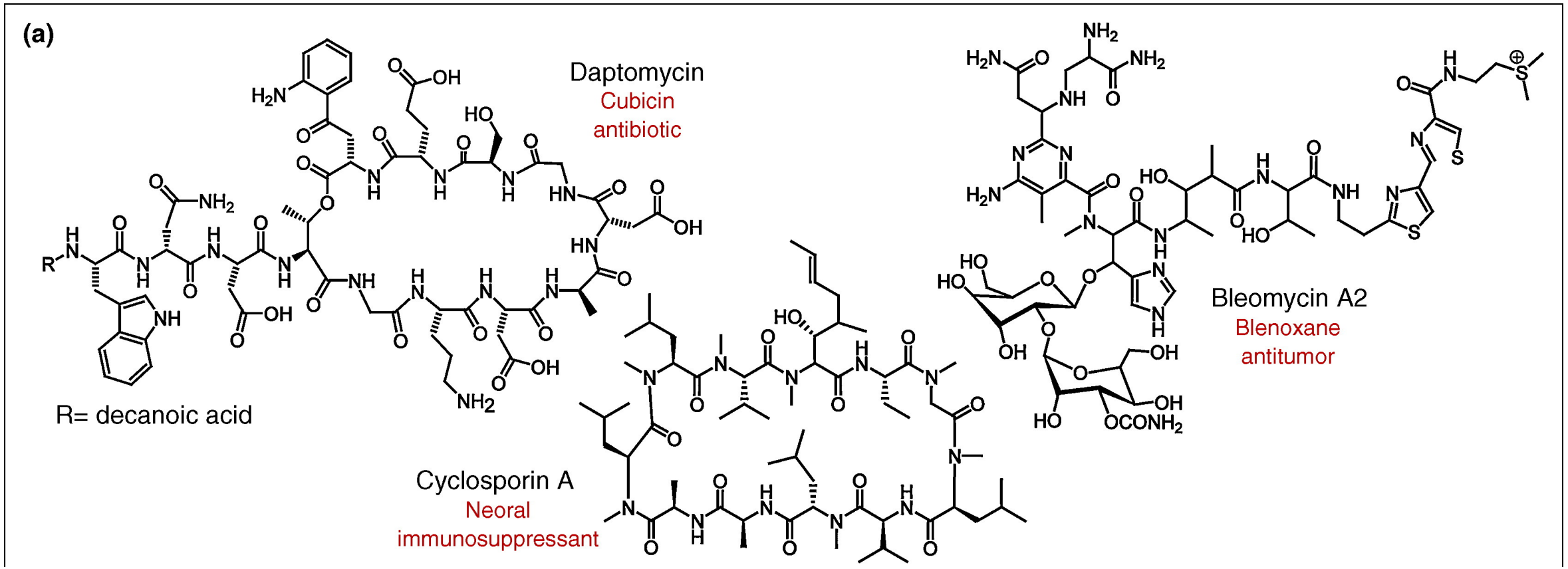

**Figure 9.23 Molecular Biology of Assemblies and Machines (© Garland Science 2016)**

# 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