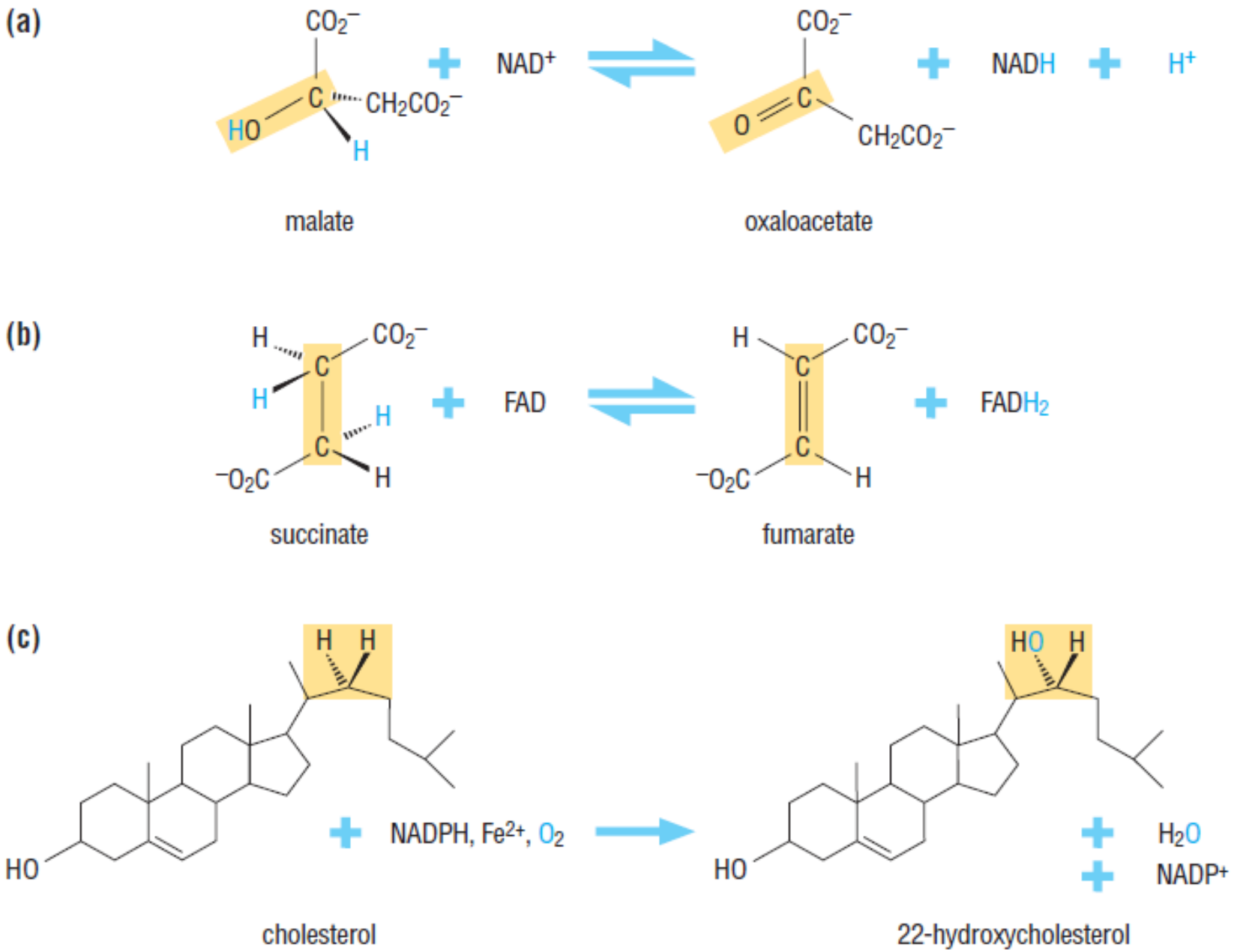
Chemical Reactions and biological transformations

- Mammalian Cell→ 10,000 protein → More than half enzymes
- Thousand reaction → most are of same type
  - Oxidation/reduction
  - Addition/elimination
  - Hydrolysis
  - Decarboxylation
- common chemical problems
  - Stable organic chemical
  - Unstable intermediates

Oxidation/reduction reactions

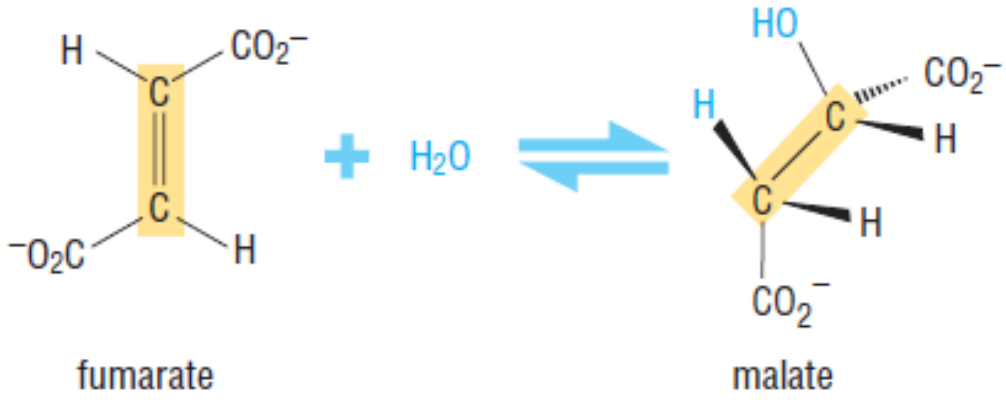
- central to metabolism
- Require cofactors
- carbon–oxygen centers reactions→ NAD, NADP
  - malate dehydrogenase
    - NAD to oxidize a –C–OH group in malate to a –C=O group, producing oxaloacetate and reduced NAD (NADH).
- Redox reactions at carbon–carbon bonds → FAD, FMN
  - Succinate dehydrogenase, converts succinate to fumarate
- Reactions at –C–H centers and N centres → Cofactor, Metal ions
  - Cytochrome P450,
    - takes specific –C–H bonds in unreactive carbon compounds and inserts molecular oxygen to produce –C–OH groups


(a)
CO2−
HO
C
CH2CO2−
H
NAD+
malate
CO2−
O
C
CH2CO2−
NADH
H+
oxaloacetate
(b)
H
CO2−
H
C
H
C
−O2C
H
FAD
succinate
H
CO2−
C
C
−O2C
H
FADH2
fumarate
(c)
H H
HO
NADPH, Fe2+, O2
cholesterol
HO H
HO
H2O
NADP+
22-hydroxycholesterol

Addition/elimination reactions

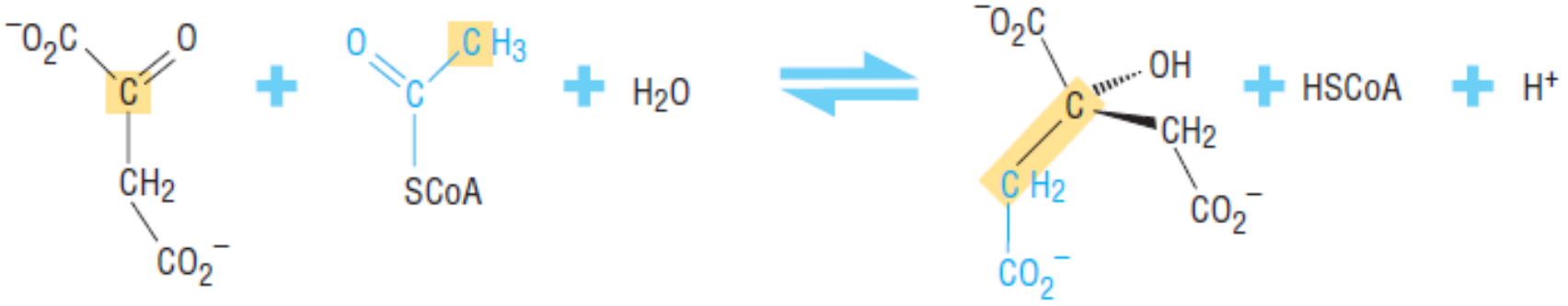
- Addition reactions
  - transfer atoms or chemical groups to the two ends of a double bond, forming a more highly substituted single bond.
- Elimination reactions
  - reverse this process, forming a new double bond.
- Many addition/elimination reactions involving C=C bonds, the transferred species is a molecule of water.
  - fumarase a molecule of water is added to the C=C of fumarate
- Aldol condensation
  - make new carbon–carbon bonds
  - involves addition of an activated carbon center (for example, the acyl moiety of acyl-coenzyme A) to the C=O carbonyl carbon and is the most common way of making a new C–C bond in biology
  - Citrate Synthase reaction
- Breaking a C–C bond → aldol Cleavage
  - aldolase catalyzes an aldol cleavage reaction

(a)



fumarate malate

(b)



oxaloacetate acetyl-CoA citrate

Hydrolysis Reactions

- The breaking of C–N, C–O, S–O, P–O and P–N bonds.
  - Degradation of biopolymers such as proteins → Proteases
  - RNA and DNA degradation → Nucleases
  - ATP to ADP → cleavage of phosphoanhydride bond
- Reactions in which compounds are formed with the loss of water include
  - Synthesis of proteins (C–N)
  - acylglycerols and
  - oligosaccharides (C–O), and
  - polynucleotides (P–O) from their monomers and
  - the formation of ATP from ADP and inorganic phosphate during respiration.
  - DNA polymerase → using activated dNTPs

(a)

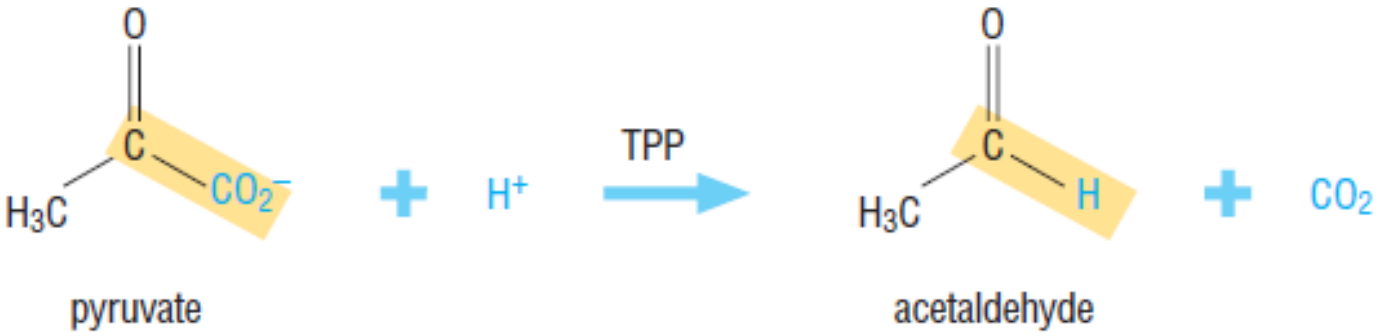

etc
H
N
O
C
H R2
N
H
etc
H R1
O
+ H2O
etc
H
N
O
C
O−
H R1
+
H2N
H R2
C
etc
O

(b)


O
R1—O
P
O−
O—R2
+ H2O
O
RO
P
O−
O−
+ ROH

Decarboxylation Reactions

- Loss of CO2
- usually assisted by cofactors.
  - The most common are pyridoxal phosphate (PLP) and
  - Thiamine diphosphate (TDP; also referred to as TPP, thiamine pyrophosphate),
  - transition-metal ions such as manganese
- Pyruvate decarboxylase
  - converts pyruvate to acetaldehyde



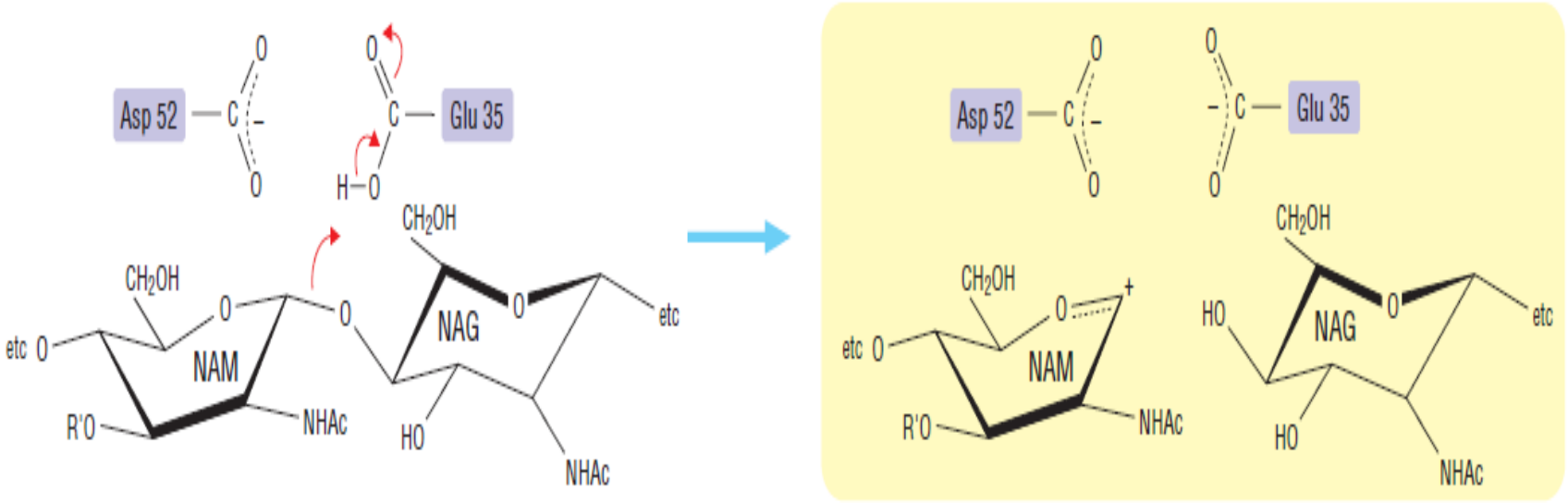
Active sites promote acid-base catalysis

- Catalysis in which a proton is transferred in going to or from the transition state is called **acid-base catalysis**
- depends on the relative proton affinities of the two groups.
- Proton affinity is measured by the **pKa value**
  - pKa can be thought of as the pH of an aqueous solution of the acid or base at which half of the molecules are protonated and the other half are deprotonated
- Strong acids lose their protons readily to water, forming hydronium ions (H_3O^+).
  - Strong acids have pK_a values of 2 or lower.
- Strong bases tend to take protons from water, forming the hydroxide ion (OH^-).
  - Strong bases have pKa values greater than about 12

- If the pKa of the group is between 4 and 7,
  - It is a weak acid (the higher the pKa the weaker the acid);
- if the pKa is between 7 and 10,
  - The group is a weak base (the lower the pKa the weaker the base).
- Enzymes can increase the efficiency of acid-base reactions by changing the intrinsic pKa values of the groups involved.
- Thus, the alpha –C–H group in lactic acid can be made more acidic (that is, its pKa can be lowered) by, for example, making a strong hydrogen bond to the –OH group attached to it.
- This hydrogen bond will tend to pull electrons away from the oxygen atom, which in turn will pull electrons away from the adjacent –C–H bond, weakening the affinity of the carbon for its hydrogen and thus lowering the pKa.
- The pKa of a weak acid such as the carboxylic acid side chain of lactic acid (pKa ~ 3.9 in water) can be raised to 7 or higher by, for example, placing the group in a nonpolar environment.

Carboxylic acid side chain of glutamate in the active site of the enzyme lysozyme

- pKa of glutamic acid 35 is raised from about 4 to above 6, and it can donate a proton to catalyze the breaking of the C–O bond in the substrate



Active site and Cofactors

- Non-amino-acid **cofactors**
- Oxidation Reduction → Cys → Redox centres
- The creation of unpaired electrons (free radicals)
- Cofactors can be as small as a metal ion or as large as a heterocyclic organometallic complex such as heme
- Cofactors that are organic compounds and assist catalysis are often referred to as **coenzymes.**
  - Cofactors may be imported into an organism from the food it eats or they can be synthesized from simple building blocks.
- Metal-ion cofactors
  - first-row transition metals and the most common are also among the most abundant in the Earth's crust.
  - Enzymes are known that use molybdenum, nickel, cobalt, and manganese, but the majority of metalloenzymes use iron, copper, zinc, or magnesium.
- The structures of typical organic cofactors
  - most of them look like pieces of RNA

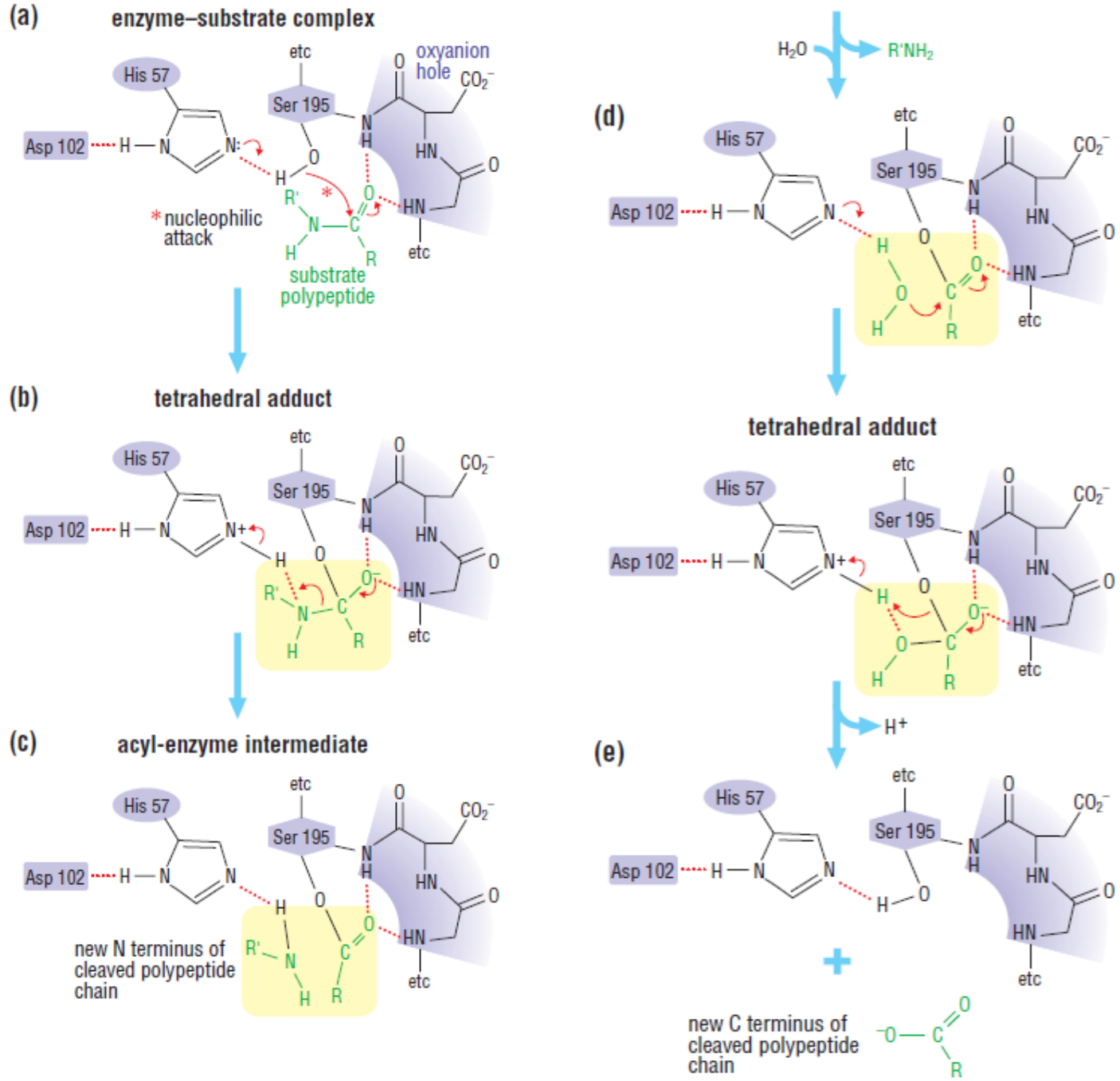
Active Site and Cofactors

- The function of organic cofactors
  - transfer specific chemical species to a substrate
  - Groups transferred range from electrons and hydride ions (H^-) to small carbon-containing fragments up to about two carbons in length
- These species are usually either extremely unstable on their own.
- would be damaging to the protein if they were not contained
- The function of the coenzyme is to make them, transfer them, and/or sequester them
- Cofactor Recycling → NAD, NADP

- amino-acid side chains in a protein can be modified to produce an organic cofactor *in situ*.
- Green fluorescent protein (GFP)
  - Fluorescent chromophore is synthesized by the protein itself from the reaction of a tyrosine side chain with neighboring serine and glycine residues
- Lysine tyrosylquinone (LTQ) in copper amine oxidase
  - LTQ is synthesized by the addition of a lysine side chain of the enzyme itself to the aromatic ring of an oxidized tyrosine residue elsewhere on the protein chain.

ACTIVE SITES EMPLOY MULTI-STEP MECHANISMS

- Most biological reactions → specific cond.
- Enzymes must circumvent the high-energy transition state, Done by
  - by stabilizing the transition state
  - enzymes may direct the reaction along a different route from substrates to products
  - often entails breaking the reaction up into a number of steps
  - Many unstable intermediates
  - intermediates can be trapped by physical (low temperature) or chemical (reduction) methods.
  - Covalent Intermediates → involve cofactors
    - Serine protease reaction
    - Water interaction with peptide backbone


(a)
enzyme–substrate complex
oxyanion hole
His 57
Asp 102
Ser 195
etc
*nucleophilic attack
substrate polypeptide
(b)
tetrahedral adduct
(c)
acyl-enzyme intermediate
new N terminus of cleaved polypeptide chain
(d)
H_2O
$R'NH_2$
tetrahedral adduct
(e)
H^+
new C terminus of cleaved polypeptide chain

- Metalloproteases → H_2O → OH^-
  - These enzymes use a metal-ion cofactor to activate water for direct attack on the substrate carbonyl
  - carboxypeptidase and thermolysin do not proceed through a stable intermediate
  - They bind a molecule of water to a metal ion, usually Zn^{2+}.
  - Binding H_2O to a transition metal ion lowers the pK_a of the bound water,
  - making it a better acid. It is thus much easier to turn metal-bound H_2O into OH^-
  - The metal-bound OH^- can attack the amide bond of a peptide substrate directly, hydrolyzing it without the need for a covalent enzyme intermediate.

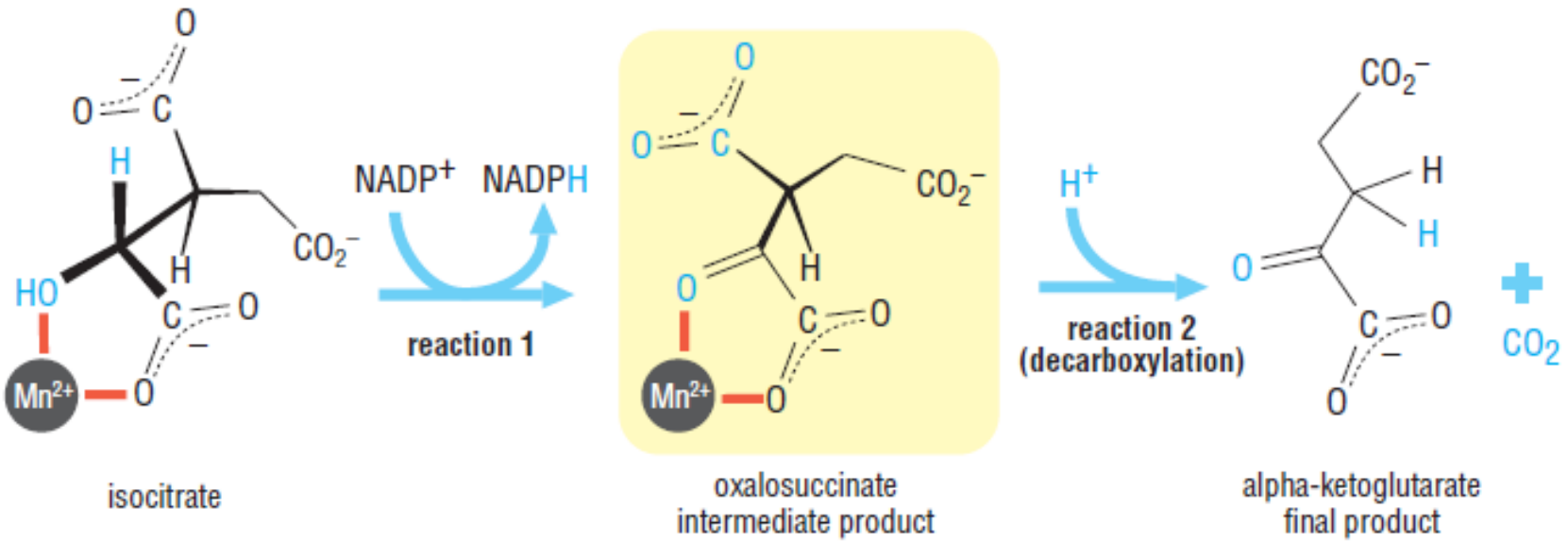
PHOSPHORYL-GROUP TRANSFER REACTIONS

- Two-step strategy
  - Catalyzed by kinases and phosphatases
  - Can occur with a wide variety of attacking groups Serine, threonine and tyrosine –OH groups, the carboxylate groups of aspartate and glutamate, cysteine –SH and the nitrogen of histidine
- Bacterial alkaline Phosphatase
  - which catalyzes the transfer of phosphate to water from many organophosphate substrates
  - a serine –OH on the enzyme first attacks the phosphorus atom
  - phosphoserine-enzyme intermediate
- Phosphoglucomutases
  - D-glucose 1-phosphate and D-glucose 6-phosphate
  - proceeds through a phosphoaspartyl anhydride intermediate
  - when the phosphate on the aspartyl group of the enzyme attacks the unphosphorylated position on the sugar and transfers the phosphate group to it.

Bifunctional or Multifunctional Enzymes

- Three classes
  - First class, the two reactions take place consecutively at the same active site
  - second, two separate chemical reactions are catalyzed by two distinct active sites, each located in a different domain some distance apart
  - In the third, two or more reactions are also catalyzed by two or more distinct active sites, but these are connected by internal channels in the protein

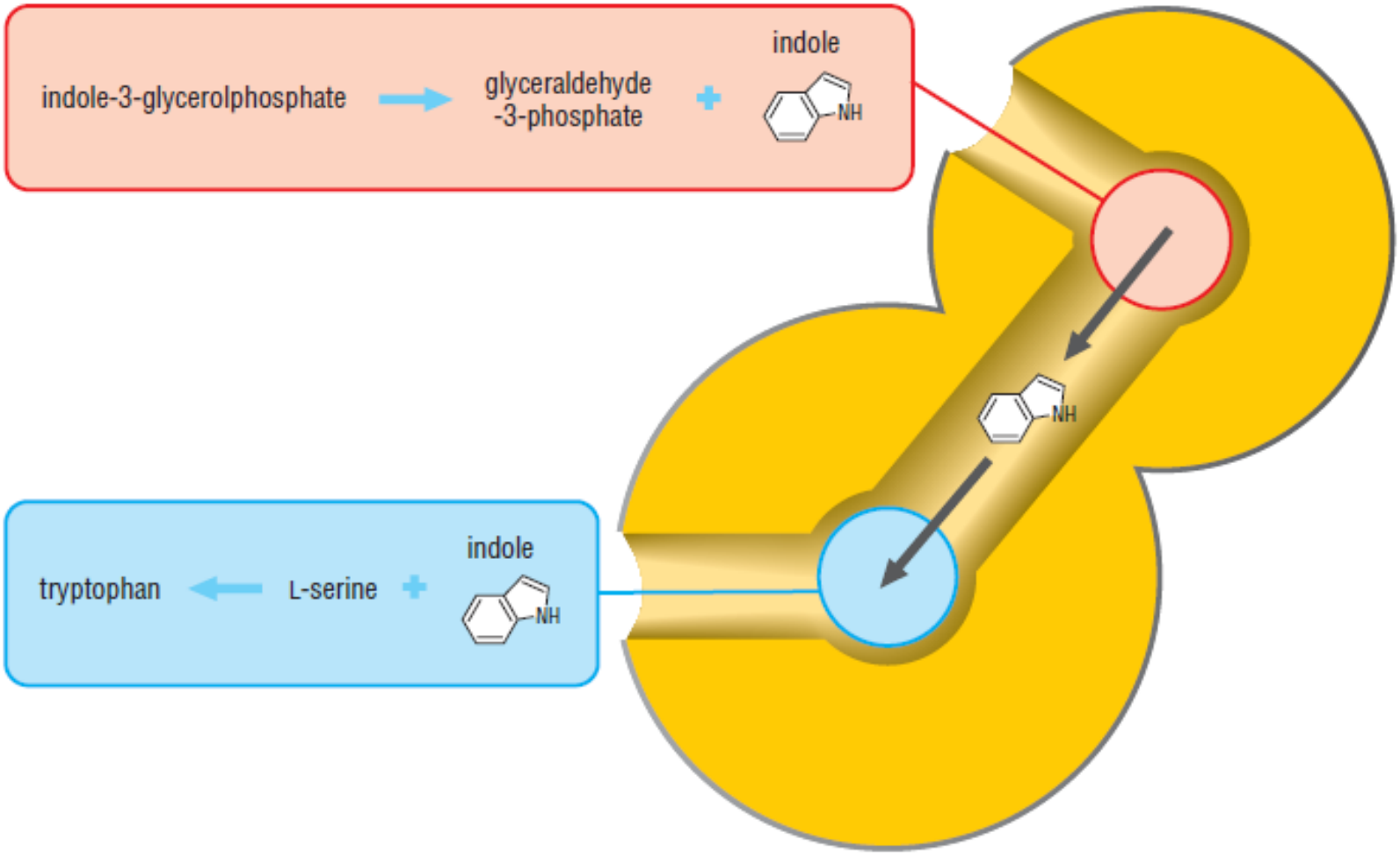
- In bifunctional enzymes that carry out two different reactions using the same active site
  - Isocitrate dehydrogenase (ICDH)
  - Isocitrate → oxalosuccinate (unstable) NADP
  - Oxalossuccinate → alpha ketoglutarate (same site) Mn



- Some bifunctional enzymes contain two active sites
- enzyme contains two independently folded domains
- First product → Dissociate → find other site
- Regulation, synthesis, two genes
- dihydrofolate reductase-thymidylate synthase
  - catalyzes two reactions in the biosynthesis of thymidine

BIFUNCTIONAL ENZYMES SHUTTLE

- Physical channel (or channels) used coz
  - first reaction product is an uncharged species
  - the reaction product is so unstable in free solution
- Tryptophan synthase
  - Tunnel is 25 Å long
  - Connects the active site of the alpha-subunit, in which indole, an uncharged molecule that might diffuse out of the cell, is generated from indole 3-glycerolphosphate, to the second active site, in which the indole is added to a molecule of acrylate, derived from serine, to produce tryptophan.


indole-3-glycerolphosphate
glyceraldehyde
-3-phosphate
indole
NH
NH
tryptophan
L-serine
indole
NH

Trifunctional enzymes

- carbamoyl phosphate synthetase
  - involved in the synthesis of 2`-deoxyUMP
  - three separate active sites connected by two tunnels through the interior of the protein
- first reaction produces Ammonia,
  - a neutral species, which travels along a tunnel to the second active site where
- it reacts with carboxyphosphate to give a carbamate intermediate
  - that would be too unstable to survive in aqueous solution. Therefore, it is transported through the interior of the protein to the third active site,
- where it is phosphorylated by ATP to give the final product.
- covers a distance of nearly 100 Å


glutamine
tunnel
ATP/HCO_3^-
carbamoyl phosphate
allosteric sites

ENZYMES ALSO HAVE NON-ENZYMATIC FUNCTIONS

- Regulatory function
  - some enzymes double as transcription factors;
  - others act as signaling proteins;
  - some are cofactors for essential reactions in protein synthesis; and
  - yet others are transported out of the cell to serve as cytokines or growth factors.
- Metabolic enzyme double as a repressor, for example, couples the expression of some genes to metabolism in a direct way
  - Folate-dependent enzyme thymidylate synthase
    - also functions as an RNA-binding protein.
    - interacts with its own mRNA to form a ribonucleoprotein complex
    - is also evidence that it can interact with a number of other cellular mRNAs, including transcripts of the *p53 tumor suppressor gene and the myc family of transcription factor genes*
    - It is also a target for several anti-cancer drugs