Chemistry 5.07, Fall 2013
Lexicon of biochemical reactions

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1. Review of electrophiles and nucleophiles in biology

| Electrophiles		Nucleophiles			
			Nucleophilic form		
H^+	Protons	$R\ddot{O}H$ ⇌	$R\ddot{O}:^{\ominus}$	$+ \; H^+$	Hydroxyl group
M^{n+}	Metal ions	$R\ddot{S}H$ ⇌	$R\ddot{S}:^{\ominus}$	$+ \; H^+$	Sulfhydryl group
$\underset{R_1}{\overset{R}{>}}C=O$	Carbonyl carbon	RNH_3^+ ⇌	$R\ddot{N}H_2$	$+ \; H^+$	Amino group
$\underset{R_1}{\overset{R}{>}}C=\overset{\oplus}{N}H-$	Imine (protonated imine) carbon (Schiff base)	(imidazolium) ⇌	(imidazole)	$+ \; H^+$	Imidazole group

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2. Carbonyl Chemistry: Mechanisms of C-C Bond Formation

- There are three general ways to form C-C bonds:
    1. Aldol reactions where an aldehyde is condensed in a reversible reaction with another aldehyde or a ketone;
    2. Claisen reaction in which a Coenzyme A thioester is condensed with an aldehyde or a ketone;
    3. Prenyl transfer reactions where two 5 carbon units are condensed to form isoprenoids.

- The aldol and claisen reactions involve carbonyl chemistry. These reactions are prevalent in glycolysis, fatty acid biosynthesis and degradation and the pentose phosphate pathway.
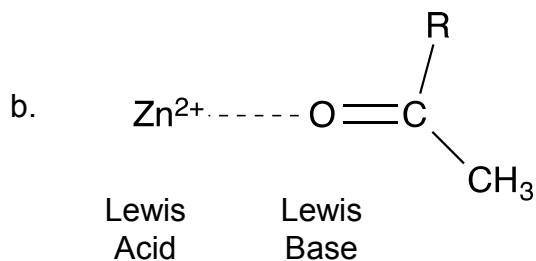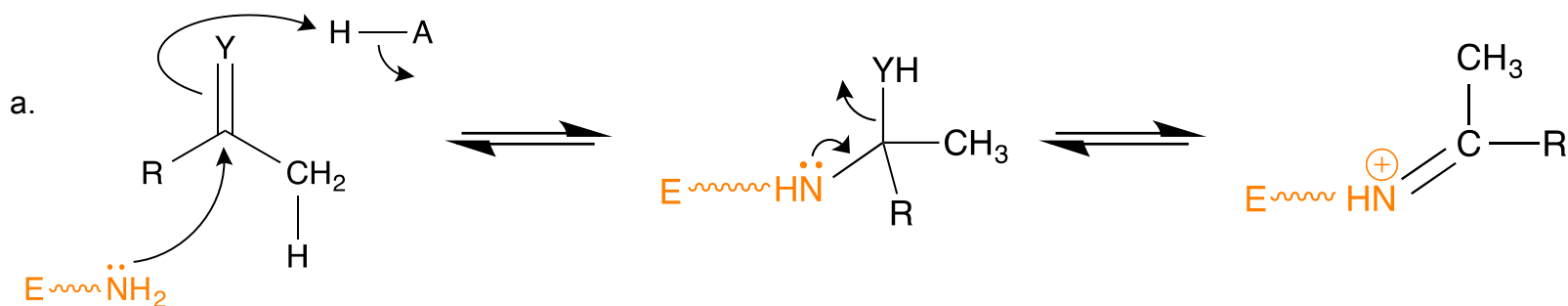
1. Aldol reaction generalizations:
    a. All aldol reactions occur with retention of configuration.
    b. All aldol reactions are reversible.
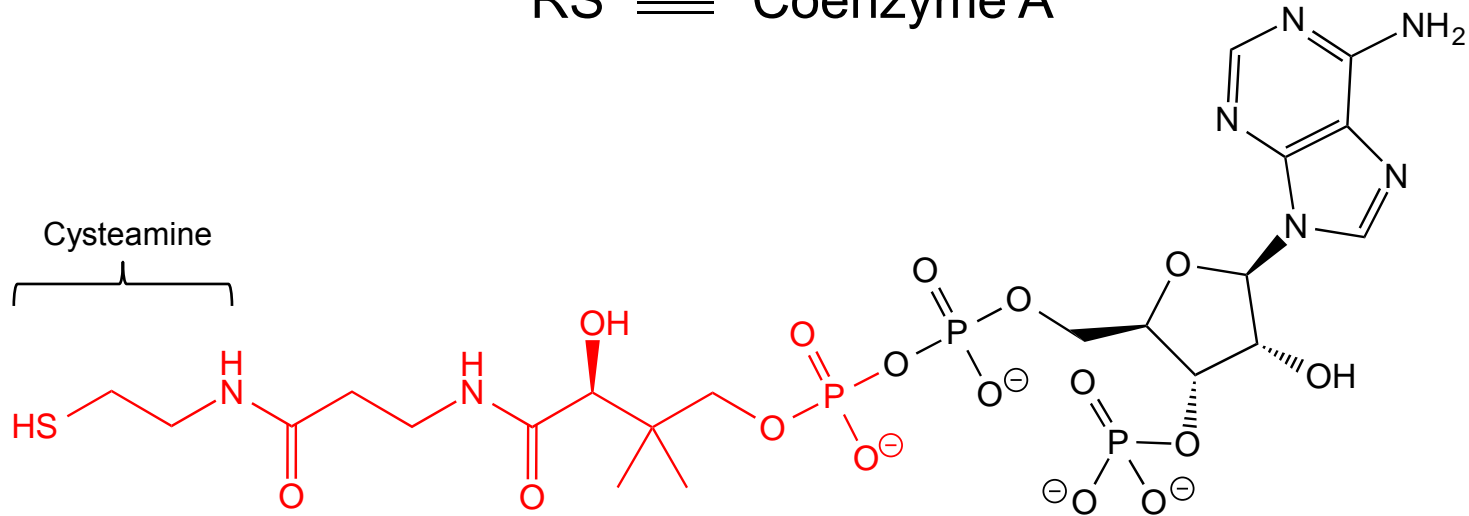   c. All aldol reactions involve carbanion intermediates.

- Generic mechanism:
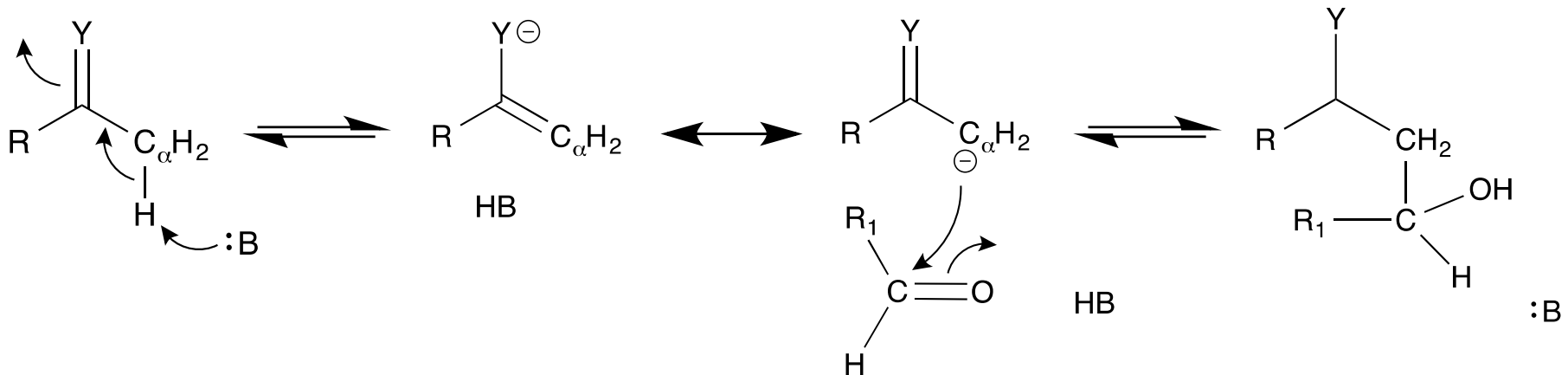
- The increase in acidity at the a hydrogens involves two mechanisms:
  a. Activation by imine formation with a lysine in the active site (class I aldolases found in eucaryotes)
  b. Use of Zn^{2+} (class II aldolases found in procaryotes)

a.

b.

Lewis Acid   Lewis Base

In addition, the "C" of $C=Y$ is activated by both mechanism (a.) and (b.) for nucleophilic attack.

RS ≡ Coenzyme A

**Coenzyme A (red = phosphopantetheine prosthetic group)**

2. Claisen reaction generalizations:
    a. All Claisen reactions occur with inversion of configuration.
    b. All Claisen involve carbanion intermediates.
    c. Most Claisen reactions involve thioesters of Coenzyme A (RS$^{\ominus}$)

•   The phosphopantetheine arm (shown in red) can also be covalently attached to a serine of an Acyl Carrier Protein (ACP).

•   Generic mechanism:

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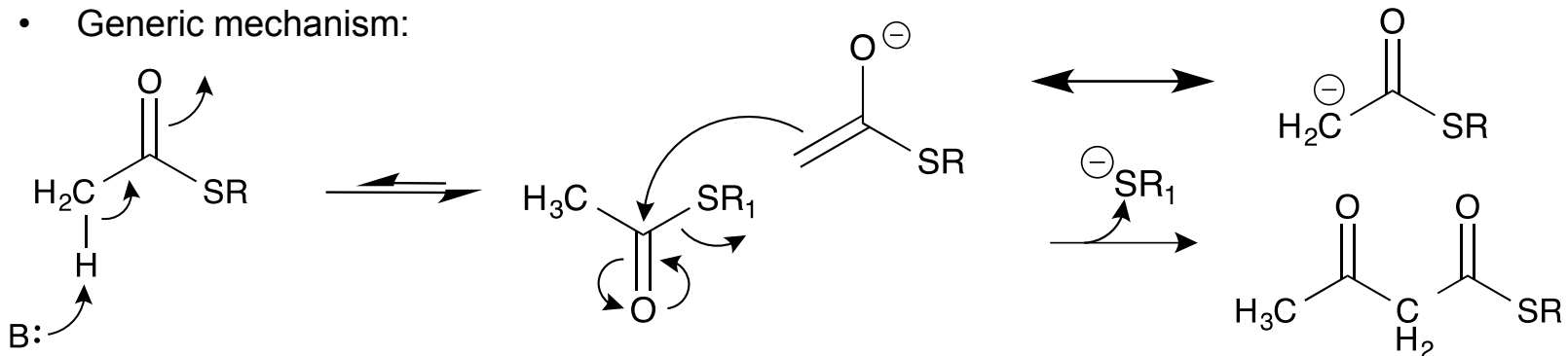
- Why has nature chosen thioesters and not oxygen esters?
    - Thioesters have two essential properties:
        - i.   They are activated for nucleophilic attack relative to oxygen esters
        - ii.   Their α hydrogens are more acidic than oxygen esters (pKa of 18 vs 22).
    - The thioester is more ketone like than the oxygen ester because in the oxygen ester, the non-bonded electrons from the oxygen of the ester can be resonance delocalized with the π electrons of the carbonyl.
        - a.   With the thioester, this delocalization is limited because of the poor electron overlap due to energetic differences.
        - b.   With the α hydrogen of the thioester, the delocalization is more favorable with the thioester than oxygen ester, for similar reasons.

a.

vs

unfavorable

b.

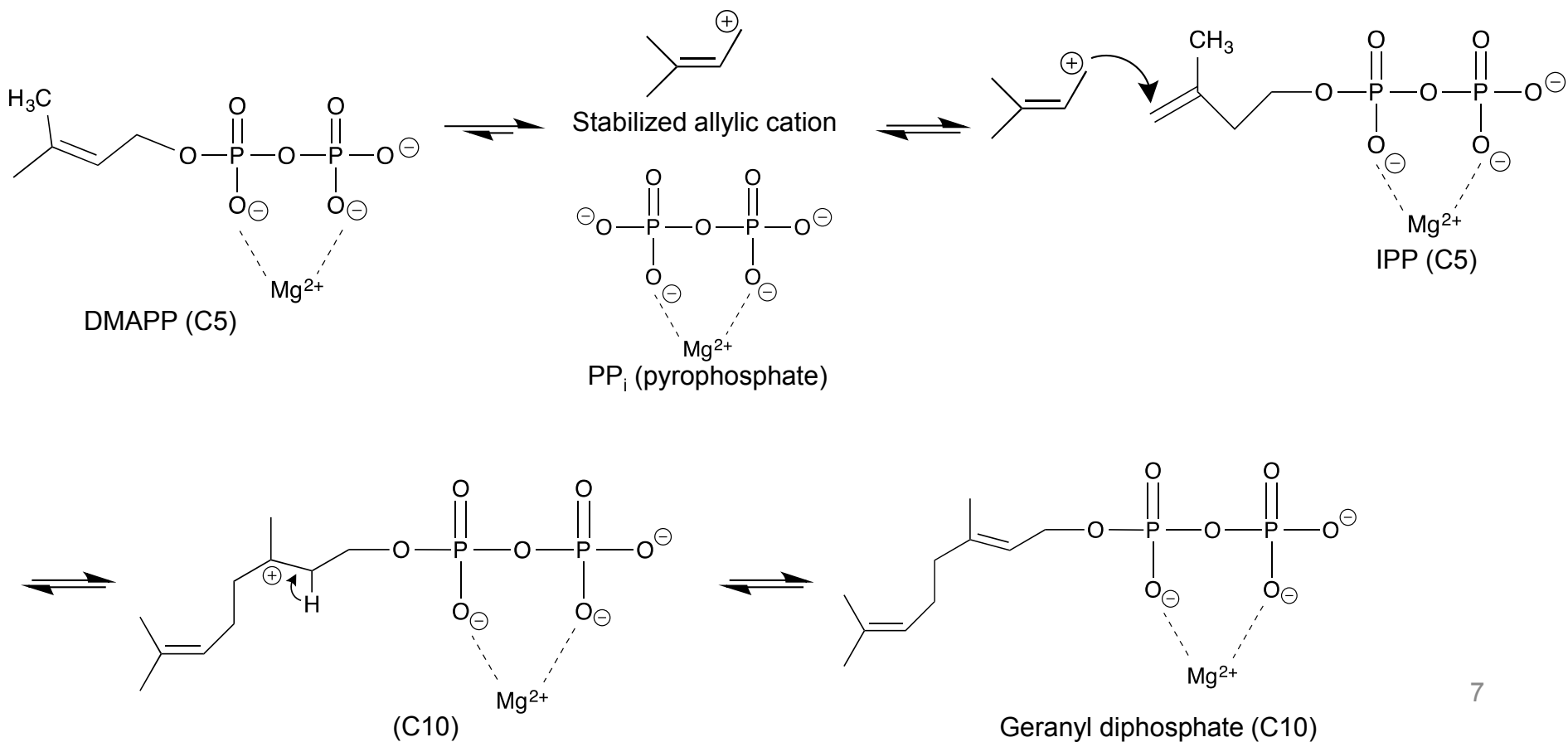pKa = 10.5   pKa = 8.5
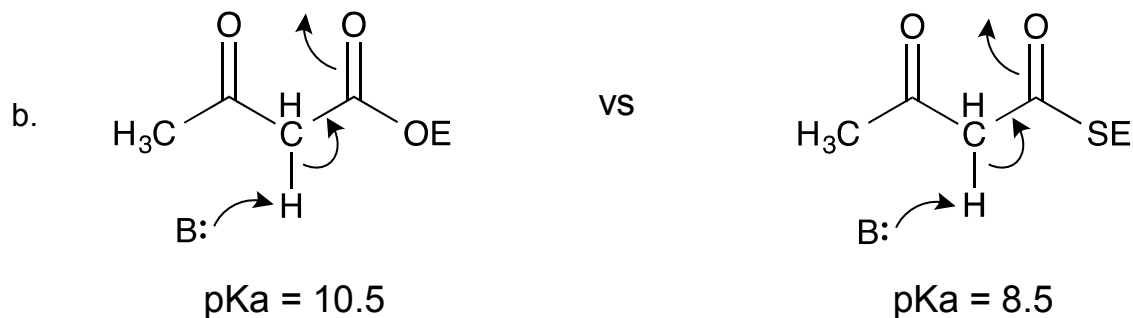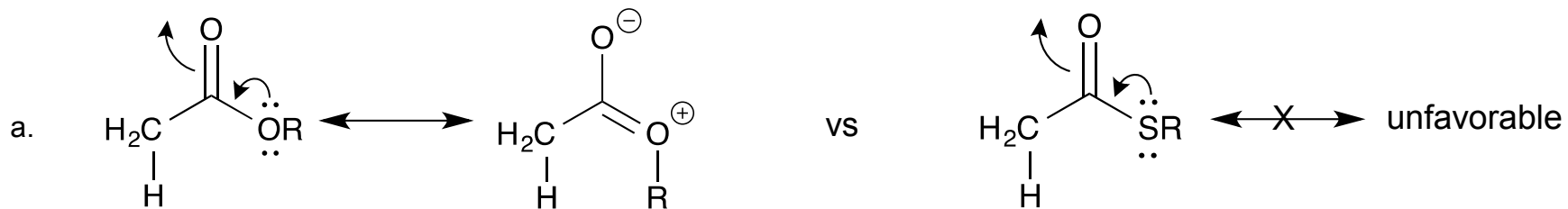
3. Prenyl transfer reactions: A third way to form C-C bonds
(not covered in 5.07)

3. Prenyl transfer reaction generalizations:
   a. All prenyl transfer reactions involve dimethylallyl pyrophosphate (DMAPP) and isopentylpyrophosphate (IPP)
   b. Most reactions involve carbo cation intermediates

- Generic mechanism:

DMAPP (C5)

Stabilized allylic cation

PP_i (pyrophosphate)

IPP (C5)

(C10)

Geranyl diphosphate (C10)

4. Redox Chemistry: Flavins (FAD, FMN, riboflavin) and nicotinamide adenine dinucleotides (NAD⁺ or NADP⁺)
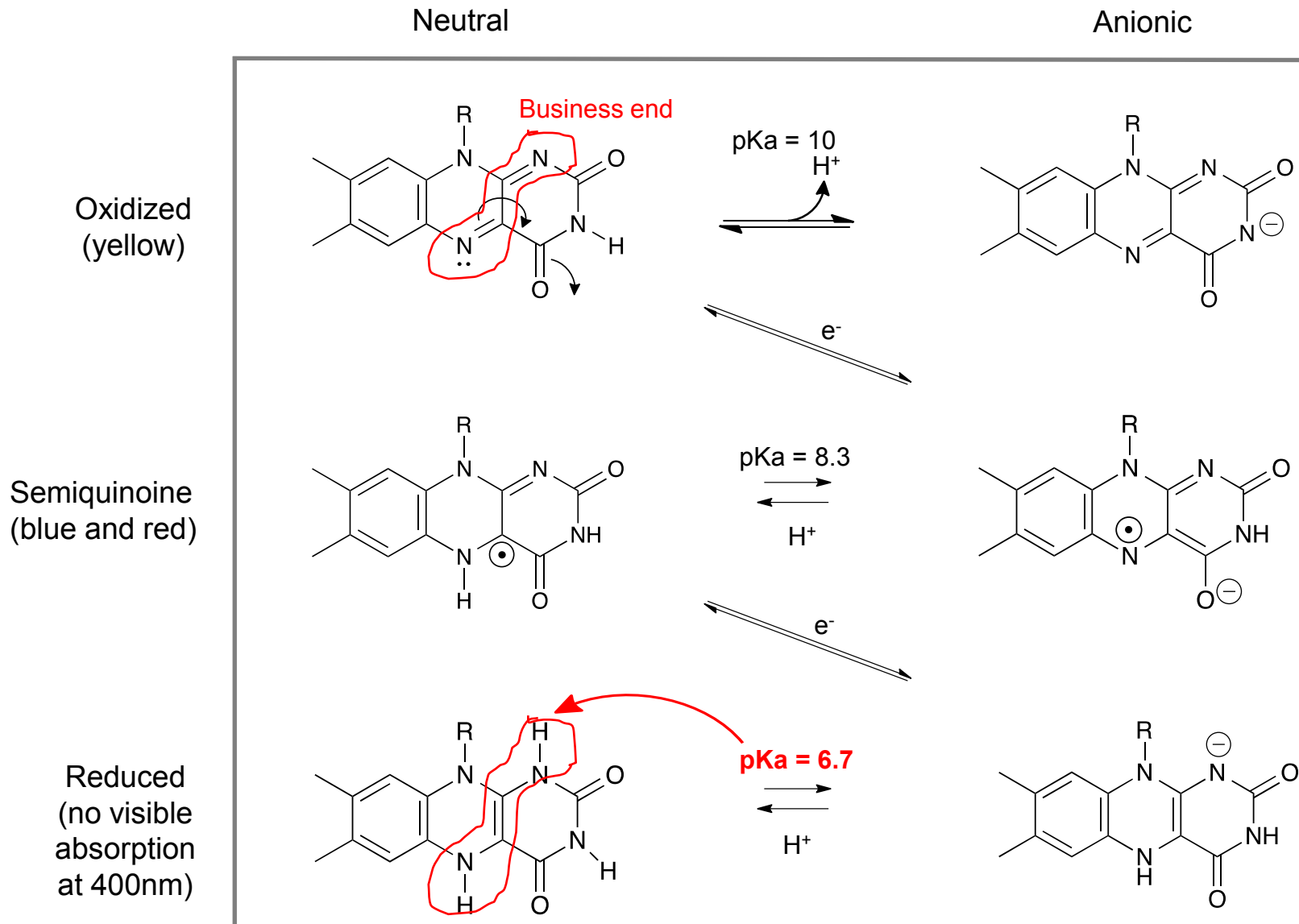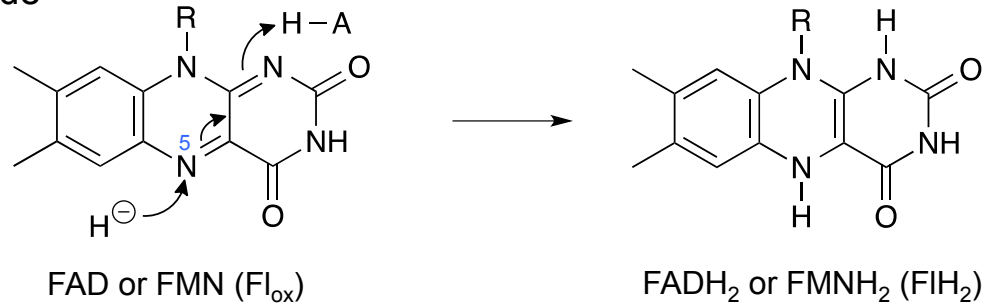
- Three forms of flavin:
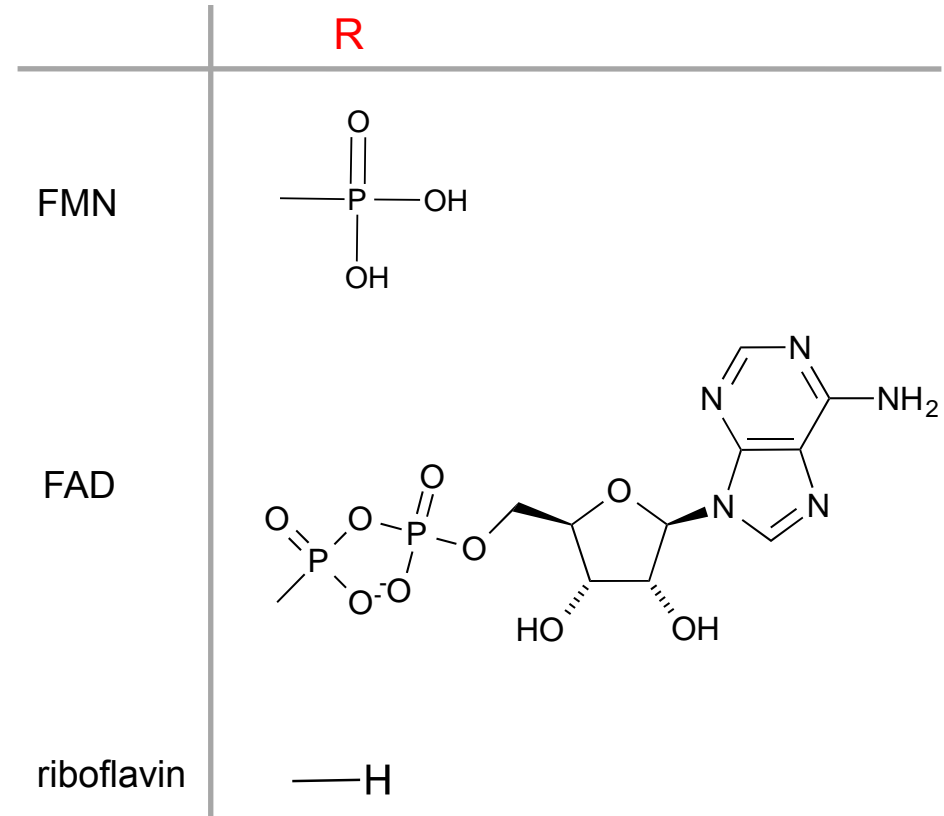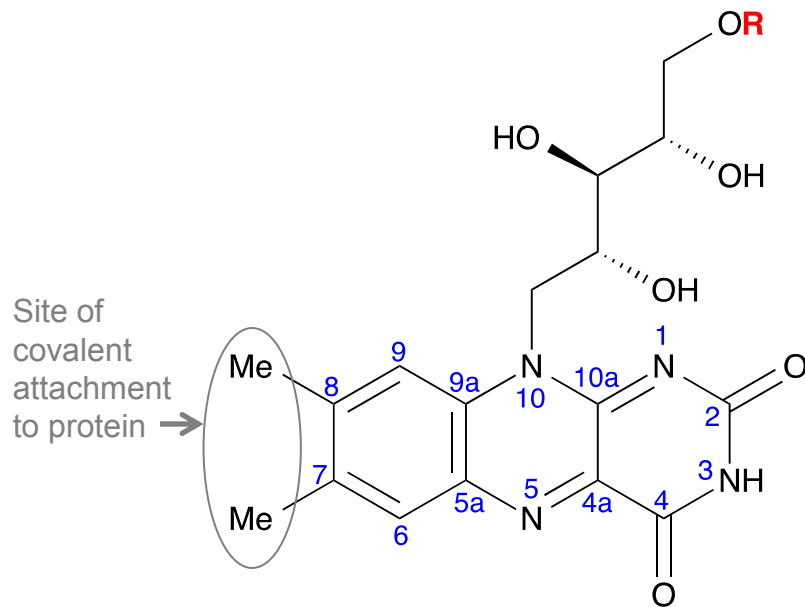
- Generalizations:
    a. FAD (FMN) are major mediators between 2 e⁻ donors and 1 e⁻ acceptors.
    b. Redox potentials can be modulated over 600 mv ($E^{o\prime}$ = -450 to +150 mv) by the protein environment.
    c. Flavins are tightly or covalently bound to most proteins, therefore they need to be reoxidized on the protein to complete the catalytic cycle.
    d. Reduced flavins ($FADH_2$ or $FMNH_2$) are very O_2 labile, that is, rapidly oxidized with O_2.
    e. Chemistry of flavins occurs at the C4a position or the N5 position. (Ex hydride from NADH is transferred to the N5 of the flavin, see example on next page.)

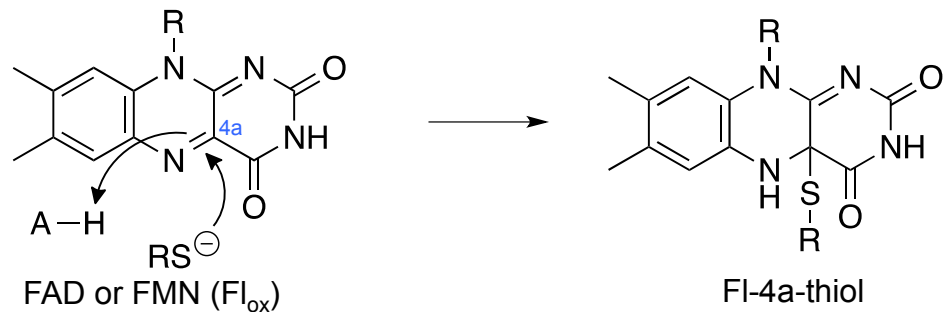# Redox states accessible to flavins under physiological conditions
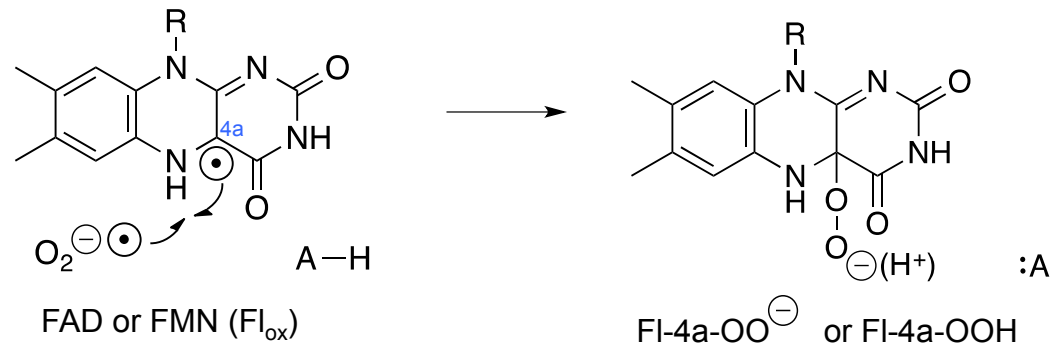
N5 and C4a chemistry of flavins
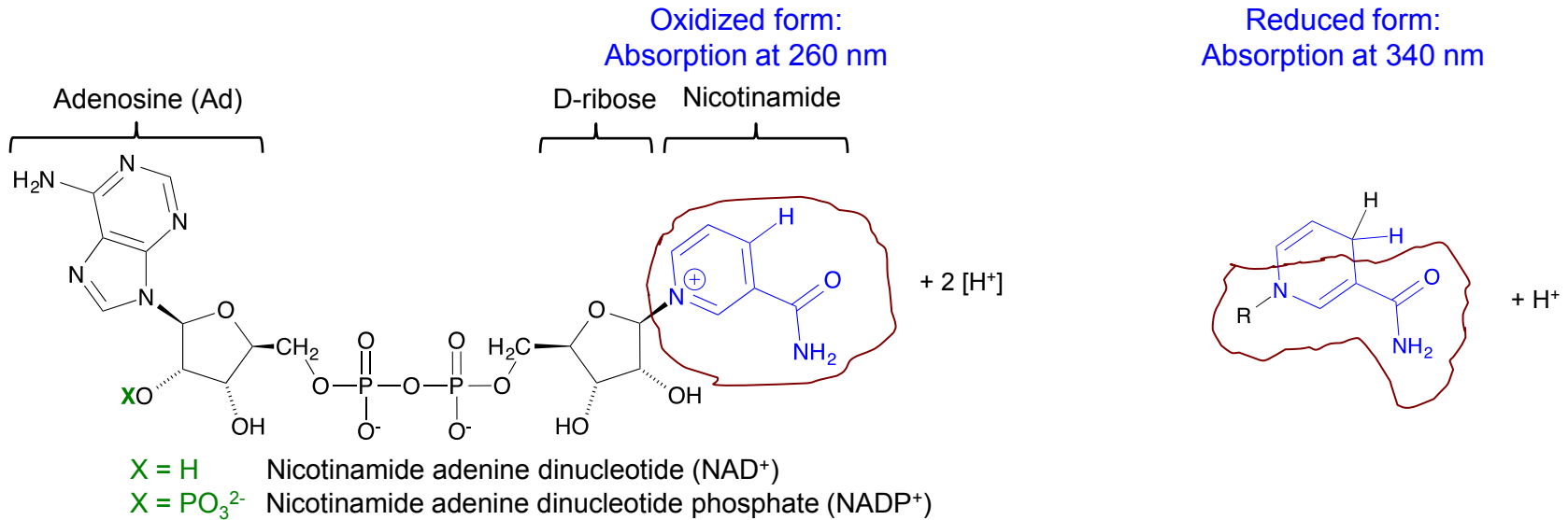
- N5 addition of hydride

FAD or FMN (Fl$_{ox}$)   FADH$_2$ or FMNH$_2$ (FlH$_2$)

- C4a addition of RS$^{\ominus}$

FAD or FMN (Fl$_{ox}$)   Fl-4a-thiol

- C4a addition of O$_2$$^{\ominus}$$^{\odot}$ to FlH$^{\odot}$ (not covered in 5.07)

FAD or FMN (Fl$_{ox}$)   Fl-4a-OO$^{\ominus}$ or Fl-4a-OOH

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NAD$^+$/NADH

Oxidized form:
Absorption at 260 nm

Reduced form:
Absorption at 340 nm

Adenosine (Ad)   D-ribose   Nicotinamide

+ 2 [H$^+$]

+ H$^+$

X = H   Nicotinamide adenine dinucleotide (NAD$^+$)
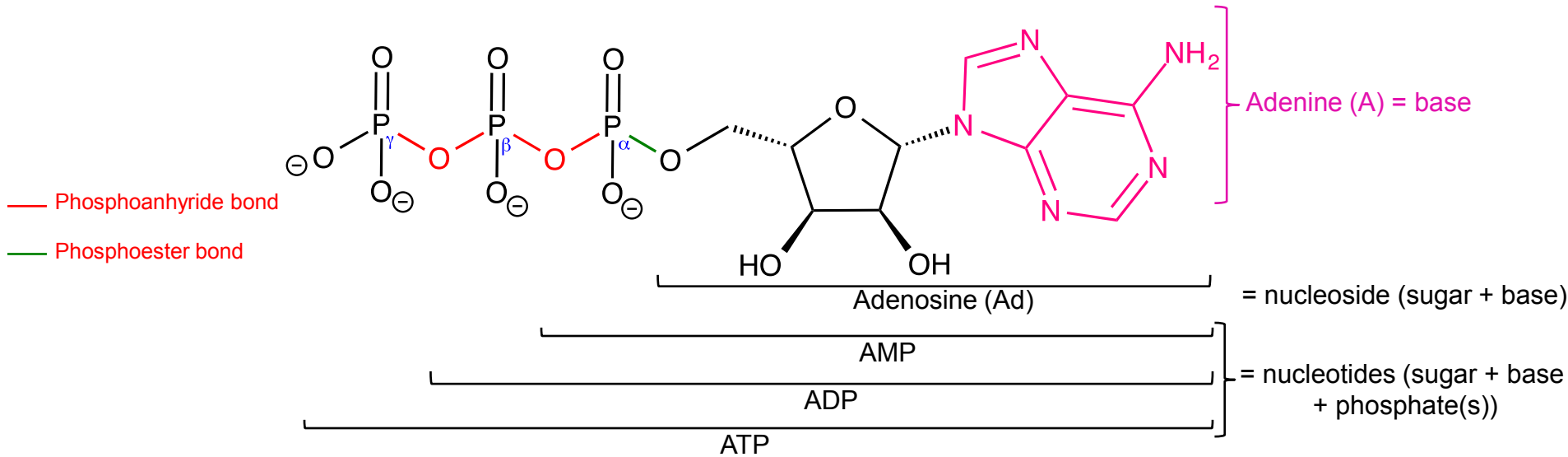X = PO$_3^{2-}$ Nicotinamide adenine dinucleotide phosphate (NADP$^+$)

- Difference in absorption is basis for spectrophotometric assay.

- Generalizations:
    a. NAD$^+$ mediates only 2 e$^-$ chemistry.
    b. Redox potential does not vary with protein environment (E$^{o\prime}$ = -320 mv).
    c. NAD functions as a substrate, comes off and on the enzyme on every turnover.
    d. NADH is not O$_2$ sensitive.
    e. The chemistry of NAD involves hydride (H$^-$) and proton (H$^+$) transfer. Don't forget the proton as it can dramatically effect equilibrium measurements.
    f. NAD in limited cases can be tightly (non-covalently) bound and be involved in masked redox chemistry, that is, it is reduced and reoxidized during conversion of substrate to product.

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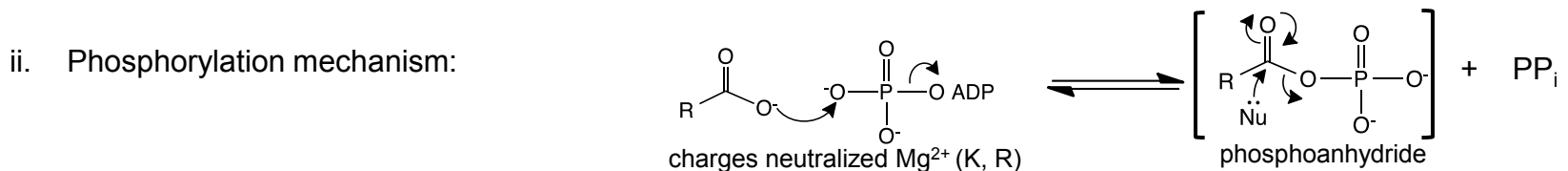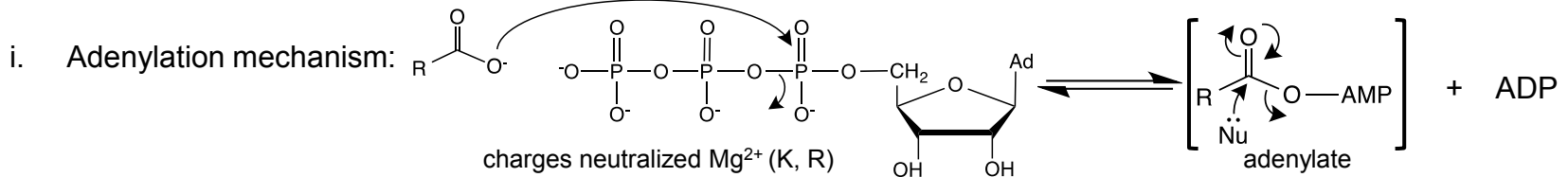
# Example of how NADH reduces flavins through N5 chemistry

- Hydride transfer mechanism:
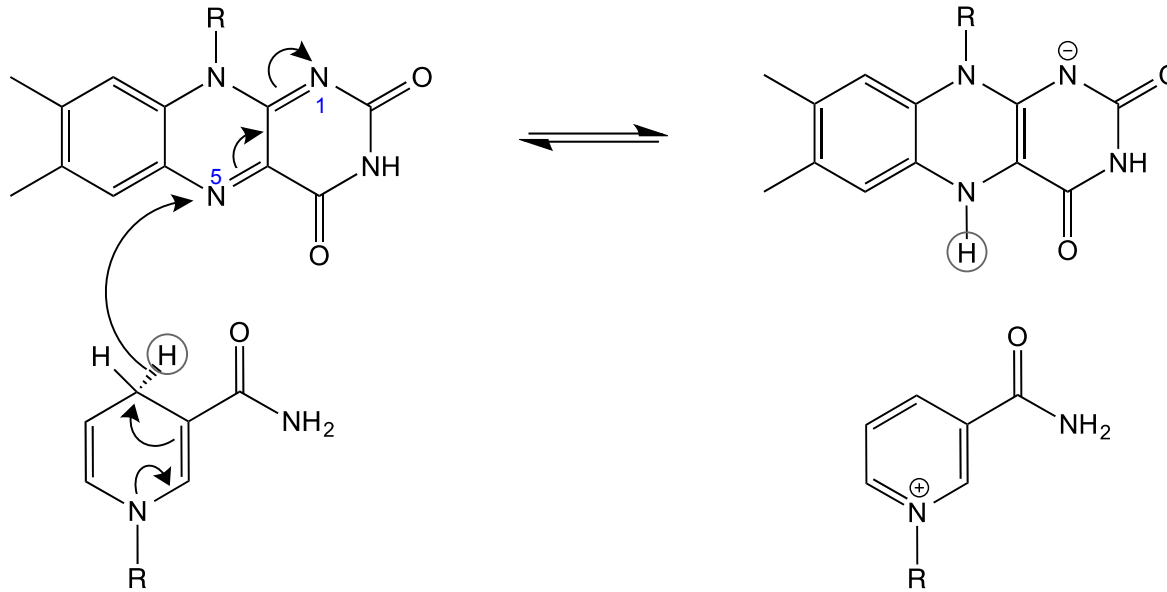
5. Mg^{2+}-ATP is the energy currency of the cell

— Phosphoanhyride bond

— Phosphoester bond

Adenine (A) = base

Adenosine (Ad) = nucleoside (sugar + base)

AMP

ADP = nucleotides (sugar + base + phosphate(s))

ATP

- ATP is kinetically stable and thermodynamically labile.

- Generalizations:
  - a. Major mechanism for dehydration of carboxylates requires either:
    - i. adenylation (attachment of AMP to make a better leaving group) or
    - ii. phosphorylation (attachment of phosphate to make a better leaving group).

i. Adenylation mechanism:

charges neutralized Mg^{2+} (K, R)

adenylate   +   ADP

ii. Phosphorylation mechanism:

charges neutralized Mg^{2+} (K, R)

phosphoanhydride   +   PP$_i$

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- ATP is almost universally coordinated to Mg^{2+}. Without neutralizing the charges on the phosphates, a nucleophile cannot attack the P. In general, nucleophiles attack either the γ or α phosphate, almost never the β phosphate.
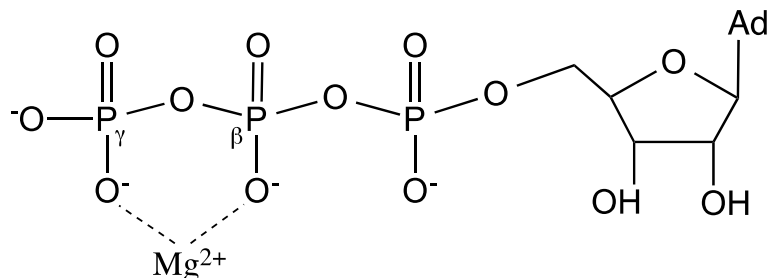
Mg^{2+} can coordinate α, β; and α, β, γ; as well as β, γ (shown)

- **Thermodynamically labile**

- The free energy of hydrolysis of ATP is large and negative and therefore ATP is frequently used in coupled reactions to drive unfavorable reactions.

- What is unique about P chemistry relative to C chemistry that you have learned about?
  1. Chemistry at phosphate can occur by associative and dissociative mechanisms, analogous to Sn1 and Sn2 reactions in carbon chemistry.
  2. In the dissociative case (Sn1 like), a metaphosphate transition state (ts) is observed (see next page). This ts is electron deficient (similar to a carbocation) and is activated toward nucleophilic attack.
  3. In the associative mechanism, a trigonal bipyramidyl (pentacoordinate) ts or intermediate ts is generated. This reaction is analogous to an Sn2 reaction. P because of its low lying 3d orbitals can form stable pentacoordinate structures (although none have been detected in biology).

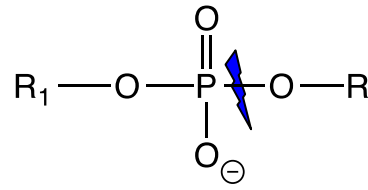# Proposed mechanisms for phosphoryl transfer
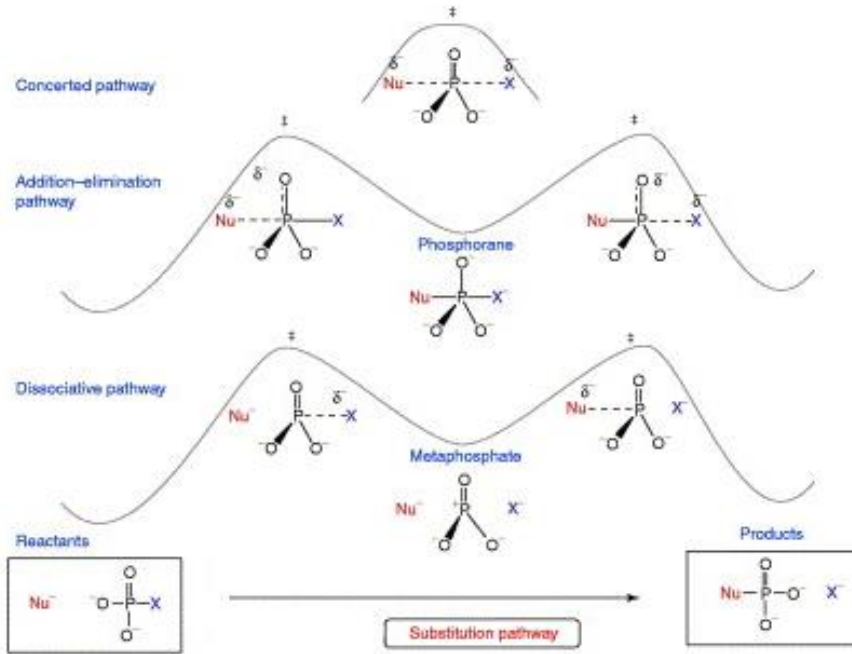
Allen, Karen N., and Debra Dunaway-Mariano. "Phosphoryl group transfer: evolution of a catalytic scaffold." *Trends in biochemical sciences* 29, no. 9 (2004): 495-503. Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.
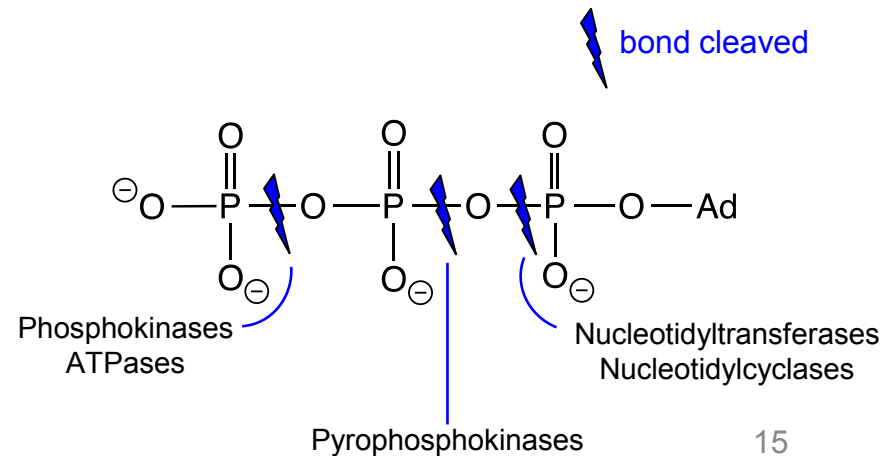
- Role in metabolism:
  - phosphoryl transfer
    - mostly in nucleic acid metabolism

bond cleaved
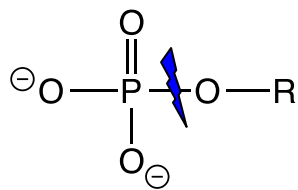
Phosphokinases
Phosphatases
Nucleotidases
Phosphomutases

Nucleases
Phosphodiesterases
Phospholipases

Phosphokinases
ATPases

Pyrophosphokinases

Nucleotidyltransferases
Nucleotidylcyclases

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6. Thiamine pyrophosphate (TPP) Vit B1

Aminopyrimidine ring
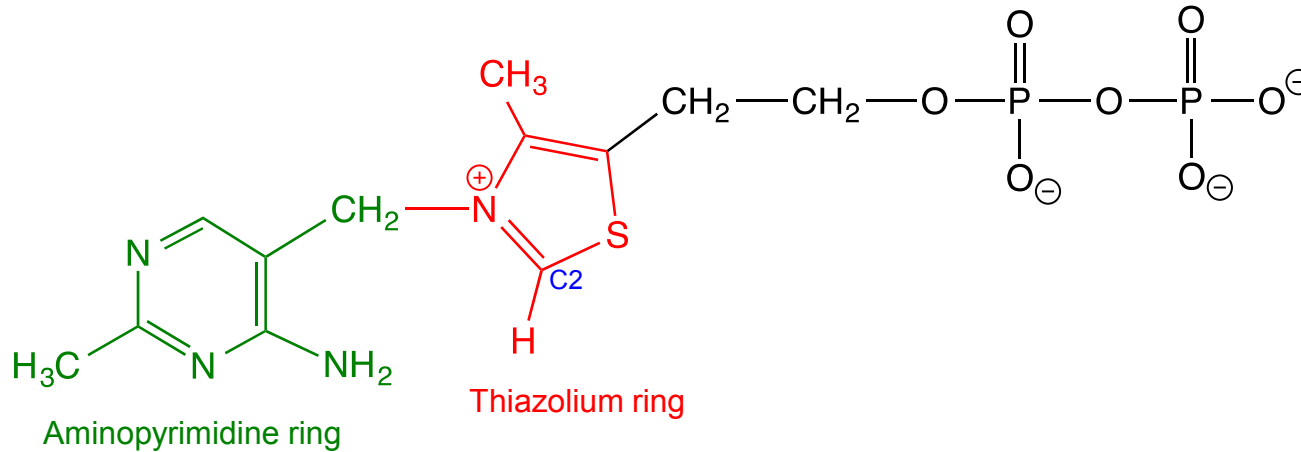
Thiazolium ring

- Role in metabolism for TPP enzymes:
  1. Oxidative decarboxylations
  2. Non-oxidative decarboxylations
  3. Aldol reactions

1. Mechanistic generalizations:
   a. Formation of an ylid on the thiazolium ring by abstraction of the C2 proton.
   b. Nucleophilic attack of the resulting carbanion on the carbonyl of the substrate to generate a tetrahedral intermediate equivalent to a β-keto acid.
   c. These types of species rapidly decarboxylate to generate hydroxyethyl-TPP.
   d. This intermediate undergoes different reactions depending on the enzyme.
      i. For non-oxidative decarboxylation, protonation occurs.
      ii. For oxidative decarboxylation, reaction with oxidized lipoic acid occurs.
      iii. For aldol reaction, reaction with an aldehyde occurs.

Example of ending via protonation

Protonation

Ending with aldehyde

Ending with oxidized lipoic acid
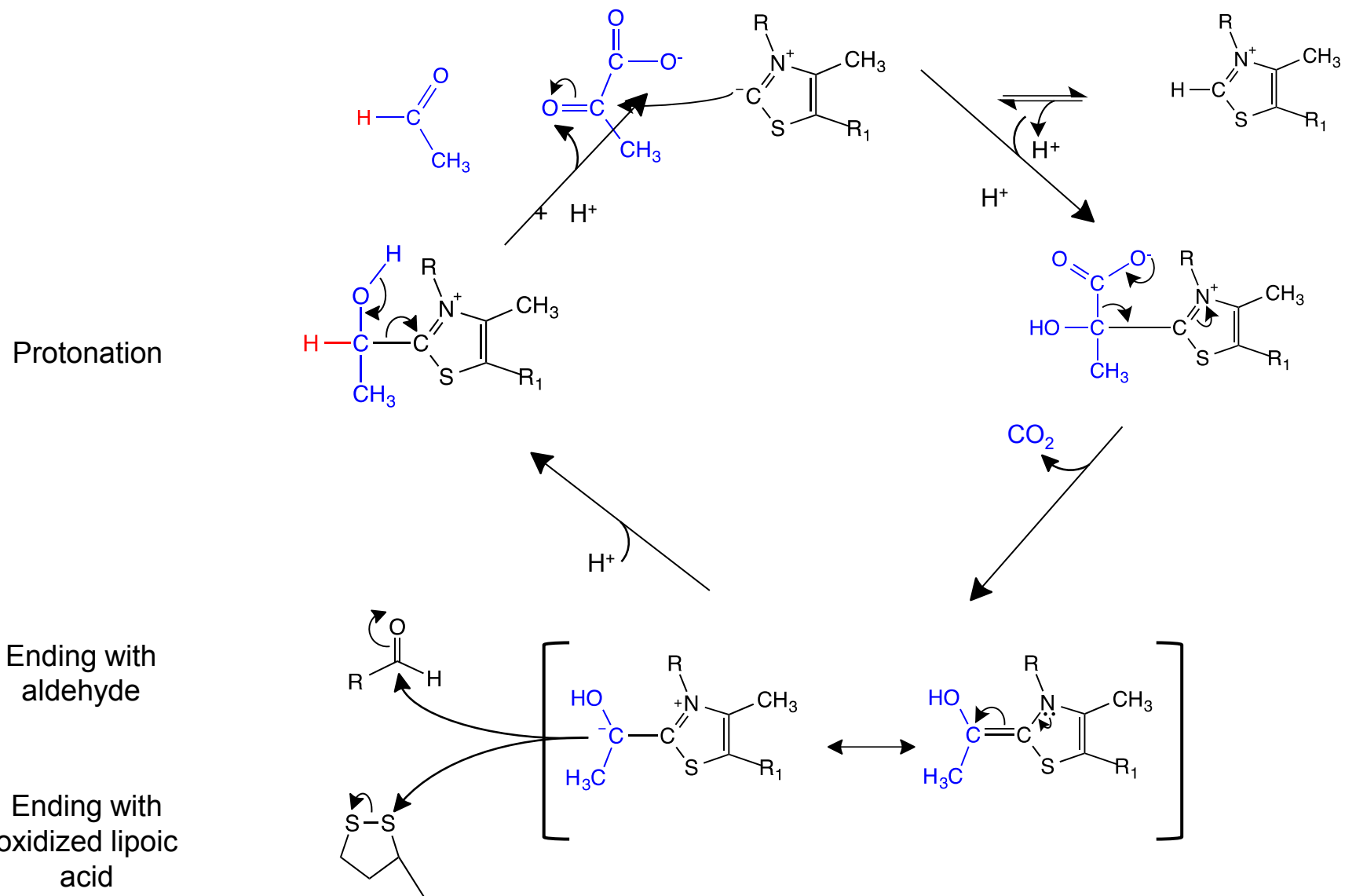
Structures of TPP enzymes reveal an unanticipated role of the pyrimidine amino group in ylid formation

Imine

Ylid
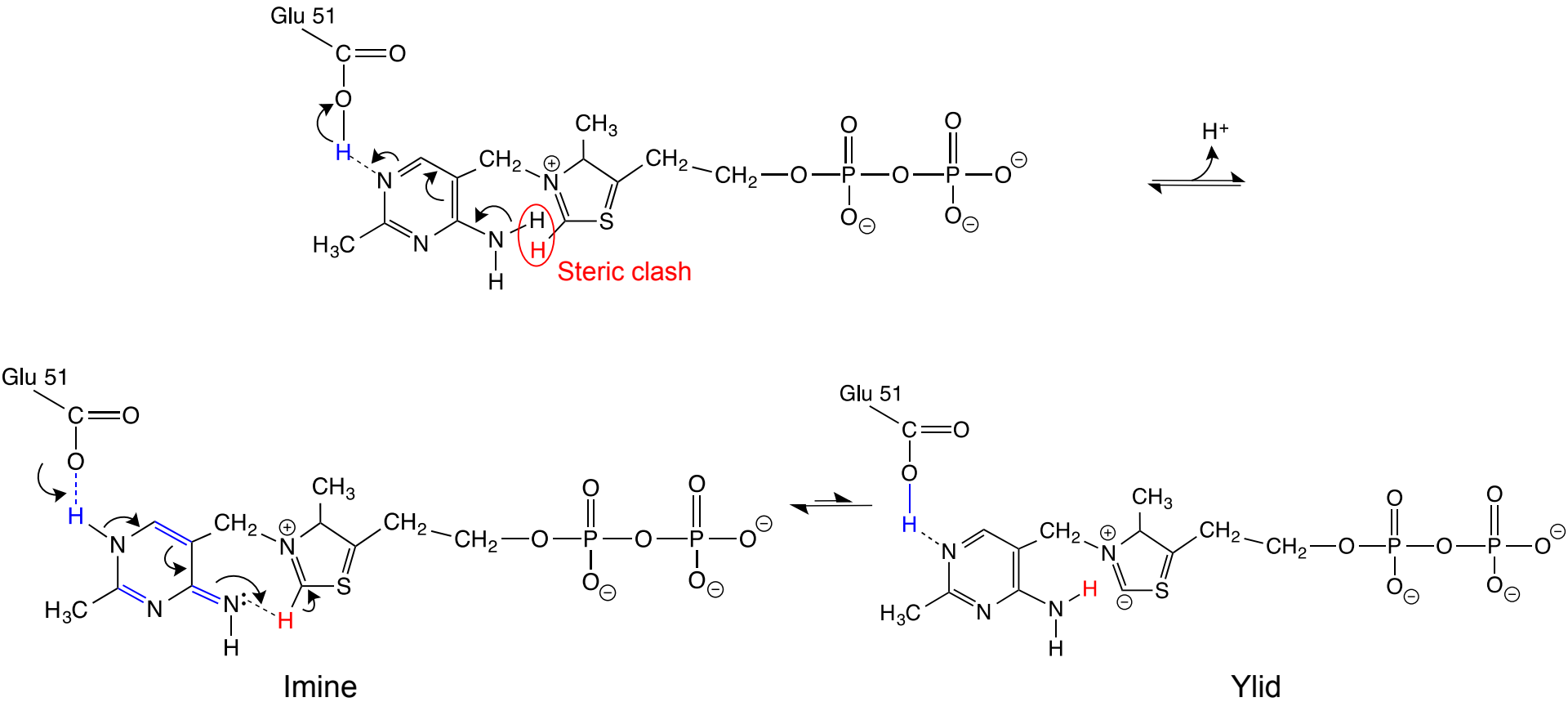
7. Lipoic acid

- Role in metabolism:
  - A redox cofactor covalently attached to proteins through a lysine
    - Examples are pyruvate dehydrogenases (PDH) and α-ketogluterate dehydrogenases (KGDH) of the TCA cycle


$2H^+ + 2e^-$

Lipoic acid

Lys residue of protein

Lipoamide

Dihydrolipoamide
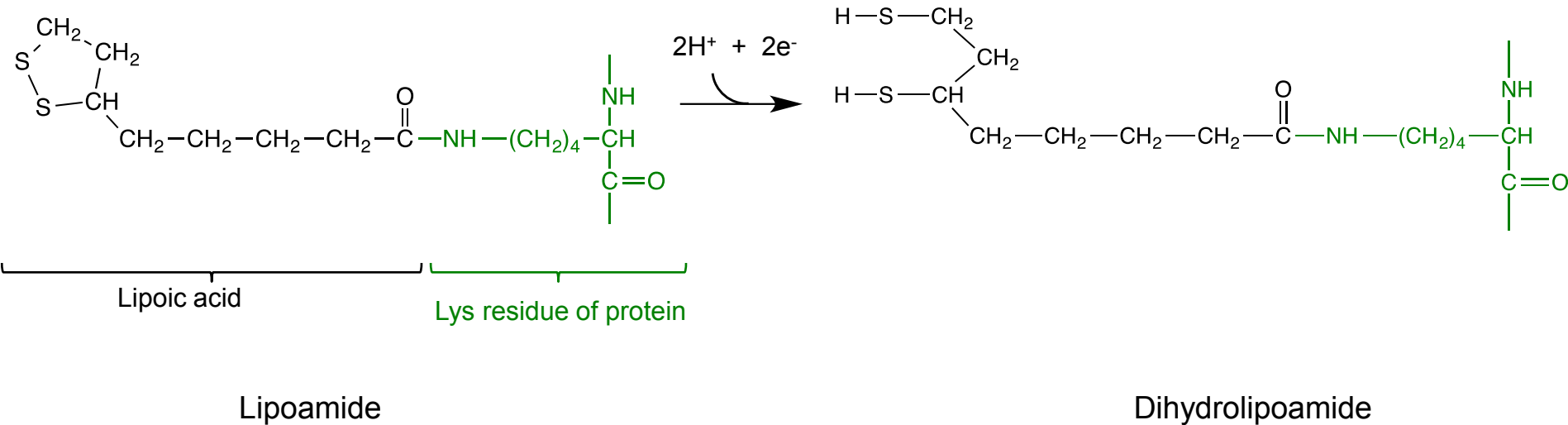
8. S-adenosylmethionine (SAM or Adomet), methylcobalamin and N-5 methyltetrahydrofolate are all methylating agents

S-Adenosylmethionine (SAM)

$$Nu - CH_3 \qquad Nu = OH, SH, NH_2, -CR_3$$
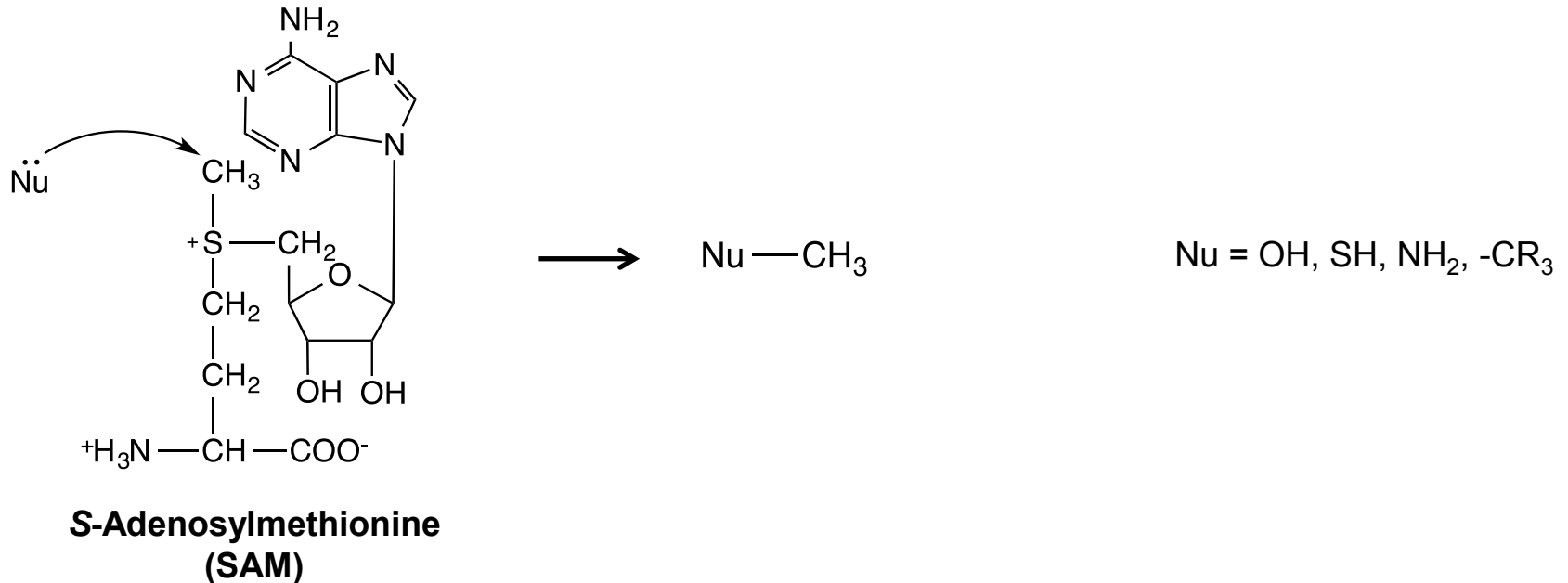
- Role in metabolism:
  - Universal methylating agent
- Mechanism generalizations:
  a. Nucleophilic attack of the activated methyl group via an Sn2 reaction. The reaction occurs with an inversion of configuration.
  b. Newly discovered role in metabolism – radical SAM superfamily (SF). In this SF, SAM binds to a unique Fe in the $[4Fe4S]^{1+}$ cluster and is reductively cleaved to generate 5'-deoxyadenosyl radical (AdoChl, 5'-dA•). This radical is involved in radical chemistry (not covered in 5.07).
     - Bioinformatics studies suggest that > 40,000 reactions will use this chemistry.

9. Pyridoxal phosphate (PLP)
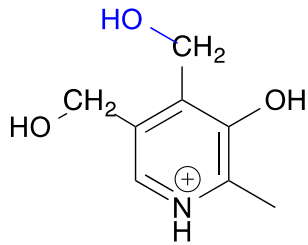
Pyridoxine
(vitamin B$_6$)
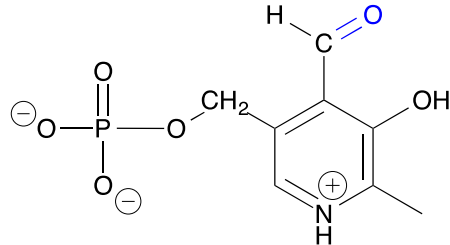
Pyridoxal-5'-phosphate
(PLP)
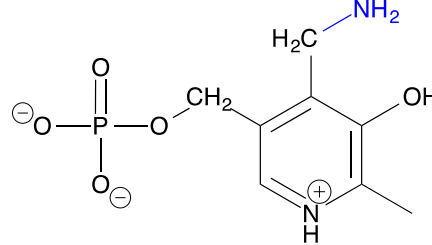
Pyridoxamine-5'-phosphate
(PMP)
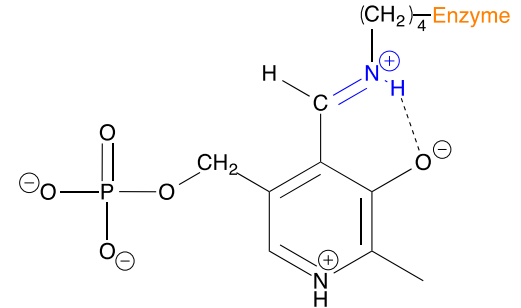
Enzyme-PLP
(Schiff base, aldimine)

- The first step in metabolism of any amino acid involves PLP.

- Generalizations:
    a. PLP is able to mediate chemistry at the α, β and γ positions of the amino acid.
        i.   At α position: transamination, epimerization, decarboxylation and aldol reactions
        ii. At β position: eliminations and replacement
        iii. At γ position: eliminations and replacements
    b. PLP is always covalently bound to the enzyme as a Schiffs base (imine) with an active site lysine (lys).
    c. The first step is a transimination reaction with the amino acid, liberating the lys which then functions as a

    general acid catalyst (gac) or general base catalyst (gbc) in further steps in substrate transformation.
    d. The second step in all PLP reactions is generation of a carbanion at the α carbon of the amino acid. This can be done by the removal of the proton from the α position or by decarboxylation of the amino acid. This intermediate is the precursor to both β and γ elimination reactions and β and γ replacement reactions.
    e. The final step in all PLP reactions is transimination to reform the Schiffs base with the lysine at the active site.

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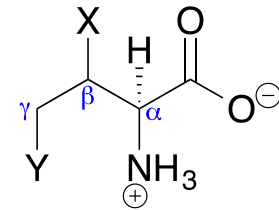
- **Dunathan's hypothesis** provides the chemical rational for the first chemistry that occurs subsequent to transimination. The bond to be cleaved (C-H or C-C bond in decarboxylation) is positioned by the enzyme, perpendicular to the plane of the π aldimine system to maximize electron overlap of the resulting anion with the pyridine ring.
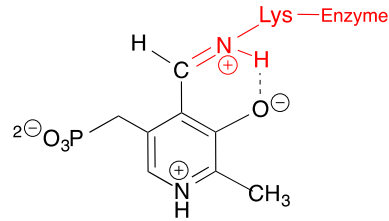
Amino acid-PLP Schiff base

Delocalized α carboanion
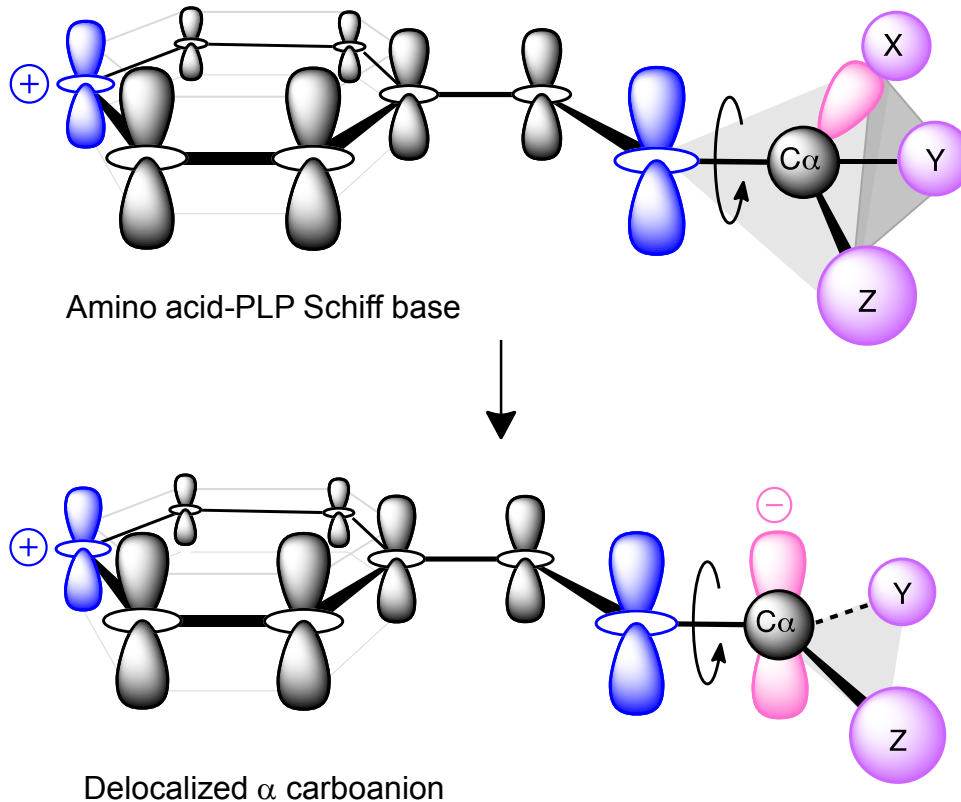
Example of the first half reaction of a transimination reaction

1. Transimination
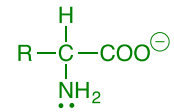
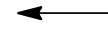α-Amino acid   Enzyme-PLP (Schiff base)   Geminal diamine intermediate
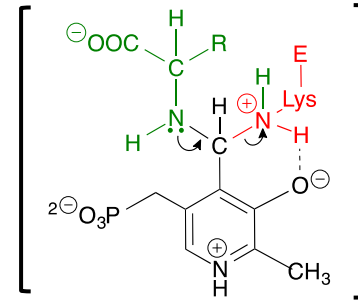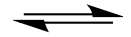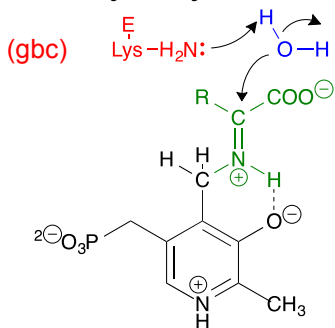
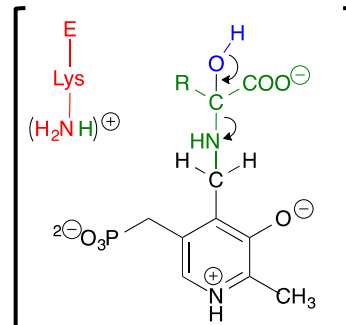2. Removal of the α hydrogen
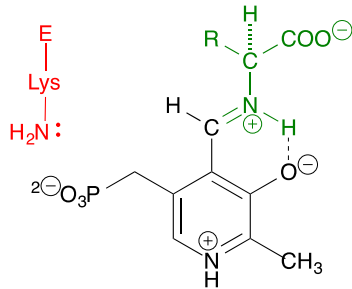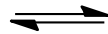

Amino acid-PLP (Schiff base, aldimine)   Resonance-stabilized intermediate
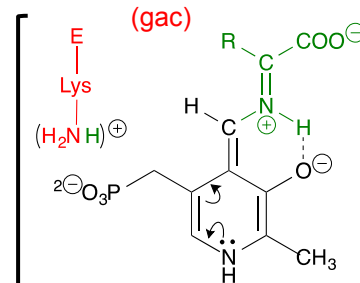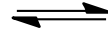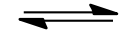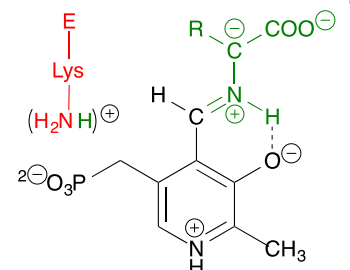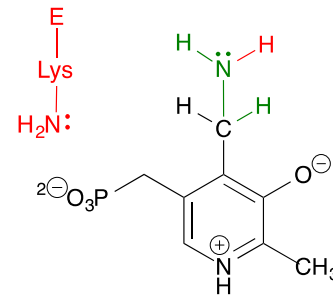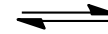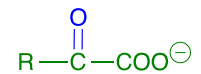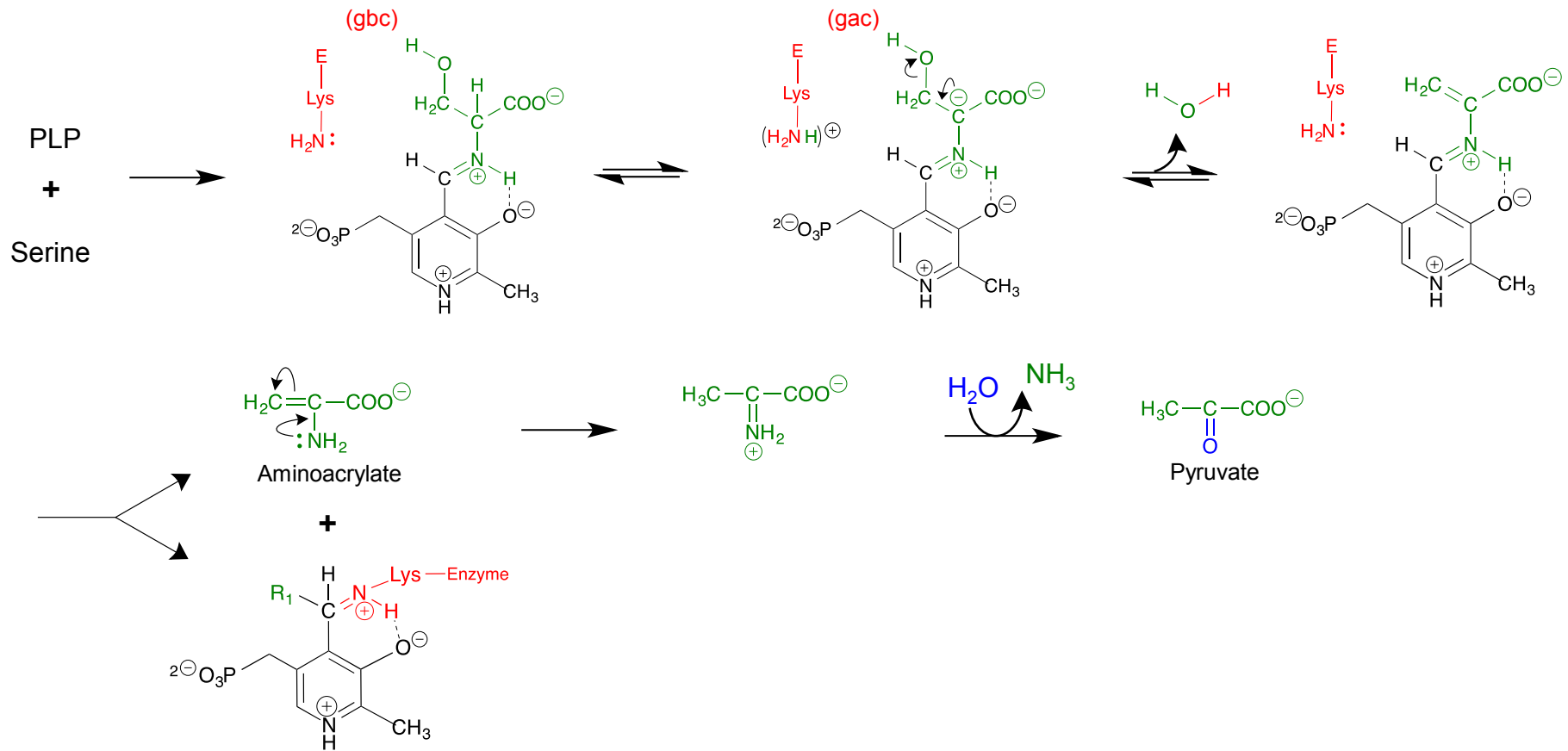
3. Hydrolysis

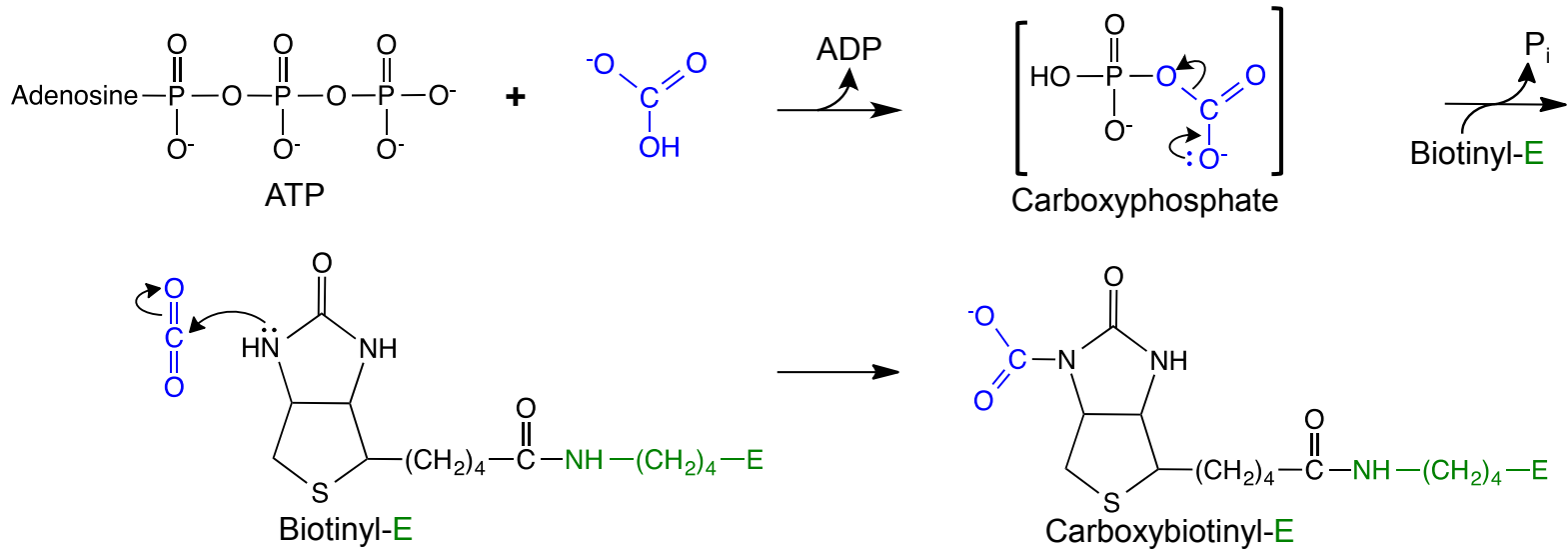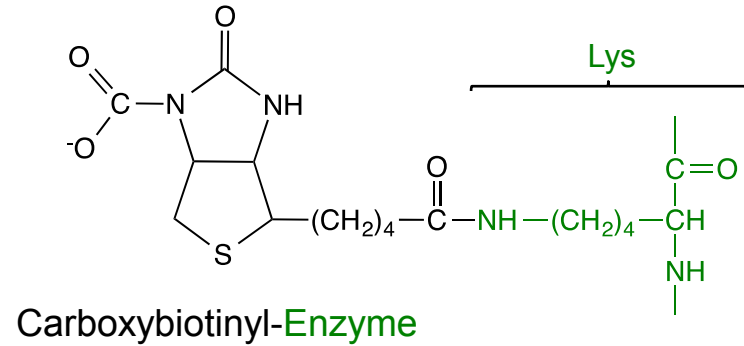Ketimine   Carbinolamine   Pyridoxamine phosphate (PMP)-Enzyme   α-Keto acid

Example of a β elimination reaction: Serine dehydratase

Aminoacrylate

Pyruvate

- The first half reaction was shown on the previous page; a second α-keto acid ($R_1-COCO_2^{\ominus}$) then converts PMP back to Enzyme-PLP and liberates the corresponding amino acid ($R_1-CH(NH_3^{\oplus})-CO_2^{\ominus}$)

- As shown in both examples, the reactions often make use of the Lys in the active site of all PLP-requiring enzymes, which functions as both a gbc and gac.

10. Biotin: carboxylation reactions involved in FA biosynthesis

Biotin

Valerate side chain

Carboxybiotinyl-Enzyme

Lys

ATP

Carboxyphosphate

Biotinyl-E

Biotinyl-E

Carboxybiotinyl-E
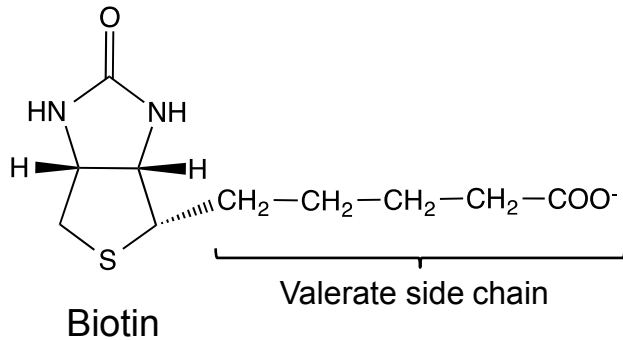
The carboxylated biotin then transfers the CO_2 to the α carbon of acetylCoA (or propionylCoA in a claisen type reaction.

11. Hemes

- As you have seen with Mb and Hb, heme is the cofactor involved in reversible O_2 binding in many organisms. Hemes are very versatile cofactors and are involved in hydroxylation of unactivated C-H bonds, epoxidation of olefins, oxidation of sulfides to sulfoxides. These types of reactions are found in the liver, which is the organ largely responsible for removal of xenobiotics (environmental toxins).

- Heme dependent enzymes also catalyze disproportionation of hydrogen peroxide to O_2 and water, removing toxic hydrogen peroxide.

- The active site of the enzyme and the residues on the distil and proximal face of the heme govern which types of reactions are observed. The distil face of the heme is the one that binds O_2 to Fe, while the proximal face in the case of Mb and Hb has a His that coordinates Fe. Cysteine and tyrosine can also coordinate Fe on the proximal face in other enzymatic systems.

H_3C—COO^- + $H_3\overset{+}{N}$—CH_2—COO^-

Acetate   Glycine
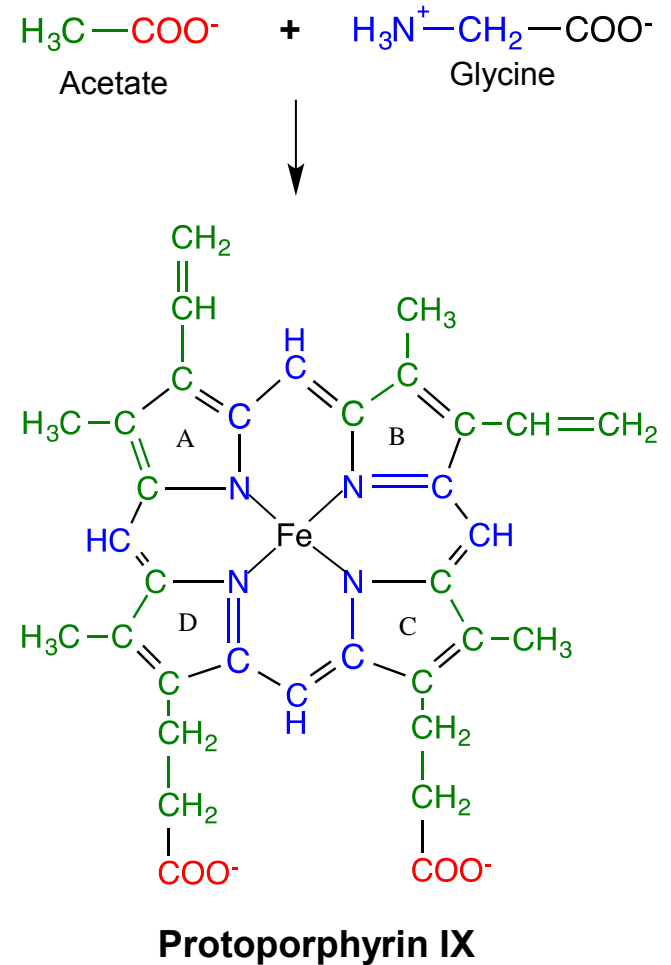
Protoporphyrin IX

Different Hemes in ET (electron transfer) respiration
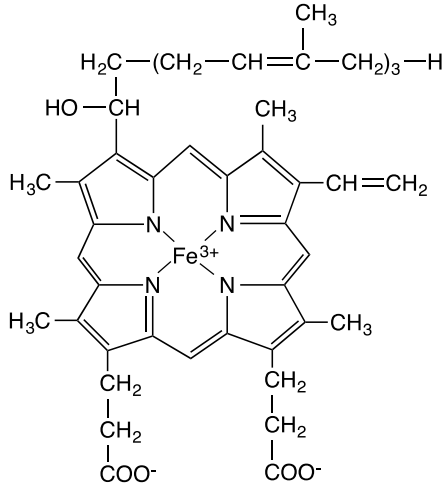
Heme *a*
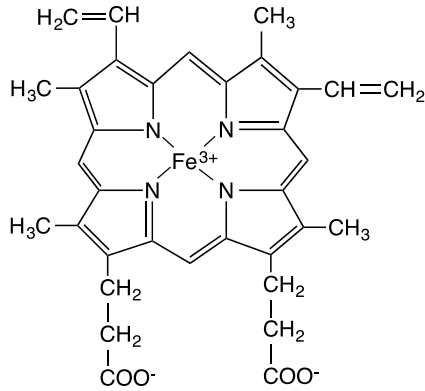
**Heme *b*
(iron-protoporphyrin IX)**
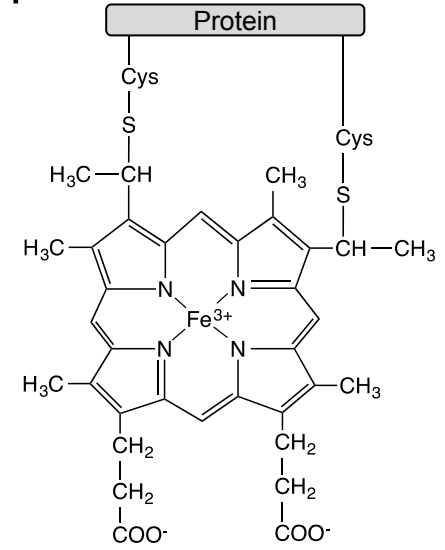
Heme *c*

Hemes *a* and *b*

His   His
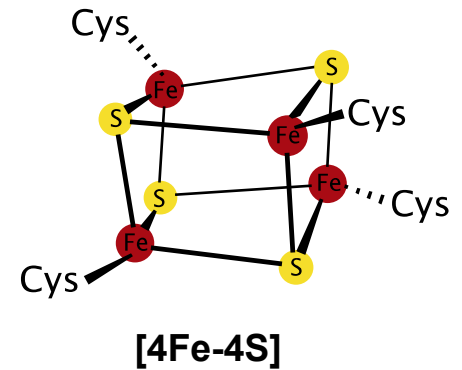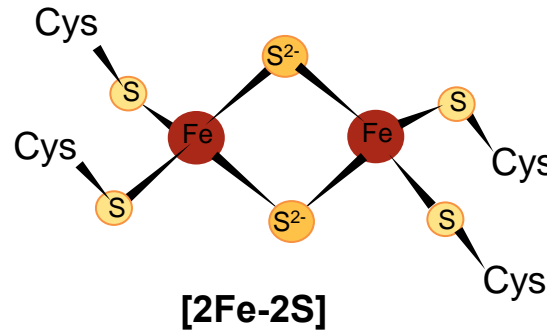
Heme *c*

Met   His
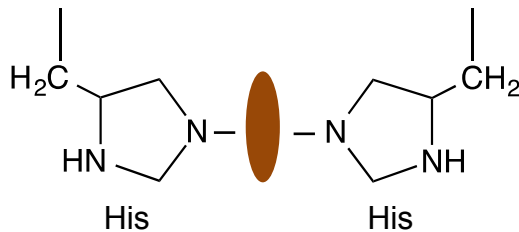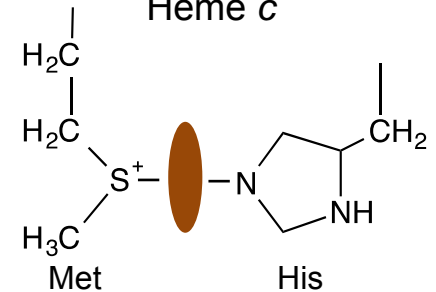
- Function: O_2 carriers, 1 e^- transfers. With Hb (Mb), the iron of the heme is in the +2 (Fe^{2+}) oxidation state. If the iron gets oxidized to ferric heme (Fe^{3+}) and superoxide ($O_2^{\bullet-}$), then the iron must be re-reduced before binding O_2. In the case of ET in respiration, the iron toggles between the +2 and +3 oxidation states.

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[Fe-S] [2Fe-2S] [4Fe-4S]
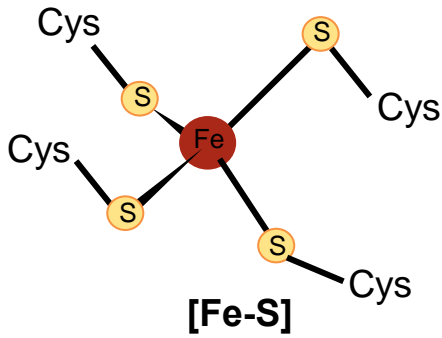
Iron sulfur clusters are involved in ET in the respiratory pathway. The irons change between the +2 and +3 oxidation states.

Other cofactors in the respiratory pathway: complex IV (cytochrome oxidase), which reduces O_2 to H_2O.

13. Coenzyme Q (ubiquinone)

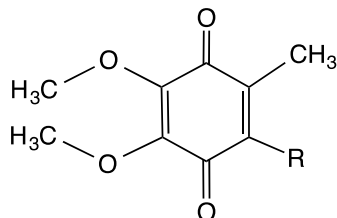**Coenzyme Q (CoQ) or ubiquinone**

R = ... Isoprenoid units
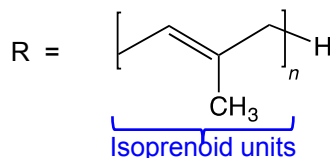
Oxidized or quinone form
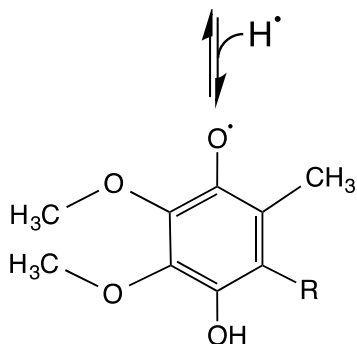
Coenzyme QH• or ubisemiquinone

Radical or semiquinone form

Coenzyme QH₂ or ubiquinol

Reduced or hydroquinone form

Functions in 1 e⁻ or 2 e⁻ transfers and is associated with membranes due to the hydrophobic tail that contains a long isoprenoid unit (see section of C-C bond formation). One electron chemistry (proton coupled electron transfer) is shown above.

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14. Electron transfer in biology over long distances
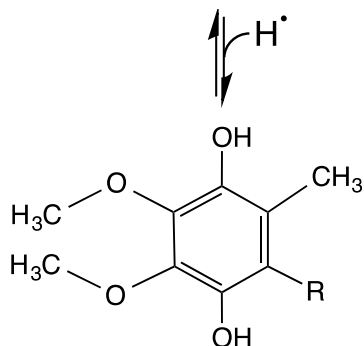
- The theory of electron transfer (ET) makes predictions about the dependence of ET rates on the structures of the electron donor (D) and electron acceptor (A) (oxidized and reduced states), the distance between D and A, and the driving force for the ET process. The e⁻ can be treated as a particle and a wave and the theories for ET are based on quantum-mechanical expressions of the wave functions of the D and A involved in electron donation and acceptance. The wave functions are coupled weakly by the media (protein) in between the A and D.

- Dealing with electron movement is distinct from dealing with proton movement because of the differences in mass of the e^- vs the H^+ (H^+ is 2000 x). Thus, while electrons can "tunnel" over 10 to 15 Å at very fast rates (10^8 s^{-1}), protons can only tunnel over 0.7 Å. Most metal clusters that you will encounter in biology (ex, respiratory chain) are spaced 10-15 Å apart and the rates are limited by physical steps, conformational gating, and not k_{ET}.
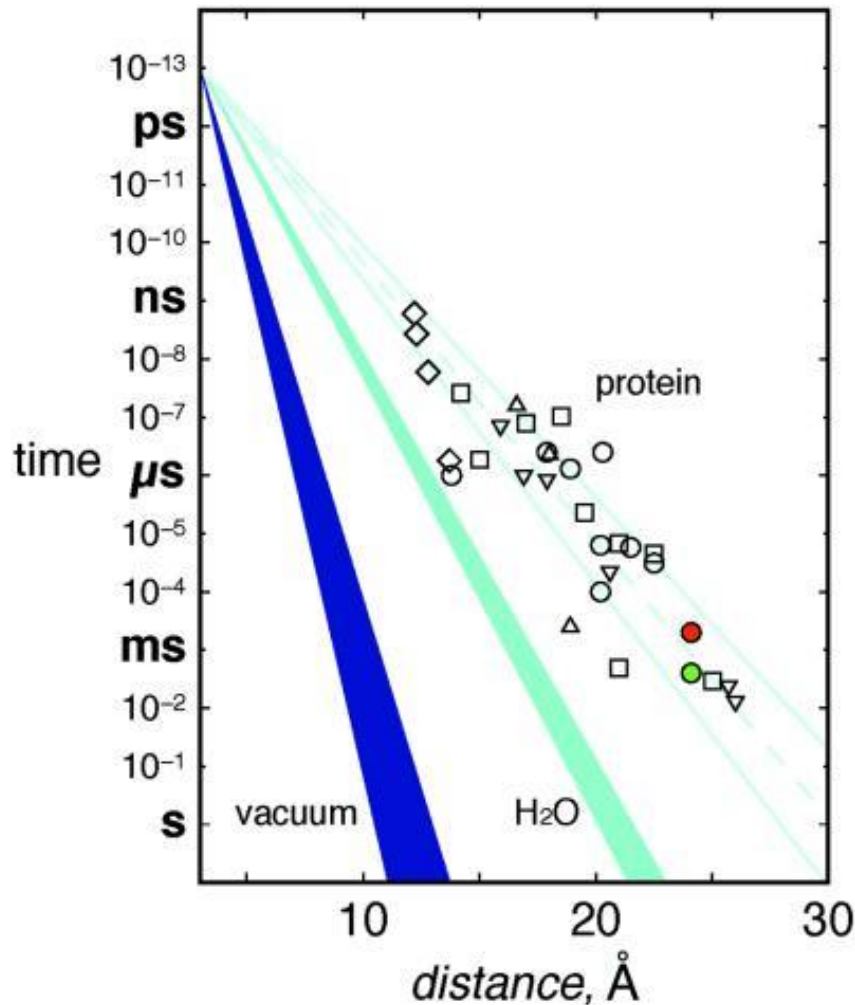
Semiclassical theory describing ET (Gray and Winkler):

$$k_{ET} = \sqrt{\frac{4\pi^3}{h^2 \lambda k_B T}} \; H_{AB}^2 \; \exp\left\{ -\frac{(\Delta G^{\circ} + \lambda)^2}{4\lambda k_B T} \right\}$$

k_{ET} is the rate constant for ET between donor (D) and acceptor (A),
ΔG° is the driving force for the reaction,
λ is the nuclear reorganization energy
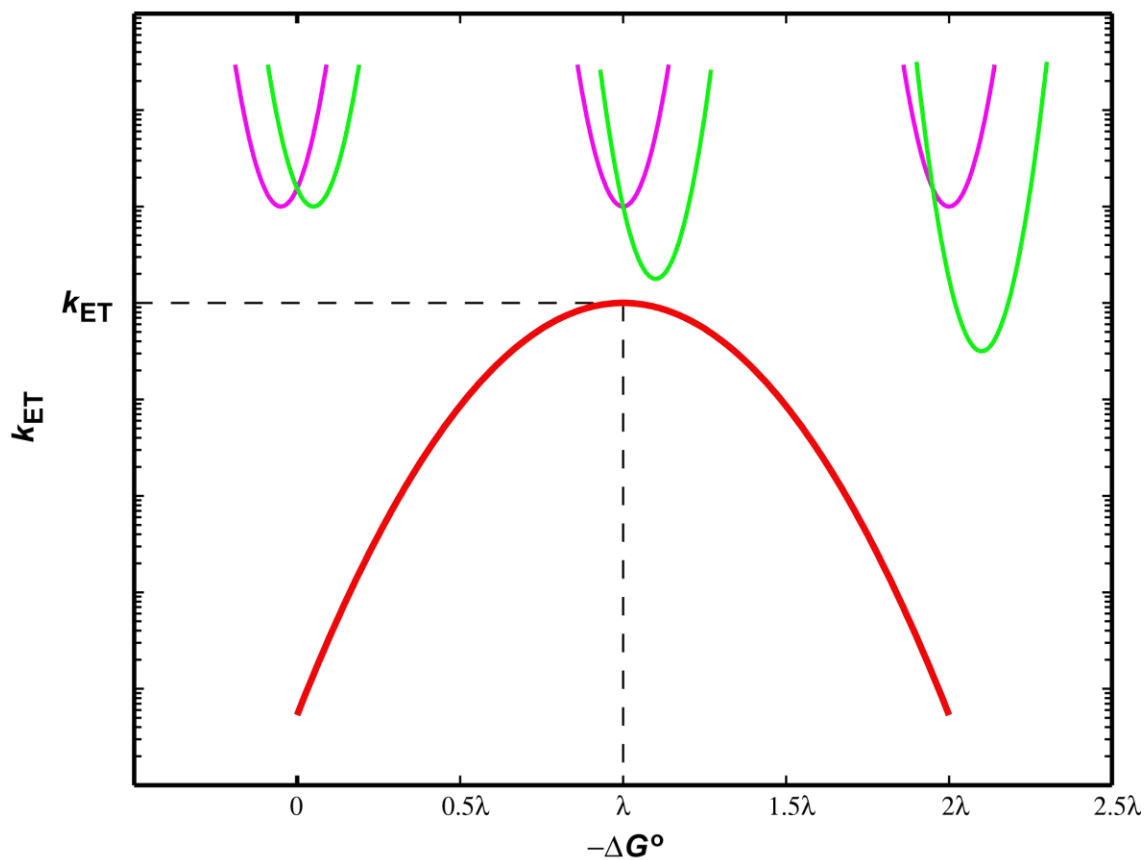
Tunneling time table for ET: k_{et} falls off exponentially with distance

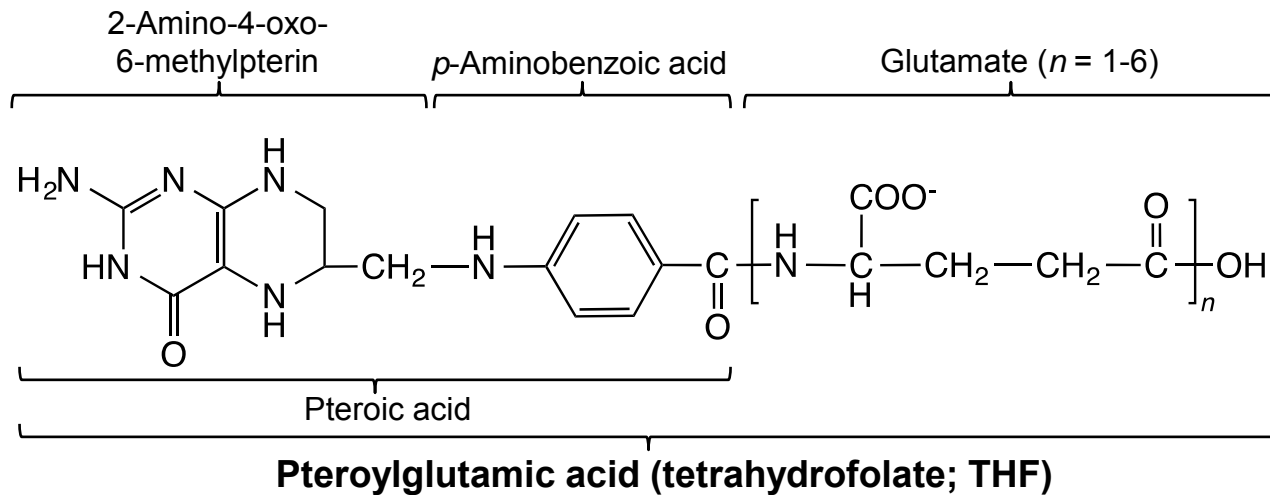$$H_{AB}(r) = H_{AB}(r_0) \exp\left\{ -\tfrac{1}{2}\beta(r-r_0) \right\}$$

The electronic coupling element (HAB) reflects the strength of the interaction between D and A at the nuclear configuration of the transition state. Hopfield's model predicts that coupling will depend exponentially on the distance, r, between redox centers.

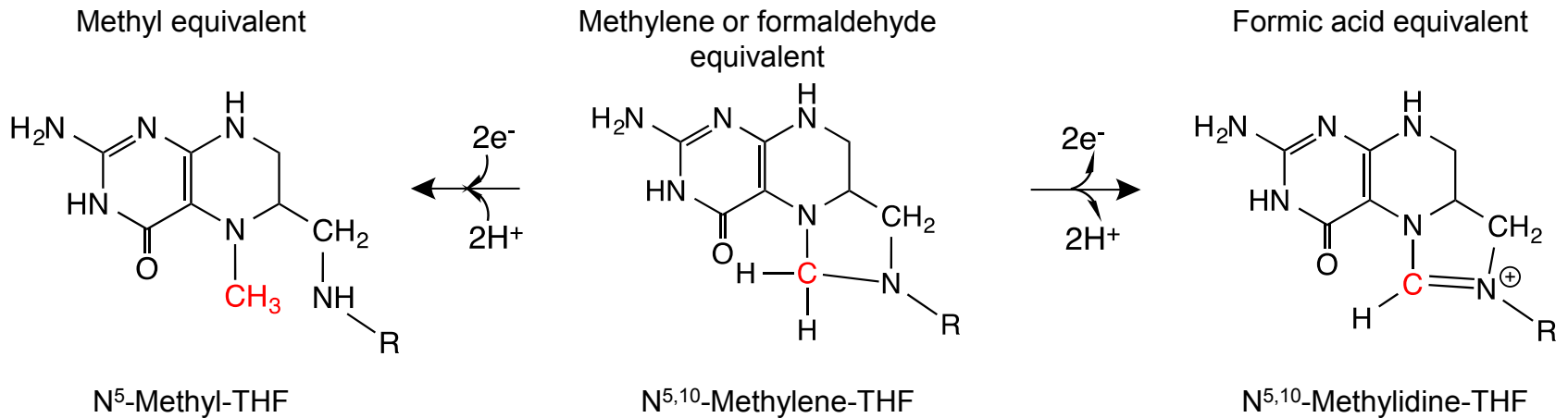β depends on the media separating D and A. For a vacuum β = 3-5 Å^{-1}; through H_2O it = 1.65Å^{-1} and for inhomogeneous proteins it has been measured by Gray/Winkler to be 1.1 Å^{-1}.

Figure 5 from Tezcan, F. Akif, Brian R. Crane, Jay R. Winkler, and Harry B. Gray. "Electron tunneling in protein crystals." *Proceedings of the National Academy of Sciences* 98, no. 9 (2001): 5002-5006.

15. Folates: One carbon transfers at all oxidation states

2-Amino-4-oxo-6-methylpterin | *p*-Aminobenzoic acid | Glutamate (*n* = 1-6)

Pteroic acid

Pteroylglutamic acid (tetrahydrofolate; THF)

All three oxidation states of C can be transferred

Methyl equivalent

Methylene or formaldehyde equivalent

Formic acid equivalent

$2e^-$

$2H^+$

$2e^-$

$2H^+$

N^5-Methyl-THF

$N^{5,10}$-Methylene-THF

$N^{5,10}$-Methylidine-THF

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- Folate is the major player in one carbon transfers. The carbon is obtained from serine. The carbon can be transferred to its acceptor in all three oxidation states. This type of reaction is prevalent in purine and pyrimidine biosynthetic pathways that we will not discuss in 5.07.

- The single carbon can be transferred as a CH_3 group from N^5-MeTHF; at the aldehyde level with $N^{5,10}$-methylene-THF; and at the acid oxidation state from N^{10}-formyl THF.

16. Methylcobalamin/Adenosylcobalamin


Corrin ring

Dimethyl-benzimidazole (DMB)

	R
Cyanocobalamin (vitamin B12, CNCbl)	CN
Methylcobalamin (MeCbl, CH$_3$-Cbl)	CH$_3$
Adenosylcobalamin (5'-deoxyadenosylcobalamin, 5'-dA-Cbl, AdoCbl)	-CH$_2$ ...
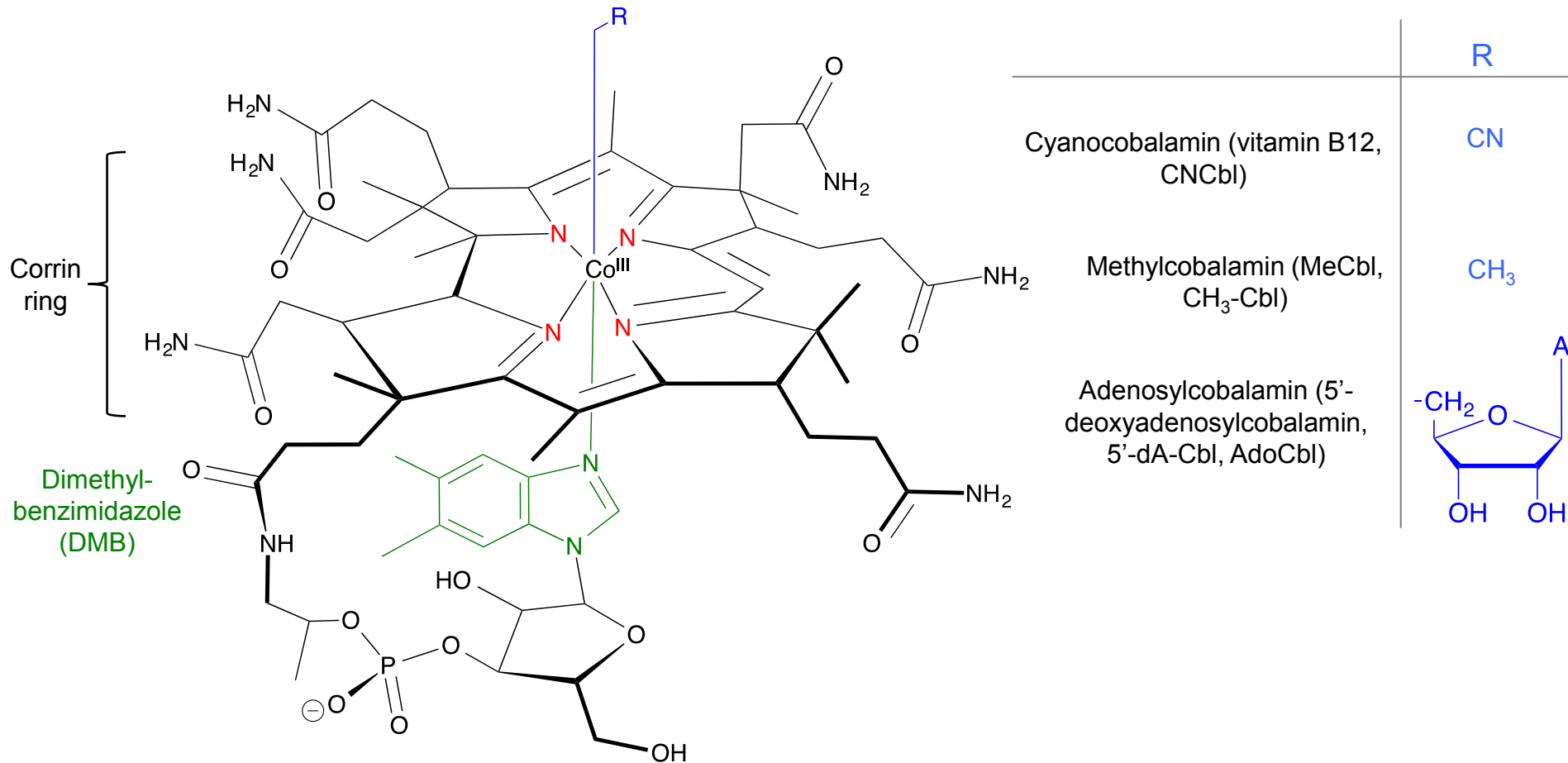
- In mammalian systems, these cofactors are not widely used, but are essential.
  a. CH$_3$-Cbl is essential for methionine biosynthesis.
  b. AdoCbl is essential for odd chain fatty acid biosynthesis.

- Mechanism generalizations:
  a. Three oxidation states are available to cobalt in the cobalamin cofactors: Co(I), Co(II), Co(III).
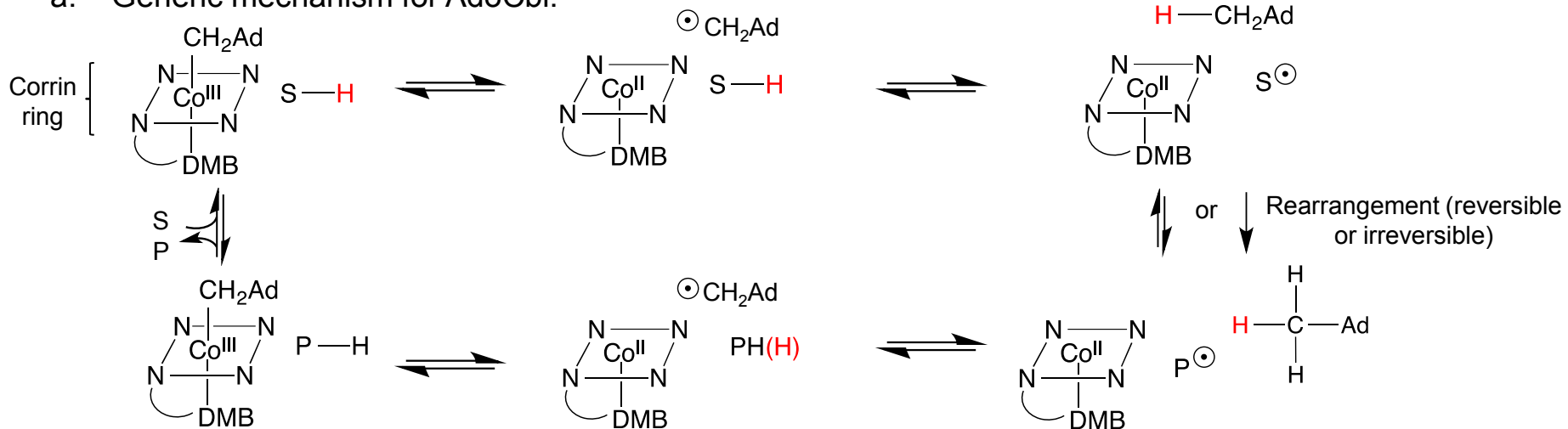
  Cob(III)alamin   Cob(II)alamin (paramagnetic)   Cob(I)alamin
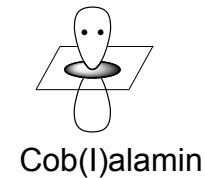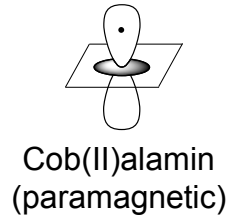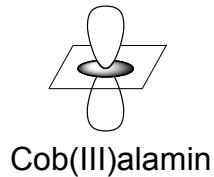
  b. All cobalamin cofactors have distinct visible spectra.

  a. For AdoCbl, homolytic cleavage of the C-Co bond to form Co^{II} shown below and on the next page with methylmalonyl-CoA mutase.
  b. For CH$_3$Cbl, heterolytic cleavage of the C-Co bond to form Co^{I} shown on the next page with methionine synthase.

  a. Generic mechanism for AdoCbl:

Corrin ring

CH$_2$Ad
N—N
Co^{III}
N—N
DMB
S—H

⇌

⊙CH$_2$Ad
N—N
Co^{II}
N—N
DMB
S—H

⇌

H—CH$_2$Ad
N—N
Co^{II}
N—N
DMB
S⊙

or / Rearrangement (reversible or irreversible)

S
P

CH$_2$Ad
N—N
Co^{III}
N—N
DMB
P—H

⇌

⊙CH$_2$Ad
N—N
Co^{II}
N—N
DMB
PH(H)

⇌

⊙CH$_2$Ad
N—N
Co^{II}
N—N
DMB
P⊙

H—C—Ad
| |
H H

- Example for AdoCbl reaction: Methylmalonyl-CoA mutase (MMCoA mutase) 2 possible mechanisms.

a.

b.
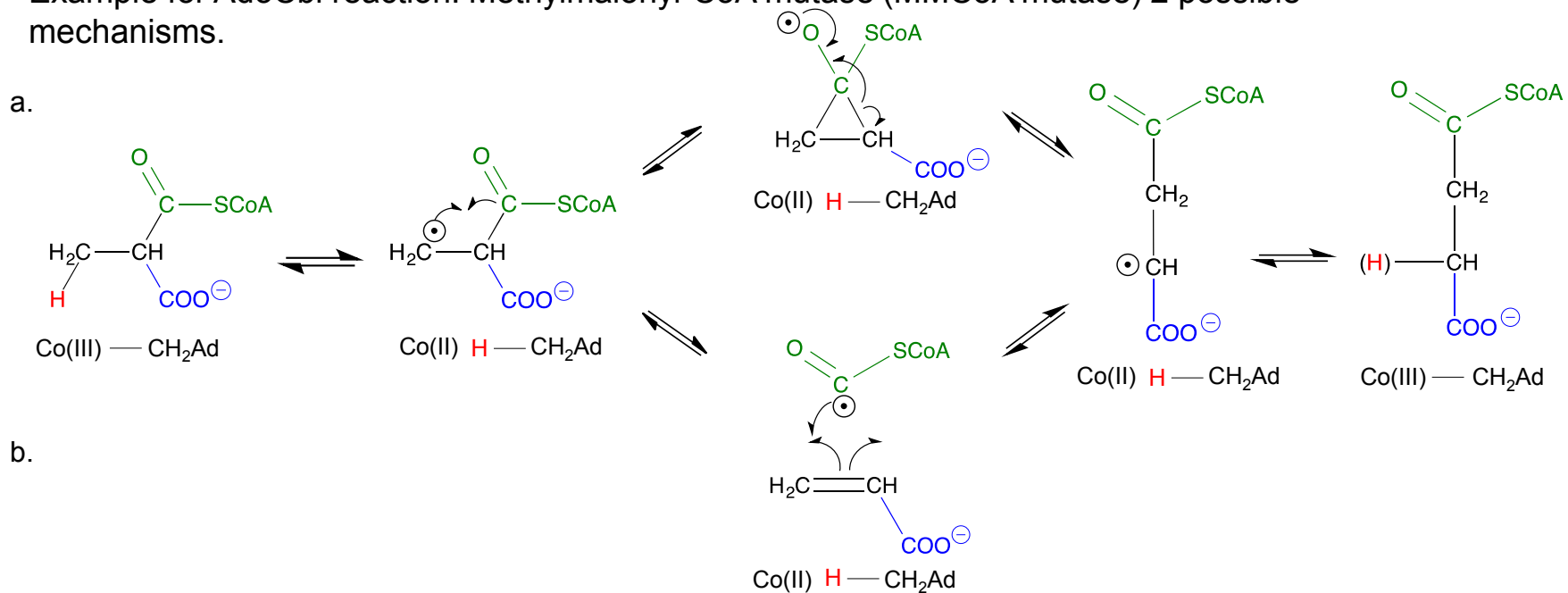
- Example for CH₃Cbl reaction: Methionine synthase (MS)
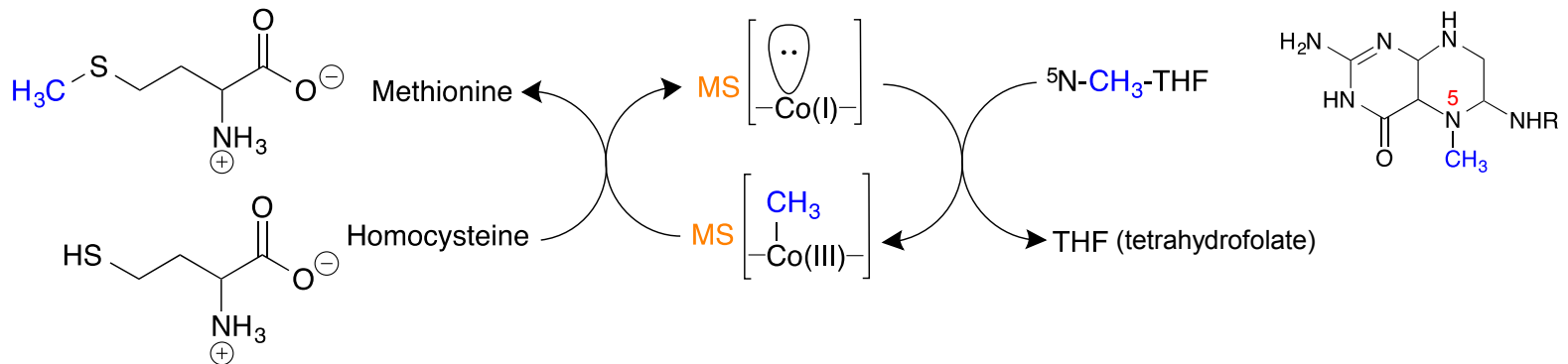
5.07SC Biological Chemistry I
Fall 2013