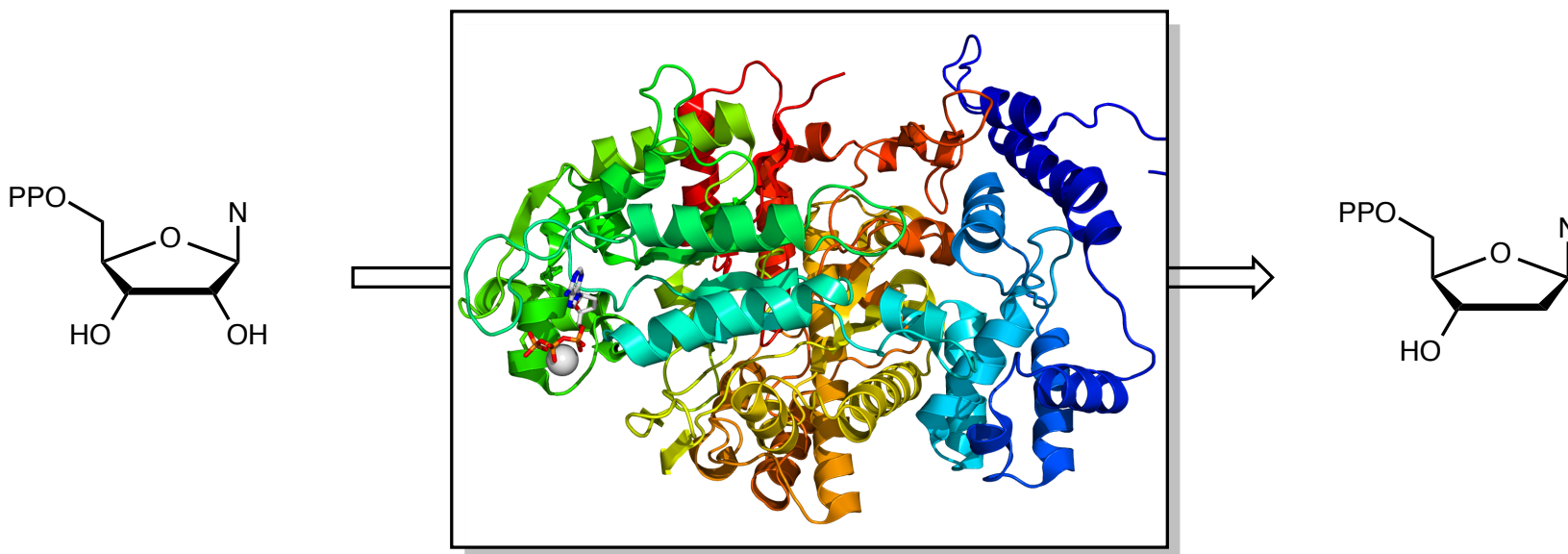


Radical Enzymes



Jian Jin
MacMillan Group Meeting
November 23, 2015

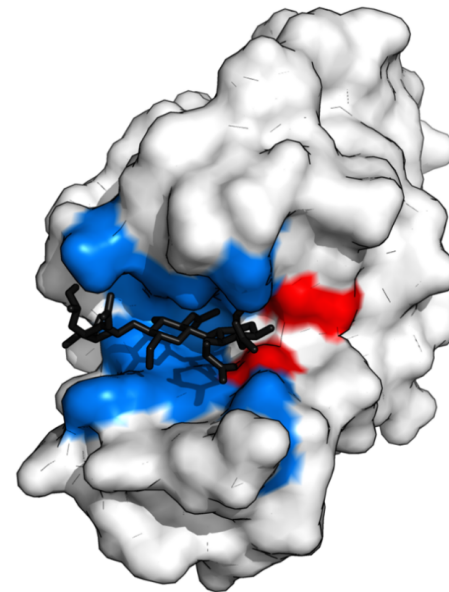
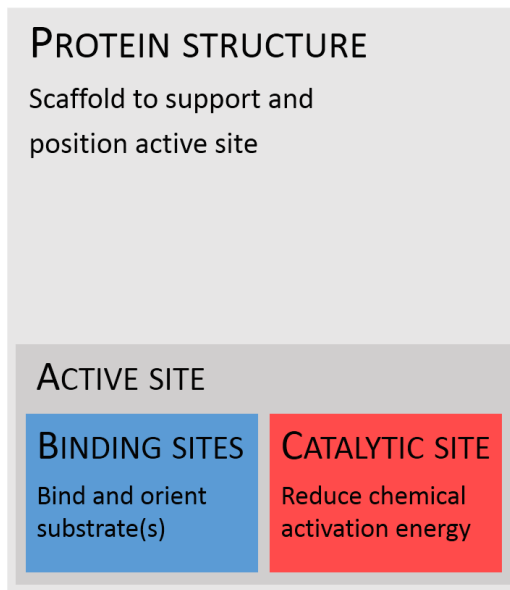
Enzymes

■ Definition

- Enzymes are macromolecular biological catalysts.
- Catalyze more than 5,000 biochemical reaction types.
- Most enzymes are proteins, a few are catalytic RNA molecules.

■ Structure

- Enzymes are linear chains of amino acids that fold to produce a three-dimensional structure.
- The sequence of the amino acids specifies the structure.
- The structure determines the catalytic activity of the enzyme.



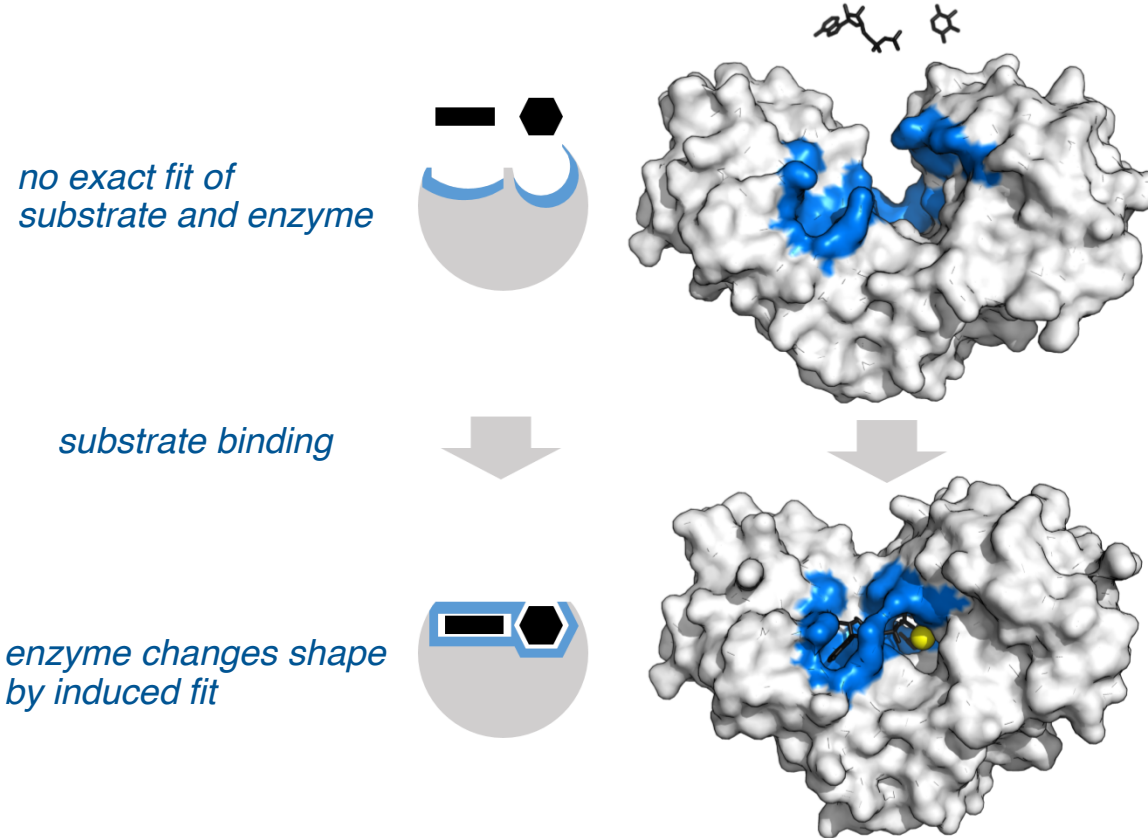
Enzymes

■ Substrate Binding

Enzymes must bind their substrates before they can catalyze any chemical reaction.

■ **"Key and lock" model:** In 1894, Emil Fischer proposed: the enzyme and the substrate fit exactly into one another.

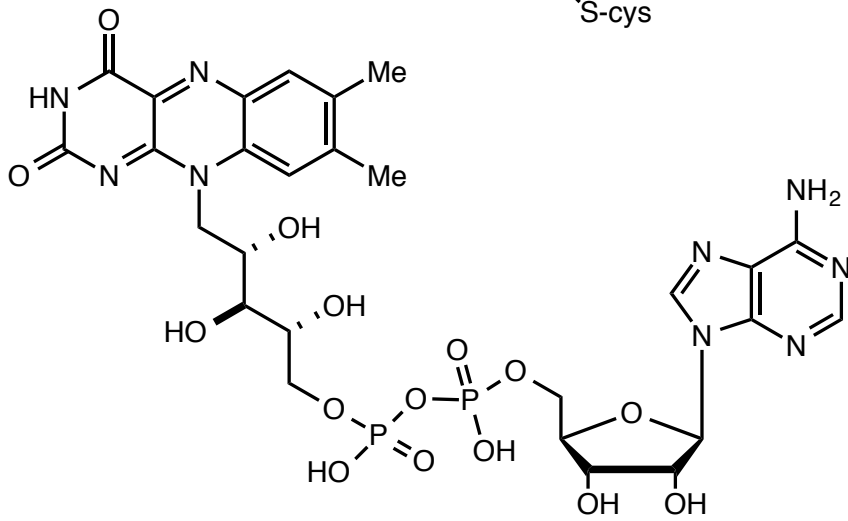
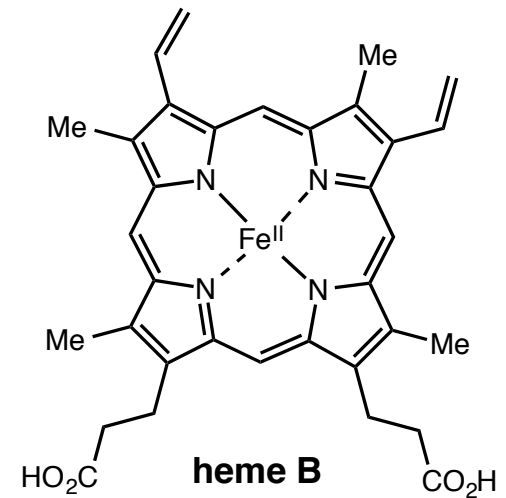
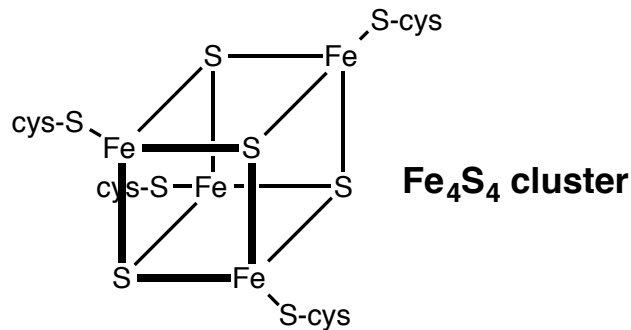
■ **Induced fit model:** In 1958, Daniel Koshland suggested a modification: the active site is continuously reshaped by interactions with the substrate.



Enzymes

■ Cofactors

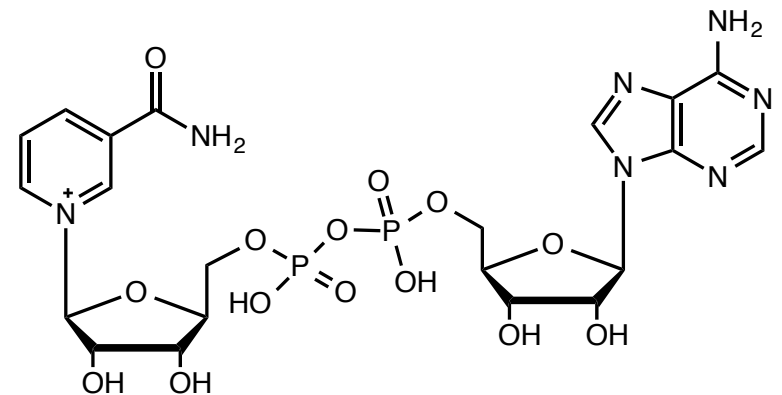
- Some enzymes do not need additional components to show full activity. Others require non-protein molecules called cofactors to be bound for activity.
- Cofactors can be either inorganic or organic compounds.



flavin adenine dinucleotide (FAD)

left half of FAD

flavin mononucleotide (FMN)



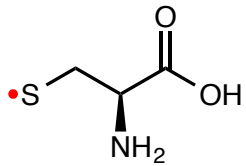
nicotinamide adenine dinucleotide (NAD)

Radical Enzymes

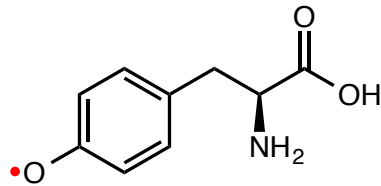
■ Definition

The term *radical enzyme* describes those enzymes that catalyze reactions in which radicals participate.

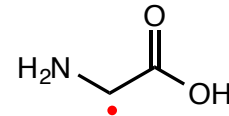
■ Radicals of Biology



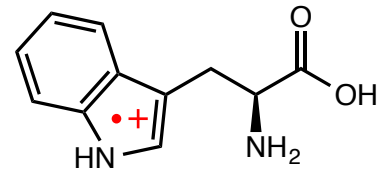
**cysteine thiyl radical
(cysteiny)**



**tyrosine oxy radical
(tyrosyl)**

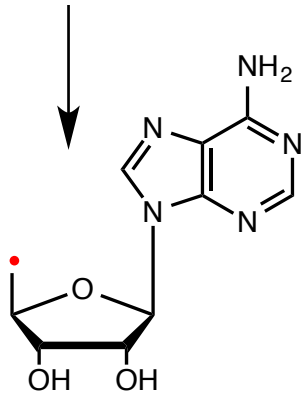


**glycine carbon radical
(glycyl)**

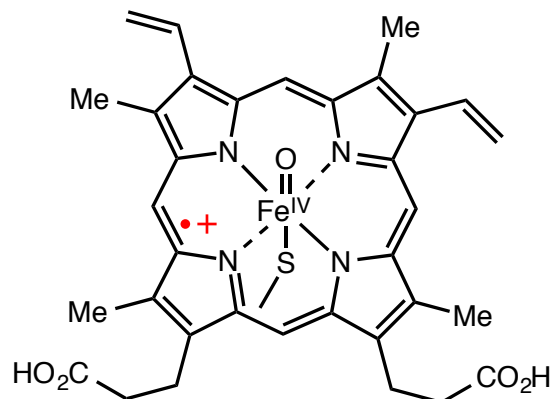


tryptophan cation radical

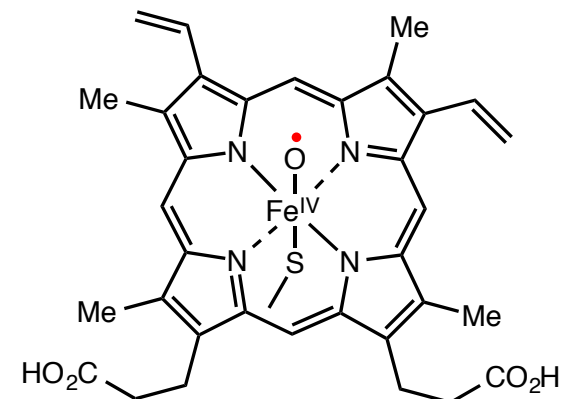
coenzyme B₁₂ / SAM



5'-deoxyadenosyl radical



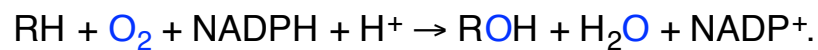
CYP450 compound I



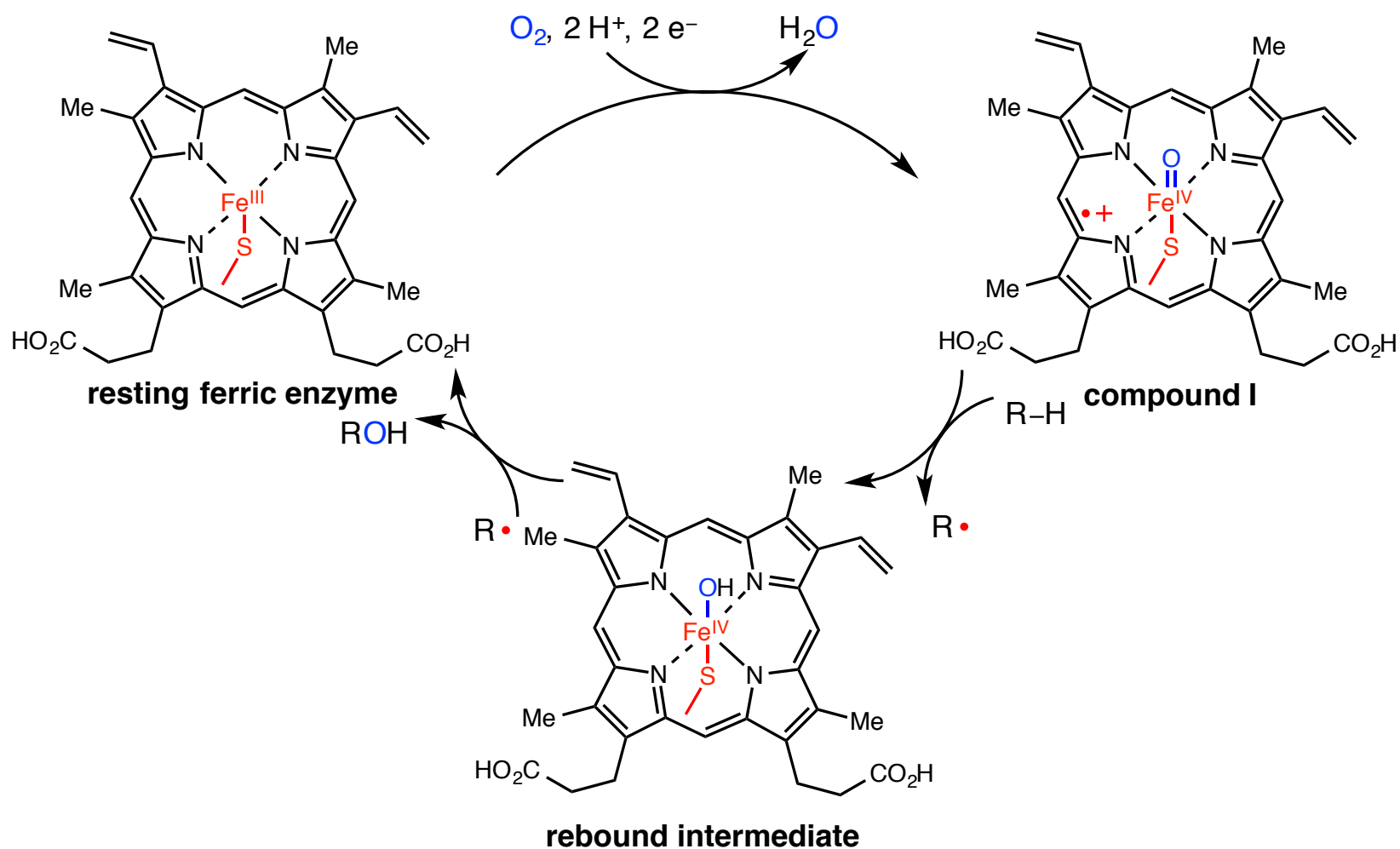
Cytochromes P450

Introduction

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction:



Oxygen Rebound Mechanism in P450-catalyzed C–H Hydroxylation



Ribonucleotide Reductases

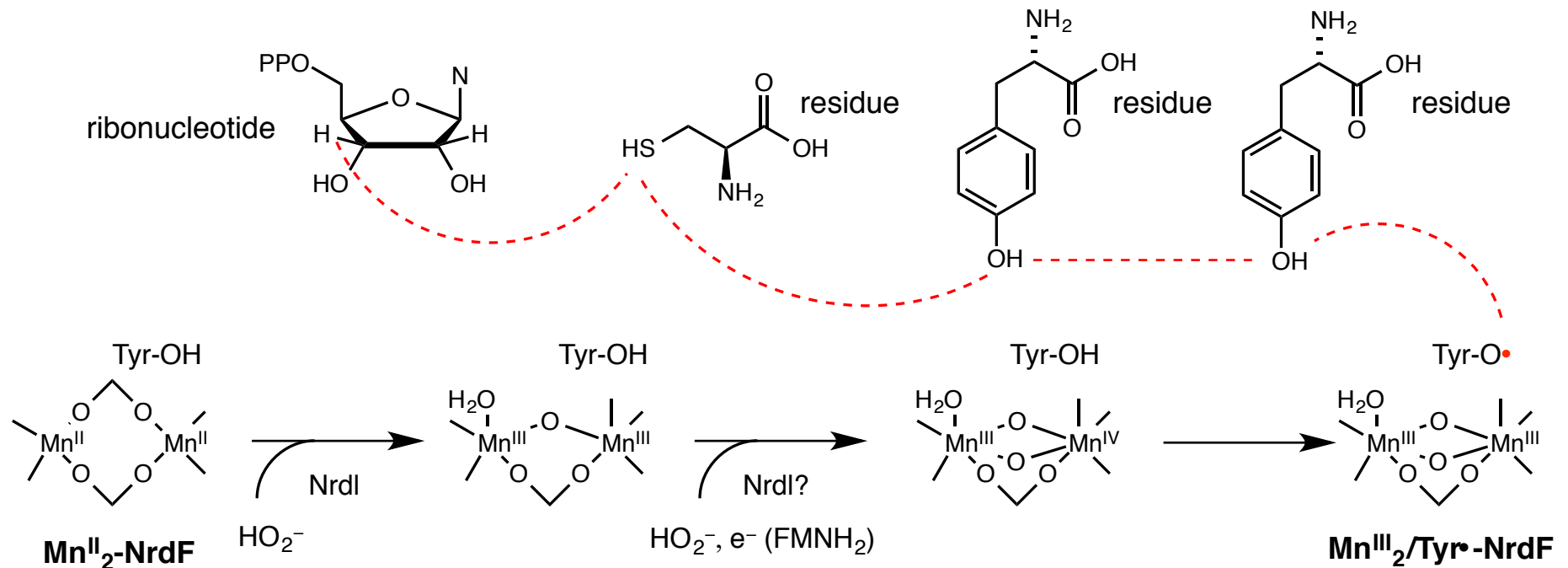
Introduction

Ribonucleotide reductases (RNR) are enzymes that catalyze the formation of deoxyribonucleotides from ribonucleotides. Deoxyribonucleotides in turn are used in the synthesis of DNA.

Classes of RNRs

- Class I enzymes:** aerobic, di-iron-oxo (class Ia) or dimanganese-oxo (class Ib) or Fe(III)-(O)₂-Mn(IV) (class Ic).
- Class II enzymes:** anaerobic but can survive when exposed to O₂, coenzyme B₁₂.
- Class III enzymes:** strictly anaerobic, SAM.

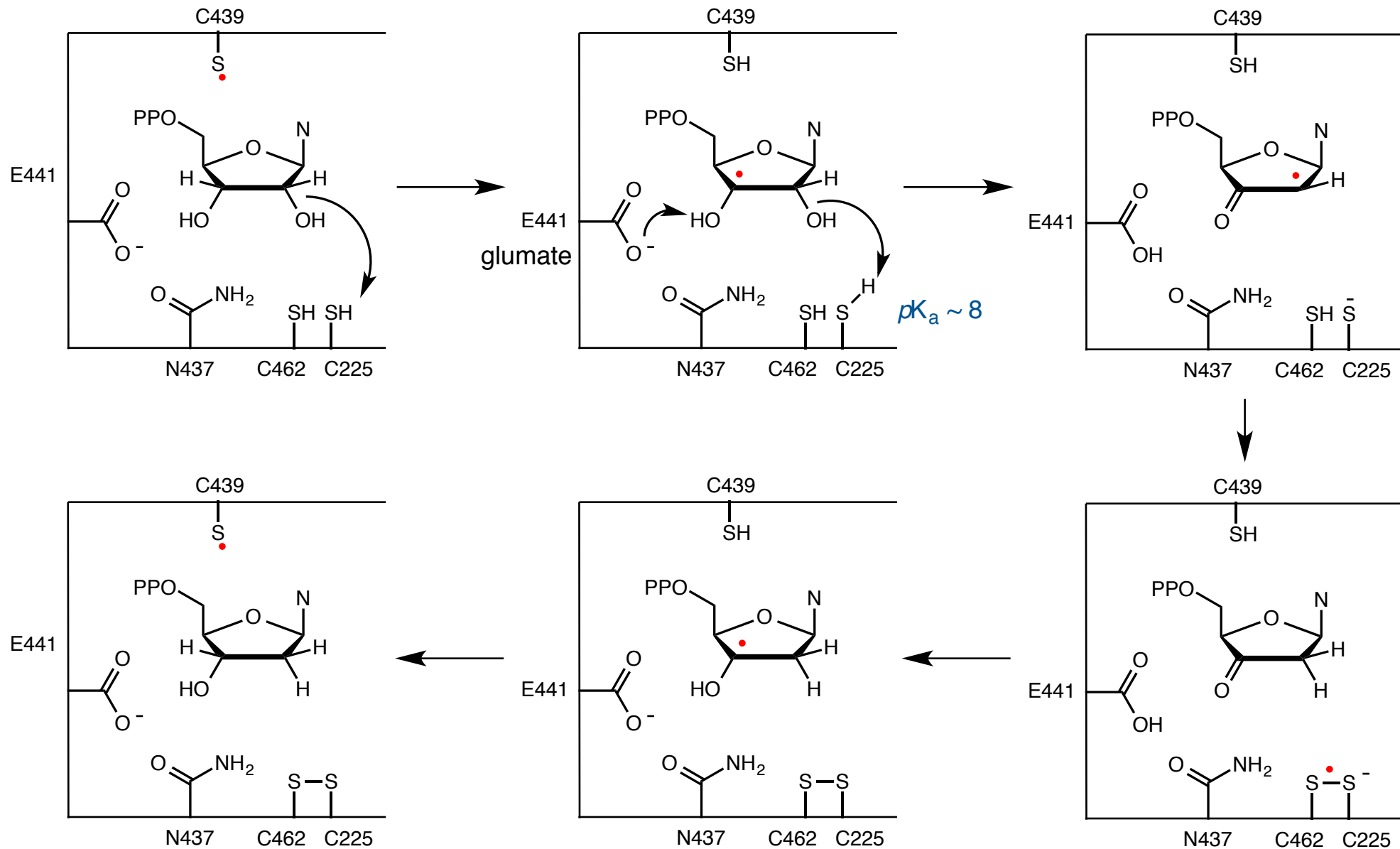
--- pathway for proton-coupled electron transfer (top)



tyrosyl radical generated by dimanganese-oxo species (bottom)

Ribonucleotide Reductases

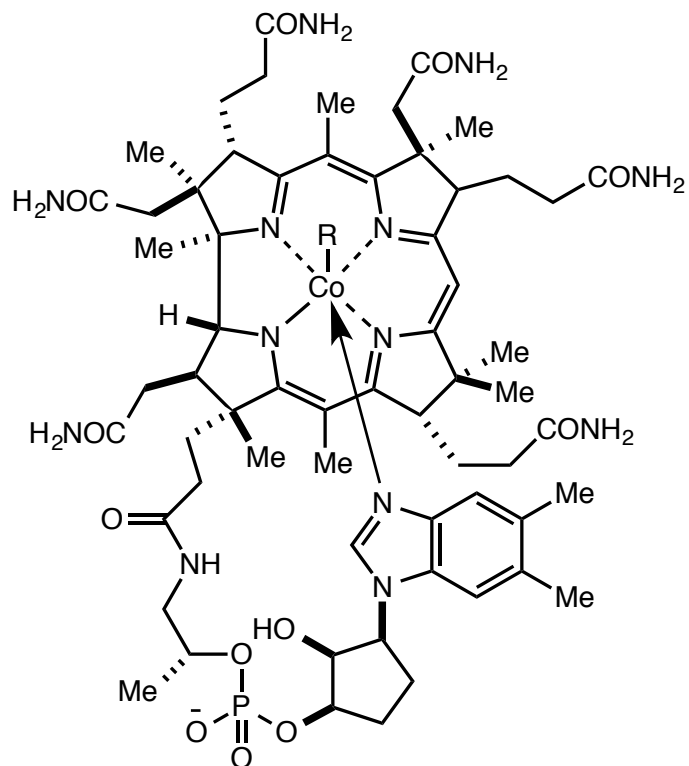
Mechanism for RNR Catalyzed Reduction of NDPs (*E. coli*)



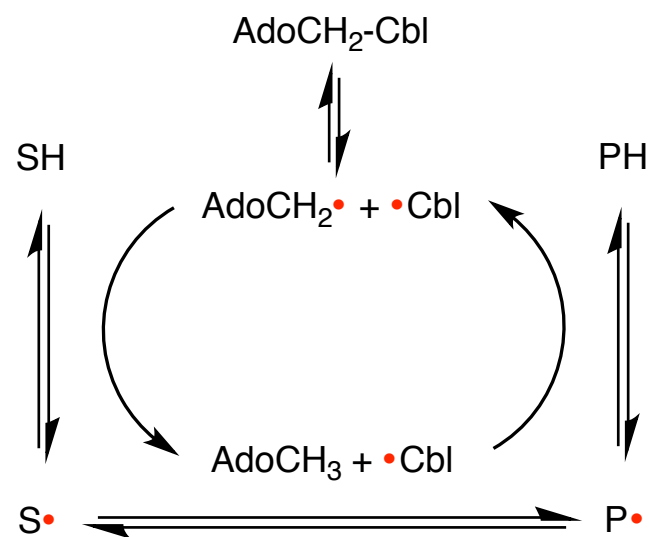
Coenzyme B₁₂-Dependent Enzymes

Introduction

- The Co-C bond is relatively weak (BDE ~ 31.2 KCal/mol), however the coenzyme B₁₂ is rather stable in water at 30 °C ($\tau_{1/2}$ 1.9 years).
- The binding to the enzyme partner and the arrival of a substrate molecule initiates cleavage of the Co-C bond.
- Coenzyme B₁₂ operates with two types of enzymes: the eliminases and the mutases.

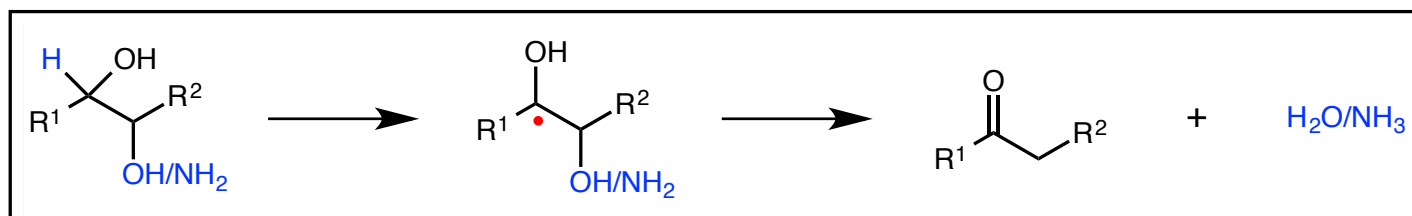


coenzyme B₁₂ (R = 5'-deoxyadenosyl)

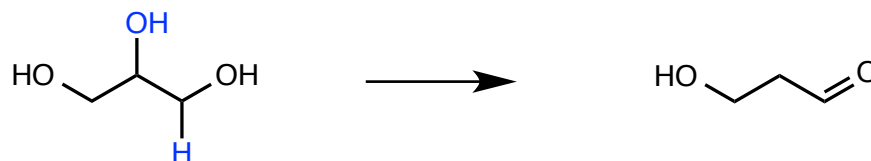


Coenzyme B₁₂-Dependent Enzymes

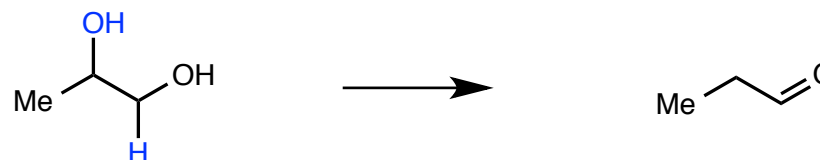
■ Eliminases



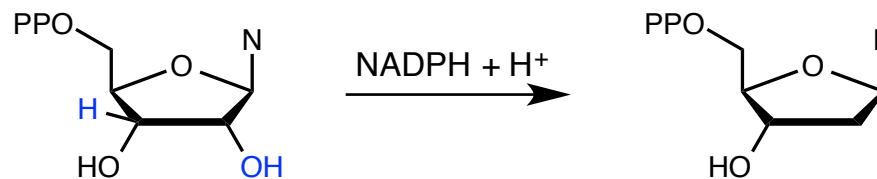
■ Glycerol dehydratase



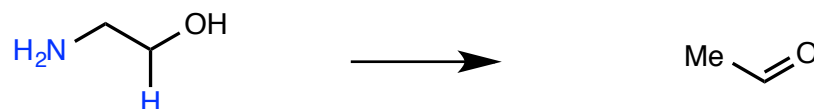
■ Propane-1,2-diol dehydratase



■ Ribonucleotide reductases

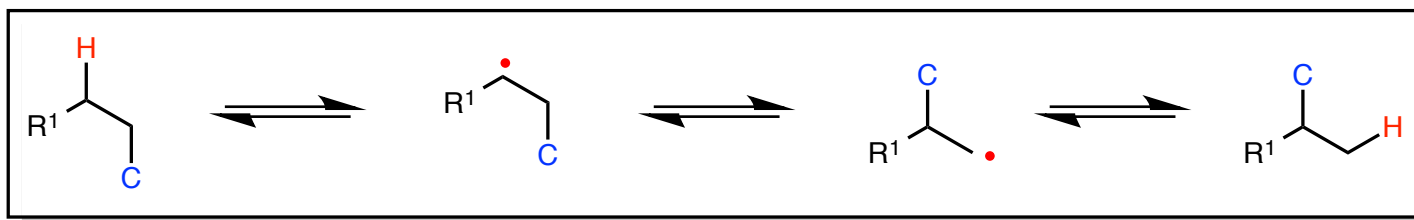


■ Ethanolamine ammonia lyase

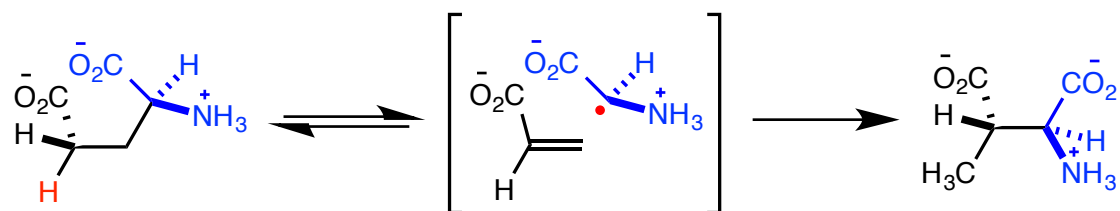


Coenzyme B₁₂-Dependent Enzymes

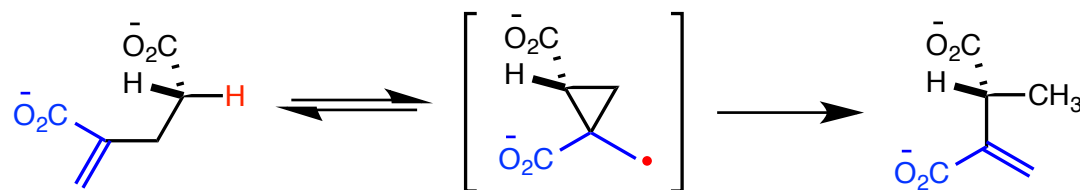
■ Carbon-skeleton Mutases



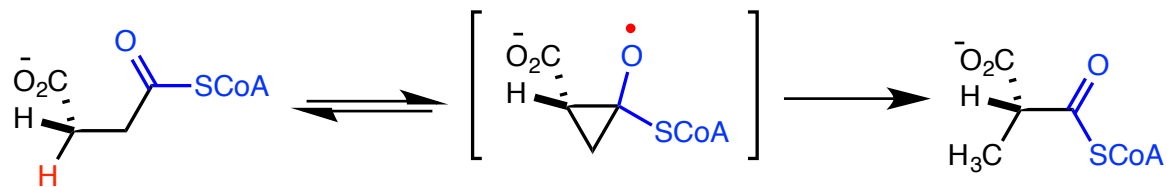
■ Glutamate mutase



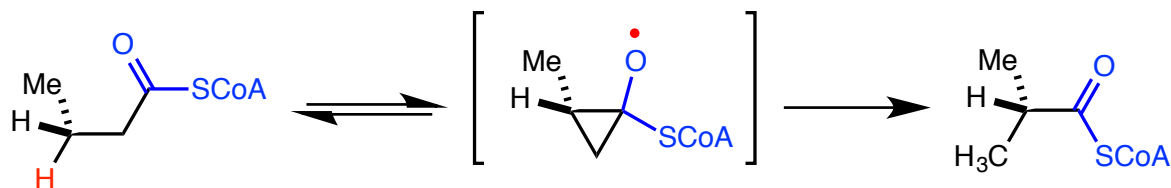
■ 2-Methylene-glutarate mutase



■ Methylmalonyl-CoA mutase

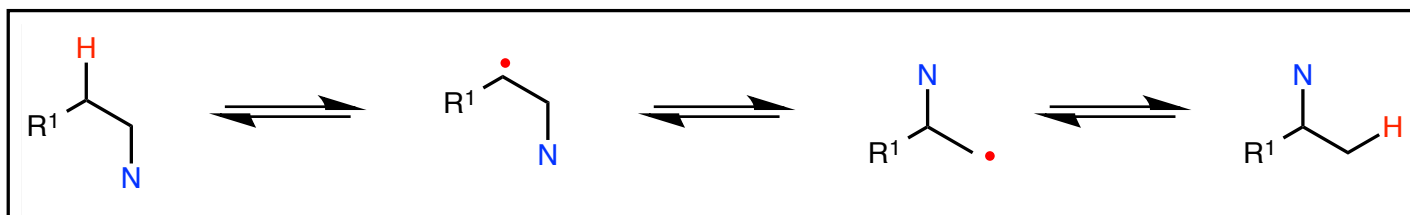


■ Isobutyryl-CoA mutase

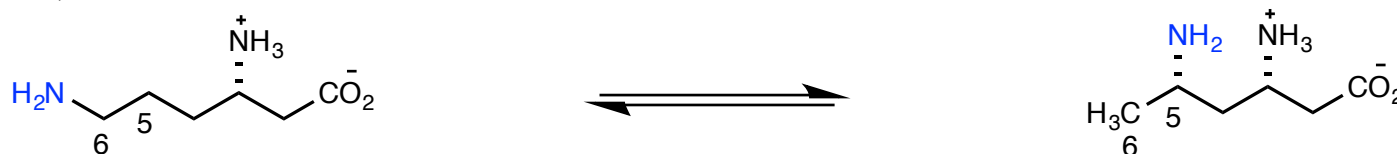


Coenzyme B₁₂-Dependent Enzymes

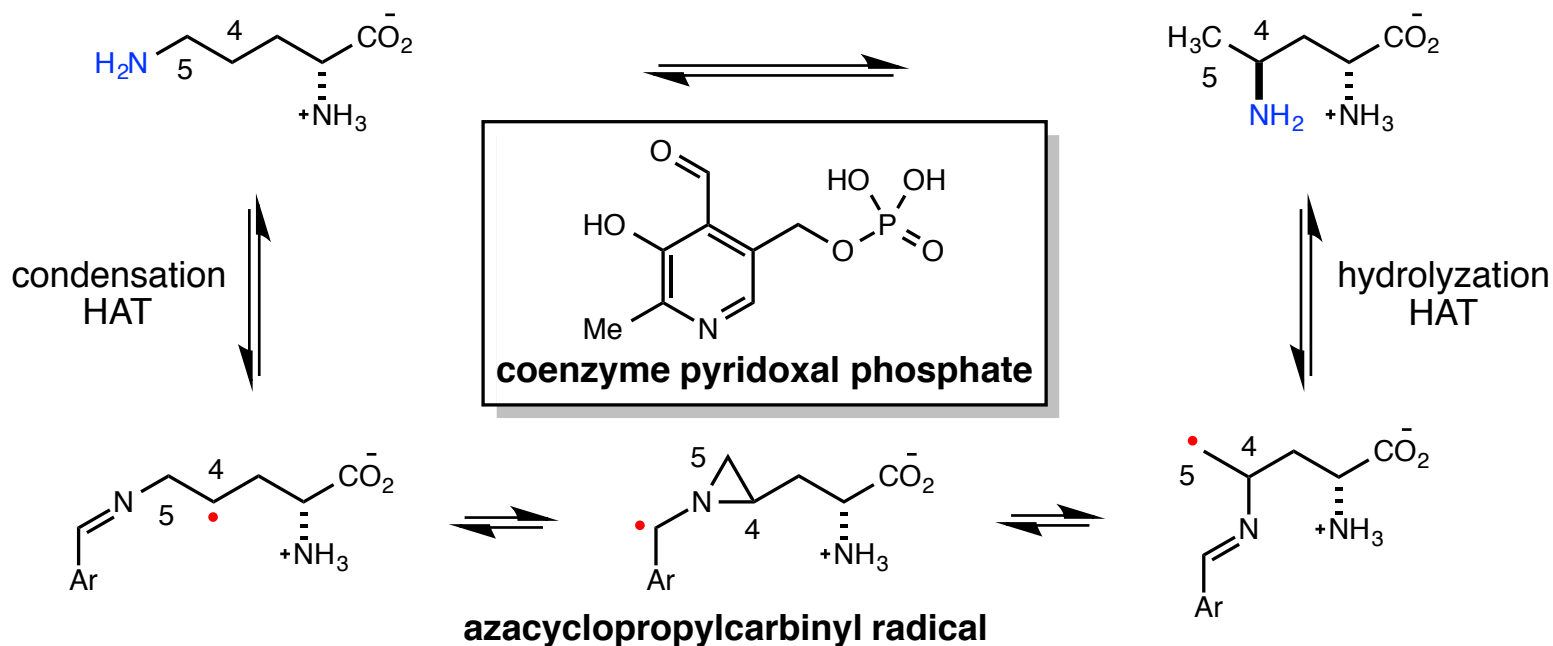
■ Amino Mutases



■ β-Lysine-5,6-aminomutase



■ Ornithine-4,5-aminomutase



SAM radical Enzymes

■ Introduction

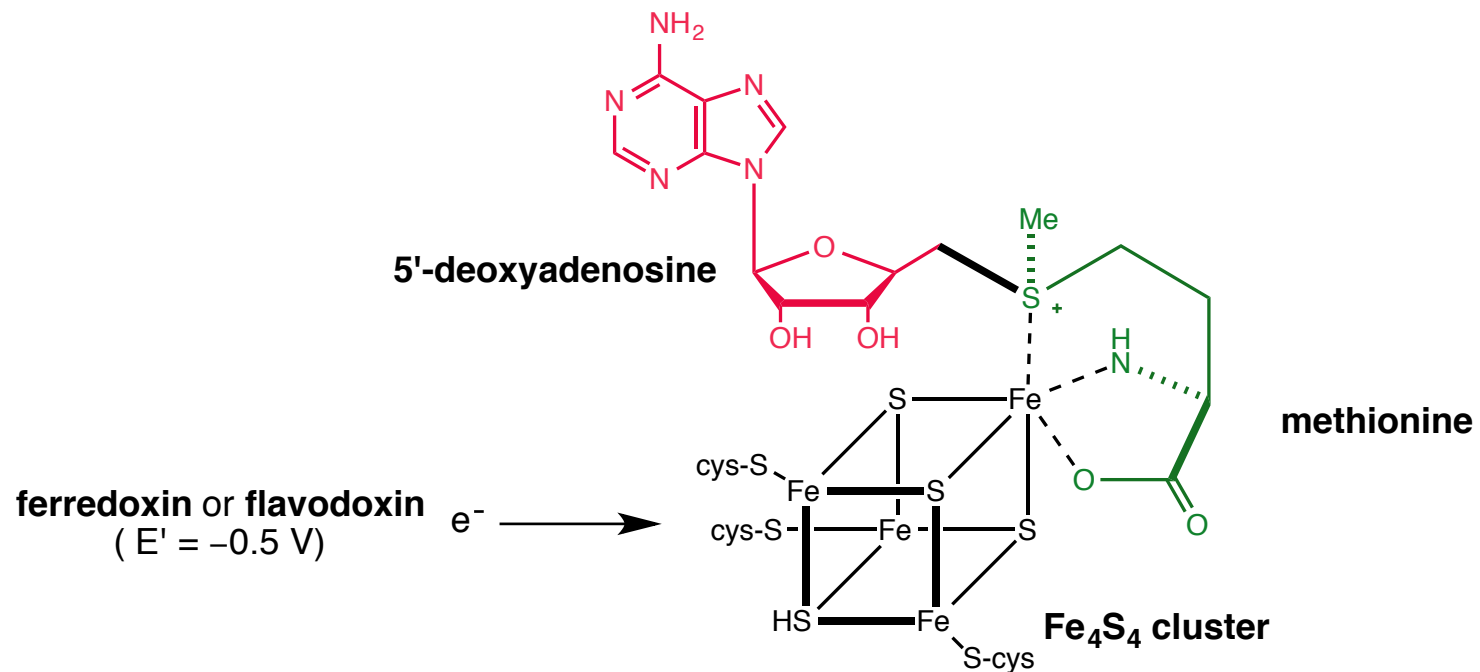
To date, there are about 40 different radical reactions catalyzed by characterized SAM radical enzymes.

The 5'-deoxyadenosyl radical initiates catalysis generally by hydrogen atom abstraction from the substrate.

■ Classes of SAM radical enzymes

■ **Recycling SAM radical enzymes:** the resulting 5'-deoxyadenosine is recycled to regenerate SAM.

■ **Irreversible SAM radical enzymes:** 5'-deoxyadenosine is dumped irreversibly.



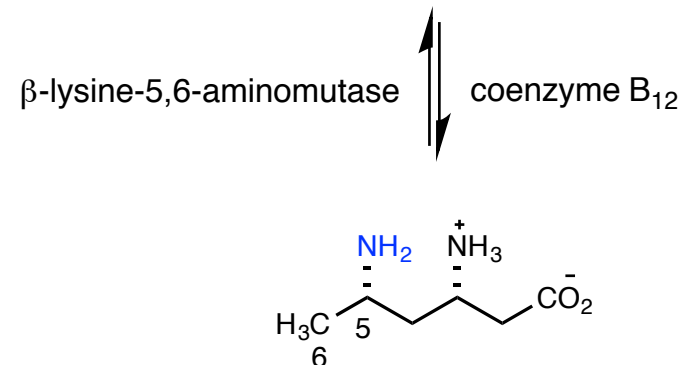
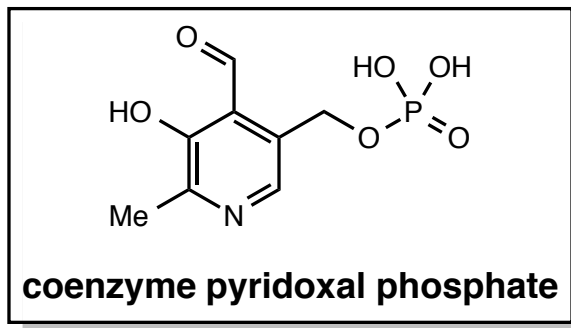
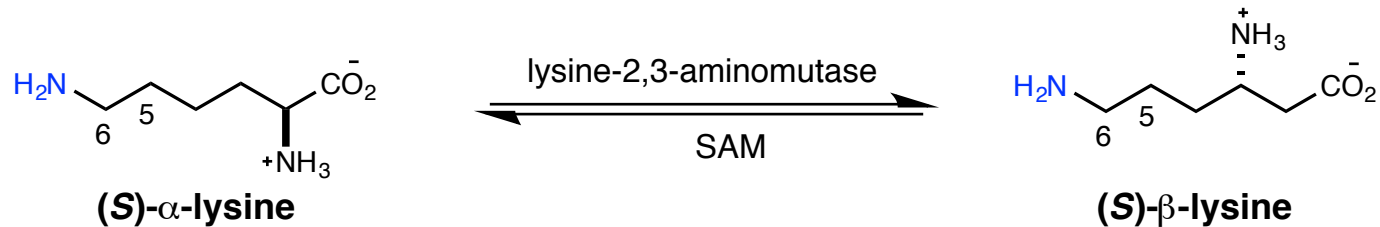
Mode of SAM (S-adenosyl methionine) binding to the fourth iron of the cluster

SAM radical Enzymes

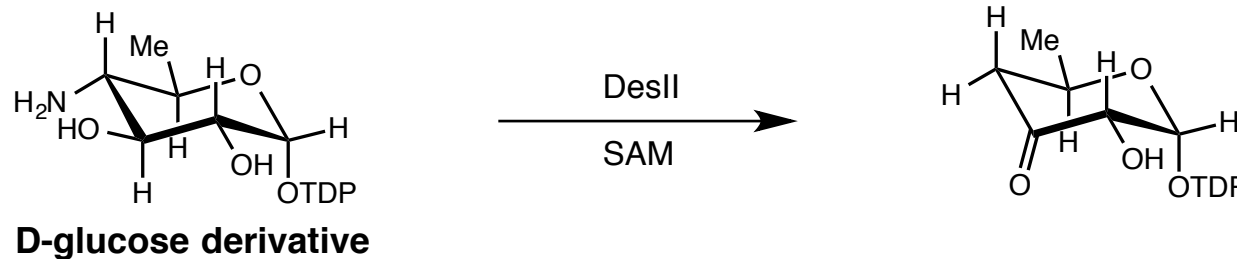
■ Recycling SAM Radical Enzymes

■ Lysine-2,3-aminomutase

In 1989, Perry A. Frey discovered hydrogen transfer from the 5'-methylene group of SAM to α - and β -lysine, which means 5-deoxyadenosyl radical is involved and could be regenerated.



■ DesII

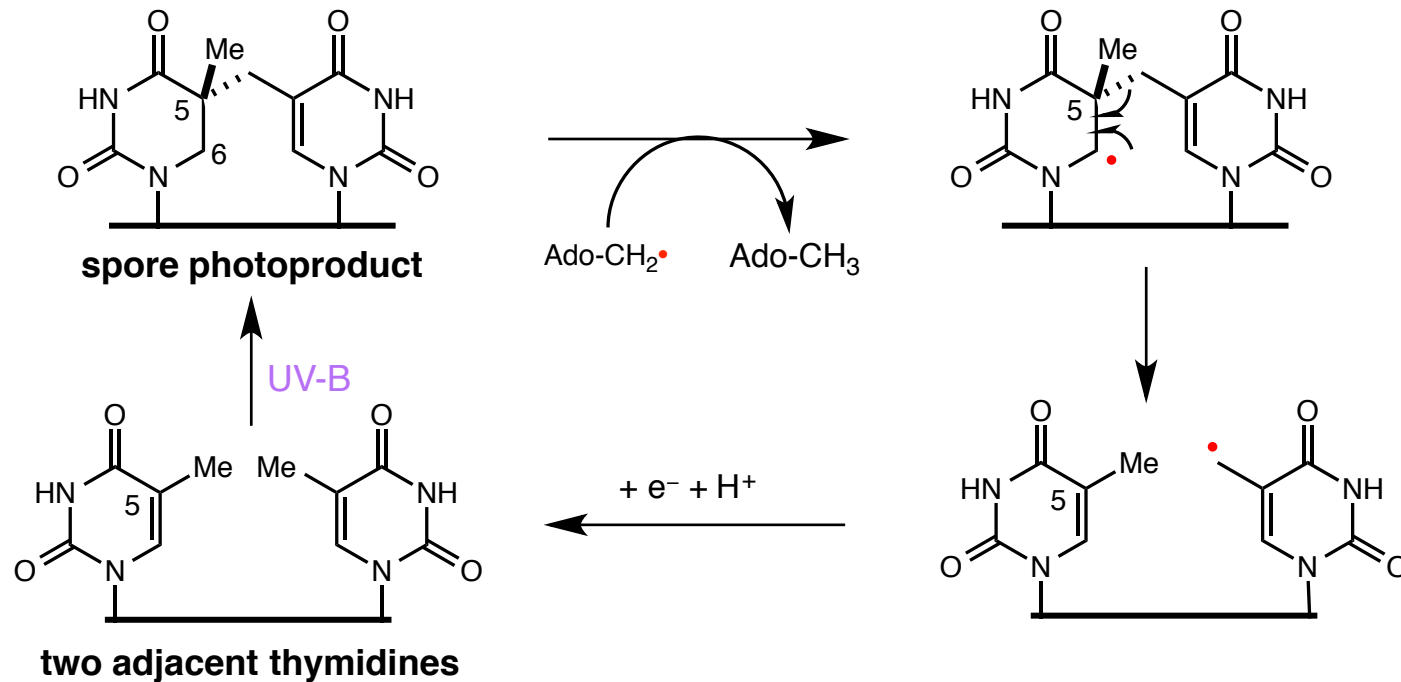


SAM radical Enzymes

■ Recycling SAM Radical Enzymes

■ Spore Photoproduct Lyase, recycling or irreversible? Recent experiments suggest No.

Spore photoproduct lyase has to repair DNA lesions that occasionally occur in bacteria germinated from spores.

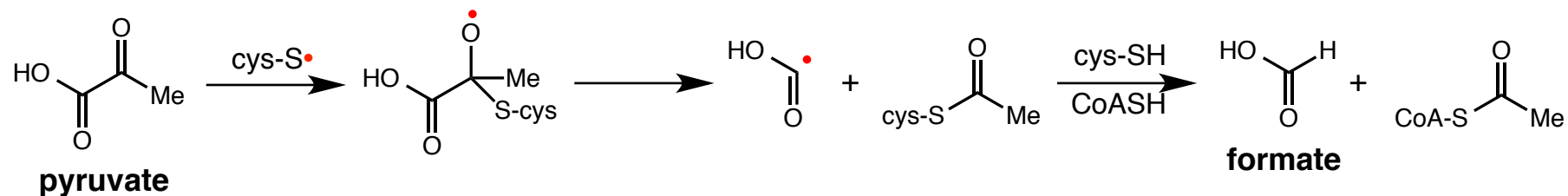


SAM radical Enzymes

■ 7 Known Irreversible SAM-Glycine-Cysteine Radical Enzymes

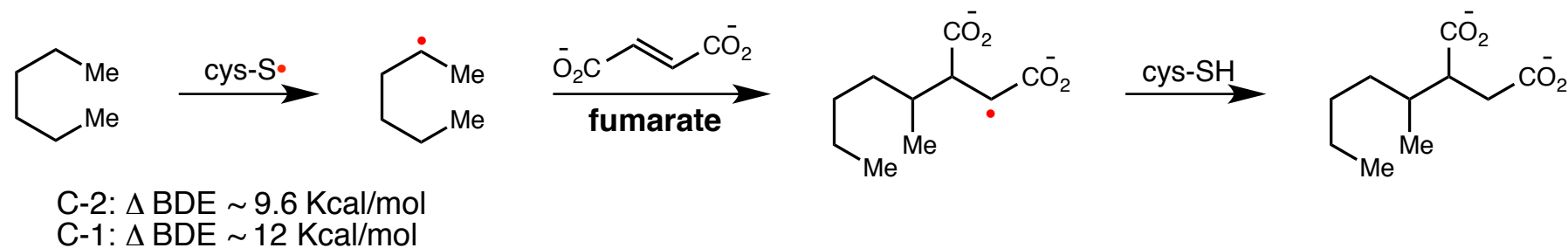
■ RNR III (refer to the RNR part)

■ Pyruvate formate lyase



■ 2-Oxobutyrate formate lyase (similar to the above)

■ 2-(1-methylpentyl)succinate synthase



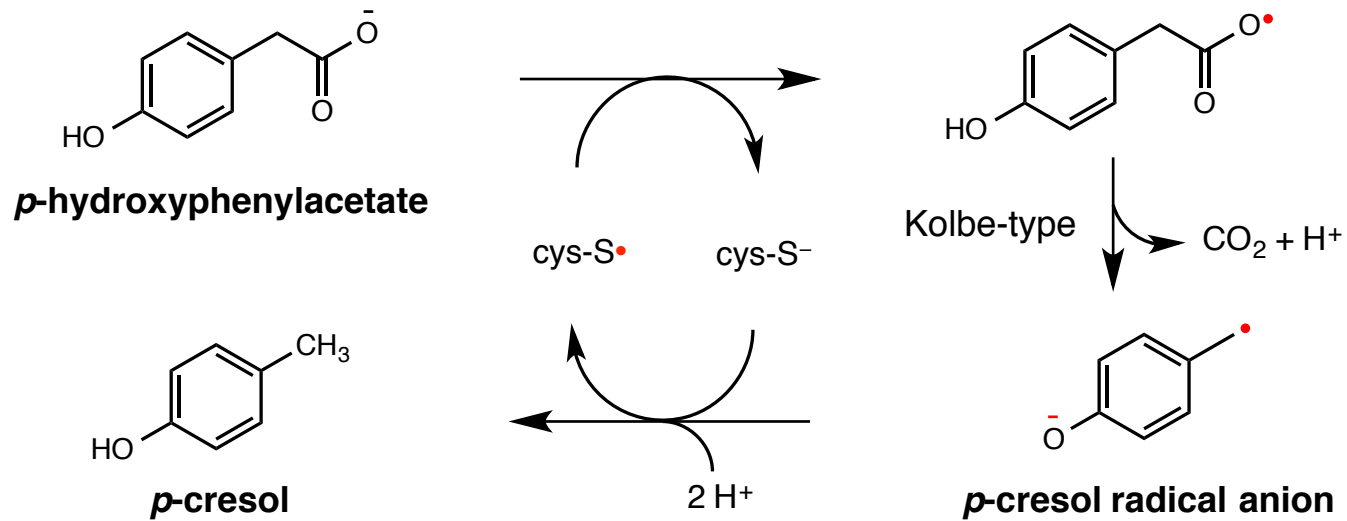
■ Benzylsuccinate synthase (similar to the above)

SAM radical Enzymes

■ 7 Known Irreversible SAM-Glycine-Cysteine Radical Enzymes

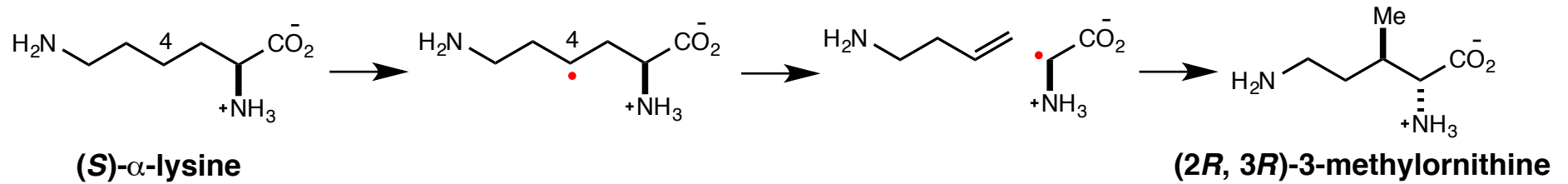
■ Glycerol dehydratase (similar to the version of coenzyme B₁₂-dependent enzyme)

■ *p*-Hydroxyphenylacetate decarboxylase

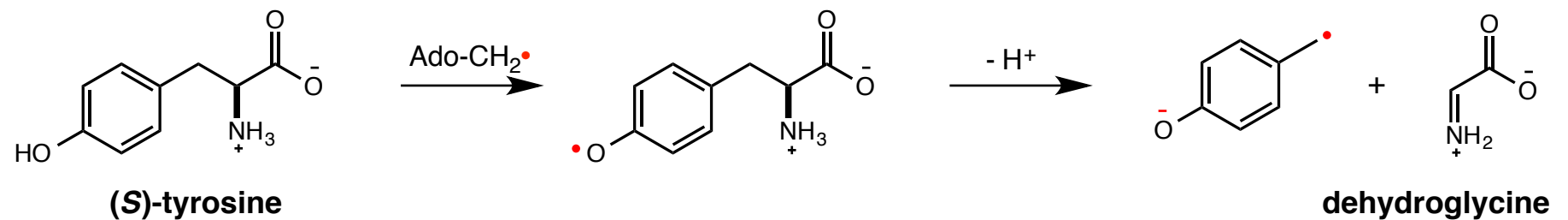


SAM radical Enzymes

■ Irreversible SAM Radical Enzymes: PylB



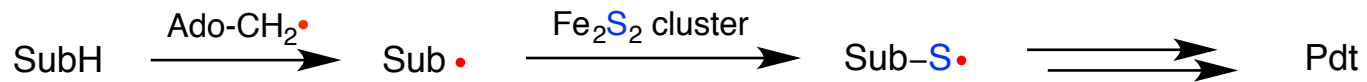
■ Irreversible SAM Radical Enzymes: HydG



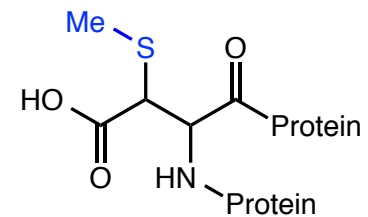
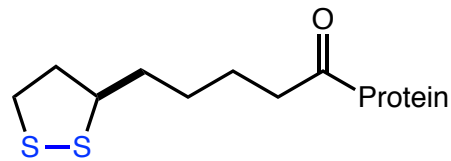
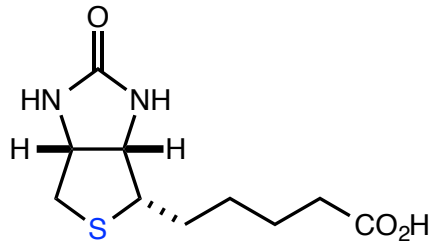
SAM radical Enzymes

■ Irreversible SAM Radical Enzymes: Sulfur insertion

The abstraction of a hydrogen atom bound to carbon is followed by insertion of sulfur derived from a second iron-sulfur cluster of SAM radical enzyme.



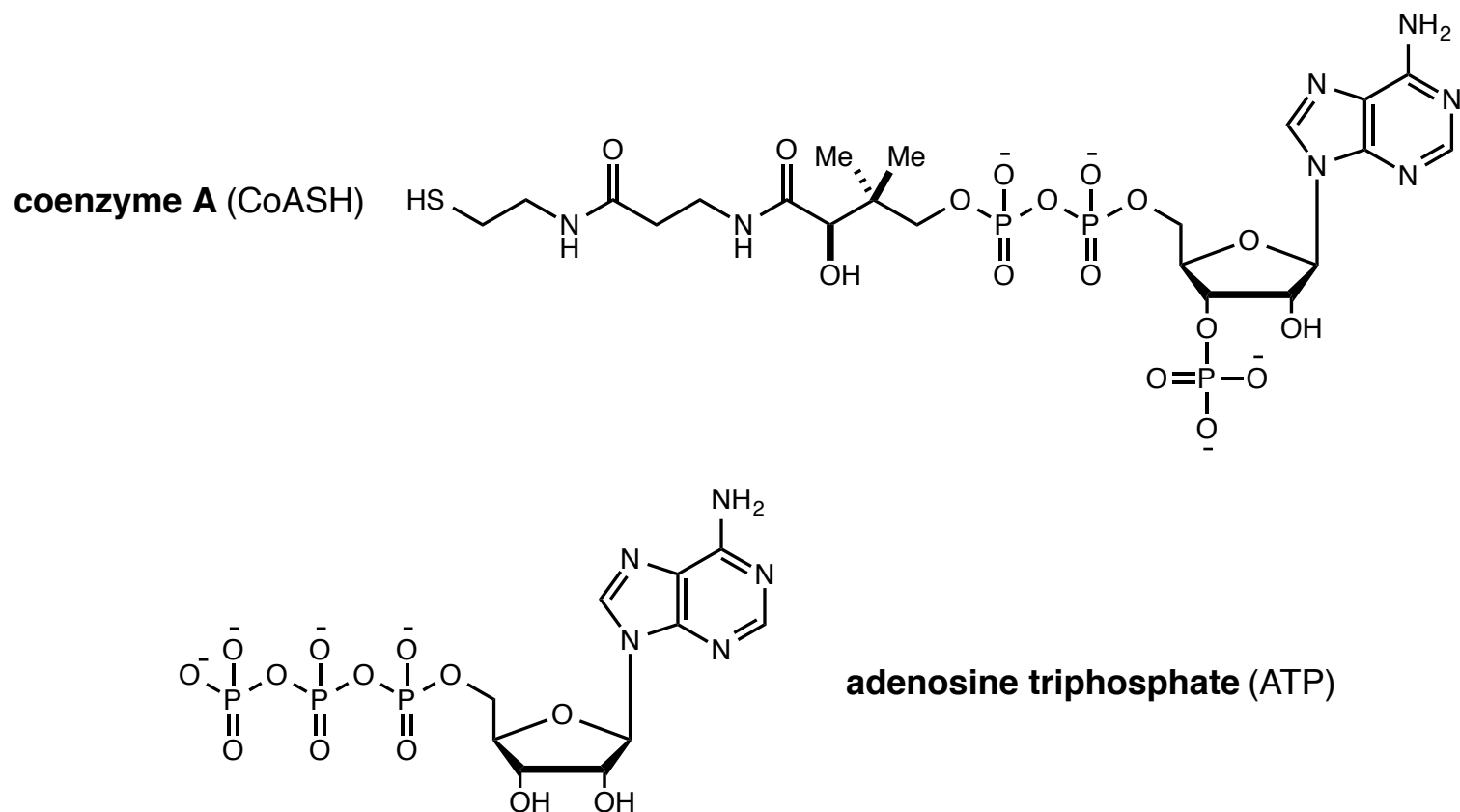
■ Examples of sulfur insertion by SAM radical enzymes



Reactions Involving Ketyl Radicals

■ Backgroup of CoASH and ATP

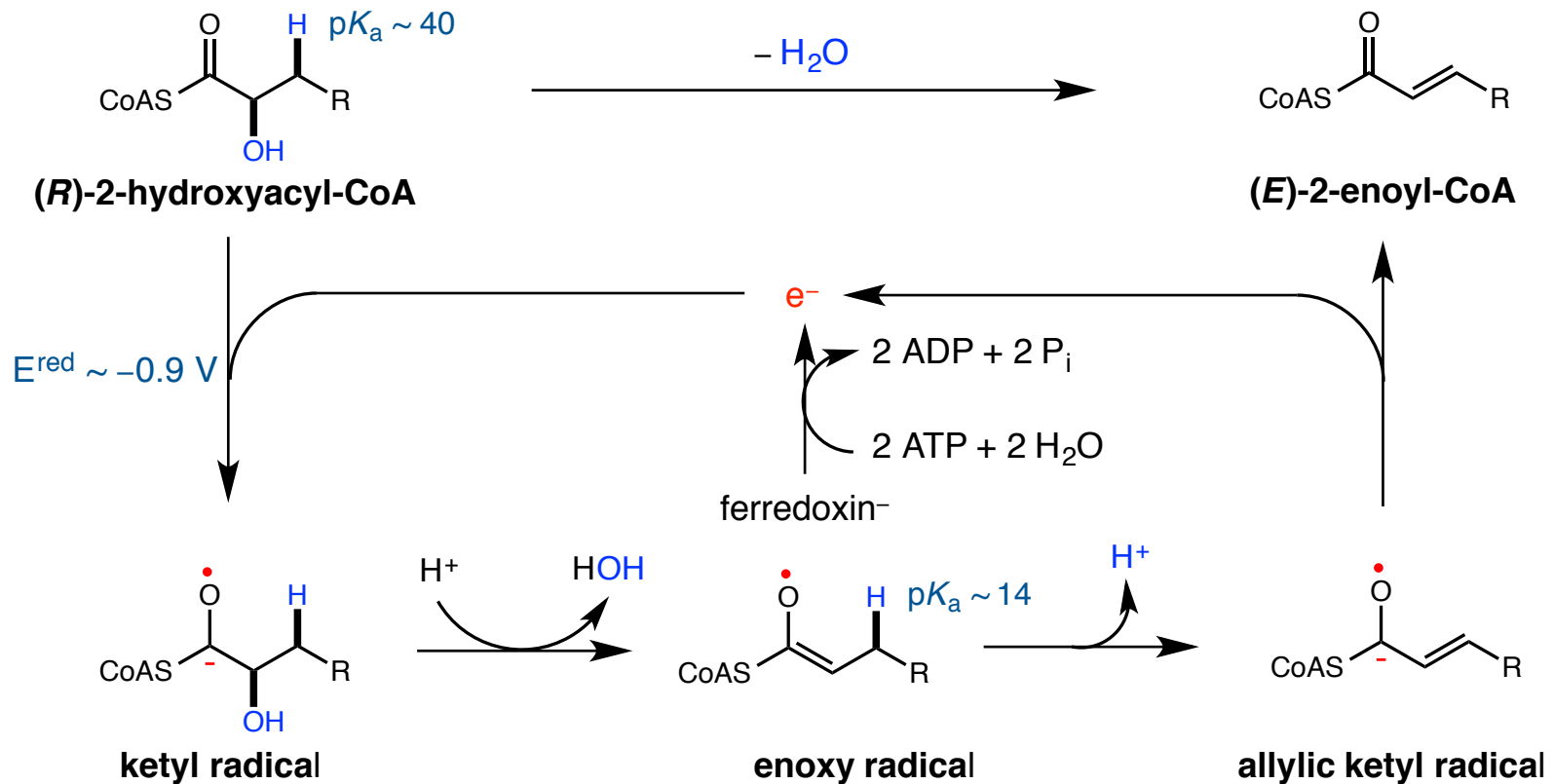
- Coenzyme A (CoASH): react with carboxylic acids to form thioesters, followed by a variety of enzyme-catalyzed transformation.
- Adenosine triphosphate (ATP): a nucleoside triphosphate used in cells, a coenzyme often called the "molecular unit of currency" of intracellular energy transfer.



Reactions Involving Ketyl Radicals

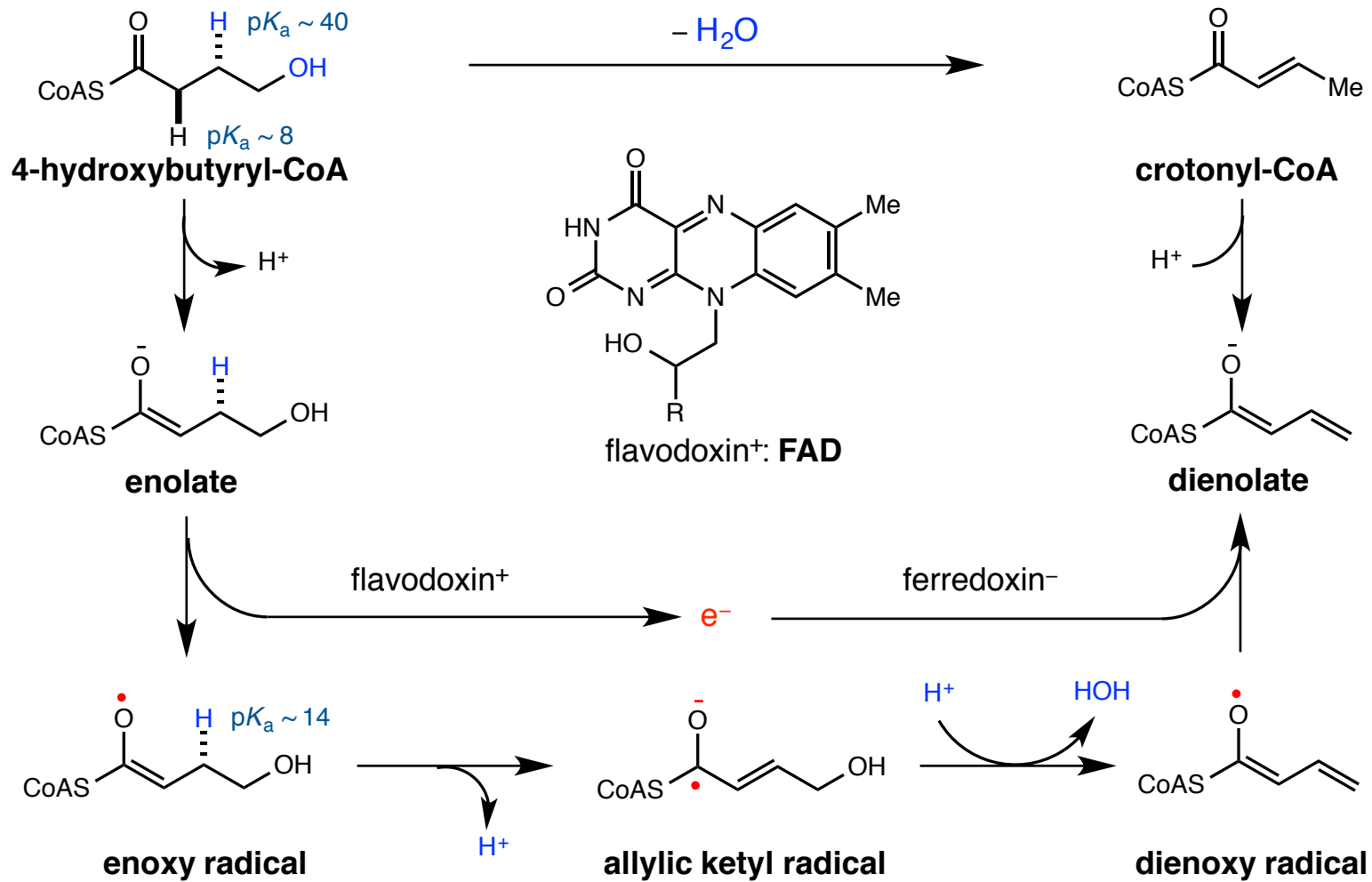
■ 2-Hydroxyacyl-CoA dehydratase

- SET causes the acidity of β -proton to increase by 26 orders of magnitude.
- Use of just one electron provides an explanation as to how an "impossible" dehydration can occur.



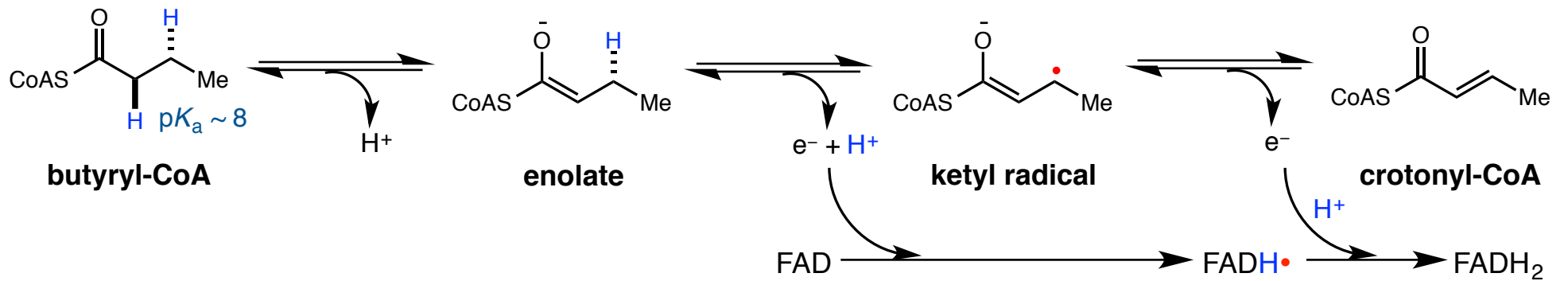
Reactions Involving Ketyl Radicals

4-Hydroxybutyryl-CoA dehydratase

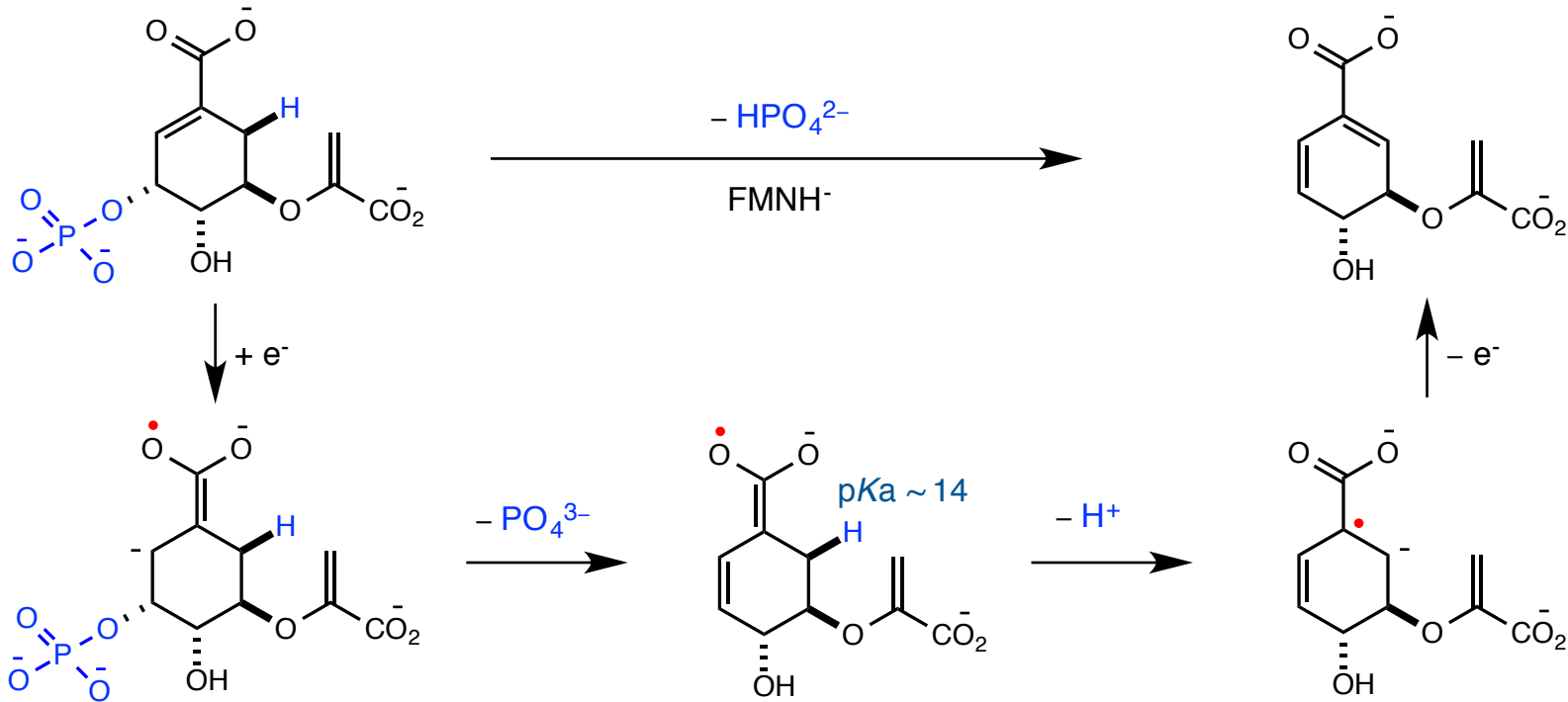


Reactions Involving Ketyl Radicals

■ Acyl-CoA Dehydrogenase

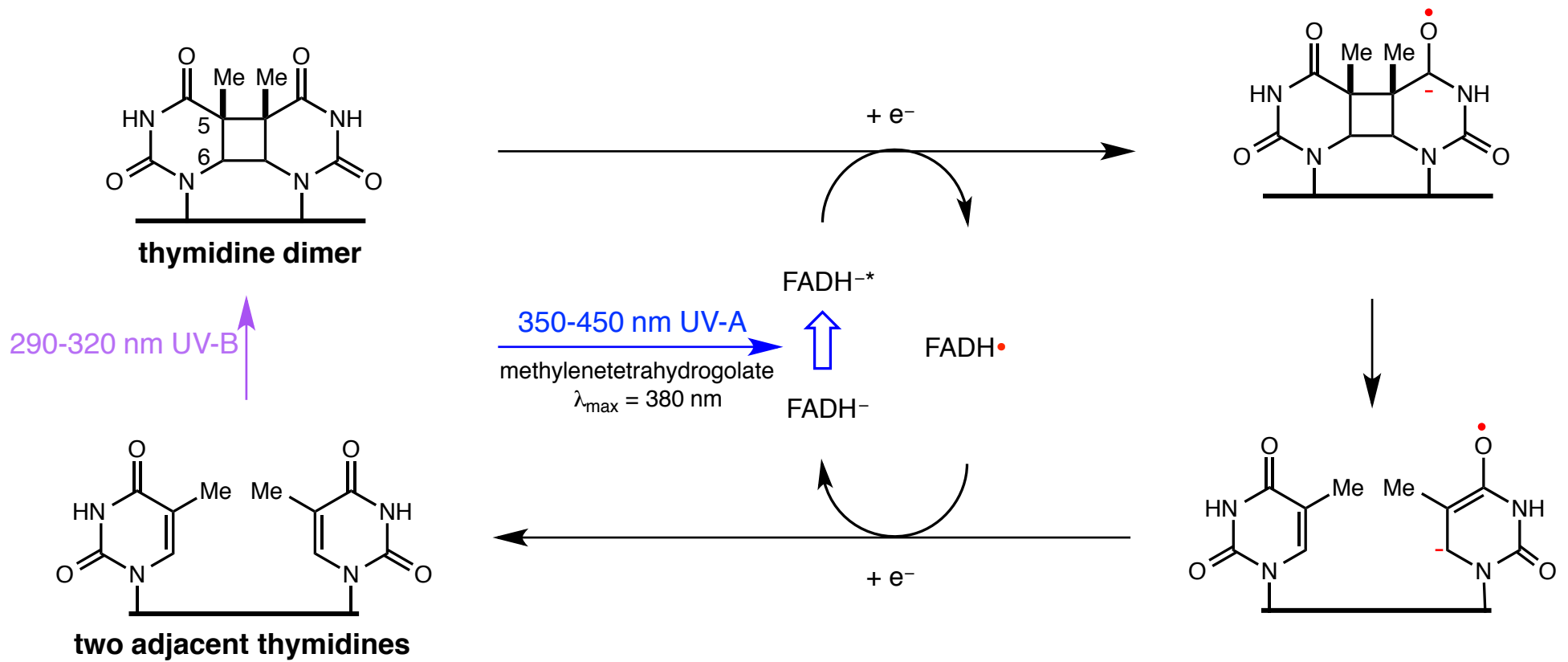


■ Chorismate Synthase



Reactions Involving Ketyl Radicals

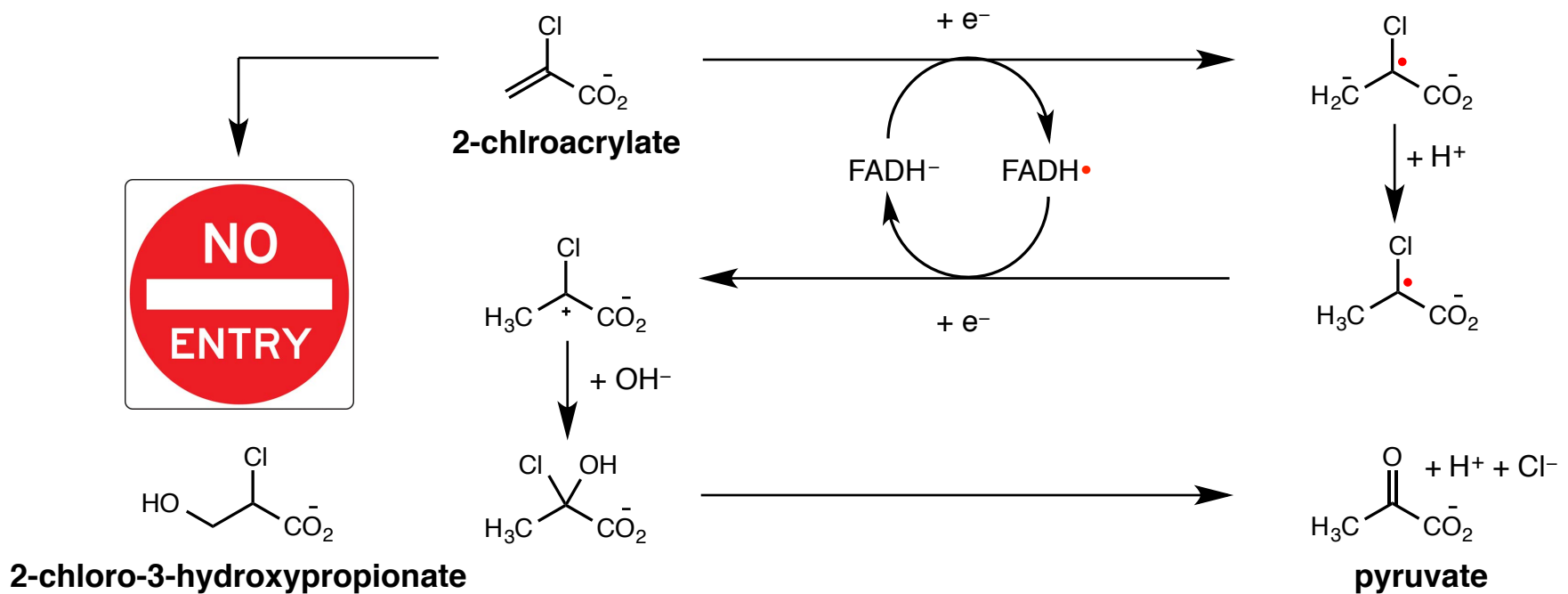
DNA photolyase



FADH₂-dependent Hydratase

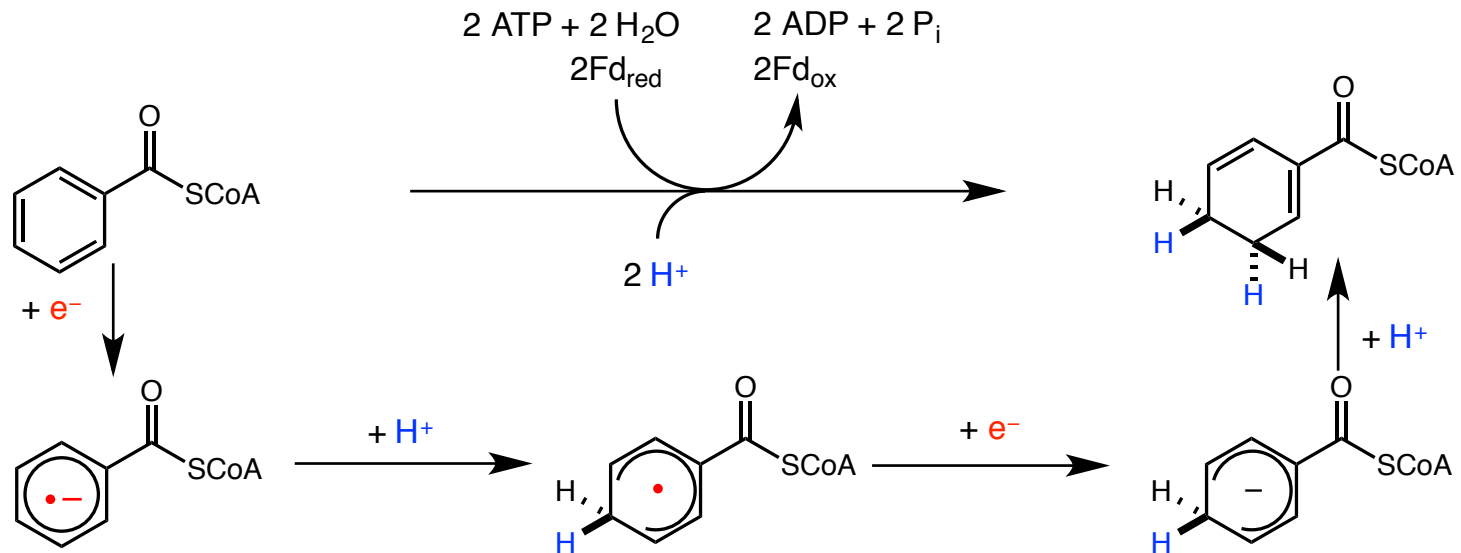
2-Haloacrylate hydratase

The hydratase catalyzes the hydration of 2-chloroacrylate to pyruvate and chloride, while a simple hydration of chloroacrylate would lead to 2-chloro-3-hydroxypropionate.

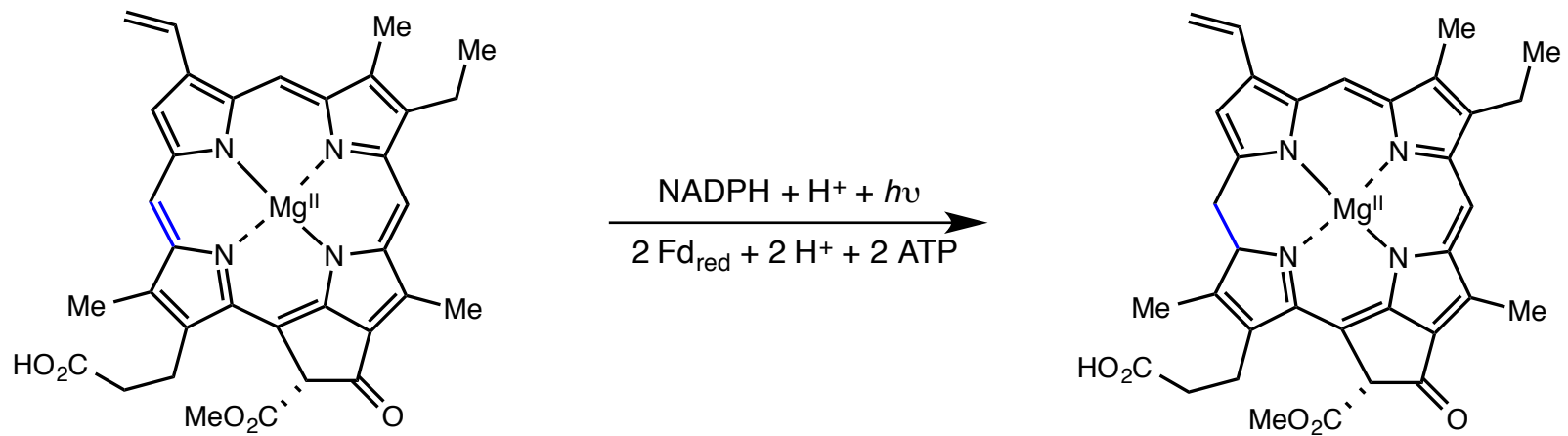


Reaction of Aromatic Compounds

■ Class I BCR



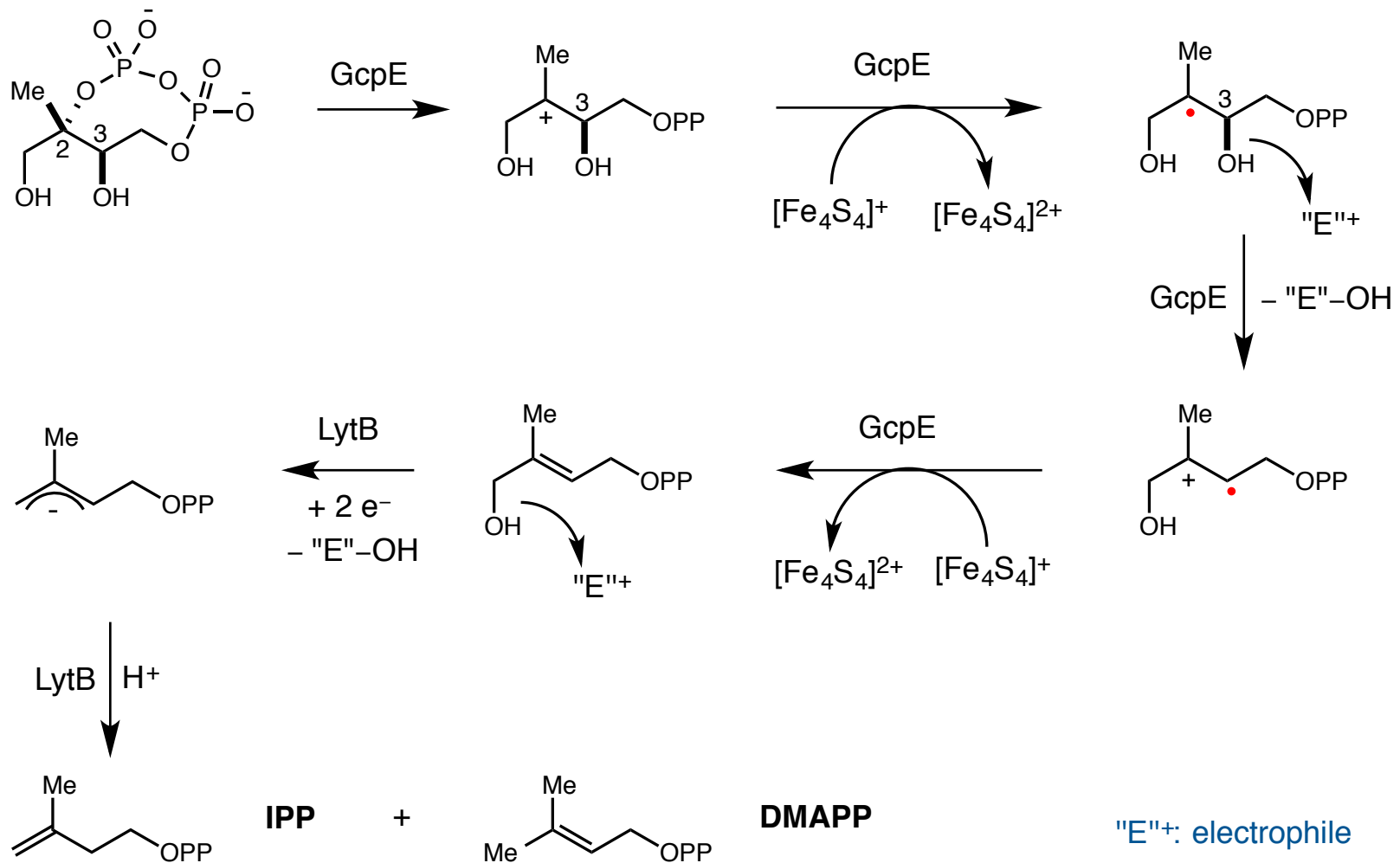
■ Protochlorophyllide reductase



Isoprenoid Biosynthesis

■ (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (GcpE) and reductase (LytB)

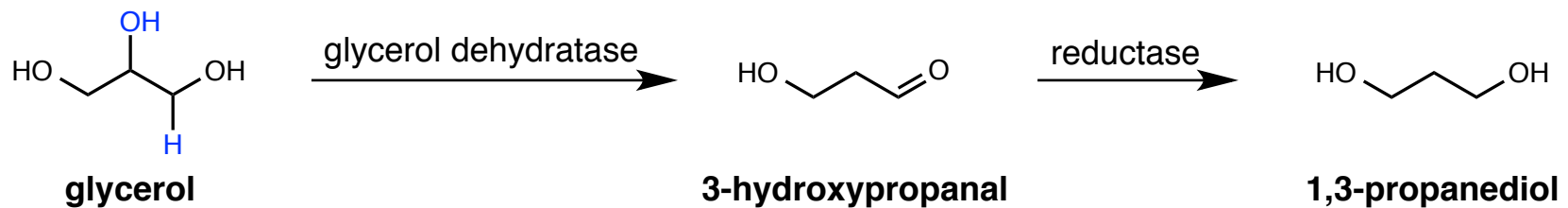
Cleavage of the cyclodiphosphate ring to a tertiary carbocation is followed by one-electron reduction to give a β -hydroxyalkyl radical that eliminates the C-3 hydroxyl group in the manner of RNR or diol dehydratase.



Biotechnological Application

■ Coenzyme B₁₂-dependent glycerol dehydratase

- The only one radical enzyme currently applied in biotechnology.
- The product 1,3-propanediol, a valuable monomer for polyesters, from which fabrics are made.
- The company Dupont advertises the fabrics as "Clothes from Corn".



From corn to polymers and fibers

DuPont™ Sorona® is made from naturally occurring starch in the kernels of corn. In the next five years, researchers plan to find ways to use starch from the entire plant.

