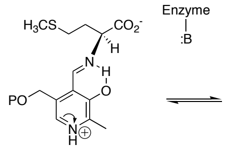
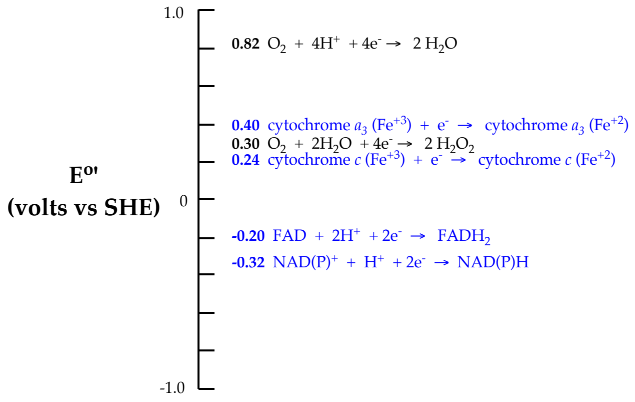
Mechanism of methionine γ-lyase



Redox Cofactors



E°'
(volts vs SHE)

1.0

0.82 $O_2 + 4H^+ + 4e^- \rightarrow 2\,H_2O$

0.40 cytochrome a_3 (Fe^{+3}) + e^- → cytochrome a_3 (Fe^{+2})

0.30 $O_2 + 2H_2O + 4e^- \rightarrow 2\,H_2O_2$

0.24 cytochrome *c* (Fe^{+3}) + e^- → cytochrome *c* (Fe^{+2})

0

-0.20 FAD + $2H^+ + 2e^-$ → $FADH_2$

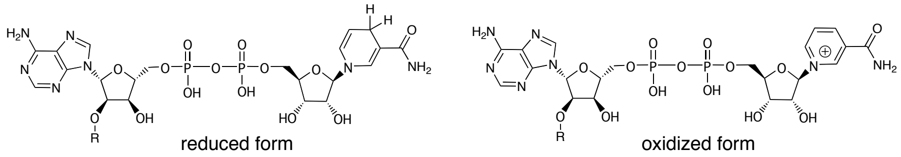
-0.32 $NAD(P)^+ + H^+ + 2e^-$ → NAD(P)H

-1.0

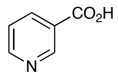
Bugg, Chapter 6, pp 115-147

Nicotinamide (pyridine dinucleotide)

Bugg, Chapter 6 pp. 117-122



R= H NADH, NAD^+
R^+ PO_3^{2-} NADPH, $NADP^+$

Vitamin B_3, nicotinic acid, niacin 

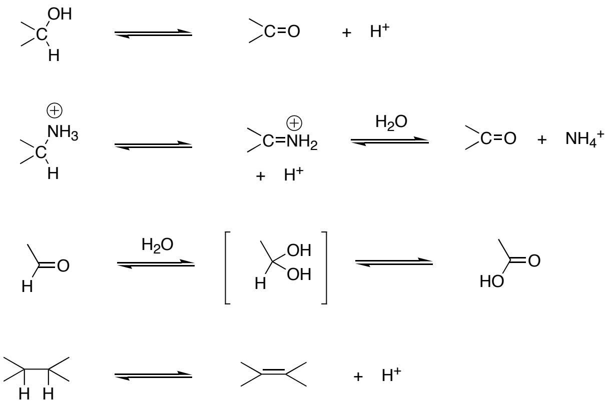
E°' NAD^+/NADH couple = - 0.32 V vs NHE

very strong reducing agent

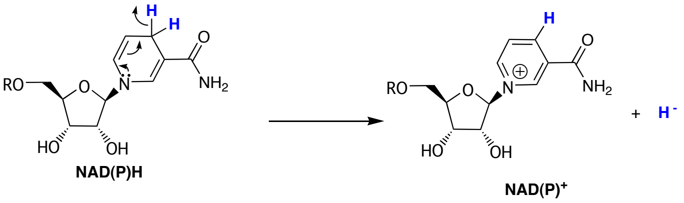
$NAD(P)^+ \rightleftharpoons NAD(P)H$ mostly involved in 2e- chemistry

H^- (hydride) source

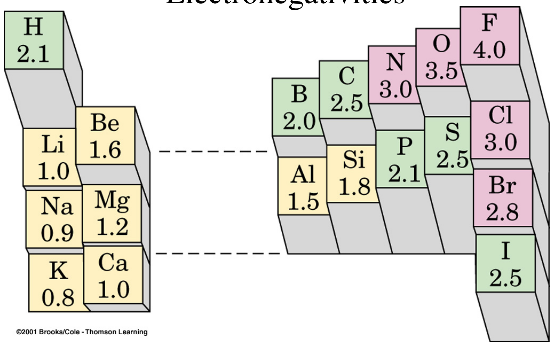
Reaction types catalyzed by $NAD(P)^+$ / NAD(P)H



NAD(P)$^+$ / NAD(P)H is a carbon hydride source!?



Electronegativities



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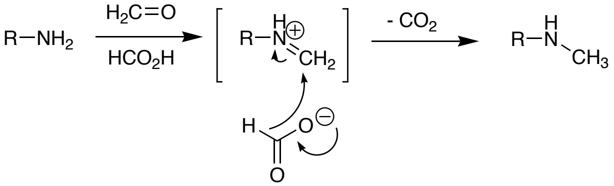
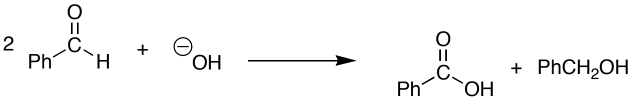
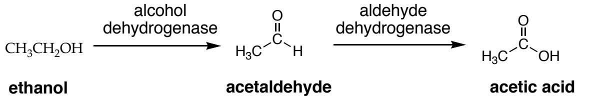
Examples of carbon hydride sources:
Eschweiler-Clark Reaction



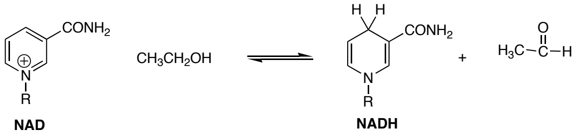
Cannizzaro Reaction



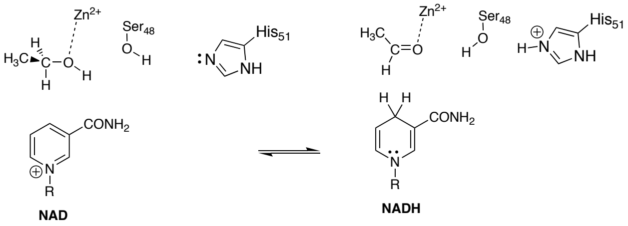
Ethanol metabolism:



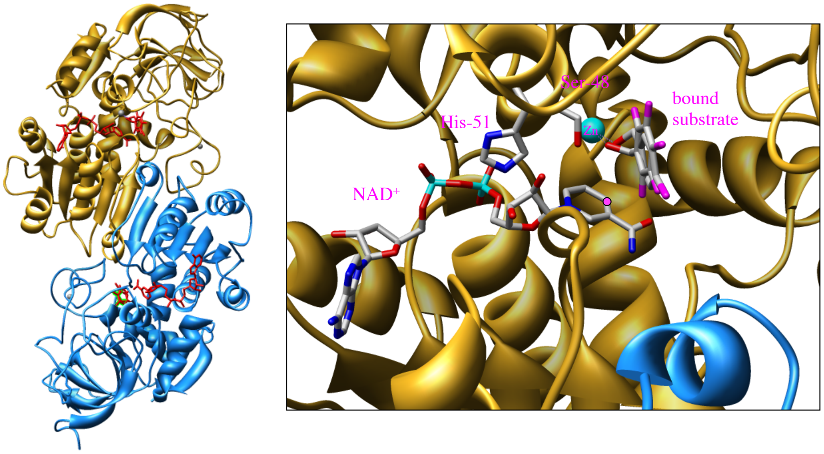
Alcohol Dehydrogenase:



- if run in T_2O or D_2O, no T or D incorporation in NADH
- if run with H_3CCD_2OH, complete D incorporation in NADH
- Results consistent with a hydride-transfer (H^-) mechanism and not a proton-transfer (H^+)



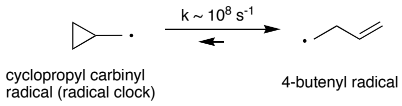
Active site of horse liver alcohol dehydrogenase

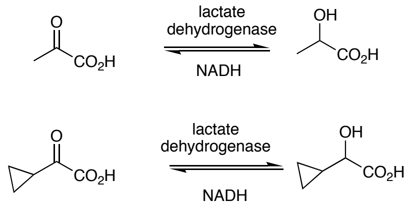


pdb code: 1HLD

Evidence for a hydride-transfer vs an electron-transfer mechanism

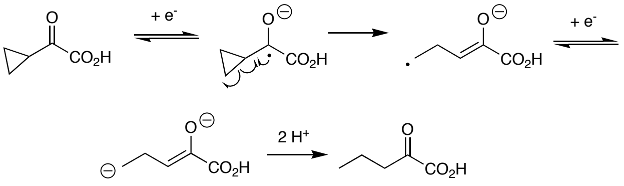
Cyclopropyl carbinyl radical ring opening as a probe for radical intermediates



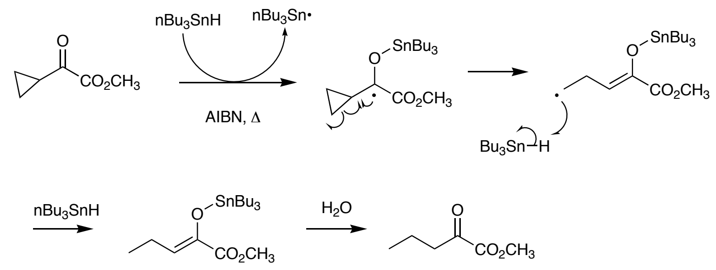


Product consistent with a hydride-transfer mechanism

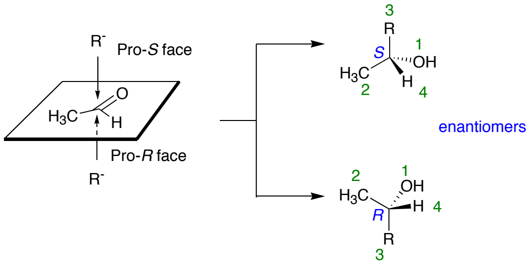
If an electron-transfer mechanism:

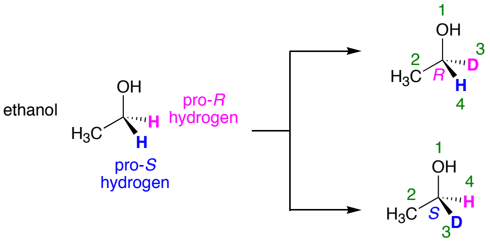


Chemical model for the electron-transfer mechanism

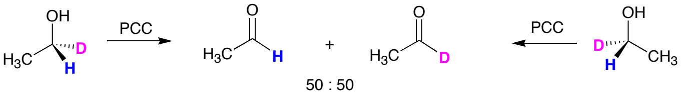


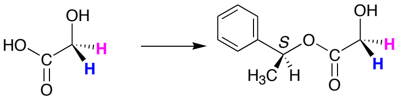
Stereochemistry of alcohol dehydrogenase:
pro-chirality



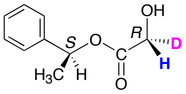


H' s are enantiotopic, chemically equivalent

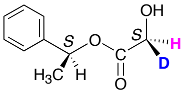




diastereotopic
NOT chemically equvalent



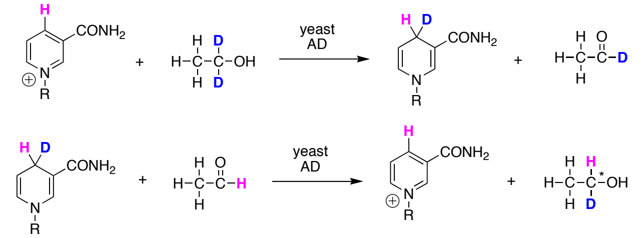
(***S***, ***R***)

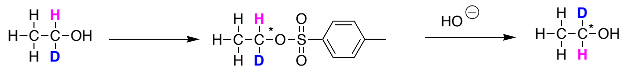


(***S***, ***S***)

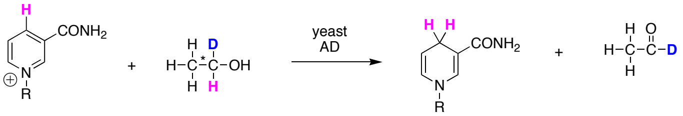
Enzymes are chiral and create a chiral environment around the substrate even though they are not covalently bound to each other.

Stereochemistry of alcohol dehydrogenase: hydride transfer is stereospecific

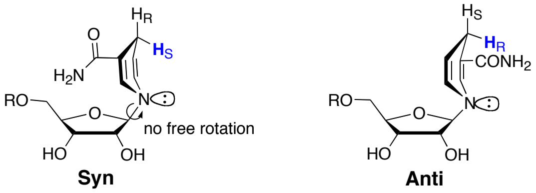


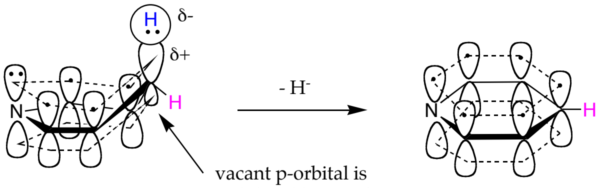


stereochemistry inverted



Conformation of the nicotinamide cofactor determines if the pro-R or the pro-S hydrogen is transferred from the cofactor

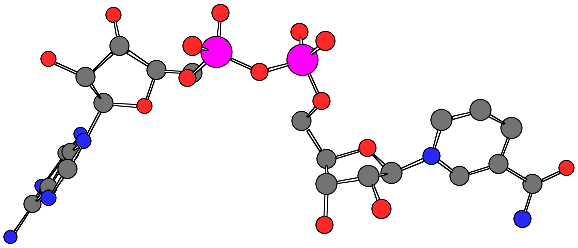




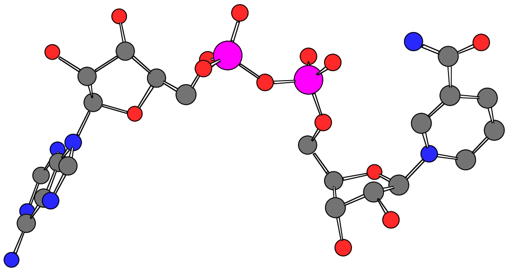
vacant p-orbital is stabilized by overlap with the π-system

Axial H of NAD(P)H is preferentially transferred

Conformation of NAD$^+$ cofactors

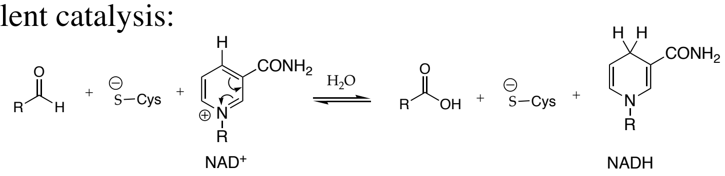


NAD$^+$ from Lactate Dehydrogenase (Pro-*R* specific)



NAD$^+$ from Glyceraldehyde-3-phosphate Dehydrogenase (Pro-*S* specific)

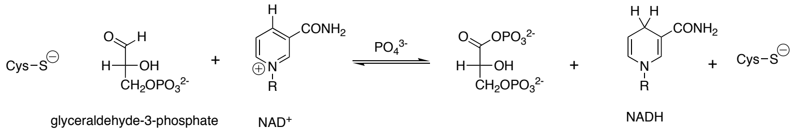
Mechanism of Aldehyde dehydrogenase
Covalent catalysis:



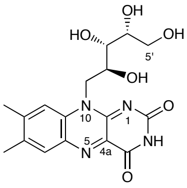
Non-covalent catalysis:

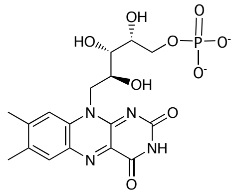
These are mechanistically equivalent to a Cannizarro Reaction

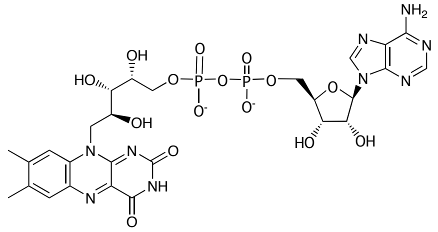
Regeneration of NADH from NAD$^+$ (glycolysis):
Glyceraldehyde-3-phosphate Dehydrogenase (G3PDH)

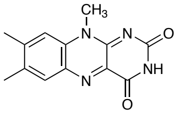


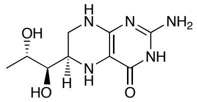
Flavin Coenzyme: Vitamin B_2, one- and two-electron transfer
Bugg, Chapter 6, pp. 122-135

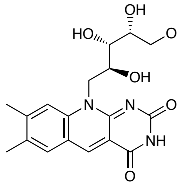

Riboflavin


Flavin Mononucleotide (FMN)


Flavin Adenine Diphosphate (FAD)

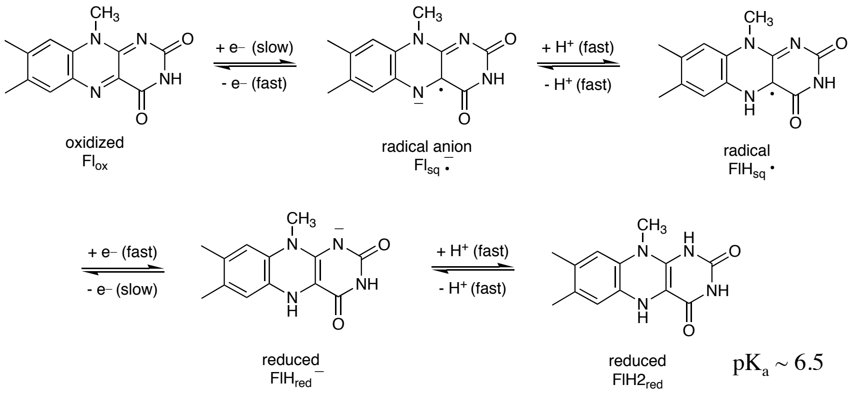

Lumiflavin


5,6,7,8-tetrahydrobiopterin


5-Deazaflavins
(more closely related to NAD)

tricyclic flavin ring system: isoalloxazine

Flavin Redox Chemistry



$pK_a \sim 6.5$

E°' ~ -0.2 V vs NHE for free flavins (two-electron couple)

The protein environment can have a large influence on the redox potential of the cofactor.

$$\begin{tikzcd}
\mathrm{Fl}_{ox} \arrow[r, shift left, "k_m"] \arrow[d, shift right, "{2e^-, H^+ \; E^{\circ\prime}}"'] & {[\,\mathrm{Fl}_{ox} \bullet \mathrm{Enzyme}]} \arrow[l, shift left, "\Delta G_1"] \arrow[d, shift right, "\Delta G_2"'] \\
\mathrm{FlH}^-_{red} \arrow[u, shift right, "{\sim 0.2\ V \; \Delta G_4}"'] \arrow[r, shift left, "k'_m"] & {[\,\mathrm{FlH}^-_{red} \bullet \mathrm{Enzyme}]} \arrow[l, shift left, "\Delta G_3"] \arrow[u, shift right, "{2e^-, H^+ \; E^{\circ\prime}}"']
\end{tikzcd}$$

thermodynamic cycle: $\Delta G_1 + \Delta G_2 = \Delta G_3 + \Delta G_4$

ΔG and E°' are related by the Nernst equation

$$\Delta G^{\circ} = -nFE^{\circ\prime} = -\frac{RT}{nF} \ln K_{eq}$$

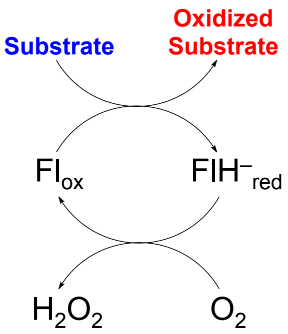
n = number of electrons

F = Faraday constant $= 23.063\ \mathrm{Kcal \bullet V^{-1} \bullet eq^{-1}}$

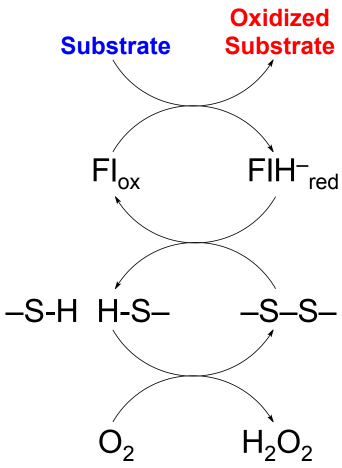
$= 96.542\ \mathrm{KJ \bullet V^{-1} \bullet eq^{-1}}$

Flavoenzymes are classified according to their re-oxidation mechanism by O_2

Direct re-oxidation

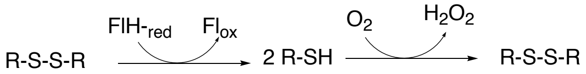


Indirect re-oxidation



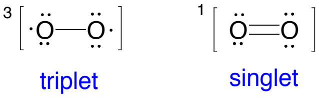
Flavoenzymes:

1. **Oxidases**: reduced flavin cofactor re-oxidized directly by O_2
2. **Dehydrogenase**: reduced flavin is re-oxidized by another group, i.e.,



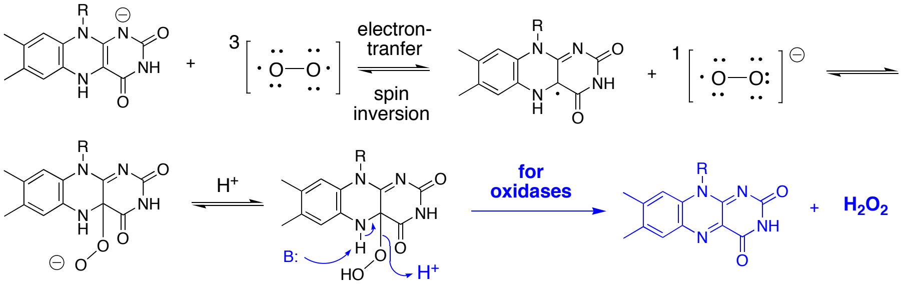
3. **Mixed Function Oxidase**: reduced flavin reacts with O_2 to give a flavin-4a-hydroperoxide (Fl-OOH), which oxidizes the substrates by transferring an oxygen atom to the substrate. Overall, O_2 is "split" with one oxygen atom being incorporated in the oxidized substrate, the other oxygen atom ends up as water.
4. **Electron-Transfer Flavoproteins** (ETF):

Oxygen:



spin: s = 2n +1
spin of an electron = ± 1/2

For oxidases and mixed function oxidases



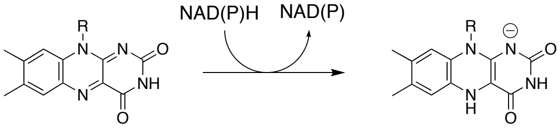
Flavin-4a-hydroperoxide

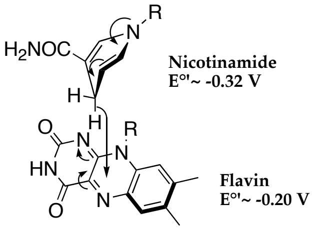
pK_a H_2O_2 ~ 11.6

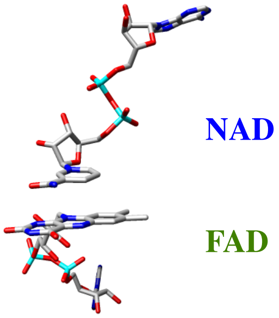
For mixed function oxidases, the flavin 4a-hydroperoxide is the oxygen-transfer (oxidizing) agent

---

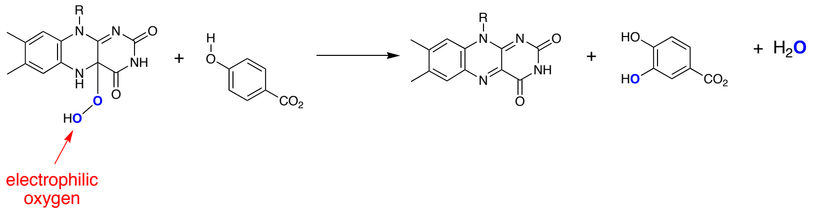
For mixed function oxidases (monooxygenases), the oxidized flavin must first be reduced to 1,5-dihydroflavin (FlH^-_{red}).
The reducing agent is often NAD(P)H and is often supplied as a separate enzyme.





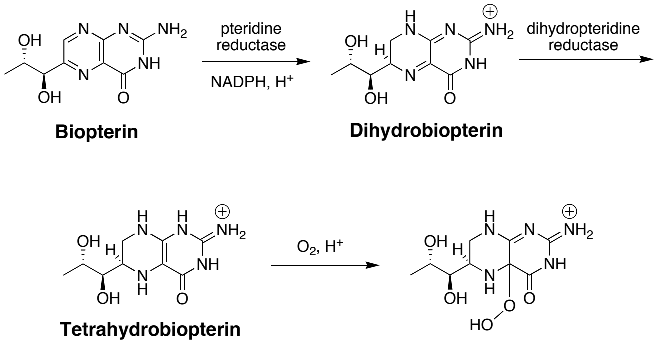


p-hydroxybenzoate hydroxylase



electrophilic oxygen

Pterins: truncated flavin coenzymes



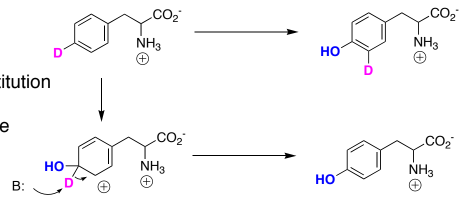
Biopterin

Dihydrobiopterin

Tetrahydrobiopterin

Bugg, p. 133-4

Phenylalanine hydroxylase: tetrahydrobiopterin dependent mixed function oxidase

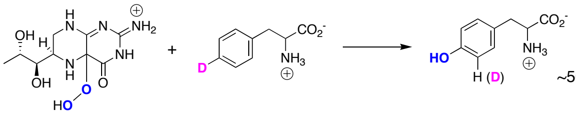


~50% D- incorporation

Electrophilic substitution analogous to p-hydroxybenzoate hydroxylase

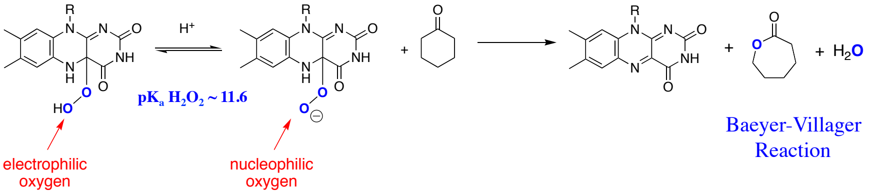
This mechanism would require NO D in the product

Mechanism of the NIH (Jerina-Daly) Shift:

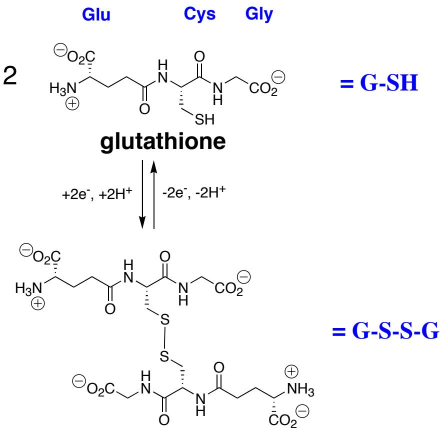


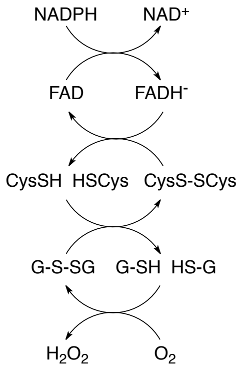
~50% D- incorporation

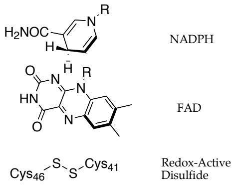
Cyclohexanone Monooxygenase: Flavin-4a-hydroperoxy anion as a nucleophilic oxidant



Glutathione Reductase: NADPH-dependent flavin dehydrogenase



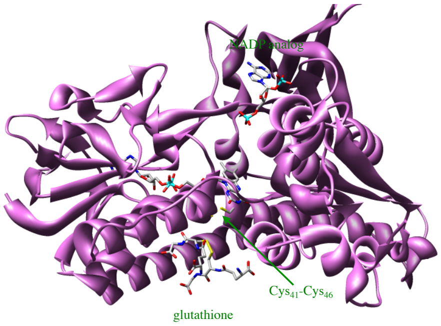


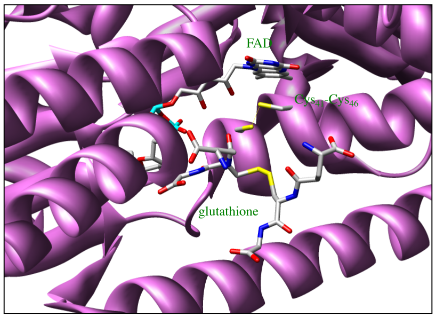


G–S–S–G Glutathione

Glutathione Reductase: mechanism involves a covalent adduct at the 4a-position of the FAD cofactor

Glutathione reductase



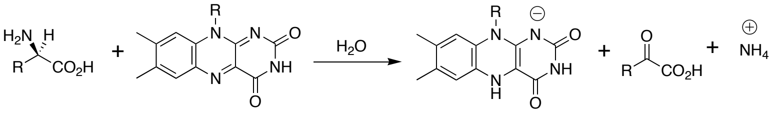


pdb code: 1GRA

Mechanism of Flavoenzyme Catalysis: D-amino acid oxidase

C-nucleophile mechanism. Amino acid enolate adds to the flavin 4a-position. The flavin then acts as a leaving group.

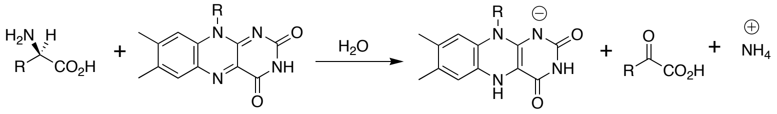
pK$_a$~ 25



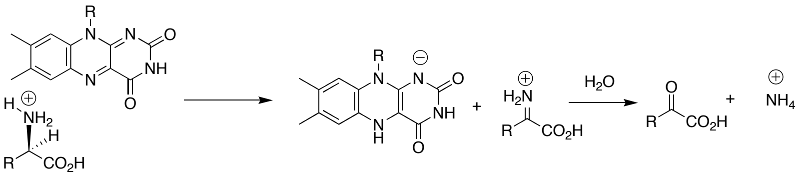
X-ray structure shows <u>NO</u> active site base

Mechanism of Flavoenzyme Catalysis: D-amino acid oxidase (con' t)

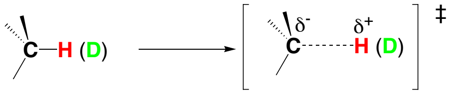
N-nucleophile mechanism



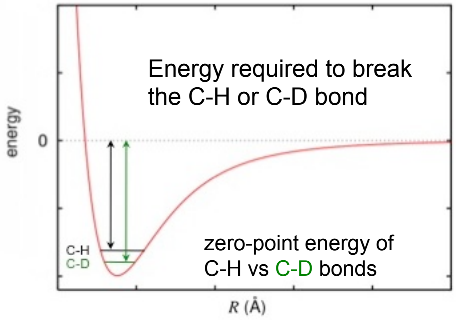
Hydride transfer mechanism: concerted, singled step mechanism, no covalent intermediate. Hydride adds directly to the flavin N5-position.



Kinetic Isotope Effect (KIE): influence of a substitution of an isotope on the rate of a reaction. Gives information on the rate limiting step of the reaction.



$$\bar{\nu} = \frac{1}{2\pi c}\left[\frac{f}{\left(\frac{m_x m_y}{m_x + m_y}\right)}\right]^{\frac{1}{2}}$$

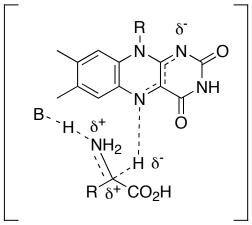


The magnitude of the KIE is dependent on the mass difference of the isotopes

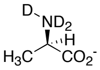
ΔG‡ for the heavier isotope (C-D) is greater, which translates into a slower reaction rate

KIEs are expressed as: $\frac{k_H}{k_D}$

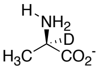
Transition state of the hydride transfer mechanism:



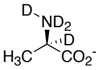
A C-H and N-H bonds are being broken in the transition state



$$\frac{k_{N\text{-}H}}{k_{N\text{-}D}} = 3.1$$ **(Solvent isotope effect)**



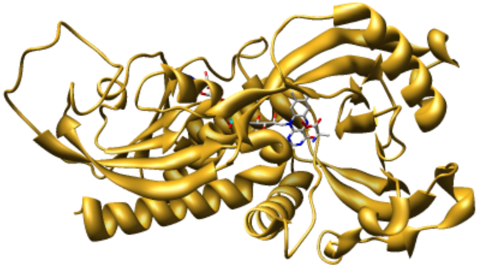
$$\frac{k_{C\text{-}H}}{k_{C\text{-}D}} = 2.9$$

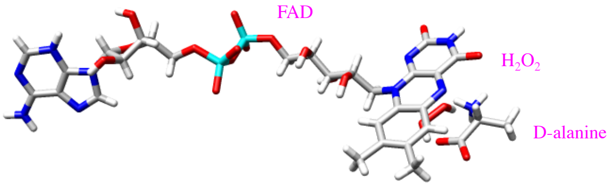


$$\frac{k_{N\text{-}H}}{k_{N\text{-}D}} \bullet \frac{k_{C\text{-}H}}{k_{C\text{-}D}} = 9.0\ (8.4)$$

Kinetic isotope effect data is consistent with two bonds being broken in the transition state for D-amino acid oxidase.

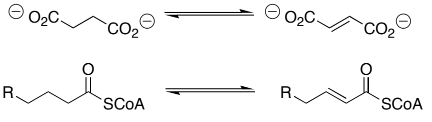
Crystal structure of D-amino acid oxidase supports the hydride transfer mechanism



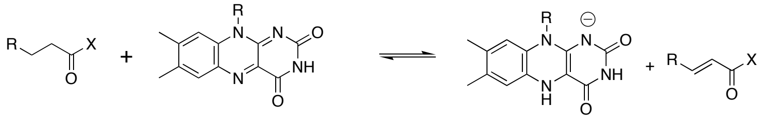


pdb code: 1C0L

Succinate and Acetyl CoA Dehydrogenase



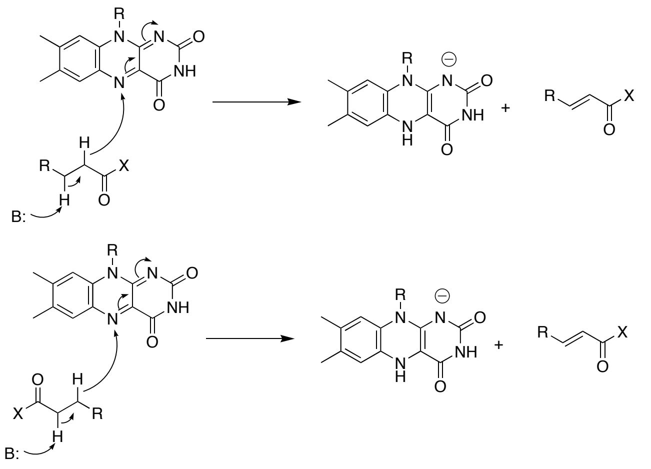
Anion mechanism



X= O^- or SCoA

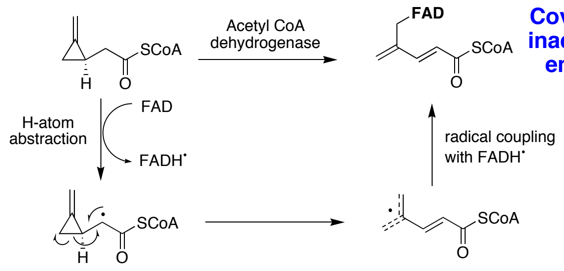
Succinate and Acetyl CoA Dehydrogenase (con' t)

Hydride-transfer mechanism



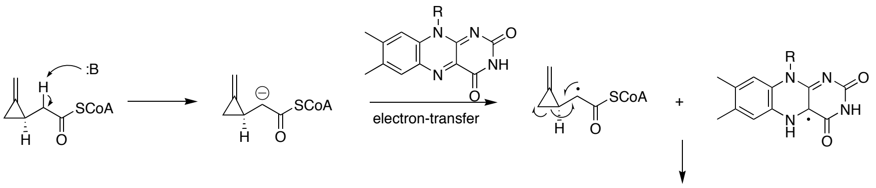
Succinate and Acetyl CoA Dehydrogenase (con’ t):

Electron-transfer mechanism



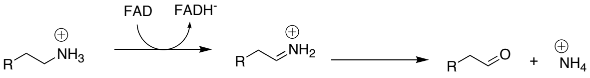
Covalently inactivated enzyme

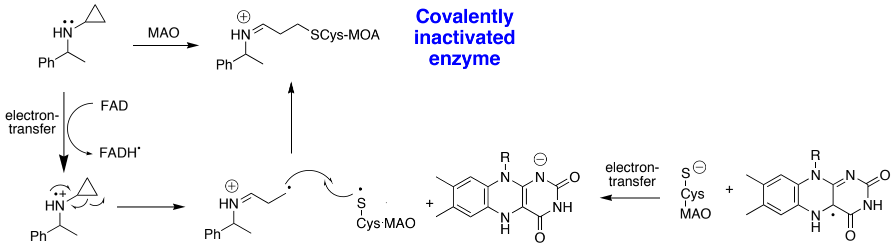
Flavins do not abstracts hydrogen atoms (H•)



Covalently inactivated enzyme

Monoamine oxidase (MAO)





Covalently inactivated enzyme

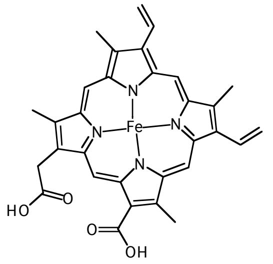
$R{-}\ddot{N}H_2 \xrightarrow{\text{electron-transfer}} R{-}\overset{\bullet+}{N}H_2$ **E°' ~ + 1.0 - 1.5 V**

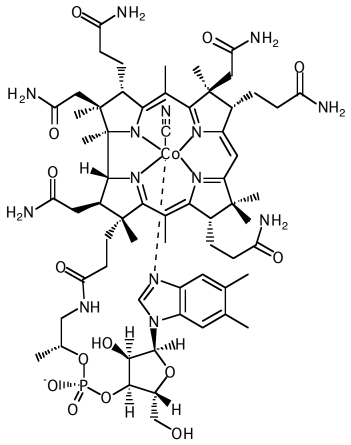
$FAD \xrightarrow{\text{electron-transfer}} FADH\bullet$ **E°' ~ - 0.2 V**

$R{-}\ddot{N}H_2 + FAD \xrightarrow{\text{electron-transfer}} FADH\bullet + R{-}\overset{\bullet+}{N}H_2$ **Thermodynamically Uphill !!! (by alot)**

There is good evidence for the N-nucleophile mechanism for MAO (slide 215)

Porphyrin vs. Corrin





Heme: Ligand for Fe(III) is a **porphyrin**, which is a fully conjugate ligand

Vitamin B$_{12}$: Ligand for Co(III) is a **corrin**, which is NOT a fully conjugate ligand

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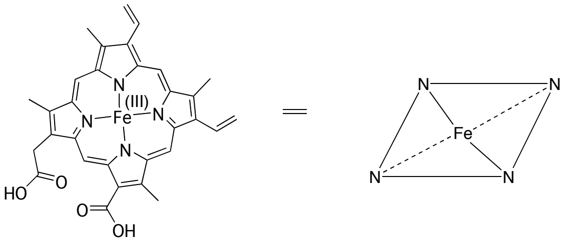
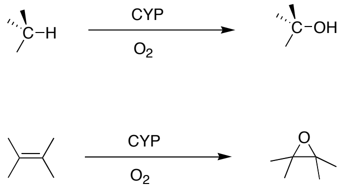
Heme (Cytochrome P450)-dependent Monooxygenases:

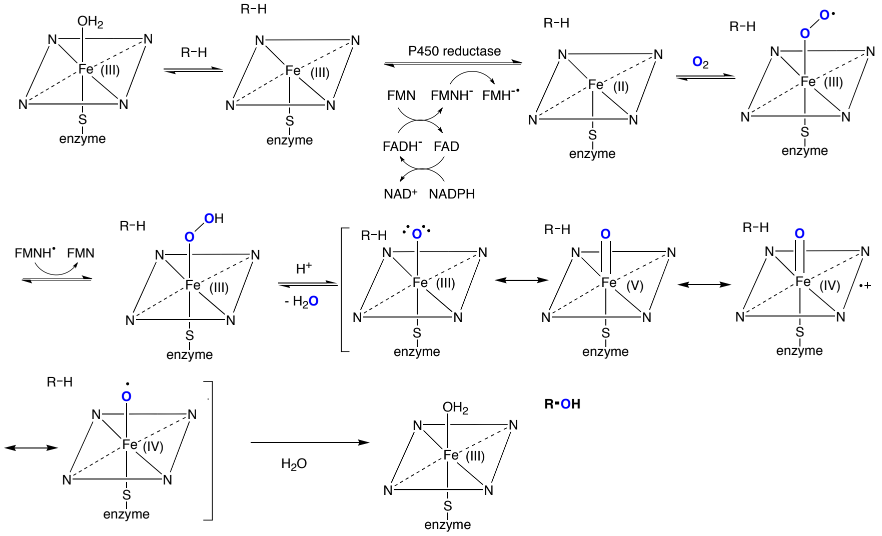
over 500 different isozyme- Superfamily

Bugg, Chapter 6, pp. 136-139



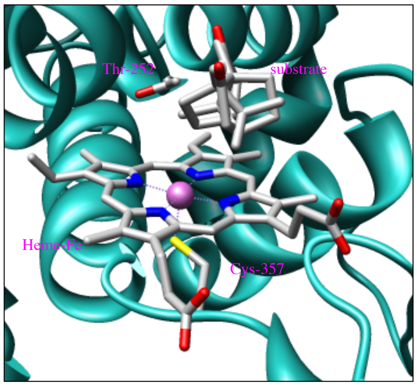


Overall mechanism of P450 hydroxylation

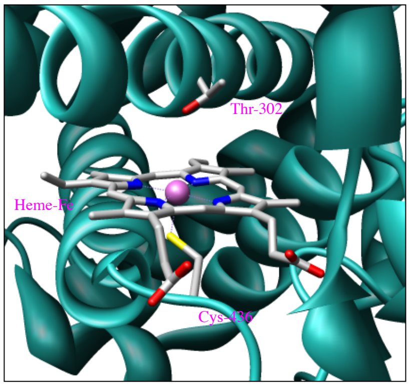


Bugg, p. 137

Active site of cytochrome P450 enzymes



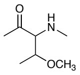
P450 cam
pdb code: 1AKD



P450 2B4
pdb code: 1PO4

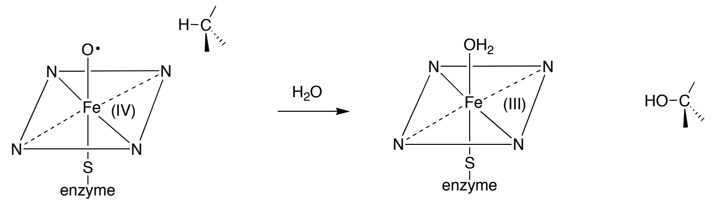
T252V or T252A mutants lose hydroxylase activity
T252X retains ~ 33% of the WT activity
suggest an intervening water molecule(s)
as the H^+ source

X= O-methylthreonine



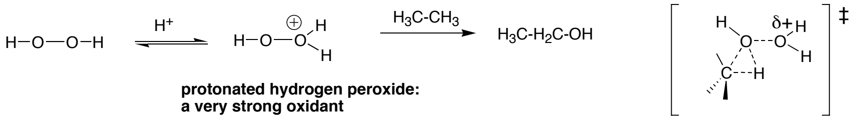
Mechanism of the hydroxylation step:

oxygen rebound mechanism (J. Groves)

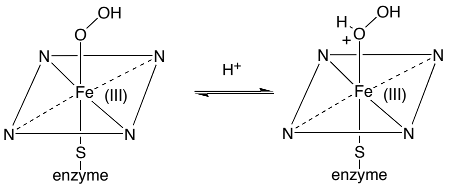


Mechanism of the hydroxylation step (con’t):

Concerted oxygen insertion (M. Newcomb)



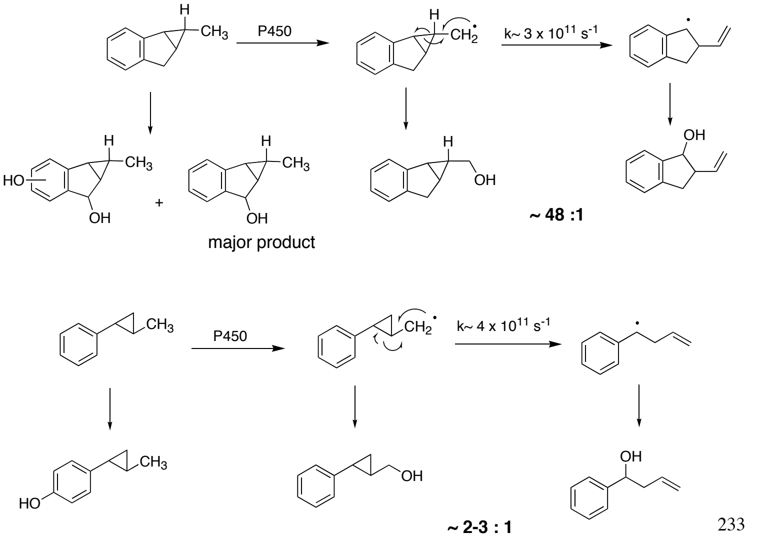
protonated hydrogen peroxide:
a very strong oxidant



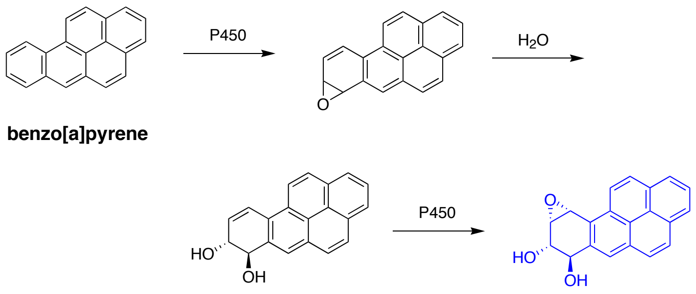
resembles protonated
hydrogen peroxides

proton may be from a conserved threonine. Not acidic enough?

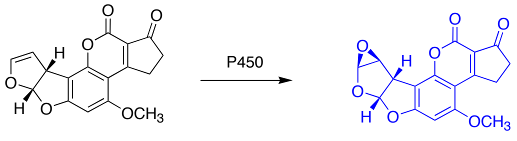
Ultra-fast Radical Clocks as mechanistic probes for P450 hydroxylation reactions: consistent, in part, with the oxygen insertion mechanism



Cytochrome P450 mediated epoxidation reactions:
activation of pro-carcinogens



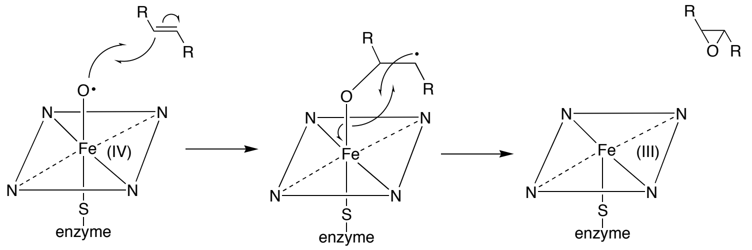
benzo[a]pyrene



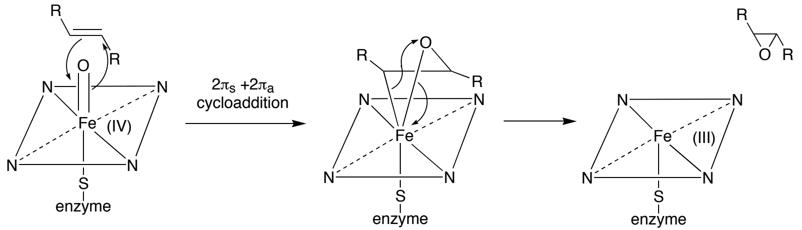
aflatoxin B_1

Mechanism cytochrome P450 epoxidation of alkenes:

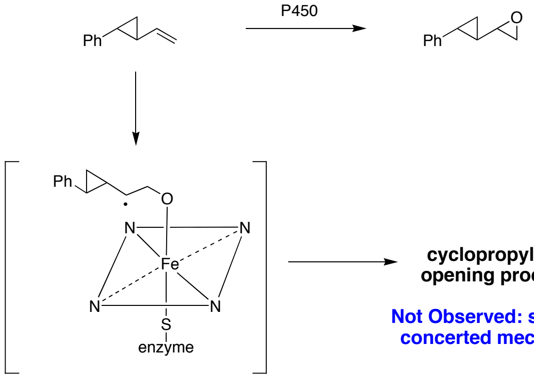
Oxygen rebound mechanism



Concerted mechanism:



Mechanistic probe for P450 alkene epoxidation:

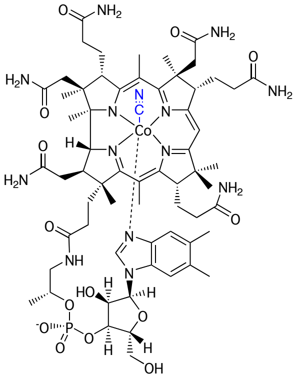


cyclopropyl ring opening products

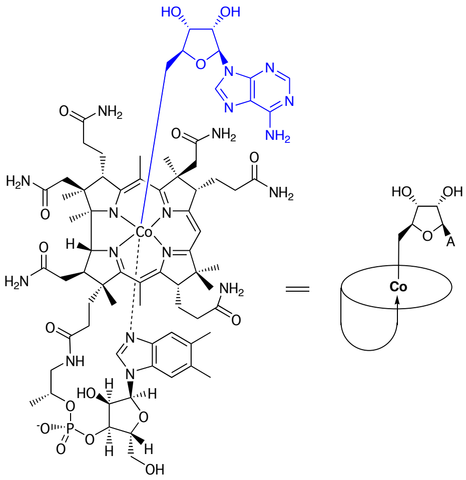
Not Observed: suggests concerted mechanism

Vitamin B_{12} Dependent Enzymes: Bugg, Chapter 11, pp 225-229

Mutase- catalyzes a rearrangement of the substrate

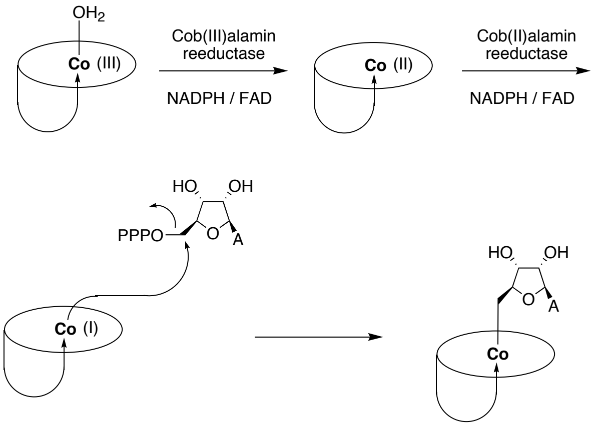


Vitamin B_{12}
(cyanocobalamin)



Coenzyme B_{12}
(adenosylcobalamin)

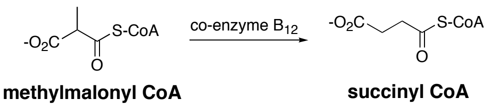
Conversion of vitamin B_{12} to co-enzyme B_{12}

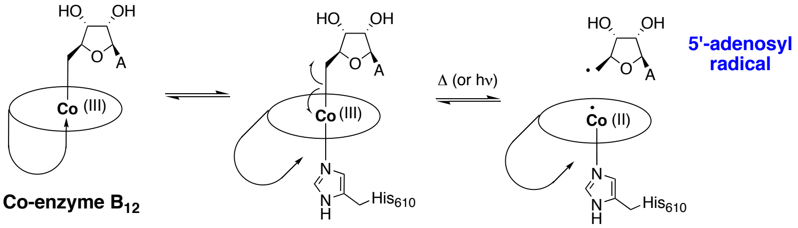


Nucleophilic cobalt

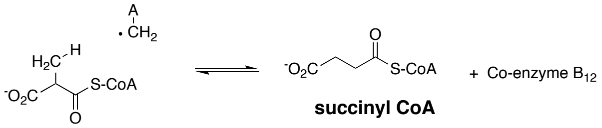
Co-enzyme B_{12}

Methylmalonyl CoA Mutase:

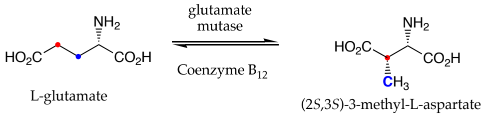


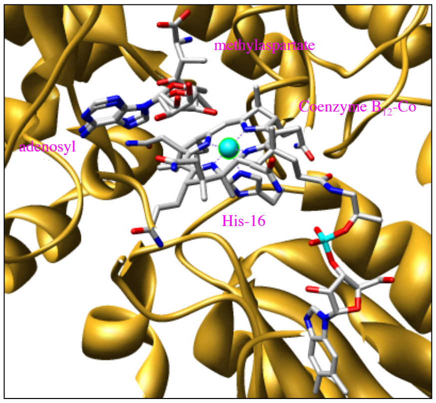


Methylmalonyl CoA Mutase (con' t):



Glutamate mutase





pdb code: 1I9C