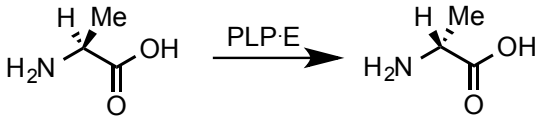
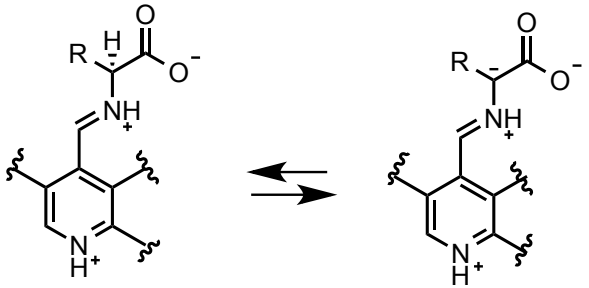
Lecture 9 - Co-factors Part II

PLP

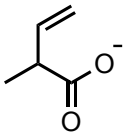
- Alanine Racemase

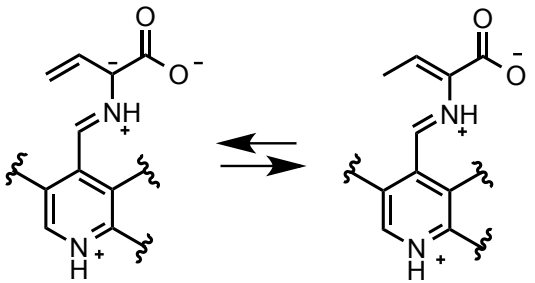


-How to inhibit the conversion of L-Ala to D-Ala?

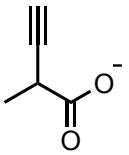


Another covalent inhibitor:



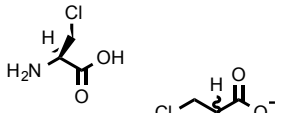


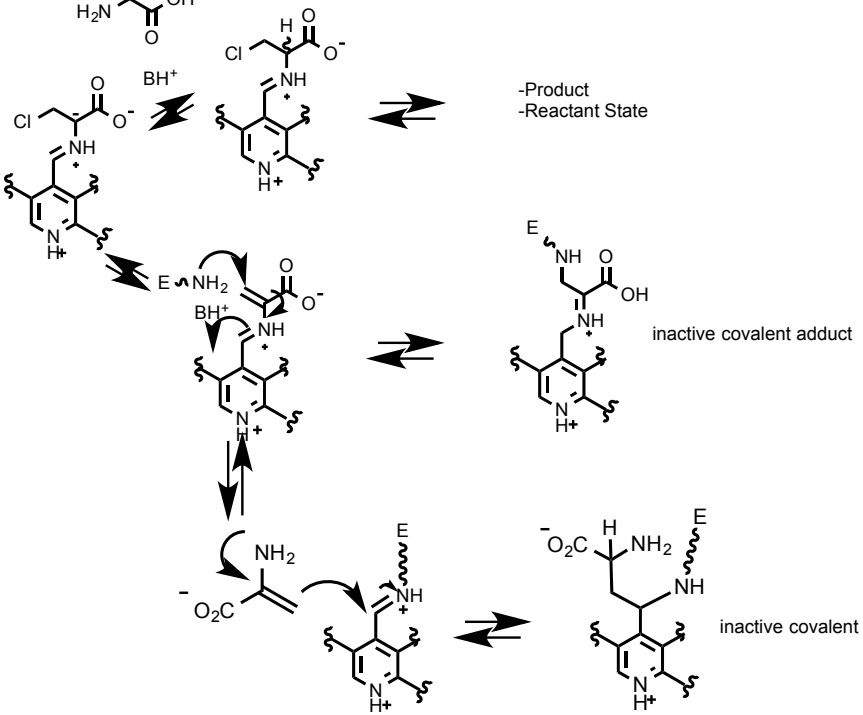
or alternatively:



Designing a mechanism based PLP inhibitor?

Use:

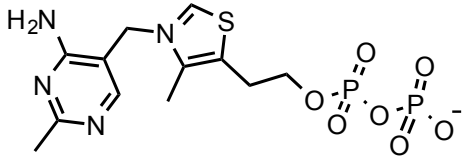




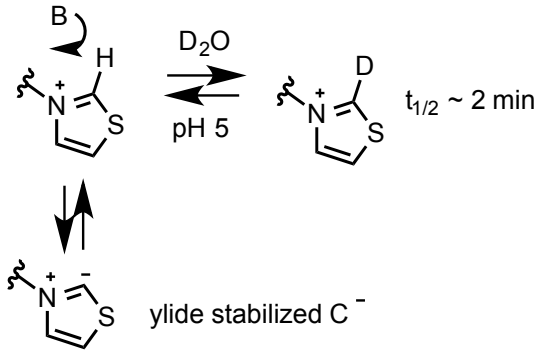
$\frac{k_{inact}}{k_{cat}} \approx$ Partitioning

Thiamine pyrophosphate (Vitamin B_1)

Like PLP, the electron-deficient heterocyclic ring of thiamine pyrophosphate (TPP) stabilizes the formation of carbanionic species.

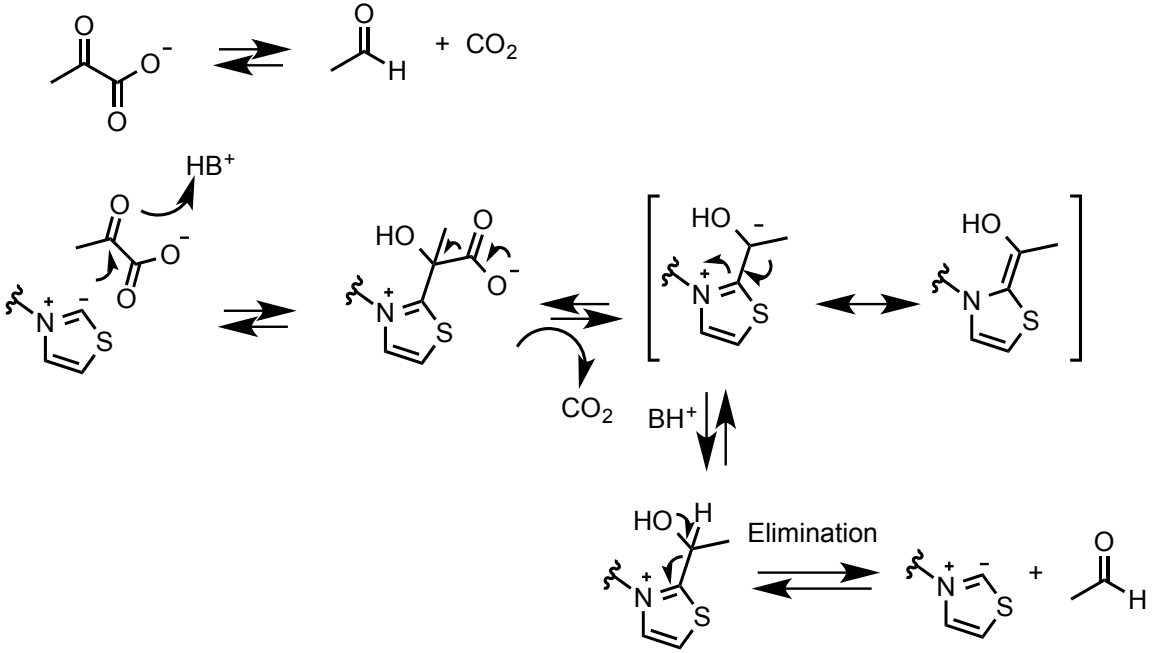


Breslow (1961) was the first to propose and provide evidence for the C-2 carbanion, showing that this proton exhanges readily in D_2O

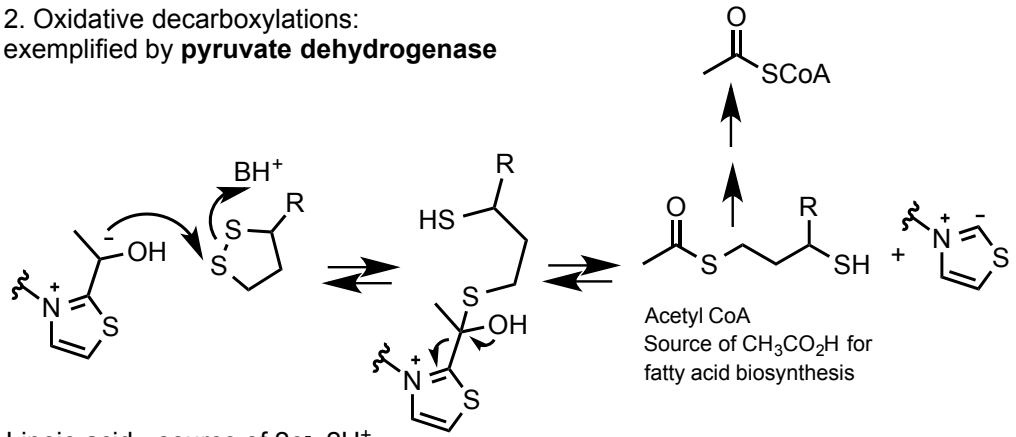


Reactions involving thiamine pyrophosphate:

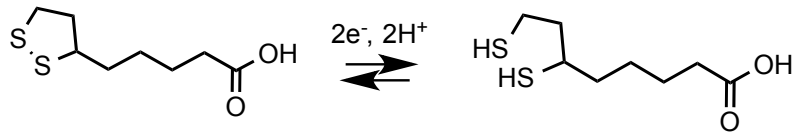
1. Decarboxylation of alpha-ketoacids: **pyruvate decarboxylase**



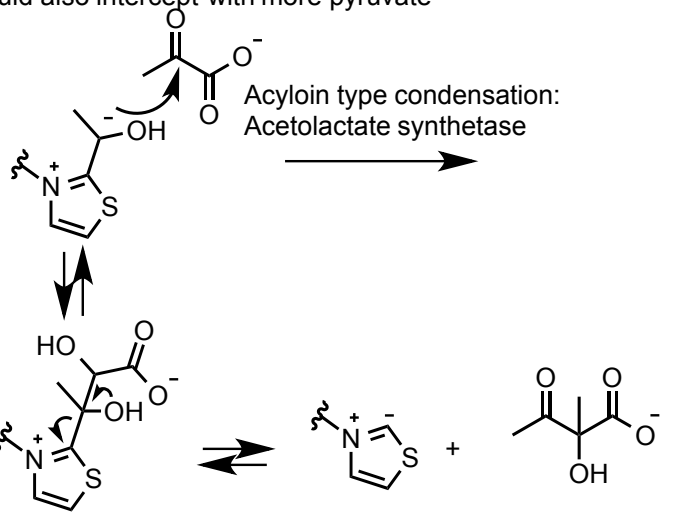
2. Oxidative decarboxylations:
exemplified by **pyruvate dehydrogenase**



Lipoic acid - source of $2e^-$, $2H^+$



-could also intercept with more pyruvate

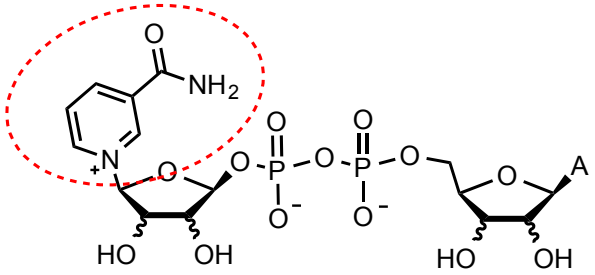


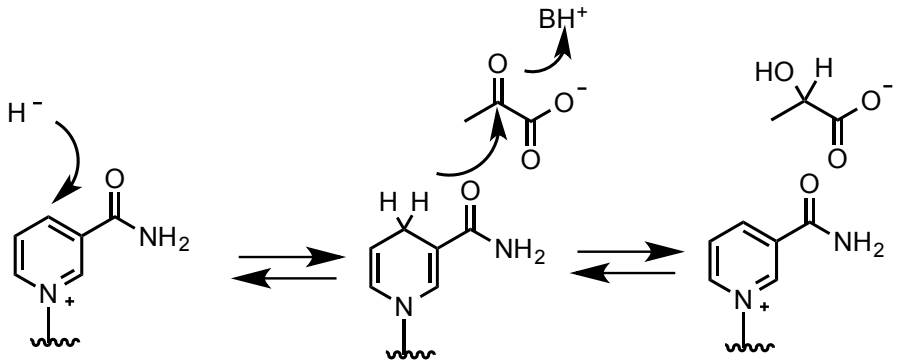
Redox Enzymes:

-NADPH/NADH ⇦ nicotinamide dependent ($2e^-$, H^+)

-FAD/FADPH ⇦ flavin dependent $1e^-$ ➔ $1e^-$ ($2e^-$,$2\ H^+$)

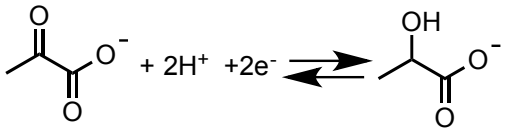
Nicotinamide adenine dinucleotide (phosphate)



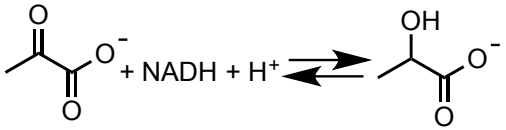


Overall Reaction:

E'_0= -0.32V

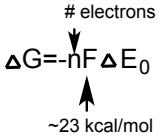


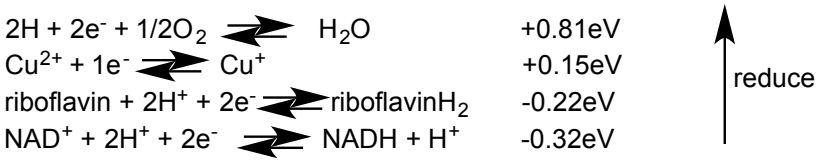
E'_0= -0.19V



$E'_0=E'_0(red) - E'_0(oxid) = -0.19V - (-0.32V) = 0.13V$

Note compounds with lower redox potential thoroughly reduce those with higher redox potentials

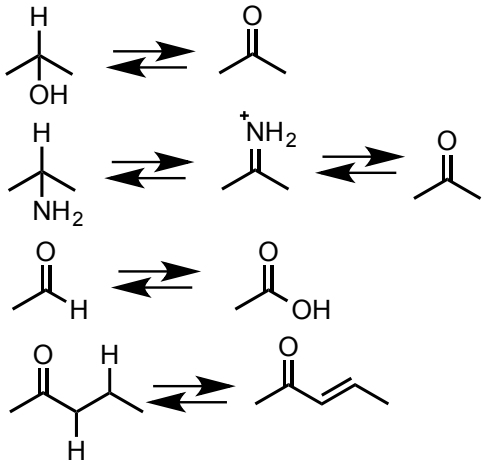




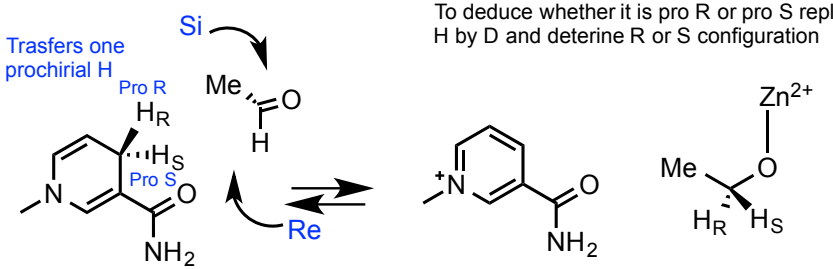
-use nicotinamide to reduce riboflavin

-molecular oxygen is reduced by riboflavin, not nicotinamide ($1e^-$ process)

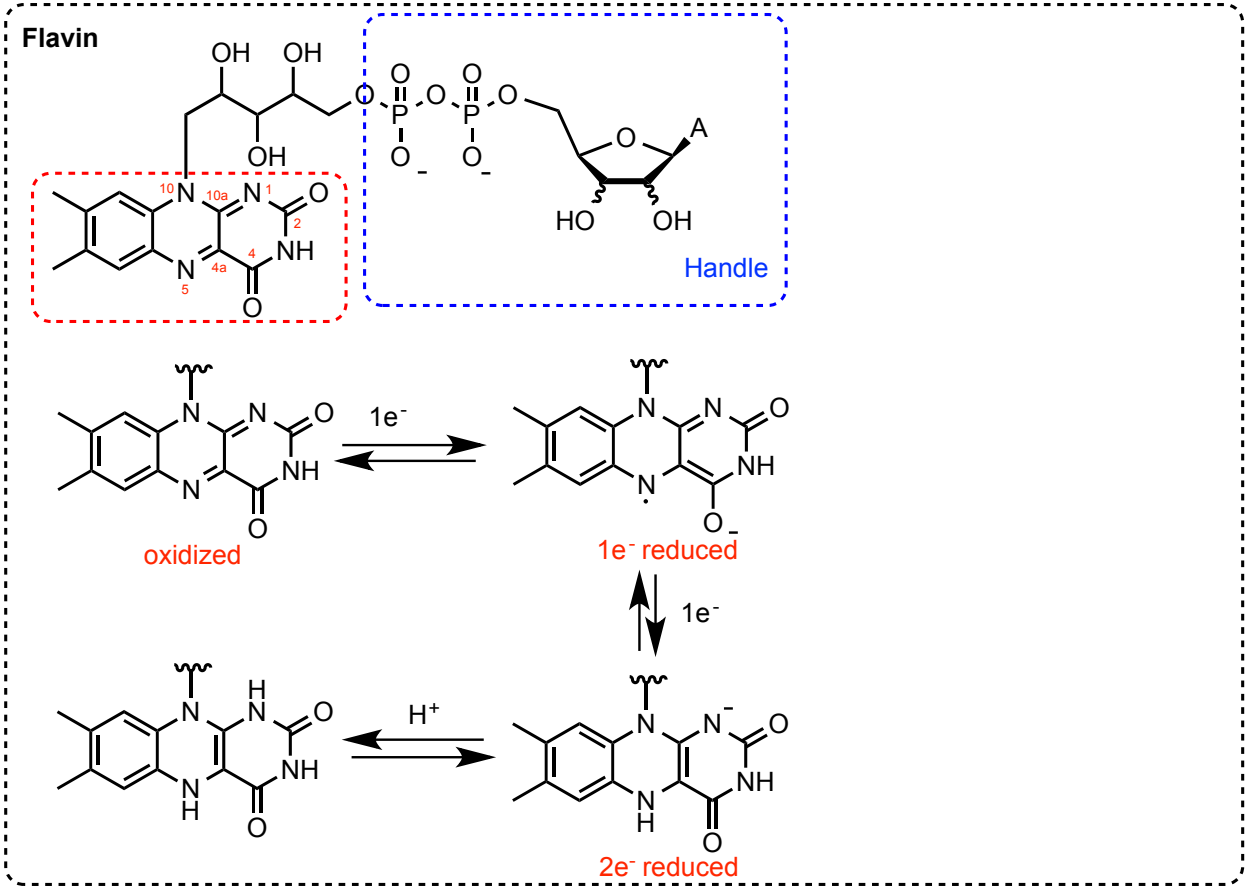
Reactions of NAD^+/$NADPH^+$ Enzymes ($2e^-$/H^+)

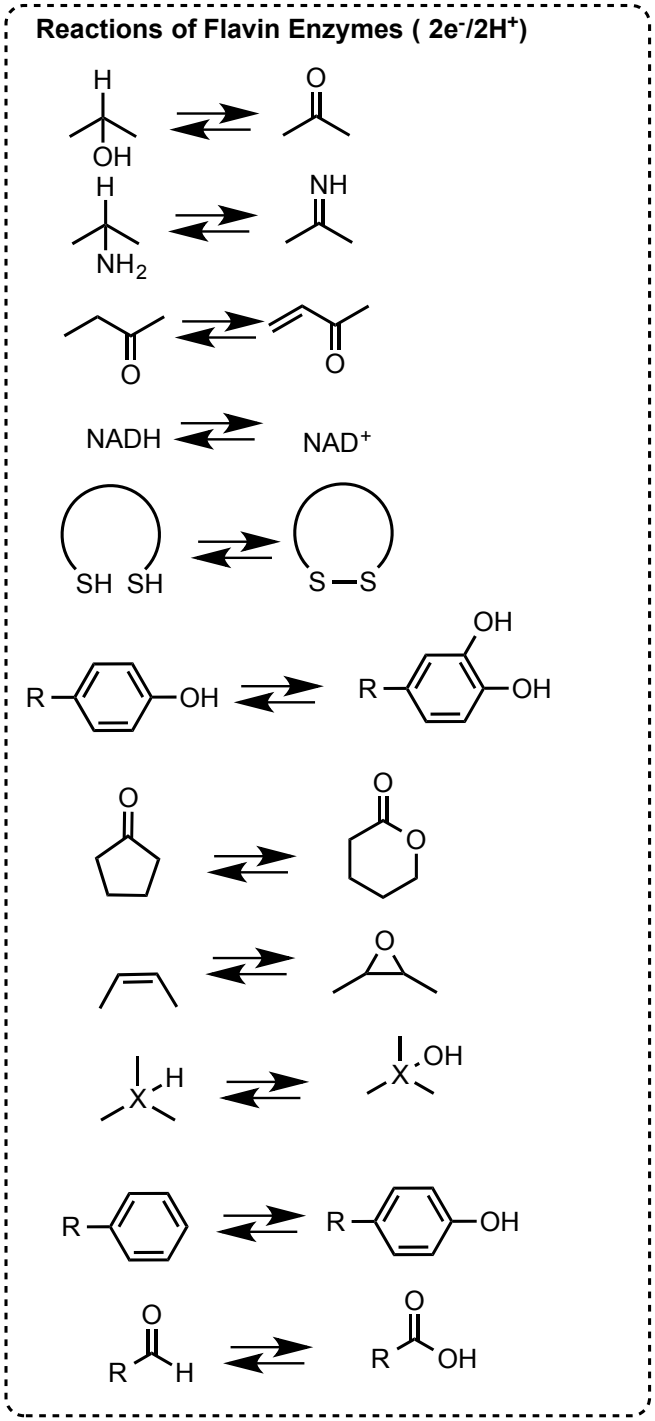


Note that these are all sterospecific

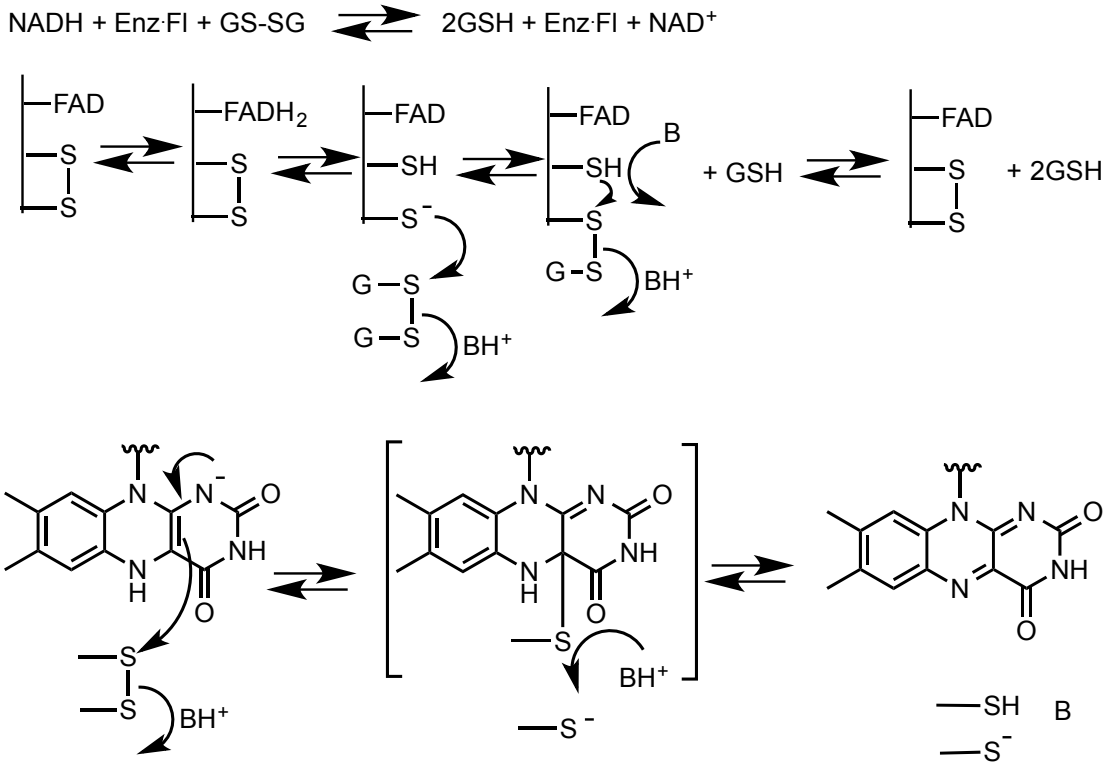


To deduce whether it is pro R or pro S replace that H by D and deterine R or S configuration


Flavin
Handle
oxidized
1e⁻
1e⁻ reduced
1e⁻
H⁺
2e⁻ reduced


Reactions of Flavin Enzymes (2e⁻/2H⁺)
NADH
NAD⁺

Reaction of glutathione (glutathione reductase)



Molecular oxygen