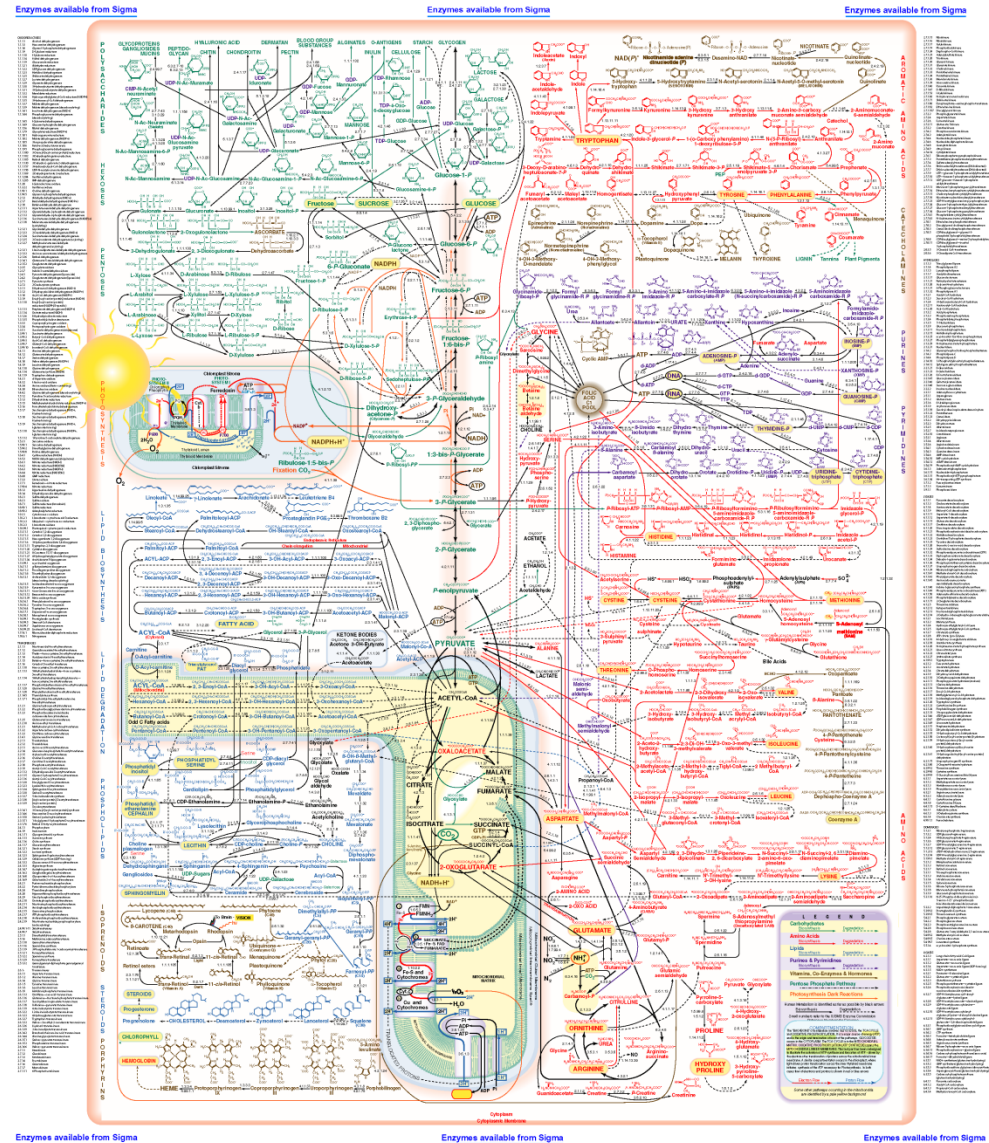
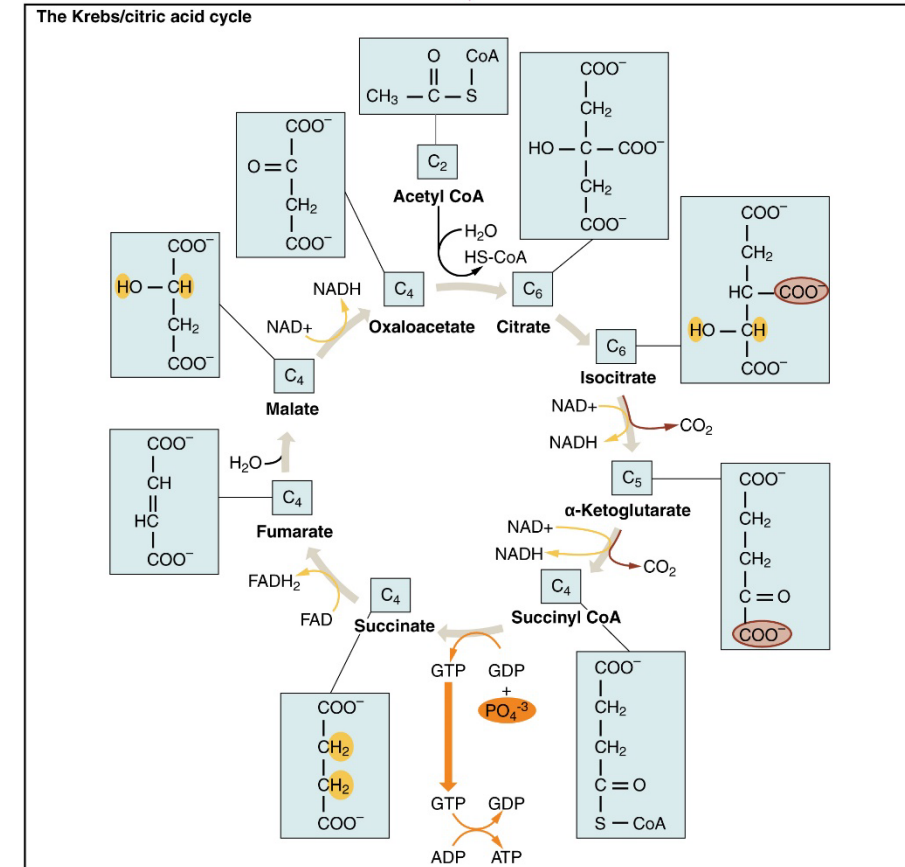
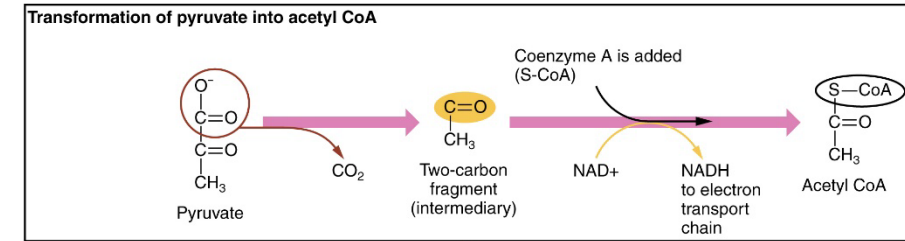
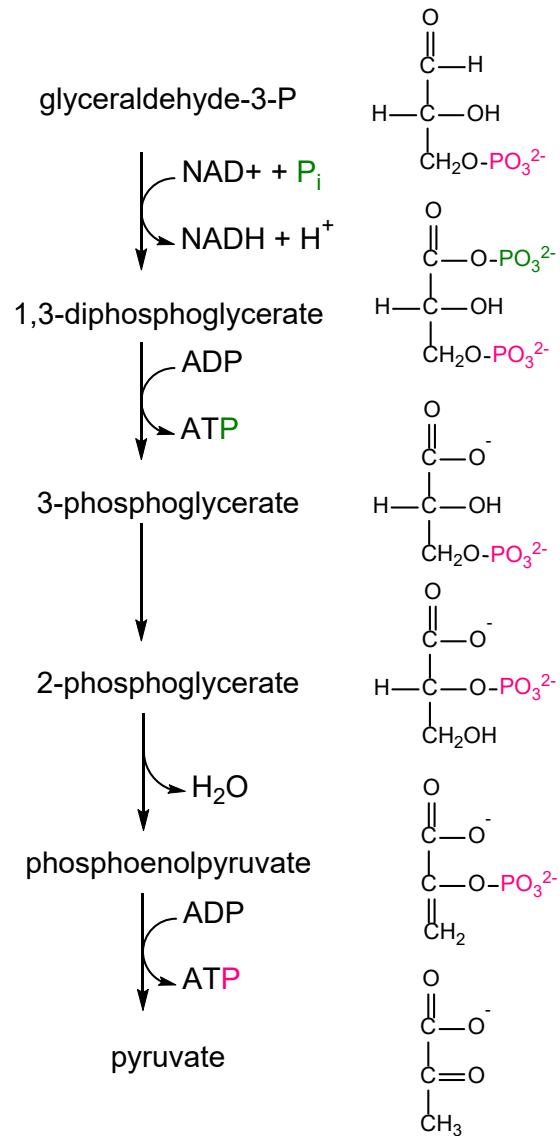
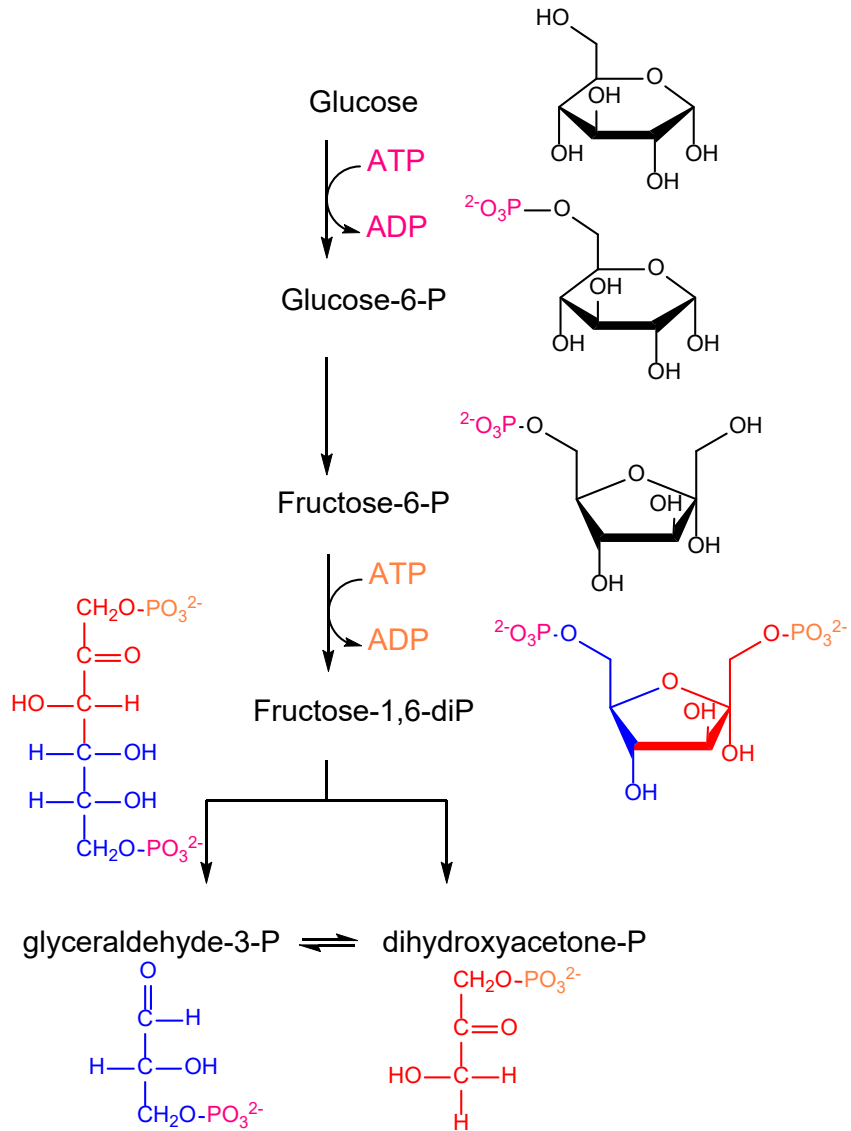


Four categories of enzymes

1. Group transfer reactions
2. Oxidations/reductions
3. **Eliminations/isomerizations/
rearrangements**
4. Reactions that make or break
C-C bonds



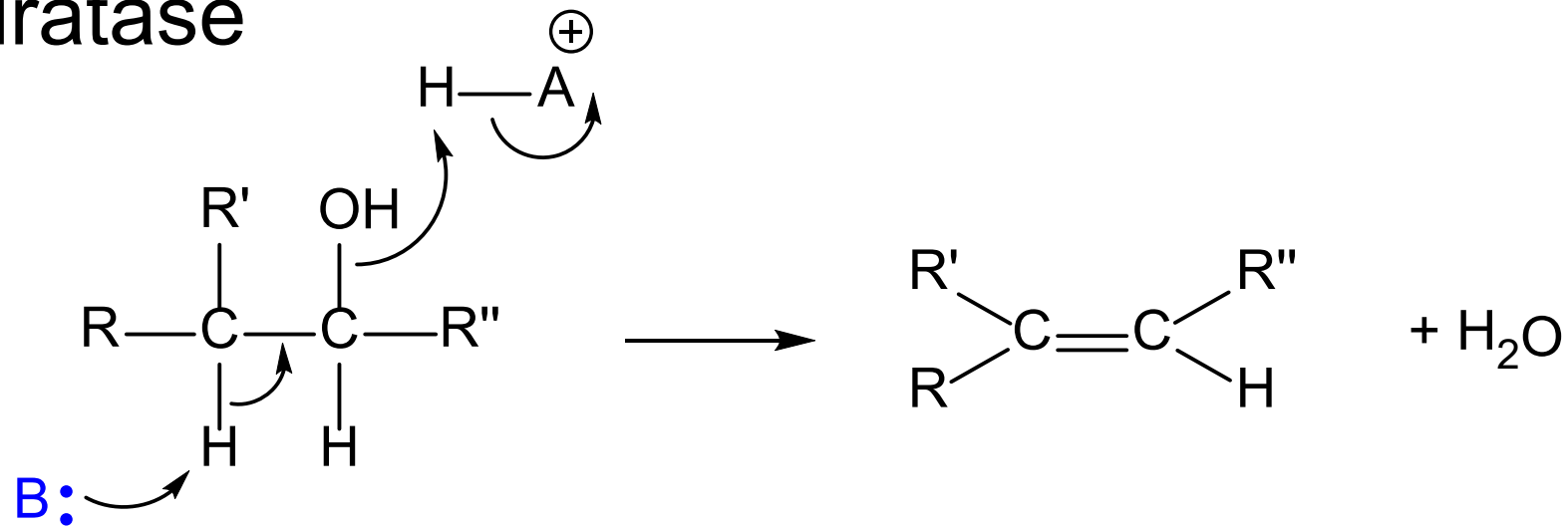


Eliminations/isomerizations/rearrangements

- reversible reactions:
 - elimination is reverse of addition
 - aldo to keto isomerization is reverse of keto to aldo isomerization
 - keep reversibility in mind

Dehydration/hydration

- “dehydratase” if water is eliminated
- draw it backwards and you have a “hydratase”
- Dehydratase followed by hydratase: aconitase
- Ex- aconitase, fumarase, enolase, enoyl CoA hydratase



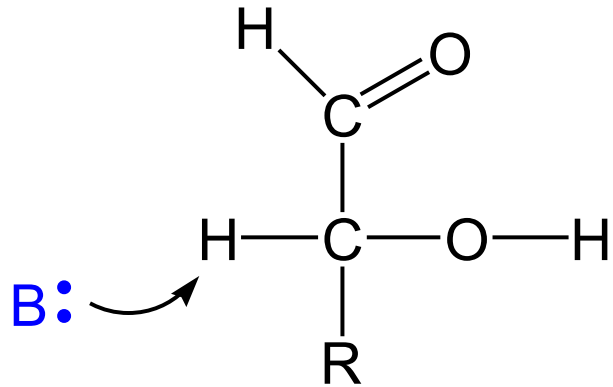
- More complicated: diol dehydratase, and more

Isomerization examples

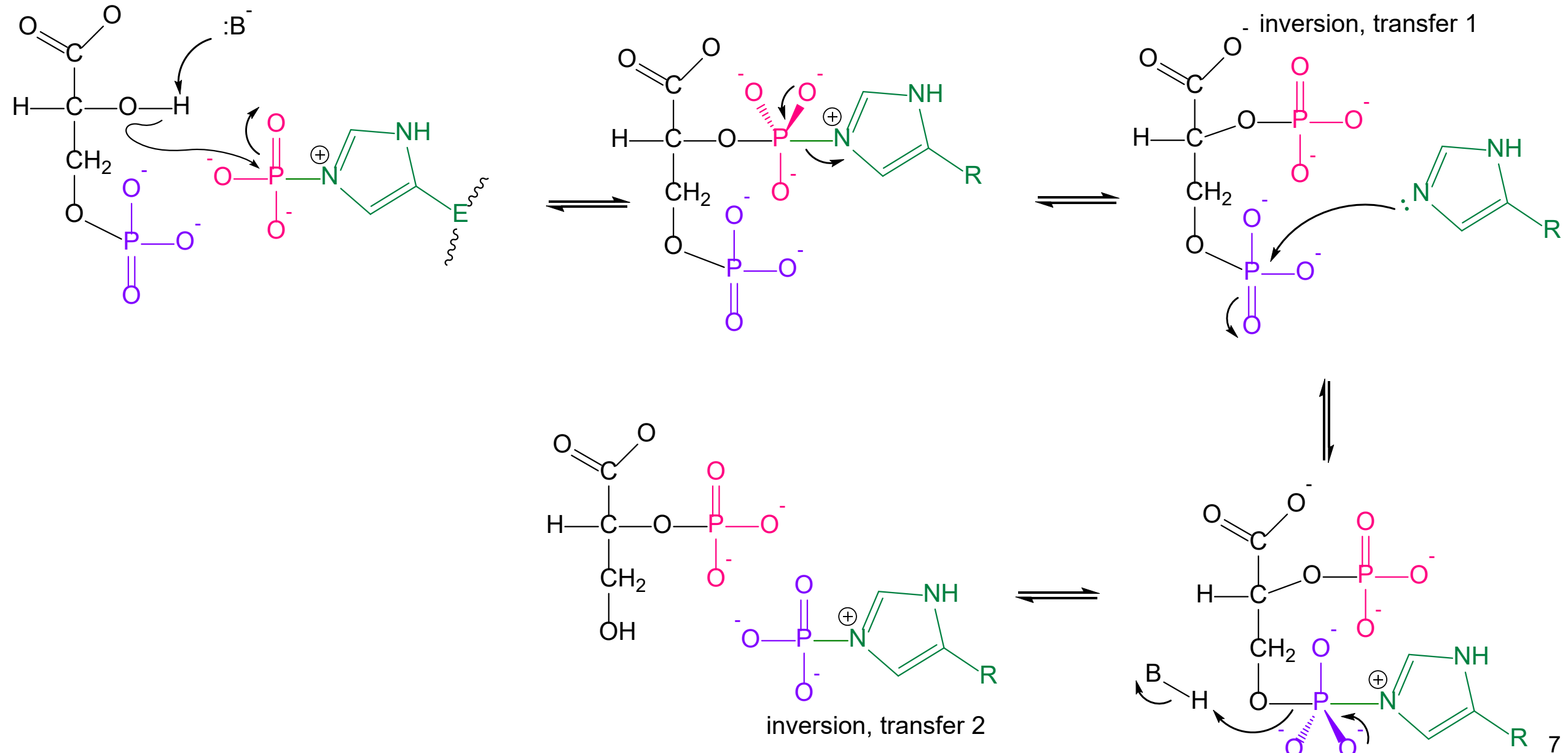
- 1,1-hydrogen shifts (racemase, epimerase)
 - E.g., methylmalonyl-CoA racemase, UDP-glc epimerase
- 1,2-hydrogen shifts: aldo to keto isomerization
 - E.g. Phosphoglucoisomerase, triose phosphate isomerase
- Movement of a phosphate group
 - Phosphoglyceromutase, phosphoglucomutase

Aldo-keto isomerization

- Cis-enediolate intermediate
- reverse would be keto to aldo sugar
- Examples: phosphoglucoisomerase, triosephosphate isomerase



Phosphoglyceromutase

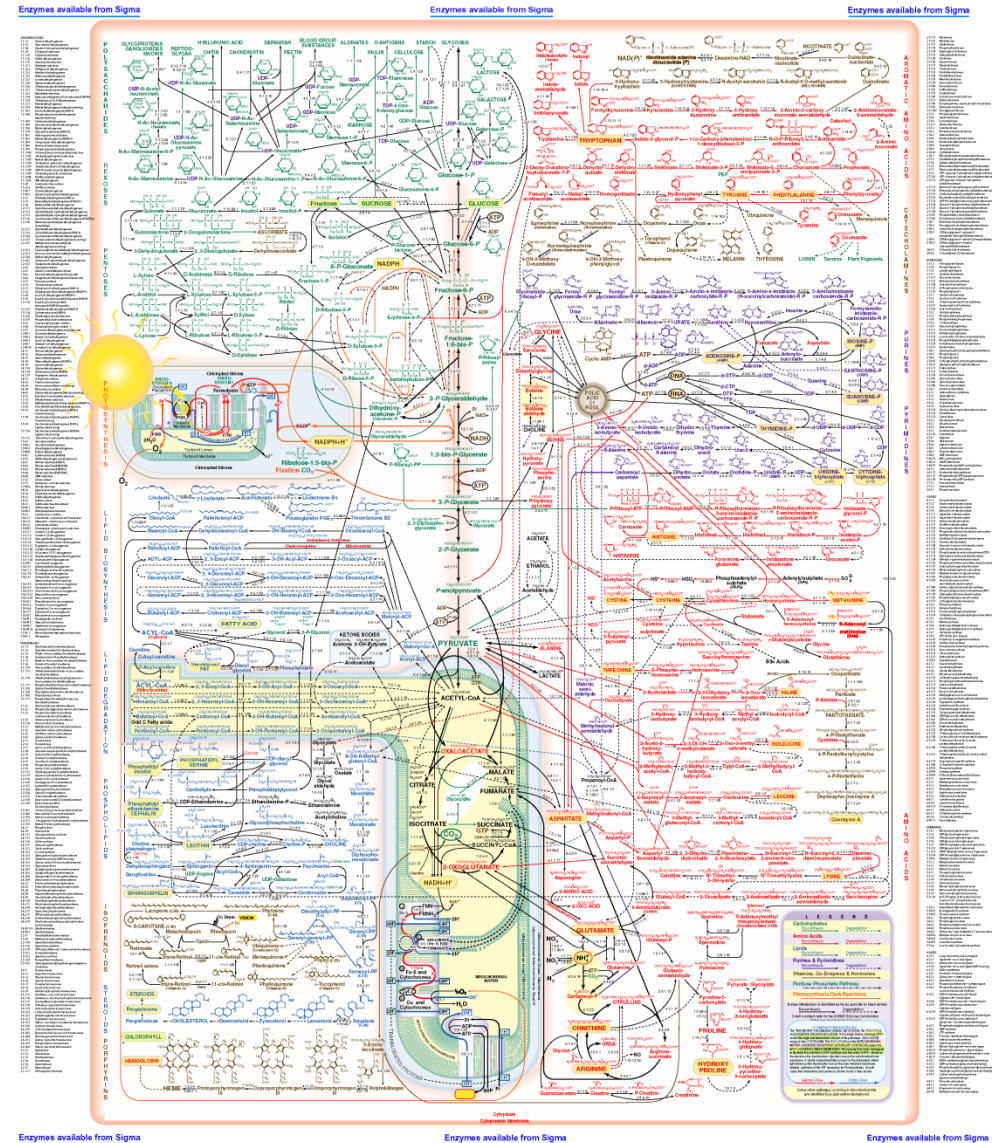


Rearrangement examples

- Alkyl migrations
- Claisen rearrangements
- 1,2-migrations using coenzyme B₁₂

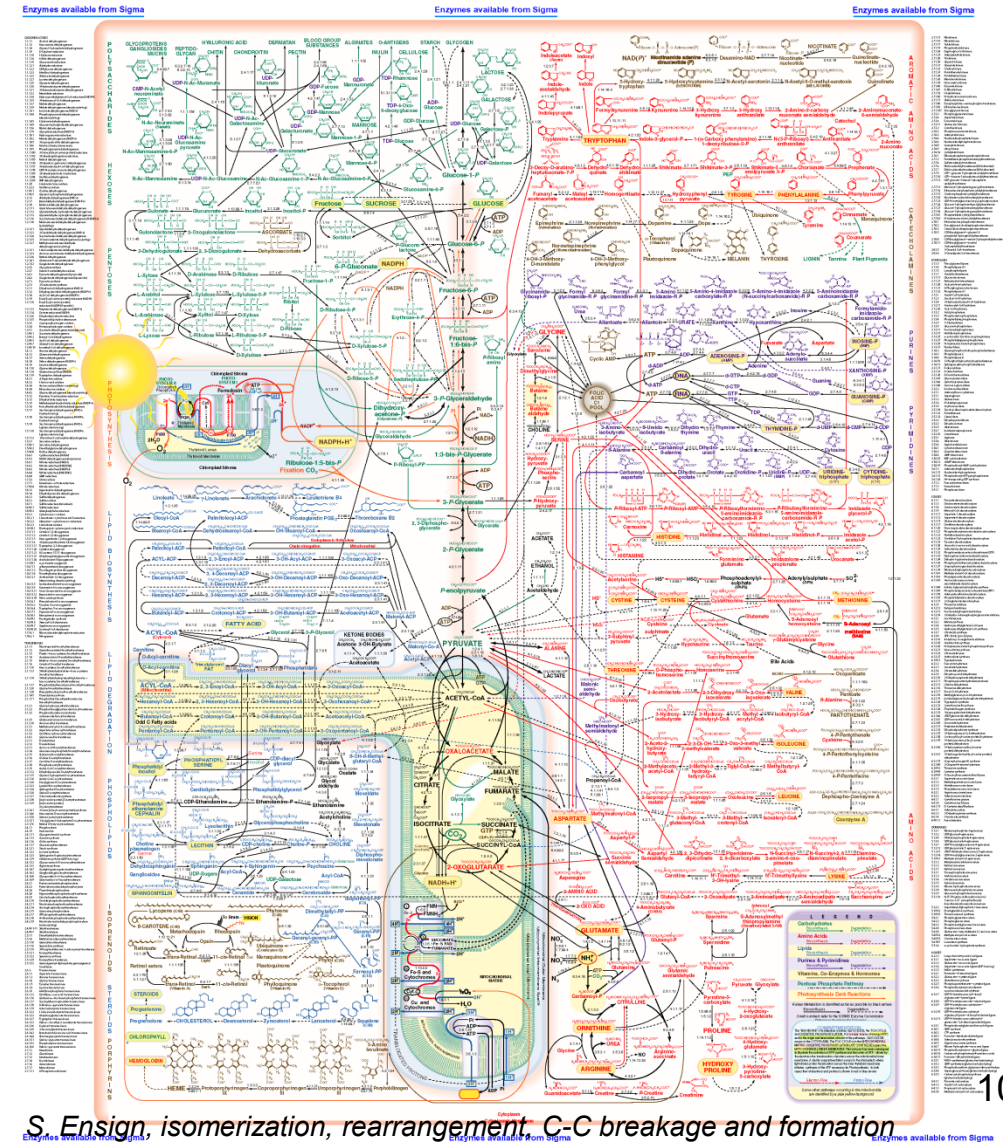
Four categories of enzymes

1. Group transfer reactions
2. Oxidations/reductions
3. Eliminations/isomerizations/
rearrangements
4. Reactions that make or break
C-C bonds



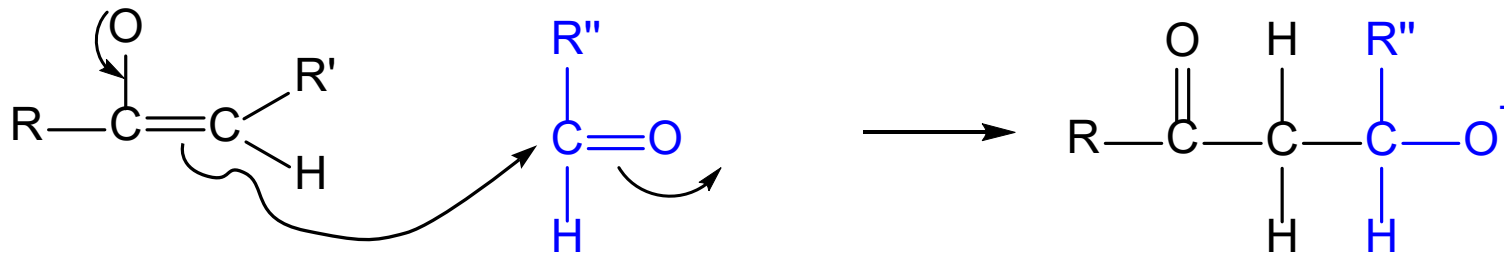
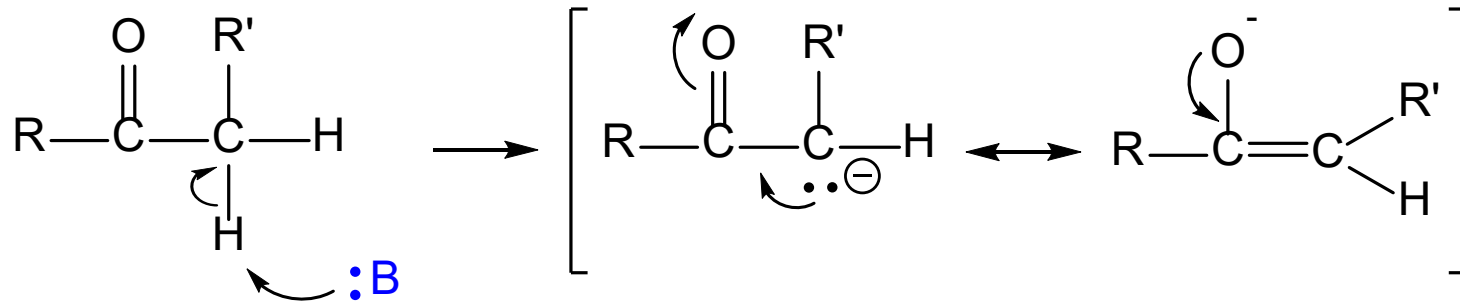
Enzymatic reactions forming or breaking carbon-carbon bonds

- Carboxylation/decarboxylation
- Aldol condensation
- Claisen condensation
- Transketolase
- C1 group transfer
- Prenyl transfer reactions



Aldol condensation

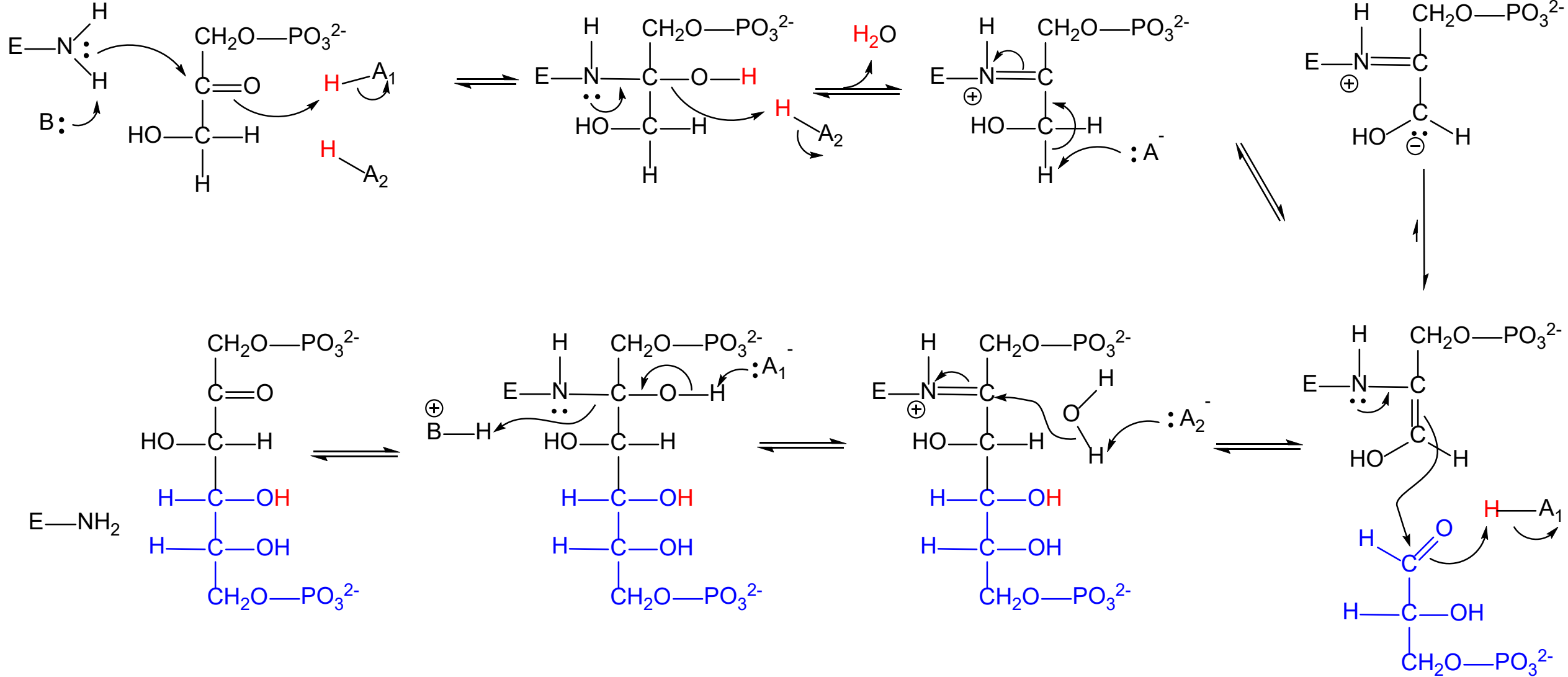
- Aldehyde + ketone or two ketones
- First step: generate resonance-stabilized carbanion on an α -carbon
- Nonenzymatic: requires strong base
- Reaction is freely reversible (aldol cleavage)



Enzymatic Aldol reactions

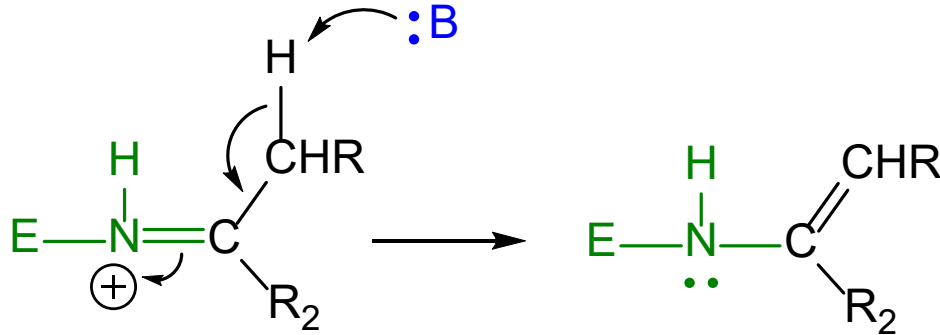
- Aldolase (glycolysis), transaldolase (pentose pathway)
 - Type I aldolase: Schiff base stabilization of enolate
 - Plants, animals, some algae and bacteria
 - Type II aldolase: Zn^{2+} stabilization of enolate
 - Fungi, some algae and bacteria
- Draw mechanisms in forward and reverse directions

Type I aldolase

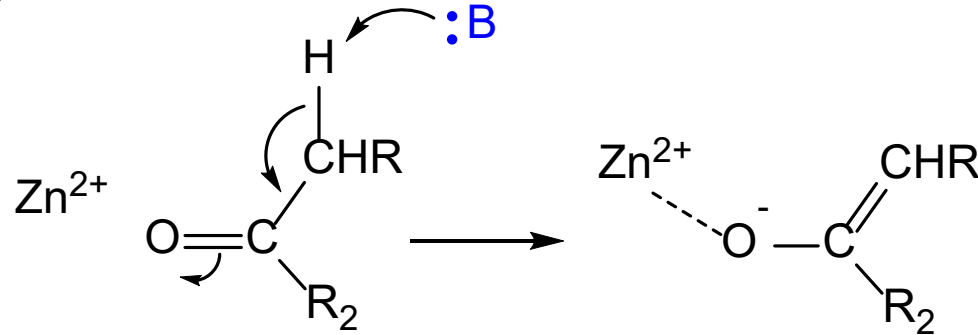


Type I and II aldolases

- Type I: Schiff base stabilization



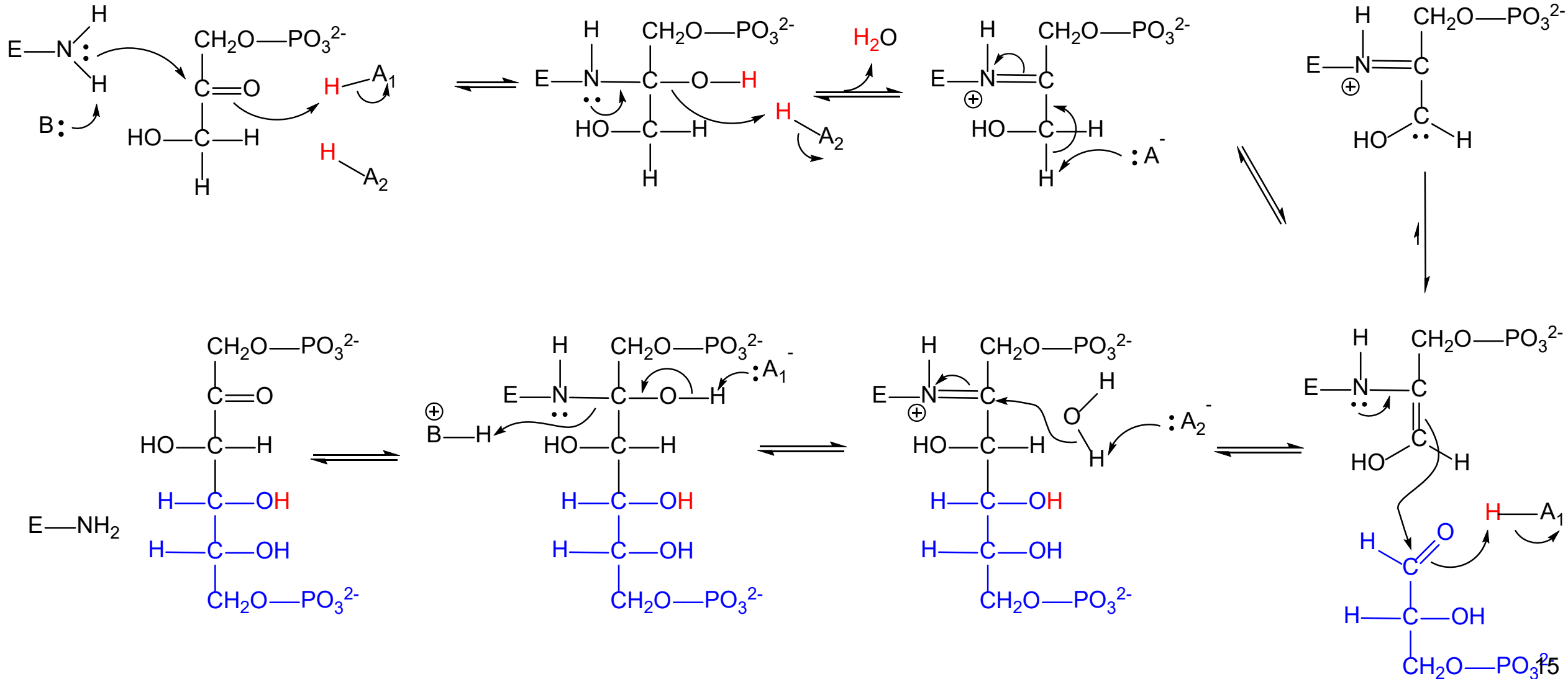
- Type II: metal ion stabilization



- How distinguish the mechanisms?

Distinguishing type I and II aldolases

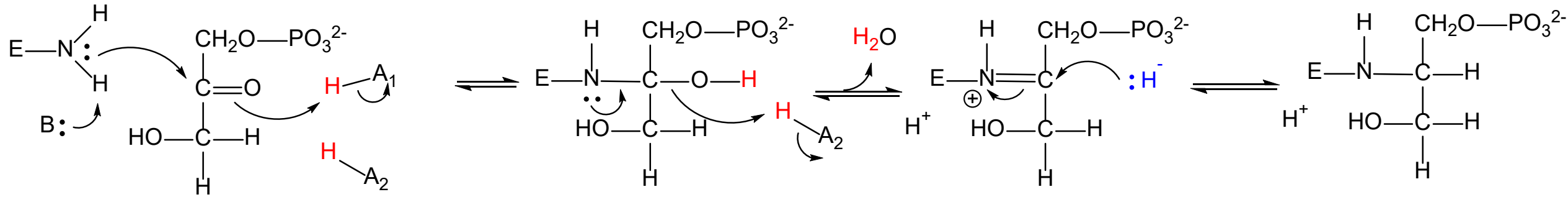
- ^{18}O exchange?
- NaBH_4 inactivation?



Distinguishing type I and II aldolases

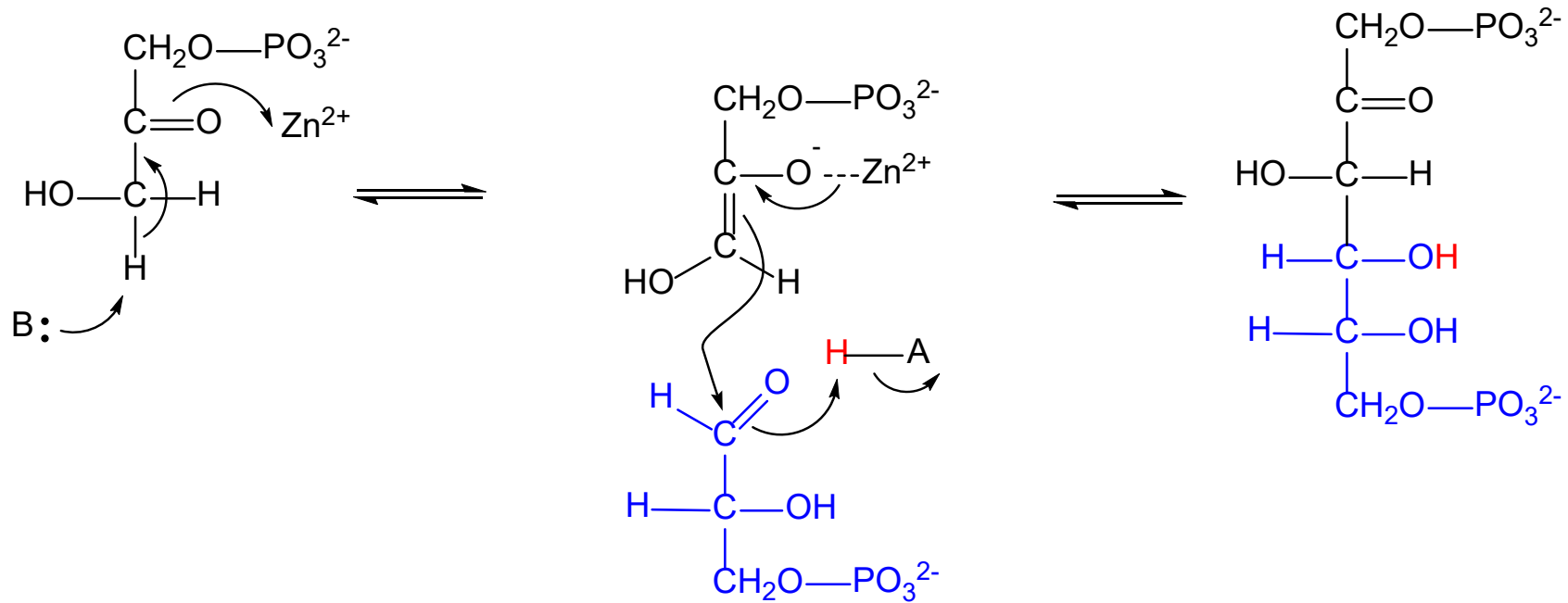
^{18}O exchange?

NaBH_4 inactivation?



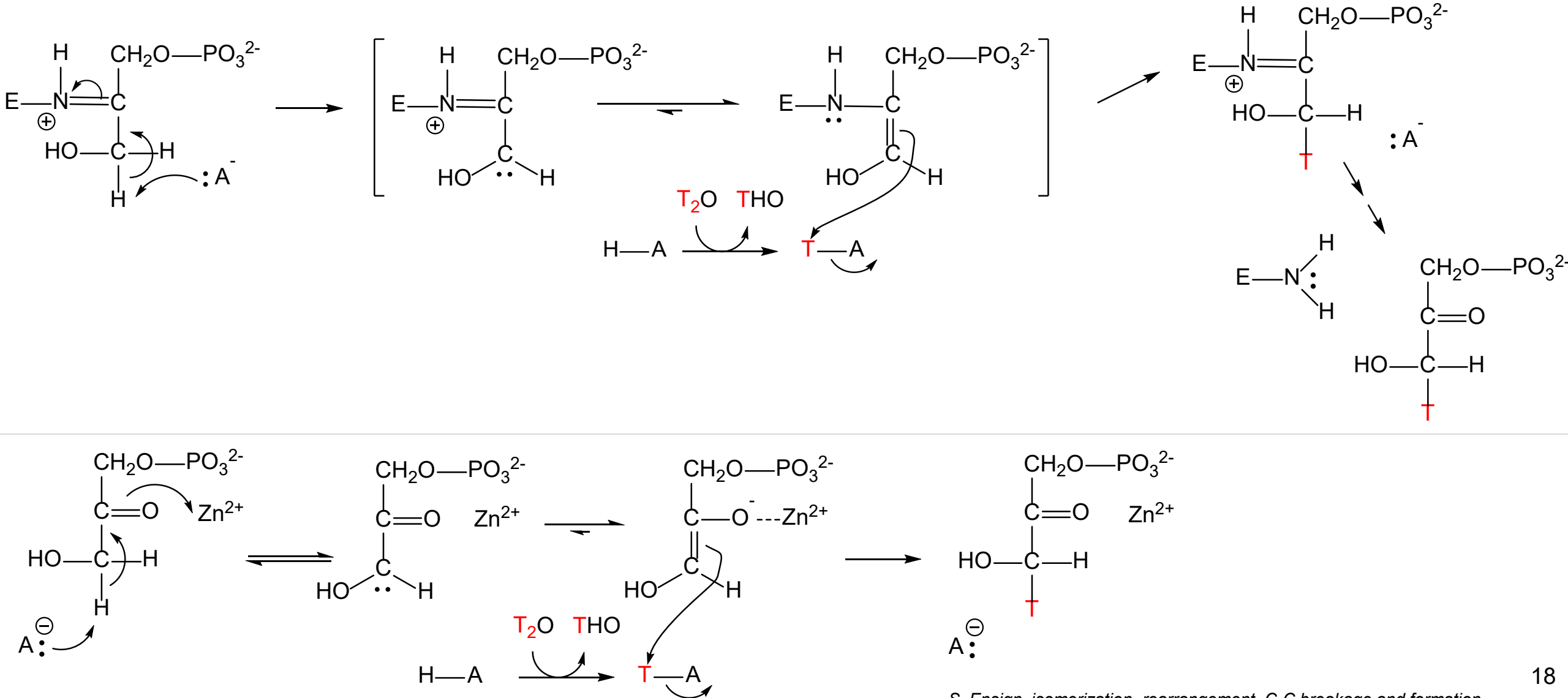
Distinguishing type I and II aldolases

- ^{18}O exchange?
- NaBH_4 inactivation?



Both type I and 2 aldolase mechanisms involve a carbanion intermediate

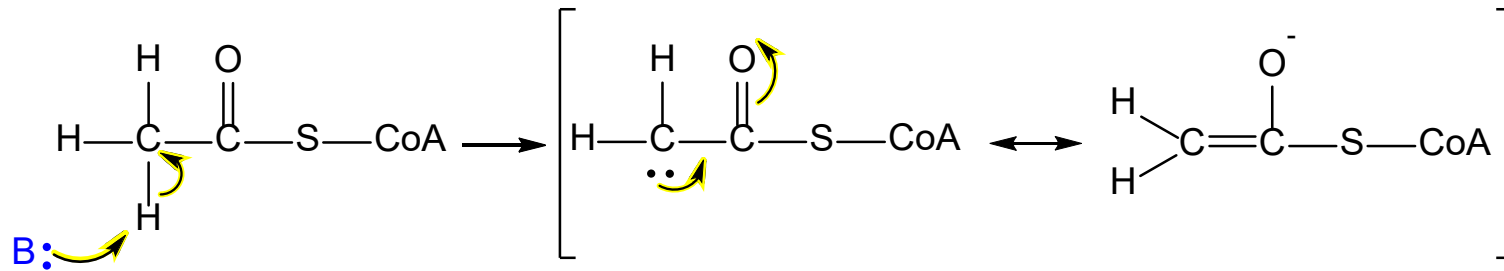
Evidence: carry out reaction in absence of second substrate, in presence of T_2O or D_2O



How do you interpret a negative result (e.g.,
lack of ^3H exchange)?

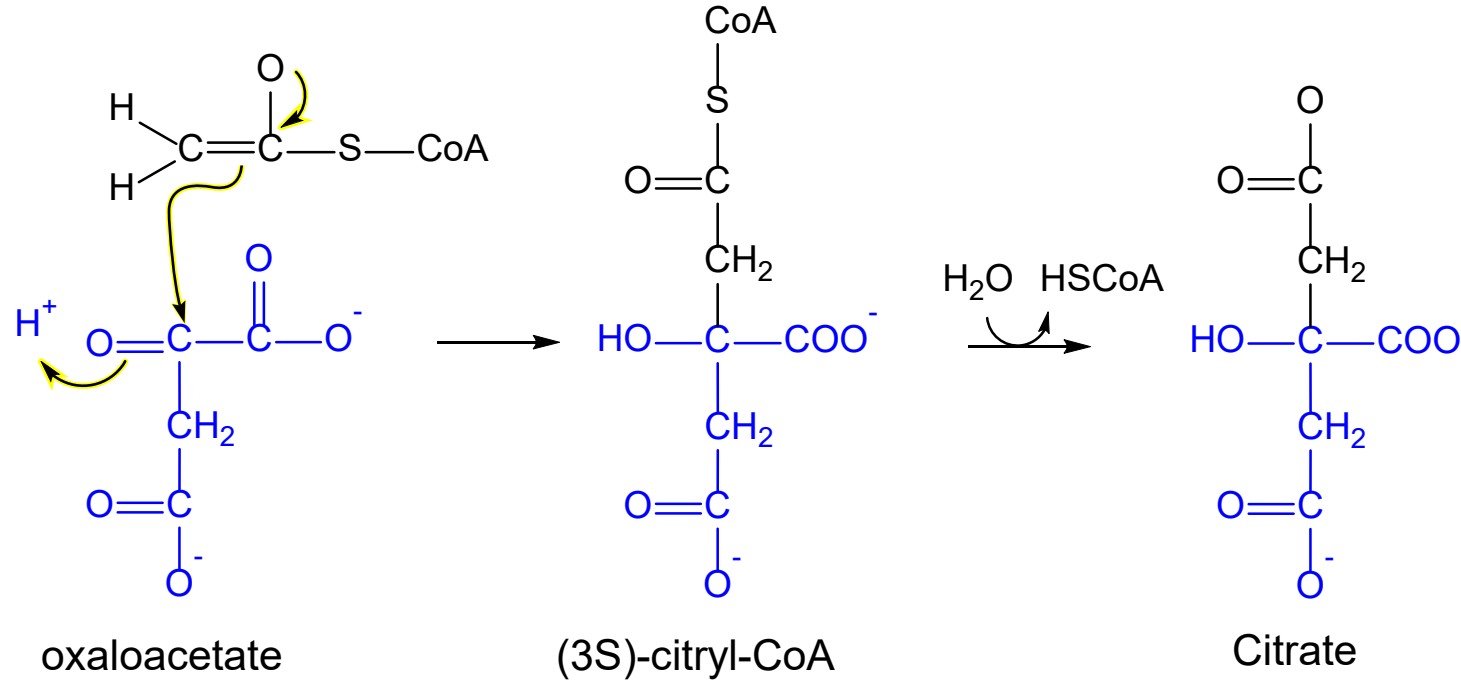
Claisen condensation/cleavage

- Ester + ester, or ester + carbonyl compound (ketone)
- First step: generate resonance-stabilized carbanion



Claisen condensation/cleavage

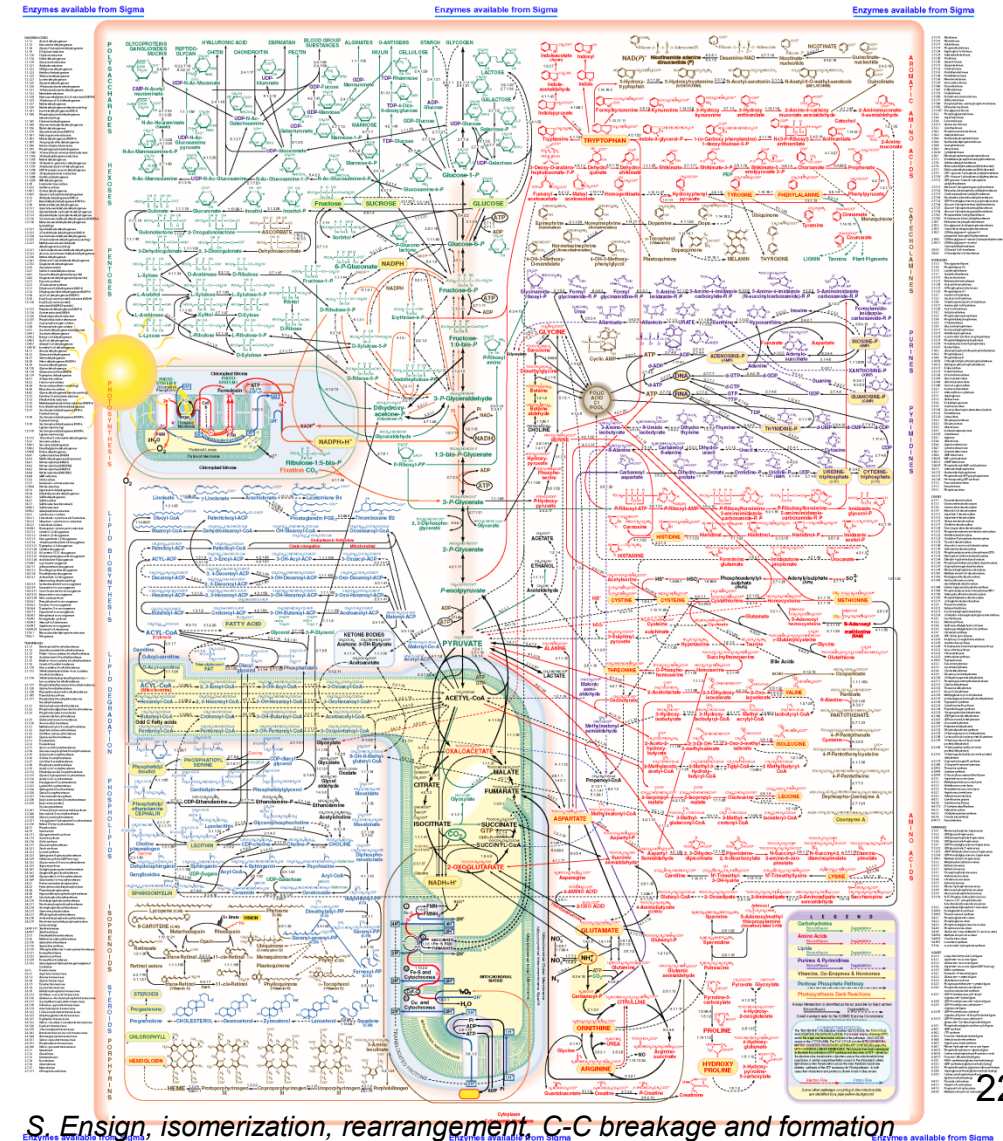
- Second step: attack of the resonance-stabilized carbanion on the electrophilic carbonyl C of OAA



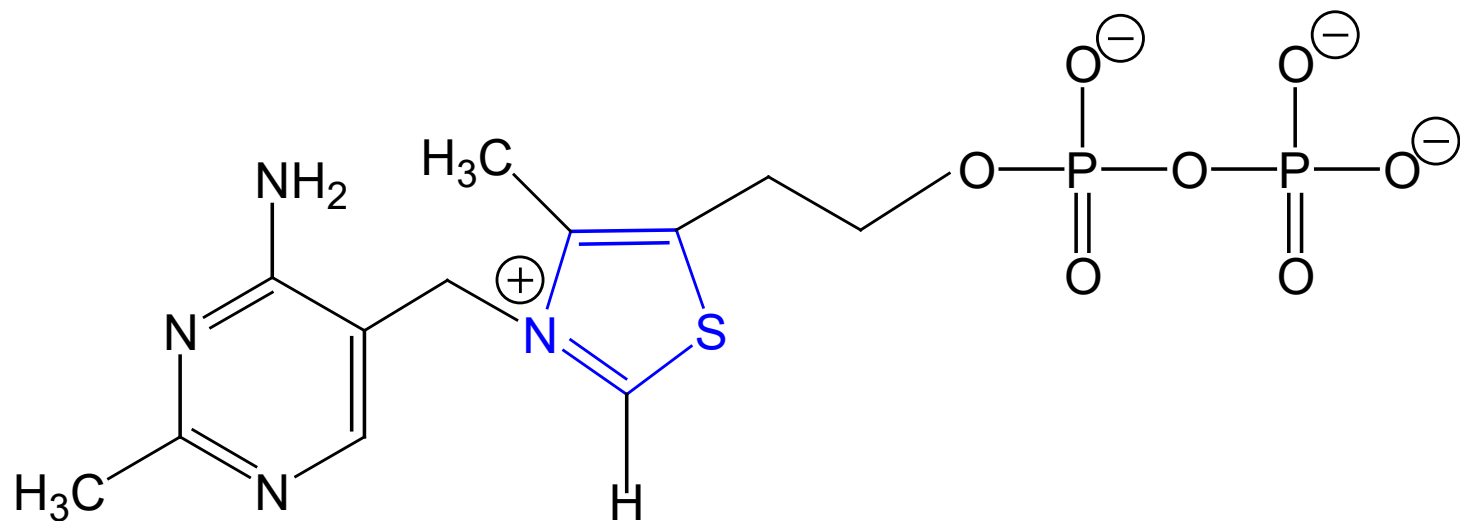
Enzymatic reactions forming or breaking carbon-carbon bonds

- Carboxylation/decarboxylation
- Aldol condensation
- Claisen condensation
- Transketolase
- C1 group transfer
- Prenyl transfer reactions

Some important coenzymes: TPP, biotin, PLP, lipoic acid, cobalamin, THF, CoA



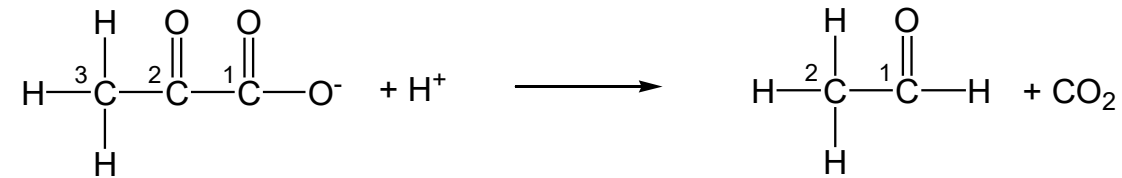
Thiamine pyrophosphate



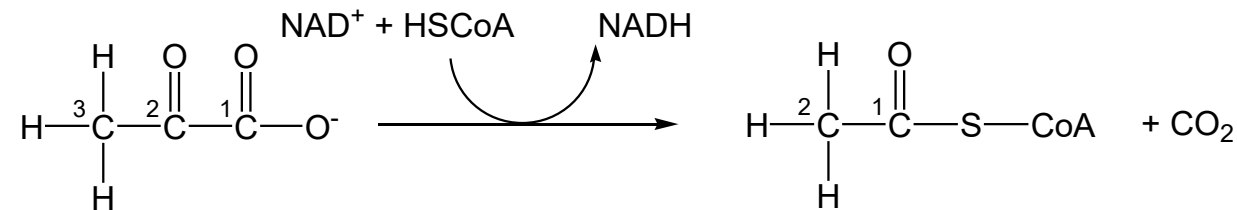
Scope of reactions

- Decarboxylations

- Nonoxidative



- Oxidative



- α -keto transfer reactions (transketolation)

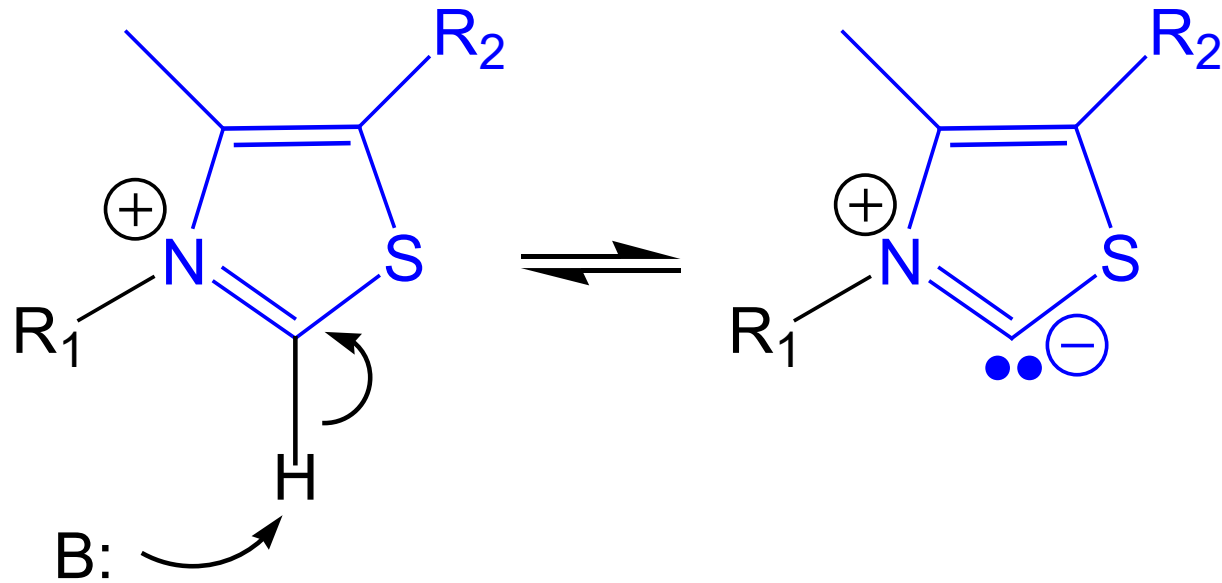
- Ketose donor + aldose acceptor $\rightarrow$ aldose + ketose

- e.g. C6 + C3 $\rightarrow$ C4 + C5

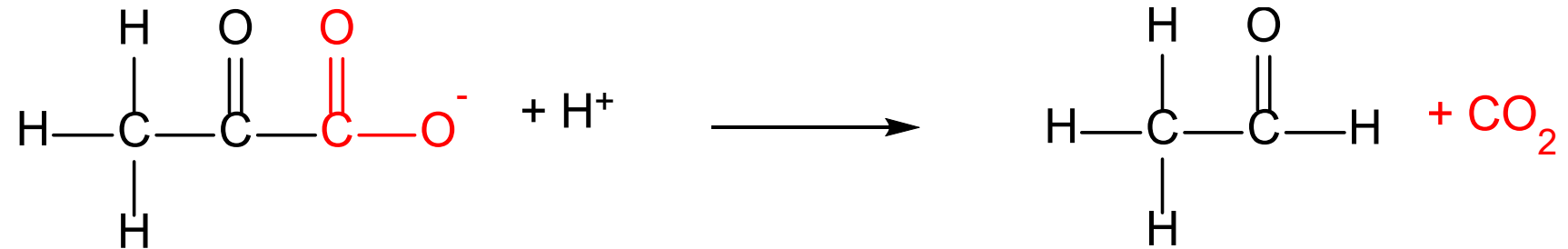
Enzymatic function of TPP

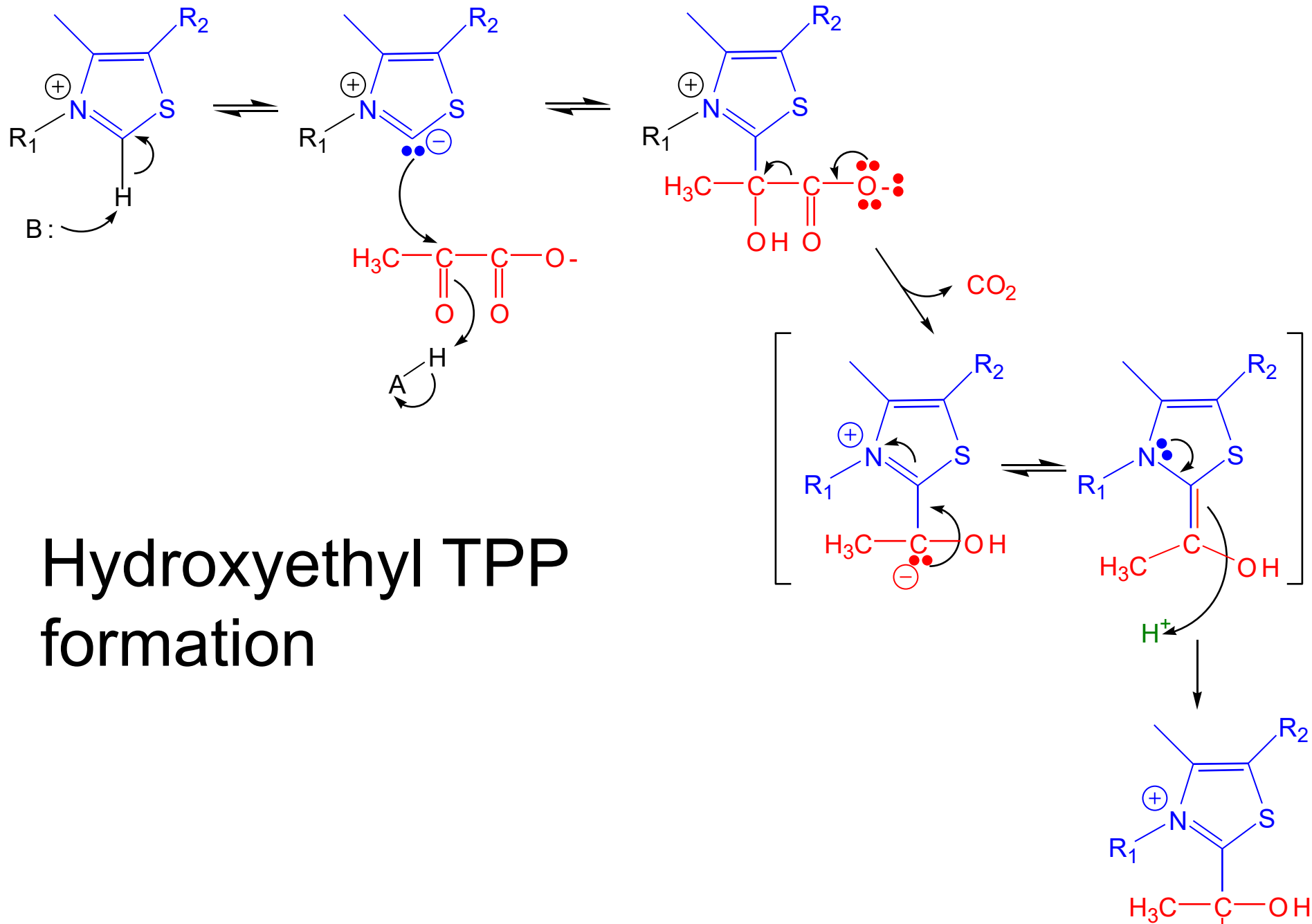
- C-C bond cleavage in substrate-TPP adduct to form a stabilized carbanion
- Condensation of carbanion with electrophilic acceptor
 - nonoxidative: H^+ , carbonyl carbon of acceptor molecule (aldehyde)
 - oxidative: oxidized lipoamide

Formation of TPP carbanion



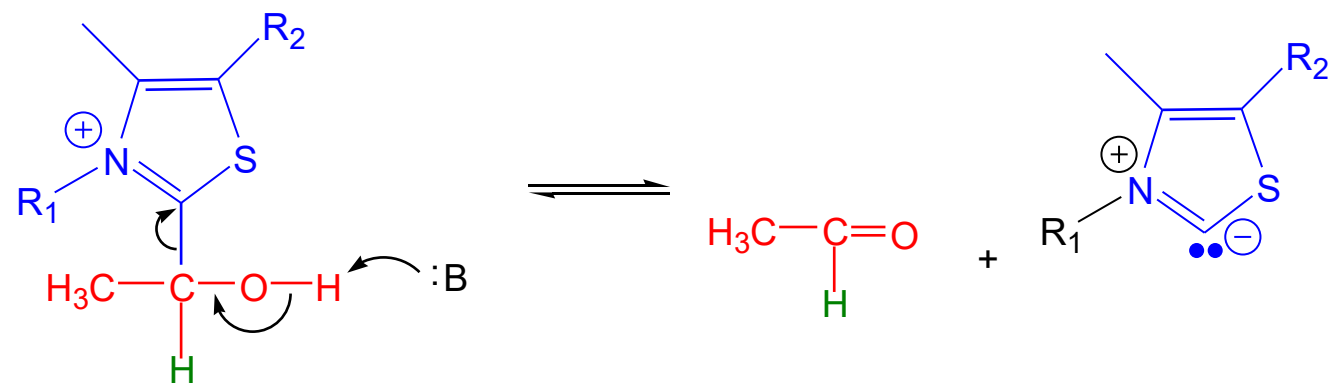
Nonoxidative decarboxylation: pyruvate decarboxylase



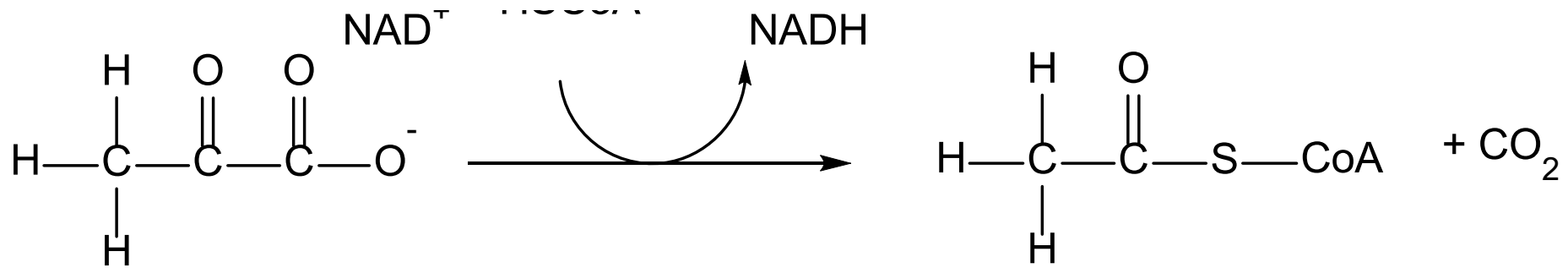


Hydroxyethyl TPP formation

HETPP breakdown to form acetaldehyde



Oxidative decarboxylation: pyruvate dehydrogenase, α -ketoglutarate dehydrogenase



Pyruvate dehydrogenase complex

Cofactor	Vitamin precursor	Function	Bound or dissociable?
Thiamine pyrophosphate	Thiamin	Decarboxylation	Bound, E1
Lipoic acid (lipoamide)		e ⁻ and acyl carrier	Bound, E2
FAD	Riboflavin	e ⁻ carrier	Bound, E3
NAD ⁺	Niacin	e ⁻ transfer	Diss. E3
Coenzyme A	pantothenate	Acyl carrier	Diss. E2

Pyruvate dehydrogenase

- A multienzyme complex consisting of three enzymes that work in unison
- E_1 : pyruvate dehydrogenase
- E_2 : dihydrolipoyl transacetylase
- E_3 : dihydrolipoyl dehydrogenase

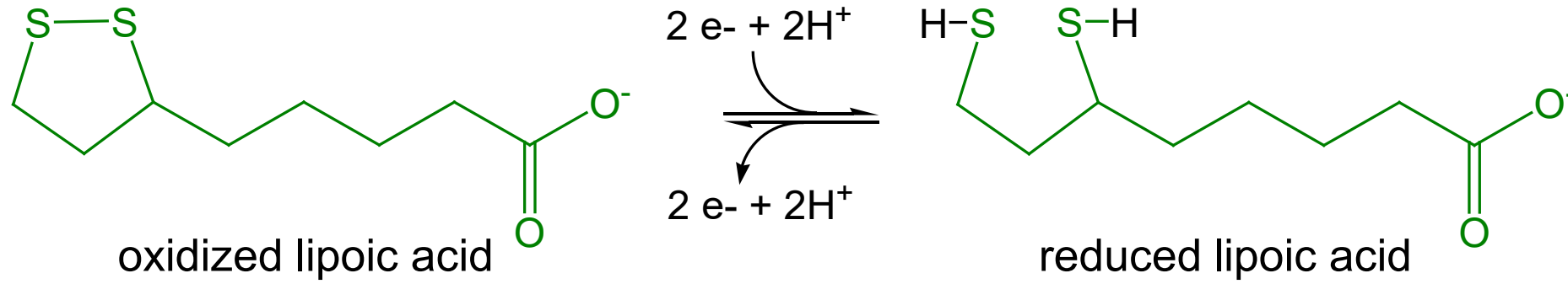
Pyruvate dehydrogenase cofactors

Cofactor	Vitamin precursor	Function	Bound or dissociable?
Thiamine pyrophosphate	Thiamin	Decarboxylation	Bound, E ₁
Lipoic acid (lipoamide)		e ⁻ and acyl carrier	Bound, E ₂
FAD	Riboflavin	e ⁻ carrier	Bound, E ₃
NAD ⁺	Niacin	e ⁻ transfer	Diss. E ₃
Coenzyme A	pantothenate	Acyl carrier	Diss. E ₂

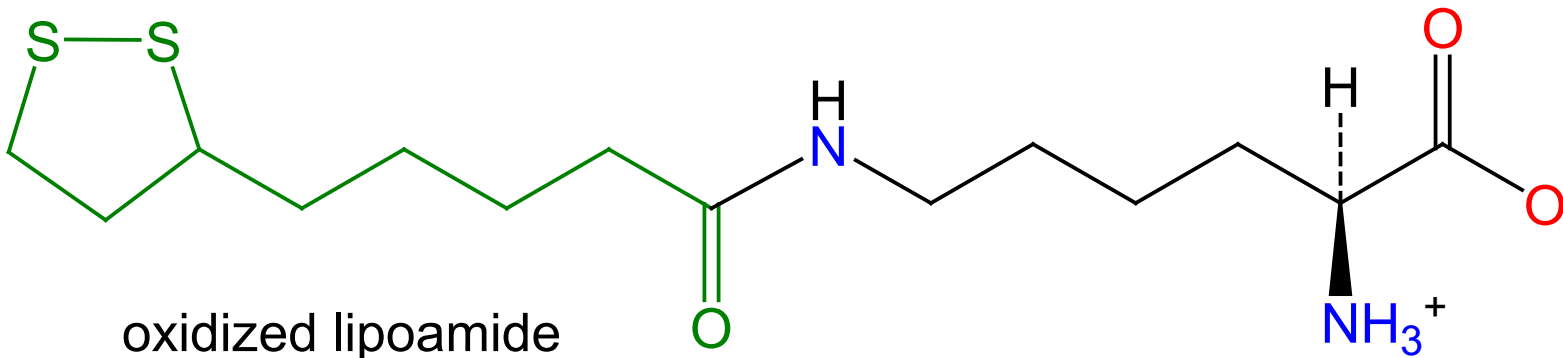
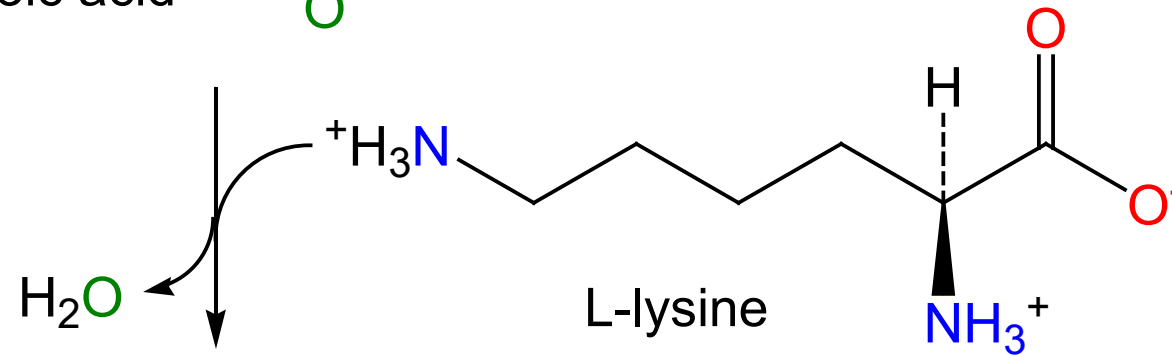
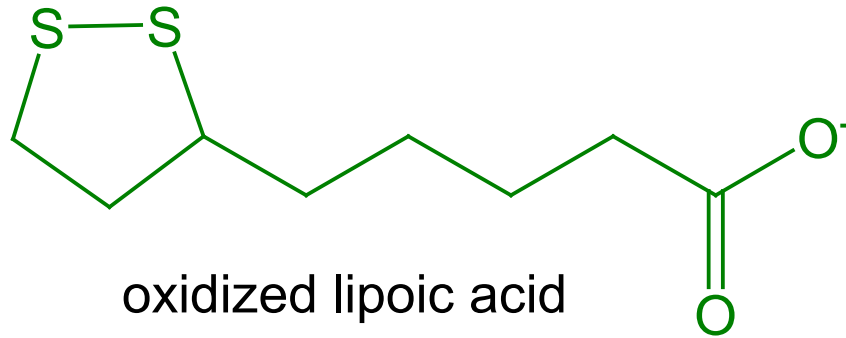
Introduction to the cofactors of the pyruvate dehydrogenase complex

- Tightly bound (catalytic):
 - TPP (noncovalent)
 - Lipoamide (covalent)
 - FAD (covalent in some enzymes, noncovalent in others)
- Substrates in the reaction (get transformed):
 - NAD^+
 - Coenzyme A

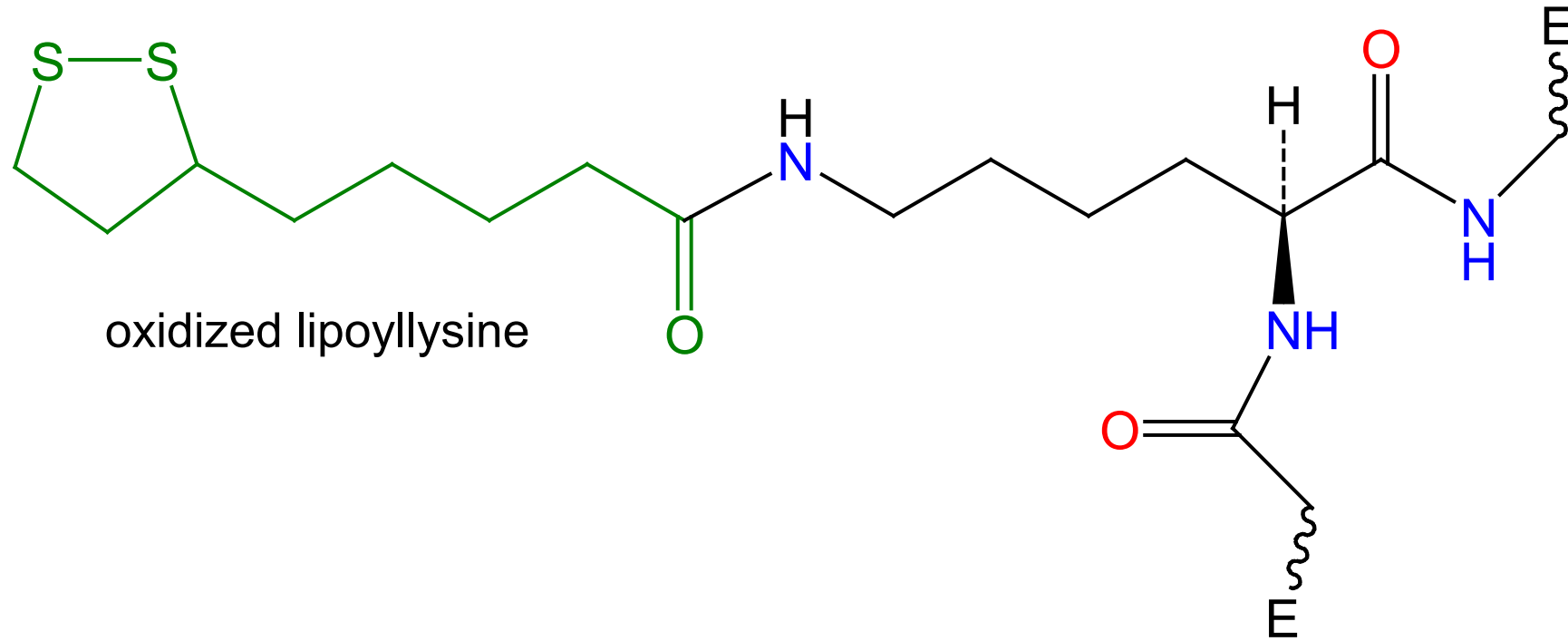
Lipoic acid



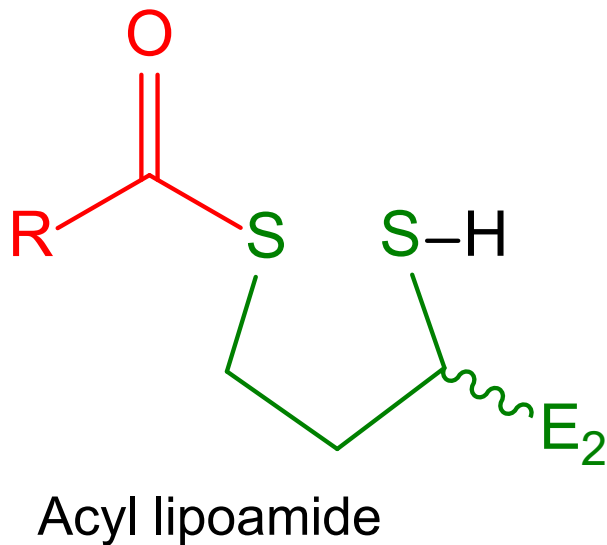
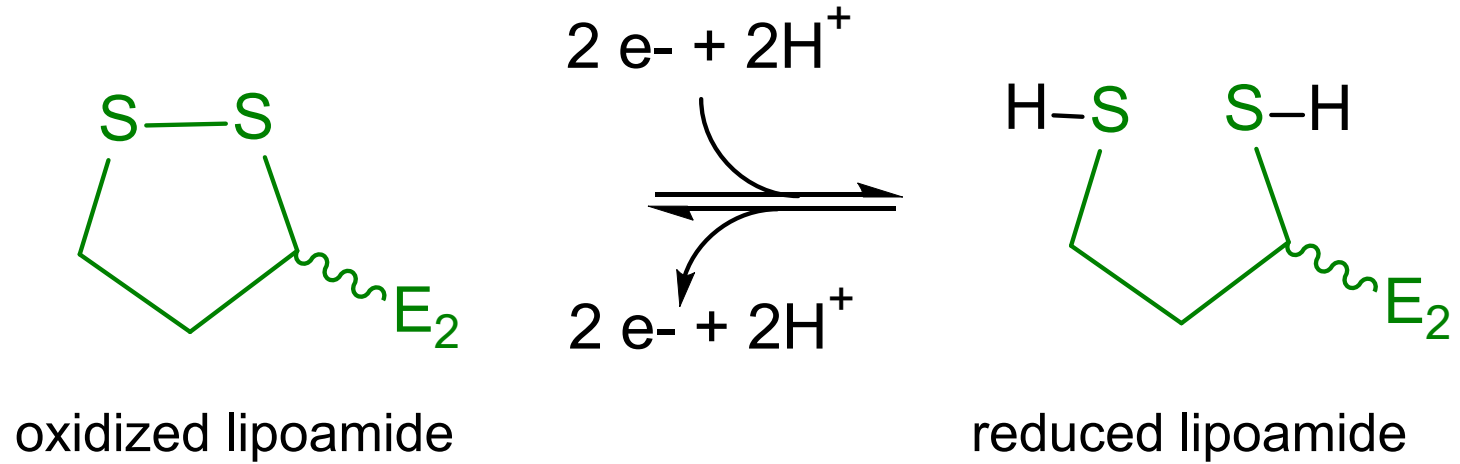
Lipoamide formation by reaction with the ϵ -amino group of lysine



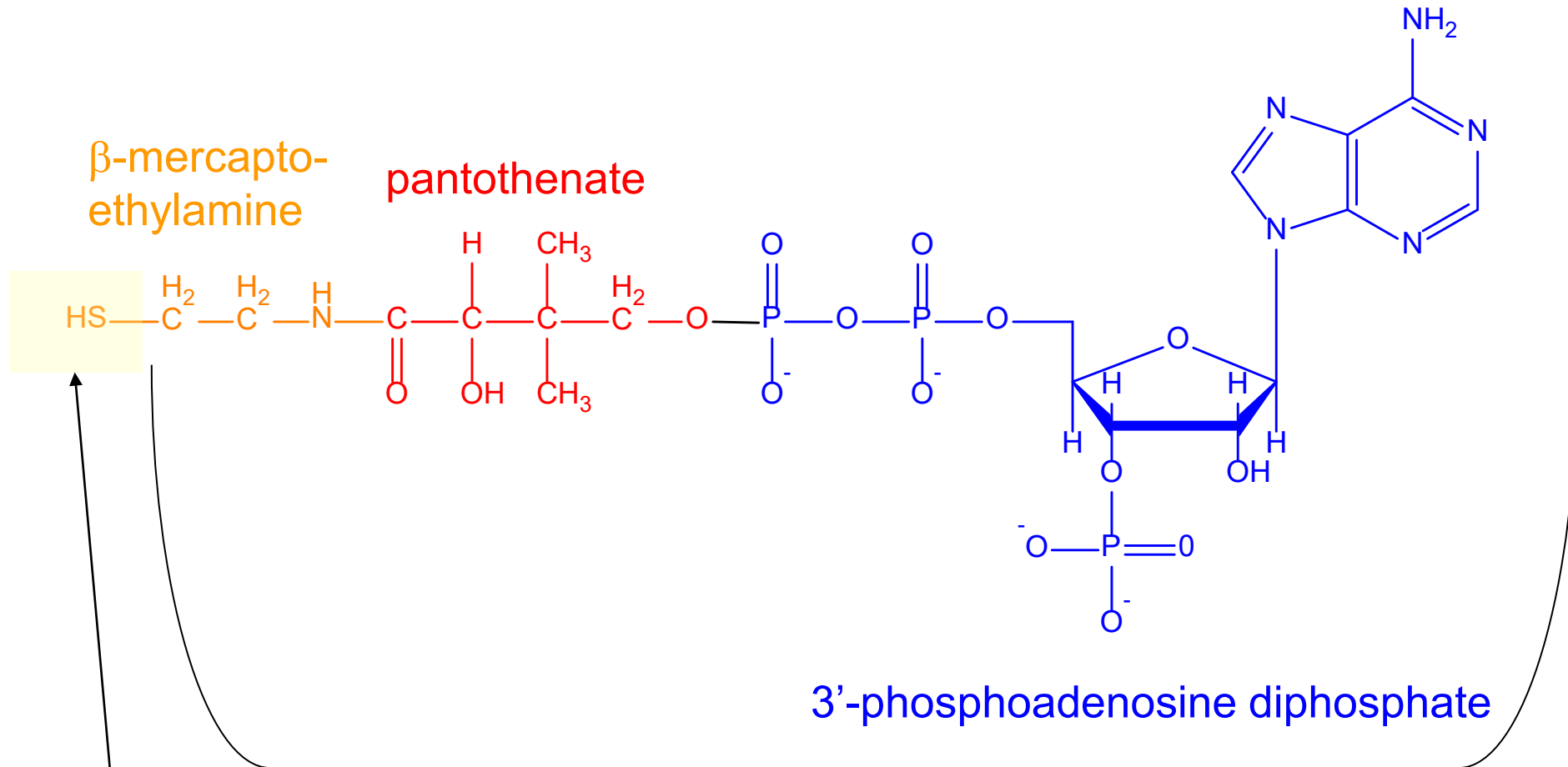
Lipoamide in a polypeptide



Biologically important forms of lipoamide



Coenzyme A, an acyl group carrier



Abbreviate all this as “CoA”

Reactive thiol group

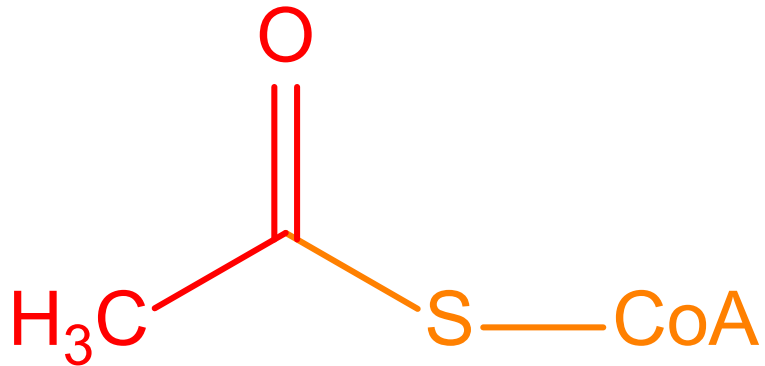
Coenzyme A, an acyl group carrier



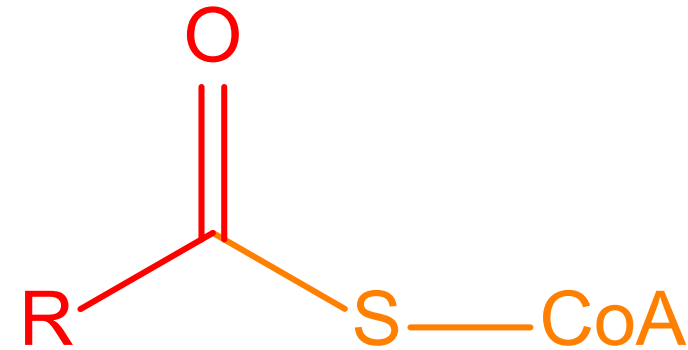
Coenzyme A



Coenzyme A thiolate

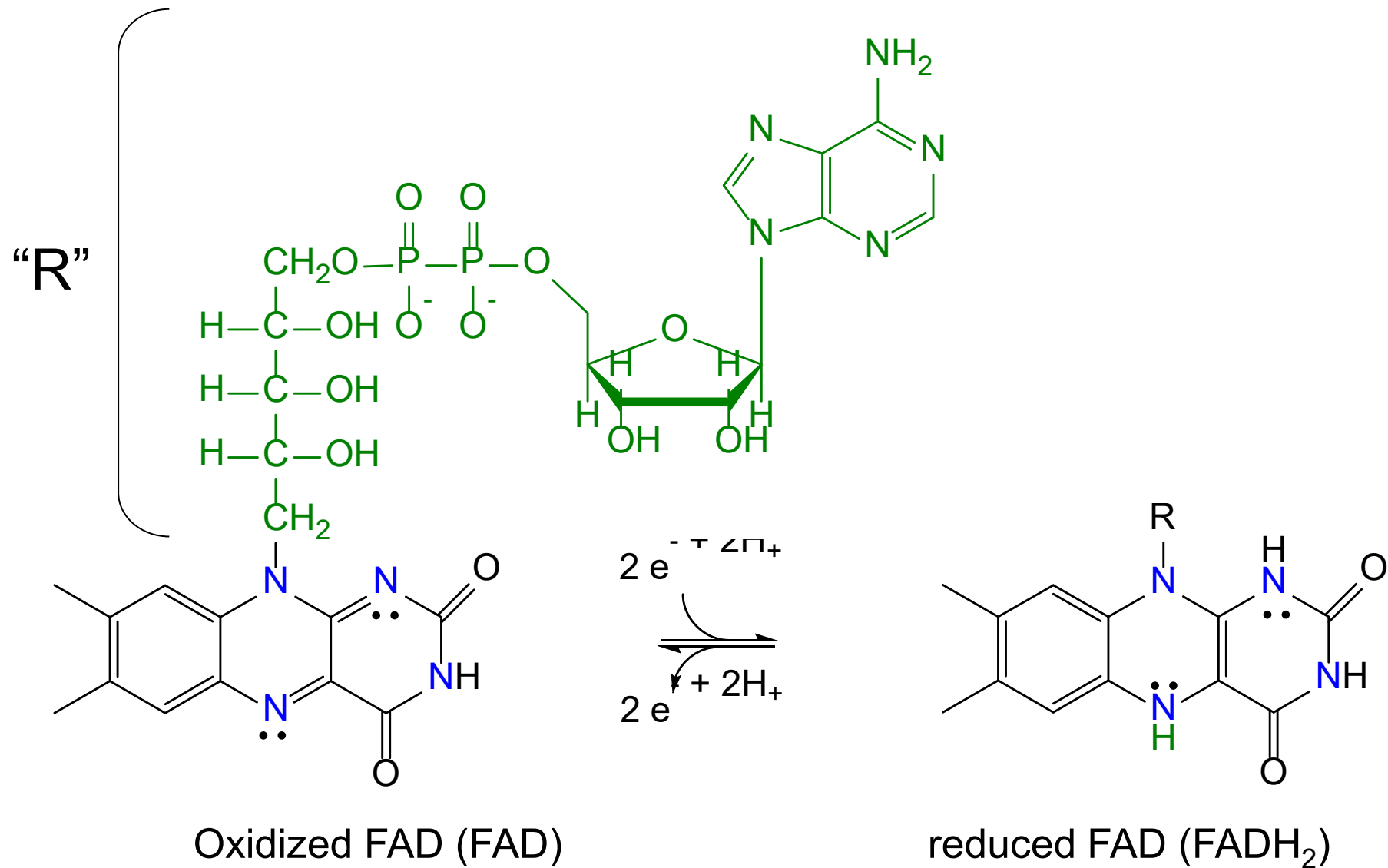


Acetyl-coenzyme A

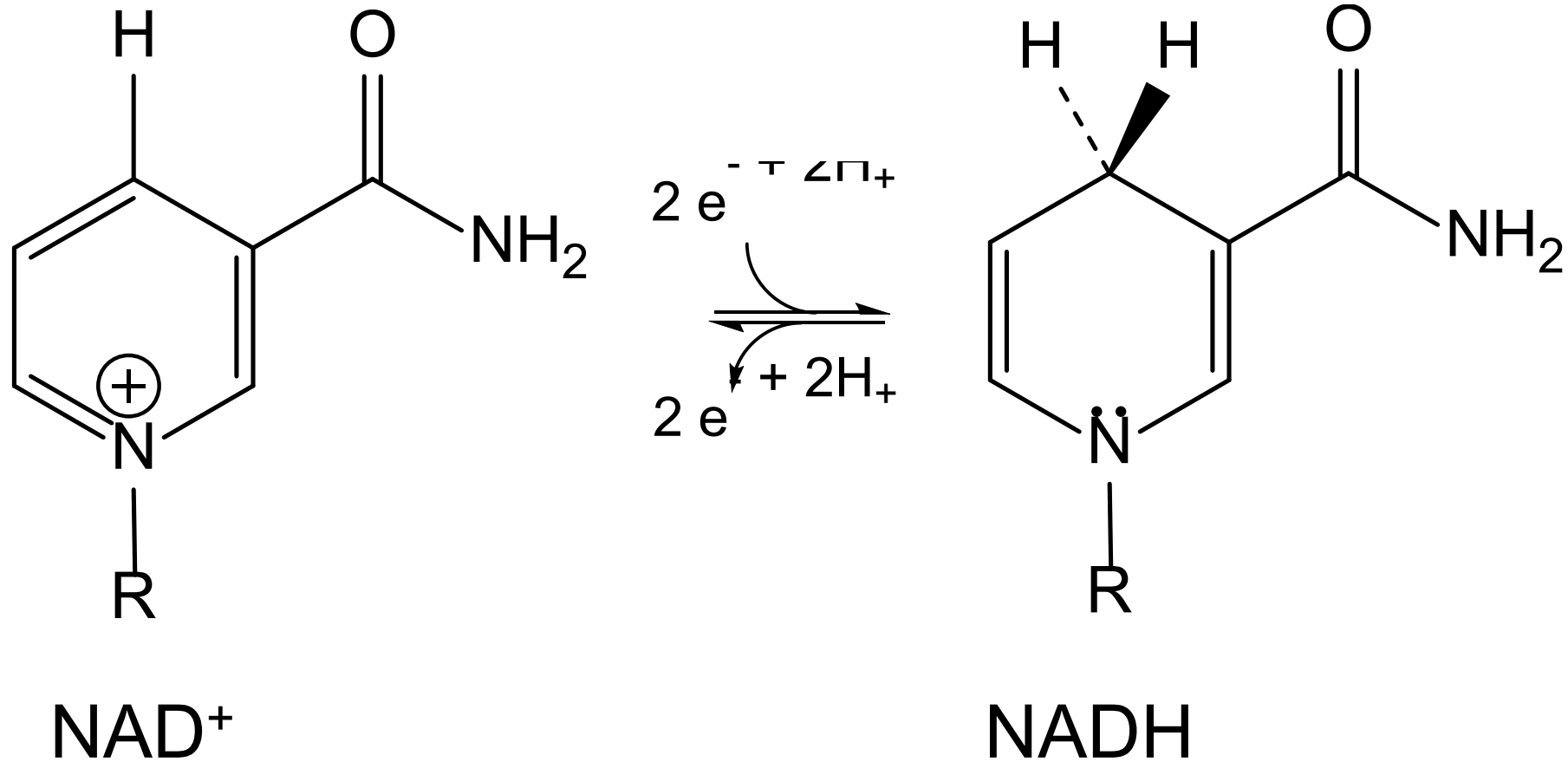


Acyl-coenzyme A

Flavin adenine dinucleotide

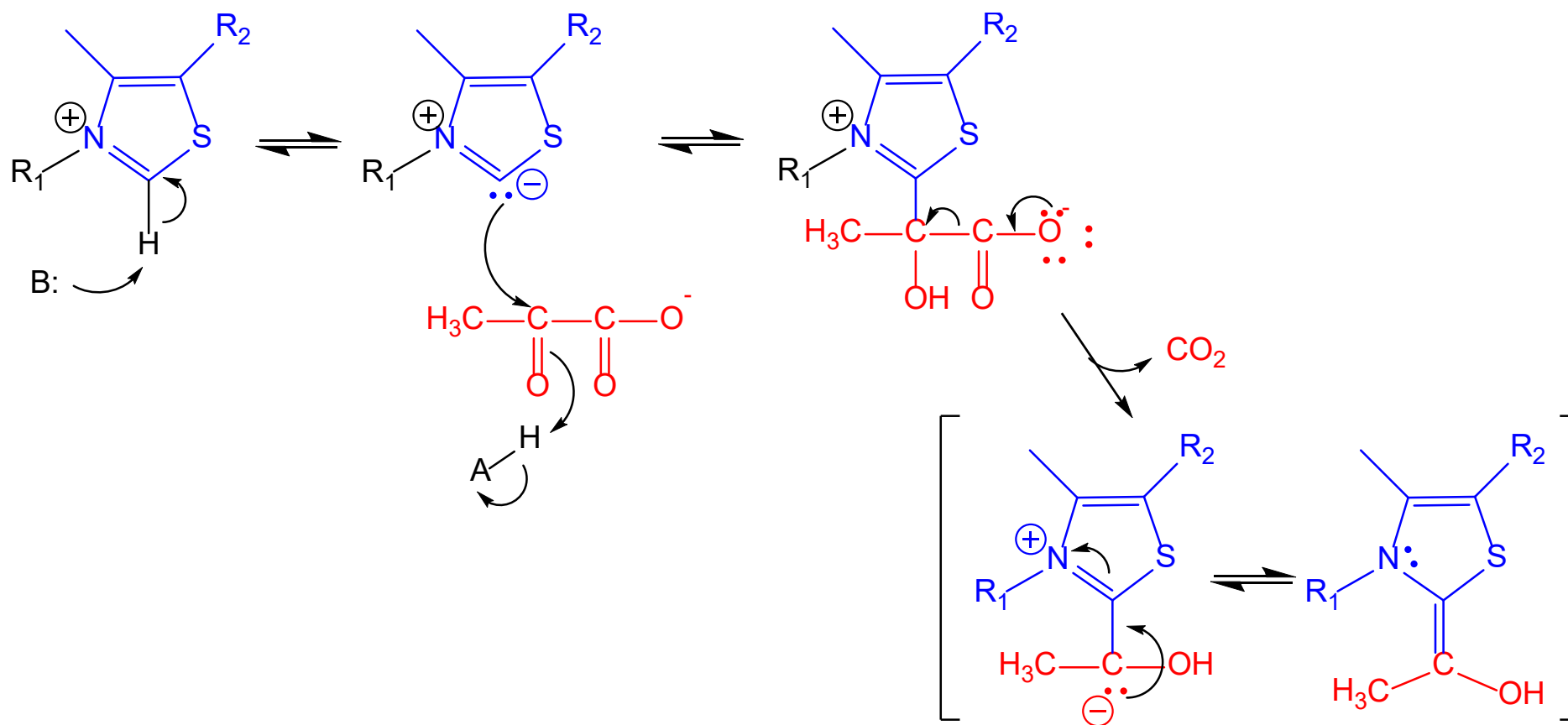


Nicotinamide adenine dinucleotide



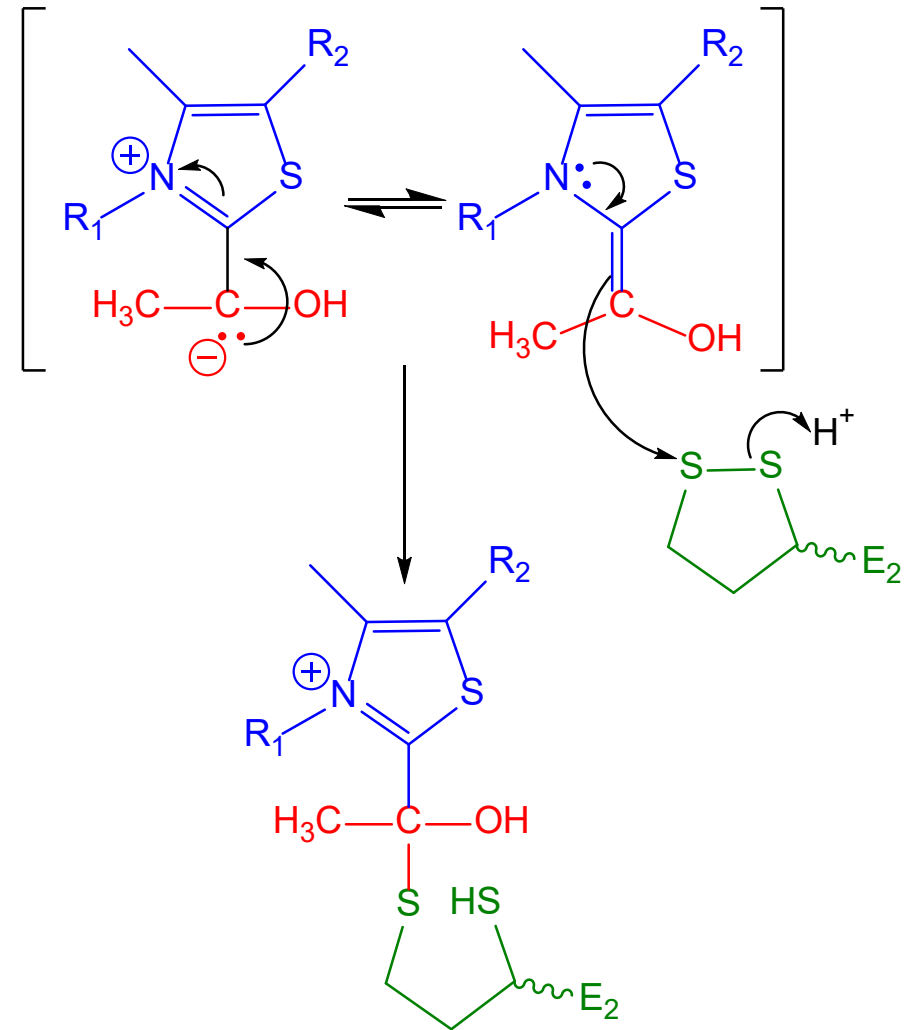
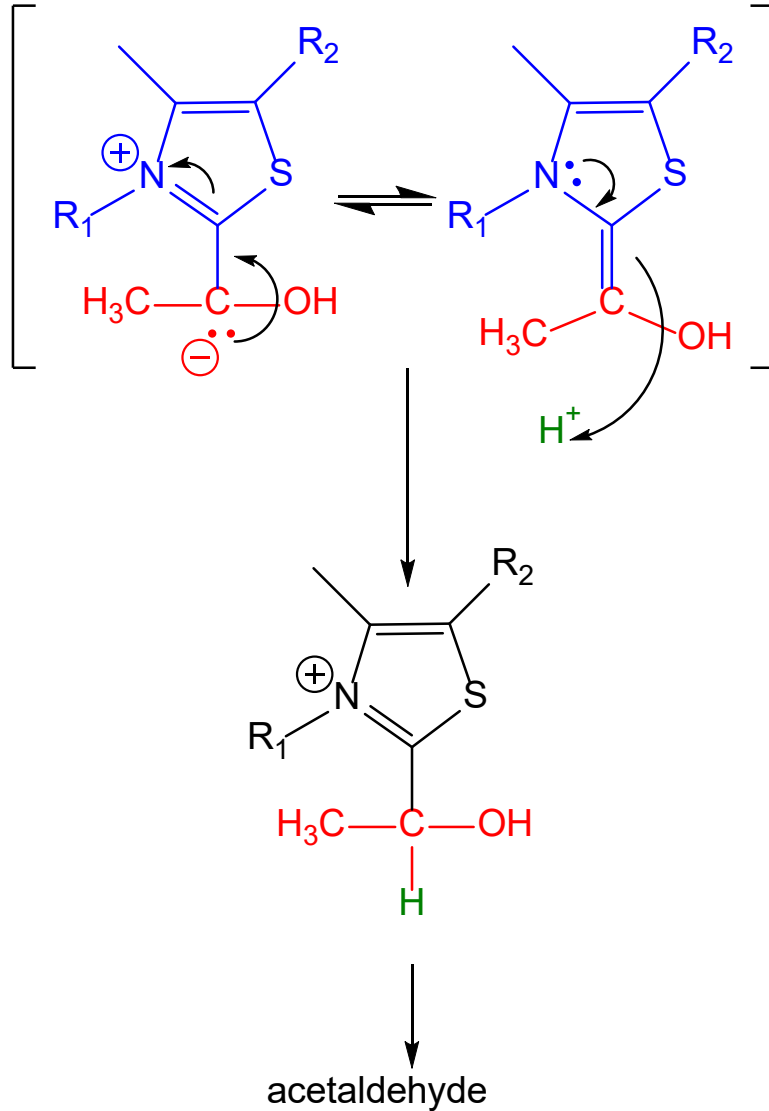
Pyruvate dehydrogenase mechanism

- The three enzyme components work in unison
- E_1 (pyruvate dehydrogenase) decarboxylates pyruvate as seen for pyruvate decarboxylase
- E_2 (dihydrolipoyl transacetylase) accepts the acetyl derived from pyruvate, and then transfers it to Coenzyme A. In the process lipoamide is reduced by two electrons
- E_3 (dihydrolipoyl dehydrogenase) reoxidizes lipoamide using FAD as the initial e^- acceptor and NAD^+ as the terminal e^- acceptor
- The lipoate group “pivots” on E_2 , translocating between E_1 and E_3 during catalysis



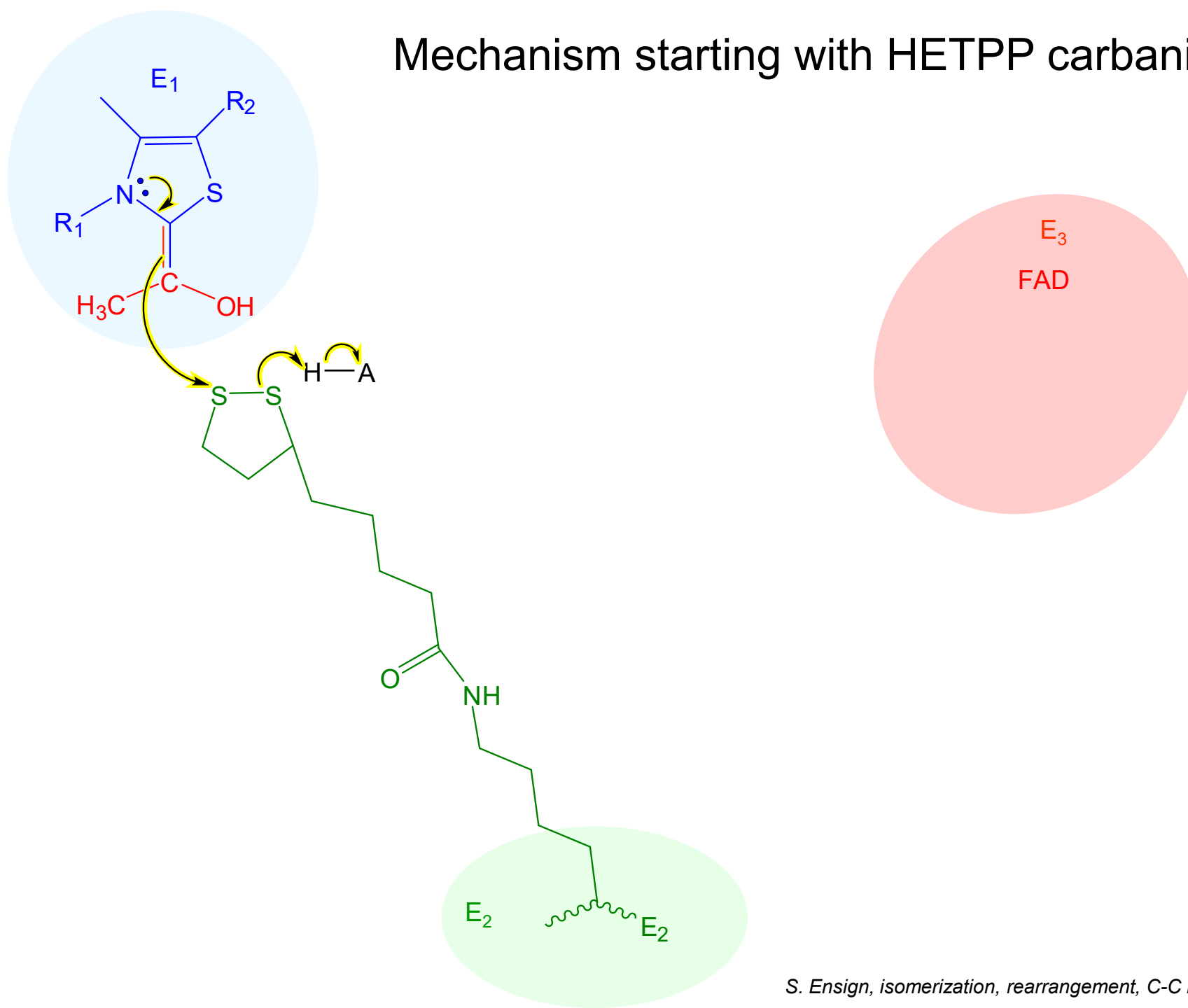
The mechanisms of pyruvate carboxylase and pyruvate dehydrogenase diverge at the level of the hydroxyethyl-TPP carbanion

In oxidative pyruvate decarboxylation, the hydroxyethyl-TPP anion is transferred to lipoamide rather than H^+

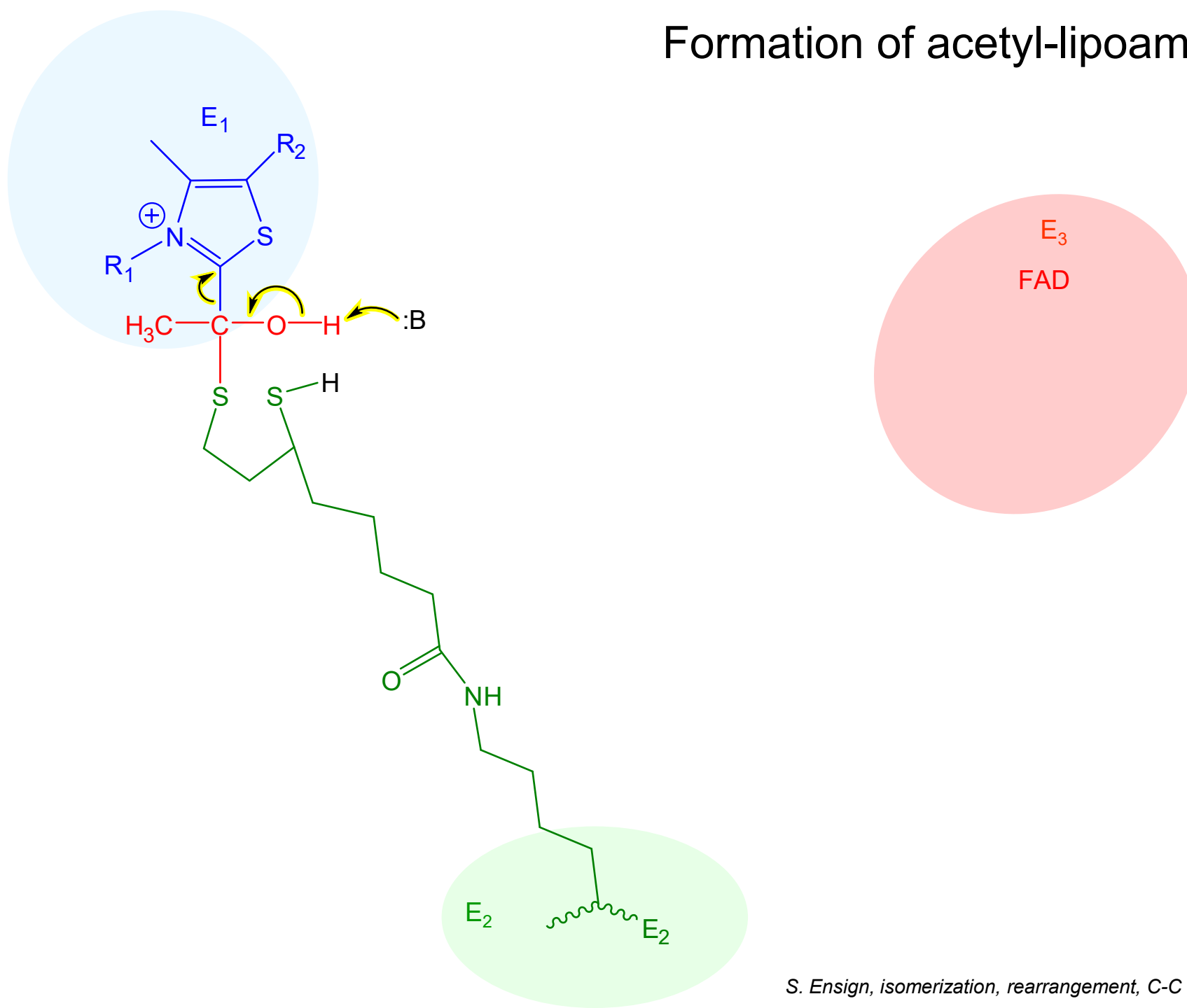


Watch how the interplay of the activities of the three subunits is mediated by the movement of the flexible linker arm connecting the “business end” of lipoamide

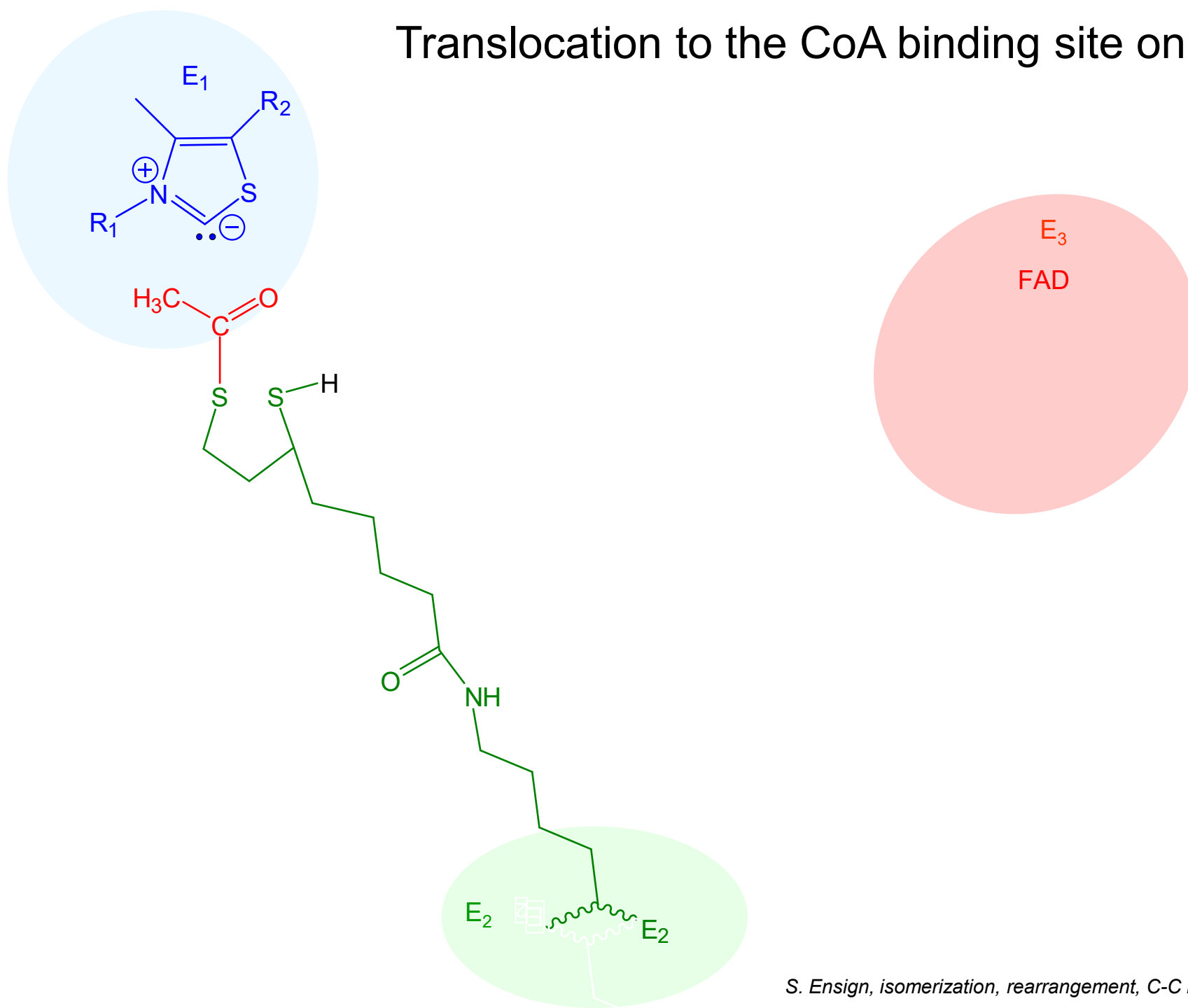
Mechanism starting with HETPP carbanion



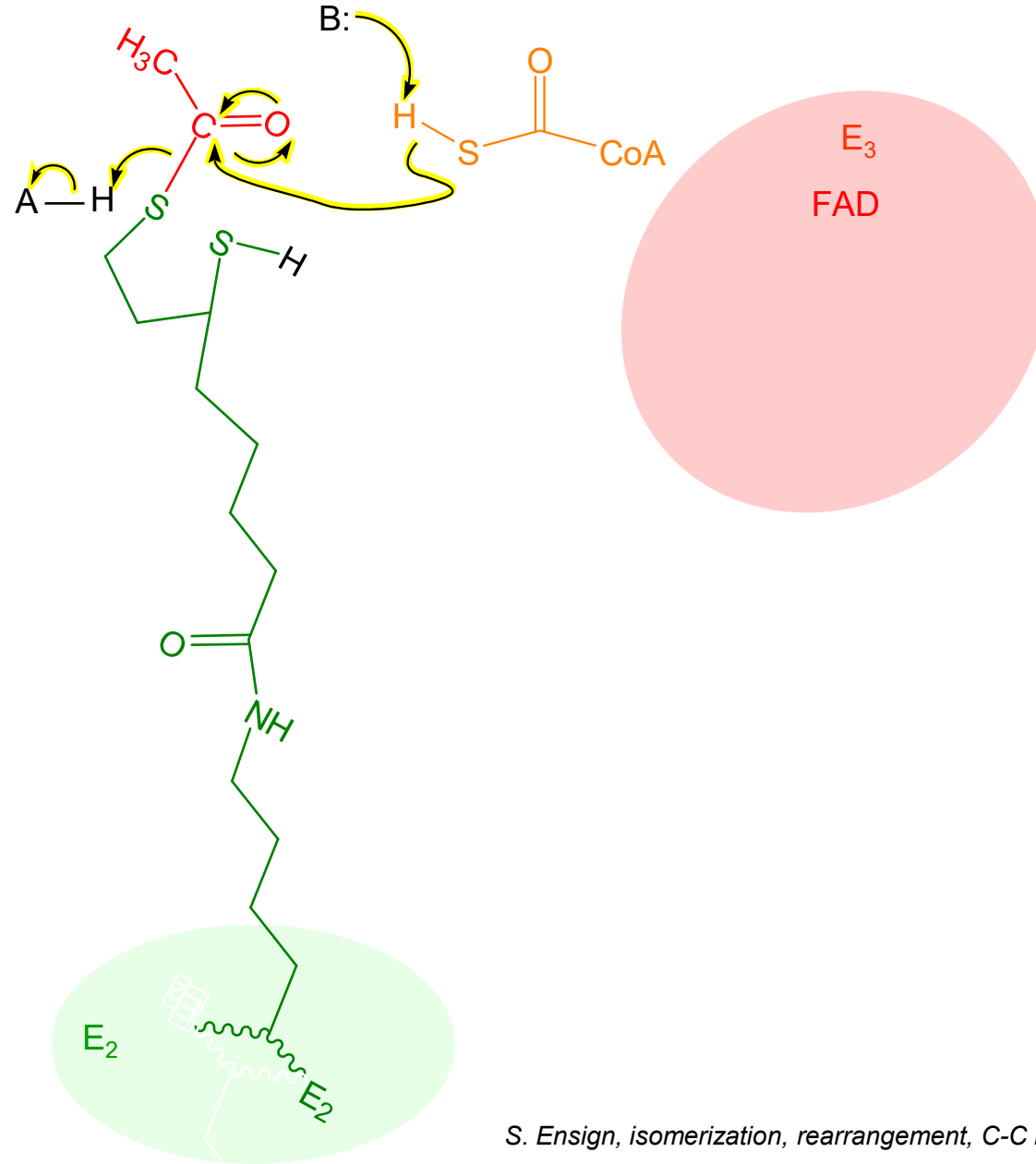
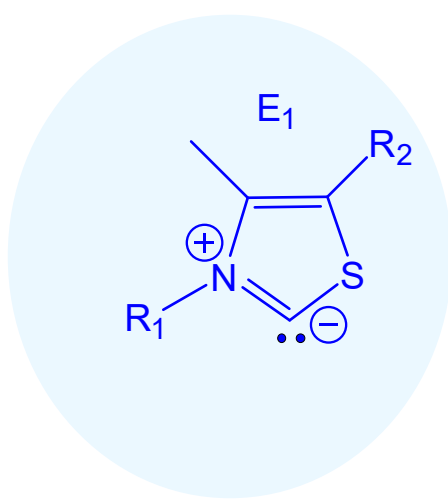
Formation of acetyl-lipoamide



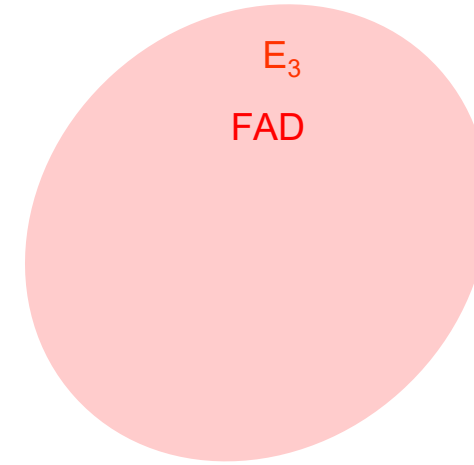
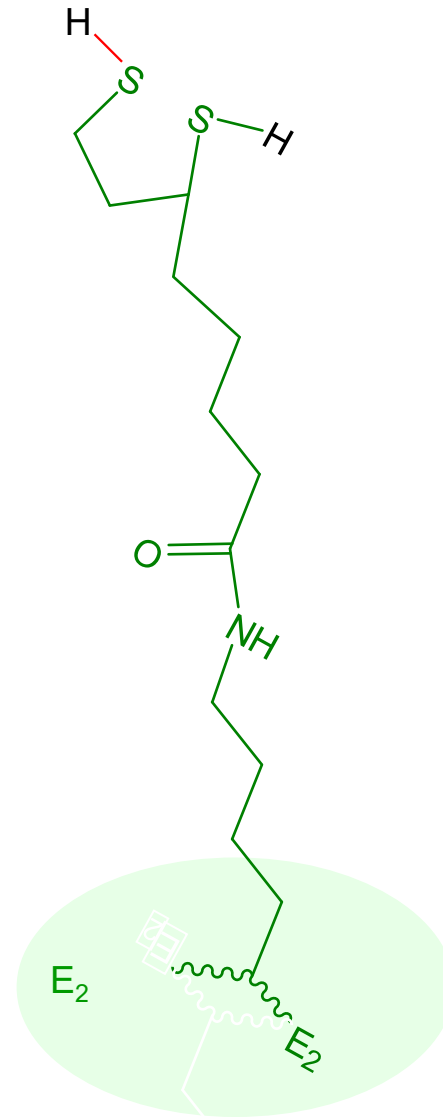
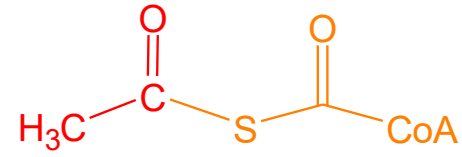
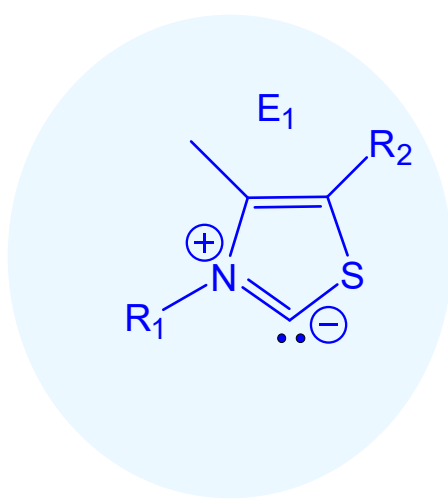
Translocation to the CoA binding site on E₂

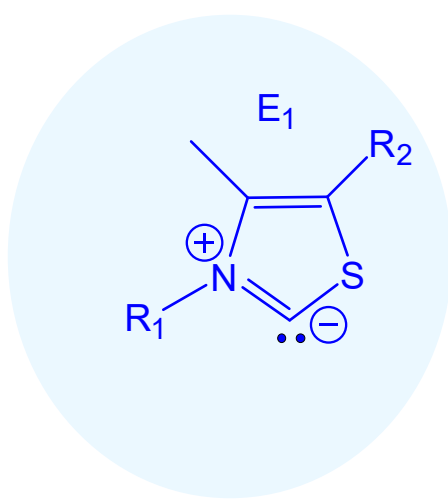


Transfer of acetyl group to CoA

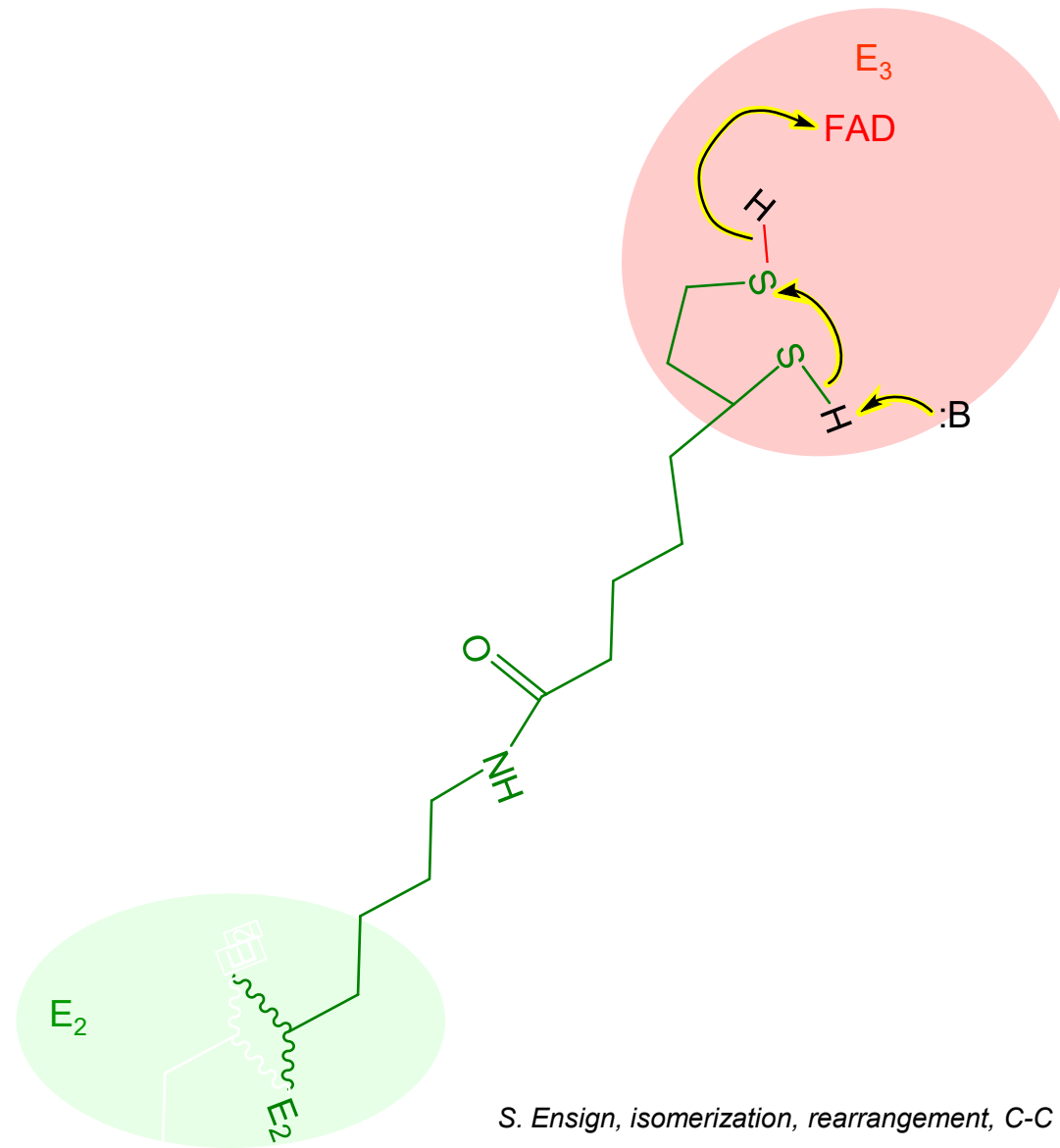


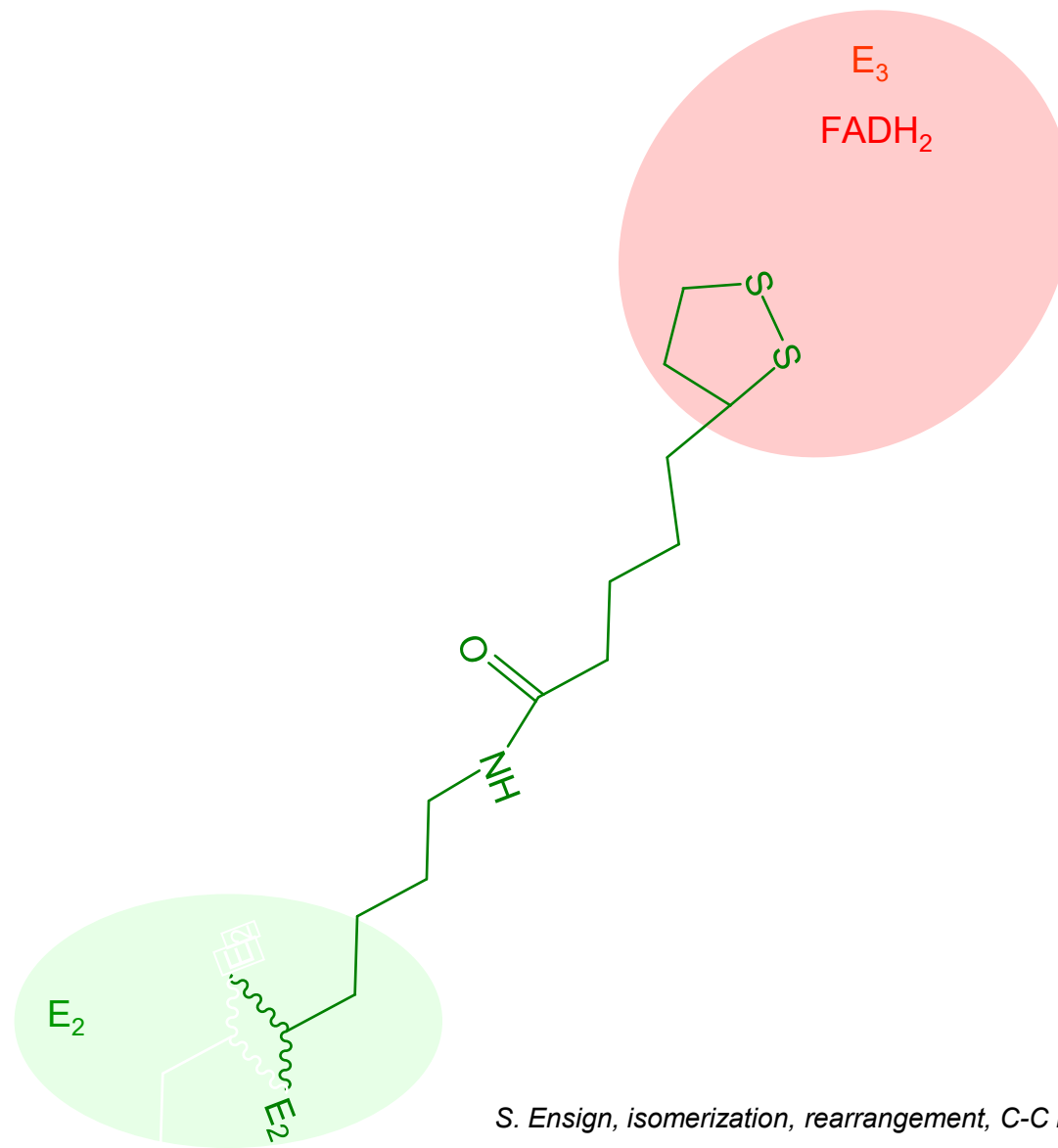
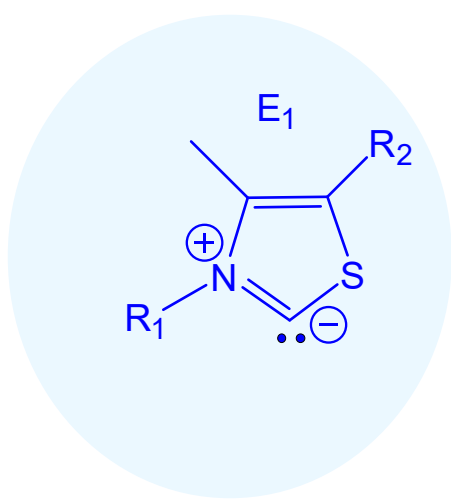
Translocation to the FAD binding site on E₃



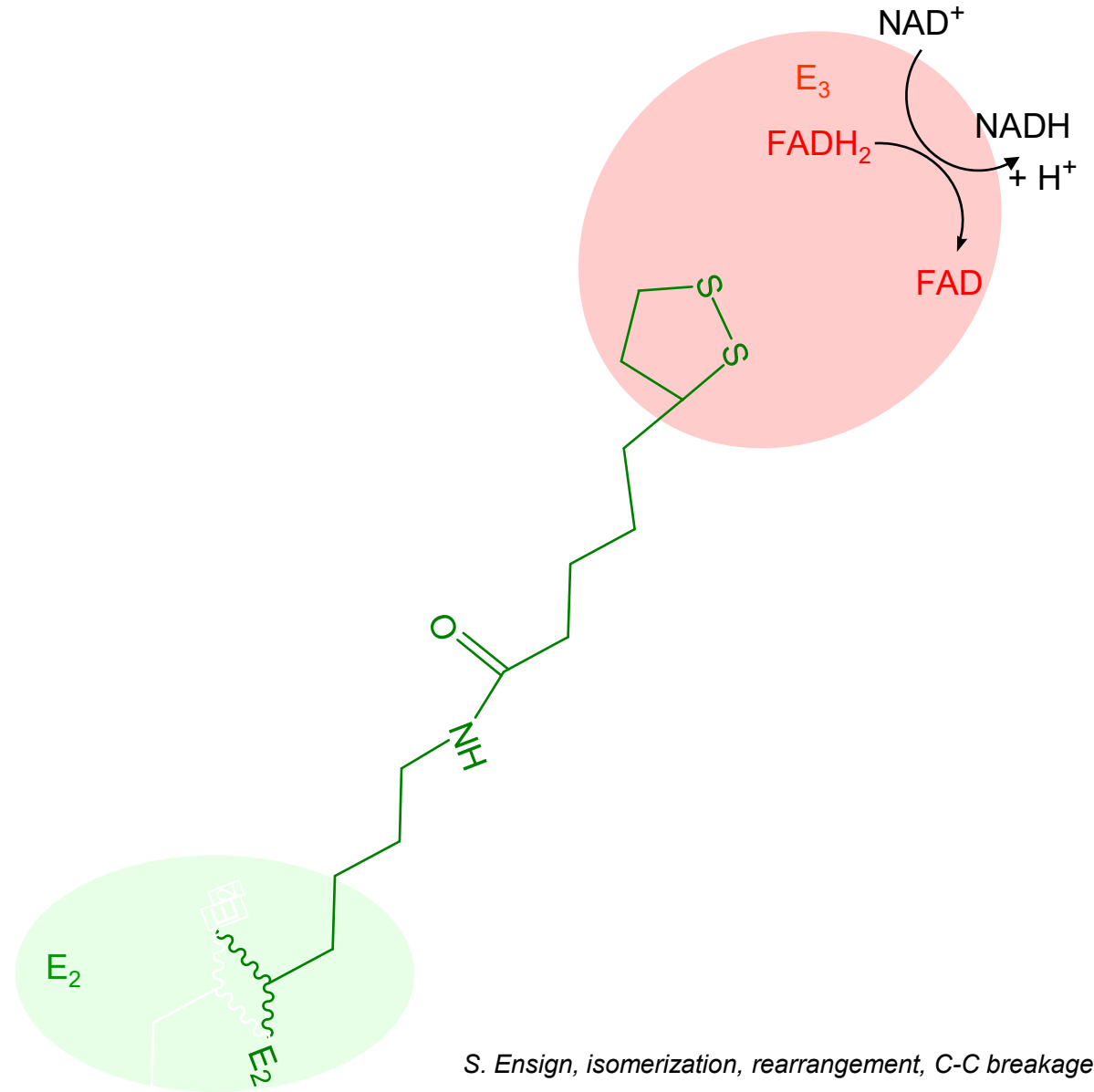
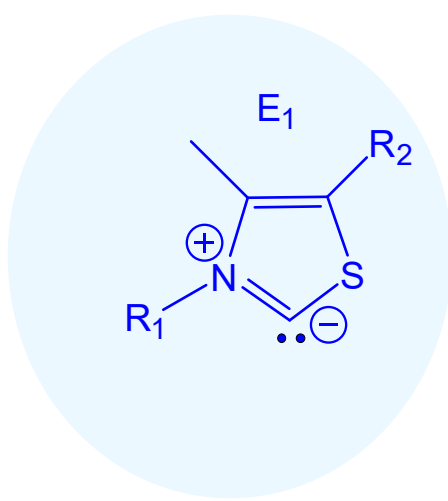


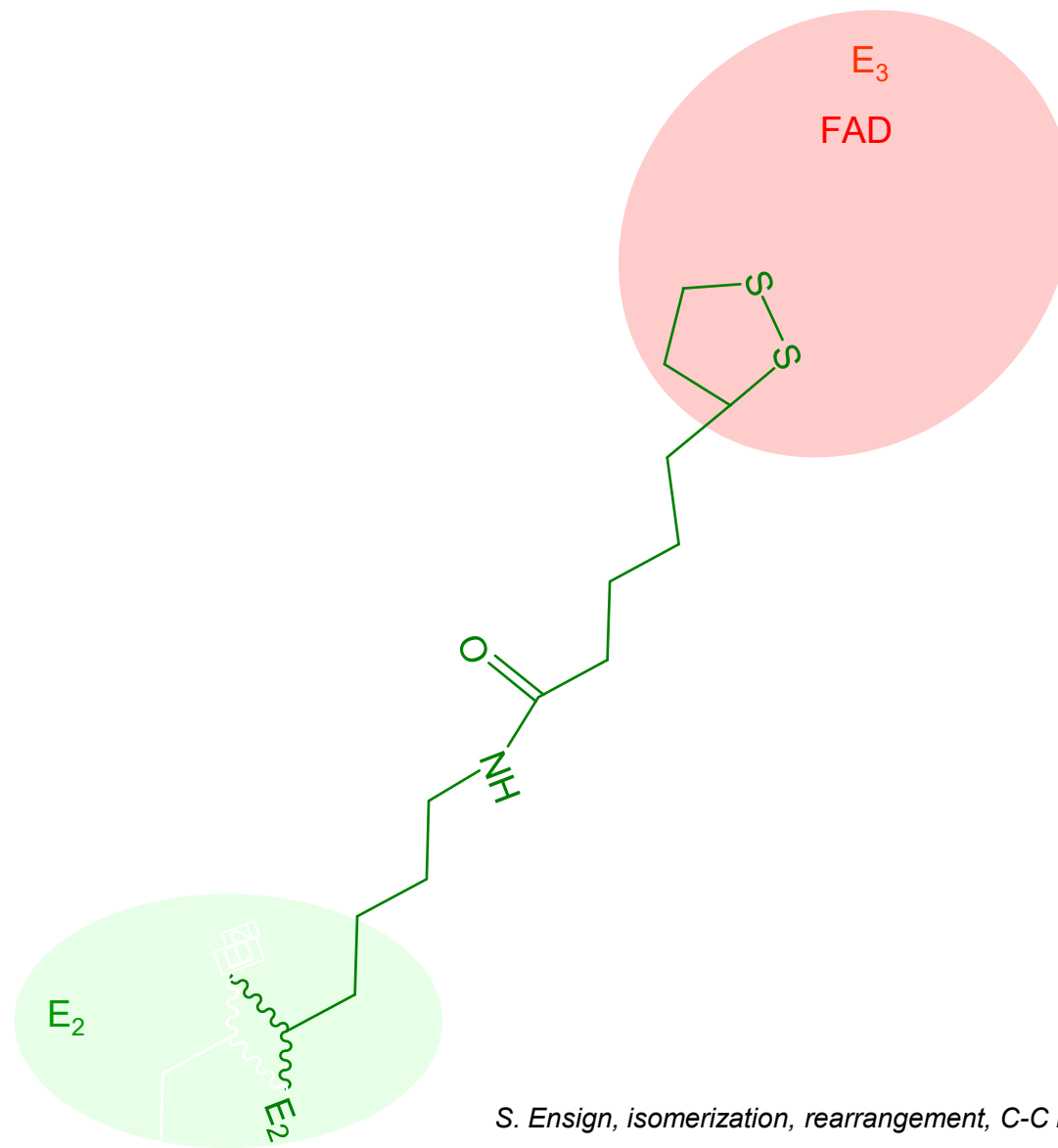
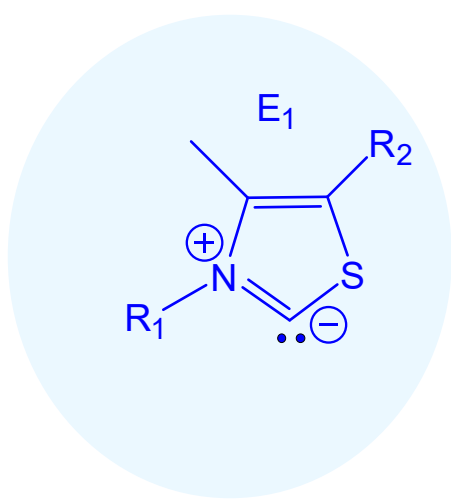
Oxidation of reduced lipoamide using FAD as e- acceptor. The mechanistic details are beyond the scope of this class

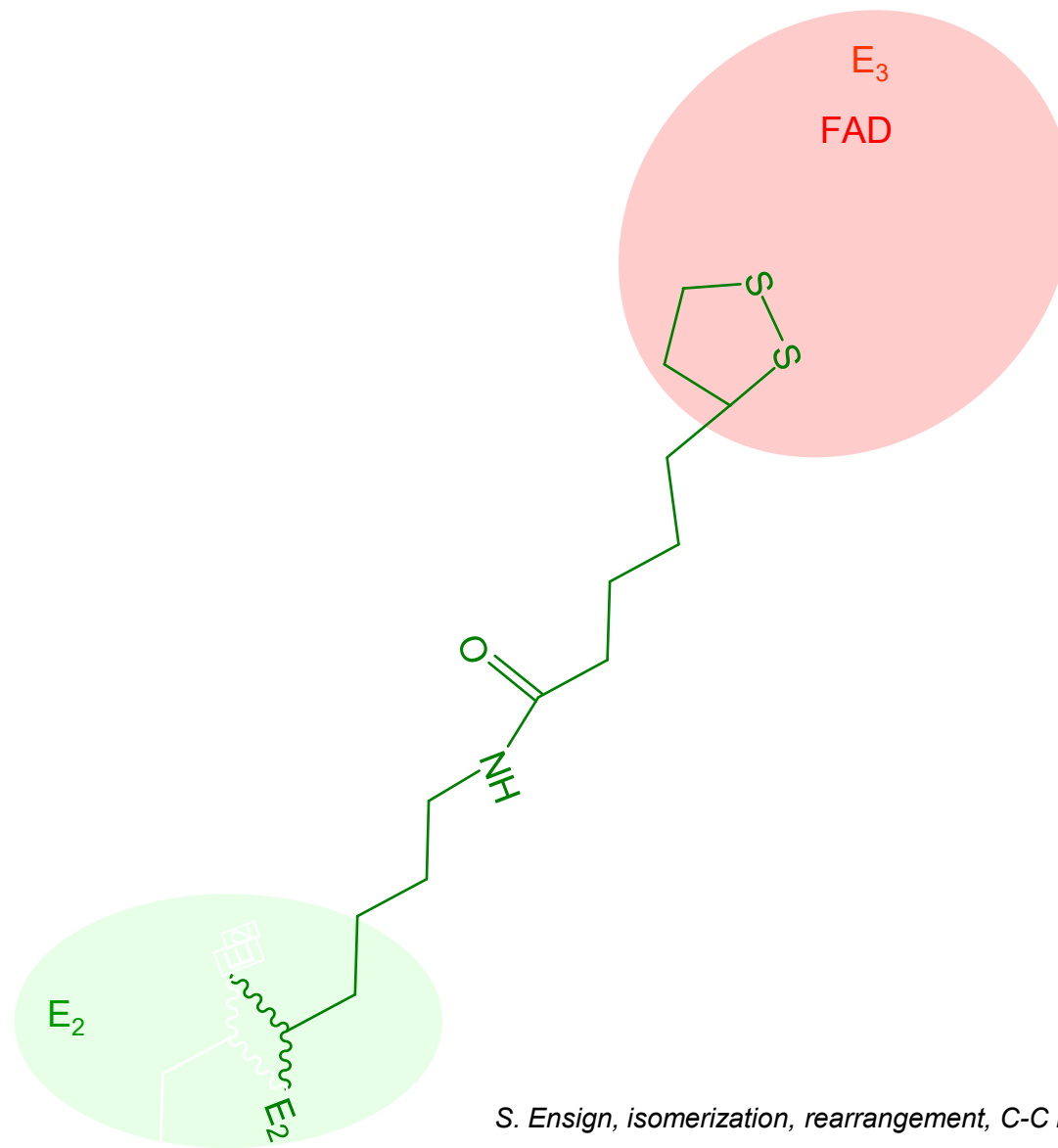
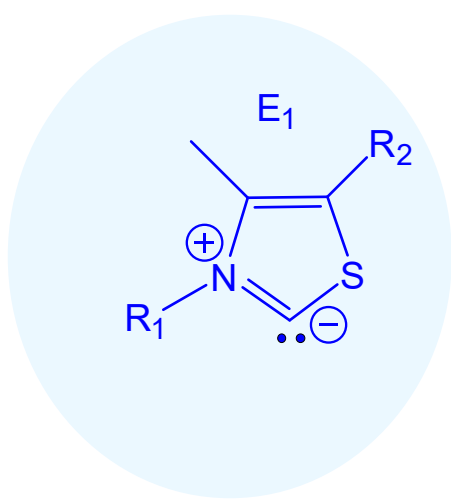


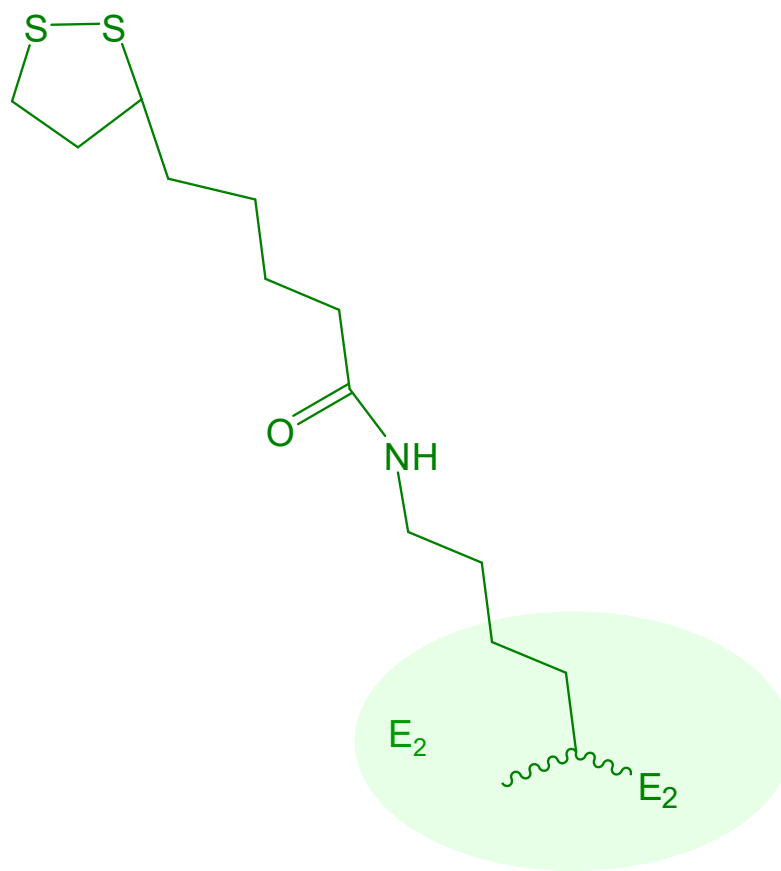
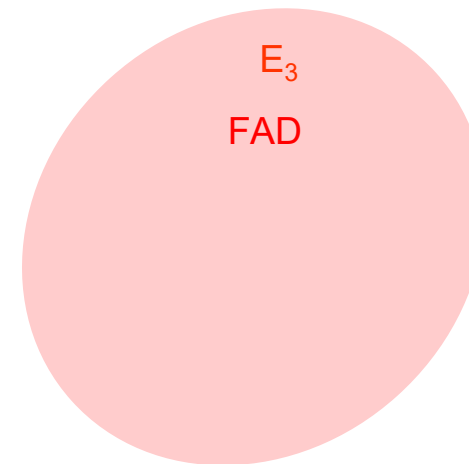
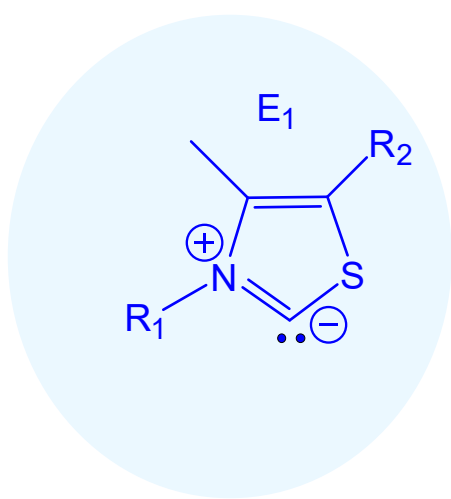


Reduction of NAD^+ by FADH_2









Carboxylation reactions in biology

- CO_2 vs bicarbonate as a substrate for carboxylation?
 - Physiological concentrations?
 - Which is a better electrophile?
- Rubisco: CO_2 is the substrate
- Biotin-dependent carboxylases: HCO_3^- is the substrate

Carboxylation reactions requiring biotin and ATP

