Engineering Enzymes for Green Chemical Synthesis

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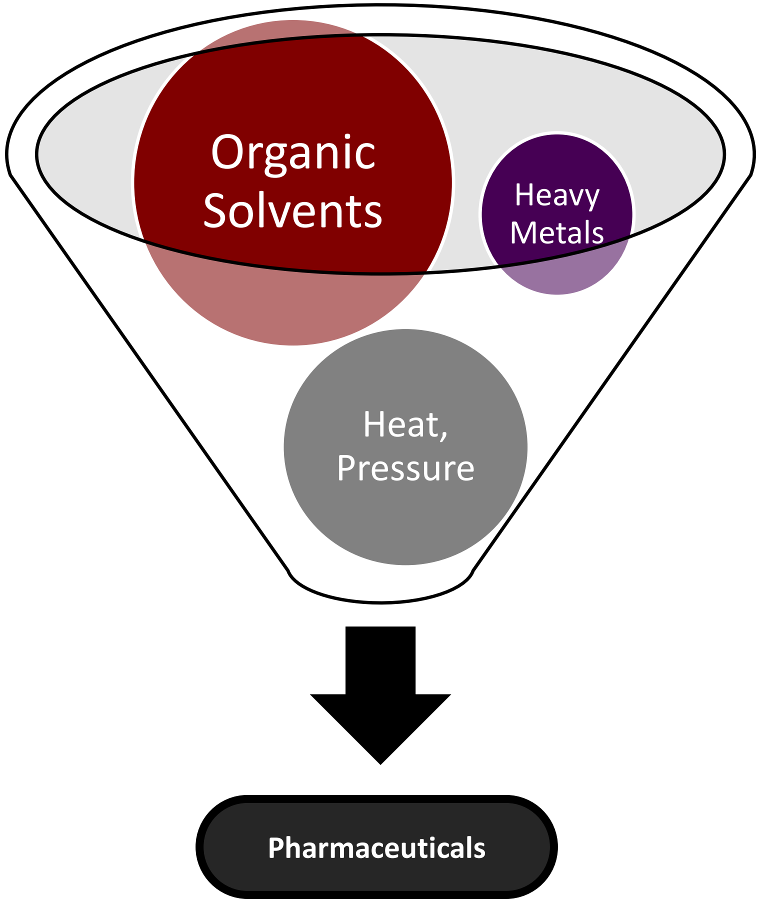
Fine chemical and pharmaceutical production is inefficient

Environmental impact of chemical industries	
<u>Industry</u>	**<u>E-factor</u>** (kg waste / kg product)
Oil refining	< 0.1
Bulk chemicals	< 1-5
Fine chemicals	5 - > 50
Pharmaceuticals	***25 - > 100***



R. A. Sheldon. Chem. Soc. Rev., 2012,**41**, 1437-1451

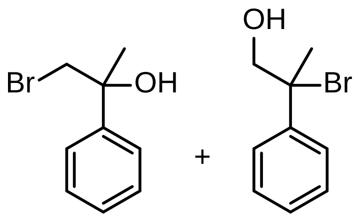
Qualities of an ideal “green” catalyst

Sustainable, non-toxic,
and biodegradable

Cost-effective

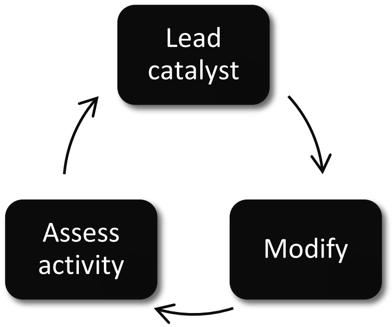
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

Exhibit high turnover

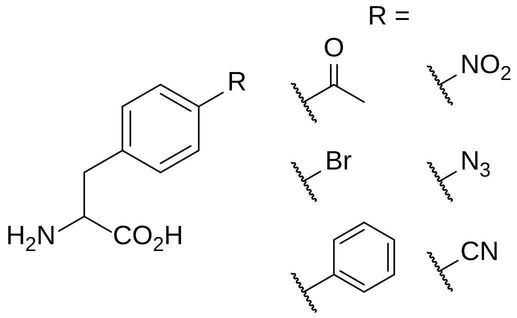


Major Minor

Selective



Amenable to systematic improvements



Flexible substrate scope

Enzymes have the potential to meet these requirements!

The power of enzyme catalysis

Enzymes are biological catalysts

Display exquisite rate enhancements

Perform under mild reaction conditions

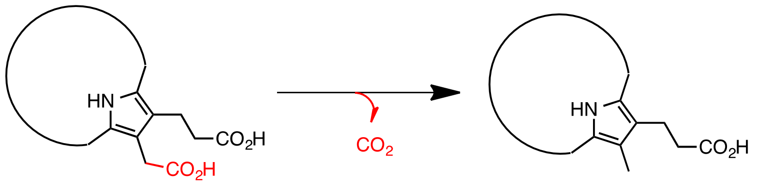
Highly selective

Evolvable

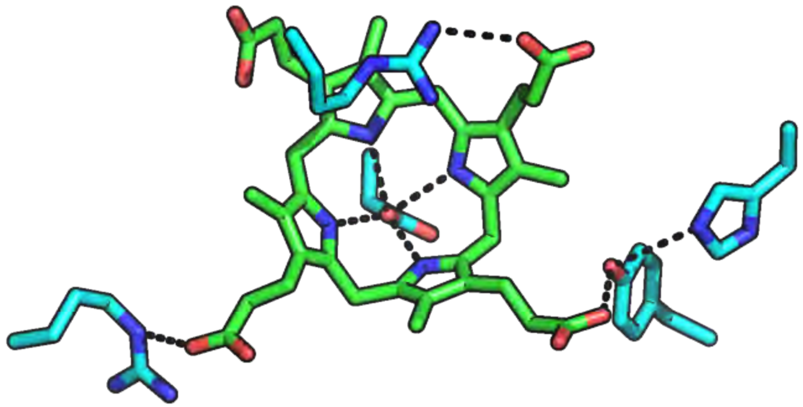
Challenge:

How do we introduce new reactions to enzymes?

Example: uroporphyrinogen decarboxylase



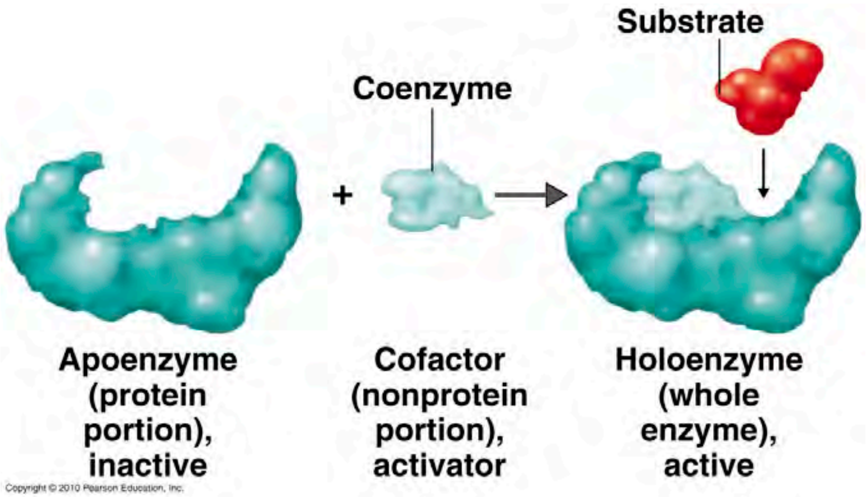
Rate enhancement ~ 10^{17}

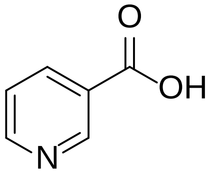


No metals involved, only amino acid side chains

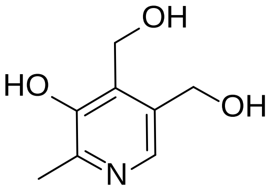
Cofactors expand enzymatic chemistry

Cofactors (or coenzymes) provide reactivity beyond that of the amino acids

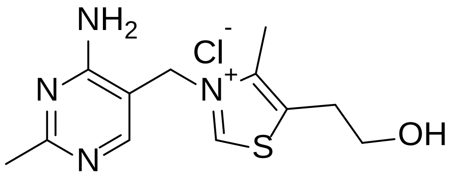




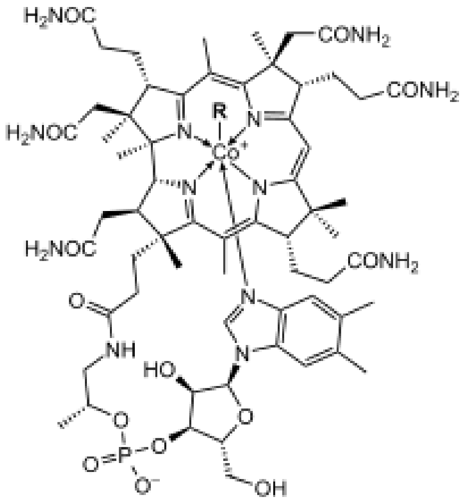
Niacin, Vitamin B_3



Pyridoxine, Vitamin B_6

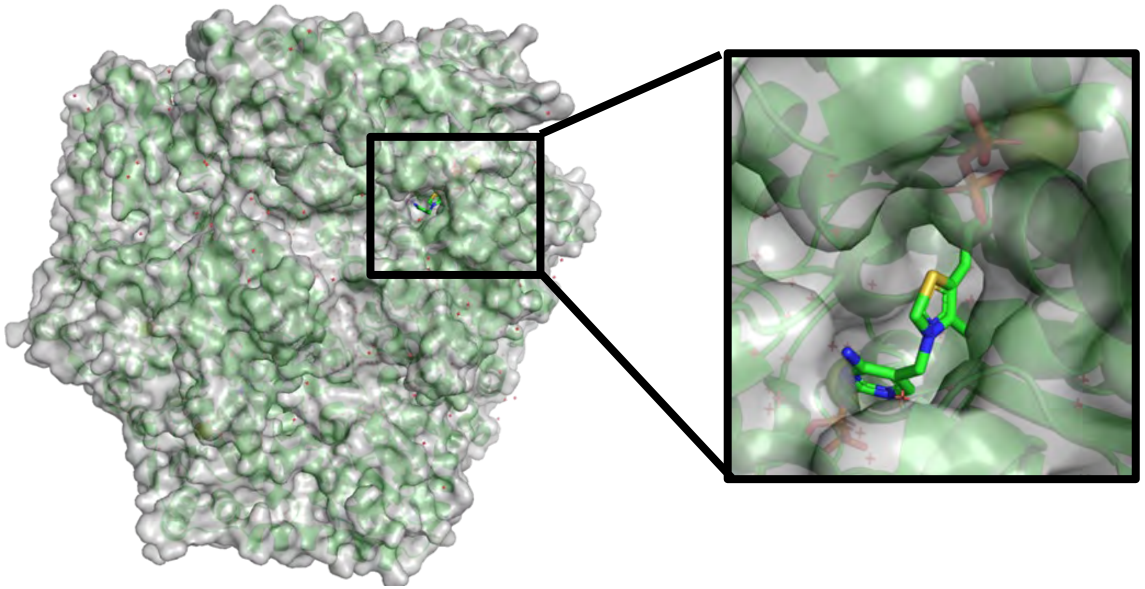


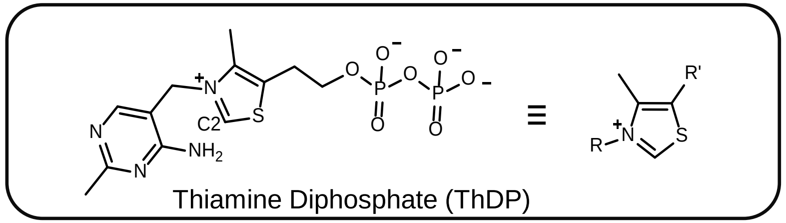
Thiamine, Vitamin B_1

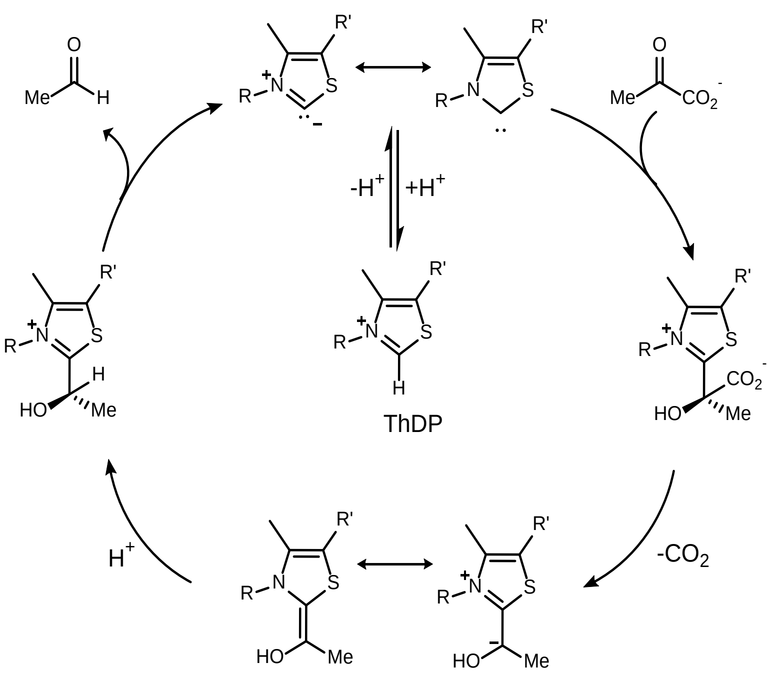


Cobalamin, Vitamin B_{12}

Thiamine-dependent enzymes are versatile catalysts





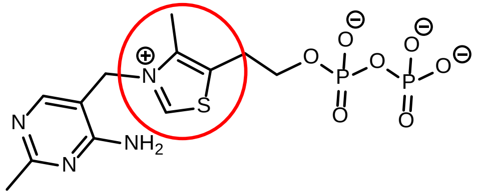


In nature, thiamine-dependent enzymes catalyze a variety of carbon-carbon bond forming or breaking reactions

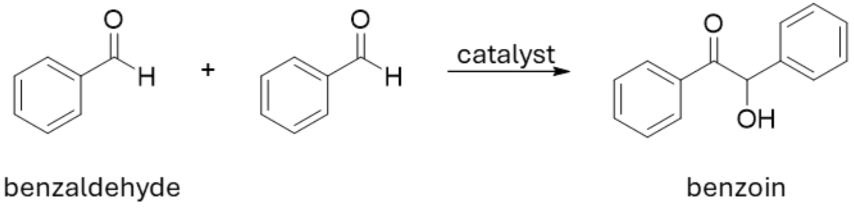
Prier, C. K.; Arnold, F. H. *J. Am. Chem. Soc.* **2015**, *137*, 13992–14006.

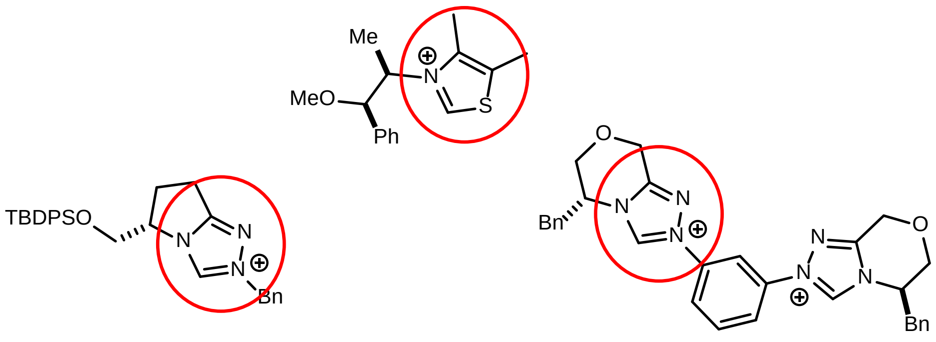
Chemistry inspired biocatalysis

Synthetic chemists have developed N-heterocyclic carbene catalysts that are similar to thiamine but catalyze many more reactions

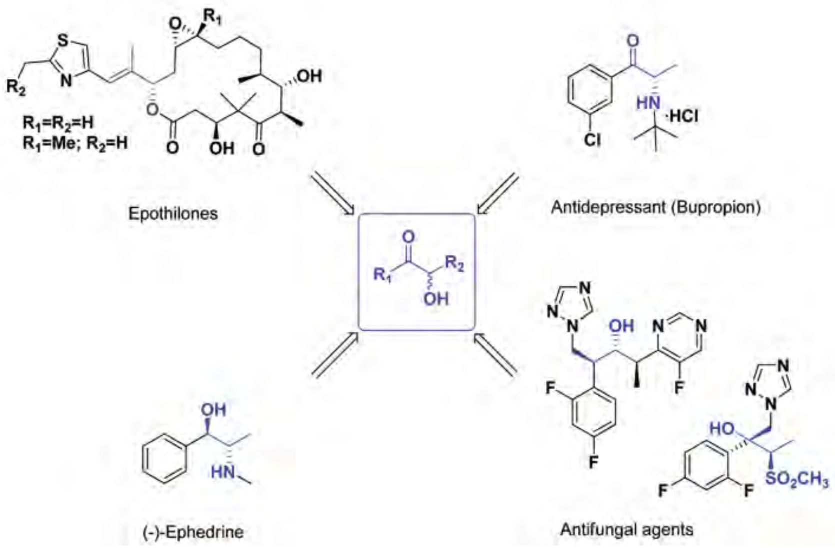


Thiamine Diphosphate





Representative Synthetic Catalysts



Can we take advantage of the similarities between chemical catalysts and thiamine to introduce new reactions to enzymes?

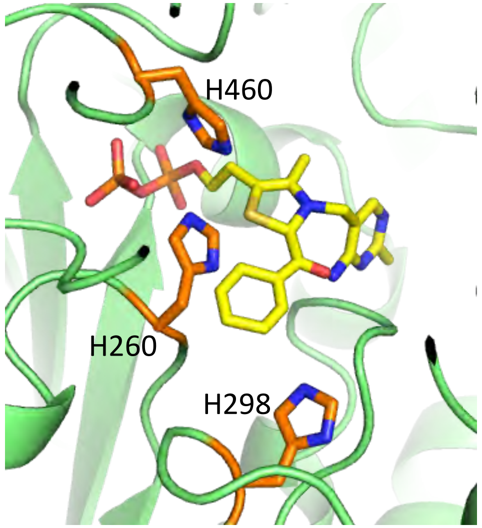
Flanigan, D. M.; Romanov-Michailidis, F.; White, N. A.; Rovis, T. *Chem. Rev.* **2015**, *115* (17), 9307–9387.
Hoyos, P., Sinisterra, J.-V., Molinari, F., Alcantara, A. R., & Dominguez de Maria, P. *Accts. Chem. Res.* **2010**, 43(2), 288–299.

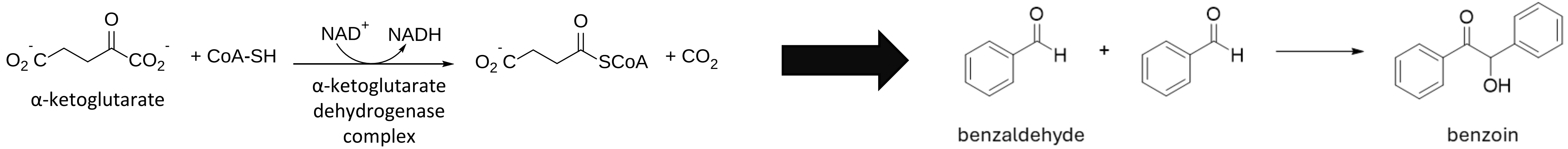
Engineering the thiamine-dependent enzyme SucA for benzoin condensation

Target: E1 subunit of the α-ketoglutarate dehydrogenase complex (SucA)

Thiamine-dependent enzyme from central metabolism

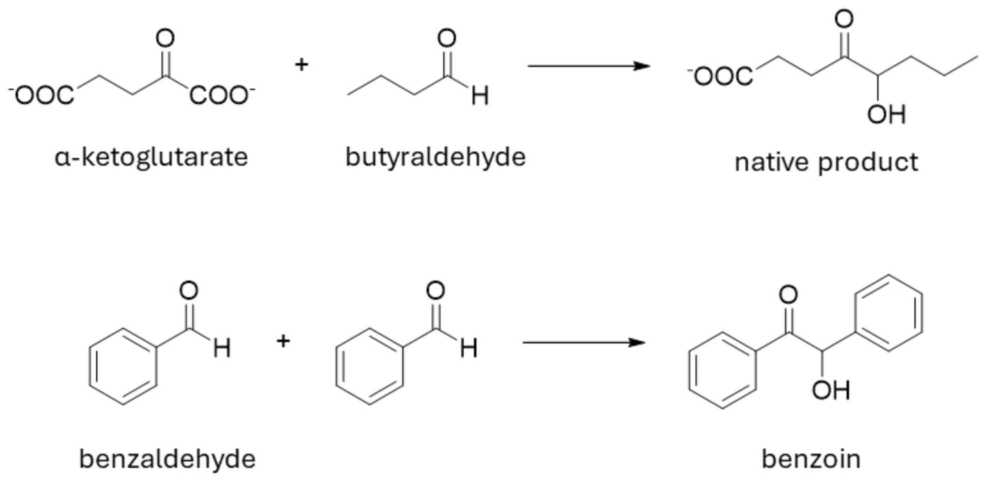
In nature, catalyzes decarboxylation of α-ketoglutarate

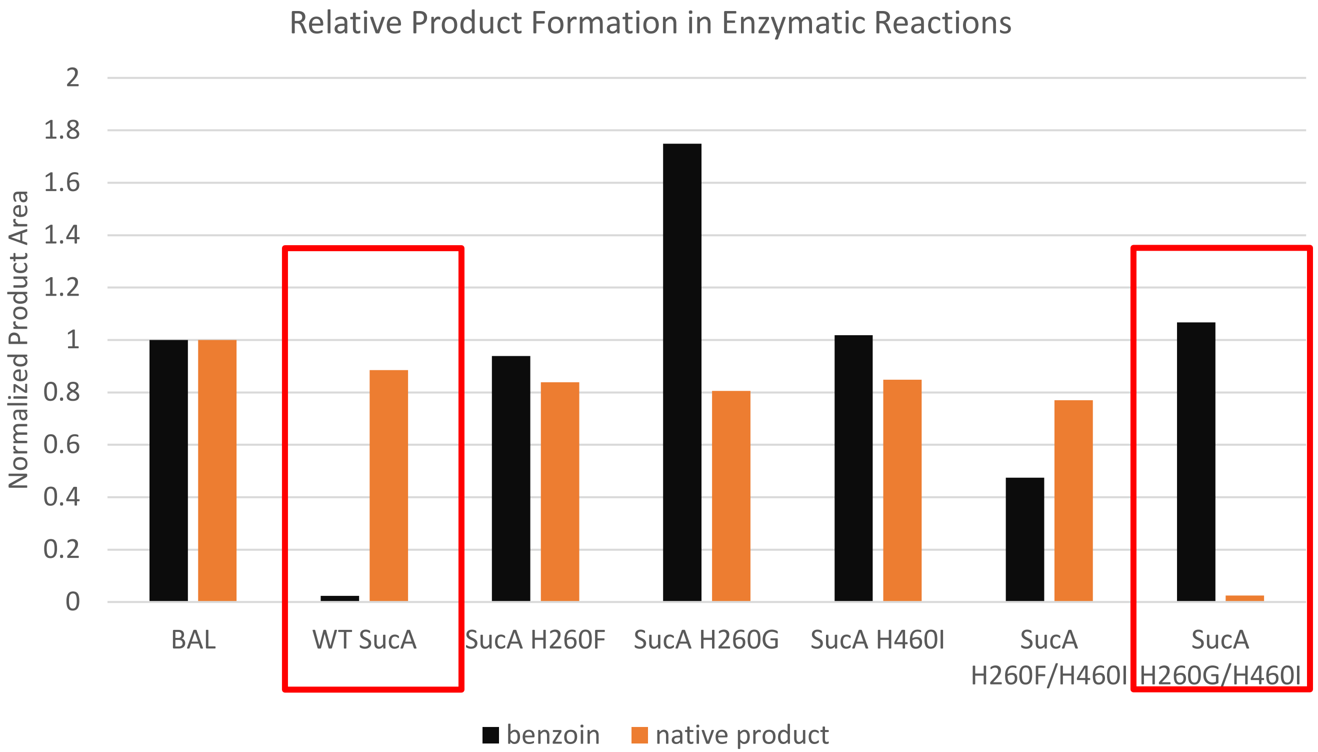




SucA variants for benzoin synthesis

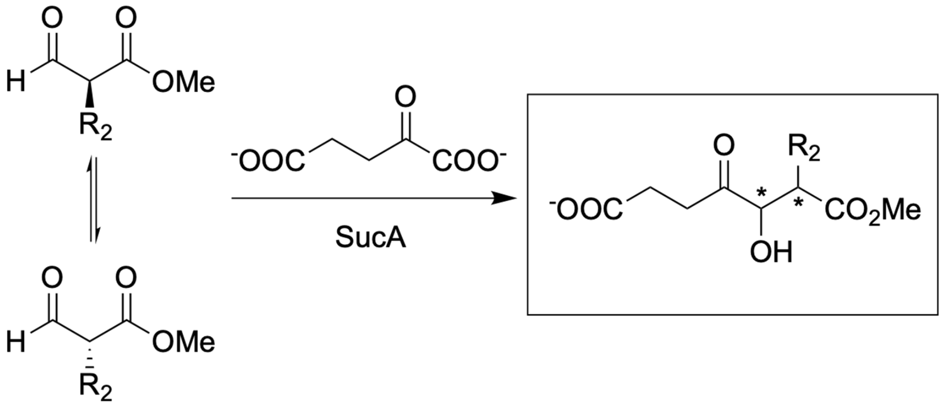
Compared activity of unmutated wild type (WT) SucA to several mutants in the “native” reaction and in benzoin synthesis

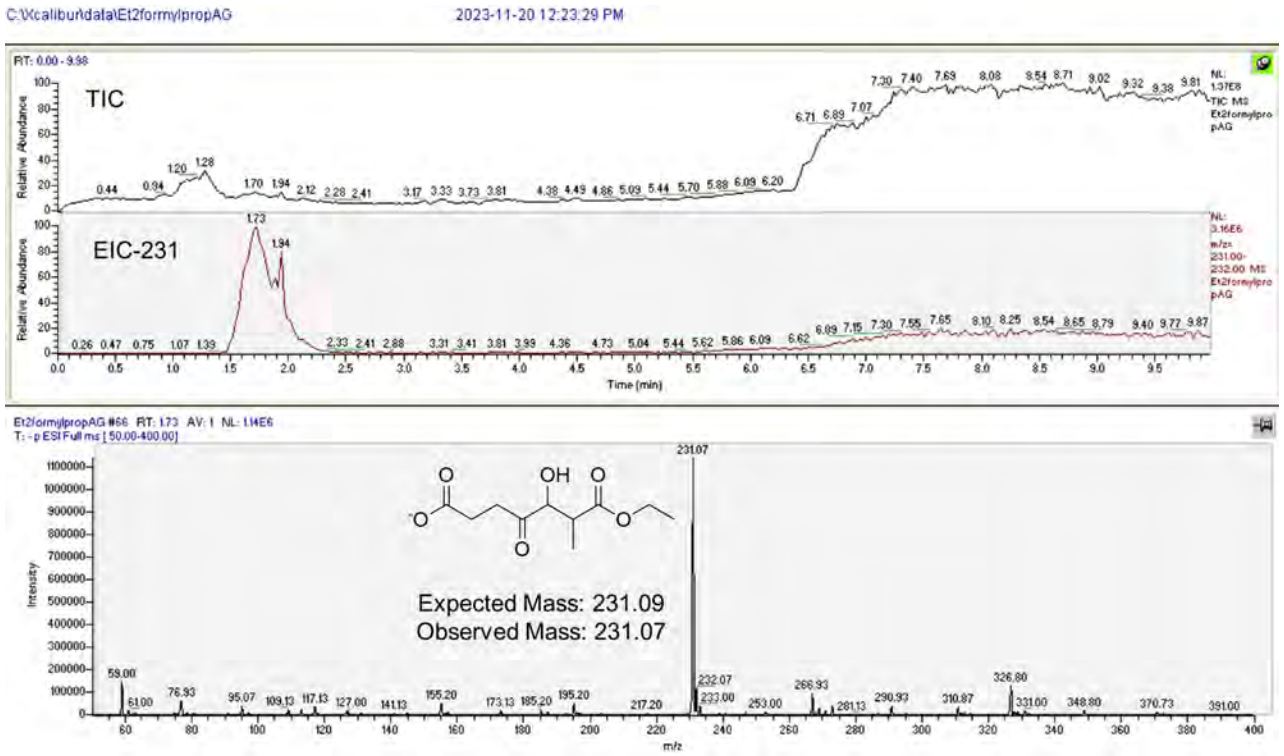




Pushing the limits of biocatalysis with SucA

Can we engineer the enzyme to make even more complex products selectively?





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