Enzymatic Carboxylations

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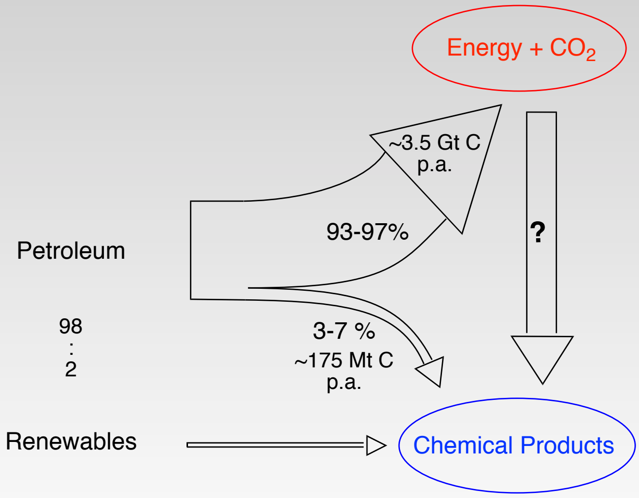
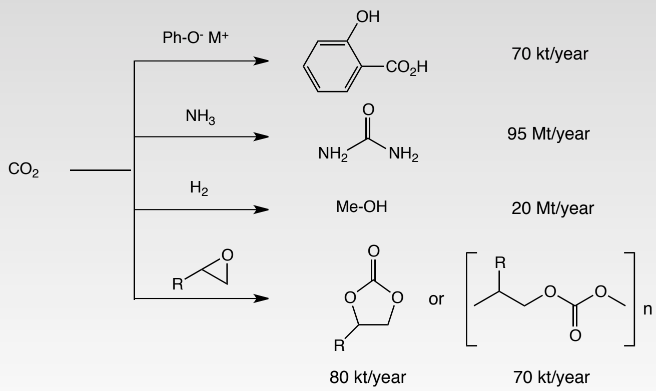
CO_2 as Raw Material for Organic Synthesis?



Projected Availability: Oil 43-67 yrs, gas 64-150 yrs, coal 200-1500 yrs (www.eia.doe.gov).

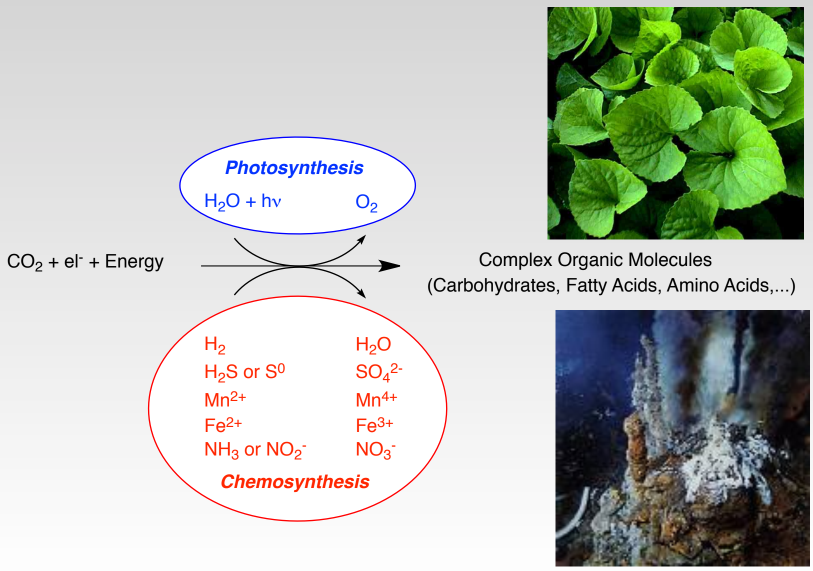
CO_2 as Raw Material for Synthesis of Chemicals

A. Behr, *Angewandte homogene Katalyse*, VCH-Wiley, **2008**, pp. 441-464; *Chem. Rev.* **2014**, *114*, 1709.



Principles of CO_2-Assimilation: Light or Dark?

Adv. Biochem. Eng. Biotechnol. **1992**, *46*, 63; *Appl. Microbiol. Biotechnol.* **2008**, *79*, 707.



Nature's Major Carboxylation Pathways:

Chem. Soc. Rev. **2010**, *39*, 313.

Chemosynthesis: e^- from H_2, H_2S, S^0, Mn^{2+}, Fe^{2+}, NO_2^-, NH_3

— Wood-Ljungdahl (chemistry can't do!)

$$2\ CO_2 + e^- + H^+ \longrightarrow CH_3\text{—}CO_2H$$

— Acyl-CoA Pathways (extreme environments)

$$2\ CO_2 + e^- + H^+ \longrightarrow CH_3\text{—}CO_2H \text{ or } O{=}CH\text{—}CO_2H$$

— Arnon-Buchanan (reverse Krebs-cycle)

$$2\ CO_2 + e^- + H^+ \longrightarrow CH_3\text{—}CO_2H$$

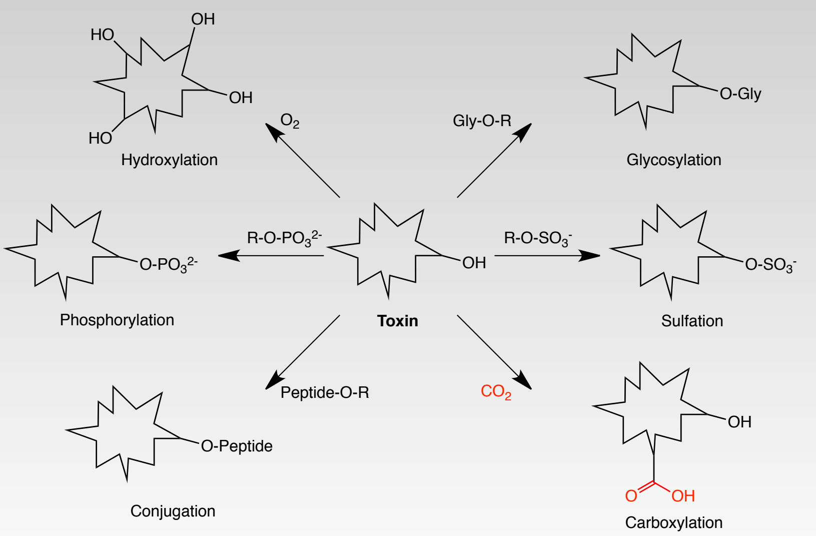
Photosynthesis: e^- from H_2O

— Calvin-Benson-Bassham (RubisCO)

$$2\ CO_2 + 2H_2O \xrightarrow{h\nu} CH_3\text{—}CO_2H + O_2$$

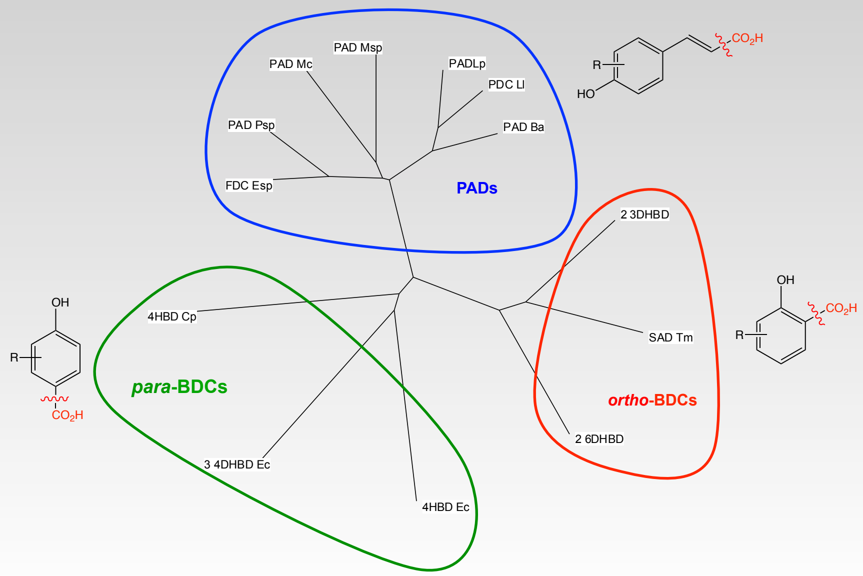
primary metabolism ⟶ highly specific enzymes ⟶ little use for biocatalysis

Detoxification: Making Things Polar

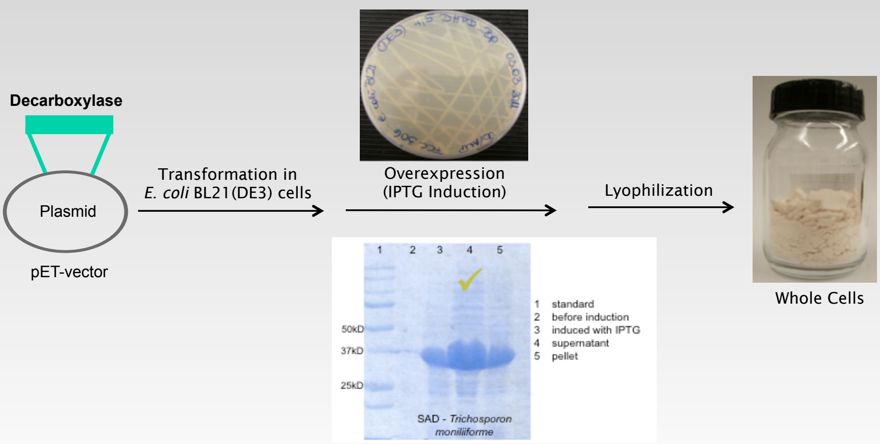


secondary metabolism ⟶ broad substrate tolerance ⟶ very useful for biocatalysis

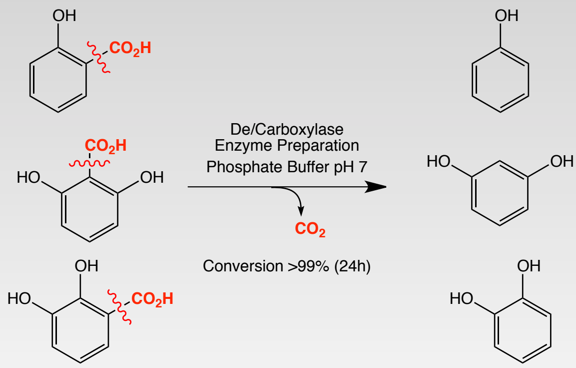
Roadmap for Navigating in Sequence Space: Phylogenetic Tree



Decarboxylases: Cloning and Overexpression



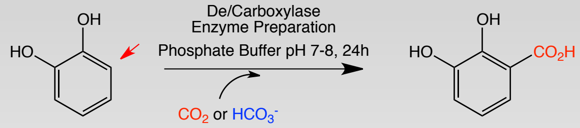
A Simple Downhill-Start: Decarboxylation



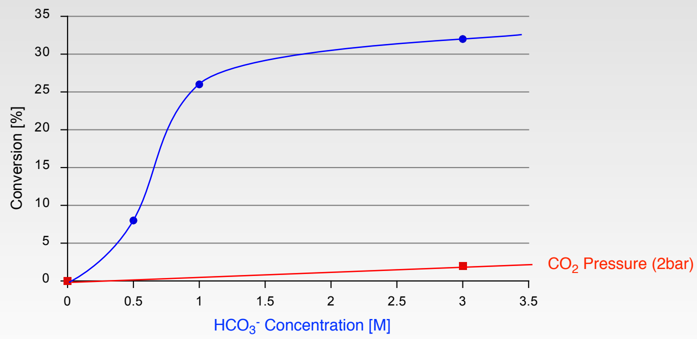
Salicylic acid decarboxylase (*Trichosporon moniliiforme*)
2,6-Dihydroxybenzoic acid decarboxylase (*Ralstonia* sp.)
2,3-Dihydroxybenzoic acid decarboxylase (*Aspergillus oryzae*)

The Uphill Challenge: Carboxylation

Chem. Lett. **2011**, *40*, 206; *Org. Lett.* **2012**, *14*, 1974; *Eur. J. Biochem.* **1998**, *257*, 495

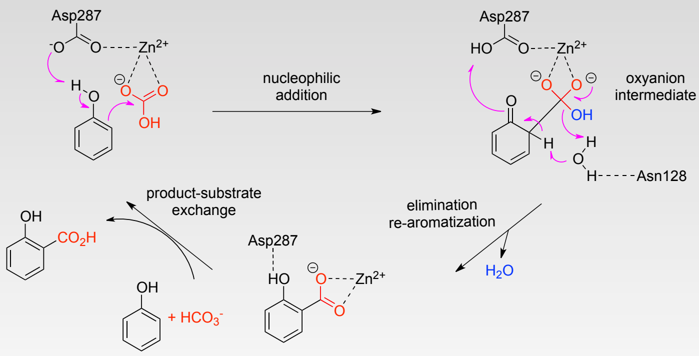


2,3-Dihydroxybenzoic acid decarboxylase (*Aspergillus oryzae*)



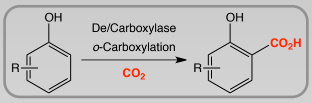
→ CO_2-Source: Bicarbonate (not CO_2)!

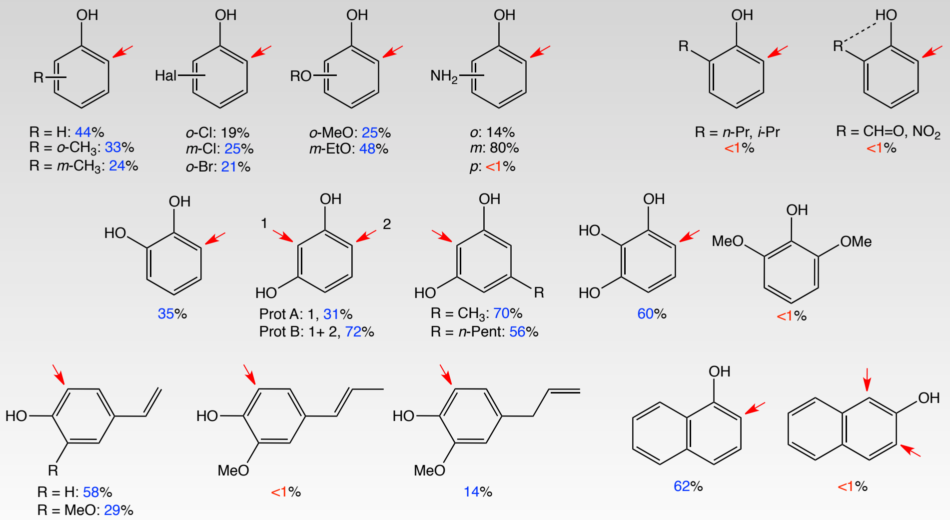
o–Carboxylation: Lewis-Acid Catalysis



o-Benzoic Acid Decarboxylases: Substrate Scope

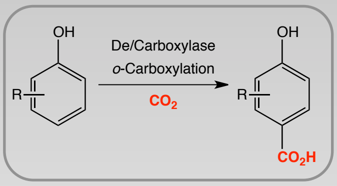
RSC Adv. **2014**, *4*, 9673.

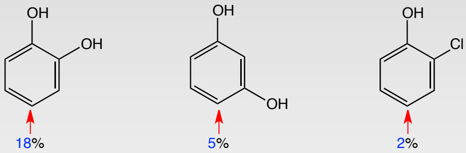




Conditions: lyophilised *E. coli* cells containing benzoic acid decarboxylase, P_i-buffer pH 8, $KHCO_3$ [3M], 30˚C, 24h.

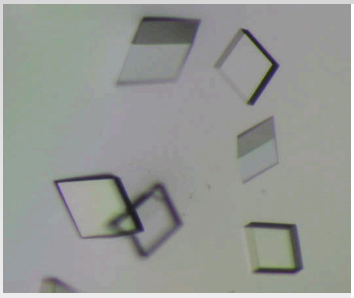
p-Benzoic Acid Decarboxylases: Substrate Scope



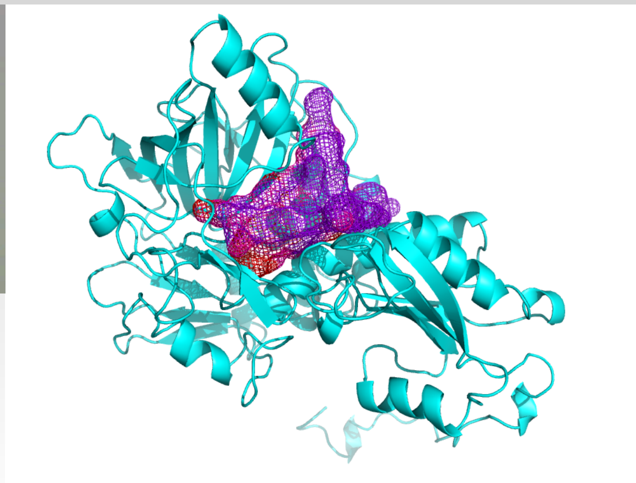


Conditions: lyophilised *E. coli* cells containing 3,4-dihydroxybenzoic acid decarboxylase, P_i-buffer pH 8, $KHCO_3$ [3M], 30˚C, 24h.

p-Carboxylase: Crystal Structure of 3,4-Dihydroxybenzoic Acid Decarboxylase



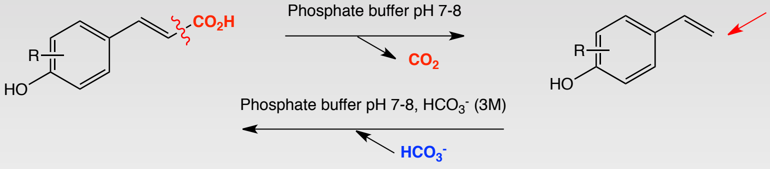
mutants at work...



Regio-Complementary Side-Chain Carboxylation?

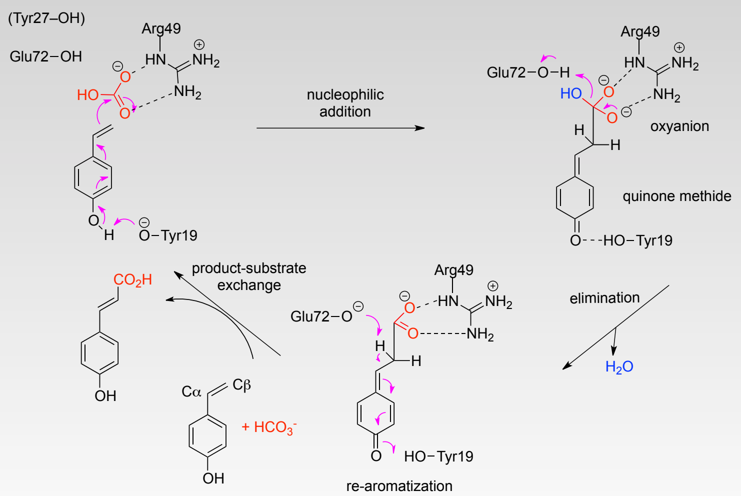
Appl. Microbiol. Biotechnol. **2013**, *97*, 1501; *Org. Lett.* **2012**, *14*, 1974.

Phenolic Acid Decarboxylases

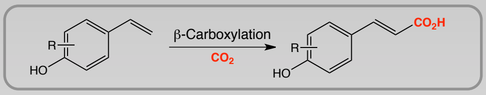


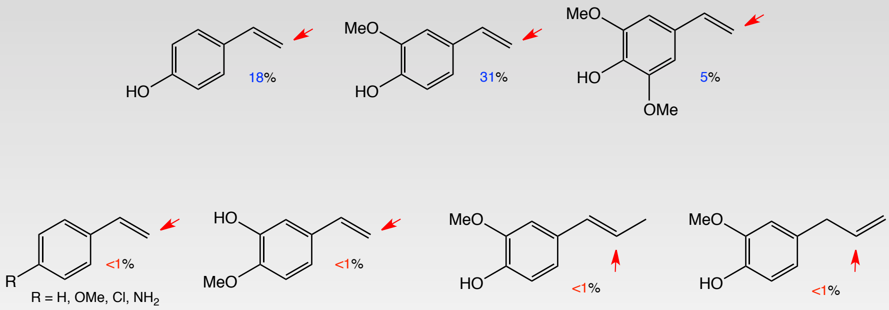
Lyophilised *E. coli* containing phenolic acid decarboxylase, P_i-buffer pH 8, $KHCO_3$ [3M].

β-Carboxylation: Acid-base Catalysis

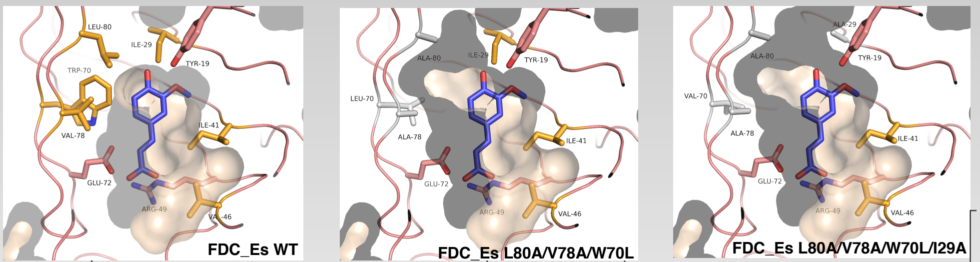


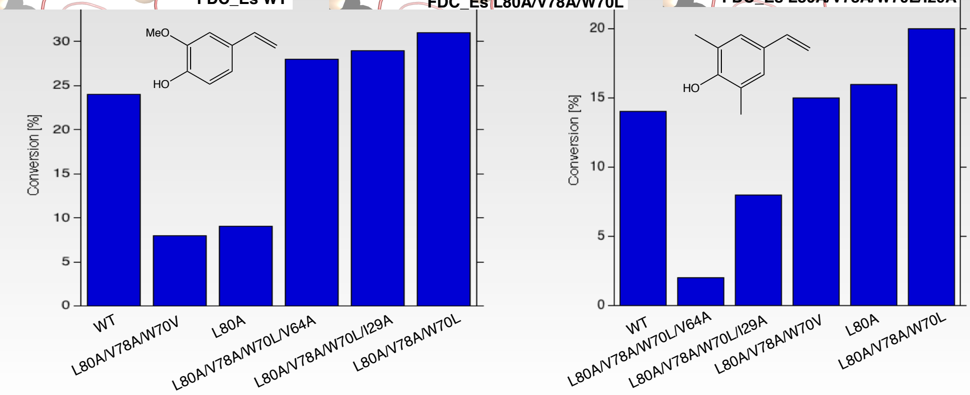
Phenolic Acid Decarboxylases: Substrate Scope





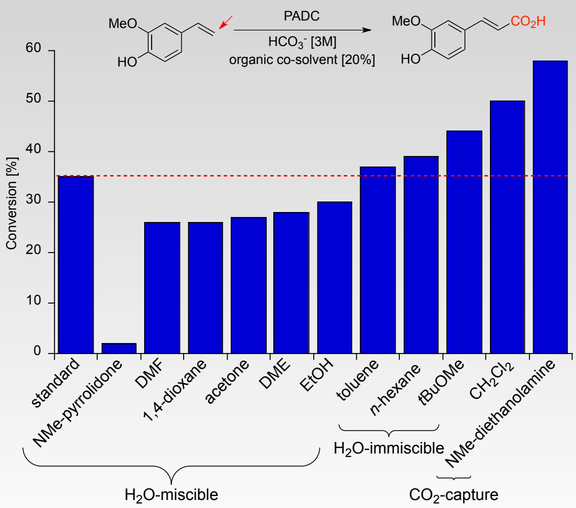
Phenolic Acid Decarboxylases: Active Site Mutants





Driving the Equilibrium: Organic Co-Solvents

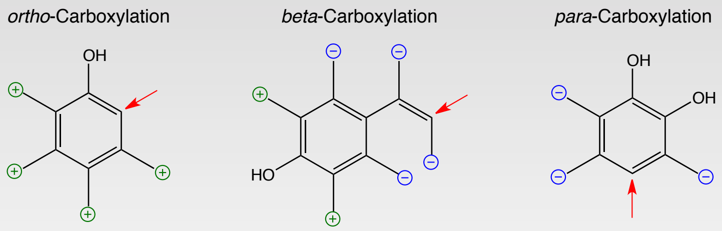
J. Biotechnol. **2013**, *168*, 264.



Regio-Complementary Enzymatic Carboxylation: Substrate Tolerance

Substituents ⊕ allowed ⊖ forbidden

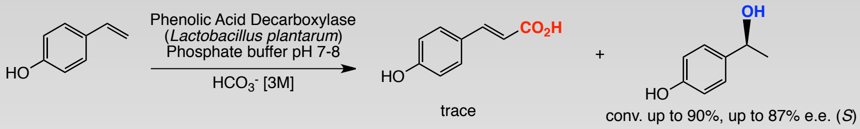
→ Carboxylation site

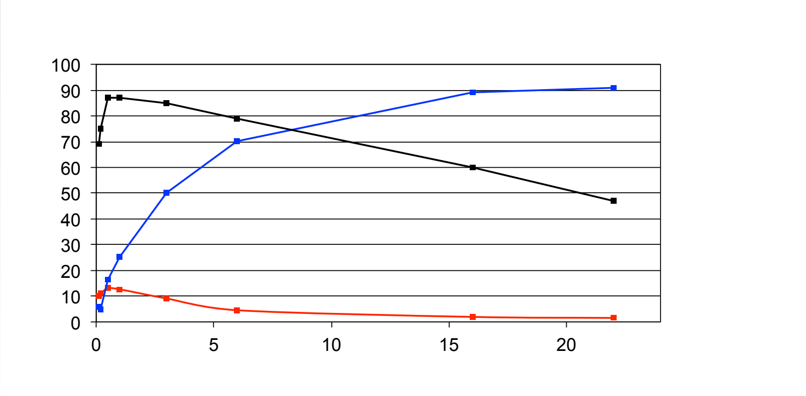


mutants at work...

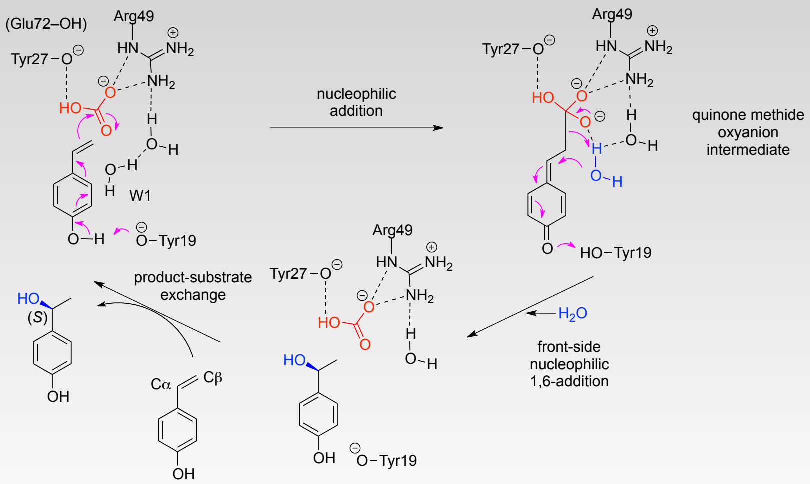
Phenolic Acid Decarboxylases: something went wrong!

Angew. Chem. Int. Ed. **2013**, *52*, 2293



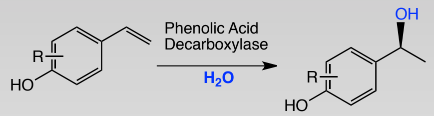


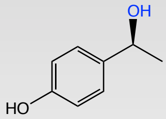
β-Carboxylation versus Hydration: Catalytic Promiscuity



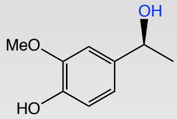
Asymmetric Hydration of Styrenes: Substrate Scope

Angew. Chem. Int. Ed. **2013**, *52*, 2293

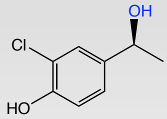




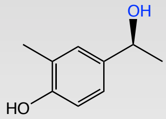
c 90%, ee 87%



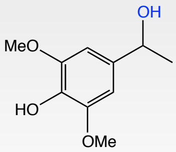
c 30%, ee 50%



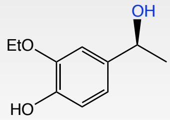
c 73%, ee 36%



c 34%, ee 71%



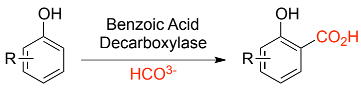
c <1%



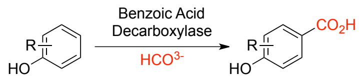
c 17%, ee 10%

Regio-Complementary Enzymatic Carboxylation:

o-Carboxylation:

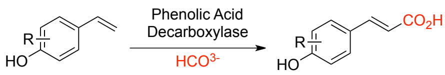


p-Carboxylation:

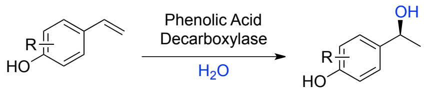


biocatalytic equivalent to Kolbe-Schmitt reaction

β-Carboxylation:



Asymmetric Enzymatic Hydration:



no chemical equivalent available

The Fab-Crew

BIOCATALYSIS	SUPPORT	STRU-BIOLOGY
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T. Reiter	B.-G. Kim	T. Pavkov
C. Wünsch	A. Liese	G. Steinkellner
	L. Pesci	
	K. Zangger	

MONEY:	FWF	FFG