CHEM-E4109
MODERN METHODS IN BIOCATALYSIS

chapter #3: oxygenative enzymes

8.3.2022

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Bioorganic
Chemistry

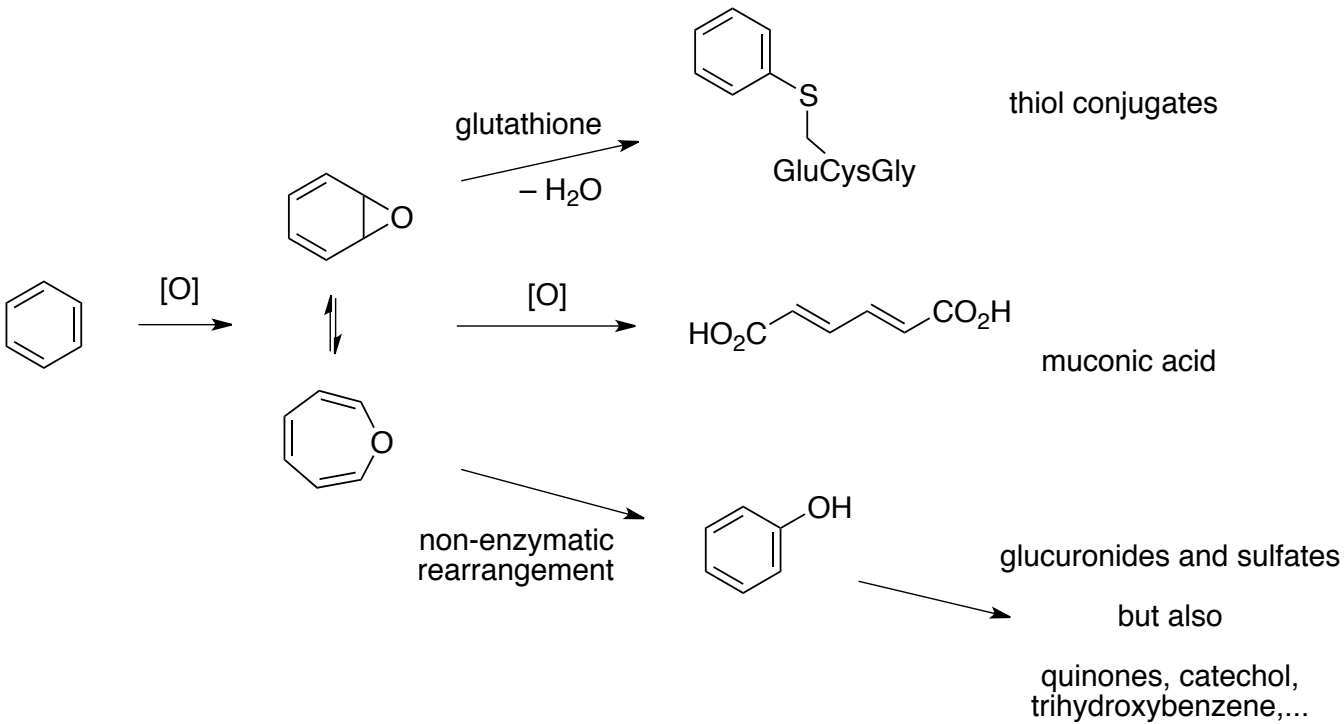
TODAY'S MENU

Content

- mono- & dioxygenases
- mechanistic comparison between iron-centred oxidizing enzymes and metal-free riboflavin-dependent biocatalysts
- blueprint for both transition metal-mediated and organocatalytic oxidations

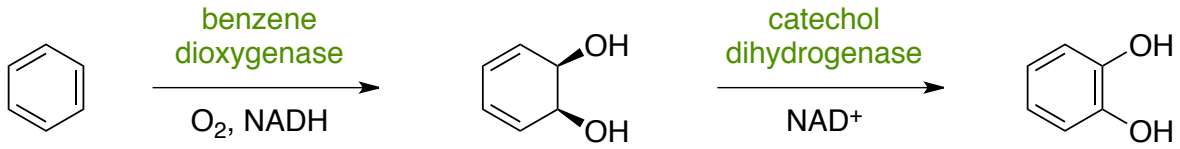
Arene dioxygenases

aromatic hydrocarbons like benzene are metabolized by mammals via initial oxygenation



Arene dioxygenases

certain strains from Pseudomonas putida accumulate cis-dihydroxycyclohexadienes



cis-dihydroxycyclohexadiene catechol

Gibson, Koch, Schuld, Kallio, *Biochem.* **1968**, *7*, 3795-3802.

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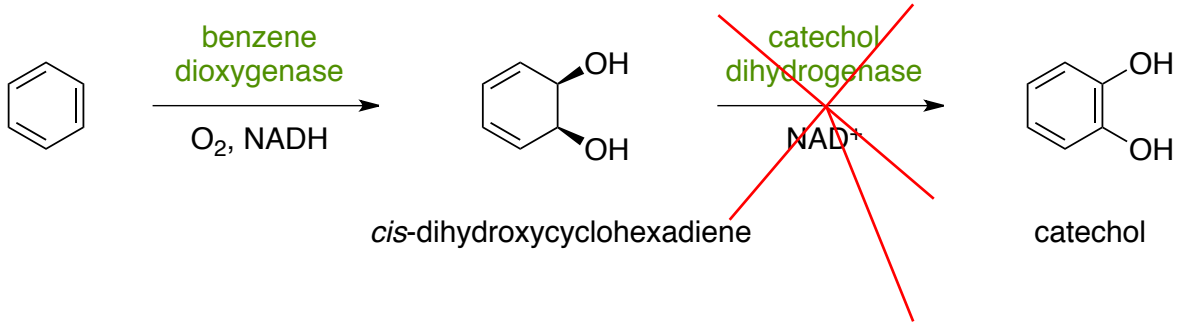

cis-dihydroxycyclohexadiene

catechol

P. putida 39/D and F1 mutants grow on toluene as carbon source but suffer from a somewhat sluggish dehydrogenase

Gibson, Koch, Schuld, Kallio, *Biochem.* **1968**, *7*, 3795-3802.

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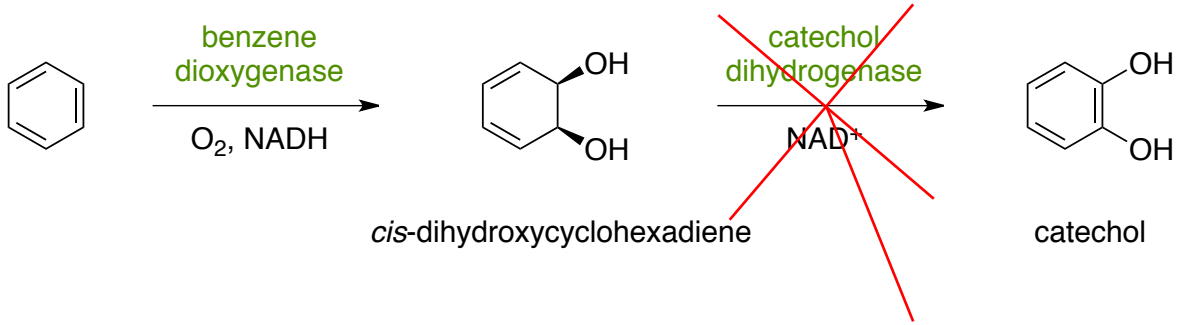
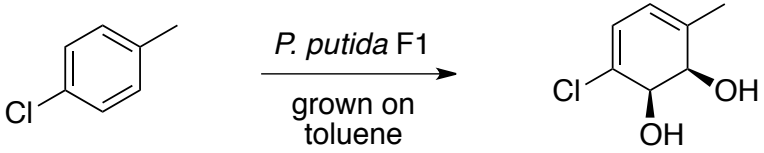


P. putida 39/D and F1 mutants grow on toluene as carbon source but suffer from a somewhat sluggish dehydrogenase

- "biosynthesis" of complex organic building blocks



diastereoselectivity: pure *cis*

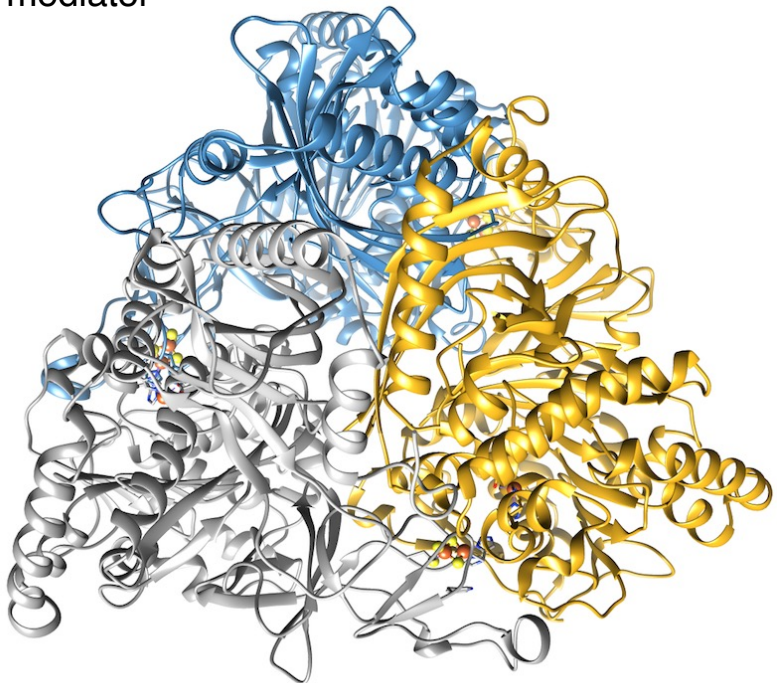
enantioselectivity: pure (+)

Gibson, Koch, Schuld, Kallio, *Biochem.* **1968**, *7*, 3795-3802.

Arene dioxygenases

Naphthalene dioxygenase (*Pseudomonas* sp. NCIB 9816-4)

- hetero-hexamer, 210 kDa, $\alpha_3\beta_3$
- 3 identical iron-binding α-subunits, two different iron species
- Rieske iron-sulfur cluster as electron mediator
- non-heme mononuclear iron
  as oxygen binding center
- requires additional reductase
  Fe-S-cluster + FAD
  (not shown)
- NADH as terminal reductant



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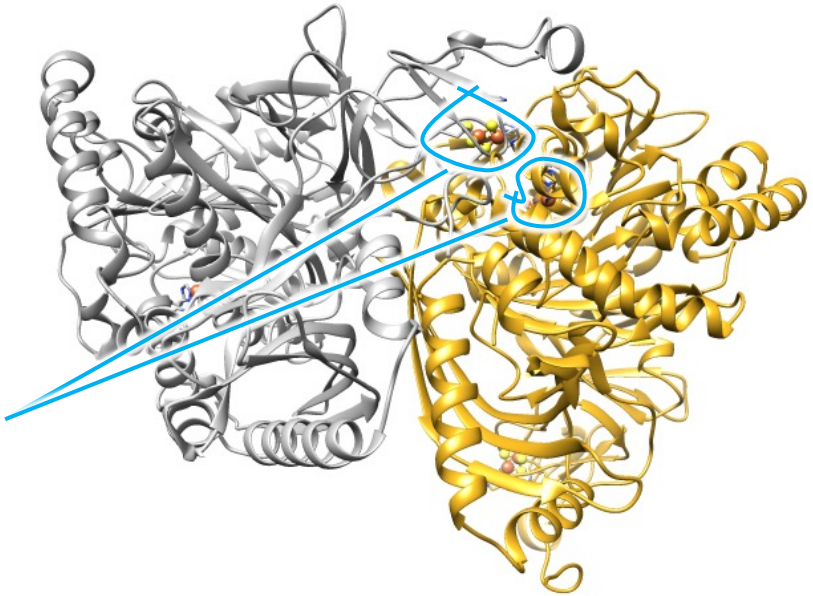
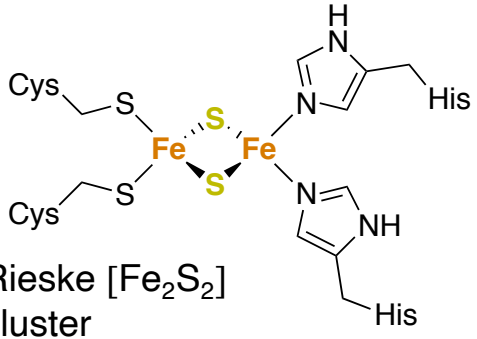


- Rieske cluster from one α-subunit
  teams up with mono-nuclear iron
  from another α-subunits

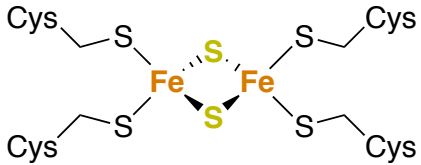
Arene dioxygenases

"biological capacitors"

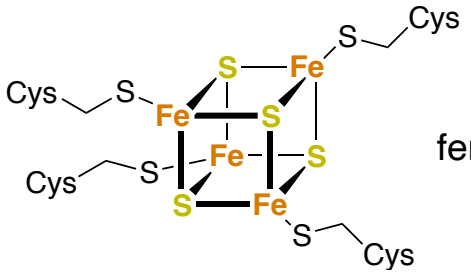
- electron transfer often proceeds via cascade processes involving organic mediators (FAD/FMN) or metallic clusters (Rieske, ferredoxin); or both



Rieske [Fe_2S_2] cluster



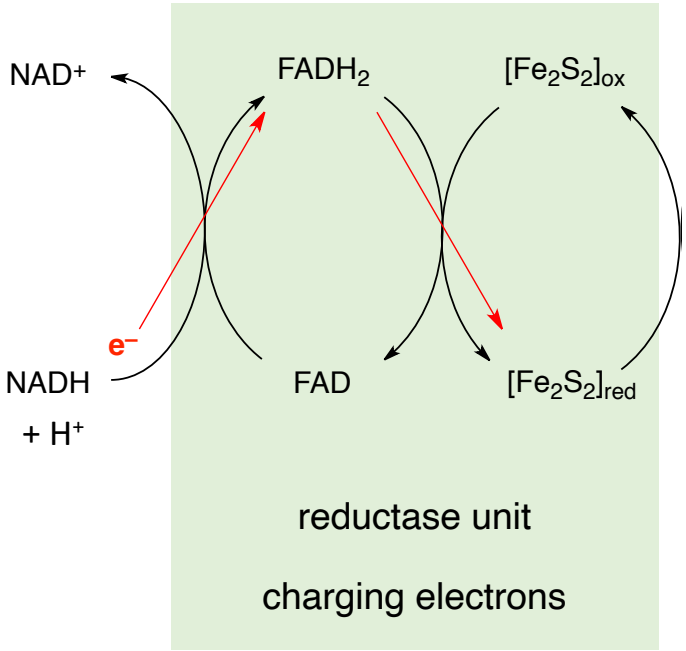
ferredoxin [Fe_2S_2] cluster



ferredoxin [Fe_4S_4] cluster

Arene dioxygenases

schematic electron flow in a naphthalene dioxygenation



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Arene dioxygenases

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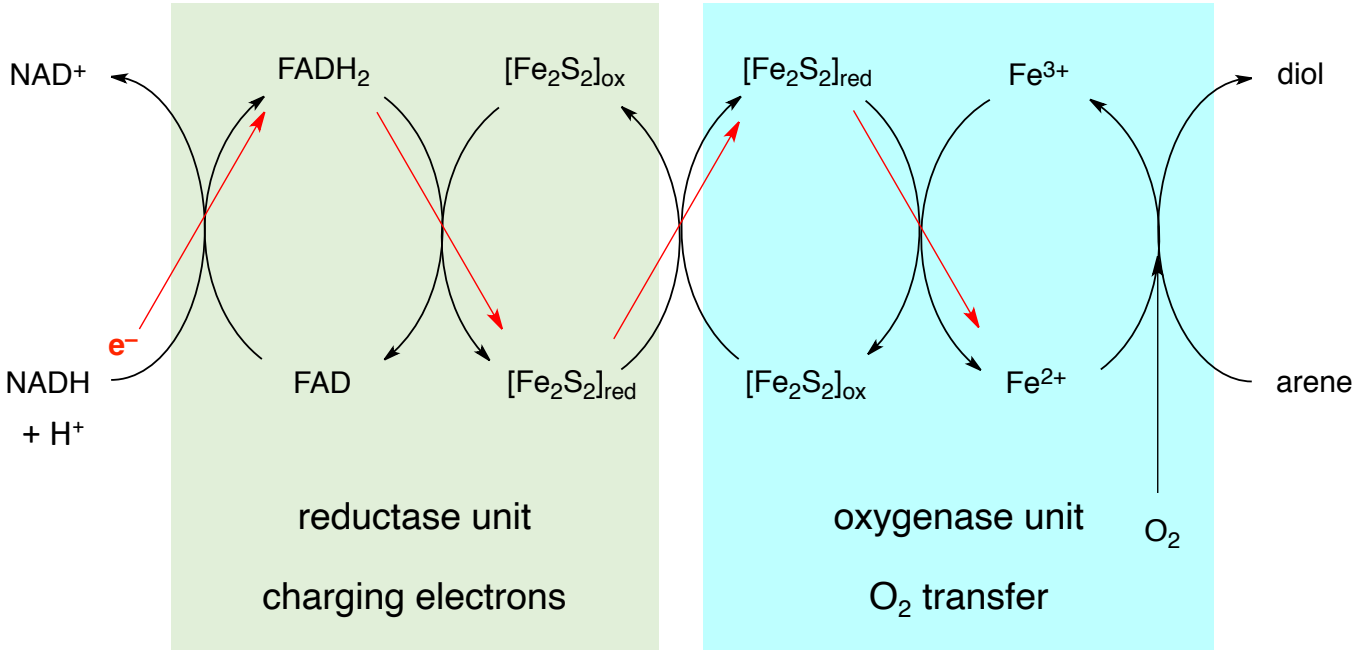

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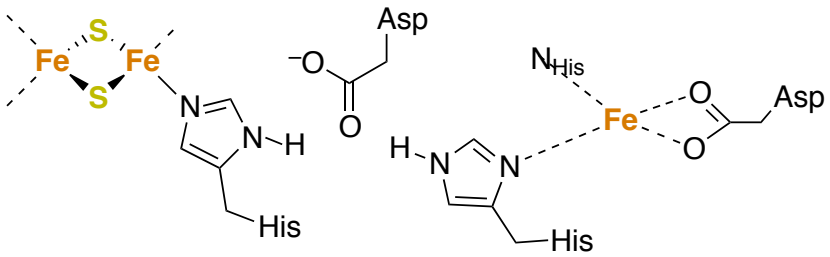
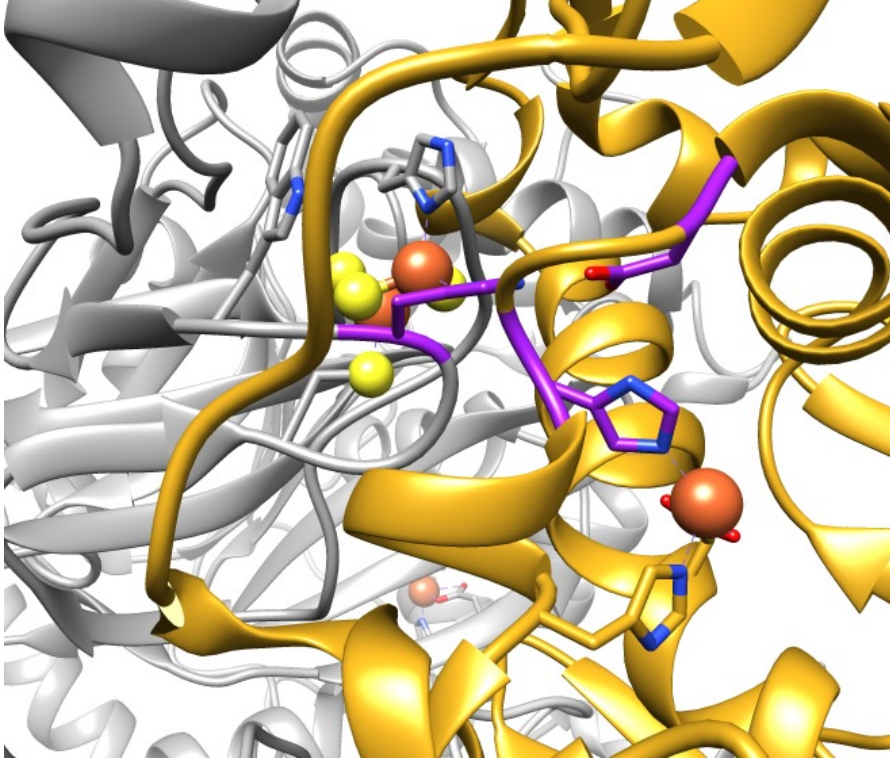
Arene dioxygenases

Mechanism

- charged [Fe_2S_2] cluster transfers electron onto mono-nuclear iron
- communication between both centres (and both subunits!) via His-Asp-His alignment (purple)
- reduced iron centre recombines with O_2





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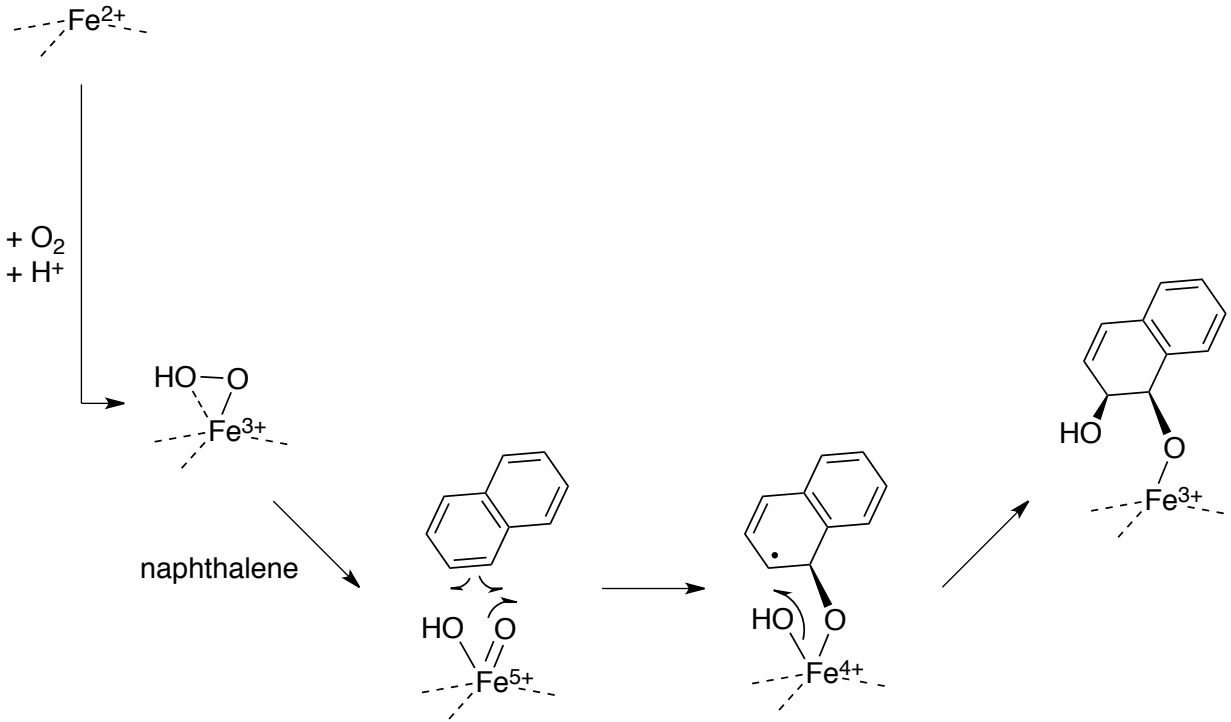
Arene dioxygenases

Mechanism: two proposed pathways for oxygen transfer



Bugg, Ramaswamy, *Curr. Opin. Chem. Biol.* **2008**, *12*, 134-140.

Arene dioxygenases

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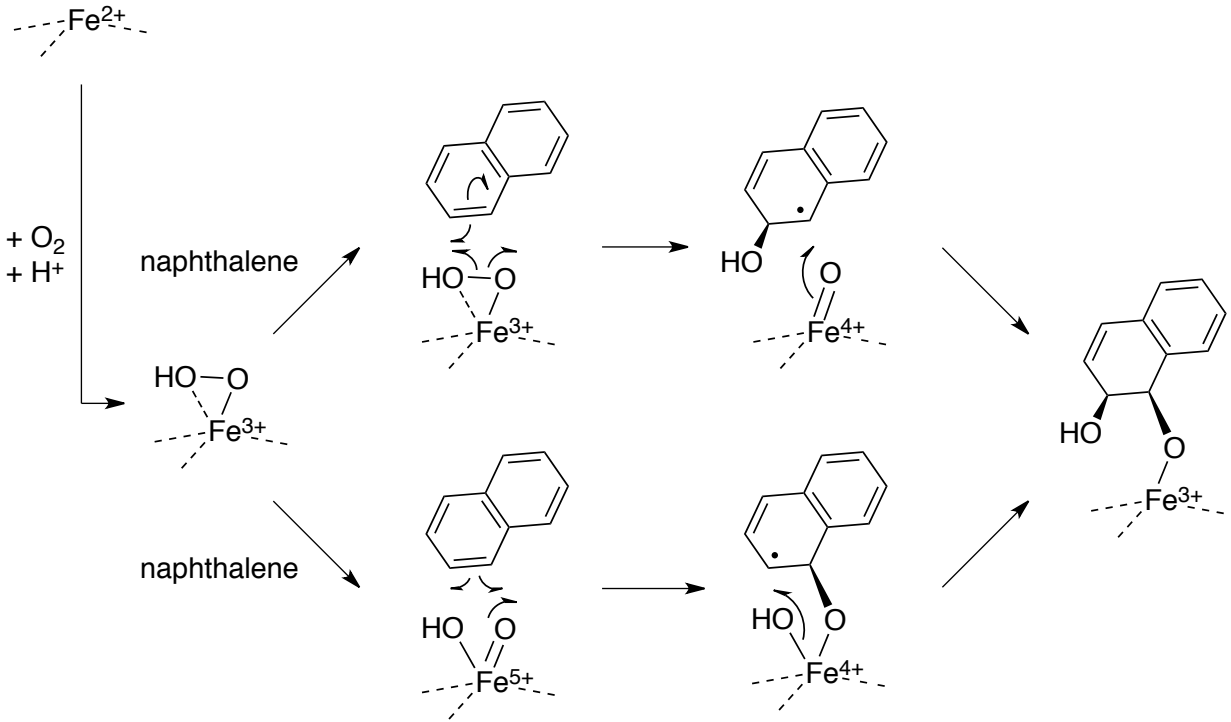

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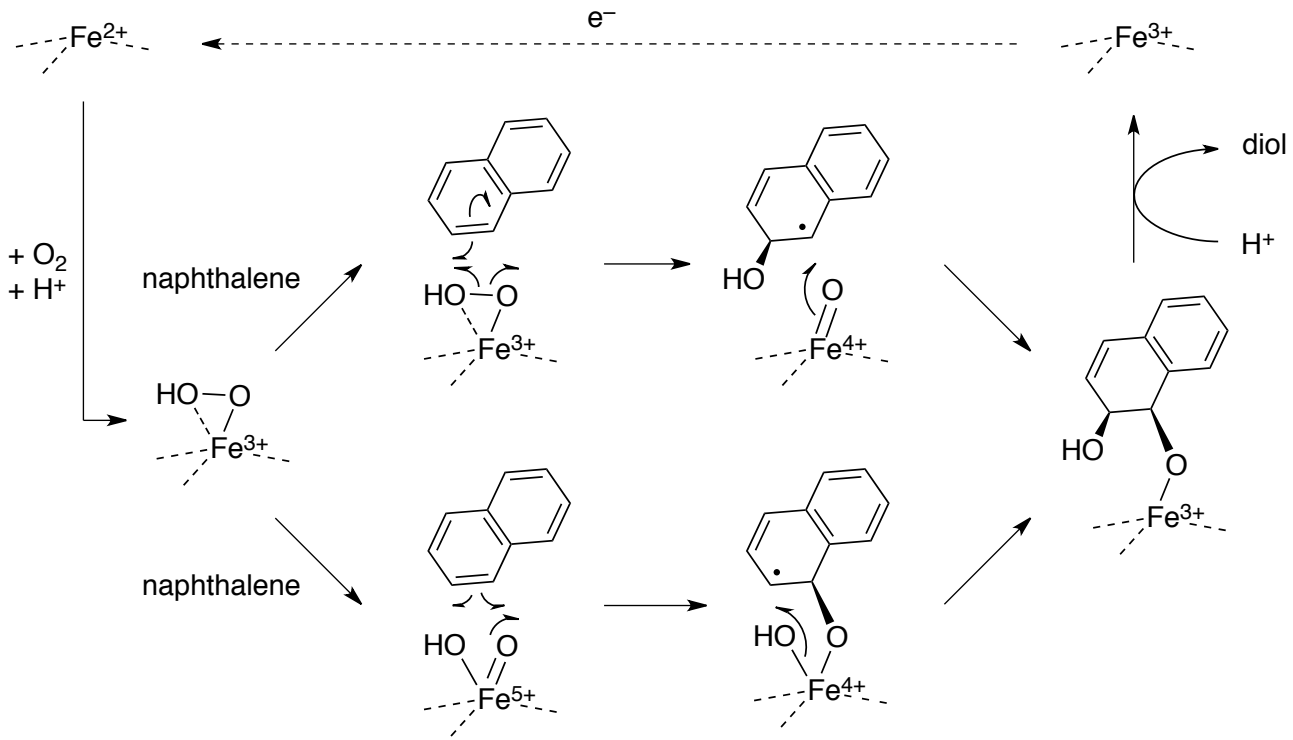
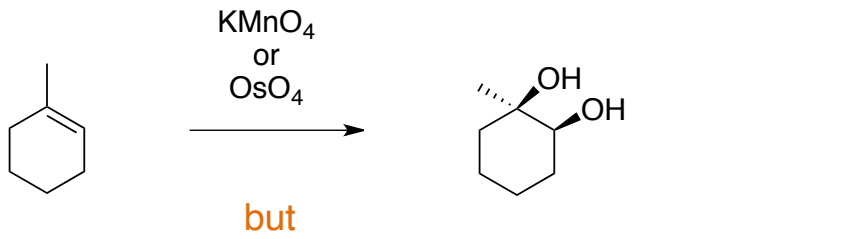


Bugg, Ramaswamy, *Curr. Opin. Chem. Biol.* **2008**, *12*, 134-140.

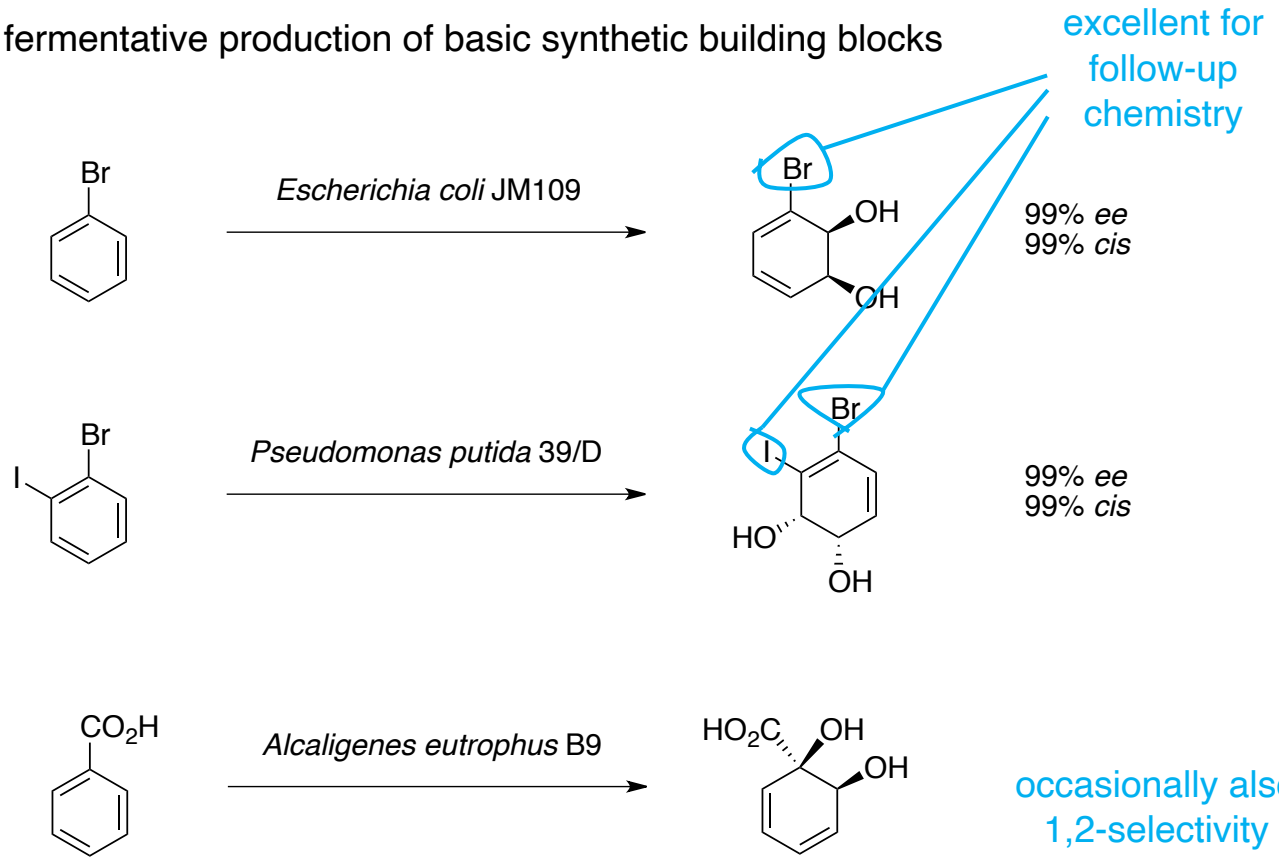
Synthetic use of arene dioxygenases

- nowadays various arene DOs with broad substrate scope known and working (benzene DO, toluene DO, naphthalene DO)
- particular knock-out mutants allow for selective production of cis-diols without catechol side products (biotech. removal of catechol dehydrogenase)
- absolutely no equivalent chemical method known!



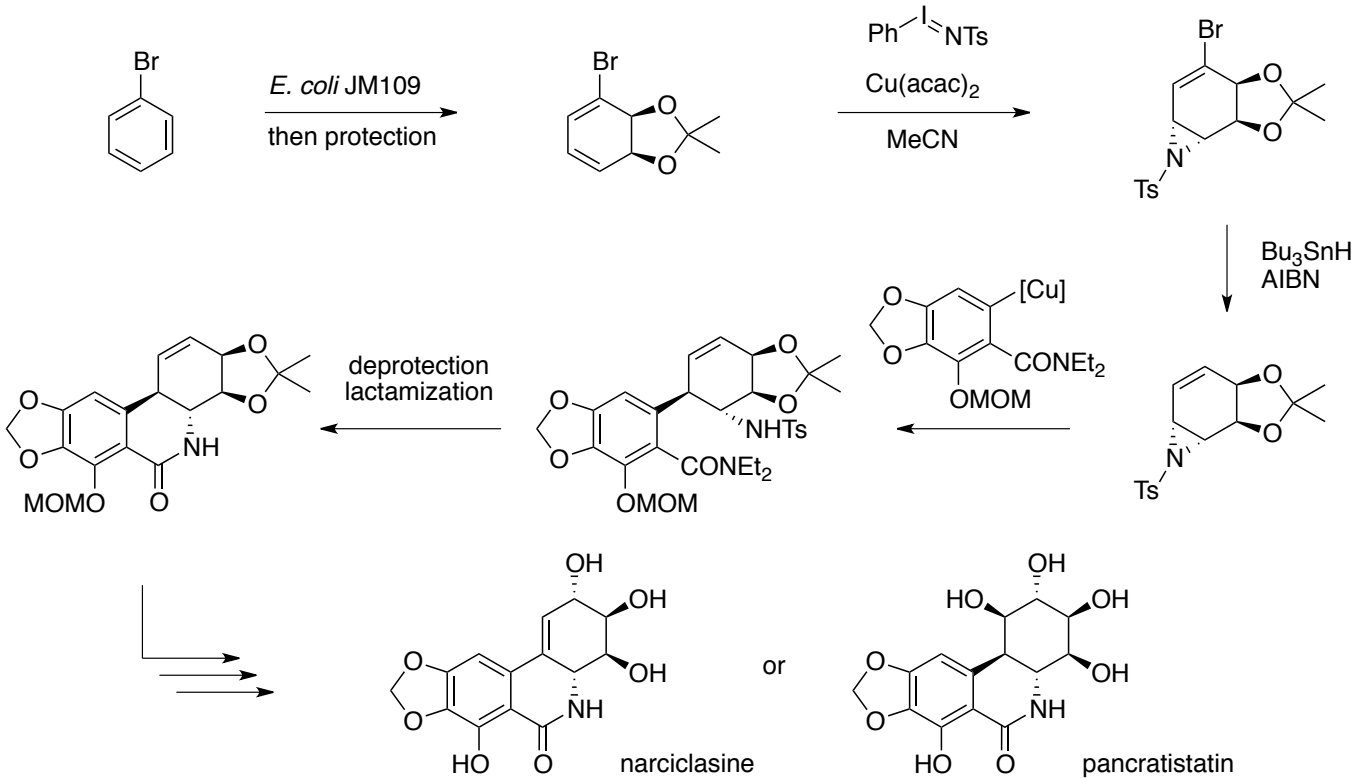
Synthetic use of arene dioxygenases

fermentative production of basic synthetic building blocks



Synthetic use of arene dioxygenases

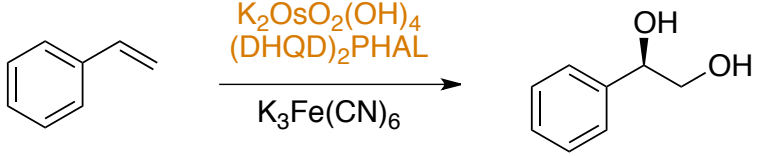
Synthesis of antitumor agents (pancratistatin family), Hudlicky 1996

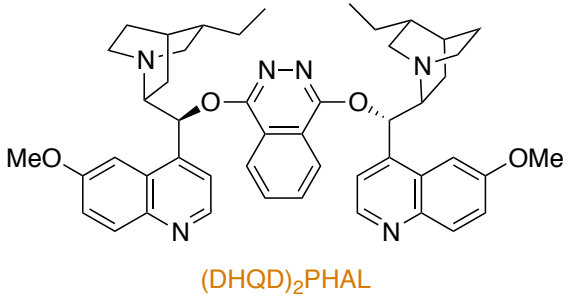


Hudlicky, Tian, Königsberger, Maurya, Rouden, Fan, *J. Am. Chem. Soc.* **1996**, *118*, 10752-10765.

Activity on non-arene substrates

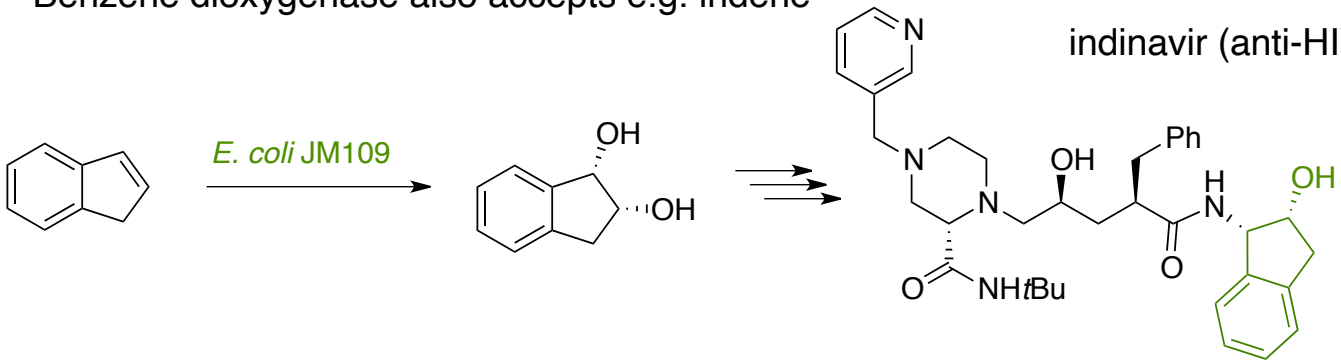
Chemical asymmetric *cis*-dihydroxylation (Sharpless dihydroxylation)





$(DHQD)_2PHAL$

Benzene dioxygenase also accepts e.g. indene



indinavir (anti-HIV)

Gally, Nestl, Hauer, *Angew. Chem. Int. Ed.* **2015**, *54*, 12952-12956.

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Monooxygenases

flavin dependent monooxygenases

- purely "organocatalytic" activation via flavin-hydroperoxide
- catalyzes oxygen-transfer processes such as sulfoxidation and N-oxygenation but also Baeyer-Villiger reactions and epoxidations of activated olefins
- NAD(P)H-dependent, without co-enzyme (reductase) requirement

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- NAD(P)H-dependent, without co-enzyme (reductase) requirement

heme-dependent monooxygenases

- iron porphyrine as terminal reactive species
- catalyzes all kinds of oxygen-transfer processes but also radical initiations, dealkylations, etc.
- NAD(P)H-dependent
- electron transfer requires often complex cascades involving flavin and ferredoxin-containing domains and additional reductases

Baeyer–Villiger monooxygenases

Baeyer-Villiger reaction

- formation of esters or lactones from acyclic or cyclic ketones, respectively
- most common oxidant: peracids
- reaction proceeds via acylperoxy adduct

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Baeyer–Villiger monooxygenases

Baeyer-Villiger reaction

- formation of esters or lactones from acyclic or cyclic ketones, respectively
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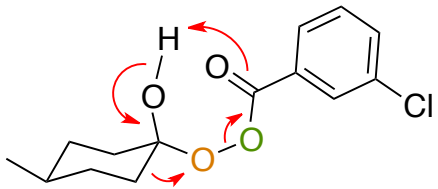
- Criegee rearrangement as key step



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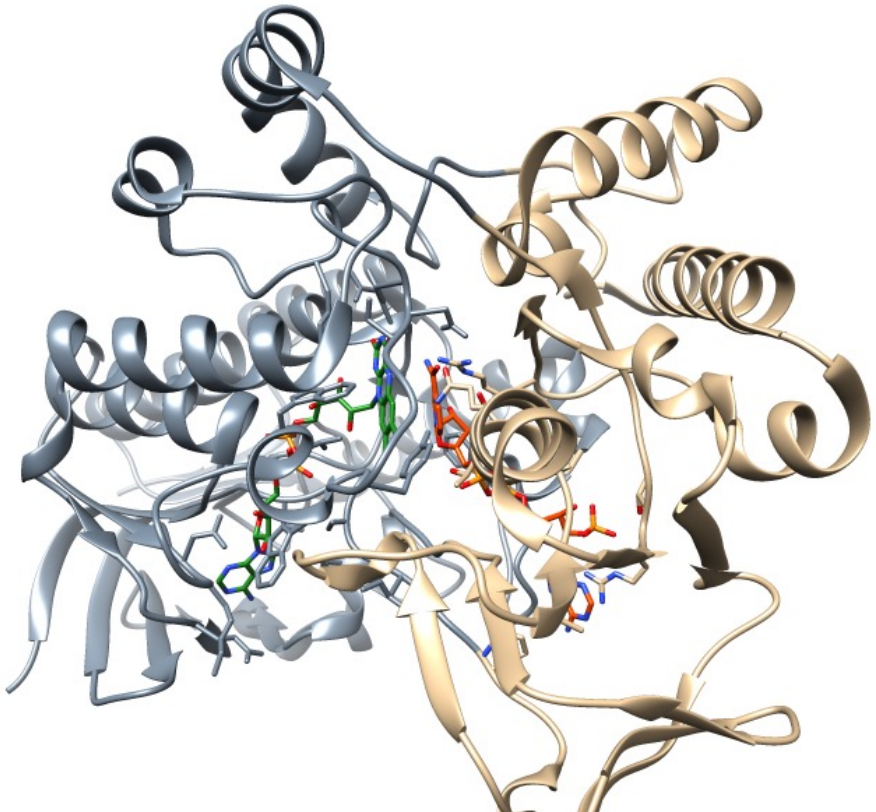
Baeyer–Villiger monooxygenases

Cyclohexanone monooxygenase (*Acinetobacter* sp. NCIB 9871)

- monomer, 59 kDa
- large FAD-binding domain
- smaller NADPH-binding domain
- flavin and nicotinamide stacked close to each other
- but quite dynamic with large domain movements



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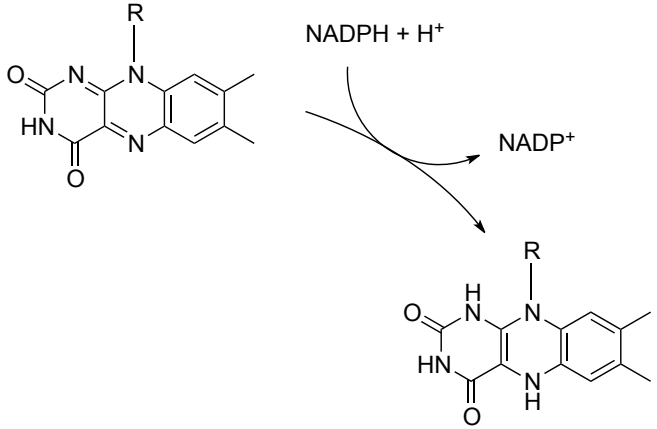
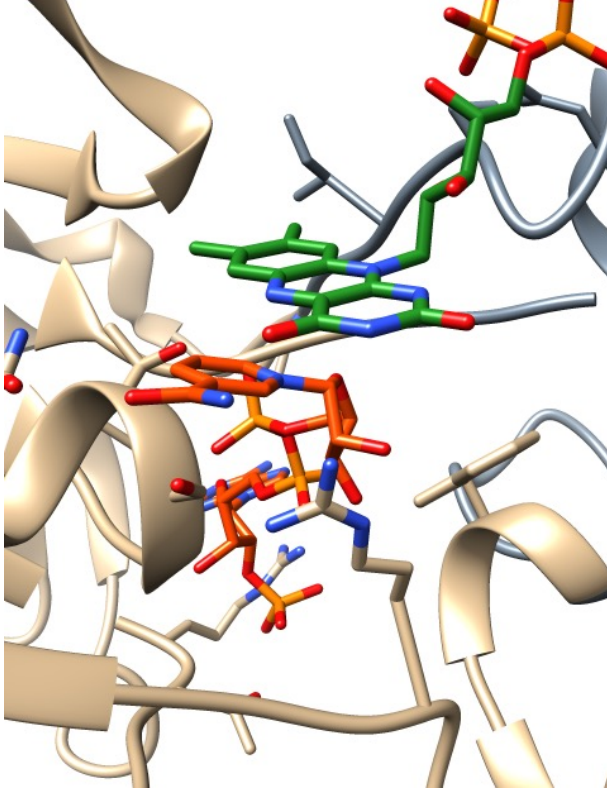
Baeyer–Villiger monooxygenases

Mechanism





Kamerbeek, Janssen, van Berkel, Fraaije, *Adv. Synth. Catal.* **2003**, *345*, 667-678.

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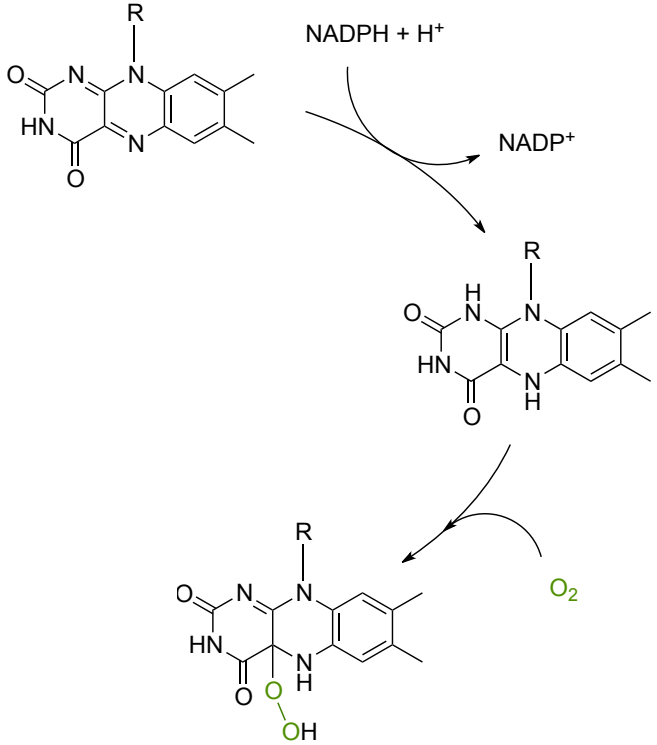

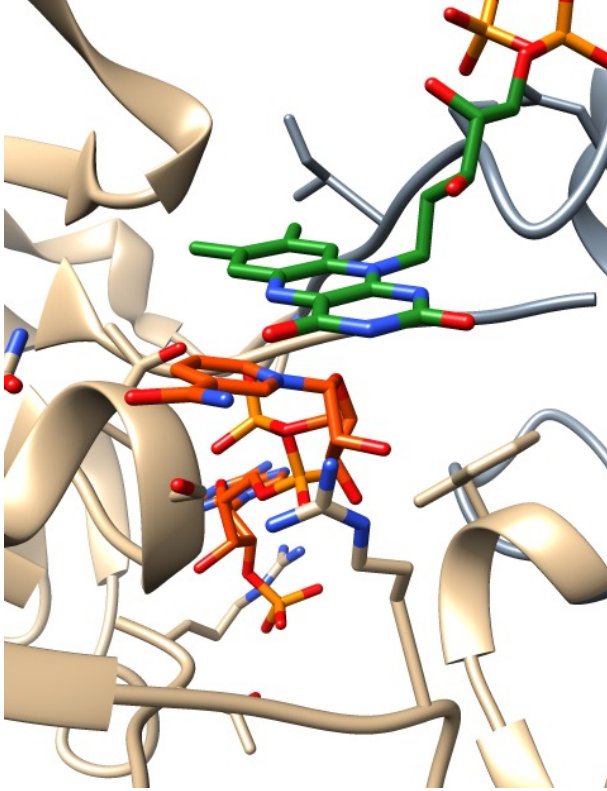

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Baeyer–Villiger monooxygenases

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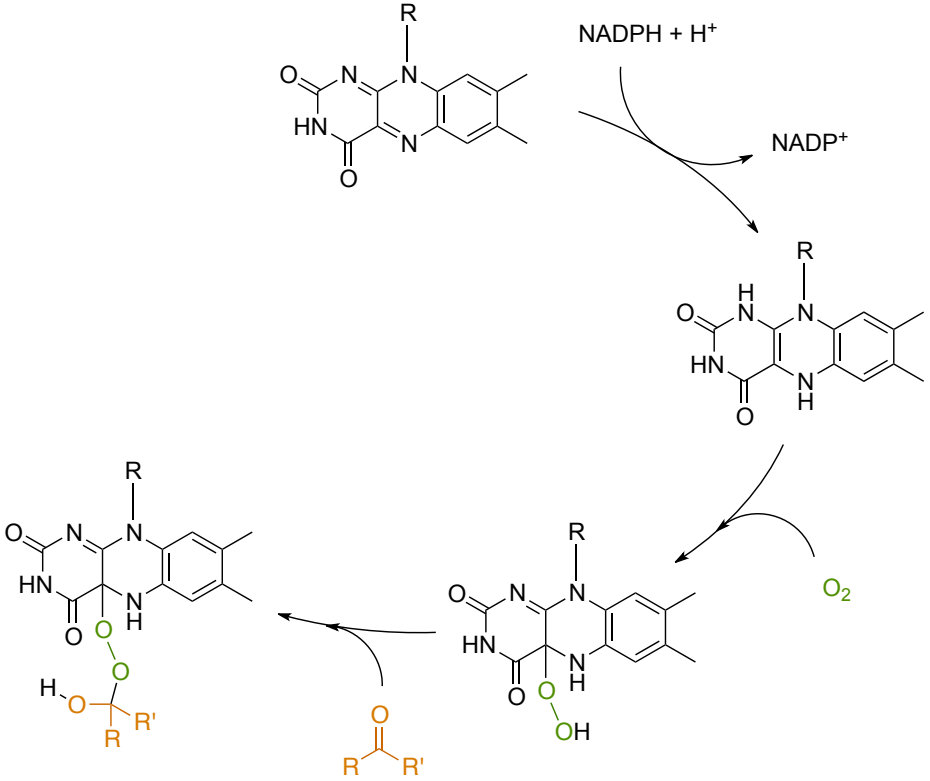

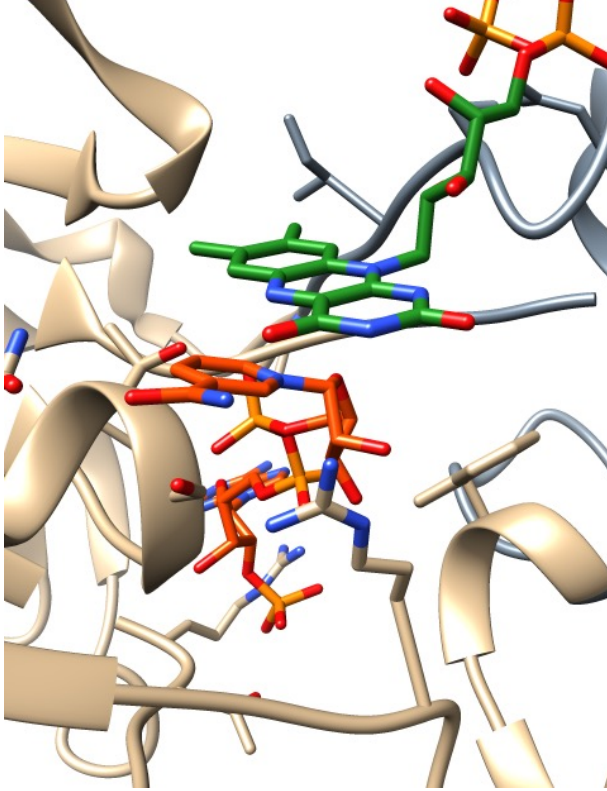

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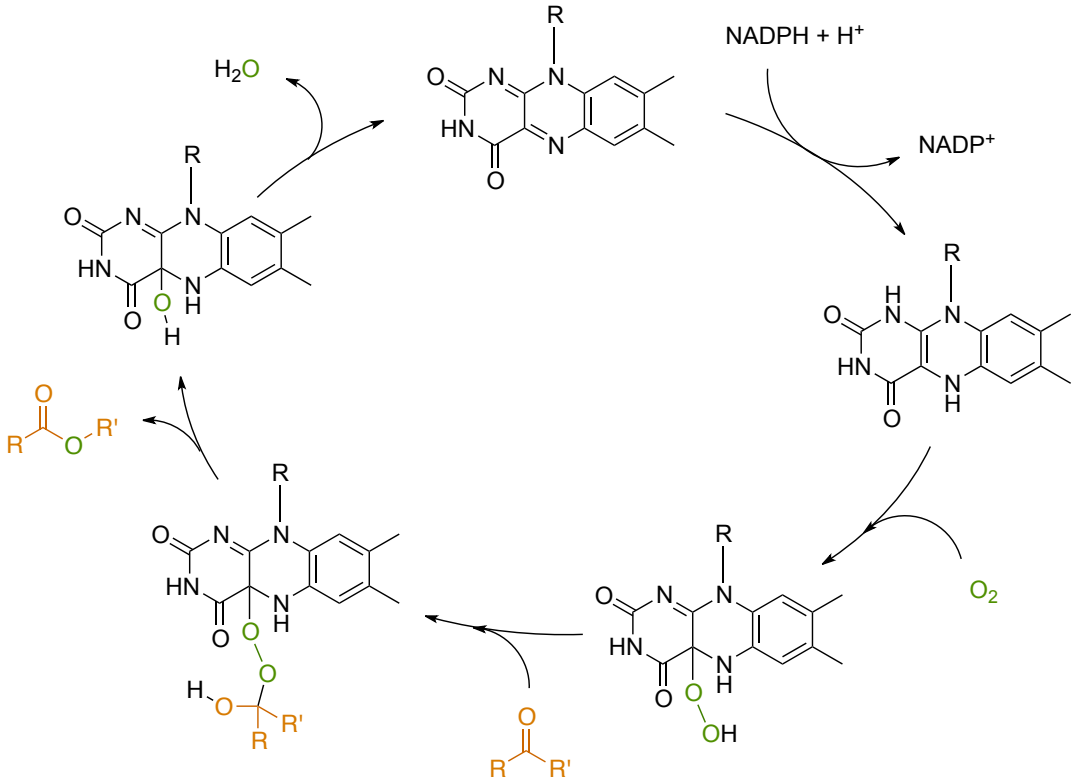

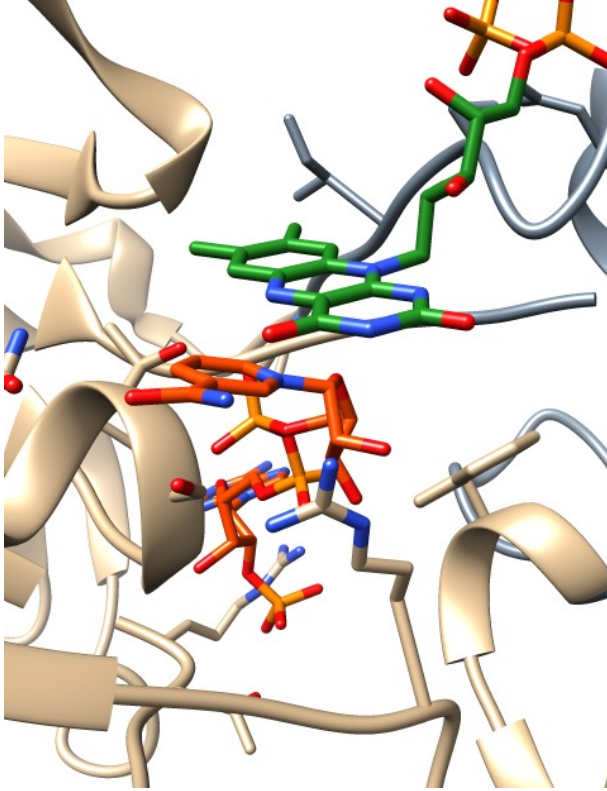

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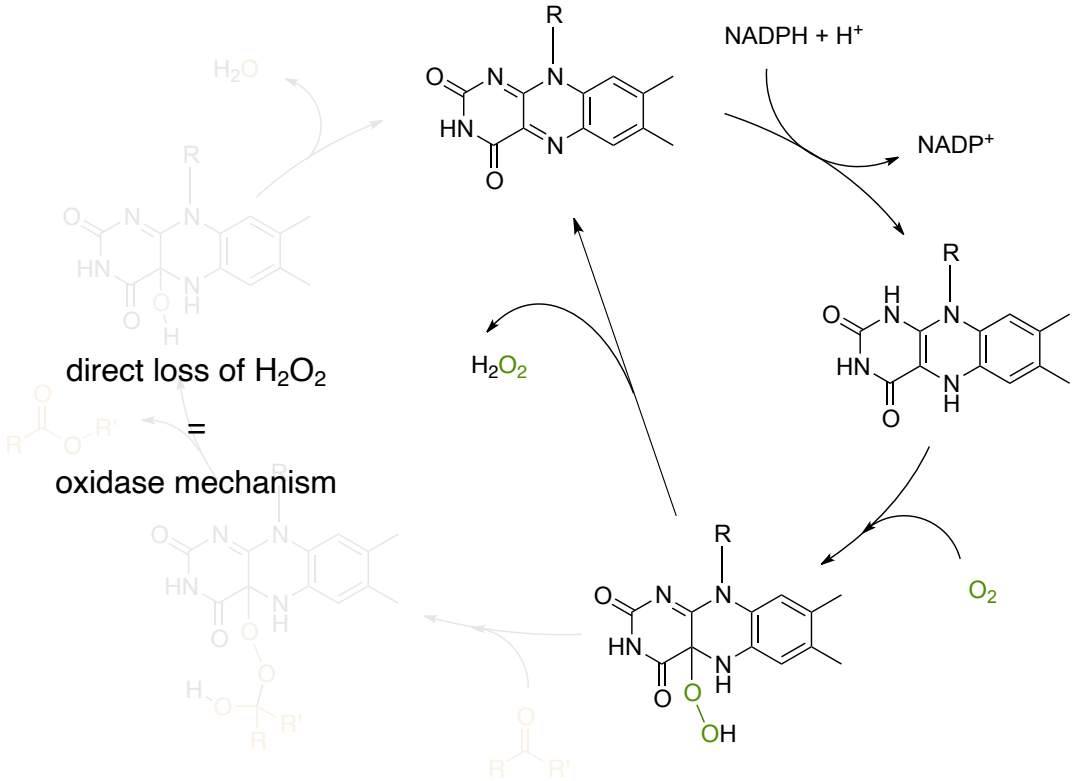

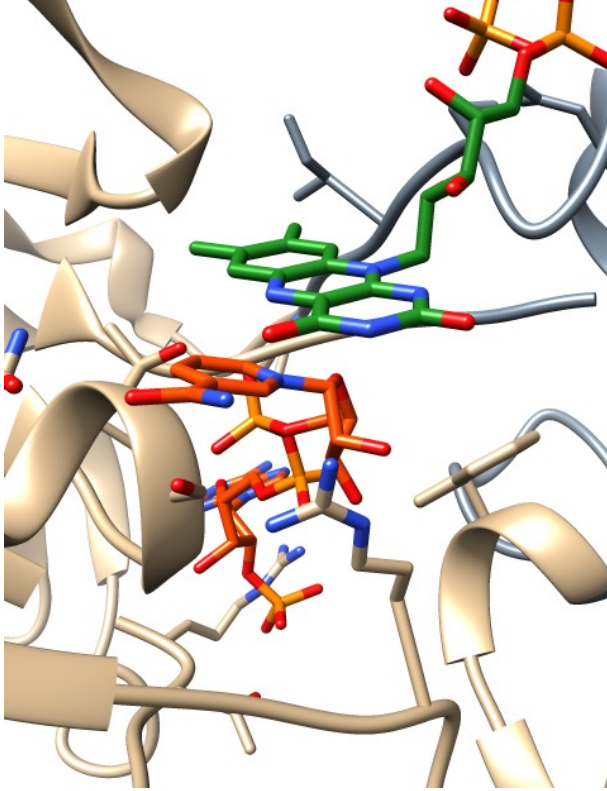

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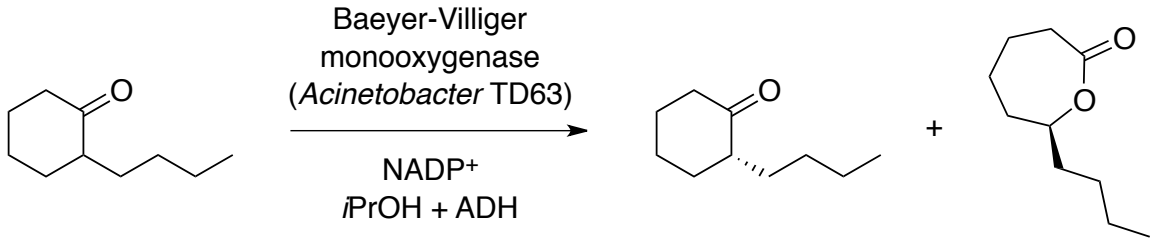
Asymmetric Baeyer–Villiger transformations

kinetic resolution of chiral ketones



36%, >98% *ee*

58%, 74% *ee*

Alphand, Furstoss, Pedragosa-Moreau, Roberts, Willetts, *J. Chem. Soc., Perkin Trans 1* **1996**, 1867-1872.

Asymmetric Baeyer–Villiger transformations

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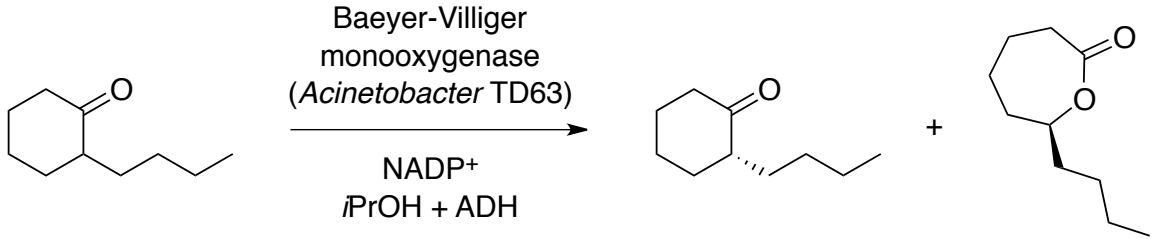
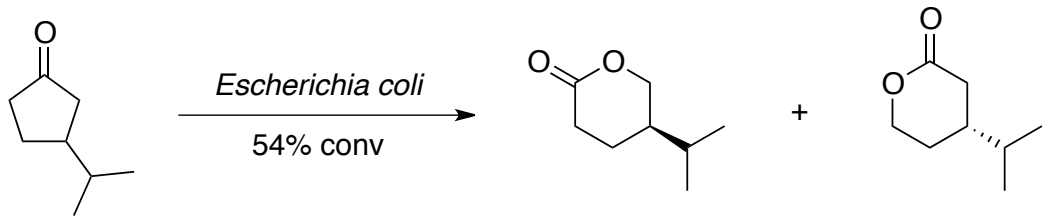


36%, >98% *ee* 58%, 74% *ee*

occasionally both enantiomers react... to give two different products



33%, 97% *ee* 67%, 93% *ee*

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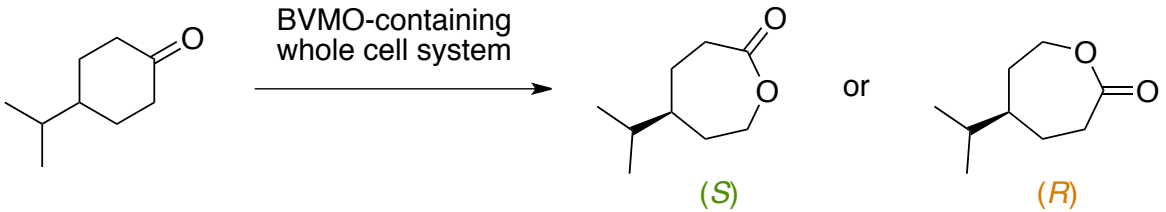
Alphand, Furstoss, Pedragosa-Moreau, Roberts, Willetts, *J. Chem. Soc., Perkin Trans 1* **1996**, 1867-1872.

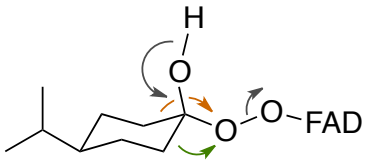
Asymmetric Baeyer–Villiger transformations

desymmetrization of achiral ketones



Acinetobacter sp. NCIB9871: 94% *ee* (*S*)

Aspergillus flavus: 84% *ee* (*R*)



Ferroni, Smit, Opperman, *J. Mol. Catal. B Enzym.* **2014**, *107*, 47-54.

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Asymmetric Baeyer–Villiger transformations

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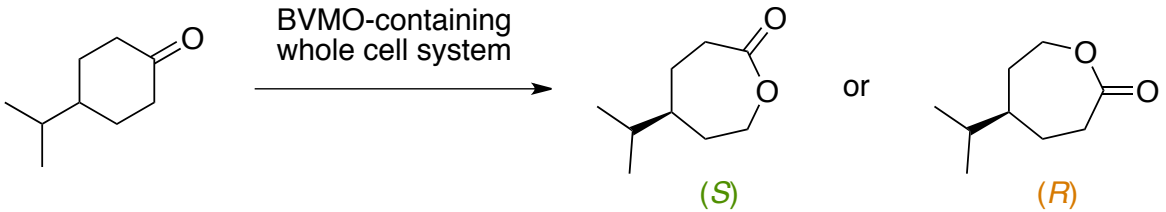

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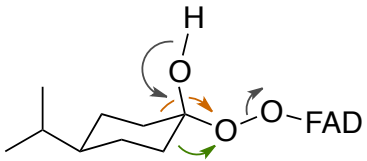

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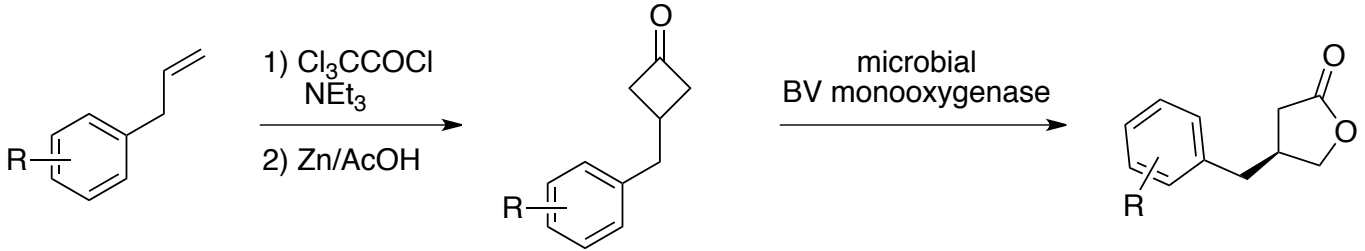
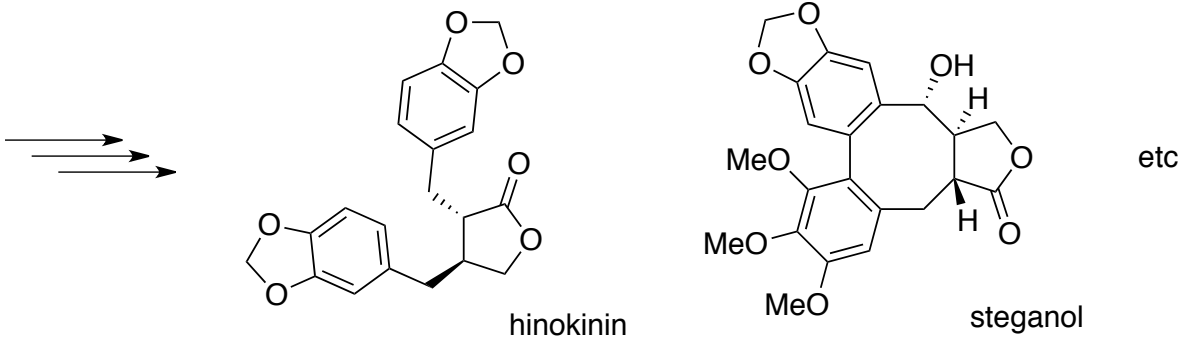
Asymmetric Baeyer–Villiger transformations

total synthesis of bioactive butyrolactones





Rudroff, Rydz, Ogink, Fink, Mihovilovic, *Adv. Synth. Catal.* **2007**, 349, 1436-1444.

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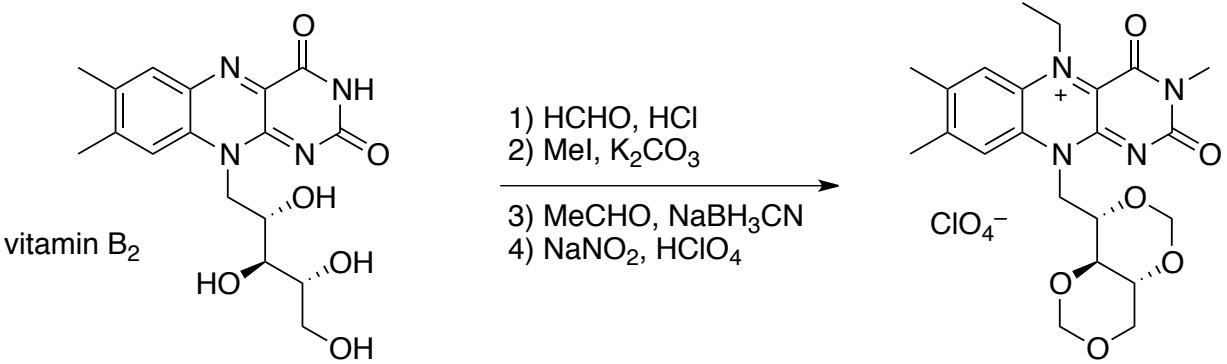
Biomimetic catalysis

modified flavins as organocatalysts



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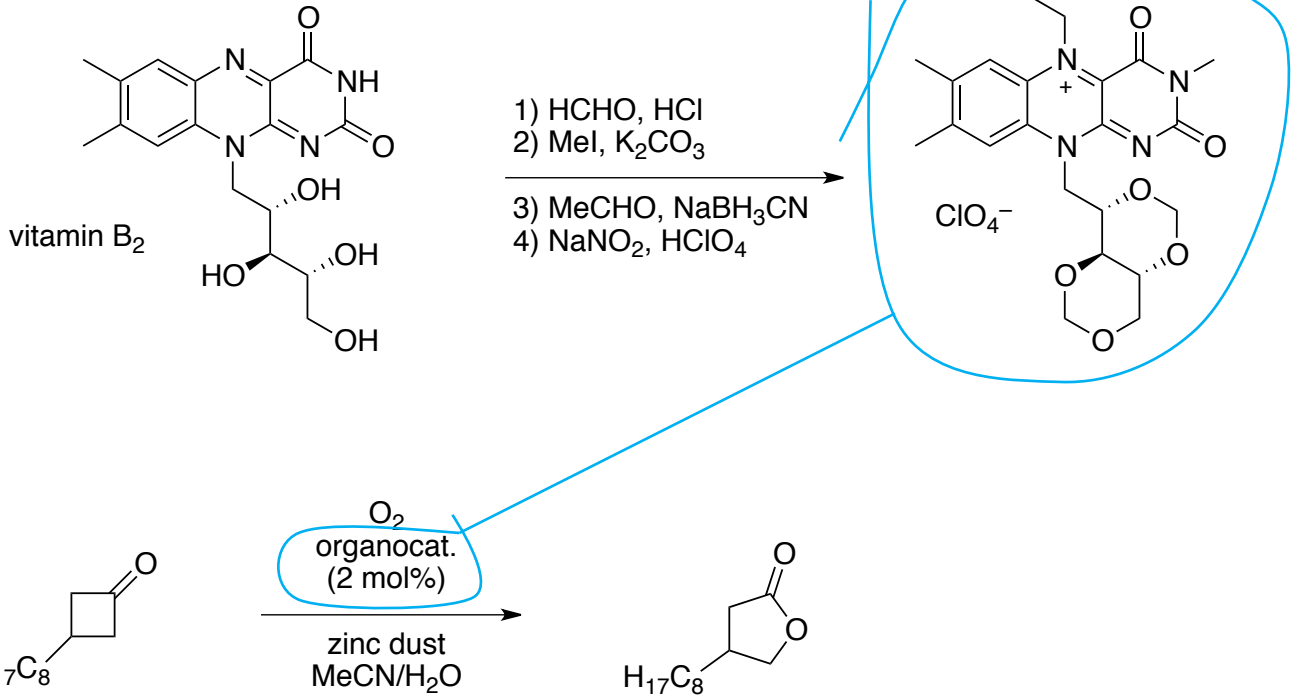

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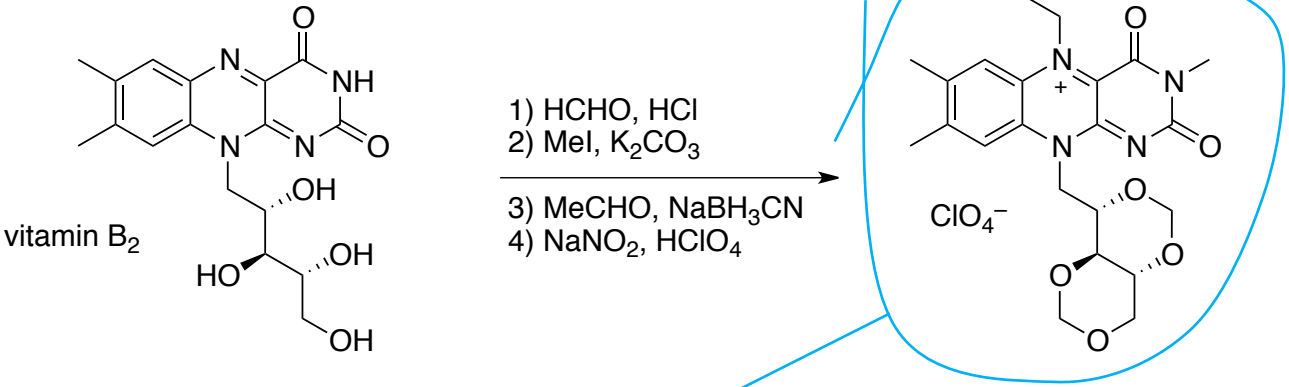

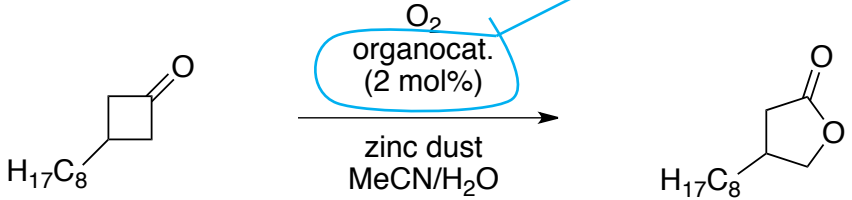

- no chiral induction (yet)
- but: more selective than other BV systems (e.g. olefins not epoxidized)

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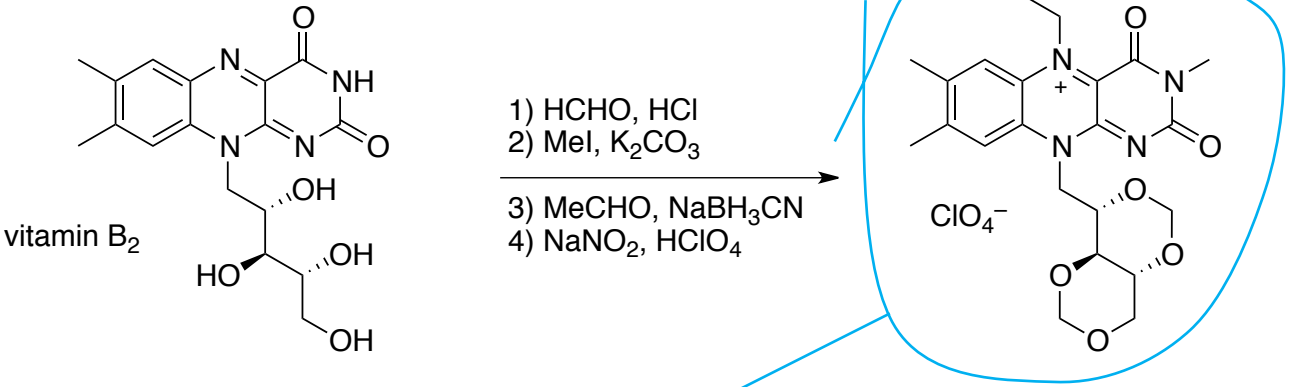

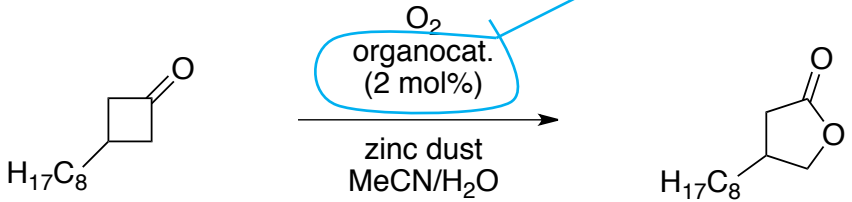

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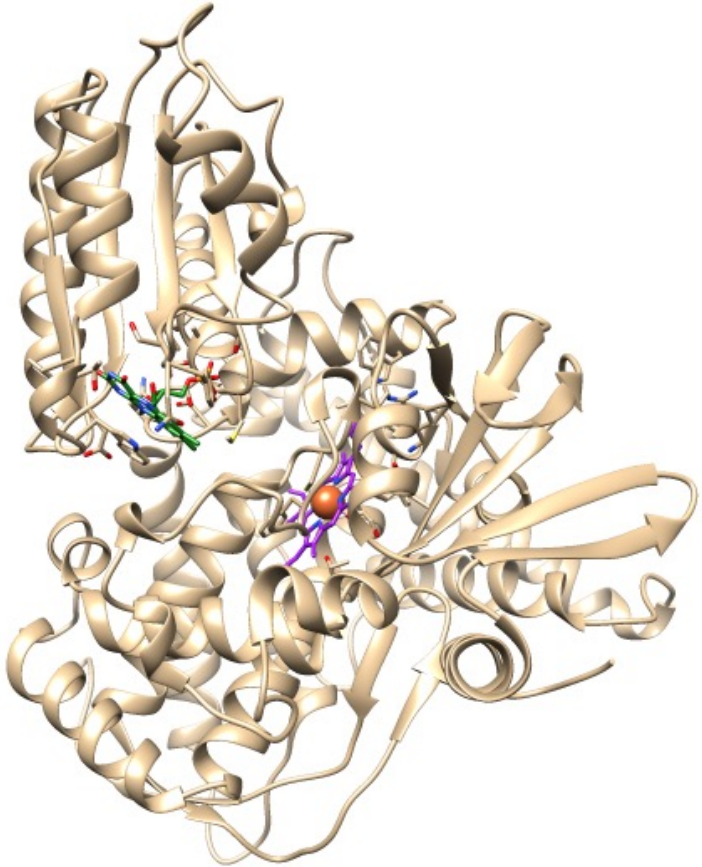
Cytochrome P450 monooxygenases

Cytochrome P450 BM3 (*Bacillus megaterium*)

- monomer, 119 kDa
- flavocytochrome
- FMN-binding domain fused to
- porphyrine-binding domain
- great exception among other CYPs
  as it requires no lengthy electron cascades
  commonly found for cytochrome systems

  (e.g. ferredoxin, ferredoxin reductase,
  cytochrome reductase,...)
- catalyzes epoxidations, sulfoxidations and
  CH-oxygenations, among others



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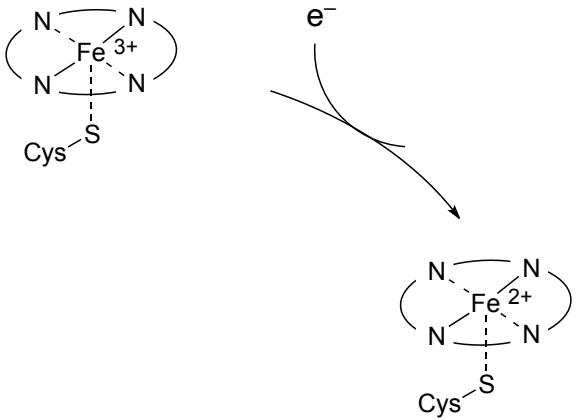
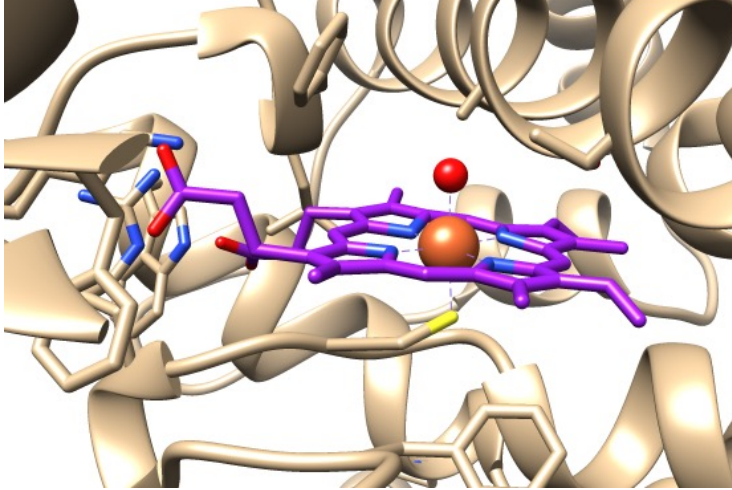
Cytochrome P450 monooxygenases

Mechanism





Meunier, de Visser, Shaik, *Chem. Rev.* **2004**, *104*, 3947-3980.

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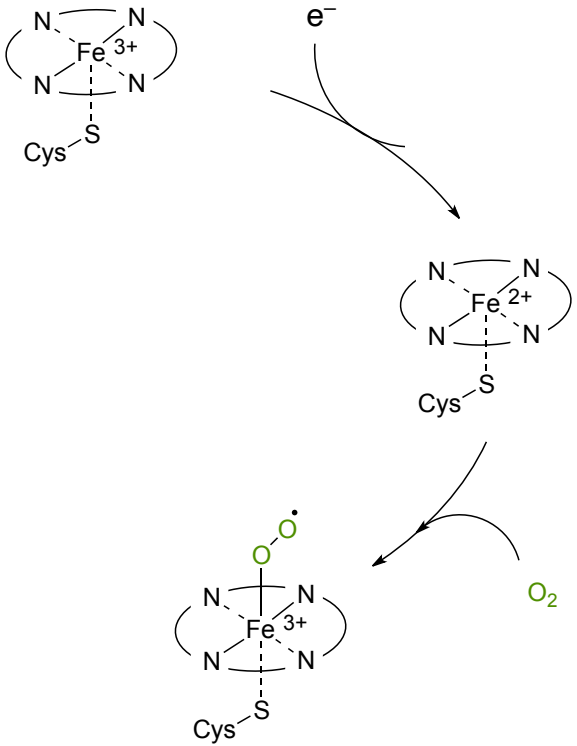

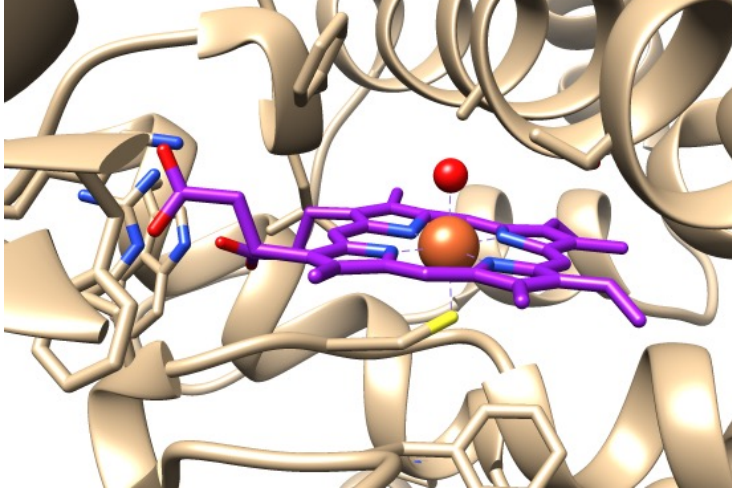

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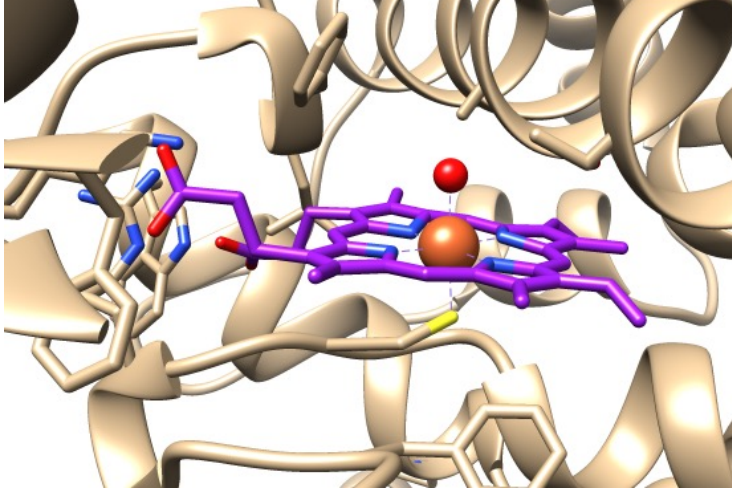

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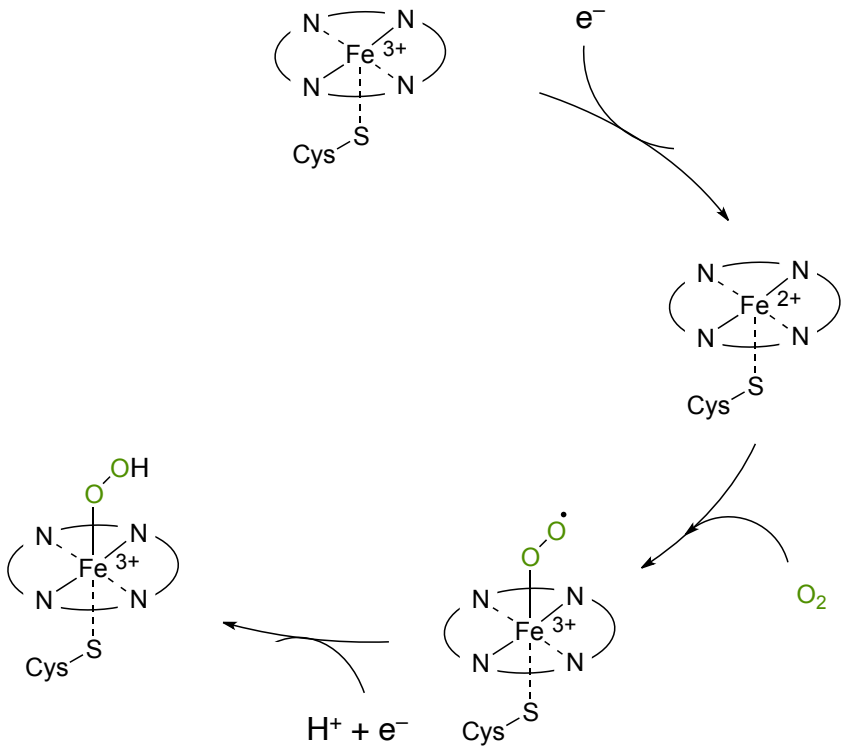

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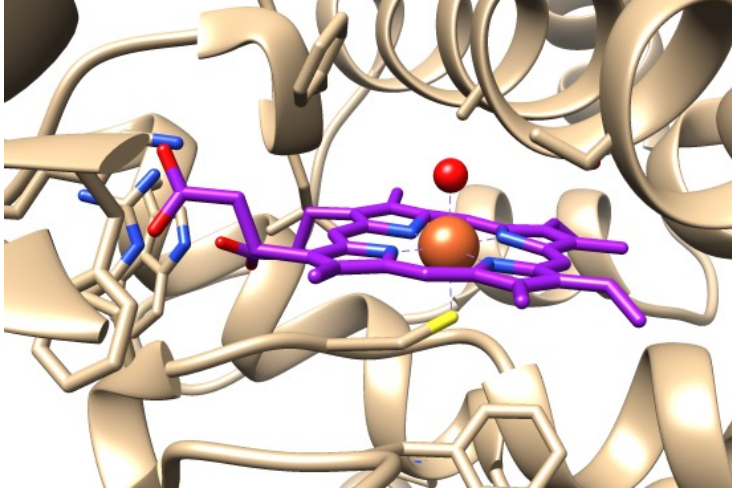

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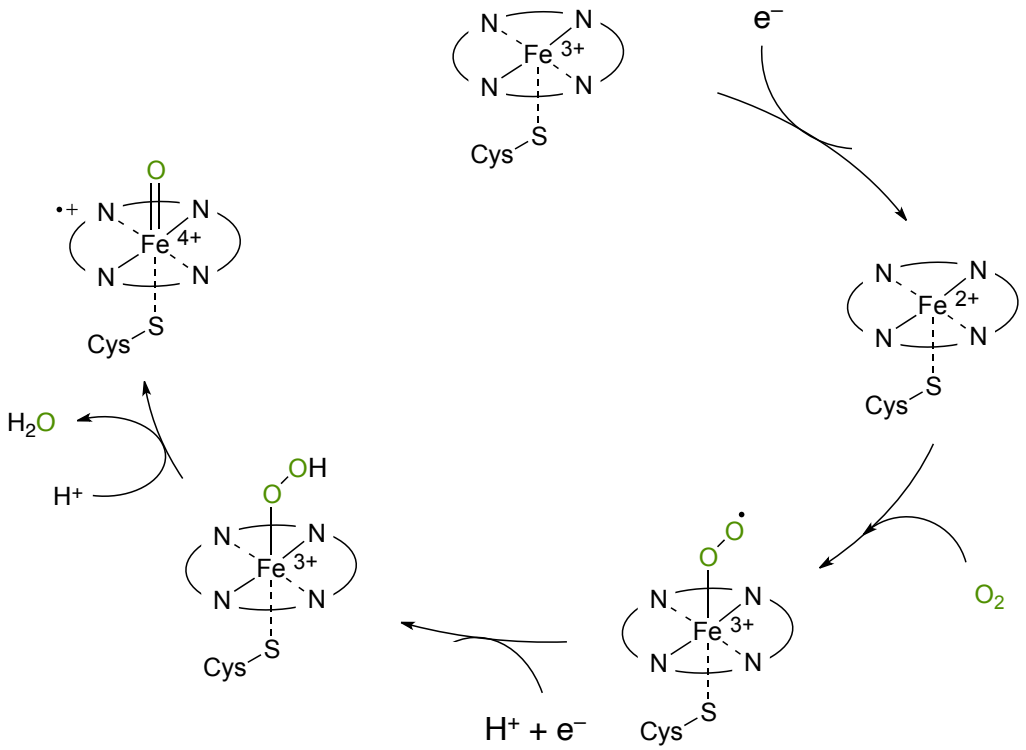

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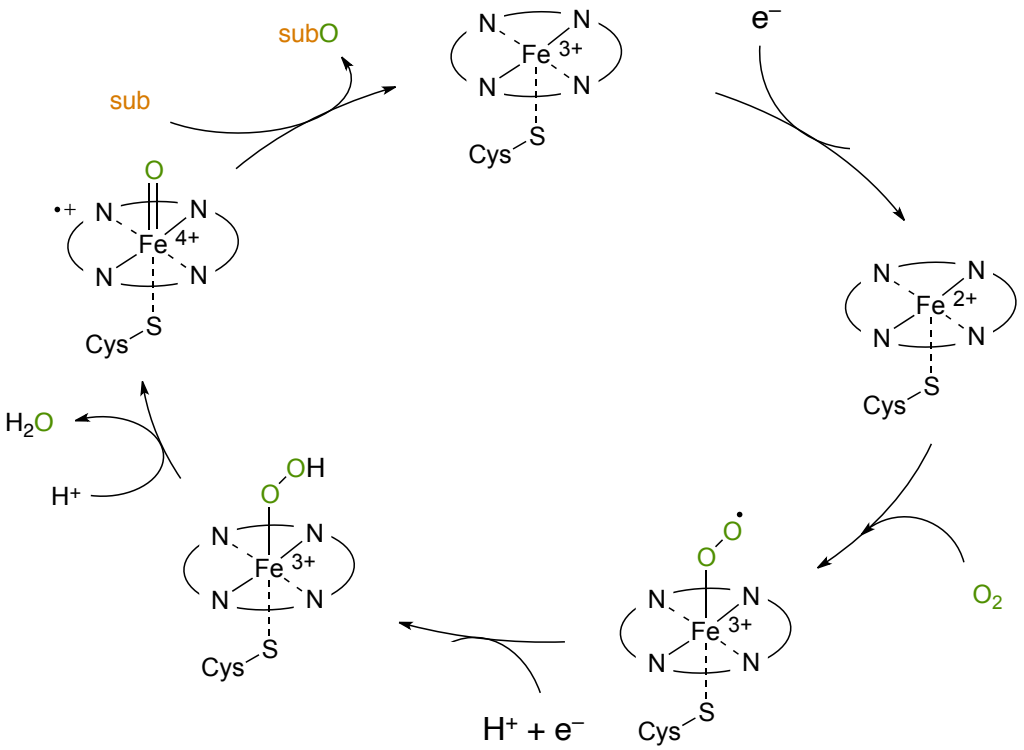

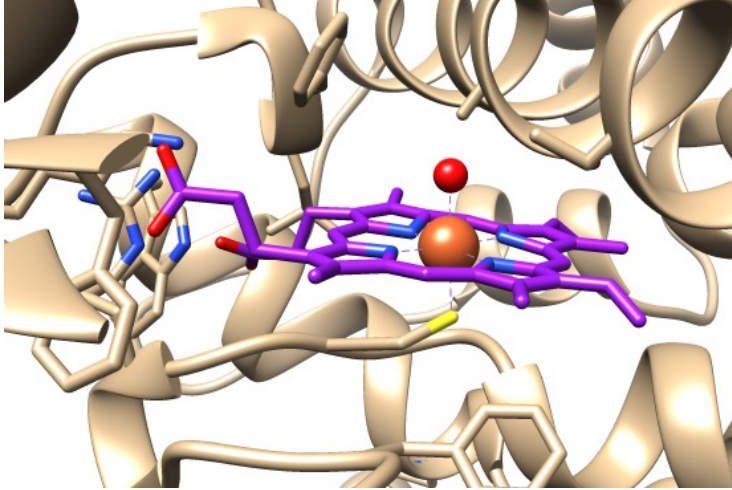

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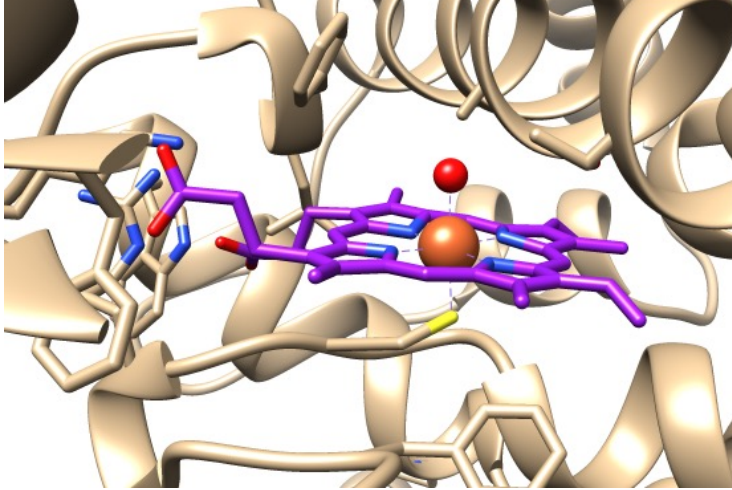

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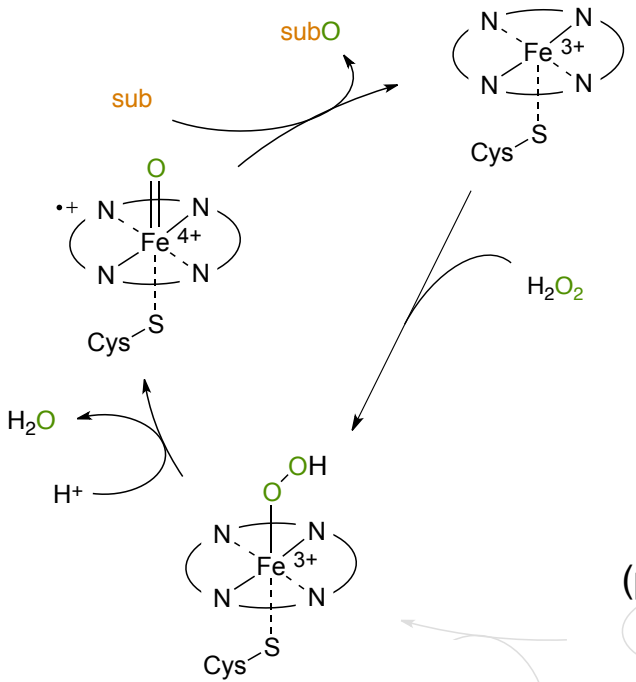

direct formation of heme hydroperoxide

=

peroxidase mechanism

(peroxide shunt pathway for P450s)

Meunier, de Visser, Shaik, *Chem. Rev.* **2004**, *104*, 3947-3980.

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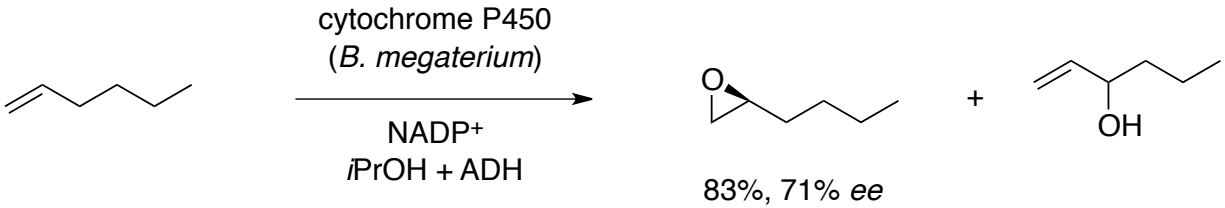
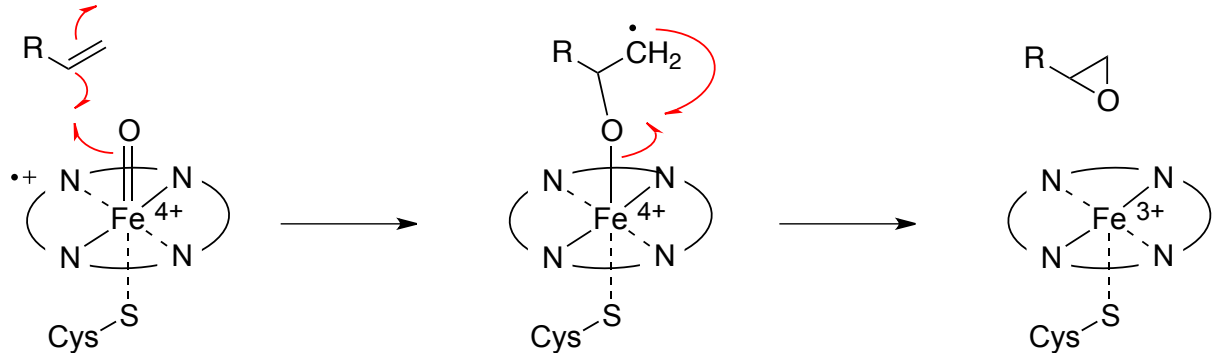
Cytochrome P450 monooxygenases

Epoxidations



proposed oxygen transfer



Kubo, Peters, Meinhold, Arnold, *Chem. Eur. J.* **2006**, *12*, 1216-1220.

Cytochrome P450 monooxygenases

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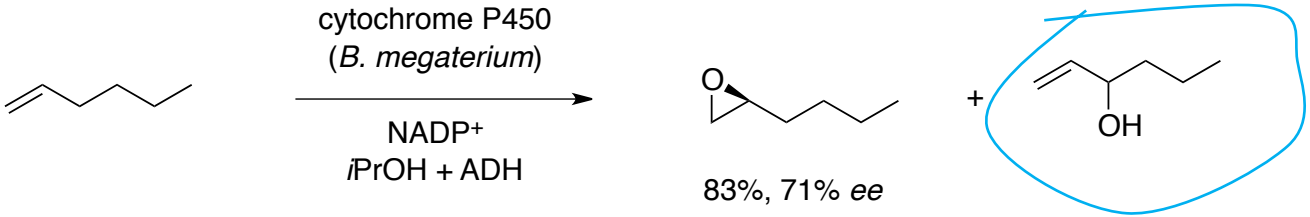

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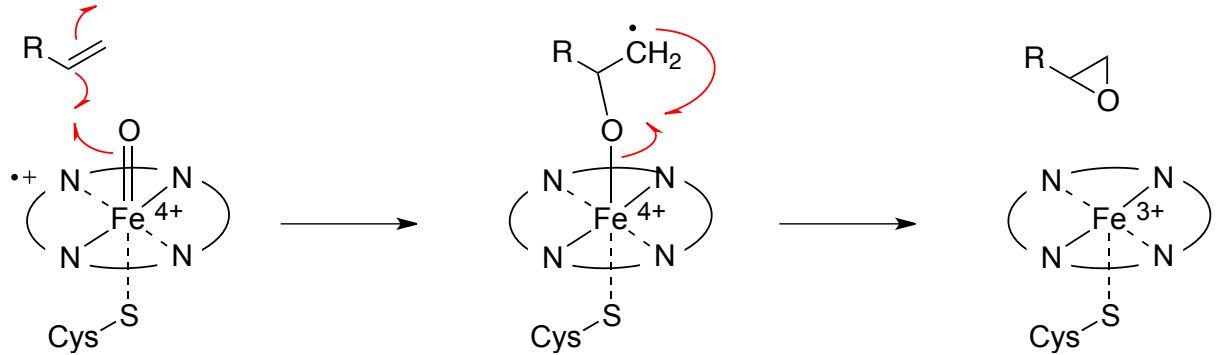
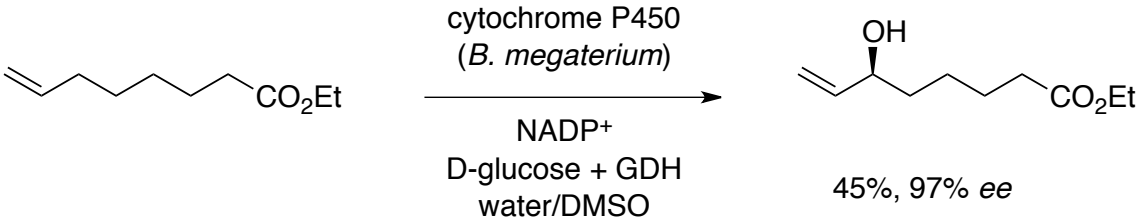


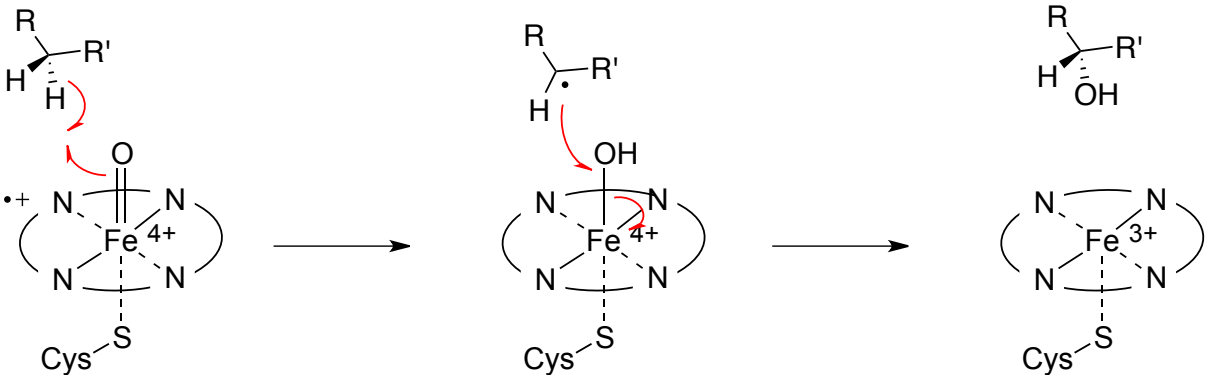
Kubo, Peters, Meinhold, Arnold, *Chem. Eur. J.* **2006**, *12*, 1216-1220.

Cytochrome P450 monooxygenases

Hydroxylations



- not limited to allylic systems, but alkane CH-hydroxylation yet untamed

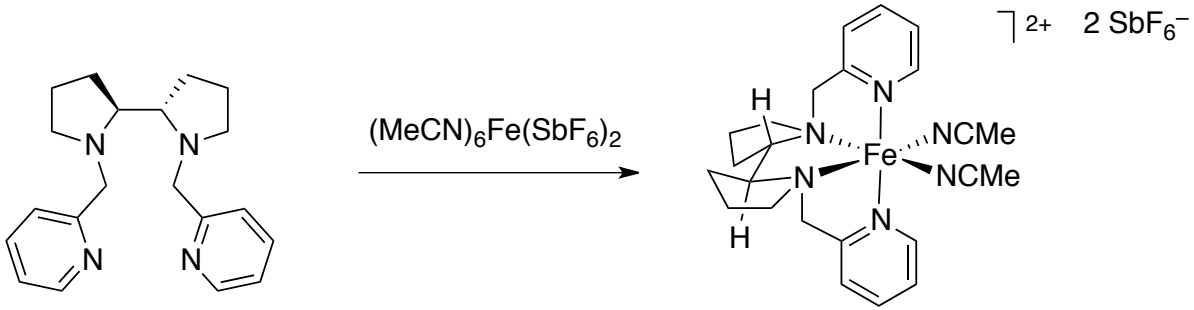


Neufeld, Henßen, Pietruszka, *Angew. Chem. Int. Ed.* **2014**, *53*, 13253-13257.

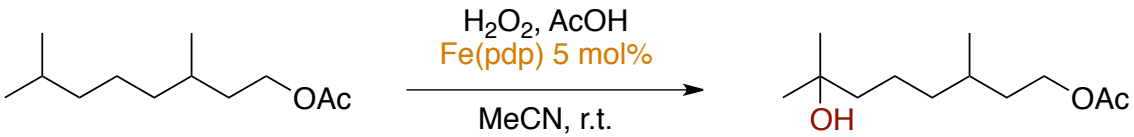
Biomimetic Iron-based hydroxylations

multiple non-heme iron complexes have been tested for epoxidation and hydroxylation

- rather successful: White-Chen catalysts (Fe(pdp))



Fe(pdp)



Gelalcha, *Adv. Synth. Catal.* **2014**, *356*, 261-299.

Chen, White, *Science* **2007**, *318*, 783-787.

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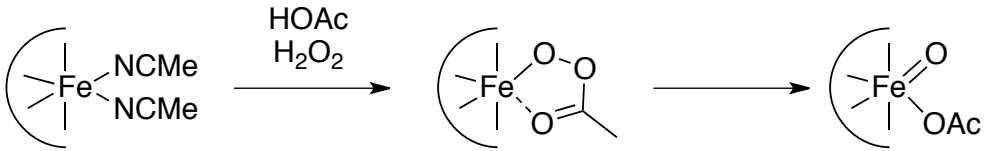
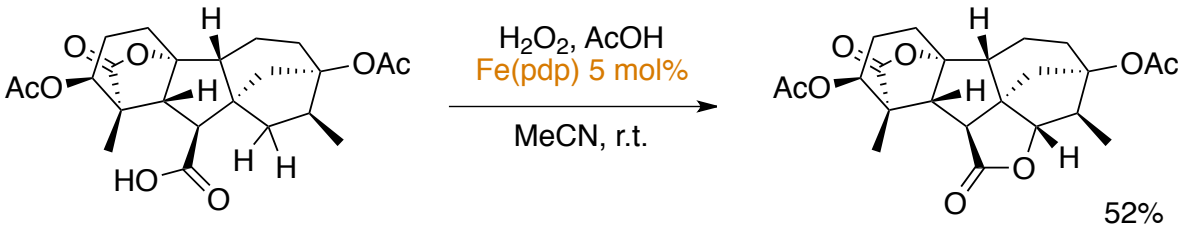


somewhat debated

✓ internal carboxylate as directing group: very high regioselectivities



52%

Chen, White, *Science* **2007**, *318*, 783-787.

SUMMARY

- dioxygenases very limited in application, but arene dihydroxylation provides unique reactivity
- molecular capacitors often involved in reductive preactivation
- both "organocatalytic" and "metal-catalyzed" versions of monooxygenases as relatively established biocatalysts also in synthetic applications