CHEM-E4109
MODERN METHODS IN BIOCATALYSIS

chapter #2: reductive enzymes

4.3.2022

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HOUSEKEEPING NOTES

Learning diaries

- first week's diary open tomorrow
- due by next Wednesday, March 9th
- this time, mostly questionnaire style

TODAY'S MENU

Content

- overview of redox enzyme subclasses
- spotlight on reductive enzymes
- mechanistic and application details on keto- and enoate reductases

Intended learning outcomes

- familiarize with various redox processes
- be able to explain key structural and mechanistic aspects of bioreductions
- evaluate strategies to drive reductive processes
- distinguish between different approaches to optically active products

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Redox Enzymes – Classification

oxidoreductases can be subdivided into several classes depending on the nature of electron donor and electron acceptor

Dehydrogenases

- remove hydrogen, hence oxidize the substrate
- require hydride-binding cofactors as electron acceptors
  = nicotinamides (NAD(P)+) or flavins (FAD/FMN) as coenzymes
- CAUTION: also reverse reaction possible
  = sometimes also called reductases

✓ provision of oxidized or reduced cofactor controls equilibrium

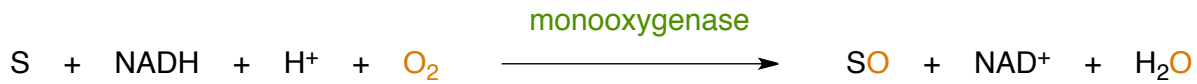
Redox Enzymes - Classification

oxidoreductases can be subdivided into several classes depending on the nature of electron donor and electron acceptor

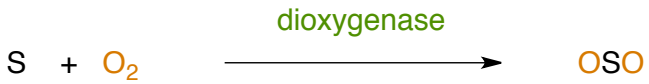
Oxygenases

- introduce one or two atoms of oxygen from O_2
- often require hydride-donating cofactors as electron sources
  = nicotinamides (NAD(P)H) or flavins ($FADH_2$/$FMNH_2$) as coenzymes

Monooxygenases



Dioxygenases



very much simplified: often associated to prereduction processes, cosubstrates to trap one oxygen atom etc...

Redox Enzymes - Classification

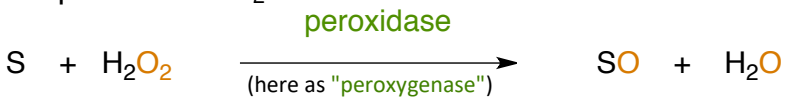
oxidoreductases can be subdivided into several classes depending on the nature of electron donor and electron acceptor

Peroxidases

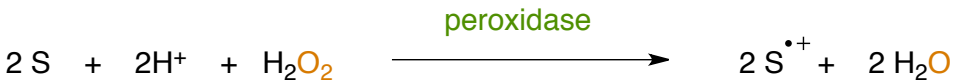
- introduce one atom of oxygen from H_2O_2

  or

- induce single-electron transfer processes (similar to some monooxygenases)
- independent of sacrificial redox cofactors as hydrogenperoxide can be considered "prereduced O_2"



or



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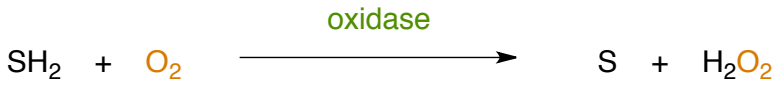
Redox Enzymes - Classification

oxidoreductases can be subdivided into several classes depending on the nature of electron donor and electron acceptor

Oxidases

- reduce O_2 to H_2O_2 (occassionally even to H_2O), thereby dehydrogenating the substrate
- redox cofactor-free "dehydrogenase"

partial O_2 reduction



full O_2 reduction

Redox Enzymes – Classification

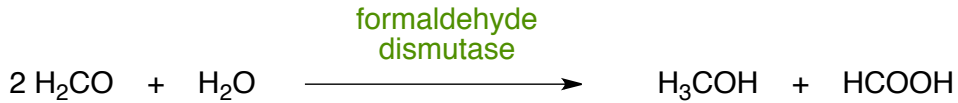
oxidoreductases can be subdivided into several classes depending on the nature of electron donor and electron acceptor

Dismutases

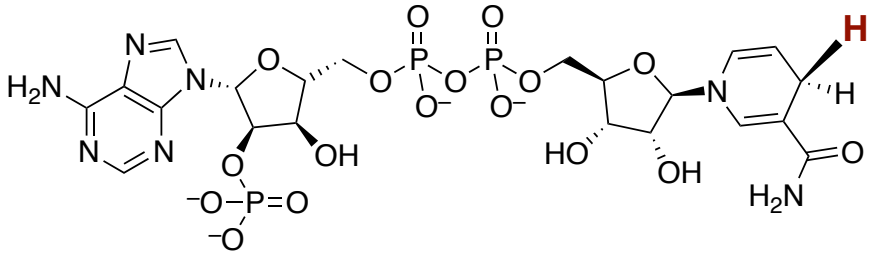
- catalyzes disproportionation of the substrate to yield an oxidized and a reduced derivative thereof

e.g. catalase

e.g. formaldehyde dismutase



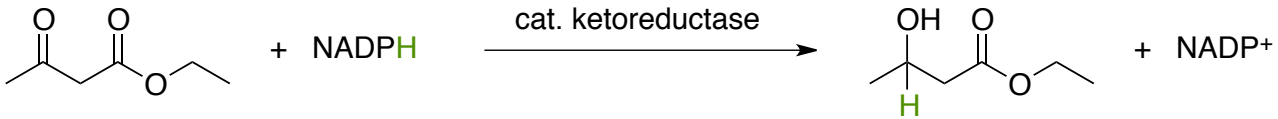
Issues related to redox cofactors



Dehydrogenases and oxygenases require NAD(P)H as stoichiometric reducing agent!

- miserable atom economy: 0.11 wt% hydride in NADPH
- high price: 100 mg NADPH = 228 EUR (Sigma Aldrich, 3.3.2022)
- relatively low stability

costs to produce 100 g of ethyl 3-hydroxybutanoate



ca. 4 € ca. 912 421 € ca. 20 €

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Issues related to redox cofactors

In order to use oxidoreductases in a non-fermentative fashion (i.e. w/ isolated enzymes), cofactor recycling is indispensable

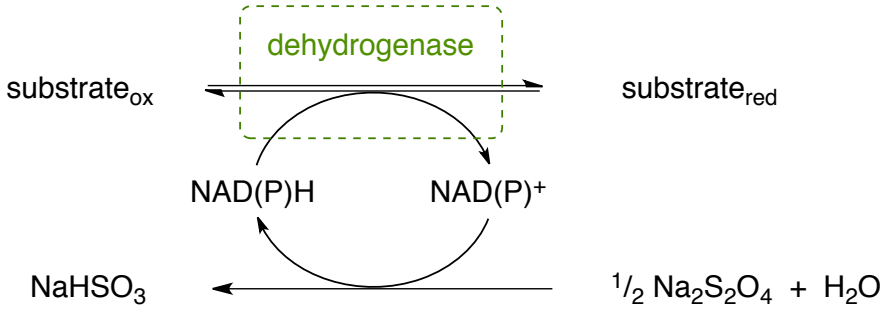
cofactor recycling = in situ reduction of NAD(P)H under consumption of a much cheaper sacrificial reducing agent

Issues related to redox cofactors

In order to use oxidoreductases in a non-fermentative fashion (i.e. w/ isolated enzymes), cofactor recycling is indispensable

<u>possibility I: chemical reduction</u>

- inorganic reducing agents (dithionite) or electrochemical reduction



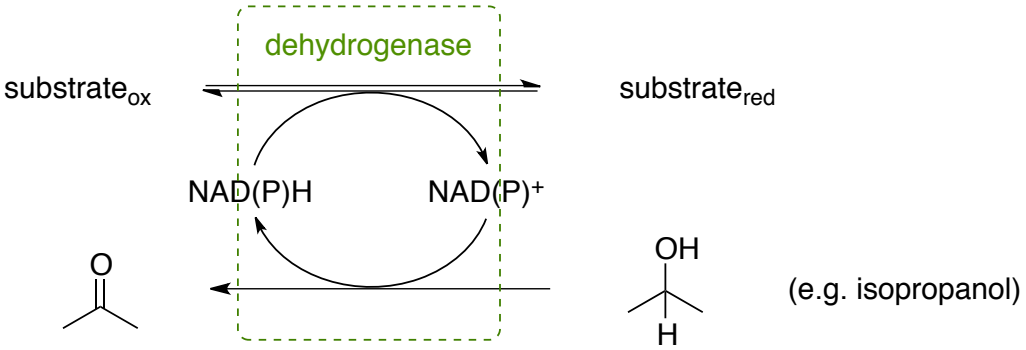
+ very cheap
– low turnover
– rapid deactivation of the proteins

Issues related to redox cofactors

In order to use oxidoreductases in a non-fermentative fashion (i.e. w/ isolated enzymes), cofactor recycling is indispensable

<u>possibility II: cosubstrate-coupled process</u>

- reduced cosubstrate that is also accepted by the enzyme pushes equilibrium



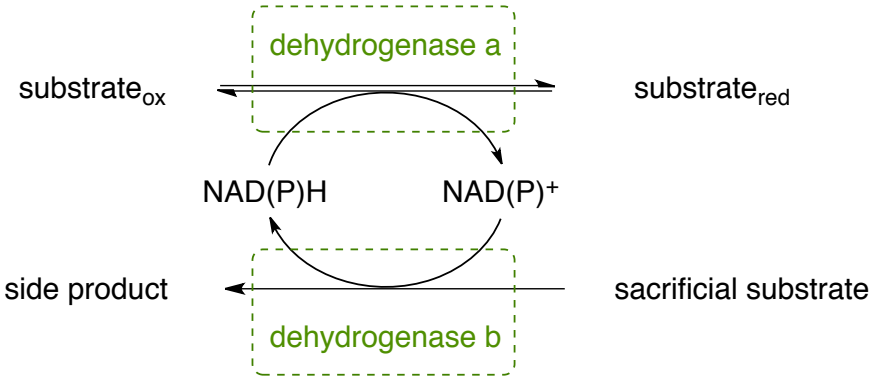
+ still pretty cheap
+ no interfering non-native processes
– enzymatic activity is distributed over two processes
– potential inhibition by high concentrations of cosubstrate/coproduct
– only applicable for dehydrogenases

Issues related to redox cofactors

In order to use oxidoreductases in a non-fermentative fashion (i.e. w/ isolated enzymes), cofactor recycling is indispensable

<u>possibility III: enzyme-coupled process</u>

- a second enzyme with a different reduced substrate is added



+ cheap reducing agents
+ minimal impairment of either enzyme
+ also well applicable with oxygenases instead of *dehydrogenase a*
– costs of additional enzymes

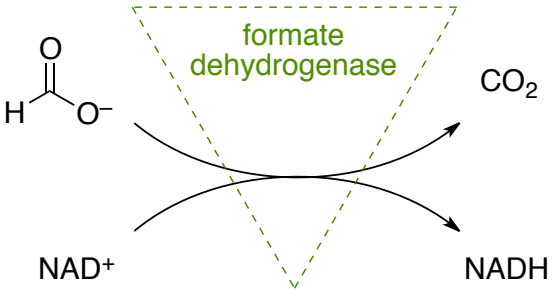
Issues related to redox cofactors

In order to use oxidoreductases in a non-fermentative fashion (i.e. w/ isolated enzymes), cofactor recycling is indispensable

<u>possibility III: enzyme-coupled process</u>

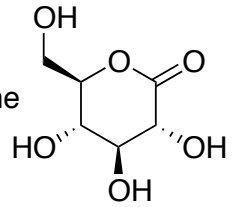
classical coenzyme systems

formate dehydrogenase

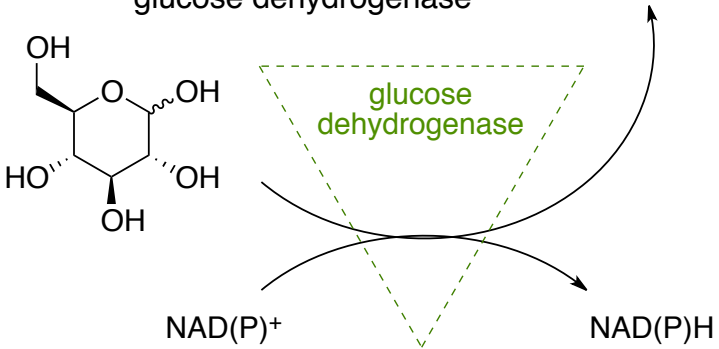


+ CO_2 formation drives equilibrium
- low specific activity
- only active on NAD^+, not $NADP^+$

gluconolactone



glucose dehydrogenase

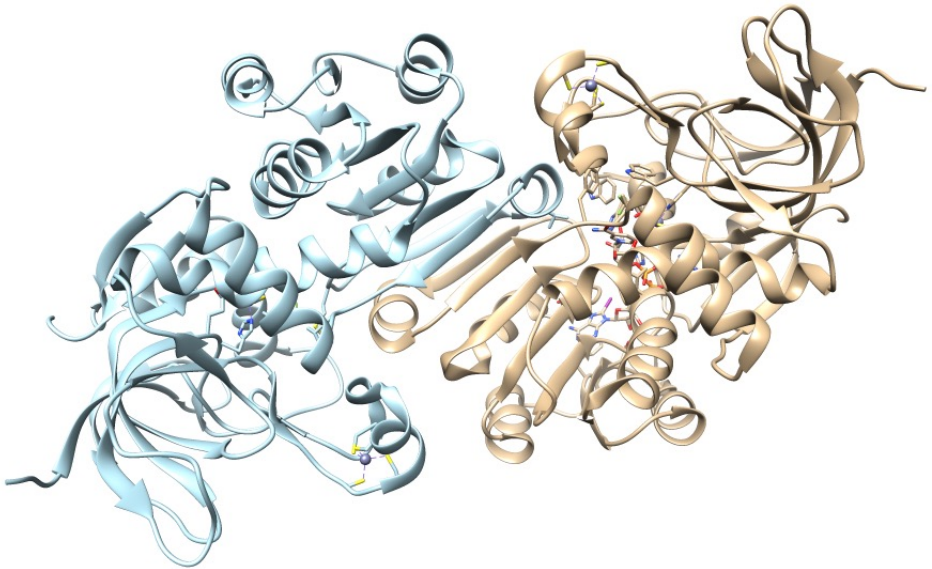


+ accepts both NAD^+ and $NADP^+$
+ hydrolysis of gluconolactone pushes equilibrium
- (gluconate as waste)

Alcohol dehydrogenases

Yeast alcohol dehydrogenase (*Saccharomyces cerevisiae*)

- evolutionarily linked to ancestral formaldehyde dehydrogenase
- homodimers, ca. 350 amino acids



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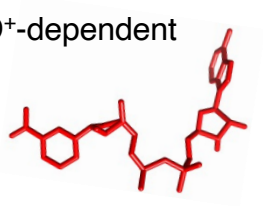
Alcohol dehydrogenases

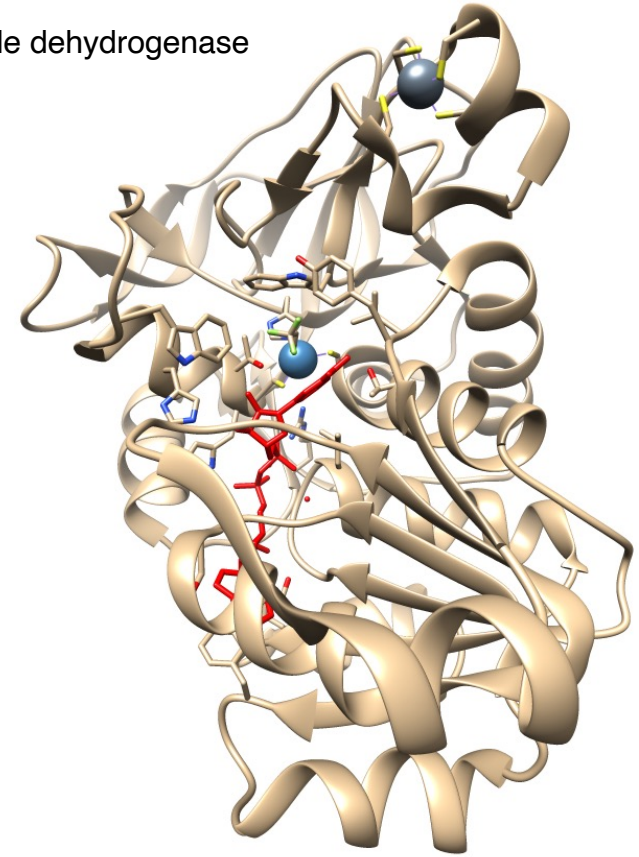
Yeast alcohol dehydrogenase (*Saccharomyces cerevisiae*)

- evolutionarily linked to ancestral formaldehyde dehydrogenase
- homodimers, ca. 350 amino acids
- zinc-containing

  (one structural Zn^{2+} + one catalytic Zn^{2+})

- NAD^+-dependent





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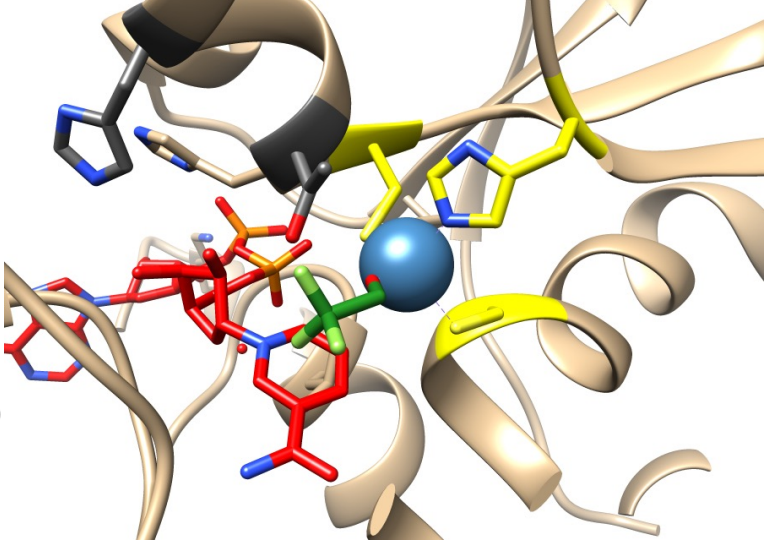

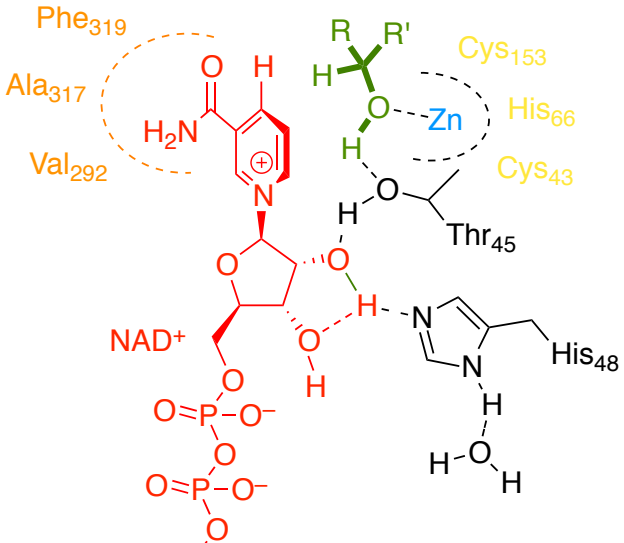

zinc binding

substrate binding

proton shuffle

nicotinamide binding

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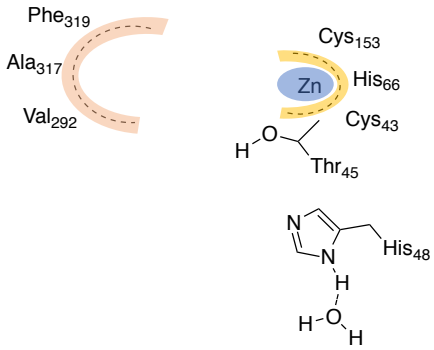
Alcohol dehydrogenases

Mechanism



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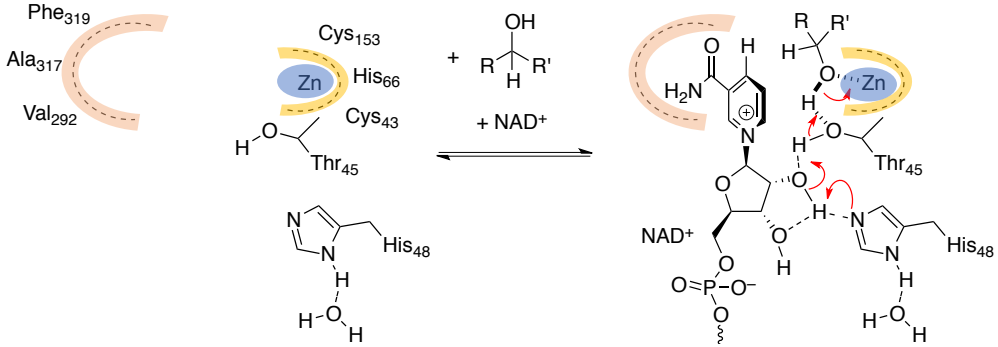
Alcohol dehydrogenases

Mechanism

1. NAD^+ binding
2. alcohol binding



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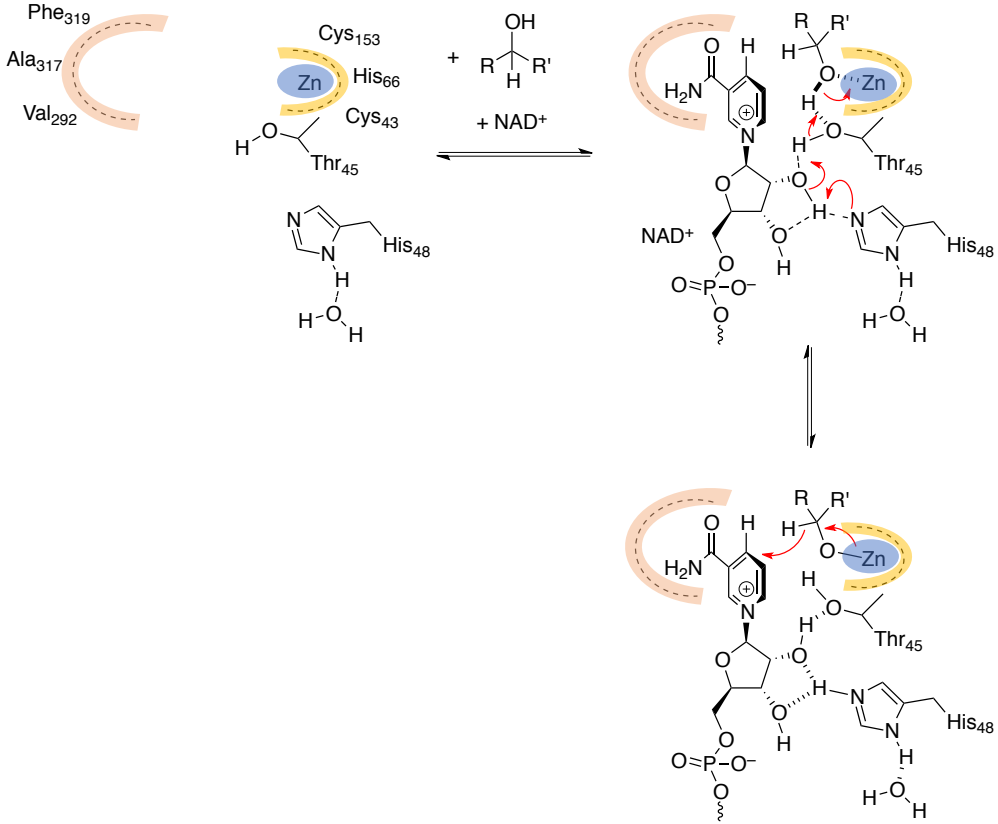
Alcohol dehydrogenases

Mechanism

1. NAD^+ binding
2. alcohol binding
3. alcohol deprotonation



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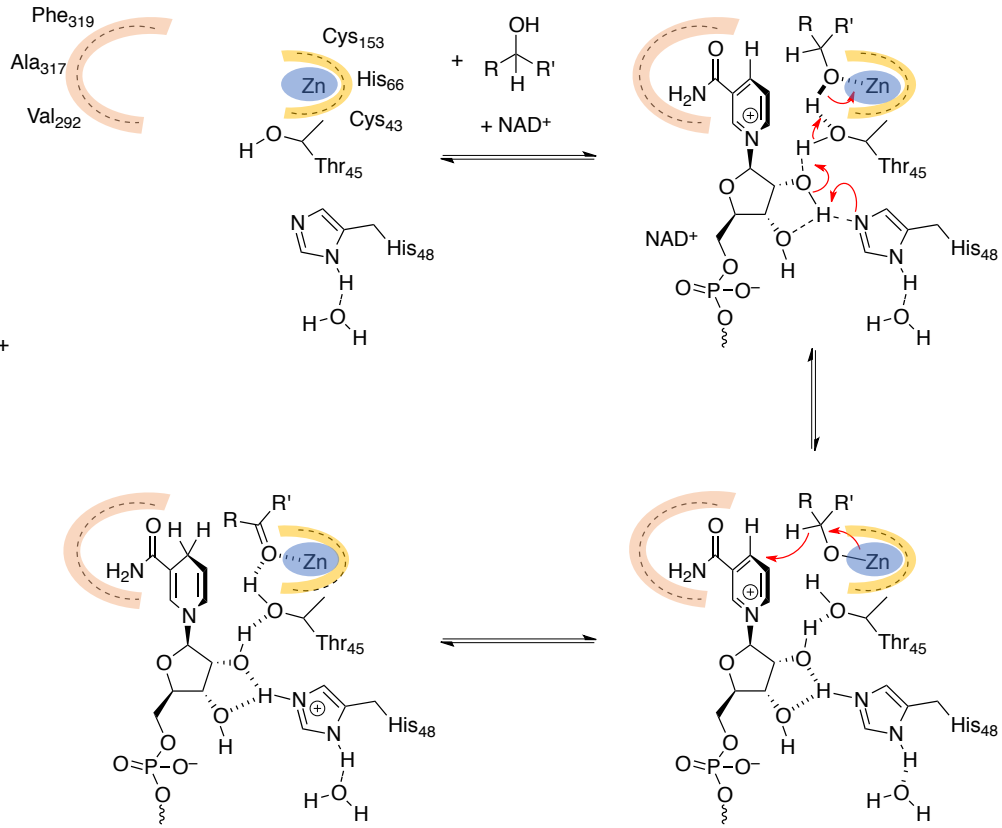
Alcohol dehydrogenases

Mechanism

1. NAD^+ binding
2. alcohol binding
3. alcohol deprotonation
4. hydride transfer from alkoxide to NAD^+



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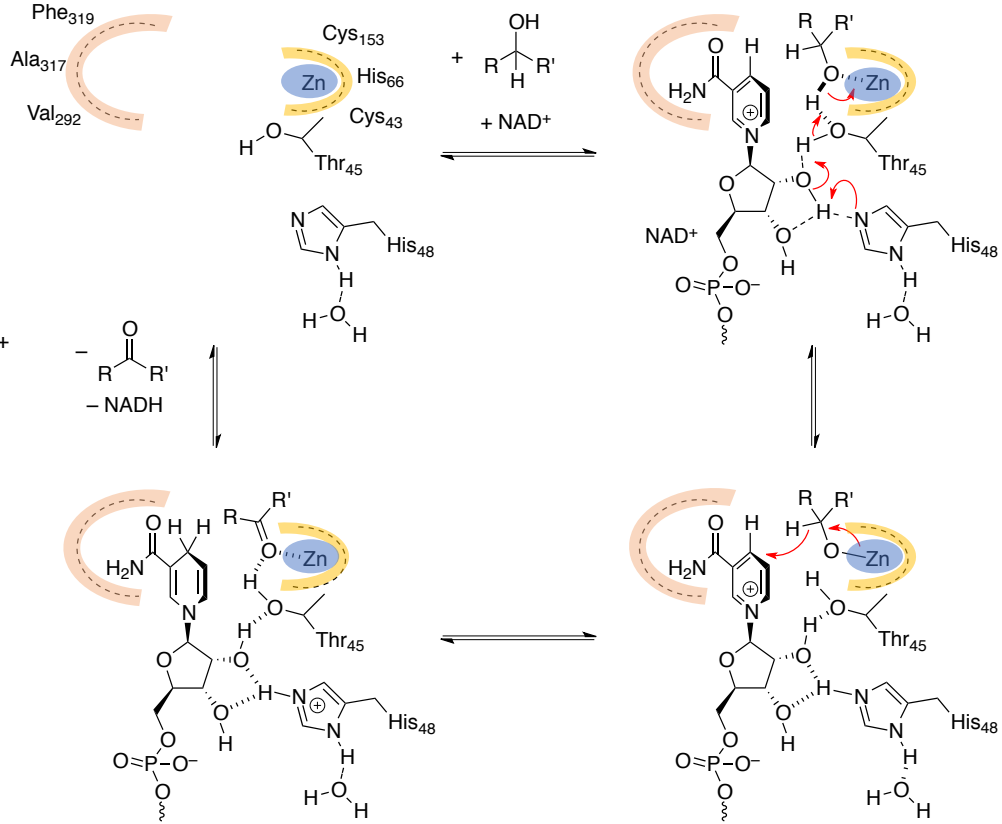
Alcohol dehydrogenases

Mechanism

1. NAD^+ binding
2. alcohol binding
3. alcohol deprotonation
4. hydride transfer from alkoxide to NAD^+
5. release of ketone
6. release of NADH



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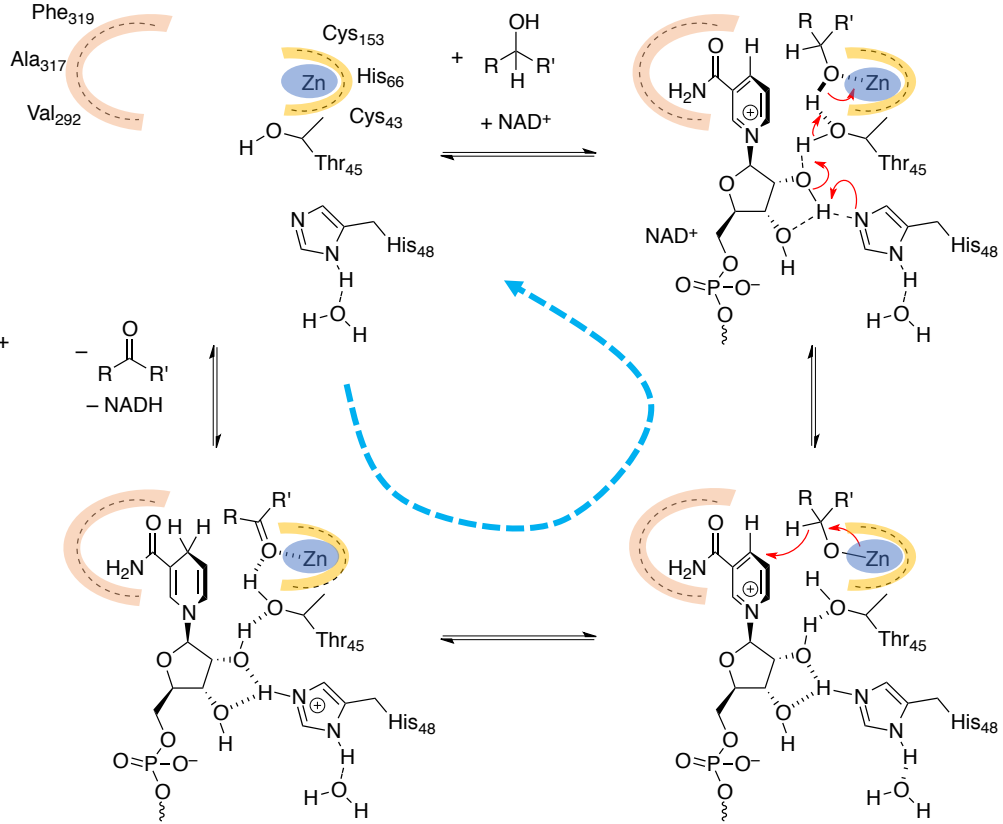
Alcohol dehydrogenases

Mechanism

1. NAD⁺ binding
2. alcohol binding
3. alcohol deprotonation
4. hydride transfer from alkoxide to NAD⁺
5. release of ketone
6. release of NADH



if run counterclockwise:
ketone reduction

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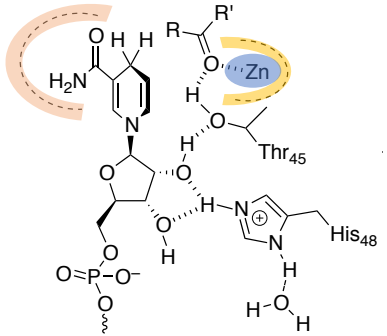
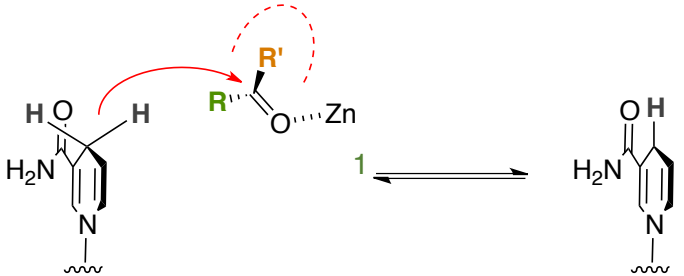
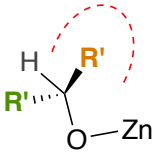
Some words about stereoselectivity

Face selectivity during hydride transfer

substrate orientation controls to which face of the carbonyl the hydrogen is transfered to



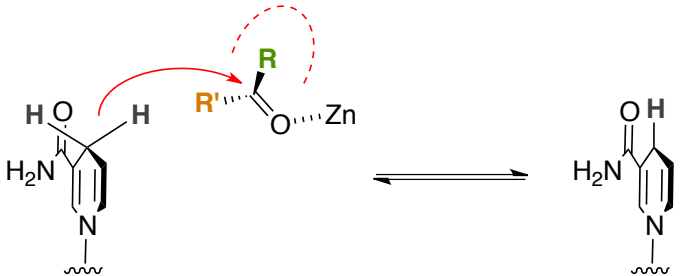


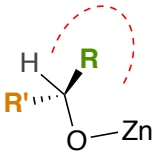


if R' < R in CIP priorities

hydrogen goes to carbonyl to *re* face forming the *S*-alcohol

or





hydrogen goes to carbonyl to *si* face forming the *R*-alcohol

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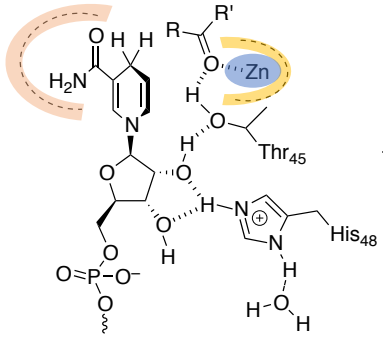
Some words about stereoselectivity

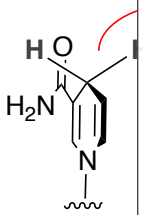
Face selectivity during hydride transfer

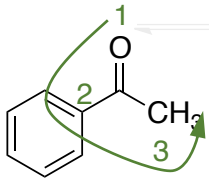
substrate orientation controls to which face of the carbonyl the hydrogen is transfered to



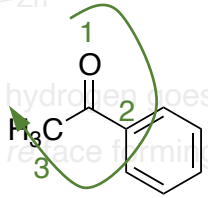
re- and *si*-face nomenclature

used to distinguish sides of prochiral elements in achiral molecules



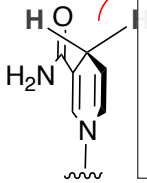


same molecule from two perspectives



counter-clockwise = *si* face

clockwise = *re* face



but: says nothing about absolute configuration of potential products

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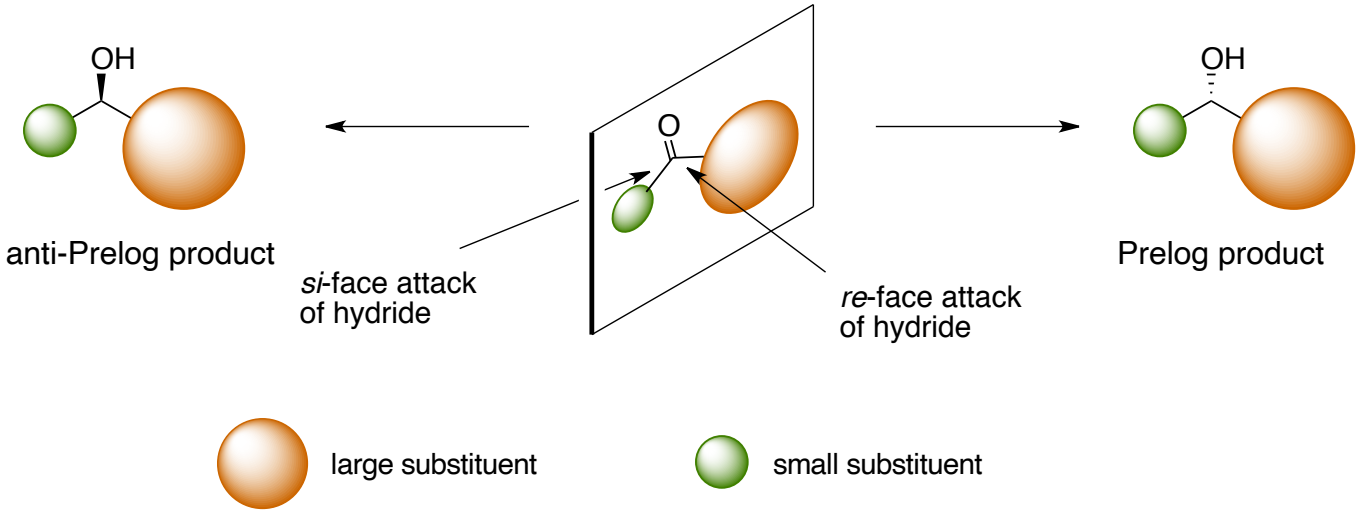
Some words about stereoselectivity

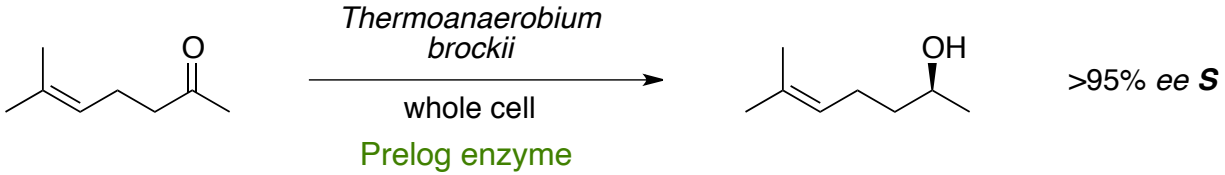
Prelog's rule to predict absolute configurations

= sterics dominate stereochemical outcome of ADH reductions and oxidations

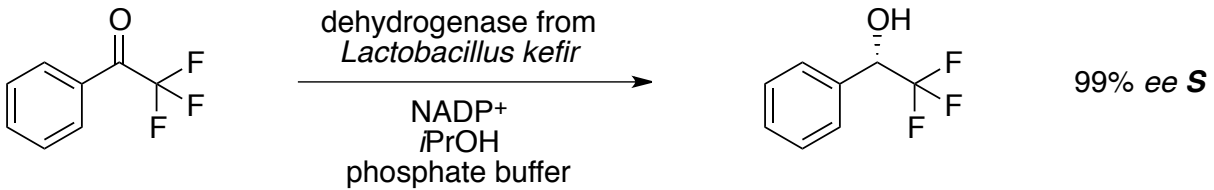


- there are both Prelog- and anti-Prelog-selective enzymes
- scope might differ much regarding the bulk of the accepted large substituent

Asymmetric ketone reductions



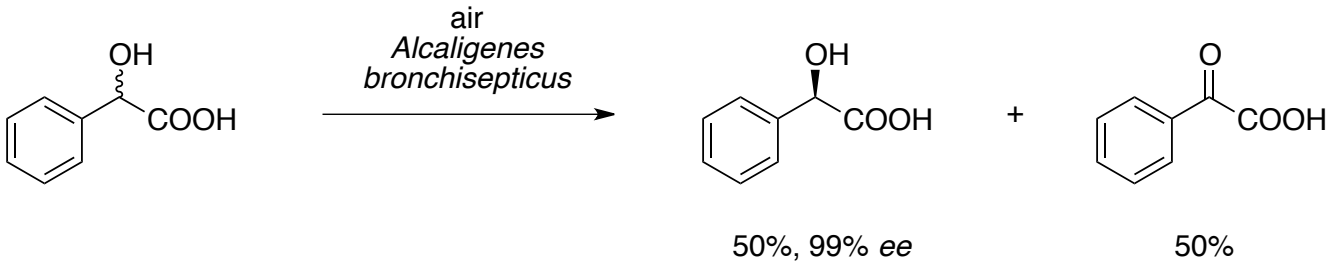
there is no R/S prediction in Prelog's rule!
R or S defined by atomic number, not sterics



Bradshaw, Hummel, Wong, *J. Org. Chem.* **1992**, *57*, 1532-1536.

Kinetic resolution & deracemization

enantioselective oxidation results in kinetic resolution



kinetic resolution =

stereoselective catalyst consumes one enantiomer faster than its mirror image

(*S*)-substrate —enzyme, k_S→ (*S*)-product or achiral product

(*R*)-substrate —enzyme, k_R→ (*R*)-product or achiral product

if $k_S >> k_R$

optically pure (*R*)-substrate can be reisolated

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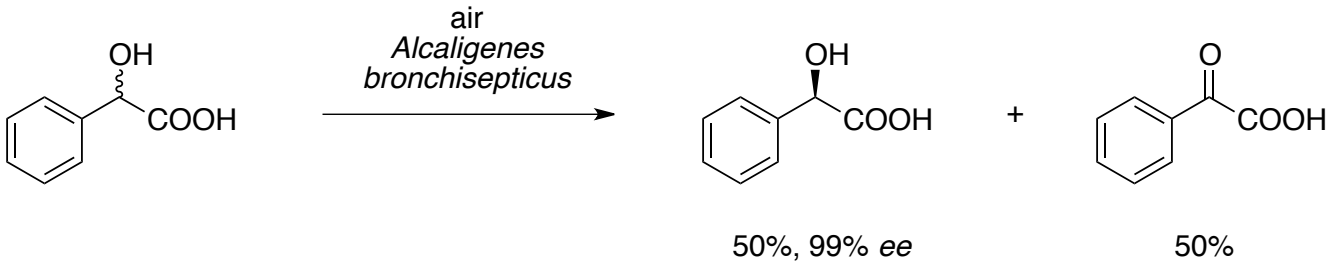
Kinetic resolution & deracemization

enantioselective oxidation results in kinetic resolution

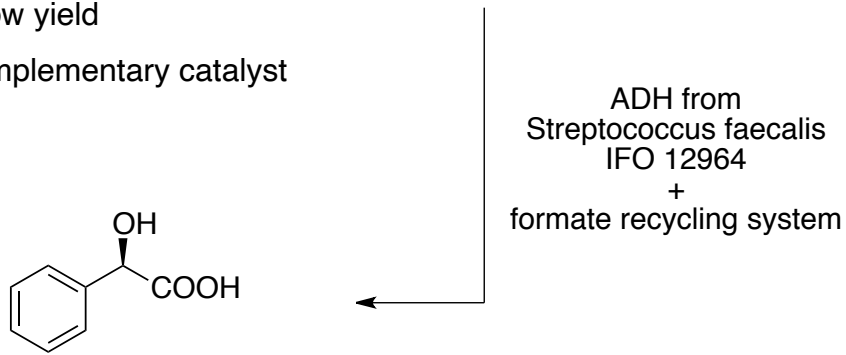


50%, 99% *ee*

50%

problem with kinetic resolutions: low yield

potential work-around: second complementary catalyst

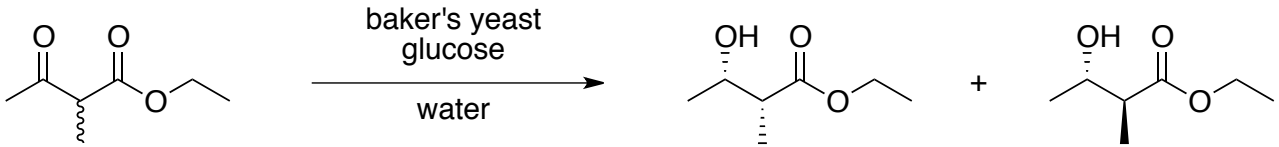


87%, 99% *ee*

Gruber, Lavandera, Faber, Kroutil, *Adv. Synth. Catal.* **2006**, *348*, 1789-1805.

Dynamic kinetic resolution

yeast reduction of racemic β-ketoesters yields enantioenriched products



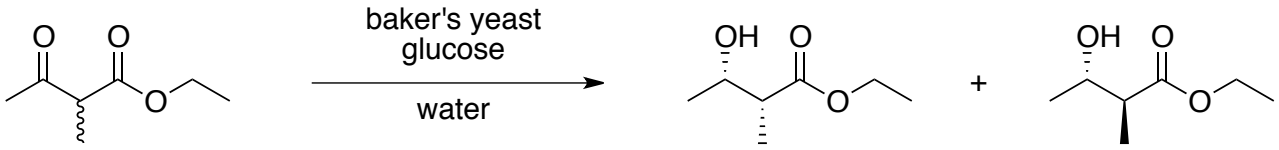
racemic

83%, 99% *ee*

17%, 99% *ee*

Dynamic kinetic resolution

yeast reduction of racemic β-ketoesters yields enantioenriched products



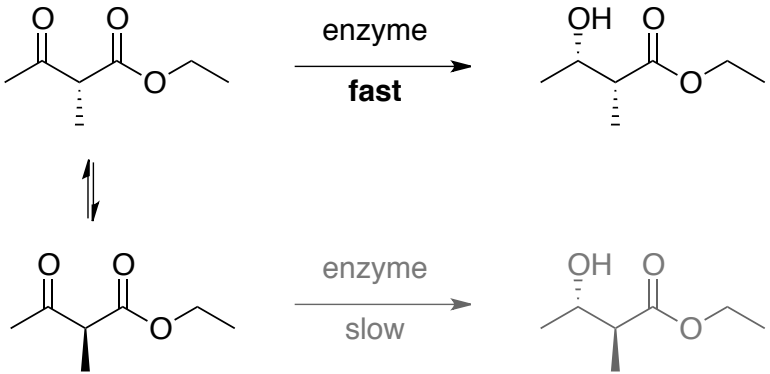
racemic

83%, 99% *ee*

17%, 99% *ee*

explanation:

high α-acidity/enolizability results in continous equilibration between (+) and (–)-enantiomer



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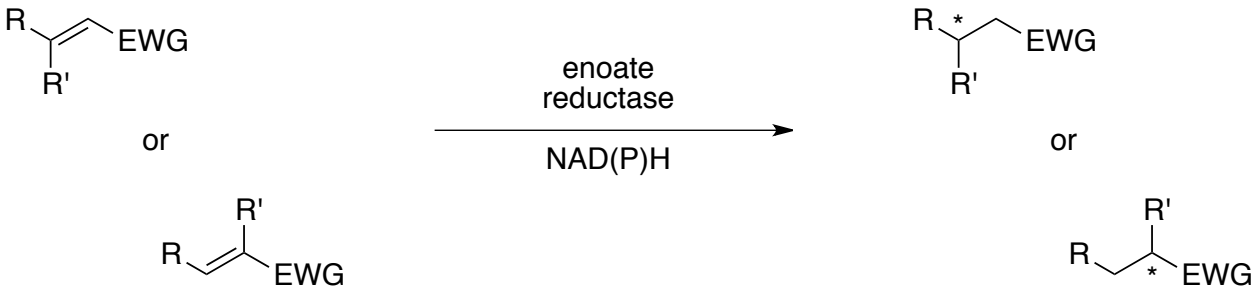
Enoate reductases

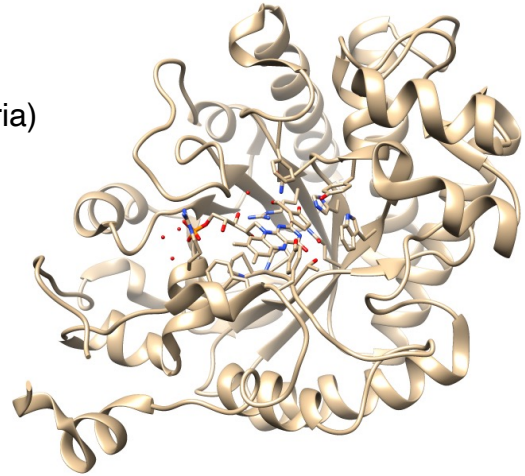
Replacement of carbonyl by polarized olefin as substrate



EWG = ester, ketone, aldehyde, nitro,...

"Old Yellow Enzymes" (found in various yeasts and bacteria)

- physiological role not entirely clear
- flavoproteins (tightly bound FMN)
- no metal cofactor
- NAD(P)H-dependent

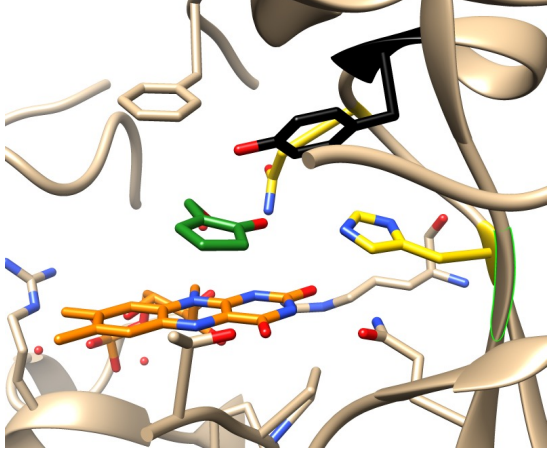


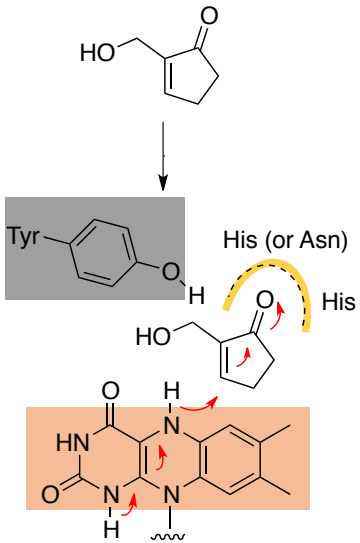
Toogood, Gardiner, Scrutton, *ChemCatChem* **2010**, *8*, 892-914.

Enoate reductases

Mechanism

1 enone binding (H-bonding to His)





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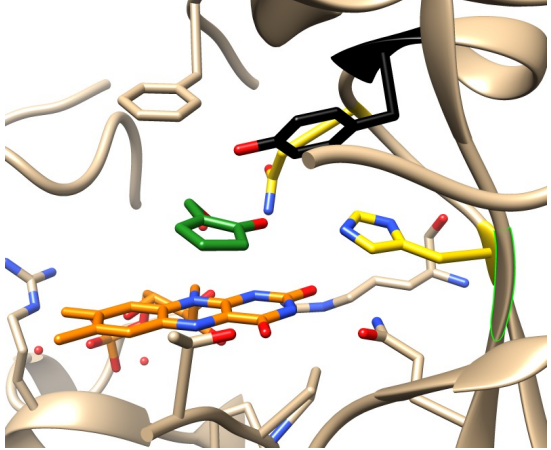
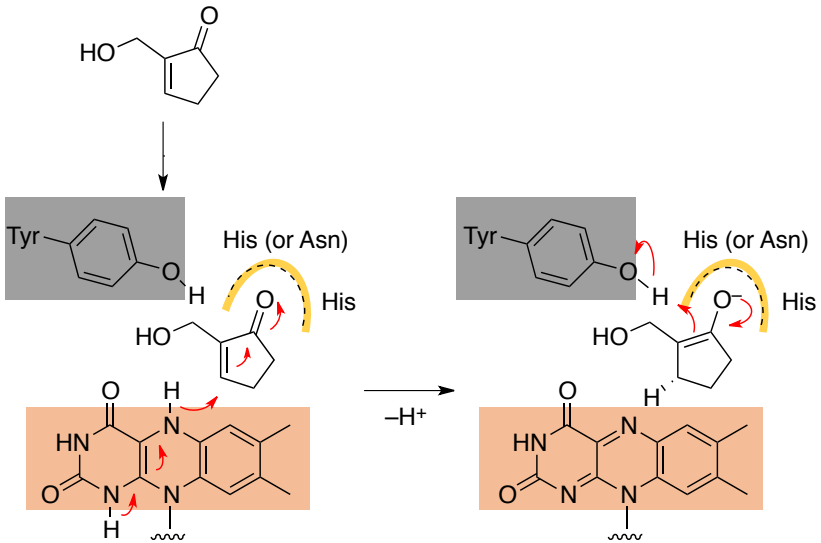
Enoate reductases

Mechanism

1. enone binding (H-bonding to His)
2. hydride transfer (from $FMNH_2$)





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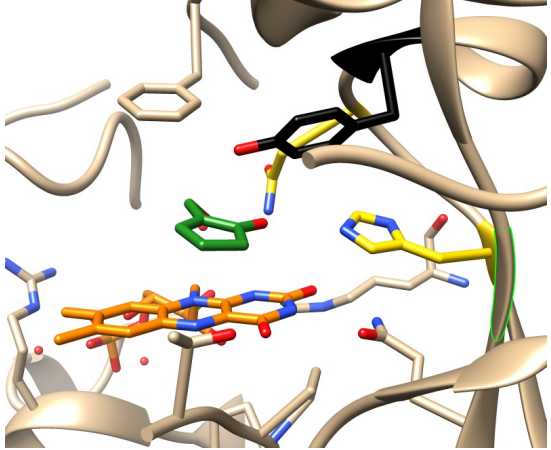
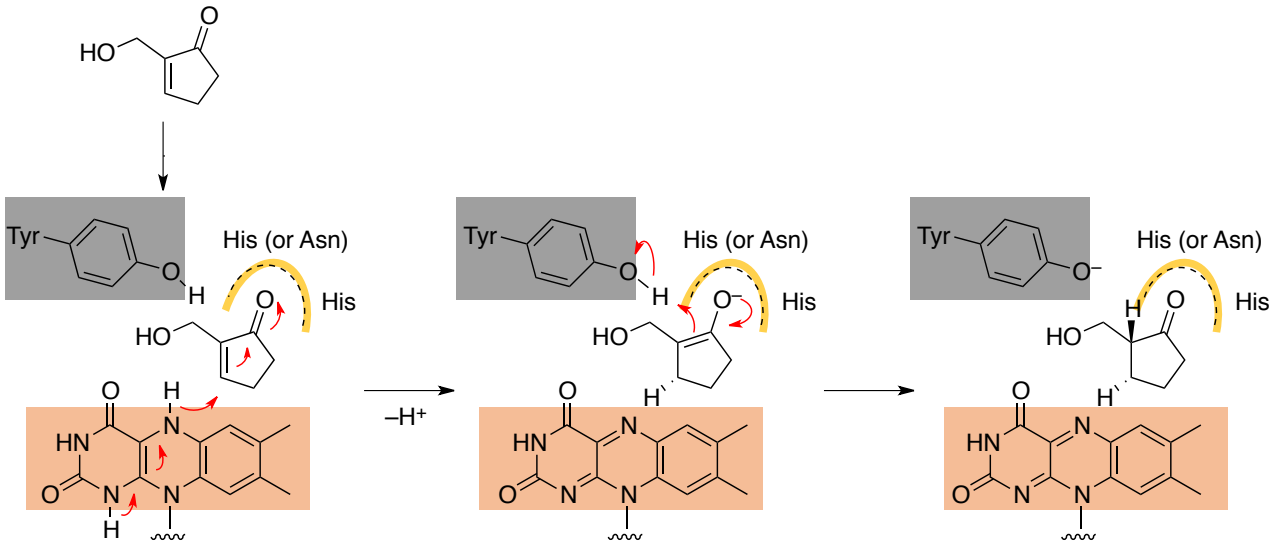
Enoate reductases

Mechanism

1. enone binding (H-bonding to His)
2. hydride transfer (from $FMNH_2$)
3. enolate protonation (from Tyr)





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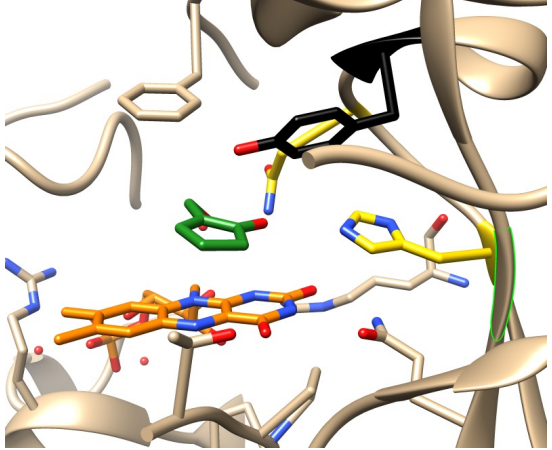
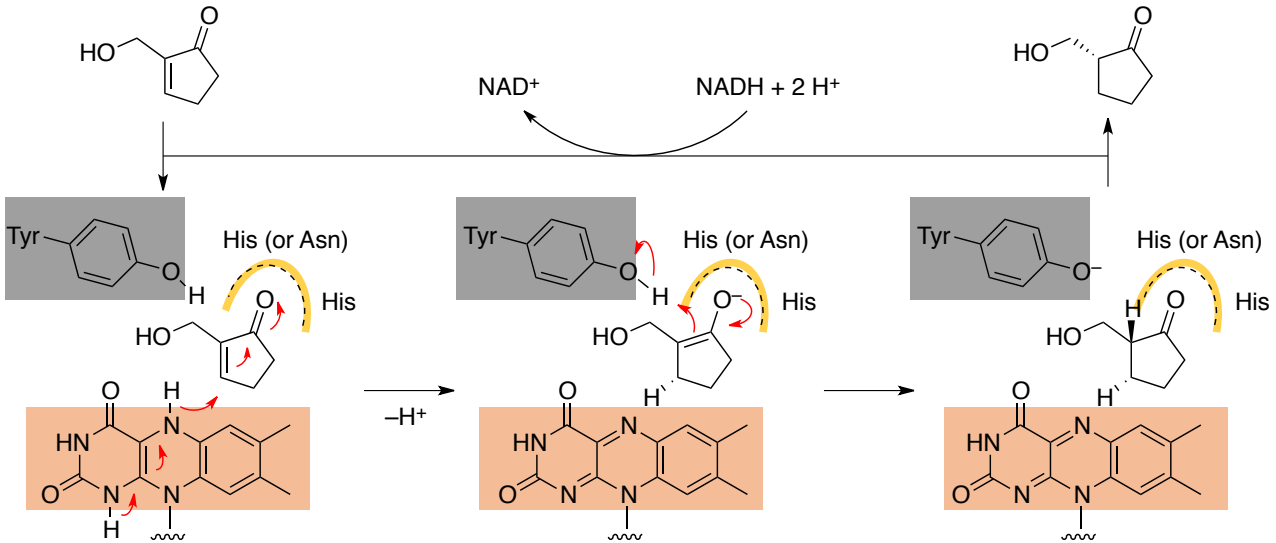
Enoate reductases

Mechanism

1. enone binding (H-bonding to His)
2. hydride transfer (from $FMNH_2$)
3. enolate protonation (from Tyr)
4. release of ketone
5. reduction of FMN to $FMNH_2$





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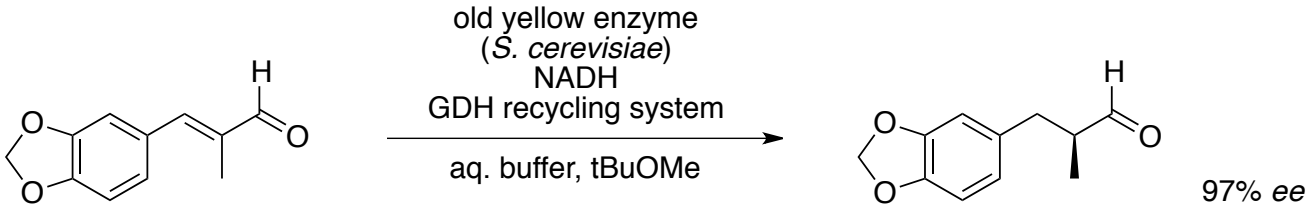
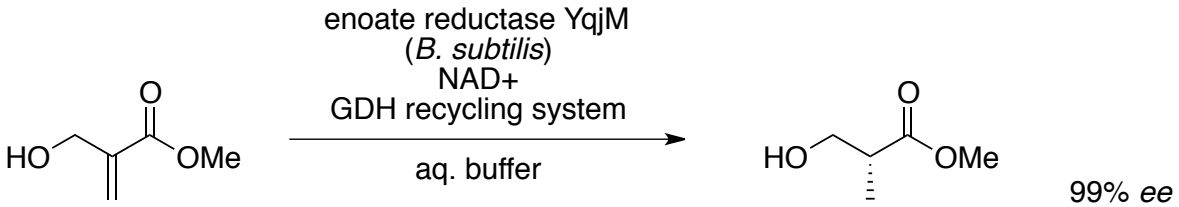
Enoate reductases

Applications





Winkler, Tasnádi, Clay, Hall, Faber, *J. Biotechnol.* **2012**, *162*, 381-389.

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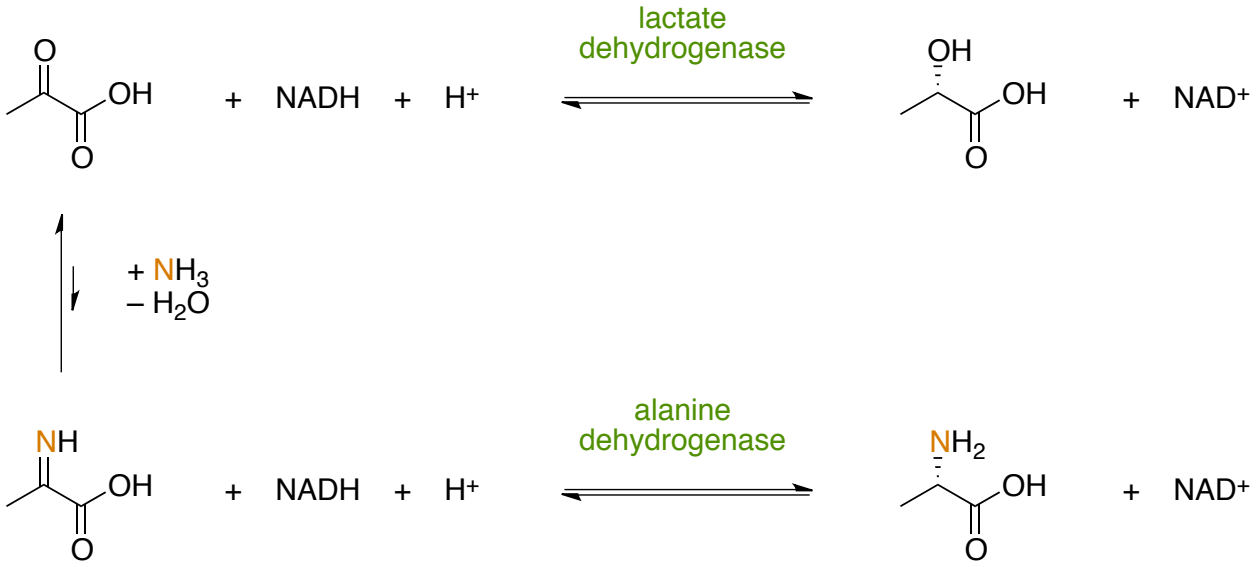
Amine dehydrogenases

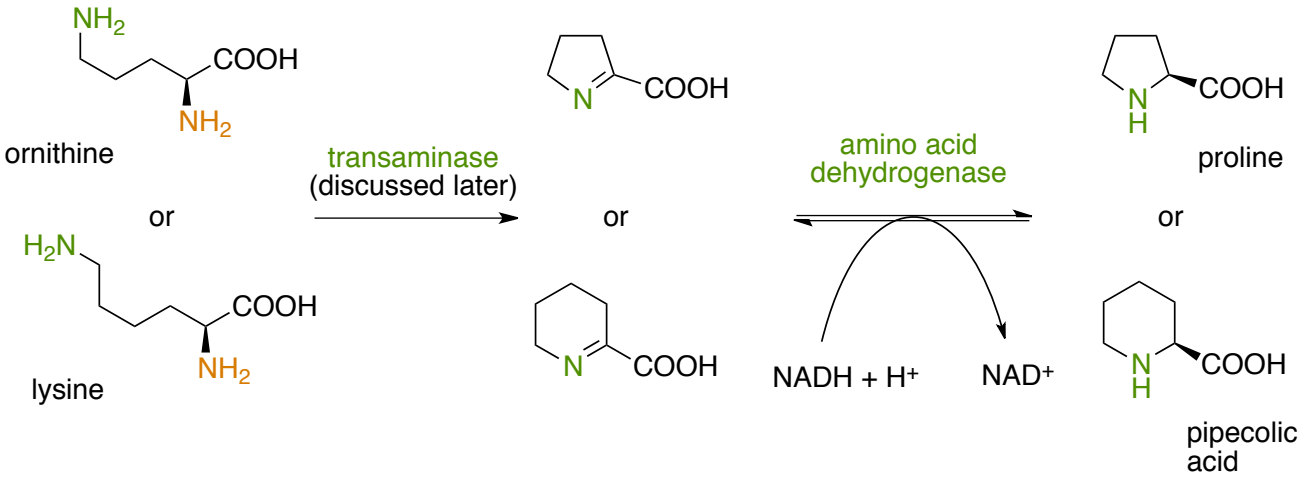
additional pre-equilibrium allows for reductive ammonia fixation



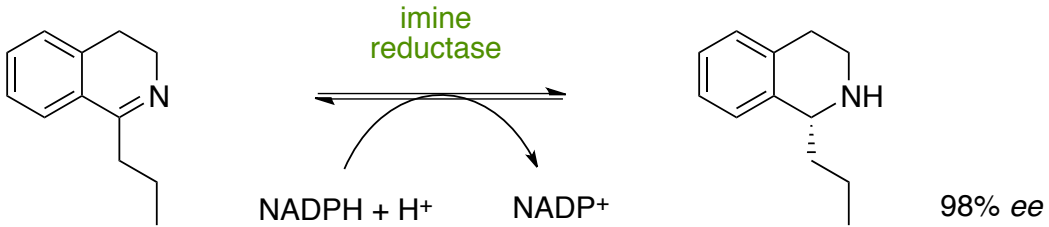
- native enzymes limited to amino acid dehydrogenases
- amino acid biosynthesis, source of ammonia
- low affinity (high K_M) favours mostly deamination

Amine dehydrogenases

e.g. biosynthesis of cyclic amino acids



but: modern engineered "imine reductase" accept non-amino acid substrates



Hussain, Leipold, Man, Wells, France, Mulholland, Grogan, Turner, *ChemCatChem* **2015**, *7*, 579-583.

Biomimetic chemistry inspired by reductases

conjugate reduction: organocatalytic enoate reductase mimics

enzymatic activation

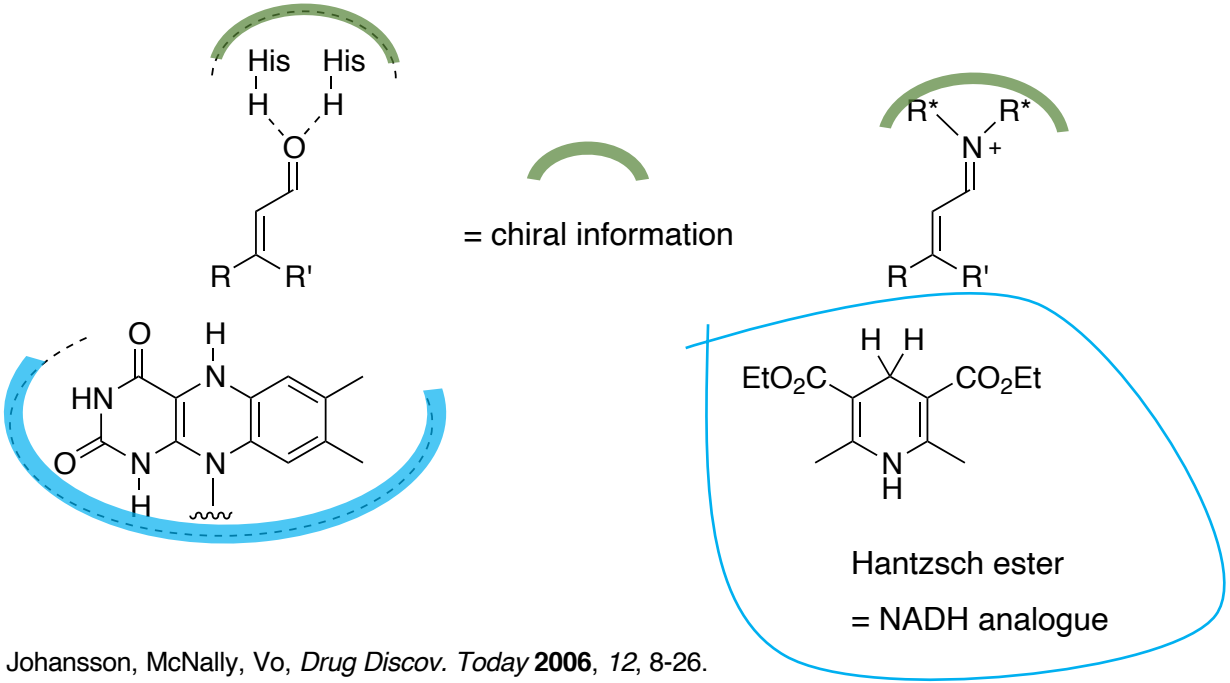
= H-bonding to carbonyl

= LUMO activation

organocatalytic activation

= iminium formation with carbonyl

= LUMO activation



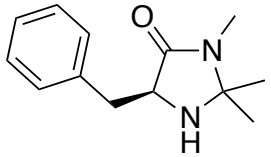
Gaunt, Johansson, McNally, Vo, *Drug Discov. Today* **2006**, *12*, 8-26.

Biomimetic chemistry inspired by reductases

conjugate reduction: organocatalytic enoate reductase mimics

chiral secondary amine organocatalysts

(MacMillan)



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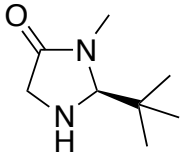
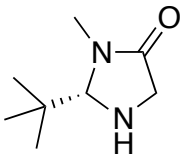
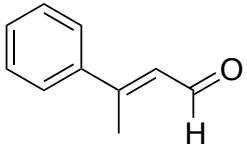


amino acid based secondary amines

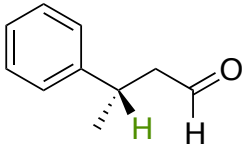




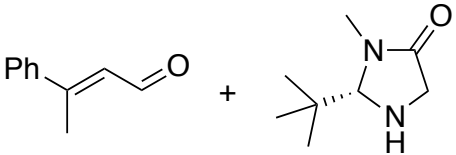
20 mol%

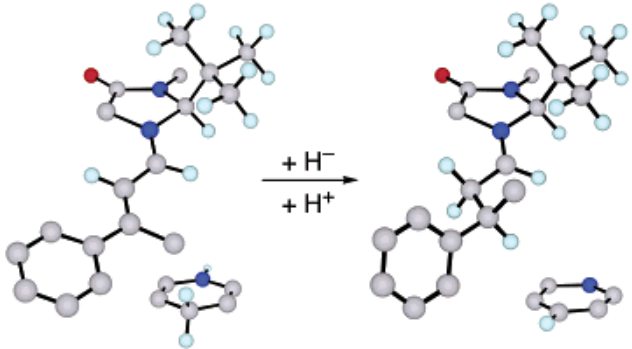
Hantzsch ester

$CHCl_3$, CF_3COOH



93% *ee*





Ouellet, Tuttle, MacMillan, *J. Am. Chem. Soc.* **2005**, *127*, 32-33.

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Biomimetic chemistry inspired by reductases

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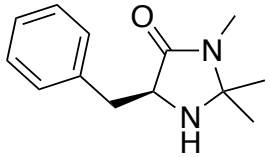
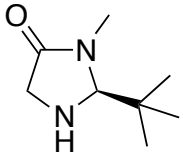
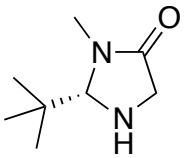
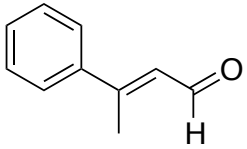


or



amino acid based secondary amines

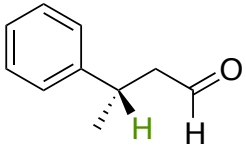




20 mol%

Hantzsch ester

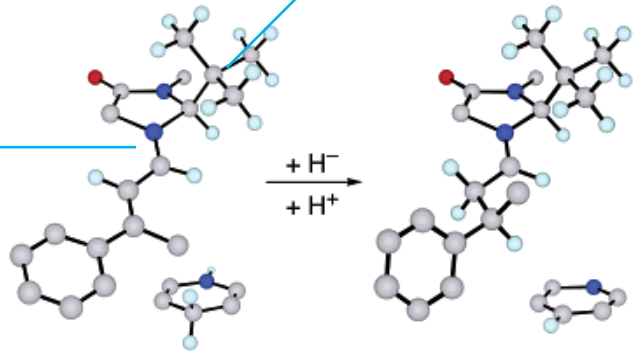
$CHCl_3$, CF_3COOH



93% *ee*

top face shielding by bulky tert-butyl

LUMO activation via condensation



Ouellet, Tuttle, MacMillan, *J. Am. Chem. Soc.* **2005**, *127*, 32-33.

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SUMMARY

- dehydrogenases and reductases are important synthetic tools
- generally require techniques for cofactor recycling
- exquiste catalysts for preparation of optically active alcohols, amines, carbonyl compounds…
  - ✓ asymmetric reductions
  - ✓ kinetic resolutions
  - ✓ dynamic kinetic resolutions