Biocatalysis

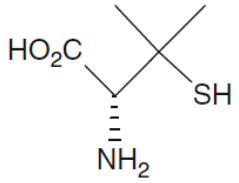
- General Principles
  - Stereoselectivity
  - Biocatalyst production
  - Biocatalyst immobilization
  - Biocatalyst modificatiln
- Hydrolytic reactions
- Redox reactions
- Addition-/elimination reactions
- Glycosyl Transfer
- Industrial applications
- Cascade processes

Biocatalysis ï General Aspects

Stereochemistry & Drug Synthesis

Å Enantiomers & Diastereomer Discrimination

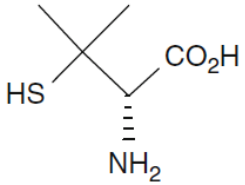
R-Enantiomer



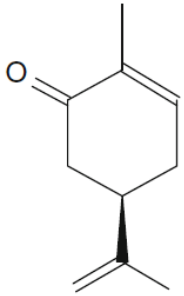
toxic

Penicillamine

S-Enantiomer

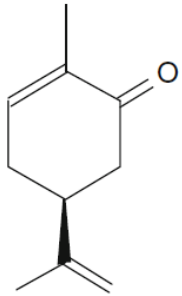


antiarthritic

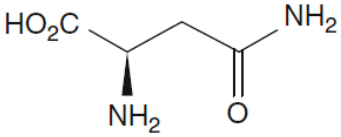


caraway scent

Carvone

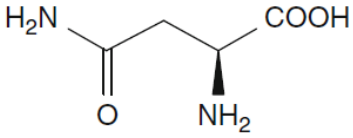


anise scent



sweet

Asparagine

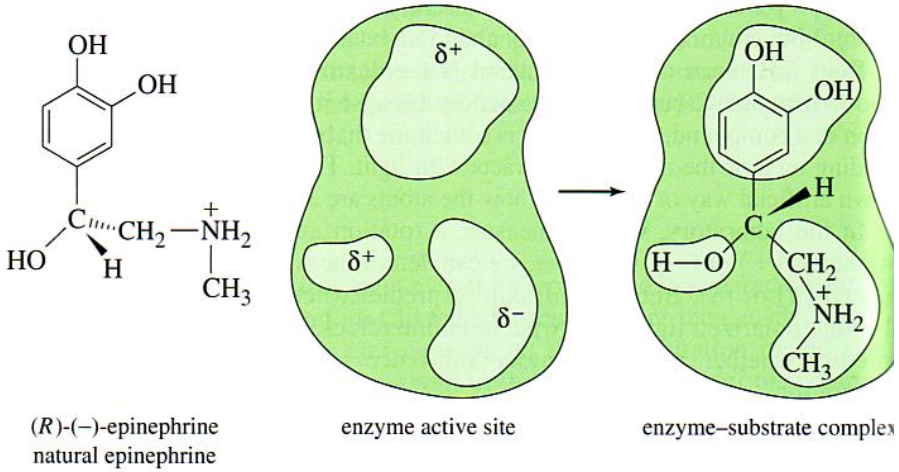


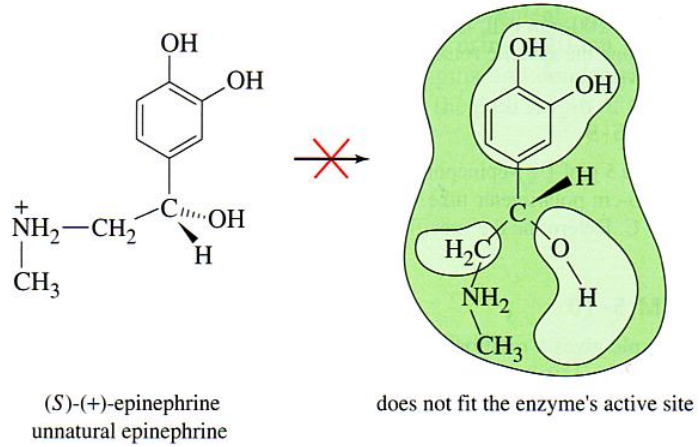
bitter

Biocatalysis ï General Aspects

Stereochemistry & Drug Synthesis

Å Enantiomers & Diastereomer Discrimination

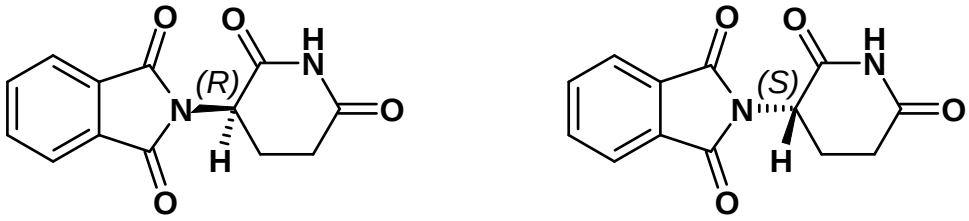




Biocatalysis ï General Aspects

Stereochemistry & Drug Synthesis

Å The Thalidomid Incident



Thalidomid:
(*R*)-enantiomer: weak analgetic
(*S*)-enantiomer: strong teratogenic side effects

Biocatalysis ï General Aspects

Pros

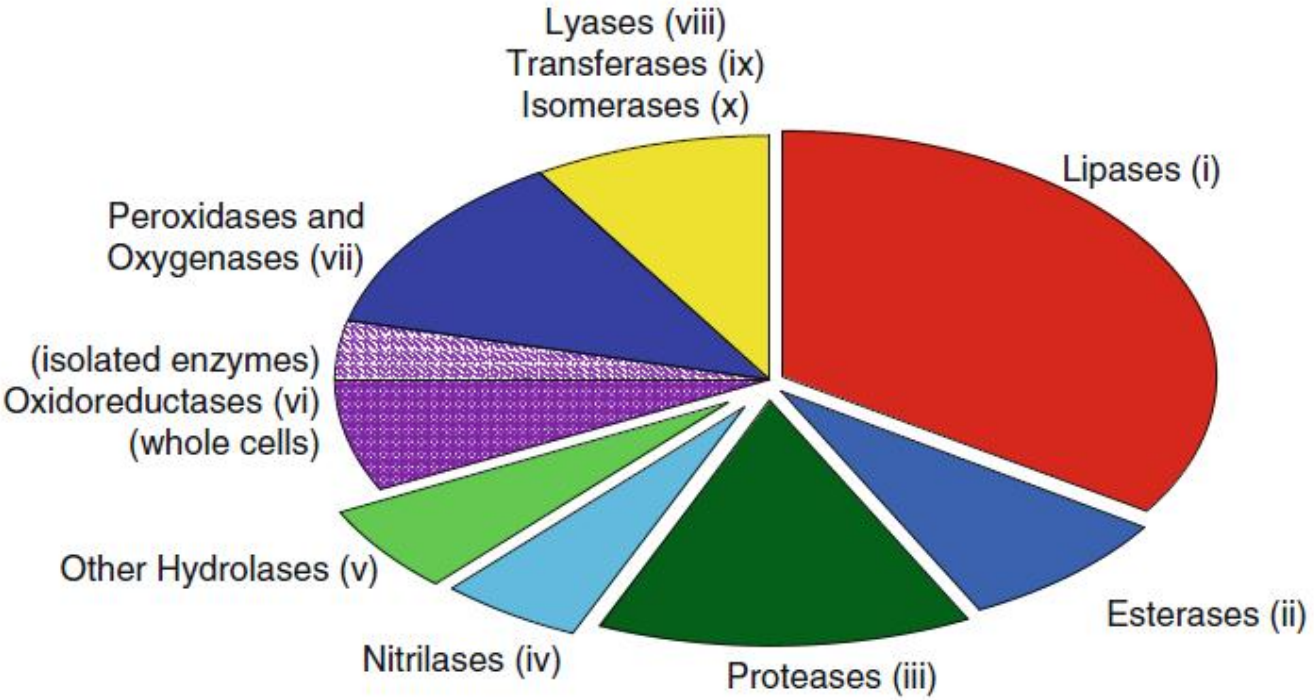
- **high enantioselectivity**
- **high regioselectivity** (incl. diastereoselectivity)
- **high chemoselectivity**
- **broad substrate tolerance**
- **high efficiency**
- **environmentally benign**
- **mild reaction conditions**
- **enzyme compatibility** (reaction cascades)

Cons

- **enantiocomplementarity**
- **cofactors**
- **low flexibility in operational parameters**
- **aqueous reaction conditions** (loss of activity in organic solvents)
- **inhibition**
- **availability**

Biocatalysis ï General Aspects

Enzymes & Transformations



(i) Ester formation, -aminolysis, -hydrolysis; (ii) ester hydrolysis; (iii) ester and amide hydrolysis, peptide synthesis; (iv) nitrile hydrolysis; (v) hydrolysis of epoxides, halogens, phosphates, and glycosides; (vi) reduction of C=O and C=C bonds; (vii) hydroxylation or C-H bonds, sulfoxidation of thioethers, epoxidation of alkenes, Baeyer-Villiger oxidation of ketones, dihydroxylation of aromatics, peroxidation; (viii) cyanohydrin formation, acyloin and aldol reaction; (ix) glycosyl and amino-group transfer; (x) Claisen-type rearrangement, isomerization of carbohydrates, racemization.

Fig. 4.1 Frequency of use of particular biocatalysts in biotransformations

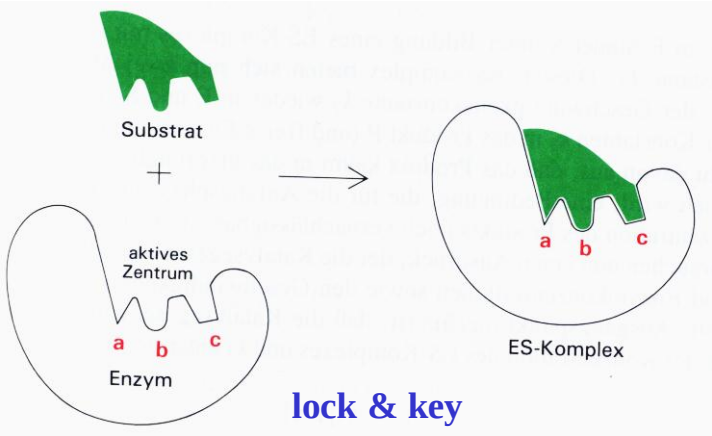
Biocatalysis ï General Aspects

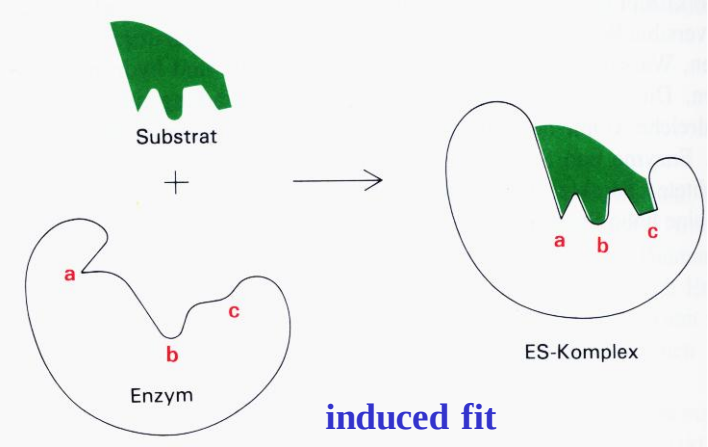
Induced-Fit-Theory

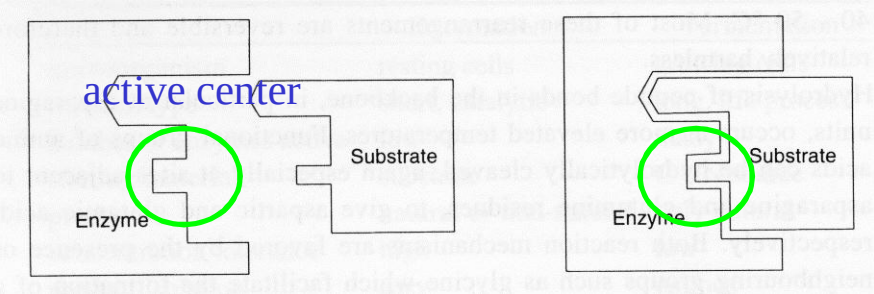
Å Koshland 1961

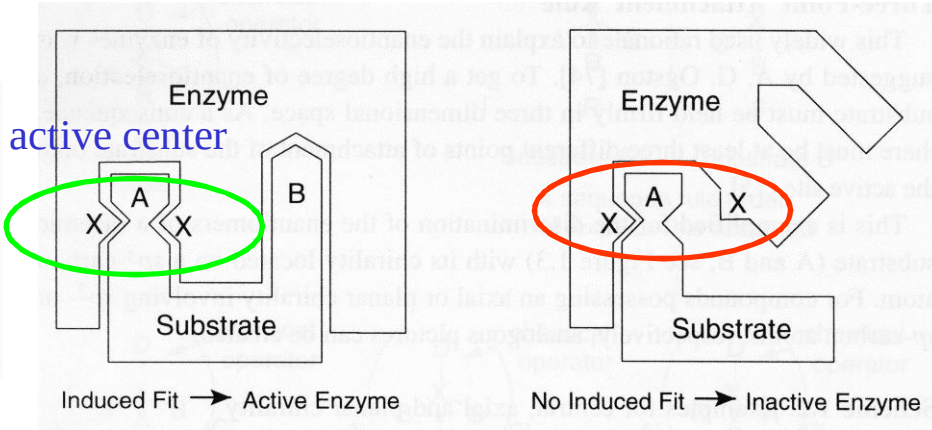
ï conformational influence by substrate & enzyme

➠ **modification of the biological activity of proteins**





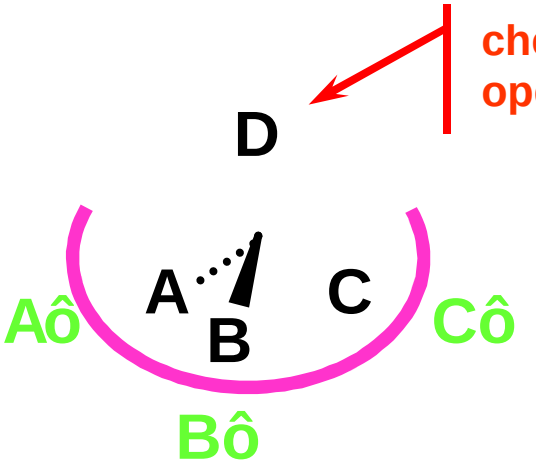


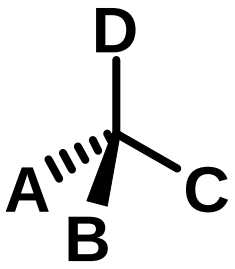


Biocatalysis ï General Aspects

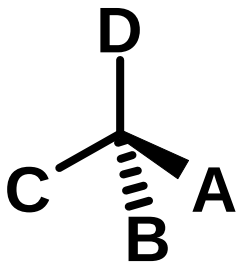
Enantioselectivity

Å Three-Point Attachment Theory (Ogston 1948)



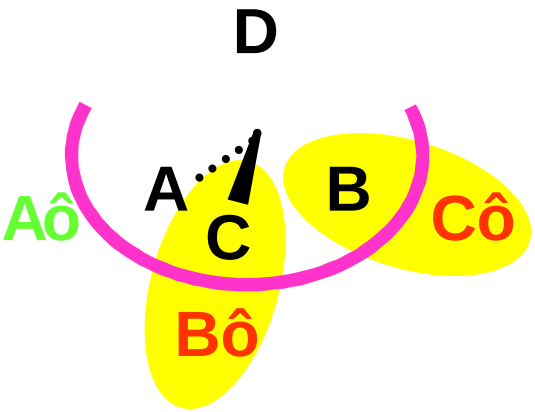


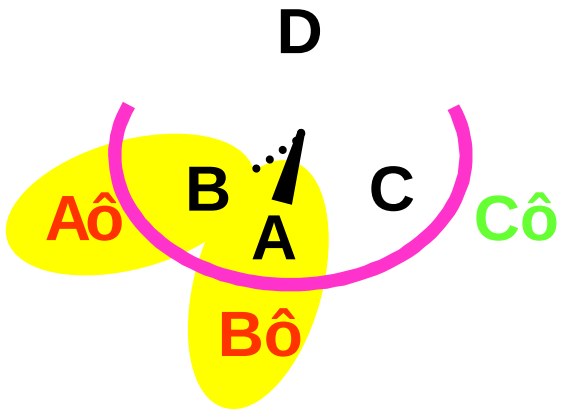
R
substrate A

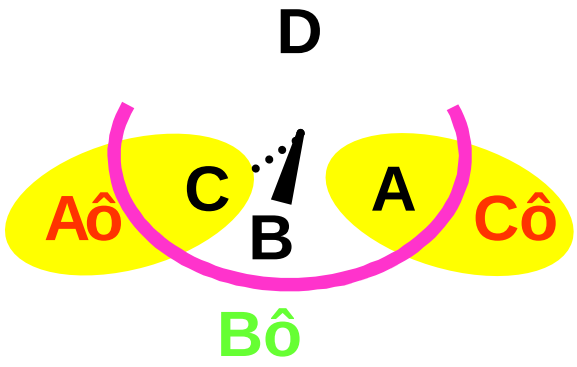


S
substrate B

sequence order A>B>C>D assumed





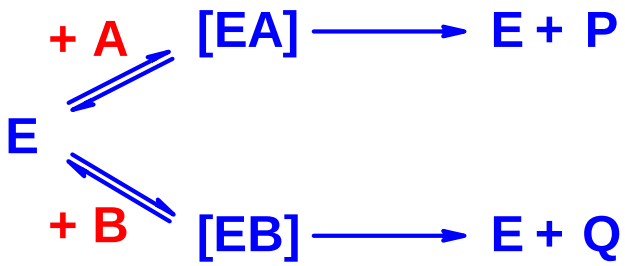


Biocatalysis ï General Aspects

Enantioselectivity

Å Three-Point Attachment Theory (Ogston 1948)

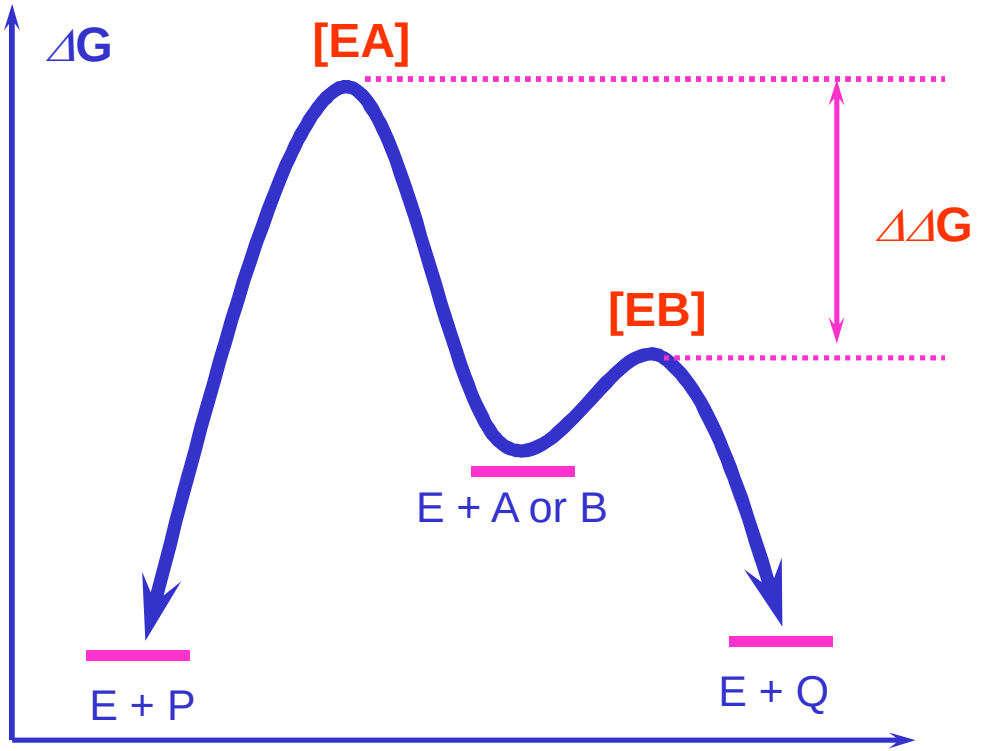
- ï optical antipodes result in diastereomeric pairs upon interact. with enzyme
- ï different energy levels of enzyme-substrate-complexes



$\Delta\Delta G = \Delta\Delta H - T\,\Delta\Delta S$

$\Delta\Delta G = -RT \ln(v_A/v_B)$

ΔΔG [kcal/mol]	vA/vB	ee %
0.118	1.2	10
0.651	3	50
1.74	19	90
2.17	39	95
3.14	199	99
4.50	1999	99.9

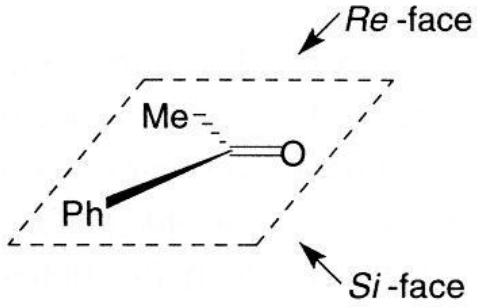


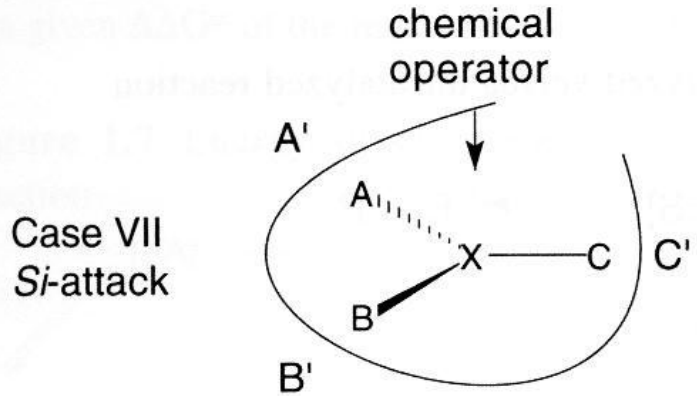
ee (%) = (P-Q)/(P+Q)

Biocatalysis ï General Aspects

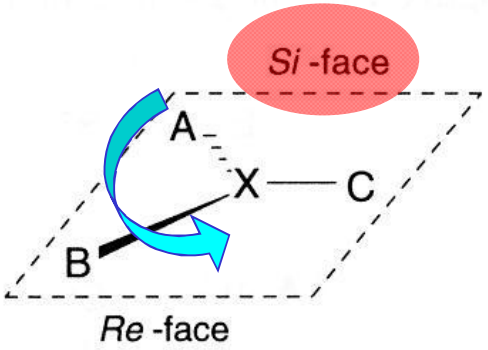
Desymmetrization

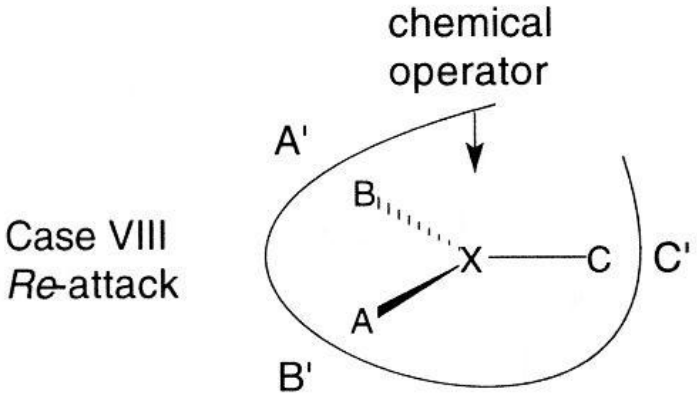
Å Enantiofacial Differentiation





Case VII
Si-attack





Case VIII
Re-attack

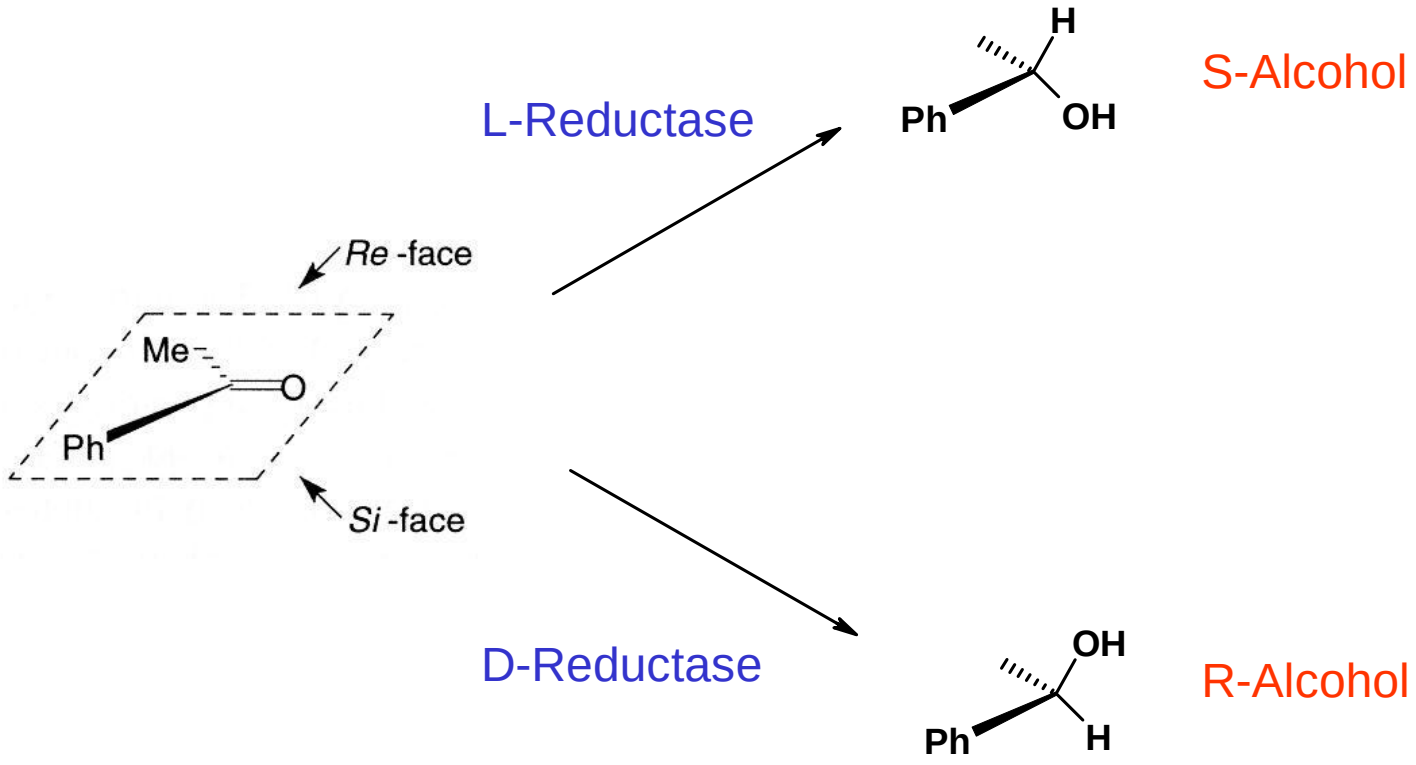
Substrate D

a sequence rule order
of A>B>C is assumed

Biocatalysis ï General Aspects

Desymmetrization

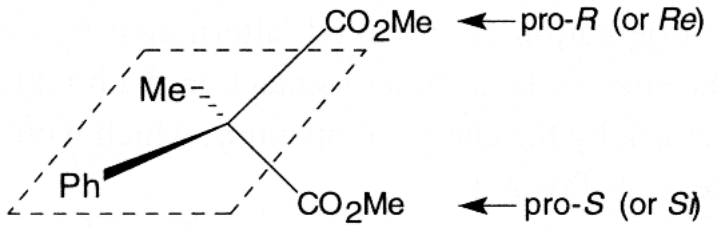
Å Enantiofacial Differentiation

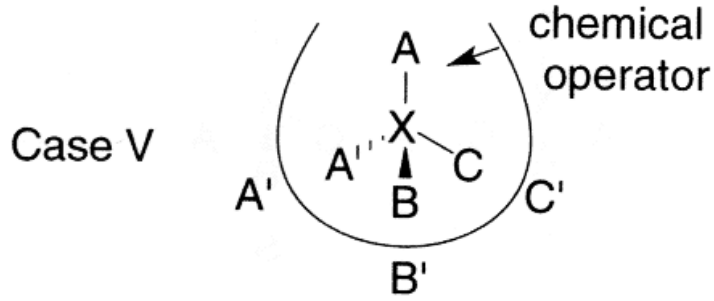


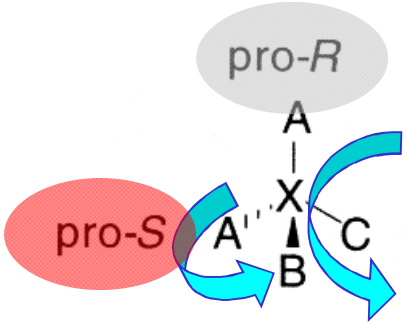
Biocatalysis ï General Aspects

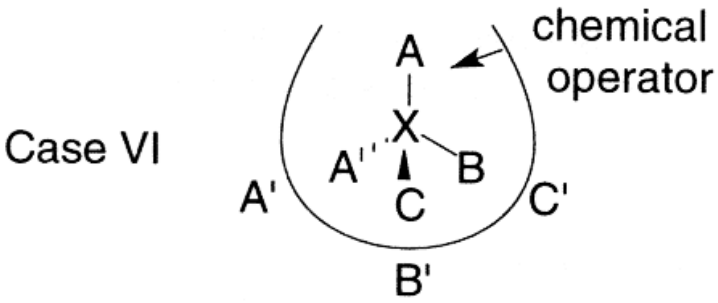
Desymmetrization

Å Enantiotopos Differentiation









Substrate C

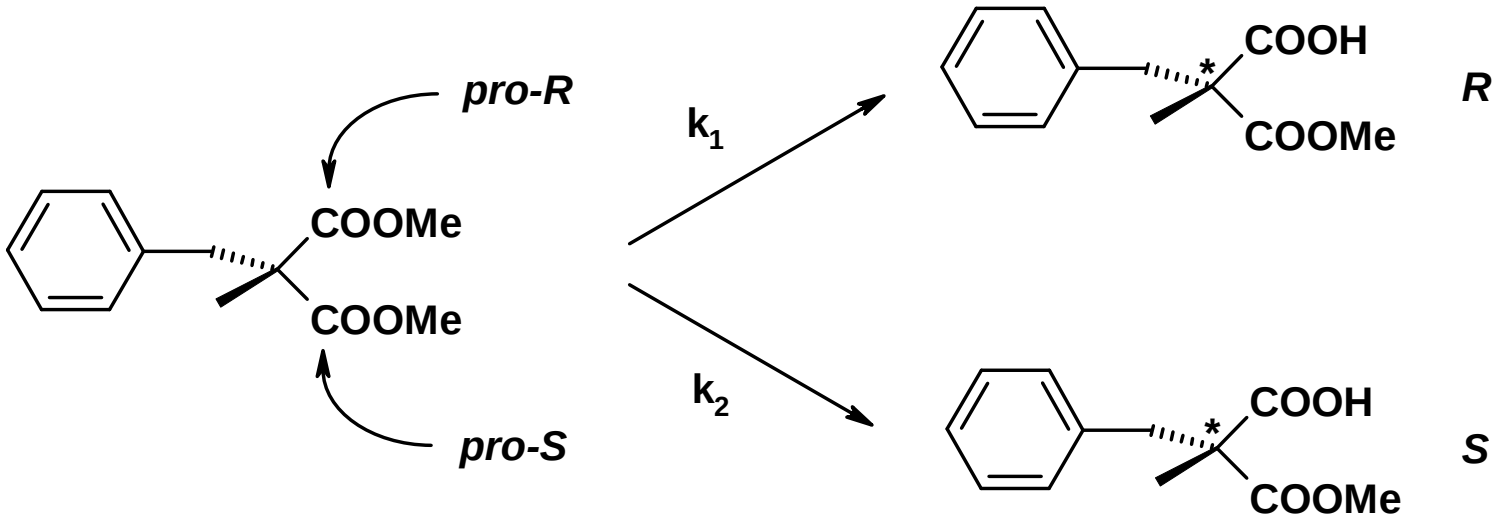
a sequence rule order of A>B>C is assumed

Biocatalysis ï General Aspects

Desymmetrization

Å Enantiotopos Differentiation

ï Single step process



selectivity $\alpha = k_1/k_2$

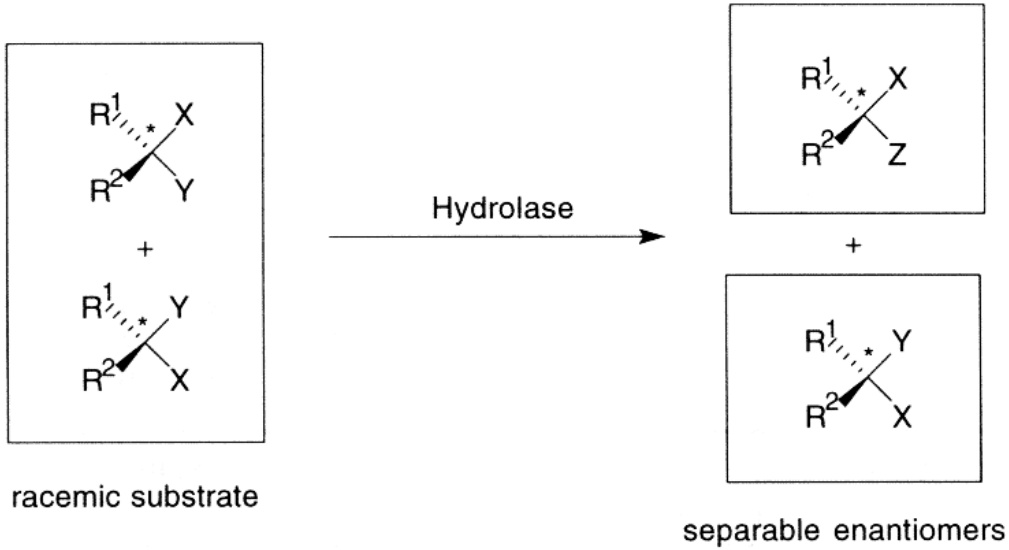
enantiomeric excess e.e. = (a-1)/(a+1) = (R - S) / (R + S)

e.e. depends on conversion

Biocatalysis ï General Aspects

Kinetic Resolution

- irreversible reaction
- reversible reaction
- sequential resolution
- dynamische resolution

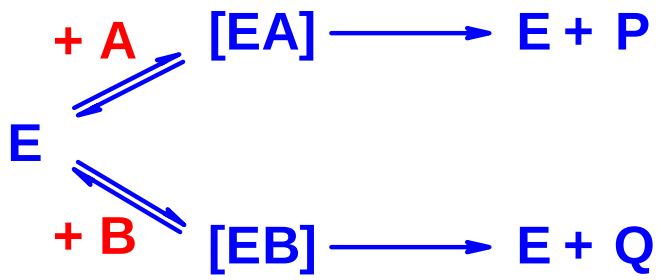


- recognition of existing chirality
- yield limitation (50%; except dynamic process)

Biocatalysis ï General Aspects

Kinetic Resolution

Å Enantiomeric Ratio E

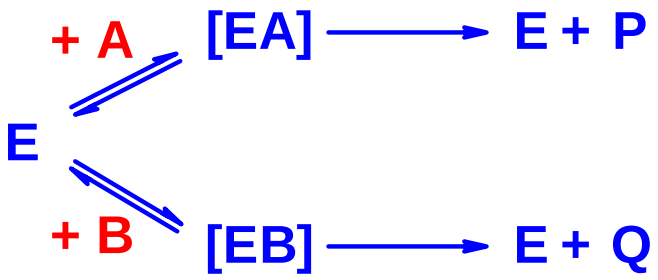


Enantiomeric Ratio $E = \frac{v_B}{v_A} = \frac{\left[\frac{k_{cat}}{K_M}\right]_A}{\left[\frac{k_{cat}}{K_M}\right]_B}$ $\Delta\Delta G^{\neq} = -RT \ln E$

Biocatalysis ï General Aspects

Kinetic Resolution

Å Enantiomeric Ratio E



ï ideal case: $k_A/k_B = \infty$ ➟ reaction stops at 50% conversion

ï real case: k_A/k_B = finite value ➟ reaction progresses beyond 50%

➟ transformation of both enantiomers depends on conversion

➟ e.e.(substrate) & e.e. (product) function of conversion

(since ratio A/B & P/Q **not** constant during whole biotransformation)

ï Mathematical model by Sharpless & Fajans (irreversible kin. ses.):

For the product

$$E = \frac{\ln[1 - c(1 + e.e._P)]}{\ln[1 - c(1 - e.e._P)]}$$

For the substrate

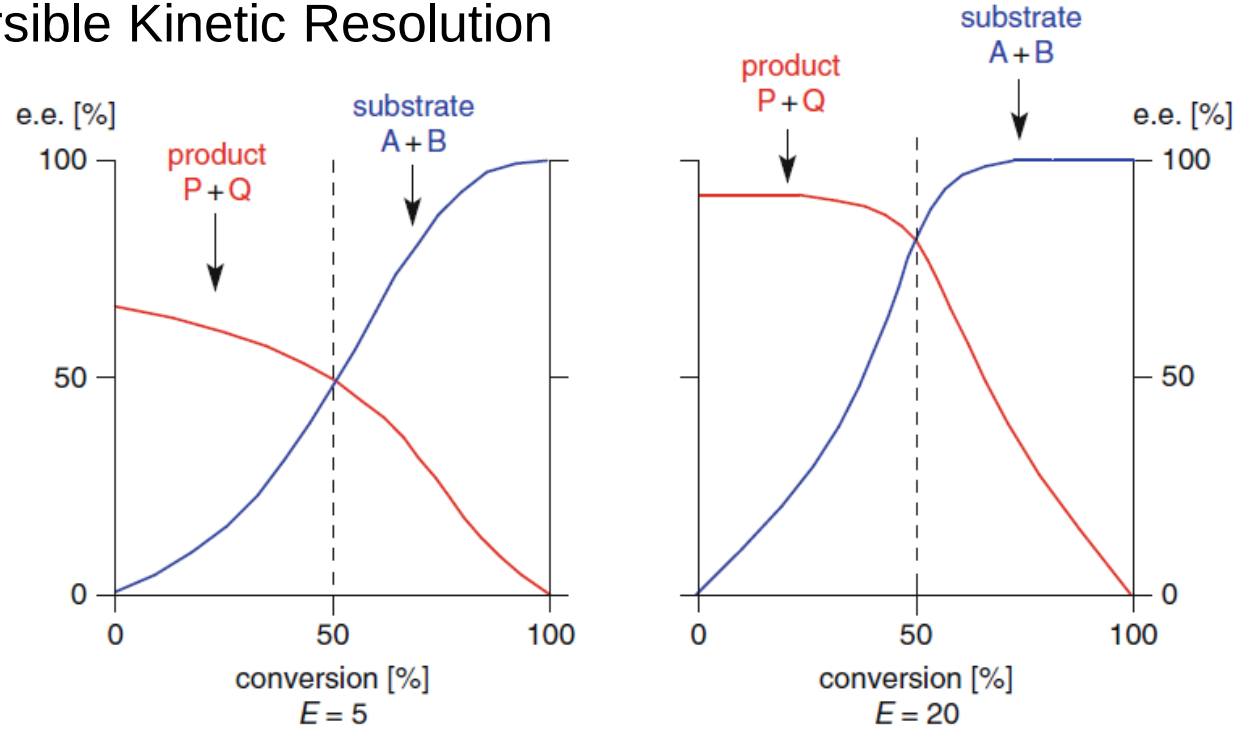
$$E = \frac{\ln[(1 - c)(1 - e.e._S)]}{\ln[(1 - c)(1 + e.e._S)]}$$

c = conversion, e.e. = enantiomeric excess of substrate (S) or product (P),
E = enantiomeric ratio

Biocatalysis ï General Aspects

Kinetic Resolution

Å Irreversible Kinetic Resolution

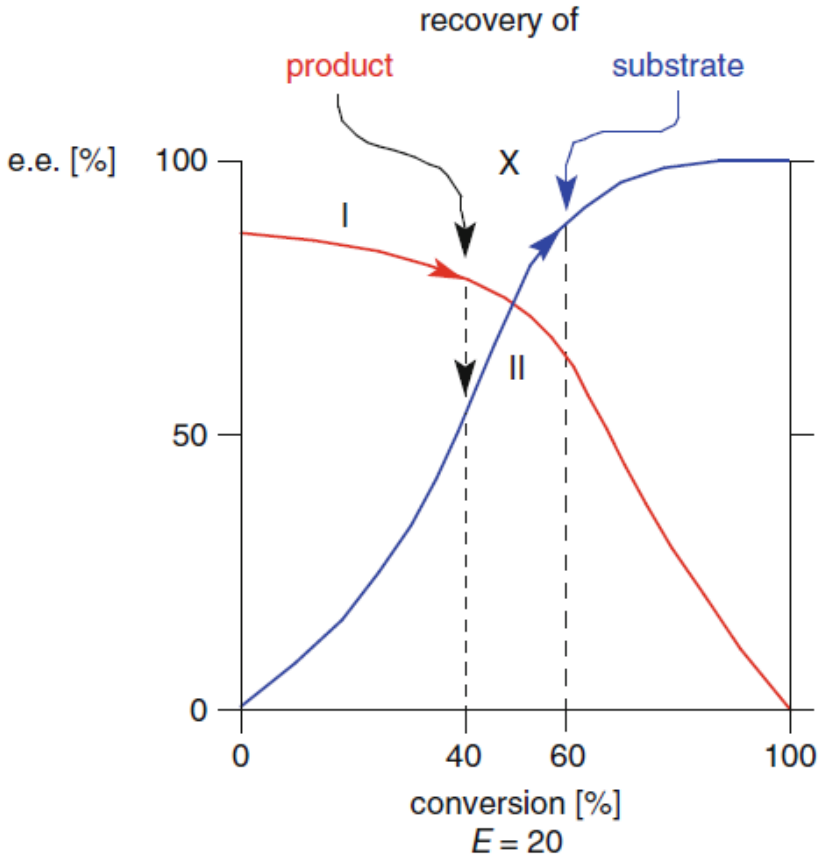


- ï e.g. hydrolysis: irreversible due to high water concentration
- ï product with high e.e. obtained before reaching 50% conversion
- ï beyond 50% decline in e.e. (high conc. of ñundesiredñsubstrate)
- ï inverted trend for substrate e.e.
- ï quality of resultion depends on E-value

Biocatalysis ï General Aspects

Kinetic Resolution

- Irreversible Kinetic Resolution
  - Substrate recovery
  - Product isolation



Biocatalysis ï General Aspects

Kinetic Resolution

- Problems in kinetic resolutions:
  - maximum yield of 50% for required enantiomer
  - remaining antipode often of no use
  - separation required (extraktion, distillation, etc.)
  - limitation of optical purity by finite E-value

- Ideal industrial process:
  - 100% yield
  - single enantiomer

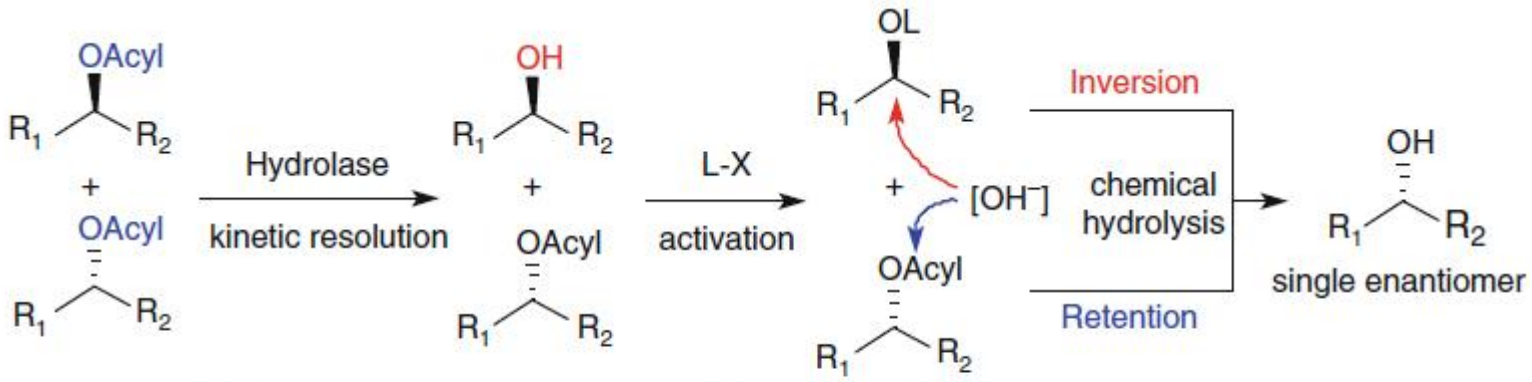
Repeated Resolution

- Racemization of unwanted antipode (mostly chemically)
- Repetition of biocatalytic resolution (iterative)
- Several additional steps
- Decrease in yields due to (mostly) forced reaction conditions

Biocatalysis ï General Aspects

Kinetic Resolution

- In-situ Inversion:
  - reaction mixture after resolution consists of
    enantiopure product
    enantiopure substrate
  - single chiral center:
    inversion by chemical activation and reaction



L = leaving group (e.g. tosylate, triflate, nitrate, Mitsunobu-intermediate)

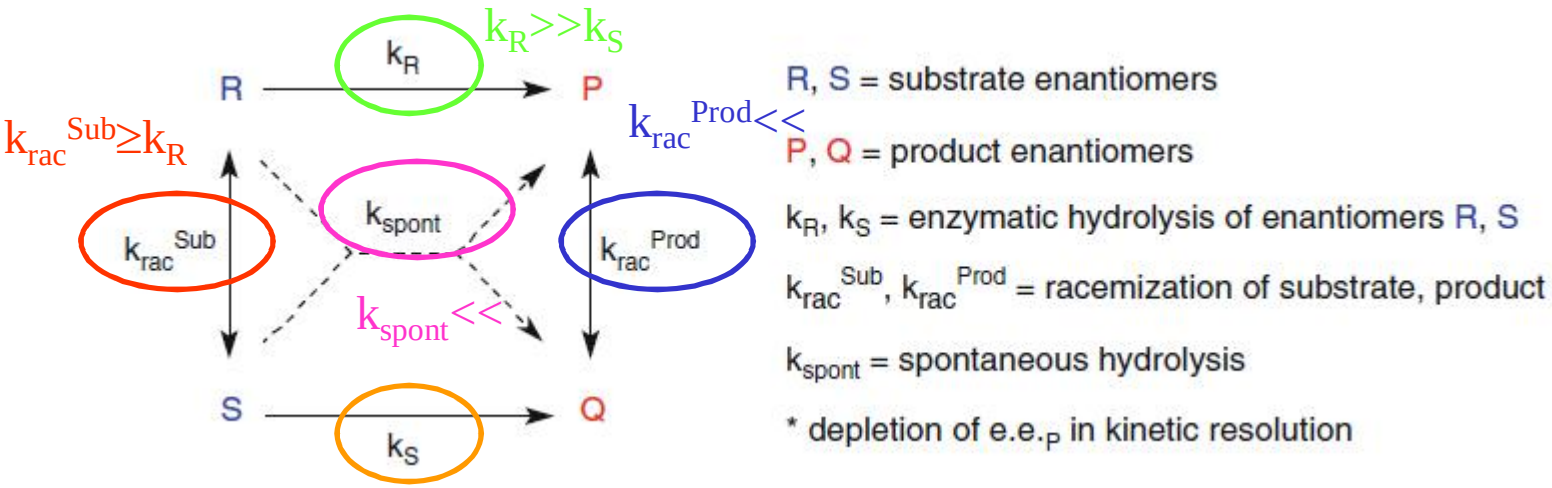
Scheme 2.9 Kinetic resolution with in-situ inversion

Biocatalysis ï General Aspects

Kinetic Resolution

Å Dynamic Kinetic Resolution

- ï classical resolution
- ï in-situ racemization of substrates
  ➟ **dynamic process**
- ï equilibrium constantly regenerated ➟ always beneficial ratio in favor of the desired enantiomer

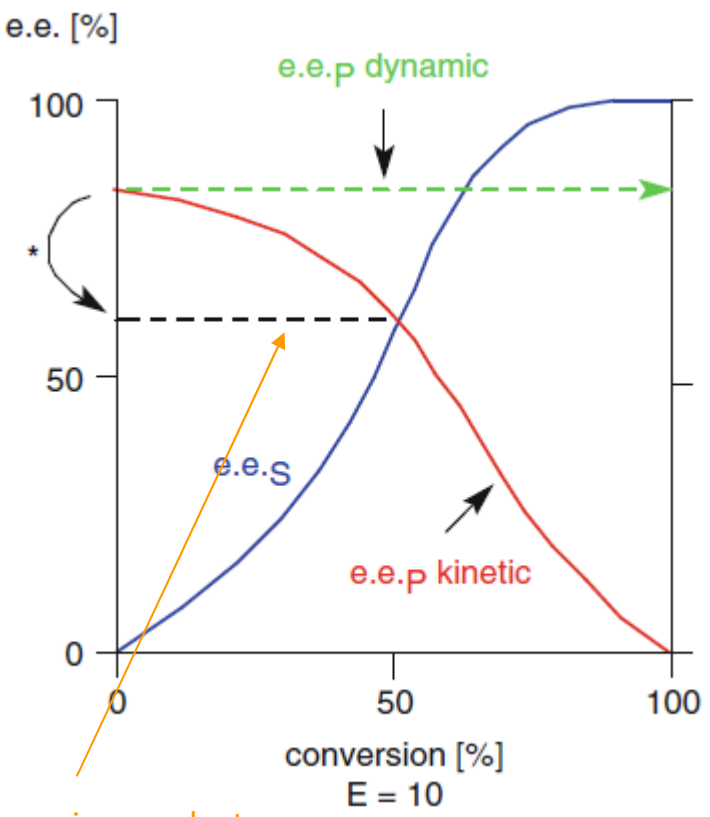


Biocatalysis ï General Aspects

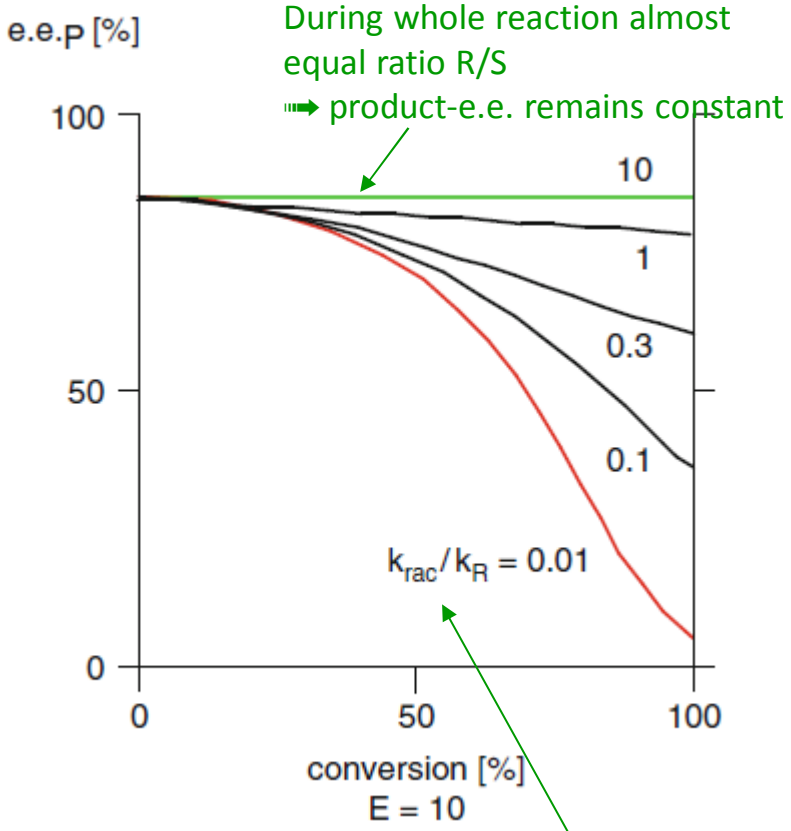
Kinetic Resolution

Å Dynamic Kinetic Resolution

ï comparison conventional resolution (E=10) with dynamic resolution



Rapidly decreasing product-e.e.
due to increasing conc. of S



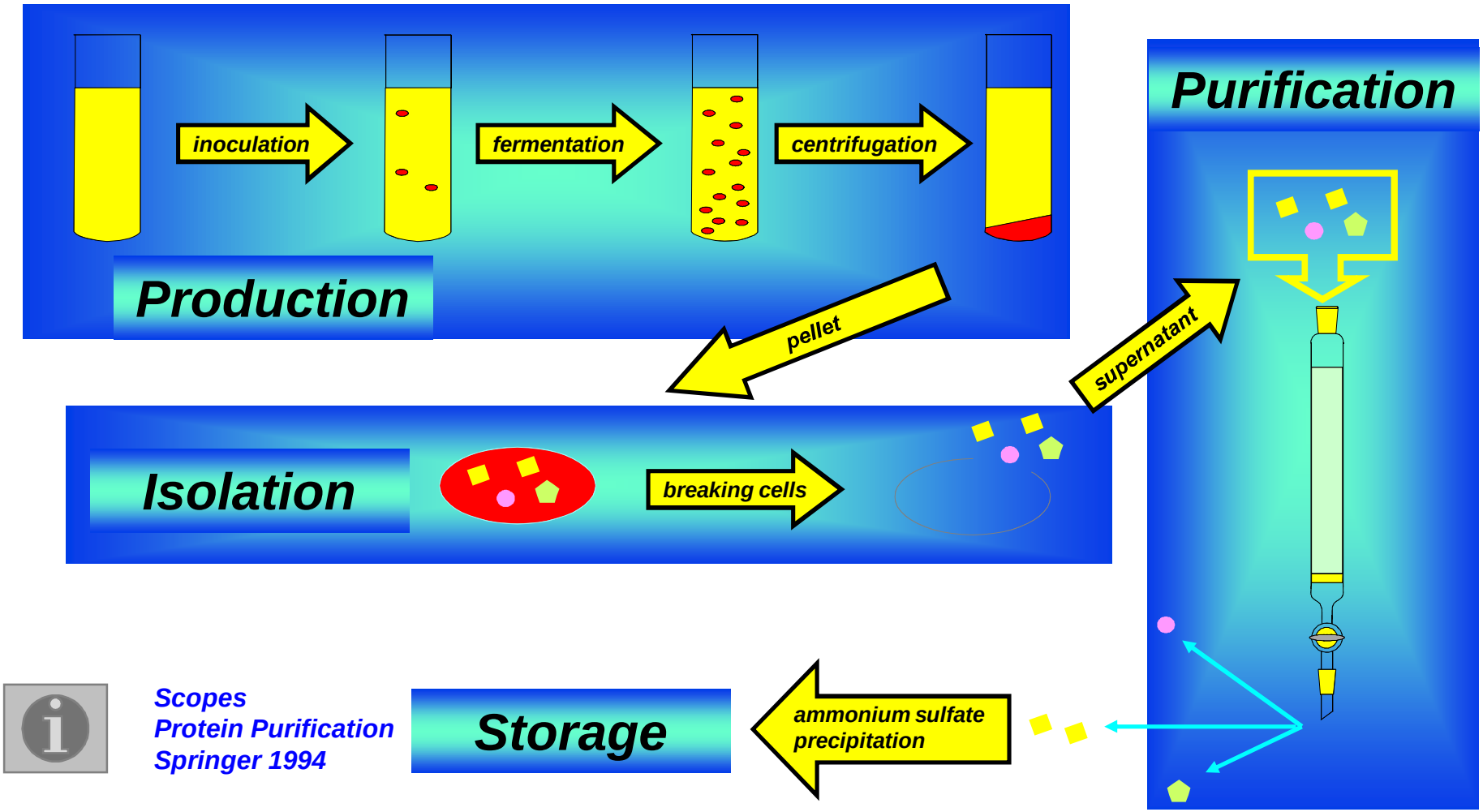
During whole reaction almost
equal ratio R/S
➠ product-e.e. remains constant

The smaller k_{rac}^{Sub}/k_R the more
similar to conventional resolution

Biocatalysis ï General Aspects

Protein Preparation

Å Work flow

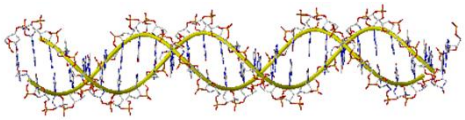


Scopes
Protein Purification
Springer 1994

Biocatalysis ï General Aspects

Protein Expression

Å Work flow



DNA source
(e.g. genomic DNA)

PCR

gene

ligation

proliferation plasmid

transfor-mation

proliferation host

Incubation plasmid isolation

plasmid amplification

digestion

manipulation of restriction sites

religation

expression plasmid

transfor-mation

expression host

storage

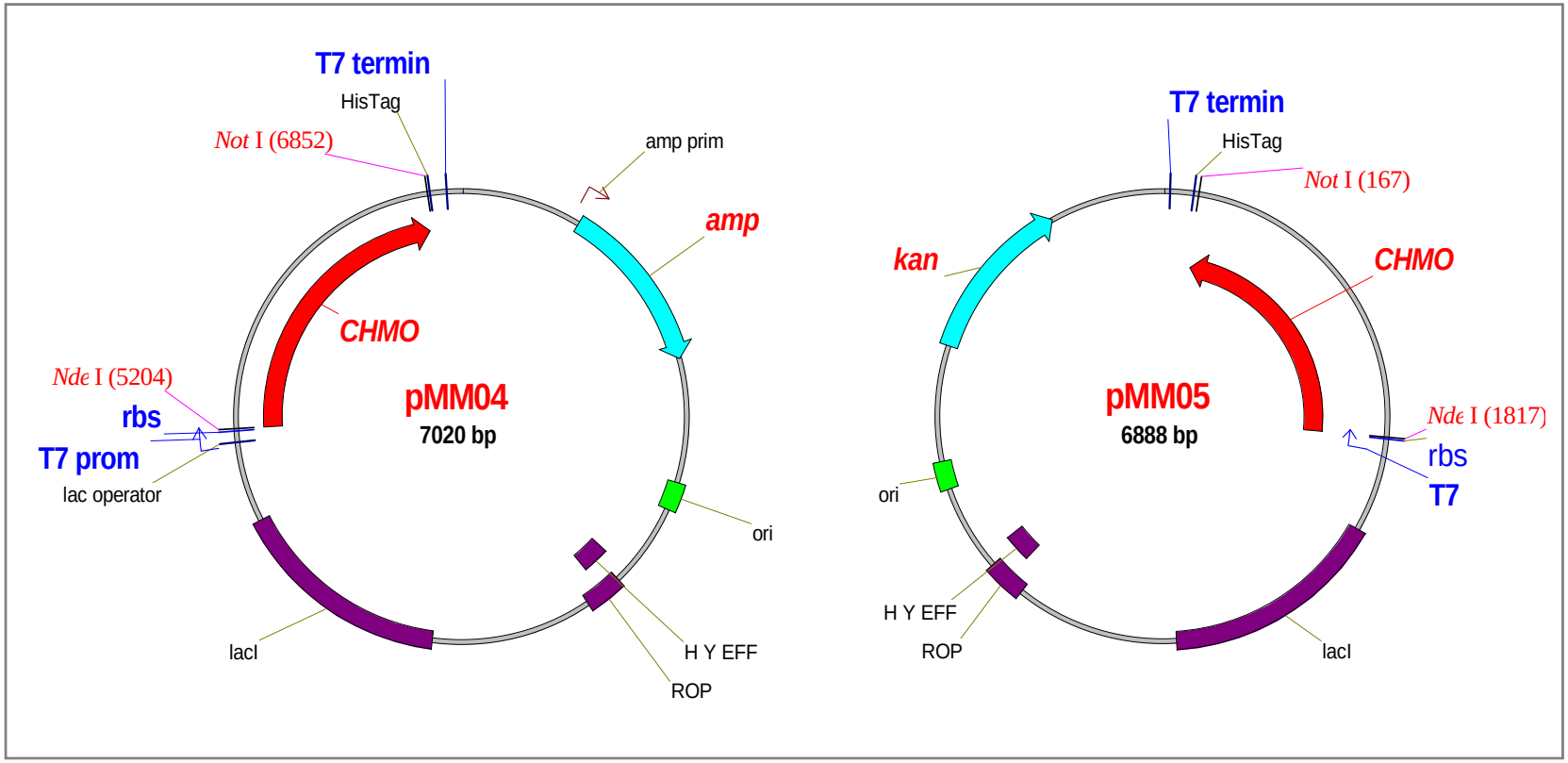
protein expression

protein isolation biotransformation

Biocatalysis ï General Aspects

Protein Expression

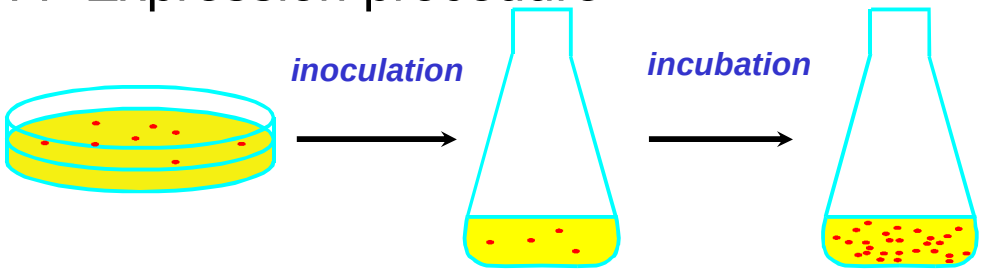
Å Expression plasmids

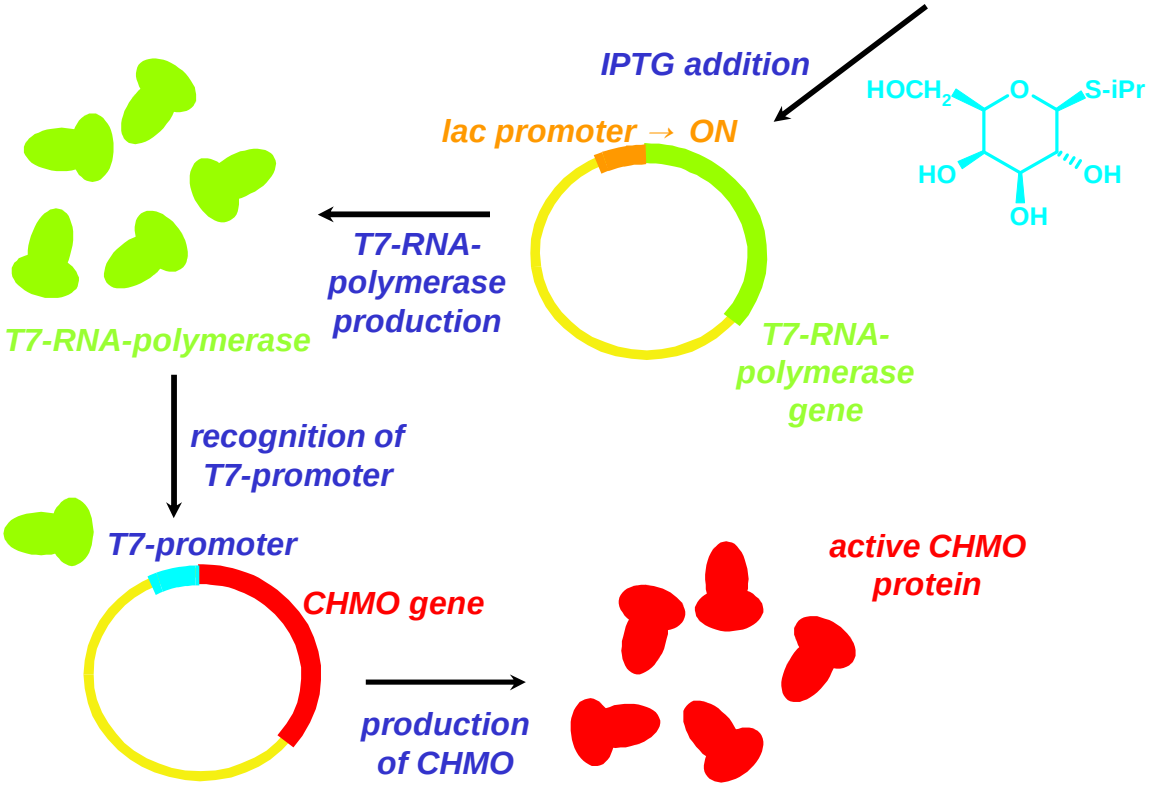


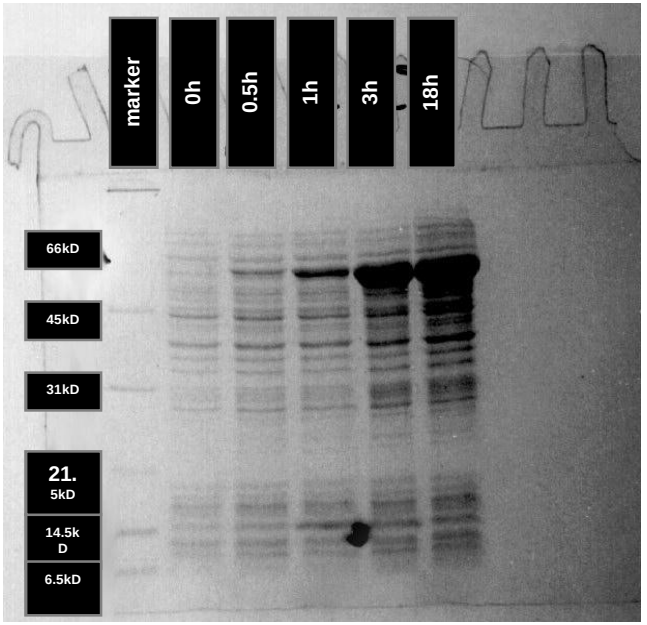
Biocatalysis ï General Aspects

Protein Expression

Å Expression procedure





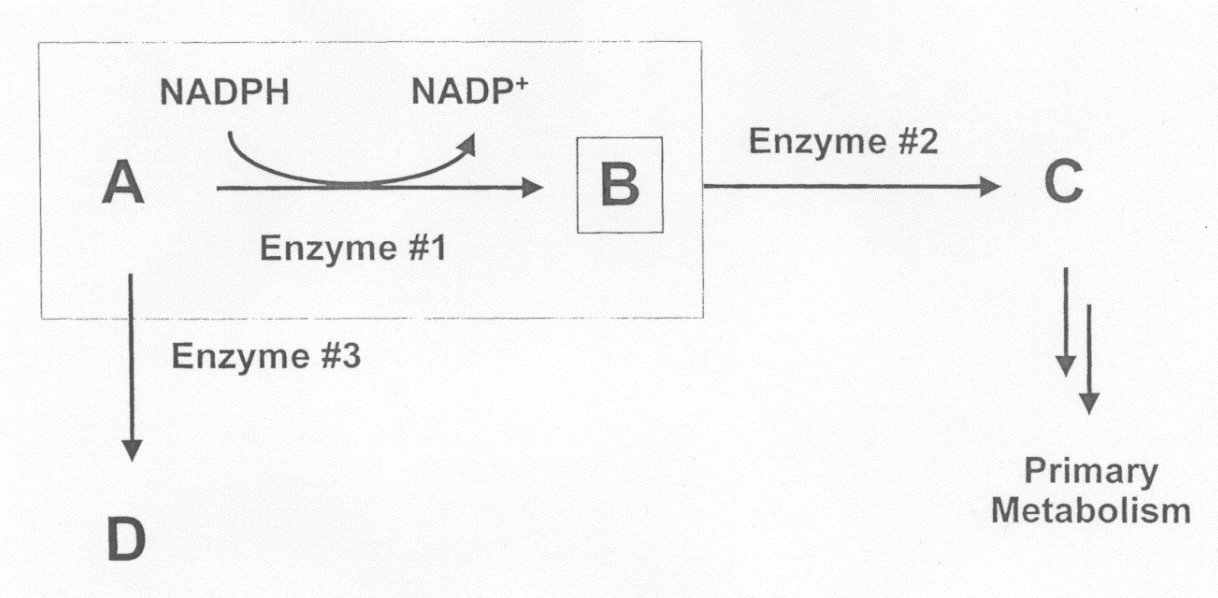


approx. 25% active protein

Biocatalysis ï General Aspects

Protein Expression

Å Whole-cell Biotransformations



- ï cofactor recycling
- ï enzyme production
- ï enzyme in natural environment
- ï cheap C-source (glucose, saccharose) for stereoselective reactions
- ï toxicity of non-natural substrates
- ï transport effects
- ï side reactions

Biocatalysis ï General Aspects

Protein Expression

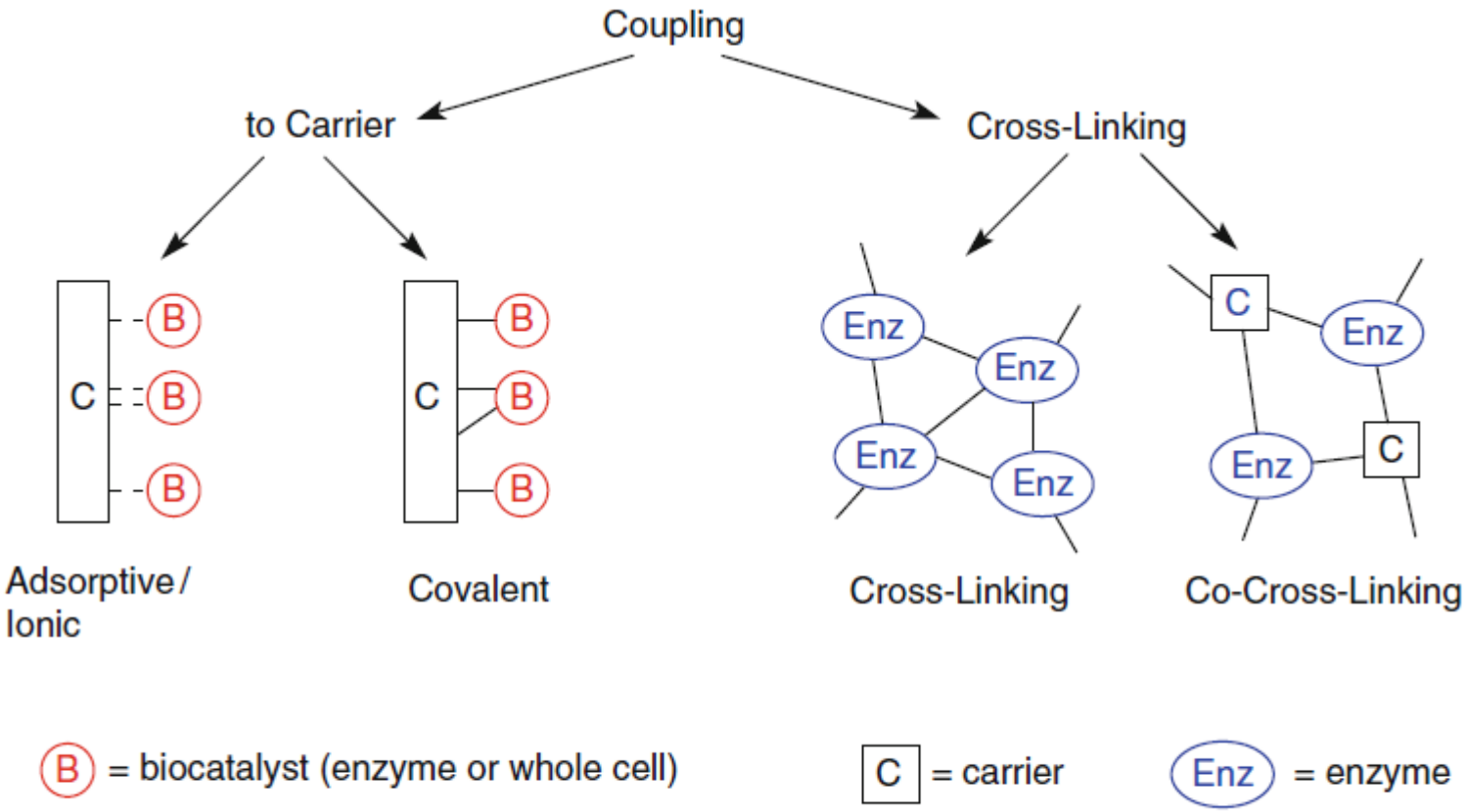
Table 1.2 Pros and cons of using isolated enzymes vs. whole cell systems

Biocatalyst	Form	Pros	Cons
Isolated enzymes	Any	Simple apparatus, simple workup, better productivity due to higher concentration tolerance	Cofactor recycling necessary, limited enzyme stabilities
	Dissolved in water	High enzyme activities	Side reactions possible, lipophilic substrates insoluble, workup requires extraction
	Suspended in organic solvents	Easy to perform, easy workup, lipophilic substrates soluble, enzyme recovery easy	Reduced activities
	Immobilized	Enzyme recovery easy	Loss of activity during immobilization
Whole cells	Any	No cofactor recycling necessary, no enzyme purification required	Expensive equipment, tedious workup due to large volumes, low productivity due to lower concentration tolerance, low tolerance of organic solvents, side reactions likely due to uncontrolled metabolism
	Growing culture	Higher activities	Large biomass, enhanced metabolism, more byproducts, process control difficult
	Resting cells	Workup easier, reduced metabolism, fewer byproducts	Lower activities
	Immobilized cells	Cell reuse possible	Lower activities

Biocatalysis ï General Aspects

Biocatalyst Immobilization

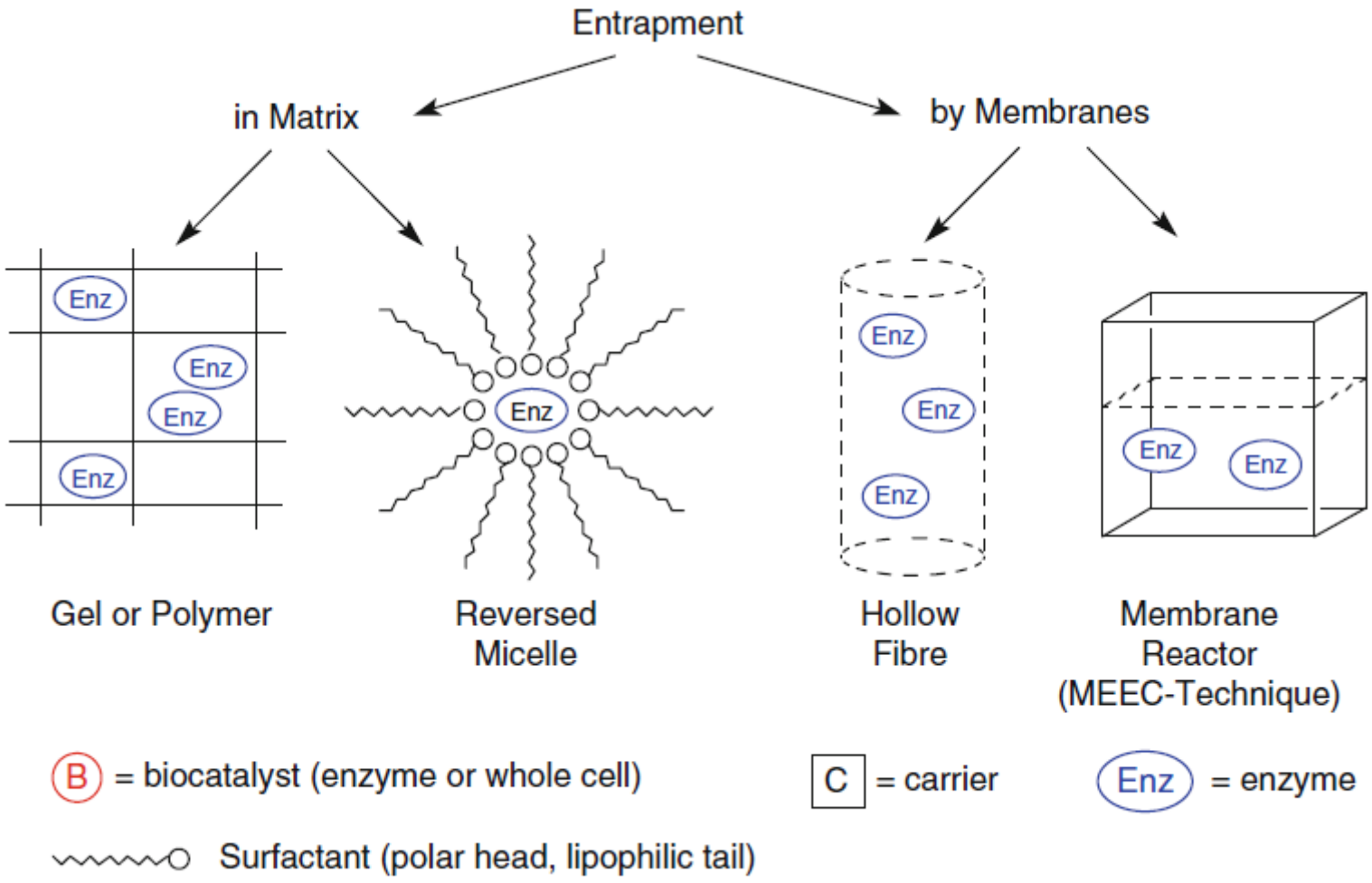
Å Coupling



Biocatalysis ï General Aspects

Biocatalyst Immobilization

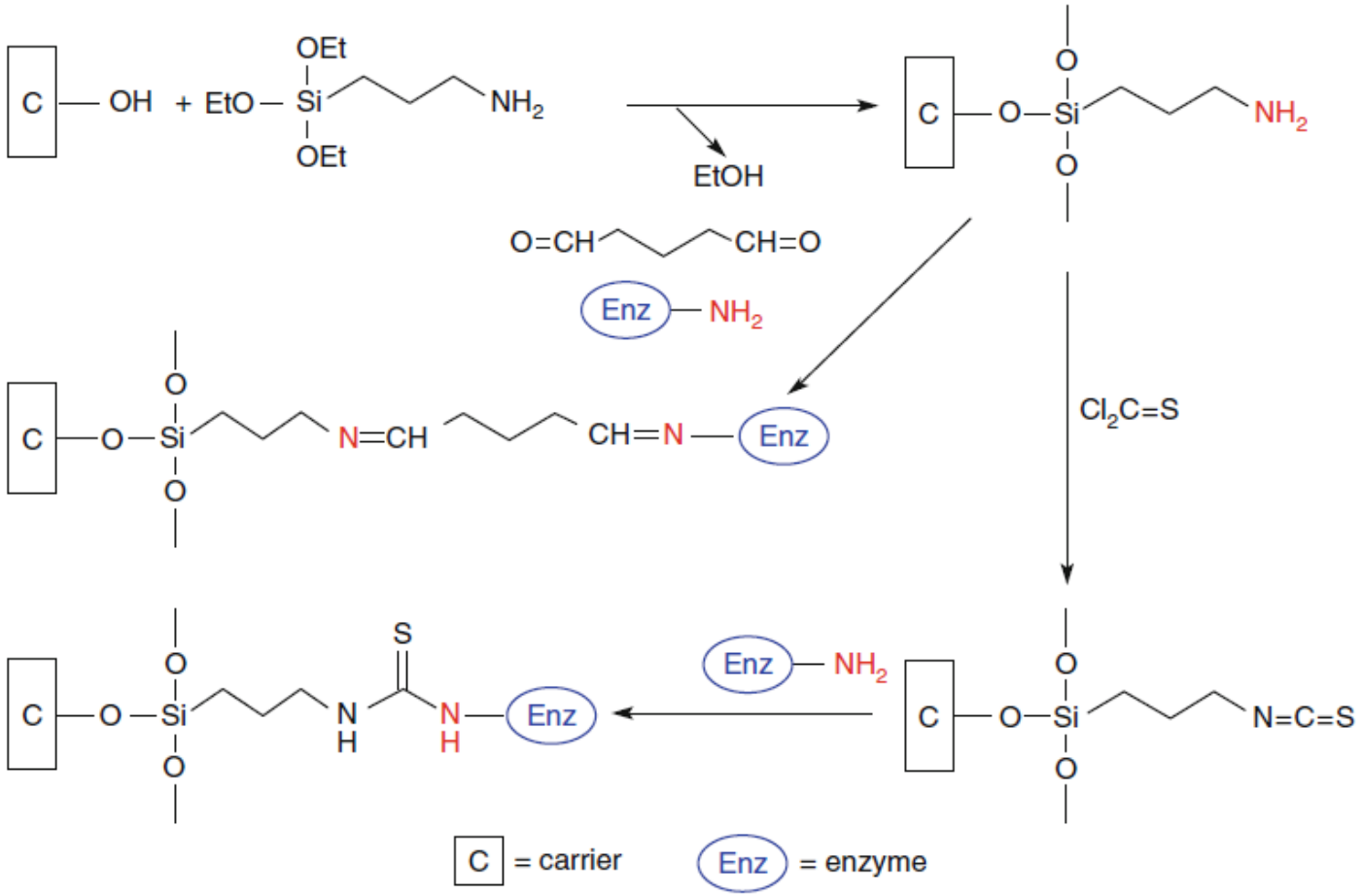
Å Entrapment



Biocatalysis ï General Aspects

Biocatalyst Immobilization

Å Covalent Linkage

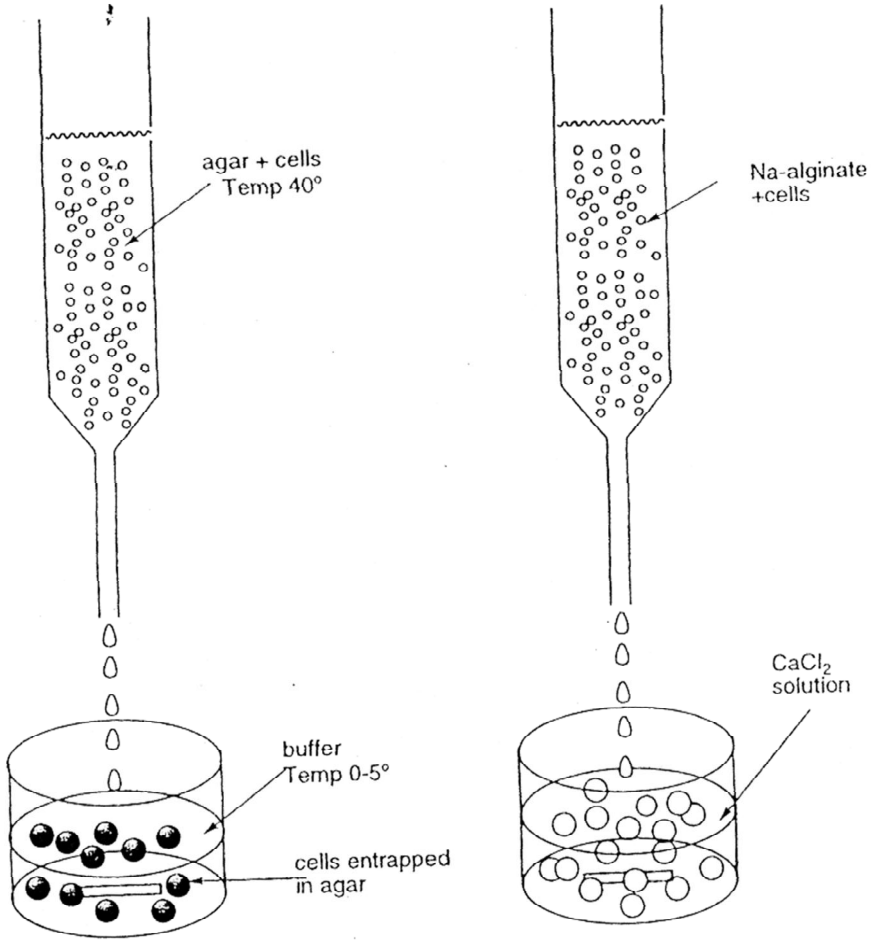


Scheme 3.34 Covalent immobilization of enzymes onto inorganic carriers

Biocatalysis ï General Aspects

Biocatalyst Immobilization

- Å Entrapment
  - ï Whole cells



Biocatalysis ï General Aspects

Protein Modification

Å Site-directed mutagenesis

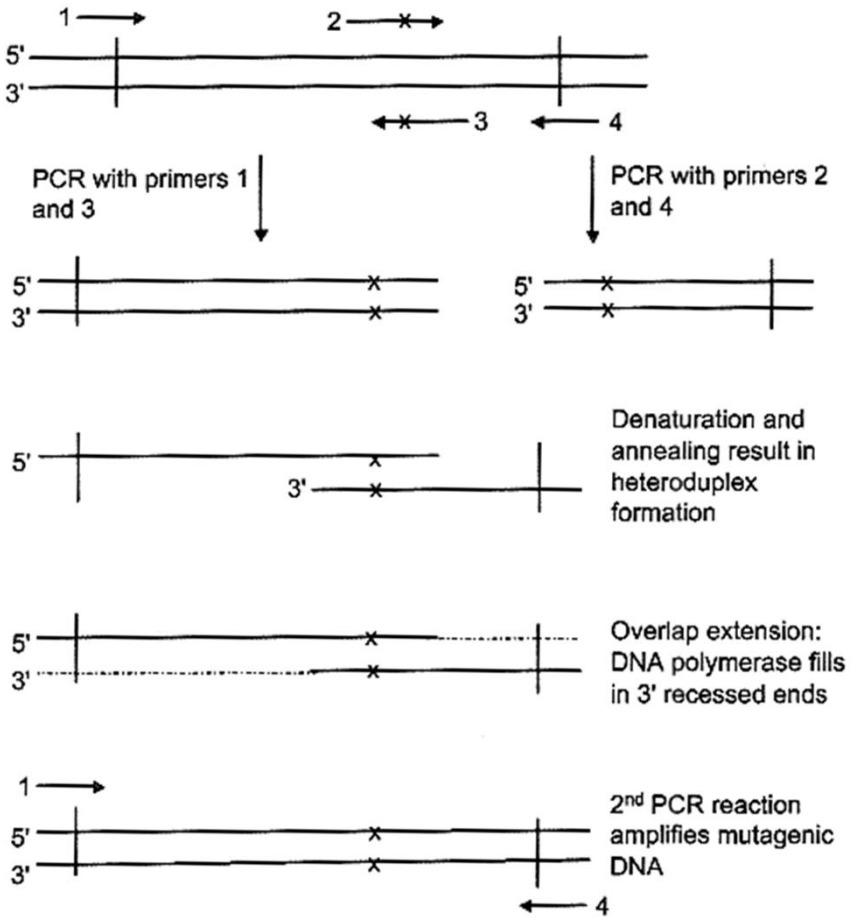


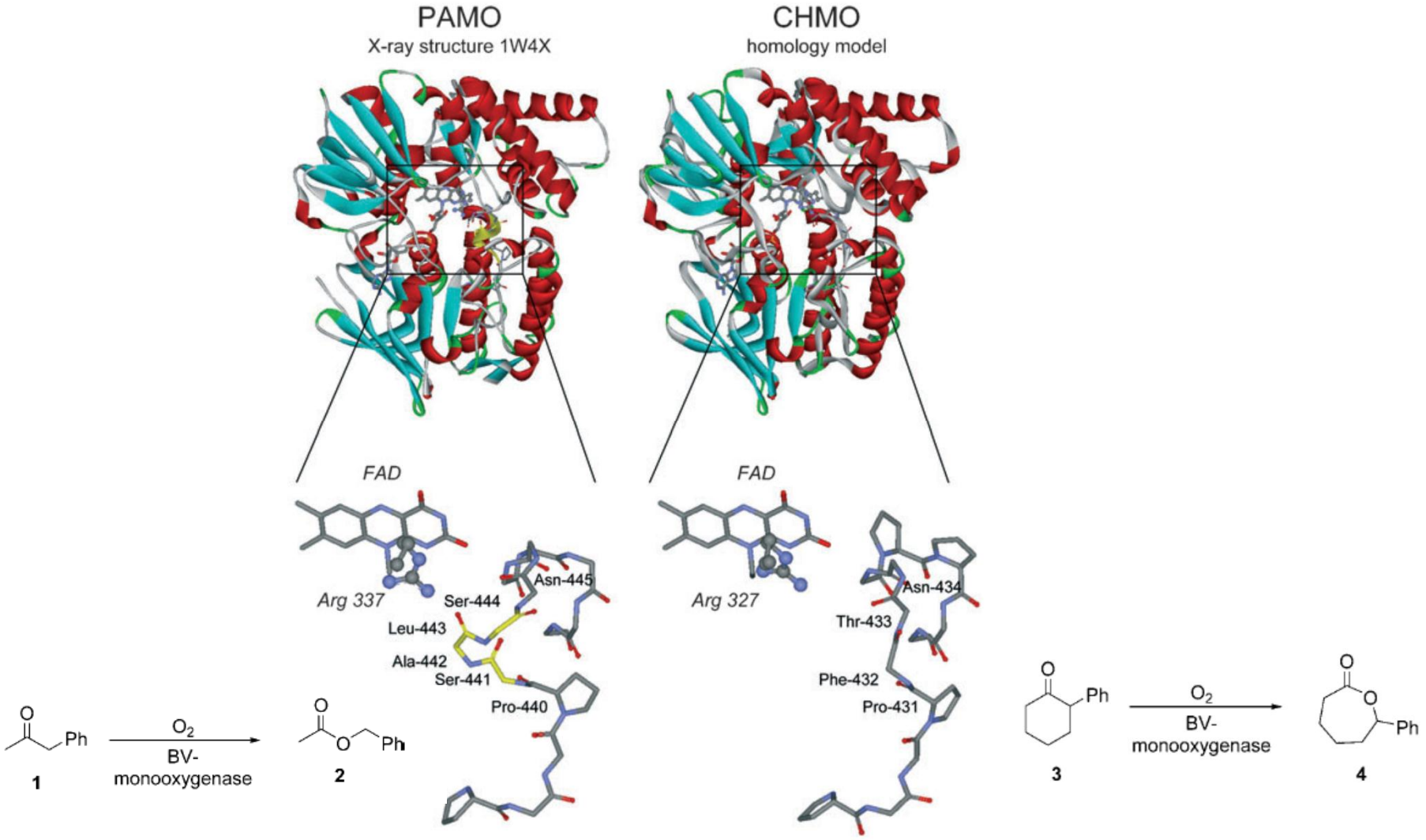
Figure 2. Overlap extension PCR method: → represents a primer, and × represents a mutagenic codon.

- ï known structure & mechanism
- ï usually: knock-out tests

Biocatalysis ï General Aspects

Protein Modification

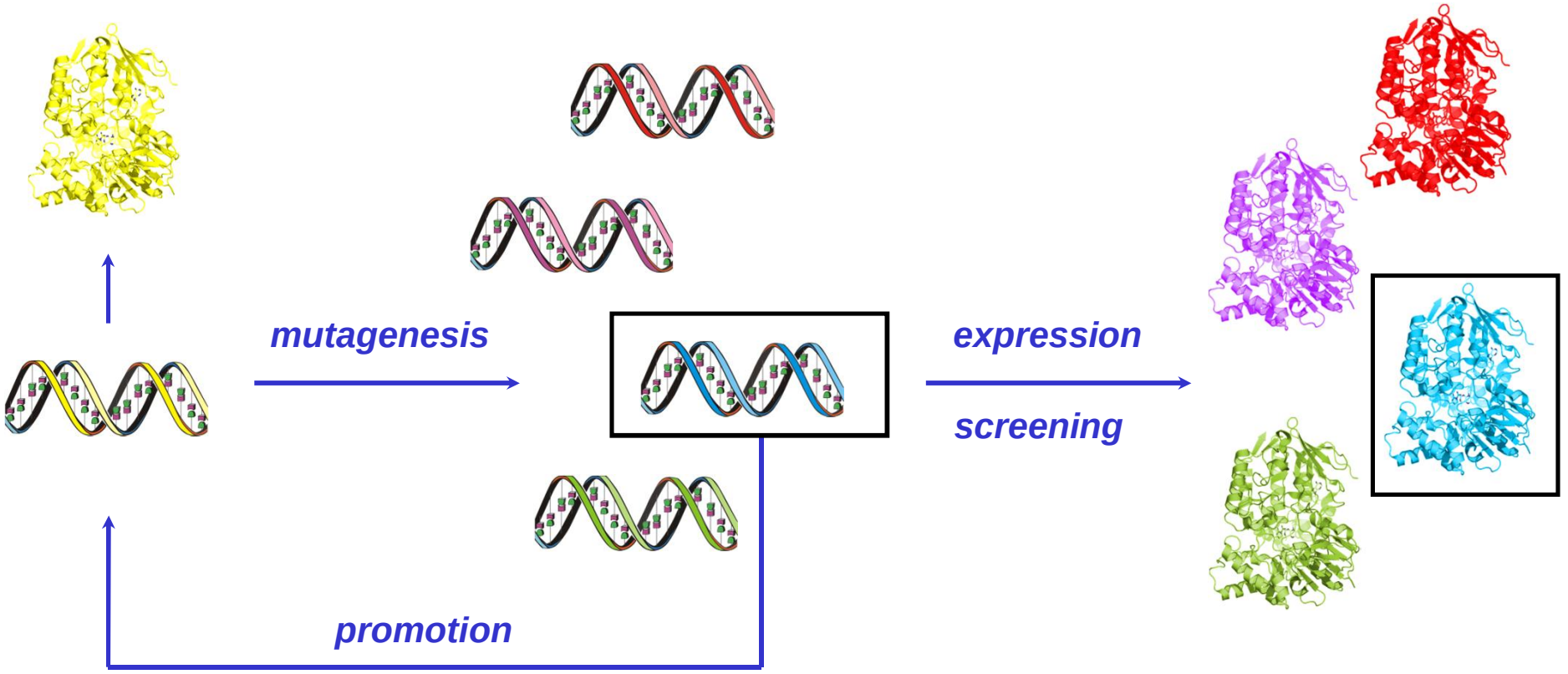
Å Site-directed mutagenesis ï rational design



Biocatalysis ï General Aspects

Protein Modification

Å Enzyme evolution



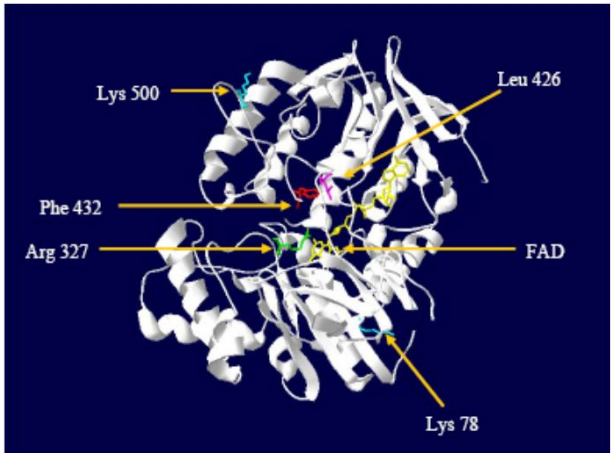
Biocatalysis ï General Aspects

Protein Modification

Å Error prone PCR (epPCR)

Figure 3.3 Differences in classical and error prone PCR.

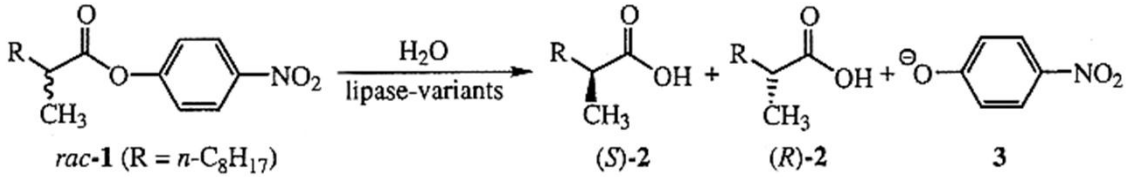
- ï operating PCR under non-ideal conditions (also saturation possible)
- ï degeneration of Code ➟ different mutation frequencies
- ï distribution of mutations randomly (remote from active site)



Biocatalysis ï General Aspects

Protein Modification

Å Error prone PCR (epPCR)



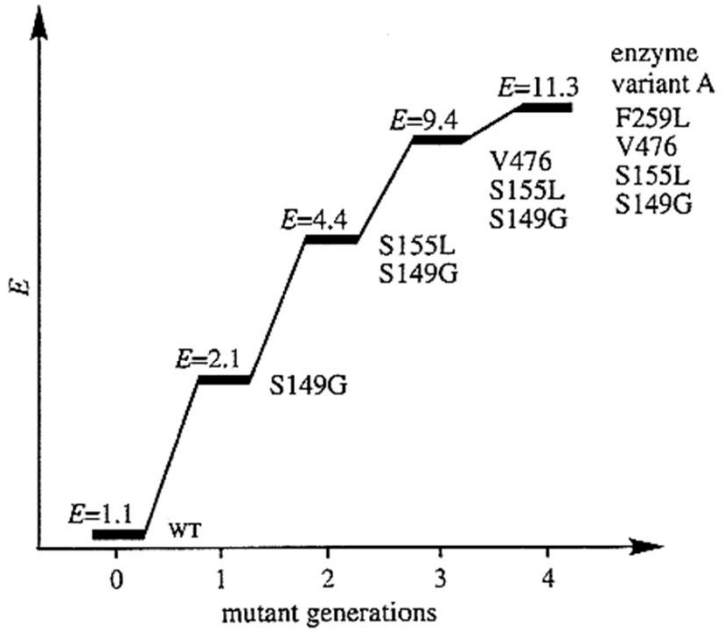


FIG. 14. Increasing the *E* values of the lipase-catalyzed hydrolysis of the chiral ester **1** by cumulative mutations caused by four rounds of epPCR (*16,22,24*).

Biocatalysis ï General Aspects

Protein Modification

Å Combinatorial Active-Site Saturation Test ï CASTing

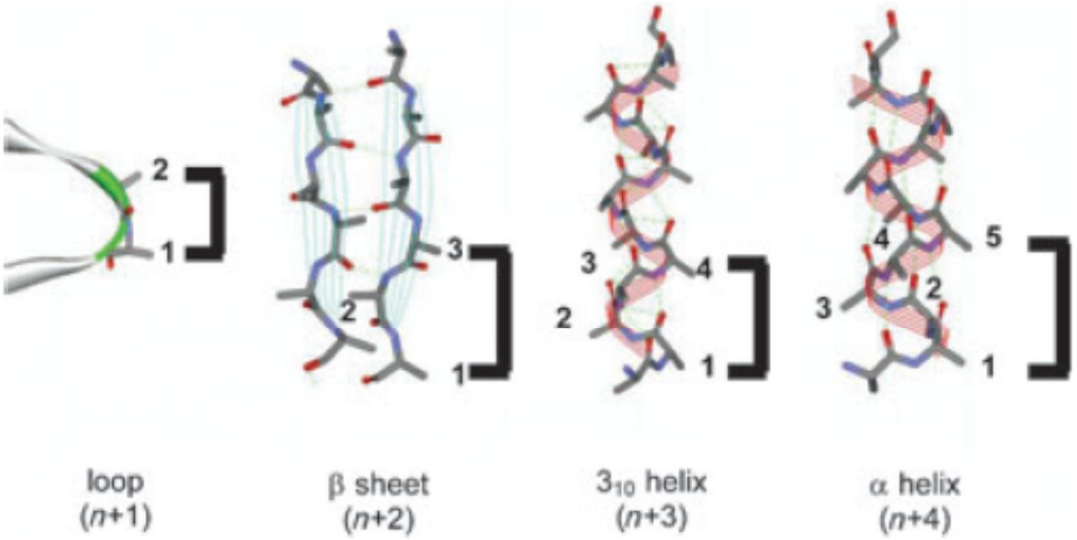


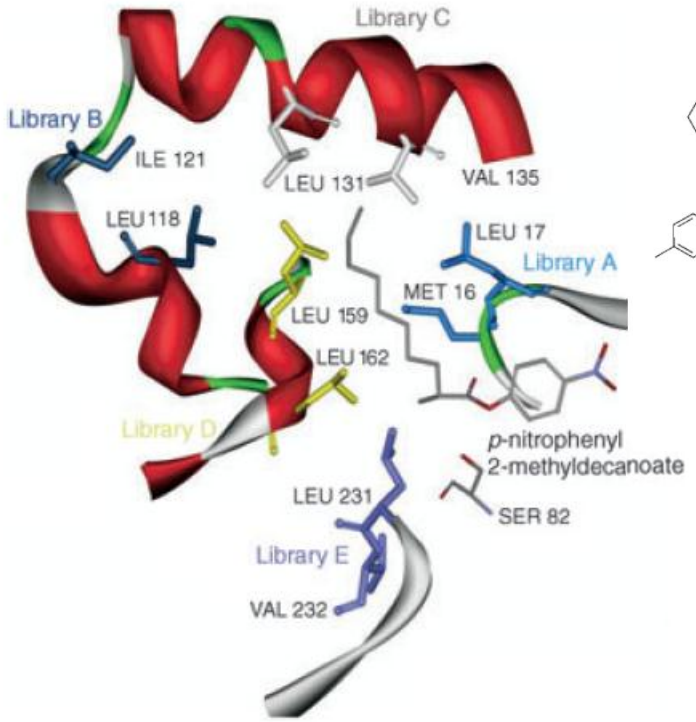
Figure 1. Structural guides in designing libraries of mutant enzymes for CASTing according to the secondary structure of proteins.

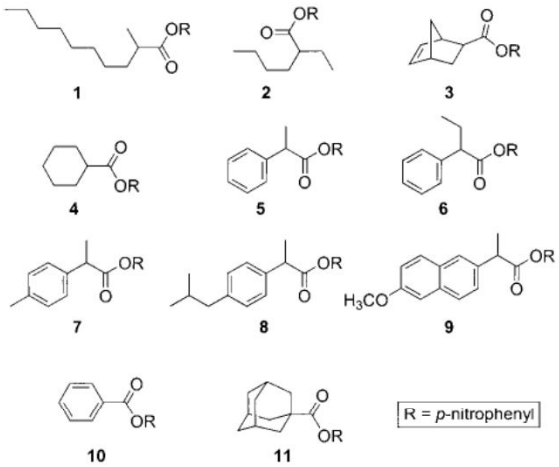
ï synergistic amino acids in spatial proximity

Biocatalysis ï General Aspects

Protein Modification

Å Combinatorial Active-Site Saturation Test ï CASTing





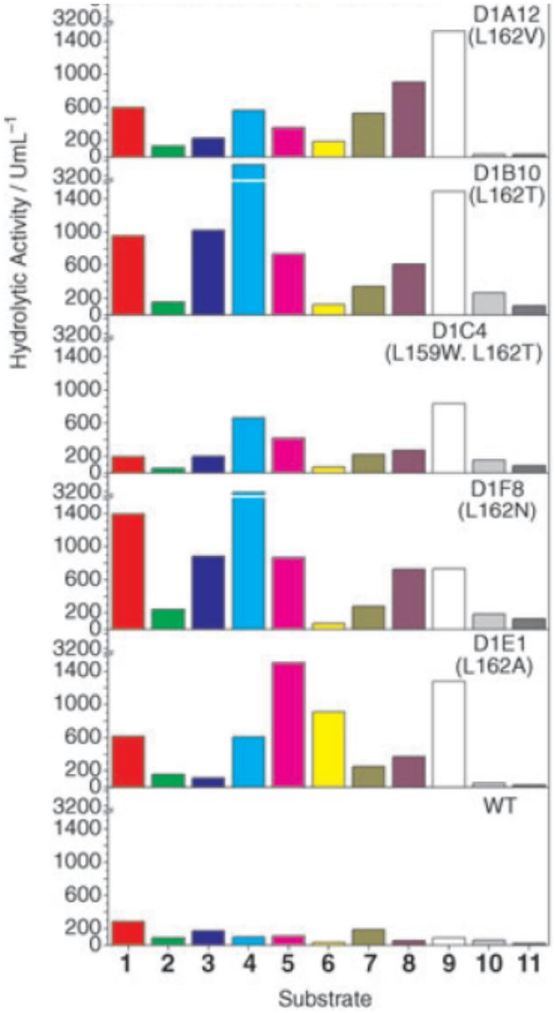


Figure 2. CASTing of the lipase from *Pseudomonas aeruginosa* leading to the construction of five libraries of mutants (A–E) produced by simultaneous randomization at two amino acid sites. (For illustrative purposes, the binding of substrate **1** is shown.)

Biocatalysis ï General Aspects

Protein Modification

Å Combinatorial Active-Site Saturation Test ï CASTing

ï combination of best sub-library candidates

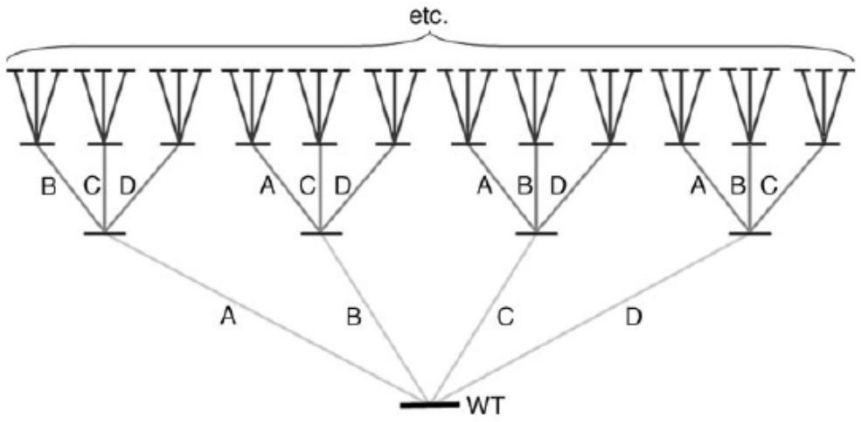
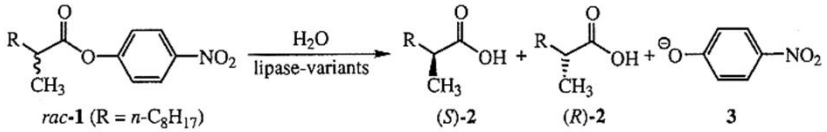


Figure 1. Schematic illustration of iterative CASTing involving (as an example) four randomization sites A, B, C, and D: Confined protein-sequence space for evolutionary enzyme optimization (redundancy in some cases is expected).



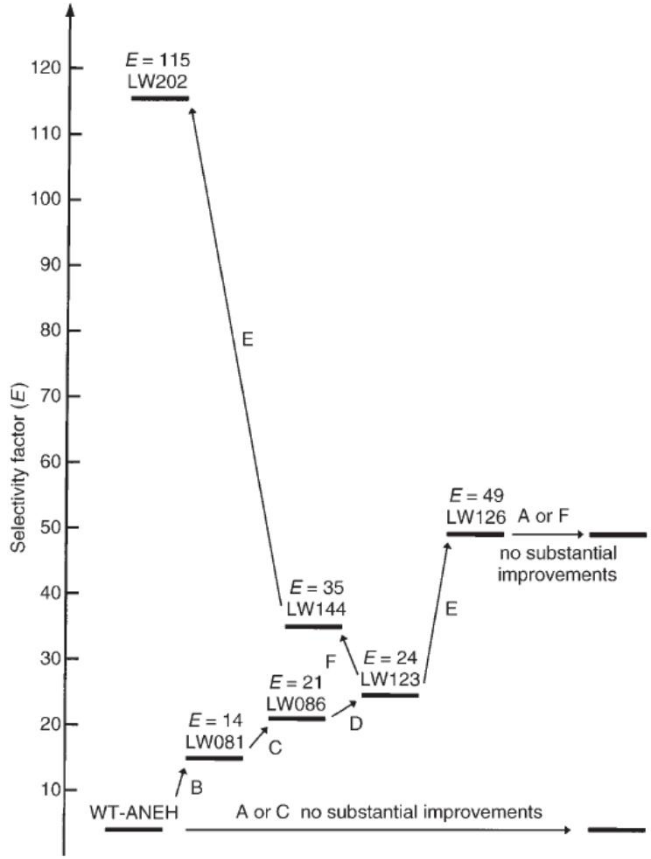


Figure 3. Iterative CASTing in the evolution of enantioselective epoxide hydrolases as catalysts in the hydrolytic kinetic resolution of *rac*-**1**.

Biocatalysis ï General Aspects

Protein Modification

Å Summary of technologies

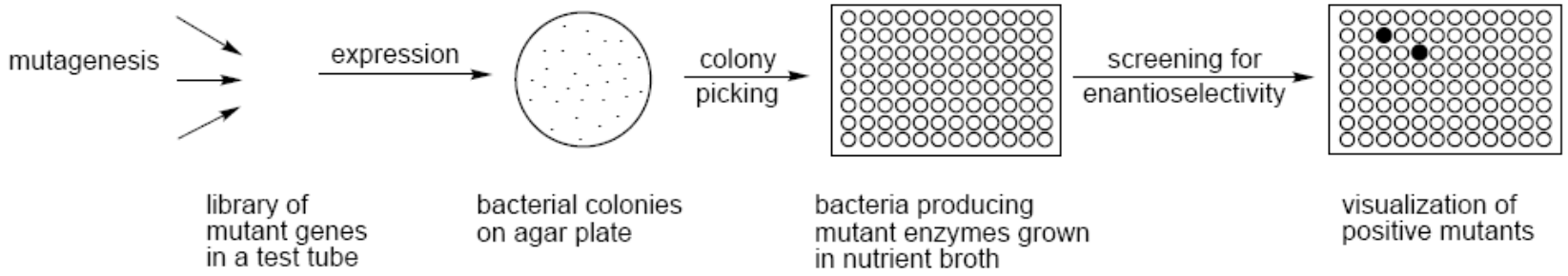
Table 3: Main library creation technologies.[108]

	Error-prone PCR	Saturation mutagenesis	Massive mutagenesis	Gene shuffling	Synthetic shuffling
Need for physical starting gene	1 gene	1 gene	1 gene	several genes	no gene
Large diversity/low cost mutant ?	yes	no	yes	yes	yes
Control over the diversity generated	very little (mutation rate)	complete	complete	little (starting sequences)	complete
Need for double strand cloning	yes	yes/no (different technologies)	no	yes	yes

Biocatalysis ï General Aspects

Protein Modification

Å Library Screening - workflow

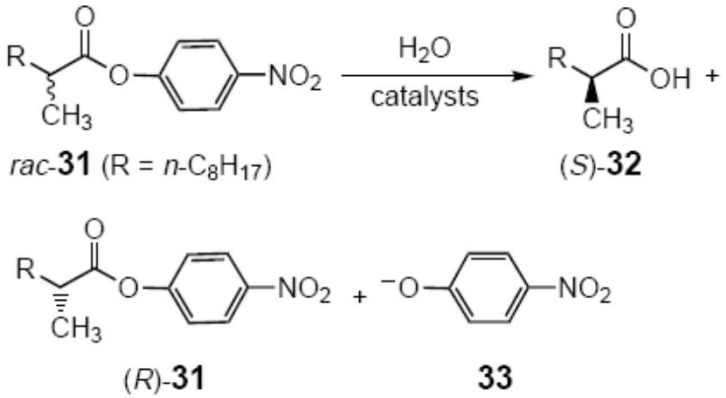


Scheme 2. Individual steps in the directed evolution of an enantioselective enzyme.[19]

Biocatalysis ï General Aspects

Protein Modification

- Å Screening Techniques
  - ï Colorimetric Screens
    - Å double experiments
    - Å high throughput



catalyst library: 30 000 mutant lipases from *P. aeruginosa*

result: *ee* = 2-8 % (*E* ≃ 1.1) $\xrightarrow{\text{evolution}}$ *ee* > 90 % (*E* = 25)

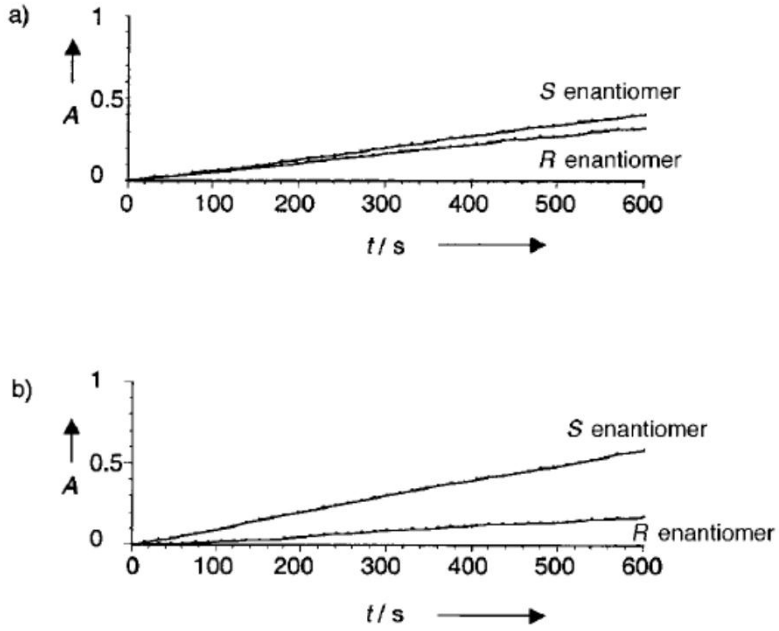


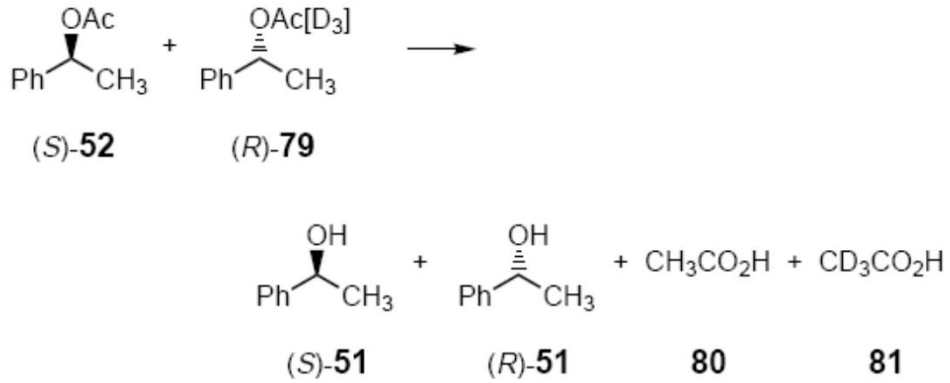
Figure 3. Course of the lipase-catalyzed hydrolysis of the (*R*)- and (*S*)-**31** as a function of time.[17] a) Wild-type lipase from *P. aeruginosa*, b) improved mutant in the first generation.

Biocatalysis ï General Aspects

Protein Modification

Å Screening Techniques

ï MS-based Screens ï ÆPseudoñRacemates



Scheme 27. Kinetic resolution of pseudo-enantiomers (*S*)-**52** and (*R*)-**79**. Screening was carried out by ESI-MS.[89]

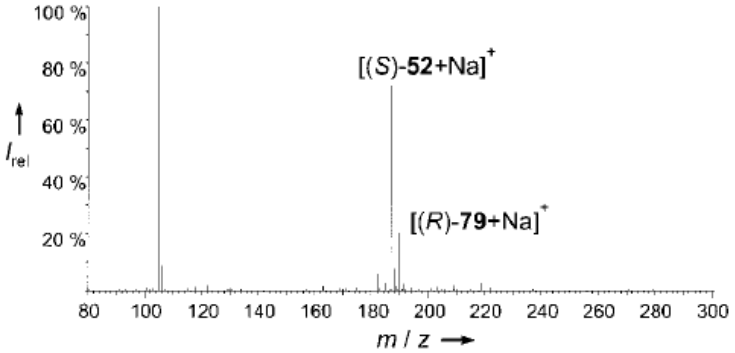


Figure 13. ESI-mass spectrum of a sample containing (*S*)-**52** and (*R*)-**79**.[89]

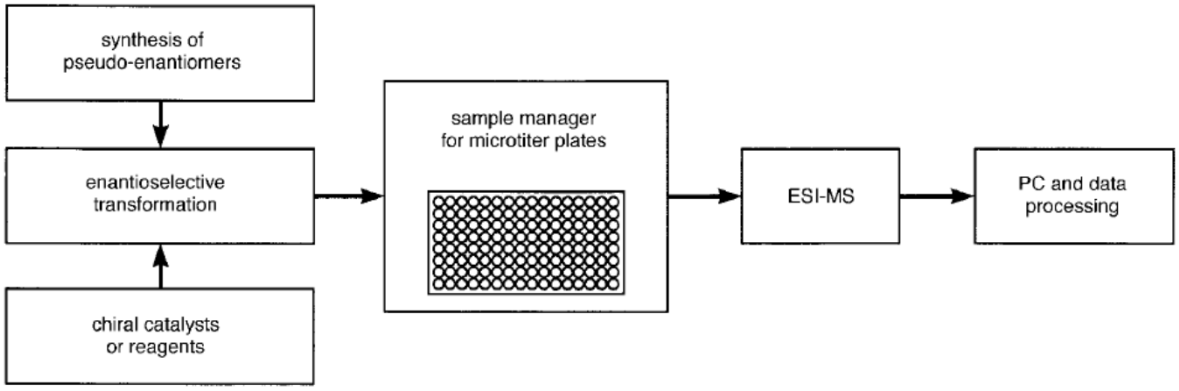


Figure 14. Experimental setup of an ESI-MS *ee*-screening system.[89]

Hydrolytic Reactions

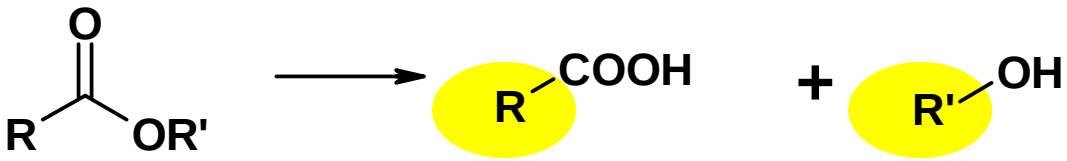
Enzyme Groups

- Esterases (cleavage of ester functionality)
  - pig liver esterase (PLE)
  - horse liver esterase (HLE)
  - acetyl choline esterase (ACE - Zitteraal)
  - *Bacillus subtilis* esterase
  - yeast (whole-cell system)
- Proteases (cleavage of amid bond)
  - α-chymotrypsin
  - pepsin
  - subtilisin
  - thermolysin
- Lipases (cleavage of triglycerids)
  - div. Candida lipases
- Nitrilases & Nitrile Hydratases
- Epoxid Hydrolyses

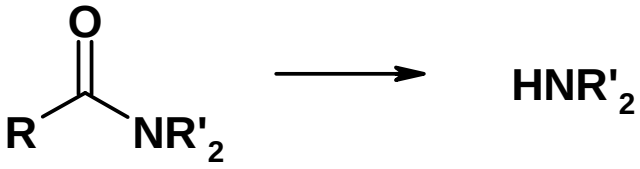
Hydrolytic Reactions

Reaction Types

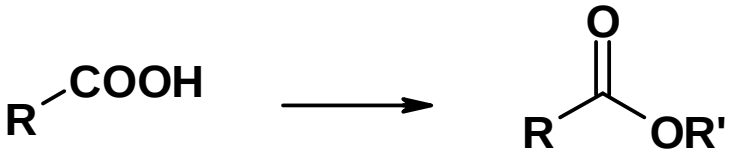
- Hydrolysis



- Deprotection



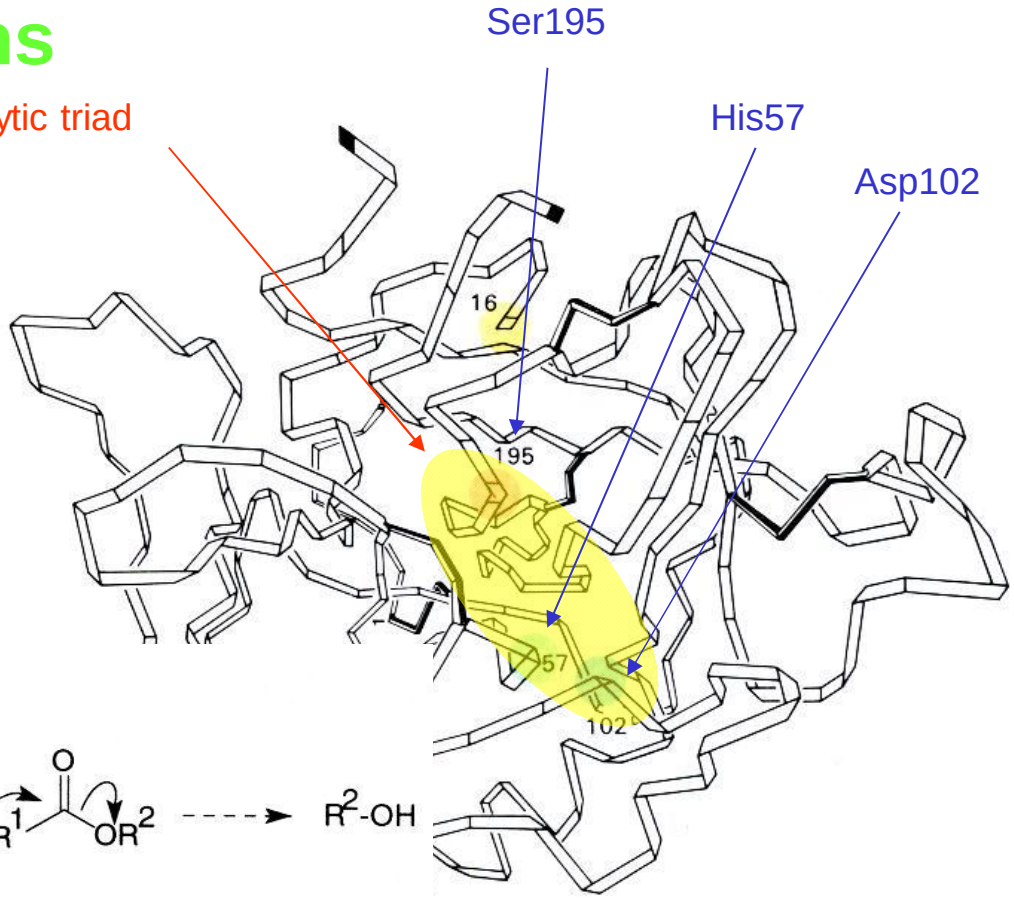
- Esterification

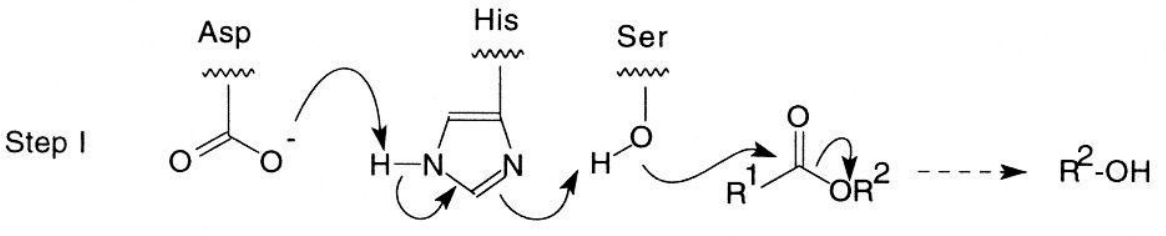


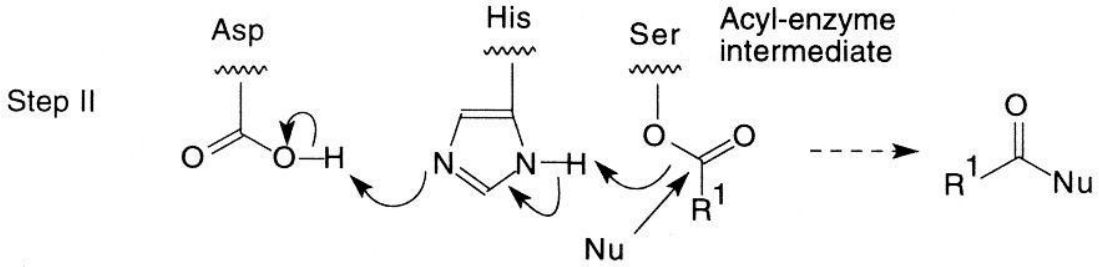
Hydrolytic Reactions

Enzyme Mechanisms

- Serin-proteases
  - e.g. chymotrypsine



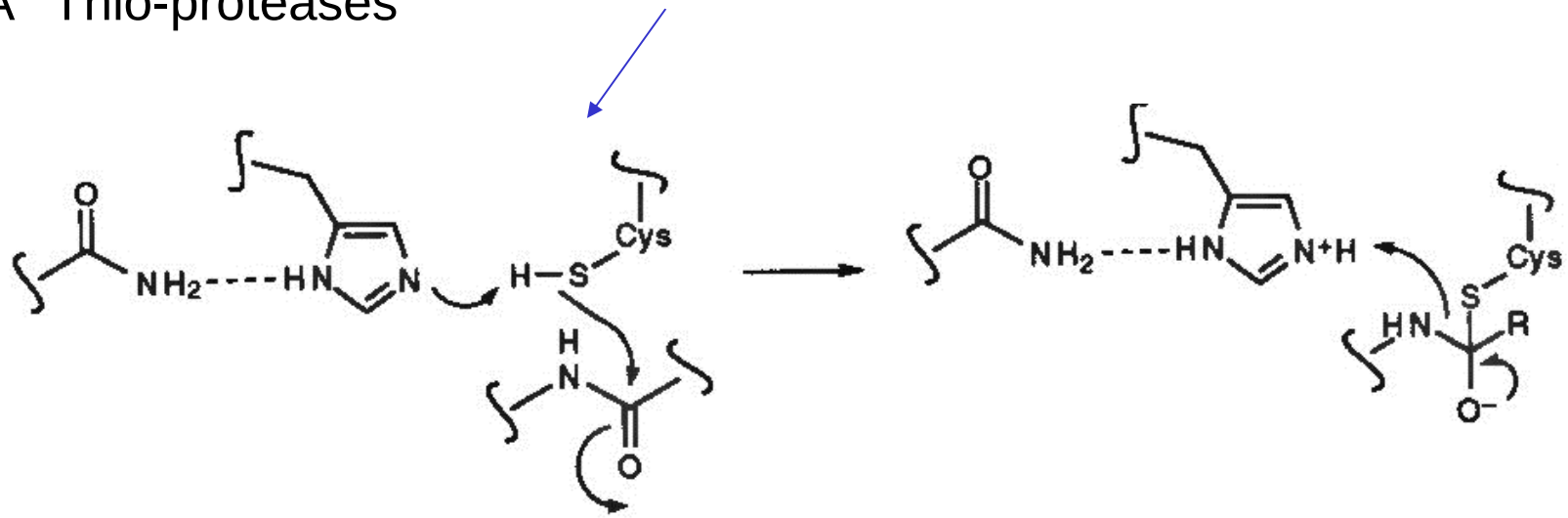




Hydrolytic Reactions

Enzyme Mechanisms

- Thio-proteases

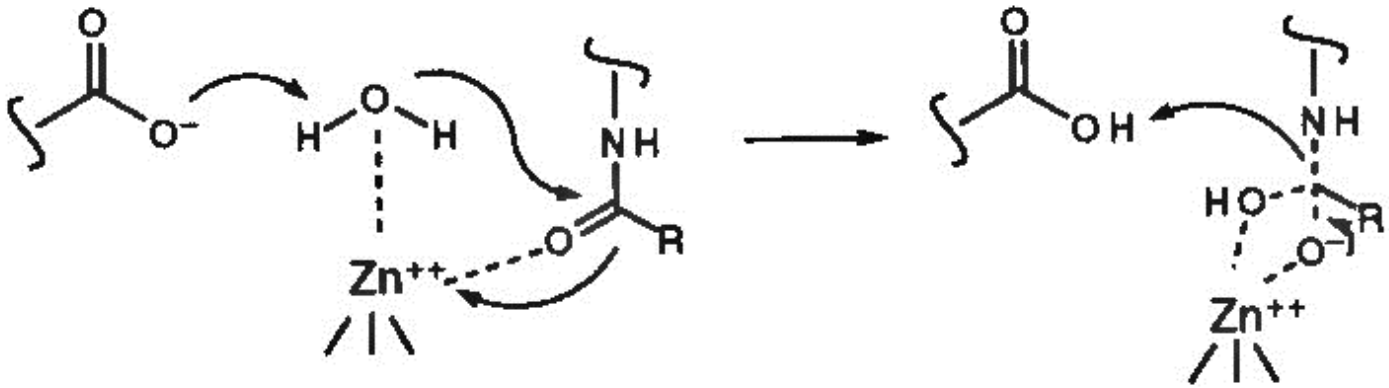


- Mechanism comparable to Ser-proteases
- examples: Papain, Cathepsin
- Minor modification of amino acids in catalytic triad upon retention of function

Hydrolytic Reactions

Enzyme Mechanisms

Å Metallo-proteases

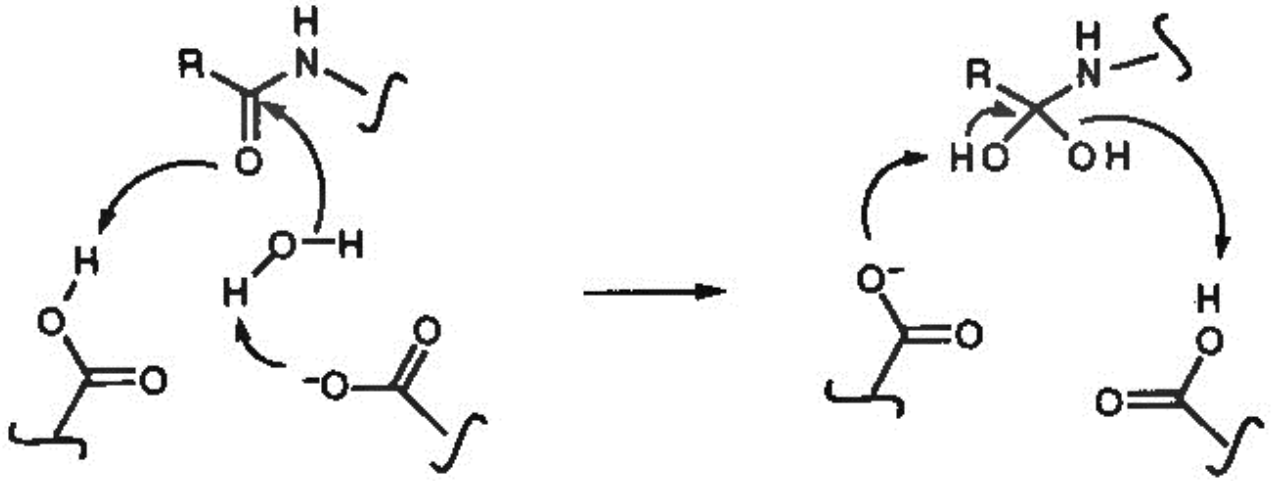


- ï Zn^{2+} as Lewis-acid
- ï no covalent intermediate
- ï examples: thermolysine, acylases

Hydrolytic Reactions

Enzyme Mechanisms

Å Aspartyl-proteases

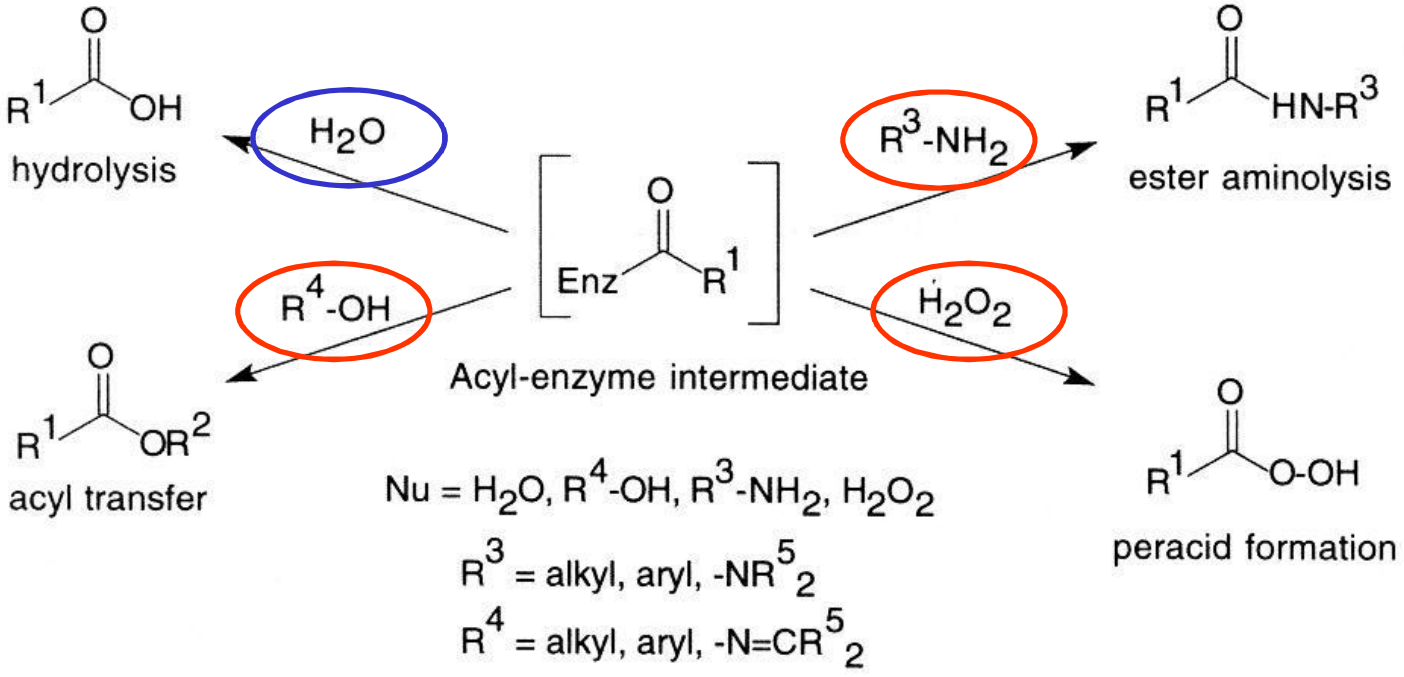


- ï 1st carboxylate = base
- ï 2nd carboxyl groupe ï general acid catalysis
- ï no covalent intermediate
- ï example: pepsin

Hydrolytic Reactions

Synthetic Applications

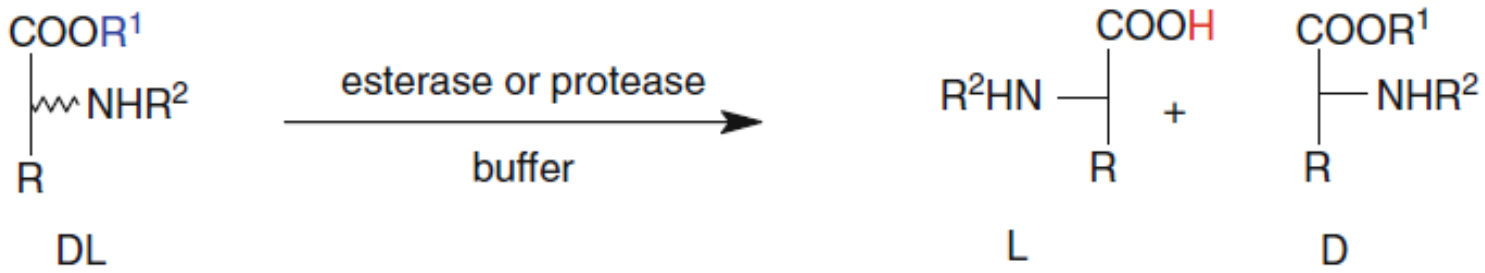
- Various nucleophiles



Hydrolytic Reactions

Amino Acid Synthesis

- Esterase Method



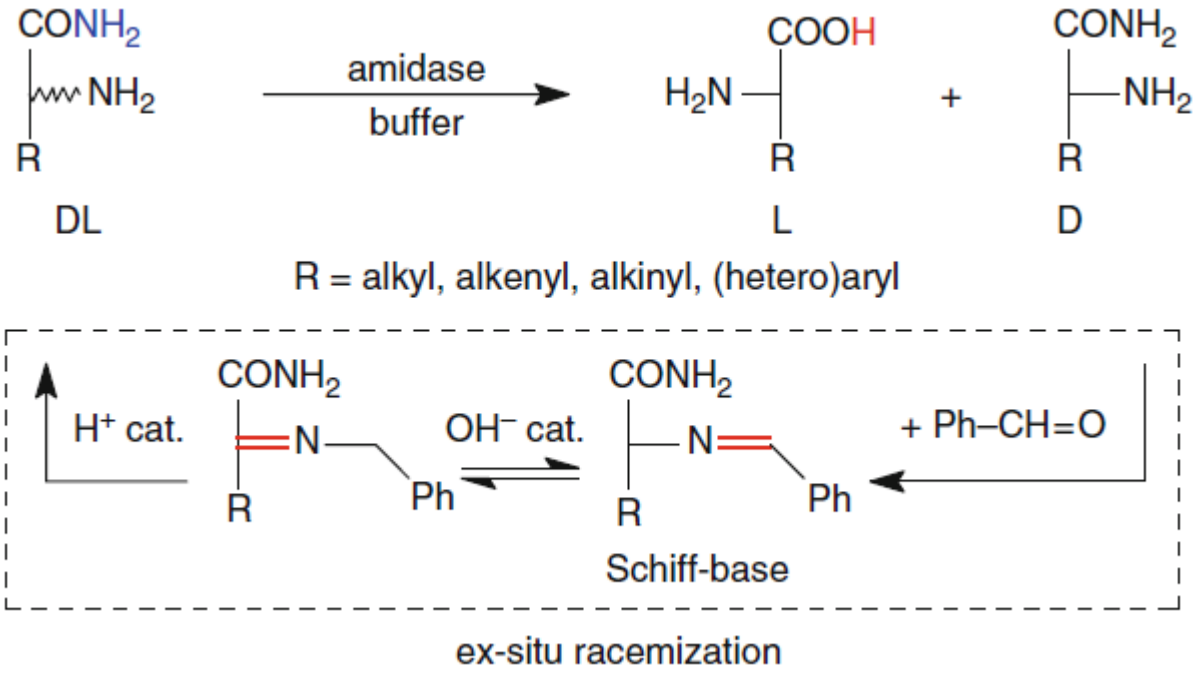
R = alkyl or aryl; R^1 = short-chain alkyl; R^2 = H or acyl

- Ester hydrolysis via:
  - Protease (cleavage of ester & amid bond possible ➟ sequential biotransformation)
  - Esterase
  - Lipase
- Most important enzyme: α-Chymotrypsin
- *Usual preferred cleavage of enantiomer most similar to natural a.a.*
- Since 1905 applied in chemistry

Hydrolytic Reactions

Amino Acid Synthesis

Å Amidase Method

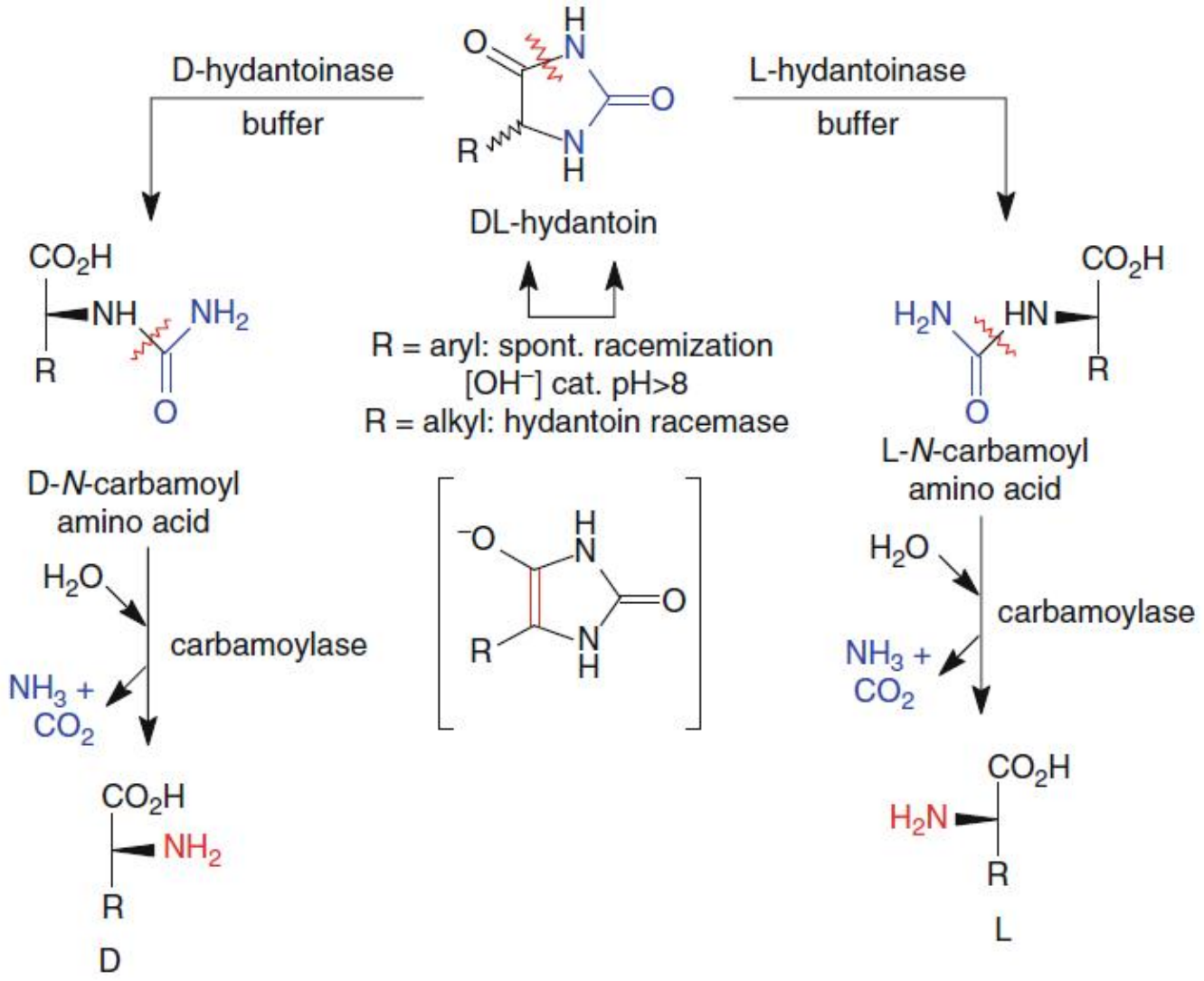


- Enzymes: mikroorganisms (Pseudomonas, Aspergillus, Rhodococcus sp.)
- Negligible chemical hydrolysis of amide products
- *separate* chemical racemization possible
- Now also with N-acylaminocarboxylic acid racemase ➟ **dynamic process**

Hydrolytic Reactions

Amino Acid Synthesis

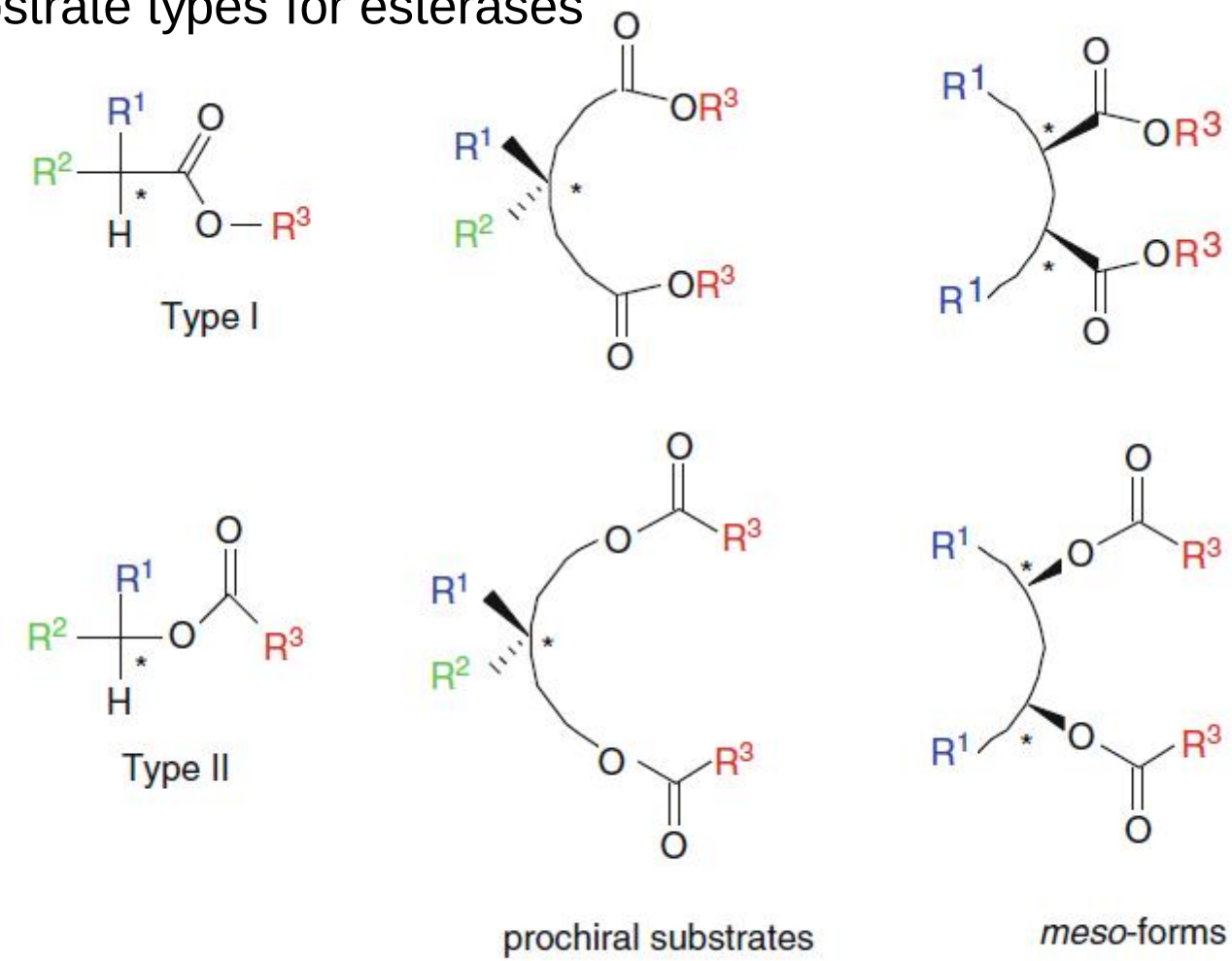
Å Hydantoinase Method



Hydrolytic Reactions

Ester Hydrolysis

Å Substrate types for esterases

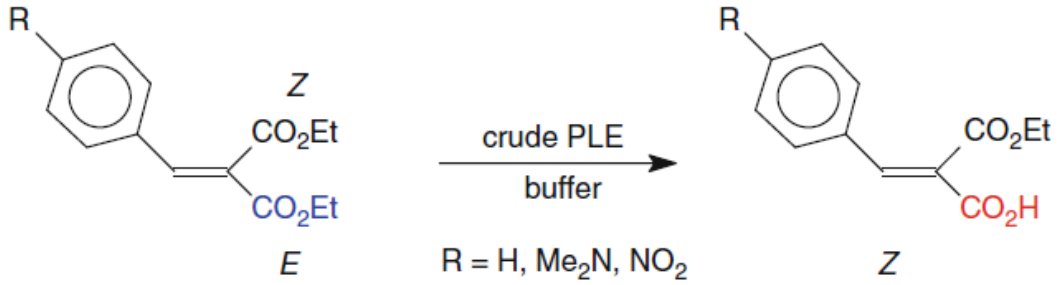


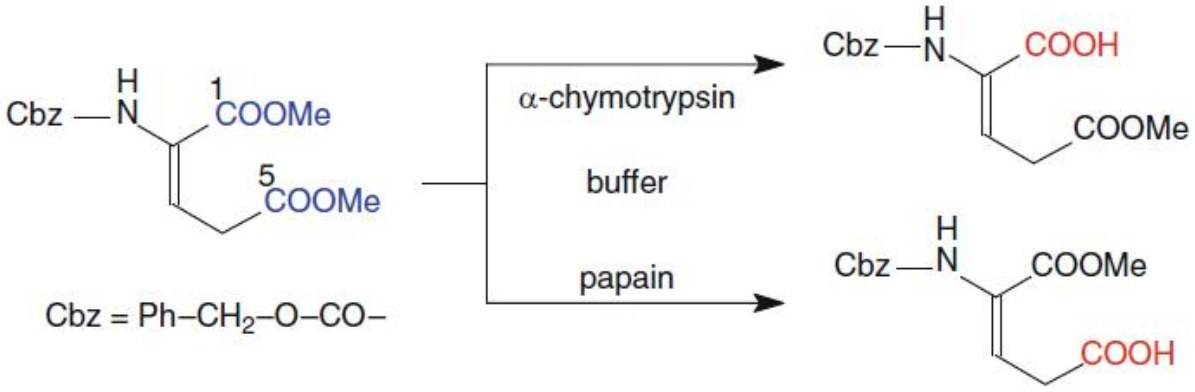
R^1, R^2 = alkyl, aryl; R^3 = Me, Et; * = center of (pro)chirality

Hydrolytic Reactions

Ester Hydrolysis

Å Substrate types for esterases

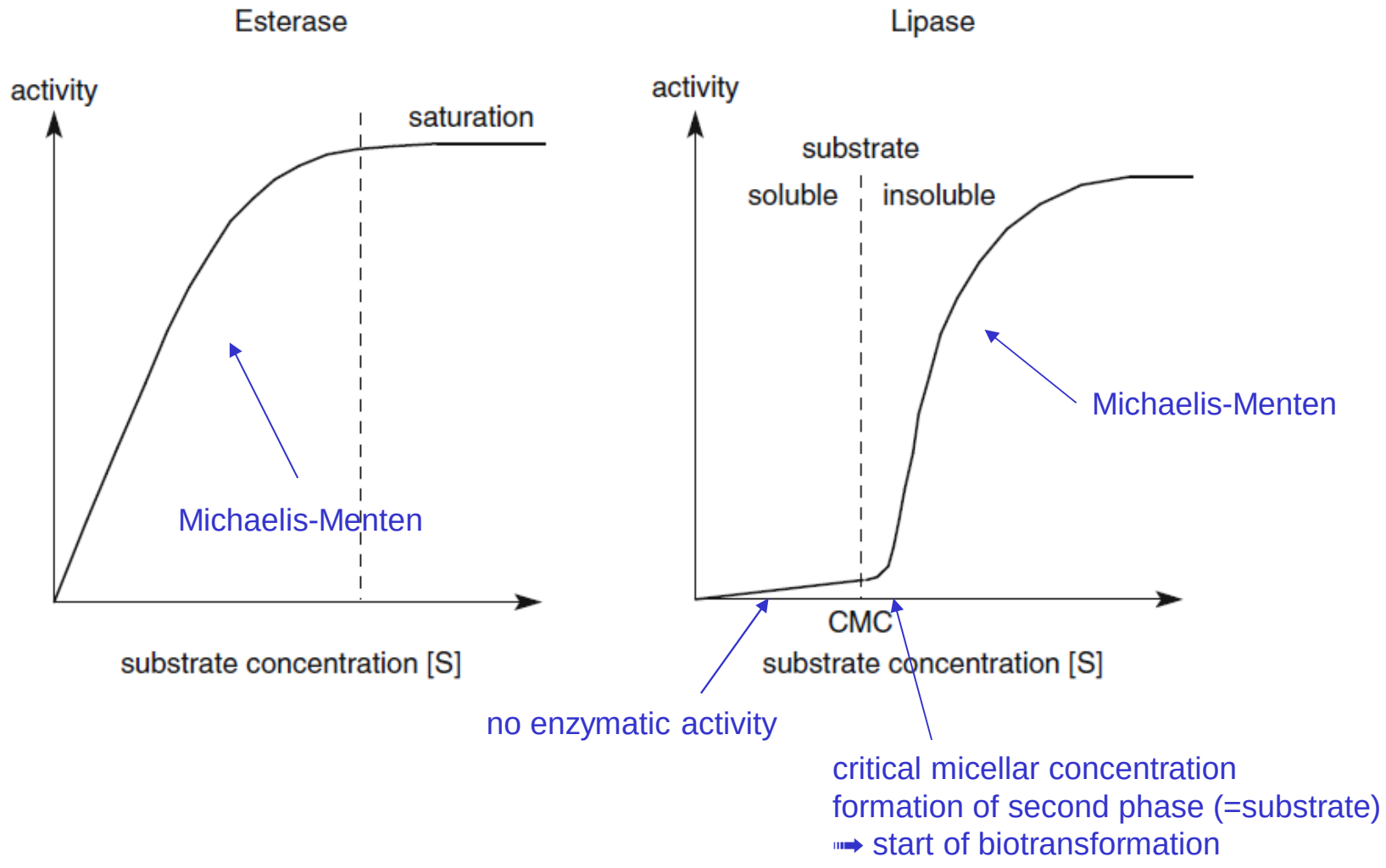




Hydrolytic Reactions

Ester Hydrolysis

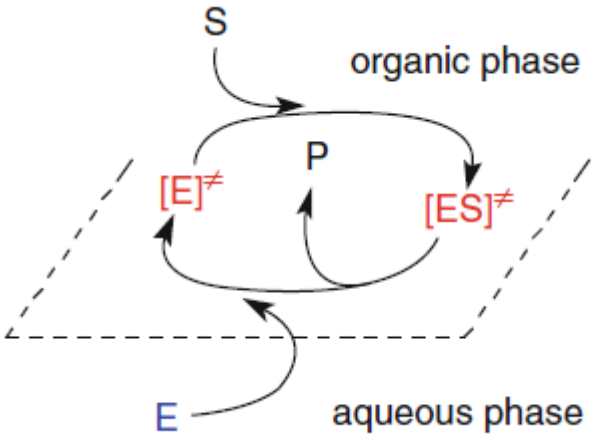
Å Lipases vs. Esterases

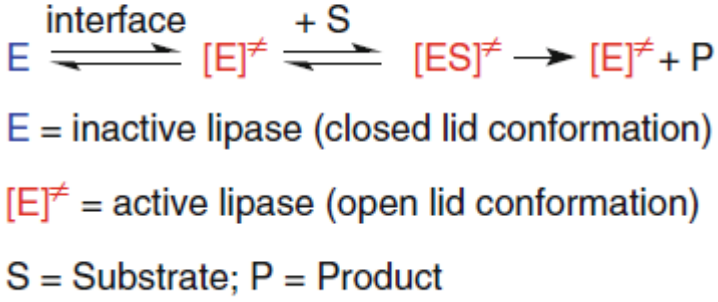


Hydrolytic Reactions

Ester Hydrolysis

Å Lipases vs. Esterases



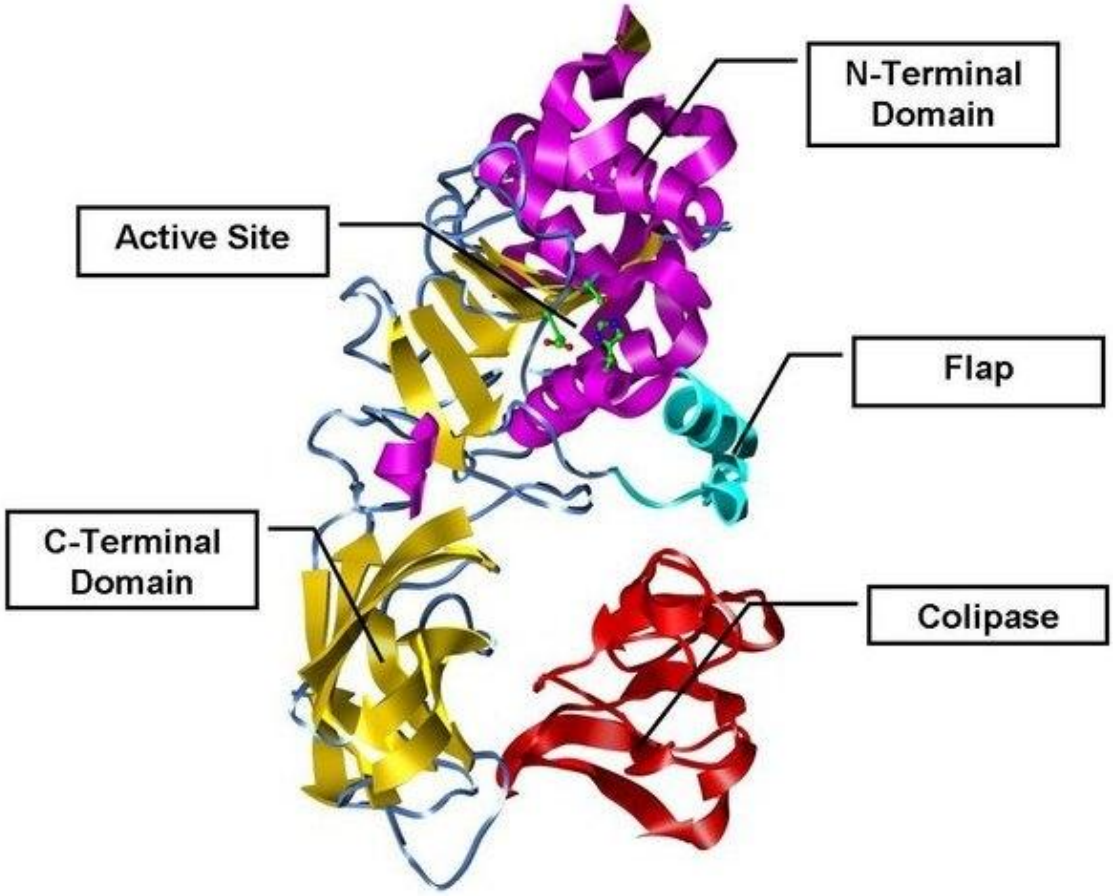


ï Lipases work best in solvent/water mixtures

Hydrolytic Reactions

Ester Hydrolysis

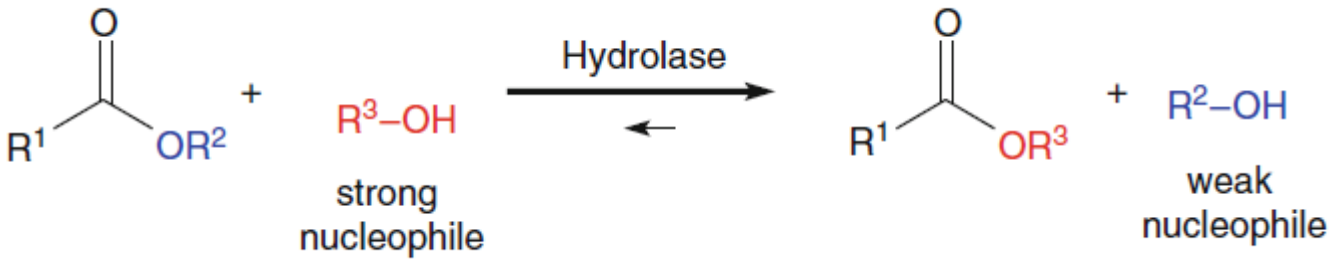
Å Lipse flap / lid



Hydrolytic Reactions

Esterification

- Principle

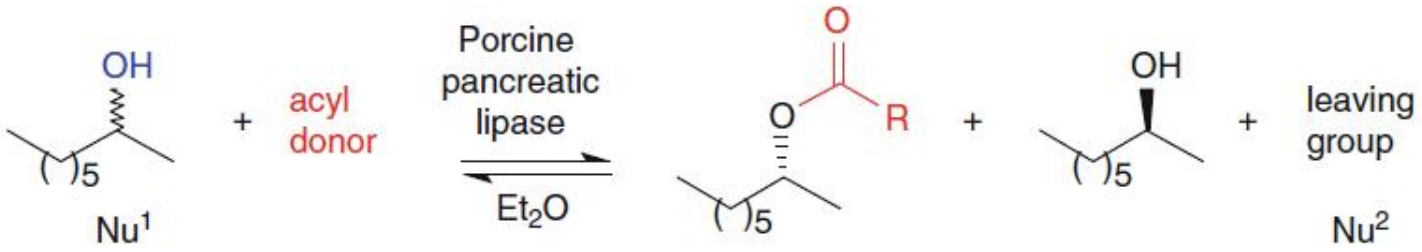


- Problem: formation of water during reaction
  - ➠ formation of aqueous interphase
  - ➠ separation of enzyme & substrate
  - ➠ incomplete conversion
- Excess acyl donor
  - removal of „bulk“water
  - pseudo-irreversible reaction
- Decrease of nucleophilicity of newly formed alcohol
  - electron withdrawing effects
  - subsequent reaction / tautomerization

Hydrolytic Reactions

Esterification

Å Leaving group alcohol



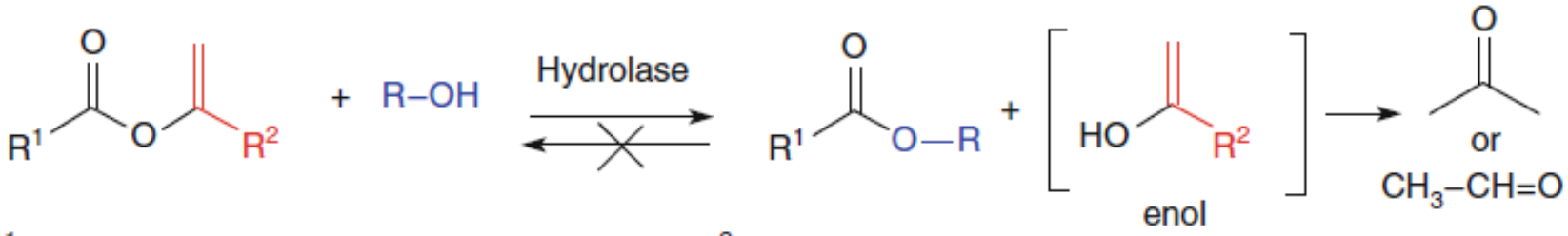
Acyl donor	R	Leaving group Nu²	Initial rate [%]
Ethyl acetate	Me	EtOH	0.3
2-Chloroethyl acetate	Me	$ClCH_2–CH_2OH$	1
Methyl butanoate	*n*-Pr	MeOH	5
Ethyl cyanoacetate	$N{\equiv}CCH_2–$	EtOH	6
Trichloroethyl trichloroacetate	$Cl_3C–$	$Cl_3C–CH_2OH$	7
Methyl bromoacetate	$BrCH_2–$	MeOH	14
Tributyrin	*n*-Pr	dibutyrin	34
Trichloroethyl butanoate	*n*-Pr	$Cl_3C–CH_2OH$	58
Trichloroethyl heptanoate	$n\text{-}C_6H_{13}–$	$Cl_3C–CH_2OH$	100

Hydrolytic Reactions

Esterification

- Enol ester acyl transfer

Enol Esters

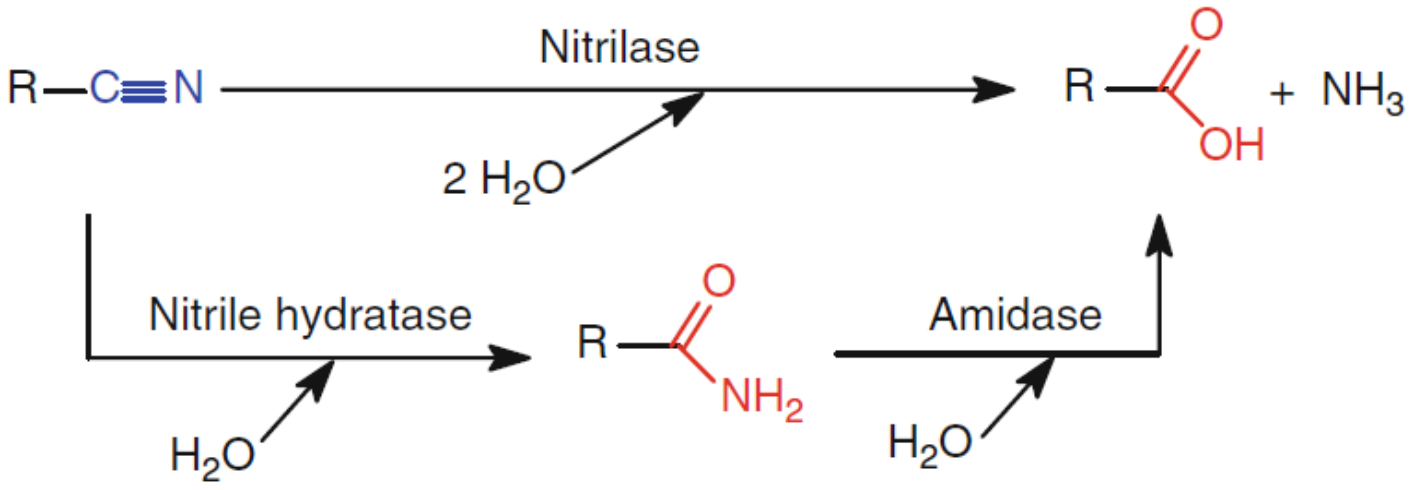


R^1 = *n*-alkyl, aryl, aryl-alkyl, haloalkyl R^2 = H, CH_3

Hydrolytic Reactions

Nitrile Hydrolysis

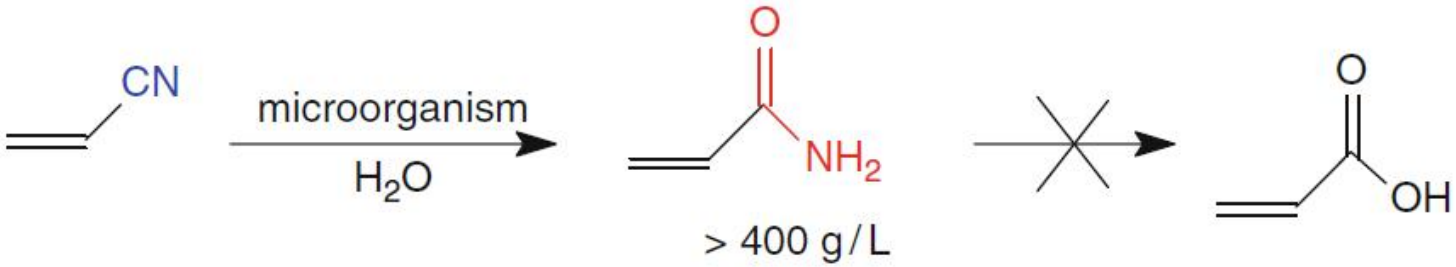
- Enzymes for Nitrile Hydrolysis
  - Nitriles are improtant C_1-building blocks in chemical industry
  - Chemical methods for hydrolysis:
    - Strong acidic or bacis ➟ incompatible with functional groups
    - High energy demands
    - Side reactions



Hydrolytic Reactions

Nitrile Hydrolysis

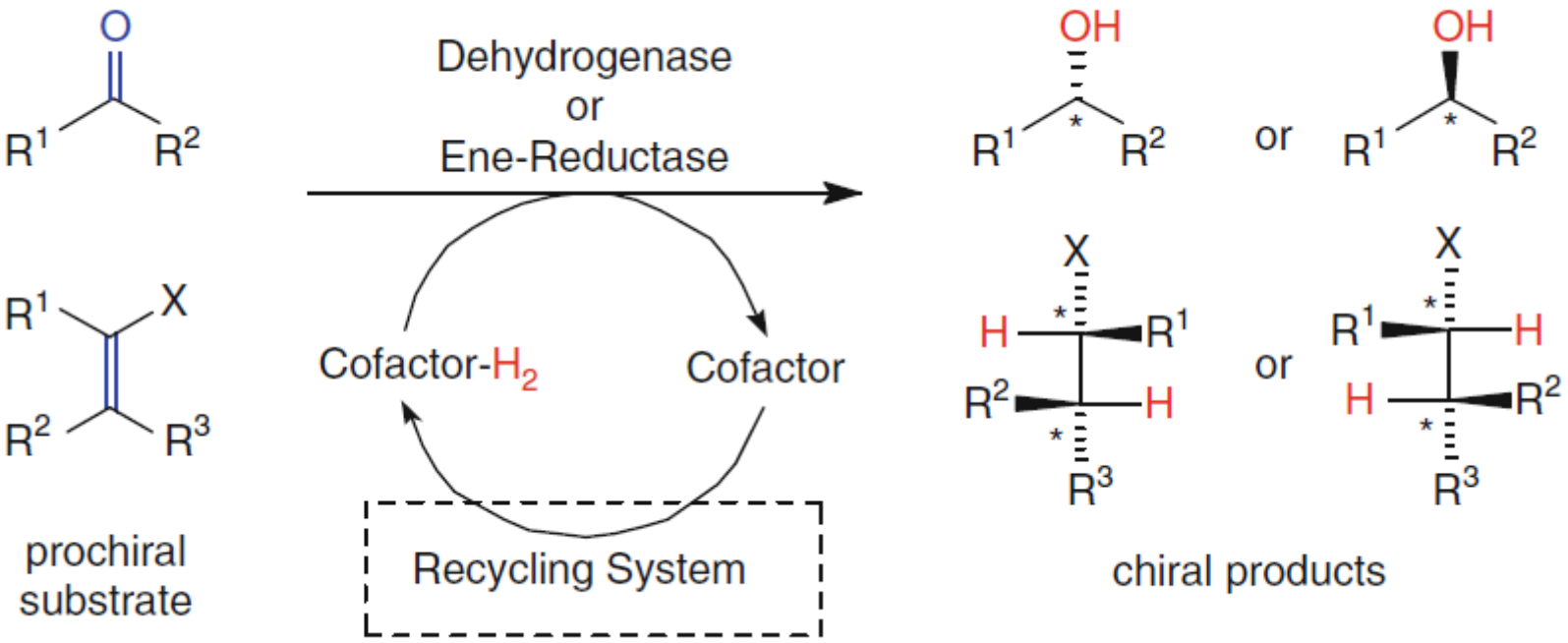
- Acrylamid Production
  - 450,000 t/a global production
  - chem. process: hydration of acrylonitrile with Cu-catalyst
  - whole-cell process yields (also lyophylized cells) >99%
    - amidase inhibition
    - 400g/L Titer
    - >100,000 t/a by biocatalysis (1997)



Rhodococcus rhodochrous
Pseudomonas chlororapis
Brevibacterium sp.

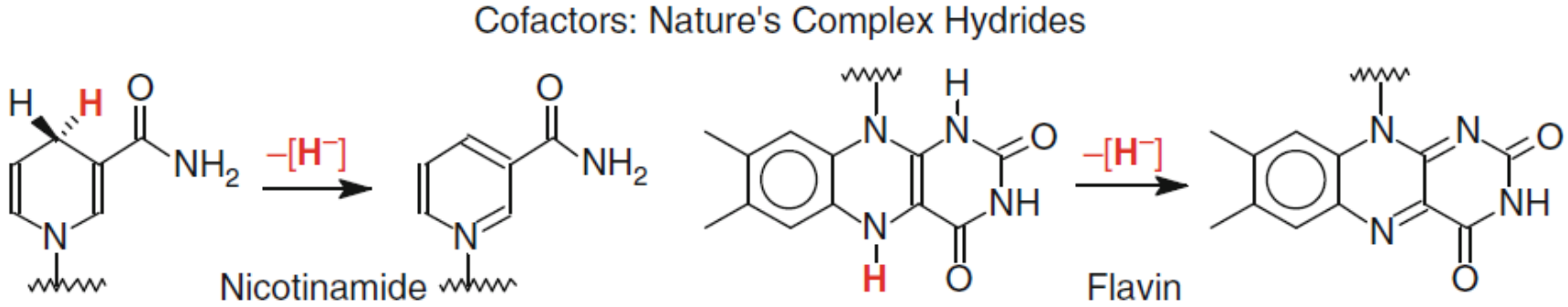
Redox Reactions

Reaction types



Redox Reactions

Cofactors

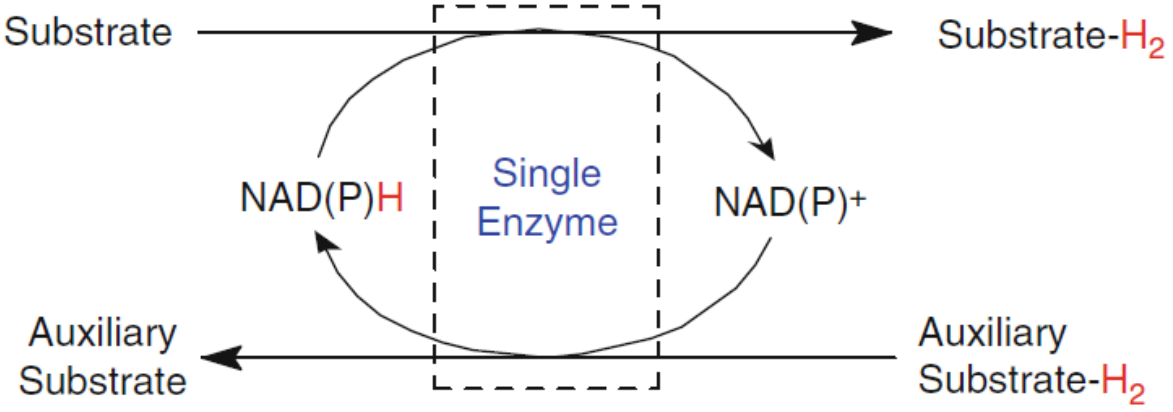


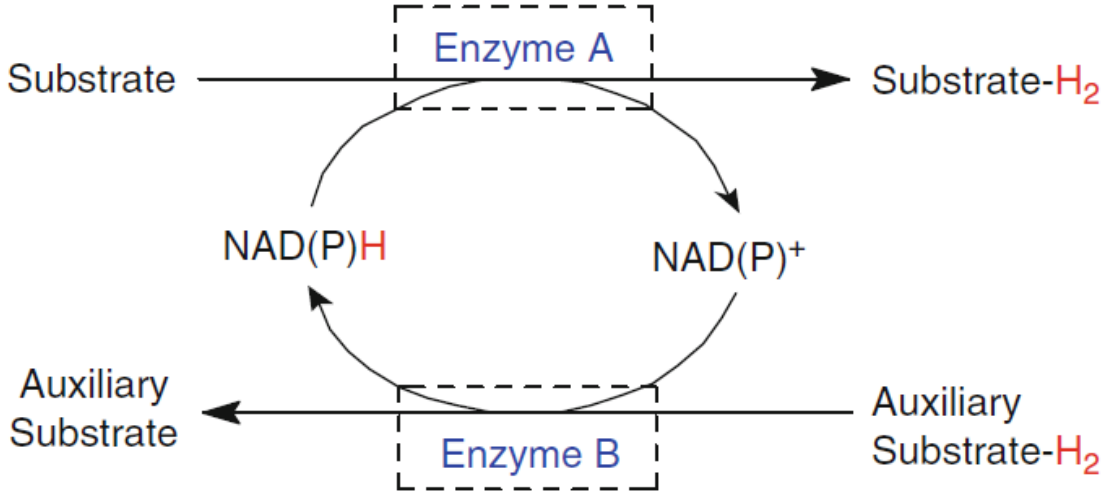
Scheme 2.110 Reduction reactions catalyzed by dehydrogenases

Redox Reactions

Cofactor Recycling

Å General Principle

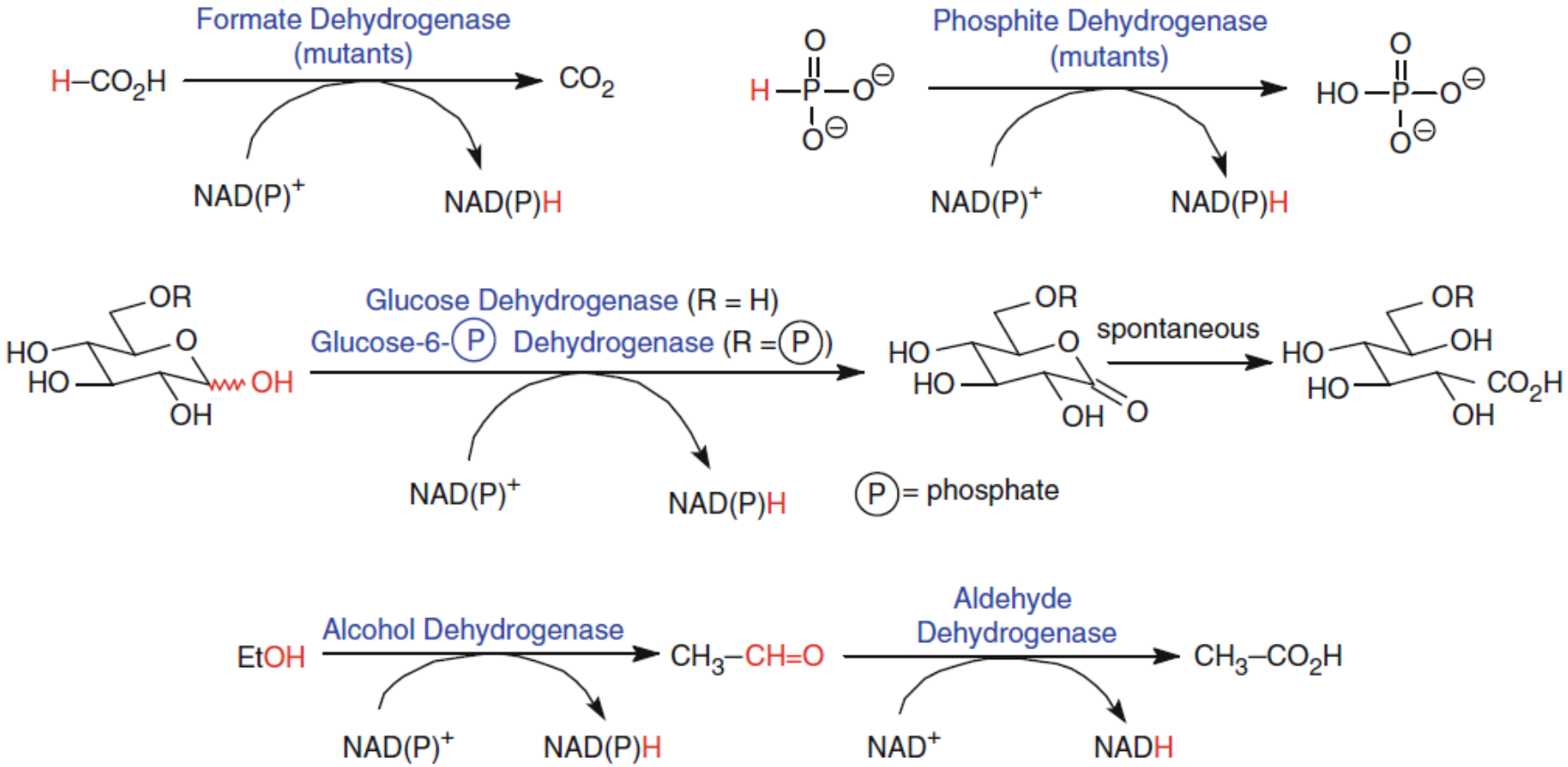




Redox Reactions

Cofactor Recycling

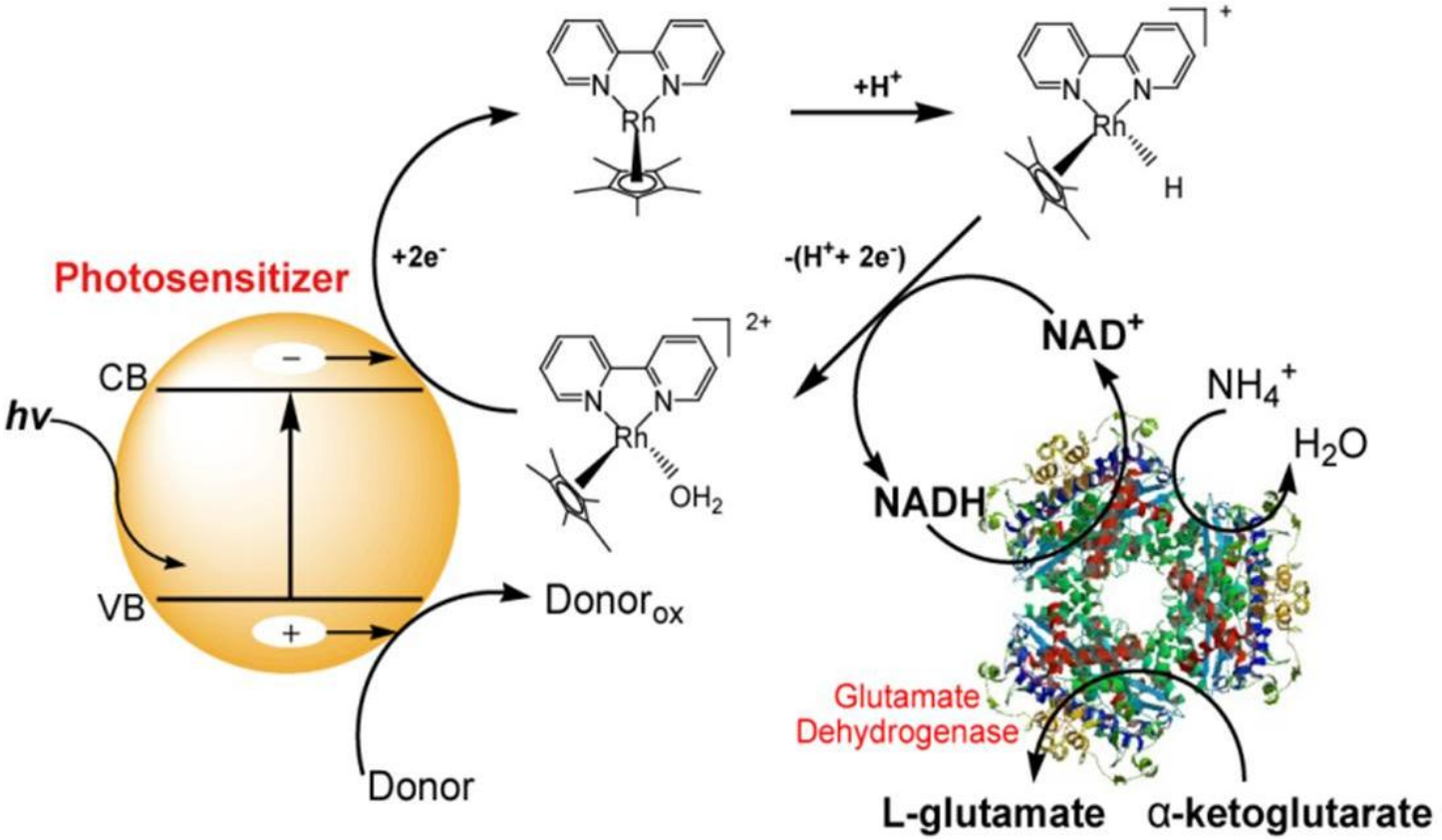
Å Recycling systems for reduced nicotinamids



Redox Reactions

Cofactor Recycling

Å Photochemistry

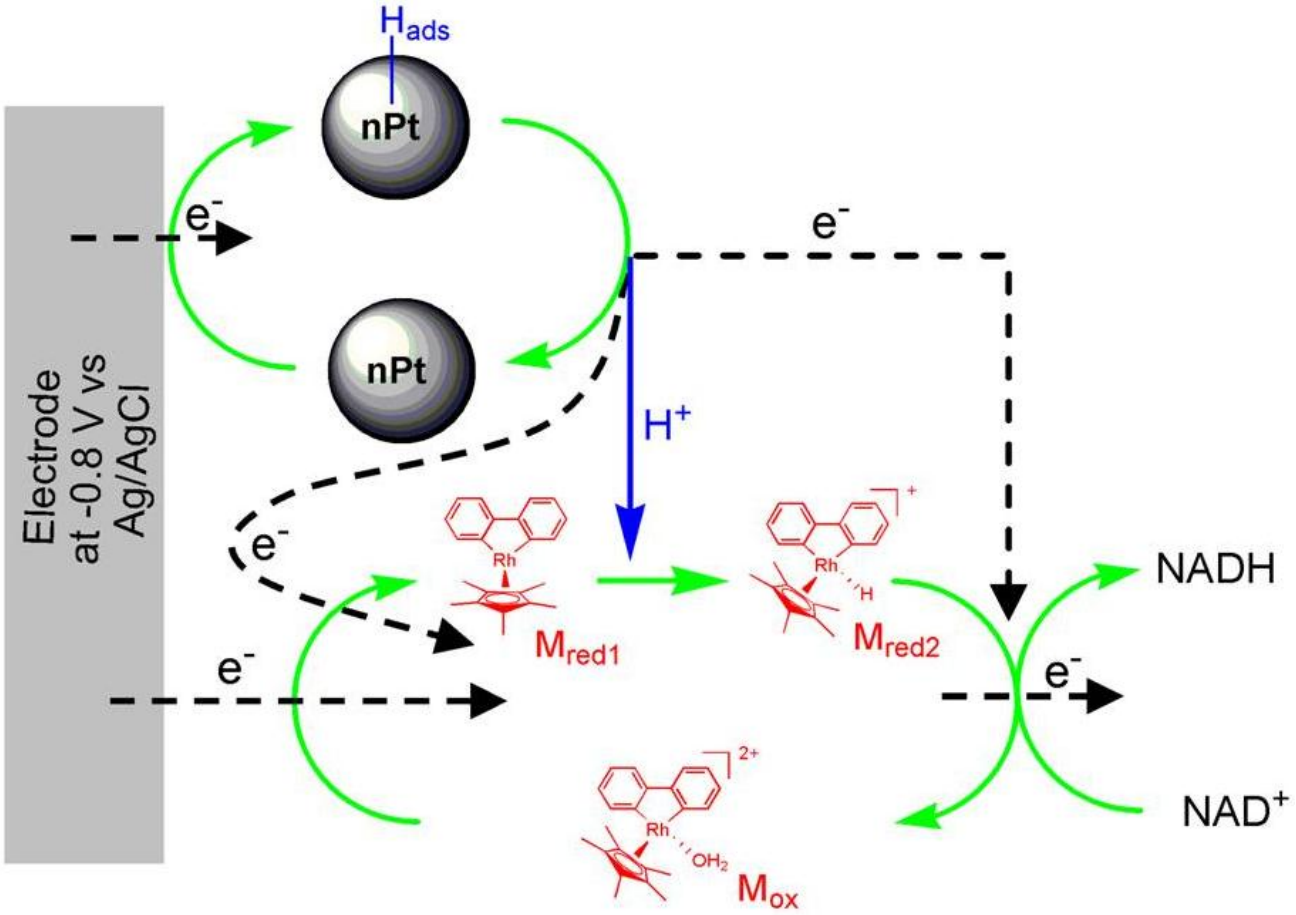


[Photochemical Cofactor Regeneration]

Redox Reactions

Cofactor Recycling

Å Electrochemistry

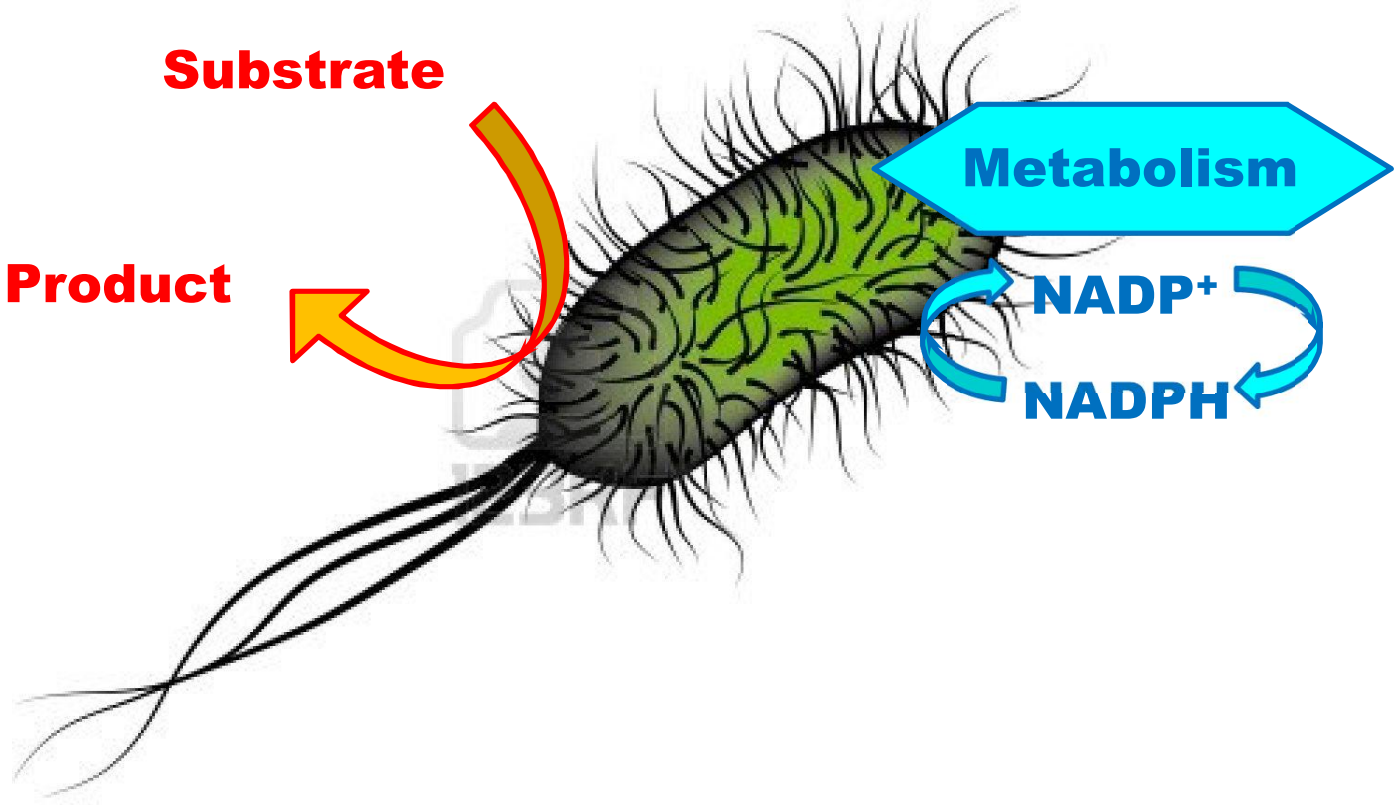


[Electrochemical Cofactor Regeneration]

Redox Reactions

Cofactor Recycling

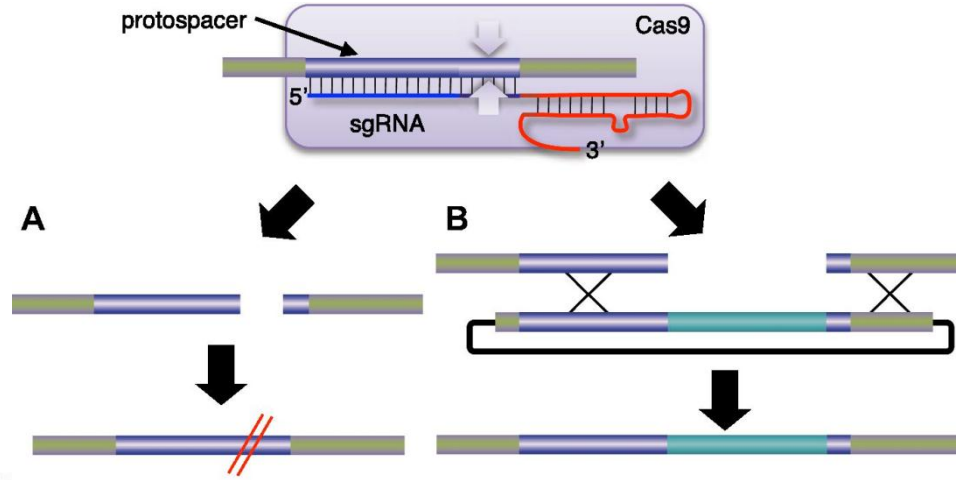
Å Whole-cell biotransformations

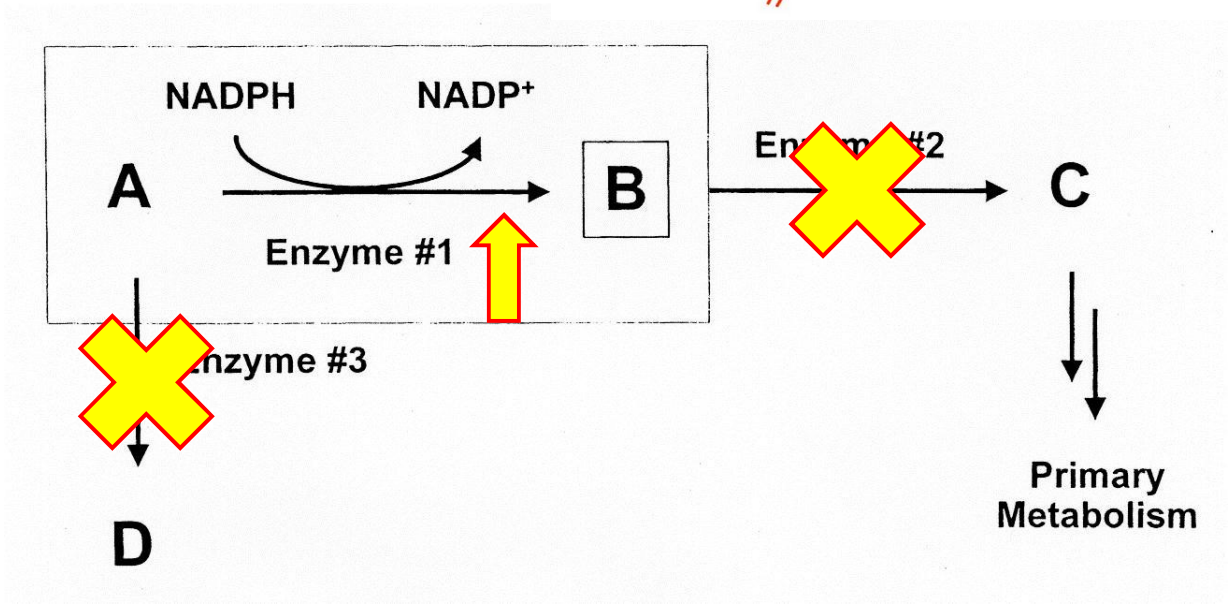


Redox Reactions

Cofactor Recycling

- Whole-cell biotransformations ï recombinant organisms
  - Genen overexpression
  - Gene knockout

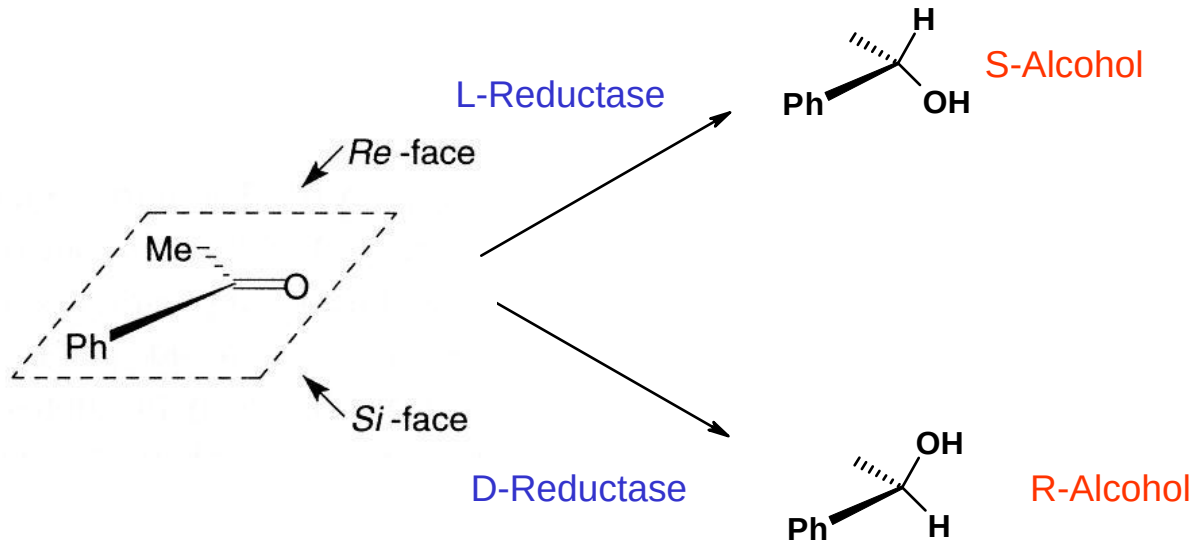




Redox Reactions

Carbonyl Reductions

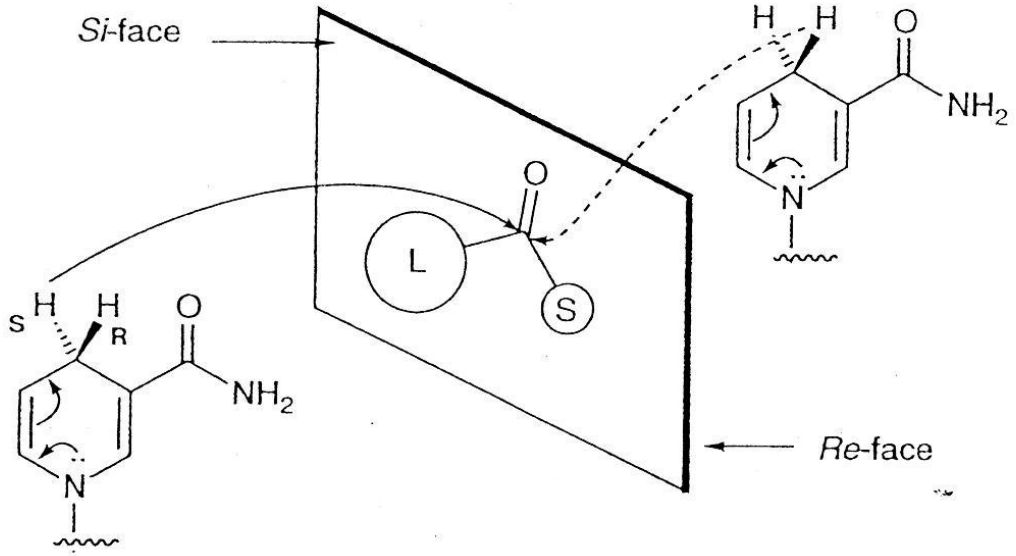
- Stereochemistry



Redox Reactions

Carbonyl Reductions

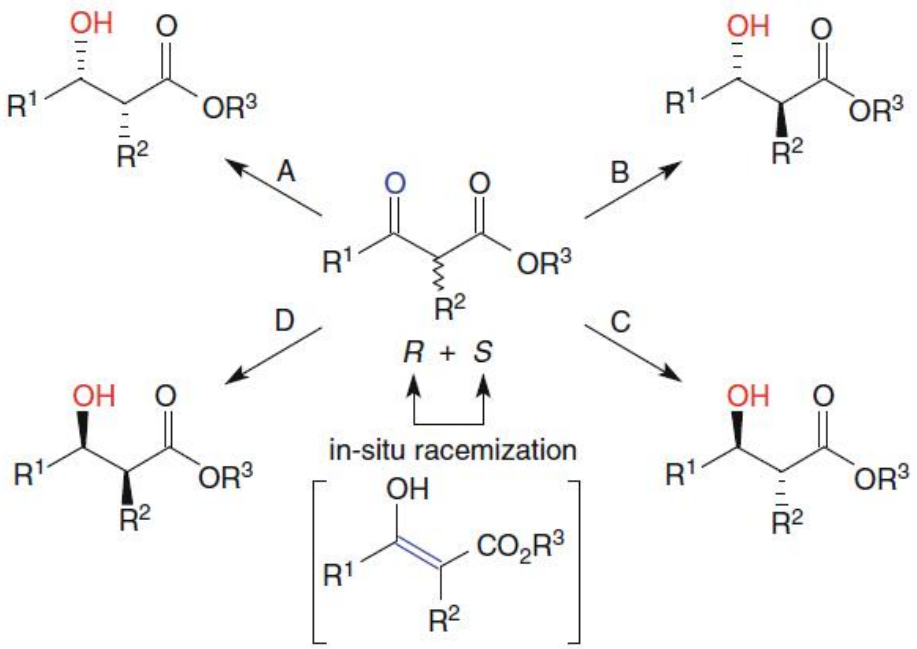
- Prelog's rule



Redox Reactions

Carbonyl Reductions

Å Dynamic Processes

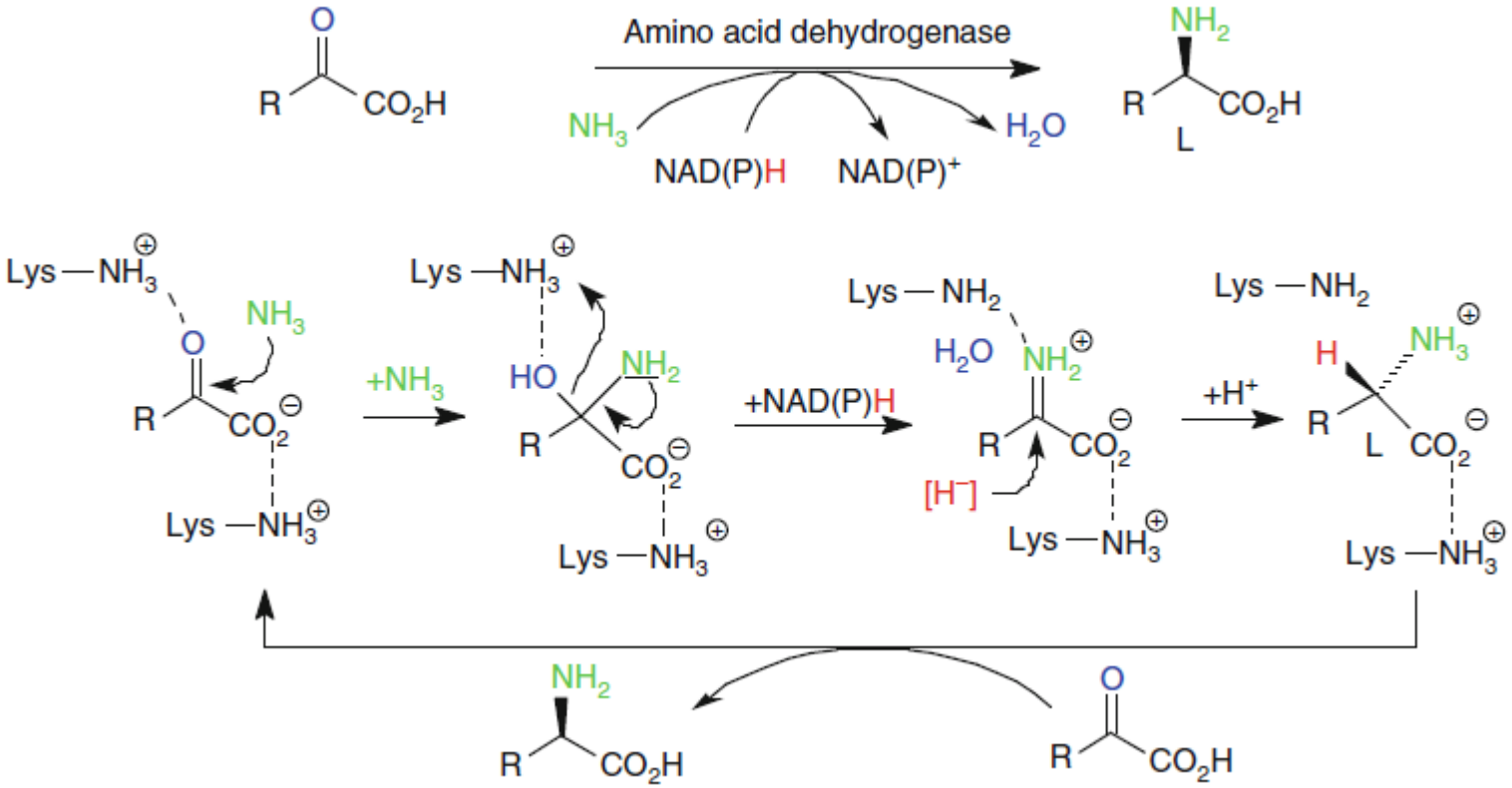


Pathway	R^1	R^2	R^3	Biocatalyst	Yield [%]	d.e. [%]	e.e. [%]	Ref.
A	Me	allyl	Et	baker's yeast	94	92	>99	[907]
A	Me	Me	*n*-Octyl	baker's yeast	82	90	>98	[908]
B	Me	Me	Et	*Geotrichum candidum*	80	>98	>98	[909]
B	Et	Me	Et	*Geotrichum candidum*	80	96	91	[910]
C	4–$MeOC_6H_4$–	Cl	Et	*Sporotrichum exile*	52	96	98	[911]
D	4–$MeOC_6H_4$–	Cl	Me	*Mucor ambiguus*	58	>98	>99	[912]

Redox Reactions

Carbonyl Reductions

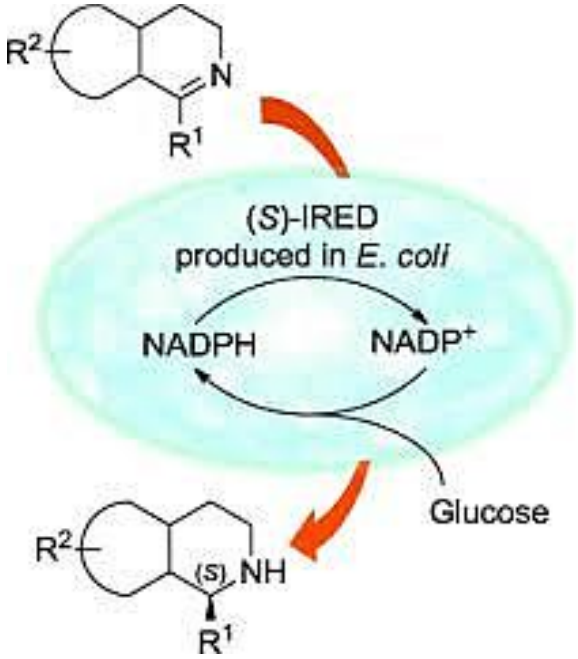
- Reductive Aminations
  - Amino acid dehydrogenase



Redox Reactions

Carbonyl Reductions

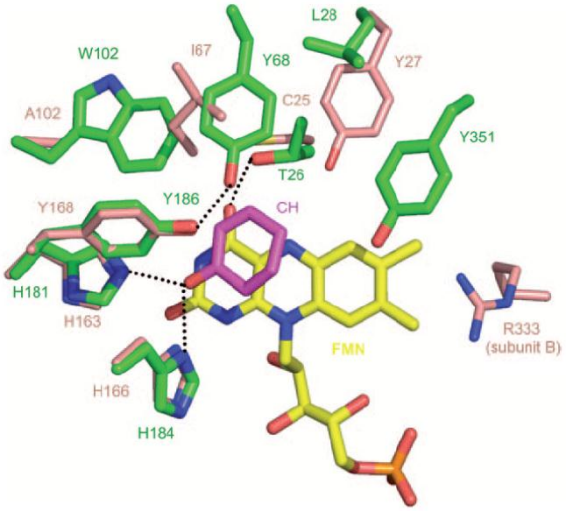
- Imine Reductases
  - Problem: imine stablity in aqueous systems

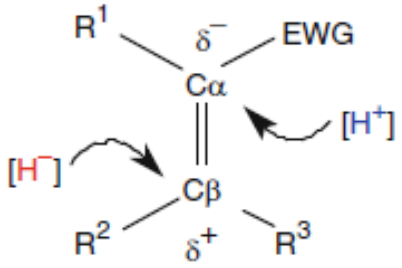


Redox Reactions

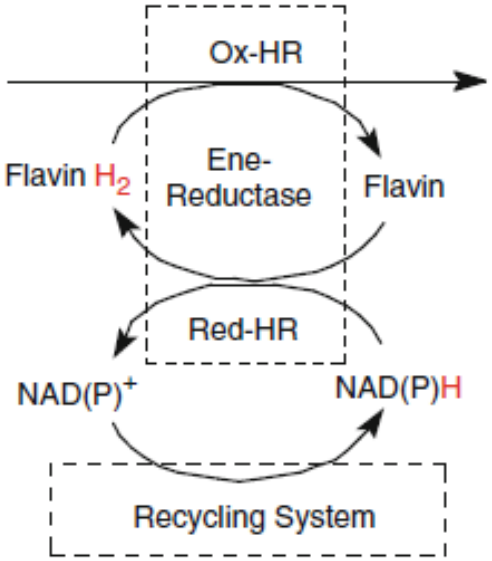
Alkene Reductions

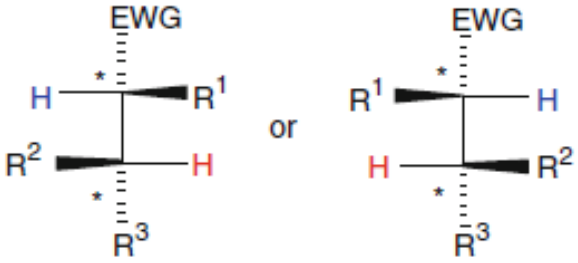
Å Enoate Reductases (Old Yellow Enzymes)





EWG = electron-withdrawing group: aldehyde, ketone, carboxylic acid ester, lactone, cyclic imide, nitro



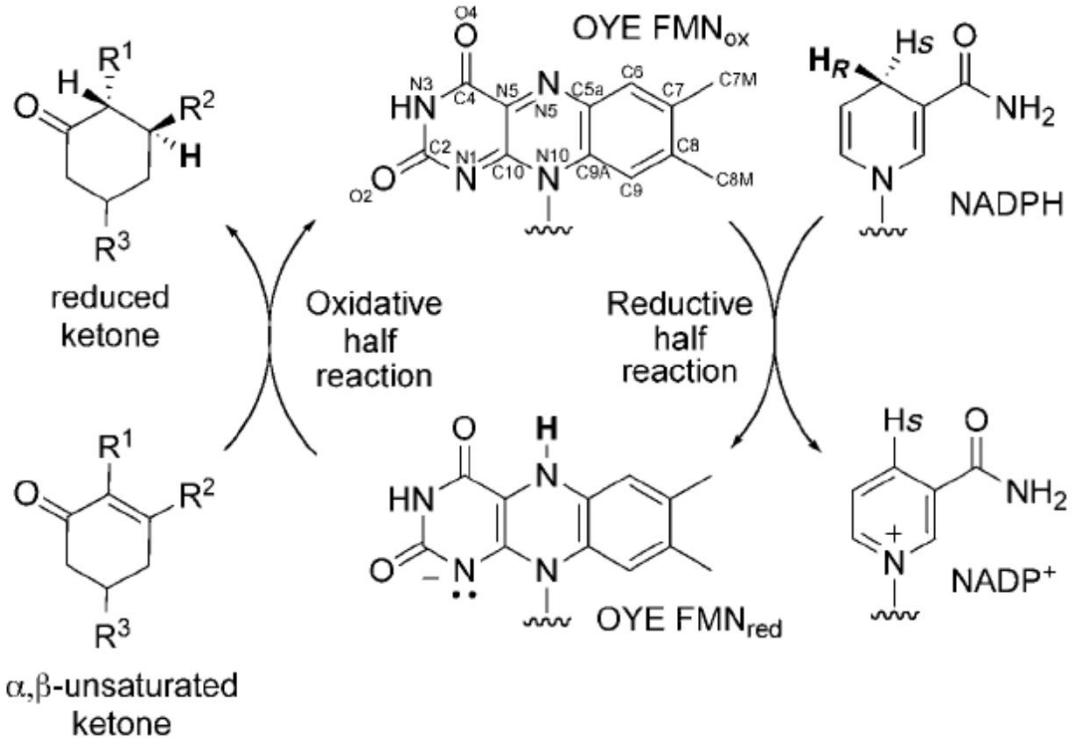


Ox-HR: oxidative half-reaction
Red-HR: reductive half-reaction
[H−] = hydride delivered from N5 of the flavin cofactor
[H+] = proton delivered via Tyr-residue

Redox Reactions

Alkene Reductions

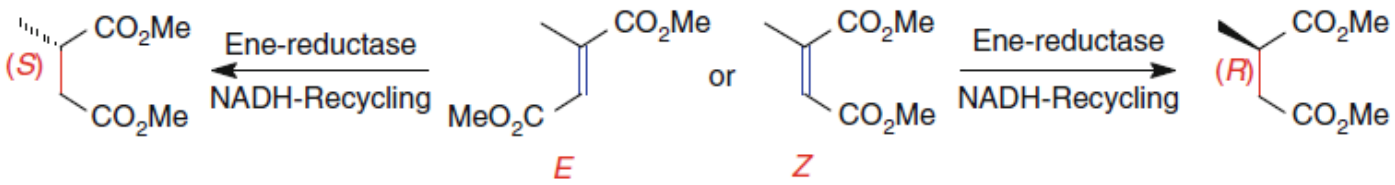
Å Enoate Reductases (Old Yellow Enzymes)



Redox Reactions

Alkene Reductions

- Enoate Reductases (Old Yellow Enzymes)
  - Alkene substitution pattern

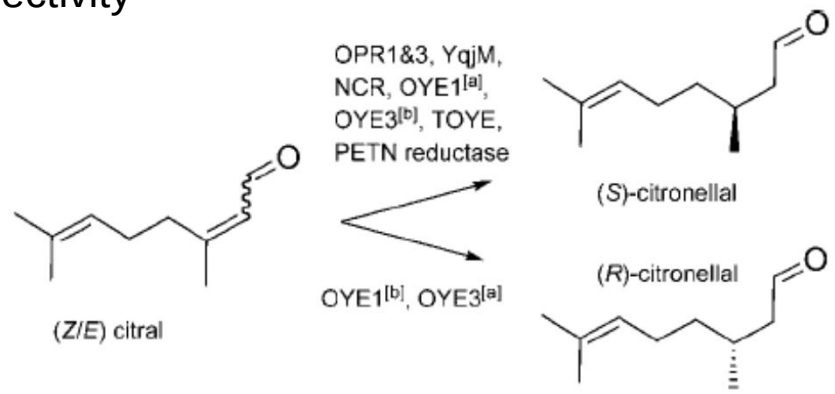


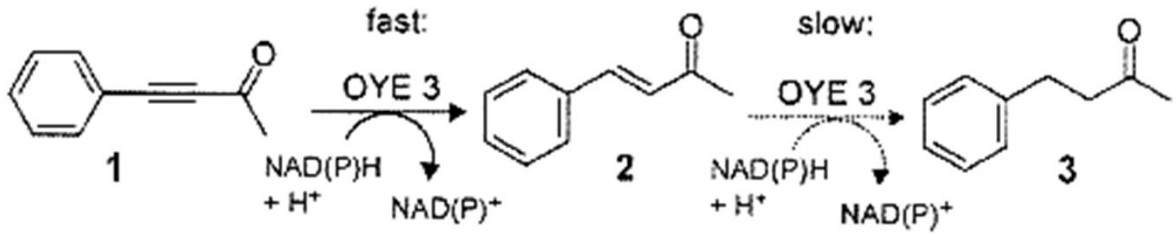
Configuration	YqjM		OPR1	
	c [%]	e.e. [%]	c [%]	e.e. [%]
Z	93	>99 (*R*)	91	>99 (*R*)
E	70	>99(***S!***)	99	>99 (*R*)

Redox Reactions

Alkene Reductions

- Enoate Reductases (Old Yellow Enzymes)
  - Chemoselectivity



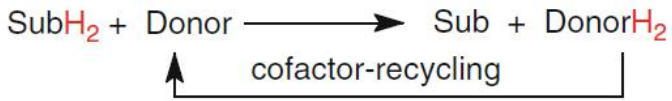


Scheme 1. Reductions catalyzed by Old Yellow Enzymes (OYEs). **1**: 4-phenyl-3-butyne-2-one; **2**: (*E*)-4-phenyl-3-butene-2-one; **3**: 4-phenyl-2-butanone; NAD(P)$^+$: nicotinamide adenine (phosphate) dinucleotide.

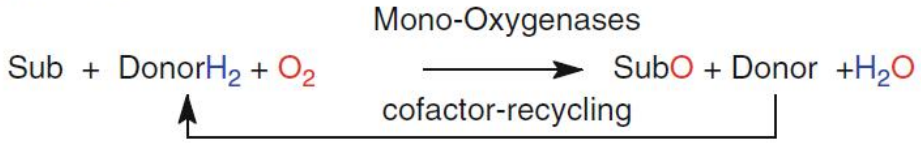
Redox Reactions

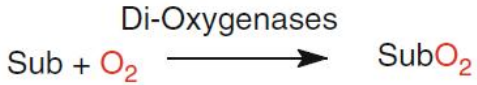
Oxidation Reactions

Dehydrogenases

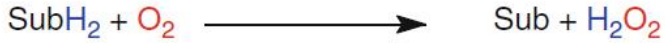


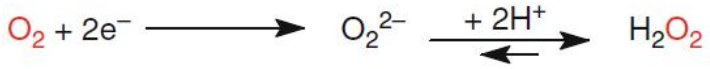
Oxygenases



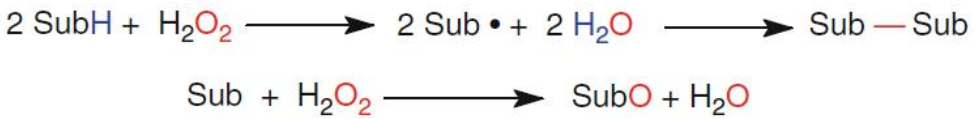


Oxidases





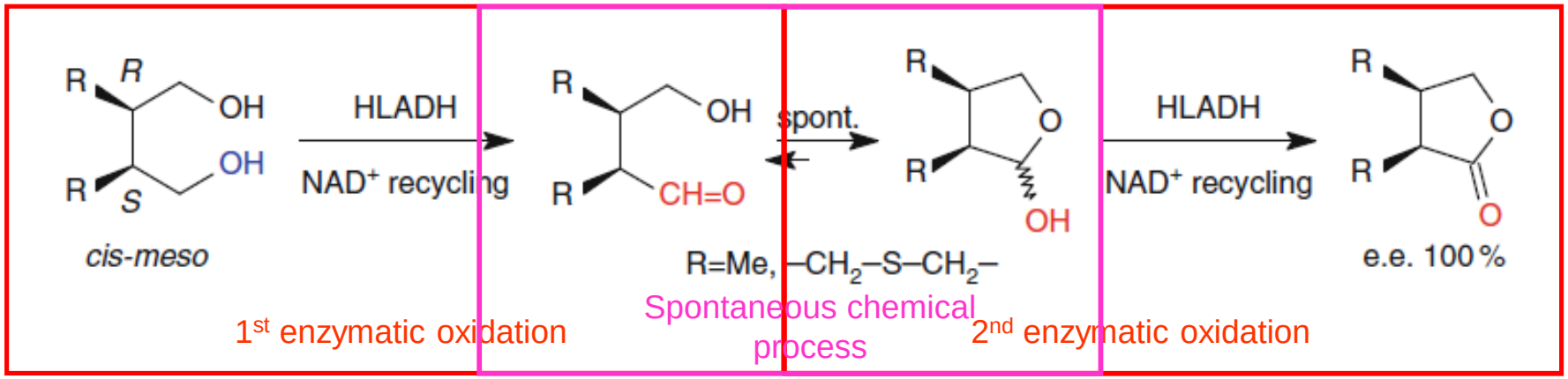
Peroxidases



Redox Reactions

Alcohol Oxidations

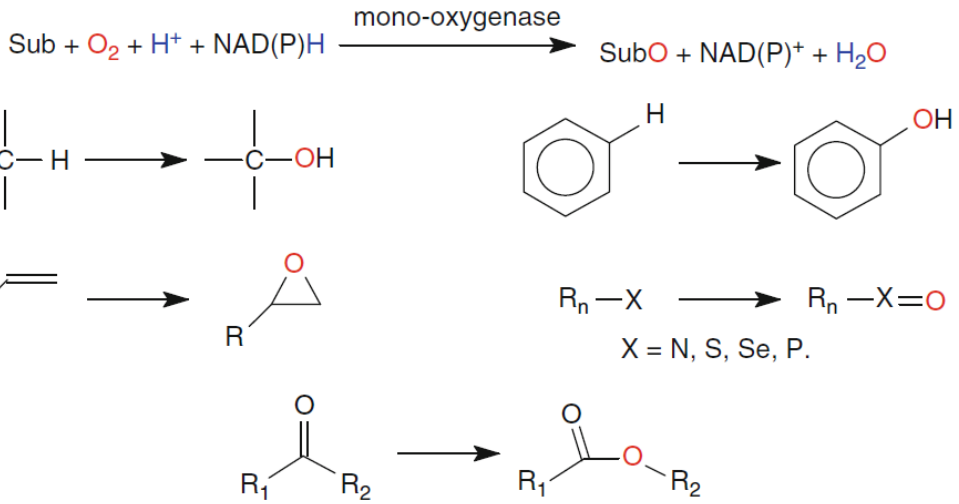
- HLADH Biooxidations
  - FMN as sacrificial substrate



Redox Reactions

Monooxygenations

Å Reaction types



Substrate	Product	Type of reaction	Type of cofactor
Alkane	alcohol	hydroxylation	metal-dependent
Aromatic	phenol	hydroxylation	metal-dependent
Alkene	epoxide	epoxidation	metal-dependent
Heteroatom[a]	heteroatom oxide	heteroatom oxidation	flavin-dependent
Ketone	ester / lactone	Baeyer-Villiger	flavin-dependent

[a]N, S, Se, or P

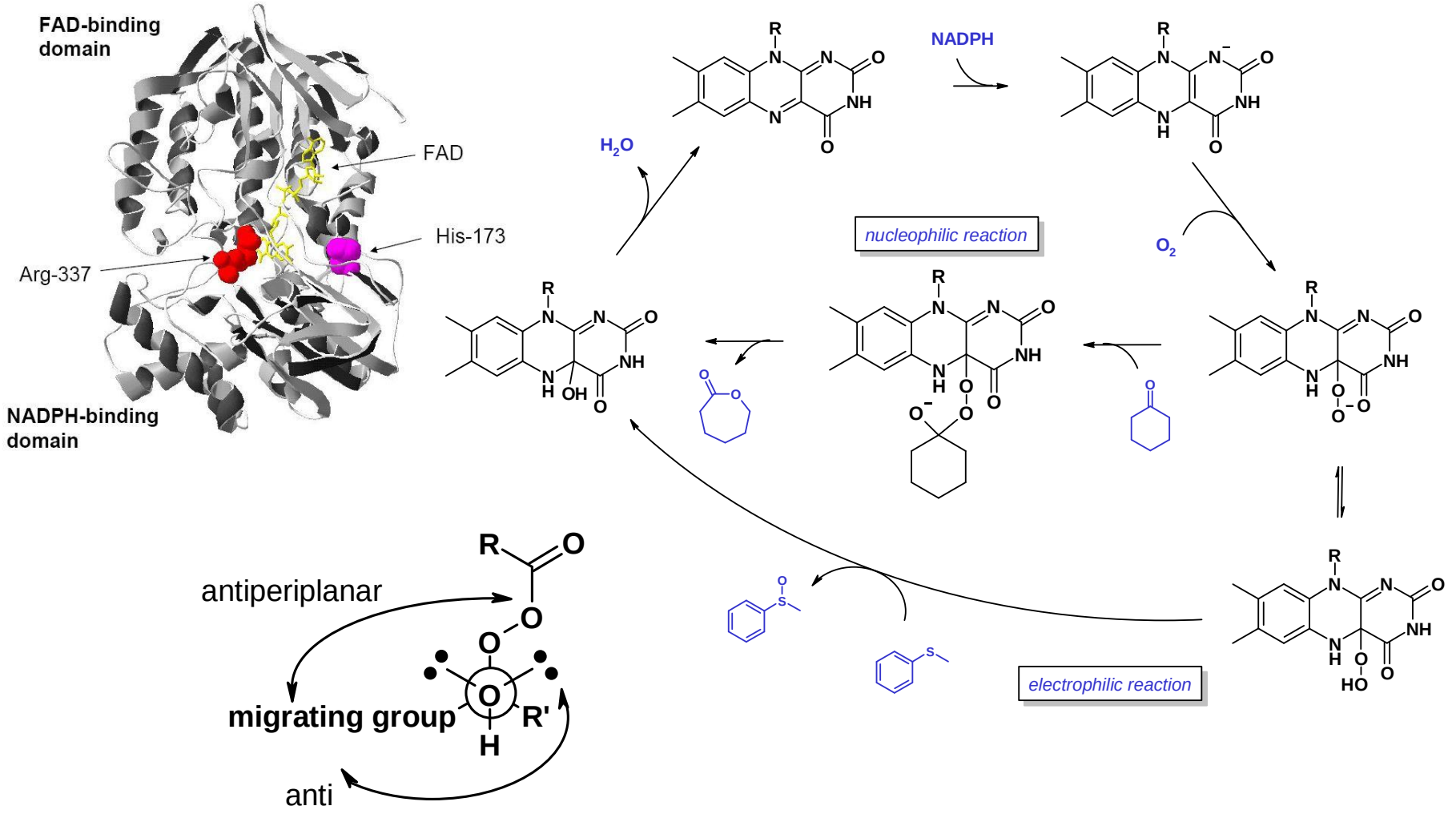
similar to chemical oxidation using peracids (**nucleophilic process**)

similar to chemical oxidation using hypervalent metals (**electrophilic process**)

Redox Reactions

Monooxygenations

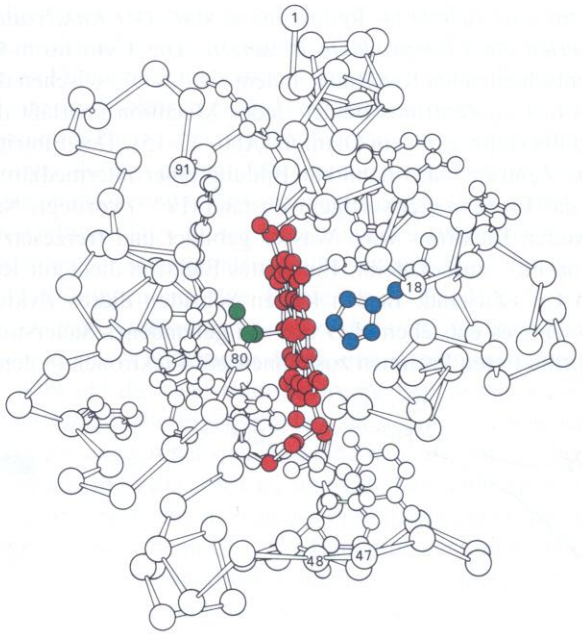
Å Flavin dependent enzymes

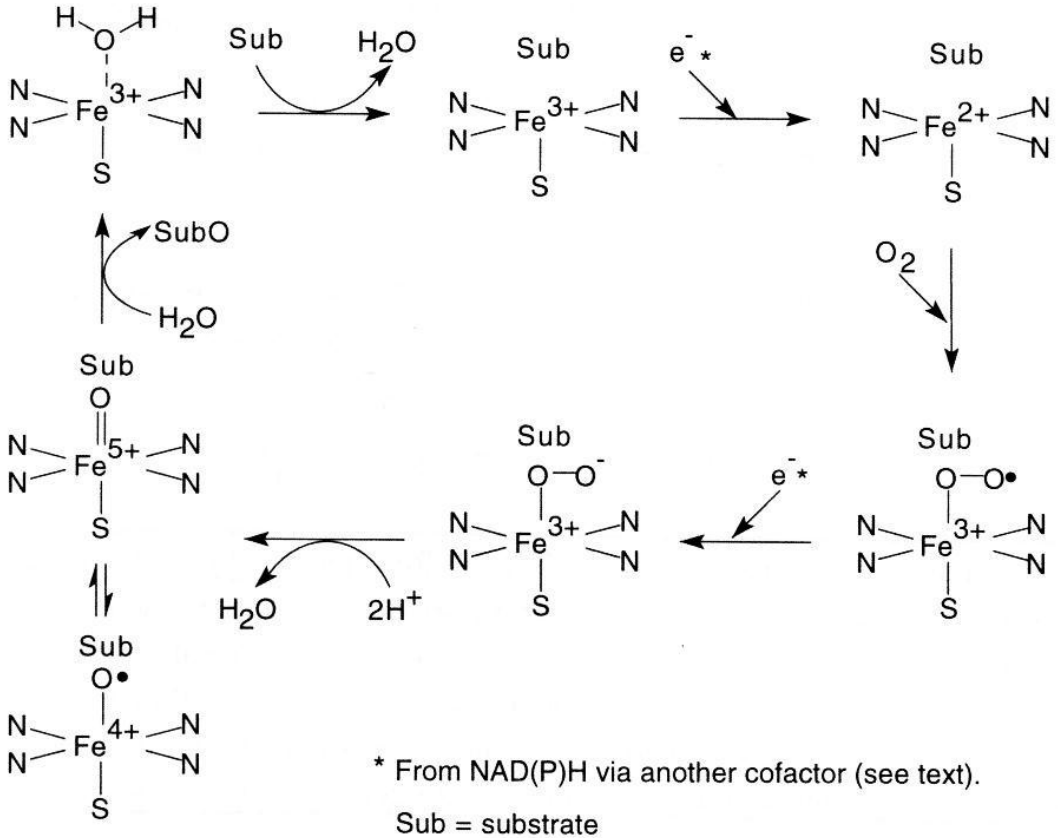


Redox Reactions

Monooxygenations

Å Cytochrome P450 enzymes

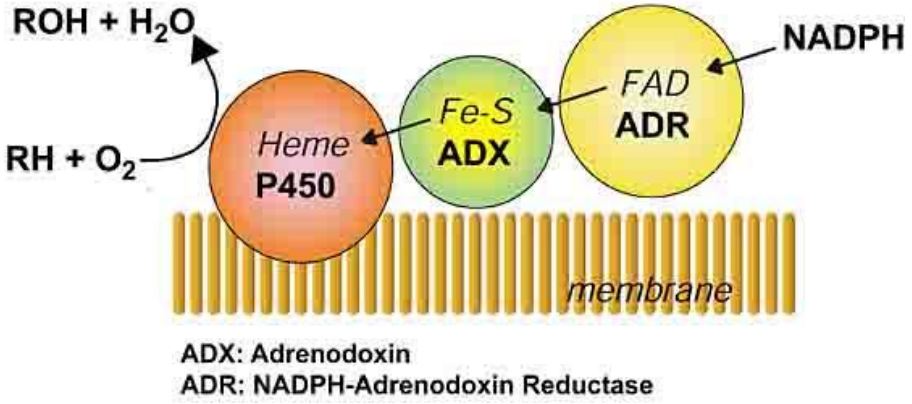




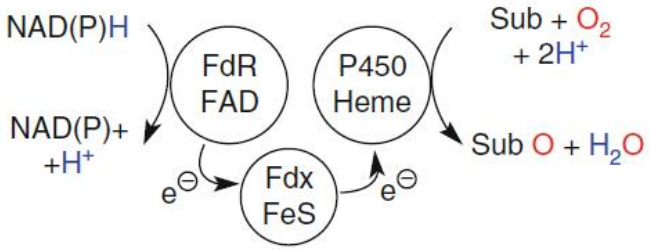
Redox Reactions

Monooxygenations

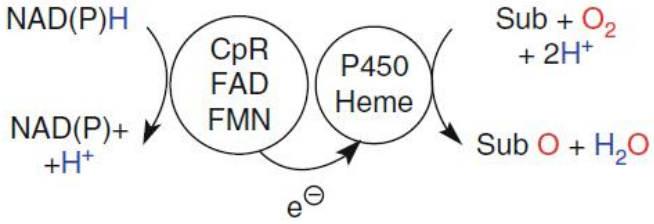
Å Cytochrome P450 enzymes



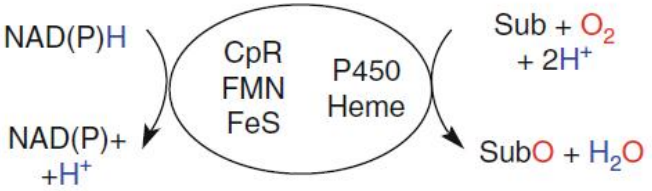
Bacterial / mitochondrial System



Microsomal System



Self-sufficient BM-3 System



FdR = Ferredoxin Reductase

FAD = Flavin adenine dinucleotide

Fdx = Ferredoxin

FeS = iron-sulfur cluster

CpR = Cytochrome P Reductase

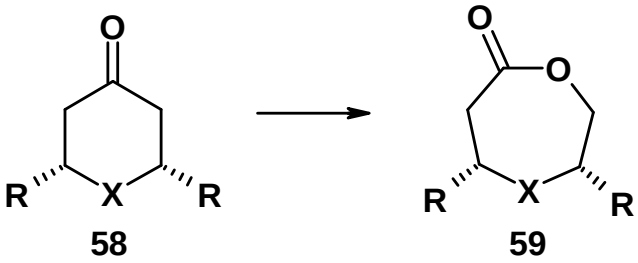
FMN = Flavin mononucleotide

P450 = Cytochrome P-450

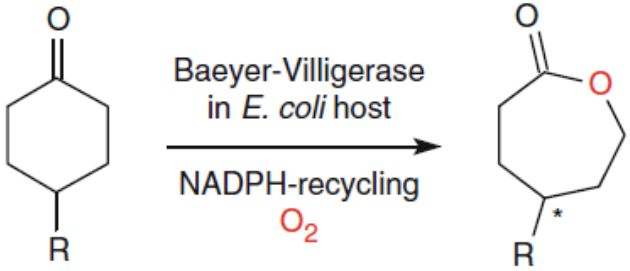
Redox Reactions

Monooxygenations

Å Baeyer-Villiger Oxidation

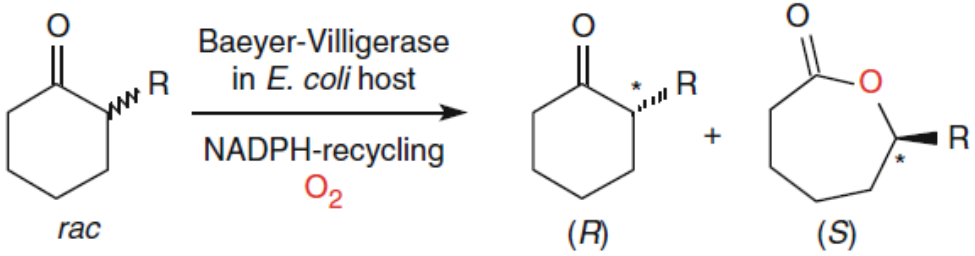


	X	R	Recomb. cells
59a	S[82]	H	48%[95]
59b	NMe	H	50%[95]
59c	NCOMe	H	39% (59%)[95]
59d	NCOOMe	H	40% (67%)[95]
59e	O	H	79%[95]
59f	O	Me	79%/> 99% *ee*[96]



(*S*): R = Me, Et, n-Pr, *i*-Pr[a]; e.e. up to > 99 %

(*R*): *n*-Bu; e.e. up to 60 %



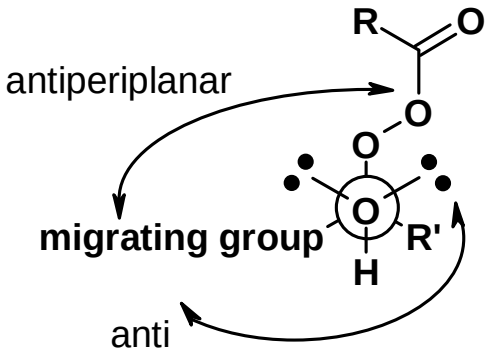
R = Me, Et, *n*-Pr, allyl[a], *n*-Bu, E 200

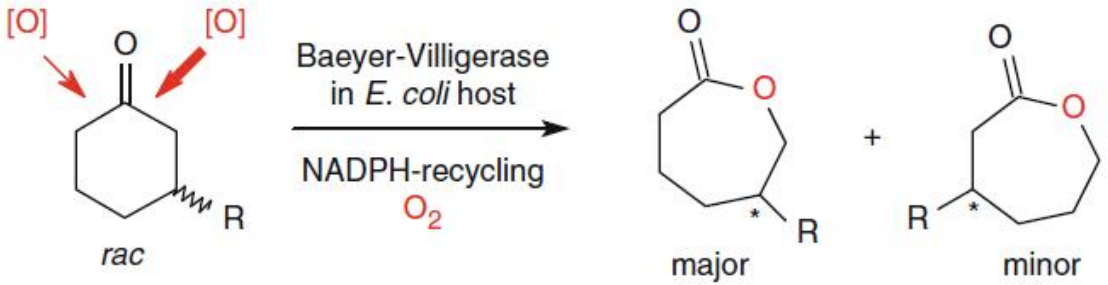
[a]Switch in sequence priority

Redox Reactions

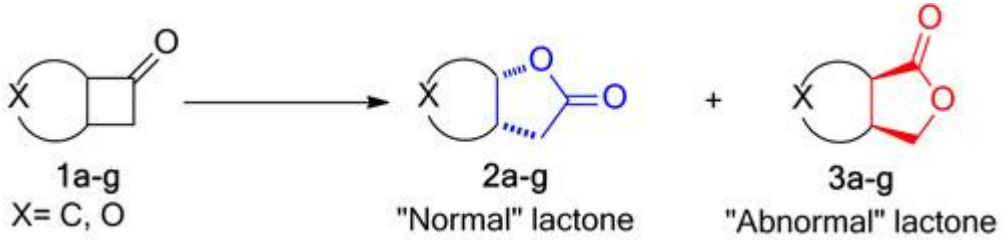
Monooxygenations

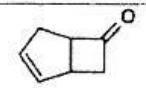
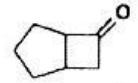
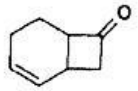
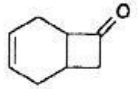
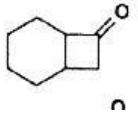
Å Baeyer-Villiger Oxidation





R	E
Me	60 (*S*)
Et	≥ 200 (*R*)
n-Pr	≥ 200 (*R*)
i-Pr	≥ 200 (*S*)[a]
n-Bu	20 (*R*) or (*S*)

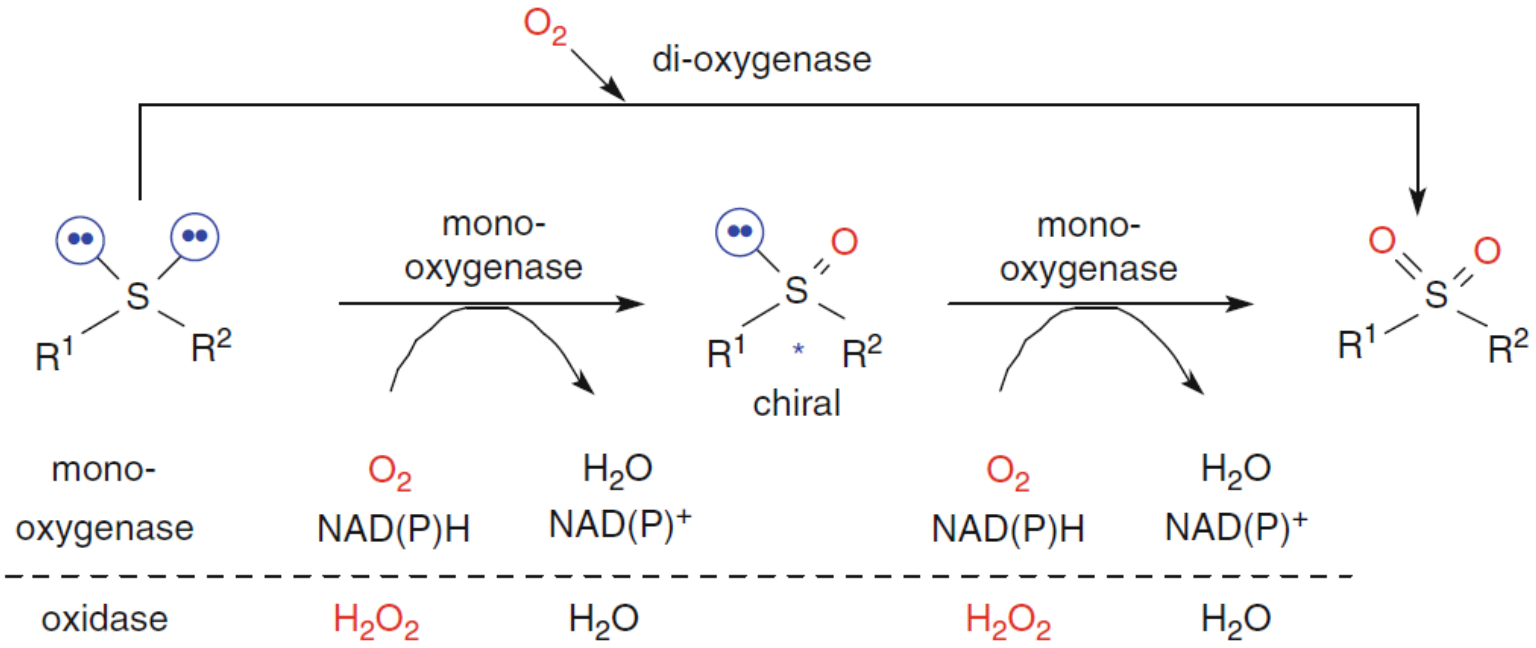


Substrate ketone	"Normal" lactone 67		"Abnormal" lactone 68
	44%/> 95% *ee* (1*S*,5*R*)[104]	a	42%/> 95% *ee* (1*R*,5*S*)[104]
	36%/> 95% *ee* (1*S*,5*S*)[104]	b	31%/> 95% *ee* (1*R*,5*S*)[104]
	43%/> 95% *ee* (1*R*,6*S*)[104]	c	37%/> 95% *ee* (1*S*,6*R*)[104]
	41%/86% *ee* (1*S*,6*S*)[104]	d	36%/> 95% *ee* (1*S*,6*R*)[104]
	52%/60% *ee* (1*S*,6*S*)[104]	e	28%/> 95% *ee* (1*S*,6*R*)[104]

Redox Reactions

Monooxygenations

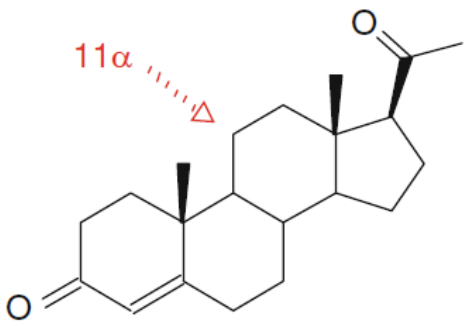
Å Heteroatom Oxidation



Redox Reactions

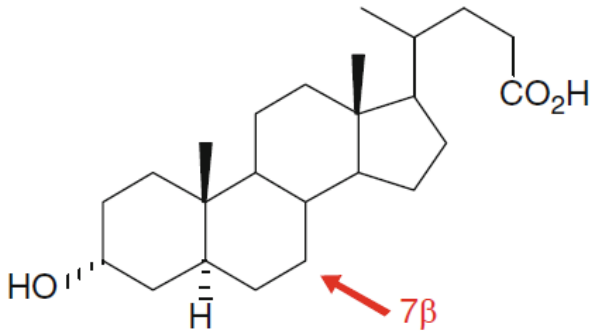
Monooxygenations

- Biohydroxylations
  - Steroid hydroxylations
    - reactivity: sec. > tert. > prim. (compare to radical reactions)
    - primarily whole-cell biotransformations
      (enzymes difficult to isolate and/or unknown)
    - sources: esp. fungi (Beauveria sp., Cunninghamella sp., Aspergillus sp.)



progesterone

Rhizopus arrhizus
Aspergillus niger



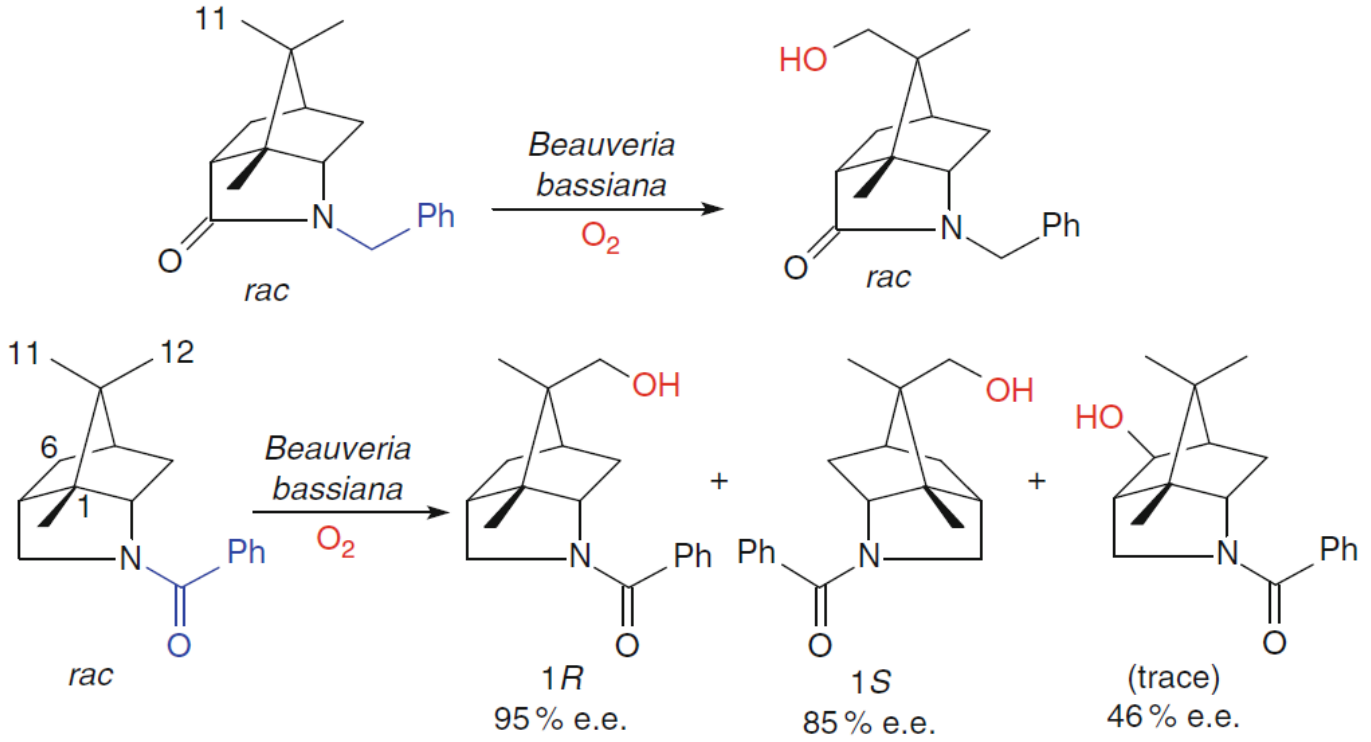
lithiocholic acid

Fusarium equiseti

Redox Reactions

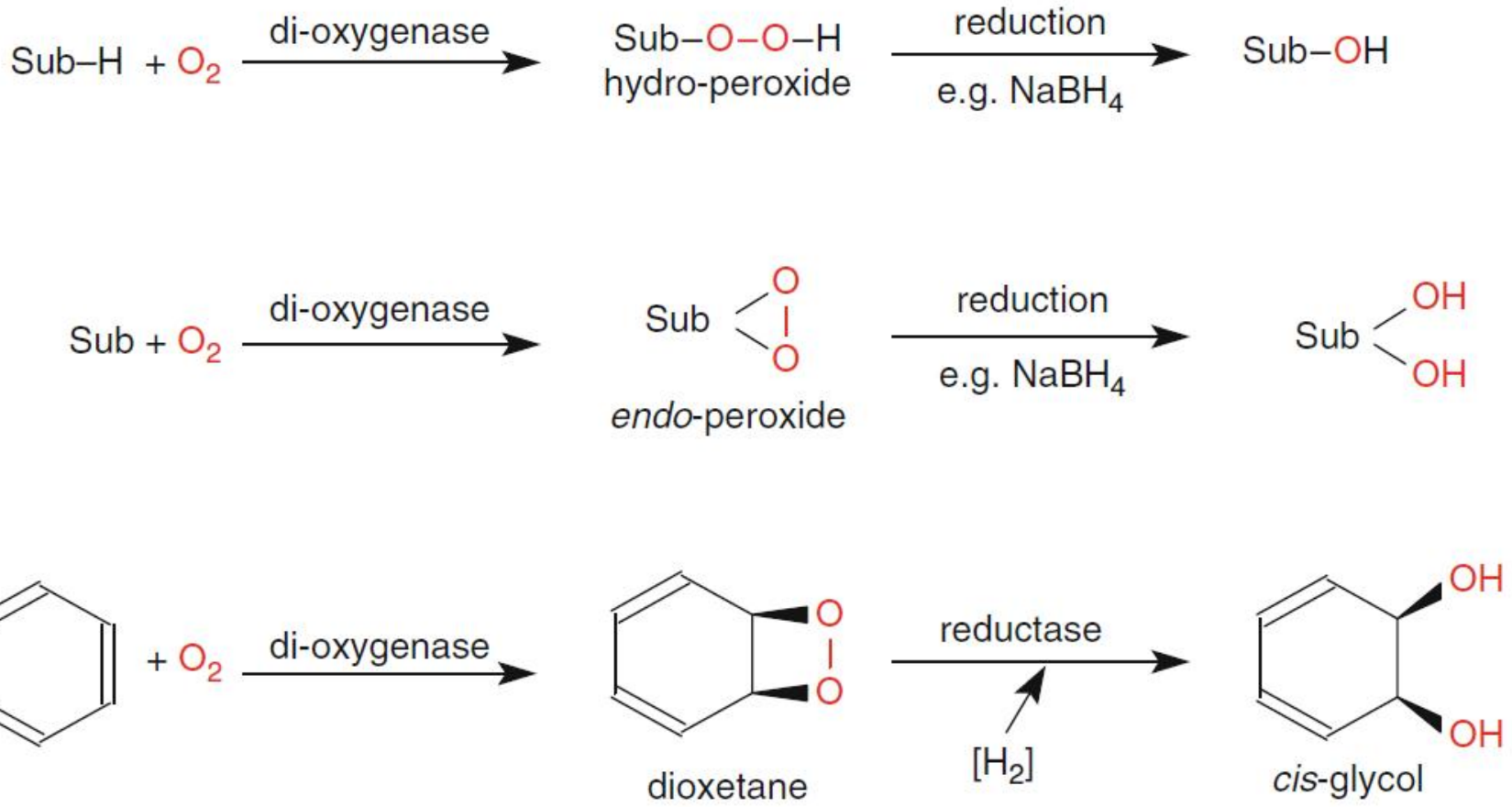
Monooxygenations

- Biohydroxylations
  - Substrate engineering



Redox Reactions

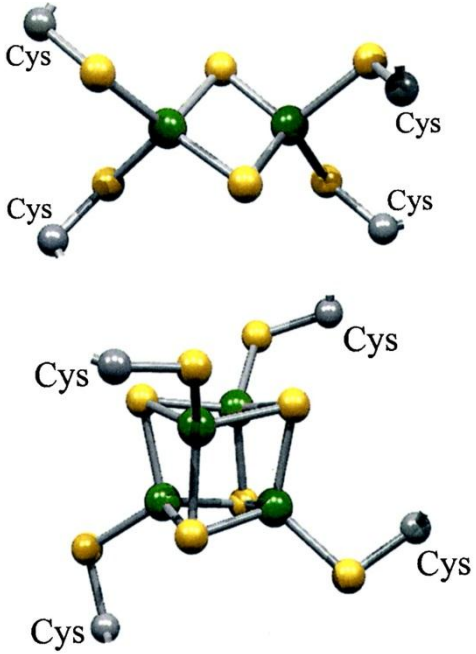
Dioxygenases

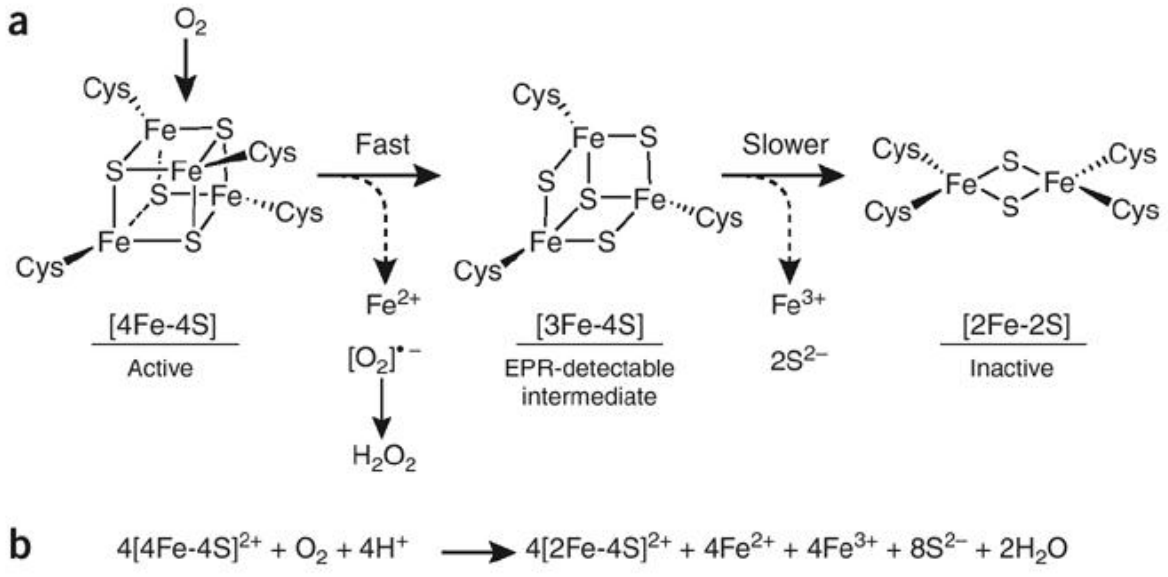


Redox Reactions

Dioxygenases

Å Fe-S Cluster Enzymes

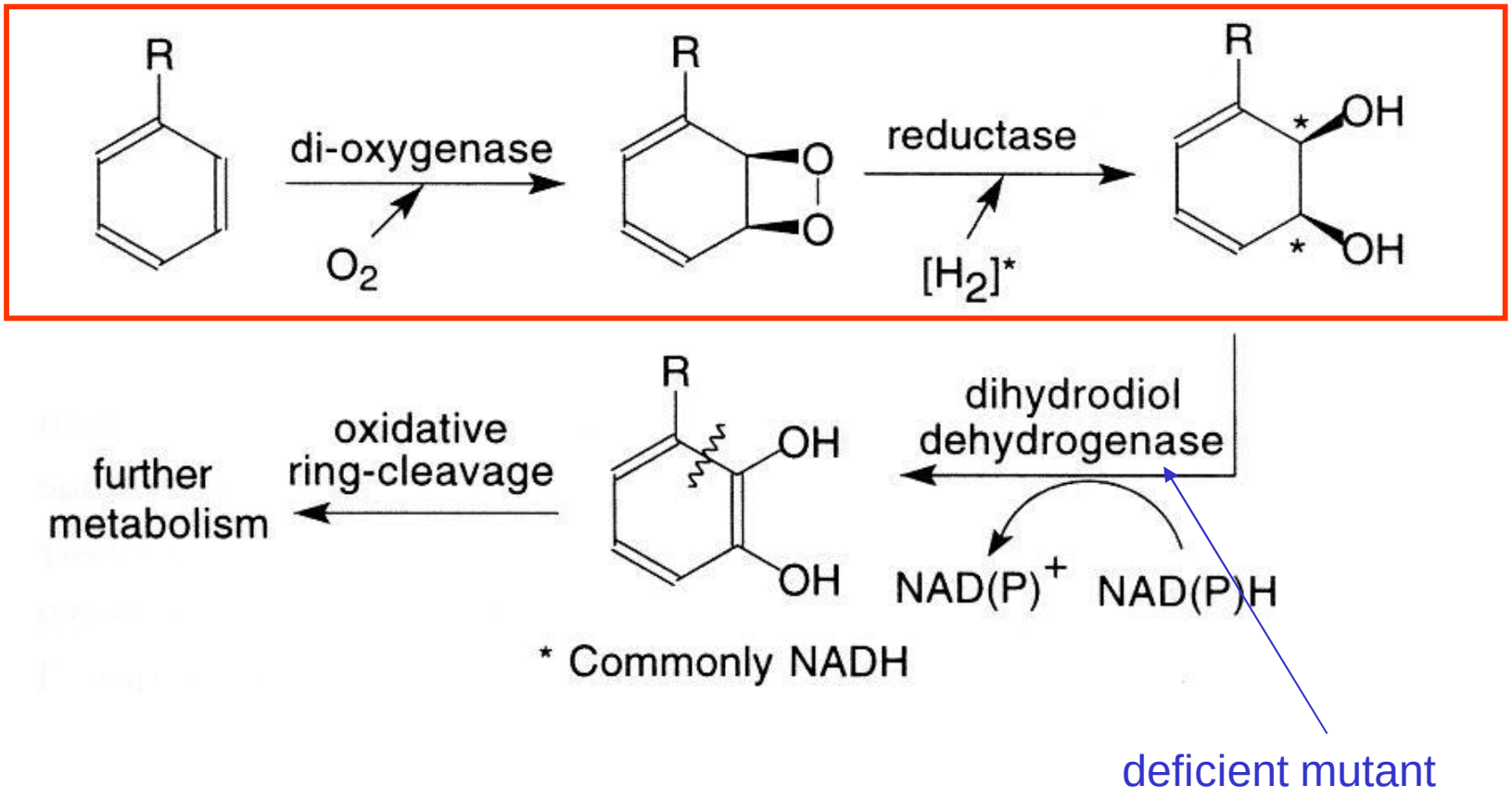




Redox Reactions

Dioxygenases

Å Aryl dioxygenations

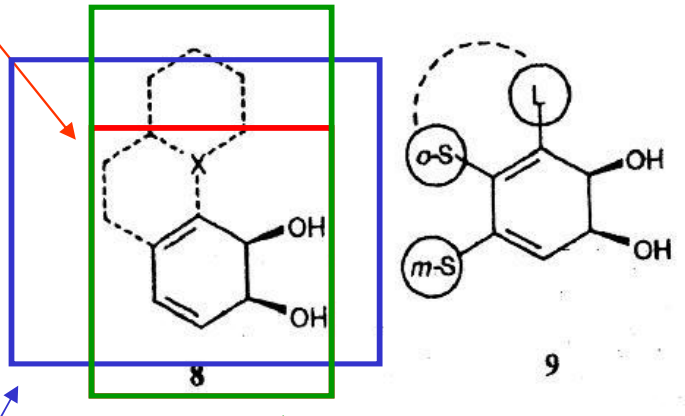


Redox Reactions

Dioxygenases

Å Aryl dioxygenations

Toluene Dioxygenase
(TDO)



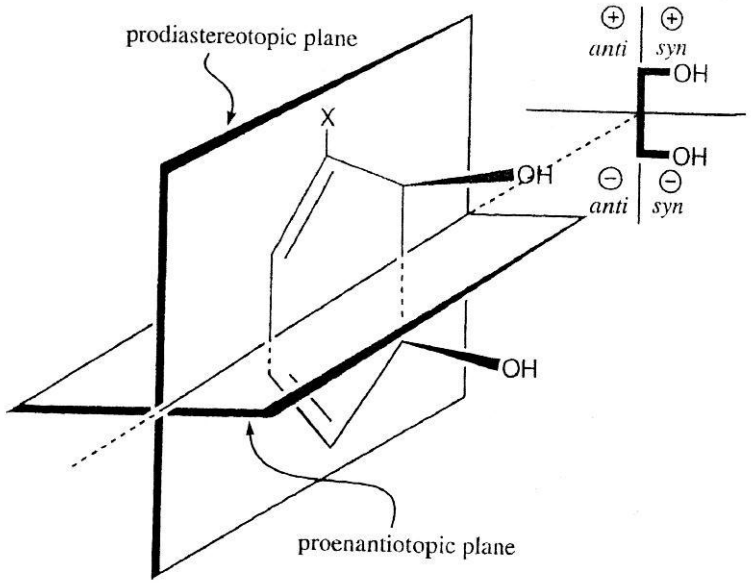
Naphthalene Dioxygenase
(NDO)

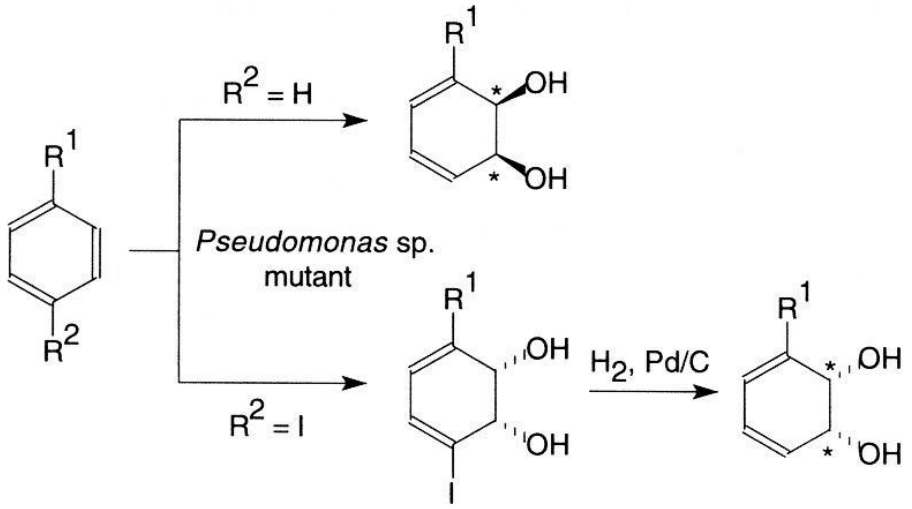
Biphenyl Dioxygenase
(BPDO)

Redox Reactions

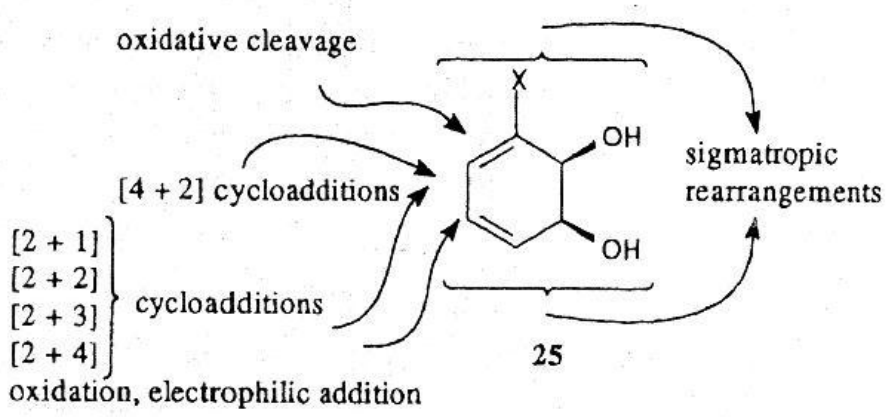
Dioxygenases

Å Aryl dioxygenations





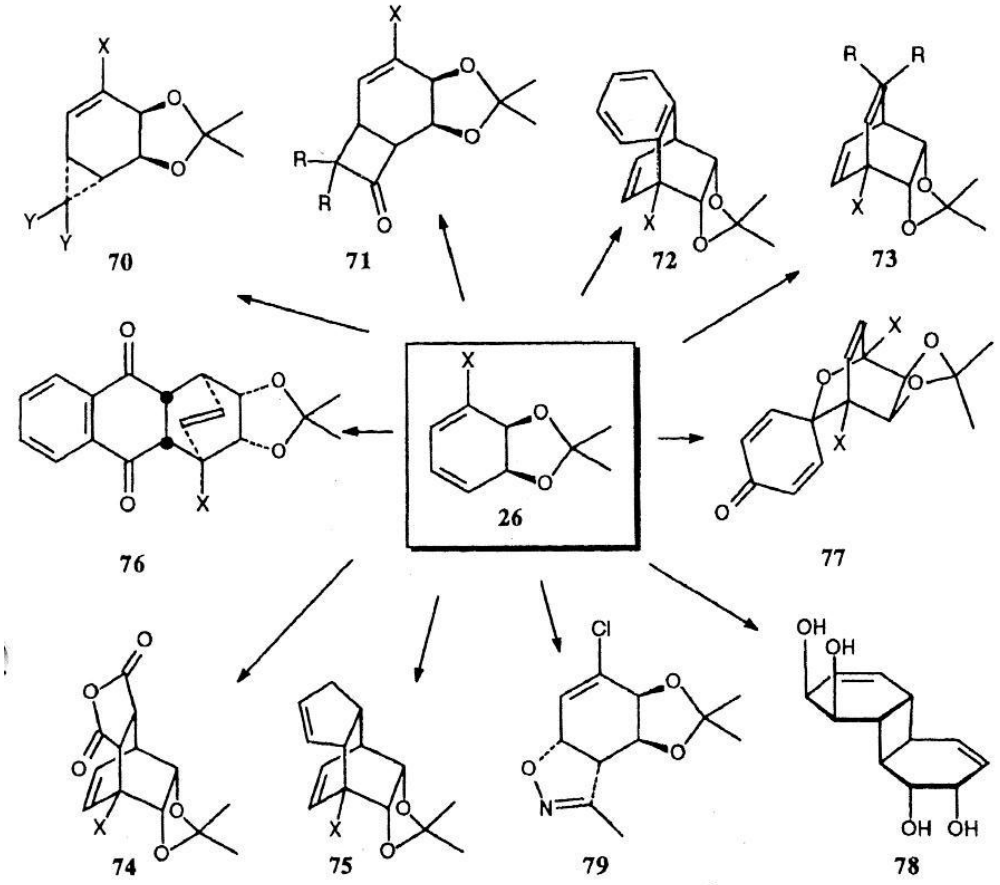
R^1	R^2
H, Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, *t*-Bu, Et-O, *n*-Pr-O, Halogen, CF_3, Ph, Ph-CH_2, Ph-CO, CH_2=CH, CH_2=CH-CH_2, HC≡C.	H
F, Me	I



Redox Reactions

Dioxygenases

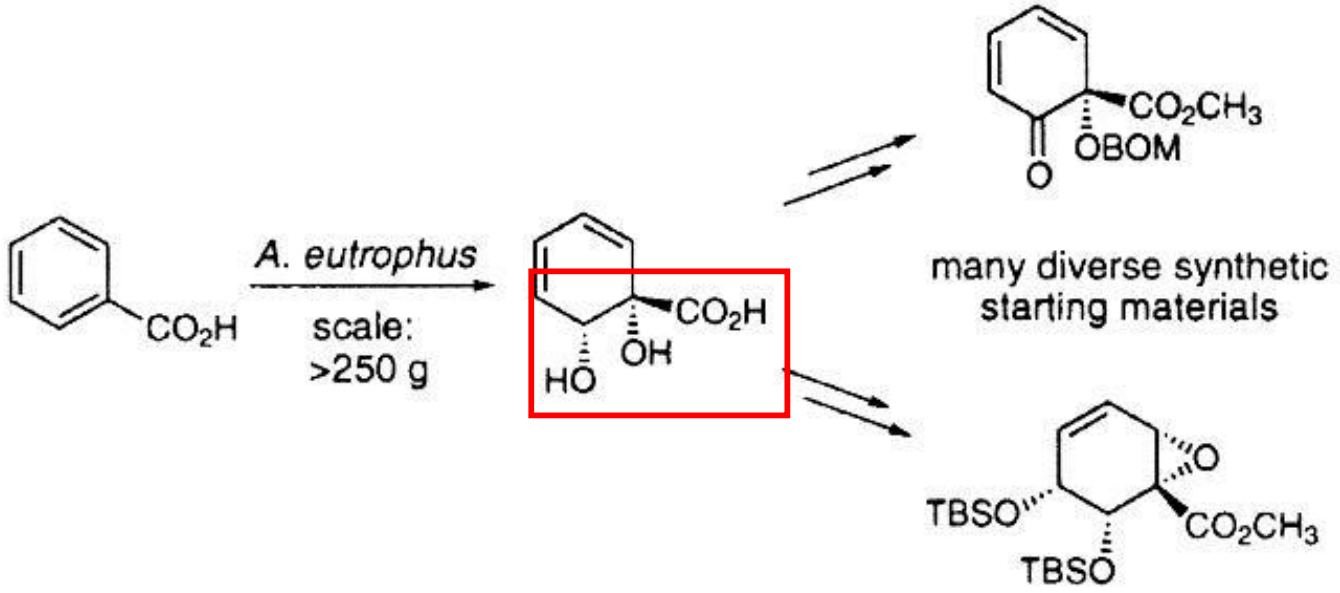
Å Aryl dioxygenations



Redox Reactions

Dioxygenases

Å Ipso-Aryl Dioxygenases

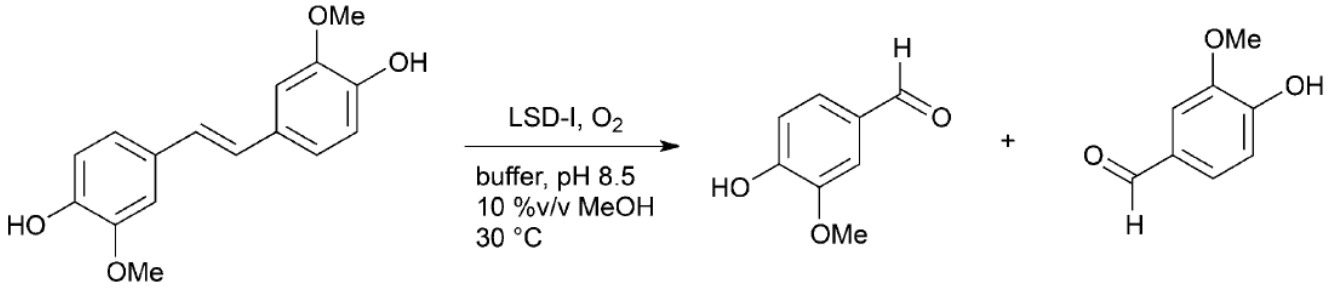


Redox Reactions

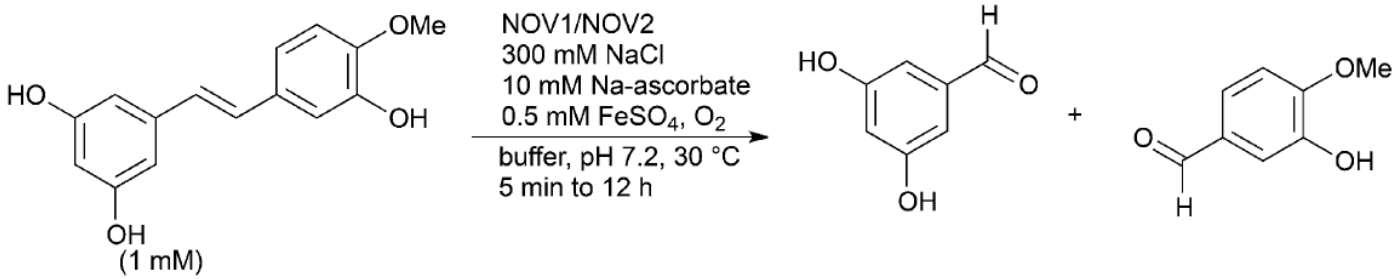
Dioxygenases

Å Alkene cleavage

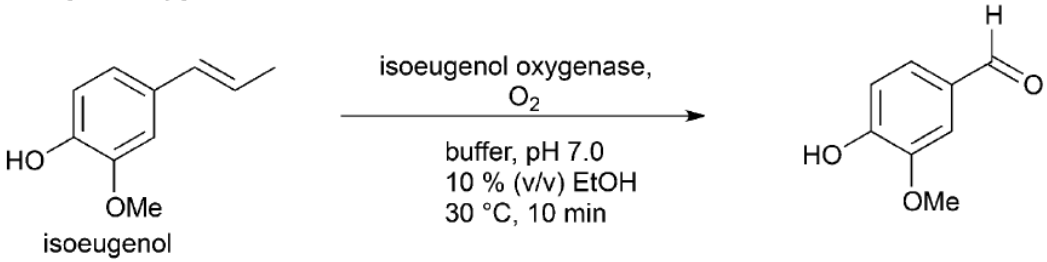
C) lignostilbene-α-β-oxygenase (LSD) from *S. paucimobilis*



D) stilbene-α-β-oxygenase (NOV_1 and NOV_2) from *N. aromaticivorans*



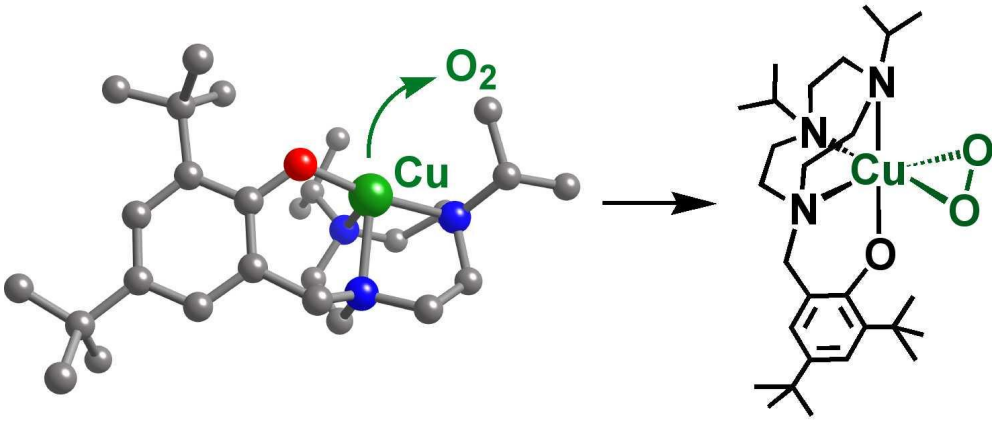
E) isoeugenol oxygenase

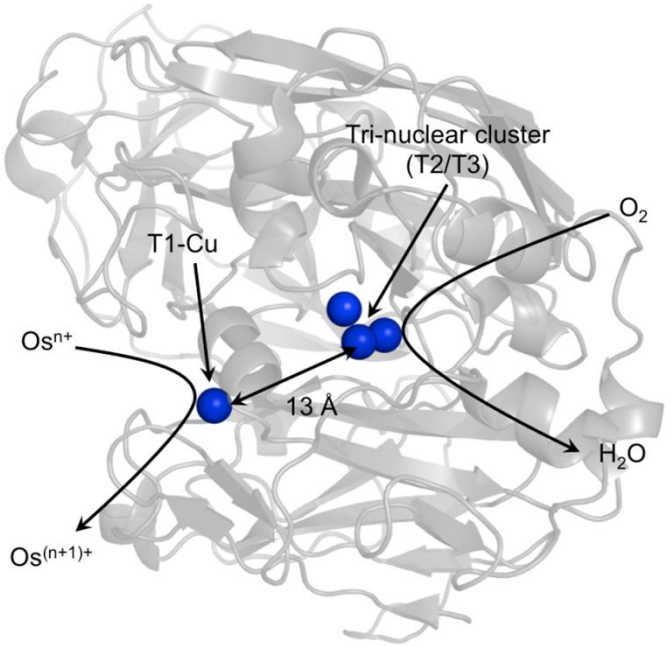


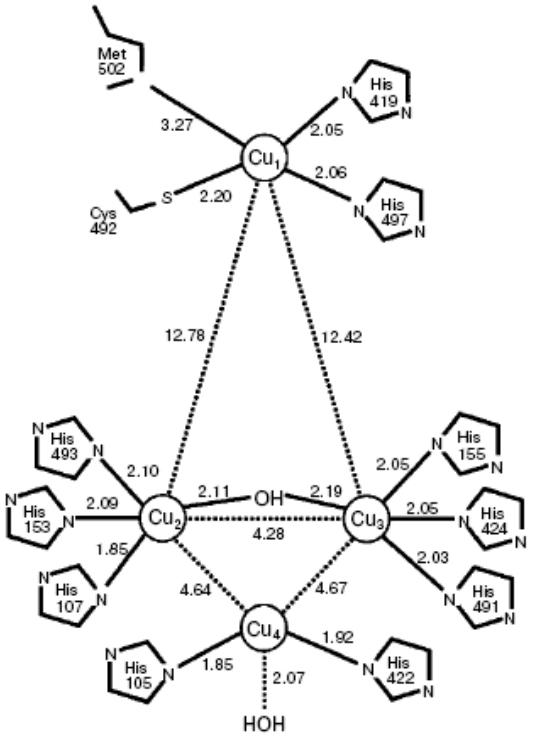
Redox Reactions

Laccases

Å Cu-containing Enzymes



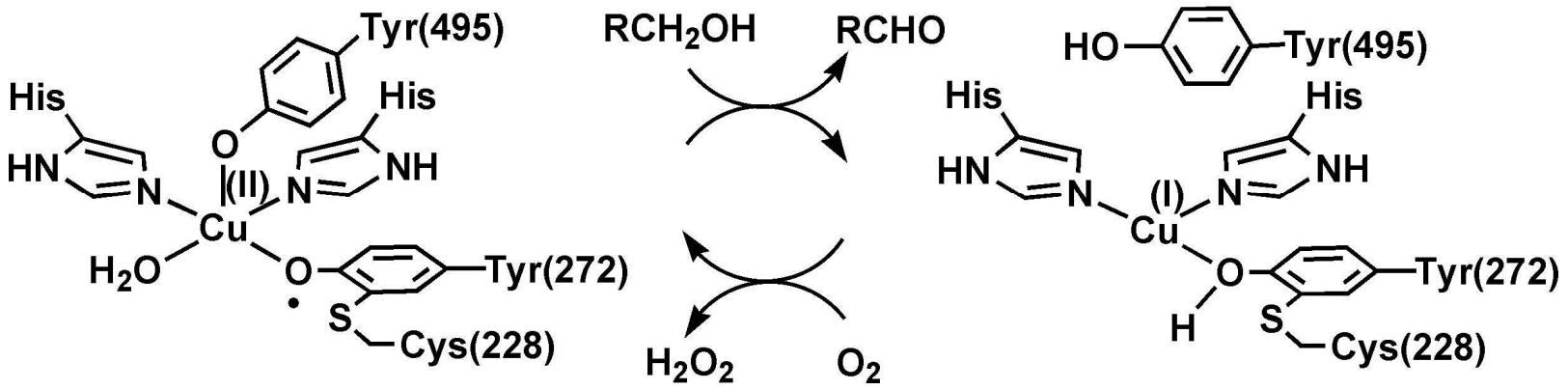


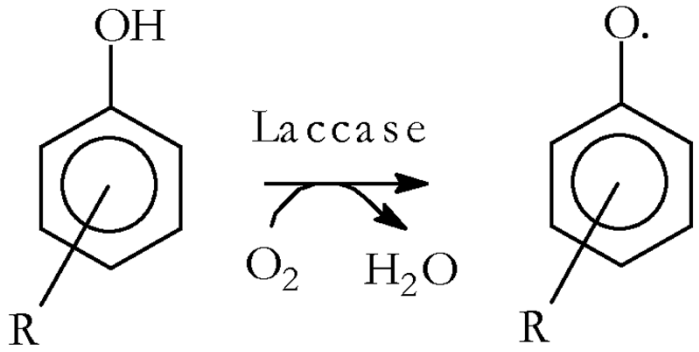


Redox Reactions

Laccases

Å Enzymatic radical chemistry

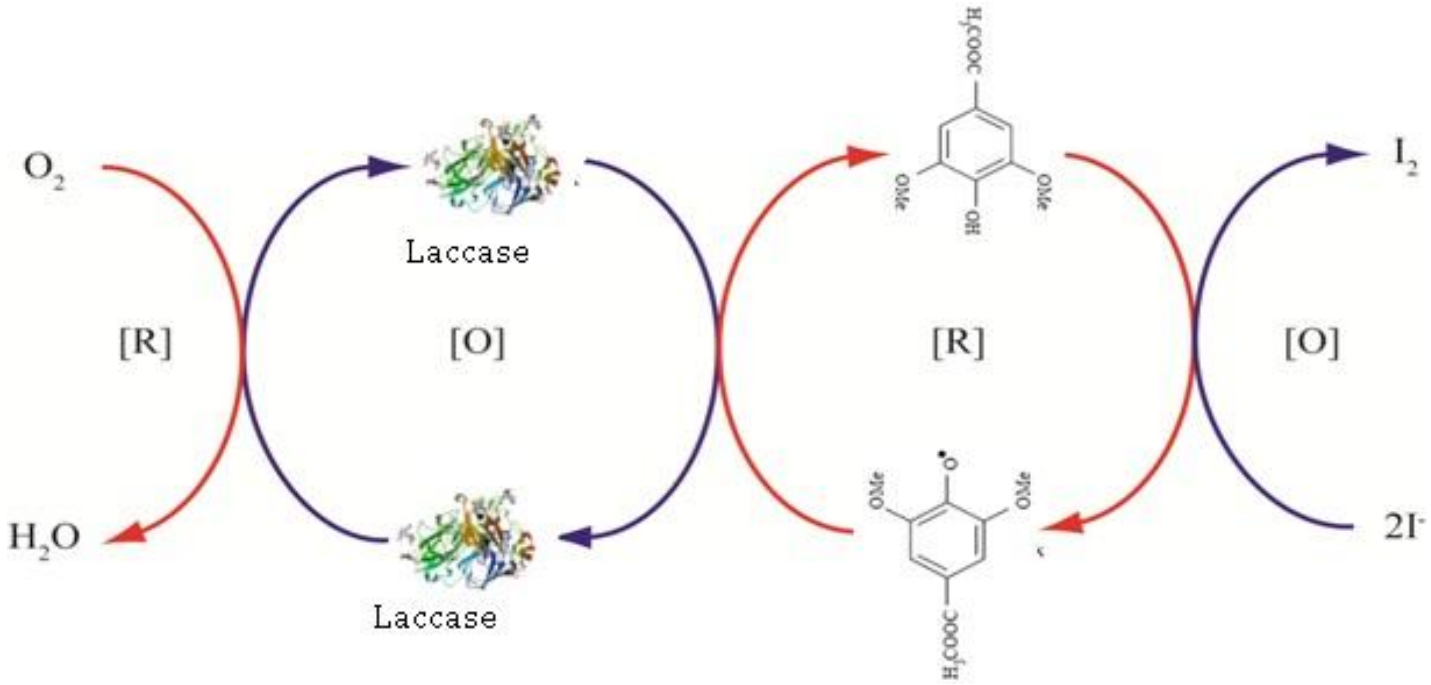




Redox Reactions

Laccases

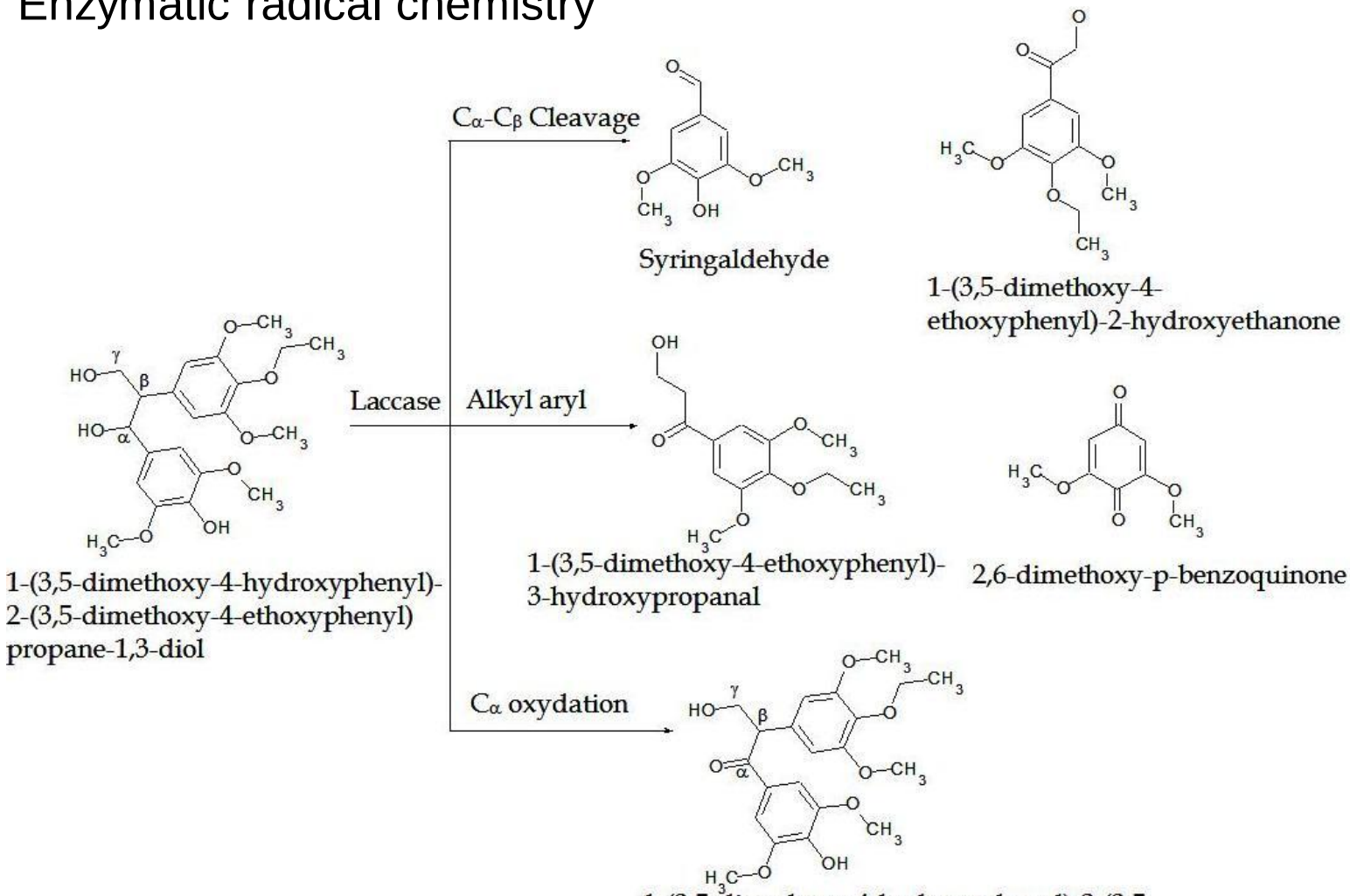
Å Enzymatic radical chemistry



Redox Reactions

Laccases

Å Enzymatic radical chemistry



Biocatalysis

Addition Reactions

- Lyases: addition of small molecules (H_2O, NH_3 etc.) across C=C or C=O

- Oxynitrilases ➟ cyano hydrin formation
- Fumarases ➟ water addition across activated double bonds
- Haloperoxidases ➟ halogenations / dehalogenations (no true lyases)

Biocatalysis

Addition Reactions

Å Oxynitrilases

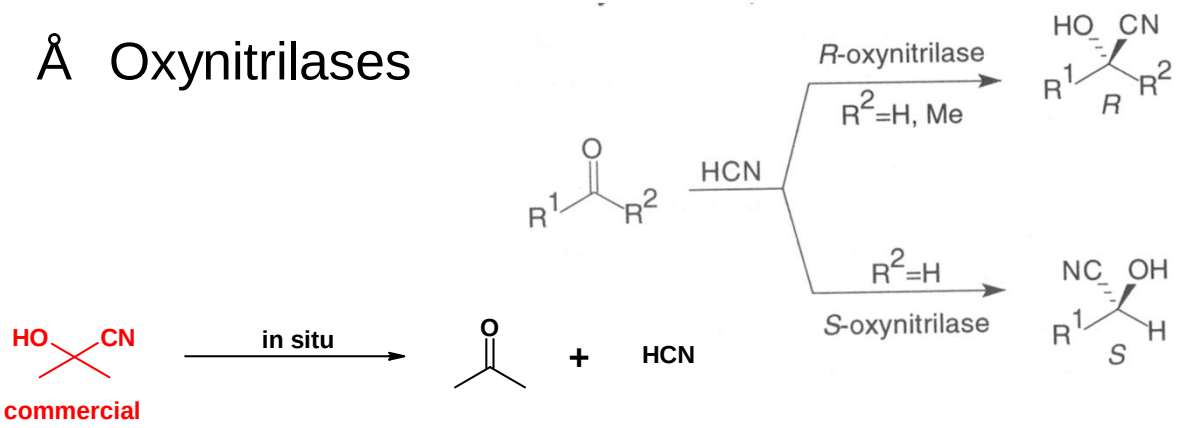


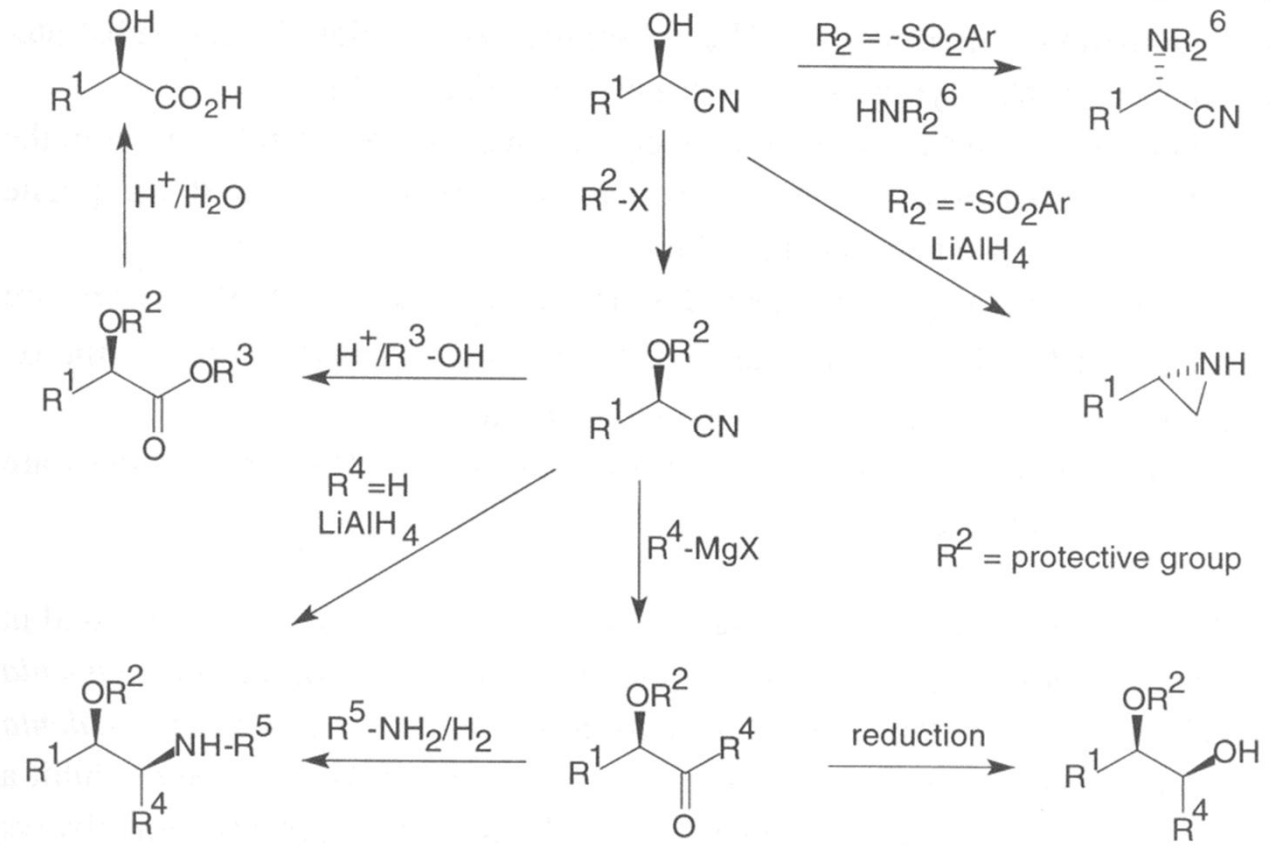
Table 14.7-1. Oxynitrilases available for organic synthesis.

Plant	Enzyme availability	Natural substrate	Substrate acceptance for syntheses	Stereo-selectivity
Prunus amygdalus	Almonds	(*R*)-Mandelonitrile	All R^1 and R^2	(*R*)
Linum usitatissimum	Flax seedlings overexpression	Acetone cyanohydrin (*R*)-2-Butanone cyanohydrin	Aliphatic aldehydes and ketones	(*R*)
Sorghum bicolor	Millet seedlings	(*S*)-4-Hydroxymandelonitrile	Aromatic aldehydes	(*S*)
Hevea brasiliensis	Rubber tree leaves overexpression	Acetone cyanohydrin	All R^1 and R^2	(*S*)
Manihot esculenta	Manioc leaves overexpression	Acetone cyanohydrin	All R^1 and R^2	(*S*)

Biocatalysis

Addition Reactions

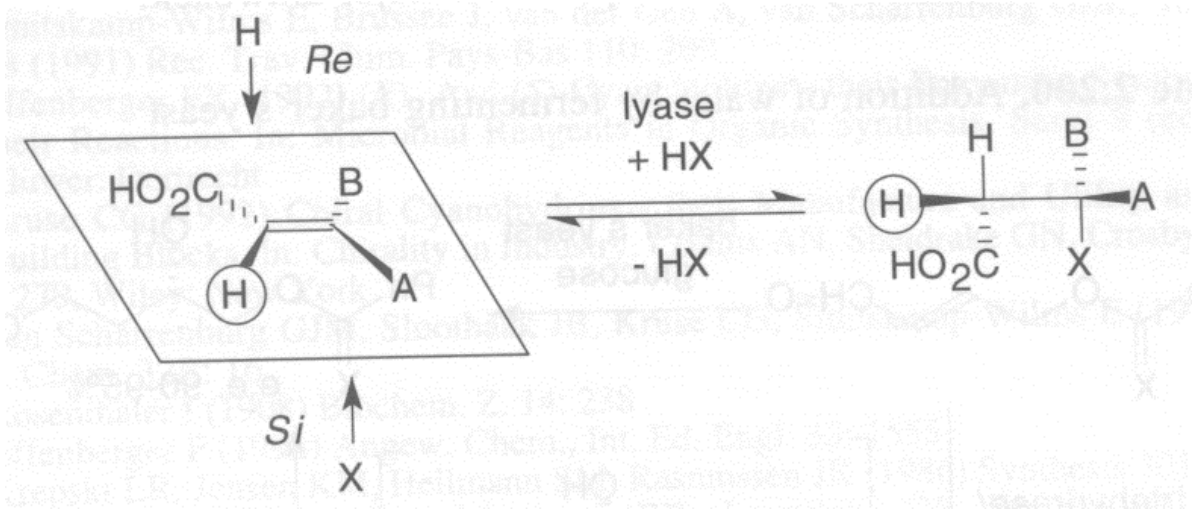
Å Oxynitrilases



Biocatalysis

Addition Reactions

Å Water/ammonia addition



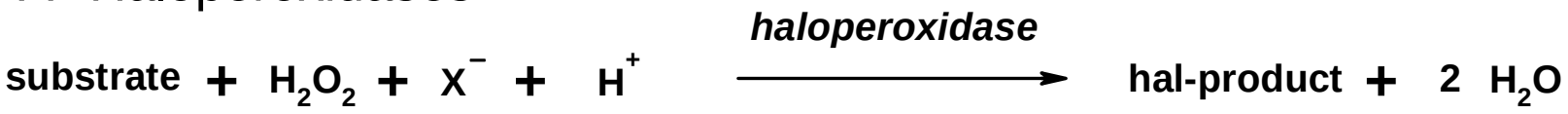
ï Activated double bond

ï anti-mechanism
nucleophile si-face, proton re-face

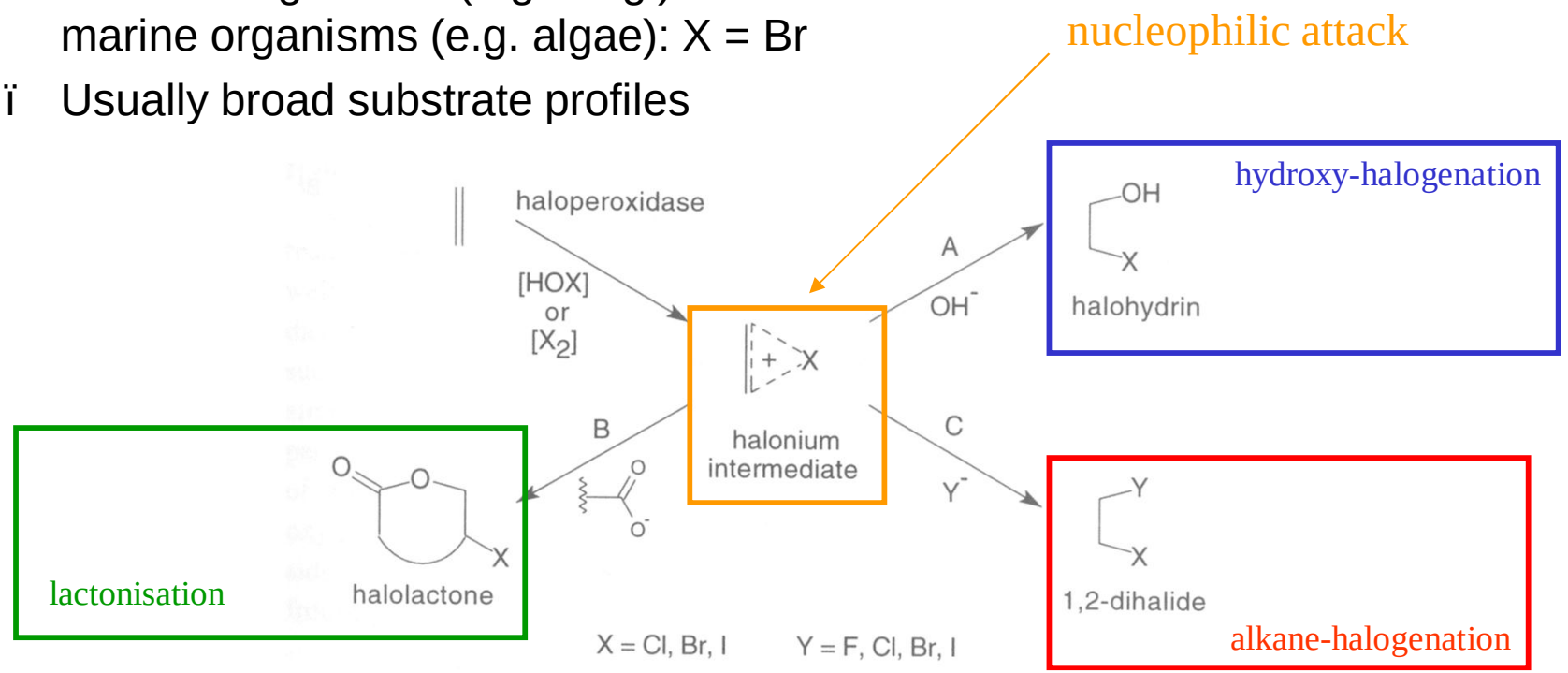
Biocatalysis

Addition Reactions

Å Haloperoxidases



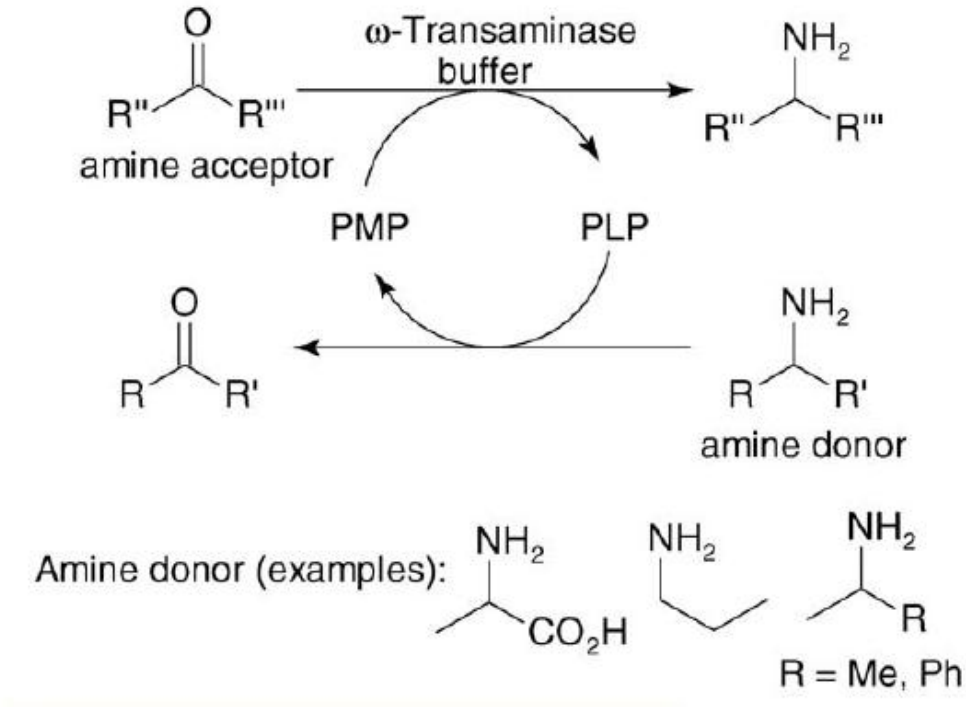
- ï No lyases
- ï Halide generates electrophilic halo species upon consumption of H_2O_2
- ï terrestrc organisms (e.g. fungi): X = Cl
  marine organisms (e.g. algae): X = Br
- ï Usually broad substrate profiles



Biocatalysis

Nitrogen Transfer

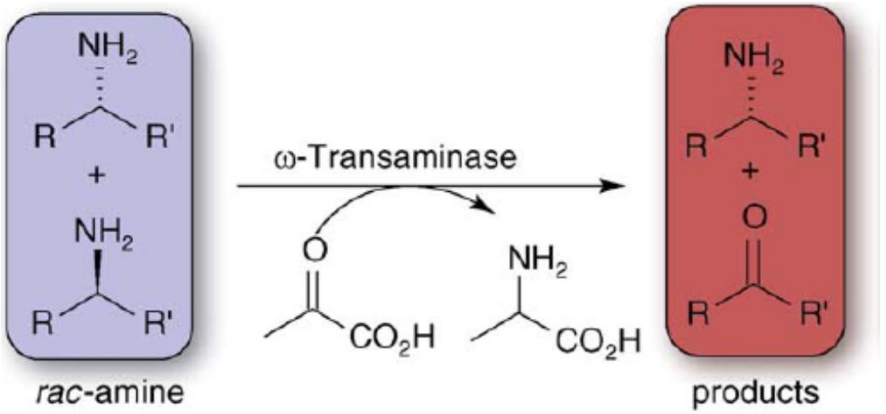
Å Transaminases



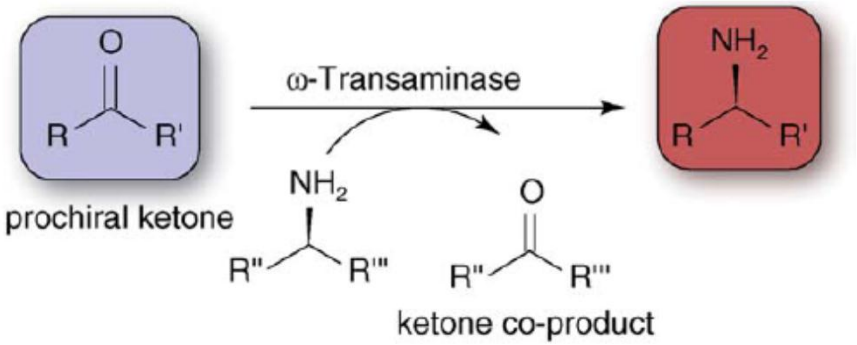
Biocatalysis

Nitrogen Transfer

- Transaminases
  - kinetic resolutions



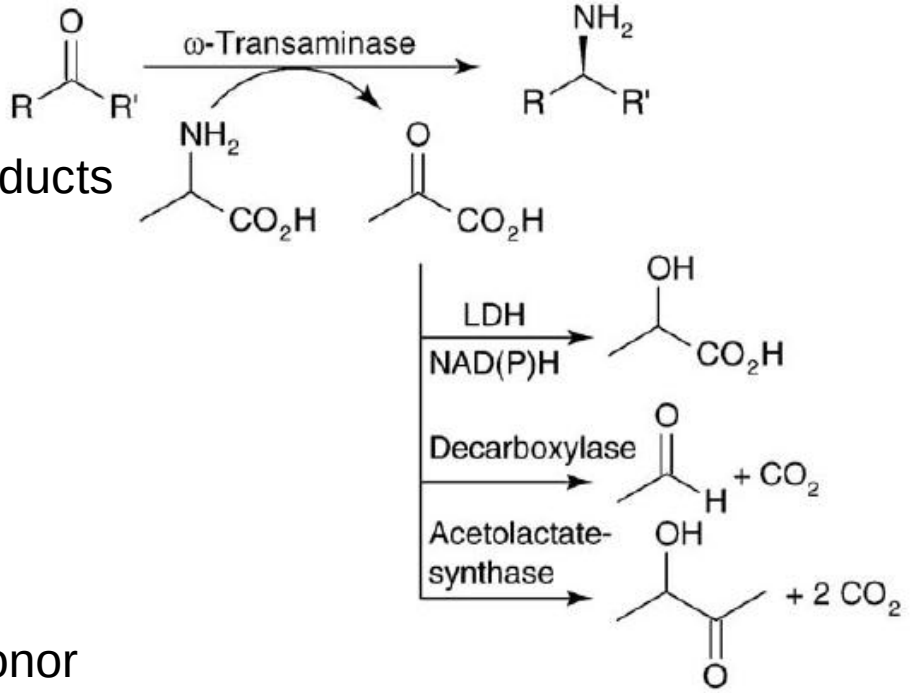
  - deracemizations



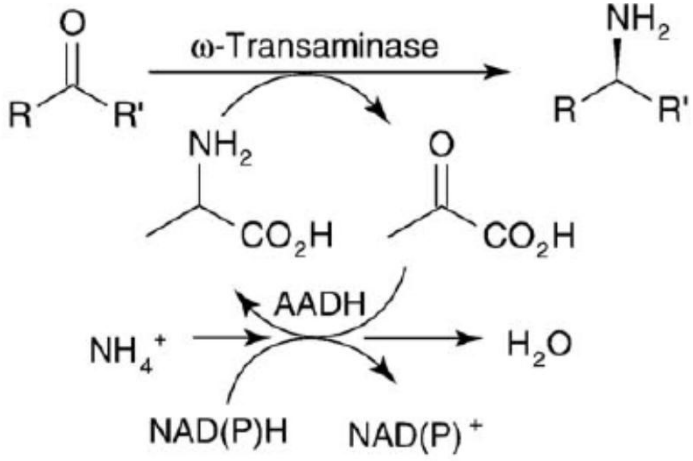
Biocatalysis

Nitrogen Transfer

- Transaminases
  - Degradation of co-products



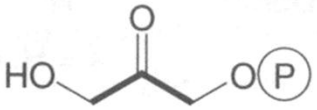
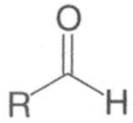
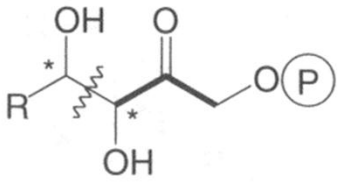
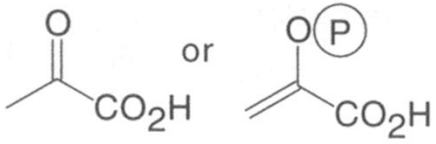
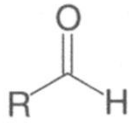
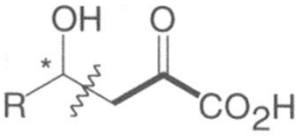
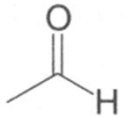
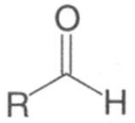
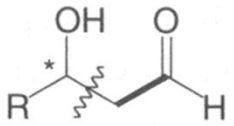
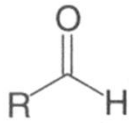
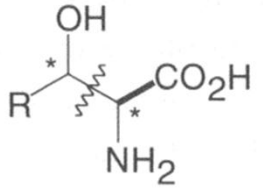
  - Recycling of amine-donor



Biocatalysis

Addition Reactions

Å Aldolases

Group	Donor (Nucleophile)	Acceptor (Electrophile)	Product
dihydroxyacetone phosphate (DHAP) dependent aldolases — I			
pyruvate dependent aldolases — II			
acetaldehyde dependent aldolases — III			
glycine dependent aldolases — IV			

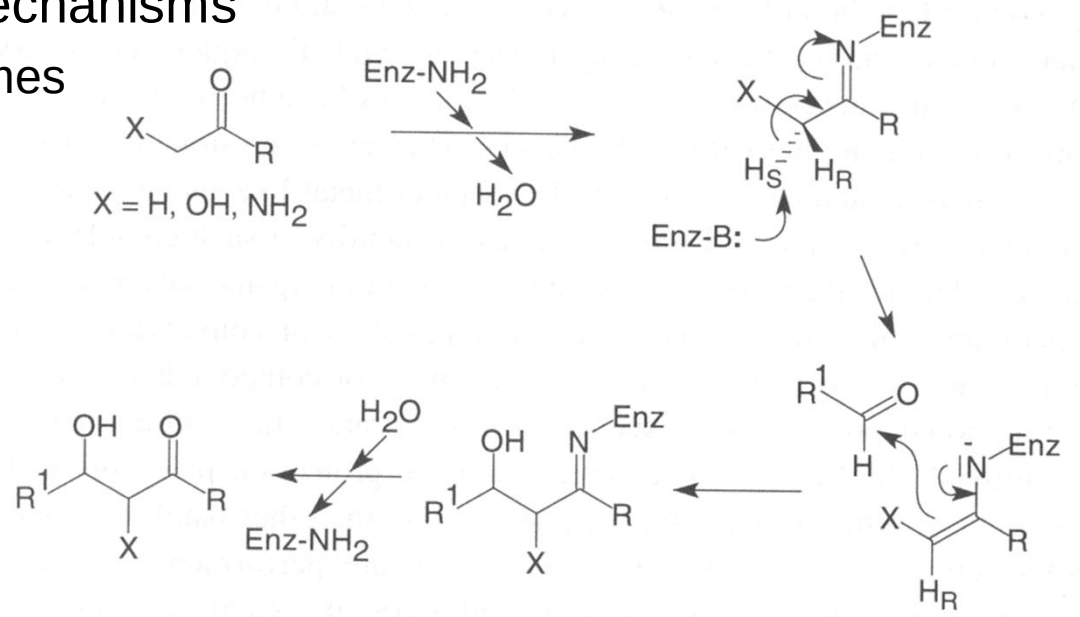
P = phosphate = new C-C bond * = newly formed stereocenter

Biocatalysis

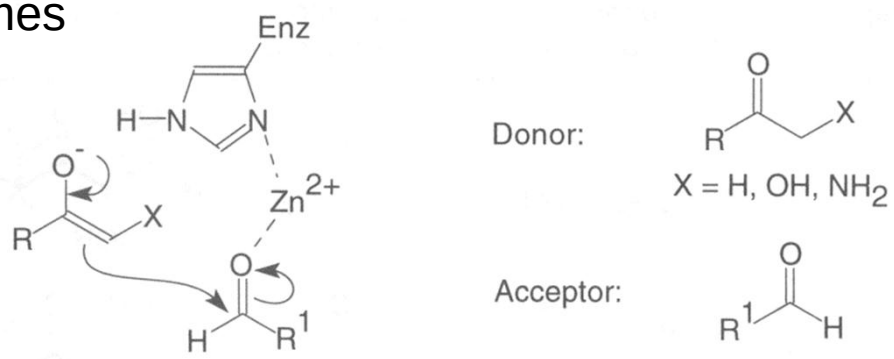
Addition Reactions

Å Aldolases ï mechanisms

ï Type I enzymes



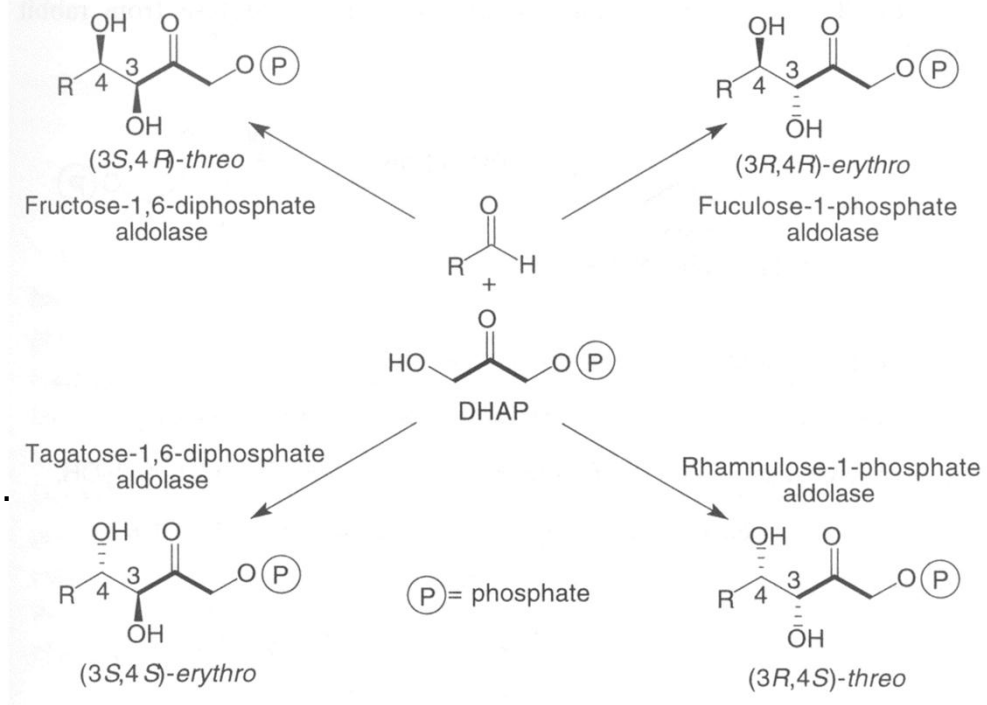
ï Type II enzymes



Biocatalysis

Addition Reactions

- Aldolasen
  - DHAP dependent aldolases



- best studied
- DHAP-donor
  - ketose-1-phosphate
- position 3 highly selective
- Pposition 4 slightly lower sel.
- complementary enzymes available

- overexperession systems for various enzymes available (otherwise difficult to isolate)
- phosphate group can be utilized upon product isolation via ion chromatography

Biocatalysis

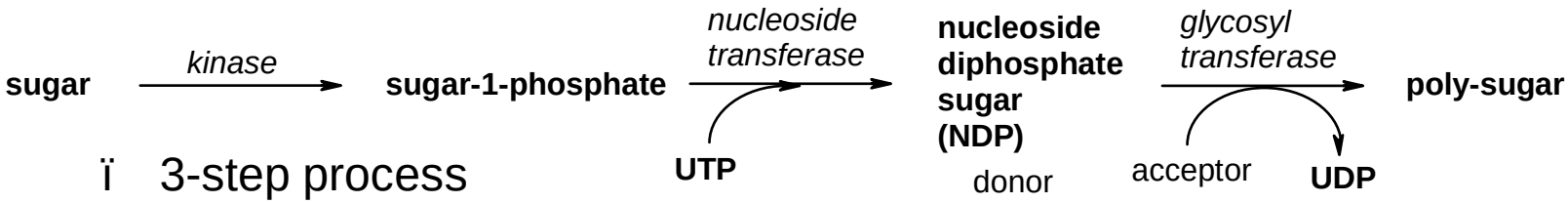
Glycosyl Transfer

- Synthesis of complex oligosaccharides
  - conventional: protecting group chemistry
- Gycosyl Transferases:
  - biosynthesis of oligosaccharides
  - activation of sugars by phosphorylation mono-/diphosphate at anomeric center
  - high specificity for substrate
  - high specificity for type of glycosidic bond
- Glucosidases:
  - hydrolytic sugar degradation ➠ low selectivity
  - production of mono-/oligosaccharides from polymers
  - glycolysis & glycogenesis in all organisms

Biocatalysis

Glycosyl Transfer

- Synthesis of oligosaccharides



  - 3-step process
    - phosphorylation by *kinase*
    - introduction of leaving group (NTP) by *nucleoside transferase* ➟ Donor
    - condensation with acceptor (mono-/oligosugar, protein, lipid) by *glyoxyl transferase*
  - high substrate specificity ➟ many enzymes in organism
    (100+ biocatalysts identified)
  - Problems
    - availability of sugar-1-phosphates
      solved by recombinant kinases
    - availability of glycosyltransferases
      unstable membrane bound multi-domain enzymes, low in-vivo conc.
      solved by recombinant cloning
    - inhibition by UDP
      ➟ in-situ recycling of UDP (conc. needs to be low)

Biocatalysis

Glycosyl Transfer

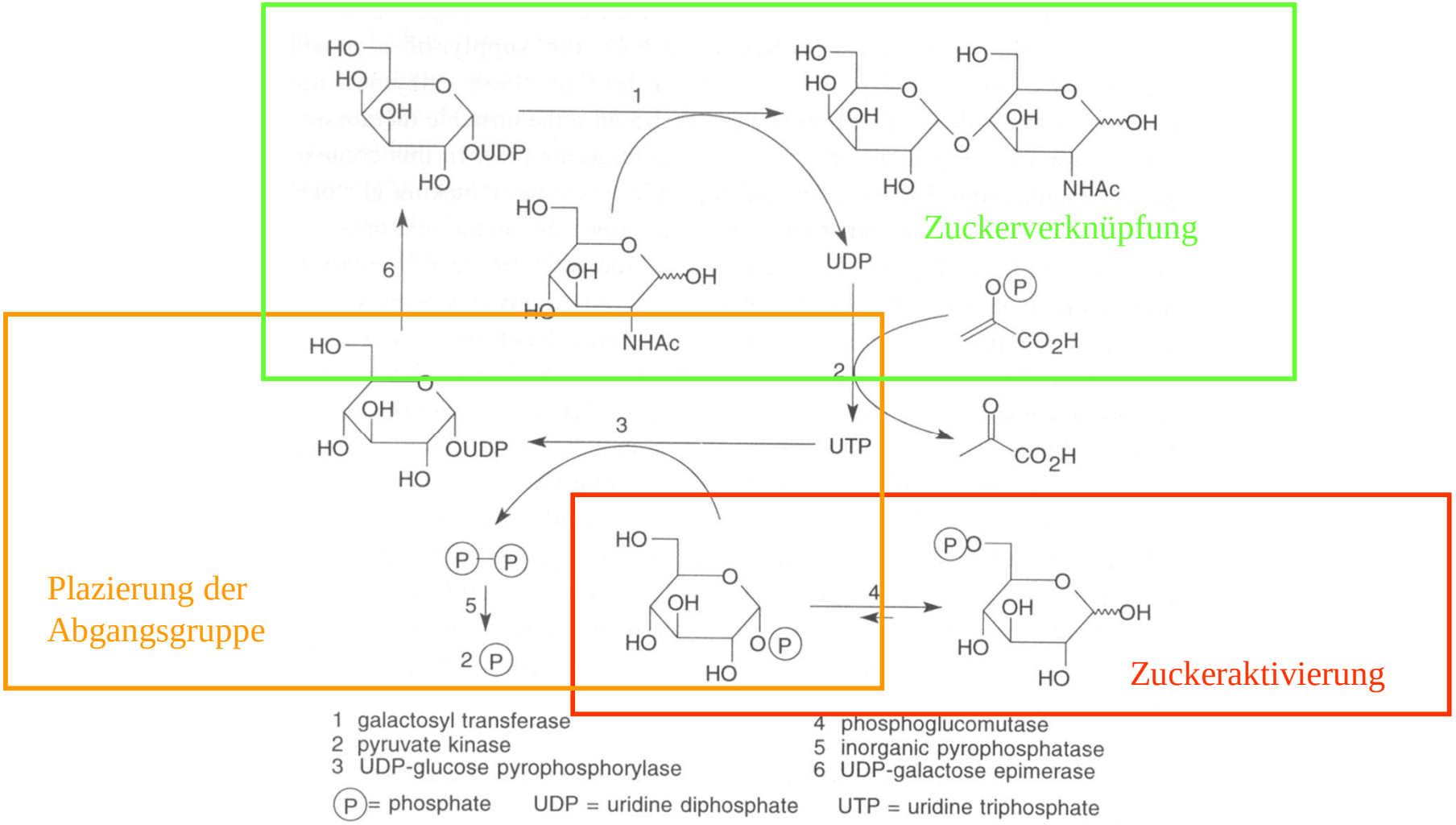
- UDP-Galactosyl (UDP-Gal) Transferase
  - best studied enzyme
  - 1-4 connection
  - donor highly specific Gal
  - acceptor specificity more promiscuous

Acceptor	Product
Glc-OH	β-Gal-(1→4)-Glc-OH
GlcNAc-OH	β-Gal-(1→4)-GlcNAc-OH
β-GlcNAc-(1→4)-Gal-OH	β-Gal-(1→4)-β-GlcNAc-(1→4)-Gal-OH
β-GlcNAc-(1→6)-Gal-OH	β-Gal-(1→4)-β-GlcNAc-(1→6)-Gal-OH
β-GlcNAc-(1→3)-Gal-OH	β-Gal-(1→4)-β-GlcNAc-(1→3)-Gal-OH

Biocatalysis

Glycosyl Transfer

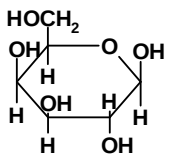
Å N-Acetyllactosamine Production



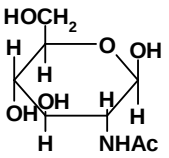
Biocatalysis

Glycosyl Transfer

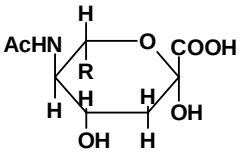
- Mixed-culture approach
  - several (recomb.) strains in bioreactor
  - detergents for material transfer
  - example: sialic acid formation



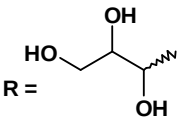
β-Gal



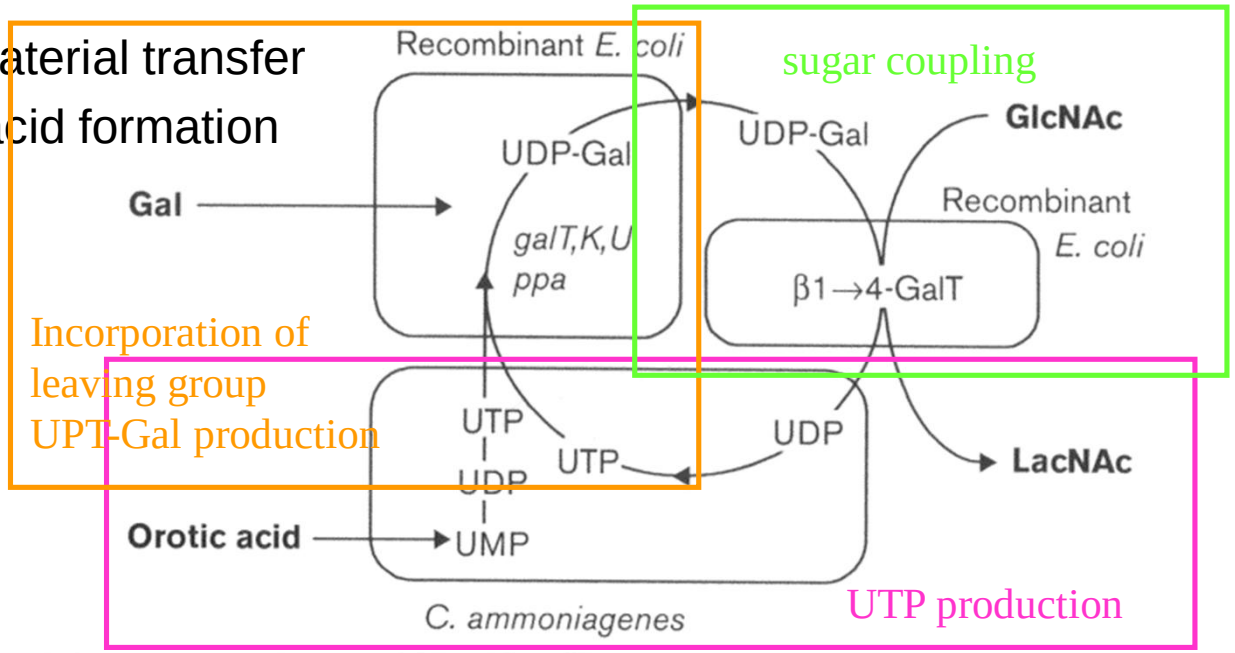
β-GlcNAc

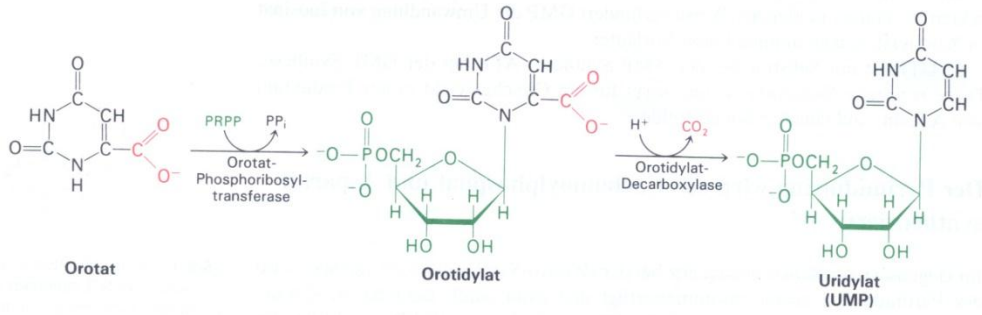


NeuAc; Sia
Sialic acid



- batch process: 123g/L

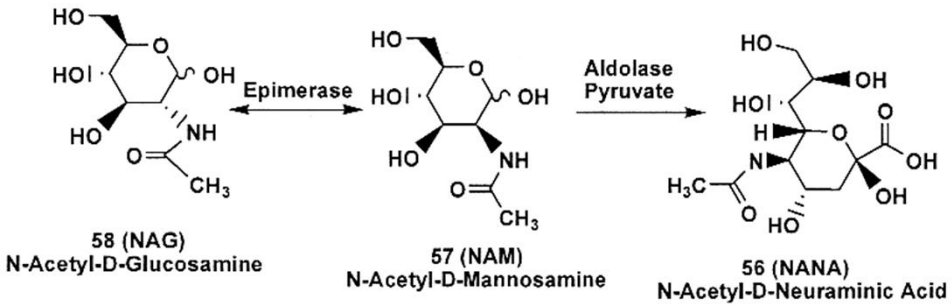




Biocatalysis

Applications in Medicinal Chemistry

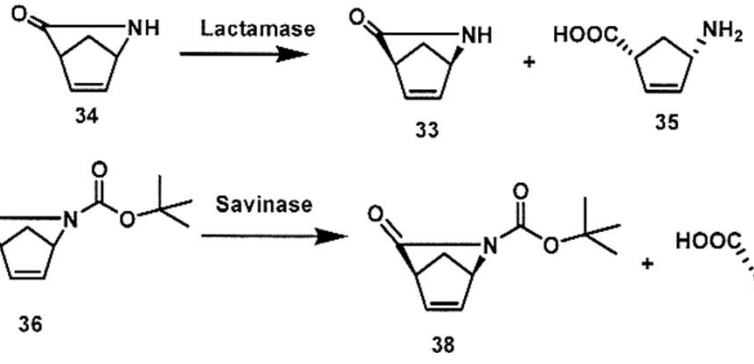
Å Anitvirals



58 (NAG)
N-Acetyl-D-Glucosamine

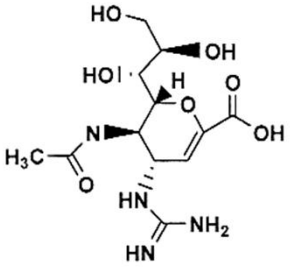
57 (NAM)
N-Acetyl-D-Mannosamine

56 (NANA)
N-Acetyl-D-Neuraminic Acid

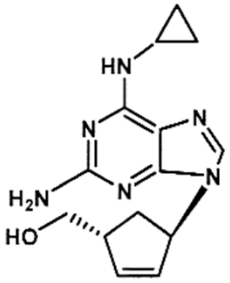


34 33 35

36 38 37



55
Relenza



Abacavir (32)

Biocatalysis

Applications

Å Cytostatics

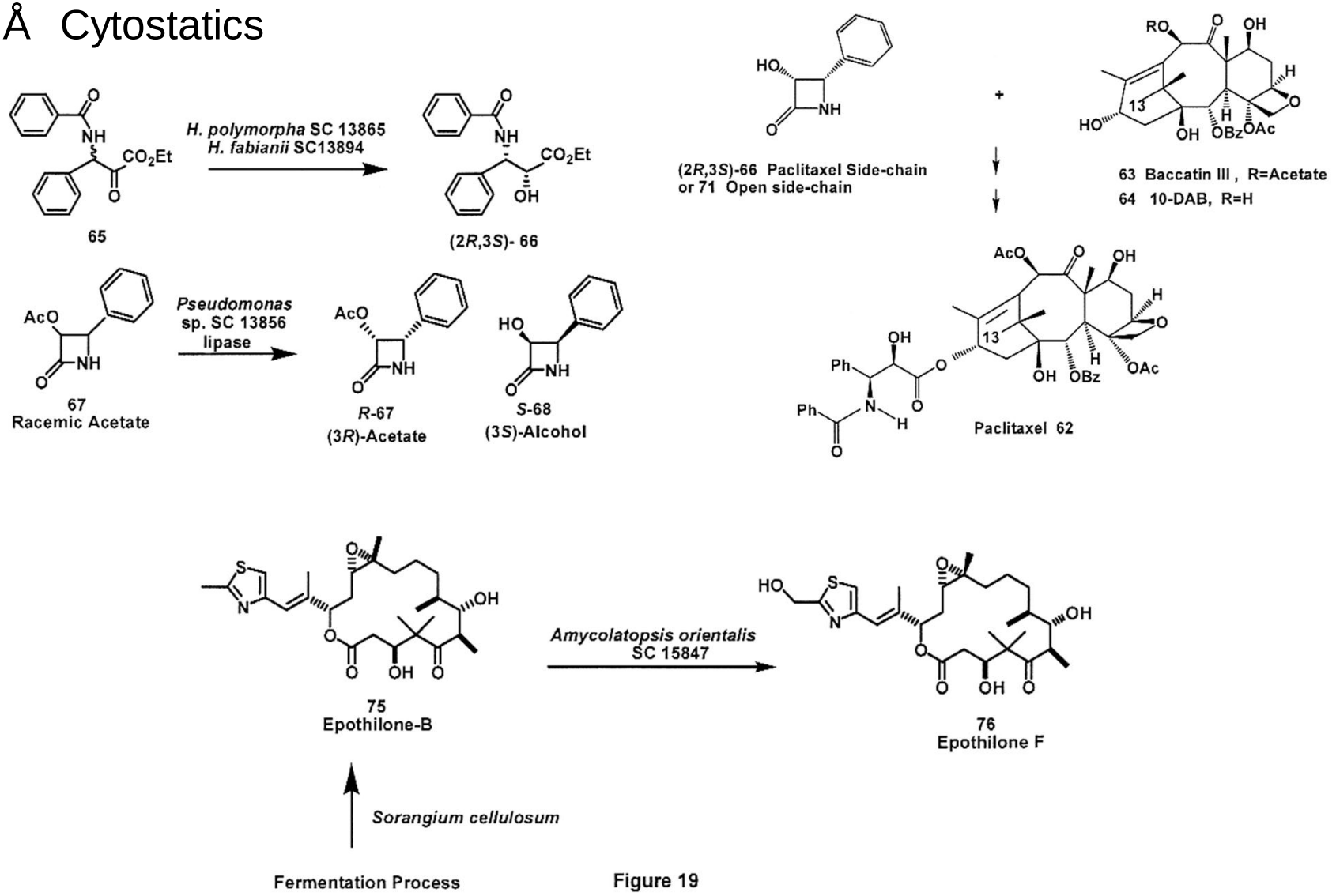


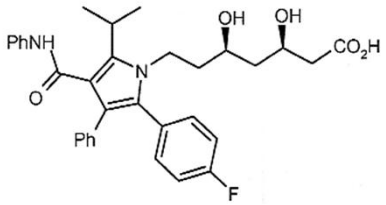
Figure 19

Biocatalysis

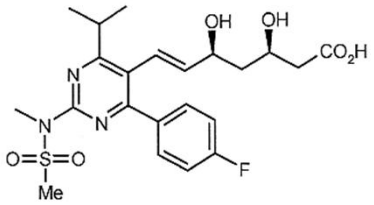
Applications

Å Lipitor

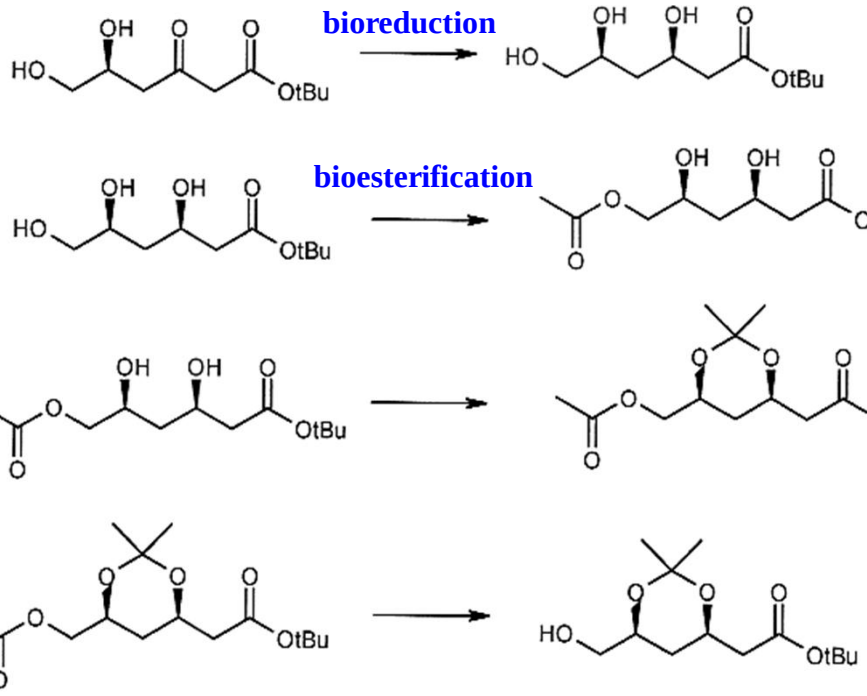
Third Generation: Synthetic HMG-CoA Reductase Inhibitors

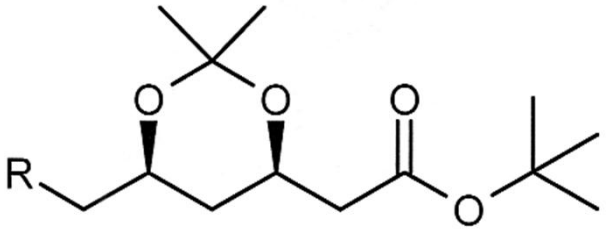


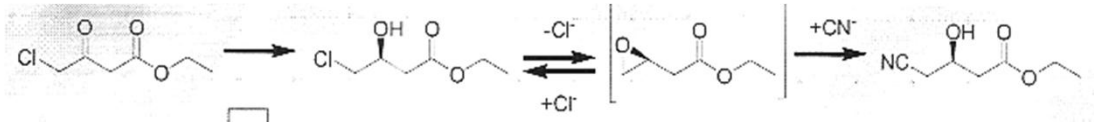
Atorvastatin



Rosuvastatin







Biocatalysis

Applications

- Lipitor