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Biocatalysis Engineering: the Big Picture

A Tutorial Review

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Biocatalysis Engineering: the Big Picture

1. Introduction: Green , Sustainable Biocatalysis
2. Substrate Engineering
3. Medium Engineering
4. Protein Engineering
5. Biocatalyst Engineering
6. Biocatalytic Cascade Processes: Cell-free Synthetic Biology
7. Reactor Engineering & Downstream Processing
8. Conclusions & Prospects

Two Types of Biotransformations

- Free enzymes
  - isolated (purified)
  - whole cells (not growing)
  - can be very high STY

- Fermentations (growing microbial cells)
  - less expensive (no enzyme isolation needed)
  - often dilute solution / low STY
  - water footprint /energy intensive
  - byproducts from enzyme impurities

Biocatalysis is Green & Sustainable

- **Enzymes are derived from renewable resources and are biodegradable (even edible sometimes)**
- **Avoids use of (and product contamination by) scarce precious metals**
- Mild conditions: ambient T & P
- High rates & highly specific : substrate, chemo-, regio-, and enantiospecific
- Higher quality product
- No special equipment needed

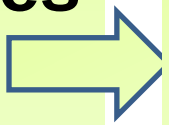
Improved Yields, Less Waste,
Better Quality, Reduced Costs

Biocatalysis & Green Chemistry

Green Chemistry Principle	Biocatalysis
1. Waste prevention	Significantly reduced waste
2. Atom economy	More atom and step economic
3. Less hazardous syntheses	Generally low toxicity
4. Design for safer products	Not relevant
5. Safer solvents and auxilaries	Usually performed in water
6. Energy efficient	Mild conditions
7. Renewable feedstocks	Enzymes are renewable
8. Step economy	No protection / deprotection
9. Catalysis	Enzymes are catalysts
10. Design for Degradation	Enzymes are biodegradable
11. Analytical Methodologies	Applicable to biocatalysis
12. Inherently safer processes	Mild and safe conditions

Biocatalysis : why now ?

1. Genome sequencing (> 20000 bacterial genomes sequenced) (more enzymes)
2. Directed evolution technologies (better enzymes)
3. Recombinant DNA technology (better production)
4. Immobilization technologies (better formulation)



Lower Costs & Shorter Time

A Revelation for Organic Chemists : Enzymes can Function in Organic Media

Abstract

"Porcine pancreatic lipase catalyzes the transesterification reaction between tributyrin and various primary and secondary alcohols in a 99 percent organic medium. Upon further dehydration, the enzyme becomes extremely thermostable. Not only can the dry lipase withstand heating at 100°C for many hours, but it exhibits a high catalytic activity at that temperature."

A. Zaks, A. M. Klibanov, *Science*, 1984, 224, 1249-1251

The Importance of Stereoisomerism in Drug Action (1980s)

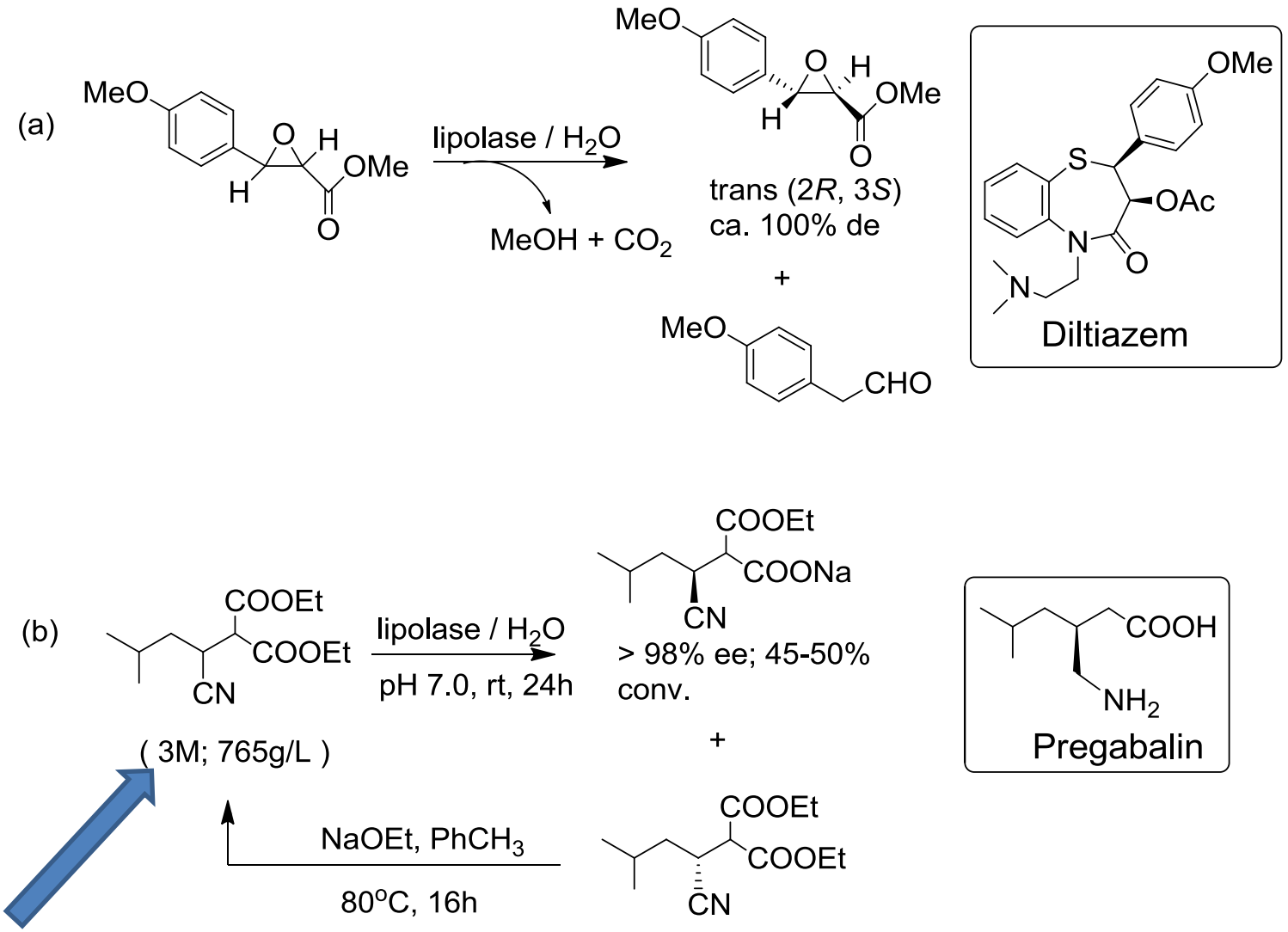
The remarkable discrepancy between, on the one hand, the high degree of purity required for pharmaceuticals and, on the other hand, the acceptance of 50% impurity, as long as isomeric ballast is involved, should be a matter for serious concern

E.J.Ariens, 1986

- Led to new FDA regulations requiring testing of both enantiomers of chiral drugs
- Created a need for cost-effective enantioselective synthesis (e.g. biocatalytic methods)

R. A. Sheldon, Chirotechnology: the Industrial Synthesis of Optically Active Compounds

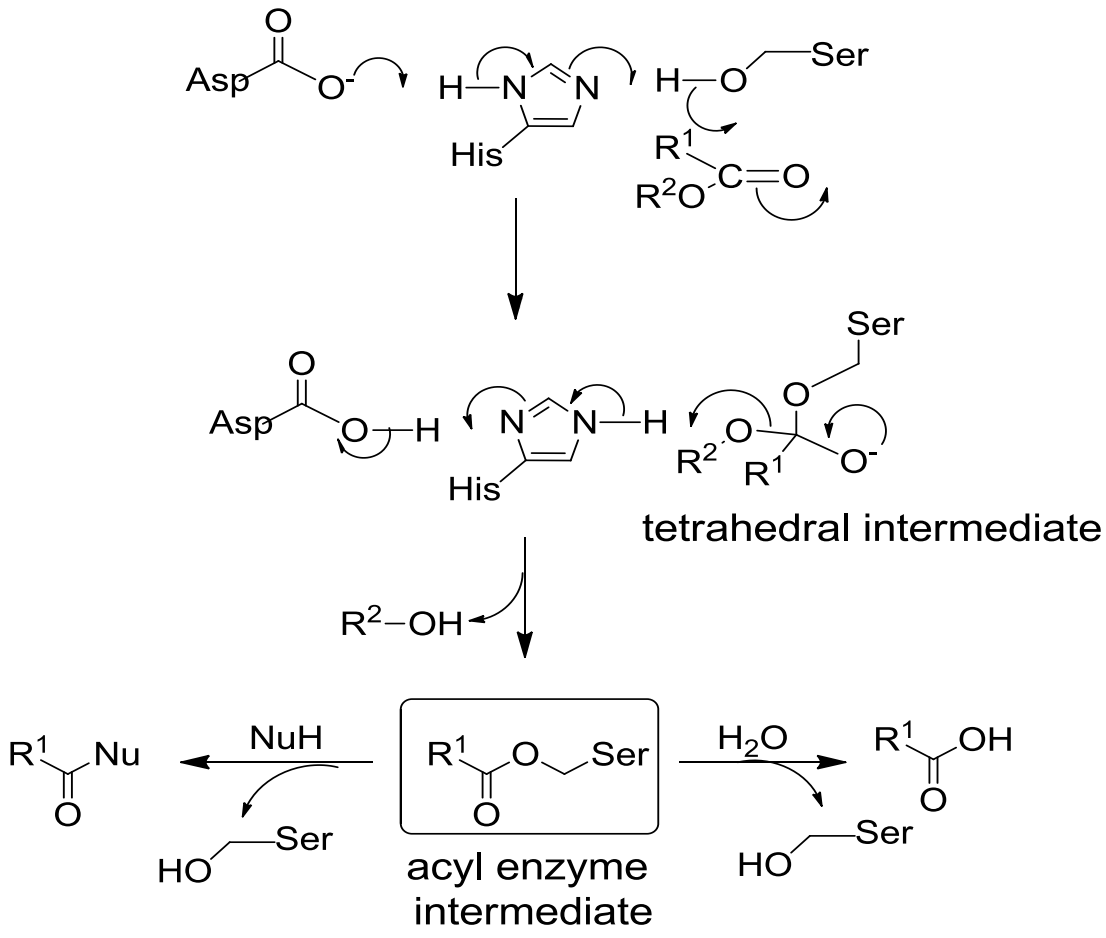
Successful Biocatalytic Processes with Commercial Enzymes



Substrate Engineering
&
Enzyme Promiscuity

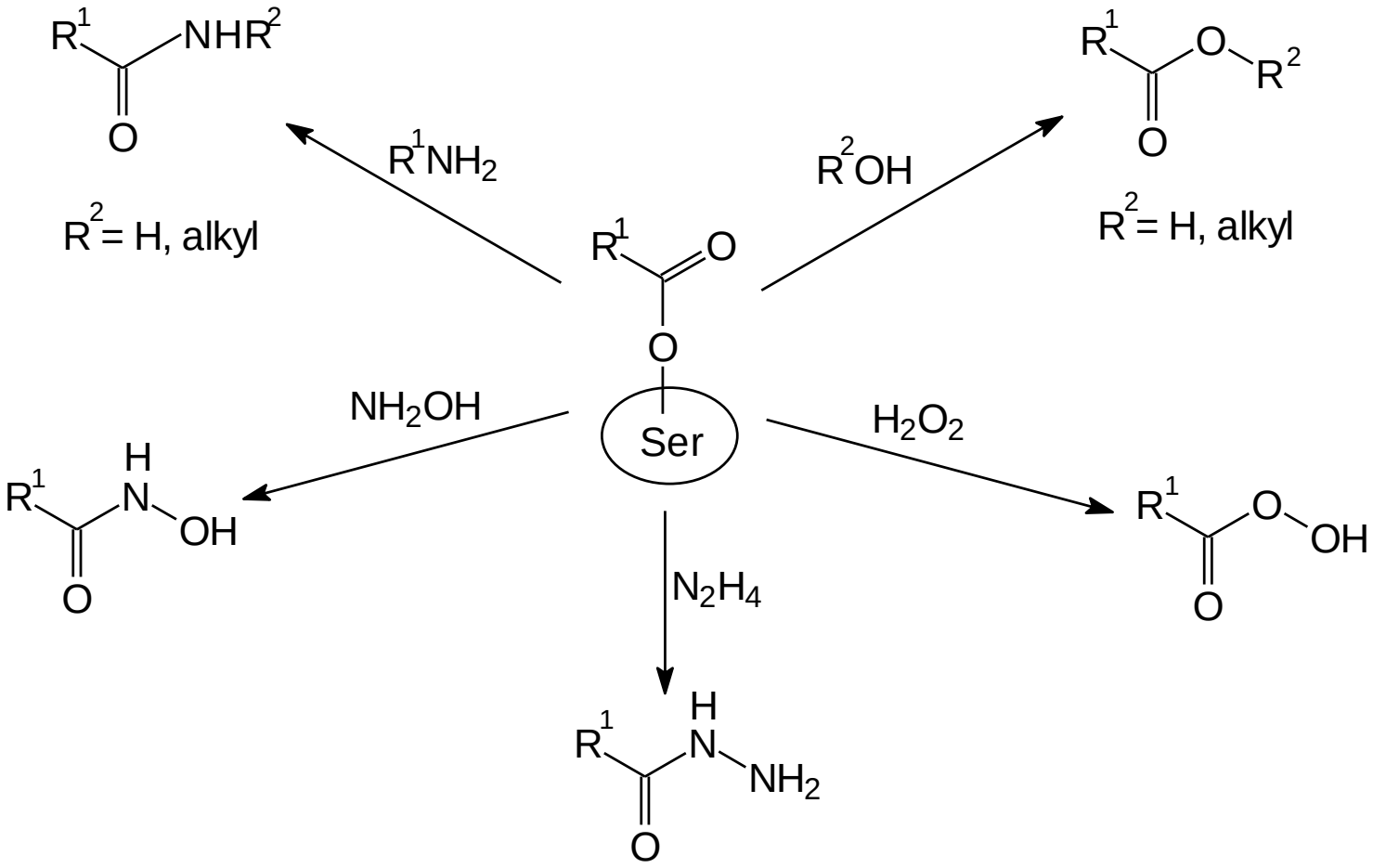
Serine Protease Mechanism

The catalytic triad (Ser /His /Asp) and the electron relay mechanism



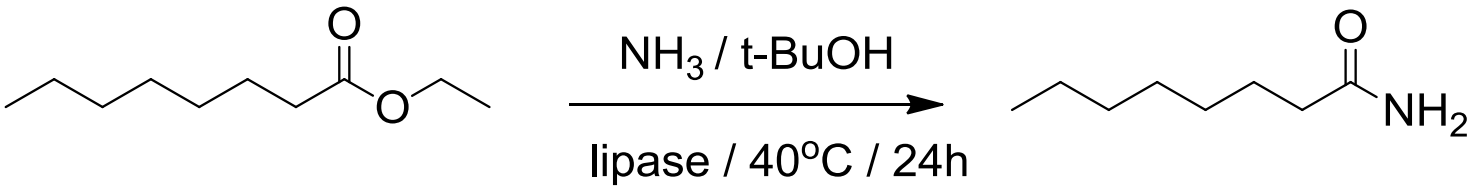
NuH = ROH, H_2O_2, RNH_2, NH_3, NH_2OH, N_2H_4

Lipases: Acyl Acceptors



de Zoete, van Rantwijk , Sheldon, *Catalysis Today,* 22, 563 (1994)

Enzymatic Ester Ammoniolysis (1)

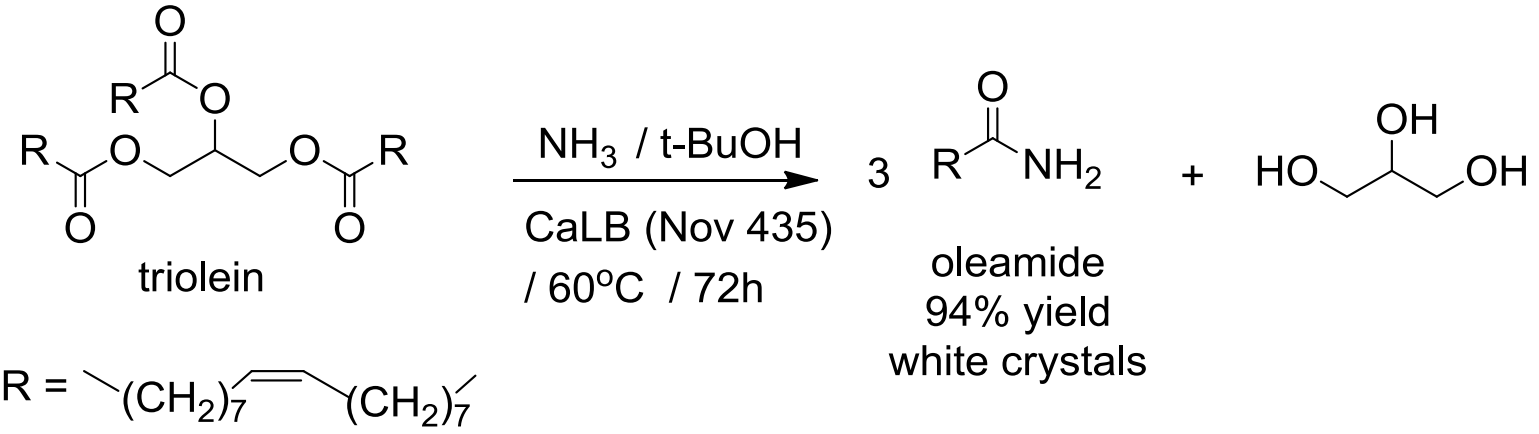


Lipase	Yield (%)	
	5 eq. NH_3	1 eq. NH_3
Candida antarctica B	95	43
Thermomyces lanuginosus	85	38

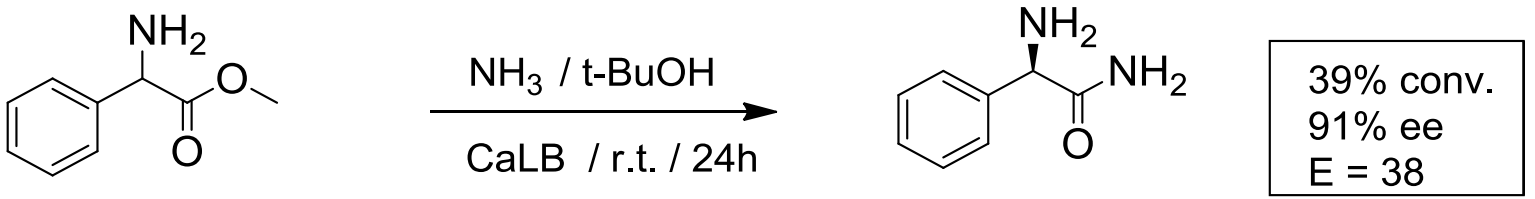
- **Mild alternative for amide synthesis**
- **Enantioselective**
- **Also with amino acids**

M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwijk, R. A. Sheldon, *Chem. Commun*., 1993, 1831-1831.

Enzymatic Ester Ammoniolysis (2)



M.C. de Zoete, A.C. Kock-van Dalen, F. van Rantwijk, R.A. Sheldon, *J. Mol. Catal. B: Enzymatic* 1996, **1**, 109-113.

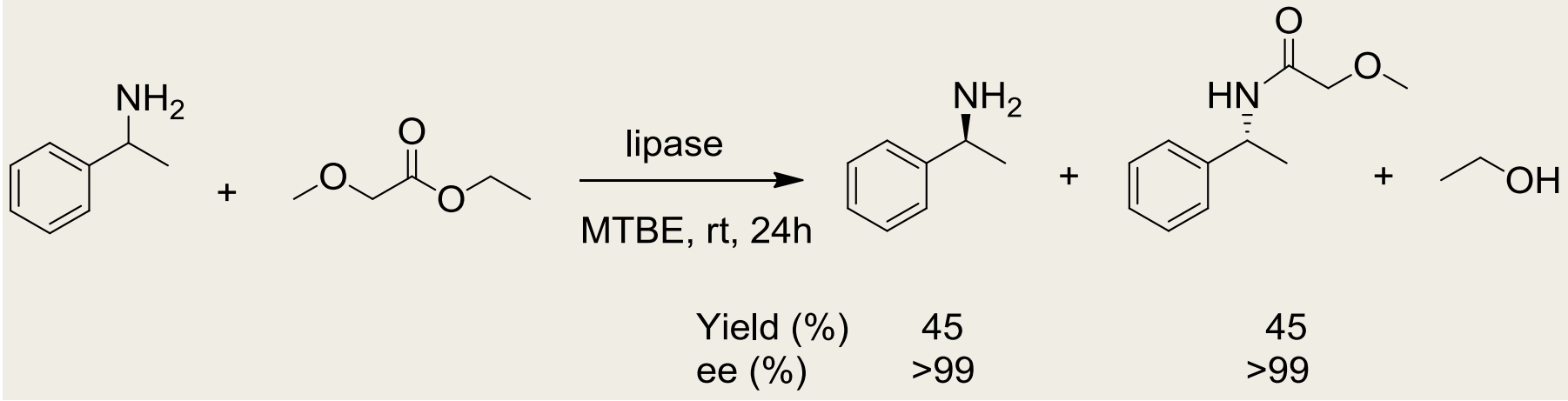


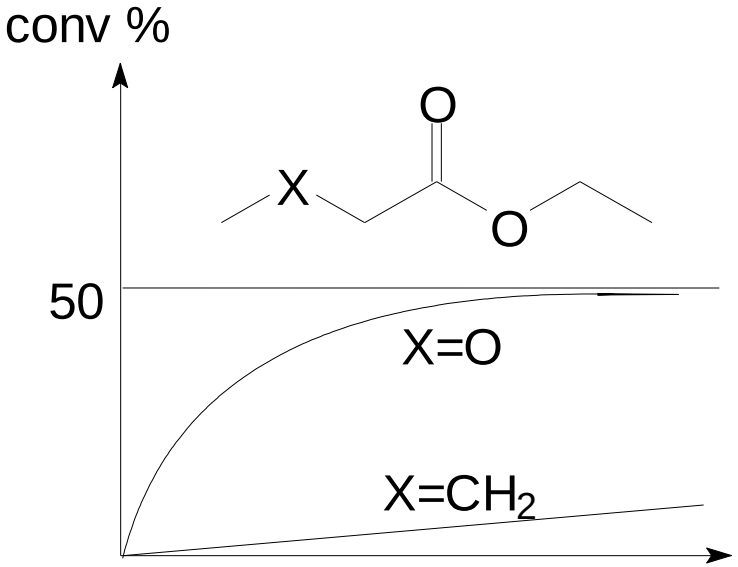
(*R*) - phenylglycine amide

M.C. de Zoete, A.A. Ouwehand, F. van Rantwijk, R.A. Sheldon, *Recl. Trav. Chim. Pays-Bas* 1995, **114** 171-174.

Resolution of Amines: BASF

Laboratory curiosity to commercial process

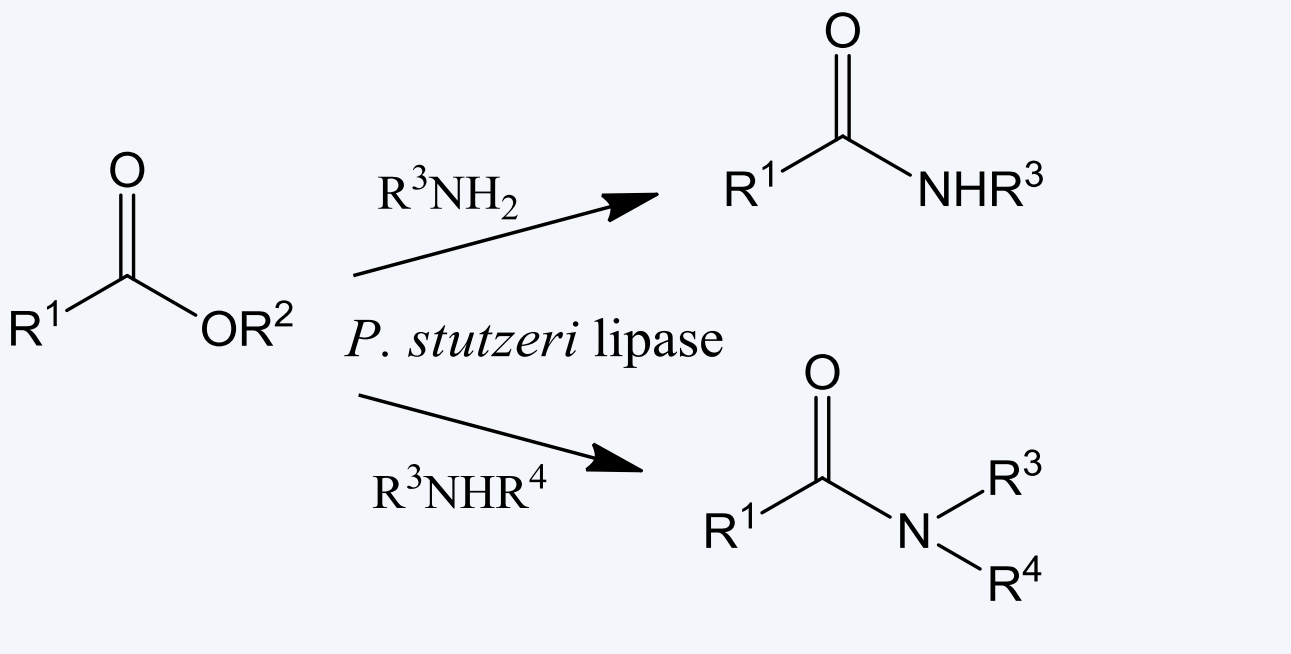




- **Engineering acyl donor**
- Separation by distillation
- Can be performed solvent-free over lipase column

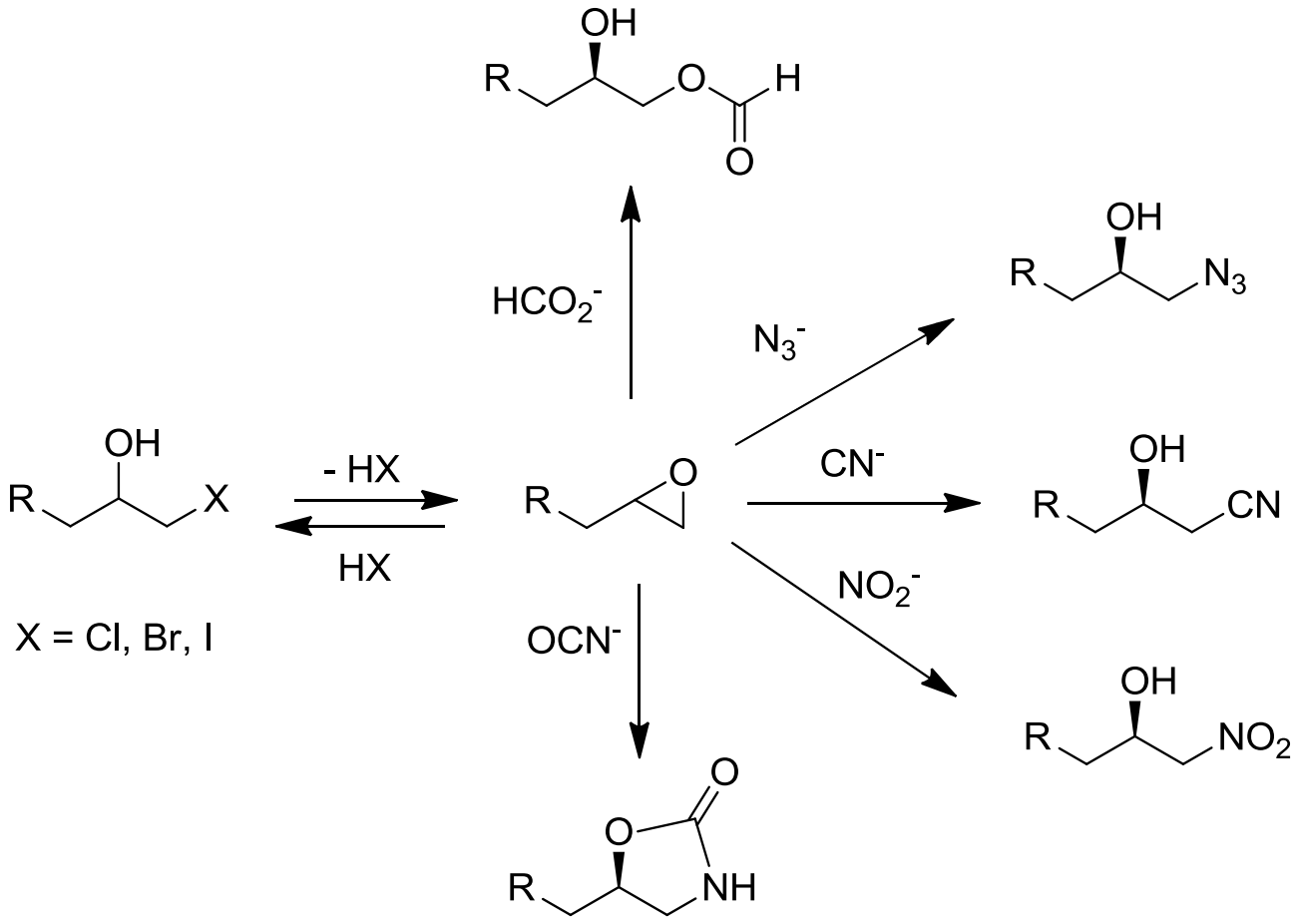
F. Balkenhohl, K. Ditrich, B. Hauer, W. Ladner, *Prakt. Chem*. 1997, 339, 381.

Broadening the Scope of Enzymatic Amidation



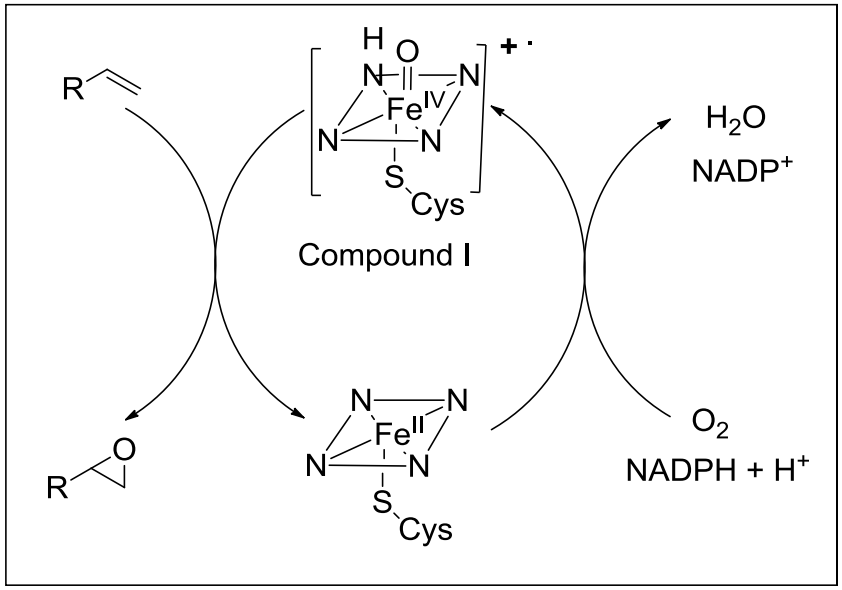
S. van Pelt, R. L. M. Teeuwen,M. H. A. Janssen, R.A. Sheldon, P. J. Dunn, R. M. R. Howard, R. Kumar, I. Martínez, J. W. Wong, *Green Chem*. 2011, 13, 1791-1798

Epoxide Ring-Opening Reactions Catalysed by Halohydrin Dehalogenase (HHDH)

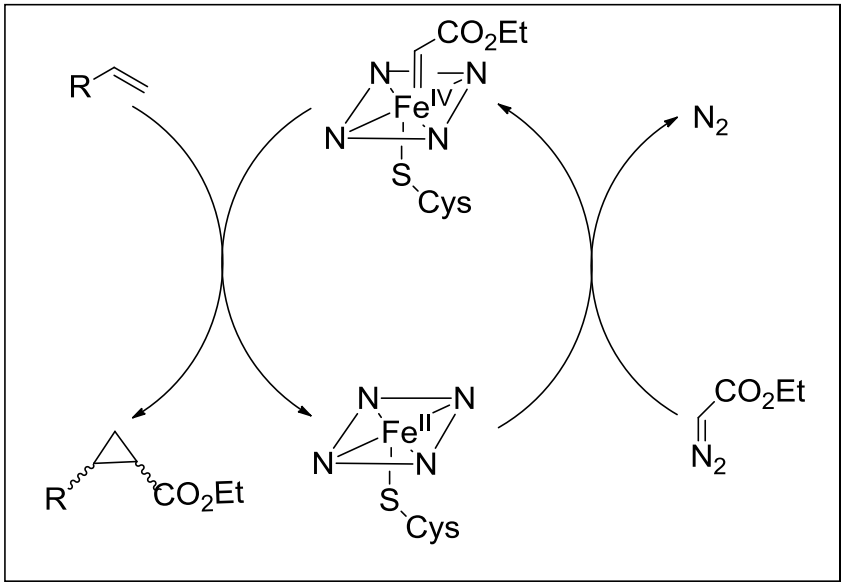


G. Hasnaoui-Dijoux, M. Majeric´ Elenkov, J. H. Lutje Spelberg, B. Hauer, D. B. Janssen, *ChemBioChem* 2008, **9**, 1048 – 1051.

Enzymatic Cyclopropanation via P450 monooxygenase Catalysed Carbene Transfer



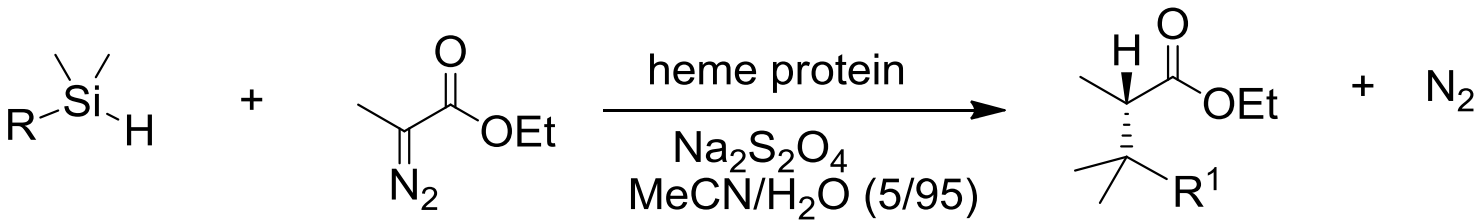
Oxene transfer (epoxidation)

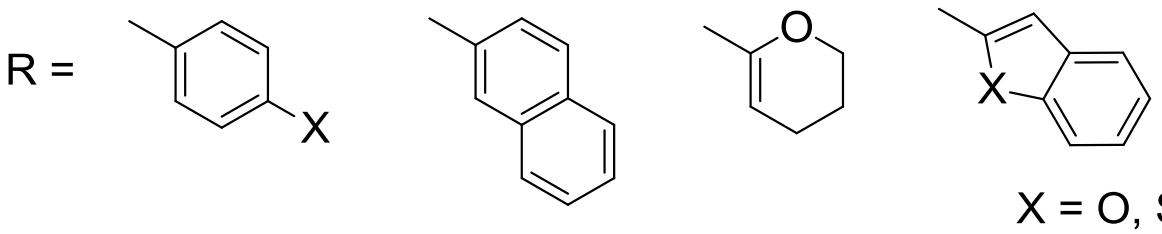


Carbene transfer (cyclopropanation)

P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, *Science,* 2013, ***339***, 307-310

Enzymatic C-Si bond Formation via Carbene Insertion in Si-H Bonds

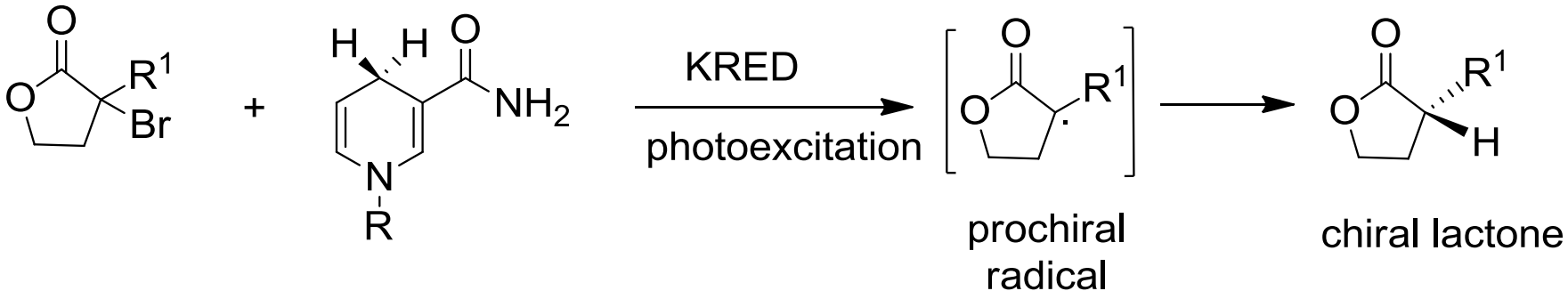




e.g. cytochrome C enhanced with directed evolution

S. B. J. Kan, R. D. Lewis, K.Chen, F. H. Arnold, *Science 2016, 354, 1048-1051.*

Accessing Non-natural Reactions of Nicotinamide Dependent Enzymes by Irradiating with Light



M. A. Emmanuel, N. R. Greenberg, D. G. Oblinsky, T. K. Hyster, Nature 2016, 540, 414-417

See also: U. T. Bornscheuer, Nature, 2016, 540, 345-346

Medium Engineering

Organic Solvents

Benefits:

- Many organic compounds sparingly soluble in water
- Some reactions, e.g. (transesterifications, amidations) not possible in water
- Easier product recovery
- Elimination of microbial contamination

But:

- Catalytic efficiencies generally two orders of magnitude lower
- Environmental issues with many volatile organic solvents (VOCs)
- Enzymes denature in polar aprotic solvents

N.B. Biocatalytic reactions can be performed, in aqueous /organic biphasic system. Substrate / product in organic phase & enzyme in aqueous phase

A. Zaks, A. M. Klibanov, *Science*, 1984, 224, 1249-1251

Non-conventional Reaction Media (1) Supercritical carbon dioxide ($scCO_2$)

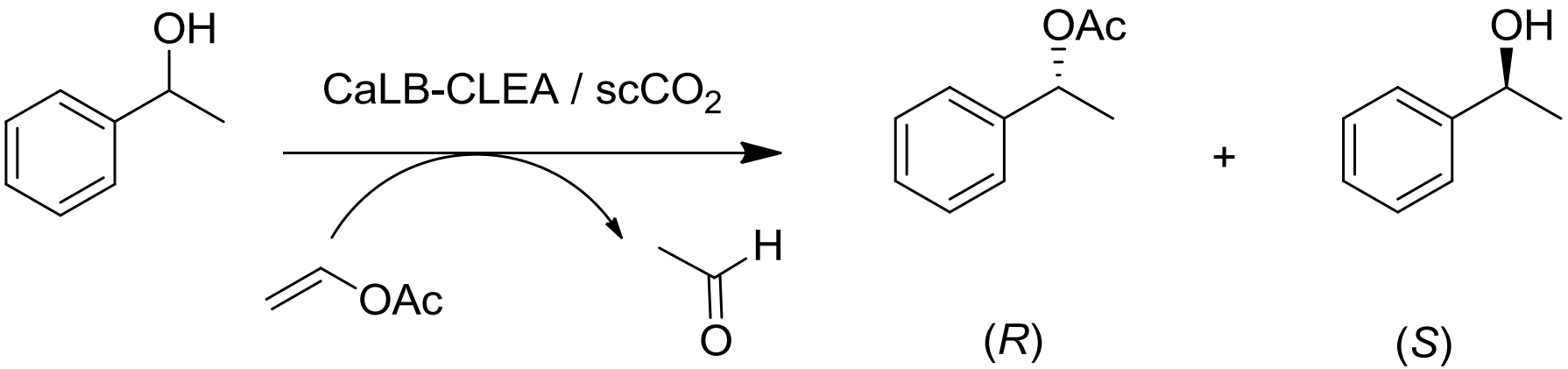
- non-flammable, non-toxic
- inexpensive
- moderate conditions (31^{o}C and 7.4 MPa)
- enzymes stable in $scCO_2$

But:

- $scCO_2$ reacts with NH_2 groups in lysine residues
- and with water to give H_2CO_3 and lower pH

A. Ballesteros, U. Bornscheuer, A. Capewell, D. Combes, J.-S. Conderet, K. König, F. N. Kolisis,A. Xenakis, *Biocat. Biotrans*. 1995, 13, 1-42.

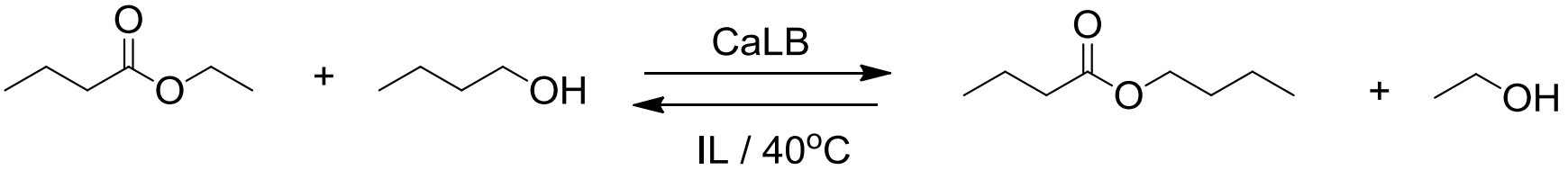
Enzymatic Transesterification in $scCO_2$

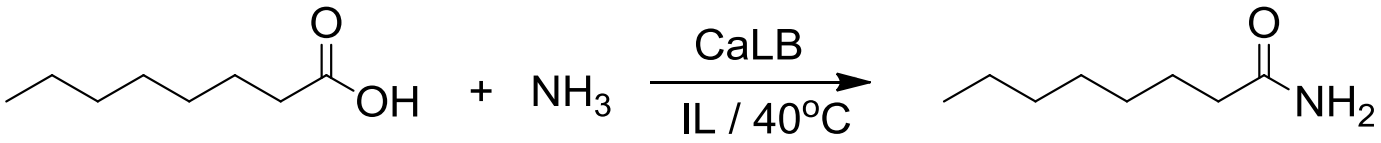


Catalyst	Conv.(%)	E
Nov435	17	280
CaLB CLEA	48	640

H.R.Hobbs, B. Kondor, P. Stephenson, R.A.Sheldon,
N. R. Thomas, M. Poliakoff, Green Chem. 2006, 6, 816-821

Enzymatic Transesterification and Amidation in Anhydrous Ionic Liquids



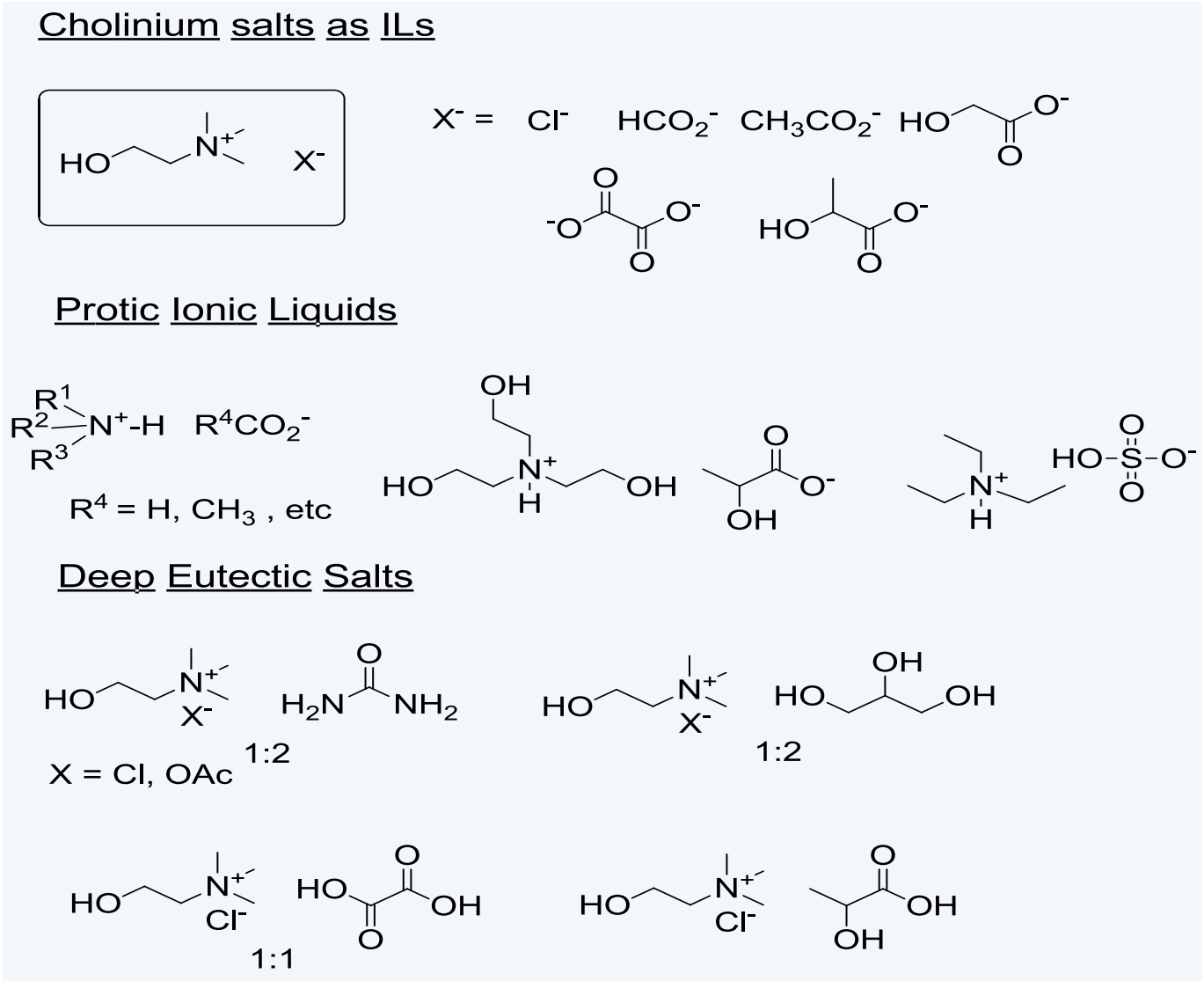


IL = [bmim][BF_4] or [bmim][PF_6]

N.B. Enzyme dried over P_2O_5

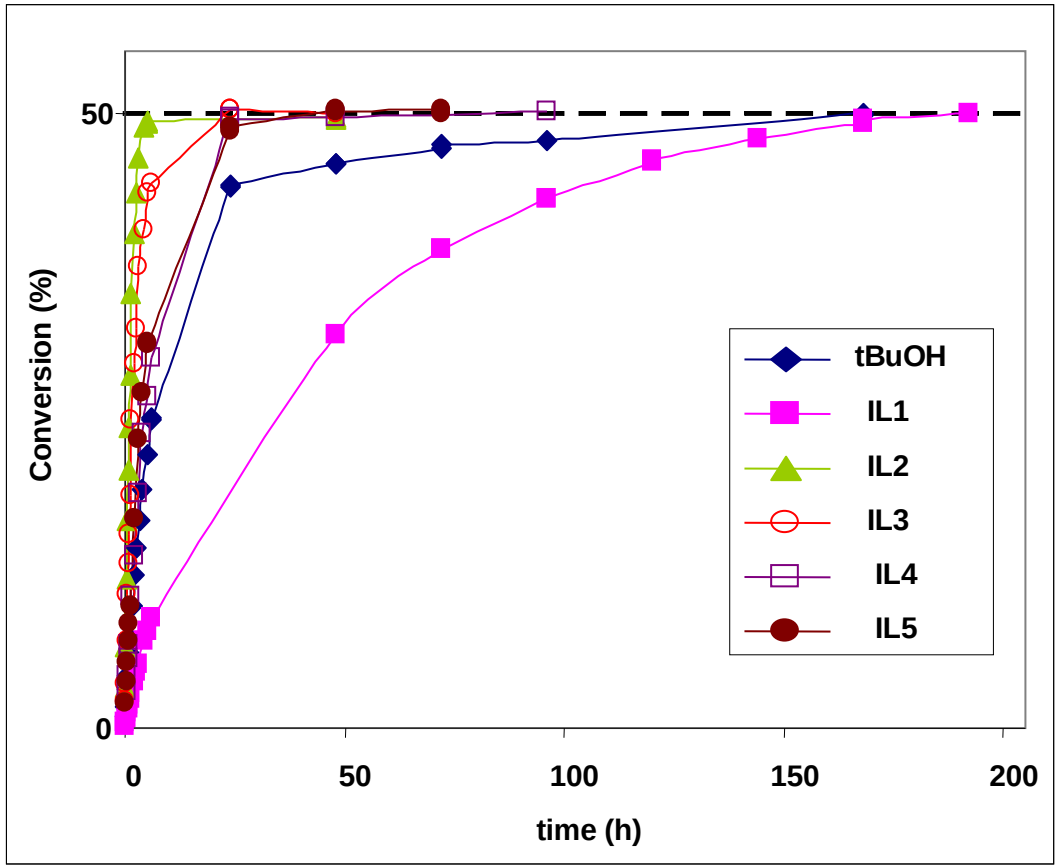
R. Madeira Lau, F. van Rantwijk, K.R. Seddon, R.A. Sheldon, *Org. Lett*. 2000, **2**, 4189-4191.

Neoteric Solvents



R. A. Sheldon, *Chem. Eur. J.*, 2016, 22, 12984-12999

CaLB CLEA in Protic Ionic Liquids (PILs)

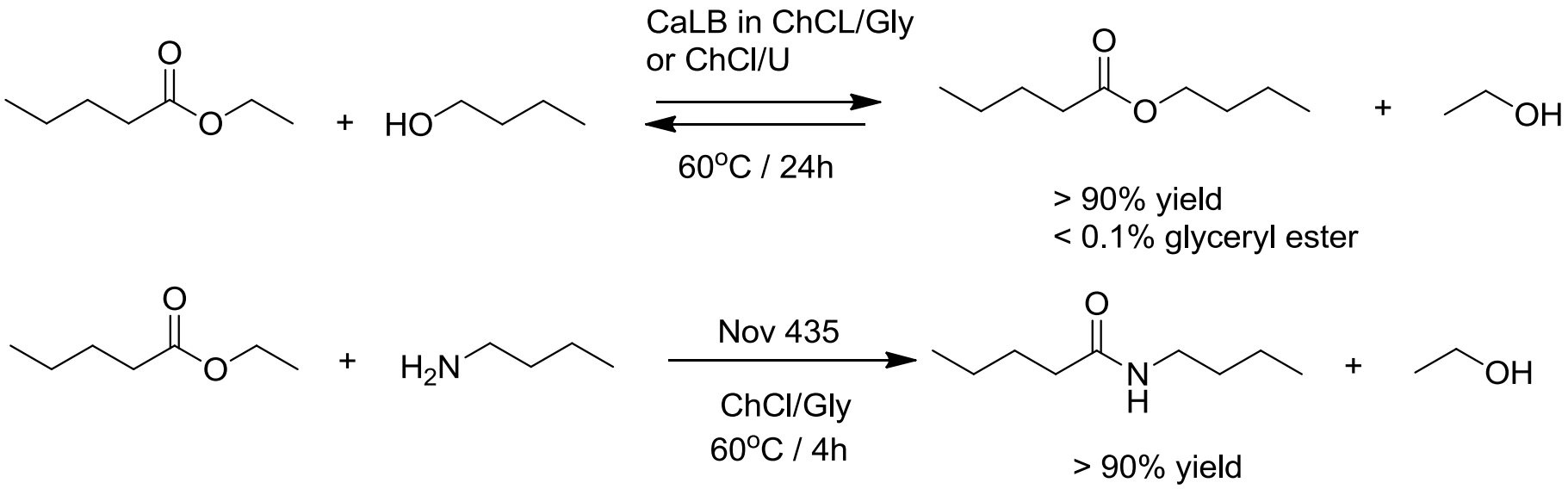


PIL	Cation	Anion
1	$C_4H_9(CH_3)_2NH^+$	$C_2H_5COO^-$
2	$C_4H_9(CH_3)_2NH^+$	$(C_8H_{17})_2CHCOO^-$
3	$C_{12}H_{25}(CH_3)_2NH^+$	$C_7H_{15}COO^-$
4	$(C_4H_9)_3NH^+$	$C_5H_{11}COO^-$
5	$(C_8H_{17})_3NH^+$	CH_3COO^-

ee > 99%

A. P. de los Ríos, F. van Rantwijk, R. A. Sheldon, *Green Chem.*. 2012, **14**, 1584-1588.

Biocatalysis in Deep Eutectic Solvents



J. T. Gorke, F. Srienc, R. Kazlauskas, *Chem Commun*., 2008, 1235-1237.

Protein Engineering

Some key references:

- R. J. Kazlauskas, U. T. Bornscheuer , *Nat. Chem. Biol., 2009, 5, 526-529*
- Protein Engineering Handbook, Vol 1-2 (2009), Vol. 3 (2012) U. T. Bornscheuer, S. Lutz, (Eds.), Wiley-VCH, Weinheim.
- Z. Sun, Y. Witmark, J. –E. Bäckvall, M. T. Reetz, *Chem. Eur. J*., 2016, 22, 5046-5054.
- H. Renata, J. Wang, F. H. Arnold, *Angew. Chem. Int. Ed*., 2015, 54, 3351-3367
- U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, *Nature*, 2012, **485**, 185-194.

Milestones in Protein Engineering

- Rational design by Site Directed Mutagenesis (SDM)

  C. A. Hutchison, S. Phillips, M. H. Edgell, S. Gillam, P. Jahnke, M. Smith, *J. Biol. Chem*., **1978**, 253, 6551-6560.

- Random mutagenesis via error-prone polymerase chain reaction (ep-PCR)

  C. Economou, K. Chen, F. H. Arnold, *Biotechnol. Bioeng*., **1992**, 39, 658-662.

- Random mutagenesis by DNA shuffling (*in vitro* recombination mimics Darwinian evolution)

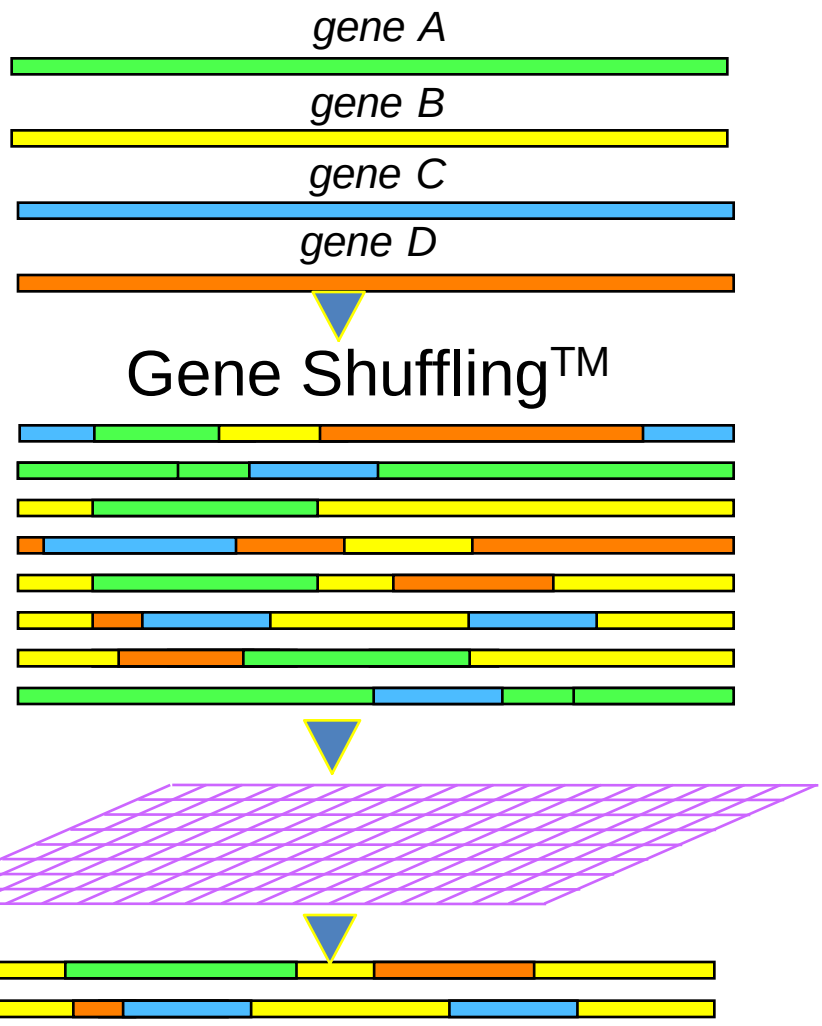
  W. P. Stemmer, *Nature*, **1994**, **370**, 389-391.

- Improving enantioselectivity by directed evolution

  M. T. Reetz, A. Zonta, K. Schimossekl, K. Liebeton, K. –E. Jaeger, *Angew. Chem. Int. Ed. Engl*., **1997**, **36**, 2830-2832.

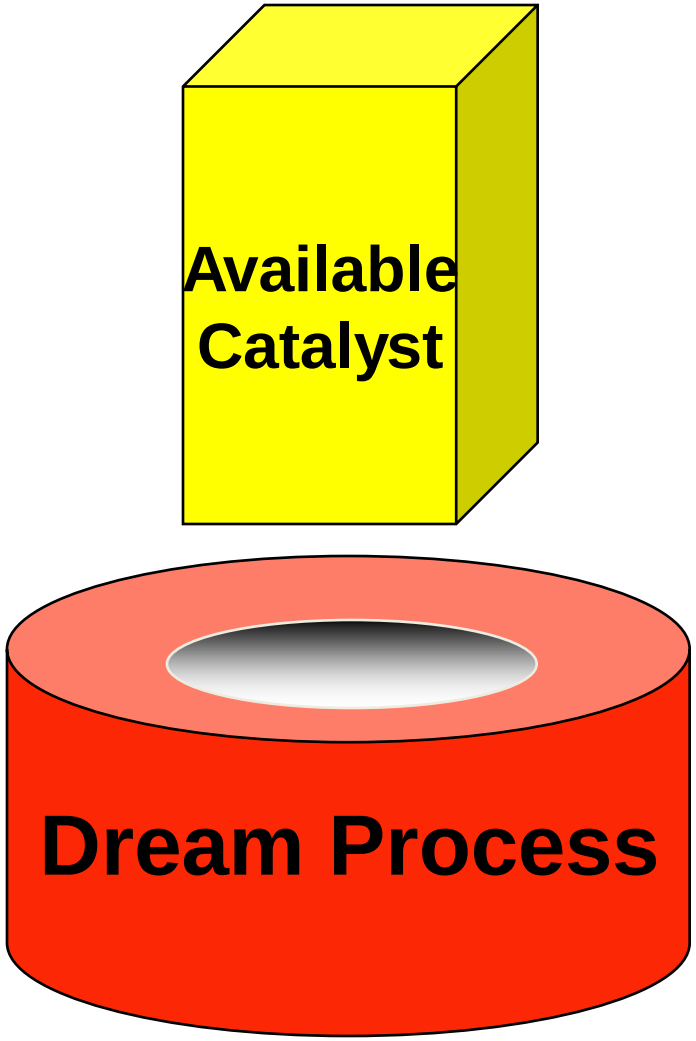
DNA Shuffling : Evolution in the Fast Lane

Pim Stemmer 1957-2013

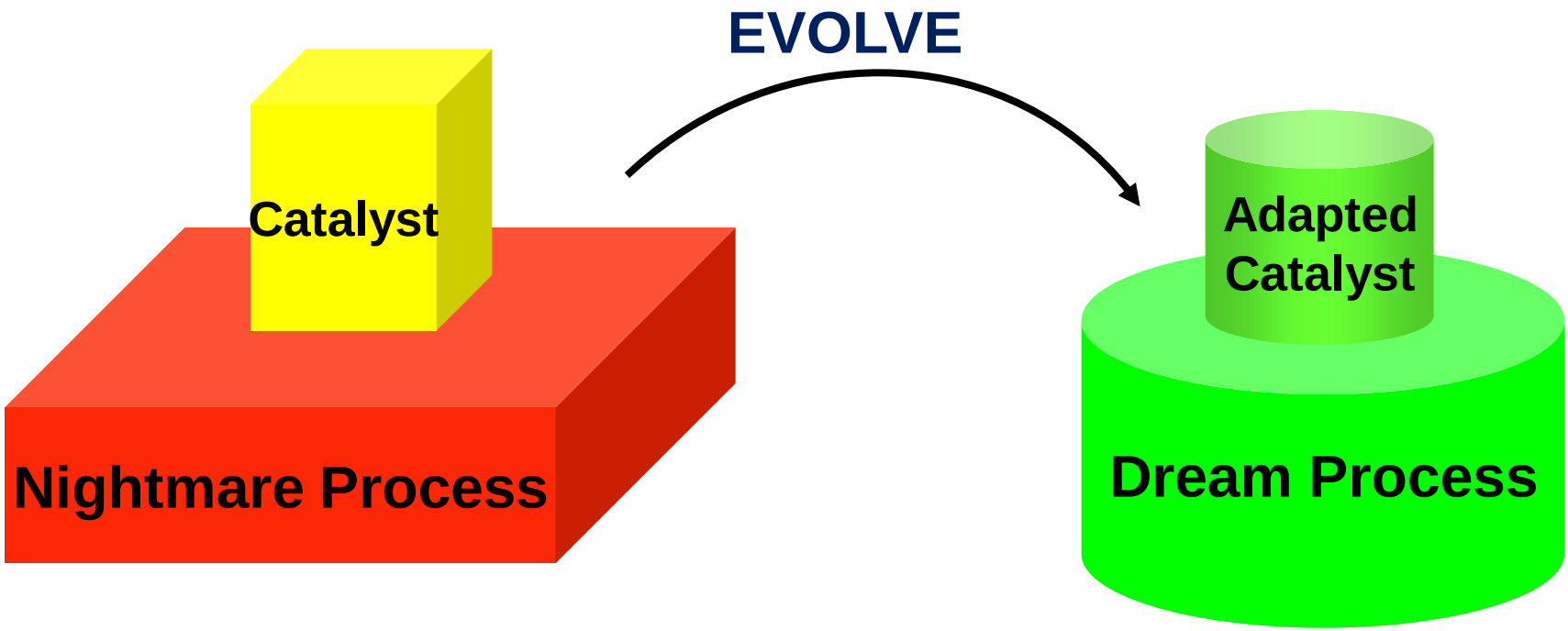


W.P.C.Stemmer, Nature, 370,389-391,1994

Historically: Adapt Process to fit Catalyst



Better: Adapt Catalyst to fit Ideal Process

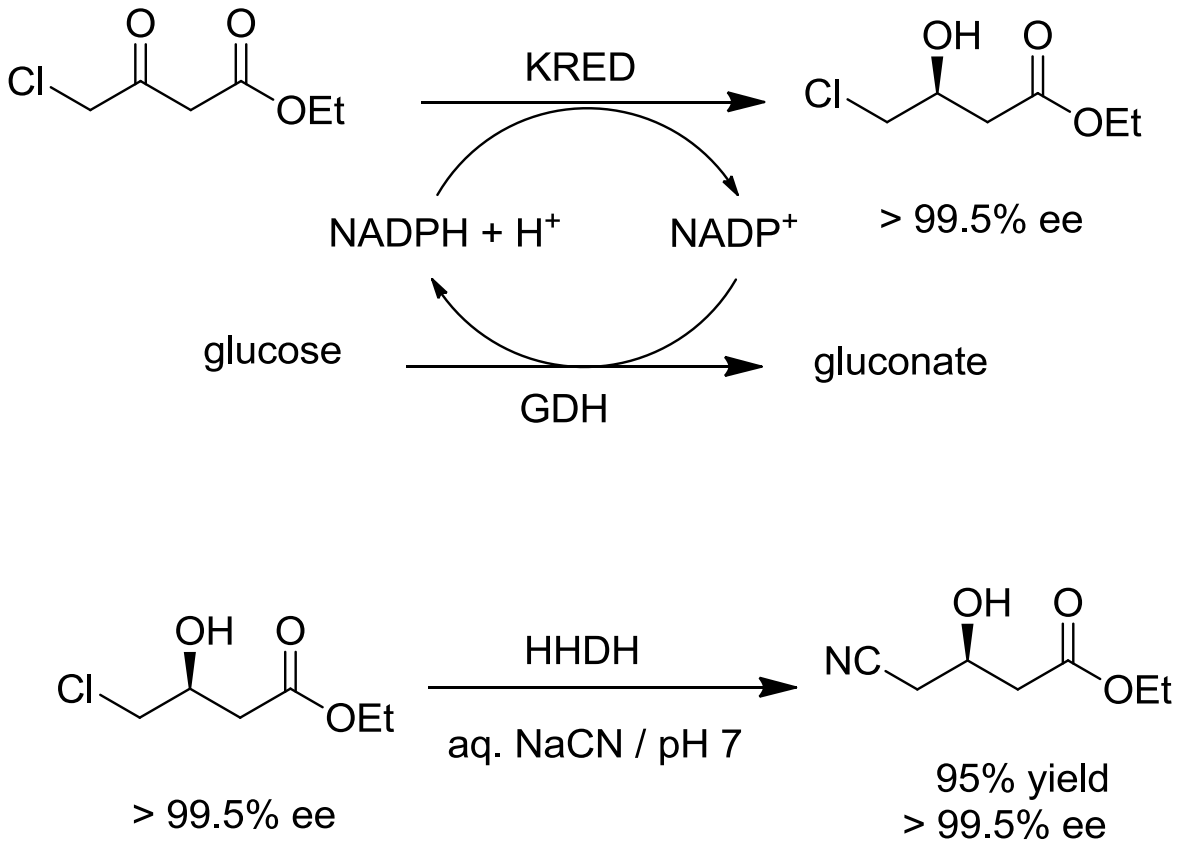


Compromise process to accommodate catalyst

Adapt catalyst to optimum process

Directed (*in vitro*)Evolution

Three Enzyme Process for Key Atorvastatin Intermediate (Codexis)



All three enzymes optimised using DNA shuffling

S. K. Ma, J. Gruber, C. Davis, L. Newman, D. Gray, A. Wang, J.Grate, G. W. Huisman, R. A. Sheldon, *Green Chem.*, 2010, **12**, 81 – 86

Evolution of a KRED/GDH Biocatalyst by DNA Shuffling (Codexis)

Parameter	Wild-type	Best Variant
TTN catalyst	3,000	>100,000
TTN NADP	4,000	>20,000
STY ($g.L^{-1}$ day^{-1})	80	600
Yield (%)	80	>95
e.e. (%)	99.8	>99.9
[Enzyme] ($g.L^{-1}$)	100	<1
[Substrate] ($g.L^{-1}$)	80	200
Reaction time (h)	24	10
Phase separation Work-up	>1h complex	ca. 1 min. very simple

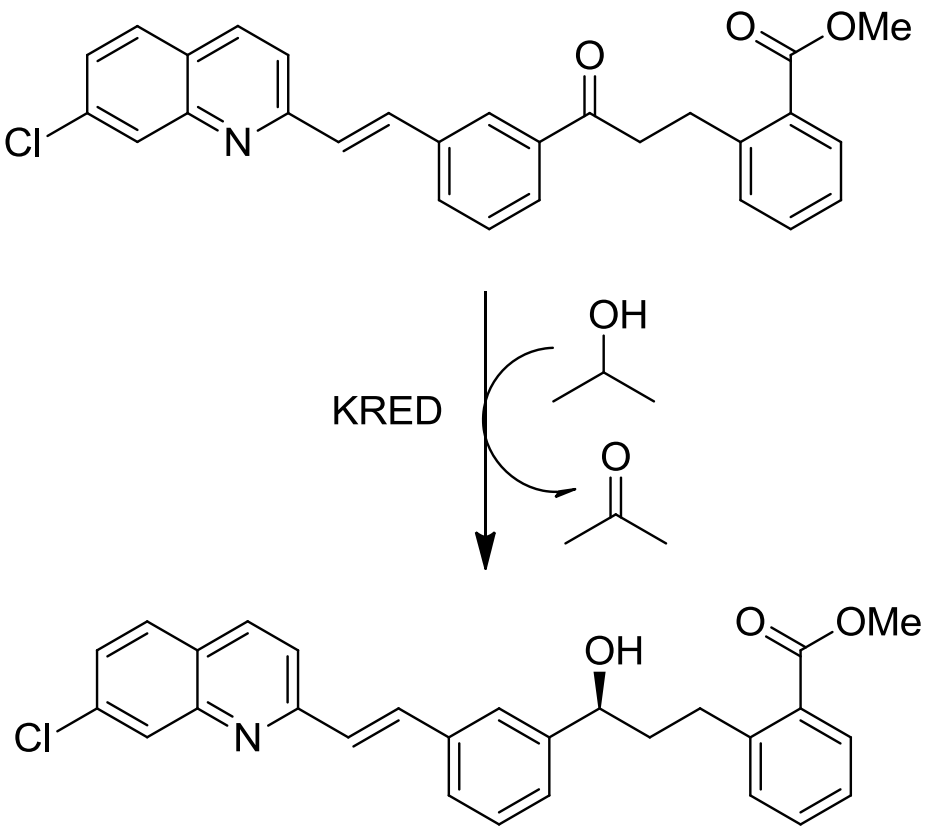
R. J. Fox, S. C. Davis, E. C. Mundorff, L. M. Newman, V. Gavrilovic, S. K. Ma, L. M. Chung, C. Ching, S. Tam, S. Muley, J. Grate, J. Gruber, J. C. Whitman, R. A. Sheldon, G. W. Huisman, Nature Biotechnol. 2007, 25, 338- 344.

Evolution of a HHDH biocatalyst by DNA shuffling

Parameter	Process design	Wild-type	Best Variant
[Substrate] ($g.L^{-1}$)	120	20	140
[Enzyme] ($g.L^{-1}$	1.5	30	1.2
Catalyst Productivity (g/g)	80	0.7	117
STY ($g.L^{-1}day^{-1}$)	>360	7	672
Isolated yield (%)	>90	67	92
Chemical purity (%)	>98	>98	>98
ee (%)	>99.5	>99.5	>99.5
Reaction time (h)	8	72	5
Phase separation (min)	<10	>60	<1

R. J. Fox, S. C. Davis, E. C. Mundorff, L. M. Newman, V. Gavrilovic, S. K. Ma, L. M. Chung, C. Ching, S. Tam, S. Muley, J. Grate, J. Gruber, J. C. Whitman, R. A. Sheldon, G. W. Huisman, Nature Biotechnol. 2007, 25, 338- 344.

Montelukast Intermediate by KRED Reduction



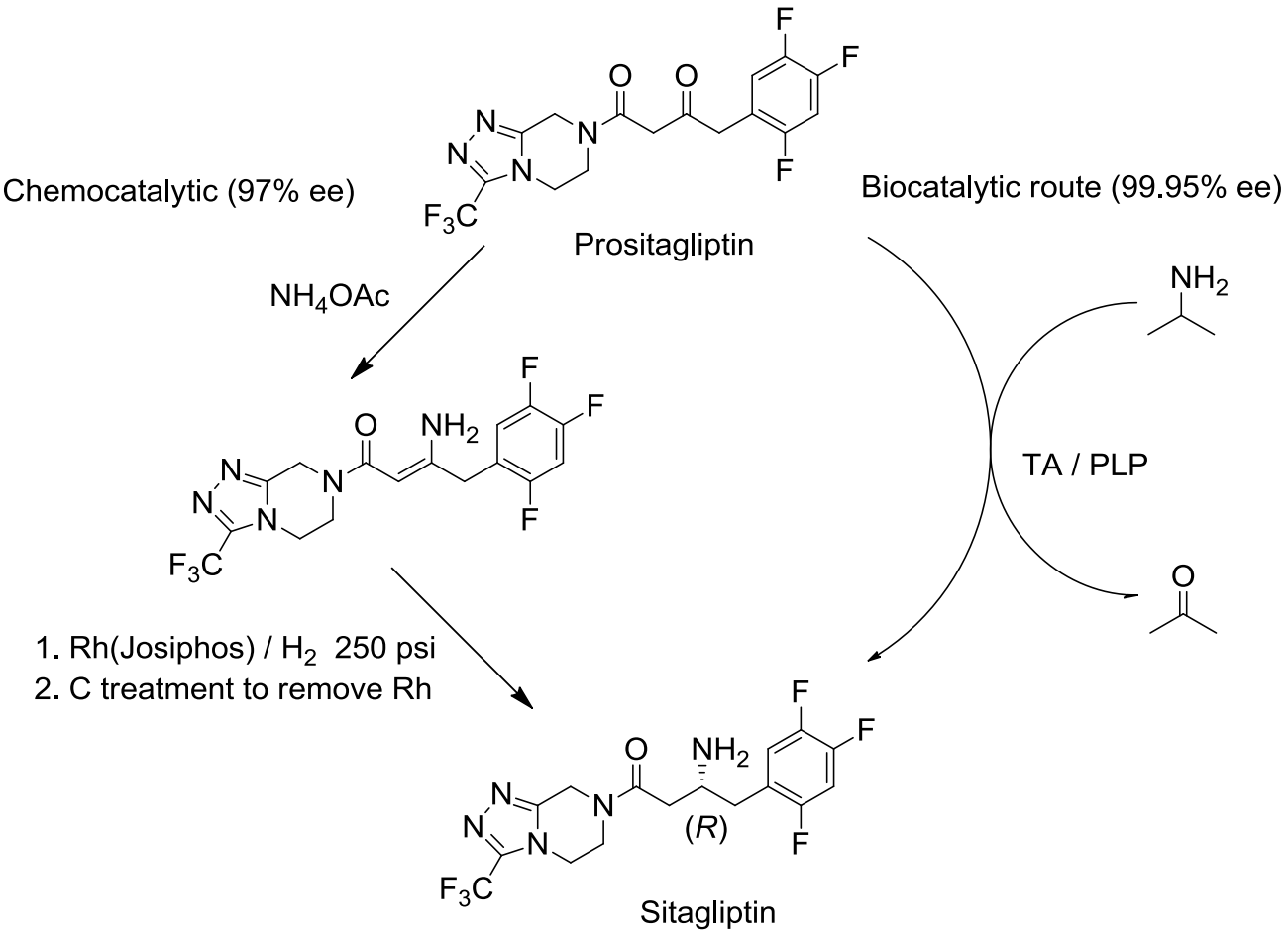
Montelukast intermediate

95% yield; >99.95 ee

- No known KRED active
- Initial screening >99.9% ee
- Activity ca. 1000x too low
- Improved with DNA shuffling
- Final variant under optimised conditions 3000x improvement
- [substrate] = 100gL^{-1}
- slurry-to-slurry (toluene/water)
- [enzyme] = 3gL^{-1}

J. Liang, J. Lalonde, B. Borup, V. Mitchell, E. Mundorff, N. Trinh, D. A. Kochrekar, R. N. Cherat, G. G. Pai, *Org. Proc. Res. Dev*. 2010, **14**, 193-198.

Synthesis of Sitagliptin by Biocatalytic Transamination (1)



C. K. Saville, J. M. Maney, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science*, 2010, **329**, 305-308

Synthesis of Sitagliptin by Biocatalytic Transamination (2)

Initial Screening:

- (*R*)-selective TA lacking activity towards prositagliptin.
- Computer-aided design of the active site and site saturation mutagenesis used to produce an active TA .
- Enzyme loading 10g.L^{-1} ; Substrate loading 2g.L^{-1} ; 0.7% conv. in 24h.
- Prositagliptin is only sparingly soluble (<1g.L^{-1}) in water, necessitating substantial amounts of DMSO as a cosolvent.

Desired Commercially Viable Process:

- 100g.L^{-1} substrate, 1M isopropylamine, >25% DMSO, >40^{o}C ; 24h
- Product with >99.9% ee.

Directed Evolution with *inter alia* DNA shuffling

- Final variant contained 27 mutations.
- 6g.L^{-1} enzyme and 100g.L^{-1} substrate in 50% DMSO at 45^{o}C,
- 92% yield and >99.95% ee (other isomer below detection level) .

See also

I. V. Pavlidis, IM. S. Weiß, M. Genz., P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, U. T. Bornscheuer, Nature Chem., 2016, 8, 1076-1082.

Biocatalyst Engineering

Enzyme Production and Formulation

1. Production

Overproduction of large amounts of the optimised enzyme at relatively low cost in a microbial host with a GRAS (Generally Regarded as Safe) status

V. Sewalt, D. Shanahan, L. Gregg, J. La Marta R. Carrillo, Ind. Biotechnol. 2016, 12(5), 295-302

2. Formulation

Enzymes are soluble in water and difficult to remove from aqueous effluents. It is possible by ultrafiltration but this can be costly.

The Challenge

- **Enzymes are soluble in water**
- **Single use is expensive**
- **How to reduce the enzyme costs?**

➡ **Immobilisation as an insoluble solid (powder)**

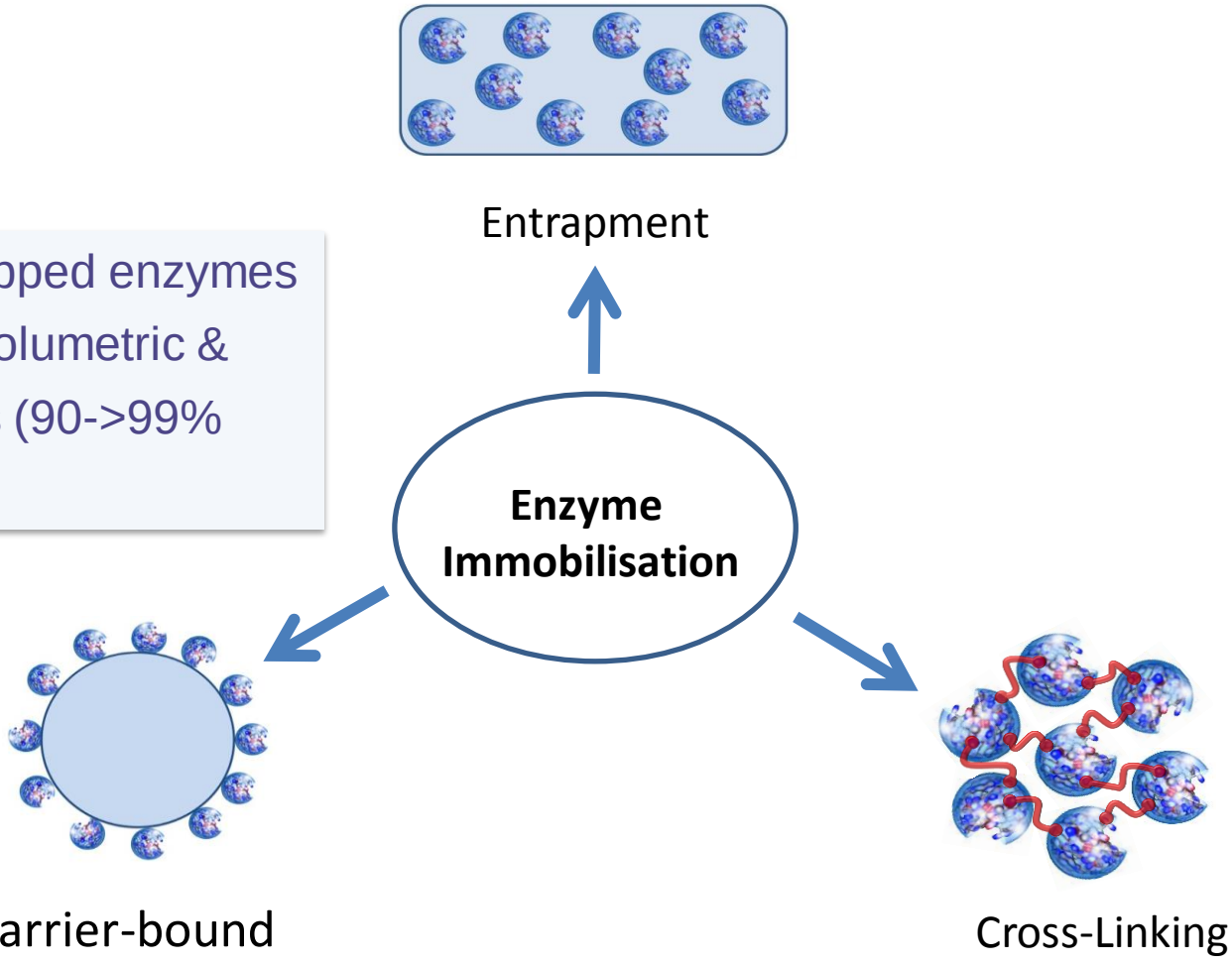
➡ **Heterogeneous catalyst**

Separate by:

- Filtration
- Centrifugation
- Column (fixed bed)

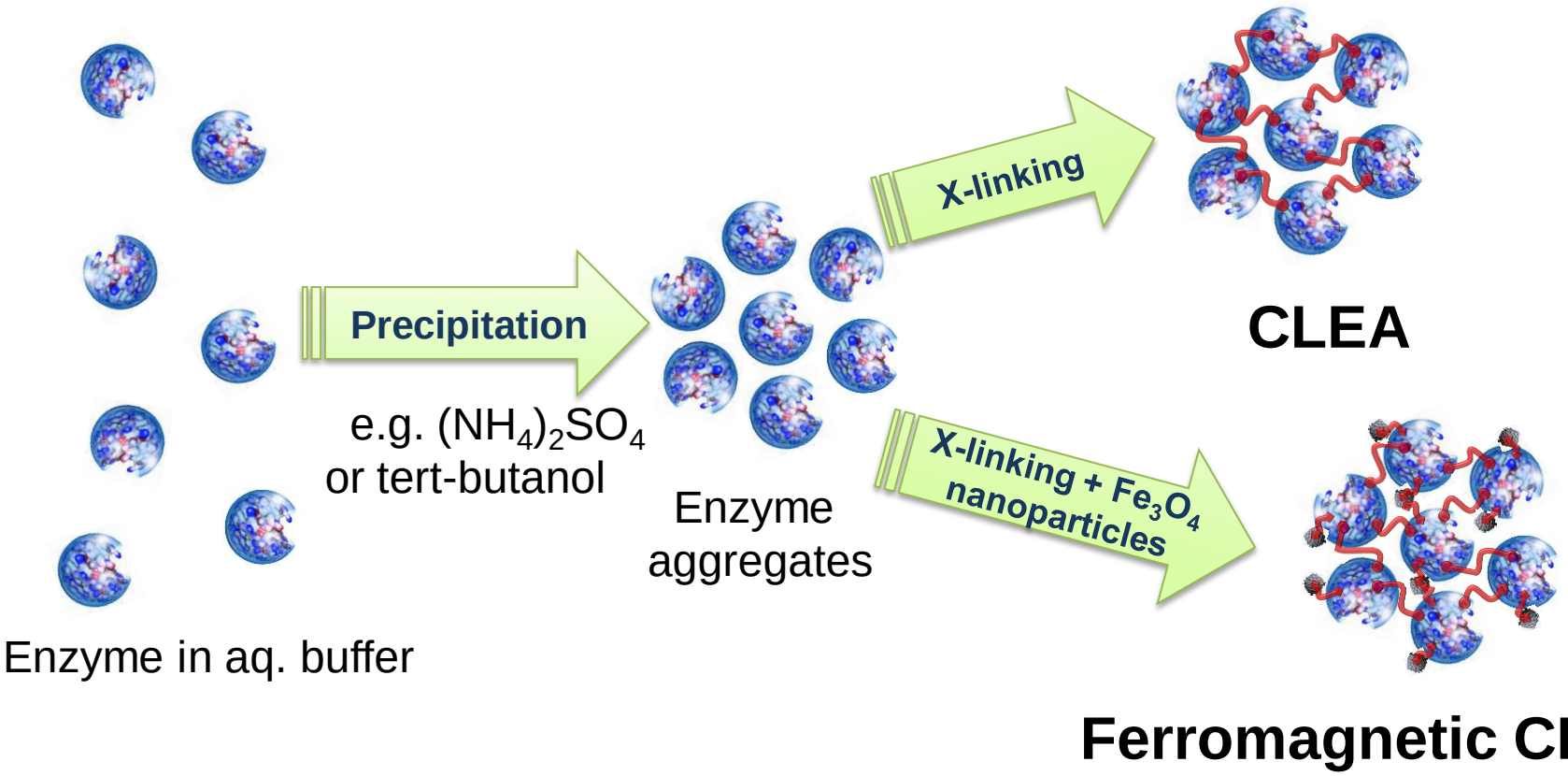
Immobilisation of Isolated Enzymes

Carrier-bound / entrapped enzymes have inherently low volumetric & catalyst productivities (90->99% non-catalytic mass)



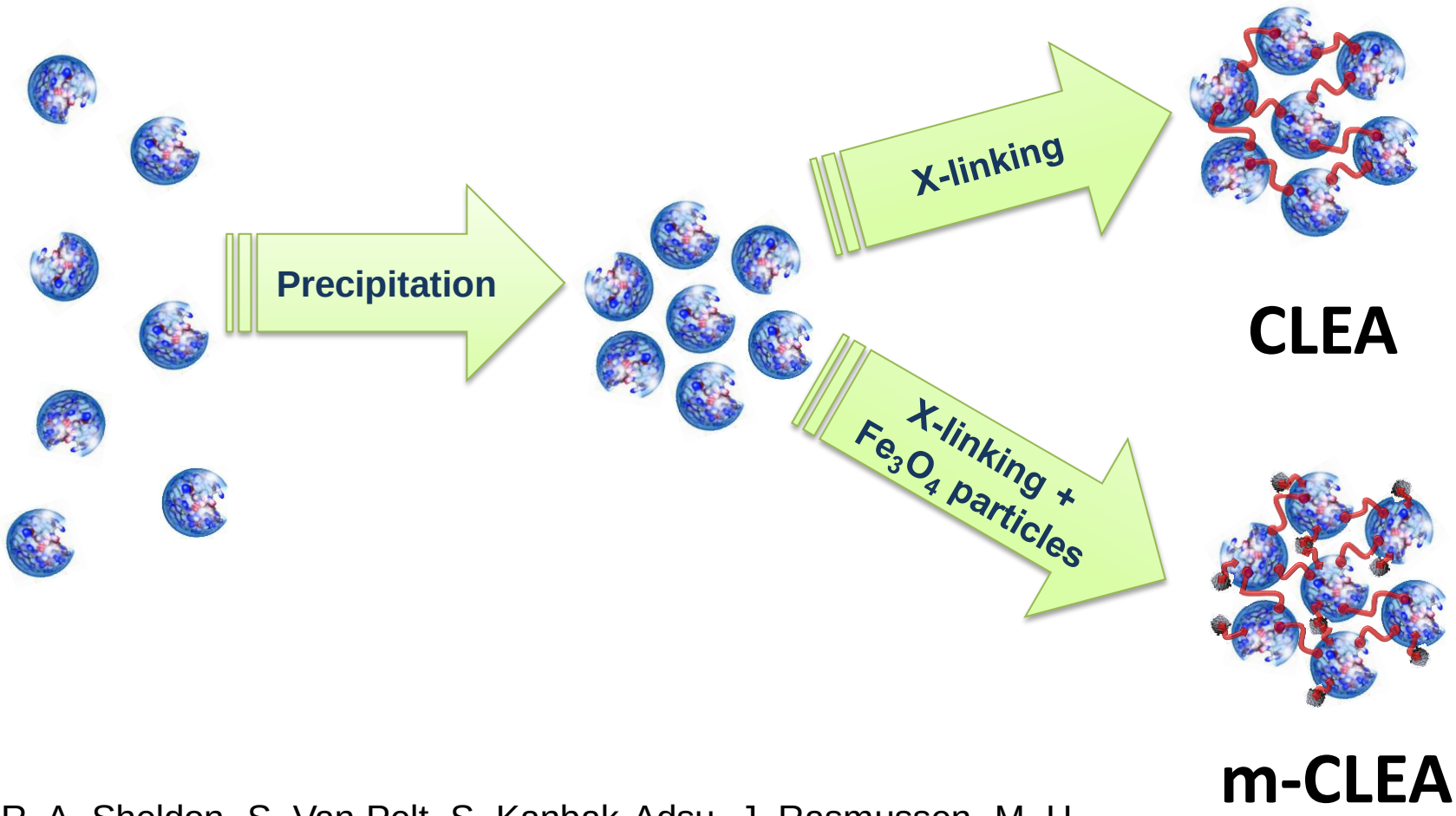
R. A. Sheldon, S. van Pelt, Chem. Soc. Revs, 2013, 42, 6223

Cross-Linked Enzyme Aggregates (CLEAs)



- R. A. Sheldon, *Appl. Microbiol. Biotechnol*. 92 (2011) 467-477;
- R. A. Sheldon, S. van Pelt, S. Kanbak-Aksu, J. Rasmussen, M. H. A. Janssen, *Aldrichim.Acta*, 46 (2013) 81-93.

Cross-linked Enzyme Aggregates (CLEAs)



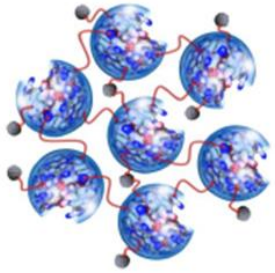
R. A. Sheldon, S. Van Pelt, S. Kanbak-Adsu, J. Rasmussen, M. H. A. Janssen, *Aldrichim. Acta*, 2013**, 46(3)**, 81-93.

Advantages of CLEAs

1. Improved properties
    - Better storage and operational stability
    - No leaching of enzyme in aqueous media
2. Cost-effective
    - No need for pure enzyme (crude cell lysate sufficient)
    - Easy recovery and recycle (easier DSP)
    - High productivities (kg product/kg enzyme)
3. Broad scope & short time to market

‘Smart’ magnetic CLEAs

- co-cross-linking enzymes with magnetic particles
- works with crude cell lysate / limited additional costs
- mechanically robust / high resistance to shear
- increased operational stability (temp. / pH)
- protease resistant

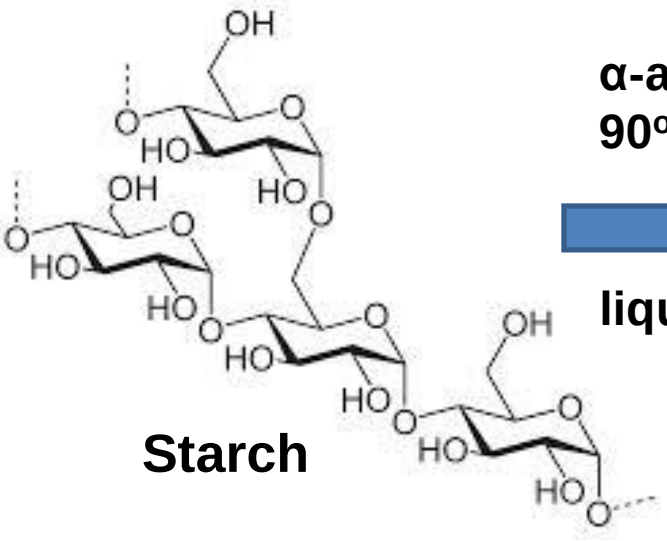


www.eclipsemagnetics.com

Separation on industrial scale using existing commercial equipment

R. A. Sheldon, M. J. Sorgedrager, B. Kondor, **2012**, WO2012/023847 A2 to CLEA Technologies B.V.

1G Bioethanol from Starch (Corn or Wheat)



Starch

α-amylase
90ºC / pH 7

liquefaction

Oligosaccharides
(5-20 glucose units)
Maltodextrins

glucoamylase
60ºC / pH 5

saccharification

D-glucose

yeast

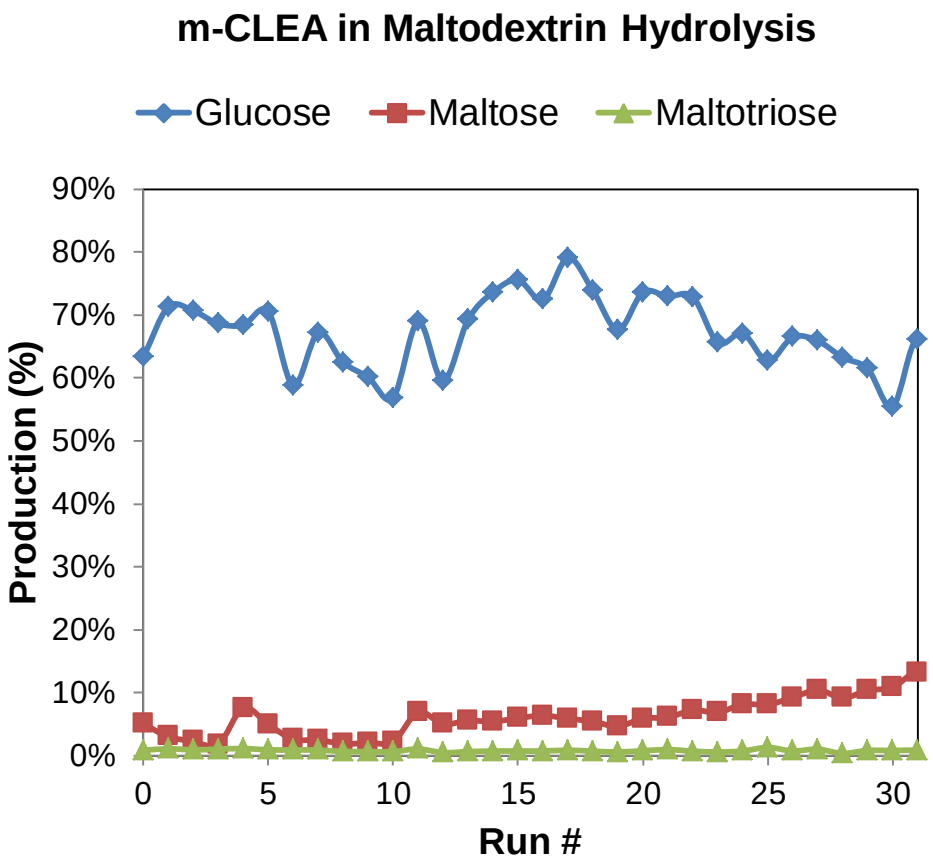
fermentation

Bioethanol

Food & Beverages
Applications

SSF = Simultaneous Saccharification and Fermentation

Maltodextrin Hydrolysis with Glucoamylase mCLEA



- Conditions: 1.0 L maltodextrin (33%), 50 °C
- Run time: 24 h per cycle

Consistent maltodextrin conversion to glucose during 31 cycles or 1 month of cumulative use!

M. Janssen, CLEA Technologies, unpublished results

Combi-CLEAs and magnetic combi-CLEAs

- Co-precipitation and aggregation of two or more enzymes
- Synergistic: Higher activities than mixture of separate CLEAs

Examples:

1. protease/lipase combi-CLEA (active and stable)
2. oxidases / catalase combi-CLEAs
3. KRED / GDH combi-CLEA
4. Glycosidase combi-CLEAs in carbohydrate conversions

1. S. S. Mahmod, et al, *Process. Biochem*. 2015, **50**, 2144
2. R. Schoevaart, et al, *Biotechnol. Bioeng*. 2004, **87**, 754.
3. C. Ning, E. et al, *J. Biotechnology*, 2014, **184**, 7-10.
4. S. Talekar, et al, Bioresour Technol. 2013, 147, 269.

Biocatalytic Cascade Processes: Cell-free Synthetic Biology

Key reviews:

- A. Bruggink, R. Schoevaart and T. Kieboom, *Org. Proc. Res. Dev*., 2003, **7**, 622.

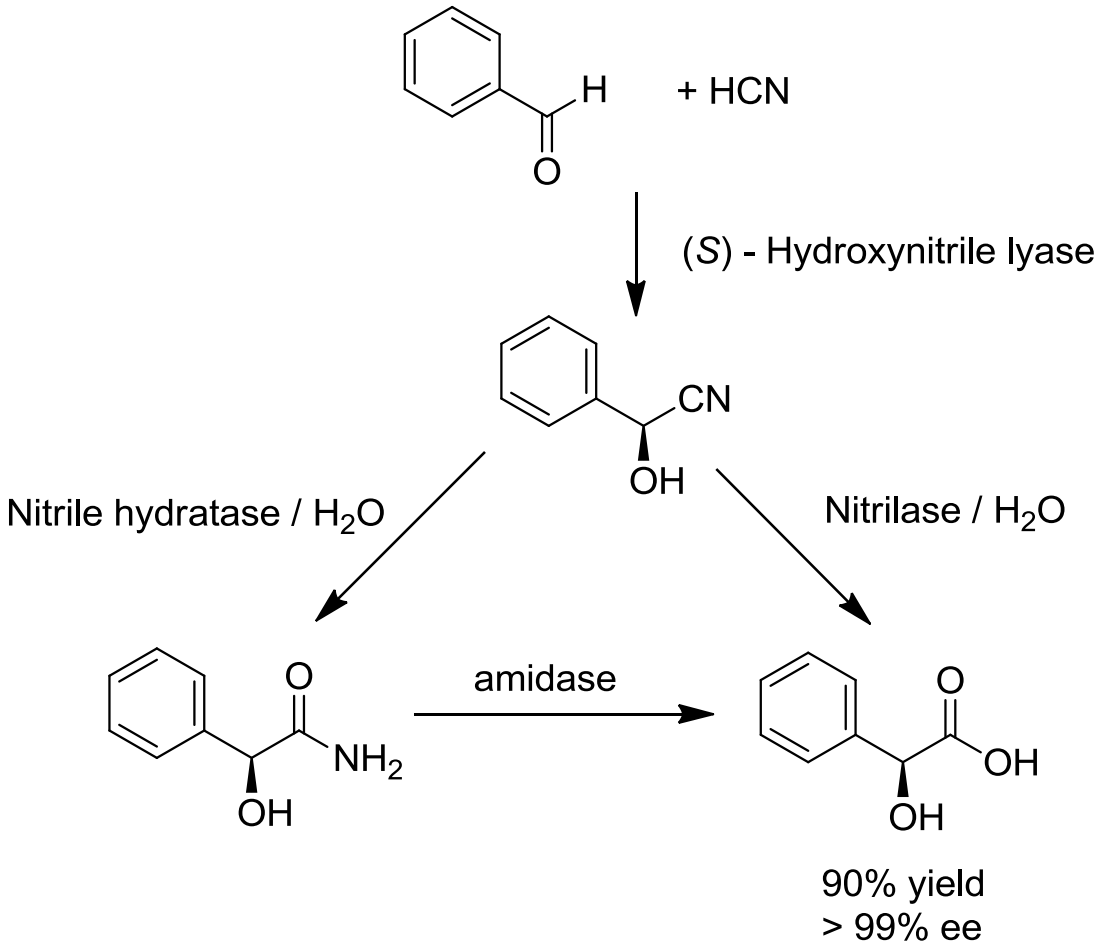
- R. Xue, J. M. Woodley, *Bioresour. Technol.*, 2012, **115**, 183-195.

- J. Muschiol, C. Peters, N. Oberleitner, M. D. Mihovilovic, U. T. Bornscheuer, F. Rudroff, *Chem. Commun*. 2015, 51, 5798-5811.

- J. H. Schrittweiser, J. Sattler, V. Resch, F. G. Mutti, W. Kroutil, *Curr. Opin. Chem. Biol*. 2011, **15**, 249-256.

- S. P. France, L. J. Hepworth, N. J. Turner, S L. Flitsch, *ACS Catalysis*, DOI: 10.1021/acscatal.6b02979;

Conversion of Benzaldehyde to *S*-Mandelic Acid with a Trienzyme Combi-CLEA

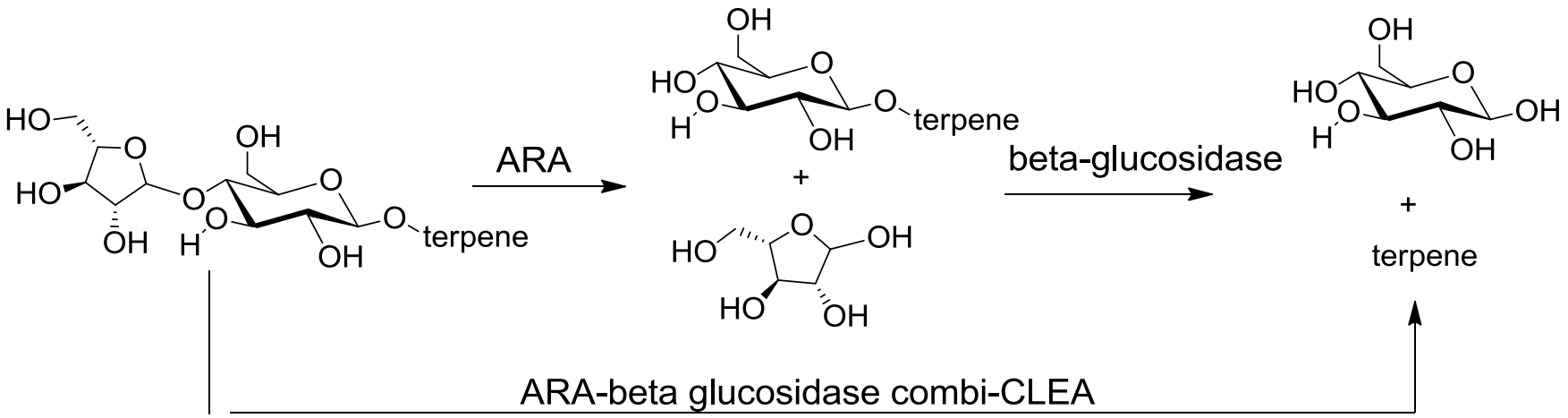


A. Chmura, S. Rustler, M. Paravidino, F. van Rantwijk, A. Stolz , R.A. Sheldon, *Tetrahedron: Asymmetry*, 2013, **24**, 1225-1232.

Aroma Enhancement in Wine using Combi-CLEA

Enzymatic hydrolysis of monoterpenyl glycosides with a combi-CLEA of:

- α-L-arabinofuranosidase (ARA)
- β—D-glucopyranosidase (β-glucosidase β-G)



K. Ahumada, A. Martinez-Gil, Y. Moreno-Simunovic, A. Illanes, L. Wilson, *Molecules*, 2016, 21, 1485

Reactor Engineering
&
Down-Stream Processing

Reactors for Biocatalytic Processes (1)

1. Stirred Tank Reactors (STR)
   - used widely in Pharma
   - separation by filtration or centrifugation
   - catalyst attrition by propeller stirrer
2. Membrane Slurry Reactor (MSR)
   - reduced catalyst attrition
   - integrates reaction / catalyst separation
3. Perfusion Basket Reactor (BR)[a]
   - “Tea Bag” concept
4. Spinning Basket Reactor[b]
5. Rotating Cell Reactor[c]
   - Spin Chem (www.spinchem.com)

a. H. Cabana, J. P. Jones, S. N. Agathos, *Biotechnol. Bioeng*., 2009, **102**, 1582.
b. G. Sheelu, G. Kavitha, N. W. Fadnavis, *J. Am. Oil. Chem. Soc*., 2008, **85**, 739
c. H. Mallin, J. Muschiol, E. Bystrom, U. T. Bornscheuer, *ChemCatChem*, 2013, **5**, 3529

Reactors for Biocatalytic Processes (2)

7. Bubble Column Reactor (BCR)
   - circumvents mechanical attrition
   - production of viscous polyol esters (emollients)
8. Fixed Bed Reactors (FBR)
   - used in oils and fats processing
   - large particles to avoid pressure drop
9. Fluidised bed reactors
   - used in petrochemicals
   - small particles but with high density
10. Magnetically Stabilised Fluidised Bed (MSFB)

L. Hilterhaus, O. Thum. A. Liese, *Org. Proc. Res. Dev.*,2008,**1 2**, 618-625

Integration of Reaction and Catalyst Separation

Pen G amidase CLEAs in a Membrane Slurry Reactor (MSR); Hydrolysis of Penicillin G to 6-APA

- **No external catalyst filtration**
- **Reduction of catalyst attrition**
- **Broad particle distribution**
- **Minimum catalyst hold up**

MSR

M. J. Sorgedrager, D.Verdoes, H. van der Meer, R. A. Sheldon, *Chim. Oggi*, 2008, **26**, 23-25.

Separation of Immobilised Enzymes from Suspended Solids

- Synthesis of semi-synthetic penicillins and cephalosporins by coupling 6APA or 7-ADCA with side-chain
- 1G and 2G Biofuels production by simultaneous saccharification and fermentation (SSF) processing of starch or lignocellulose

1. Sieve bottom reactor[a]
2. Magnetic CLEAs in standard STR[b,c]
   - Magnetic separation with standard commercial equipment

a. A. Bruggink (Ed.), *Synthesis of β-lactam antibiotics: chemistry, biocatalysis and process integration, Springer 2001.*

b. W. Kopp, T. P. Da Costa, S. C. Pereira, M. Jafelicci, R. C. Giordano, R. F. C. Marques, F. M. Araujo-Moreira, R. L. C. Giordano, *Process Biochem*. 2014, **49**, 38.

c. A. Bhattacharya, B. L. Pleschke, *J. Mol. Catal. (B): Enzymatic*, 2015, **115,** 140-150.

Conclusions and Prospects

- Biocatalysis benefited enormously from advances in molecular biology and biotechnology
- Biocatalysis is green and sustainable
- Mainstream technology for pharma, specialties and some commodity chemicals
- Important in transition from fossil resources to bio-based economy (hydrocarbon to carbohydrates as feedstocks)
- Holistic approach of *Biocatalysis Engineering* will play an important role

The best is yet to come!