Biocatalysis : Historical Development and Recent Applications

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LSPN Seminars

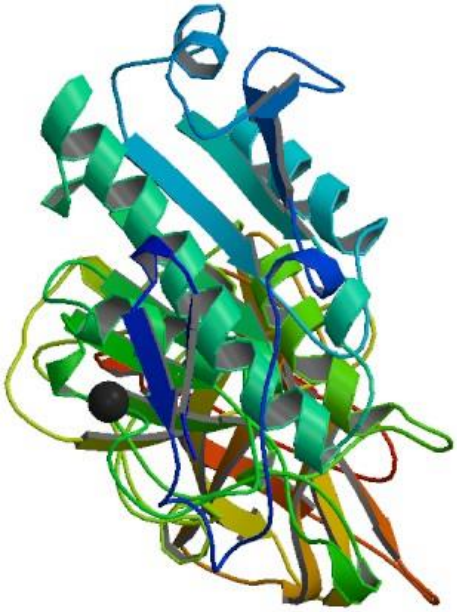
2nd August 2018

Plan

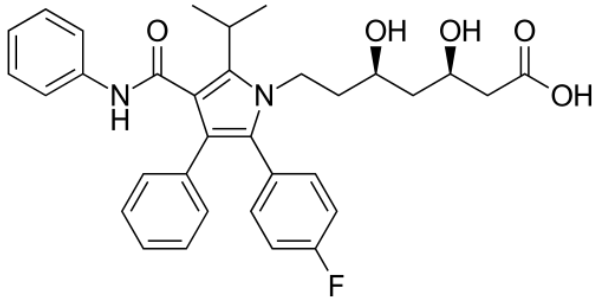
1) **Introduction**
2) **Historical Milestones**
3) **Applications of Wild-type Enzymes to Organic Synthesis : Examples**
4) **Protein Engineering : Rationale Design & Directed Evolution**
5) **Applications and Highlights of the Recent Literature**
6) **Conclusion & Outlook**

1) Introduction

- Enzyme = in-yeast
- Proteins
- Nature's catalysts

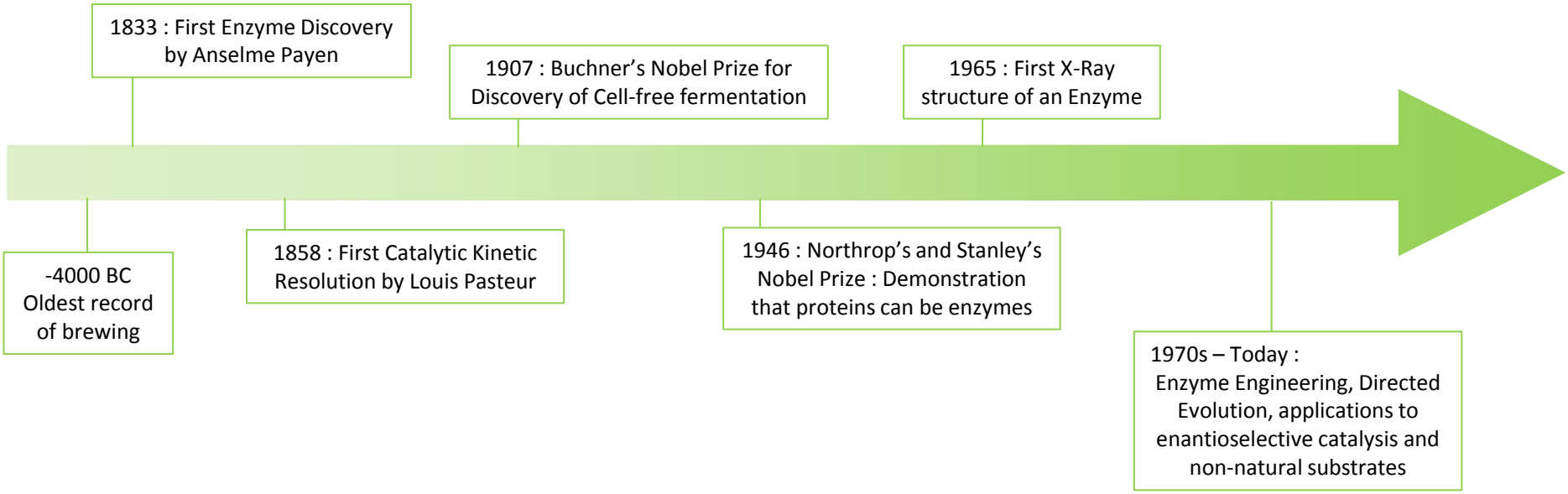


Pancreatic Lipase (Guinea-Pig)
PDB entry 1GPL



Atorvastatin, Lipid-lowering agent
1996-2012 world's best selling drug

2) Historical Milestones



2) Historical Milestones

1833 : First Enzyme Discovery by Anselme Payen

1907 : Buchner's Nobel Prize for Discovery of Cell-free fermentation

1965 : First X-Ray structure of an Enzyme

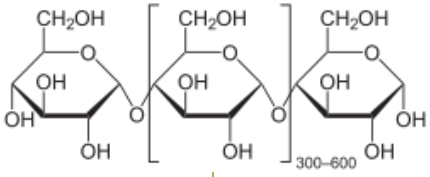
-4000 BC Oldest record of brewing

1858 : First Catalytic Kinetic Resolution by Louis Pasteur

1946 : Northrop's and Stanley's Nobel Prize : Demonstration that proteins can be enzymes

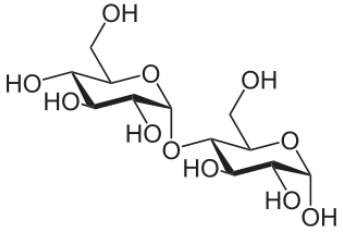
1970s – Today : Enzyme Engineering, Directed Evolution, applications to enantioselective catalysis and non-natural substrates

Anselme Payen



Starch

Diastase



Maltose

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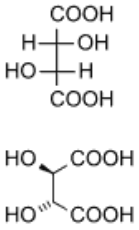
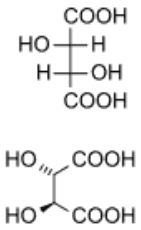
Louis Pasteur

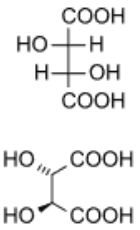


L-(+)-tartaric Acid



D-(-)-tartaric Acid

Yeast



D-(-)-tartaric Acid

First report of Catalytic Kinetic Resolution : Pasteur, L., *C.R. Seances Acad. Sci.*,**1858**, *46*, 615

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-4000 BC
Oldest record of brewing

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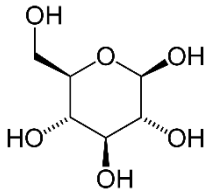
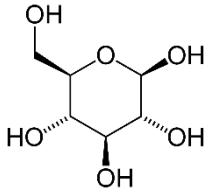
Eduard Buchner

Pasteur



Yeast → Alcohol

Buchner



Yeast-extracts → Alcohol

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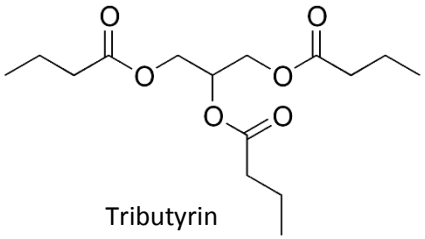
Zaks and Klibanov (MIT), 1984 :



Tributyrin

Porcin Pancreatic Lipase

Organic Media, High temperatures

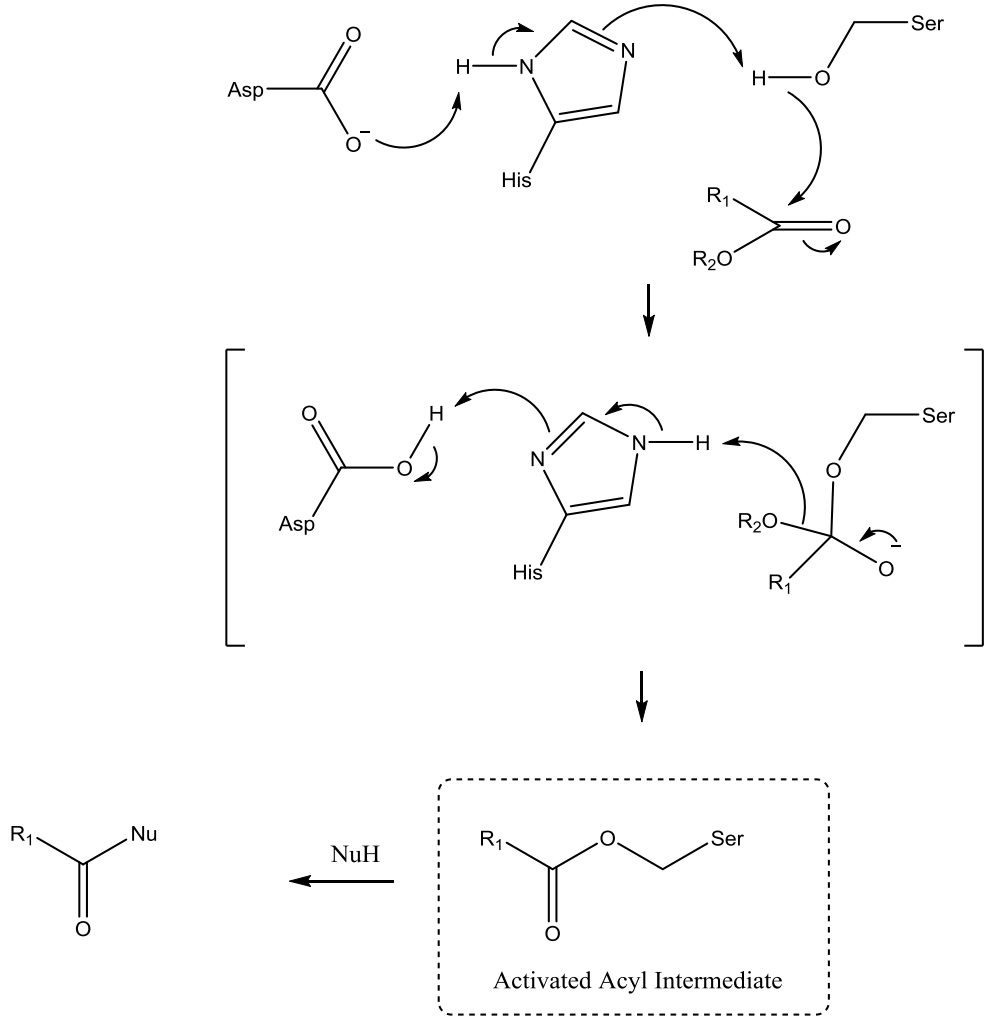
Hexane, Acetone, THF, Toluene…

- Transesterification
- Esterification
- Amidation
- Thioesterification

Enzymatic catalysis in organic media at 100°C, Zaks A., Klibanov A.M., *Science,* **1984**, *224*, 1249-51
Enzyme-catalyzed processes in organic solvents, Zaks A., Klibanov A.M., *PNAS,* **1985**, *82*, 3192-96

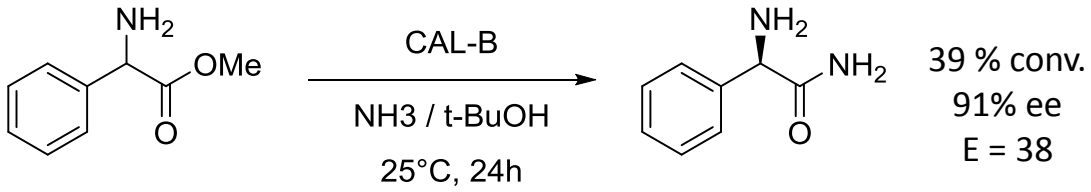
3) Wild-type Enzymes : Lipases

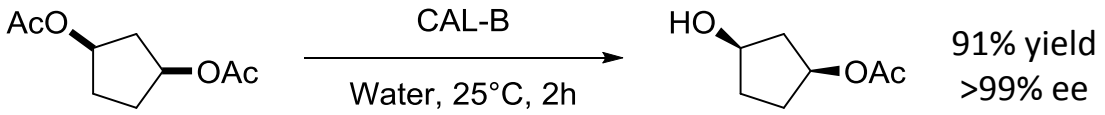
- Lipases (Serine Proteases)
- Catalytic Triad
- Electron-relay mechanism

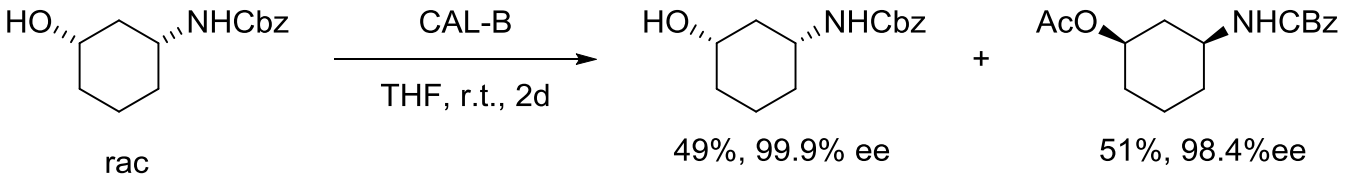


Chem. Soc. Rev., **2017**, *46*, 2678-2691

3) Wild-type Enzymes : Lipases



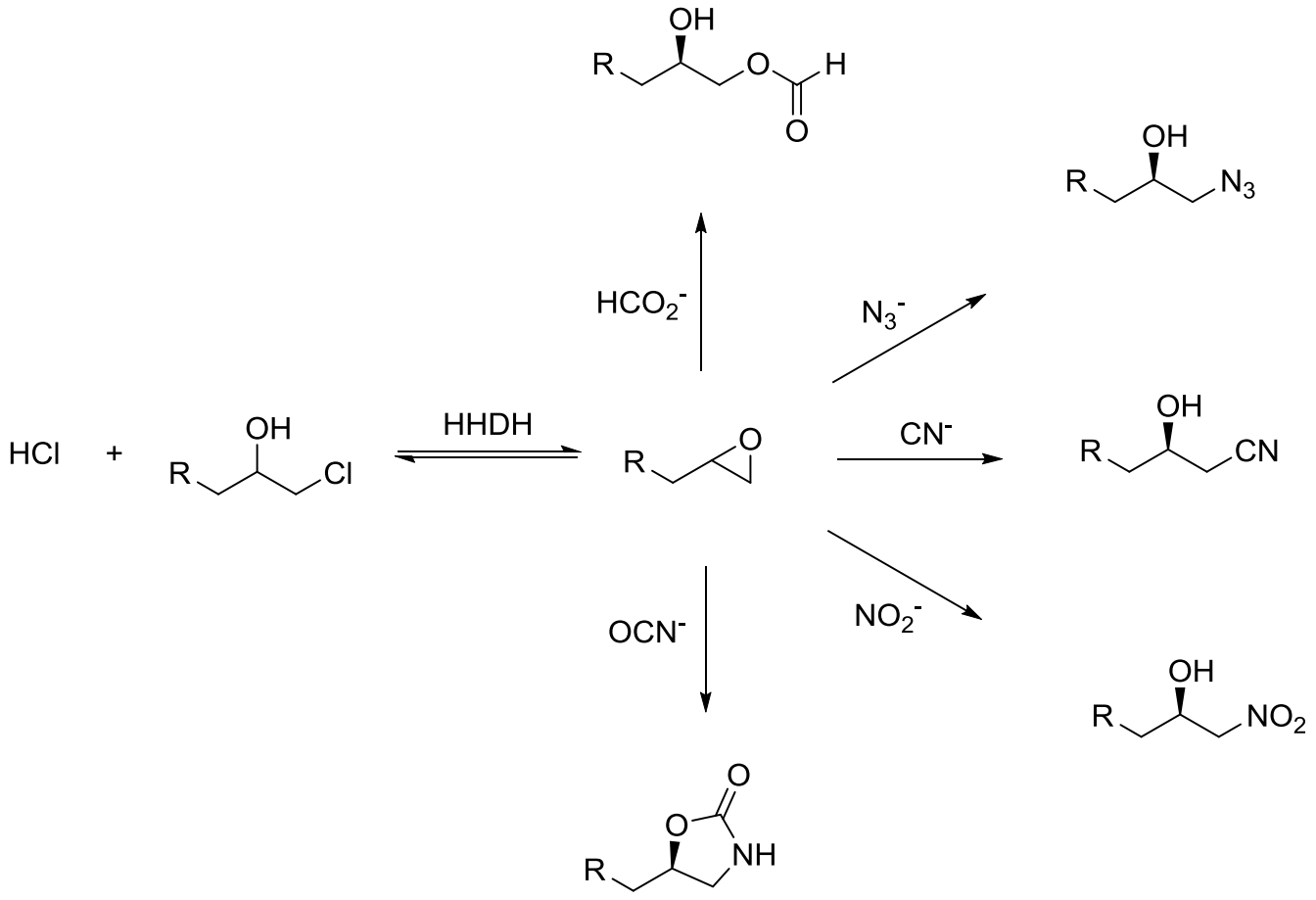




Chem. Soc. Rev., **2017**, *46,* 2678-2691
Tetrahedron Lett. **1992**, *33*, 7287–7290
OPRD, **2011**, *15*, 294-300

3) Wild-type Enzymes : HHDH

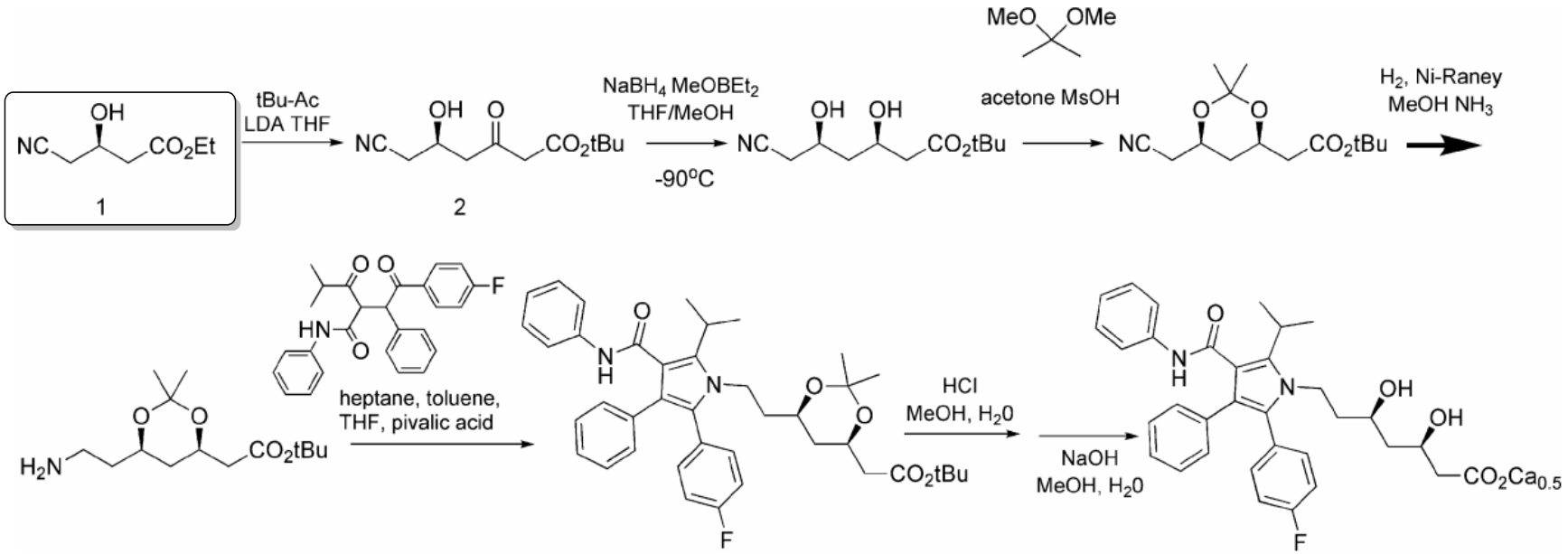
- Halohydrin dehalogenases

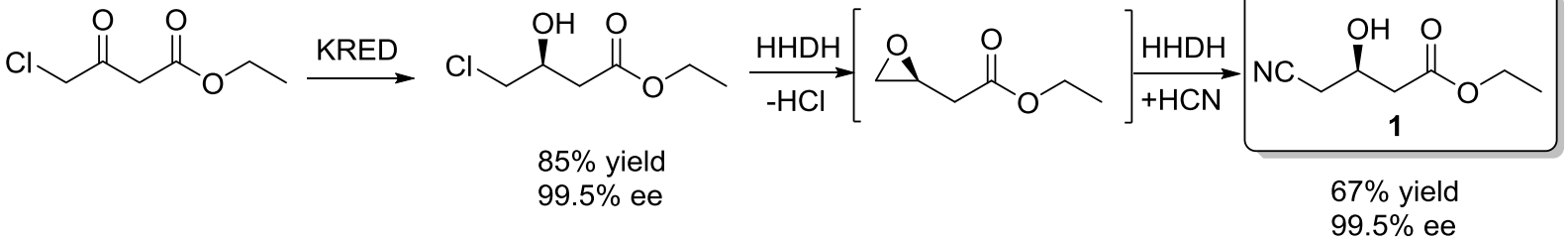


ChemBioChem., **2008**, *9,* 1048-1051

3) Wild-type Enzymes : HHDH

Synthesis of **Atorvastatin** using wild-type enzymes

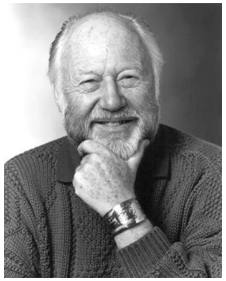




Green Chem., **2010**, *12, 81-86*

4) Protein Engineering

Rational design



Michael Smith

Known Protein Structure (X-Ray)

Site-directed mutagenesis (SDM)

If the mechanism is known, specific amino acids can be rationally exchanged
e.g. steric hindrance

Smith, *J. Biol. Chem*., **1978**, *253*, 6551–6560
Also see : *J. Biol. Chem*., **2006**, *281*, e31

Directed Evolution

Frances. H. Arnold

No structural information required

Random mutagenesis, creation of a mutant library, screening for desired properties, selection of the best mutants. Darwinian-type evolution.

F. H. Arnold, *Biotechnol. Bioeng.,* **1992**, *39*, 658–662
Also see : ACIE, **2018**, *57*, 4143 –4148

Combination of both

General Wisdom :

Directed Evolution can help identifying amino acids that should be substituted to improve performance

In many cases, but not all :

Additional effect of beneficial mutations

Mutations closer to the active more effectively influence the catalytic properties of enzymes

Chem. Rev., **2011**, *111*, 4141-4164
Trends Biotechnol., **2005**, *23*, 231
Curr. Opin. Chem. Biol. **2005**, *9*, 195

5) Applications of Designed Enzymes

A) KRED : Ketone Reductases

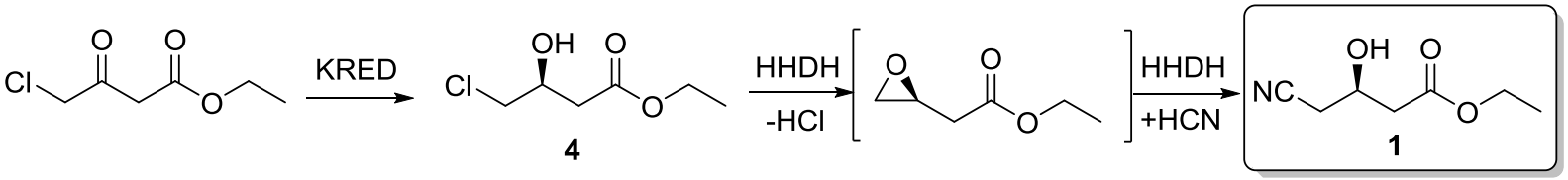
B) Transaminases

C) Diels-Alderase

D) Olefin Cyclopropanation

5) Applications of Designed Enzymes : KREDs

Atorvastatin : Wild-type enzymes were subjected to directed evolution techniques to improve process efficiency



Overall yield >90%

KRED

Parameter	Process design	Initial process	Final process
Substrate loading/g L^{-1}	160	80	160
Reaction time/h	<10	24	8
Biocatalyst loading/g L^{-1}	<1	9	0.9
Isolated yield/%	>90	85	95
Chemical purity/%	>98 (GC)	>98	>98
E.e. of **4**/%	>99.5	>99.5	>99.9
Phase separation of organic product phase from aqueous phase containing enzyme/min	<10	>60	<1
Space–time yield/$g_{product}$ L^{-1} d^{-1}	>384	80	480
Catalyst yield ($g_{product}/g_{cat}$)	>160	9	178

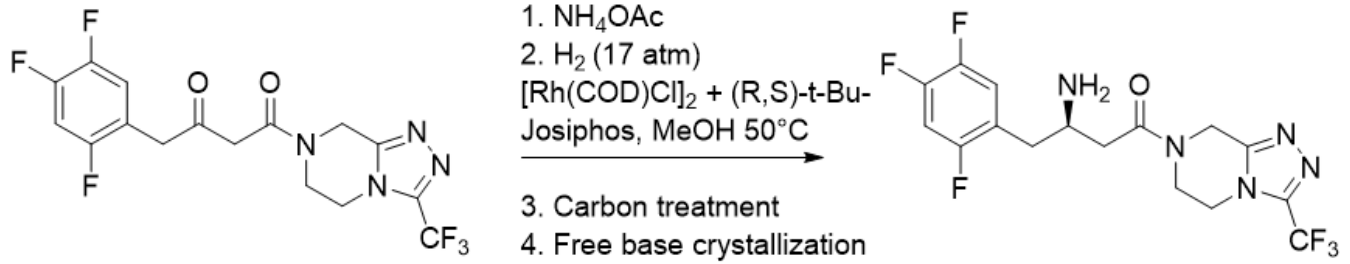
HHDH

Parameter	Process design	Initial process	Final process
Substrate loading/g L^{-1}	≥120	20	140
Reaction time/h	8	72	5
Biocatalyst loading/g L^{-1}	1.5	30	1.2
Isolated yield/%	>90	67	92
Chemical purity/%	>98	>98	>98
E.e. of **1**/%	>99.5	>99.5	>99.5
Phase separation of organic product phase from aqueous phase containing enzyme/min	<10	>60	<1
Space–time yield/$g_{product}$ L^{-1} d^{-1}	>360	7	672
Catalyst yield ($g_{product}/g_{cat}$)	80	0.7	117

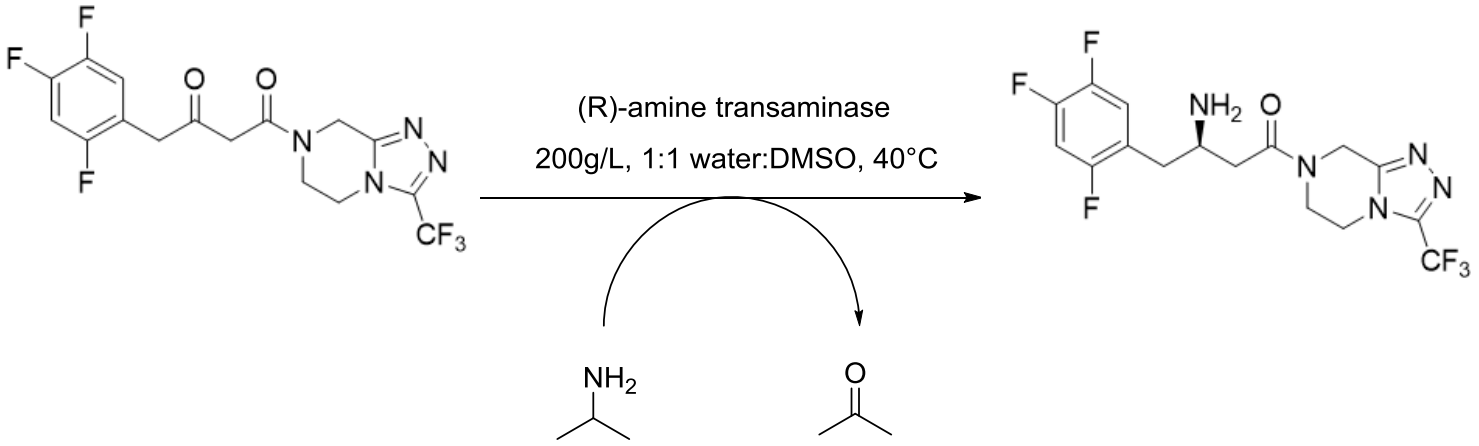
Green Chem., **2010**, *12, 81-86*

5) Applications of Designed Enzymes : Transaminases

Sitaglyptin Synthesis : Chemocatalytic route vs. Enzymatic route



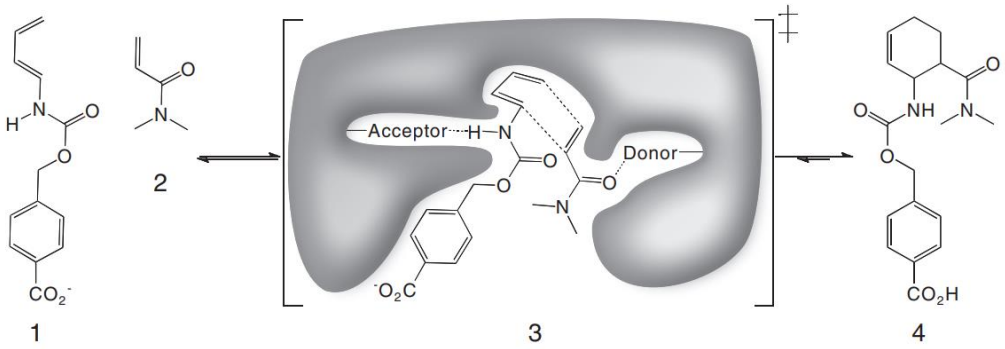
91% yield
99.5% ee

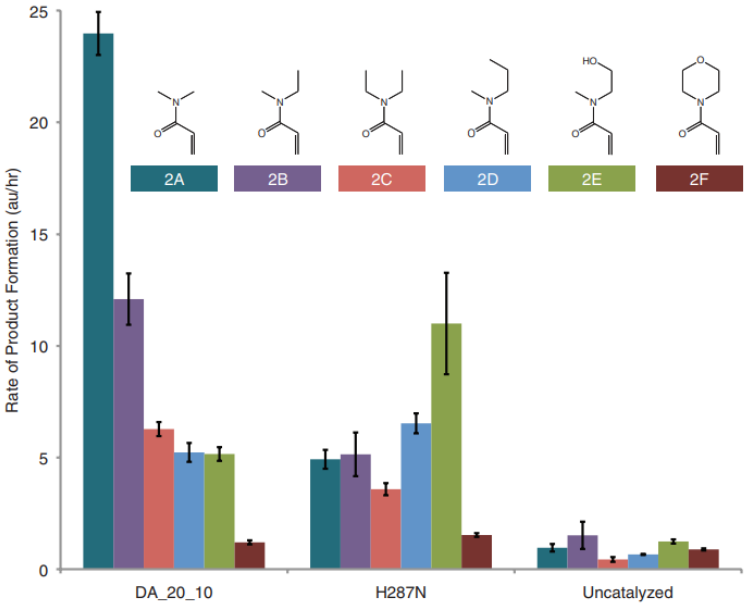


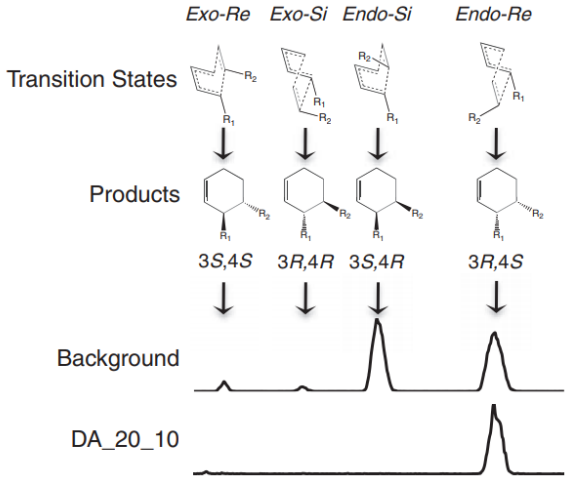
92% yield
>99.95% ee

ACIE, **2011**, *50, 1974-1976*

5) Applications of Designed Enzymes : Diels-Alderase

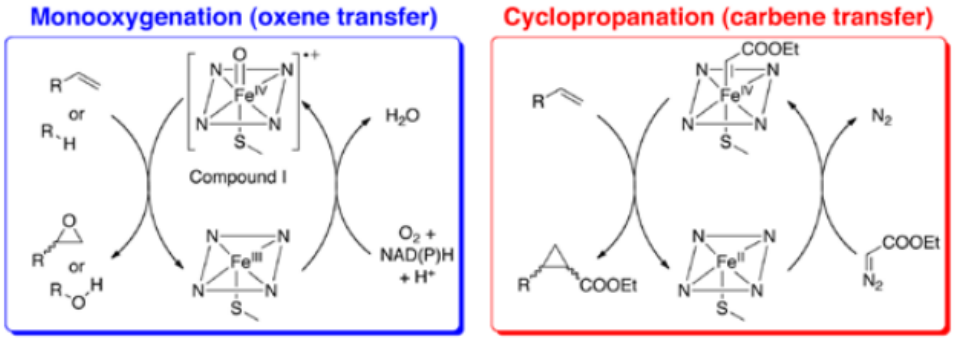


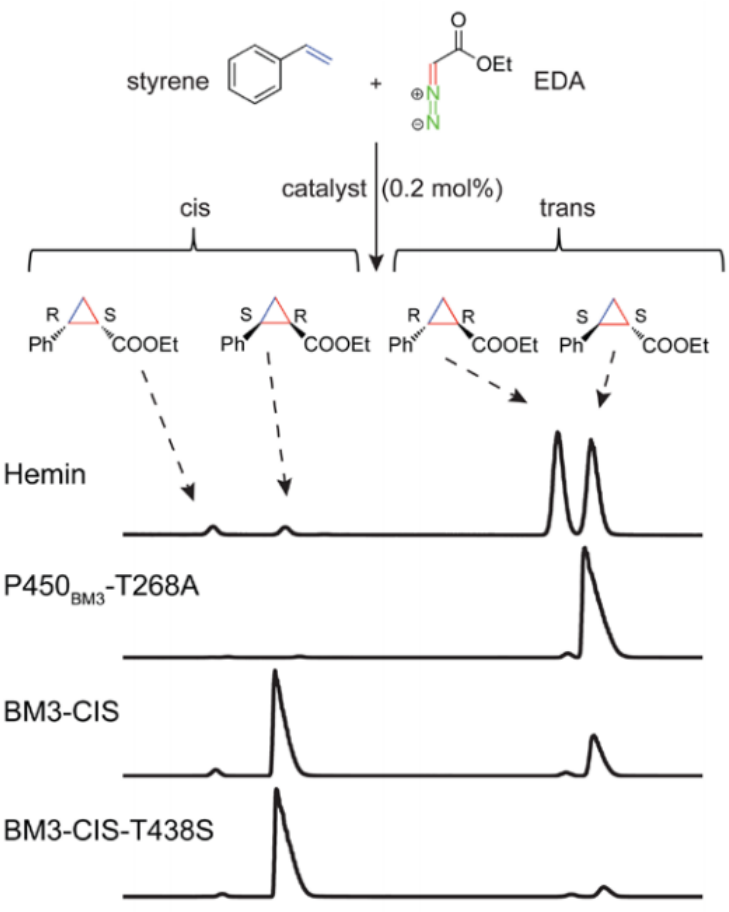




D. Baker, *Science*, **2010**, *329, 309-313*

5) Applications of Designed Enzymes : Cyclopropanation





F. Arnold, *Science*, **2012**, *1231434*