



# **Biocatalysis:** A valuable tool for cost savings in the synthesis of pharma intermediates and APIs

Oliver May  
Competence Manager Biocatalysis  
San Francisco, January 26th

# Content



## *Technology:*

- Introduction to Biocatalysis and Biodiversity

## *Application:*

- Divers and Challenges
- Examples

## *Summary:*

- Two take home messages





## Some Statements about Biocatalysis

***“The overall impact [of biocatalysis] on the chemical process industry will be slight. Organic chemistry will continue as its mainstream.”***

*R. L. Hinman, A Role for Biotech in Producing Chemical? Bio/Technology 1991, 9, 533 (1991)*

**Only 10 years later...**

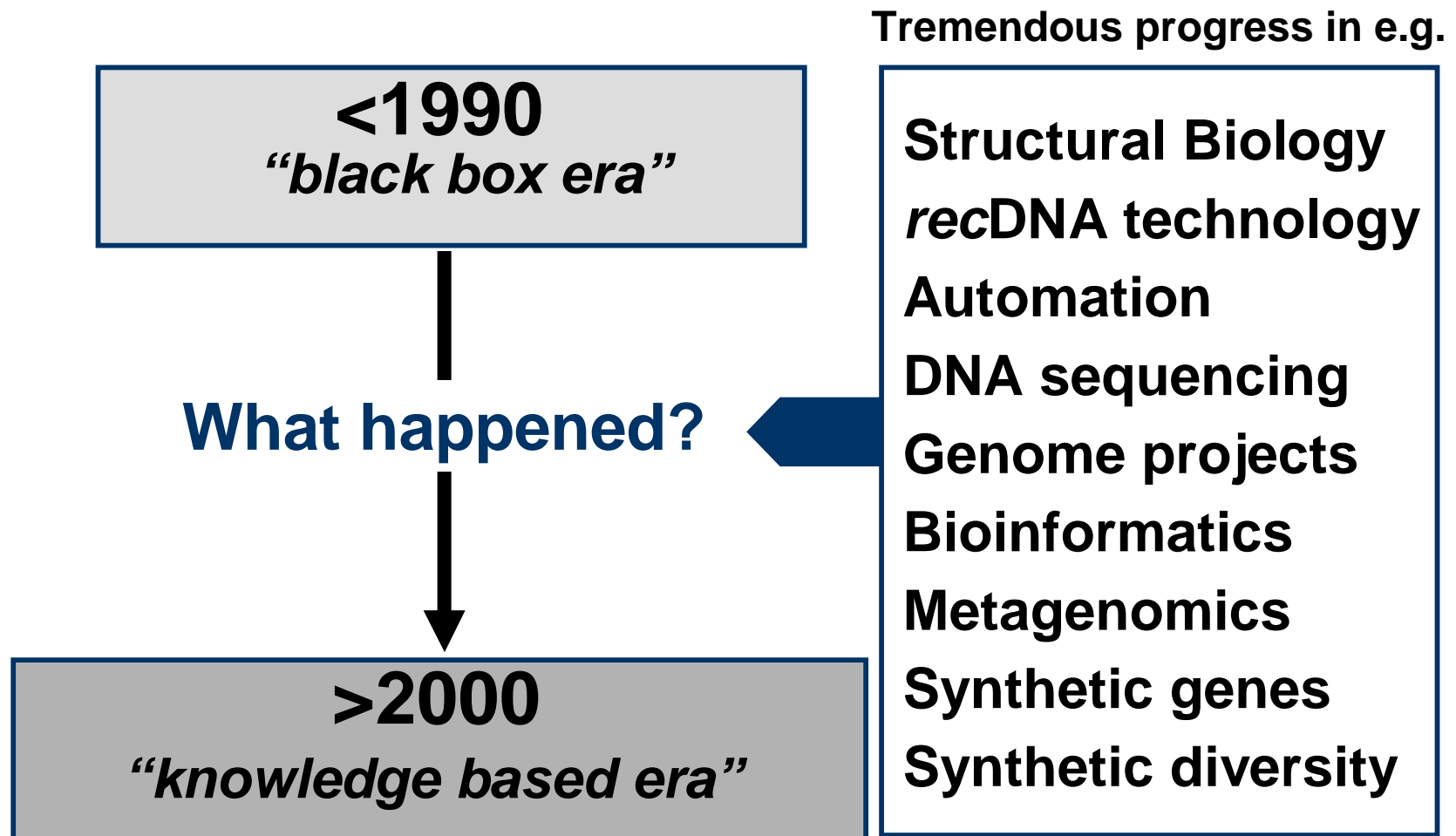


***“In biocatalysis, we are no longer limited by our ignorance. We are only limited by our imagination.”***

*Frances H. Arnold  
Caltech, 1999*

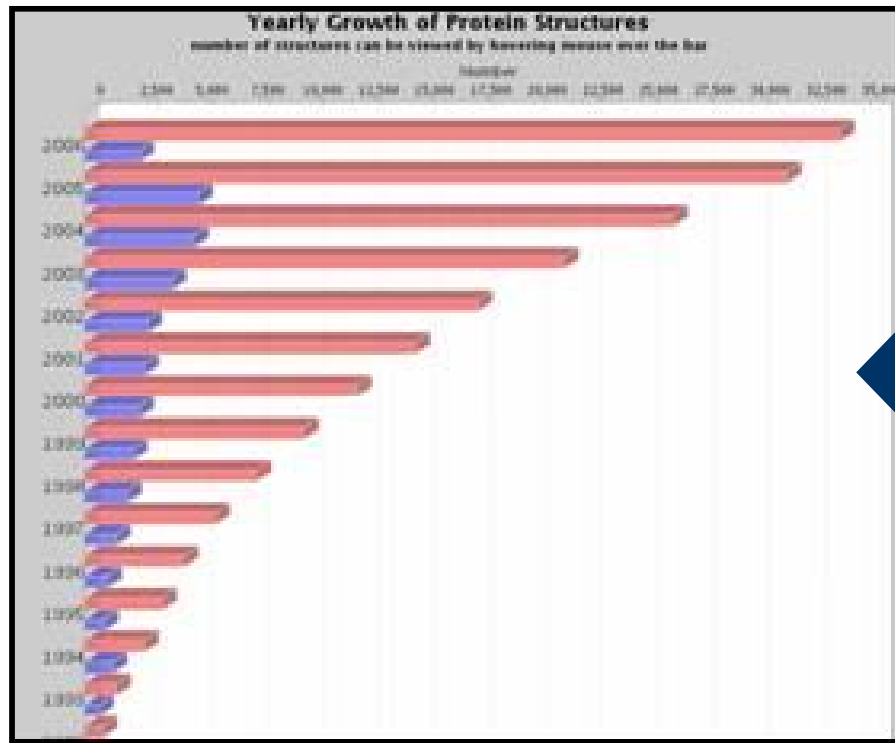


# Biocatalysis is emerging...





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## **Structural Biology**

**recDNA technology**

**Automation**

**DNA sequencing**

**Genome projects**

**Bioinformatics**

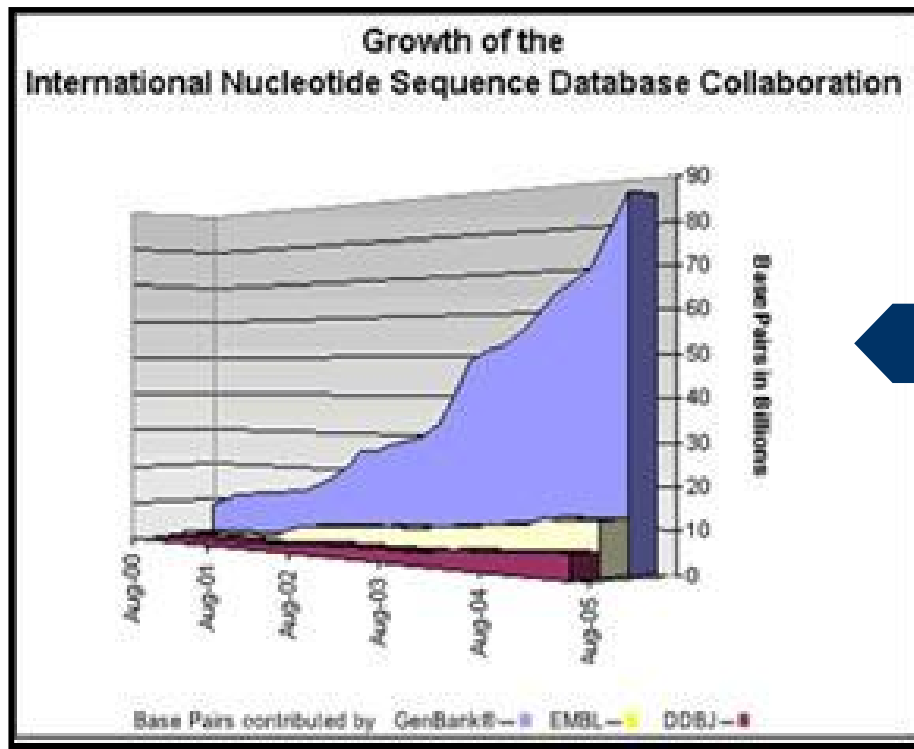
**Metagenomics**

**Synthetic genes**

**Synthetic diversity**



# Biocatalysis is emerging...



**Structural Biology**  
**recDNA technology**  
**Automation**  
**DNA sequencing**  
**Genome projects**  
**Bioinformatics**  
**Metagenomics**  
**Synthetic genes**  
**Synthetic diversity**

# Biocatalysis is emerging...



Several 200 l ocean water from Sargasso Sea:

over **1.2 million** previously unknown genes discovered

**Structural Biology**  
**recDNA technology**  
**Automation**  
**DNA sequencing**  
**Genome projects**  
**Bioinformatics**  
**Metagenomics**  
**Synthetic genes**  
**Synthetic diversity**

Venter et al. *Science* (2004): Vol. 304. no. 5667, pp. 66 - 74



# Our Biocatalysis Toolbox is rapidly growing

**Past**



**Present**



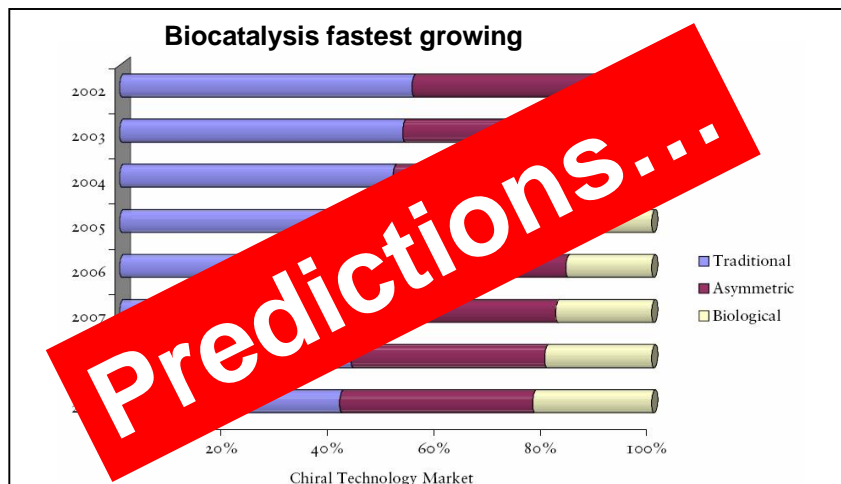
**Future**



# Trends in Biocatalysis



The number of **applications will be growing** due to significant advances in technology (e.g. access to biodiversity, enzyme engineering,...)



# About Predictions



***“In forecasting the future of scientific research there is one quite general law to be noted. The unpredicted always happens.***

***So one can be quite sure that the future will make any detailed prediction look rather silly.”***

**J.B.S. Haldane (1892-1964)  
in “The Future of Biology”**



# About Predictions



***“I believe in horses.  
The automobile is a transient phenomenon.”***

**Wilhelm II, last German emperor (1905)**

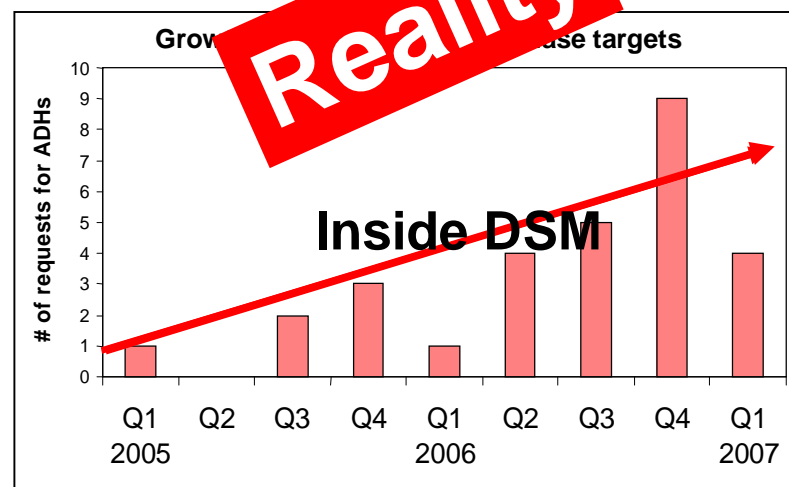
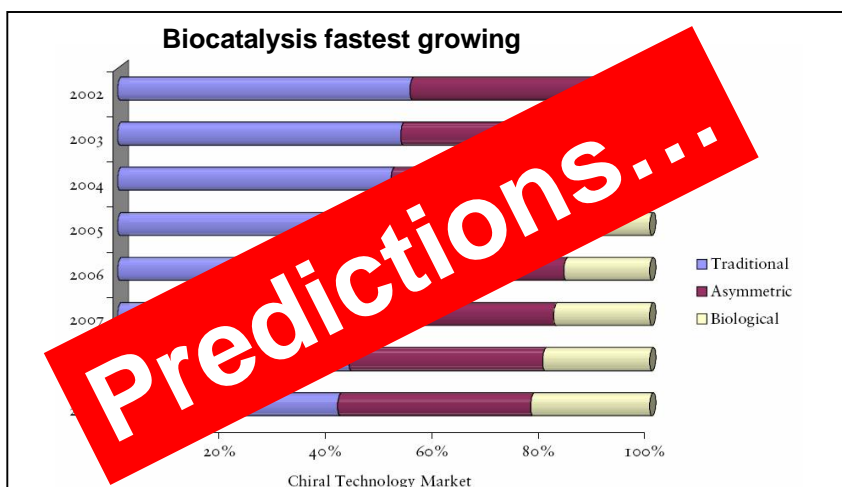
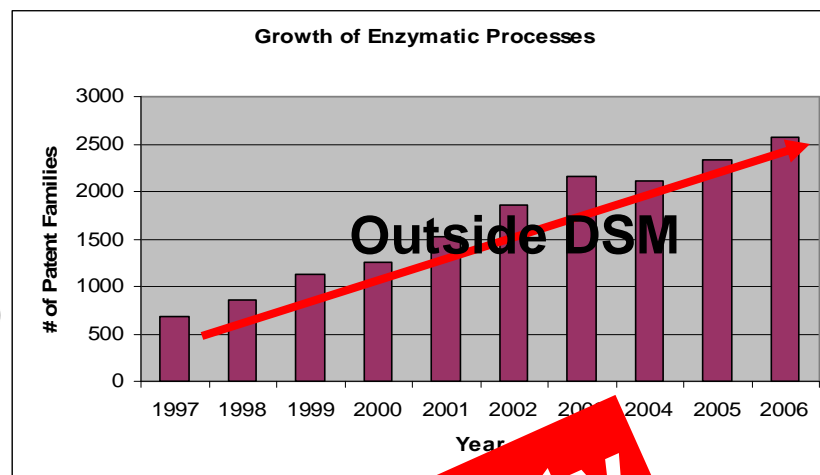


**First invented car (Carl Benz, 1886)**

# Trends in Biocatalysis



The number of applications will be **growing** due to significant advances in technology (e.g. access biodiversity, enzyme engineering,...)



**Reality**

# From DSM track record to our believe in Bio



Product	Process	Food	Pharm & Fine Chem
Aspartame	Biocatalysis: Protease	•	•
Citric Acid	Fermentation	•	•
Vit. C	Biotransformation: sorbitol → sorbose	•	•
Vit. B2	Fermentation: Metabolic Engineering	•	•
D-p-Hydroxyphenylglycine	Biocatalysis: Hydantoinase / Carbamoylase	•	•
6-APA	Fermentation & Biocatalysis: Acylase	•	•
7-ADCA	Fermentation & Biocatalysis: Metabolic Engineering, Acylase	•	•
Cephalexin	Biocatalysis: Acylase	•	•
Agro application	Biocatalysis: Hydroxynitrile Lyase	•	•

→ **>100 products and applications**

Production of Fine Chemicals, Anti-infectives and Vitamins – Commercial products ranging from 100 tpa to 100,000 tpa

Product	Process
Citric Acid	Fermentation
Vit. C	Biotransformation: sorbitol → sorbose
Vit. B2	Fermentation: Metabolic Engineering
Aspartame	Biocatalysis: Protease
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# DSM Biocatalysis

## Competence in Depth & Breadth unmatched!



Combined strengths and experience makes DSM unique in biocatalysis

Strain devel.  
Enzyme devel.  
Fermentation.



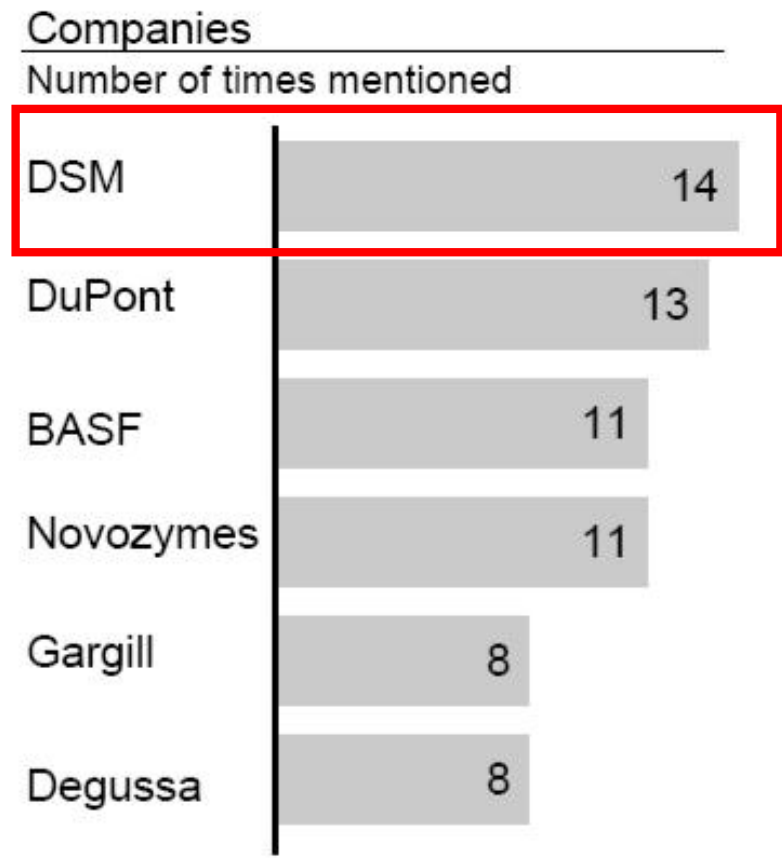
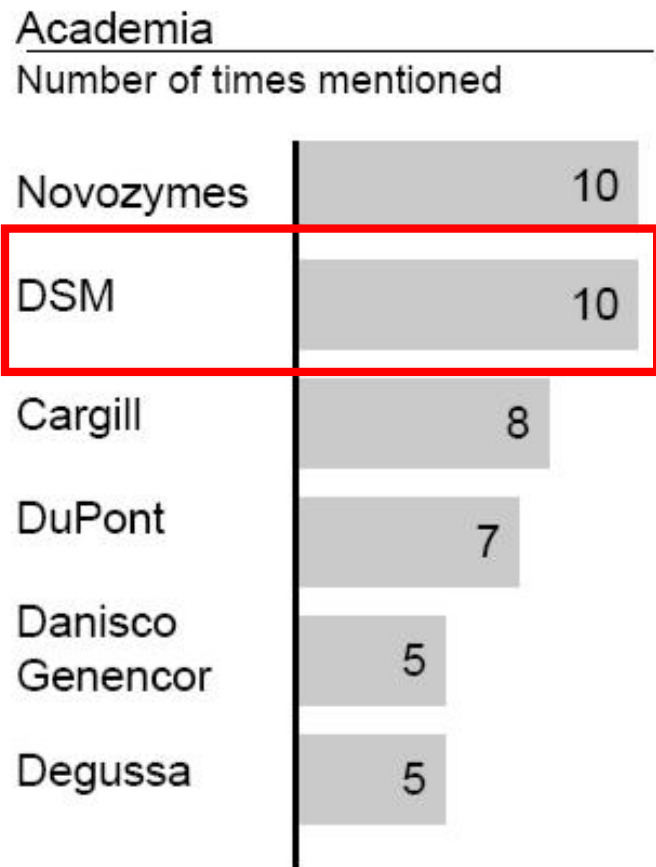
Enzyme manufacturing

Chemistry  
Route scouting  
Platform screening

Enzyme application  
Regulatory (cGMP)



# Outside world: Which Companies will be successful in Industrial Biotechnology in the Long Run?



Industrial Biotech Expert Survey 2006  
McKinsey&Company



# Content

## *Technology:*

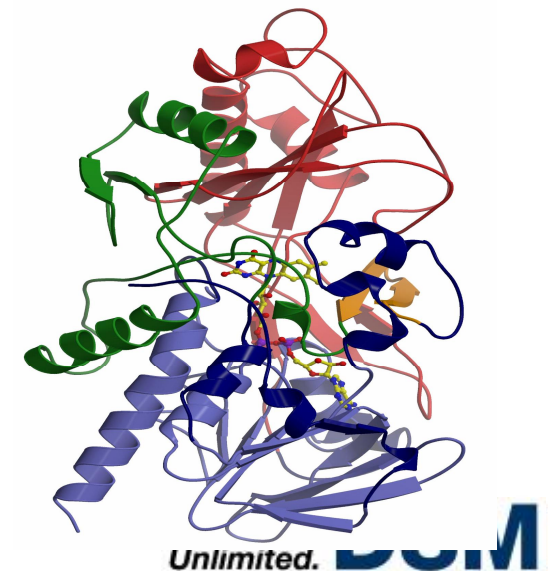
- Introduction to Biocatalysis and Biodiversity

## *Application:*

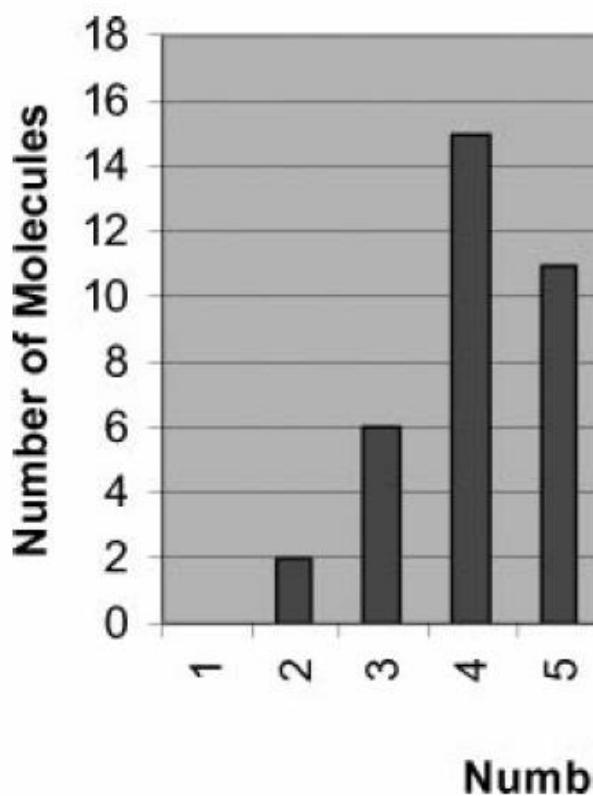
- Divers and Challenges
- Examples

## *Summary:*

- Two take home messages



# Driver: Cut down steps – Improve efficiency



**DSM Route Scouter**

**FDA APPROVAL**

ROUTE OPTIONS: enzymes included!

ARRIVAL TIME: 2 months earlier

DISTANCE: 3 steps less

**Find a Shortcut with DSM Enzymes**

More Than 1000 Enzymes Updated with PharmaPLE™ Reductases Aldolases

DSM

Source: *Org.Biomol.Chem.*, 2006, 4, 2337-2347

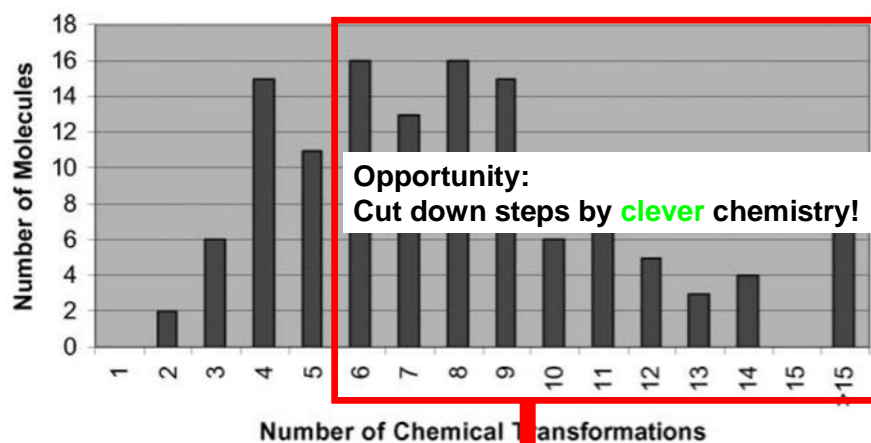
Unlimited. **DSM**



# Pharma Need:

efficient manufacturing processes for very complex products

Pharma Syntheses are Multi-step Syntheses!



Source:  
*Org.Biomol.Chem.*, 2006,  
4, 2337-2347

**Asymmetric Synthesis**

(e.g. HCN addition, ketone reduction, transamination, NH<sub>3</sub> addition)



**100% yield**  
**>99.9% ee**



**Desymmetrization**  
(e.g. nitrile, ester, epoxide hydrolysis)



**Dynamic kinetic resolution & deracemization**

(e.g. hydrolysis of hydantoins, esters or acylation of alcohols, redox cycles for amines and alcohols)



# Examples of established pharma processes based on enzymatic transformations

Product	Reaction Type	Enzyme Platform
Dorzolamide;	reduction;	<i>ketoreductase</i>
Nelfinavir;	desymmetrization;	<i>epoxide hydrolase</i>
Pregabalin;	stereoselective hydrolysis;	<i>lipase</i>
Cholest. Ester; (highlighted)	C-C bond formation;	<i>transaminase</i>
Pyrethrin; (highlighted)	stereoselective hydrolysis;	<i>esterase</i>
Cardiovascular; (highlighted)	asymmetric amide addition;	<i>amidase</i>
Cardiovascular; (highlighted)	asymmetric NH <sub>3</sub> addition;	<i>transaminase</i>
Antibiotic; (highlighted)	dynamic kinetic resolution;	<i>hydantoinase</i>
Blood Pressure; (highlighted)	stereoselective hydrolysis;	<i>hydrolase (esterase)</i>

**2nd Generation processes**

**Many 100% concepts...**

**Increasing number of different platforms applied**

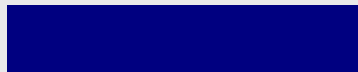
--- DSM Examples

- **Substitution due to significant cost savings!**
- **First time right is still exception**



# Typical Development Timelines

**Substrate / product  
preparation**



*ca. 2-4 weeks*

**Develop analytics  
(%ee, yield)**



*ca. 2 weeks*

**Enzyme  
screening**



*ca. 2-4 weeks*



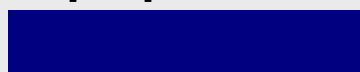
**Identification of  
enzyme which**

- Accepts substrate
- Shows desired selectivity



# Typical Development Timelines

Substrate / product preparation



ca. 2-4 weeks

Develop analytics (%ee, yield)



ca. 2 weeks

Go / no go for

Catalyst preparation

- Reaction engineering
- Process development
- Sample supply
- Large-scale quotation

**Timelines not different to any other technology!**

Finishing

ca. 2-4 weeks

ca. 1,5-2,5 months

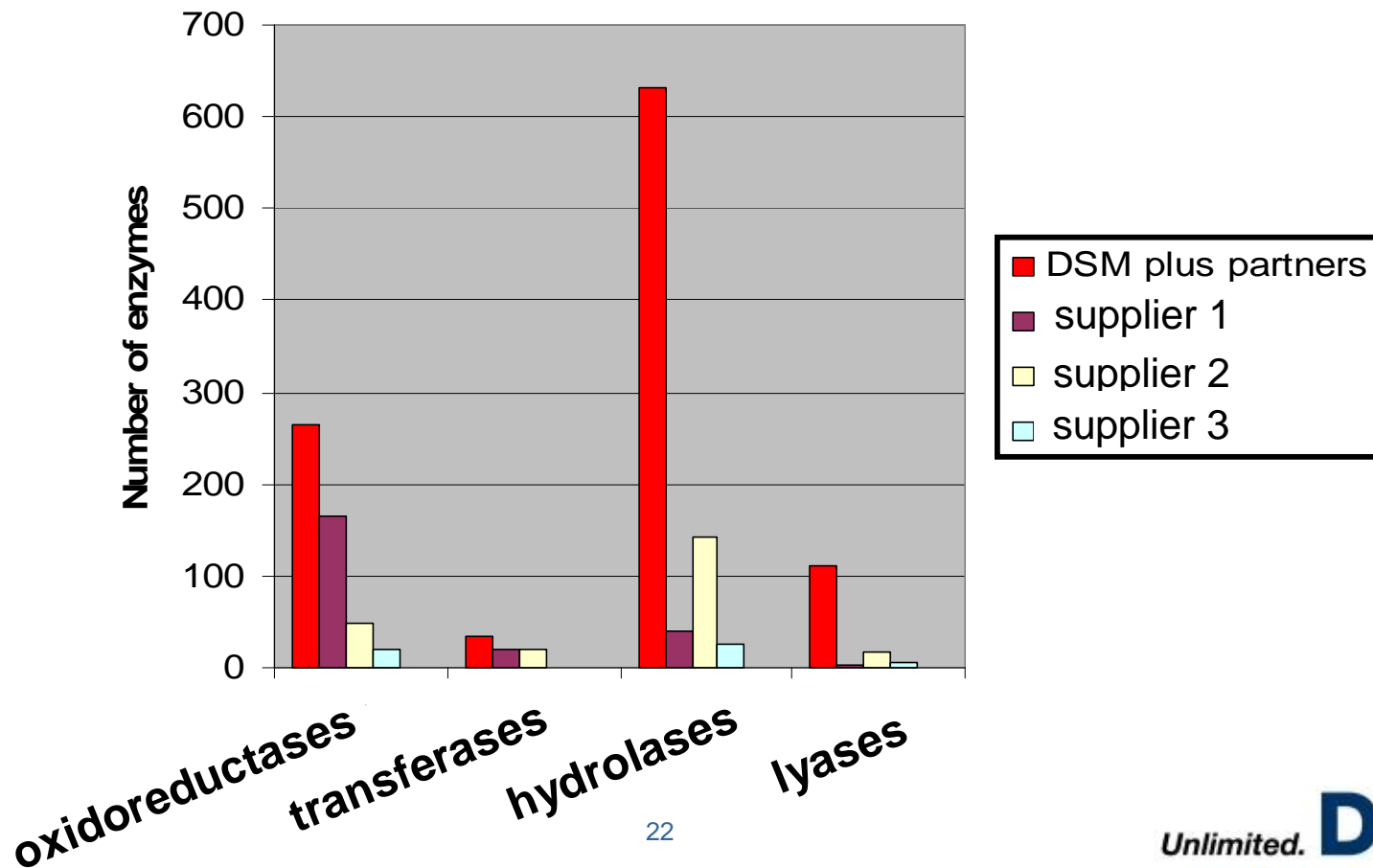
ca. 2-3 months

Unlimited. **DSM**

# Success factors



- **Broadest collection of off-the-shelf enzymes**



# Success factors

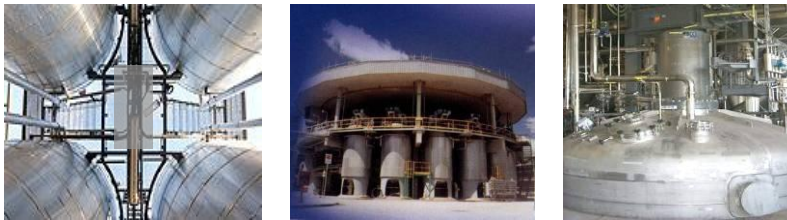


- **Broadest collection of off-the-shelf enzymes established and expanded @ DSM through:**
    - In-house development (Corporate Research)
    - Partnering (Verenium, IEP, Libragen,...)
    - Use of suppliers (Codexis, Novozymes, Amano,...)
    - Academic network (Graz, Manchester, IBOS, B-BASIC...)
- 
- **Increase chance to identify suited enzyme**
  - **Reduce development cost**
  - **Timelines fit to pharma needs**

# Success factors



- **Broadest collection of off-the-shelf enzymes**
- **Secure supply at any scale (and with FTO!)**



**In-house enzyme production (Capua >1.400.000 l)**



**Broad range of proprietary Production organisms (pluGbugs)**

# Success factors



- Broadest collection of off-the-shelf enzymes
- Secure supply at any scale (and with FTO!), fast scale-up
- Experience (also regulatory) in large-scale implementation

Biocatalysis Platforms	Product Classes
L-Amidases	$\alpha$ -Amino acids, $\alpha$ -Hydroxy acids
D-Hydantoinase / D-Carbamoylase	$\alpha$ -Amino acids
R- and S-hydroxynitrile lyases	$\alpha$ -/ $\beta$ -Amino acids, $\alpha$ -H...
Ammonia lyases	$\alpha$ -Amino acids
Acyases	$\alpha$ -/ $\beta$ -Amino acids, Amines, Amino-nitriles
Lipases, Esterases	Carboxylic acids, Alcohols, Amines
Proteases	
Aldolases	1,2-Diols, Aminoalcohols, Hydroxyesters
Epoxide hydrolases	Epoxides, 1,2-Diols
Racemases, Isomerases	Racemization
Haloperoxidases	Alcohols, Epoxides, Sulfoxides
Hydratases	$\alpha$ - / $\beta$ -Hydroxy acids
Nitrilases	$\alpha$ - / $\beta$ -Amino acids, $\alpha$ -Hydroxy acids
Reductases & Dehydrogenases	Alcohols, other chiral intermediates

**>25 industrial processes implemented**



# Success factors



- **Broadest collection of off-the-shelf enzymes**
- **Secure supply at any scale (and with FTO!), fast scale-up**
- **Experience (also regulatory) in large-scale implementation**
- **Interdisciplinary route scouting (including all options!)**



# DSM: Leader in Industrial Biocatalysis



## Examples

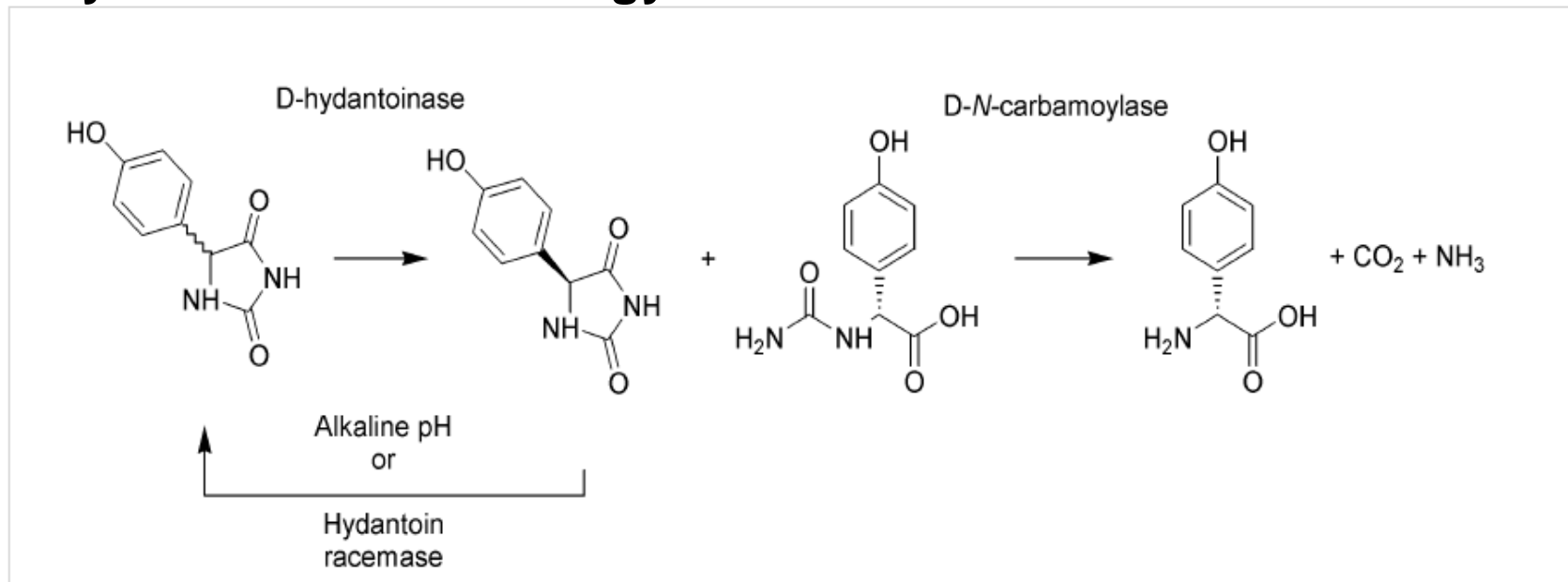
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Ammonia lyases	$\alpha$ -Amino acids
Acyases	$\alpha$ -/ $\beta$ -Amino acids, Amines, Amino-nitriles
Lipases, Esterases	Carboxylic acids, Alcohols, Amines
Proteases	
Aldolases	1,2-Diols, Aminoalcohols, Hydroxyesters
Epoxide hydrolases	Epoxides, 1,2-Diols
Racemases, Isomerases	Racemization
Haloperoxidases	Alcohols, Epoxides, Sulfoxides
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Reductases & Dehydrogenases	Alcohols, other chiral intermediates

**>25 industrial processes implemented**

# Example for Dynamic Kinetic Resolution



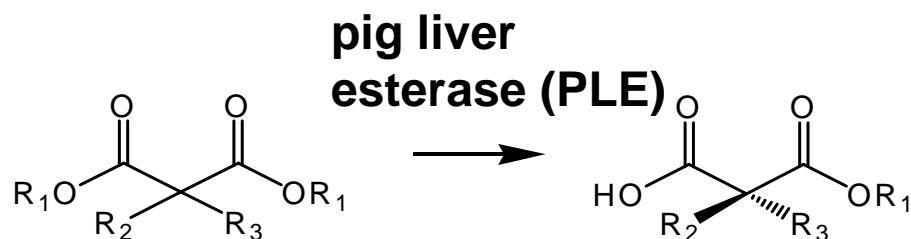
## Hydantoinase Technology



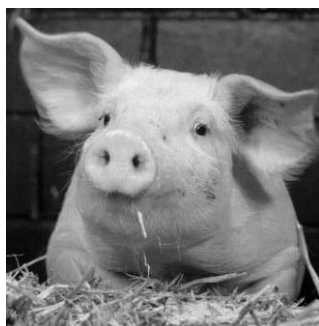
Source	Stereo-Specificity	Substrate Specificity
<i>Agrobacterium radiobacter</i>	<i>R</i>	Broad (Racemization)

Now also *S*!

# Example for Enzymatic Desymmetrization



**DSM has exclusive access to a  
non-animal derived  
porcine liver esterase!**



R <sub>2</sub>	R <sub>3</sub>	Yield	ee
<i>t</i> -Bu	Me	90 %	96 % ( <i>R</i> )
Me <sub>3</sub> -Si-CH <sub>2</sub> -	Me	95 %	98 % ( <i>R</i> )
Me <sub>3</sub> -Si-CH <sub>2</sub> -	H	92 %	10 ( <i>R</i> )
Naphthyl-	Me	95 %	> 95 % ( <i>R</i> )
Isobutyl-	Me	97 %	94 % ( <i>R</i> )
(4- <i>t</i> -butyl)-phenyl-	Me	83 %	> 97 % ( <i>R</i> )
Phenyl	Me	90 %	81 % ( <i>R</i> )
(4-methoxy-2-nitro)- phenoxy-	Me	78 %	80.5 % ( <i>R</i> )
Adamantylmethyl-	Me	80 %	> 98 % ( <i>R</i> )
3-biphenyl-	Me	80 %	> 98 % ( <i>R</i> )
Phenylallyl-	Me	88 %	96 % ( <i>R</i> )



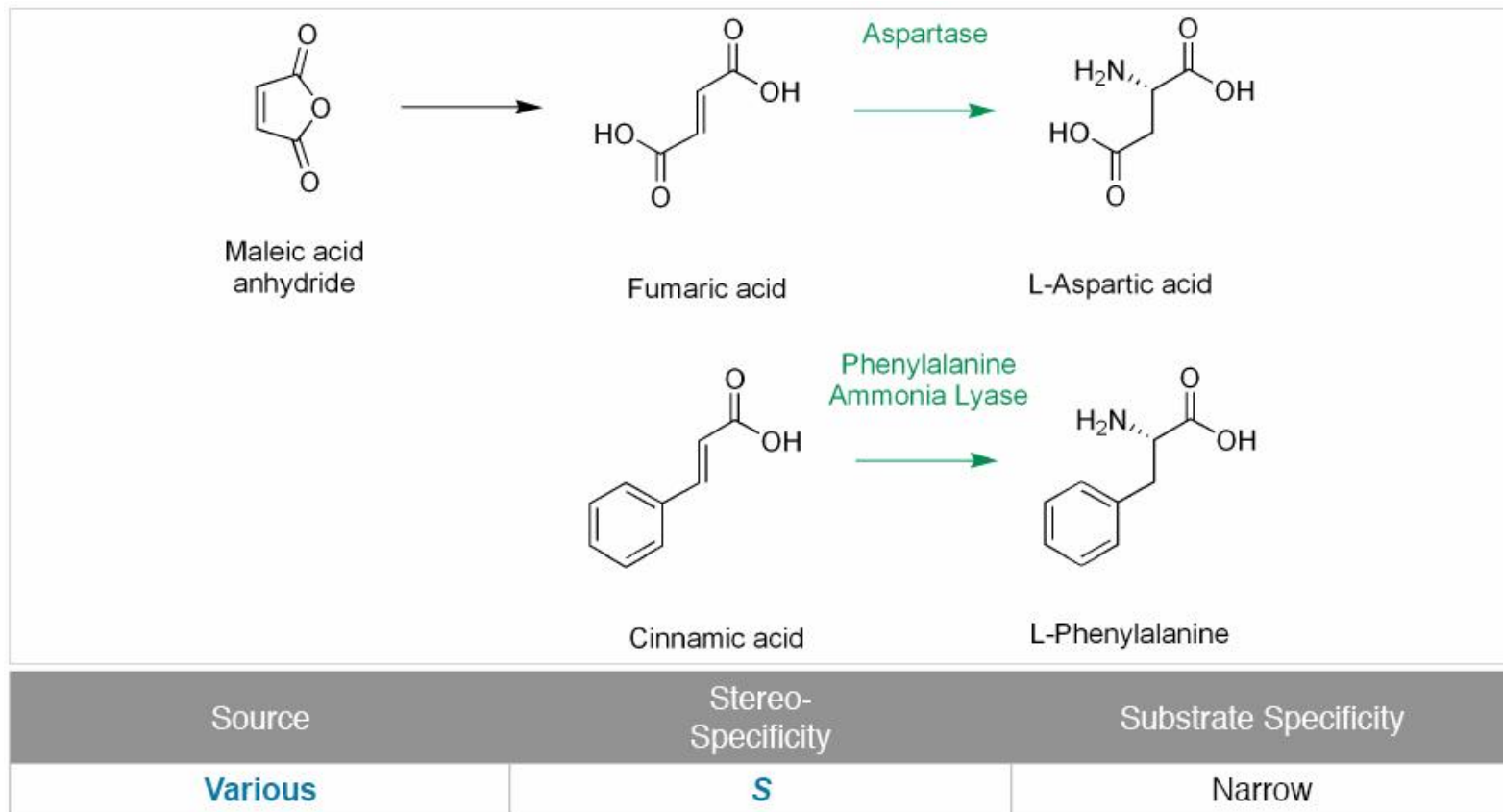
# DSM Innovation Award 2007



# Example for Asymmetric Synthesis (1)



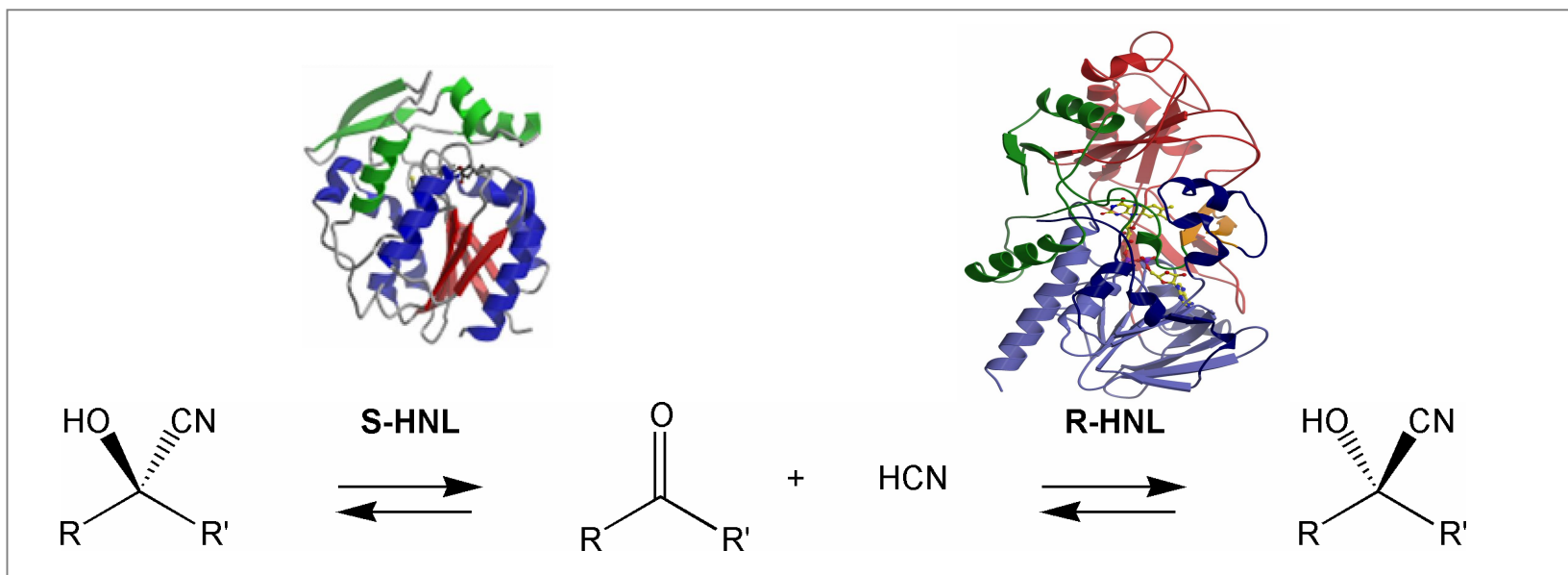
## Ammonia Lyase Technology



# Example for Asymmetric Synthesis (2)

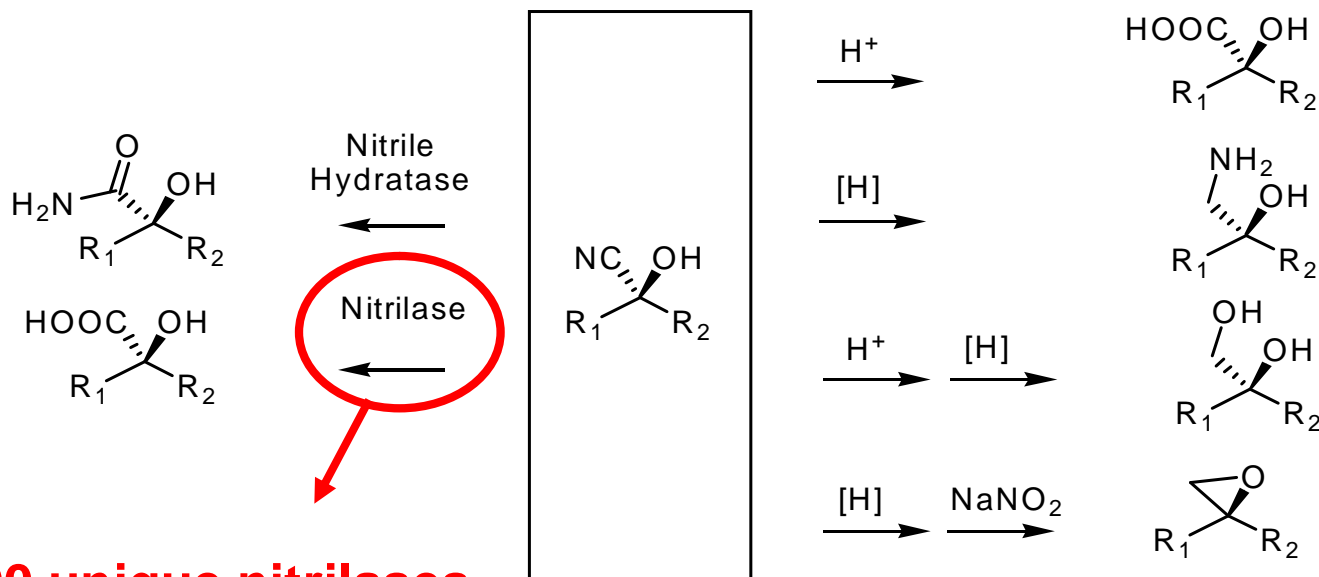
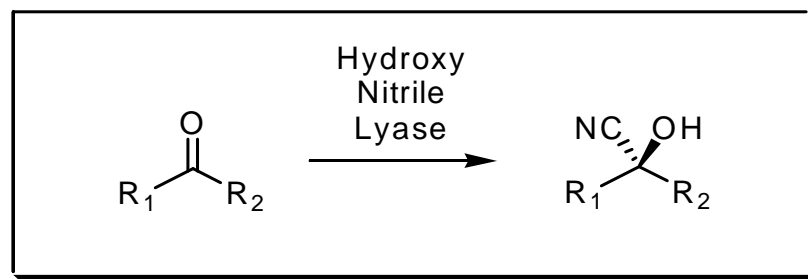


## Hydroxynitrile Lyase Technology



Source	Stereo-Specificity	Substrate Specificity
<b>Almonds</b>	<b>R</b>	Aliphatic, aromatic
<b>Rubber Tree</b>	<b>S</b>	Aliphatic, aromatic

# Platform offers Access to a wide Spectrum of Intermediates

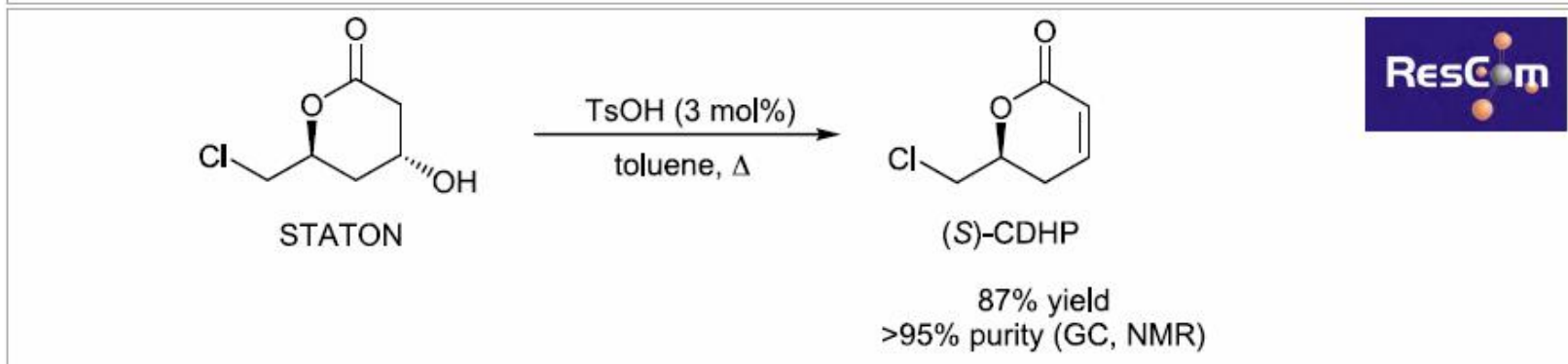
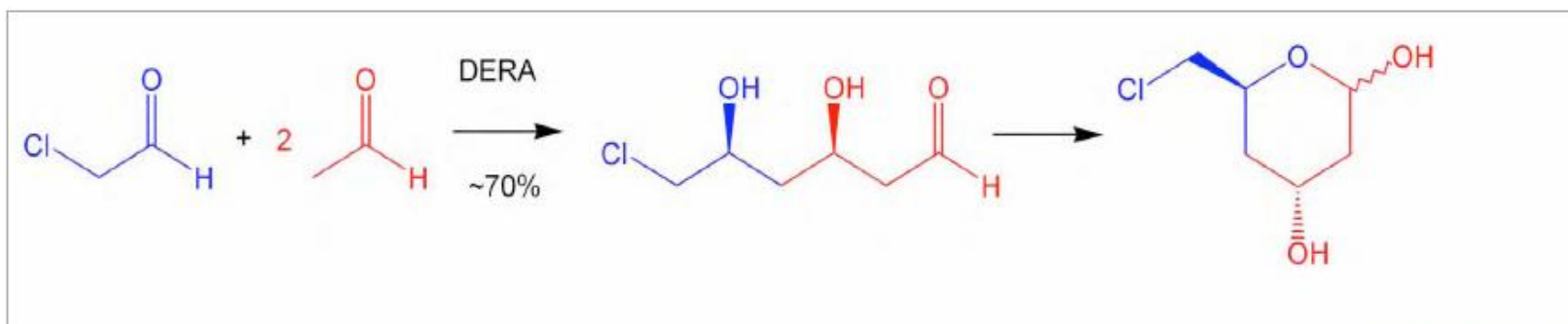


**>100 unique nitrilases  
in our screening platform**

# Example for Asymmetric Synthesis (3)



## Aldolase Technology

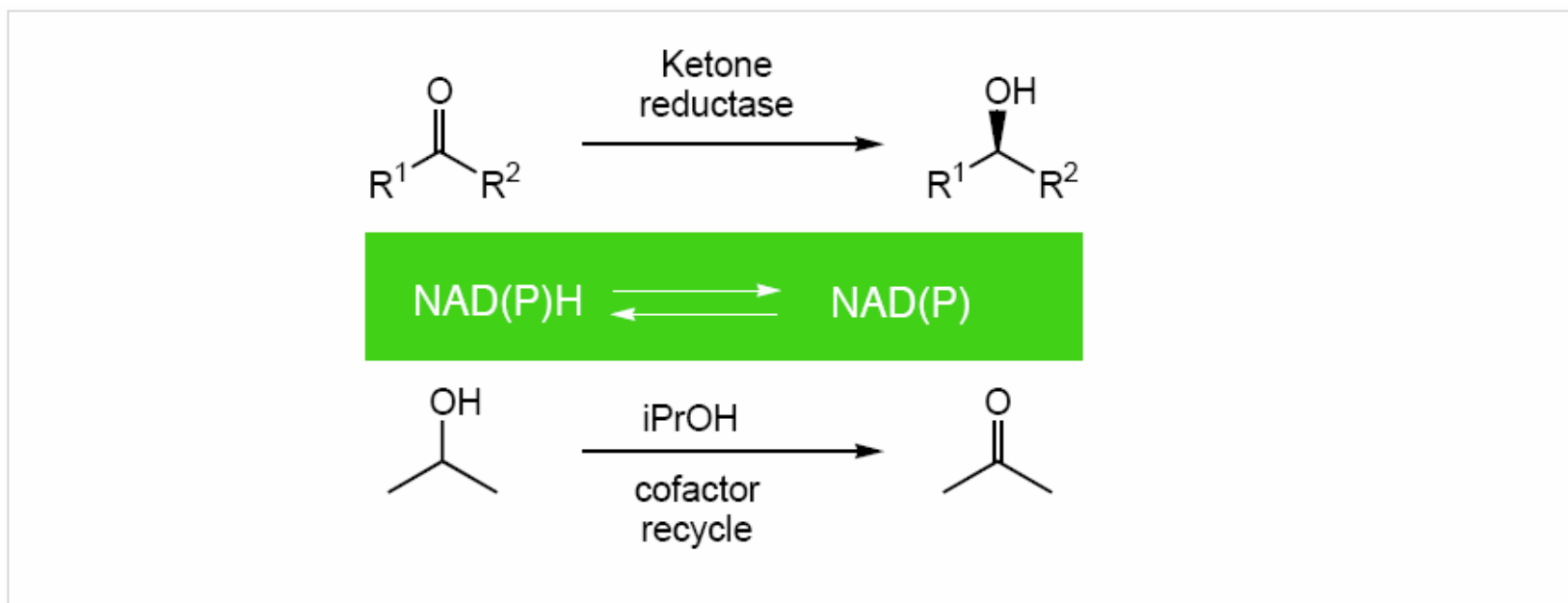


Source	Stereo-Specificity	Substrate Specificity
<i>E. coli</i>	3 <i>S</i> , 5 <i>R</i>	Broad (aldehydes)

# Example for Asymmetric Synthesis (4)



## Dehydrogenase Technology

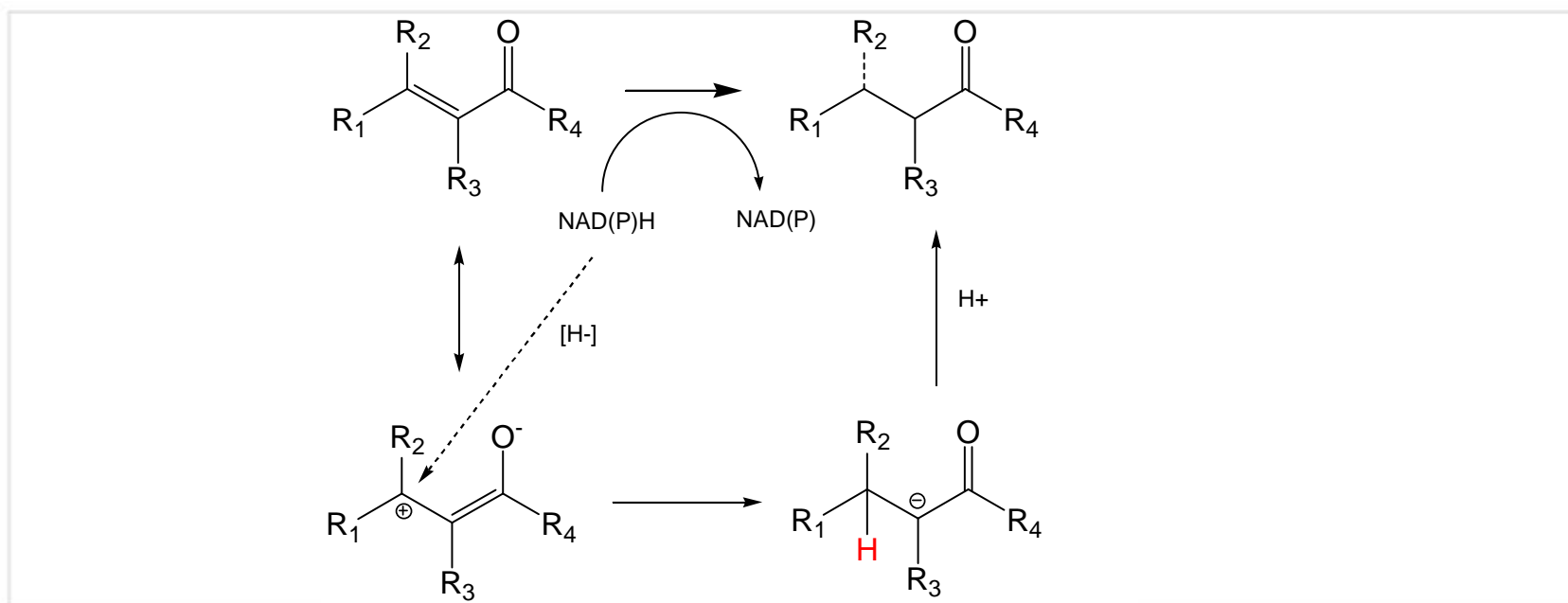


Source	Stereo-Specificity	Substrate Specificity
Various	<i>R</i>	Aliphatic, aromatic
Various	<i>S</i>	Aliphatic, aromatic

# Example for Asymmetric Synthesis (5)



## Enone Reductase Technology



Source	Stereo-Specificity	Substrate Specificity
Various	E/Z (R/S)	broad



# Content

## *Technology:*

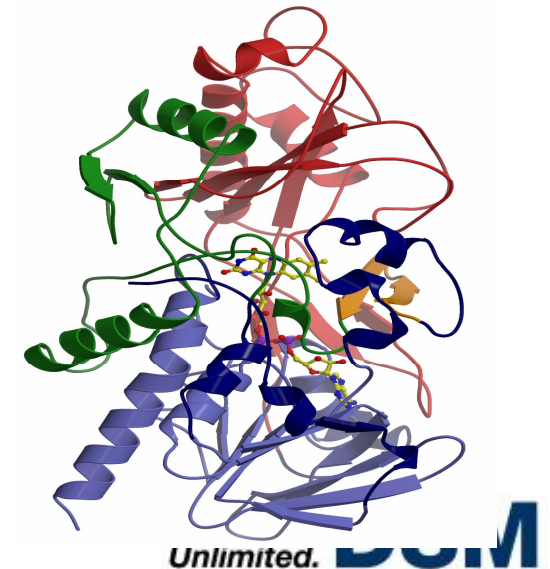
- Introduction to Biocatalysis and Biodiversity

## *Application:*

- Divers and Challenges
- Examples

## *Summary:*

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# Conclusions



- **Toolbox of Biocatalysis is rapidly developing**
  - More versatile due to better access to biodiversity
  - Proven and well established within DSM
- **Don't be afraid to kiss the “Biocatalysis Frog”**
  - It can be fast...
  - It can be highly rewarding...
  - **If you are still “afraid to kiss”, ask us...**





She doesn't need  
to understand

**biocatalysis.**

She needs to feel better.

*Unlimited.*

**DSM**



**For further information about biocatalysis,  
please contact:**

**Name**     **Dr. Oliver May**  
**Phone**    **+31 46 47 60551**  
**E-mail**    **[oliver.may@dsm.com](mailto:oliver.may@dsm.com)**

**Unlimited. DSM**