

# Strain and process development for the production of APIs

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Materials Valley Workshop

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**EVONIK**  
INDUSTRIES

# Die Geschäftsfelder



## Chemie

- Fokus: Spezialchemie
- Zählt zu den weltweit führenden Unternehmen
- 10,8 Mrd. € Umsatz 2007
- Führende Marktpositionen bei mehr als 80% des Umsatzes
- Marktorientierte Forschung & Entwicklung und modernes Innovationsmanagement
- Mehr als 100 Produktionsstandorte in rund 30 Ländern



## Energie

- Fokus: Steinkohlekraftwerke und erneuerbare Energien
- Technologisch führende Position bei Planung, Bau und Betrieb von Steinkohlekraftwerken
- 2,8 Mrd. € Umsatz 2007
- Fünftgrößter Stromerzeuger in Deutschland
- International erfolgreich mit Kraftwerken in der Türkei, Kolumbien, Philippinen
- Starke Stellung in Deutschland bei Biomasse und Geothermie



## Immobilien

- Fokus: Wohnimmobilien
- Eine der größten privaten Wohnungsgesellschaften in Deutschland
- Rund 60.000 eigene Wohneinheiten und 50% an der THS mit mehr als 75.000 Wohnungen
- 0,4 Mrd. € Umsatz 2007
- Aktiver Bestandshalter mit starker regionaler Fokussierung

# Evonik Degussa GmbH in figures



## Key Figures 2008:

Turnover:	11.5 Billion Euro
EBITDA:	1.6 Billion Euro
Employees	31 700

### Technical Specialties

Industrial  
Chemicals

Inorganic  
Materials

### Consumer Solutions

Consumer  
Specialties

Health and  
Nutrition

### Specialty Materials

Coatings and  
Additives

Performance  
Polymers

# Research & Development



**Research, development and innovation are key elements in our sustainable development growth strategy**



→ € 307 Mio R & D expenditure

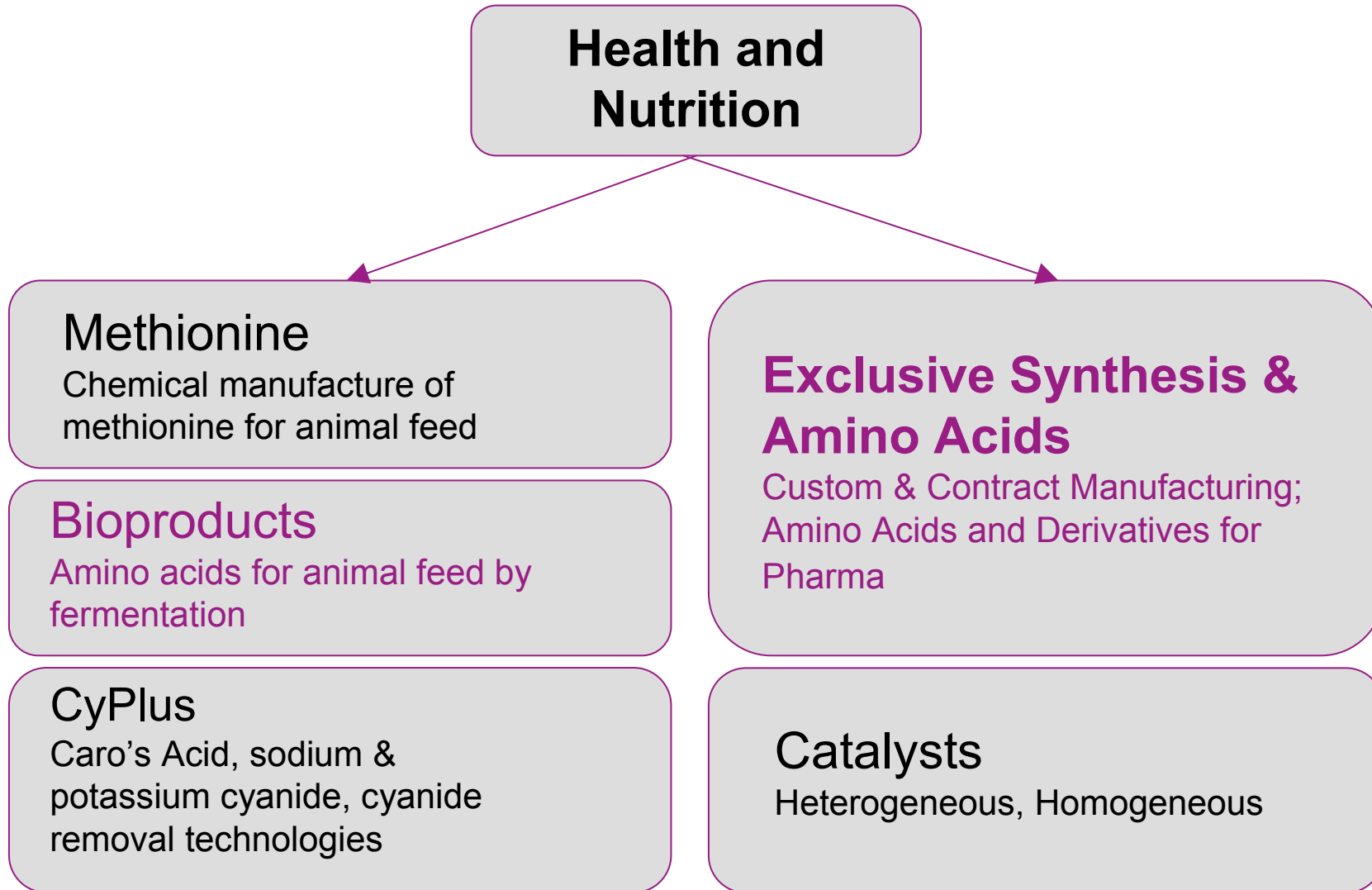
→ 2.8% research-to-sales ratio  
(7% ratio in Exclusive Synthesis)

→ Over 2,300 employees in research

→ 35 research sites

as of December 31, 2007

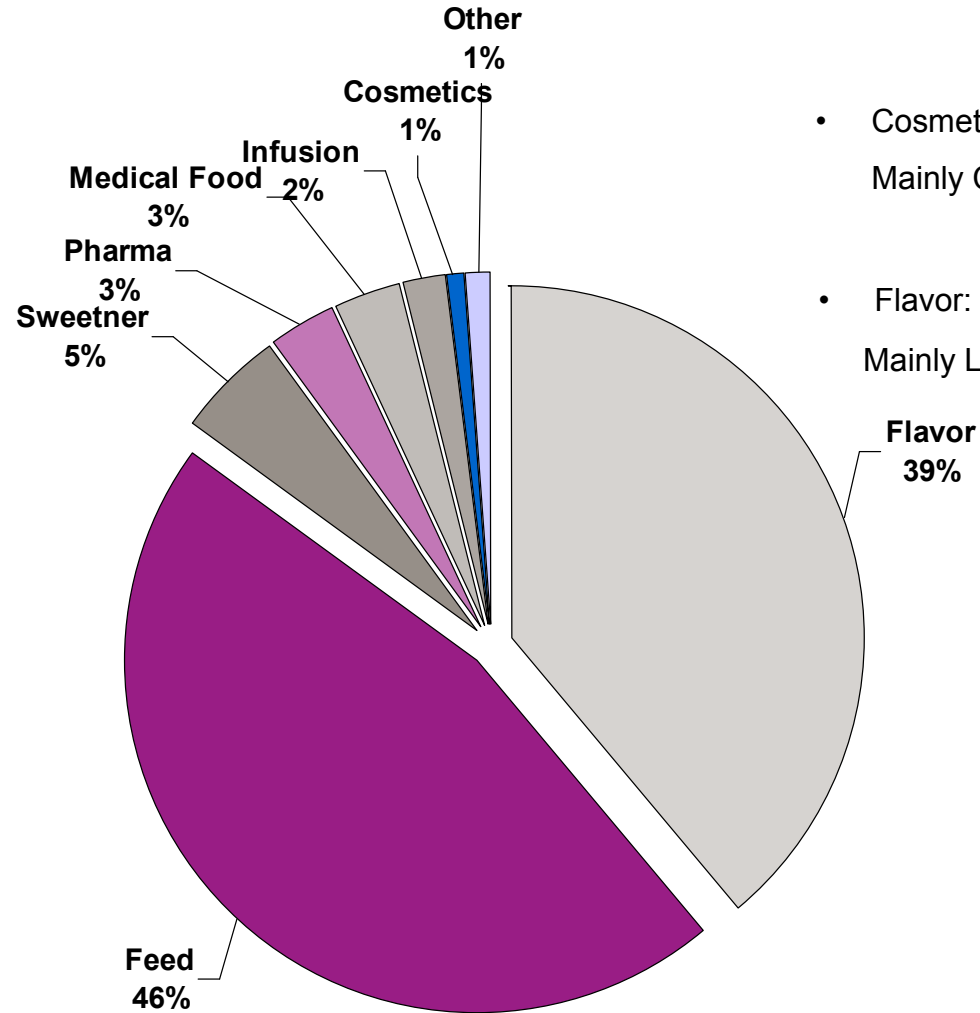
# Health & Nutrition Business Unit



# World Market Amino Acids



- **Infusion:**  
Amino acids highly purified used as APIs
- **Pharma:**  
Amino acids as building blocks for Peptides and APIs and also none natural amino acids
- **Sweetner:**  
Mainly Aspartam (dipeptide Phe-Asp)
- **Feed:**  
Mainly D/L-Methionine, L-Lysine, L-Threonine, and L-Tryptophan



- **Cosmetics:**  
Mainly Cys, Asn and Gly
- **Flavor:**  
Mainly L-Glutamate

Total amino acid market is 5,5-6 billion€

# Access to APIs



## Raw material basis:

Extraction plants/animal derived



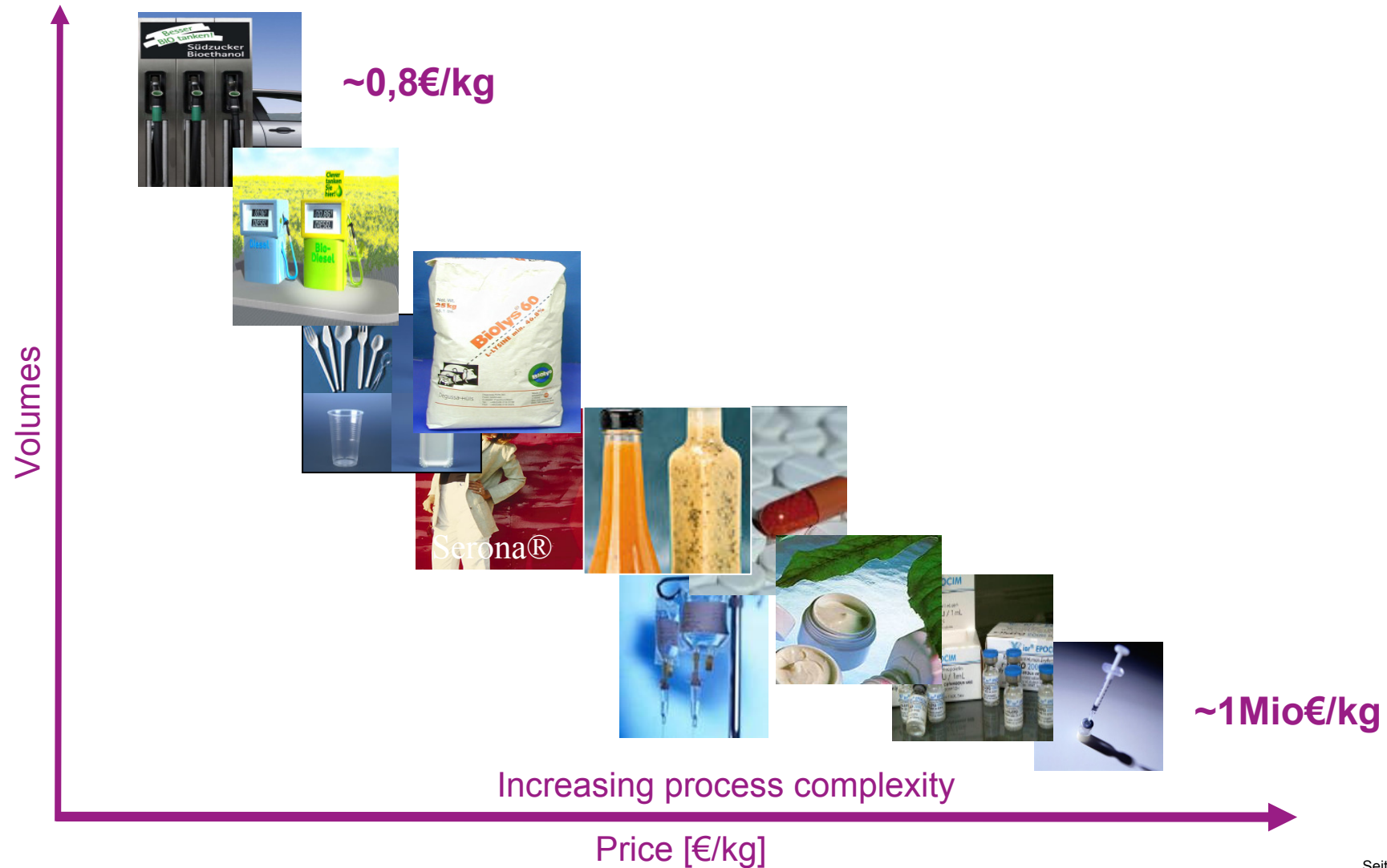
Chemical or biochemical synthesis



Fermentation

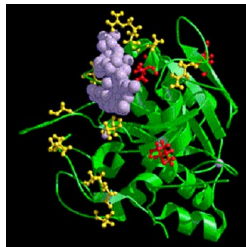


# Biotechnology: Annual capacities from grams to multi-tones scale

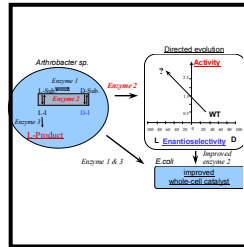




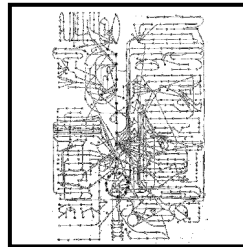
# Biological Production Systems



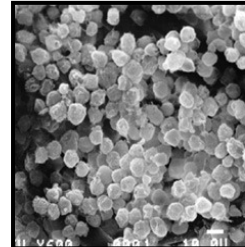
isolated enzymes



whole cell catalysts



microbial fermentation



mammalian cells



transgenic plants



transgenic animals

chemistry

biology

growth

embryonic

simple

complex

short development times

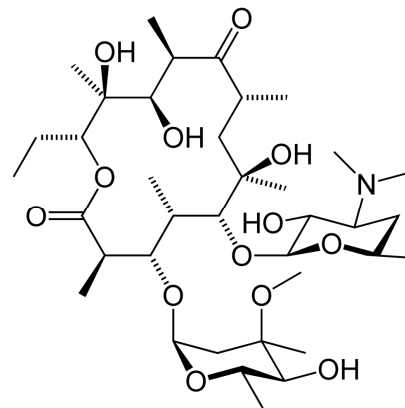
long development times

# Different molecular structures of APIs via biotechnology



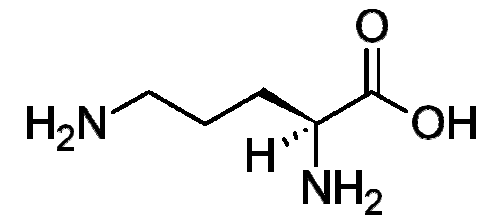
**Erythropoietin**

= Protein



**Erythromycin**

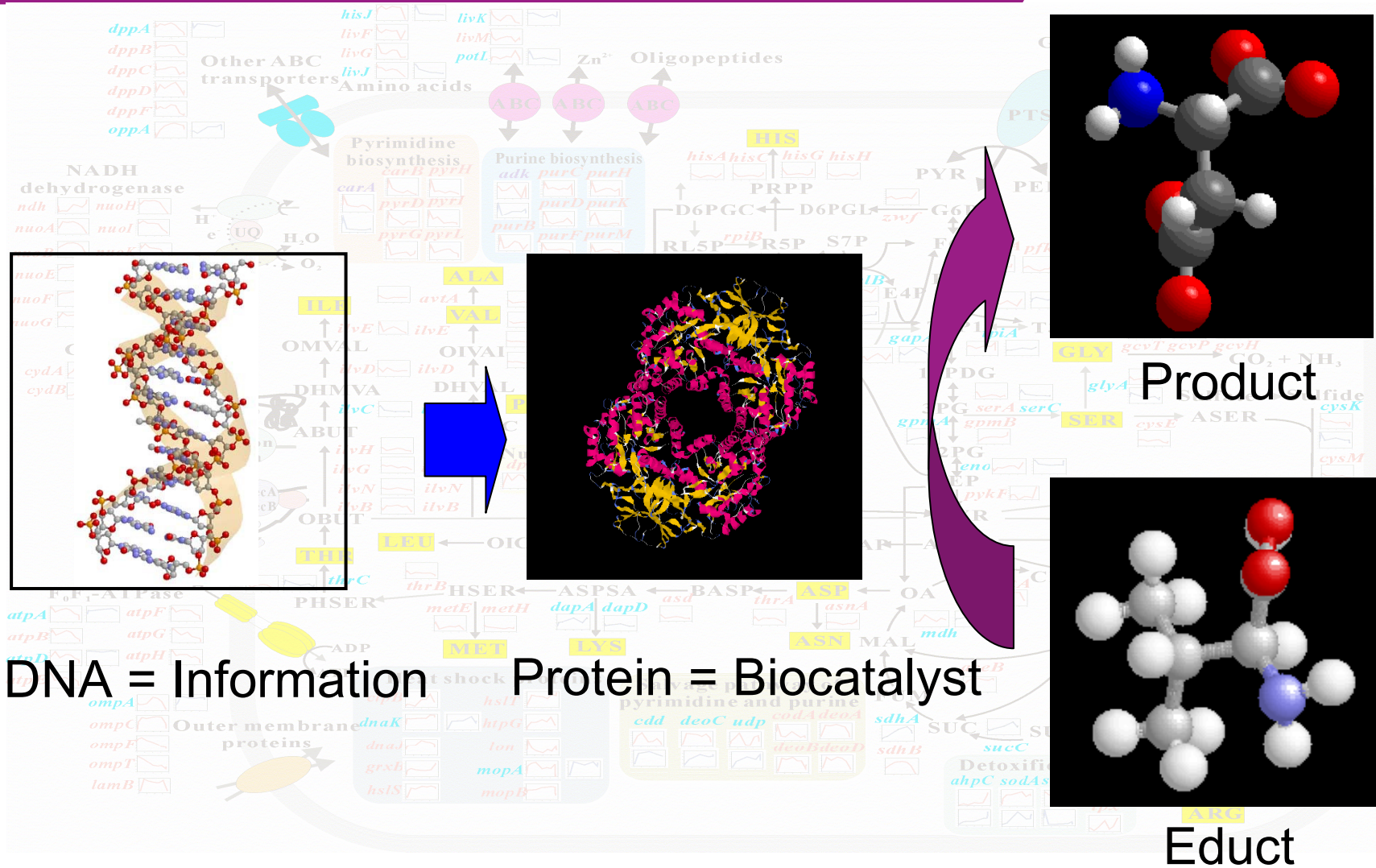
= Secondary metabolite



**L-Ornithine**

= Primary metabolite

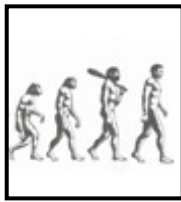
# Biotransformations: Proteins as “technical” catalysts



DNA = Information

Protein = Biocatalyst

# Steps in development of a biocatalytic process



Product

Substrate

Screening for Biocatalysts with relevant activity

Evolution of Biocatalysts

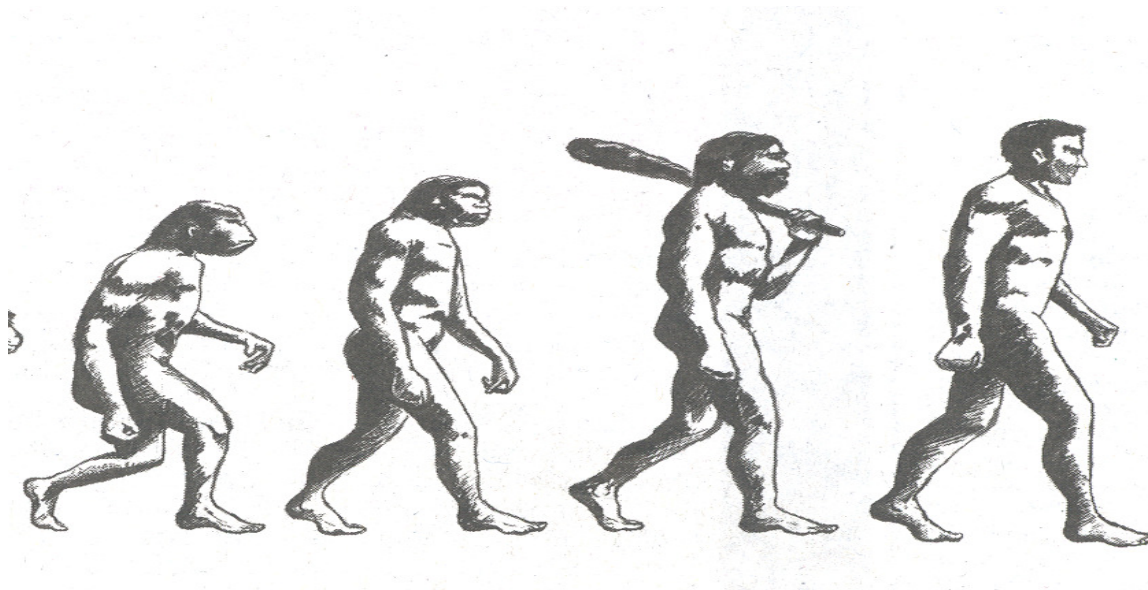
Producing Biocatalysts

Applying Biocatalysts

Integration in chemical Production

<ul style="list-style-type: none"> <li>• Screening enzyme collection</li> <li>• Screening strain collection</li> <li>• Screening expression libraries</li> <li>• Screening genome data bases</li> <li>• Bioinformatics</li> </ul>	<ul style="list-style-type: none"> <li>• Molecular biology</li> <li>• Expression systems (yeast, fungi, E. Coli, Bacillus sp)</li> <li>• Directed evolution</li> <li>• Assay development</li> <li>• High-throughput-screening</li> <li>• Bioinformatics</li> </ul>	<ul style="list-style-type: none"> <li>• Fermentation in batch, fed-batch and continuous mode</li> <li>• Production of whole cell biocatalysts</li> <li>• Isolation of enzymes</li> </ul>	<ul style="list-style-type: none"> <li>• Choosing suitable reactor system</li> <li>• Immobilisation</li> <li>• recycling and reuse of biocatalyst</li> <li>• Mathematical modeling</li> </ul>	<ul style="list-style-type: none"> <li>• Process optimization and siting</li> <li>• Large scale production using native or immobilized enzymes or whole cells</li> </ul>
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# Evolution in the lab.....

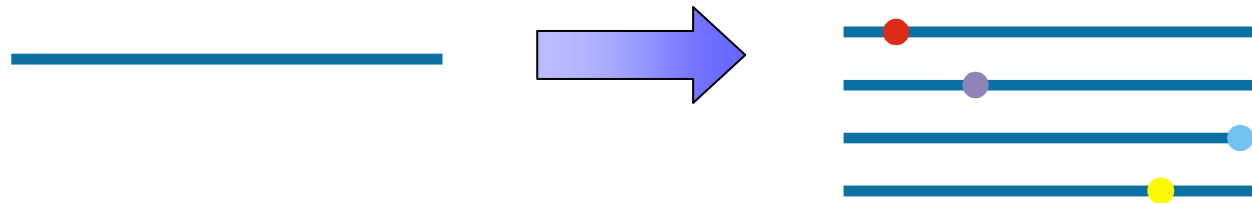


Can we accelerate it?  
How can we do it in the Lab?

# ... via artificial manipulation of the DNA sequence and selection

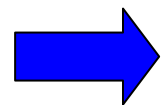
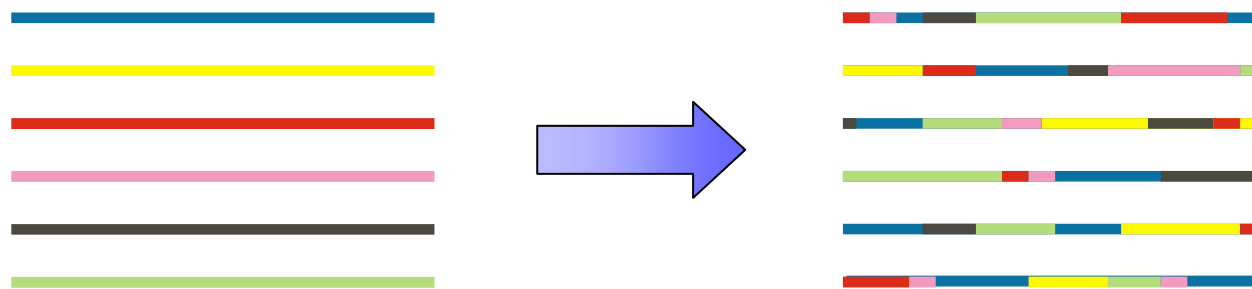
**Random:**

- Error prone PCR
- Chemical mutagenesis



**Recombination of DNA Fragments from different species:**

- Shuffling
- STEP

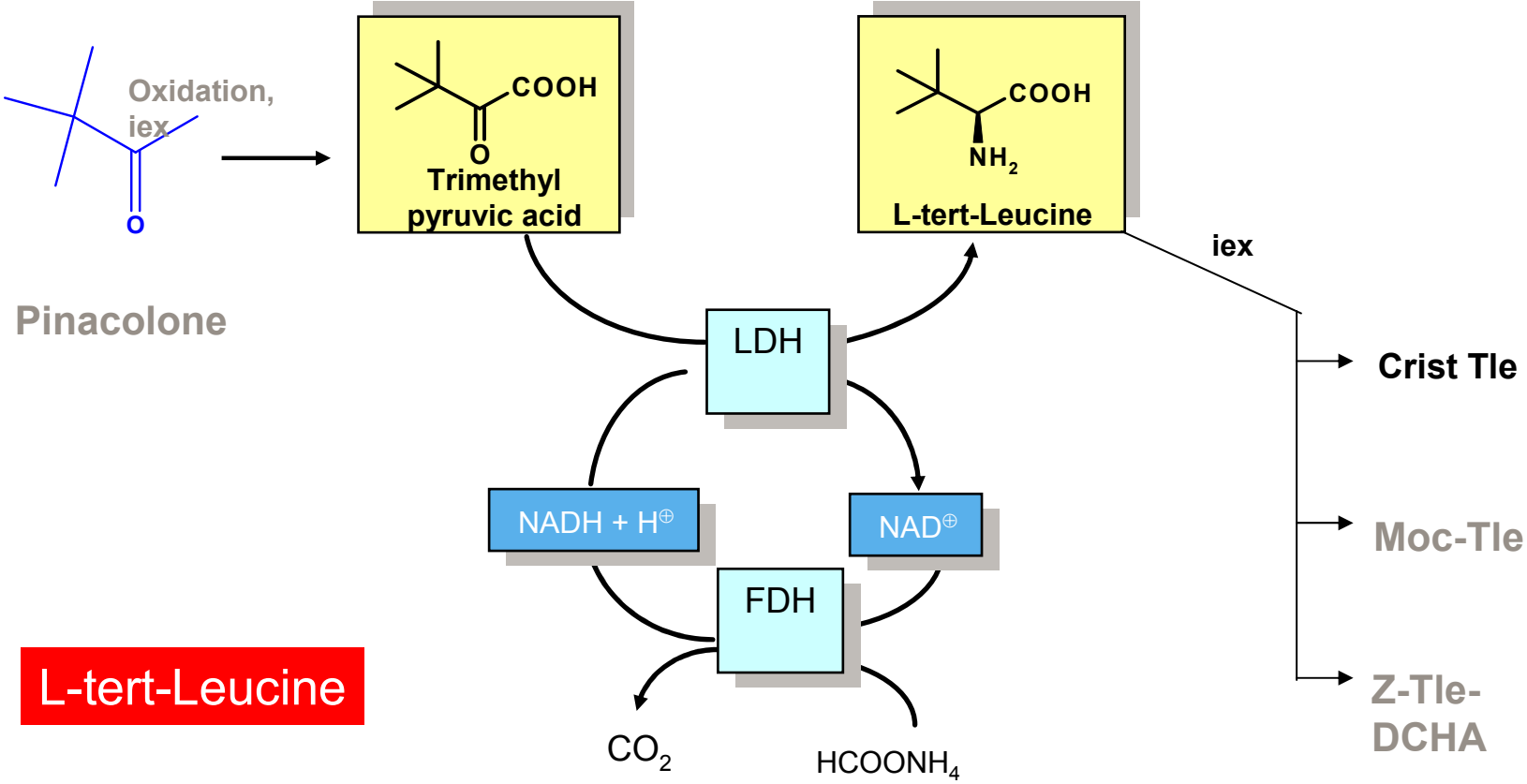


**Proteins with new properties**

# Example for enzymatic synthesis

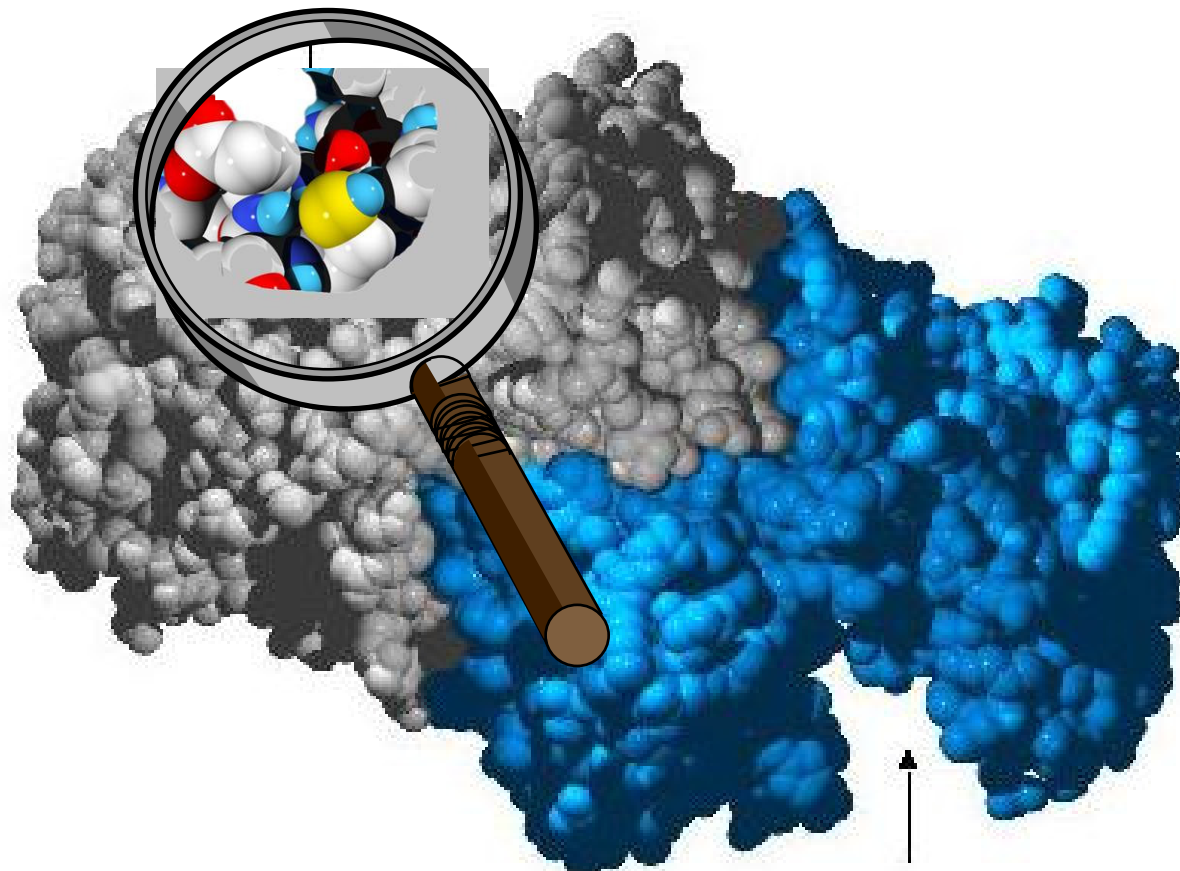


## Process with continuous Cofactor regeneration



# Limited stability of FDH under process conditions

**Reason: Cysteine residues in active binding site of the enzyme will be oxidized under process conditions**

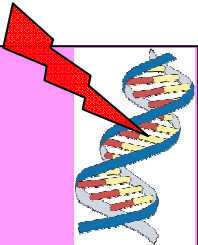




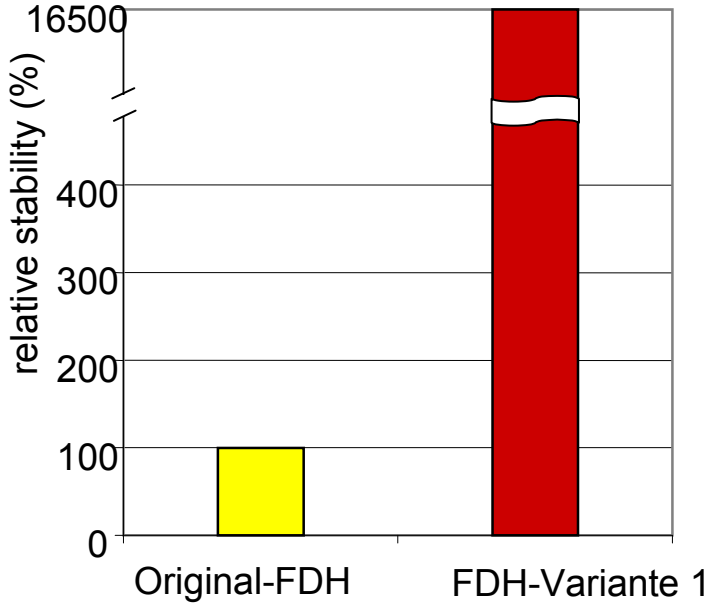
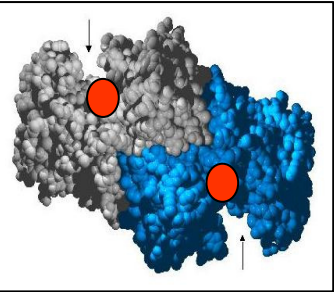
# FDH with improved process stability



Targeted Evolution:  
Mutante FDH Cys 23 Ala

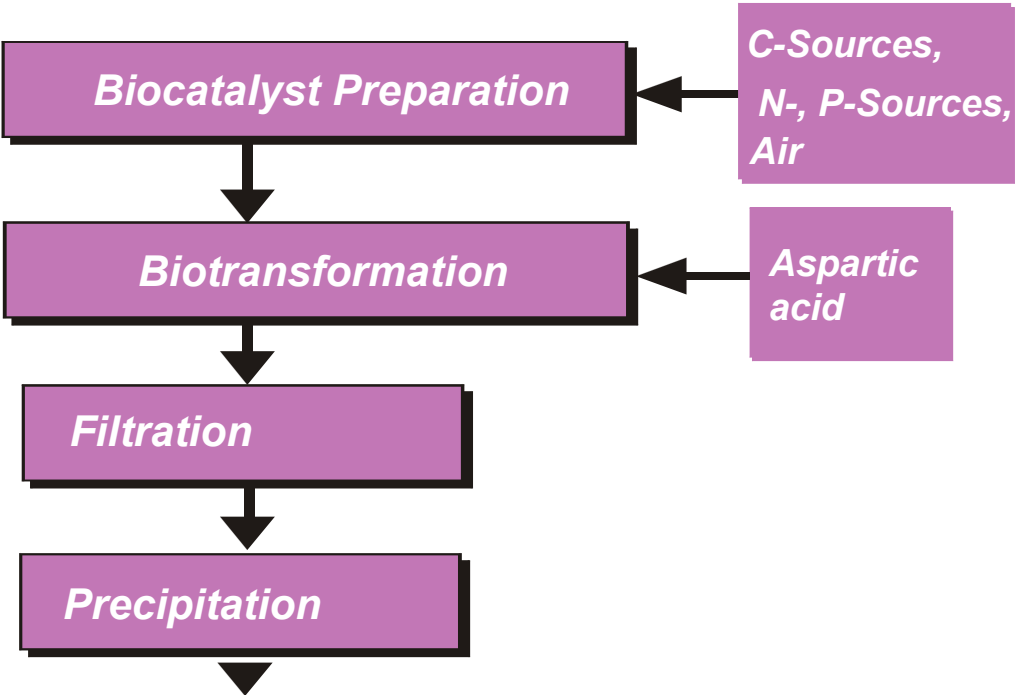
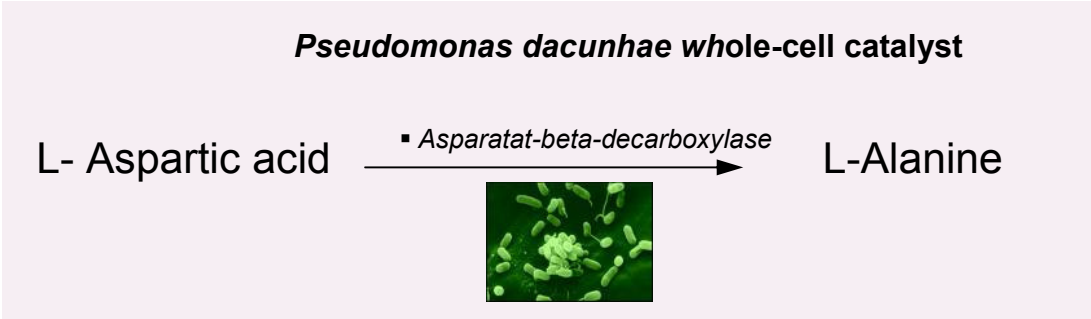


Expression, purification and  
characterisation

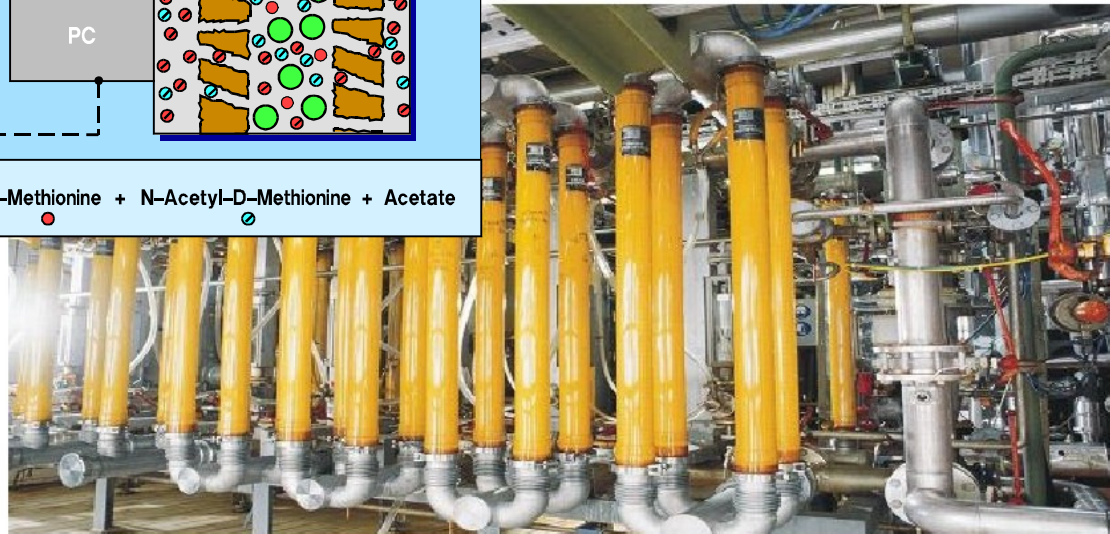
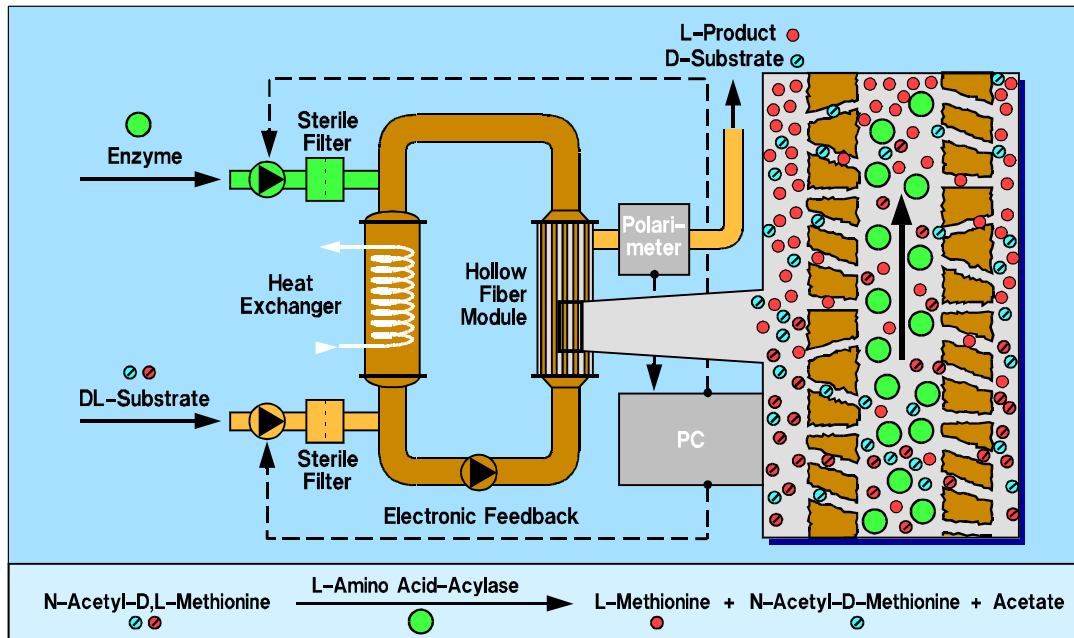


H. Slusarczyk, M. Pohl, M.-R. Kula (1997) US Patent 6,242,234  
H. Slusarczyk, S. Felber, M.-R. Kula, M. Pohl (2000) Eur. J. Biochem., 267, 1280-1289

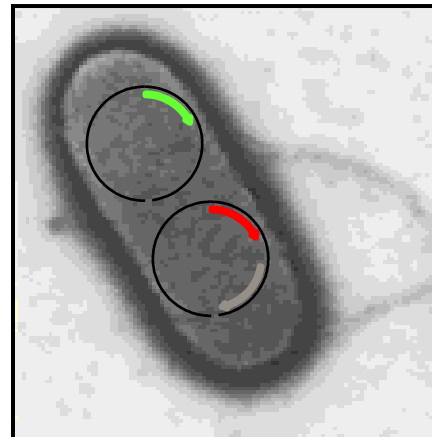
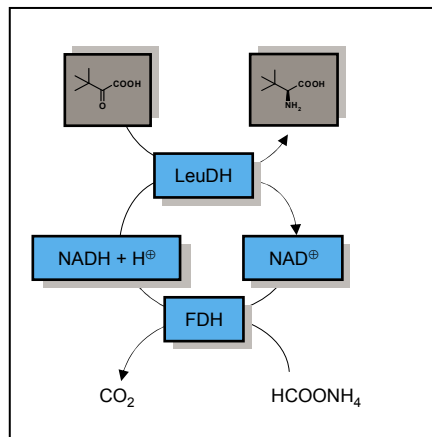
# Enzymatic Decarboxylation



# Applied Biocatalysis



# Prizes for Biocatalysis



**Enzyme Membrane Reactor (EMR)**  
 (development together with Kula and Wandrey)

**Technology transfer prize of the BMBF for Kula and Wandrey**

**Dehydrogenase Process**  
 (development together with Kula and Pohl)

**Future Prize of the German President for Kula and Pohl**

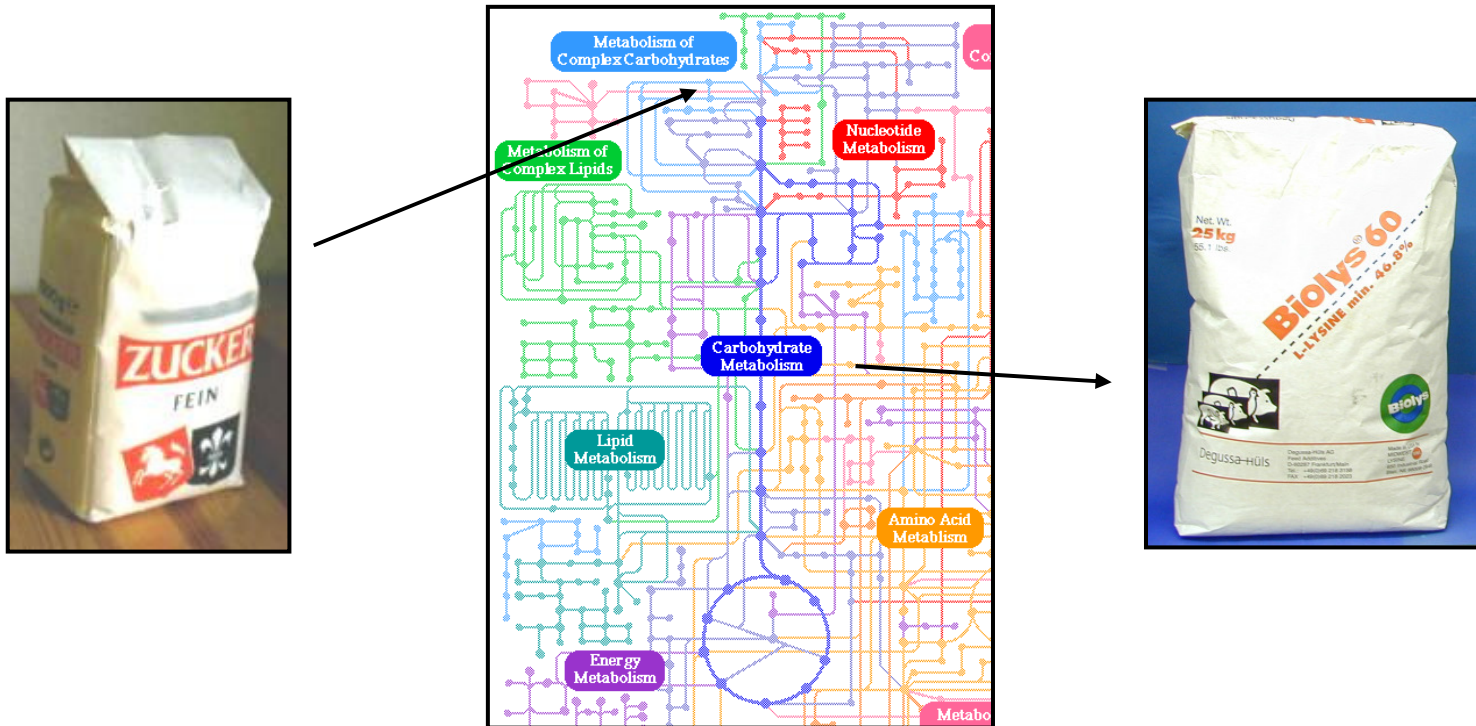
**Hydantoinase Process**  
 (development together with Syldatk and Arnold)

**Fine Chemicals Product of the Year Award by Frost & Sullivan**

**β-Amino Acids**

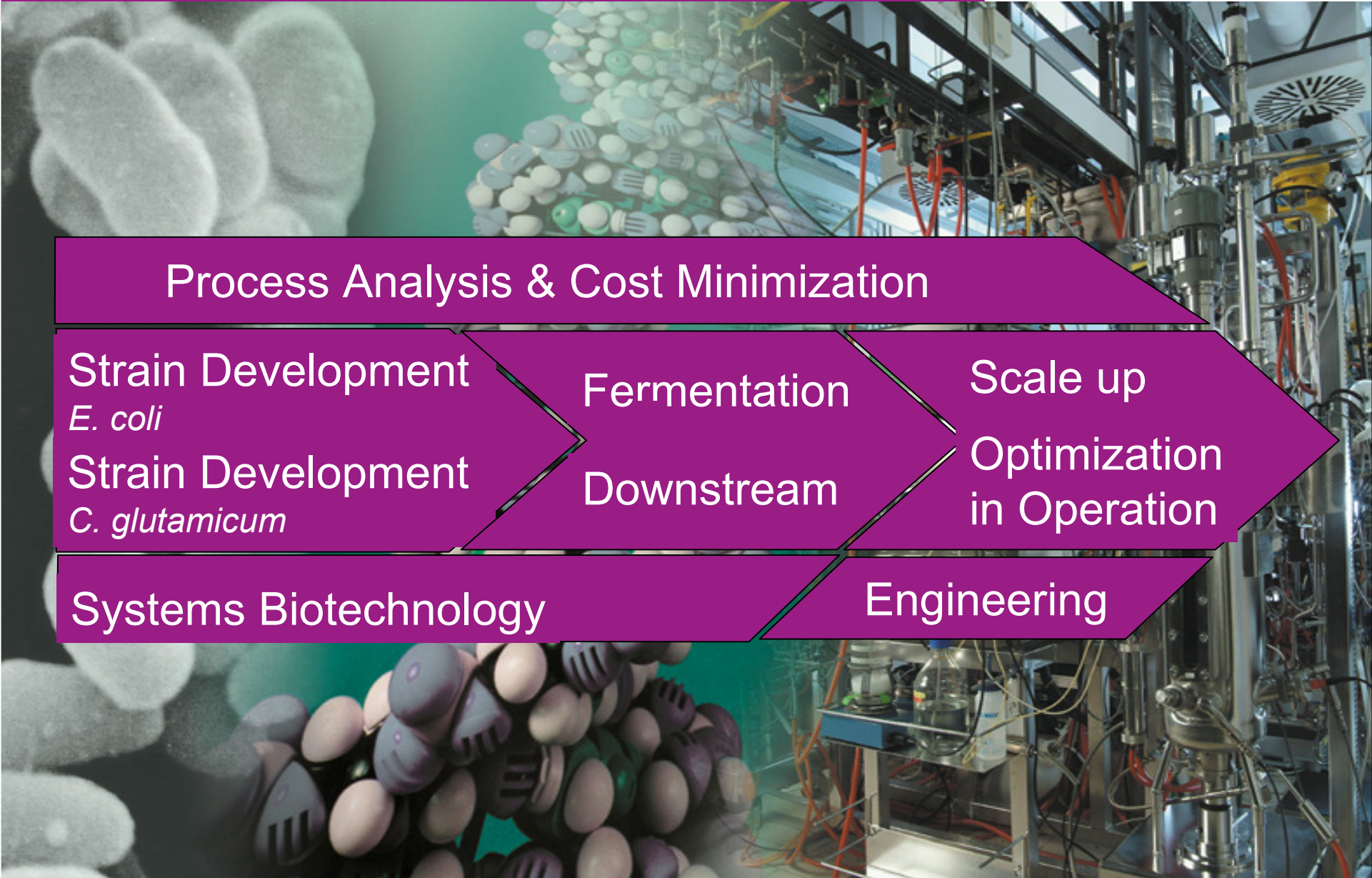
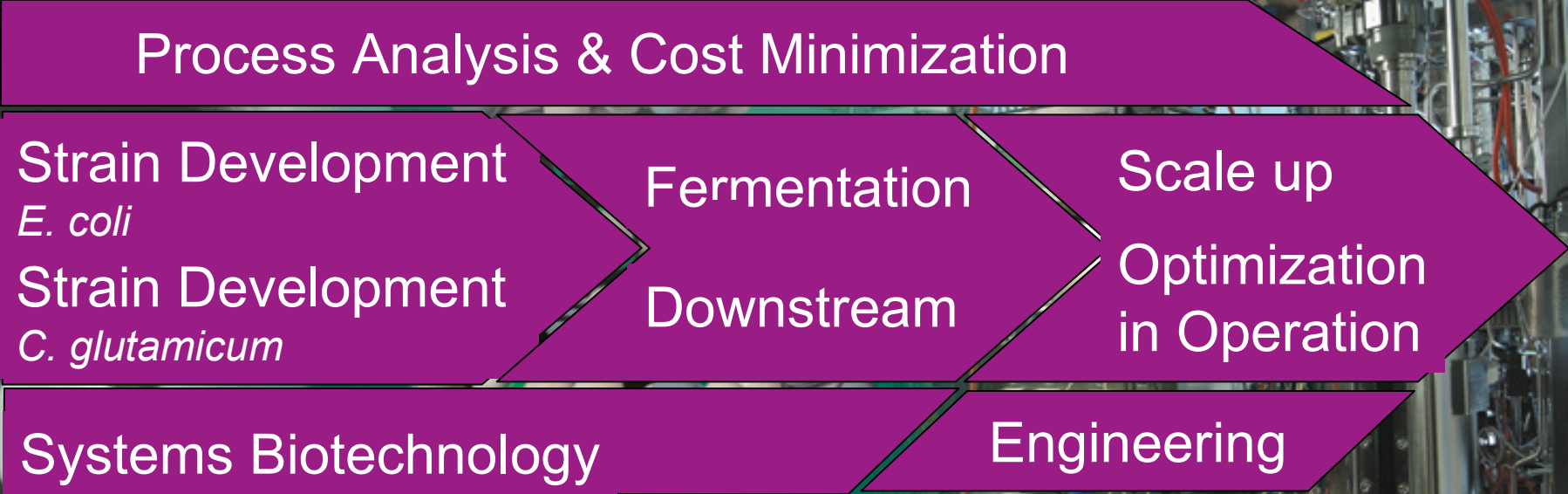
**Innovation Award for New Product of Degussa**

# Fermentation



Using the whole metabolic network of a microorganismus

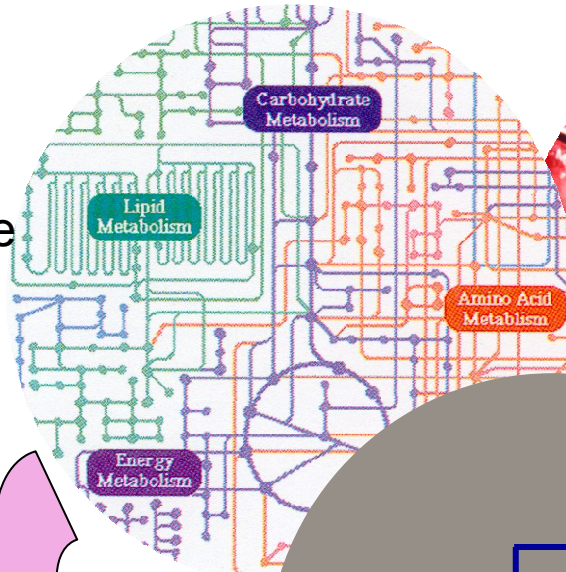
# Strain and fermentation development



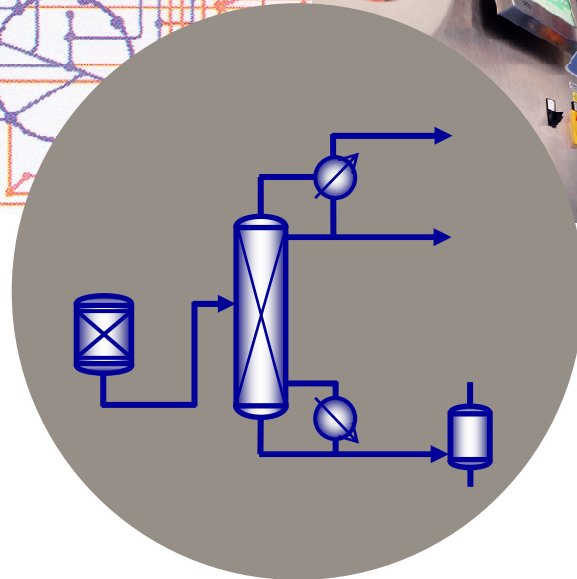
# Increased Interaction Strain- Fermentation - Downstream



Strain  
Performance

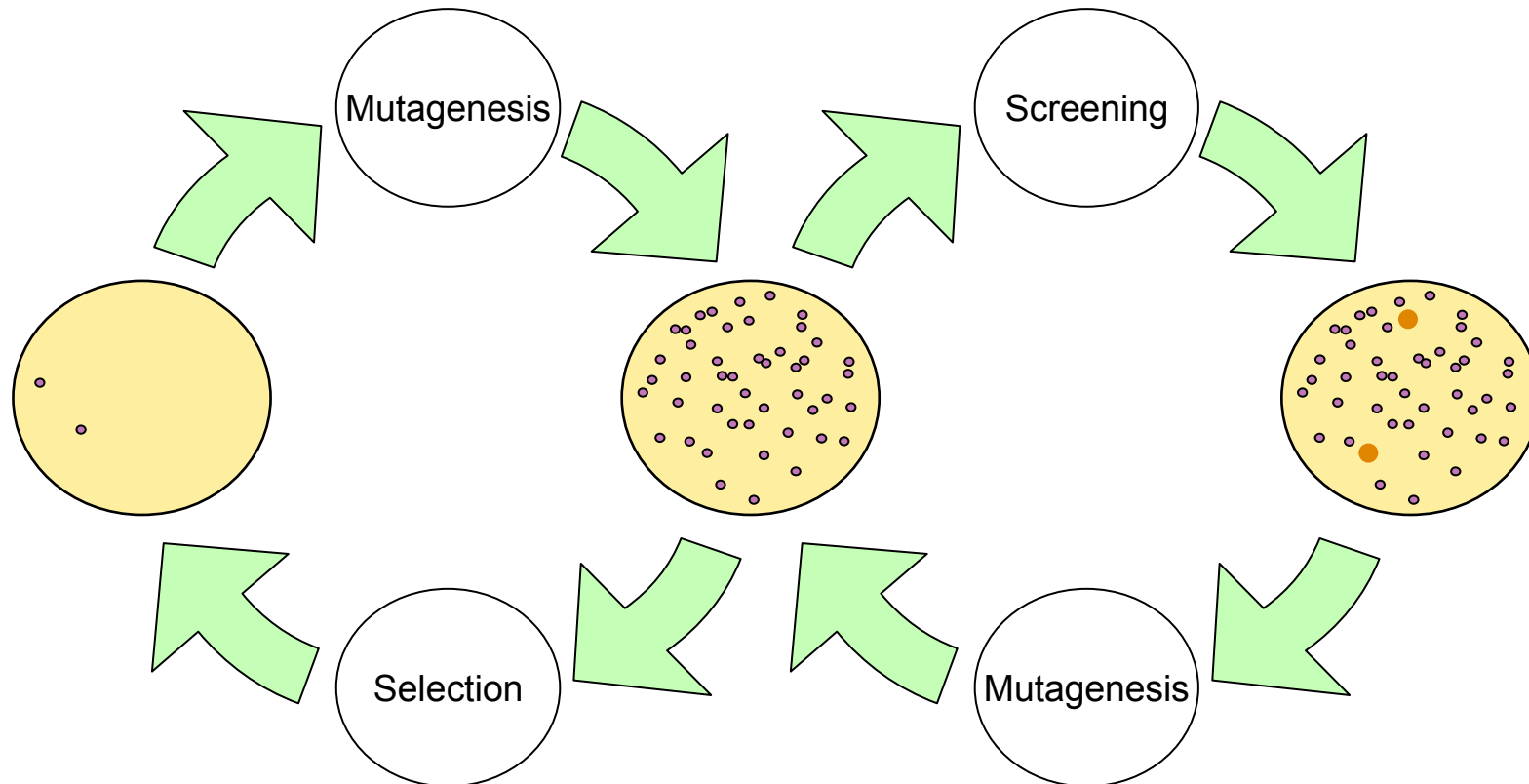


Fermentation  
Performance



DSP  
Performance

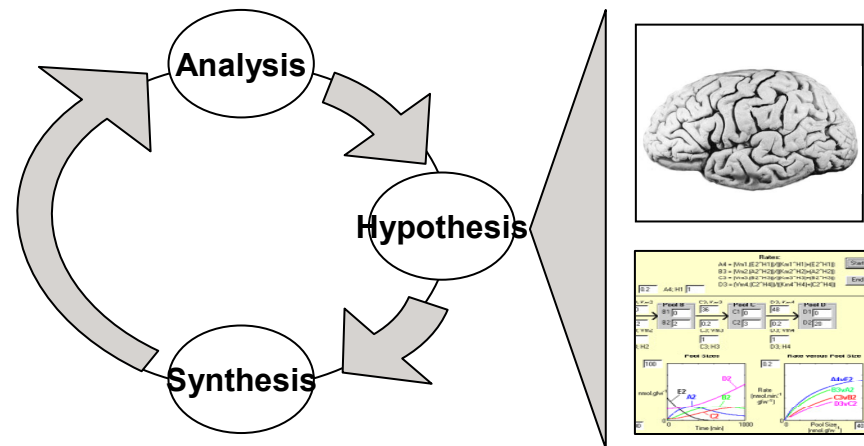
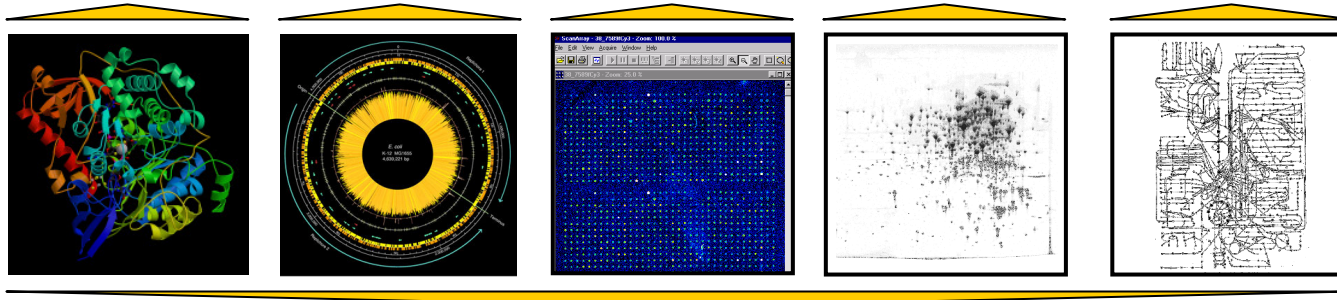
# Today strain development is still based on a combination of classical strain ...



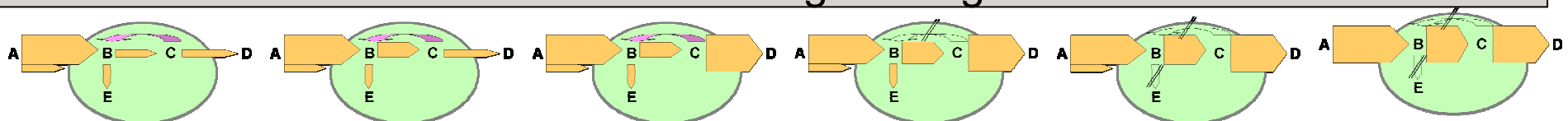


# ... and rational strain development

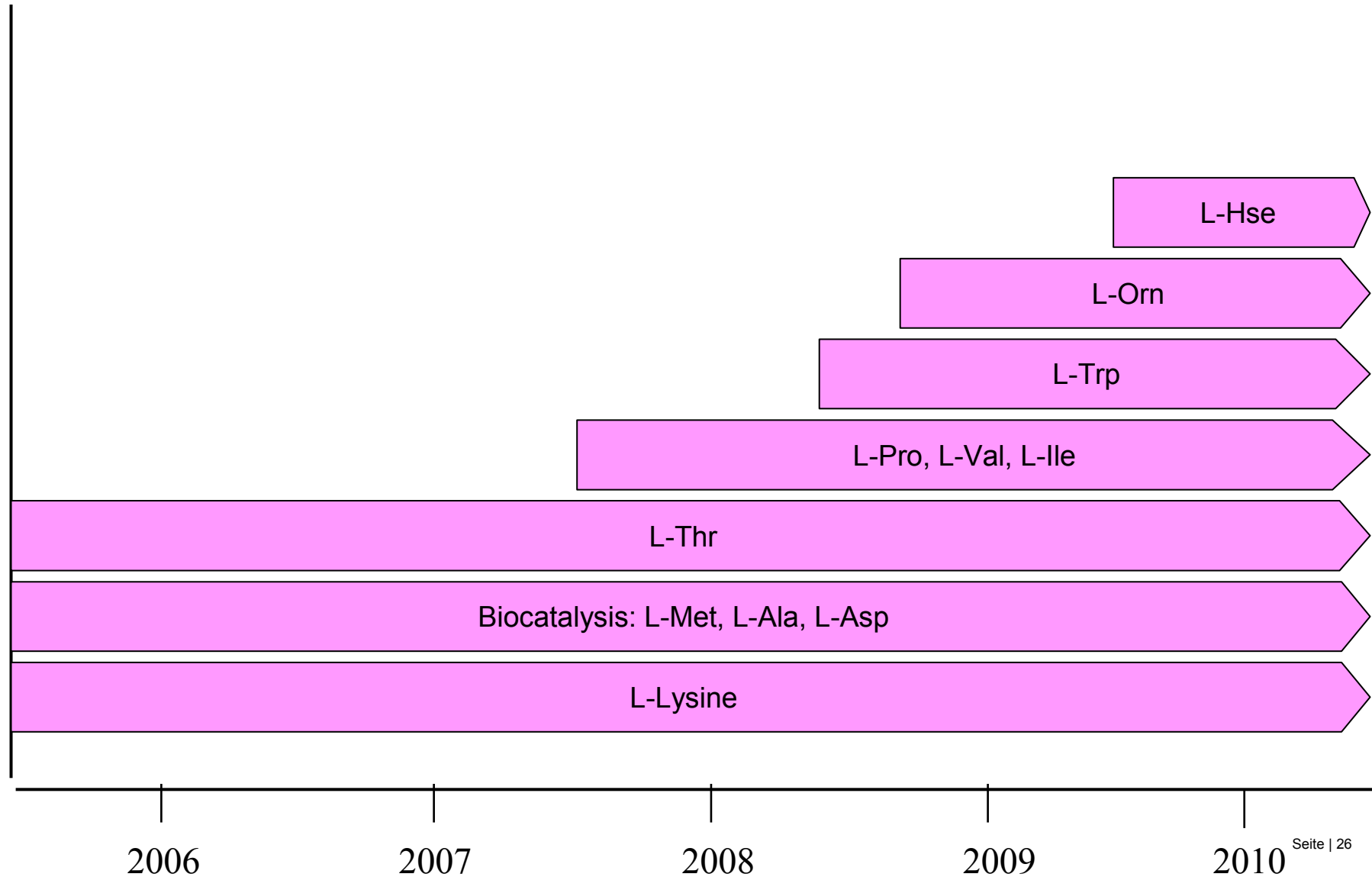
## Systems Biology



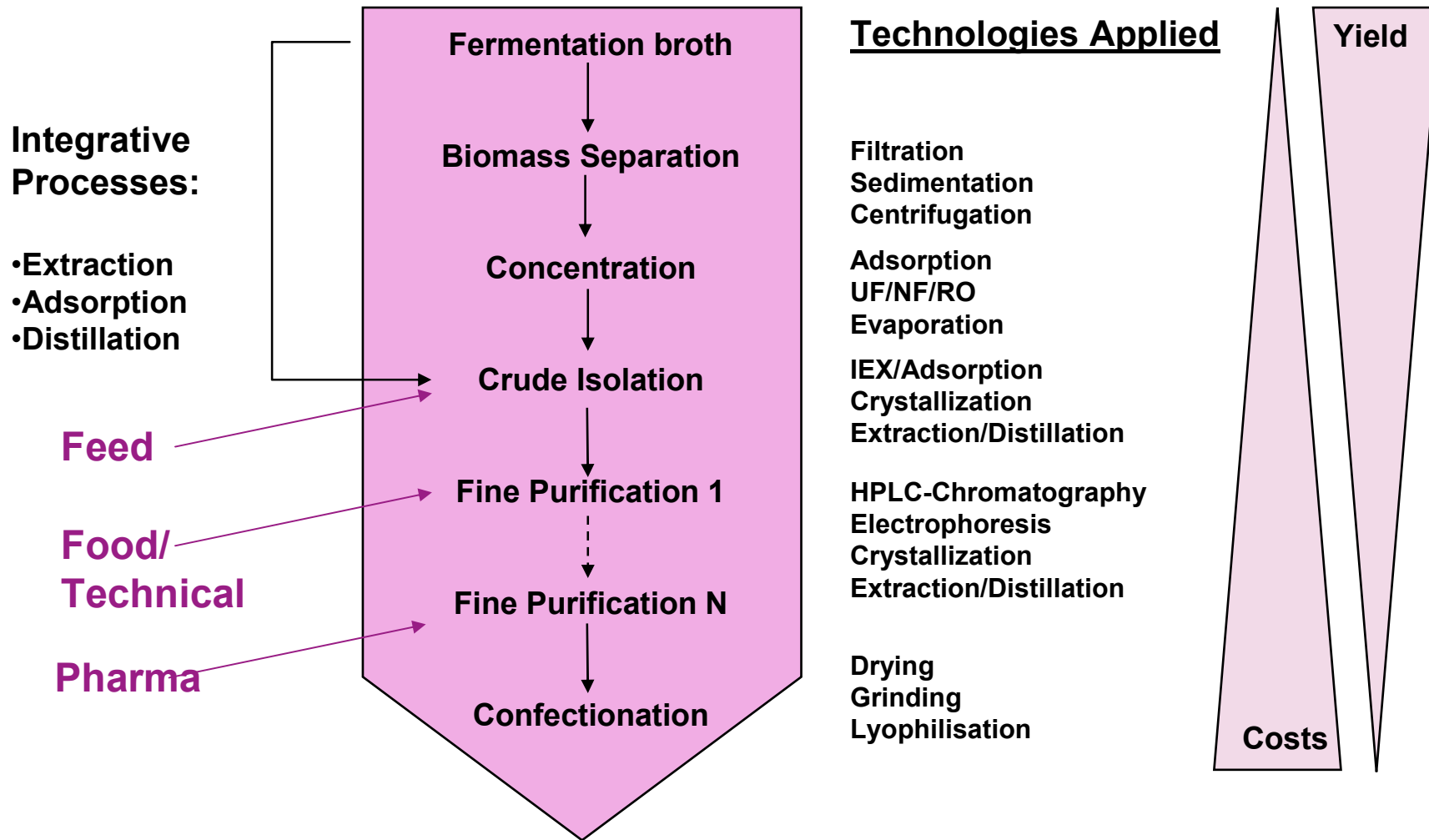
## Metabolic Engineering



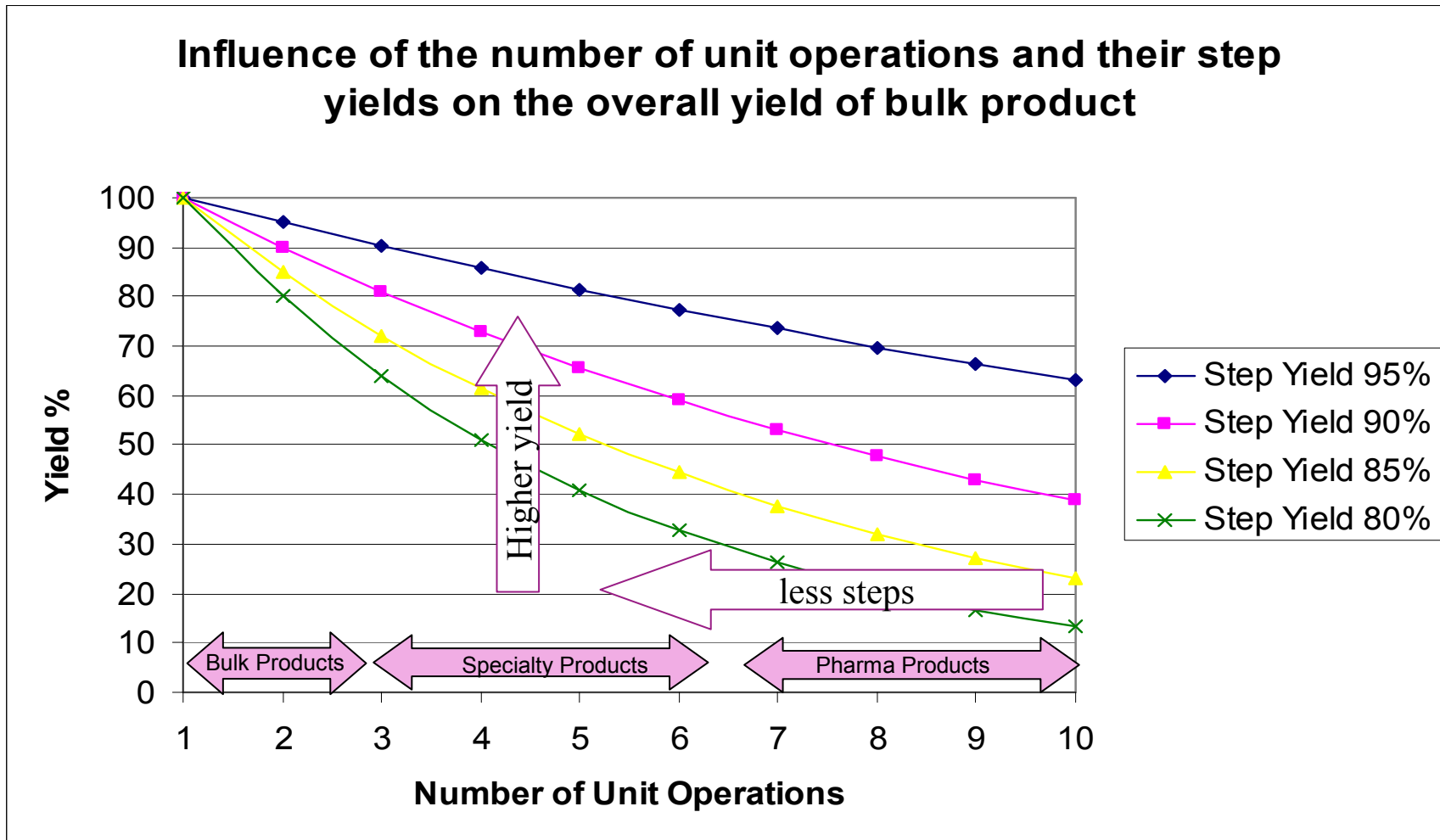
# Amino acids produced by Evonik via biotechnology



# General DSP Flow Chart



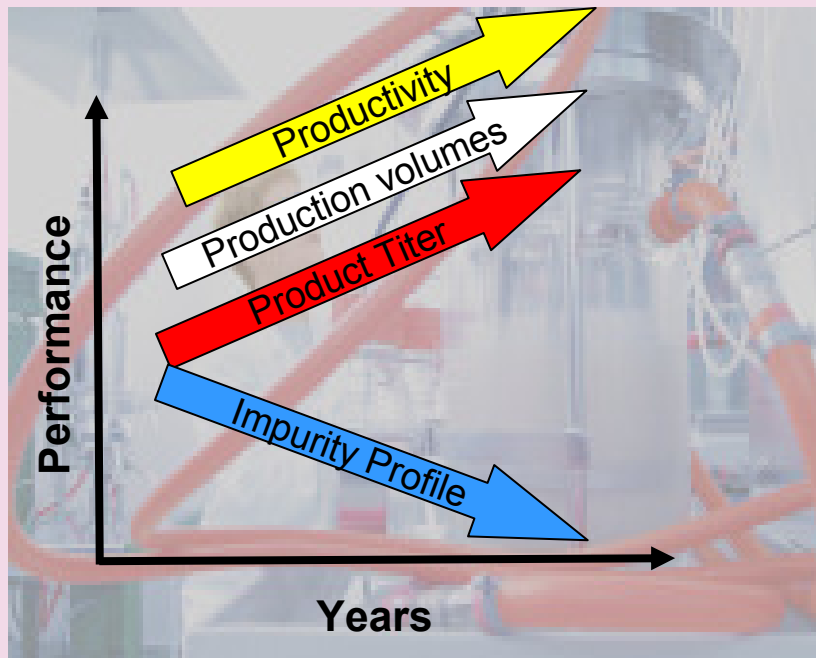
# Need for less but high yield processing steps



# Performance Increase Bioprocesses



## Fermentation Process Development



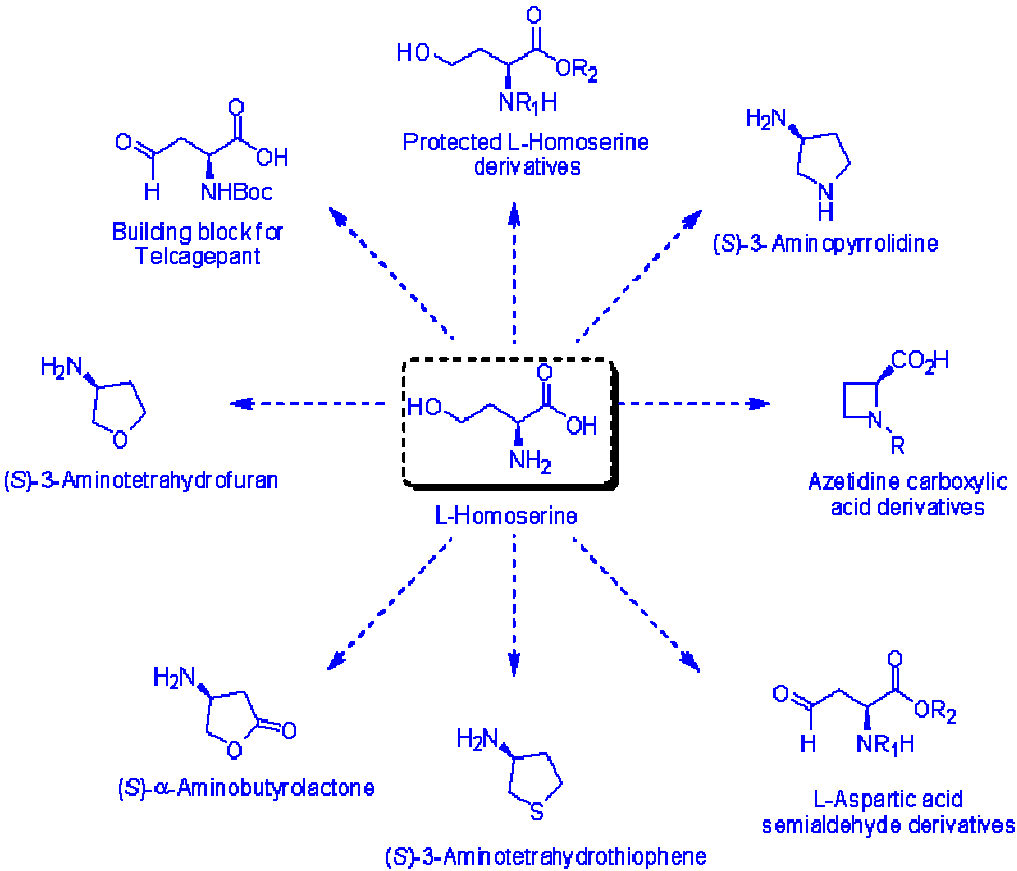
## DSP requirements

- High productivity unit operations
- Improvement of separation selectivity
- Unit operations able to handle high concentrations
- Minimization of diluting process steps
- Minimization of waste generation
- Easy scalable process steps
- Reduction of process steps

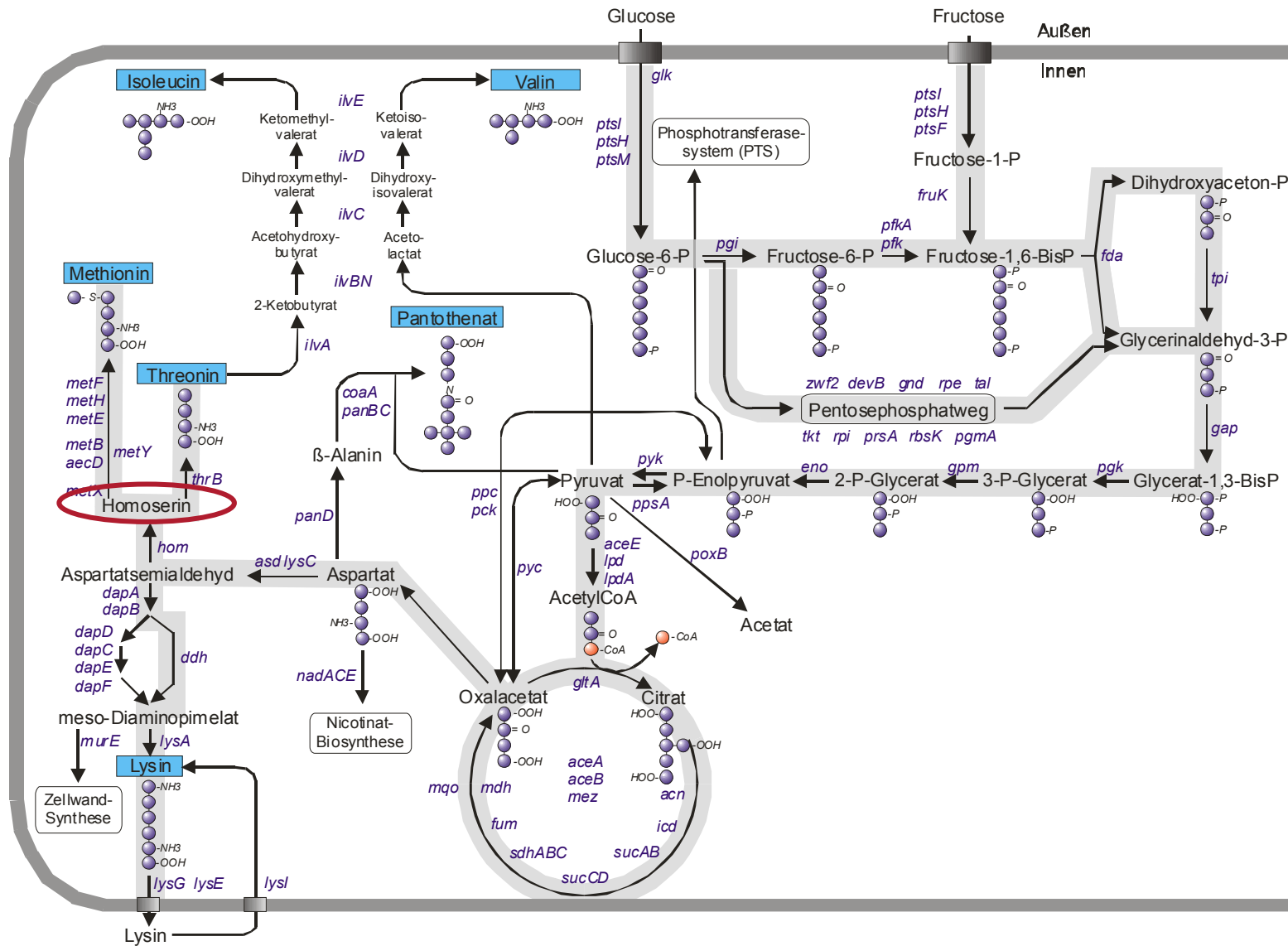
# Homoserine Product Tree



Homoserine: A universal building block based on renewable resources



# Homoserine is a primary metabolite

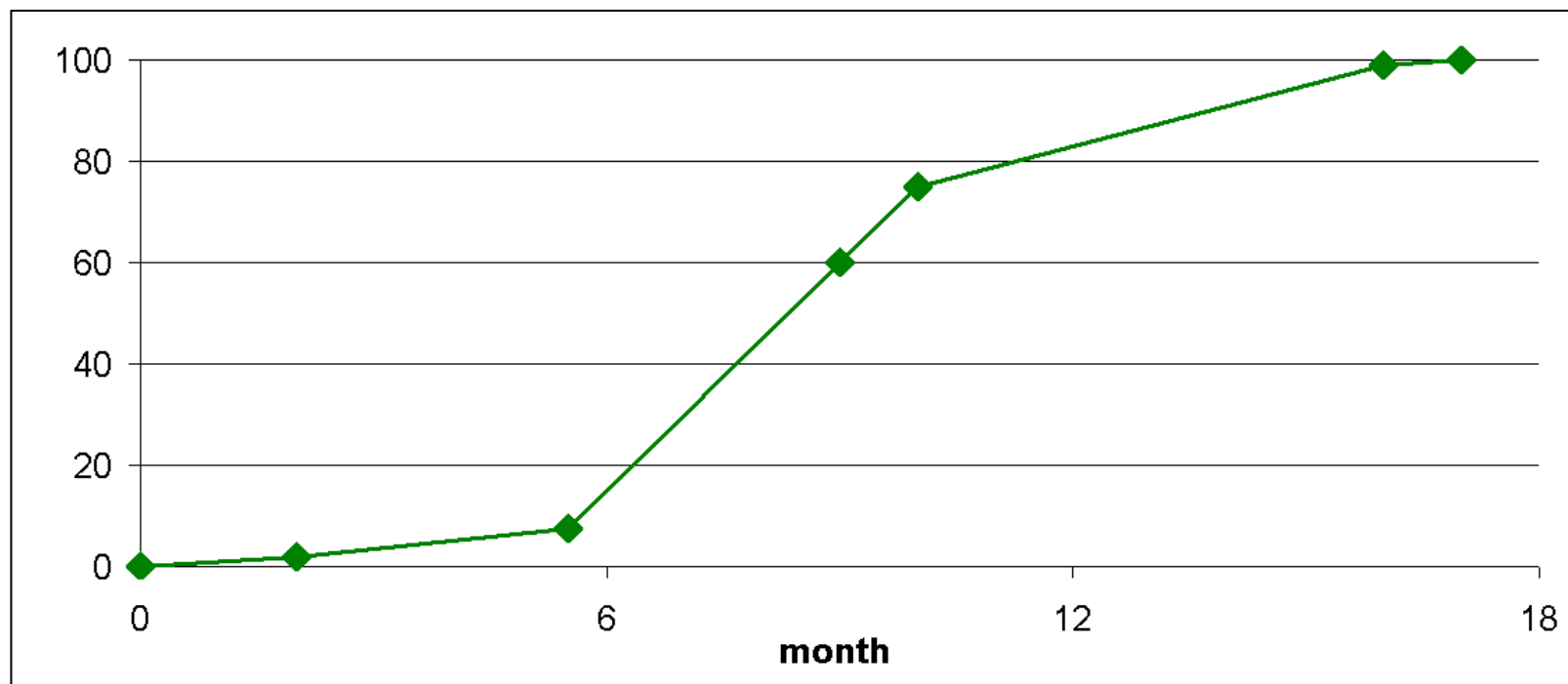


# L-Homoserine

## Development of *E. coli* Based L-Homoserine Process



- strain development based on a high performance *E. coli* threonine producer
- high yield, low byproducts, robust fermentation
- process transferred into production 2009





# Science-to-Business Center Biotechnology



## Science to Business Center „Bio“

- Focus on sustainable production processes – fermentation and biocatalysis - mainly based on renewable feedstock
- Development of new and highly competitive routes to established products as well as innovative biomaterials and functional products for life science and skin & hair care markets
- Start operation in January 2007
- Co-funded by Evonik Degussa and the German and EU governments and their agencies



This project is co-financed by the European Union and is financially supported by the state of North-Rhine Westphalia.



# Science-to-Business Biotechnology

## Vision and Mission

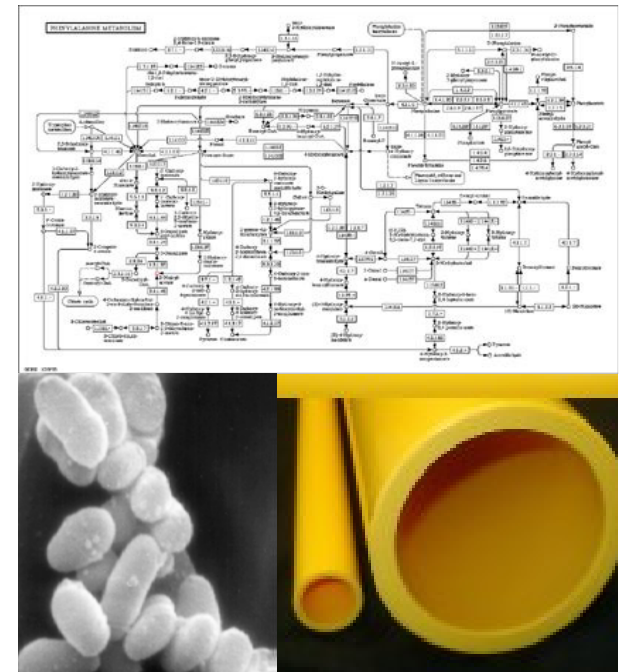


### Vision

- Biotechnology is an established, competitive technology to contribute and save Evoniks future chemical business

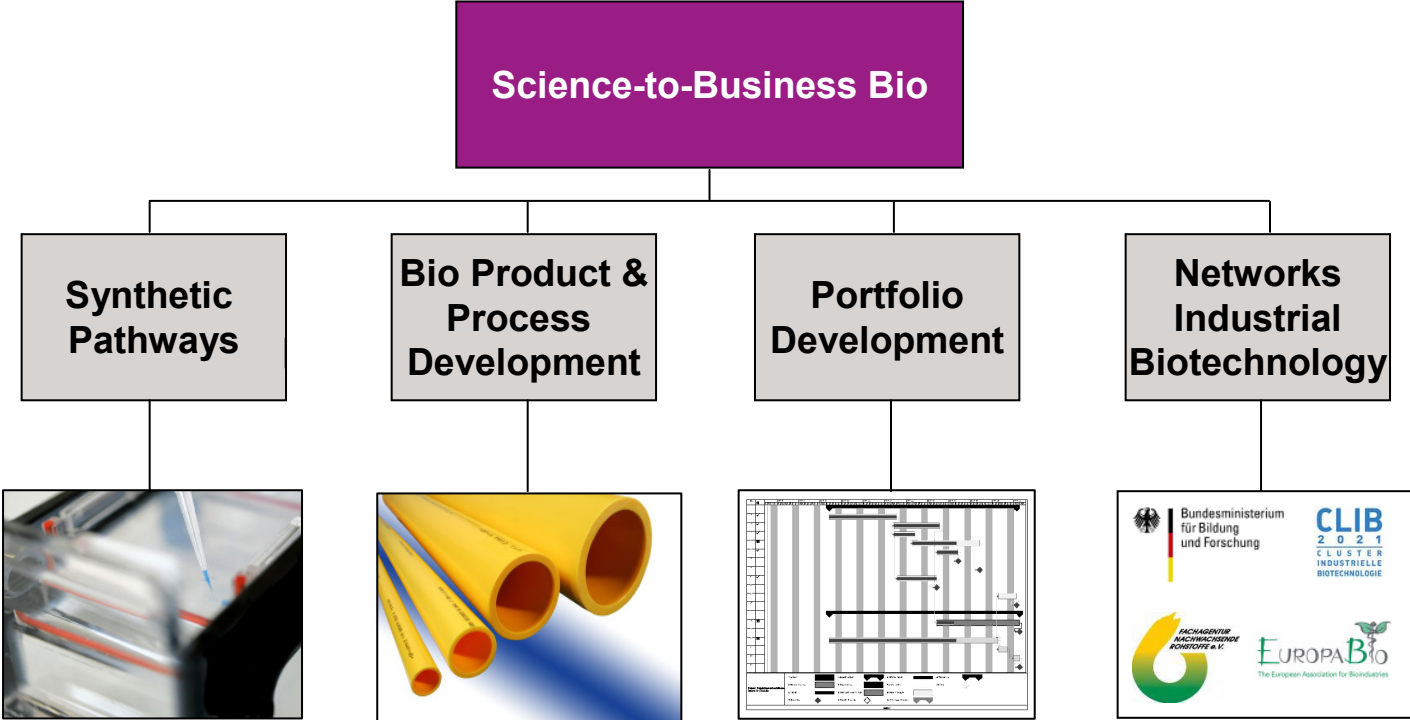
### Mission

- Generation of a „factory in a cell“ via new synthetic pathways
- Synthesis of bio-derived materials with superior performance or significant cost advantage



# Science-to-Business Bio Profile

## Biotechnology Competence Structure



**Many thanks for your attention...**