Applied enzymology – e-learning course

Extended sylabus + presenations

Applied enzymology = Enzyme technologies

OECD (Organization for economic cooperation and development) in **2001** – ET are sustainable part of industrial development

Interdisciplinary – from direct industrial applications to production of new pharmaceuticals and research tools

OECD: Moving Toward More Sustainable Industries

• **processes and production systems which:**

− save costs and are more profitable because they are less wasteful of materials and energy (resulting in less emissions of greenhouse gases, persistent organic chemicals and other pollutants).

− enable greater and more efficient utilization of renewable resources (energy, chemicals and materials), lessening our dependence on non-renewable resources such as petroleum and reducing associated greenhouse gas emissions.

• **products which are**:

− better performing, more durable and don't persist after their useful life.

− less toxic, more easily recyclable and more biodegradable than their conventional counterparts.

− derived as much as possible from renewable resources and contribute minimally to net greenhouse gas emissions.

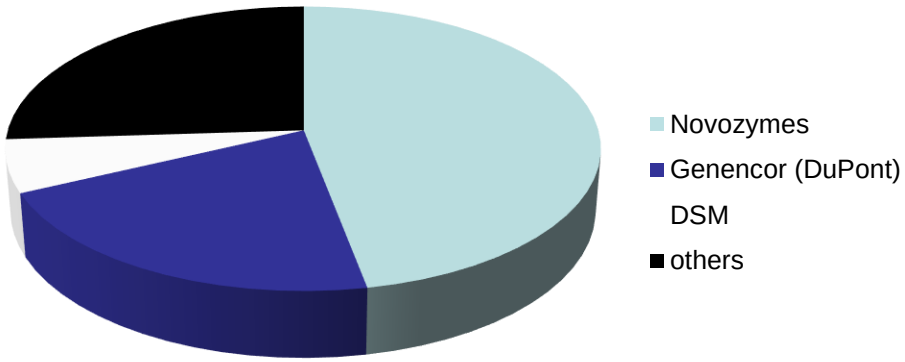
1. Intro

- History
- Why enzymes should be used?
- …. And which?
- Composition of technological enzyme preparations
- Determination of enzyme activity
- Enzyme sources
- Searching for new enzymes

History of enzyme research and application

1833- diastase from germinating barley
1834- enzyme hydrolysis of starch (Berzelius)
1839 – 1897 - 60 years of discussion on the role of yeast in fermentation
1894 - takadiastase (A.oryzae)
1908 - Otto Rőhm – Oropon – leather industry
1914 - Burnus – first biodetergent
1963 - Alcalase – bacterial protease Novo
1964 - enzymes in food industry
1984 - recombinant enzymes

World producers of technical enzyme preparations



Year	Sales (bil. USD)
1983	0.4
2000	1.5
2010	**3.3**
2016	**5.0**
Prediction 2021	**6.3**

Why use enzymes in technologies (and elsewhere)?

- Possibility to control the process, higher conversion
- Specificity – lower risk of side products
- Lower costs (technological equipment)
- Positive effect on the environment:

  - ✓ „More friendly“ waste products
  - ✓ Lower energy consumption
  - ✓ Renewable raw materials (MO production)

Factors affecting usage of enzymes in technology

1. Substrate accessibility, nonhomogeneity of technological substrates

2. Reaction conditions:

in laboratory	x	technology
↓		
optimal		↑↓ temperature ↑ substrate conc. (inhibition by products) ↑ viscosity ↑↓ pH polarity of the environment

Most frequently used enzymes?

80% Hydrolases

Proteases

50%

Glycosidases

50%

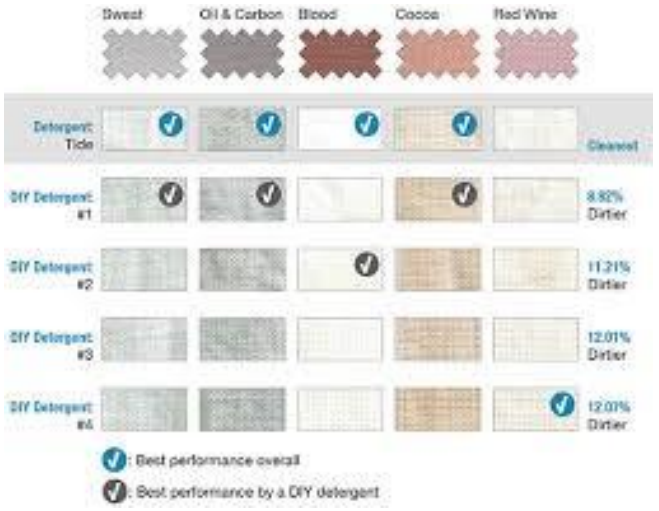
Enzyme	Yield (%)	Used in
Bacterial proteases	35	detergens
Glucoamylase	13	Starch industry
Glucoseisomerase	12	Starch industry
Bacterial amylases	10	Starch industry
Pectolytic enzymes	9	Preservation industry
Rennets	9	Dairy industry
Fungal amylases	3	Bakery
Fungal proteases	2	Bakery
Others	7	miscelaneous

Determination of the activity of technical enzymes

Problems:
- Technical enzymes are not pure proteins
- Substrates are not chemically pure compounds
- Reaction conditions are often far from optimal

Solutions:
- Synthetic substrates as models, standard conditions (EC recommednation) – fals results
- Standard substrates but conditions mimicking technological process
- Ignore EC recommendation and use special method based on the process (substrate,conditions)

ex. washing tests



Preparation of enzymes for sale

1. use of additives
2. the controlled use of covalent modification,
3. enzyme immobilisation

Composition of technical enzyme preparations

Form: liquid, powder, immobilized

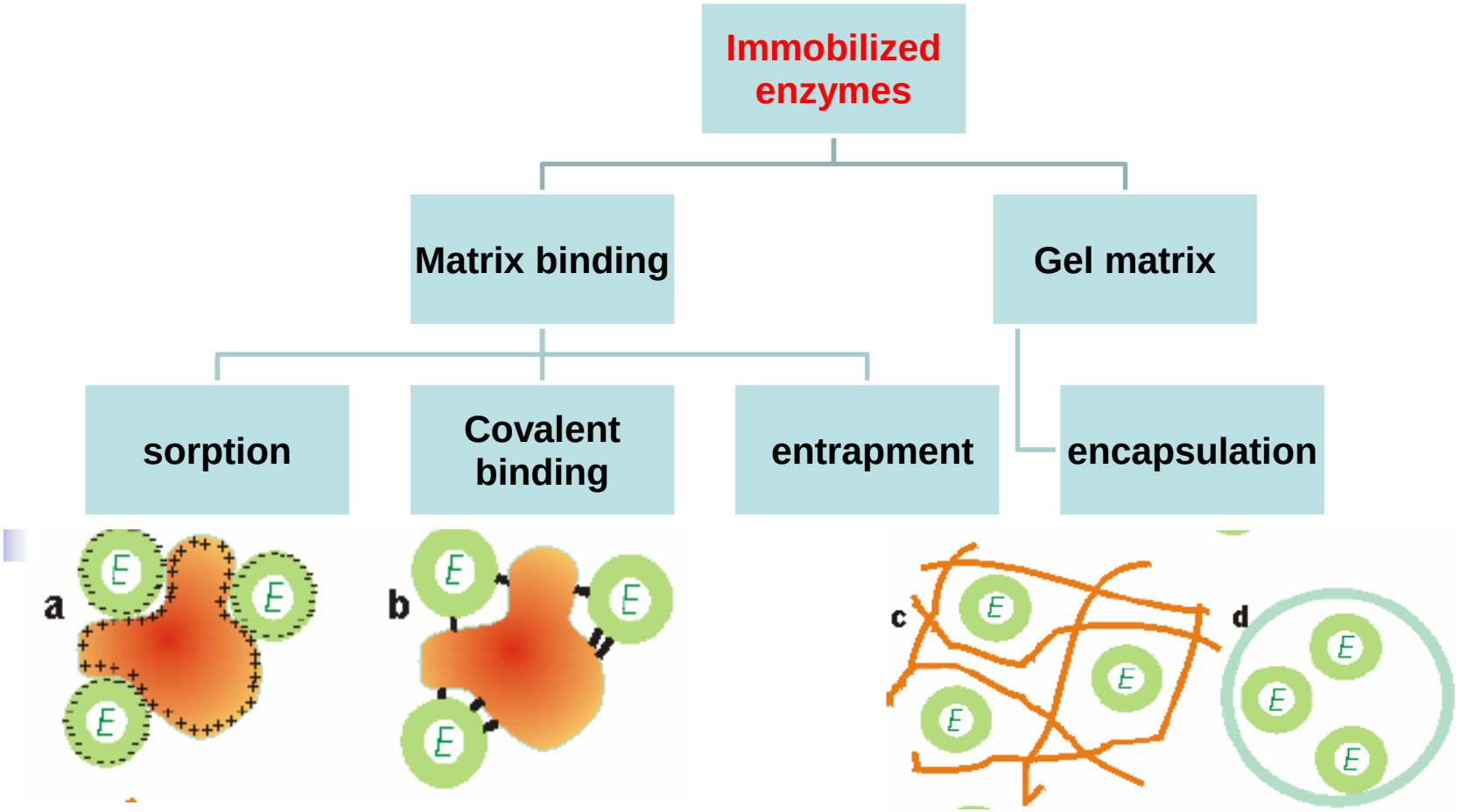
Formulation of technical preparation – „tailoring“

component	Yield (%)
Proteins and aminoacids	10 - 15
Active protein (enzyme)	1 - 5
polysaccharides	5 - 12
sacharides	2 - 40
Inorganic salts	3 - 40
Preservation	0 - 0,3

Immobilized enzymes

IUPAC definition - enzymes, physically restricted or localized, maintaining its activity and can be used repeatedly and continually

Heterogenous catalyzer

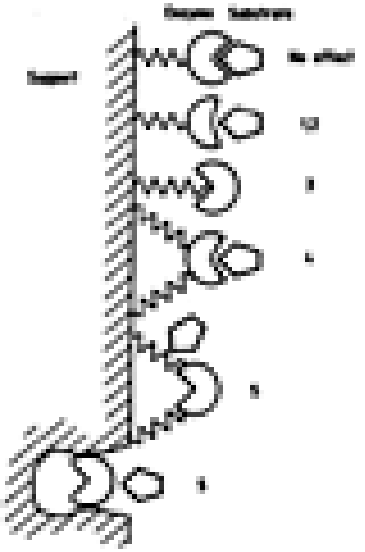


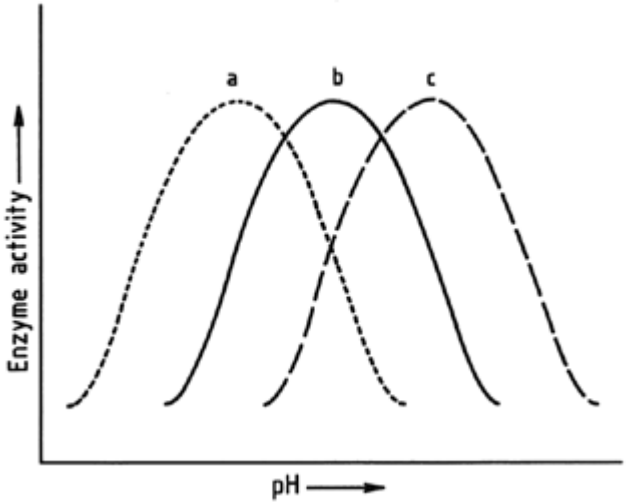
Types of matrices used

- natural polymers
    - polysacharides – dextran, carageenan, cellulose
    - proteins - gelatine
- synthetic polymers - polystyrene, polyacrylates, hydroxylakylmethakrylates, atd.)
- anorganic matrices (minerals, charcoal, porous glass, porous metal oxides)

Influence of immobilization on enzyme characteristics:

1. Inactivation by reactant and products of immobilization reaction
2. Conditions of immobilization reaction
3. Binding is fixing an enzyme molecule in inactive conformation
4. Binding reaction proceeds with active site side chains
5. Orientation of enzyme molecule on the surface limits the substrate access
6. Influence of the functional group of the matrix





a) Matrix with positive charge

b) Native enzyme

c) Matrix with negative charge

Enzyme resources

Animal – production time,

- lagislation restriction

Plant – production time

- climate

Microbial – 95% or more of all enzymes used

- short porduction time, renewable resources,
- legislation restriction – low number of MO approved, but many strains of approved MO
- recombinant technologies
- extremophile MO

recombinant DNA technology

Amplification of DNA using plasmids

Tools for manipulation with genes:

- Plasmid – vector
- Restriction endonucleases

Restriction endonukleases

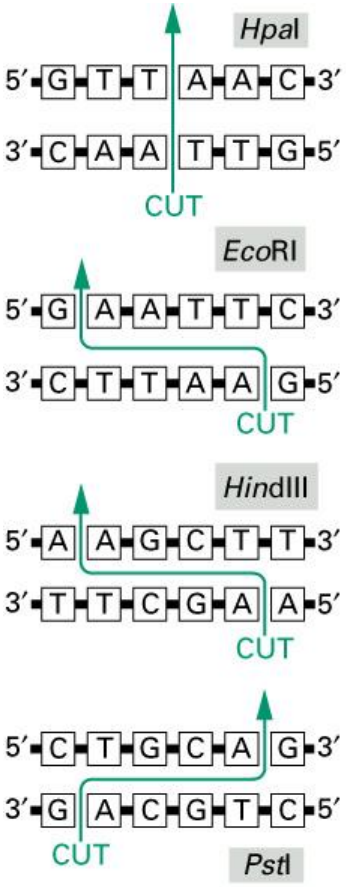
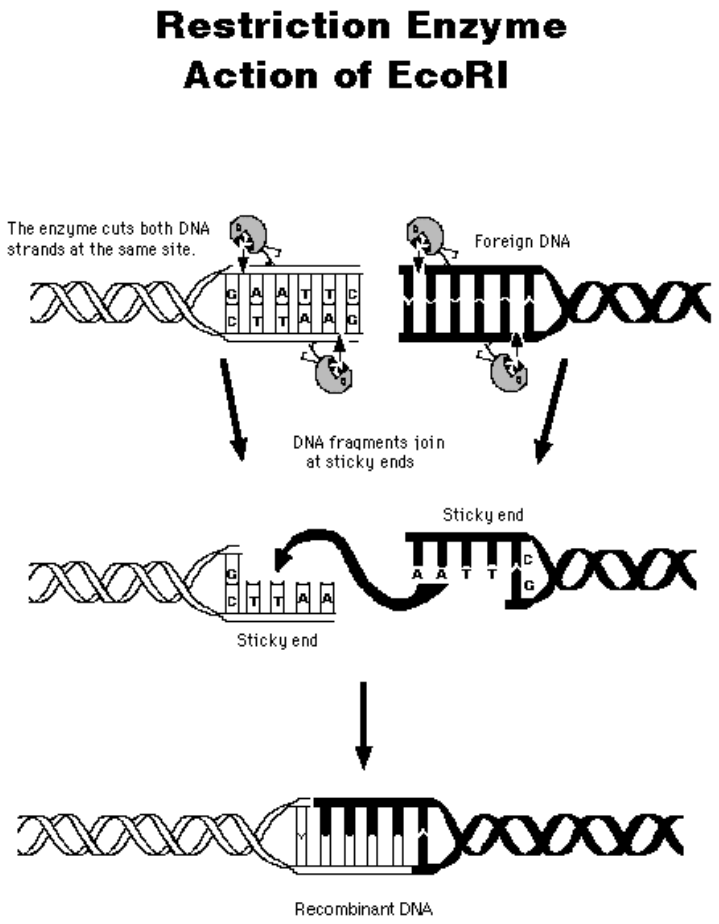
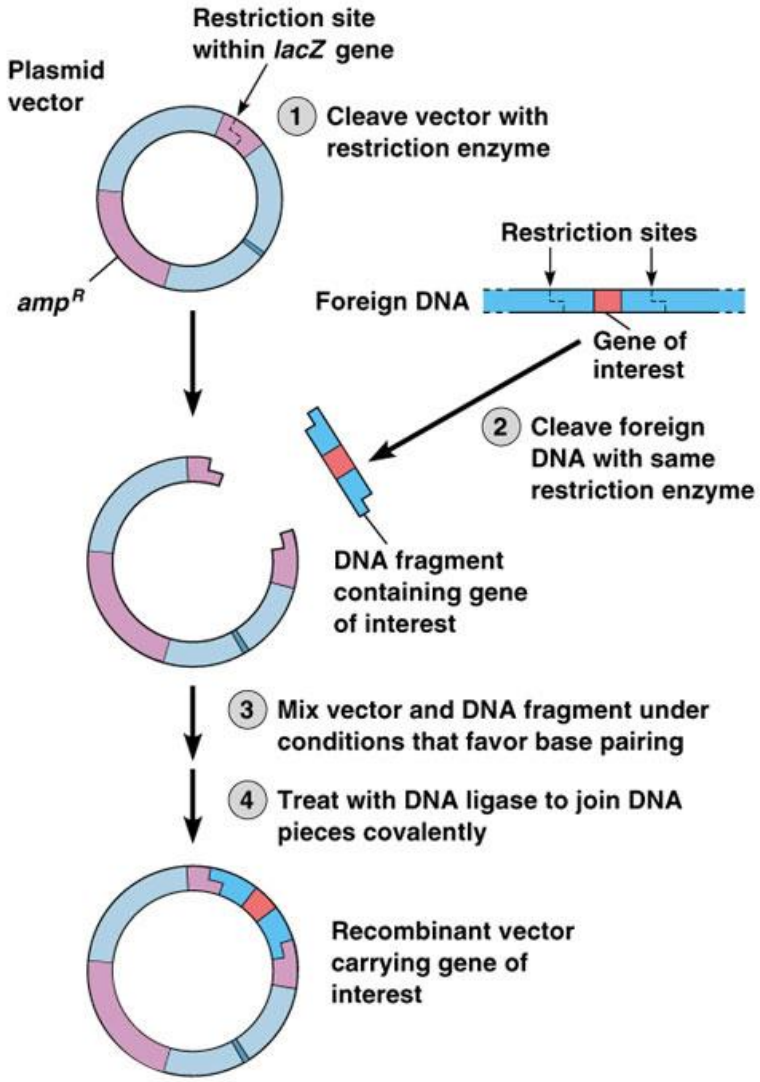


Figure 8–21. Molecular Biology of the Cell, 4th Edition.



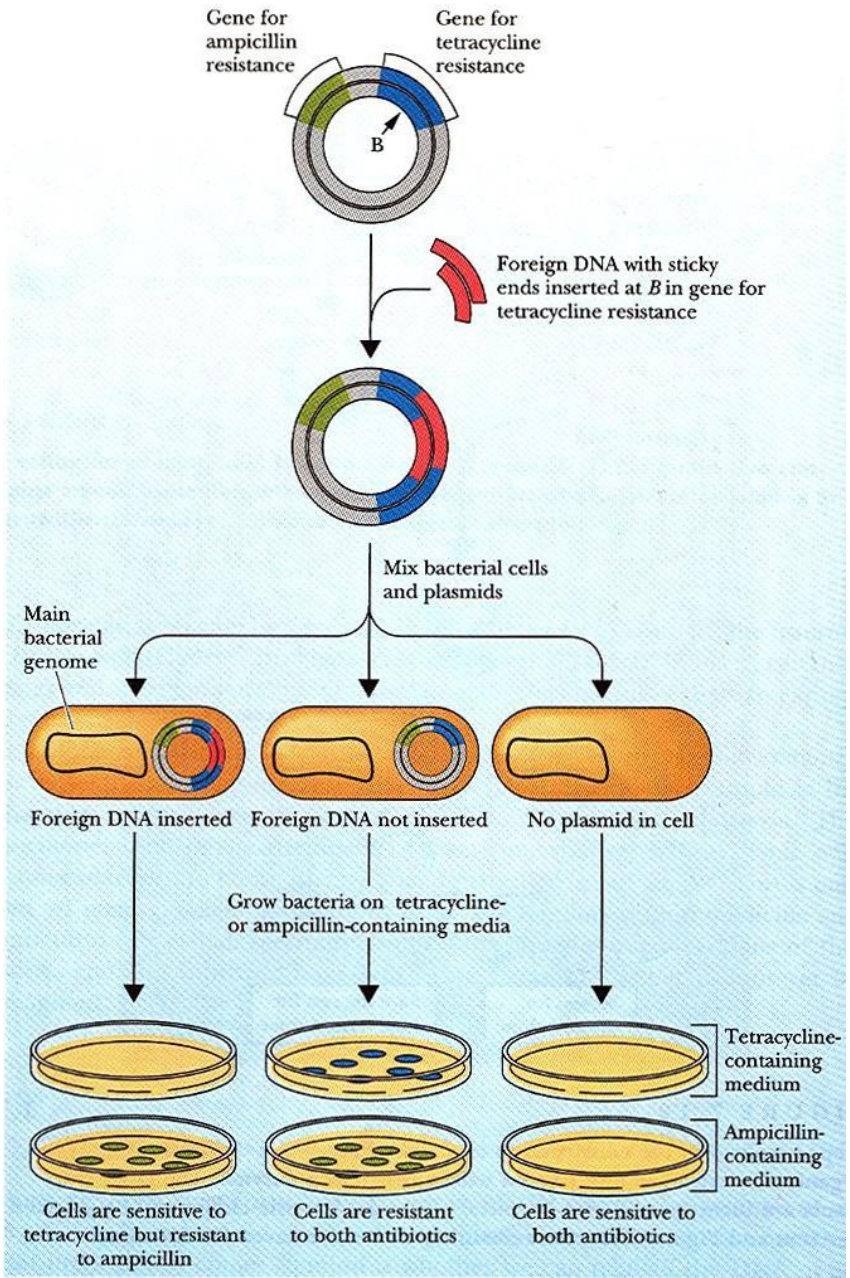
Madam I´m Adam

Preparation of plasmid (vector) with DNA of our interst – recombinant DNA



(b) Preparation of recombinant plasmid vector

Selection of plasmids containing inserted DNA



1. Suitable plasmid (resistence toward ampicilline and tetracycline
2. Insertion of DNA
3. Transformation of cells (bacterial)
4. Selection of cells bearing plasmid with the insert

Extremophiles – good chance and perspective

MO	conditions	source	Enzymes, application
thermophilic	50 – 110˚C	geothermal regions	Amylases xylanases proteases DNA polymerases
Psychrophilic	5 – 20 ˚C	Glacier sediments	Proteases, glycosidases lipases
Acidophilic	pH < 2	solfatars	Sulphur oxidation
Alkalophilic	pH > 9	sludges	lipases proteases
Halophilic	3 – 20% salt	Salt lakes	Biotransformations (additives, drugs)
Barophilic	High pressure	Submarine sediments	

Enzyme production

1. Choose the entire enzyme
2. Choose the relevant source
3. Construction of overexpressing MO (typically producing 50g/l)
4. Optimization of cultivation conditions
5. Optimization of the isolation and purification procedure
6. Formulation of stable preparation

Searching for new enzymes

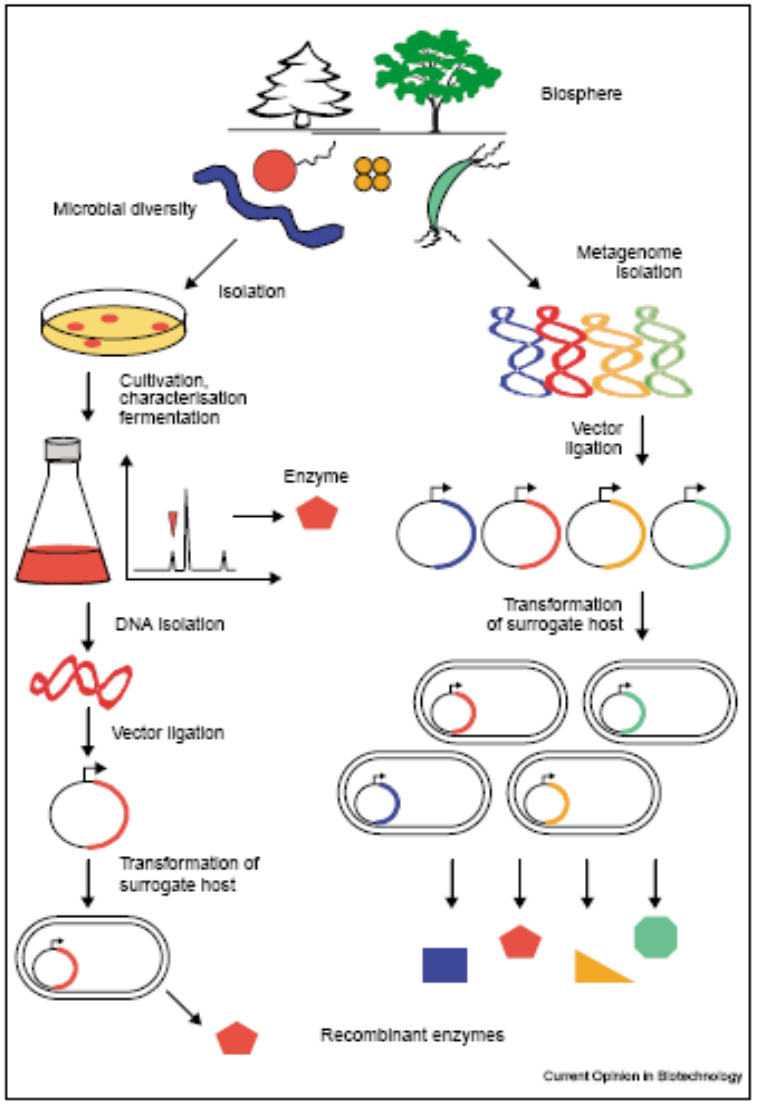
„Classical“ approach

Production MO:

Selection pressure – substrate as the only source of C

Detection of enzyme activity

Problem: Only approx 1% MO can be cultivated *in vitro*

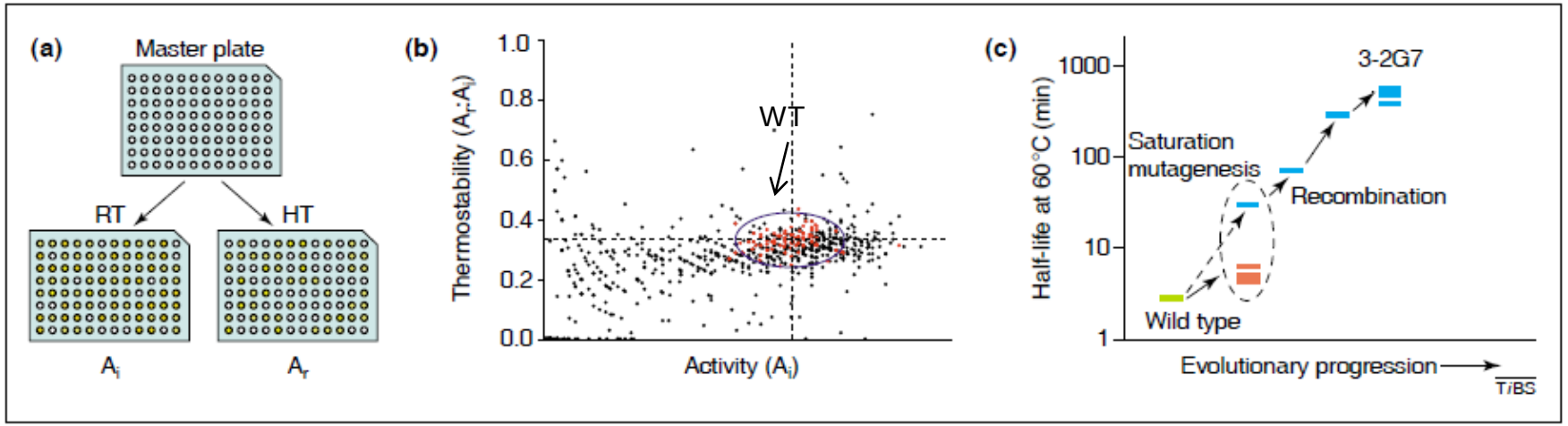


Metagenomic approach

„Development“ of new enzymes

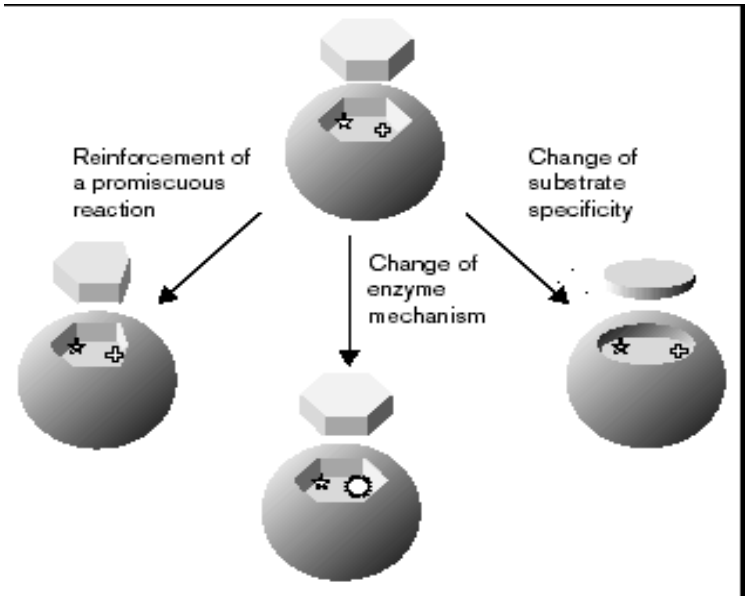
Directed (oriented) evolution:
Mimics the process of natural evolution – mutagenesis (Ethyl methanesulfonate – EMS), recombination, gene edditing (CrisprCas system)

Example: Increasing termostability of subtilisin from the psychrophilic MO



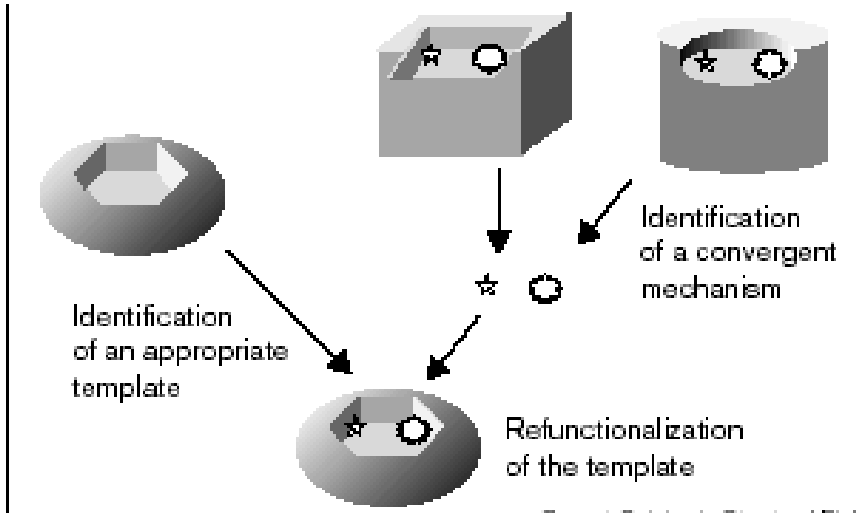
2. Protein engineering - “Retailoring of enzymes”

1. Change of catalytic activity of existing enzyme protein

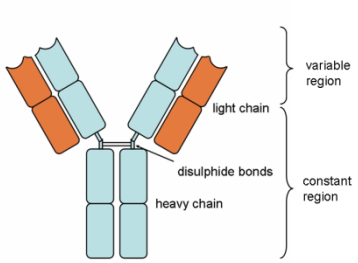


2. Formation of a new biocatalyst:

template + **functional parts**

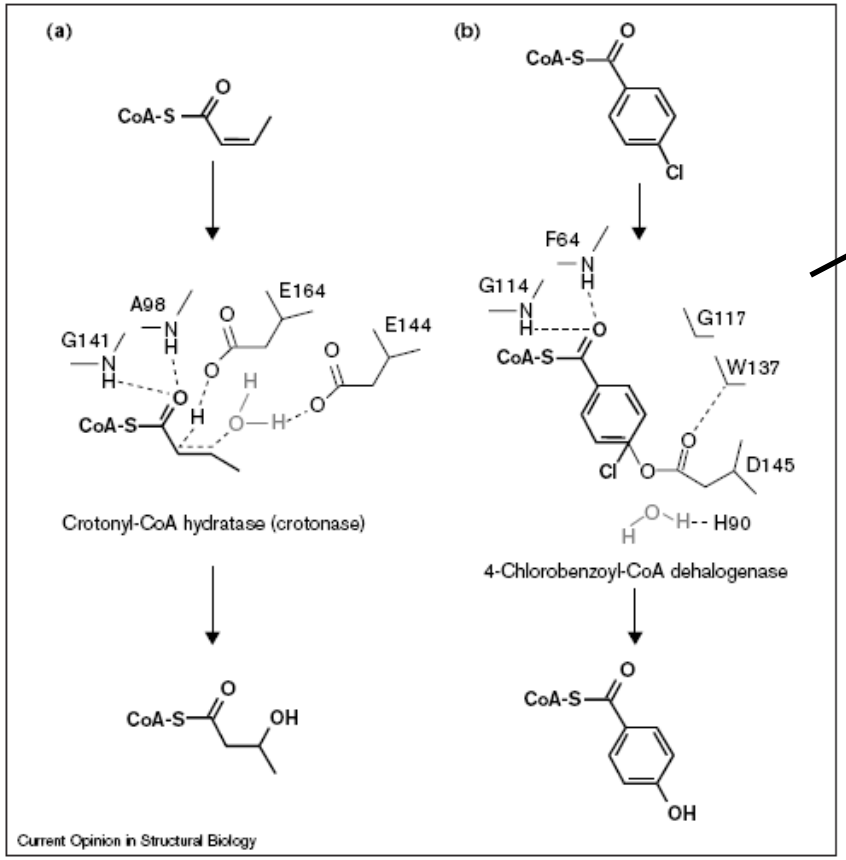


Ex.



abzyme

Example:

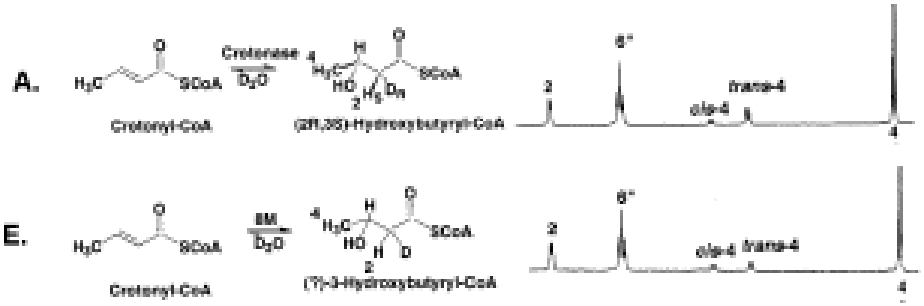


8 site directed mutations:

2 catalytic residues

1 substrate binding AA

5 remodeling catalytic loop



Biosynthesis of lys and trp,
fermentation of butyric acid

Risks in using enzymes:

- Catalytic activity?
- Allergic reactions?
- toxic metabolites (mycotoxins, antibiotics)

Joint FAO/WHO Expert committee on Food Additives (**JECFA**) differentiate:

- enzymes from animal edible parts and tissues used as food, from edible parts of plants and microorganisms traditionally accepted as foods or food components,
- enzymes from non - pathogenic MO
- enzymes from other MO

Enzyme status

- additives
- part of technological process

Enzyme status in technologies differes according to national legislations

In feed industry generally as for foods, in other industry – chemicals