Main applications of enzymes in industry

Major biotransformations at industrial scale 1.

Fine-pharma chemistry

Production scale [tpy]	Product	Enzyme	Reactor	Company
> 1 000 000	high-fructose corn syrup (HFCS)	glucose isomerase	fixed-bed, IME	various
> 100 000	lactose-free milk	lactase	fixed-bed, IME	various
> 10 000	acrylamide	nitrilase	batch reactor	Nitto Co.
	cocoa butter*	lipase (CRL)	fixed-bed, IME	Fuji Oil
> 1,000				
	nicotinamide	nitrilase	3-stage batch	Lonza Guangzhou
	D-pantothenic acid	aldonolactonase		Fuji Pharma-ceuticals
	(S)-chloropropionic acid	lipase		Dow Chemical
	6-aminopenillanic acid	penicillin amidase	fixed-bed, IME	various
	7-aminocephalo-sporanic acid	glutaryl amidase	Kundl/Hoechst	
	aspartame®	thermolysin	soluble enzyme	Tosoh/DSM
	L-aspartate	aspartase	fixed-bed, IME	various
	D-phenylglycine	hydantoinase/ (carbamoylase)	resting cells	Kanegafuchi
	D-*p*-OH-phenyl-glycine	hydantoinase/ carbamoylase	resting cells	Recordati

Major biotransformations at industrial scale 2.

Production scale [tpy]	Product	Enzyme	Reactor	Company
> 100	ampicillin	penicillin amidase	stirred IME	DSM–Gist Brocades
	L-methionine, L-valine	aminoacylase	EMR	Degussa (Rexim)
	L-carnitine	dehydrase/ hydroxylase	whole cells	Lonza
	L-dopa	β-tyrosinase		Ajinomoto
	L-malic acid	fumarase	fixed-bed, IME	Tanabe
	(*S*)-methoxyiso-propylamine	lipase	repeated batch	BASF
	(*R*)-HPOPS	hydroxylase	batch reactor	BASF
	(*R*)-mandelic acid	nitrilase	batch reactor	BASF
	L-alanine	L-aspartate-β-decarboxylase	IME	various

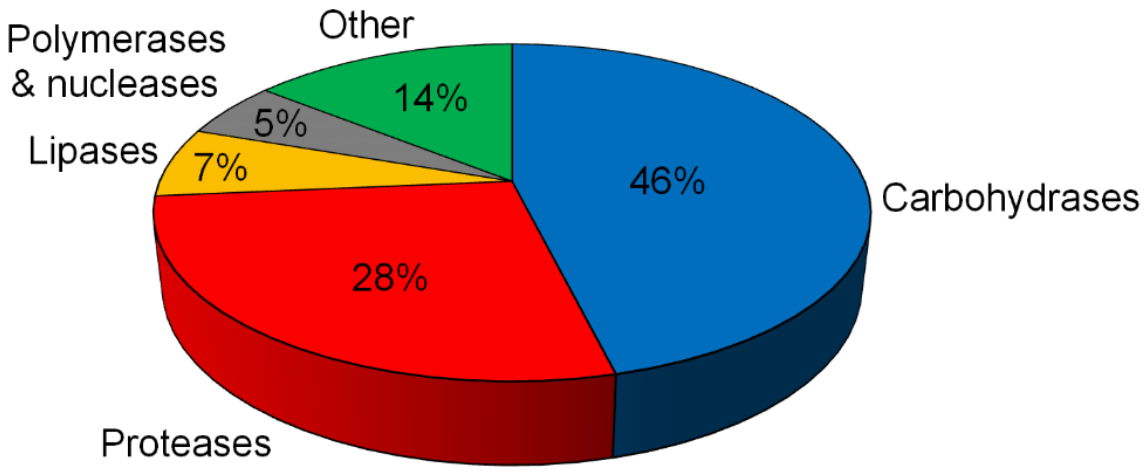
tpy: (metric) tons per year; IME: immobilized enzyme reactor; EMR: enzyme membrane reactor; L-dopa: 3,4-dihydroxyphenylalanine; (*R*)-HPOPS: (*R*)-2-(4-hydroxyphenoxy)propionic acid.

* Operation depends on the price of substrate palm oil vis-à-vis competing sources.

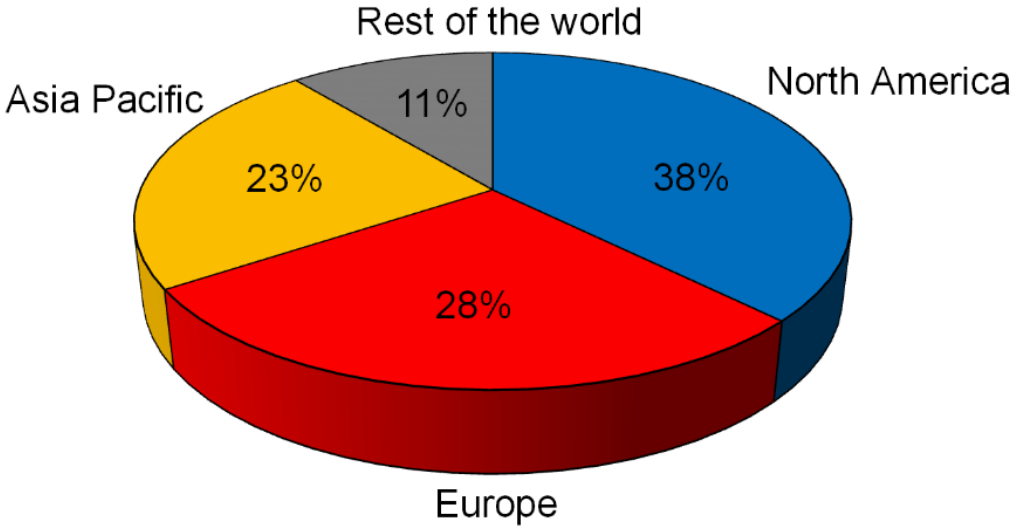
Enzyme market

The **biocatalyst market for specialty enzymes**, where pharmaceuticals are the prominent application, is accounted only for **$230 M**. Although these figures do not take into account own production and usage of enzymes by companies, it is evident that **specialty enzymes represent a limited portion of the global industrial enzyme market**, which in 2013 was valued at around **$4.4 B** and by 2020 it is expected to reach 111.7 kt by volume. This growth is motivated by the increasing industrialization, the growing environmental concerns but especially by the evident benefits coming from the use of enzymes in multiple technical applications in the food, environmental, biomedical and energy sectors.

2013 Global enzyme market revenue by product



2013 Global enzyme market revenue by region



Total 2013: 4412 USD million

Data extracted from the Grand View Research Enzyme Market Report

hydrolases

isomerases

transferases

oxidoreductases

lyases

Industry	Enzyme class	Application
Detergent (laundry and dish wash)	Protease	Protein stain removal
	Amylase	Starch stain removal
	Lipases	Lipid stain removal
	Cellulase	Cleaning, color clarification, anti-redeposition (cotton)
	Mannanase	Mannanan stain removal (reappearing stains)
Cellulose/Starch	Amylase	Starch liquefaction and saccharification
	Cellulase	Cellulose saccharification
	Xylanase	Viscosity reduction
	Pullulanase	Saccharification
	Glucosidase	Saccharification
	Glucose isomerase	Glucose to fructose conversion
	Cyclodextrin-glycosyltransferase	Cyclodextrin production

hydrolases

isomerases

transferases

oxidoreductases

lyases

Food (including dairy)	Protease	Milk clotting, infant formulas (low allergenic), flavor
	Lipase	Cheese flavor
	Lactase (β-galactosidase)	Lactose removal (milk)
	Pectin methyl esterase	Firming fruit-based products
	Pectinase	Fruit-based products
	Transglutaminase	Modify visco-elastic properties
Baking	Amylase	Bread softness and volume, flour adjustment
	Xylanase	Dough conditioning
	Lipase and phospholipase	Dough stability and conditioning (*in situ* emulsifier)
	Glucose oxidase	Dough strengthening
	Lipoxygenase	Dough strengthening, bread whitening
	Protease	Biscuits, cookies
	Transglutaminase	Laminated dough strengths

hydrolases

isomerases

transferases

oxidoreductases

lyases

Beverage	Pectinase	De-pectinization, mashing
	Amylase	Juice treatment, low calorie beer
	β-Glucanase	Mashing
	Acetolactate decarboxylase	Maturation (beer)
	Laccase	Clarification (juice), flavor (beer), cork stopper treatment
Textile	Cellulase	Denim finishing, cotton softening
	Amylase	De-sizing
	Pectate lyase	Scouring
	Catalase	Bleach termination
	Laccase	Bleaching
	Peroxidase	Excess dye removal

hydrolases

isomerases

transferases

oxidoreductases

lyases

Pulp and paper	Lipase	Pitch control, contaminant control
	Protease	Biofilm removal
	Amylase	Starch-coating, de-inking, drainage improvement
	Xylanase	Bleach boosting
	Cellulase	De-inking, drainage improvement, fiber modification
Fats and oils	Lipase	Transesterification
	Phospholipase	De-gumming, lyso-lecithin production
Leather	Protease	Bating (macerazione)
Personal care	Lipase	De-pickling
	Amyloglucosidase	Antimicrobial (combined with glucose oxidase)
	Glucose oxidase	Bleaching, antimicrobial
	Peroxidase	Antimicrobial

Adapted from: Ole Kirk et al., Current Opinion in Biotechnology 2002, 13, 345.

Classification of enzymes used in organic synthesis.

Class	Enzyme	Common reaction
1. Oxidoreductases	Dehydrogenases	Oxidation of **alcohols** and aldehydes, reduction of **aldehydes and ketones**; oxidation of **C-C** single bonds, reduction of **C=C** double bonds
	Oxidases	Oxidation of alcohols and amines
	Mono- and dioxygenases	Hydroxylation, sulphoxidation, epoxidation, Baeyer-Villiger oxidation,
	Peroxidases	Oxidation, epoxidation, halohydration
2. Transferases	Kinases	Phosphorylation (ATP-dependent)
	Sulphotransferases	Formation of sulphate esters
	Glycosyltransferases	Glycosidic bond formation
	Transketolases	Ketol (α-hydroxyketones) group transfer

Class	Enzyme	Common reaction
3. Hydrolases	Esterase, lipases	Ester hydrolysis / synthesis
	Amidohydrolases (amidases or acylases)	Amide hydrolysis / synthesis
	Proteases	Peptide bond hydrolysis / synthesis
	Glycosidases	Glycosidic bond formation/hydrolysis
	Nitrilase (nitrile aminohydrolase)	Hydrolysis of nitrile to carboxylate
	Epoxide hydrolases	Hydrolysis of epoxides
	Phosphatases	Hydrolysis of phosphate esters
	Dehalogenases	C-halide hydrolysis

Class	Enzyme	Common reaction
4. Lyases	Aldolases	Aldol reaction (C–C bond)
	Oxynitrilase	Cyanohydrine formation
5. Isomerases	Glucose isomerase	Isomerisation of carbohydrates,
	Mandelate racemase	Racemisation
6. Ligases		Not used at present for practical applications

Main coenzymes required by enzymes used in biocatalysis

Coenzyme	**Reaction type**
Flavines	Oxygenation
Thiamine pyrophosphate	Decarboxylation, transchetolization
$NAD(P)^+$ /NAD(P)H	Hydrogenation/dehydrogenation
$NAD(P)^+$ /NAD(P)H	Oxygenation
ATP	Phosphorylation
Pyridoxal- phosphate	Modification of aminoacids
Metal-phorphyrin complexes	Peroxidation, oxygenation

Several **cofactors** can be **recycled effectively**, including nucleoside triphosphates such as ATP in phosphoryl transfer reactions, (NAD^+/NADH and $NADP^+$/NADPH) in oxidoreductions, acetylCoA in acyl transfer reactions, and sugar nucleotides in glycosyl transfer reactions.

Many cofactor dependent reactions have been applied on preparative or industrial scales.

Nevertheless, hydrolases still remain the most widely employed enzymes due to their large availability and to the fact that they do not need organic coenzymes.

Oleochemistry

Lipases in the synthesis of esters:

- **Biodiesel**
- **Surfactants**
- **Emollients**
- **Food ingredients**

Lipases and Transformation of Fats and Oils

The basic oleochemicals are:
fatty acids (ca. 52%), the respective methyl esters (ca. 11%), amines (ca. 9%), and alcohols (ca. 25%).

Besides food applications, these are used for the production of important chemical products: **surfactants, lubricantsand coatings** but also **biofuels.**

Vegetable Oils production: 80 B Kg

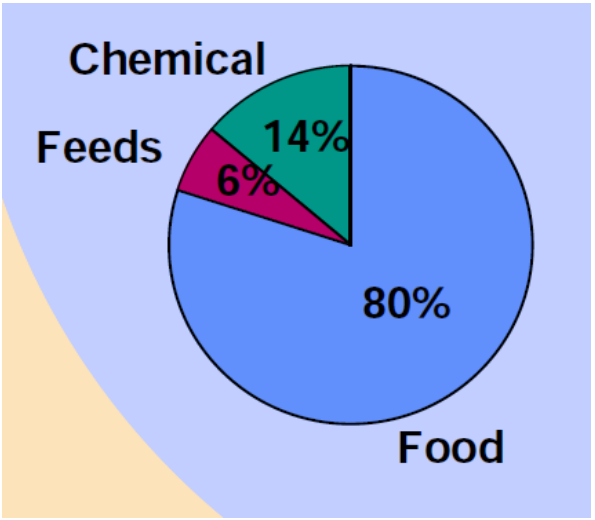
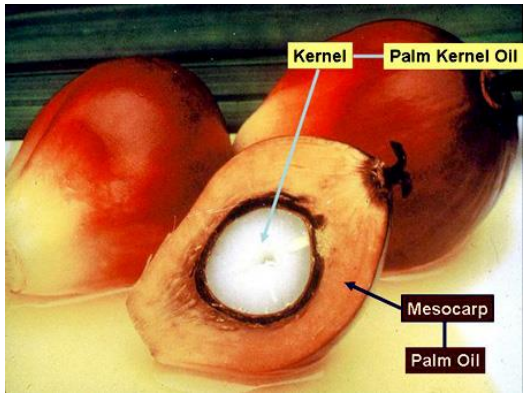


Figure 1. Production of oils and fats that are important as feedstock for the oleochemical industry in 1999/2000 and 2009/2010.[18,19]

- **palm kernel oil,**
- **palm oil,**

highly saturated vegetable fats

African oil palm
Elaeis guaineensis



Palm kernel oil:

- semi-solid at room T, more saturated than palm oil and comparable to coconut oil. Used in commercial cooking because of its low cost, and because it remains stable at high cooking temperatures and can be stored longer than other vegetable oils.(fewer C=C)
- is high in lauric acid which has been shown to raise blood cholesterol levels

semisolid at room temperature

Palm oil: tens of millions of tons of palm oil is produced annually, accounting for over 30% of the world's vegetable oil production. This single vegetable oil is found in approximately 40-50% of household products in many developed countries like Australia. Palm oil can be present in a wide variety of products, including baked goods, shampoo, cosmetics, washing detergents and toothpaste.

Saturated fatty acids

palm mesocarp oil is 49% ,
palm kernel oil 81%
coconut 86%

Fatty acid content of palm kernel oil

Type of fatty acid	
Lauric saturated C12	48.2%
Myristic saturated C14	16.2%
Palmitic saturated C16	8.4%
Capric saturated C10	3.4%
Caprilic saturated C8	3.3%
Stearic saturated C18	2.5%
Oleic monounsaturated C18:1	15.3%
Linoleic polyunsaturated C18:2	2.3%
other	0.4%

Fatty acid content of palm oil

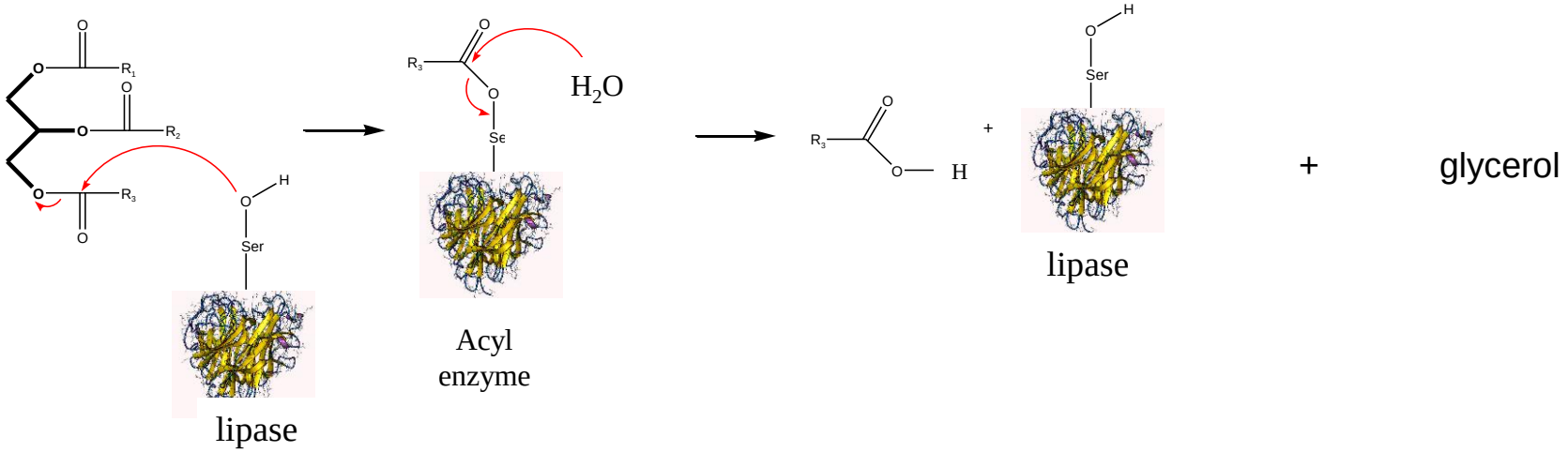
Type of fatty acid	
Mirystic saturated C14	1.0%
Palmitic saturated C16	43.5%
Stearic saturated C18	4.3%
Oleic monounsaturated C18	36.6%
Linoleic polyunsaturated C18	9.1%
Other/Unknown	5.5%

Lipases and phospholipases used for processing of lipids

Enzyme or Microorganism	Application	Examples	Ref.
Lipases	structured triglycerides	cocoa-butter equivalent, betapol	[155–158]
	enrichment/incorporation of specific fatty acids	PUFA from fish oils	[159, 160]
	ester synthesis	emollient esters	[161, 162]
	biofuels	FAMEs[b]	[163, 164]
Phospholipases	removal of fatty acids in sn1- or sn2-position (PLA_1 or PLA_2)[b]	degumming of oils	[165]
	removal of phosphate groups (PLC)[b]	degumming of oils	[166, 167]
	head-group exchange (PLD)[b]	synthesis of phosphatidyl-serine	[168]

[a] FAME: fatty acid methyl ester. [b] PUFA: poly unsaturated fatty acid; AA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; PL: phospholipase.

Lipase hydrolyze (digest) triglycerides at mild conditions

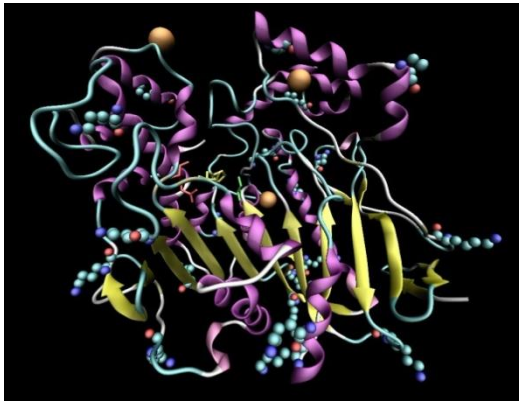


Advantages of industrial applications (coco nuts oil, olive oil…):

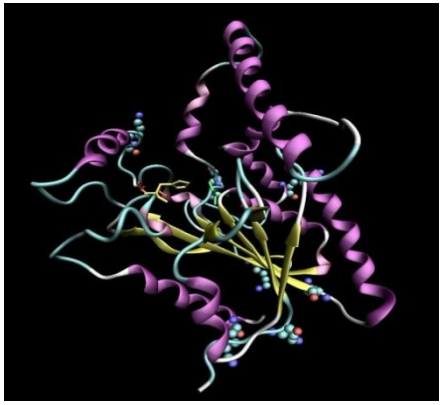
a) reduction of wastes and side products
b) mild and neutral reaction conditions leading to highest quality products
c) higher purity of the glycerol obtained as secondary product

Lipases for ydrolysis, esterification/transesterification:

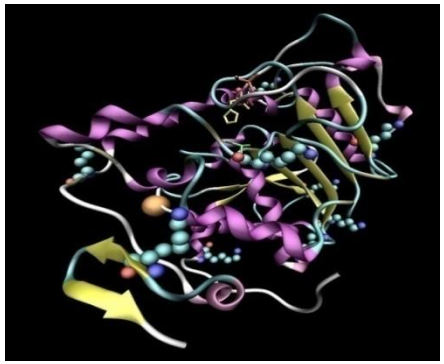
Burkholderia cepacia (Ps. cepacia) (33 kDa)



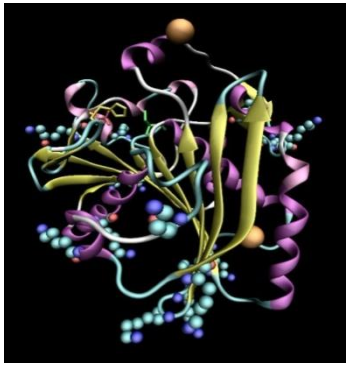
Candida rugosa (o cylindracea) (57 kDa)



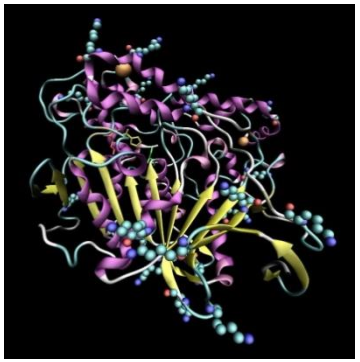
Pseudomonas aeruginosa (33 kDa)



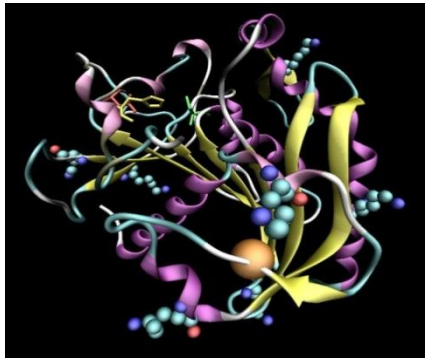
Candida antarctica B (33 kDa)



Rhizopus niveus (30 kDa)



Geotrichum candidum (60 kDa)



Humicola lanuginosa (60 kDa)

Table 3. Important commercially available lipases.

Origin	Code[a]	*M* [kDa] (rounded)	Specificity (remarks)	Applications
of mammalian origin				
human pancreatic lipase	HPL	50	*sn*-1,3	
human gastric lipase	HGL	50	*sn*-3 (acid-stable)	
porcine pancreatic lipase	PPL	50	*sn*-1,3	organic synthesis, digestive aid
guinea pig pancreatic lipase	GPL-RP2	48	*sn*-1,3 (phospholipase A1 activity)	
of fungal origin				
Candida rugosa	CRL	60	nonspecific	organic synthesis
Candida antarctica B	CAL	60	*sn*-1,3	organic synthesis
Geotrichum candidum	GCL	60	cis-Δ^9 (unsaturated fatty acids)	oleochemistry
Humicola lanuginosa	HLL	30	nonspecific	detergents
Rhizomucor miehei	RML	30	*sn*-1,3	cheese manufacturing
Aspergillus oryzae	AOL			cheese manufacturing
Penicillium camembertii	PEL	30	*sn*-1,3	monoglycerides
Rhizopus delemar	RDL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
Rhizopus oryzae	ROL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
Rhizopus arrhizus	RAL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
of bacterial origin				
Pseudomonas glumae	PGL	33	nonspecific	detergent enzyme, organic synthesis
Burkholderia cepacia	PCL/BCL	33	nonspecific	organic synthesis
Pseudomonas pseudoalcaligenes	PPL	33	*sn*-1,3	detergents
Pseudomonas mendocina	PML	33	*sn*-1,3	detergents
Chromobacterium viscosum	CVL	33	*sn*-1,3	organic synthesis
Bacillus thermocatenulatus	BTL-2	43	*sn*-1,3 (thermoalkalophilic)	
Fusarium solani (hydrolyzes cutin)	FSL	22		detergents

[a] Other appreviations for lipases used in this article: PSL (*Pseudomonas species* lipase), PFL (*Pseudomonas flourescens* lipase), HLL (human lipoprotein lipase), LPL (lipoprotein lipase). Lipases can be obtained commercially from many suppliers. Important original producers are Novo-Nordisk (Baegsvard, Denmark), Genencor International B. V. (Delft, The Netherlands), Boehringer-Mannheim (Mannheim, Germany), and Amano Co. (Nagoya, Japan).

Specificità di substrato

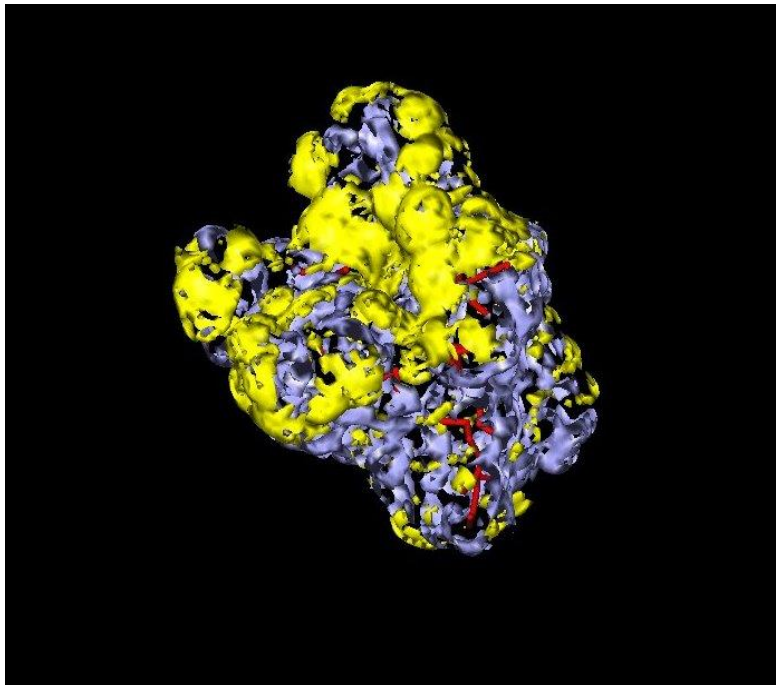
Lipasi possono essere classificate sulla base delle loro specificità di substrato. Ad esempio, lipasi da *Mucor miehei, Ryzopus delemar*, e pancreas suino attaccano preferenzialmente le **posizioni primarie** dell'ossidrile di glicerolo (1,3) e si dice che sono 1,3-specifici.

D'altra parte, lipasi da *Candida. rugosa, Chromobacterium viscosum*, e da semi di ricino, non sono specifiche rispetto alla posizione. La lipasi da *Geotrichum candidu*m è selettiva verso acidi grassi **cis-insaturi** (Δ9), come l'acido oleico.

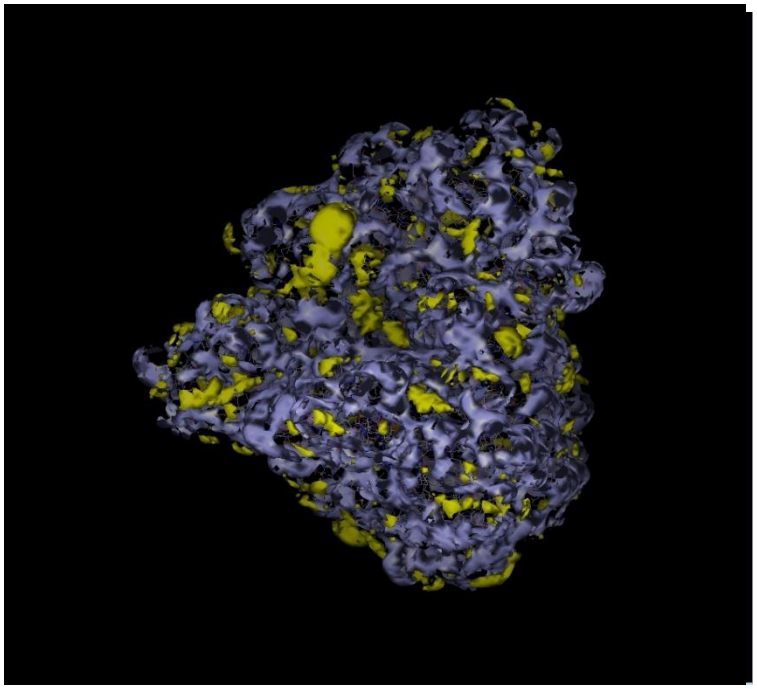
Lipasi da *Mucor miehei* discriminano gli acidi polinsaturi, come ad esempio γ-linolenico e docosaesaenoico.

Così, l'idrolisi dei lipidi a diversi tipi di acidi grassi, in posizioni differenti, porterà a idrolisi che sono specifiche per ciascun particolare tipo di lipasi. Quindi, la regio specificità di substrato e la selettività delle lipasi può essere vantaggiosamente sfruttata per il loro utilizzo nella determinazione strutturale dei trigliceridi e per una sintesi specifica e definita di mono-e / o digliceridi

- Lipases are proteins with wide hydrophobic areas on the surface
- Lipases have evolved to be active on insoluble and hydrophobic substrates



A lipase: large hydrophobic surface



A general hydrolytic enzyme (amidase): hydrophylic surface

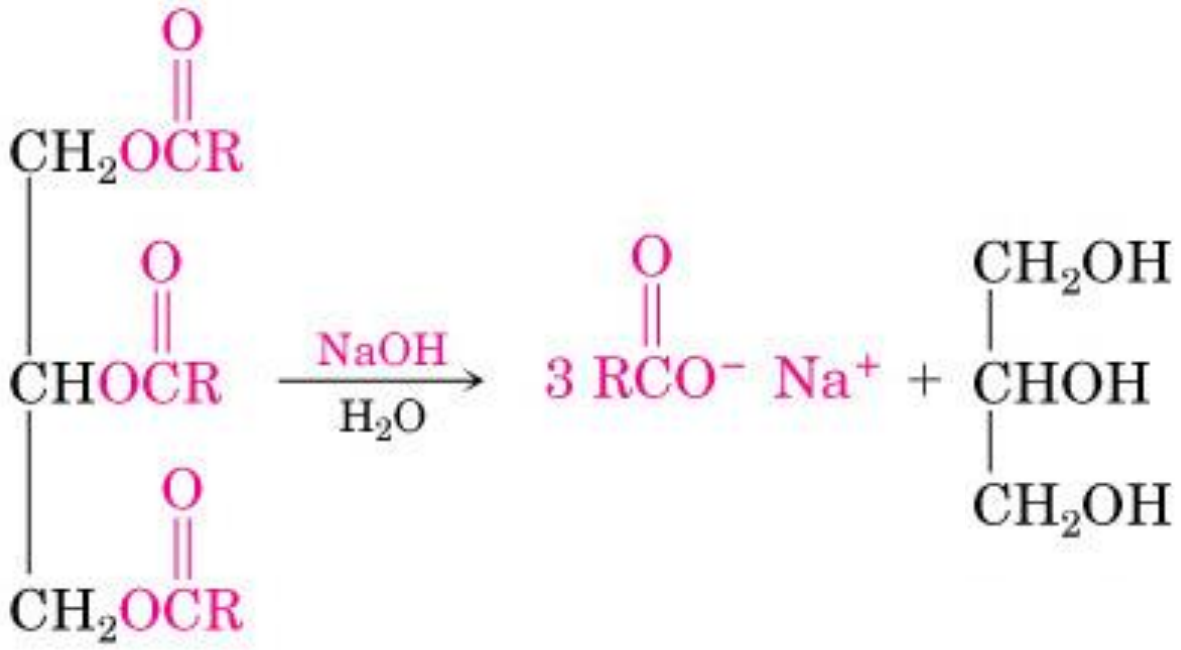
hydrophobic

hydrophilic

Idrolisi di trigliceridi catalizzata da lipasi

Un processo di importanza industriale è l'idrolisi di oli vegetali, come l'olio d'oliva o l’olio di cocco, per produrre acidi grassi e glicerolo, i quali trovano applicazioni diffuse, soprattutto in saponi e detergenti, cosmetici, prodotti farmaceutici, e alimenti.

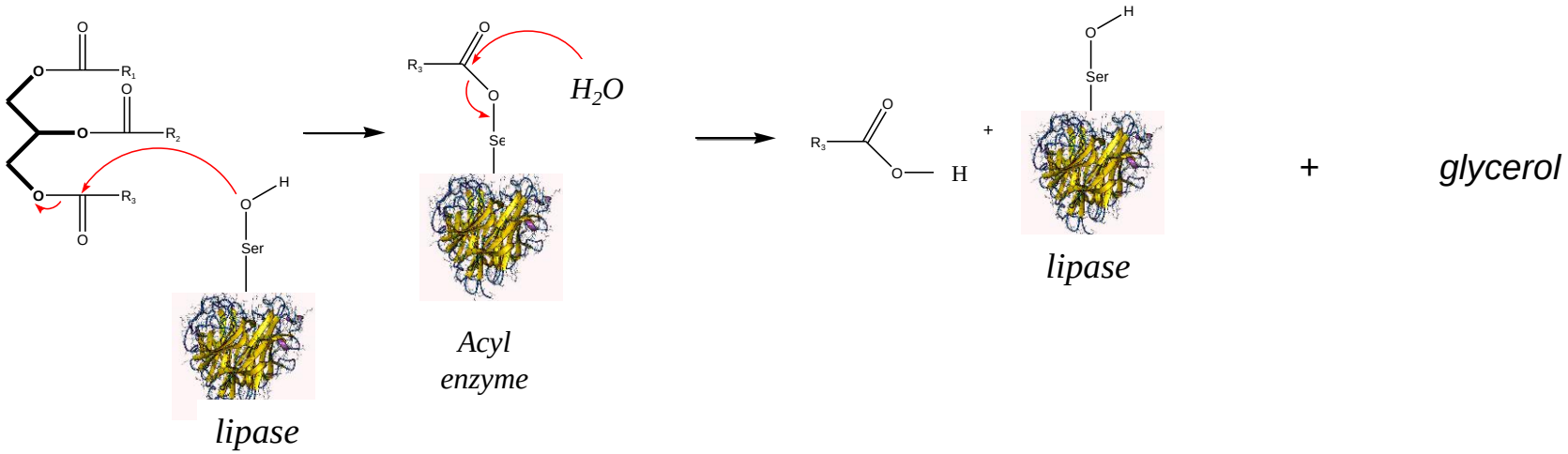
The chemical hydrolysis of oils requires strong basic reagents



Triglyceride

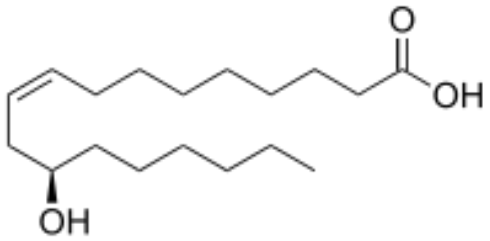
Impiego delle lipasi nella trasformazione di grassi e oli: Idrolisi

Questo processo impiega la capacità delle lipasi di idrolizzare, in presenza di acqua, i lipidi in modo da ottenere acidi grassi e glicerolo, molecole che hanno entrambe importanti applicazioni industriali. Ad esempio, gli acidi grassi vengono utilizzati per la produzione di sapone. Lipasi utilizzate per questo scopo sono quelle da *Candida rugosa*, da seme di ricino (processo già operativo su scala commerciale) e *Pseudomonas fluorescens*.



Idrolisi di trigliceridi catalizzata da lipasi: vantaggi

L' idrolisi enzimatica può essere utilizzata per ottenere acidi grassi instabili da oli che contengono acidi grassi insaturi. Questo è generalmente difficile da conseguire con mezzi convenzionali a causa delle alte temperature (200-240°C) e pressioni utilizzate, che potrebbero portare ad un' indesiderata ossidazione dei lipidi. Ciò è ampiamente dimostrato dalla produzione di acido ricinoleico, un materiale di partenza prezioso per la produzione di un'ampia varietà di prodotti tecnici: l'acido ricinoleico non può essere ottenuto da olio di ricino con processi convenzionali a causa di reazioni collaterali, come la disidratazione, interesterificazione, ecc. Questi possono essere evitati utilizzando una lipasi presente nei semi di ricino e che viene utilizzata per l'idrolisi dell'olio di ricino.



acido ricinoleico

Idrolisi di trigliceridi catalizzata da lipasi: vantaggi

Al contrario la **scissione enzimatica di trigliceridi è effettuata a pressione e temperatura ambiente (40-60 ° C),** consentendo un costo energetico inferiore.

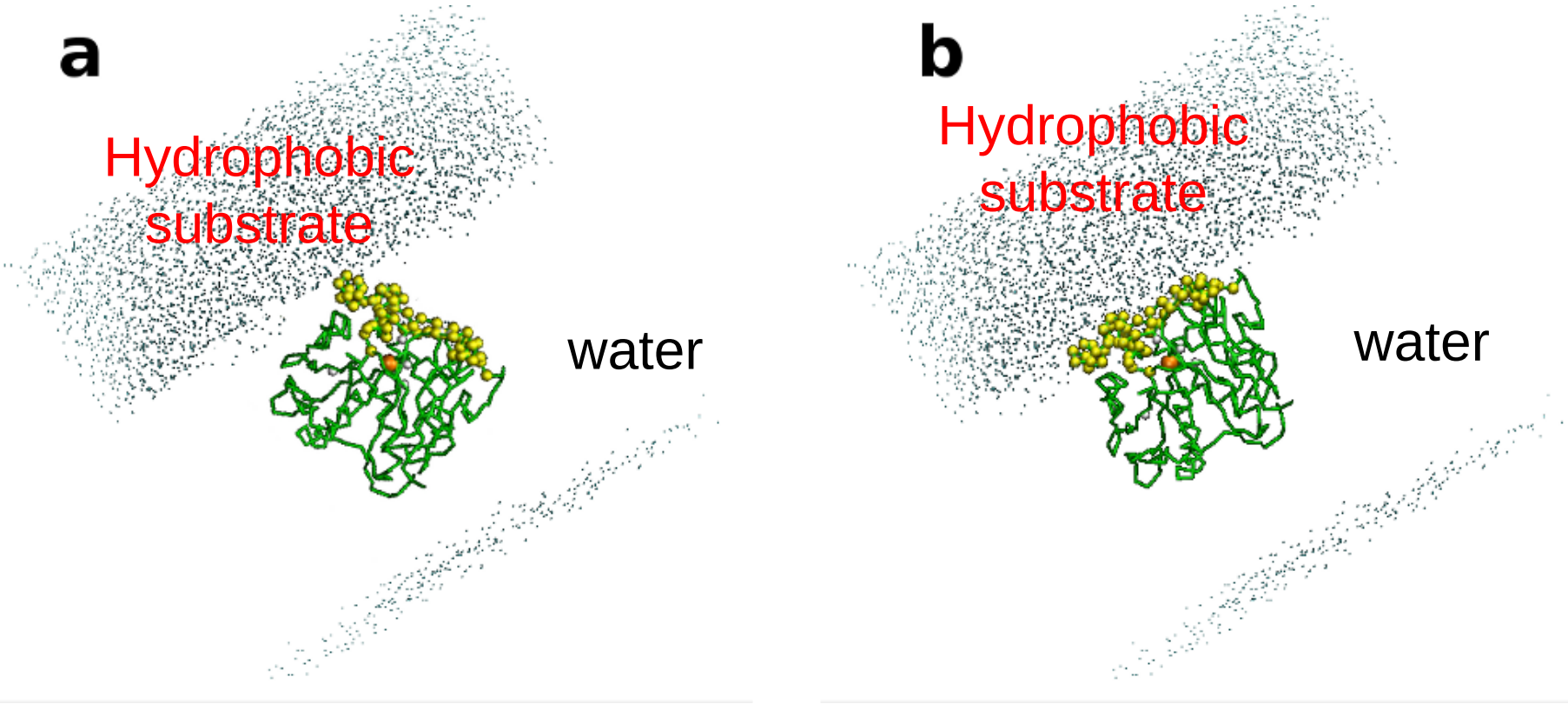
Il costo complessivo del processo enzimatico determina un vantaggio economico dovuto al fatto che **gli impianti di reazione non devono essere molto resistenti alla corrosione né resistere a condizioni di reazione molto aggressive.**

I prodotti che derivano da bioprocessi enzimatici hanno anche un **odore e un colore migliore e di solito sono più puri,** poiché, grazie alla selettività delle lipasi e alle blande condizioni ambientali vengono evitate reazioni secondarie rispetto ai processi convenzionali. Ciò ha il vantaggio di ridurre ulteriormente i costi, grazie alla **riduzione del numero di procedure di estrazione** e purificazione, inoltre le temperature di reazione inferiori garantiscono una minor degradazione termica.

Lipases in organic media are generally used immobilized in order to prevent protein aggregation and to maximize dispersion/accessible surface

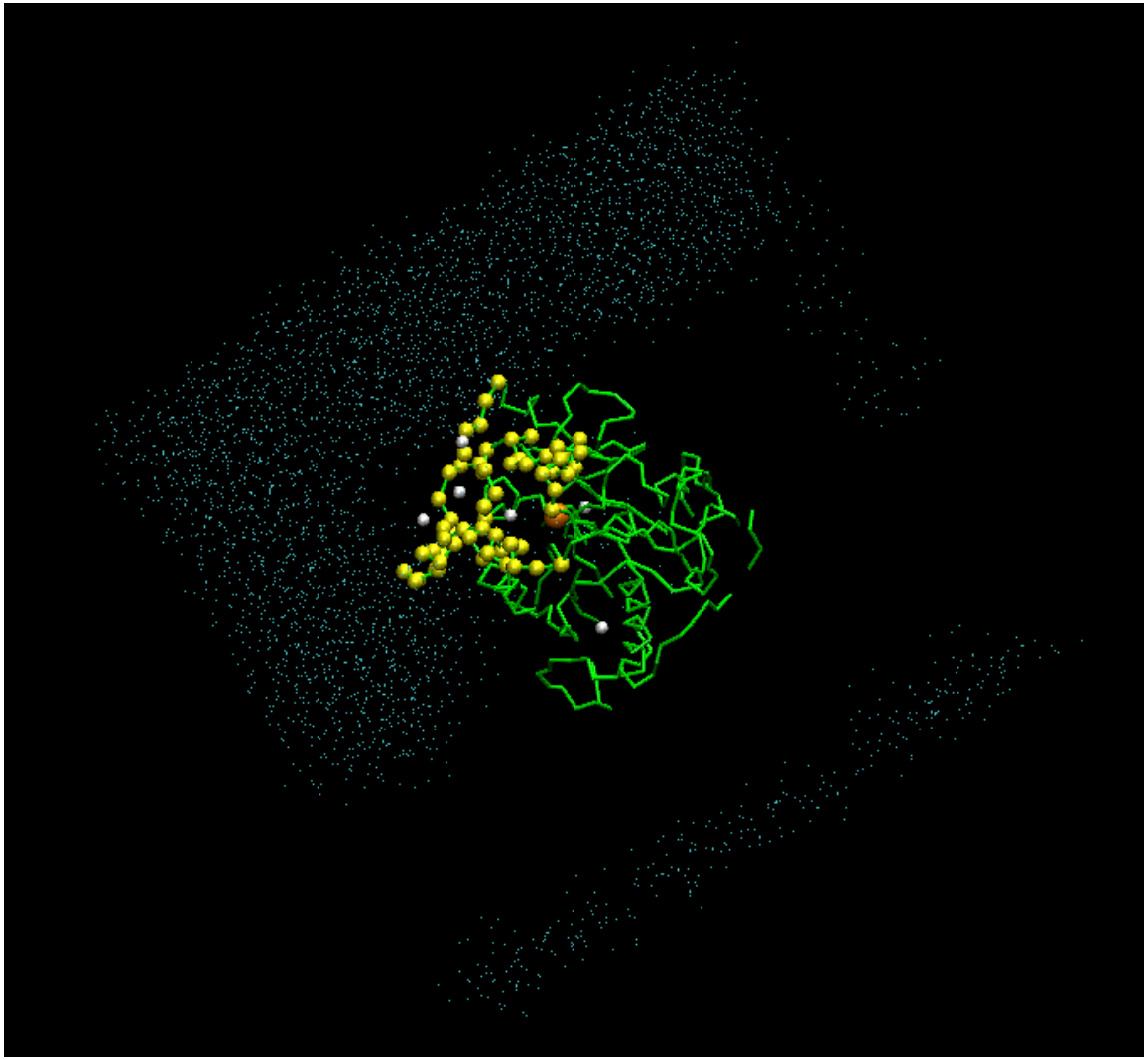
Lipase immobilization is quite challenging

Lipases approach and hydrolyze insoluble hydrophobic substrates



Lipases are active at the interface:

Simulation of Water-Octane Interface



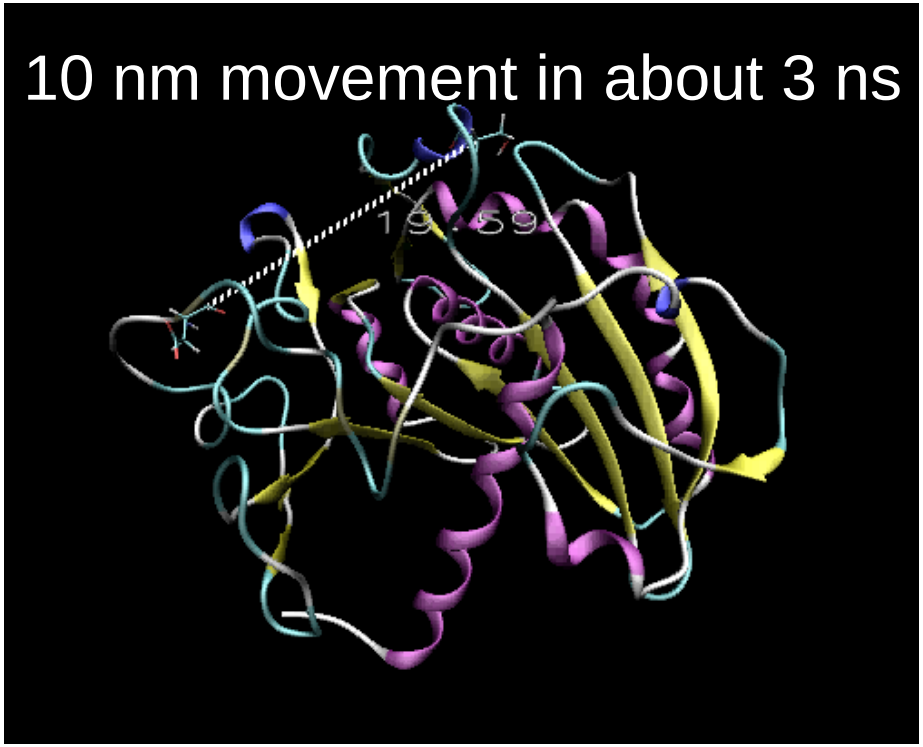
GROMACS
(MARTINI)

Martini FF - Protein

Pseudomonas cepacia lipase

Lipases modify their conformations while approaching the triglycerides.

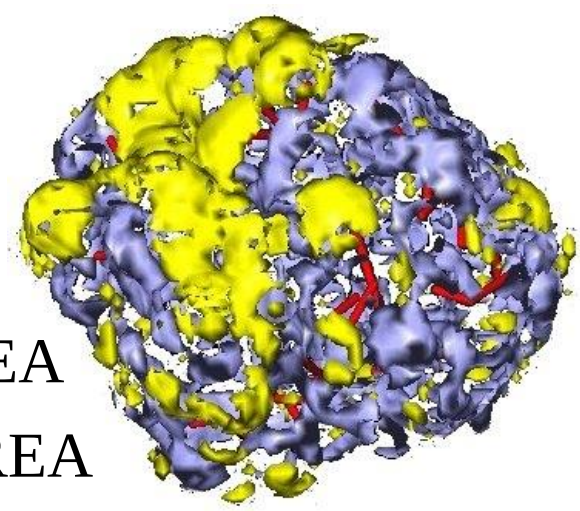
The hydrophobic active site becomes exposed and accessible



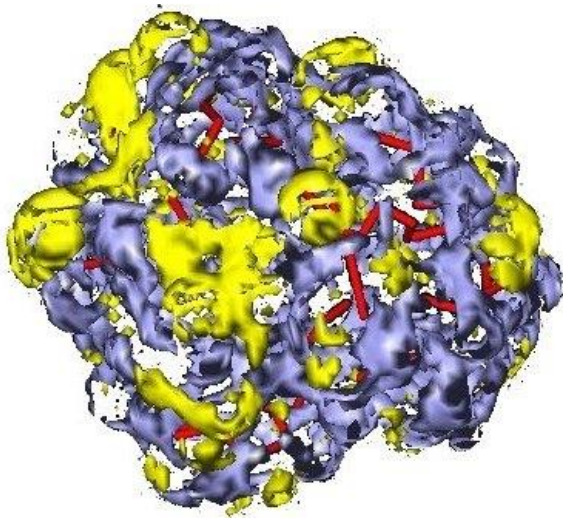
Humicola lanuginosa lipase (30KDa)

HYDROPHILIC AREA

HYDROPHOBIC AREA



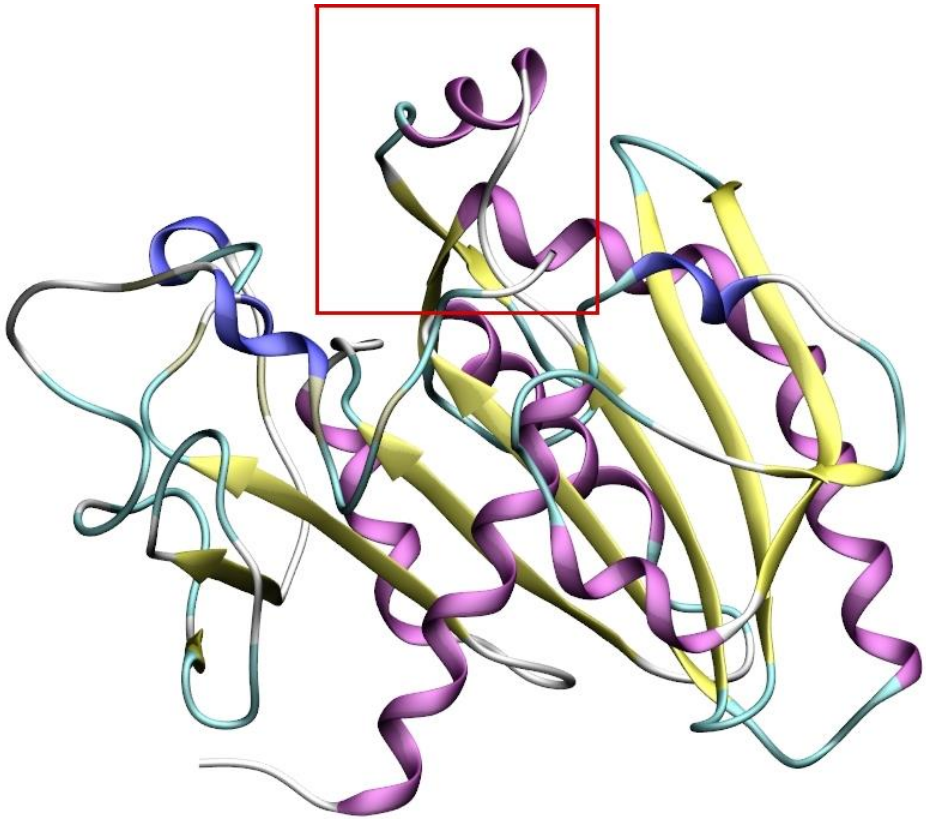
OPEN CONFORMATION

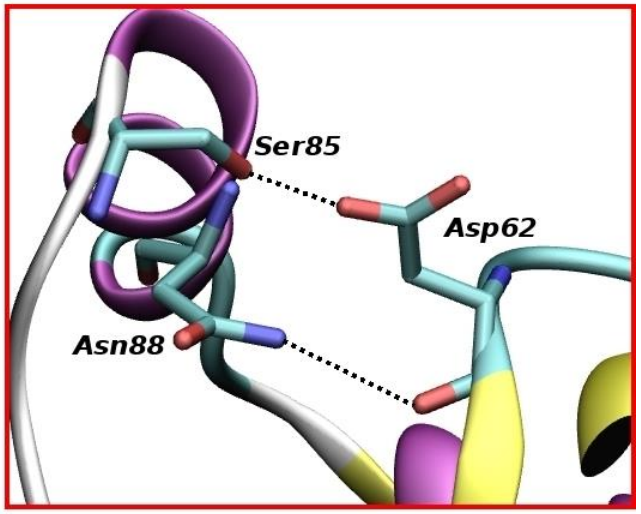


CLOSED CONFORMATION

Humicola lanuginosa LIPASE

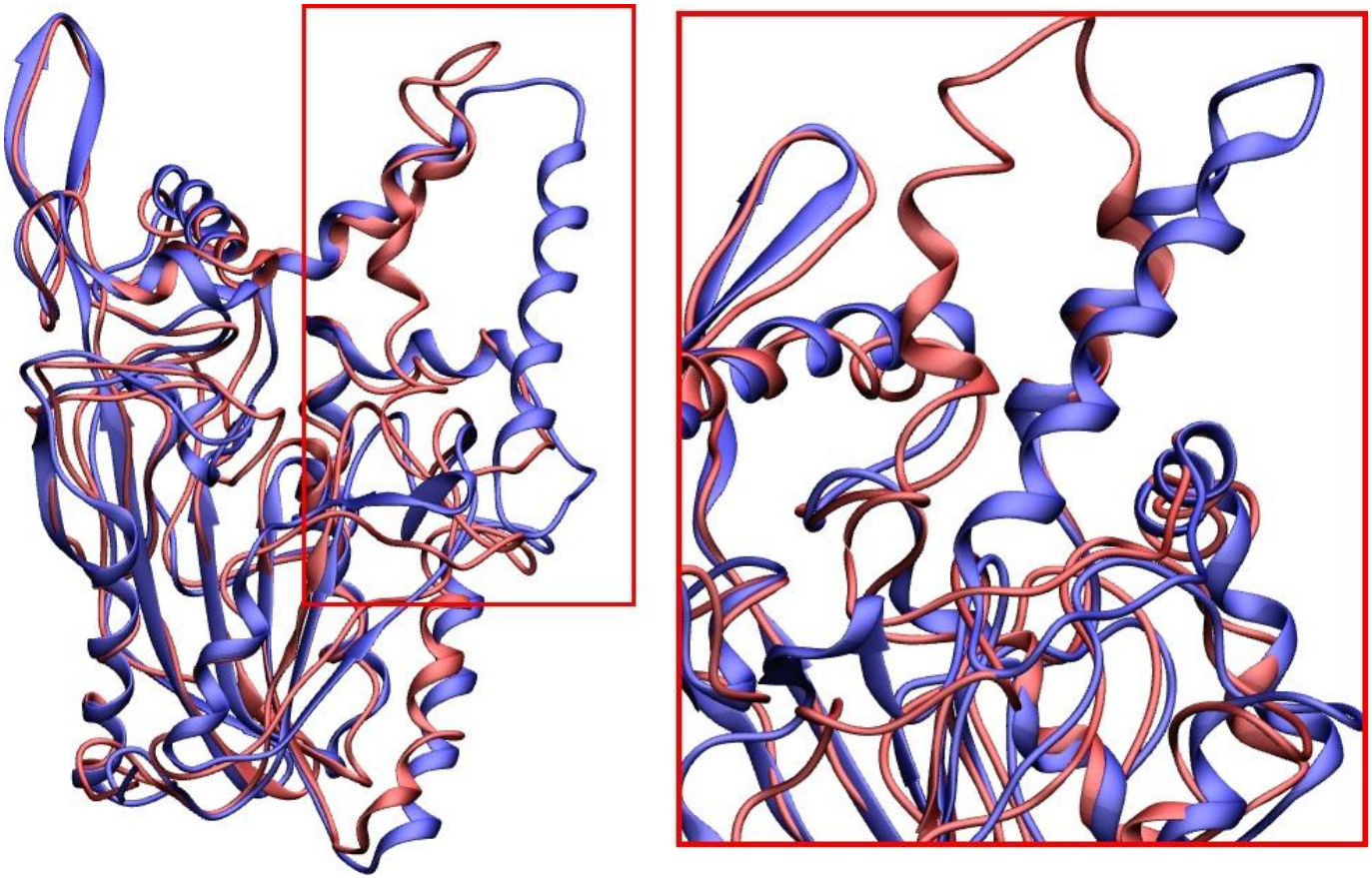
LID Stabilization in hydrophobic environment – open conformation





Pseudomosas cepacia LIPASE: what happens in water?

MD Simulation results: lid partially closed after 10 ns

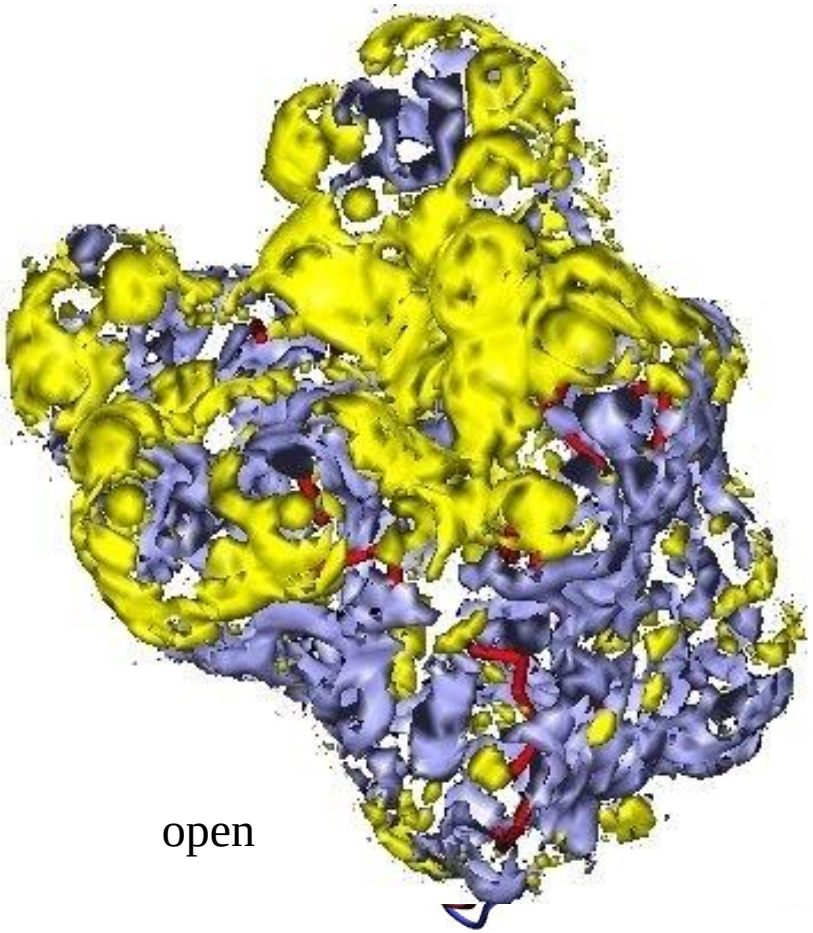


Open conformation

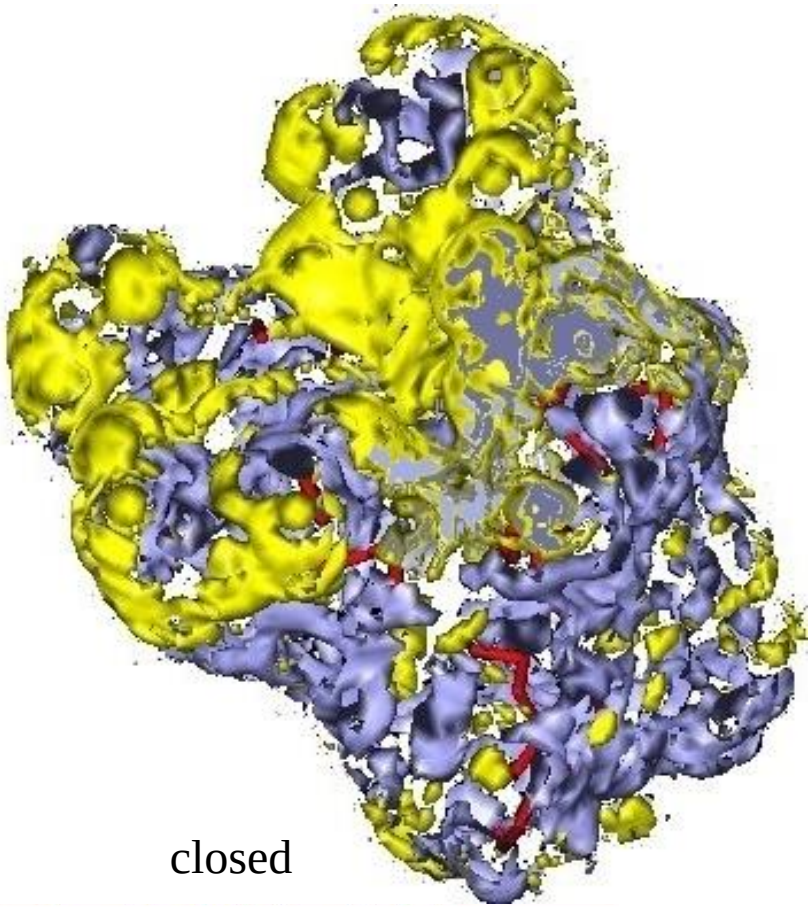
Closed conformation

Pseudomosas cepacia LIPASE

MD Simulation in water: lid partially closed after 10 ns



open



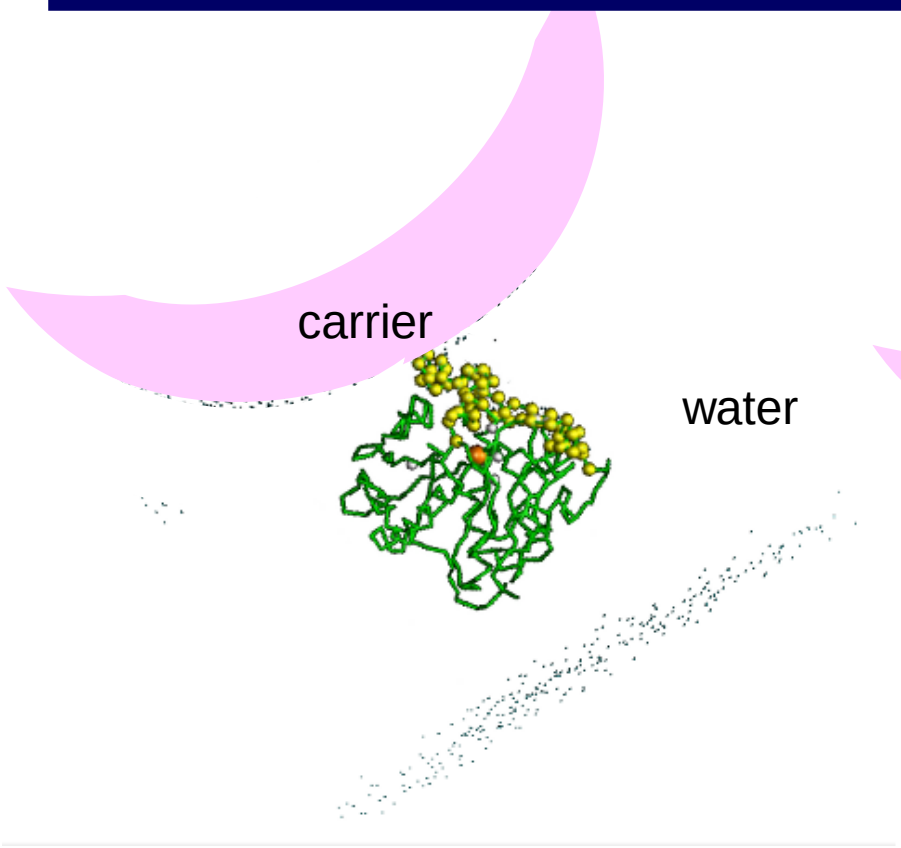
closed

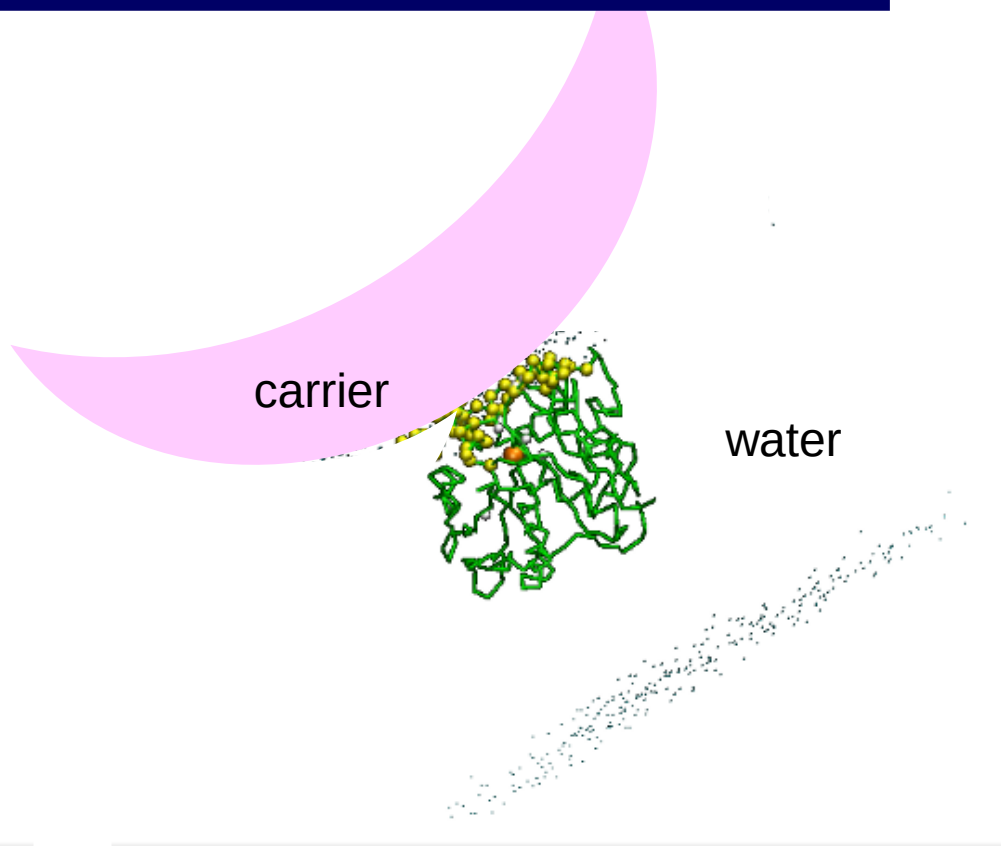
HYDROPHOBIC AREA

HYDROPHILIC AREA

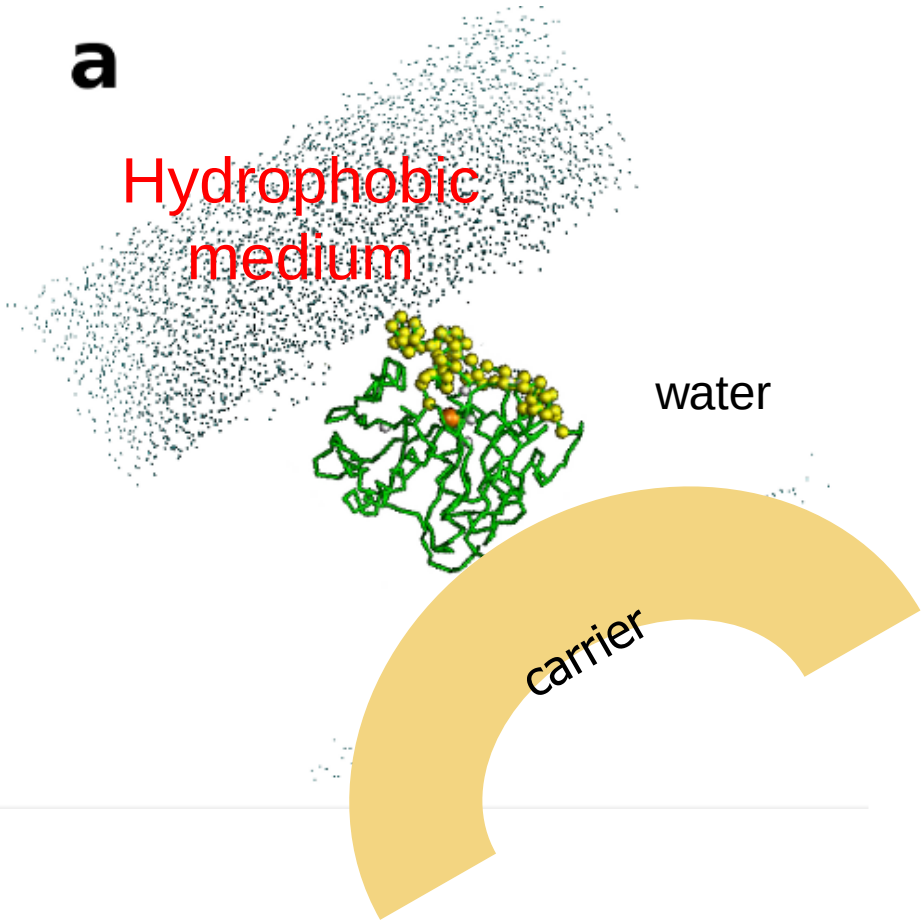
Immobilizzazione di lipasi

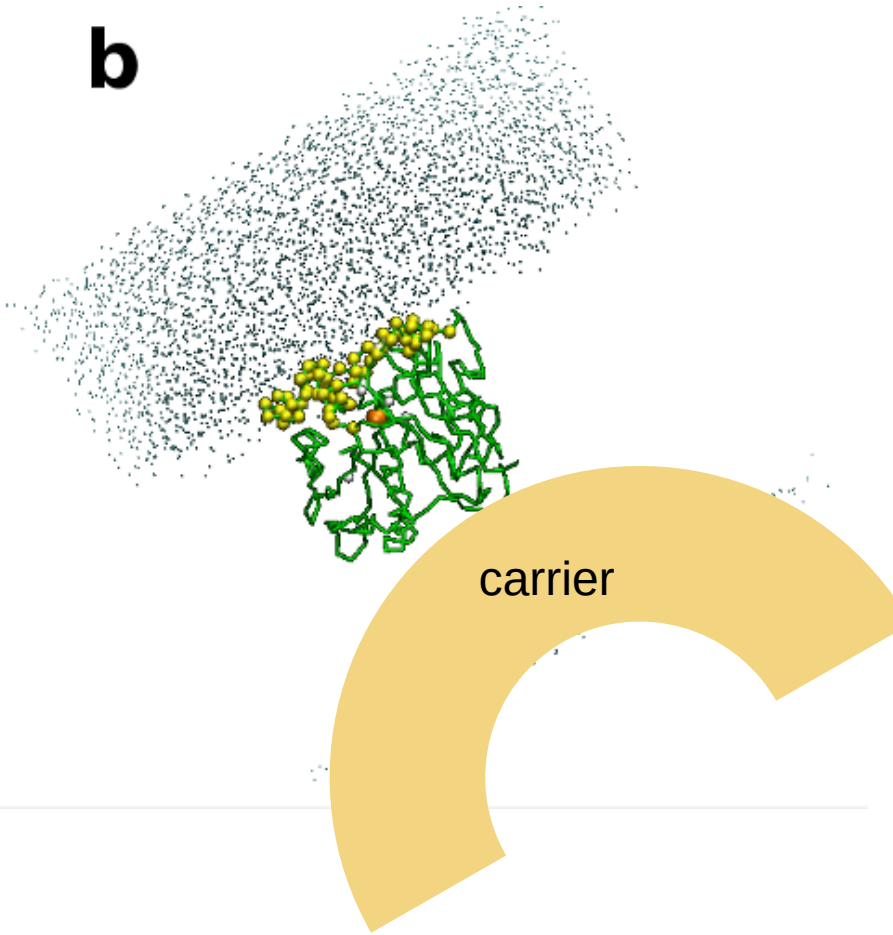
Immobilization of lipases on organic hydrophobic resins + aqueous buffer: the active site will point towards the carrier. Wrong orientation!



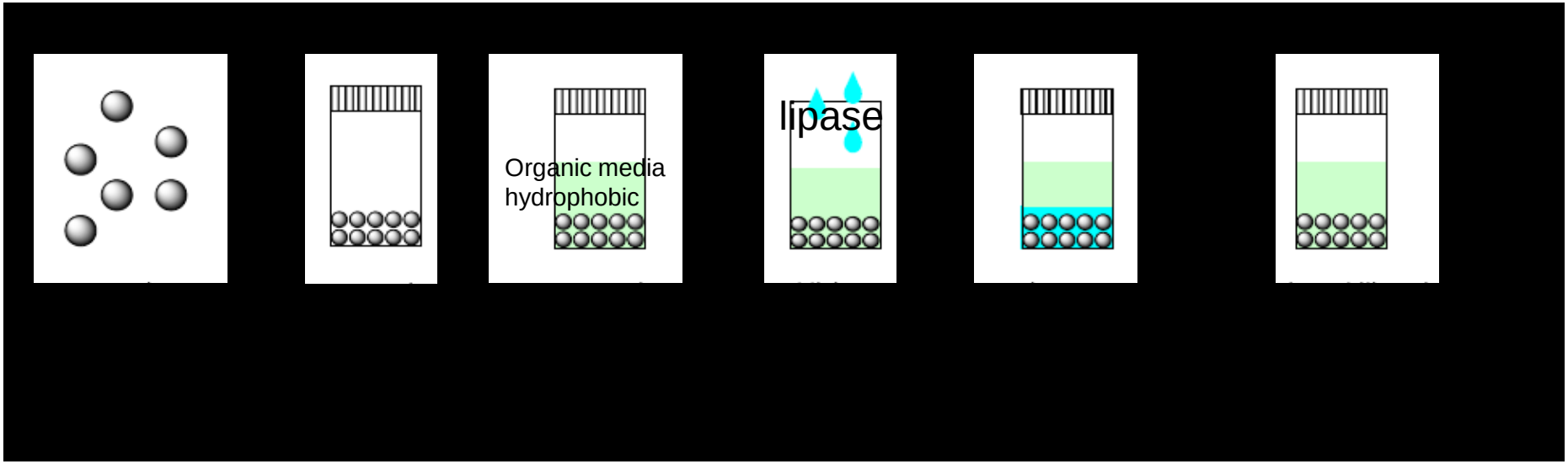


Immobilization in hydrophobic bulk medium

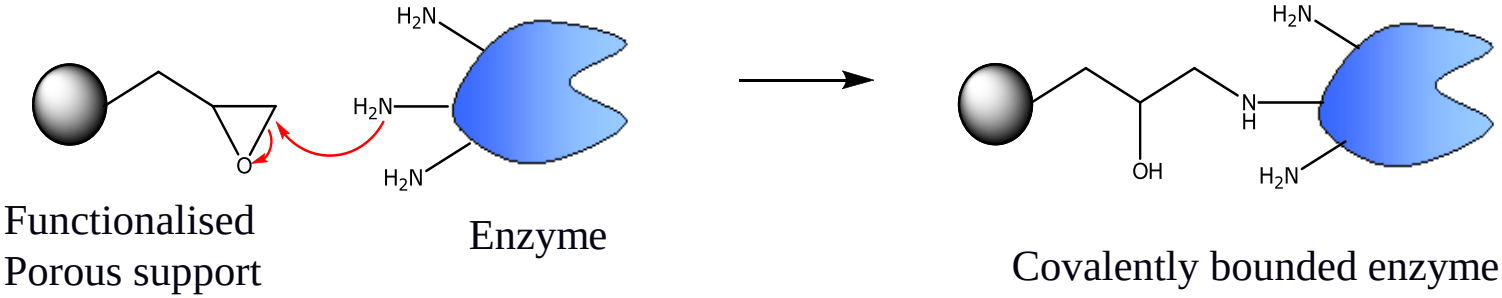




Lipase immobilization in hydrophobic media (also oil)



Covalent immobilisation on epoxy-acrylic resins



Functionalised
Porous support

Enzyme

Covalently bounded enzyme

Adv. Synth. Catal. 2011, *353*, 2466 – 2480

Chem. Soc. Rev., 2013, DOI: 10.1039/C3CS35464D

Patent WO2012085206 A1

Covalent immobilization of lipase from *Rhizopus oryzae*

(Loading: 26000U/g dry)

Immobilization Carrier	Immobilization medium	Hydrolytic activity (U/g dry)	Immobilization yield (% hydrolytic U recovered)
epoxy methacrylate	Toluene	11000	40
epoxy methacrylate	Phosphate buffer	0	0
epoxy methacrylate	Buffer+Toluene	2800	10

Patent WO2012085206 A1

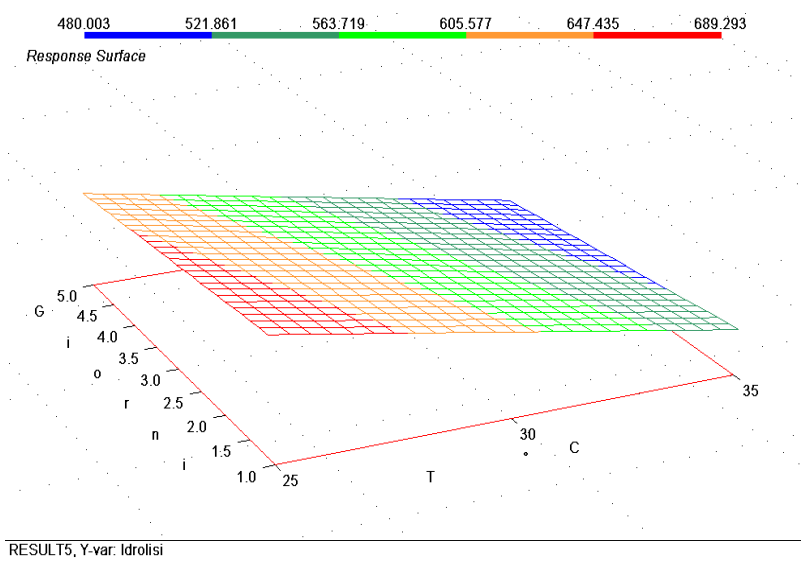
* Activity of adsorbed in buffer: 270÷ 3000 U/g dry

Lipase	Medium of immobilization	Hydrolytic activity (U/gdry)	Protein loaded (%)
P. cepacia	Organic hydrophobic	>5000	>90
P. cepacia	buffer	<500	>90
CaLB	Organic hydrophobic	>1000	>90
CaLB	buffer	<700	>90

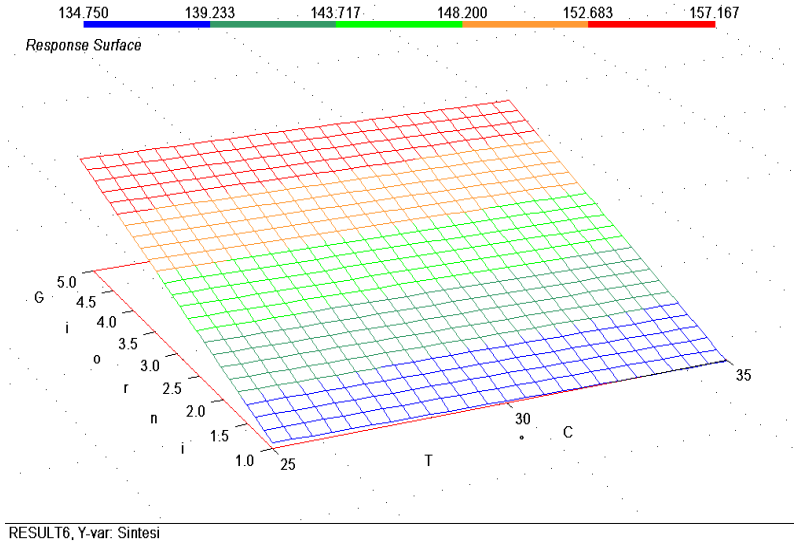
Chem. Soc. Rev., 2013, 42, 6262-6276.
Adv. Synth. Catal. 2011, *353*, 2466 – 2480

Variables affect the efficiency of the immobilised enzymes as a function of the final application

2-D graphic representation of dependency of Y variables on experimental factors

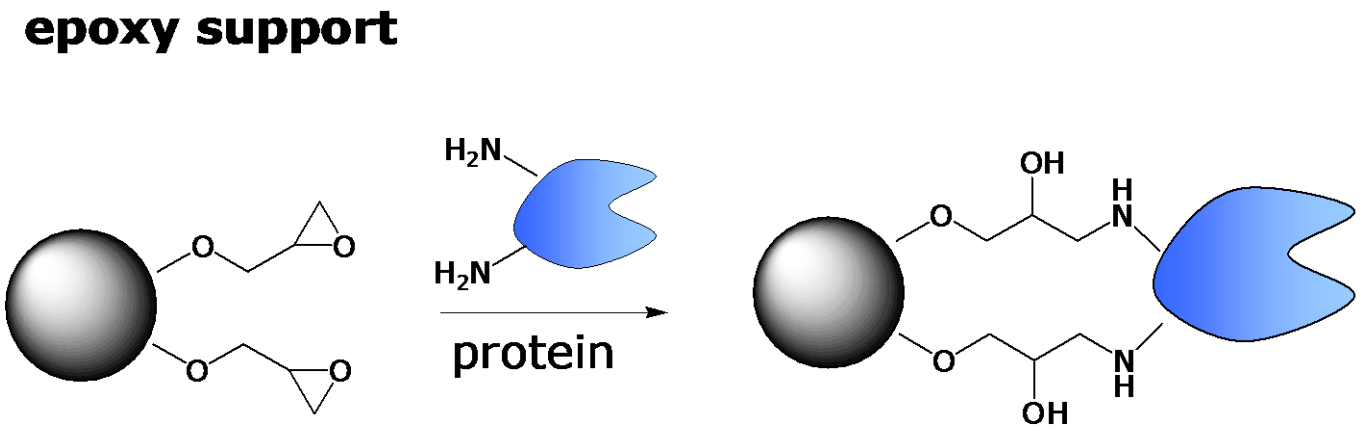


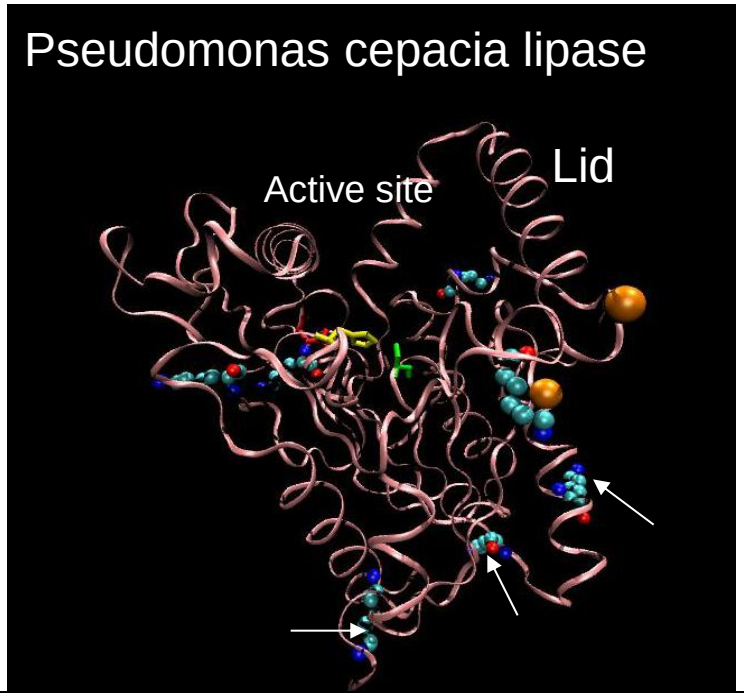
Hydrolytic activity

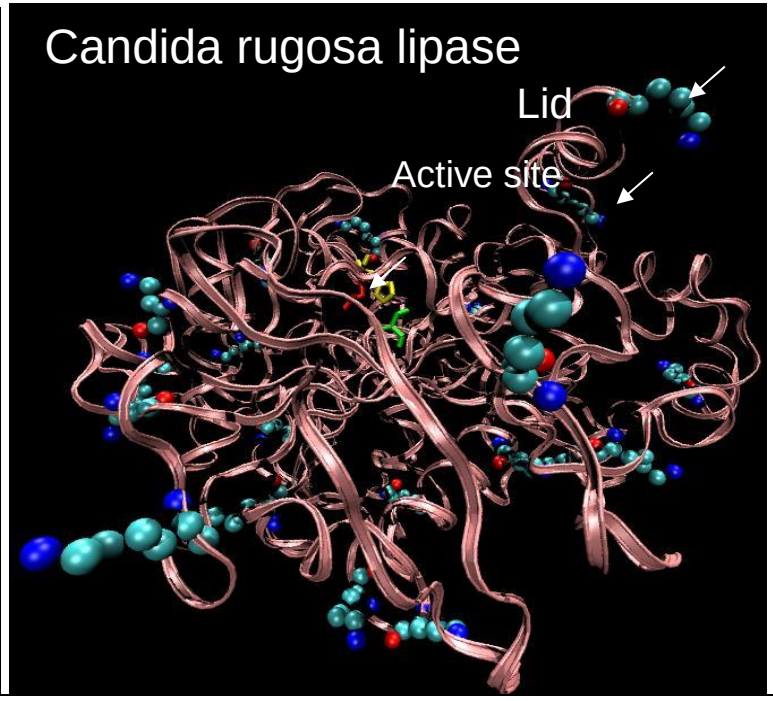


Synthetic activity

Structural comparison of lipases: Lys on surface





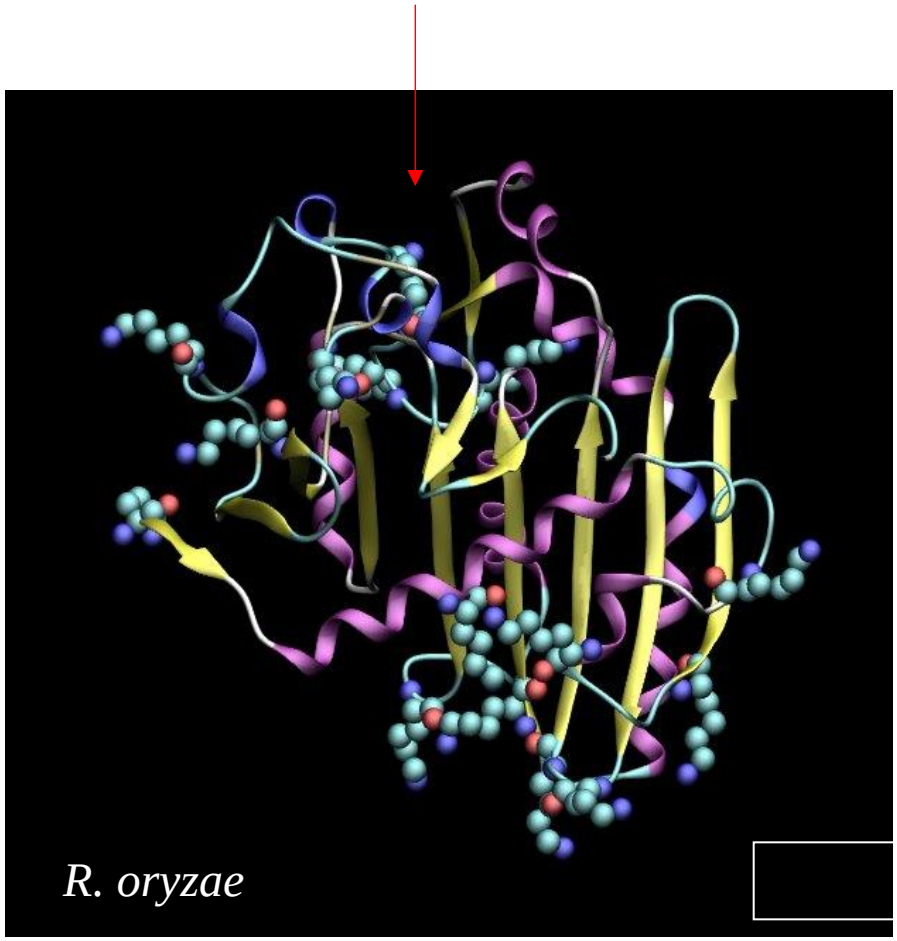


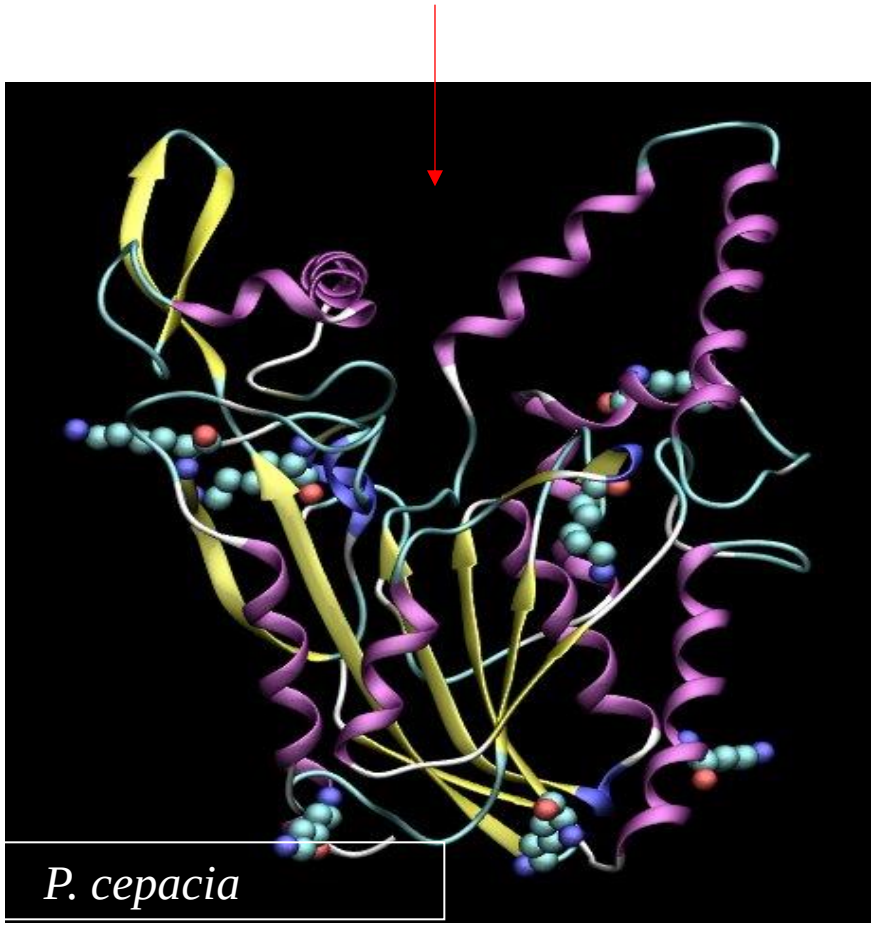
Lys residues on the surface

Glycosylation sites (theoretical)

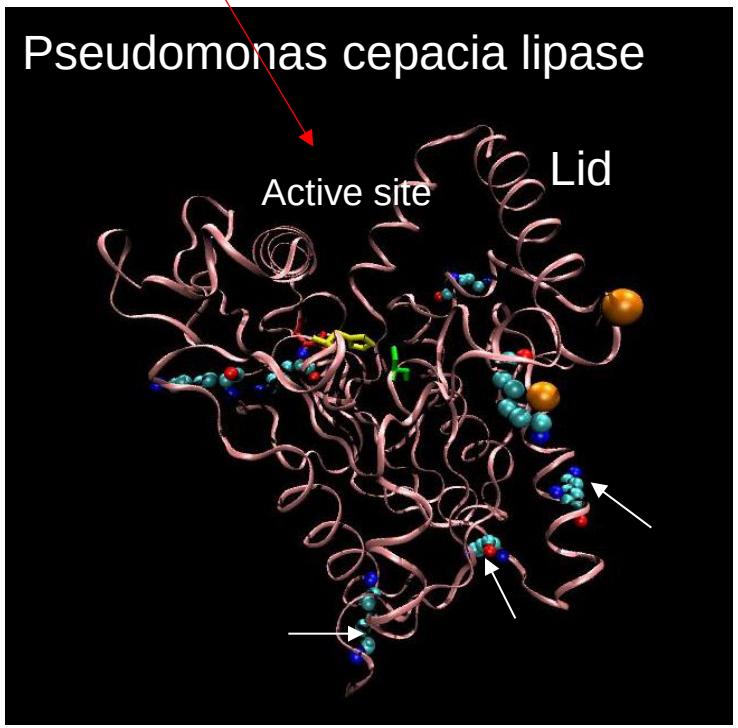
Arrows indicate crucial Lys residues on the surface (space-filling representation)

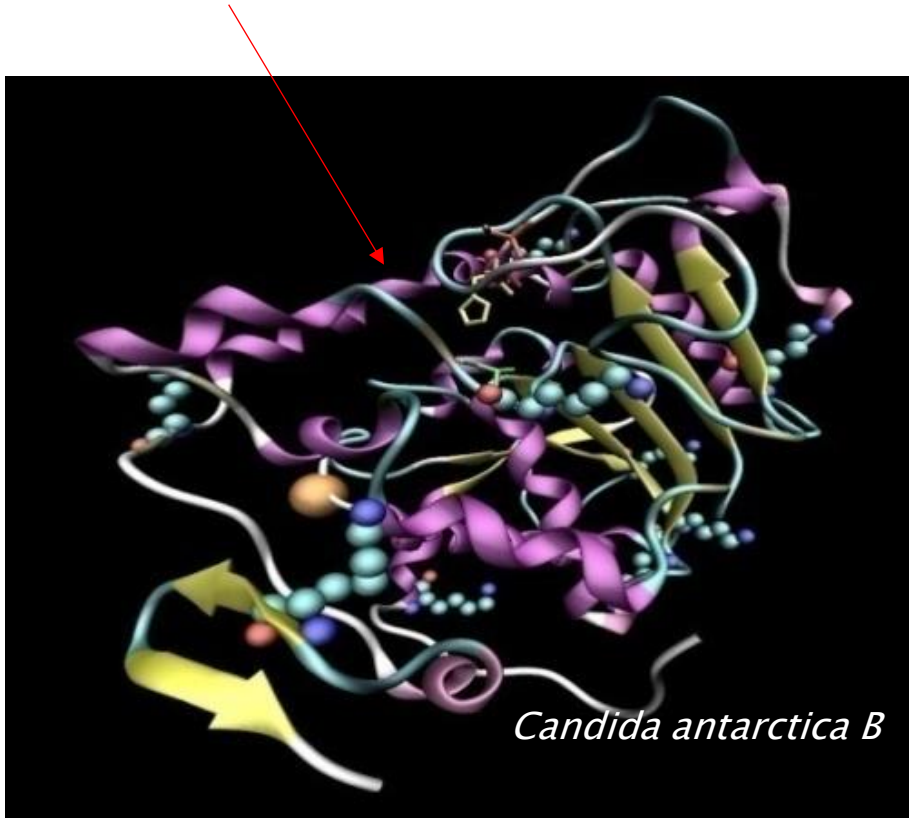
Structural comparison of lipases: Lys on surface



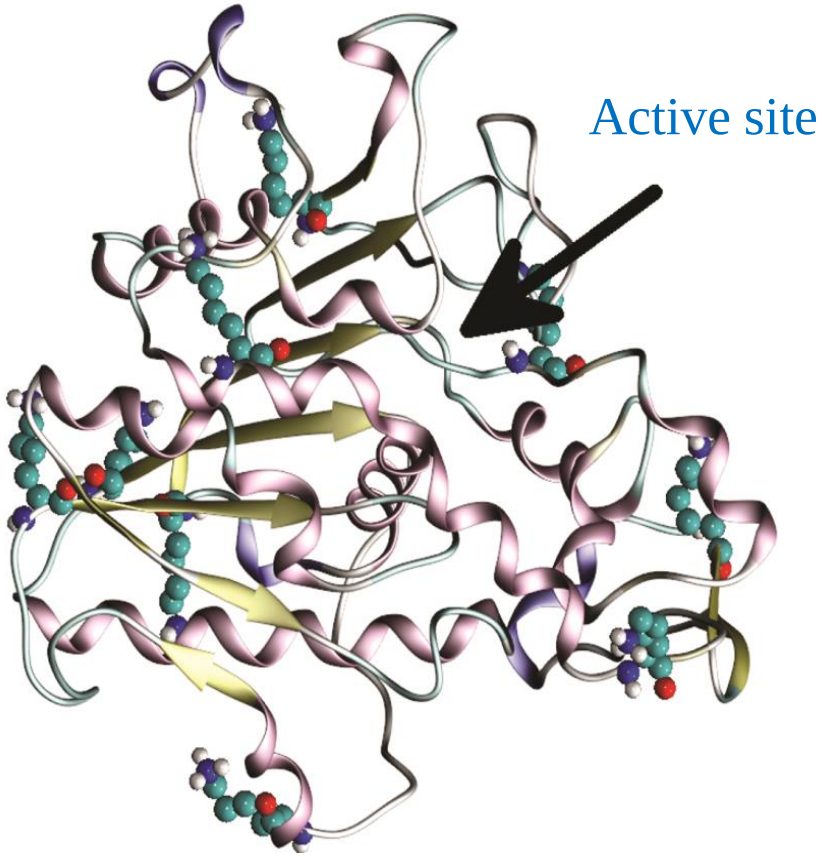


Structural comparison of lipases: Lys on surface





Lipase B from Candida antarctica: the lid is very small. There is no evidenti conformational modification nor interfacial activation in the presence of hydrophobic phases (esterase like enzyme)



Adv. Synth. Catal. 2011, *353*, 2466 – 2480

Native lipases available on the market

Source	Commercial Name	Supplier
Aspergillus niger lipase	Lipase AS	Amano
Candida antarctica lipase B	Lipozyme CALB L	Novozymes
Candida cylindracea	Lipase OF	Meyto Sangyo Co. Ltd.
Candida lipolytica	-	Amano
Candida rugosa	Lipase AY “Amano” 30	Amano
Candida rugosa	-	Sigma
Candida rugosa	-	Meyto Sangyo Co. Ltd.
Chromobacterium viscosum	-	Asahi Chemical Industry Co.
Geotrichum candidum	Amano GC-4	Amano
Humicola insolens	Lipozyme TL 100L	Novozyme
Papaya latex lipase	Carica papaya latex	Sigma
Penicillium camembertii	Lipase G	Amano
Penicillium expansum	-	Shenzhen Leveking Bioenineering
Penicillium roqueforti	Lipase R “Amano”	Amano
Porcine pancreas Type II	-	Sigma
Pseudomonas cepacia lipase	Lipase PS “Amano” SD	Amano
Pseudomonas fluorescens lipase	*Pseudomonas fluorescens* (AK)	Amano
Rhizomucor miehei	Palatase 20000	Novozyme
Rhizopus delemar	-	Seikagaku Kogyo Co.
Rhizopus japonicus	Lipase A-10FG	Nagase Biochem., Ltd.
Rhizopus niveus	Newlase F	Amano
Rhizopus oryzae	Amano F-AP15	Amano

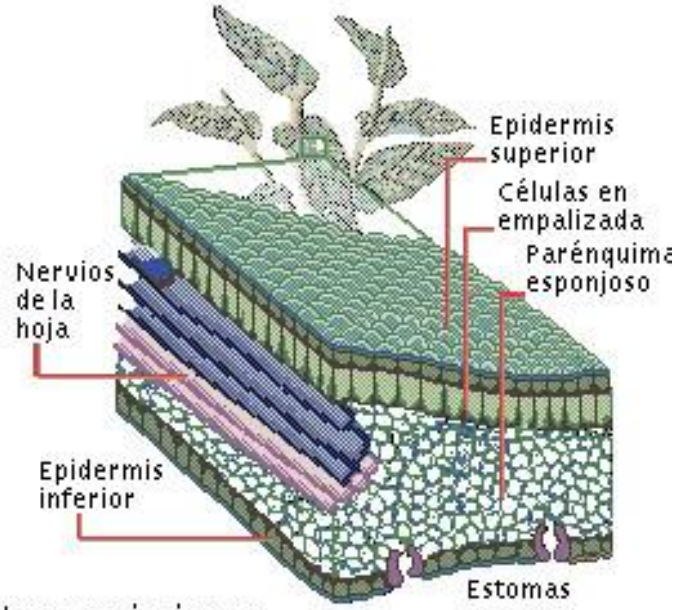
Some examples of immobilized lipases reported in the literature

Source	Product and Supplier	Method	Support
C. antarctica	Novozym®435 Sprin-CALB Lipobond	Adsorption	Acrylic resin
Porcine pancreas	- (Sigma)	Ionic interaction	Ionic resin
P. cepacia	Lipase PS IM (Amano)	Deposition	Celite
P. cepacia	Lipase PS-C (Amano)	Deposition	Ceramic
R. miehei	Lipozyme® RM-IM (Novozymes)	Ionic interaction	Duolite A568
T.lanuginosus	Lipozyme®TL IM (Novozymes)	Granulate with binder	Silica

Cutinases

Cutina

- La **cutina** è la sostanza cerosa idrofoba presente in maggior quantità all'interno della cuticola protettiva che ricopre le parti esterne dei tessuti tegumentari delle piante prevenendone la disseccazione.



CUTIN ACIDS

C_{16} - FAMILY

$CH_3(CH_2)_{14}COOH$

$CH_2OH(CH_2)_{14}COOH$

$CH_2OH(CH_2)_x CHOH(CH_2)_y COOH$

$(y = 8, 7, 6, \text{or } 5 \quad x + y = 13)$

C_{18} - FAMILY*

$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$

$CH_2OH(CH_2)_7CH{=}CH(CH_2)_7COOH$

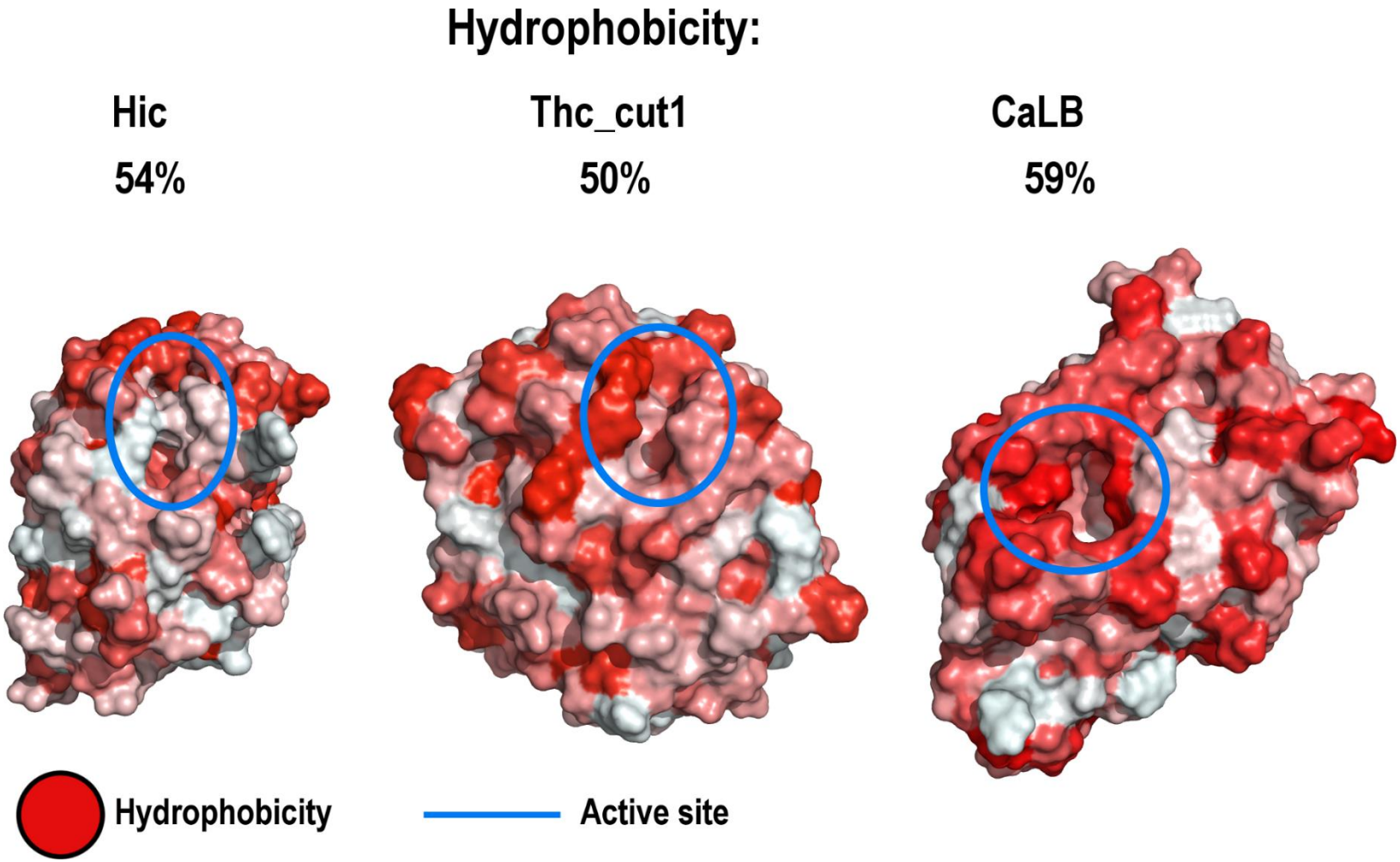
$CH_2OH(CH_2)_7CH\text{-}CH(CH_2)_7COOH$ (epoxide: O bridging the CH–CH)

$CH_2OH(CH_2)_7CHOH\text{-}CHOH(CH_2)_7COOH$

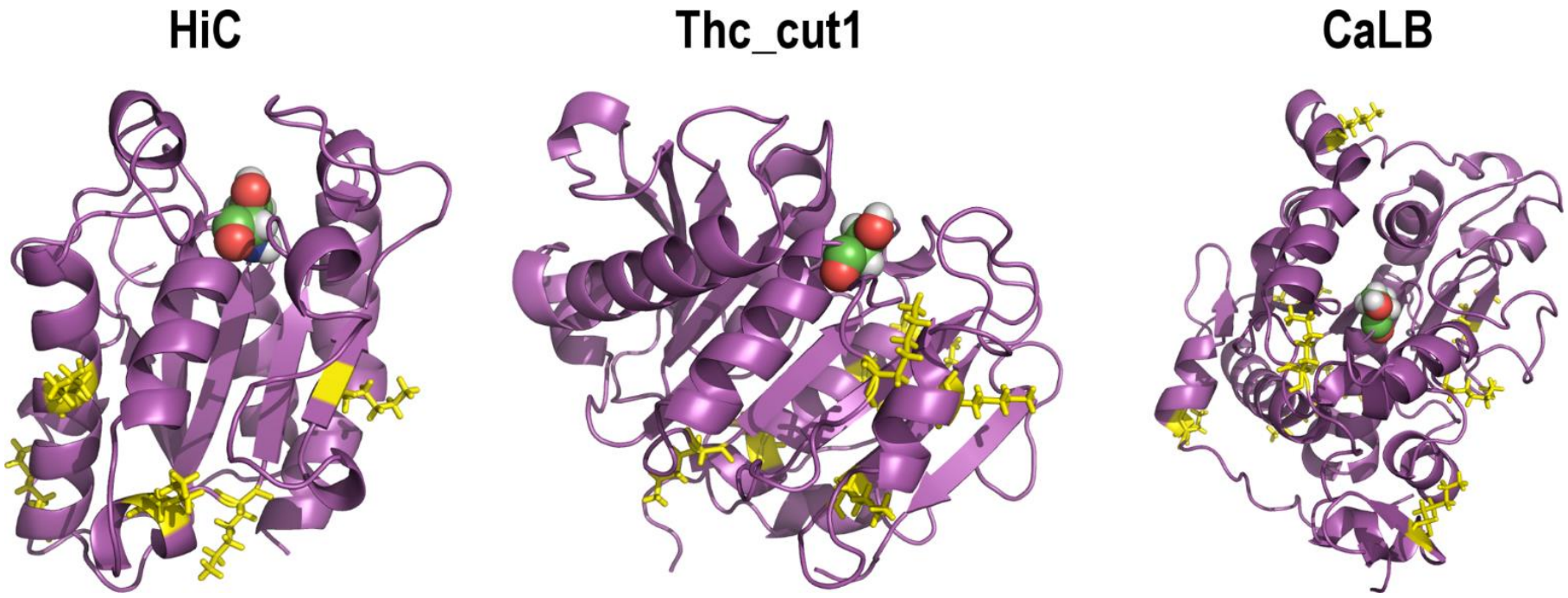
* Δ^{12} UNSATURATED ANALOGS ALSO OCCUR

Fig. 4 Structure of the most common major monomers of cutin.

Comparison: cutinases and lipase from Candida antarctica (CALB)



Comparison: cutinases and lipase from Candida antarctica (CALB)



Lipases in food industry

Transesterificazione di trigliceridi in campo alimentare

Specialty esters

-Structured triglycerides containing long chain fatty acids are an important class of raw materials for emulsions since their sensory properties vary with the fatty acid composition (content, position and saturation degree of fatty acids).

Replacement of a fatty acid with a different one to confer different properties

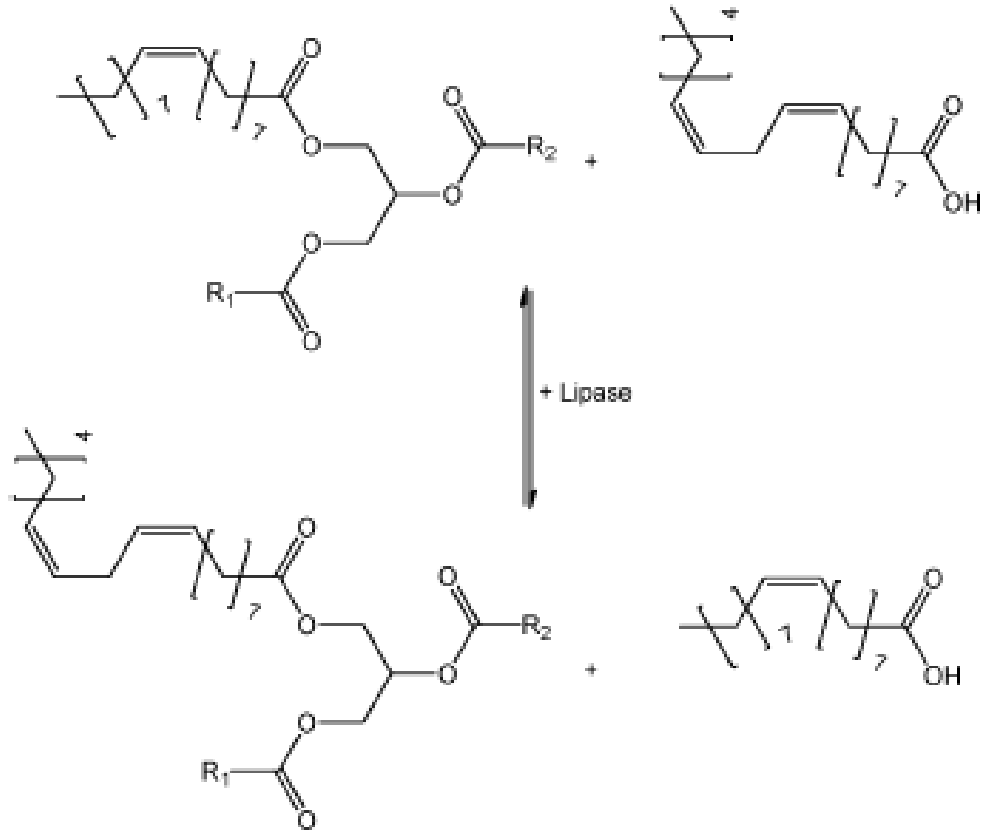
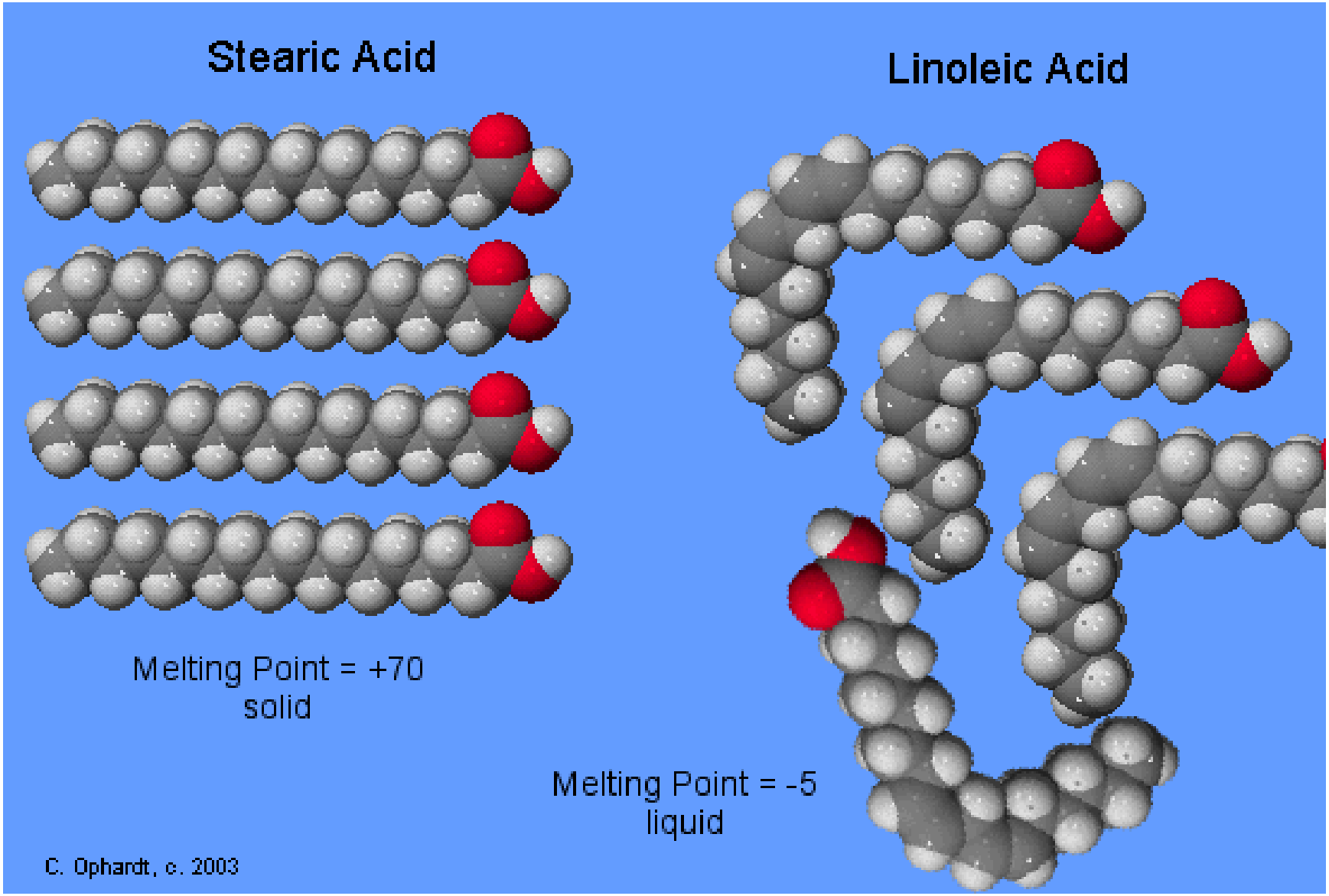


Fig. 2 Representative reaction scheme for the preparation of structured triglycerides. Here, an oleic acid moiety is replaced by a linoleic acid moiety.

Unsaturated fatty acids have lower melting point



Le proprietà dei trigliceridi dipendono dalla composizione in acidi grassi

Tabella 26.2 Grammi di acido grasso per 100 g di trigliceride, per alcuni grassi e oli*

	Acidi grassi saturi			Acidi grassi insaturi	
Grasso o olio	**Laurico (12:0)**	**Palmitico (16:0)**	**Stearico (18:0)**	**Oleico (18:1)**	**Linoleico (18:2)**
Grasso umano	–	24.0	8.4	46.9	10.2
Grasso di manzo	–	27.4	14.1	49.6	2.5
Grasso di burro	2.5	29.0	9.2	26.7	3.6
Olio di cocco	45.4	10.5	2.3	7.5	tracce
Olio di mais	–	10.2	3.0	49.6	34.3
Olio di oliva	–	6.9	2.3	84.4	4.6
Olio di palma	–	40.1	5.5	42.7	10.3
Olio di arachide	–	8.3	3.1	56.0	26.0
Olio di soia	0.2	9.8	2.4	28.9	50.7

* Sono riportati solo gli acidi grassi più abbondanti; altri acidi grassi sono presenti in minor quantità.

The possibility of using lipases in low-water environments has made possible their application for the production of structured triacylglycerols by enzymatic inter- and transesterifications.

This mild and selective approach has been utilized for producing

- **cocoa-butter equivalent**
- **and infant-formula substitutes.**
- **triacylglycerols containing polyunsaturated fatty acids (PUFA),**

Lipases in the synthesis of structured esters

The possibility of using lipases in low-water environments has made possible their application for the production of structured triacylglycerols by enzymatic inter- and transesterifications.

This mild and selective approach has been utilized for producing

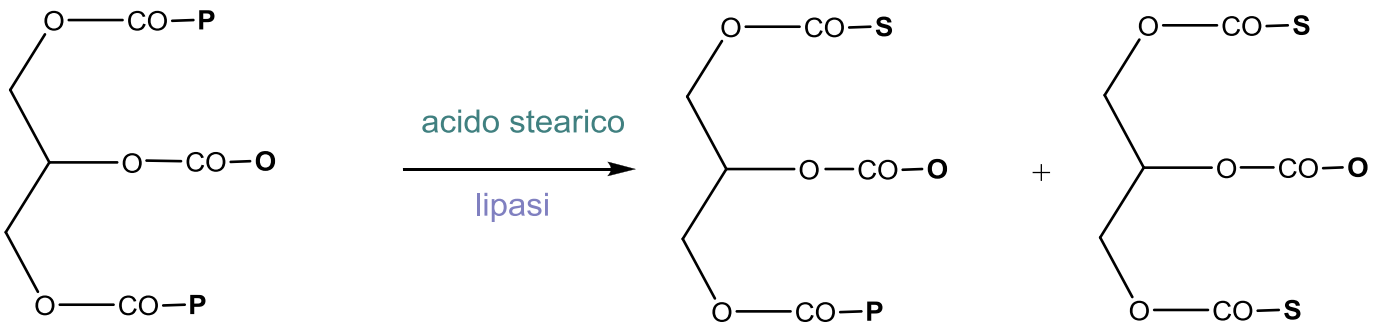
- **cocoa-butter equivalent**
- **and infant-formula substitutes.**
- **triacylglycerols containing polyunsaturated fatty acids (PUFA)**

Synthesis of cocoa butter analogues

Cocoa butter has a melting point close to 37°C.
It is mainly composed of SOP and SOS triacylglycerols (where S = stearic and O = oleic, P = palmitic).

Palm oil mid-fraction is liquid at room temperature an rich of POP triacylglycerol (where P = palmitic acid) and can be converted into cocoa butter by chemo- and regiospecific lipases able to introduce stearic acid in position sn1 and sn3. The biotransformation can be carried out in solvent-free medium, where palm oil is used in large excess.

Transesterification catalyzed by lipase from Mucor mihei



Palm oil

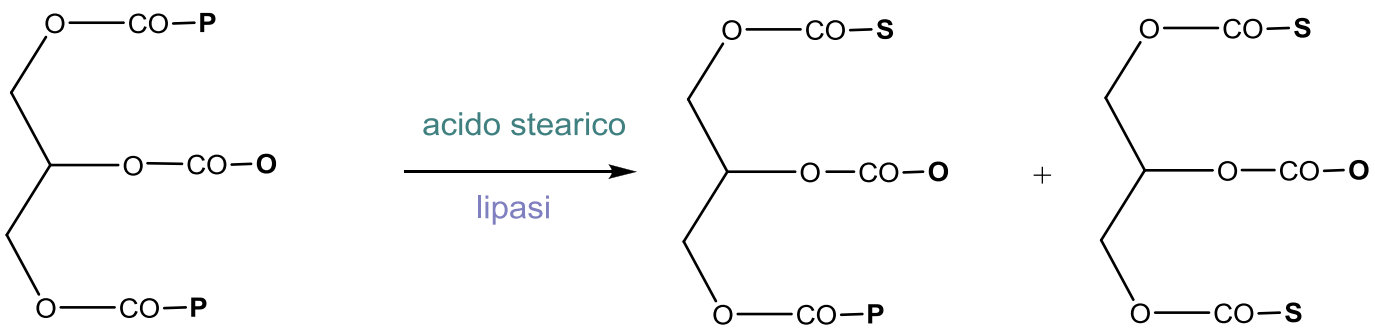
Cocoa butter analogue

P: palmitic – saturated 16Carbon atoms
O: oleic – unsaturated
S: stearic – saturated 18 Carbon atoms

The biotransformation at industrial level

The lipase is immobilized onto porous silica granulates which are insoluble in oil.

The immobilized lipase Lipozyme® TL IM viewed through a light microscope. The enzyme is bound to a silica carrier

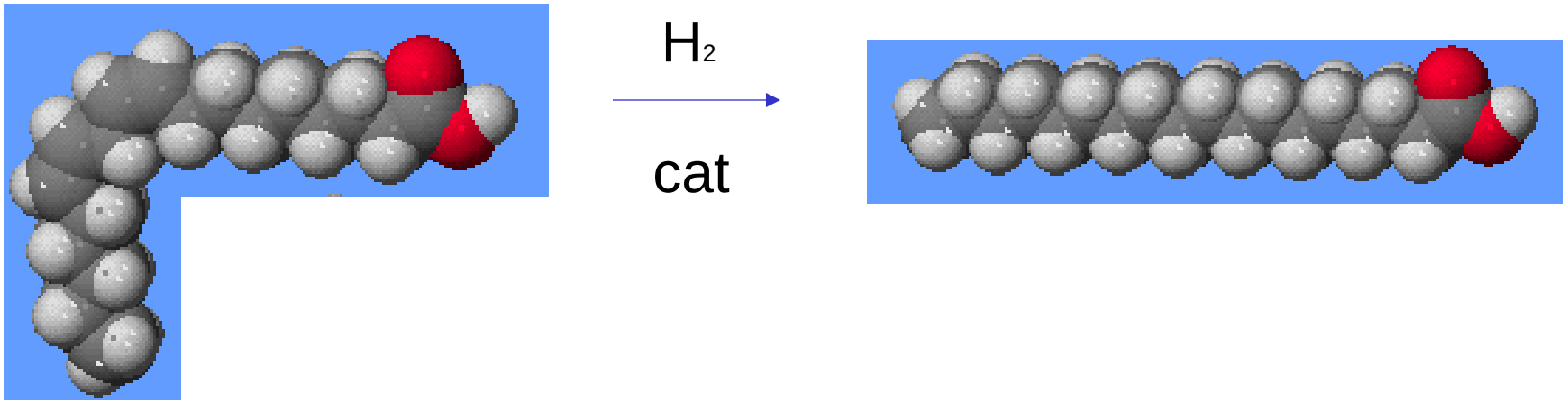


olio di palma

burro di cacao

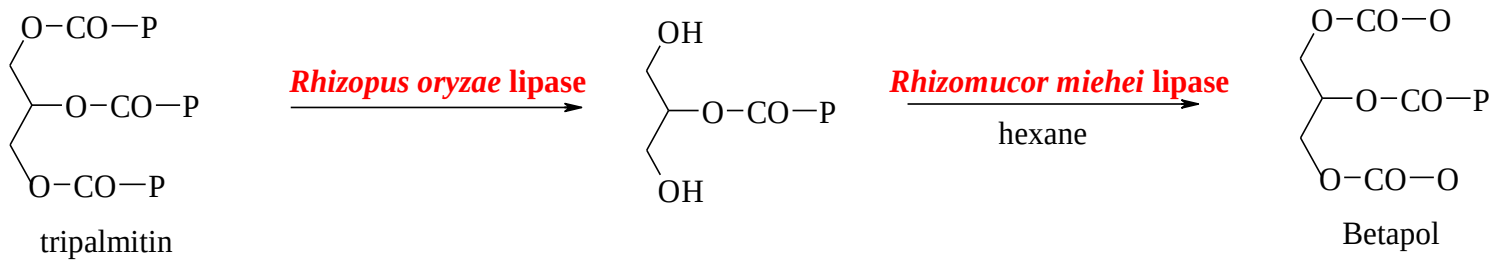
The chemical alternative: hydrogenated fatty acids

Hydrogen and a metal catalyst operating at about 100°C and under vacuum.
Unsaturated fatty acids are converted in saturated fatty acids



The observation that lipases selectively catalyze both hydrolysis and esterifications has been further exploited for the obtainment of Betapol® from tripalmitin (palm oil). Betapol® consists of triglyceride fatty acids commonly found in vegetable and animal fats. A similarity to human milk fat indicated a potential use in **infant formulae** as well as for food use in general.

Tripalmitin is firstly hydrolysed with a lipase from Rhizopus oryzae able to remove only the fatty acids in position 1 and 3. The resulting 2-mono-palmitin has been esterified with oleic acid using the Rhizomucor miehei lipase in organic solvent

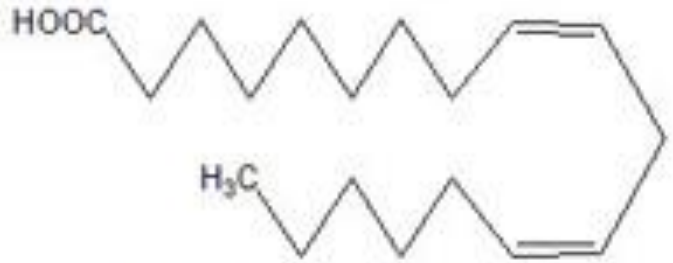


P: palmitic – saturated 16Carbon atoms
O: oleic – unsaturated

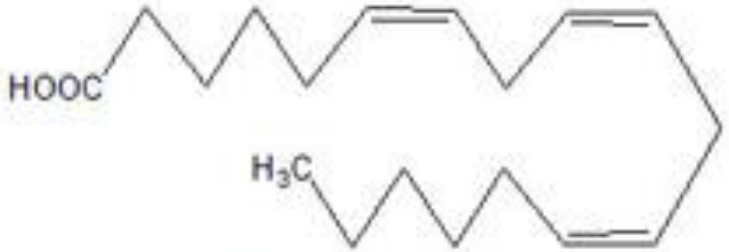
Lipases have been extensively used for the hydrolysis and transesterification of triacylglycerols. Chemo- and regio-selective hydrolysis of triacylglycerols has been exploited for enrichment of specific fatty acids, such as **polyunsaturated fatty acids (PUFA) from fish oils**.

PUFAs are increasingly used as food additives, pharmaceuticals and nutraceuticals because of their metabolic benefits. Many PUFAs are essential for normal synthesis of lipid membranes and prostaglandins.

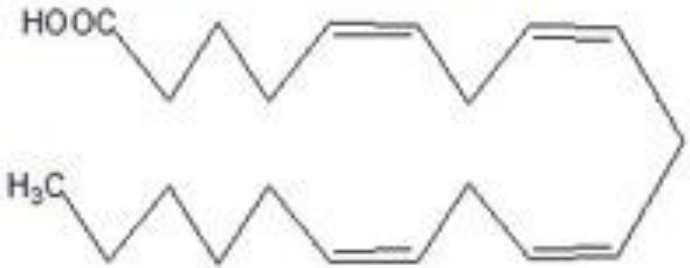
Omega-6 Fatty Acids



Linoleic Acid (18:2n-6)

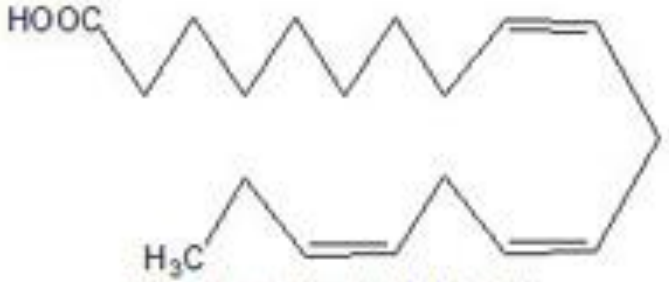


γ-Linolenic Acid (18:3n-6)

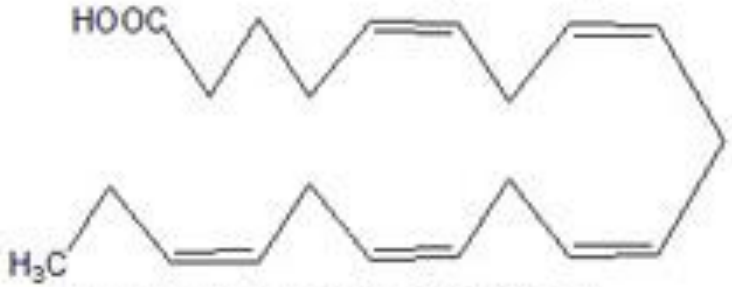


Arachidonic Acid (20:4n-6)

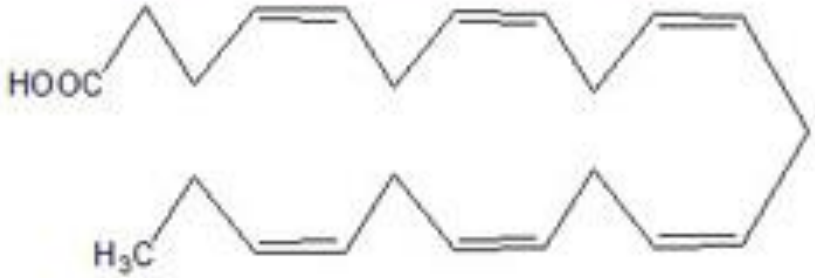
Omega-3 Fatty Acids



α-Linolenic Acid (18:3n-3)



Eicosapentaenoic Acid (20:5n-3)



Docosahexaenoic Acid (22:6n-3)

PUFA: alternative sources

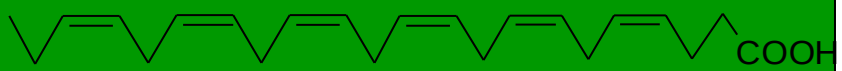
Piante e cianobatteri

Gamma-linolenic acid (GLA) C18:3(n-6) GLA

Arachidonic acid (AA) C20:4(n-6) AA

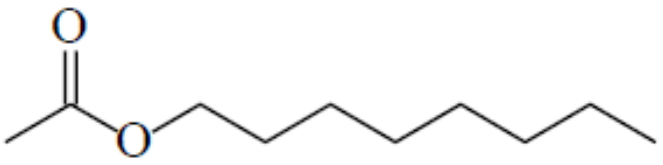
Eicosapentaenoïc acid (EPA) C20:5(n-3) EPA

Docosahexaenoïc acid (DHA) C20:6(n-3) DHA



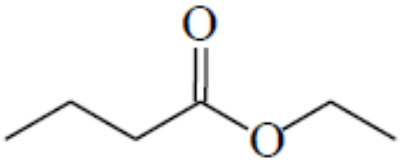
Prodotti da microalghe eucariotiche

Esters synthesised by lipases used as aromas in food and pharma sector



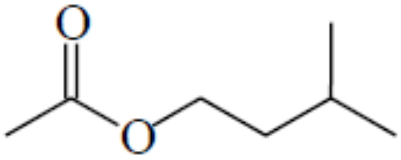
ottil etanoato
(ottil acetato)

arancia



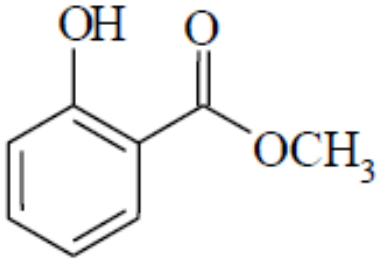
etil butanoato
(etil butirrato)

ananas



3-metilbutil etanoato
(isopentil acetato, isoamil acetato)

banana



metil 2-idrossibenzoato
(metil salicilato)

menta

L'Organizzazione Internazionale delle Industrie degli aromi definisce gli ingredienti aromatizzanti naturali come ***"quelli ottenuti mediante appropriate trasformazioni fisiche, enzimatiche o microbiologiche da un materiale di origine vegetale o animale, allo stato naturale o previa trasformazione per il consumo umano "***. Quindi composti ottenuti da sintesi di substrati naturali, biologici, sono considerati come naturali, mentre gli stessi materiali prodotti chimicamente non lo sono. La produzione di una varietà di prodotti da processi naturali assume grande significato per il consumatore finale che attribuisce al prodotto “naturale” una maggiore sicurezza e genuinità, siano essi prodotti di consumo alimentare o prodotti cosmetici (ad esempio creme). Questi prodotti determinano in modo sostanziale un aumento dei prezzi. Così, la fabbricazione di prodotti vari mediante catalisi di lipasi si rivela essere remunerativa per l'industria.

Enzyme modified cheese (EMC)

E’ noto che lo sviluppo del tipico sapore del “ Blue Cheese” è dovuto agli enzimi del Pencillium Roqueforti. Questi enzimi possono essere utilizzati in creme di formaggio e sostituti del formaggio, per conferire un sapore di latte ad un ampia gamma di prodotti alimentari trasformati, per promuovere la formazione del sapore di panna nella materia grassa butirrica del latte trattato, per la produzione sapori specifici in burro e margarina .

Il latte o il formaggio vengono incubati ad alte temperature in presenza di miscele enzimatice o microbiche al fine di sintetizzare in-situ gli aromi

Altre applicazioni delle lipasi in campo alimentare

Table 4—Lipase applications in the food industry[14]

Food industry	Action	Product of application
Dairy foods	Hydrolysis of milk fat, cheese ripening, modification of butter fat.	Development of flavouring agents in milk, cheese and butter
Bakery foods	Flavour improvement	Shelf-life propagation.
Beverages	Improved aroma	Alcoholic beverages, e.g. sake, wine
Food dressings	Quality improvement	Mayonnaise, dressings and whippings
Health foods	Transesterification	Health foods
Meat and fish	Flavour development	Meat and fish product, fat removal
Fats and oils	Transesterification, hydrolysis	Cocoa butter, margarine, fatty acids, glycerol, mono and diglycerides

Table 5—Examples of lipase in cheese production[37]

Cheese type	Lipase source
Romano	Kid/lamb pre-gastric
Domiati	*Mucor miehei*
Feta	
Camembert	*Penicillium camemberti*
Mazarella	Calf/kid pre-gastric
Parmesan	
Provolone	
Fontina	*Mucor miehei*
Ras	
Romi	
Roque fort	*Penicillium roqueforti*
Cheddar	*Aspergillus oryzae/A. niger*
Manchego	
Blue	

Idrolisi di trigliceridi catalizzata da lipasi

L’idrolisi parziale dei trigliceridi per aumentare il contenuto di monogliceridi viene ottenuta mediante l'aggiunta di lipasi alla pasta del pane: ciò porta ad un ritardo del raffermamento.