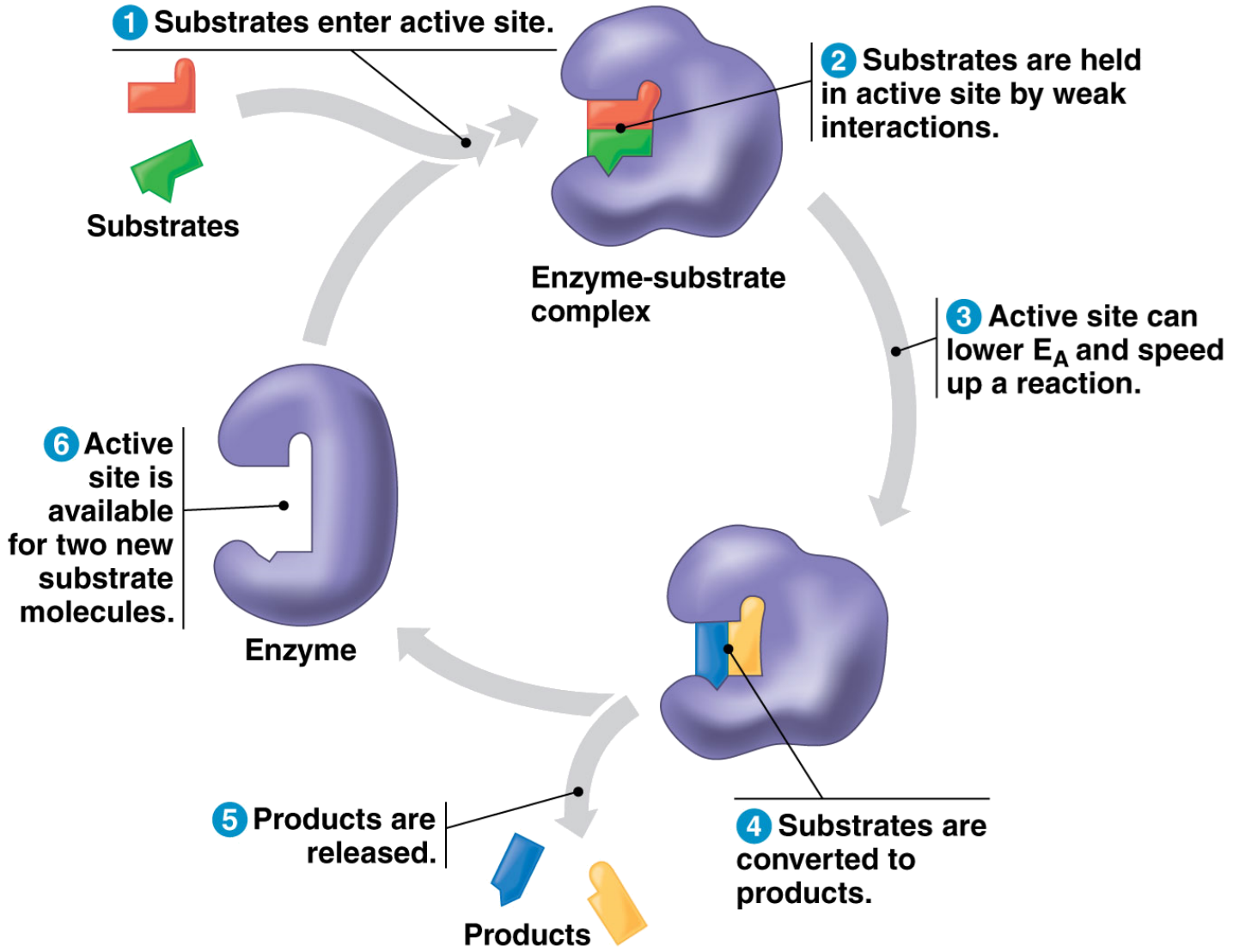
CYCLOLAB

The Cyclodextrin Company

Cyclodextrins in Biotechnology

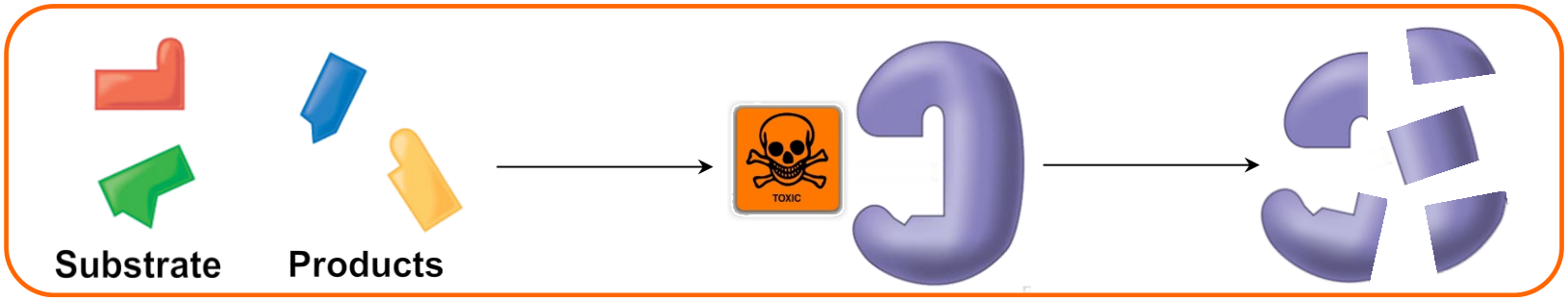
Milo Malanga

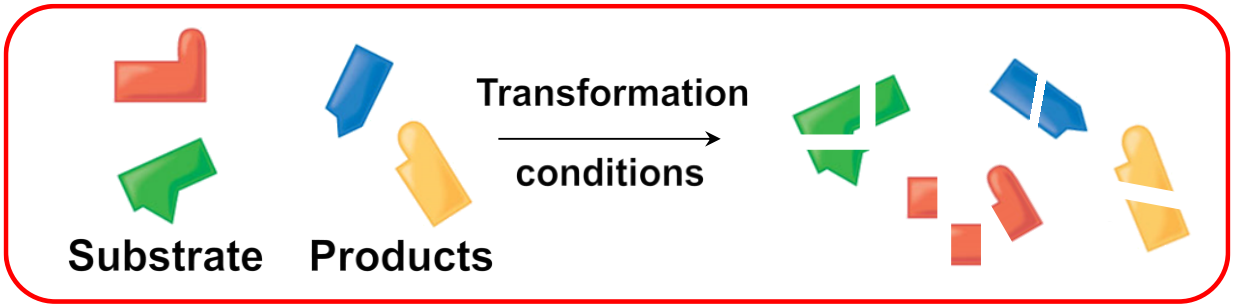
Biotechnology processes



Challenges in Biotechnology

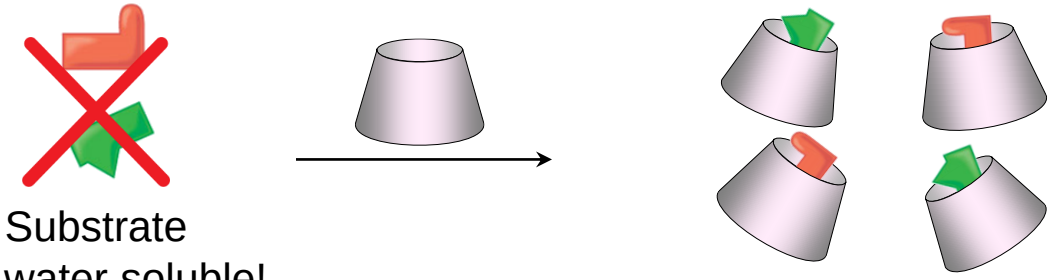
Not water soluble!



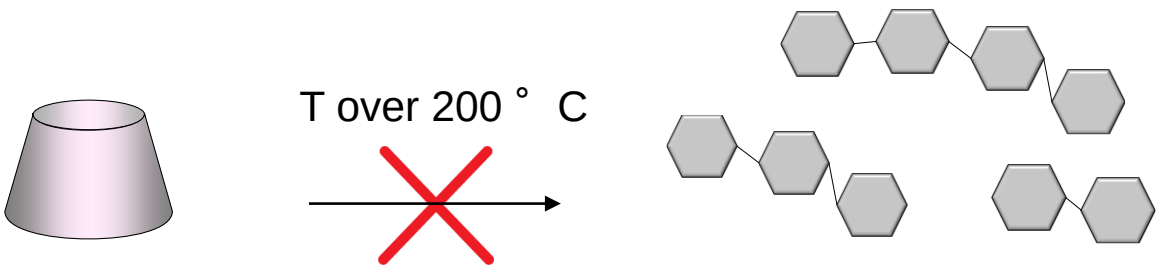


CDs in Biotechnology processes

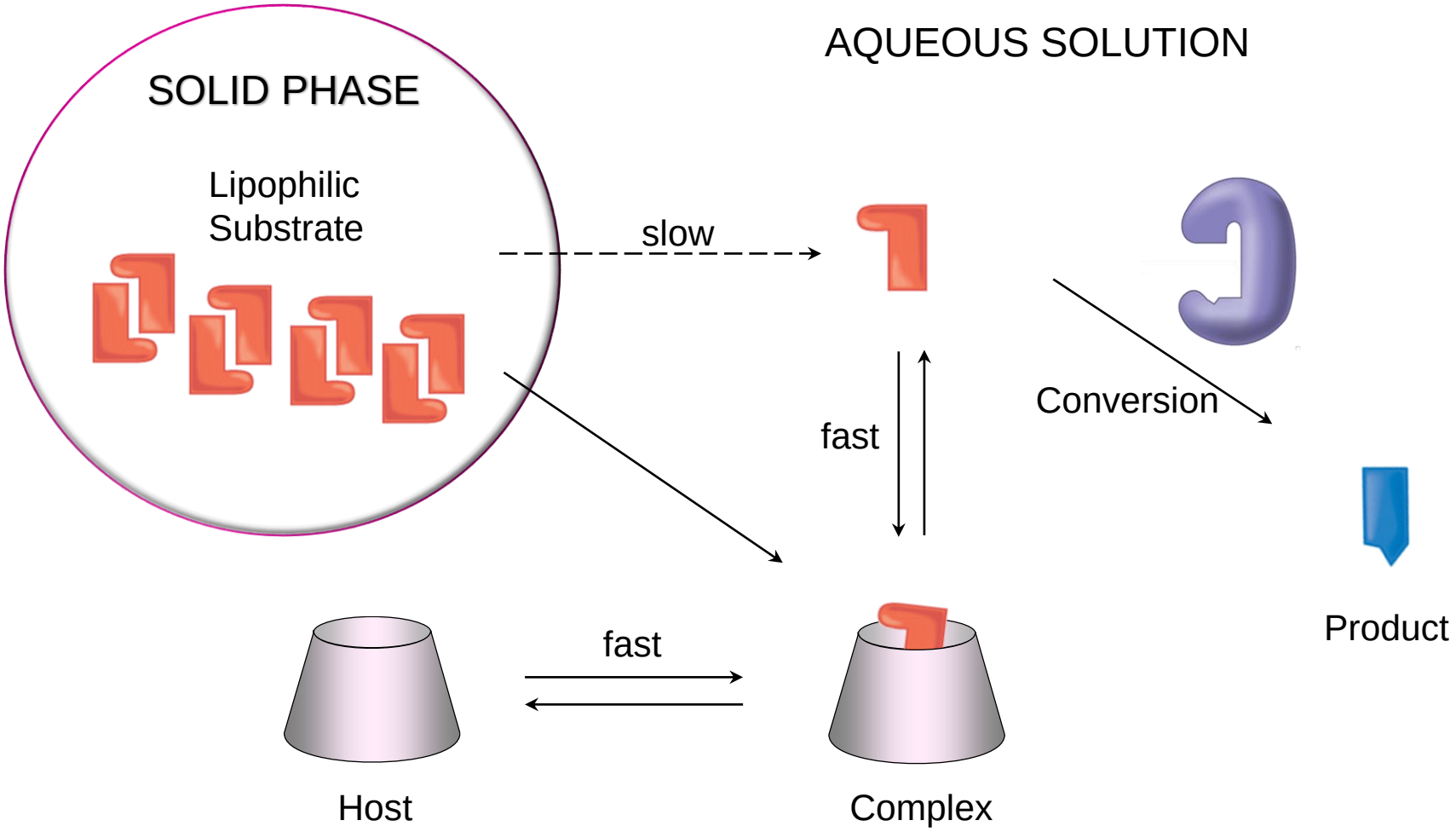
Solubilization and protection



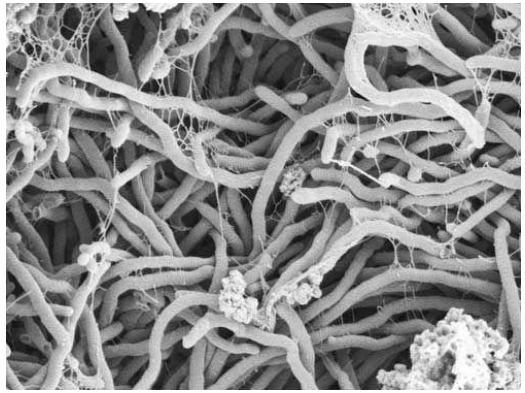
Stable and inert



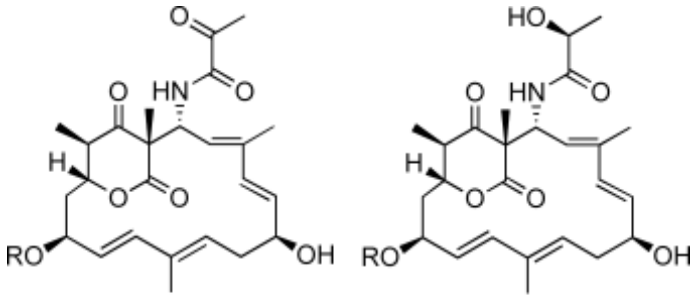
CDs in Biotechnology processes



Biosynthesis by Fermentation



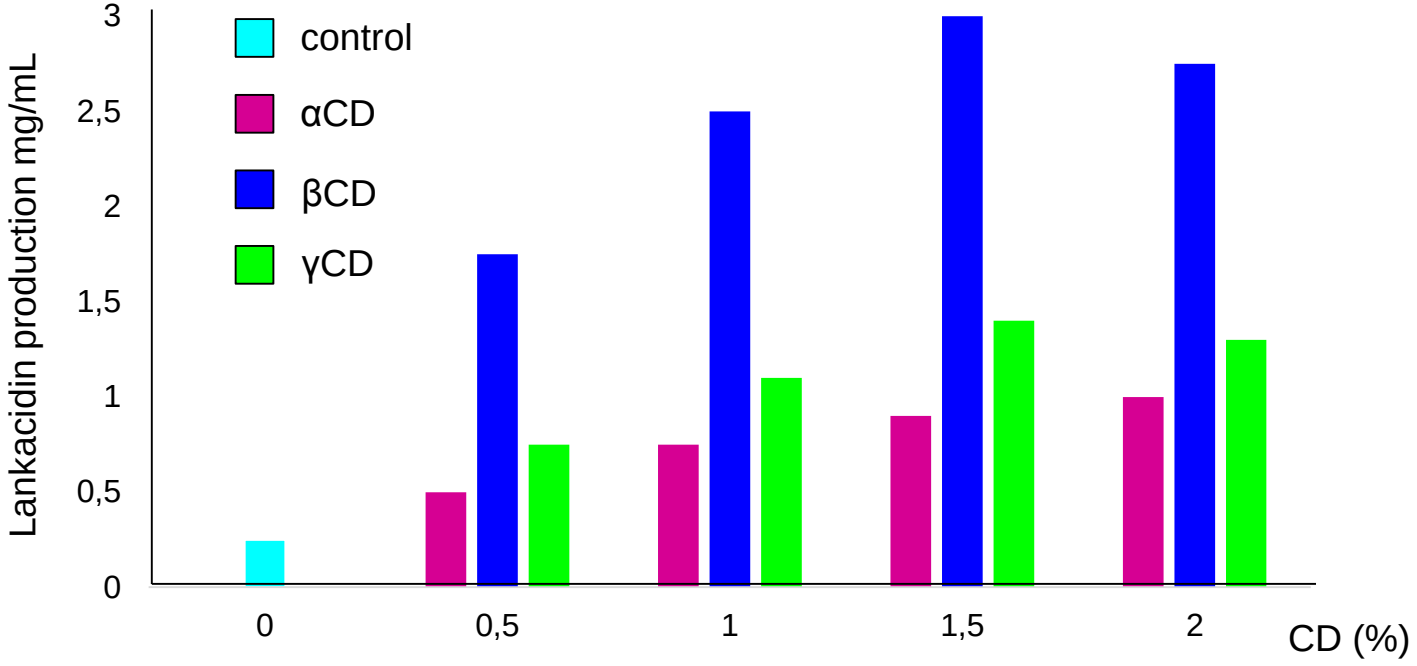
Streptomyces rochei volubilis



Lankacidin A (R=Ac)
Lankacidin C (R=H)

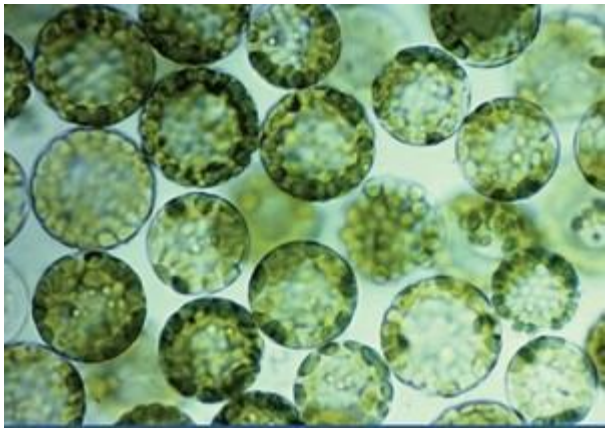
Lankacidinol A (R=Ac)
Lankacidinol C (R=H)

Macrolides Antitumor-Antibiotic

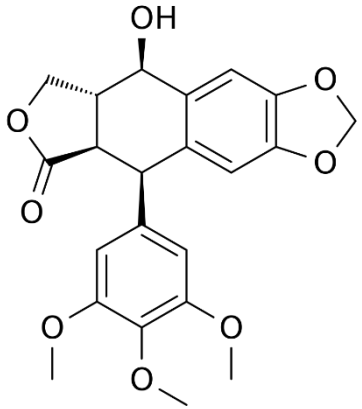


Biosynthesis by Fermentation

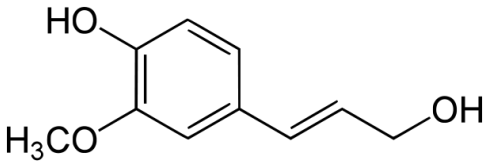
Podophyllum hexandrum



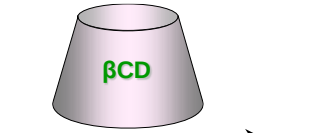
Root cell cultures

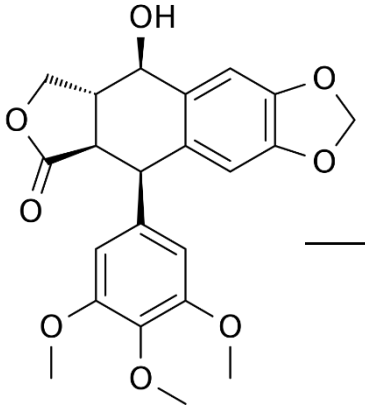


Podophyllotoxin
(Condylox ® - Wartec ®)
(Cathartic, purgative, antiviral
vescicant, antihelminthic activity)

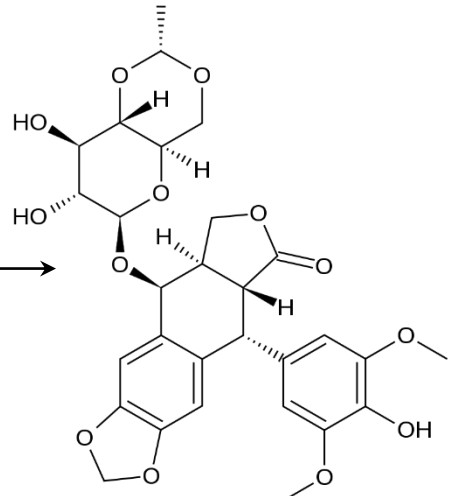


Conyferyl alchol
(poorly water soluble!)





Podophyllotoxin



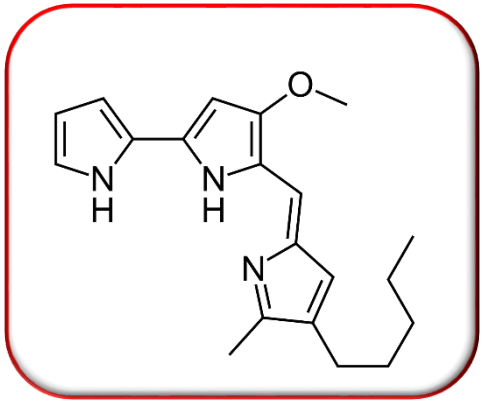
Etoposide
(topisomerase inhibitor)

Biosynthesis by Fermentation

Serratia marcescens
Gram-negative prokaryote

Secondary metabolism

Feedback inhibition!



Prodigiosin - red pigment
(insoluble in water)

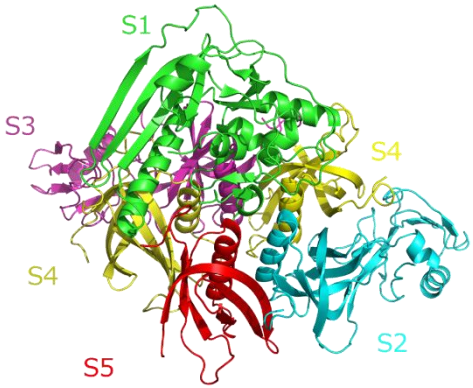
antibacterial
antifungal
antiprotozoal
antimalarial
immunosuppressive
and anticancer properties

CD	Yield increasing
Alpha	+ 68%
Beta	+ 92%
Gamma	- 16%
HPBCD	- 90%
TRIMEB	- 35%
DIMEB	- 5%

Vaccine Production

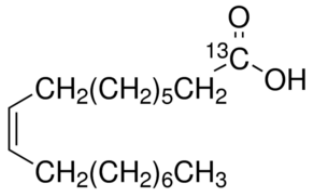
SEM of Bordetella pertussis

Production

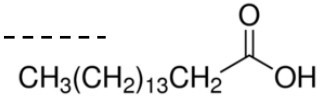


Pertussis toxin

inhibition



Oleic acid



Palmitic acid

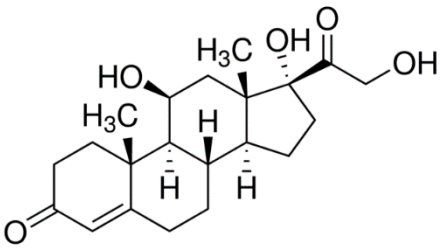
Bordatella pertussis cell growth

Inoculum size cells in 5 µL	0	α	β	γ	DIMEB
10^3	-	-	-	-	++
10^4	-	-	-	-	+++
10^5	-	-	-	-	+++
10^6	-	++	+	+	+++
10^7	-	+++	++	++	+++

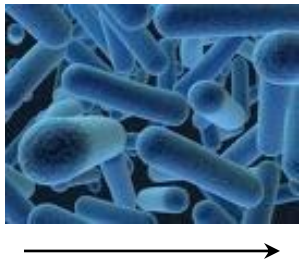
- no growth + < 100 colonies ++ 10^2 to 10^3 colonies +++ full growth

DIMEB increases pertussin toxin production 100-fold!

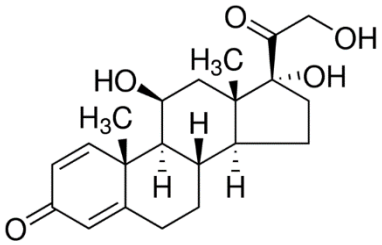
Microbiological Substrate Conversion



Hydrocortisone

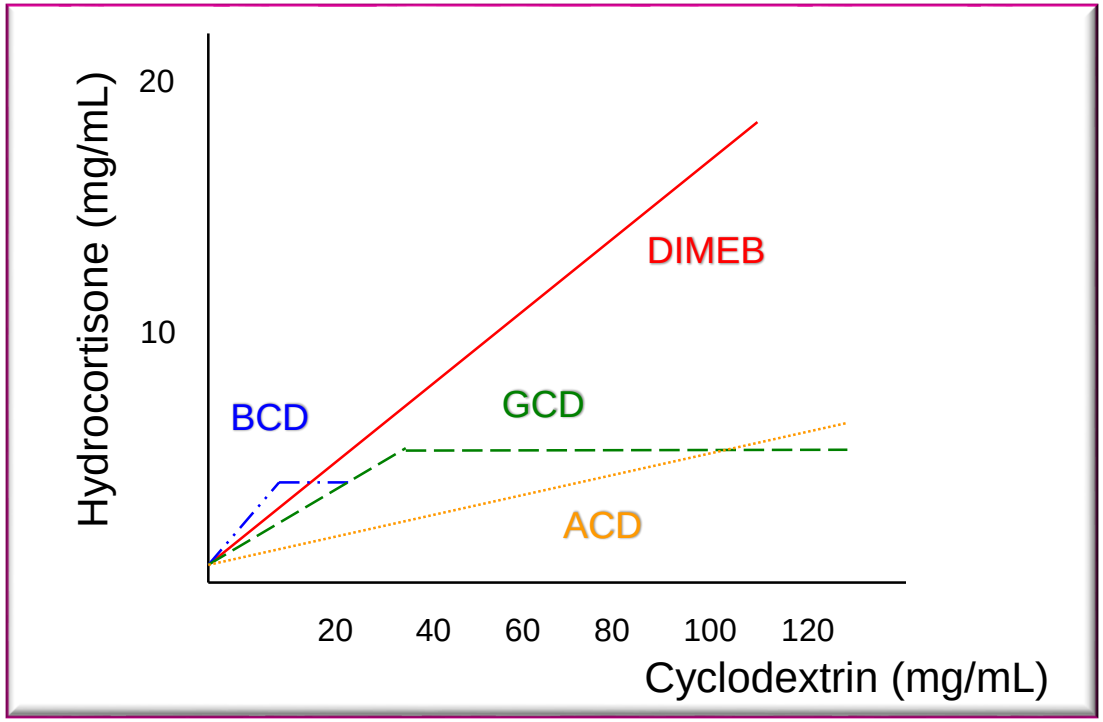


Anthrobacter simplex

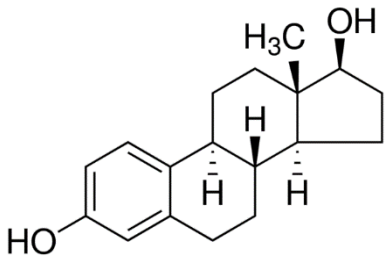


Prednisolone

Aqueous solubility hydrocortisone: 0.4 mg/mL

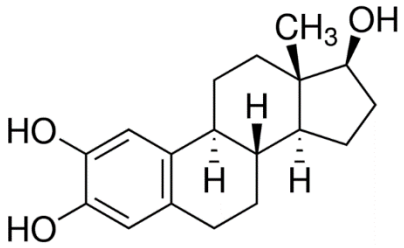


Microbiological Substrate Conversion



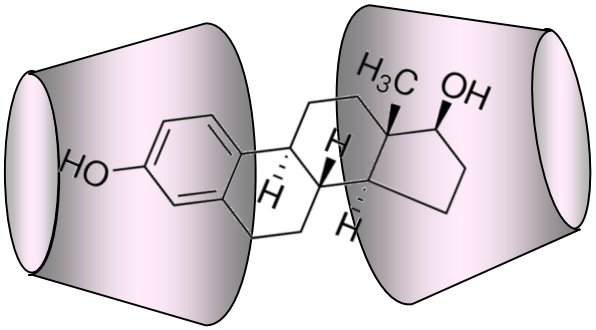
17-β-estradiol

Mucuna Pruriens
(phenoloxidase)



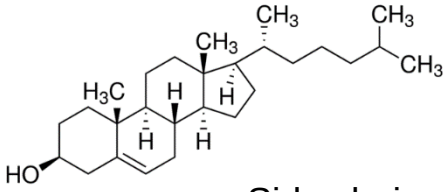
4-hydroxy-estradiol

Aqueous solubility 17-β-estradiol: 30 µg/mL $\xrightarrow{\beta CD}$ 17 mg/mL



BCD increases 4-hydroxy-estradiol production 100-fold!

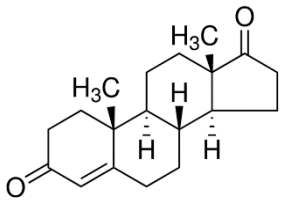
Microbiological Substrate Conversion



Cholesterol

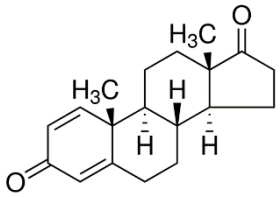
Side chains degradation

Micobacterium sp.



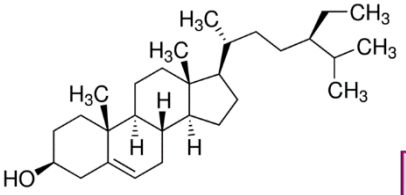
4-Androstene-3-17-dione

+



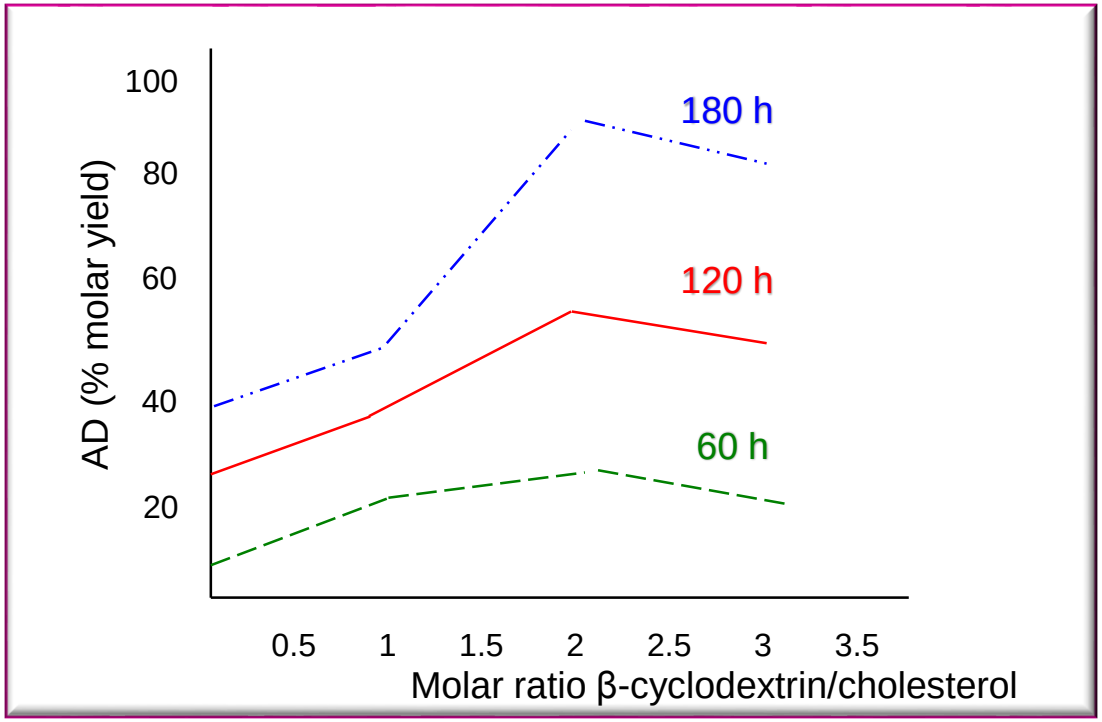
1,4-Androstadiene-3-17-dione

New steroid drugs

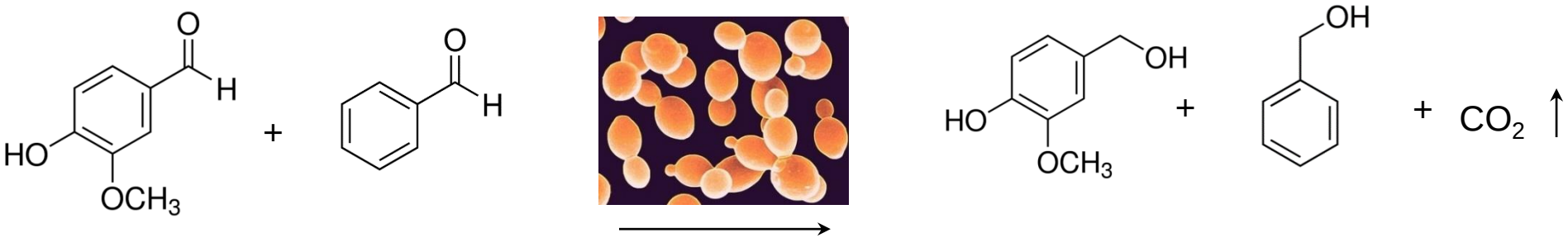


β-Sitosterol

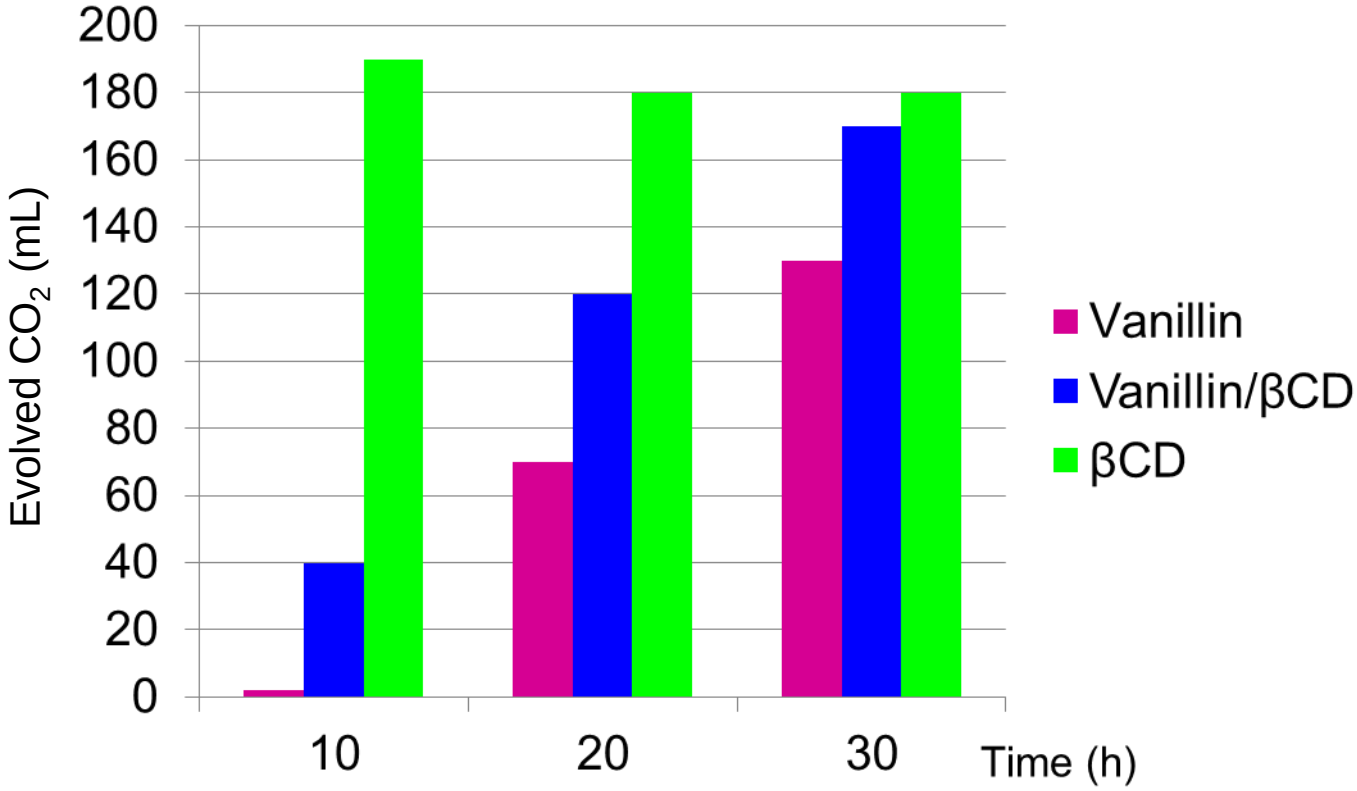
Feedback inhibition



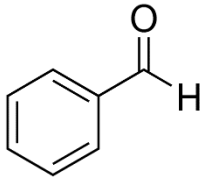
Microbiological Substrate Conversion



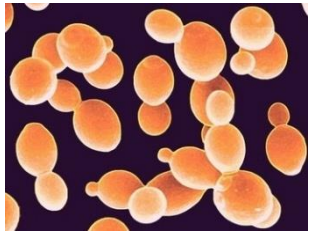
Vanillin + Benzaldehyde → (Saccharomyces cerevisiae) Vanillyl alcohol + Benzyl alcohol + CO_2



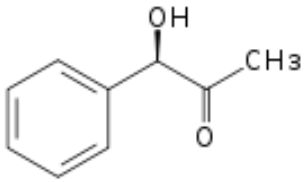
Microbiological Substrate Conversion



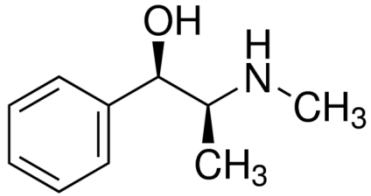
Benzaldehyde



Saccharomyces cerevisiae (ATCC-834)



L-phenylacetyl carbinol



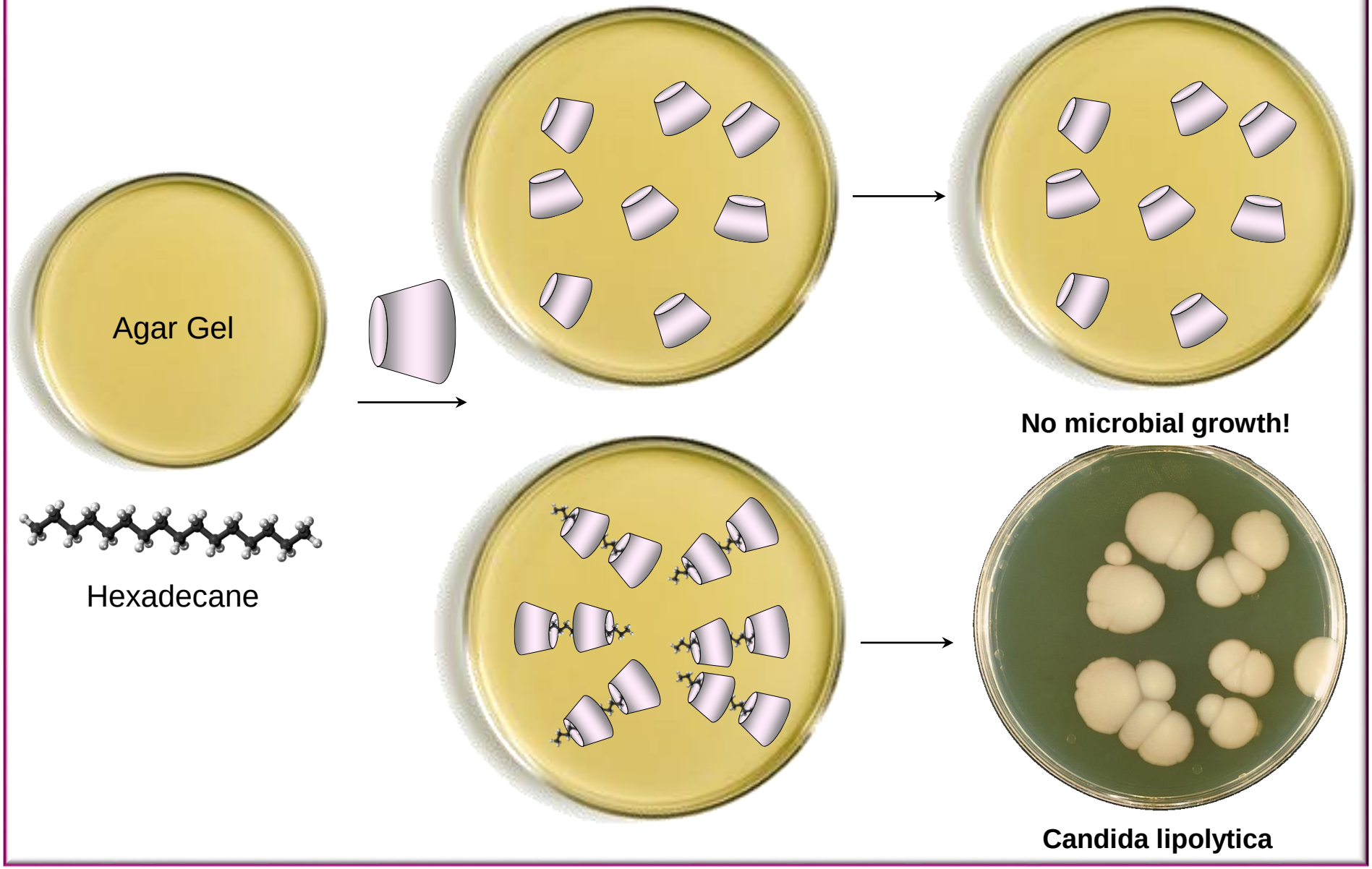
L-ephedrine

L-phenylacetyl carbinol concentration (g/L) in the fermentation medium

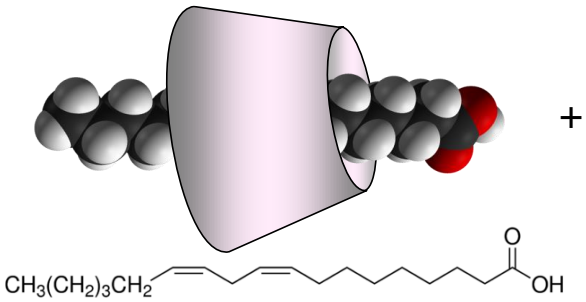
βCD (w/v)	Fermentation time (h) 8	12	16
0	4.0	6.5	8.5
0.5	8.5	10.0	9.0
1.0	9.0	11.5	10.5

βCD improves the solubility of benzaldehyde and reduces its toxicity. βCD also stimulates the microbial growth!

CDs in Microbiological Cultivation

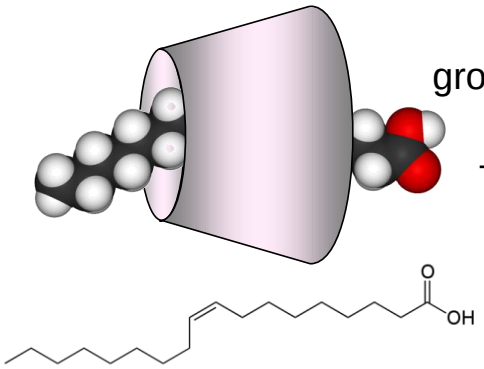


CDs in Tissue Cultures



Linoleic acid/βCD complex

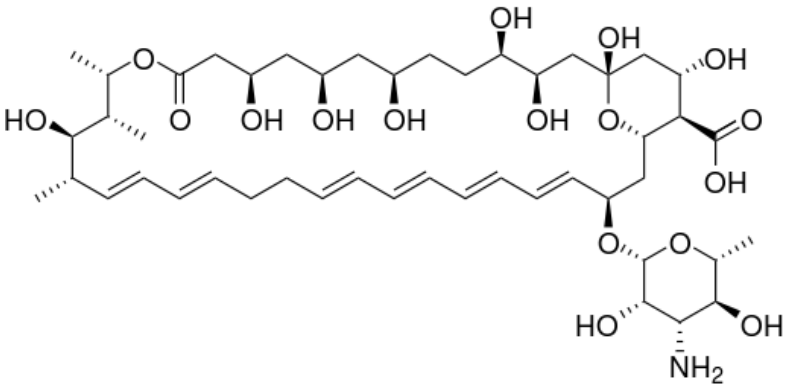
+



Oleic acid/βCD complex

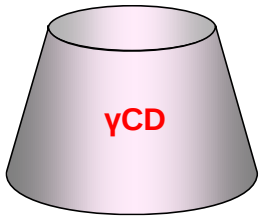
growth-enhancing effect (10 x)

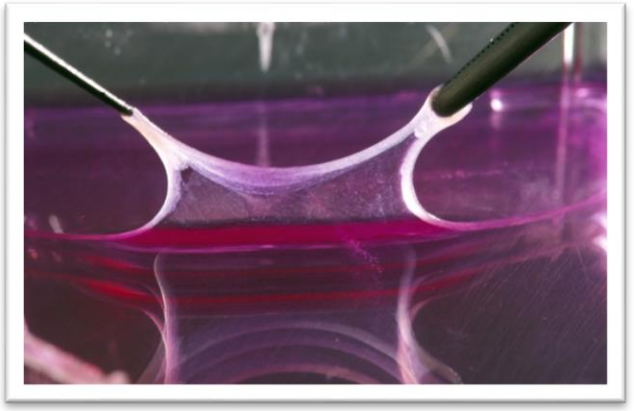
Human lymphoblast cell



Nystatin-polyene antifungal drug
(insoluble and easely oxidized in water)

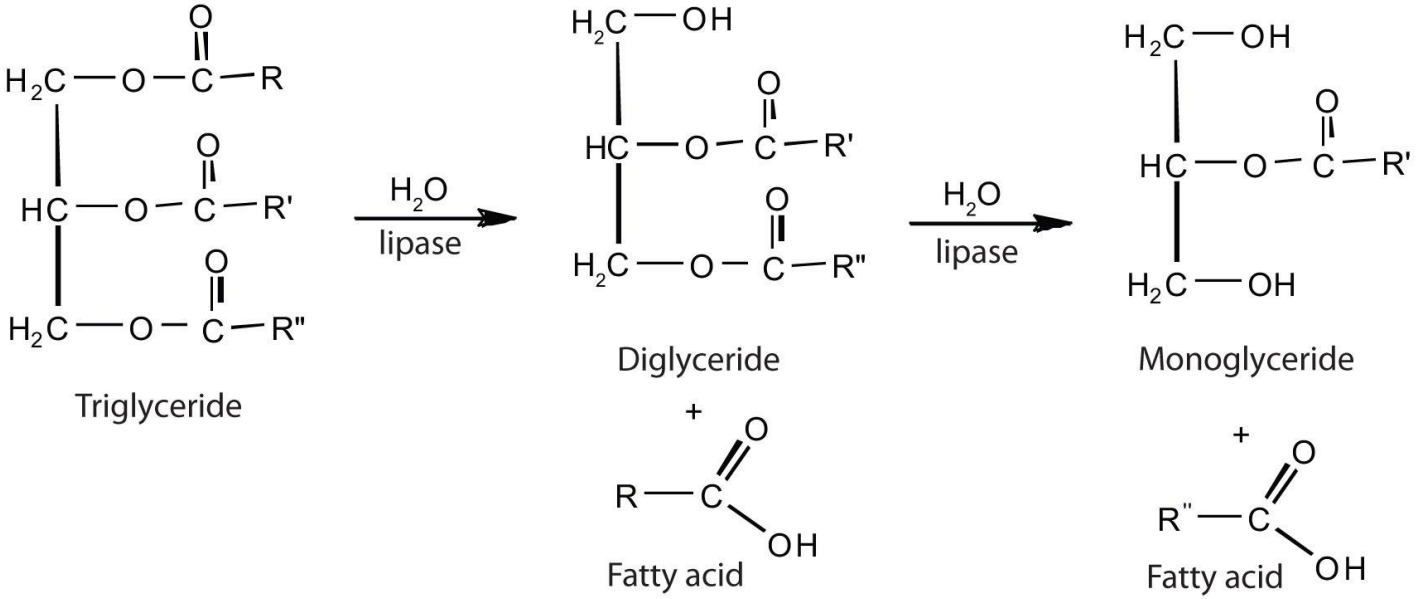
+





Mammalian tissue

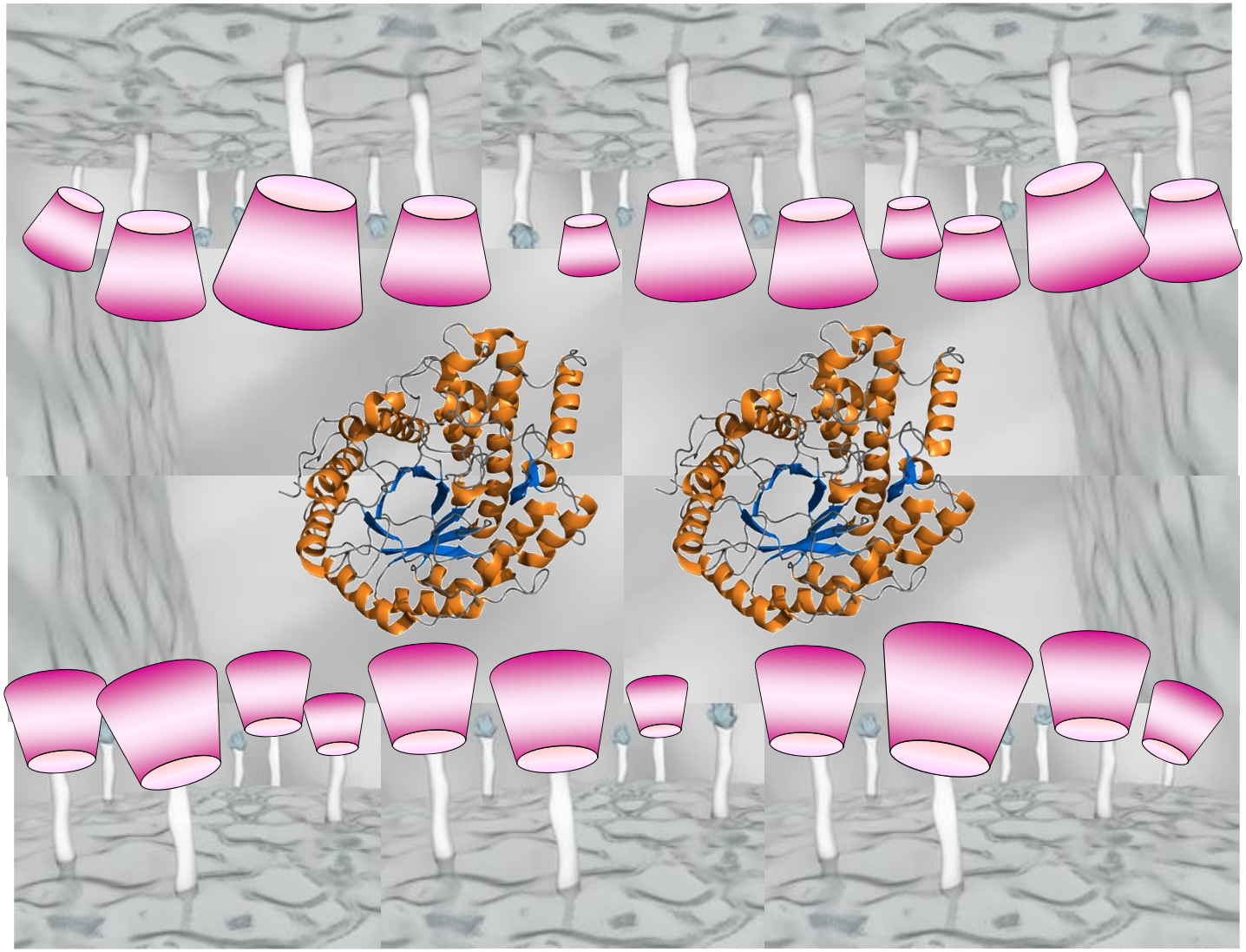
Enzyme Reaction of Lipids



Enzyme hydrolysis of olive oil by hog pancrease

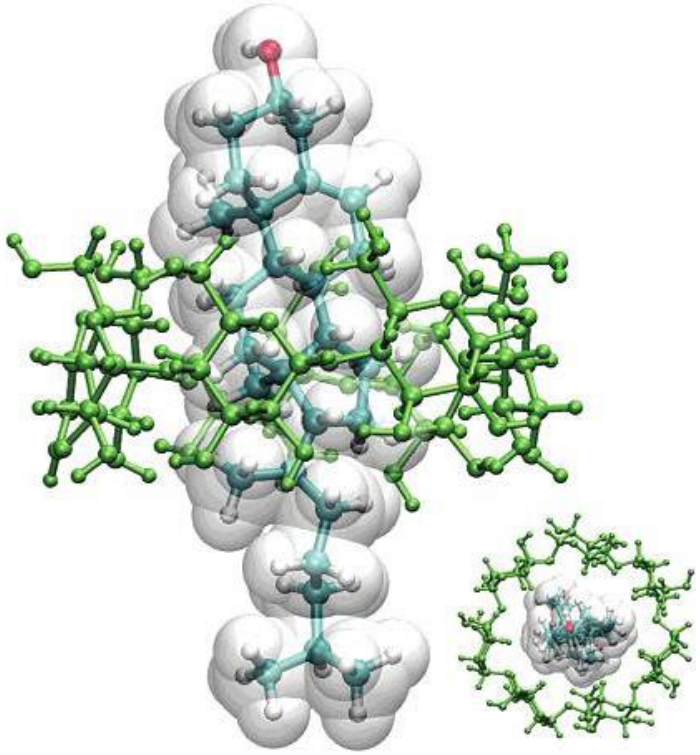
Reaction time (h)	Consumed 0.005 N NaOH (mL) Control (no emulsifier)	Hog bile added	DIMEB added
0.5	0	0.82	1.74
1.0	0.36	1.17	2.00
2.0	0.48	1.37	2.20
19.0	0.63	2.62	4.77
Reaction rate acceleration		~ 4 x	~ 7.4 x

Isolation of Components from Mixtures



β-amylase entrapped in a sepharose 6B gel coupled with αCD
(3 mg of enzyme in 1 mL sedimented gel!)

Isolation of Components from Mixtures



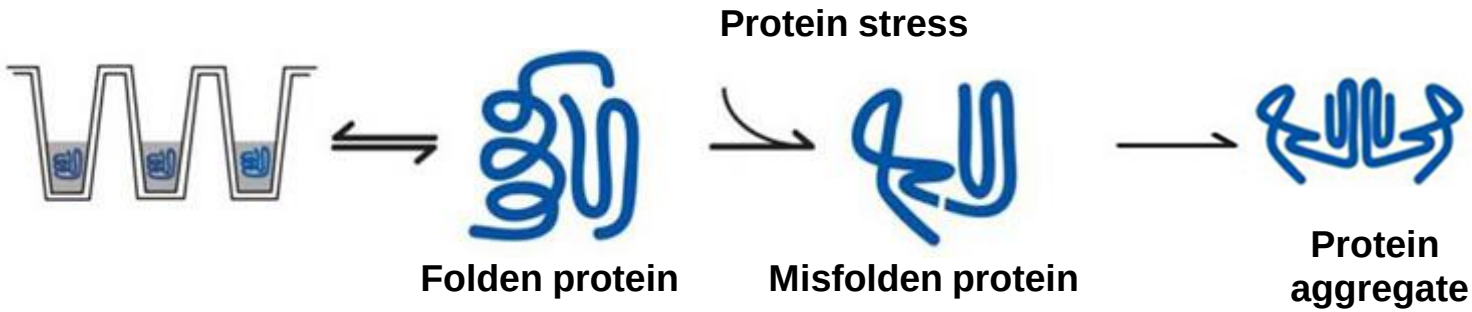
β-CD Cholesterol complex

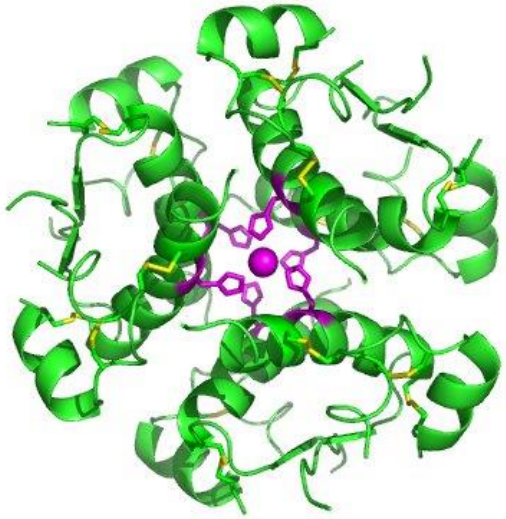
Example of Cholesterol immobilization:
Marketed low Cholesterol Food

Balade® products (Butter and Cream) by Corman Co.

(Roderbourg, et al (CORMAN) Eur. Patent 0 387 708, 1990.)

Interaction of CDs with Proteins





Insulin (folded hexamer)

Saline Buffer

pH=7.4

Two weeks

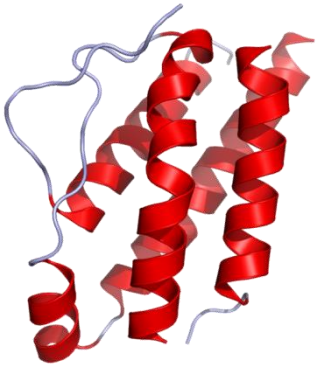
Insulin aggregation

40% HPBCD

Two months

Insulin in solution!

Interaction of CDs with Proteins



Interleukin-2

Freee drying →

Redissolution in buffer →

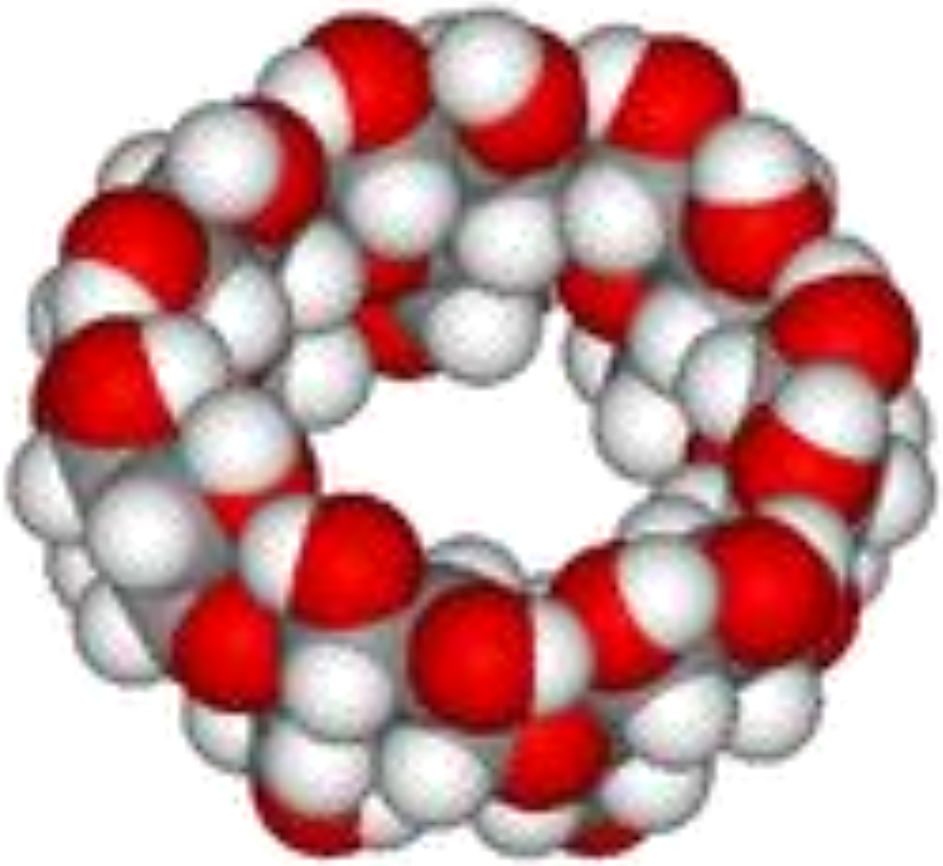
Interleukin-2 aggregation

Citrate buffer, sucrose →

0.5 % HPBCD

Conclusions

- *CDs enhance the solubility of complexed substrates and reduce their toxicity*
- *CDs do not damage the microbial cells or the enzymes*
- *CDs intensify the enzymatic conversion of lipophilic substrates*
- *The yield of product-inhibited ferementations can be improved by CDs*
- *Organic toxic compounds are tolerated by microbes in higher concentrations*
- *Compounds in small amounts can be isolated simply from complicxated mixtures*
- *CDs complexes can substitute for mammalian serum in tissue cultures*
- *Unstable and/or insoluble proteins can be dissolved and stabilized in aqueous solution*



Thank you for your kind attention!