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Project Title: *Cooperation and partnership strategy for the enhancement of the education quality of strategic master Chemistry of Advanced Materials in line with Green Chemistry requirements – Green Chemistry of Advanced Materials*

E-SUPPORT FOR THE REDESIGNED COURSES ENCLOSING KNOWLEDGE FROM MODERN, UP-TO-DATE, GREEN AND SUSTAINABLE CHEMISTRY FIELDS

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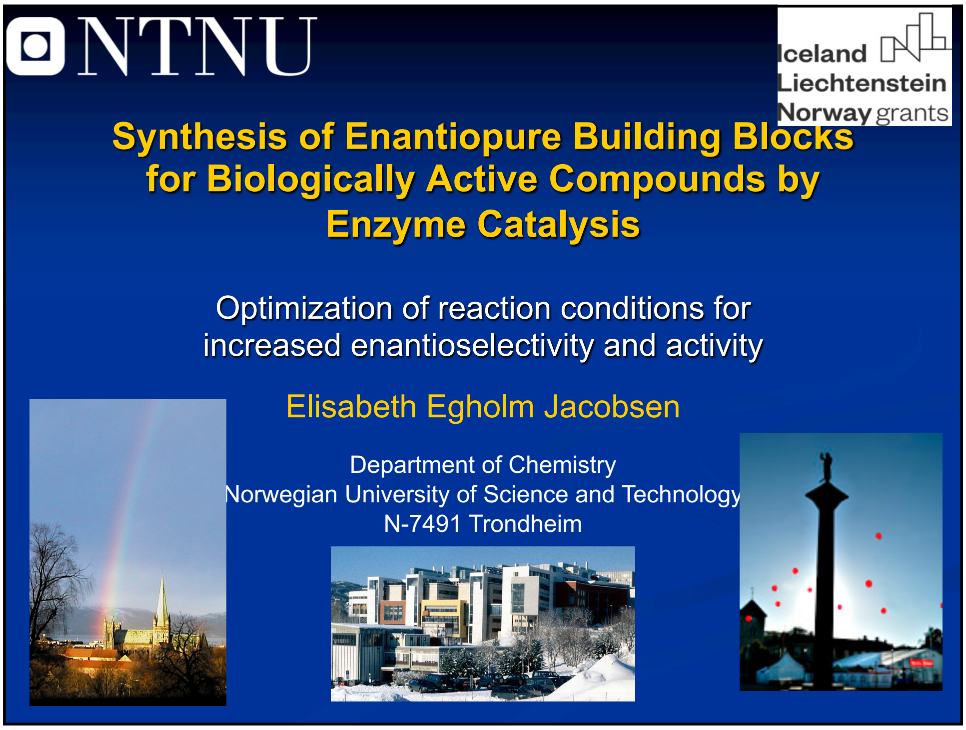
BIOCATALYSIS
(BIOCATALYTIC MATERIALS)

Assoc. Prof. Elisabeth Jacobsen



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Synthesis of Enantiopure Building Blocks for Biologically Active Compounds by Enzyme Catalysis

Optimization of reaction conditions for increased enantioselectivity and activity

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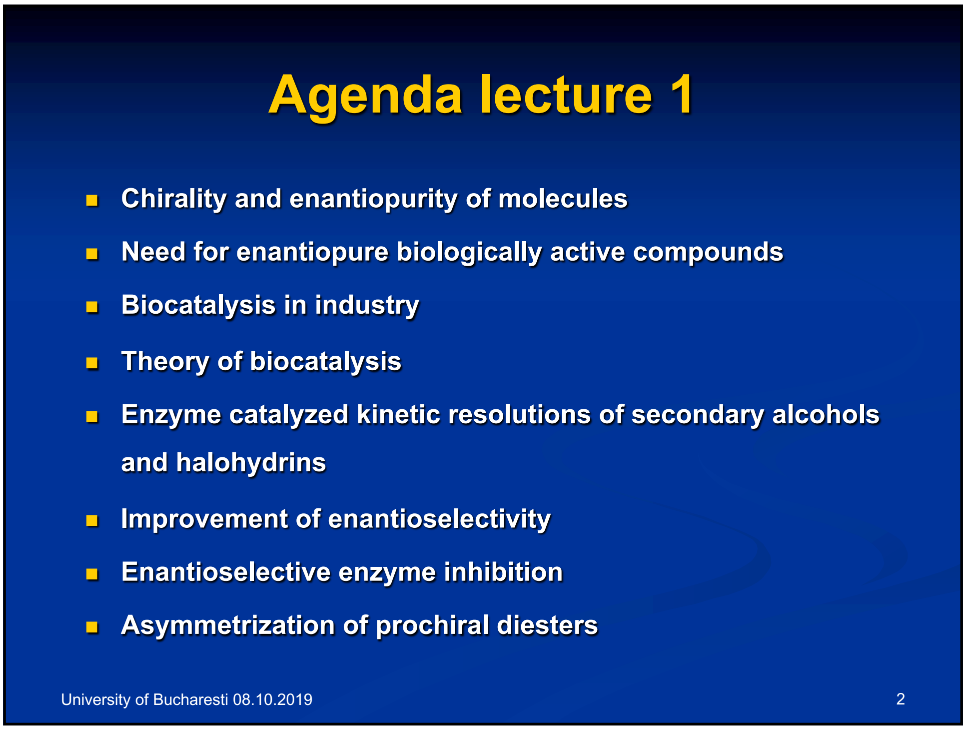
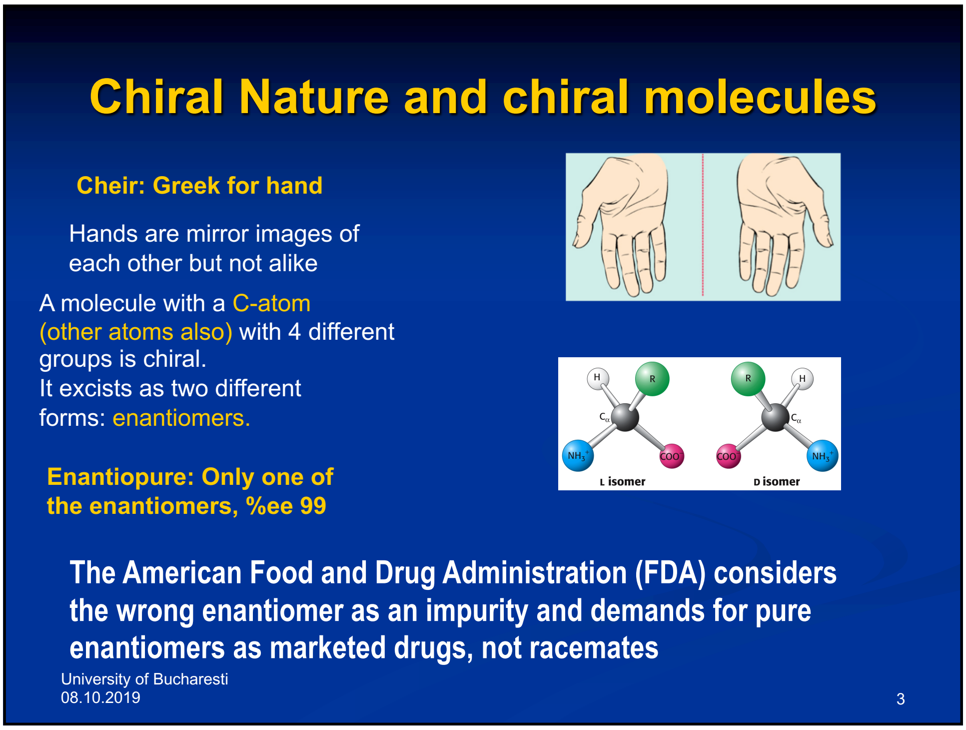


Agenda lecture 1

- Chirality and enantiopurity of molecules
- Need for enantiopure biologically active compounds
- Biocatalysis in industry
- Theory of biocatalysis
- Enzyme catalyzed kinetic resolutions of secondary alcohols and halohydrins
- Improvement of enantioselectivity
- Enantioselective enzyme inhibition
- Asymmetrization of prochiral diesters



Chiral Nature and chiral molecules

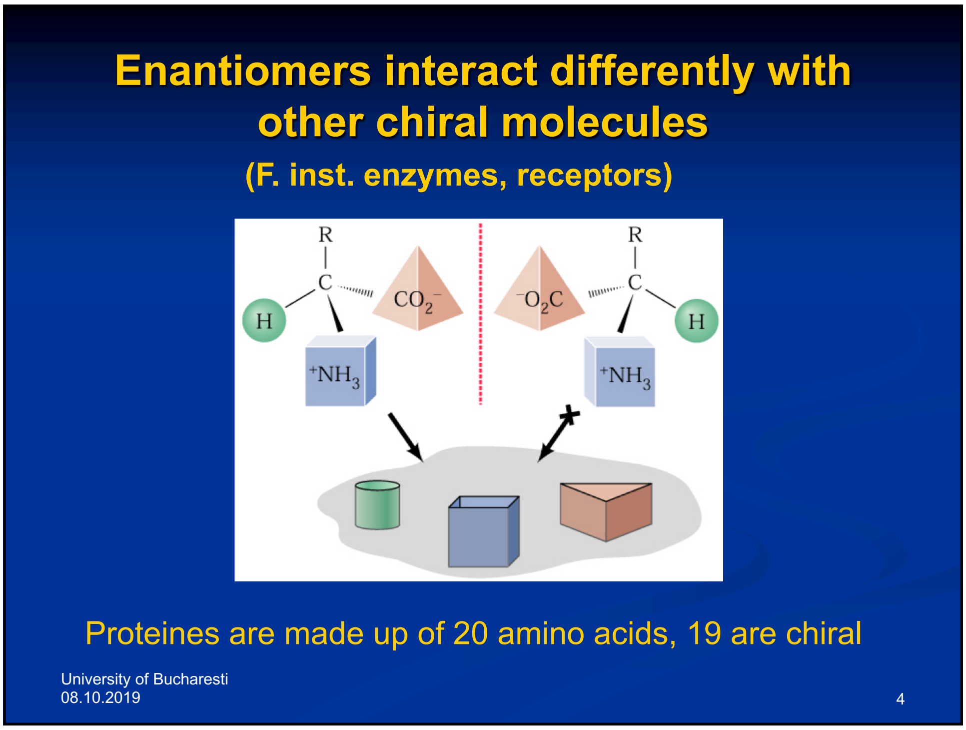
Cheir: Greek for hand

Hands are mirror images of each other but not alike

A molecule with a C-atom (other atoms also) with 4 different groups is chiral.
It excists as two different forms: enantiomers.

Enantiopure: Only one of the enantiomers, %ee 99

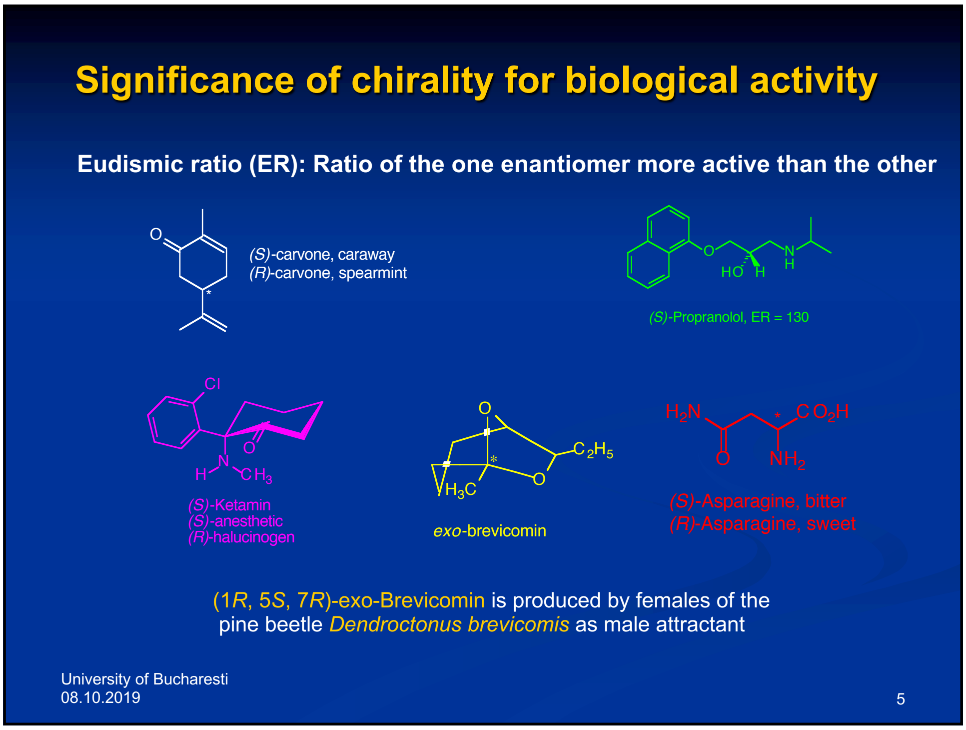
The American Food and Drug Administration (FDA) considers the wrong enantiomer as an impurity and demands for pure enantiomers as marketed drugs, not racemates



Enantiomers interact differently with other chiral molecules

(F. inst. enzymes, receptors)

Proteines are made up of 20 amino acids, 19 are chiral



Significance of chirality for biological activity

Eudismic ratio (ER): Ratio of the one enantiomer more active than the other

(*S*)-carvone, caraway
(*R*)-carvone, spearmint

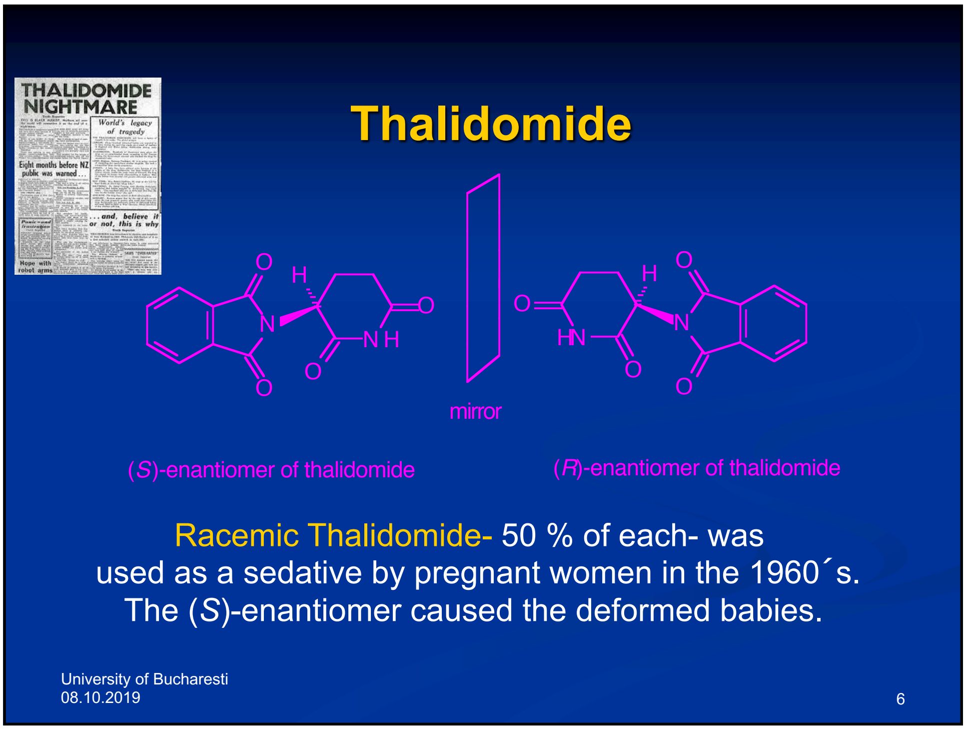
(*S*)-Propranolol, ER = 130

(*S*)-Ketamin
(*S*)-anesthetic
(*R*)-halucinogen

exo-brevicomin

(*S*)-Asparagine, bitter
(*R*)-Asparagine, sweet

(1*R*, 5*S*, 7*R*)-exo-Brevicomin is produced by females of the pine beetle *Dendroctonus brevicomis* as male attractant

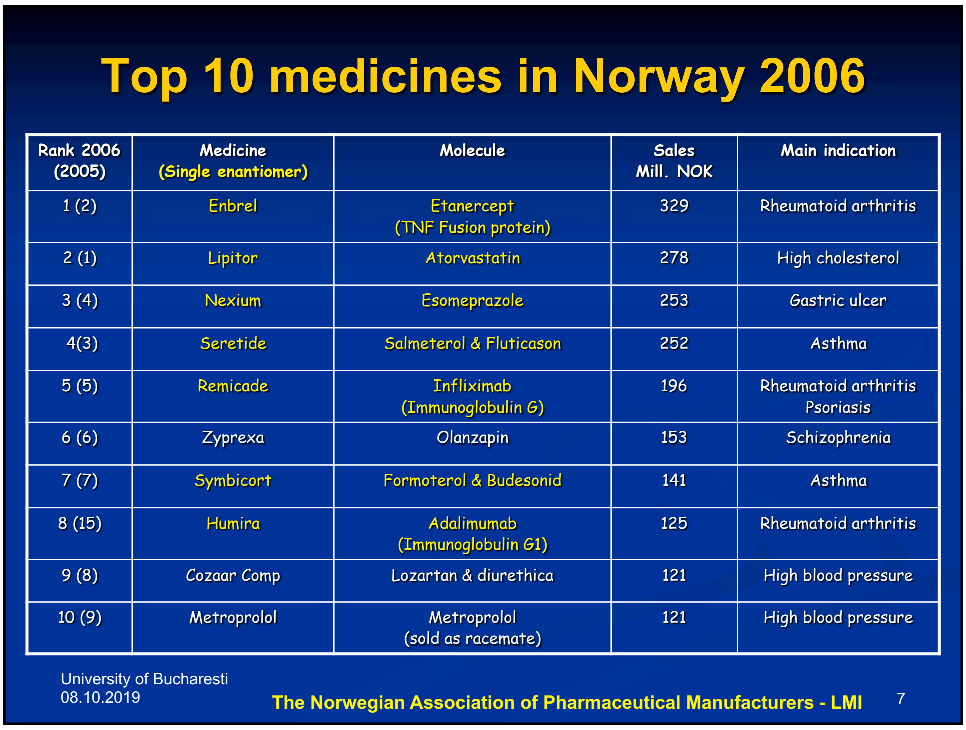


Thalidomide

(*S*)-enantiomer of thalidomide

(*R*)-enantiomer of thalidomide

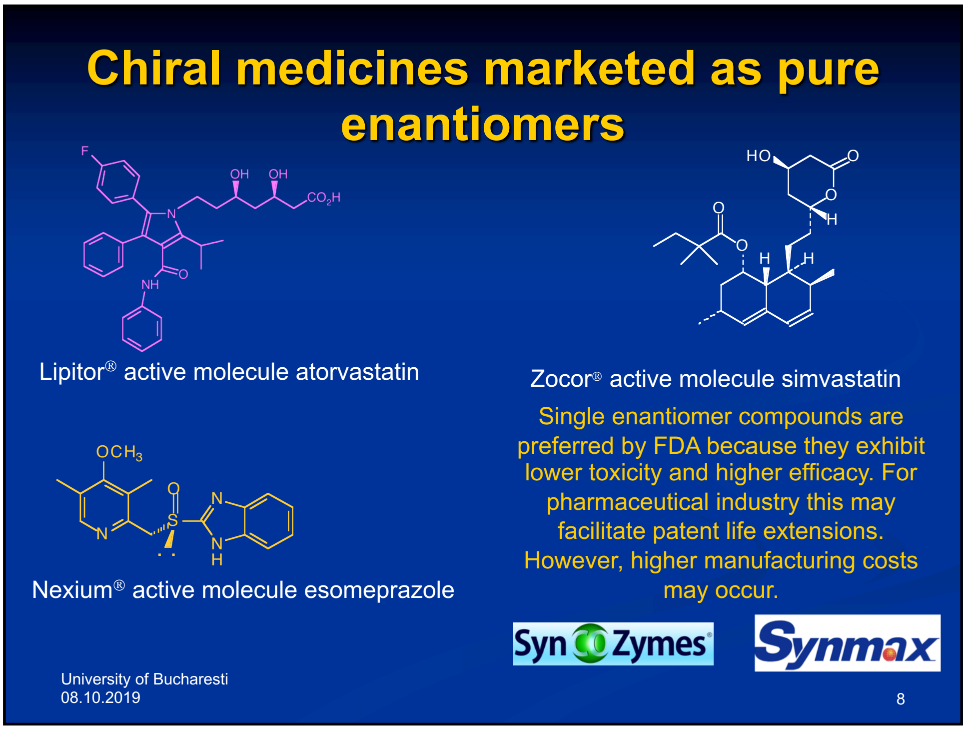
Racemic Thalidomide- 50 % of each- was used as a sedative by pregnant women in the 1960´s. The (*S*)-enantiomer caused the deformed babies.



Top 10 medicines in Norway 2006

Rank 2006 (2005)	Medicine (Single enantiomer)	Molecule	Sales Mill. NOK	Main indication
1 (2)	Enbrel	Etanercept (TNF Fusion protein)	329	Rheumatoid arthritis
2 (1)	Lipitor	Atorvastatin	278	High cholesterol
3 (4)	Nexium	Esomeprazole	253	Gastric ulcer
4(3)	Seretide	Salmeterol & Fluticason	252	Asthma
5 (5)	Remicade	Infliximab (Immunoglobulin G)	196	Rheumatoid arthritis Psoriasis
6 (6)	Zyprexa	Olanzapin	153	Schizophrenia
7 (7)	Symbicort	Formoterol & Budesonid	141	Asthma
8 (15)	Humira	Adalimumab (Immunoglobulin G1)	125	Rheumatoid arthritis
9 (8)	Cozaar Comp	Lozartan & diurethica	121	High blood pressure
10 (9)	Metroprolol	Metroprolol (sold as racemate)	121	High blood pressure

The Norwegian Association of Pharmaceutical Manufacturers - LMI



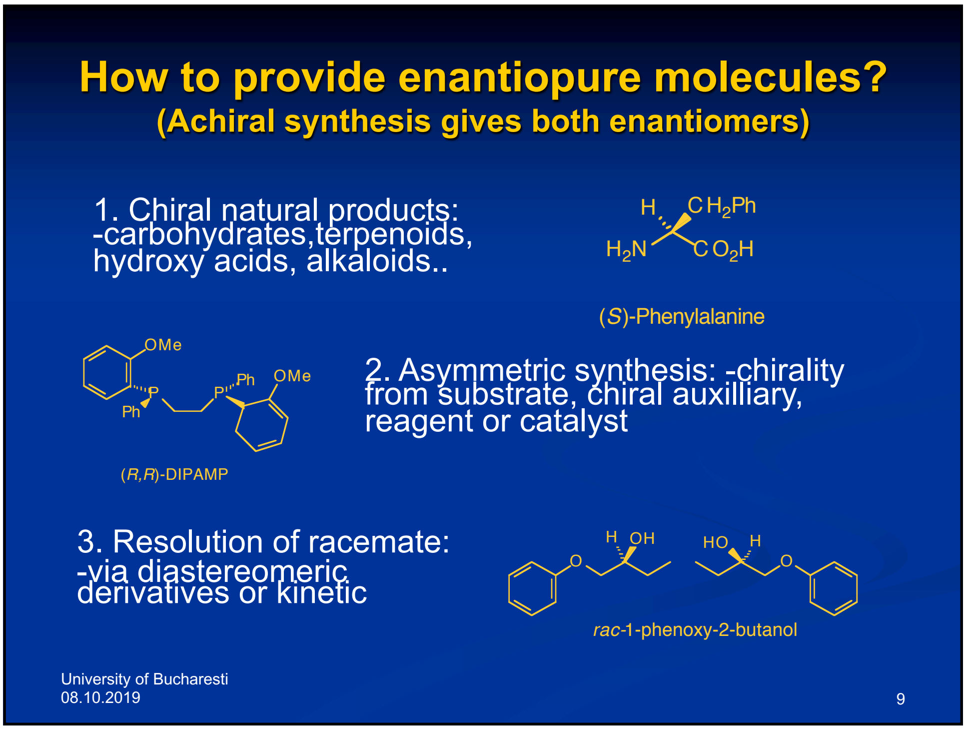
Chiral medicines marketed as pure enantiomers

Lipitor® active molecule atorvastatin

Zocor® active molecule simvastatin

Single enantiomer compounds are preferred by FDA because they exhibit lower toxicity and higher efficacy. For pharmaceutical industry this may facilitate patent life extensions. However, higher manufacturing costs may occur.

Nexium® active molecule esomeprazole



How to provide enantiopure molecules?

(Achiral synthesis gives both enantiomers)

1. Chiral natural products: -carbohydrates,terpenoids, hydroxy acids, alkaloids..

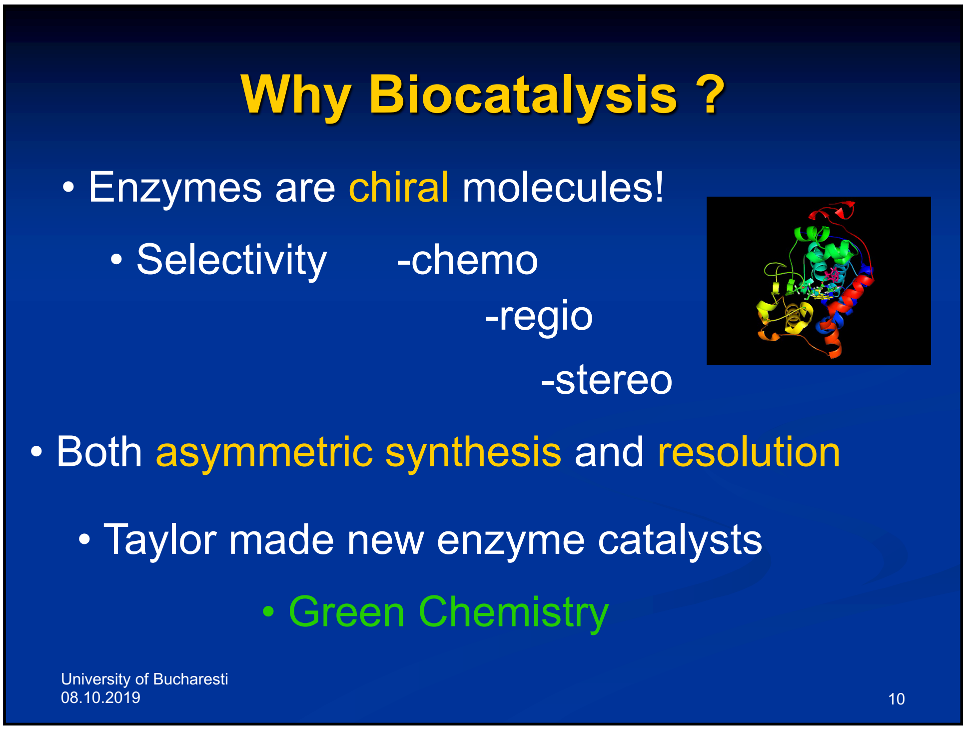
(*S*)-Phenylalanine

2. Asymmetric synthesis: -chirality from substrate, chiral auxilliary, reagent or catalyst

(*R*,*R*)-DIPAMP

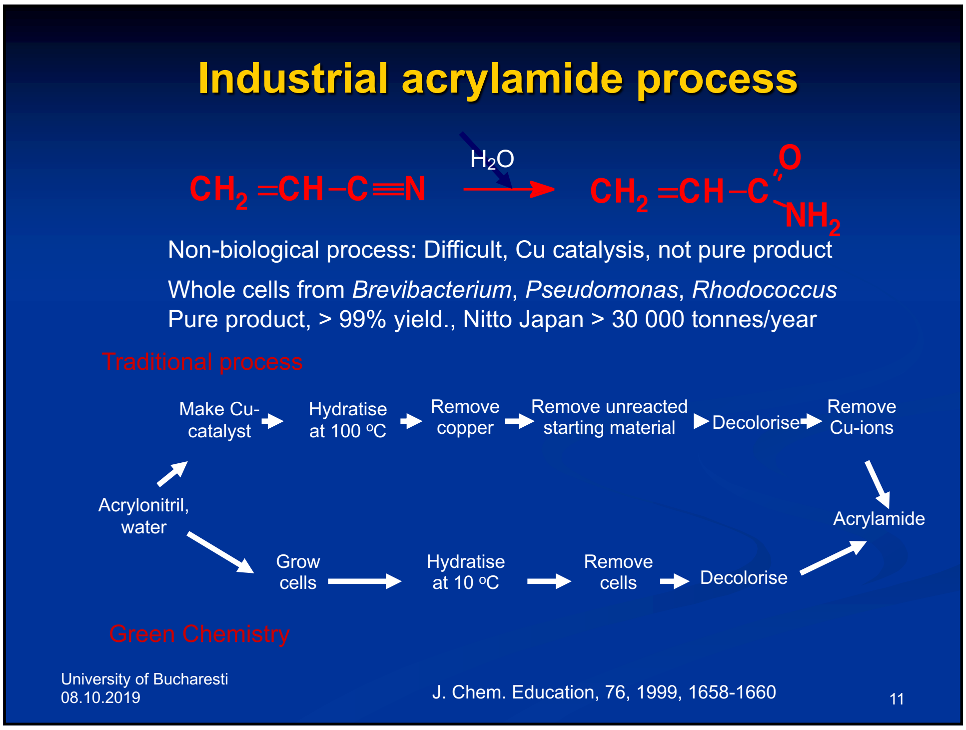
3. Resolution of racemate: -via diastereomeric derivatives or kinetic

rac-1-phenoxy-2-butanol



Why Biocatalysis ?

- Enzymes are chiral molecules!
  - Selectivity -chemo
    -regio
    -stereo
- Both asymmetric synthesis and resolution
  - Taylor made new enzyme catalysts
    - Green Chemistry



Industrial acrylamide process

$$CH_2{=}CH{-}C{\equiv}N \xrightarrow{H_2O} CH_2{=}CH{-}C(=O)NH_2$$

Non-biological process: Difficult, Cu catalysis, not pure product

Whole cells from *Brevibacterium*, *Pseudomonas*, *Rhodococcus*
Pure product, > 99% yield., Nitto Japan > 30 000 tonnes/year

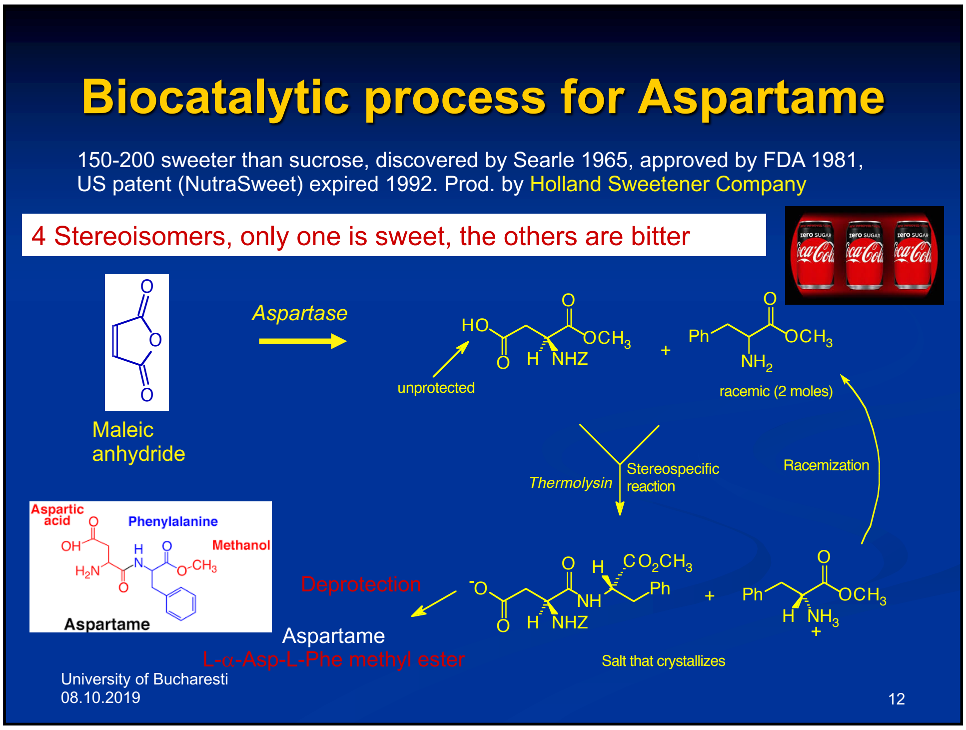
Traditional process

Acrylonitril, water → Make Cu-catalyst → Hydratise at 100 °C → Remove copper → Remove unreacted starting material → Decolorise → Remove Cu-ions → Acrylamide

Acrylonitril, water → Grow cells → Hydratise at 10 °C → Remove cells → Decolorise → Acrylamide

Green Chemistry

J. Chem. Education, 76, 1999, 1658-1660

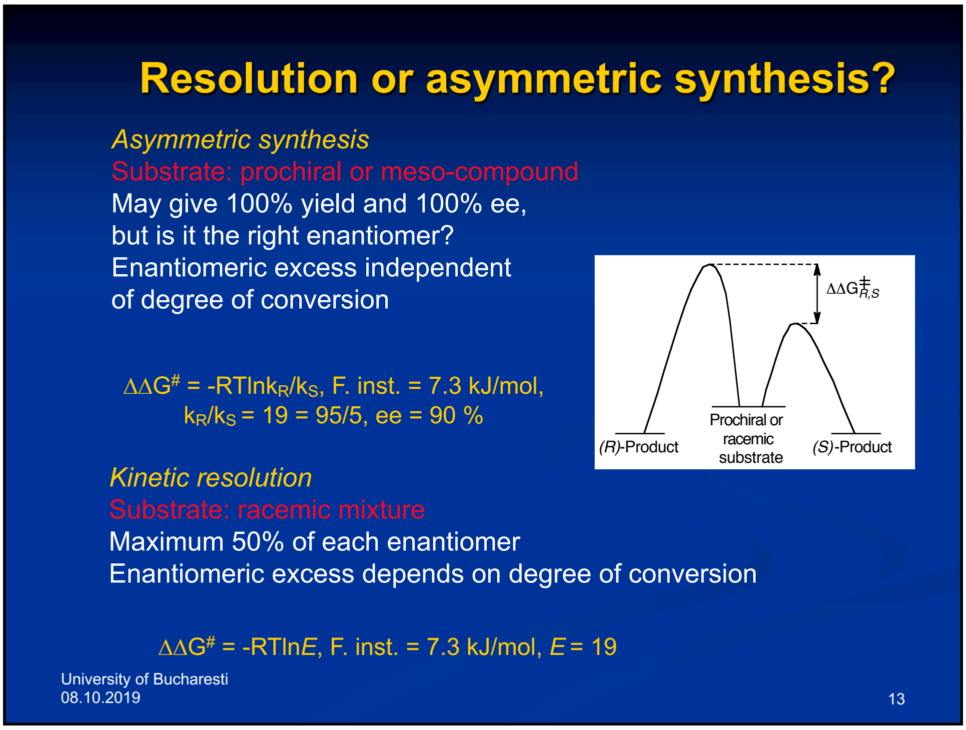


Biocatalytic process for Aspartame

150-200 sweeter than sucrose, discovered by Searle 1965, approved by FDA 1981, US patent (NutraSweet) expired 1992. Prod. by Holland Sweetener Company

4 Stereoisomers, only one is sweet, the others are bitter

Aspartame
L-α-Asp-L-Phe methyl ester



Resolution or asymmetric synthesis?

Asymmetric synthesis

Substrate: prochiral or meso-compound

May give 100% yield and 100% ee,
but is it the right enantiomer?
Enantiomeric excess independent
of degree of conversion

$\Delta\Delta G^{\#} = -RT\ln k_R/k_S$, F. inst. = 7.3 kJ/mol,
$k_R/k_S = 19 = 95/5$, ee = 90 %

Kinetic resolution

Substrate: racemic mixture

Maximum 50% of each enantiomer
Enantiomeric excess depends on degree of conversion

$\Delta\Delta G^{\#} = -RT\ln E$, F. inst. = 7.3 kJ/mol, $E = 19$

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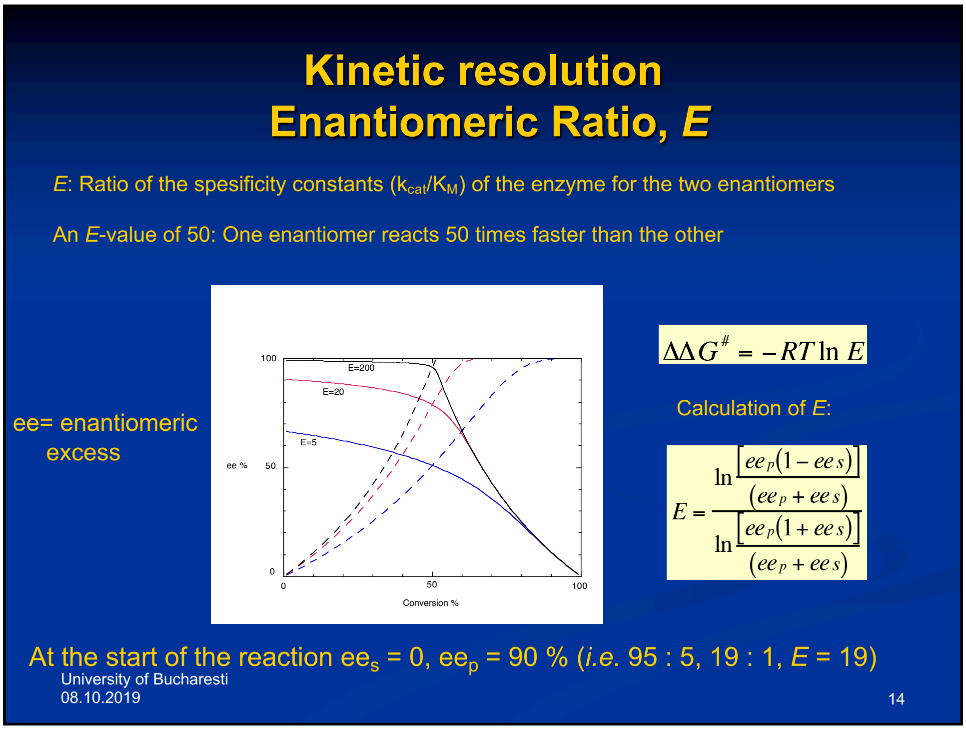
13



Kinetic resolution
Enantiomeric Ratio, *E*

E: Ratio of the spesificity constants (k_{cat}/K_M) of the enzyme for the two enantiomers

An *E*-value of 50: One enantiomer reacts 50 times faster than the other

ee= enantiomeric excess

$$\Delta\Delta G^{\#} = -RT\ln E$$

Calculation of *E*:

$$E = \frac{\ln \frac{\left[ee_p(1-ee_s)\right]}{(ee_p+ee_s)}}{\ln \frac{\left[ee_p(1+ee_s)\right]}{(ee_p+ee_s)}}$$

At the start of the reaction $ee_s = 0$, $ee_p = 90$ % (*i.e.* 95 : 5, 19 : 1, *E* = 19)

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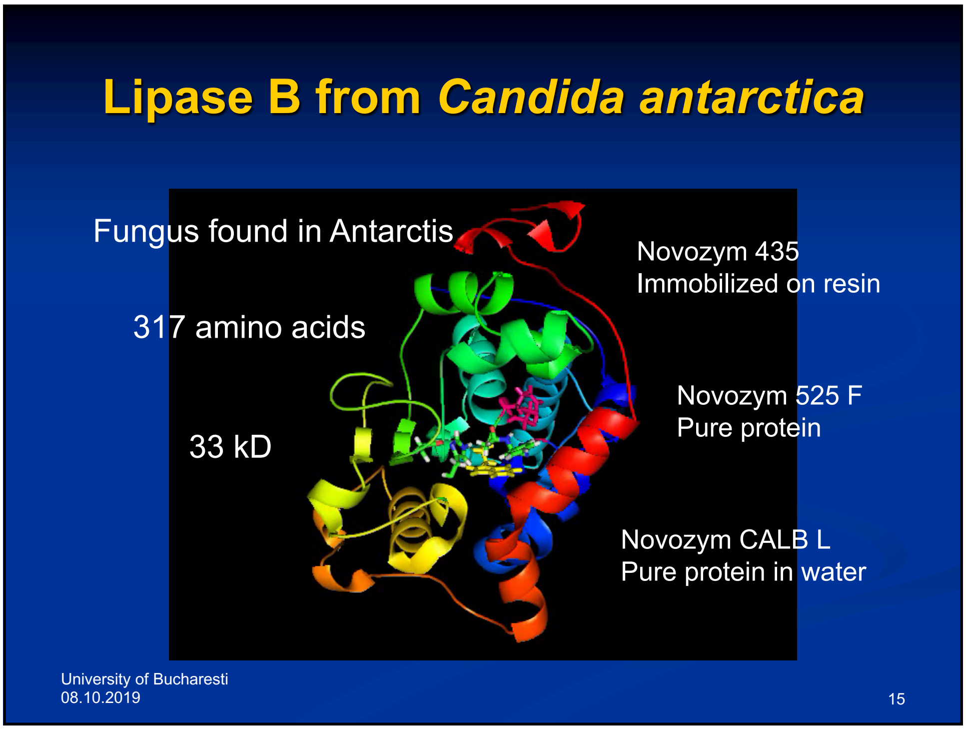

Lipase B from *Candida antarctica*

Fungus found in Antarctis

317 amino acids

33 kD

Novozym 435
Immobilized on resin

Novozym 525 F
Pure protein

Novozym CALB L
Pure protein in water

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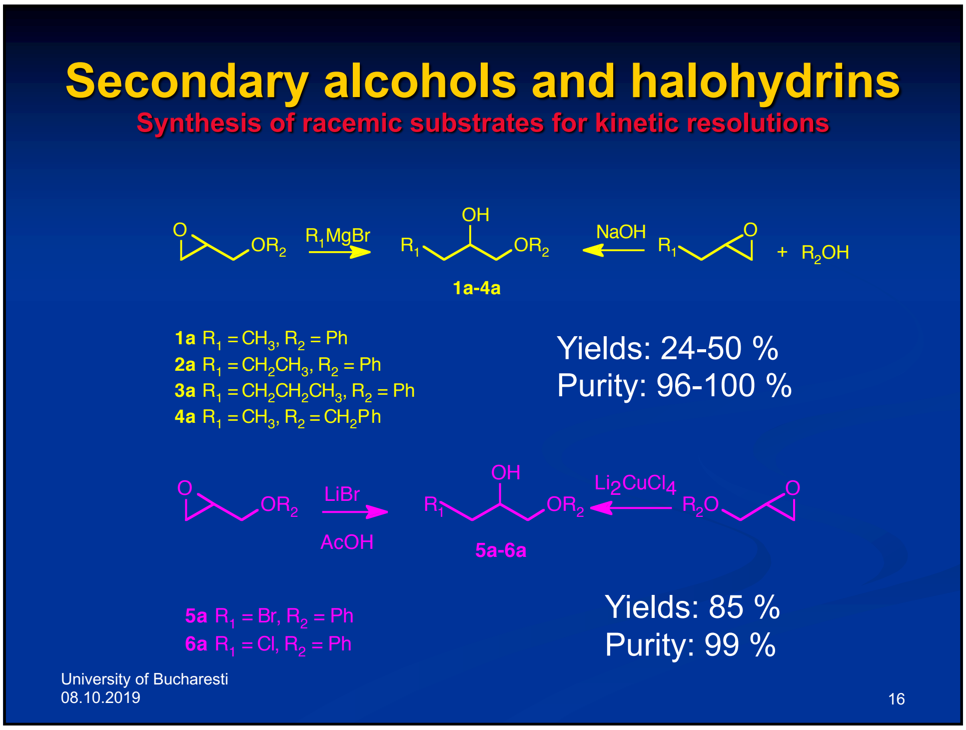

Secondary alcohols and halohydrins

Synthesis of racemic substrates for kinetic resolutions

1a $R_1 = CH_3$, $R_2 = Ph$
2a $R_1 = CH_2CH_3$, $R_2 = Ph$
3a $R_1 = CH_2CH_2CH_3$, $R_2 = Ph$
4a $R_1 = CH_3$, $R_2 = CH_2Ph$

Yields: 24-50 %
Purity: 96-100 %

5a $R_1 = Br$, $R_2 = Ph$
6a $R_1 = Cl$, $R_2 = Ph$

Yields: 85 %
Purity: 99 %

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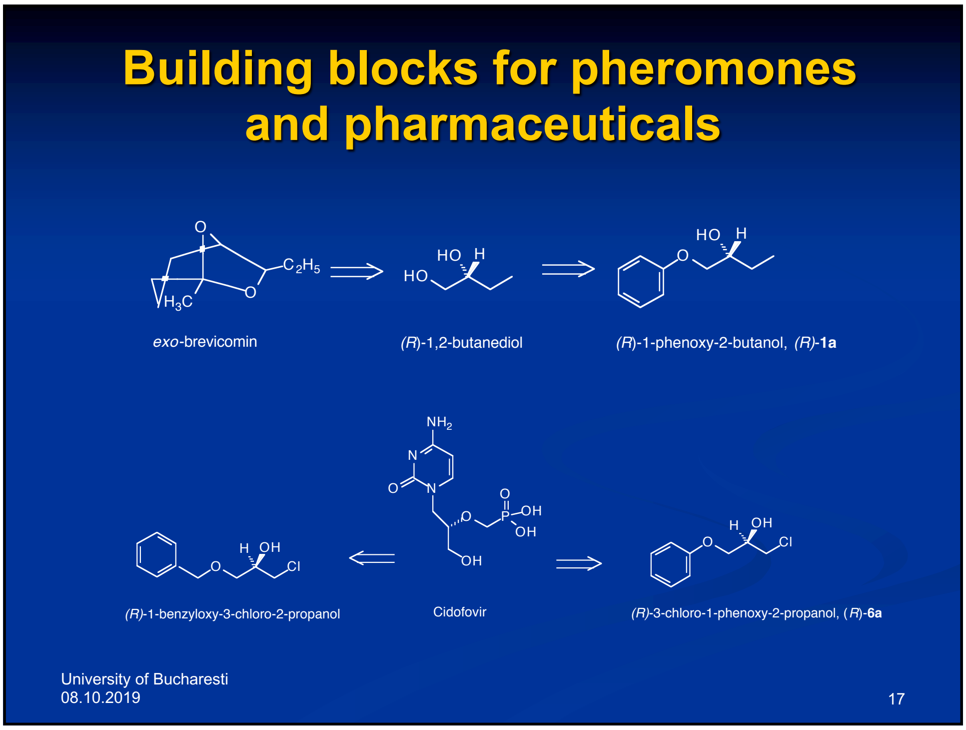

Building blocks for pheromones and pharmaceuticals

exo-brevicomin

(*R*)-1,2-butanediol

(*R*)-1-phenoxy-2-butanol, (*R*)-**1a**

(*R*)-1-benzyloxy-3-chloro-2-propanol

Cidofovir

(*R*)-3-chloro-1-phenoxy-2-propanol, (*R*)-**6a**

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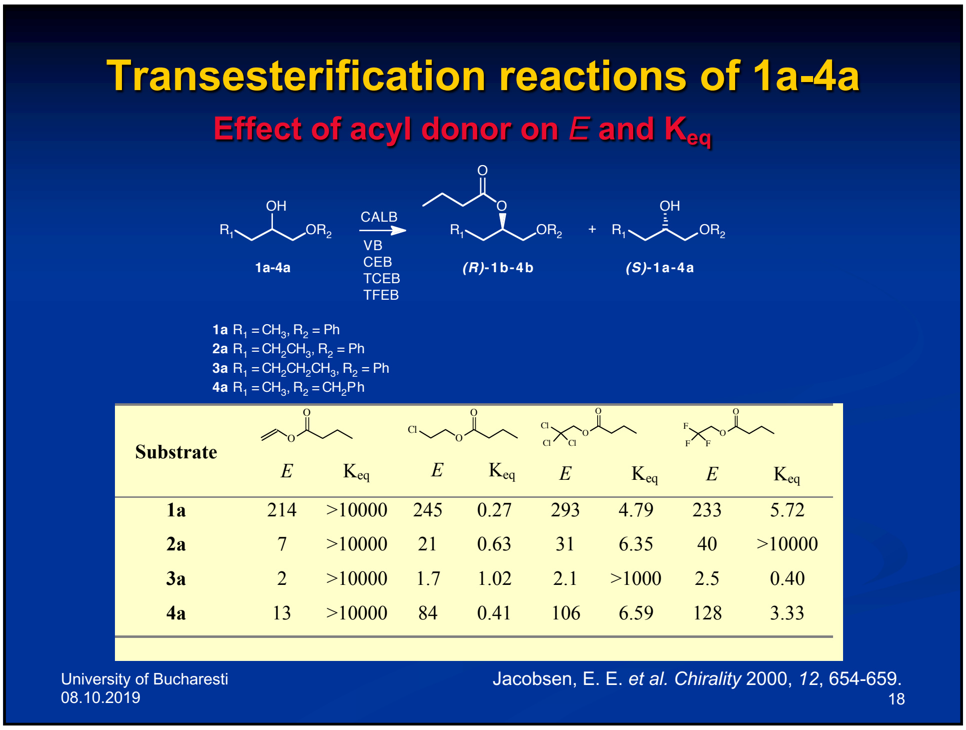

Transesterification reactions of 1a-4a

Effect of acyl donor on *E* and K_{eq}

1a-4a

VB
CEB
TCEB
TFEB

(*R*)-**1b-4b**

(*S*)-**1a-4a**

1a $R_1 = CH_3$, $R_2 = Ph$
2a $R_1 = CH_2CH_3$, $R_2 = Ph$
3a $R_1 = CH_2CH_2CH_3$, $R_2 = Ph$
4a $R_1 = CH_3$, $R_2 = CH_2Ph$

Substrate	*E*	K_{eq}	*E*	K_{eq}	*E*	K_{eq}	*E*	K_{eq}
1a	214	>10000	245	0.27	293	4.79	233	5.72
2a	7	>10000	21	0.63	31	6.35	40	>10000
3a	2	>10000	1.7	1.02	2.1	>1000	2.5	0.40
4a	13	>10000	84	0.41	106	6.59	128	3.33

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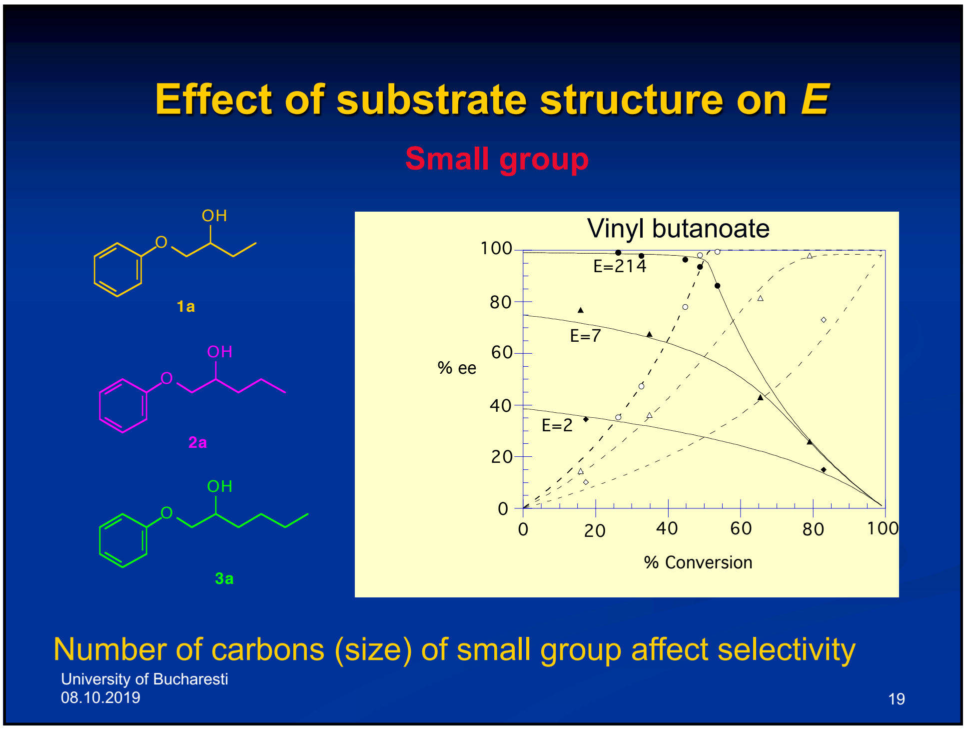
Jacobsen, E. E. *et al. Chirality* 2000, *12*, 654-659.



Effect of substrate structure on *E*

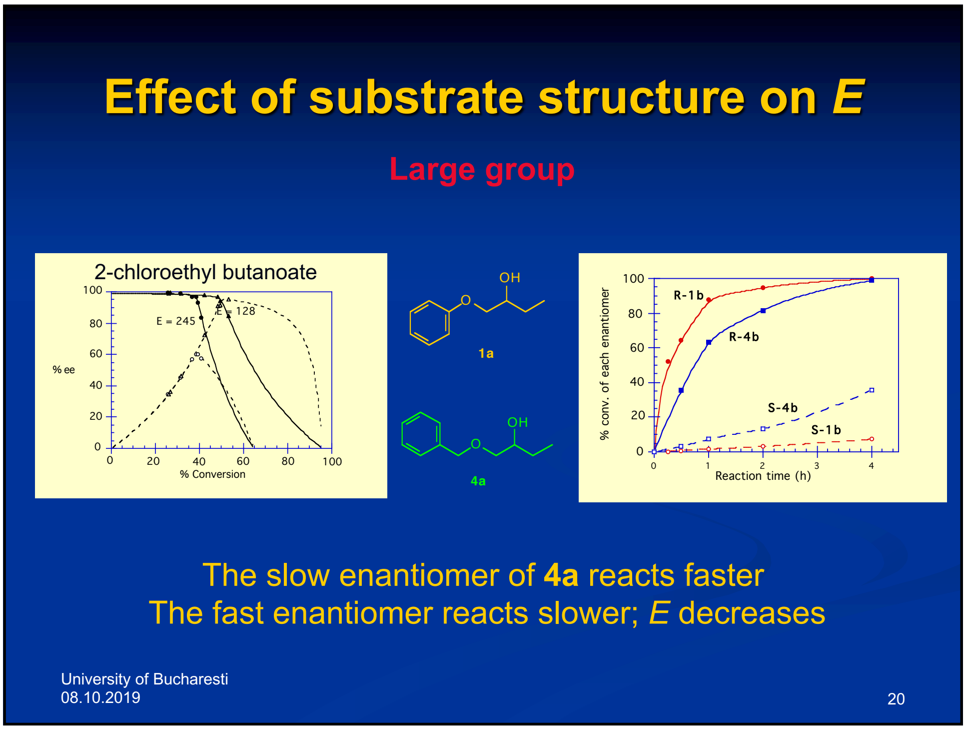
Small group

1a

2a

3a

Number of carbons (size) of small group affect selectivity



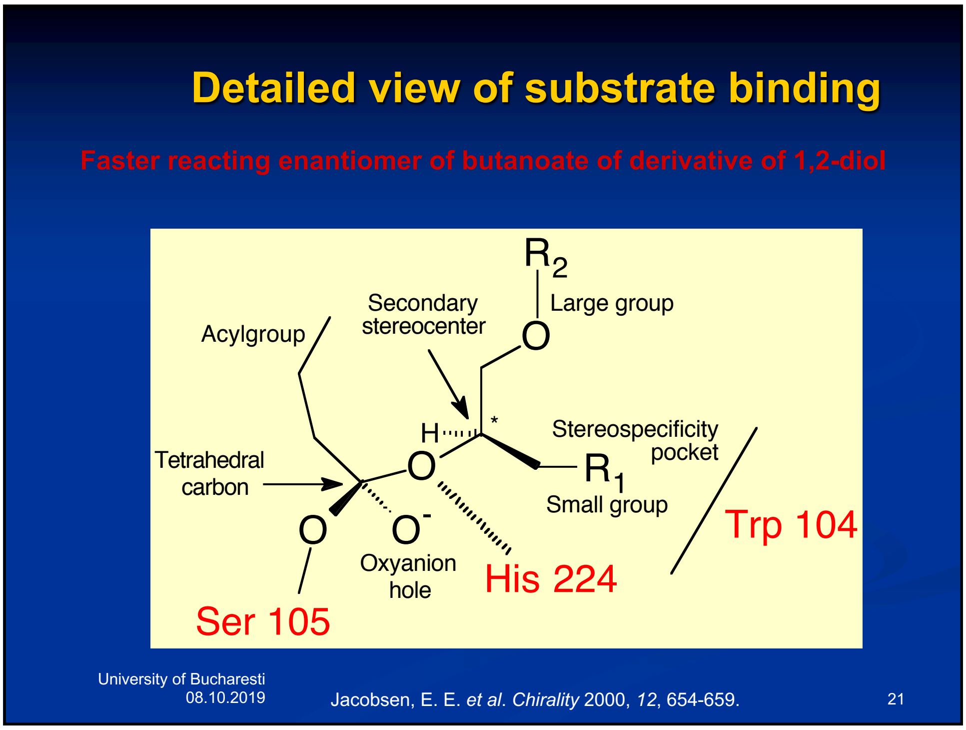
Effect of substrate structure on *E*

Large group

1a

4a

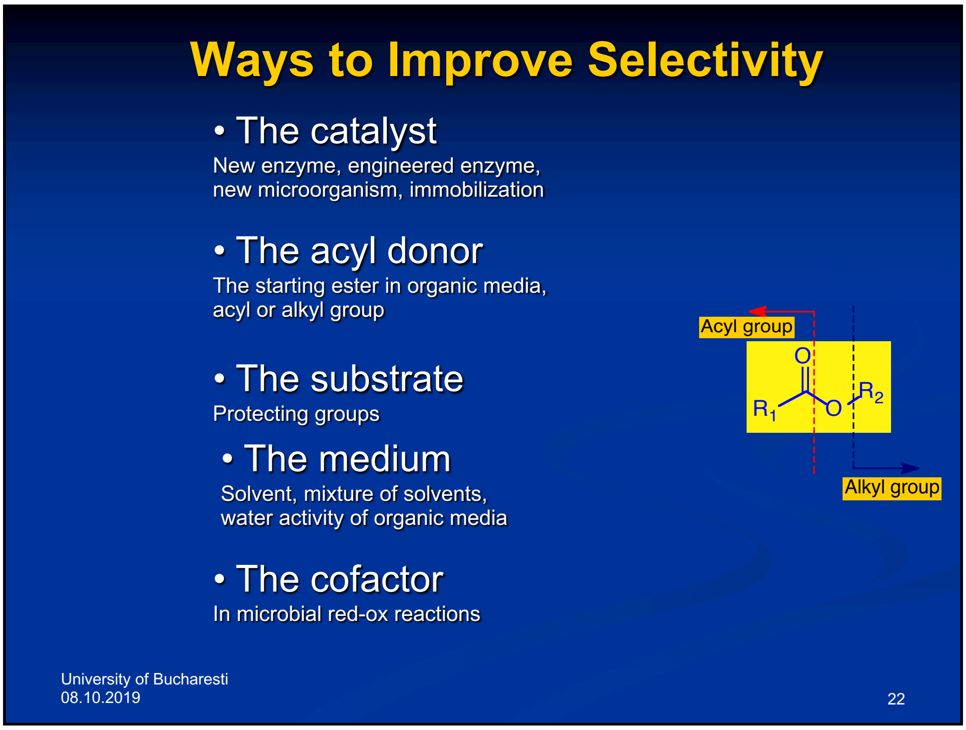
The slow enantiomer of **4a** reacts faster
The fast enantiomer reacts slower; *E* decreases



Detailed view of substrate binding

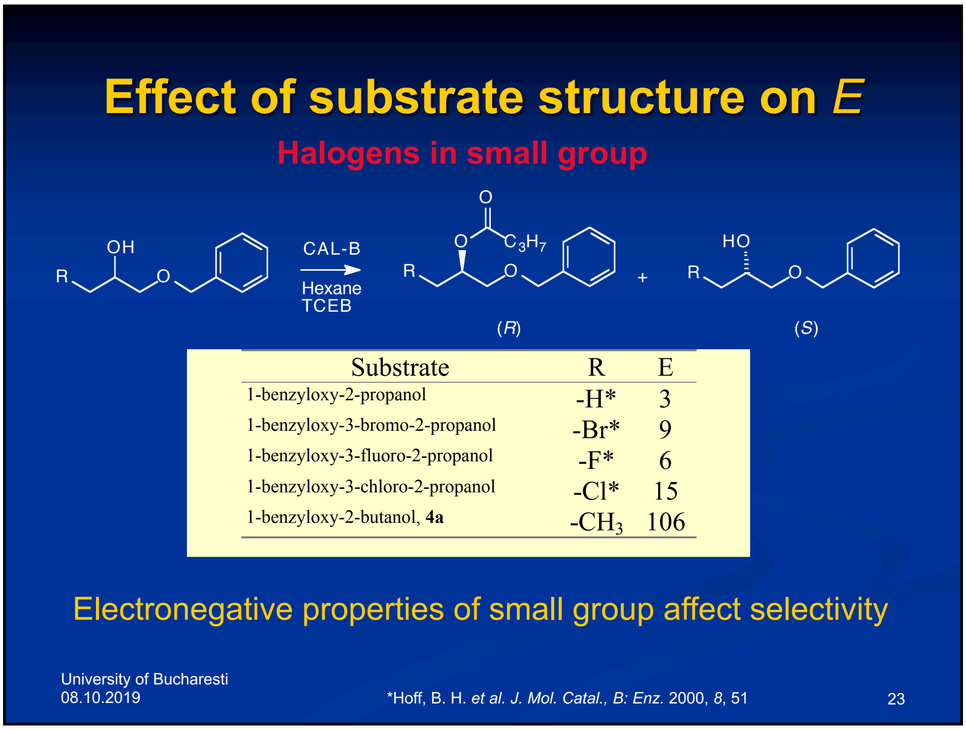
Faster reacting enantiomer of butanoate of derivative of 1,2-diol

Jacobsen, E. E. *et al.* *Chirality* 2000, *12*, 654-659.



Ways to Improve Selectivity

- The catalyst
  New enzyme, engineered enzyme, new microorganism, immobilization
- The acyl donor
  The starting ester in organic media, acyl or alkyl group
- The substrate
  Protecting groups
- The medium
  Solvent, mixture of solvents, water activity of organic media
- The cofactor
  In microbial red-ox reactions



Effect of substrate structure on *E*

Halogens in small group

Substrate	R	E
1-benzyloxy-2-propanol	-H*	3
1-benzyloxy-3-bromo-2-propanol	-Br*	9
1-benzyloxy-3-fluoro-2-propanol	-F*	6
1-benzyloxy-3-chloro-2-propanol	-Cl*	15
1-benzyloxy-2-butanol, **4a**	$-CH_3$	106

Electronegative properties of small group affect selectivity

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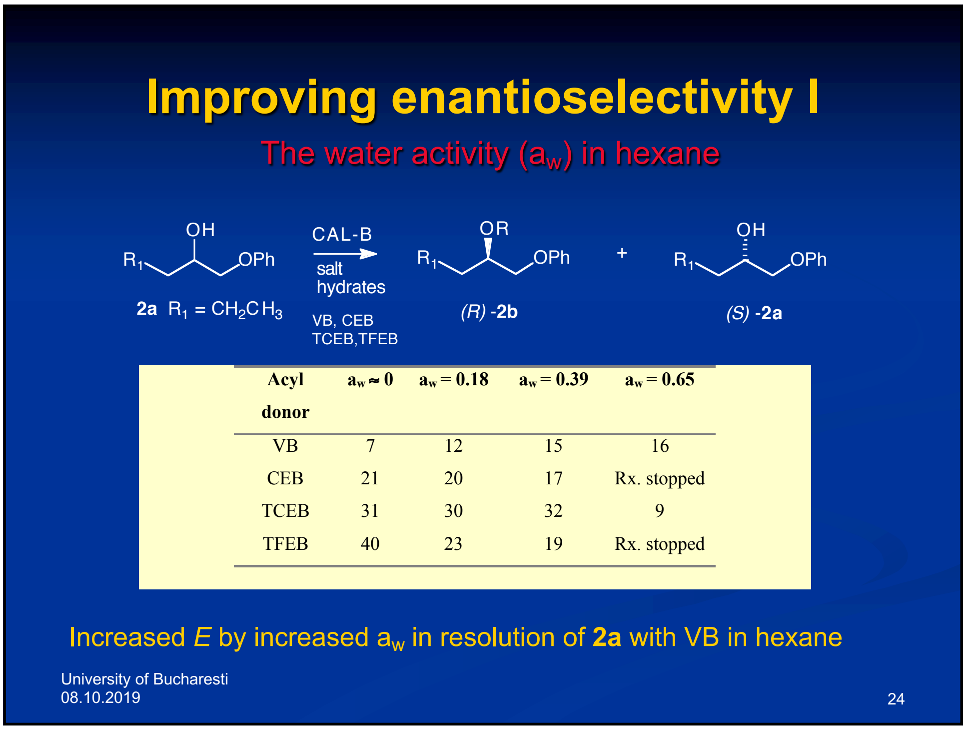
*Hoff, B. H. *et al. J. Mol. Catal., B: Enz.* 2000, *8*, 51

23



Improving enantioselectivity I

The water activity (a_w) in hexane

Acyl donor	**$a_w \approx 0$**	**$a_w = 0.18$**	**$a_w = 0.39$**	**$a_w = 0.65$**
VB	7	12	15	16
CEB	21	20	17	Rx. stopped
TCEB	31	30	32	9
TFEB	40	23	19	Rx. stopped

Increased *E* by increased a_w in resolution of **2a** with VB in hexane

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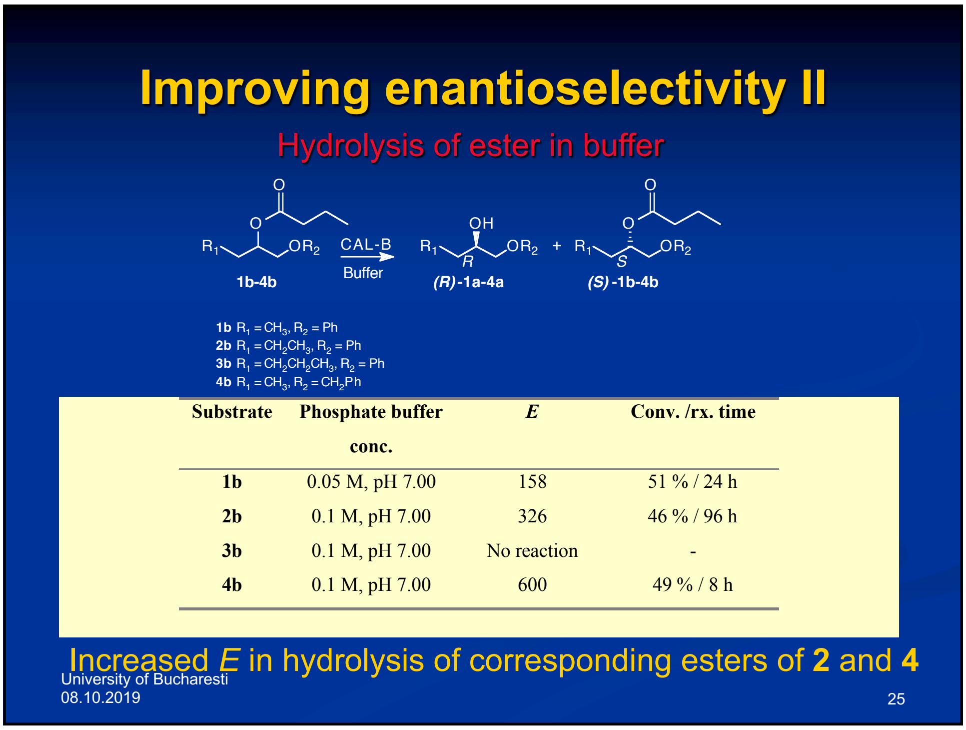

Improving enantioselectivity II

Hydrolysis of ester in buffer

1b $R_1 = CH_3$, $R_2 = Ph$
2b $R_1 = CH_2CH_3$, $R_2 = Ph$
3b $R_1 = CH_2CH_2CH_3$, $R_2 = Ph$
4b $R_1 = CH_3$, $R_2 = CH_2Ph$

Substrate	Phosphate buffer conc.	*E*	Conv. /rx. time
1b	0.05 M, pH 7.00	158	51 % / 24 h
2b	0.1 M, pH 7.00	326	46 % / 96 h
3b	0.1 M, pH 7.00	No reaction	-
4b	0.1 M, pH 7.00	600	49 % / 8 h

Increased *E* in hydrolysis of corresponding esters of **2** and **4**

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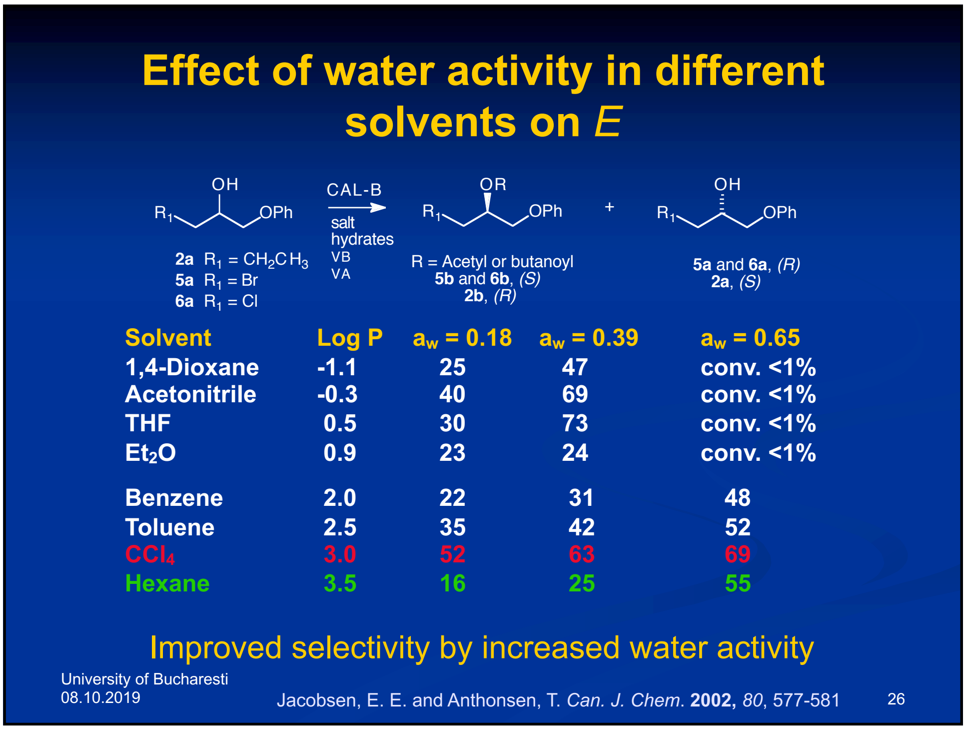

Effect of water activity in different solvents on *E*

2a $R_1 = CH_2CH_3$
5a $R_1 = Br$
6a $R_1 = Cl$

R = Acetyl or butanoyl
5b and **6b**, *(S)*
2b, *(R)*

5a and **6a**, *(R)*
2a, *(S)*

Solvent	Log P	$a_w = 0.18$	$a_w = 0.39$	$a_w = 0.65$
1,4-Dioxane	-1.1	25	47	conv. <1%
Acetonitrile	-0.3	40	69	conv. <1%
THF	0.5	30	73	conv. <1%
Et_2O	0.9	23	24	conv. <1%
Benzene	2.0	22	31	48
Toluene	2.5	35	42	52
CCl_4	3.0	52	63	69
Hexane	3.5	16	25	55

Improved selectivity by increased water activity

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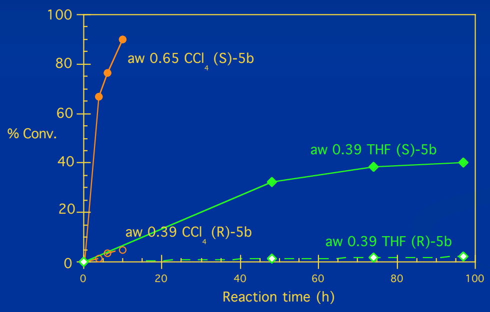
Jacobsen, E. E. and Anthonsen, T. *Can. J. Chem.* **2002**, *80*, 577-581

26

Effect of a_w in different solvents on reaction rate

Esterification of **5a** in CCl_4 and THF



No conversion of **5a** in THF when a_w = 0.65

5a in THF a_w 0.39: 40 % conv. in 100 h *E* = 73

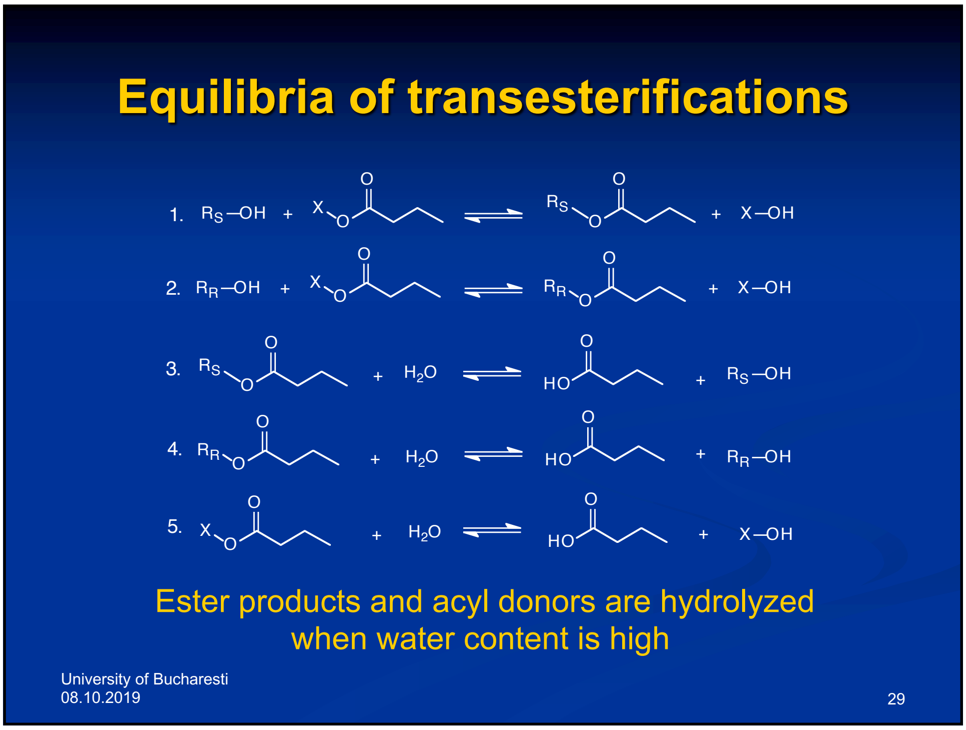
5a in CCl_4 a_w 0.65: 90 % conv. in 5 h *E* = 69

Water activity *vs.* water content

Water content (ppm, μg water per g solvent) Karl Fisher method

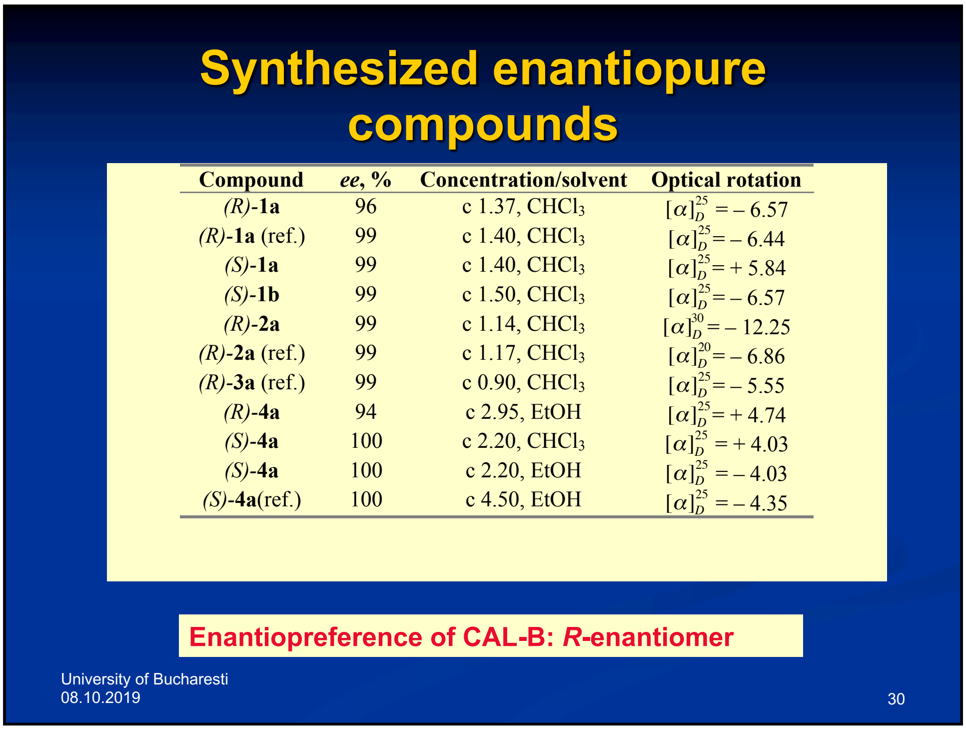
Solvent	**Log P**	**a_w=0.18**	**a_w=0.39**	**a_w=0.65**
1,4-Dioxane	**-1.1**	**360**	**433**	**5820**
Acetonitrile	**-0.3**	**1641**	**1862**	**12391**
THF	**0.5**	**882**	**904**	**13003 (1.3%)**
Et_2O	**0.9**	**471**	**543**	**7980**
Benzene	**2.0**	**134**	**179**	**627**
Toluene	**2.5**	**138**	**142**	**510**
CCl_4	**3.0**	**45**	**55**	**84**
Hexane	**3.5**	**27**	**28**	**74**

Increase of water activity results in higher increase of water content in polar solvents than in apolar solvent



Equilibria of transesterifications

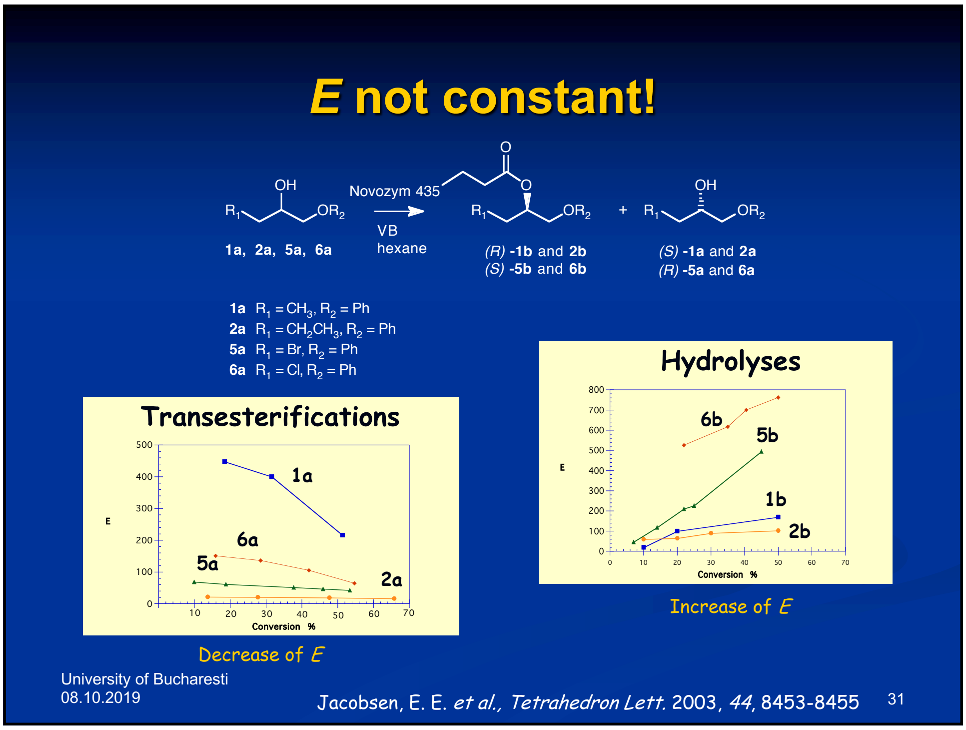
Ester products and acyl donors are hydrolyzed when water content is high



Synthesized enantiopure compounds

Compound	*ee*, %	Concentration/solvent	Optical rotation
(R)-**1a**	96	c 1.37, $CHCl_3$	$[\alpha]_D^{25} = -6.57$
(R)-**1a** (ref.)	99	c 1.40, $CHCl_3$	$[\alpha]_D^{25} = -6.44$
(S)-**1a**	99	c 1.40, $CHCl_3$	$[\alpha]_D^{25} = +5.84$
(S)-**1b**	99	c 1.50, $CHCl_3$	$[\alpha]_D^{25} = -6.57$
(R)-**2a**	99	c 1.14, $CHCl_3$	$[\alpha]_D^{30} = -12.25$
(R)-**2a** (ref.)	99	c 1.17, $CHCl_3$	$[\alpha]_D^{20} = -6.86$
(R)-**3a** (ref.)	99	c 0.90, $CHCl_3$	$[\alpha]_D^{25} = -5.55$
(R)-**4a**	94	c 2.95, EtOH	$[\alpha]_D^{25} = +4.74$
(S)-**4a**	100	c 2.20, $CHCl_3$	$[\alpha]_D^{25} = +4.03$
(S)-**4a**	100	c 2.20, EtOH	$[\alpha]_D^{25} = -4.03$
(S)-**4a**(ref.)	100	c 4.50, EtOH	$[\alpha]_D^{25} = -4.35$

Enantiopreference of CAL-B: *R*-enantiomer



E not constant!

1a, 2a, 5a, 6a

(R) -**1b** and **2b**
(S) -**5b** and **6b**

(S) -**1a** and **2a**
(R) -**5a** and **6a**

1a $R_1 = CH_3$, $R_2 = Ph$
2a $R_1 = CH_2CH_3$, $R_2 = Ph$
5a $R_1 = Br$, $R_2 = Ph$
6a $R_1 = Cl$, $R_2 = Ph$

Transesterifications

Decrease of *E*

Hydrolyses

Increase of *E*

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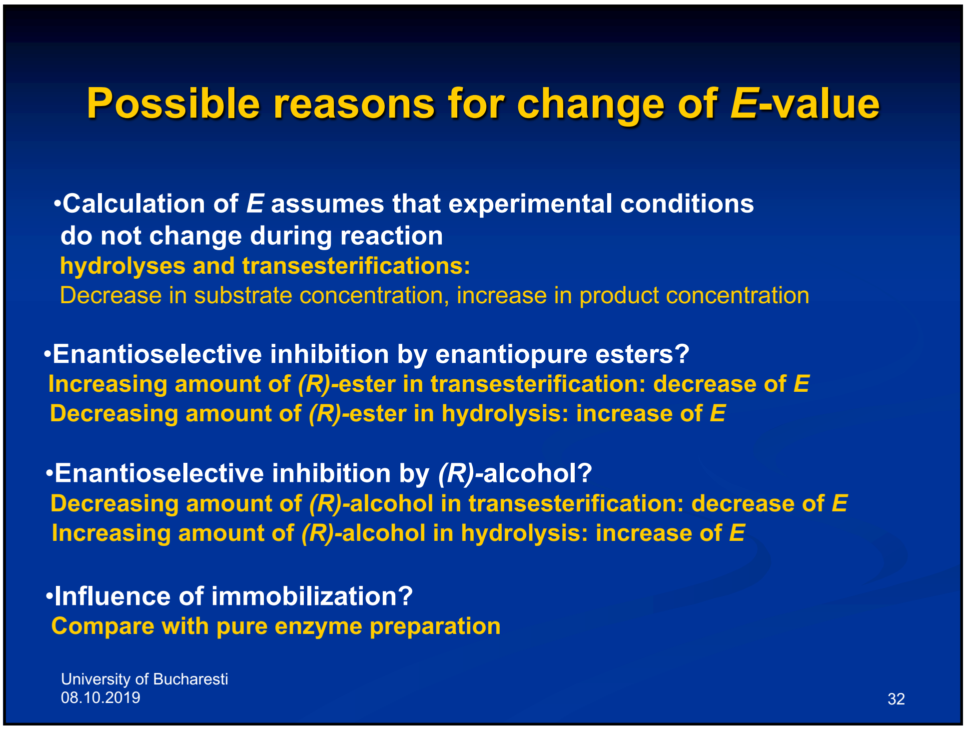
Jacobsen, E. E. *et al., Tetrahedron Lett.* 2003, *44*, 8453-8455

31



Possible reasons for change of *E*-value

- **Calculation of *E* assumes that experimental conditions do not change during reaction**
  hydrolyses and transesterifications:
  Decrease in substrate concentration, increase in product concentration

- **Enantioselective inhibition by enantiopure esters?**
  Increasing amount of *(R)*-ester in transesterification: decrease of *E*
  Decreasing amount of *(R)*-ester in hydrolysis: increase of *E*

- **Enantioselective inhibition by *(R)*-alcohol?**
  Decreasing amount of *(R)*-alcohol in transesterification: decrease of *E*
  Increasing amount of *(R)*-alcohol in hydrolysis: increase of *E*

- **Influence of immobilization?**
  Compare with pure enzyme preparation

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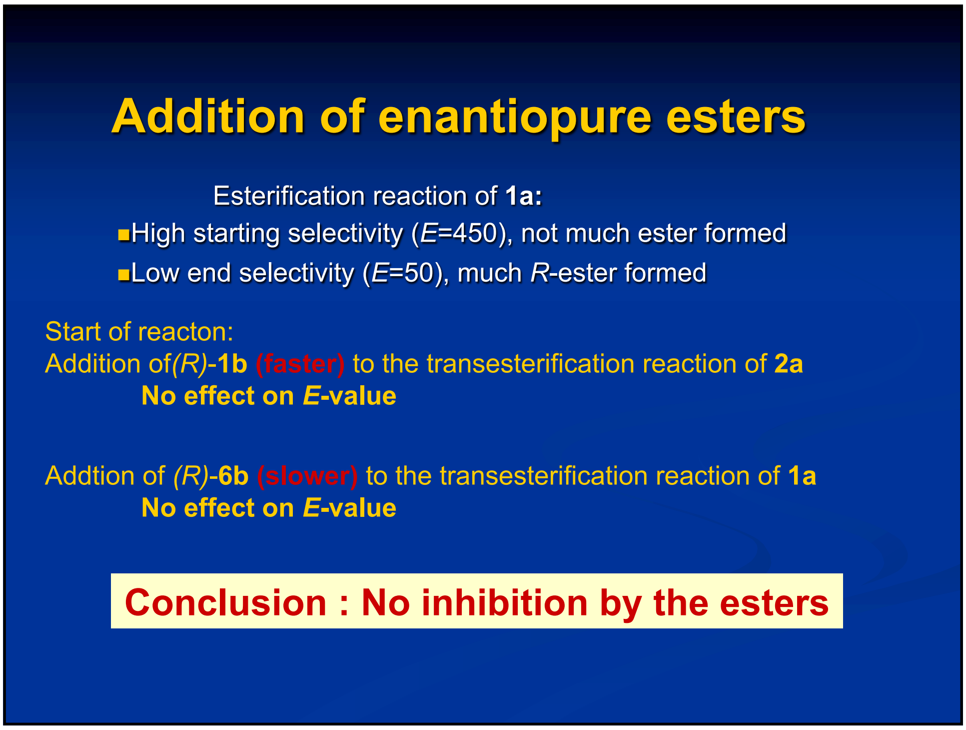
32



Addition of enantiopure esters

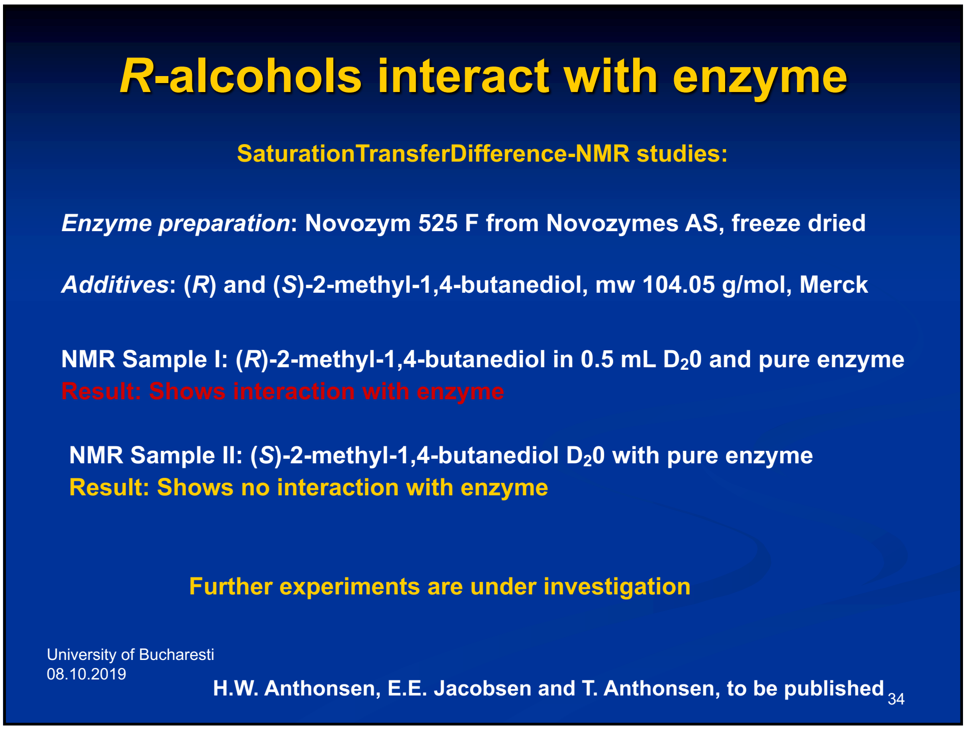
Esterification reaction of **1a:**

- High starting selectivity (*E*=450), not much ester formed
- Low end selectivity (*E*=50), much *R*-ester formed

Start of reacton:
Addition of*(R)*-**1b** (faster) to the transesterification reaction of **2a**
No effect on *E*-value

Addtion of *(R)*-**6b** (slower) to the transesterification reaction of **1a**
No effect on *E*-value

Conclusion : No inhibition by the esters



R-alcohols interact with enzyme

SaturationTransferDifference-NMR studies:

***Enzyme preparation*: Novozym 525 F from Novozymes AS, freeze dried**

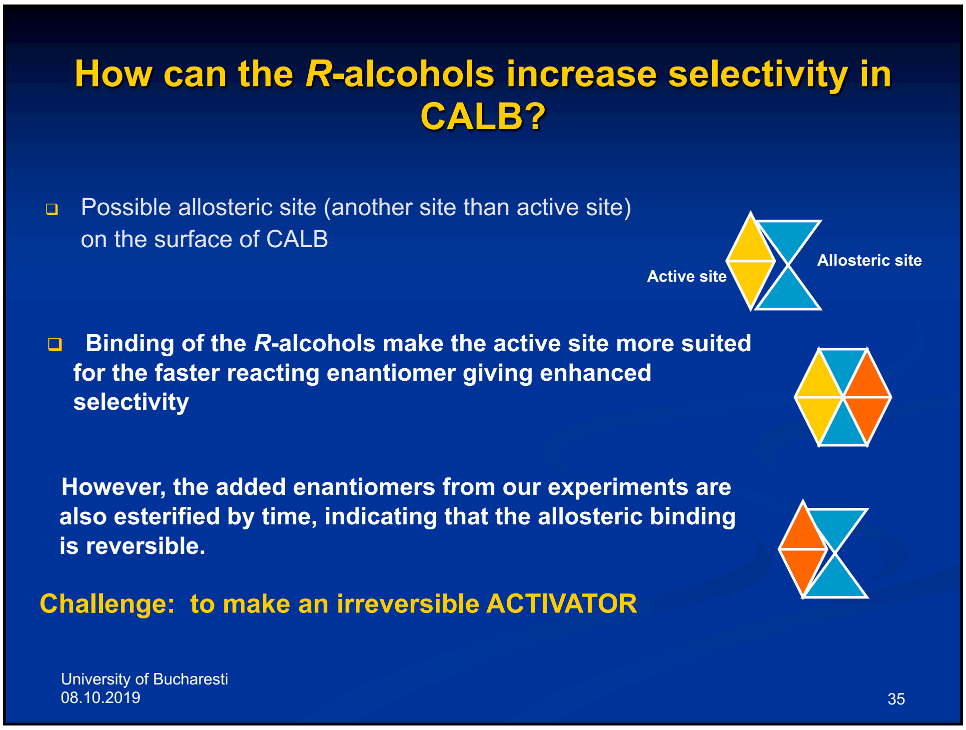
***Additives*: (*R*) and (*S*)-2-methyl-1,4-butanediol, mw 104.05 g/mol, Merck**

NMR Sample I: (*R*)-2-methyl-1,4-butanediol in 0.5 mL D_20 and pure enzyme
Result: Shows interaction with enzyme

NMR Sample II: (*S*)-2-methyl-1,4-butanediol D_20 with pure enzyme
Result: Shows no interaction with enzyme

Further experiments are under investigation

H.W. Anthonsen, E.E. Jacobsen and T. Anthonsen, to be published



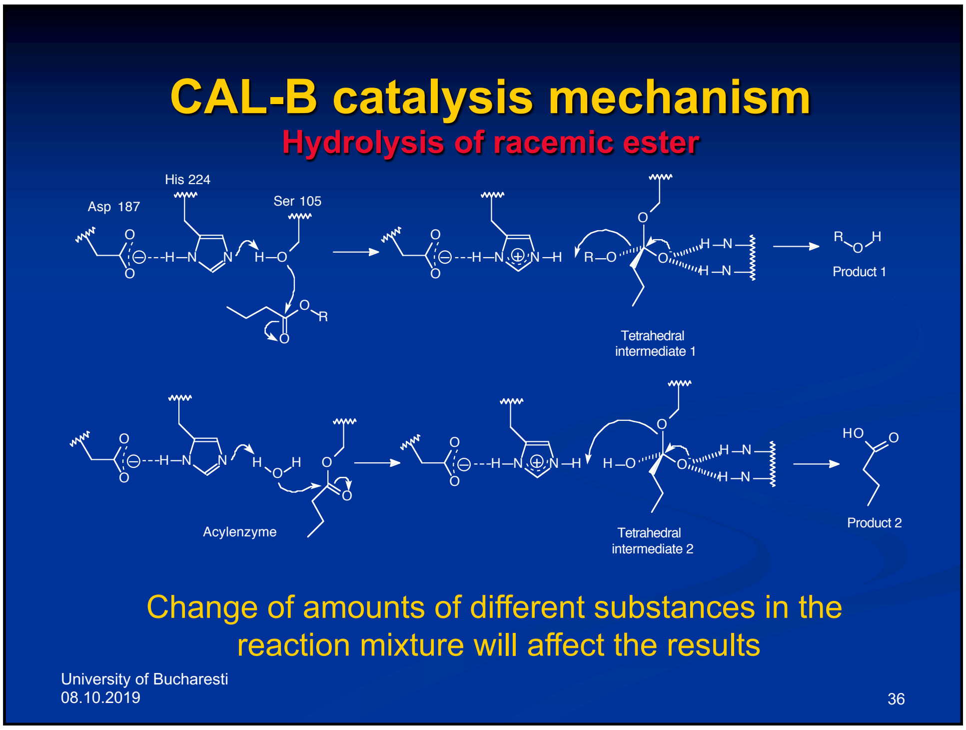
How can the *R*-alcohols increase selectivity in CALB?

- Possible allosteric site (another site than active site) on the surface of CALB

- **Binding of the *R*-alcohols make the active site more suited for the faster reacting enantiomer giving enhanced selectivity**

However, the added enantiomers from our experiments are also esterified by time, indicating that the allosteric binding is reversible.

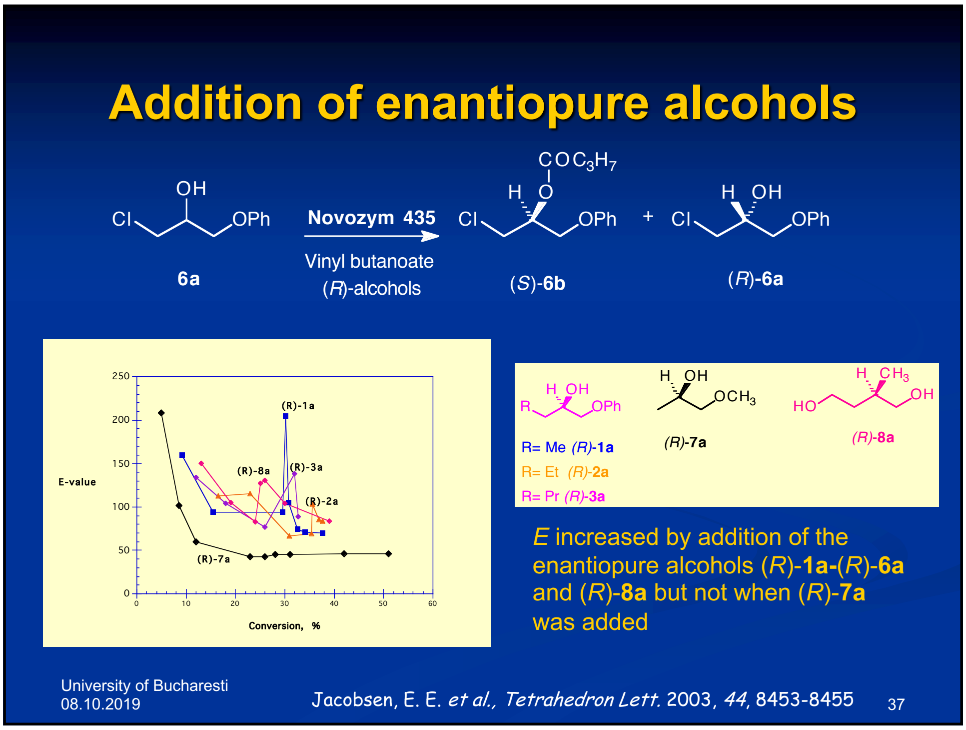
Challenge: to make an irreversible ACTIVATOR



CAL-B catalysis mechanism

Hydrolysis of racemic ester

Change of amounts of different substances in the reaction mixture will affect the results



Addition of enantiopure alcohols

6a → **Novozym 435**, Vinyl butanoate, (*R*)-alcohols → (*S*)-**6b** + (*R*)-**6a**

R= Me (*R*)-**1a**
R= Et (*R*)-**2a**
R= Pr (*R*)-**3a**
(*R*)-**7a**
(*R*)-**8a**

E increased by addition of the enantiopure alcohols (*R*)-**1a**-(*R*)-**6a** and (*R*)-**8a** but not when (*R*)-**7a** was added

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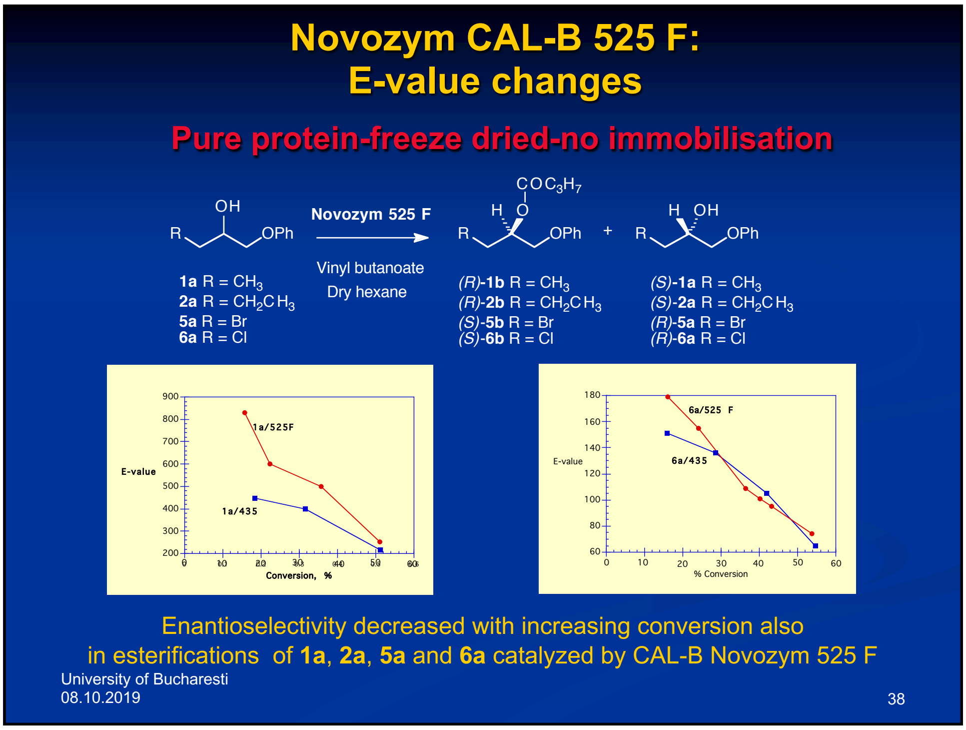
Jacobsen, E. E. *et al., Tetrahedron Lett.* 2003, *44*, 8453-8455

37



Novozym CAL-B 525 F: E-value changes

Pure protein-freeze dried-no immobilisation

Novozym 525 F
Vinyl butanoate
Dry hexane

1a R = CH_3
2a R = CH_2CH_3
5a R = Br
6a R = Cl

(*R*)-**1b** R = CH_3
(*R*)-**2b** R = CH_2CH_3
(*S*)-**5b** R = Br
(*S*)-**6b** R = Cl

(*S*)-**1a** R = CH_3
(*S*)-**2a** R = CH_2CH_3
(*R*)-**5a** R = Br
(*R*)-**6a** R = Cl

Enantioselectivity decreased with increasing conversion also in esterifications of **1a**, **2a**, **5a** and **6a** catalyzed by CAL-B Novozym 525 F

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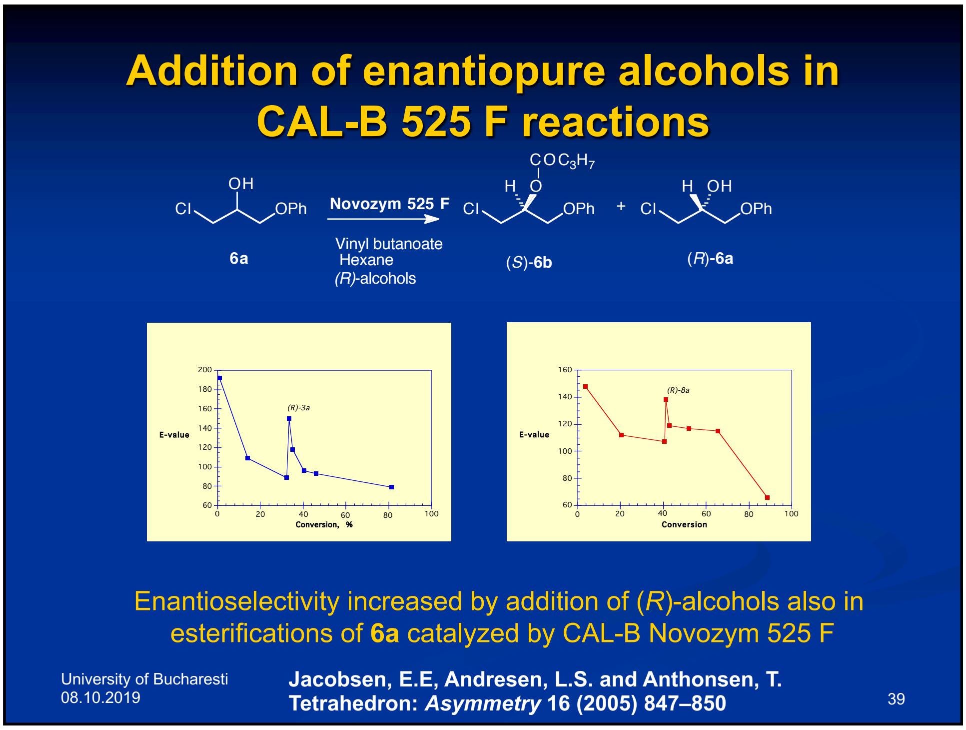
38



Addition of enantiopure alcohols in CAL-B 525 F reactions

Enantioselectivity increased by addition of (*R*)-alcohols also in esterifications of **6a** catalyzed by CAL-B Novozym 525 F

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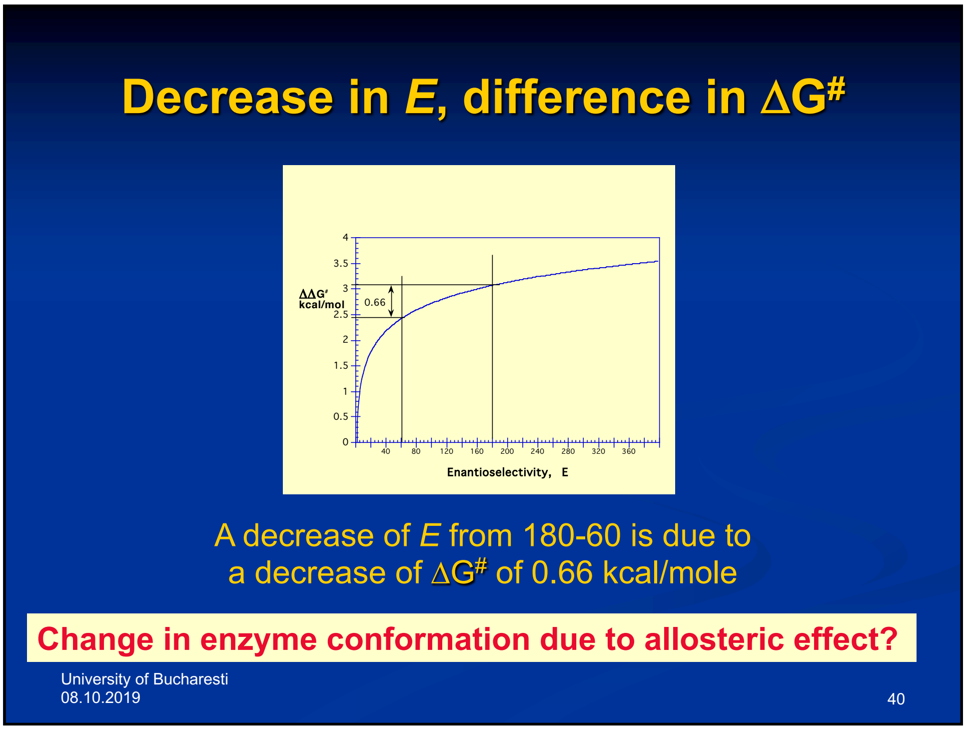
Jacobsen, E.E, Andresen, L.S. and Anthonsen, T.
Tetrahedron: *Asymmetry* 16 (2005) 847–850

39



Decrease in *E*, difference in $\Delta G^{\#}$

A decrease of *E* from 180-60 is due to a decrease of $\Delta G^{\#}$ of 0.66 kcal/mole

Change in enzyme conformation due to allosteric effect?

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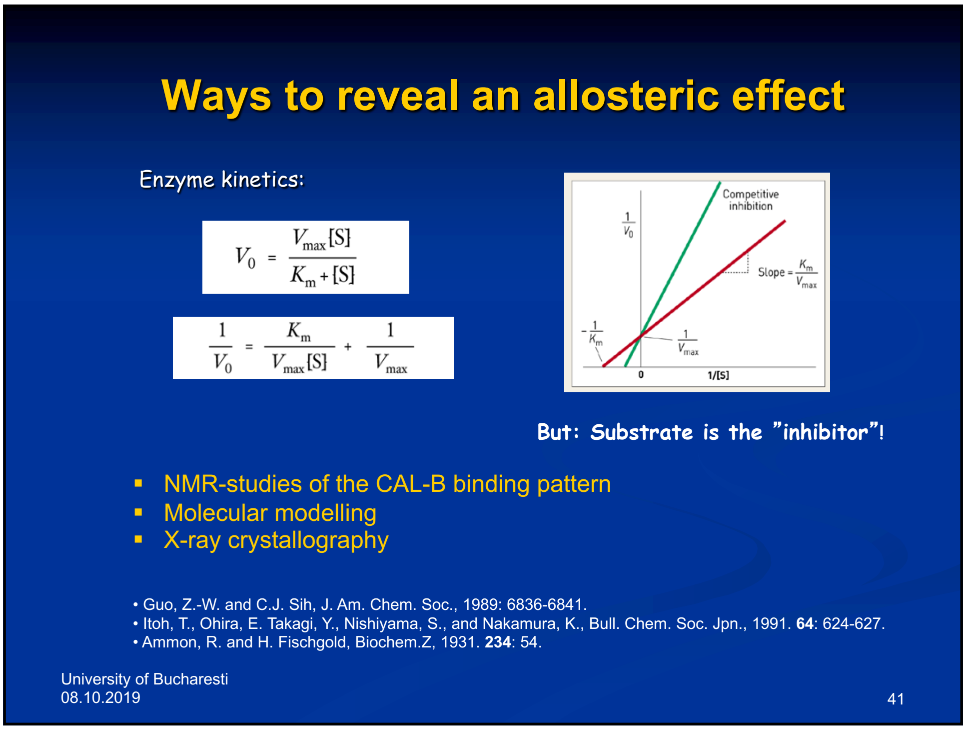
40



Ways to reveal an allosteric effect

Enzyme kinetics:

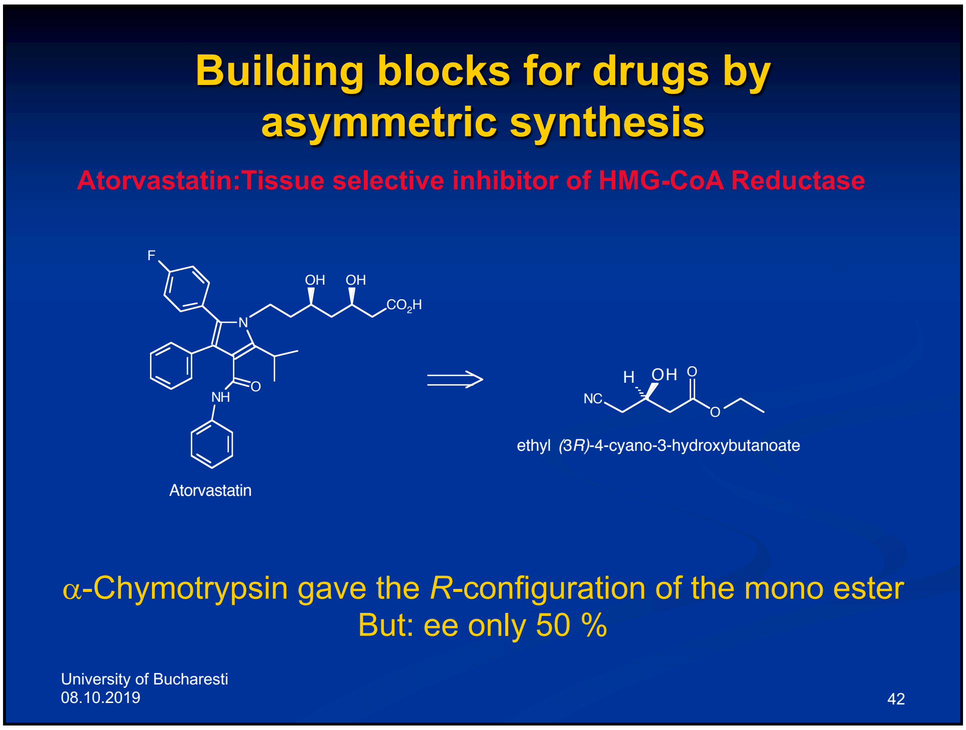
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

But: Substrate is the "inhibitor"!

- NMR-studies of the CAL-B binding pattern
- Molecular modelling
- X-ray crystallography

- Guo, Z.-W. and C.J. Sih, J. Am. Chem. Soc., 1989: 6836-6841.
- Itoh, T., Ohira, E. Takagi, Y., Nishiyama, S., and Nakamura, K., Bull. Chem. Soc. Jpn., 1991. **64**: 624-627.
- Ammon, R. and H. Fischgold, Biochem.Z, 1931. **234**: 54.



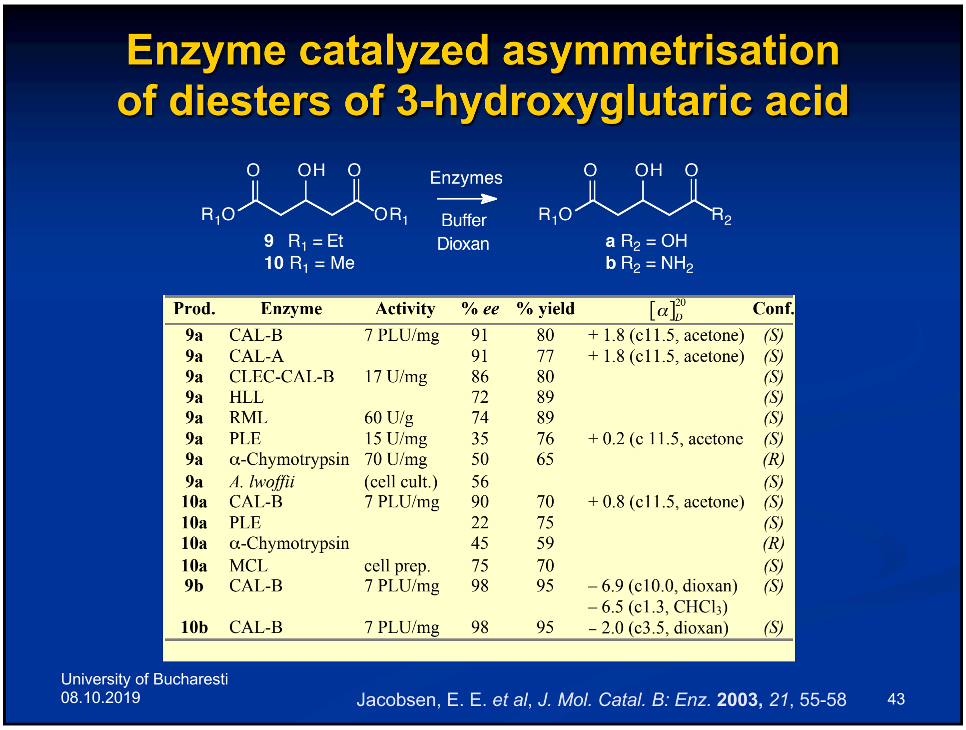
Building blocks for drugs by asymmetric synthesis

Atorvastatin:Tissue selective inhibitor of HMG-CoA Reductase

Atorvastatin

ethyl (3*R*)-4-cyano-3-hydroxybutanoate

α-Chymotrypsin gave the *R*-configuration of the mono ester
But: ee only 50 %



Enzyme catalyzed asymmetrisation of diesters of 3-hydroxyglutaric acid

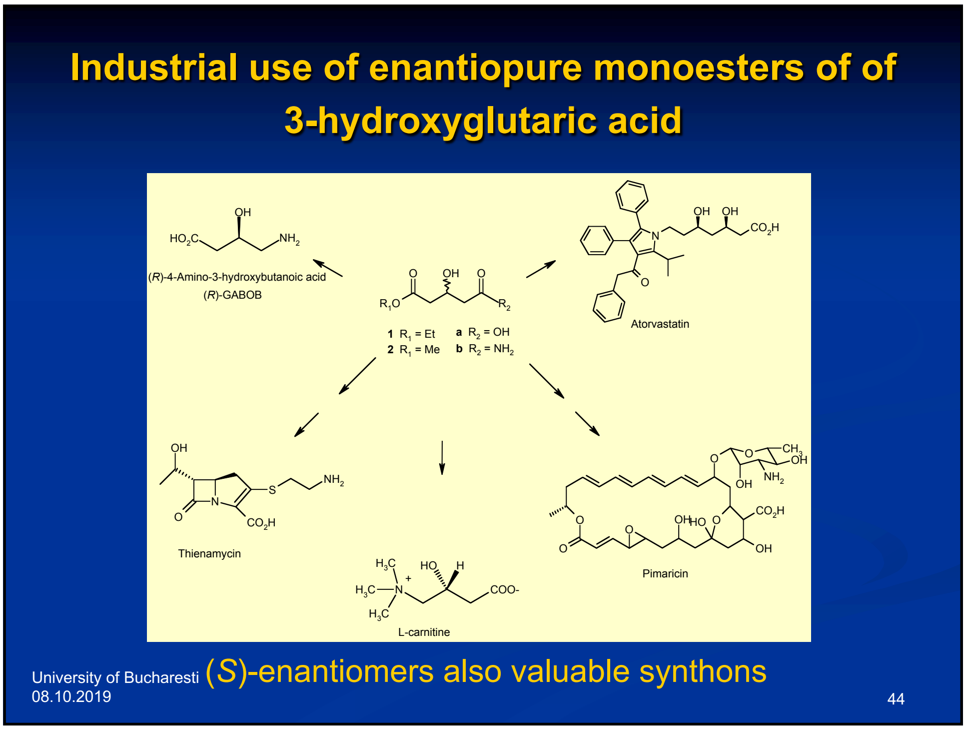
9 R_1 = Et
10 R_1 = Me

Enzymes
Buffer
Dioxan

a R_2 = OH
b $R_2 = NH_2$

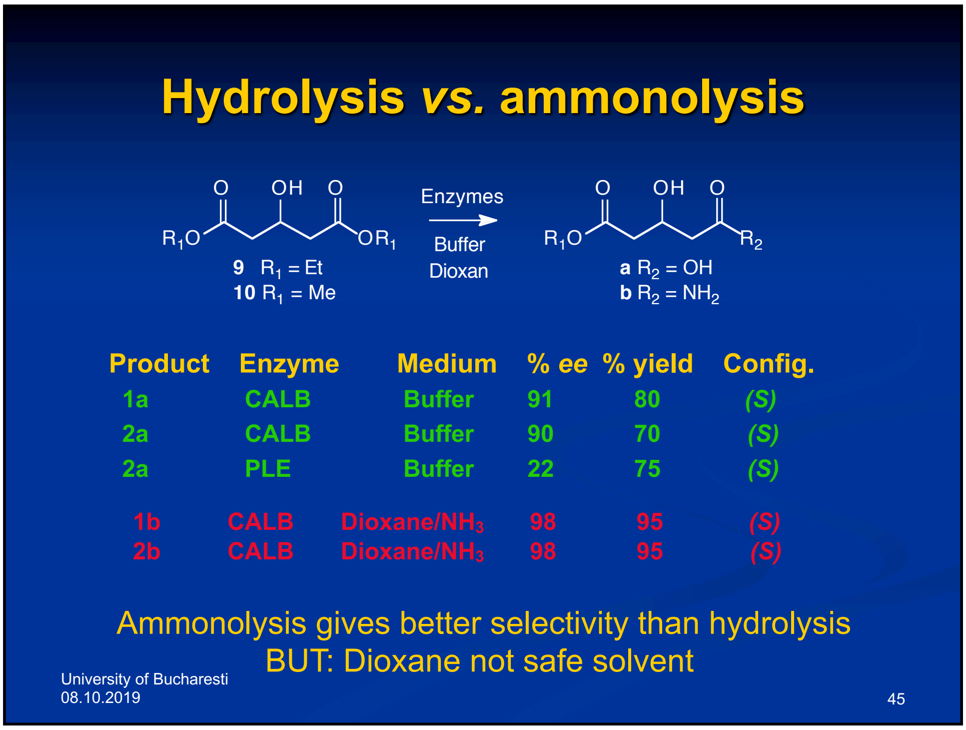
Prod.	Enzyme	Activity	% *ee*	% yield	$[\alpha]_D^{20}$	Conf.
9a	CAL-B	7 PLU/mg	91	80	+ 1.8 (c11.5, acetone)	*(S)*
9a	CAL-A		91	77	+ 1.8 (c11.5, acetone)	*(S)*
9a	CLEC-CAL-B	17 U/mg	86	80		*(S)*
9a	HLL		72	89		*(S)*
9a	RML	60 U/g	74	89		*(S)*
9a	PLE	15 U/mg	35	76	+ 0.2 (c 11.5, acetone	*(S)*
9a	α-Chymotrypsin	70 U/mg	50	65		*(R)*
9a	*A. lwoffii*	(cell cult.)	56			*(S)*
10a	CAL-B	7 PLU/mg	90	70	+ 0.8 (c11.5, acetone)	*(S)*
10a	PLE		22	75		*(S)*
10a	α-Chymotrypsin		45	59		*(R)*
10a	MCL	cell prep.	75	70		*(S)*
9b	CAL-B	7 PLU/mg	98	95	– 6.9 (c10.0, dioxan) – 6.5 (c1.3, $CHCl_3$)	*(S)*
10b	CAL-B		7 PLU/mg	98	95	– 2.0 (c3.5, dioxan)	*(S)*

Jacobsen, E. E. *et al*, *J. Mol. Catal. B: Enz.* **2003,** *21*, 55-58



Industrial use of enantiopure monoesters of of 3-hydroxyglutaric acid

(*S*)-enantiomers also valuable synthons



Hydrolysis *vs.* ammonolysis

Enzymes

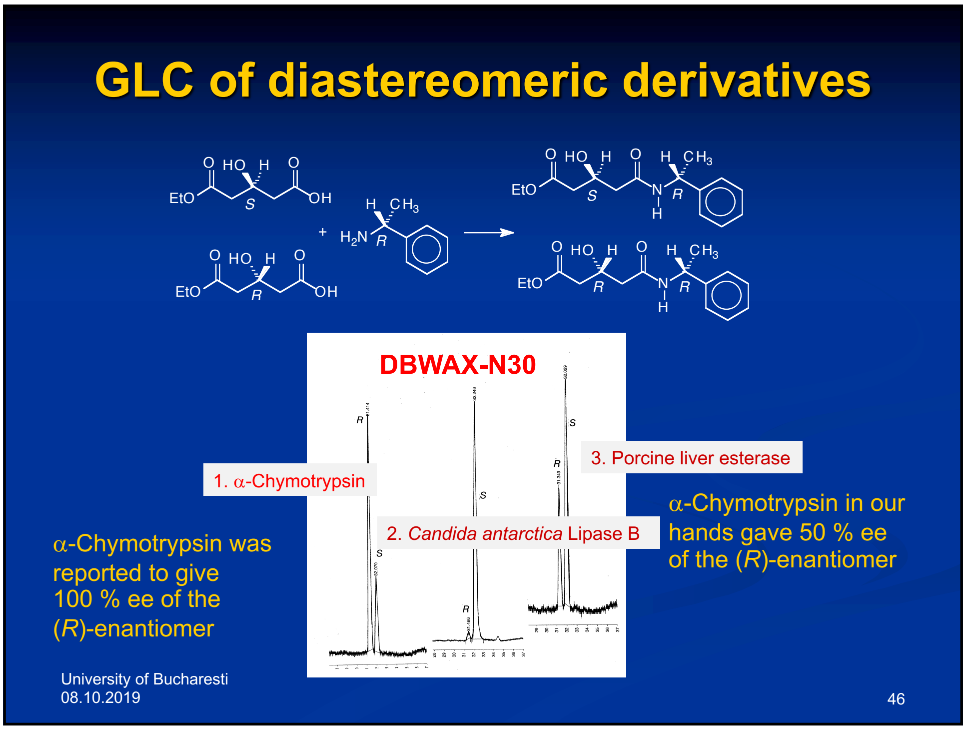
Buffer
Dioxan

9 R_1 = Et
10 R_1 = Me

a R_2 = OH
b R_2 = NH_2

Product	Enzyme	Medium	% *ee*	% yield	Config.
1a	CALB	Buffer	91	80	*(S)*
2a	CALB	Buffer	90	70	*(S)*
2a	PLE	Buffer	22	75	*(S)*
1b	CALB	Dioxane/NH_3	98	95	*(S)*
2b	CALB	Dioxane/NH_3	98	95	*(S)*

Ammonolysis gives better selectivity than hydrolysis
BUT: Dioxane not safe solvent



GLC of diastereomeric derivatives

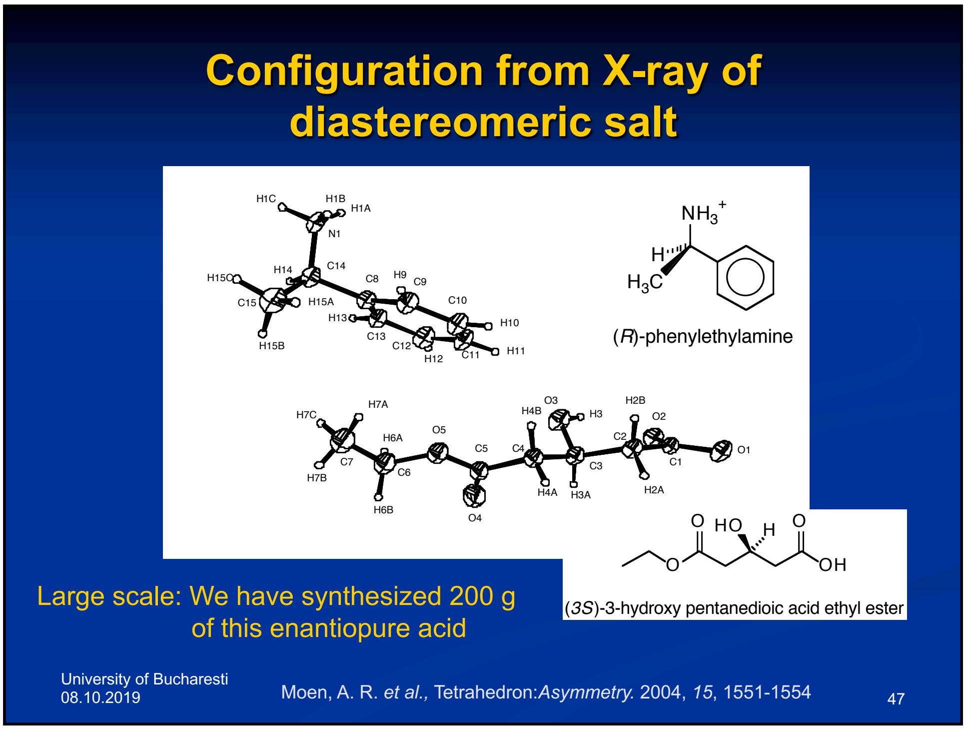
1. α-Chymotrypsin

2. *Candida antarctica* Lipase B

3. Porcine liver esterase

α-Chymotrypsin was reported to give 100 % ee of the (*R*)-enantiomer

α-Chymotrypsin in our hands gave 50 % ee of the (*R*)-enantiomer



Configuration from X-ray of diastereomeric salt

(*R*)-phenylethylamine

(3*S*)-3-hydroxy pentanedioic acid ethyl ester

Large scale: We have synthesized 200 g of this enantiopure acid

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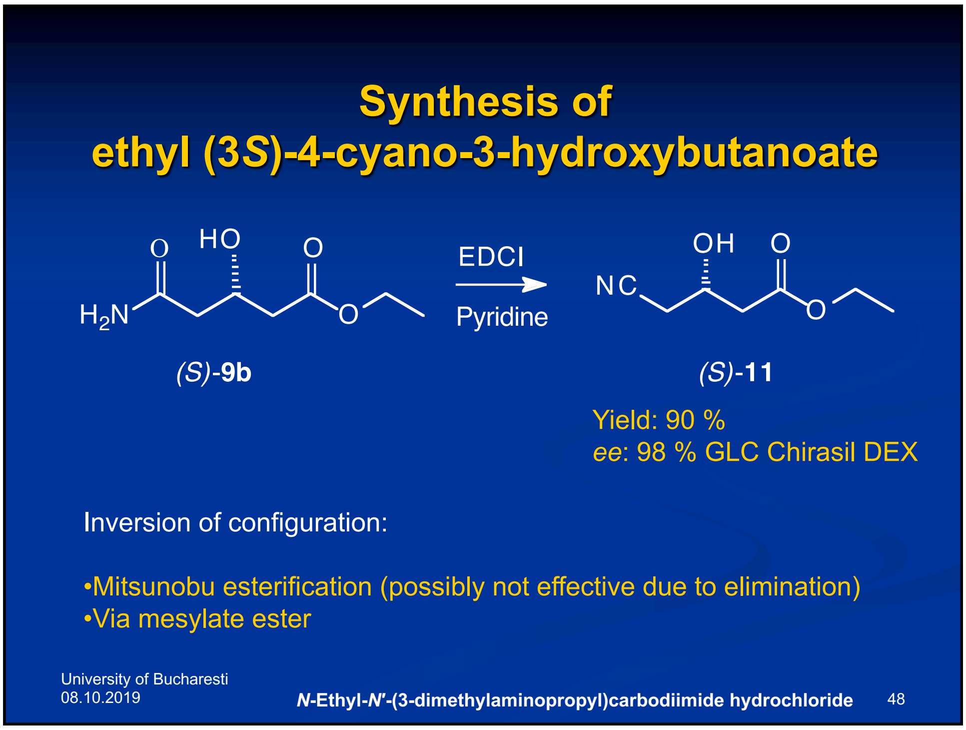
Moen, A. R. *et al.*, Tetrahedron:*Asymmetry.* 2004, *15*, 1551-1554

47



Synthesis of ethyl (3*S*)-4-cyano-3-hydroxybutanoate

(*S*)-**9b**

(*S*)-**11**

Yield: 90 %
ee: 98 % GLC Chirasil DEX

Inversion of configuration:

- Mitsunobu esterification (possibly not effective due to elimination)
- Via mesylate ester

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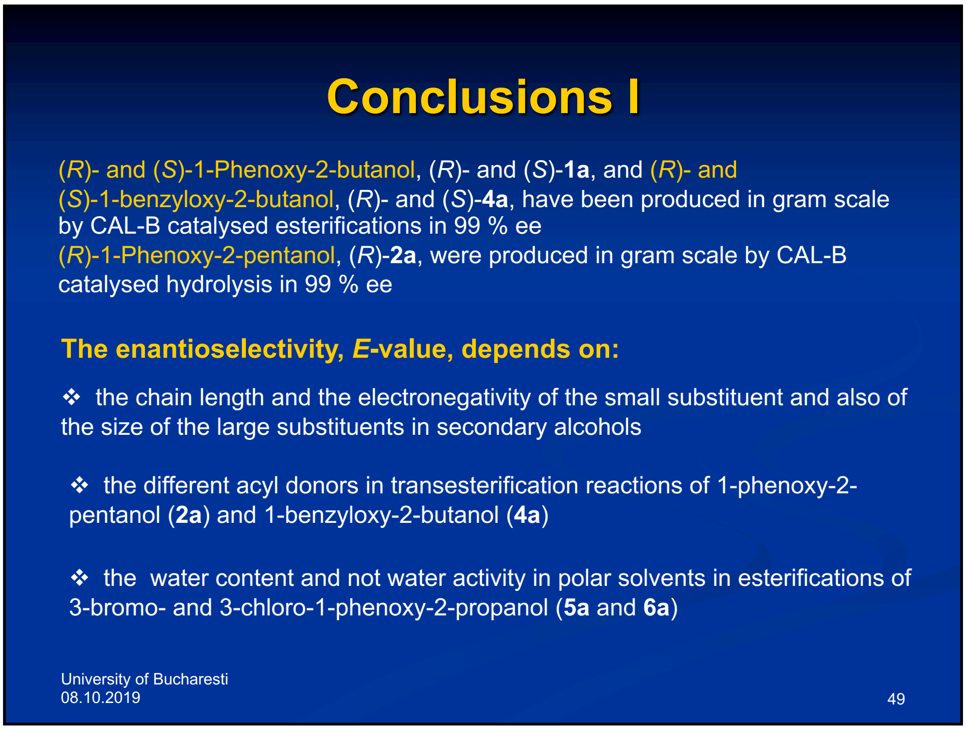
***N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride**

48



Conclusions I

(*R*)- and (*S*)-1-Phenoxy-2-butanol, (*R*)- and (*S*)-**1a**, and (*R*)- and (*S*)-1-benzyloxy-2-butanol, (*R*)- and (*S*)-**4a**, have been produced in gram scale by CAL-B catalysed esterifications in 99 % ee
(*R*)-1-Phenoxy-2-pentanol, (*R*)-**2a**, were produced in gram scale by CAL-B catalysed hydrolysis in 99 % ee

The enantioselectivity, *E*-value, depends on:

❖ the chain length and the electronegativity of the small substituent and also of the size of the large substituents in secondary alcohols

❖ the different acyl donors in transesterification reactions of 1-phenoxy-2-pentanol (**2a**) and 1-benzyloxy-2-butanol (**4a**)

❖ the water content and not water activity in polar solvents in esterifications of 3-bromo- and 3-chloro-1-phenoxy-2-propanol (**5a** and **6a**)

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49

Chemo-enzymatic synthesis of enantiopure stiripentol and

β-antagonists atenolol, metoprolol, practolol and pindolol and β-agonist clenbuterol precursor

Elisabeth Jacobsen and Fredrik Blindheim
Department of Chemistry
Norwegian University of Science and Technology
Trondheim, Norway

Iceland
Liechtenstein
Norway grants

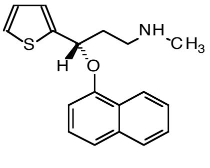
The Research Council of Norway

Enantiopure drugs in Trondheim

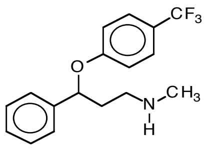
both enantiomers synthesised by lipase catalysed kinetic resolution (99 % ee)

Non-tricylic antidepressants
Inhibits serotonin re-uptake

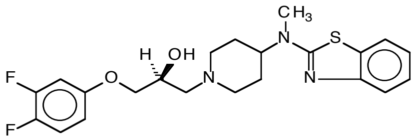
Treatment of ischemic stroke



(*S*)-(+)-Duloxetine
ER=2
Cymbalta by Lilly



Fluoxetine
Prozac, Fontex
Racemate by Lilly

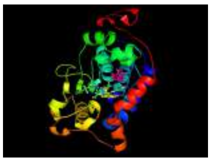


(2*S*)-Lubeluzole
Prosynap by Johnsen & Johnsen

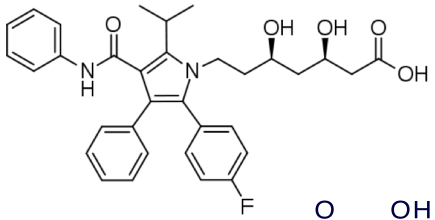
Liu et al. *J. Chem. Soc. Perkin Trans.* 1, **2000**, 1767 and *Chirality,* 13, **2001**, 135

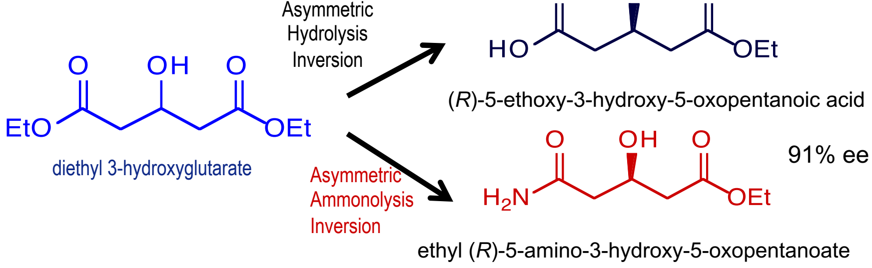
Chiral side chain of Atorvastatin

by asymmetrisation of prochiral diethyl 3-hydroxyglutarate



Lipase B *from Candida antarctica*





diethyl 3-hydroxyglutarate

Asymmetric Hydrolysis Inversion

(*R*)-5-ethoxy-3-hydroxy-5-oxopentanoic acid

Asymmetric Ammonolysis Inversion

91% ee

ethyl (*R*)-5-amino-3-hydroxy-5-oxopentanoate

Jacobsen *et al. J. Mol. Catal. B, Enzymatic*, 21, **2003**, 55-58, Riise Moen *et al. Tetrahedron: Asymmetry.* **2004**, 15, 1551-1554

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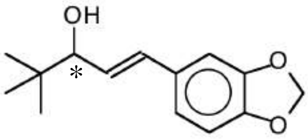
rac-Stiripentol –

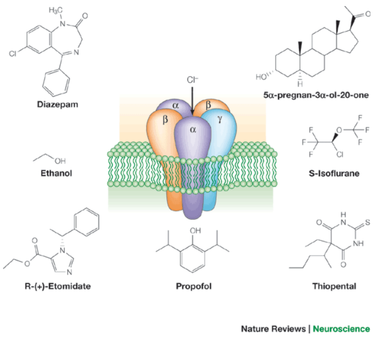
allylic aromatic alcohol

1 stereocenter



Diacomit™ by Bioodex

- unlike other antiepileptic drugs
- potent for children´s epilepsy; infants with Dravet syndrome
- under investigation since 1970´s, used in France and Canada since 1995, FDA appr. 2008
- used together with valproate and clobazam (onfi),
- also useful in autism treatment



Nature Reviews | Neuroscience

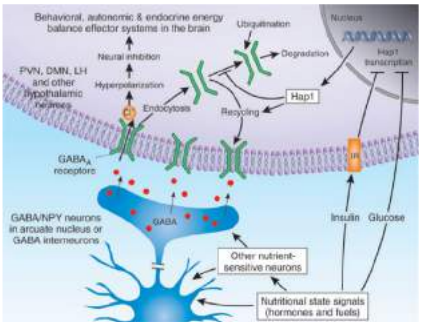
- acts directly on the $GABA_A$ receptor as a positive allosteric modulator at the α3 subunit: increases γ-aminobutyric acid in brain

- also indirect mechanism; inhibits liver microsomal enzymes (CYT P450): increases plasma conc. of other drugs

Fischer, J. *Neuropharmacology* **2009,** *56*, 190-197, Chiron, C. *Expert Opi. Investig. Drugs* **2005,** *14*, 905-911

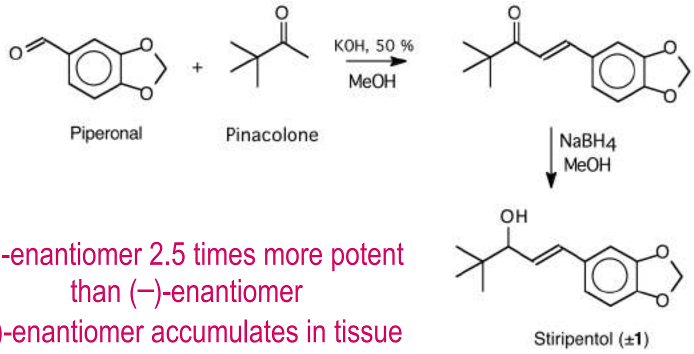
Epilepsy

- central nervous system pathology characterised by seizures, affects 1-2 %
- treated by several drugs, f. inst acetozolamide, clonacepam, carbamacepine, pregabalin, clobazam, valproate, stiripentol
- several act on gamma aminobutyric acid receptor (GABA)
- epilepsy patiens may have mutations in $GABA_A$



Stiripentol synthesis

(±)-1-(benzo[d][1,3]dioxol-5-yl)-4,4-dimethylpent-1-en-3-ol ((±)-**1**)

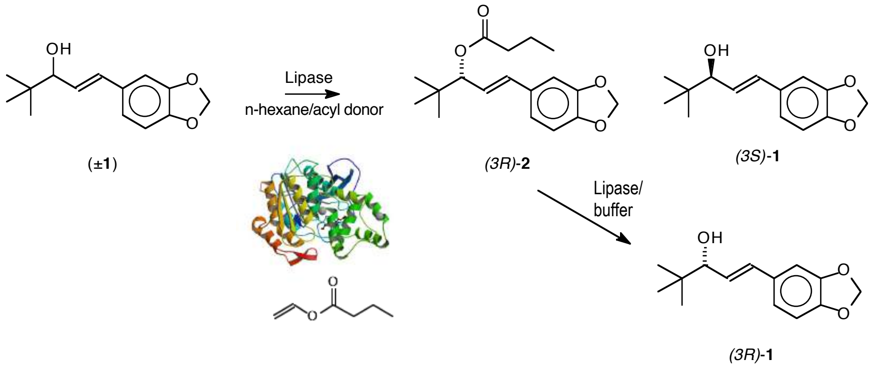


(+)-enantiomer 2.5 times more potent than (−)-enantiomer
(−)-enantiomer accumulates in tissue
However, racemisation observed in rats

Yield: 8.0 g, 79 %

Vallet F. M. J. US 3910959, **1975**, Trojnar, M. K.et al., *Pharmacological Reports* **2005**, 154-160 , Arends, R.H *et al. Epilepsy Res.* **1994**, 18,91-96, Davies, N.M , **2004**

(R)-Stiripentol from esterification and hydrolysis



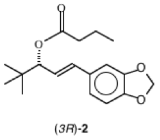
***Candida antarctica* lipase A (Novozym 735) and vinyl butanoate, *E*-value 24**

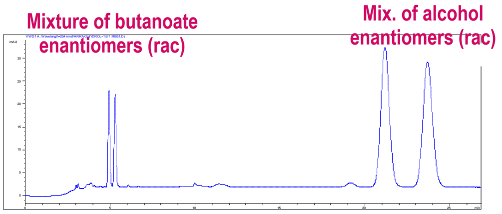
E-value: Ratio of the spesificity constants (k_{cat}/K_M) of the enzyme for the two enantiomers

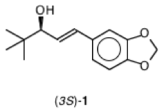
An *E*-value of 24: One enantiomer reacts 24 times faster than the other

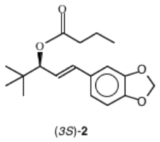
Jacobsen, E.E. et al *Lipase Catalysed Kinetic Resolution of Stiripentol. Int. J. Chem*. **2012** , *4* (1) 7-13

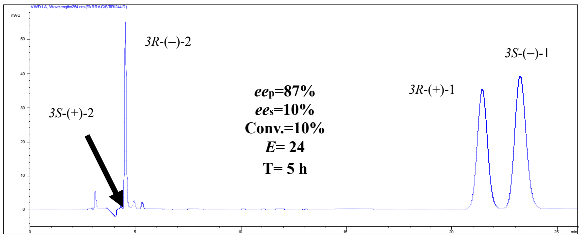
Chiral HPLC Chirasil OD-H, n-Hex/MTBE/2-PrOH

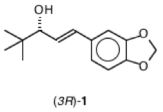

(3R)-2




(3S)-1


(3S)-2

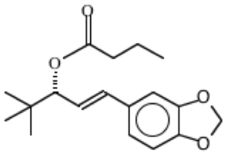



(3R)-1

$$E = \frac{\ln \frac{[ee_P(1-ee_S)]}{(ee_P+ee_S)}}{\ln \frac{[ee_P(1+ee_S)]}{(ee_P+ee_S)}}$$

enantiomers identified from lipase preference and from optical rotation value comparisons

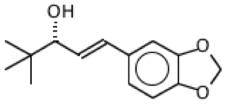
ee profile of kinetic resolution of *rac*-1, 106 h
E-value 24



(3*R*-**2**

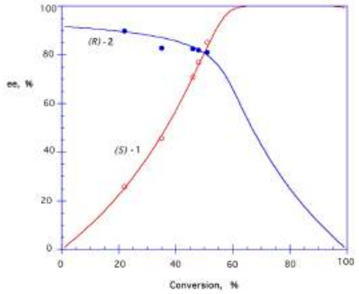
Ester product
(3*R*)-**2**, ee_p, 87 % *ee*

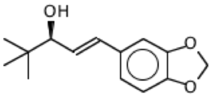
↓ CALA
Ph 7



(3*R*)-**1**

(3*R*)-**1**, 94 % *ee*





(3*S*)-**1**

Remaining alcohol
(3*S*)-**1**, ee_s, 86 % *ee*

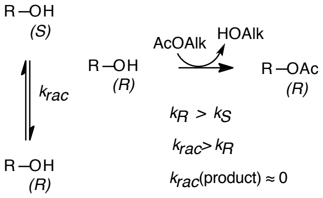
50 % waste?

Low *E*-value:
Low yield of high *ee*-enantiomer product
Always high *ee* of unconverted enantiomer!

Deracemization

100 % yield and 100 % ee from racemate

1. Dynamic Kinetic Resolution



$k_R > k_S$

$k_{rac} > k_R$

k_{rac}(product) ≈ 0

(*R*)- is the faster reacting enantiomer
(*S*)- is racemized

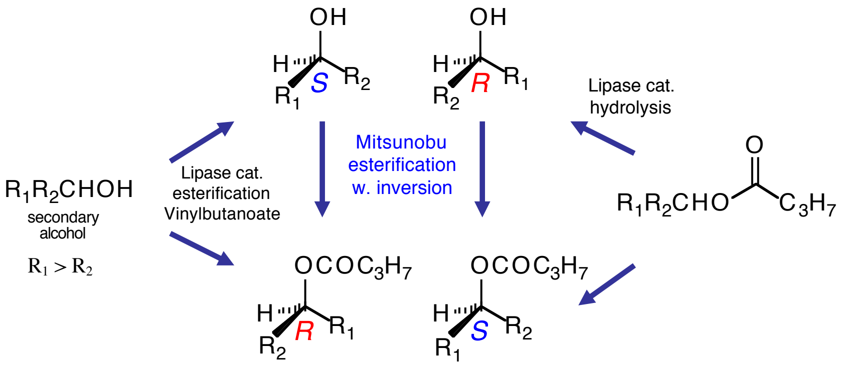
The enzyme always encounters a racemic mixture

2. Stereoinversion by Mitsunobu esterification

(*R*)- Substrate is converted to (*R*)-product

(*S*)- Substrate is converted to the same (*R*)-product in another reaction without separation (”one pot”)

Stereoinversion



R_1R_2CHOH
secondary
alcohol

$R_1 > R_2$

Lipase cat.
esterification
Vinylbutanoate

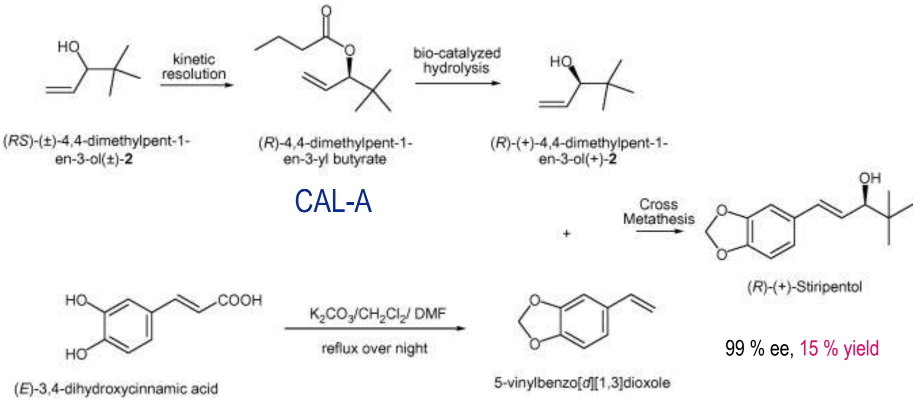
Mitsunobu
esterification
w. inversion

Lipase cat.
hydrolysis

R is the preferred
enantiomer both in
hydrolysis and esterification

Liu & Anthonsen, *Chirality*, 14, **2002**, 25

(*R*)-Stiripentol by combination of lipase catalysed resolution and alkene metathesis



(*RS*)-(±)-4,4-dimethylpent-1-en-3-ol(±)-**2**

(*R*)-4,4-dimethylpent-1-en-3-yl butyrate

(*R*)-(+)-4,4-dimethylpent-1-en-3-ol(+)-**2**

CAL-A

(*R*)-(+)-Stiripentol

(*E*)-3,4-dihydroxycinnamic acid

5-vinylbenzo[*d*][1,3]dioxole

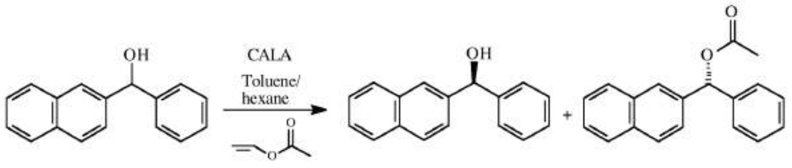
99 % ee, 15 % yield

El-Behairy, M et al. *Tetrahedron: Asymmetry* 24, **2013** 285–289

Lipase A from *Candida antarctica*, CALA

R-selective catalyst in resolutions of secondary and tertiary alcohols with bulky groups around the stereocenter

Resolution of phenyl-(2-naphthyl) methanol by CALA, *E*-value 67:



6 (*S*)-6 (*R*)-7

Tjosås, F., Anthonsen, T., Jacobsen, E. E. *ARKIVOC* **2008,** *vi*, 81-90.

Crystal structure of CALA (441 amino acids) has been determined:

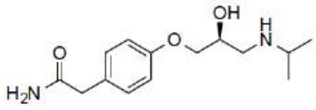
Ericsson, D. J.et al. *J. Mol. Biol.* **2008,** *376*, 109-119.

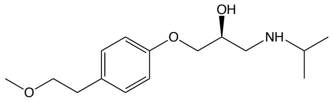
Conclusions

- (*R*)-Stiripentol has been synthesised in 94% *ee* in 60 % yield by kinetic resolution of *rac*-Stiripentol catalysed by lipase A from *Candida antarctica.*
- (*R*)-Stiripentol was also synthesised by a combination of lipase catalysed resolution and alkene metathesis in 99 % *ee* and 15 % yield.
- Wanted: 99 % *ee* AND 60 % yield!
- Lipase catalysis is a green, mild and enantioselective method to obtain pure enantiomers both from prochiral substrates and from secondary alcohols etc

Enantiopure cardioselective β-blockers atenolol and metoprolol

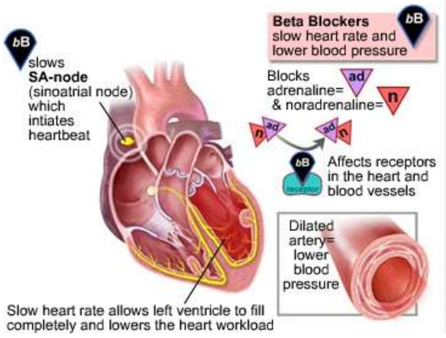
Only the *S*-enantiomers have the desired effect





Atenolol is manufactured as Atpure® with enantiopure active pharmaceutical ingredient (API).

Mylan® has the racemic API

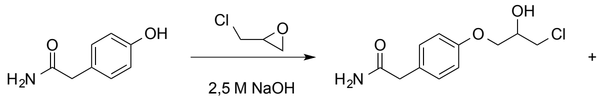


Metoprolol was the 14th most sold drug in Norway in 2017, and the most sold drug against hypertension in USA.

Marketed as Toprol-XL® by Astra Zeneca and as Lopressor® by Novartis, both with racemic API.

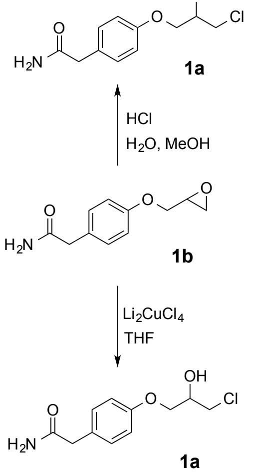
http://www.cardiachealth.org/heart-disease-treatment/heart-disease-medications/blood-pressure-medications/beta-blockers

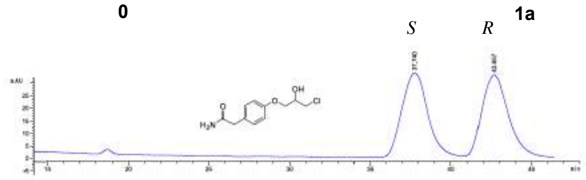
Organic synthesis of racemic building block for atenolol- the halogen will subsequently be substituted by an amine



0

1a



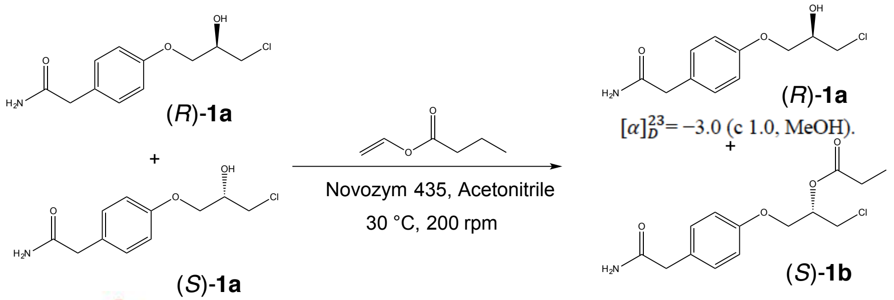


Enantiomers of **1a** were separated by HPLC on a Chiralcel OD-H column, eluent hexane:2-propanol (83:17, v:v) flow 1.000 mL min^{-1}, UV 254 nm.

Yield (80%):

1a sticky and slimy compound

Kinetic resolution of racemic building block by CALB



(*R*)-**1a**

(*S*)-**1a**

Novozym 435, Acetonitrile

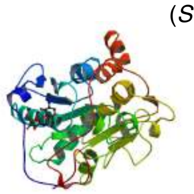
30 °C, 200 rpm

(*R*)-**1a**

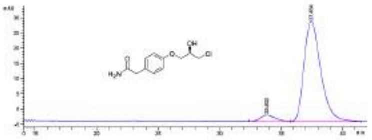
$[\alpha]_D^{23} = -3.0$ (c 1.0, MeOH).

(*S*)-**1b**

both ester and alcohol in 98-99 % *ee*



Lipase B
Candida antarctica (CALB)
Novozym 435

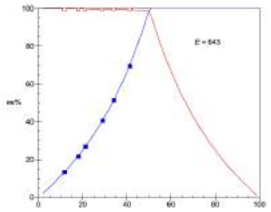


HPLC chromatogram of (*R*)-**1a** after separation from (*S*)-**1b** by flash chromatography.

Enzyme preference determines the right enantiomer:

the *S*-enantiomer will be converted

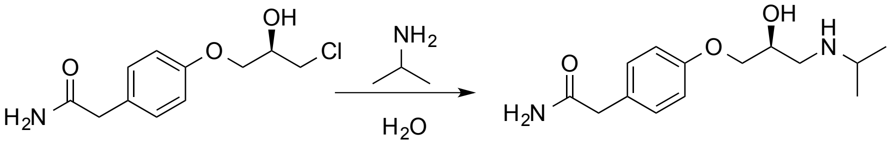
Enantiopure (*S*)-atenolol



$$E = \frac{k_{sp}^{R}}{k_{sp}^{S}} = \frac{(k_{cat}/K_M)^R}{(k_{cat}/K_M)^S}$$

$$E = \frac{\ln\frac{[ee_P(1-ee_S)]}{(ee_P+ee_S)}}{\ln\frac{[ee_P(1+ee_S)]}{(ee_P+ee_S)}}$$

High *E*-value (>200):
One enantiomer is converted 200 times faster to the ester than the other

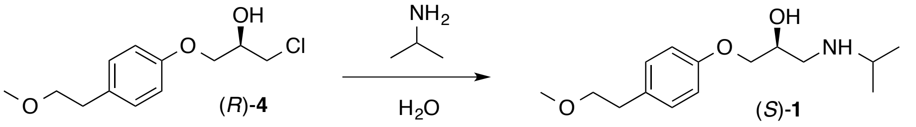


(*R*)-**1a**, 99 % *ee*

(*S*)-atenolol, 99 % *ee*

Lund, I.T., Bøckmann, P.L, Jacobsen, E.E. Tetrahedron **2016**, *72*, 7288-7294

Enantiopure (*S*)-metoprolol, (*S*)-1

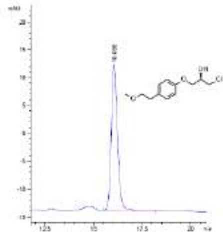


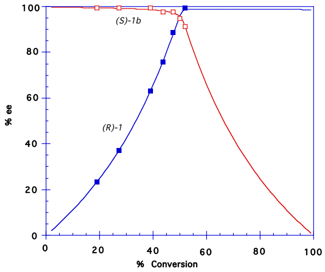
(*R*)-**4**

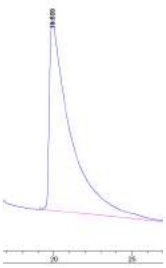
ee >98.5%

(*S*)-**1**

ee >99%, 62% yield







E= >200

$[\alpha]_D^{20} = -39.78°$(c 0.88, MeOH).

Blindheim, F.H. Bøckmann, P.L, Jacobsen, E.E. Manuscript in preparation

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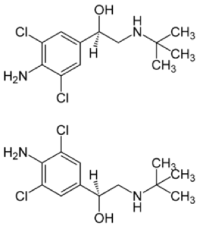
(*R*)-clenbuterol, a β$_2$-agonist - opposite of β-blocker



Widely used as medication for horses [1]

Ventipulmin®

Reduces fat and increase muscle mass.

«Superclen» (clen+tyroxine)

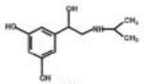
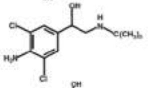
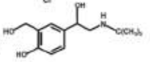
Clenbuterol is a β$_2$-agonist which has been widely used as bronchodilator in treatment of asthma and chronic obstructive pulmonary disease in Spain, Germany, Italy and Austria [2,3].

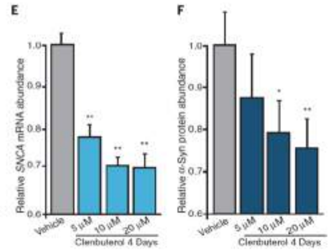
- **HOWEVER it is on the WADA list! Alberto Contador tested positive on clenbuterol**

The *R*-(−)enantiomer is responsible for this activity and the *S*-(+)-enantiomer has no effect.

[1] Norton JL, Jackson K, Chen JW, et al. *J Vet Intern Med* **2013,** *27*, 1523.
[2] Boner, AL, Vallone, G., Brighenti, C., Schiassi, M., Miglioranzi, P. and Richelli, C. *Pediatr. Pulmonol.* **1988**, *4,* 197.
[3] Papiris, S. Galvotti,. V. and Sturani, C. *Respiration* **1986**, *49*, 101.

β2-Adrenoreceptor is a regulator of the α-synuclein gene driving risk of Parkinson’s disease

No.	Name	Class	Structure	FDA approved	Indication	Blood-brain penetrant
1.	Metaproterenol	β2–Adrenoreceptor Agonist		Yes	Asthma	No
2.	Clenbuterol	β2–Adrenoreceptor Agonist		No	Asthma	Yes
3.	Salbutamol	β2–Adrenoreceptor Agonist		Yes	Asthma	Yes

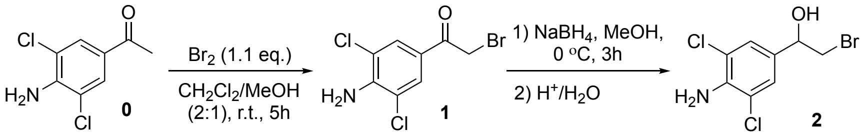


β2AR agonist clenbuterol lowered the expression of SNCA mRNA (E) and α-Syn protein (F) in a dose-dependent manner in neuroblastoma cells

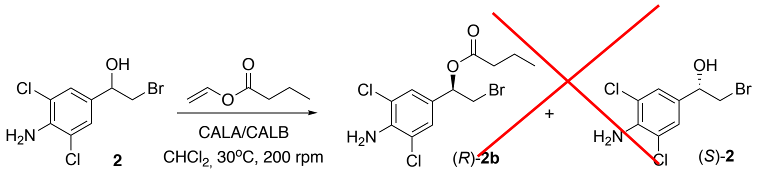
However, which of the enantiomers of clenbuterol is causing this effect??
We are under way of making them sepately-just wait for it!

Mittal *et al*., Science **2017**, *357*, 891-898

1-(4-Amino-3,5-dichlorophenyl)-2-bromoethan-1-ol (**2**)



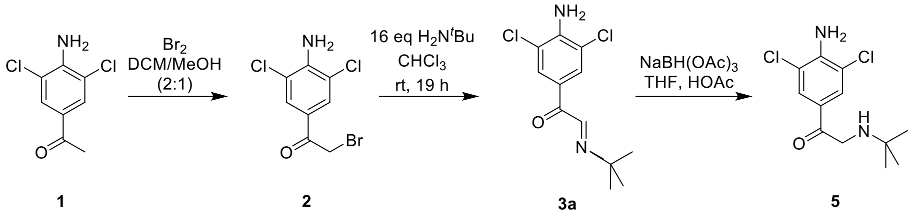
Transesterification of **2** with lipases



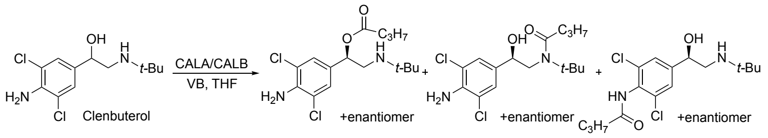
Several lipases used in kinetic resolutions-no conversion

Let´s synthesise racemic clenbuterol and perform kinetic resolution

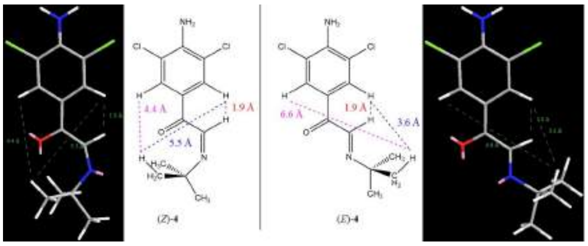
However, problems with imine formation by addition of *t*-butylamine



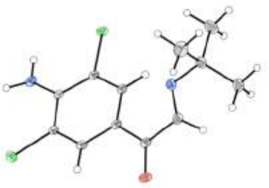
Transesterification of clenbuterol with lipases



(Z)- and (E)-**4** modelled with their optimal geometries (minimal energy calculation - MM2), and distances measured in Chem3D (PerkinElmer Inc.)

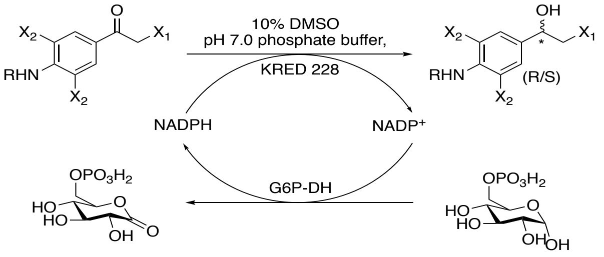


(*E*)-**4** determined by X-ray analysis



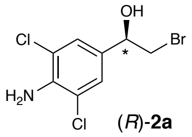
(*E*)-**4**

Building bock for (*R*)-clenbuterol in 93% ee by KRED 228



1: X_1 = H, X_2 = Cl, R = H
2: X_1 = Br, X_2 = Cl, R = H
4: X_1 = H, X_2 = H, R = H
5: X_1 = H, X_2 = H, R = Ac
6: X_1 = H, X_2 = Cl, R = Ac
7: X_1 = Br, X_2 = H, R = Ac

1a: X_1 = H, X_2 = Cl, R = H n.r.
(*R*)-**2a**: X_1 = Br, X_2 = Cl, R = H 93% ee
4a: X_1 = H, X_2 = H, R = H n.r.
(*S*)-**5a**: X_1 = H, X_2 = H, R = Ac 73% ee
(*S*)-**6a**: X_1 = H, X_2 = Cl, R = Ac 98% ee
(*R*)-**7a**: X_1 = Br, X_2 = H, R = Ac 35% ee



(*R*)-**2a**

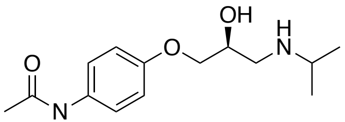
Ketoreductase, KRED228

SynZymes
尚科生物医药

Blindheim, F.H., Hansen, M.B., Evjen, S., Zhu, W. and Jacobsen, E.E. *Chemo-Enzymatic Synthesis of Enantiopure Synthons as Precursors for (R)-Clenbuterol and other β-2 agonists.* **Catalysts, 2018,** 8 (11), 516 doi:10.3390/catal8110516

Enantiopure cardioselective β-blockers practolol and pindolol

Only the *S*-enantiomers have the desired effect

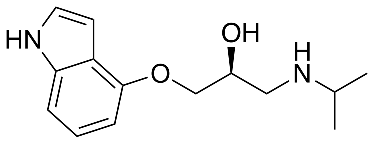


(*S*)-Practolol, (*S*)-**5**

Practolol
Selective β1-antagonist
First β1-selective β-blocker in treatment of cardiovascular diseases (1970s)

1. effective treatment of hearth failure
2. arrhythmic hearth rate
3. critical side-effects: oculomucocutaneous syndrome (dry eyes)

-Then withdrawn from the market.



(*S*)-Pindolol, (*S*)-**7**

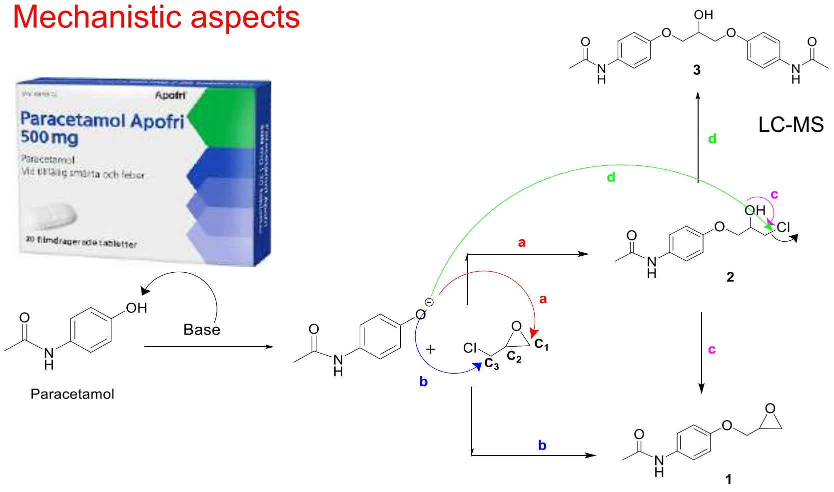
Pindolol
β1-antagonist that reduces high blood pressure.

1. also used in treatment of angina pectoris
2. also been studied for use in treatment of depression as an add-on therapy to selective serotonin re-uptake inhibitors (SSRIs).

http://www.cardiachealth.org/heart-disease-treatment/heart-disease-medications/blood-pressure-medications/beta-blockers

Step 1 Practolol:
N-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide, **2**

Mechanistic aspects



Step 1: Different concentrations of NaOH

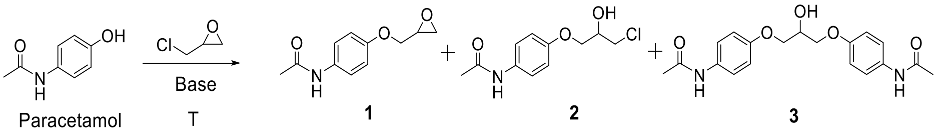
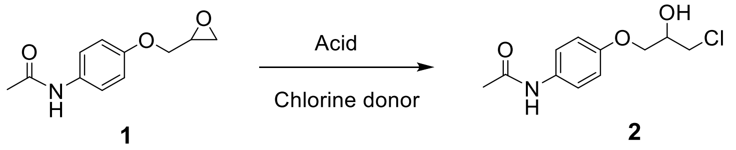


TABLE 1. Measured areas (amount) by GC, DB 1701 COLUMN, 100-270ºC/10ºC

Base	Equivalent	T	Time [h]	Amount **1** [%]	Amount **2** [%]	Amount **3** [%]
NaOH	0.1	r.t.	48	29	38	10
NaOH	0.5	r.t.	8	37	43	8
NaOH	1.0	r.t.	7	81	12	4
NaOH	2.0	r.t.	7	36	0	64
NaOH	10.0	r.t.	18	0	3	24

2 eq. Epichlorohydrin

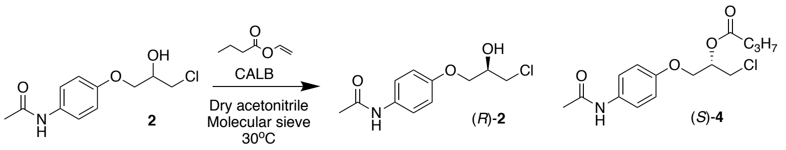
Step 2: Addition of LiCl and Acetic Acid



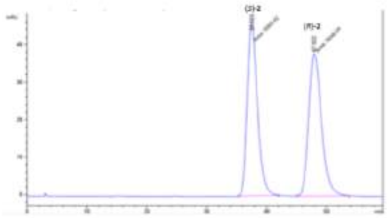
Equivalents LiCl	Equivalents Acetic acid	Time [h]	Amount **2** in GC [%]	Yield [%]
1.0	6.0	120	82	-
2.0	6.0	120	>99	43
4.0	6.0	48	82	-
4.0	10.0	26	98	62

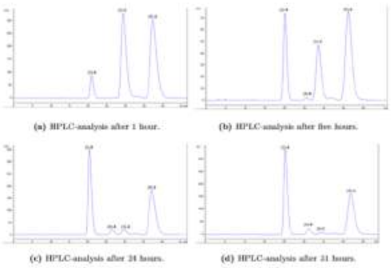
Similar results for pindolol building block, **6**

Transesterification of **2** with CALB



Novozym 435: 93%ee
Syncozymes CAL-B: 96% ee




(a) HPLC-analysis after 1 hour.
(b) HPLC-analysis after five hours.
(c) HPLC-analysis after 24 hours.
(d) HPLC-analysis after 31 hours.

Chiralcel OD-H colum, hexane-2-propanol (85:15), 1 mL/min

ee-values and *E*-values

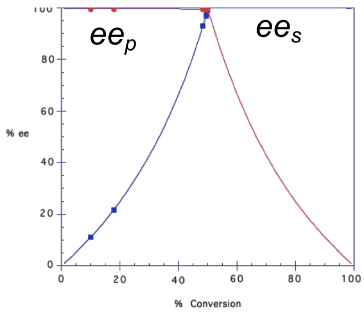
Plotting ee-values into *E & K Calculator* : average *E*-value

$$E = \frac{\left(k_{cat}/K_M\right)_R}{\left(k_{cat}/K_M\right)_S}$$

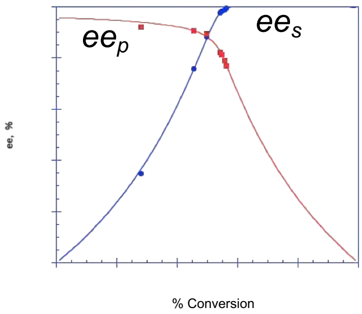
$$\Delta\Delta G^{\#} = -RT \ln E$$

$$\boxed{E = \frac{\ln \frac{\left[ee_p(1-ee_s)\right]}{(ee_p+ee_s)}}{\ln \frac{\left[ee_p(1+ee_s)\right]}{(ee_p+ee_s)}}}$$

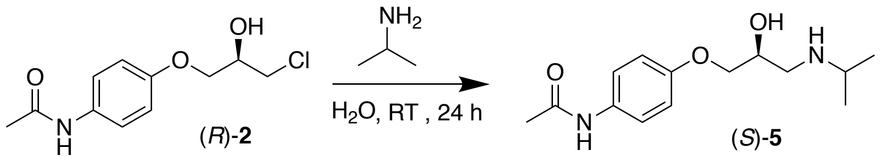
Practolol E=1000



Pindolol E=46



Synthesis of (*S*)-practolol, (*S*)-**5**



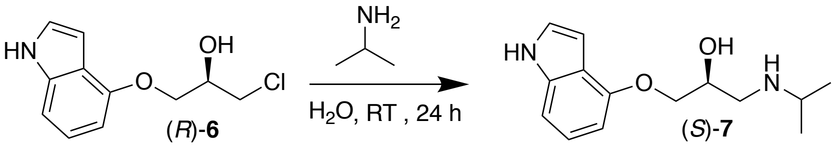
(*R*)-**2**
ee=94%

(*S*)-**5**
ee=96%
Yield=16%

$[\alpha]_D^{20} = 3.99$

Hansen, Mari B., Austli, Guro B., Jacobsen, Elisabeth E. *Manuscript in preparation*

Synthesis of (*S*)-pindolol, (*S*)-**7**



(*R*)-**6**

ee: 96%
Yield: nd

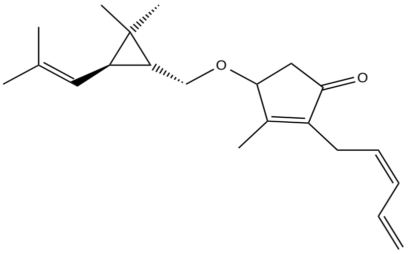
(*S*)-**7**

ee: 96%
Yield: nd

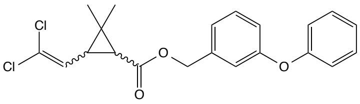
- Yield not determined due to residue of isopropylamine-will be improved
- Optical rotation not determined

Dale, Oskar and Jacobsen, Elisabeth E. *Manuscript in preparation*

Insect killers: Active ingredients pyrethrin and permethrin, inhibit insects´ central nervous system



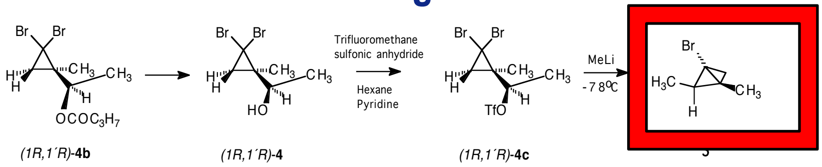
Pyrethrin-I from *Chrysanthemum cinerariaefolium*



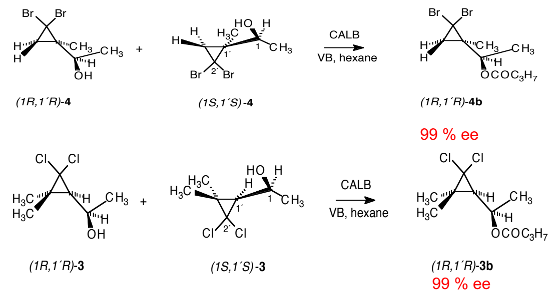
Permethrin has four stereoisomeres. Industrial process

Martel, J. The development and manufacture of pyrethroid insecticides In: *Chirality in Industry*; Collins, A. N.; Sheldrake, G. N.; Crosby, J. Eds.; John Wiley & Sons: New York, **1992**

Enantiopure dihalobicyclobutanes – new building blocks



(1R,1´R)-**4b** (1R,1´R)-**4** (1R,1´R)-**4c**

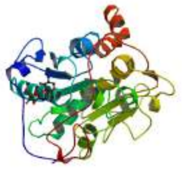


(1R,1´R)-**4** (1S,1´S)-**4** (1R,1´R)-**4b**

99 % ee

(1R,1´R)-**3** (1S,1´S)-**3** (1R,1´R)-**3b**

99 % ee



Lipase B
Candida antarctica

Jacobsen, E.E.; *et. al.* Enantiopure dihalocyclopropyl alcohols and esters by lipase catalyzed kinetic resolution *J. Biotechnol*. **2013**, *168*, 284-288

Thank you for your attention!

The Research Council of Norway (contract Grant Number 202903/11)
and EEA grants (contract Grant Number18-COP-0041) are thanked for funding

Iceland
Liechtenstein
Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

UNIVERSITY OF BUCHAREST
VIRTUTE ET SAPIENTIA

NTNU
Norwegian University of Science and Technology

ADVANCED POLYMERS

Dr. Vegar Ottesen
Dr. Solon Oikonomopoulos
Dr. Sulalit Bandyopadhyay

Nanotechnology - introduction

Vegar Ottesen, PhD.
vegar.ottesen@ntnu.no

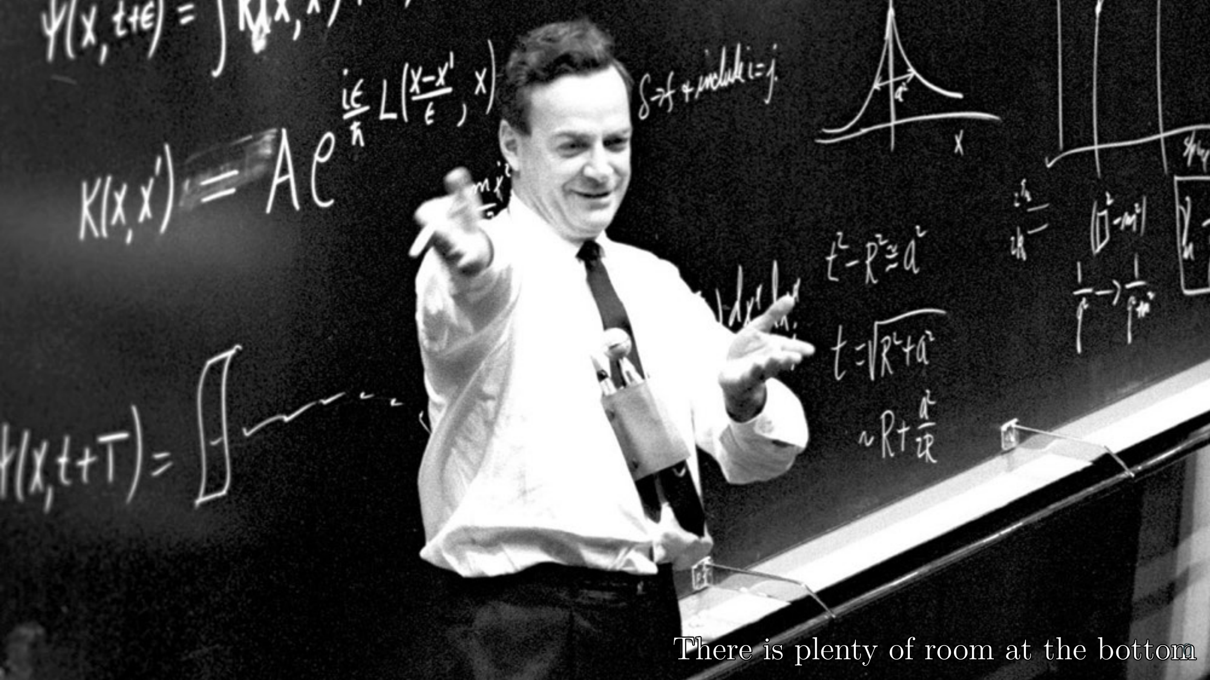
September 28, 2020

Dept. of Chemical Engineering, NTNU

Welcome!

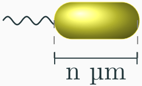
NTNU
Faculty of Natural Sciences

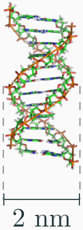
What is Nanotechnology?

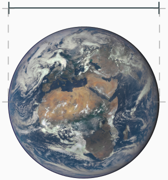


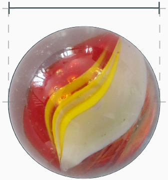
There is plenty of room at the bottom

Nano and micro

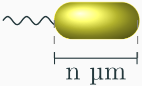


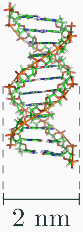




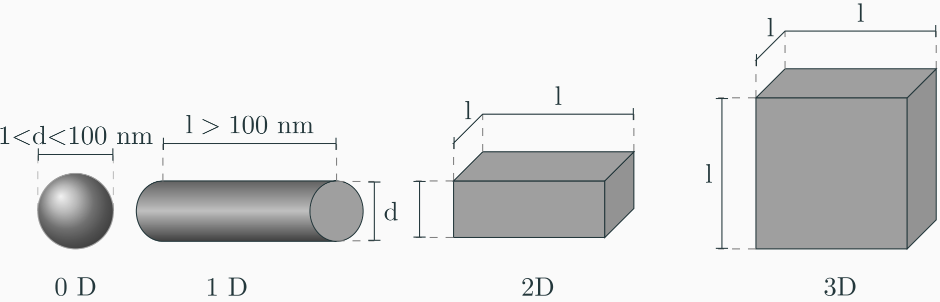


Terms





Terms



$$\mathrm{SSA} = \frac{\mathrm{A}}{\mathrm{V}\rho}$$

$$[\mathrm{SSA}] = \frac{\mathrm{m}^2}{\mathrm{g}}$$

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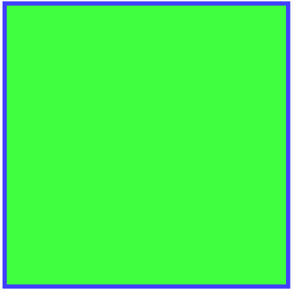
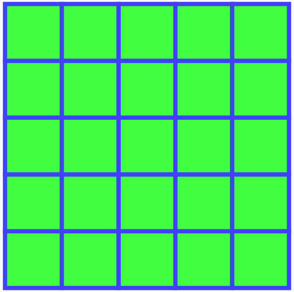


$$\mathrm{SSA} = \frac{\mathrm{A}}{\mathrm{V}\rho}$$

$$[\mathrm{SSA}] = \frac{\mathrm{m}^2}{\mathrm{g}}$$



Bandgap

$$E_{\text{nano}} = E_{\text{g,bulk}} + \frac{h^2\pi^2}{2mr_{\text{nano}}^2} \tag{1}$$

Bandgap

$$\mathrm{E_{nano}} = \mathrm{E_{g,bulk}} + \frac{\mathrm{h}^2\pi^2}{2\mathrm{m}\mathrm{r}^2_{\mathrm{nano}}} \tag{1}$$

Melting temperature

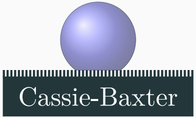
Why?

Bandgap

$$E_{\mathrm{nano}} = E_{\mathrm{g,bulk}} + \frac{h^2\pi^2}{2mr^2_{\mathrm{nano}}} \tag{1}$$

Melting temperature

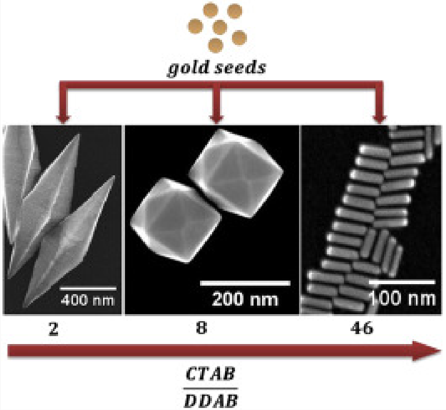
Interactions with environment



Top-Down
vs.
Bottom-Up

Production?

Nucleation and growth



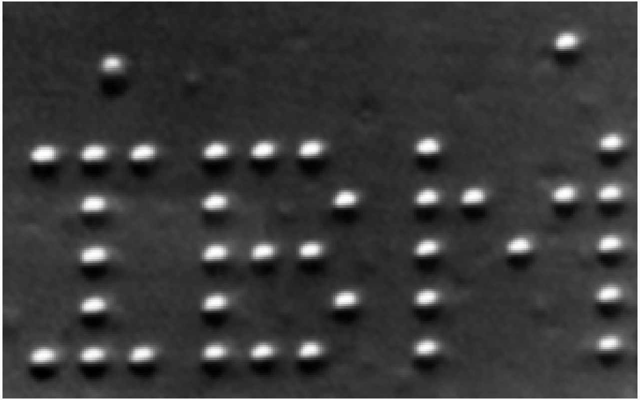
Sulalit Bandyopadhyay, Mat. Today, 2017

Production?

Bottom-up

Nucleation and growth

Scanning Probe Microscopy (SPM)

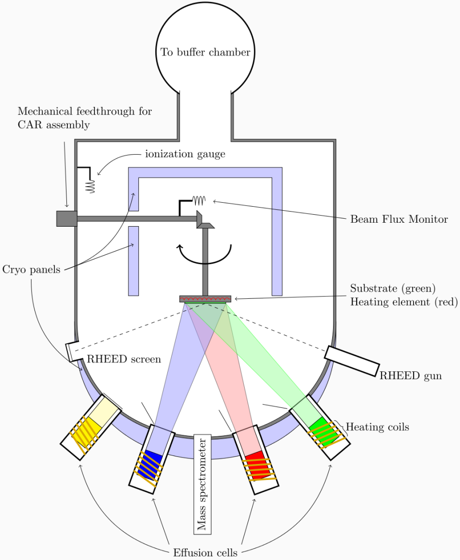


Production?

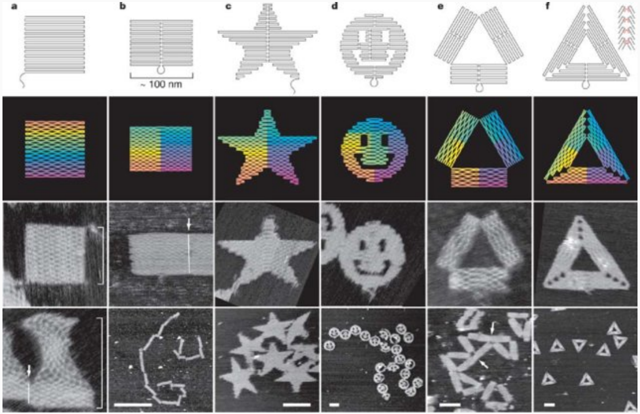
Nucleation and growth

SPM

Molecular Beam Epitaxy (MBE)



Nucleation and growth
SPM
MBE
Polymer origami



Rothemund, Nature, 2006

Production?

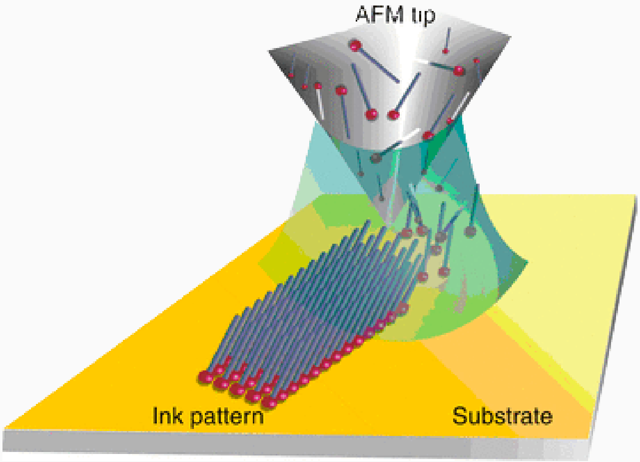
Nucleation and growth

SPM

MBE

Polymer origami Lithography

- Dip-pen, Soft lithography...



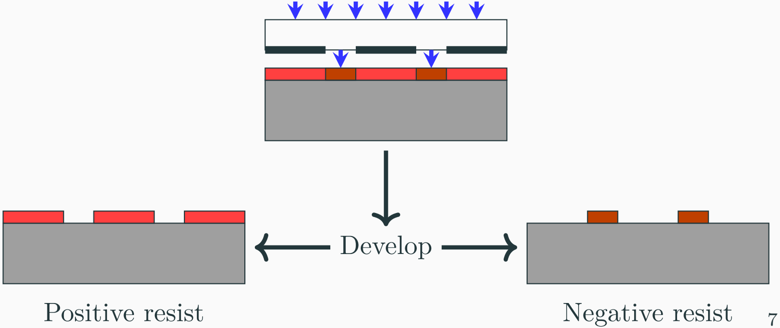
Smith et al, Nano Letters, 2003

Production?

Top-down

Lithograhpy

- Photolithography, EBL, SCIL, NIL...



Production?

Top-down

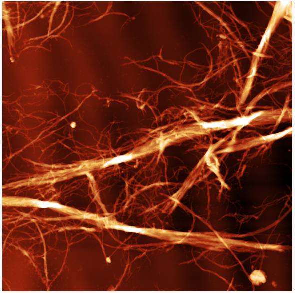
Lithograhpy

- Photolithography, EBL,
- SCIL, NIL...

Forces

- Shear, impact

Production?



Top-down

Lithograhpy

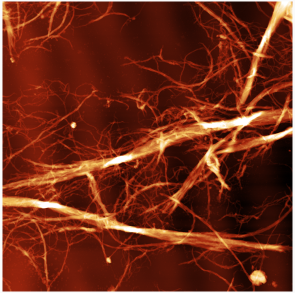
- Photolithography, EBL, SCIL, NIL...

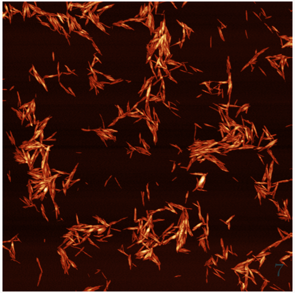
Forces

- Shear, impact

Production?

- Top-down
- Lithograhpy
    - Photolithography, EBL, SCIL, NIL...
- Forces
    - Shear, impact
- Partial dissolution
    - Acids, bases





Characterization

Statistical

- X-Ray Scattering (WAXS/SAXS)
- Neutron Scattering
- Nuclear Magnetic Resonance (NMR)

Microscopy

$$d = \frac{\lambda}{2NA}, d > 0.25\mu m$$

STED, PALM, `STORM`, SIM...

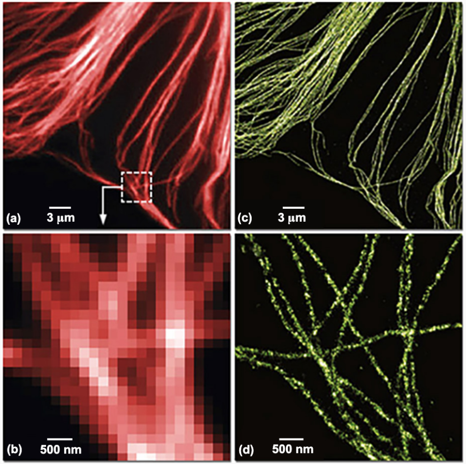
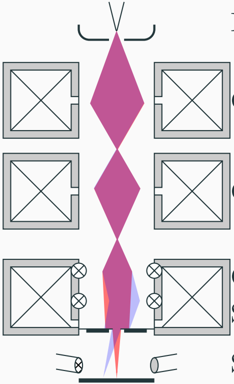


Image: Nikon



Electron gun

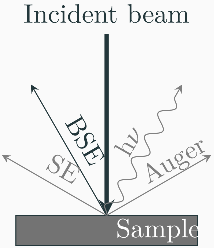
Condenser Lens

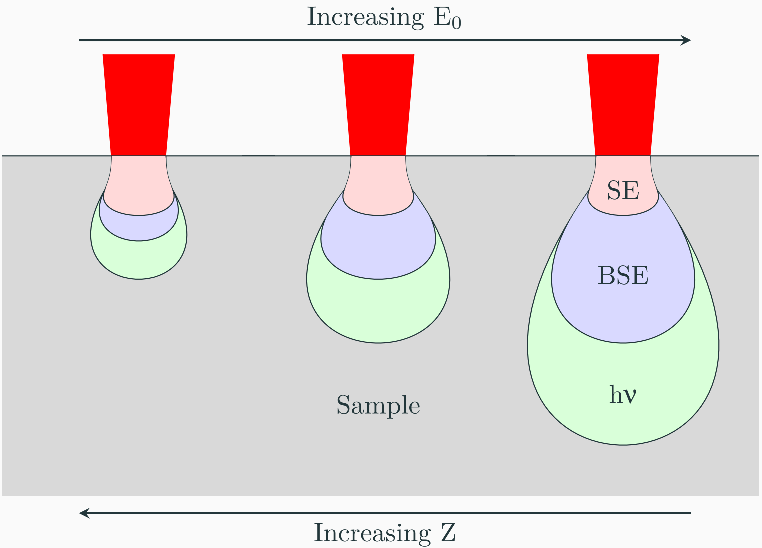
Condenser Lens

Objective lens

Scanning coils

Sample and detectors





Increasing E_0

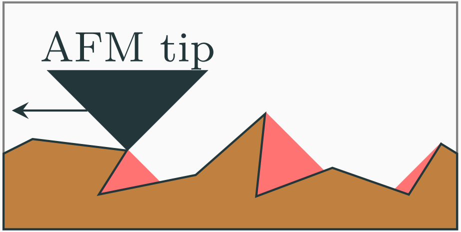
SE

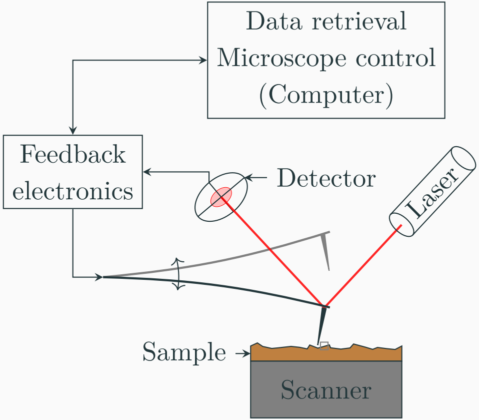
BSE

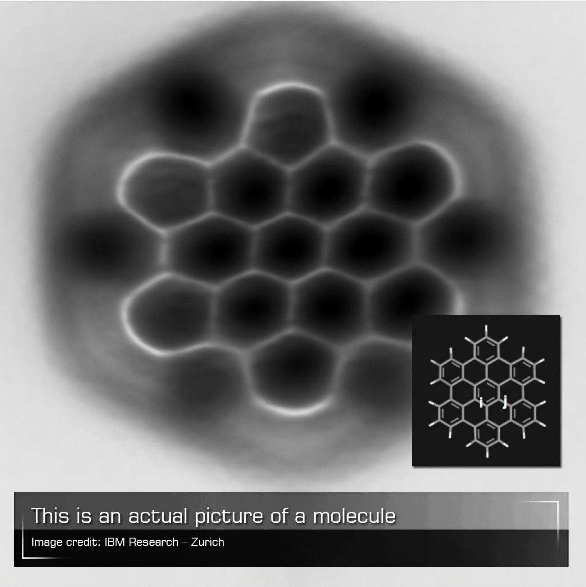
hν

Sample

Increasing Z


AFM tip


Data retrieval
Microscope control
(Computer)
Feedback
electronics
Detector
Laser
Sample
Scanner



This is an actual picture of a molecule

Image credit: IBM Research – Zurich

summary

1-3D $\in \{1, 100\}$ nm

summary

1-3D $\in \{1, 100\}$ nm

novel properties

summary

1-3D $\in \{1, 100\}$ nm

novel properties

Build from ground up

Produce from larger whole

summary

1-3D $\in \{1, 100\}$ nm
novel properties
Build from ground up
Produce from larger whole
Demanding characterization

Polymeric Nanomaterials – An Overview

Sulalit Bandyopadhyay

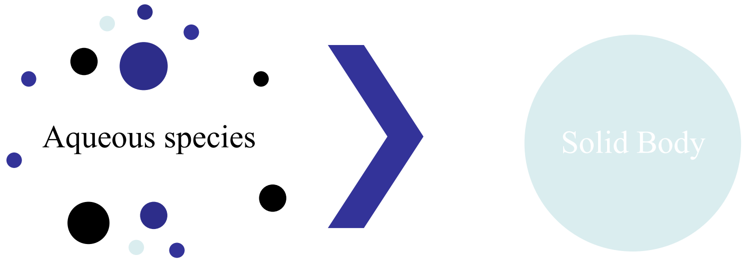
https://www.precisionnanosystems.com/areas-of-interest/formulations/polymeric-nanoparticles

Overview

- **Classical Nucleation Theory**
  - Nucleation
  - Growth
  - Size Control
- **Synthesis of Polymeric NPs**
  - Nanoprecipitation
  - Flash Nanoprecipitation
  - Precipitation polymerization

Classical Nucleation Theory

Crystallization From Solution



Aqueous species

Solid Body

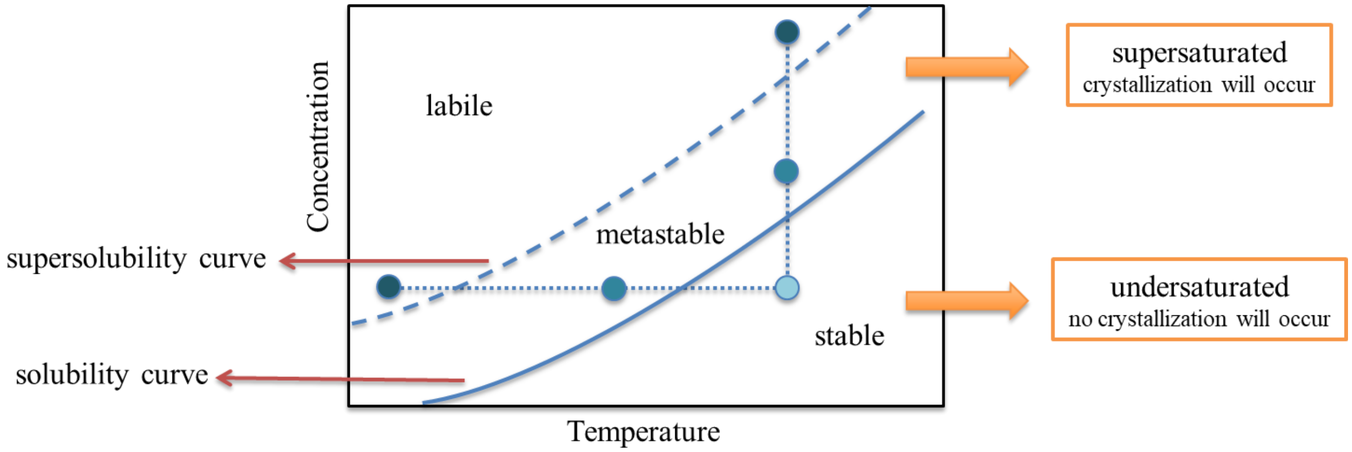
What is the driving force?

Lowering Free Energy

Crystallization is a phase transition process during which matter is transformed from a *high free energy*, disordered state to a crystal phase characterized by the regular arrangement of its building units to a *low free energy* state.

Solubility & Supersaturation

- A **saturated solution** is in thermodynamic equilibrium with the solid phase at a specified $T > c = c^*$
- Solutions where $c > c^*$ can be prepared (exm: by slow cooling), such solutions are called **supersaturated**
- The state of supersaturation is essential for all crystallization operations!!



supersaturated
crystallization will occur

undersaturated
no crystallization will occur

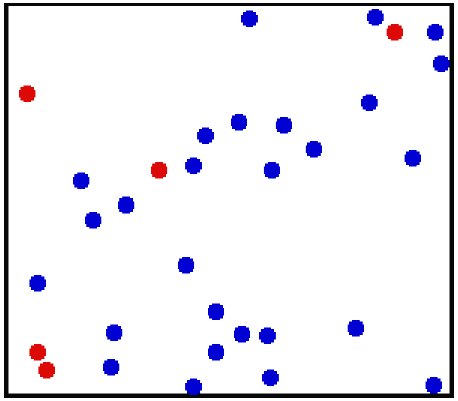
Stable zone: undersaturated- crystallization is impossible

Metastable zone: **supersaturated-** spontaneous crystallization is probable

Labile zone: **supersaturated-** uncontrolled spontaneous crystallization

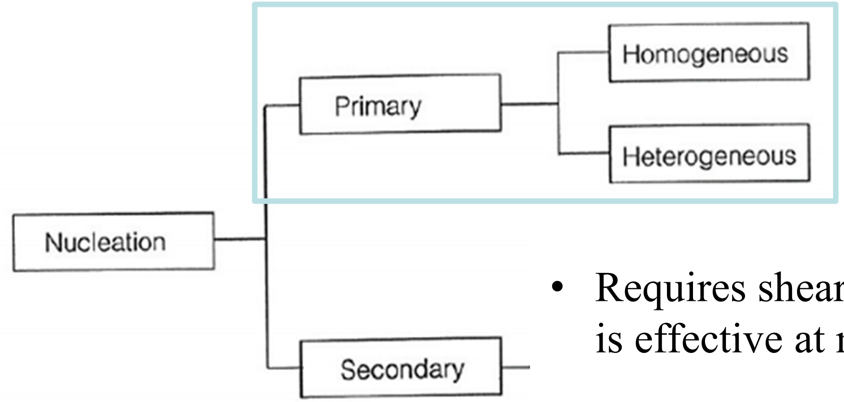
Nucleation

- First step in the formation of the new phase
- According to CNT, nuclei form via the dynamic and stochastic association of monomeric units that overcome a free energy barrier at a critical size and result in a phase separation in the system.



- Stochastic process
- Brownian motion
- Ions/molecules come together and apart until the formation of stable nucleus

Types of Nucleation

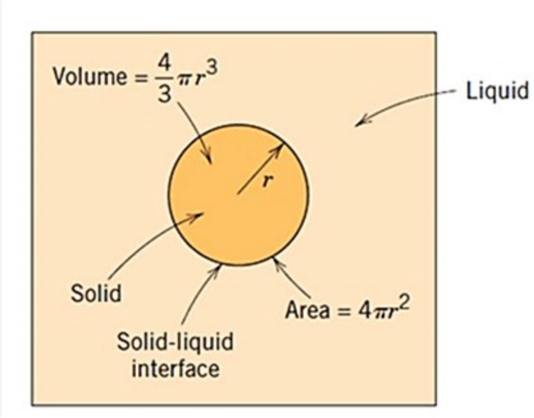


- Requires shear force to be active on particles that is effective at micron size range.

- Negligible for nanoparticle formation.

Primary Homogeneous Nucleation

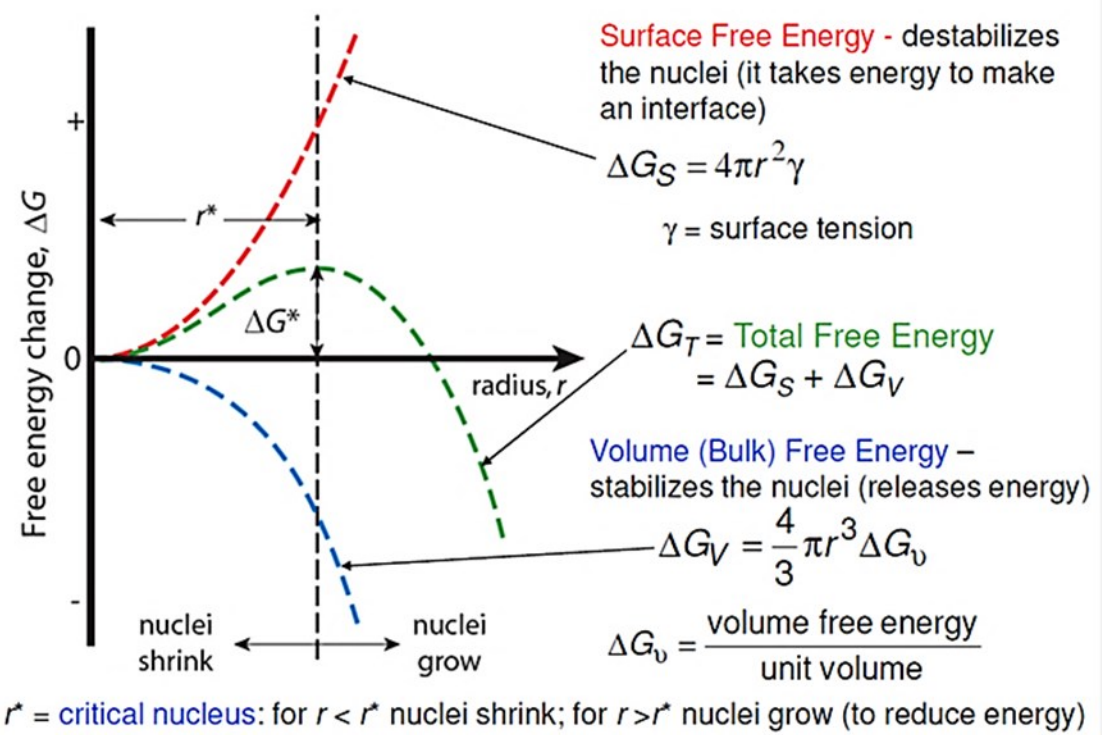
A crystal forms via ***formation of a stable nucleus*** with a critical size where its bulk energy balances the energetic costs of creating a surface; and subsequently grows via ***monomer-by-monomer attachment of its building units***, that can be atoms, molecules or ions.



$$\Delta G = \Delta G_V + \Delta G_S$$

$$\Delta G \underset{(-)}{=} \underset{(+)}{\Delta G_v} V + \gamma A$$

Primary Homogeneous Nucleation



Surface Free Energy - destabilizes the nuclei (it takes energy to make an interface)

$$\Delta G_S = 4\pi r^2 \gamma$$

γ = surface tension

ΔG_T = Total Free Energy $= \Delta G_S + \Delta G_V$

Volume (Bulk) Free Energy – stabilizes the nuclei (releases energy)

$$\Delta G_V = \frac{4}{3}\pi r^3 \Delta G_\upsilon$$

$$\Delta G_\upsilon = \frac{\text{volume free energy}}{\text{unit volume}}$$

r^* = critical nucleus: for $r < r^*$ nuclei shrink; for $r > r^*$ nuclei grow (to reduce energy)

Primary Homogeneous Nucleation

- The nucleation rate, J, can be expressed as an Arrhenius reaction equation, commonly used for thermally activated processes

$$J = A\exp\left(\frac{-\Delta G^{*}}{kT}\right)$$

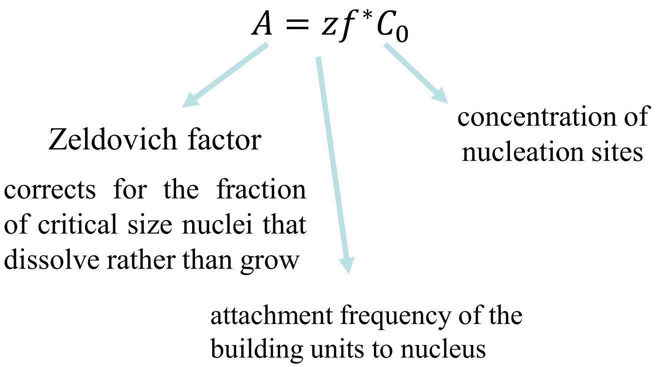
$$J = A\exp\left[-\frac{16\pi v_{m}{}^{2}\gamma^{3}}{3\upsilon^{2}\left(kT\right)^{3}(\ln S)^{2}}\right]$$

Effecting variables: γ, T, S

Kinetic Factor

The pre-exponential factor A

- deals with the kinetic factors of nucleation



$$A = zf^{*}C_{0}$$

Zeldovich factor

corrects for the fraction of critical size nuclei that dissolve rather than grow

attachment frequency of the building units to nucleus

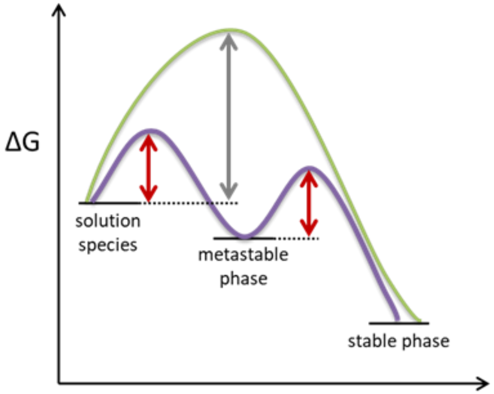
concentration of nucleation sites

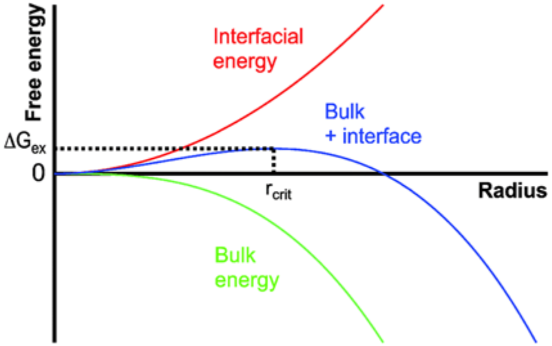
- The energy barrier for homogeneous nucleation is usually too high to enable precipitation (i.e. critical radius is too large).
- Thus, in practice primary homogeneous nucleation almost never occurs!

D. Kashchiev, G. M. van Rosmalen: **Review: Nucleation in solutions revisited (2003)**

Ostwald's Rule of Stages

- Thermodynamically metastable phases associated with ***lower energy barriers of formation*** can initially appear in a supersaturated system as a ***coaction of thermodynamic and kinetic factors***





metastable phase -> higher solubility -> lower bulk free energy

lower interfacial energy

kinetically favorable

- Given the sufficient time and the free energy of activation, they eventually transform into the more stable phase

$$\Delta \mathrm{G} = G_{stable} - G_{metastable} < 0$$

- The driving force for this transformation is the minimization of the total free energy of the system

Size Enlargement

- Enlargement of crystalline particles in solution is described by two processes according to the classical theory:
  1. crystal growth – monomer addition
  2. agglomeration – crystal growth during contact which forms a bridge between two crystals

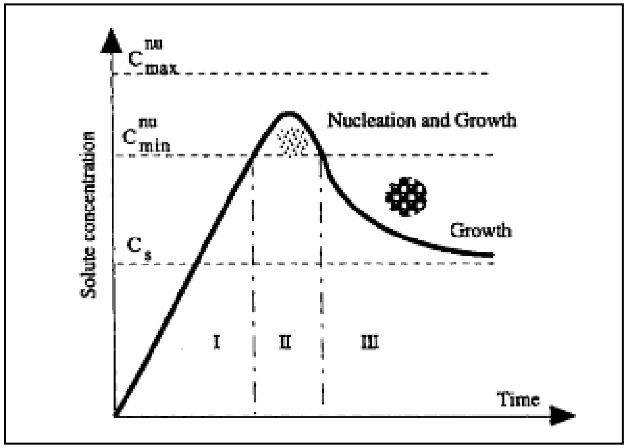
- The driving force for crystal growth is the lower chemical potential of the growth units as constituents of the solid, in equilibrium with its solution, than as solution species
- Thus, the activity based supersaturation can be defined in the same way for both processes and used as a measure of the driving force:

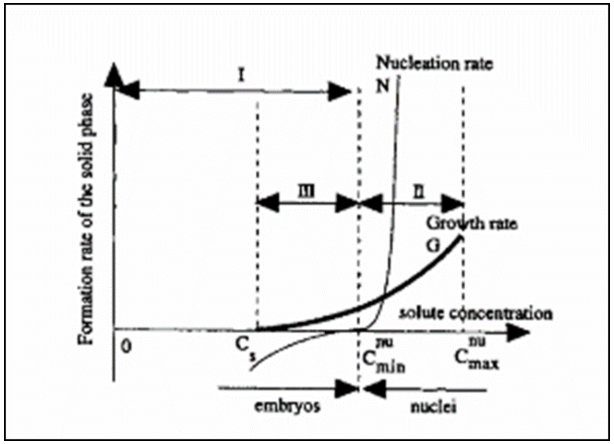
$$S = \left(\frac{IAP}{K_{sp,a}}\right)^{1/v}$$

Size Enlargement

- Although the fundamental driving force for nucleation and growth processes is the same, they follow different energy landscapes.

LaMer Diagram





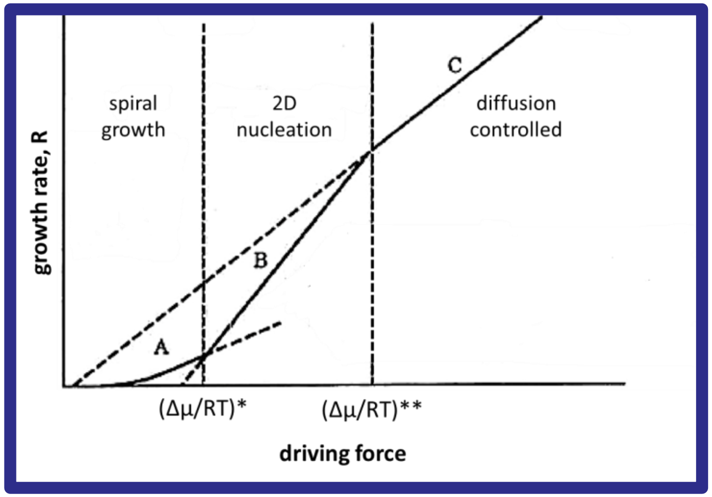
- When the activity/concentration of the solute is reduced below the value required for nucleation, only growth will be responsible for the relaxation of the supersaturated system towards $\Delta\mu = 0$ at $S = 1$.

Growth Rate

Growth rate law: $R = k(S-1)^g$

Diffusion controlled	
Rough growth	**g=1**
Reaction controlled	
Spiral growth	**g=2**
Polynuclear growth	**g > 2**

- Experimental determination of the growth kinetics supported by microscopic examination of crystals often allows the determination of the prevailing growth mechanism.



- Whichever the prevailing growth mechanism, the constant, k, is proportional to the solute concentration, i.e., soluble compounds grow faster than slightly soluble ones

Teng, H. H. et. al; *Geochimica et Cosmochimica Acta*, 64, **2000**, 2255-2264.

Particle Size and Size Distribution

Uniform Size Distribution

Nucleation Stage

For the synthesis of NPs with uniform size distribution, it is best if all nuclei are formed at the same time.

↓

When formed under the same conditions, all nuclei would have the same or similar size

↓

In addition, they will all have the same subsequent growth

Growth Stage

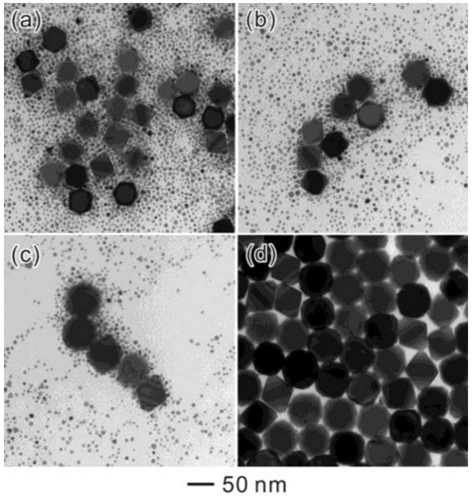
- The size distribution of particles can be further altered during the growth process
- Different growth mechanisms exert dissimilar outcomes on the size distribution in accordance with the correlation between the growth rate and the particle size

The formation of uniformly sized nanoparticles can be achieved if both processes are appropriately controlled.

Particle Size and Size Distribution

Ostwald Ripening

- When solid particles are dispersed in their own saturated solution **(aging)** there is a tendency for the smaller particles to dissolve and the solute to be deposited on the larger particles
- Particle size distribution ultimately changes towards that of a monosized dispersion



TEM images of Pd octahedra obtained after ripening for different periods of time: (a) 6 h, (b) 24 h, (c) 48 h, and (d) 72 h.

Zhang et al., DOI: 10.1039/c5sc01787d

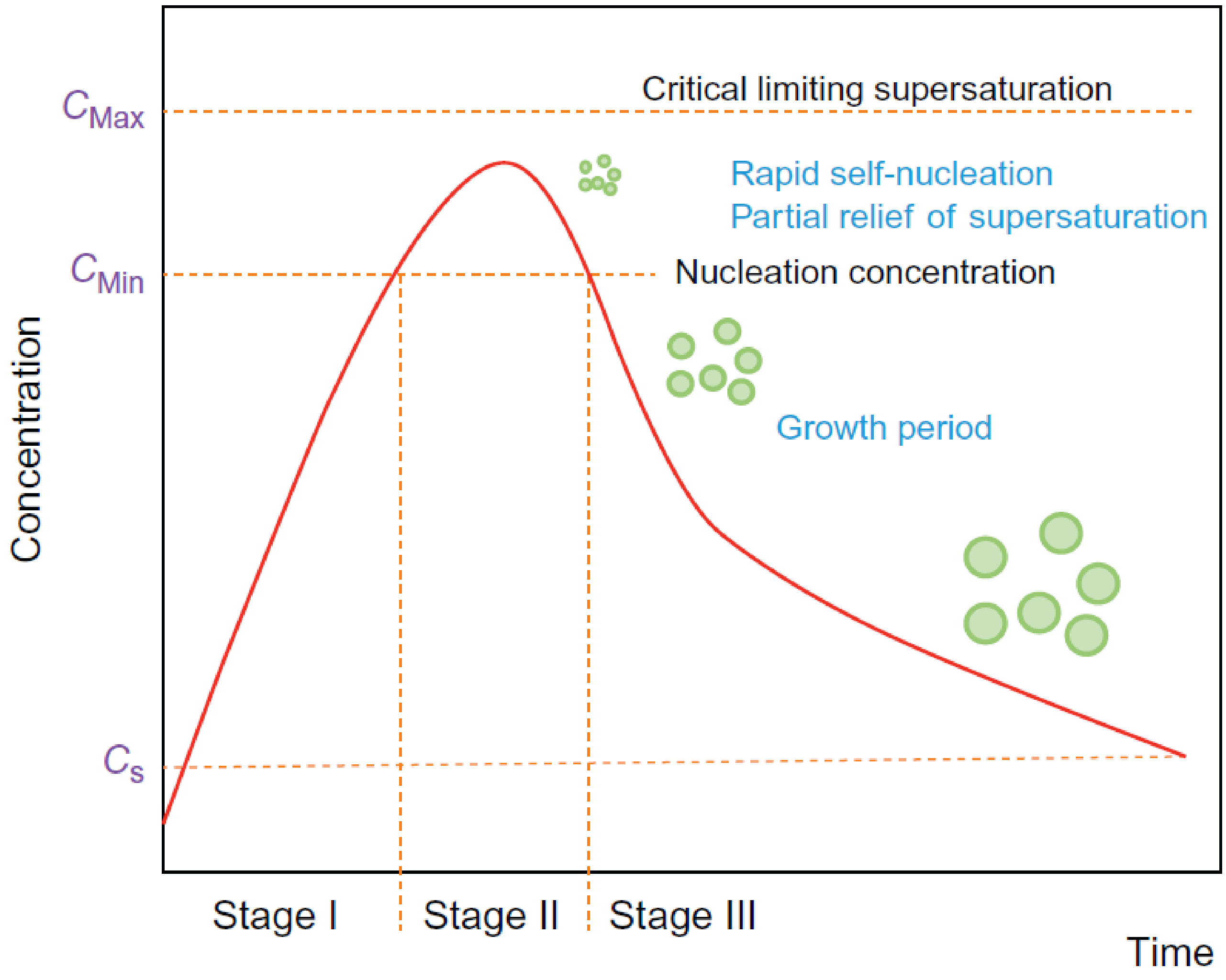
Recap

1. Supersaturation
2. Nucleation
3. Crystal growth
4. Secondary growth

$$S = \frac{c}{c^*}$$

$$\sigma = \frac{\Delta c}{c^*} = S - 1$$

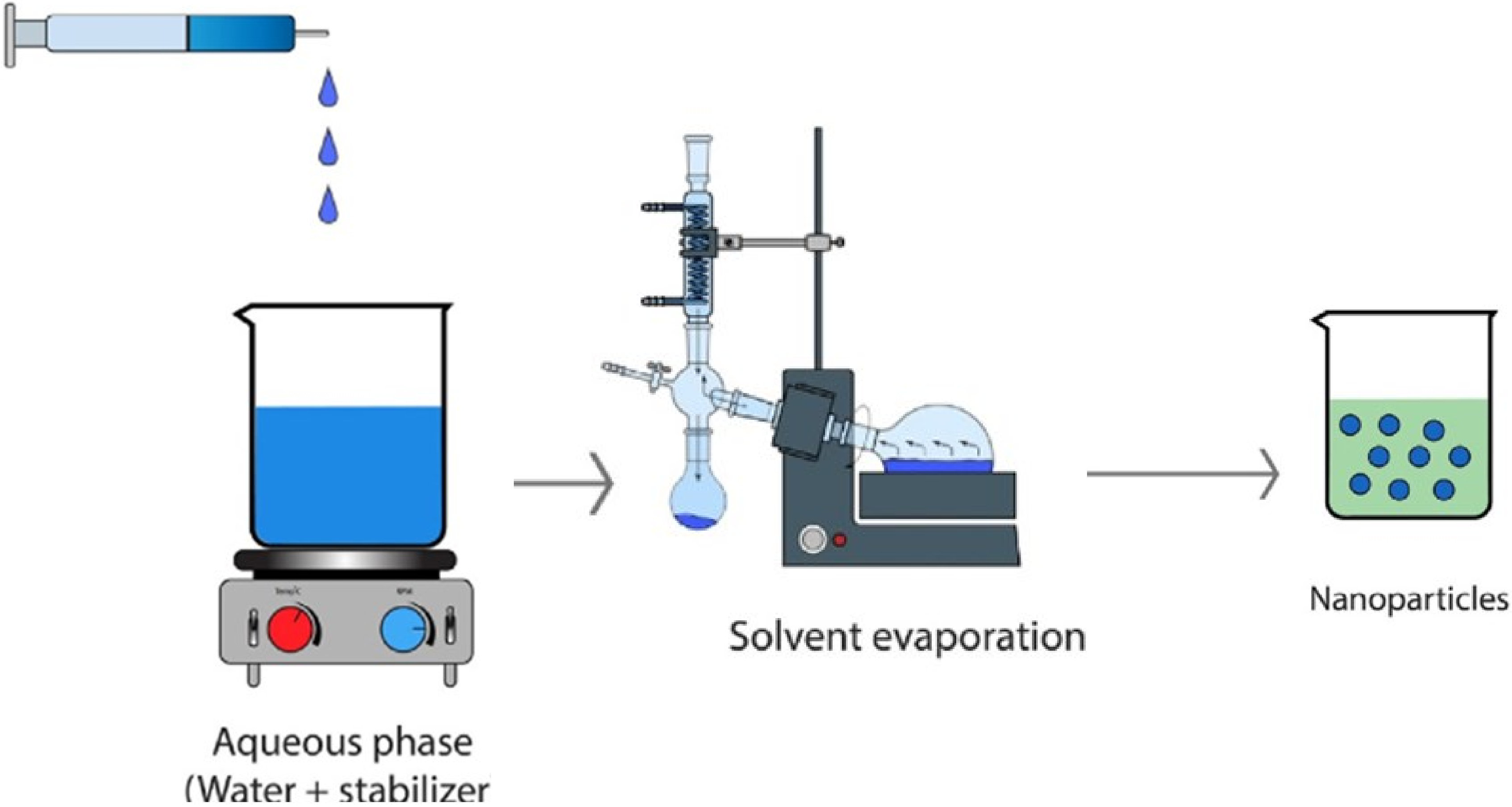
Recap



Lamer’s Diagram

Synthesis of Polymeric NPs

Nanoprecipitation



Rivas et al; International Journal of Pharmaceutics, **2017**, 66-81

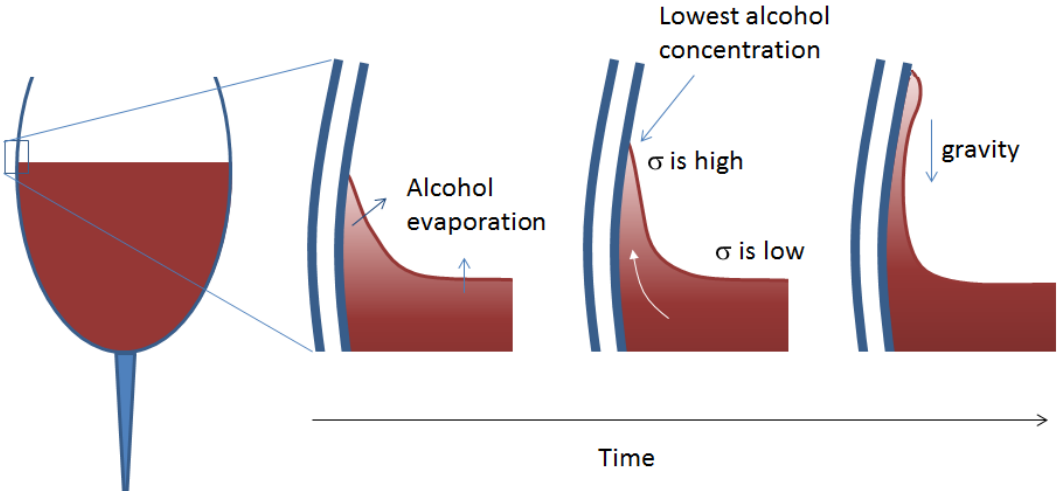
Nanoprecipitation

- Method to synthesize polymeric NPs.
- Components:
  - Solvent; containing polymer
  - Non solvent; containing surfactant
- Organic solvent can be removed by evaporation at ambient temperature or a rotavapour.
- Aqueous phase can be removed using ultracentrifugation or freeze-drying.
- Patented by Fessi et al. in 1989.
- Was originally designed to encapsulate hydrophobic drugs, however research has been conducted with hydrophilic drugs.

Rivas et al; International Journal of Pharmaceutics, **2017**, 66-81

Gibb's Marangoni Effect

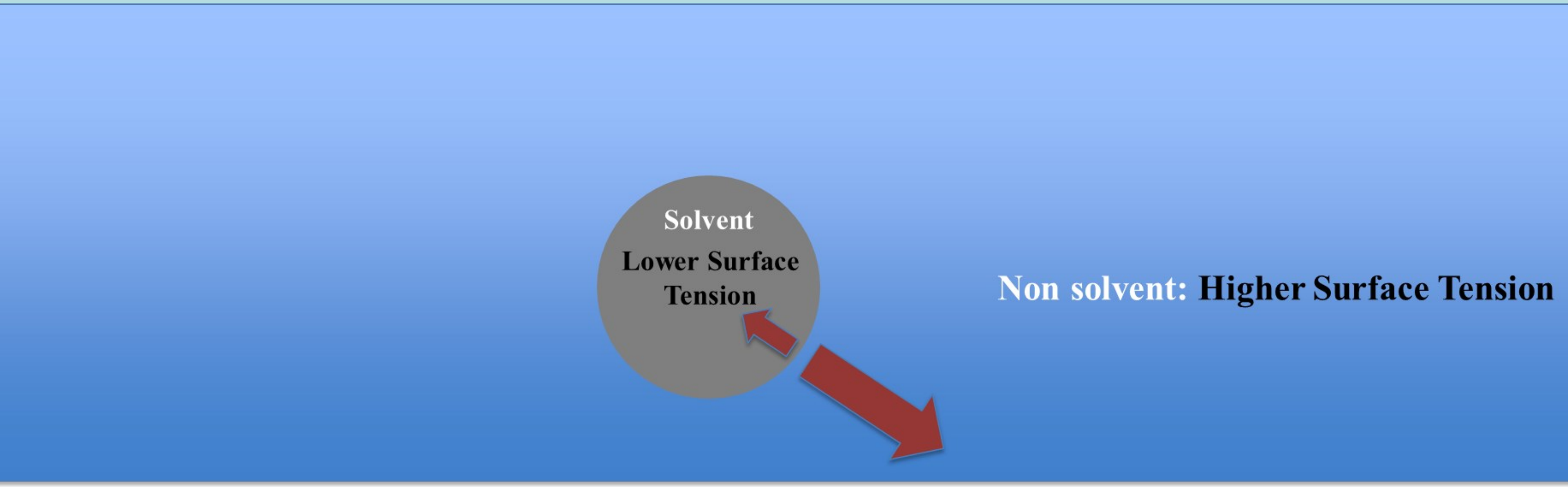
- Mass transfer along an interface between two fluids due to a gradient of the surface tension.
- First identified by James Thomson: **Tears of wine phenomenon**



https://www.comsol.com/multiphysics/marangoni-effect

Mechanism

- Surface tension gradient tends to contract or stretch the interface resulting from a **contractile force**.
- **Greater the surface tension: greater is the contractile force**



Mechanism

Nucleation and Growth Theory

- Three Stages:
  - Nucleation
  - Growth
  - Aggregation
- Supersaturation determines nucleation rate, that is further determined by fluid dynamics and phase mixing.
- Higher mixing rate; Smaller NP size

Gibbs-Marangoni Effect

- Difference in surface tension that causes interfacial turbulence and thermal inequalities.
- Thus, vortices are continuously formed and at the interface of both liquids.
- Diffusion of solvent to a region of high surface tension causes precipitation of the polymer resulting in NPs due to **aggregation**.
- NPs can be stabilised using a surfactant.

https://doi.org/10.1007/978-3-319-41421-8_2

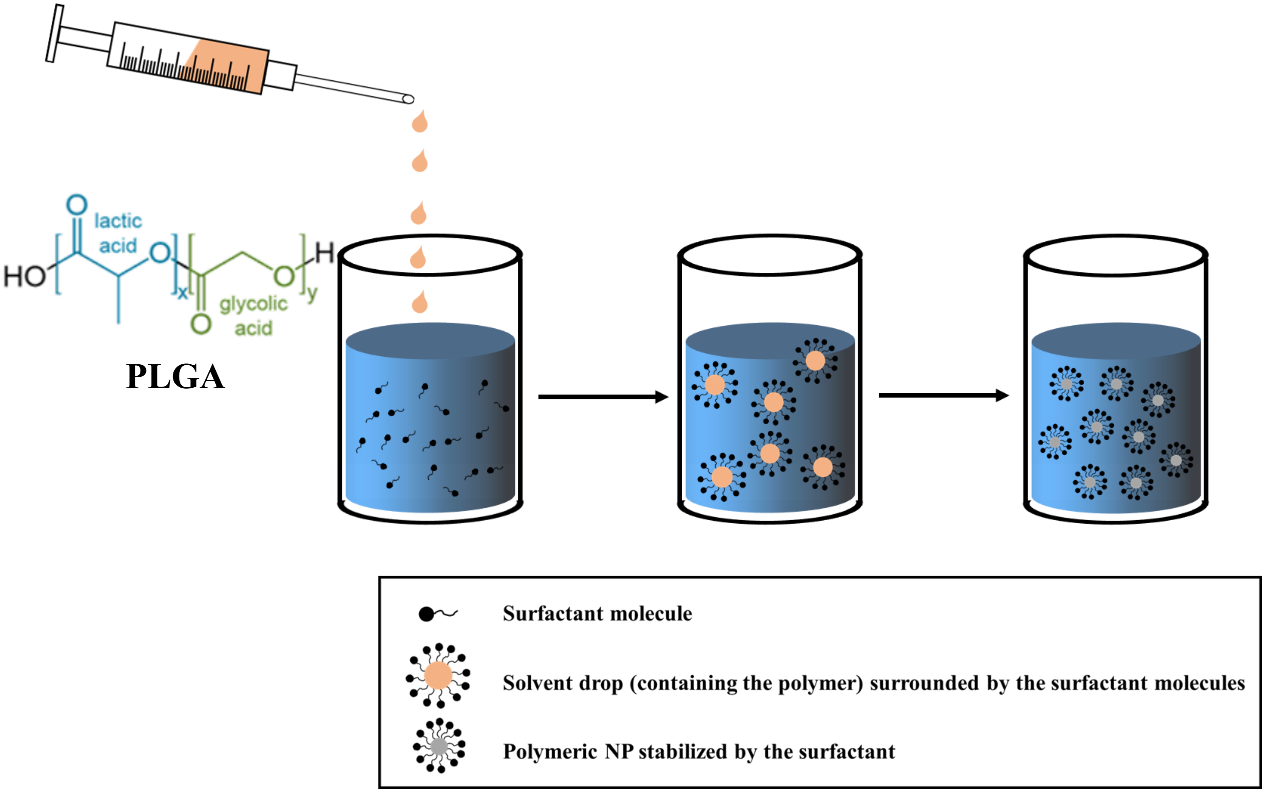
Parameters

- Stirring Rate
- Injection Rate
- Fraction of non-solvent phase
- Surfactant concentration
- Polymer concentration
- Polymer molecular weight
- Solvent selection
- Temperature

Lince et al; Journal of Colloid and Interface Science, **2008**, 505-515; Miladi et al., Nanoprecipitation Process: From Particle Preparation to In Vivo Applications, **2016**, 17-53

This is a drop of a **water-alcohol mixture**.

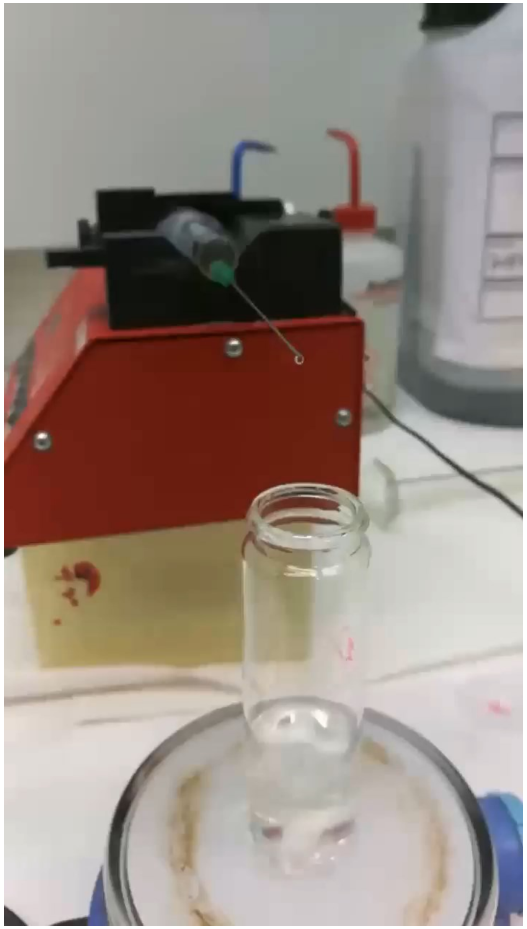
Polymer Coated Magnetic NPs



Surfactant molecule

Solvent drop (containing the polymer) surrounded by the surfactant molecules

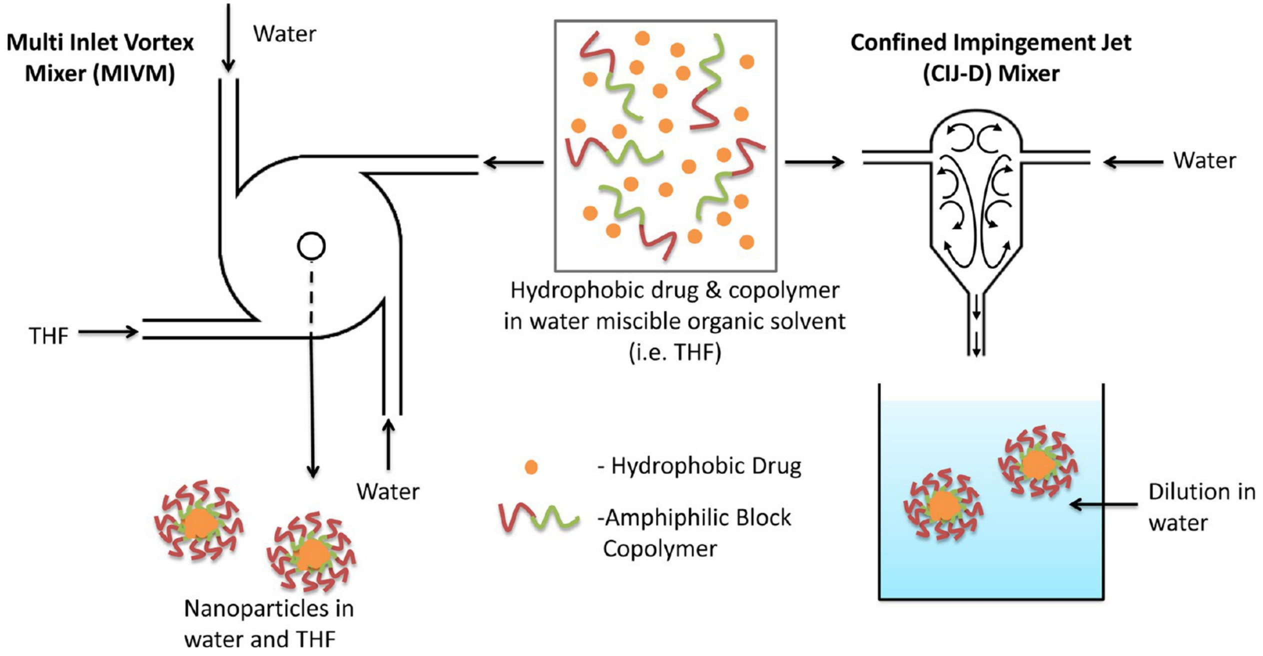
Polymeric NP stabilized by the surfactant



2. Flash Nanoprecipitation

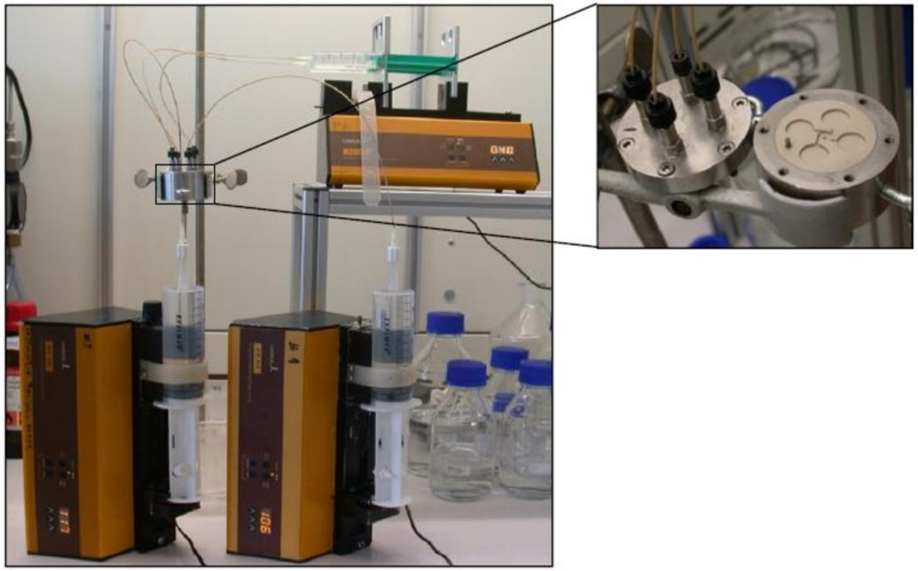
- Rapid micromixing
  - Solvent , anti-solvent mix at a time scale shorter than formation of NPs
- Creation of high supersaturation
- Leads to precipitation of dissolved hydrophobic components
- Mixing time must be less than induction time for polymer aggregation and induction time for nucleation & growth.
- Kinetically controlled process
- Narrow particle size distribution

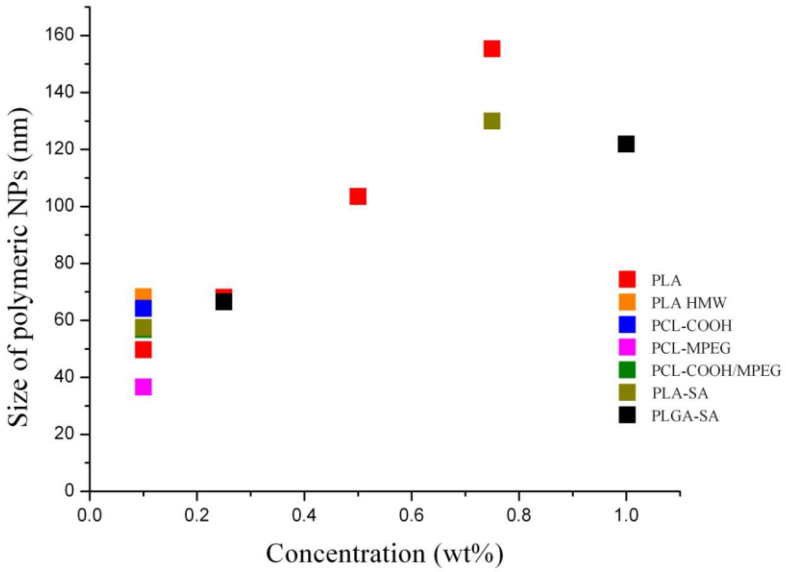
MIVM Setup



Pustulka et al. , Mol. Pharmaceutics, **2013**, 4367-4377

MIVM Setup





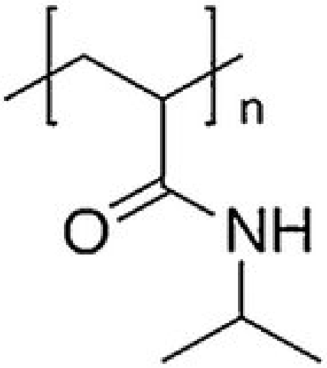
Multi Inlet Vortex Mixer (MIVM) setup for Flash Nanoprecipitation.

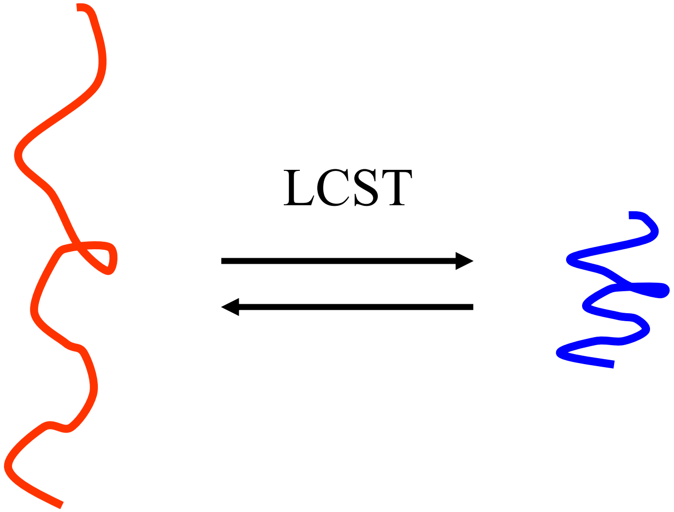
Hydrodynamic sizes of polymeric NPs as a function of concentration.

Sulalit Bandyopadhyay, MSc Thesis, 2010

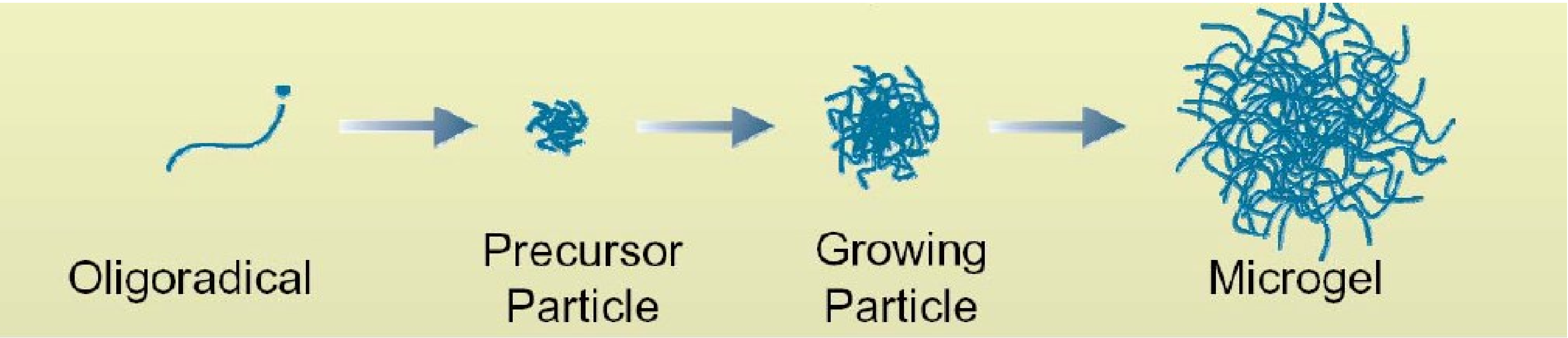
Precipitation Polymerization

- Polymers like Poly(N-isopropyl acrylamide) (PNIPAm) dissolved in water undergo a coil-to-globule transition at temperatures exceeding its lower critical solution temperature (LCST) (~32 ^{0}C)
- The LCST/cloud point can be tuned by altering the composition of a PNIPAm-containing block copolymer
- LCST also affected by the mobility of the PNIPAm chains





Mechanism

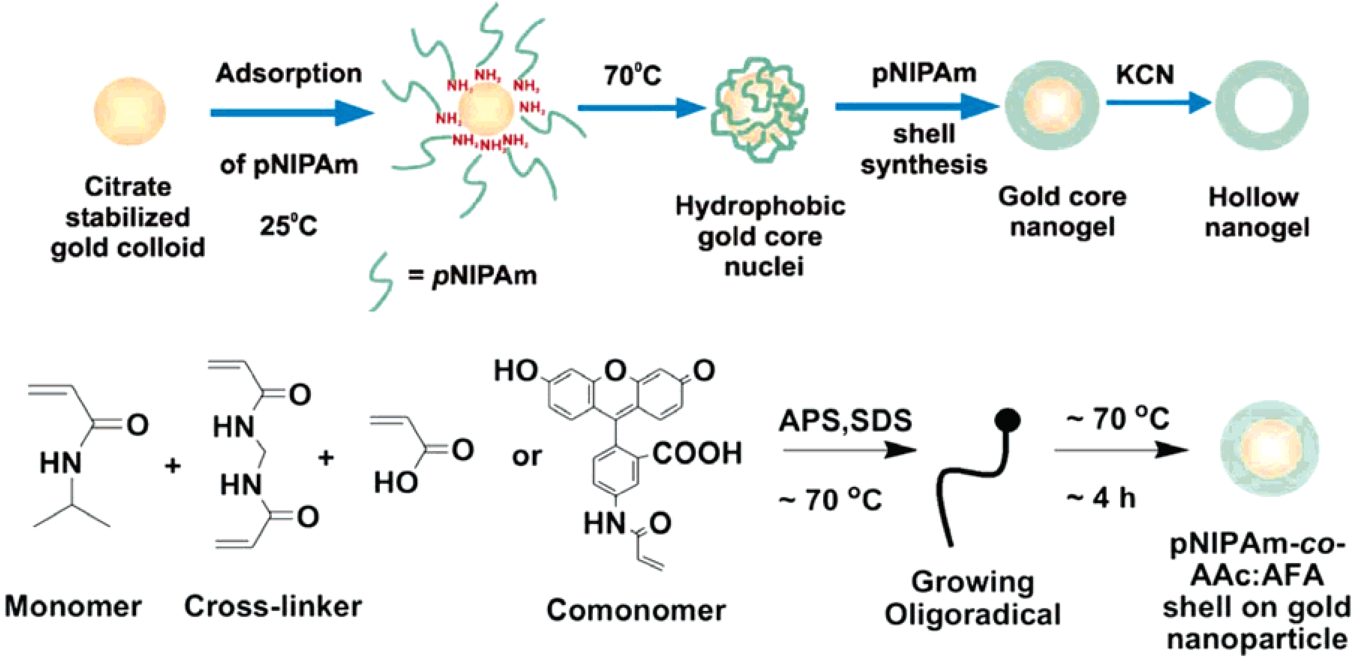


A nucleation and growth process

- Monomer + cross linking agent in water
- T > LCST
- Homogeneous nucleation on collapsed oligomers
- Oligomer and monomer addition, aggregation
- Low PDI, control of charge, size, cross-link density

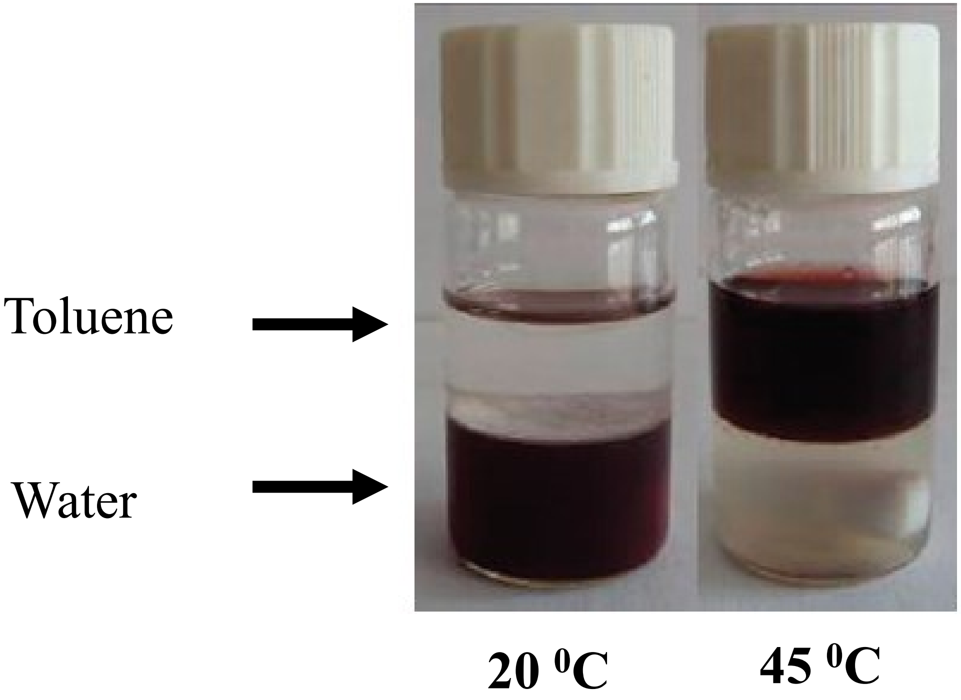
Singh, N., Lyon, L.A, *Chem. Mater.* **2007,** *19,* 719-726

Au-pNIPAm



Singh, N., Lyon, L.A, *Chem. Mater.* **2007,** *19,* 719-726

Phase Transfer



Polarity and phase behavior of the Au NP constructs tunable via temperature

Wen, Y., Jiang, X., Yin, G., and Yin, J., *Chem. Commun.*, **2009**, 6595-6597

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NANOMATERIALS

Assoc. prof. Elisabeth Jacobsen

Dr. Vegar Ottesen

Dr. Sulalit Bandyopadhyay

Zeeshan Ali, PhD student

Nesrine Bali, PhD student

Katharina Zürbes, PhD student

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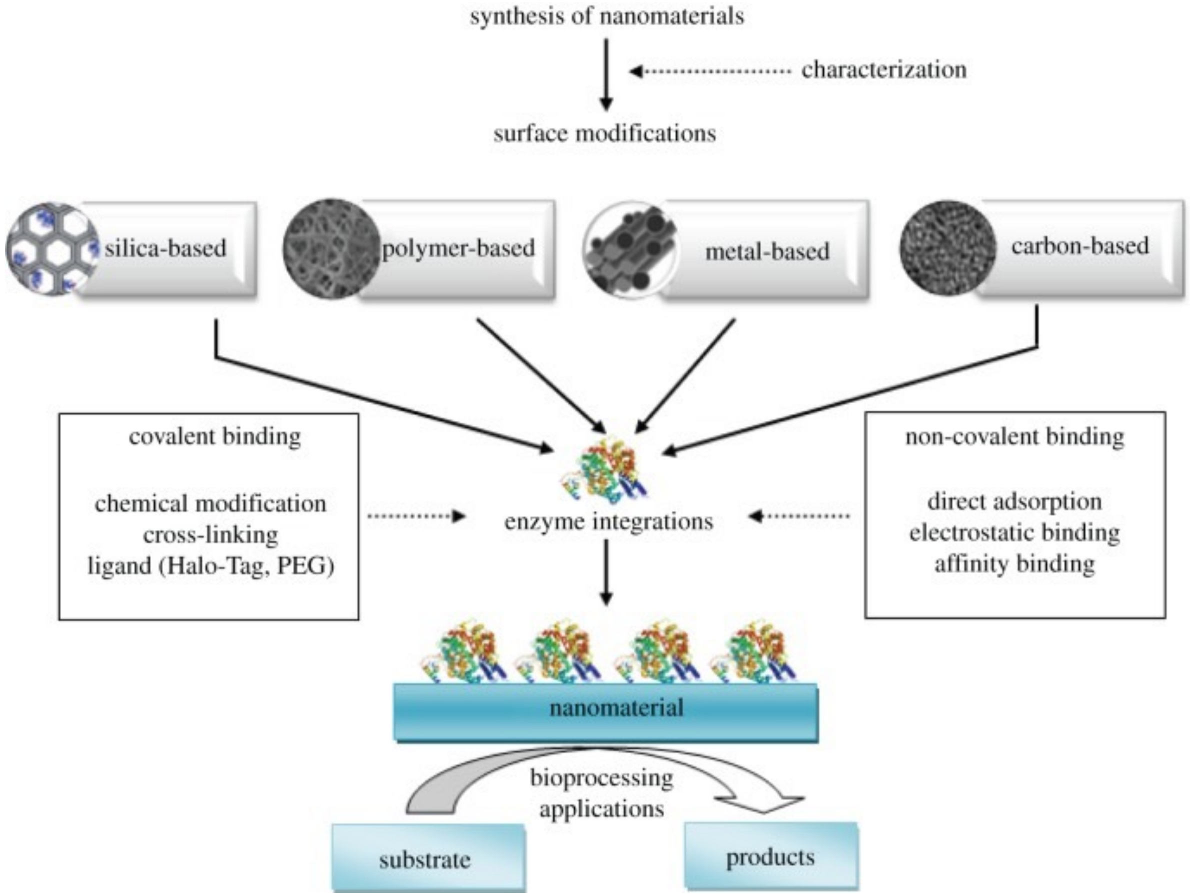
I. Applications from bio-nanocatalysis

Thursday April 8th *10:15 - 12:00* Romania time



Misson *et al*, 2015

Norwegian University of Science and Technology

Agenda

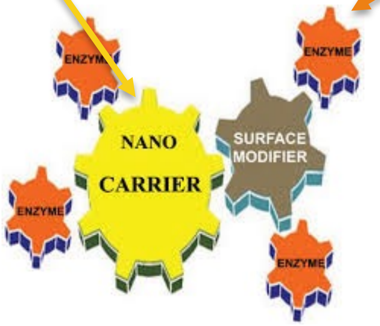
- Definitions
- Benefits of coupling enzymes to nanostructures:

(is it still a nanostructure and is it green chemistry??)

1. Increasing effectiveness?
2. Increasing stability?
3. Sustainability (re-useable)?

Fossil based?

Bio based?



- Cascade reactions with biocatalysis and nanocat.
- Production of biofuels by Biocat/Nanotech
- Production of agrochemicals by Biocat/Nanotech

Definition of biocatalysis

Biocatalysis is “the use of natural substances to speed up (catalyze) chemical reactions”.

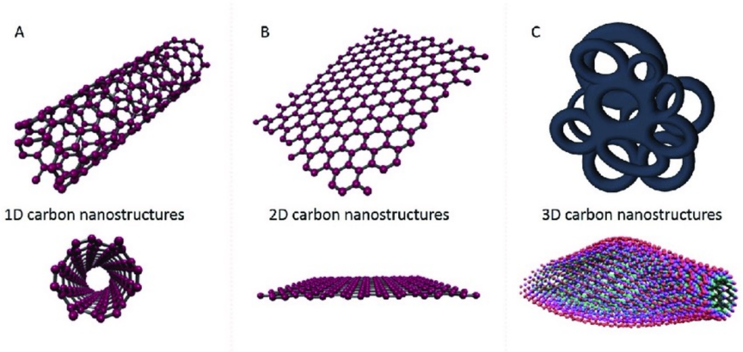
In most cases, a group of proteins called enzymes will be carrying out the catalysis, but a combination of enzymes as well as cells can be used.

These enzymes can be taken from the cell, either from the original cell or from a different cell that was modified to produce the enzyme.

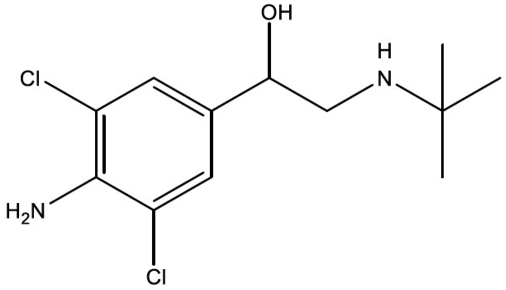
Definition of nanostructure

A nanostructure is a structure of intermediate size between microscopic and molecular structures.

Nanostructural detail is microstructure at nanoscale.



Carbon nanostructures-large polymers!



Clenbuterol- a molecule based on benzene- why not nanostructure?

In describing **nanostructures**, it is necessary to differentiate between the number of dimensions in the volume of an object which are on the nanoscale. (Wikipedia)

Definition of nanotechnology

Nanotechnology is the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications.

Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this small scale.

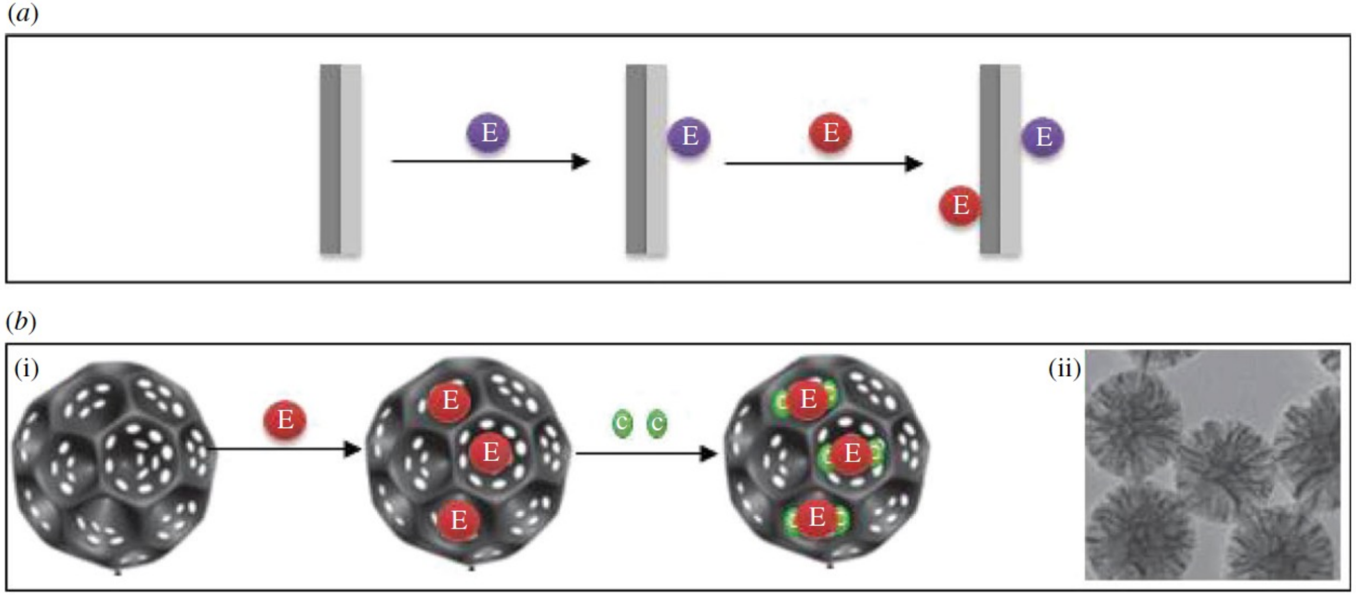
National Nanotechnology Initiative (NNI)

Nanofibers (NF´s=polymers) + enzymes

Immobilization onto solid supports may reduce enzyme activity, f. inst bovine serum albumin (BSA), penicillin acylase and β-galactosidase. This belief may not be true when enzymes are immobilized onto nanocarriers.

NF´s offer a high surface-to-volume ratio to show a high adsorption capacity of enzyme loading:

enzymes penetrate the polymer and attach into inner region of polymer – giving optimal substrate diffusion



(a) Side-by-side hybrid nanofibers promote immobilization of two enzymes to perform simultaneous reactions.
(b) Schematic illustration of dendrimer-like nanoporous silica for the co-immobilization of enzyme with cofactors or other biomolecules (i), TEM image of dendrimer-like nanopores silica (ii)

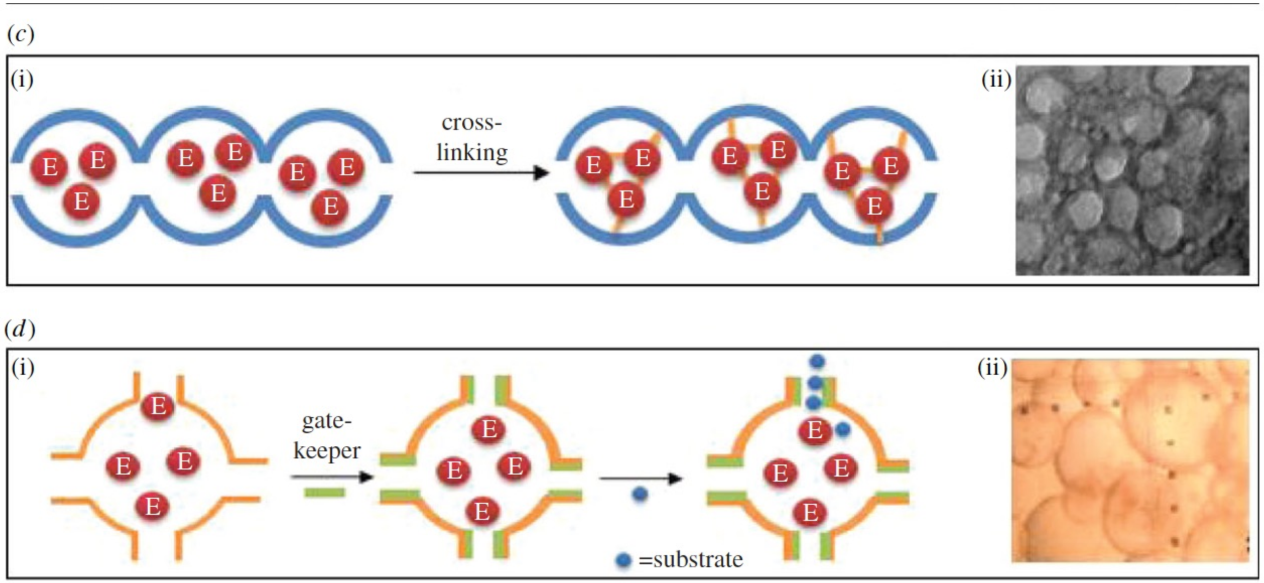
Du *et al* 2013

Nanocages (mesoporous silika) + enzymes

Enzymes can be attached to
- fibre surface or
- enclosed inside the pores.

However, enzyme leaching can be an issue if only the enzymes are attached by physical adsorption. Enzyme reactivity could be affected due to conformational change or is reduced by exposing to cross-linking reagents.

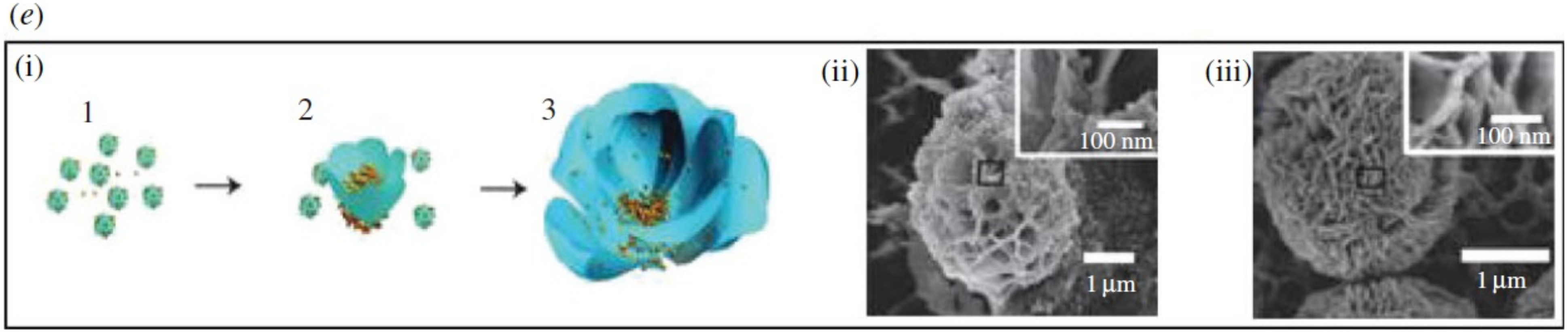
To encapsulate the enzyme molecules inside a nanoscale container is a promising approach to maintain enzyme activity
- substrates can freely diffuse into and out of the container



(c) Schematic illustration (i) and TEM image (ii) of ship-in-a-bottle pore structures to retain and stabilize enzymes inside the nanocages. (cross linking w glutaraldehyde) Pore size only large enough for diffusion of small molecules, not enzymes.
(d) Schematic illustration (i) and optical micrograph (ii) of nanocages with substrate-diffusion gatekeepers to prevent enzyme leaching.

Lee *et al.* 2005 , Liu *et al.* 2013

Nanoflowers + enzymes

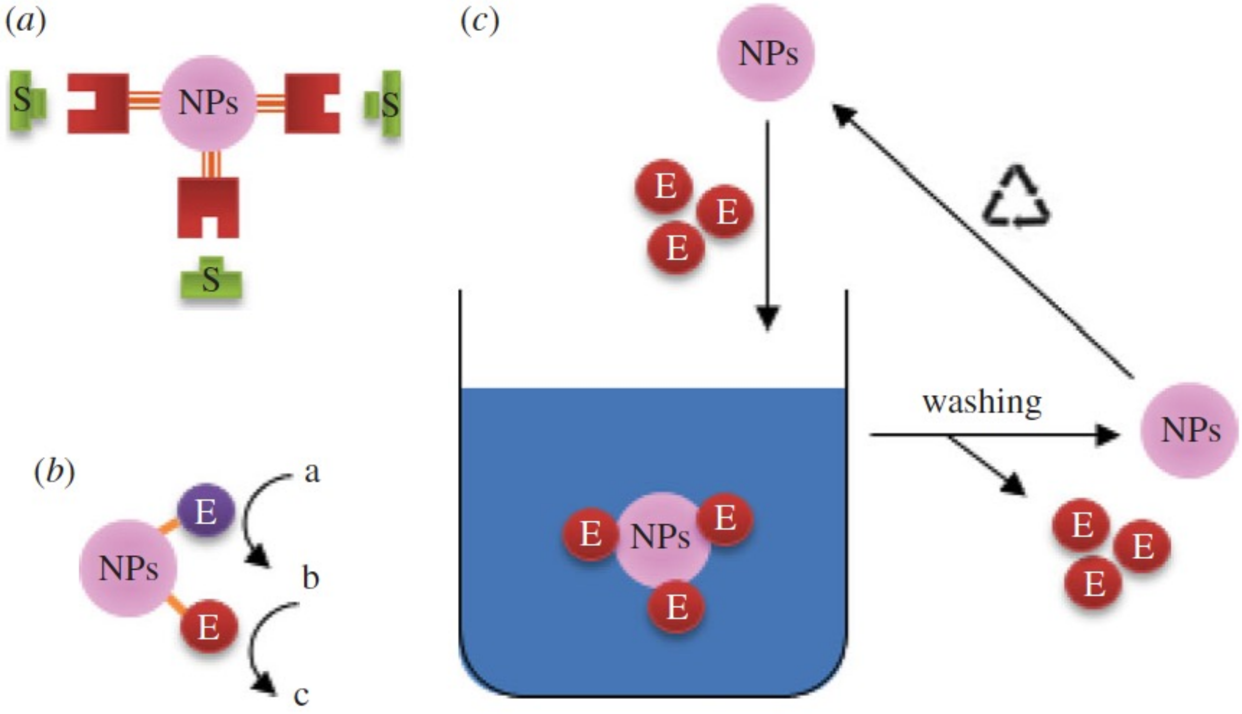


(e) Schematic diagram (i) and SEM images of the formation of Bovine Serum Albumin (BSA)-incorporated $Cu_3(PO_4)_2.3H_2O$ nanoflowers (spheres in nanoflowers' core as protein molecules) at 12 h (ii) and 3 days (iii)

The hybrid nanoflower exhibit enhanced enzymatic activity and stability compared with free enzymes, which may be attributed to the confinement of the enzyme in the core of the nanoflower.

Enzymes : α-lactalbumin, laccase, carbonic anhydrase and lipase Ge *et al*, 2012

Engineering performance of NanoBioCatalysts in bioprocess applications.



(a) Enhancing enzyme activity by stabilizing the enzyme reactive sites towards the substrate.

(b) Accelerating biocatalysis through cascade reactions of the co-immobilized enzymes in one-pot medium.

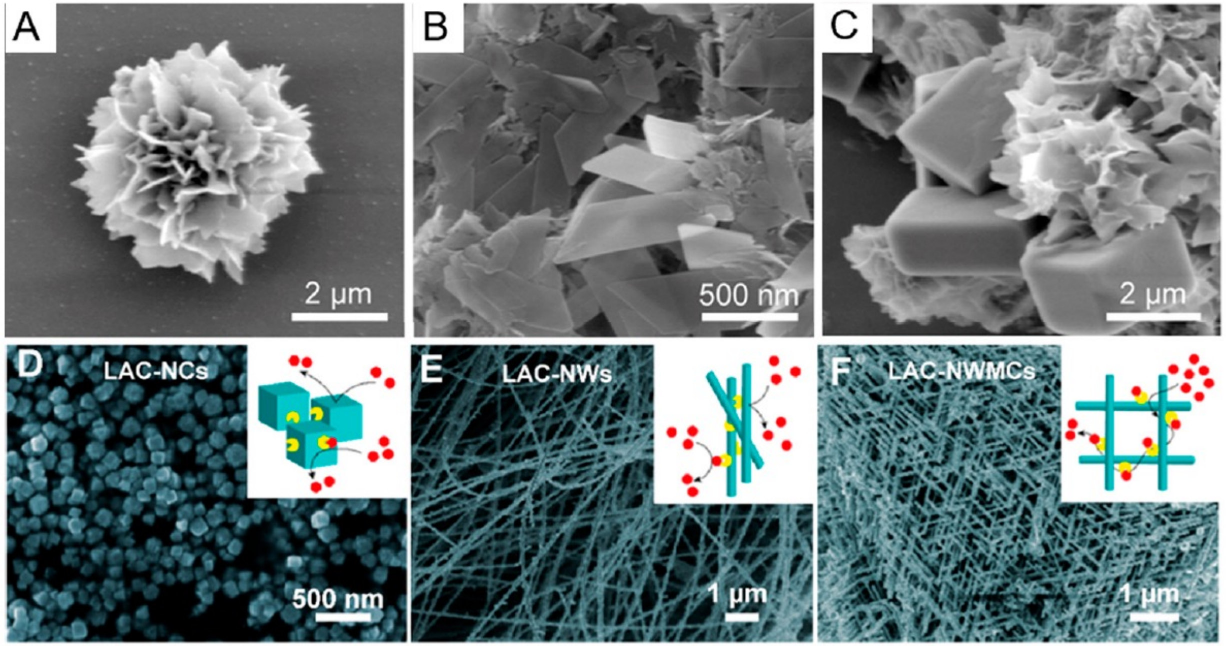
(c) Recycling the nanocarriers after the enzyme activity decays.

f. Inst Integration of magnetic technology with the enzyme immobilization on the nanocarriers can enhance recoverability and reusability of the NBC´s

Misson *et al*, 2015

Coupling of the α-amylase and laccase to nanostructures

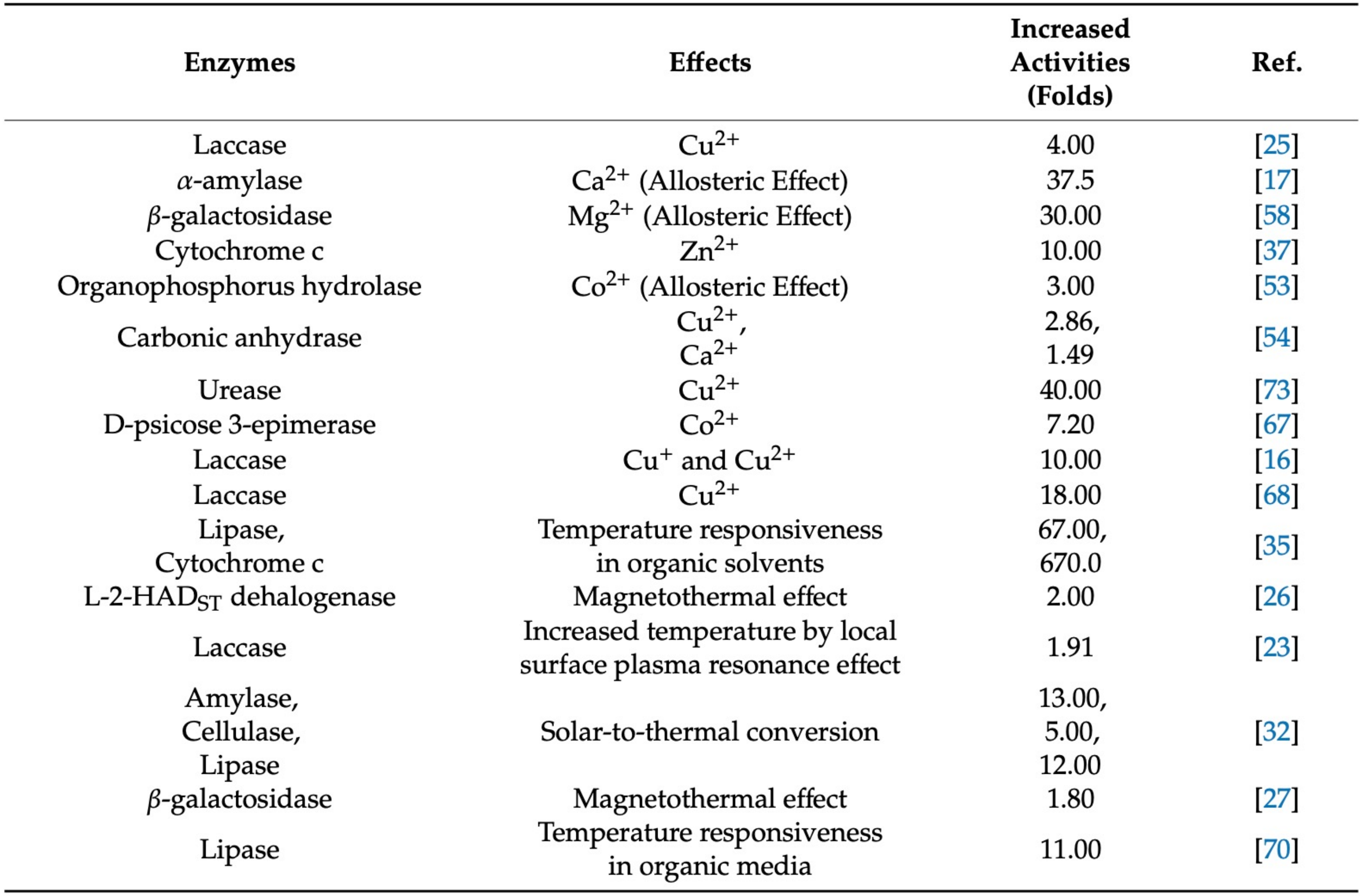
SEM images of $CaHPO_4$-α-amylase nanobiocatalysts, (A) nanoflowers, (B) nanoplates, and (C) parallel hexahedrons. (Wang *et al*, 2013, (ref [17] in An *et al* 2010))



SEM images of Cu_2O–laccase nanobiocatalysts, (D) nanocubes, (E) nanowires, and (F) nanowire mesocrystal, insets are the schematic illustrations of the plausible substrate diffusion pathways for these hybrid materials. (Li *et al*, 2018, ref [16] in An *et al* 2010)

An *et al* 2018 (review)

Effects of metal ion and temperature on enhanced activities of immobilized enzymes

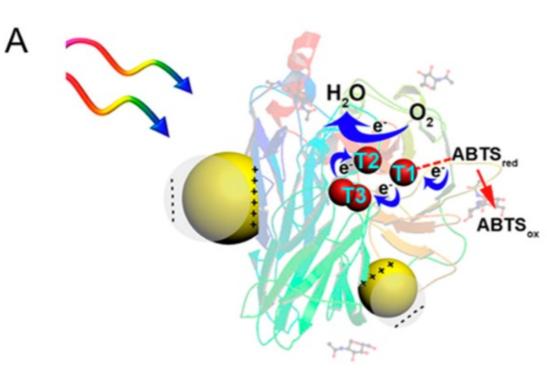


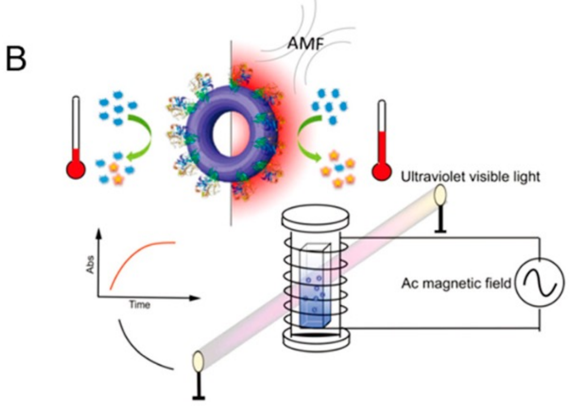
Enzymes	Effects	Increased Activities (Folds)	Ref.
Laccase	Cu^{2+}	4.00	[25]
α-amylase	Ca^{2+} (Allosteric Effect)	37.5	[17]
β-galactosidase	Mg^{2+} (Allosteric Effect)	30.00	[58]
Cytochrome c	Zn^{2+}	10.00	[37]
Organophosphorus hydrolase	Co^{2+} (Allosteric Effect)	3.00	[53]
Carbonic anhydrase	Cu^{2+}, Ca^{2+}	2.86, 1.49	[54]
Urease	Cu^{2+}	40.00	[73]
D-psicose 3-epimerase	Co^{2+}	7.20	[67]
Laccase	Cu^{+} and Cu^{2+}	10.00	[16]
Laccase	Cu^{2+}	18.00	[68]
Lipase, Cytochrome c	Temperature responsiveness in organic solvents	67.00, 670.0	[35]
L-2-HAD$_{ST}$ dehalogenase	Magnetothermal effect	2.00	[26]
Laccase	Increased temperature by local surface plasma resonance effect	1.91	[23]
Amylase, Cellulase, Lipase	Solar-to-thermal conversion	13.00, 5.00, 12.00	[32]
β-galactosidase	Magnetothermal effect	1.80	[27]
Lipase	Temperature responsiveness in organic media	11.00	[70]

An *et al* 2018 (review)

Experimental set ups

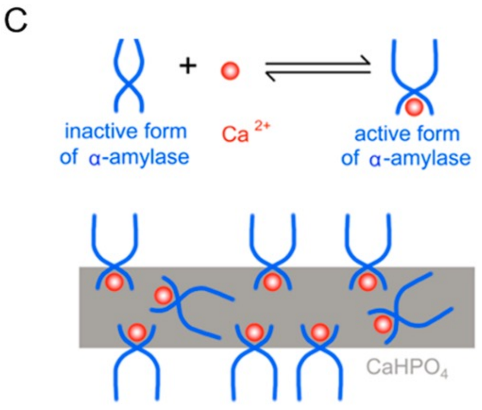
A. Au–laccase hybrids with enhanced electron transfer

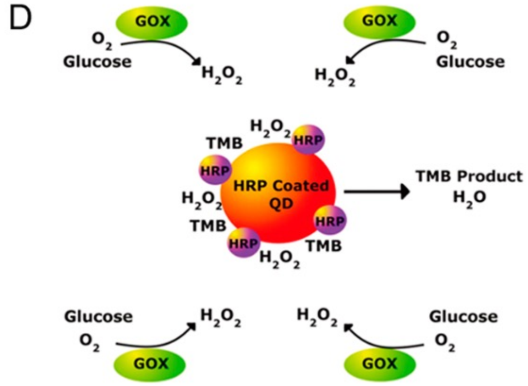




B. Experimental set-up β-Gal hybrids

C. The α-amylase-$CaHPO_4$ nanoflower nanobiocatalyst. Ca^+ binds to allosteric sites in inactive α-amylase and generates active α-amylase





D. Diagram of the GOX/HRP–CdSe/ZnS QDs system with enhanced coupled enzymatic activity.

An *et al* 2018 (review)

Magnetic nanoparticles

Magnetic nanoparticles (MNP) suitable as supports to enzymes due to:

- low toxicity
- flexible surface modification by chemical reactions
- large enzyme capacity
- good reusability [30-in de Jesús Rostro-Alanis].

Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) widely used:

- low toxicity
- stability
- availability
- low environmental impact
- small size
- super-paramagnetic properties
- ease of separation from the reaction media [31–33-in de Jesús Rostro-Alanis.].

de Jesús Rostro-Alanis *et al*, 2016

Nanostructure characteristics enable design of robust biocatalysts

Main advantages of nanomaterials for immobilization of enzymes:

1. possibility of fine tuning the biological activity by designing specific materials

2. high surface area - allows for a high loading of enzyme.

Desirable characteristics of Nanostructured materials for support for enzymes:

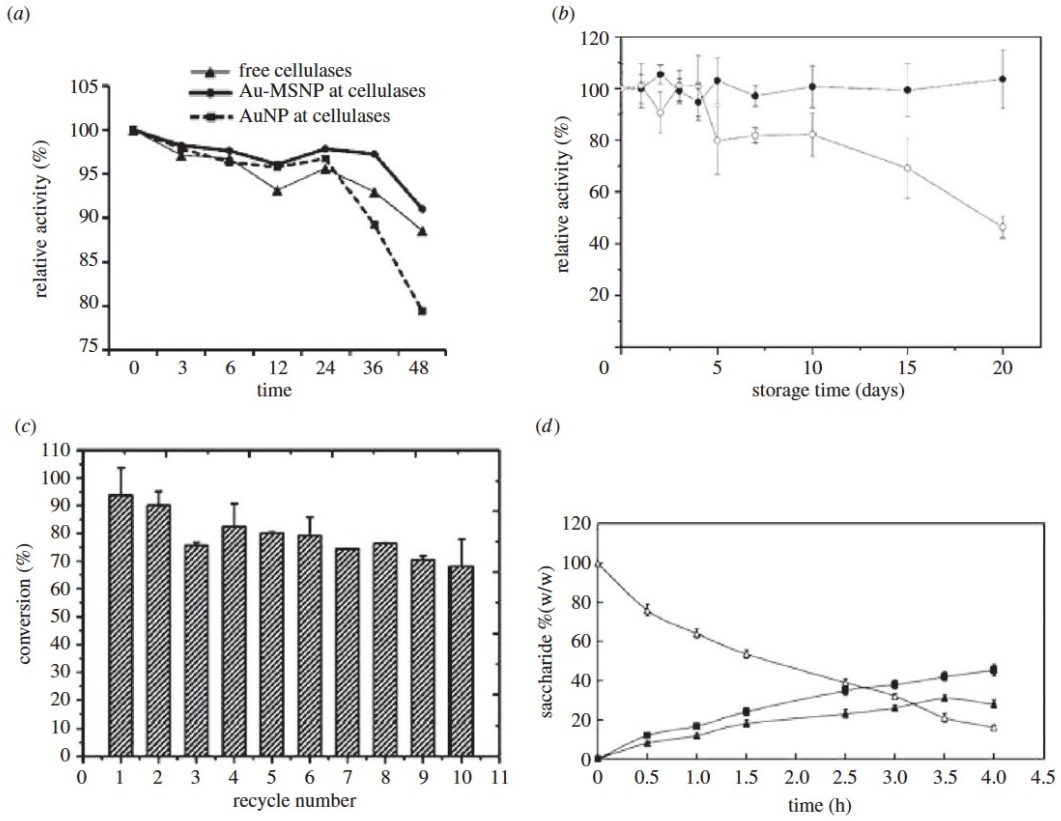
1. pore diameter on the scale of nanometers (5–100 nm)
2. hardness
3. defined geometry
4. hydrophobicity/hydrophilicity ratio
5. conductivity
6. magnetic properties

de Jesús Rostro-Alanis *et al*, 2016

Applications of NBC´s in bioprocesses.

(a) Extension of enzyme activity from 24 to 36 h by immobilized cellulases on Au-magnetic silica NP´s [136] in Misson 2015.

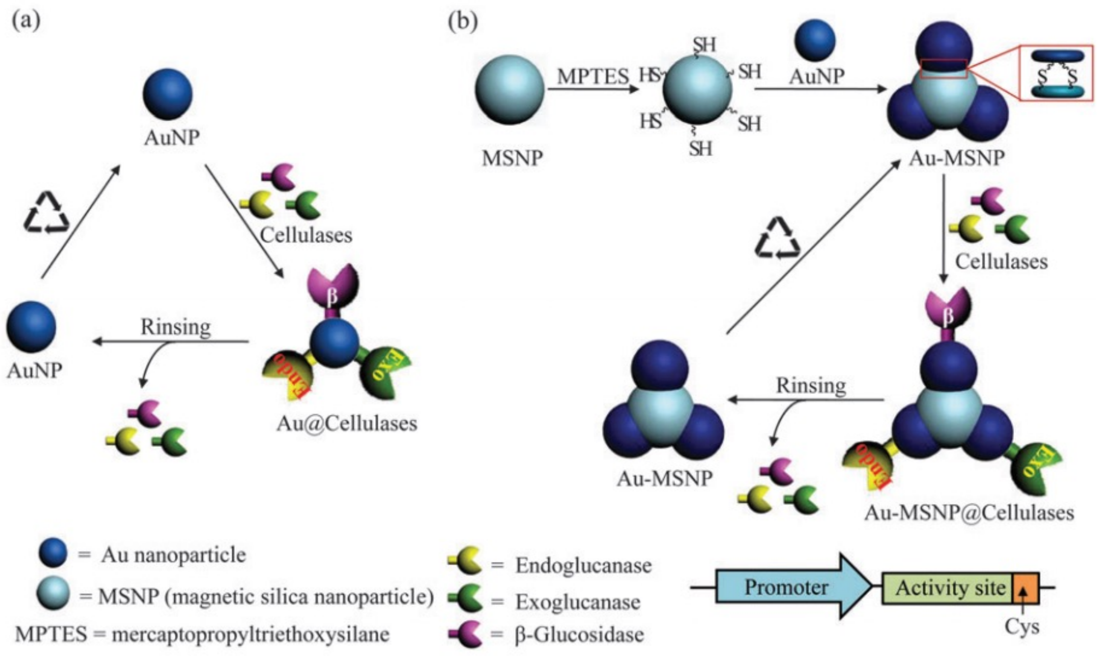
(c) Recyclability of lipase-nanoporous gold biocomposite for catalytic conversion of soybean oil to biodiesel [66] in Misson 2015.



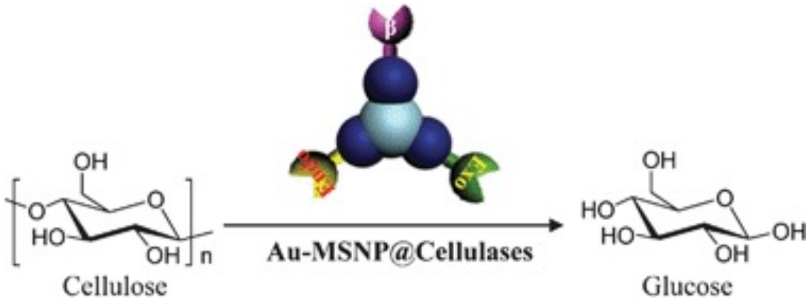
(b) Storage stability of free lipase (unfilled circles) and immobilized lipase (filled circles) on polyacrylonitrile nanofibrous membrane [145] in Misson 2015.

(d) Synthesis of GalactoOligoSaccharide (GOS) from lactose conversion by β-galactosidase-nanospheres (unfilled triangles, lactose; filled triangles, total GOS; filledsquares,monosaccharide) [146] in Misson 2015.

Co-immobilization of three cellulases on Au-doped magnetic silica nanoparticles for the degradation of cellulose



Scheme 1 Overall schemes for the synthesis of the cellulases immobilized on (a) **AuNP** and (b) **Au-MSNP**.



Cho *et al*, 2012

Large scale biocatalysis/nanocatalysis POTENSIAL

Immobilized enzymes used for large-scale industrial processes:

1. glucose isomerase for production of fructose corn syrup(HFCS) (107 tons per annum)
2. lipase for transesterification of food oils (105 tons per annum)
3. penicillin G acylase for antibiotic modification (104 tons per annum)

HOWEVER:

Studies on the development and application of nanocarrier-based NBC´s for bioprocesses still carried out in laboratory-scale bioreactor

A successful case using NBC´s in LARGE SCALE industrial bioprocesses has not been found in the literature so far.

Discussion: HOW CAN THIS UPSCALING BE POSSIBLE?

References nanobiocatalysis- from An *et al*, 2018

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Cascade enzyme catalysis-nanocatalysis

One pot combination

Benefits over traditional prosesses:

1. Avoiding purification and isolation of intermediates

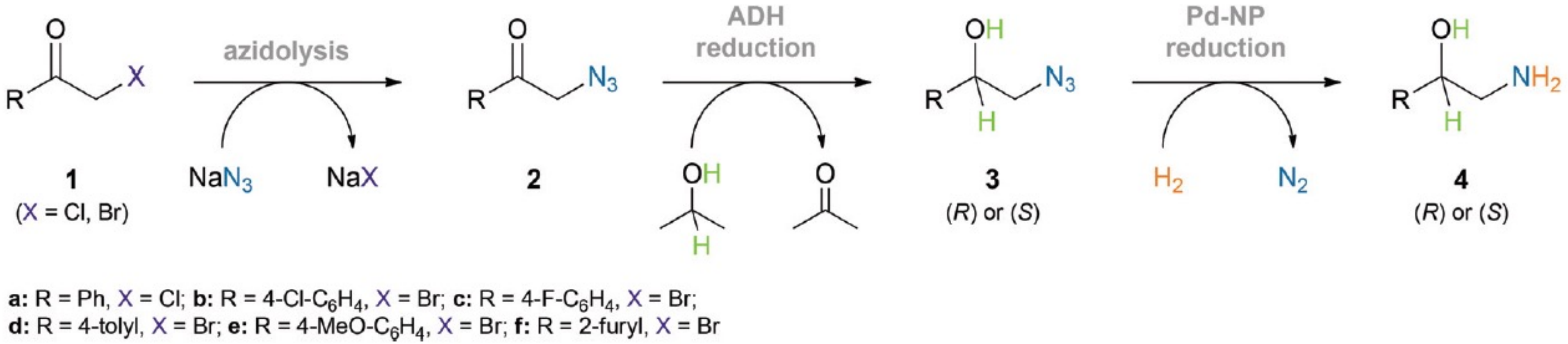
2. low ecological footprint, quantified by
   - the *E*-factor
   - solvent demand

The ideal *E*-factor is zero.
Kilograms of raw materials in, minus kilograms of desired product, divided by kilograms of product out.

Sheldon, 2017

Cascade enzyme catalysis-nanocatalysis

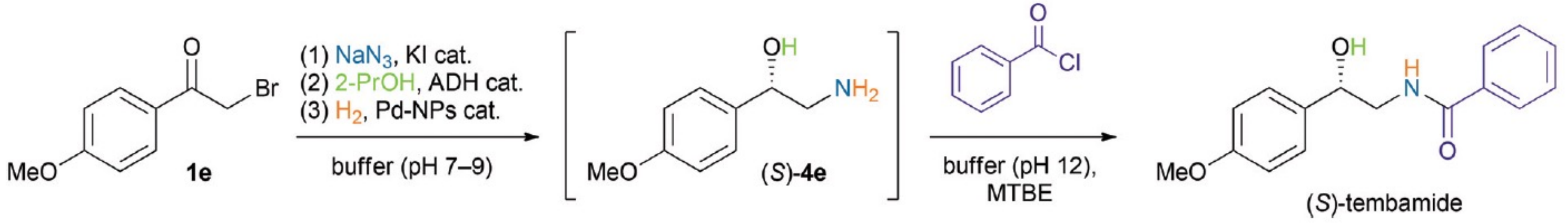
One pot combination



Scheme: Chemo-enzymatic approach towards optically pure 1,2-amino alcohols via azidolysis, alcohol dehydrogenase (ADH) catalysed asymmetric reduction, and Pd nanoparticle (Pd-NP) catalysed azide hydrogenation

Schrittwieser *et. al,* 2013

Antiviral natural product (*S*)-tembamide (**1**), 73% yield, *ee* >99%



Scheme: Asymmetric synthesis of (*S*)-tembamide in a chemo-enzymatic four-step one-pot sequence.

Table: Environmental impact comparison of catalytic asymmetric syntheses of tembamide

Article	Steps[a]	Asymmetric key step	Yield[b] [%]	*E*-factor[c]	Solvent[d] [mL g^{-1}]
Present work	4 (1)	Asymmetric ketone reduction (ADH)	73	11.1	309
Lee *et al.* 2007	5 (5)	Asymmetric ketone reduction (Rh catalyst)	62	57.8	1600
Baeza *et al.* 2005	3 (2)	Asymmetric cyano-*O*-phosphorylation (Lewis acid/Brønsted base catalyst)	65	23.3	1031
Kamal *et al.* 2004	5 (4)	Enantioselective transesterification (lipase)	42	114.9	1801
Yadav *et al.* 2001	3 (2)[e]	Asymmetric ketone reduction (carrot root)	85	97.5	826
Brown *et al.* 1993, 1994	3 (3)	Asymmetric hydrocyanation (peptide catalyst)	72	14.6	483

Schrittwieser *et. al,* 2013

One-Pot Combination of Metal- and Bio-Catalysis in Water
Synthesis of enantiopure molecules

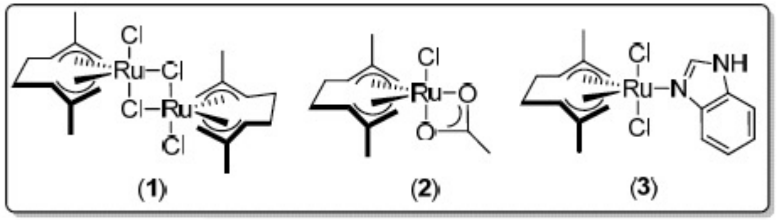
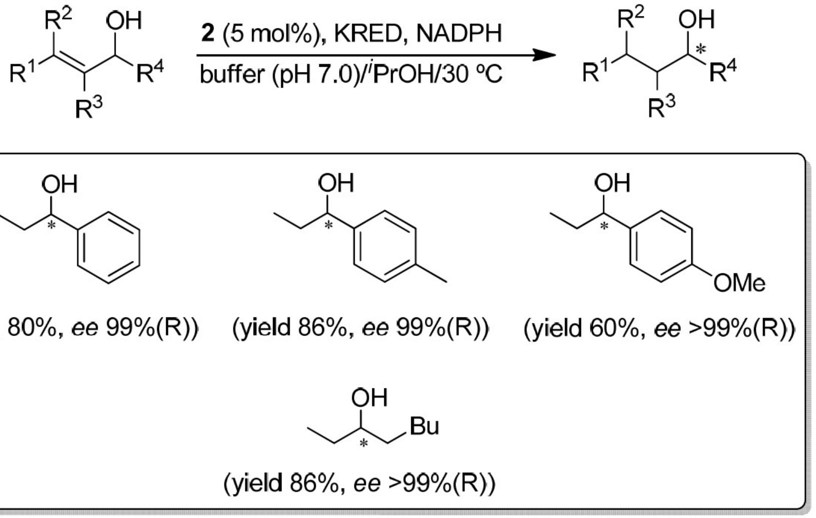
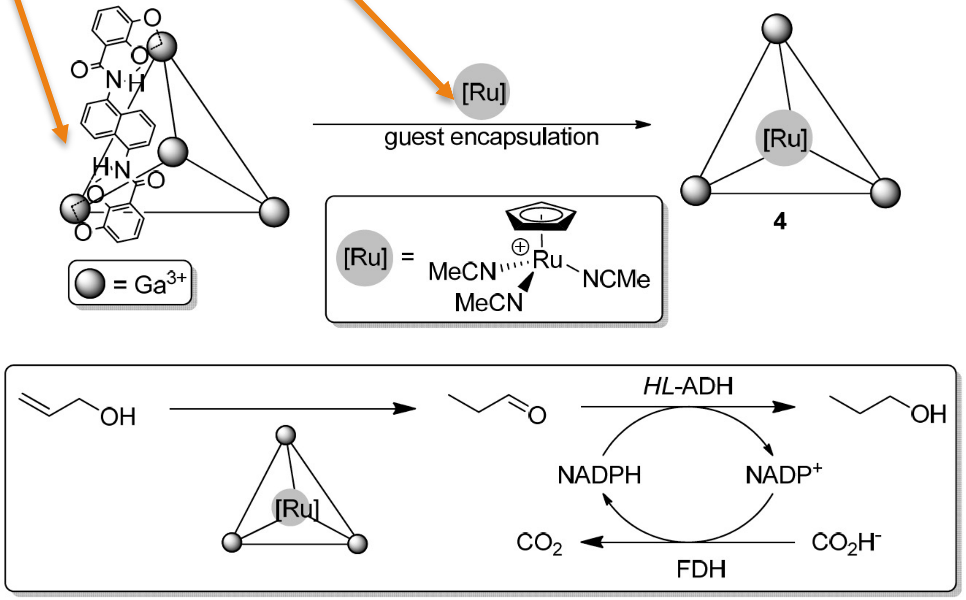


Fig. Highly-efficient and selective bis(allyl)-ruthenium(IV) catalysts (**1**–**3**) for the redox isomerization of allylic alcohols in water and under mild reaction conditions.



Ríos-Lombardía *et al*, 2018

Host –guest (nano) metal complexes + biocatalyst



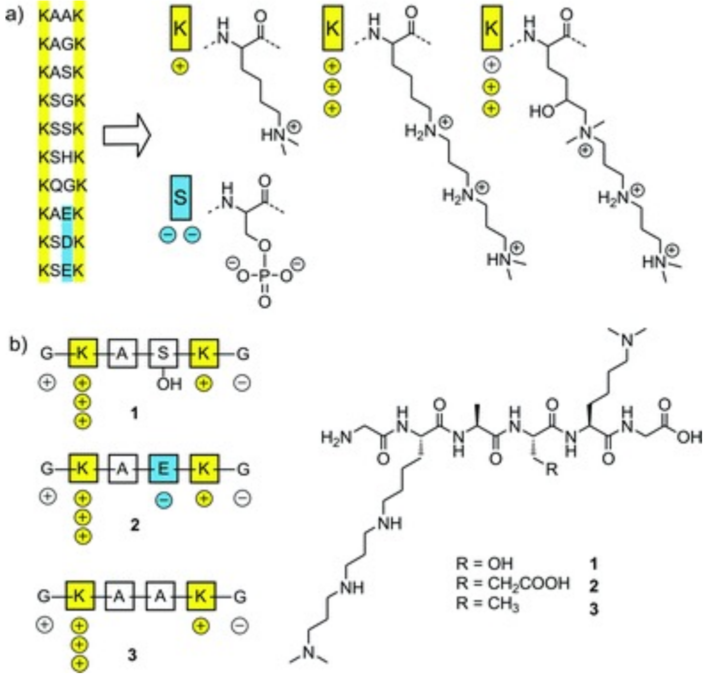
Synthesis of Ru(II)-host-guest Complex **4** and the design of a tandem isomerization/bioreduction of the allylic alcohol 2-propenol

Ríos-Lombardía *et al*, 2018

Another promising application of nanotechnology in the biofuel industry is enzyme (biocatalysts) immobilization during lipase-catalyzed biodiesel and cellulosic ethanol production processes (Kim *et al*., 2018).

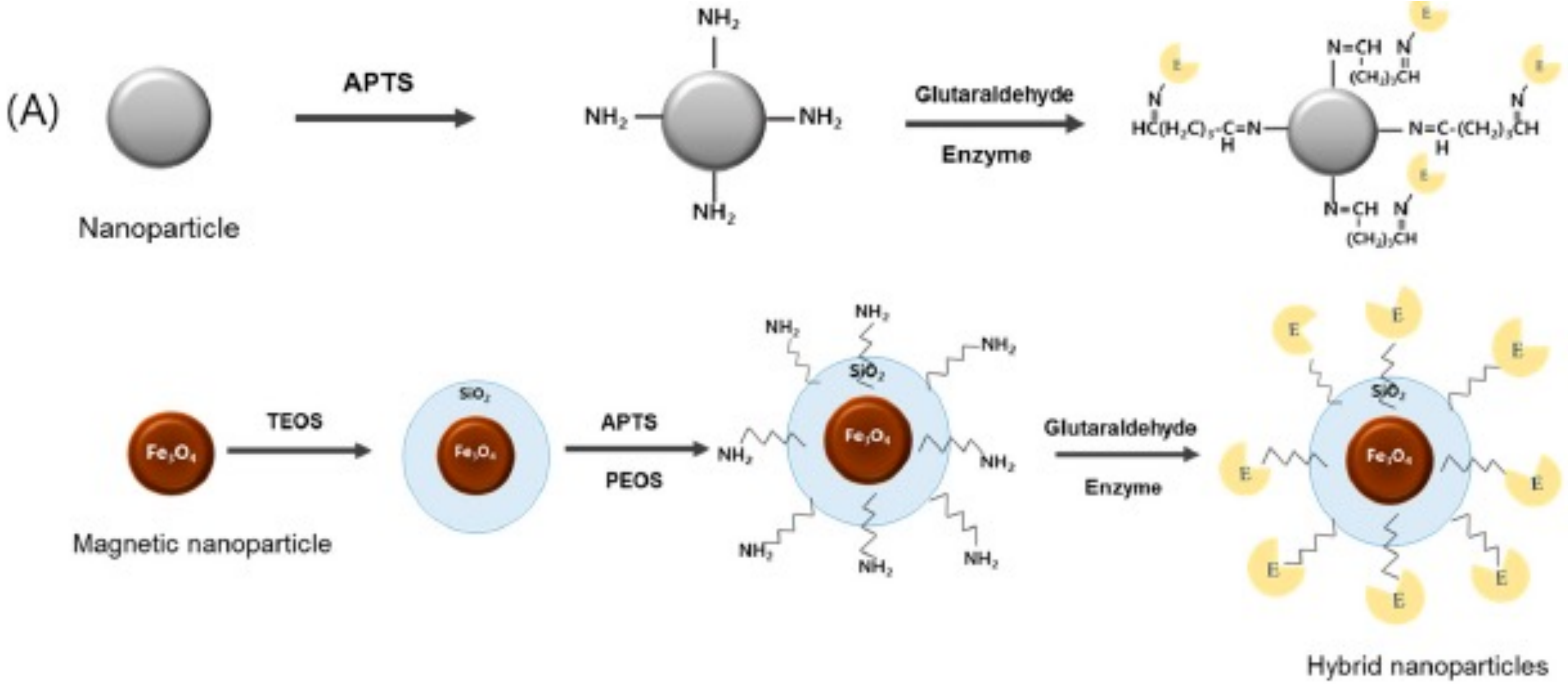
The benefits nanostructures offer in this domain include large surface area for high enzyme loading, higher enzymatic stability, and possibility of enzyme reusability, which could reduce the operational cost of large-scale biofuel production plants (Trindade, 2011).

Examples of the techniques developed for enzymes immobilization using nanotechnology are nano-encapsulation, self-entrapment with silaffin, and adsorption.



Silaffin polypeptide Sil-3 with increasing polarity from top to bottom

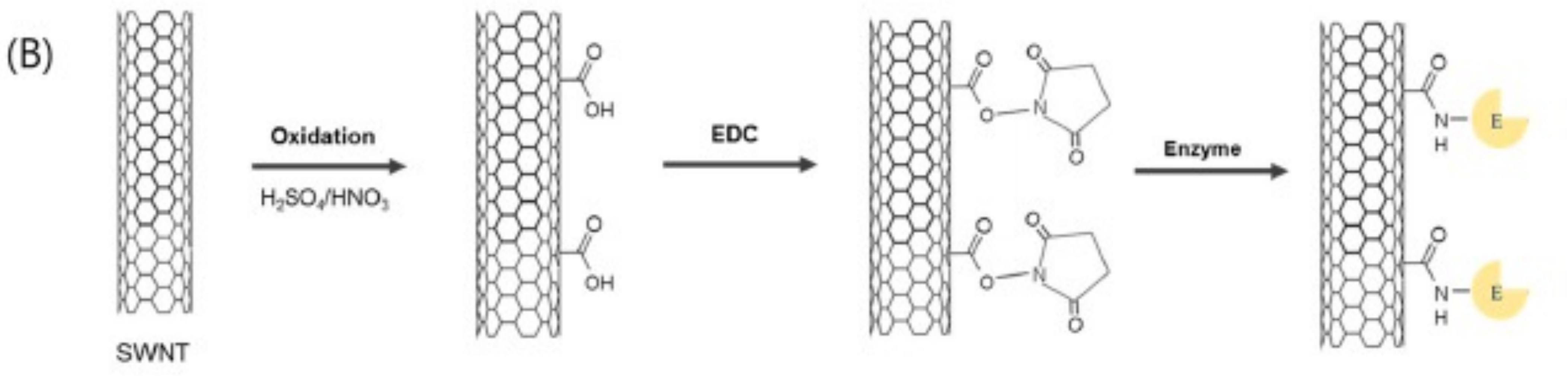
Enzyme immobilization techniques to nanoparticles -For biofuel production



APTS: 3-aminopropyltriethoxysilane, TEOS: tetra-ethoxy silane, PEOS: poly-ethoxy silane

Kim *et al*, 2013, Netto et al, 2009

Nanotubes + enzymes *via* linker

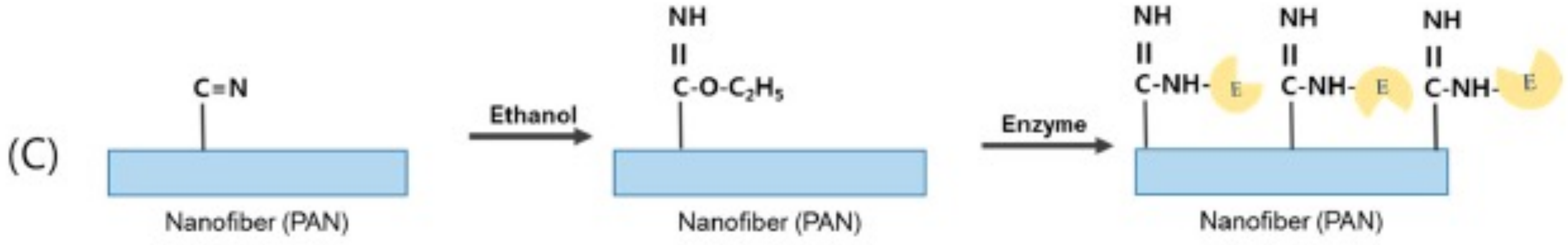


Single walled NT

EDC: N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride

Ji *et al.* 2010

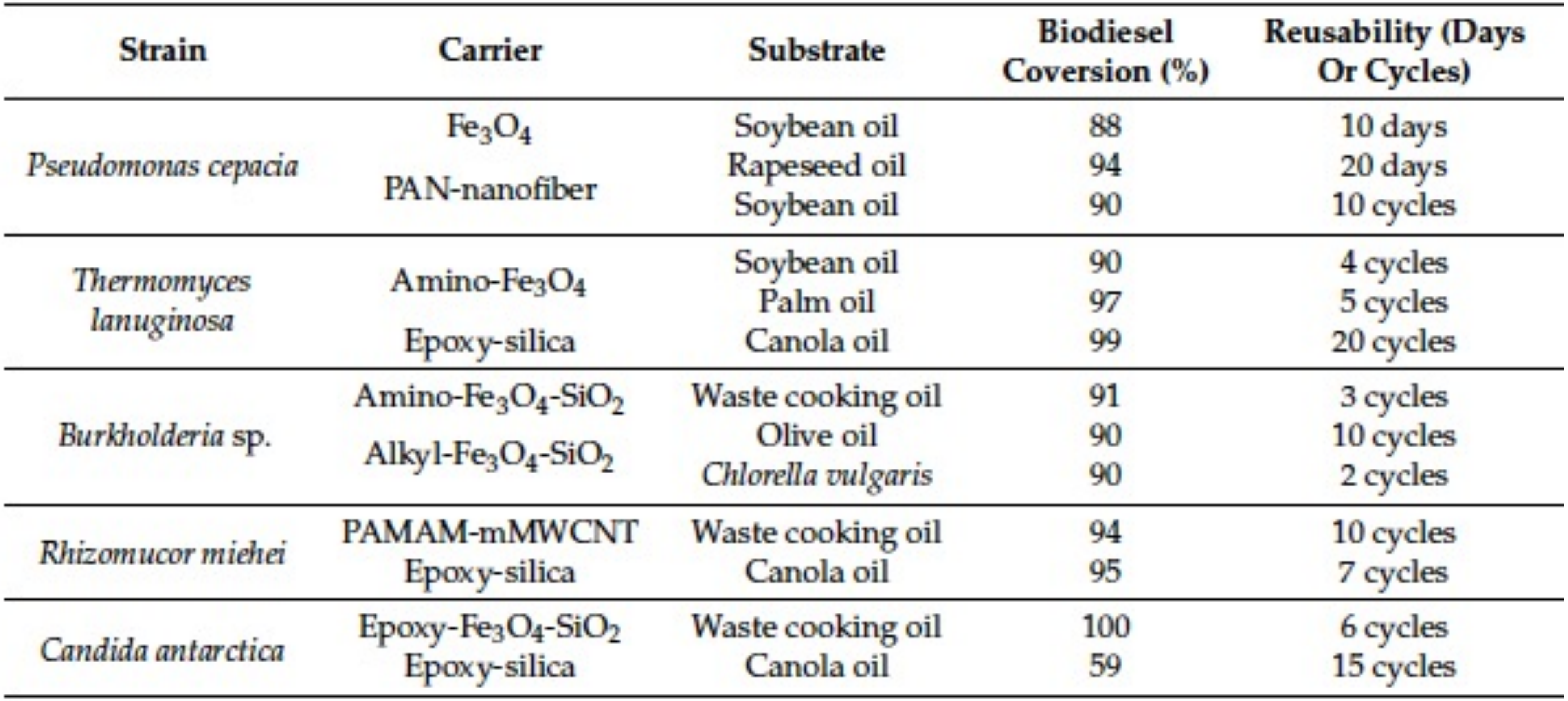
Nanofibers + enzyme *via* linker



PAN: polyacrylonitrile polymer

Li *et al*, 2007

Applications



Strain	Carrier	Substrate	Biodiesel Coversion (%)	Reusability (Days Or Cycles)
Pseudomonas cepacia	Fe_3O_4	Soybean oil	88	10 days
	PAN-nanofiber	Rapeseed oil	94	20 days
		Soybean oil	90	10 cycles
Thermomyces lanuginosa	Amino-Fe_3O_4	Soybean oil	90	4 cycles
		Palm oil	97	5 cycles
	Epoxy-silica	Canola oil	99	20 cycles
Burkholderia sp.	Amino-Fe_3O_4-SiO_2	Waste cooking oil	91	3 cycles
	Alkyl-Fe_3O_4-SiO_2	Olive oil	90	10 cycles
		Chlorella vulgaris	90	2 cycles
Rhizomucor miehei	PAMAM-mMWCNT	Waste cooking oil	94	10 cycles
	Epoxy-silica	Canola oil	95	7 cycles
Candida antarctica	Epoxy-Fe_3O_4-SiO_2	Waste cooking oil	100	6 cycles
	Epoxy-silica	Canola oil	59	15 cycles

Nano-immobilized lipase in packed-bed reactors

POTENTIAL for industrial biodiesel production

- high enzyme loading
- multiple reuses
- effective protection from enzyme denaturation

Goal:

- The integrated development of a high enzyme and nano-immobilization technique will play a key role in cost-effective biodiesel production

Further investigations necessary:

- scale-up of the biodiesel production process using nano-immobilized lipase necessary to implement these technologies on an industrial level.

Kim *et al*, 2018

Importance of engineered nanoparticles (ENP´s) for agrochemicals

- Engineered nanoparticles (ENPs) (polymers, carbon-based, inorganic, zero-valent metal NPs, etc.), have unique physicochemical properties:

  I. Novel approach to boost the efficiency of agrochemical remediation.
  II. Acting as biocides or as nanocarriers of particular conventional agrochemicals, ENP´s increase risk–benefit assessment of remediation.
  III. NPs used to assist alternative remediation processes such as phytoremediation and bioremediation.

- Use of highly efficient specific nanofertilizers and nanopesticides
- Engineering of nanodimensional devices for precise monitoring, so-called nanosensing of environmental parameters, and the right-time
- Efficient application of needed agro measures.
- The final goal is the creation of integrated agriculture supported by the development of nanotechnology and the evolution of efficient advanced agriculture and precision farming.

Boritzev *et al, chapter 19* 2020

Bioremediation: Degradation with NP and microorganisms

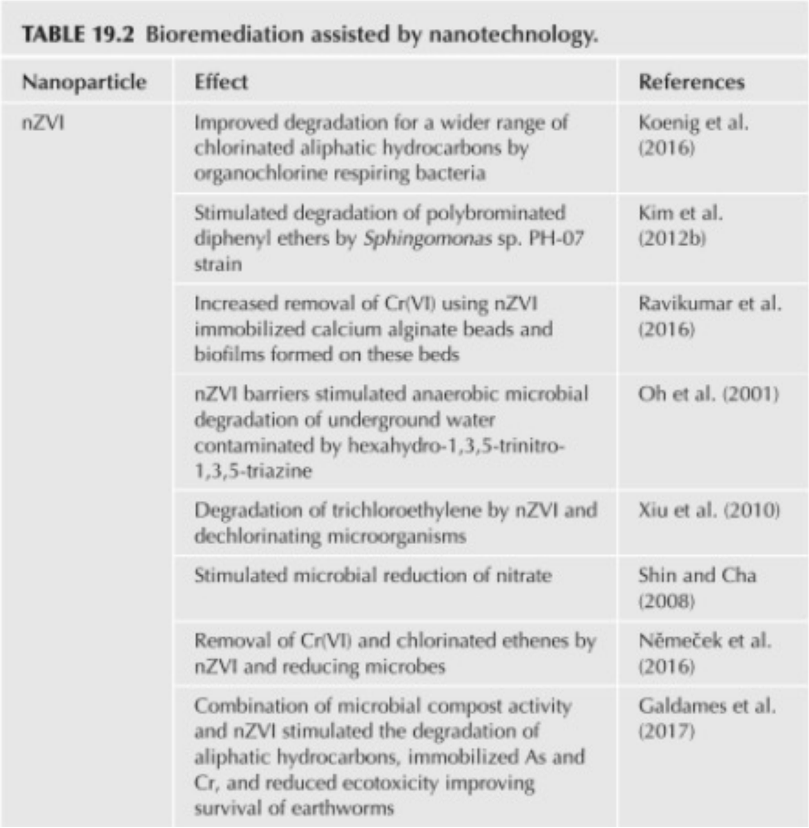
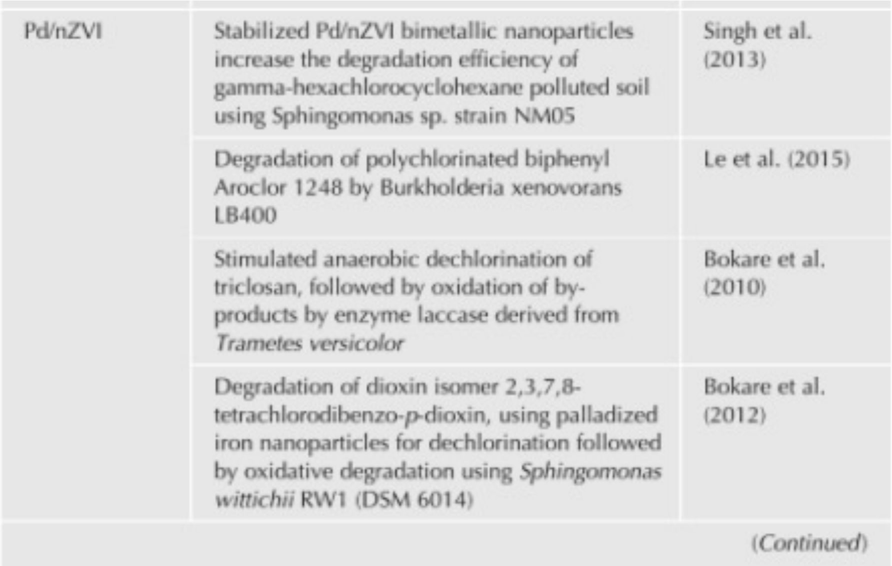


TABLE 19.2 **Bioremediation assisted by nanotechnology.**

Nanoparticle	Effect	References
nZVI	Improved degradation for a wider range of chlorinated aliphatic hydrocarbons by organochlorine respiring bacteria	Koenig et al. (2016)
	Stimulated degradation of polybrominated diphenyl ethers by *Sphingomonas* sp. PH-07 strain	Kim et al. (2012b)
	Increased removal of Cr(VI) using nZVI immobilized calcium alginate beads and biofilms formed on these beds	Ravikumar et al. (2016)
	nZVI barriers stimulated anaerobic microbial degradation of underground water contaminated by hexahydro-1,3,5-trinitro-1,3,5-triazine	Oh et al. (2001)
	Degradation of trichloroethylene by nZVI and dechlorinating microorganisms	Xiu et al. (2010)
	Stimulated microbial reduction of nitrate	Shin and Cha (2008)
	Removal of Cr(VI) and chlorinated ethenes by nZVI and reducing microbes	Němeček et al. (2016)
	Combination of microbial compost activity and nZVI stimulated the degradation of aliphatic hydrocarbons, immobilized As and Cr, and reduced ecotoxicity improving survival of earthworms	Galdames et al. (2017)

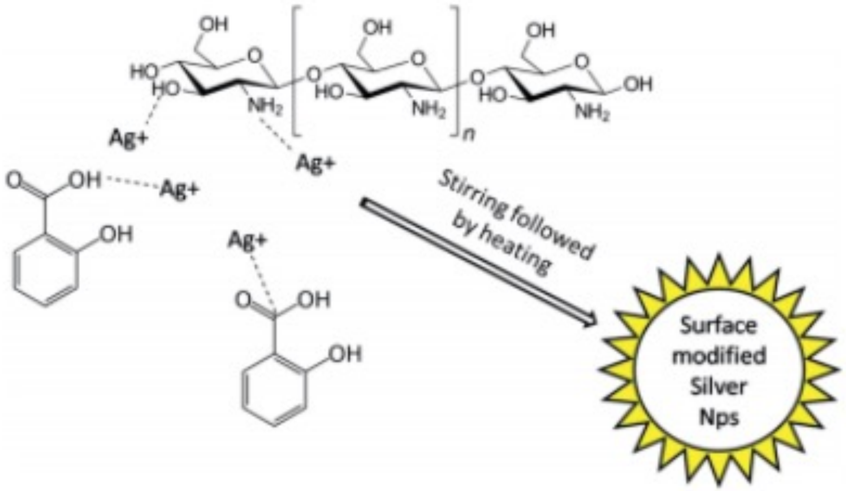


Pd/nZVI	Stabilized Pd/nZVI bimetallic nanoparticles increase the degradation efficiency of gamma-hexachlorocyclohexane polluted soil using Sphingomonas sp. strain NM05	Singh et al. (2013)
	Degradation of polychlorinated biphenyl Aroclor 1248 by Burkholderia xenovorans LB400	Le et al. (2015)
	Stimulated anaerobic dechlorination of triclosan, followed by oxidation of by-products by enzyme laccase derived from *Trametes versicolor*	Bokare et al. (2010)
	Degradation of dioxin isomer 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, using palladized iron nanoparticles for dechlorination followed by oxidative degradation using *Sphingomonas wittichii* RW1 (DSM 6014)	Bokare et al. (2012)

(Continued)

Borisev *et al*, 2020

Agrochemical degradation with nanoparticles



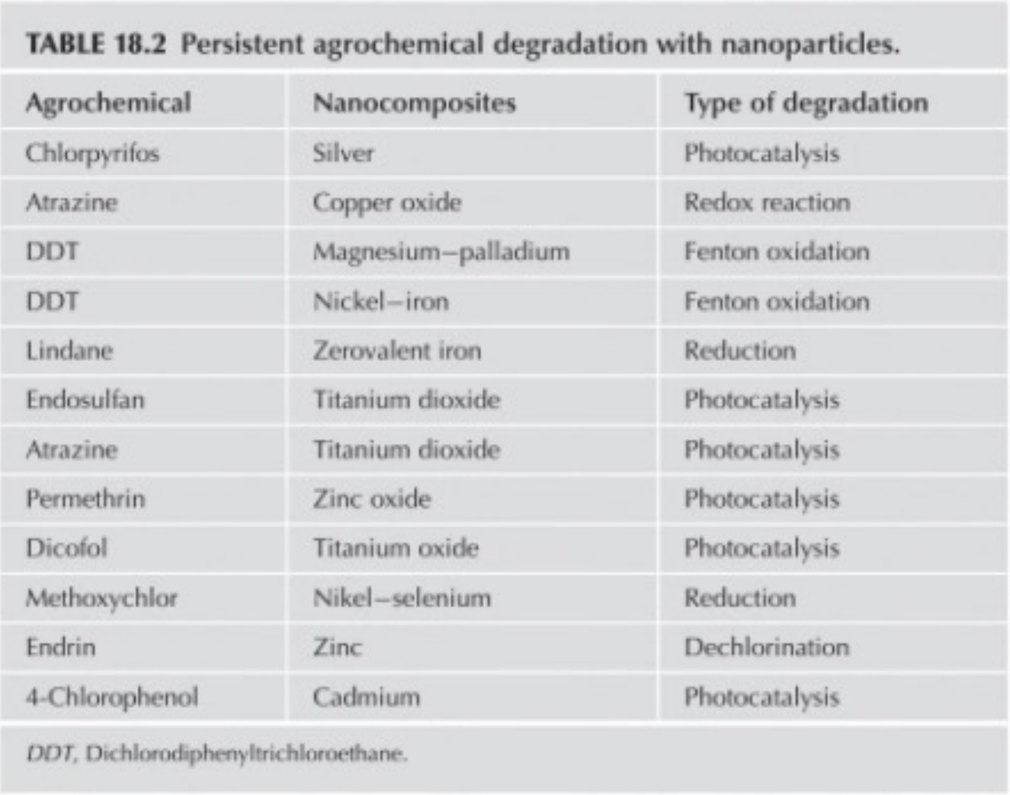


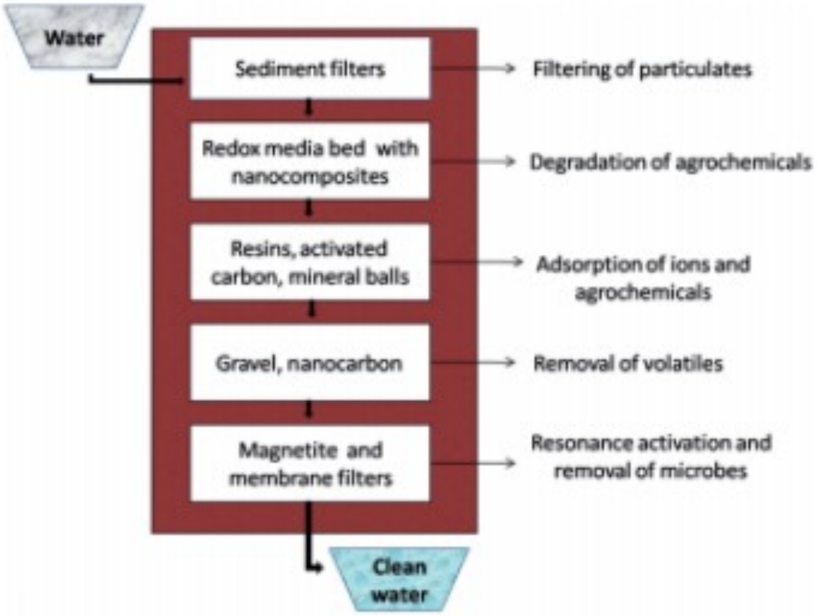
TABLE 18.2 Persistent agrochemical degradation with nanoparticles.

Agrochemical	Nanocomposites	Type of degradation
Chlorpyrifos	Silver	Photocatalysis
Atrazine	Copper oxide	Redox reaction
DDT	Magnesium–palladium	Fenton oxidation
DDT	Nickel–iron	Fenton oxidation
Lindane	Zerovalent iron	Reduction
Endosulfan	Titanium dioxide	Photocatalysis
Atrazine	Titanium dioxide	Photocatalysis
Permethrin	Zinc oxide	Photocatalysis
Dicofol	Titanium oxide	Photocatalysis
Methoxychlor	Nikel–selenium	Reduction
Endrin	Zinc	Dechlorination
4-Chlorophenol	Cadmium	Photocatalysis

DDT, Dichlorodiphenyltrichloroethane.

Sebastian *et al*, 2020

Mulitistep removal of agrochemicals from water



Chlordane, dieldrin, endrine, toxaphene: persistent in agriculture residues, soil particles and in water present in irrigation channels.

Can migrate into deeper layers of soil resulting in ground water pollution.

Nanoparticles degrade persistent agrochemicals: photocatalytic reactions creating electron-hole pairs, results in the formation of free radicals (such as hydroxyl radicals)
* then the free radicals direct secondary reactions that end up in degrading the chemicals

Sebastian *et al*, 2020

Conclusions

Process-related traits of NanoBioCatalysts (NBC´s) are not fully understood.

Exploitation of NBC technologies still in the infant stage in the bioprocessing industry.
Success of NBC technology in the large-scale manufacturing processes relies on:

1. specific activity under the process conditions;
2. stability of the NBC´s when exposed to pH/temperature variations, organic solvents, high shear stress and other harsh environments;
3. reusability of biocatalysts
4. high throughput for large-scale processes.

It is economically and technically crucial that the NBC´s are able either to maintain stable activities in the long term in a continuous process, or to be recycled for re-use in a batch operation process for many runs in which the NBCs are separated from the reaction media after the reaction is completed.

For a continuous operation, stability of enzyme activity as well as reduction of enzyme leakage are the main targets for immobilization, while for batch operations, recyclability and constant enzyme activity of NBC´s are the key challenges.

Future improvements of NBC´s

To further promote the applications of nanobiocatalysts, the following urgent challenges need to be addressed:

1. an evolutional nanobiocatalyst that can permit the simple recycle and reuse of enzymes
2. minimizing the “dead areas” of nanobiocatalysts in catalytic reactions to make the hybrid systems more economically friendly
3. improving the biocompatibility and stability of nanobiocatalysts for in vivo and in vitro biomedical applications
4. smart nanobiocatalysts that can respond efficiently to remote stimuli for modulating the activities of nanobiocatalysts on demand.

An *et al* 2018 (review)

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Borišev, I[1]Borišev, M[2]JovićD[1]Župunski, M[2]ArsenovD[2]Pajević S[2]DjordjevicA[1] In Agrochemicals Detection, Treatment and Remediation Pesticides and Chemical Fertilizers **2020**, Chapter 19, 487-533

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NNI: https://www.nano.gov/

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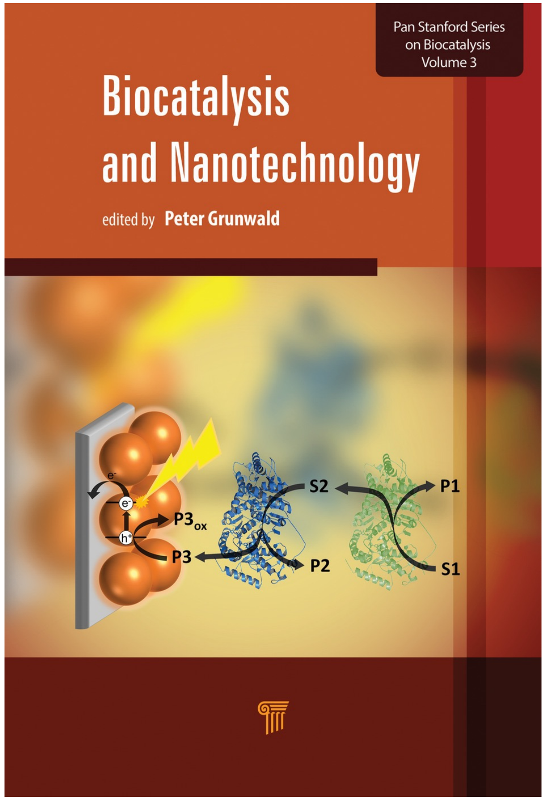
Biocatalysis and nanotechnology

Friday April 9th *14:15 - 16:00* Romania time



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Biocatalysis and Nanotechnology

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Norwegian University of Science and Technology

Elisabeth E. Jacobsen, Department of chemistry, NTNU, Trondheim, Norway

NTNU

CONFRONTING THE BIG 3

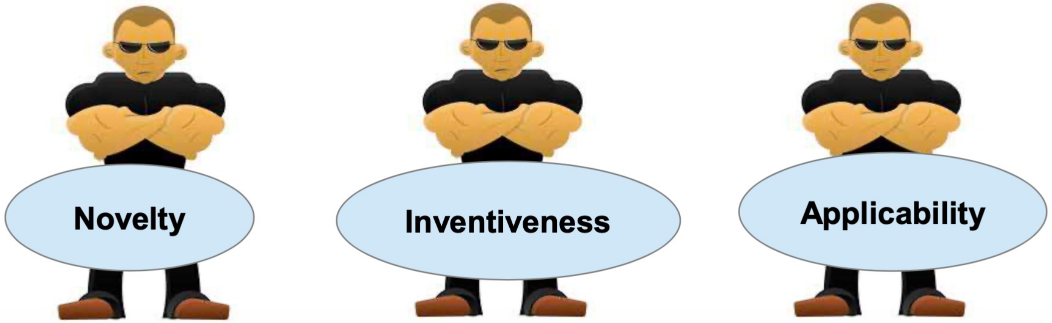
Patent Law at the forefront of Bionanotechnology

Novelty – inherent properties of a known material vs unique properties at the nano-scale.

Inventive Step/non-obviousness – it may be obvious to make materials smaller, but the properties at nanoscale may not be obvious

Industrial applicability – the scope of the nanobiotech inventions in industry is huge. The nano product itself may not be patentable, but the process for making it may be patentable.

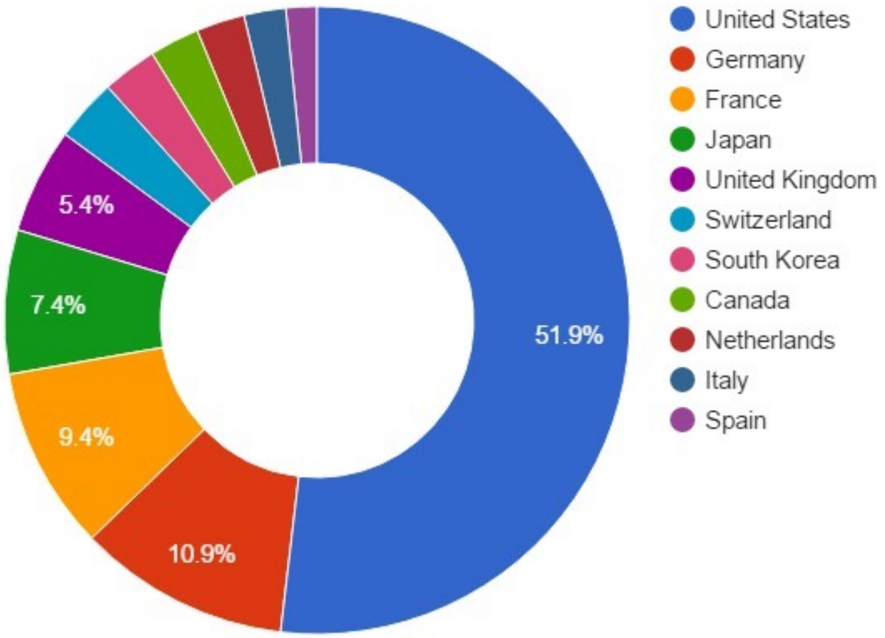
But analysts wonder whether undue experimentation would be necessary to teach those with 'ordinary skill' how to make and use the invention, in the future.



Patents in Bionanotechnology

United States is leading the charge in bionanotechnology applications.

In part due to nanotechnologies having received **recognition** and **national funding** in the early 2000´s by the NNI. WIPO explains that US corporations are pushing many of these therapies forward:

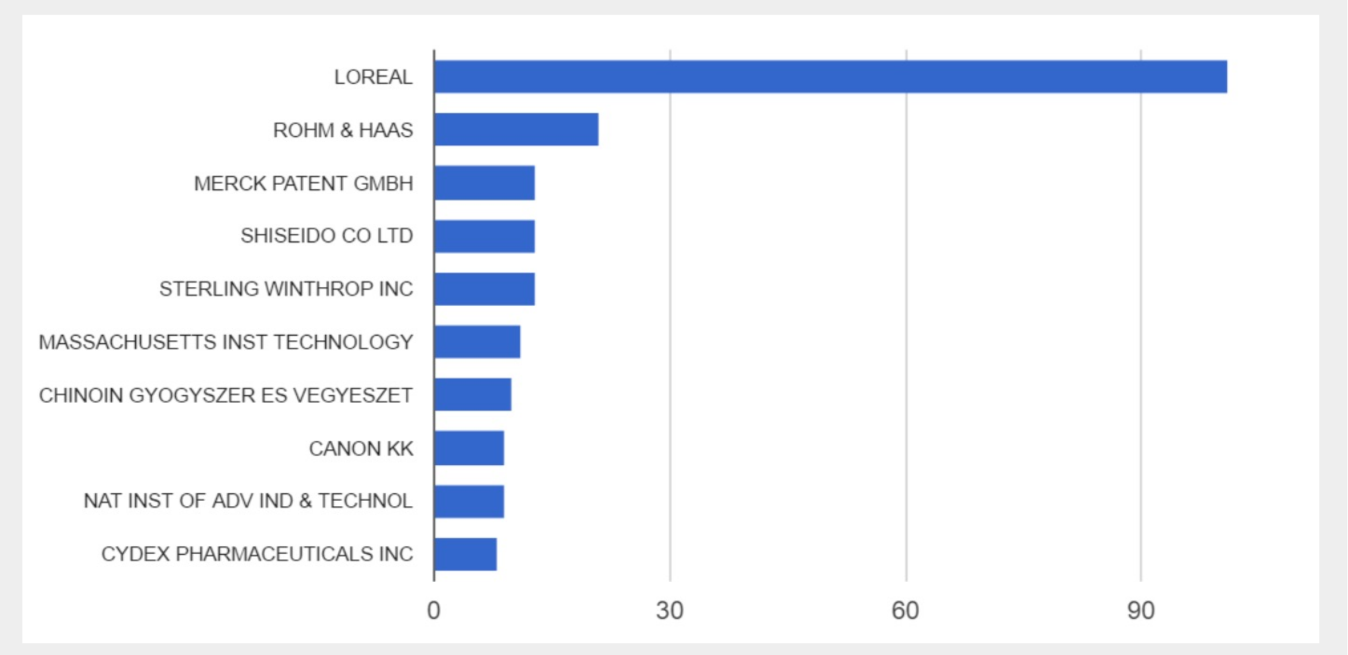


Bionanotechnology Patent Applications by Country

World
Intellectual
Property
Organisation
WIPO

"As of 2013, a few hundred nano-related medical therapies had been approved or had entered clinical trials in the United States".

Patent filers of Japanese Applications



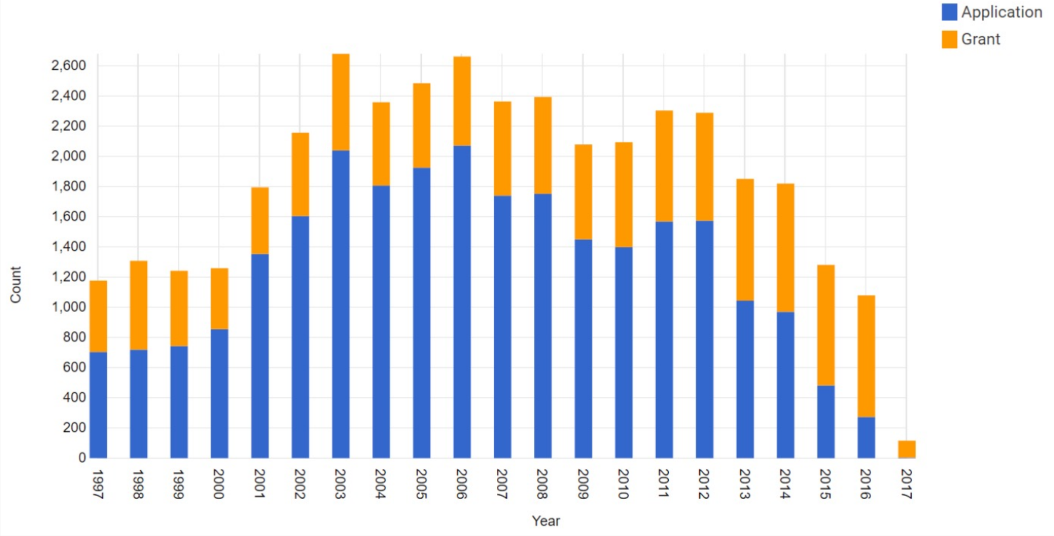
Source: ktMINE

China: World´s second largest economy- and largest in GDP

Out of over 49,000 patents and patent applications, Chinese entities own fewer than 1% of bionanotechnology applications and grants. It could be that China is focusing their current nanoscience efforts in the electronics and semiconductor space. But this data suggests China will face limitations to advance innovation in bionanotechnology applications.

Total patents WIPO

Compound annual Growth Rate (CAGR)

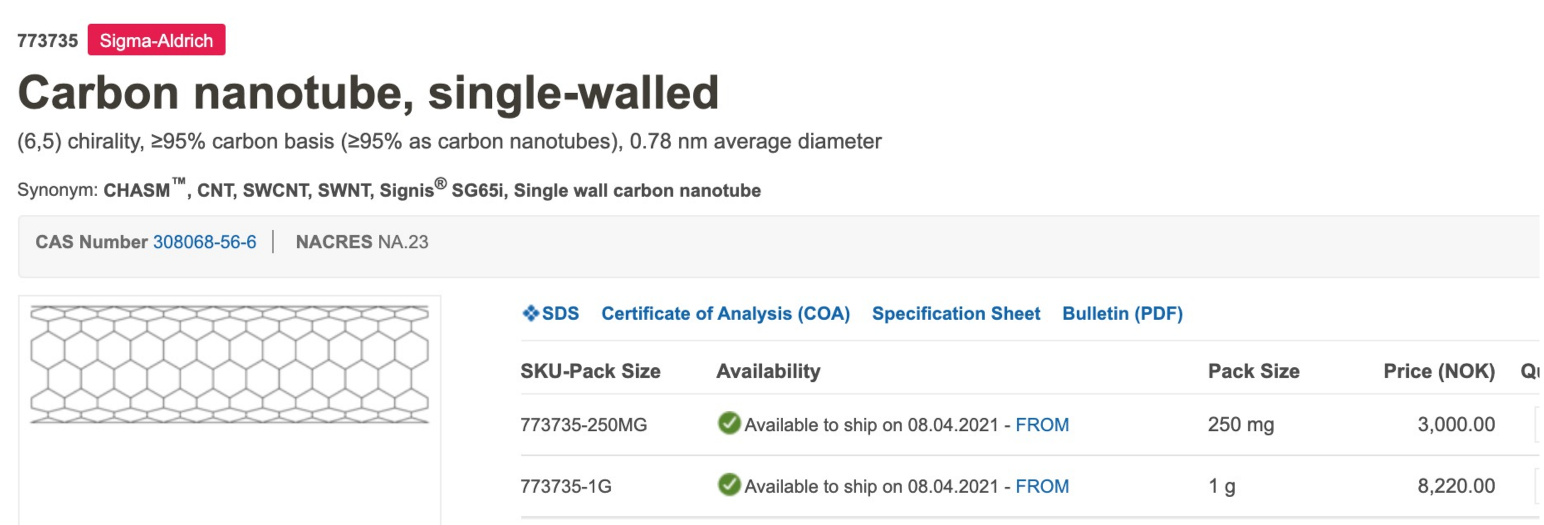


Source: ktMINE: CPC classifications A61, C12N AND B81, B82

“The global nanotechnology market is expected to grow at a CAGR of around 17% during the forecasted period of 2017-2024”. (Research and Markets)

Graphene oxide, nanotubes and enzyme prices

Amount of GO	Form	Amount of paste	Price (USD)
2 Kg	Aqueous acidic paste	10 Kg	3000
1 Kg	Aqueous acidic paste	5 Kg	1600
0			



773735 Sigma-Aldrich

Carbon nanotube, single-walled

(6,5) chirality, ≥95% carbon basis (≥95% as carbon nanotubes), 0.78 nm average diameter

Synonym: **CHASM™, CNT, SWCNT, SWNT, Signis® SG65i, Single wall carbon nanotube**

CAS Number 308068-56-6 | **NACRES** NA.23

SKU-Pack Size	Availability	Pack Size	Price (NOK)	Qu
773735-250MG	✓ Available to ship on 08.04.2021 - FROM	250 mg	3,000.00	
773735-1G	✓ Available to ship on 08.04.2021 - FROM	1 g	8,220.00	

300 EUR
822EUR/G

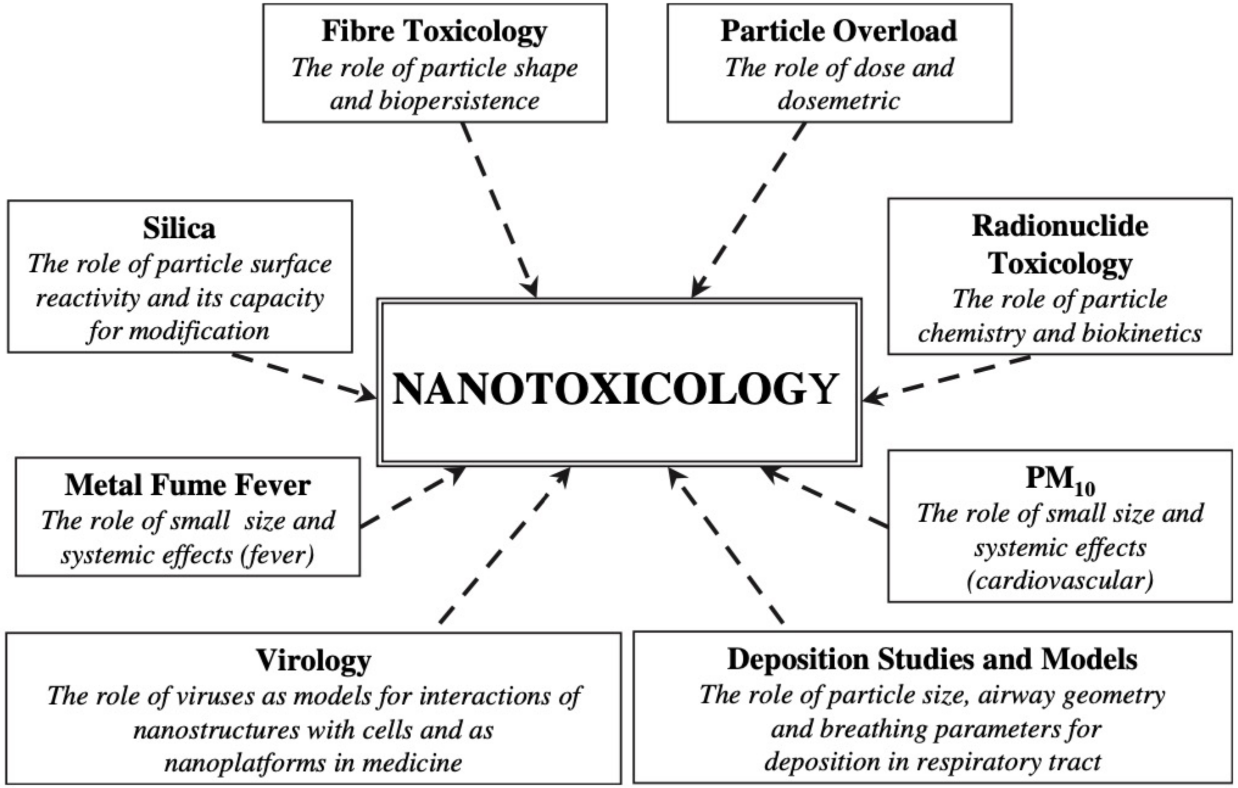
Novozym ® 435 market price > 1000 USD/KG

Nanobiocatalysis- a subarea of enzyme biotechnology
Advantages and disadvantages of various immobilization techniques

Immobilization technique	Advantages	Disadvantages
Adsorption immobilization	• Simple and low-cost • Reversible • Little or no damage to biocatalyst • No additional coupling agent or enzyme modification is required • k_{cat} and k_m values remain substantially unchanged • Higher catalytic activity of immobilized enzymes	• Based on weak and reversible interactions between carrier and enzymes • High probability of enzyme leaching and desorption • Loss of enzyme activity with time • No control over packing density of the immobilized enzymes • Low stability
Covalent binding Immobilization	• Strong and stable binding • Prevention of enzyme leaching • Improved thermostability	• Often results in enzyme deactivation • Decrease in substrate affinity of immobilized enzymes • Conformational restriction
Entrapment Immobilization	• Protection of enzyme from effect of mechanical sheer, hydrophobic solvents, and gas bubbles. • Suitability for continuous operation • Simple downstream processing • Retain protein integrity and efficacy	• Lower enzyme loading • Limitation of mass transfer
Cross-Linking Immobilization	• Support matrix is not required • High enzyme stability • Decrease in desorption • Ease of recycling and reuse	• Loss of enzyme activity via conf • Decrease in diffusion rate

Singh, N, Dhanya, BS, Verma, ML *Materials Science for Energy Technologies* **2020**, *3*, 808-824

Foundations of Nanotoxicology



Fibre Toxicology
The role of particle shape and biopersistence

Particle Overload
The role of dose and dosemetric

Silica
The role of particle surface reactivity and its capacity for modification

Radionuclide Toxicology
The role of particle chemistry and biokinetics

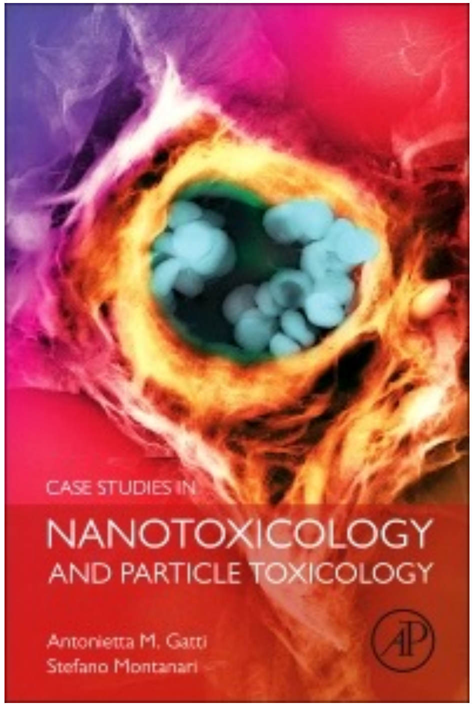
NANOTOXICOLOGY

Metal Fume Fever
The role of small size and systemic effects (fever)

PM_{10}
The role of small size and systemic effects (cardiovascular)

Virology
The role of viruses as models for interactions of nanostructures with cells and as nanoplatforms in medicine

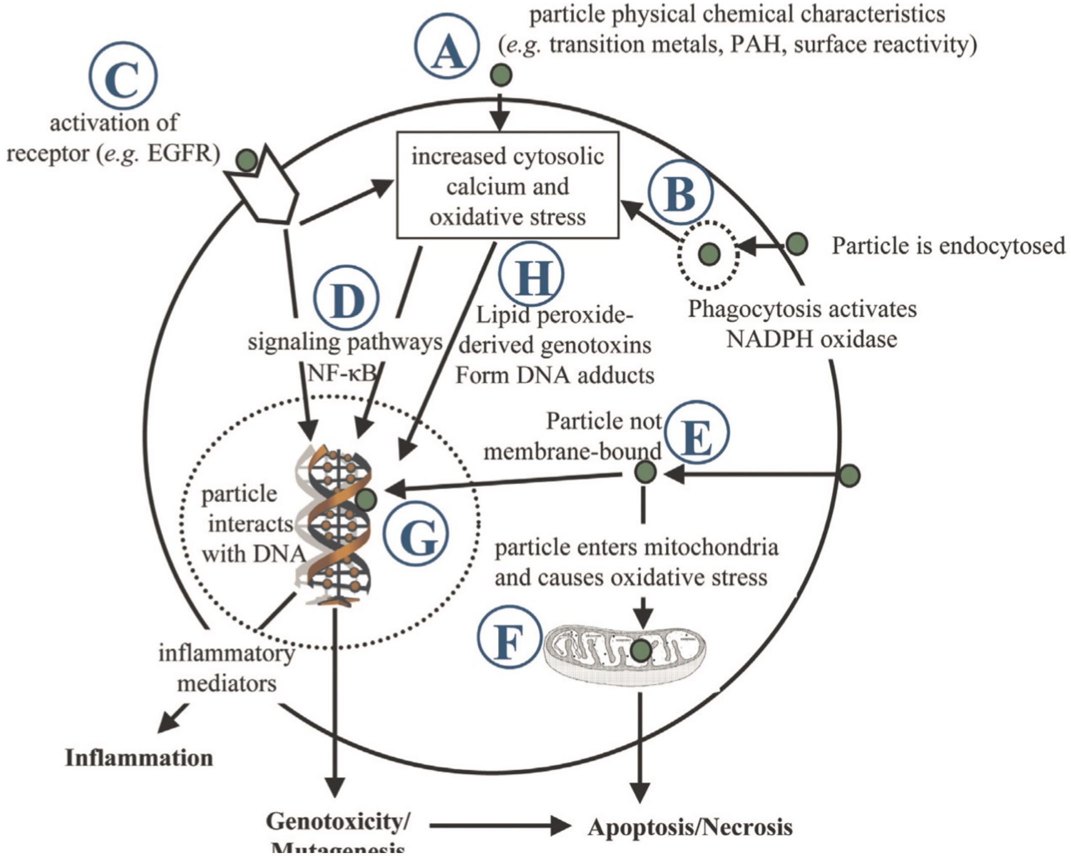
Deposition Studies and Models
The role of particle size, airway geometry and breathing parameters for deposition in respiratory tract



Oberdorster, G , Stone, V, Donaldson, K. *Nanotoxicology*, **2007**; *1* (1), 2-25

Hypothetical cellular interactions of NP

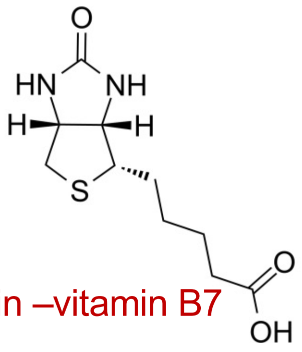
The cell



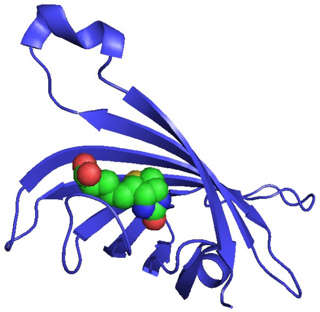
Oberdorster *et al*, 2007

5. Biological Strategies in Nanobiocatalyst Assembly

Ian Dominic F. Tabañag, and Shen-Long Tsai

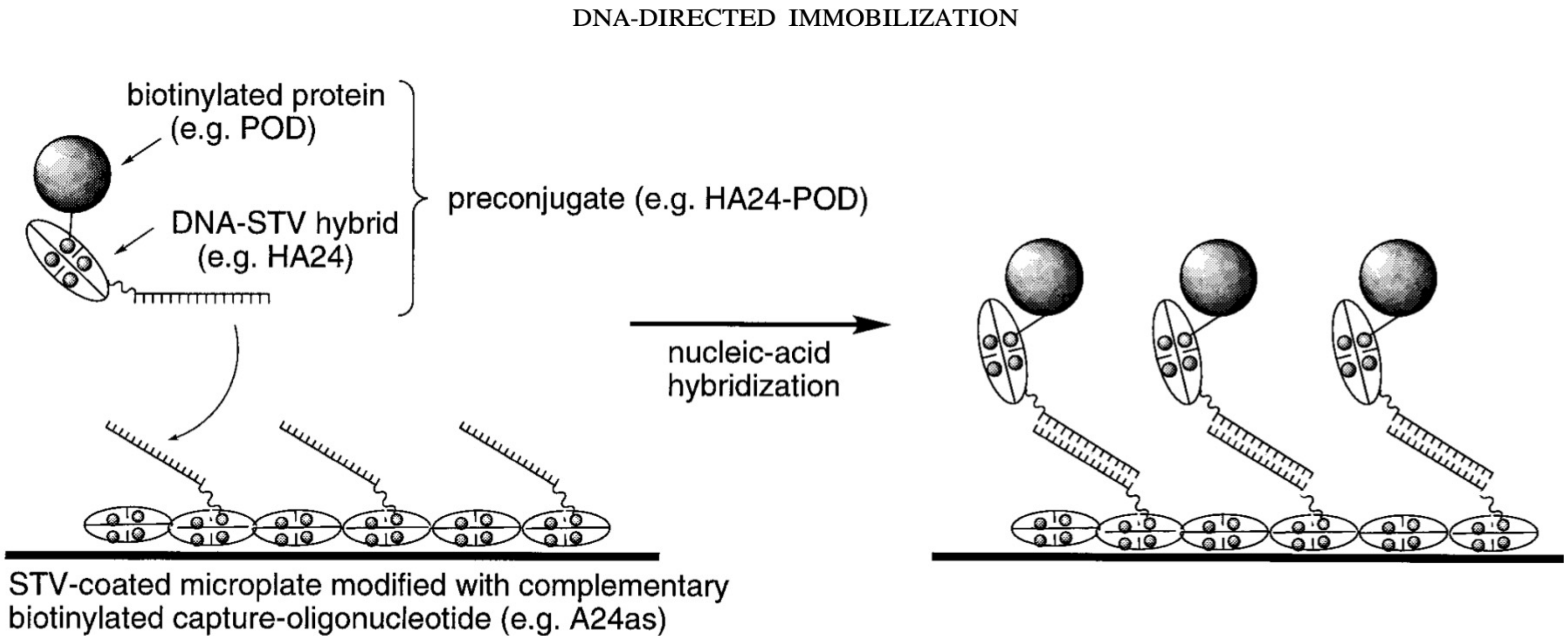


Biotin –vitamin B7



Streptavidin w biotin

DDI Process



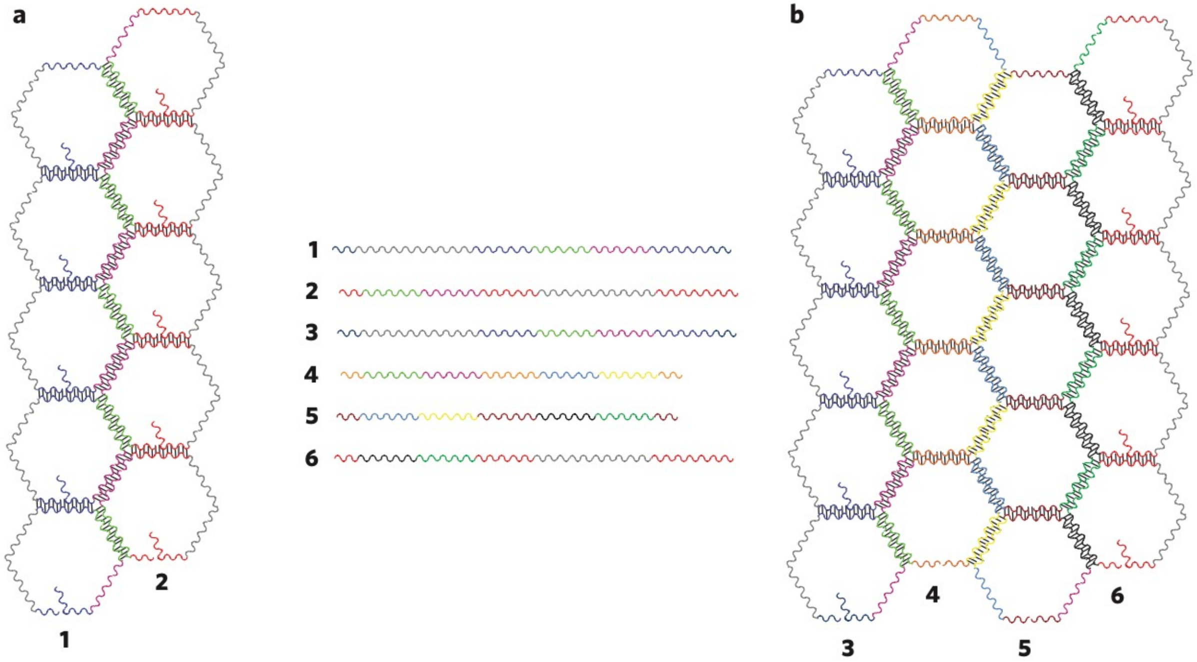
Advantages: -Mild reversible technique

For biosensing and biomedical diagnostics, and fundamental studies in biology and medicine

Wilner, OI, Weizmann, Y, Gill, R, Lioubashevski, O, Freeman, R and Willner, I. *Nature Nanotechnology* **2009**, *4*, 249-254

Assembly of hexagon-like DNA strips and their structural imaging.

A: Two-hexagon DNA strip assembly

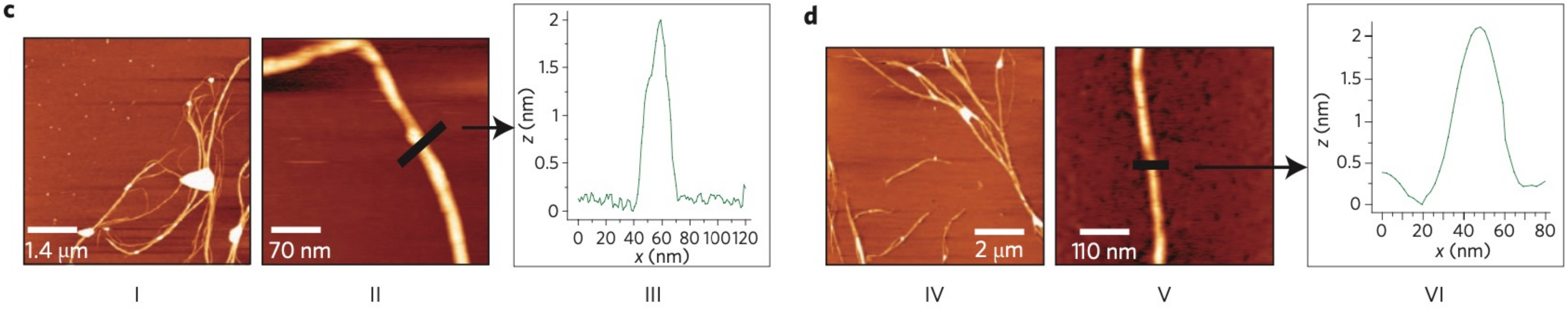


B: Four-hexagon DNA strip assembly.

Wilner *et al*, 2009

Atomic Force Images

C: AFM images of the two-hexagon strip: (I) large-scale image that includes a collection of strips, (II) image of a single strip, and (III) cross-sectional analysis of a single strip.



D: AFM images of the four-hexagon strip: (IV) large-scale image that includes several strips, (V) image of a single strip, and (VI) cross- sectional analysis of a single strip.

Cascade enzyme reactions

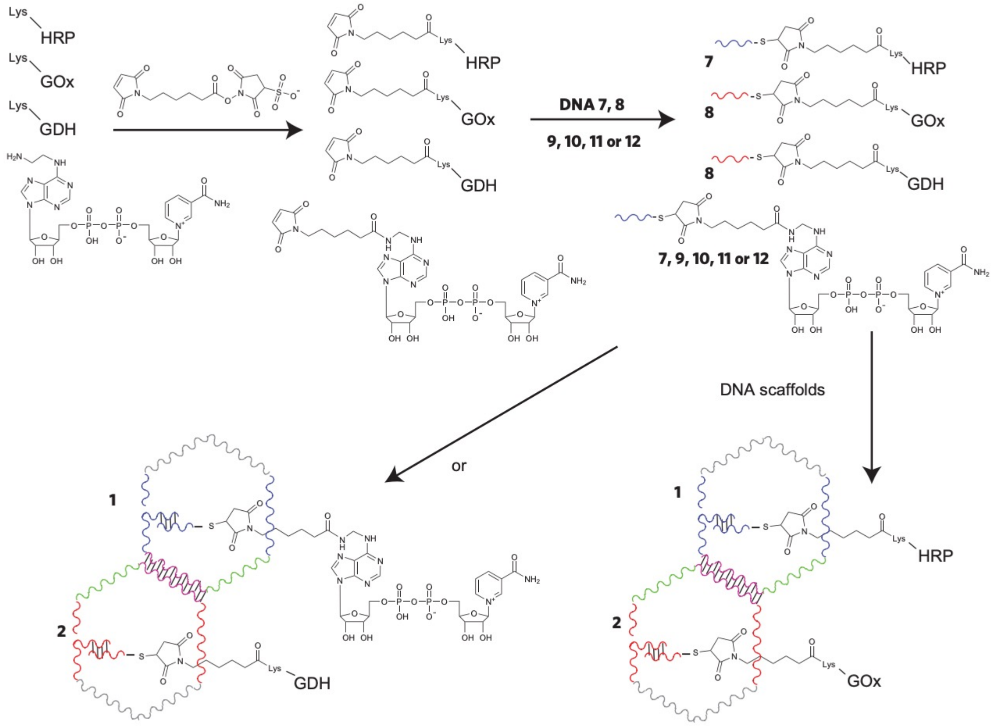
- two enzymes or a cofactor-enzyme pair are added to the scaffold
- shows that enzyme cascades or cofactor-mediated biocatalysis can proceed effectively
- similar processes are not observed in diffusion-controlled homogeneous mixtures of the same components.
- because relative position of the two enzymes or the cofactor-enzyme pair is determined by the topology of the DNA scaffold, it is possible to control the reactivity of the system through the design of the individual DNA strips.

Advantage: self-organization of complex multi-enzyme cascades.

Enzymes on 1D and 2D DNA scaffolds

Enzymes immobilised on:

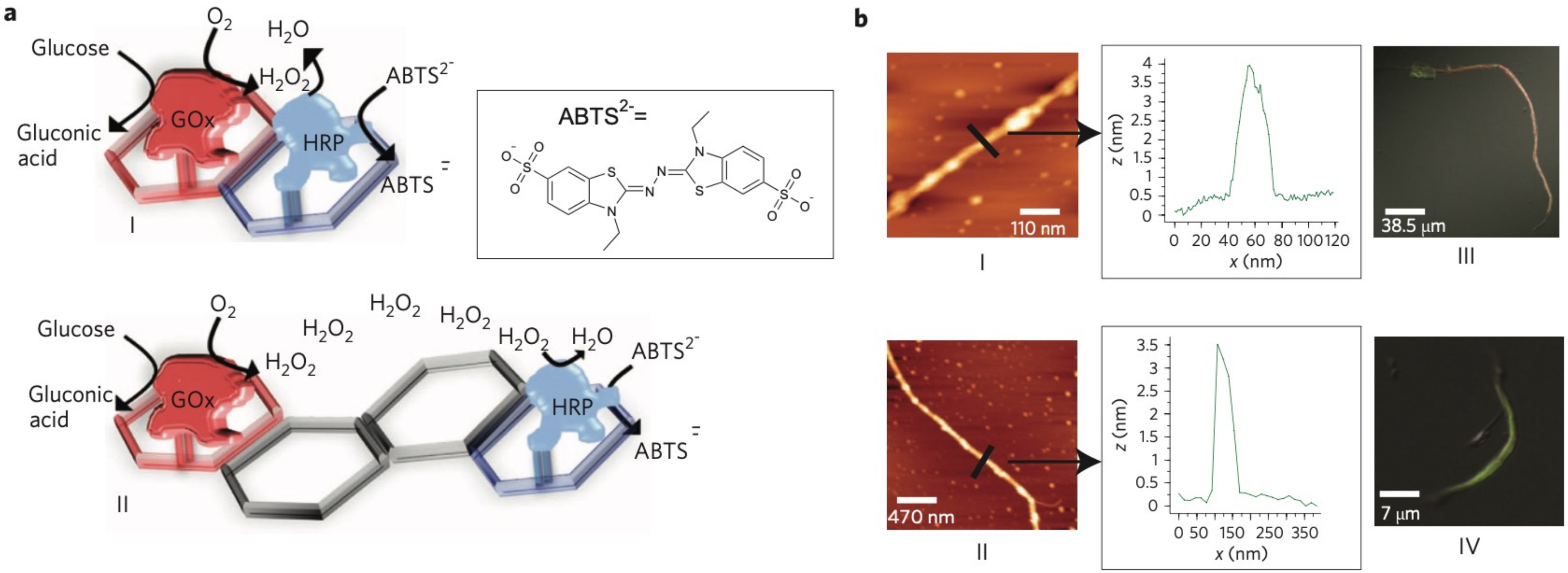
Enzymes:
Glucose oxidase (GOX) and horseradish peroxidase (HRP)
Glucose dehydrogenase (GDH)



Advantages:
-Increased flexibility and enzyme activity

Wilner *et al*, 2009

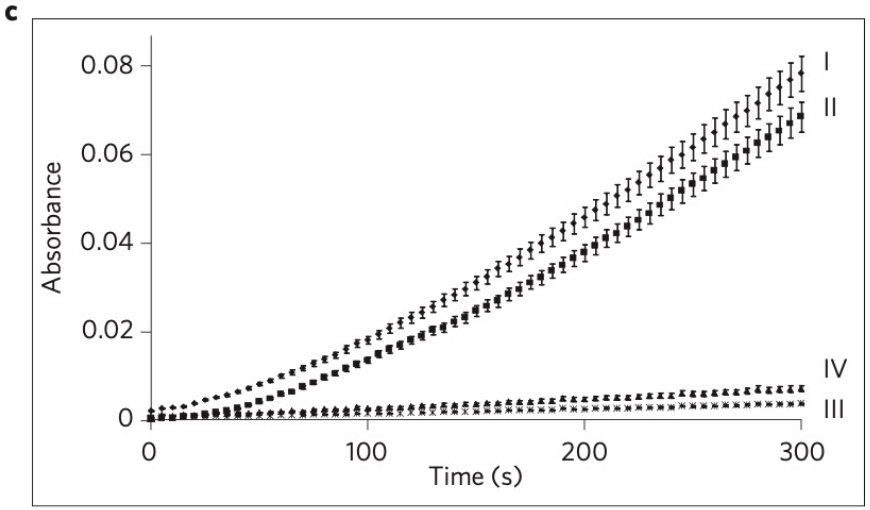
Assembly of enzyme cascades or cofactor–enzyme cascades on hexagon-like DNA scaffolds, their imaging and their functional characterization

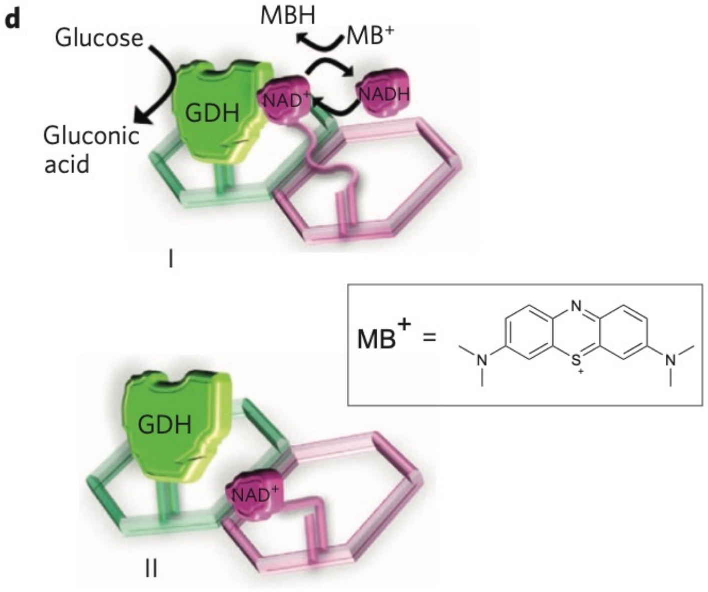


The primary enzyme GOx biocatalyses the oxidation of glucose to gluconic acid, with the concomitant formation of H_2O_2. The latter product acts as substrate for HRP, mediating the oxidation of 2´ 2´ azino-bis[3-ethylbenzthiazoline-6-sulphonic-acid], $ABTS^{2-}$, to the coloured product, $ABTS^{-}$

Wilner *et al*, 2009

C: Time-dependent absorbance changes as a result of the oxidation of $ABTS^{2-}$ by the GOx–HRP cascade in the presence of (I) the two-hexagon scaffold, (II) the four-hexagon scaffold, (III) in the absence of any DNA, and (IV) in the presence of foreign calf thymus DNA.

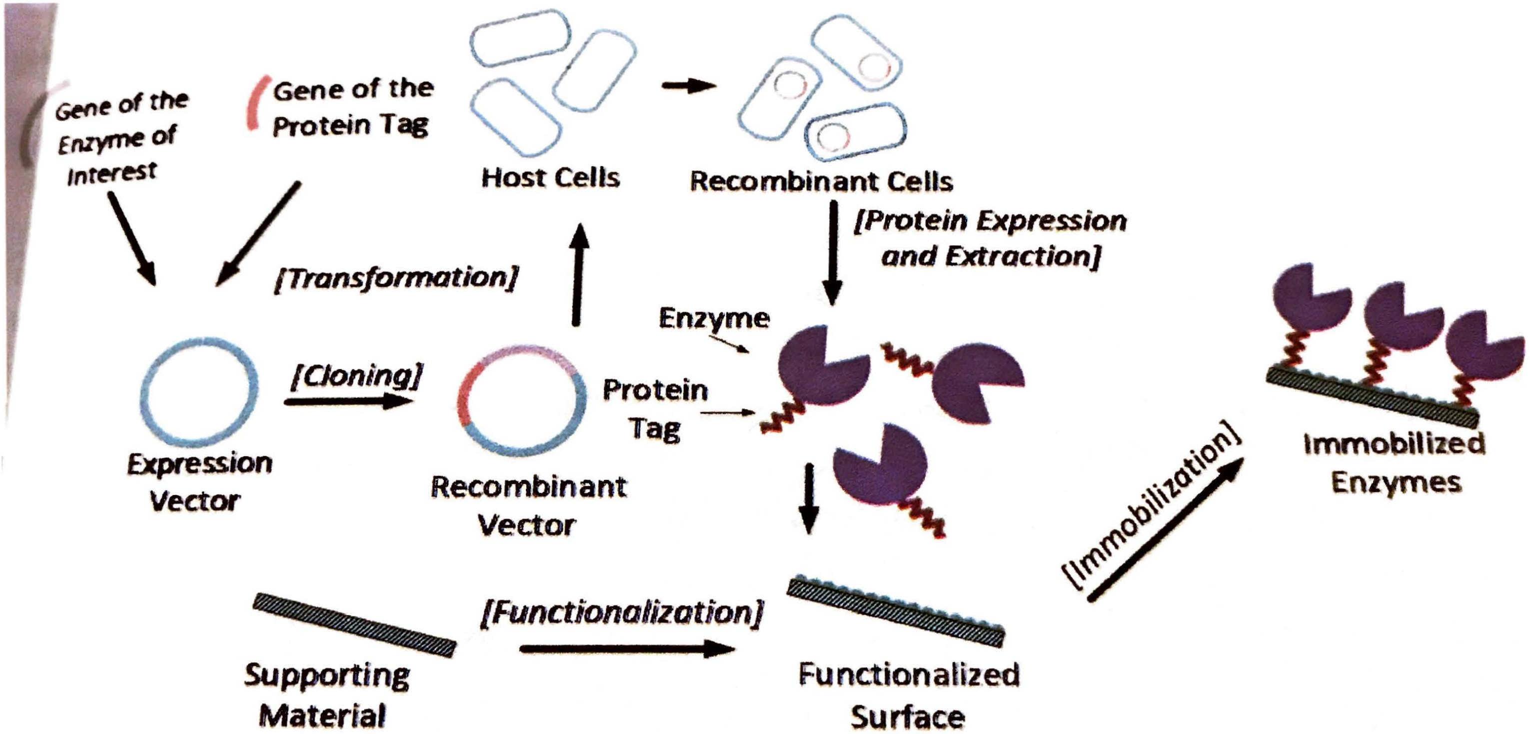




D: Assembly of the NAD+/GDH system on the two-hexagon scaffold using different lengths of tethers linking the NAD+ cofactor to the scaffold.

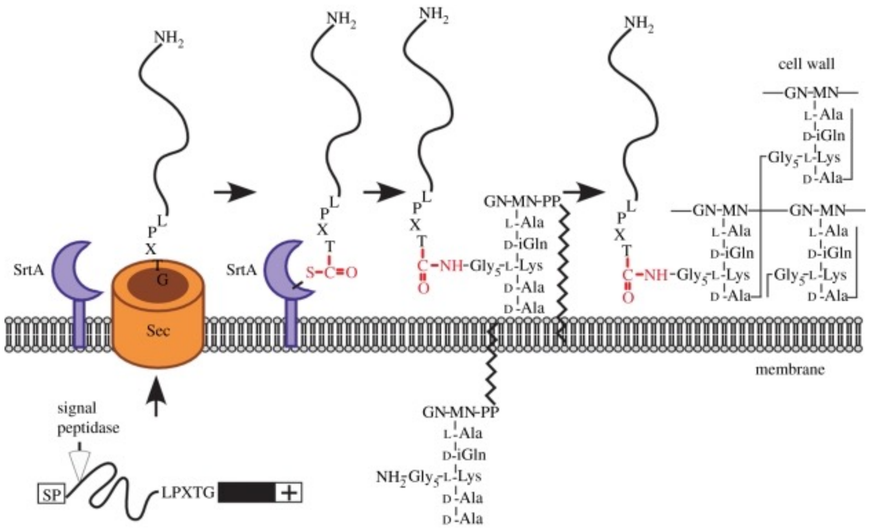
Wilner *et al*, 2009

Enzyme immobilisation *via* protein affinity tags



Advantage: No need for protein purification steps after protein expression and extraction of the transformed cells

Enzyme assisted covalent immobilisation



Sortase A-enzyme used by Gram-positive bacteria to anchor surface proteins to the cell wall between a C-terminal tag

Advantages for bionanocatalysis: Specific and mild-and no need for ligands.-conjugating enzyme instead

Mostly addition of tags to N and C terminals-HOWEVER: If these terminals are near active-the site active site may be blocked.

To avoid this: Must use unnatural amino acids (with unique functional groups) in the synthesis of proteins

Schneewind. O and Missiakas, DM *Phil. Trans. R. Soc. B* **2012**, *367*, 1123-1139
Parthasarathy, R, Subramanian, S, Boder, ET *Bioconjugate Chem*. **2007**, *18*, 469-476

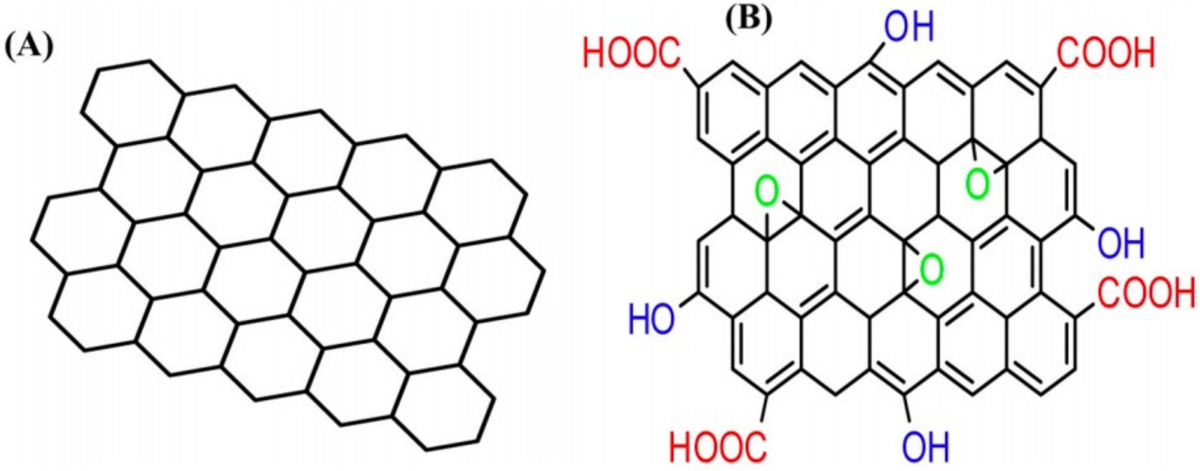
6. Graphene-Based Nanobiocatalytic System (GBN´s)

Michaela Patilaa , George Orfanakisa , Angeliki C. Polydera, Ioannis V. Pavlidis, and Haralambos Stamatis

Application of graphene oxide (GO) for biomolecule immobilisation

Utilised for:

- Biofuel production
- Degradation of pollutants
- In situ protein digestion
- Biosensing

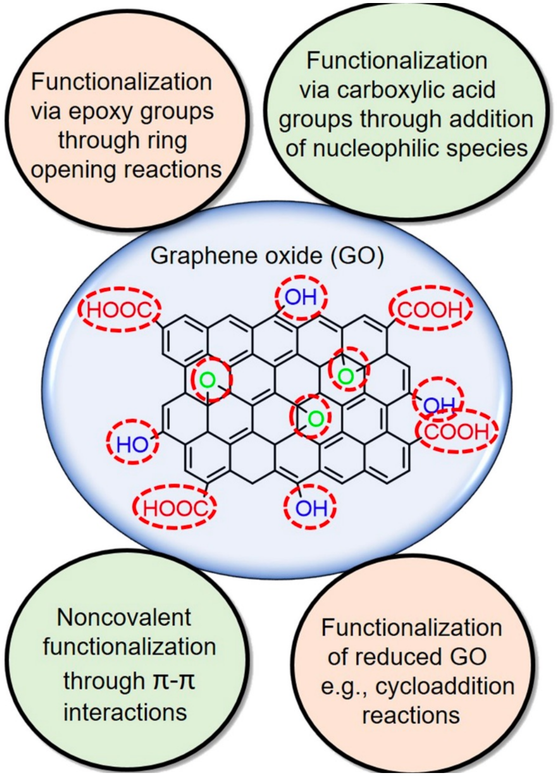


Adeel M. *et al*. *Int J of Biol Macromolecules* **2018**, *120*, 1430-1440

Graphene based nanomaterials as enzyme immobilisation supports

Strategies to immobilise enzymes onto graphene:

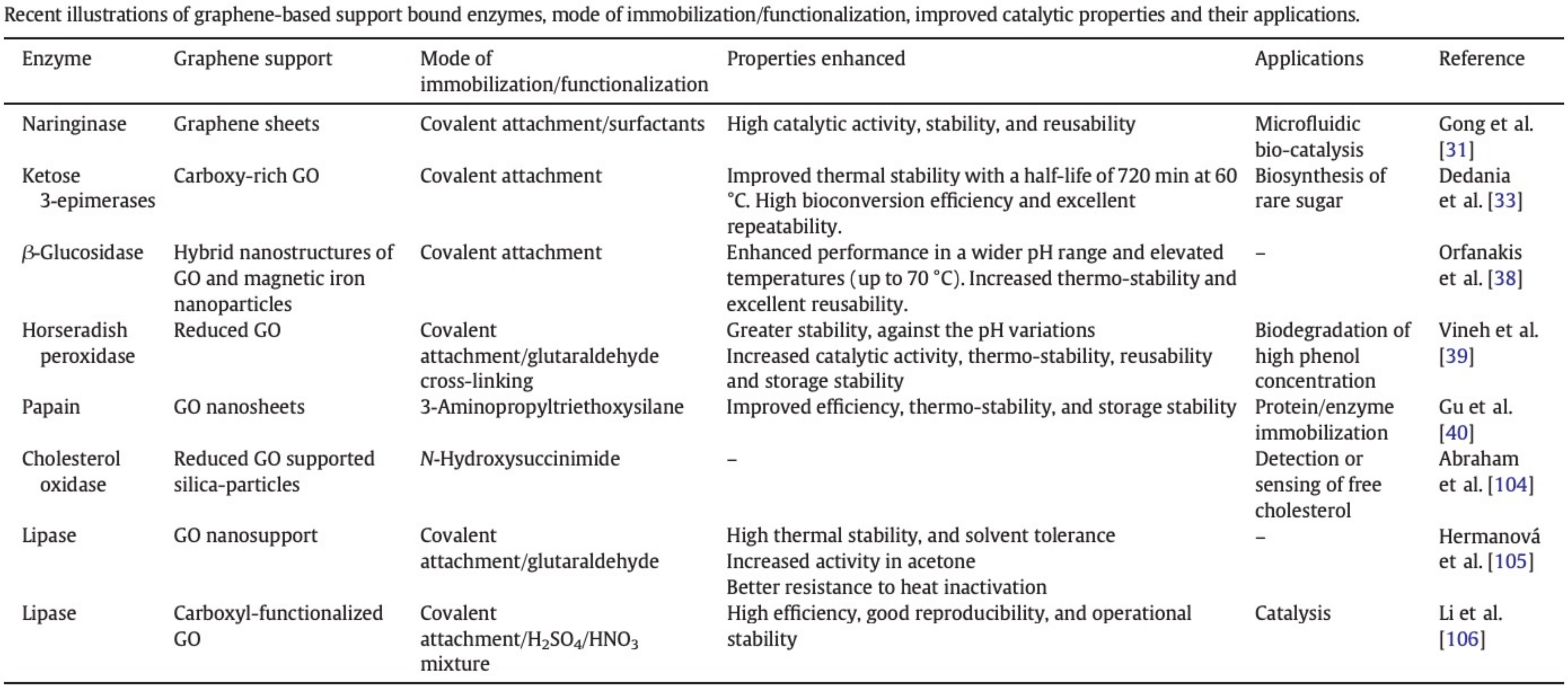
Physical adsorption, covalent attachment, site spesific affinity interactions, gluteraldehyde as linker



Advantages:
Surface chemistry of the nanomaterials affect the catalytic properties and conformation of the enzymes

Adeel *et al*. **2018**

Graphene-based support bound enzymes, mode of immobilization/functionalization, improved catalytic properties and their applications

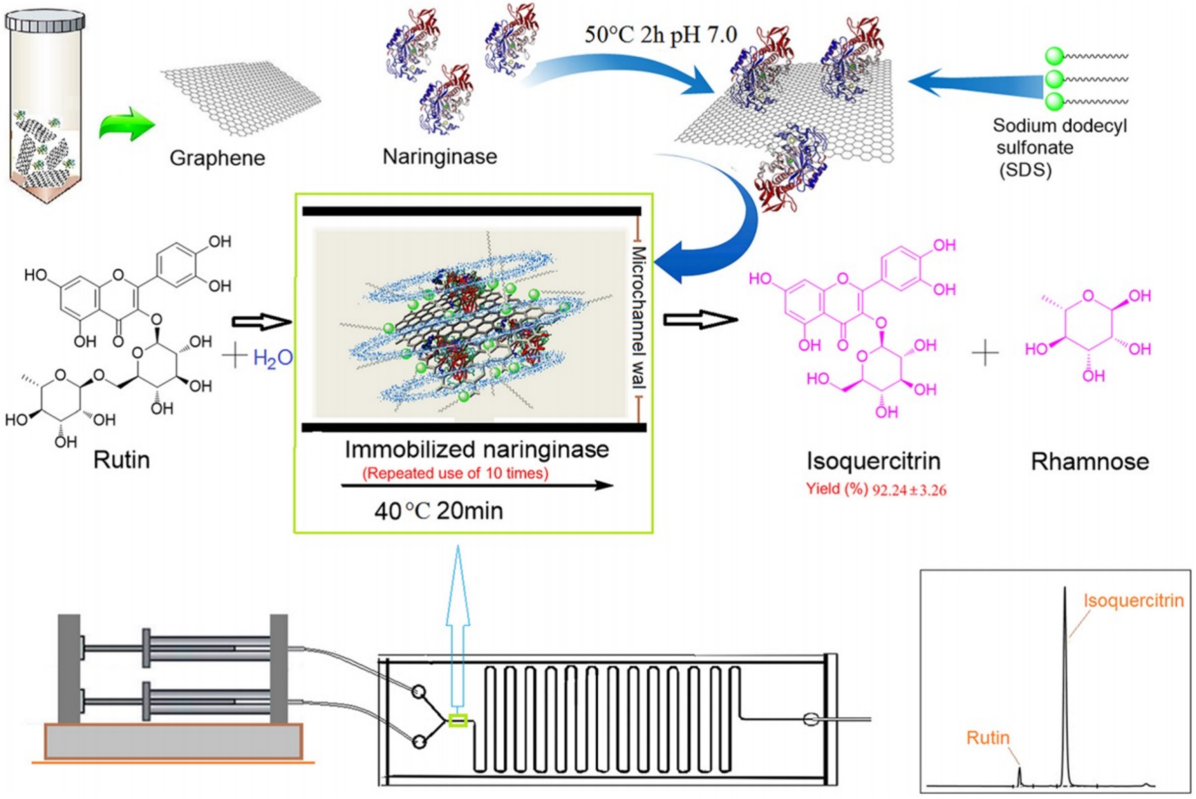


Recent illustrations of graphene-based support bound enzymes, mode of immobilization/functionalization, improved catalytic properties and their applications.

Enzyme	Graphene support	Mode of immobilization/functionalization	Properties enhanced	Applications	Reference
Naringinase	Graphene sheets	Covalent attachment/surfactants	High catalytic activity, stability, and reusability	Microfluidic bio-catalysis	Gong et al. [31]
Ketose 3-epimerases	Carboxy-rich GO	Covalent attachment	Improved thermal stability with a half-life of 720 min at 60 °C. High bioconversion efficiency and excellent repeatability.	Biosynthesis of rare sugar	Dedania et al. [33]
β-Glucosidase	Hybrid nanostructures of GO and magnetic iron nanoparticles	Covalent attachment	Enhanced performance in a wider pH range and elevated temperatures (up to 70 °C). Increased thermo-stability and excellent reusability.	–	Orfanakis et al. [38]
Horseradish peroxidase	Reduced GO	Covalent attachment/glutaraldehyde cross-linking	Greater stability, against the pH variations Increased catalytic activity, thermo-stability, reusability and storage stability	Biodegradation of high phenol concentration	Vineh et al. [39]
Papain	GO nanosheets	3-Aminopropyltriethoxysilane	Improved efficiency, thermo-stability, and storage stability	Protein/enzyme immobilization	Gu et al. [40]
Cholesterol oxidase	Reduced GO supported silica-particles	*N*-Hydroxysuccinimide	–	Detection or sensing of free cholesterol	Abraham et al. [104]
Lipase	GO nanosupport	Covalent attachment/glutaraldehyde	High thermal stability, and solvent tolerance Increased activity in acetone Better resistance to heat inactivation	–	Hermanová et al. [105]
Lipase	Carboxyl-functionalized GO	Covalent attachment/H_2SO_4/HNO_3 mixture	High efficiency, good reproducibility, and operational stability	Catalysis	Li et al. [106]

Adeel *et al*. **2018**

Biosynthesis diagram of isoquercitrin in a microchannel reactor with a fluid and unsinkable immobilized enzyme

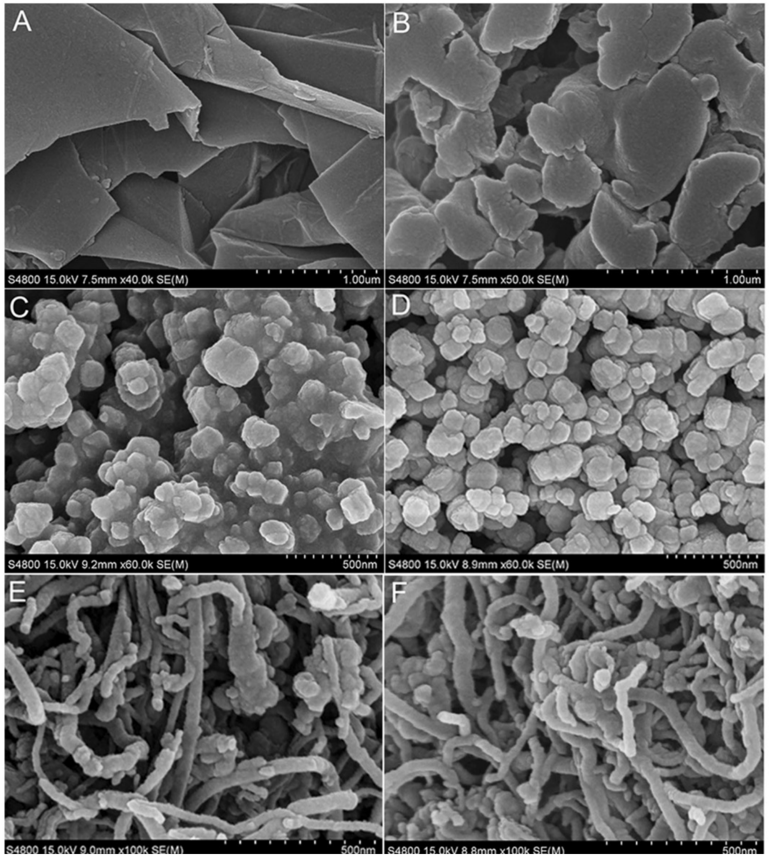


Gong, A, *et al, Scientific Reports* **2017**, *7*, 4309

SEM images:Graphene immobilisation

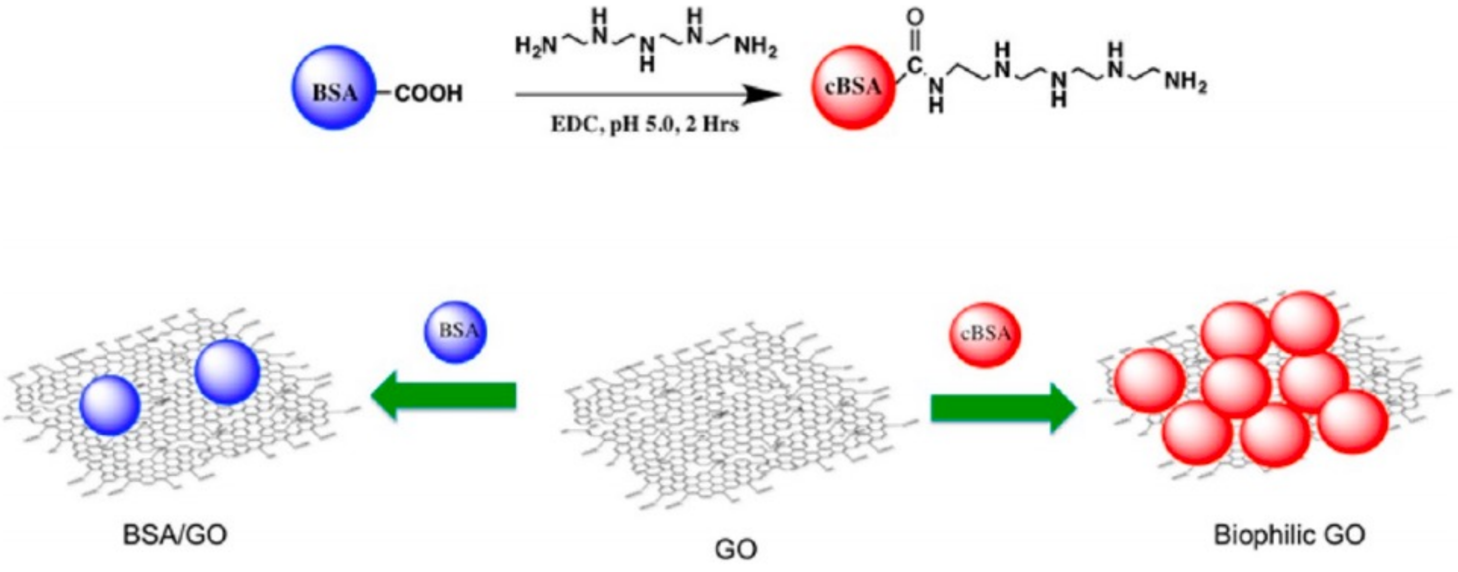
SEM photos of pure graphene (A and B), Fe_2O_3 (C and D) and carbon nanotube nanoparticle (E and F) before and after immobilizing.

Reaction condition:
enzyme solution (20 g/L) dissolved by disodium hydrogen phosphate-citrate buffer (pH 7); graphene nanoparticles mass (10mg) added in 2mL of enzyme solution, mixture stirred at 120 rpm in an incubator shaker for 3h, reaction temperatures 50 °C.



Cationisation of Bovine Serum Albumin (BSA)

Reaction by BSA side chains -COOH with tetraethylene-pentamine (TEPA) via carbodiimide coupling.

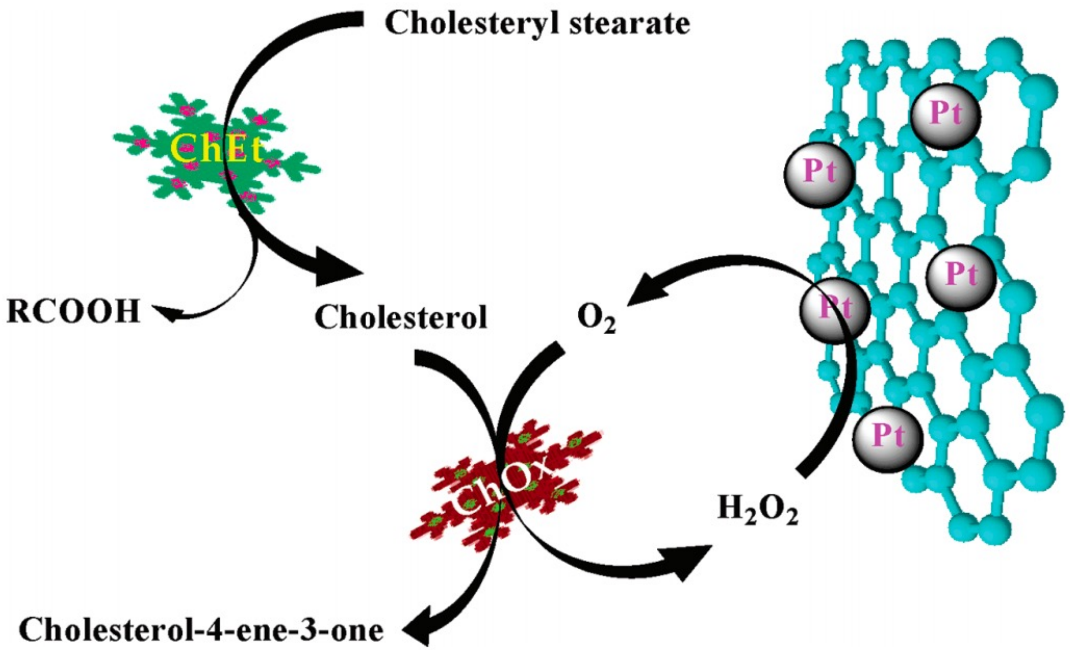


Drawback of physical adsorption: enzyme leakage.

Advantage: Covalent linking of enzyme to nanocatalyst.

Enzyme based biosensors

Biosensing of cholesterol ester with GNS-nPt-based biosensor



The enzyme ChEt hydrolyses the cholesterol ester to cholesterol and ChOx catalyzes the oxidation of cholesterol. The Pt nanoparticles on the surface of GNS can effectively sense the enzymatically generated H_2O_2

Dey, RS and Raj, CR *J. Phys. Chem. C*, **2010**,114 (49)

Graphene based enzymatic bioelectrodes and biofuel cells

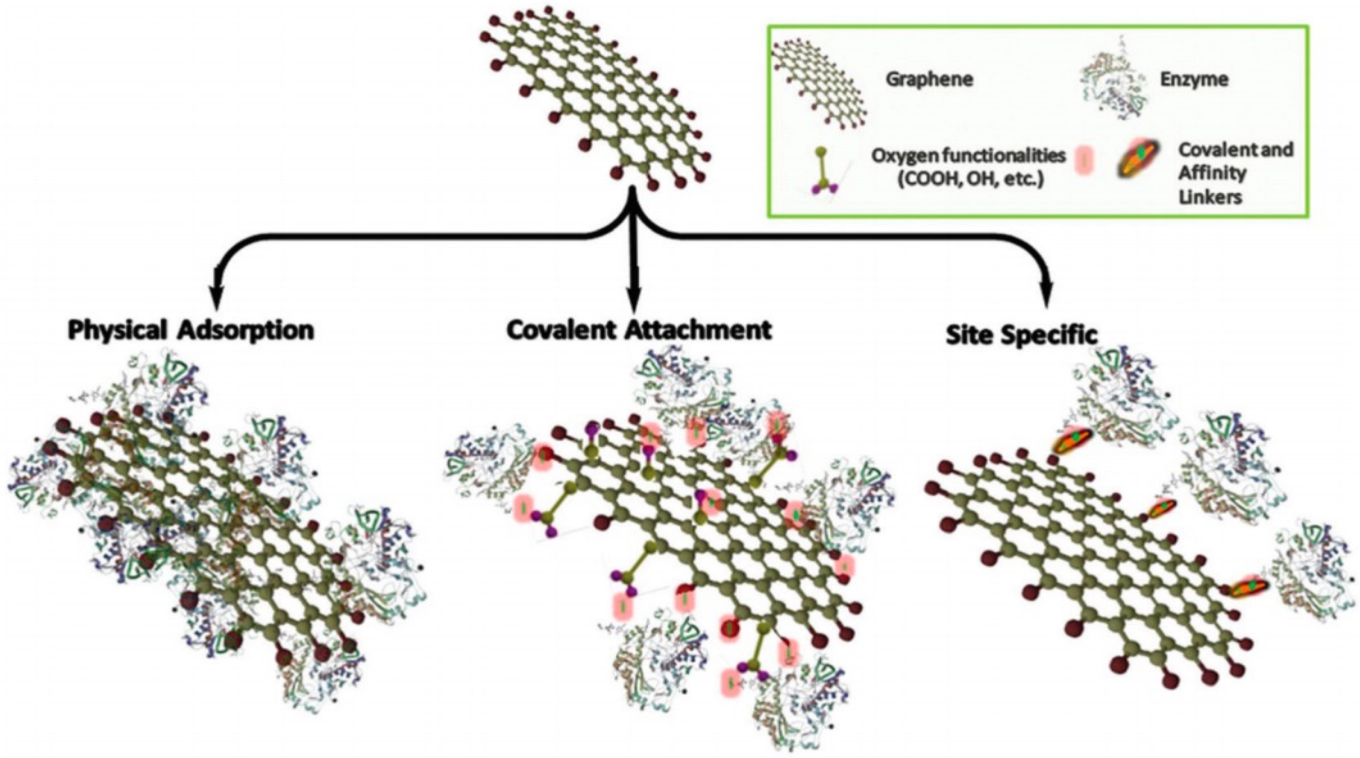


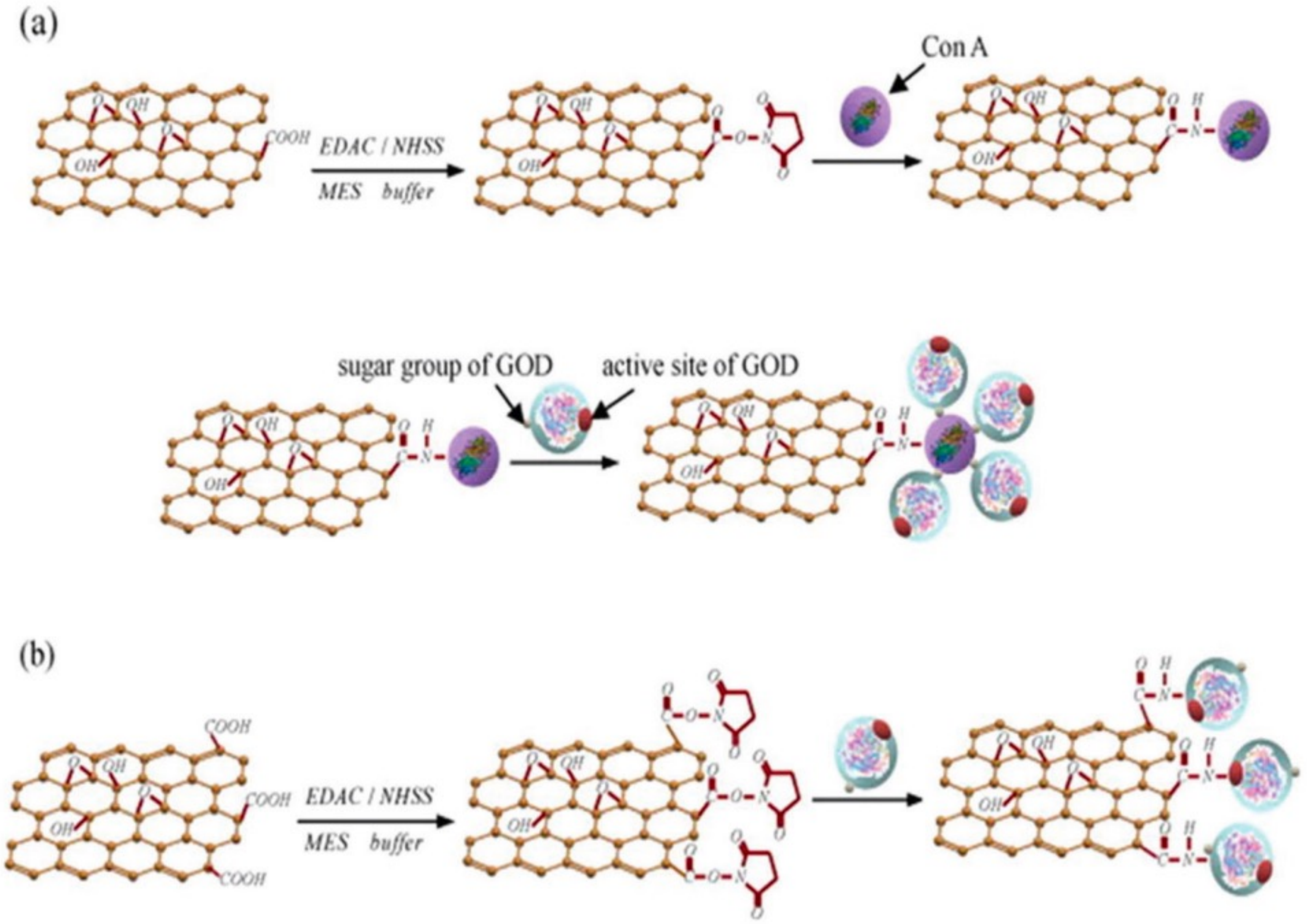
Illustration of enzyme immobilization methods onto graphene

Karimi *et al*, 2015

A: Comparison between site specific oriented (a)

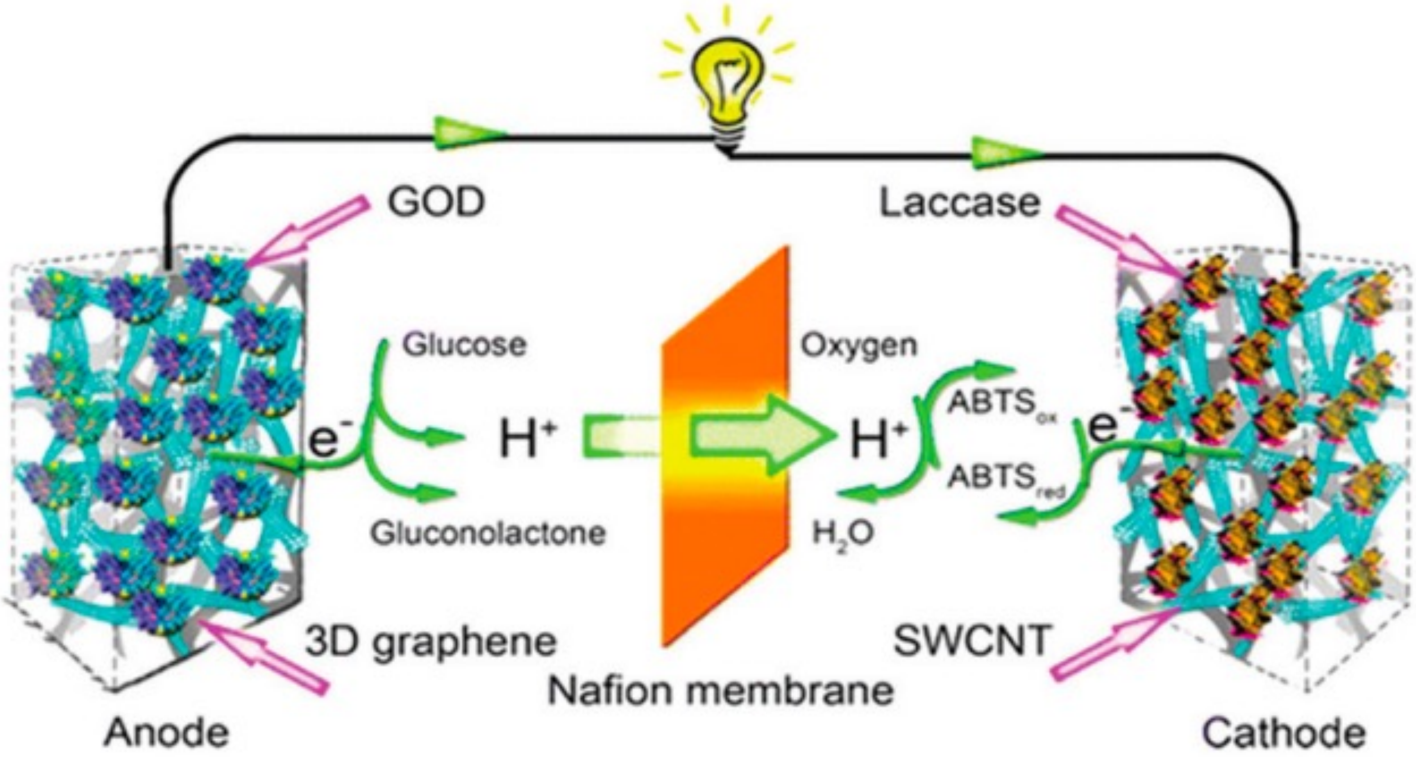
B: and random covalent immobilization of GOx on graphene *via* concanavalin A (Con A)

(GOD refers to GOx in the figure).



Zhou, LY, Jiang, Y J, Gao, J, Zhao, XQ, Ma L and Zhou, Q. L. *Biochem. Eng. J.*, **2012**, *69*, 28-31

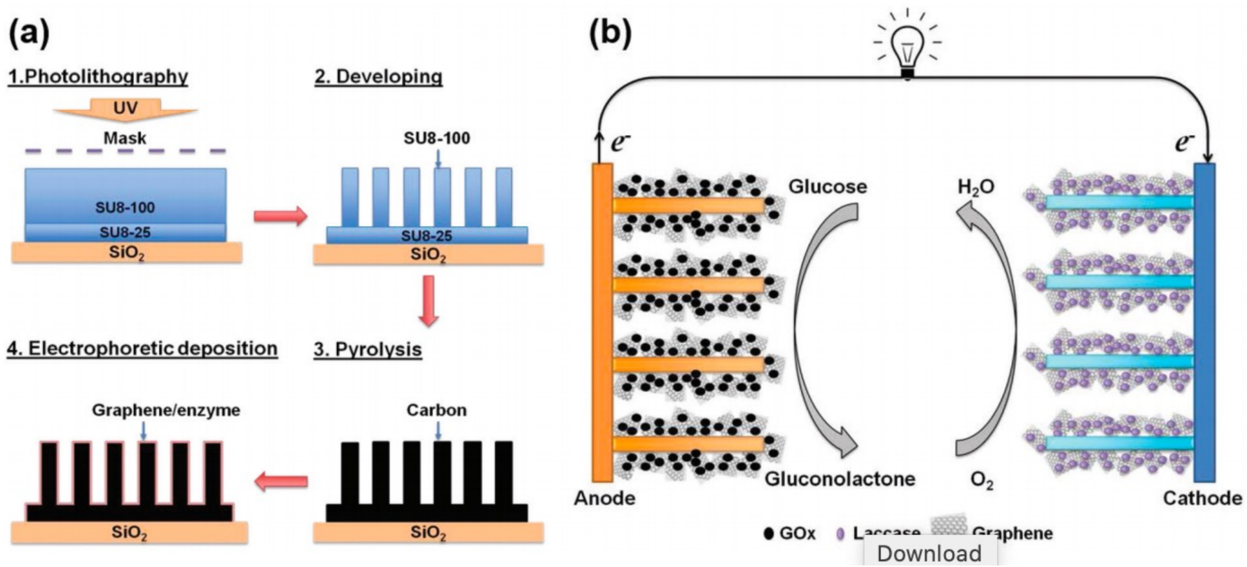
Enzymatic biofuel Cells (EBFC) based on 3D graphene-SWCNT hybrid electrodes.



Prasad, KP, Chen, Y and Chen, P*ACS Appl. Mater. Inter-faces*, **2014**, *6*, 3387-3393.

Carbon microelectromechanical systems C-MEMS

A: Fabrication of EBFC based on C-MEMS micropillar arrays.

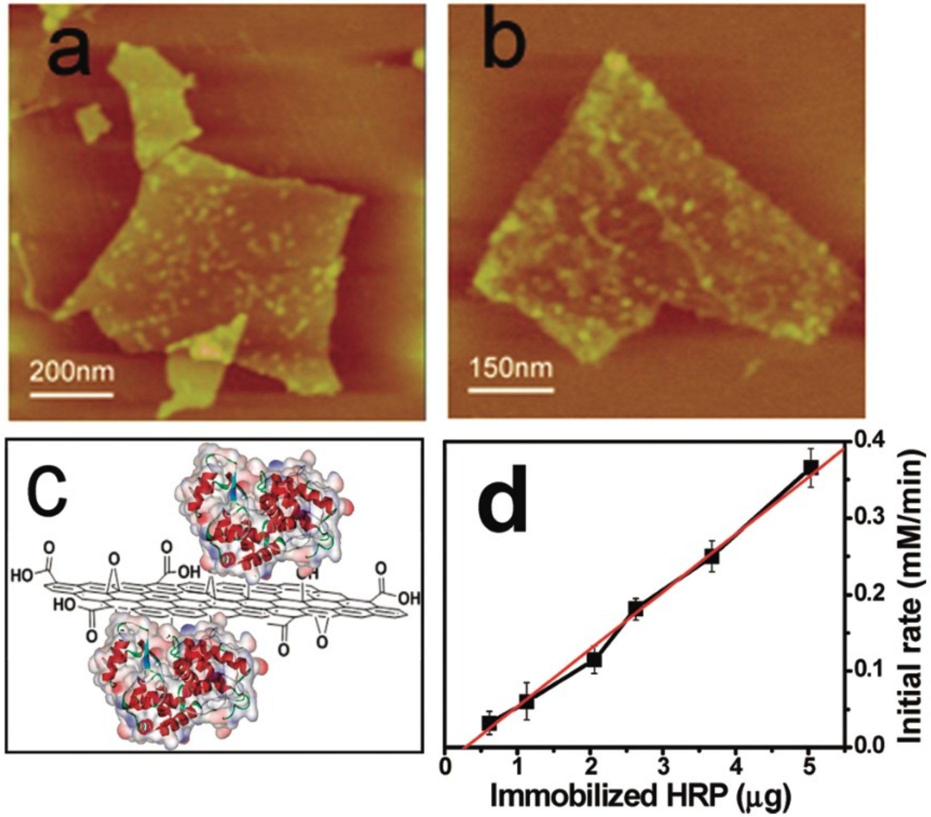


B: Illustration of the EBFC with graphene/enzyme-encrusted 3D carbon micropillar arrays (not to scale).

Song, Y, Chen C and Wang C *Nanoscale*, **2015**, *7*, 7084-7090

7. Immobilization of Biocatalysts onto Nanosupports: Advantages for Green Technologies

Alan S. Campbell, Andrew J. Maloney, Chenbo Dong, and Cerasela Z. Dinu

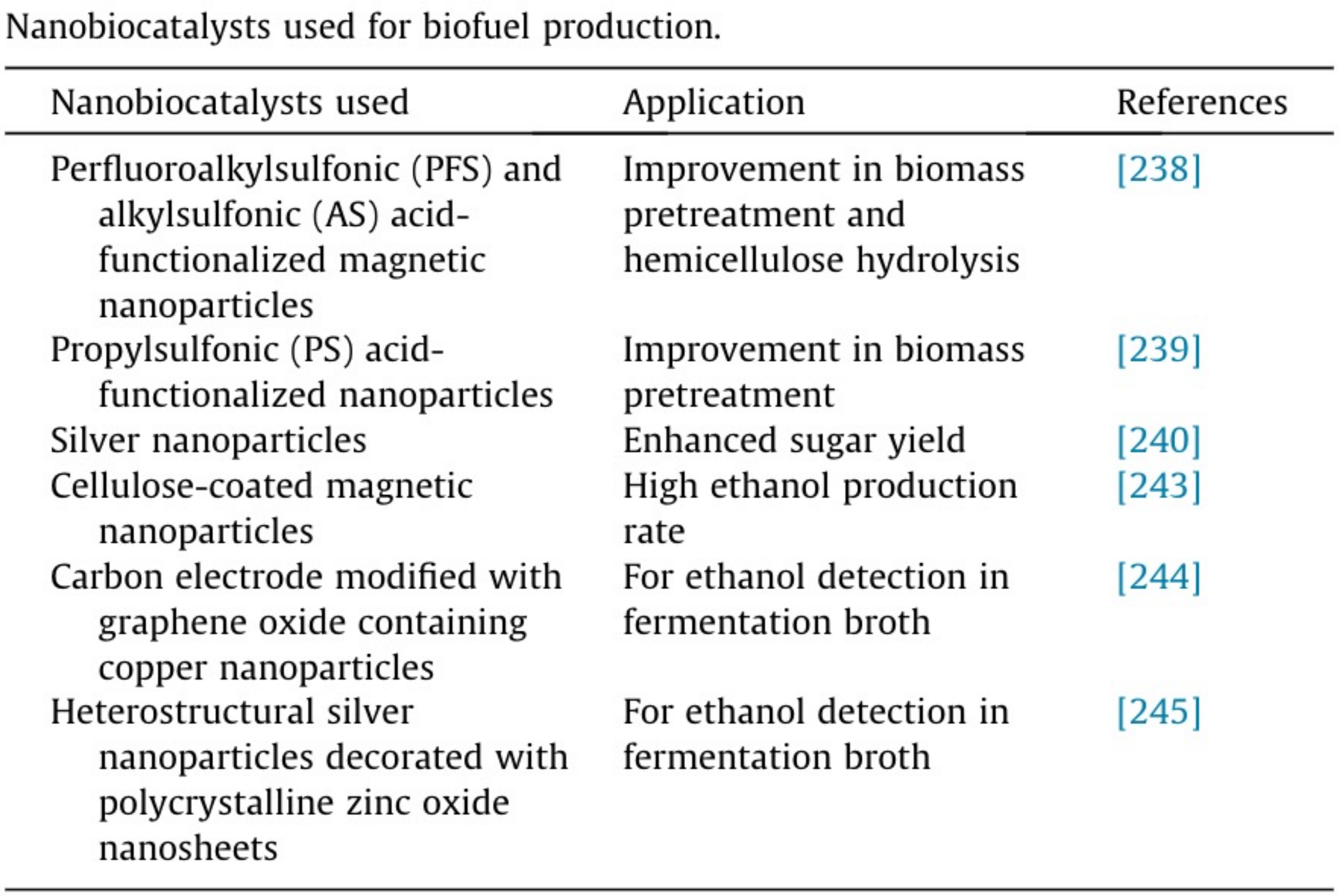


Tapping mode AFM images of the GO-bound HRP with
(a) lower and
(b) higher enzyme loadings acquired in a liquid cell.

(c) Schematic model of the GO-bound HRP.
(d) Initial reaction rates of GO-bound HRP versus HRP concentration.
GO Graphene Oxide

Zhang *et al*, *Langmuir* **2010**, *26* (9), 6083-6085

9. Potential Applications of Nanobiocatalysis for Industrial Biodiesel Production *Avinesh Byreddy and Munish Puri*



Nanobiocatalysts used for biofuel production.

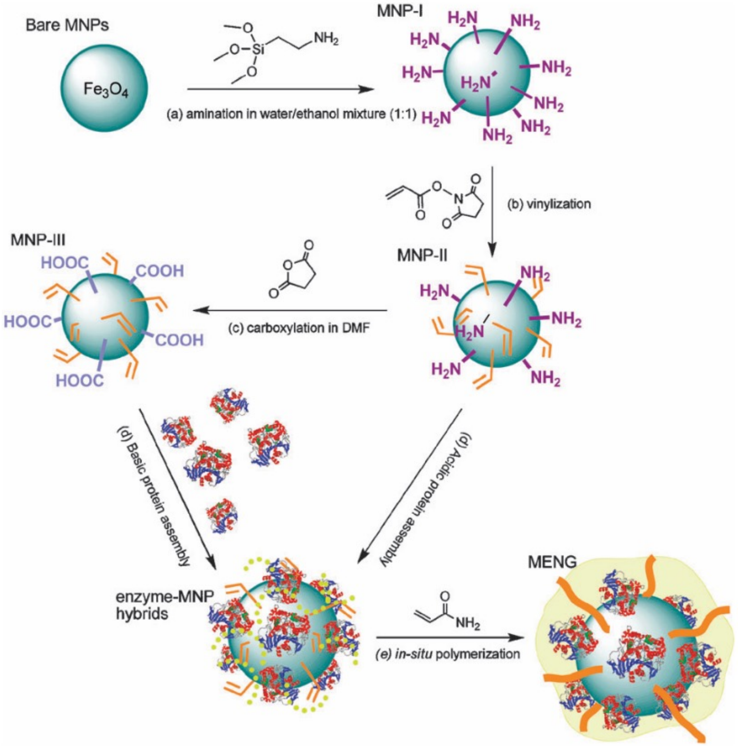
Nanobiocatalysts used	Application	References
Perfluoroalkylsulfonic (PFS) and alkylsulfonic (AS) acid-functionalized magnetic nanoparticles	Improvement in biomass pretreatment and hemicellulose hydrolysis	[238]
Propylsulfonic (PS) acid-functionalized nanoparticles	Improvement in biomass pretreatment	[239]
Silver nanoparticles	Enhanced sugar yield	[240]
Cellulose-coated magnetic nanoparticles	High ethanol production rate	[243]
Carbon electrode modified with graphene oxide containing copper nanoparticles	For ethanol detection in fermentation broth	[244]
Heterostructural silver nanoparticles decorated with polycrystalline zinc oxide nanosheets	For ethanol detection in fermentation broth	[245]

Singh *et al*, 2020

11. Recent Advances in Nanostructured Enzyme Catalysis for Chemical Synthesis in Organic Solvents

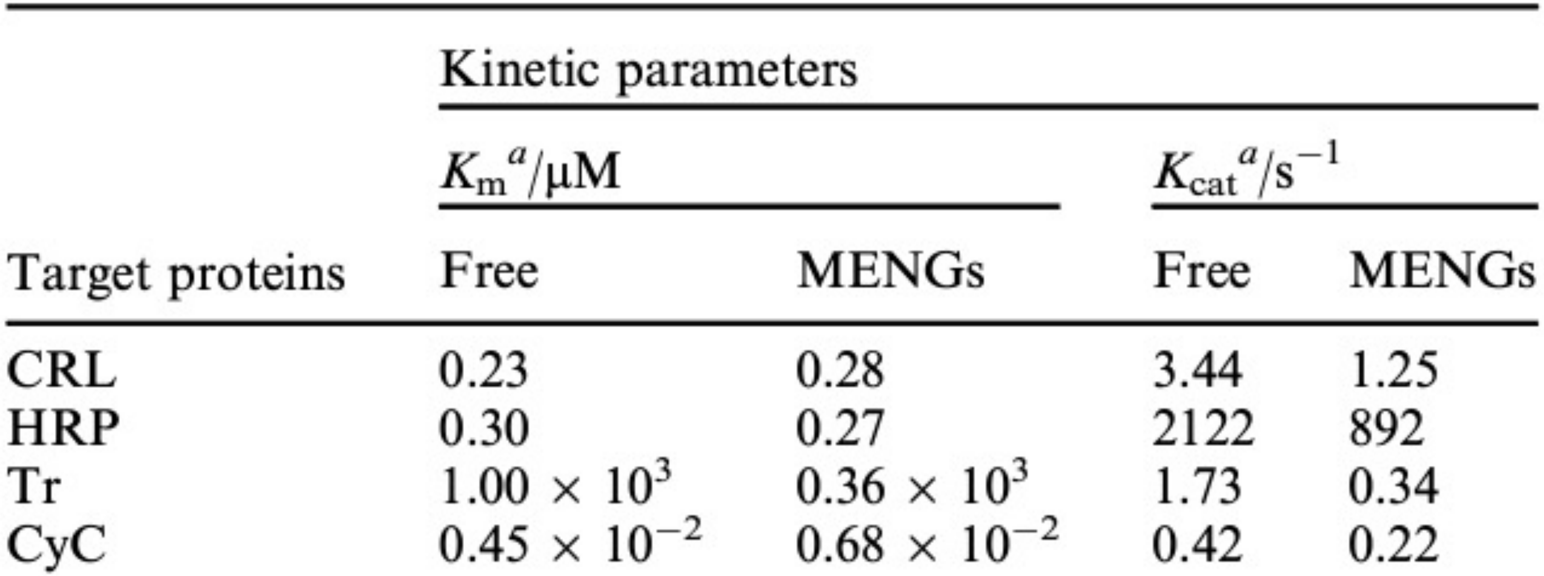
Zheng Liu, Jun Ge, Diannan Lu, Guoqiang Jiang, and Jianzhong Wu

Synthetic route of step-by-step fabrication of magnetic enzyme nanogels (MENG´s)



Lin *et al, Chem. Commun.*, **2012**, *48*, 3315-3317

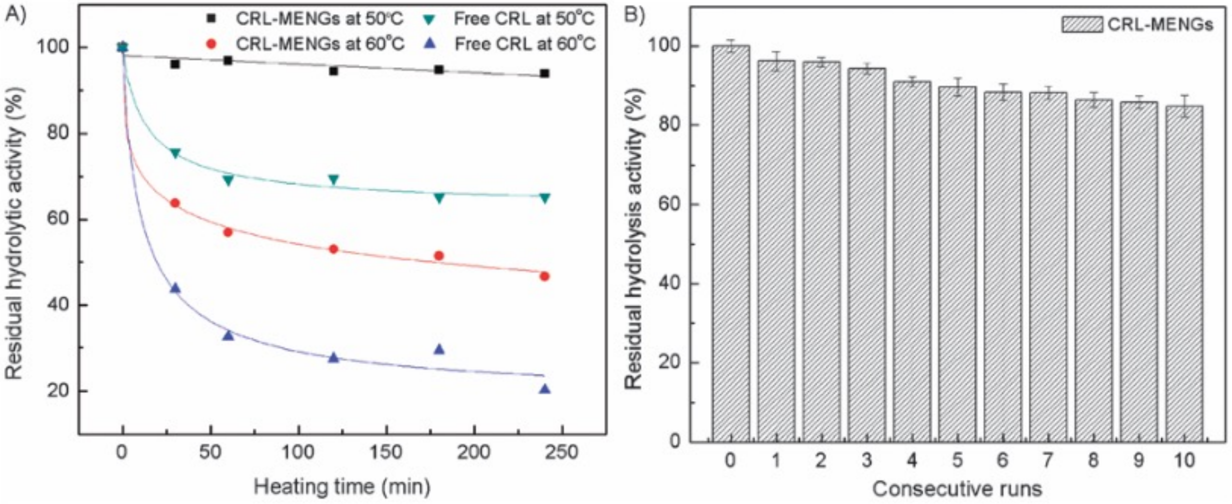
Kinetics parameters, Michaelis constant (K_m) and transformation efficiency (K_{cat}), of free enzymes and MENs



	Kinetic parameters			
	$K_m{}^a/\mu M$		$K_{cat}{}^a/s^{-1}$	
Target proteins	Free	MENGs	Free	MENGs
CRL	0.23	0.28	3.44	1.25
HRP	0.30	0.27	2122	892
Tr	1.00×10^3	0.36×10^3	1.73	0.34
CyC	0.45×10^{-2}	0.68×10^{-2}	0.42	0.22

After encapsulation within the magnetic polyacrylamide nanogel, the Kcat values of the MENGs decreased to 30–35% of the original values determined for their free counterparts. The slight increase in K_m and the decrease in K_{cat} values, except in the case of trypsin-MENGs, may be attributed to spatial hindrance in accessing the active site of the enzyme and additional mass-transport resistance by the polyacrylamide network

Thermal inactivation of enzyme activity Candida rugosa lipase-CRL



(A) Thermal inactivation kinetics of CRL in the free form and MENG´s at 50^{o}C and 60^{o}C

(B) recycling of the CRL-MENG´s in aqueous media, in which CRL-MENG´s were recovered by a bench magnet for 10 consecutive runs.

What’s next for bionanotechnology?

- Judging by the applicant countries of emerging nanotechnologies, we can continue to expect aggressive innovation from the above countries.

- However, whether or not China is planning to enter the fray has yet to be seen. They are certainly far behind in bionanotechnologies. In an area with a death of granted patents, it is crucial for large patenting venues to compare and determine the patentability of nanotechnologies moving forward.

- Bionanotechnology inventions will not only involve emerging methods of drug delivery, medical products, pharmaceuticals, but also the tools with which scientists study and even manufacture items at a nanoscale.

- It is not a question of if, but when, will bionanotechnologies disrupt a long-established industry with billions of dollars on the line.

https://www.ktmine.com/future-bionanotech-told-by-nanotechnology-patents/

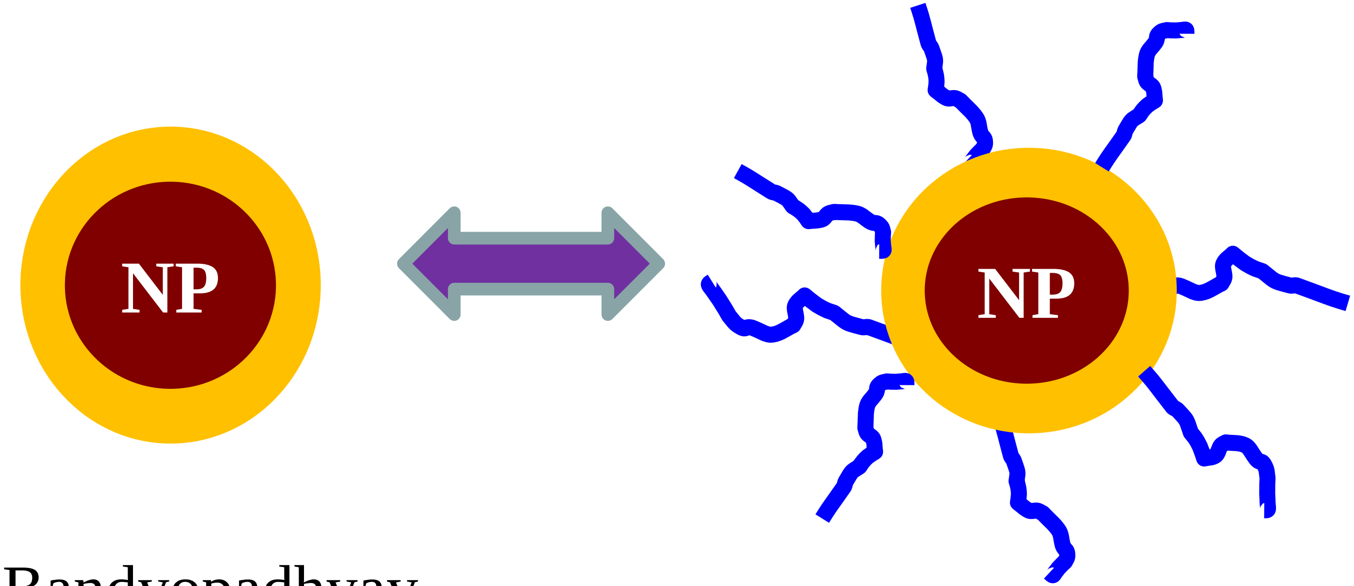
DISCUSSION

- What can we as researchers bring to the table of bionanotechnology?

  - Continue the research in all areas
  - Inform the industry about the advantages-10-15 years scope
  - Inform the society about the advantages
  - Industry should look into bionanotechnology based

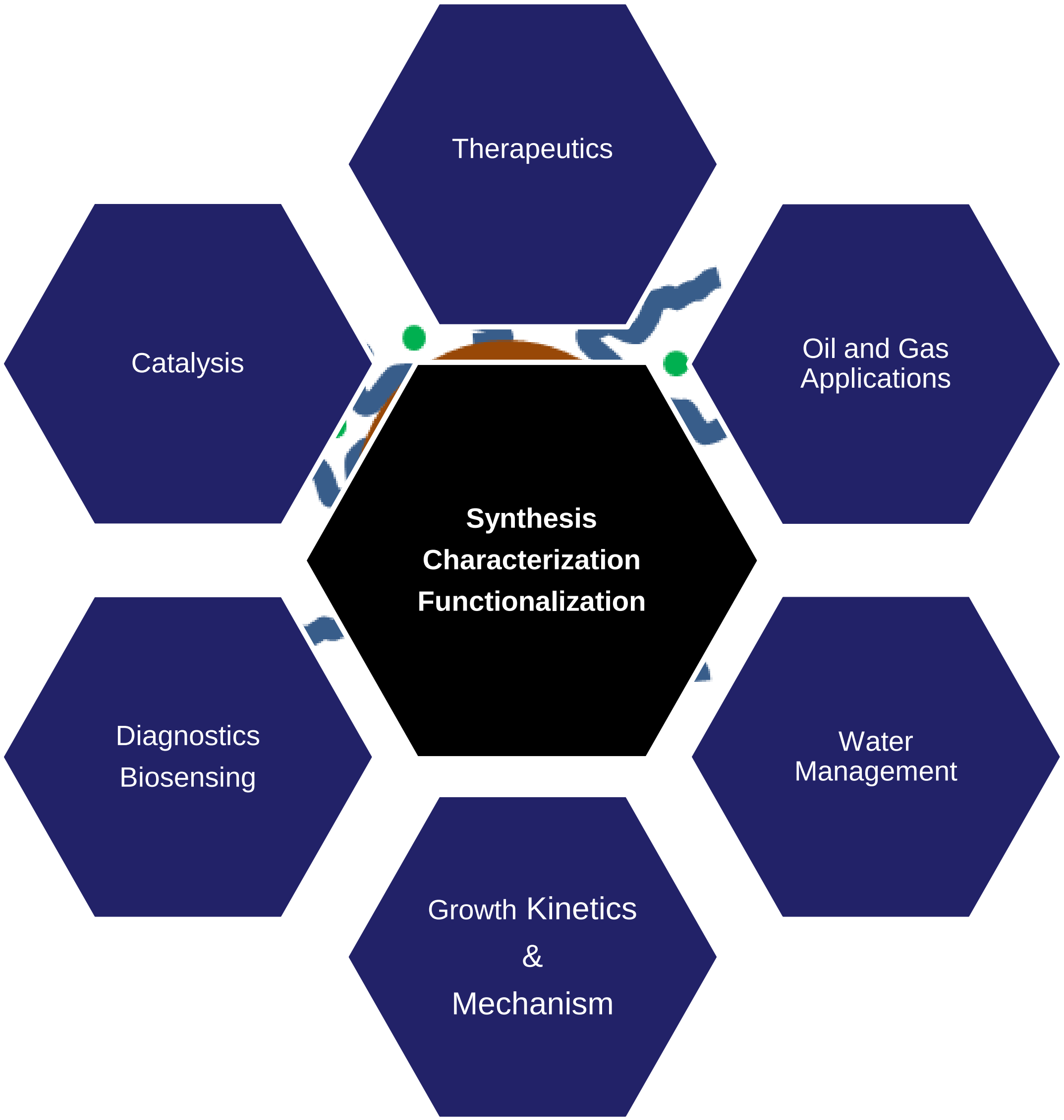
  cost effective processes

Synthesis and Functionalisation of Nanoparticles



Sulalit Bandyopadhyay,
Associate Professor,
Department of Chemical Engineering, NTNU.
sulalit.bandyopadhyay@ntnu.no

NTNU
Norwegian University of
Science and Technology



Therapeutics

Catalysis

Oil and Gas Applications

Synthesis
Characterization
Functionalization

Diagnostics
Biosensing

Water Management

Growth Kinetics
&
Mechanism

Reading Material

- Fabrication and Application of Nanomaterials, S. Bandyopadhyay, McGraw-Hill Education.

- Mora-Huertas, C. E., Fessi, H., & Elaissari, A. (2011). ***Advances in colloid and interface science***, *163*(2), 90–122.

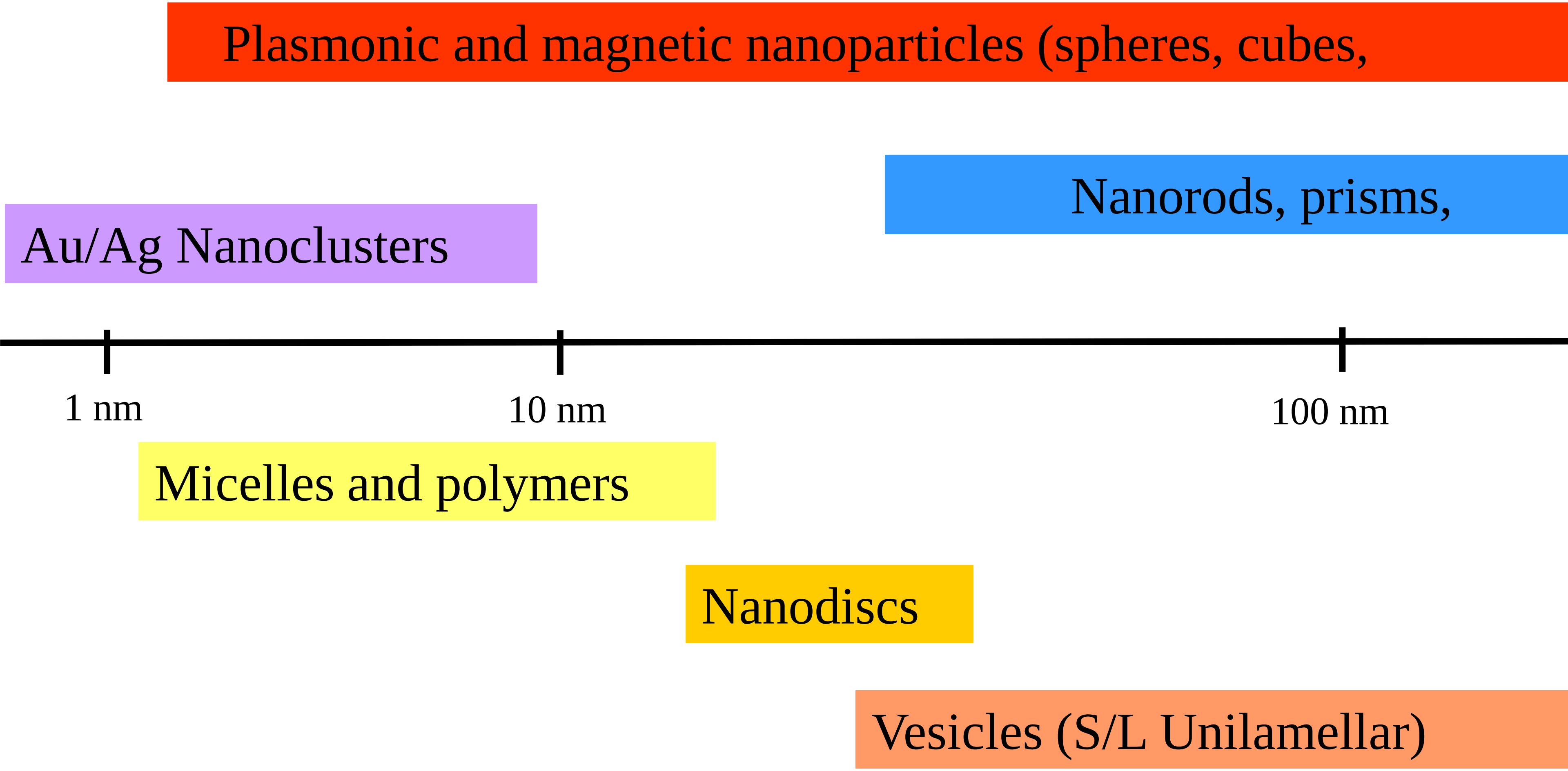
- Bally, Florence, et al.(2012) ***Polymer**,* 53(22), 5045-5051.

- Saad, Walid S., and Robert K. Prud’homme. (2016) ***Nano Today,*** 11(2), 212-227.

Topics

- **Metallic NPs**
  - Synthesis Methods
    - Reduction of Metallic Precursors
    - Thermal Decomposition
    - Colloidal Templating
    - One pot methods
  - Anisotropic NPs
- **Functionalization of NPs**
- **Polymer-based NPs**
  - Nanoprecipitation
  - Flash Nanoprecipitation
  - Precipitation Polymerization

Nanomaterials



Plasmonic and magnetic nanoparticles (spheres, cubes,

Nanorods, prisms,

Au/Ag Nanoclusters

1 nm

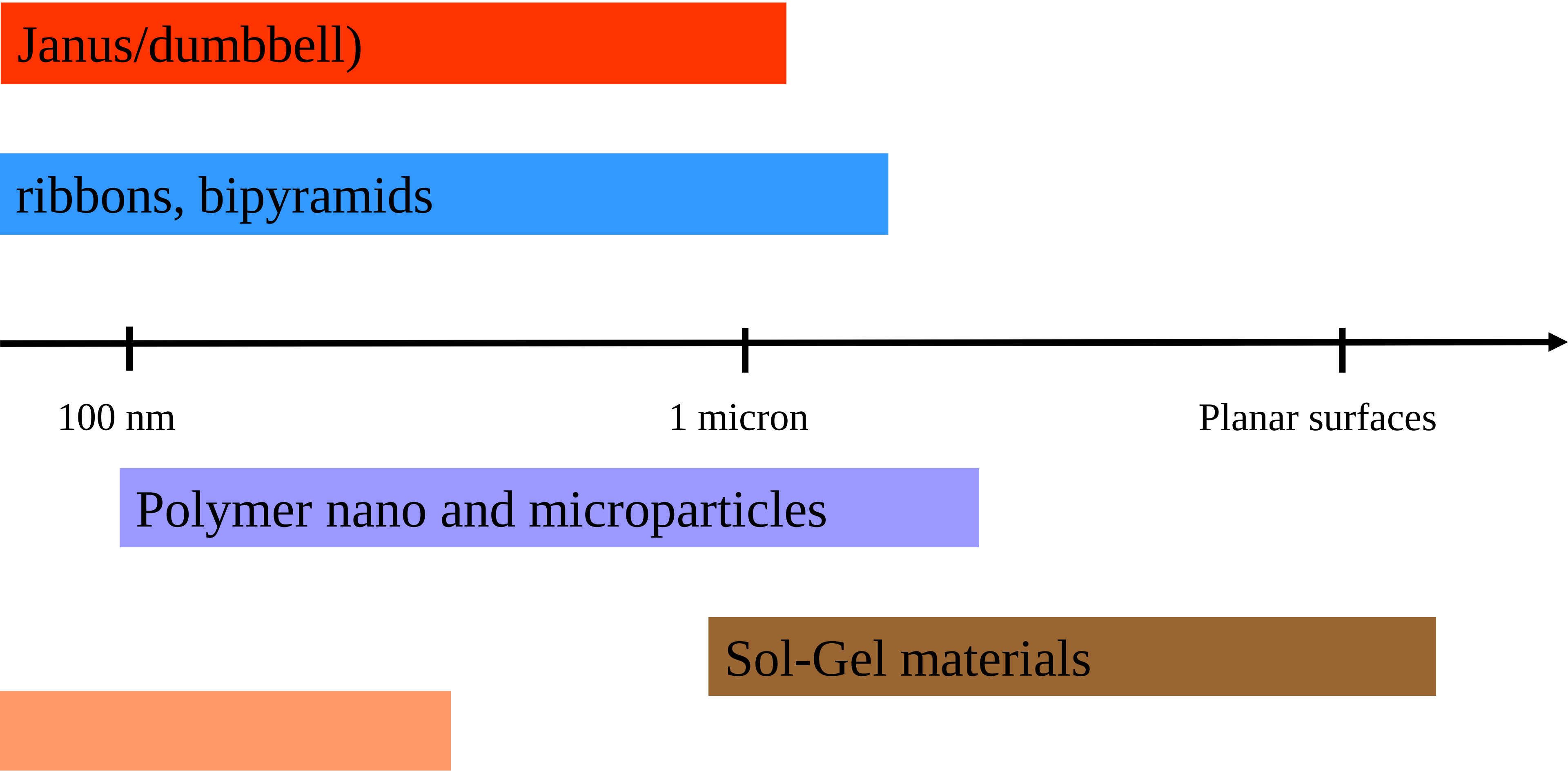
10 nm

100 nm

Micelles and polymers

Nanodiscs

Vesicles (S/L Unilamellar)



Janus/dumbbell)

ribbons, bipyramids

100 nm

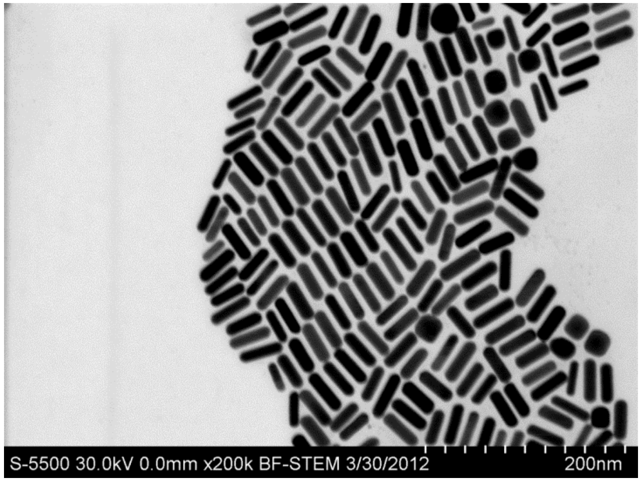
1 micron

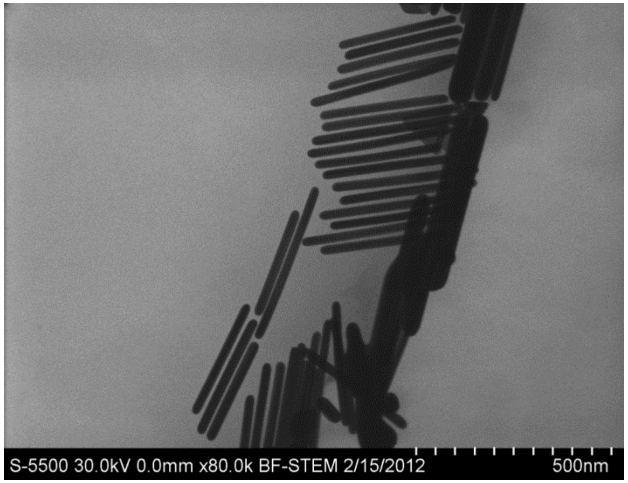
Planar surfaces

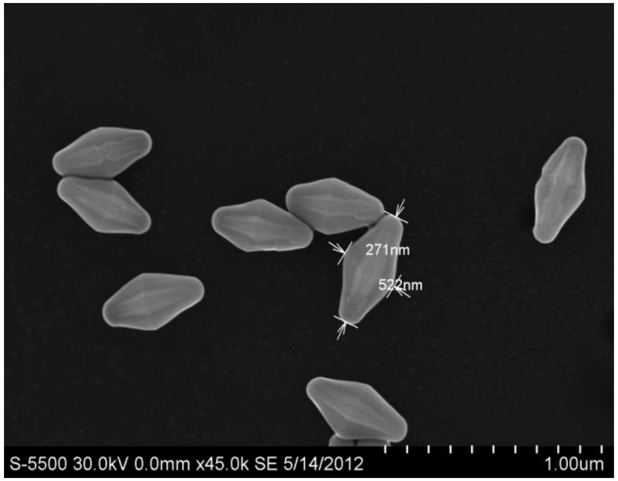
Polymer nano and microparticles

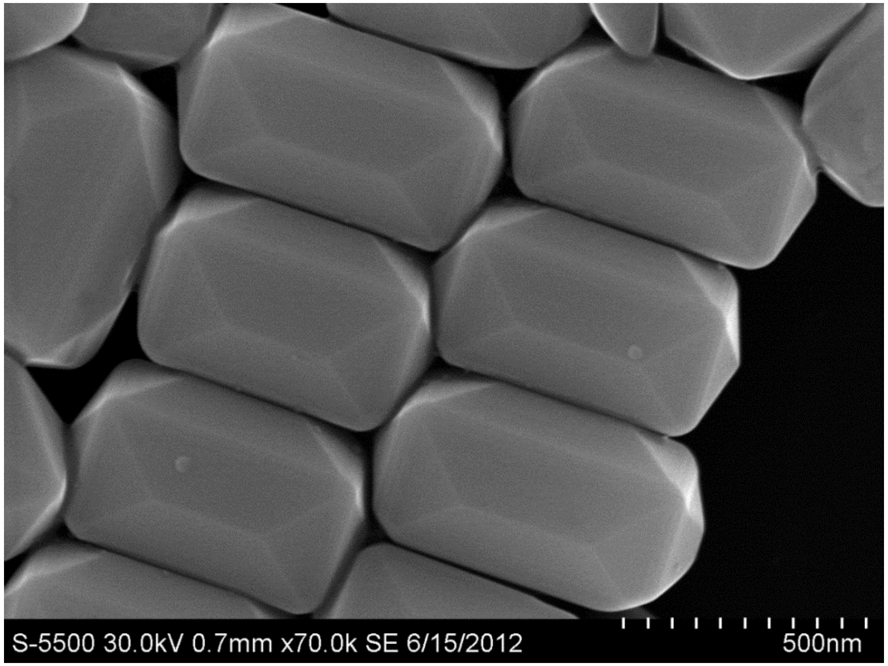
Sol-Gel materials

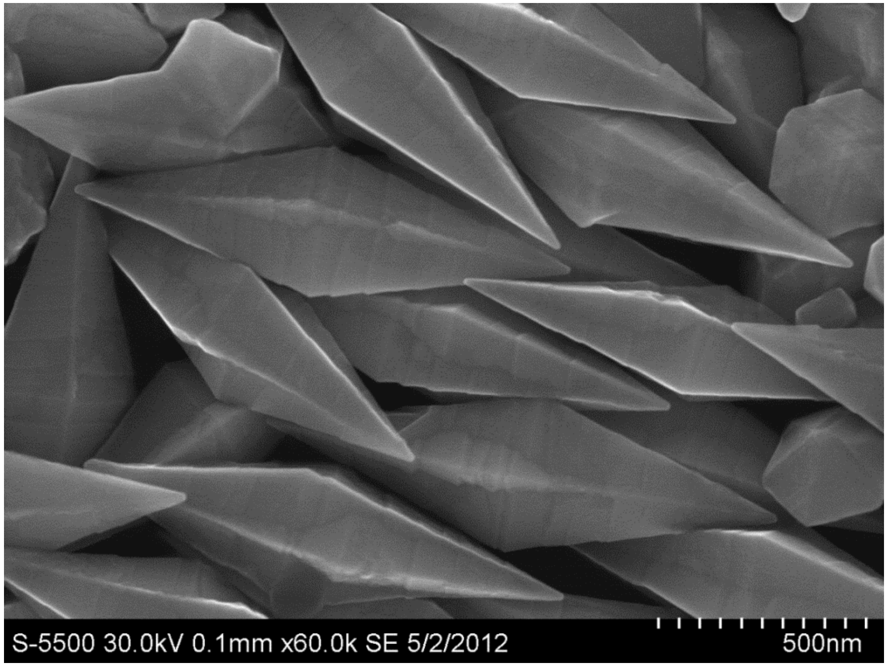
Library of NPs



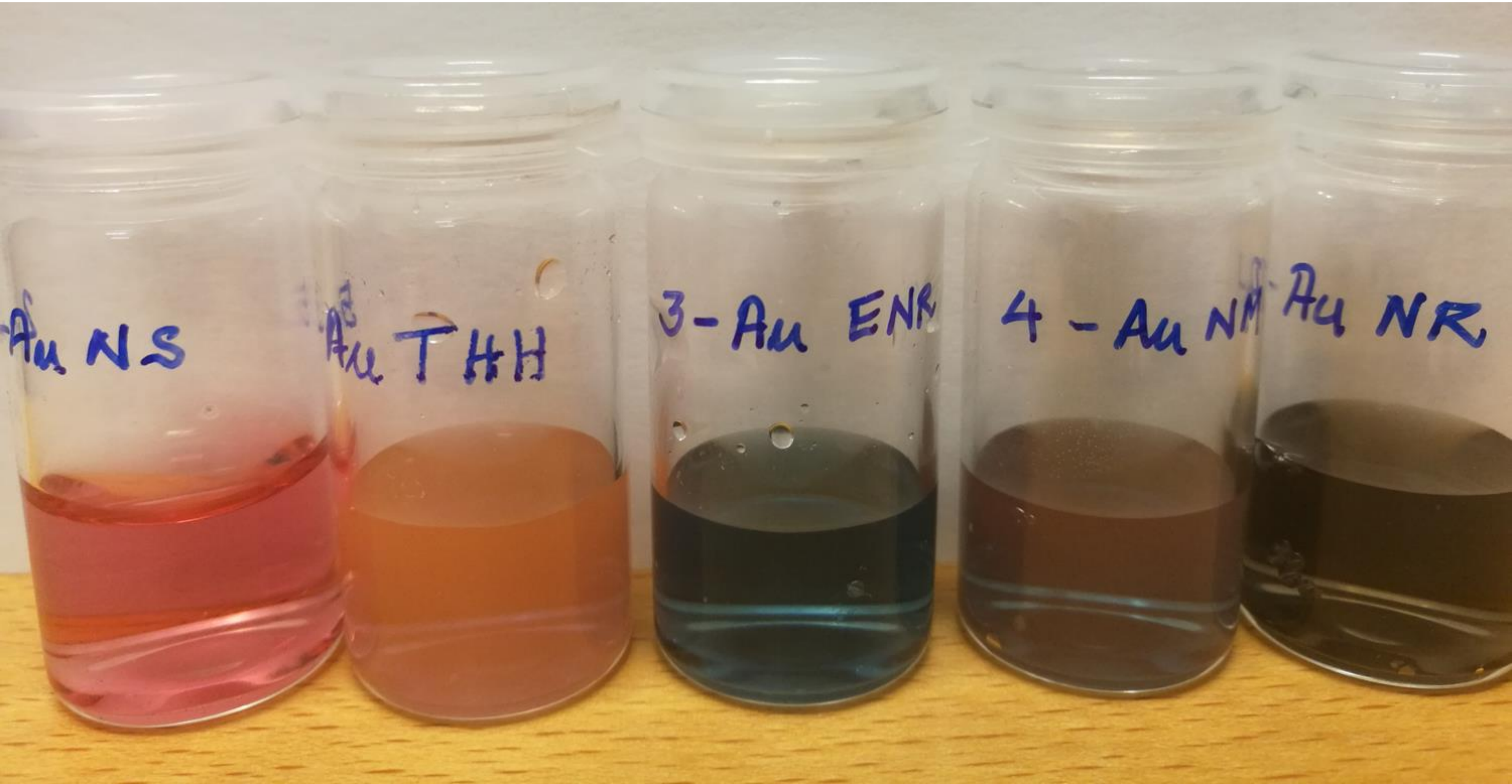




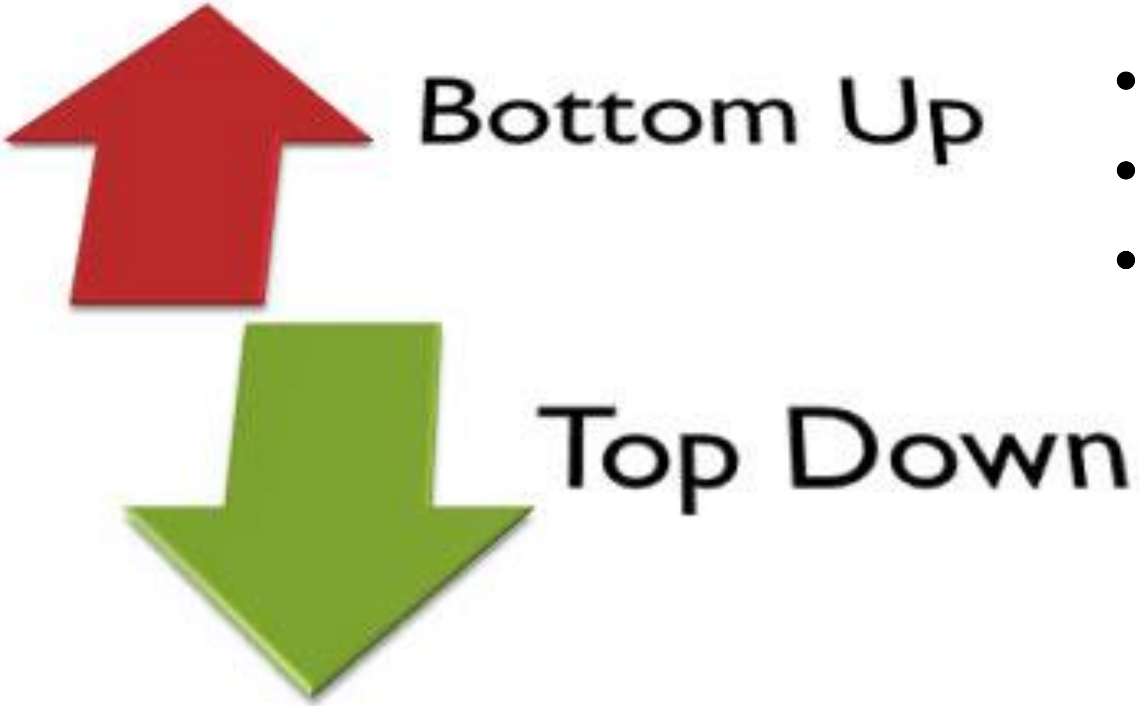




Synthesis of Metallic NPs



Synthesis Approaches



- Heterogeneity
- PSD
- Defects, Impurities

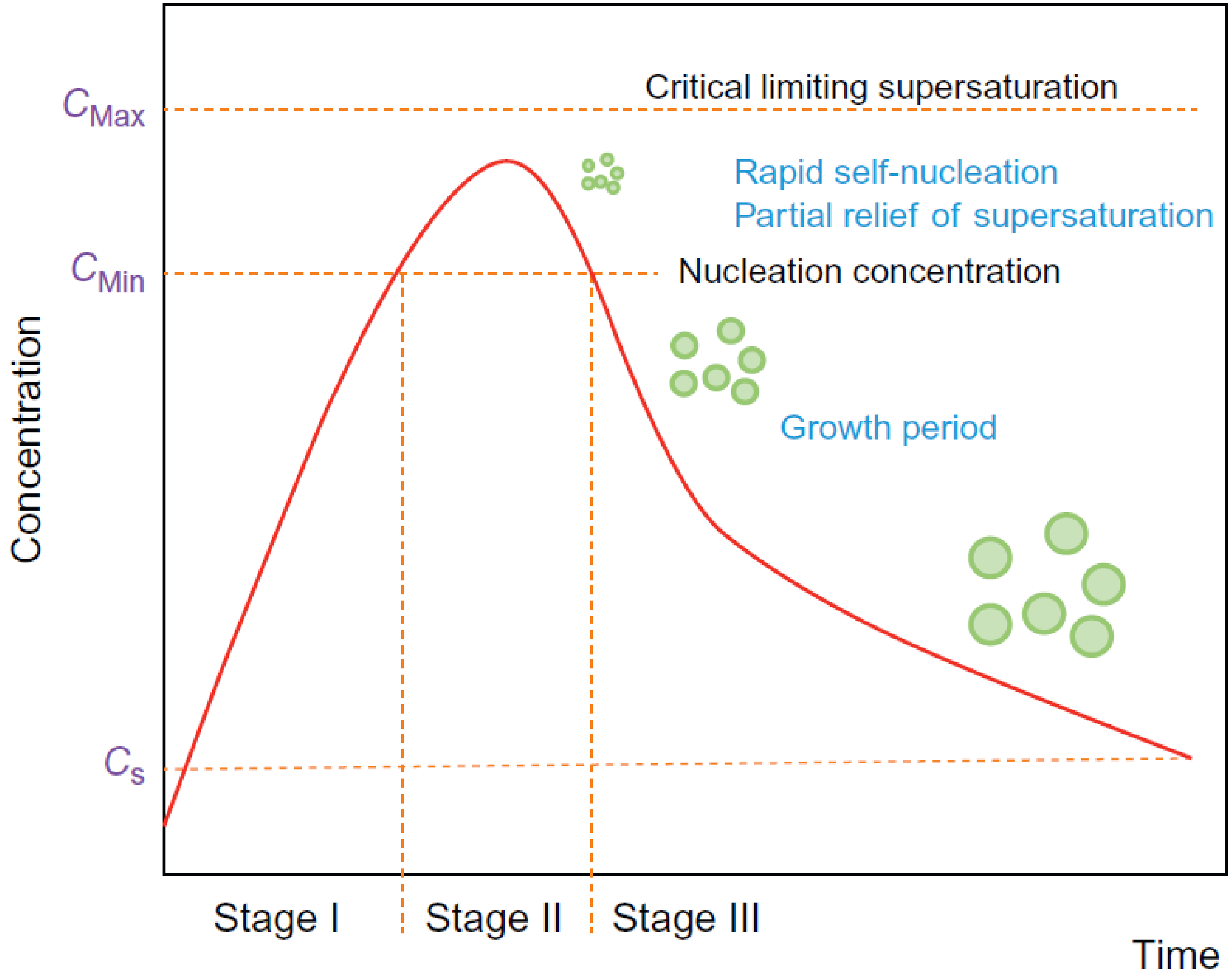
Classical Nucleation Theory

1. Supersaturation
2. Nucleation
3. Crystal growth
4. Secondary growth

$$S = \frac{c}{c*}$$

$$\sigma = \frac{\Delta c}{c*} = S - 1$$

Lamer’s Diagram

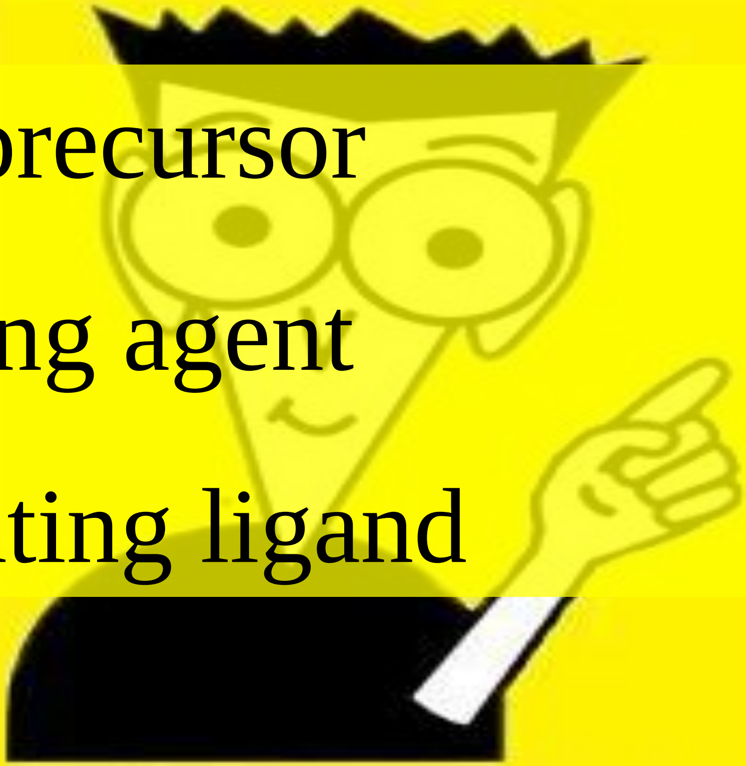


Crystallization

~~&~~

~~Precipitation~~

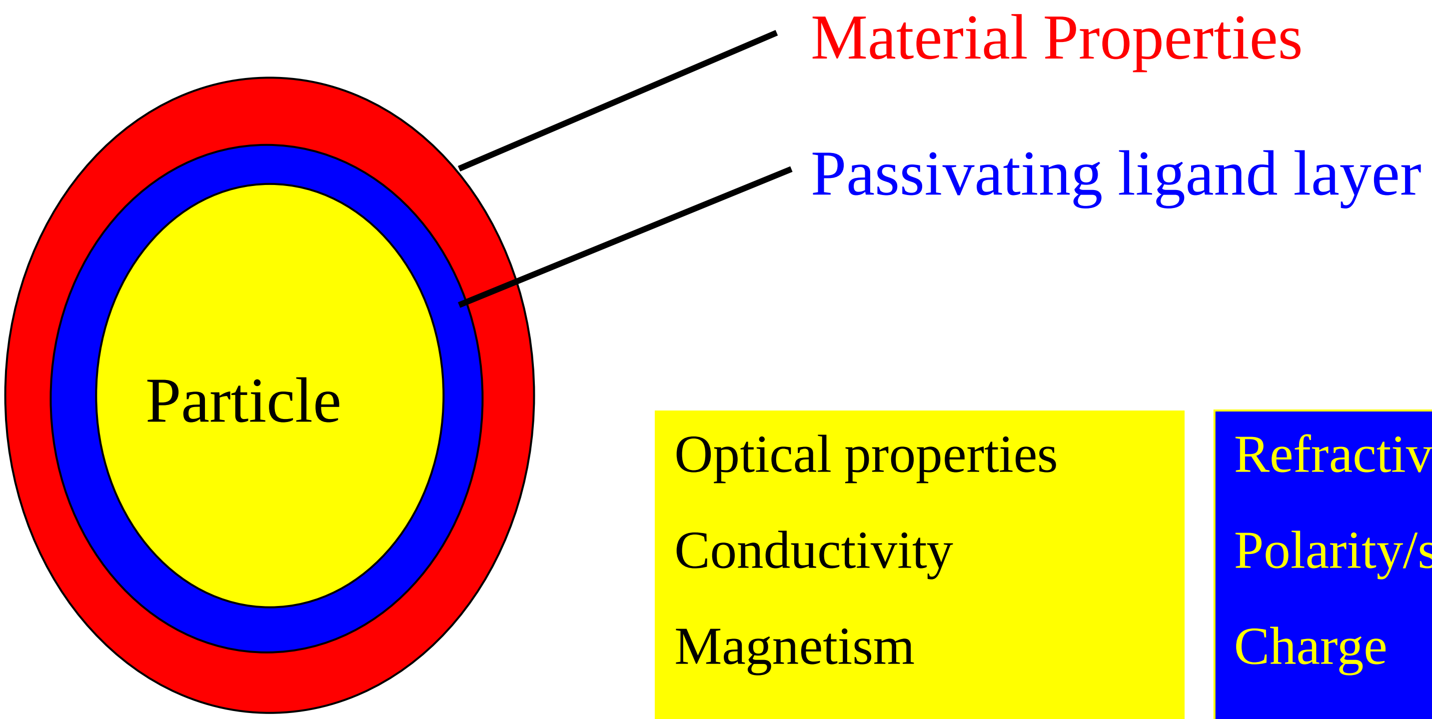
Crystallization FOR DUMMIES



1. Metal precursor
2. Reducing agent
3. Passivating ligand

Why?

Properties of Metallic NPs



Material Properties

Passivating ligand layer

Particle

- Optical properties
- Conductivity
- Magnetism
- Catalytic activity

- Refractive index
- Polarity/solubility
- Charge
- Functionality

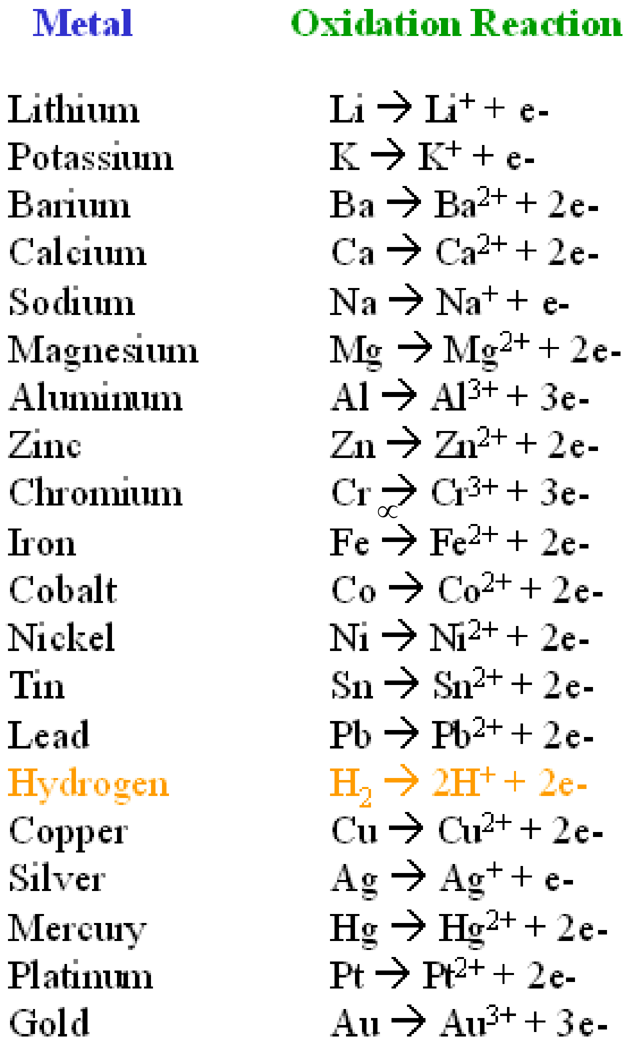
Functionality/application

Rule of Thumb

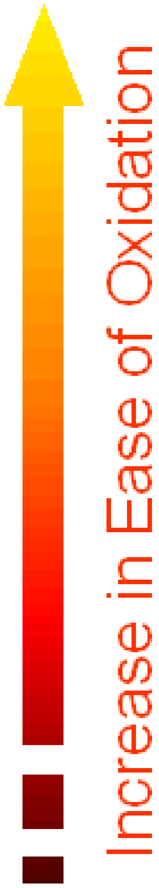
Redox potential

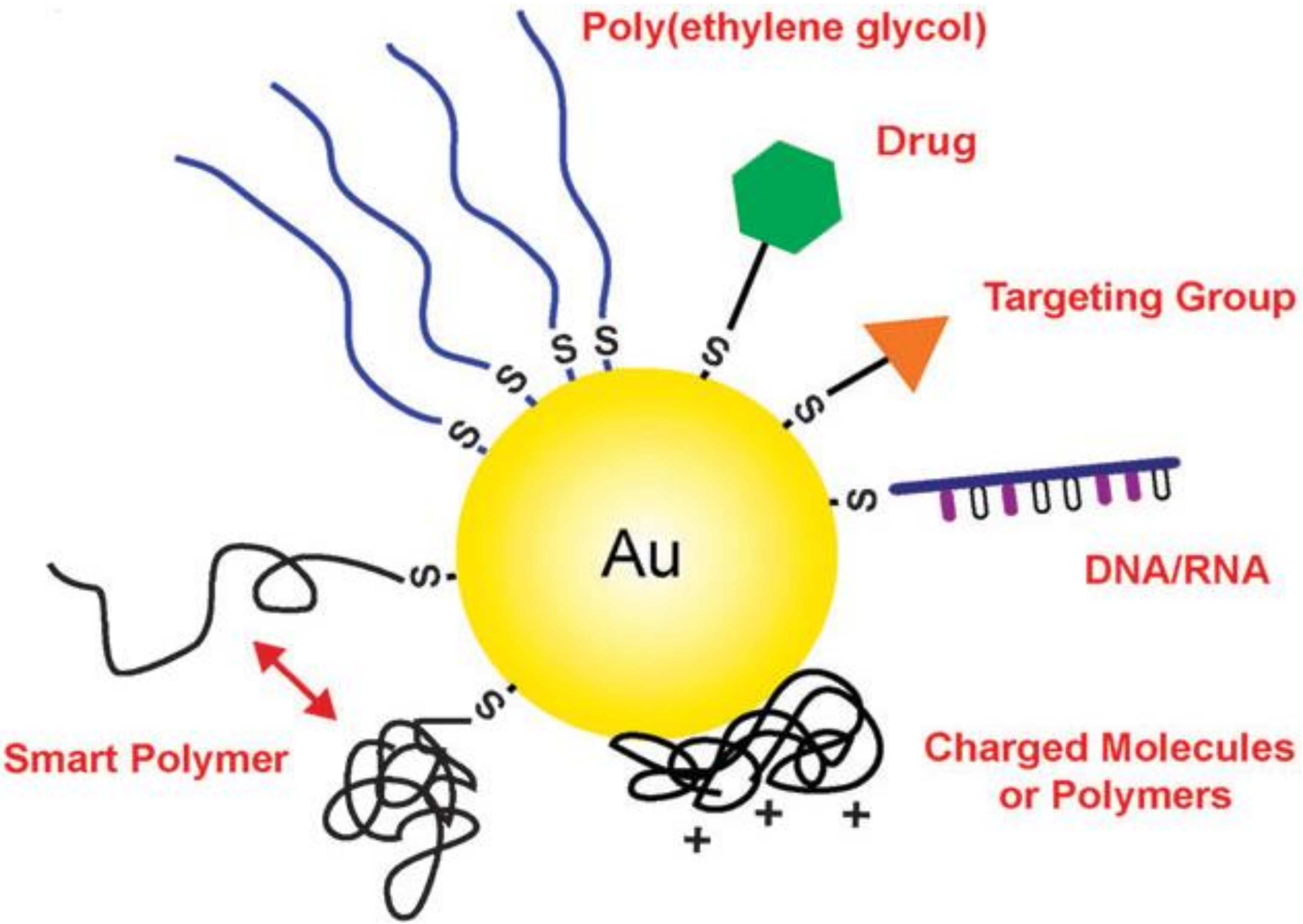
$$\propto^{-1}$$

Particle Size



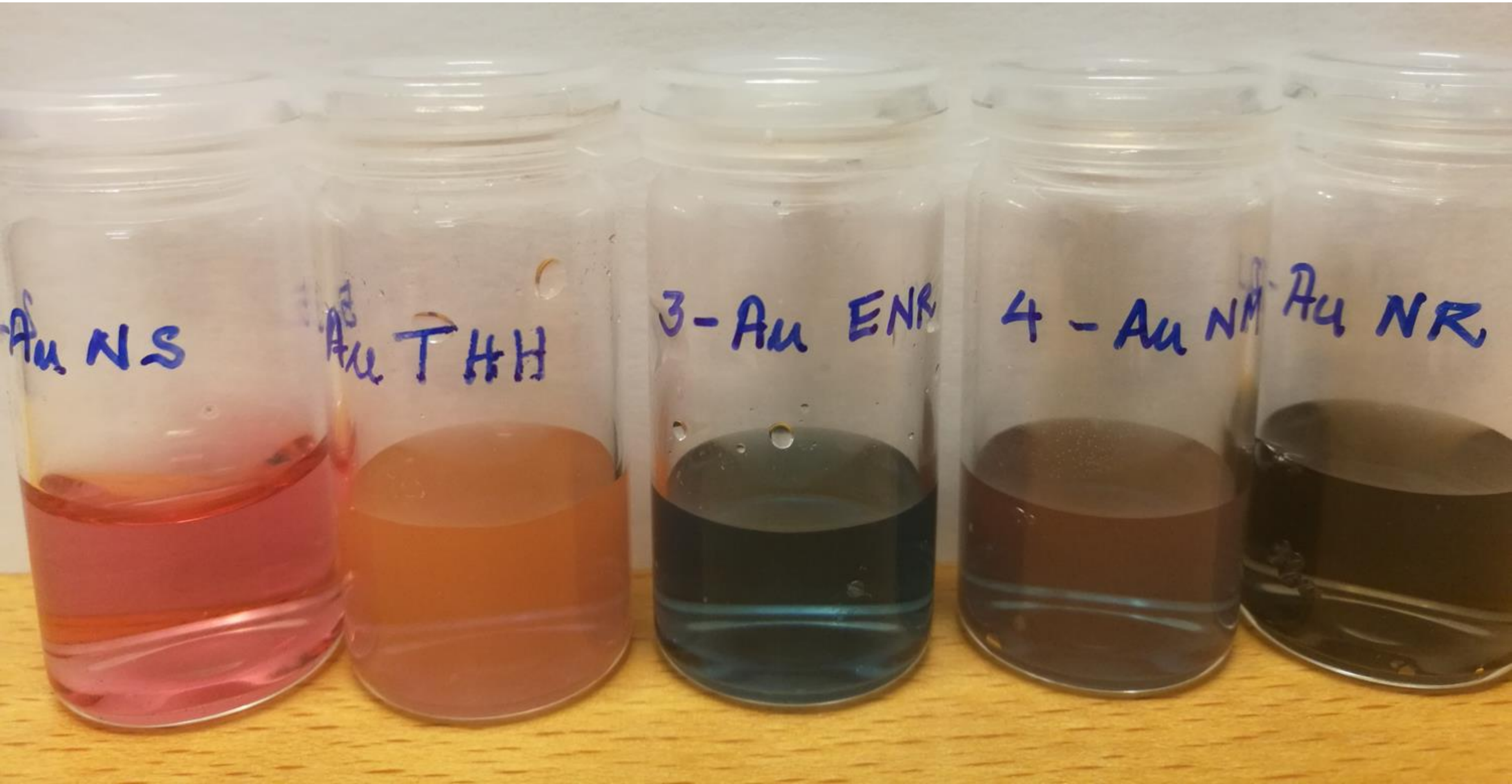
Metal	Oxidation Reaction
Lithium	$Li \rightarrow Li^{+} + e^{-}$
Potassium	$K \rightarrow K^{+} + e^{-}$
Barium	$Ba \rightarrow Ba^{2+} + 2e^{-}$
Calcium	$Ca \rightarrow Ca^{2+} + 2e^{-}$
Sodium	$Na \rightarrow Na^{+} + e^{-}$
Magnesium	$Mg \rightarrow Mg^{2+} + 2e^{-}$
Aluminum	$Al \rightarrow Al^{3+} + 3e^{-}$
Zinc	$Zn \rightarrow Zn^{2+} + 2e^{-}$
Chromium	$Cr \rightarrow Cr^{3+} + 3e^{-}$
Iron	$Fe \rightarrow Fe^{2+} + 2e^{-}$
Cobalt	$Co \rightarrow Co^{2+} + 2e^{-}$
Nickel	$Ni \rightarrow Ni^{2+} + 2e^{-}$
Tin	$Sn \rightarrow Sn^{2+} + 2e^{-}$
Lead	$Pb \rightarrow Pb^{2+} + 2e^{-}$
Hydrogen	$H_2 \rightarrow 2H^{+} + 2e^{-}$
Copper	$Cu \rightarrow Cu^{2+} + 2e^{-}$
Silver	$Ag \rightarrow Ag^{+} + e^{-}$
Mercury	$Hg \rightarrow Hg^{2+} + 2e^{-}$
Platinum	$Pt \rightarrow Pt^{2+} + 2e^{-}$
Gold	$Au \rightarrow Au^{3+} + 3e^{-}$



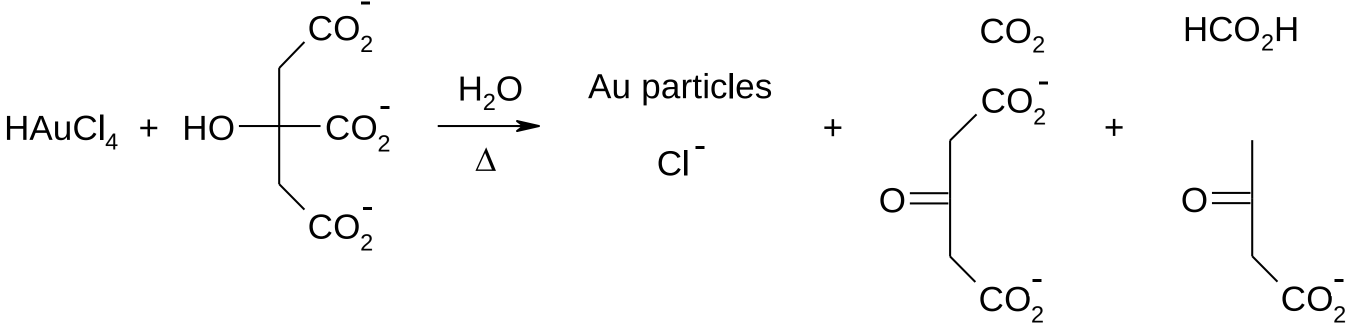


Xia, Y. et al., *Chem Soc Rev*, **2011**, 44-56

1. Reduction of Metallic Precursors in Solution



Turkevich Reaction (Citrate Reduction of Chloride Precursor)



- Aqueous phase synthesis
- Citrate acts both as reducing agent and passivating ligand
- Most common synthesis method (commercially available)
- Synthesis temperature typically 100 °C with refluxing conditions

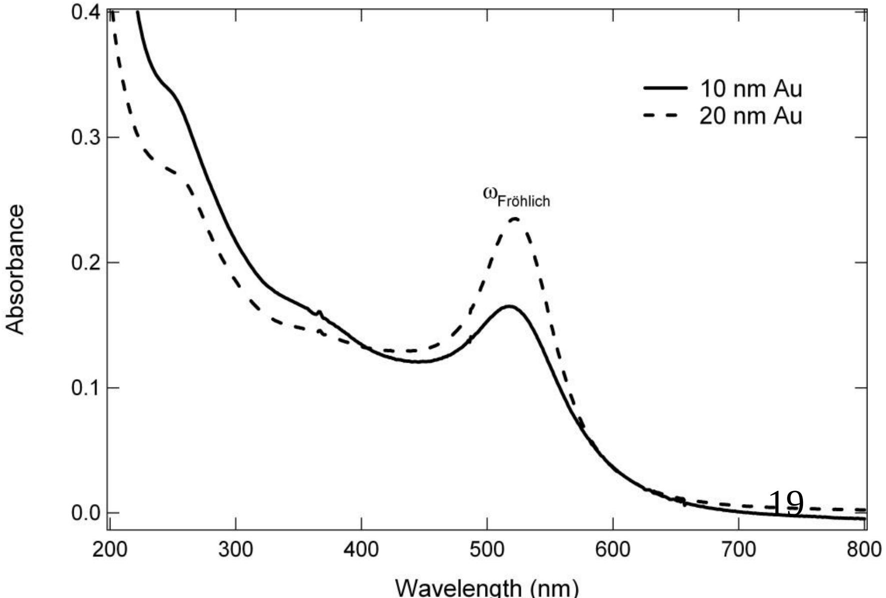
Turkevich, J. et al., *Discussions of the Faraday Society*, **1951**, 55-&

Turkevich, J. et al., *J. Phys. Chem.*, **1953**, 57, 670-673

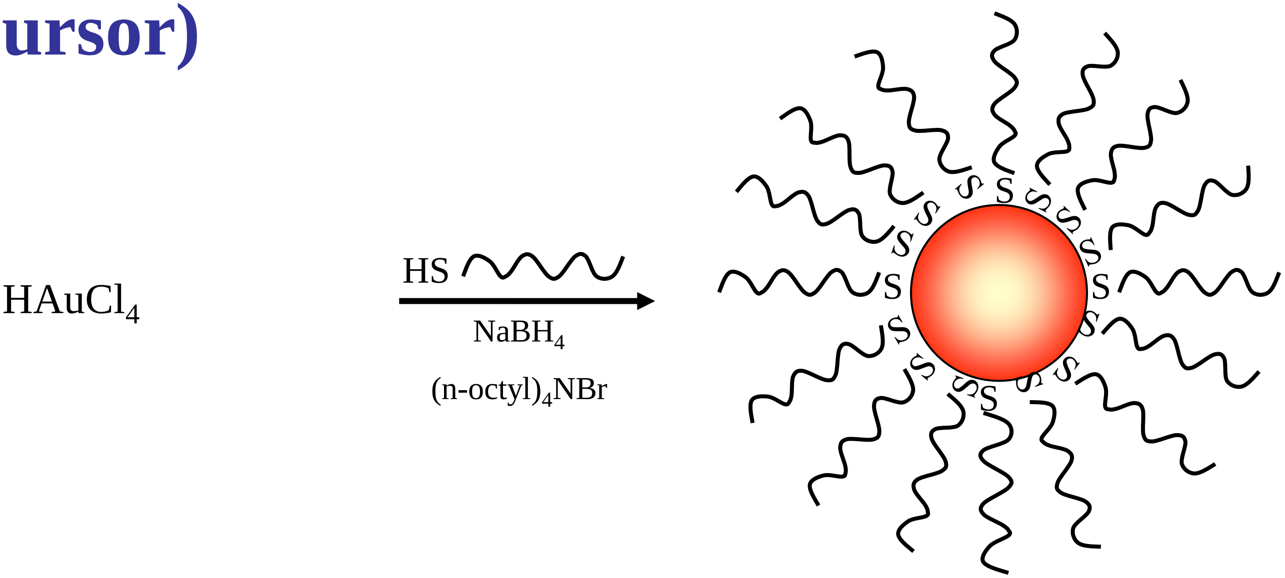
Turkevich, J. et al., *J. Colloid Sci.*, **1954**, 9, S26-S35

Reduction of Metallic Precursors in Solution
Turkevich Reaction

- Readily available in sizes from 2-200 nm
- Wide array of surface functionalities available by simple ligand exchange reactions
- Citrate can be displaced by thiols, isothiocyanates and phosphines
- Susceptible to flocculation/aggregation by changes in solvent conditions



Brust Reaction (BH_4^- Reduction of Chloride Precursor)

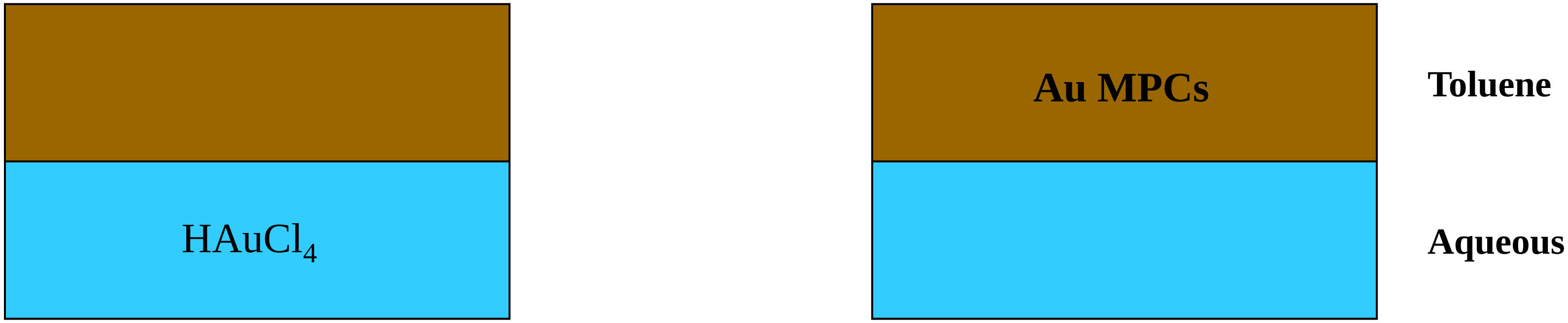


$HAuCl_4$

HS

$NaBH_4$

$(n\text{-octyl})_4NBr$



$HAuCl_4$

Au MPCs

Toluene

Aqueous

Brust, M. et al., *Chem. Commun.*, **1994**, 801-802

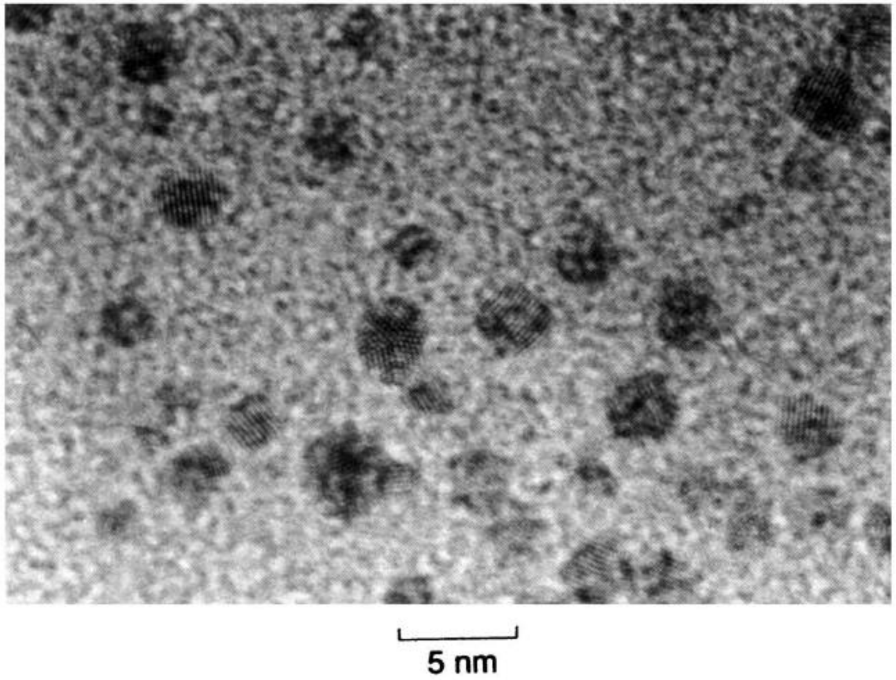
Brust, M. et al., *Chem. Commun.*, **1995**, 1655-1656

Reduction of Metallic Precursors in Solution

Brust Reaction

$$AuCl_4^-{}_{(aq)} + N(C_8H_{17})_4 + (C_6H_5Me) \rightarrow N(C_8H_{17})_4 + AuCl_4^-(C_6H_5Me) \qquad (1)$$

$$mAuCl_4^-(C_6H_5Me) + nC_{12}H_{25}SH(C_6H_5Me) + 3\,me^- \rightarrow 4mCl^-{}_{(aq)} + (Au_m)(C_{12}H_{25}SH)_n(C_6H_5Me) \qquad (2)$$



- Typically 1.5-8 nm particle size
- Very stable NPs.
- Access to a wide variety of surface functionalities through ligand exchange.
- Both polar and non-polar solvents.

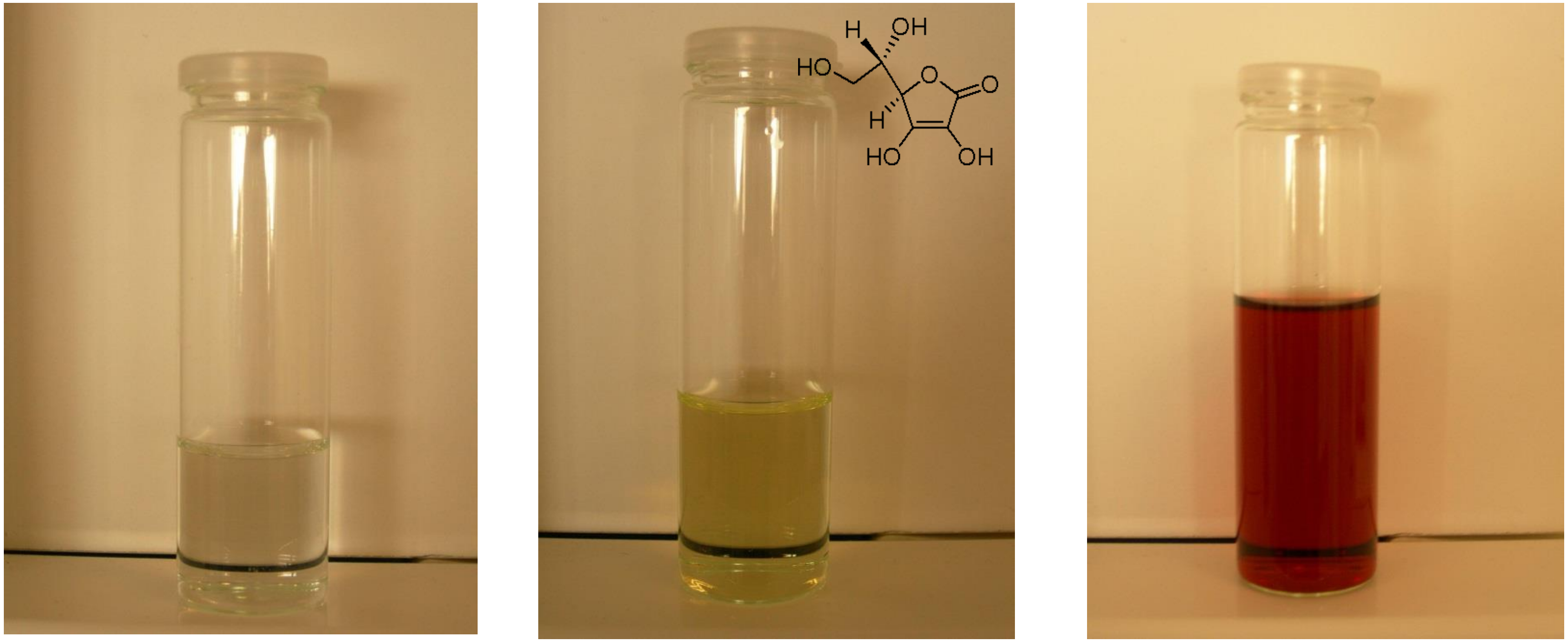
Brust, M. et al., *Chem. Commun.*, **1994**, 801-802

Brust, M. et al., *Chem. Commun.*, **1995**, 1655-1656

Goia reaction (Iso-ascorbic Acid Reduction of Chloride Precursor)

Reduction of auric acid with iso-ascorbic acid;

$$2\ HAuCl_4 + 3\ C_6H_8O_6 \rightarrow 2\ Au^0 + 3\ C_6H_6O_6 + 8\ HCl$$



Andreescu, D., Sau, T.K. and Goia, D.V., *J. Colloid Interface Sci.*, **2006**, 298, 742-751

Reduction of Metallic Precursors in Solution

Goia Reaction

- *Stabilizer-free* meaning ascorbic acid acts as passivating ligand as for citrate
- Room temperature, very rapid nucleation and growth
- Aqueous phase synthesis
- Ascorbic acid can be displaced from surface by thiols etc.
- Particle size tunable through pH, reactant ratios, concentration
- 30-100 nm if ”stabilizer free” system at room temperature
- 80 nm to 5 μm if prepared in the presence of gum arabic at very high Au conc.

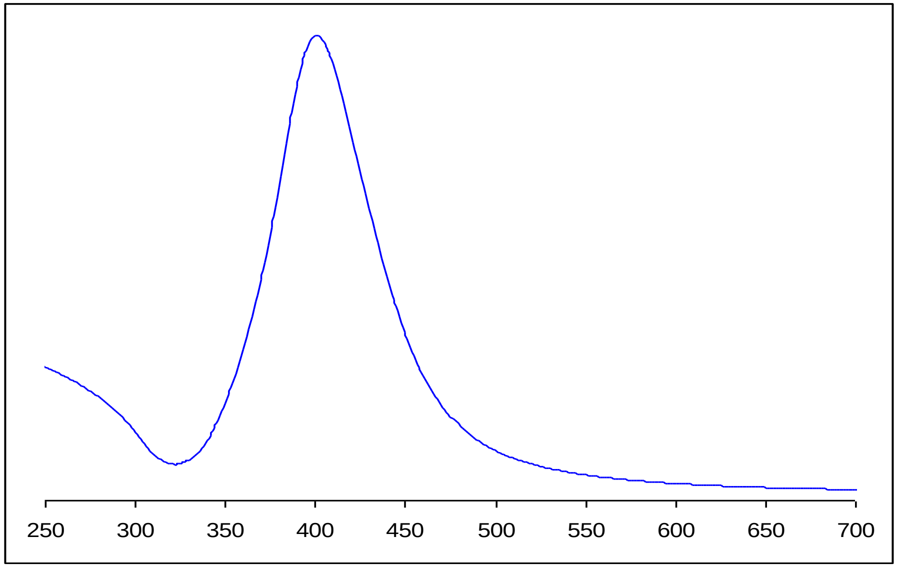
***Stabilizer-free* Goia reaction standard together with Turkevich particles.**

Andreescu, D., Sau, T.K. and Goia, D.V., *J. Colloid Interface Sci.*, **2006**, 742-751

Goia, D.V., Matijevic, E., *Colloids and Surfaces A*, **1999**, 139-152

Reduction of Metallic Precursors in Solution

Goia Reaction



- Reduction of silver nitrate by iso-ascorbic acid
- Slightly larger particles (40-70 nm)
- pH must be above 10

Co-Precipitation

- Metal salt precursors in aqueous solutions.
- Addition of a reducing agent (base).
- The products are generally insoluble species formed under conditions of high supersaturation.
- Nucleation is a key step, and a large number of small particles will be formed.
- Secondary processes, such as Ostwald ripening and aggregation, dramatically affect the size, morphology, and properties of the products.
- The supersaturation conditions necessary to induce precipitation are usually the result of a chemical reaction.

Co-Precipitation

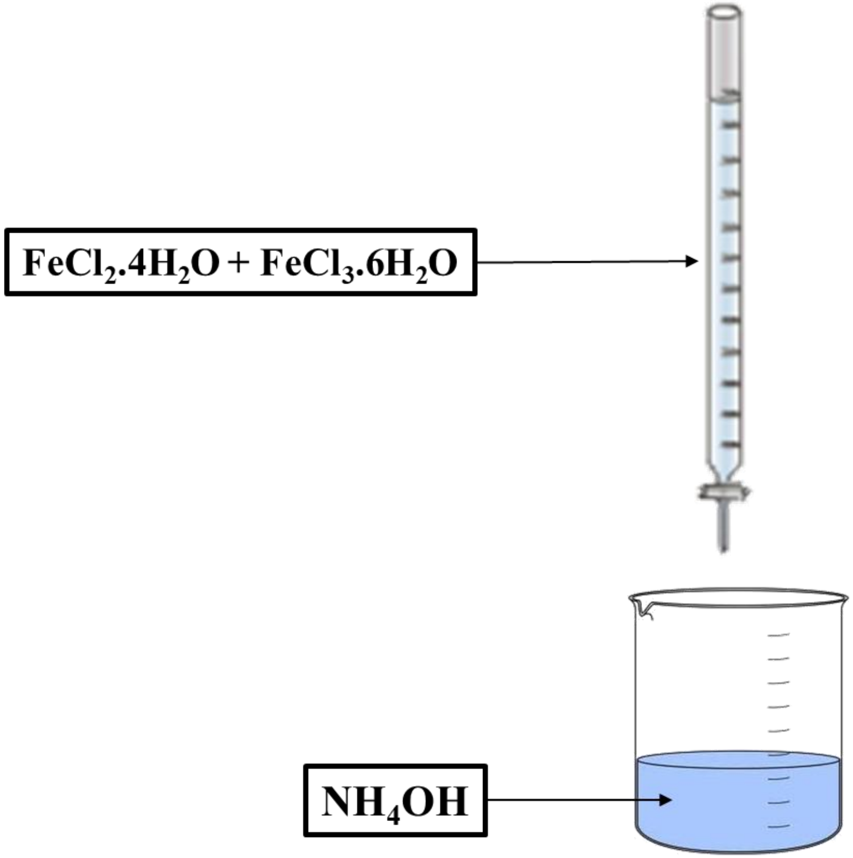
Advantages

- Simple and rapid preparation
- No toxic intermediates
- No use of organic solvents
- Does not require precursor complexes
- Proceeds at low temperatures
- Scalable
- Reproducible

Disadvantages

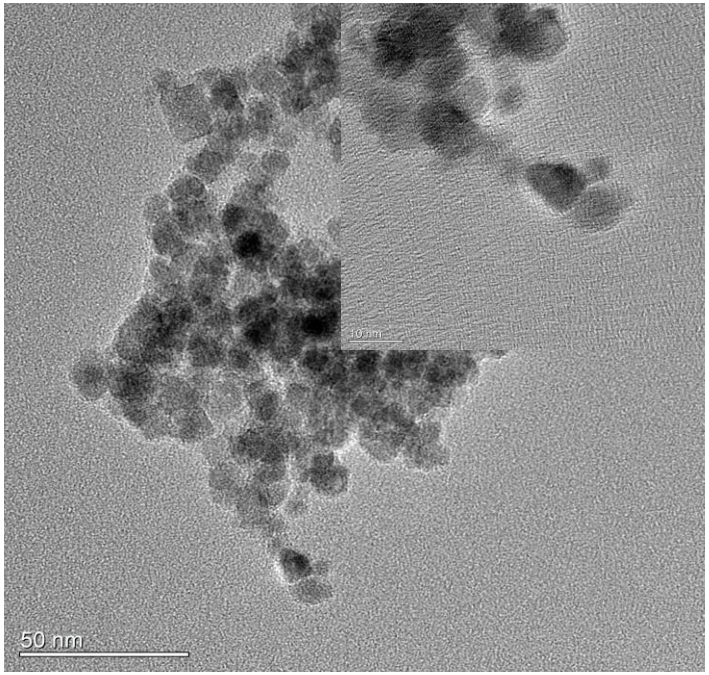
- Particles with a broad size distribution.
- Trace impurities can also get precipitated with product.

Co-Precipitation



$FeCl_2.4H_2O + FeCl_3.6H_2O$

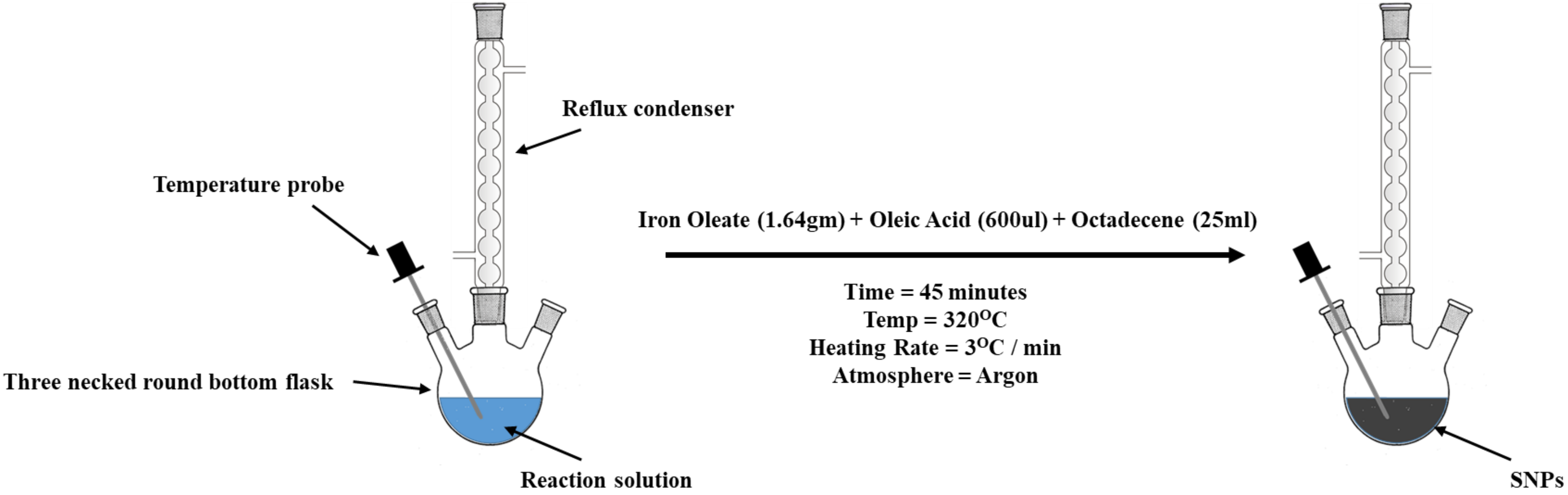
NH_4OH



Courtesy: Anuvansh Sharma, PhD Candidate

2. Thermal Decomposition

- Preparation of Metallic Precursor.
- Thermal decomposition of Metallic Precursor.



Iron Oleate (1.64gm) + Oleic Acid (600ul) + Octadecene (25ml)

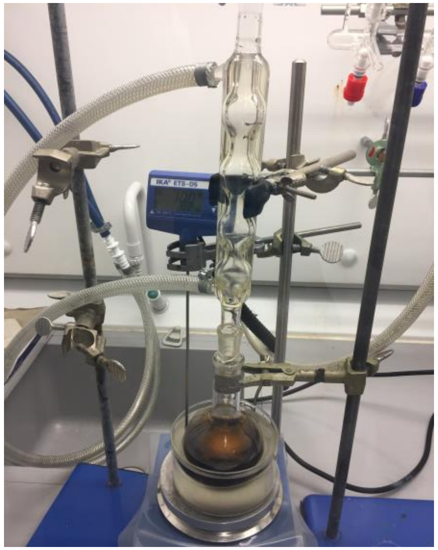
Time = 45 minutes
Temp = 320^{O}C
Heating Rate = 3^{O}C / min
Atmosphere = Argon

Courtesy: Anuvansh Sharma, PhD Candidate

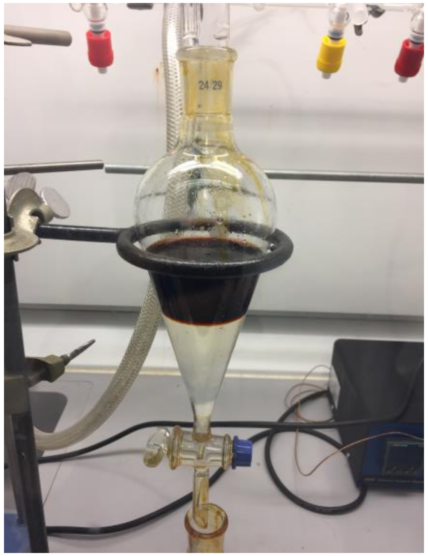
Iron Oxide NPs

Preparation of Metallic Precursor

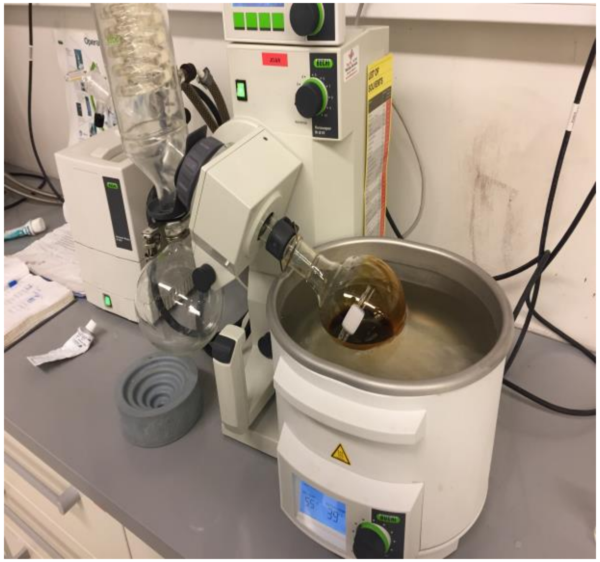
I



II



III

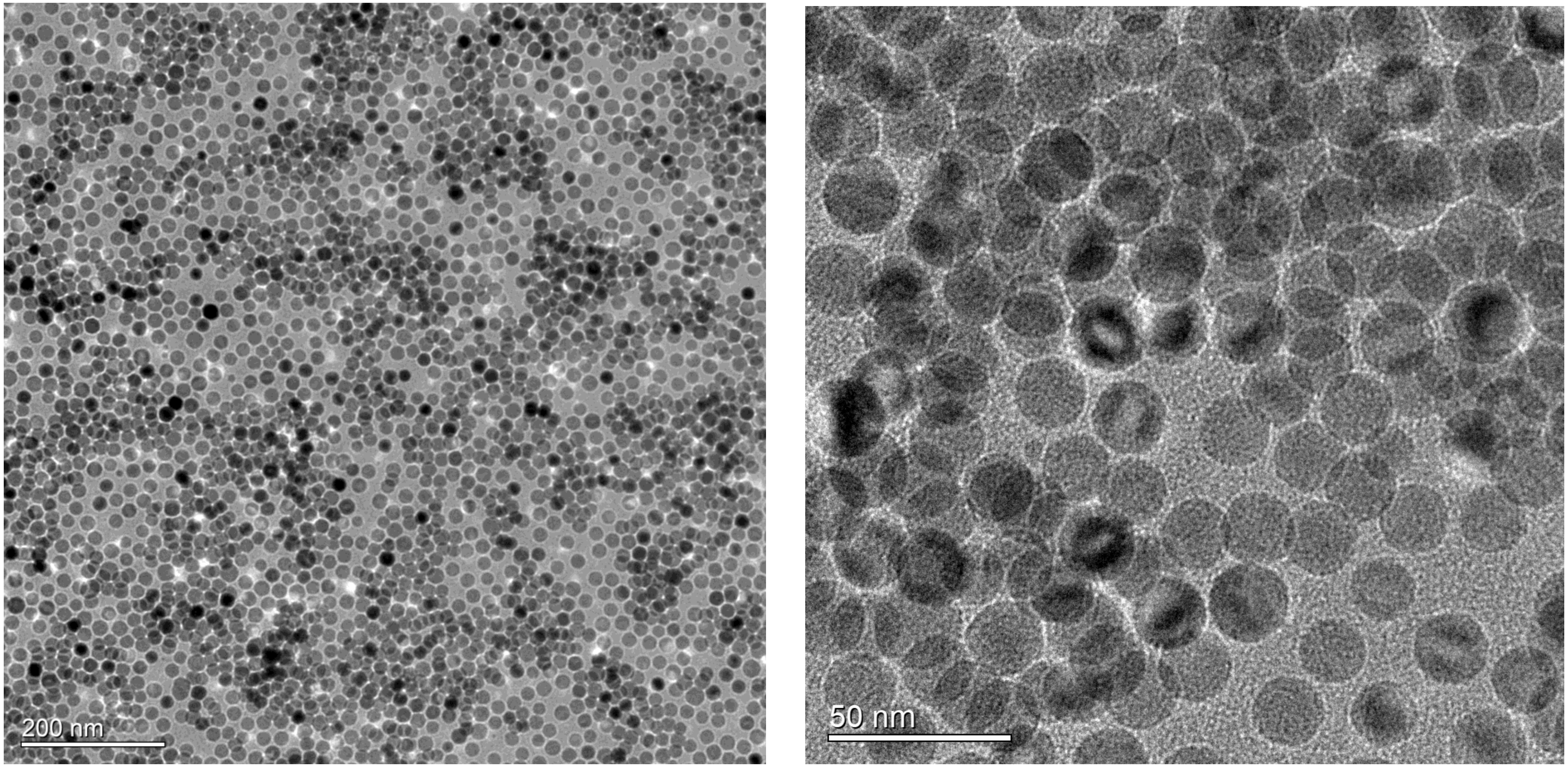


- $FeCl_3.6H_2O$ + Na-Oleate

  Solvents: Water + Hexane + Ethanol
- Mixture is refluxed at 70^{O}C for 4 hours with vigorous stirring. (Fig I)
- Red organic phase washed with water. (Fig II)
- Evaporation of solvent. (Fig III)

Courtesy: Anuvansh Sharma, PhD Candidate

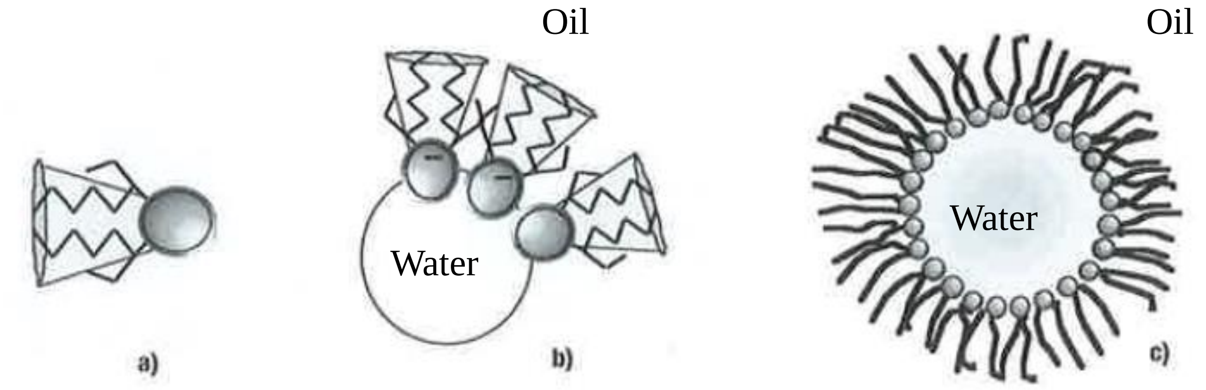
Iron Oxide NPs



Courtesy: Anuvansh Sharma, PhD Candidate

3. Colloidal Templating: Reverse Micelles as Spherical Nanoreactors

Formation of reverse micelles



Surfactant with small polar head and acyl chains

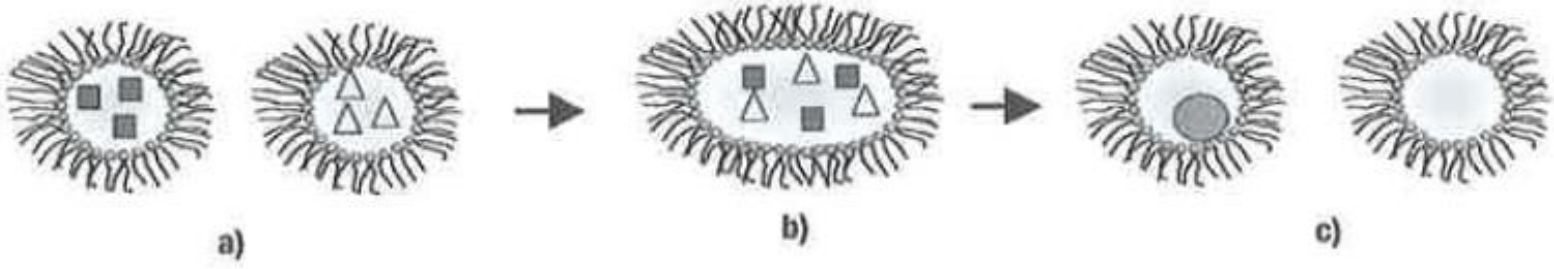
Surfactants join together in non-polar solvents…

..forming reverse micelles

Often microemulsions

Colloidal Templating

General principle: Exchange of aqueous cores



Reverse micelles subject to Brownian motion

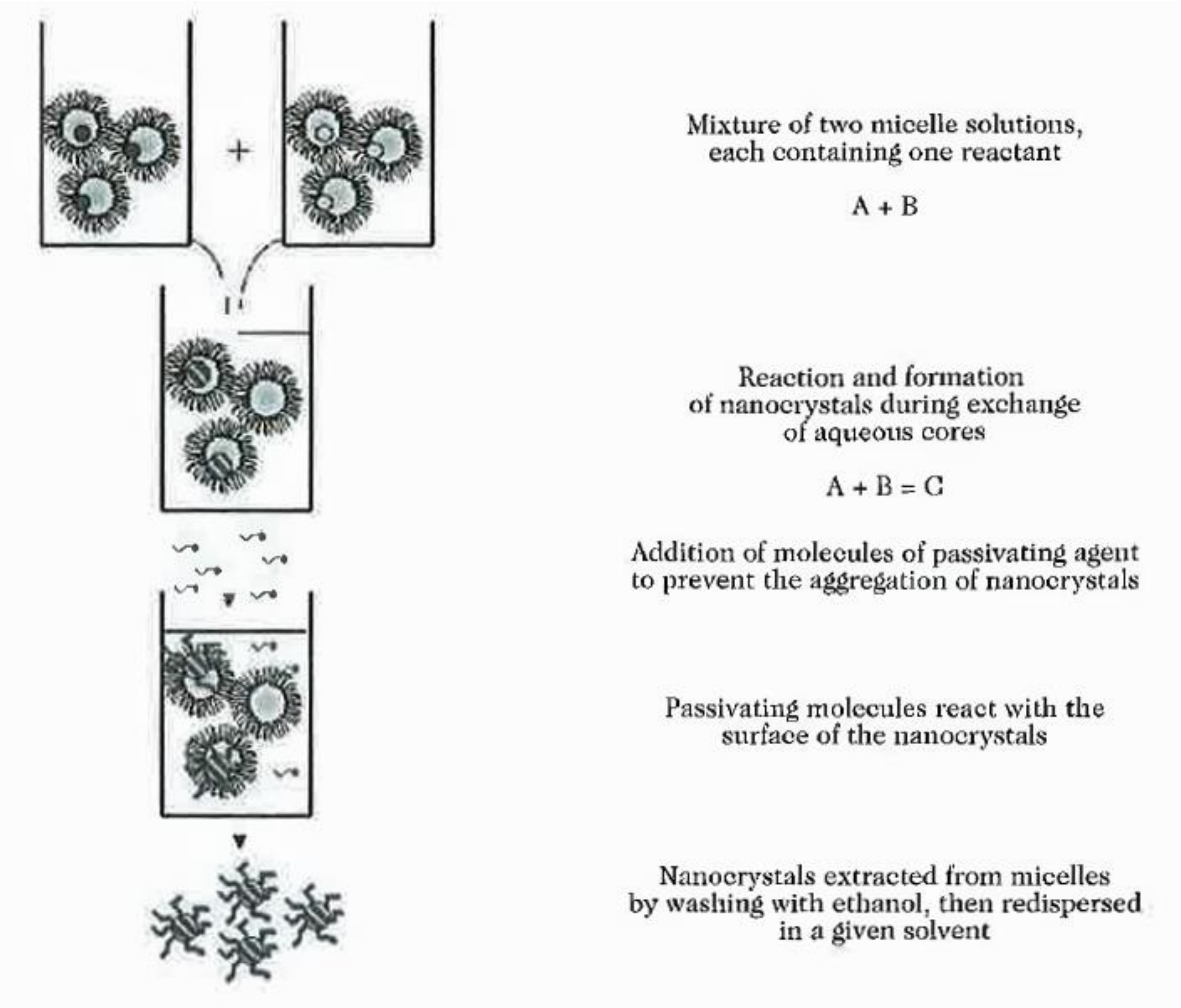
Collapsed reverse micelles and mixing of aqueous content

Formation of two micelles, identical to the initial micelles

■ Metal precursor

△ Reducing agent

Colloidal Templating



Mixture of two micelle solutions, each containing one reactant

A + B

Reaction and formation of nanocrystals during exchange of aqueous cores

A + B = C

Addition of molecules of passivating agent to prevent the aggregation of nanocrystals

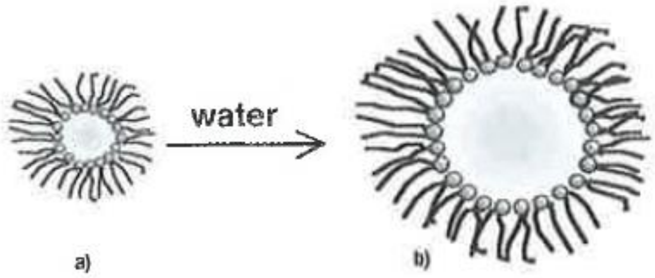
Passivating molecules react with the surface of the nanocrystals

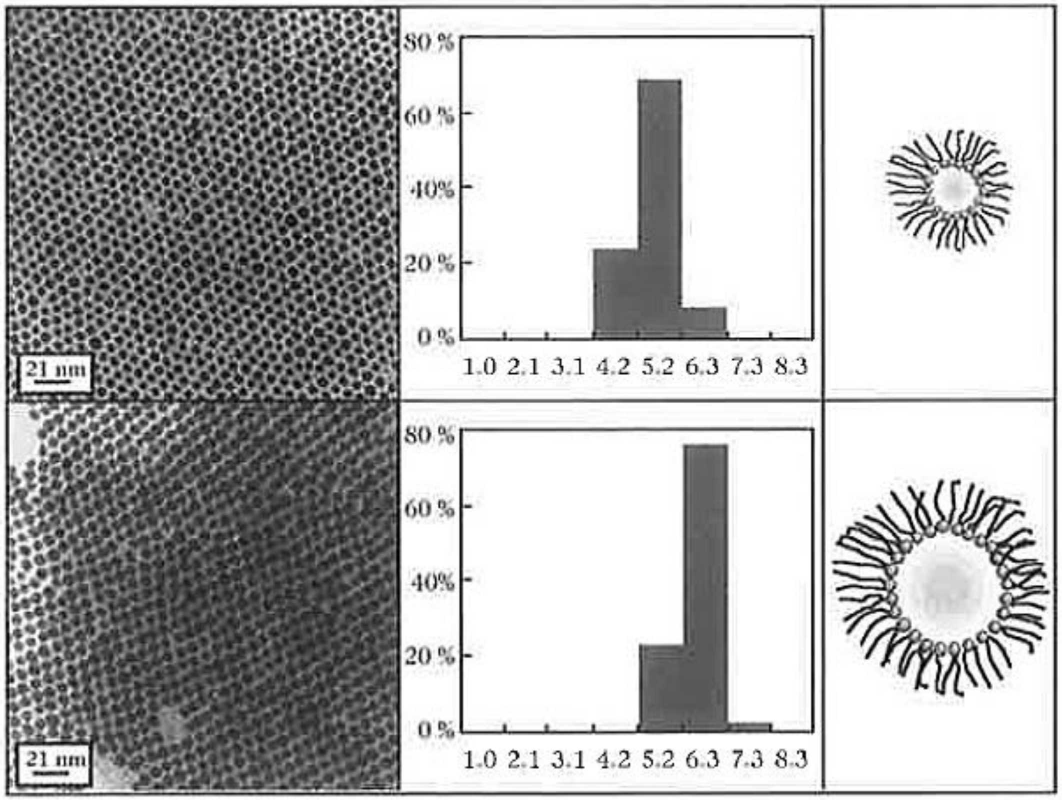
Nanocrystals extracted from micelles by washing with ethanol, then redispersed in a given solvent

Bréchignac C, Houdy P, Lahmani M. Nanomaterials and Nanochemistry;Springer, 2006.

Colloidal Templating

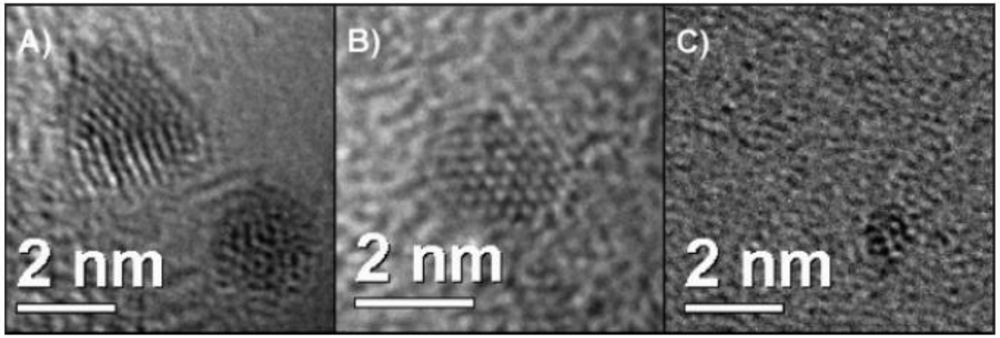
Controlling the size of the reverse micelles controls the size of the nanoparticles, as shown here for silver nanocrystals

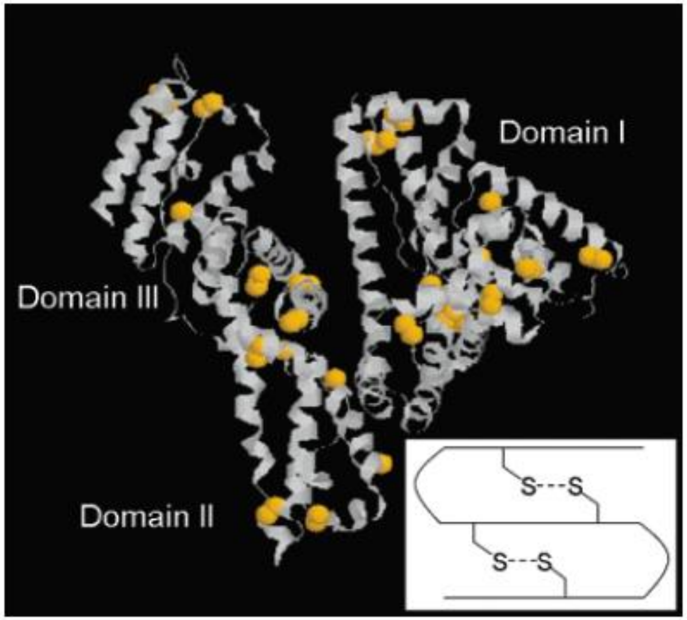




4. One-pot Synthesis Using Globular Proteins

$HAuCl_4$ + $NaBH_4$ (reducing agent) + Bovine Serum Albumin (BSA)

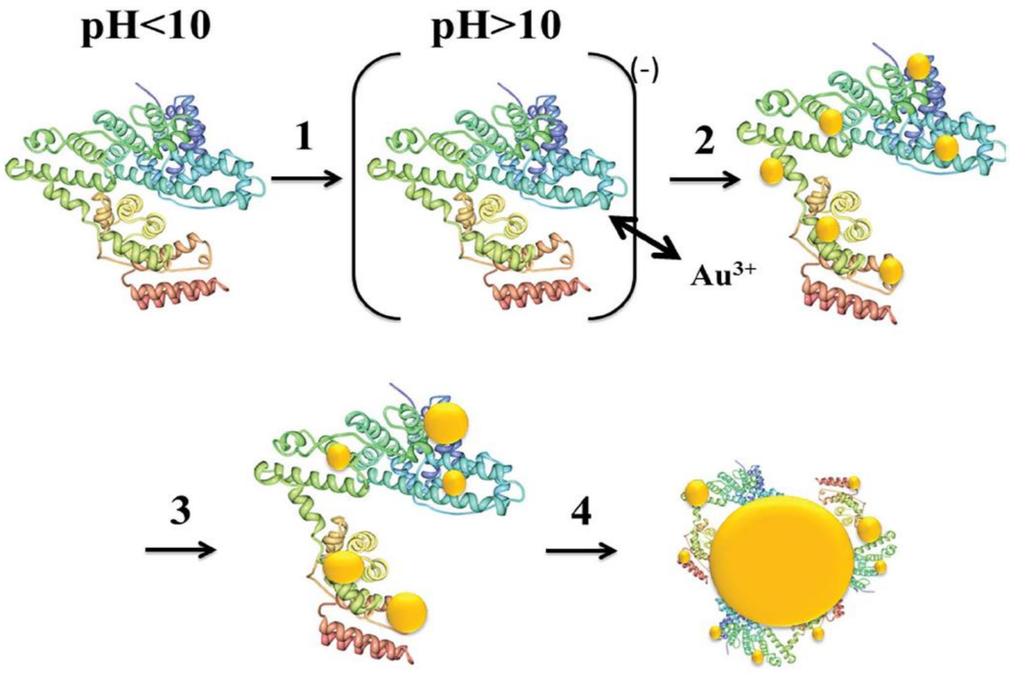




Au NPs embedded in the protein

Burt, J.L. et al., *Langmuir*, **2004**, 20, 11778-11783

One-Pot Synthesis Using Globular Proteins



Proposed mechanism for growth of AuNPs stabilized by BSA.

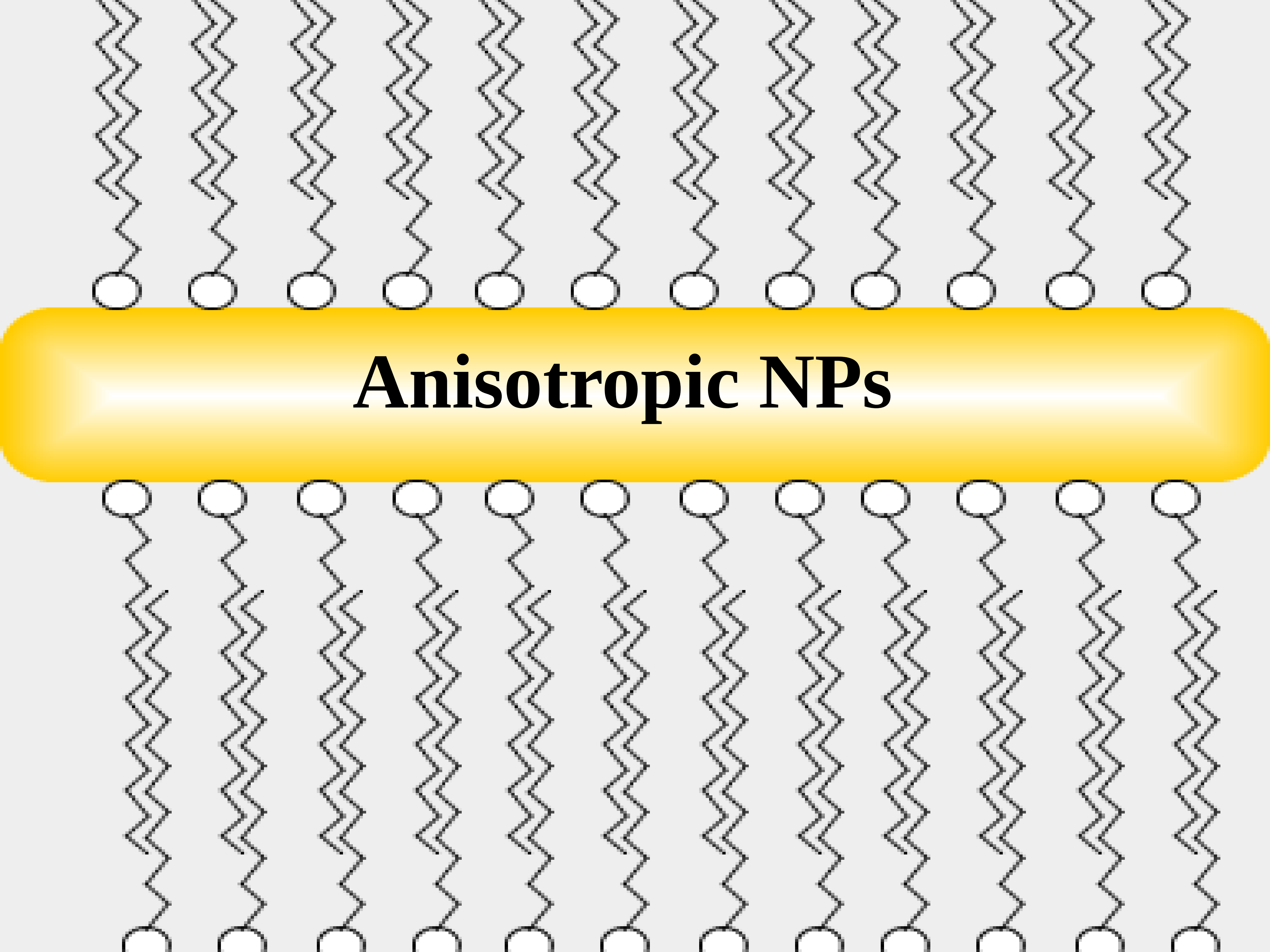
1. Increasing the pH to above 10 causes BSA to be negatively charged which induces electrostatic interactions with the gold ions.

2. Vis AuNCs that emit in the visible region of the EM spectrum start to form.

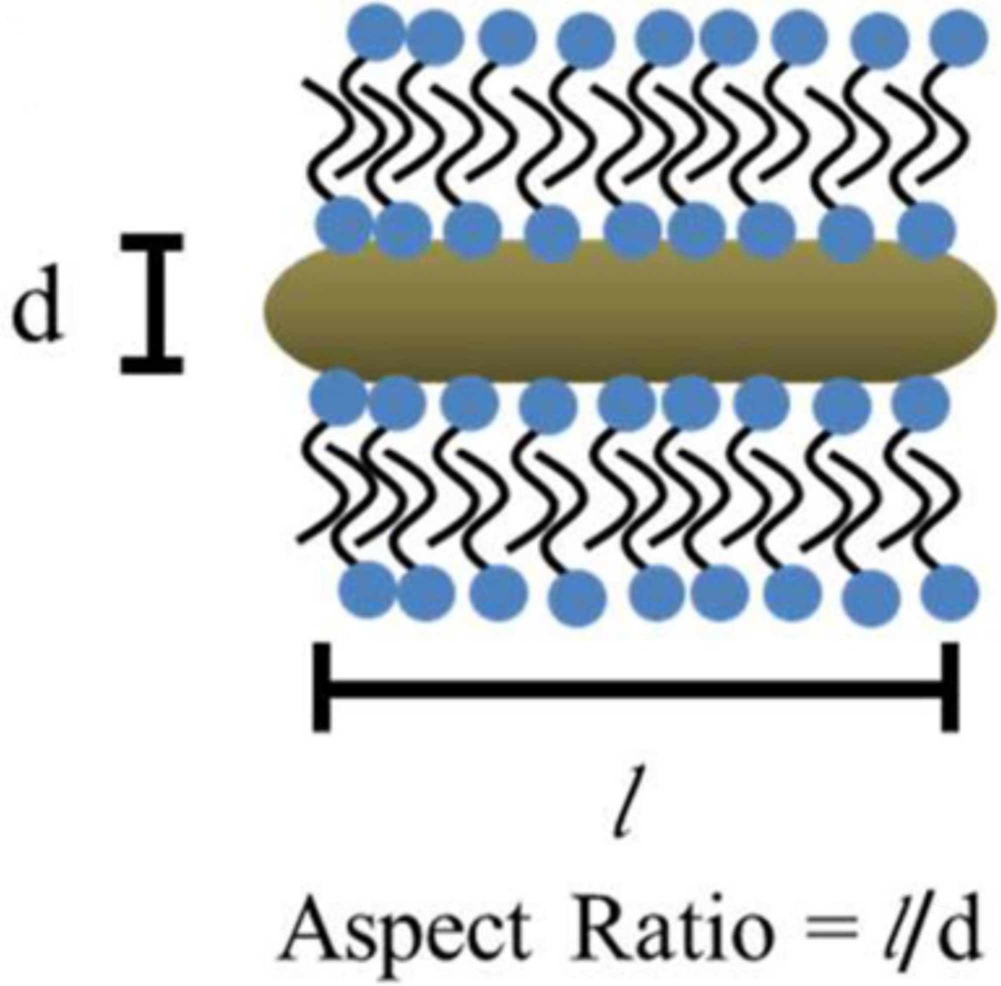
3. Depending on time and concentration of $HAuCl_4$, larger Near IR AuNCs.

4. AuNPs are formed via a shuffling of Near IR AuNCs.

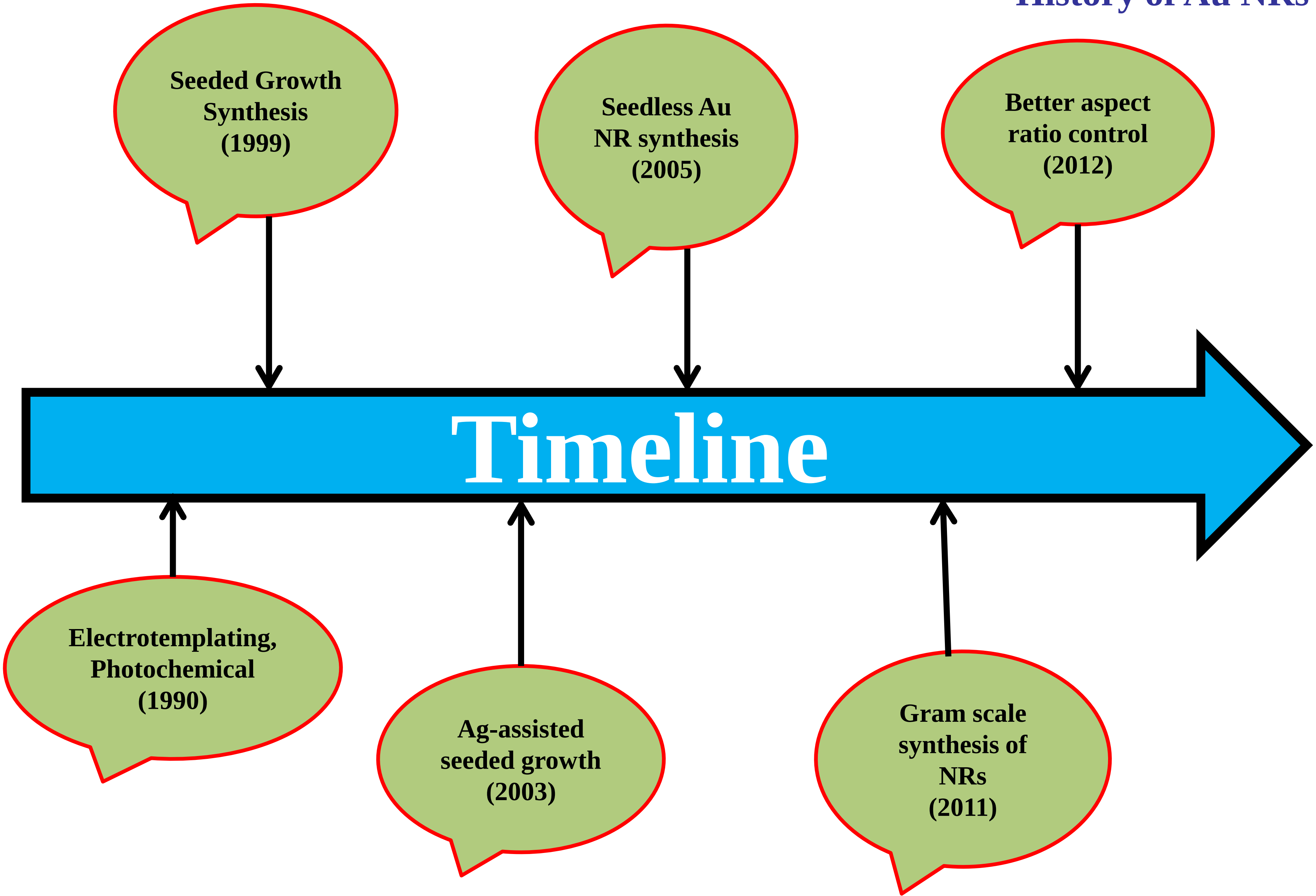
McDonagh, B.H. et al., *RSC Advances*, **2015**, 5, 101101–101109


Anisotropic NPs

Aspect Ratio



History of Au NRs



Seeded Growth Synthesis (1999)

Seedless Au NR synthesis (2005)

Better aspect ratio control (2012)

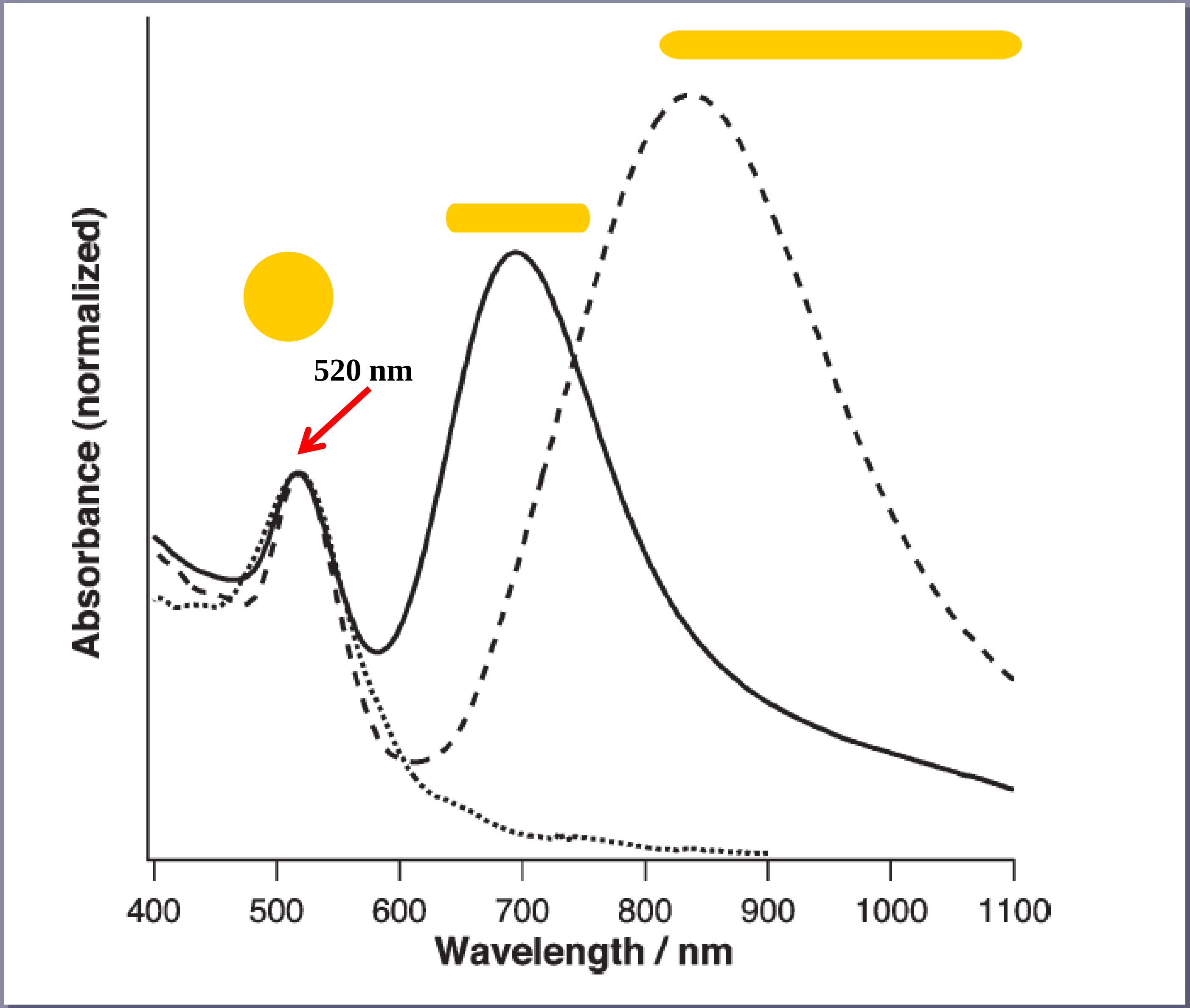
Timeline

Electrotemplating, Photochemical (1990)

Ag-assisted seeded growth (2003)

Gram scale synthesis of NRs (2011)

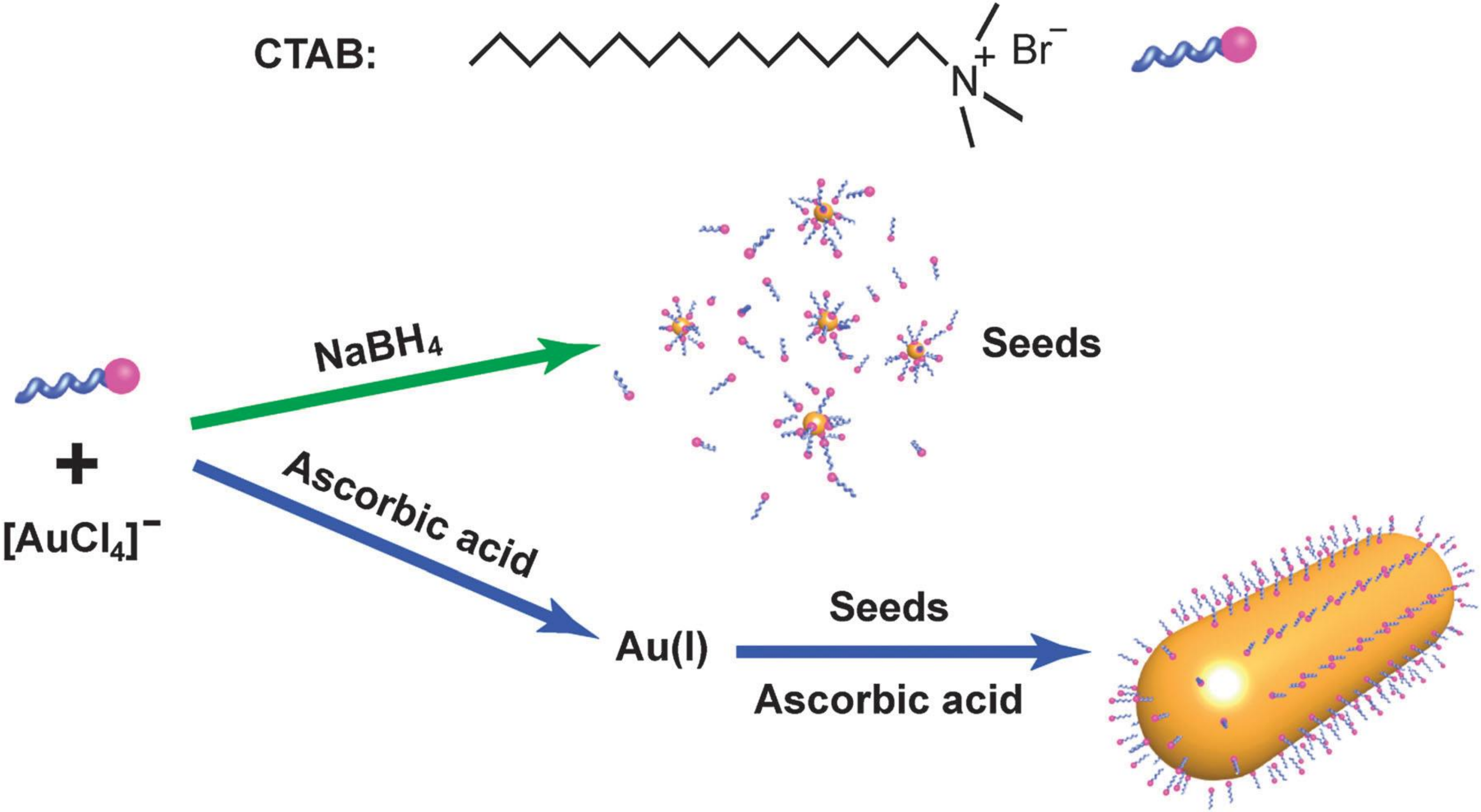
Localized Surface Plasmon Resonance (LSPR)



Okamoto et al., *J. Mater. Chem.*, 2006,3920-3928

Larger extinction coefficient (ε)	**Smaller** extinction coefficient (ε)
Scatter **more** light at longitudinal plasmon λ	Scatter **less** light at longitudinal plasmon λ
Better performance in optical imaging	Improved efficiency in photothermal applications

Seeded Growth Synthesis (In a Nutshell)



Wang et al, *Chem. Soc. Rev.*, **2013**, 42, 2679

Factors

- Size and surface chemistry of the seed

Size increases, decrease in AR

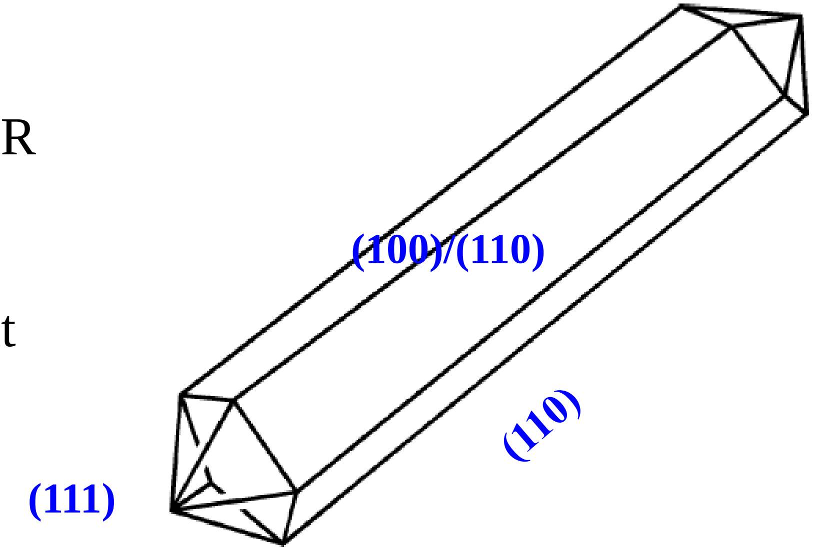
Surface charge effects dispersity of AR

- Chain length of the directing agent

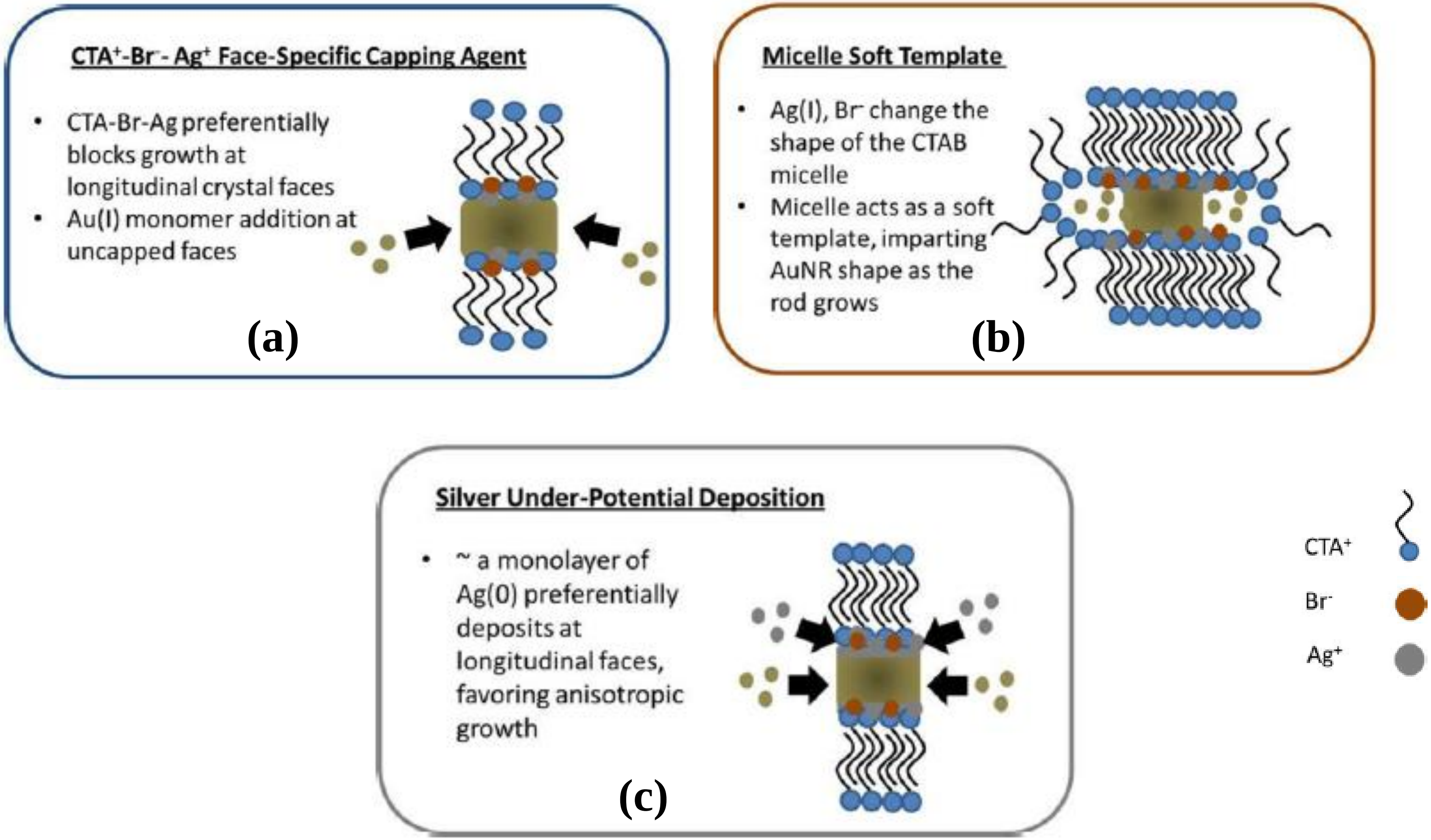
Length< CTAB, lower AR

- Nature of the counterion

Cl^- growth is inhibited



Growth Mechanism



CTA$^+$-Br$^-$- Ag$^+$ Face-Specific Capping Agent

- CTA-Br-Ag preferentially blocks growth at longitudinal crystal faces
- Au(I) monomer addition at uncapped faces

(a)

Micelle Soft Template

- Ag(I), Br$^-$ change the shape of the CTAB micelle
- Micelle acts as a soft template, imparting AuNR shape as the rod grows

(b)

Silver Under-Potential Deposition

- ~ a monolayer of Ag(0) preferentially deposits at longitudinal faces, favoring anisotropic growth

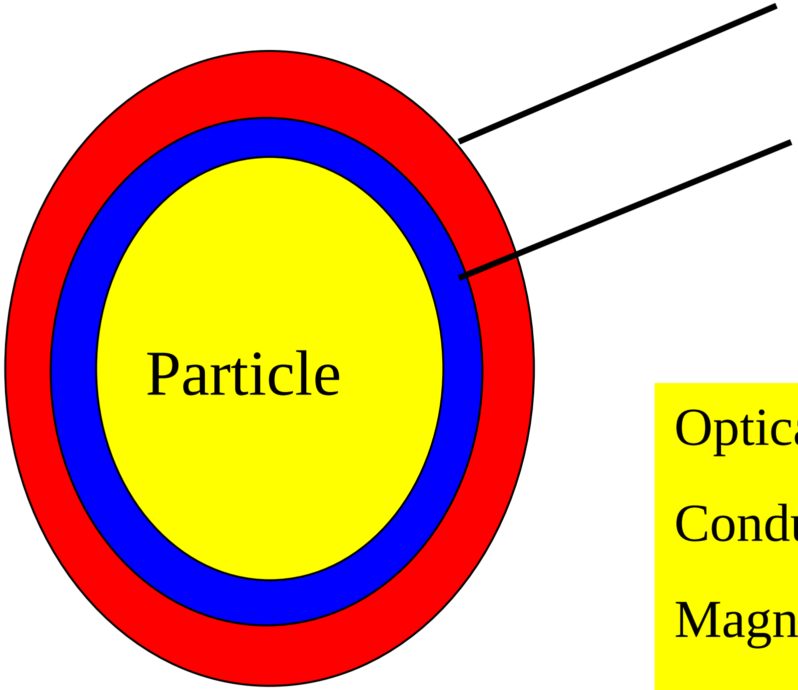
(c)

Murphy et al, *Chem*, *Mater.*, **2013**,1250-1261

Functionalization of NPs

Functionalization – Why?

- Ag and Au NPs are not stable in solutions without a surface layer of a passivating ligand
- This imparts an electrostatic and/or steric stabilization to the colloidal sol.



Material Properties

Passivating ligand layer

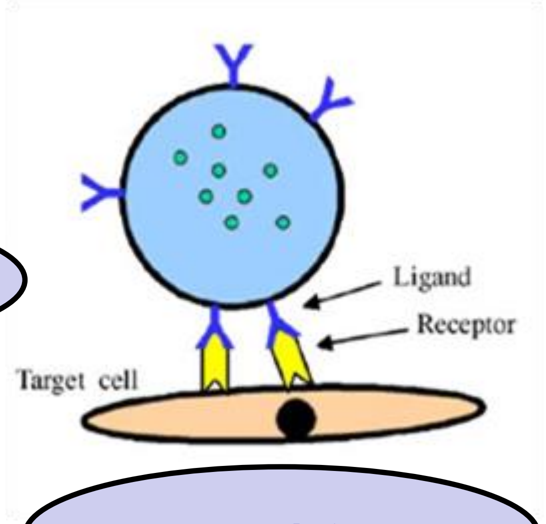
Optical properties
Conductivity
Magnetism
Catalytic activity

Refractive index
Polarity/solubility
Charge
Functionality

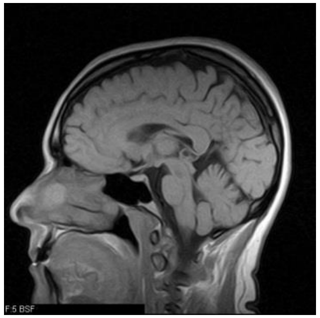
Functionality/application

Functionalization – Why?

Drug Delivery

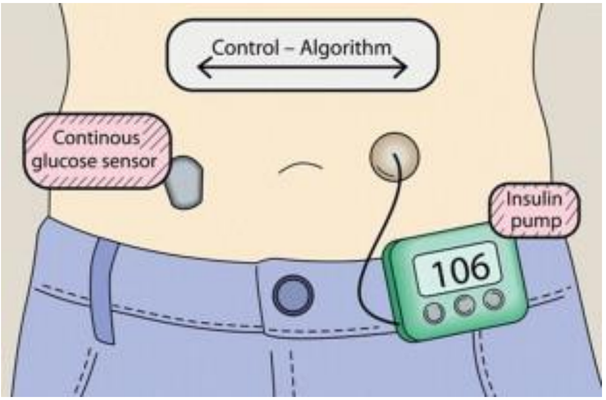


Imaging



Catalysis

Biosensors



Environmental Applications

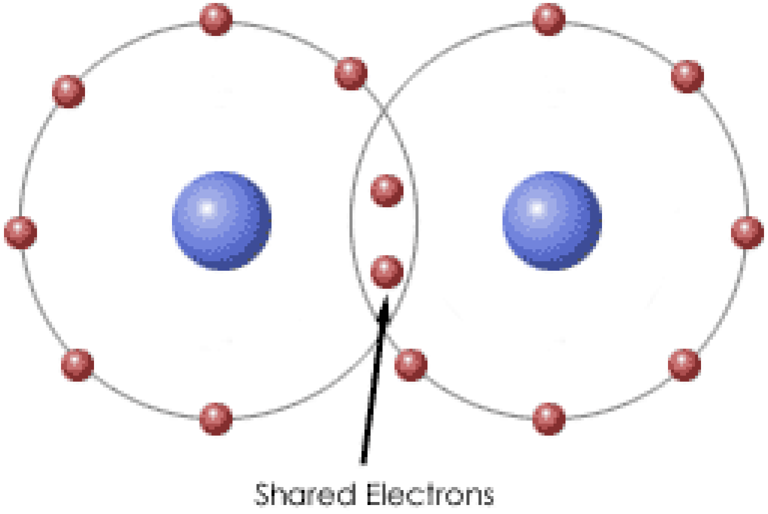
And many more…

Approaches to Functionalization

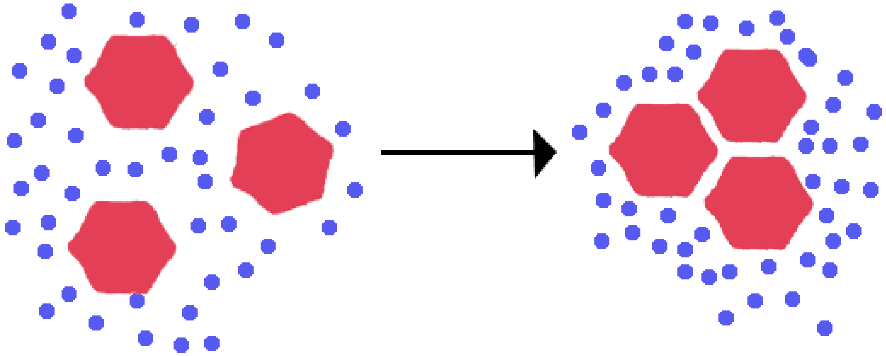
1. **Direct (*in situ*) Functionalization**
   - Done during synthesis
   - Examples:
     - Turkevich Reaction
     - Brust Reaction
2. **Post - Synthesis Functionalization**
   - Functionalization after synthesis
   - Factors
     - Binding to or replacing the exisiting surface ligand
     - Intermolecular forces
     - No of attachment sites, thickness and density of surface layer
     - No. of different surface groups required

Approaches to Functionalization
Post - Synthesis Functionalization

- Examples:
  - Non – specific binding
  - Displacement
  - Layer – by – layer (LBL)
  - Protein binding
  - Grafting polymers, etc.



Covalent

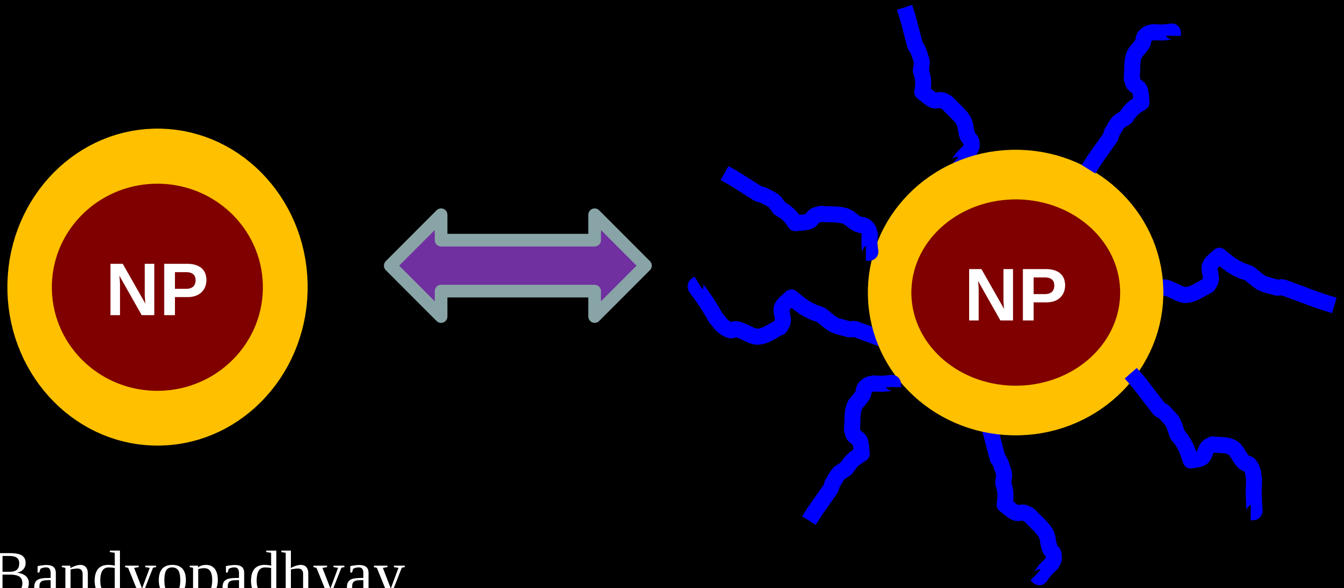


Hydrophobic

Reader’s Digest Version:

- Huge library of NPs of different shapes and sizes can be synthesized using solution methods.
- Metallic Precursor + Reducing Agent + Passivating Ligand.
- Strength of the reducing agent controls particle size.
- Synthesis strategy dependent on:
  - Properties of NPs desired.
  - Simplicity of the process.
  - Further application downstream.
- Control of particle size – supersaturation, nucleation, growth.

Synthesis and Functionalisation of Polymer-based NPs



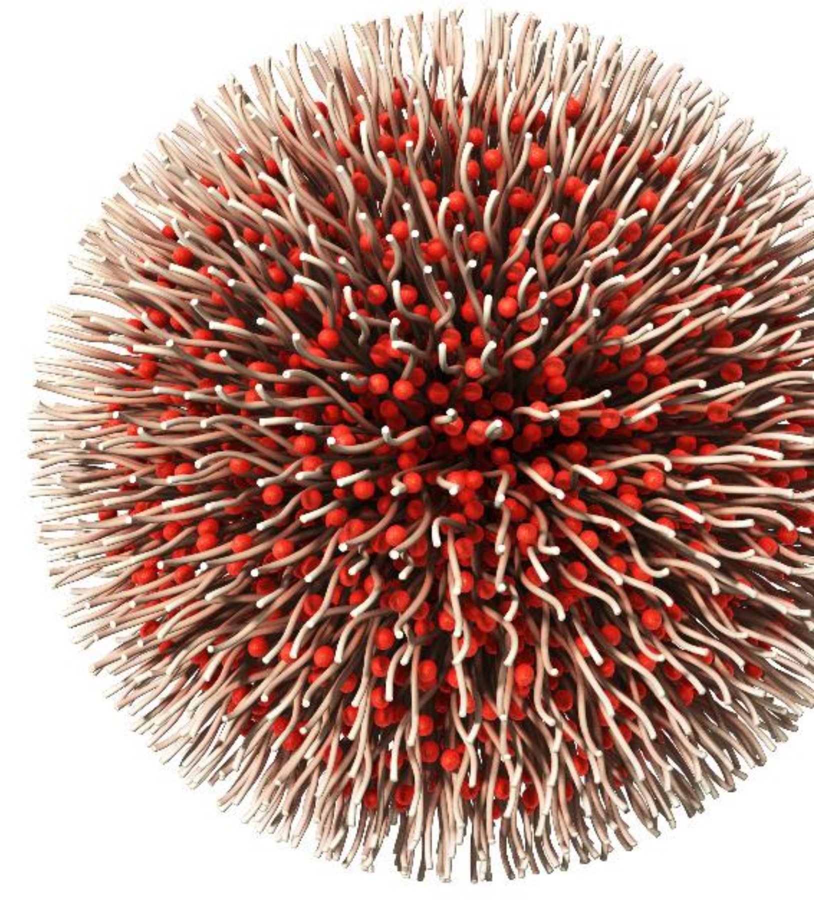
Sulalit Bandyopadhyay,
Associate Professor,
Department of Chemical Engineering, NTNU.
sulalit.bandyopadhyay@ntnu.no

NTNU
Norwegian University of
Science and Technology

Topics

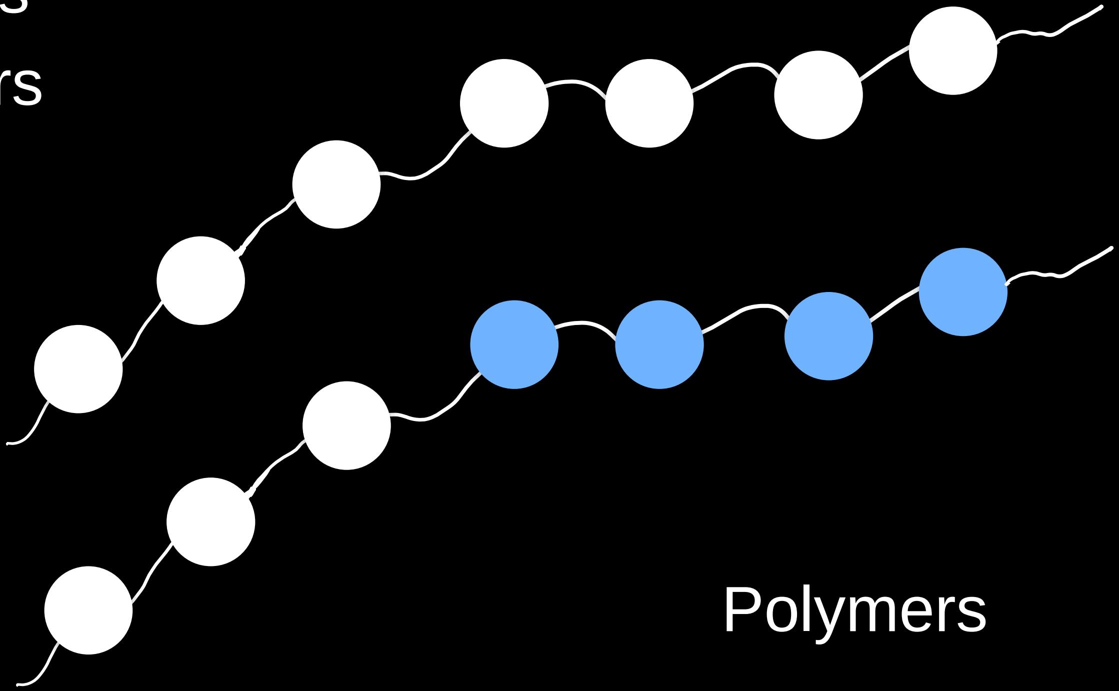
- **Metallic NPs**
  - Synthesis Methods
    - Reduction of Metallic Precursors
    - Thermal Decomposition
    - Colloidal Templating
    - One pot methods
  - Anisotropic NPs
- **Functionalization of NPs**
- **Polymer-based NPs**
  - Nanoprecipitation
  - Flash Nanoprecipitation
  - Precipitation polymerization

Polymer-based NPs



Polymer-based NPs

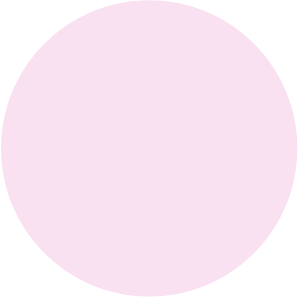
- Sub-micron particles
- Pre-formed polymers
  - Self- Assembly
  - Precipitation



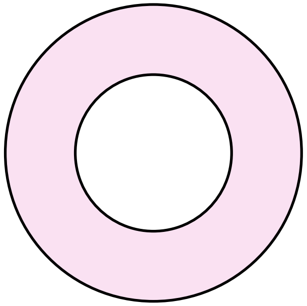
Polymers

● Monomer

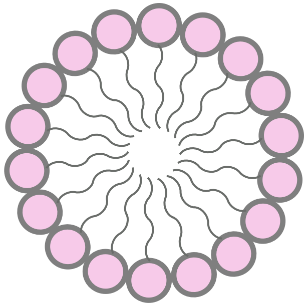
Classification



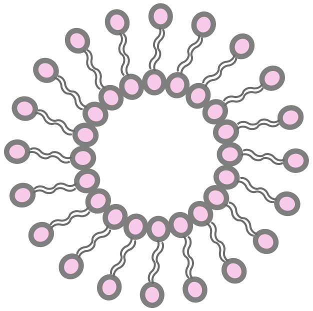
Nanospheres/ NPs



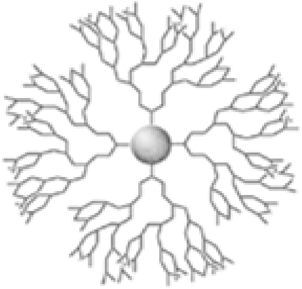
Nanocapsules



Polymeric Micelles

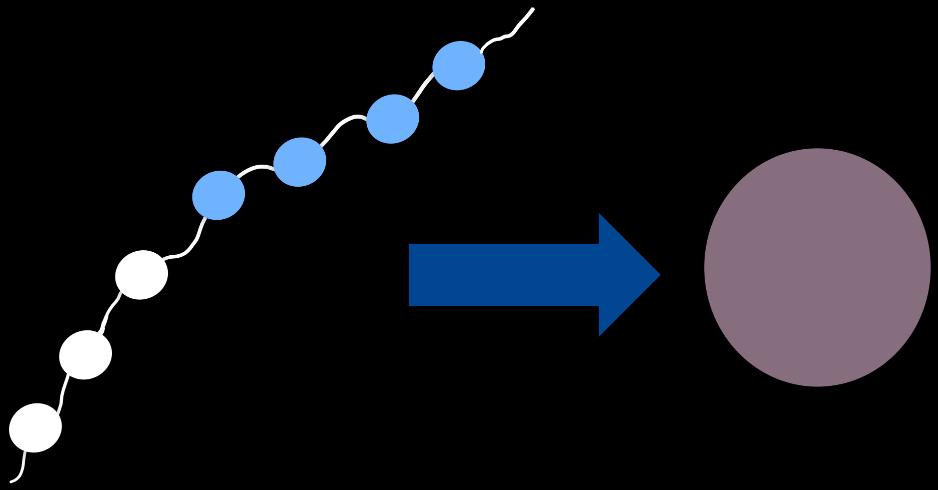


Polymerosomes



Dendrimer

Synthesis Methods



Categories

Emulsion based processes

- 2 Step Process
- Preparation of an emulsion
- Particle formation by precipitation or cross-linking

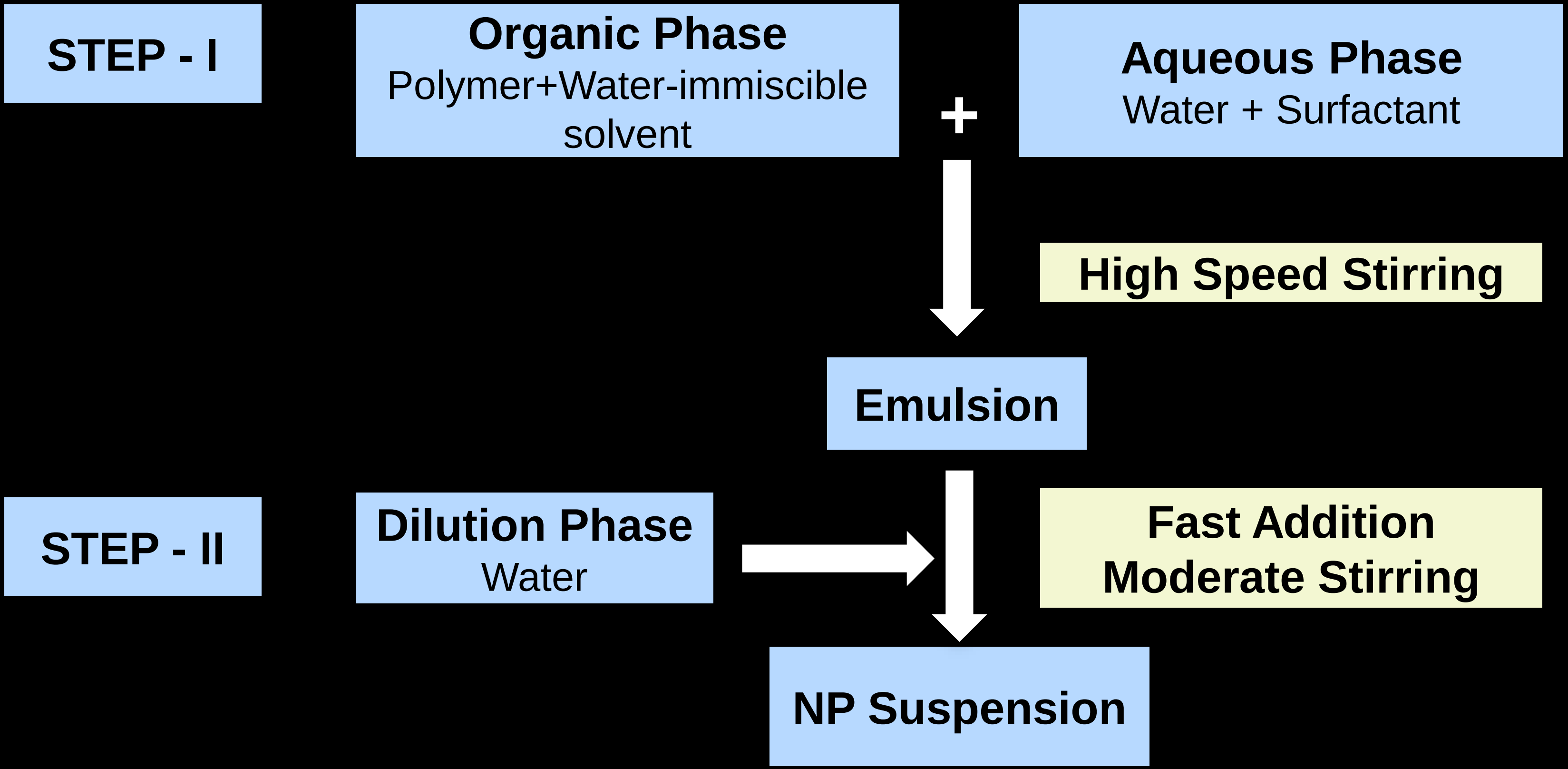
- Eg: Emulsification – Diffusion, Emulsification - Evaporation

Solvent - Displacement

- Single Step Process
- Polymer precipitation or self-assembly or poly-electrolyte complexes

- Eg: Nanoprecipitation, layer-by-layer technique

Emulsification-Diffusion



STEP - I

Organic Phase
Polymer+Water-immiscible solvent

+

Aqueous Phase
Water + Surfactant

High Speed Stirring

Emulsion

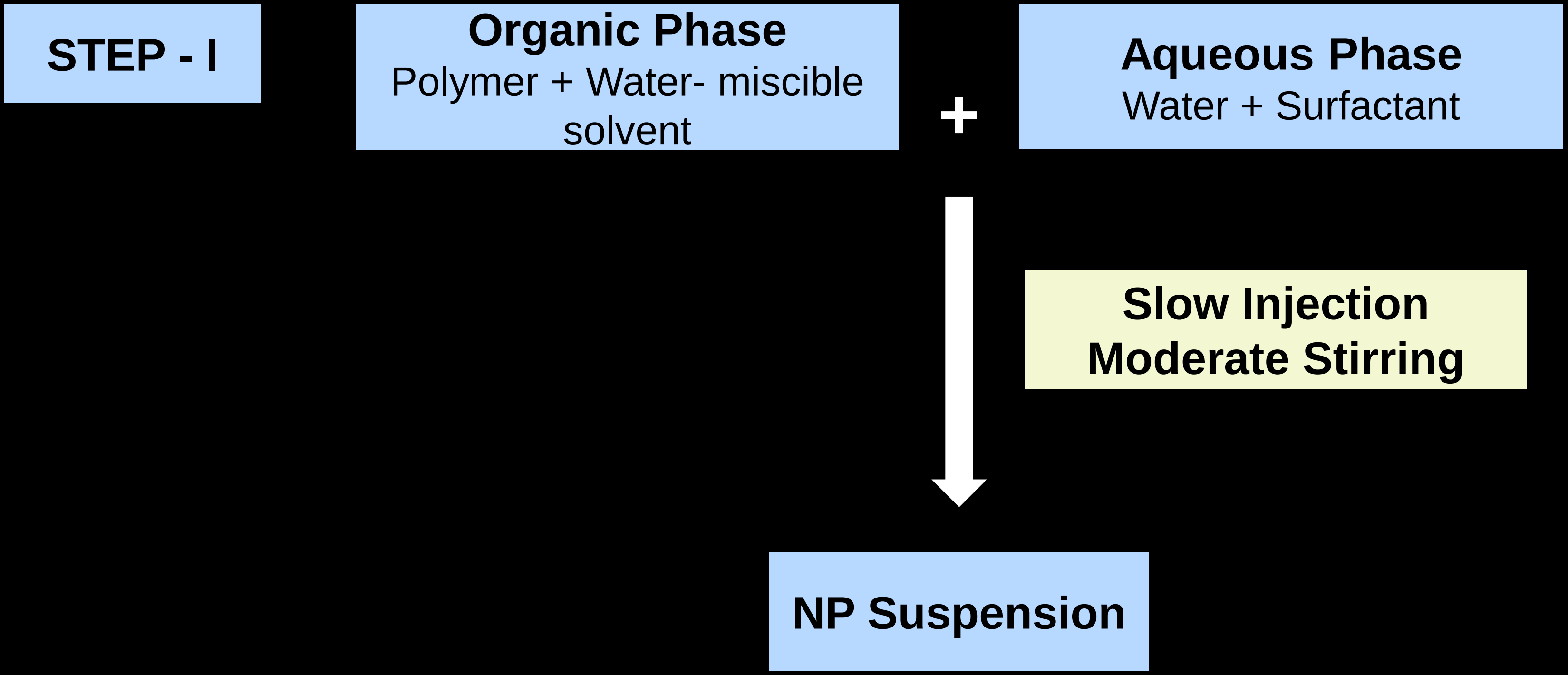
STEP - II

Dilution Phase
Water

Fast Addition
Moderate Stirring

NP Suspension

Solvent Displacement



STEP - I

Organic Phase
Polymer + Water- miscible solvent

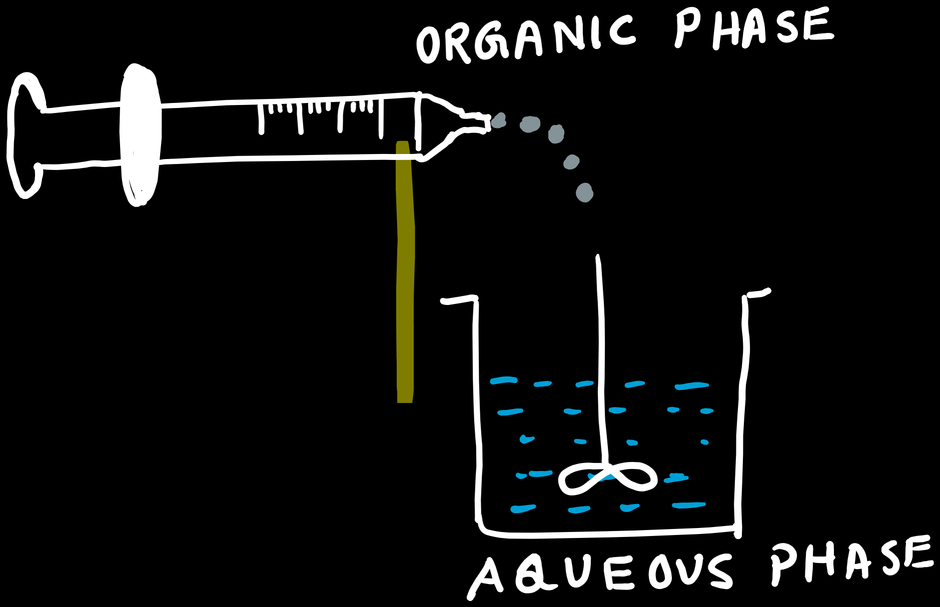
\+

Aqueous Phase
Water + Surfactant

Slow Injection
Moderate Stirring

NP Suspension

Nanoprecipitation



Process Overview

- Method to synthesize polymeric NPs.
- Components:
  - Solvent; containing polymer
  - Non solvent; containing surfactant
- Organic solvent can be removed by evaporation at ambient temperature or a rotavapour.
- Aqueous phase can be removed using ultracentrifugation or freeze-drying.
- Patented by Fessi *et al*. in 1989.
- Was originally designed to encapsulate hydrophobic drugs, however research has been conducted with hydrophilic drugs.

Mechanism

Mechanical Mechanisms

- Gibb’s Marangoni Effect
- Interfacial tension driven
  - Variations in interfacial tension at the solvent/non-solvent interface
  - Disturbances in mechanical equilibrium
- Factors:
  - Physico-chemical properties of organic phase
  - Interaction with aqueous medium

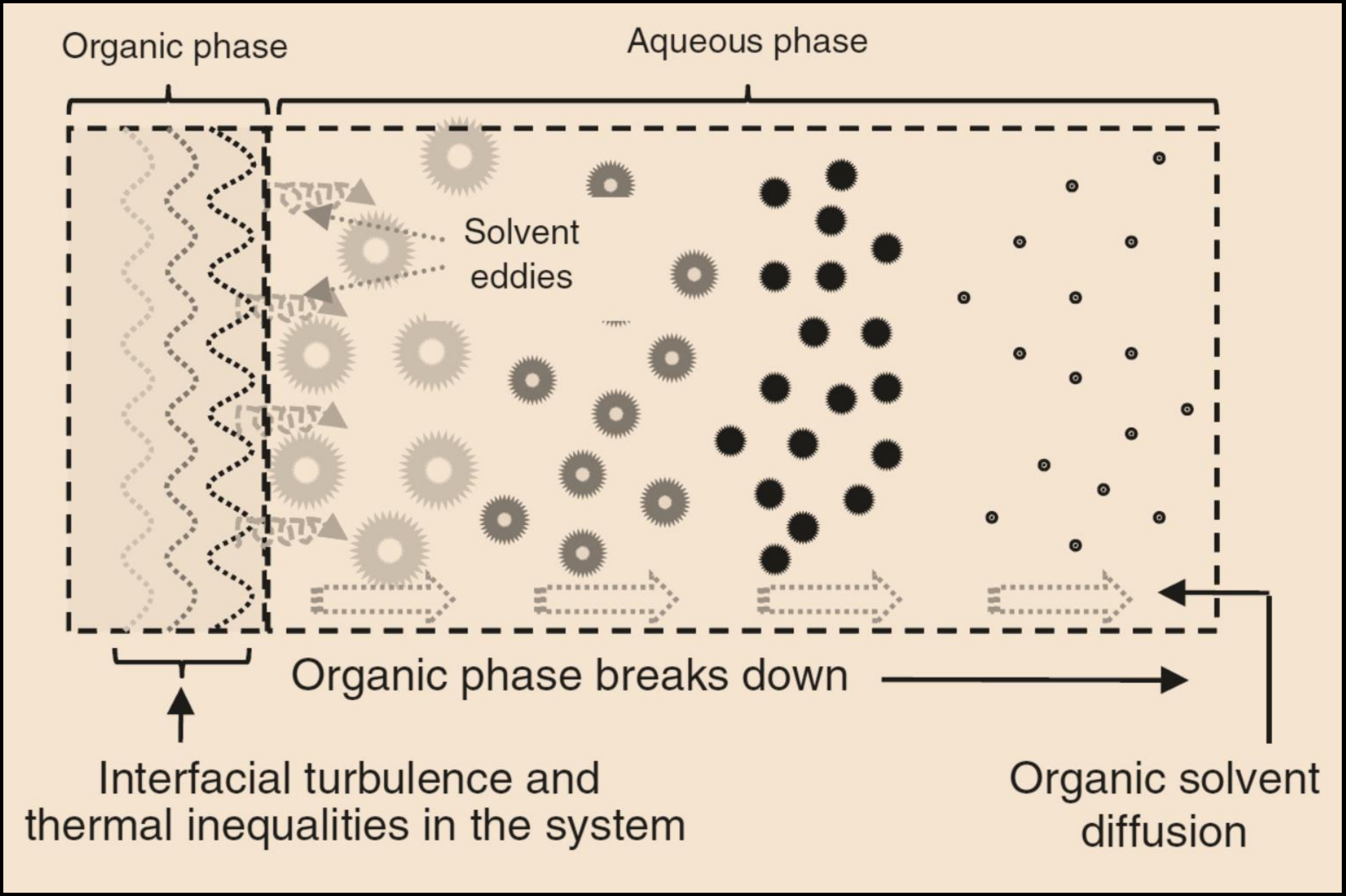
Chemical Instability

- Supersaturation driven
  - Nucleation and growth
  - Size enlargement
- Factors:
  - Composition of polymer/solvent/non-solvent system
  - Interaction between particles formed
  - Physico-chemical properties of dispersive medium

Gibb’s Marangoni Effect

$$\gamma_{ORG} < \gamma_{AQU}$$

- Interfacial turbulence, thermal inequalities
- Solvent Eddy formation - Interfacial convective flows
- Increasing mass-exchange rate between phases
- Violent spreading (miscible system)
- Organic phase breaks into smaller droplets
- Solvent flows away from low interfacial tension region
- Polymer precipitates


Organic phase
Aqueous phase
Solvent eddies
Organic phase breaks down
Interfacial turbulence and thermal inequalities in the system
Organic solvent diffusion

Gibb’s Marangoni Effect

$$Ma = \frac{\Delta\gamma\Delta C}{\eta D_{AB}}$$

$\Delta\gamma =$ rate of change of interfacial tension

$\Delta C =$ concentration gradient

$\eta =$ viscosity of the organic phase

$D_{AB} =$ diffusion coefficient of the organic phase into aqueous phase

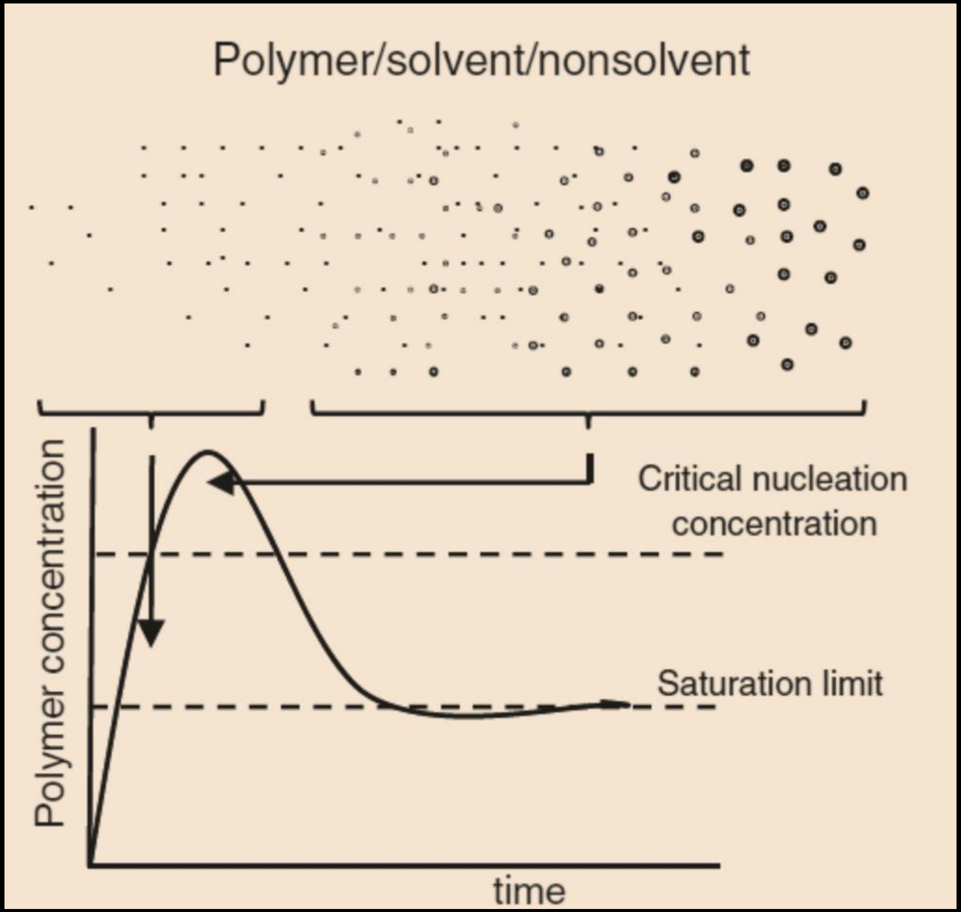
$Ma =$ Marangoni number

Chemical Instability

- Polymer solution in contact with water
- Solvent diffuses into aqueous phase
- Local S

$$S = \frac{C_{POLYMER}}{C^{*}_{POLYMER}}$$

- Nucleation & Growth – further size enlargement
- Blending rate and molecular diffusion are extremely rapid, in comparison to the nucleation rate


Polymer/solvent/nonsolvent
Critical nucleation concentration
Saturation limit
Polymer concentration
time

Chemical Instability

Nucleation:

$$J = \frac{2D}{d^5}\exp\left(-\frac{16\pi\gamma^3\nu^2}{3k_B^3T^3\left[\ln(S)\right]^2}\right)$$

$J =$ nucleation rate

$D =$ molecular diffusion of the polymer molecule

$d =$ molecular diameter

$k_B =$ Boltzmann constant

$T =$ temperature

$\gamma =$ interfacial tension between already formes particles and the solution

$\nu =$ polymer molecular volume

$S =$ supersaturation

Chemical Instability

Growth:

$$G = \frac{2k_m M_W C}{\rho}(S-1)$$

$G =$ growth rate
$M_W =$ molecular weight of the polymer
$C =$ concentration of the polymer

$k_m =$ mass transfer coefficient
$\rho =$ density of the polymer

Aggregation:

- Size of the particles
- Probability of encounters due to Brownian motion (perikinetic aggregation)
- Probability of encounters due to fluid motion (orthokinetic aggregation).

Chemical Instability

Aggregation:

Perikinetic :

- dynamic viscosity of the dispersive medium
- T
- Particle size

Orthokinetic :

- Particle size
- Shear rate

Controlling Size - Steps

- Growing process
- Aggregation phenomena
- Stabilizing agent

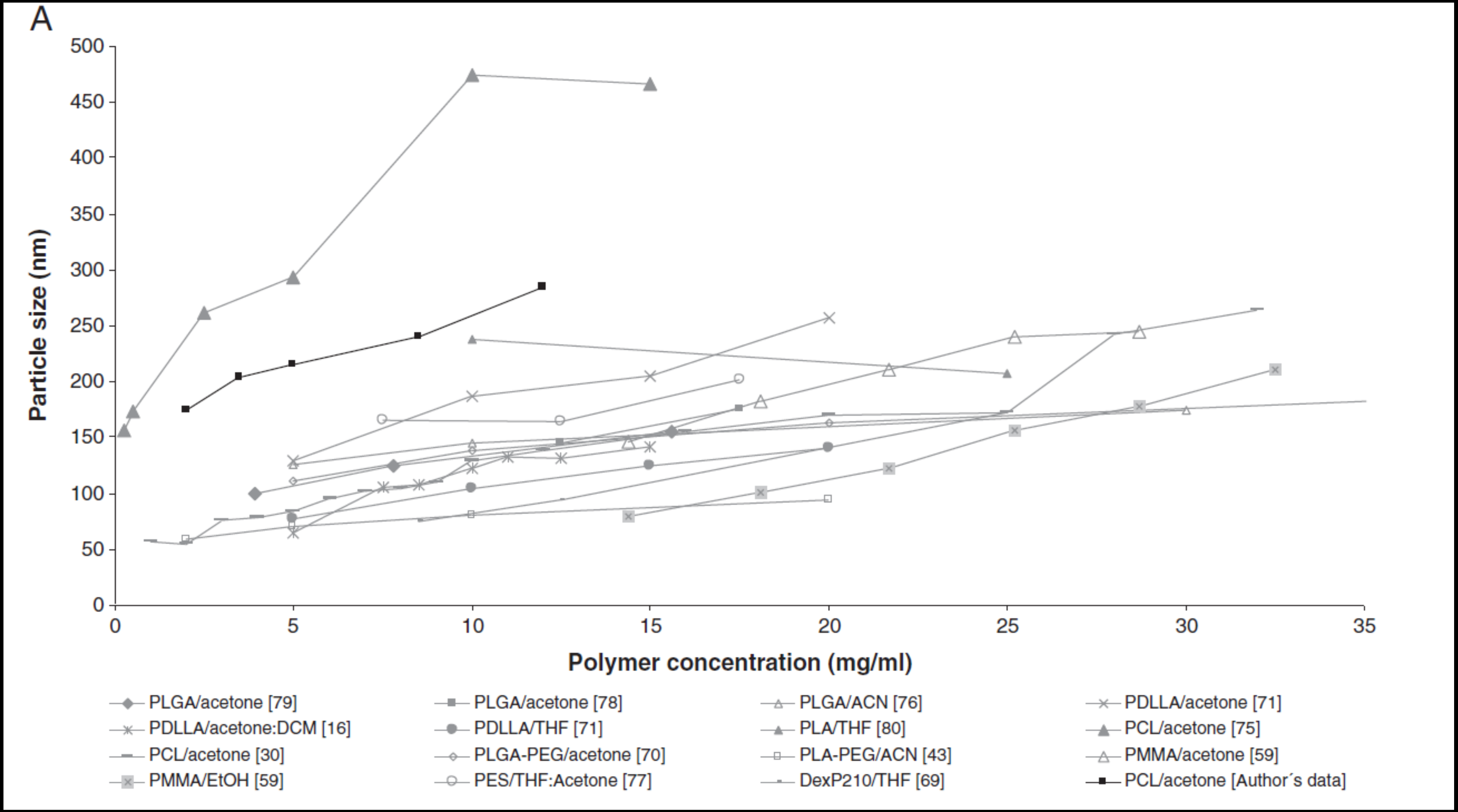
Controlling Size - Factors

Operating Variables

- Organic/aqueous phase ratio
- Organic phase addition method
- Stirring system
- Temperature
- Final stirring time

System Components

- Nature of the polymer
- Polymer concentration
- Nature of the stabilizing agent
- Stabilizing agent concentration
- Nature of the solvent
- Stabilizing agent concentration
- Nature of the solvent


A
Particle size (nm)
500
450
400
350
300
250
200
150
100
50
0
Polymer concentration (mg/ml)
0
5
10
15
20
25
30
35
PLGA/acetone [79]
PLGA/acetone [78]
PLGA/ACN [76]
PDLLA/acetone [71]
PDLLA/acetone:DCM [16]
PDLLA/THF [71]
PLA/THF [80]
PCL/acetone [75]
PCL/acetone [30]
PLGA-PEG/acetone [70]
PLA-PEG/ACN [43]
PMMA/acetone [59]
PMMA/EtOH [59]
PES/THF:Acetone [77]
DexP210/THF [69]
PCL/acetone [Author´s data]

Size of Polymeric NPs

Polymer Concentration

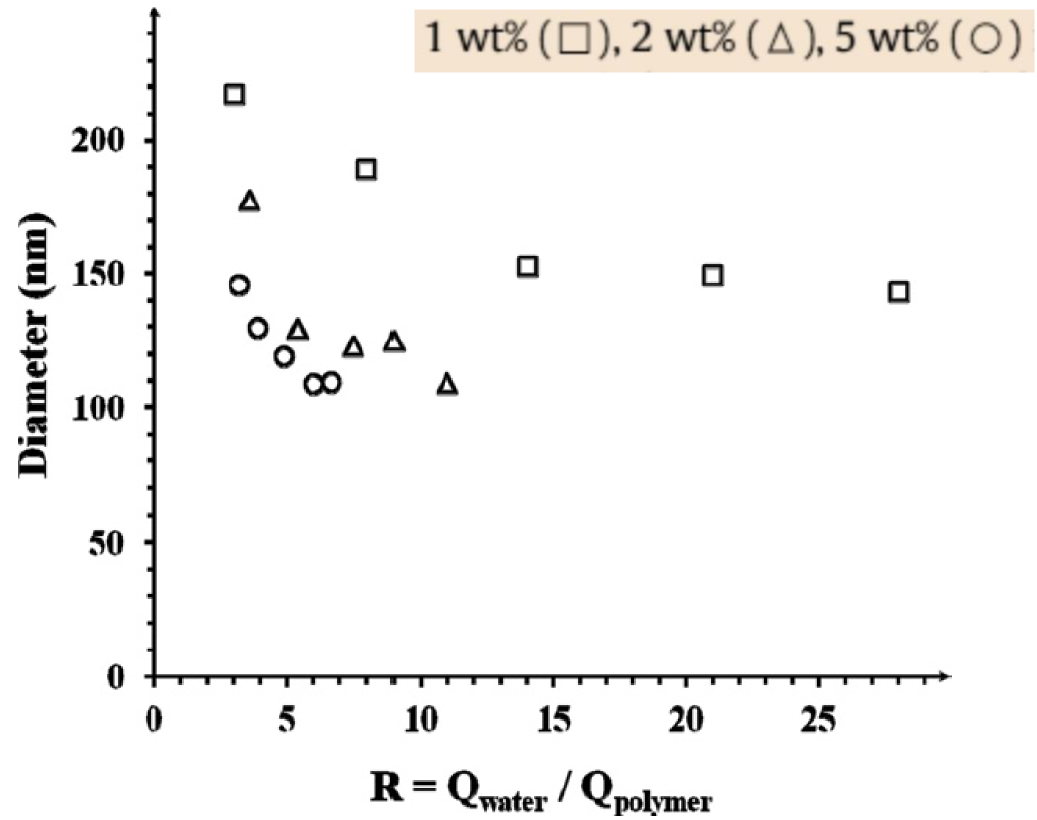
- Size increases with increase in the polymer concentration

Why?

- No. of polymer chains increase per volume, increases J – more no. of nuclei
- Higher polymer-polymer interaction/ association

$$J = \frac{2D}{d^5}\exp\left(-\frac{16\pi\gamma^3 v^2}{3k_B^3 T^3\left[\ln(\boxed{S})\right]^2}\right)$$

- Increase in viscosity of organic phase
  - Resistance to mass transfer
  - Decrease in diffusion rate of organic phase into aqueous phase



R: Proportion of non-solvent to solvent

Size of Polymeric NPs

Polymer Concentration and R

- Increasing R, decreases particle size
- At a certain R, size decreases with increasing polymer concn.

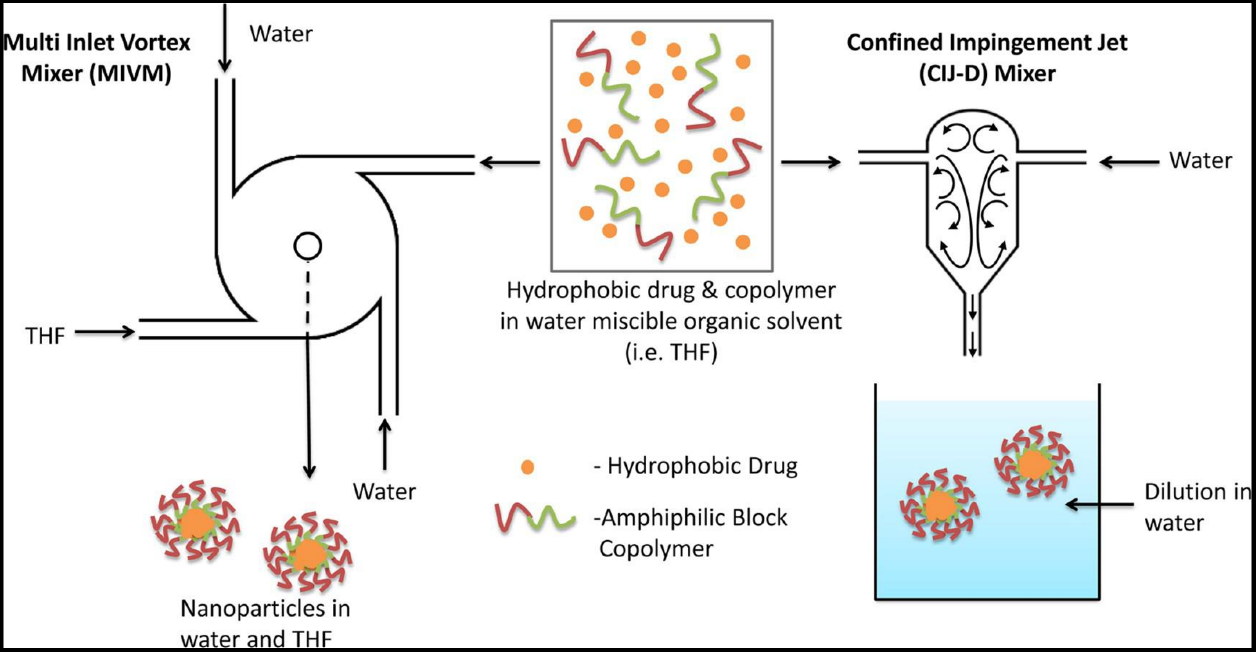
Why?

- High R, high S – smaller particle size (Chemical Instability)
- High R, potential interface increases, more droplets formed during phase separation. Local concentration of the polymer decreases, smaller NPs (Mechanical Mechanisms)

Flash Nanoprecipitation

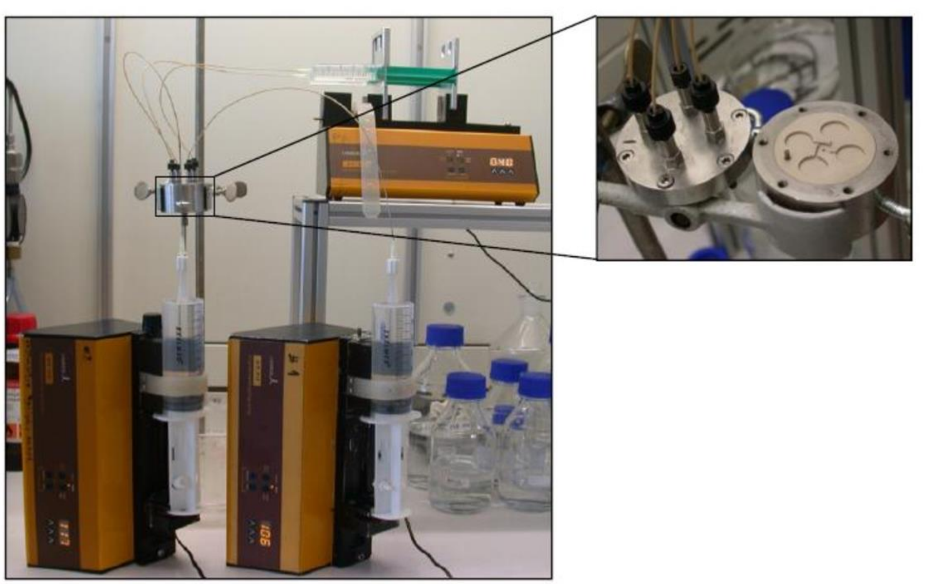
- Rapid micromixing
  - Solvent , anti-solvent mix at a time scale shorter than formation of NPs
- Creation of high supersaturation
- Leads to precipitation of dissolved hydrophobic components
- Mixing time must be less than induction time for polymer aggregation and induction time for nucleation & growth.
- Kinetically controlled process
- Narrow particle size distribution

MIVM Setup

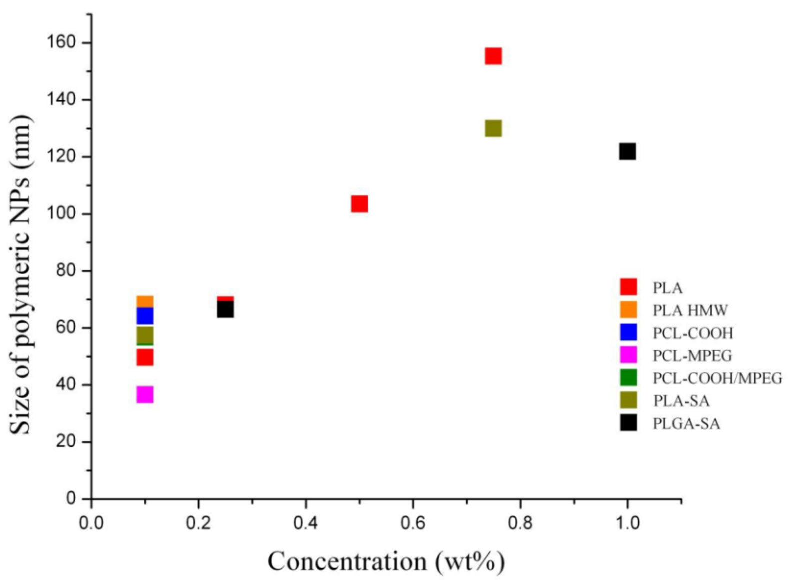


Pustulka et al. , Mol. Pharmaceutics, **2013**, 4367-4377

MIVM Setup



Multi Inlet Vortex Mixer (MIVM) setup for Flash Nanoprecipitation.

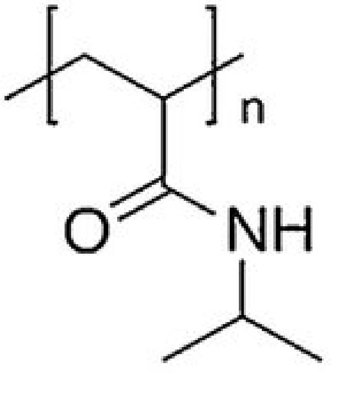


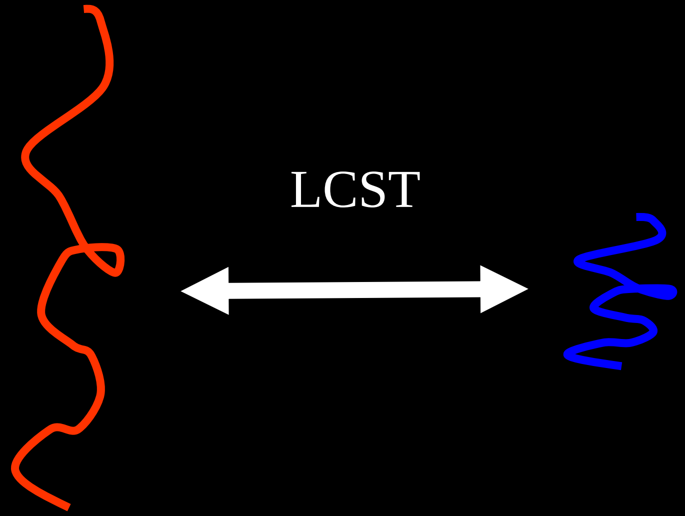
Hydrodynamic sizes of polymeric NPs as a function of concentration.

Sulalit Bandyopadhyay, MSc Thesis, 2010

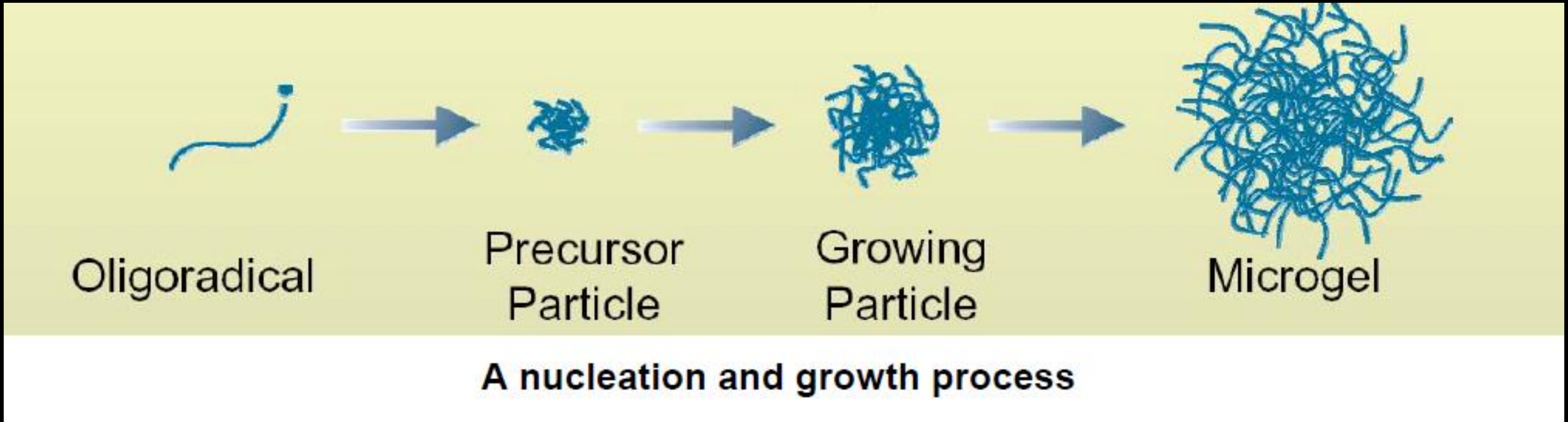
Precipitation Polymerization

- Polymers like Poly(N-isopropyl acrylamide) (PNIPAm) dissolved in water undergo a coil-to-globule transition at temperatures exceeding its lower critical solution temperature (LCST) (~32 ^{0}C)
- The LCST/cloud point can be tuned by altering the composition of a PNIPAm-containing block copolymer
- LCST also affected by the mobility of the PNIPAm chains





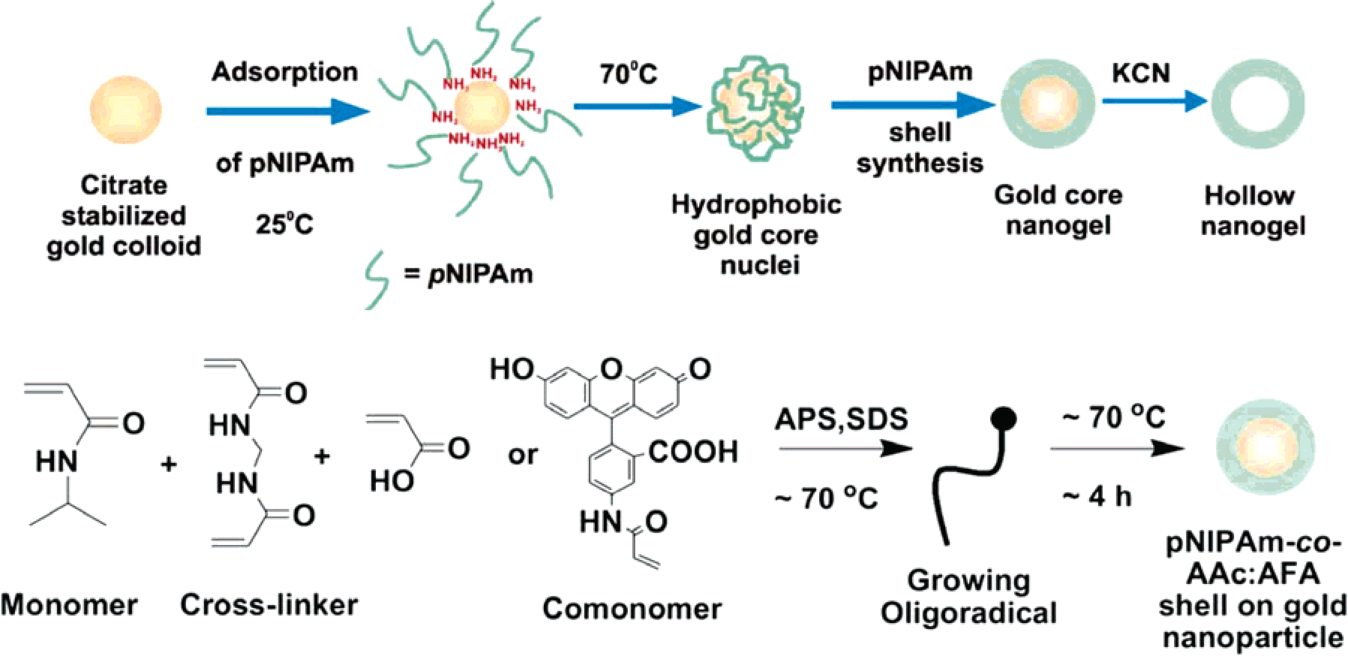
Mechanism



- Monomer + cross linking agent in water
- T > LCST
- Homogeneous nucleation on collapsed oligomers
- Oligomer and monomer addition, aggregation
- Low PDI, control of charge, size, cross-link density

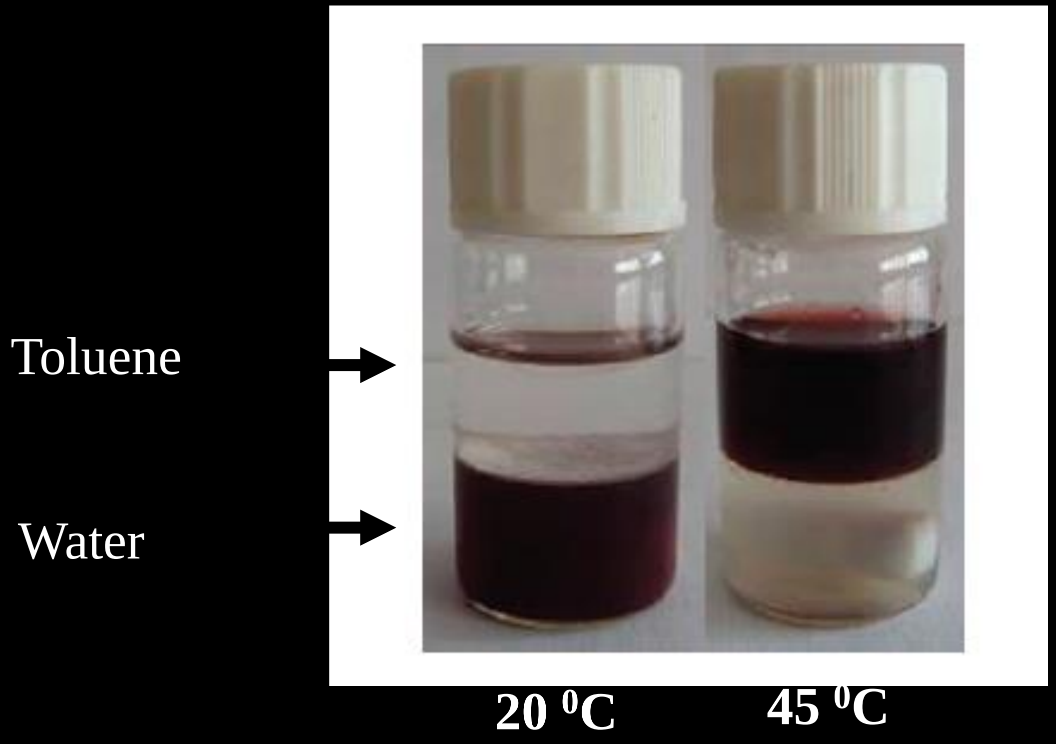
Singh, N., Lyon, L.A, *Chem. Mater.* **2007,** *19*, 719-726

Au-pNIPAm



Singh, N., Lyon, L.A, *Chem. Mater.* **2007,** *19*, 719-726

Phase Transfer



Polarity and phase behavior of the Au NP constructs tunable via temperature

Wen, Y., Jiang, X., Yin, G., and Yin, J., *Chem. Commun.*, **2009**, 6595-6597

Reader’s Digest Version:

- Nanoprecipitation & Flash Nanoprecipitation
  - Fundamentals
  - Mechanisms
  - Parameters
- Nanogels
  - Mechanism
  - Functionalization

Nanomaterials Characterization

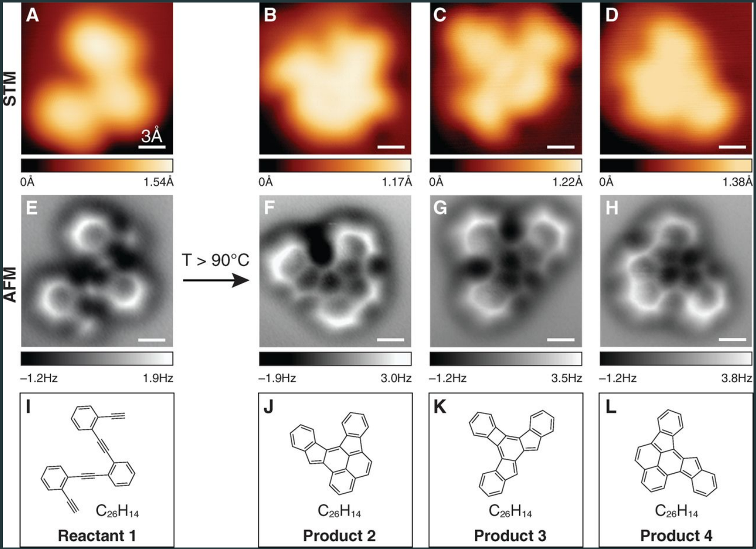
By Atomic Force Microscopy

Vegar Ottesen, vegar.ottesen@ntnu.no

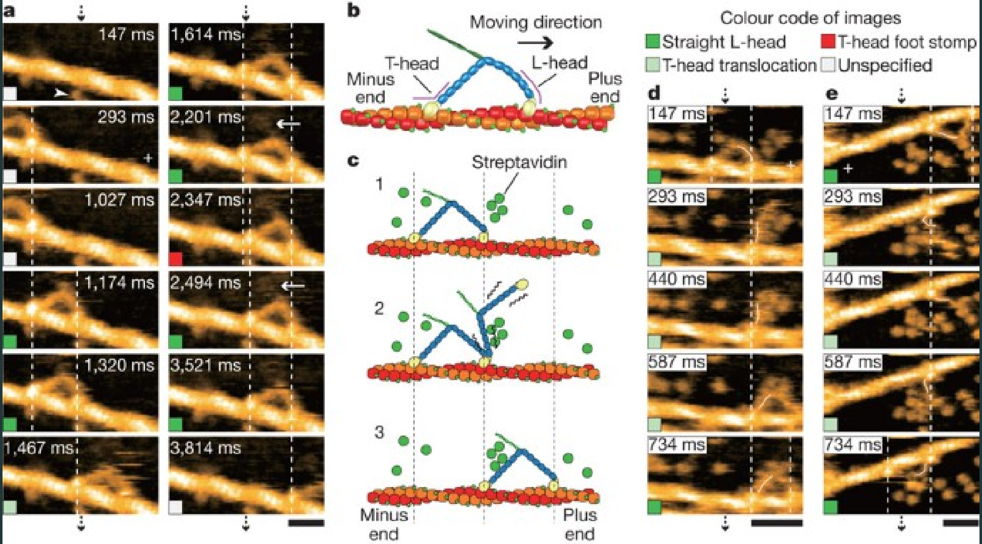
Spring, 2021

Dept. of Chemical Engineering, NTNU

AFM:
Atomic Force Microscopy



Oteyza et al. 2013



Myosin walking on Actin. Kodera et al. 2010

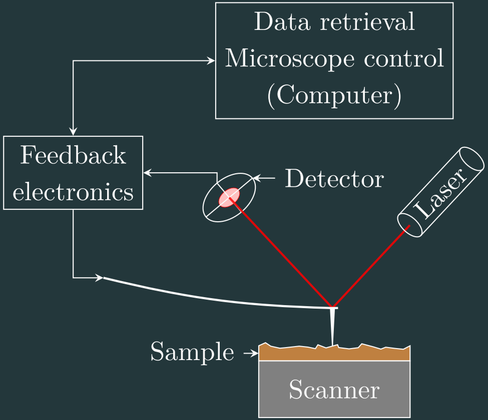
AFM:

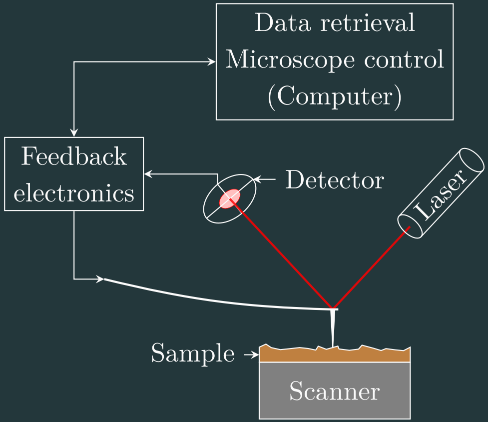
Atomic Force Microscopy

OR:

Artifact Fabrication Machine

Principle


Data retrieval
Microscope control
(Computer)
Feedback
electronics
Detector
Laser
Sample
Scanner



Data retrieval
Microscope control
(Computer)

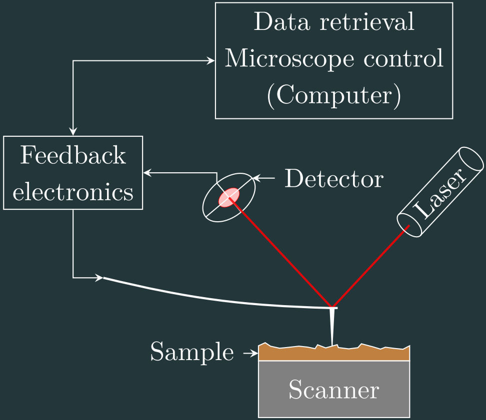
Feedback
electronics

Detector

Laser

Sample

Scanner



Data retrieval
Microscope control
(Computer)

Feedback
electronics

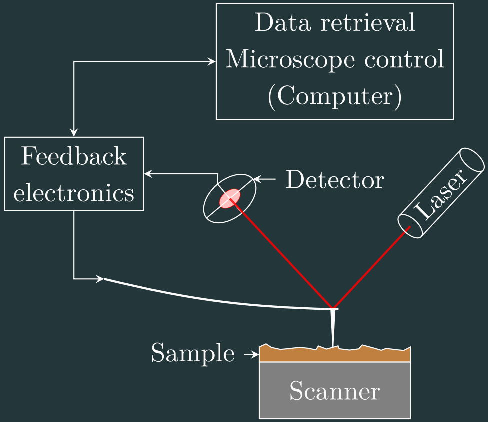
Detector

Laser

Sample

Scanner

displacement in y



displacement in x

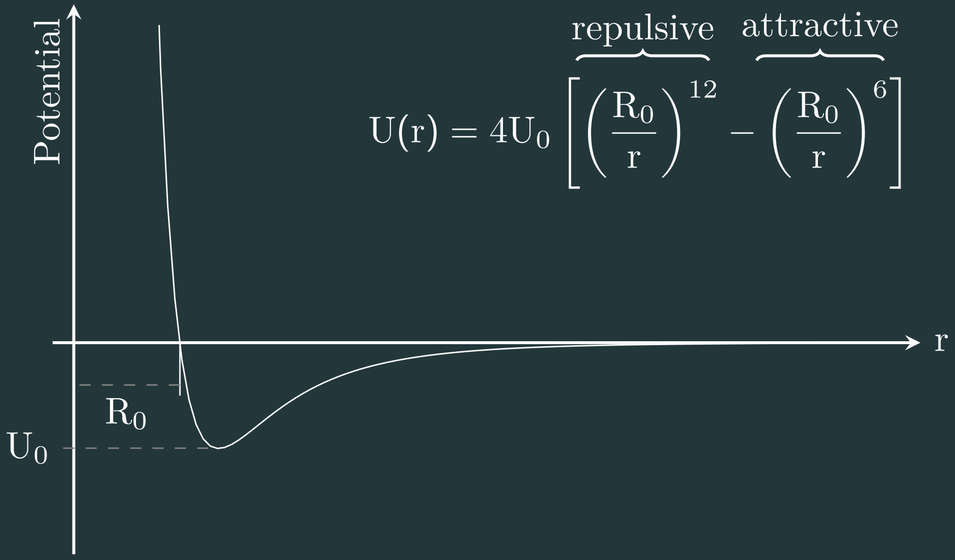
Vacuum, air, liquid

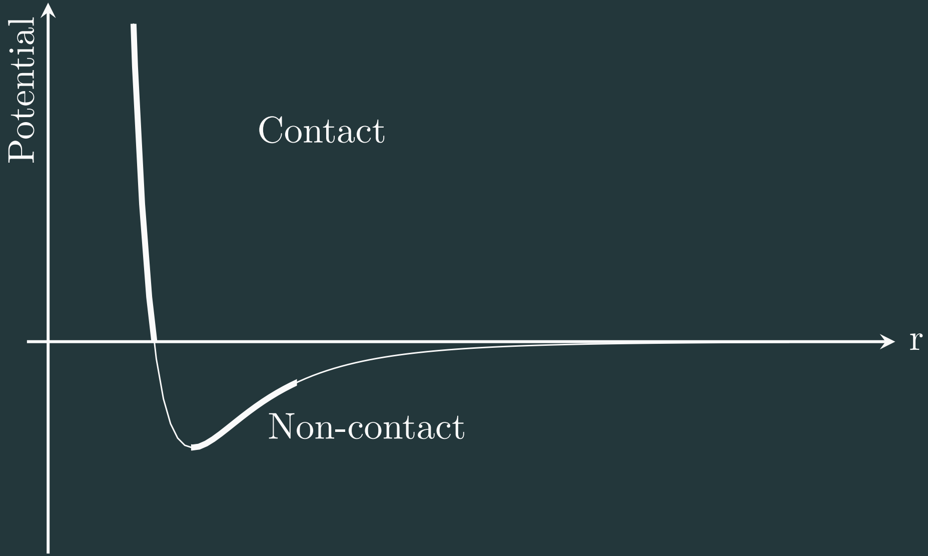
- Topography
- Mechanical properties
- Chemistry
- Magnetism
- Electrical/semiconductors
- Lithography
- +++

Typically slow BUT: fast AFM can image proteins in action

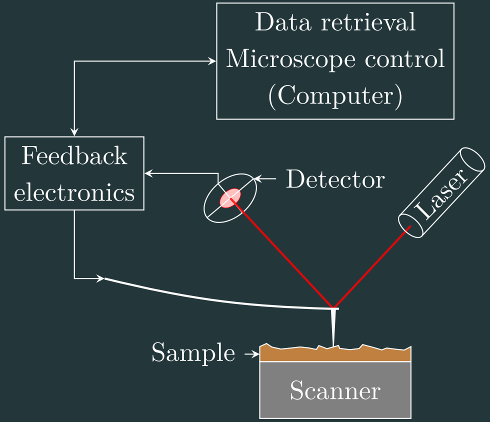
Achievable resolution: Below atomic scale.

$$U(r) = 4U_0 \left[\overbrace{\left(\frac{R_0}{r}\right)^{12}}^{\text{repulsive}} - \overbrace{\left(\frac{R_0}{r}\right)^{6}}^{\text{attractive}} \right]$$




Potential
Contact
r
Non-contact

Contact



Data retrieval
Microscope control
(Computer)

Feedback electronics

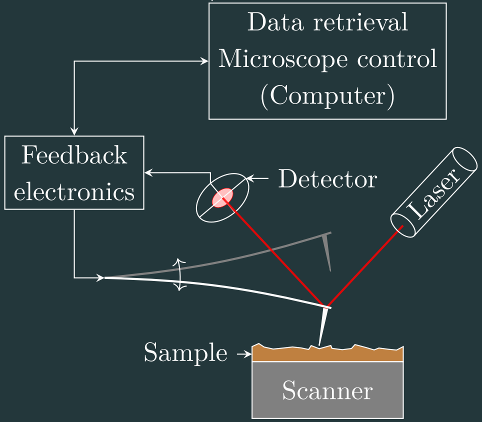
Detector

Laser

Sample

Scanner

Tapping/non-contact



Data retrieval
Microscope control
(Computer)

Feedback electronics

Detector

Laser

Sample

Scanner

Contact

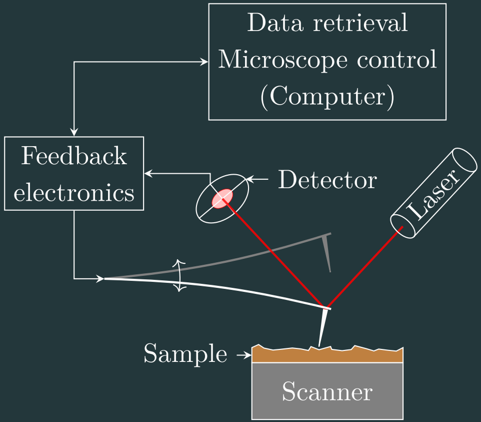
- Harsh
- Any environment
- Simple
- Lithography

Tapping

- Gentle
- Any environment
- Less simple
- Most common

Non-Contact

- Very Gentle
- Not liquid
- Not simple
- Forces, not topography



Data retrieval
Microscope control
(Computer)

Feedback
electronics

Detector

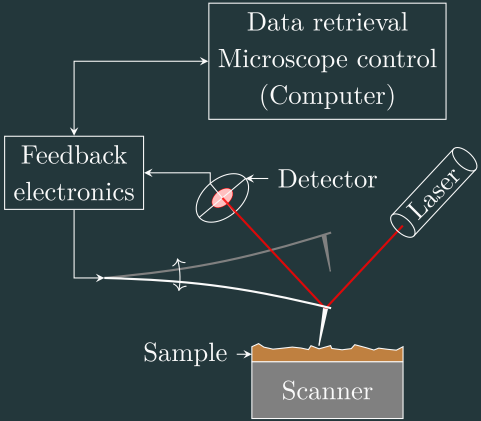
Laser

Sample

Scanner

Oscillating cantilever

Piezo-driven stage



Data retrieval
Microscope control
(Computer)

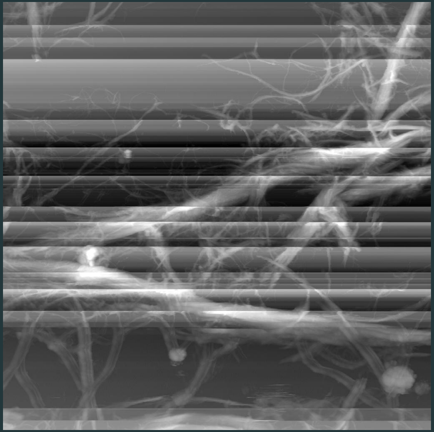
Feedback
electronics

Detector

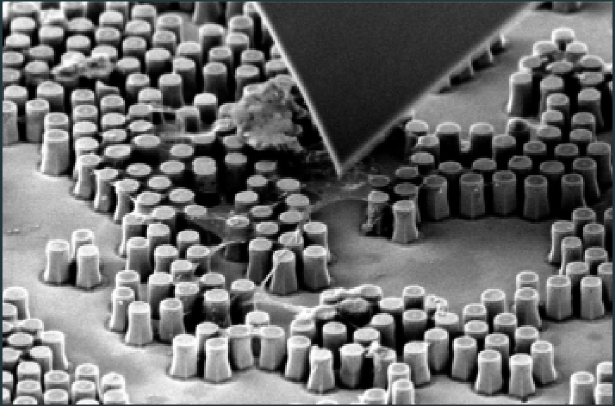
Laser

Sample

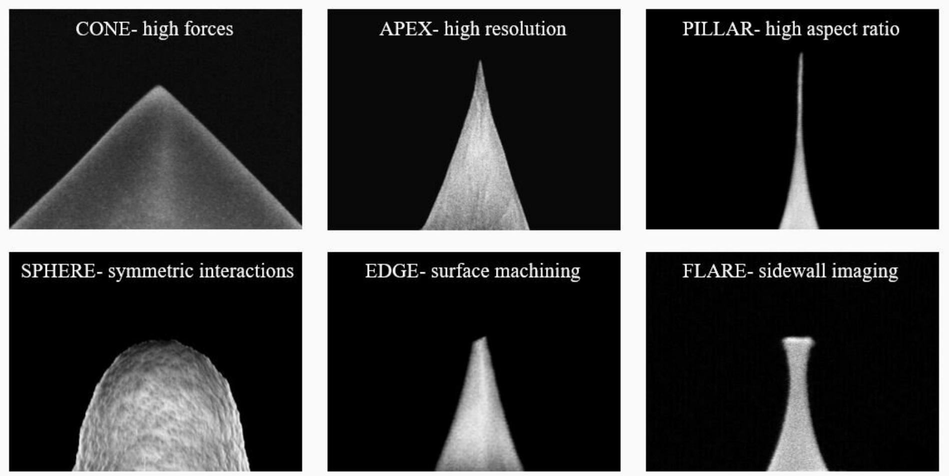
Scanner



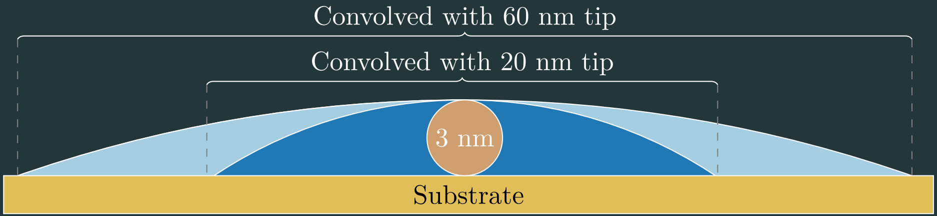
The tip

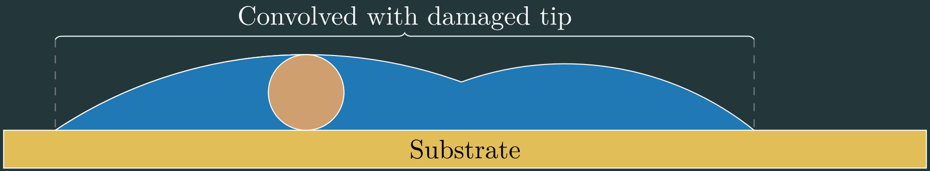


Source:Azo materials

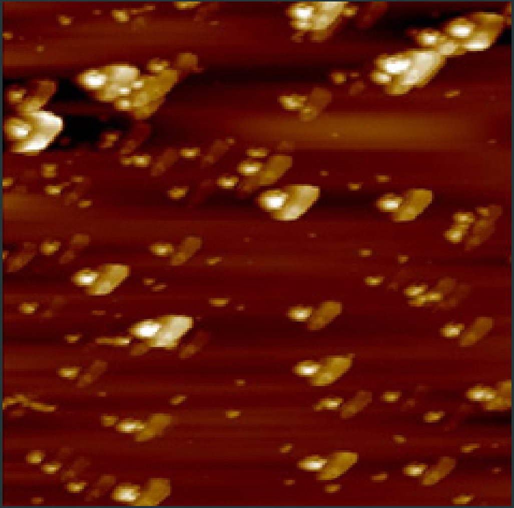


Source: Adama Innovations

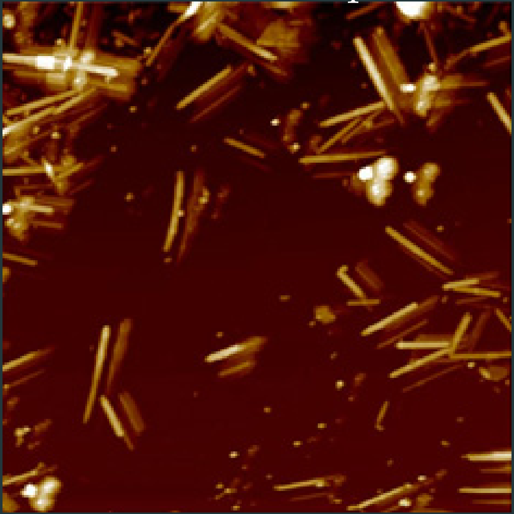

Convolved with 60 nm tip
Convolved with 20 nm tip
3 nm
Substrate


Convolved with damaged tip
Substrate

Contamination



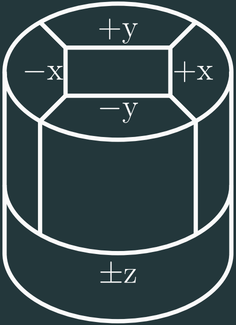
Double tip

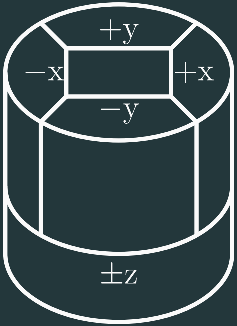


Images: Myscope.com

Geometry is essential

The Scanner


+y
−x
+x
−y
±z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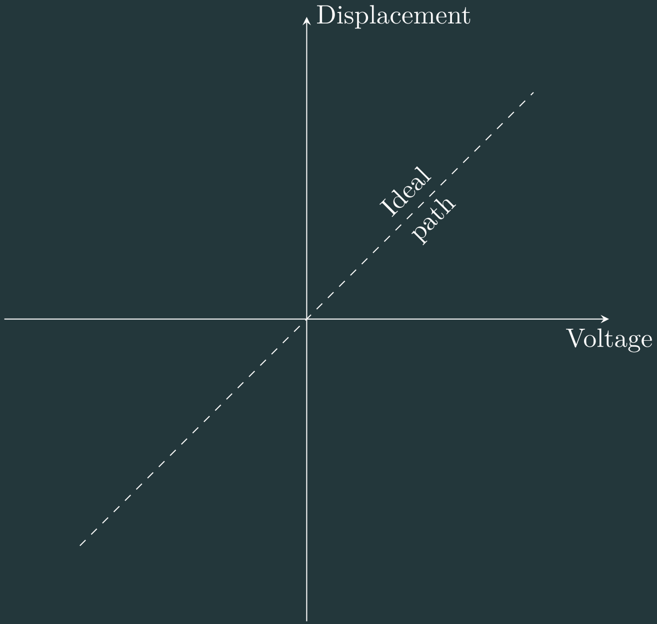
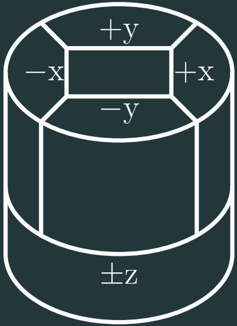

Displacement
Ideal path
Voltage


+y
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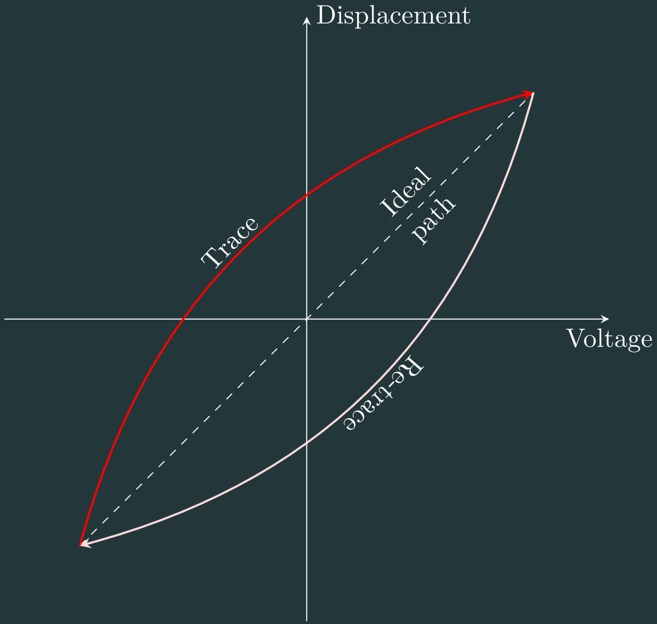
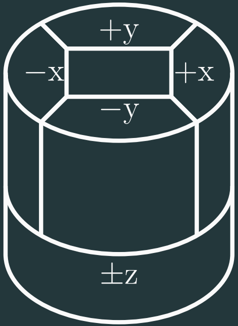
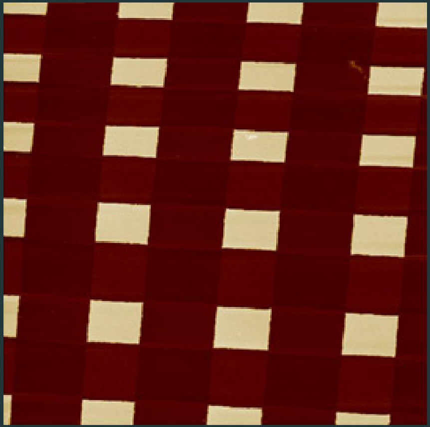
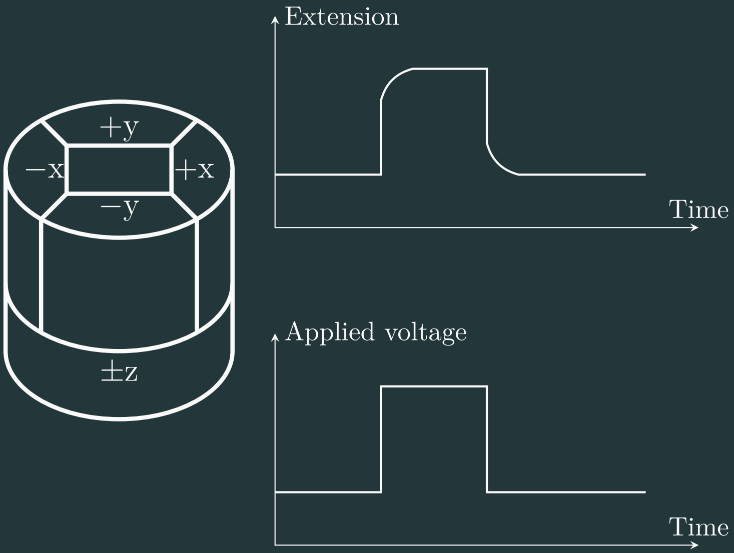
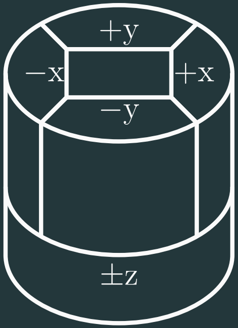

Displacement
Voltage
Trace
Ideal path
Re-trace





Myscope.com


+y
−x
+x
−y
±z
Extension
Time
Applied voltage
Time


+y
−x
+x
−y
±z


Curved image surface

Software correction (to an extent)

Imaging

Channels

Error

Height

Inphase

Horizontal

Adhesion

Dissipation

Young modulus

Conductivity

...

Channels

Error

Height

Inphase

Horizontal Dissipation

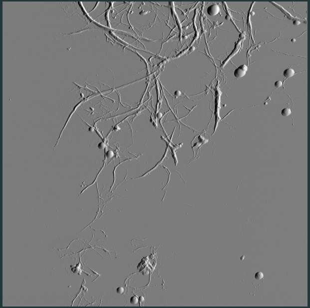
Dissipation

Young modulus

Conductivity

...

Error



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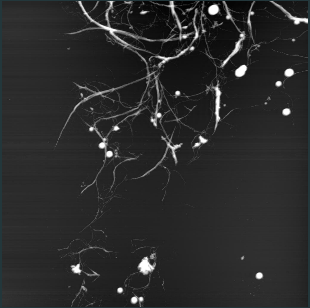
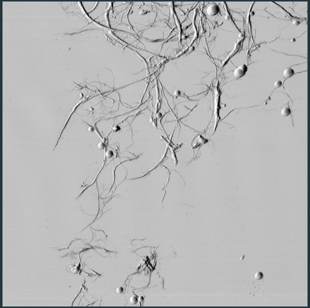
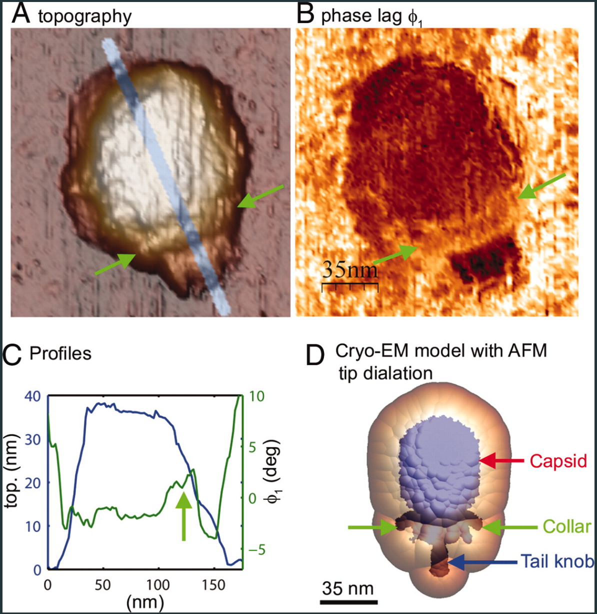


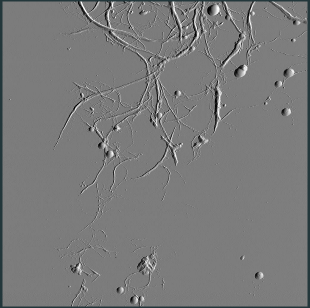
Phase





From Melcher et al. 2009

Error



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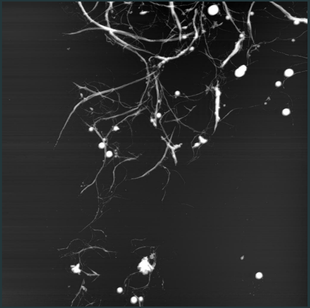

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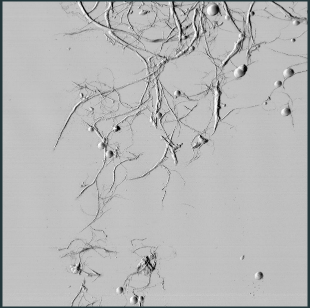

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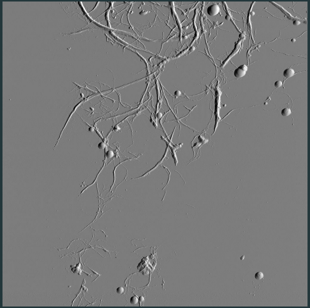

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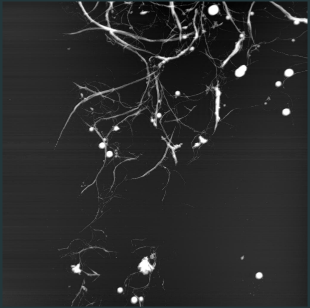
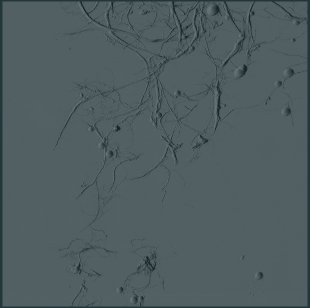
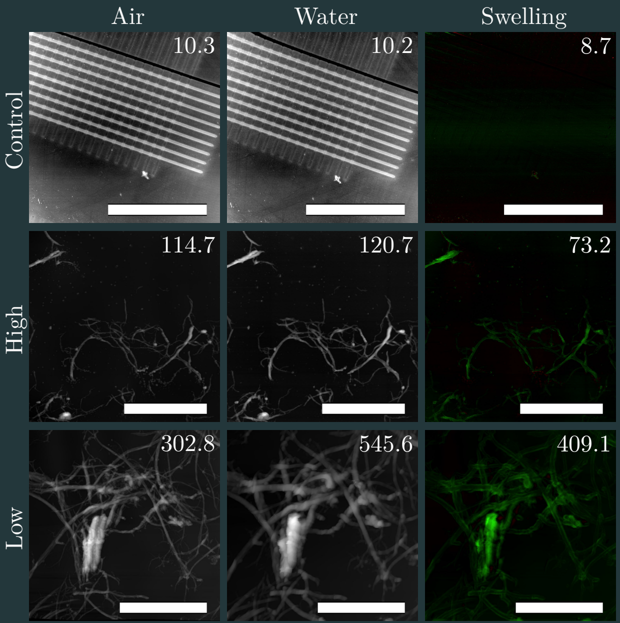


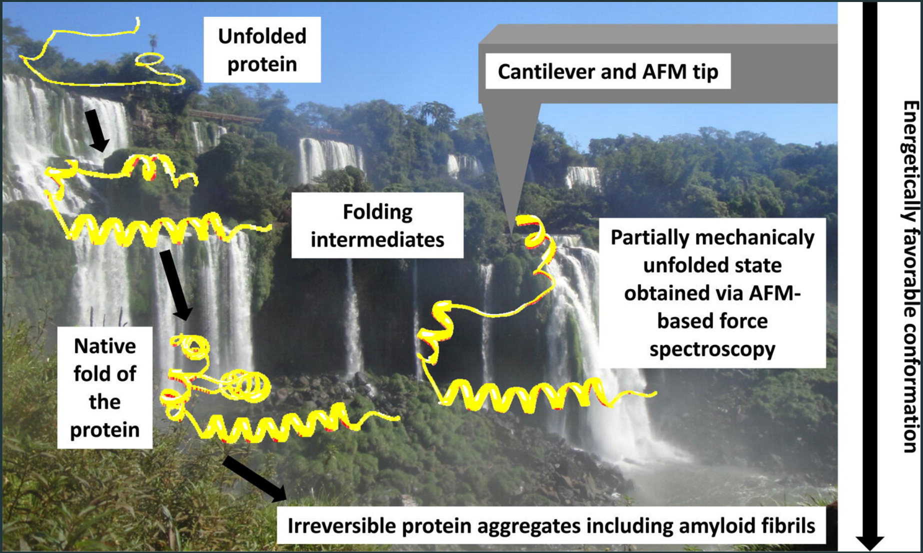
Phase



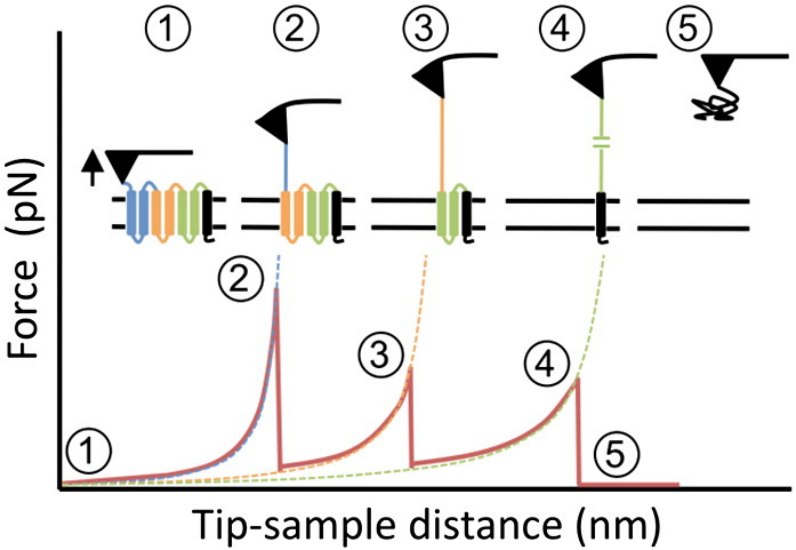


From Ottesen & Syverud 2020

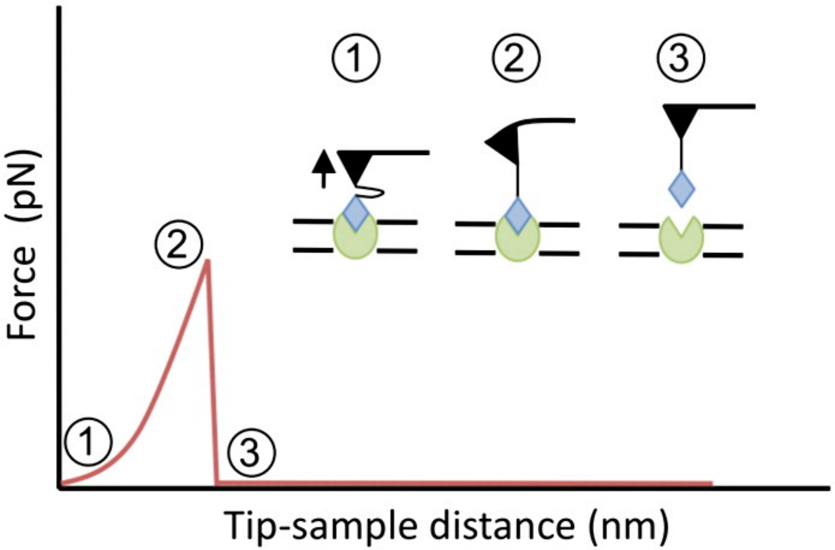
Spectroscopy



//Carvalo et al. 2013



Whithed & Park, 2014



Whithed & Park, 2014

Summary

AFM: Atomic Force Microscopy

Sharp tip rastered across sample

Deflection, twist, oscillation: Basis for signal

Works in air, liquid, vacuum

Can resolve even covalent bonds

Contact, Tapping, Non-contact

Tip

Sharp tip affixed to larger cantilever

Tip geometry affects resolution

Tip geometry/chemistry must fit experiment

Tip care essential

Scanner

Piezo-electric, often below sample

Hysteresis, creep, scanner geometry may skew image

Mostly software corrected

Imaging

Various channels w. different data

Available channels depend on setup

Spectroscopy

Functional tip, test molecular interactions

Examples: Protein folding, protein interactions

General:

Versatile surface technique

Supreme resolution

Easy to use, difficult to master

?

Iceland
Liechtenstein
Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

UNIVERSITY OF BUCHAREST
VIRTUTE ET SAPIENTIA

NTNU
Norwegian University of
Science and Technology

NATURAL PRODUCTS CHEMISTRY

Assoc. prof. Elisabeth Jacobsen

Natural product chemistry

Introduction
Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU
Spring 2022

Textbook: K. B. G. Torssell: Natural Product Chemistry, 2. utg., Apotekersocieteten/Taylor & Francis, 1997

Natural products

- All chemical compounds that are found in nature, produced by living cells.
- Ancient science:
  - Extracts from nature were used for preparation of food, coloring, fibers, toxins, medicine and stimulants
- Late 18th century:
  - Natural products were separated, purified and analyzed

Organic natural product chemistry

- Occurence of natural products
    - (sources and amounts)
- Structure and stereochemistry
- Physicochemical properties
- Function in the organism
- Biosynthesis
- Chemical synthesis (for structural evidence)

Why is natural product chemistry interesting today?

One reason: antibacterial and anticancer medicine

Natural products were precursors for 54% of new drugs on the market from 1981-2002

David J. Newman, Gordon M. Cragg and Kenneth M. Snader, *J. Nat. Prod*, 2003, *66*, 1022-1037

Primary and secondary metabolites

Metabolism = **anabolism** (synthesis of molecules needed by the organism) + **catabolism** (breakdown of molecules for energy)

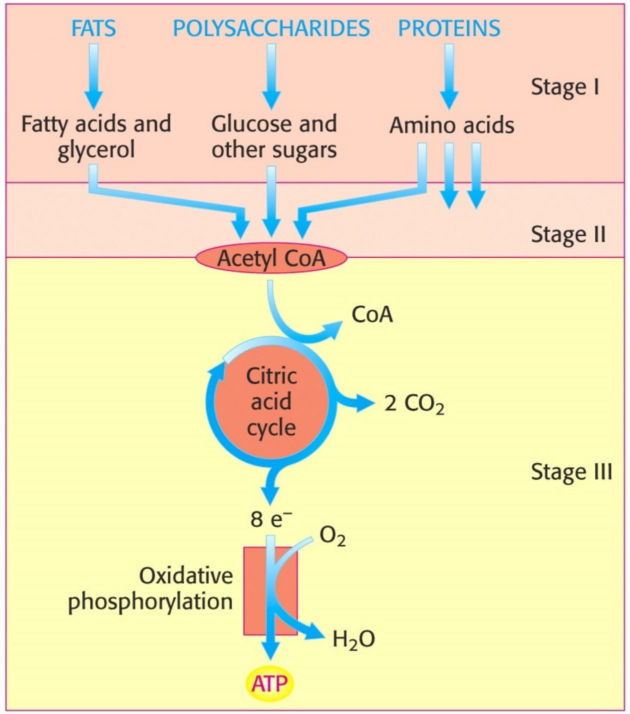
Primary metabolites

- Compounds that are essential for life
- Photosynthetic processes

$CO_2 + H_2O \rightarrow \text{carbohydrates} + O_2$

- Starting materials for secondary metabolites

- Low molecular weight carboxylic acids (Krebs cycle)
- α-amino acids
- Carbohydrates
- Fats
- Protein
- Nucleic acids

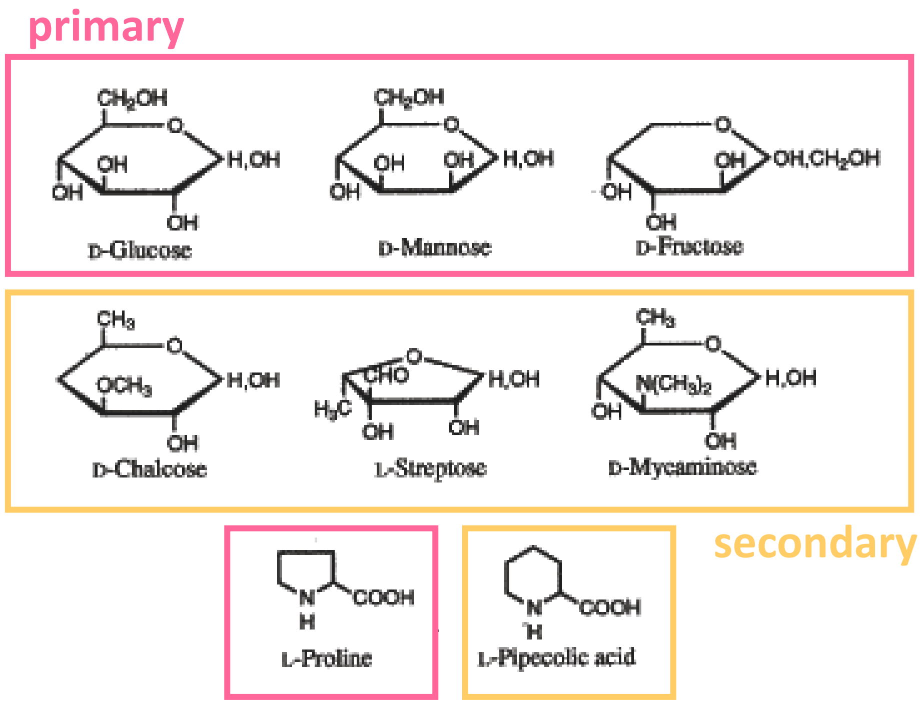


Primary and secondary metabolites

Metabolism = **anabolism** (synthesis of molecules needed by the organism) + **catabolism** (breakdown of molecules for energy)

Secondary metabolites

- Non-essential, «specialized» molecules
- Often characteristic for groups of organisms
- Natural product chemistry
- No sharp line between primary and secondary metabolites



Examples of secondary metabolite functions

Attract other individuals

Pheromones, pigments, aroma
Reproduction & propagation

Repel other individuals

Bad taste, smell, toxicity, phytoalexins
Defence & protection

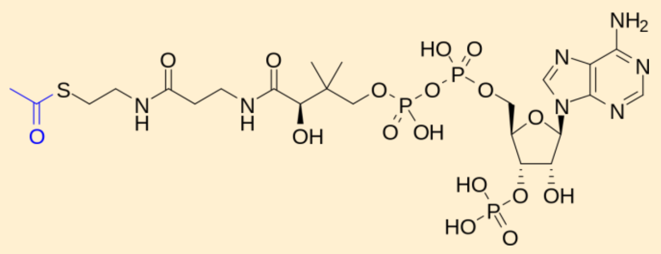
Modify membranes

Protecting the organism from the environment (temperature, etc.)

Aid in metabolism

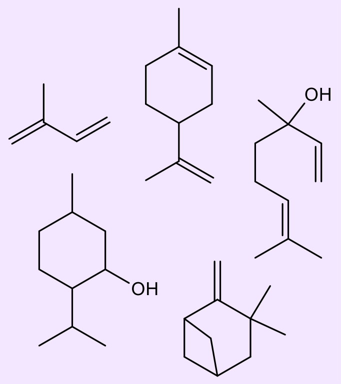
Prosthetic groups/coenzymes, vitamins, hormones, etc.

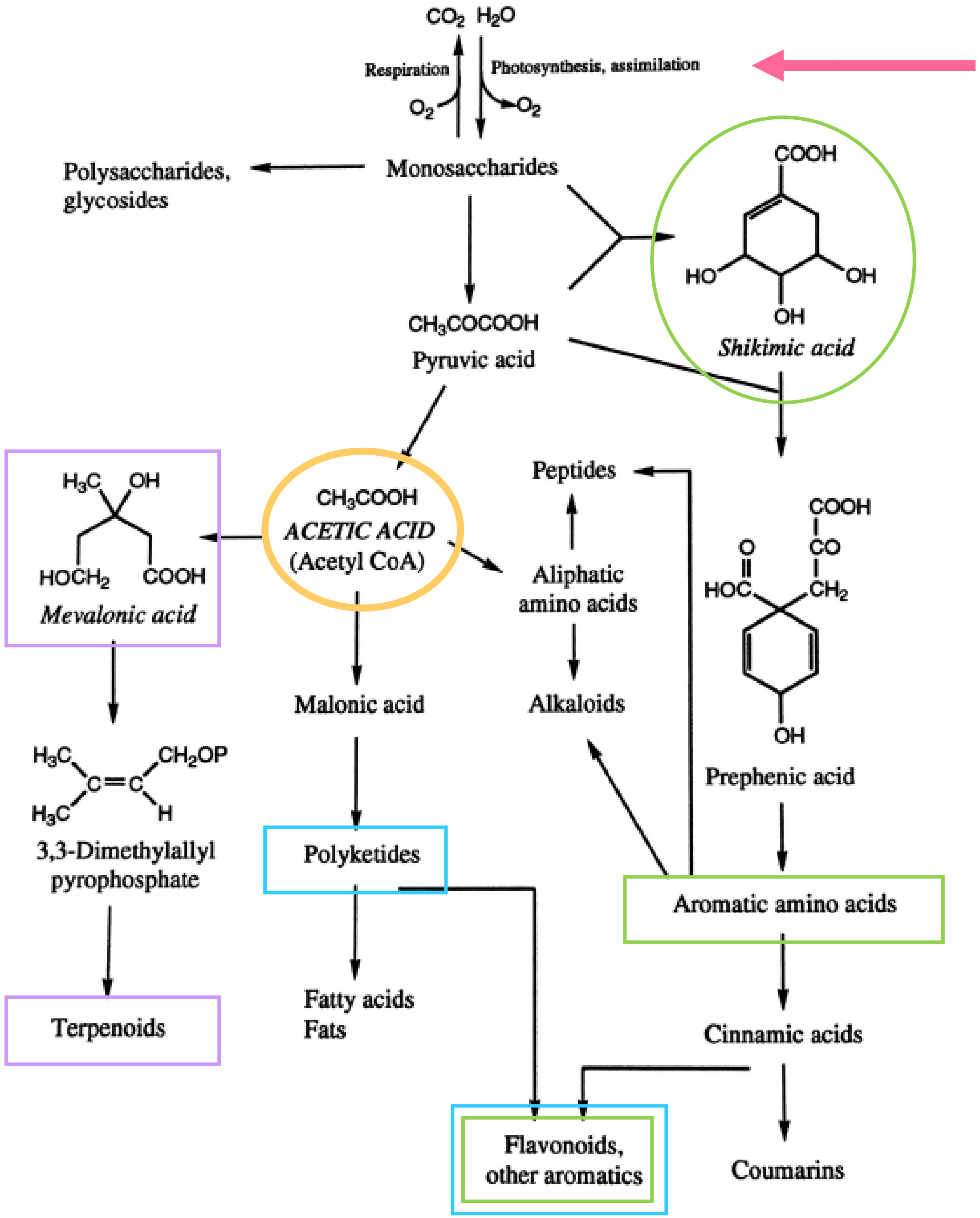
Main streams of secondary metabolism



Acetyl CoA

Terpenes/terpenoids





CO_2 H_2O

Respiration

Photosynthesis, assimilation

O_2 O_2

Polysaccharides, glycosides

Monosaccharides

$CH_3COCOOH$

Pyruvic acid

Shikimic acid

CH_3COOH

ACETIC ACID

(Acetyl CoA)

Mevalonic acid

Peptides

Aliphatic amino acids

Alkaloids

Prephenic acid

Malonic acid

3,3-Dimethylallyl pyrophosphate

Polyketides

Aromatic amino acids

Terpenoids

Fatty acids

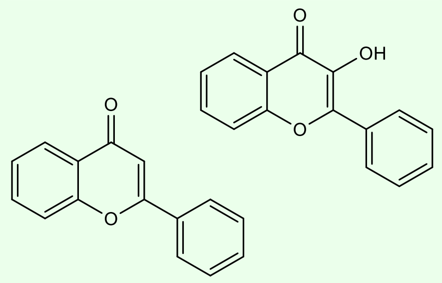
Fats

Cinnamic acids

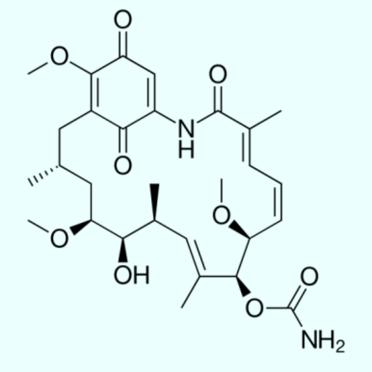
Flavonoids, other aromatics

Coumarins

Primary metabolism



Flavones/flavonoids



Geldanamycin (a polyketide)

We will go through:

3: Carbohydrates and primary metabolites

4: The shikimic acid pathway

5: The polyketide pathway

6: The mevalonic acid pathway & the terpenes

7: Amino acids, peptides and proteins

8: The alkaloids

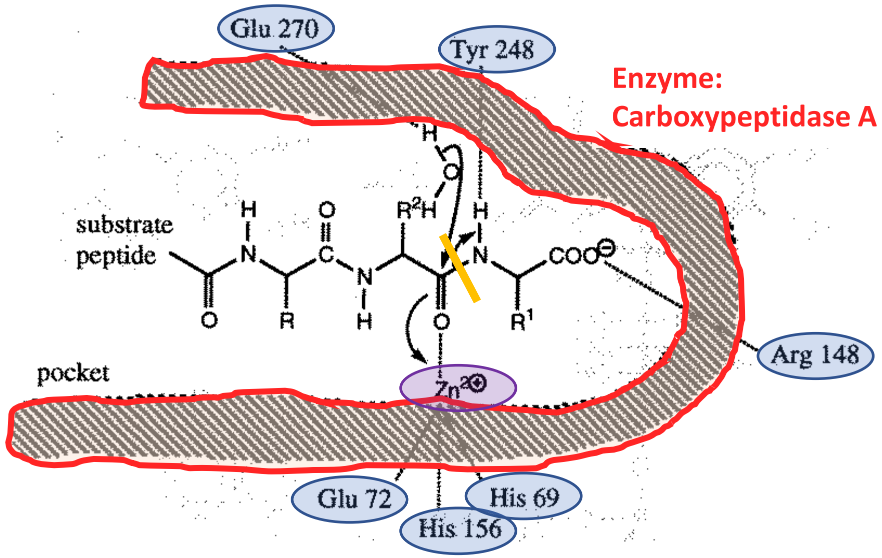
9: The N-heteroaromatics

Biochemical reactions (reminders)

- Enzymatic reactions: can easily form enantiopure compounds, because of the chiral structure of enzymes.

Example: Hydrolysis of a terminal amino acid of a peptide.

- This enzyme is a chain of 307 amino acids.
- Through hydrogen bonding and other types of bonds, the substrate is ideally placed for the reaction.
- The dipolar character of the carbonyl bond is enhanced thanks to Zn^{2+}.
- Protonation of the amino function lead the cleavage of the peptide bond.



Biochemical reactions (reminders)

- A lot of enzymatic reactions require coenzymes. They will act as carriers of some necessary groups.

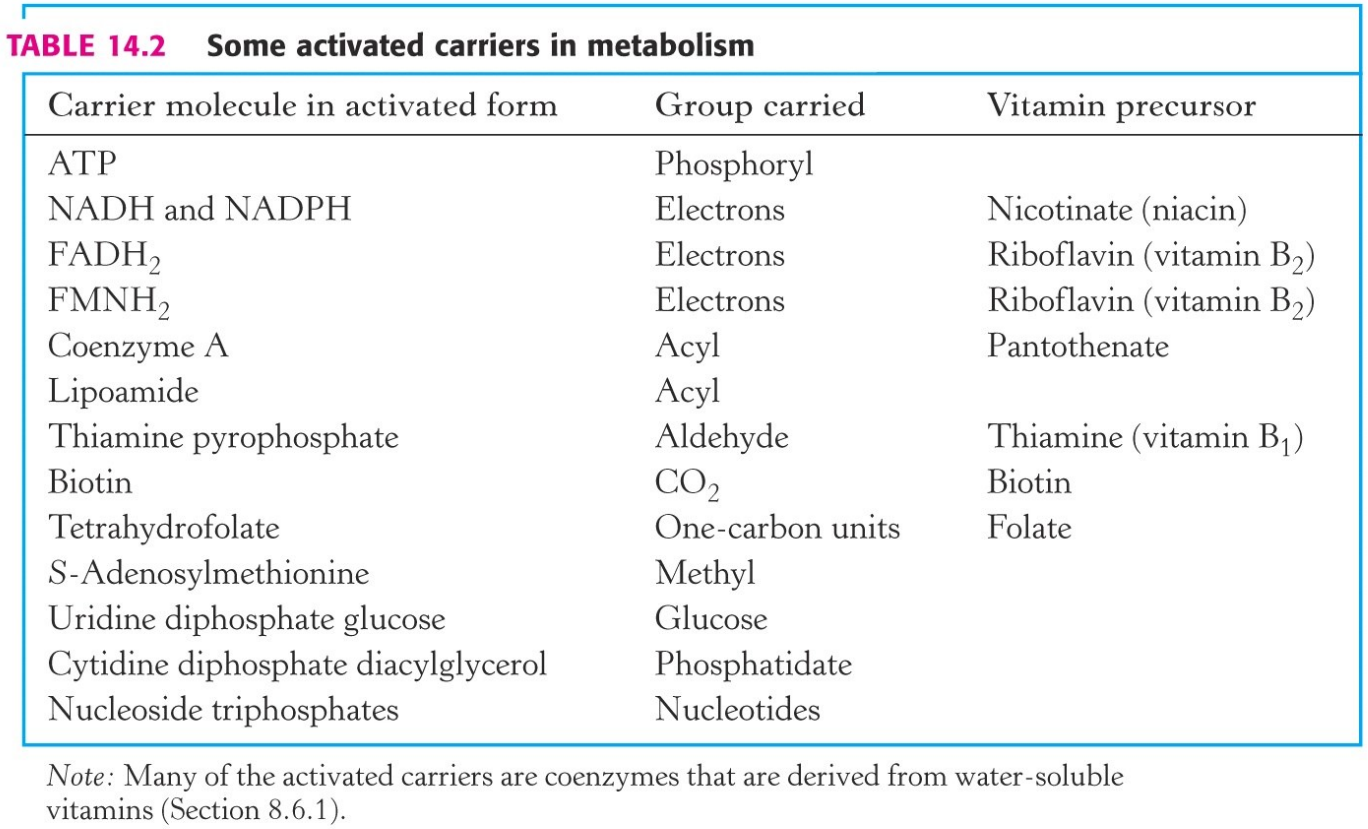


TABLE 14.2 **Some activated carriers in metabolism**

Carrier molecule in activated form	Group carried	Vitamin precursor
ATP	Phosphoryl	
NADH and NADPH	Electrons	Nicotinate (niacin)
$FADH_2$	Electrons	Riboflavin (vitamin B_2)
$FMNH_2$	Electrons	Riboflavin (vitamin B_2)
Coenzyme A	Acyl	Pantothenate
Lipoamide	Acyl	
Thiamine pyrophosphate	Aldehyde	Thiamine (vitamin B_1)
Biotin	CO_2	Biotin
Tetrahydrofolate	One-carbon units	Folate
S-Adenosylmethionine	Methyl	
Uridine diphosphate glucose	Glucose	
Cytidine diphosphate diacylglycerol	Phosphatidate	
Nucleoside triphosphates	Nucleotides	

Note: Many of the activated carriers are coenzymes that are derived from water-soluble vitamins (Section 8.6.1).

Biochemical reactions

- Several types of reactions can be distinguished:

Carbon-carbon coupling (Claisen and Michael)

In enzyme promoted acylation with thioesters for instance.

Electrophilic substitutions

In C-, N- and O-alkylations with S-adenosyl methionine and phospahtes

Eliminations

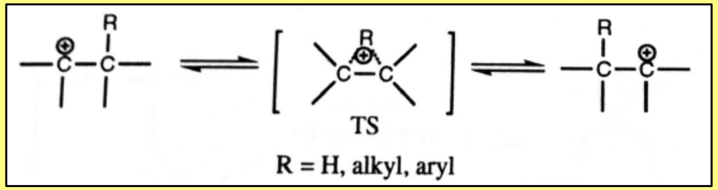
The nucleophile group of an enzyme (-OH,-NH$_2$,-SH) will lead to the elimination of, for instance, water or ammonia.

Oxidations/reductions

Cofactors like NAD$^+$ and FAD are used as electron carrier/receiver.

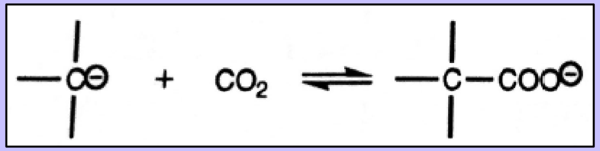
Carbonium anion rearrangements

Happens for instance in terpene biosynthesis



Carboxilation/decarboxylation

Happens for instance in fatty acid synthesis.



Elucidation of metabolic sequences

- Intermediate are only present in very small quantities in normal organisms.
- Use of defective organisms (obtained for ex. by UV or X-rays irradiation):
  - In mutant **1**, D will accumulate, and E will be needed for the mutant to grow
  - The filtrate of **1** will allow **2** to grow, but the opposite is false.
  - The order of the sequence can be proven this way.

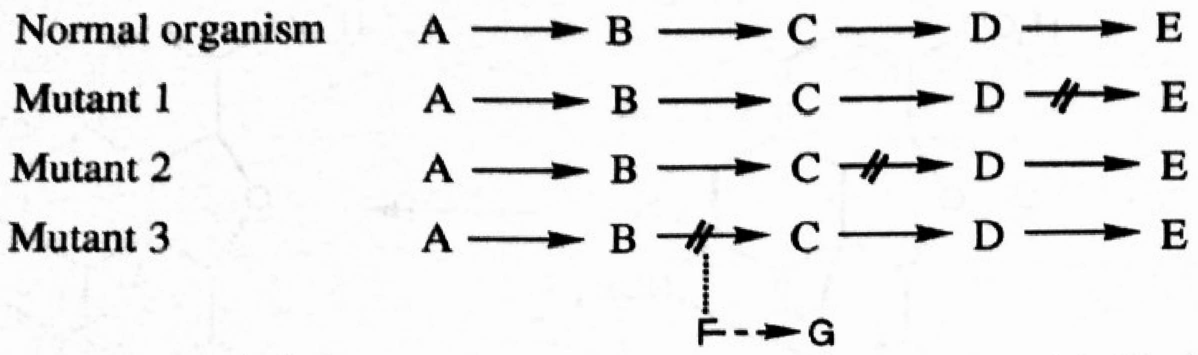
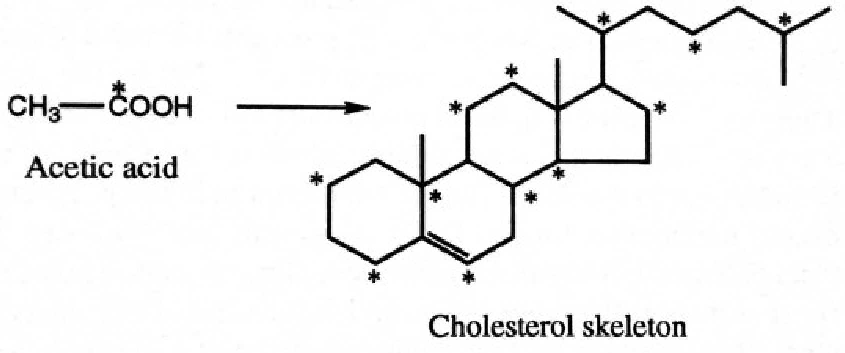


Fig. 9 Mutants with defective enzymes at different points along a metabolic chain

Elucidation of metabolic sequences

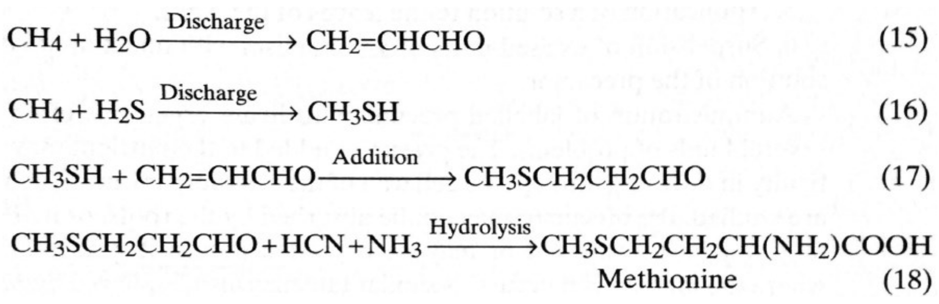
- Use of isotopes:
  - Radioactive substrates are given to the organism.
  - In a sequence A→E, radioactivity will first appear in A, then B,...
  - Allow us to know precisely where a certain carbon in a final product comes from (isotope labeling).
  - The pathway from acetic acid to cholesterol was found by degrading a labeled cholesterol molecule.
  - C^{13}-NMR and H-NMR are powerful tools to analyze labeled compounds



Prebiotic chemistry

How were the first amino-acids created on earth?

- An electrical discharge in a mixture of basic compounds (water, methane, ammonia and nitrogen) gives amino-acids in a low yield.



$$CH_4 + H_2O \xrightarrow{\text{Discharge}} CH_2{=}CHCHO \tag{15}$$

$$CH_4 + H_2S \xrightarrow{\text{Discharge}} CH_3SH \tag{16}$$

$$CH_3SH + CH_2{=}CHCHO \xrightarrow{\text{Addition}} CH_3SCH_2CH_2CHO \tag{17}$$

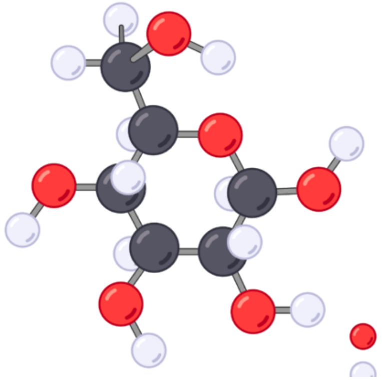
$$CH_3SCH_2CH_2CHO + HCN + NH_3 \xrightarrow{\text{Hydrolysis}} \underset{\text{Methionine}}{CH_3SCH_2CH_2CH(NH_2)COOH} \tag{18}$$

- A lot of amino-acids may have been formed from oligomerization of hydrocyanic acid.
- The preference for the L form of chiral compounds in living beings doesn’t have any good explanation.

Iceland
Liechtenstein
Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

Chapter 3
Carbohydrates and primary metabolites

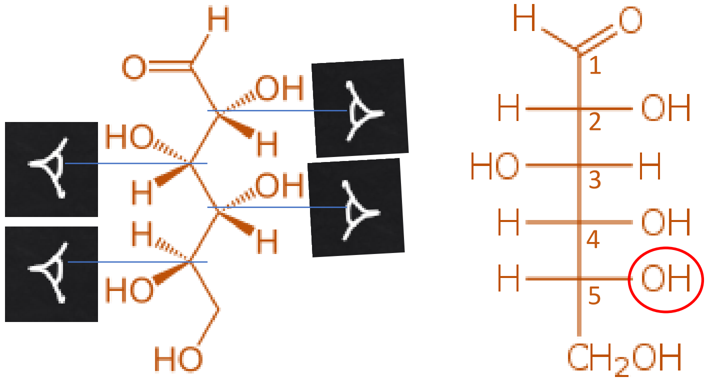


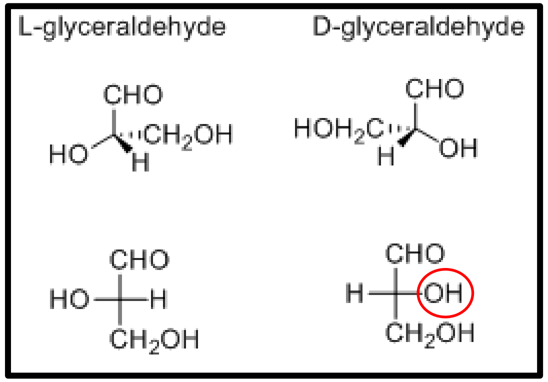
Elisabeth Jacobsen and Lucas Boquin, NTNU

Spring 2022

Classification of carbohydrates

- Number of carbon atoms in one unit (most common: hexoses)
- Aldehyde or keto function
- Number of units (monosaccharides, disaccharides,...)
- Fischer representation:

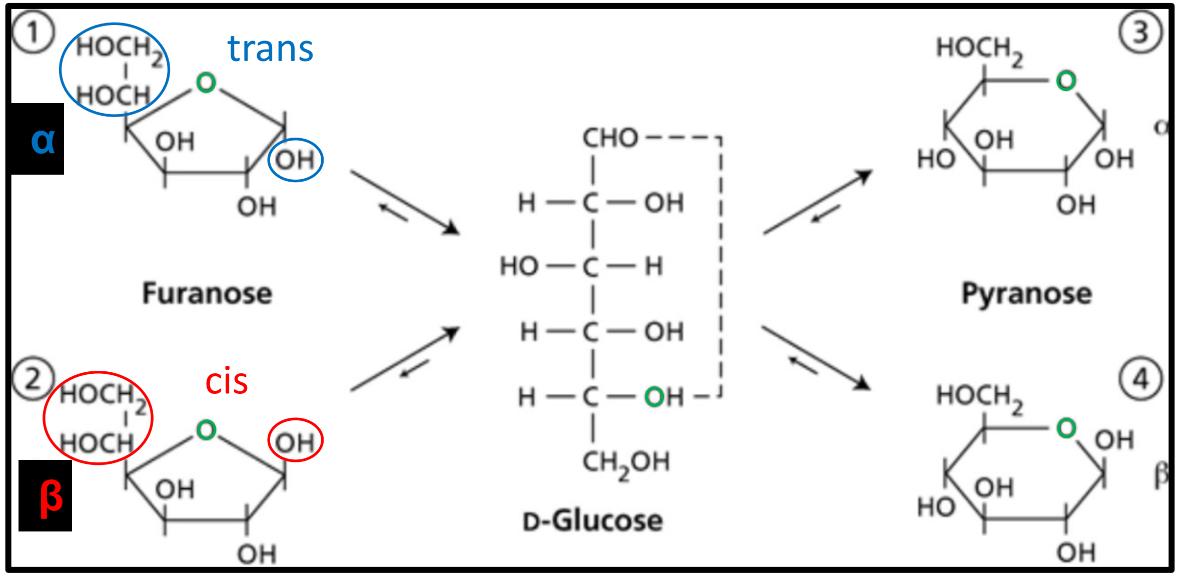




Conformations and stereoisomerism

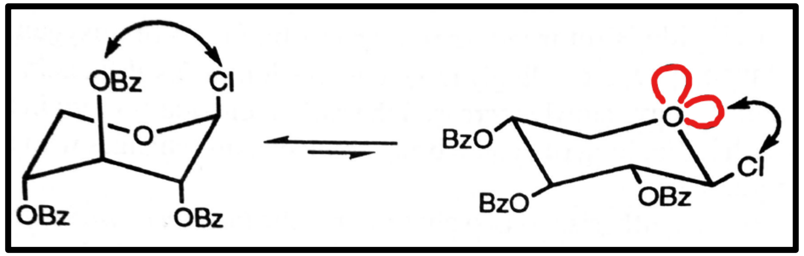
- The open form of hexoses are in equilibrium with their hemiacetal/hemiketal form.

α / β : Different physical properties



6-membered rings:
In chair and boat form

- The preferred isomer isn't always the one with bulky groups in equatorial position.



Carbohydrate biosynthesis in plants: Photosynthesis

- 1- Light reaction

energy rich

$$H_2O + NADP^+ + P + ADP \xrightarrow{h\nu} {}^{1}/_{2}O_2 + NADPH + ATP + H^+$$

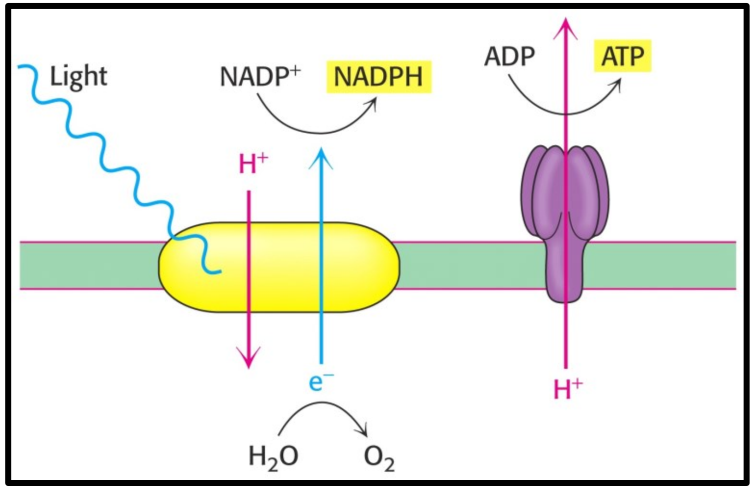
reducing agent

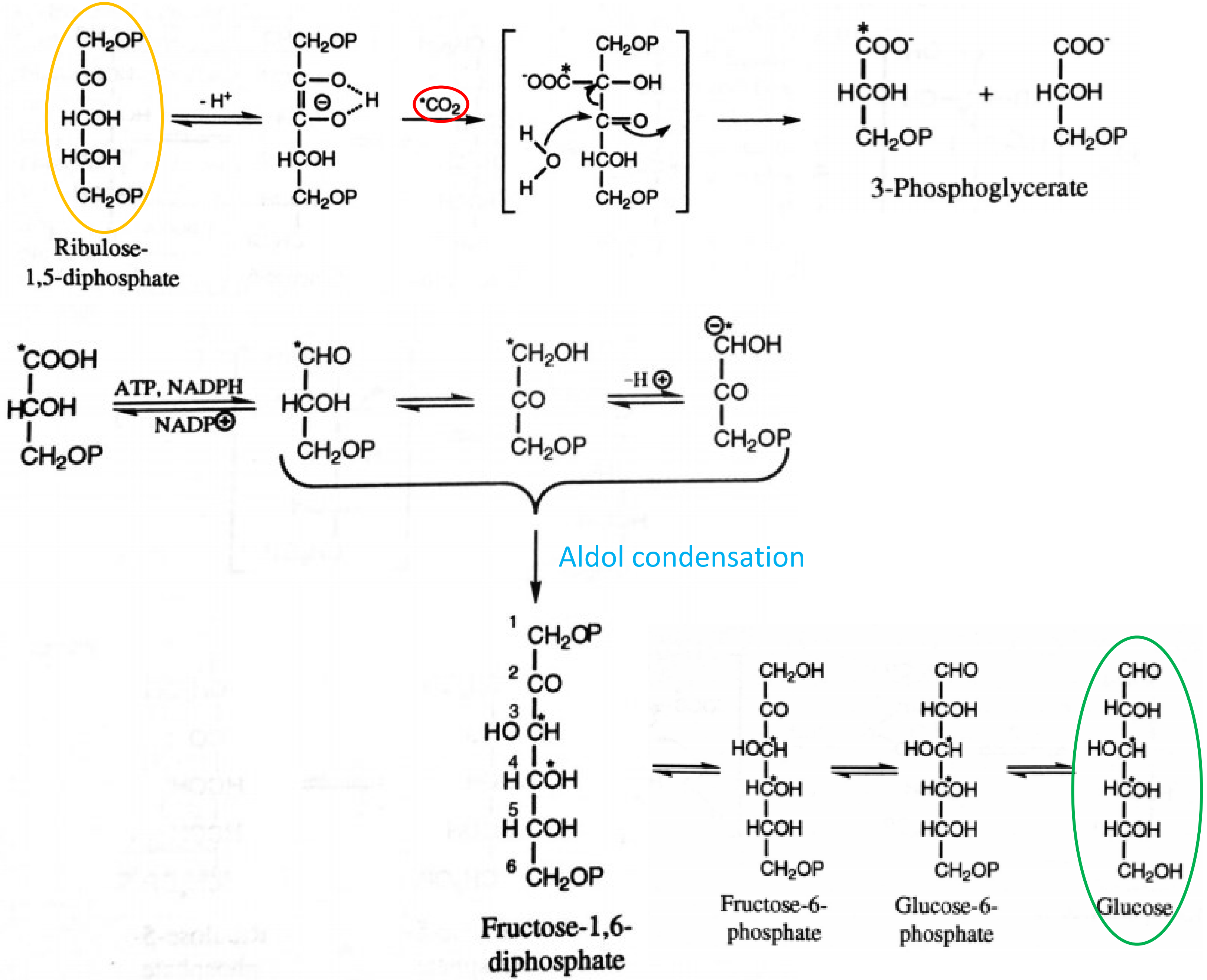
ATP will be used to phosphorylate some groups, making them more reactive.

The oxygen here comes from water, and not CO_2.

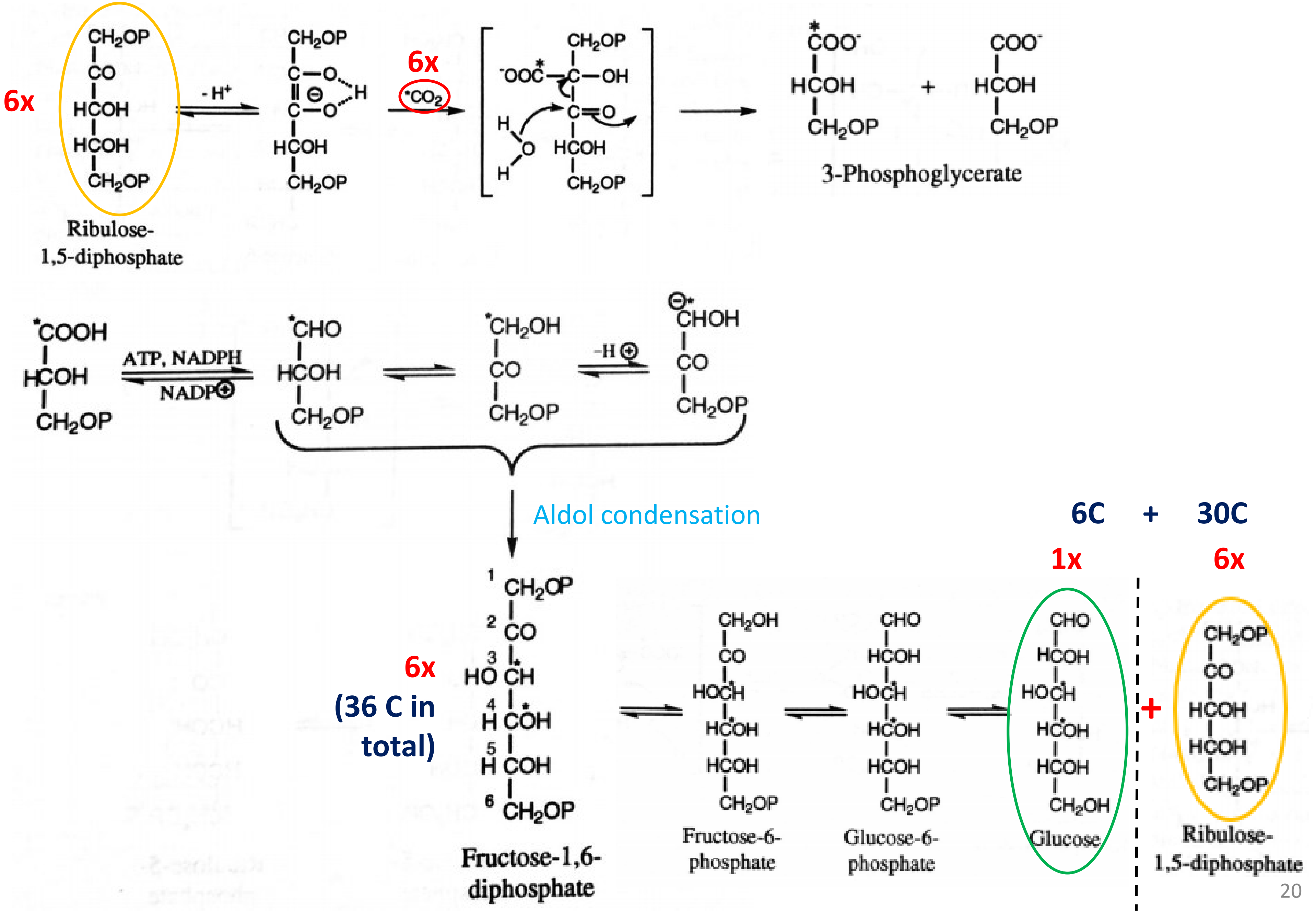
$$2H_2O \xrightarrow{h\nu} 4\,H^+ + 4e^- + O_2$$

- 2- Dark reaction: CO_2 is reduced with the help of NADPH and ATP.
  - Very complex mechanism





Aldol condensation


6x
Ribulose-1,5-diphosphate
- H⁺
6x
*CO₂
3-Phosphoglycerate
ATP, NADPH
NADP⊕
-H⊕
Aldol condensation
6x
(36 C in total)
Fructose-1,6-diphosphate
Fructose-6-phosphate
Glucose-6-phosphate
6C + 30C
1x
6x
Glucose
+
Ribulose-1,5-diphosphate

Carbohydrate biosynthesis in plants: Photosynthesis

- 1- Light reaction

$$H_2O + NADP^+ + P + ADP \xrightarrow{h\nu} {}^1\!/_2O_2 + \underset{\text{reducing agent}}{NADPH} + \overset{\text{energy rich}}{ATP} + H^+$$

ATP will be used to phosphorylate some groups, making them more reactive.

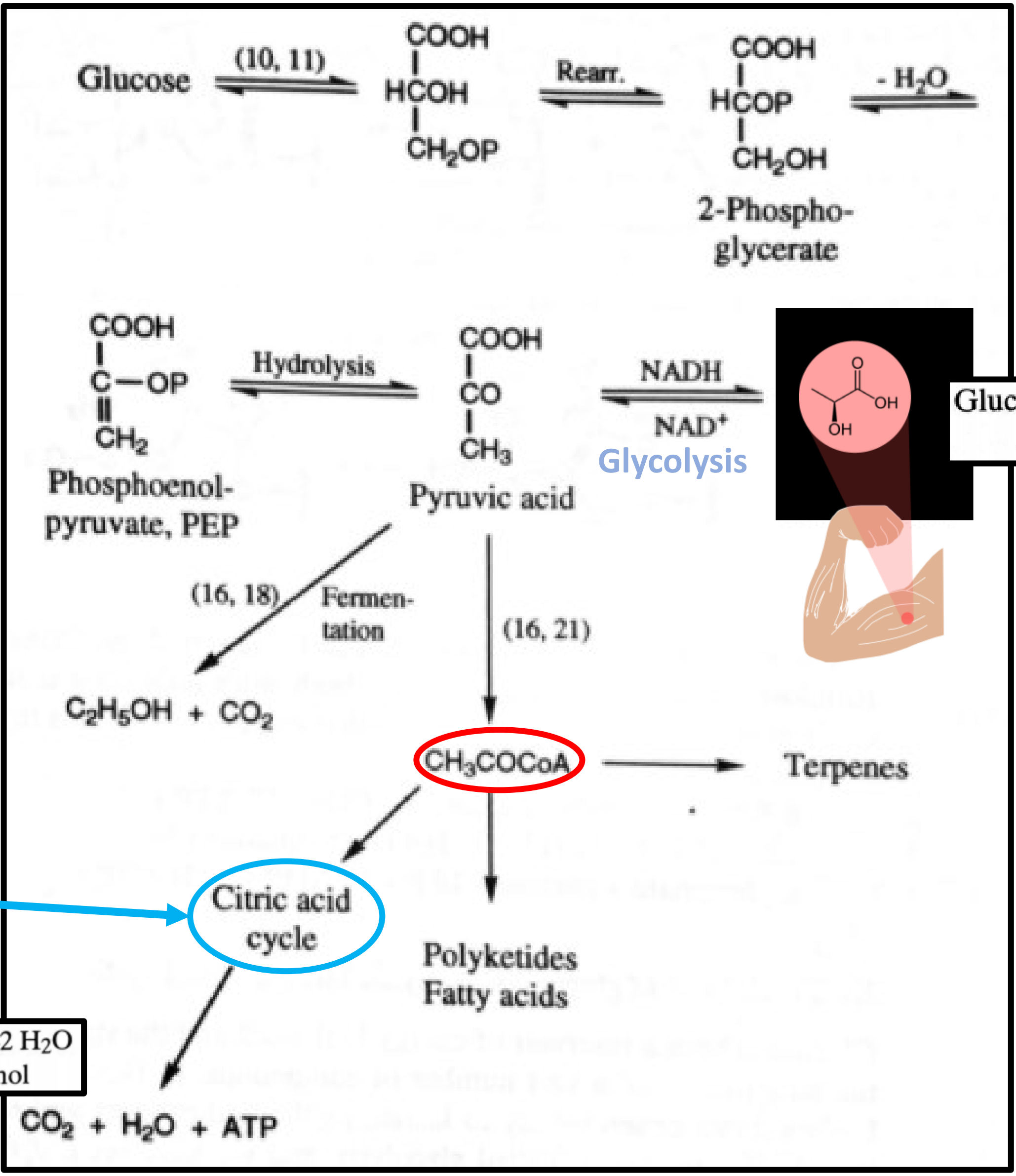
The oxygen he r, and not CO_2.

$$2H_2O \xrightarrow{h\nu} 4\,H^+ + 4e^- + O_2$$

- 2- Dark reactio DPH and ATP.

$$6\ \text{Ribulose-1,5-diphosphate} + 6\ CO_2 + 18\ ATP + 12\ NADPH + 12\ H^{\oplus} + 12\ H_2O \rightarrow 6\ \text{ribulose-1,5-diphosphate} + \text{glucose} + 18\ P + 18\ ADP + 12\ NADP^{\oplus}$$

Breakdown of glucose

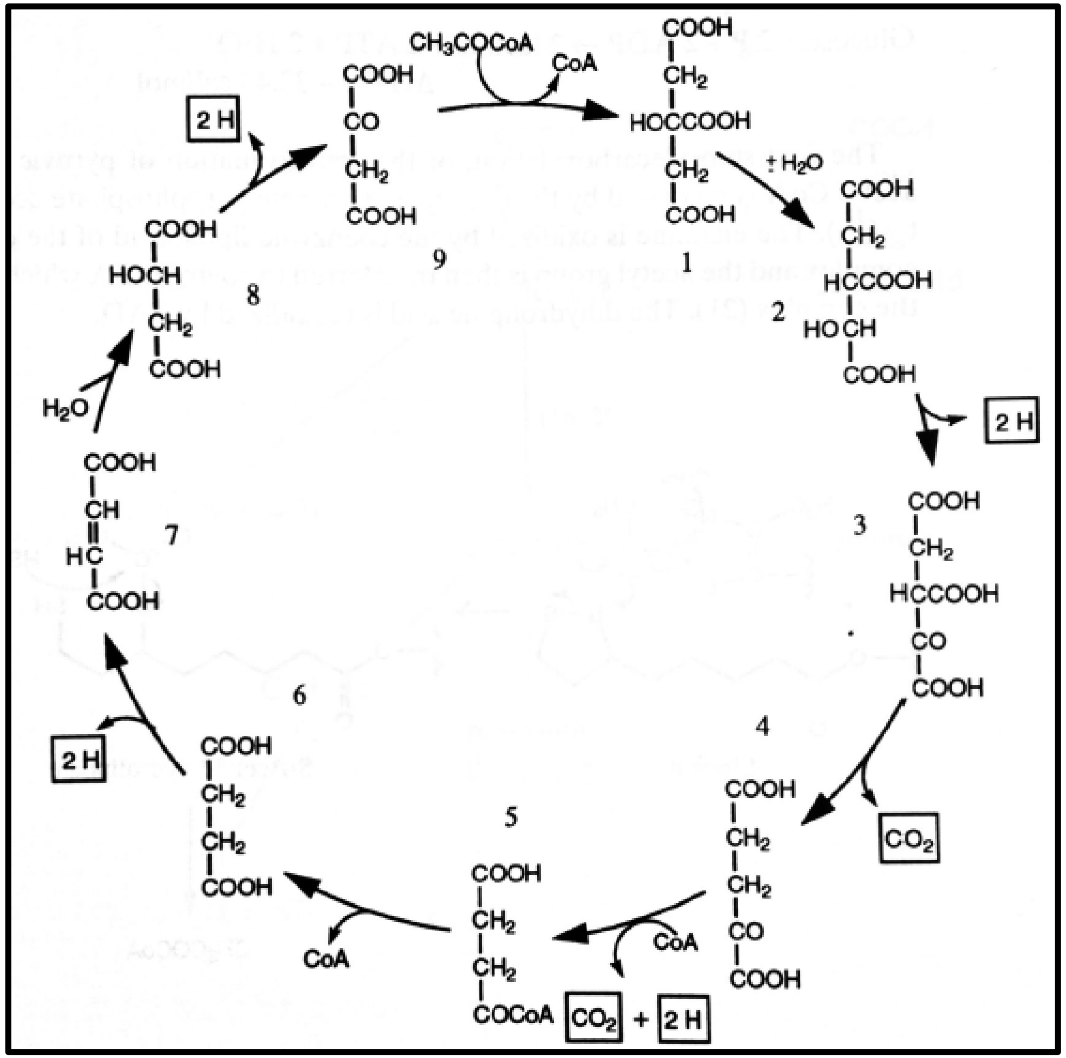


$$\text{Glucose} + 2\ \text{P} + 2\ \text{ADP} \rightarrow 2\ \text{lactate} + 2\ \text{ATP} + 2\ H_2O$$
$$\Delta G^{O'} = -32.4\ \text{kcal/mol}$$

Coupled with oxidative phosphorylation, the citric acid cycle is the essence of respiration

$$\text{Glucose} + 6\ O_2 + 36\ \text{P} + 36\ \text{ADP} \rightarrow 6\ CO_2 + 36\ \text{ATP} + 42\ H_2O$$
$$\Delta G^{O'} = -423\ \text{kcal/mol}$$

The citric acid cycle

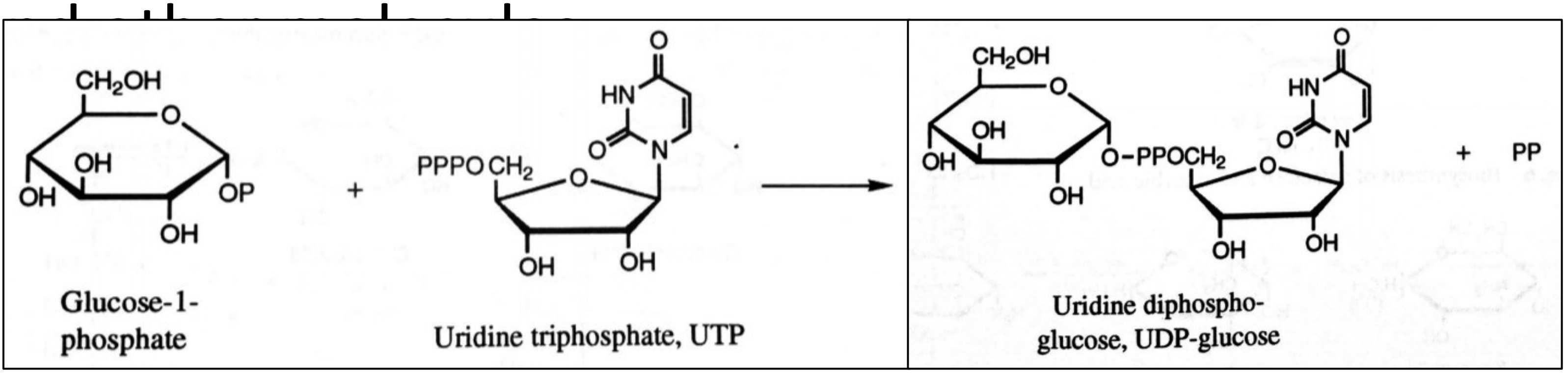


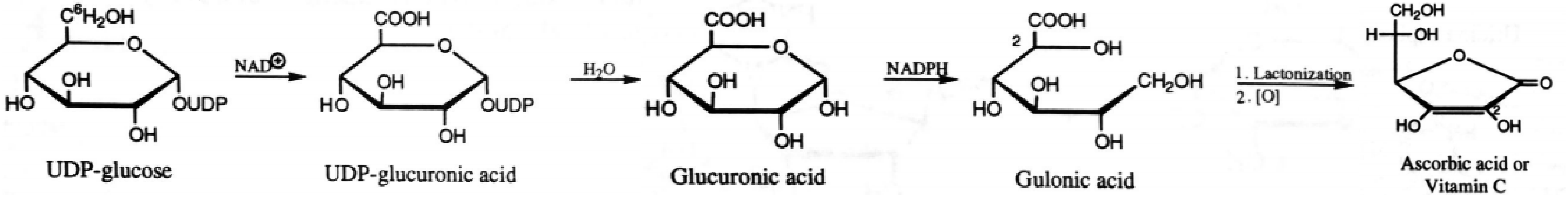
- 1 molecule of acetyl CoA is consumed.
- 8 H and 2 CO_2 are released.
- The 8 H are oxidized to water with production of free energy and ATP

- The citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant.
- It removes electrons from acetyl CoA and uses these electrons to form NADH and $FADH_2$, which will be reoxidized in oxidative phosphorylation.

Formation of monosaccharides

- Glucose is the precursor of other hexoses, disaccharides, polysaccharides, glycosides a





Example: Biosynthesis of vitamin C

Disaccharides and polysaccharides

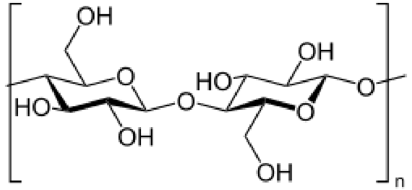
- Disaccharides:
  - 2 monosaccharides joined by an acetal or ketal link
  - Classified according to their reducing power
    - Non-reducing: The carbonyl function is blocked as acetal (for instance sucrose)
    - Reducing: For instance lactose

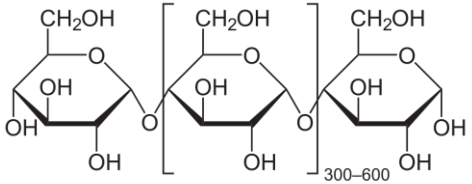
sucrose

lactose

◇ Polysaccharides:

◇Long chain of linear or branched monosaccharides, used as storage (starch,





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Chapter 4-1
The Shikimic Acid Pathway

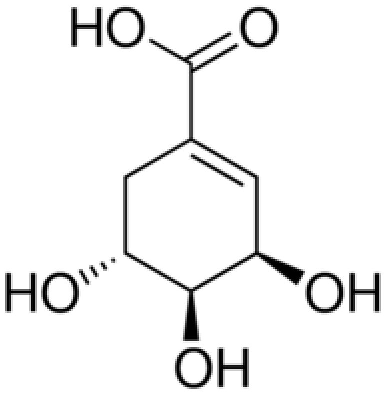
Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

Spring 2022

Shikimic acid

Shikimic acid could replace the aromatic amino acids in *E.coli* mutants

- It has to be an intermediate in their biosynthesis



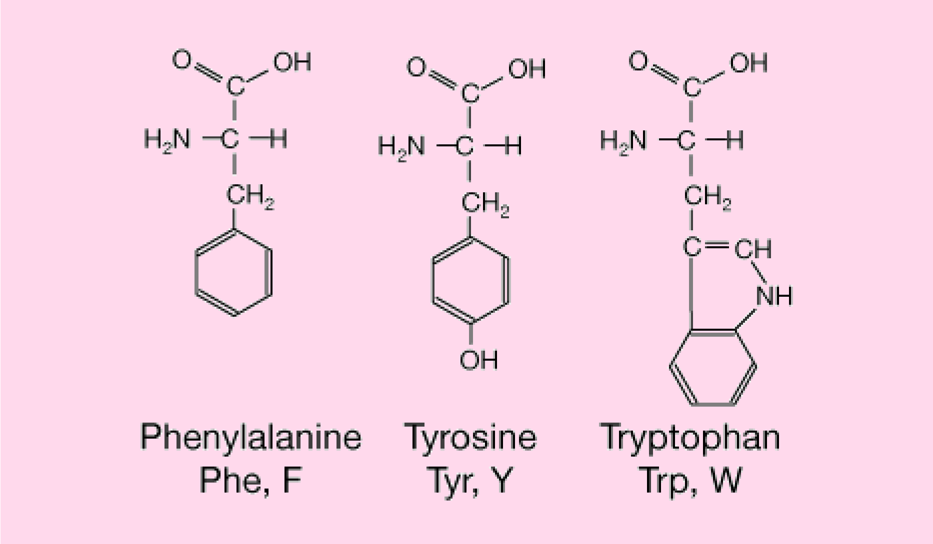
Shikimic acid

Illicium anisatum

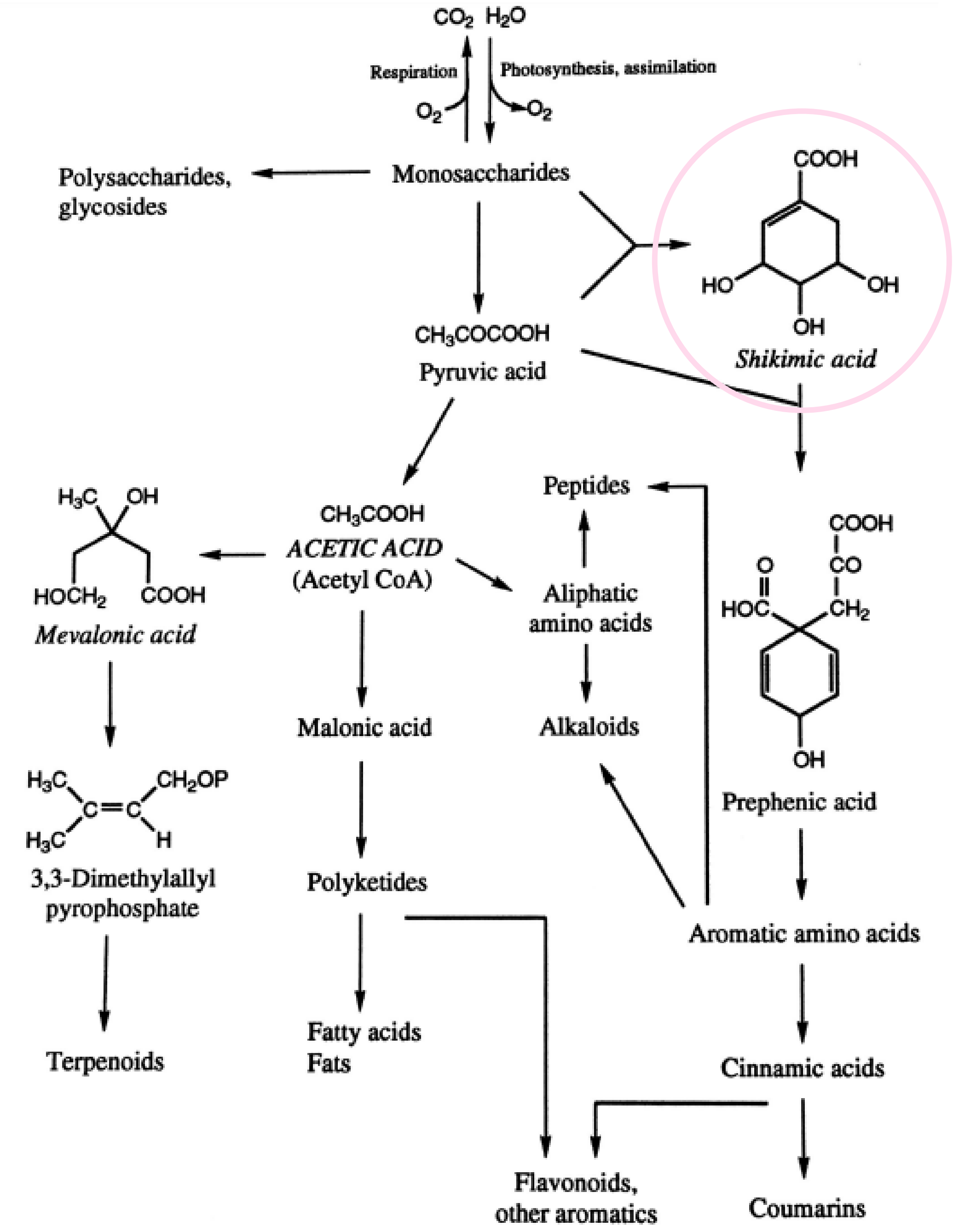
Shikimic acid was first isolated from japanese star anise, which is where it got its name.

Shikimic acid pathway

Biosynthesis of aromatic amino acids



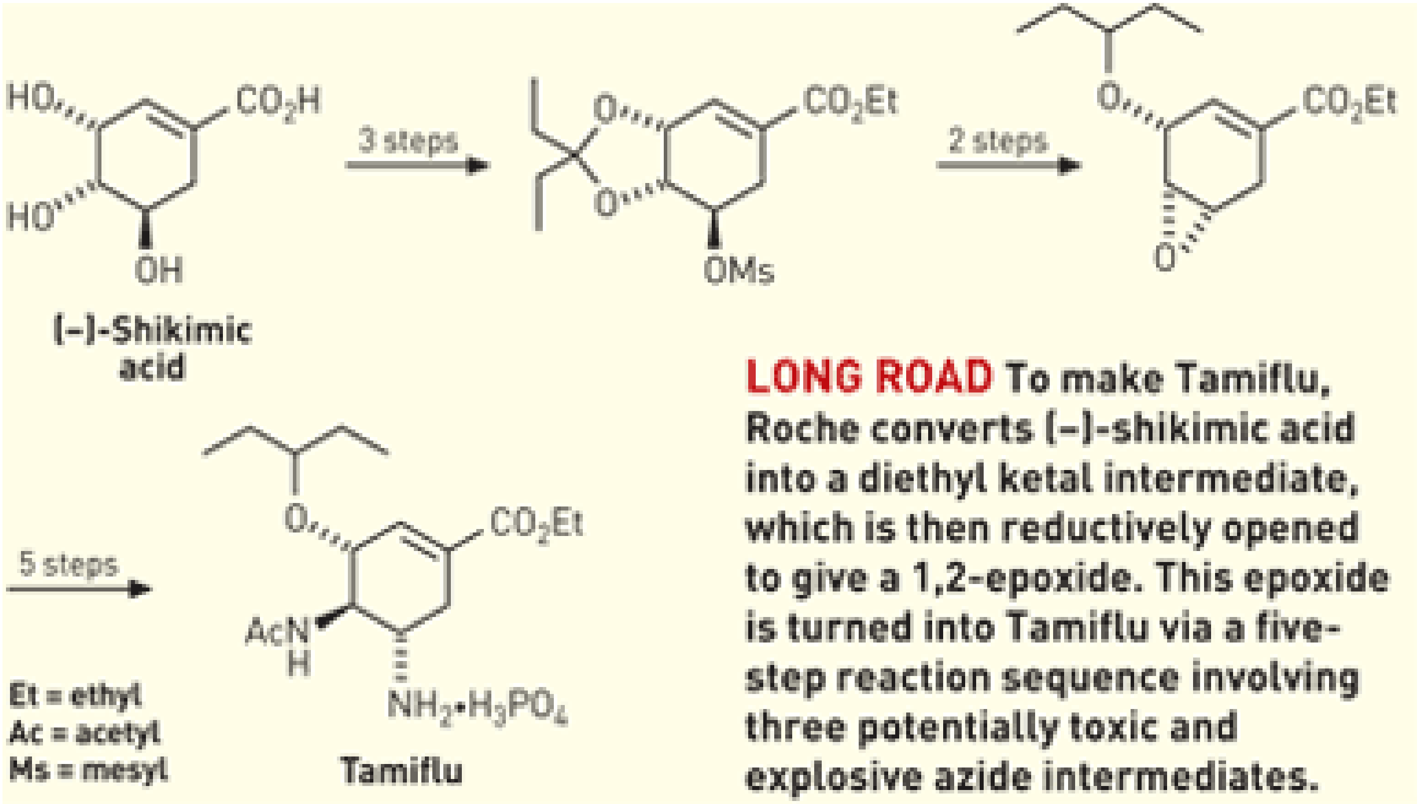
This biosynthetic pathway is present in bacteria, funghi and higher plants – but not in mammals.



Tamiflu

Tamiflu = oseltamivir phosphate

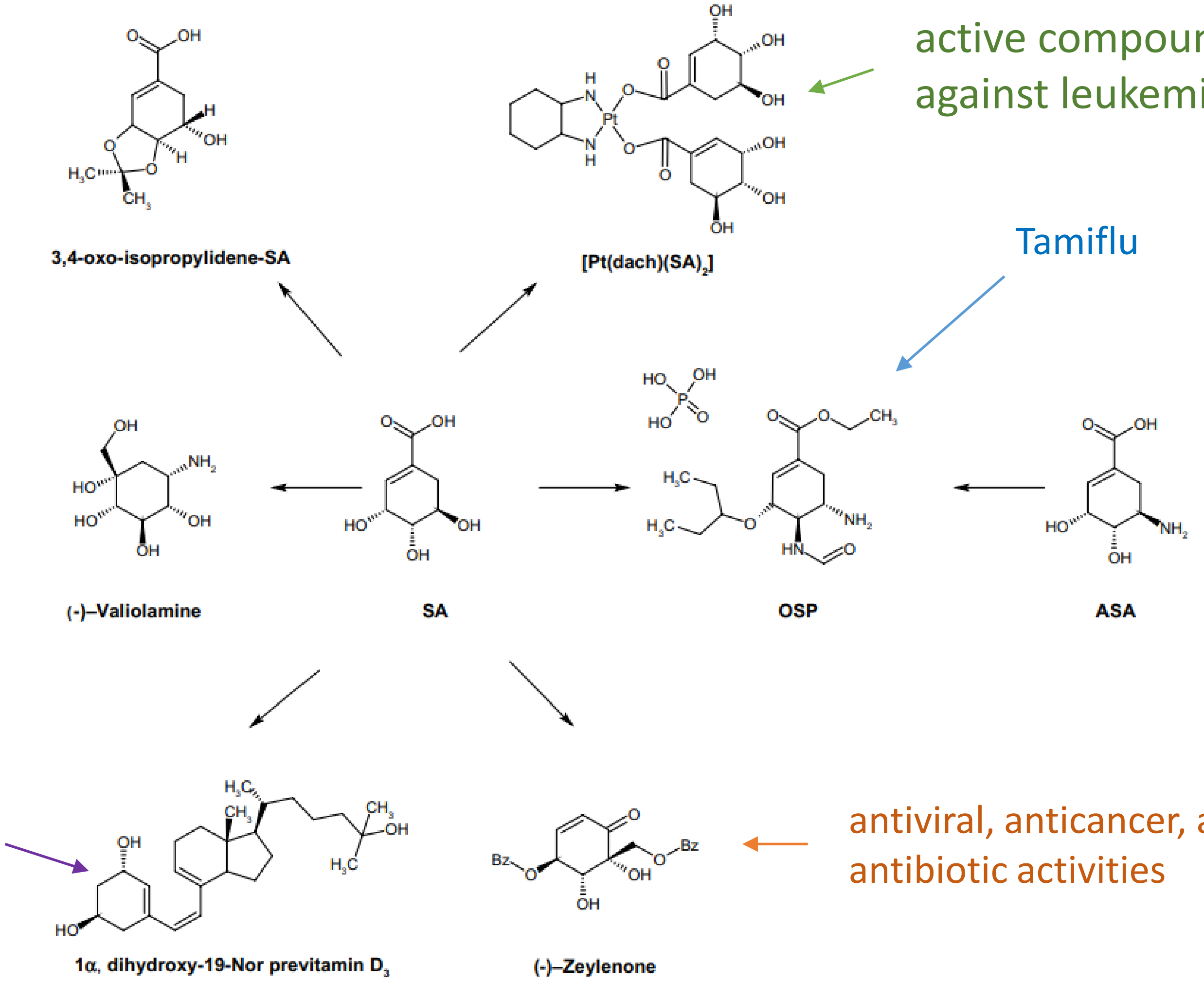
Important drug for bird flu (and other influenza viruses).



LONG ROAD To make Tamiflu, Roche converts (–)-shikimic acid into a diethyl ketal intermediate, which is then reductively opened to give a 1,2-epoxide. This epoxide is turned into Tamiflu via a five-step reaction sequence involving three potentially toxic and explosive azide intermediates.

Chem. & Eng. News Aug. 29, 2005, Vol 28, No 35, p 22

Pharmaceutical products derived from SA



active compound against leukemia

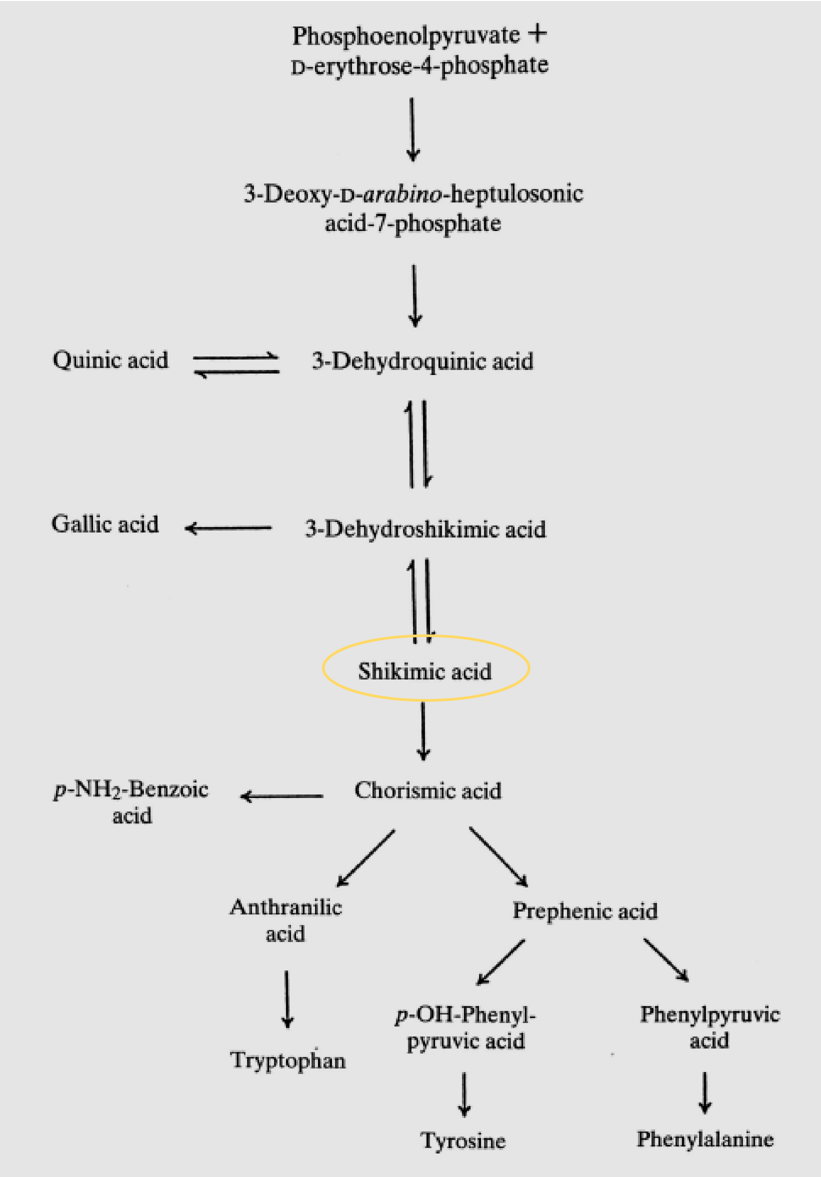
Tamiflu

promise for the treatment of osteoporosis

antiviral, anticancer, and antibiotic activities

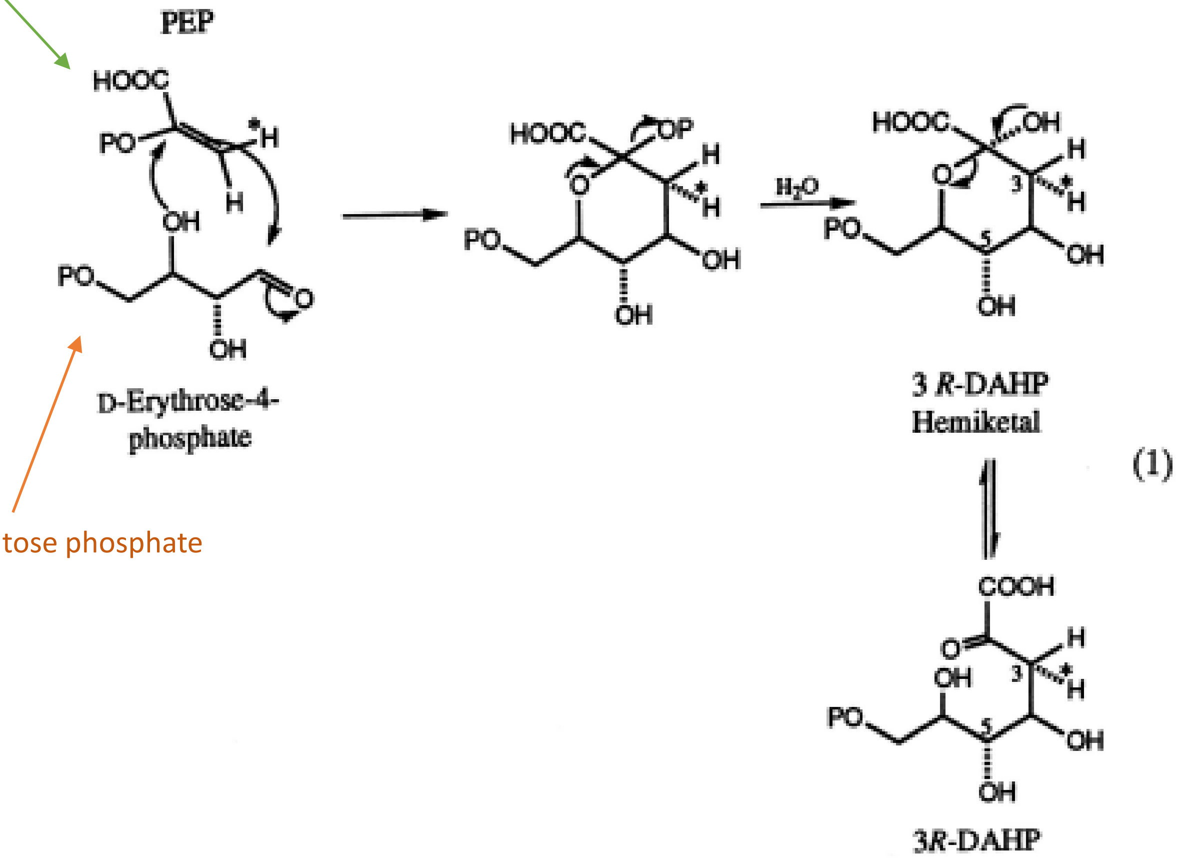
Díaz Quiroz *et al.* Annu Rep Med Chem 2014; 4:35-46

Shikimic acid pathway



Step 1:
Condensation of D-Erythrose-4-phosphate with phosphoenol pyruvate (PEP)

- Catalyzed by DAHP synthases
- Stereospecific reaction
  - Si face of PEP adds to Re face of erythrose-4-phosphate.
- Gives 3-deoxy-D-arabino-heptosulonic acid-7-phosphate (DAHP)



From glycolysis

From pentose phosphate pathway

3-deoxy-D-arabino-heptosulonic acid-7-phosphate

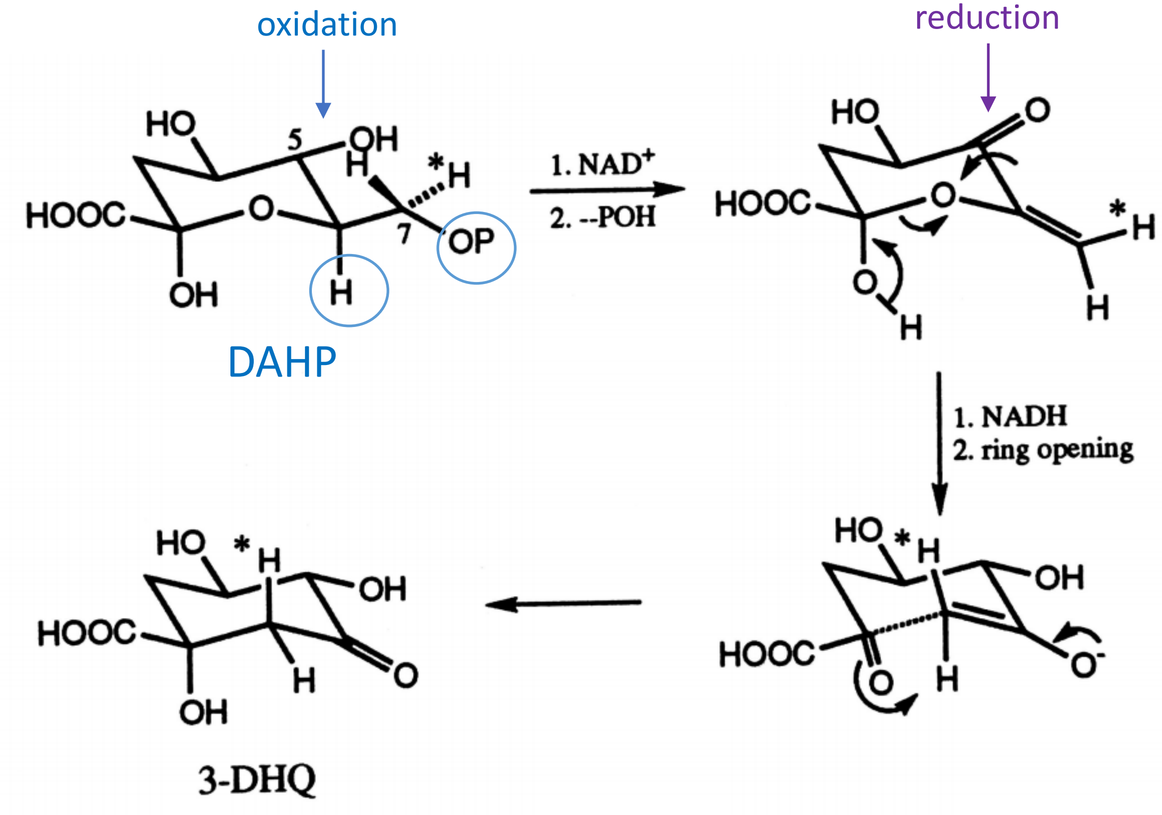
Step 2:
Formation of 3-dehydroquinic acid (DHQ)

- Catalyzed by DHQ synthase
- NAD+/NADH

α-β-elimination of phosphate group (cis elimination)
Oxidation at C5

Reduction at C5
Ring opening

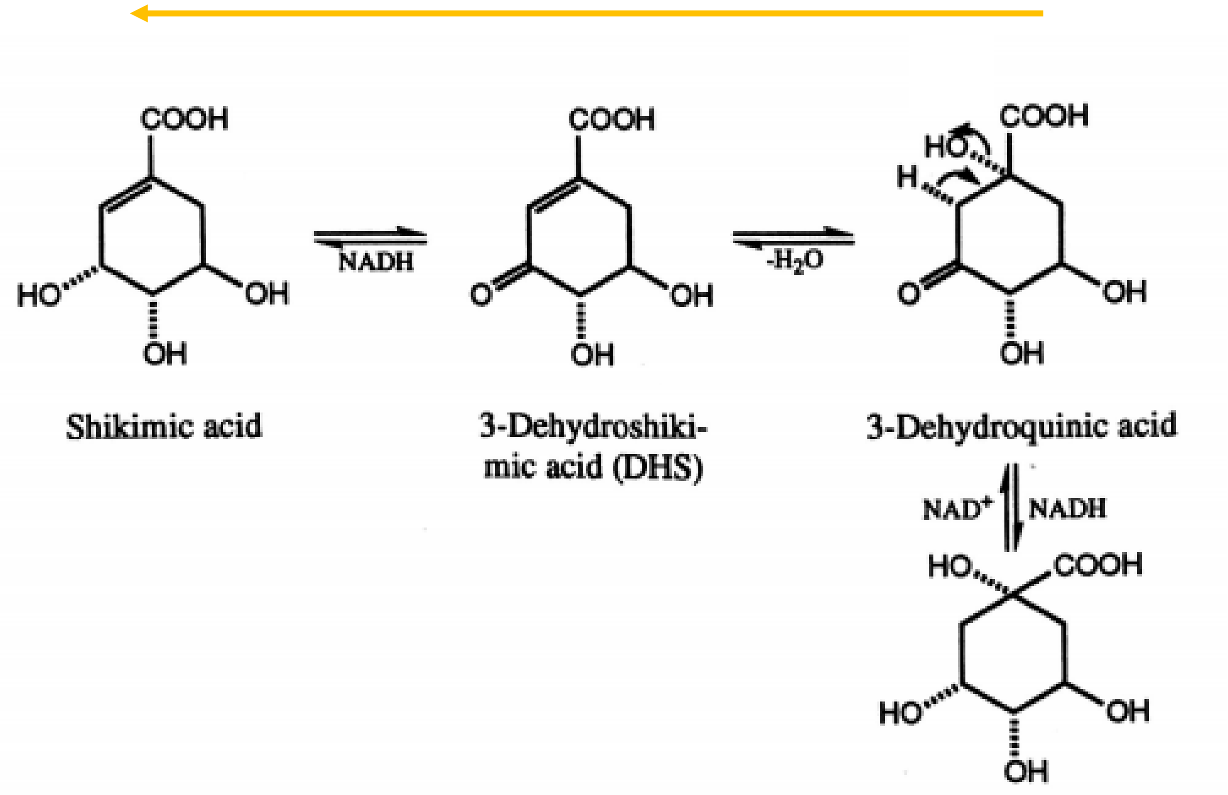
Ring closing forms 3-dehydroquinic acid.



Step 3:
Dehydration and reduction

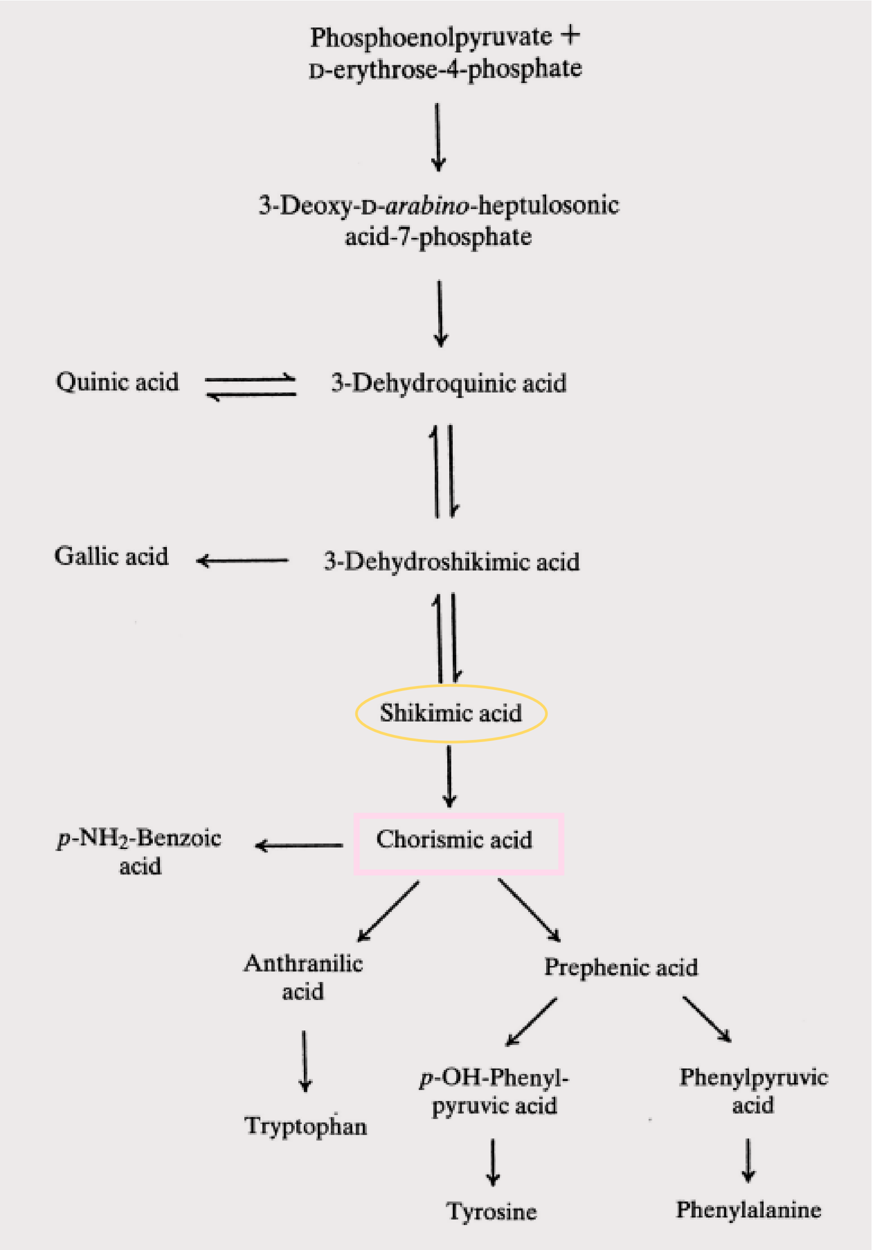
- Catalyzed by DHQ dehydratase and shikimic acid dehydrogenase

Quinic acid
Abundant in nature
Once it is formed it is not so easily metabolized
Except many microorganisms that can convert it back to DHQ



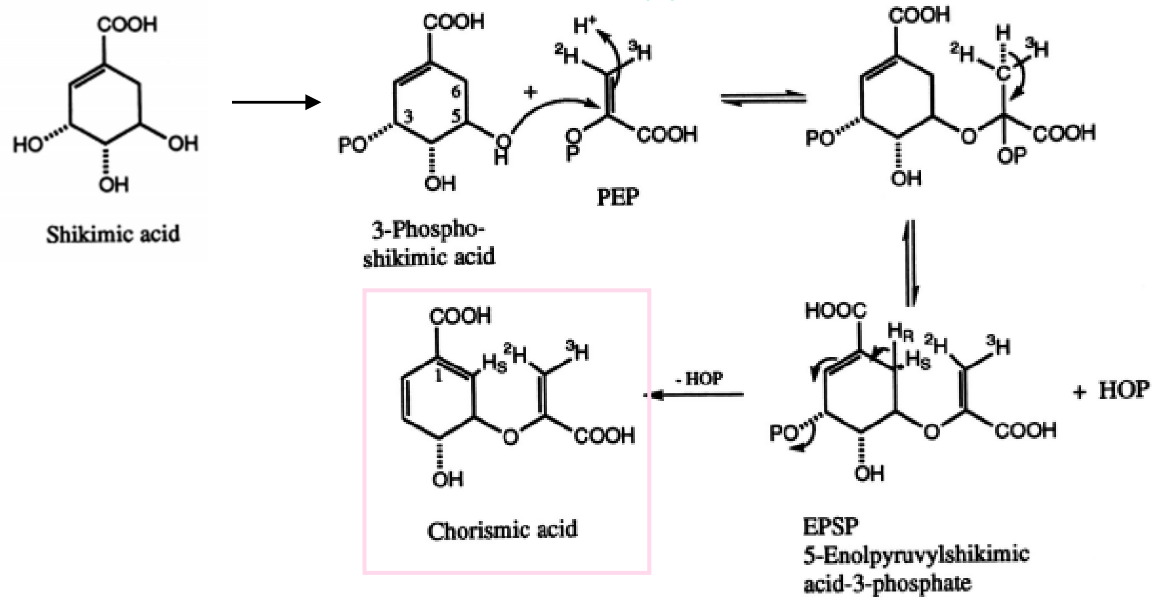
Quinic acid

Conversion of shikimic acid to chorismic acid



Phosphorylation
(creates a better LG for elimination that follows)

Addition of phosphoenol pyruvate (PEP)



Eliminations of phosphoric acid (HOP)

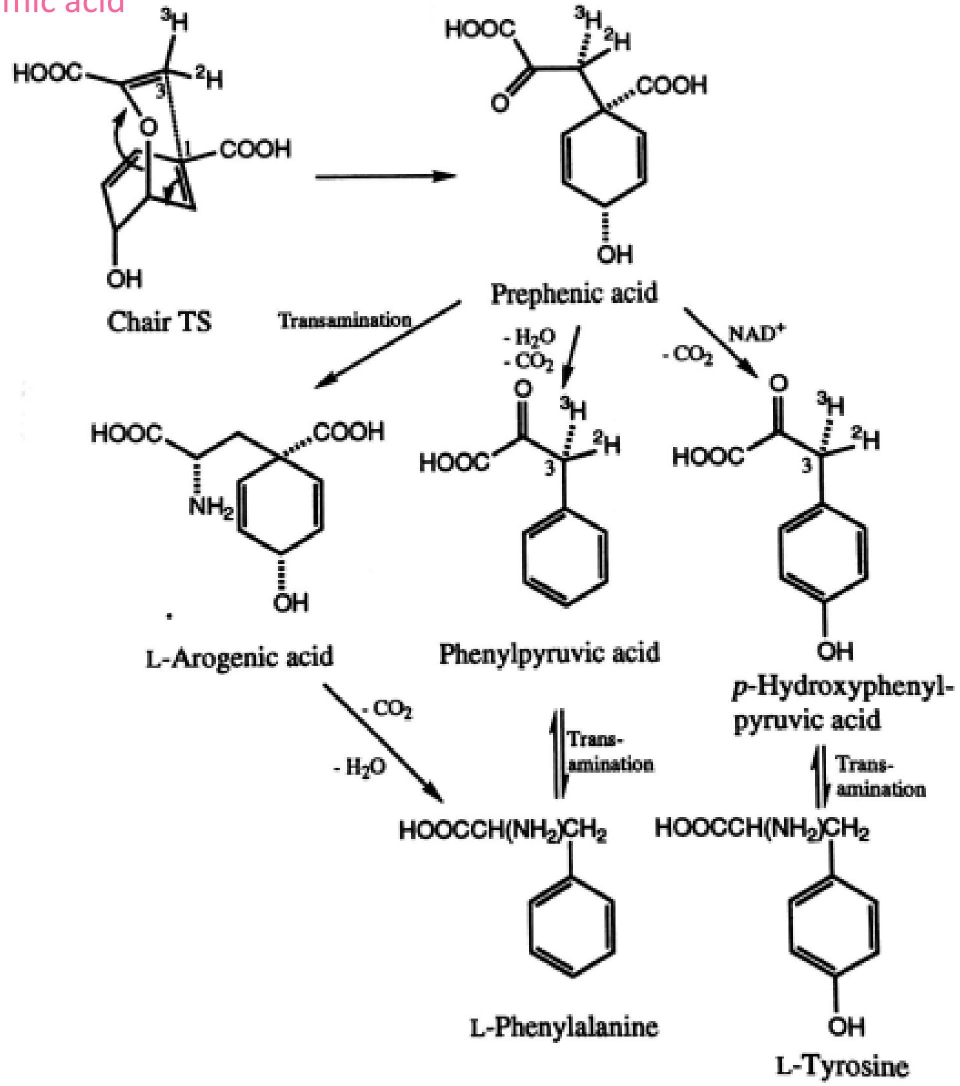
Biosynthesis of aromatic amino acids

Claisen rearrangement to prephenic acid

L-phenylalanine
Rapid *in vitro* conversion to phenylpyruvic acid
Transamination to phenylalanine

Some organisms can do transamination directly from prephenic acid

L-tyrosine
Conversion to *p*-hydroxyphenylpyruvic acid
Transamination to tyrosine

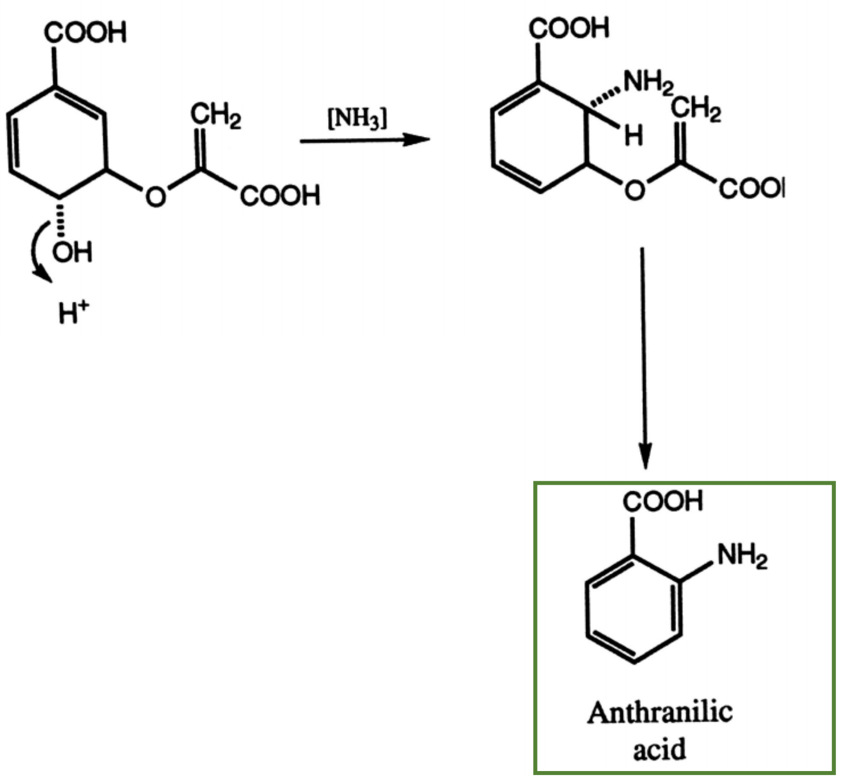


Chorismic acid

Biosynthesis of aromatic amino acids

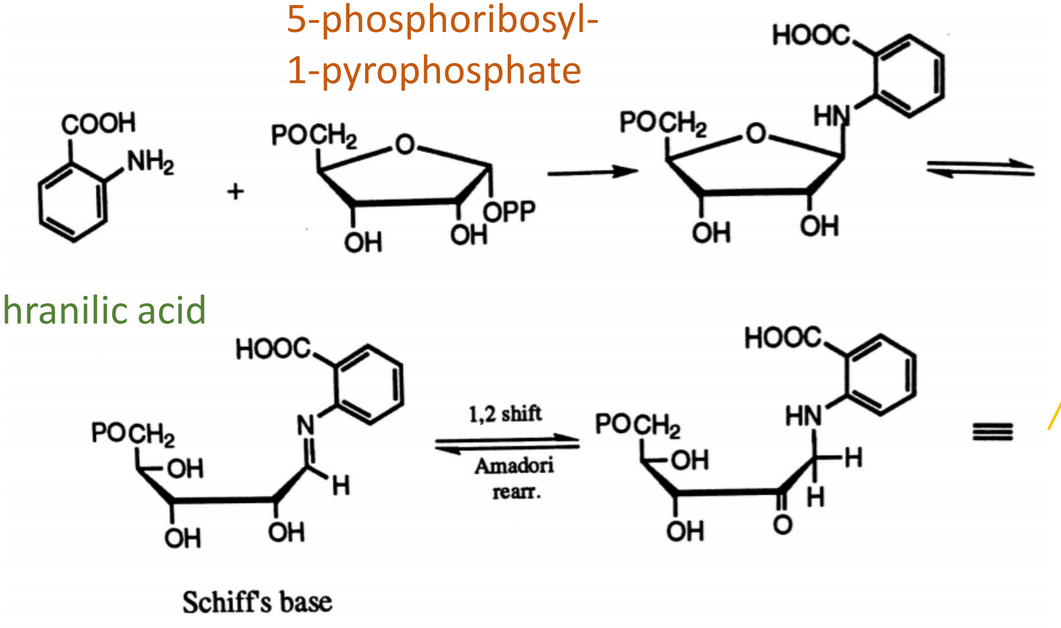
Conversion of chorismic acid to anthranilic acid:

L-tryptophan biosynthesis



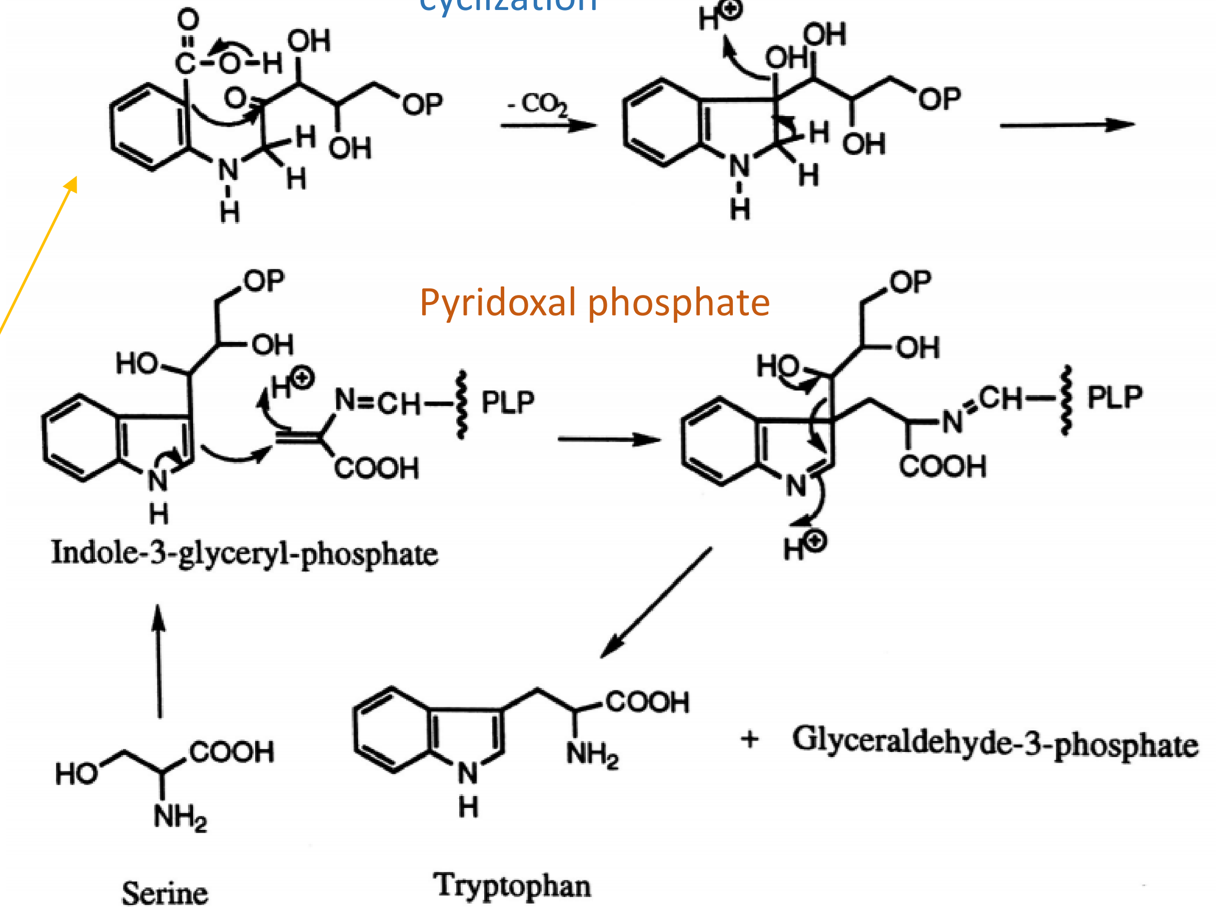
Biosynthesis of aromatic amino acids

L-tryptophan biosynthesis



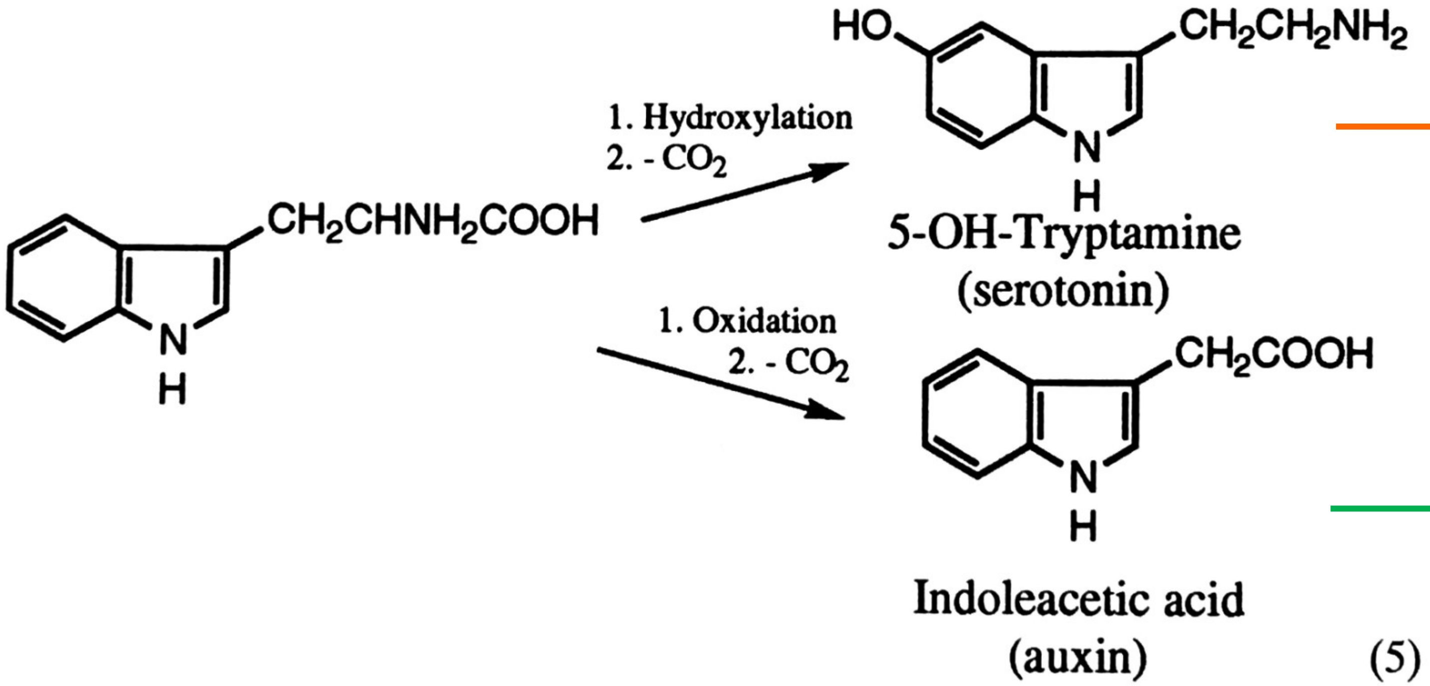
5-phosphoribosyl-1-pyrophosphate

anthranilic acid



cyclization

Pyridoxal phosphate



5-OH-Tryptamine
(serotonin)

Indoleacetic acid
(auxin)

(5)

Neurotransmitter

Plant growth hormone

Biological hydroxylation – redox reactions

- Oxidation and reduction of carbonyls

$$-CH_2OH \rightleftharpoons -CHO \rightleftharpoons -COOH$$

- Oxygen insertion into unactivated C-H bonds

Catalyzed by:

- Dehydrogenases (without molecular O_2)

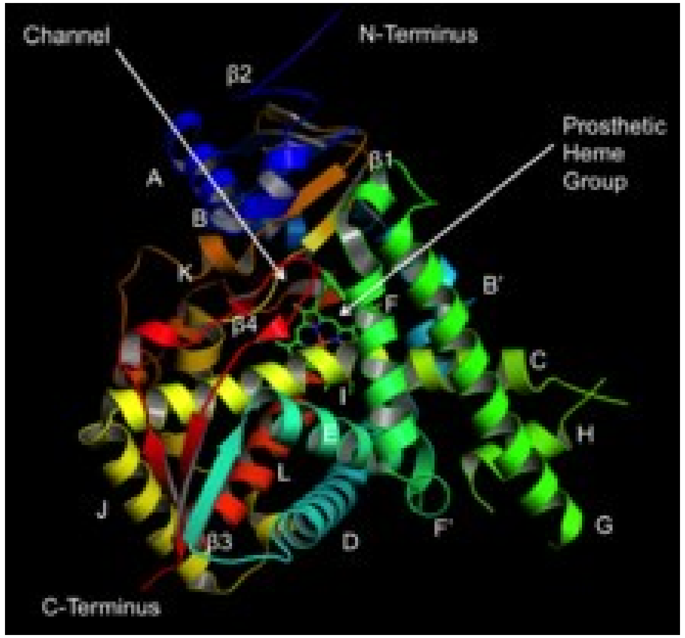
  $RH_2 + NAD^+ \leftrightarrow R + NADH + H^+$

- Oxidases (electron transfer to O_2)

  $RH_2 + O_2 \leftrightarrow R + H_2O_2$

- Oxygenases (incorporating molecular oxygen)

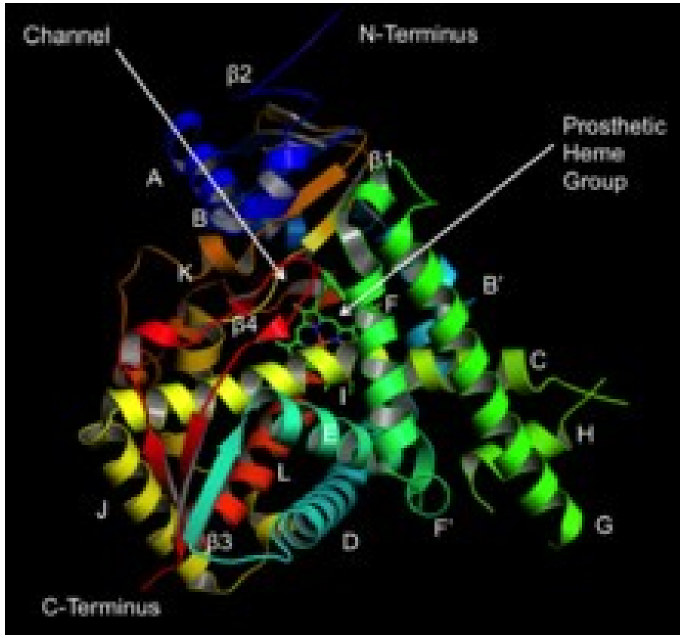
  $RH_2 + O_2 + NADPH + H^+ \rightarrow RHOH + H_2O + NADP^+$



Cytochrome P450s are monooxygenases

Biological hydroxylation

- Monooxygenases are especially important
  - Can oxidize non-activated hydrocarbons
    - Steroids
    - Fatty acid α-oxidation
    - Hydroxylation of aromatics
      - i.e. Conversion of phenylalanine to tyrosine
    - Oxidation of amino groups to nitro groups
    - Dealkylation of amines, ethers, thioesters
    - etc.



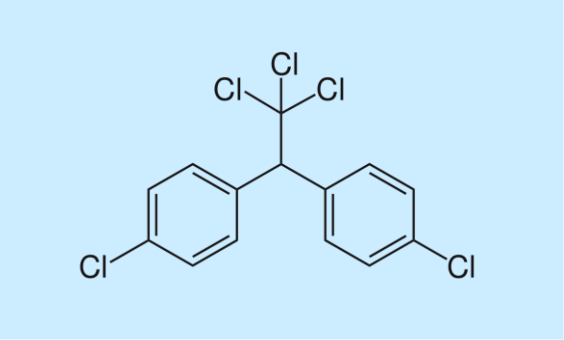
Cytochrome P450s are monooxygenases

Biological hydroxylation

- Drug metabolism
  - Oxidizing foreign substances make them more polar, easier to excrete

Example: DDT is hard to oxidize

- Accumulated in fat cells – dangerous!
- DDT is a pesticide – banned in the 70s



Dichlorodiphenyl-trichloroethane (DDT)

Spraying DDT in Oregon, USA, 1955

ENVIRONMENT

Malaria Control

Resurging use of the banned pesticide DDT to prevent malaria poses dilemma for health, environment

by ***Bette Hileman***

JULY 24, 2006 | APPEARED IN **VOLUME 84, ISSUE 30**

Late last year, the U.S. Agency for International Development (**USAID**), after a long hiatus, announced that it would fund DDT spraying on the inside walls of houses to prevent malaria. For many years, USAID had supported the DDT spraying, but after the pesticide was banned in developed countries, USAID stopped funding its use.

Spraying DDT on interior walls presents a dilemma. It may be the cheapest, most effective way to reduce malaria deaths in some parts of Africa. But the DDT intended for interior spraying may end up on crops, endangering wildlife and beneficial insects. Also, new evidence indicates that prenatal exposure to DDT may retard child development and lead to preterm birth.

Beginning in 1945, DDT was used extensively to eradicate malaria-carrying mosquitoes from all of southern Europe and the southern U.S. It was also widely employed for malaria control in Asia, Latin America, and Africa. By 1966, according to the **U.S. National Academy of Sciences**, DDT had saved 500 million lives.

DDT was banned in the developed world in the early 1970s because of its environmental effects. Thereafter, DDT spraying for mosquito control ceased in most of Africa, though its use was continued relatively unnoticed in South Africa, Botswana, Indonesia, and India. Declining foreign aid budgets also contributed to dwindling efforts to control mosquitoes with other insecticides or with alternative methods in less developed countries.

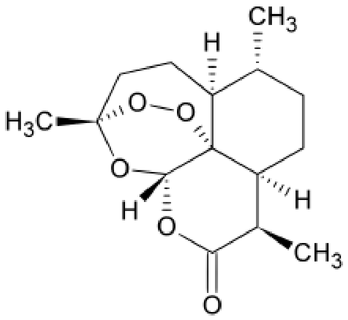
LINE OF DEFENSE

[+]Enlarge

Credit: USAID

Lambda-cyhalothrin insecticide is being sprayed on the walls of a house in Angola to prevent malaria. Spraying in Angola started last December.

> ”Malaria parasites have become resistant to the commonly used, inexpensive drugs chloroquine and sulfadoxine-pyrimethamine. The newer, more effective artemisinin drugs are prohibitively expensive for poor African patients.”



Artemisinin

Chem & Eng News, July 24, 2006, Volume 84, Number 30, pp. 30-31

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NTNU
Norwegian University of
Science and Technology

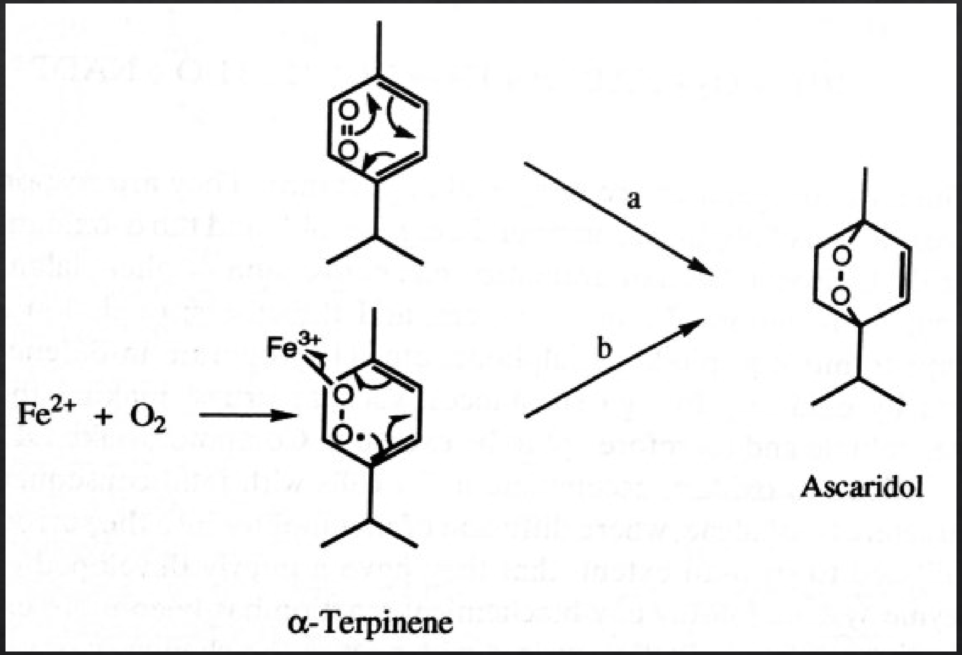
Chapter 4-2
The shikimic acid pathway

Elisabeth Jacobsen and Lucas Boquin, NTNU

Spring 2022

Oxidation in nature

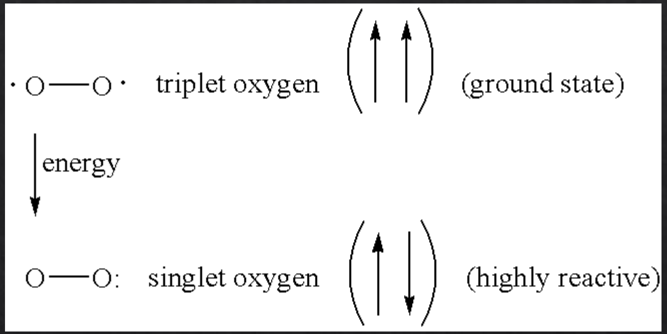
- Singlet oxygen $^1O_2$ is a short lived and very reactive molecule which is thought to participate in oxidations in nature.
- Triplet oxygen $^3O_2$ is nevertheless the most plausible oxidating agent.



Possible pathways to the biosynthesis of ascaridol from α-Terpinene

(a) Using $^1O_2$

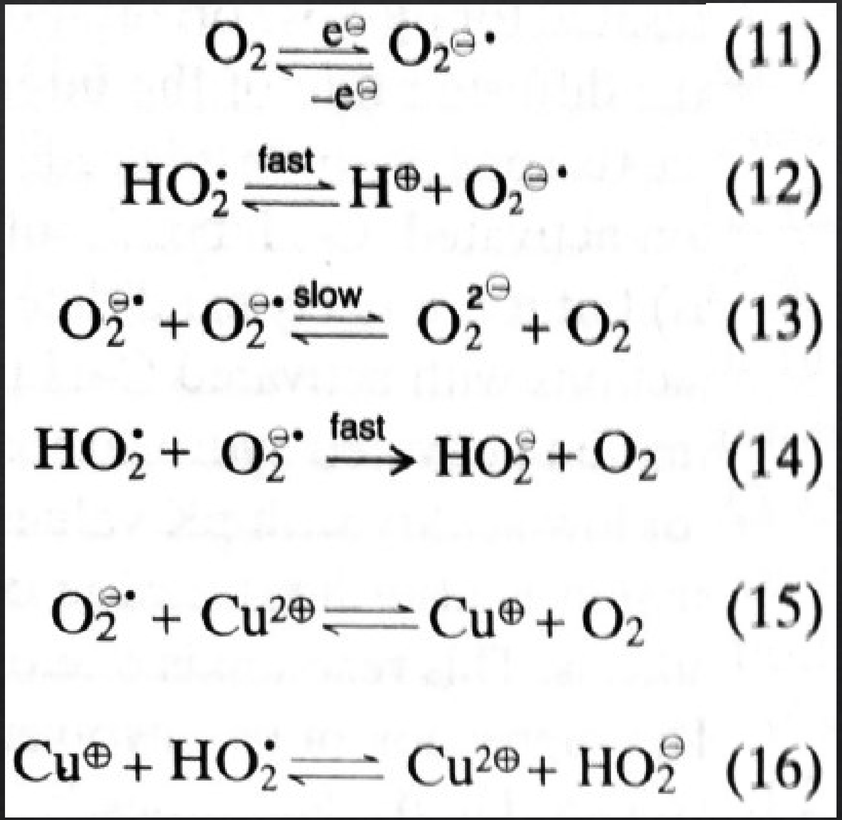
(b) Using $^3O_2$ (iron catalyzed)



Reduction of oxygen to water

(11) Formation of the superoxide radical ion (**not an oxidant**)
(12) Hydrogen peroxide radical formation (at physiological pH, this acidic form is barely present).
(13-16) Impurities and moisture cause a slow formation of peroxide and oxygen (electron transfer). This is accelerated with the protonated specie, and with copper ions.

$O_2^{-\cdot}$: Good nucleophile and mild reducing agent (ex: reaction with alkyl bromide)
$HO_2^{\cdot}$: Strong oxidant, hydrogen abstractor (on unsaturated hydrocarbons, or activated saturated hydrocarbons)



$$O_2 \underset{-e^{\ominus}}{\overset{e^{\ominus}}{\rightleftharpoons}} O_2^{\ominus\bullet} \quad (11)$$

$$HO_2^{\bullet} \overset{\text{fast}}{\rightleftharpoons} H^{\oplus} + O_2^{\ominus\bullet} \quad (12)$$

$$O_2^{\ominus\bullet} + O_2^{\ominus\bullet} \overset{\text{slow}}{\rightleftharpoons} O_2^{2\ominus} + O_2 \quad (13)$$

$$HO_2^{\bullet} + O_2^{\ominus\bullet} \xrightarrow{\text{fast}} HO_2^{\ominus} + O_2 \quad (14)$$

$$O_2^{\ominus\bullet} + Cu^{2\oplus} \rightleftharpoons Cu^{\oplus} + O_2 \quad (15)$$

$$Cu^{\oplus} + HO_2^{\bullet} \rightleftharpoons Cu^{2\oplus} + HO_2^{\ominus} \quad (16)$$

Involvments of $\mathbf{O_2^{-\cdot}}$ in biological hydroxylation

- Peroxide is often the first intermediate in aliphatic hydroxylation.
- Example: Biosynthesis of prostaglandins (very important molecules in animals, having diverse hormone-like effects in animals)
  - A radical is formed by hydrogen abstraction, leading to a cyclazation
  - A peroxide is formed, and a peroxidase allow the O-O cleavage.
  - Rearrangements lead to prostacyclin, thromboxane or $PGF_{2\alpha}$

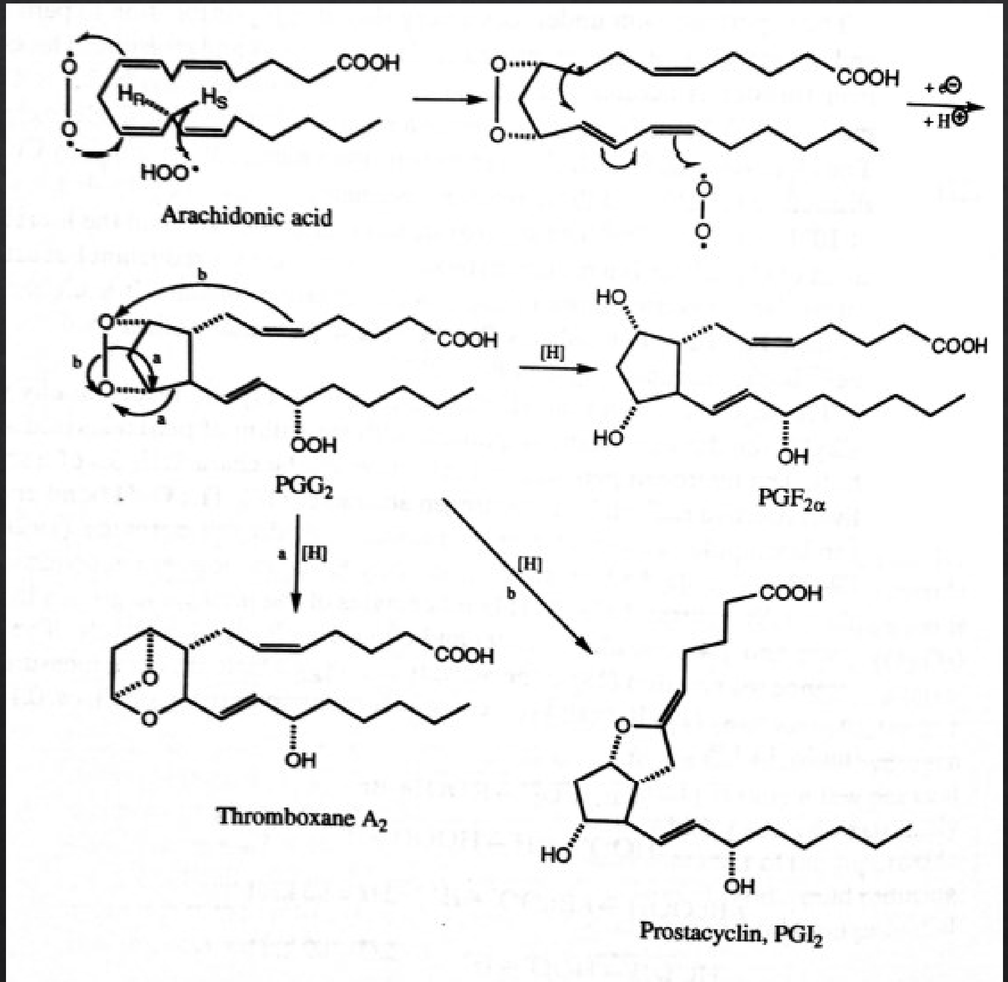
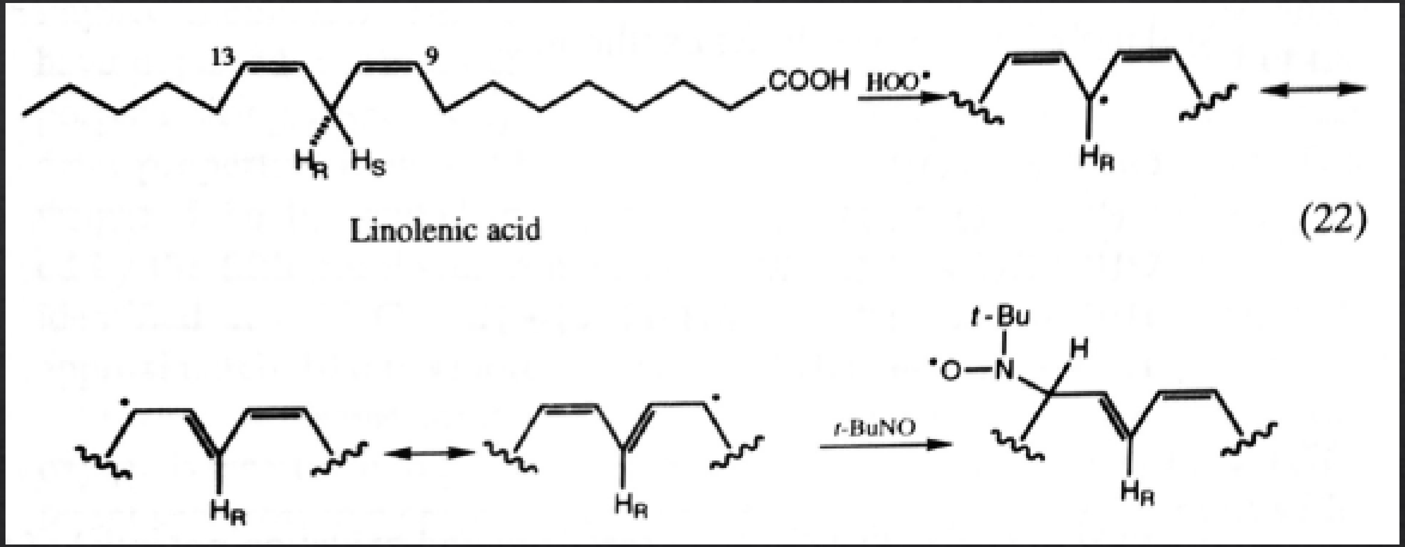


Fig. 5 Biosynthesis of prostaglandins and thromboxanes from arachidonic acid

How can we know that radicals are formed ?

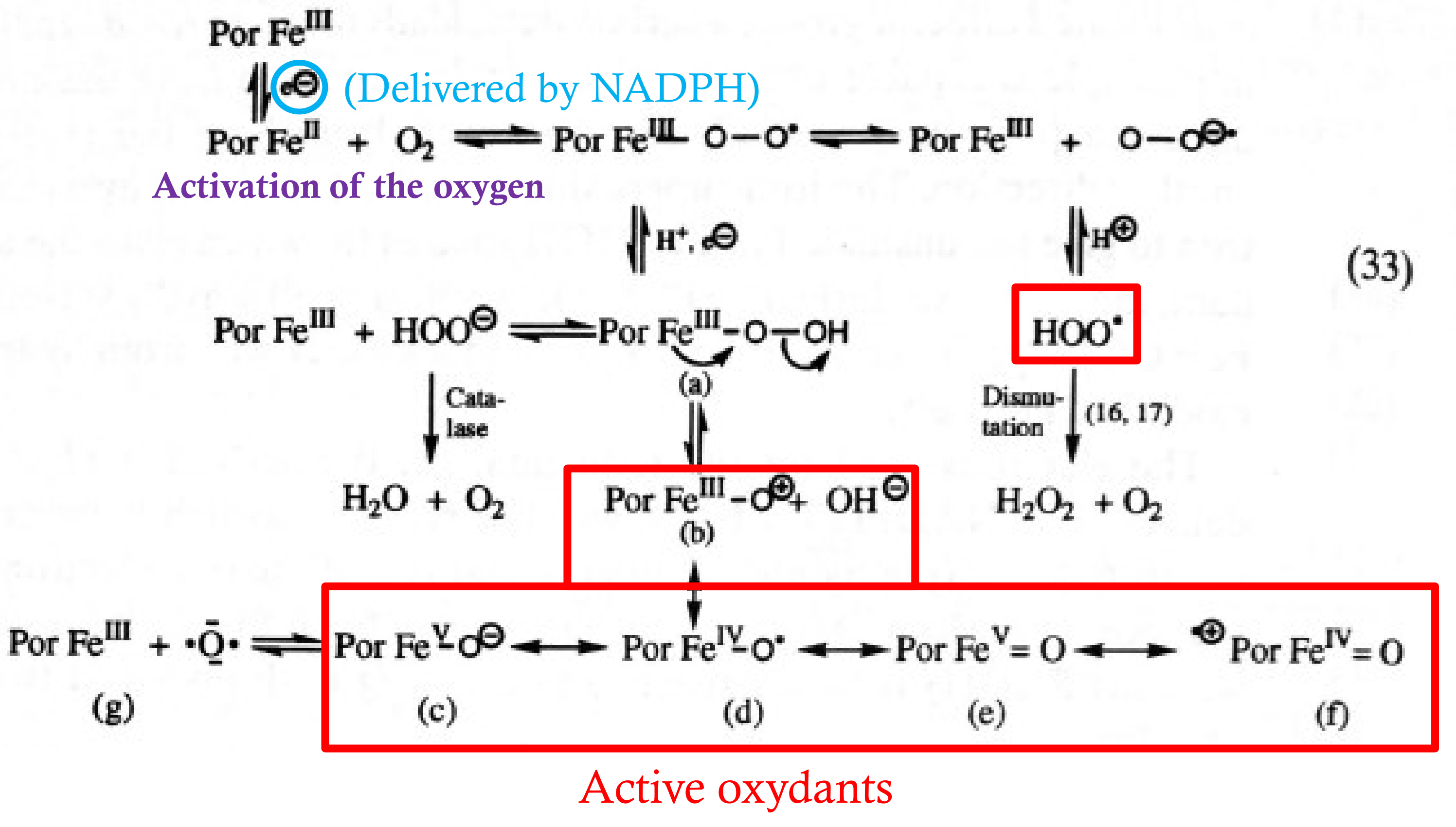


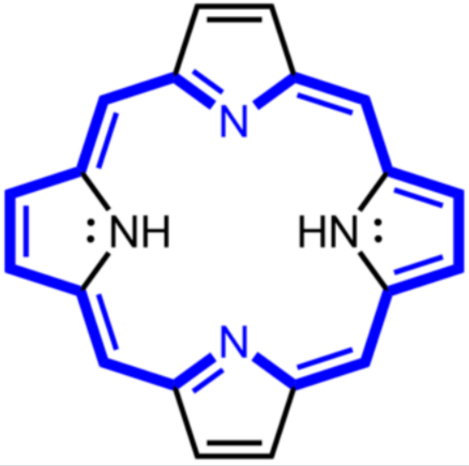
2-methyl-2-nitrosopropane is added as scavenger to a mixture of linolenic acid, oxygen and lipoxygenase.

It competes with oxygen for the radical.

Hydroxylation of non-activated aliphatics

- Iron-porphyrin complexes are playing a major role.





Exemple of porphyrin

$$\mathrm{PorFe^{IV}-O^{\bullet} + H_2O_2 \rightarrow PorFe^{IV}OH + HOO^{\bullet}}$$

$\mathrm{PorFe^{IV}}$ can break non-activated C-H bonds

Oxidation of aromatics

- Oxidation of aromatics happens during their detoxifying mechanism.
- The epoxide is just an intermediate, but can still be present in significant quantities, especially in some polycyclic aromatics.
- Epoxides of polycyclic aromatics display carcinogenic properties…

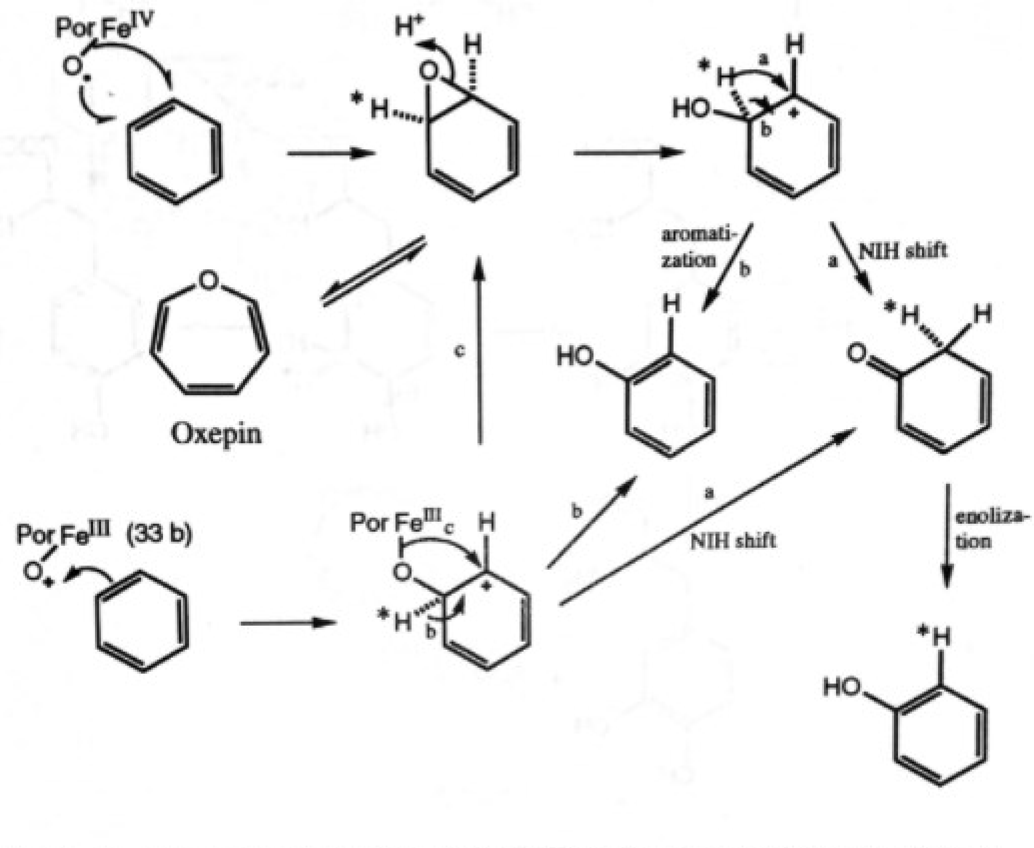
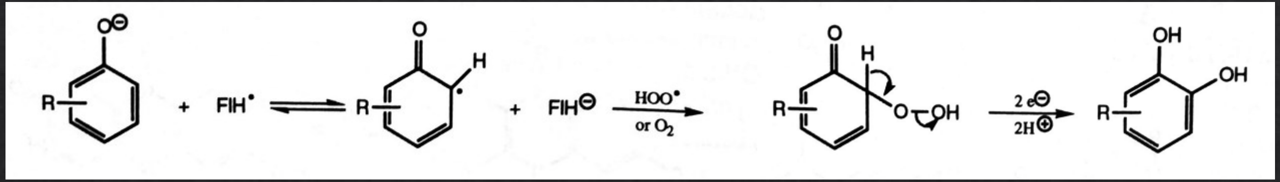


Fig. 8 Possible mechanistic pathways for P450 mediated aromatic hydroxylations

Flavin dependent oxygenases

- Flavins act as converters between 2 e^- oxidants (NAD^+), and 1 e^- oxidants ($PorFe^{3+}$)
- It is oxidized to its quinoid form by oxygen.
- The blue radical is also an active species in hydroxylation of phenols and indoles



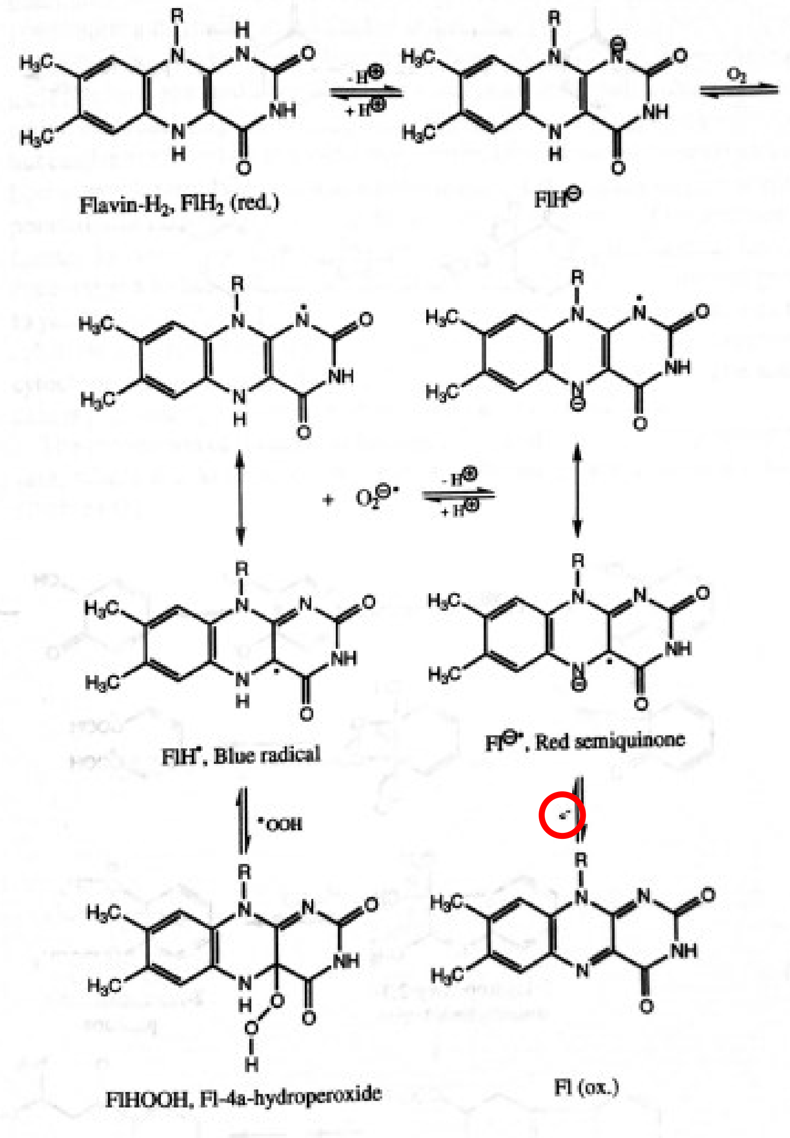


Fig. 11 Stepwise one-electron transfers; oxidation of flavin-H$_2$ by oxygen

Flavin dependent oxygenases

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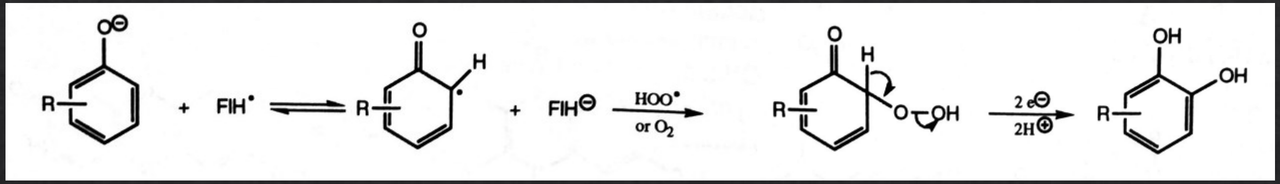
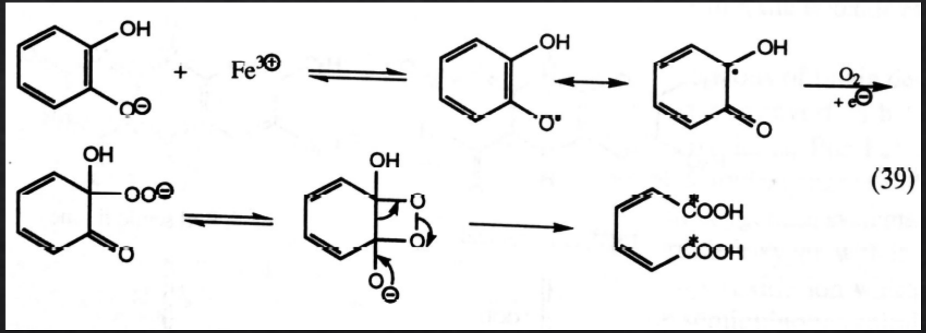


- This is part of the degradation of aromatics (phenol → catechol → ring opening):



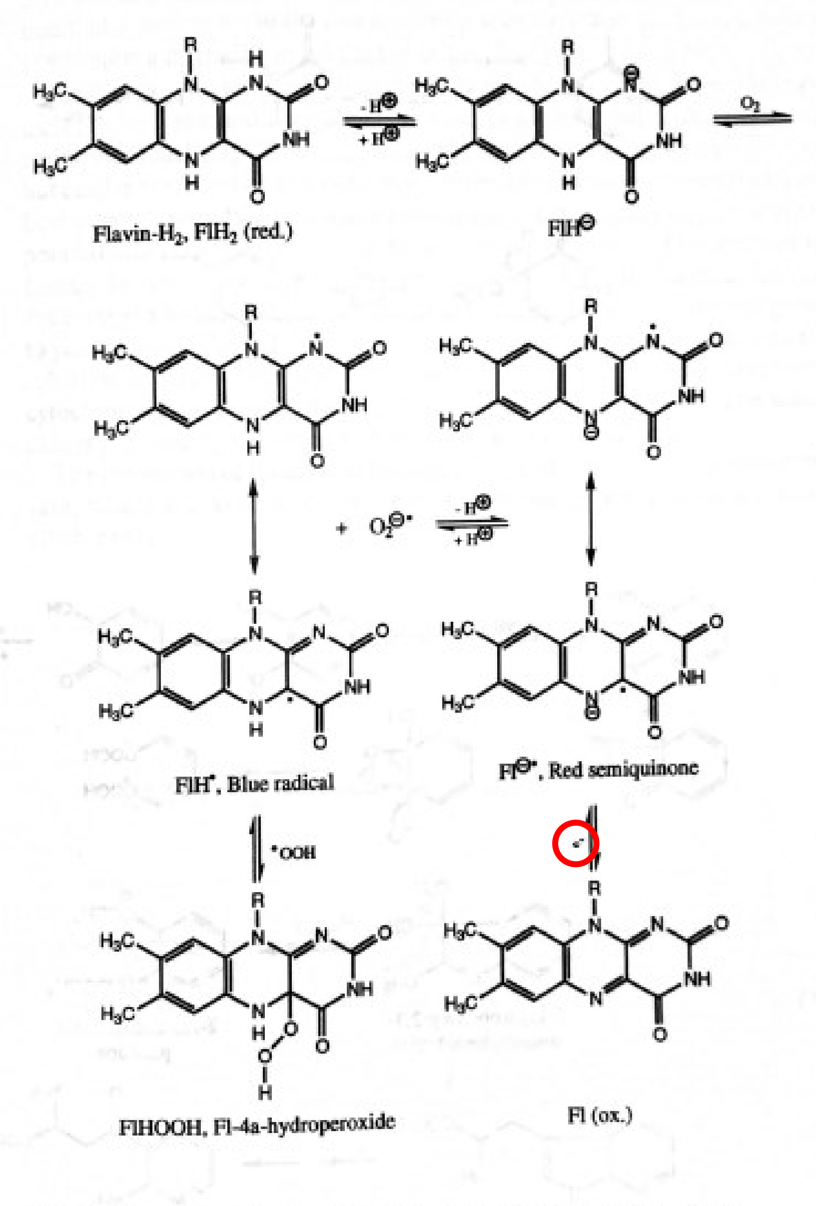
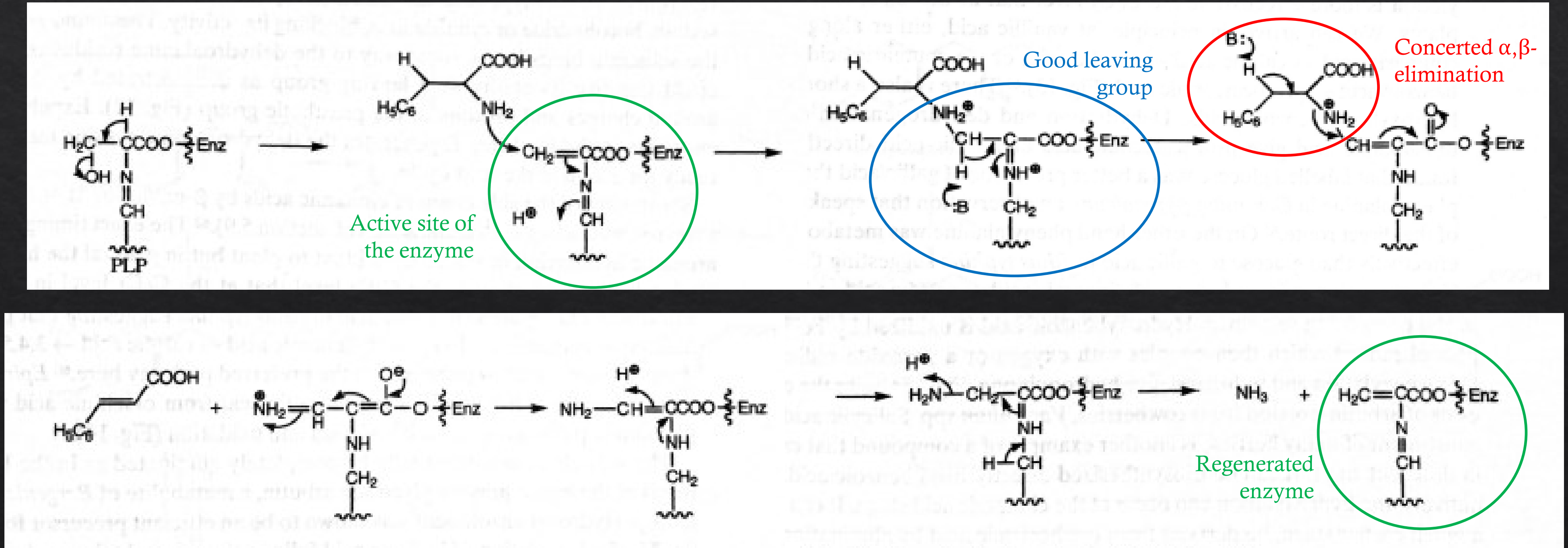


Fig. 11 Stepwise one-electron transfers; oxidation of flavin-H_2 by oxygen

Cinnamic and benzoic acid

- Cinnamic acids are widespread in nature and are formed from phenylalanine by enzymatic elimination of ammonia followed by aromatic hydroxylation and methylation.
- Enzyme: PAL (phenylalanine ammonia lyase)


Active site of the enzyme

Good leaving group

Concerted α,β-elimination

Regenerated enzyme

Fig. 12 Hypothetical mechanism for PAL activity

Biosynthetic network of cinnamic and benzoic acids

- The starting material is phenylalanine.

- Different paths are available for the synthesis of, for instance, vanillic acid:
    - Passing by coumaric acid
    - Or by benzoic acid

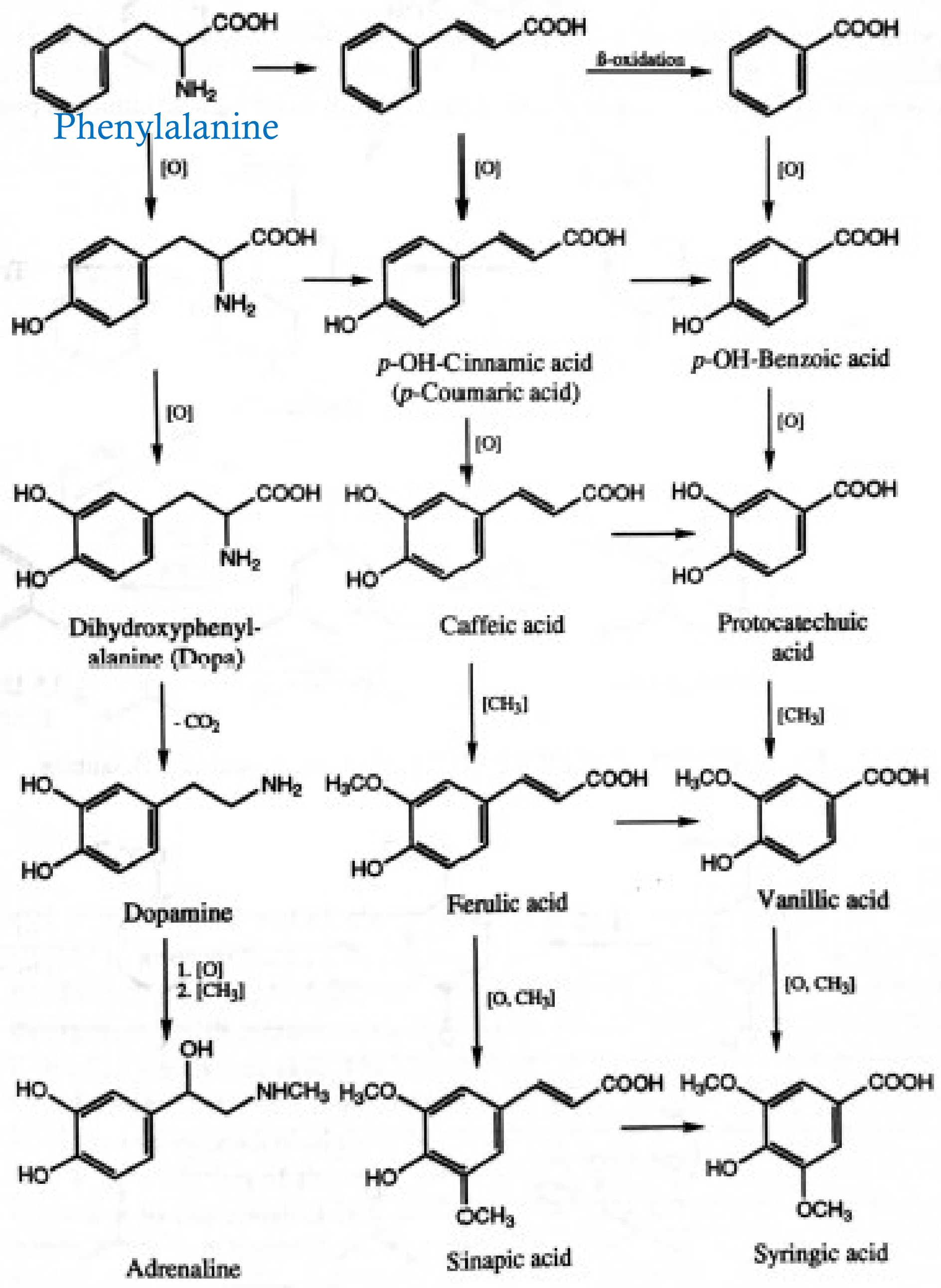


Fig. 13 Biosynthetic network of cinnamic and benzoic acids and biosynthesis of adrenaline

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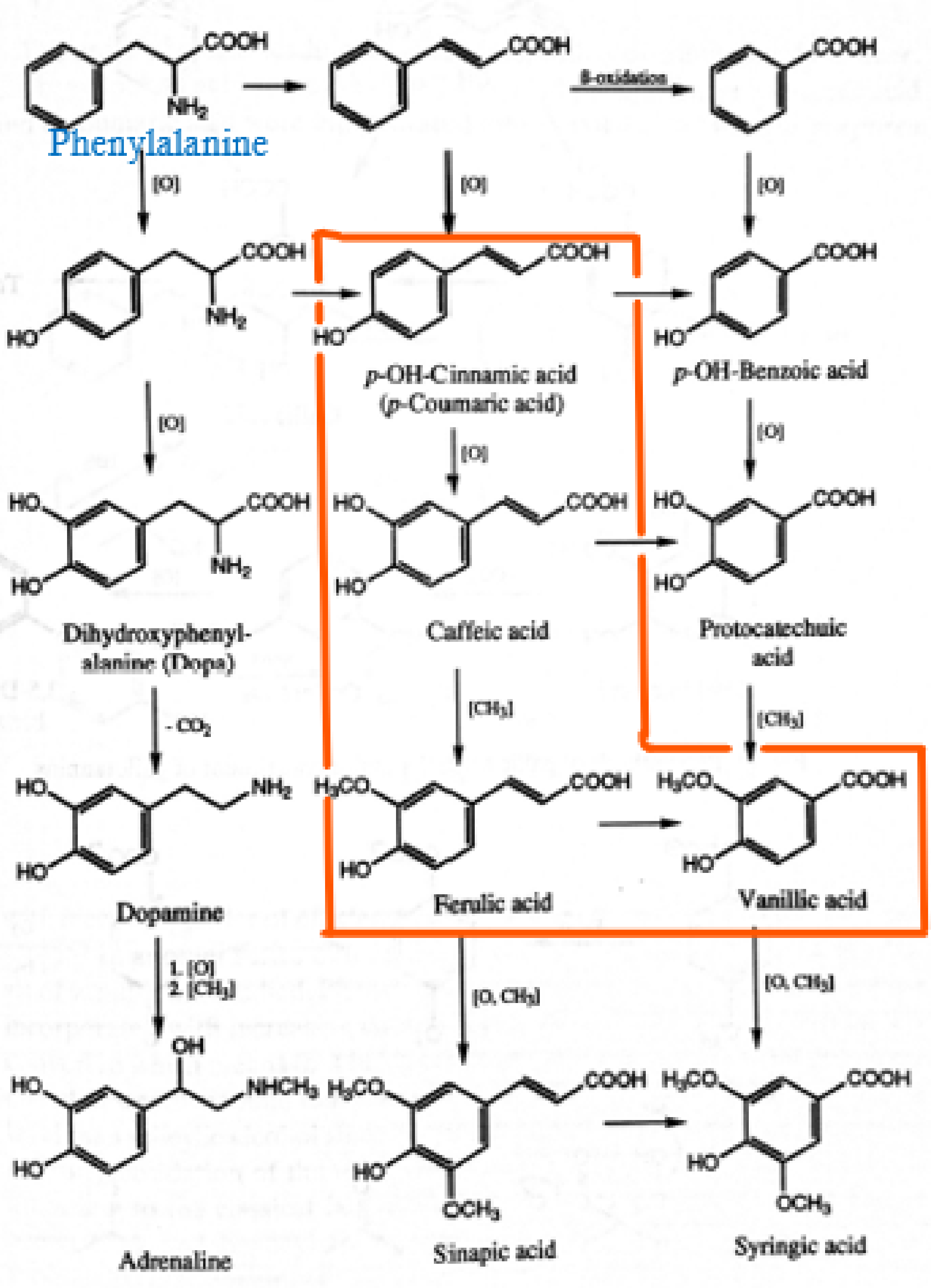

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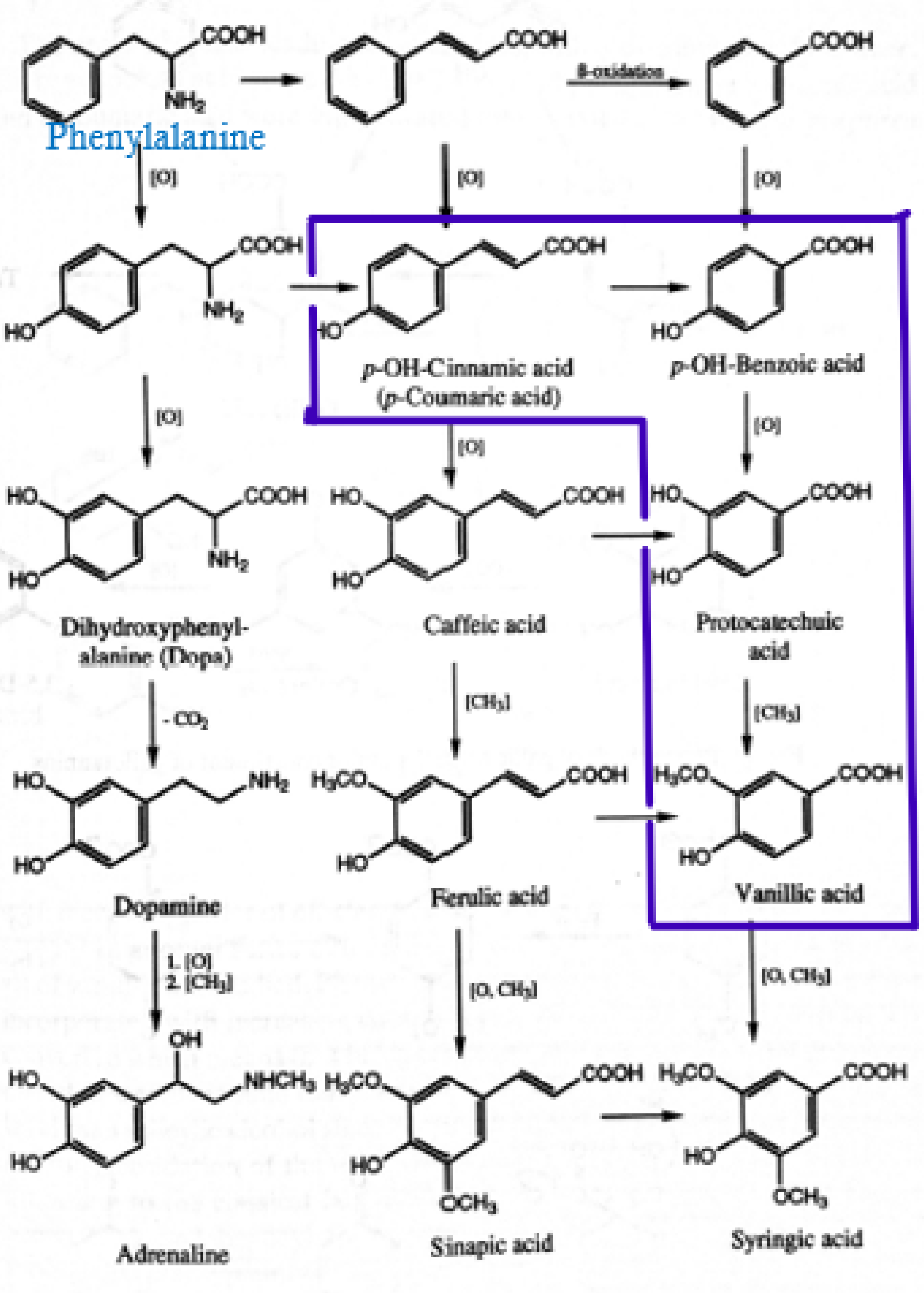


Fig. 13 Biosynthetic network of cinnamic and benzoic acids and biosynthesis of adrenaline

Biosynthetic network of cinnamic and benzoic acids

- Dehydration and dehydrogenation of 3-dehydroshikimic acid leads directly to gallic acid.
- Glucose is a better precursor of gallic acid than phenylalanine in *Geranium pyrenaicum*, but not in *Rhus typhina*

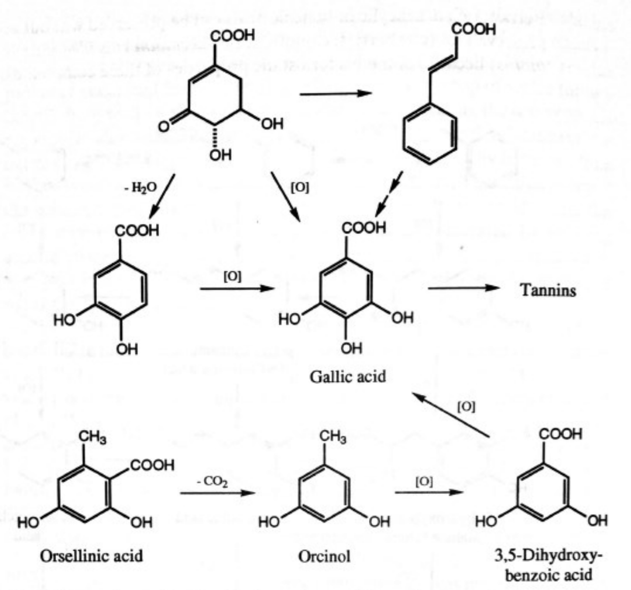


Fig. 14 Biosynthesis of gallic acid, the major constituent of gallotannins

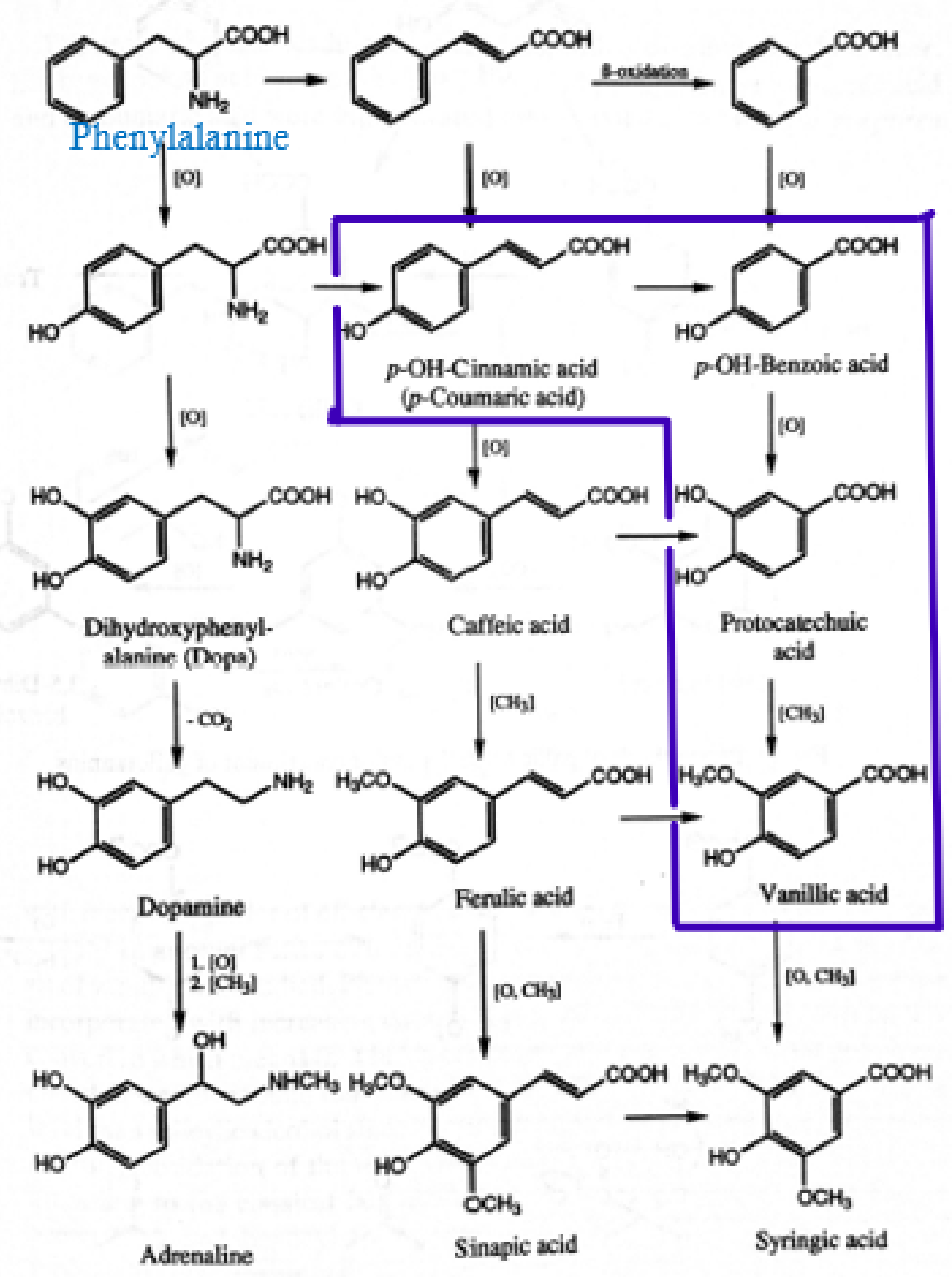


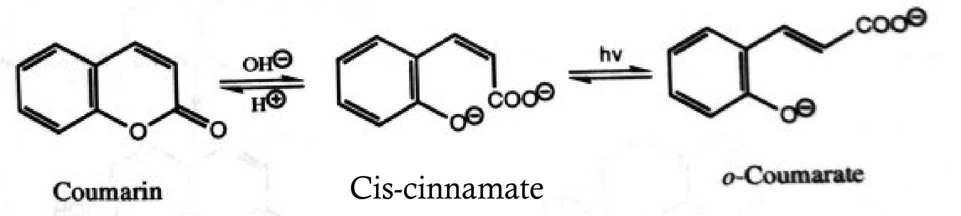
Fig. 13 Biosynthetic network of cinnamic and benzoic acids and biosynthesis of adrenaline

Cinnamic and benzoic acid derivatives

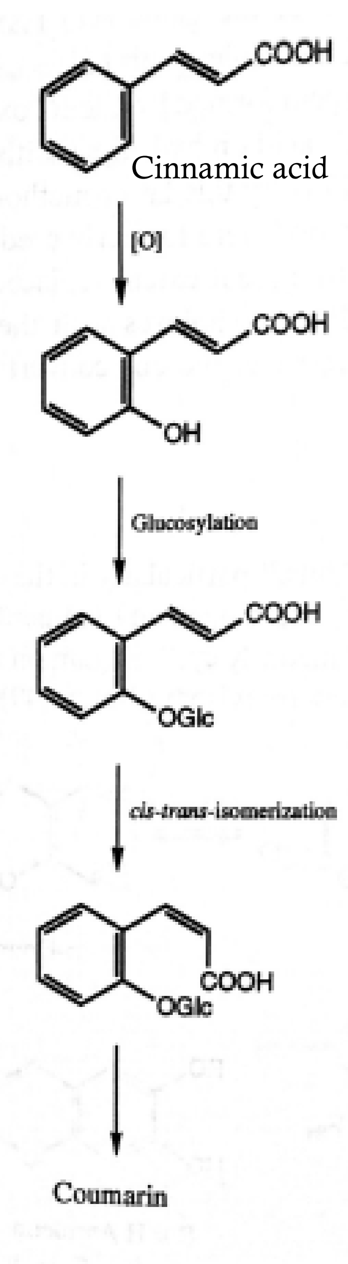
- In Vanilla planifolia, phenylalanine, cinnamic acid, and ferulic acid were better substrate to vanillin than vanillic acid.
- In higher plants, free benzoic acids are poorly reduced.

Coumarins

- Coumarins are lactones which open on treatment with base and cyclize again on acidification. Irradiation causes cis-trans isomerization of the cis-cinnamate.

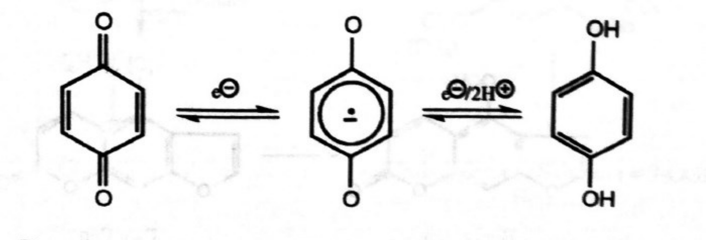


- Coumarins derive from shikimic acid via cinnamic acids.



Quinones

- Quinones include some pigments, antibiotics, coenzymes, and vitamin K.
- They can serve as one-electron transfer agents.



- Quinone biosynthesis are very diverse, and two structurally resembling quinones can have very different origins.

From the polyketide pathway

From skikimic acid

Plumbago europaea

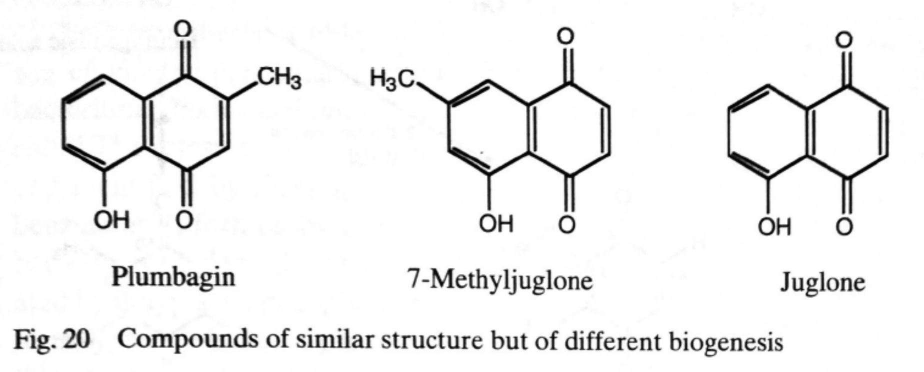


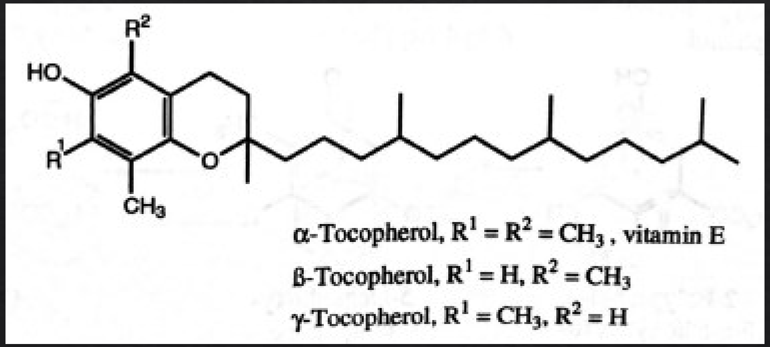
Fig. 20 Compounds of similar structure but of different biogenesis

Juglans regia

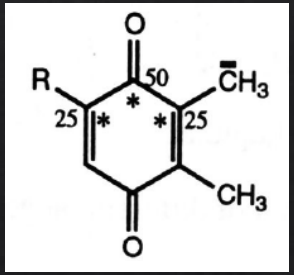
Quinones

- In bacterias, p-Hydroxybenzoic acid is obtained by elimination of pyruvic acid from chorismic acid
- In plants and mammals, it is obtained by degradation of phenylalanine.

- Vitamin E is structurally related to plastoquinones



α-Tocopherol, $R^1 = R^2 = CH_3$, vitamin E
β-Tocopherol, $R^1 = H$, $R^2 = CH_3$
γ-Tocopherol, $R^1 = CH_3$, $R^2 = H$



Example of plastoquinone (derives from tyrosine)

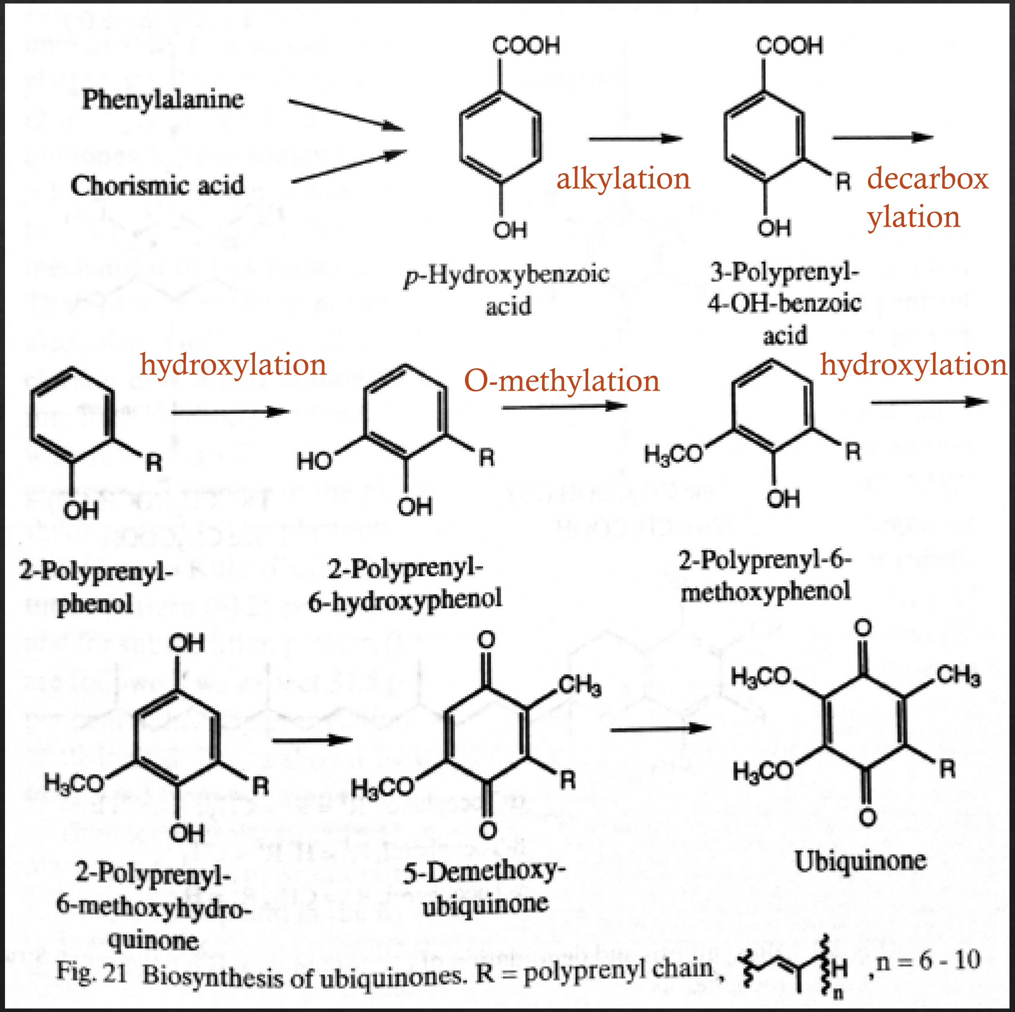
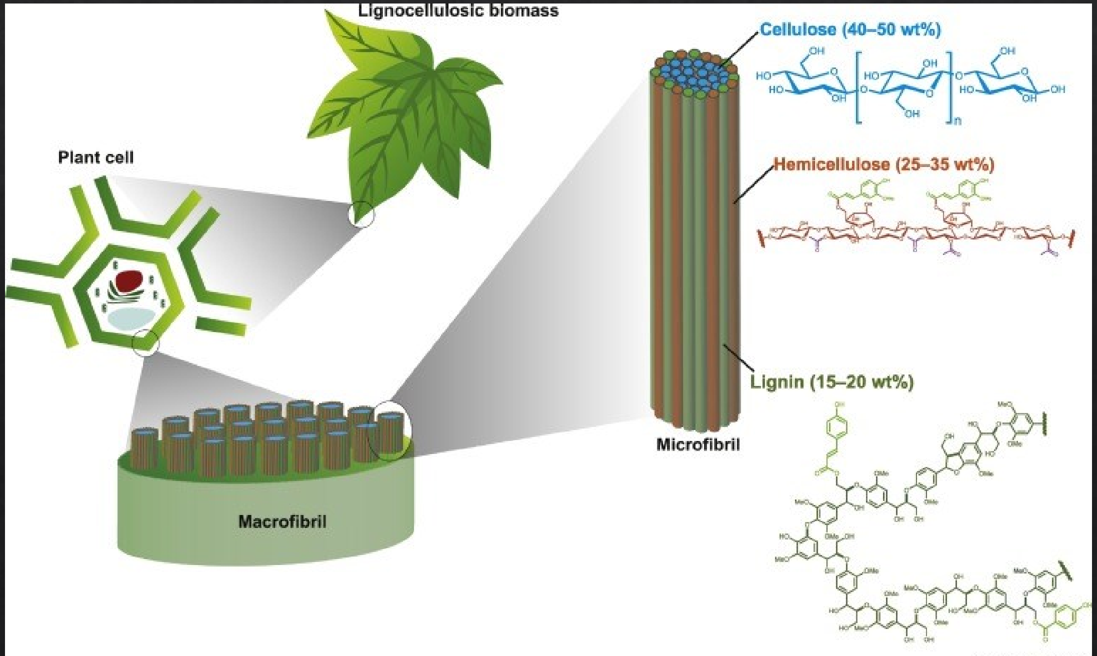


Fig. 21 Biosynthesis of ubiquinones. R = polyprenyl chain, n = 6 - 10

Lignin constituents

- Lignin is a polymeric network of aromatic building blocks present in all woody tissues.
- It acts as a matrix for the cellulose fibers and allows strength and stability of the cell wall.



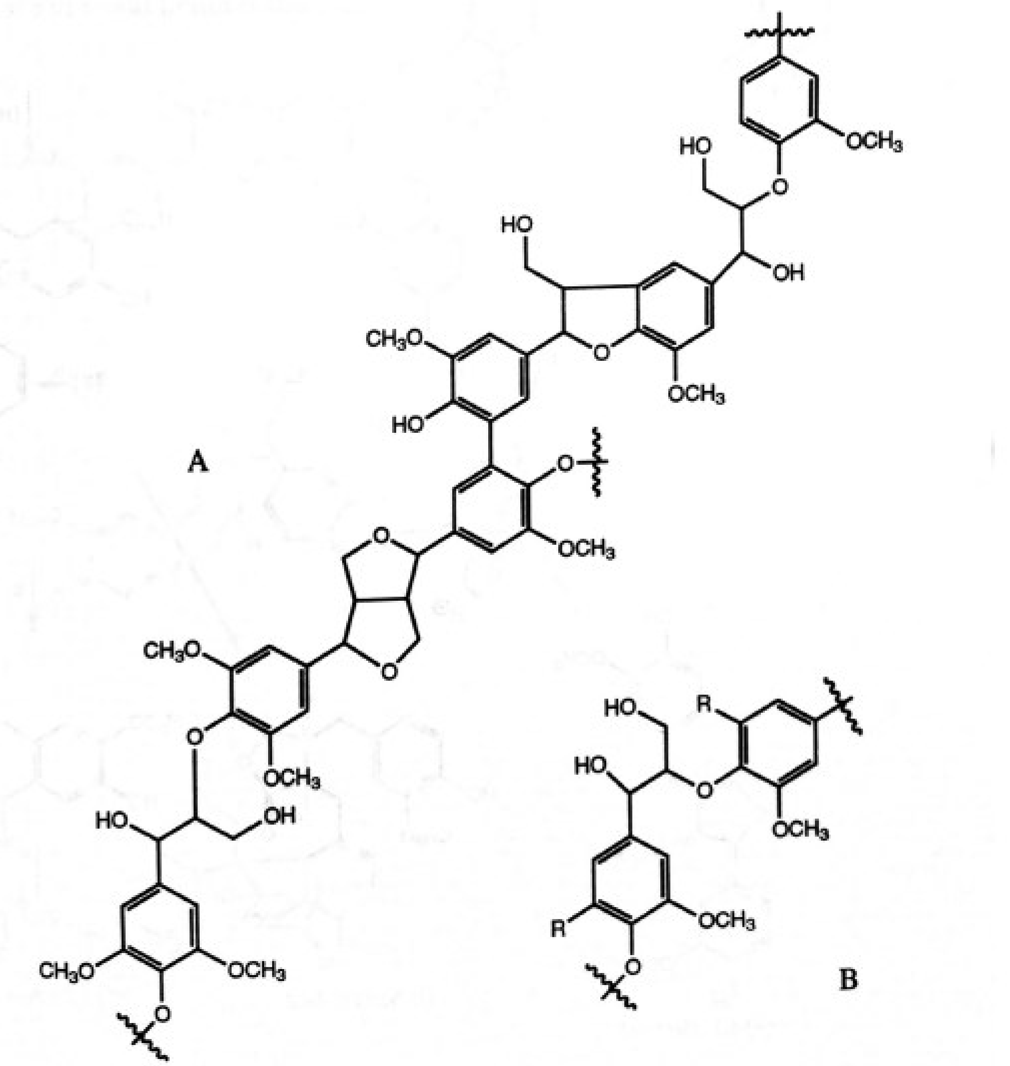
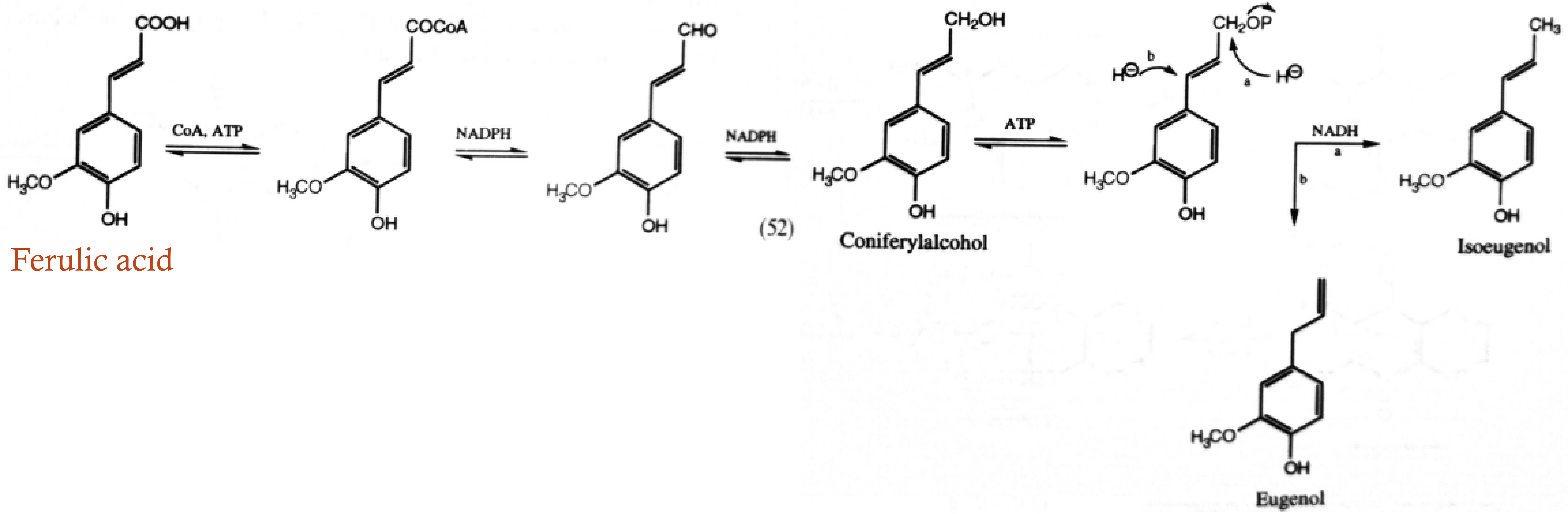
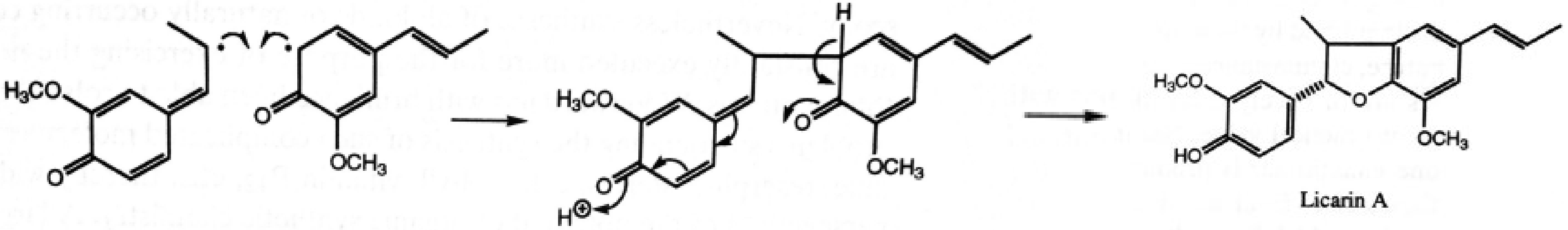


Fig. 26 A. Hypothetical lignin model. B. The most frequently occurring substructure. R = H, OCH_3

Lignin constituents



Ferulic acid



Synthesis of natural products in the lab

- The use of cheap optically active starting material, like sugars, is of importance.

- Shikimik acid can be synthesized from the easily available D-arabinose.
- Shikimik acid contains 3 asymmetric centres, with the same absolute configuration as the 3 asymmetric centres of D-arabinose.

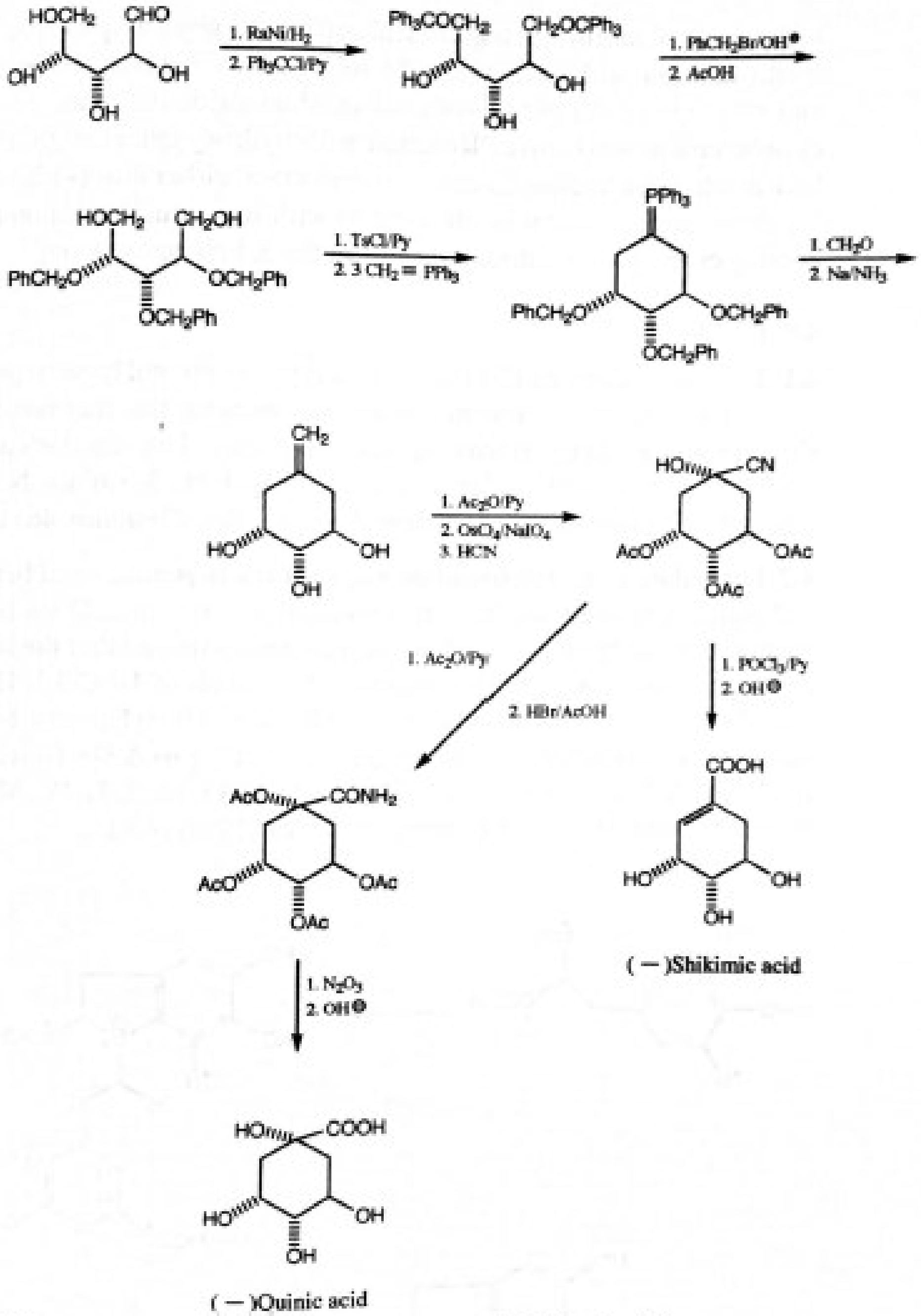


Fig. 29 Total synthesis of (−)quinic acid and (−)shikimic acid

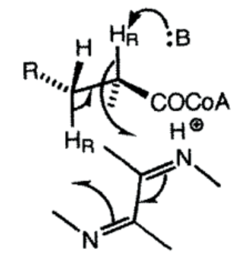
Chapter 5-2
The polydetide pathway

Elisabeth Jacobsen and Lucas Boquin, NTNU

Spring 2022

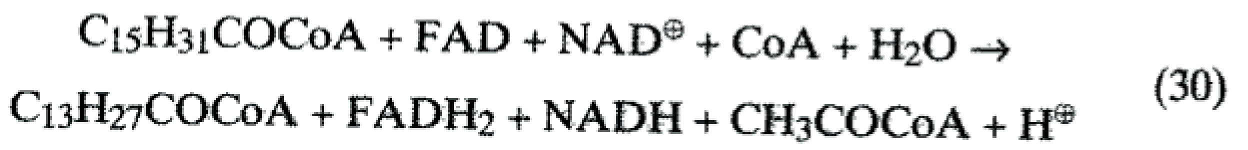
β-Oxidation:
How the body uses the energy stored in fatty acids

- 1- The fatty acid in transported into the mitochondria and is esterified with CoA
- 2- Dehydrogenation (anion formation, oxidation by FAD, final H abstraction)

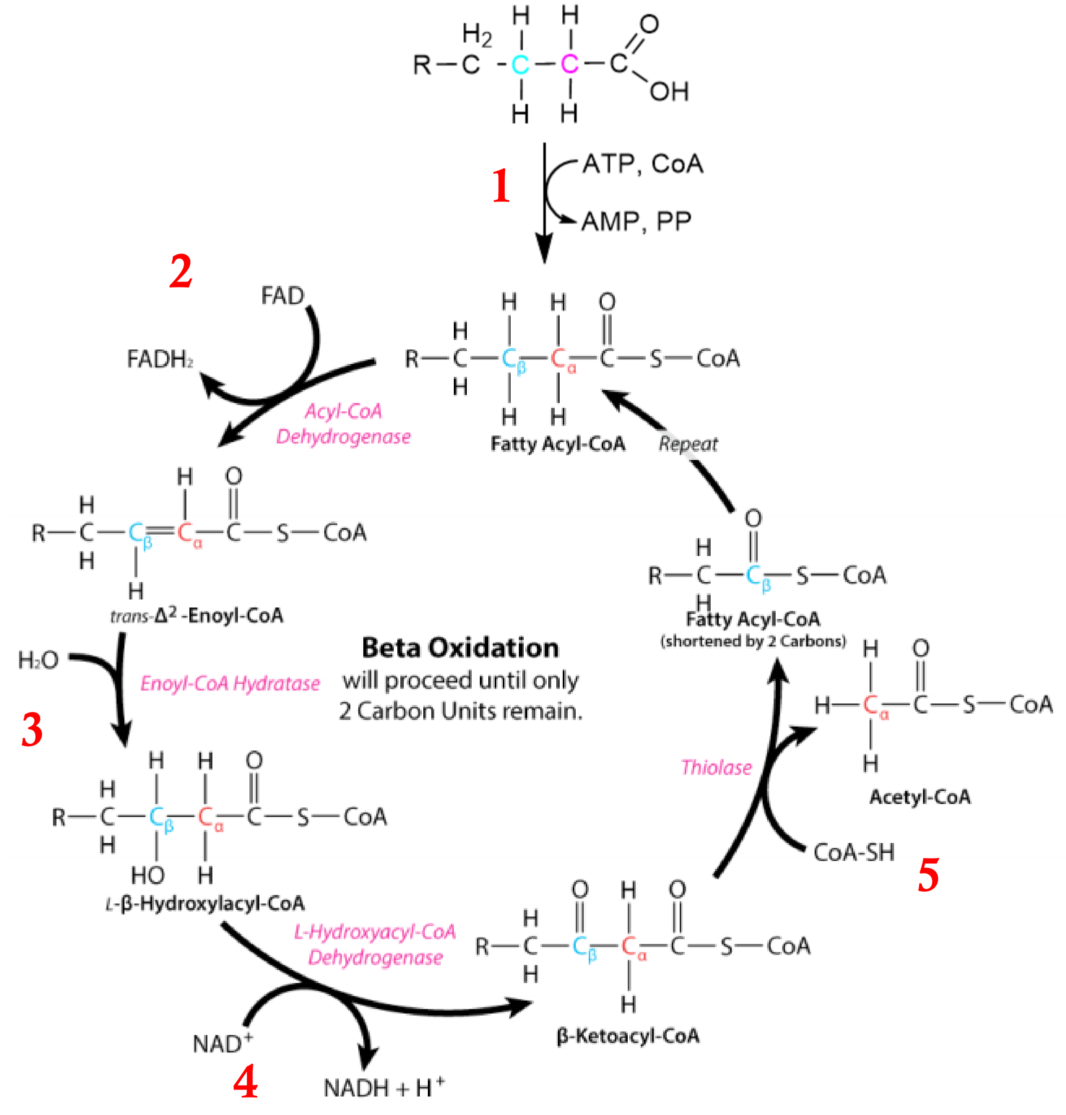


- 3- Stereospecific hydration
- 4- Oxidation by NAD+ to a keto fatty acid
- 5- Thiolysis by another CoA molecule

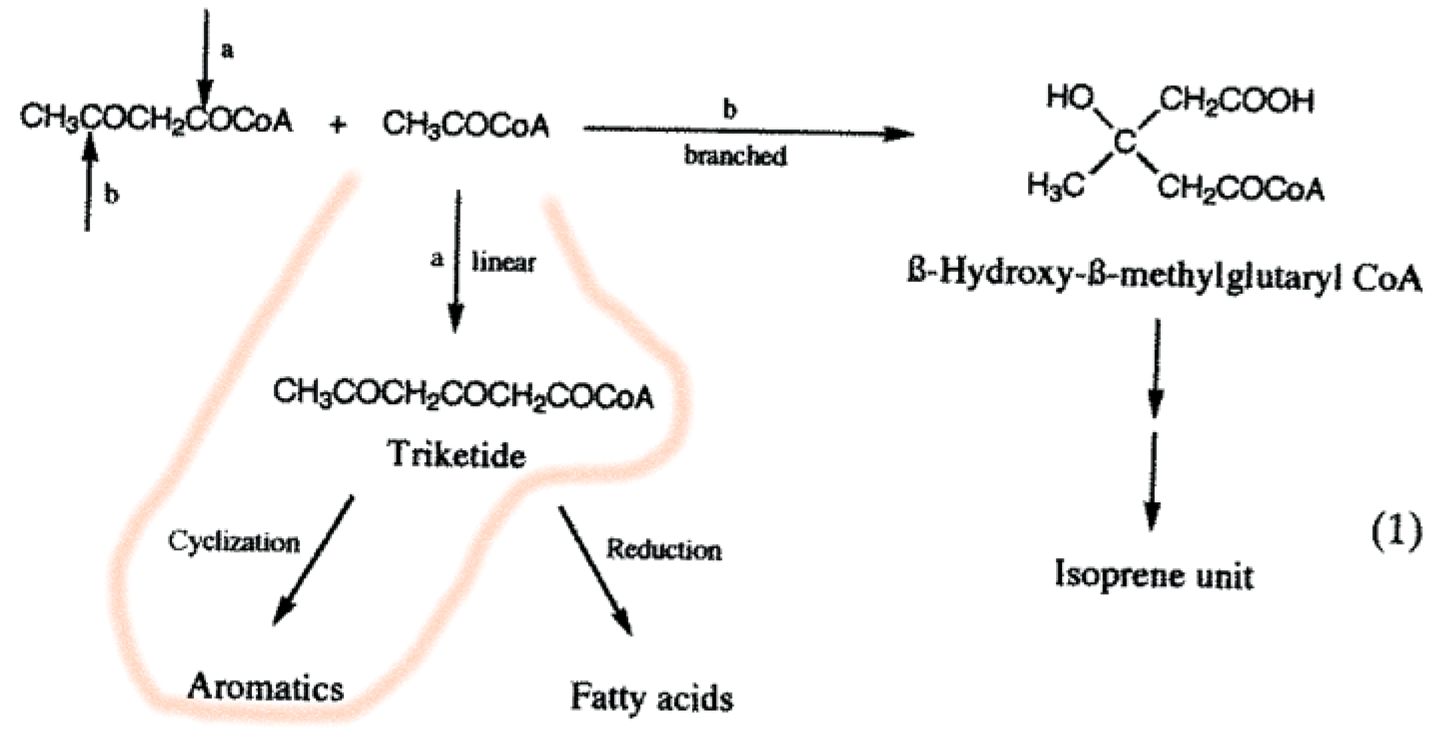
<u>Overall equation (for one cycle):</u>



$$C_{15}H_{31}COCoA + FAD + NAD^{\oplus} + CoA + H_2O \rightarrow C_{13}H_{27}COCoA + FADH_2 + NADH + CH_3COCoA + H^{\oplus} \qquad (30)$$



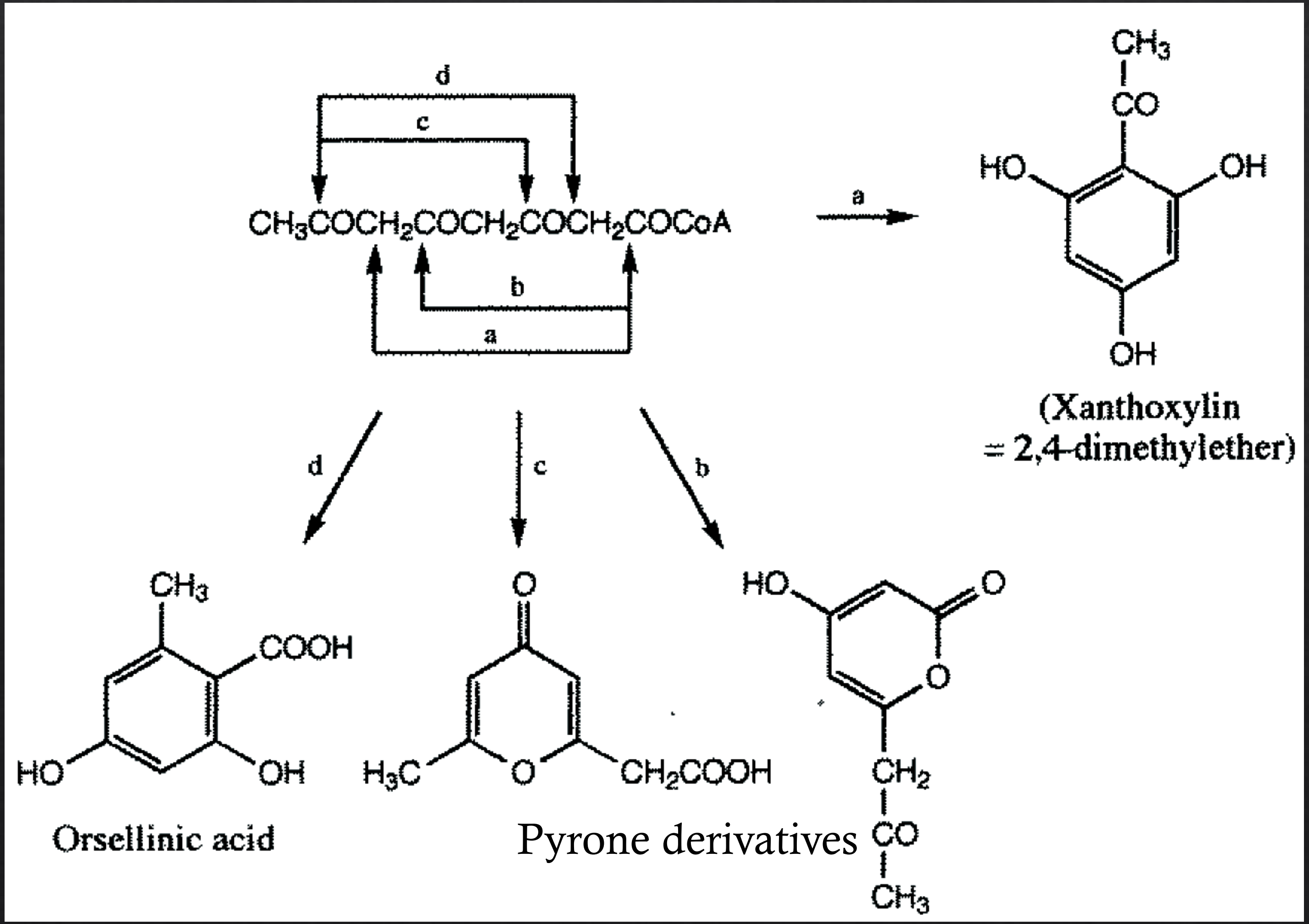
Formation of aromatics from polyketides



- Further condensations before reduction lead to the very reactives β-polyketoesters. They can be temporarly stabilized by bonding on an enzyme for further building.
- They undergo cyclization, leading to aromatics.

Formation of aromatics from polyketides

- Tetraketides can cyclize in different ways:

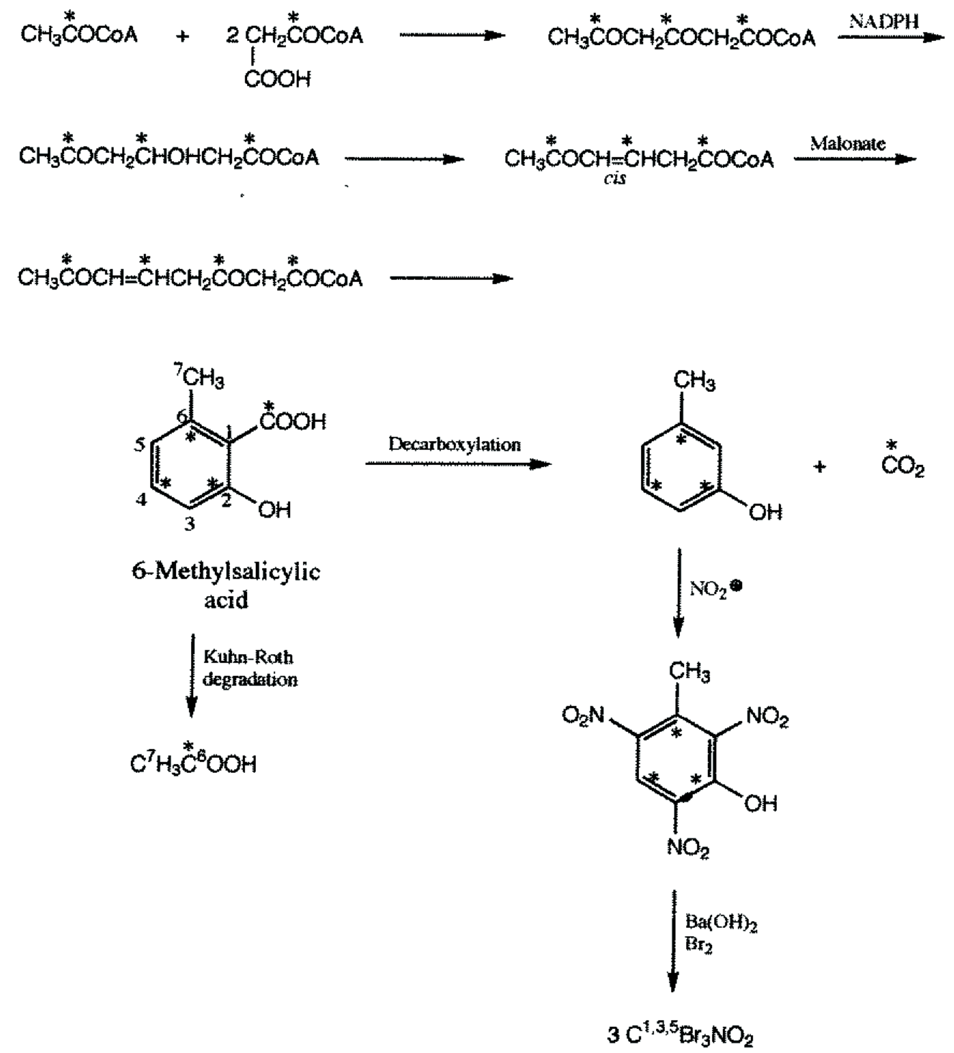


A lot of metabolites can be synthesized, depending on:

- The chain initiating unit
- The number of acetyl CoA involved
- The mode of cyclization
- The condensation of separately synthesized polyketides
- The secondary processes (halogenation, alkylation, redox reactions,...)

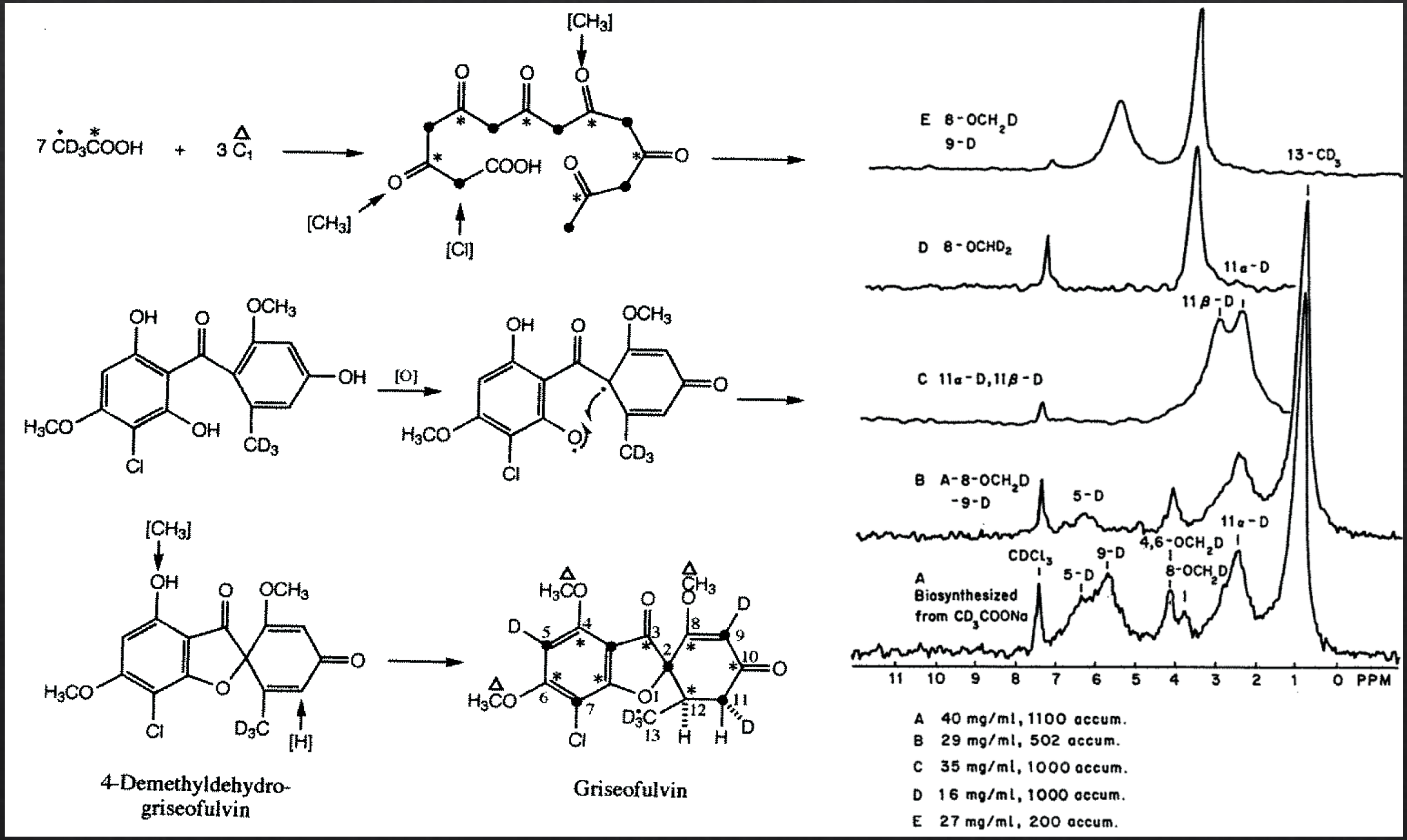
Confirmation of the acetate hypothesis

- Birch was the first to formulate the acetate hypothesis.
- First confirmations:
- By feeding the plant with marked starting materials, and then chemically degrading the product.
  - The Kuhn-Roth degradation allow us to analyze C6 and C7 activity.
  - The other pathway allow us to verify the absence of activity on carbons 1,3 and 5, and the activity of the CO_2 released.



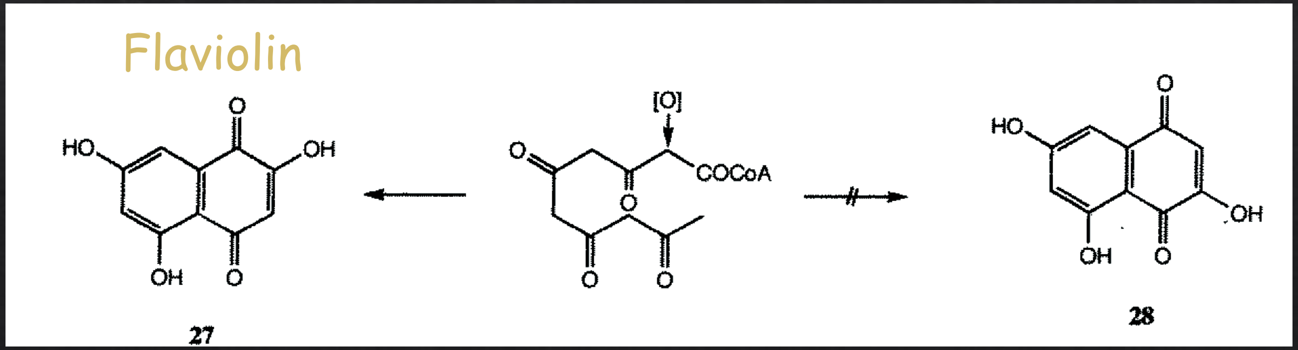
Confirmation of the acetate hypothesis

- Chemical degradation is a very complex process, especially for big molecules.
- Today, ^{2}H-,^{3}H- and ^{13}C- labelled compounds can be synthesized, and analyzed by NMR.
    - ^{3}H-NMR: Slightly negative NOE effect, which can allow us to measure the isotopic content in a molecule.
    - ^{2}H-NMR: Inexpensive, stable, low natural abondance (shorter relaxation time), no NOE, but low sensitivity
    - ^{13}C-NMR: Stable, but higher natural abondance and low sensitivity. Some techniques can increase signal intensity and maintain some hydrogen coupling information (off-resonance technique)



Derivation of structure

- Usually, aromatic products coming from polyketide have a pattern of meta-hydroxy substitution. Those coming from shikimic acid have ortho-hydroxy substitution. Structure can often help to determine the origin of a molecule, even if it is not always accurate.
- Two structures of Flaviolin were considered, and one intermediate was known. Structure 28 is not likely to be the good one.

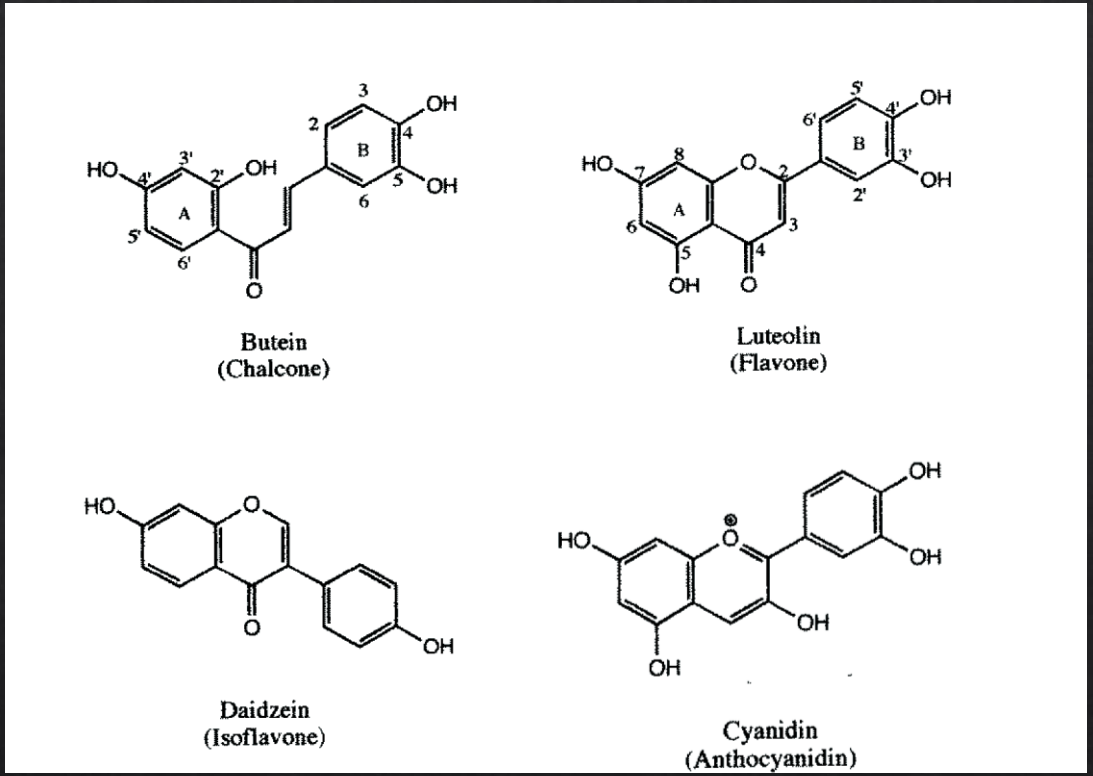


Flavonoids

Flavonoids are coloring substances present in flowers and fruits.

Dahlia

Soybeans (daidzu (大豆) in japanese)



Butein
(Chalcone)

Luteolin
(Flavone)

Daidzein
(Isoflavone)

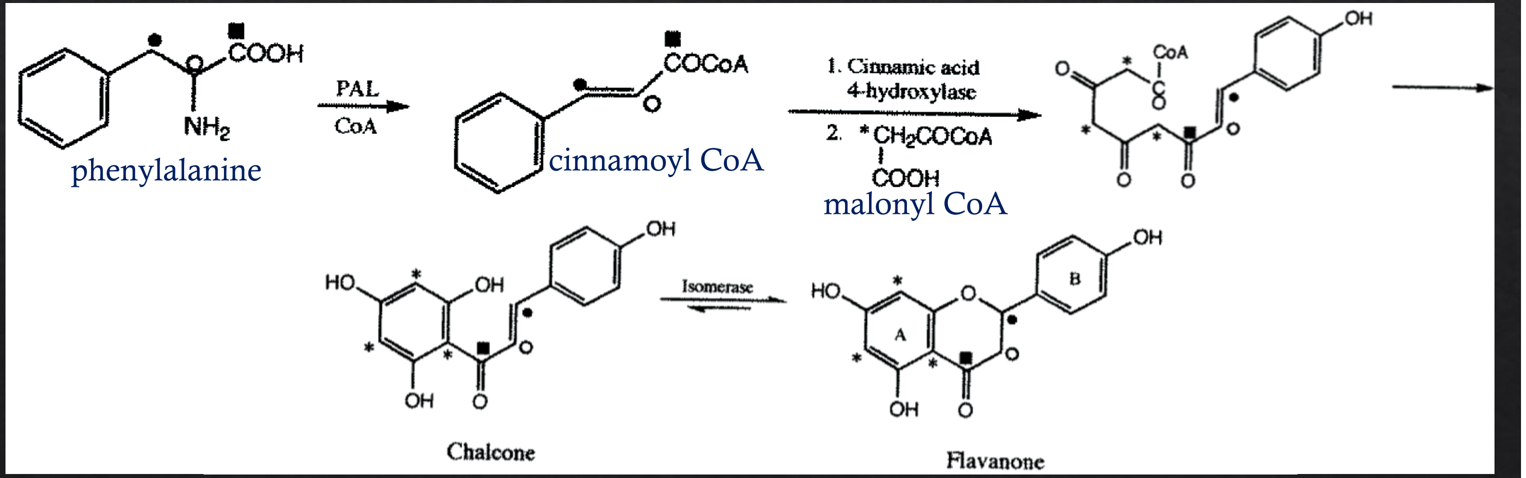
Cyanidin
(Anthocyanidin)

Reseda luteola

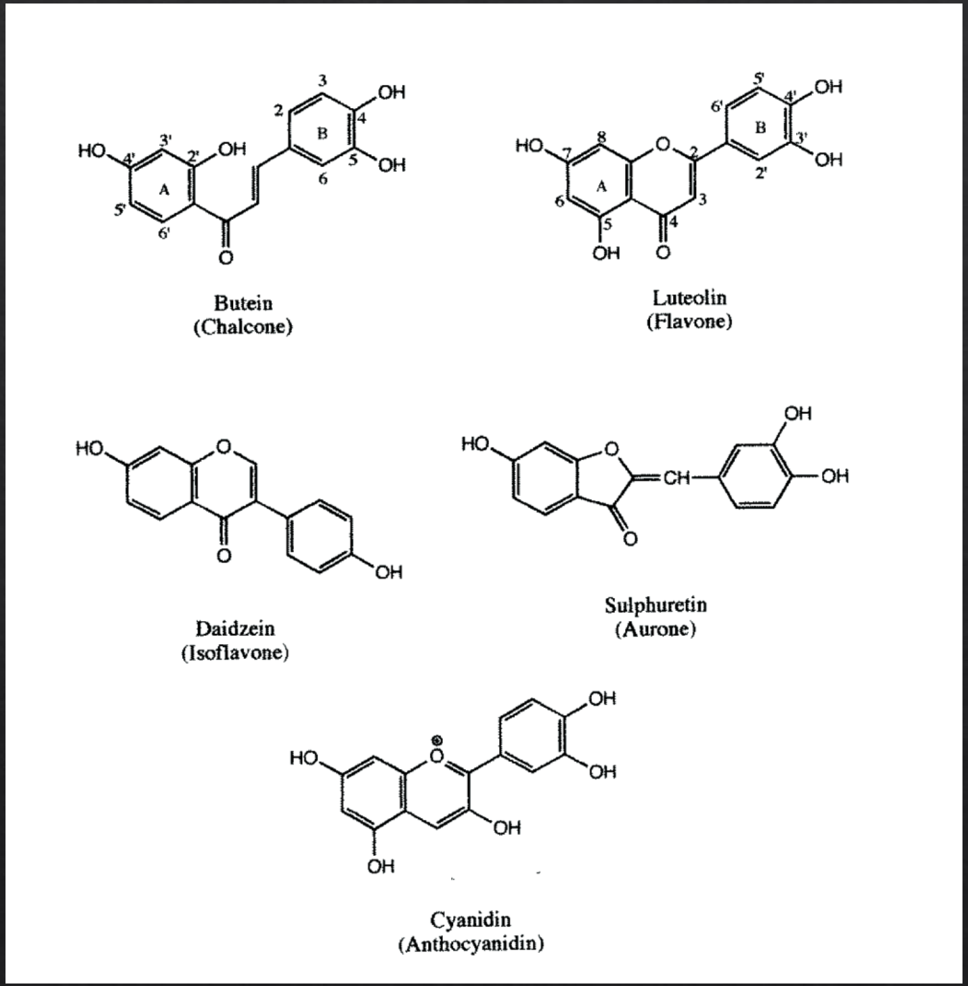
Ipomoea purpurea

Flavonoids

- Flavonoids are coloring substances present in flowers and fruits.
  - Flavones: yellow or orange
  - Antocyanidins: Red, purple or blue
- They are composed of two hydroxylated aromatic rings, A and B, joint by a three carbon fragment.

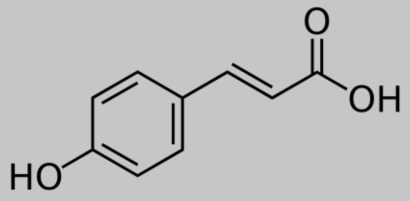


Biosynthesis of the basic flavone skeleton

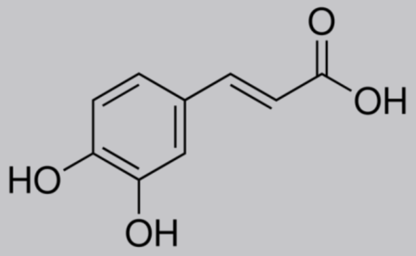


Flavonoids

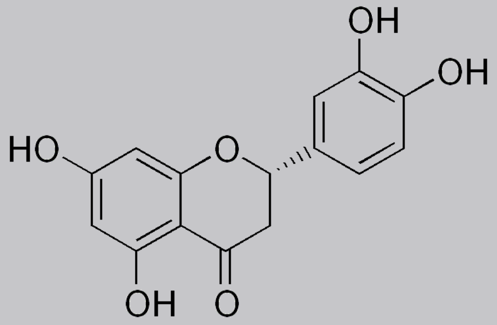
- In *Haplopappus gracilis*, p-hydroxycinnamic acid is used efficiently at pH 8 to synthesize eriodictyol, but at pH 7, caffeic acid is preferred.



p-Hydroxycinnamic acid



Caffeic acid

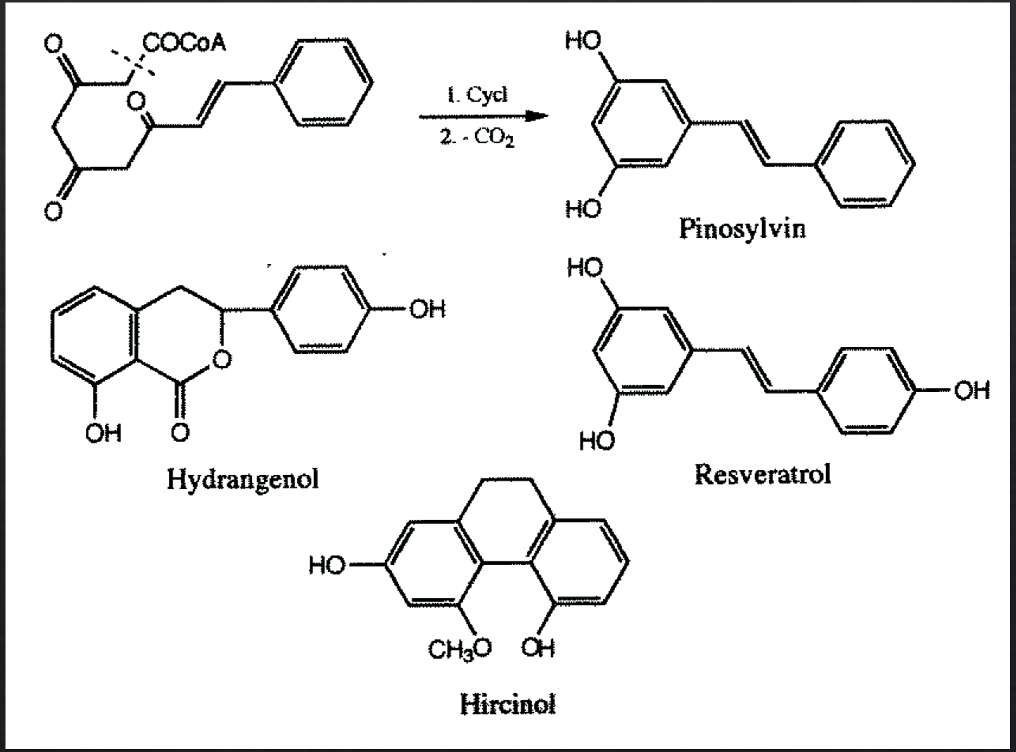


Eriodictyol

Stilbenes

- Stilbenes are structurally close to flavonoids, and their biosynthesis results from a slightly different folding of the polyketide. They are often formed by plants in response to microbial attack or stress.

Hydrangea macrophylla (hortensia)

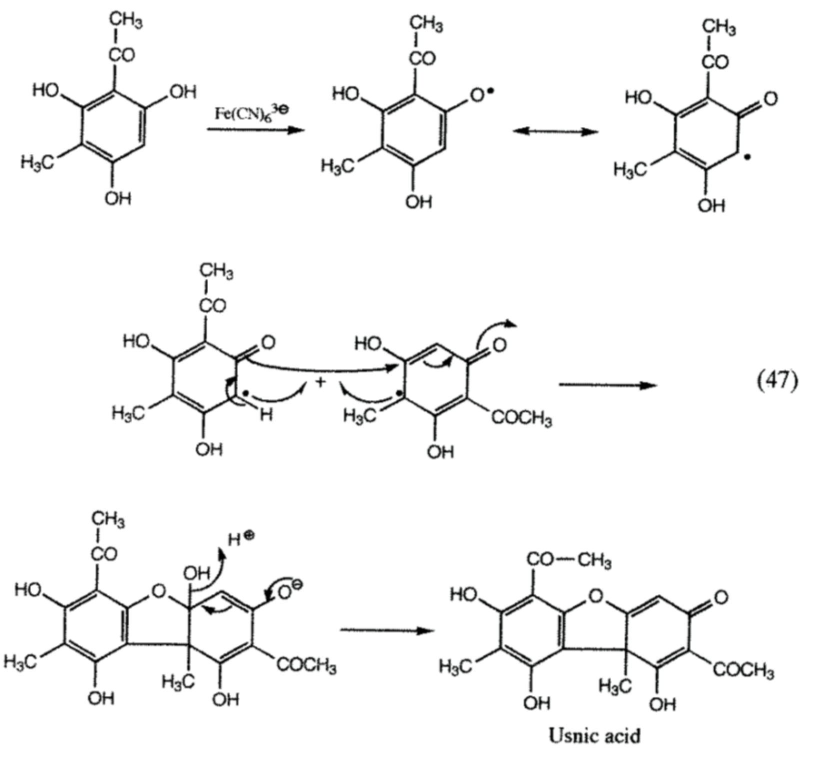


Norway spruce (Gran på norsk)

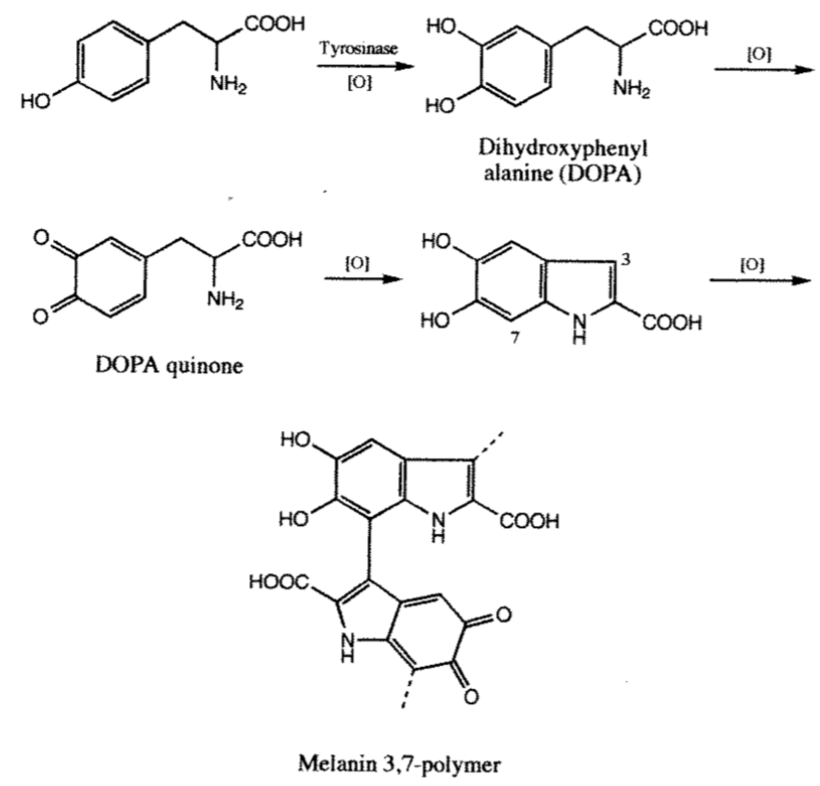
Pinosylvin: Present in pine trees. Fungitoxin protecting the wood from fungal infection.

Oxidative coupling of phenols

- Some enzymes can catalyze oxidative coupling of phenols, by one-electron transfers. For instance, melanine is formed by enzymatic oxidation of tyrosine.



Usnic acid



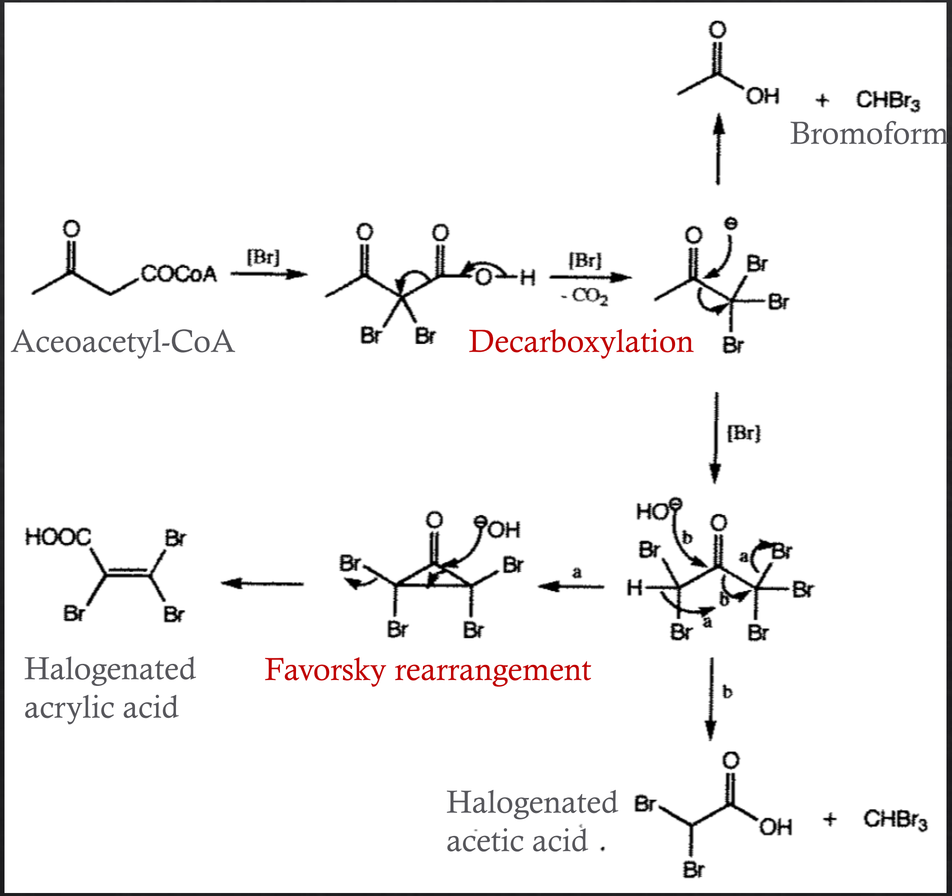
Dihydroxyphenyl alanine (DOPA)

DOPA quinone

Melanin 3,7-polymer

Halogen compounds

- Iodine, fluorine and bromine compounds are extremely rare in nature.
- Chloro compounds are more common, biosynthesized mainly by microorganisms and marine organisms.
- Halogenation has a potentiating effect on biological activities of a lot of compounds.
  - Chloromycetin has a 100-times more antibacterial effect than the acetyl analogue.
- The capability of seaweeds to accumulate bromine and iodine, and the low oxidation potential of these halogens, explains the occurrence of some iodine and bromide-containing metabolites.



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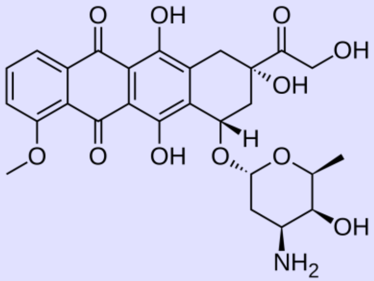
Chapter 5-1
The Polyketide Pathway

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

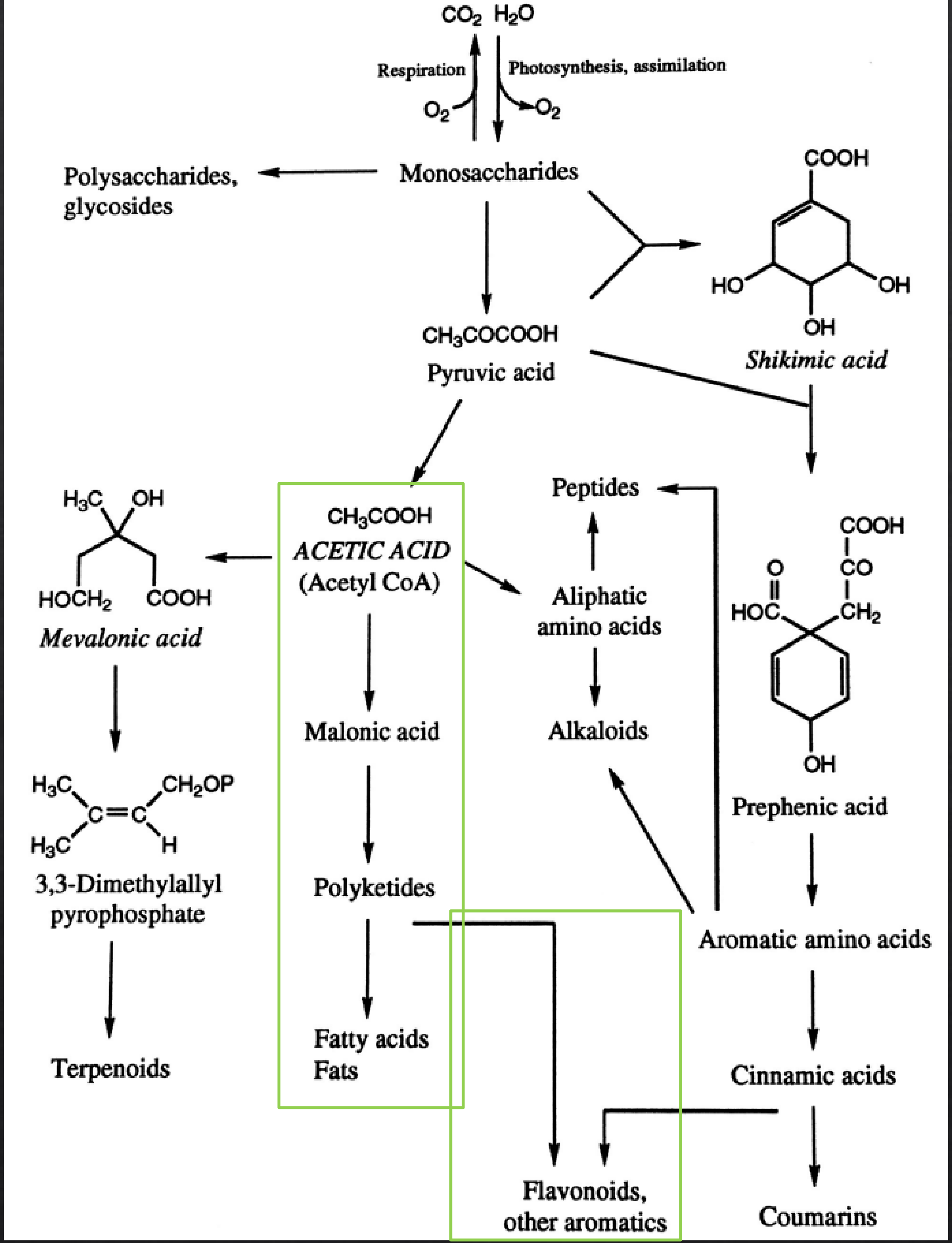
Spring 2022

Polyketide synthesis:

- In bacteria, fungi and plants, some marine animals
- Polyketide synthases (PKSs)
- Large group of molecules with very diverse properties

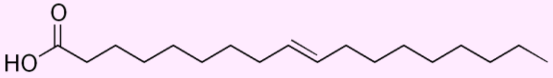


Doxorubicin



Fatty acid synthesis:

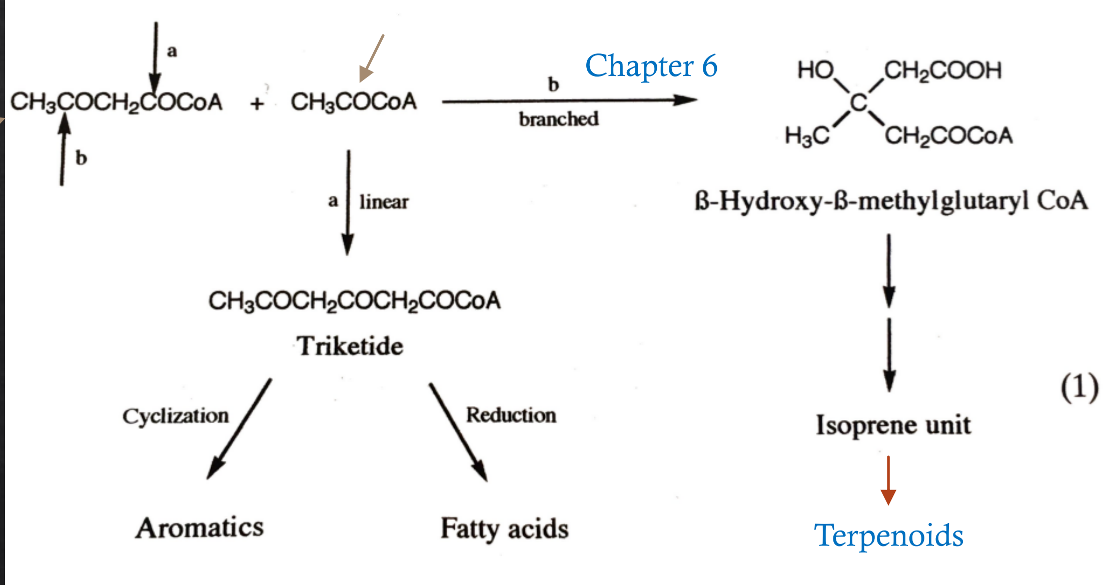
- In animals, bacteria, fungi, plants
- Fatty acid synthases (FASs)
- Relatively small group of molecules



Oleic acid

Polyketides

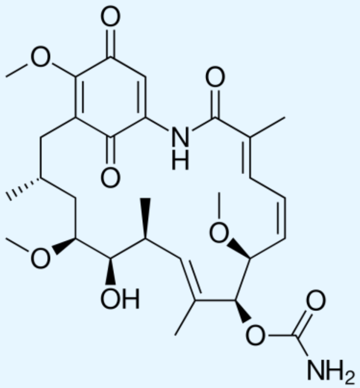
- Natural compounds containing alternating carbonyl and methylene groups ($-CO-CH_2-$)
- Secondary metabolites
- Derived from repeated condensation of acetyl coenzyme A (via malonyl coenzyme A)



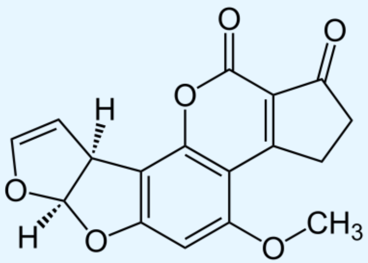
Malonyl-CoA

Chapter 6

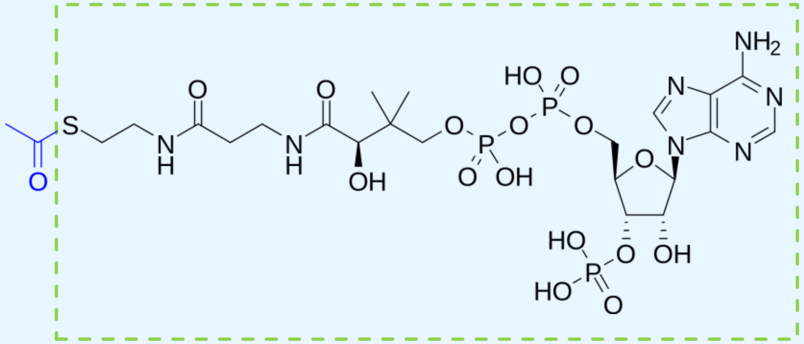
Terpenoids



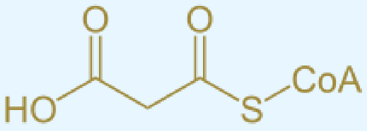
Geldanamycin
antibiotic



Aflatoxin B1
carcinogenic



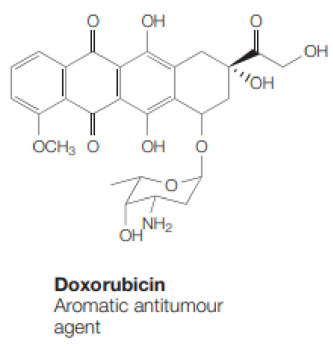
Acetyl CoA



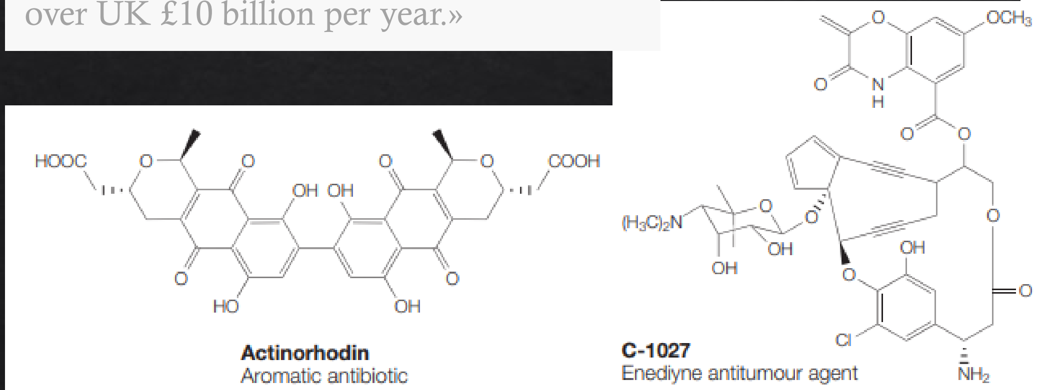
Malonyl-CoA

Polyke

«**Polyketide natural products are among the most important microbial metabolites in human medicine, targeting both acute and degenerative diseases.**
They are in clinical use as antibiotics (erythromycin A, rifamycin S), anticancer drugs (doxorubicin, epothilone), cholesterol-lowering agents (lovastatin), antiparasitics (avermectin), antifungals (amphotericin B), insecticides (spinosyn A) and immunosuppressants (rapamycin). Polyketide-derived pharmaceuticals comprise **20% of the top-selling drugs**, with combined worldwide revenues of over UK £10 billion per year.»

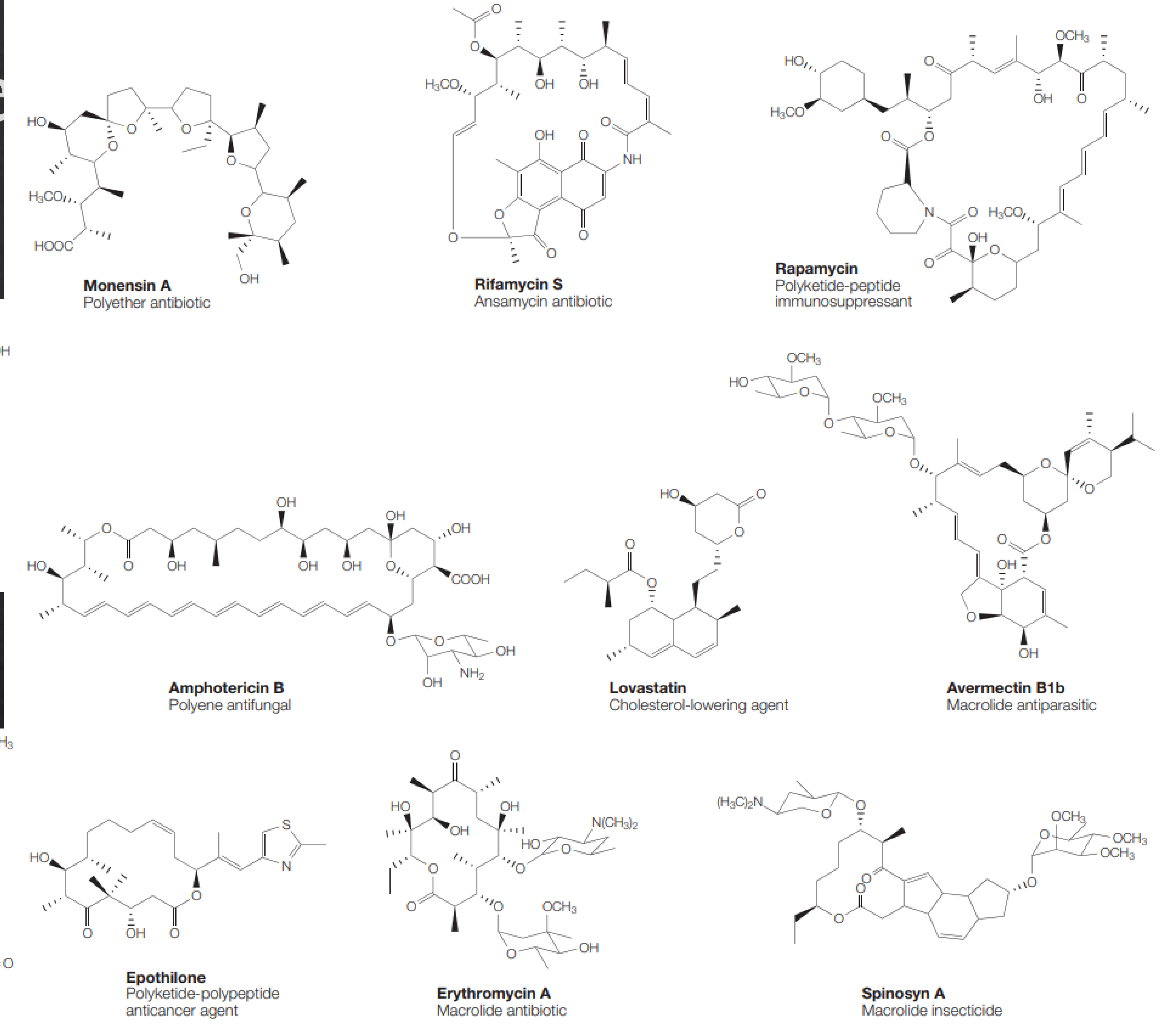


Doxorubicin
Aromatic antitumour agent



Actinorhodin
Aromatic antibiotic

C-1027
Enediyne antitumour agent



Monensin A
Polyether antibiotic

Rifamycin S
Ansamycin antibiotic

Rapamycin
Polyketide-peptide immunosuppressant

Amphotericin B
Polyene antifungal

Lovastatin
Cholesterol-lowering agent

Avermectin B1b
Macrolide antiparasitic

Epothilone
Polyketide-polypeptide anticancer agent

Erythromycin A
Macrolide antibiotic

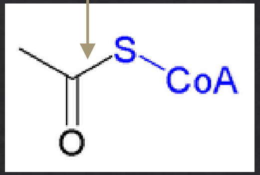
Spinosyn A
Macrolide insecticide

Nature Reviews Microbiology, 2005, volume 3, pp. 925–

Fatty acids and fats

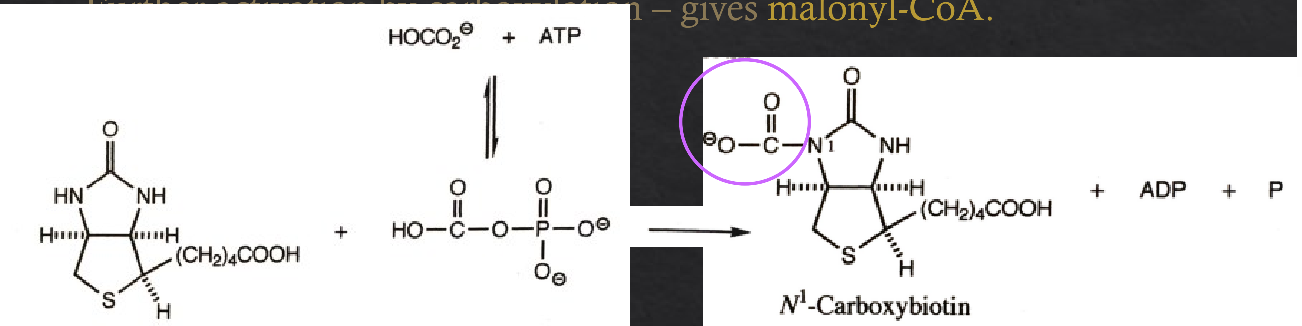
This happens in animals, bacteria, fungi, plants

High-energy bond, makes acetyl-CoA more active in condensations.



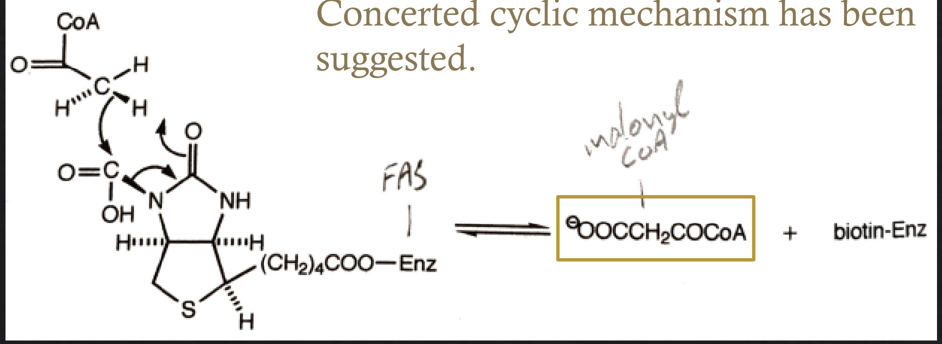
But it is not reactive enough for **fatty acid synthase (FAS)** to use.

Further activation by carboxylation – gives malonyl-CoA.



CO₂ carrier

Concerted cyclic mechanism has been suggested.



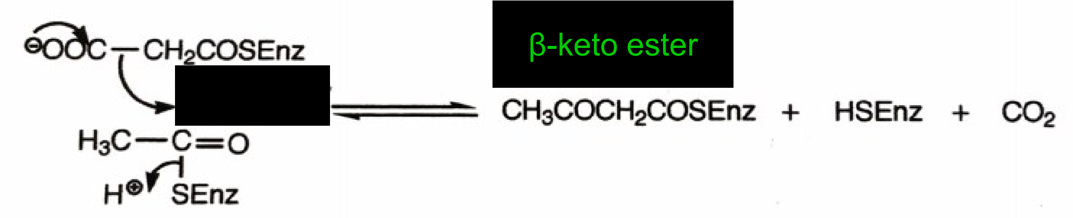
Acetyl-CoA and malonyl-CoA attaches to the acyl carrier protein on FAS.

$CH_3COCoA + HSEnz \rightleftharpoons CH_3COSEnz + CoA$

[redacted] [redacted]

$^{\ominus}OOCCH_2COCoA + HSEnz \rightleftharpoons {}^{\ominus}OOCCH_2COSEnz + CoA$

Claisen condensation



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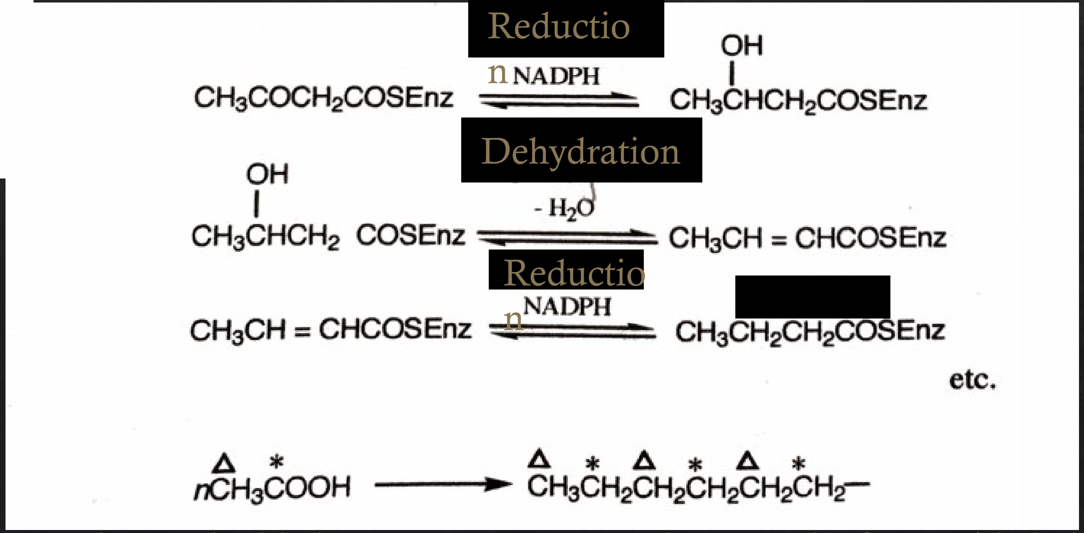


$CH_3COCH_2COSEnz \rightleftharpoons CH_3CH(OH)CH_2COSEnz$

$CH_3CH(OH)CH_2COSEnz \xrightarrow{-H_2O} CH_3CH{=}CHCOSEnz$

$CH_3CH{=}CHCOSEnz \rightleftharpoons CH_3CH_2CH_2COSEnz$

[redacted]

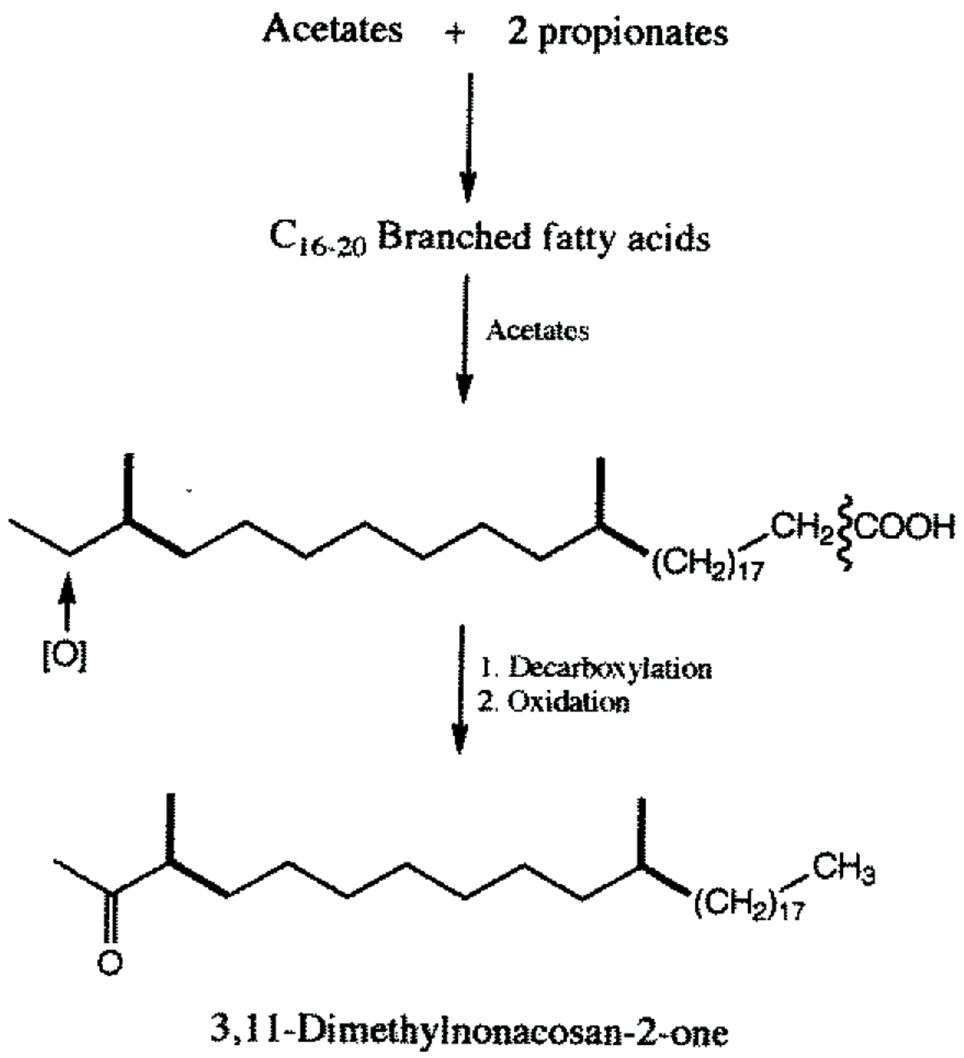
$n\overset{\Delta}{C}H_3\overset{*}{C}OOH \longrightarrow \overset{\Delta}{C}H_3\overset{*}{C}H_2\overset{\Delta}{C}H_2\overset{*}{C}H_2\overset{\Delta}{C}H_2\overset{*}{C}H_2—$

For each acetyl added, we get two more carbons on the fatty acid chain.

Fatty acids usually have an even number of carbons.

Long-chain fatty acids and hydrocarbons

- Branched fatty acids have been identified as intermediates in synthesis of long-chain fatty acids and hydrocarbons
- 3,11-dimethylnonacosan-2-one is the pheromone of the female German cockroach



L

- Lipids are compounds that are soluble in hydrocarbons
- Fats, waxes, phosphoglycerides
- Common fatty acids: palmitic, stearic and oleic acid

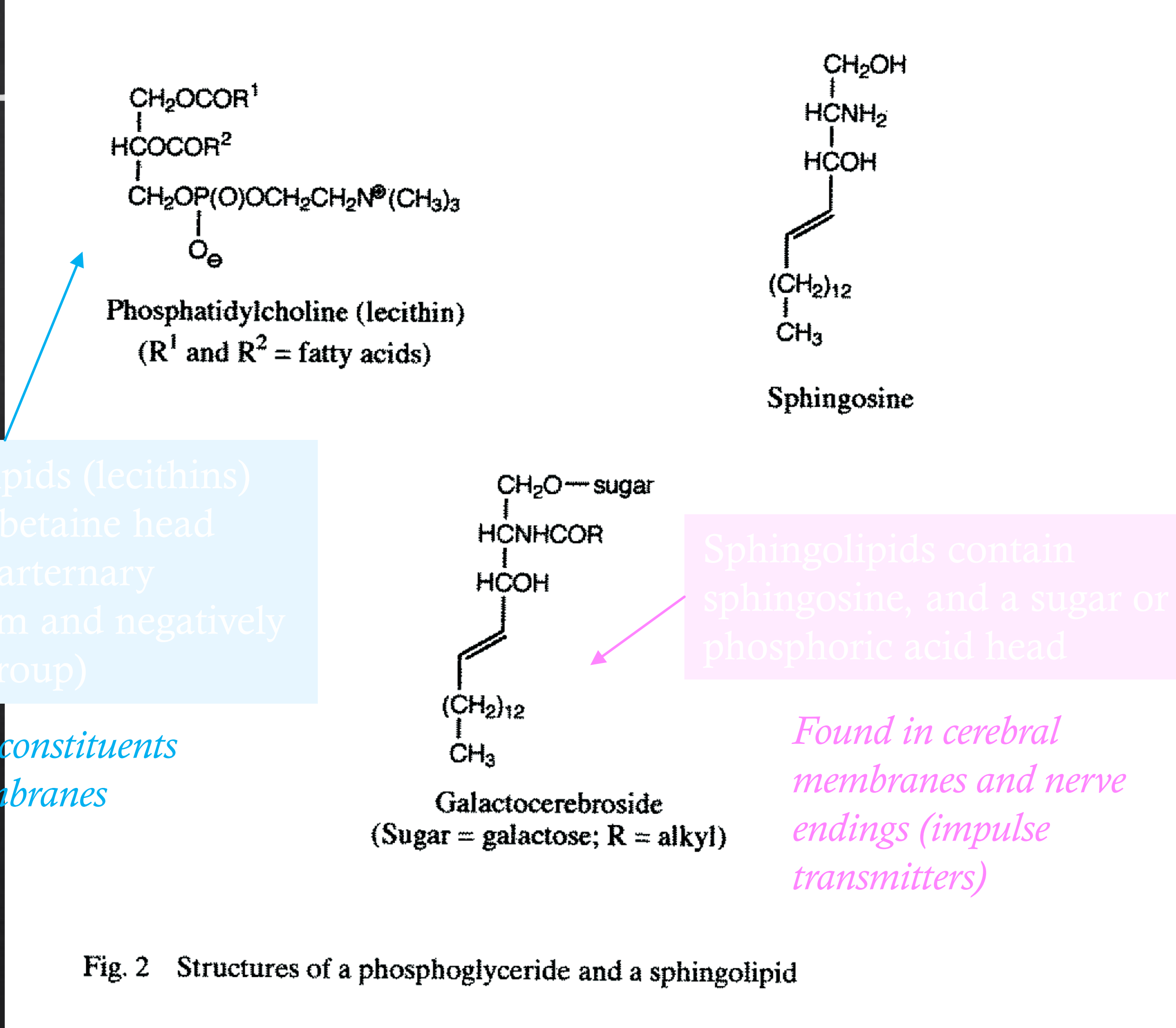


Fig. 2 Structures of a phosphoglyceride and a sphingolipid

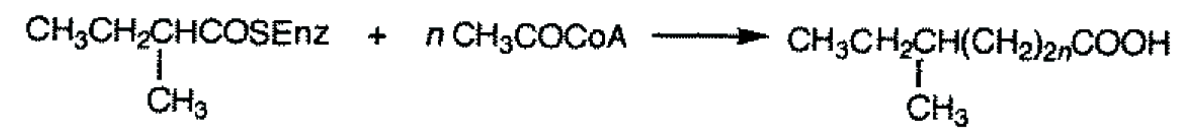
Phospholipids (lecithins) contain a betaine head group (quarternary ammonium and negatively charged group)

Important constituents of cell membranes

Sphingolipids contain sphingosine, and a sugar or phosphoric acid head

Found in cerebral membranes and nerve endings (impulse transmitters)

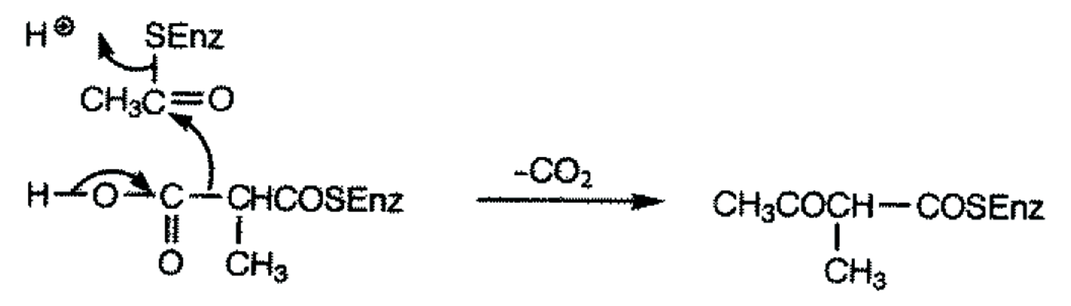
Branched fatty acids



$$\mathrm{CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCOSEnz} + n\,\mathrm{CH_3COCoA} \longrightarrow \mathrm{CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}H(CH_2)_{2n}COOH}$$

Branched starting material
Isobuturyl-CoA or α-metylbutyryl-CoA

OR



$$\mathrm{H{-}O{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}\underset{\displaystyle CH_3}{\underset{|}{C}}HCOSEnz} + \mathrm{CH_3C(SEnz){=}O} \xrightarrow{-\mathrm{CO_2}} \mathrm{CH_3CO\underset{\displaystyle CH_3}{\underset{|}{C}}H{-}COSEnz}$$

Condensation of alkylated malonyl-CoA

$$\mathrm{CH_3CH_2COCoA} + {}^{\ominus}\mathrm{OOC{-}biotin{-}Enz} \longrightarrow \mathrm{CH_3CH}\begin{cases}\mathrm{COO^{\ominus}}\\ \mathrm{COCoA}\end{cases}$$

Methylmalonyl CoA

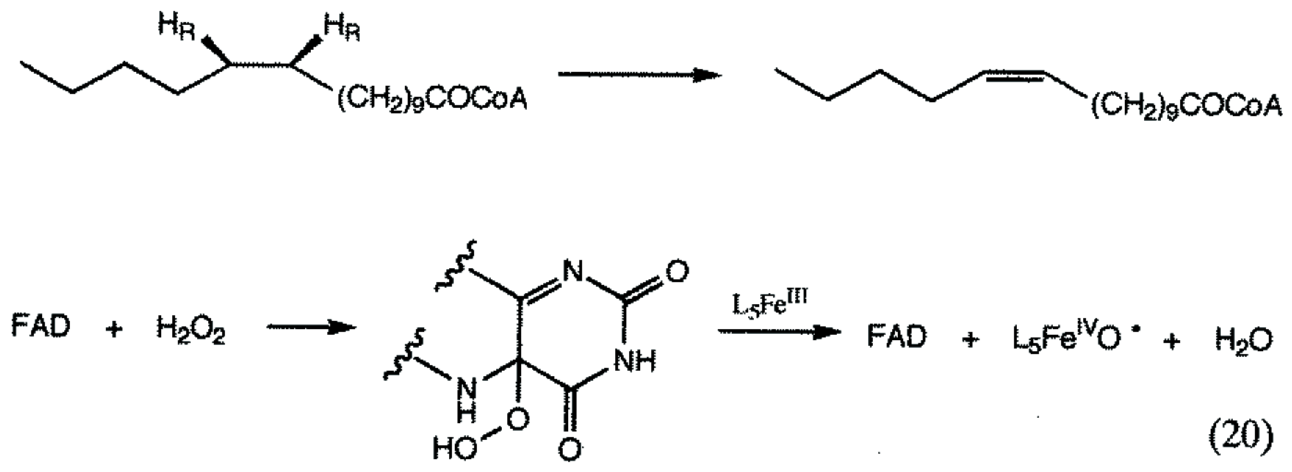
Alkylation of malonyl-CoA occurs by carboxylation of acetic acid homologues

Unsaturated fatty acids & prostagladins

- Unsaturation is introduced differently in aerobic and anaerobic environments

Aerobic
Double bond is introduced into the saturated fatty acid

Requires oxygen, FAD, NADPH and a dehydrogenase



(20)

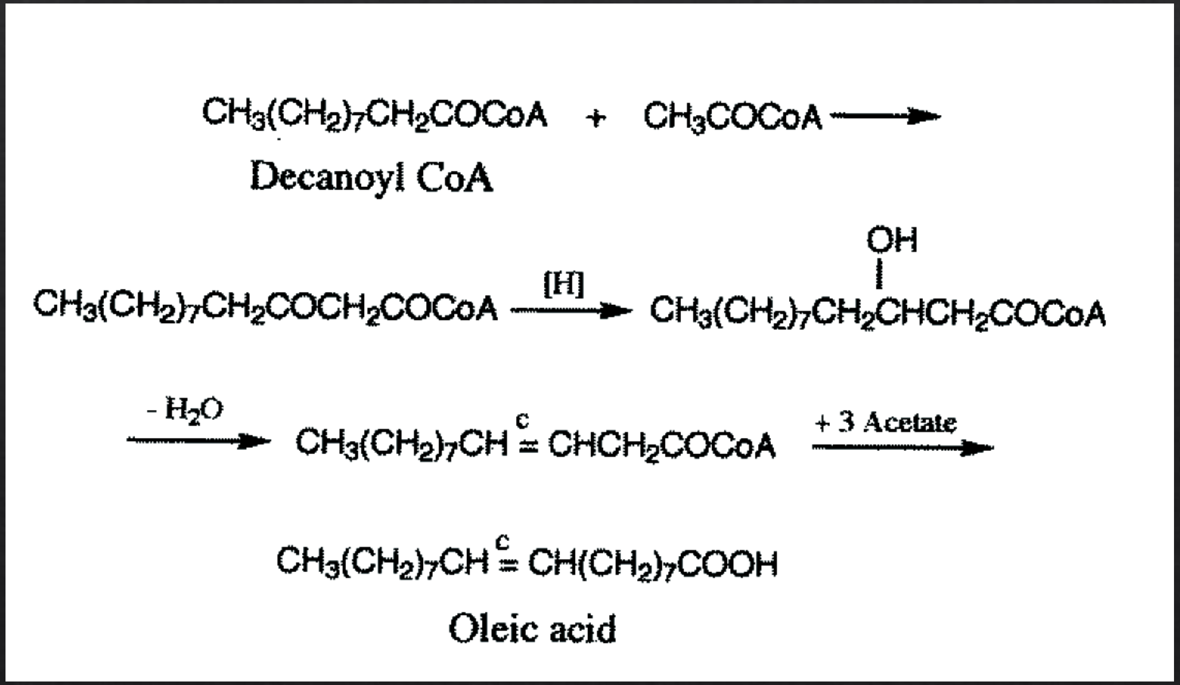
Unsaturated fatty acids & prostagladins

- Unsaturation is introduced differently in aerobic and anaerobic environments

Anaerobic

Double bond comes from dehydrated hydroxyalkanoylthioesters (intermediates in the fatty acid synthesis)

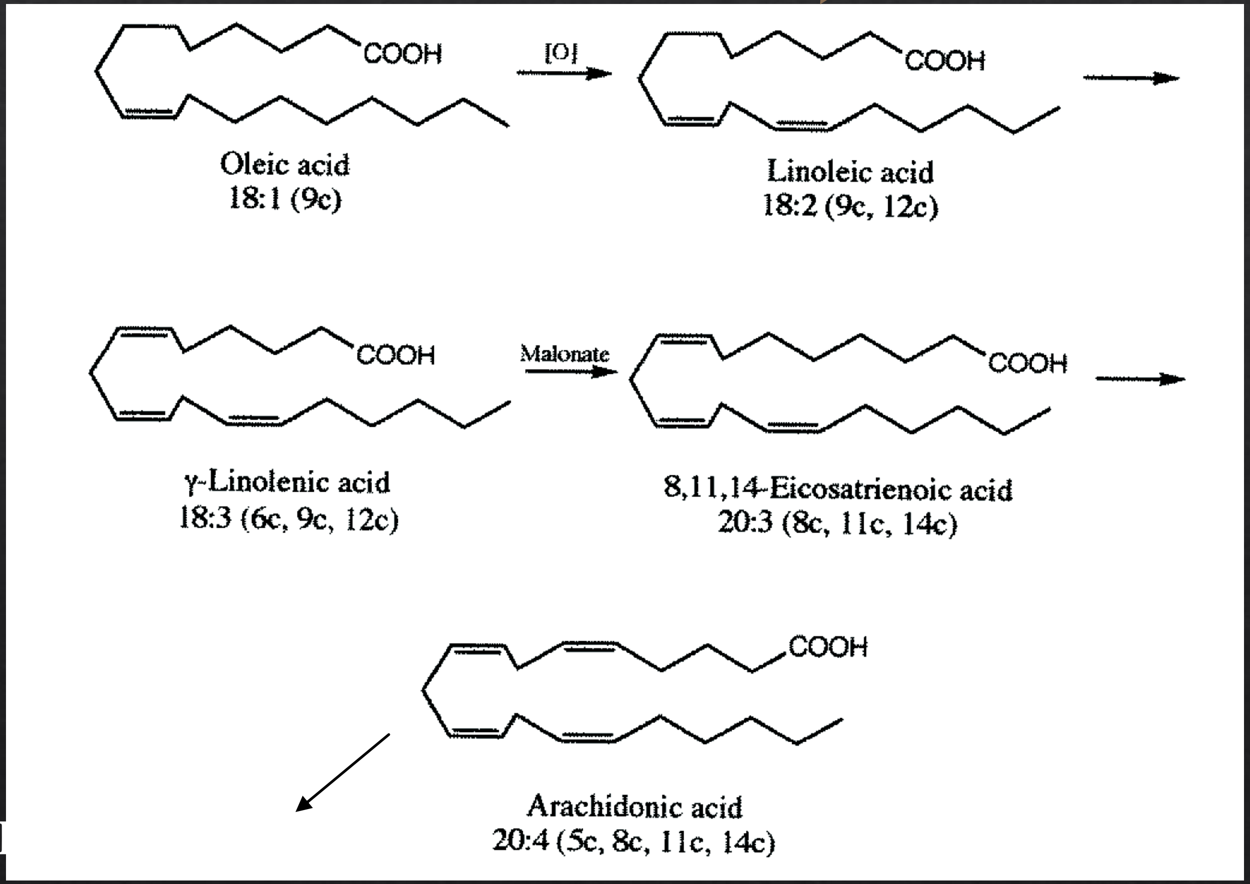
More «economic»



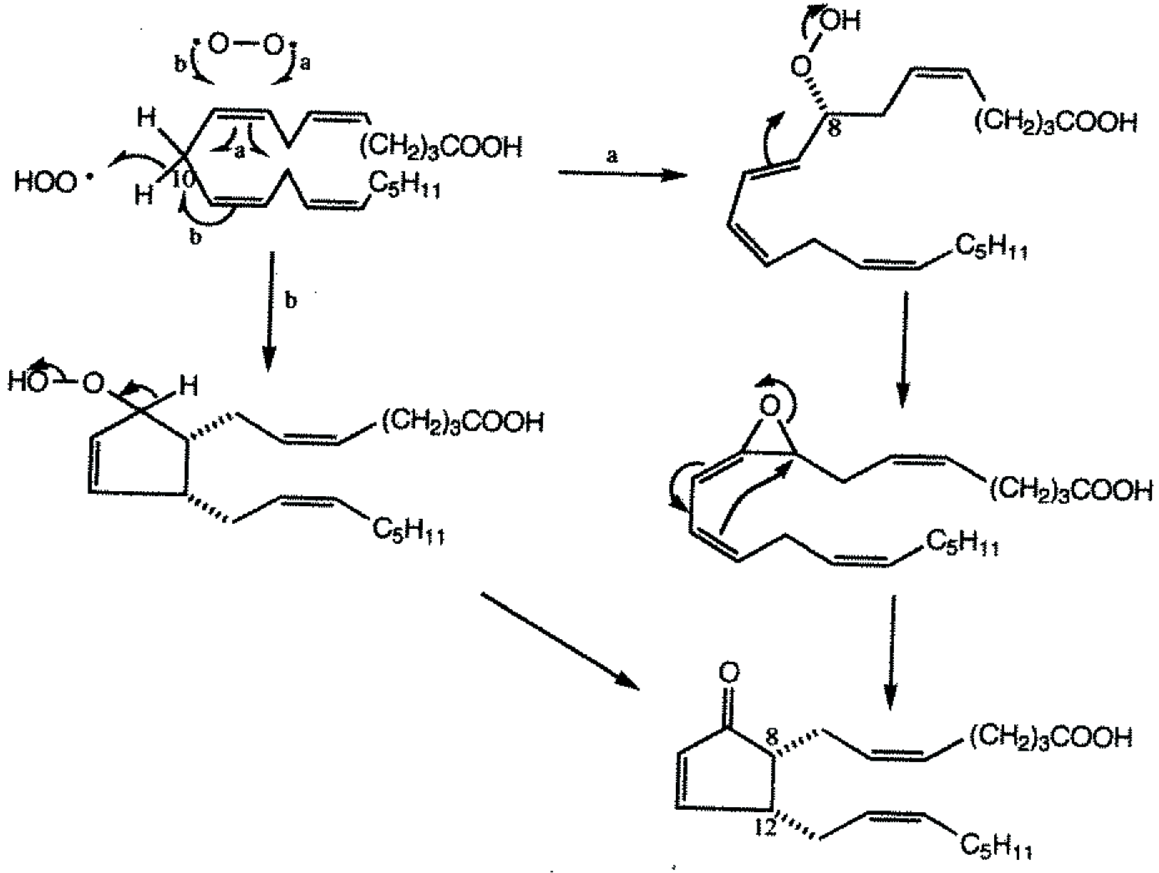
Further desaturation of oleic acid gives linoleic and linolenic acids

Unsaturated fatty acids & prostagladins

- **Arachidonic acid** is a precursor for prostagladin hormones
  - Biosynthesized from oleic acid
- Prostagladins
  - Control blood pressure, contractions of smooth muscle, gastric acid secretion, platelet aggregation
  - Medical properties



Essential fatty acid for mammals



Preclavulone A

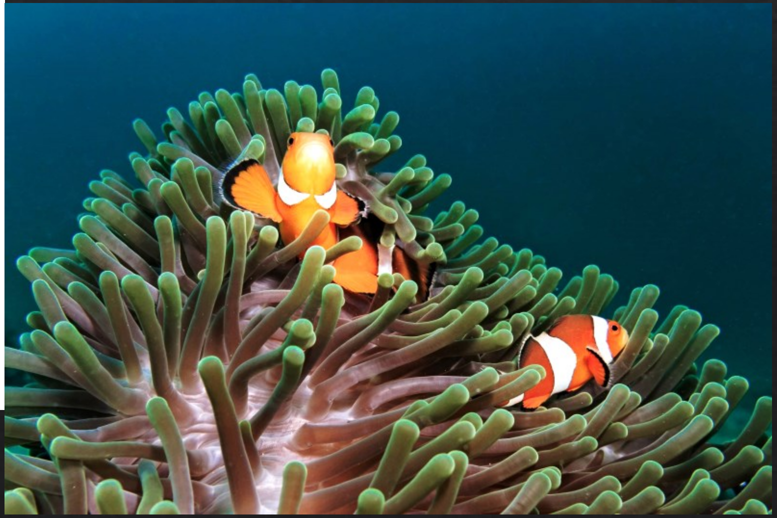
Example:

Biosynthesis of preclavulone A

In corals

Attack at C10 of arachidonic acid

Catalyzed by a lipooxygenase



Acetylene (ethyne) compounds


Umbrelliferae


Compositae


Basidiomycetes

- Often unstable, sensitive to light, heat and oxygen
- Formed from polyketide products
  - Further desaturation of fatty acids

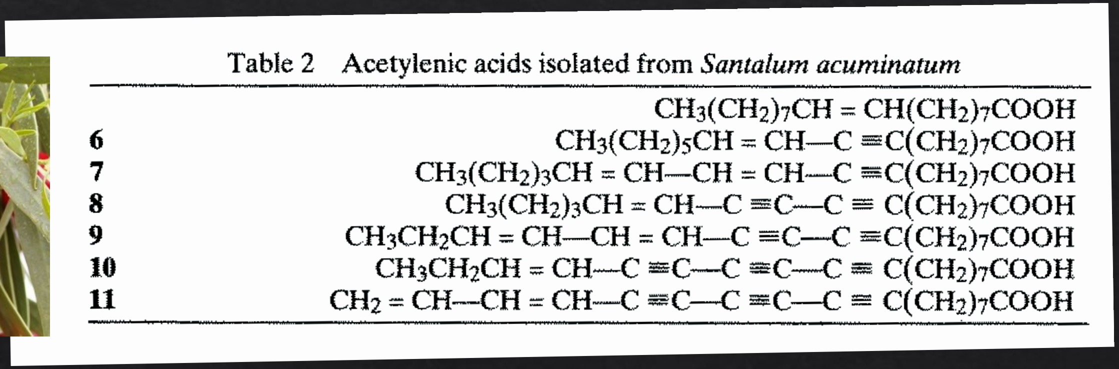
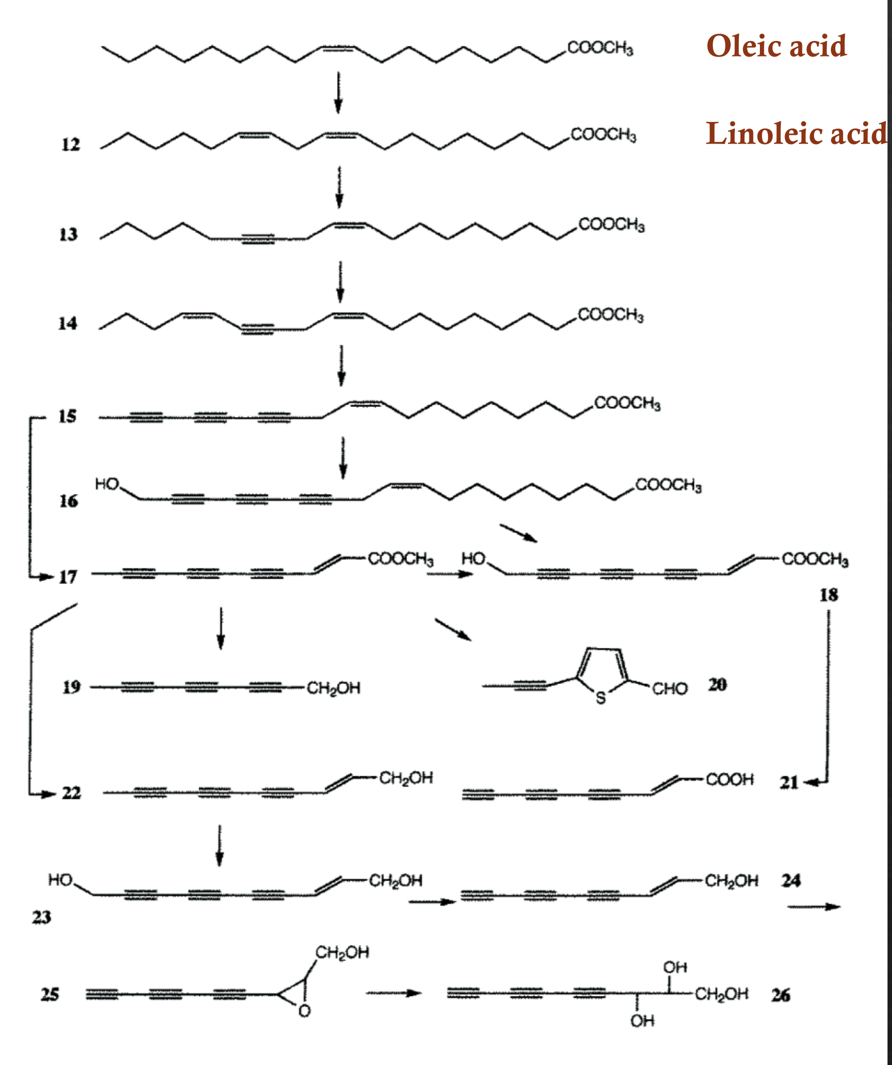


Table 2 Acetylenic acids isolated from *Santalum acuminatum*

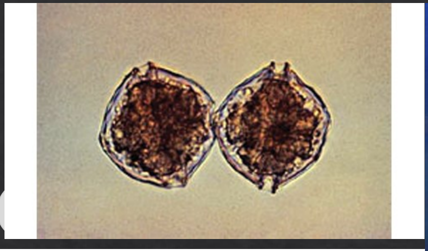
	$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$
6	$CH_3(CH_2)_5CH = CH{-}C \equiv C(CH_2)_7COOH$
7	$CH_3(CH_2)_3CH = CH{-}CH = CH{-}C \equiv C(CH_2)_7COOH$
8	$CH_3(CH_2)_3CH = CH{-}C \equiv C{-}C \equiv C(CH_2)_7COOH$
9	$CH_3CH_2CH = CH{-}CH = CH{-}C \equiv C{-}C \equiv C(CH_2)_7COOH$
10	$CH_3CH_2CH = CH{-}C \equiv C{-}C \equiv C{-}C \equiv C(CH_2)_7COOH$
11	$CH_2 = CH{-}CH = CH{-}C \equiv C{-}C \equiv C{-}C \equiv C(CH_2)_7COOH$

Sequential dehydrogenation catalyzed by desaturases

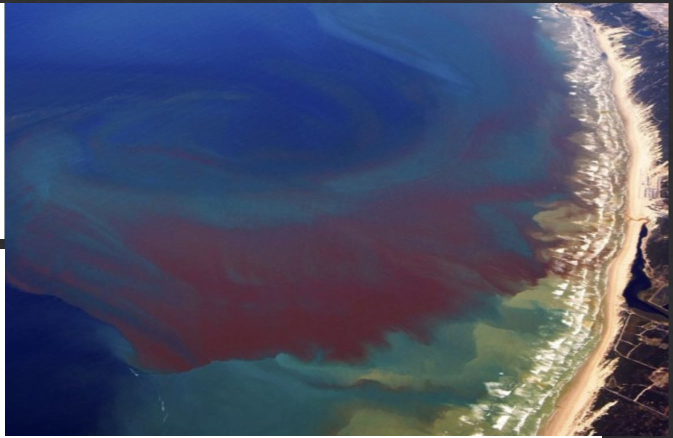

Oleic acid

Linoleic acid

Polyeth

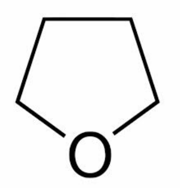


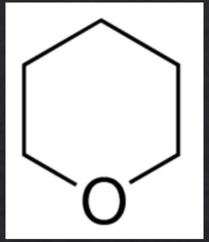
Gymnodinium breve

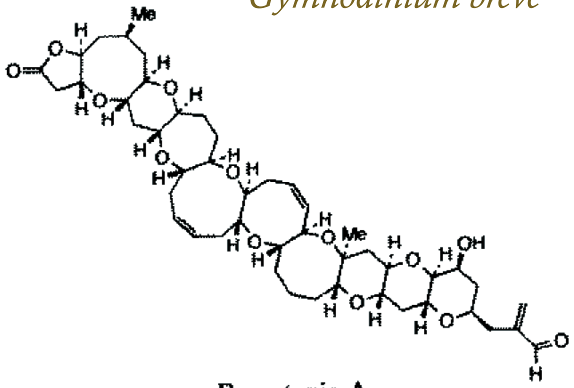


Brevetoxin A is a toxic compound that causes the red tide in the Gulf of Mexico

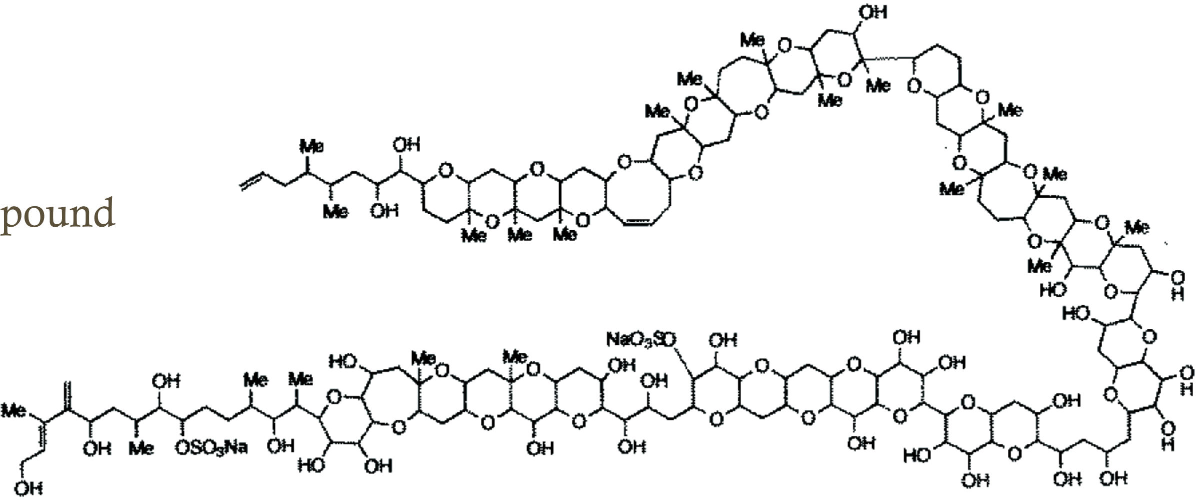
- Contain tetrahydrofuran a
  tetrahydropyrane rings







Brevetoxin A



Maitotoxin

Gambierdiscus toxicus

Maitotoxin is the largest monomeric organic compound known

Mw = 3422 g/mol

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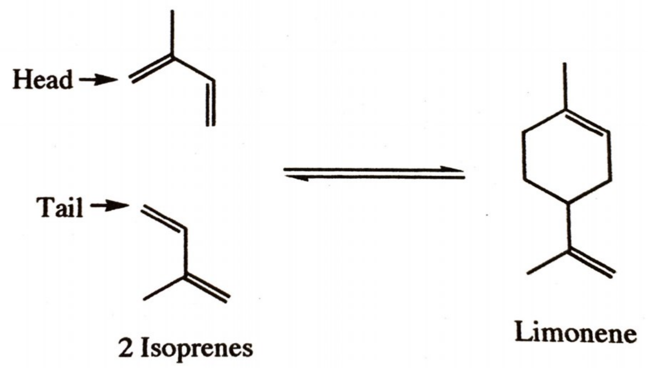
Chapter 6-2
The Mevalonic Acid Pathway
The Terpenes

Elisabeth Jacobsen and Lucas Boquin, NTNU

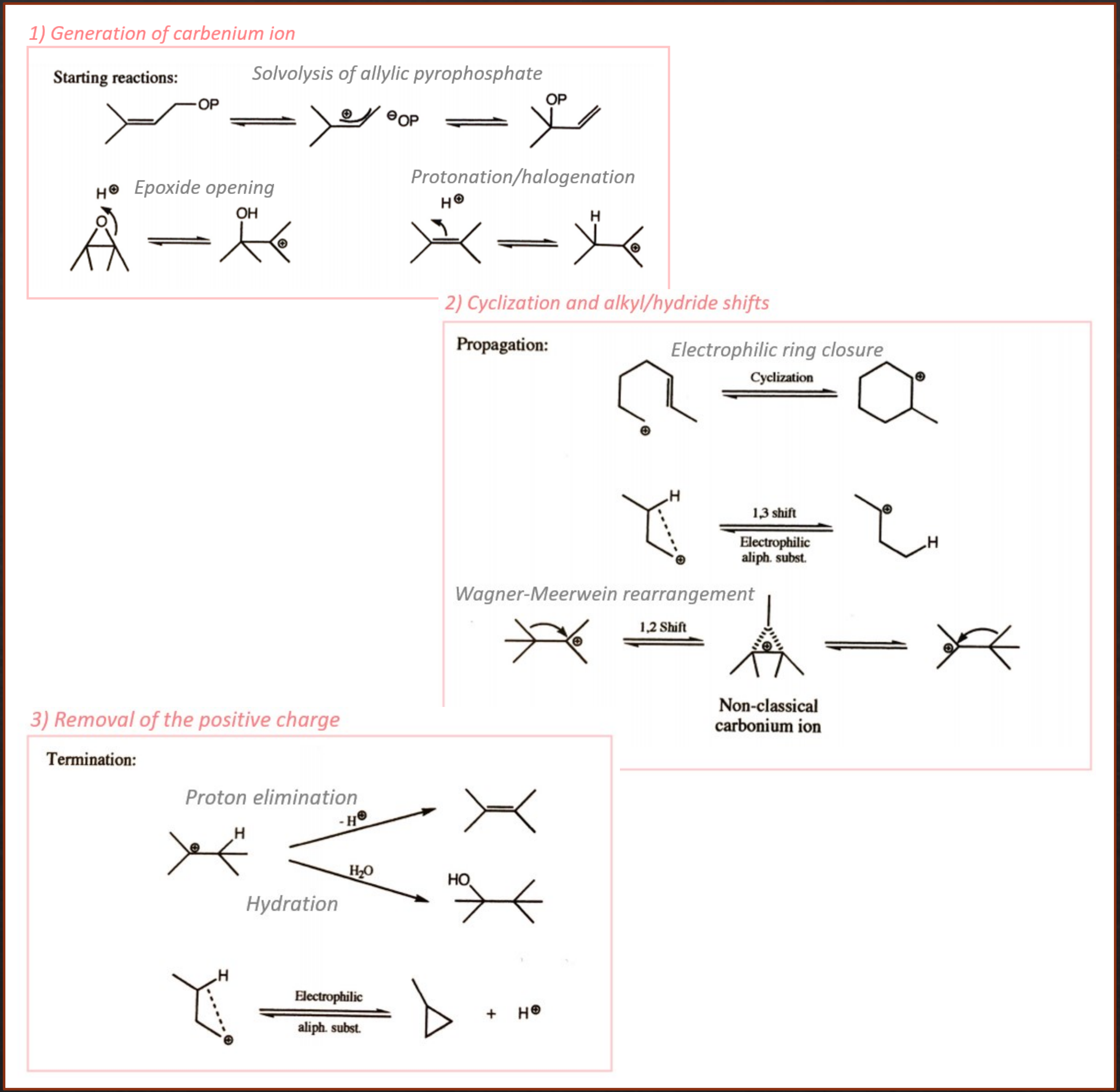
Spring 2022

Previously…

- Terpenes can be broken down into C_5 units called isoprenes

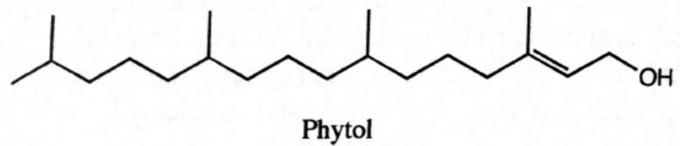


Hemiterpenes	C_5
Monoterpenes	C_{10}
Sesquiterpenes	C_{15}
Diterpenes	C_{20}
Sesterterpenes	C_{25}
Triterpenes	C_{30}
Tetraterpenes	C_{40}
Polyterpenes	C_5 x2000



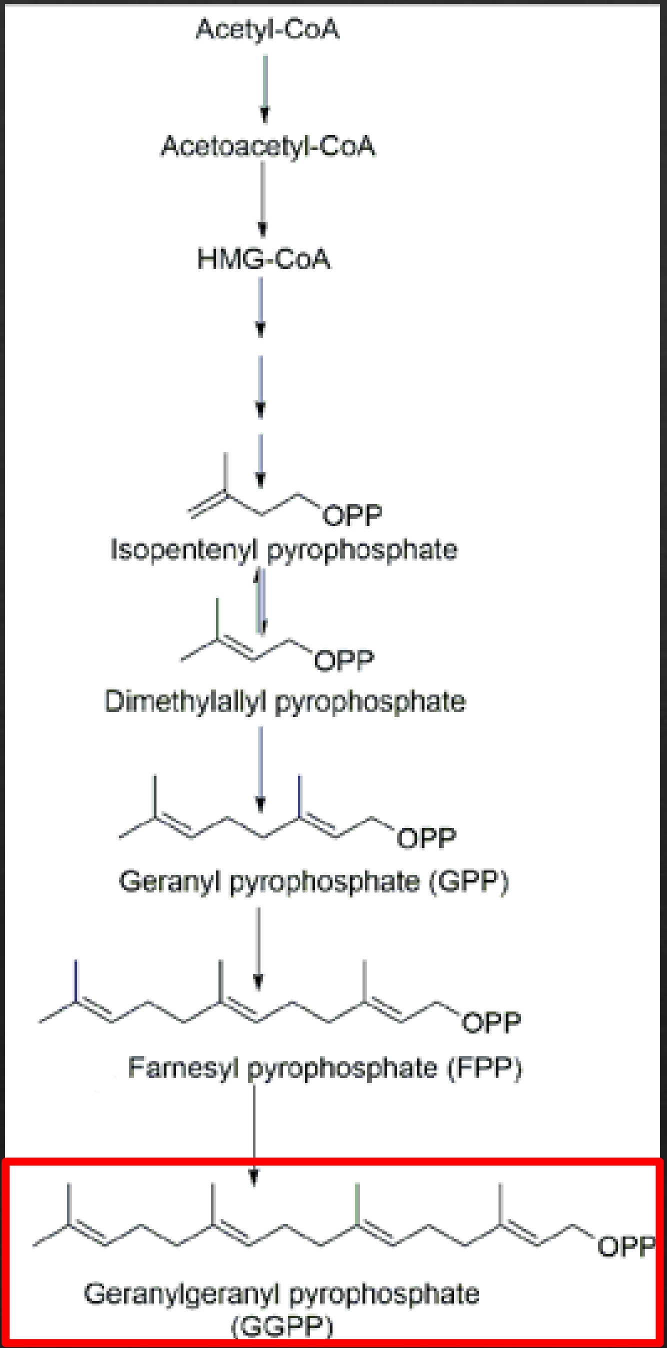
Diterpenes (C_{20})

- Formed from geranylgeranyl pyrophosphate (GGPP)
- Some linear diterpenes exist:



Phytol

Phytol forms the lipophilic side chain of chlorophyll in plants.



Diterpenes (C_{20})

- Formed from geranylgeranyl pyrophosphate (GGPP)
- Some linear diterpenes exist, but most of the diterpenes are mono- (rare), di-, tri- and tetracyclic derivatives.

Sclareol is present in *Salvia sclarea*. It is used in the perfume industry, and in the treatment of leukemia

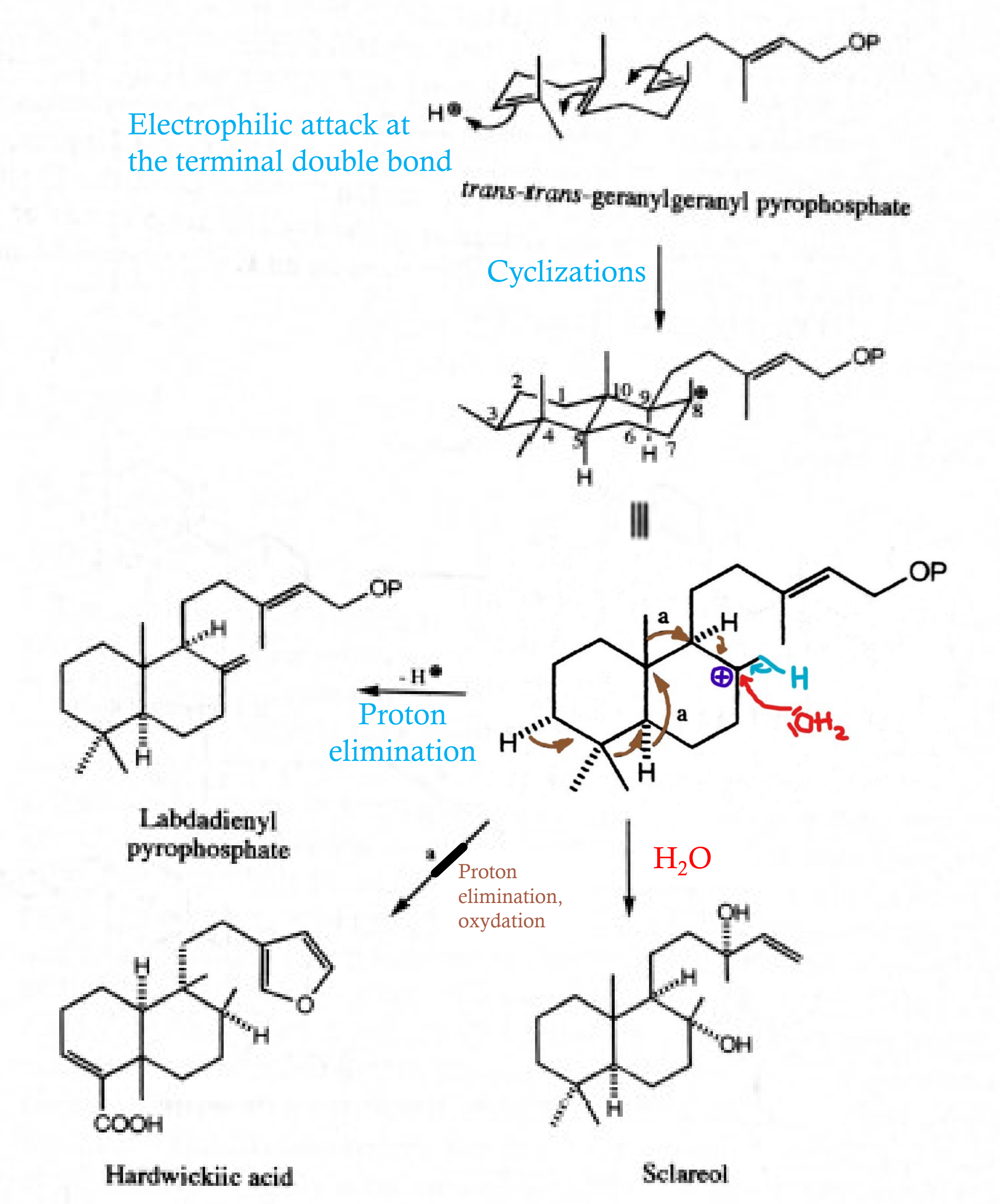


Fig. 19 Cyclization of all-*trans*-geranylgeranyl pyrophosphate in a chair–chair conformation to bicyclic diterpenes

Diterpenes (C_{20})

- A lot of tricyclic diterpenes are formed from the solvolysis of labdadienyl pyrophosphate.

Abietic acid is a widely distributed molecule in *Coniferae*. It is used, among others, in soaps and in lacks.

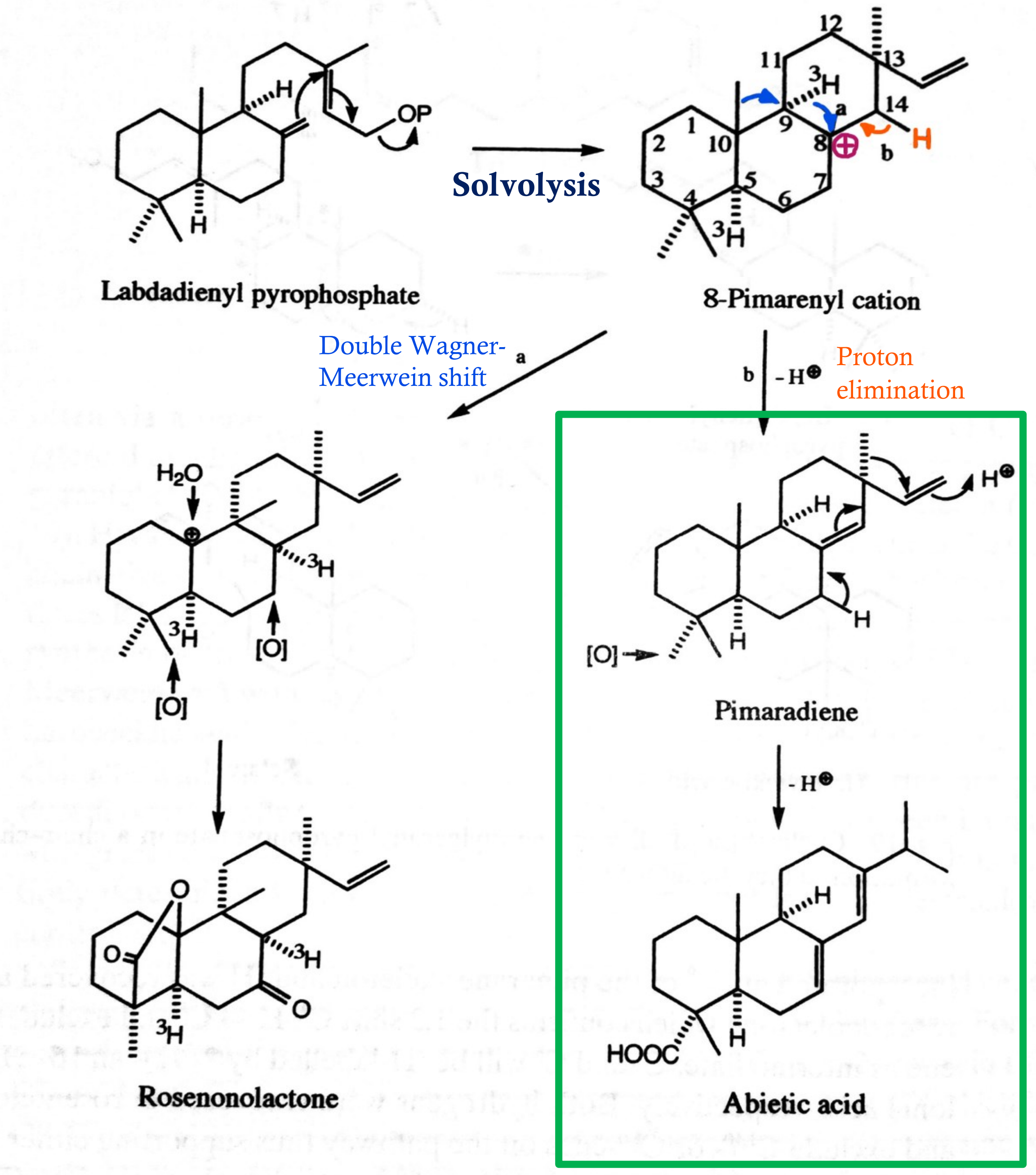


Fig. 20 Biosynthesis of tricyclic diterpenes

Diterpenes (C_{20})

- Tetracyclic diterpenes can also be formed from labdadienyl pyrophosphate. A lot of them are used as hormones.
- Finally, macrocyclic diterpenes also exist.
  - In nasutiterme termites, cambrenes serve as scent-trail pheromones.
  - Certain cambrenes have tumor inhibitor effects

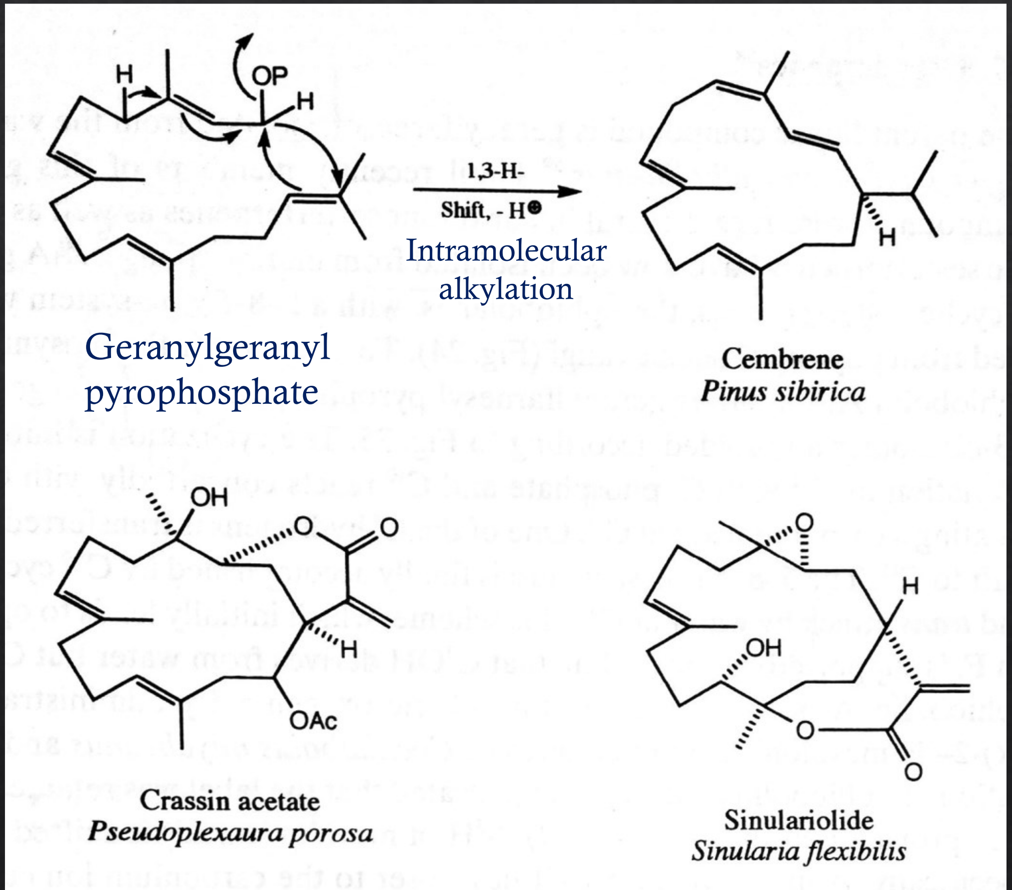
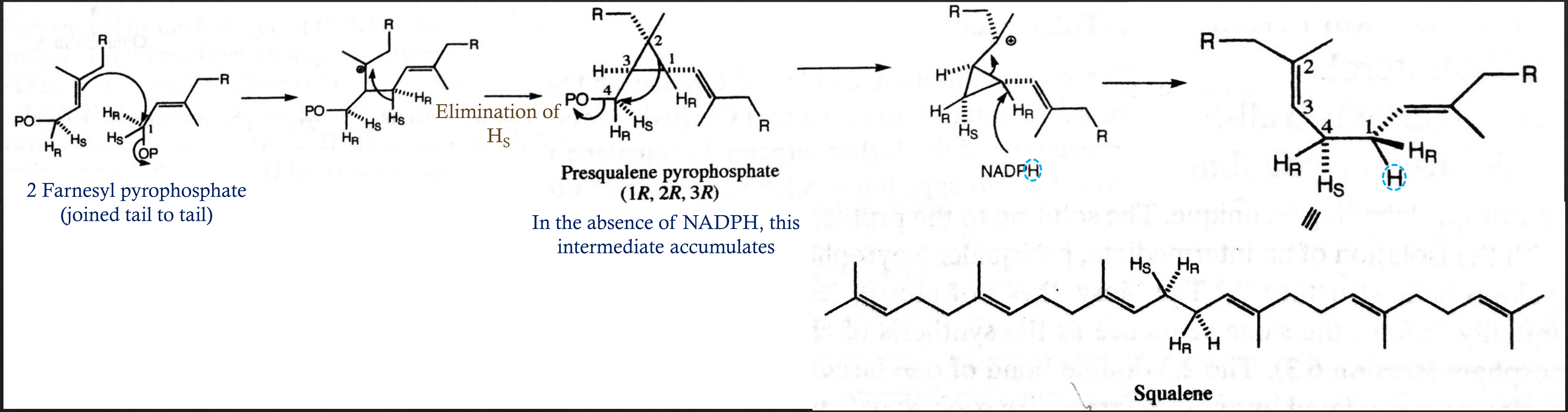


Fig. 23 Macrocyclic diterpenes

Squalene (Triterpenes, C_{30})

- Squalene is a rare C_{30} hydrocarbon that is thought to be the precursor of a lot of higher terpenoids. It is, for instance, an intermediate for the biosynthesis of cholesterol.



2 Farnesyl pyrophosphate (joined tail to tail)

Presqualene pyrophosphate (1*R*, 2*R*, 3*R*)

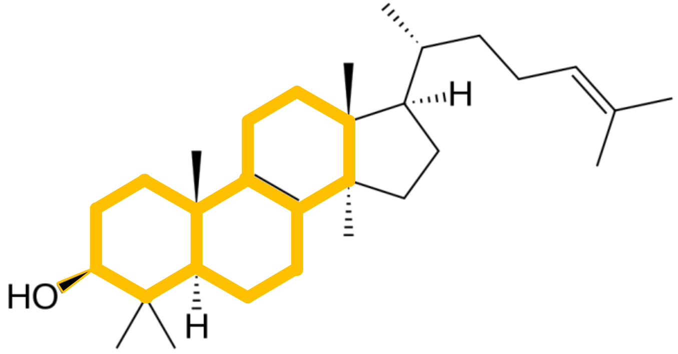
In the absence of NADPH, this intermediate accumulates

Squalene

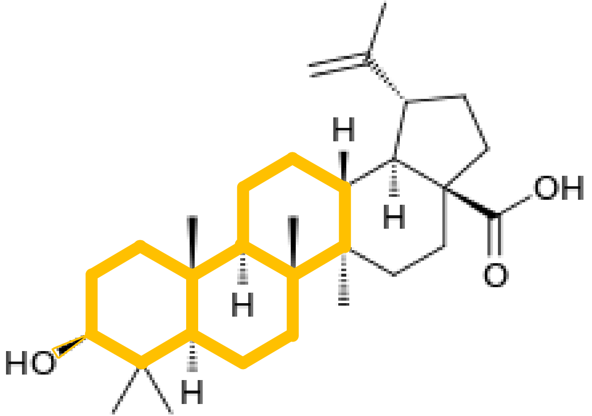
- Fording squalene then allow the formation of a lot of triterpenes.

Triterpenes, C_{30}

- Triterpenes have few skeletal variations; they have usually three 6-membered rings, and an equatorial hydroxy group on the first ring.
- They can be divided into two groups: tetracyclic triterpenes and pentacyclique triterpenes.



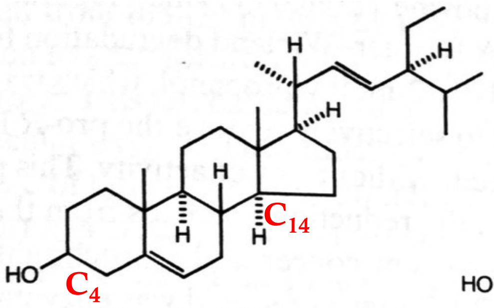
Lanosterol is the precursor of all steroids produced by animals and fungi



Butelonic acid has antimalarial, anti-inflammatory, and potentially anticancer properties

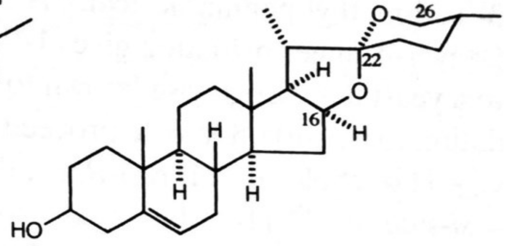
Steroids

- Most of steroids come from squalene, but have lost the carachteristic squeleton of terpenes because of extensive degradation (oxidative cleavage, ring openings, wagner-meerwein shifts, introduction of additional hydroxyl and olefinic groups,…)
- The fundamental secondary modification leading to steroids is selective C_4 and C_{14} demethylation.



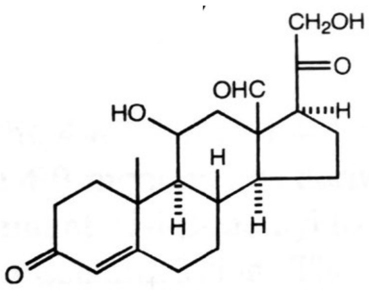
Stigmasterol (Phytosterol)

Sterols are essential structural component of cell membranes (like cholesterol in animals)



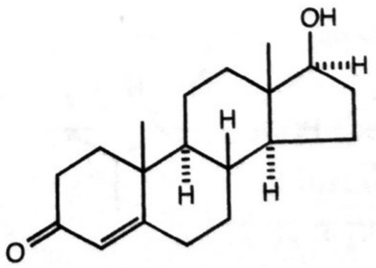
Diosgenin (Sapogenin)

Sapogenin can protect plants against microbes, fungi, and other organisms



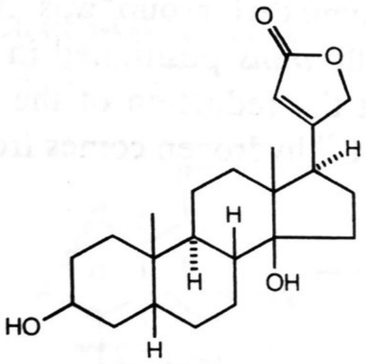
Aldosterone (Adrenal steroid)

Adrenal steroids are produced by the adrenal glands. They regulate the metabolism of sugars and proteins, and the water level in the kidney. A lot of them are involved in stress response processes.



Testosterone (Male sex hormone)

In both sexes, testosterone plays an important role in health and well-being. Women have less of this hormone than men, but are more sensitive to it.



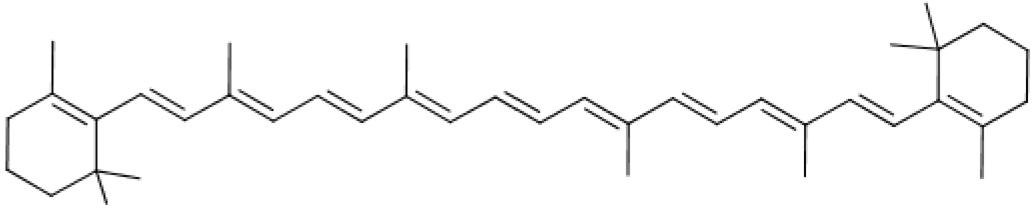
Digitoxigenin (Cardiac aglycone)

Cardiac glycosides have powerful and specific actions on the heart muscle. They are used in heart ailment treatments.

Carotenes (C_{40})

- Their biosynthesis is similar to the one of squalene: Tail-to-tail coupling of two pyrophosphate (here: geranylgeranyl pyrophosphate).
- They are yellow-red conjugated polyene pigments, and are present in egg yolks, carrots, tomatoes, yellow automn leaves, algae,…

During the autumn, the first pigment in leaves to degrade is chlorophyll. The main remaining pigments (carotenes), give the characteristic yellow color to the leaves.



β-Carotene is transformed through oxidative fission into vitamin A (retinol) and retinal, which play important role in vision.

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Chapter 6-1
The Mevalonic Acid Pathway
The Terpenes

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

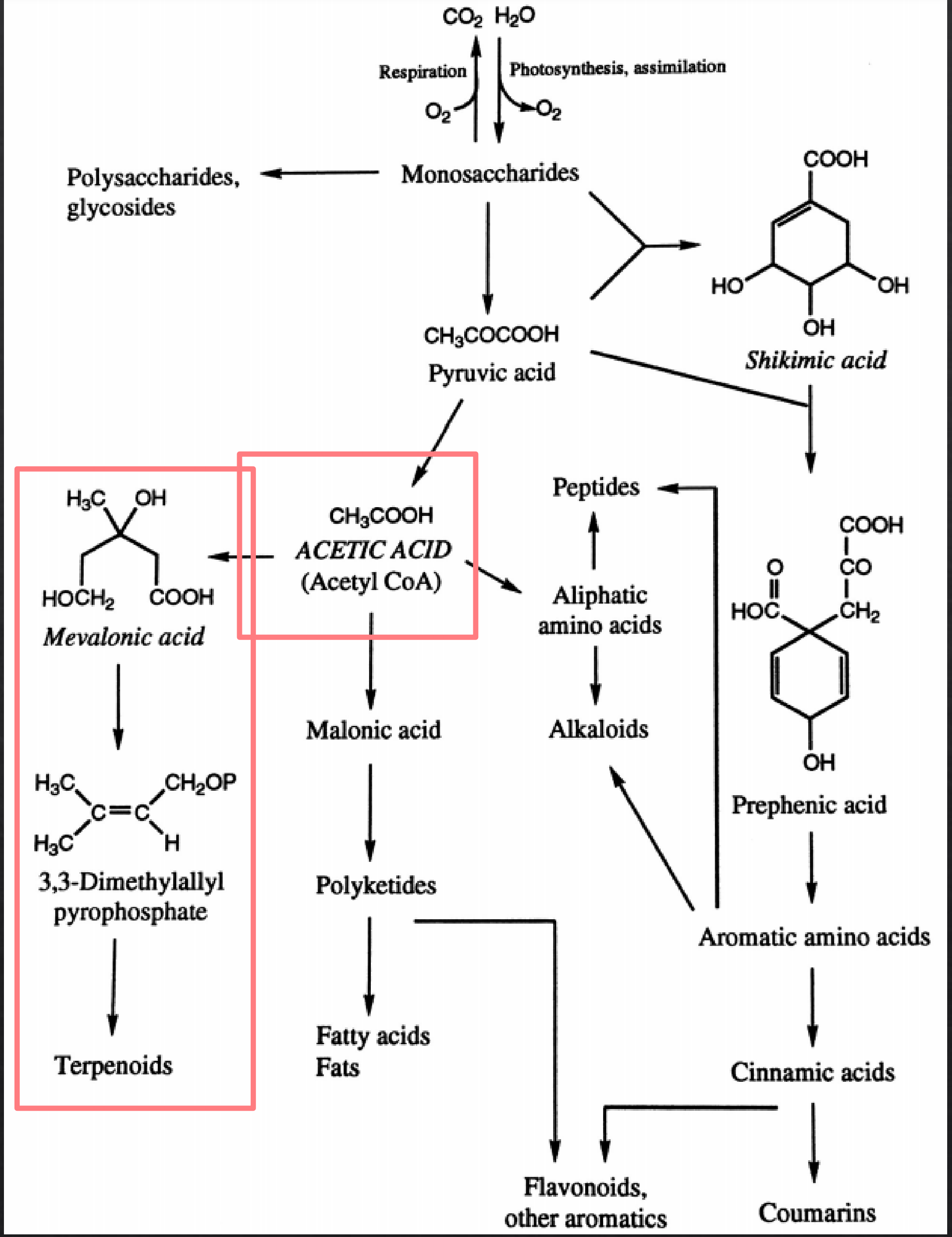
Spring 2022

Mevalonic acid pathway is present in most organisms

Gives the precursors for different end products in each organism

- Animals (steroid hormones, cholesterol)
- Plants (terpenes, terpenoids)

Most organisms produce terpenes through the Mevalonic acid pathway, but there is also an alternative non-mevalonic acid pathway (that we will not discuss).



Terpenes

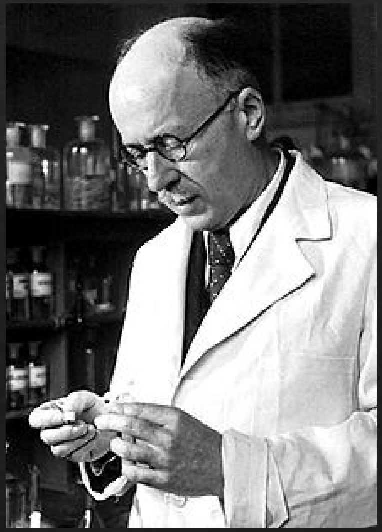
- Terpenes are secondary metabolites (primarily from plants)
  - $(C_5H_8)_n$ - isoprene (isopentenyl) units
  - Terpenoids (aka. isoprenoids) are terpenes with additional functional groups (typically oxygen)

The name «terpene» comes from the terebinth tree (*Pistacia terebinthus*)

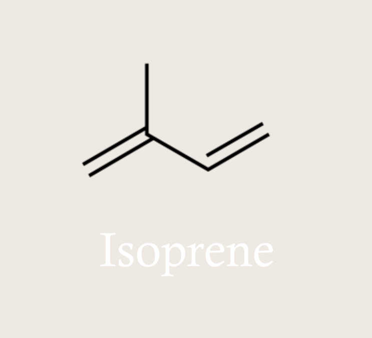
The isoprene rule

Hemiterpenes	C_5
Monoterpenes	C_{10}
Sesquiterpenes	C_{15}
Diterpenes	C_{20}
Sesterterpenes	C_{25}
Triterpenes	C_{30}
Tetraterpenes	C_{40}
Polyterpenes	C_5 x2000

Steroids (C_{27}) do not follow the isoprene rule



Leopold Ruzicka: The Nobel Prize in Chemistry 1939, for his work on terpenes and being the first to synthesize male sex hormones



Isoprene

Applications o es

Terpenes function as protective or attractive substances in plants and some animals

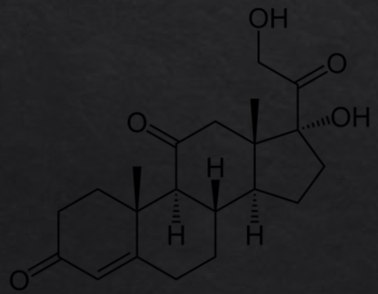
Perfume industry
Essential oils

Painting industry
Turpentine/terpentine

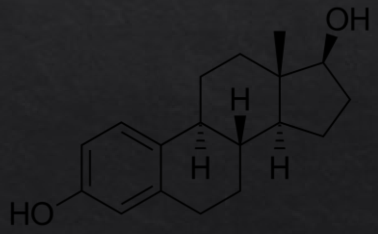
Natural rubber
Polyisoprene

Some thermites fire glue-like, toxic terpene mixtures at predators from «guns» on their head

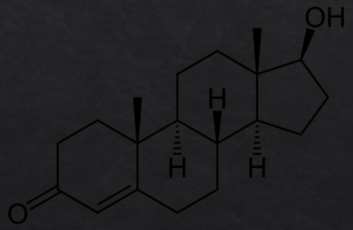
J. Sobotník et al. (2010) Journal of Insect Physiology 56 1012–1021



Cortisone

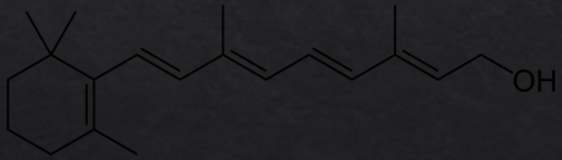


Estrogen

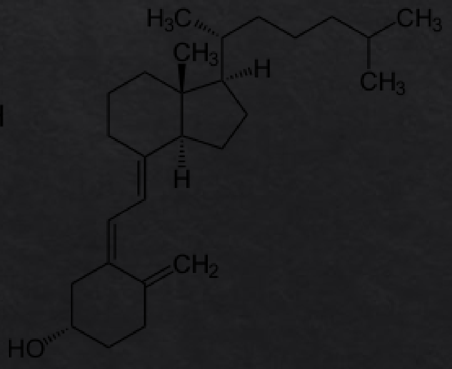


Testosterone

Hormones

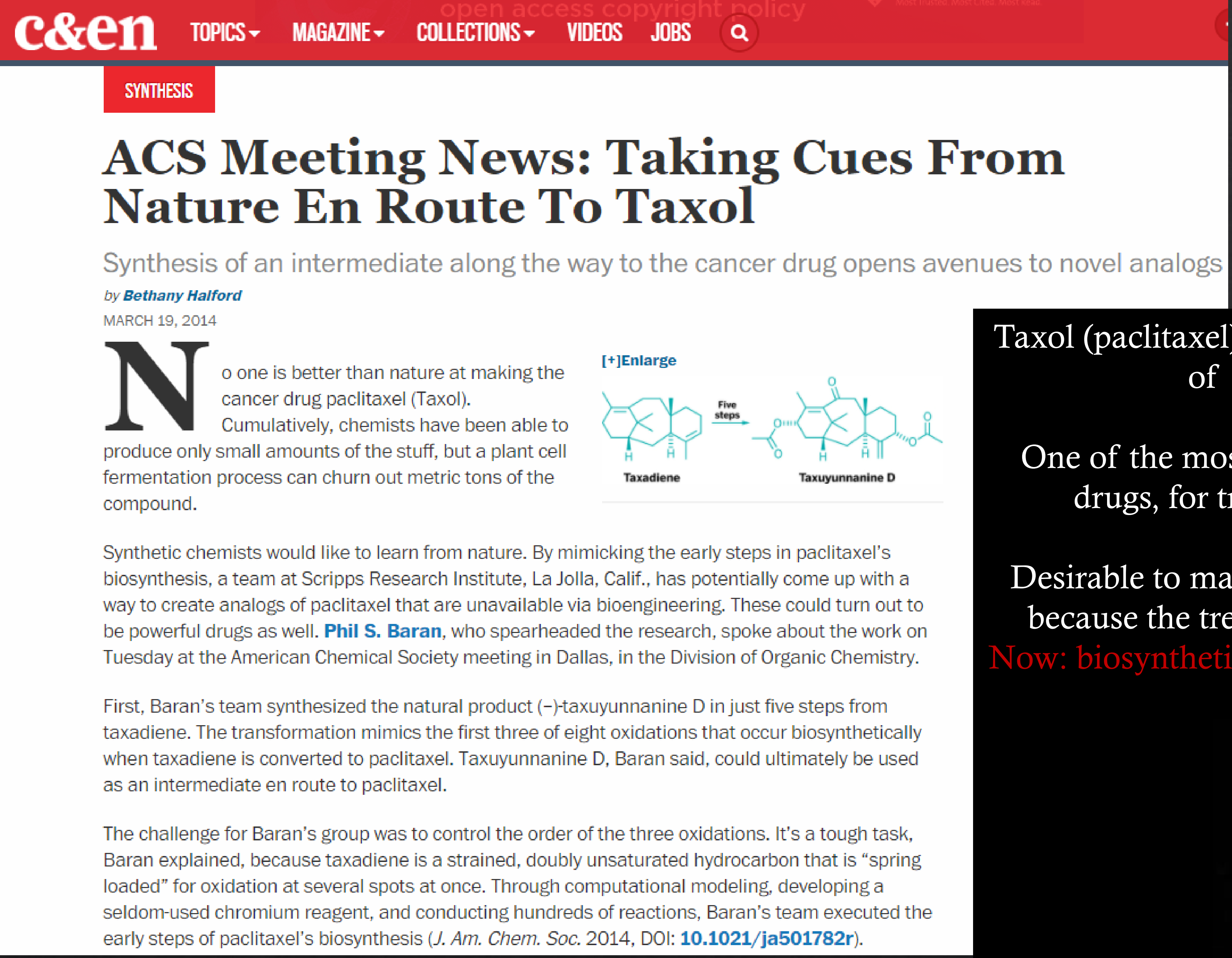


Retinol (vitamin A)



Cholecalciferol (vitamin D3)

Vitamins



ACS Meeting News: Taking Cues From Nature En Route To Taxol

Synthesis of an intermediate along the way to the cancer drug opens avenues to novel analogs

by **Bethany Halford**

MARCH 19, 2014

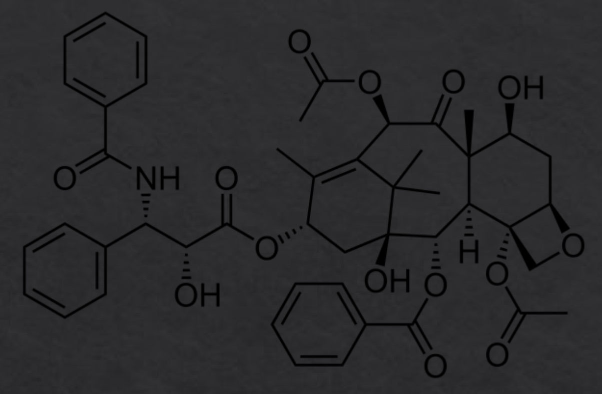
No one is better than nature at making the cancer drug paclitaxel (Taxol). Cumulatively, chemists have been able to produce only small amounts of the stuff, but a plant cell fermentation process can churn out metric tons of the compound.

Synthetic chemists would like to learn from nature. By mimicking the early steps in paclitaxel's biosynthesis, a team at Scripps Research Institute, La Jolla, Calif., has potentially come up with a way to create analogs of paclitaxel that are unavailable via bioengineering. These could turn out to be powerful drugs as well. **Phil S. Baran**, who spearheaded the research, spoke about the work on Tuesday at the American Chemical Society meeting in Dallas, in the Division of Organic Chemistry.

First, Baran's team synthesized the natural product (−)-taxuyunnanine D in just five steps from taxadiene. The transformation mimics the first three of eight oxidations that occur biosynthetically when taxadiene is converted to paclitaxel. Taxuyunnanine D, Baran said, could ultimately be used as an intermediate en route to paclitaxel.

The challenge for Baran's group was to control the order of the three oxidations. It's a tough task, Baran explained, because taxadiene is a strained, doubly unsaturated hydrocarbon that is "spring loaded" for oxidation at several spots at once. Through computational modeling, developing a seldom-used chromium reagent, and conducting hundreds of reactions, Baran's team executed the early steps of paclitaxel's biosynthesis (*J. Am. Chem. Soc.* 2014, DOI: **10.1021/ja501782r**).

Chem. & Eng. News March 19., 2014, ISSN 0009-2347



Taxol (paclitaxel) was typically derived from the bark of the tree «Pacific yew».

One of the most popular naturally derived cancer drugs, for treating primarily breast cancer.

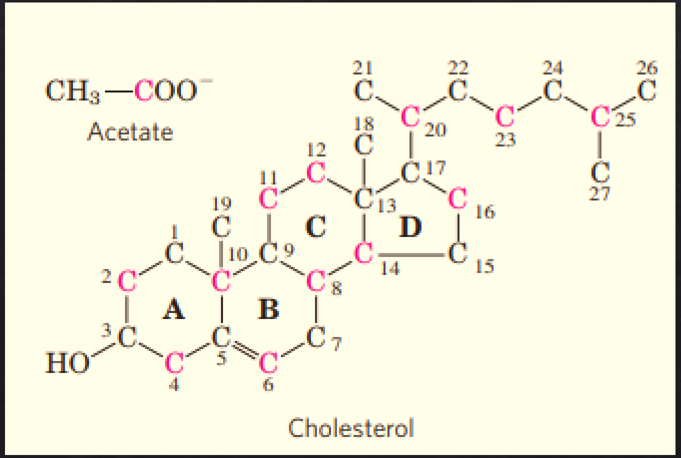
Desirable to make it from more renewable sources, because the trees die when the bark is harvested.
Now: biosynthetic routes are becoming more popular.

Cholesterol structure

Many terpenes are not UV active
UV spectroscopy did not work well

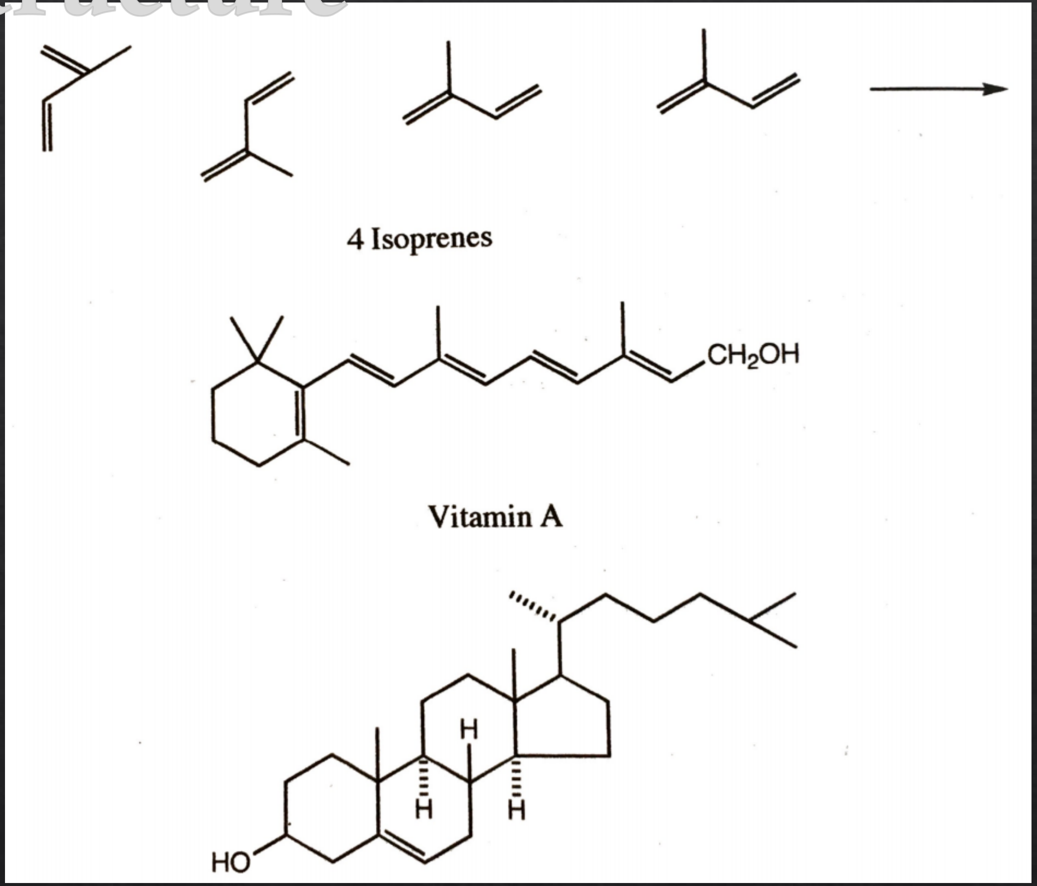
Cholesterol

- Structure defined in 1932
  - XRD



Origin of carbon atoms in cholesterol

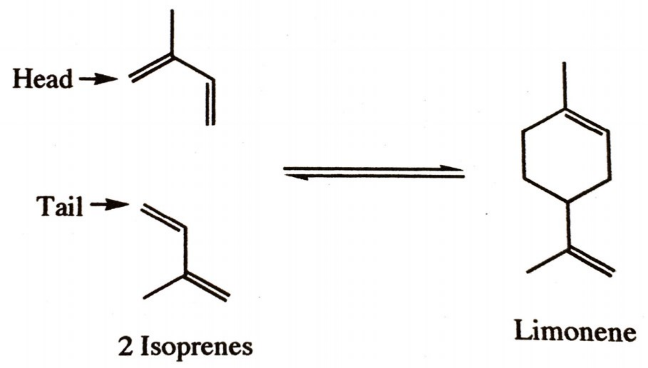
Lehninger Principles of Biochemistry, 6th ed. 2013



Cholesterol

Cholesterol-lowering drugs (statins) inhibit the mevalonic acid pathway

Mevalonic acid p



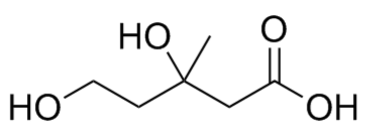
Limonene can be synthesized by a Diels-Alder reaction of two isoprene units, but isoprene is not the starting material in biosynthesis of limonene and other terpenes.

In 1956, mevalonic acid was isolated, and i was shown how mevalonic acid can be a building block in terpene biosynthesis

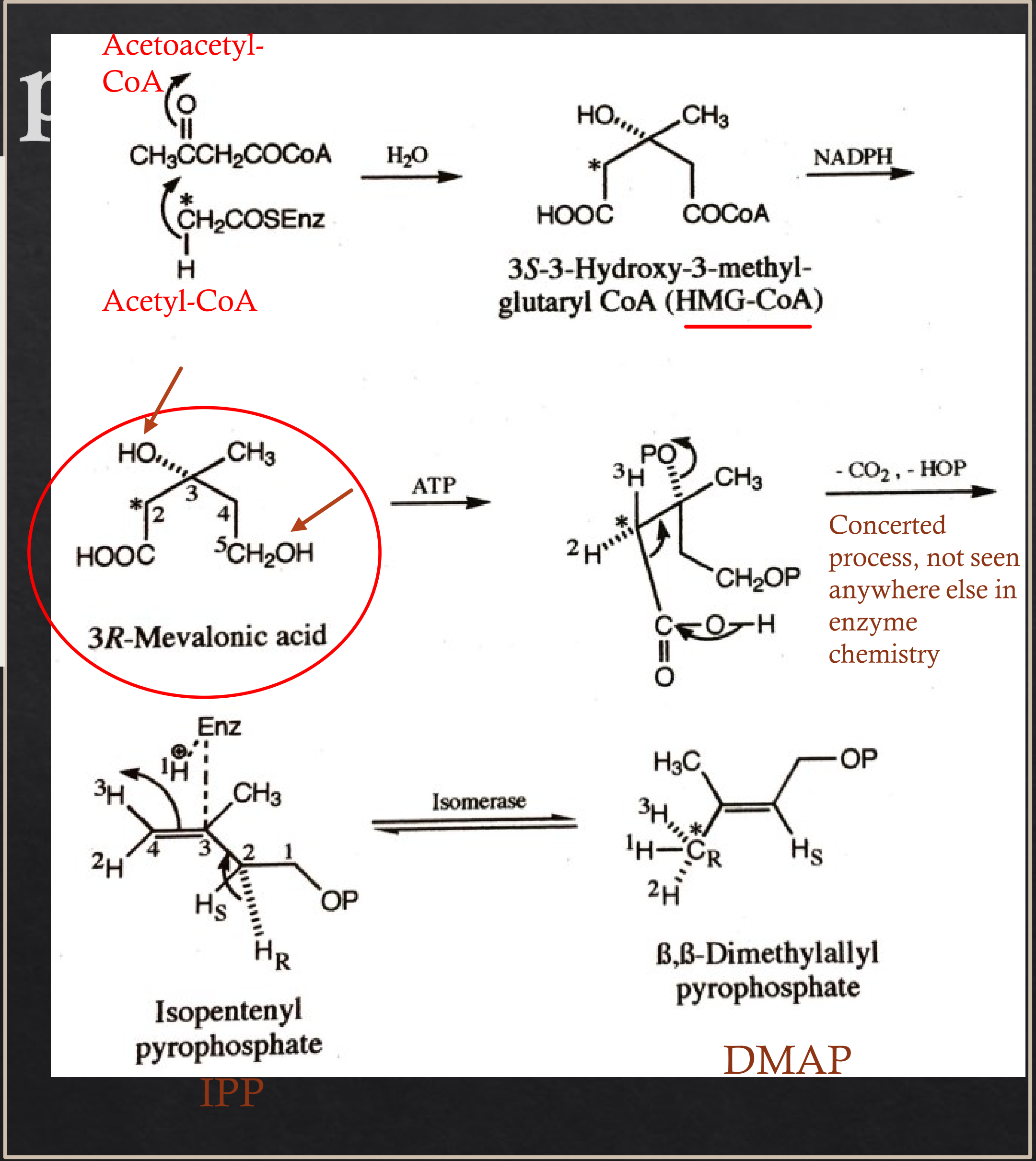
First part is the same in all organisms

Second part happens differently in eukaryotes and archaea (here: archaea)

End products are the same



Biosynthesis of starter units of terpenes: DMAP/IP



Acetoacetyl-CoA

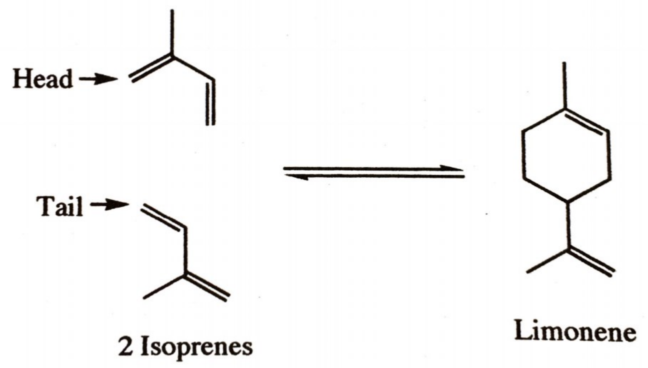
Acetyl-CoA

Concerted process, not seen anywhere else in enzyme chemistry

IPP

DMAP

Mevalonic acid p



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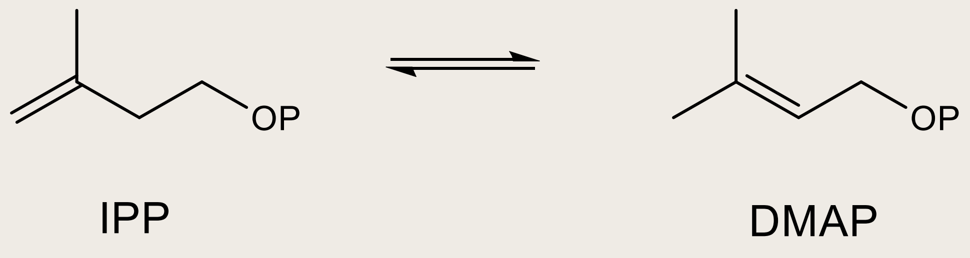
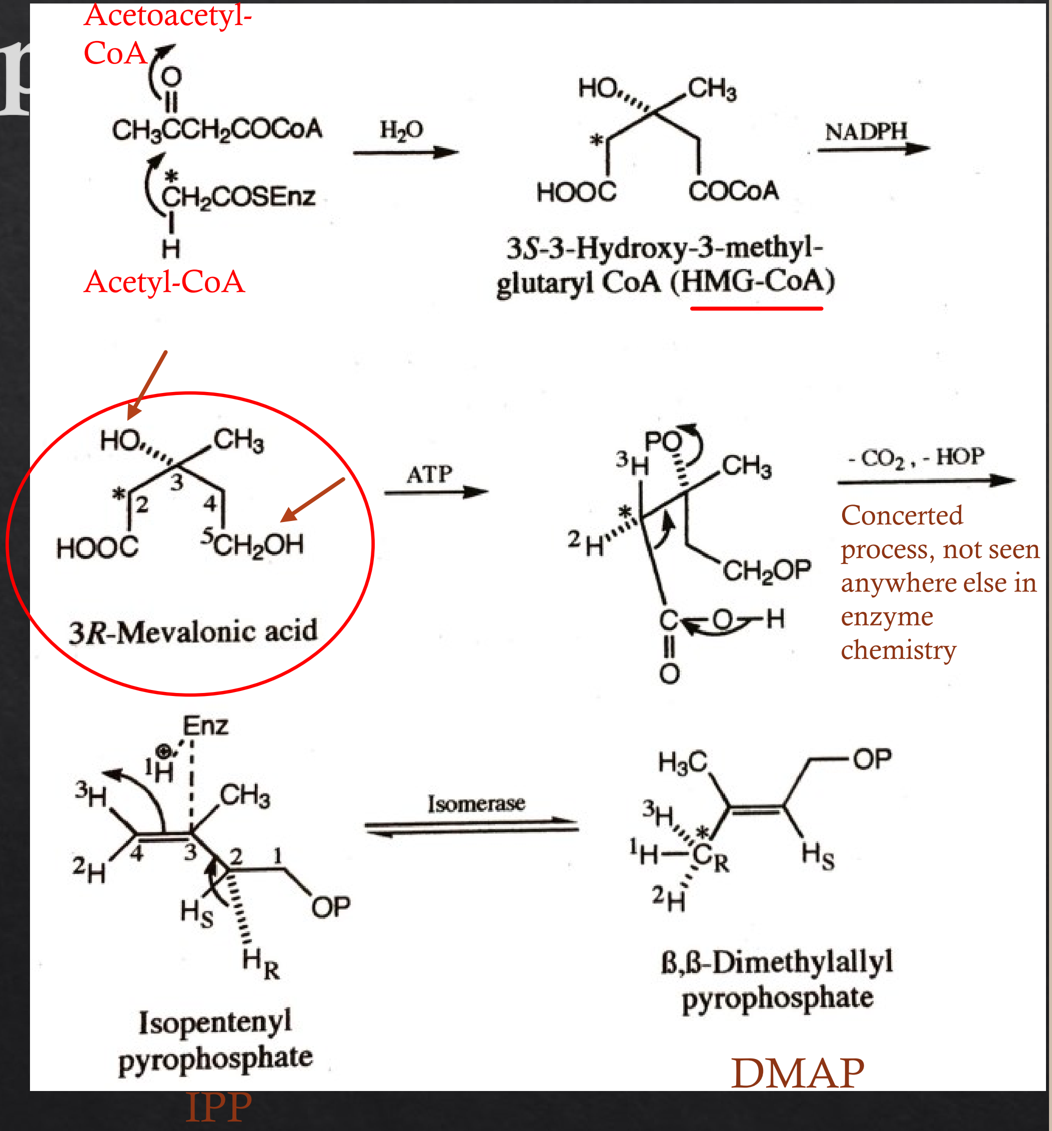


«Activated isoprenes»

Biosynthesis of starter units of terpenes: DMAP/IP



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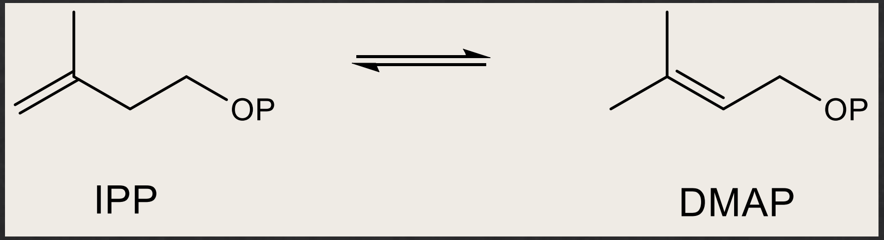
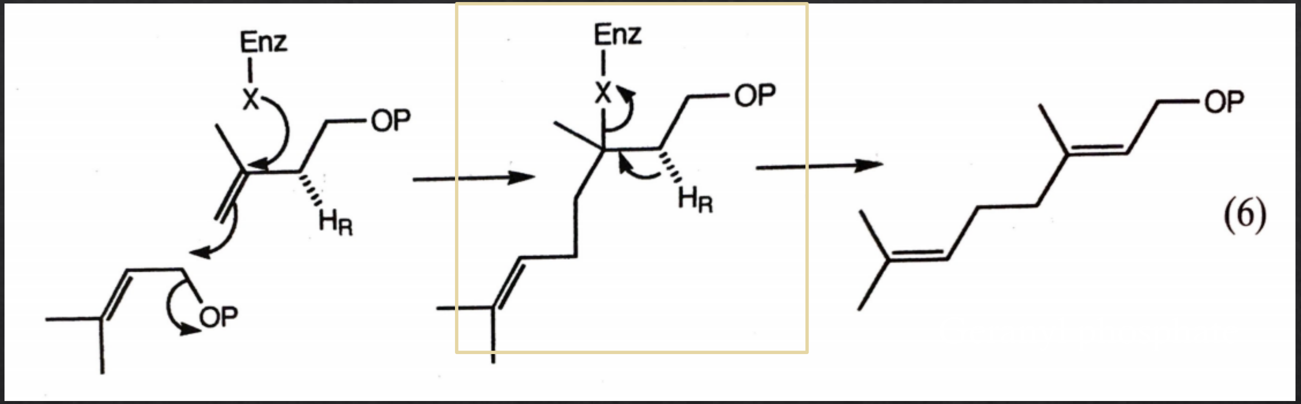
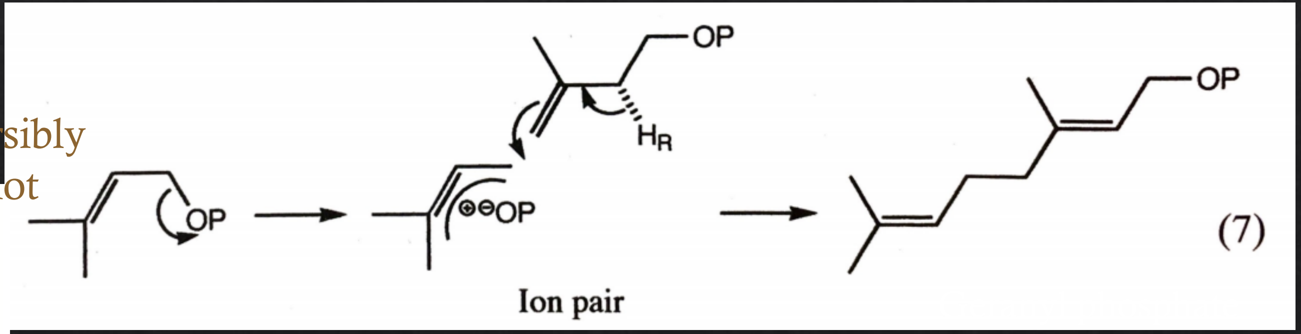
Biosynthesis of terpenes



«Activated isoprenes»

Two proposed mechanisms for further reaction between IPP and DMAP:

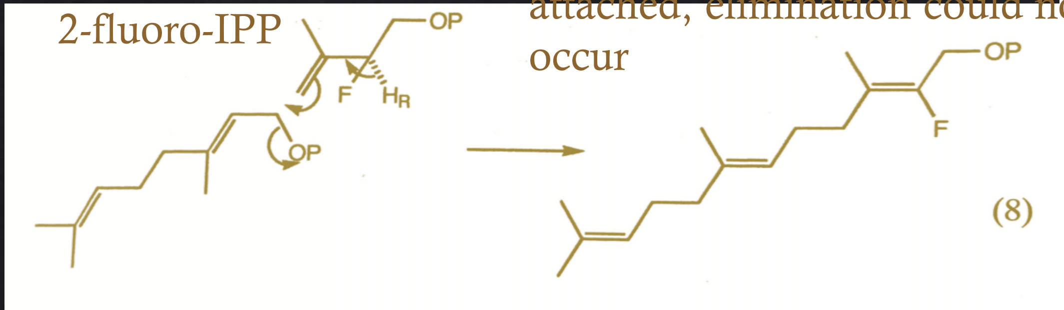




Trans-1,2-addition, and *trans*-1,2-elimination – enzyme functions as a nucleophile

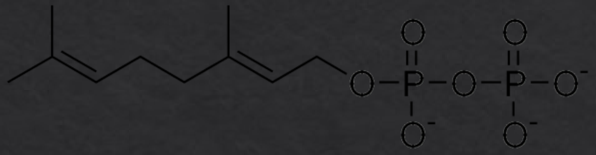
Ionization of DMAP, and addition of IPP to the allylic cation

2-fluoro-IPP would be irreversibly attached, elimination could not occur

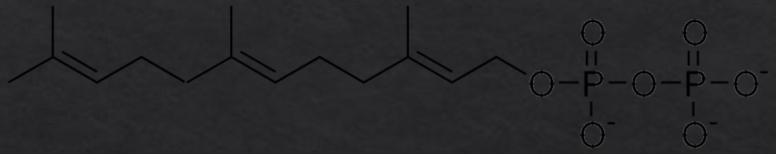


Enzyme activity was not irreversibly lost

4 phases of terpene biosynthesis



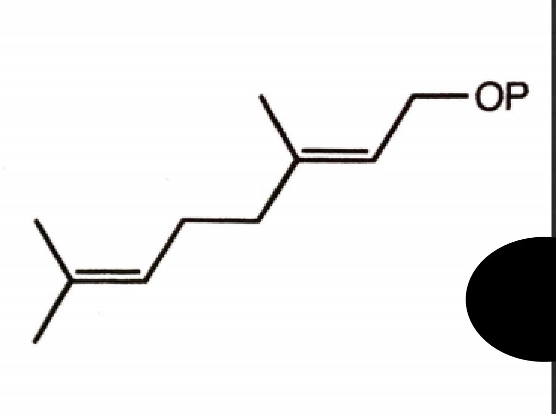
Geranyl pyrophosphate



Farnesyl pyrophosphate

1) Synthesis of IPP and DMAP (the mevalonic acid pathway)
2) Linear polymerization of the isoprene units
   - Gives geranyl PP, farnesyl PP, geranylgeranyl PP, etc.
   - (Linear polyprenyl precursors)
3) Folding, cyclization, rearrangement of polyprenyl precursors.
4) Functionalization to create terpenoids

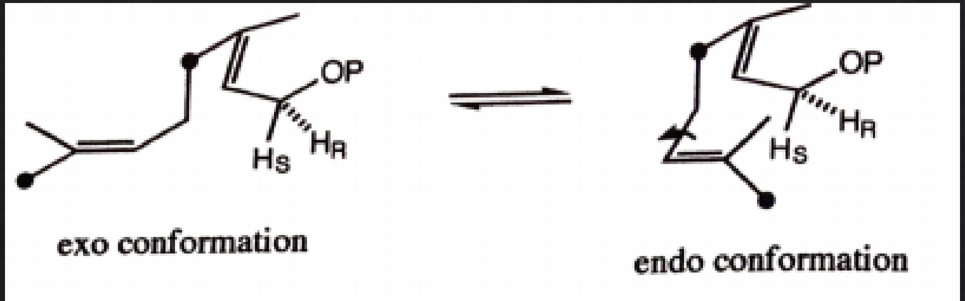
Biosynthesis o - monoter

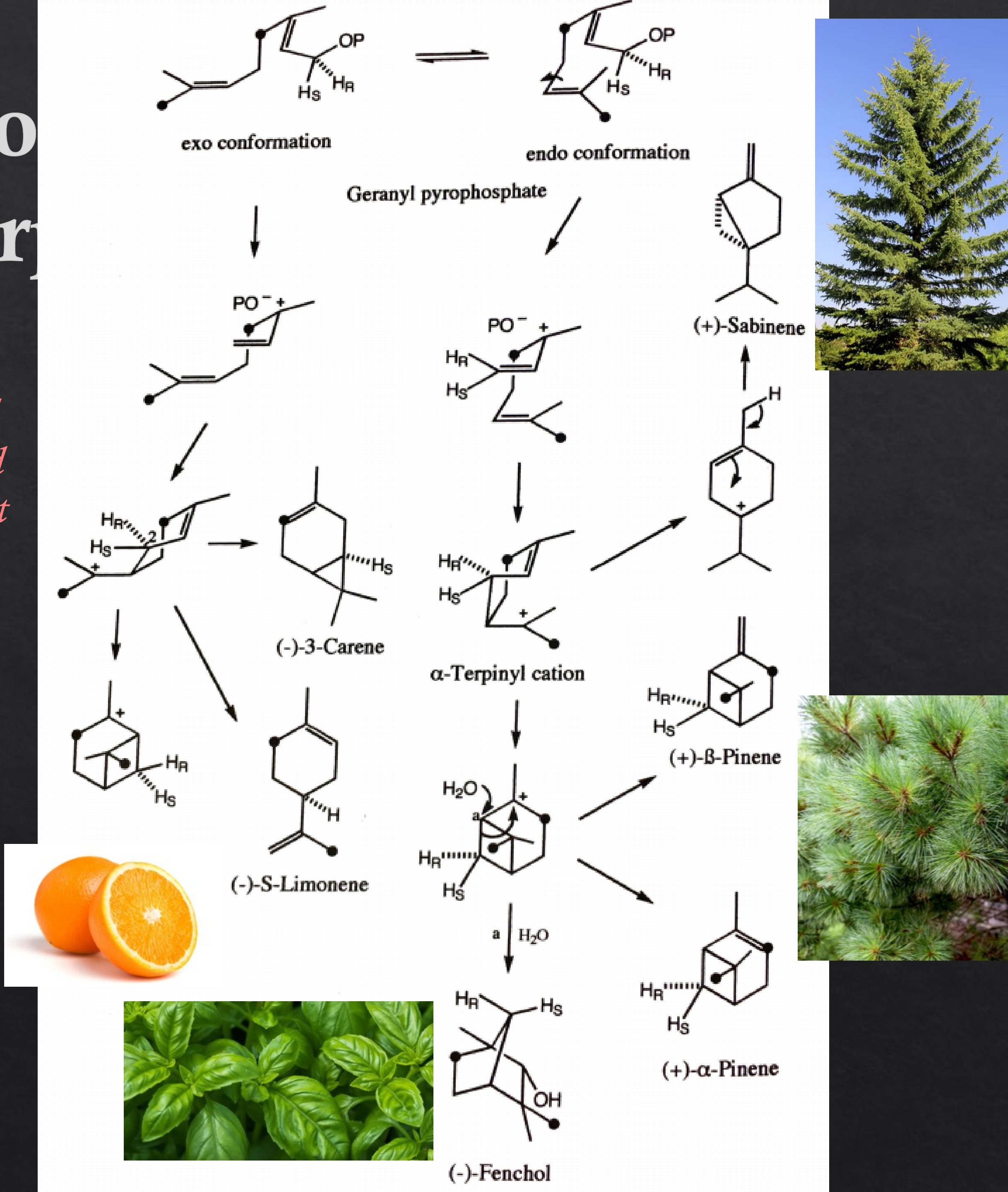


Geranyl phosphate

Starting material for monoterpenes

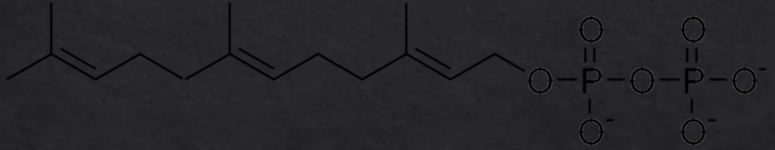
The products from cyclization of geranyl pyrophosphate depend on the enzymes present in the plant



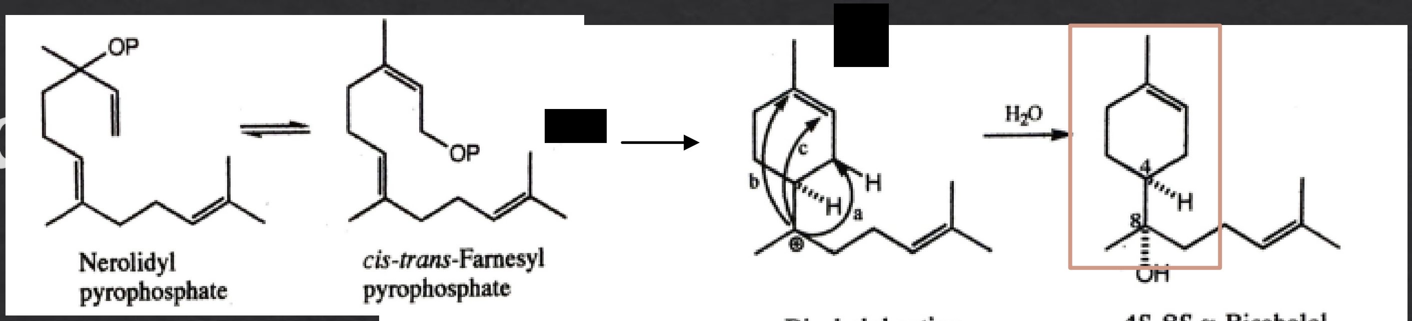


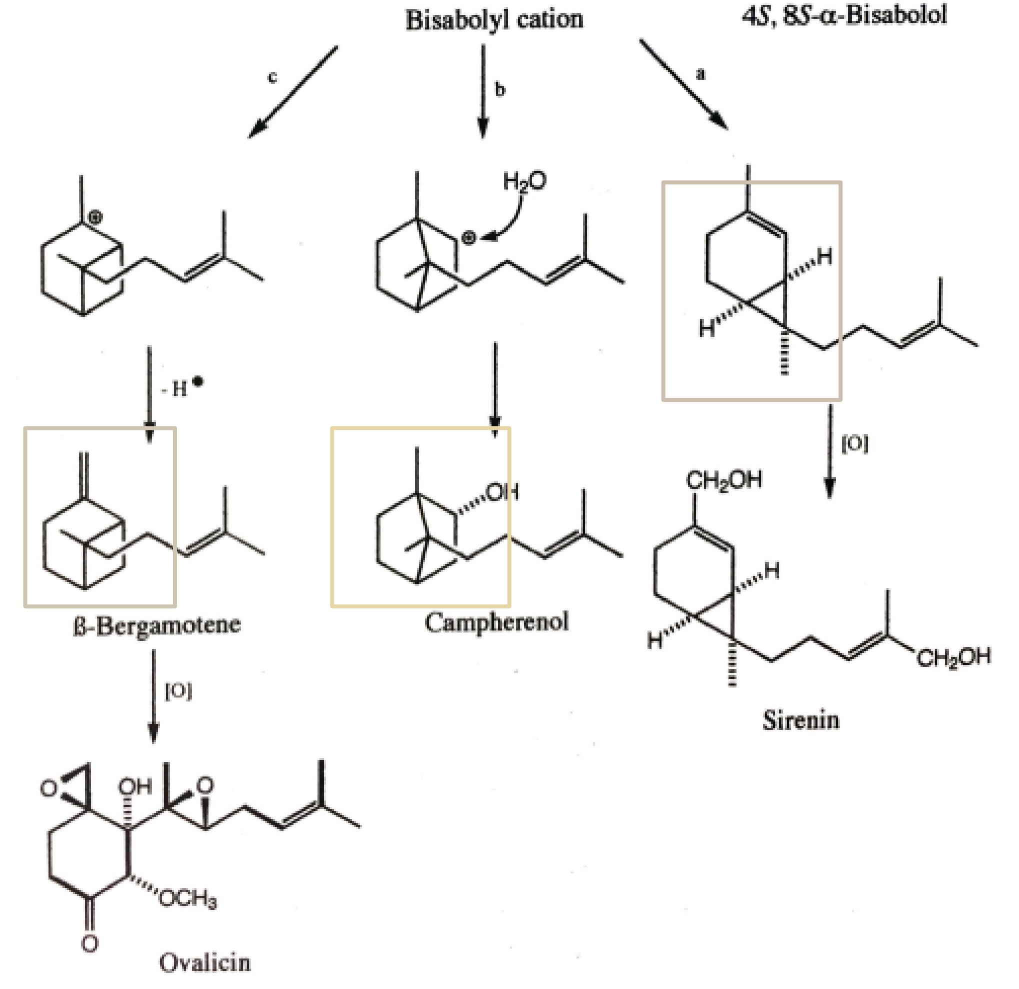
Ses

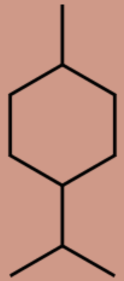
- Longer chain lenght – more structural variety
- Largest group of terpenes
- 50 different carbon skeletons



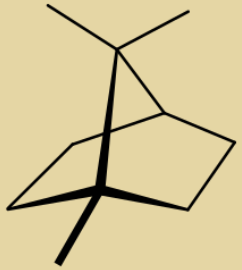
Farnesyl pyrophosphate = starting material for sesquiterpenes



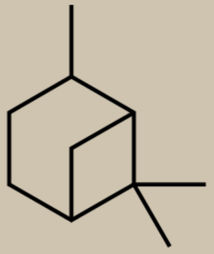




menthane



bornane

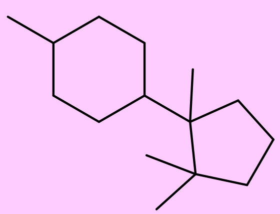


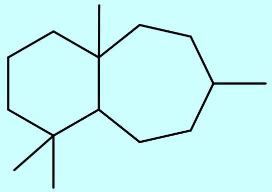
pinane

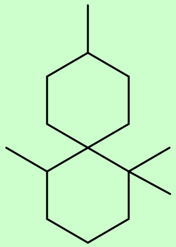
carane

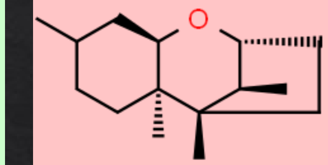
Sesquiterpe

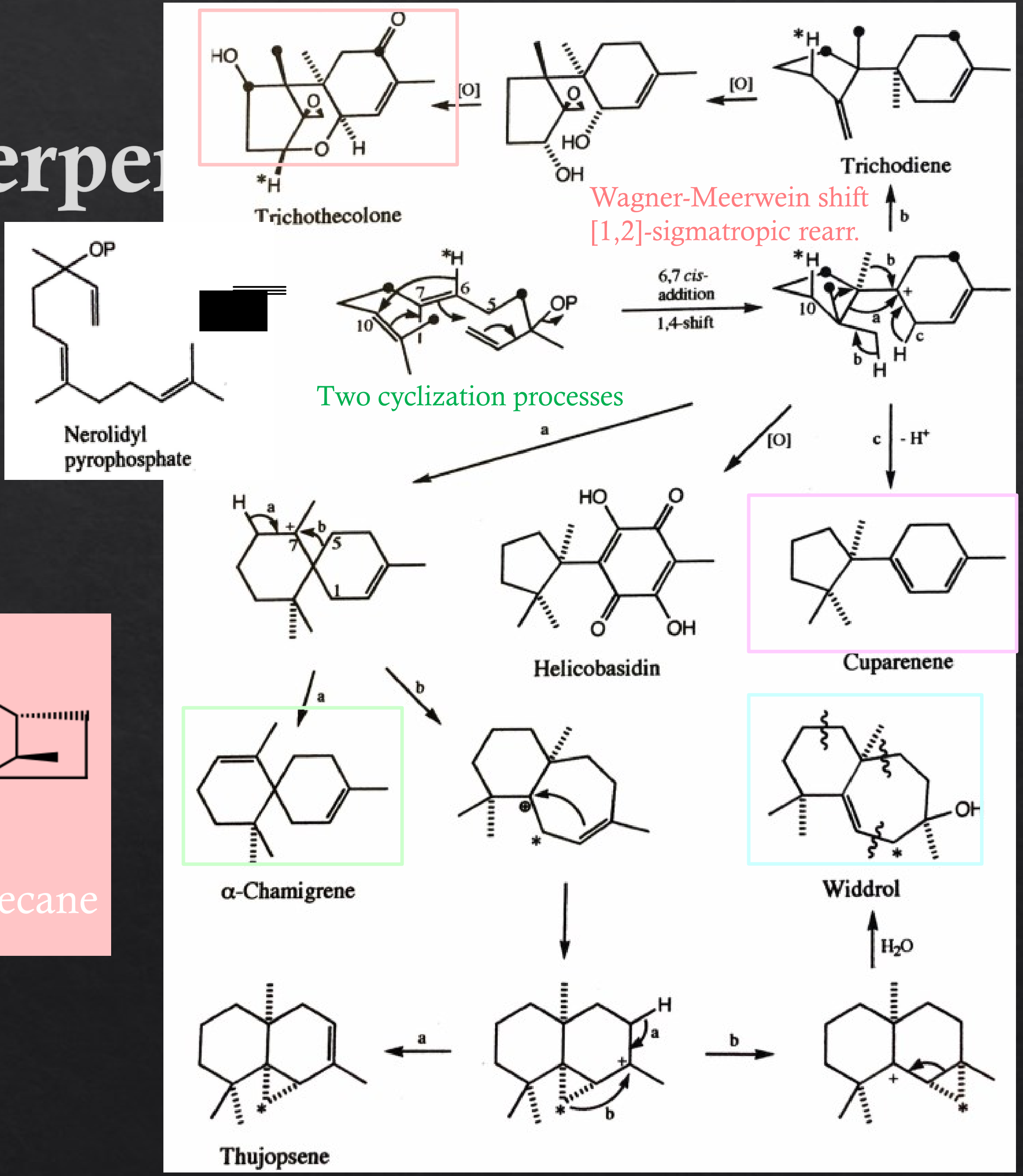
- Hydride and alkyl shifts
  - via carbocation intermediates
  - Gives even more structure variation


cuparane


widdrane


chamigrane


trichothecane


Wagner-Meerwein shift
[1,2]-sigmatropic rearr.

Two cyclization processes

Iceland Liechtenstein Norway grants
“Working together for a green, competitive and inclusive Europe”

GREENCAM for tomorrow

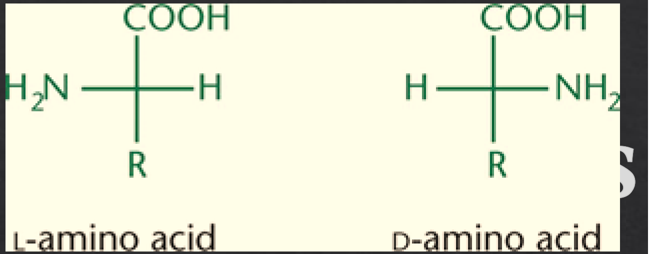
University of Bucharest
Virtute et Sapientia

NTNU
Norwegian University of Science and Technology

Chapter 7
Amino Acids, Peptides and Proteins

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

Spring 2022



Amino acids contain:
- amino group (-NH$_2$)
- carboxyl group (-COOH)

Amino acids in proteins are L-α-amino acids
- 20 common amino acids

10 of these are essential t humans
- More complex biosynthesis

300 non-protein amino acids

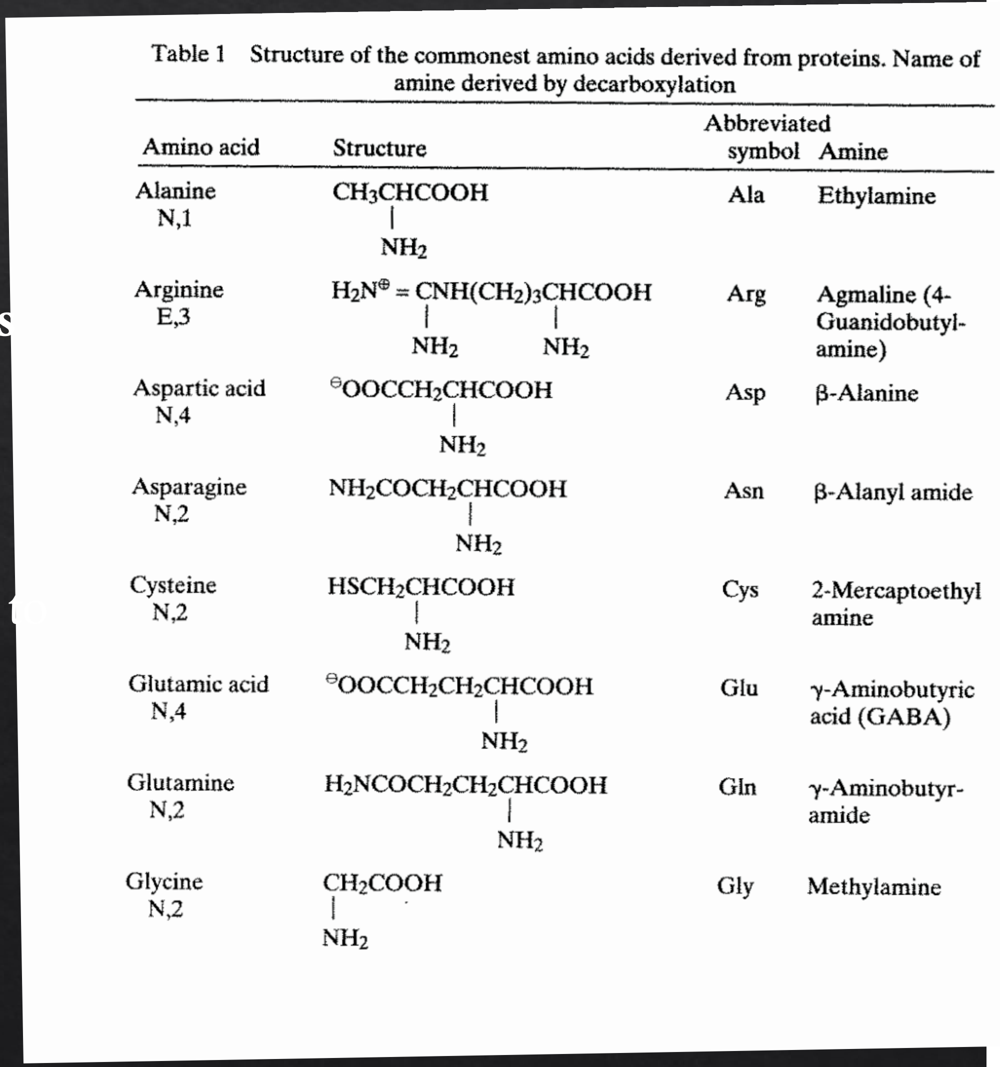


Table 1 Structure of the commonest amino acids derived from proteins. Name of amine derived by decarboxylation

Amino acid	Structure	Abbreviated symbol	Amine
Alanine N,1	$CH_3\underset{NH_2}{CH}COOH$	Ala	Ethylamine
Arginine E,3	$H_2N^{\oplus}=\underset{NH_2}{C}NH(CH_2)_3\underset{NH_2}{CH}COOH$	Arg	Agmaline (4-Guanidobutyl-amine)
Aspartic acid N,4	$^{\ominus}OOCCH_2\underset{NH_2}{CH}COOH$	Asp	β-Alanine
Asparagine N,2	$NH_2COCH_2\underset{NH_2}{CH}COOH$	Asn	β-Alanyl amide
Cysteine N,2	$HSCH_2\underset{NH_2}{CH}COOH$	Cys	2-Mercaptoethyl amine
Glutamic acid N,4	$^{\ominus}OOCCH_2CH_2\underset{NH_2}{CH}COOH$	Glu	γ-Aminobutyric acid (GABA)
Glutamine N,2	$H_2NCOCH_2CH_2\underset{NH_2}{CH}COOH$	Gln	γ-Aminobutyr-amide
Glycine N,2	$\underset{NH_2}{CH_2}COOH$	Gly	Methylamine

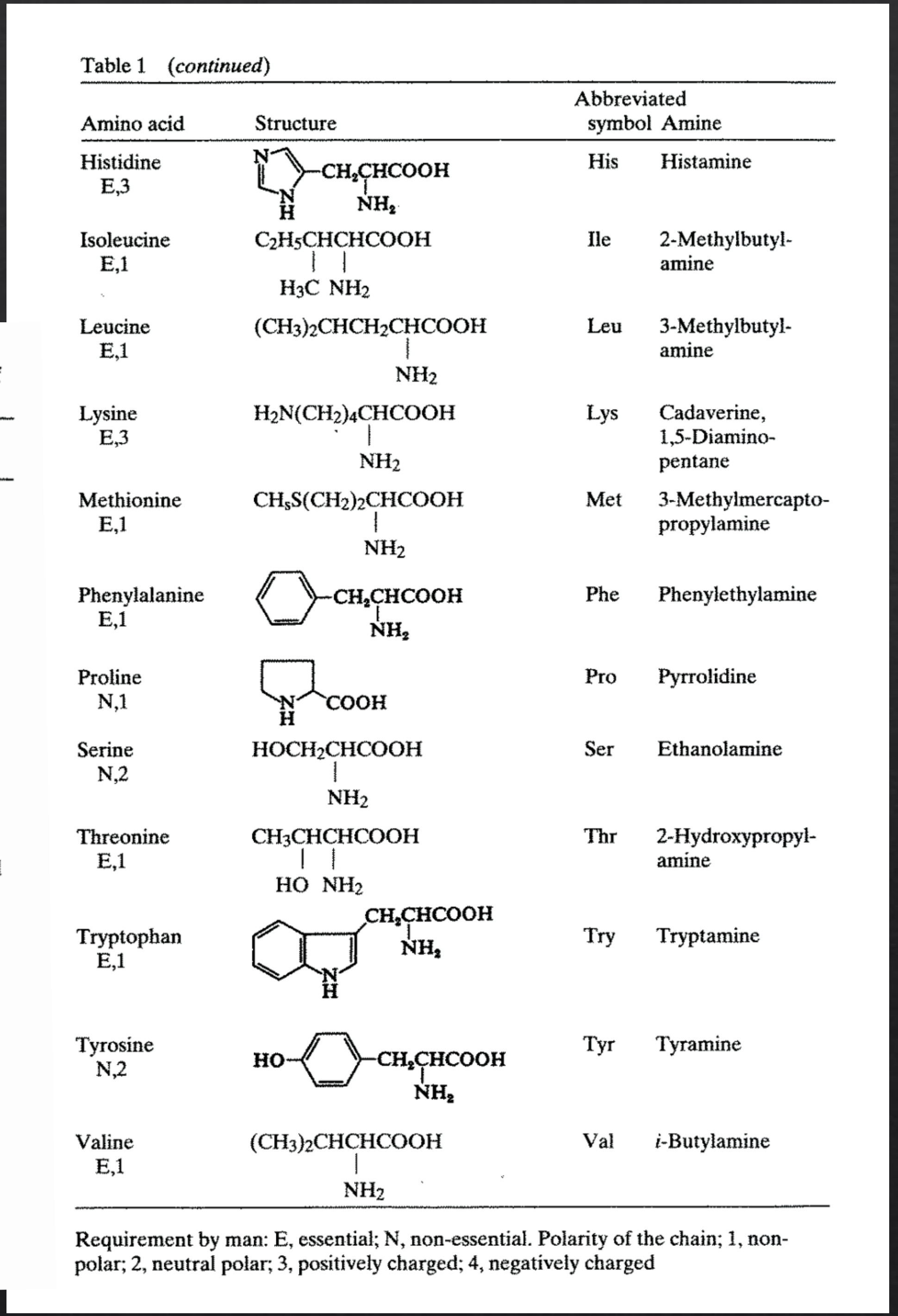


Table 1 *(continued)*

Amino acid	Structure	Abbreviated symbol	Amine
Histidine E,3	$-CH_2\underset{NH_2}{CH}COOH$	His	Histamine
Isoleucine E,1	$C_2H_5\underset{H_3C}{CH}\underset{NH_2}{CH}COOH$	Ile	2-Methylbutyl-amine
Leucine E,1	$(CH_3)_2CHCH_2\underset{NH_2}{CH}COOH$	Leu	3-Methylbutyl-amine
Lysine E,3	$H_2N(CH_2)_4\underset{NH_2}{CH}COOH$	Lys	Cadaverine, 1,5-Diamino-pentane
Methionine E,1	$CH_3S(CH_2)_2\underset{NH_2}{CH}COOH$	Met	3-Methylmercapto-propylamine
Phenylalanine E,1	$-CH_2\underset{NH_2}{CH}COOH$	Phe	Phenylethylamine
Proline N,1	N H COOH	Pro	Pyrrolidine
Serine N,2	$HOCH_2\underset{NH_2}{CH}COOH$	Ser	Ethanolamine
Threonine E,1	$CH_3\underset{HO}{CH}\underset{NH_2}{CH}COOH$	Thr	2-Hydroxypropyl-amine
Tryptophan E,1	$CH_2\underset{NH_2}{CH}COOH$ N H	Try	Tryptamine
Tyrosine N,2	$HO-\ \ -CH_2\underset{NH_2}{CH}COOH$	Tyr	Tyramine
Valine E,1	$(CH_3)_2CH\underset{NH_2}{CH}COOH$	Val	*i*-Butylamine

Requirement by man: E, essential; N, non-essential. Polarity of the chain; 1, non-polar; 2, neutral polar; 3, positively charged; 4, negatively charged

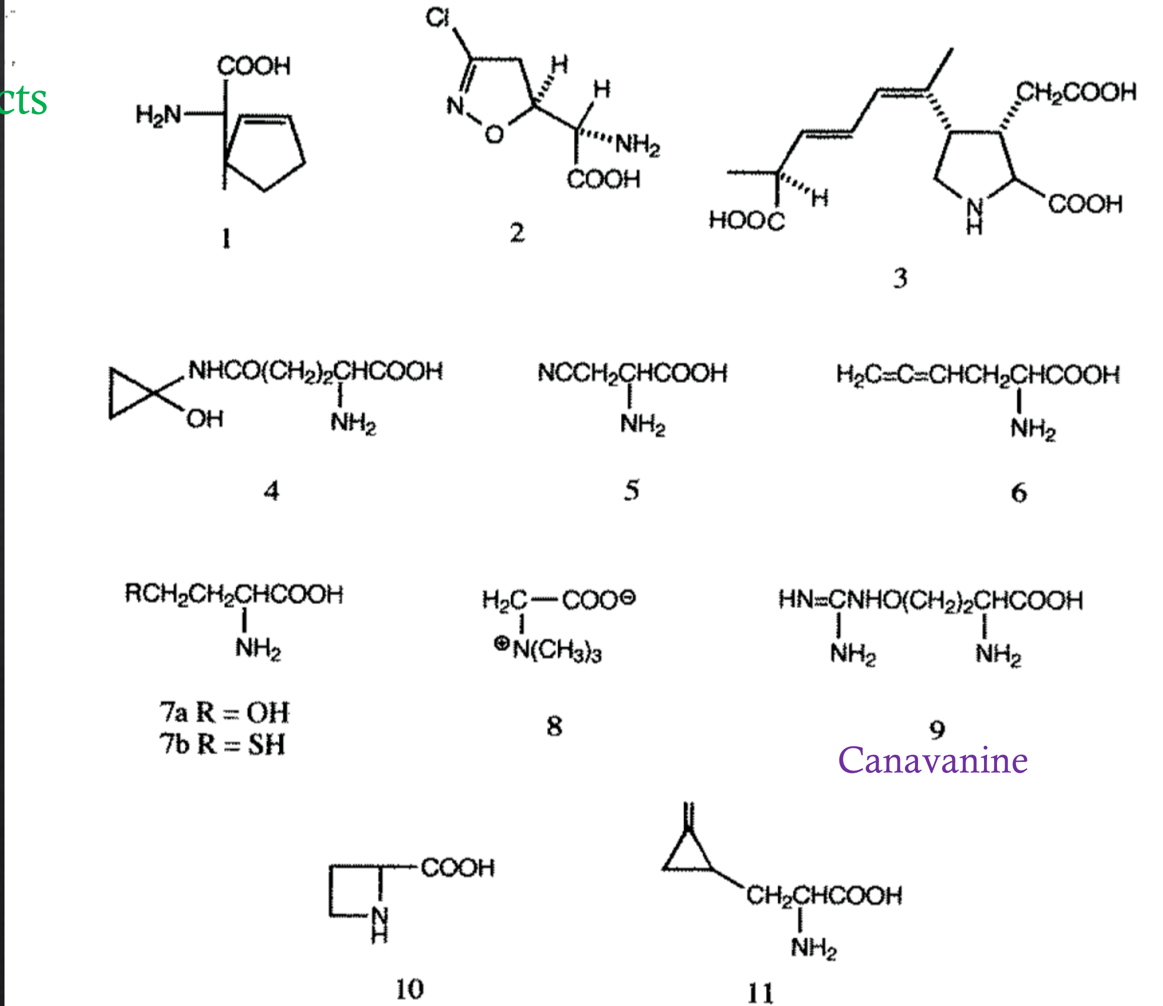
Examples of non-protein amino acids

Blood sugar depressing effects

Blighia sapida
National fruit of Jamaica,
poisonous if not cooked
properly

Hypoglycin A binds irreversibly to coenzyme A, inhibiting many enzymes

=> Depletes glucose stores



Canavanine

Hypoglycin A

Phytotoxic effects

avanine
ts growth
of
thyrus

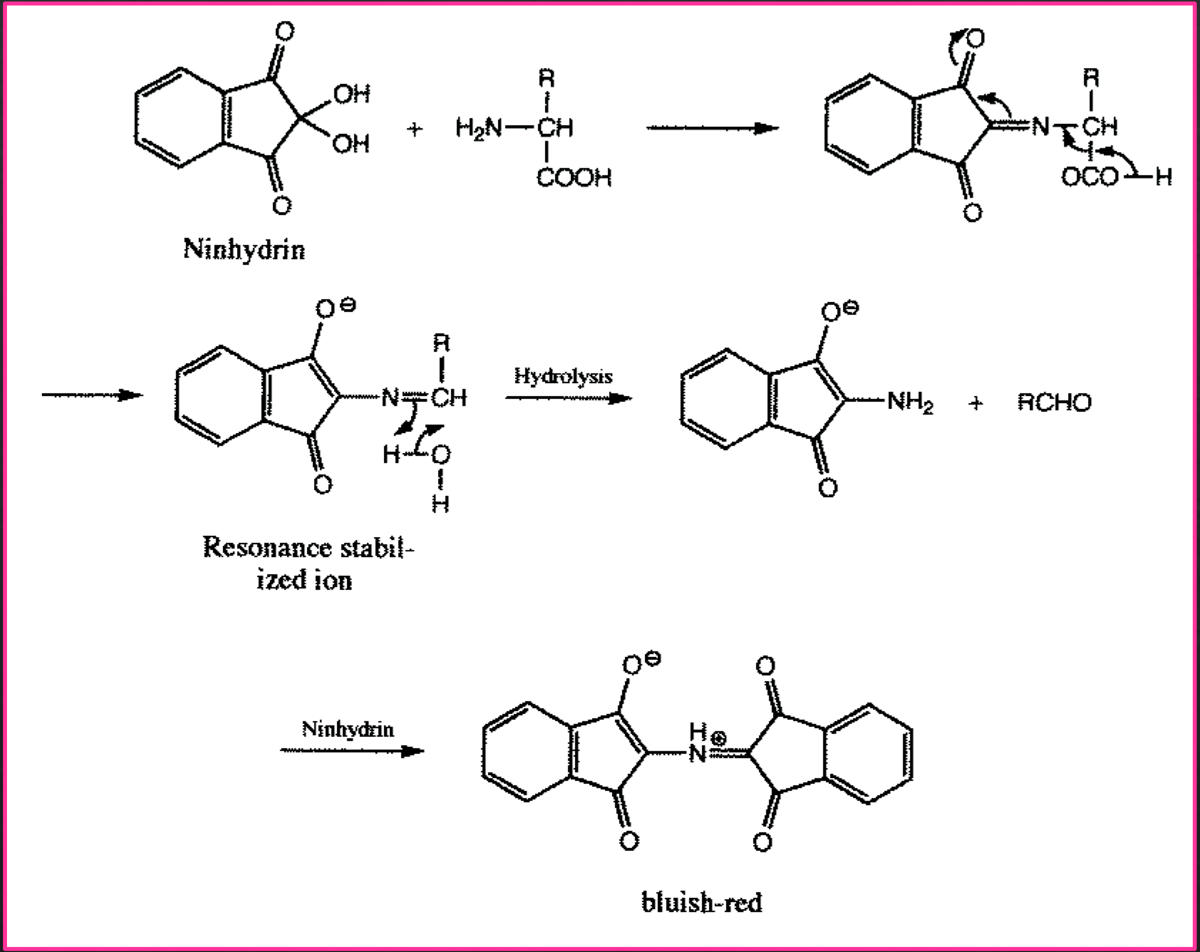
Vicia bengalensis
Produces canavanine

Detection of amino acids

- Forensic chemistry
  - Detection of fingerprints on paper

Ninhydrin can be sprayed on the paper, followed by heating

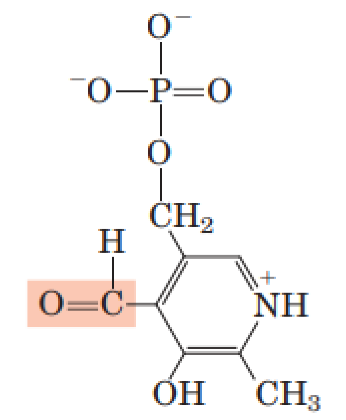
If amino acids are present, they form a purple complex with ninydrin

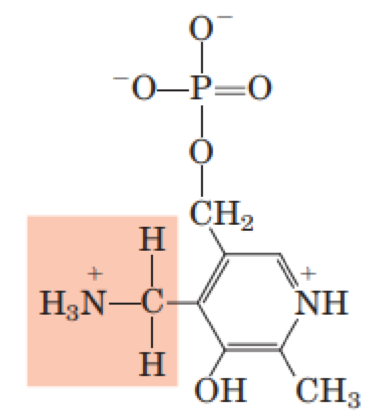


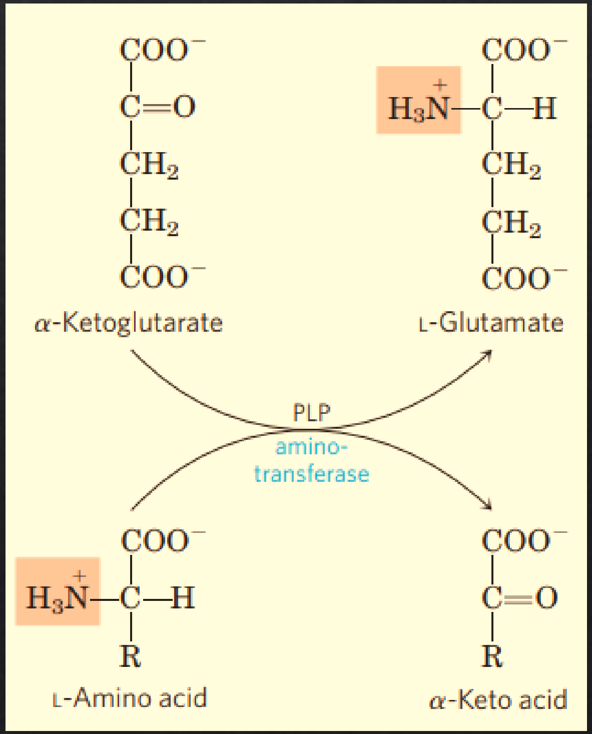
J. Chem. Educ. **2020** *97* (2), 571-573

Pyridoxal phosphate

- Cofactor in transamination reactions
  - Temporary carrier of amino groups
- Important for both synthesis and degradation of amino acids
- Glutamic acid is often the amino group donor (or acceptor) in transaminations


Pyridoxal phosphate (PLP)

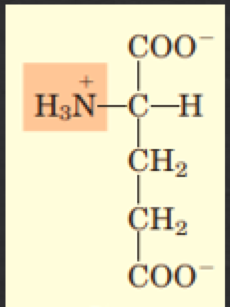

Pyridoxamine phosphate



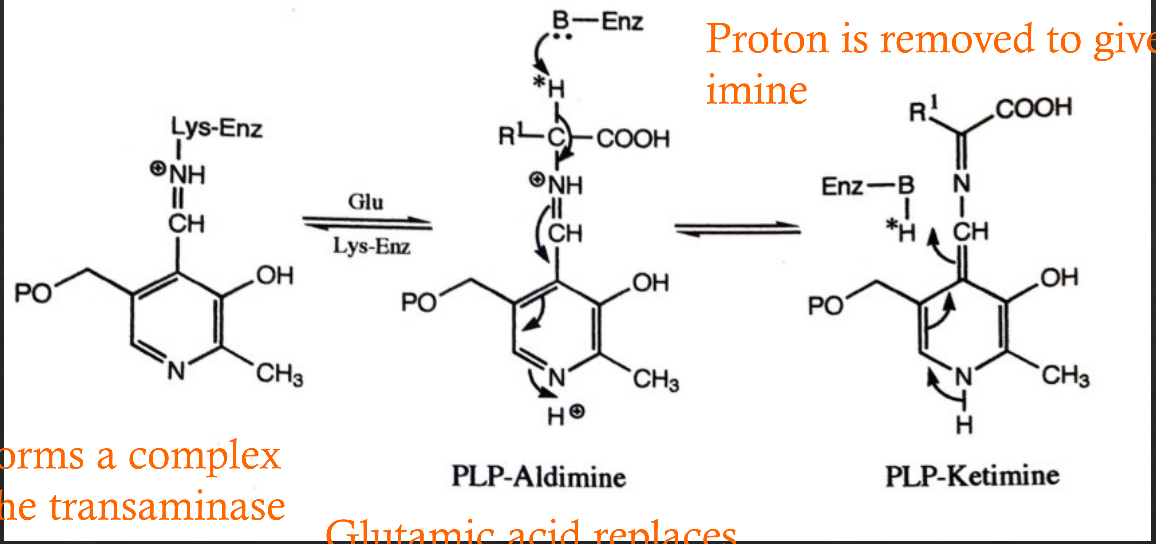
Lehninger Principles of Biochemistry, 6th ed. 201

Pyridoxal phosphate (PLP)

Glutamic acid is the NH3 donor



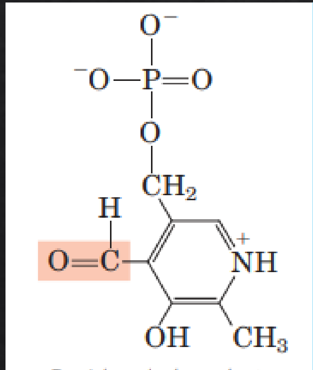
L-Glutamate



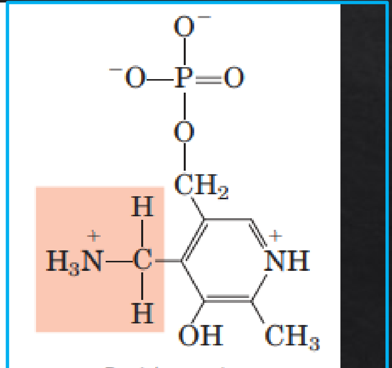
Proton is removed to give imine

PLP forms a complex with the transaminase

Glutamic acid replaces Lys

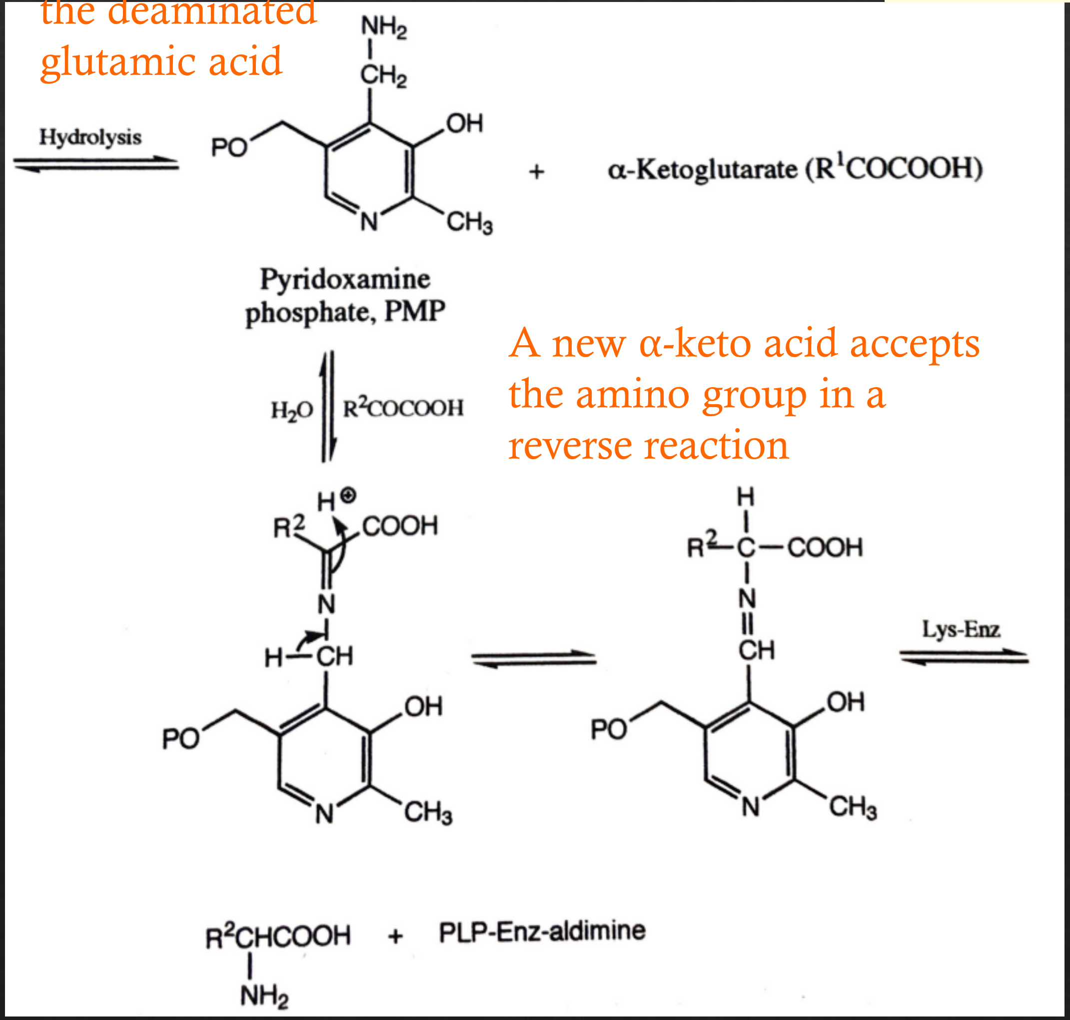


Pyridoxal phosphate (PLP)



Pyridoxamine phosphate

Hydrolysis removes the deaminated glutamic acid

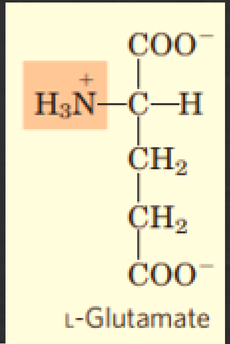


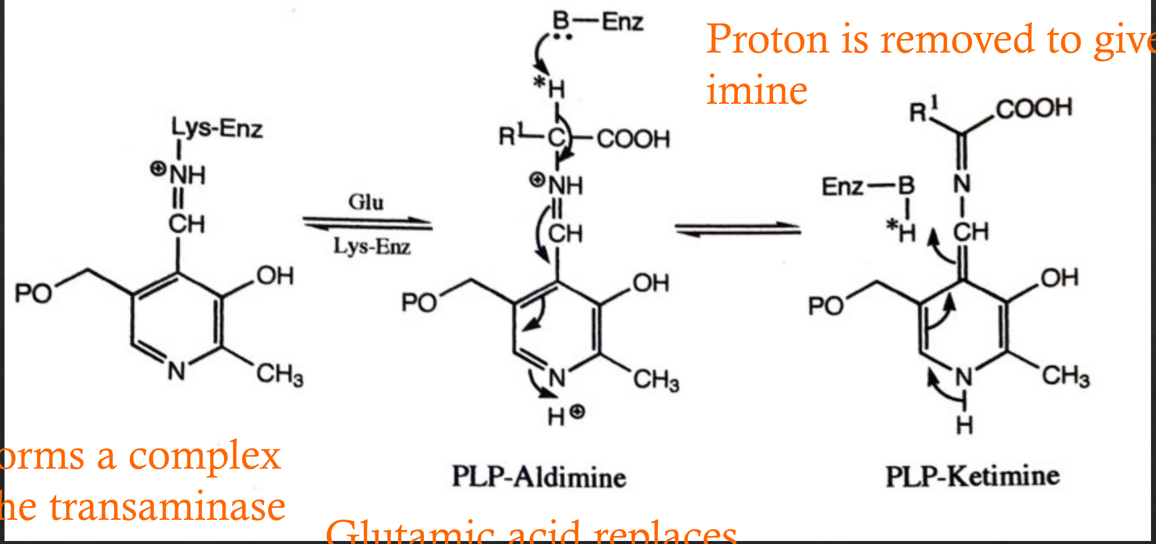
A new α-keto acid accepts the amino group in a reverse reaction

Lehninger Principles of Biochemistry, 6th ed. 2013

Pyridoxal phosphate (PLP)

Glutamic acid is the NH_3 donor





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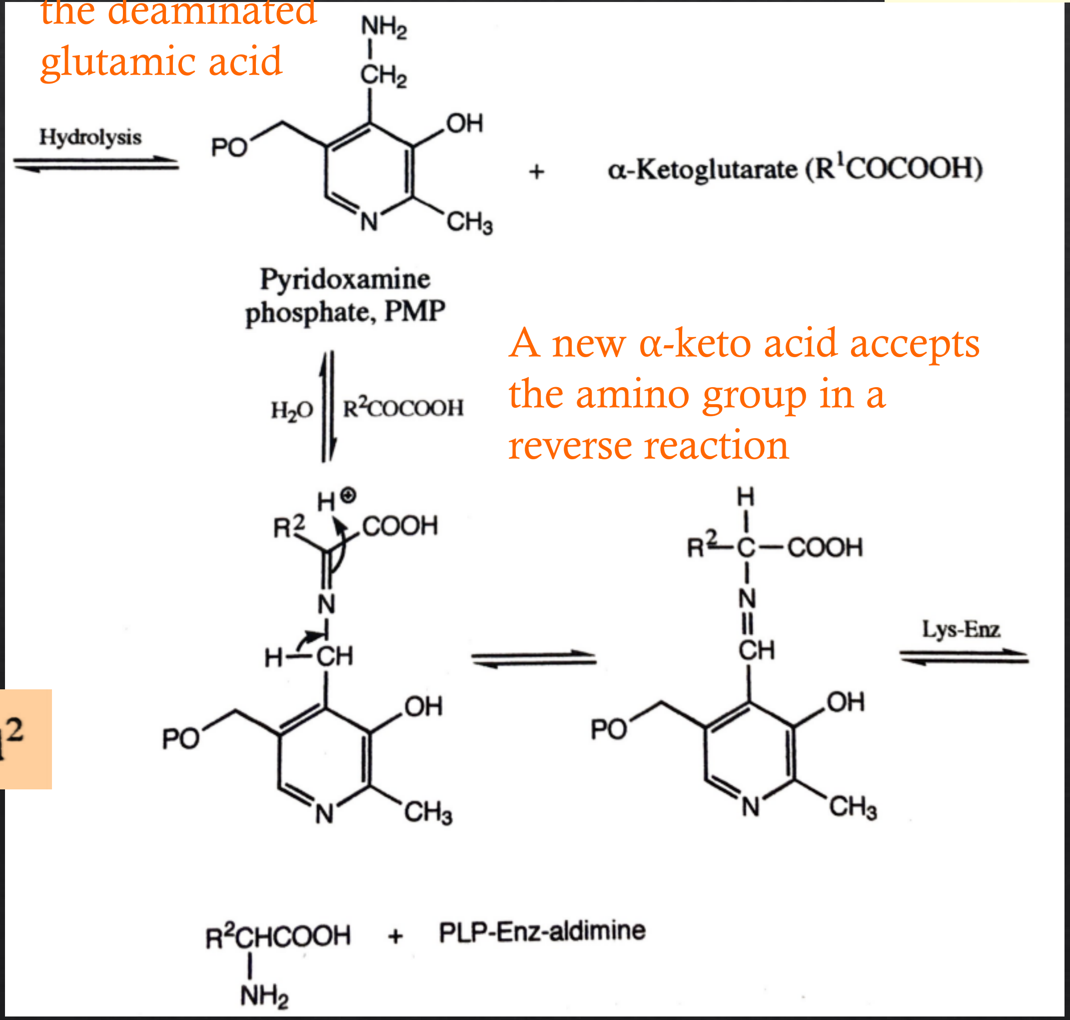
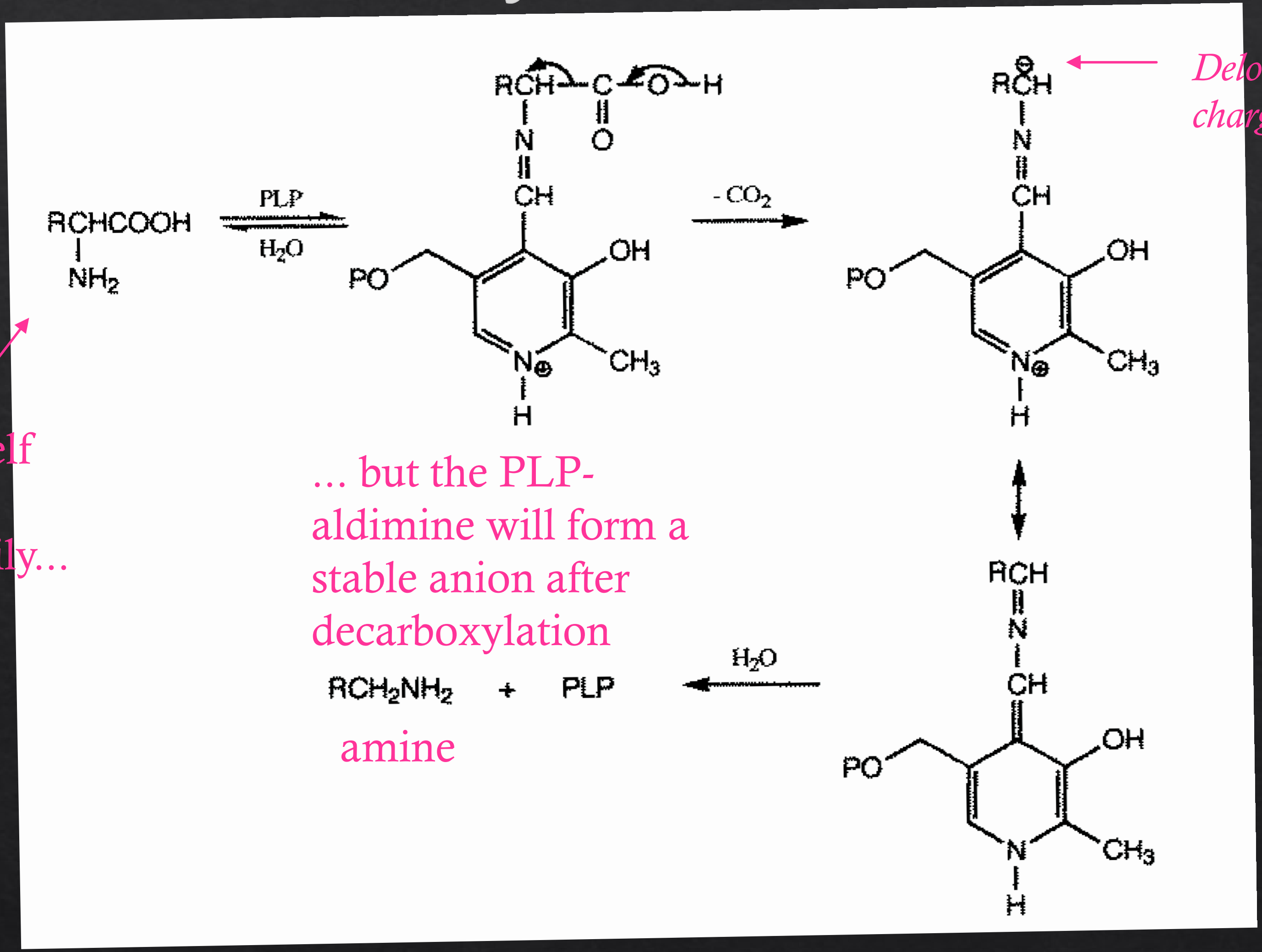


A new α-keto acid accepts the amino group in a reverse reaction

Summary of transamination reactions:

Amino acid^1 + Keto acid^2 $\rightleftharpoons$ Keto acid^1 + Amino acid^2

Decarboxylation of amino acids



Delocalized negative charge

The amino acid itself will not be decarboxylated easily…

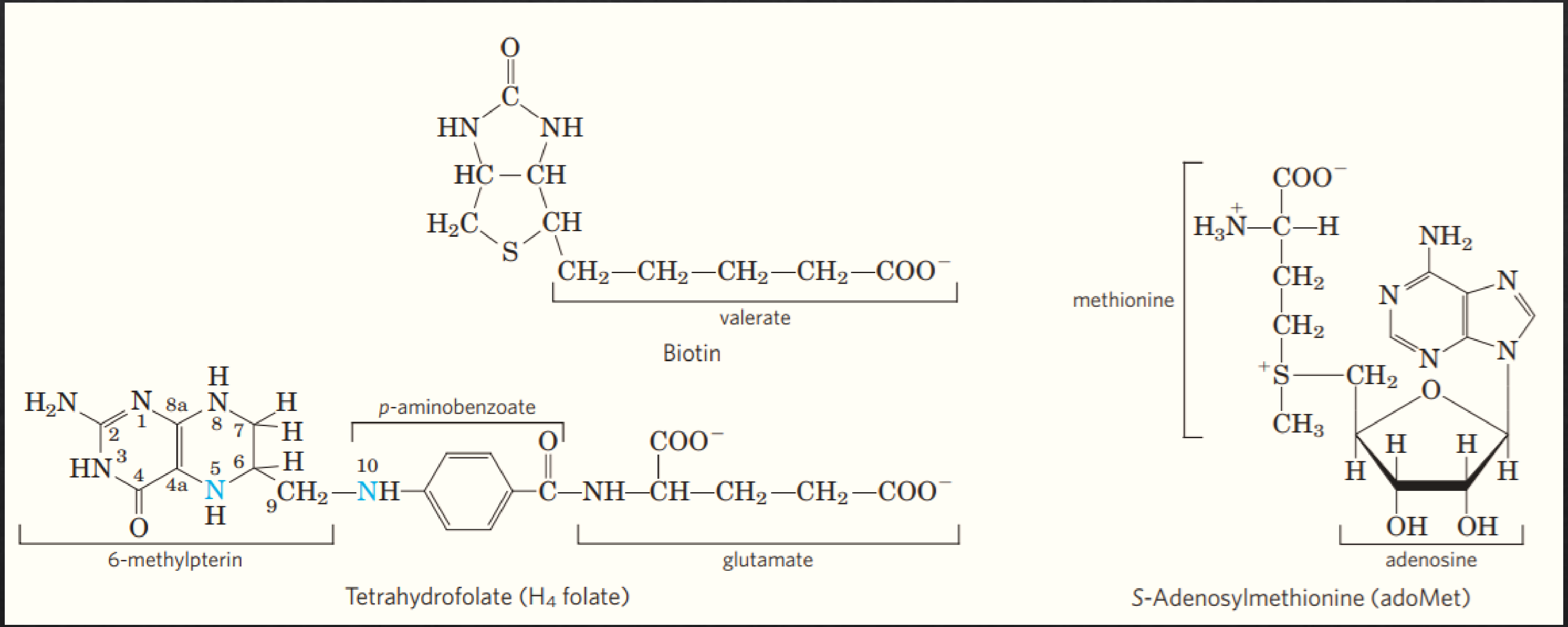
… but the PLP-aldimine will form a stable anion after decarboxylation

amine

This is an important biosynthetic route to amines

Cofactors for transfer of one-carbon fragments

- Important cofactors in amino acid catabolism



Biotin

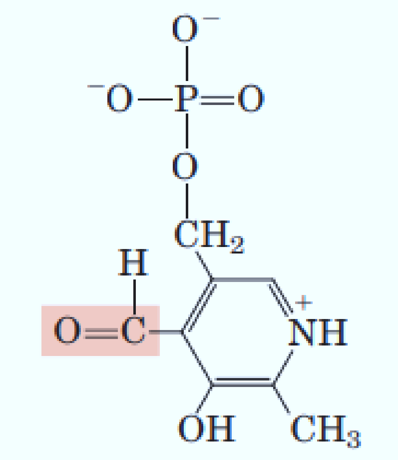
Tetrahydrofolate (H_4 folate)

S-Adenosylmethionine (adoMet)

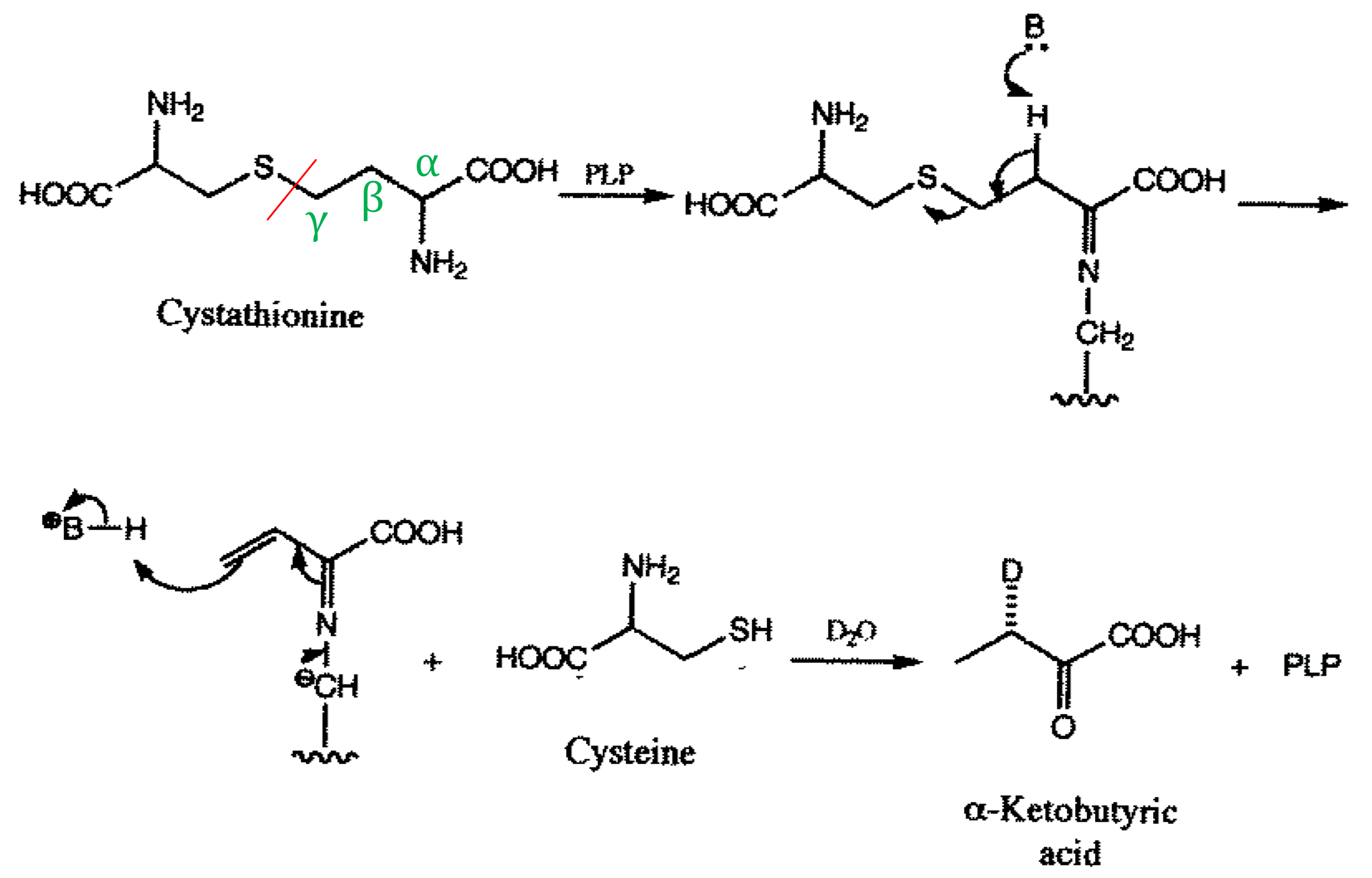
Lehninger Principles of Biochemistry, 6th ed. 2013

Biosynthesis of cysteine in mammals – role of PLP

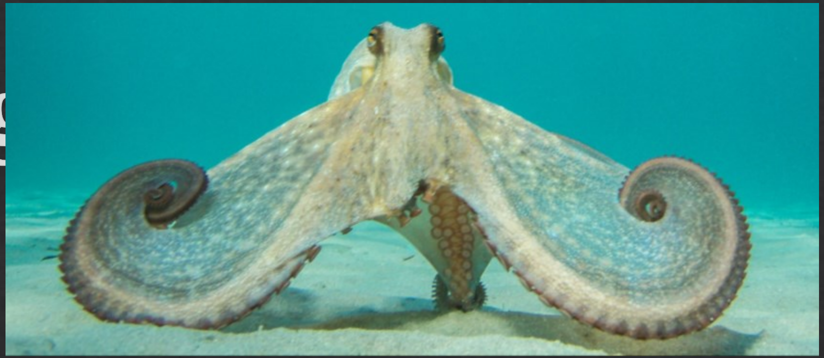
PLP activates cleavage of cystathionine in the γ position



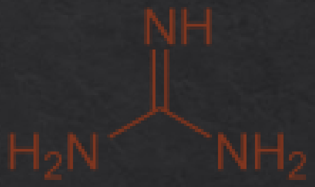
Pyridoxal phosphate (PLP)



The guanidino functional

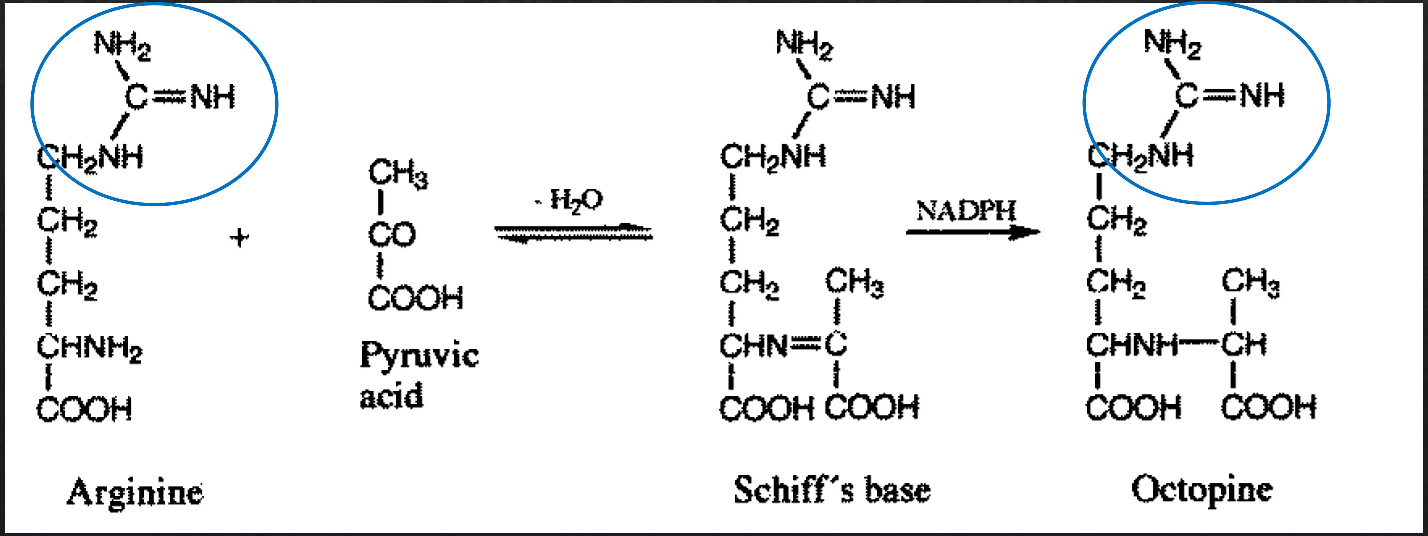


Common functional group in marine metabolites



Guanidine

Example: **Biosynthesis of octopine**



Octopine is an analog of lactic acid in the octopus.

Plants can also produce it if infected with the bacteria *agrobacterium tumifaciens,* which changes the DNA of the plant and induces tumor growth in the infected plant.

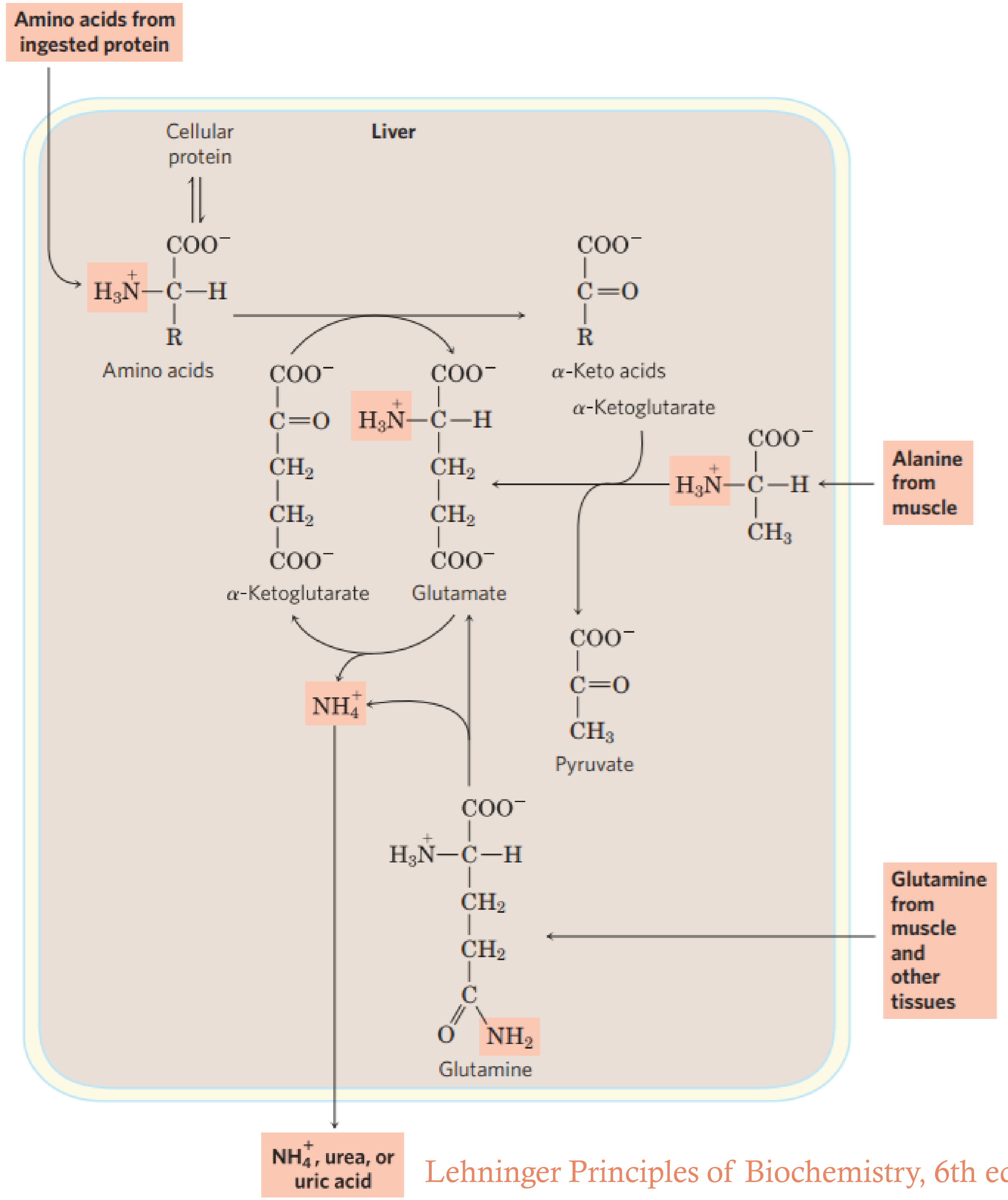
The ure

- NH3 is toxic, removed in urea cycle

1 - NH3 from different tissue in the vertebrates is transferred to the liver as glutamine and alanine

2 - The amino group gets transferred to α-ketoglutarate to form glutamic acid

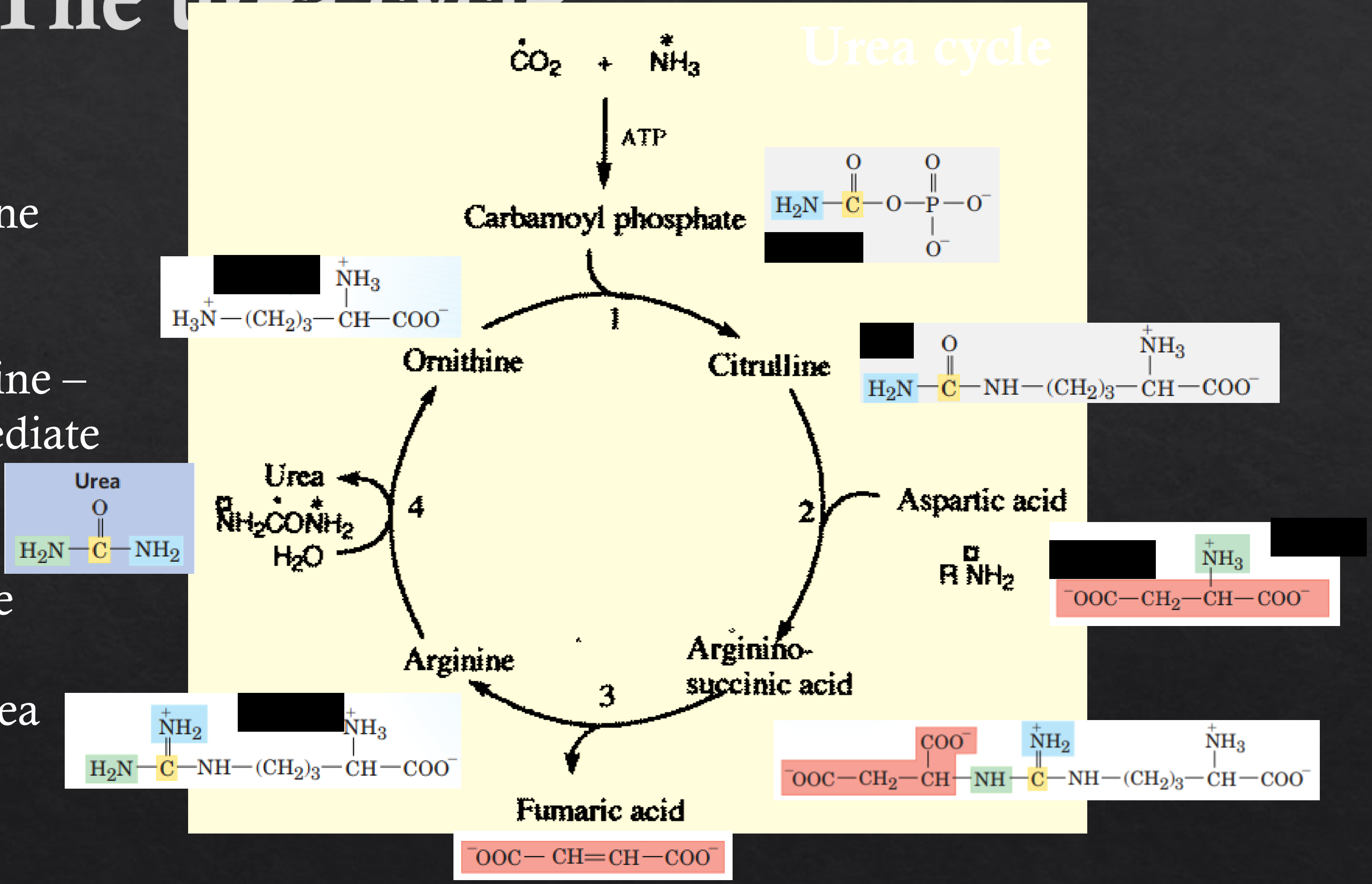
3 – Glutamic acid releases the amino group which then enters the urea cycle



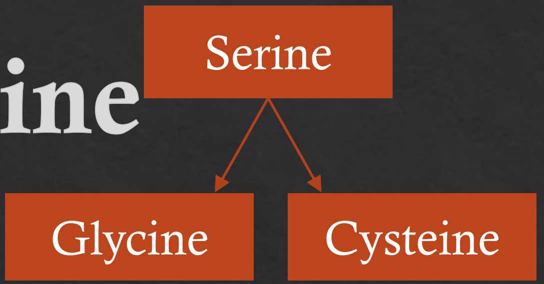
Lehninger Principles of Biochemistry, 6th ed. 20

The urea cycle

1. Formation of citrulline from carbamoyl phosphate and ornithine
2. Aspartic acid enters to form argininosuccinic acid from citrulline – through a citrulline-AMP intermediate (requires ATP)
3. Release of fumarate gives arginine
4. Hydrolysis of arginine releases urea

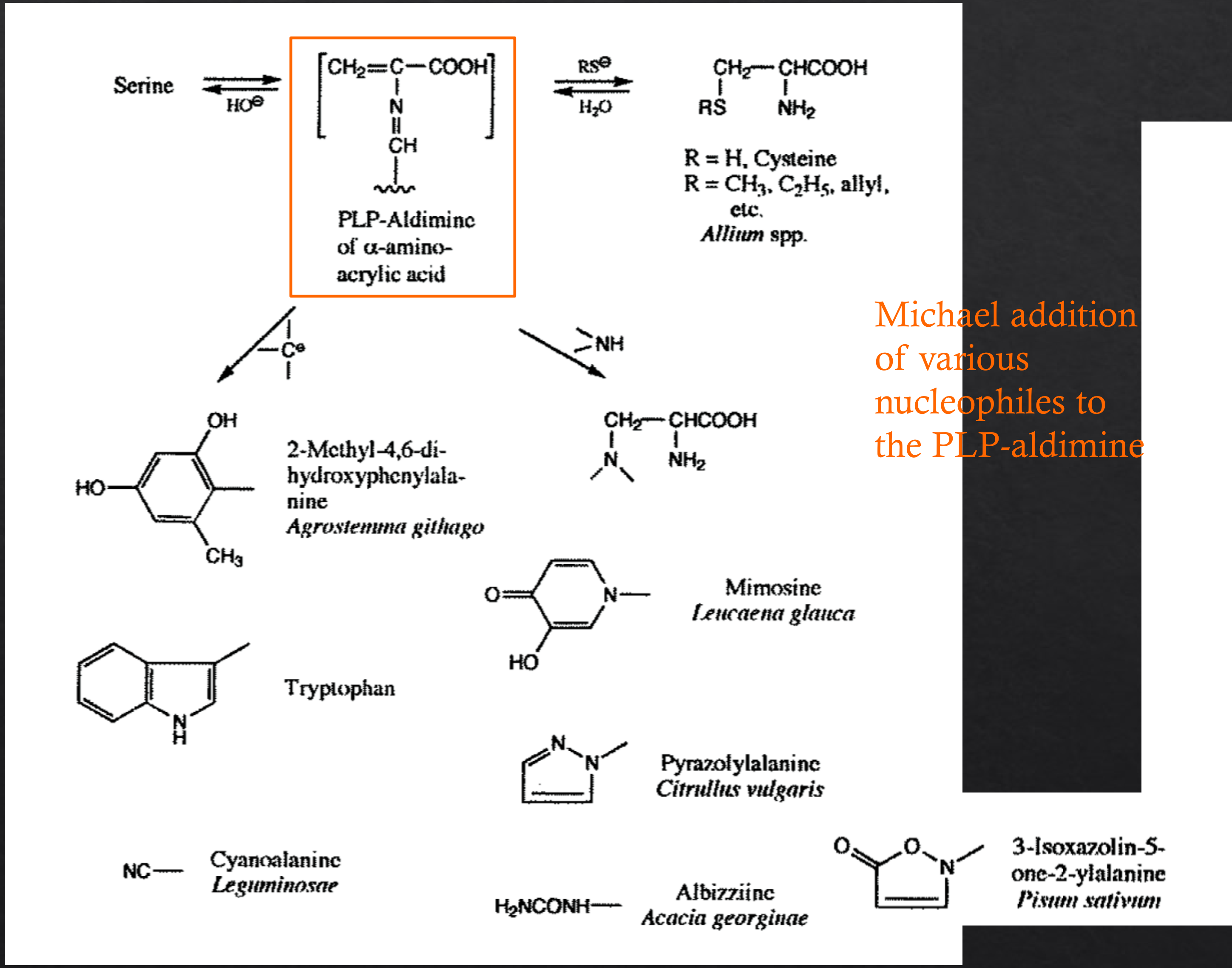


Secondary products of serine and cysteine

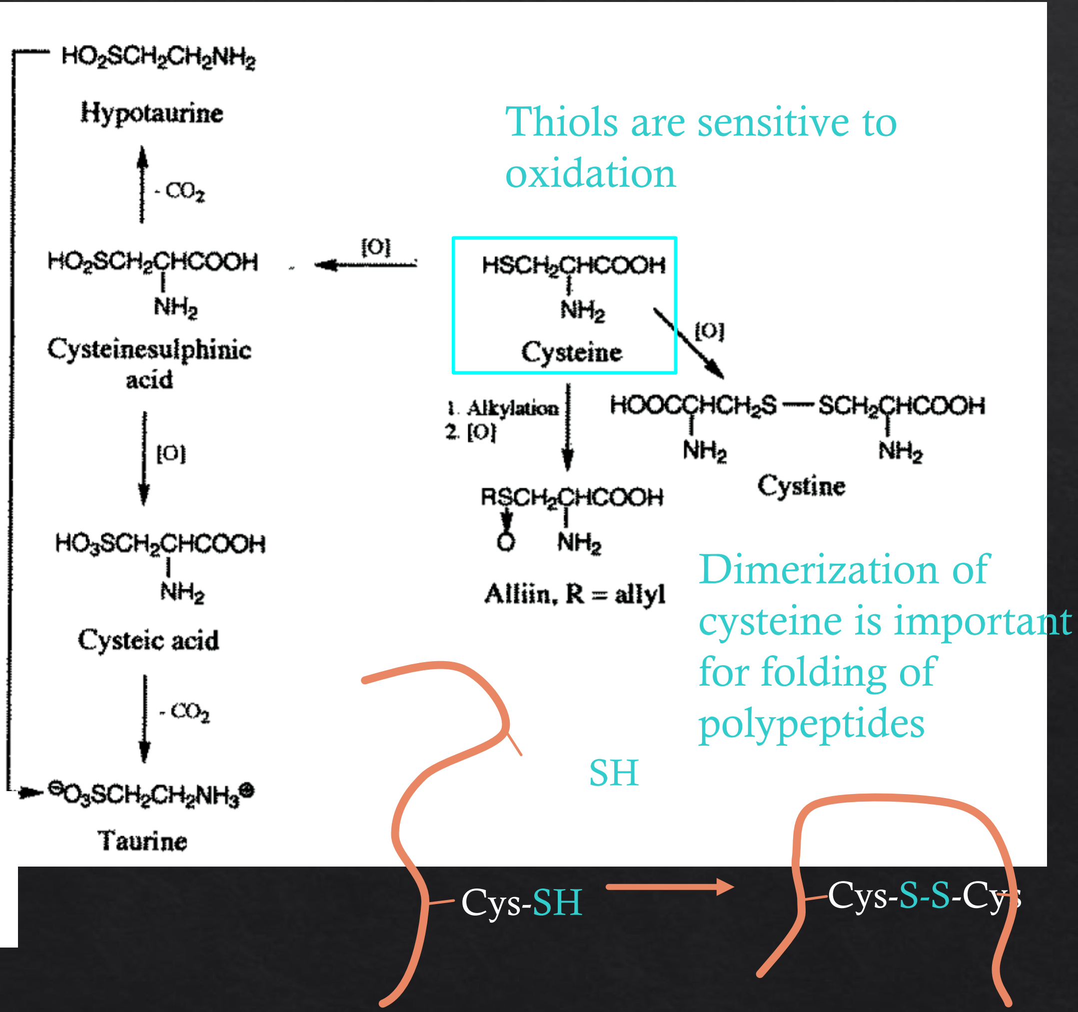

Serine

Glycine

Cysteine



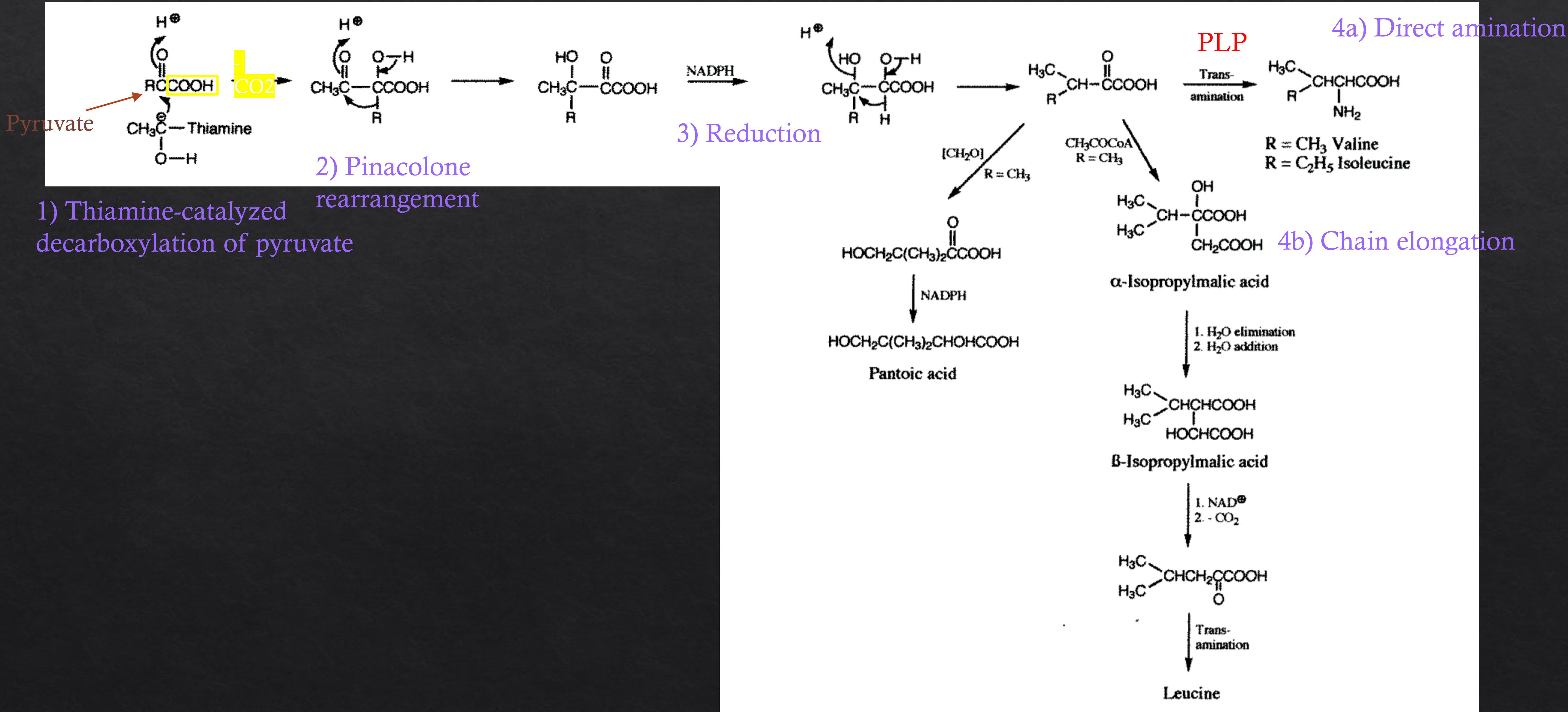
Michael addition of various nucleophiles to the PLP-aldimine



Thiols are sensitive to oxidation

Dimerization of cysteine is important for folding of polypeptides

Biosynthesis of leucine, valine and isoleucine



1) Thiamine-catalyzed decarboxylation of pyruvate

2) Pinacolone rearrangement

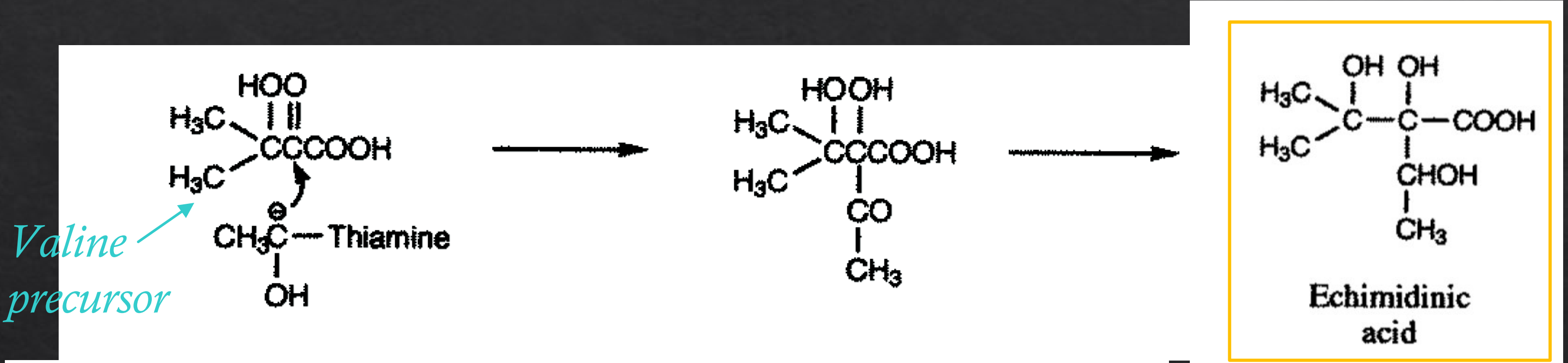
3) Reduction

4a) Direct amination

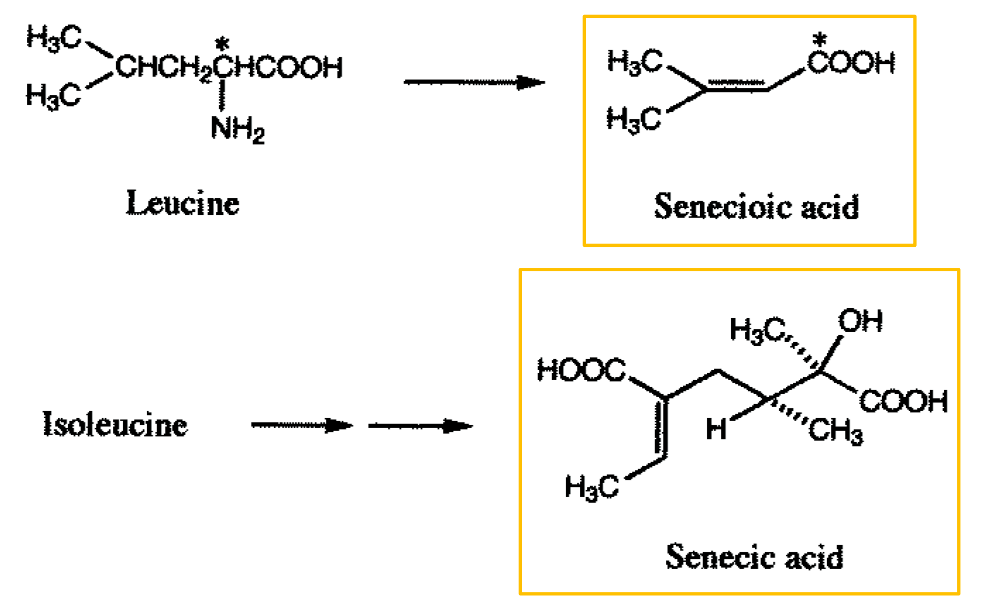
4b) Chain elongation

Secondary products of valine, isoleucine and leucine

Necic acids are often derived from these amino acids (not from acetate, as originally suspected):

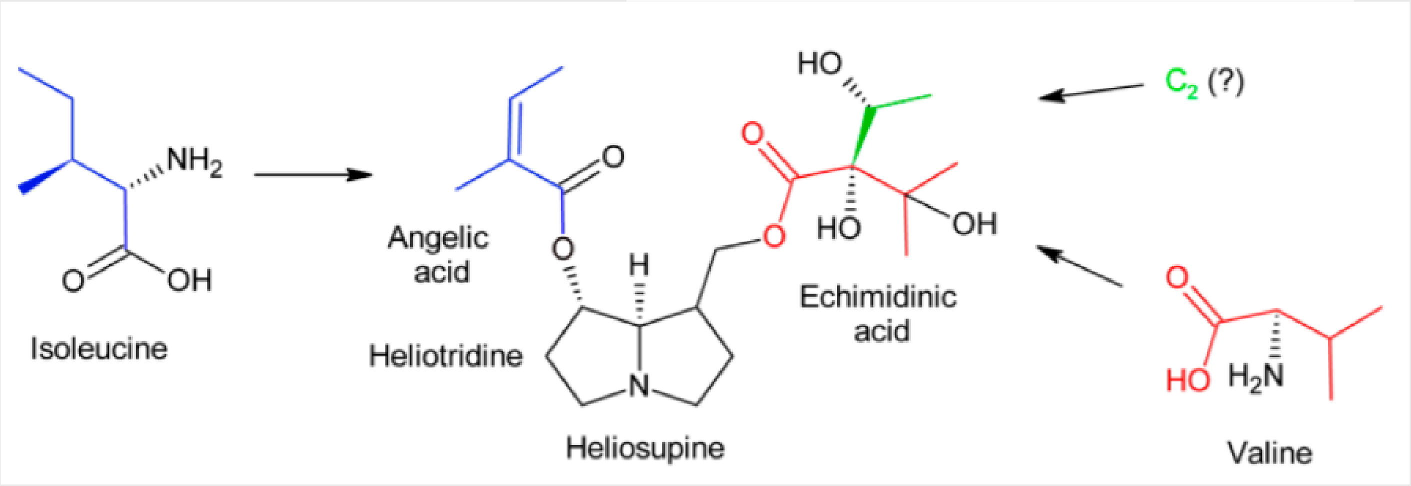


Valine precursor



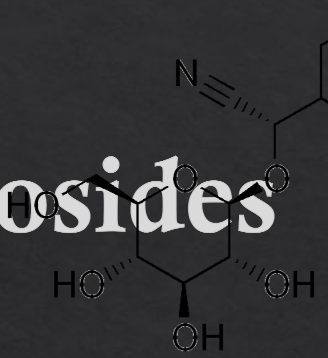
Necic acids are important building blocks of pyrrolizidine alkaloids such as heliosupine

(Plant defence chemicals, highly toxic compounds)



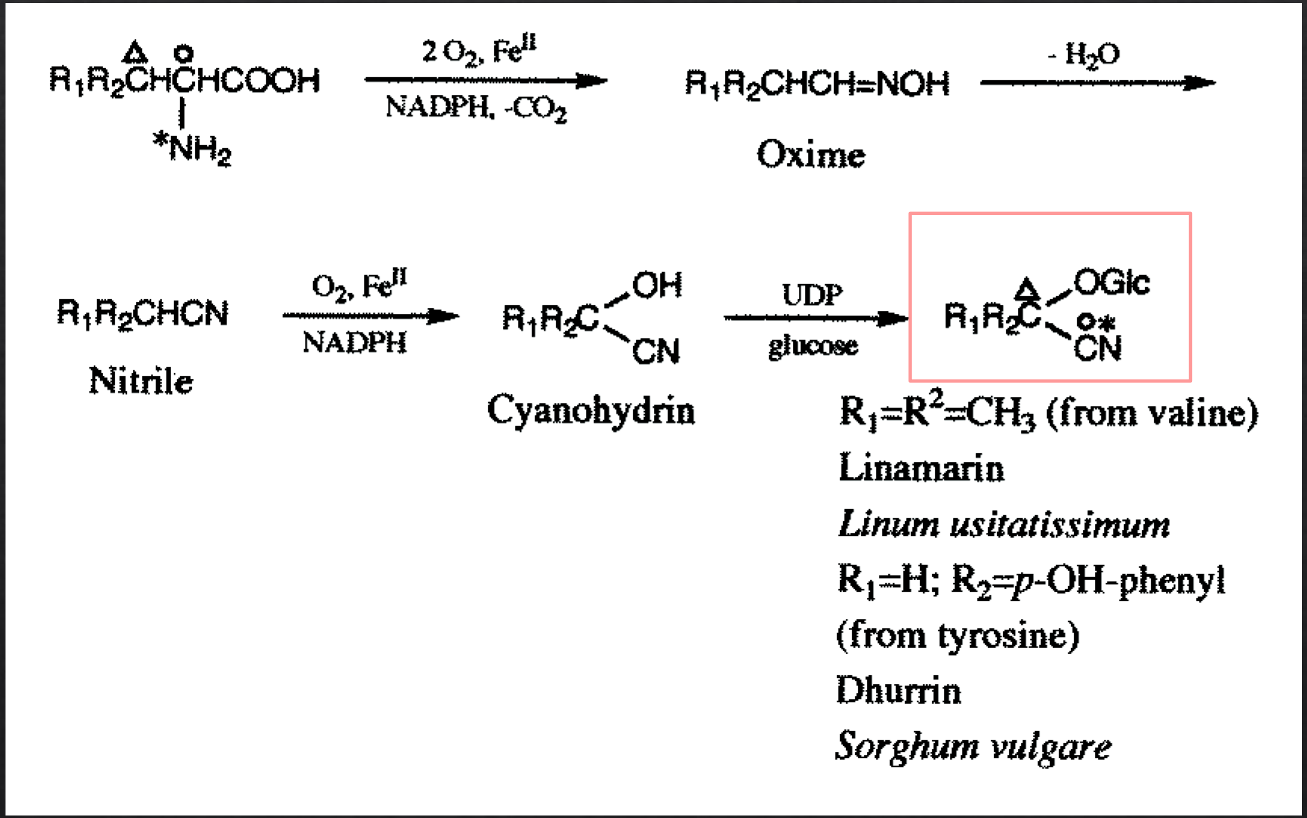
Schramm *et al. (2019)* Molecules, 24, 498

Cyanogenic glycosides



Dhurrin

Sorghum vulgare



Amino acids → Cyanogenic glycosides → HCN

Cyanogenic glycosides:

Precursors for HCN in some plants

Decomposition gives sugar, ketone/aldehyde and HCN

Defence mecanism:

Tissue damage in plant releases enzymes that catalyze decomposition => HCN released

Vetter J. (2017) Plant Cyanogenic Glycosides. Plant Toxins. Toxinology. Springer, Dordrecht.

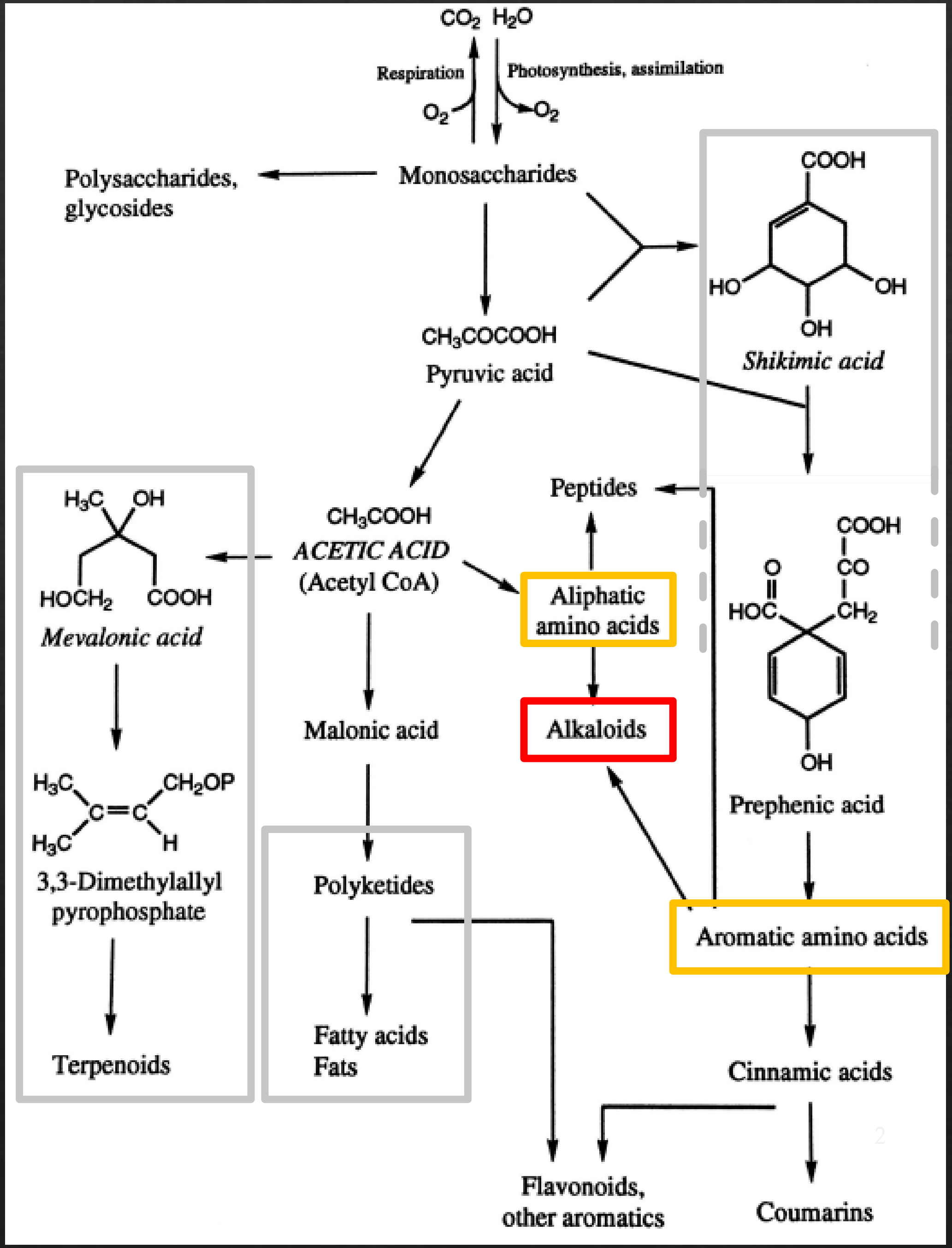
Chapter 8-1
The alkaloids

Elisabeth Jacobsen and Lucas Boquin, NTNU

Spring 2022

What are alkaloids?

- An alkaloid is a cyclic organic compound that contains nitrogen in a negative oxidation state and is of limited presence among living organisms. It doesn't include simple amides.
- It is almost always derived from an amino acid combined with building blocks from the shikimic acid, polyketide, or mevalonic acid pathways.



CO_2 H_2O

Respiration

Photosynthesis, assimilation

O_2

Polysaccharides, glycosides

Monosaccharides

$CH_3COCOOH$

Pyruvic acid

Shikimic acid

Peptides

CH_3COOH

ACETIC ACID

(Acetyl CoA)

Mevalonic acid

Aliphatic amino acids

Alkaloids

Malonic acid

Prephenic acid

3,3-Dimethylallyl pyrophosphate

Polyketides

Aromatic amino acids

Terpenoids

Fatty acids

Fats

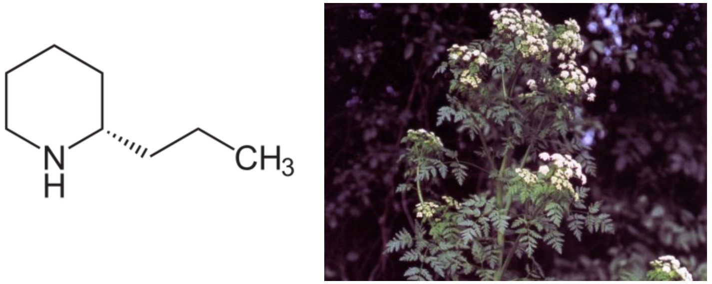
Cinnamic acids

Flavonoids, other aromatics

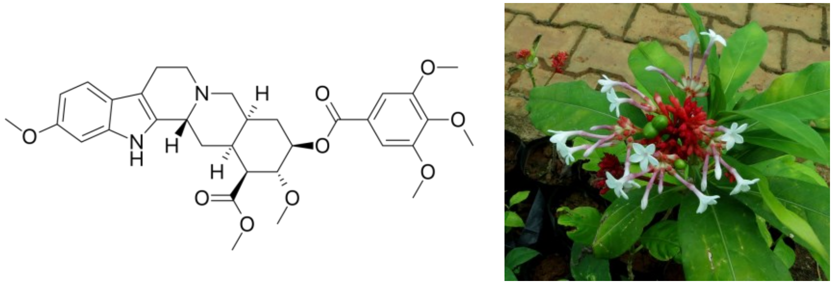
Coumarins

What are alkaloids?

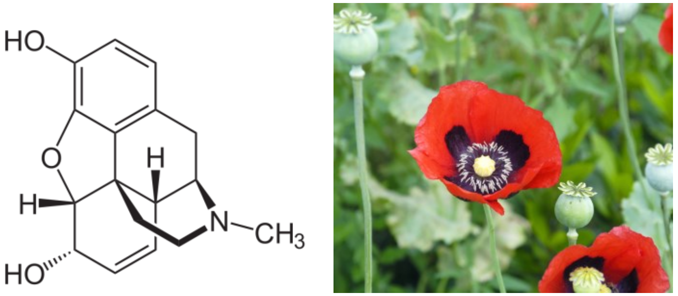
Caffeine (*Coffea*)



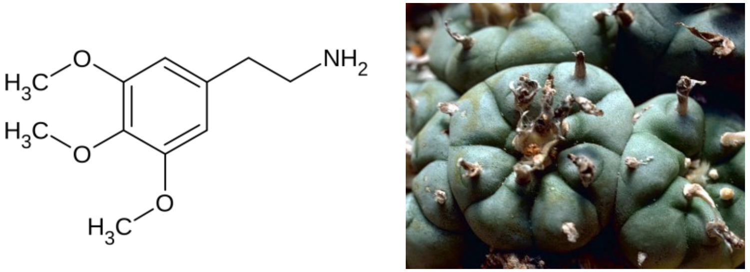
Coniine (*Conium maculatum*)



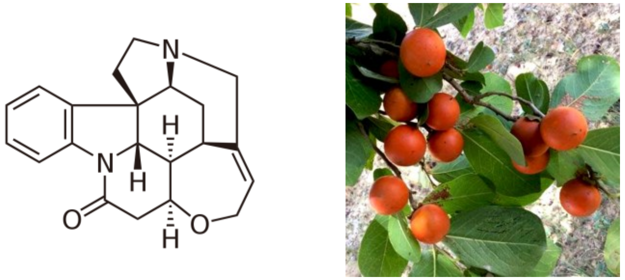
Reserpine (*Conium maculatum*)



Morphine (*Papaver somniferum*)

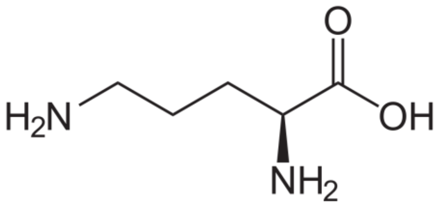


Mescaline (*Lophophora williamsii*)

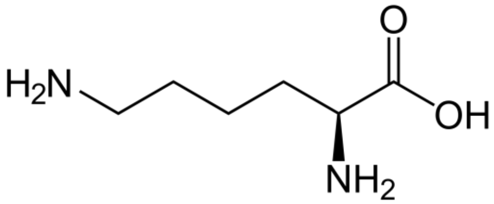


Strychnine (*Strychnos nux-vomica*)

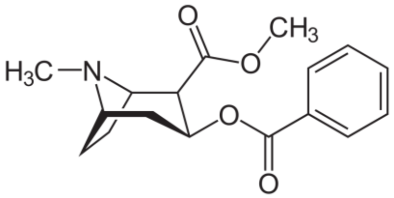
The pyrrolidine and piperidine alkaloids



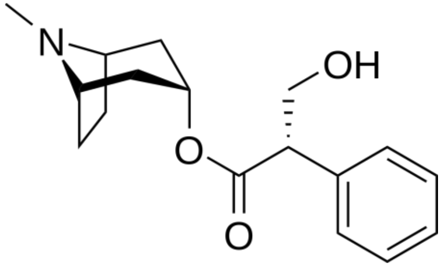
Ornithine



Lysine



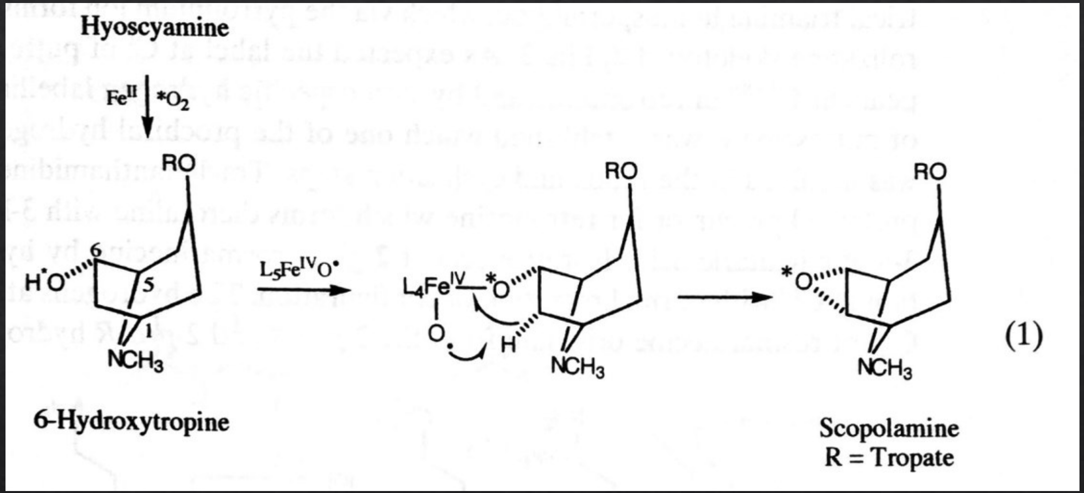
Cocaine (*Erythroxylum coca)*



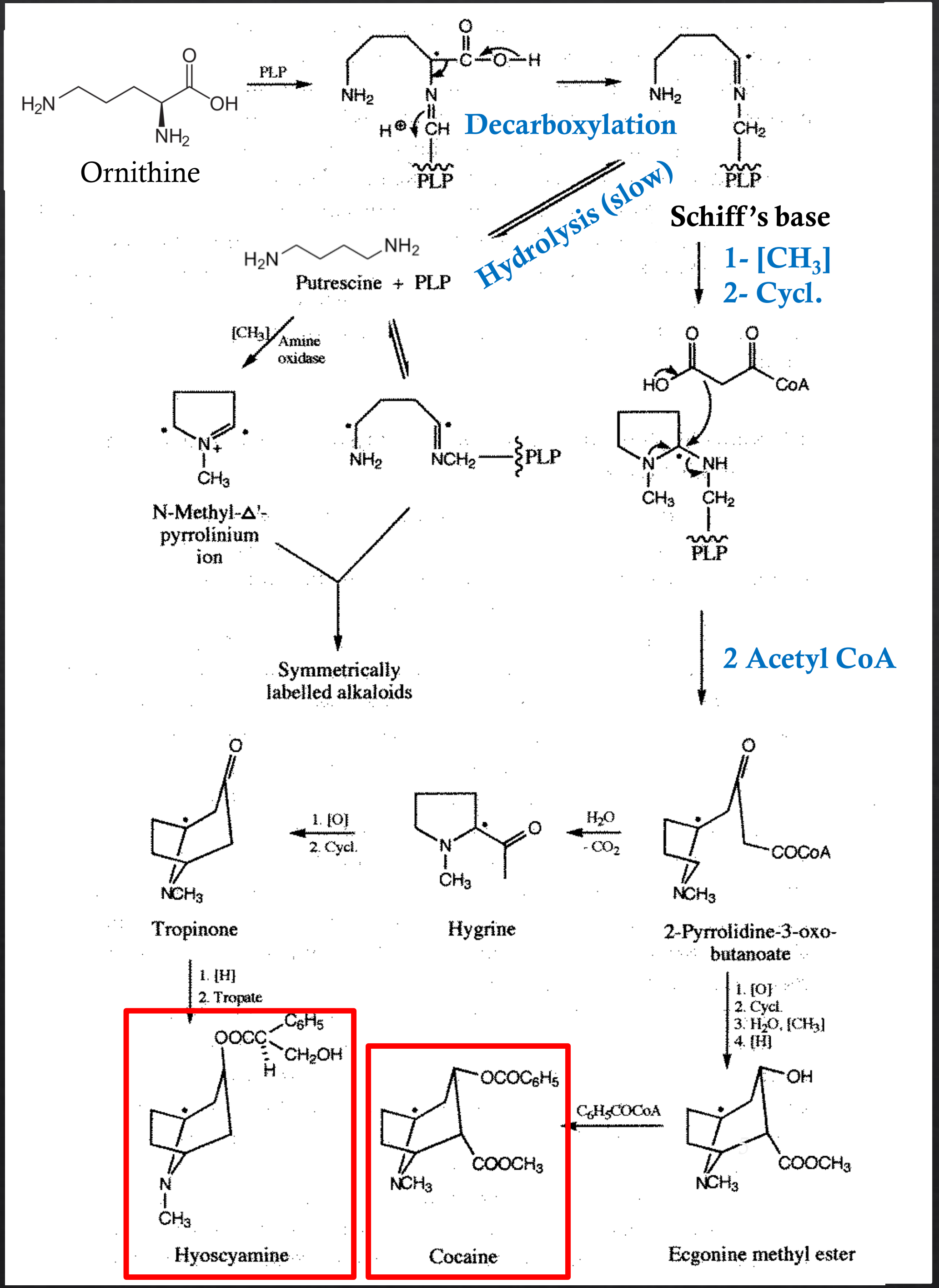
Hyoscyamine (*Solanaceae)*

The pyrrolidine and piperidine alkaloids

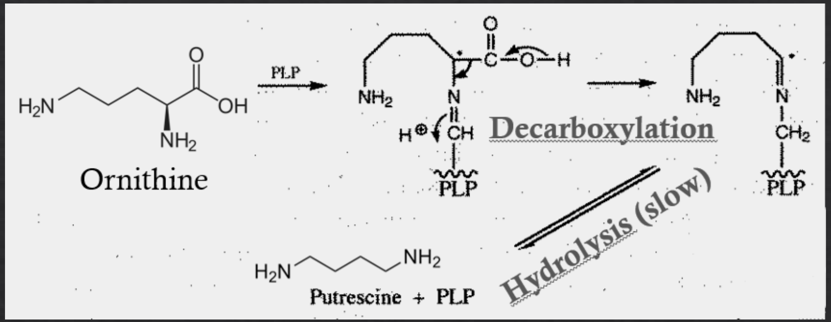
- Putrescine is accepted by the plant as a precursor, but the asymmetric incorporation of ornithine shows that putrescine isn't on the main pathway.



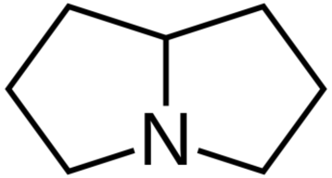
- To form scopolamine, an epoxide is added on the hyoscyamine structure via an unusual pathway involving a non-heme iron catalyst and molecular oxygen.



The pyrrolidine and piperidine alkaloids



- Condensation of two molecules of putrescine leads to pyrrolizidine alkaloids.
- They are produced by plants as a defense mechanism against insect herbivores. Many insects build up those alkaloids in their bodies, and use them to produce useful compounds (such as pheromones in queen butterfly)



Pyrrolizidine

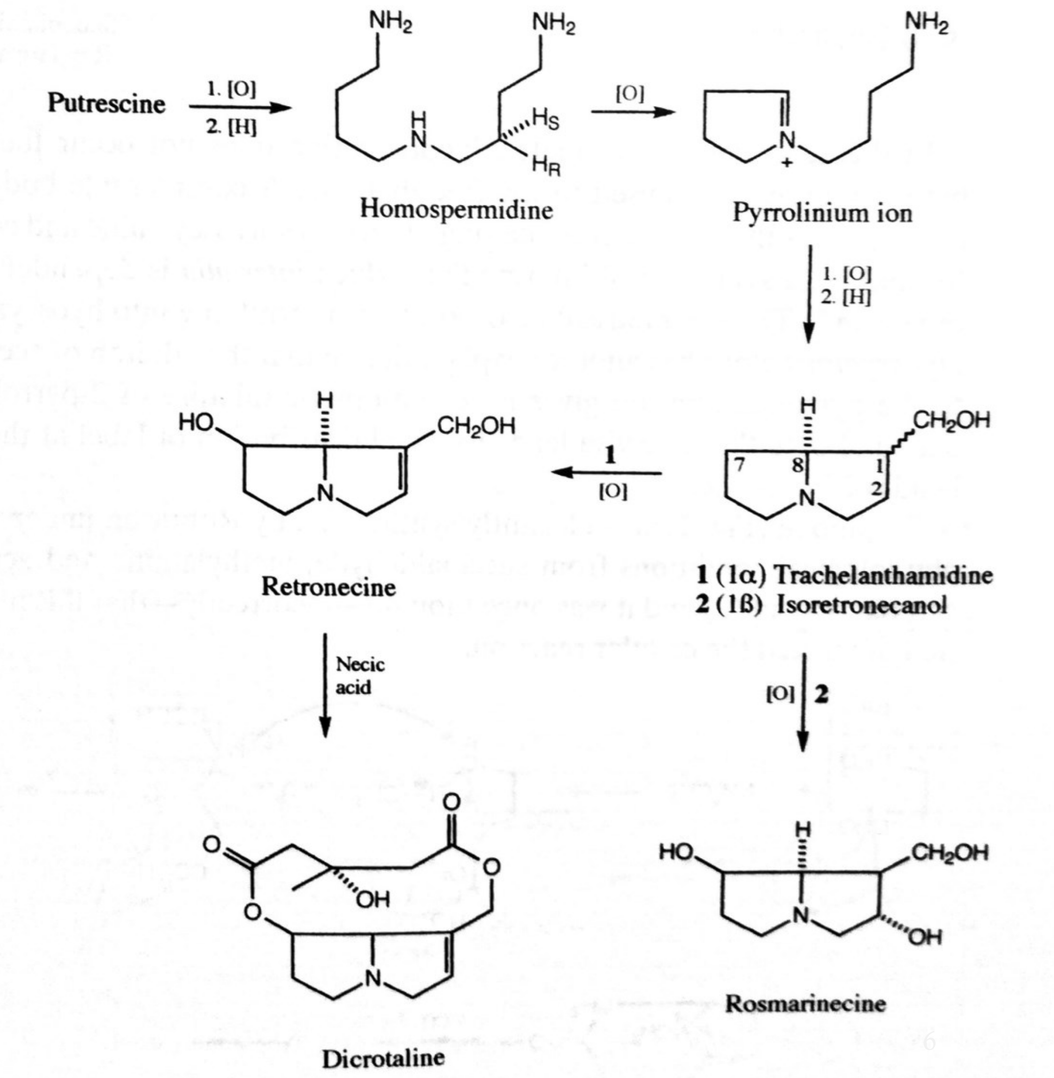


Fig. 2 Biosynthesis of pyrrolizidine alkaloids

The pyrrolidine and piperidine alkaloids

- Pelletierine, a vermifuge, is biosynthesized from lysine, in a pathway similar to the one leading to cocaine.
- Other alkaloids similar in structure follow a completely different path:

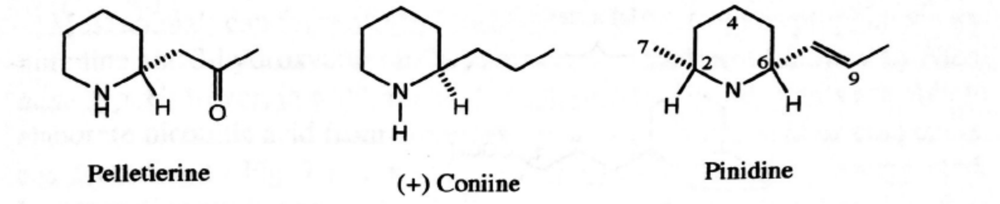
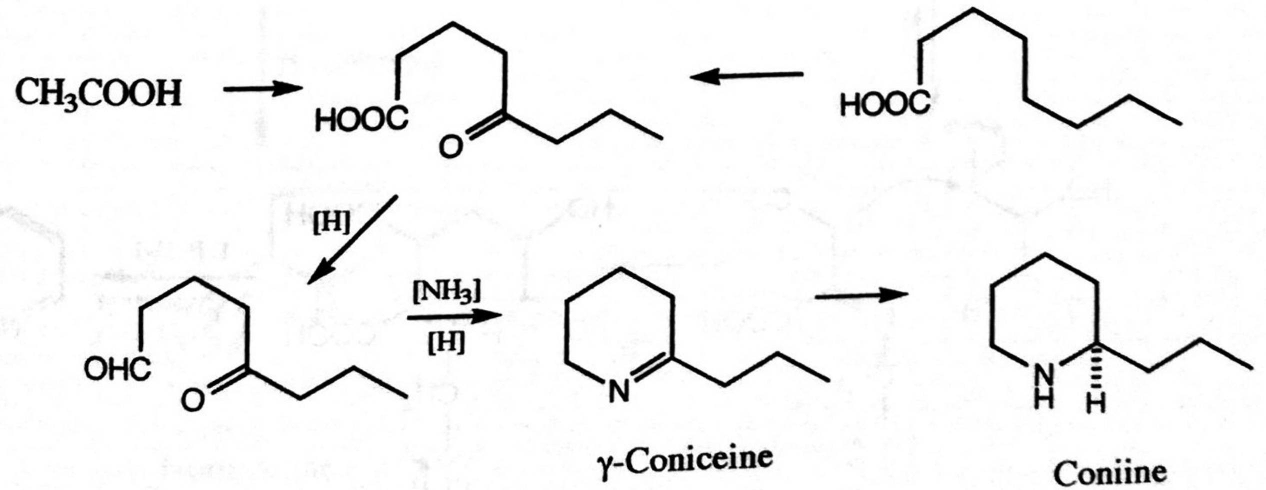


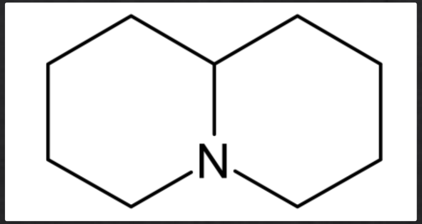
Fig. 4 Structure of piperidine alkaloids of different biogenesis. Pelletierine comes from lysine, whereas coniine and pinidine come from acetate



Here, the nitrogen atom doesn't come from an amino acid, but directly from ammonia

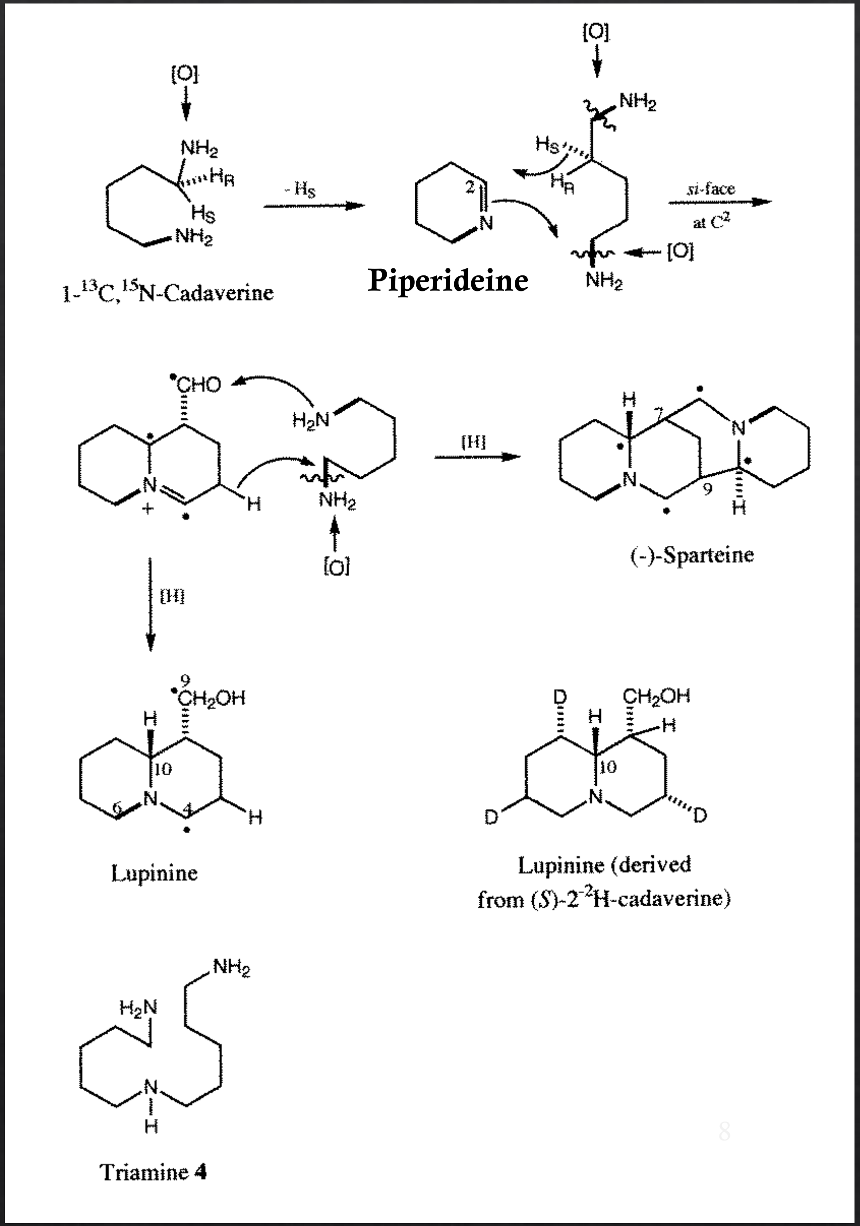
The pyrrolidine and piperidine alkaloids

- Quinolizidine alkaloids are formed from cadaverine (coming from lysine) and piperidine.

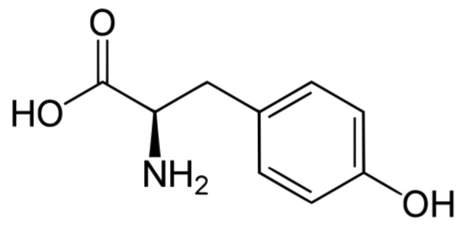


Quinolizidine

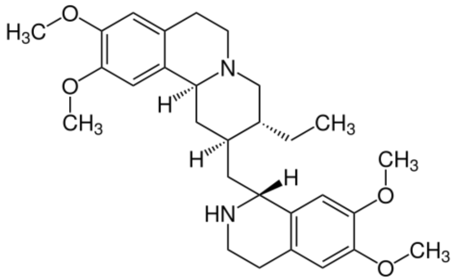
- Sparteine is an antiarrhytmic agent found in *Cytisus scoparius.*



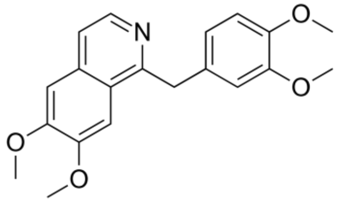
Alkaloids derived from tyrosine



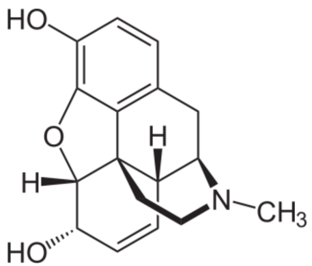
Lysine



Emetine (*Carapichea ipecacuanha*)



Papaverine

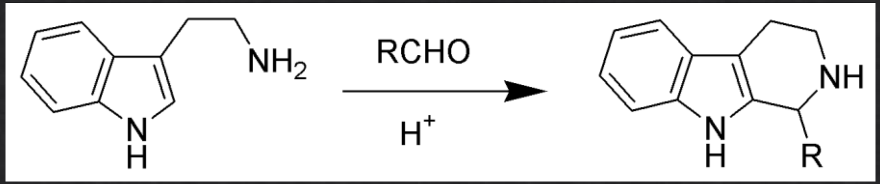


Morphine

(*Papaver somniferum*)

Alkaloids derived from tyrosine

- Fundamental principles:
  - Aromatic hydroxylation, O-methylation, and decarboxylation of the amino-acid.
  - Pictet-Spenger condensation with a carbonyl compound



  - Phenol coupling
- Bebeerine and its derivative tubocurarine are the main active components of the arrow poison prepared by the indians of the Amazon.

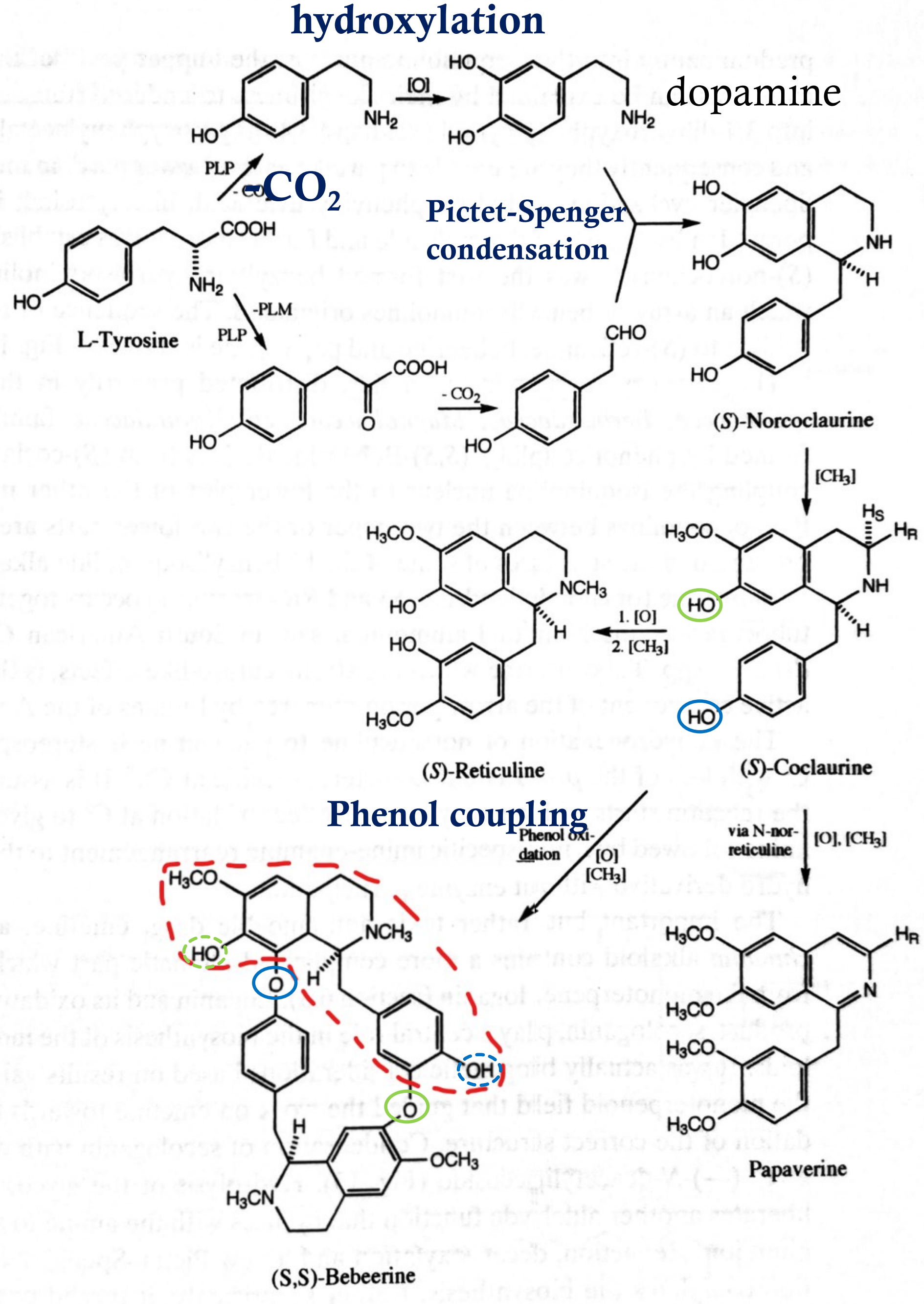


Fig. 12 Biosynthesis of (S)-reticuline, papaverine and (S,S)-bebeerine

Alkaloids derived from tyrosine

- The rather complicated aliphatic part of emetine comes from the monoterpene loganine.
- Secologanin on the scheme comes from the oxidative fission of loganine.

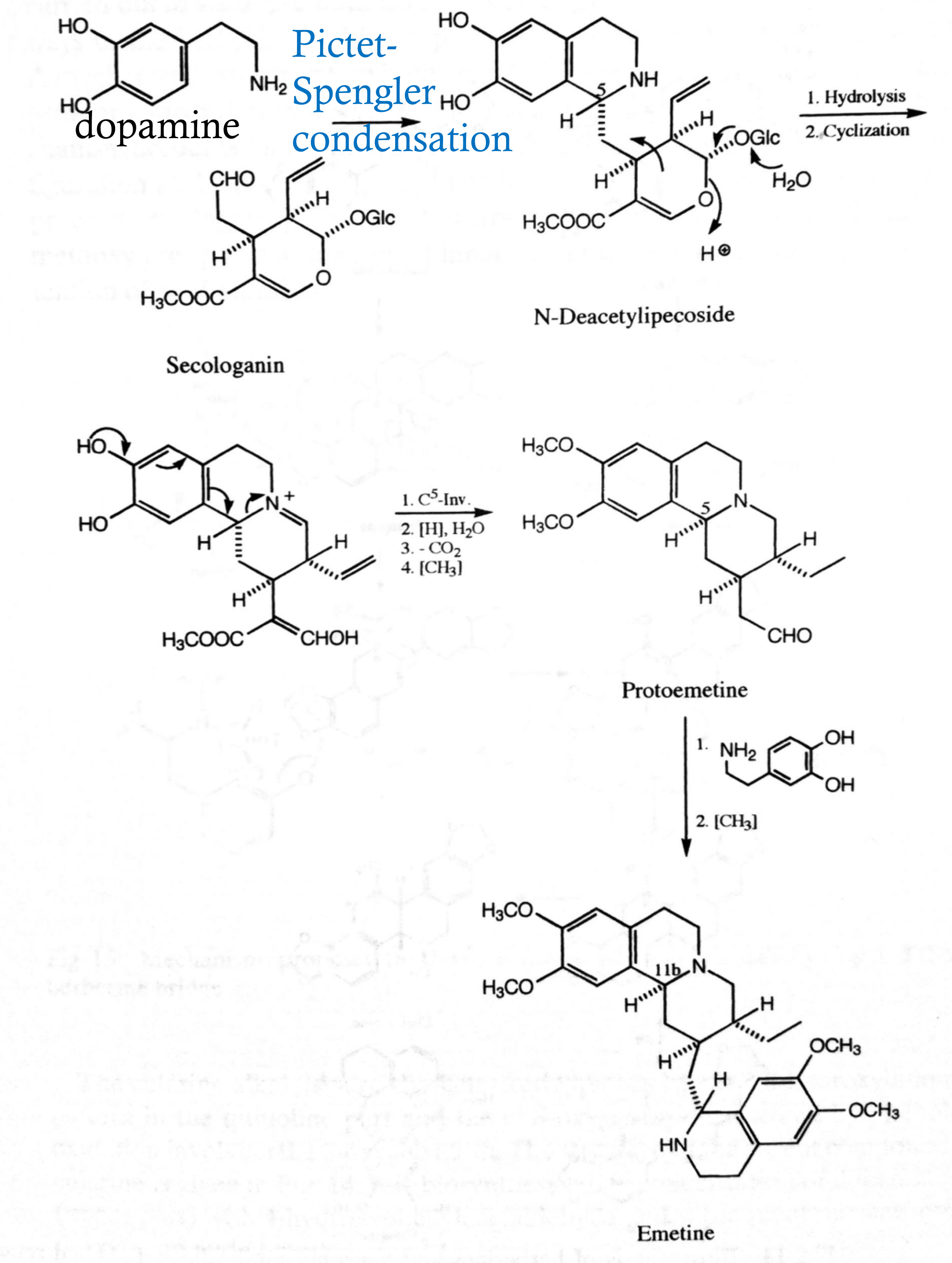
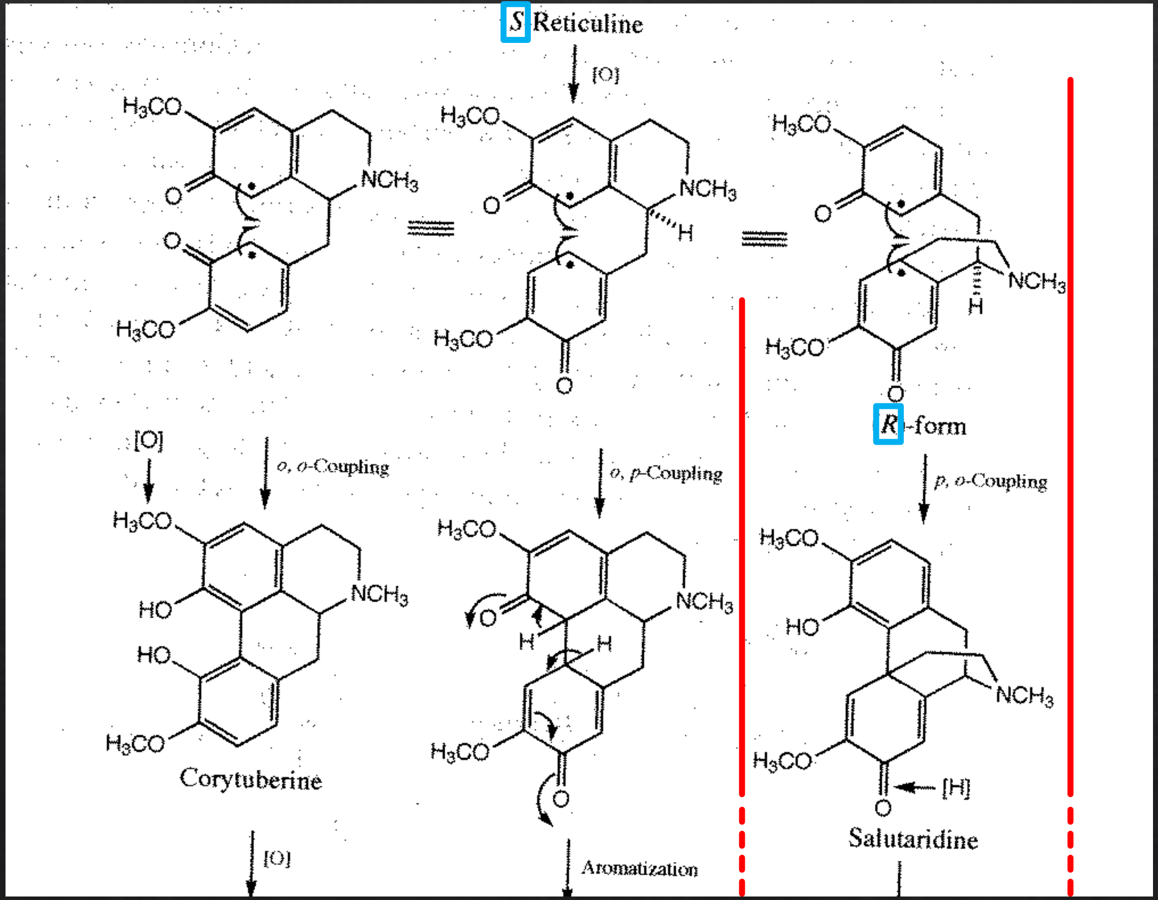
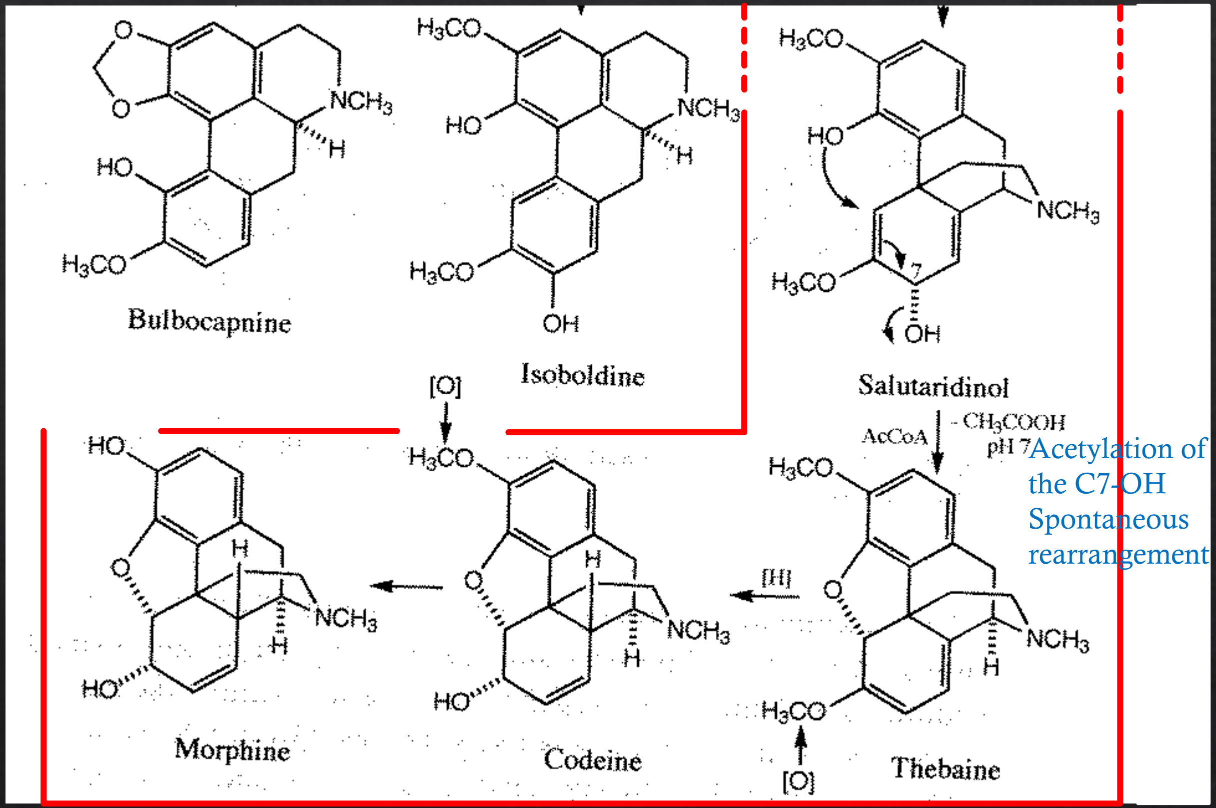


Fig. 13 Biosynthesis of emetine

Alkaloids derived from tyrosine

- Morphine is biosynthesized from reticuline, which we saw two slides ago.





Acetylation of the C7-OH Spontaneous rearrangement

- The enzyme systems capable of converting codeine into morphine are also present in mammalian tissues

Alkaloids derived from tyrosine

- Corydine, glaucine and dicentrine have similar structure, so chemists could imagine this kind of biosynthesis, from reticuline.
- Actually, those alkaloids aren’t formed from reticuline, but from norlaudanosoline.

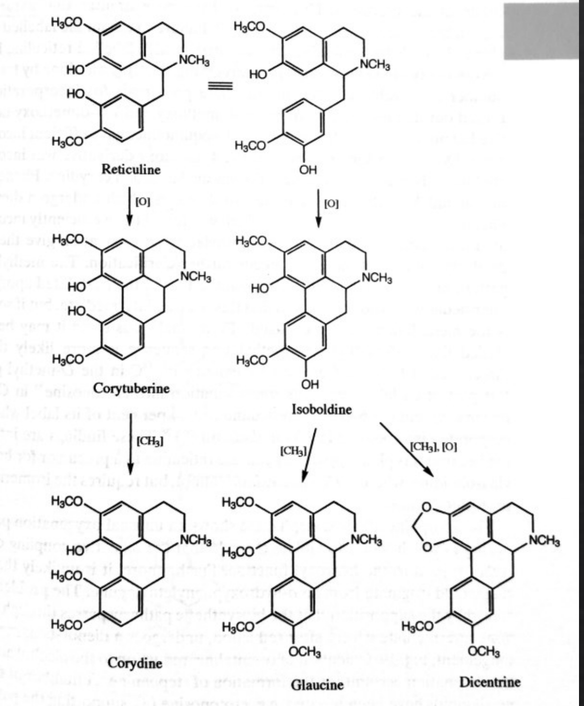


Fig. 17 Disproved routes to corydine, glaucine, and dicentrine in *Dicentra eximia*

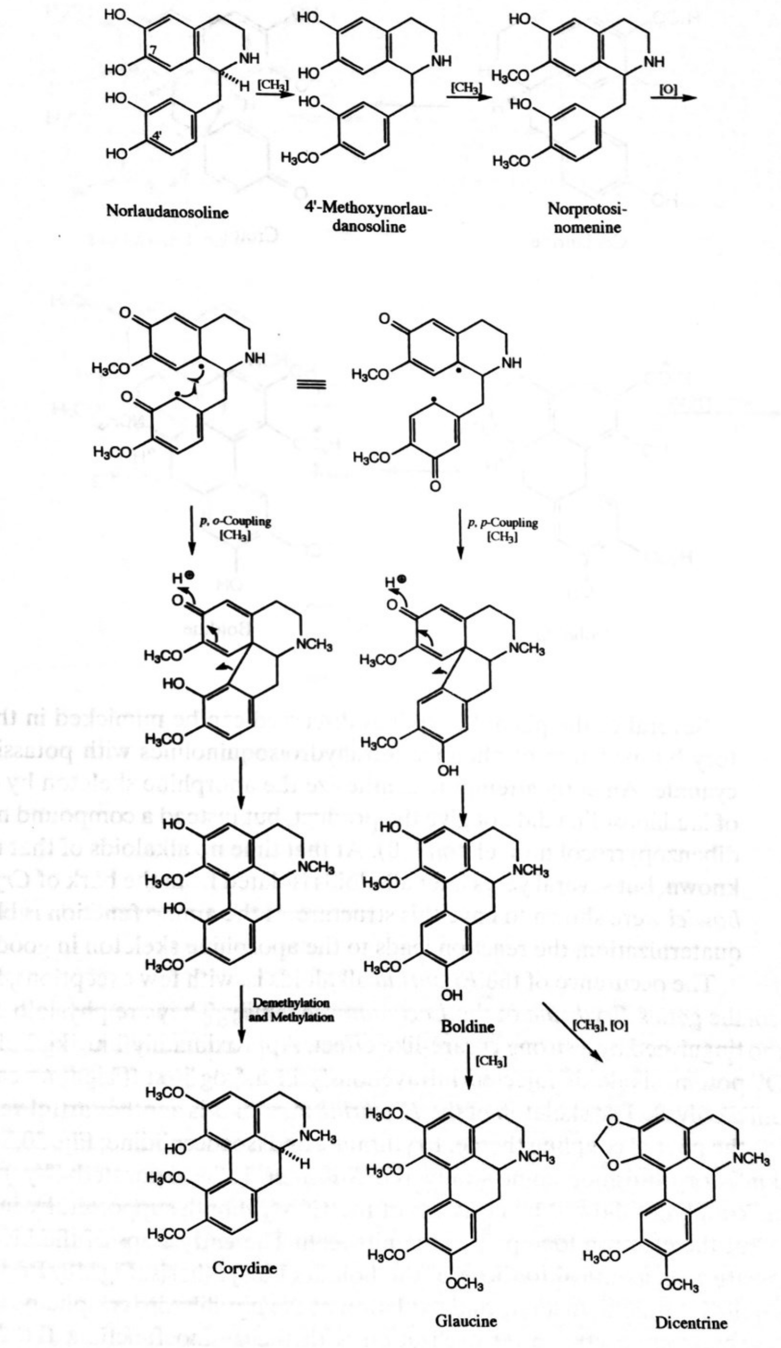


Fig. 18 Biosynthesis of corydine, glaucine and dicentrine

Iceland
Liechtenstein
Norway grants
“Working together for a green, competitive and inclusive Europe”

GREENCAM for tomorrow

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VIRTUTE ET SAPIENTIA

NTNU
Norwegian University of Science and Technology

Chapter 8-2
The Alkaloids

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

Spring 2022

Alkaloids derived from tryptophan

- «The indole alkaloids»
- Primarily from three plant families:

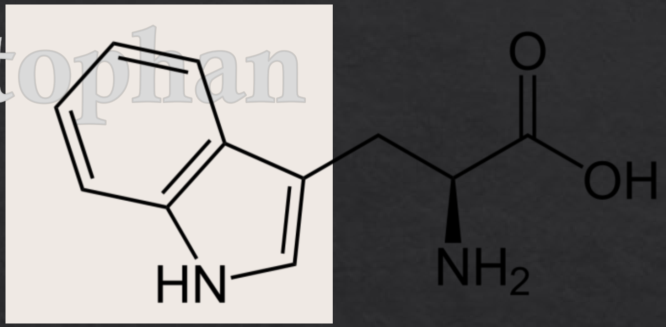

Apocynaceae.


Loganiaceae


Rubiaceae

- Neurophysiological action
- Sometimes the indole nucleus is modified

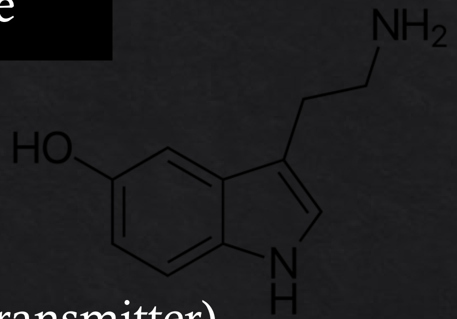
Indole



Tryptophan

Quinoline

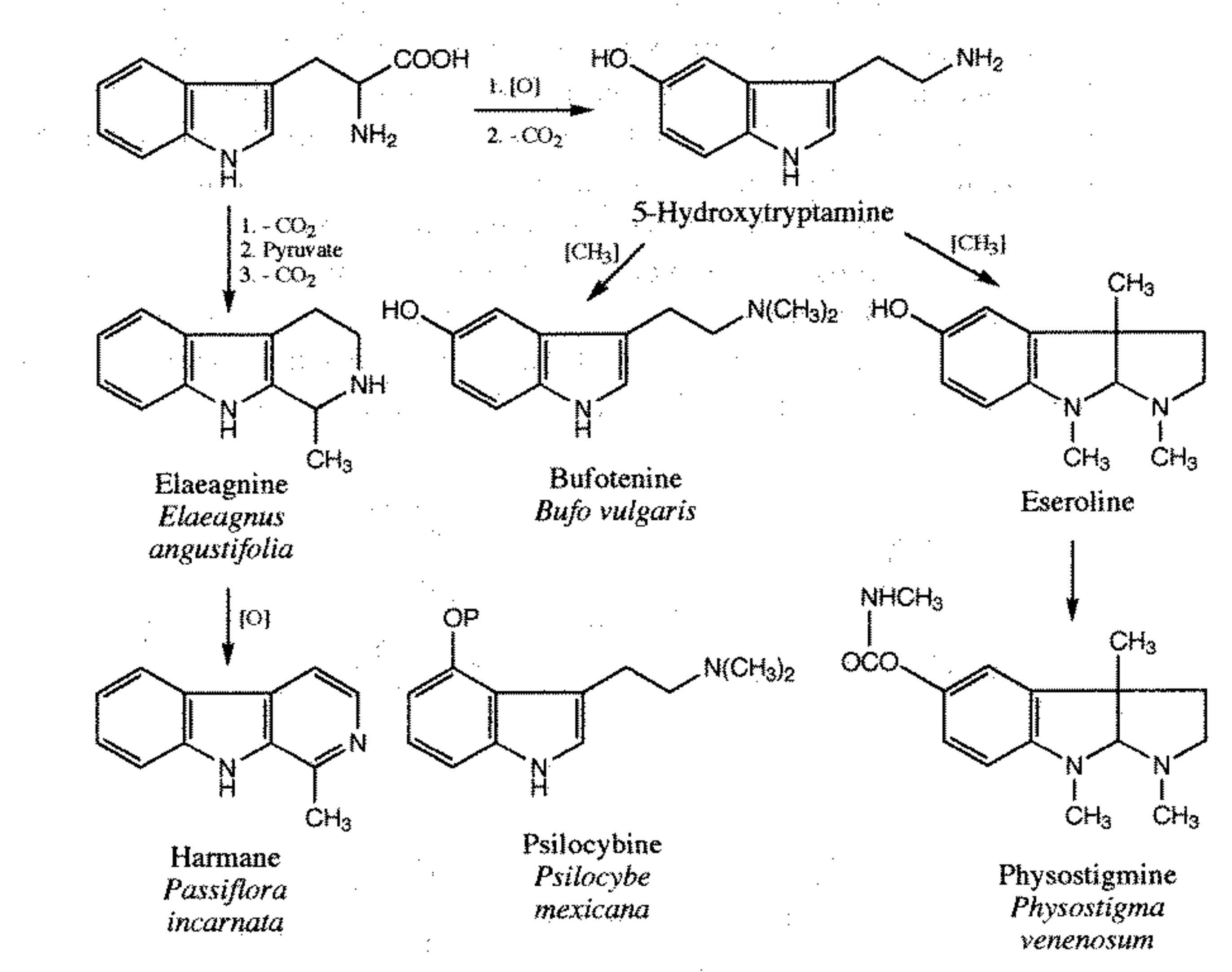
Isoquinoline



Serotonin (neurotransmitter)

Alkaloids derived from tryptophan

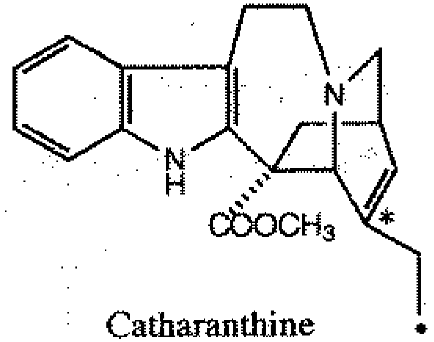
Biosynthesis of son
simple tryptophan
alkaloids:



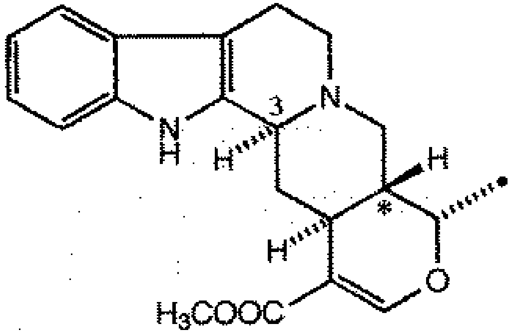
Catharanthus roseus

Alkaloids derived from tryptophan

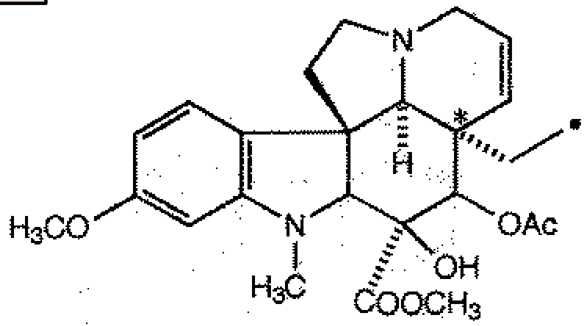
Where does the aliphatic part of more complex indole alkaloids come from?



Catharanthine
Iboga type



Ajmalicine
Corynanthe type



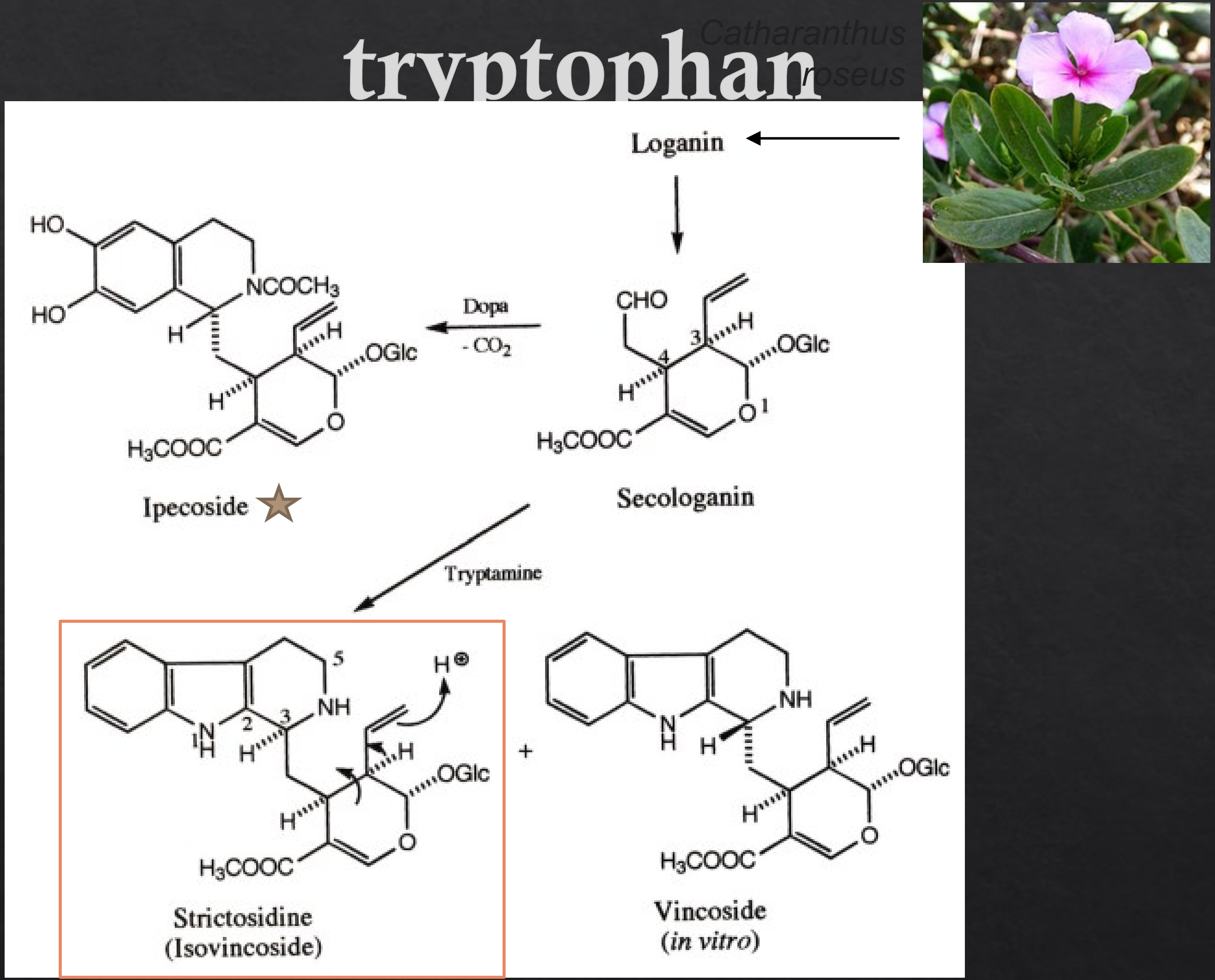
Vindoline
Aspidosperma type

Monoterpene hypothesis:

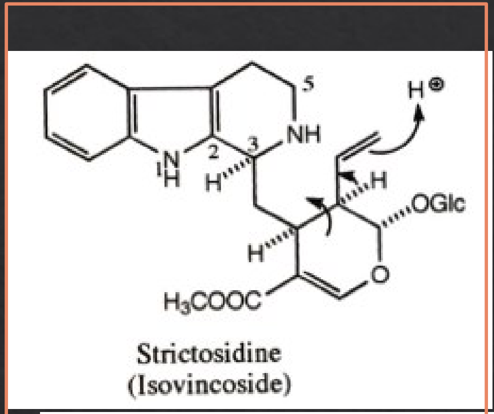
Mevalonate (in the form of geranyl phosphate) is incorporated to give a variety of indole alkaloids

Proved by incorporating 13C labelled geranyl phosphate

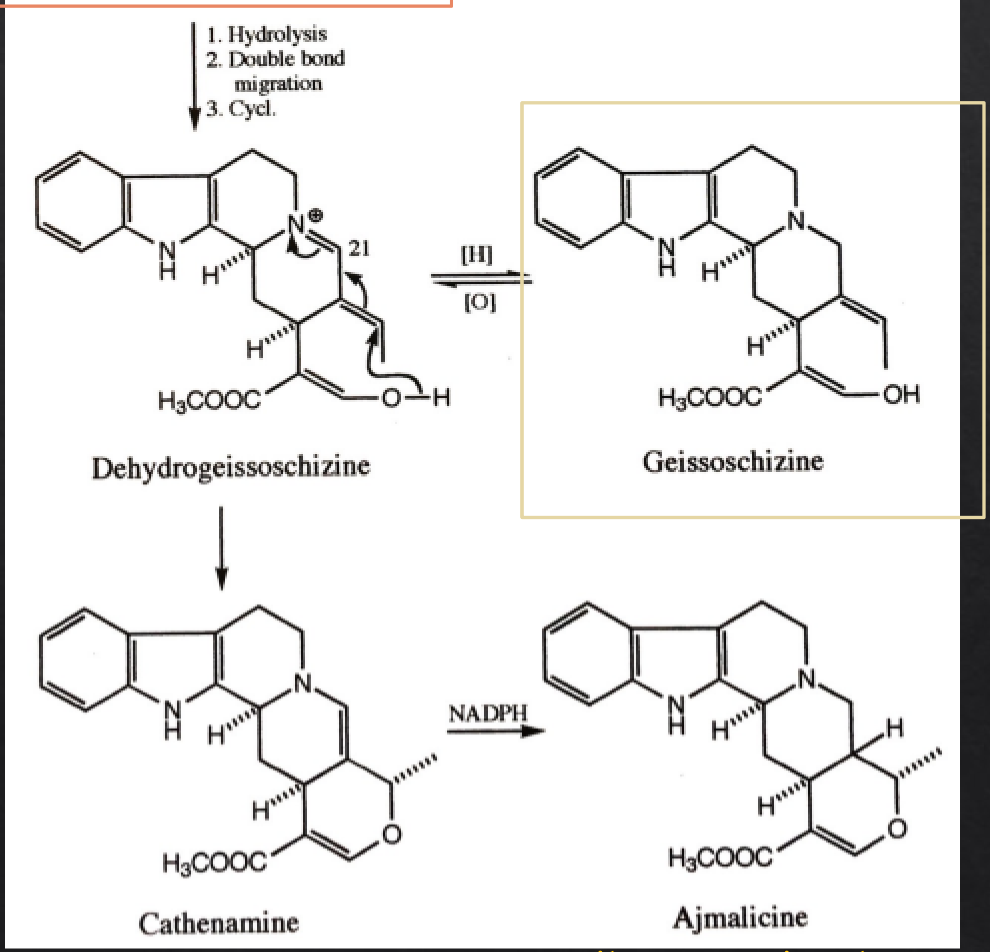
Alkaloids derived from tryptophan



Catharanthus roseus



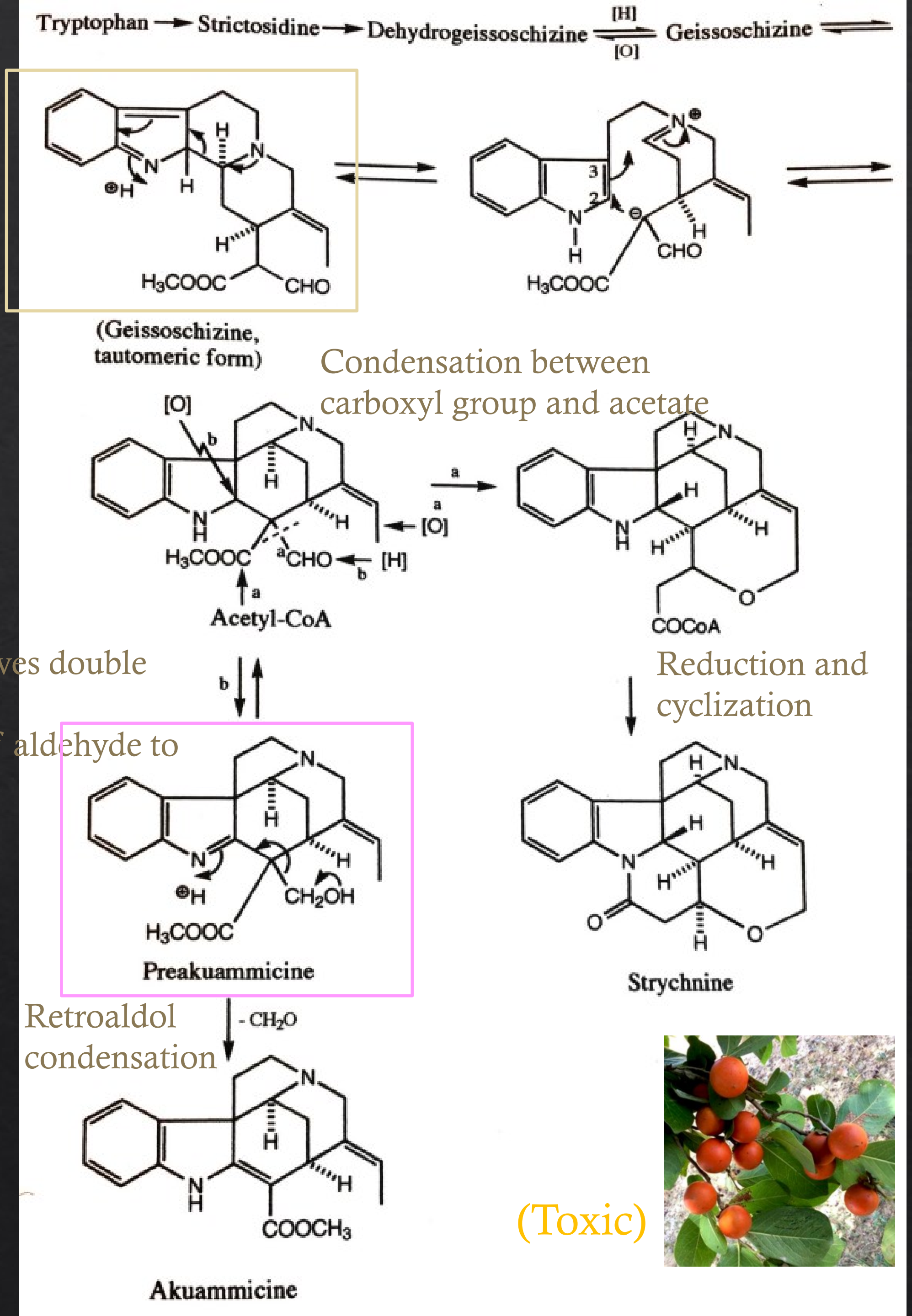
Strictosidine is a «universal intermediate» in the biosynthesis of indole alkaloids



antihypertensive drug

Alkaloids derived from tryptophan

- Geisoschizine is also an important intermediate



Condensation between carboxyl group and acetate

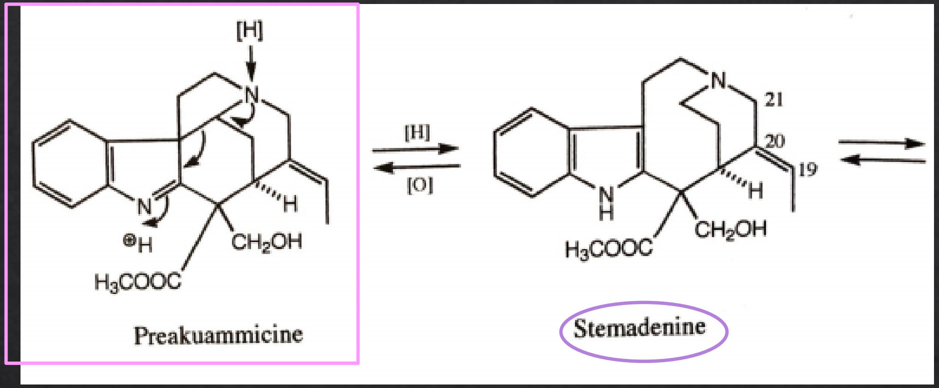
Oxidation gives double bond
Reduction of aldehyde to alcohol

Reduction and cyclization

Retroaldol condensation

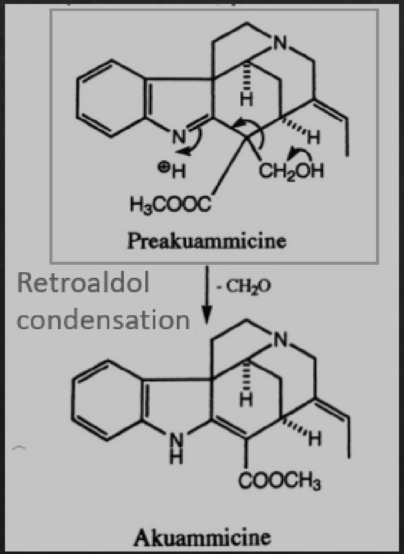
(Toxic)

Alkaloids derived from tryptophan



Preakuammicine

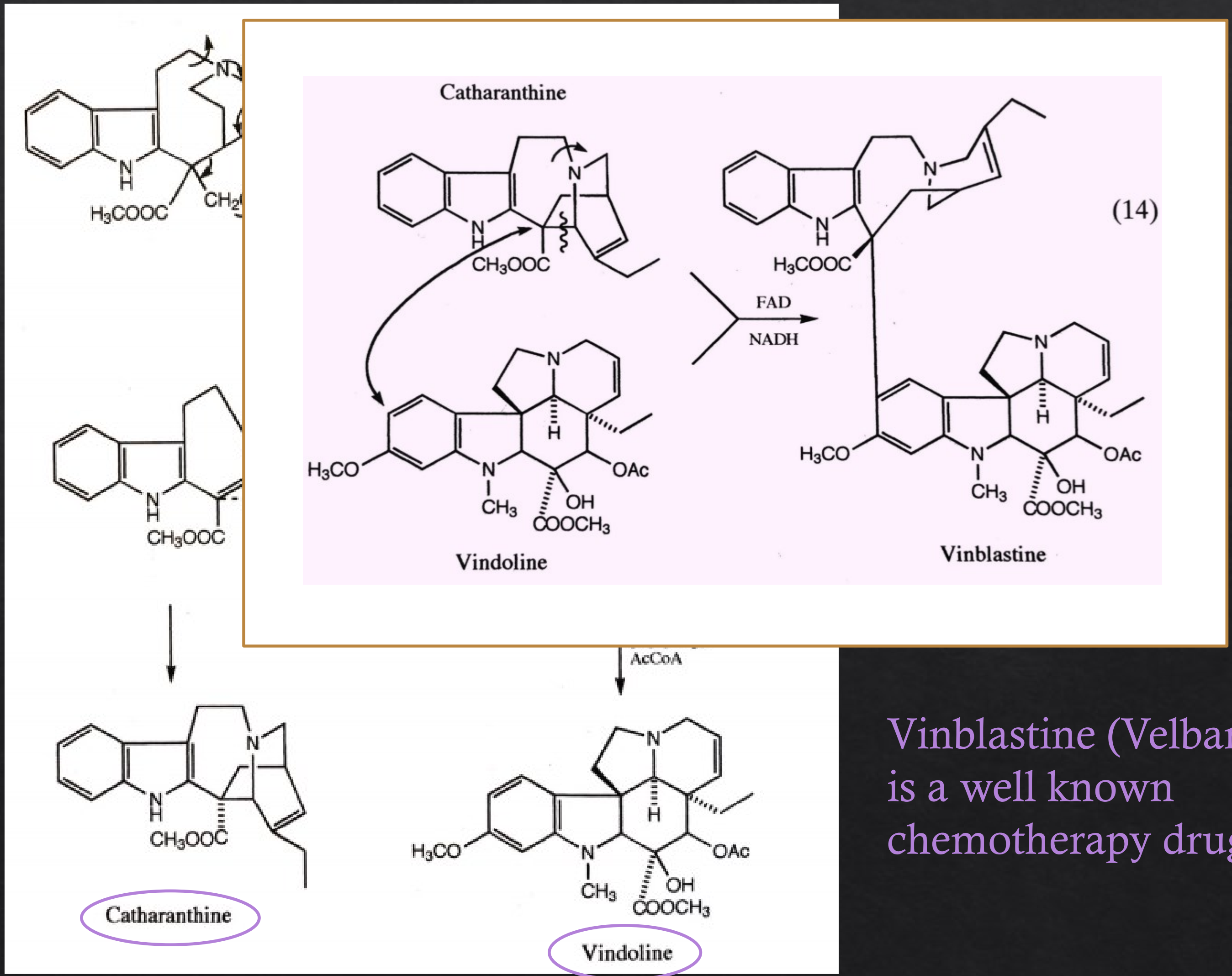
Stemadenine



Preakuammicine

Retroaldol condensation

Akuammicine



Catharanthine

Vindoline

Vinblastine

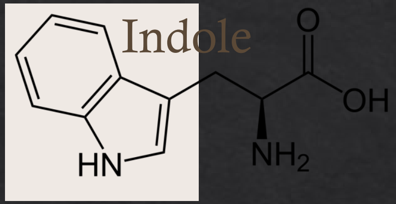
Catharanthine

Vindoline

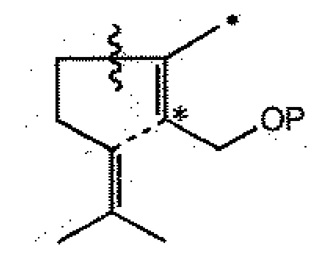
Vinblastine (Velban) is a well known chemotherapy drug

Summary of tryptophan (indole) alkaloids

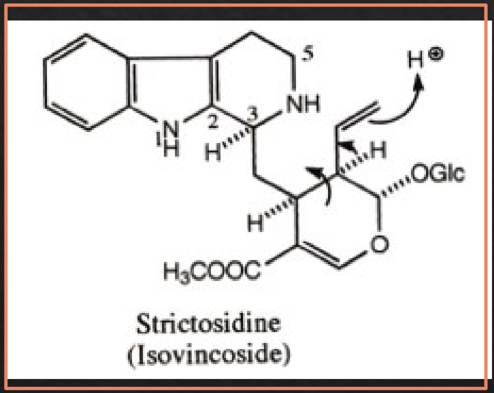
- Indole part comes from tryptophan
- Rest comes from mevalonic acid as geranyl phosphate
- Important intermediates:
    - Strictosidine
    - Geissoschizine
    - Preakuammicine



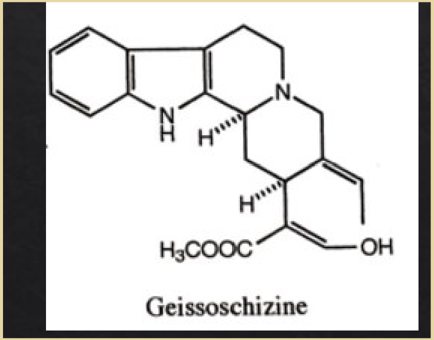
Tryptophan



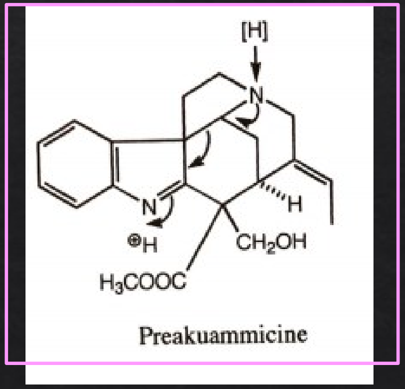
Geranyl phosphate



Strictosidine (Isovincoside)

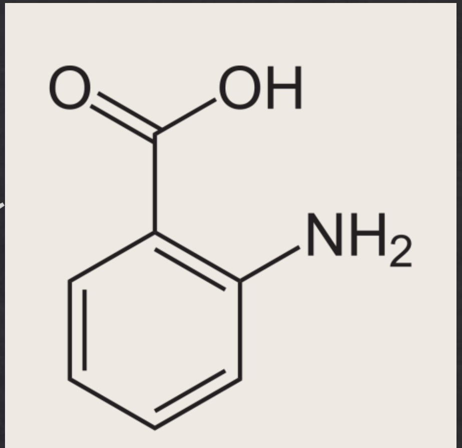


Geissoschizine



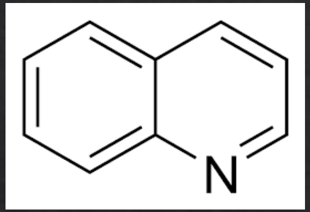
Preakuammicine

Alkaloids derived from anthranilic a

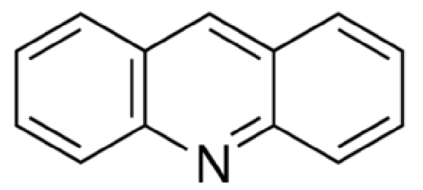


Anthranilic acid

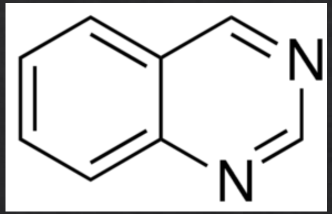
- Quinoline, acridine and quinazoline skeletons



Quinoline



Acridine



Quinazoline

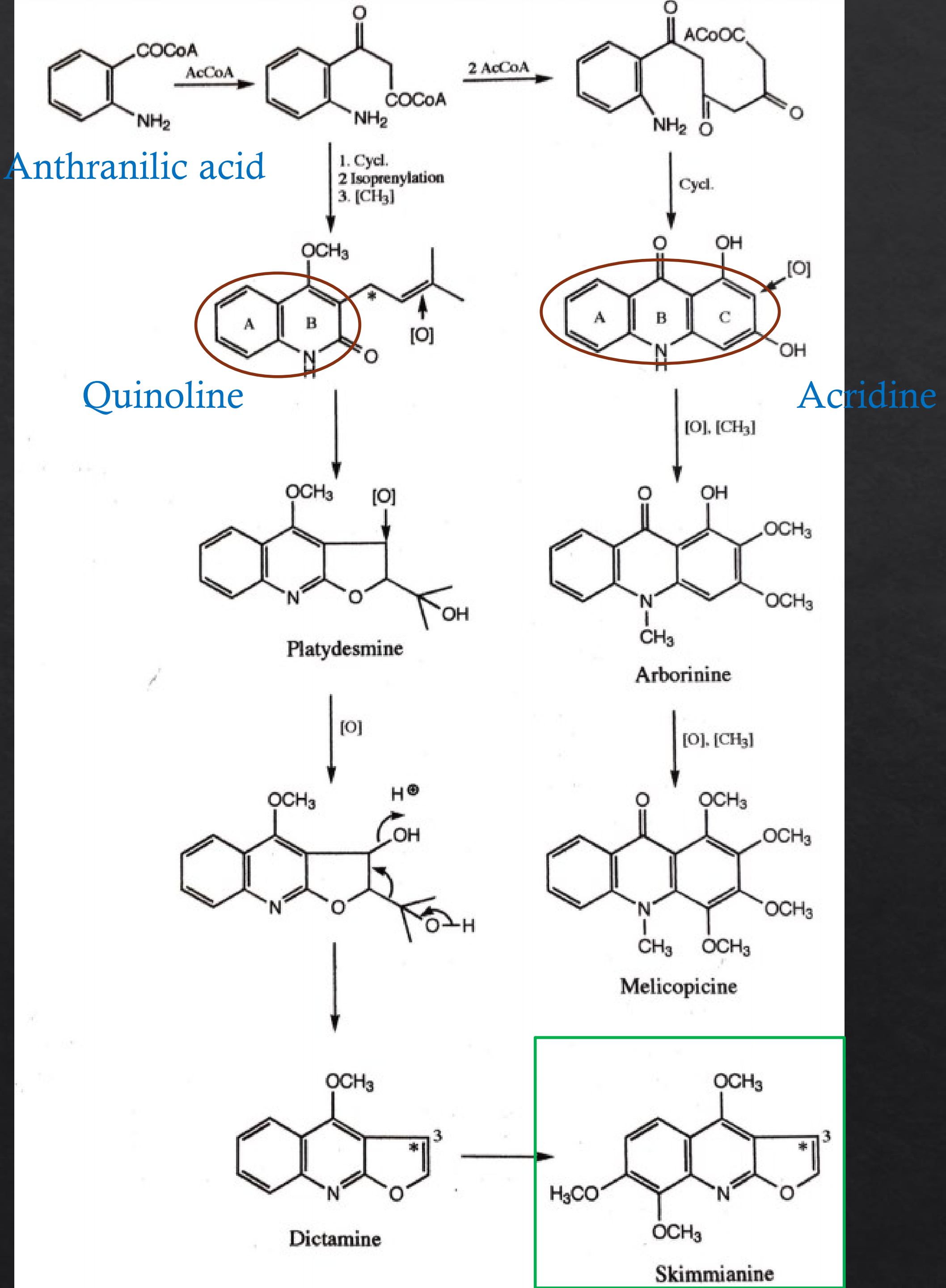
- Anthranilic acid comes from shikimic acid, and is a precursor to tryptop

Often found in the *Rutaceae*
(rue) family

Alkaloids derived from anthranilic acid

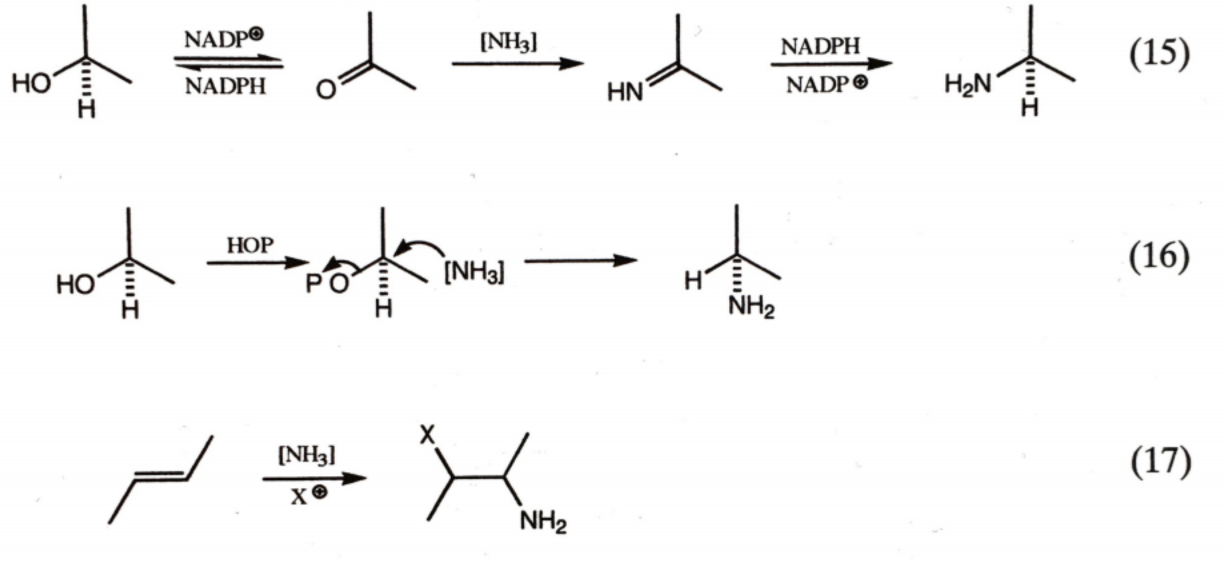
The skimmianine alkaloids

1. Activation of the anthranilic acid with acetyl CoA
2. Chain elongation with acetyl or malonyl CoA.
3. Cyclization gives quinoline and acridine skeletons
4. Further derivatization



Terpene derived alkaloids

- Amination of terpenes/terpenoids



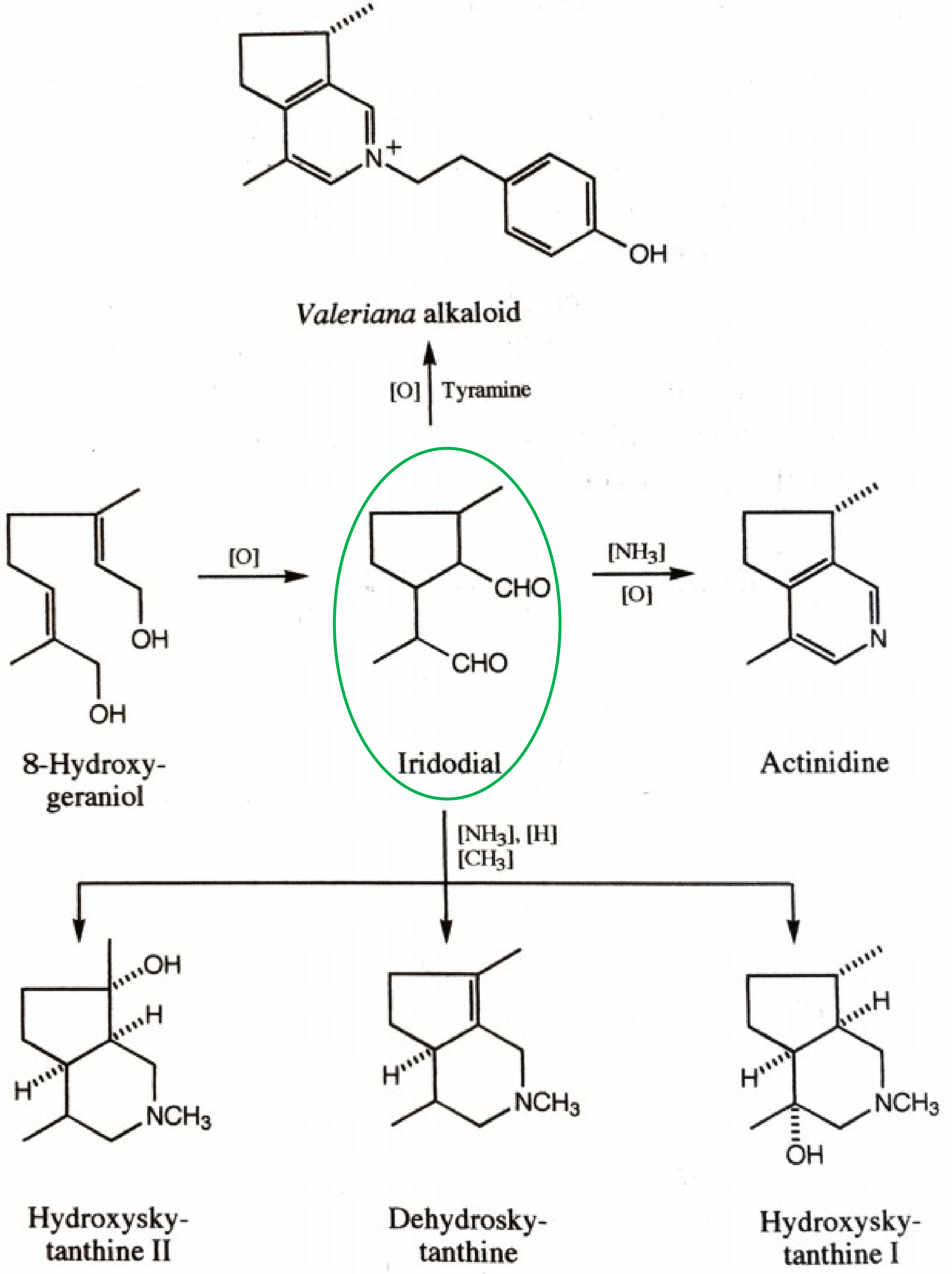
Redox process (oxidation, amination, reduction)

Substitution

Addition

Terpene derived

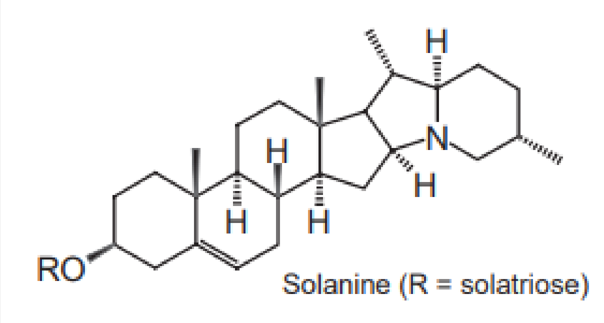
- Many monoterpene alkaloids are derived from iridoids of varying oxidation levels
- *Valeriana officinalis* and *Actinidia polygama* attract cats because they contain specific monoterpenoids and alkaloids
- Actinidine affects the EEG of cats



Terpene derived alkaloids

STEROID ALKALOIDS

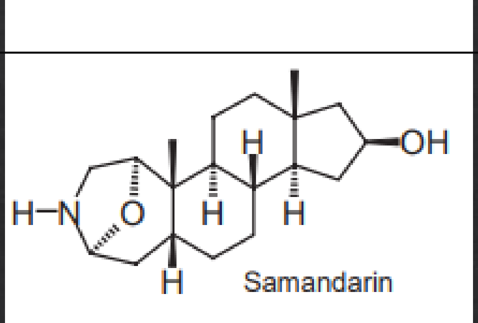
- Metabolites of *Solanaceae, Liliaceae* and *Buxaceae* families



Solanine (R = solatriose)

Found in nightshades
Very toxic
Used in pesticides, asthma treatment and for common cold

«Symptoms include nausea, diarrhoea, vomiting, stomach cramps, burning of the throat, headaches and dizziness. Other adverse reactions, in more severe cases, include hallucinations, loss of sensation, paralysis, fever, jaundice, dilated pupils and hypothermia. Solanine overdose can be fatal.»



Samandarin

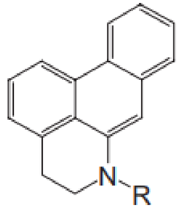
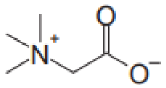
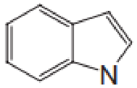
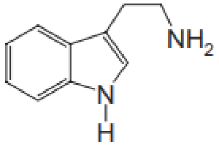
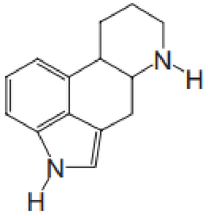
Found in the skin of the fire salamander (*Salamandra salamandra)*
Extremely toxic, causes strong muscle convulsion (shaking) and hyperventilation in vertebrates

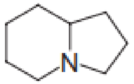
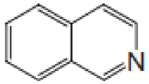
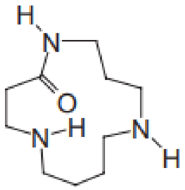
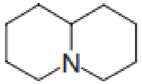
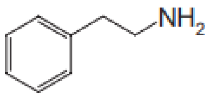
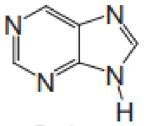
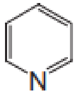
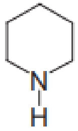
Solanaceae (nightshades)

Liliaceae (lily family)

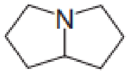
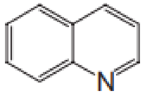
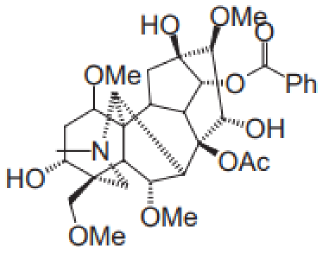
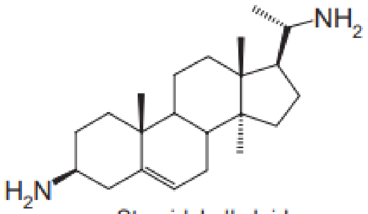
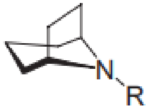
Buxaceae (box family)

Sarker & Nahar, *Chemistry for Pharmacy Students,* Wiley 2007

Class/structural types	Generic structure	Examples
Aporphine (Tyrosine derived)	 Aporphine R = Me Noraporphine R = H	Boldine
Betaines	 Betaine	Choline, muscarine and neurine
Imidazole	 Imidazole	Pilocarpine
Indole (Tryptophan derived)	 Indole	
Tryptamines	 Tryptamine	Moschamine, moschamindole, psilocybin and serotonin
Ergolines	 Ergoline	Ergine, ergotamine and lysergic acid

Class/structural types	Generic structure	Examples
Indolizidine	 Indolizidine	Swainsonine and castanospermine
Isoquinoline (Tyrosine derived)	 Isoquinoline	Codeine, berberine, morphine, papaverine, sanguinarine and thebaine
Macrocyclic spermines and spermidines	 Spermine	Celabenzine
Norlupinane (Lysine derived)	 Norlupinane	Cytisine and lupanine
Phenethylamine (Phenylalanine derived)	 Phenylethylamine	Ephedrine and mescaline
Purine	 Purine	Caffeine, theobromine and theophylline
Pyridine and (Nicotinic acid derived)	 Pyridine  Piperidine	Arecoline, coniine, nicotine, piperine, sparteine and trigonelline

Sarker & Nahar, *Chemistry for Pharmacy Students,* Wiley 2007

Class/structural types	Generic structure	Examples
Pyrrole and pyrrolidine (Ornithine derived)	 Pyrrole  Pyrrolidine	Hygrine, cuscohygrine and nicotine
Pyrrolizidine	 Pyrrolizidine	Echimidine and symphitine
Quinoline (Tryptophan/anthranilic acid derived)	 Quinoline	Cinchonine, brucine, quinine and quinidine
Terpenoidal/steroidal Terpenoidal	 Aconitine	Aconitine
Steroidal	 Steroidal alkaloid	Batrachotoxin, conanine, irehdiamine A, solanine, samandarine and tomatillidine
Tropane (Ornithine derived)	 Tropane R = Me Nortropane R = H	Atropine, cocaine, ecgonine, hyoscine and scopalamine

Sarker & Nahar, *Chemistry for Pharmacy Students,* Wiley 2007

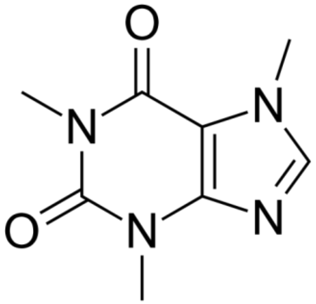
Chapter 9:
N-Heteroaromatics

Elisabeth Jacobsen and Lucas Boquin, NTNU

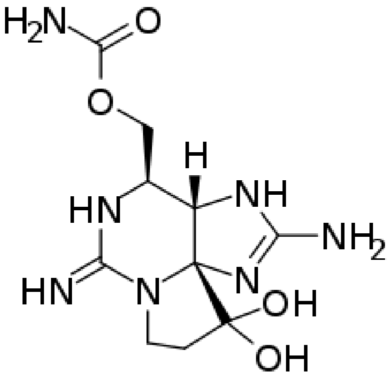
Spring 2022

N-Heteroaromatics

- A lot of N-heteroaromatics are essential for life and reproduction, and are part of the primary metabolism.
- Some N-heteroaromatics are products of the secondary metabolism, such as caffeine, and saxitoxin

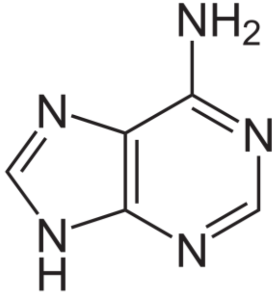


Caffeine

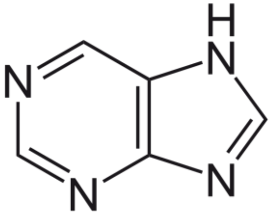


Saxitoxin
Fatally poisonous toxin produced
by some algaes, and occasionally
accumulated in clams and shellfishes

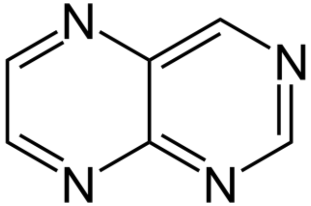
N-Heteroaromatics



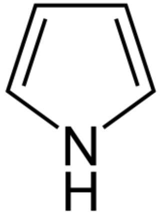
Pyrimidine



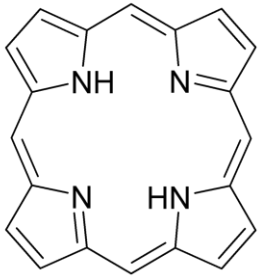
Purine



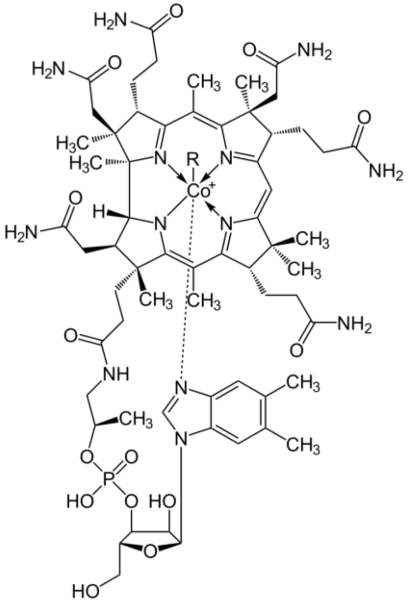
Pteridine



Pyrrole



Porphin

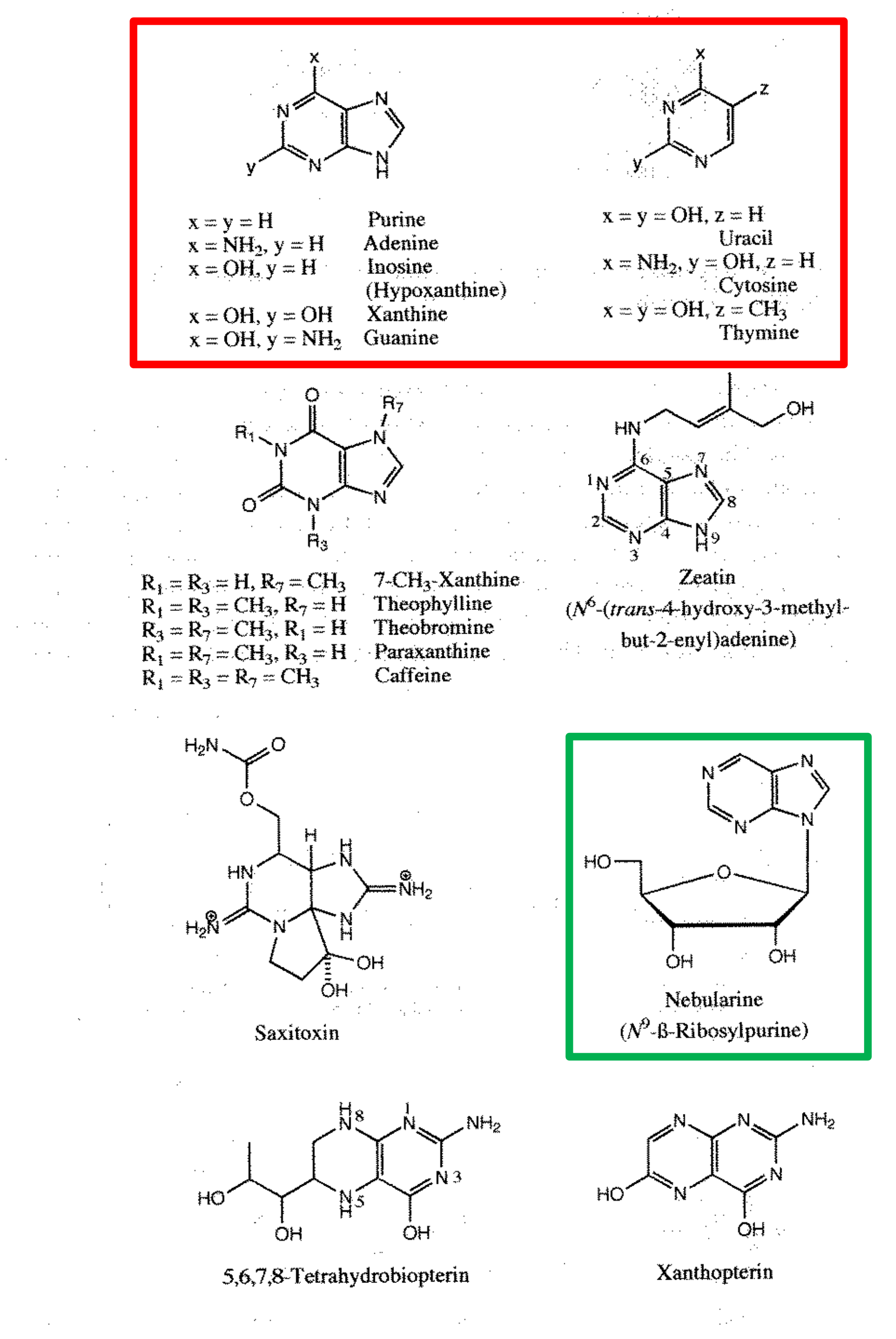


Cobalamin (vitamin B12)

Pyrimidines, purines and pteridines

- The major bases in RNA and DNA are purine derivatives (adenine, guanine, cytosine, uracil (only in RNA), thymine (only in DNA)…)
- The free bases are only present in trace amounts in the cells.
- In some fungis, nucleosides are produced in larger quantities and have antibiotic properties (such as neburaline)

Clitocybe nebularis



$x = y = H$ Purine
$x = NH_2, y = H$ Adenine
$x = OH, y = H$ Inosine (Hypoxanthine)
$x = OH, y = OH$ Xanthine
$x = OH, y = NH_2$ Guanine

$x = y = OH, z = H$ Uracil
$x = NH_2, y = OH, z = H$ Cytosine
$x = y = OH, z = CH_3$ Thymine

$R_1 = R_3 = H, R_7 = CH_3$ 7-CH_3-Xanthine
$R_1 = R_3 = CH_3, R_7 = H$ Theophylline
$R_3 = R_7 = CH_3, R_1 = H$ Theobromine
$R_1 = R_7 = CH_3, R_3 = H$ Paraxanthine
$R_1 = R_3 = R_7 = CH_3$ Caffeine

Zeatin
(N^6-(*trans*-4-hydroxy-3-methyl-but-2-enyl)adenine)

Saxitoxin

Nebularine
(N^9-ß-Ribosylpurine)

5,6,7,8-Tetrahydrobiopterin

Xanthopterin

Biosynthesis of purines

- This biosynthesis shows, again, that structural information can be misleading when finding the natural synthesis pathway.

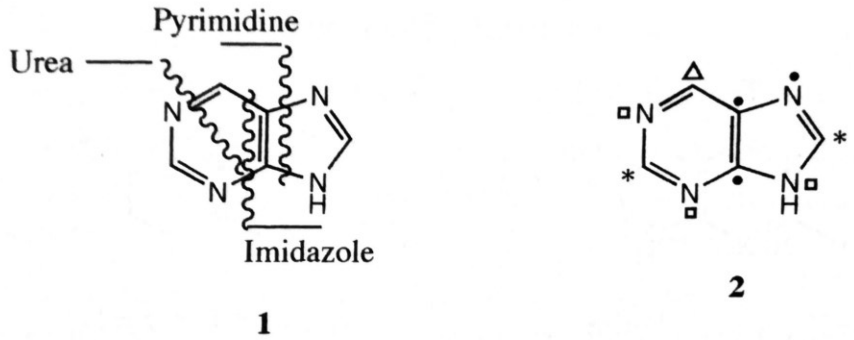
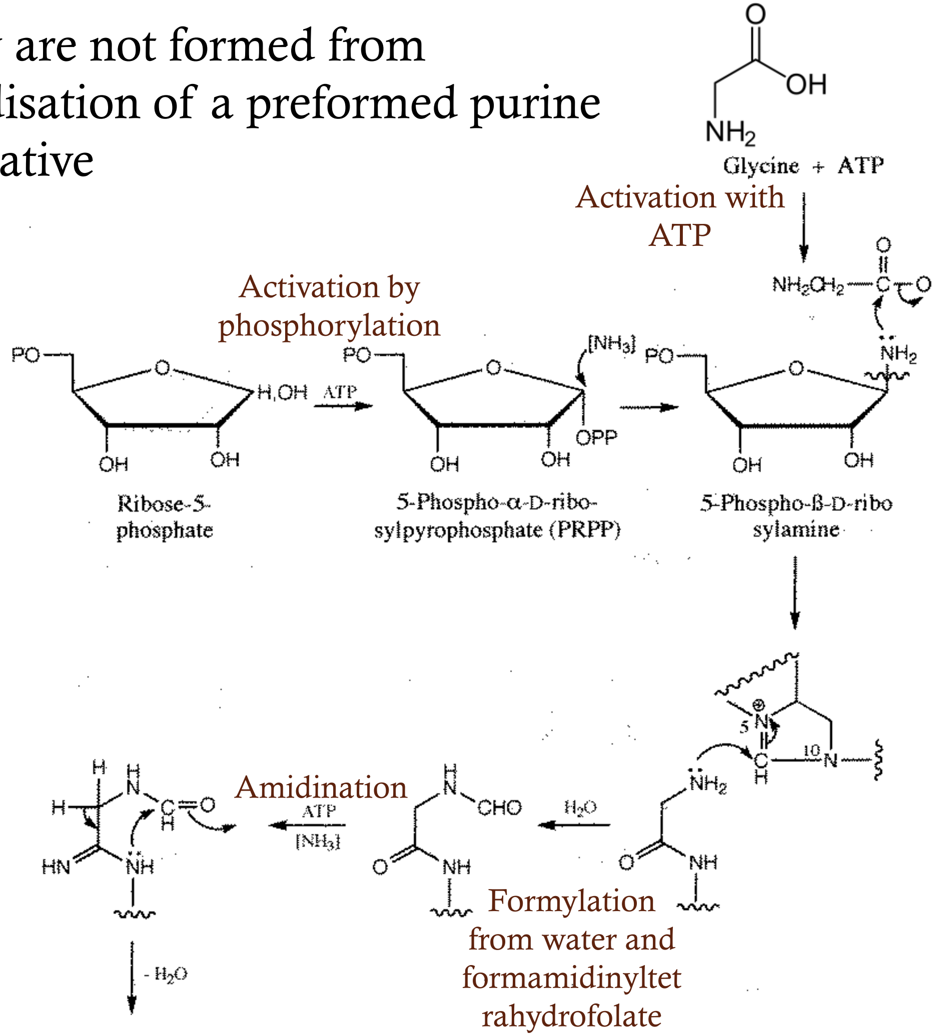


Fig. 3 **1**, Imaginable precursors of the purine ring; **2**, precursors of the purine ring
• Glycine, * Formate, □ $[NH_3]$, △ CO_2

Biosynthesis of purine nucleotides

They are not formed from ribodisation of a preformed purine derivative

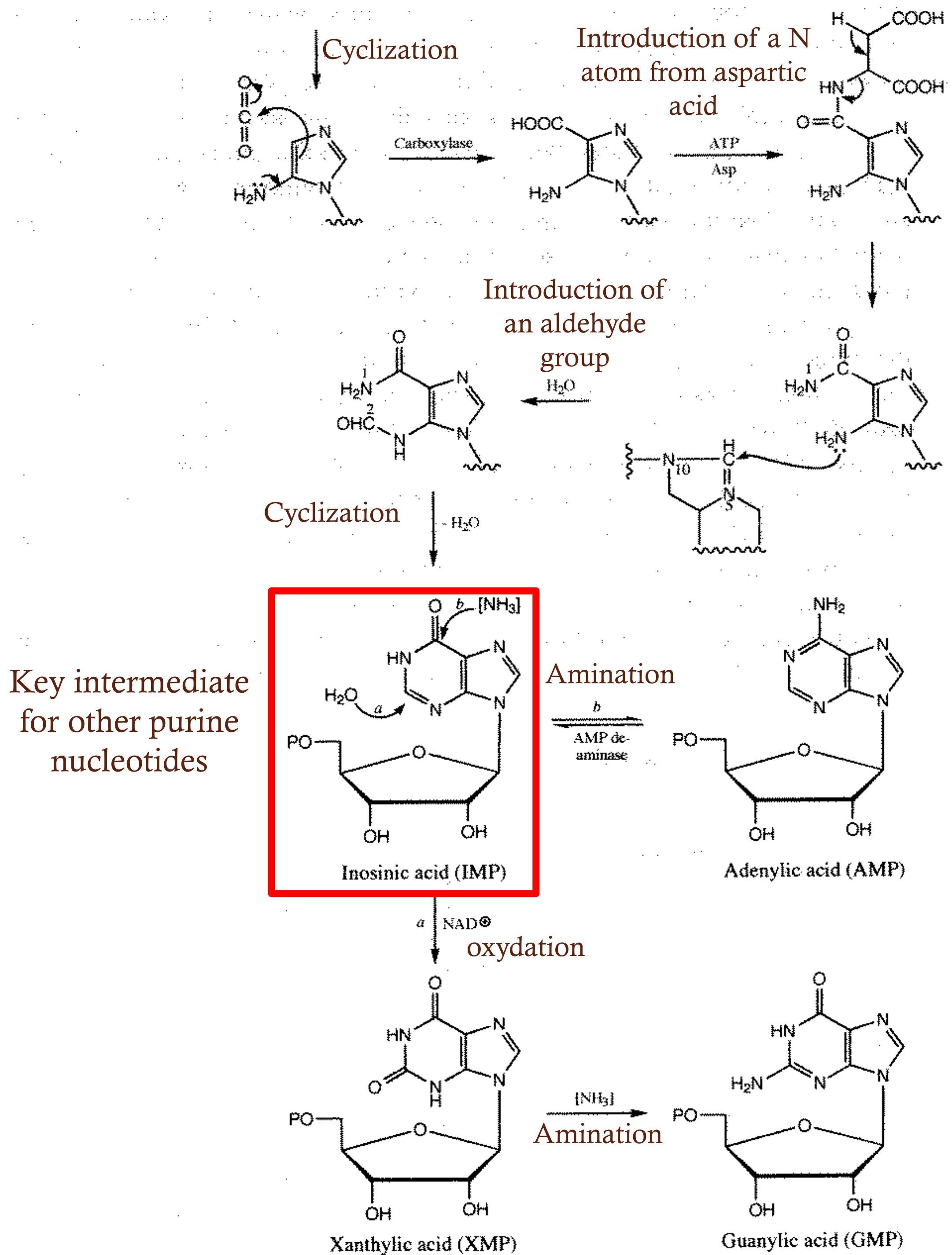


Activation with ATP

Activation by phosphorylation

Amidination

Formylation from water and formamidinyltetrahydrofolate



Cyclization

Introduction of a N atom from aspartic acid

Introduction of an aldehyde group

Cyclization

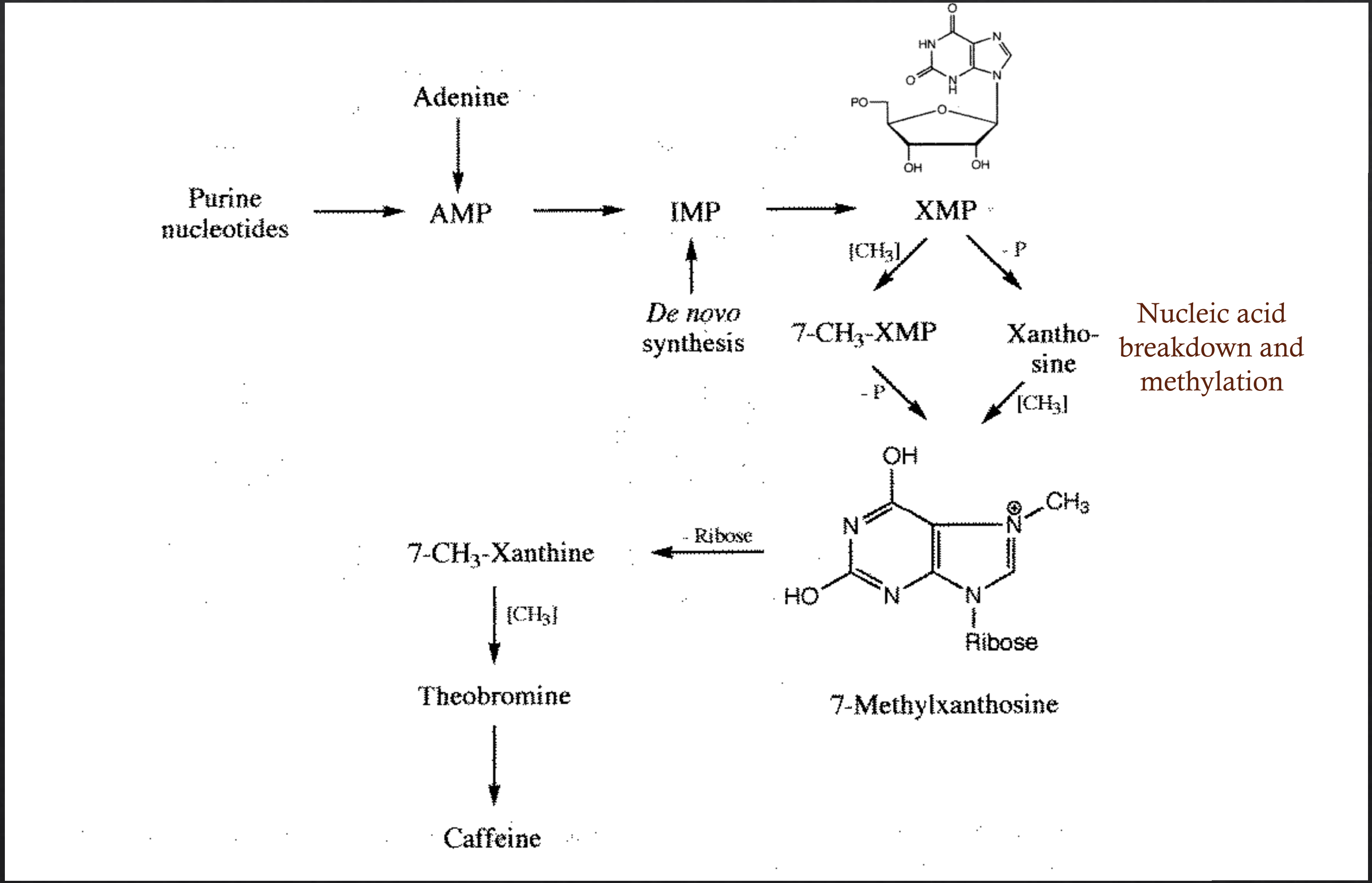
Key intermediate for other purine nucleotides

Amination

oxydation

Amination

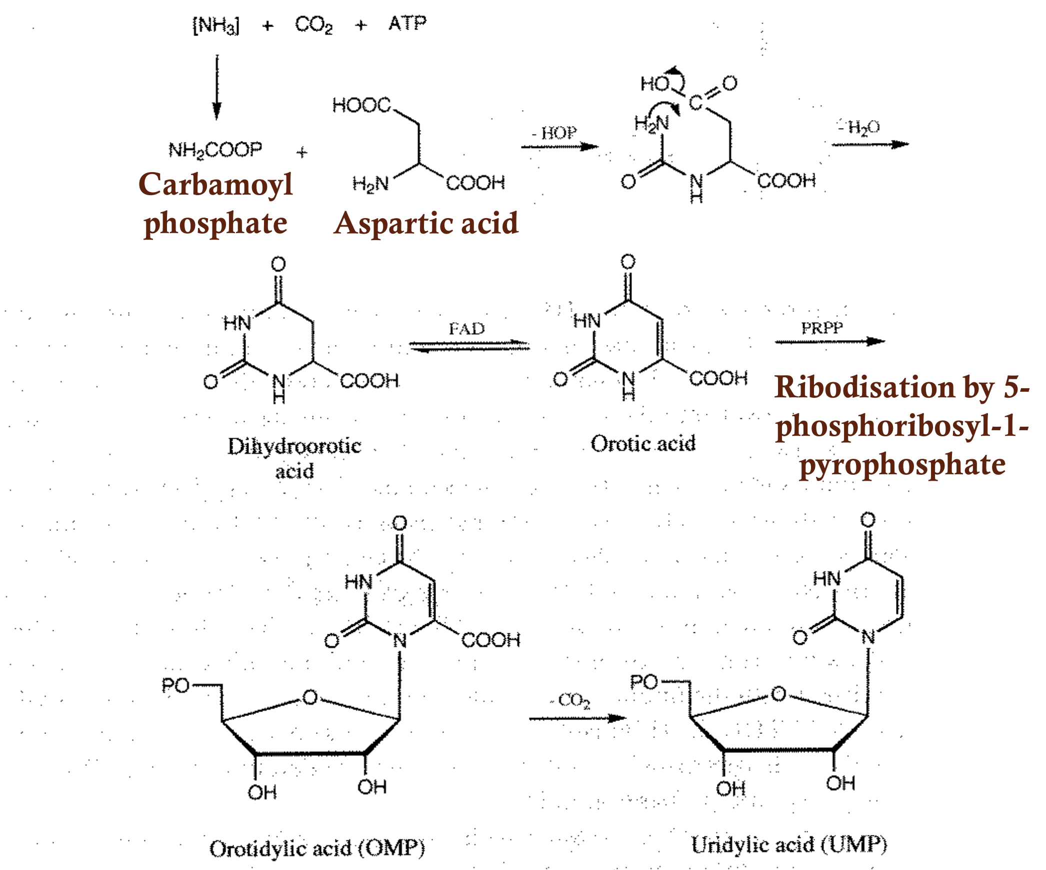
Biosynthesis of purine alkaloids



Nucleic acid breakdown and methylation

Biosynthesis of pyrimidine nucleotides

- Pyrimidine nucleotides are formed from ribodisation of a preformed pyrimidine derivative, and has a simpler biosynthesis.



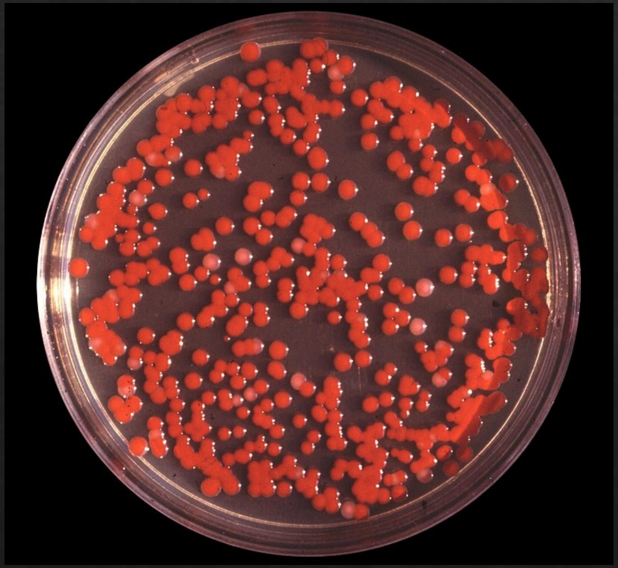
Carbamoyl phosphate

Aspartic acid

Ribodisation by 5-phosphoribosyl-1-pyrophosphate

Pyrroles and porphyrins

- Pyrrole derivatives are rare in nature. An example is prodigiosin, produced by the bacteria *Serratia marcescens.*



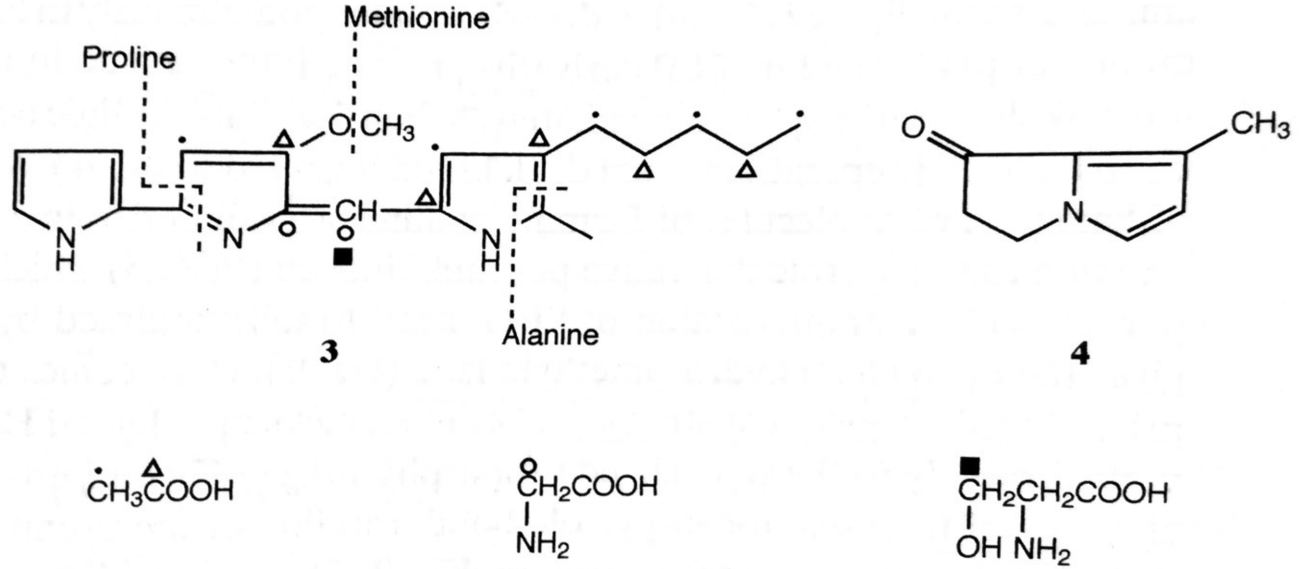
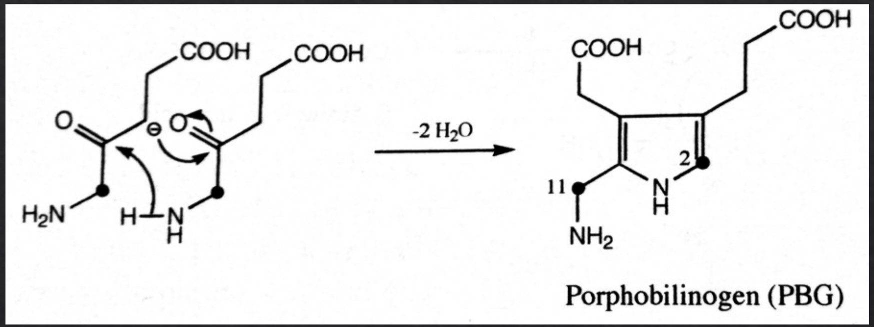


Fig. 8 Pyrrole derivates. **3**, Labelling pattern in prodigiosin observed from feeding experiments; **4**, a pheromone in *Danaus* spp. derived from a pyrrolizidine alkaloid

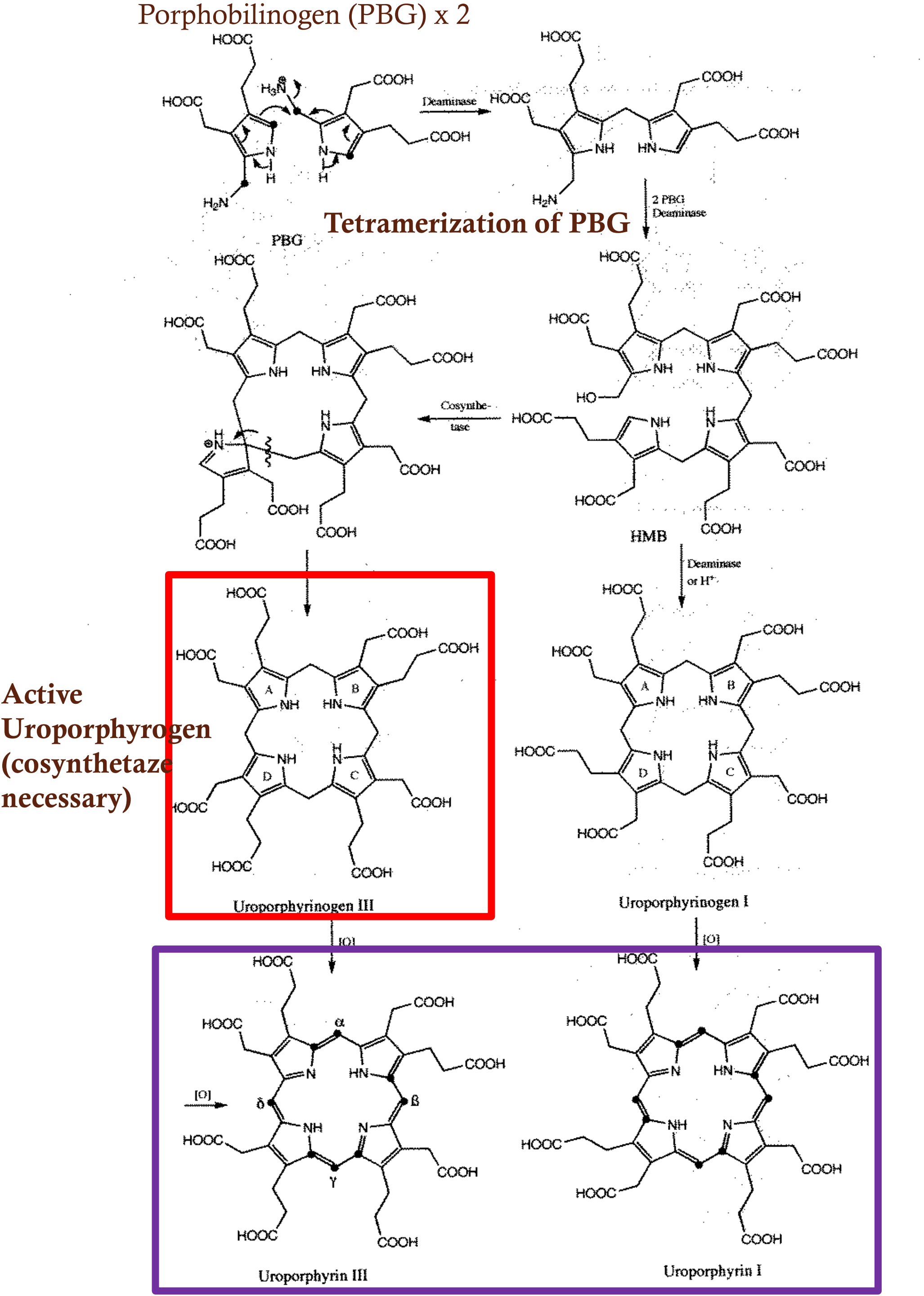
Biosynthesis of uroporphyrogen

- Porphobilinogen are synthesized from the dimerization of 5-aminolevulinic acid, which comes from succinyl CoA and glycine.



Porphobilinogen (PBG)

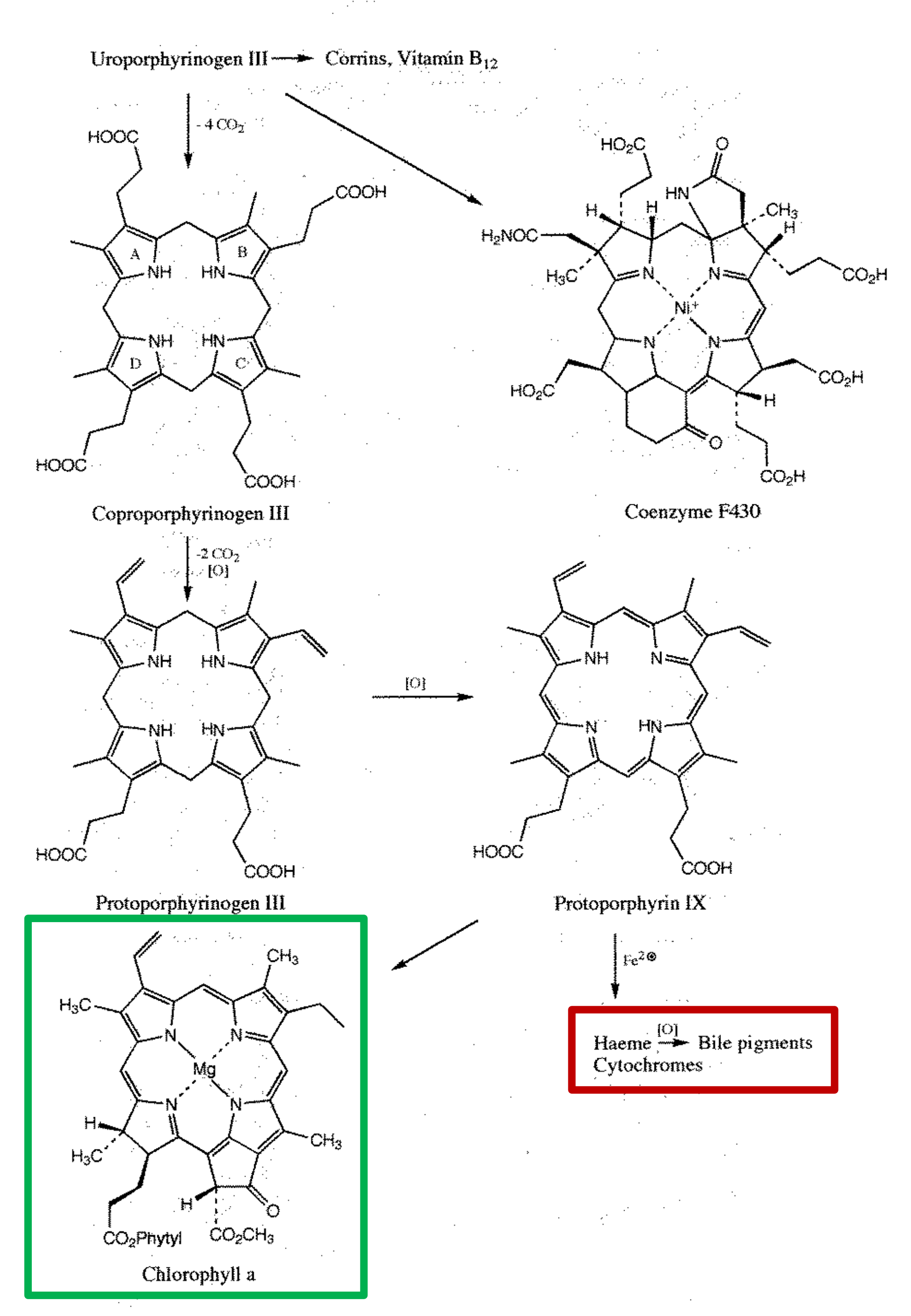
- Cosynthetaze is needed to complete the biosynthesis of uroporphyrogen. Otherwise, it leads to its inactive version.
- Uroporphyrin I and III are strongly colored compound (conjugated 18-pi-systems).

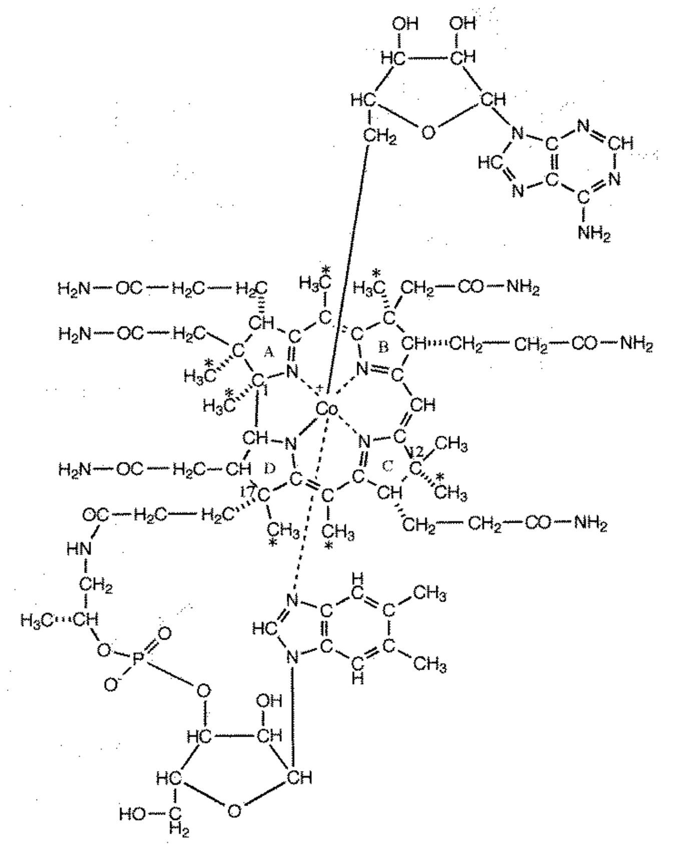


Porphobilinogen (PBG) x 2

Tetramerization of PBG

Active Uroporphyrogen (cosynthetaze necessary)





Cobalamin (vitamin B12)
The structure was elucidated in 1946 using X-ray crystallography.