Skin xenobiotic metabolism and review of structural consideration

Prof. Jean-Pierre Lepoittevin
University of Strasbourg

IDEA pre- and pro-haptens Workshop
Brussels, October 16-17, 2019

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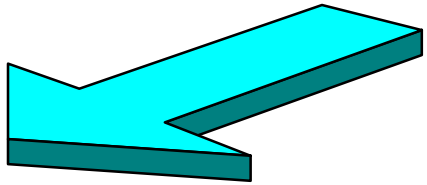
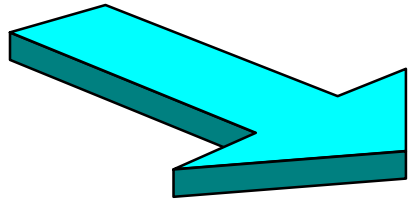
Prohaptens ?

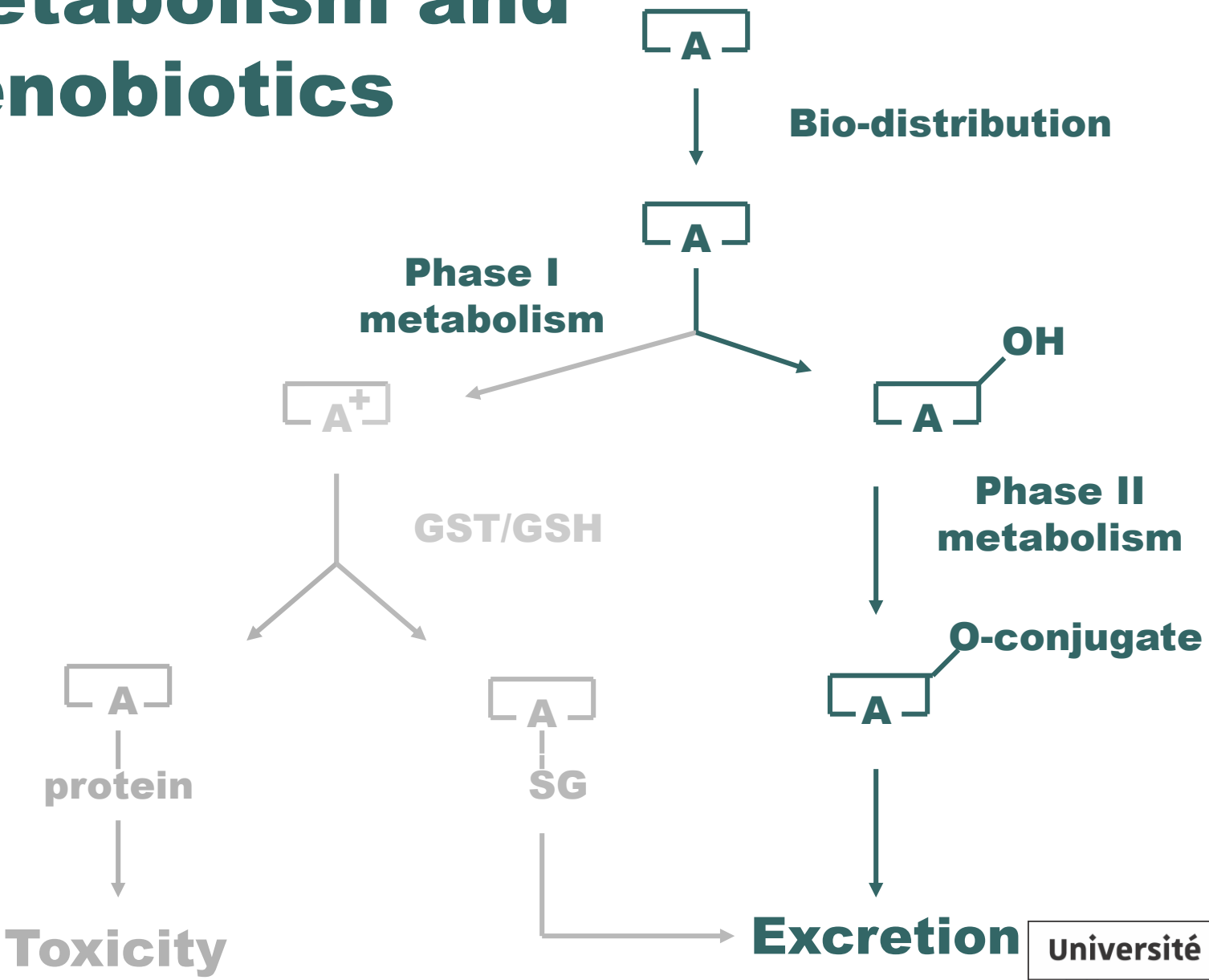


Activated by skin metabolism…

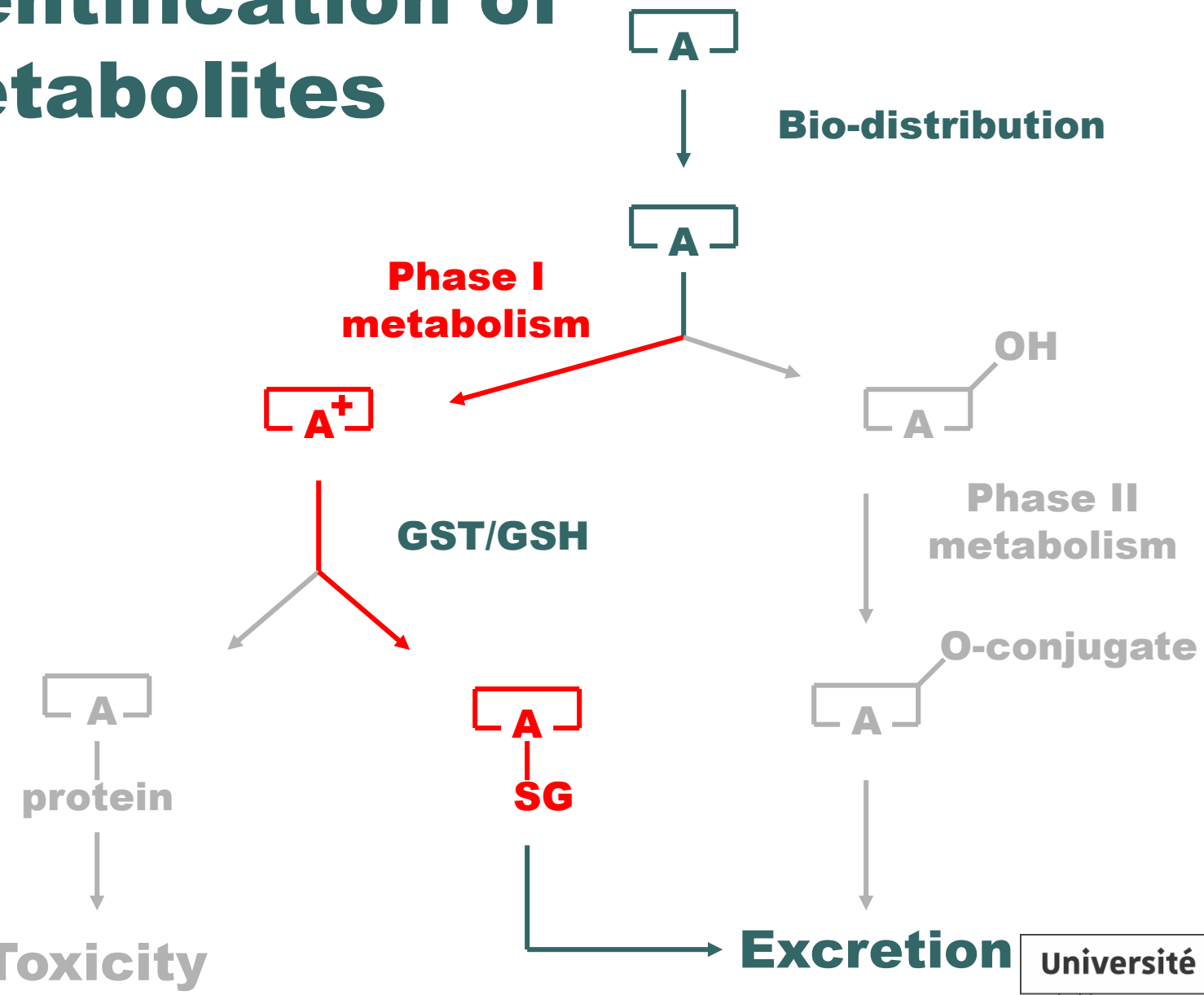


Reactive metabolites…

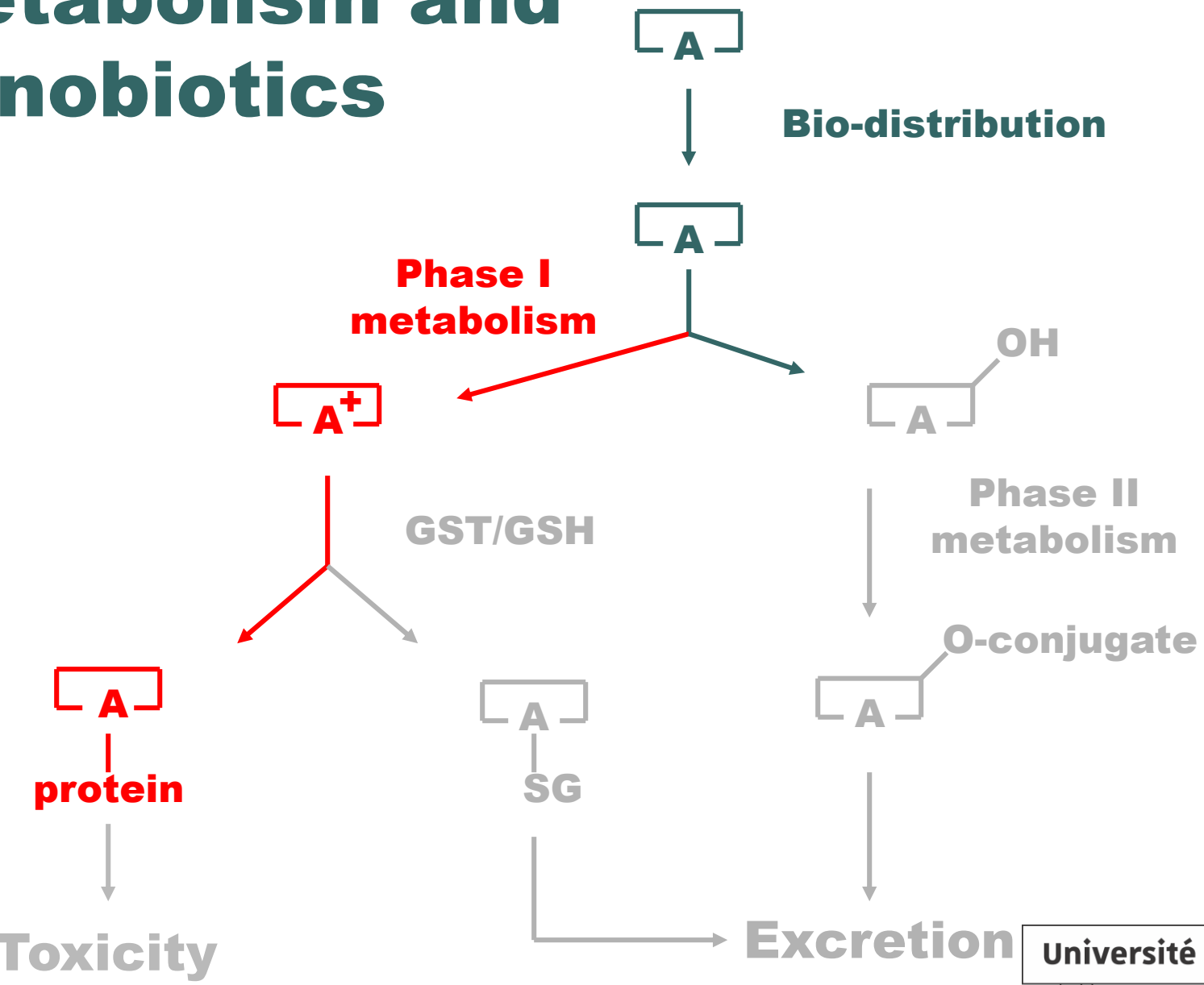
Metabolism and xenobiotics



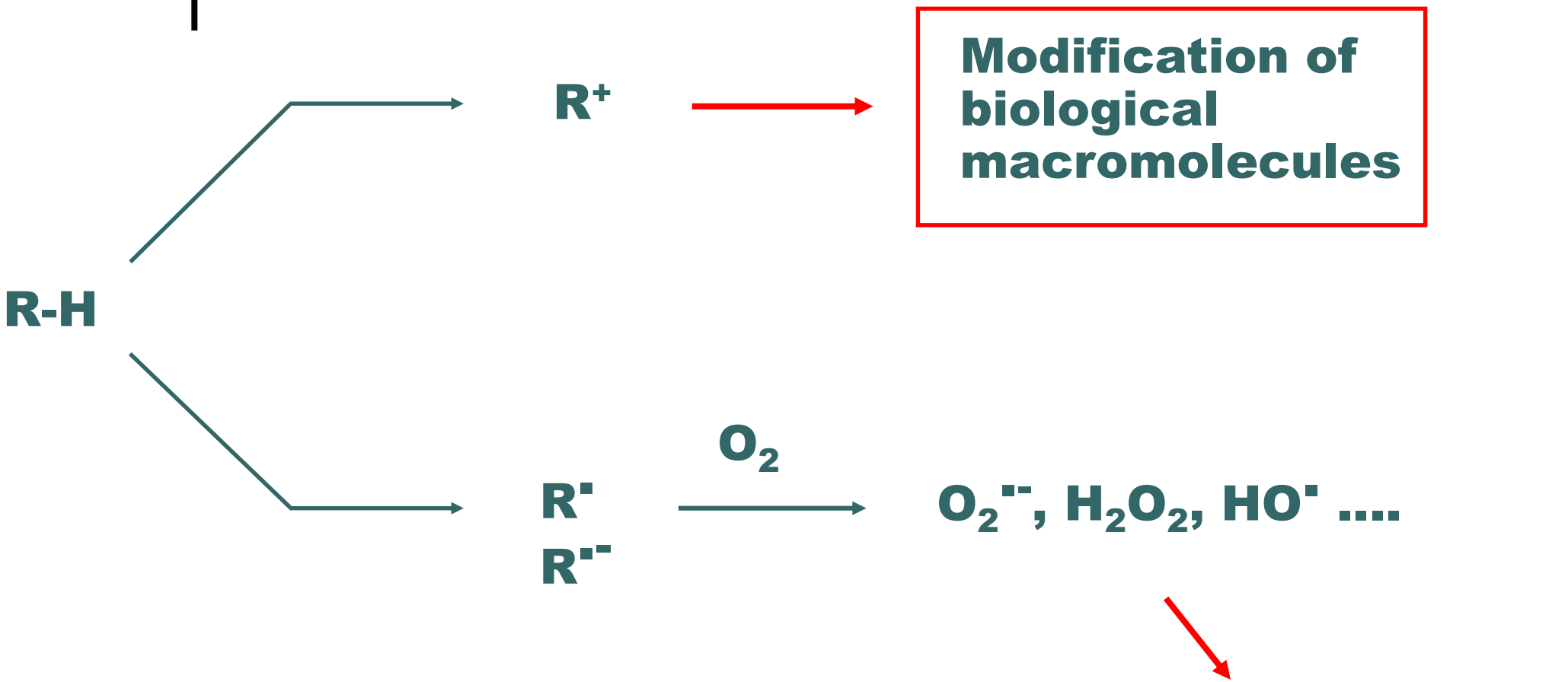
Identification of metabolites



Metabolism and xenobiotics



Metabolism of xenobiotics



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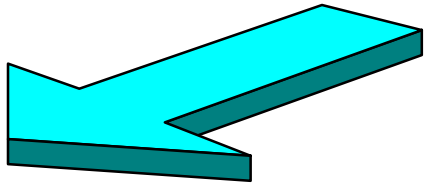
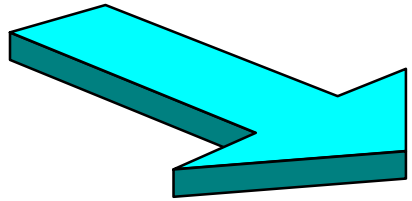
Metabolic studies



Liver metabolism…



Skin metabolism…

Metabolism of xenobiotics

Main enzymatic systems identified in Human epidermis…

EC 1	Oxydoreductases	Cytochromes P450 (**CYPs**)	1A1/1B1, 2B6/2E1, 3A5/3A7…
		Alcohol / Aldehyde deshydrogenases (**ADH** / **ALDH**)	EC 1.1.1.1 / EC 1.2.1.3
		Peroxidases	EC 1.11.x
EC 2	Transferases	Catechol-*O*-methyl transferases (**COMT**)	EC 2.1.1.6
		N-acetyltransferases (**NAT**)	EC 2.3.1
		Glucuronosyltransferases (**UGT**)	EC 2.4.1.17
		Glutathion S-transferases (**GST**)	EC 2.5.1.18
		Sulfotransferases (**SULT**)	EC 2.8.2.x
EC 3	Hydrolases	Esterases (**ES**)	EC 3.1.x

Eilstein et al. Arch. Toxicol. 2014, 88, 1681–1694

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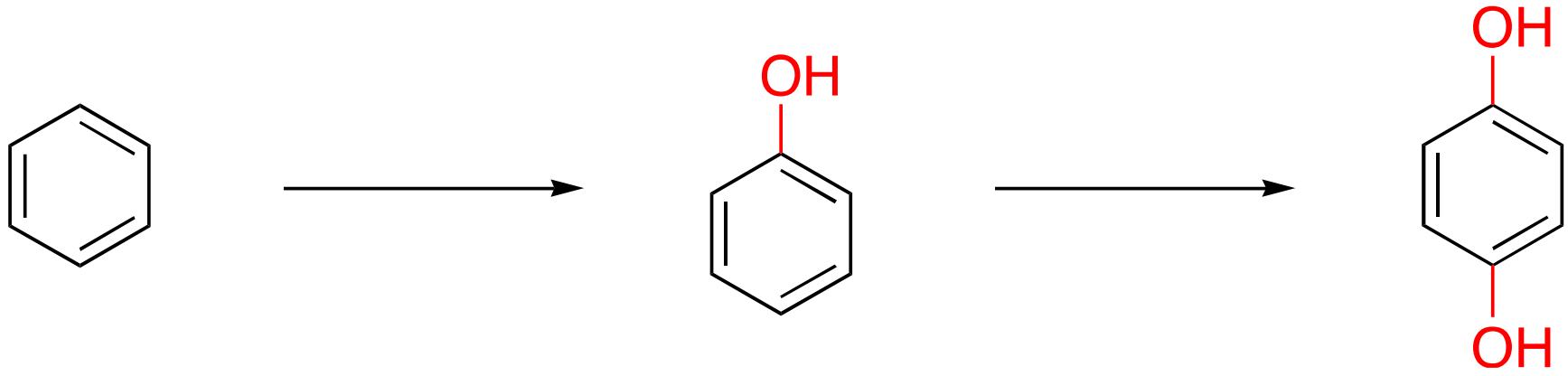
Mono-oxygenases in the skin

- CYP 1-4 are involved in the metabolism…
- All « liver » CYP have not yet be found in the skin…
- CYP 1A1 responsible for the metabolism of aromatic rings (immuno-histochemistry)
- CYP 2B1 and CYP 2B6 responsible of dealkylation (ARN-messenger)
- CYP 2B12 responsible of lipid epoxidation

Some transformations operated by CYP…

- **Hydroxylation of aromatic systems**



- **Hydroxylation of alkanes**

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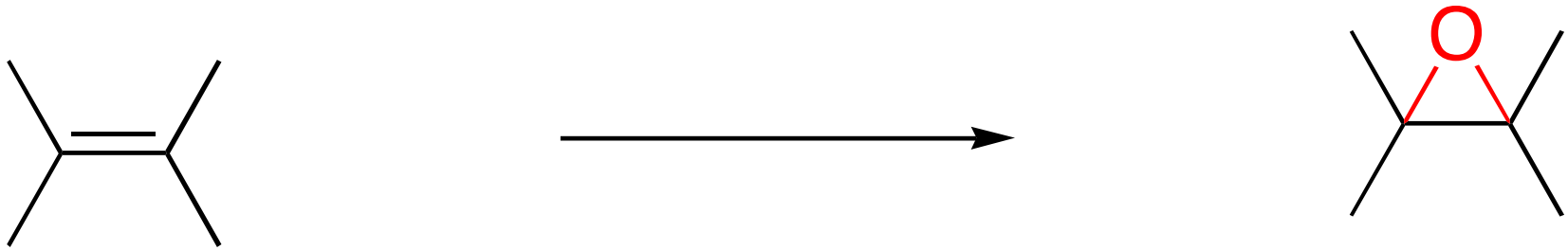
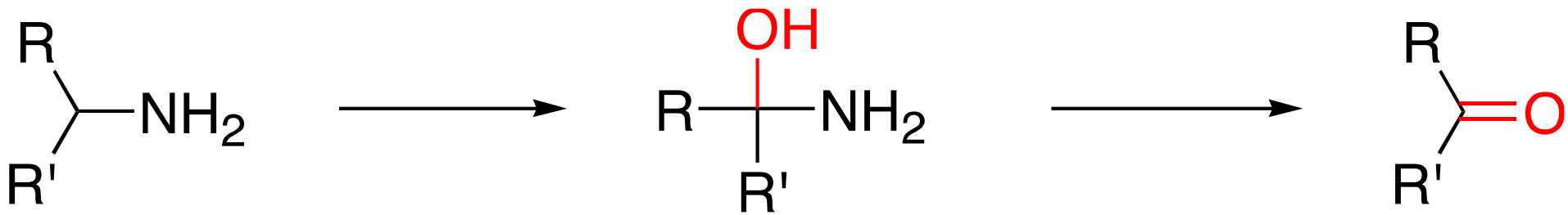
Some transformations operated by CYP…

- **Epoxidation**

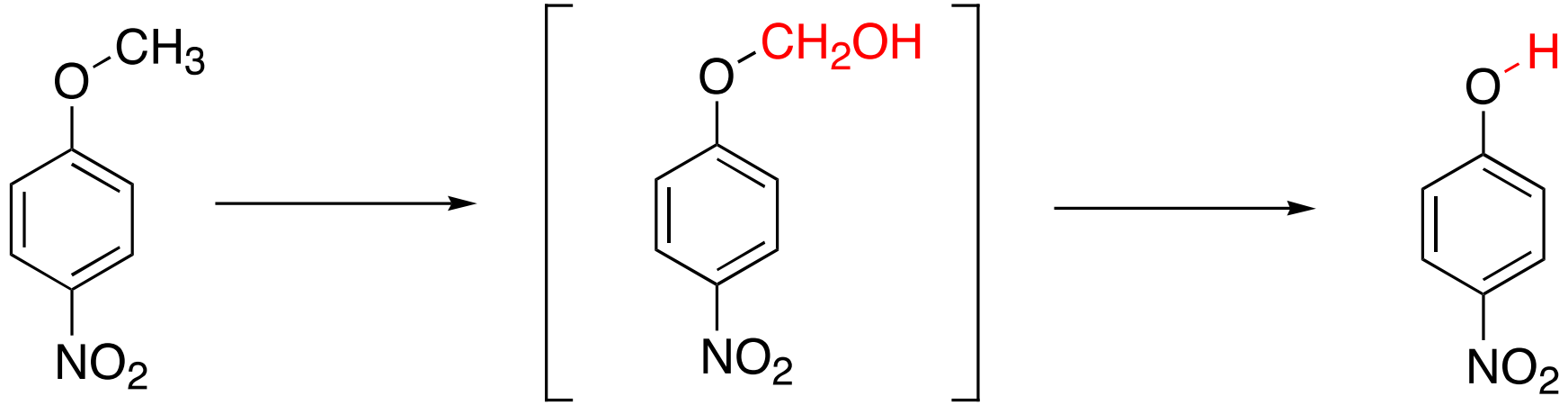


- **Deamination**



Some transformations operated by CYP…

- ***O*-Dealkylation**

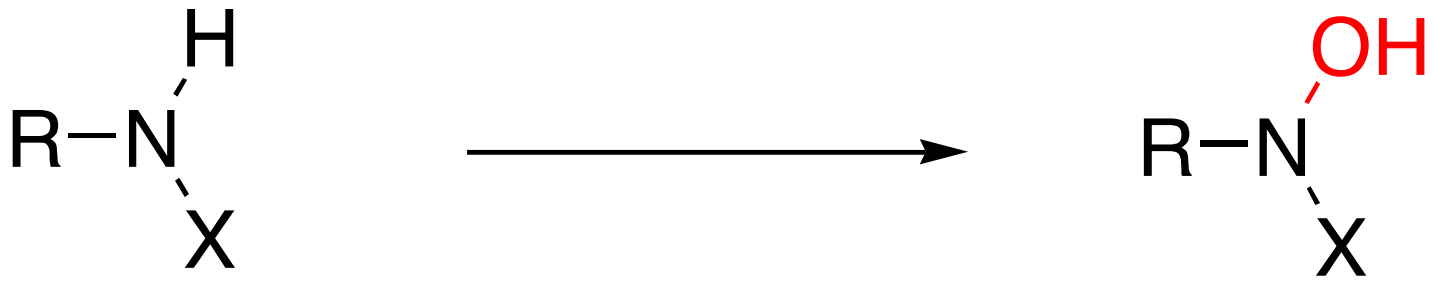


Flavin monooxygenases (FMO)

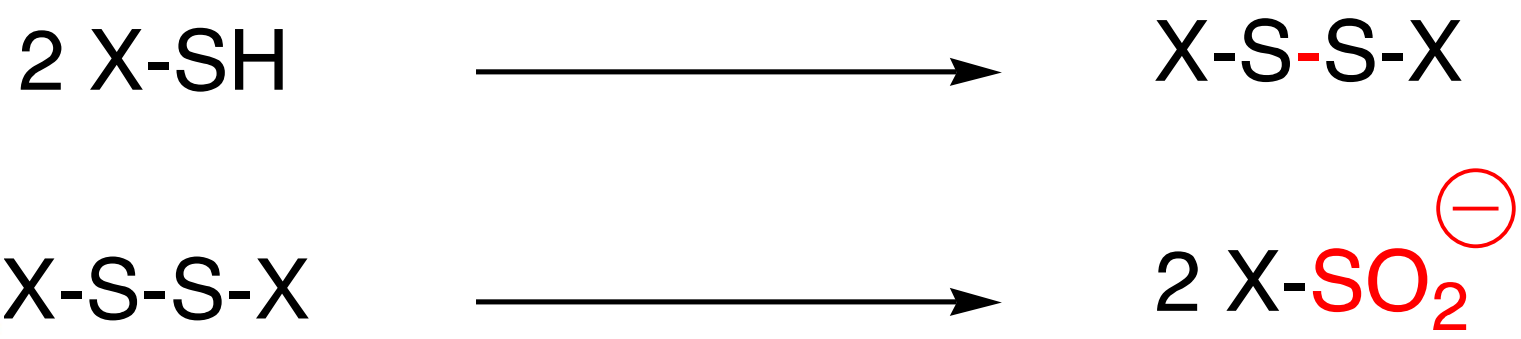
- Involves into the oxidation of nitrogen, sulfur and phosphorus atoms…
- A competition can exist between CYPs and FMOs…
- The CYP/FMO ratio varies from one organ to another one
  - Liver: 85% CYP / 15% FMO
  - Skin: 33% CYP / 66% FMO

Flavin monooxygenases (FMO)

- **Oxidation of amines**



- **Oxidation of thiols**



Alcohol and aldehyde dehydrogenases (ADH et AlDH)

- Oxido-reduction systems present in many organs…
- Oxidation of alcohols into aldehydes or ketones…
- Source of oxidant: NAD^+ co-factor…
- 5 classes of ADH have been identified and 3 expressed in the skin at the protein level

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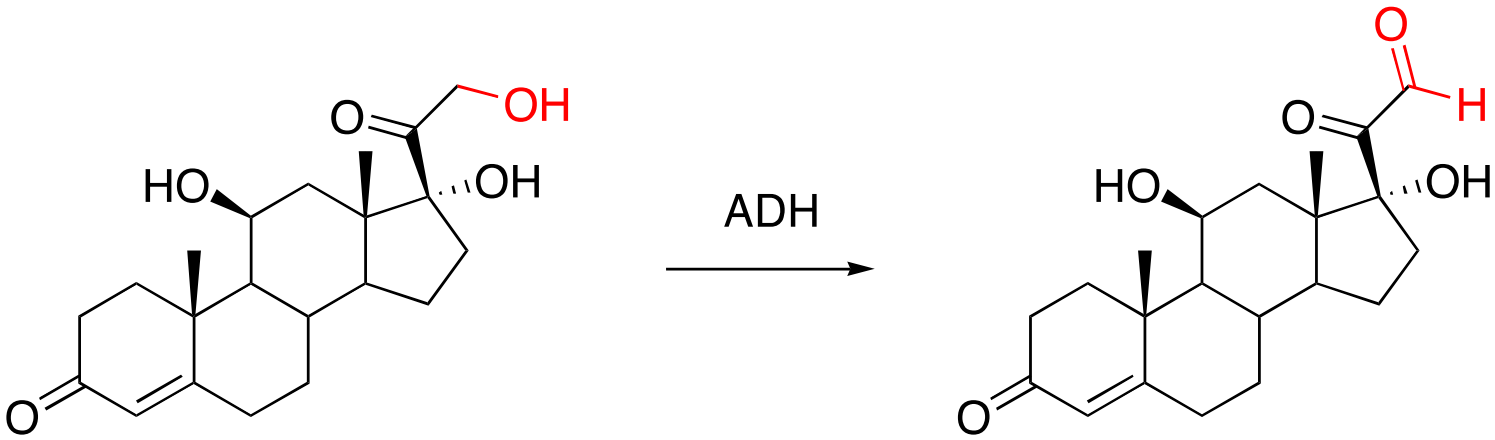
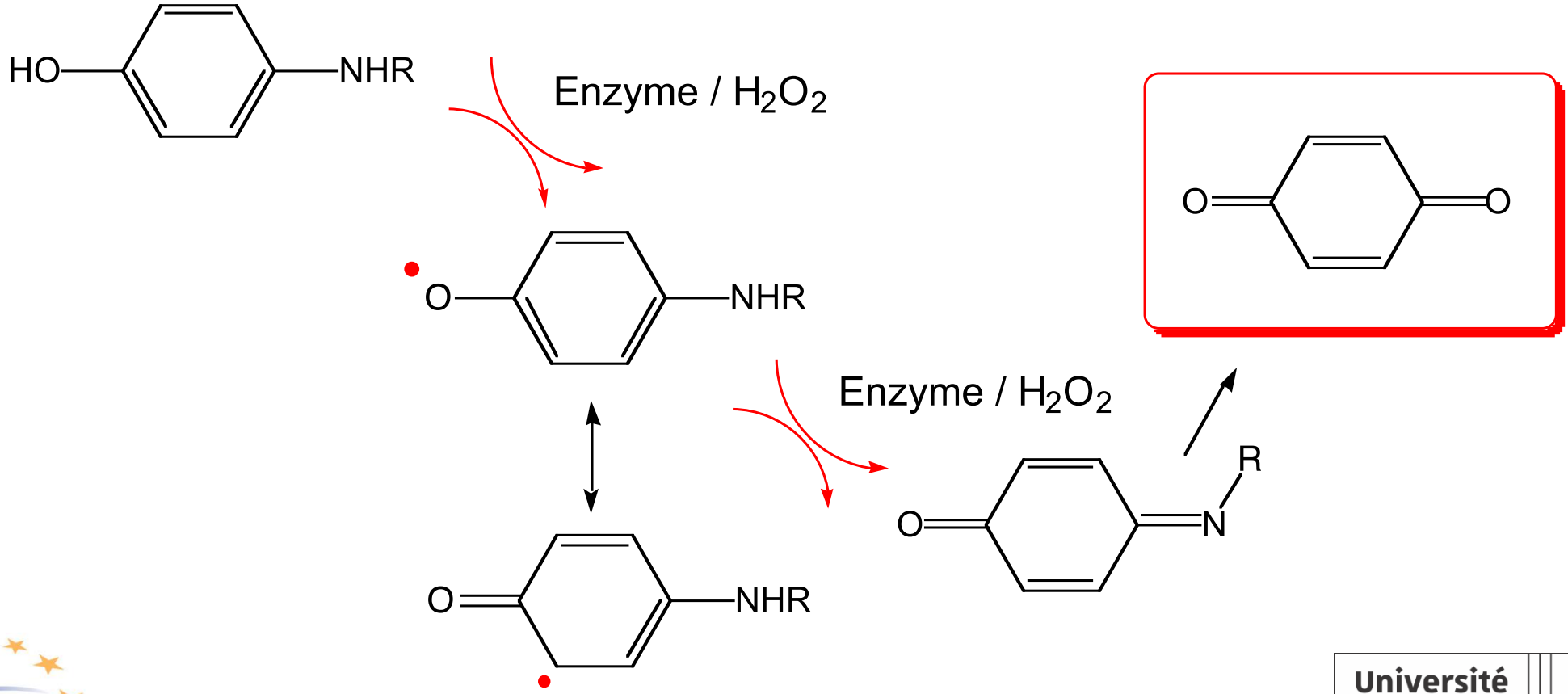
Example of pro-haptens that can be activated by ADH…



Hydrocortisone

21-DHC

Peroxidase/H_2O_2 and phenols/aromatic amines…



Contact Dermatitis, 1998, 39, 293–303
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CONTACT DERMATITIS
ISSN 0105-1873

Fragrances and other materials in deodorants: search for potentially sensitizing molecules using combined GC-MS and structure activity relationship (SAR) analysis

S. C. RASTOGI[1], J.-P. LEPOITTEVIN[2], J. D. JOHANSEN[3], P. J. FROSCH[4], T. MENNÉ[3], M. BRUZE[5], B. DREIER[4], K. E. ANDERSEN[6] AND I. R. WHITE[7]

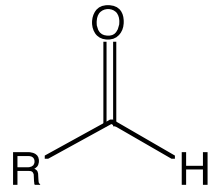
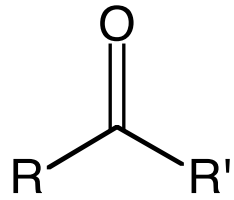
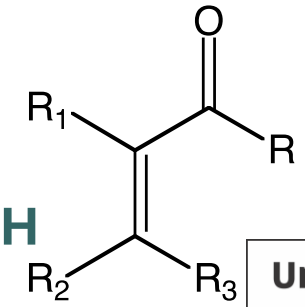
[1]Department of Environmental Chemistry, National Environmental Research Institute, Roskilde, Denmark; [2]Laboratoire de Dermatochimie associé au CNRS, Université Louis Pasteur, Strasbourg, France; [3]Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark; [4]Department of Dermatology, Der Städtische Kliniken, Dortmund, and University of Witten/ Herdecke, Germany; [5]Department of Occupational Dermatology, University Hospital, Malmö, Sweden; [6]Department of Dermatology, Odense University Hospital, Denmark; [7]St. John's Institute of Dermatology, London, UK

Fragrances and other materials in deodorants

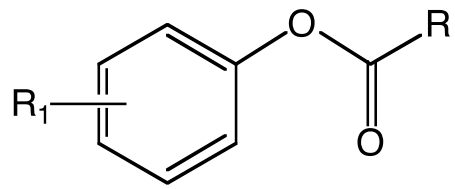
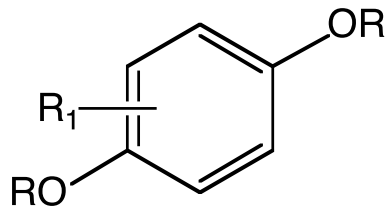
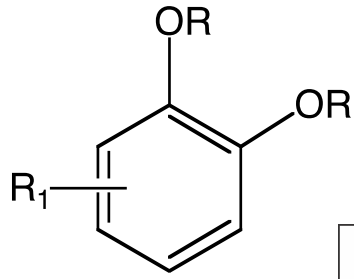
- 71 deodorants (spray and roll-on) were analyzed by GC-MS...
- 226 molecules were identified...
- 84 molecules were found to contain at least one strutural alert...
- These molecules can be grouped into 9 main chemical families...

Contact Dermatitis, 1998, *39*, 293-303

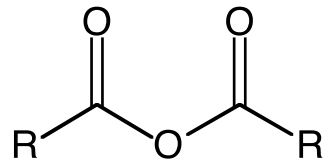
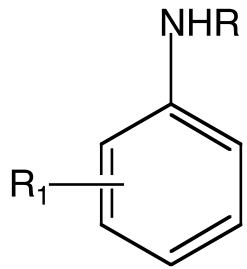
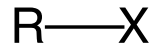
Identified strutures

Type	Fonction	Substituents	Structure
1	Aldehydes	R = alkyl, aryl	
2	Ketones	R, R' = alkyl, aryl	
3	α,β-unsaturated esters, ketones aldehydes, amides	R = H, C, N, O (not OH) R1 = not heteroatomes R2 = not aryl except R = H	

Identified structures

Type	Fonction	Substituents	Structure
4	phenyl esters	R = alkyl, aryl R1 = all	
5	hydroquinones	R = H, alkyle R1 = all	
6	catechols	R = H, alkyl R1 = all	

Identified structures

Type	Fonction	Substituents	Structure
7	anhydrides	R = all	
8	Aromatic amines	R = alkyl, aryl R1 = all	
9	haloalkanes	R = alkyl X = Cl, Br, I	

Specificities of fragrance chemicals…

Among 84 molecules containing at least one structural alert, 70 were included in the « aldehyde, ketone or α,β unsaturated aldehyde and ketone » family … or can be converted into an « aldehyde, ketone or α,β unsaturated aldehyde and ketone » …

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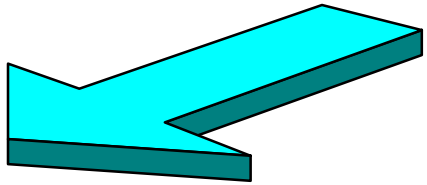
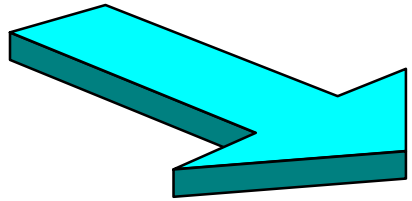
Pitfalls in metabolic studies



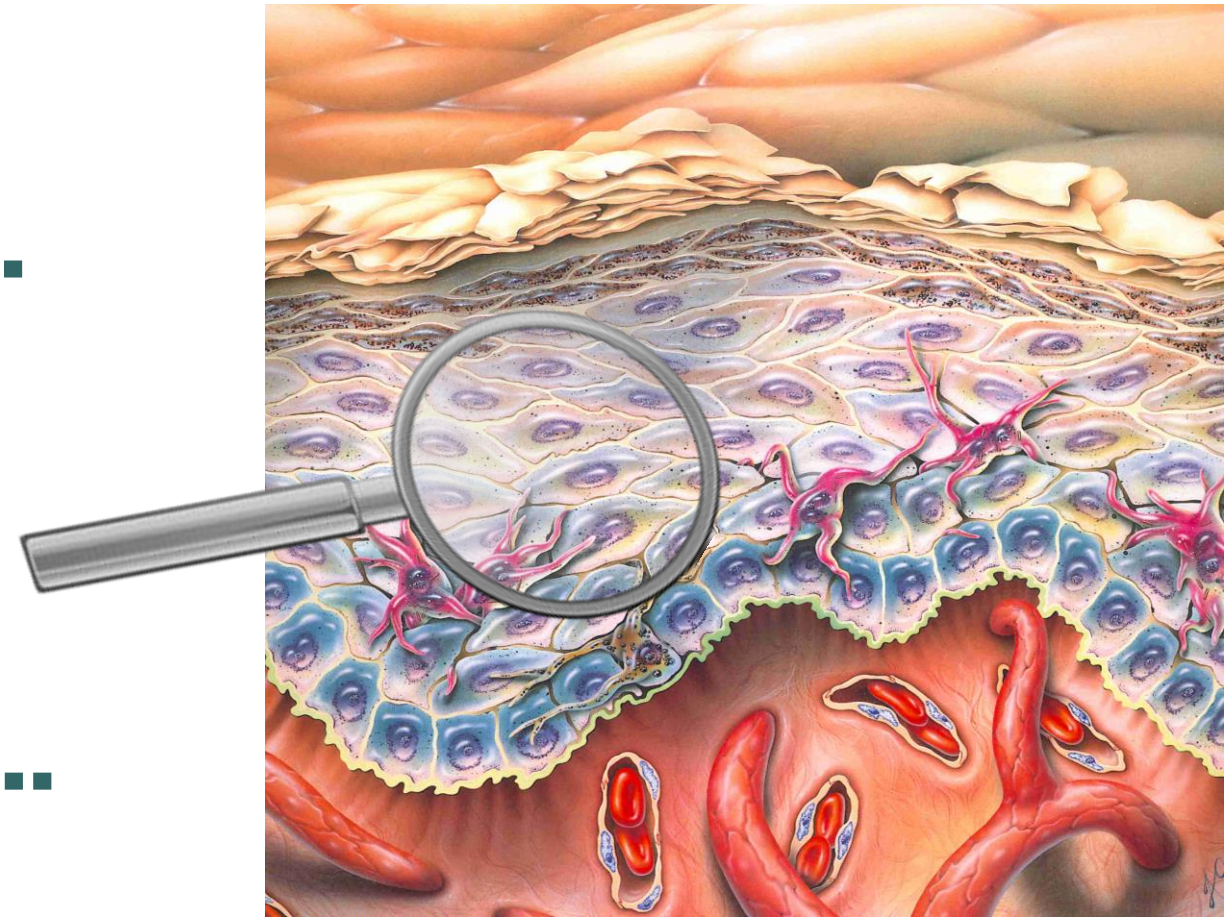
Liver vs skin…



Highly reactive intermediates…

What happen *in situ*?

- Direct observation…
- Non invasive approach…
- Problem of a complex environment…



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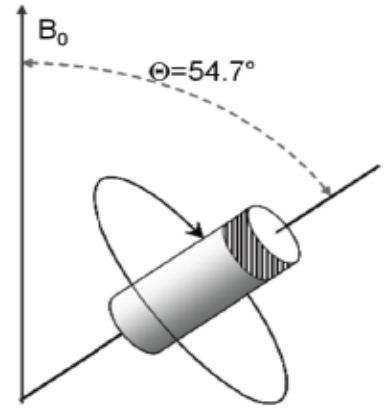
HRMAS NMR

- High-Resolution Magic Angle Spinning "HRMAS" Nuclear Magnetic Resonance…
- Bring to zero inhomogeneity associated with the sample…
- Well adapted to soft solids…



Is it possible to follow and quantify *in situ* the toxication/detoxication balance of chemicals in Reconstructed Human Epidermis ?

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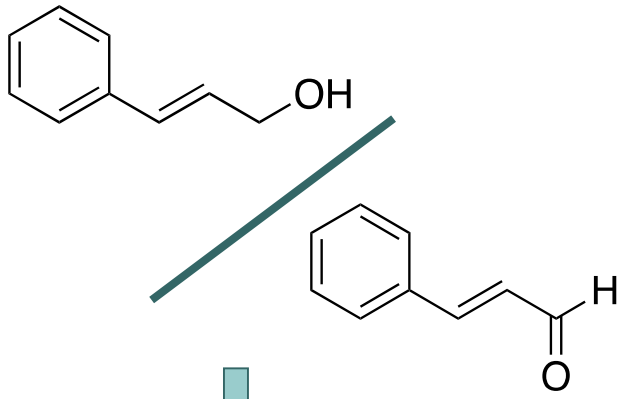
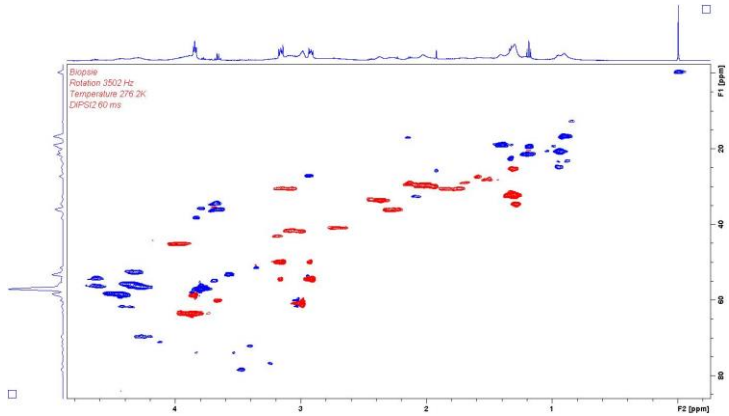
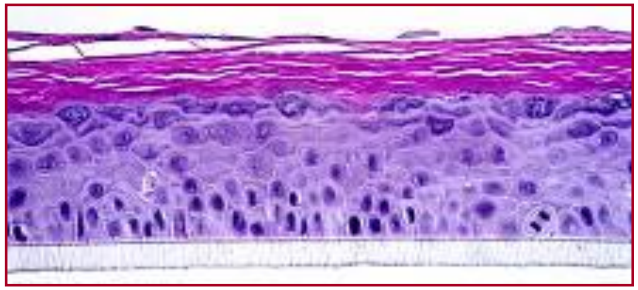
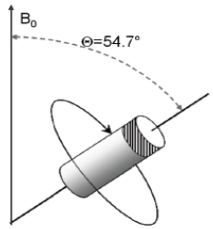
Cinnamyl derivatives









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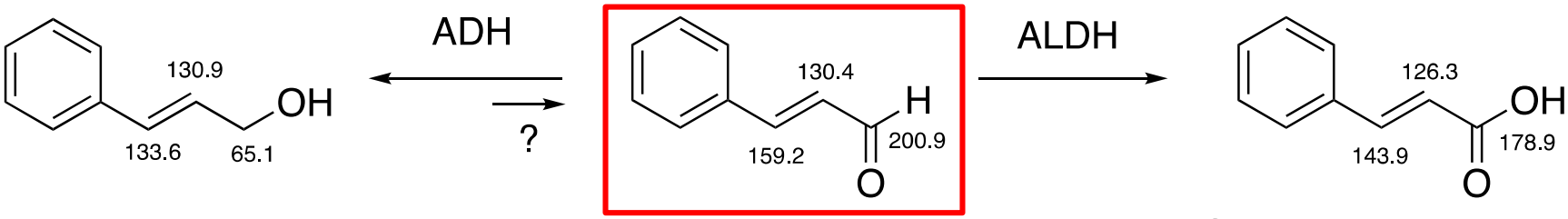
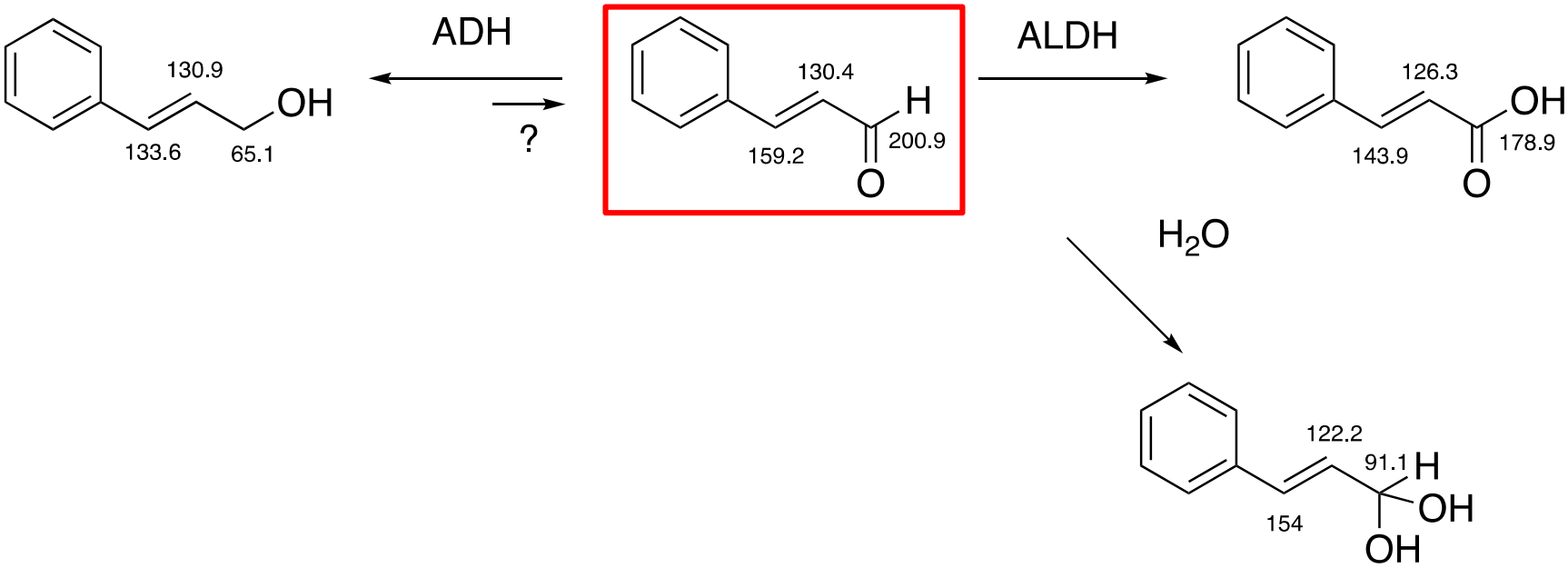
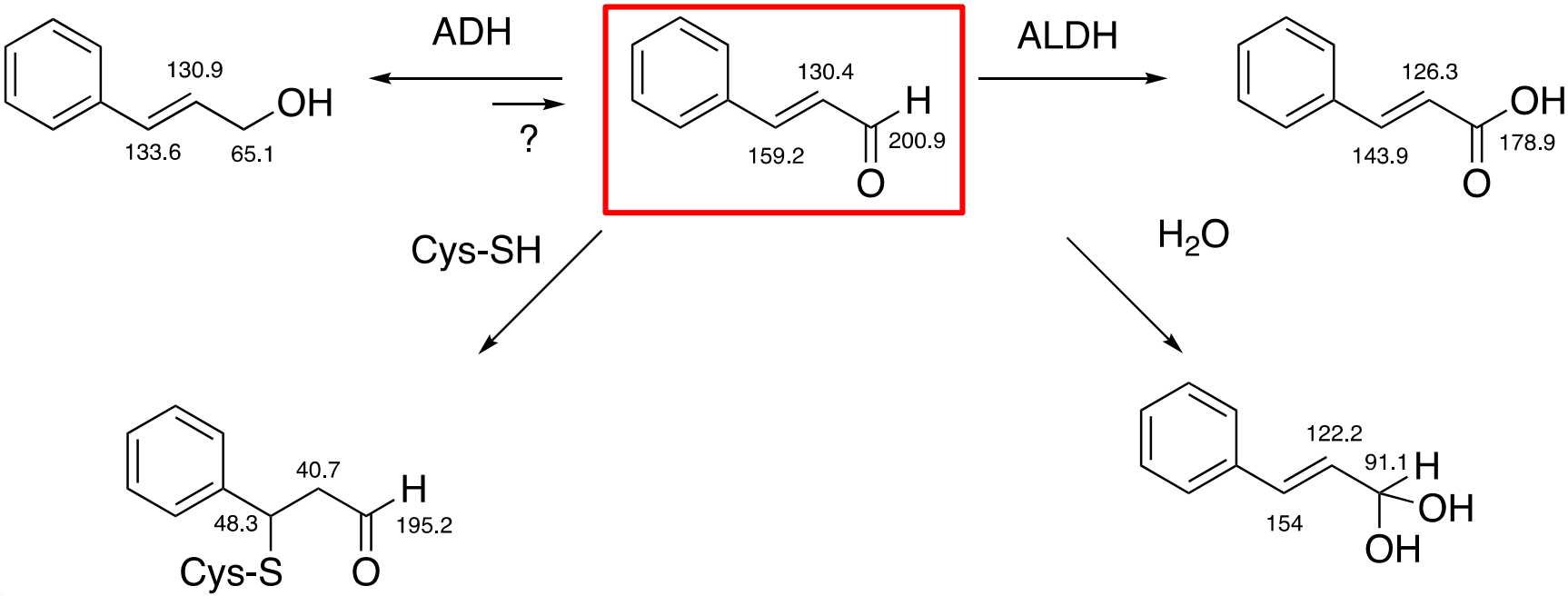
Cinnamyl derivatives



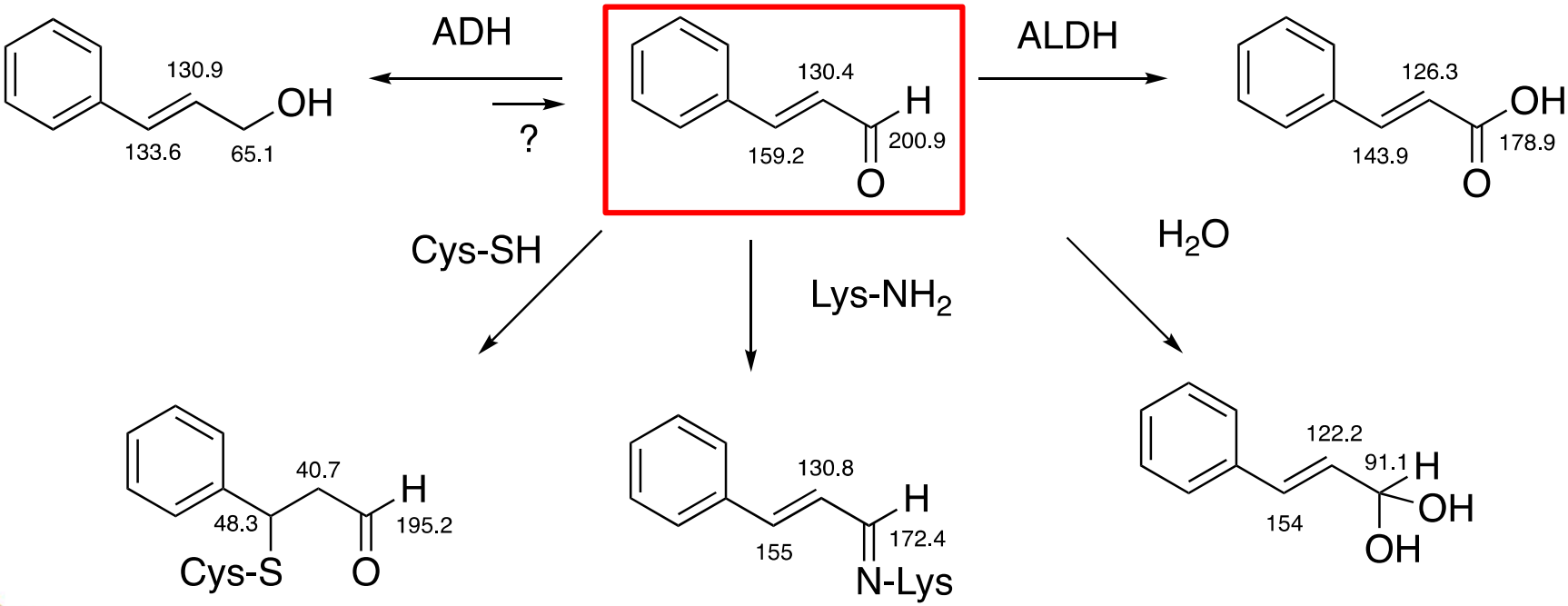
Cinnamyl derivatives



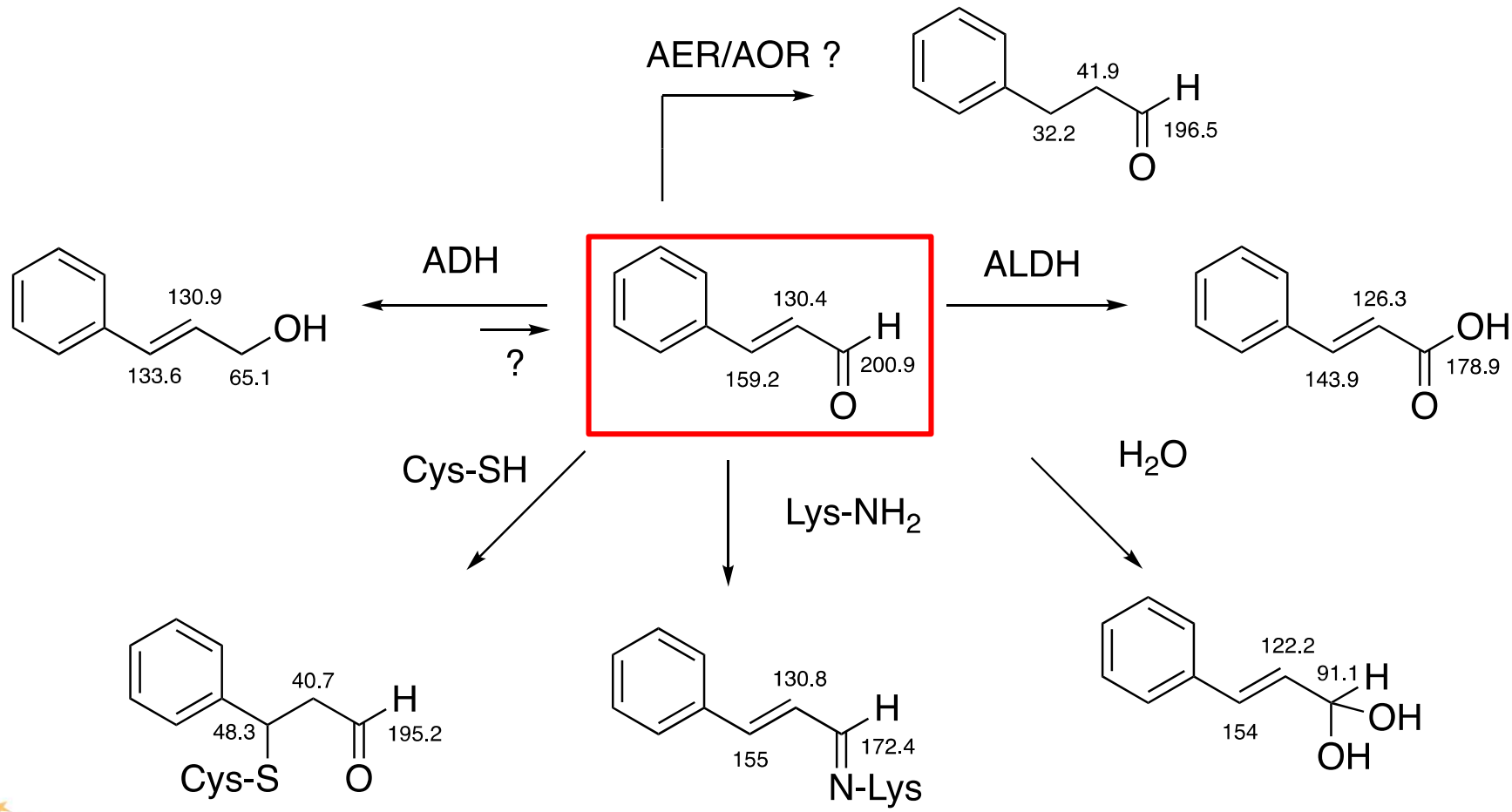
Cinnamyl derivatives



Cinnamyl derivatives

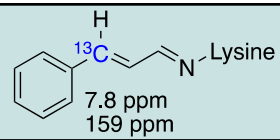
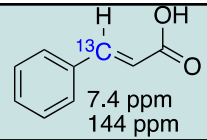
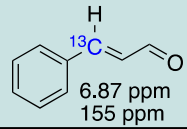
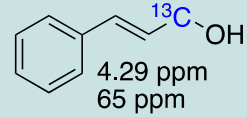
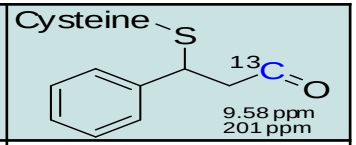


Cinnamyl derivatives



Cinnamaldehyde

Concentrations with time of ^{13}C signals expressed in nmol/mg of RHE [0.8M]

					
30 mn	**6.1**	**1.25**	**144**	**0.4**	**6.8**
1 h	**4.8**	**0.62**	**34.2**	**0.7**	**5.1**
2 h	**3.2**	**0.91**	**4.6**	**0.3**	**2.6**
8 h	**1.3**	**0.47**	_	**0.2**	**0.7**

NB: Cinnamic acid detected in the culture medium (concentration: 0.42 nmol/mg)

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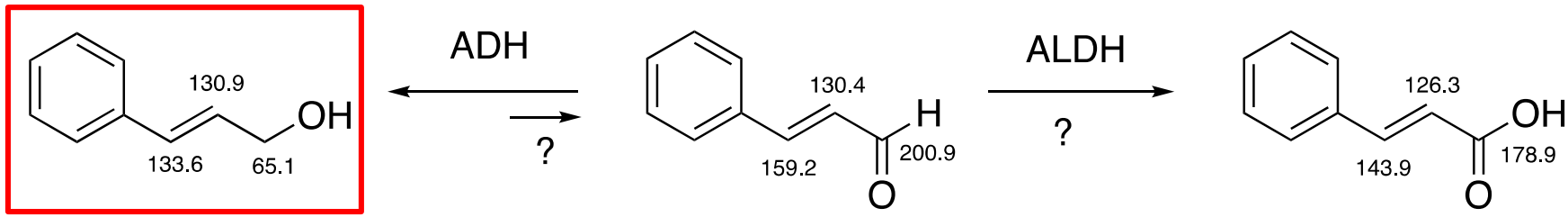
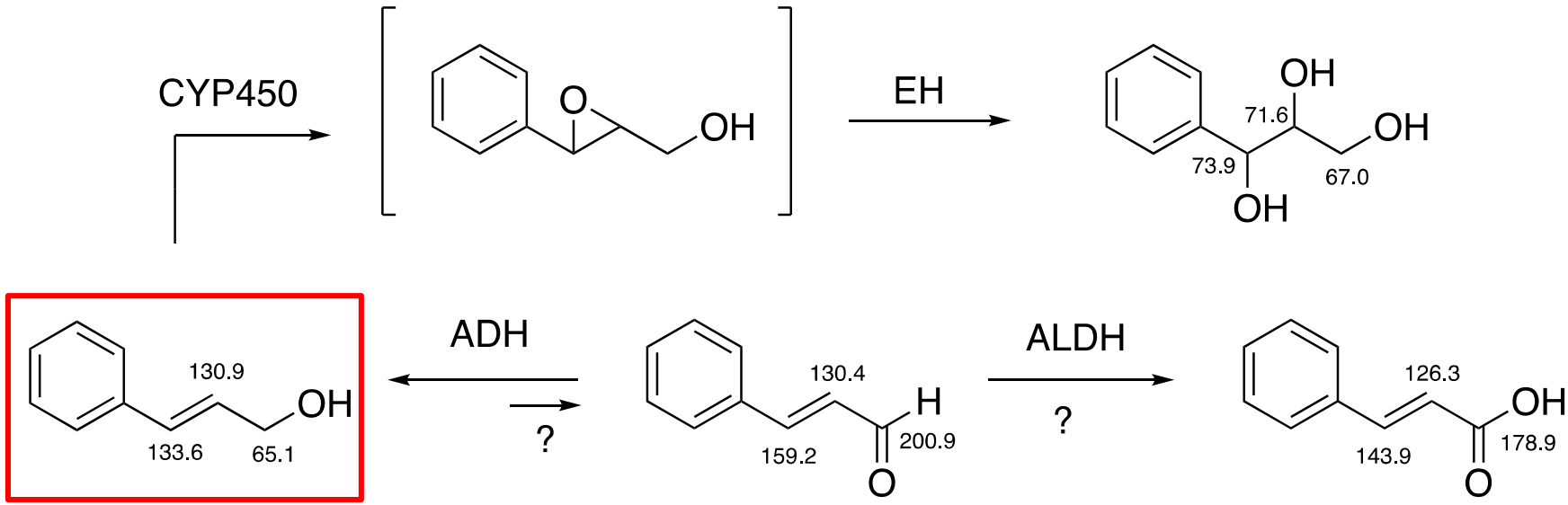
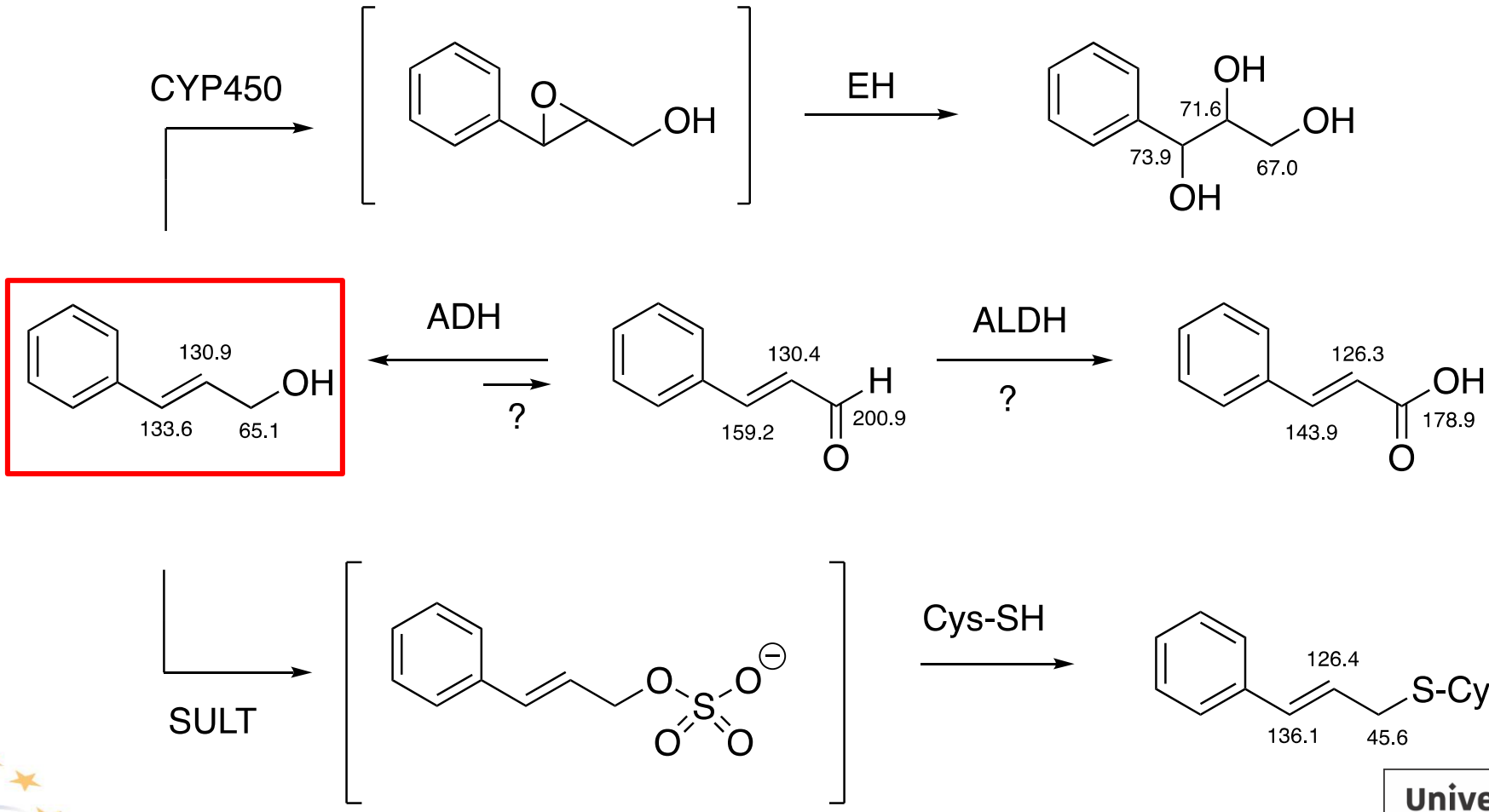
Cinnamyl derivatives



Cinnamyl derivatives



Cinnamyl derivatives



Conclusions/Perspectives…

- The detoxication/toxication balance as well as the reactivity of skin sensitizers can be observed and quantified *in situ*,
- Chemical reactions in RHE were found much faster than in solution (hours vs days),
- Most prohaptens are identified by alternative methods: Re*gul Toxicol Pharmacol*, 2016, 82, 147-155.

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Acknowledgments

- Camille DEBEUCKELAERE
- Dr Eric MOSS
- Dr Marie BETOU
- Dr François-Marie MOUSSALLIEH
- Dr Valérie BERL
- Dr Elena GIMENEZ-ARNAU
- Dr Karim ELBAYED
- Plateforme CARMEN / ICube
- Région Alsace
- RIFM
- Cosmetics Europe
- Centre National de la Recherche Scientifique
- Université de Strasbourg

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