Some views on the prehapten paradigm…

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University of Strasbourg

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

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What do we know?

- **Allylic hydroperoxides are formed at air exposure…**

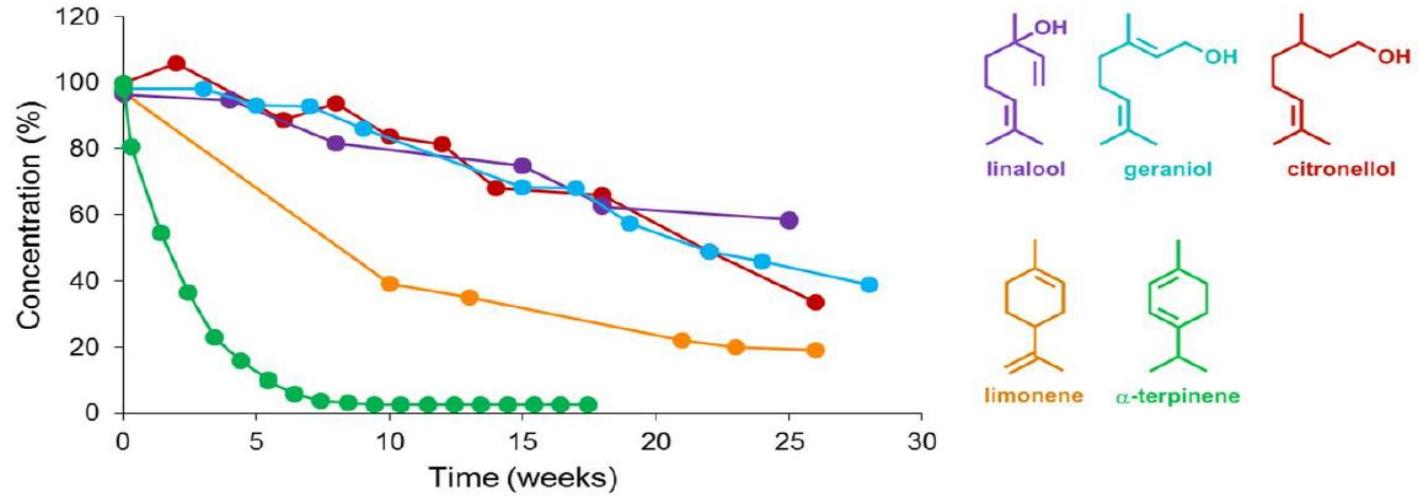
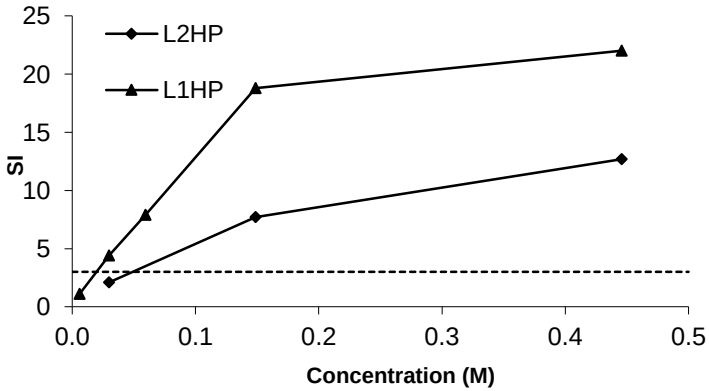


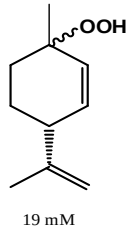
Figure 7.1 Degradation of α-terpinene (●), limonene (●), citronellol (●), linalool (●), and geraniol (●) over time comparing their different oxidation rate.

From Johanna Rudbäck's Thesis, Gothenburg University, 2014

What do we know?

- **Allylic hydroperoxides derived from terpenes (mono-, sesqui- and di-) are potent skin sensitizers…**

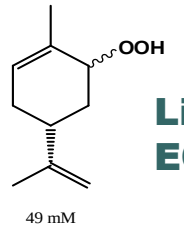




19 mM

Limonene-1-OOH
EC3= 19 mM (0.33%)

82.5 μg/cm^2



49 mM

Limonene-2-OOH
EC3= 49 mM (0.83%)

208 μg/cm^2

S. Johansson et al. ***Chem. Res. Toxicol.*** **2008, *21,* 1536–1547**

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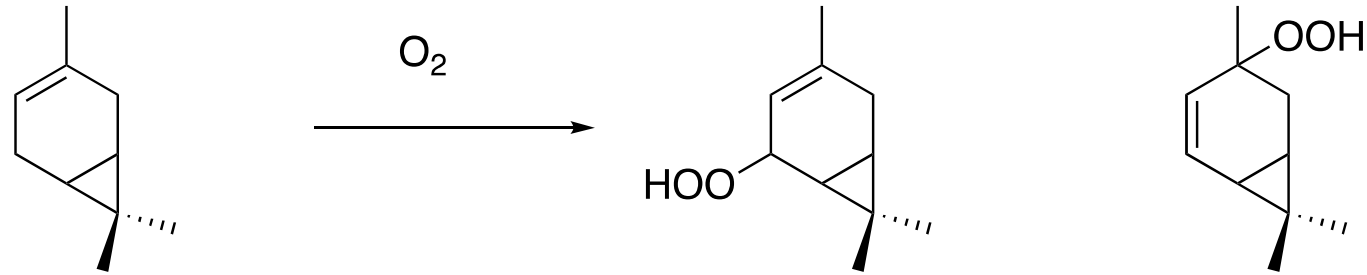
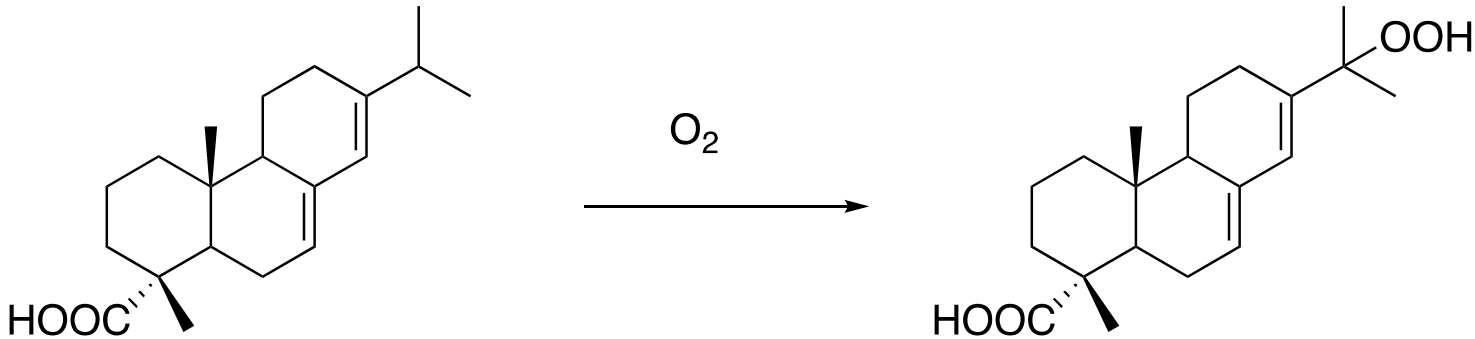
What do we know?

- **Exposure to allylic hydroperoxides can induce occupational "Allergic Contact Dermatitis"**



Δ-3-carene

Δ-3-carene hydroperoxides



Abietic acid

15-hydroperoxoabietic acid

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What do we know?

- **The “turpentine story”**
  - **Occupational exposure has been reported in painters, varnishers and in the ceramic industry since the 30’s,**
  - **Contact allergy was found to be correlated to the presence of Δ-3-carene in turpentine oil (French vs Swedish),**
  - **Contact allergy was found to be correlated to the oxidation state of Δ-3-carene (pure vs oxidized),**
  - **On 100 patients sensitized to turpentine, 25 reacted to concentrations of Δ-3-carene hydroperoxide lower than 50 ppm among which 9 reacted to concentrations lower than 10 ppm (down to 1 ppm).**

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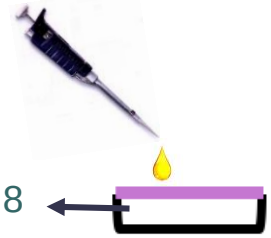
What do we know?

- Allylic hydroperoxides degrade, through the formation of radicals, into secondary oxidation products…
  - that are sometimes haptens (ascaridol),
  - that are sometimes weak sensitizers or non-sensitizers (limonene).

What do we know?

- **Radicals are also formed in the epidermis following exposure to allylic hydroperoxides:**

1) Spin-trap incubation

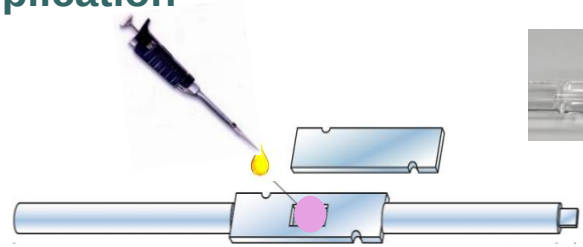


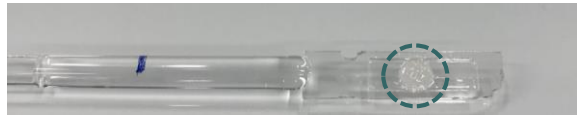
Hepes 10 mM pH 6.8

Incubation 15 min
Spin-trap 250 mM (20 µL)
Hepes - DMSO (v:v)
37 ° C, 5 % CO_2

2) Target compound application

100 mM - 50 mM
acétone (20 µL)





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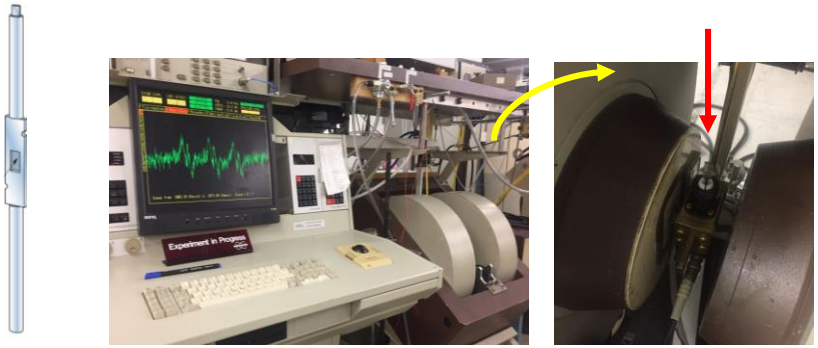
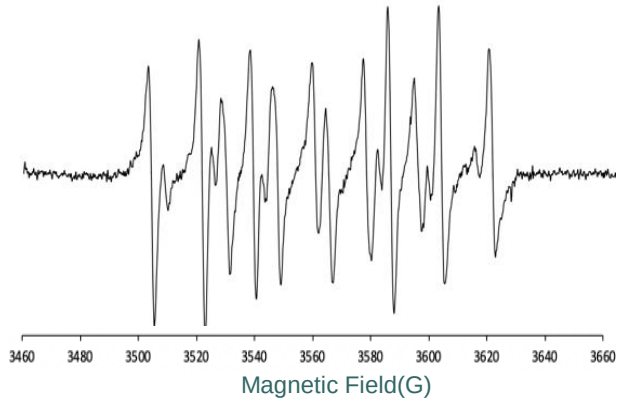
What do we know?

- **Radicals are also formed in the epidermis following exposure to allylic hydroperoxides:**

3) Acquisition



4) Signal treatment



Kuresepi *et al. Free Radic Res* **2018**, 52, 171
Sahli *et al*. *Arch Toxicol* **2019**, 93, 1337; *Free Radic Res* **2019**, 53, 737

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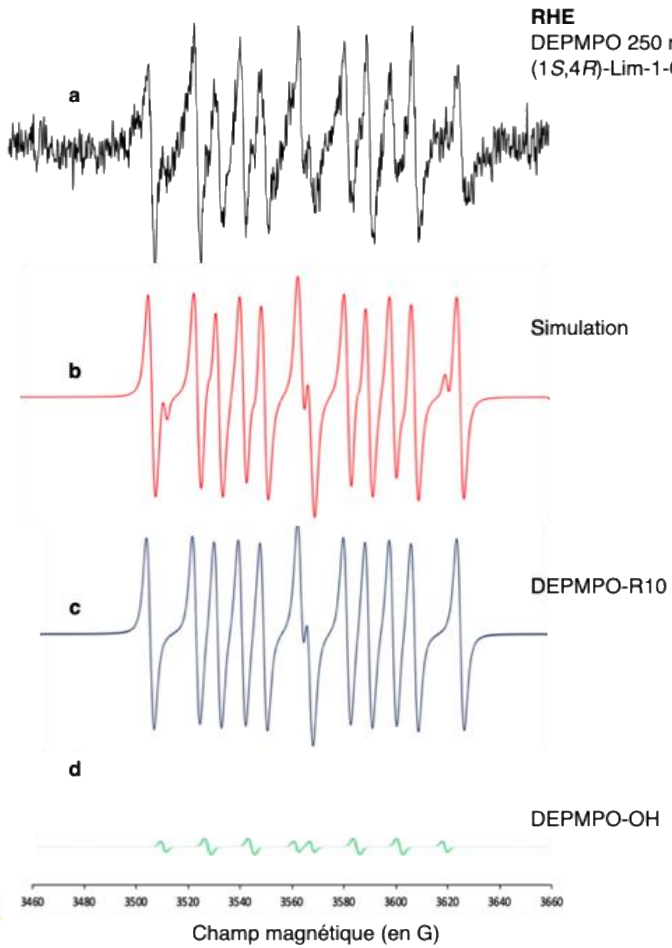
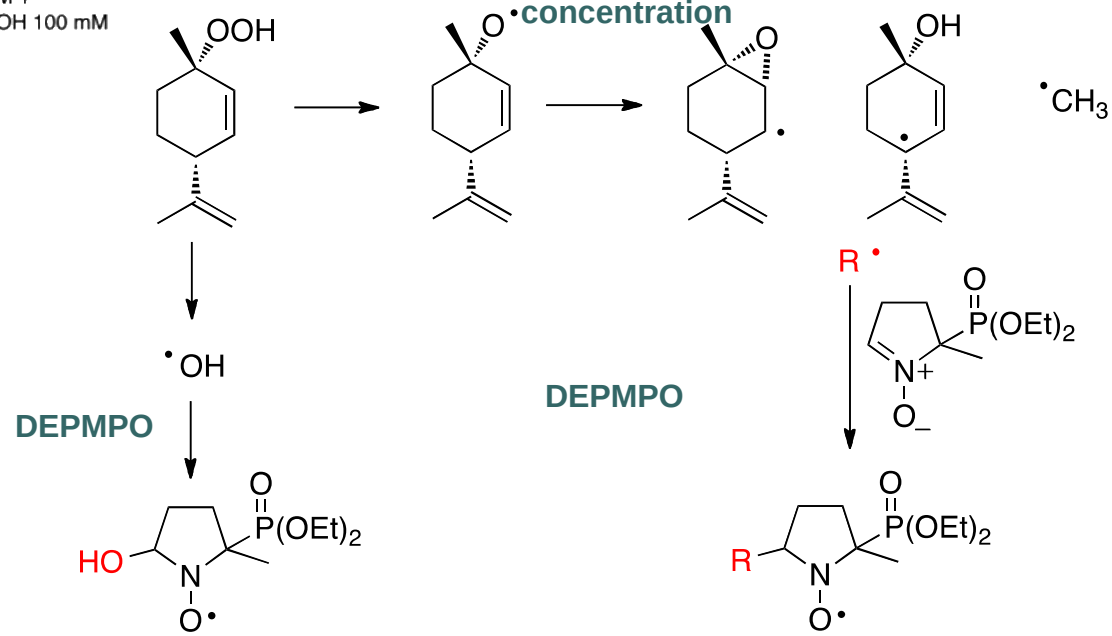
What do we know?

Lim-1-OOH:



- short life radicals
- not enough concentration



- longer life radicals
- detectable by EPR
- hyperfine coupling constants = f(**R'**)
- exact carbon radical trapped not yet identified

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What do we ignore?

- Can radicals derived from hydroperoxides significantly modify epidermal proteins to induce sensitization?
- Is the Adverse Outcome Pathway for skin sensitization developed for haptens fully relevant for prehaptens?

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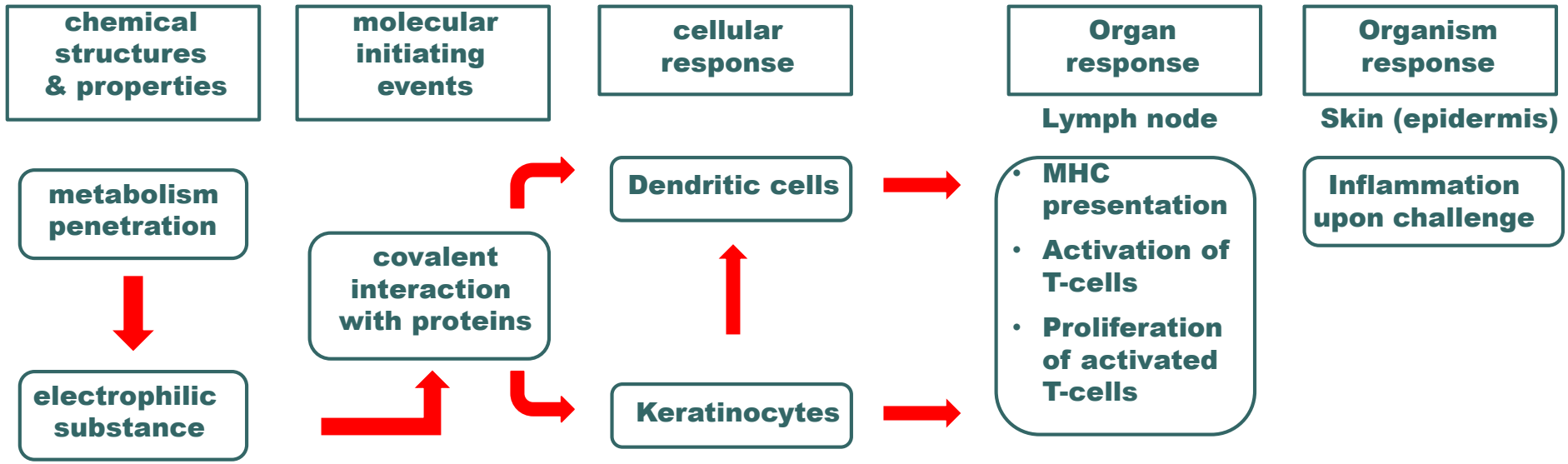
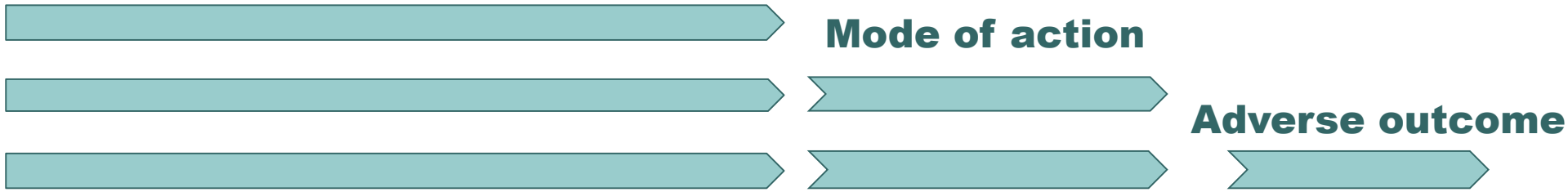
Adverse Outcome Pathway (AOP)



Toxicity pathway



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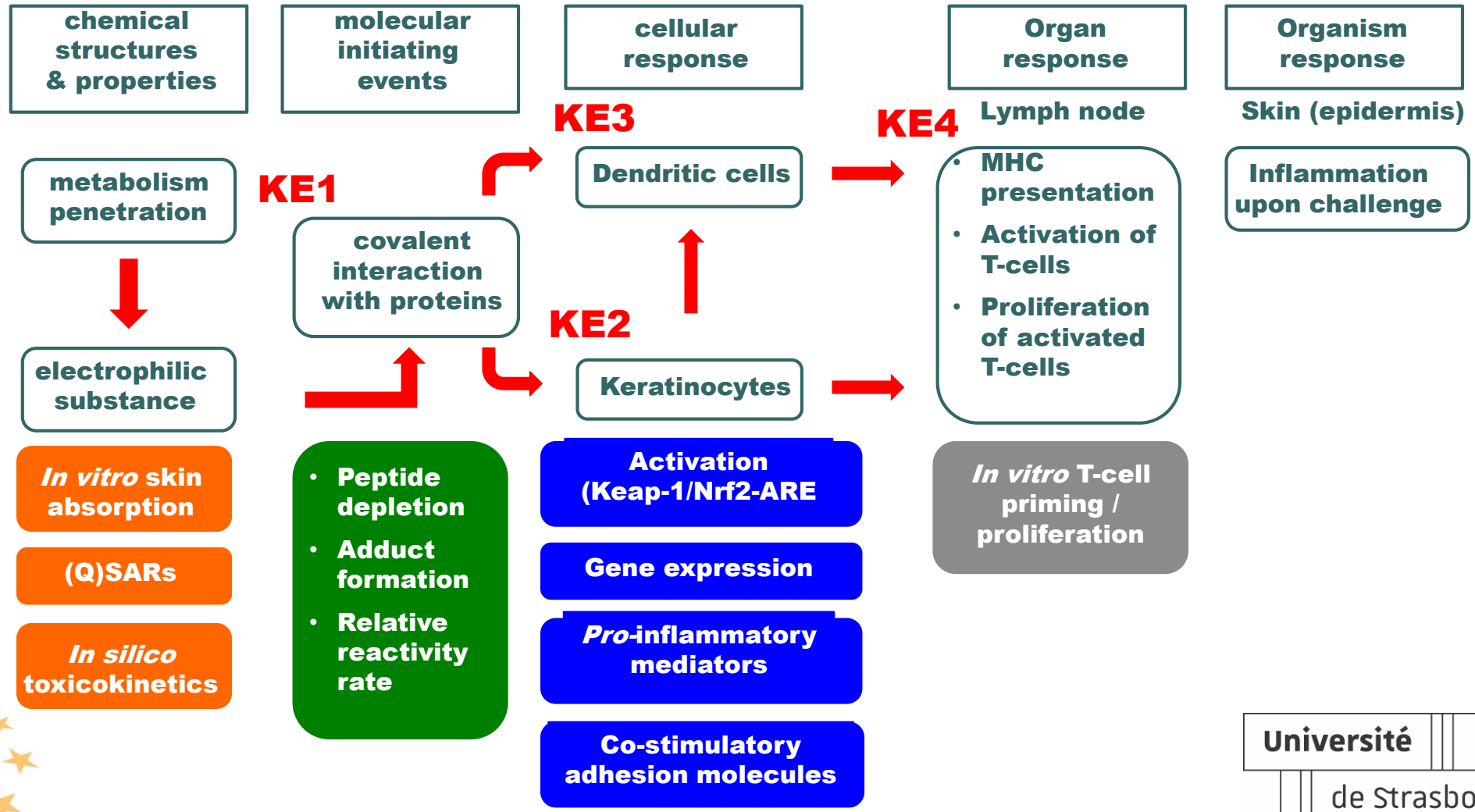
Adverse Outcome Pathway (AOP)



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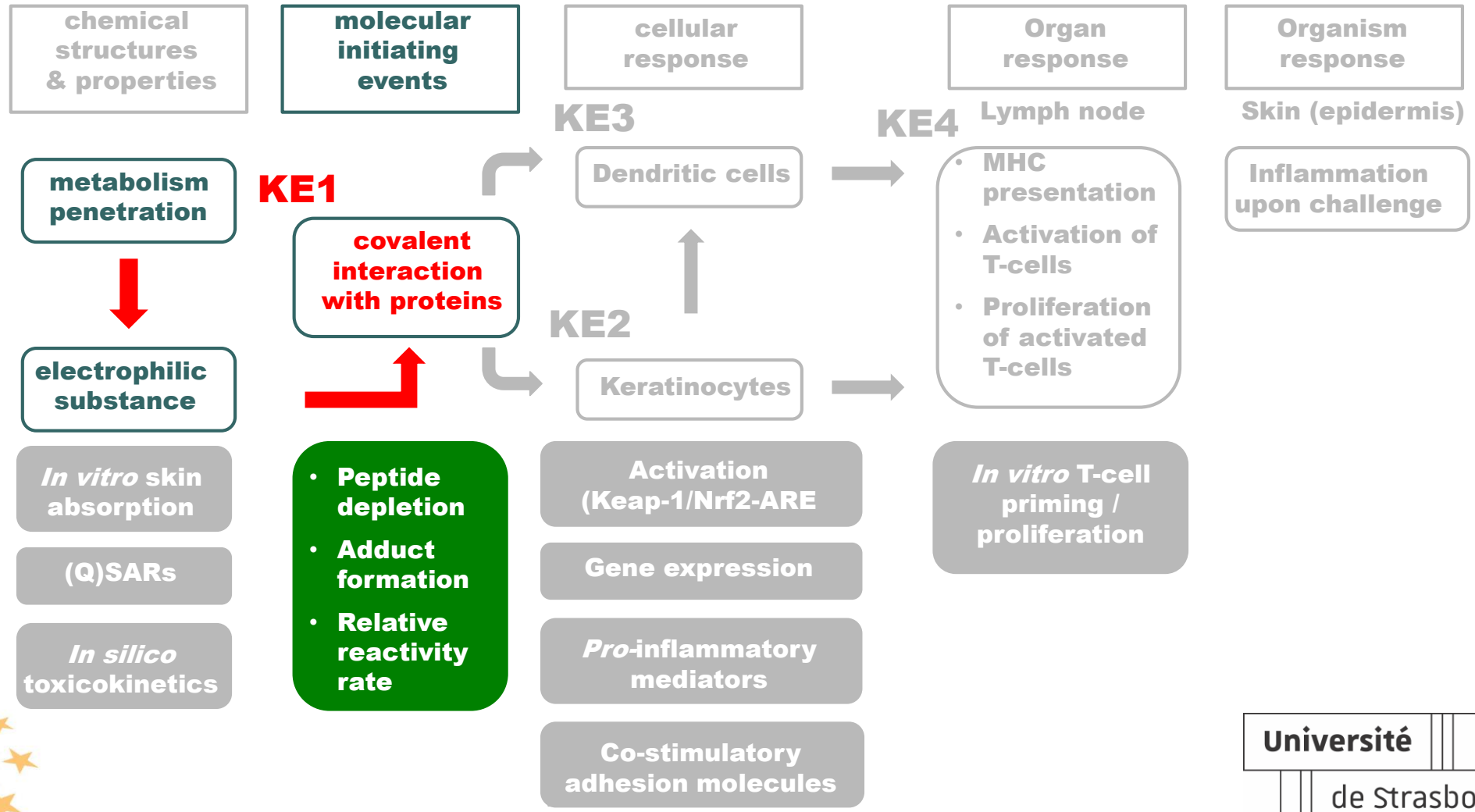

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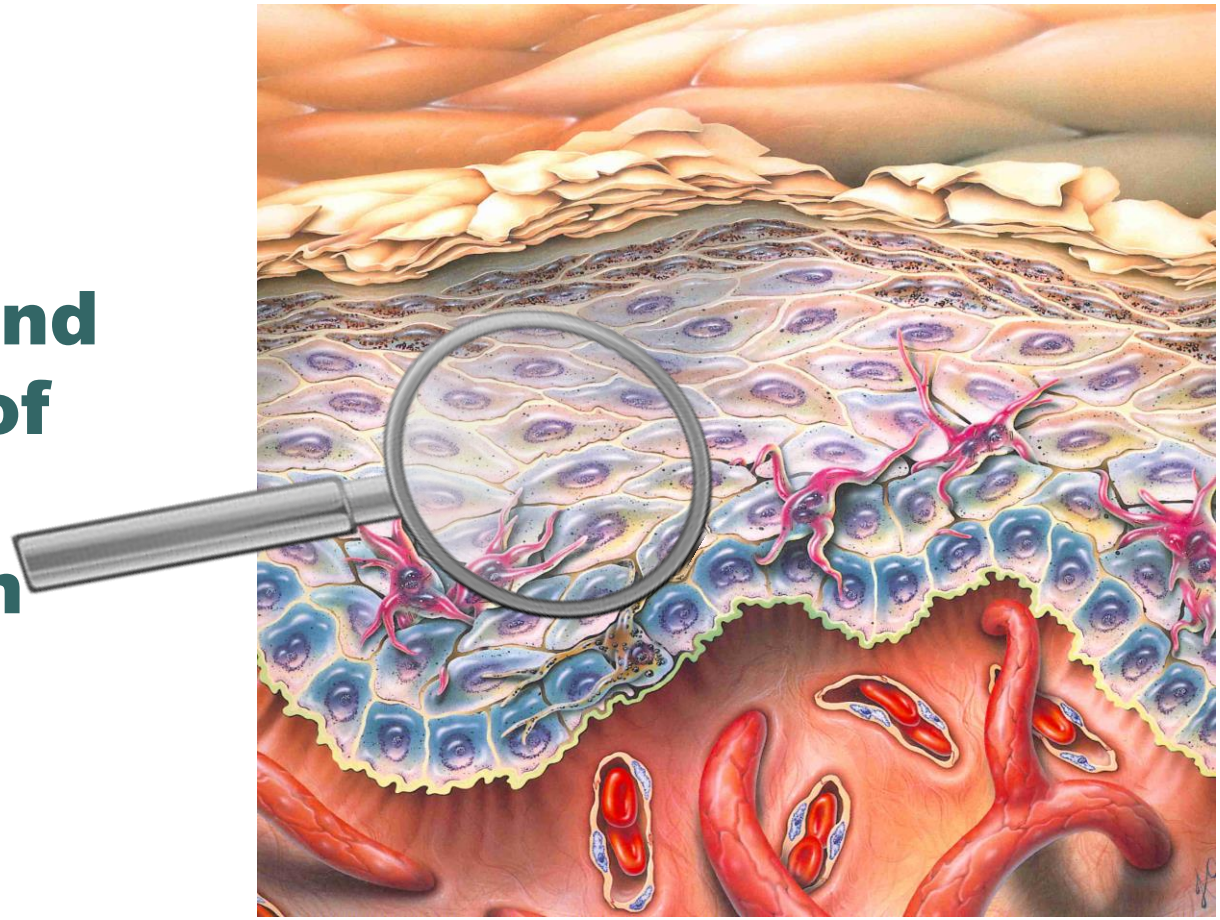
What happen *in situ*?

- Non invasive approach,
- Identification and quantification of adducts,
- High-Resolution Magic Angle Spinning (HRMAS) NMR.



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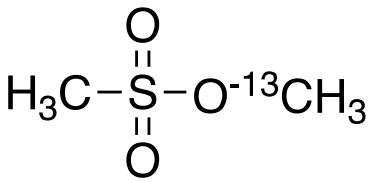
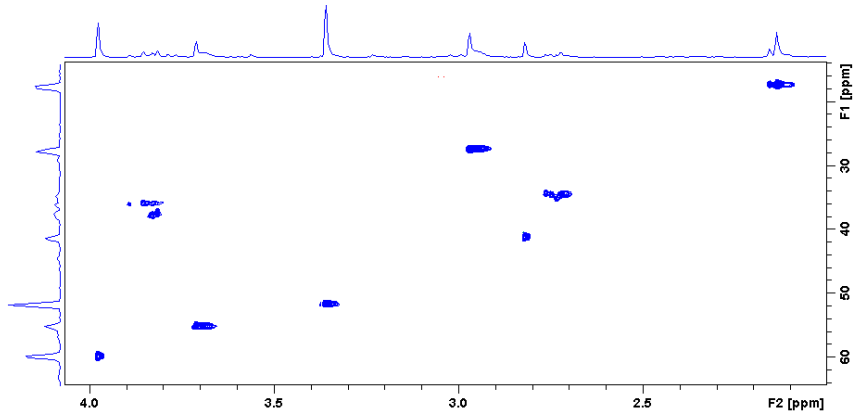
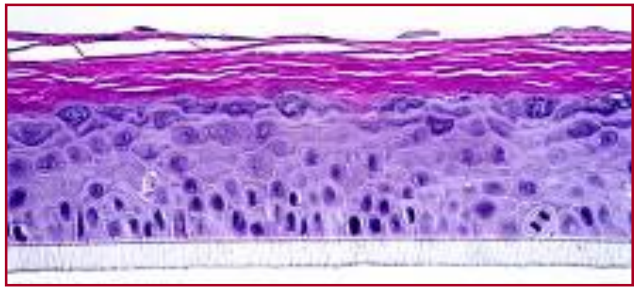
Methyl methanesulfonate







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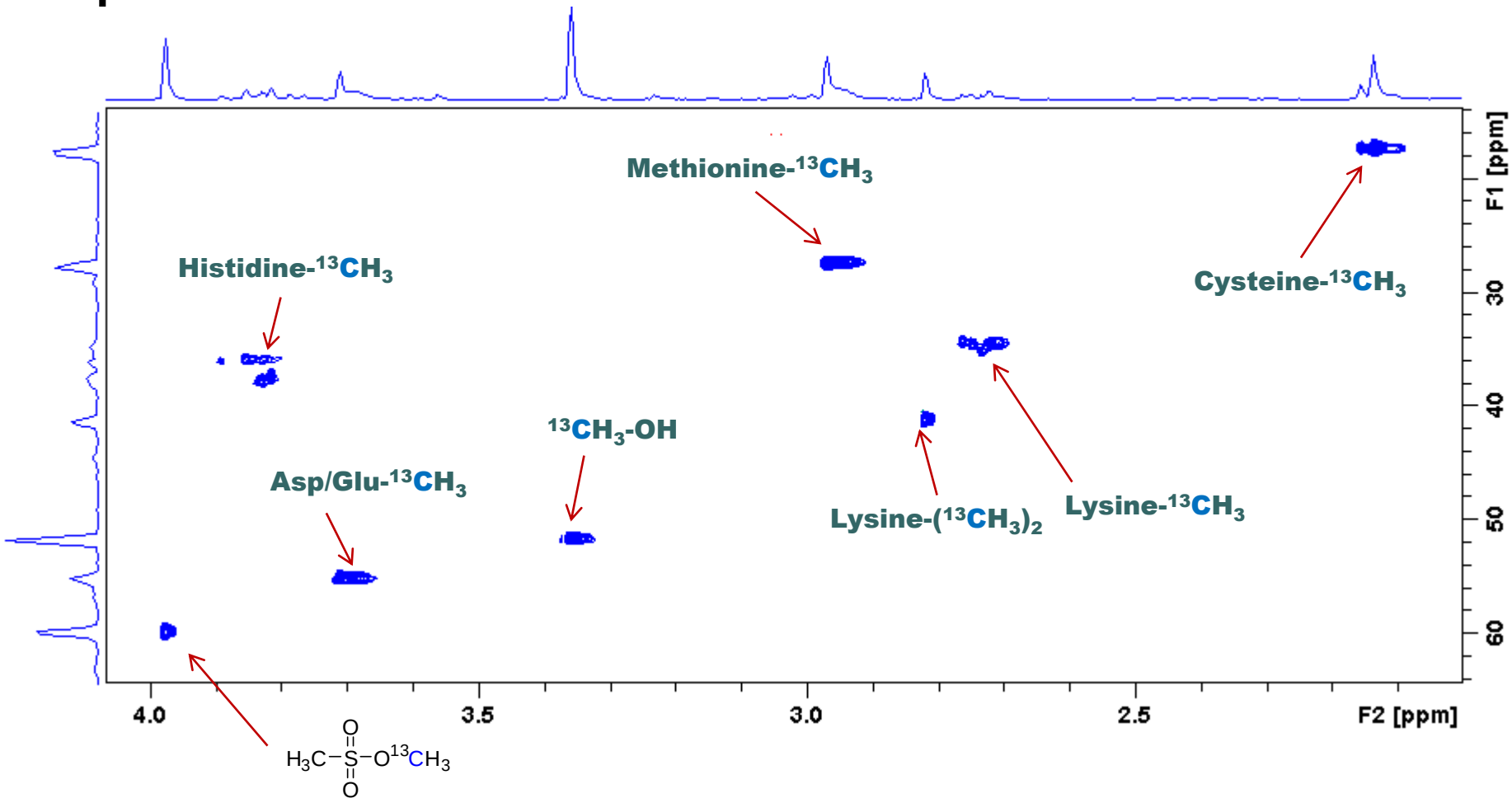
Methyl methanesulfonate



2D HSQC experiments

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Quantitative HRMAS on RHE

Concentrations of methyl signals [0.4M] as a function of time expressed in nmol/mg of RHE

Time	MMS	$^{13}CH_3$-OH	His	Asp/Glu	Met	Lys	Cys	Total adducts
1h	9.2	0.3	2.1	0.5	0.3	ND	1.8	4.7
2h	4.5	0.4	1.6	0.6	0.5	ND	2.0	4.7
4h	2.5	0.6	2.2	0.8	0.5	0.5	2.3	6.2
8h	1.9	0.9	1.9	0.9	0.4	0.5	2.0	5.8
24h	0.6	1.1	1.5	0.7	0.4	0.4	0.6	3.7

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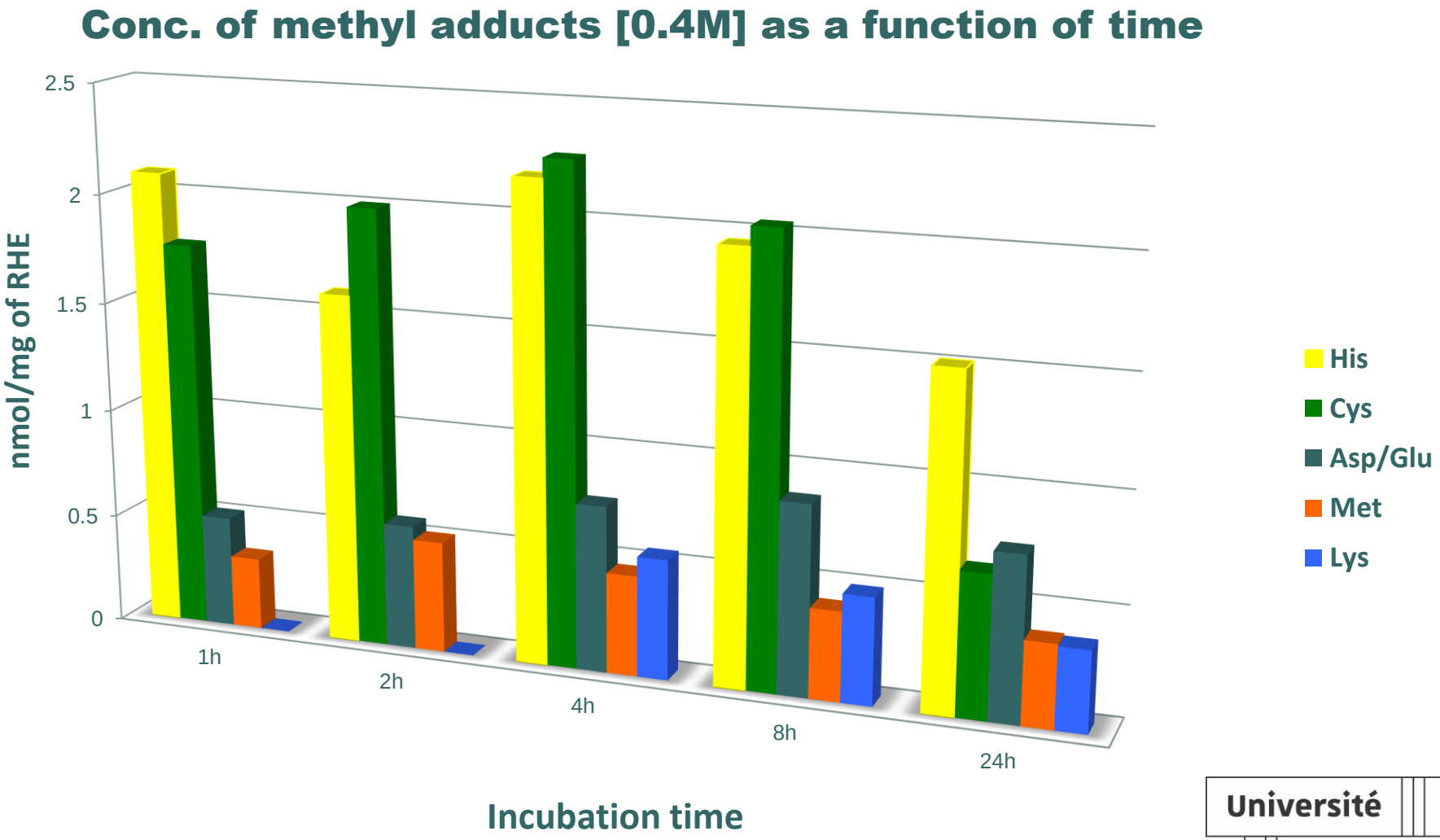
Quantitative HRMAS on RHE



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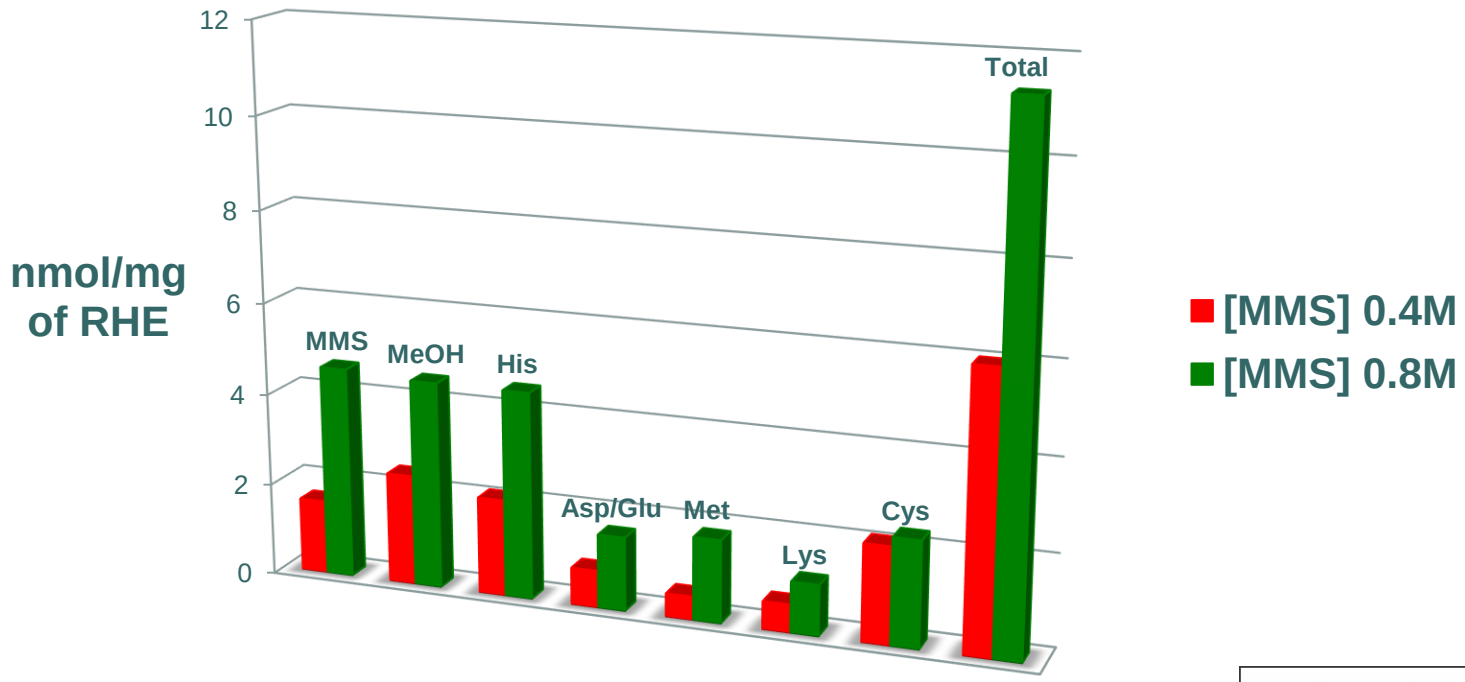
Quantitative HRMAS on RHE

Concentrations of methyl signals (8h of exposure) expressed in nmol/mg of RHE as a function of applied [conc].

	MMS	$^{13}CH_3$-OH	His	Asp/Glu	Met	Lys	Cys	Total
0.2	0.6	0.3	1.3	0.4	0.5	0.3	1.0	3.5
0.4	1.6	2.4	2.1	0.8	0.5	0.6	2.1	6.0
0.6	1.8	2.7	2.8	0.9	0.9	0.6	1.9	7.0
0.8	4.6	4.5	4.5	1.6	1.8	1.1	2.3	11.3
1	5.7	6.5	6.5	2.4	2.2	1.4	2.1	14.7

Quantitative HRMAS on RHE

Concentrations of methyl signals (8h of exposure) expressed in nmol/mg of RHE as a function of applied [conc].



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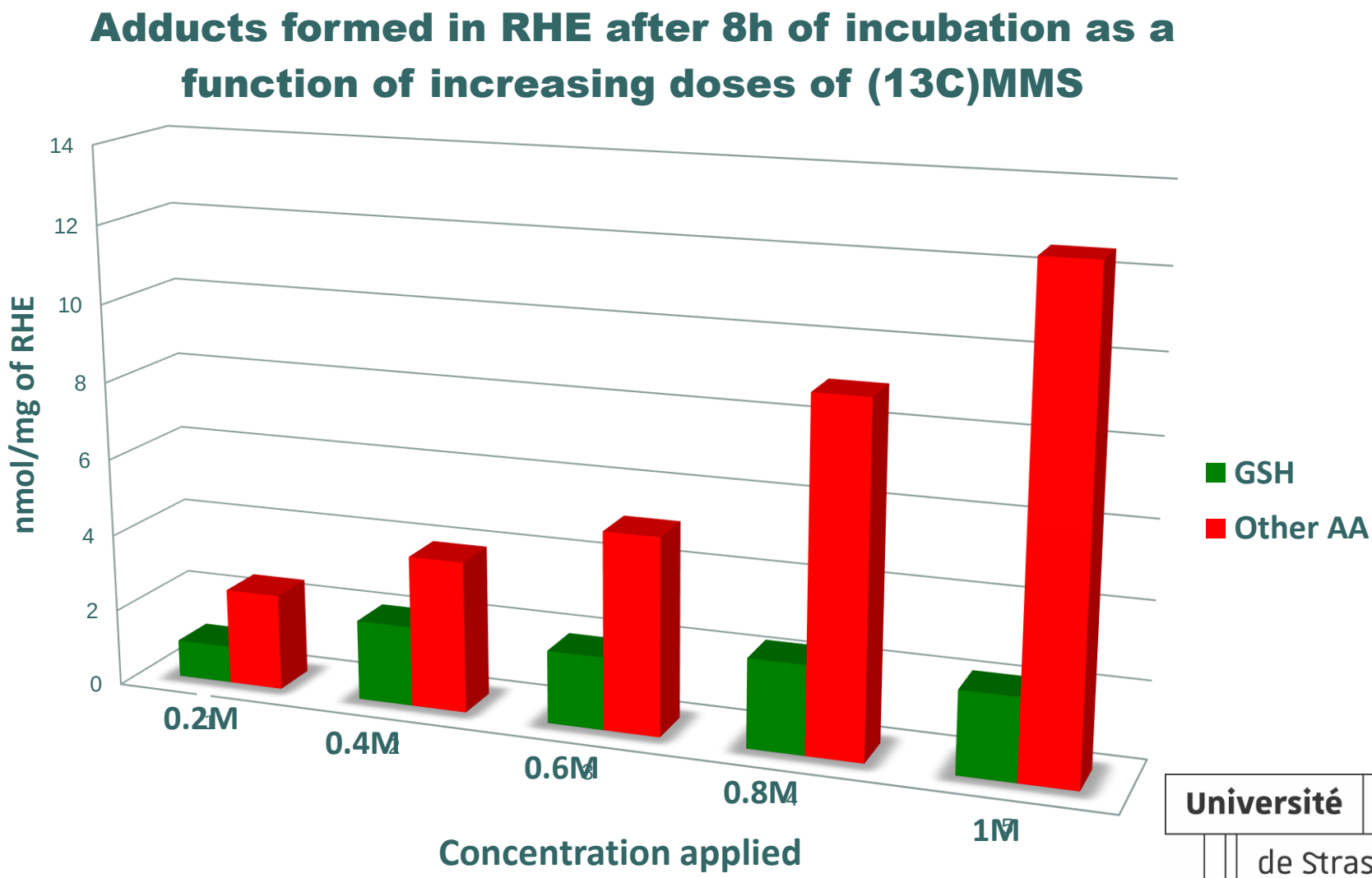
Quantitative HRMAS on RHE



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Adverse Outcome Pathway…

- KE1, as defined in the AOP for skin sensitization, seems relevant for haptens directly reacting with nucleophiles,
- A significant amount of adducts (between 2-12 nmol/mg of RHE in relation with the concentration of exposure) is formed in the epidermis,
- This is in good agreement with DPRA data showing a parallel relation between the reactivity and the potency.

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Reactivity of a prehaptens in RHE

- *p*-Phenylenediamine [106-50-3]
  - EC_3 = 0.16
- Direct Peptide Reactivity Assay
  - Pep-Cys = 93.0 +/- 6.8
  - Pep- Lys = 23.5 +/- 0.9

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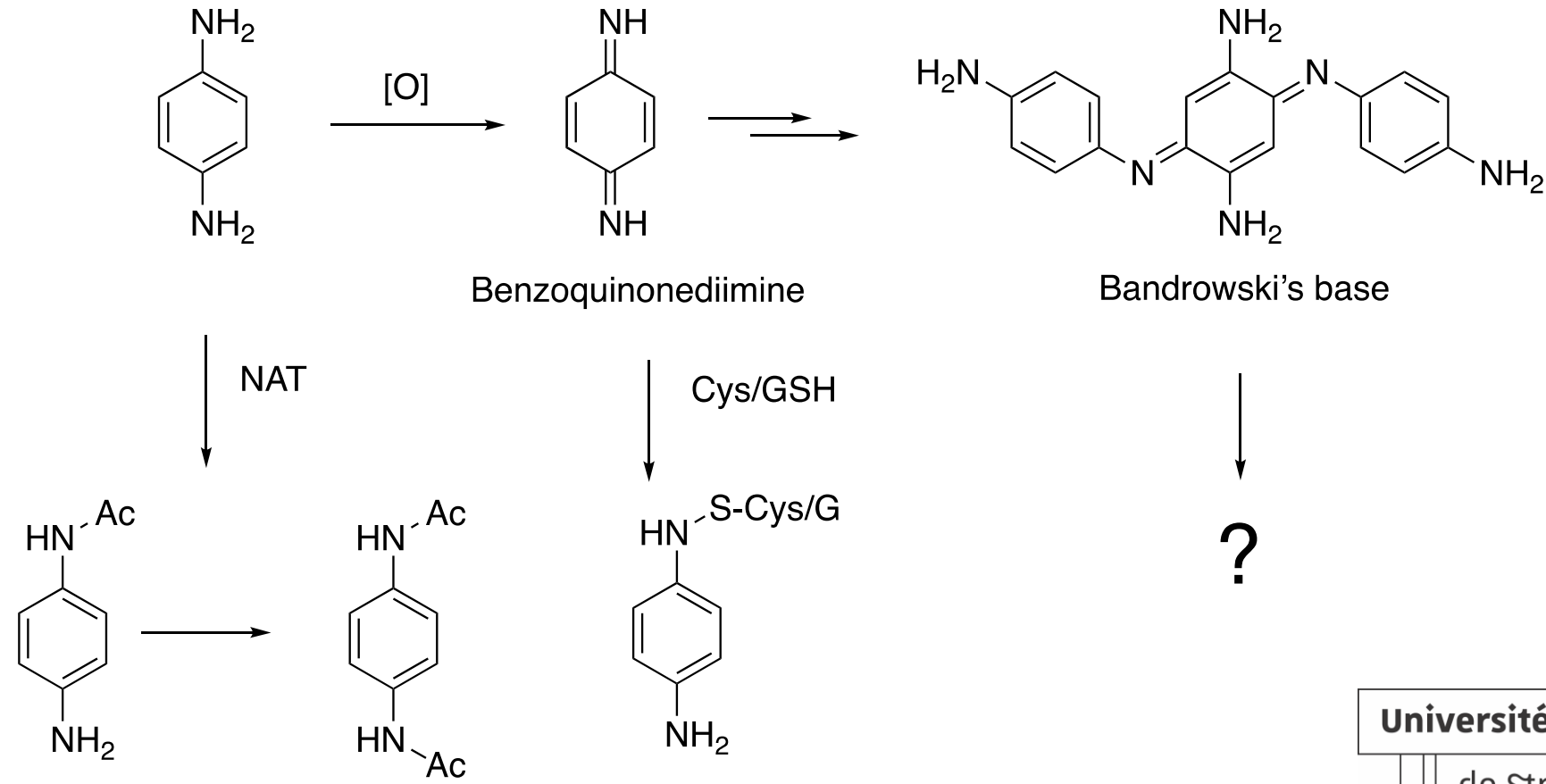
1,4-phenylenediamine (PPD)

- **Subjected to oxidation and epidermal metabolism**

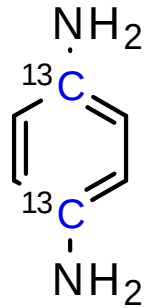


1,4-phenylenediamine (PPD)

- **RHE were treated with 30µL of PPD (O.8 M in acetone) and incubated for various time periods: 1h, 8h, 24h, 48h.**

RHE

+



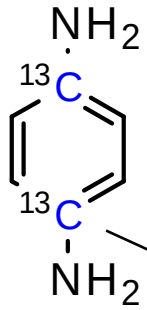
30 µl, 0.8 M

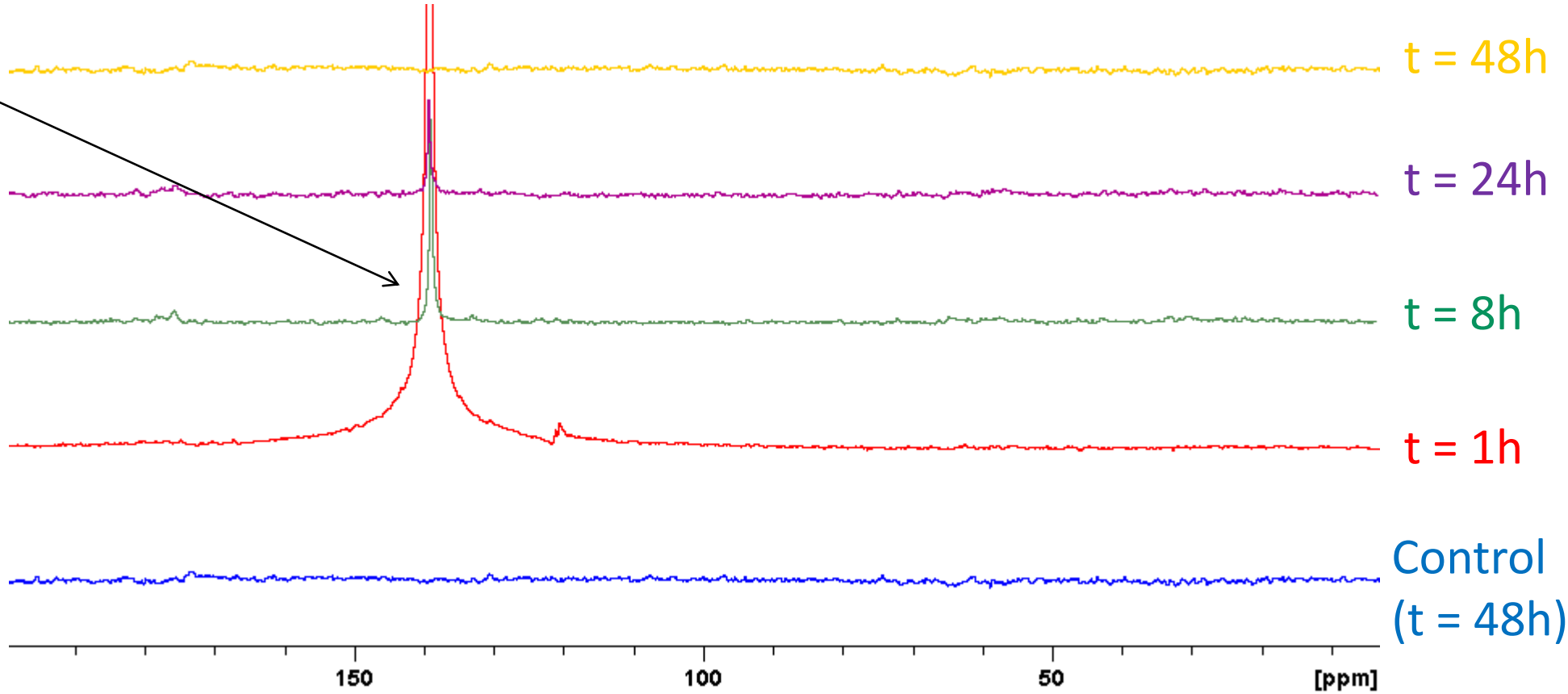
Incubation (37 ° C, 5%CO_2)
≠ times

Modified RHE

HRMAS

Behavior of 1,4-(^{13}C)PPD in RHE



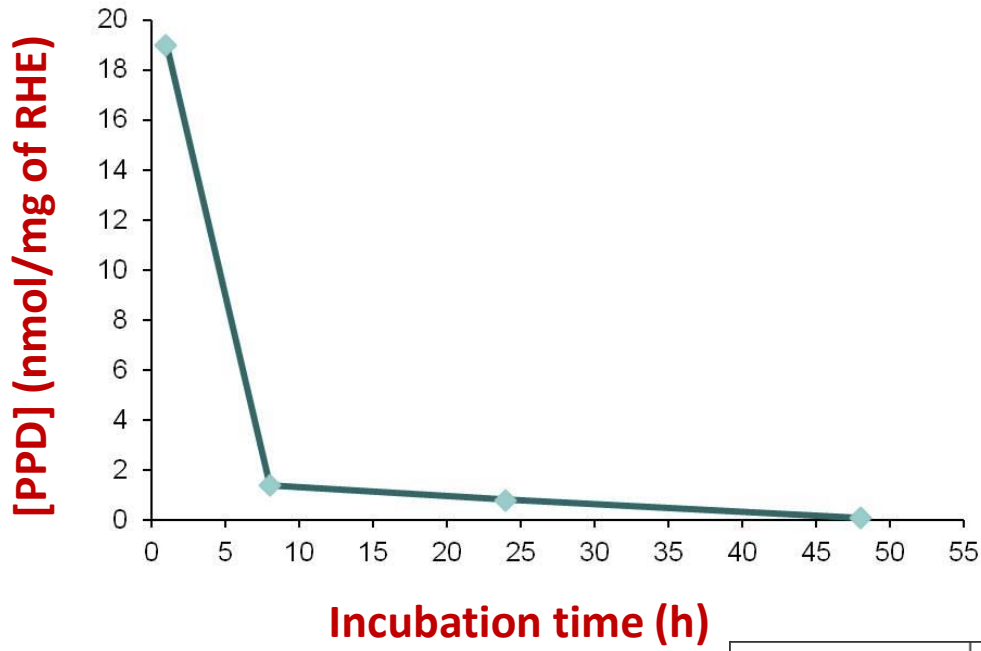


1D ^{13}C experiments

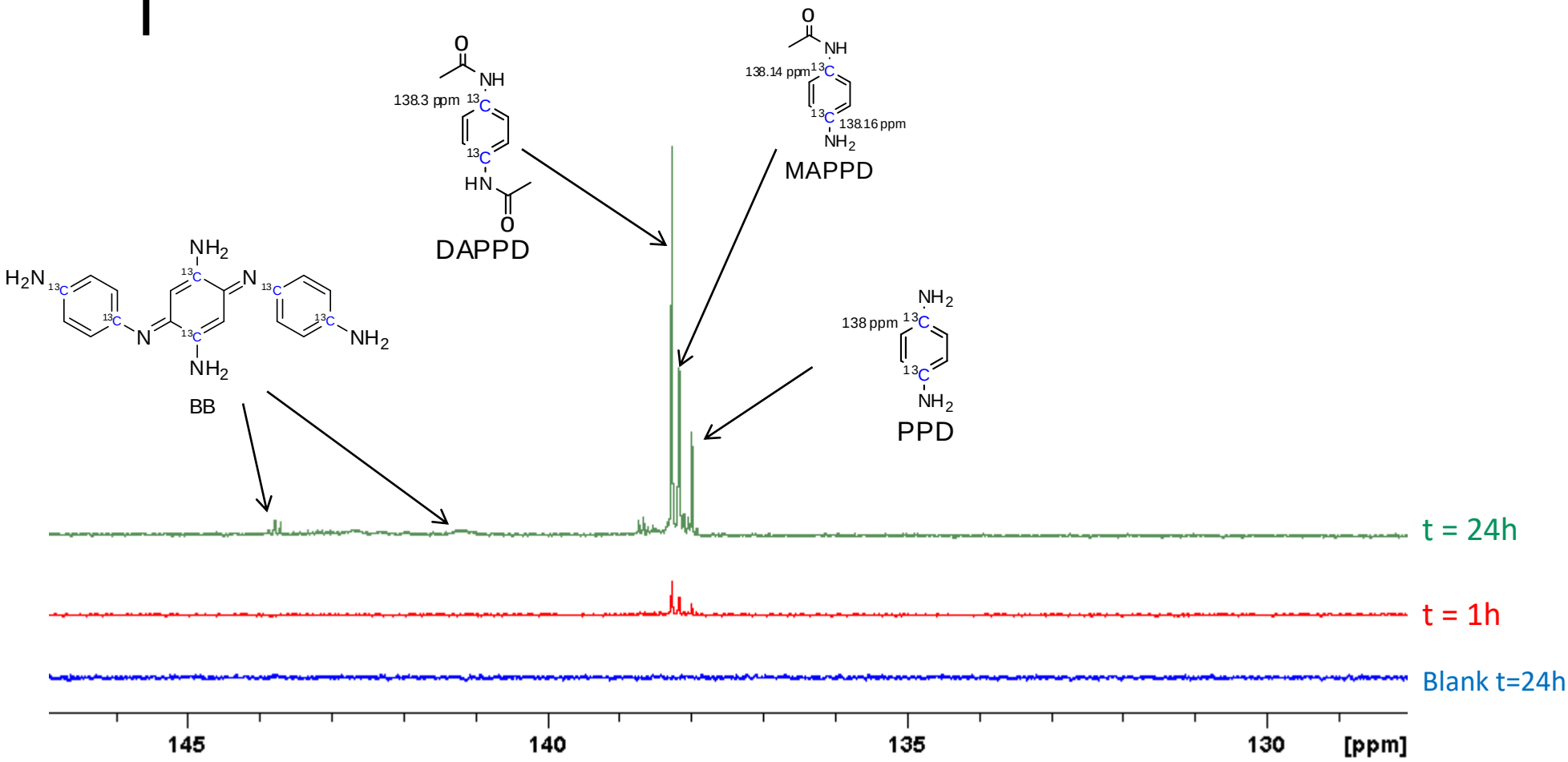
Behavior of 1,4-(^{13}C)PPD in RHE

- **Absolute concentrations of the remaining 1,4-(^{13}C)PPD in RHE with time calculated using 1D HMBC**

	1,4- (^{13}C)-PPD (nmol/mg of RHE)
1 h	19
8 h	1.4
24 h	0.8
48 h	0.1

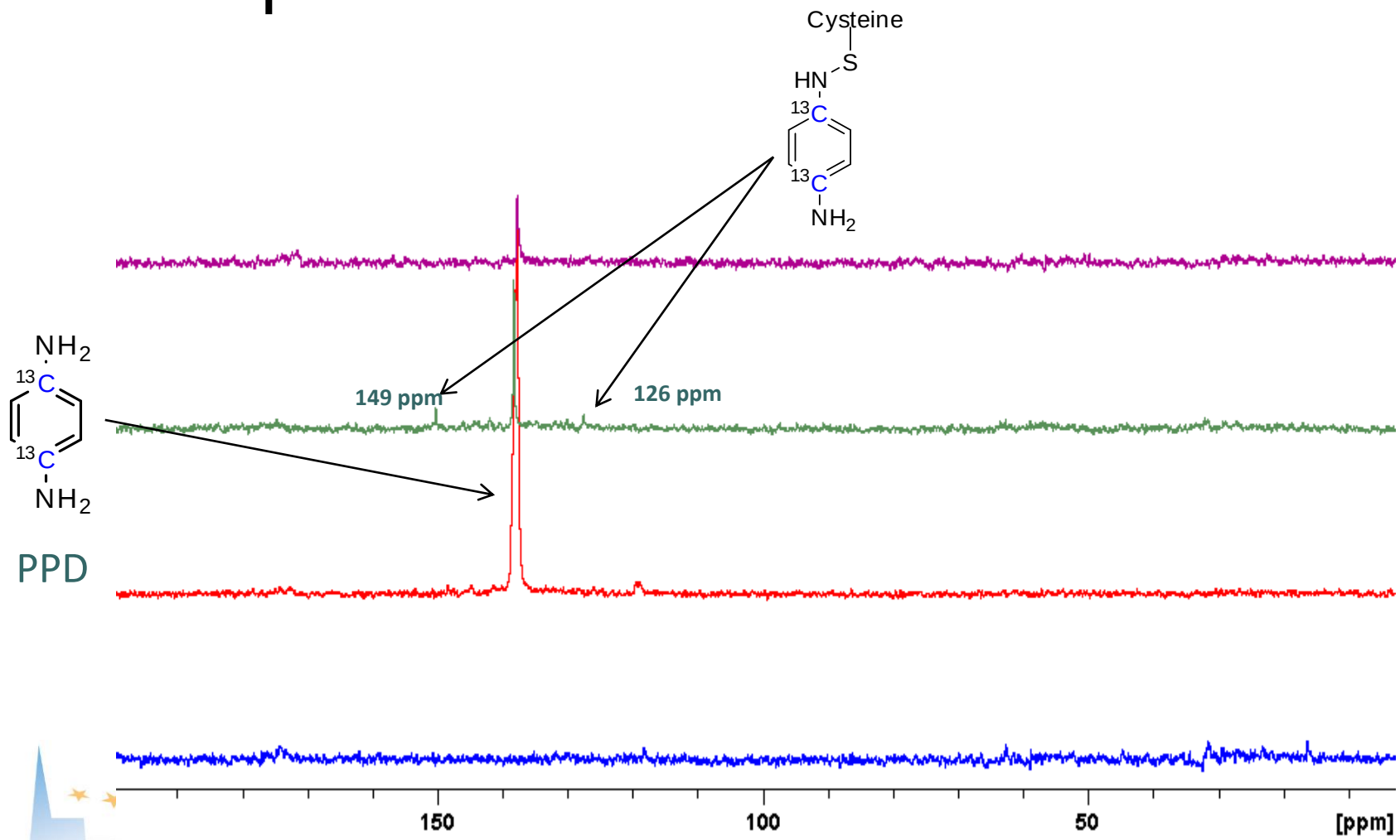


Behavior of 1,4-(^{13}C)PPD in RHE



^{13}C NMR of the culture medium – RHE incubated with PPD for 1 and 24h

Behavior of 1,4-(^{13}C)PPD in RHE under oxidative conditions (H_2O_2)



1D ^{13}C experiments

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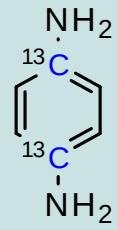
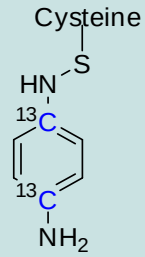
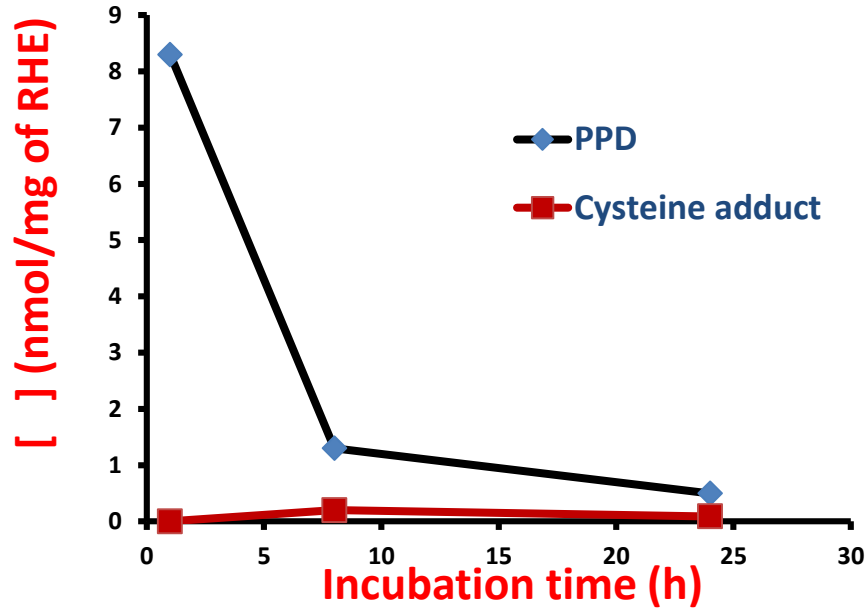
Behavior of 1,4-(^{13}C)PPD in RHE under oxidative conditions (H_2O_2)

Absolute concentrations in RHE with time calculated using 1D HSQC NMR

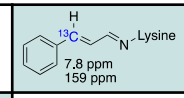
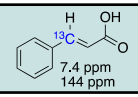
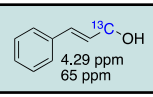
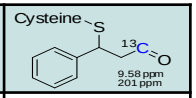
		
1 h	8.3	
8 h	1.3	0.2
24 h	0.5	0.09

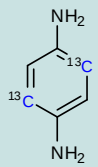
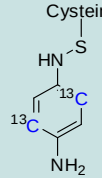


Compared absolute concentrations in RHE

Concentrations with time expressed in nmol/mg of RHE after exposure to a solution [0.4M]

	Histidine-$^{13}CH_3$	Methionine-$^{13}CH_3$	Lysine-$^{13}CH_3$	Cysteine-$^{13}CH_3$
1h	2.09	0.33		1.77
8h	1.95	0.41	0.49	2.04
24h	1.51	0.38	0.37	0.65

				
1 h	4.8	0.62	0.7	5.1
2 h	3.2	0.91	0.3	2.6
8 h	1.3	0.47	0.2	0.7

		
1 h	8.3	
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24 h	0.5	0.09

Adverse Outcome Pathway…

- KE1, as defined in the AOP for skin sensitization, seems non-relevant for PPD, a prehapten needing to be oxidized to become reactive,
- A very low amount of adducts is formed in the epidermis,
- There is no parallel relation between the reactivity and the potency,

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Adverse Outcome Pathway…

- All prehaptens have in common to form oxidative chemicals once activated,
- Could, for such chemicals, a low amount of adducts be sufficient to induce sensitization?
- Shall we change of paradigm for this sub-category of skin sensitizers?

Acknowledgments

- Dr Eric MOSS
- Dr Marie BETOU
- Dr Hassan SROUR
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