Haptenation in HaCaT cells: comparison between DNCB and cinnamaldehyde

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ERGECD Liverpool 2024

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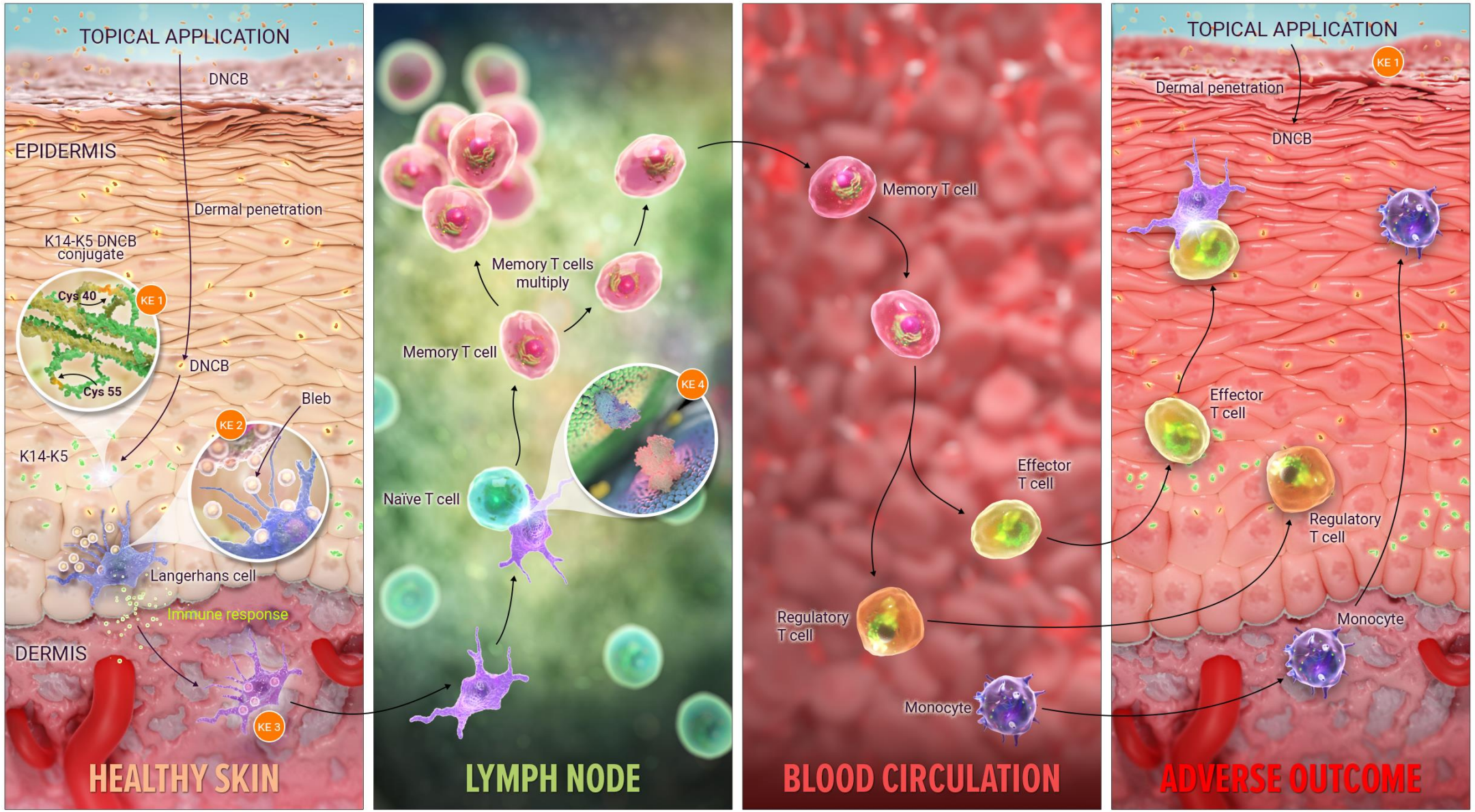
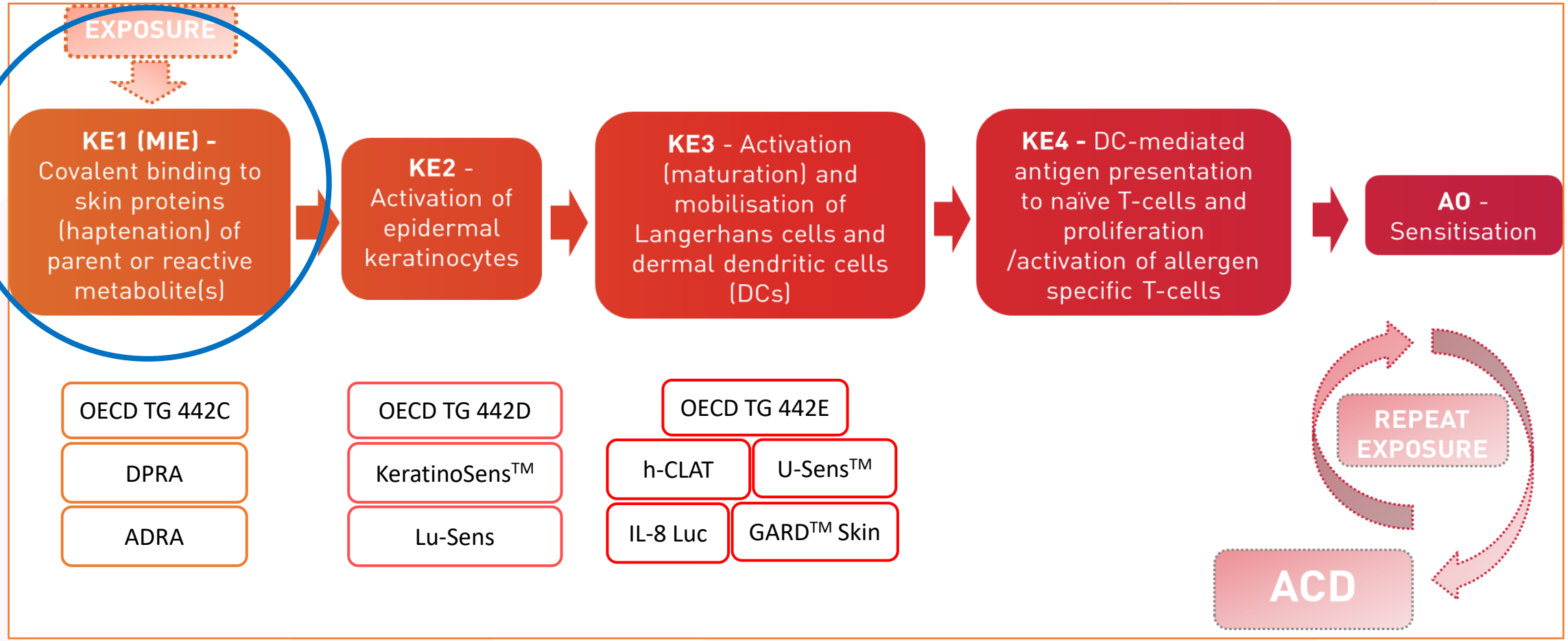
SKIN SENSITISATION OVERVIEW



Imagery: NEXU Science Communication

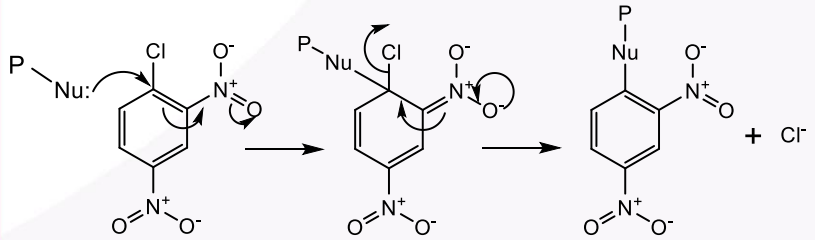
Adverse Outcome Pathway for Skin Sensitisation



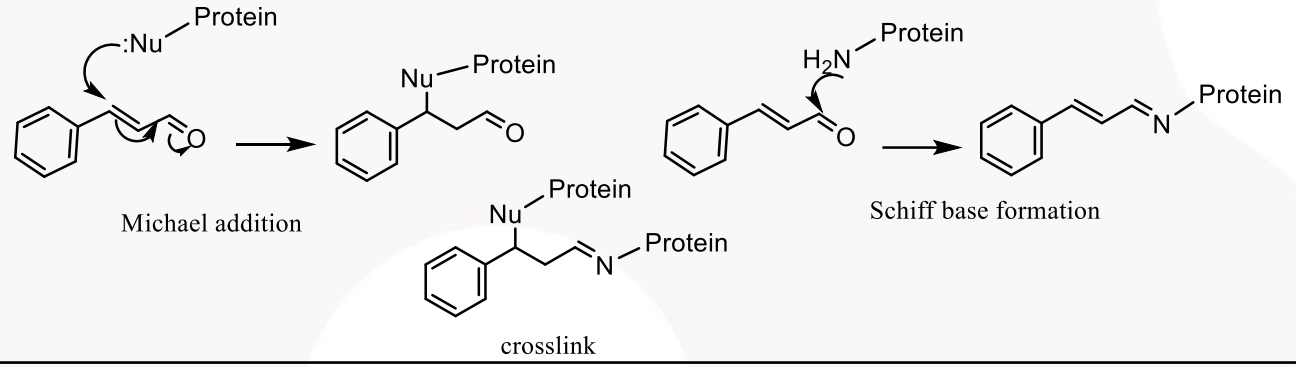
OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

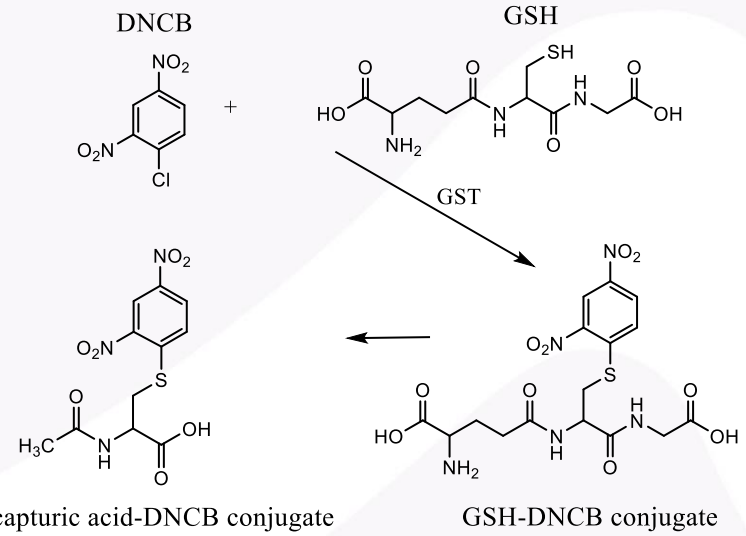
DNCB and cinnamaldehyde: Reactivity to protein nucleophiles and metabolism

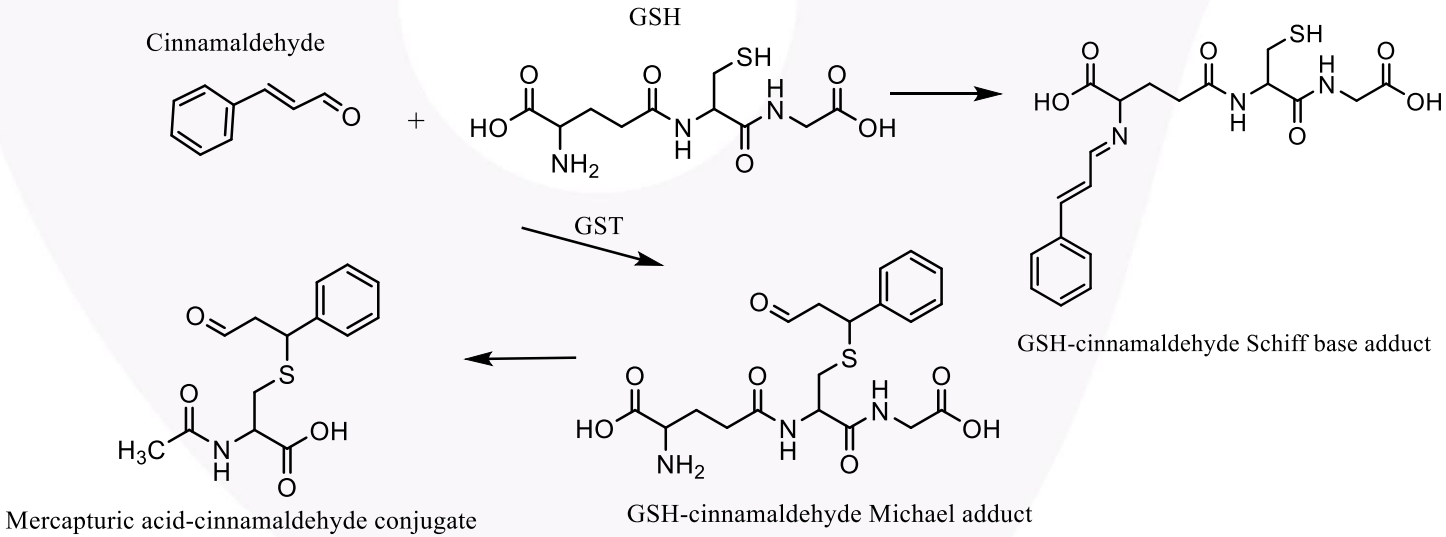
DNCB

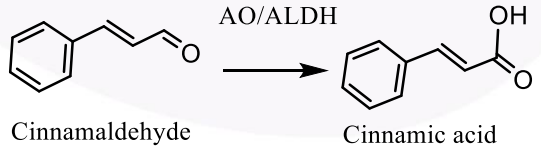


Cinnamaldehyde

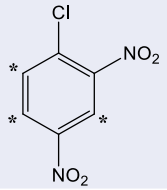
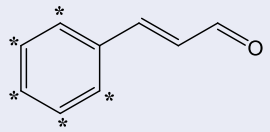




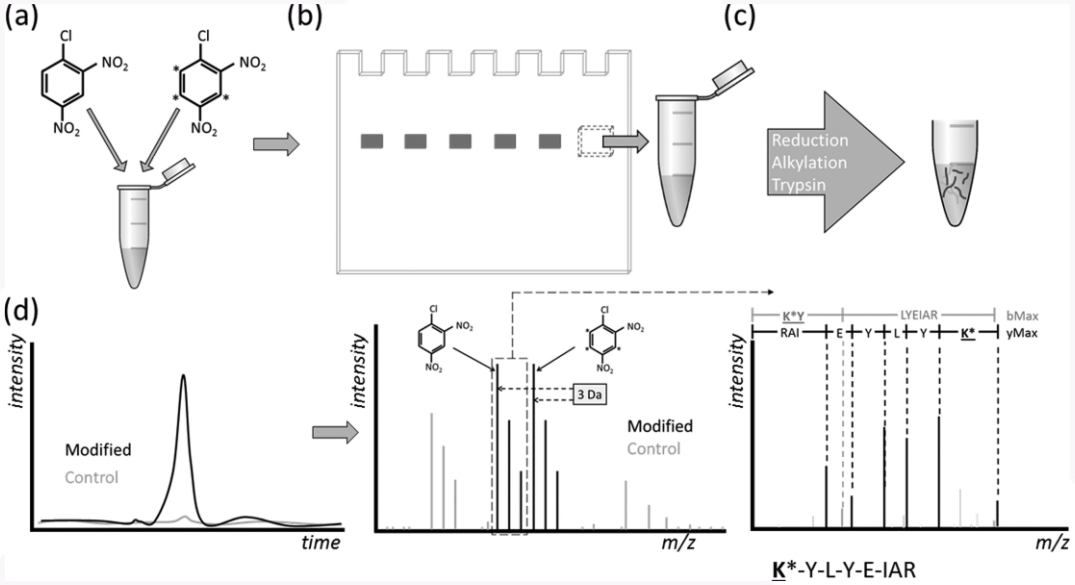




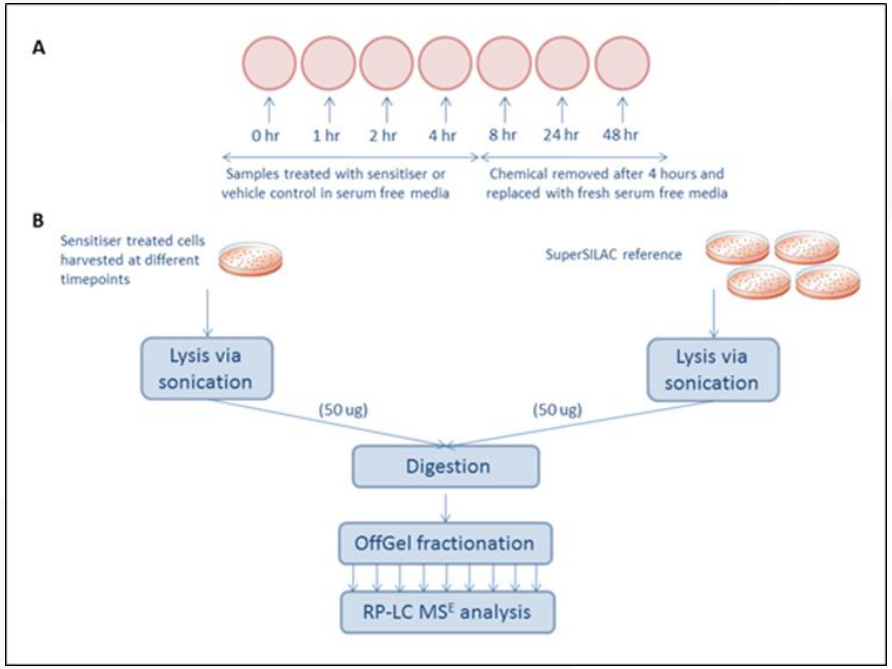
Method overview

Chemical	Structure	Potency (EC3 (%))	Δmass	Mechanism	Nucleophile
1-chloro-2,4-dinitrobenzene (DNCB)		Extreme (0.05)	+166.0015 (+169.0195)	S_NAr	Cys, His, Lys, Tyr
Cinnamaldehyde		Moderate (3.0)	+114.047 (+119.078)	Schiff base	Arg, Lys
			+132.0575 (+137.0885)	Michael adduct; acylation	Arg, Cys, His, Lys

Stable isotope labelling technique



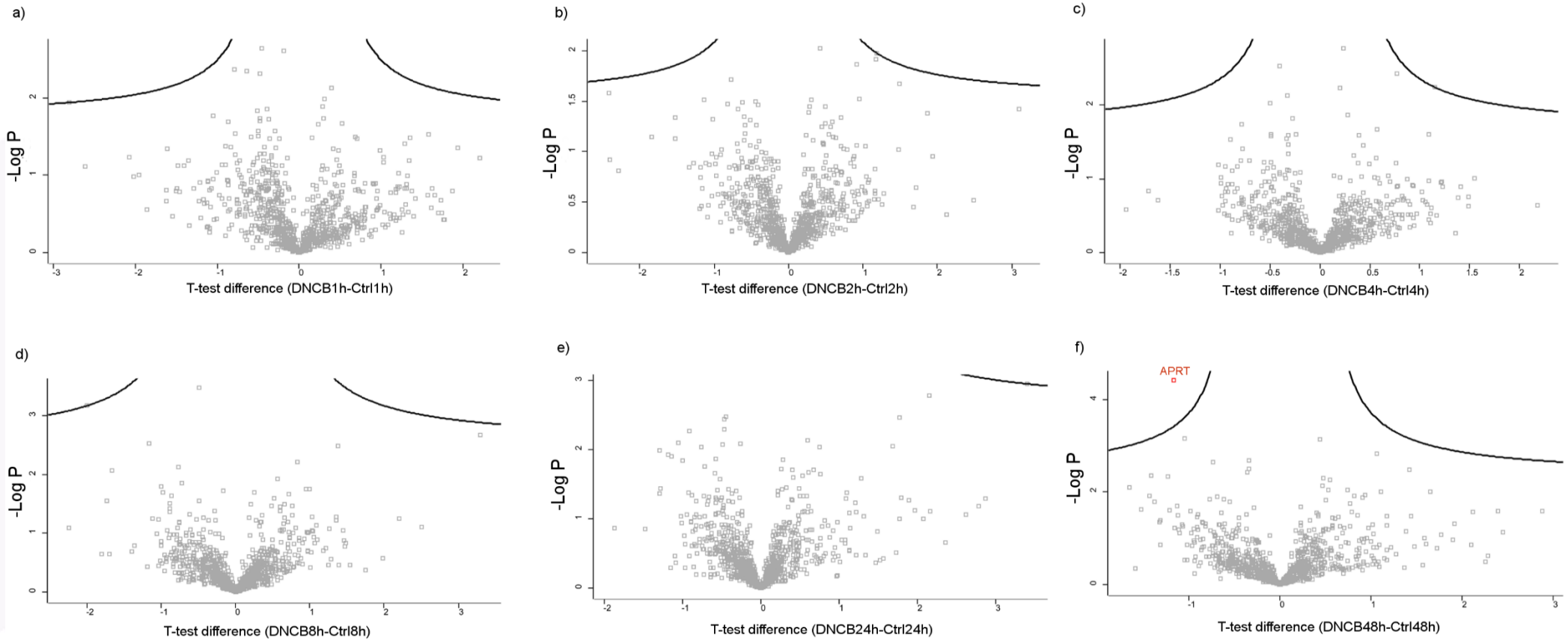
Cell treatment overview



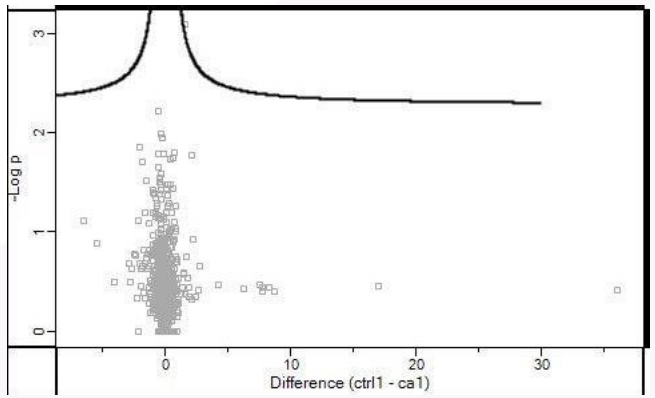
Parkinson *et al.* (2014) Stable Isotope Labelling Method for the Investigation of Protein Haptenation by Electrophilic Skin Sensitisers, Tox Sci, 142(1):239-49.

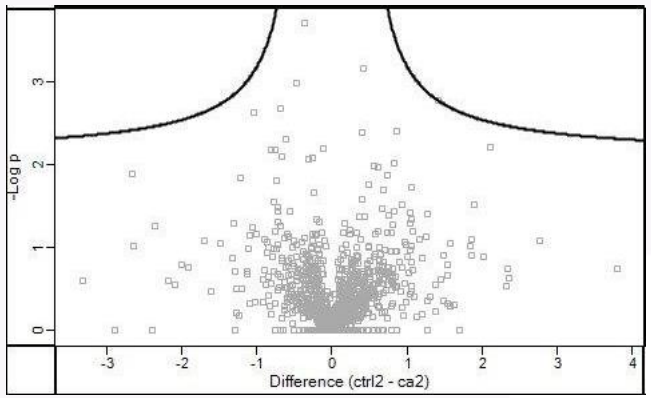
Parkinson *et al.* (2020), Proteomic analysis of the cellular response to a potent sensitiser unveils the dynamics of haptenation in living cells, Toxicology 445, pp1-10; 152603

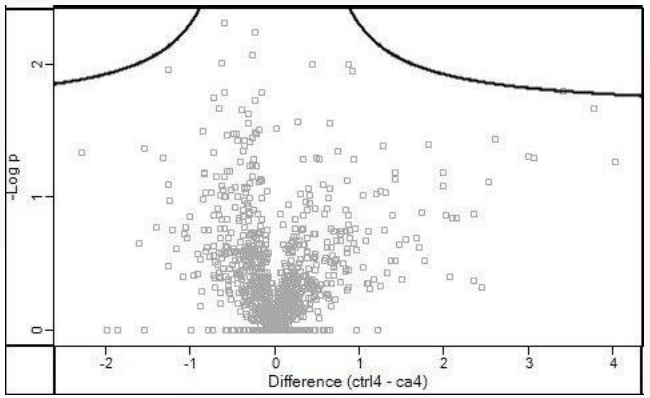
No change in protein expression throughout 48h experiment (DNCB)

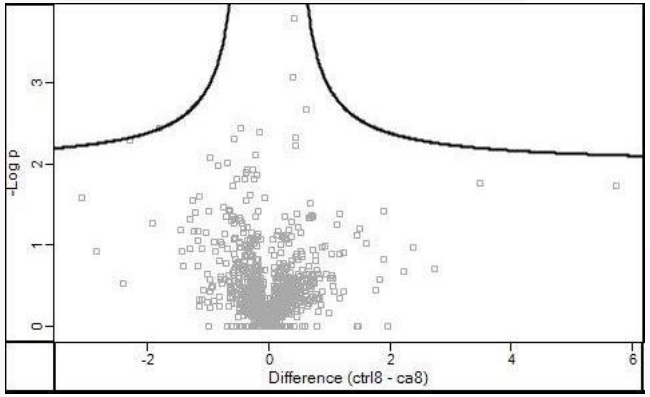


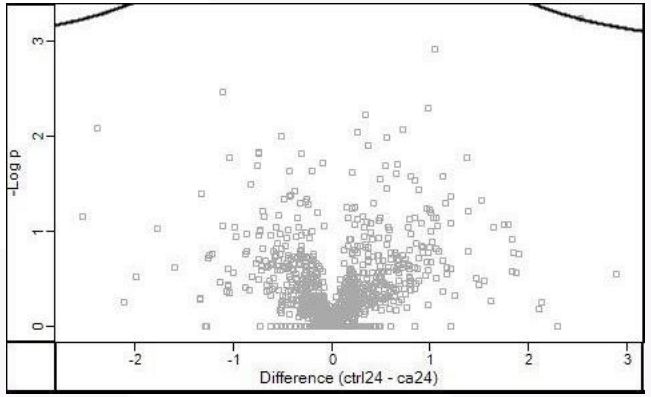
No change in protein expression throughout 48h experiment (cinnamaldehyde)

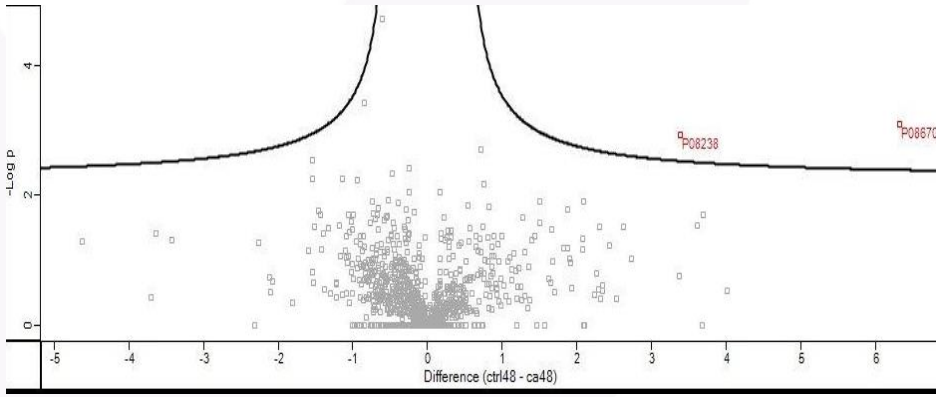






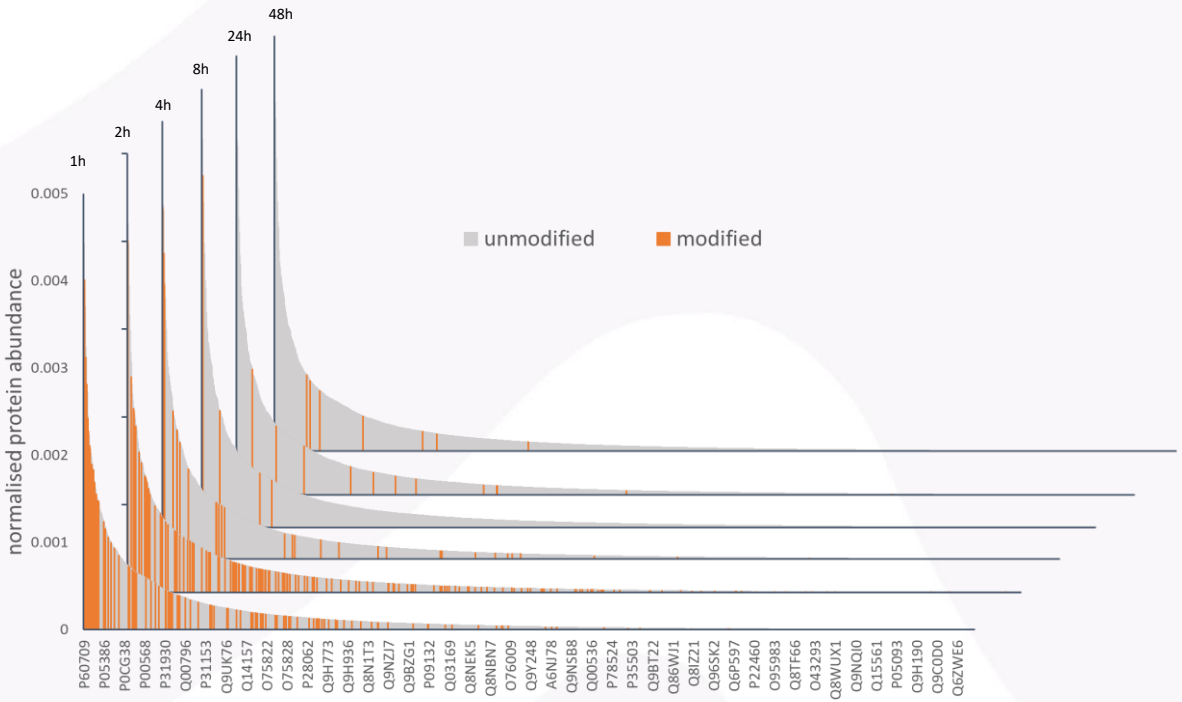




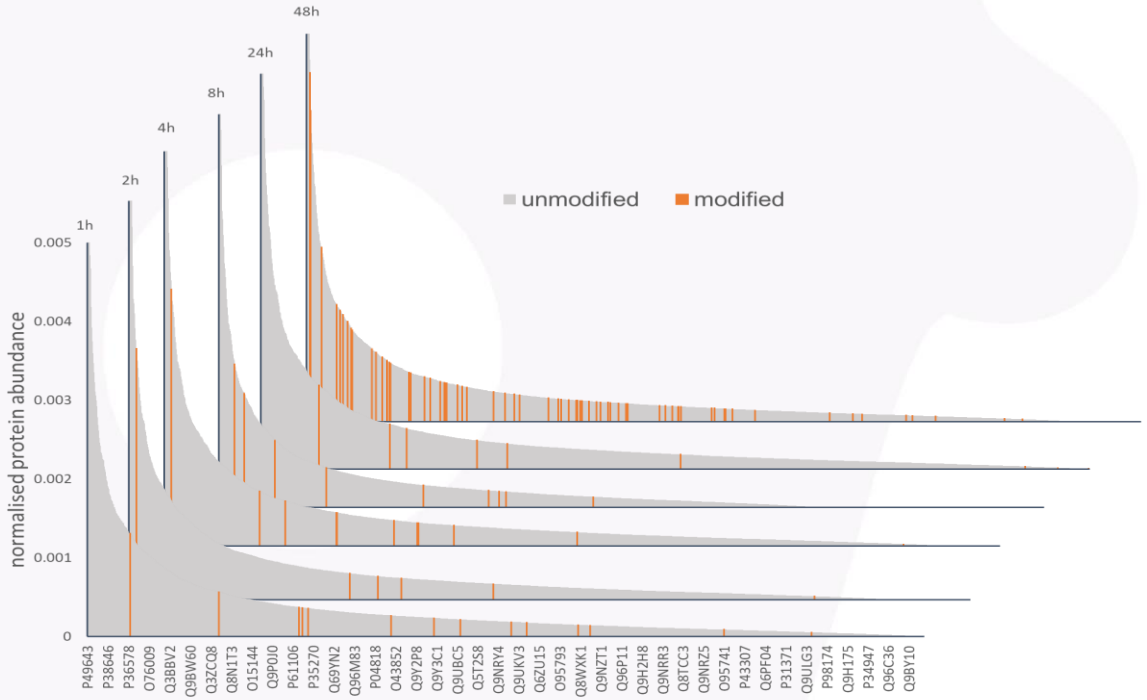


The Dynamics of Haptenation by DNCB and cinnamaldehyde in HaCaT cells

DNCB



Cinnamaldehyde



Parkinson et al (2020), Toxicology 445, pp1-10; 152603

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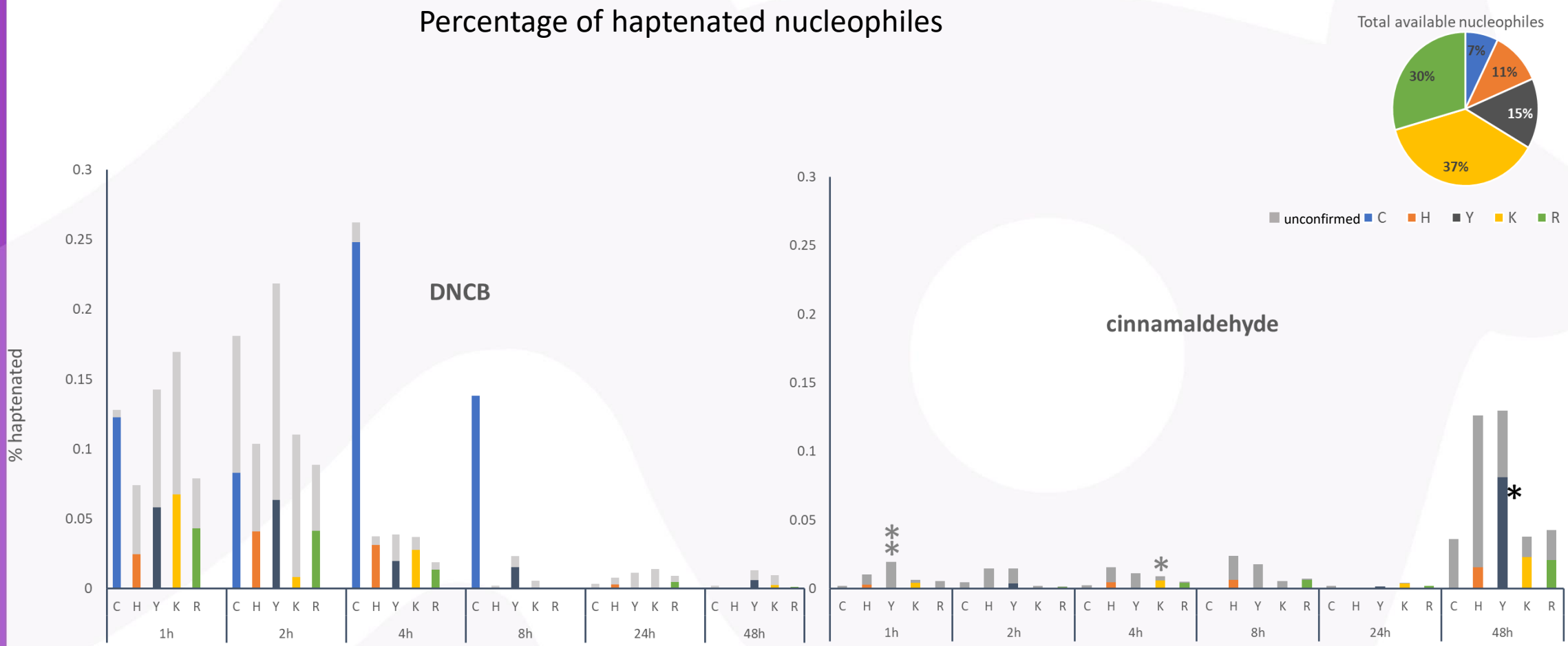
DNCB and cinnamaldehyde haptenation dynamics differ



Parkinson et al (2020), Toxicology 445, pp1-10; 152603

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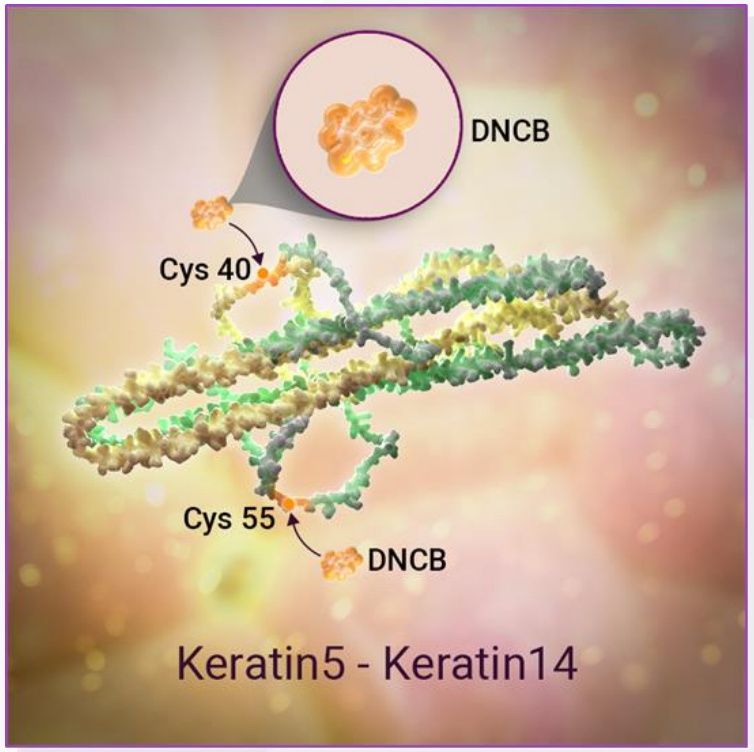
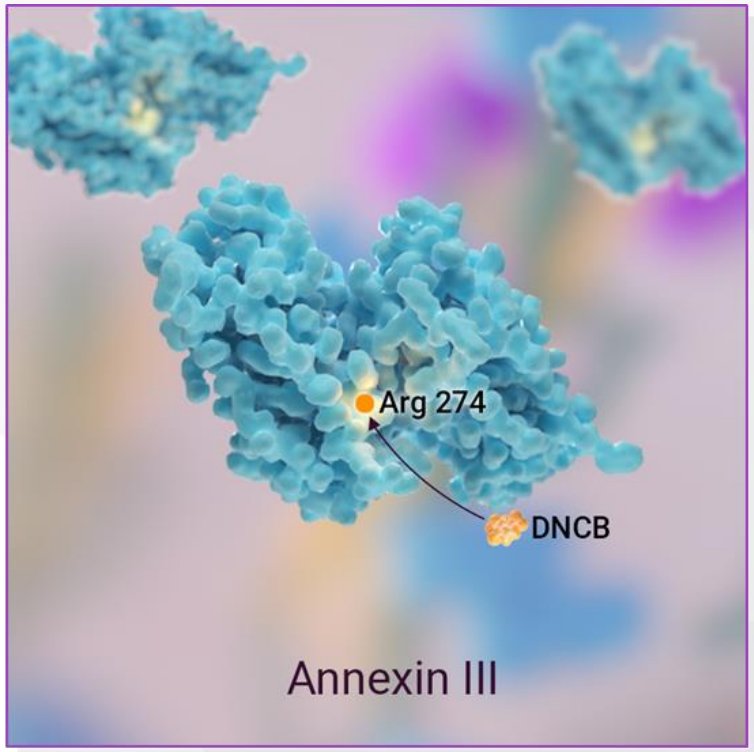
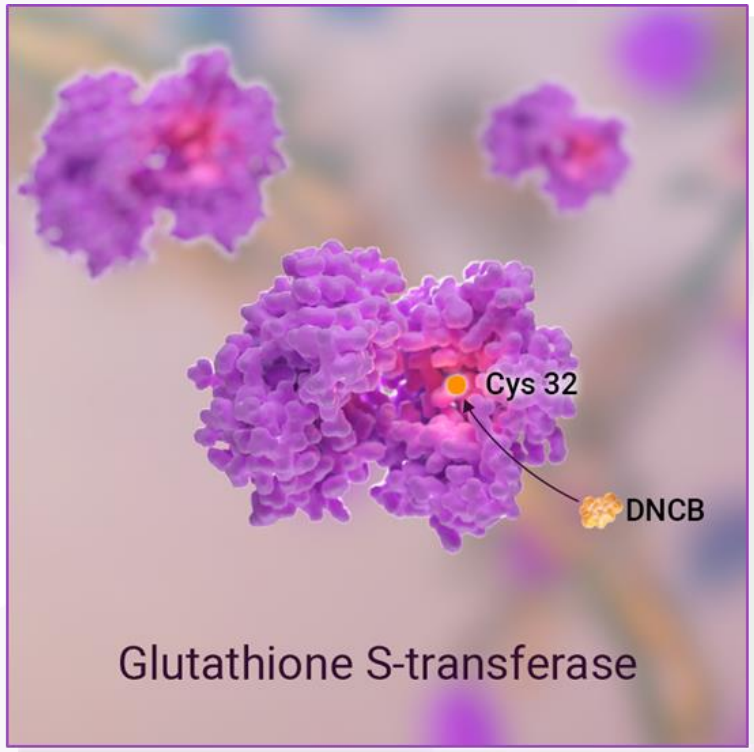
Typical DNCB haptenated proteins in HaCaT cells

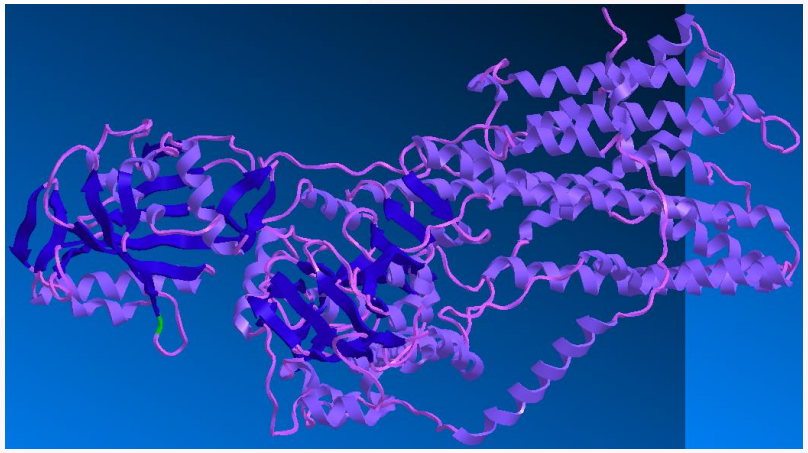




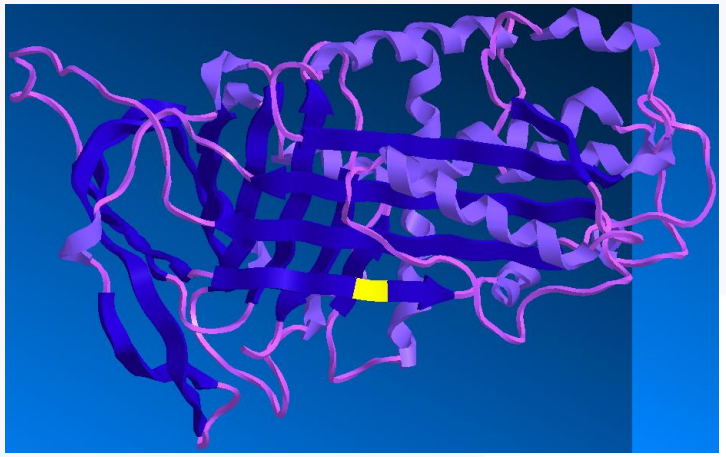


Imagery: NEXU Science Communication

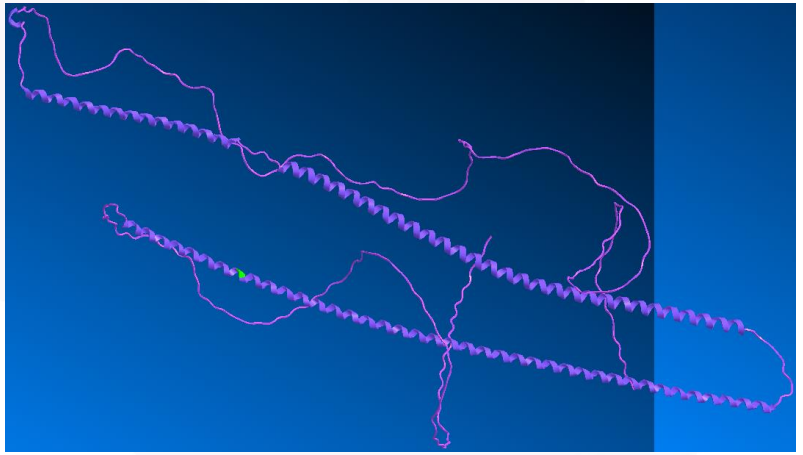
Cinnamaldehyde haptenated proteins in HaCaT cells



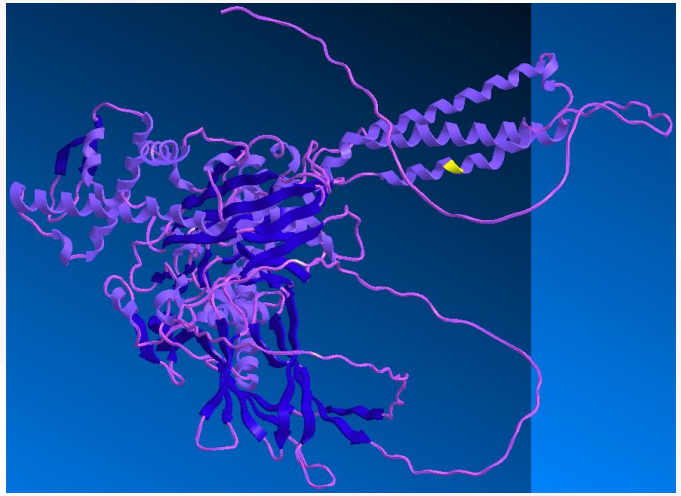
1h – Calcium transporting ATPase type 2C Schiff base @Lys490



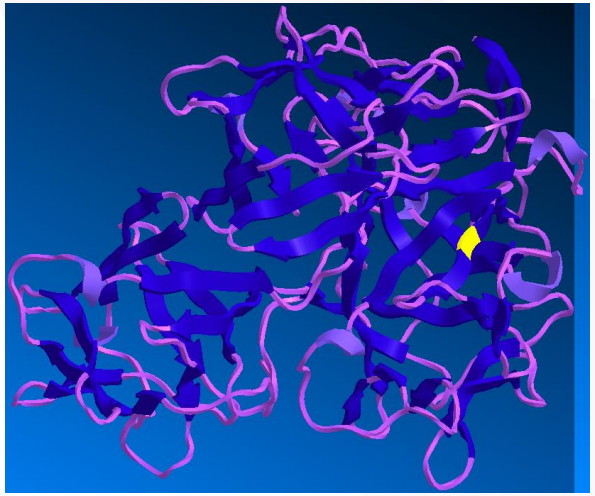
4h – Serpin B5 Schiff base @Lys280



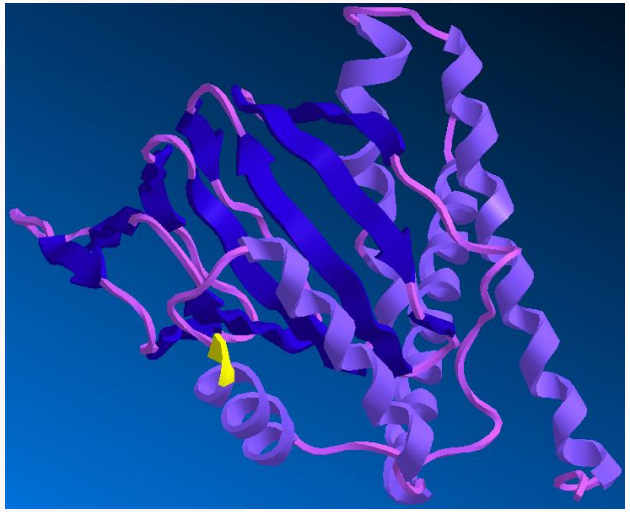
48h – K5 Schiff base @Tyr453



1h – Heat shock protein 70 kDa Schiff base @Lys776



48h – Fascin (actin binding) Michael addition @Tyr186



48h – Sigma non-opioid intracellular receptor Schiff base @Tyr49

Conclusions, future work in research and potential use in RA

- **Cinnamaldehyde shows different dynamic profile of haptenation in living HaCaT cells when compared to DNCB**
  - No overall change in differential protein expression for non-cytotoxic concentrations of either chemical
  - Level of haptenation by cinnamaldehyde lower than DNCB
  - DNCB haptenates Cys residues - no confirmed Cys adducts for cinnamaldehyde
  - DNCB haptenation peaks at 4h – cinnamaldehyde haptenation barely detectable at all timepoints except for 48h
- **Phase II metabolism – concomitant and likely faster than haptenation**
  - Can simple assays be developed to be used in addition to reactivity assays and improve our prediction of sensitising potency?
- **Are all haptenation events reversible?**
  - To what extent and can this be measured?
- **Assays do not have to be complicated to be useful in risk assessment!**

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Thank you:

SEAC, Unilever:

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Gavin Maxwell

University of Southampton:

Erika Parkinson
Scott Adams
Alex Lester
Paul Skipp

NexuCreative, Dublin:

Eoin Winston
Frank Munnelly

Thank you for your attention!

Questions?

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