

Does Ageing need Chemistry?

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Why Chemistry?

- **Synthesis** - more, better, faster
 - Synthesis gives mg to g scale
 - Analogues give different / improved activity
 - Ability to add to media or food directly
- **Analysis** - from titration to Mass Spectrometry
 - What is it and how much is there?

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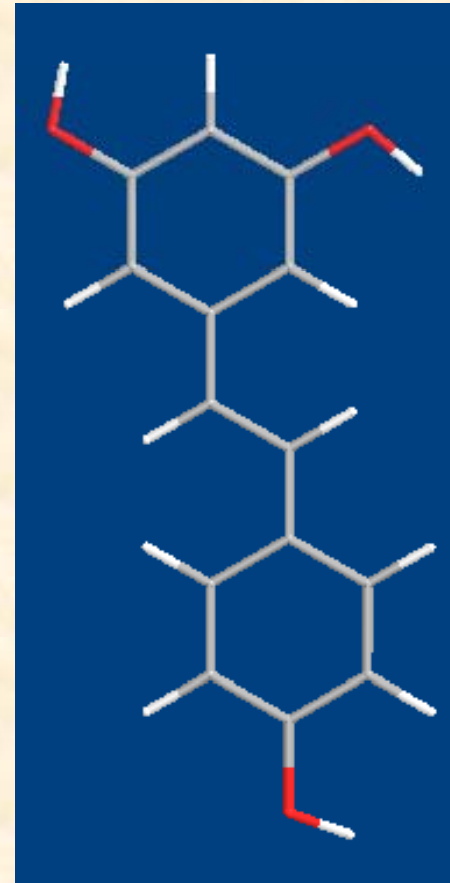
What can Chemistry do?

- Is resveratrol an anti-ageing compound?
 - Synthesis
 - In vitro* activity of Resveratrol and metabolites
 - Analysis
 - Resveratrol oxidation and isomerisation
- Why do fruit flies die?
 - Analysis
 - What molecular damage occurs as the animals age?

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Resveratrol

- Extends lifespan?
- SIRT1 activator
- DNA intercalator
- Topoisomerase inhibitor
- Antioxidant
- Pro-oxidant
- Antiviral
- Antiproliferative



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Is cytostasis anti-ageing?

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Resveratrol & primary cells

MRC5 fibroblasts, 10-100 μ M Resveratrol for 48h

- Effect on growth

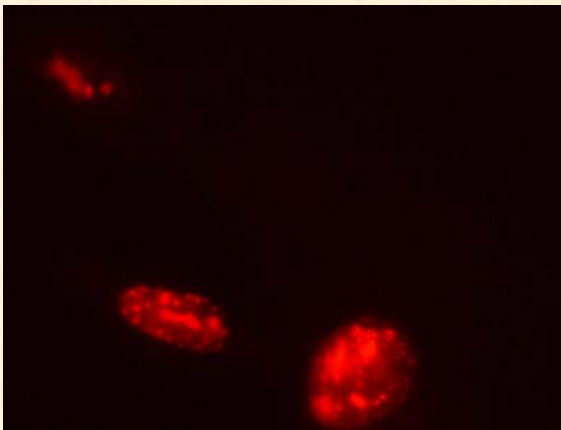
- Cells fixed and stained for pKi67
- Determine growth fraction

- Effect on phenotype

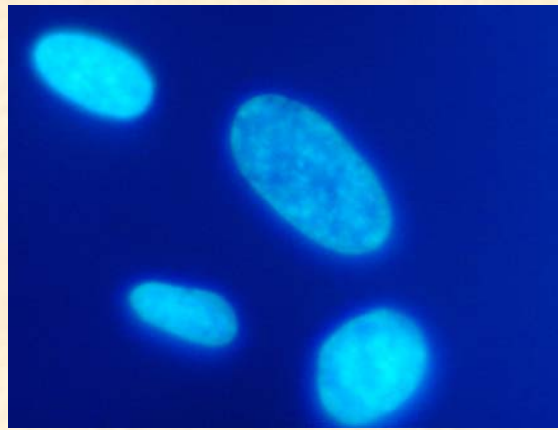
- Induction of senescence by SA- β -galactosidase staining

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Results



Ki67



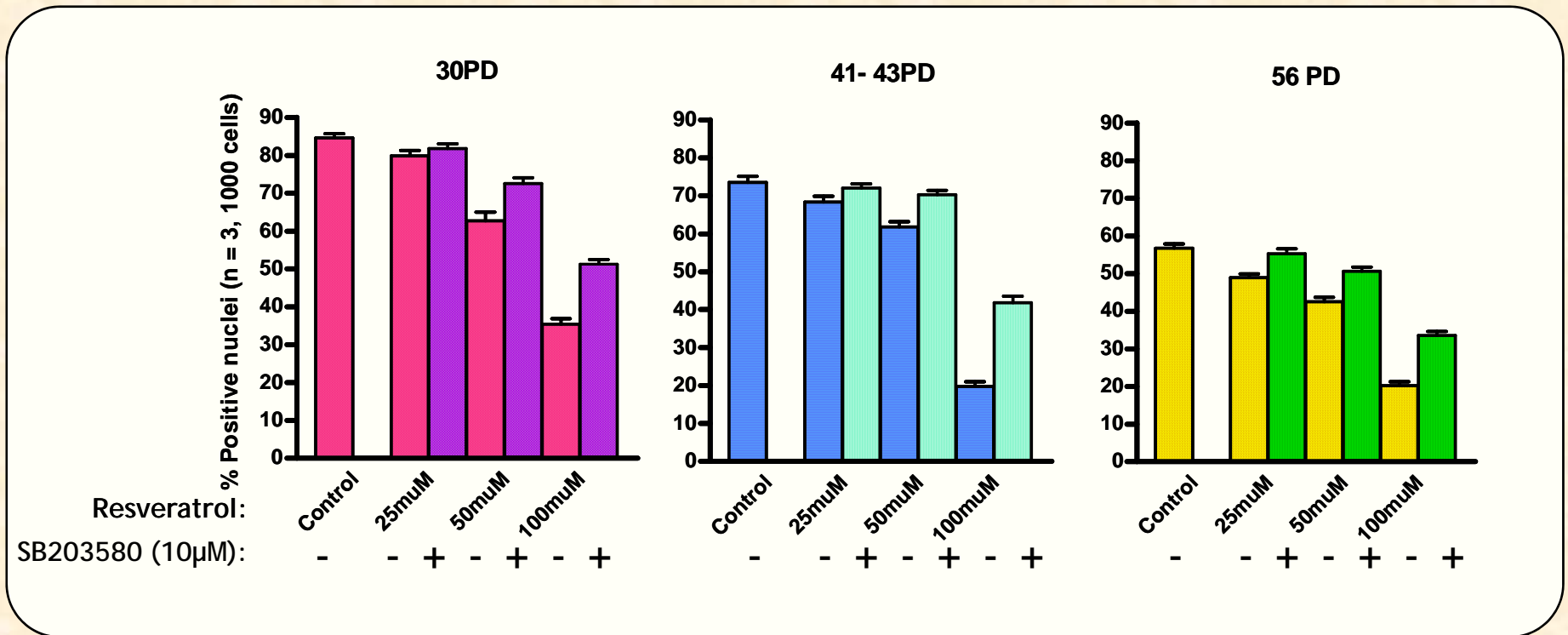
DAPI



SA-β-Gal

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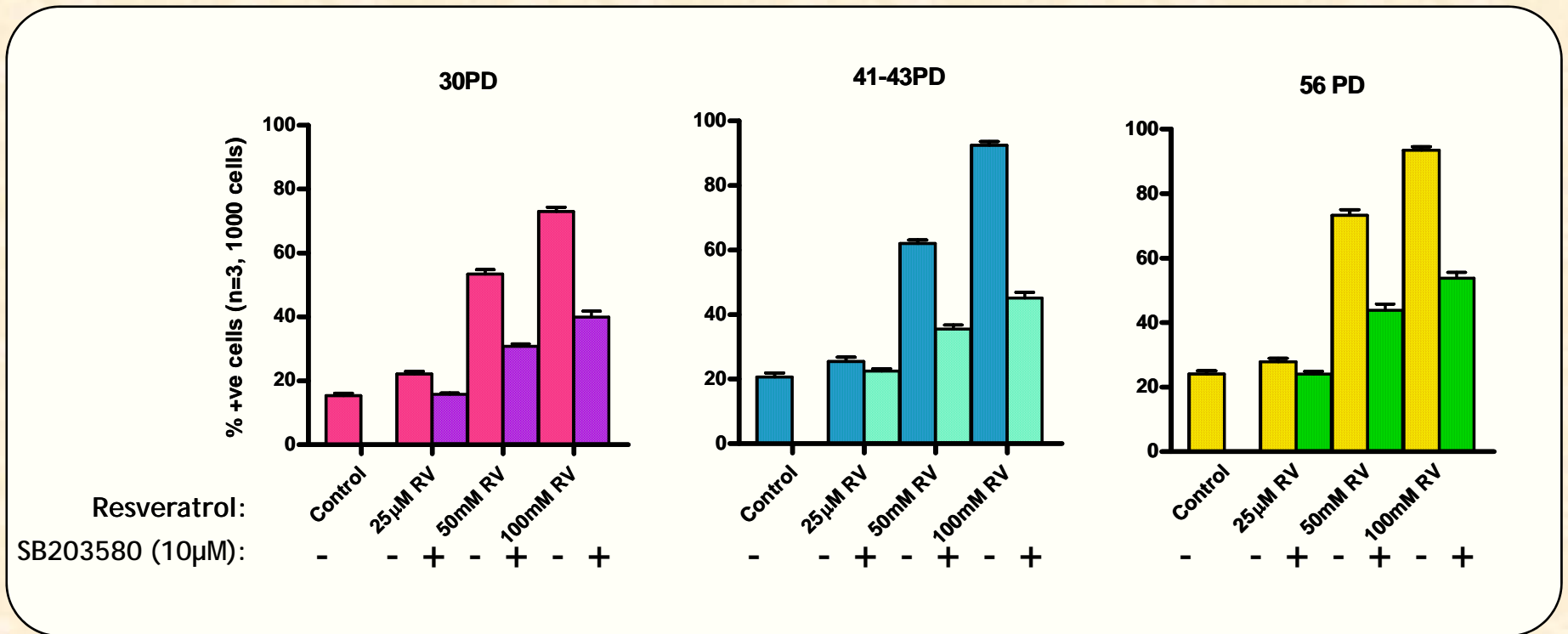
Resveratrol *in vitro*



Resveratrol induces cytostasis

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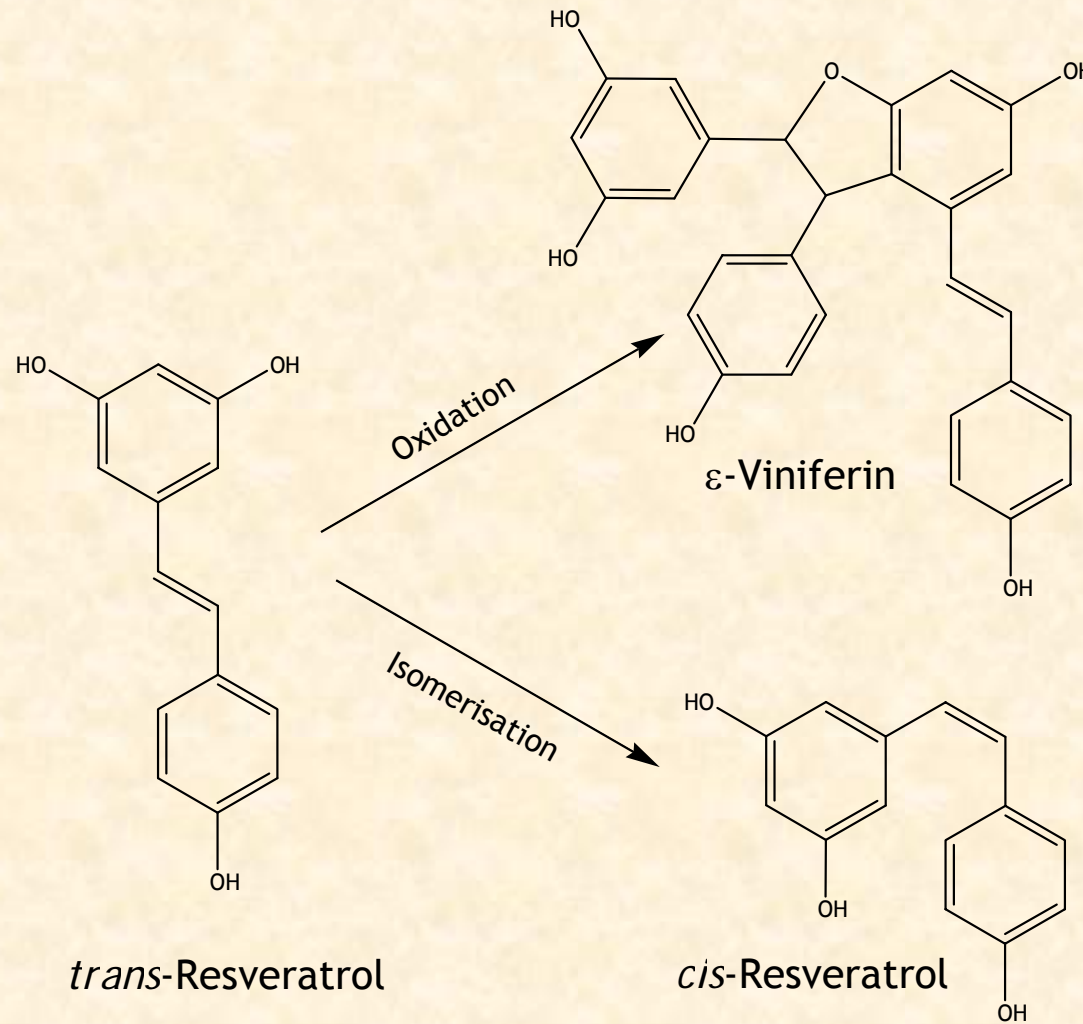
Resveratrol *in vitro*



Resveratrol triggers senescence

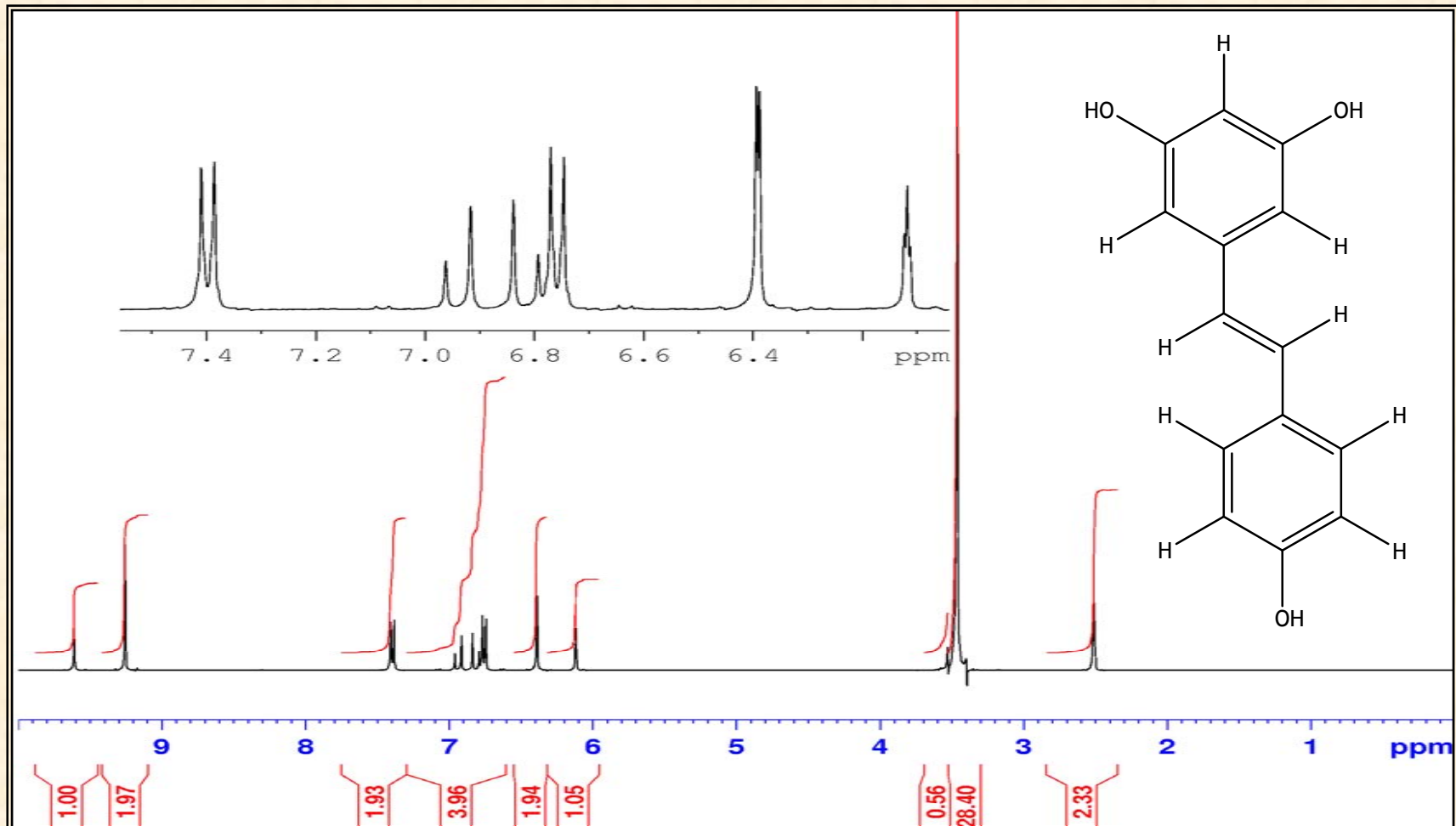
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Is it really Resveratrol?



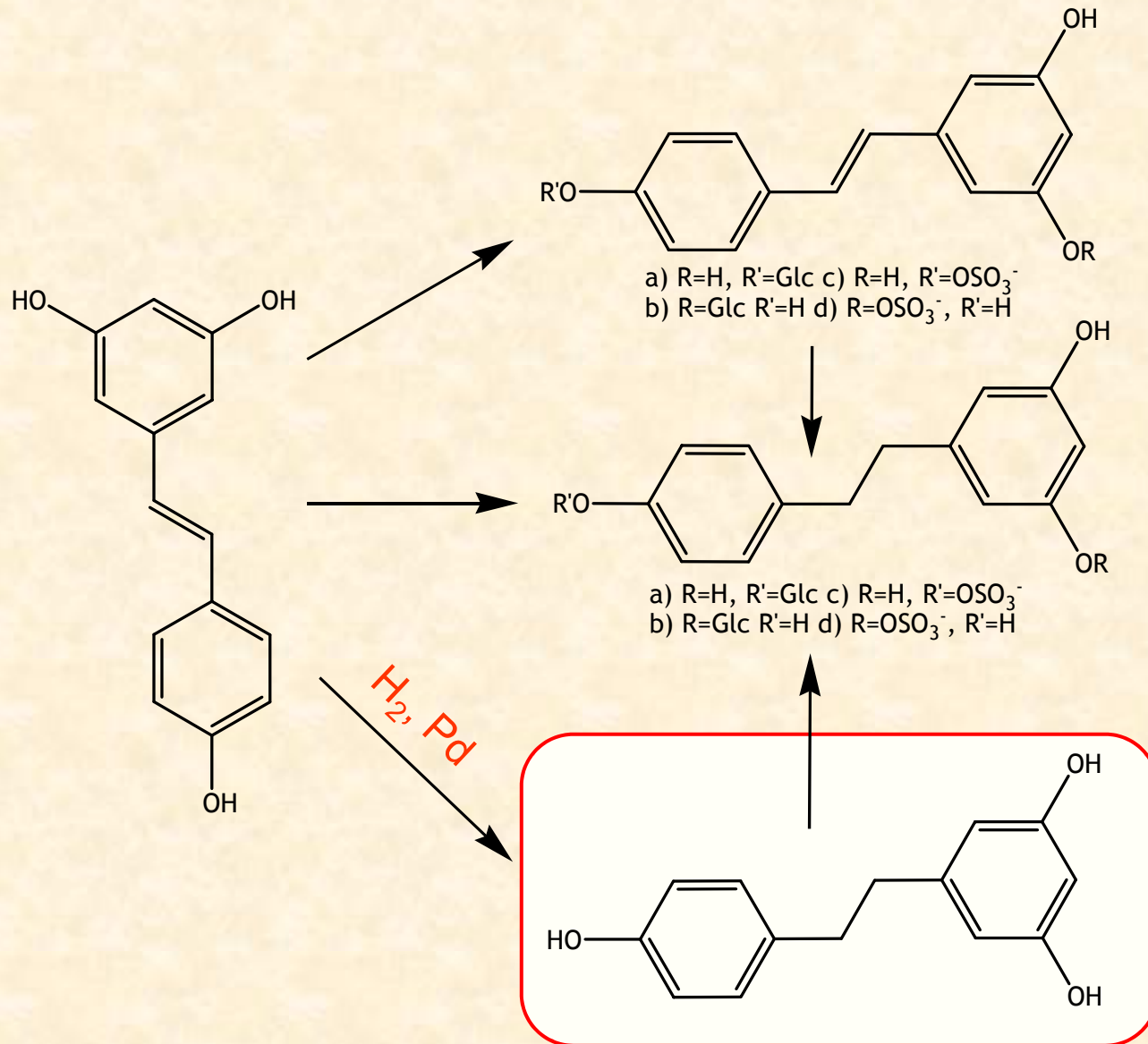
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Resveratrol ^1H NMR

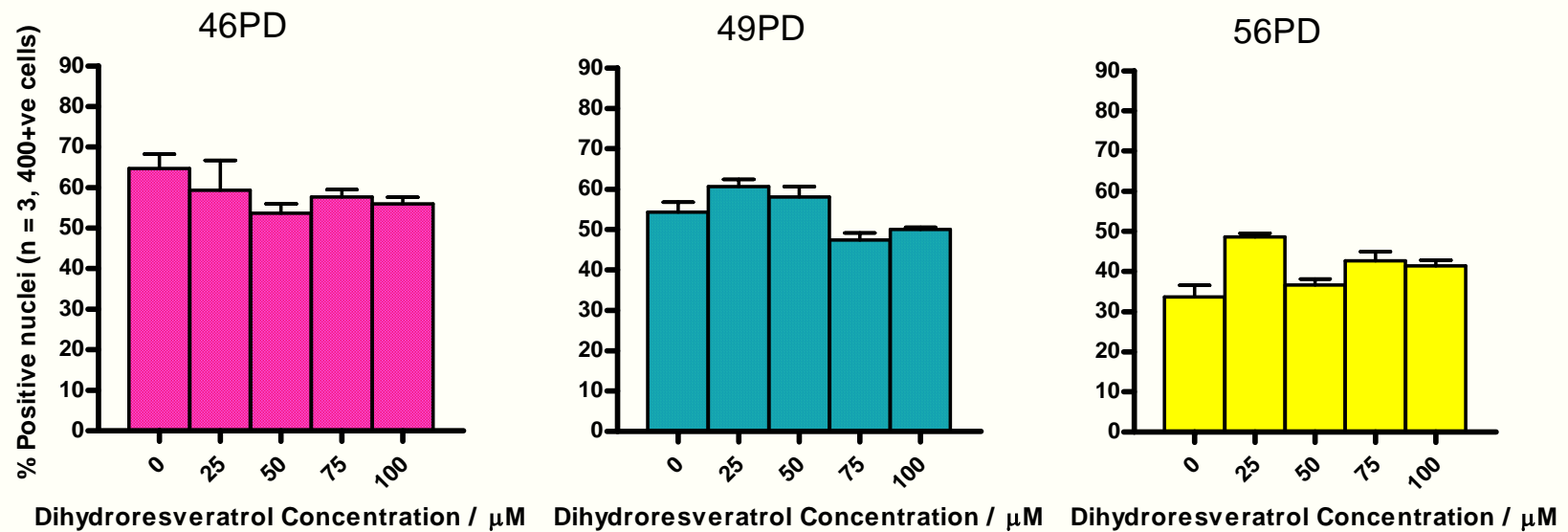


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Resveratrol metabolites



Dihydroresveratrol *in vitro*



Ki 67 staining

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Resveratrol - Conclusions

- Resveratrol induces p38 MAP kinase dependent senescence

 Phenocopy of Werner's Syndrome!

- Dihydroresveratrol is inactive
- Resveratrol must be acting through alternative mechanisms

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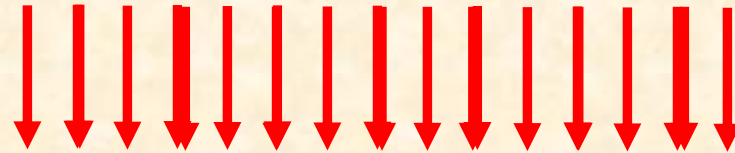


“Green” theory of Ageing

Undamaged molecules, healthy tissue

INCREASED TEMPERATURE

A B C D E F etc



REPAIR
PROCESSES

REPAIR
PROCESSES

Damaged molecules, dysfunctional tissue

DIETARY
RESTRICTION

DIETARY
RESTRICTION

AGEING

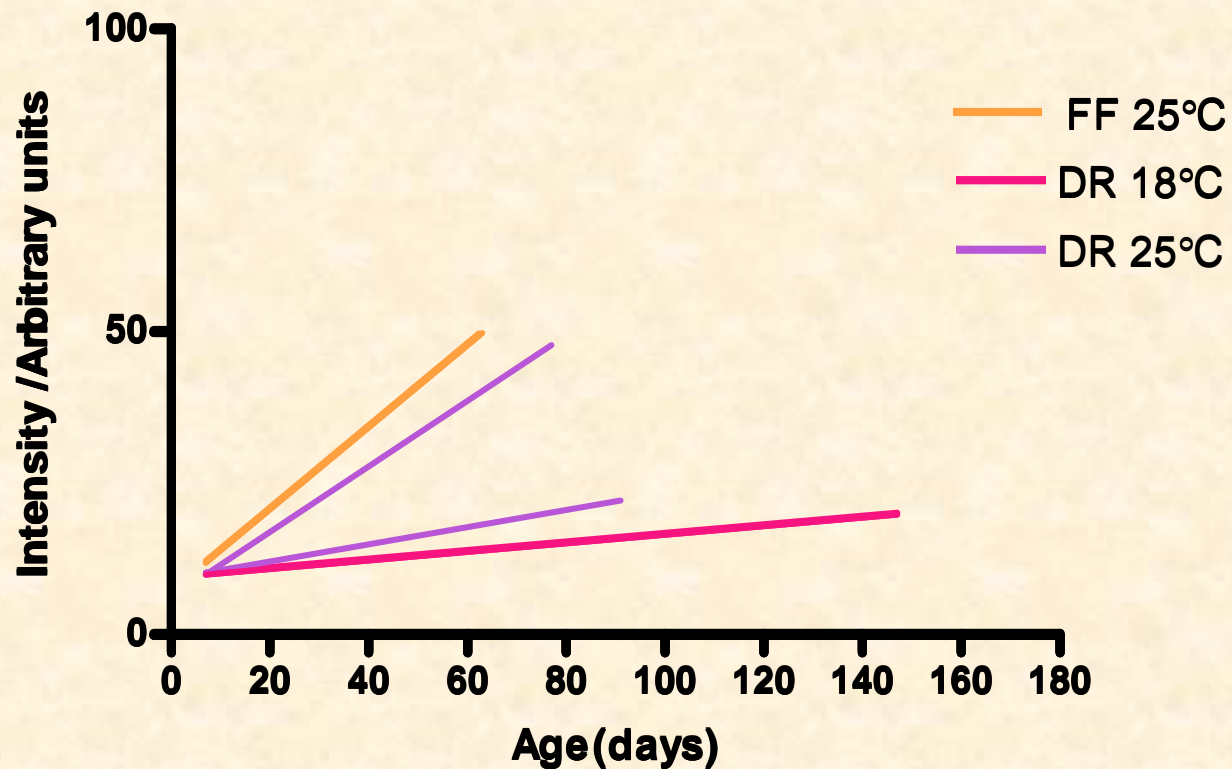
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D. Gems, JJ. McElwee, *Mech. Ageing. Dev.* (2005) 126: 381

Do flies recycle?

Predictions from green theory

Predicted trends in AGE fluorescence



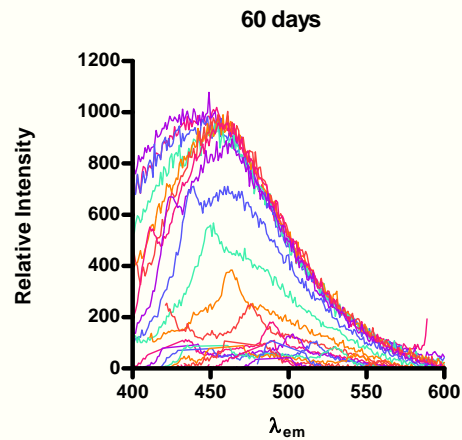
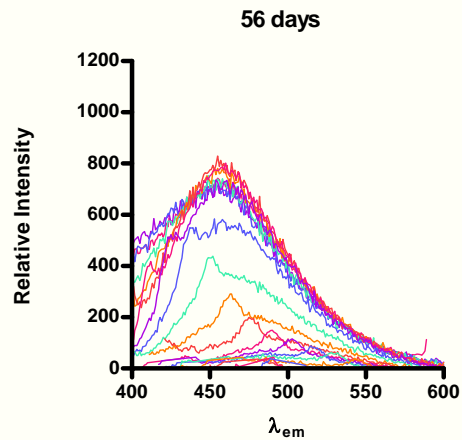
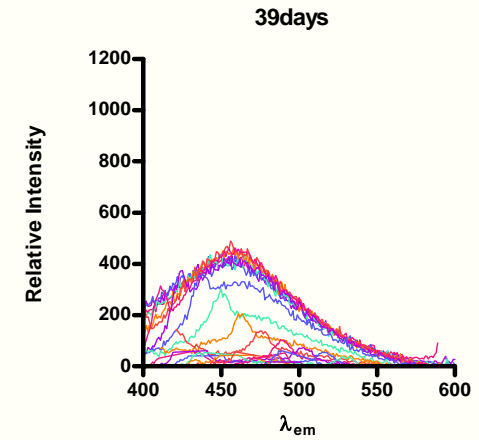
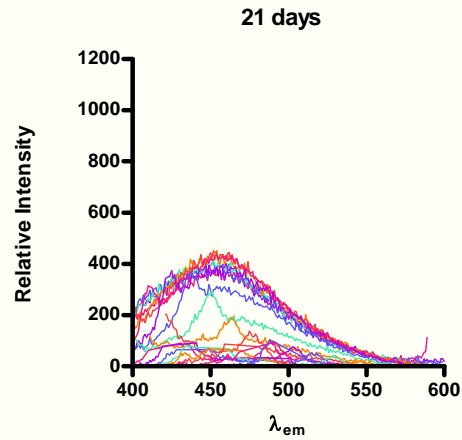
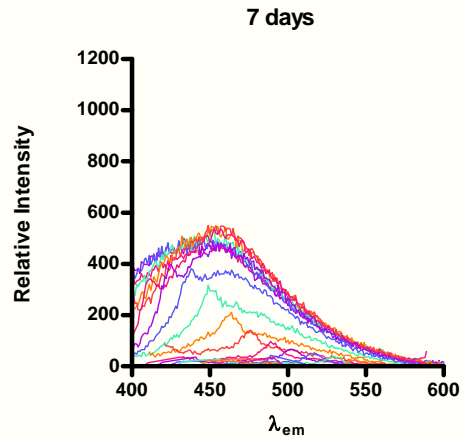
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Do fruit flies recycle?

- Chemical analysis of whole flies
- Variant of metabolomics
- Methods selected for AGE detection
 - Longitudinal data (7/14 day sampling)
 - Live flies collected, frozen & digested
 - Spectrophotometric, NMR and MS analysis
- Age-related signals identified
- Effect of lifespan modifications assessed

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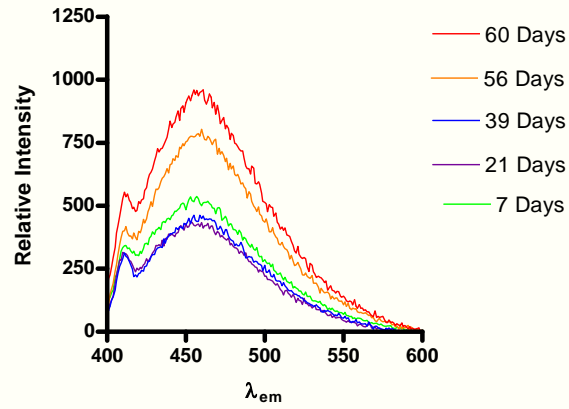
Fluorimetry : 3D scans



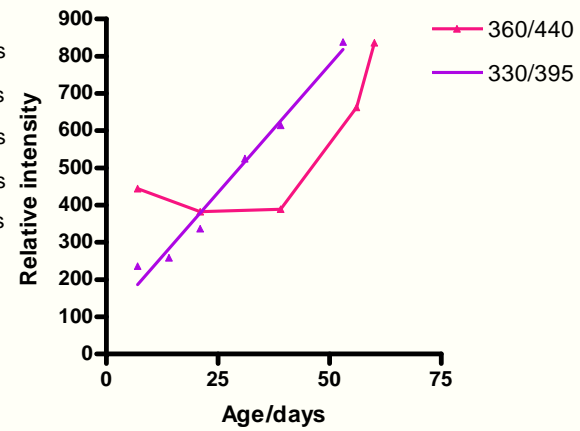
- | | |
|-----------|-----------|
| EX_300.00 | EX_410.00 |
| EX_310.00 | EX_420.00 |
| EX_320.00 | EX_430.00 |
| EX_330.00 | EX_440.00 |
| EX_340.00 | EX_450.00 |
| EX_350.00 | EX_460.00 |
| EX_360.00 | EX_470.00 |
| EX_370.00 | EX_480.00 |
| EX_380.00 | EX_490.00 |
| EX_390.00 | EX_500.00 |
| EX_400.00 | |

Fluorimetry : AGE wavelegnth

Graph of relative emission intensities versus wavelength for excitation at 360nm

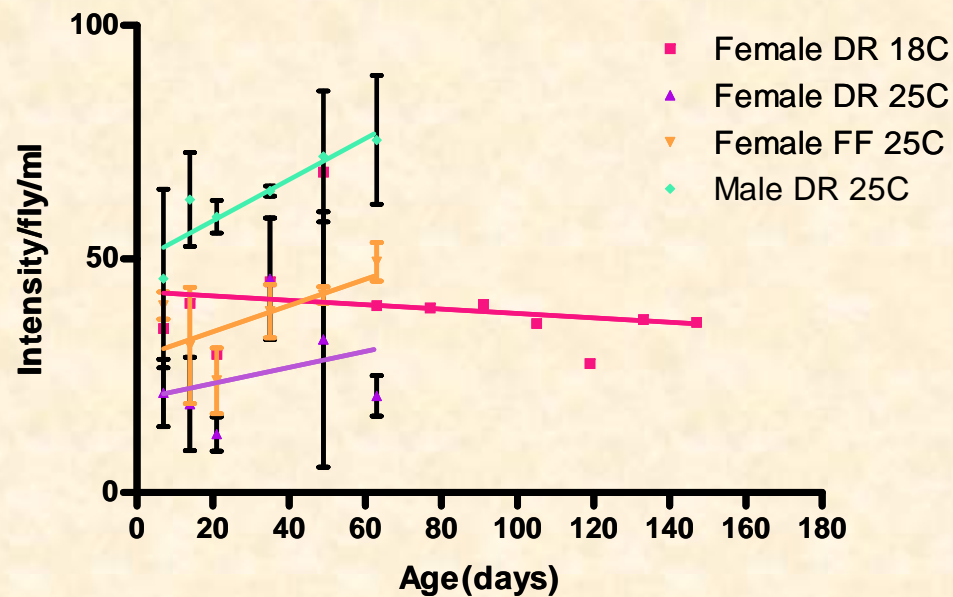


Graph of Fluorescence intensity vs Age



Fluorimetry : Effect of T and DR

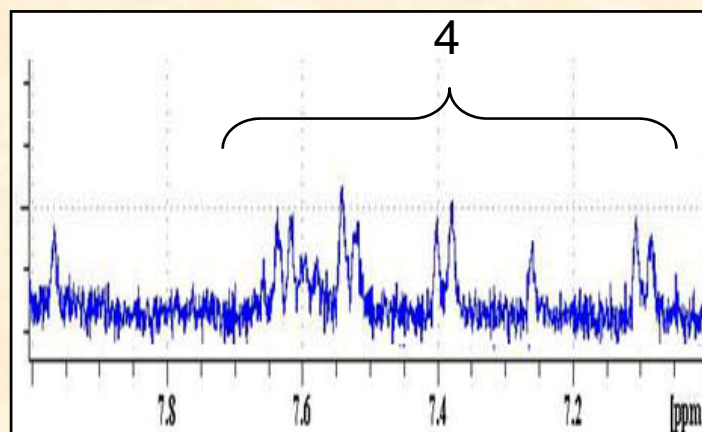
Fluorescence intensity at $\lambda_{ex} = 360\text{nm}$, $\lambda_{em} = 430\text{nm}$ vs Age



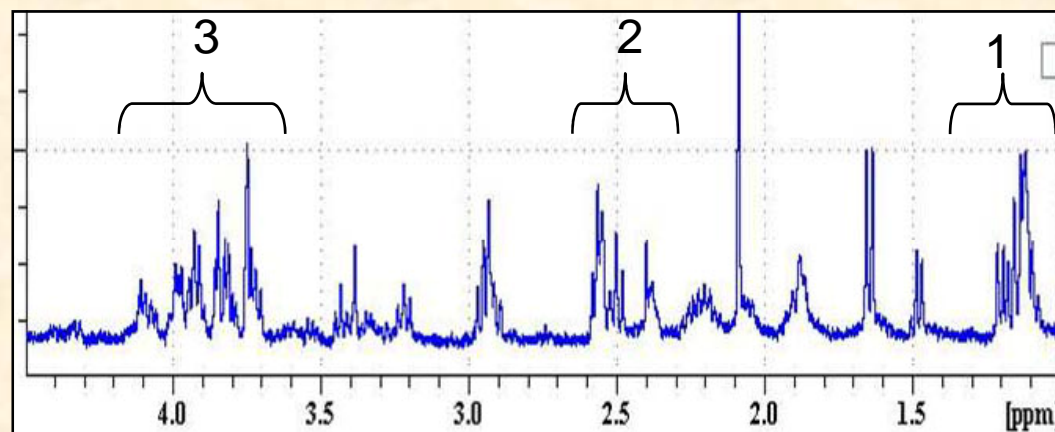
- Significant increases in AGE-associated fluorescence over lifespan for: a) fully fed b) male (even when DR)
- No increase in DR flies, although 25° C scattered

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^1H NMR (1)



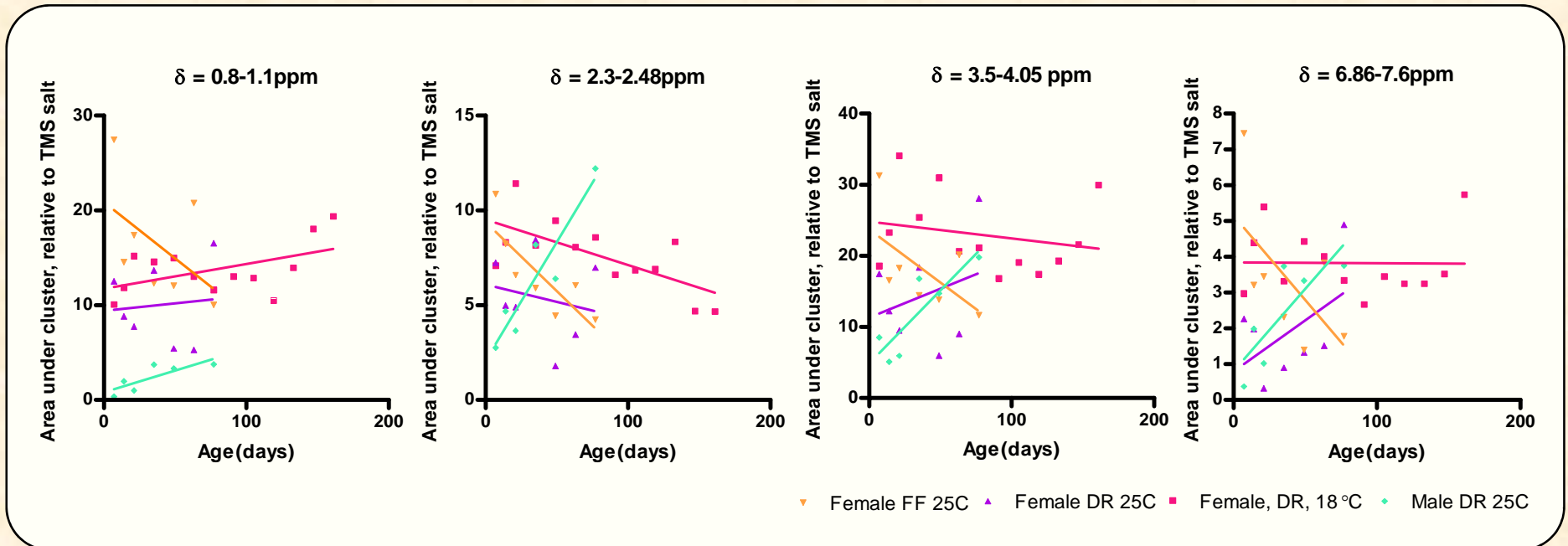
Aromatic Region



Benzylic Region

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^1H NMR (2)



- Male DR, 25°C upward trend all clusters ($p < 0.05$)

- $\delta = 2.3-2.48\text{ppm}$

| Conditions | Female FF, 25°C | Female DR, 25°C | Female DR, 18°C | Male DR, 25°C |
|-------------|-------------------|--------------------|---------------------|-------------------|
| Slope | -0.07 ± 0.022 | -0.018 ± 0.039 | -0.024 ± 0.0077 | 0.125 ± 0.025 |
| Y-intercept | 9.4 ± 1.0 | 6.1 ± 1.7 | 9.5 ± 0.71 | 2.1 ± 1.0 |

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Do flies recycle? - Conclusions

Analytical signals from acid-stable compounds show:

- Slower rate of change for DR than FF
- More scatter for 25°C than 18°C - treadmilling
- DR males behave like FF females, correlating with male lifespan unchanged by DR

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Acknowledgements

Glycation in Drosophila

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Resveratrol

Angela Sheerin

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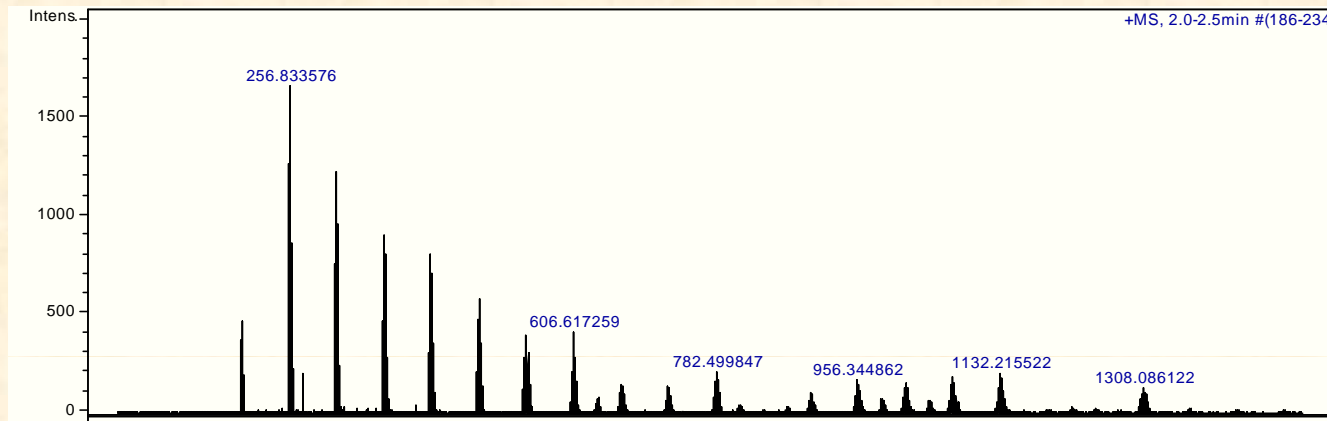
Noel See-Yau Fong

Funders

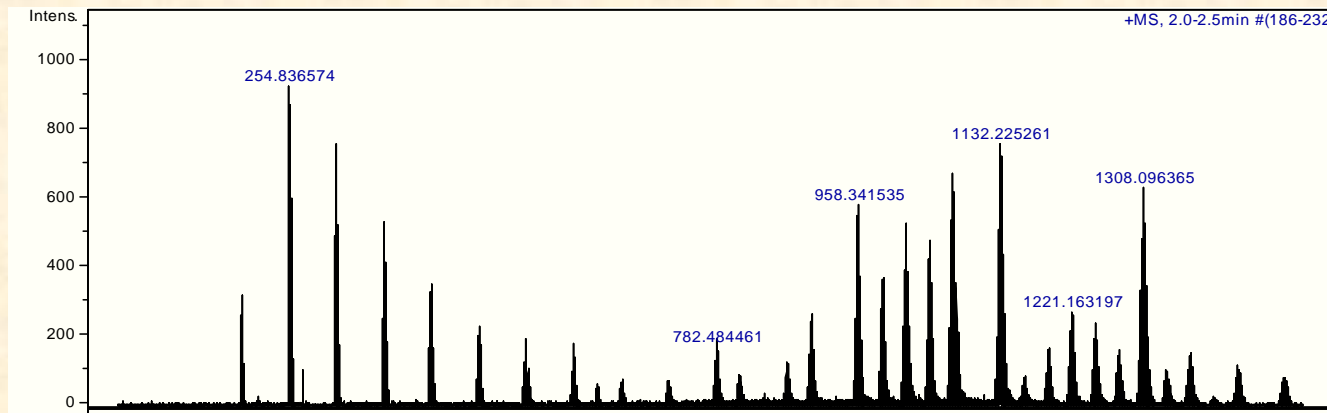


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Mass spectrometry



Typical mass spectrum of digest of young (7 days) flies



Typical mass spectrum of digest of old (133 days) flies

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