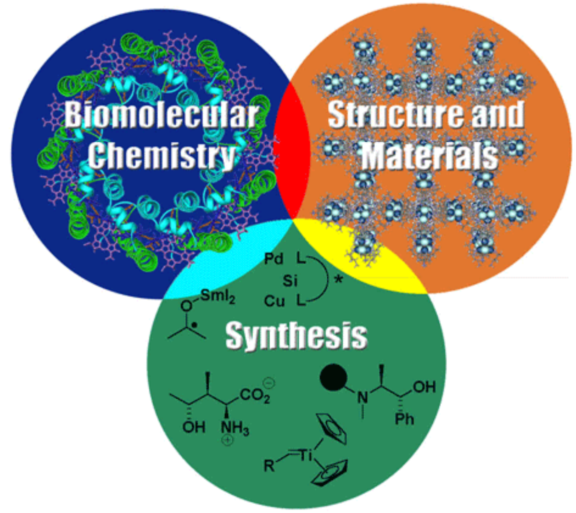
University of Glasgow

VIA VERITAS VITA



Chemical Biology: *Oxidative Processes of Ageing and the Potential of Antioxidants*

37th Annual Meeting of American Aging Association
Boulder, Colorado, 31st May 2008

Dr Richard C. Hartley
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WestCHEM

Chronological and Biological ageing do not always equate…..

57

43

61

52

The Root of the Problem

- Oxidative Stress leads to Damage
- Damage decreases ability to deal with further stress
- Responsible for cellular ageing
- Causes or exacerbates diseases of old age
  - neurodegeneration
  - stroke
  - heart disease
  - diabetes

Reactive Oxygen Species

Couple (Oxidant / Antioxidant)	E^{O} / mV	
$HO^{\bullet}$, H^+ / H_2O	2310	**Highly oxidising**
$RO^{\bullet}$, H^+ / ROH	1600	
$HOO^{\bullet}$, H^+ / H_2O_2	1150	↓
$ROO^{\bullet}$, H^+ (lipid peroxyl radical) / ROOH	1060	
$O_2^{\bullet -}$, $2H^+$ (superoxide anion) / H_2O_2	940	
$PUFA^{\bullet}$, H^+ / PUFA-H (polyusaturated fatty acid)	600	
α-tocopheroxyl$^{\bullet}$, H^+ / α-tocopherol (Vit E)	500	
$Ascorbate^{\bullet}$, H^+ / ascorbate monoanoin (Vit C)	282	**Highly reducing**

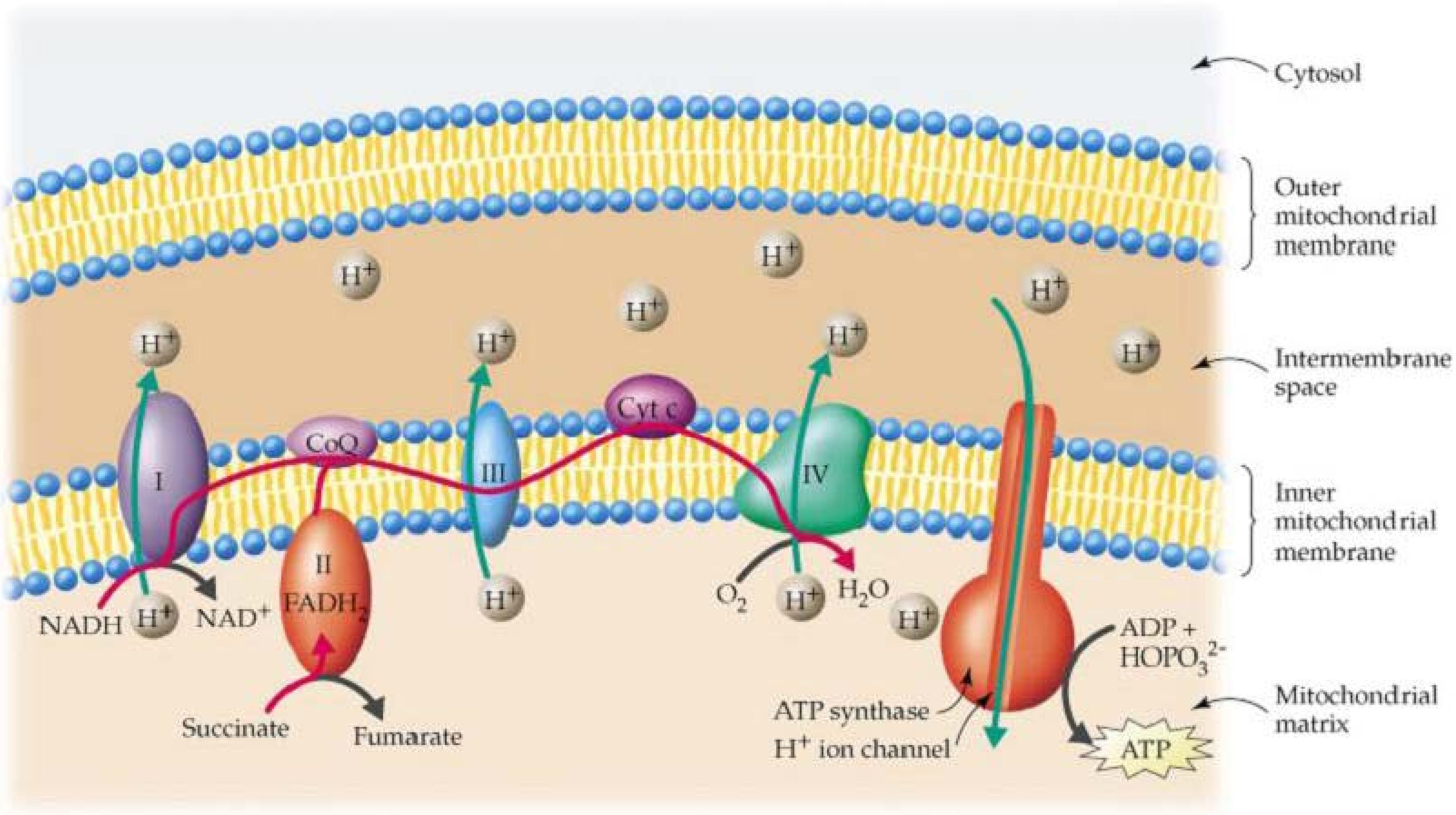
Buttner, G. *Arch. Biochem. Biophys.*, **1993**, 535

Cellular Ageing (Senescence):

- Damage to genes and the biological clock (telomeres)

- Damage to membranes
- Damage to metabolic capacity - oxidative damage to mitochondria

Reactive Oxygen Species
– produced in mitochondria
– damage mitochondria including mitochondrial DNA



Chemical Biology

Detect and identify the contributors to oxidative stress

- EPR spectroscopy - the only technique that sees radicals directly

Locate and quantify the oxidizing species

- Probes targeted to Mitochondria

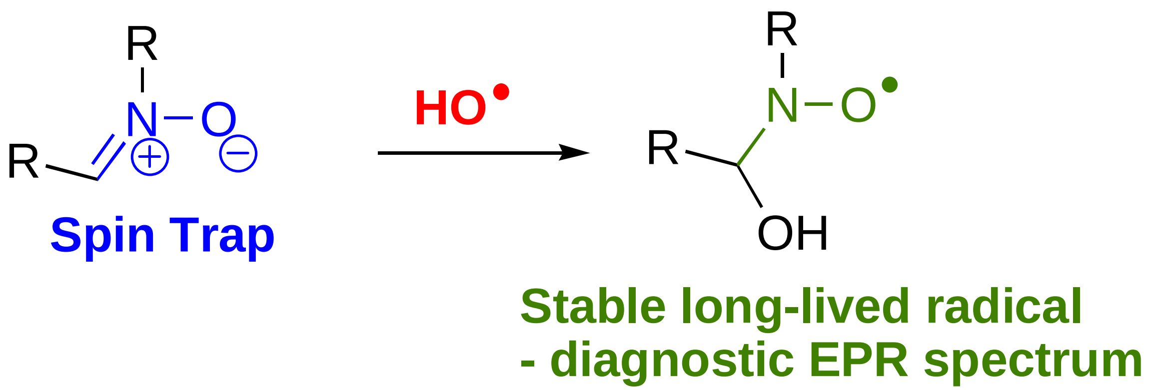
Ameliorate oxidative stress

- therapeutic antioxidants that scavenge ROS:

Track compounds claimed to be good for you

- Assess whether antioxidants found in food can be biological antioxidants *in vivo*.

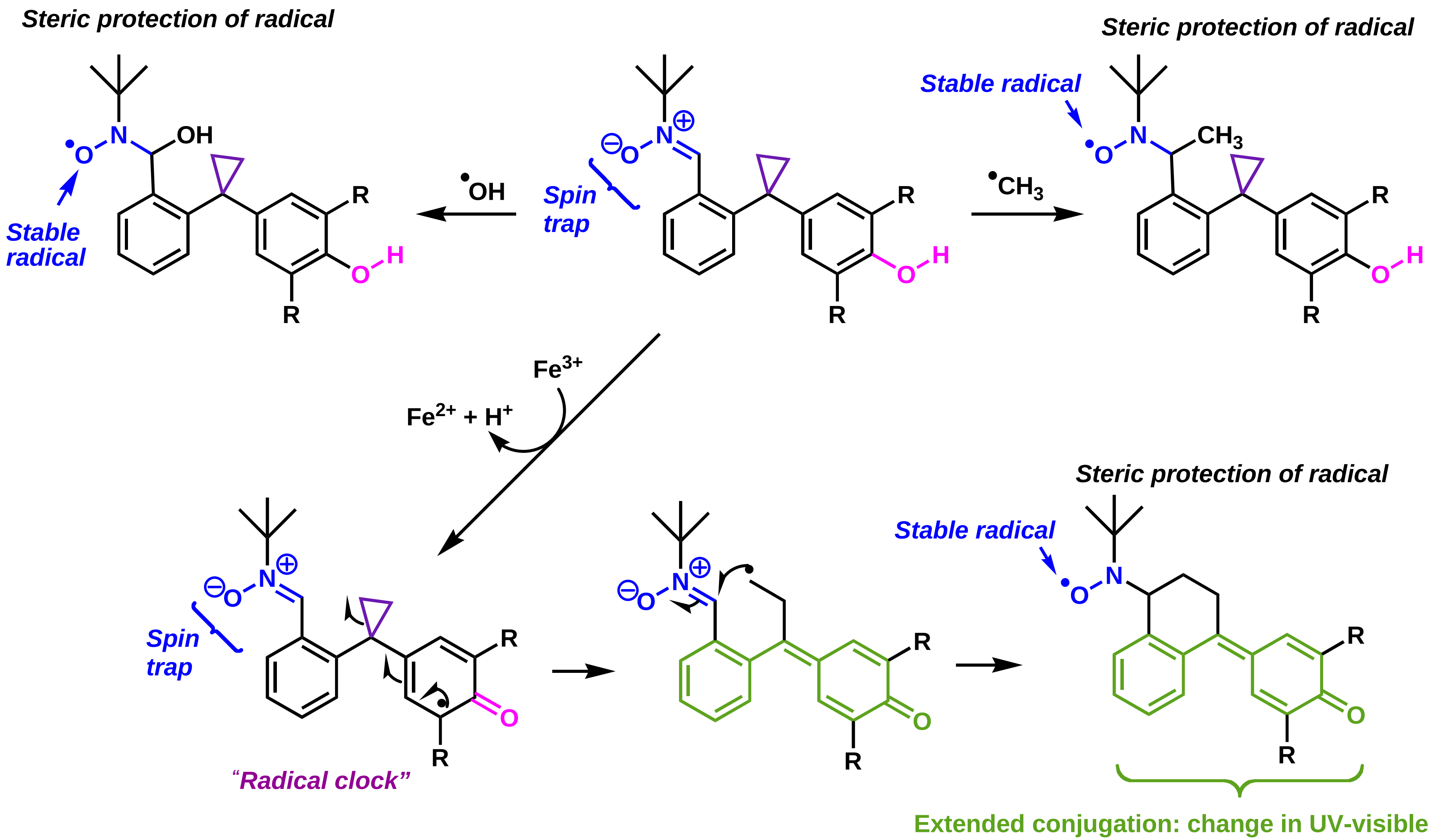
Detect and Identify ROS: Spin traps and EPR Spectroscopy.



Detects ROS:
In vitro and in vivo
Only sees the radicals

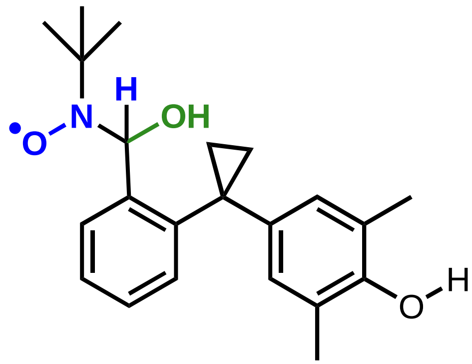
How can different contributors to oxidative stress be detected selectively?

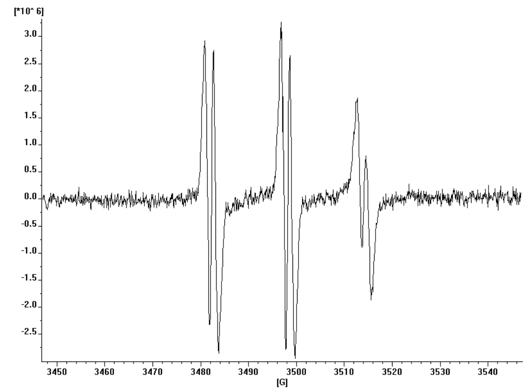
Differential Radical Detection and Double Display



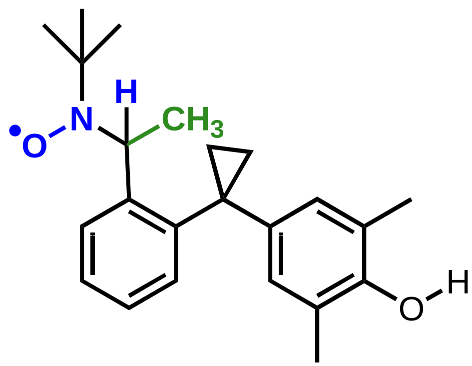
Caldwell, S. T.; Quin, C.; Edge, R.; Hartley, R. C. *Org. Lett.* **2007**, *9*, 3499-3502

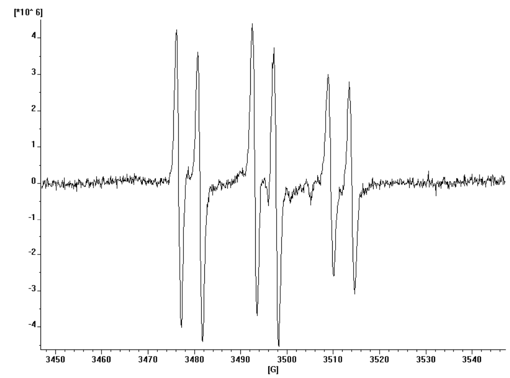
Trapping carbon-centred and oxygen-centred radicals



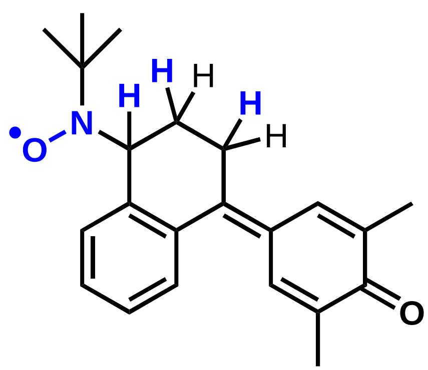


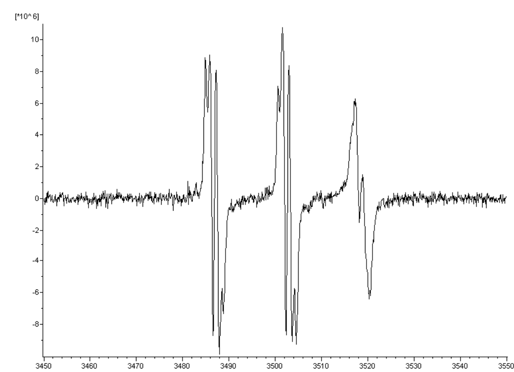
g = 2.0059
A_N = 15.93 G (t)
A_H^{β} = 1.85 G (d)





g = 2.0056
A_N = 16.32 G (t)
A_H^{β} = 4.57 G (d)





g = 2.0055
A_N = 15.70 G (t)
A_H^{β} = 1.42 G (d)
$A_H^{\gamma/\delta}$ = 1.05 and 0.99 G (dd)

Locate and quantify ROS:

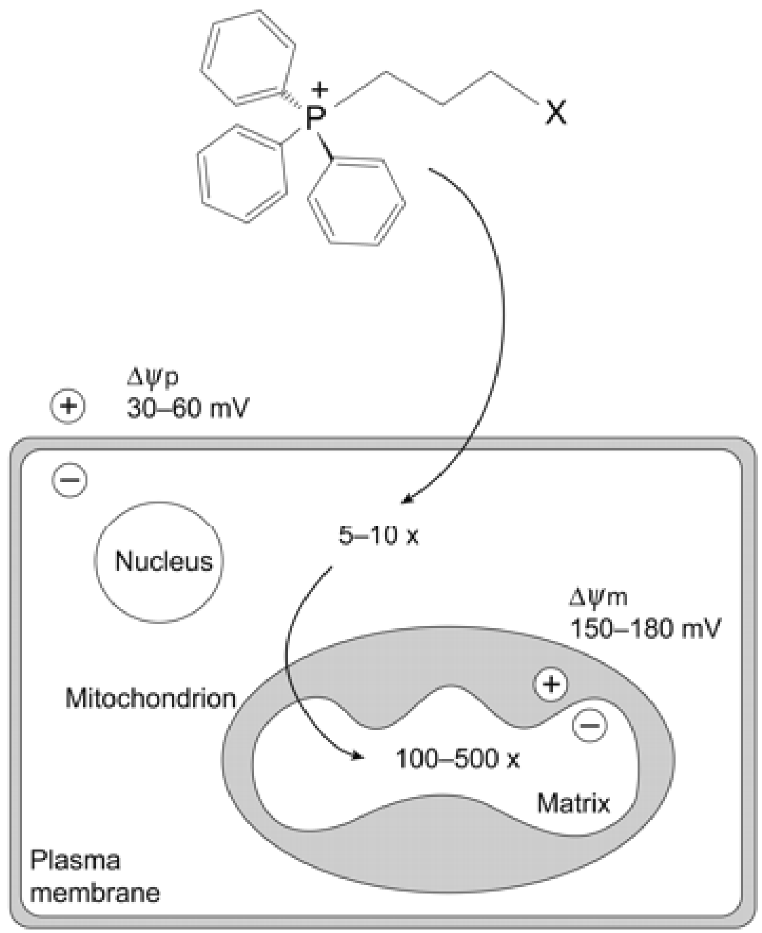
Target Mitochondria

- *Main source of ROS*

- *Mitochondrial dysfunction involved in*
  - *neurodegenerative diseases*
  - *ageing.*

See: Lin, M. T.; Beal, M. F. *Nature* **2006**, *443*, 787-795

Use membrane potential:



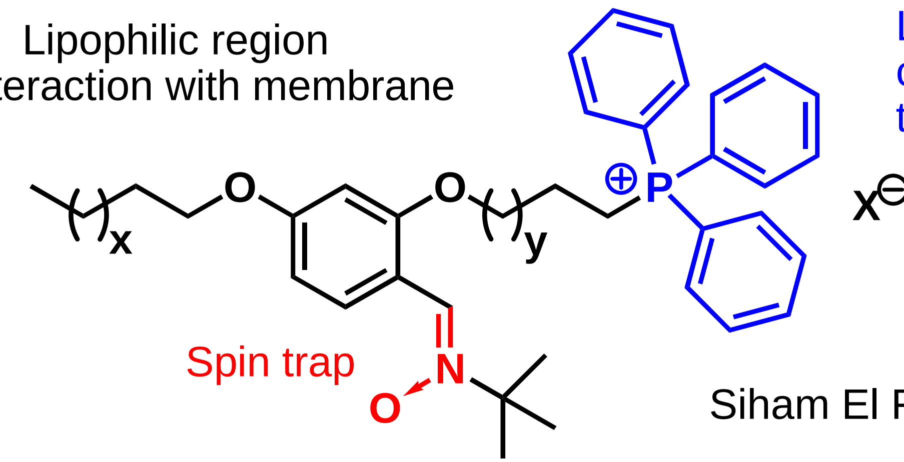
(a) pH within the matrix about 1.4 units lower than in the cytosol

(b) Large membrane potential ($\Delta\psi$) of –150 to –180 mV (negative inside the matrix) across the inner membrane of mitochondrion.

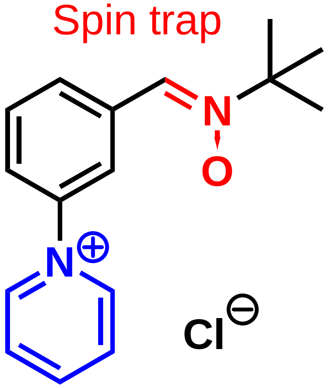
(c) Both contribute to Δp so that this is about 230 mV in active mitochondria.

See: Murphy, M. P. et al. *J. Biol. Chem.* **2003**, 278, 48534-48545

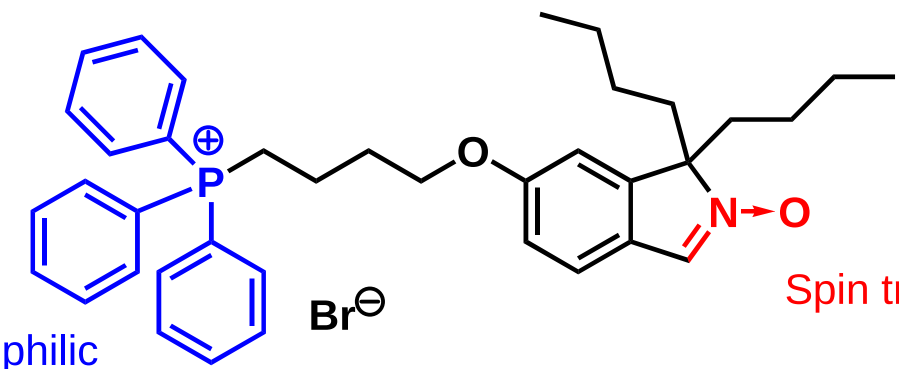
Lipophilic cations:
radical detectors/antioxidants targeted to mitochondria



Siham El Fangour

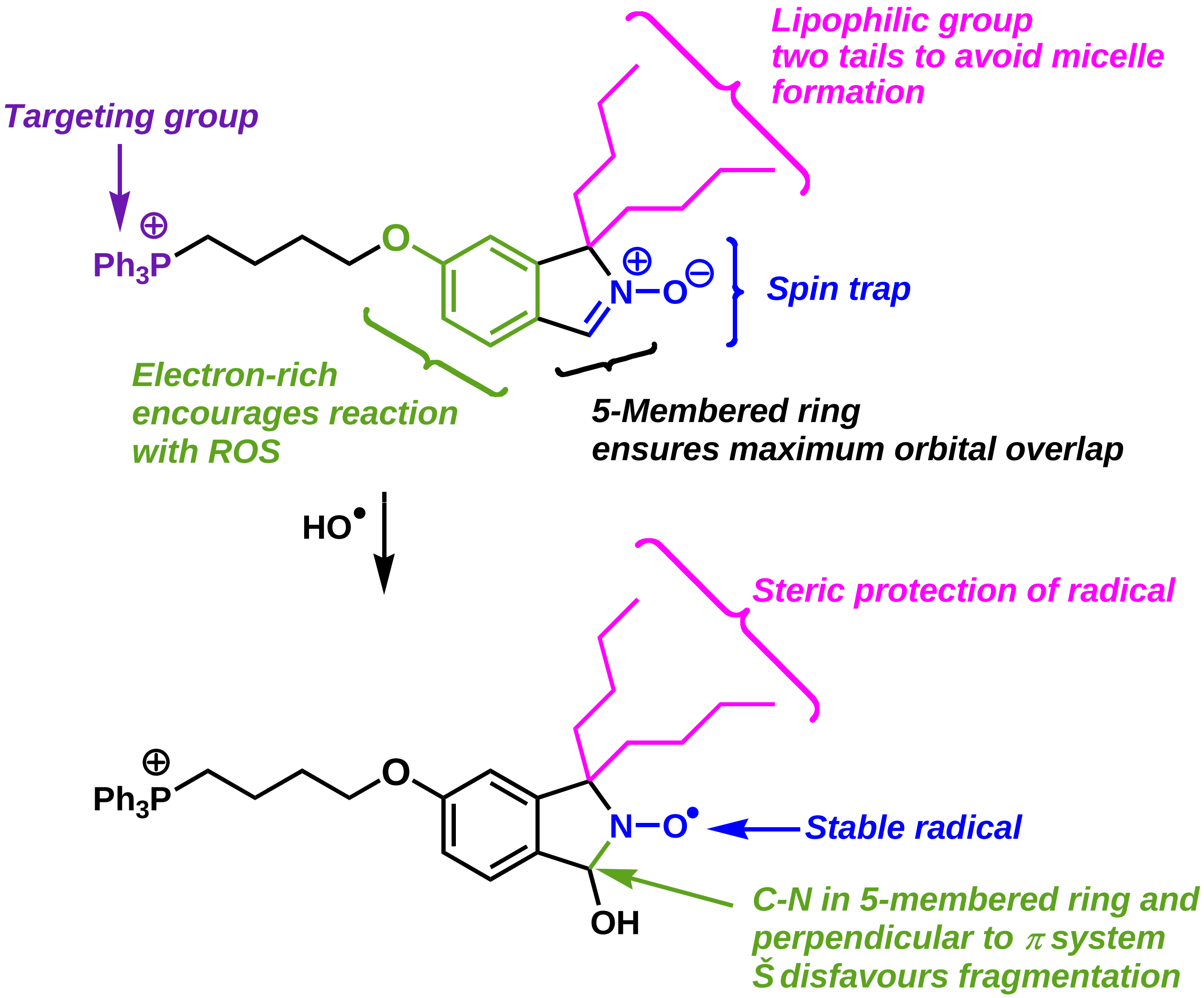


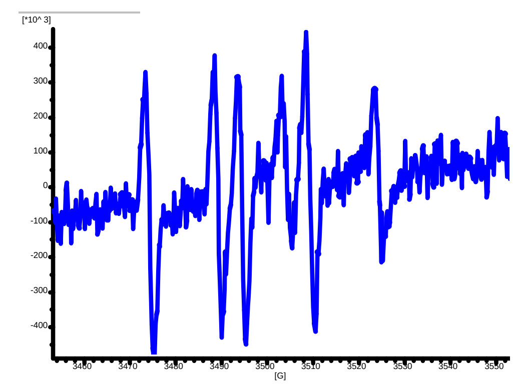
Linsey Robertson



Caroline Quin

MitoSPIN:

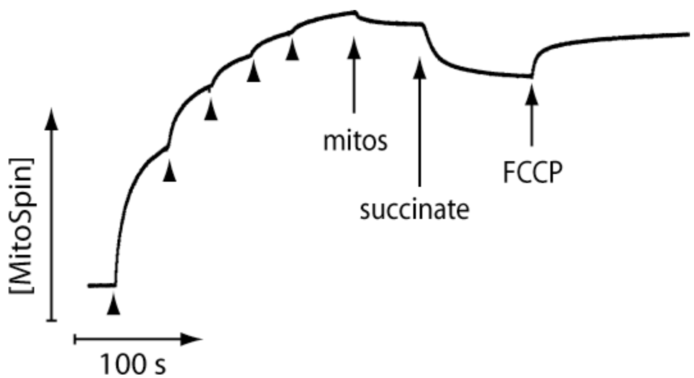




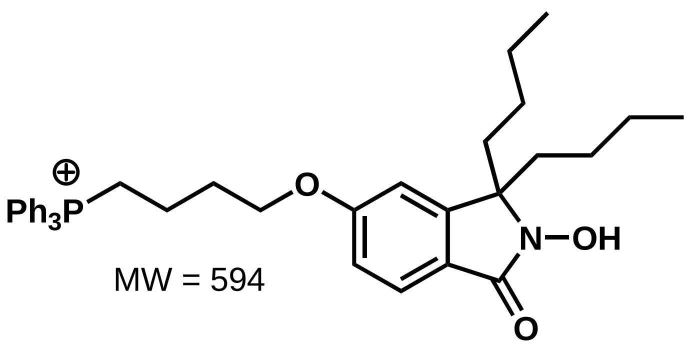
EPR spectrum

MitoSPIN

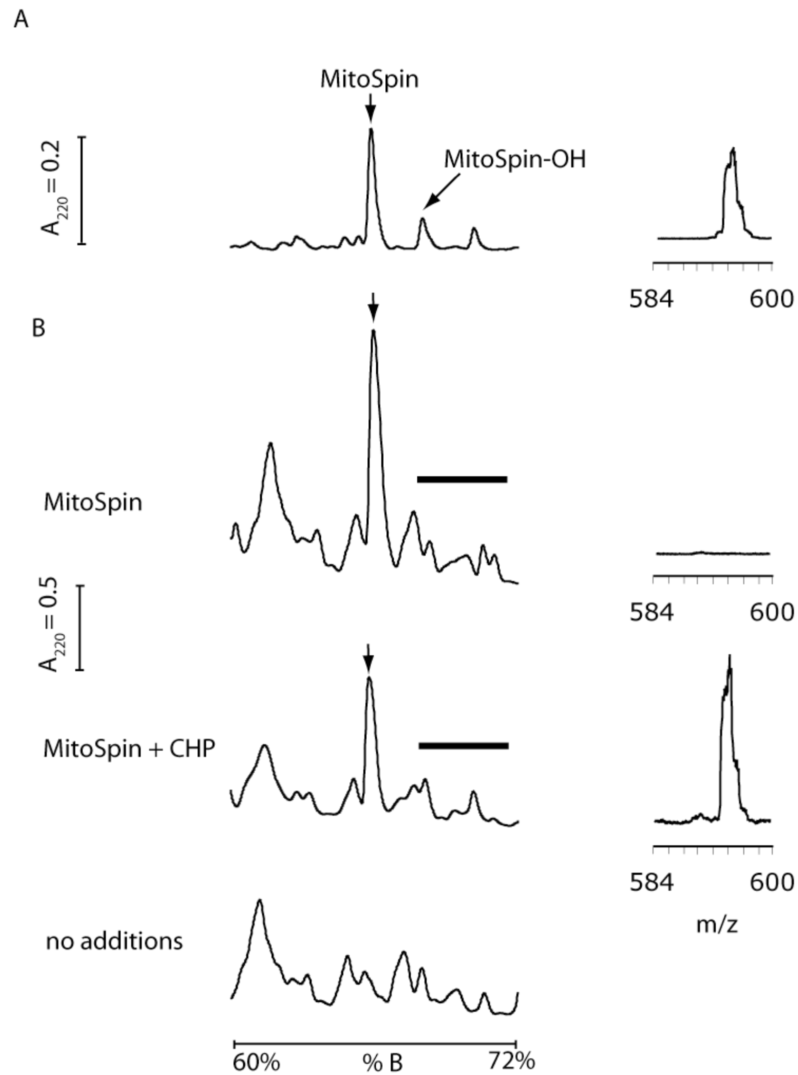
Accumulates 1000-fold in active mitochondria



Is oxidized to MitoSPIN-OH by hydroxyl radicals



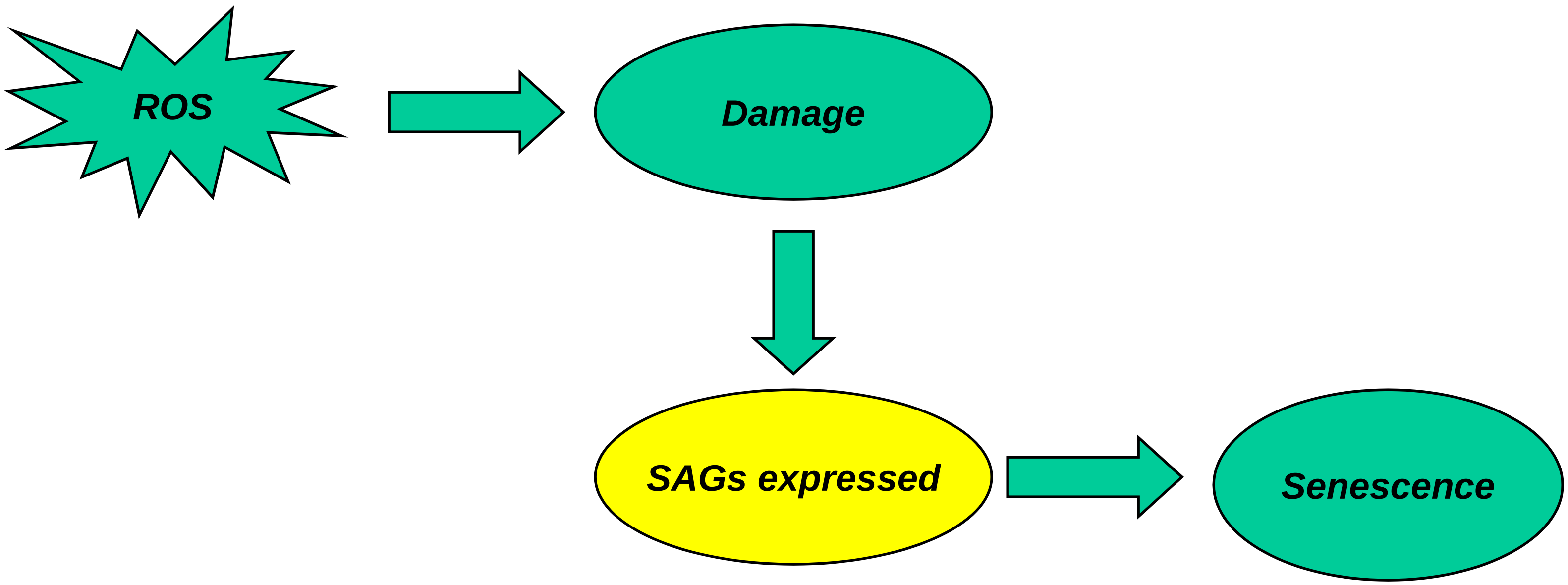
Can be used to measure ROS in mitochondria by ESI-MS



Michael Murphy, Jan Trnka, MRC Human Nutrition Unit, Cambridge

Ameliorate oxidative stress and slow ROS-associated senescence

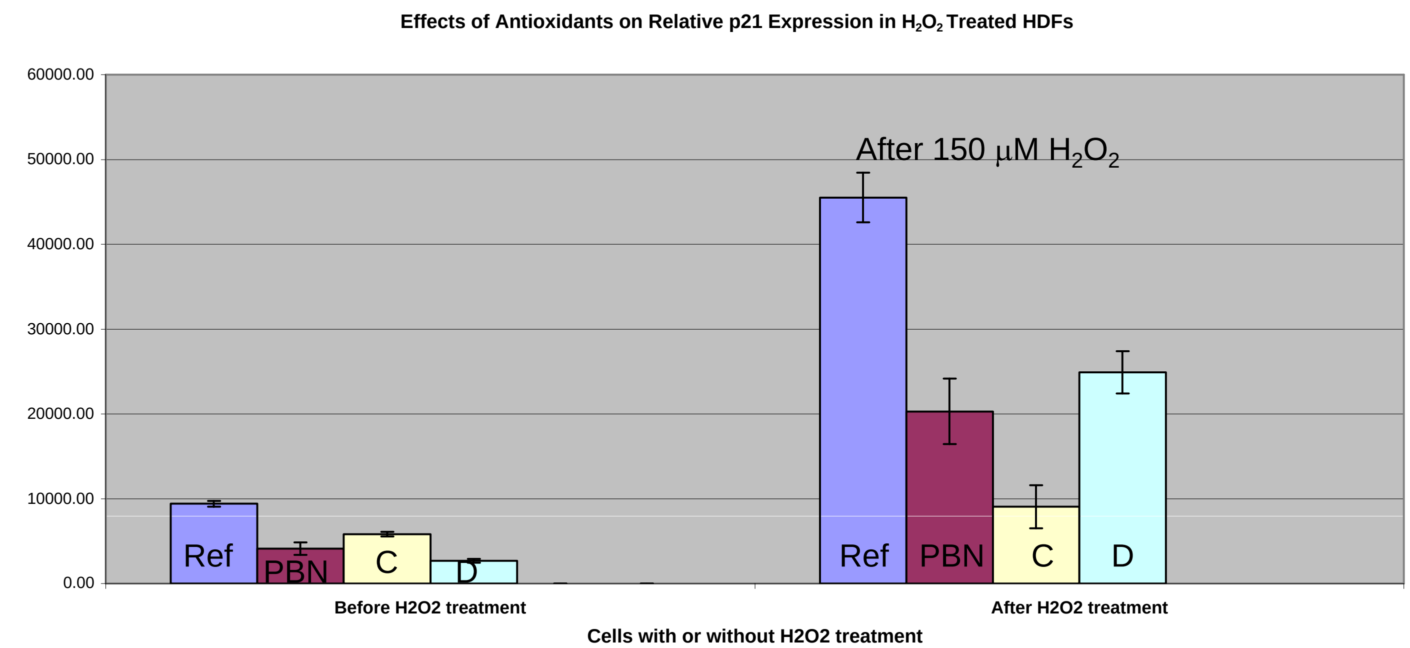
- Oxidative stress increases expression of senescence associated genes



Antioxidants protect primary Human Diploid fibroblasts from an oxidative assault?

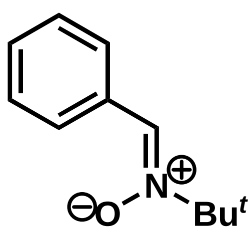
p21 expression in HDF cells

p21 inhibits cyclin dependent kinases in response to p53 and thus initiates senescence

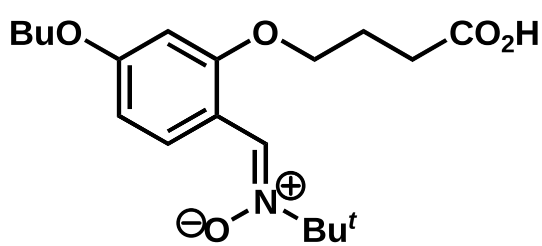


Real Time PCR (Taqman) analyses of DNA damage response gene expression. Mean expression levels are shown relative to HPRT

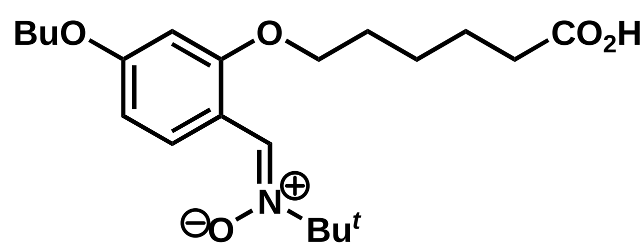
expression.



PBN (@ 750 µM)



Nitrone C (@ 400 µM)

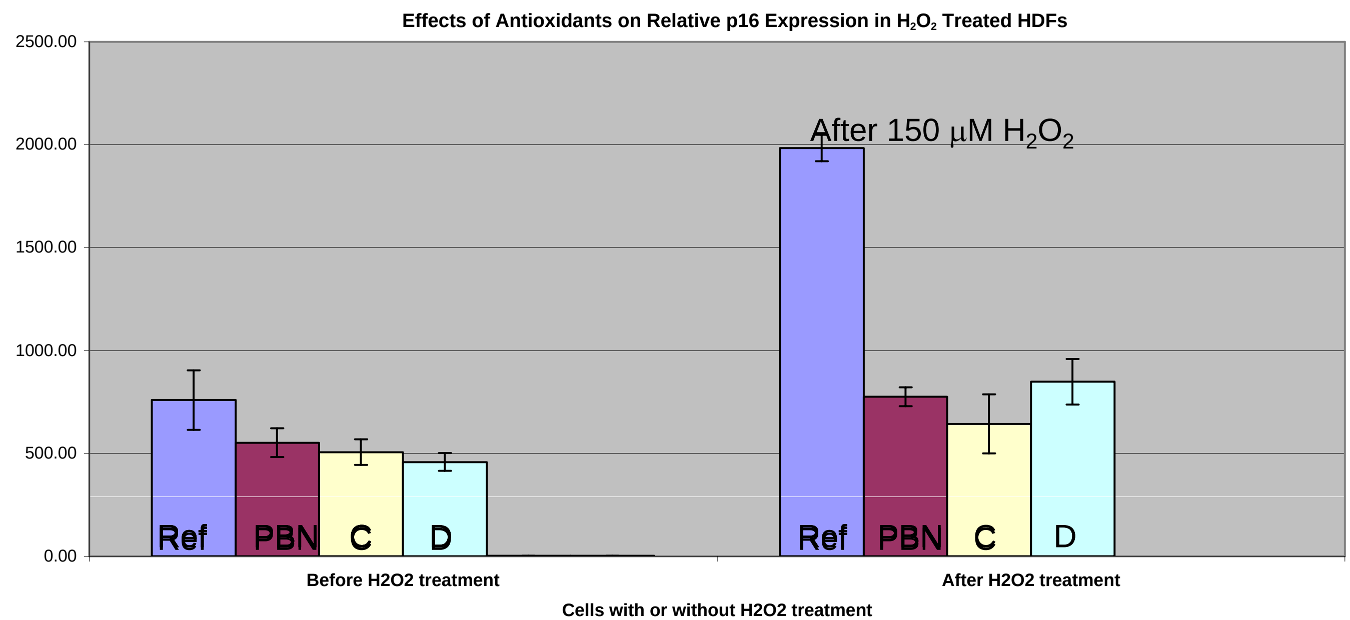


Nitrone D (@ 300 µM)

Elaine Brown, Alan MacIntyre, Nadeem Ashraf, Kelly Lamb, W. D. George, E. Sklavounou and **Paul Shiels**

p16 expression in HDF cells

Involved in long term maintenance of senescence



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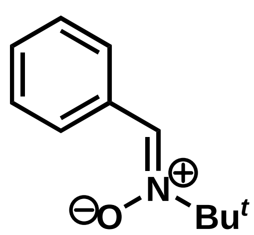
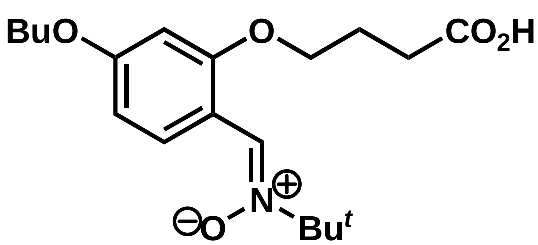


PBN (@ 750 μM)



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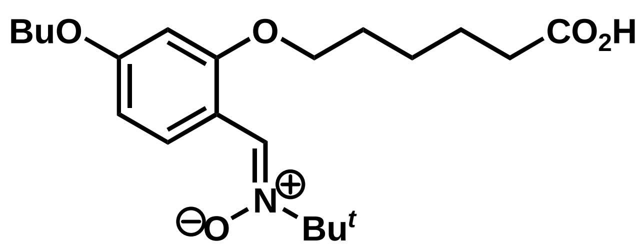


Nitrone D (@ 300 μM)

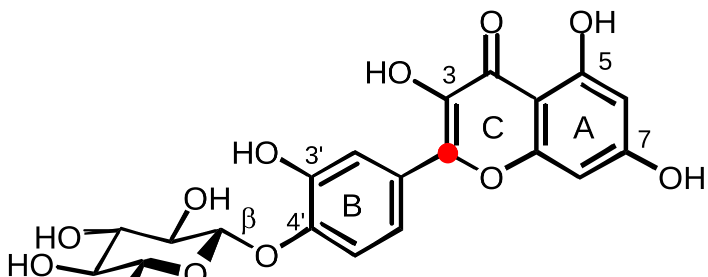
Sklavounou, E.; Hay, A.; Lamb, K.; Brown, E.; MacIntyre, A.; George, W. D.; Hartley R. C.; Shiels, P. G. *BBRC* **2006**, *347*, 420-427.

Tracking dietary compounds and metabolites

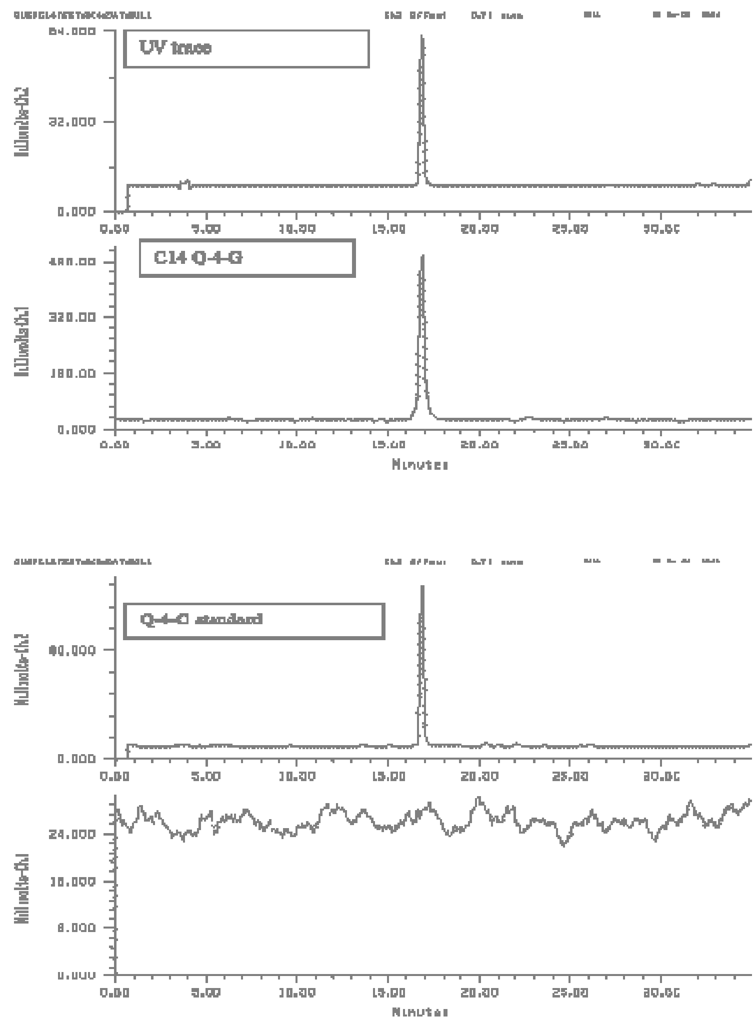
– isotopic labelling

Are flavonoids biological antioxidants?

Isotopic labelling to determine uptake, distribution and metabolism of drugs and natural products



Quercetin-4'-glucoside



W. Mullen,R. C. Hartley, A. Crozier, *Jouranl of Chromatography A* **2003**, *1007*, 21-29. W. Mullen, B. A. Graf, S. T. Caldwell, R. C. Hartley, G. G. Duthie, C. A. Edwards, M. E. J. Lean, and A. Crozier, *J. Agric. Food Chem.* **2002**, *50*, 6902-6909. S. T. Caldwell, A. Crozier, and R. C. Hartley, *Tetrahedron* **2000**, *56*, 4101-4106.

Distribution of Radioactivity in Rats 60 min after Ingestion of [2-^{14}C]Quercetin-4'-Glucoside.

Tissue/fluid	Total radioactivity	Concentration of radioactivity
Intestine and contents	54,530 ± 4,728 ***(93.6%)***	2,117 ± 162
Plasma	1,644 ± 157 ***(2.8%)***	110 ± 10
Red blood cells	4 ± 4 ***(-)***	0 ± 0
Liver	684 ± 240 ***(1.2%)***	37 ± 313
Kidneys	468 ± 295 ***(0.8%)***	147 ± 109
Spleen	6 ± 1 ***(-)***	7 ± 1
Brain	1 ± 1 ***(-)***	0 ± 0
Lungs	38 ± 10 ***(0.07%)***	21 ± 5
Heart	18 ± 2 ***(0.03%)***	14 ± 2
Muscle	839 ± 310 ***(1.4%)***	4 ± 2
Testes	25 ± 34 ***(0.04%)***	8 ± 2

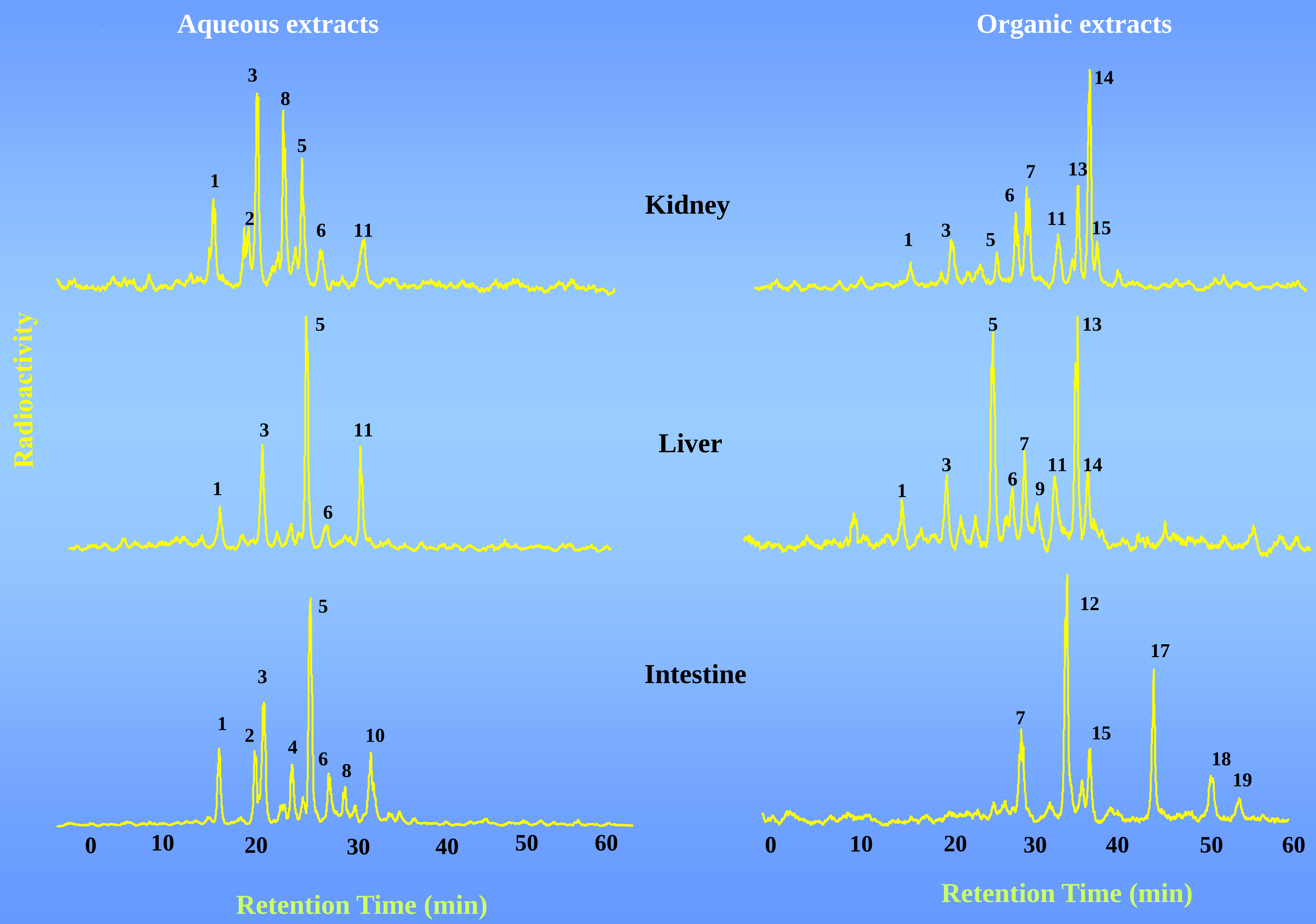
Radioactivity expressed as dpm x 10^3 per tissue/fluid per rat ± standard deviation (n = 3) and in parenthesis as a percentage of the recovered radioactivity.

3 mg of quercetin glucoside per 430 g rat (58.6 x 10^6 dpm)

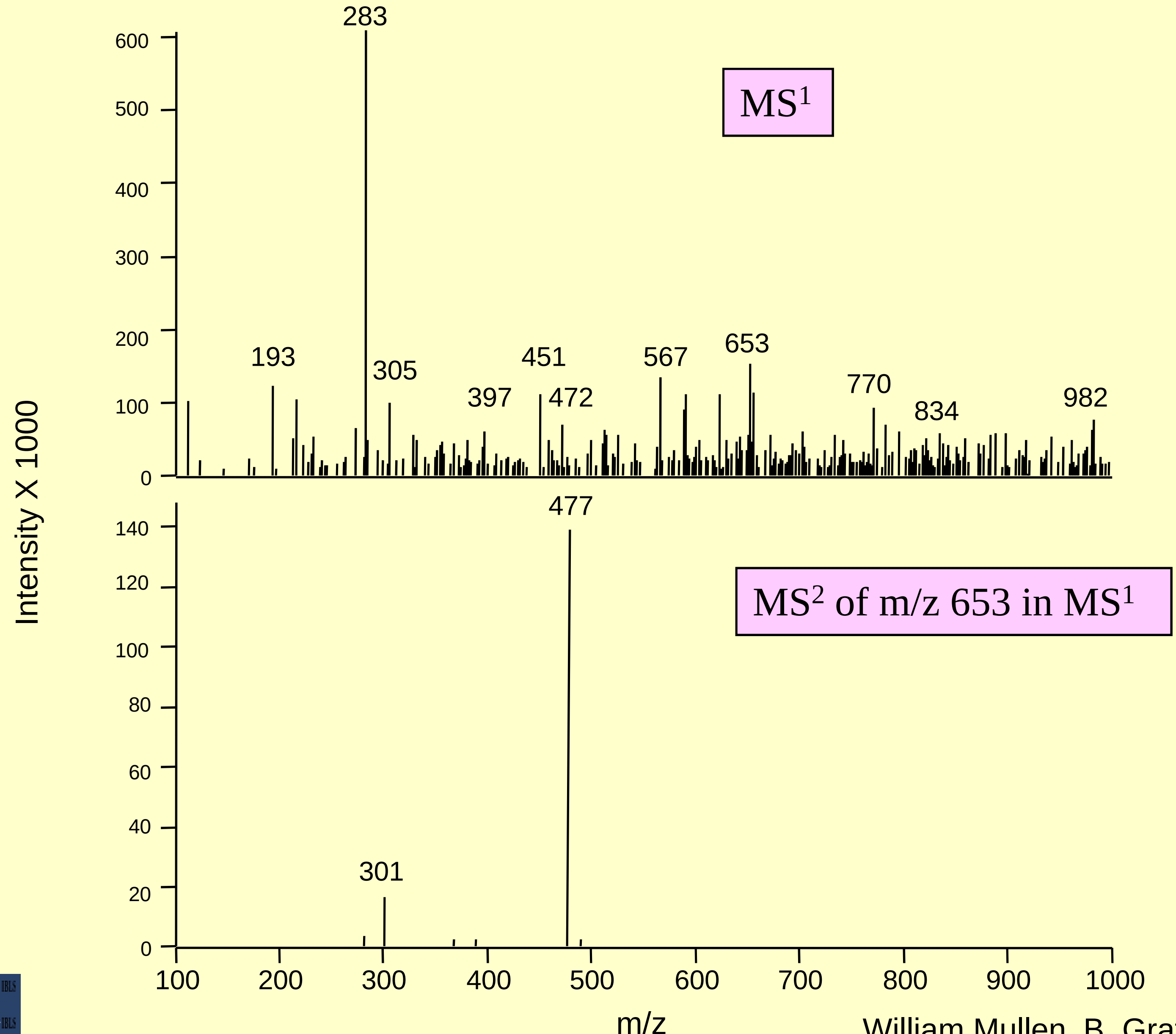
Institute of Biomedical and Life Sciences

William Mullen, B. Graf, **Alan Crozier**

METABOLISM OF [2-^{14}C]QUERCETIN-4'-GLUCOSIDE IN RATS



Tandem MS of a quercetin diglucuronide metabolite from rat intestine



Institute of Biomedical and Life Sciences

William Mullen, B. Graf, **Alan Crozier**

Identification of Metabolites of [2-^{14}C]Quercetin-4'-Glucoside Detected in Rats.

Peak	Rt (mins)	Compound	$[M-H]^-$ (m/z)	Fragment ions (m/z)
1	16.7	Quercetin diglucuronide	653	477 (M^{--}-GlcUA), 301[Q](M^--GlcUA-GlcUA)
2	20.6	Unknown		
3	21.6	Methylquercetin diglucuronide	667	491 (M^{--}-GlcUA), 315 [MQ](M^--GlcUA-GlcUA)
4	23.7	Quercetin diglucuronide	653	477 (M^--GlcUA), 301[Q](M^--GlcUA-GlcUA)
5	24.3	Methylquercetin diglucuronide	667	491 (M^{--}-GlcUA), 315 [MQ](M^--GlcUA-GlcUA
6	25.1	Quercetin diglucuronide	653	477 (M^--GlcUA), 301[Q](M^--GlcUA-GlcUA)
7	26.4	Quercetin diglucuronide	653	477 (M^--GlcUA), 301[Q](M^--GlcUA-GlcUA)
8	28.3	Quercetin diglucuronide	653	477 (M^--GlcUA), 301[Q](M^--GlcUA-GlcUA)
9	29.6	Quercetin-3-glucuronide	477	301 [Q], (M^--GlcUA)
10	30.3	Quercetin glucuronide sulphate	557	477 (M^--SO_3), 381 (M^--GlcUA), 301 [Q](M^--SO_3-GlcUA)
11	32.6	Methylquercetin glucuronide sulphate	571	491 (M^--SO_3), 315 [MQ](M^--SO_3-GlcUA)
12	34.5	Quercetin-4'-glucoside	463	301 [Q](M^--Glc)
13	35.3	Methylquercetin glucuronide	491	315 [MQ](M^--GlcUA)
14	36.5	Methylquercetin glucuronide	491	315 [MQ](M^--GlcUA)
15	37.4	Methylquercetin glucuronide	491	315 [MQ](M^--GlcUA)
16	37.4	Quercetin glucuronide	477	301 [Q](M^--GlcUA)
17	44.6	Quercetin	301	
18	51.1	Methylquercetin sulphate	395	315 [MQ](M^--SO_3)
19	51.2	Quercetin sulphate	381	301 [Q]](M^--SO_3)

HPLC-RC retention times, negative ion MS-MS fragmentation patterns and identities of metabolites detected in rat tissues 60 min after oral ingestion of [2-^{14}C]quercetin-4'-glucoside. Peak number refer to peaks in Figure 1. Q – quercetin, MQ – methylquercetin, GlcUA – glucuronyl unit; Glc – glucosyl unit; $[M-H]^-$ - molecular ion.

W. Mullen, R. C. Hartley, A. Crozier, *Jouranl of Chromatography A* **2003**, *1007*, 21-29. W. Mullen, B. A. Graf, S. T. Caldwell, R. C. Hartley, G. G. Duthie, C. A. Edwards, M. E. J. Lean, and A. Crozier, *J. Agric. Food Chem.* **2002**, *50*, 6902-6909.

Chemical Biology

Detect and identify ROS

Locate and quantify ROS

Ameliorate oxidative stress

Track and assess antioxidants

University of Glasgow

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EPSRC

Caroline Quin