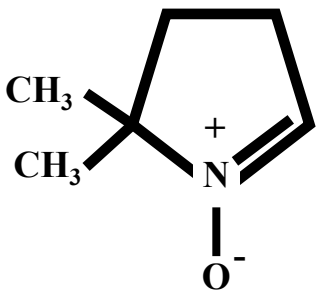
EXPERTIMENTAL TECHNIQUES

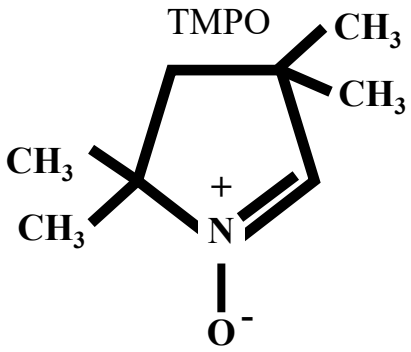
FREE RADICAL DETECTION BY ELECTRON PARAMAGNETIC RESONANCE (EPR)

DMPO



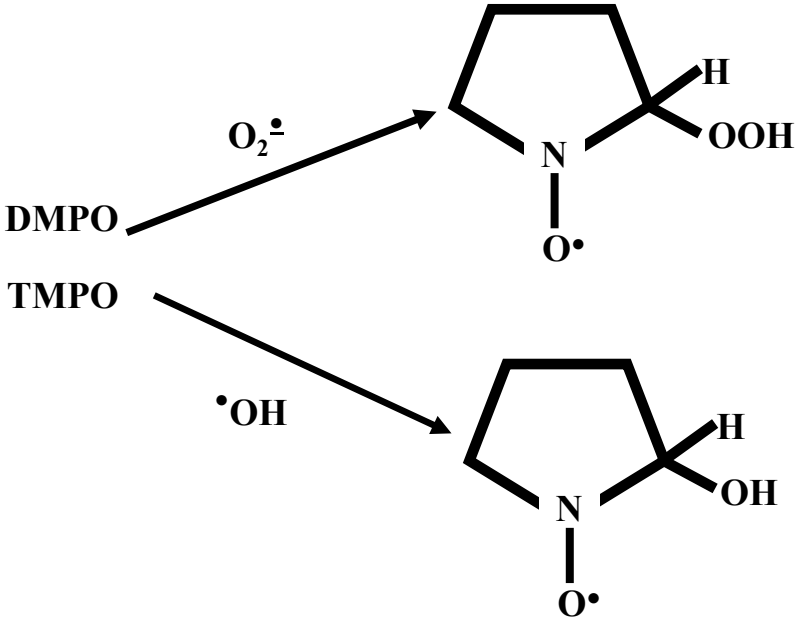
5,5 dimethyl pyrroline-1-oxide

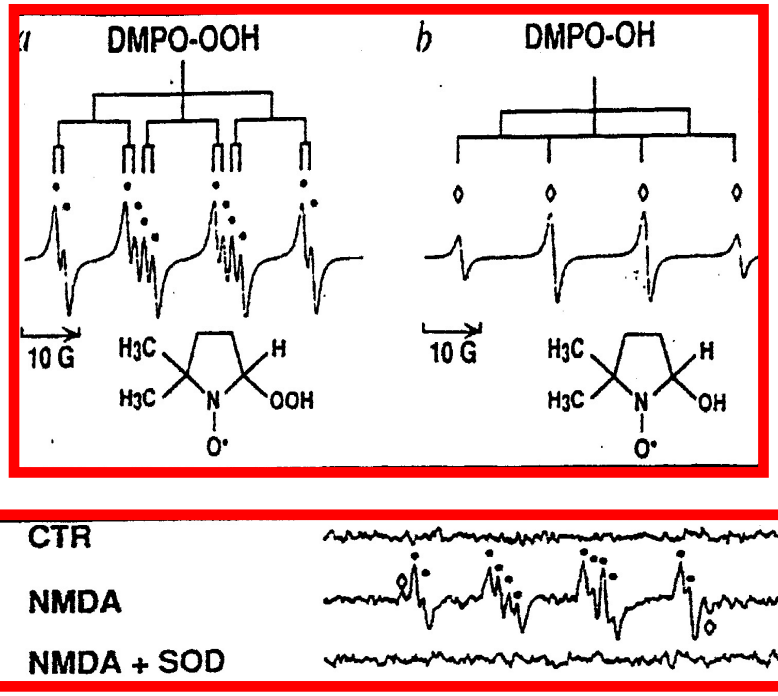
TMPO



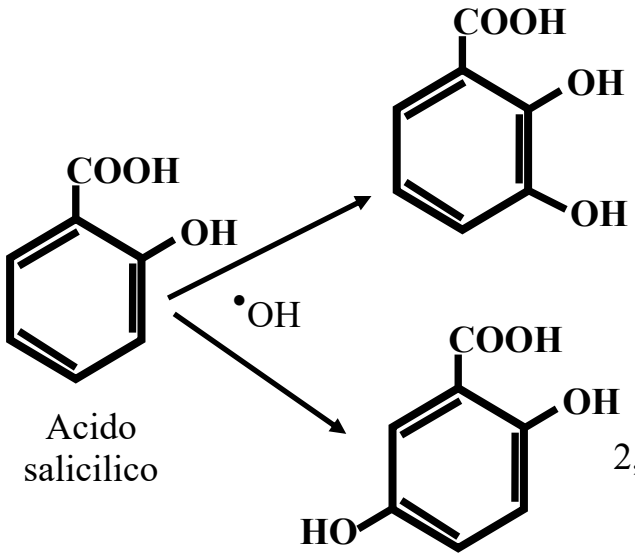
3,3,5,5 tetramethyl pyrroline-1-oxide

https://www.azom.com





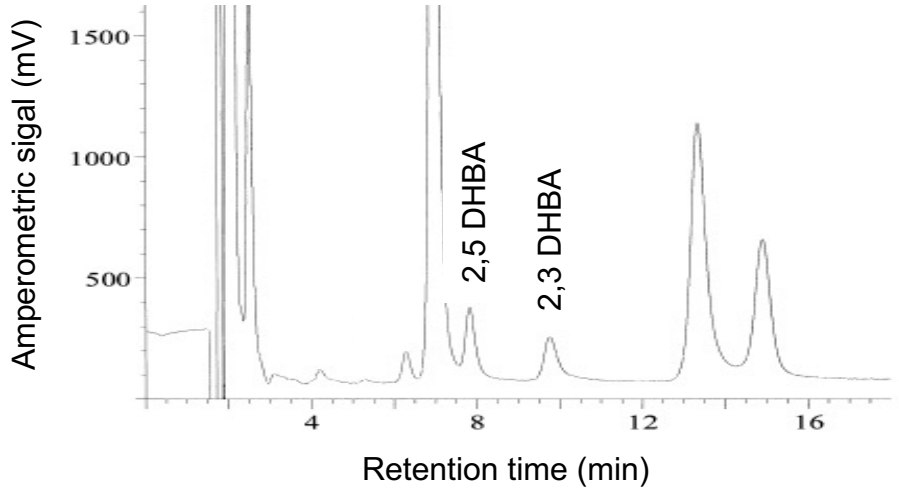
MEASUREMENT OF •OH BY SALICILIC ACID BY HPLC ANALYSIS WITH AMPEROMETRIC DETECTION



Acido salicilico

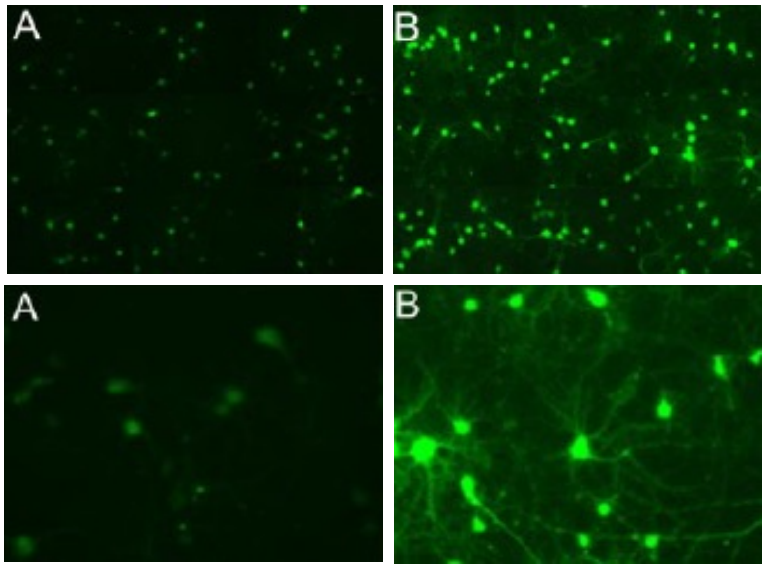
2,3 dihydroxybenzoic acid (2,3 DHBA)

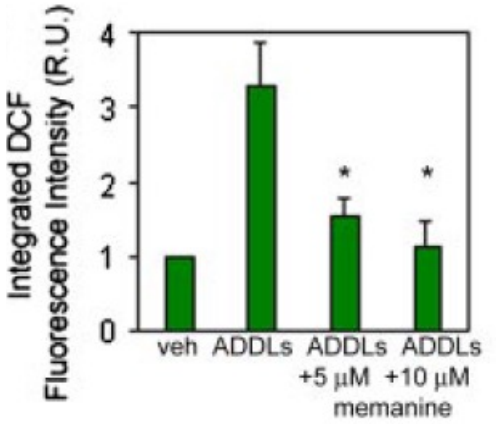
2,5 dihydroxybenzoic acid (2,5 DHBA)



ROS DETECTION WITH FLUORESCENT PROBES

Incubation of biological material with probes (e.g. CM-H2 DCFDA) that become fluorescent in the presence of ROS. Analysis with confocal microscopy.





Adapted from: De Felice et al., Journal of Biological Chemistry 2007, 282: 11590-11601

AMPEROMETRIC NO MEASUREMENT

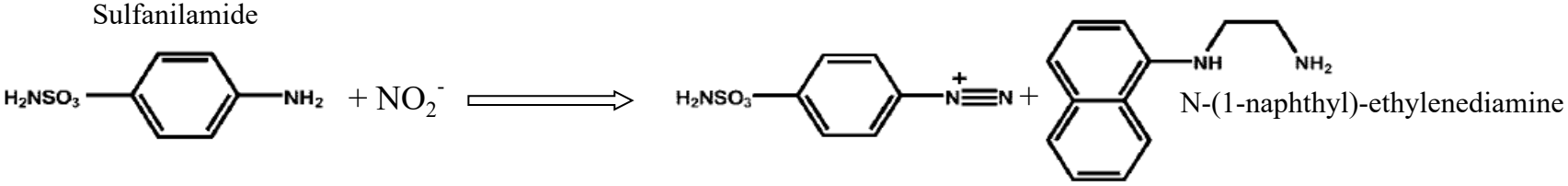
Electrode size:
2mm – 100 nm diameter

Detection limit 0.5 nM

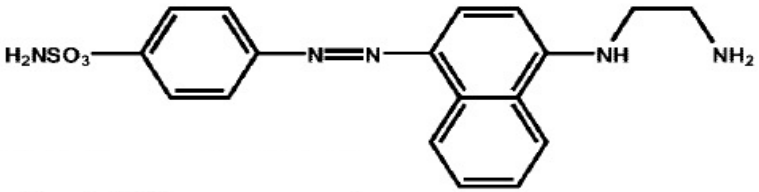
NO DETECTION BY GRIESS REACTION (NO_2^- AND NO_3^-)

- Samples containing nitrites e nitrates
- Reduction of nitrates to nitrites by nitrate reductase
- Griess reagent [sulfanilamide + N-(1-naphthyl)-ethylediamine] and spectrophotometric detection

Sulfanilamide



N-(1-naphthyl)-ethylenediamine

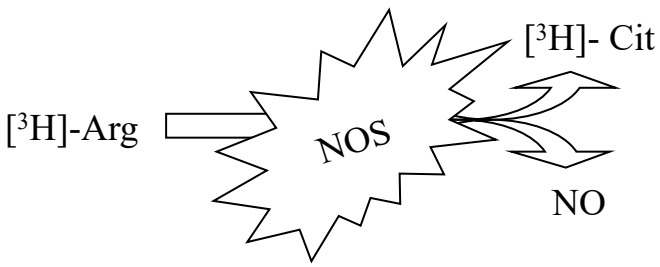


Diazonium compound λ 540 nM (violet)

NO DETECTION BY MEASURING L-CITRULLINE

L-arginine is transformed into l-citrulline + NO by NOS with a stoichiometry 1:1. Therefore, citrulline is a measure of NO production and can be detected by hplc

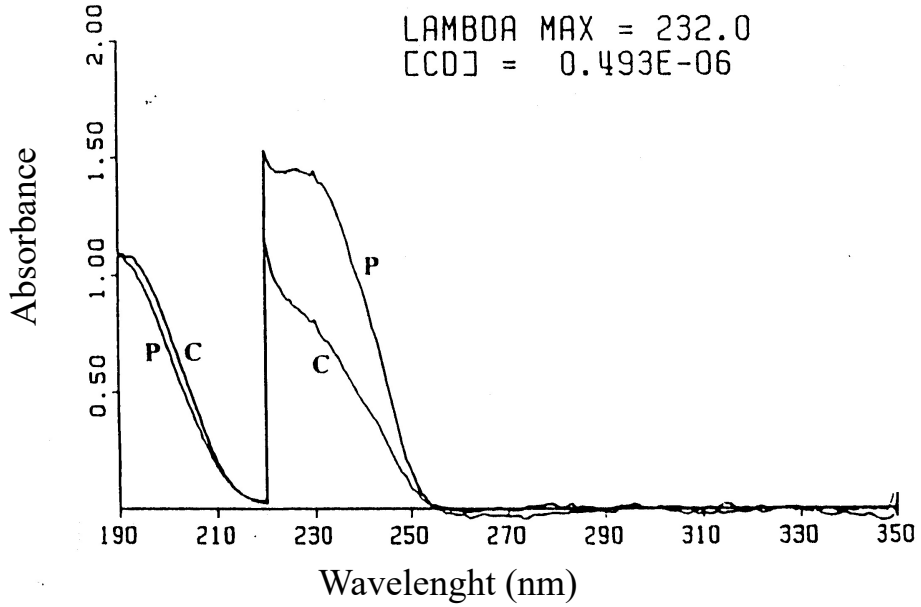
NO DETECTION BY MEASURING [^{3}H]CITRULLINE



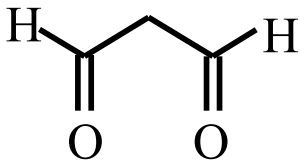
The biological model is incubated with [^{3}H]-arginine that will be transformed by NOS into [^{3}H]-citrulline + NO.
Separation of the two tritiated amino acids by ion exchange chromatography and quantification of radioactive arginine and citrulline by liquid scintillation counting.

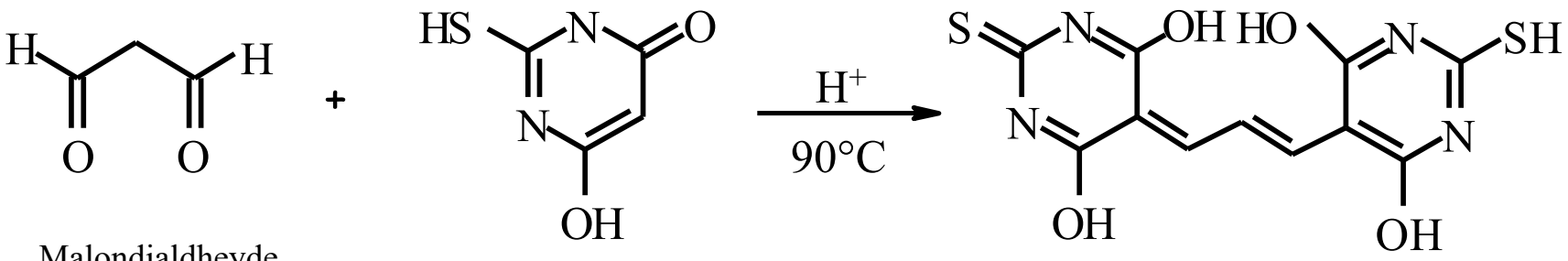
DETECTION OF LIPID PEROXYDATION BY CONJUGATED DIENES QUANTIFICATION

Conjugated dienes can be quantified by UV spectrophotometric analysis



DETECTION OF LIPID PEROXYDATION BY MALONDIALDEHYDE QUANTIFICATION





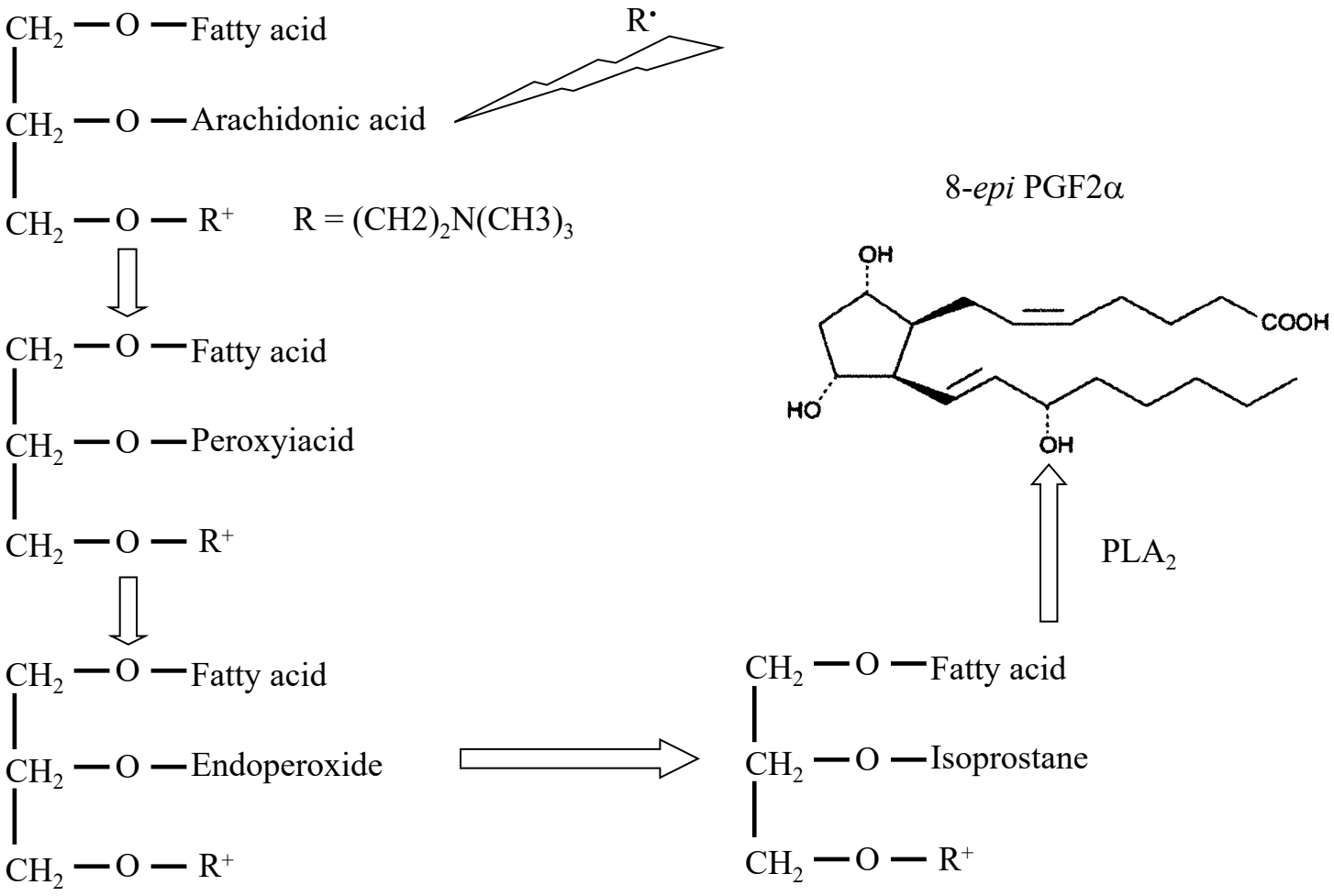
Malondialdheyde

Thiobarbituric acid

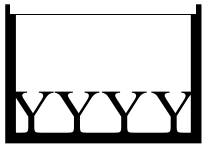
λ_{max} 532 nm (pink-violet)

Malondialdheyde reacts with thiobarbituric acid to produce a chromophore that is quantified by spectrophotometer analysis

DETECTION OF LIPID PEROXYDATION BY EIA ISOPROSTANE QUANTIFICATION



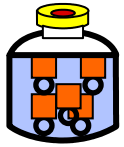
DETECTION OF LIPID PEROXYDATION BY EIA ISOPROSTANE QUANTIFICATION



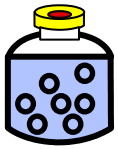
Mouse anti-rabbit IgG coated multiwell plate

Buffered solution

Anti 8-isoprostane rabbit antiserum (Anti-8-iso-As)

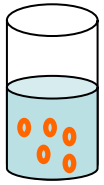


8-isoprostane linked to acetylcholinesterase (8-iso-AchEst)



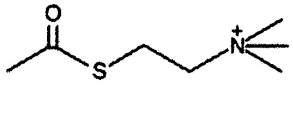
8-isoprostane Standard (8-isoSTD)

Ellman reagent



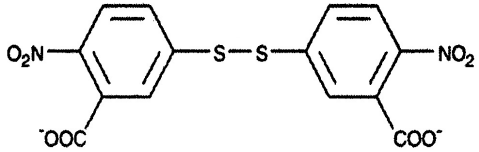
Sample with unknown amounts of 8-isoprostane (8-iso-unk)

Ellman reagent



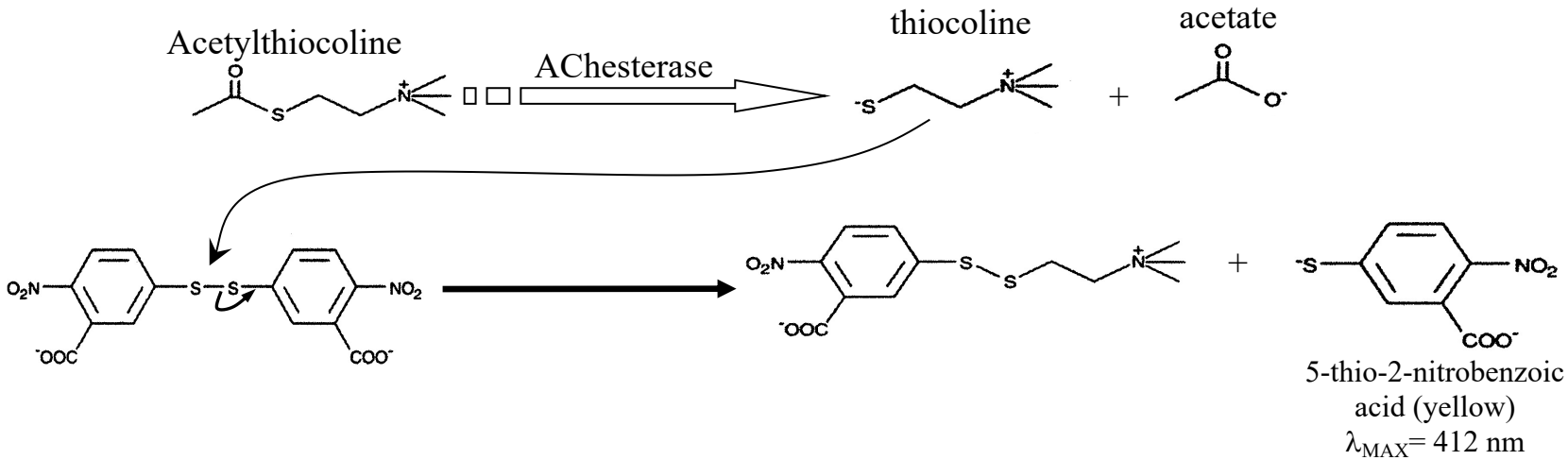
Acetyltiocholine

\+



5,5'-dithio-bis-2-nitrobenzoic acid

Ellman reagent

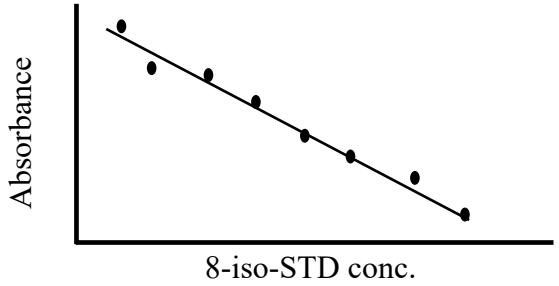


Procedure

All the wells necessary for the analysis are filled with buffer solution followed by the addition of the rabbit Anti-8-iso-As that will be bound by the mouse anti-rabbit IgG at the bottom of the well. At this point, 8-iso-AchEst is added that will be bound by the Anti-8-iso-As.

For the calibration curve, 8-9 different known concentrations of 8-iso-STD are added, whereas in other wells a certain amount of the unknown samples is added that contains 8-iso-unk. The 8-iso-STD will displace the 8-iso-AchEst from the Anti-8-iso-As in a concentration-dependent manner (the higher the 8-iso-STD concentration, the more 8-iso-AchEs id displaced) and the displaced 8-iso-AchEs will be washed away. Similarly, displacement of 8-iso-AchEs will occur in the wells where 8-iso-unk has been added.

At this point, the Ellman reagent is added. Since the intensity of the yellow color (therefore, the absorbance) is due to the activity of AchEst present in the well, absorbance will be inversely proportional to the concentration of 8-iso-STD.



For unknown samples, once the value of absorbance has been measured for each sample, the concentration of the respective endogenous 8-iso will be obtained by the interpolation with the calibration curve

- Detection of protein carbonyls PROTEIN-C=O

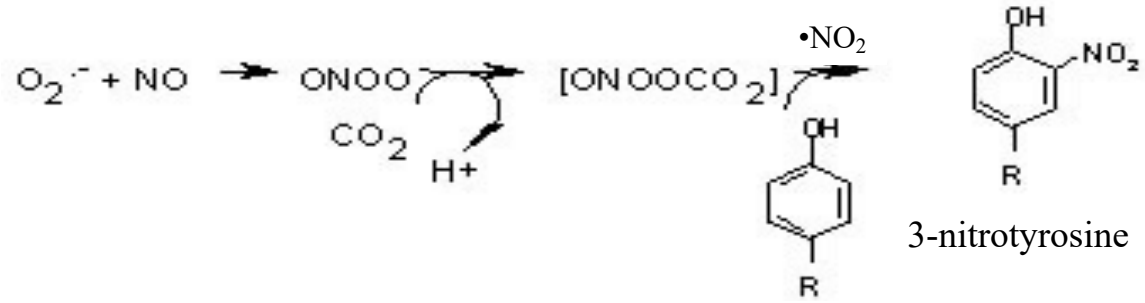
  a) direct oxidation (Pro, Lys, Arg, Thr)

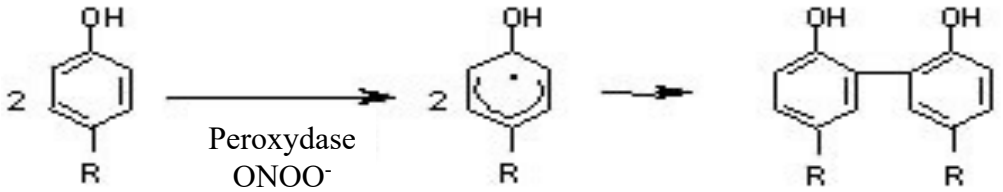
  b) lipoperoxydation (aldehydes His, Lys, Cys)

1) Reaction with dinitrophenylhydrazine (DNPH) and spectrophotometric detection at 360 nm

2) ELISA

- Detection of tyrosine oxidation products (HPLc, GC-MS, ELISA)

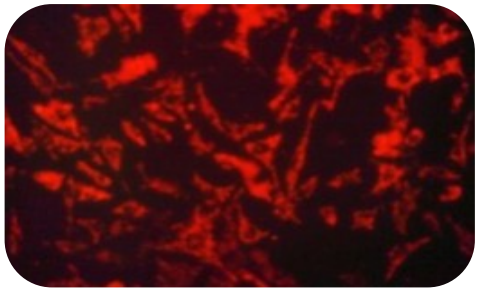


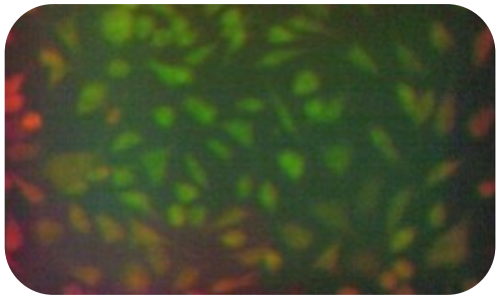


MITOCHONDRIAL DAMAGE

MITOCHONDRIAL MEMBRANE POTENTIAL (MMP) ASSAY

Cultured cells are incubated with the fluorescent die JC-10 that accumulates in healthy mitochondria (polarized membrane) where it forms reversible red-fluorescent dimeric aggregates.
When mitochondria are damaged, MMP collapse induces the release of JC-10 that returns to its green-fluorescent monomeric form.



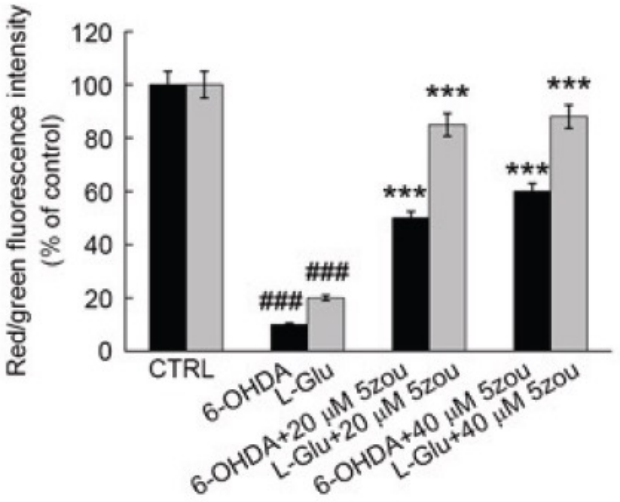


MOLECULAR MEDICINE REPORTS 16: 1133-1138, 2017

Investigation of the neuroprotective effects of a novel synthetic compound via the mitochondrial pathway

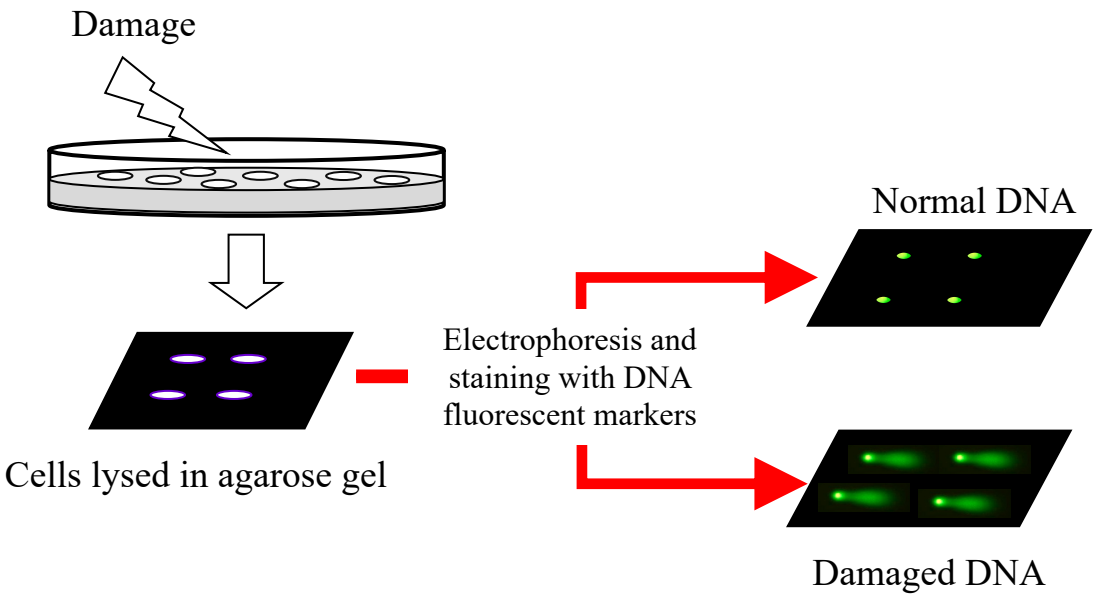
DI WANG[1-3*], SHUANG HU[2*], JUNRONG ZHANG[2], QIUYUE LI[2], XINYU LIU[2] and YU LI[1]

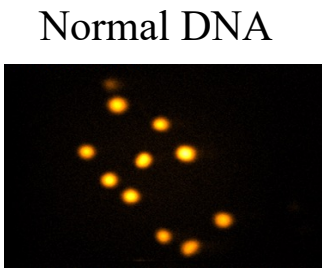
[1]Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, Jilin 130118; [2]School of Life Sciences, Jilin University, Changchun, Jilin 130012; [3]Zhuhai College of Jilin University, Jilin University, Zhuhai, Guangdong 519041, P.R. China

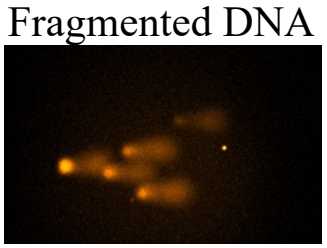


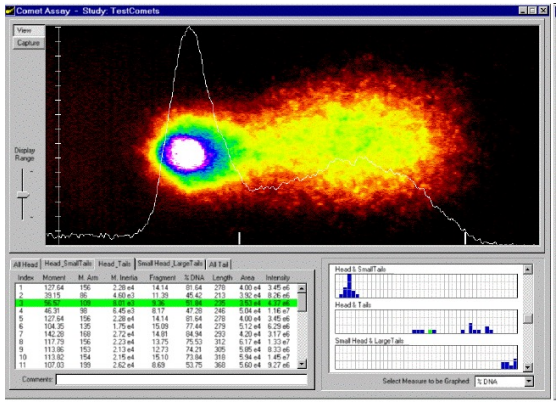
OXIDATIVE DAMAGE TO DNA

- 8-hydroxy-2’-deoxyguanosine (8OHdG) detection by HPLC, GC, ELISA
- Comet assay (DNA strand breaks)



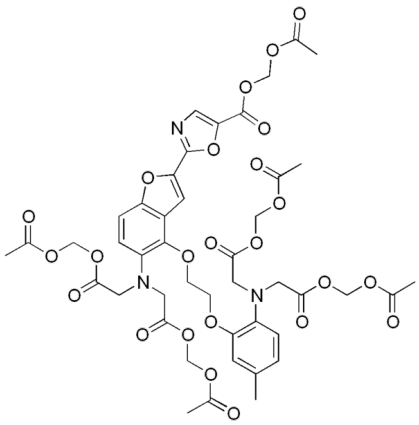




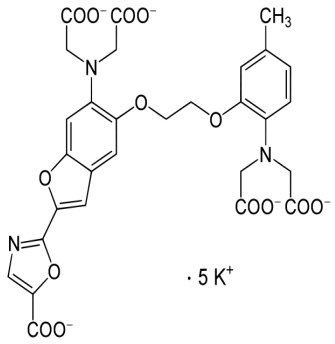


The image can be analysed using dedicated softwares able to quantify different aspects of the damaged genetic material

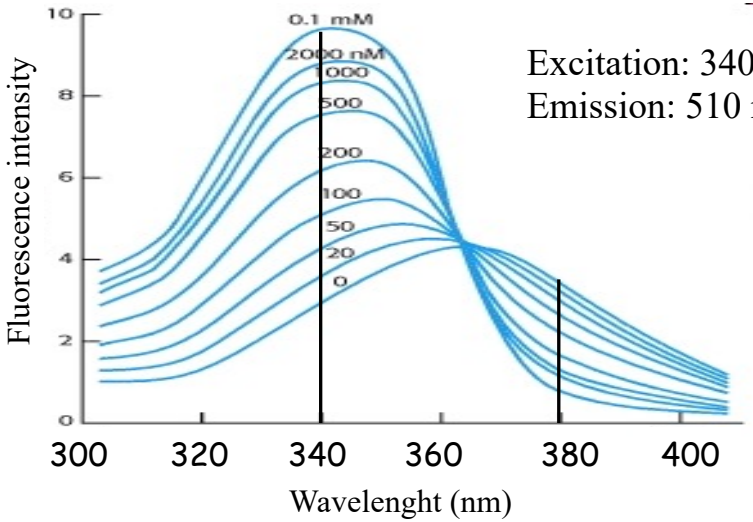
INTRACELLULAR CALCIUM DETECTION



FURA-2
acetoxymethyl ester
(cell permeable)

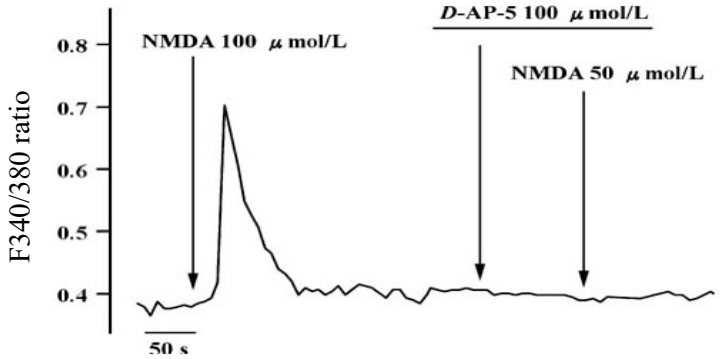


FURA-2

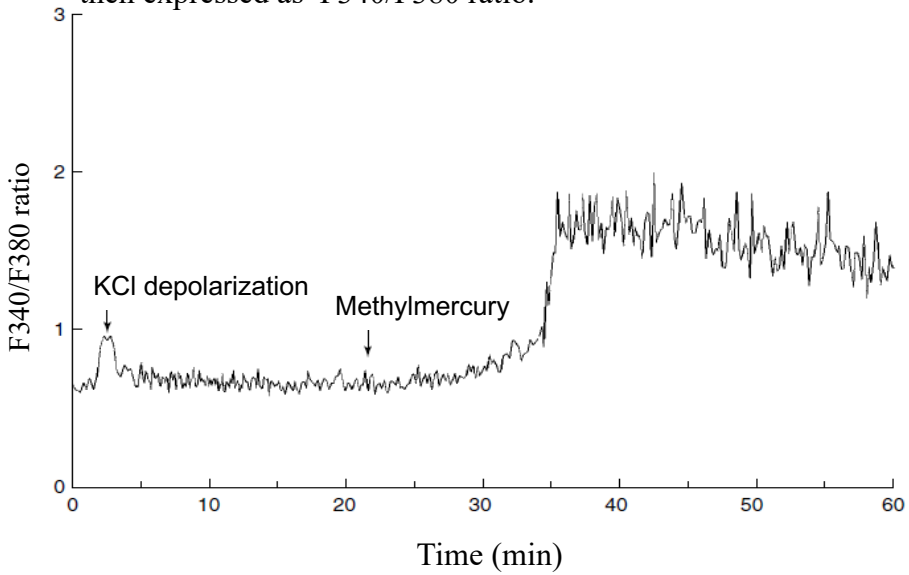


Excitation: 340 and 380 nm
Emission: 510 nm

As shown in the graph, increasing Ca^{2+} concentrations cause the increase of fluorescence intensity (F) when exciting samples at 340 nm and the decrease of F when exciting samples at 380 nm. Data are then expressed as F340/F380 ratio.



Adapted from: Hu et al., *Acta Pharmacol. Sin.* 2004, 25, 714



Adapted from: Limke & Atchison, *Current Protocols in Toxicology, 2009, Vol. 42, 12.15.1*