Chemical aspects of the cell

Chemical probes that trigger the cell environment and response for pH, reactive oxygen species

Compounds working as probes

Many applications require the use of indirect detection techniques

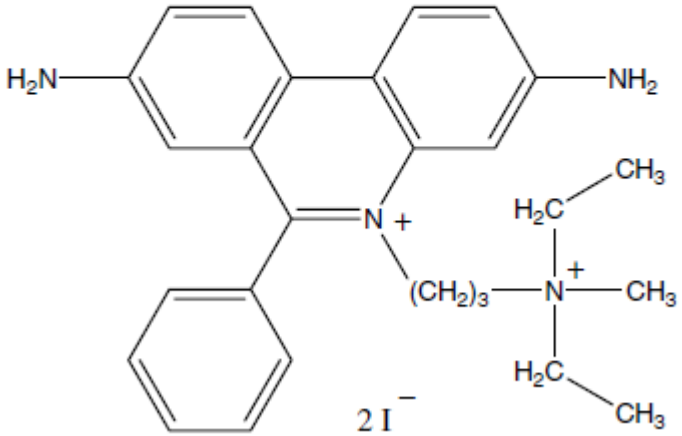
In this respect, chemical probes have an increasing role to detect different cell processes, some of them will be shown here, just to give a brief idea about this topic.

Examples of DNA staining dyes

PROPIDIUM IODIDE

CAS Registry Number 25535-16-4

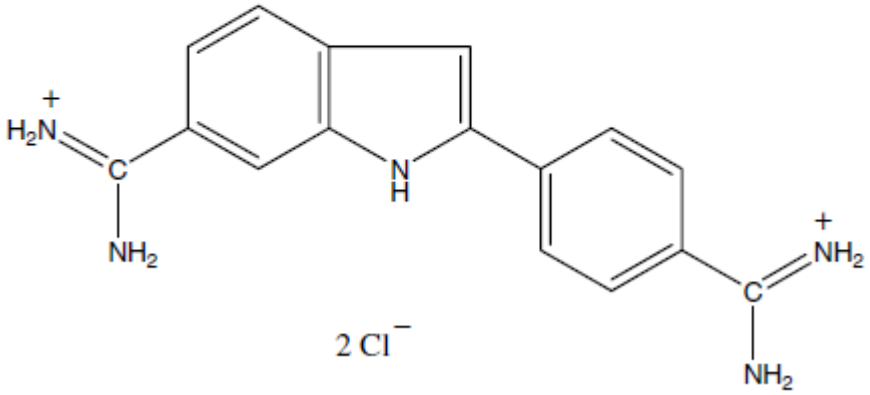
Chemical Structure



DAPI

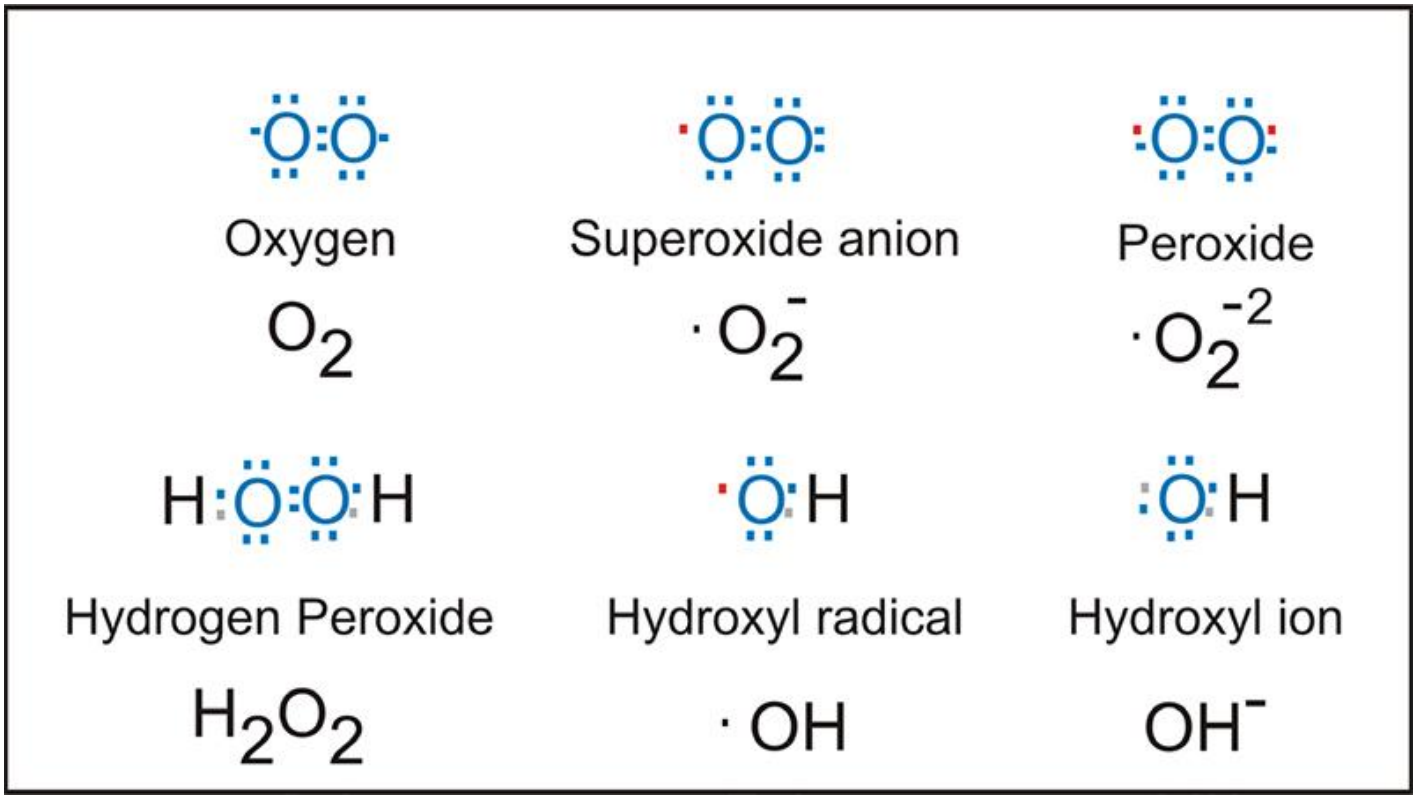
CAS Registry Number 28718-90-3

Chemical Structure



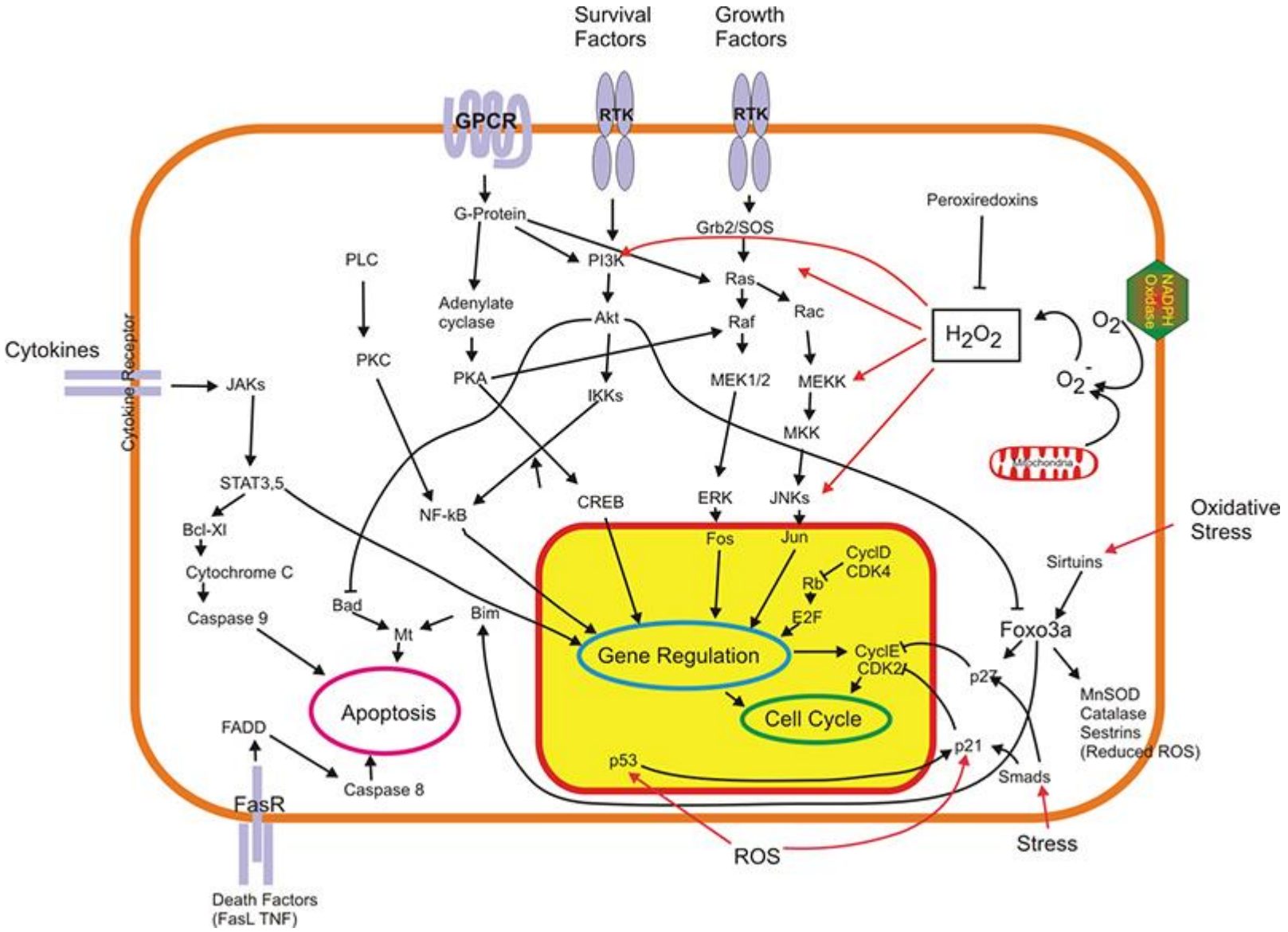
Sabnis, R. W. Handbook of Biological Dyes and Stains- Synthesis and Industrial Applications, 2010, John Wiley & Sons Inc., 521 pp.

Production of reactive oxygen species (ROS)



https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

Reactive oxygen species (ROS)



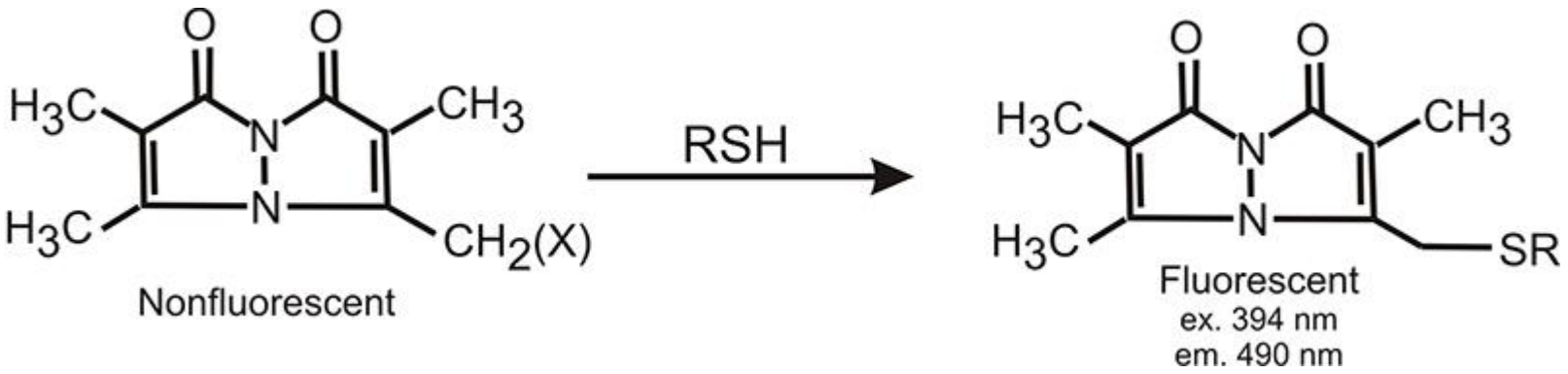
https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

Mechanisms to reduce ROS level

Glutathione oxidase

Eq. 3. $GSSH + NADPH + H^+ \rightarrow 2\,GSH + NADP^+$

Detection of proteins with thiols, including glutathione

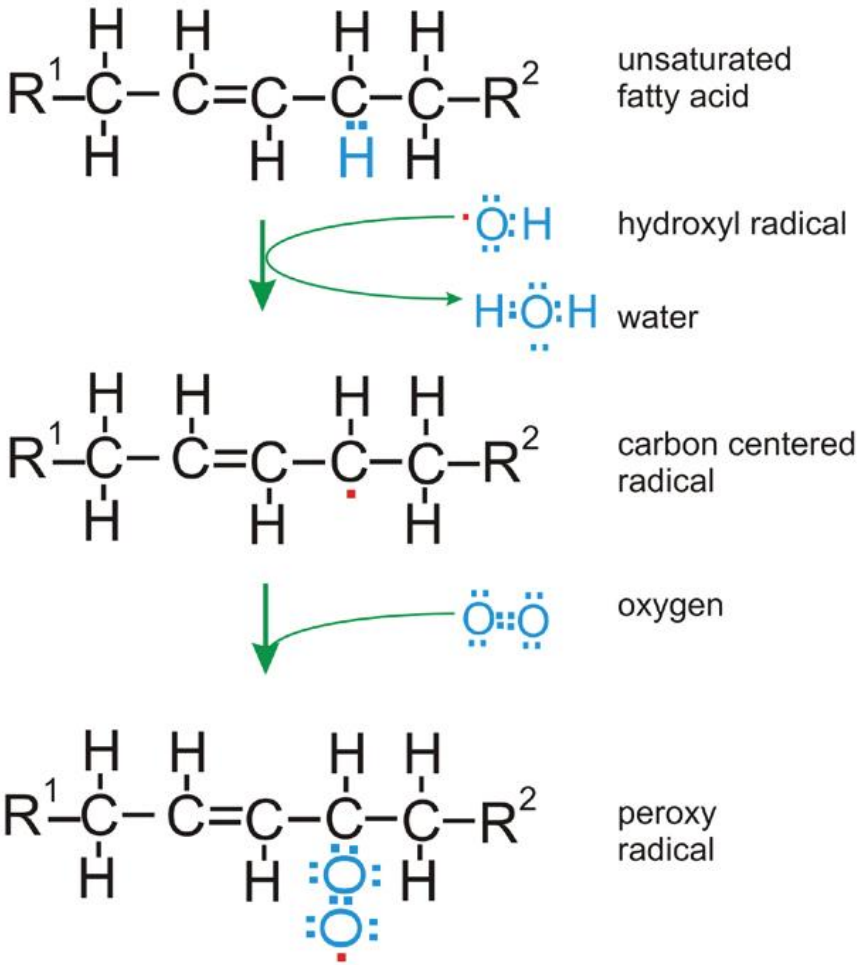


X = Br: Monobromobimane
X = Cl: Monochlorobimane

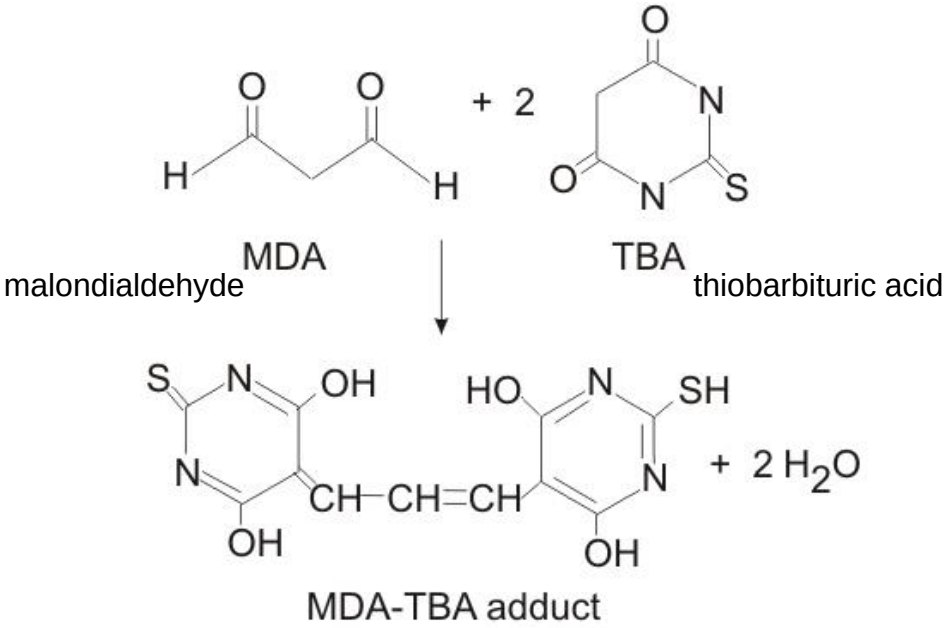
https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

Mechanisms to reduce ROS level

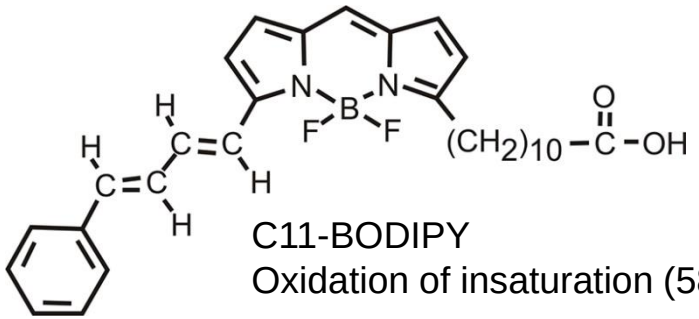
Peroxidation of lipids



Detection tools



Colorimetric assay: 532 nm
Fluorescence: 530 nm (excitation) and 550 nm (emmision)

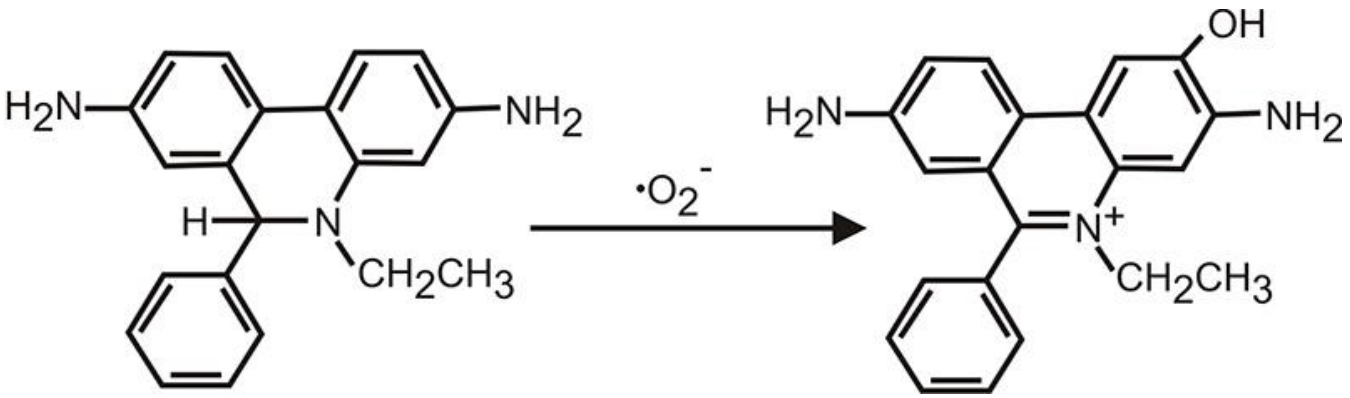


C11-BODIPY
Oxidation of insaturation (581/591)

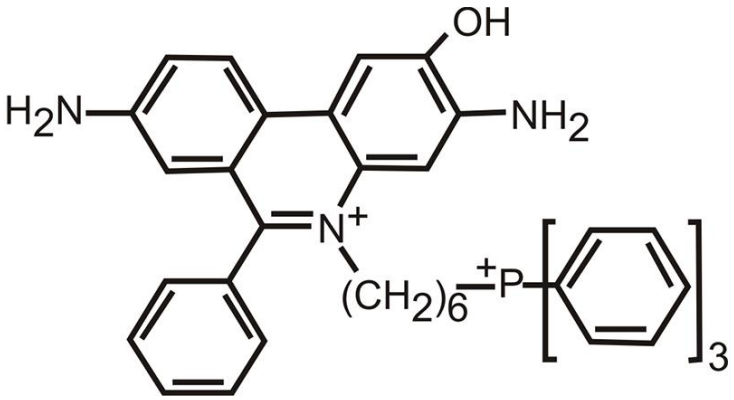
https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

Detection of superoxide

Oxidation of Dihydroethidium to 2-Hydroxyethidium by Superoxide

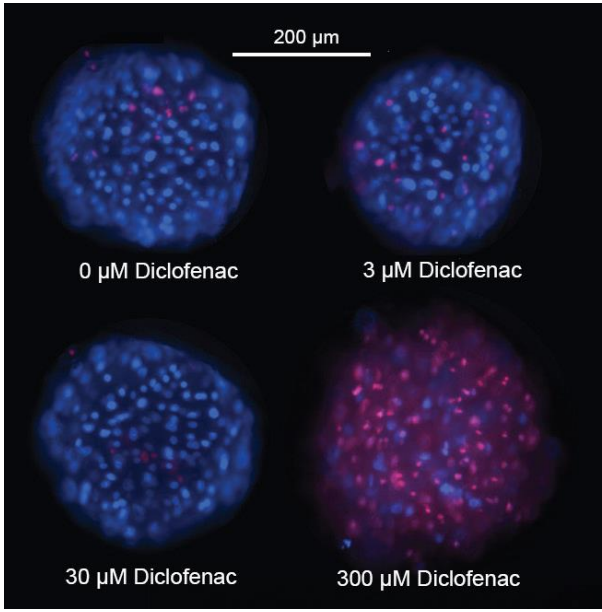


Oxidized MitoSox™ Red mitochondrial superoxide indicator



(510/590)

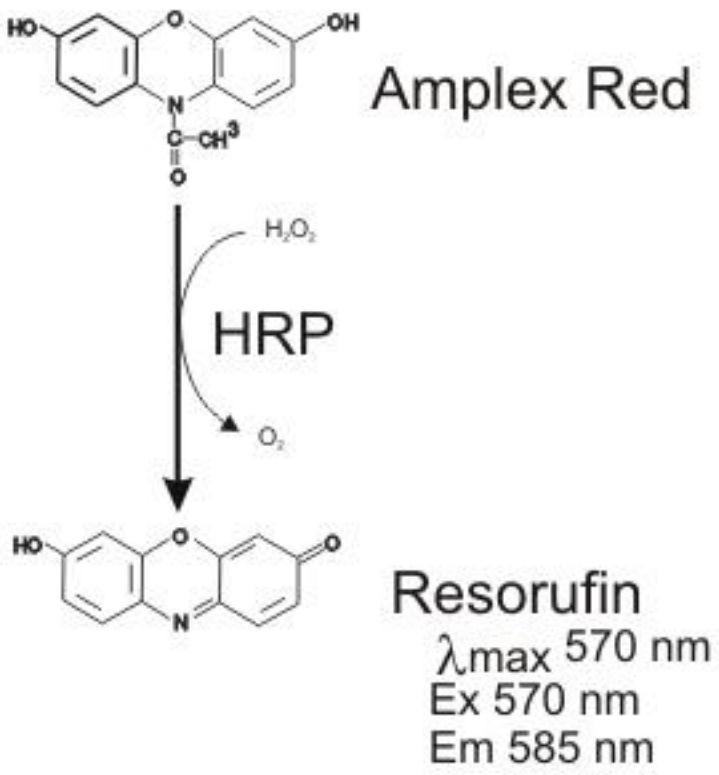
Hoechst 33342 : blue
MitoSox: red



https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

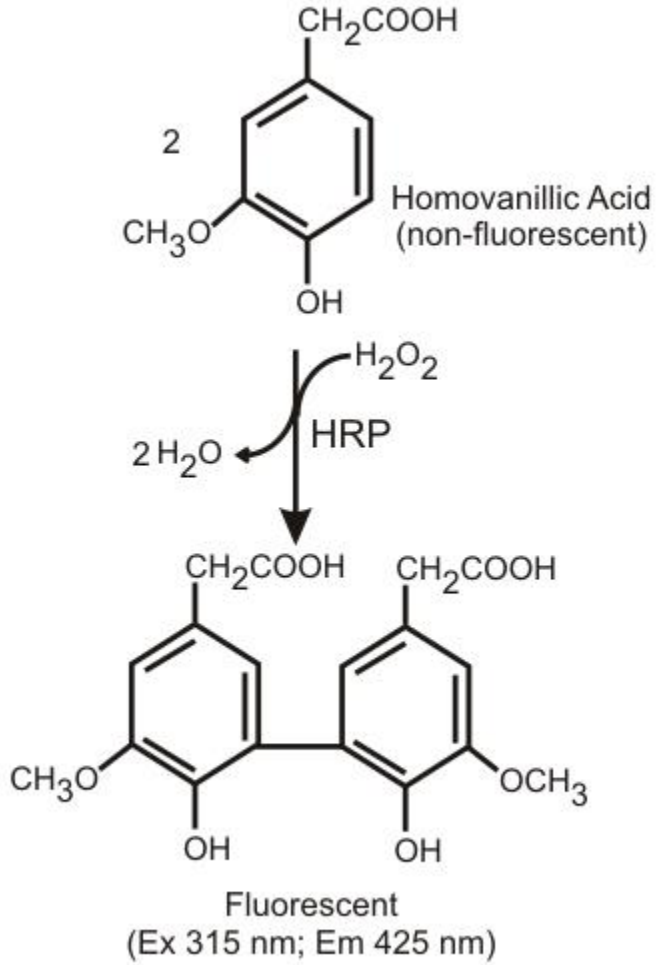
Detection of hydrogen peroxide

Conversion of Amplex Red to resorufin by HRP using H_2O_2



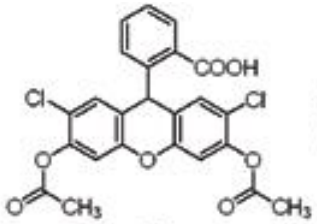
HRP: Horseradish peroxidase

Dimerization of homovanillic acid by the action of HRP and H_2O_2

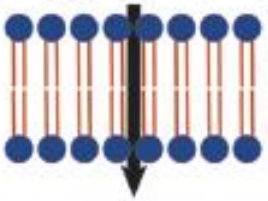


https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

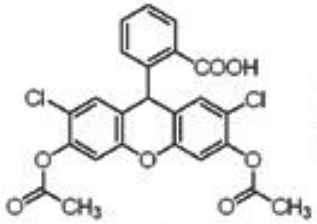
Detection of reactive oxygen species (ROS)



DCFH-DA
(Non-Fluorescent)

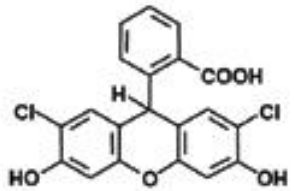


Penetration of
Cell Membrane



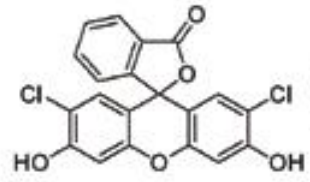
DCFH-DA
(Non-Fluorescent)

Cellular
Esterase



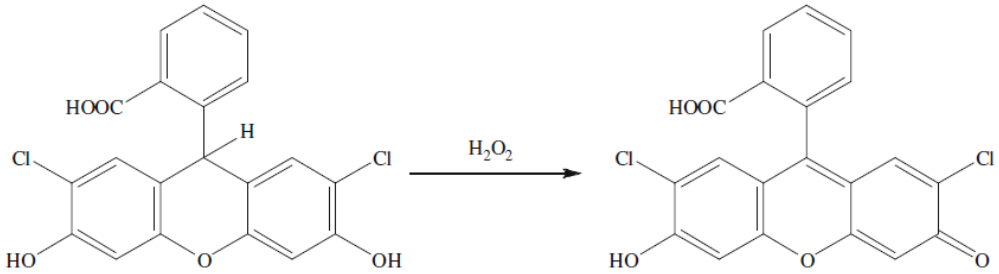
DCFH
(Non-Fluorescent)

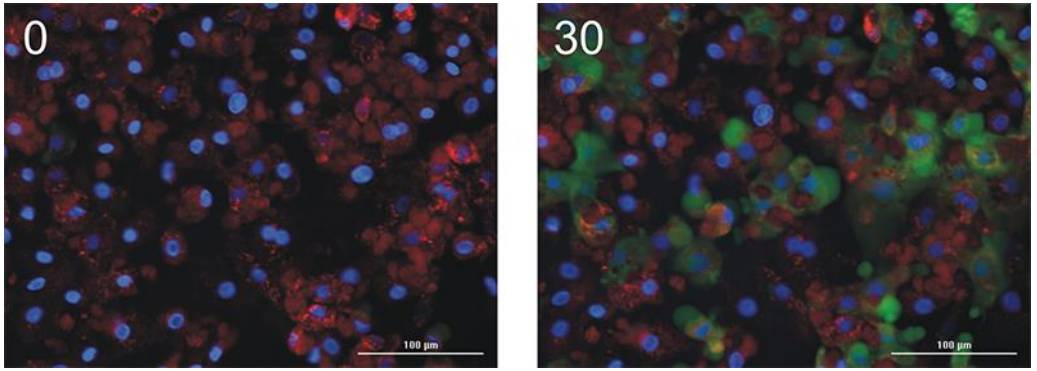
ROS



DCF
(Fluorescent)

2,7-Dichlorodihydrofluorescein (DCFH) FL of product (λ_{ex} = 498 nm, λ_{em} = 522 nm) pM–nM Can be oxidized by other ROS and HRP





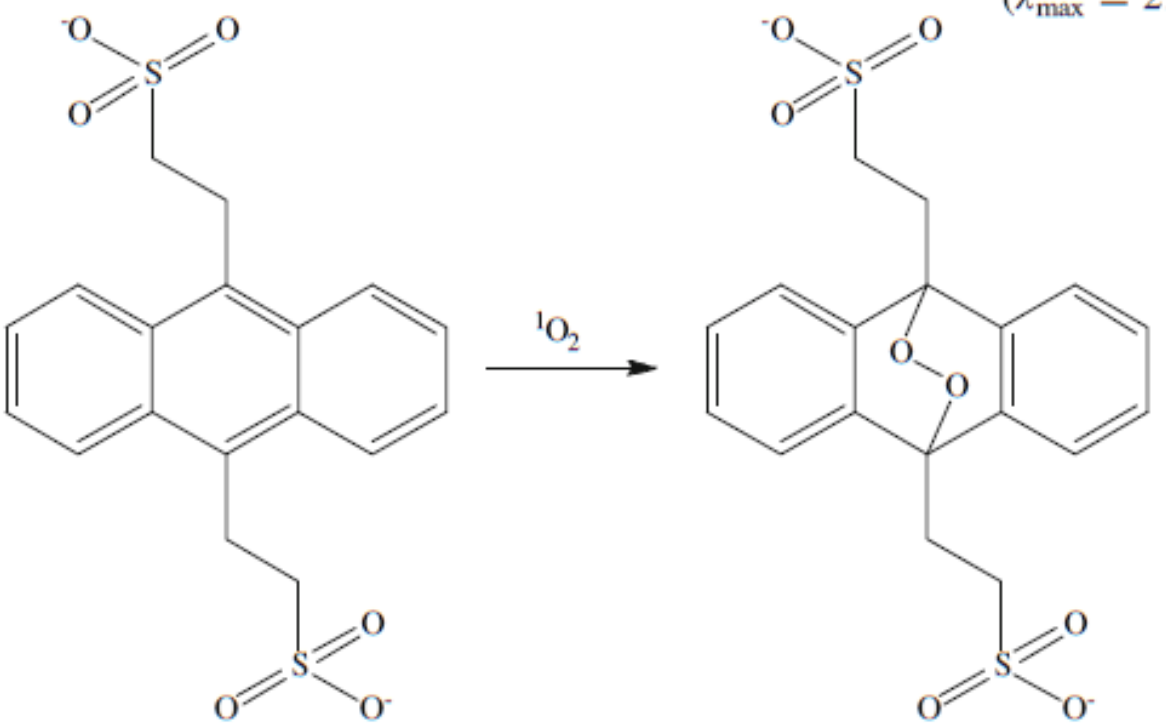
Hoechst 33342 (blue), mitochondria were stained with MitoTracker® Red (Red); and oxidized DCF reagent is visualized in green.

https://www.cellbiolabs.com/reactive-oxygen-species-ros-assay

Detection of singlet oxygen ($^1O_2$)

Anthracene-9,10-bis(ethanesulphonate) (AES)

Absorbance of AES (λ_{max} = 399 nm, $\varepsilon_{399} = 1.26 \times 10^4\ M^{-1}\ cm^{-1}$) and endoperoxide product with HPLC–UV (λ_{max} = 216 nm)



It can detect ·OH

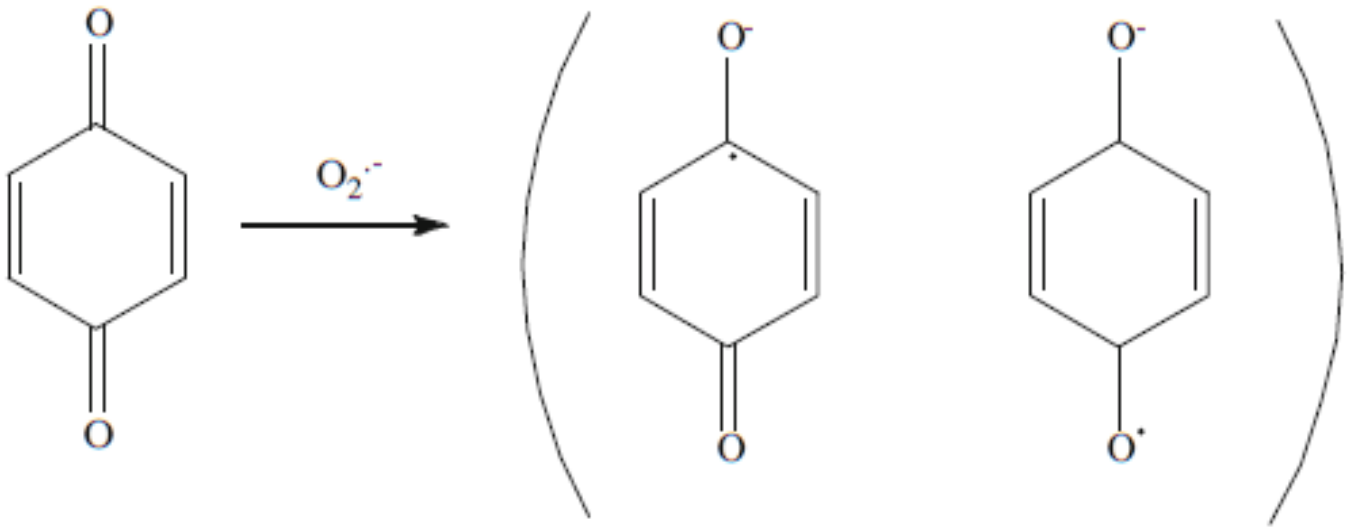
... and many more.

Burns, J.M.; *et al*. *Aquat. Sci.* **2012**, *74*, 683-734

Detection of singlet oxygen ($^1O_2$)

1,4-Benzoquinone

Absorbance of semiquinone($\lambda_{max} = 430$ nm, $\varepsilon_{430} = 6{,}100\ M^{-1}\ cm^{-1}$)



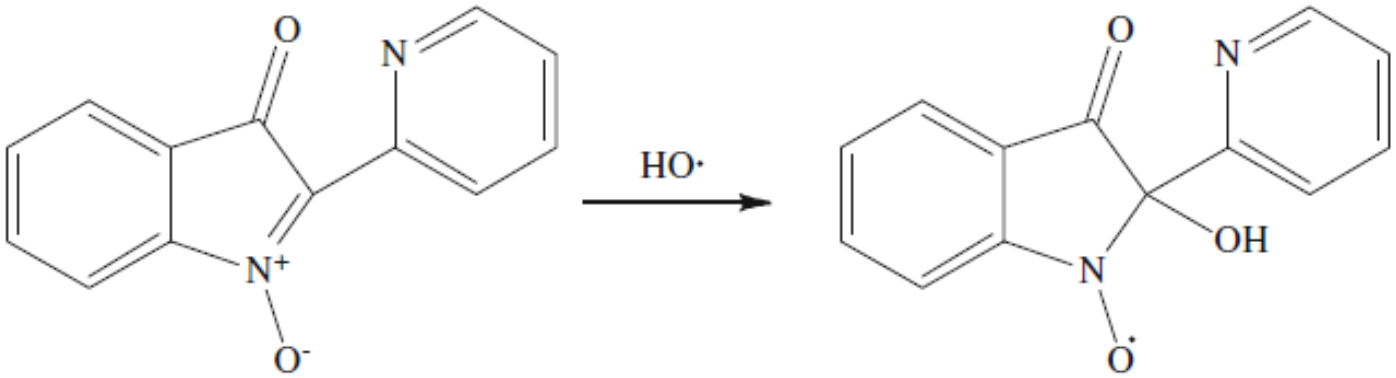
Interference from e_{aq}^- and CO_2^-

... and many more.

Burns, J.M.; *et al*. *Aquat. Sci.* **2012**, *74*, 683-734

Detection of hydroxyl radical (·OH)

2-(2-Pyridyl)-3H-indol-3-one *N*-oxide	EPR spin trapping—signal increase due to adduct	μM	No interference from $O_2^{\cdot-}$



... and many more.

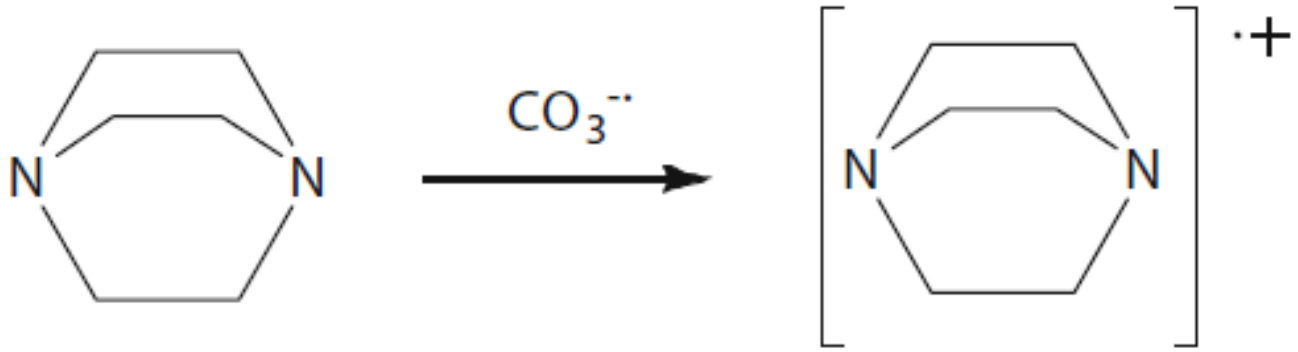
EPR: Electron Paramagnetic Resonance Spectroscopy

Burns, J.M.; *et al*. *Aquat. Sci.* **2012**, *74*, 683-734

Detection of carbonate radical ($CO_3^{\cdot-}$)

1,4-Diaza-bicyclo[2.2.2]octane (DABCO)

Absorbance of radical cation ($\lambda = 465$ nm, $\varepsilon_{465} = 2.1 \times 10^3\ M^{-1}\ cm^{-1}$)



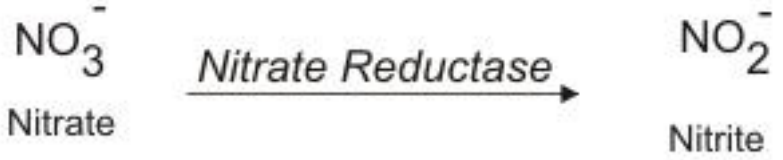
Radical cation has a $t_{1/2} \leq$ ms

... and many more.

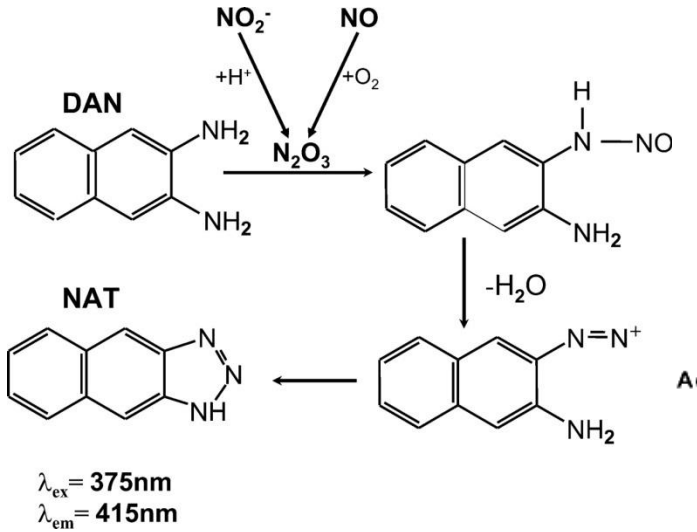
Burns, J.M.; *et al*. *Aquat. Sci.* **2012**, *74*, 683-734

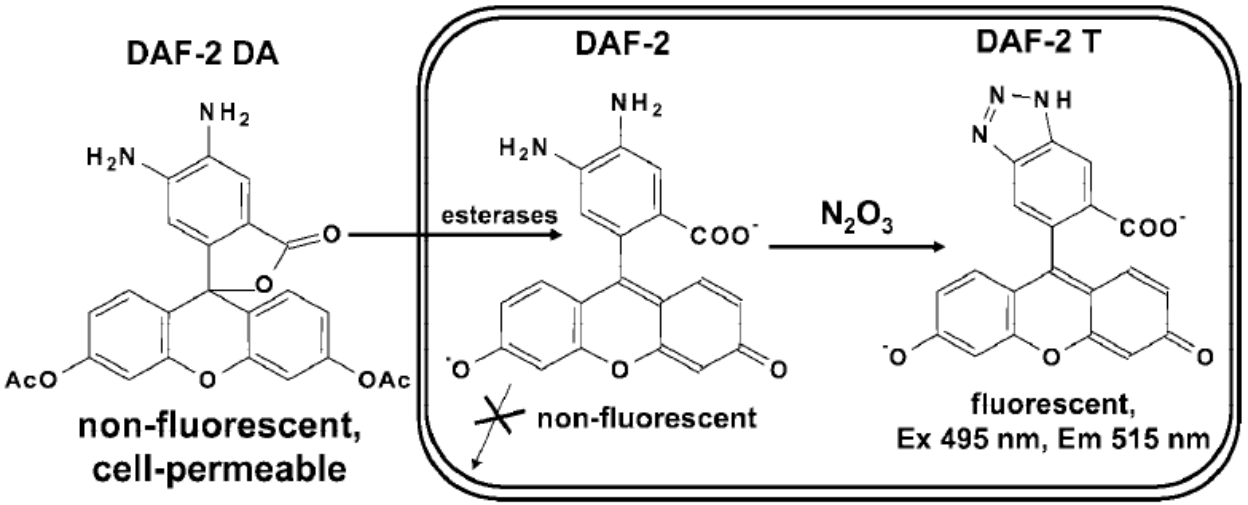
Detection of nitric oxide (NO·)

NO· has a very short life time and it is converted to nitrate than nitrile:



Indirect detection:





Tarpey, M.M.; Wink, D.A.; Grishman, M.B. *Am. J .Physiol. Regul. Integr. Comp. Physiol.* **2004**, *286*, R431-R444

Different types of ROS and RNS produced in the cell

Reactive Oxygen Species (ROS)

Radicals:	
$O_2^{\cdot -}$	Superoxide
$OH^{\cdot}$	Hydroxyl
$RO_2^{\cdot}$	Peroxyl
$RO^{\cdot}$	Alkoxyl
$HO_2^{\cdot}$	Hydroperoxyl

Non-Radicals:	
H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite

Reactive Nitrogen Species (RNS)

Radicals:	
$NO^{\cdot}$	Nitric Oxide
$NO_2^{\cdot}$	Nitrogen dioxide

Non-Radicals:	
$ONOO^-$	Peroxynitrite
ROONO	Alkyl peroxynitrites
N_2O_3	Dinitrogen trioxide
N_2O_4	Dinitrogen tetroxide
HNO_2	Nitrous acid
NO_2^+	Nitronium anion
NO^-	Nitroxyl anion
NO^+	Nitrosyl cation
NO_2Cl	Nitryl chloride

Dhawan, V. N.K. Reactive Oxygen and Nitrogen Species: General Considerations. In: *Studies on Respiratory Disorders*, Oxidative Stress in Applied Basic Research and Clinical Practice, Ganguly et al. (eds.), Springer, New York, 2014

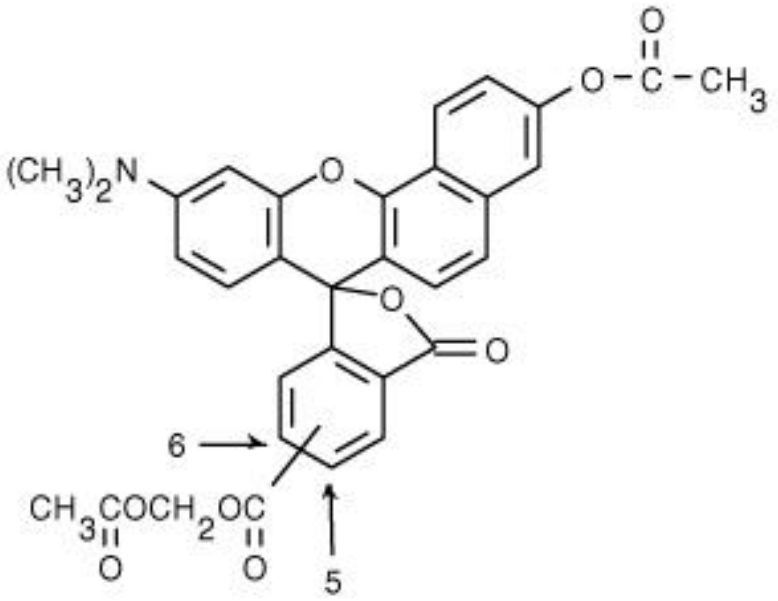
Enzymatic and nonenzymatic antioxidants

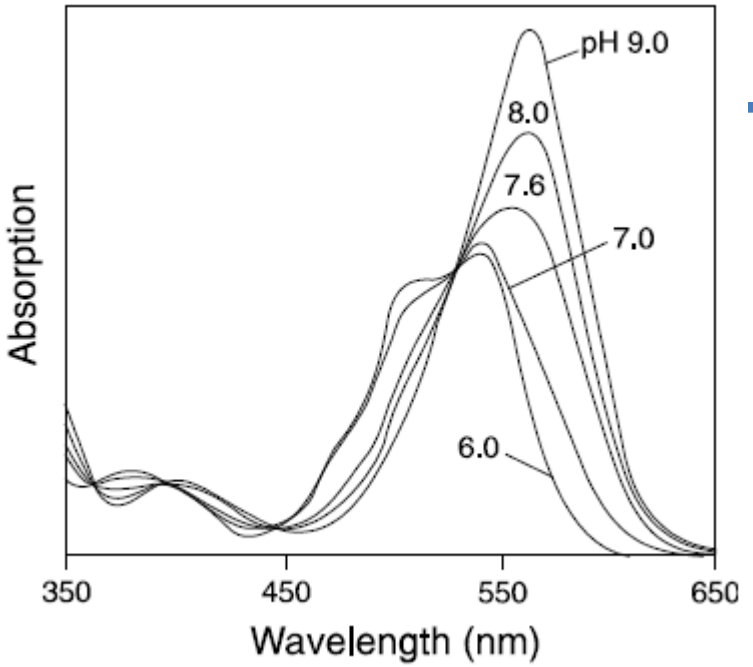
Enzymatic antioxidants	Nonenzymatic antioxidants
Thioredoxin (Trx)	Vitamins C, E, A
Peroxiredoxins (Prx)	Thiols
Glutaredoxin (Grx)	β-Carotene
Glutathione peroxidase (Gpx)	Polyphenols
Reduced glutathione (GSH)	NAC
Oxidized glutathione (GSSG)	Zinc, selenium
Glutathione reductase (GR)	Glutathione
Extracellular glutathione peroxidase (eGPx)	Uric acid
Catalase	Lycopene
Peroxidase	Allyl sulfide
Superoxide dismutase	Indoles
	Gallic acid
	Hesperitin
	Catechin
	Chrysin

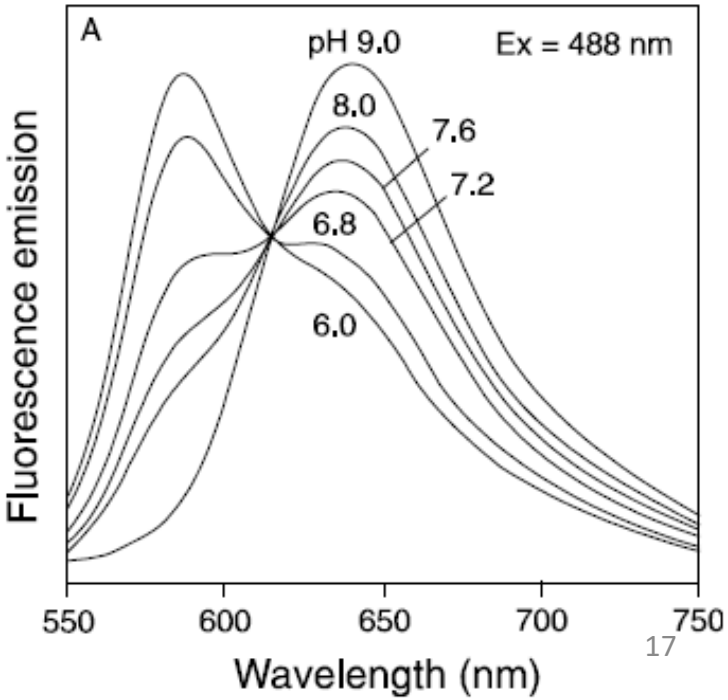
Dhawan, V. N.K. Reactive Oxygen and Nitrogen Species: General Considerations. In: *Studies on Respiratory Disorders*, Oxidative Stress in Applied Basic Research and Clinical Practice, Ganguly et al. (eds.), Springer, New York, 2014

pH indicators

5-(and-6)-Carboxy SNARF™-1





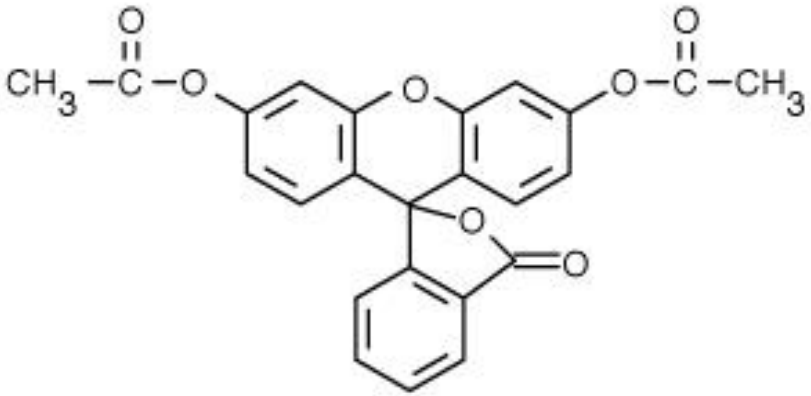


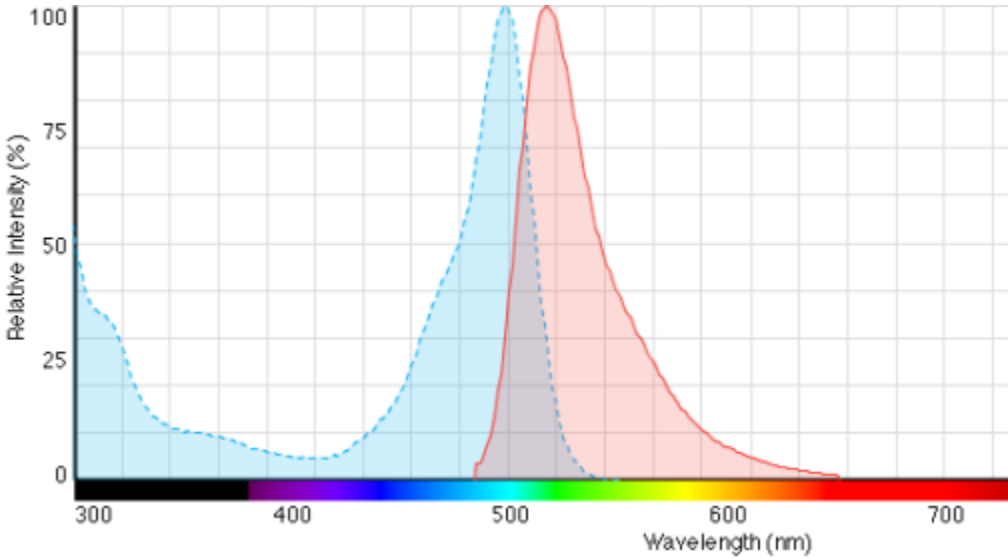
Parent Fluorophore	pH Range	Typical Measurement
SNARF indicators	6.0–8.0	Emission ratio 580/640 nm

https://www.thermofisher.com/order/catalog/product/C1270

pH indicators

Fluorescein diacetate





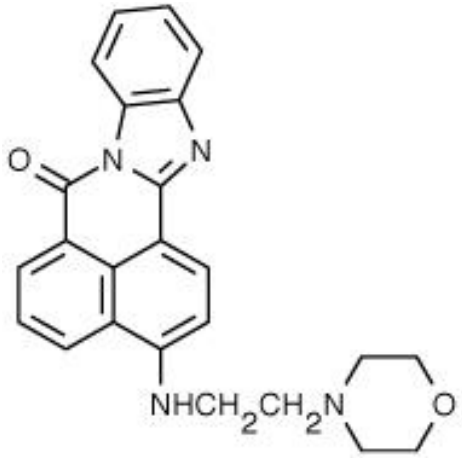
Parent Fluorophore	pH Range	Typical Measurement
Fluoresceins and carboxyfluoresceins	6.0–7.2	Excitation ratio 490/450 nm

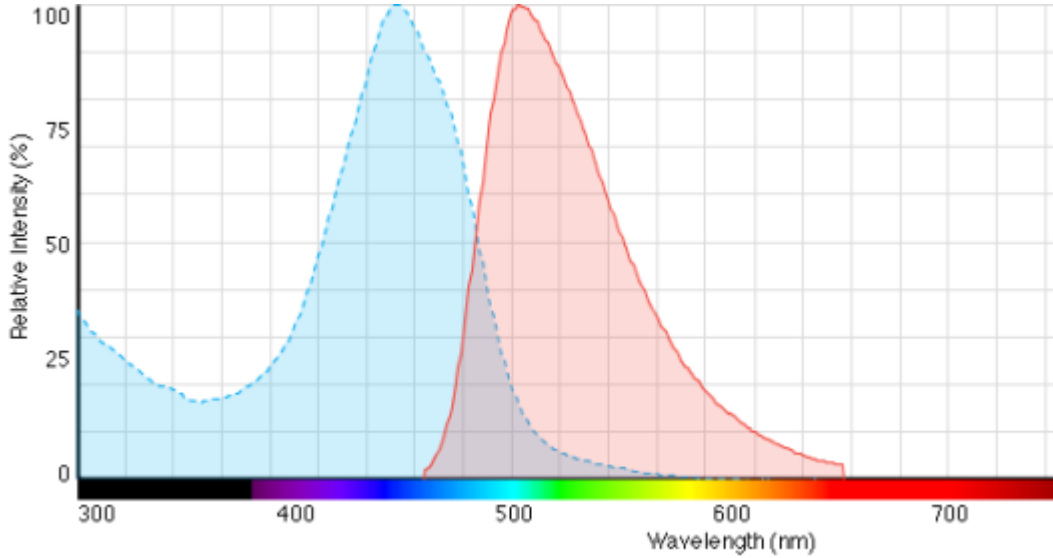
Also used for cell viability assays.

https://www.thermofisher.com/order/catalog/product/C1270

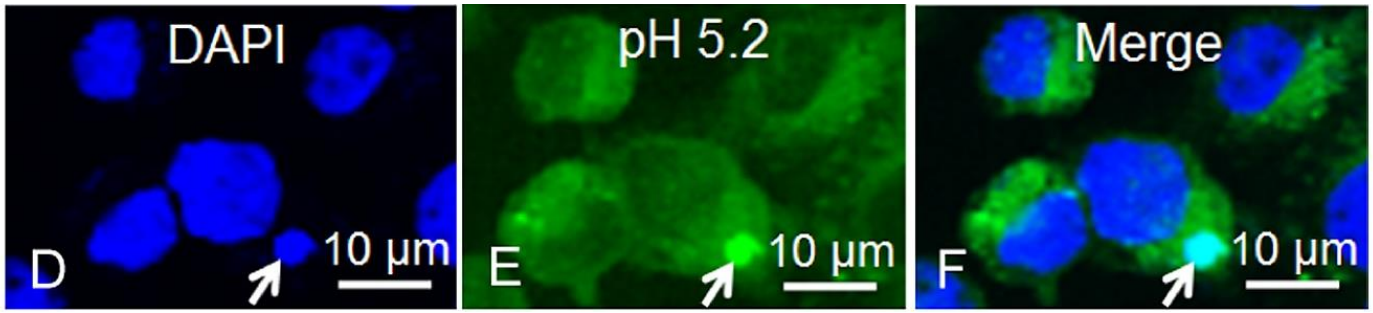
pH indicators

LysoSensor Green DND-189





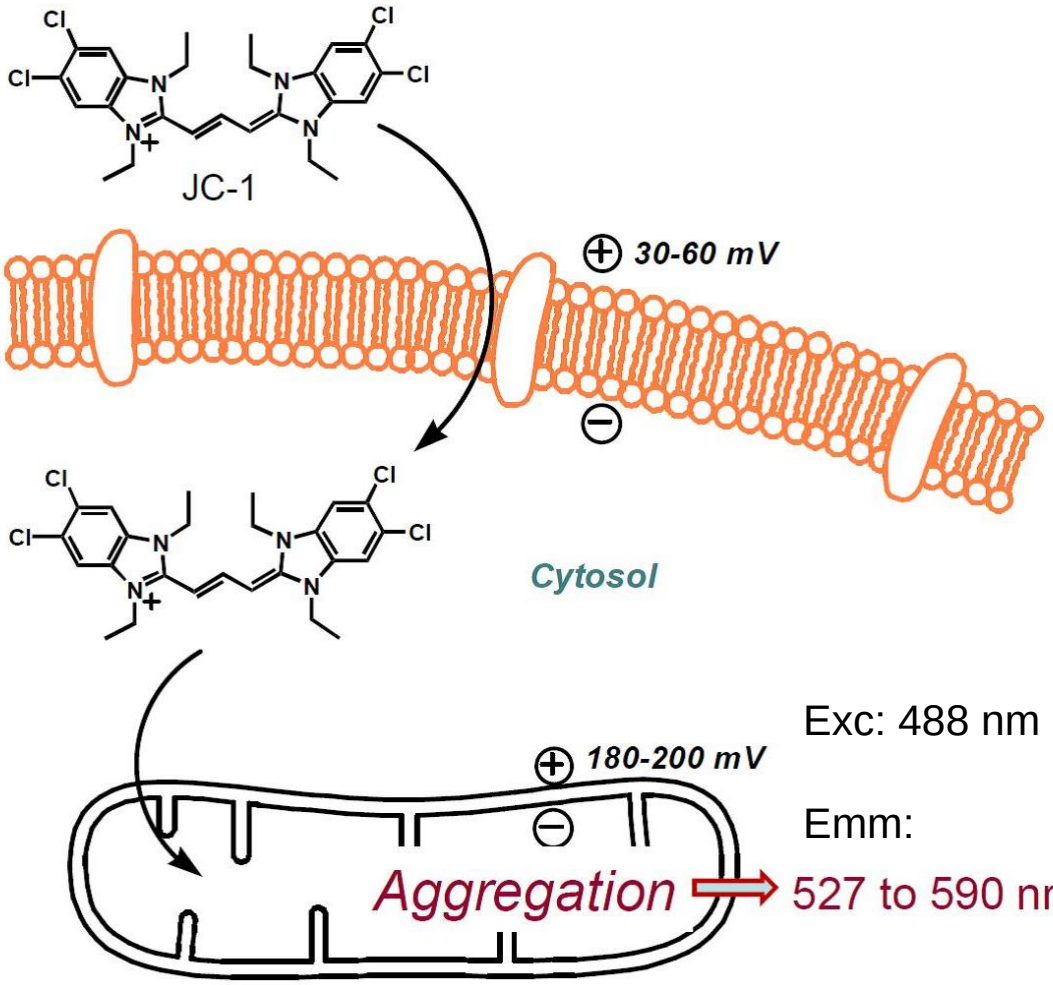
Parent Fluorophore	pH Range	Typical Measurement
LysoSensor Green DND-189	4.5–6.0	Single emission 520 nm



https://www.thermofisher.com/order/catalog/product/L7535
Cheng, Y.; *et al*. *PLoS ONE* **2014**, *9*, e88461

Apoptosis indicator

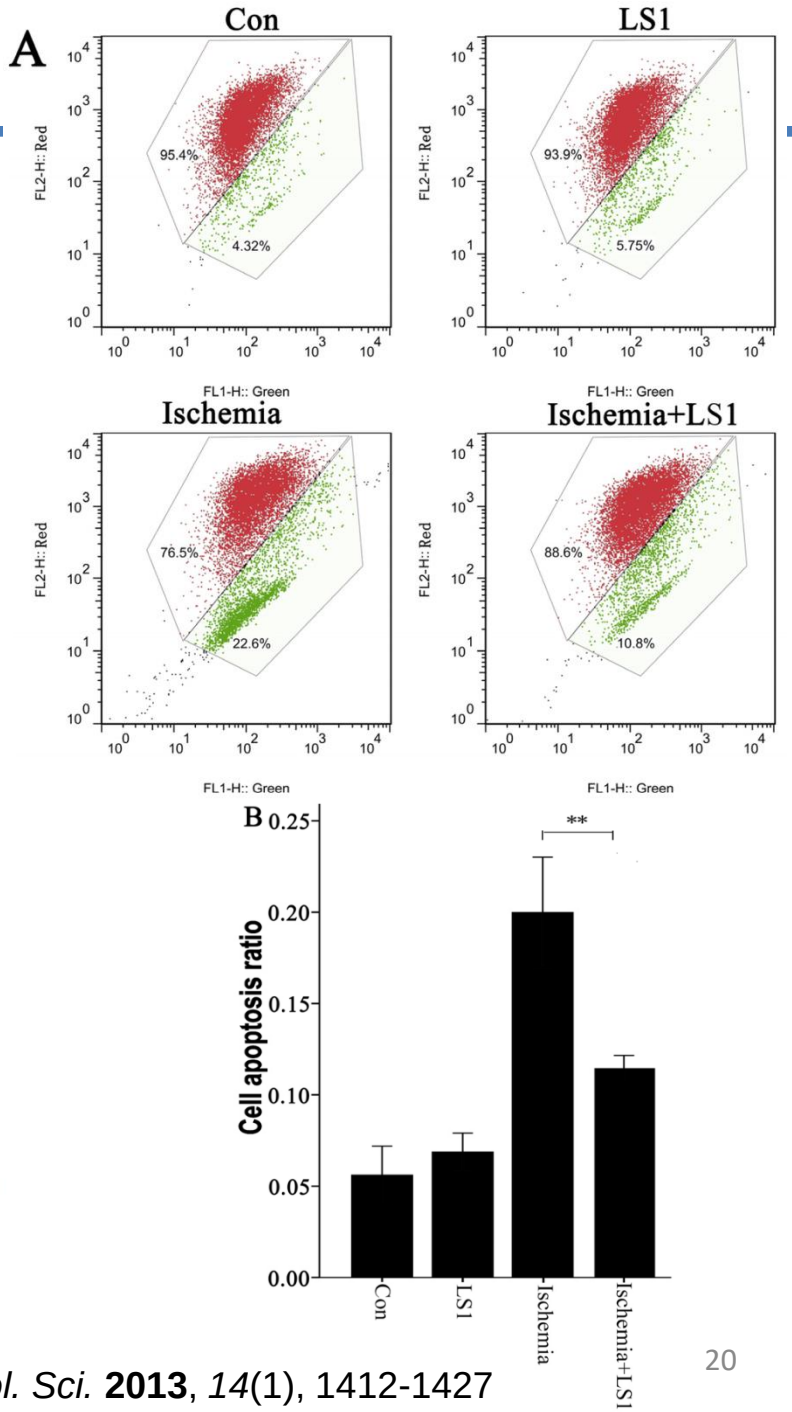
JC-1 dye



Exc: 488 nm

Emm:

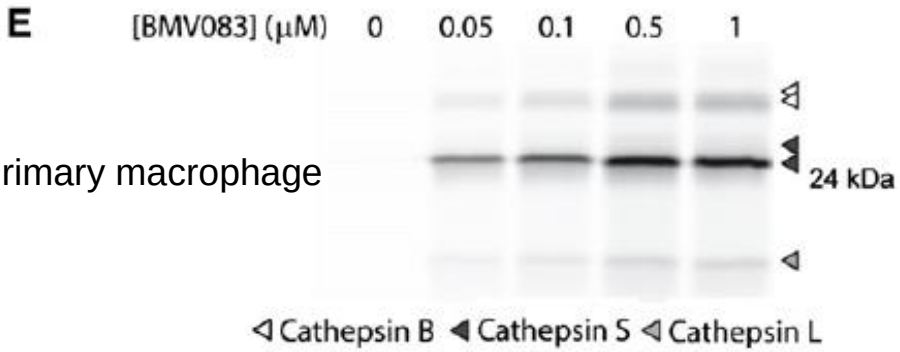
527 to 590 nm



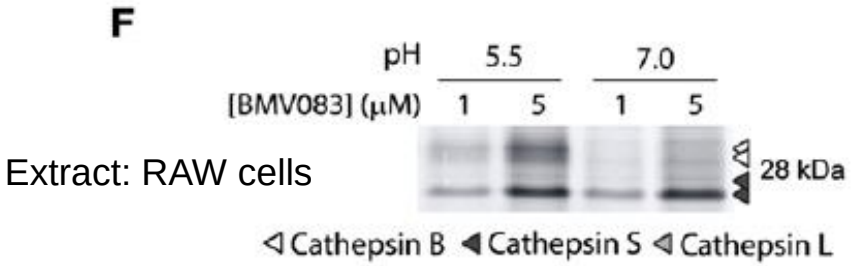
Tian, S.; *et al. Int. J. Mol. Sci.* **2013**, *14*(1), 1412-1427

Example of indicator of enzyme activity

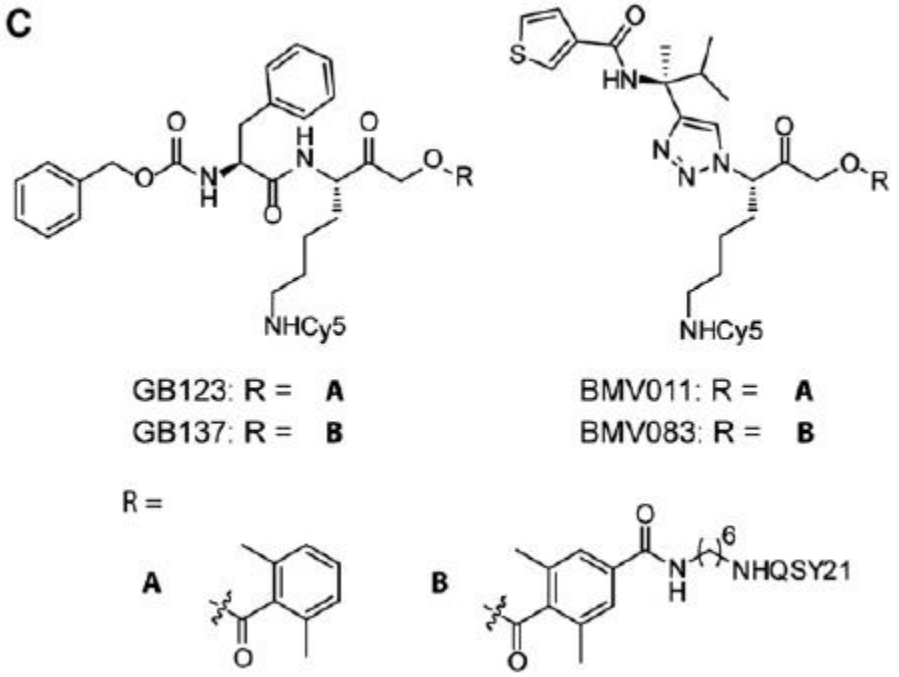
Probing for cysteine protease activity



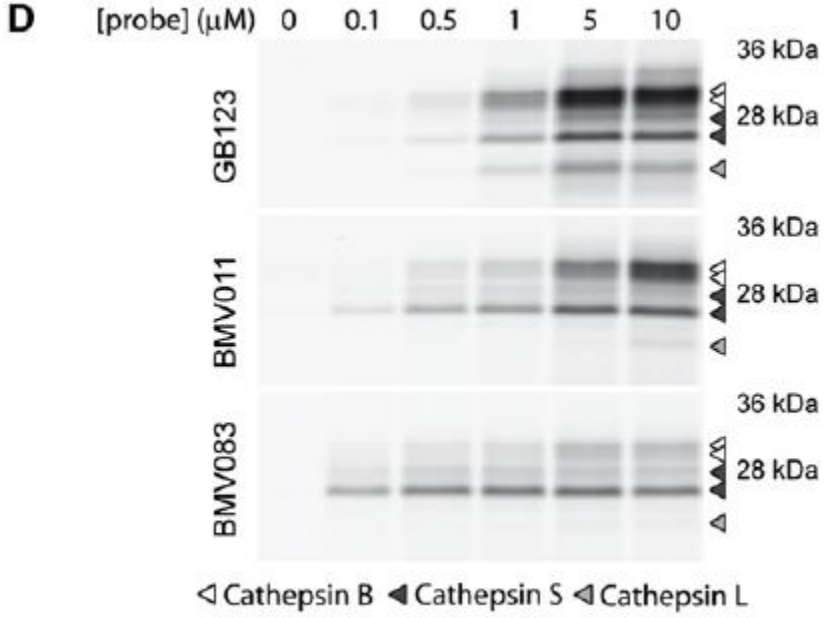
Primary macrophage



Extract: RAW cells



Living RAW cells



Verdoes, M.; *et al. Chem. Biol.* **2012**, *19*, 619-628