Recent advances in reactive oxygen species detection methods

Silvia Sorce

Journal Club, 1st April 2014

Outline

- Introduction to ROS biology
- How to detect ROS
- The Neurinox consortium

Definitions (I)

Free Radicals:

- Any species that contains one or more unpaired electrons

$R_3C^{\cdot}$	Carbon-centered
$R_3N^{\cdot}$	Nitrogen-centered
$R\text{-}O^{\cdot}$	Oxygen-centered
$R\text{-}S^{\cdot}$	Sulfur-centered

Non-Radicals:

- Species that have strong oxidizing potential

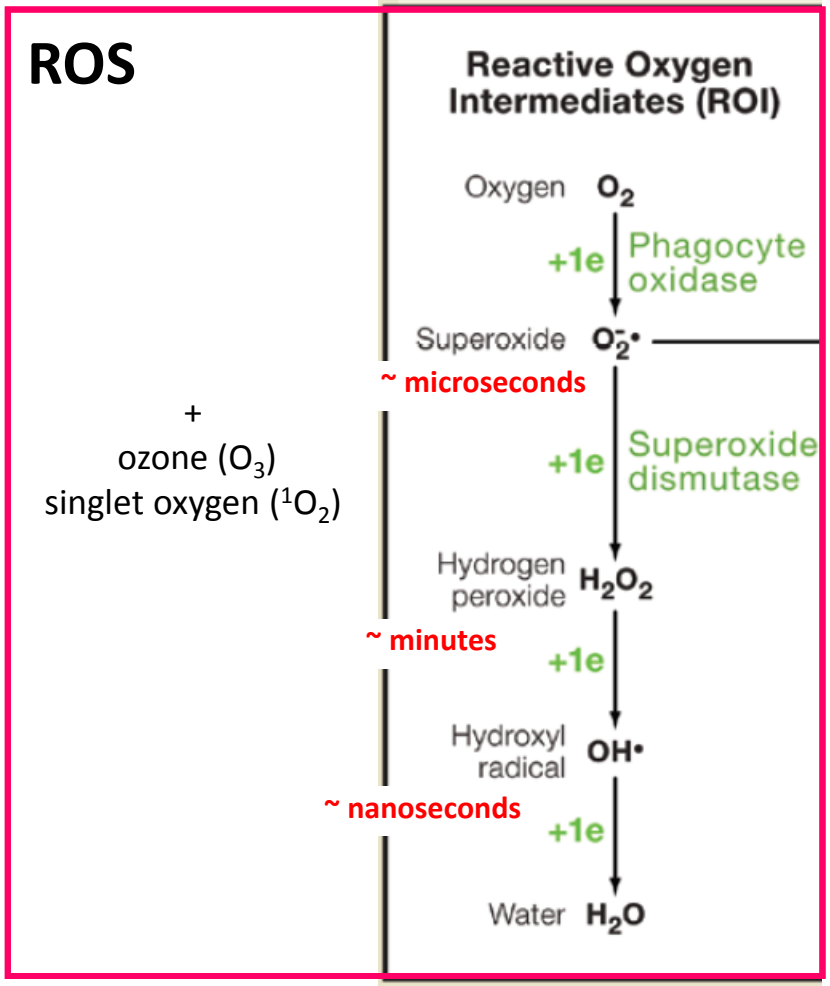
H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite

Definitions (II)

Term	Definition
Oxidation	Gain in oxygen Loss of hydrogen Loss of electrons
Reduction	Loss of oxygen Gain of hydrogen Gain of electrons
Oxidant	Oxidizes another chemical <u>by taking</u> electrons, hydrogen, or by adding oxygen
Reductant	Reduces another chemical <u>by supplying</u> electrons, hydrogen, or by removing oxygen

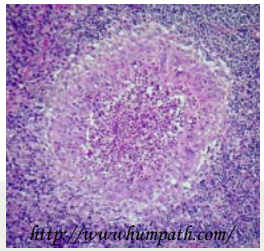
Reactive Oxygen Species

ROI: 1 electron reduction products of O_2
en route to the production of water



952 Cell *140*, March 19, 2010 ©2010 Elsevier Inc. DOI 10.1016/j.cell.2010.03.008

Historical milestones in ROS biology (I)



Timeline | **A sampling of milestones in ROS biology***

1908 — Warburg discovers increased consumption of oxygen by sea urchin eggs following fertilization[134]. The biological importance only becomes apparent in 1978

1928 — Fungal glucose oxidase is the first enzyme found to produce ROS, namely H_2O_2 (REF. 135)

1929 — Fleming discovers that *Penicillium notatum* releases an antibacterial product. The search for other antibiotics from fungi is hindered by contamination with fungal glucose oxidase, which produces H_2O_2 that contributes additional antibiotic activity[136]. It is discovered 78 years later that many antibiotics themselves, including β-lactams such as penicillin, partly work by causing bacteria to generate ROS

1959 — Phagocytosing PMNs are found to consume large amounts of oxygen by a non-mitochondrial process[137]

1961 — The first ROS identified as being produced by a mammalian cell is H_2O_2 in phagocytosing PMNs[138], which explains their increased oxygen consumption

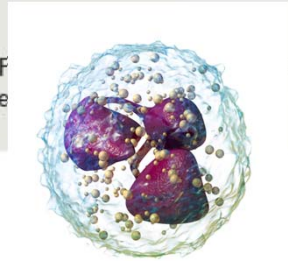
1969 — Discovery of superoxide dismutase from diverse tissues indicates production of superoxide[139]

1973 — The hypothesis that some cells can produce superoxide is confirmed. These cells are found to be PMNs[140]

1974 — PMNs from patients with CGD, who have increased susceptibility to infection, are found to produce little or no superoxide. This suggests the first biological function for ROS: the killing of pathogens. Production of ROS represents the first defined host defence mechanism[141]

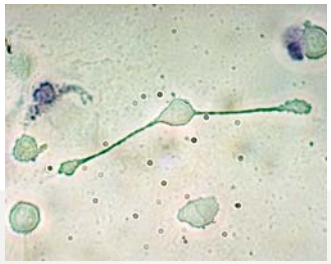
1975 — Non-activated macrophages are found to relieve the dependence of lymphoid cells on exogenous thiols for their survival *in vitro*. By contrast, immunologically activated macrophages, which were soon found to produce ROS (see 1977), do not fulfil this accessory cell function. The lack of trophic support by activated macrophages contributes to their apparent ability to kill lymphoma cells[90]

CGD, chronic granulomatous disease; F dministration; IFNγ, interferon-γ; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species. *This Timeline is an incomplete citations.



Nathan and Cunningham-Bussel, NATURE REVIEWS IMMUNOLOGY, 2013

Historical milestones in ROS biology (II)



Macrophages are identified as the second mammalian cell type to produce ROS. ROS production increases markedly following immunological activation and this is associated with enhanced antimicrobial activity[142]

Leukocyte-derived H_2O_2 is confirmed to kill many kinds of pathogens, a process that is facilitated by myeloperoxidase[145]

IFNγ is defined as a major inducer of ROS production and antimicrobial function in mouse macrophages[146]

Prophylaxis with IFNγ is tested in children with CGD and found to markedly reduce the incidence of infections[149]. The FDA soon approves recombinant IFNγ for clinical use[66]

Human tumour cell lines are found to produce H_2O_2 spontaneously at rates comparable to PMNs[110]. These were the first mammalian, non-myeloid cells found to produce large amounts of ROS. It is hypothesized that tumour-derived ROS contribute to oncogenesis and cancer progression

Additional isoforms of ROS-producing NADPH oxidases are identified in non-myeloid cells and implicated in malignant transformation[150]

1977 1978 1980 1983 1986 1988 1991 1999 2007

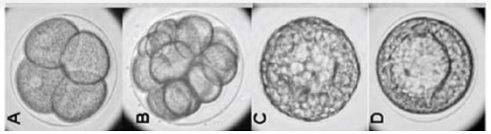
A non-myeloid cell is identified that undergoes an H_2O_2-producing respiratory burst like that of PMN and macrophages: the sea urchin egg undergoing fertilization. Besides explaining Warburg's 1908 observation, this shows a second biological function of ROS: oxidative crosslinking of tyrosine residues to produce the fertilization membrane, preventing polyspermy[143]

A superoxide-producing, cytochrome-containing activity is discovered in neutrophil membranes[144]

IFNγ is defined as a principal inducer of ROS production and antimicrobial function in humans[147]

A subunit of the superoxide-producing NADPH oxidase enzyme discovered in 1978 is cloned on the basis of its being mutated in patients with CGD. This is the first enzyme identified the function of which seems to be the *de novo* generation of ROS, as opposed to making ROS as a by-product or turning one ROS into another[148]

Antibiotics with diverse primary targets are found to kill bacteria partly by inducing them to produce ROS[129]. Thus, a major form of natural host defence against infection and a principal form of medical treatment for infection converge on the generation of ROS



Nathan and Cunningham-Bussel, NATURE REVIEWS IMMUNOLOGY, 2013

Sources of ROS

Box 1 | **Sources of ROS and mediators of their catabolism**

Exogenous sources of ROS

- Smoke
- Air pollutants
- Ultraviolet radiation
- γ-irradiation
- Several drugs

Endogenous sources of ROS

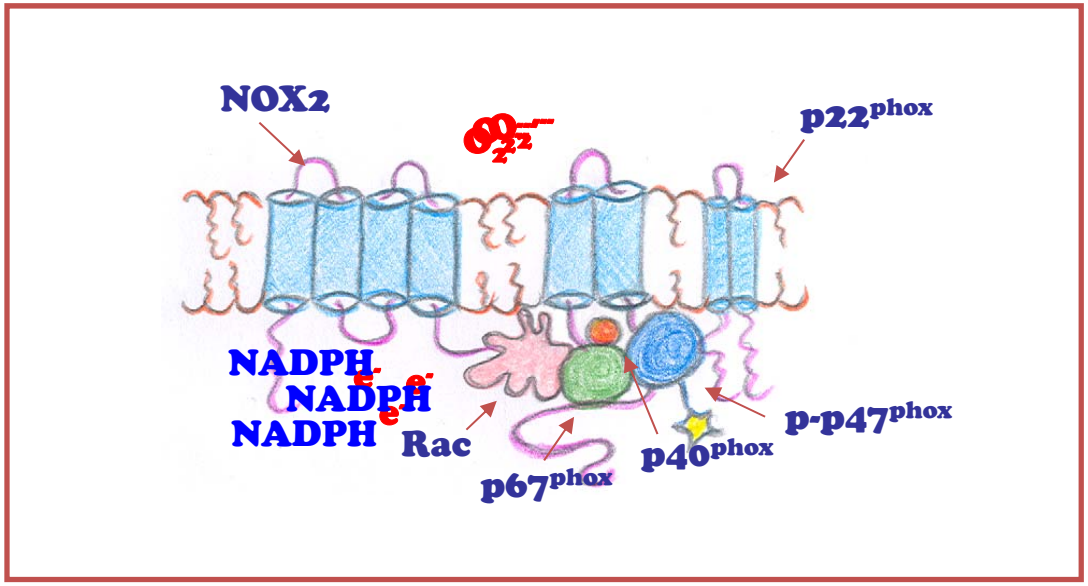
- NADPH oxidases
- Mitochondria
- ER flavoenzyme ERO1
- Xanthine oxidase
- Lipoxygenases
- Cyclooxygenases
- Cytochrome P450 enzymes
- Flavin-dependent demethylase
- Polyamine and amino acid oxidases
- Nitric oxide synthases
- Free iron or copper ions
- Haem groups
- Metal storage proteins

ER, endoplasmic reticulum; ROS, reactive oxygen species. Sources reviewed in REFS 10–12.

de novo ROS generation

Nathan and Cunningham-Bussel, NATURE REVIEWS IMMUNOLOGY, 2013

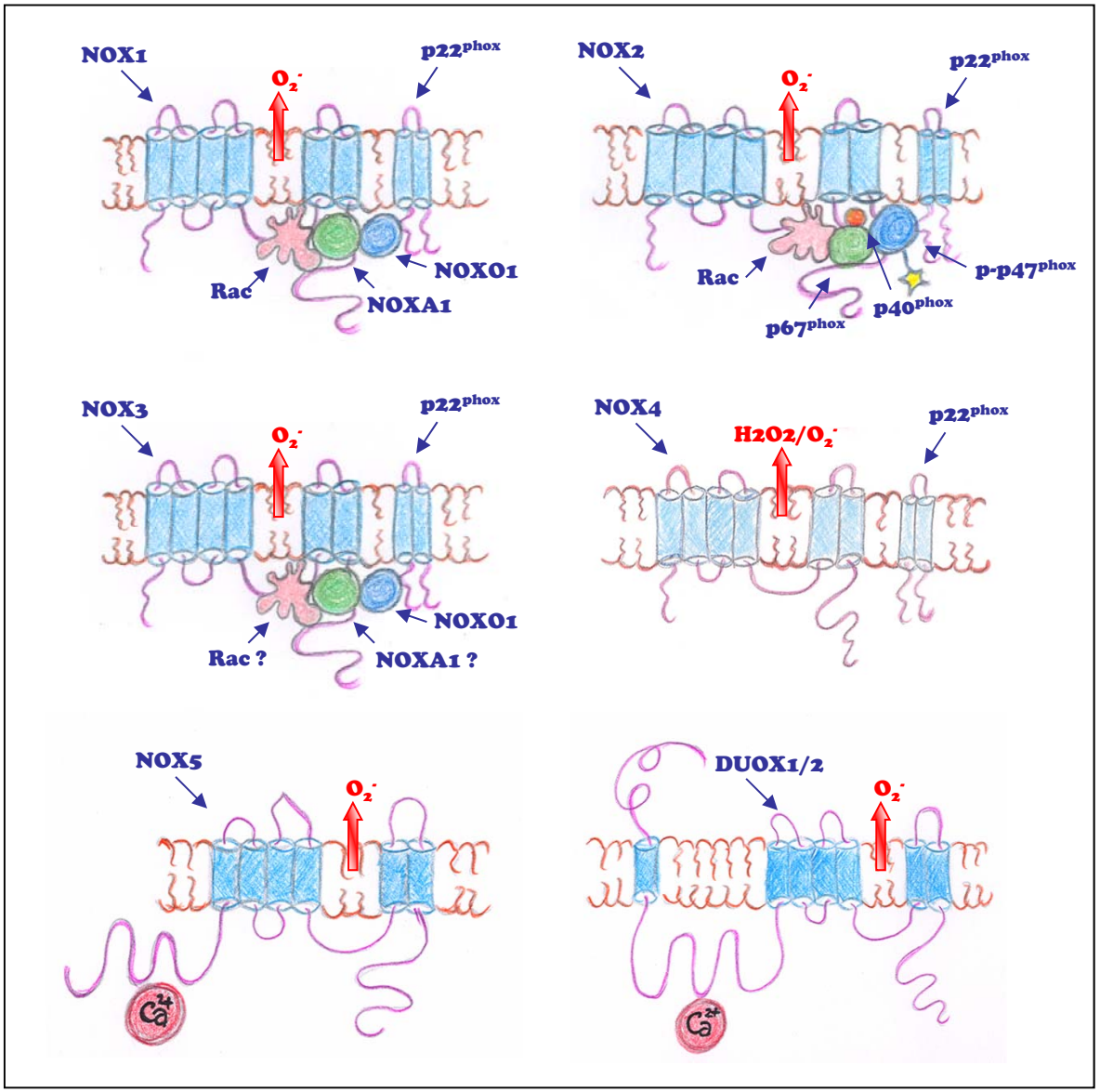
NOX2: the prototype isoform



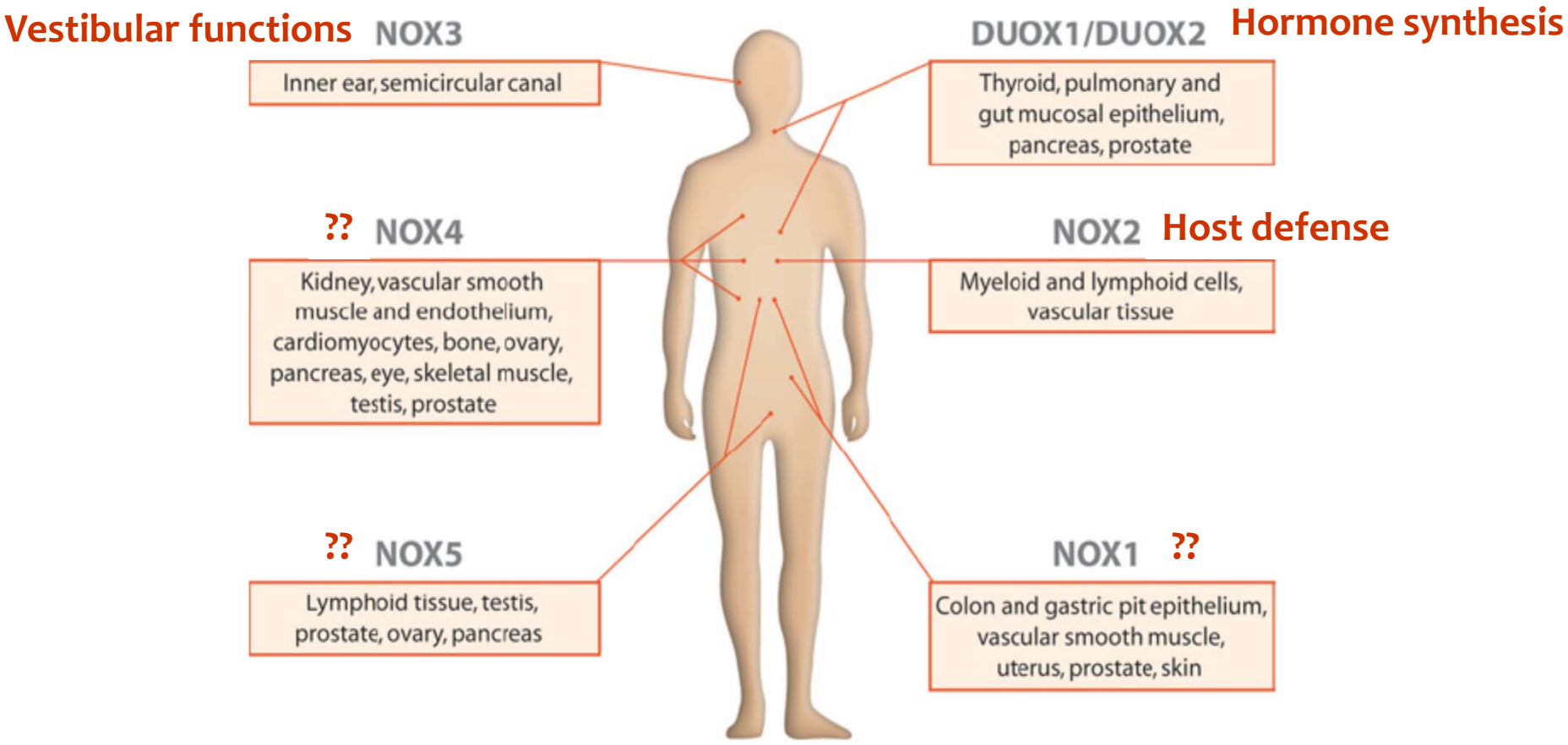
→ NOX2 works like an electron transport chain

→ Product of the enzymatic reaction: superoxide → dismutated to H_2O_2

NOX family


NOX1
p22phox
O2-
Rac
NOXO1
NOXA1
NOX2
p22phox
O2-
Rac
p-p47phox
p67phox
p40phox
NOX3
p22phox
O2-
Rac ?
NOXO1
NOXA1 ?
NOX4
H2O2/O2-
p22phox
NOX5
O2-
Ca2+
DUOX1/2
O2-
Ca2+

NOX expression and physiological functions



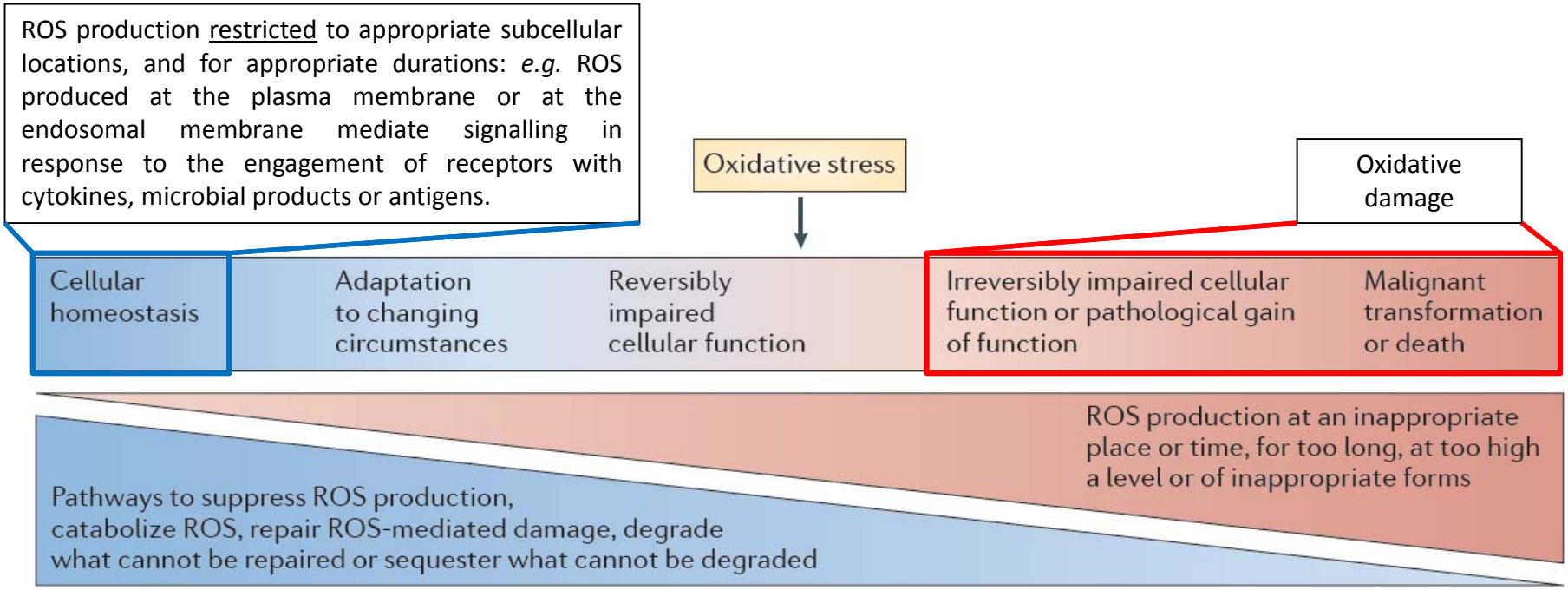
Quinn *et al.*, *Clinical Science* 2006; Bedard and Krause, *Physiol Rev*, 2007

Oxidative stress

Definition:

old : excessive production of ROS

emerging concept: aberration in redox signaling and control



Nathan and Cunningham-Bussel, NATURE REVIEWS IMMUNOLOGY, 2013

ROS detection methods

Direct measurements:

- Electron spin resonance (ESR) spectroscopy

Indirect measurements:

- Using probes which react with oxidants

- Analysing oxidation products *e.g.* proteins, DNA…

Probes for indirect detection methods

in vitro-ex vivo assays:

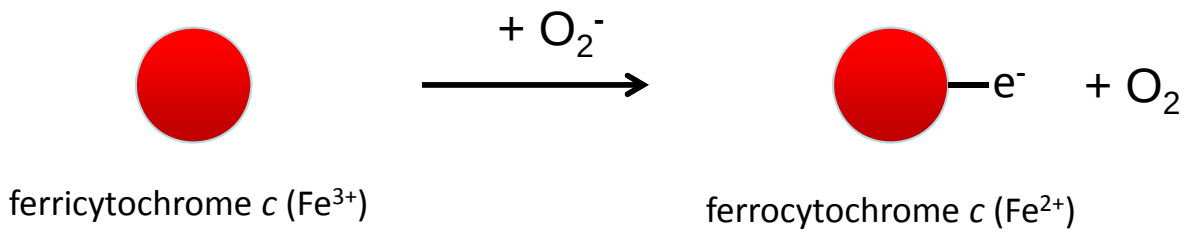
absorbance	luminescence	fluorescence
- Cytochrome C reduction	- Lucigenin	- DHE
- NBT	- Luminol	- DCFH-DA
	- L-012	- Amplex red
	- MCLA	- DHR123
		- “masked” probes

mostly performed using a plate reader:

in vivo live imaging:

- Peroxy Caged luciferin (luminescence)
- Lucigenin/luminol (luminescence)
- Qcy-7 (fluorescence)

Absorbance 1: Cytochrome *c* reduction



SAMPLES: phagocytes > cell lysates/membranes > tissue segments

REAGENTS: buffer composed of (mmol/L): NaCl, 145; KCl, 4.86; NaH_2PO_4, 5.7; $CaCl_2$, 0.54; $MgS0_4$, 1.22; glucose, 5.5; deferoxamine mesylate, 0.1; 50 µmol/L of acetylated ferricytochrome c; manganese superoxide dismutase (100 U/mL); catalase (125 U/mL)

Strenghts:

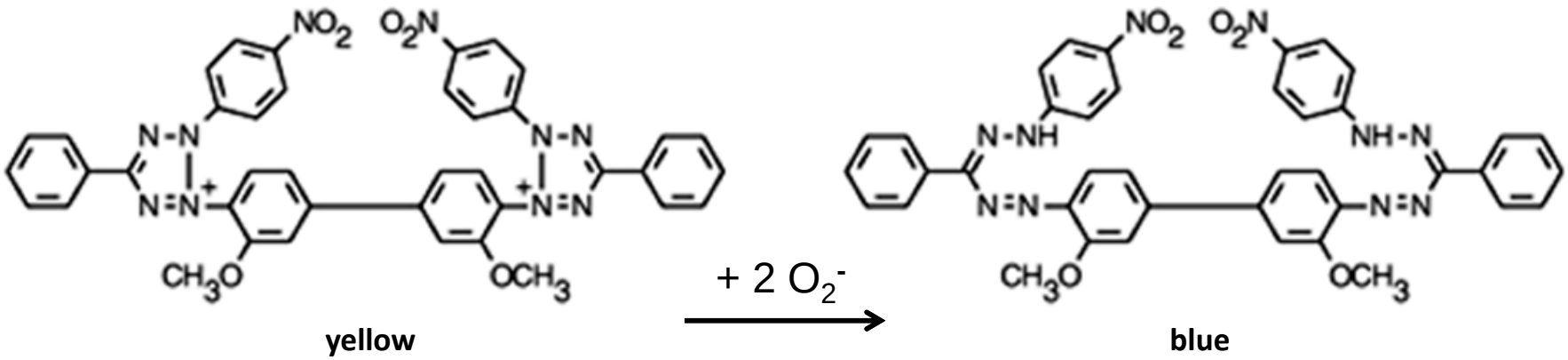
- Easy
- Quite accurate estimates of O_2^- in picomolar range

Caveats:

- Not very sensitive → good detection only for large amounts *e.g.* phagocytes
- Specificity → can be directly reduced by enzymes and other molecules/can be re-oxidized → + SOD and catalase
- Only detects extracellular O_2^-

Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

Absorbance 2: Nitroblue tetrazolium (NBT)



SAMPLES: intact cells

METHOD: Monoformazan can be detected spectrophotometrically at 550nm.
However, the monoformazan usually precipitates and most methods dissolve the crystals in cells by addition of a solvent such as dimethylsulfoxide and then measure the blue color at 630nm.

Strenghts:

- Very simple → diagnosis for CGD
- Intracellular O_2^- production

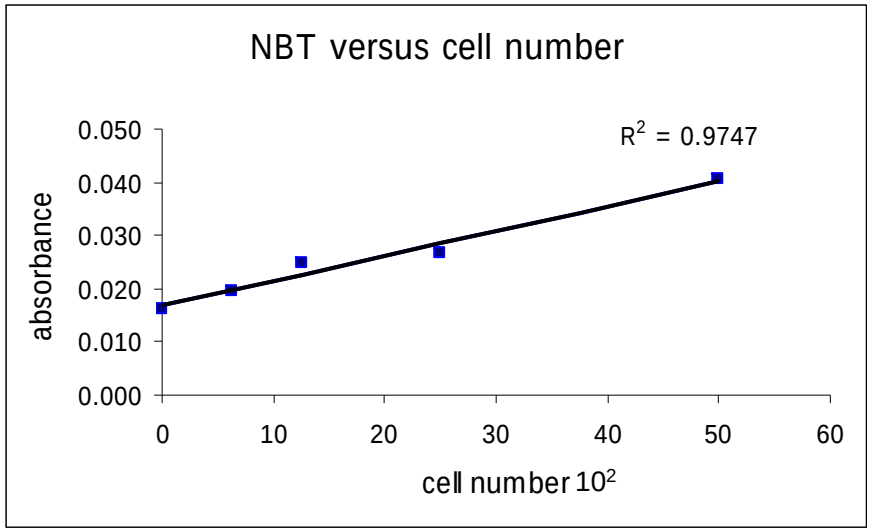
Caveats:

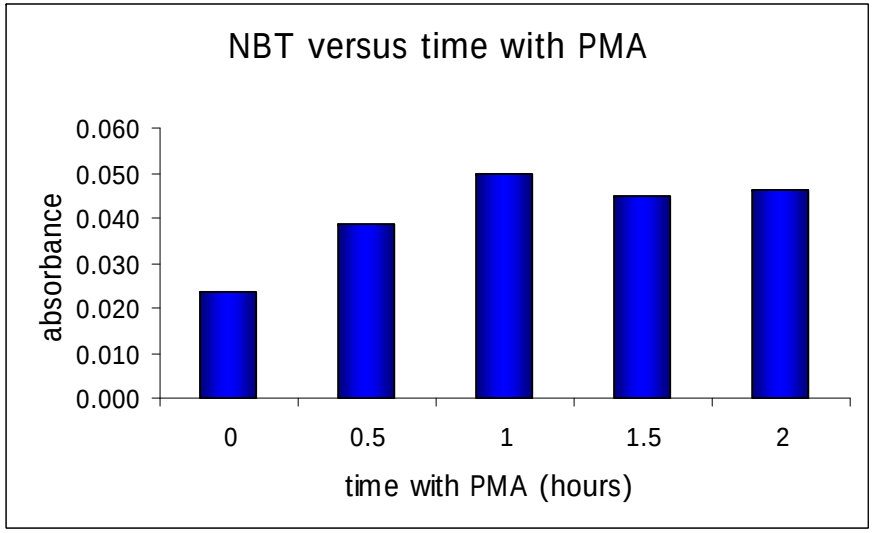
- Specificity → can be directly reduced by cellular enzymes, cross-reactivity with NOS
- Not very sensitive → good detection only for large amounts *e.g.* phagocytes

Alternative: **WST-1** (sulfonated tetrazolium salt) → can be reduced by O_2^- to a water soluble formazan with low background, moderate sensitivity

Maghzal *et al.,* Free Radic Biol Med. 2012

Absorbance 2: Nitroblue tetrazolium (NBT) → example





- 0.1 uM PMA final
- 1 mg/mL NBT
- In HBSS, 200 uL volume
- Incubation for 1h

- Centrifuged
- removed NBT solution
- Suspended pellet in 20 uL isopropanol and 20 uL DMSO

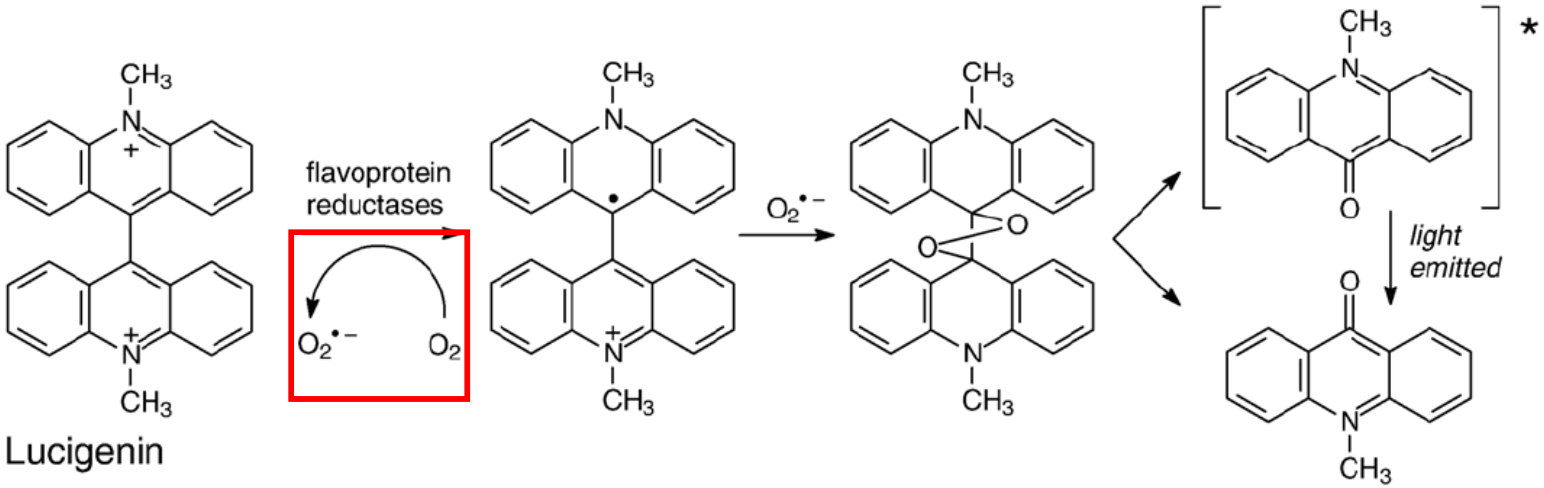
- Read absorbance at 570 nm

- Only about 2X difference between no cells and 50 000

- No further reaction after 1 hour?

Courtesy of V. Jaquet, University of Geneva

Chemiluminescence 1: lucigenin



SAMPLES: intact cells > tissue segments > homogenates, purified membrane

REAGENTS: Krebs/HEPES buffer; 5 μm lucigenin

Strenghts:

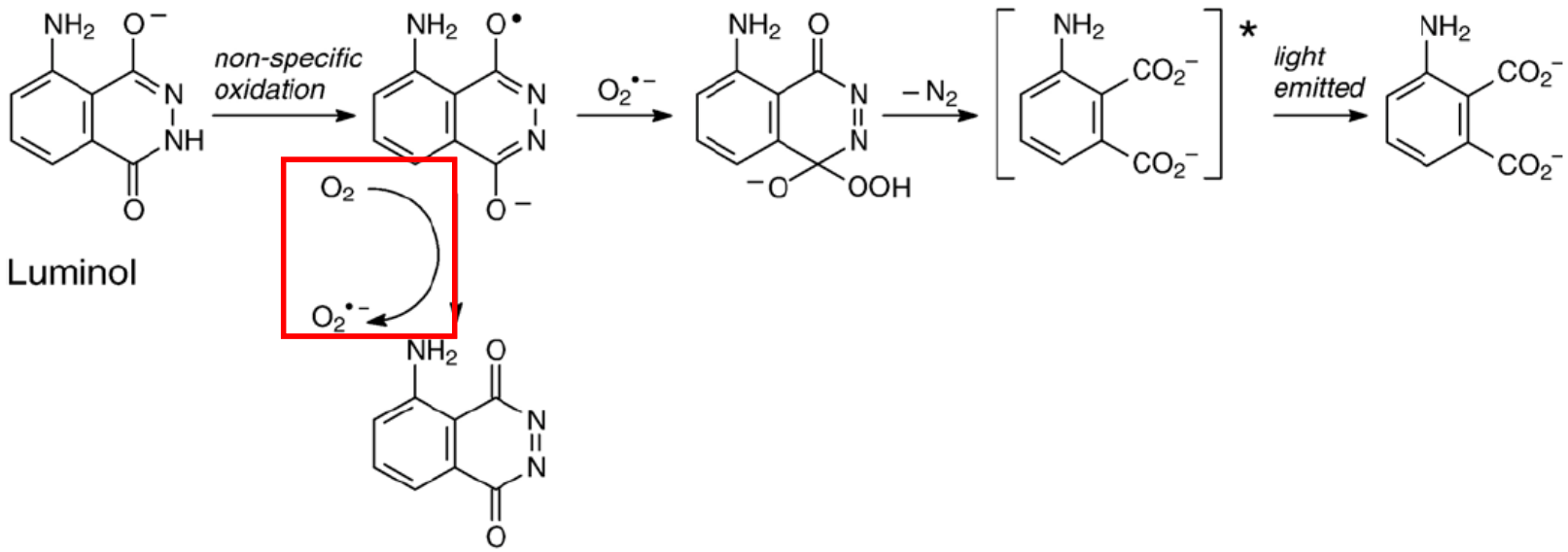
- Simple, not expensive
- Very sensitive, minimal toxicity
- Selective for O_2^- production

Caveats:

- Redox cycling: possible artifact, even when low lucigenin concentration are used
- Extracellular production only

P. Wardman; Free Radic Biol Med. 2007; Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

Chemiluminescence 2: luminol



SAMPLES: intact cells > tissue segments

REAGENTS: Krebs/HEPES buffer; 5 μm lucigenin

Strenghts:

- Simple, not expensive
- Sensitive, minimal toxicity, cell permeable
- Extracellular + Intracellular ROS production

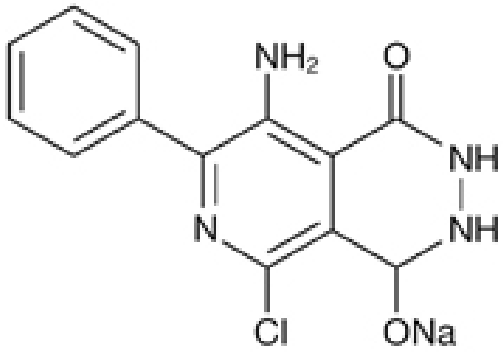
Caveats:

- Needs peroxidase for the reaction to occur
- Not specific: can react with different ROS
- pH-dependent

P. Wardman; Free Radic Biol Med. 2007; Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

Chemiluminescence 3: L-012 and MCLA

L-012



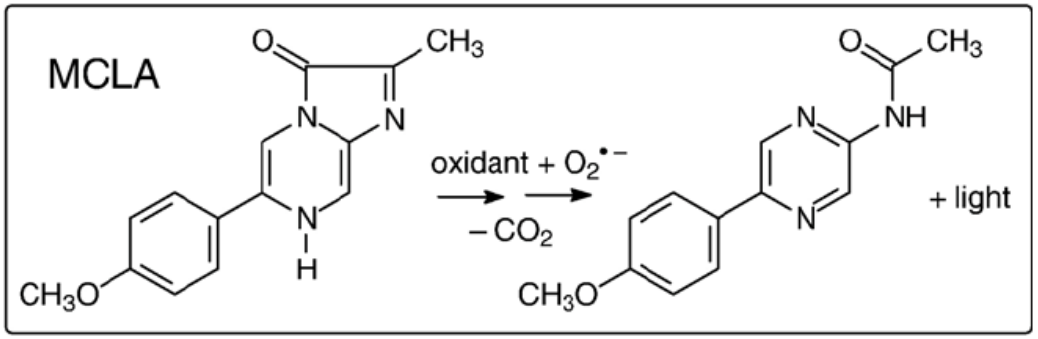
SAMPLES: in vivo, purified membrane cells

Strenghts:

- Improved version of luminol/lucigenin
- 100 times more sensitive than luminol

Caveats:

- Not specific: can react with different ROS
- Peroxidase-dependent
- Redox cycling



SAMPLES: purified membranes, cells

Strenghts:

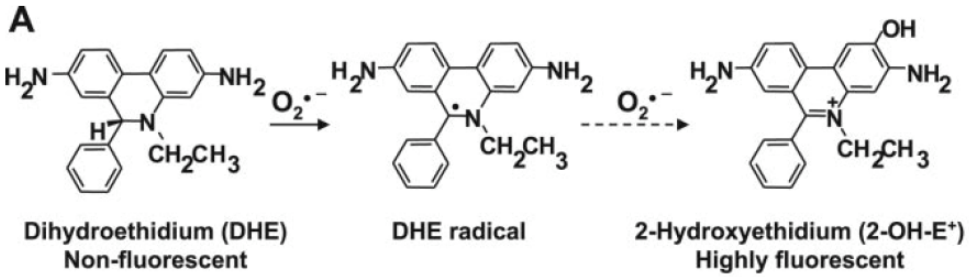
- Selective for O_2^- production
- more sensitive than most detection systems

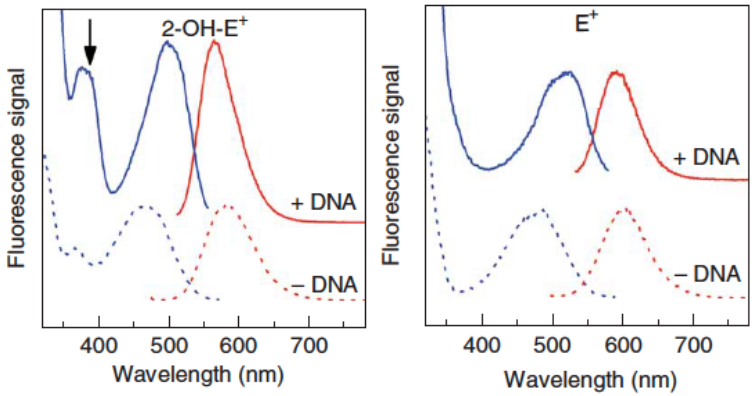
Caveats:

- Extracellular production only
- Subject to autooxidation → high background

P. Wardman; Free Radic Biol Med. 2007; Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

Fluorescence 1: Hydroethidine/Dihydroethidium (HE/DHE)





SAMPLES: intact cells, tissues in situ and ex vivo, in vivo

Strenghts:

- Cell permeable →Intracellular ROS production
- Specific product (2-hydroxyethidium) generated on reation with O_2^-

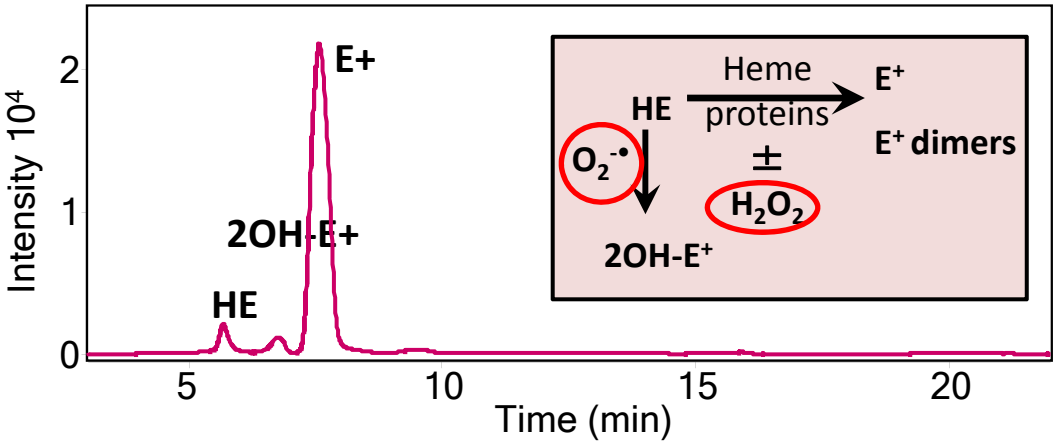
Caveats:

- Sensitive to light and oxygen → dim light/argon-purged buffers/dark tubes
- Reacts with many ROS
- Oxidized to Ethidium→intercalates with DNA → red fluorescence, similar excitation/emission spectra as 2-hydroxyethidium
- Specific products can be detected only by HPLC-based methods

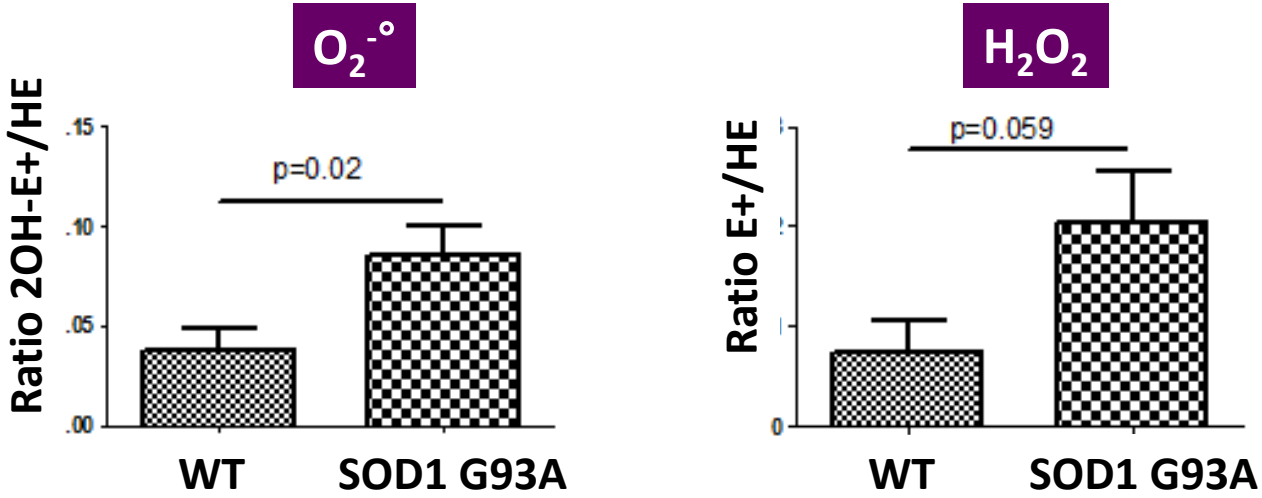
Zielonka *et al.,* Nat prot, 2007; Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

Fluorescence 1: Hydroethidine/Dihydroethidium (HE/DHE) → example

ROS levels in SOD1(G93A) spinal cord



Quantitative LC/MS analysis of products of hydroethidine oxidation

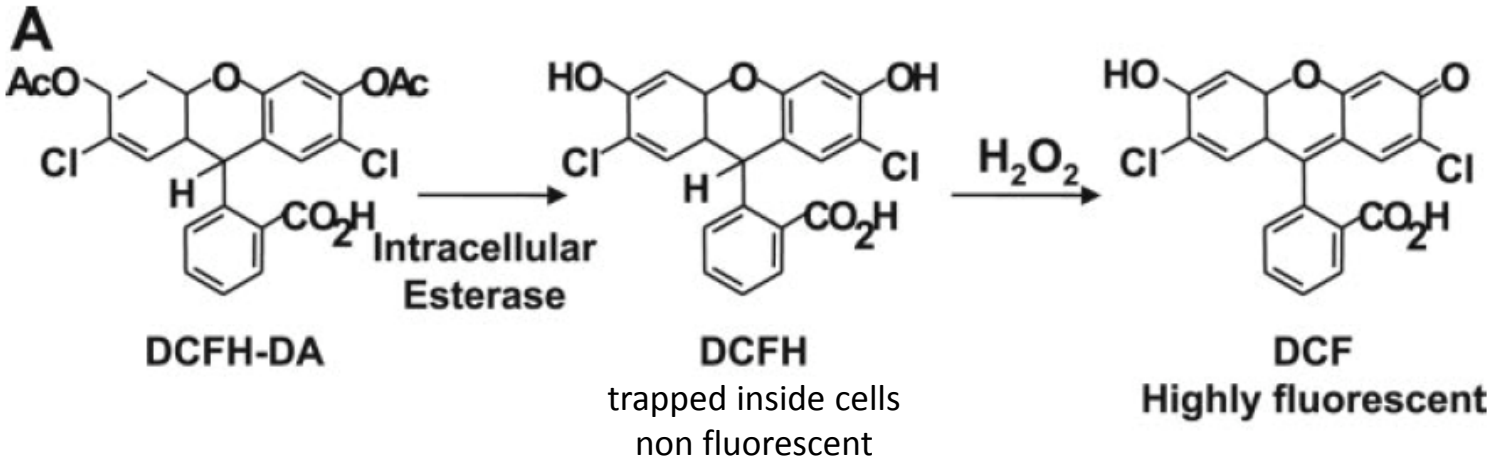


- Age: 120 days
- N=6 each group
- Males
- Intraspinal injection of HE

Increased levels of $O_2^{-\bullet}$ and H_2O_2 are observed in the spinal cord of SOD1G93A mice

Courtesy of V. Jaquet, University of Geneva

Fluorescence 2: Dichlorofluorescein diacetate (DCFH-DA)



SAMPLES: intact cells, frozen tissue sections

Strenghts:

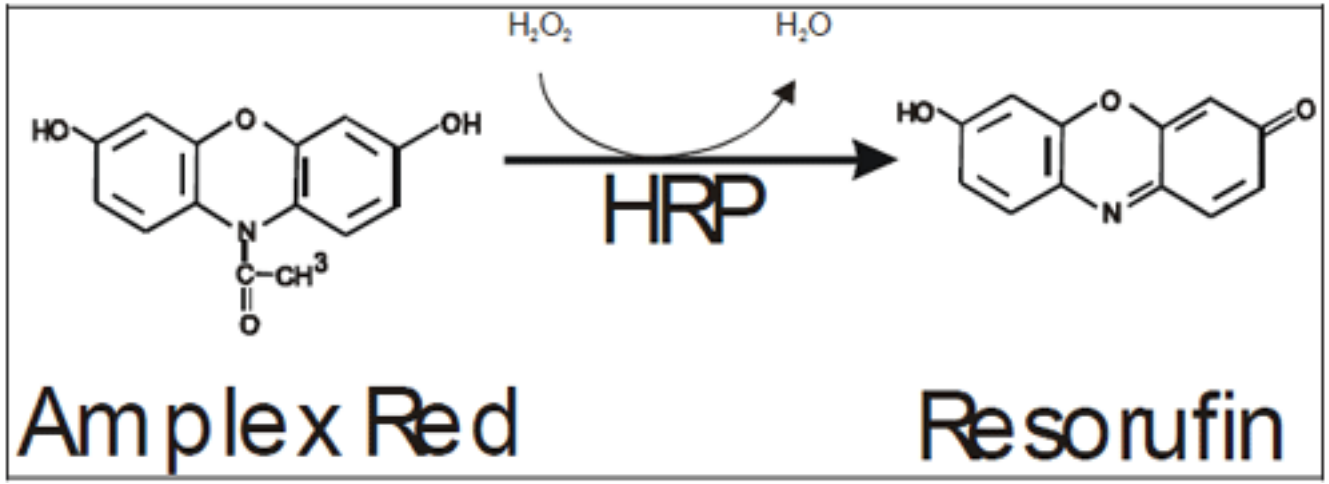
- Cell permeable →Intracellular ROS production
- Highly fluorescent

Caveats:

- Autooxidation, Redox cycling and production of O_2^-
- Not selective for H_2O_2, Reacts with many ROS
- Reaction with peroxidases

Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

Fluorescence 3: Amplex red



N-acetyl-3,7-dihydroxyphenoxazine,

Pink
Fluorescent at 587nm

SAMPLES: intact cells

Strenghts:

- Highly fluorescent, low background
- High sensitivity
- Specific for H2O2

Caveats:

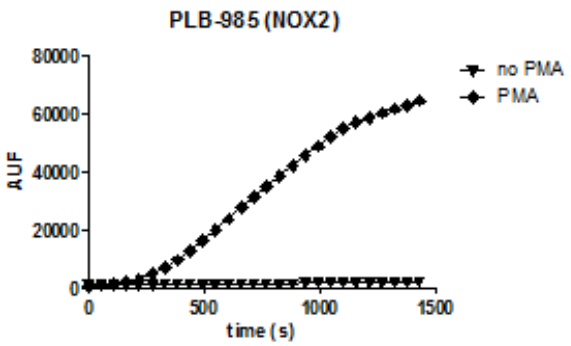
- Extracellular H2O2 only, HRP does not penetrate cells
- Reaction with endogenous peroxidases

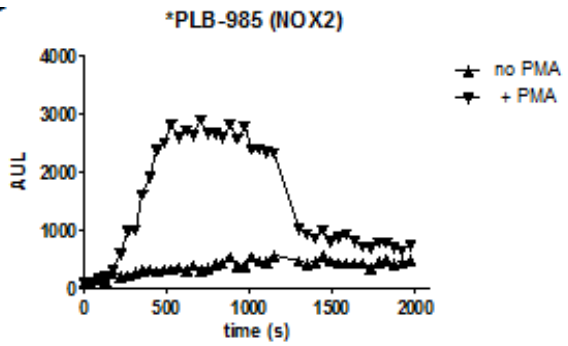
Dikalov *et al.,* Hypertension, 2007; Maghzal *et al.,* Free Radic Biol Med. 2012

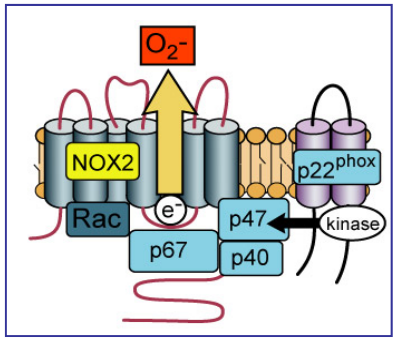
Examples: Amplex red *vs* MCLA

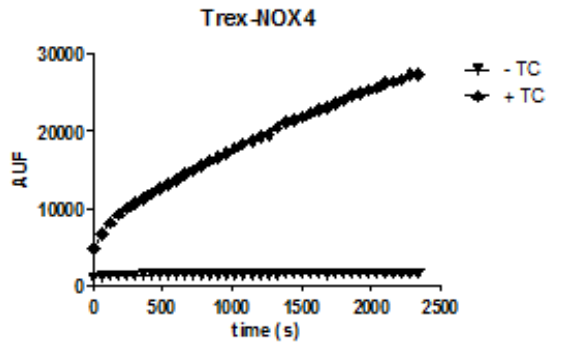
H_2O_2 production
Amplex red fluorescence

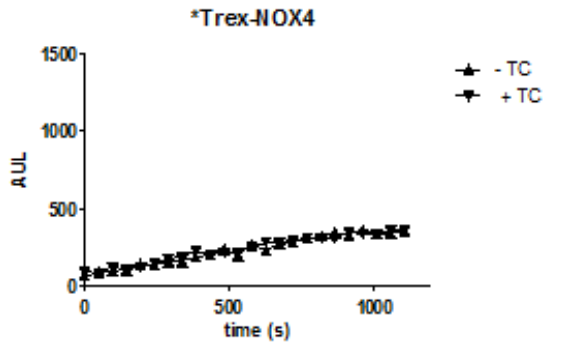
$O_2^{\bullet -}$ production
MCLA luminescence

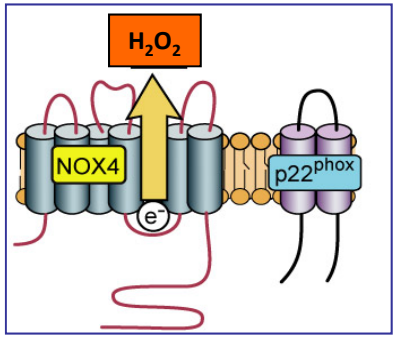








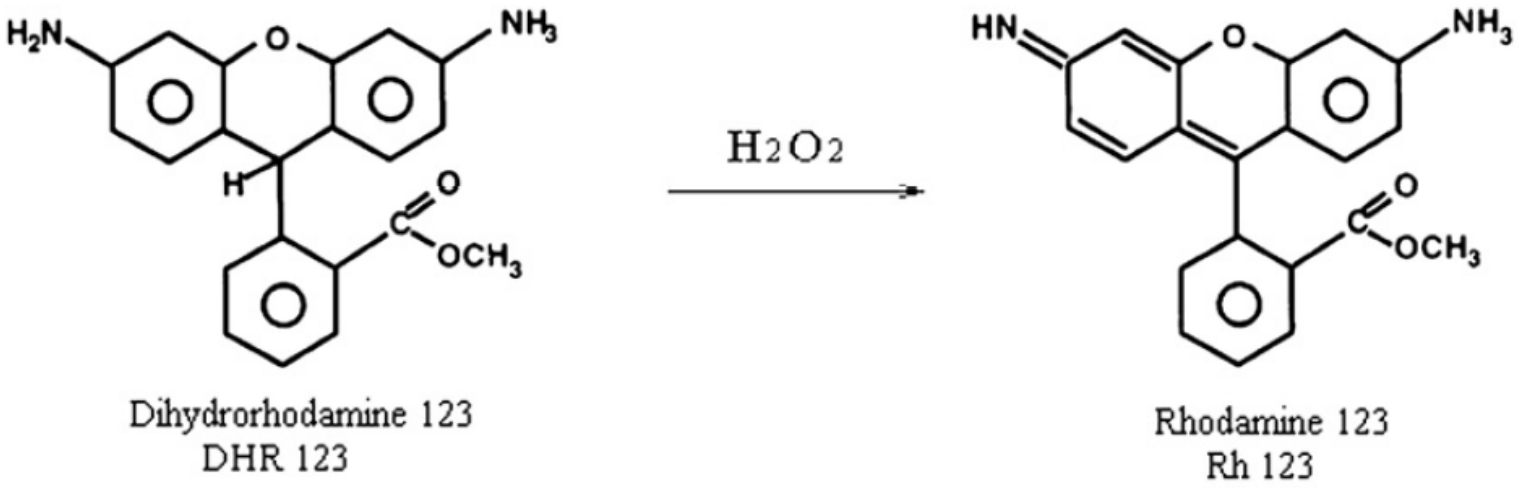




PLB-985: human myeloid cell line, differentiated in granulocytes
Trex-NOX4: HEK cells overexpressing NOX4 only upon tetracycline (TC) exposure

Courtesy of V. Jaquet, University of Geneva

Fluorescence 4: Dihydrorhodamine (DHR) 123



wavelength 530 nm

SAMPLES: intact cells

Strenghts:

- Highly fluorescent, intracellular
- Can be used for flow cytometry on whole blood → diagnosis of CGD
- Blood specimens as small as 0.1 ml can be used → ideal for use in neonates and young children

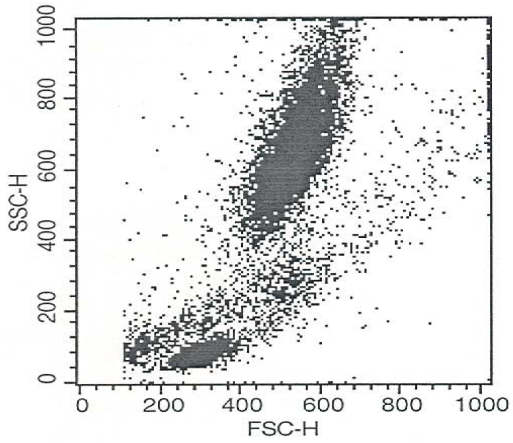
Caveats:

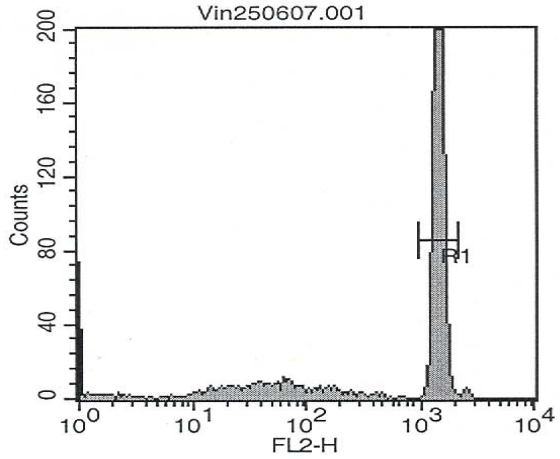
- Specificity
- Sensitivity

Richardson *et al.,* J Immunol Methods. 1998; Qin *et al.,* Cell Biology International 32 (2008)

Fluorescence 4: Dihydrorhodamine (DHR) 123 → human whole blood assay

Propidium iodide

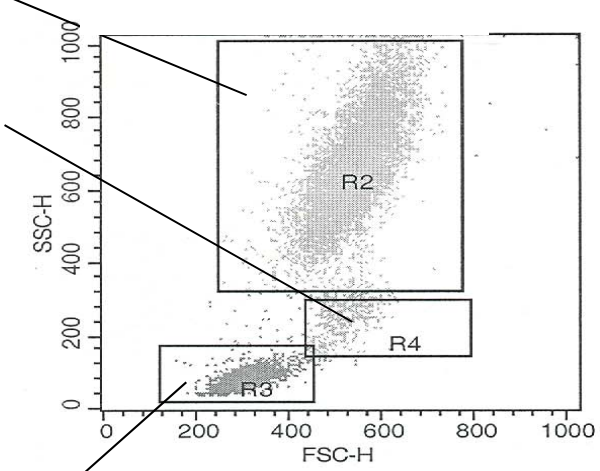




Granulocytes

Monocytes

B lymphocytes

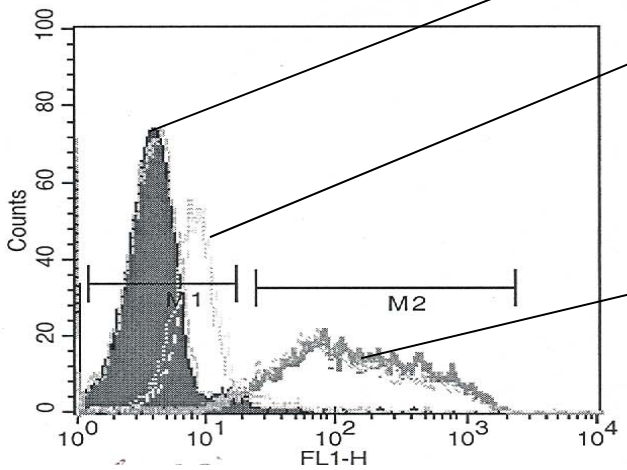


R2

No PMA

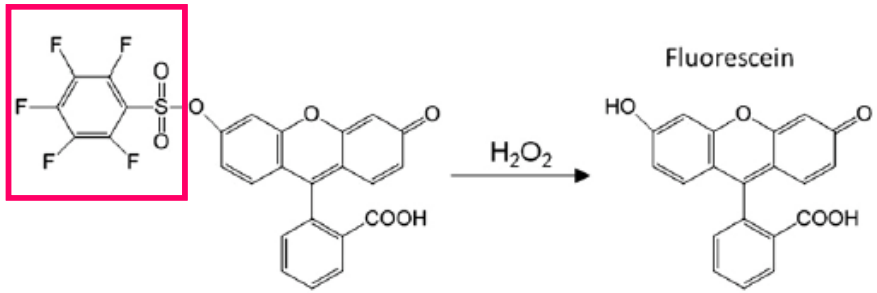
DPI + PMA

PMA



Courtesy of V. Jaquet, University of Geneva

Fluorescence 5: probes unmasked by H_2O_2



Benzene-sulfonyl derivatives

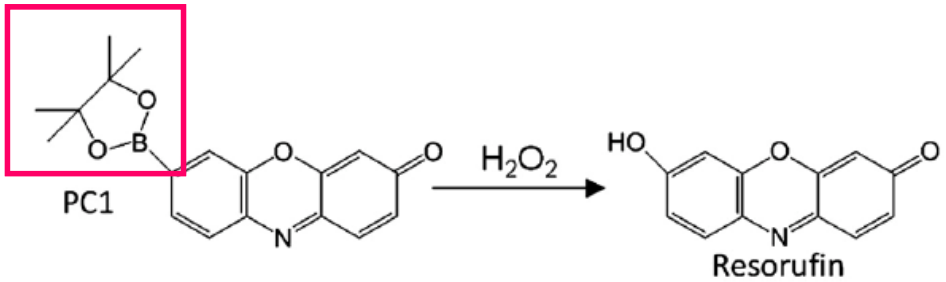
1. pentafluorobenzenesulfonyl-fluorescein
2. Bis(2,4-dinitrobenzenesulfonyl) fluorescein

Strenghts:

- 1. Not dependent on peroxidase
- 2. Selective for O_2^-

Caveats:

- 1. Not specific
- 2. can react with GSH



Aryl boronates derivatives

(Chang's group, University of California, Berkeley)

Strenghts:

- Can be trapped inside cells → live imaging
- Not dependent on peroxidase

Caveats:

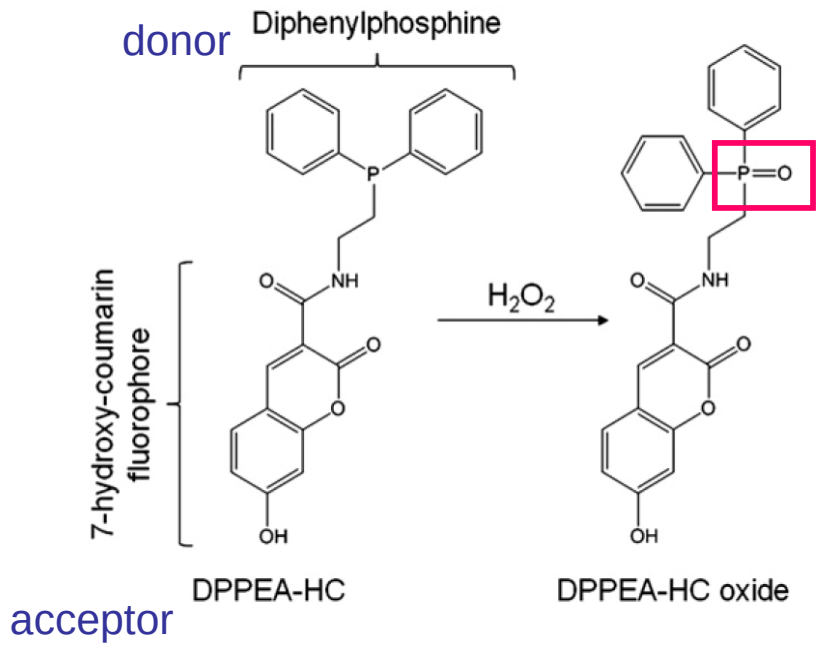
- Reaction is accelerated at higher pH
- Selectivity for H_2O_2 has been questioned
- High background fluorescence

Lippert *et al.*, Acc Chem Res. 2011 Maghzal *et al.,* Free Radic Biol Med. 2012 c

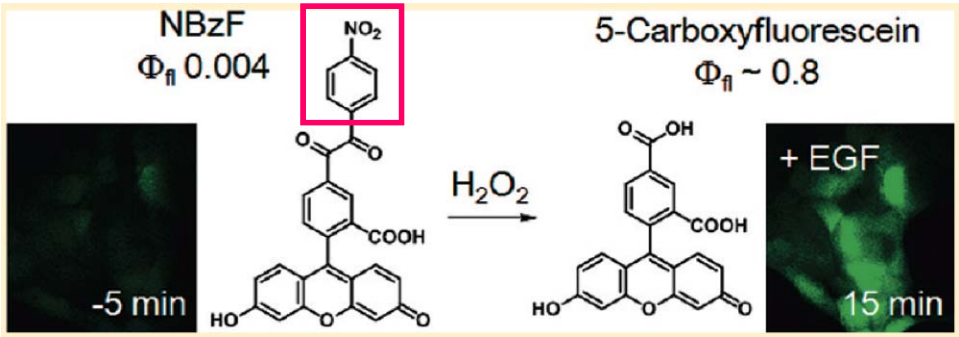
Fluorescence 5: probes unmasked by H_2O_2 → PET and FRET approaches

photo-induced electron transfer (PET)-based probe

1: DPPEA_HC



2: NBzF



→ Live cell imaging
→ Non selective

Fluorescence resonance energy transfer (FRET)-based probe

Ratio peroxyfluor

Albers *et al.*, , J. Am. Chem. Soc., 2006; Abo *et al.*, J. Am. Chem. Soc., 2011; Maghzal *et al.,* Free Radic Biol Med. 2012

Probes for indirect detection methods

in vitro-ex vivo assays:

absorbance	luminescence	fluorescence
- Cytochrome C reduction	- Lucigenin	- DHE
- NBT	- Luminol	- DCFH-DA
	- L-012	- Amplex red
	- MCLA	- DHR123
		- “masked” probes

mostly performed using a plate reader:

in vivo live imaging:

- Peroxy Caged luciferin (luminescence)
- Lucigenin/luminol (luminescence)
- Qcy-7 (fluorescence)

In vivo imaging of hydrogen peroxide production in a murine tumor model with a chemoselective bioluminescent reporter

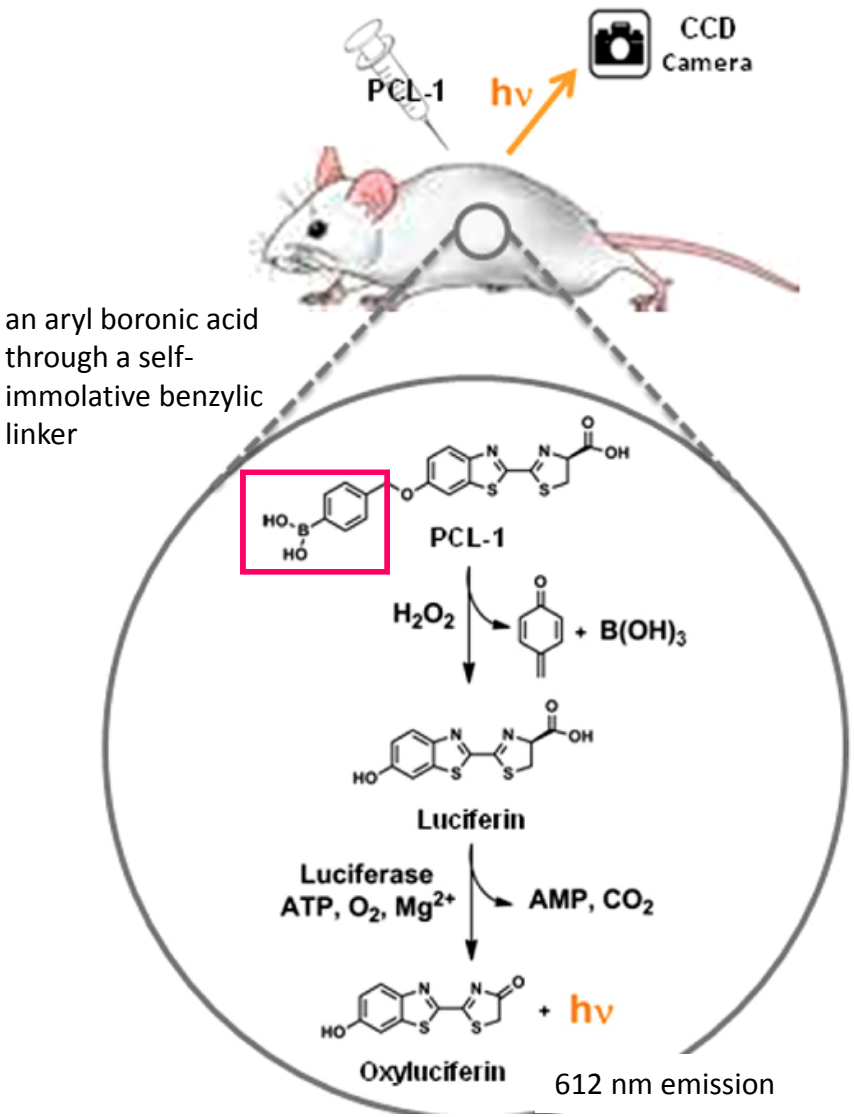
Genevieve C. Van de Bittner[a], Elena A. Dubikovskaya[a], Carolyn R. Bertozzi[a,b,c,d], and Christopher J. Chang[a,b,1]

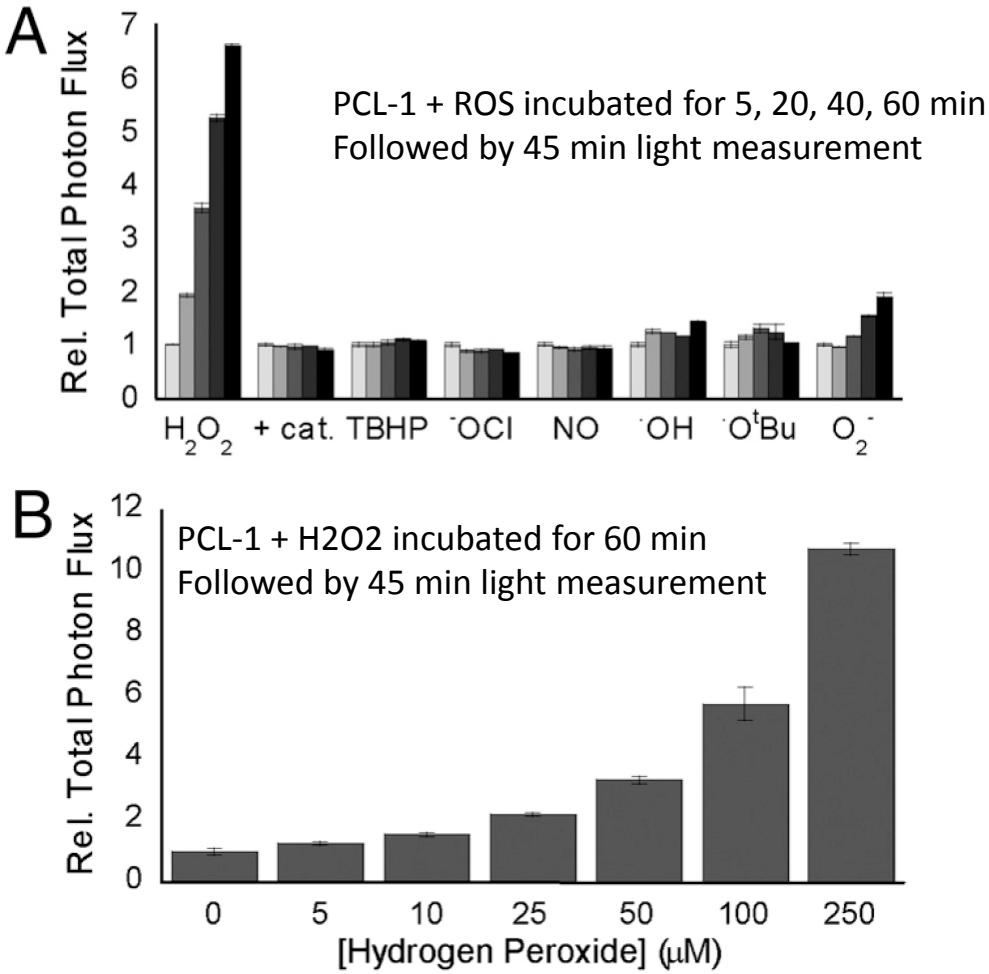
[a]Department of Chemistry, [b]Howard Hughes Medical Institute, and [c]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720; and [d]The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Edited by Doug Neckers, Spectra Group Ltd., Millbury, OH, and accepted by the Editorial Board October 15, 2010 (received for review August 29, 2010)

In vivo live luminescence 1: Peroxy Caged luciferin 1 (I)

- small, membrane-diffusible → extra- and intracellular detection
- mobility of probes throughout the organism
- No removal of fur
- greater signal-to-noise contrast ratio compared to fluorescence
- increased sensitivity





Van de Bittner *et al.*, PNAS, 2011

In vivo live luminescence 1: Peroxy Caged luciferin 1 (II)

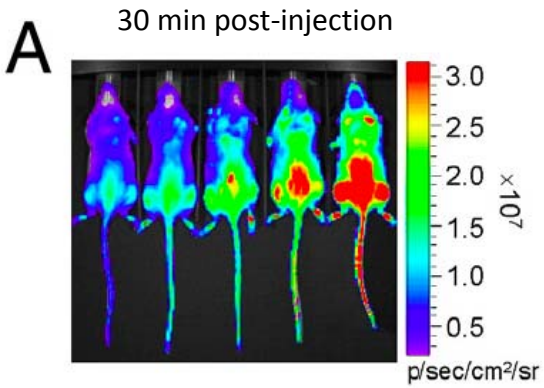
Unshaven FVB-luc+ mice that ubiquitously express firefly luciferase (CAG promoter)

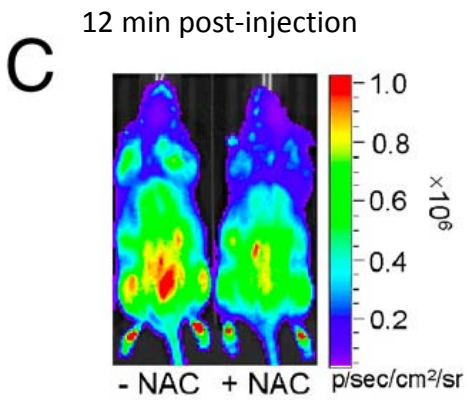
- Available from Jackson Lab
- Bioluminesence is detected in heart, spleen, muscle, pancreas, skin, thymus and bone marrow
- luciferase expression is generally greater in males than females

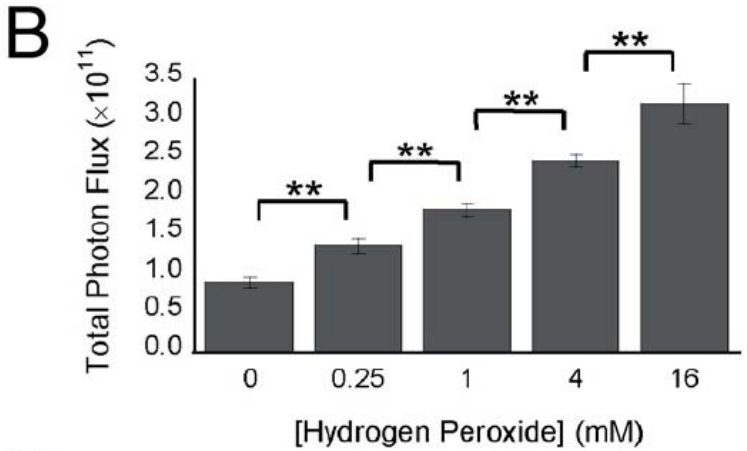
i.p. injection of several concentrations of H2O2

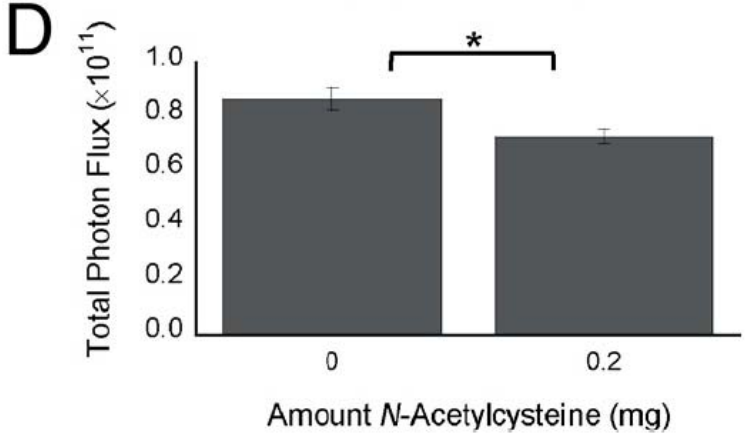
subsequent i.p. injection of PCL-1

Real-time imaging of bioluminescent signal for 1h









Van de Bittner *et al.*, PNAS, 2011

In vivo live luminescence 1: Peroxy Caged luciferin 1 (III)

- androgen-sensitive prostate cancer cells (LNCaP)
- In dissociated cell culture, LNCaPs respond to testosterone by increasing their proliferation rate and elevating their ROS production
- …and In vivo ? → LNCaP-luc+ tumor xenograft in immunodeficient SCID hairless outbred mice (SHO),
  i.p. injection of 3 × 10^6 LNCaP-luc cells (100 µL of 1:1 PBS:Matrigel) in adult SHO mice
  Incubation: 4 weeks

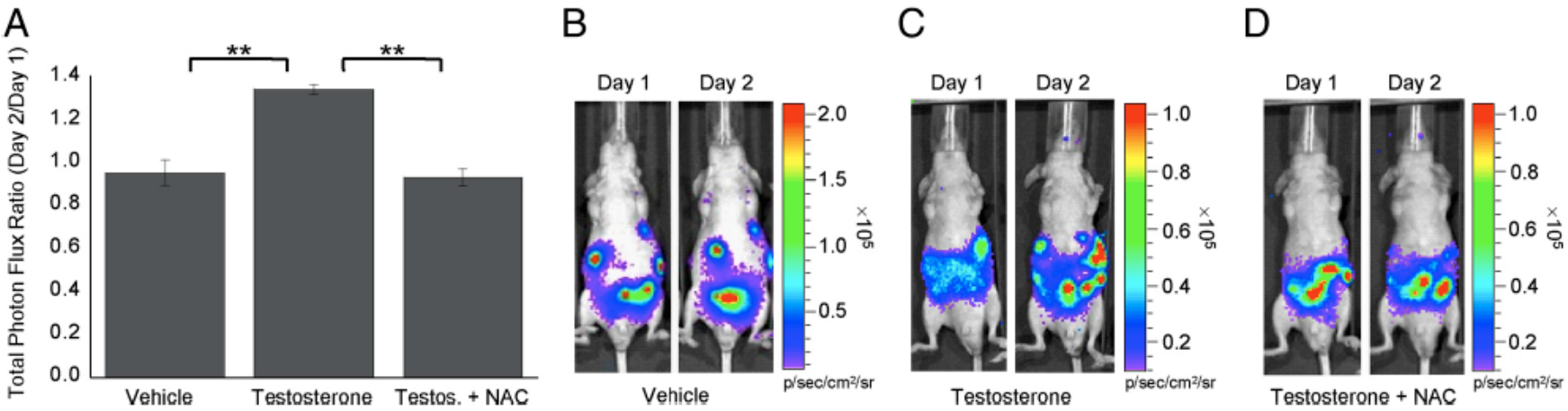


Fig. 5. Bioluminescent signal from SHO mice with LNCaP-luc tumors. (*A*) Ratios of total photon fluxes for mice injected with PCL-1 (i.p., 0.5 µmol in 50 µL of 1 : 1 DMSO : PBS) on day 1 and PCL-1 (i.p., 0.5 µmol in 50 µL of 1 : 1 DMSO : PBS) plus the vehicle (i.p., 50 µL of sesame oil), testosterone propionate (i.p., 3 mg in 50 µL of sesame oil), or testosterone propionate (i.p., 3 mg in 50 µL of sesame oil) and NAC (i.p., 0.2 mg in 100 µL of PBS) on day 2. Sesame oil and testosterone were injected 1.5 h prior to PCL-1 on day 2, and NAC was injected immediately prior to PCL-1 on day 2. Statistical analyses were performed with a two-tailed Student's *t* test. $^{**}P < 0.005$ ($n = 5$), and error bars are ±SD. Representative images from one mouse in each experiment are shown (*B–D*).

Van de Bittner *et al.*, PNAS, 2011

Chemistry & Biology
Resource

In Vivo Imaging of Inflammatory Phagocytes

Jen-Chieh Tseng[1,*] and Andrew L. Kung[1,2,3]
[1]Lurie Family Imaging Center
[2]Department of Pediatric Oncology
Dana-Farber Cancer Institute, Boston, MA 02215, USA
[3]Present address: Columbia University Medical Center, New York, NY 10032, USA
*Correspondence: jen-chieh_tseng@dfci.harvard.edu
http://dx.doi.org/10.1016/j.chembiol.2012.08.007

In vivo live luminescence 2: lucigenin and luminol (I)

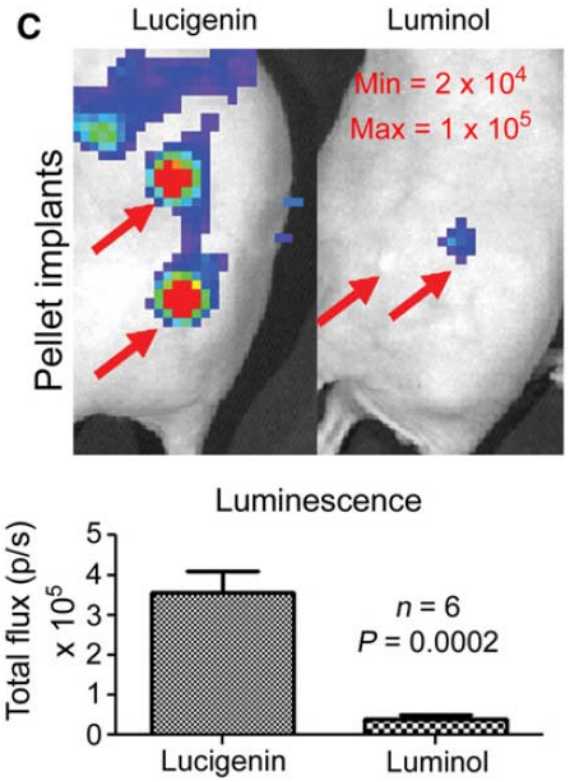
Long-term inflammation model:
s.c. implant of estrogen-releasing pellets onto NCr nude mice
→ Imaging 4 months after pellet implant with lucigenin (25 mg/kg i.p.) or luminol (100 mg/kg i.p.)

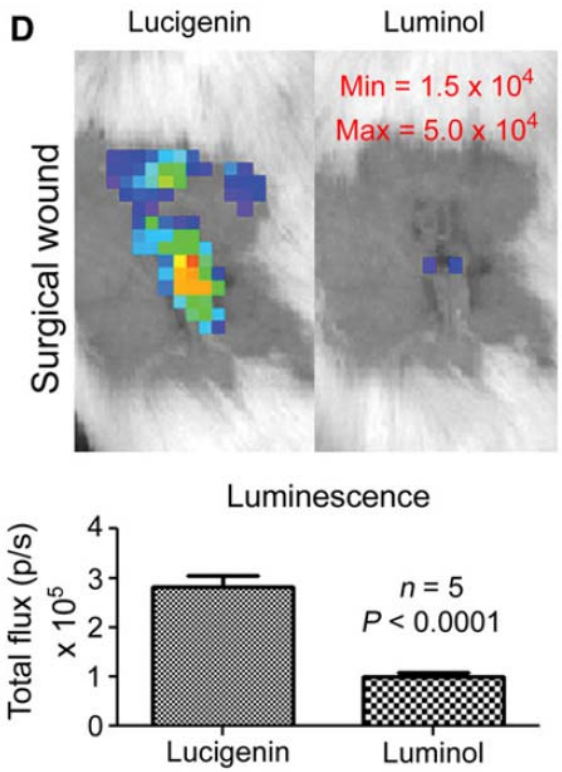
Short-term inflammation model:
surgical lacerations to the skin

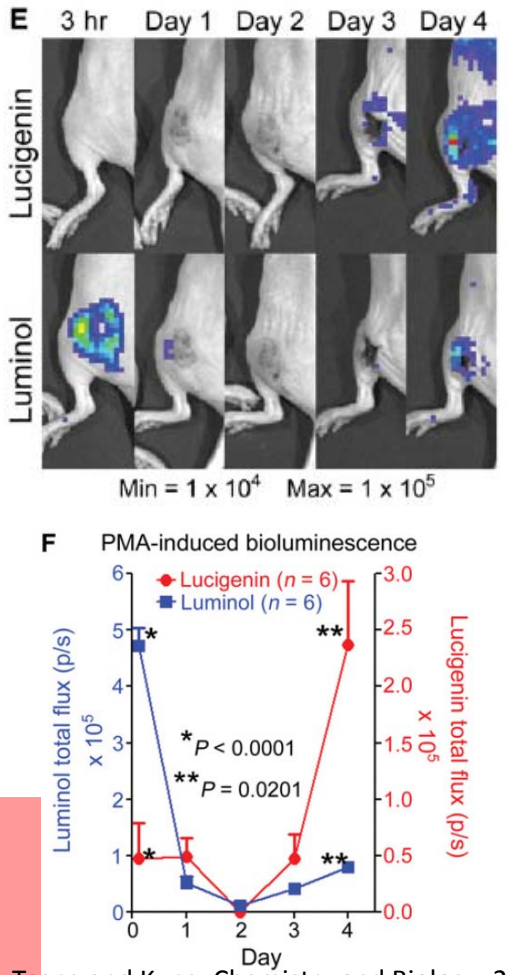
→ Imaging 4 days after surgery during healing phase with lucigenin (25 mg/kg i.p.) or luminol (100 mg/kg i.p.)

Short-term inflammation model:
s.c. injection of 50 µg PMA

→ Longitudinal imaging starting 3 hours after surgery for 4 days with lucigenin (25 mg/kg i.p.) or luminol (100 mg/kg i.p.)







Luminol bioluminescence → acute phase of inflammation
Lucigenin bioluminescence → late phases of inflammation

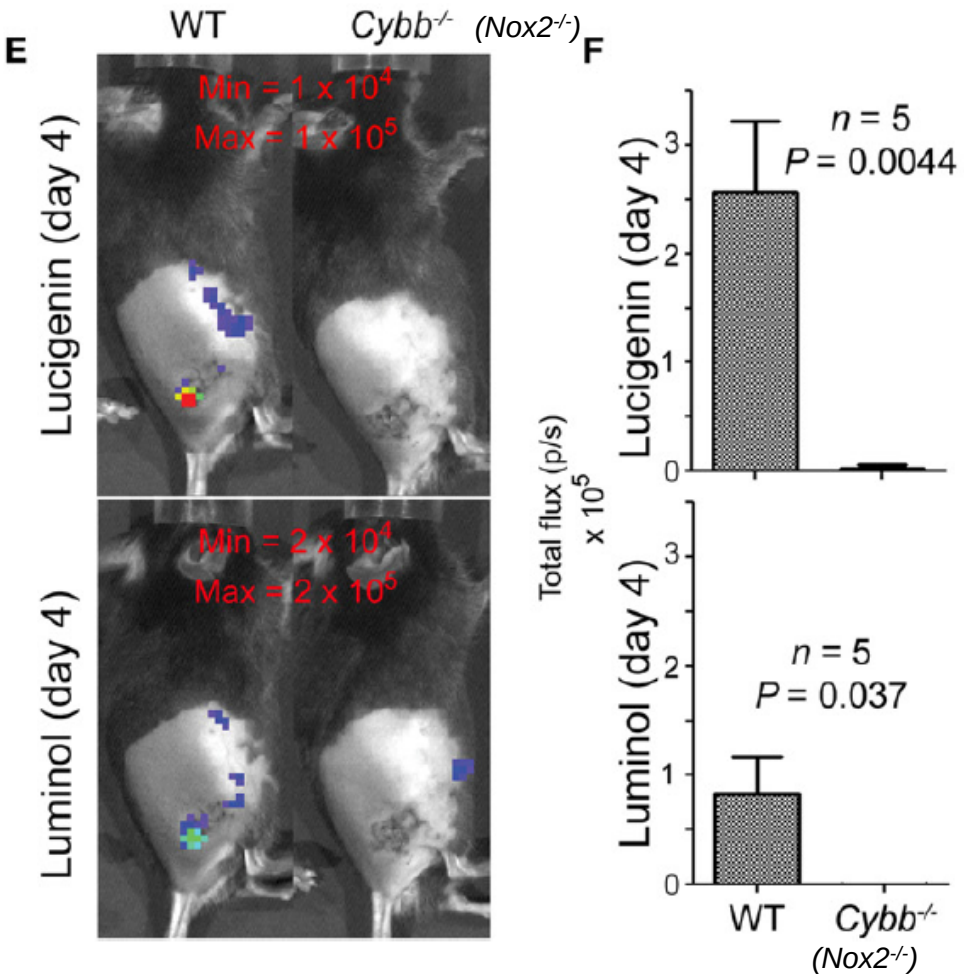
luminescence from these different substrates are mediated through distinct cell types and mechanisms

Tseng and Kung, Chemistry and Biology, 2012

In vivo live luminescence 2: lucigenin and luminol (II)

Short-term inflammation model: surgical lacerations to the skin

→ Imaging 4 days after surgery during healing phase with lucigenin (25 mg/kg i.p.) or luminol (100 mg/kg i.p.)

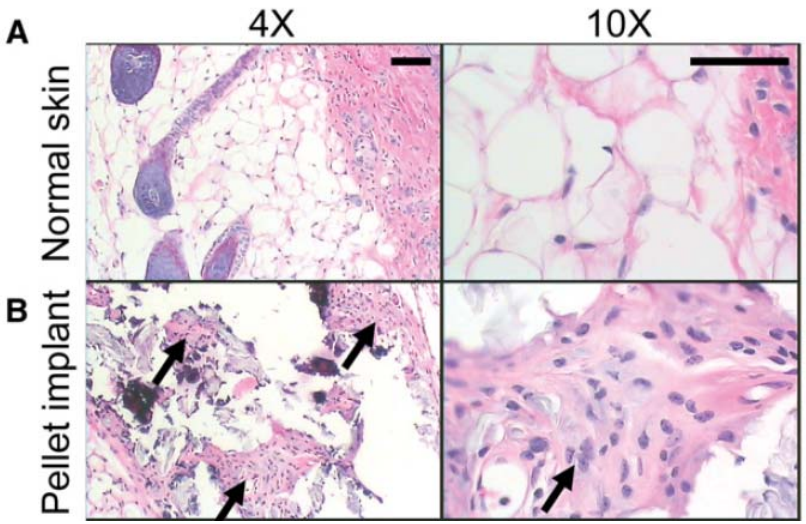


Tseng and Kung, Chemistry and Biology, 2012

In vivo live luminescence 2: lucigenin and luminol (III)

Long-term inflammation model:
s.c. implant of estrogen-releasing pellets
onto NCr nude mice

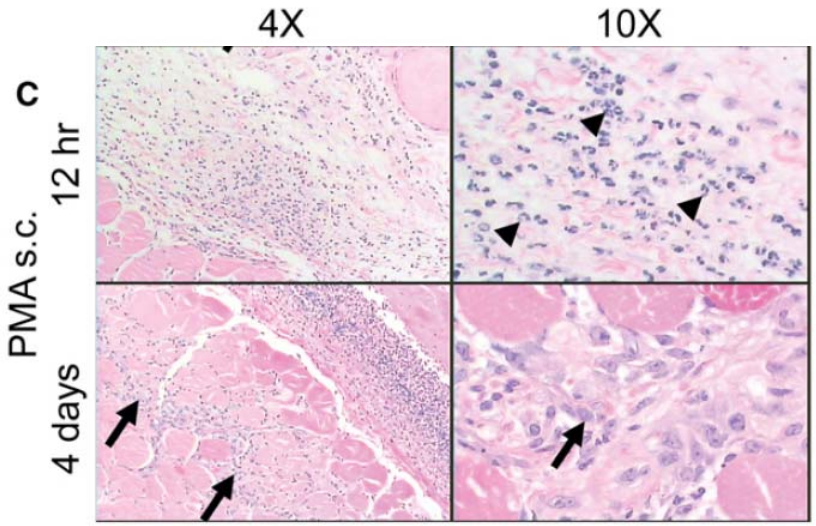
lucigenin > luminol



H&E staining revealed massive tissue-infiltrating macrophages and granuloma formation (arrows), with very few neutrophils.

Short-term inflammation model:
s.c. injection of 50 μg PMA

Luminol > lucigenin (acute phase)
Lucigenin> luminol (late phase)



Twelve hours after injection → massive neutrophil infiltration (arrowheads)
Four days after injection → infiltration of macrophages (arrows)

luminol bioluminescence → neutrophils in the acute phase
lucigenin bioluminescence → macrophages in the chronic phase of inflammation

!!!!! redox-cycling issue: lower oxygen pressure in tissue (10 mm Hg, compared with 150 mm Hg in the atmosphere) and higher affinity to endogenous superoxide anion (O2-, with a negative charge opposite to LC+), make it unlikely to occur in phagocytes during in vivo imaging

Tseng and Kung, Chemistry and Biology, 2012

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ARTICLE

pubs.acs.org/JACS

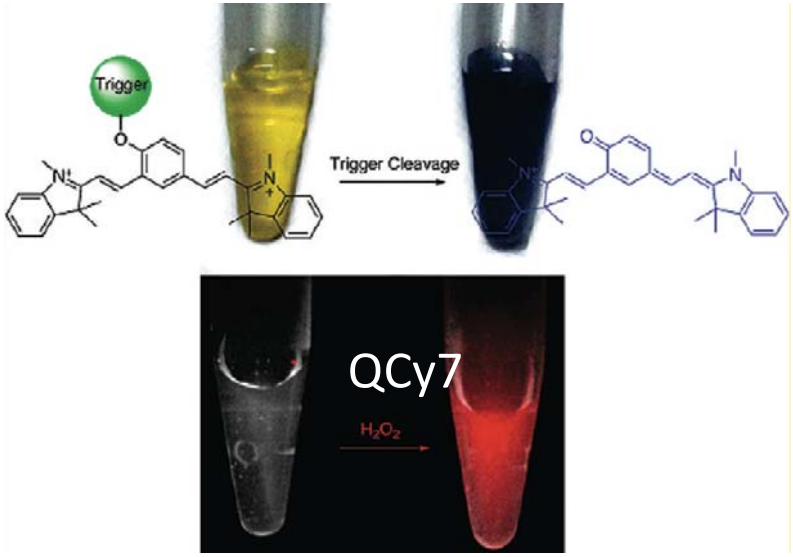
A Unique Paradigm for a Turn-ON Near-Infrared Cyanine-Based Probe: Noninvasive Intravital Optical Imaging of Hydrogen Peroxide

Naama Karton-Lifshin,[†] Ehud Segal,[‡] Liora Omer,[‡] Moshe Portnoy,[†] Ronit Satchi-Fainaro,[*,‡] and Doron Shabat[*,†]

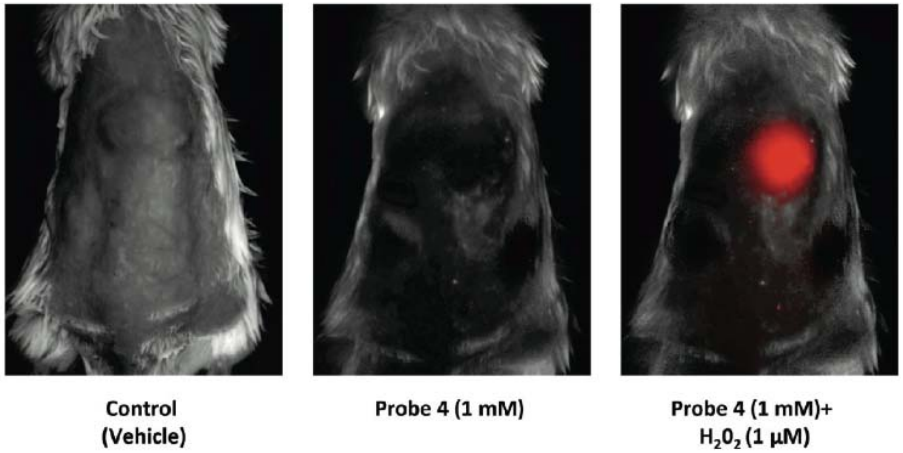
[†]School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences and [‡]Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

S *Supporting Information*

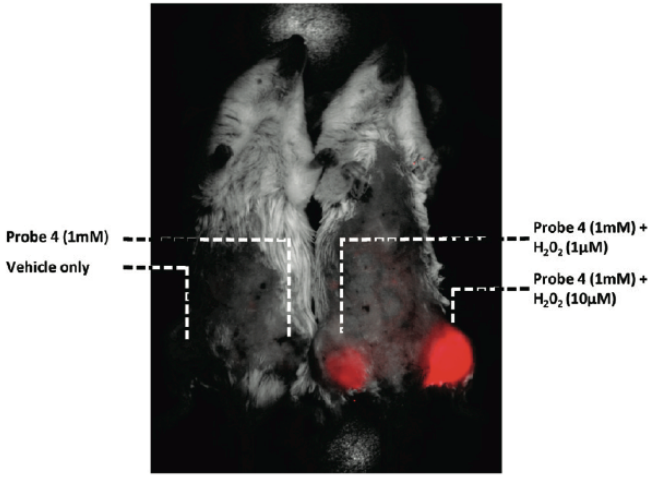
In vivo live fluorescence: QCy7



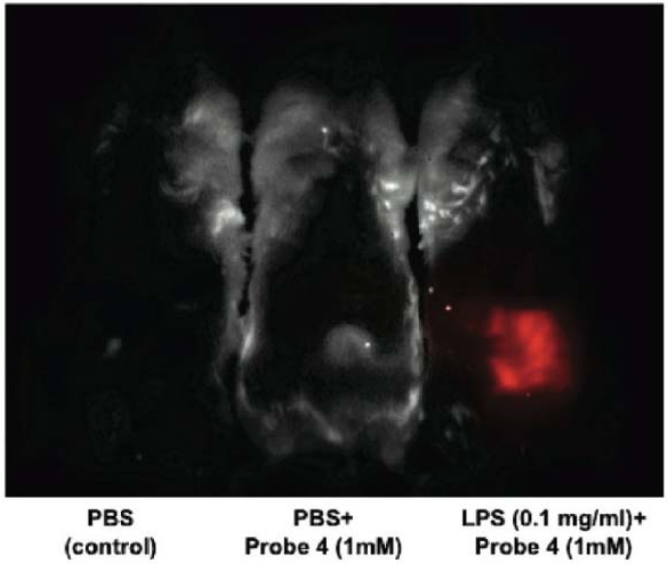
Water-soluble QCy7: Cy-7 + phenyl boronic acid→ near infrared fluorescence upon reaction with H2O2



In vivo imaging of <u>exogenous</u> hydrogen peroxide. Shaved Balb/c mice. CRI Maestro image. Excitation at 595 nm, emission cutoff filter of 635 nm.



the intensity of fluorescence depends on H2O2 concentration



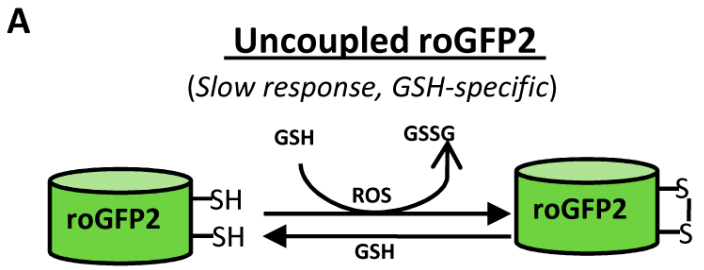
In vivo imaging of <u>endogenous</u> hydrogen peroxide 6h post-i.p. LPS-induced inflammatory response.

Karton-Lifshin *et al.*, J. Am. Chem. Soc., 2011

Genetically encoded biosensors

1. **roGFPs**: surface-exposed residues on the Aequorea victoria green fluorescent protein (GFP) were substituted with cysteines in appropriate positions to form disulfide bonds
   → ratiomeric H_2O_2 determinationn

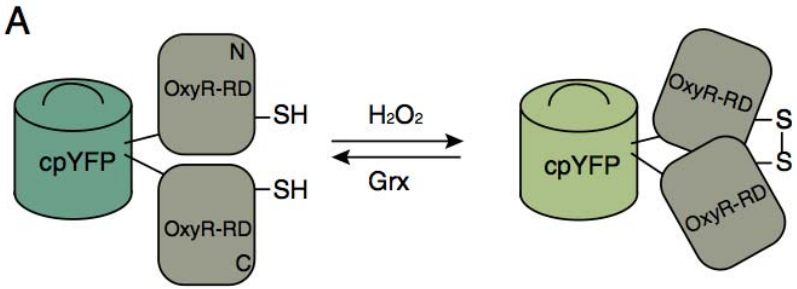
! Can be induced by other oxidants, slow reaction
Improved by coupling with glutaredoxins (Grx1-roGFP) or peroxidases (e.g. Orp1-roGFP)



Bhaskar et al., PLoS Pathog 10(1): e1003902.

2. **Hyper**: insertion of circularly permuted yellow fluorescent protein (cpYFP) into OxyR,a bacterial sensor of H2O2: disulfide bond formation upon oxidation and conformational change → fluorescence

→ ratiometric, highly selective for H2O2
! Reversible reaction, depends on glutathione system, pH-sensitive



3. **cpYFP**: discovered by serendipity as selective sensor for O_2^- →mechanism not understood

→ can be targeted to cellular compartments
→ Need transfection

Maghzal *et al.*, Free Radic Biol Med. 2012

General considerations

Table 1. The ideal fluorescent ROS detecting probe ....does not exist!

Characteristic	Rationale
Chemoselectivity	ROS-type chemoselectivity and no cross-reactivity with other ROS to avoid disambiguity of the type of ROS involved in the reaction; Based on the innate chemical nature of the ROS type
Membrane pearmibility	Good membrane permeability but little diffusion of the product to allow for localization of the reaction
Sensitivity	Good sensitivity (nano-micro range of ROS concentration) to detect signaling concentration of the ROS
Defined spectral peaks	Narrow peaks of excitation and emission spectra to allow simultaneous detection of more than one probe
Photostability	Little photooxidation and photobleaching to facilitate imaging on the microscope
Post-fixation retention	Retention after fixation to allow for simultaneous detection of the dye and the antibody for colocalization studies
Linear response	Linear relationship between the fluorescent signal to the ROS concentration to allow for quantitative studies of the ROS generation
Signal-to-noise ratio	Low fluorescence of the ROS-unbound form to avoid false signal from the accumulation of the probe in the cell
Bioorthogonality	Bioorthogonality and nontoxicity of the probe not to interfere with other biological processes
In vivo capability	Possibility of the probe usage in the *in vivo* studies to permit of the redox reaction studies in the animal models
Two-photon microscopy	Compatibility with two-photon microscopy to allow for deep tissue penetration imaging and prolonged observation without specimen damage

- **Understand underlying principles involved and how these may be affected by potential biological changes**
- **Use a combination of approaches**
- **Use appropriate controls and Interpret data with care, recognizing the limitations of the assay**

Analysing oxidation products

Biomarkers of Oxidative Stress Study **(http://www.niehs.nih.gov/research/resources/databases/bosstudy/index.cfm)**

Assays that measure oxidation of lipids, proteins, DNA and a group of antioxidants.

Lipid peroxidation assays: lipid hydroperoxides, TBARS, MDA, isoprostanes, various HETEs.

Protein oxidation assays: protein carbonyls, various tyrosine products, methionine sulfoxidation.

DNA oxidation assays: 8-OH-dG, oxidation changes by the Comet assay, M_1G.

Antioxidants: Ascorbic acid, tocopherols, GSH, GSSG, uric acid, TAC (total antioxidant capacity)

http://www.neurinox.eu/

SEVENTH FRAMEWORK PROGRAMME

Neurinox

Search

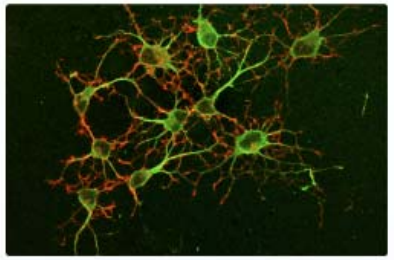
EUROPEAN COMMISSION Research & Innovation

Latest Publications | Neurinox Approach | News and events

- Home
- About NEURINOX +
- Patient information +
- Educational material +
- Opportunities for Industry partnerships
- Publications +
- Project documentation
- News and events
- Contact

Welcome to the official website of the NEURINOX project!

NEURINOX aims at identifying novel therapeutic targets for neuroinflammatory diseases, by focusing on NADPH oxidases (NOX). NOX enzymes catalyse the formation of reactive oxygen species (ROS) and are key regulators of neuroinflammation. Establishment of chronic neuroinflammation is characterised by either increased or decreased NOX activity. **The NEURINOX project aims at elucidating the links between neuroinflammation, NOX enzyme activity and neurodegenerative diseases (ND).** It aims at **validating NOX as therapeutic targets** using animal models of neuroinflammation, human samples, prospective clinical studies and, in case of success of preclinical evaluation of NOX inhibitors in models of **amyotrophic lateral sclerosis (ALS),** an early **clinical trial with ALS patients.**



With a total budget of 15.4 M€ and a funding support of 11.4 M€ for 5 years from the European Union's Seventh Framework Programme (FP7) for Research and Technology Development, NEURINOX is coordinated by University of Geneva and includes several SMEs, internationally renowned research groups and clinical institutions with extensive experience in NOX research and neurodegenerative diseases.

NeuriNOX consortium: the aim

to develop novel therapeutic approaches for the treatment of inflammatory neurodegenerative diseases focusing on NOX enzymes

NEURINOX research activities include:

- studies of NOX activity in animal models and in human patient samples

- development and validation of the effect of NOX regulating drugs (small molecules) in animal models

- a phase I-II clinical trial with a NOX regulating drug to determine safety and efficacy on a small population of ALS patients.

NeuriNOX consortium: 13 members

experimental research

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE
Switzerland
Read more

Universität Zürich
Switzerland
Read more

Karolinska Institutet
Sweden
Read more

Institut des Neurosciences GRENOBLE
France
Read more

clinical research

Italy
Read more

Platforms: transcriptomic, proteomic, ROS measurements

NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
Greece
Read more

ACADEMY OF ATHENS
FOUNDATION FOR BIOMEDICAL RESEARCH
Greece
Read more

Victor Chang Cardiac Research Institute
Australia
Read more

Model development

SYNAPCELL
France
Read more

neurix
Switzerland
Read more

Small molecule development

genkyotex
Switzerland
Read more

redoxis
Sweden
Read more

management

ARTTIC
France
Read more

Acknowledgements

Neurinox

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE
Read more ▸

Victor Chang
Cardiac Research Institute
Read more ▸

Vincent Jaquet

Roland Stocker
Ghassan Maghzal
Magda Lam

Thank you for your attention!

Questions/comments?