PROGETTO “LOMBARDIA ECCELLENTE”

Obiettivi e Contesto della ricerca

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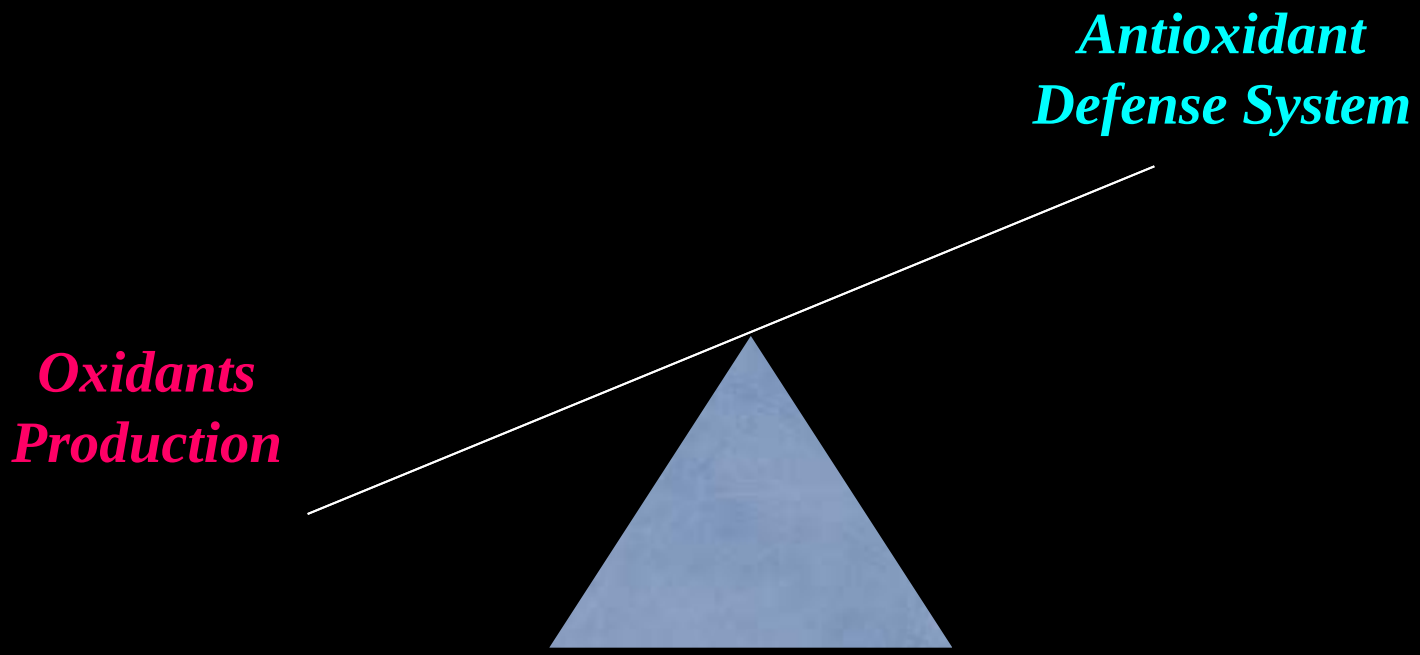
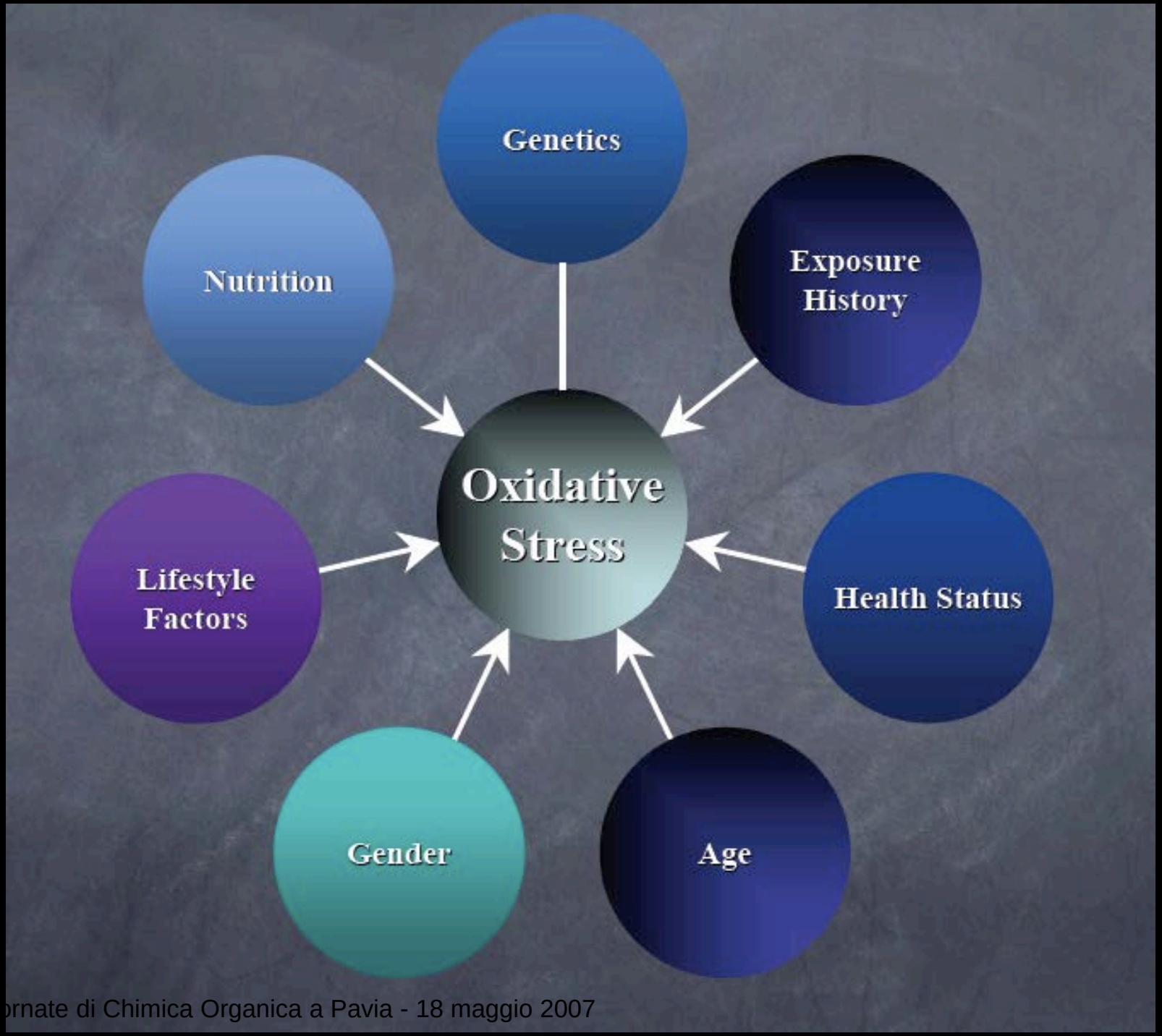
“ The paradox of aerobic life, or the “Oxygen Paradox”, is that humans, animals, and plants cannot exist without oxygen, yet oxygen is inherently dangerous to their existence.”

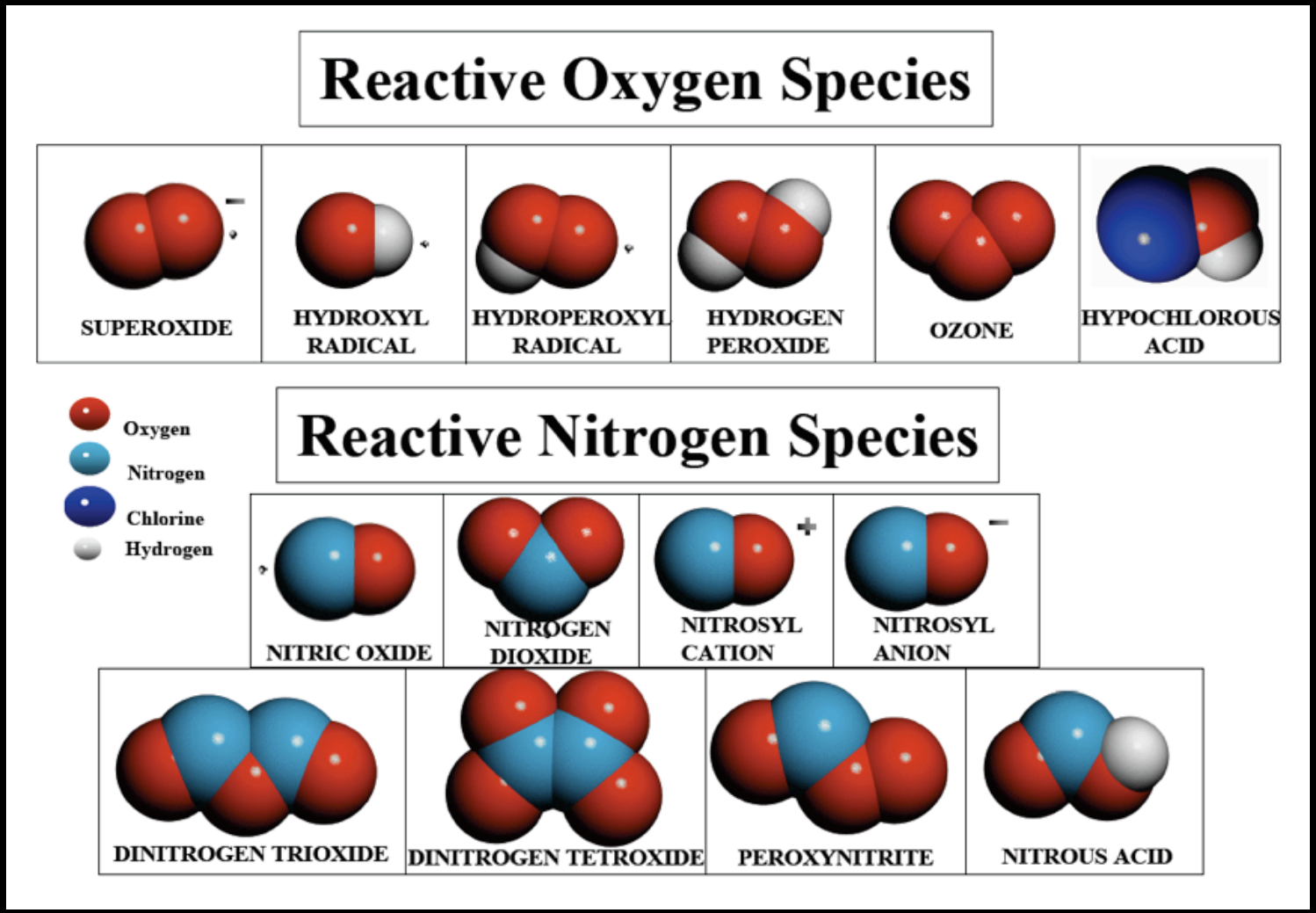
Oxidative Stress

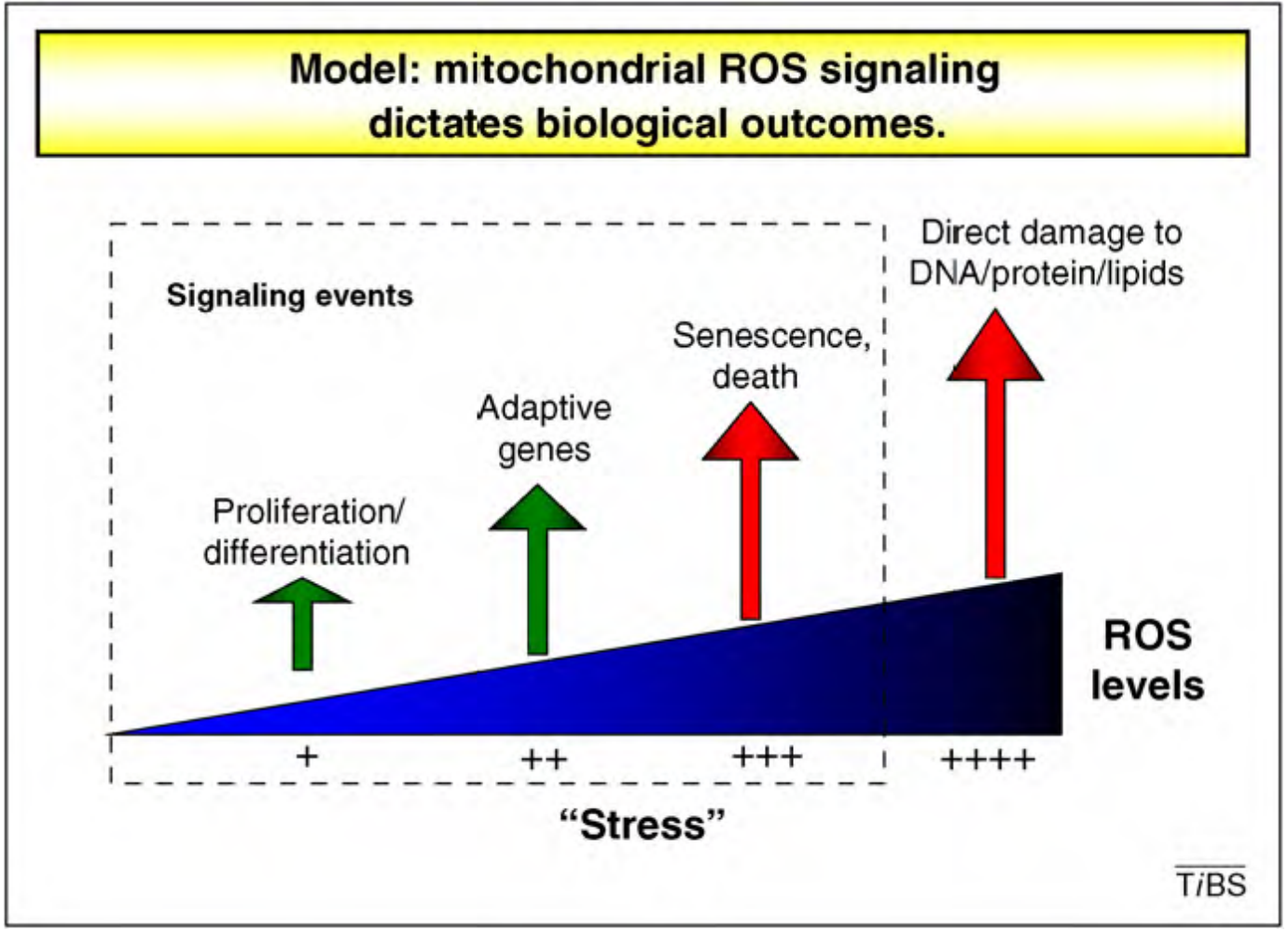


Altered homeostatic balance resulting from oxidant insult.


Genetics
Nutrition
Exposure History
Oxidative Stress
Lifestyle Factors
Health Status
Gender
Age

Oxidative Stress





Mitochondrial ROS levels are crucial for biological outcomes. Low levels of mitochondrial ROS production are required for cellular processes such as proliferation and differentiation. An induction in ROS production will lead to adaptive programs including the transcriptional upregulation of antioxidant genes. Even higher levels of ROS will signal the initiation of senescence, apoptosis and necrosis. Non-signaling, irreversible damage to cellular components is only observed under the highest levels of cellular ROS.

Oxidative Stress - Human Health

The Good

Inflammation protects the body

Destroys invading pathogens

Dissolves damaged tissue

The Bad

Chronic or prolonged inflammation
Allergies and Autoimmune Diseases

Oxidative Stress - Human Health

The Ugly ***collateral damage results in…..***

Asthma
Rheumatoid Arthritis (RA)
Multiple Sclerosis (MS)
Systemic Lupus Erythematosus (SLE)
Chronic Obstructive Pulmonary Disease (COPD)

Aging processes

Neurodegeneration

Parkinson Disease

Alzheimer Disease

Huntington Disease

Circulatory Diseases

Cancer

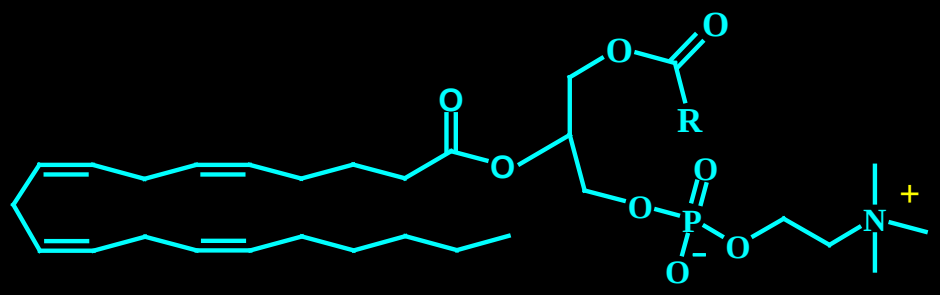
Oxidative Stress and Human Health

Free radical-mediated oxidative damages to tissue biomolecules, e.g. lipids, proteins, and DNA, are believed to play a key role in the pathophysiology of severe human diseases.

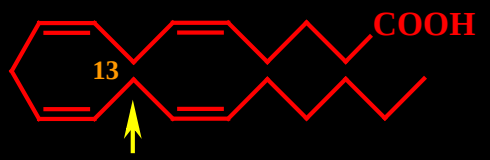
“Antioxidant compounds are considered to be beneficial to maintain human healthy conditions”

Lipid peroxidation

Peroxidation of cell membrane lipids is a central feature of oxidtive stress and occurs in various tissues of humans and other living organisms, promoting the formation of important biologically and physiologically active compounds

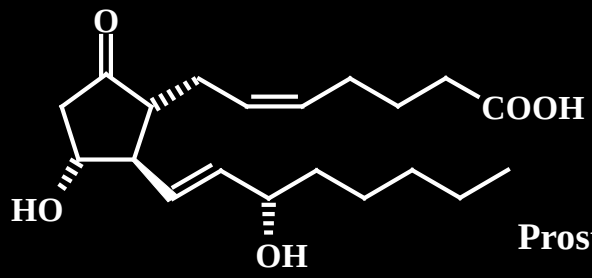


Lipid peroxidation: enzyme and free-radical promoted routes



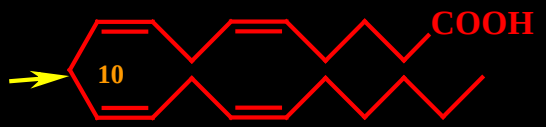
Arachidonic acid (AA)

COX 1/2



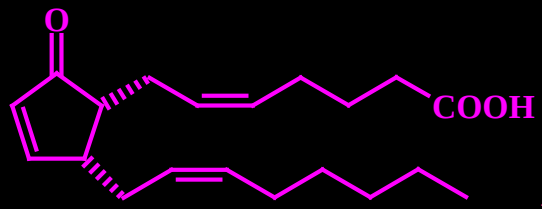
PGE_2

Prostaglandins (mammals)



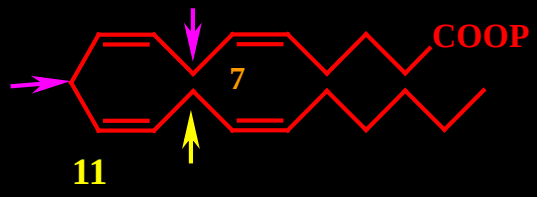
(AA)

(8*R*)-LOX/AOS/AOC



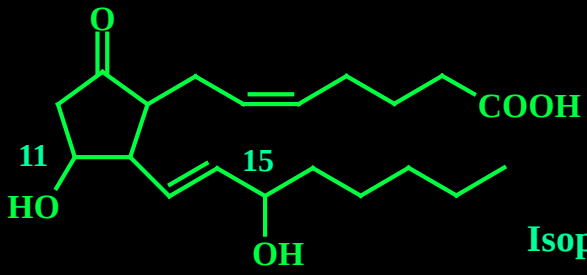
Preclavulone A

Preclavulones (corals)



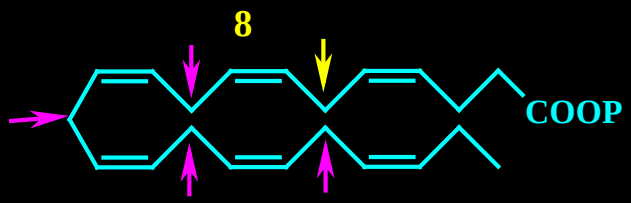
(AA)

ROS / O_2



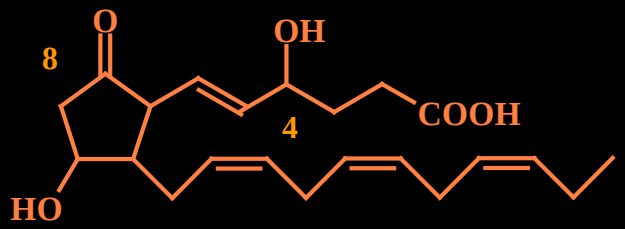
$15\text{-}E_2\text{-IsoP}$

Isoprostanes (mammals)



Docosahexaenoic acid (DHA)

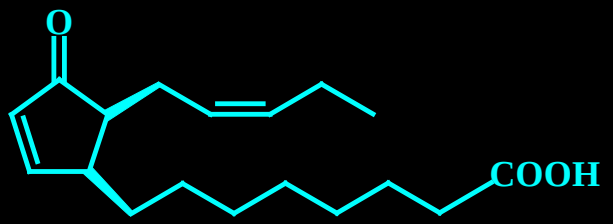
ROS / O_2



$4\text{-}E_4\text{-NP}$

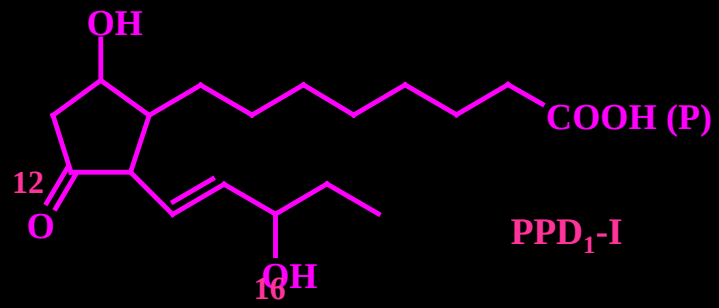
Neuroprostanes (neuronal tissues)

α-Linolenic acid



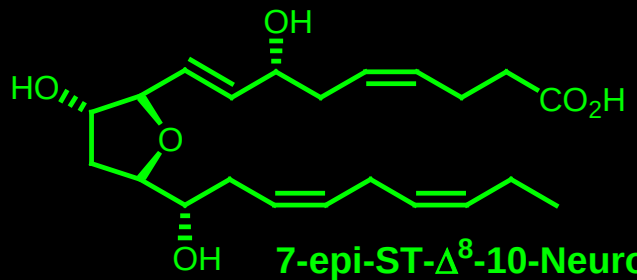
Jasmonates (plants) 12-oxo-PDA

α-Linolenic acid



Phytoprostanes (plants)

Docosahexaenoic acid (DHA)

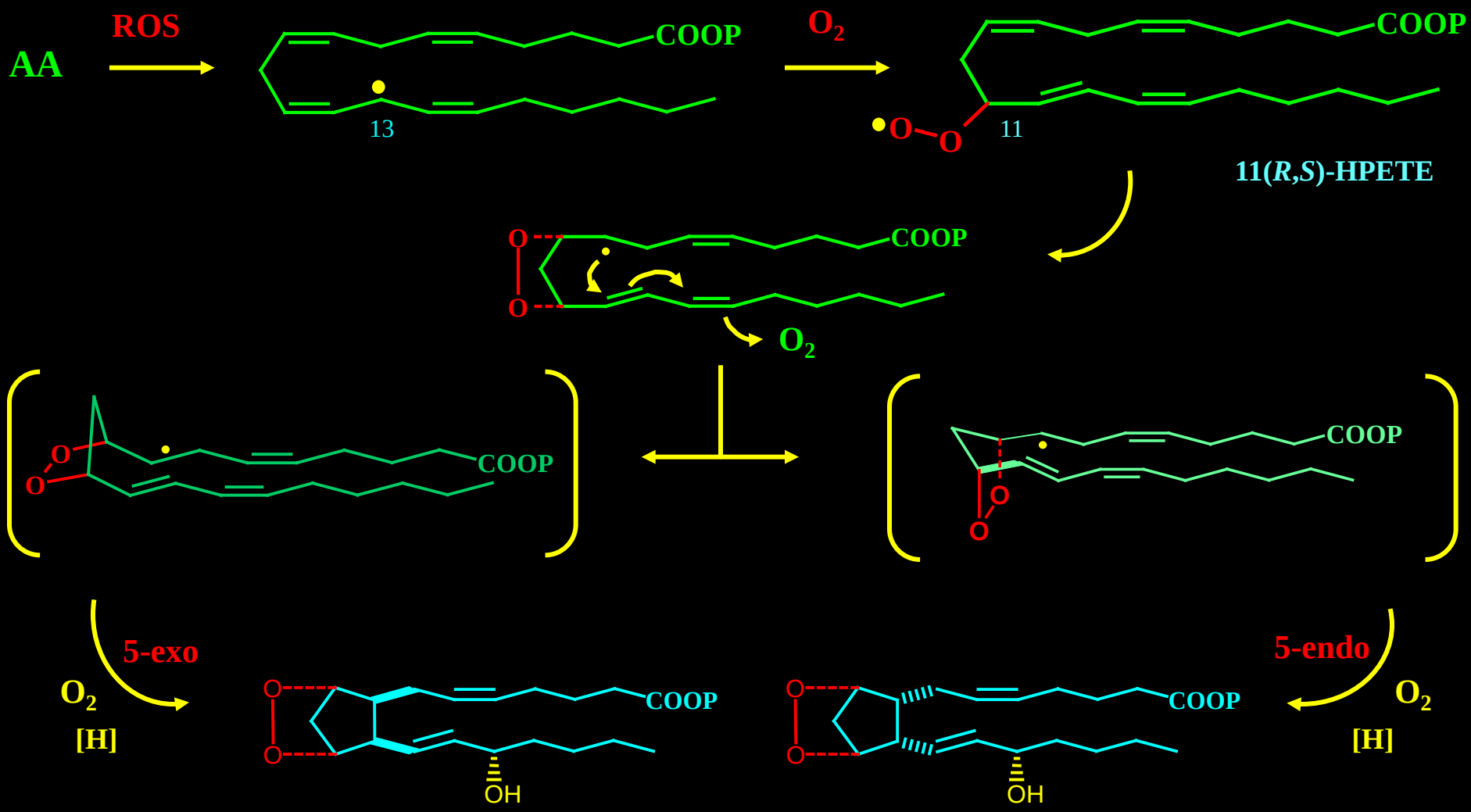


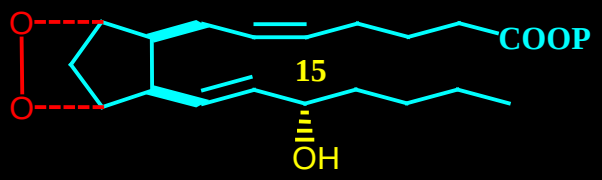
7-epi-ST-Δ8-10-NeuroF Neurofuran

Isoprostanes: J.D. Morrow et al. *Anal. Biochem.* **1990**, *184*, 1; *Curr. Med. Chem.* **2003**, *10*, 1723.
Neuroprostanes: J. D. Morrow et al. *J. Biol. Chem.* **1998**, *273*, 13605; L. J. Roberts et al. *Chemistry and Physics of Lipids* **2004**, *128*, 173.
Clavulones: A. N. Grechkin *J. Lipid Mediators Cell Signaling* **1995**, *11*, 205; A. F. Rowley et al. *J. Exp. Biol.* **2005**, *208*, 3.
Jasmonates: M. H. Beale and J. L. Ward *Nat. Prod. Rep.* **1998**, 533.
Phytoprostanes: M.. Mueller et al. *The Plant Journal* **2003**, *34*, 363; *Current Opinion in Plant Biology* **2004**, *7*, 441.

Radical route to Isoprostanes

Produced in abundance in vivo in quantities far exceeding COX-derived PGs



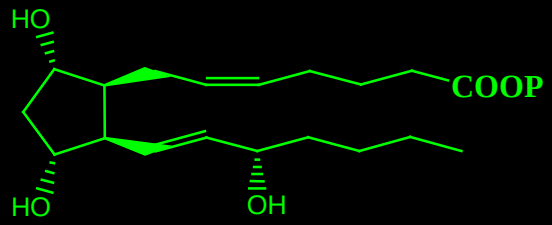


15-H_{2t}-Isop (8-*iso*-PGH_2)

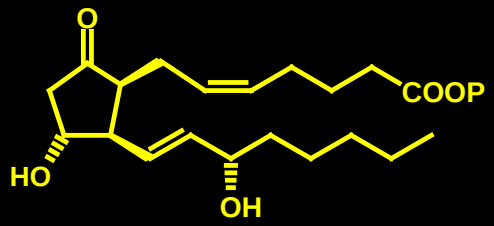
[H]

Rearrang.

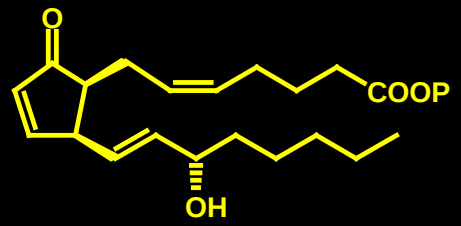
Cyclopentenone isoprostanes



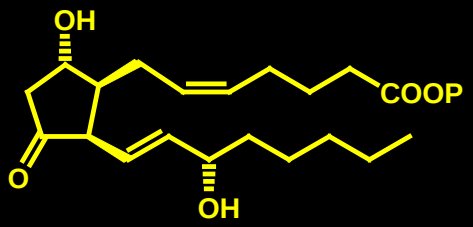
15-F_{2t}-Isop (8-*iso*-$PGF_{2\alpha}$)



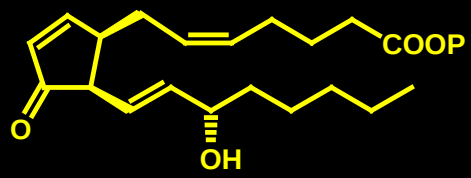
15-E_{2t}-IsoP



15-A_{2t}-IsoP



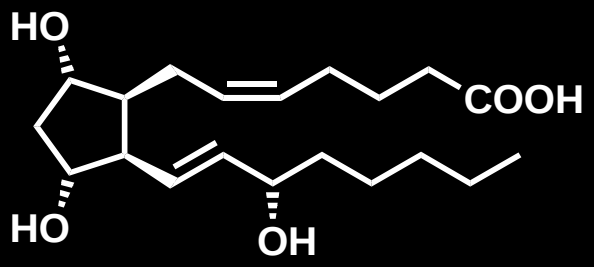
15-D_{2t}-IsoP



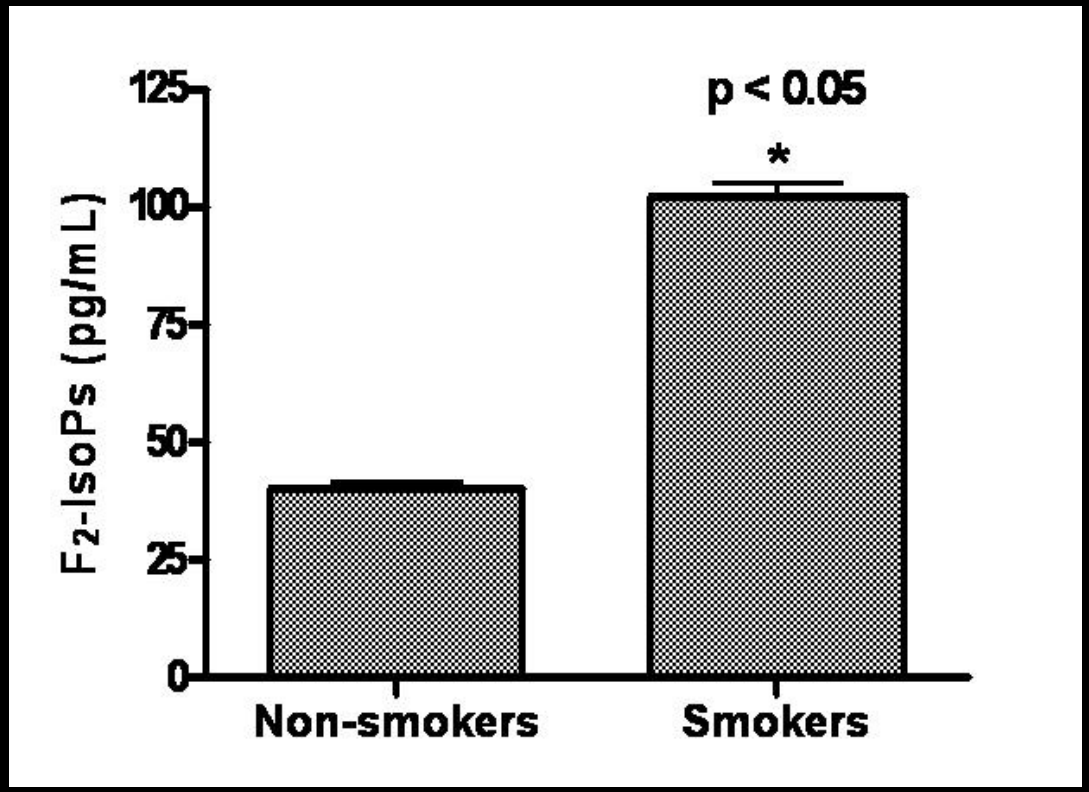
15-J_{2t}-IsoP

(a) Rokach, J.; Khanapure, S. P.; FitzGerald, G. A. *Synthesis* **1998**, 569;
(b) Morrow, J. D.; Roberts II, J. L. et al. *Biochim. Biophys. Acta* **1999**, *1436*, 550;
(c) Morrow, J. D.; Roberts II, J. L. et al. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9383;
(d) Morrow, J. D.; Roberts II, J. L. et al. *J. Biol. Chem.* **1999**, *274*, 10863.
(e) Morrow, J. D et al. *Antioxidants and Redox Signaling* **2005**, *7*, 210.

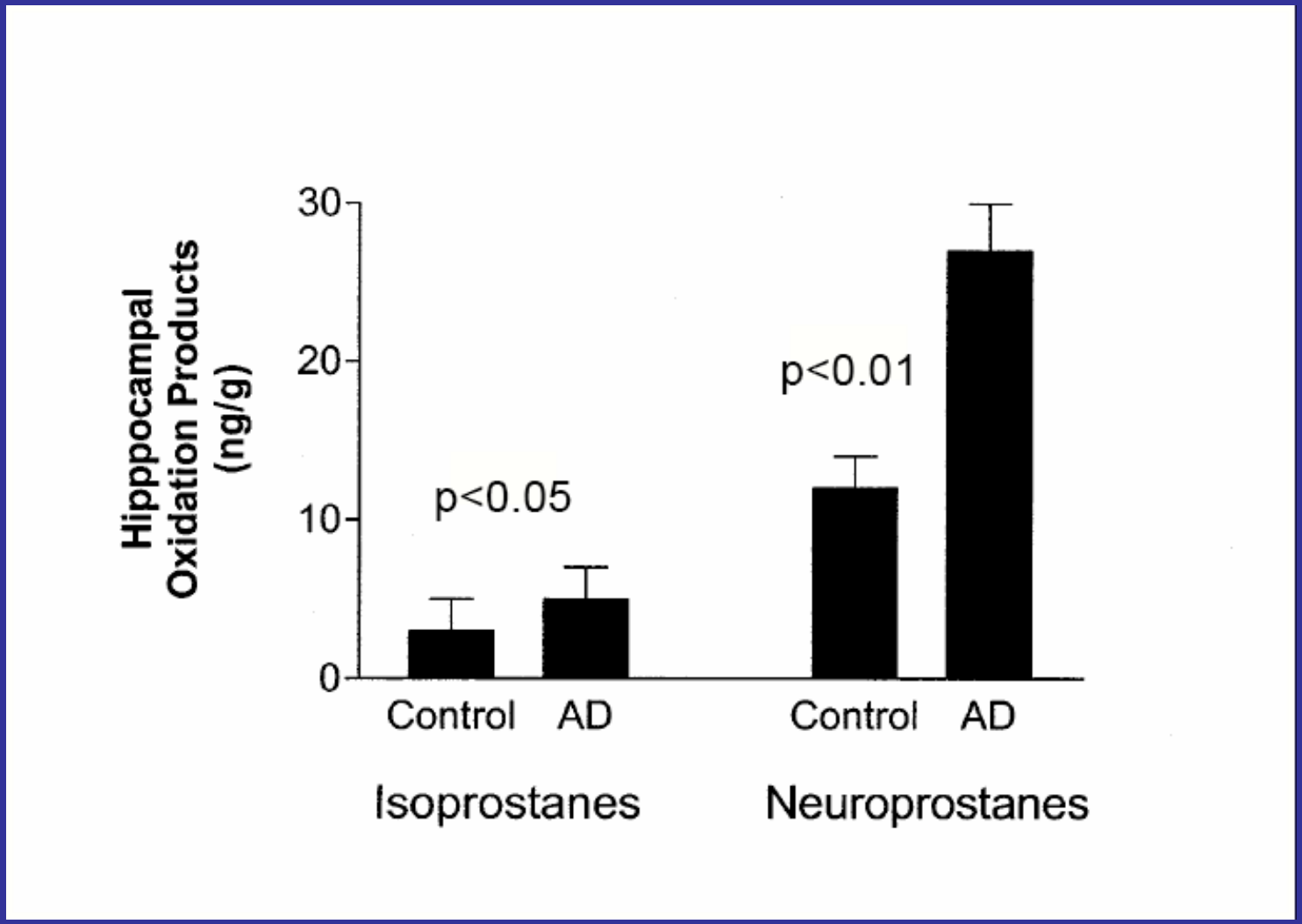
Isoprostanes in Smokers



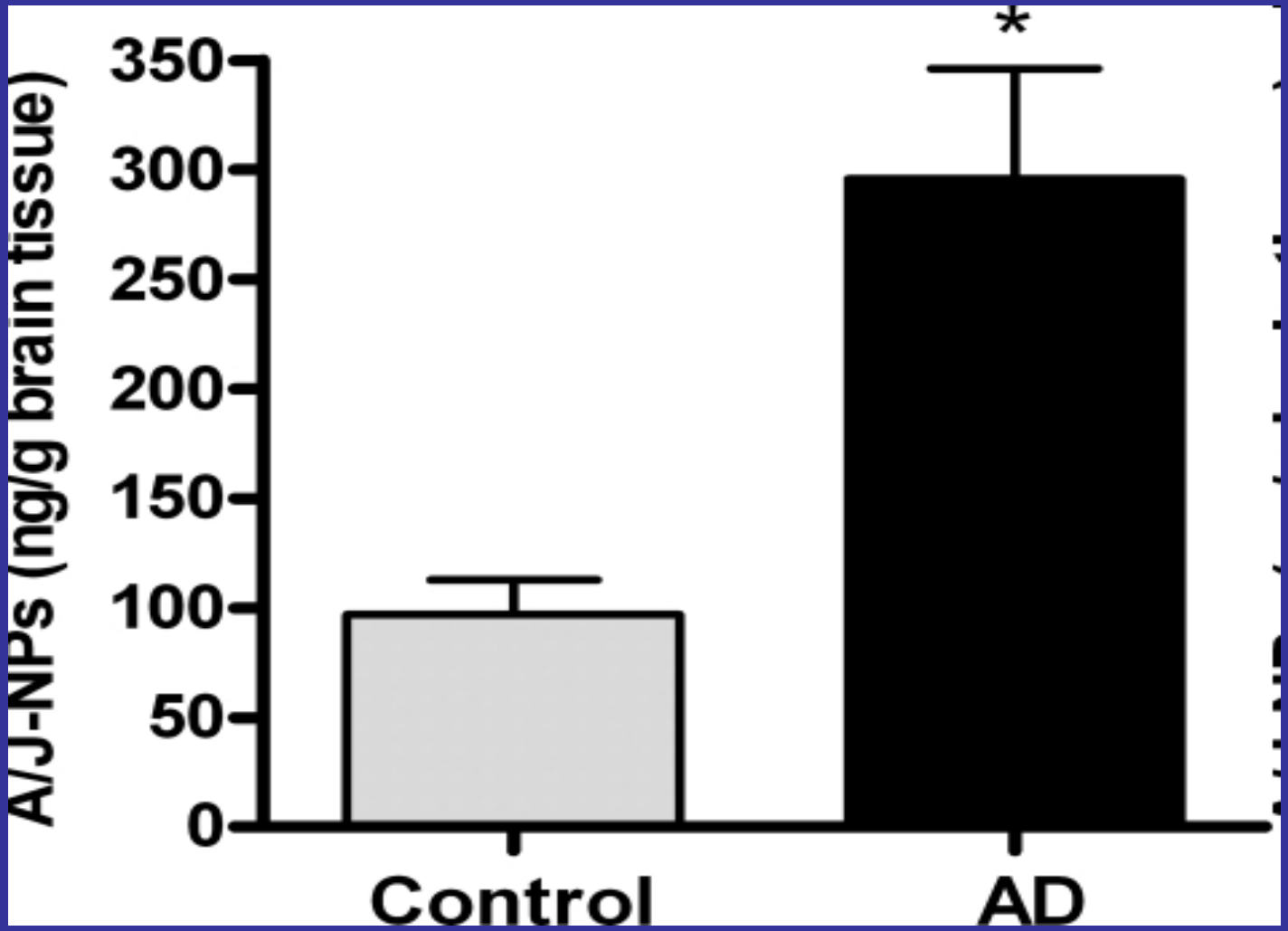
15-F_{2t}-IsoP



Neuroprostanes Vs Isoprostanes in Alzheimer Disease



A_4/J_4 Neuroprostanes in Human Brain affected by AD



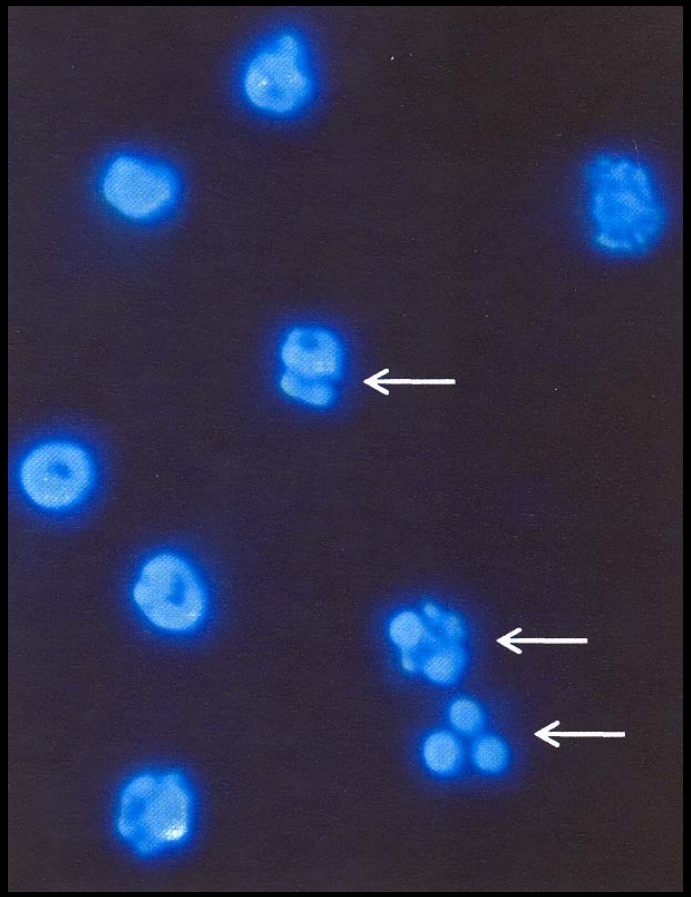
Cyclopentenone PG-Like Compounds: What is their biological significance?

Biomarkers

vs

Biological Activity

15-A_{2t}-IsoP Induces Apoptosis in Neurons

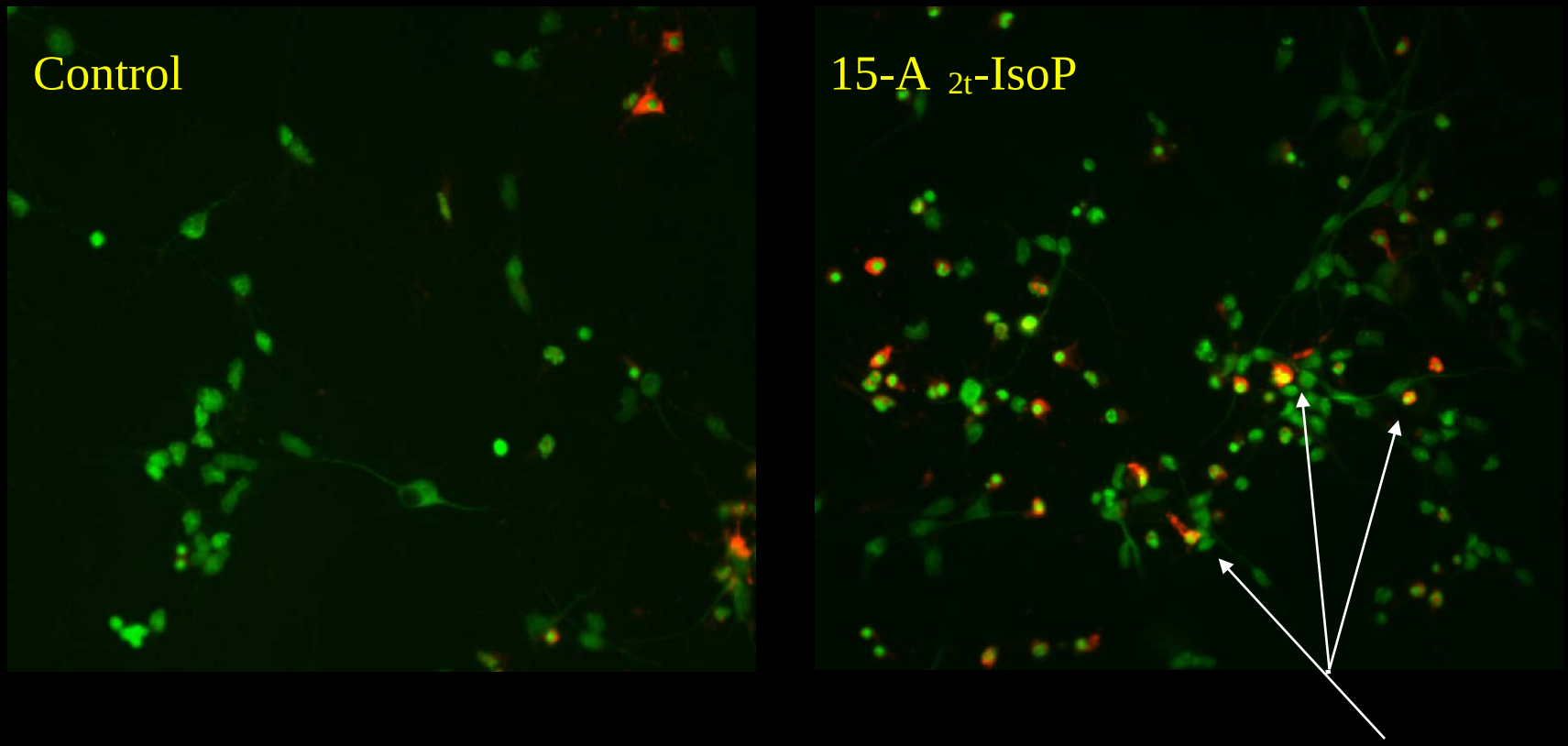


30 μM 15A_{2t}-Isop for 24h

4’-6-Diamidino-2-phenyl indole (DAPI) nuclear staining: abundance of asymmetric chromatin formation consistent with apoptotic cell death

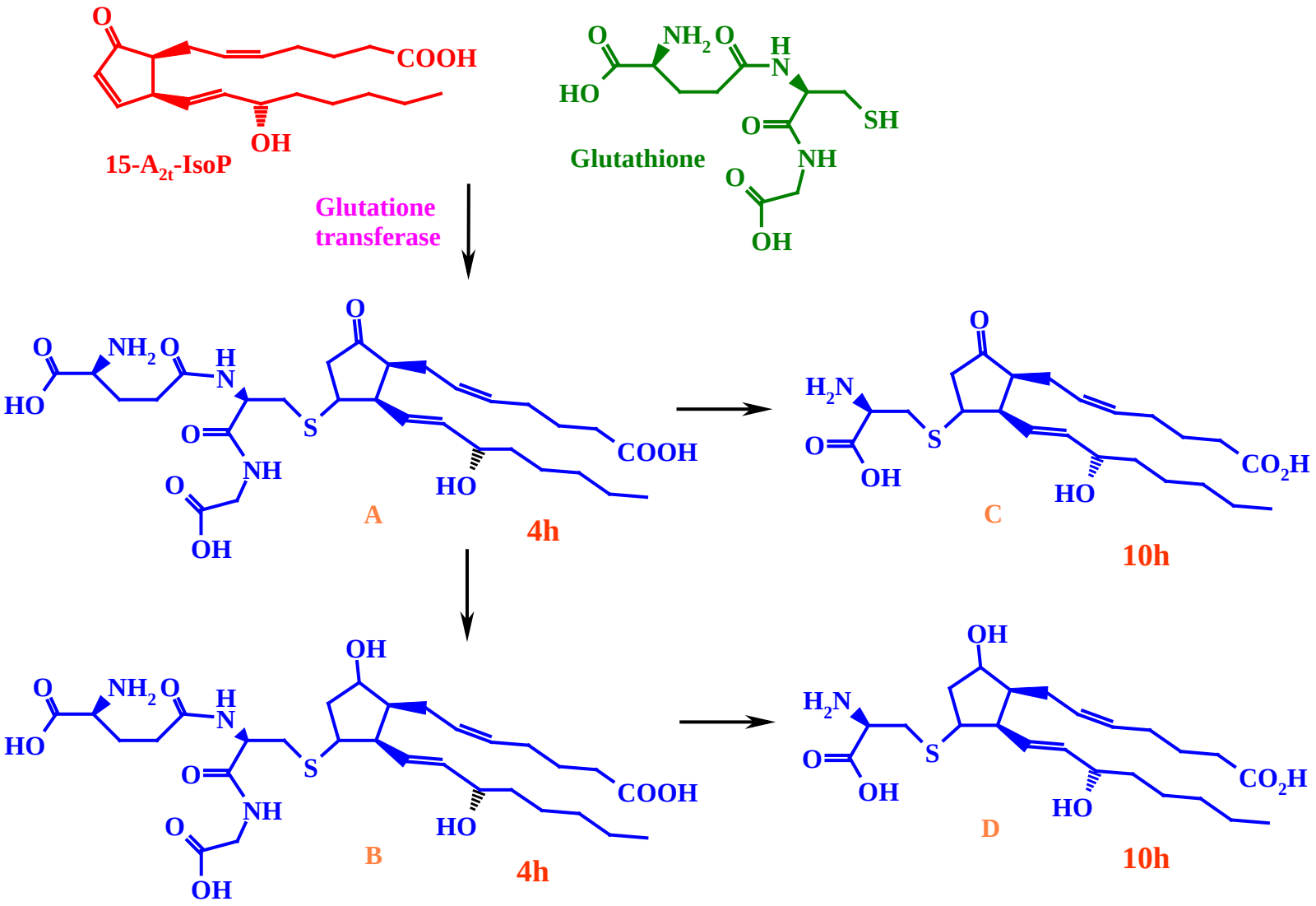
Journal of Neurochemistry, 2006, *97*, 1301

Caspase-3 is Activated by 15-A_{2t}-IsoP



Journal of Neurochemistry, 2006, 97, 1301

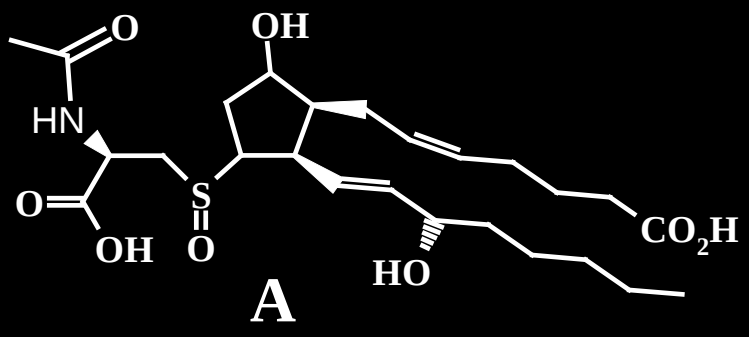
Conjugation of 15-A_{2t}-Isoprostane with Glutathione in HepG2 Cells



G. L. Milne, G. Zanoni, A. Porta, S. Sasi, G. Vidari, E. S. Musiek, M. L. Freeman, J. D. Morrow *Chem. Res. Toxicol*. **2004**, *17*, 17
G. L. Milne, L. Gao, A. Porta, G. Zanoni, G. Vidari, J. D. Morrow *J. Biol. Chem*. **2005**, *280*, 25178.

Biological activities of 15-A_{2t}-IsoP and 15-J_{2t}-IsoP

15-A_{2t}-IsoP was proven to have proangiogenic effects.
It is potently neurotoxic, activates a series of novel signalling molecules in neurons, and potentiates neurodegeneration caused by other insults.
Other studies indicate that conjugation with GSH represents a major route of metabolic disposition of 15-A_{2t}-IsoP *in vivo*. Compound A was identified in rats as the major urinary metabolite of 15-A_2-IsoP.
The conjugation renders 15-A_2-IsoPs biologically inactive and may well constitute a primary detoxification process in cells and tissues.



A

A_4-Neuroprostanes: *potent anti-inflammatory mediators*

- A_4/J_4-NPs readily form Michael adducts with GSH and key intracellular proteins.

- in RAW macrophages A_4/J4-NPs potently suppress LPS-induced nitric oxide production and NF-*K*B mediated expression of the pro-inflammatory enzymes inducible nitric oxide synthase (iNOS) and COX-2.

- A_4-NPs also completely block NF-*K*B activation induced by the pro-inflammatory cytokines TNFα and IL-1β

- NF-*K*B signalling is not inhibited by A_4-NPs at the receptor level; instead, the TNFα or LPS-induced phosphorylation of I*K*Bα by IKK and its subsequent proteasomal degradation , hence the translocation of the transcription factor NF-*K*B to the nucleus, are suppressed

- perturbation of IKK function and subsequent inhibition of NF-*K*B activation are a consequence, at least in part, of the Michael addition of the IKKβ Cys-179 thiol group to the cyclopentenone moiety of A_4-NPs

My warmest thanks !!

The B2 group: past, present,….and future