SFRBM

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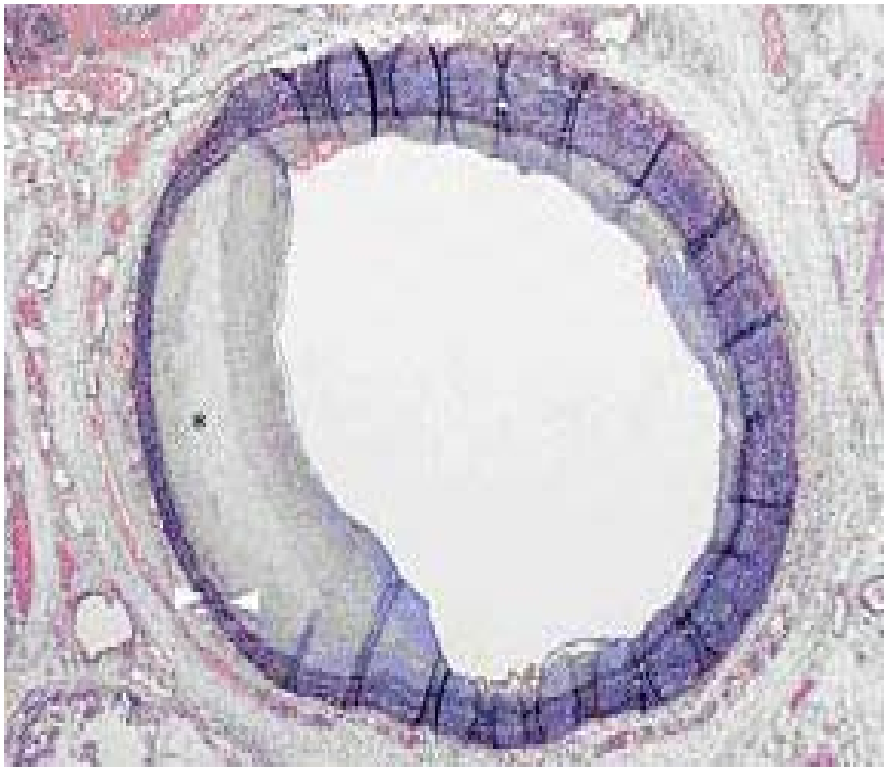
MARRIOTT INDIANAPOLIS

2008 Sunrise Free Radical School
Presentation by:

Bruce Freeman, Ph.D.

Lipid Oxidation

Bruce Freeman, PhD
Department of Pharmacology and Chemical Biology
University of Pittsburgh School of Medicine
freerad@pitt.edu



Brief History – Milestones in Lipid Oxidation

- BCE Fat was subjected to base hydrolysis to make soap
- 1800 Walnut oil shown to consume 150x its volume in oxygen
- 1818 “Cholesterine” described in gallstones
- 1827 Fat, in addition to protein and CHO, reported as key dietary constituent
- 1834 Chloroform, diethyl ether utilized for lipid extractions
- 1835-1850 Free fatty acids purified, chain length/unsaturation described
- 1860 French patent awarded for margarine
- 1861 Lipases first described in plant seeds, pancreatic fluids
- 1881 Hydroxy fatty acid derivatives first described
- 1886 Diene nature of linoleic acid (18:2) and triene nature of linolenic acid (18:3) first described
- 1895 Diesel built first engine that ran on peanut oil
- 1895 First proposal red cell membranes made of lipids, 2 molecules thick
- 1899 Acetone used to separate phospholipids from cholesterol, glycerides

Brief History – Milestones in Lipid Oxidation

- 1902 Patent issued for conversion of unsaturated fatty acids and glycerides into saturated fats
- 1903 Cobra venom shown to hydrolyze phosphatidylcholine
- 1909 Arachidonic acid (20:4) first isolated from liver lipids
- 1913 Lipid soluble vitamins first recognized as necessary
- 1922 “Antioxygenes" identified as phenolic substances preventing the oxidation butter and other vegetable oils
- 1926 Term “lipid” first used
- 1930 Dietary essentiality of long chain polyunsaturated fatty acids recognized
- 1932 Description of a fatty acid oxidase activity ("lipoxydase") in soybean
- 1935 Eicosapentaenoic and docosahexaenoic (20:5, 22:6) first identified in sardine oil, term “steroids” first coined, tocopherol structure ID’ed
- 1939 Inositol and inositol phosphate lipids first described
- 1943 Hydroperoxides identified as constituents of “rancid” fat
- 1950 18:2 observed to be a precursor of 20:4 – fatty acid elongation

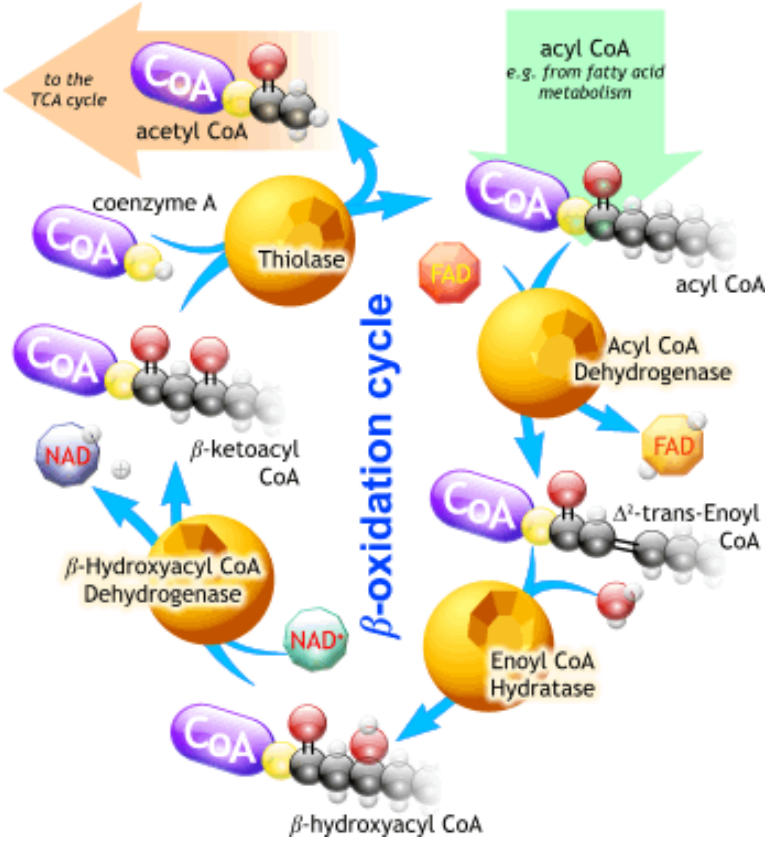
Brief History – Milestones in Lipid Oxidation

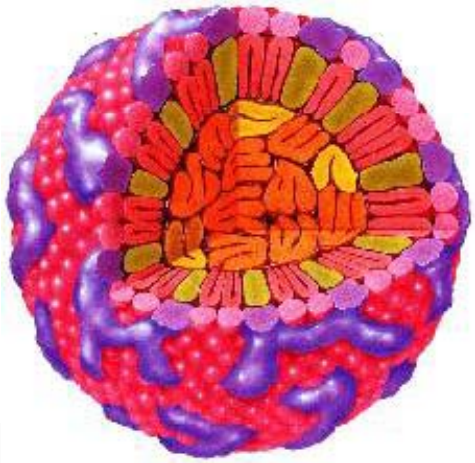
- 1951 TLC, GLC, alumina column chromatography and rotavaps used!!
- 1952 Trans fatty acids were detected in biological specimens
- 1953 Acetylcholine was observed to initiate diglyceride release from membrane PL, mitochondrial β-oxidation described
- 1957 First detection of prostaglandins, 1964 20:4 conversion to PGE2
- 1965 Artificial membranes (liposomes) produced
- 1972 Serum cholesterol, LDL and triglyceride levels linked with CAD, platelet activating factor (PAF, alkyl acetyl PC) first described, fluid mosaic model of cell membrane proposed
- 1975 "Phosphatidyl inositol effect" linked with regulation of cell Ca levels
- 1978 EPA (20:5) linked with inhibition of thrombosis, atherosclerosis
- 1979 Leukotrienes (OH derivatives of 20:4, SRS-A) first described
- 1980 Free radical oxidation of membrane phospholipids described
- 81-84 Oxidation of lipoproteins results in pro-atherogenic foam cell formation
- 1982 Nobel Prize for prostaglandins, leukotrienes, thromboxanes
- 1985 Nobel Prize for regulation of cholesterol metabolism

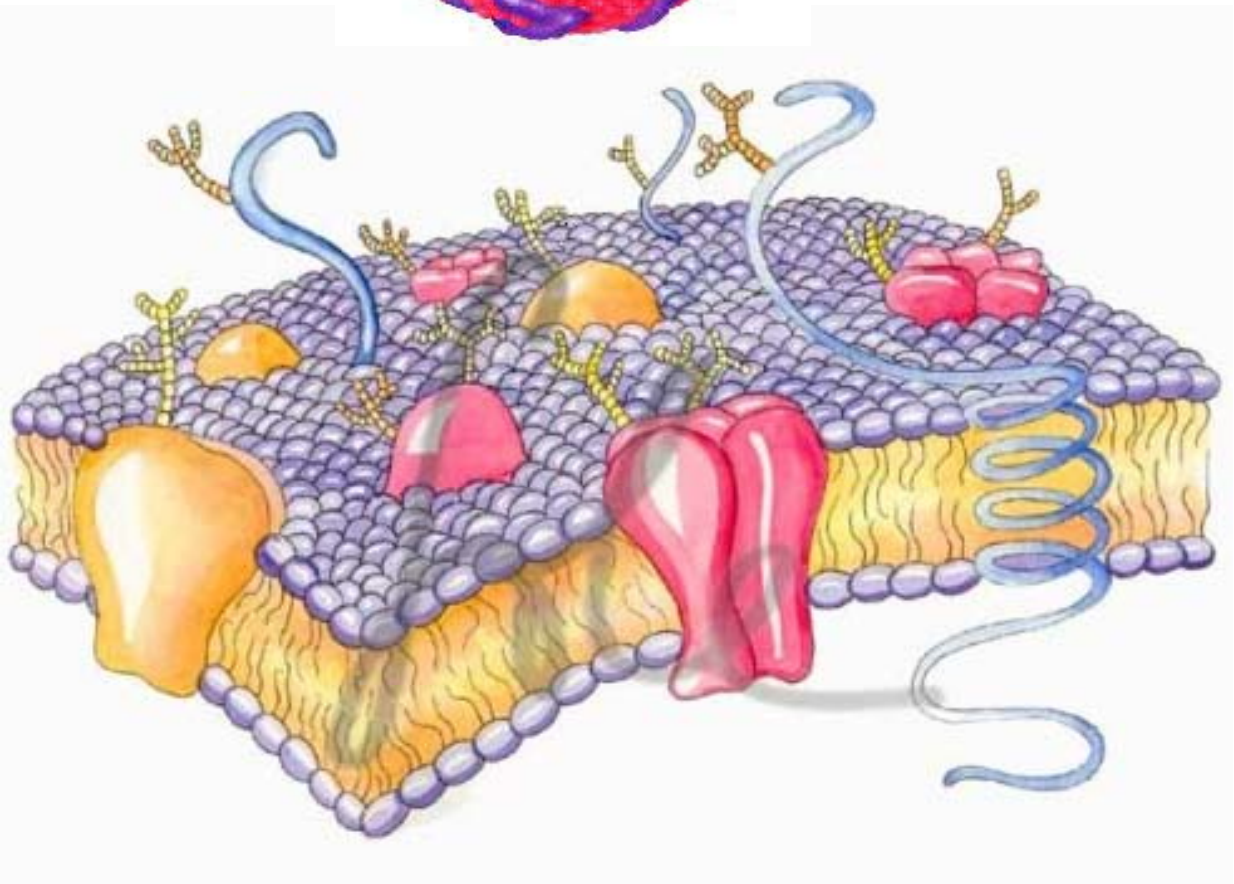
Brief History – Milestones in Lipid Oxidation

- **1985 Fish oil reported to reduce intimal proliferation after vein grafting**
- **1987 Conjugated linoleic acid (CLA) described as anti-cancer, cardioprotective**
- **1990 Isoprostanes described as free radical oxidation products of 20:4**
- **1990 Peroxisome proliferator activated receptors and other nuclear lipid receptors described**
- **1995 2-arachidonyl glycerol described as ligand for cannabinoid receptor**
- **96-99 F4-neuroprostanes, isothromboxanes and F3-isoprostanes described**
- **1999 Nitrogen dioxide observed to induce cis-trans isomerization of 20:4**
- **2003 Hydroxytriene derivatives of 20:5, 20:6 shown to be anti-inflammatory**
- **02-08 Electrophilic FA shown to be anti-inflammatory, PPARγ ligands**

Lipids First Viewed as Metabolic Intermediates And Membrane Structural Components

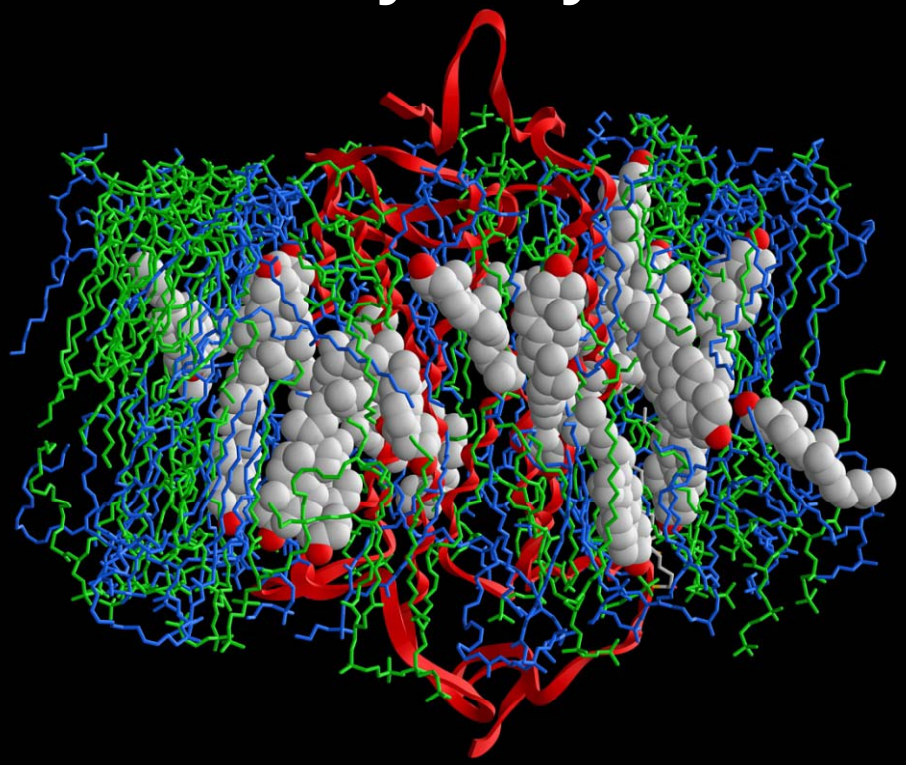




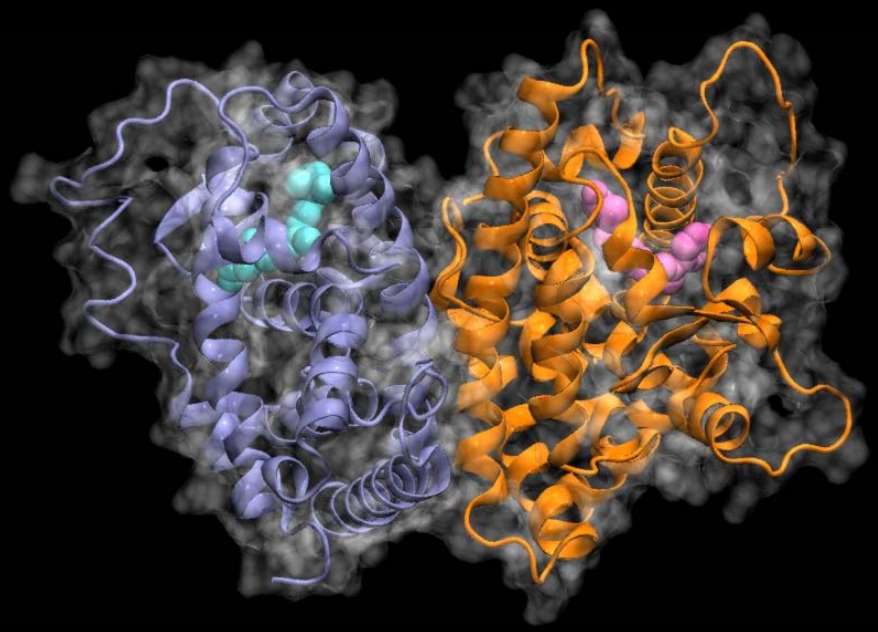


New View

Enzymatic and Autocatalytic Oxidation of Lipids Yields Products that Mediate Cell Signaling via Discrete Reactions with Receptors, Transcription Factors, Protein Kinases and Key Enzymes of Intermediary Metabolism



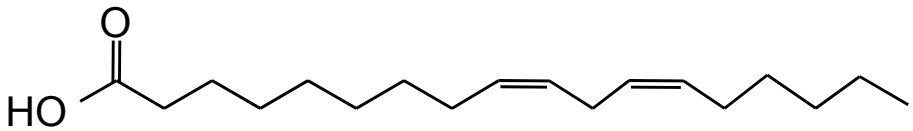
G-Protein Coupled Receptors



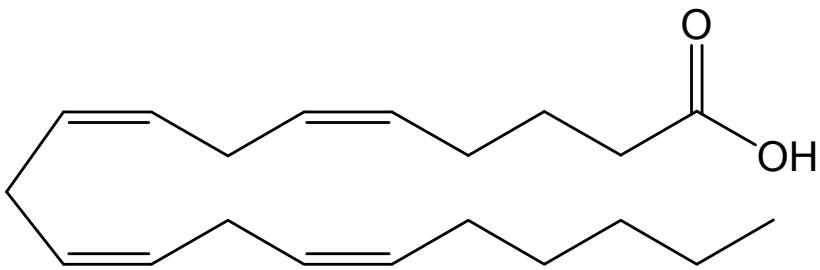
Peroxisome Proliferator Activated Receptor – Retinoid X Receptor Heterodimer

Fatty Acids

Fatty Acids have common and IUPAC names and numerous acronyms



Linoleic Acid
Octadeca-9,12-dienoic acid
18:2 *m/z* 279
LA

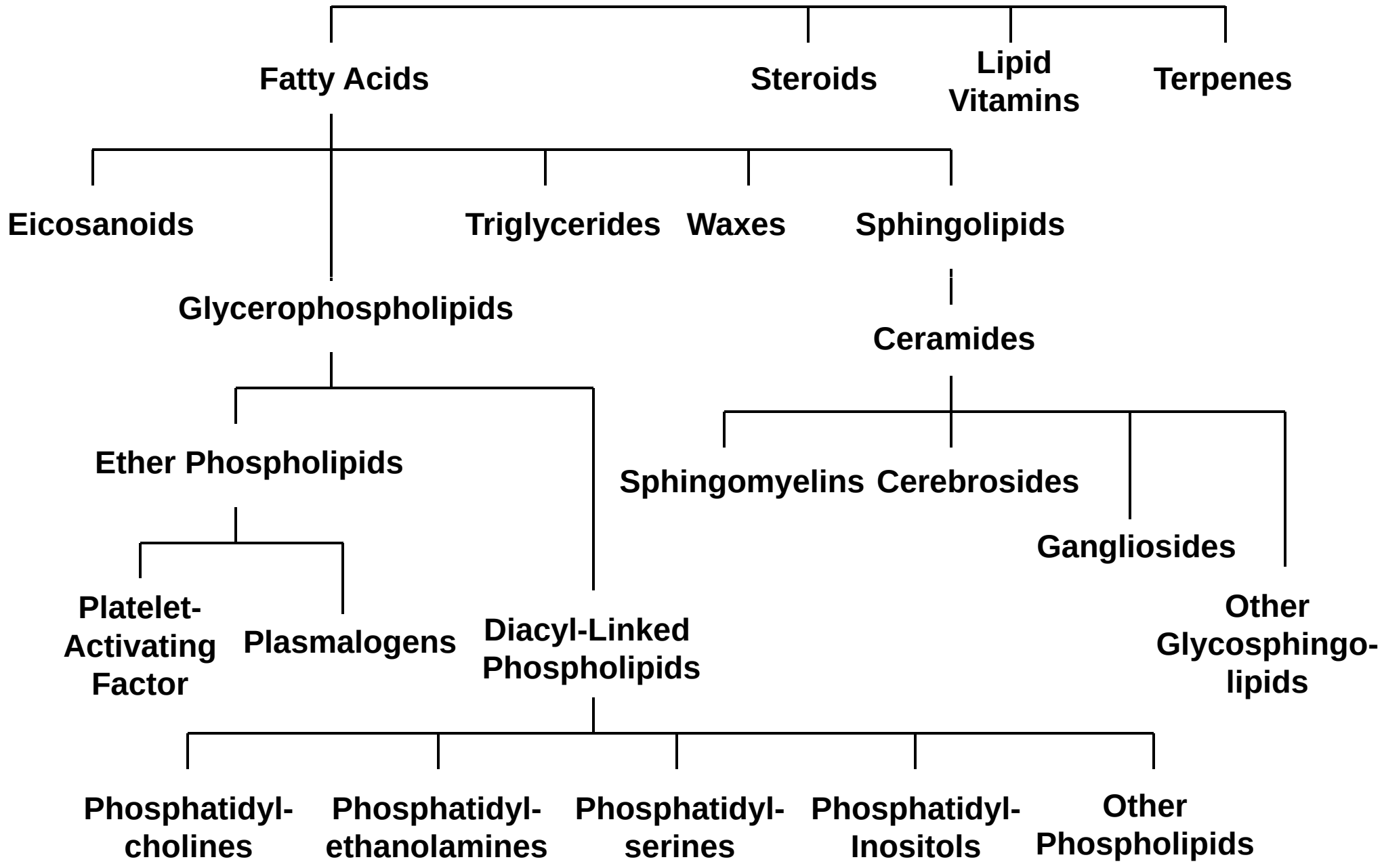


Arachidonic Acid
Eicosatetraenoic Acid
Dodeca-5,8,11,14-tetraenoic acid
20:4 *m/z* 303
AA

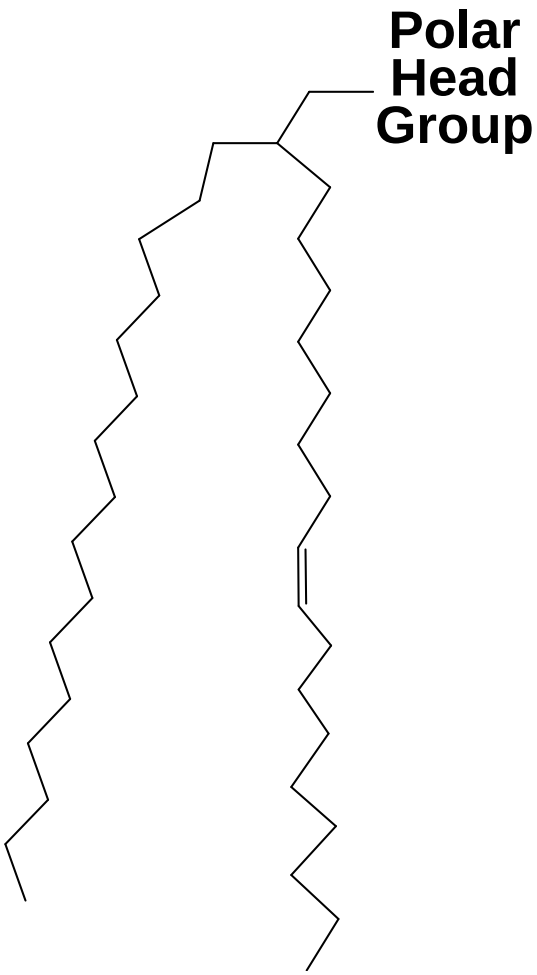
Myristic acid	**(14:0) *m/z* 227**	**Linolenic Acid**	**(18:3) *m/z* 277**
Palmitic acid	**(16:0) *m/z* 255**	**Arachidic Acid**	**(20:0) m/z 311**
Stearic acid	**(18:0) *m/z* 283**	**Eicosapentaenoic acid**	**(20:5) *m/z* 301**
Oleic acid	**(18:1) *m/z* 281**	**Docosahexaenoic acid**	**(22:6) *m/z* 327**

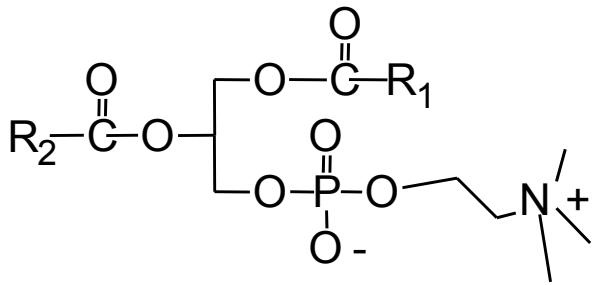
m/z values for negative ion mode

Lipids - ~180,000 different species/cell

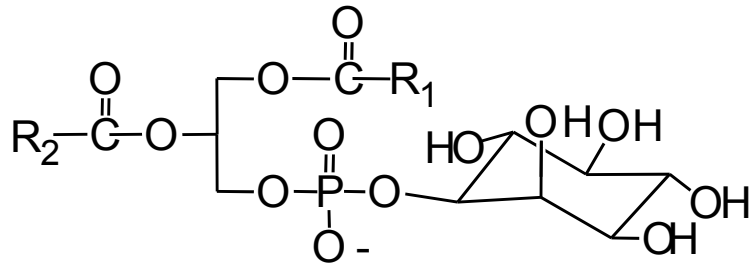


Phospholipid Structures

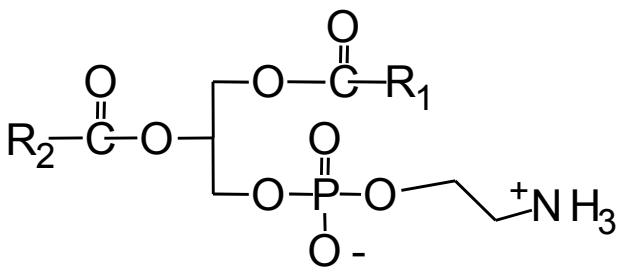




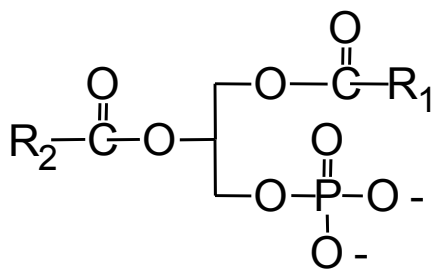
Phosphatidylcholine



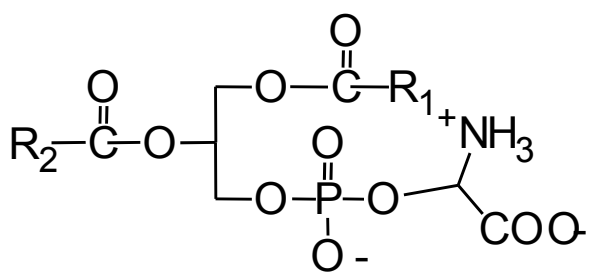
Phosphatidylinositol



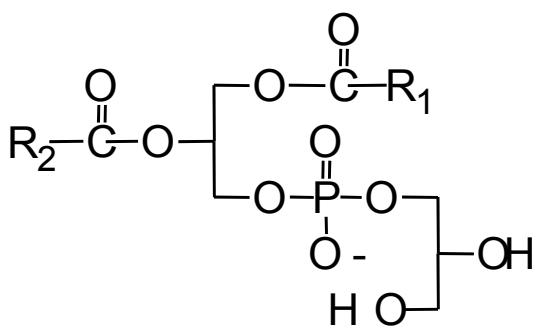
Phosphatidylethanolamine



Phosphatidic acid

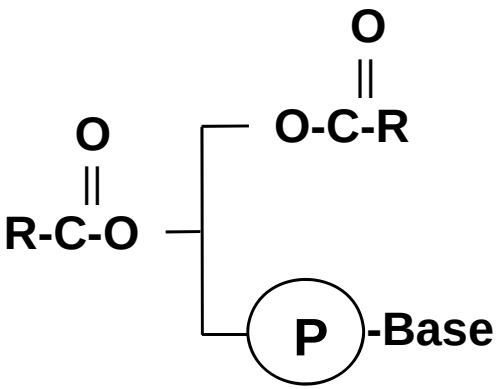


Phosphatidyl serine

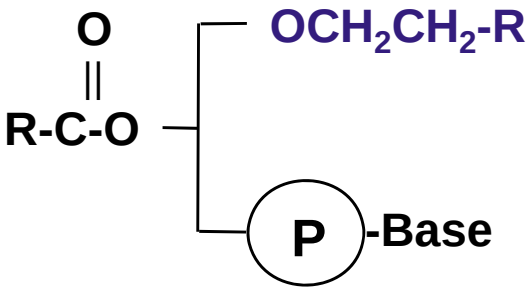


Phosphatidylglycerol

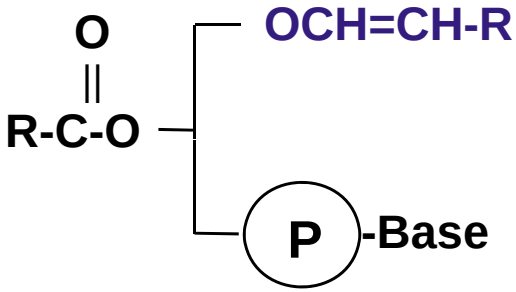
Glycerolipid Bond Type at the *sn*-1 Carbon



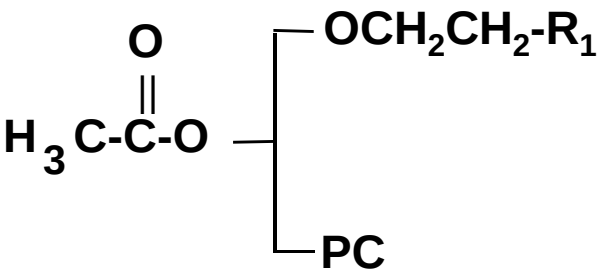
Diacyl-



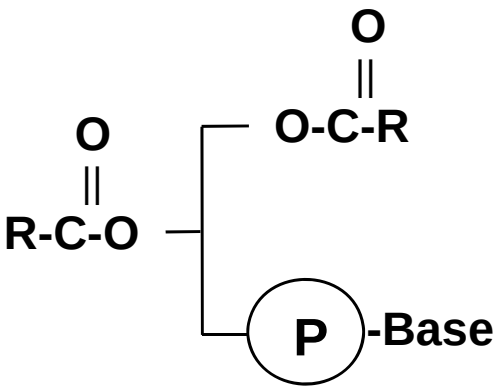
1-*O*-alkyl-2-acyl-



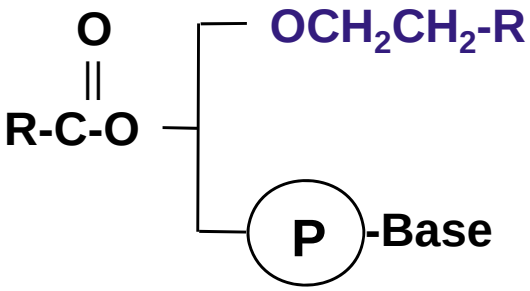
1-*O*-alk-1'-enyl-2-acyl-
(plasmalogen)



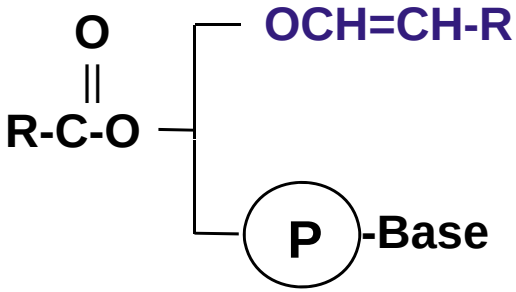
Glycerolipid Bond Type at the *sn*-1 Carbon



Diacyl-



1-*O*-alkyl-2-acyl-



1-*O*-alk-1'-enyl-2-acyl-
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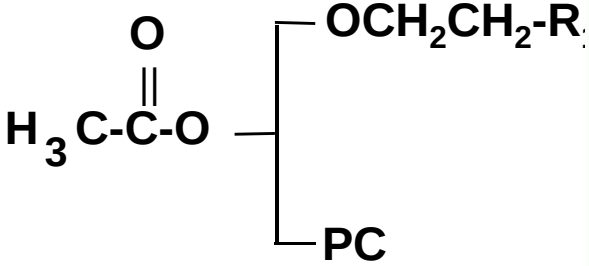
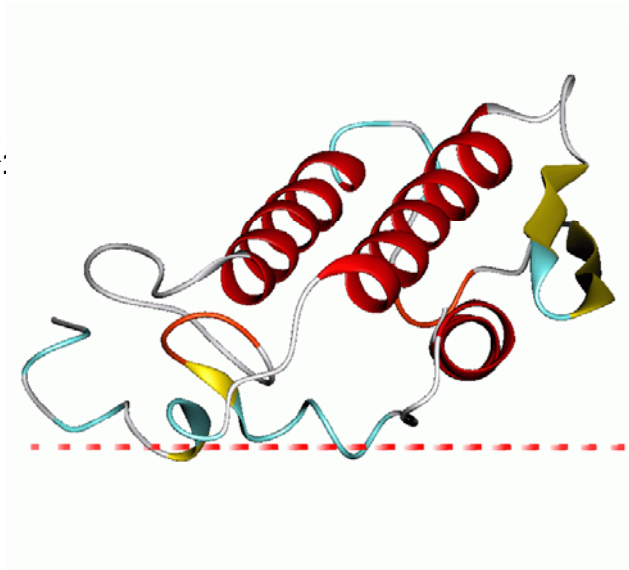
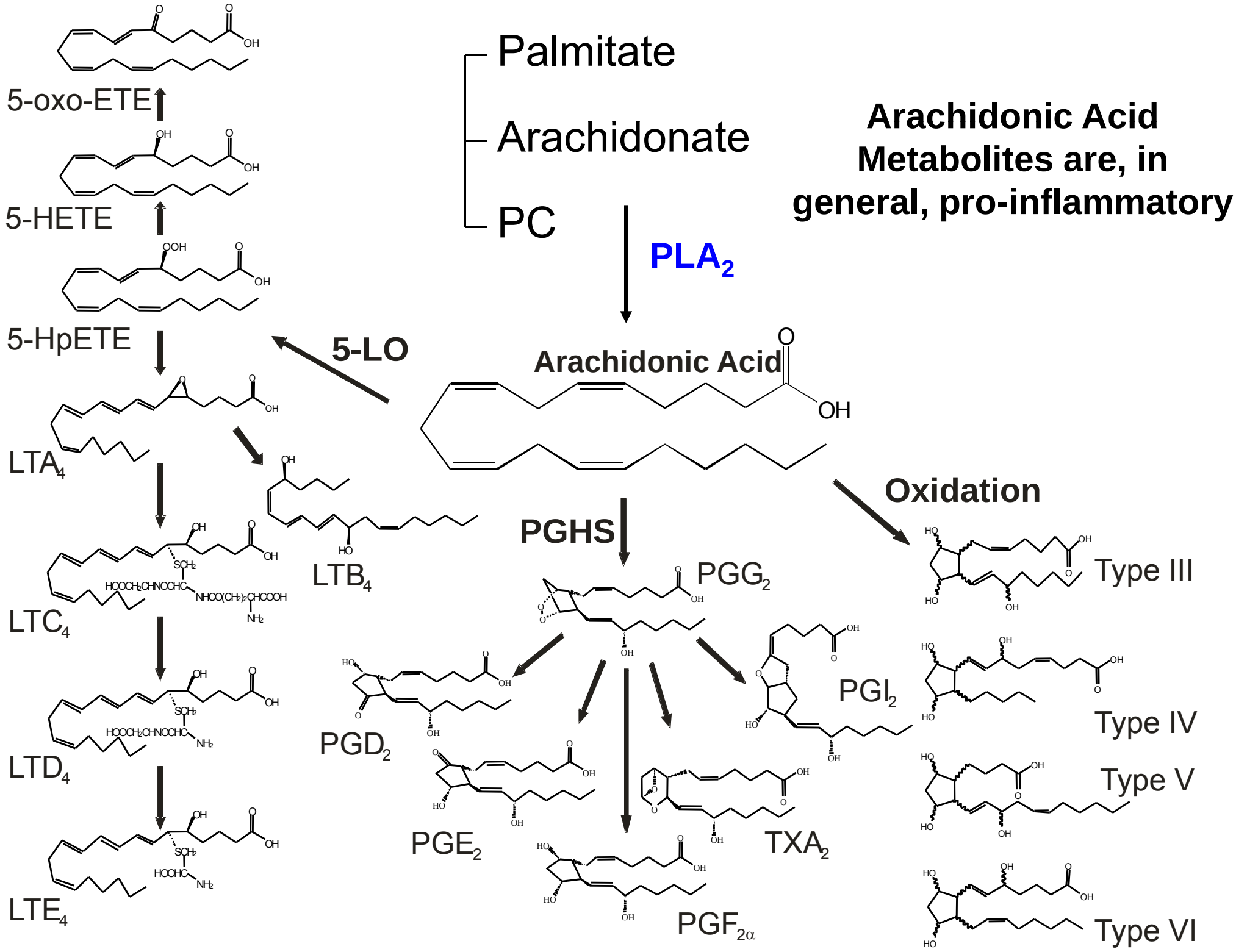


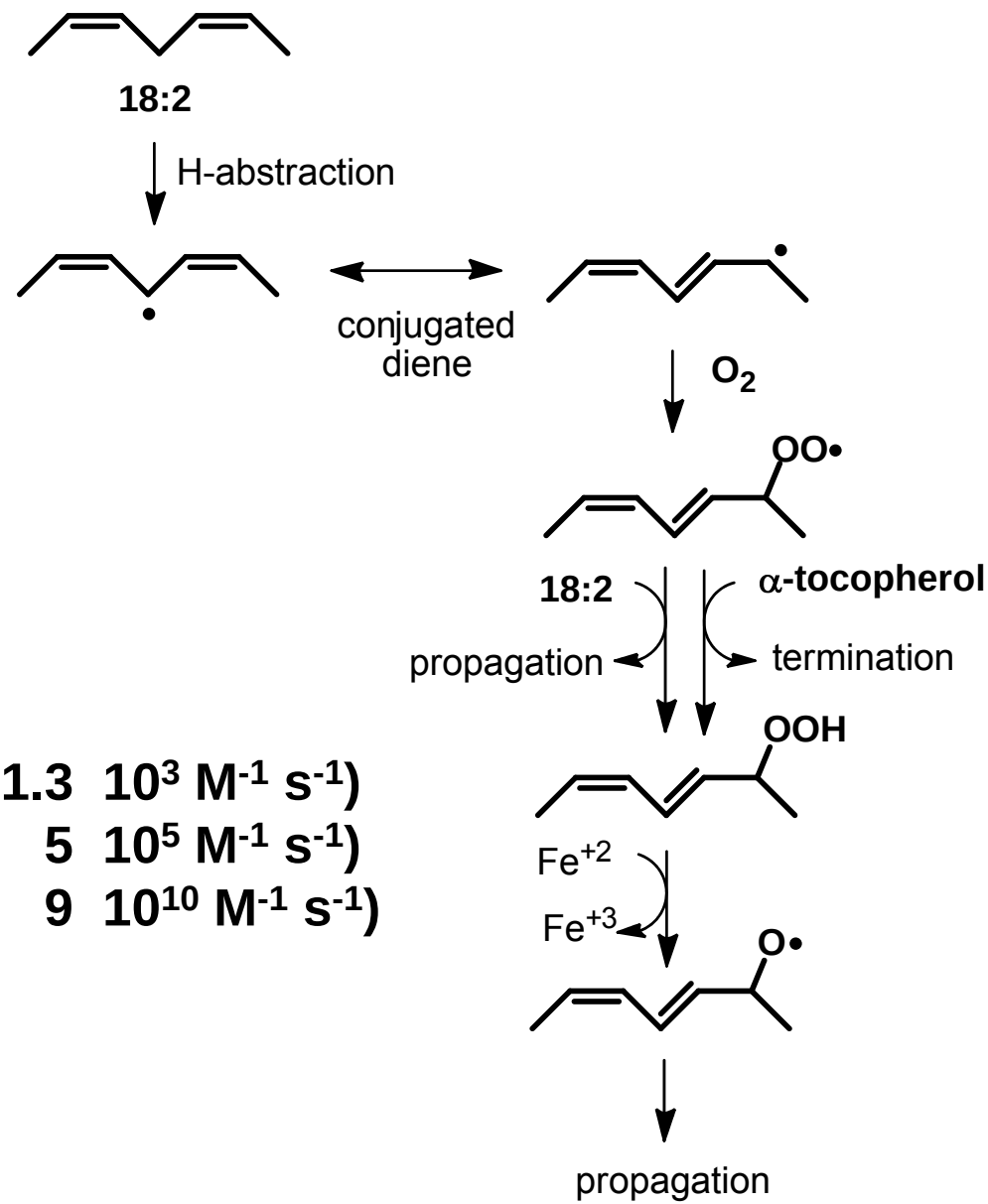


Phospholipase A_2
Preferentially hydrolyzes oxFA at sn-2 position

FRBM 1985, 1:263–271


Arachidonic Acid Metabolites are, in general, pro-inflammatory
Palmitate
Arachidonate
PC
PLA2
Arachidonic Acid
5-LO
5-oxo-ETE
5-HETE
5-HpETE
LTA4
LTB4
LTC4
LTD4
LTE4
PGHS
PGG2
PGD2
PGE2
PGF2α
PGI2
TXA2
Oxidation
Type III
Type IV
Type V
Type VI

Peroxidation of Unsaturated Fatty Acids

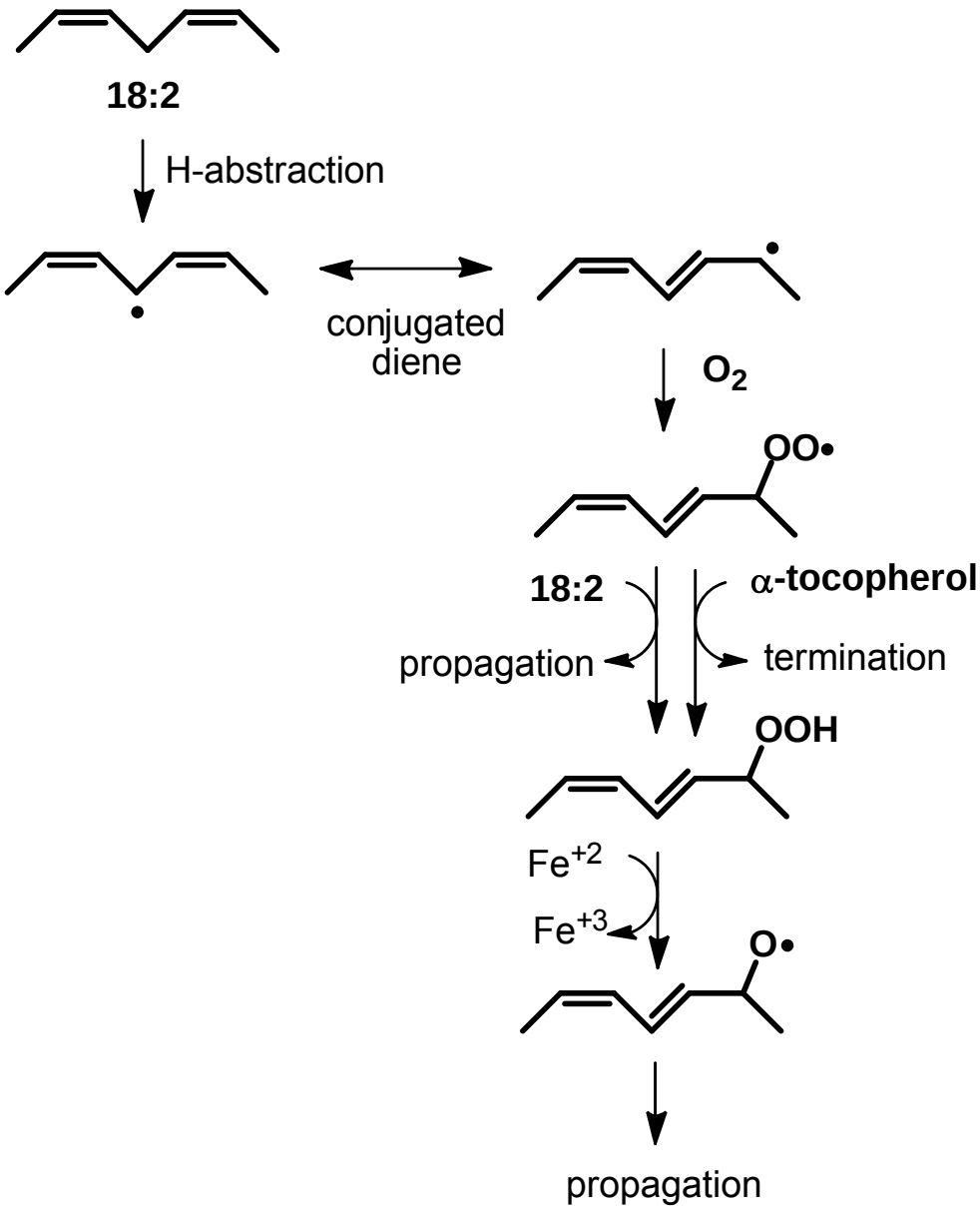


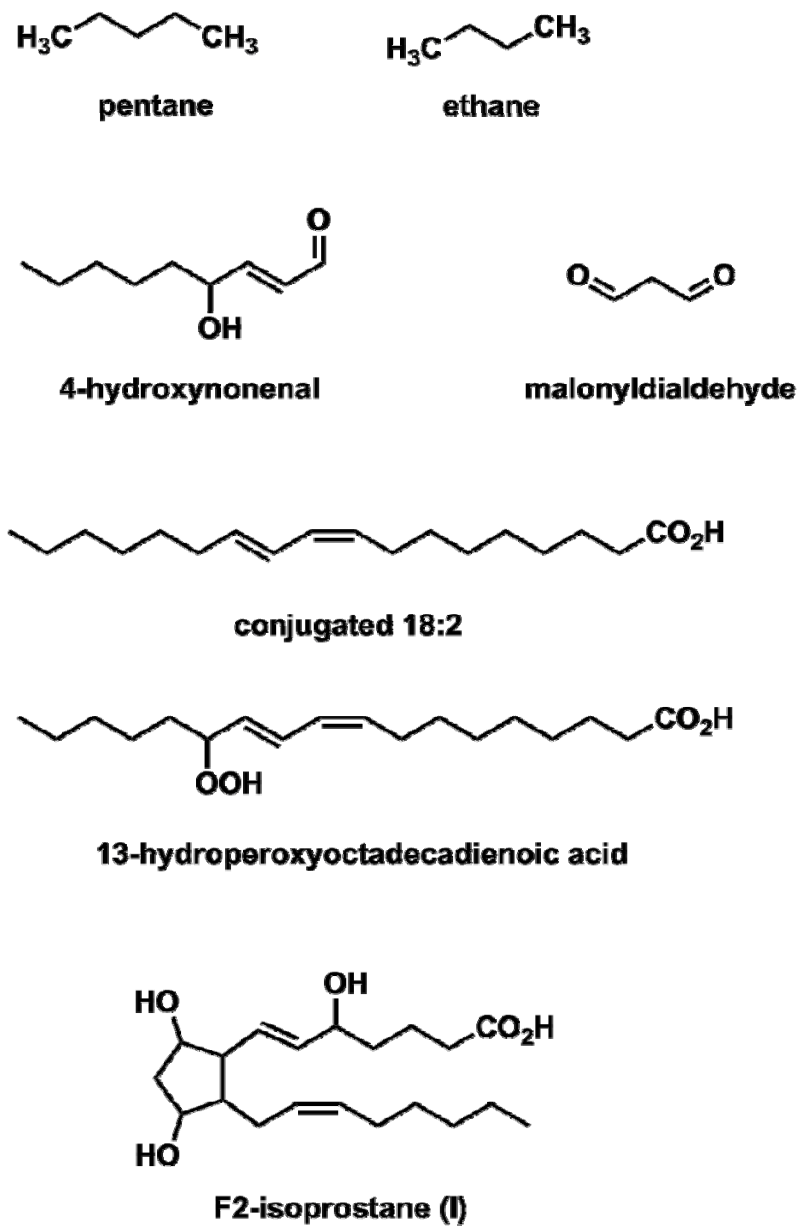
Propagation **(k =1.3 10^3 M^{-1} s^{-1})**

α-TH - LOO· **(k = 5 10^5 M^{-1} s^{-1})**

NO· - LOO· **(k = 9 10^{10} M^{-1} s^{-1})**

Principal Peroxidation Products





Iron Enhances Lipid Peroxidation

- **Iron and other transition metals catalyze the oxidation of biomolecules**
- **Iron oxidizes unsaturated fatty acids indirectly *via* the generation of iron-oxo complexes, partially reduced oxygen species and lipid alkoxyl and peroxyl radicals**
- **Agents that reduce Fe(II) to Fe(II) accelerate lipid oxidation**
- **Peroxynitrite-induced lipid oxidation is Fe-independent**

$$\text{Fe (II)} + O_2 \longrightarrow \text{Fe (III)} + \mathbf{O_2^{\bullet -}}$$
$$2\, O_2^{\bullet -} + 2H^+ \longrightarrow \mathbf{H_2O_2} + O_2$$
$$H_2O_2 + \text{Fe (II)} \longrightarrow \text{Fe(III)} + \mathbf{HO^\bullet} + OH^-$$

$$\mathbf{ROOH} + \text{X-Fe (II)} \longrightarrow \mathbf{RO^\bullet} + OH^- + \text{X-Fe(III)}$$
$$\mathbf{RO^\bullet} + LH \longrightarrow ROH + \mathbf{L^\bullet}$$
$$\mathbf{L^\bullet} + O_2 \longrightarrow \mathbf{LOO^\bullet}$$

Cholesterol Oxidation

Free radical-mediated reactions

Two epimeric ChOOHs are generated:
3β-hydroxycholest-5-ene-7α-hydroperoxide (7α-OOH)
3β-hydroxycholest-5-ene-7β-hydroperoxide (7β-OOH).

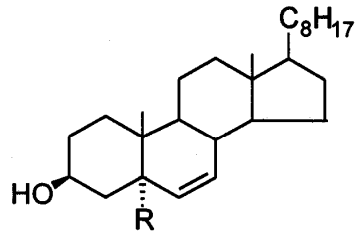
These are accompanied by:
cholest-5-ene-3β,7α-diol (7α-OH)
cholest-5-ene-3β,7β-diol (7β-OH)
5,6-epoxide epimers, and the 7-ketone.

CE oxidation can give rise to species modified in either the cholesteryl or fatty acyl moiety, or both.

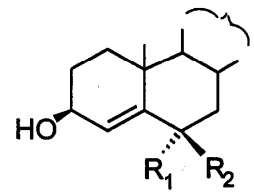
Singlet oxygen-mediated reactions

Three ChOOHs are formed *via* ene-type addition:
3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α-OOH, major
3β-hydroxycholest-4-ene-6α-hydroperoxide (6α-OOH)
3β-hydroxycholest-4-ene-6β-hydroperoxide (6β-OOH).

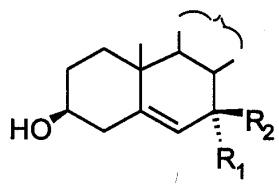
5α-OOH, 6α-OOH and 6β-OOH can be chemically or enzymatically reduced to 5α-OH, 6α-OH and 6β-OH, respectively.



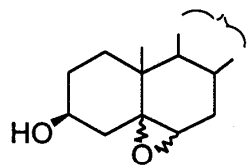
	R
5α-OOH	OOH
5α-OH	OH



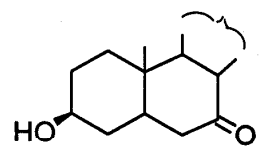
	R_1	R_2
6α-OOH	OOH	H
6β-OOH	H	OOH
6α-OH	OH	H
6β-OH	H	OH



	R_1	R_2
7α-OOH	OOH	H
7β-OOH	H	OOH
7α-OH	OH	H
7β-OH	H	OH



5,6-epoxide



7-ketone

Thanks to A. Girotti

Nitric Oxide Plays an Inextricable Role in Regulating Lipid Oxidation

- **NO accelerates the rate and increases the breadth of reactions that transduce “redox signaling”**

- **NO promotes covalent target molecule modification by serving as a precursor for oxidizing, nitrosating and nitrating species**

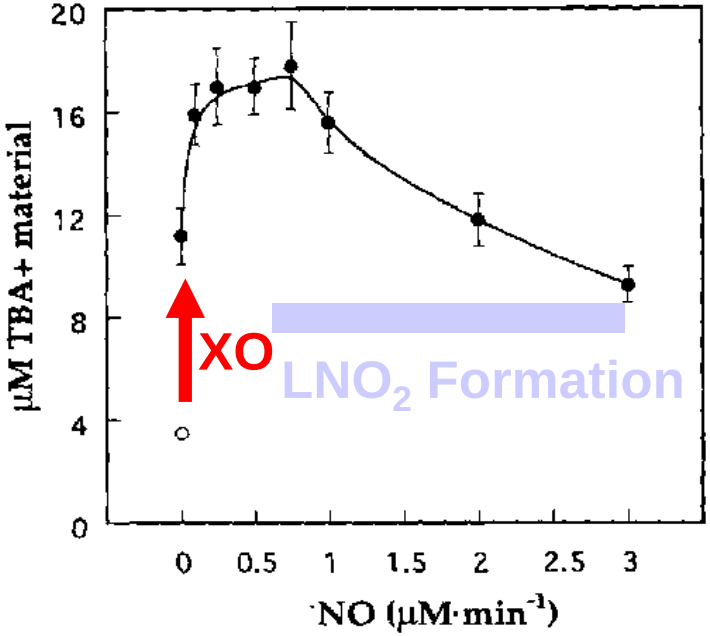
- **NO is lipophilic, concentrates in membranes, lipoproteins**

- **NO modulates both the gene expression and catalytic activity of cyclooxygenases and and lipoxygenases**

- **NO participates in reactions that both stimulate and inhibit lipid peroxidation, depending on the underlying oxidative mileu**

- **Lipid peroxidation processes catalytically consume NO and can impact on cGMP-dependent signaling**

Nitric Oxide Modulates $O_2^{\cdot -}/H_2O_2/Fe$ – Induced Fatty Acid Oxidation



- NO switches from oxidative to nitrosative/nitrative chemistry

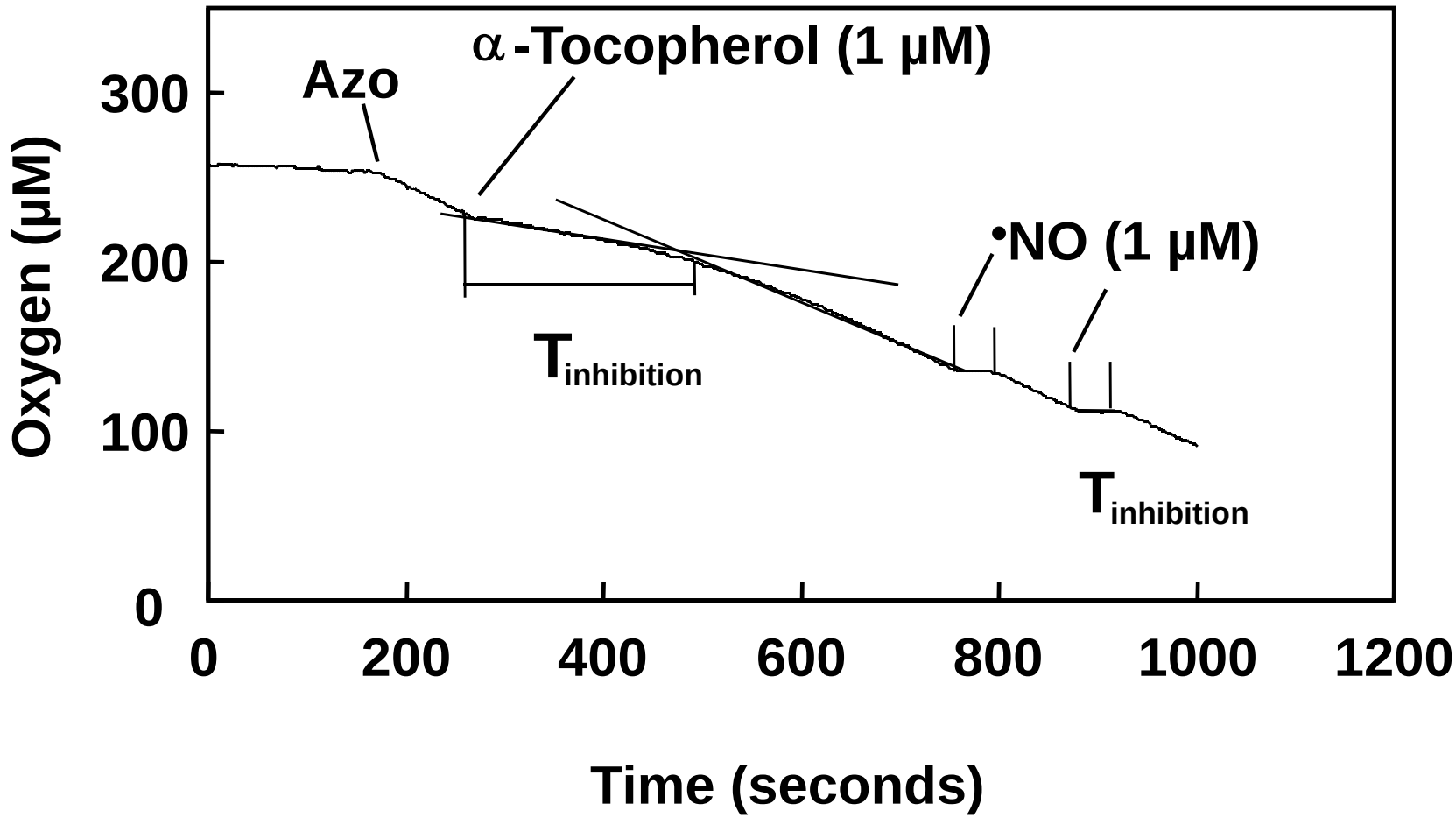
$$O_2\bullet^{-} \xrightarrow{\bullet NO} ONOO^{-} \xrightarrow{H^+} ONO\bullet \quad \bullet OH$$

$$ONOO^{-} \xrightarrow{CO_2} ONO\bullet \quad \bullet OCO_2$$

$$\bullet NO + LOO^{\bullet} \rightarrow LOONO \rightarrow \rightarrow LOOH, LNO_2, L(O)NO_2, NO_2^{-}$$

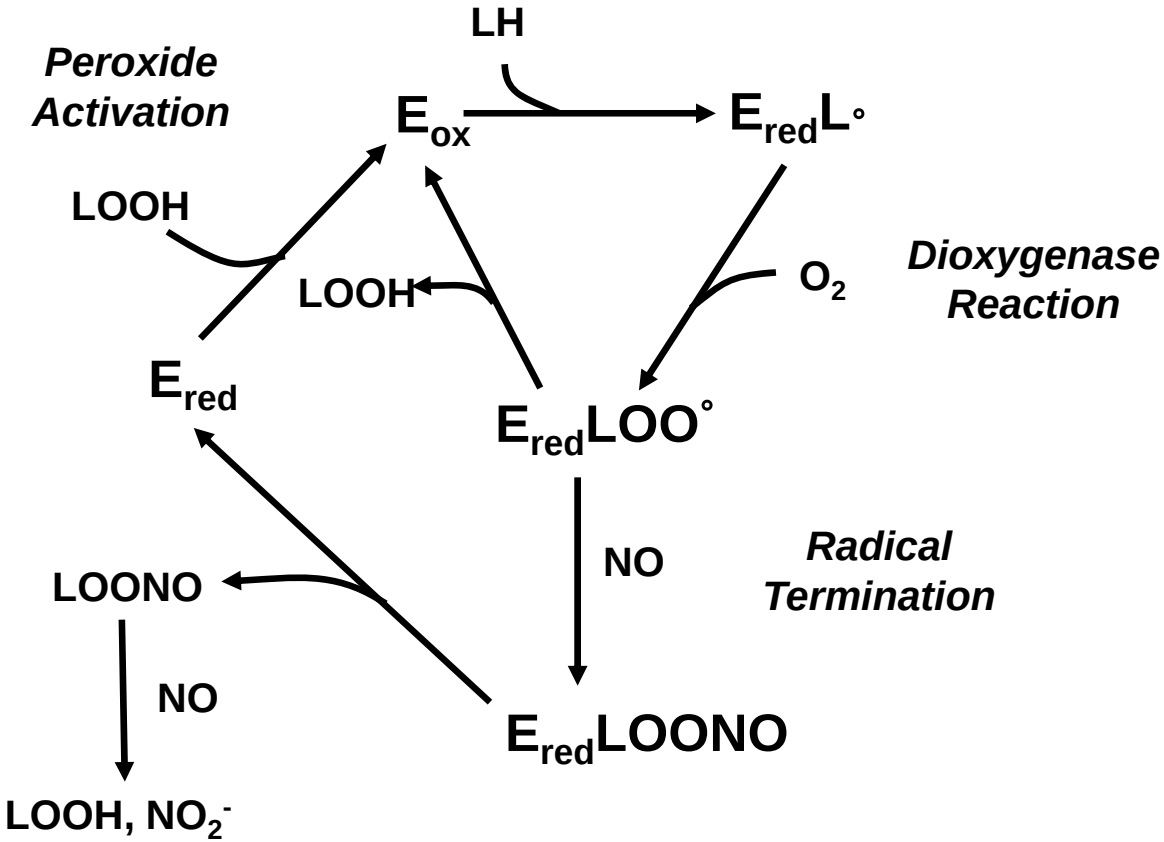
J Biol Chem 269:28066-28075, 1994

Oxygen is Consumed by Lipid Oxidation



Biochemistry 1997, 36:15216

Lipoxygenase Consumes ˙NO via Reaction With Enzyme-Bound Peroxyl Radical Intermediate

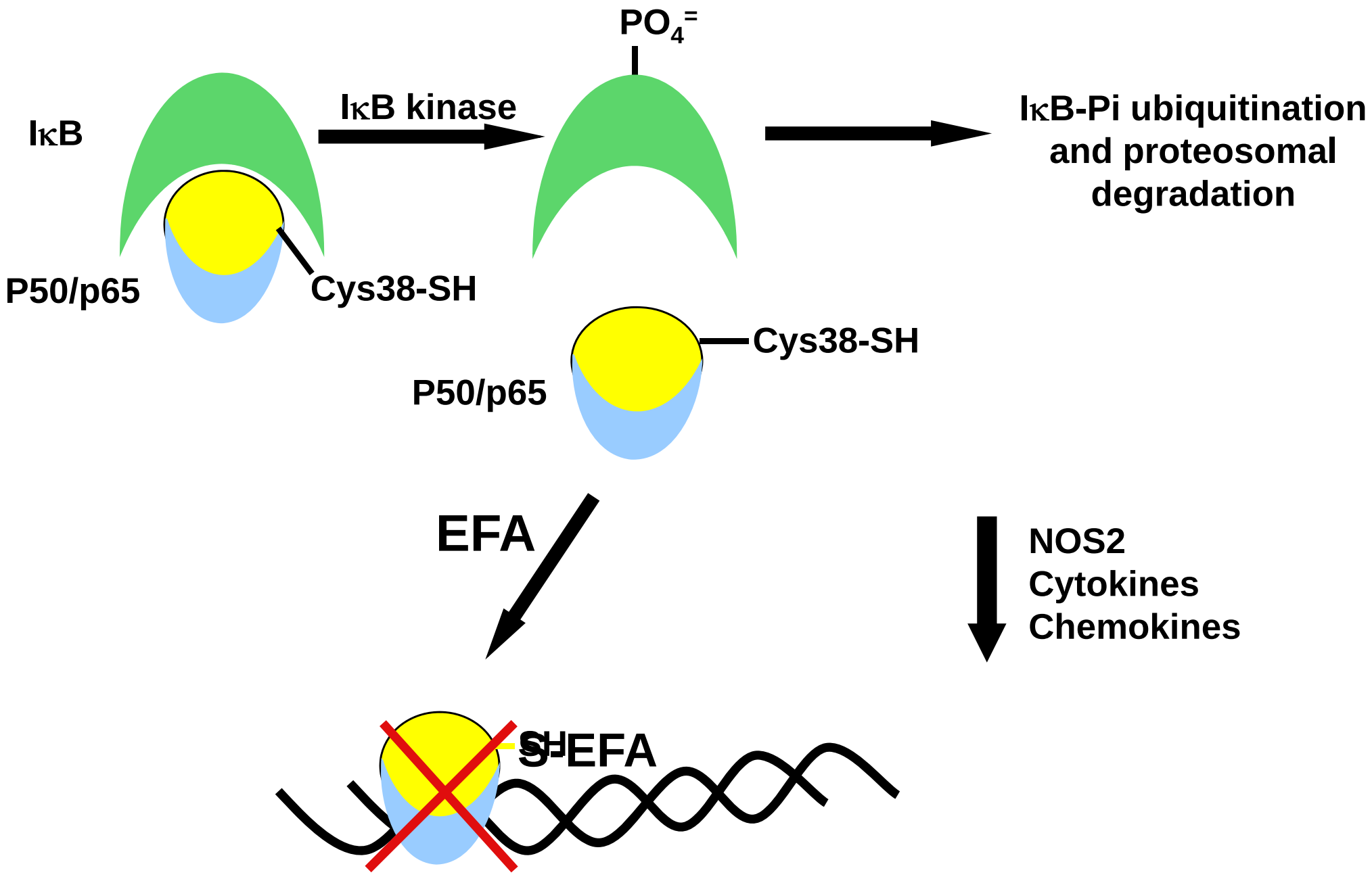


JBC 274:20083-91, 1999; PNAS 98:8006-11, 2001

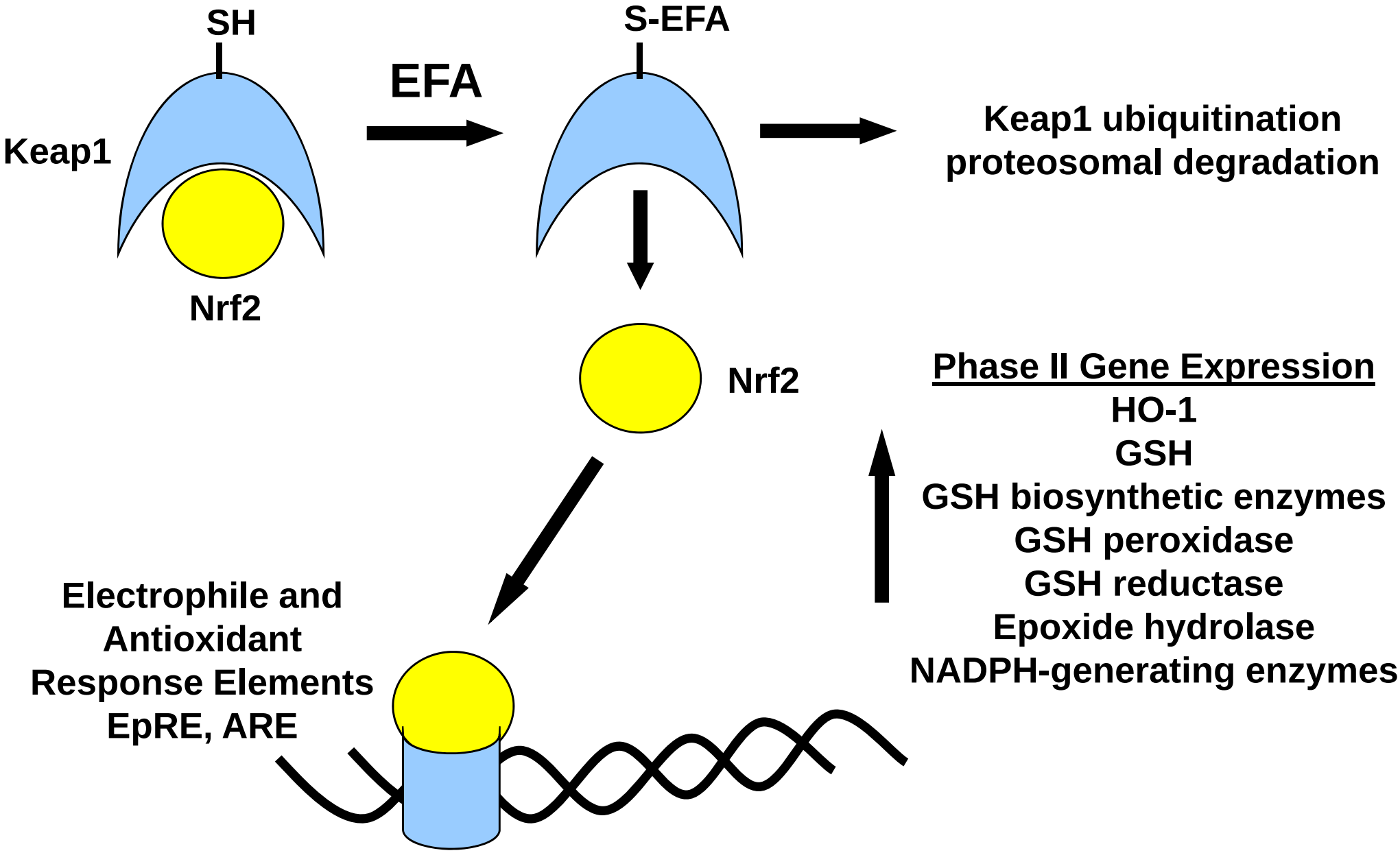
New Perspective
Lipid Oxidation Products Modulate the Expression of “Adaptive” Genes

Organisms have evolved a set of proteins that sense oxidized, electrophilic species (such as lipid oxidation products) that in turn activate adaptive signaling mechanisms mediated by receptors, transcription factors and protein kinases

Electrophilic Fatty Acids Inhibit NF-κB Signaling



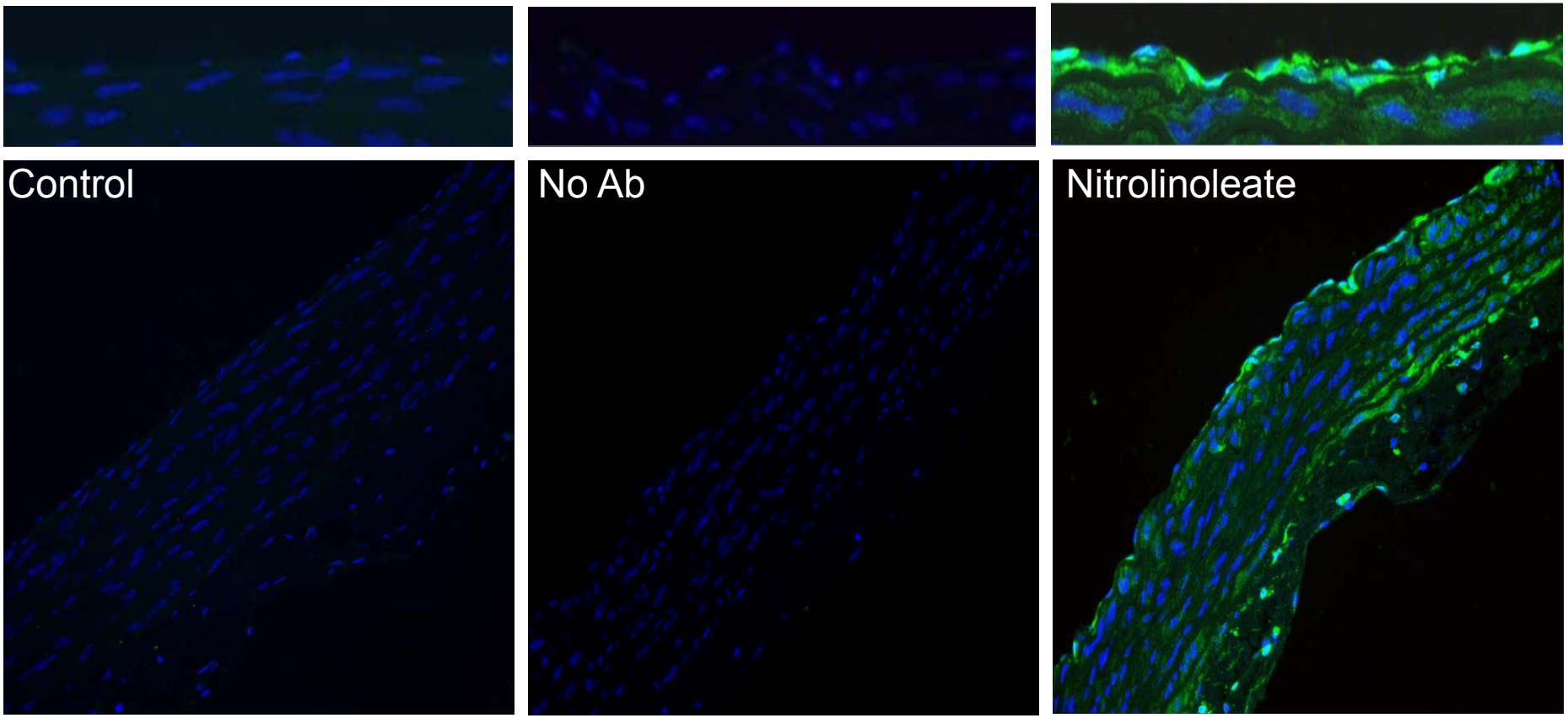
Electrophilic Fatty Acids Induce Phase II Gene Expression



Electrophilic FA Induce HO-1 Expression

Rodent Aorta

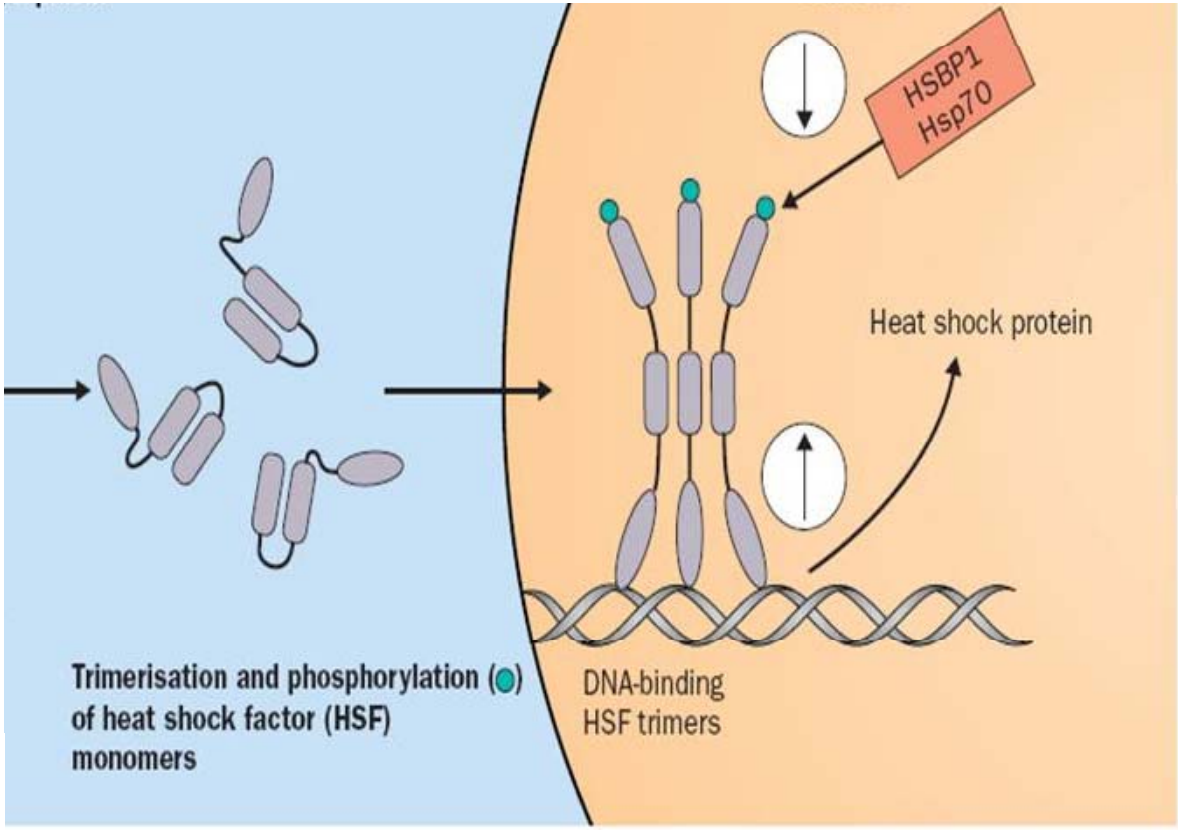
$$\text{Heme} \xrightarrow{\text{HO-1}} \text{CO, Bilirubin, Biliverdin}$$

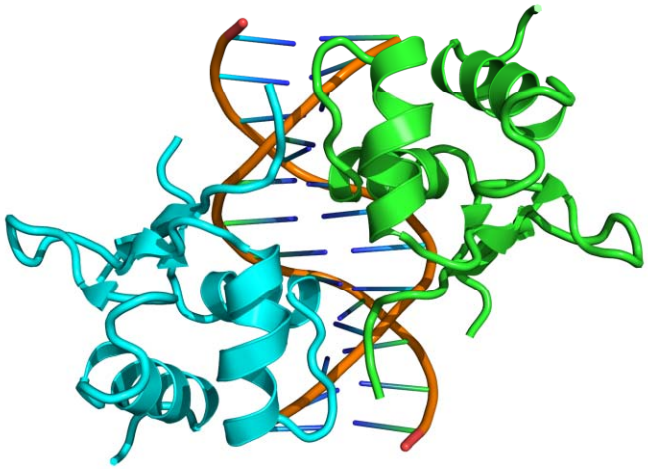


PNAS 2006, 103:4299

Electrophilic Fatty Acids Induce Heat Shock Factor-Dependent Gene Expression

Electrophiles
H_2O_2
ONOO
@ Cys35, Cys105

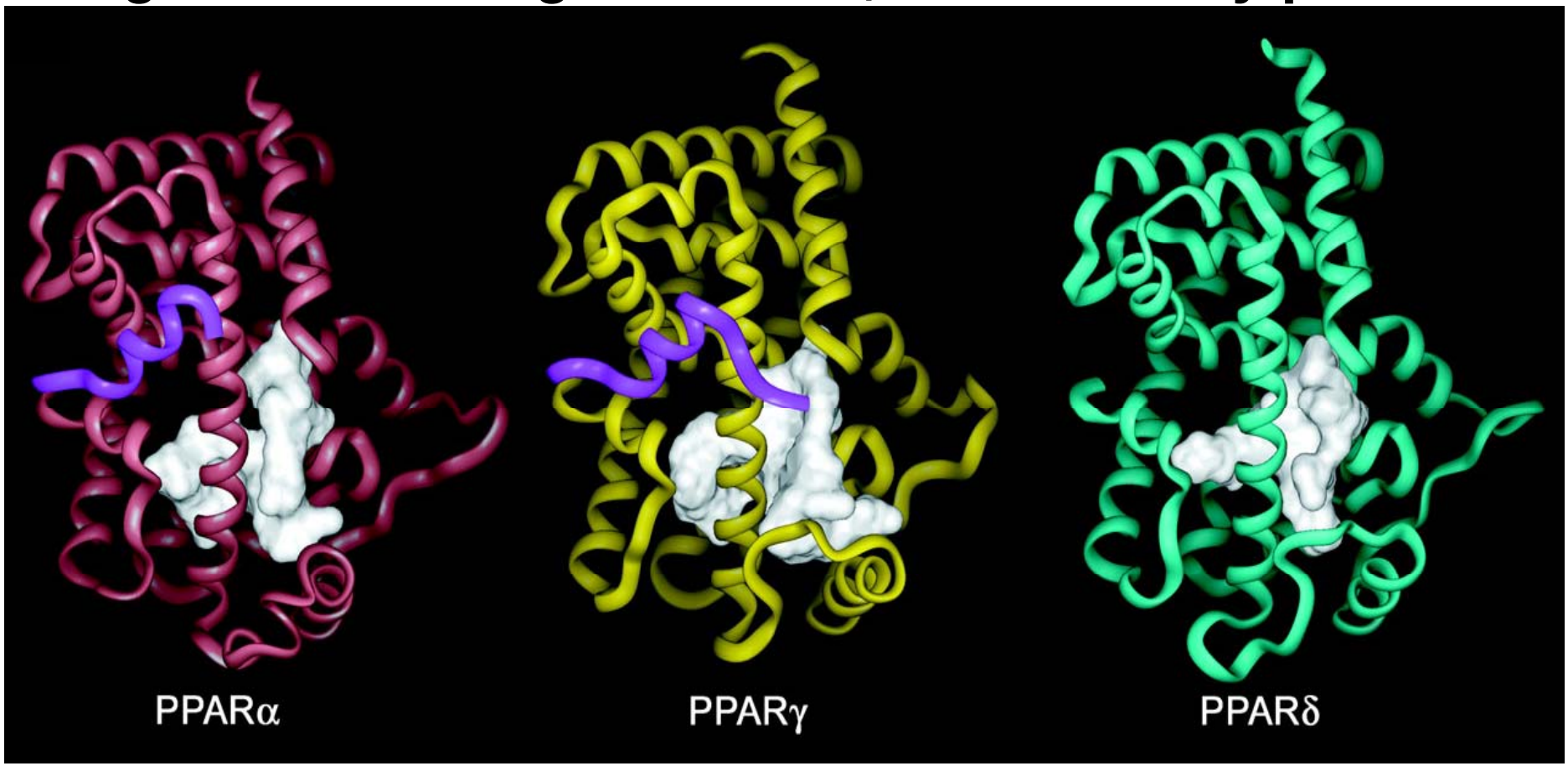




HSF-60
HSF-70
HSF-90

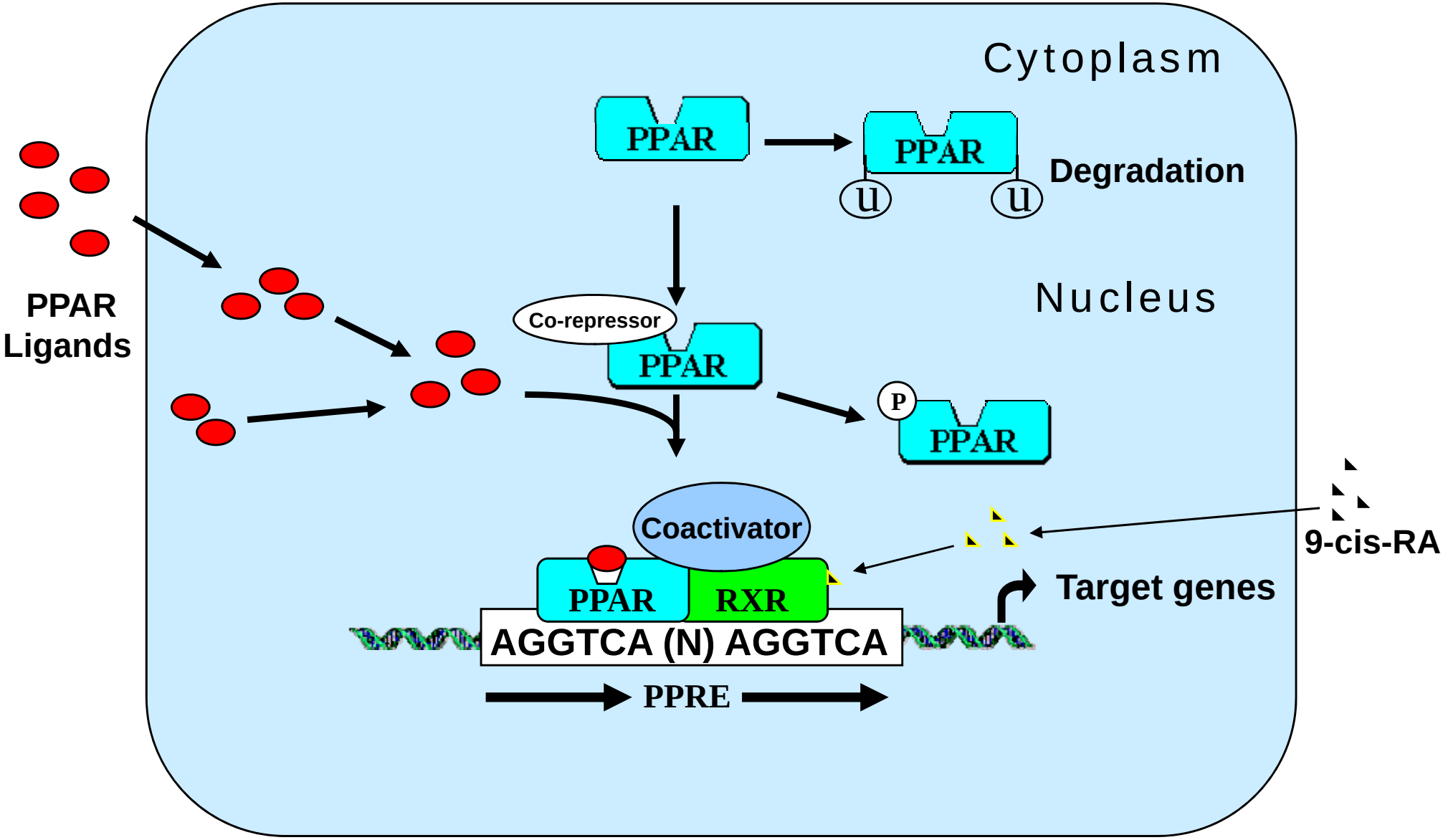
Peroxisome Proliferator-Activated Receptors

Bind lipophilic ligands, regulate nuclear transcription of genes encoding metabolic, inflammatory proteins



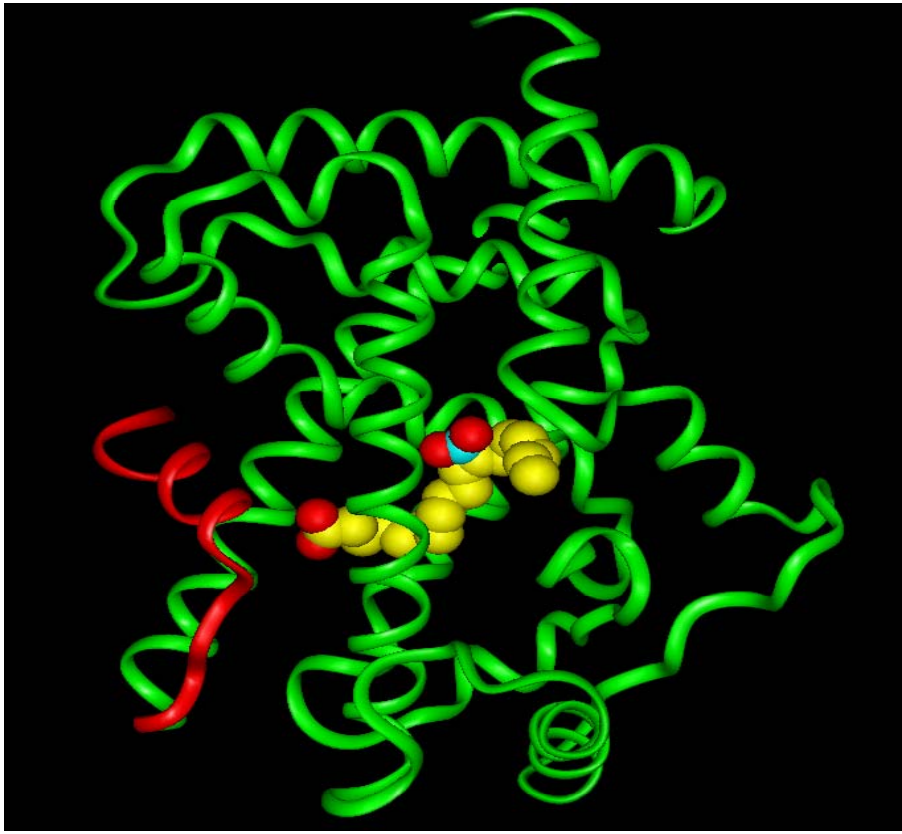
Saturated fatty acids	**Oxidized fatty acids**	**Sat/Unsat Fatty Acids**
Fibrates	**Thiazolidinediones (Rosiglitazone)**	**Tyr analogs with carboxylate, acyl, N-substituents**

PPAR-Dependent Genes



PPARγ Helix 12

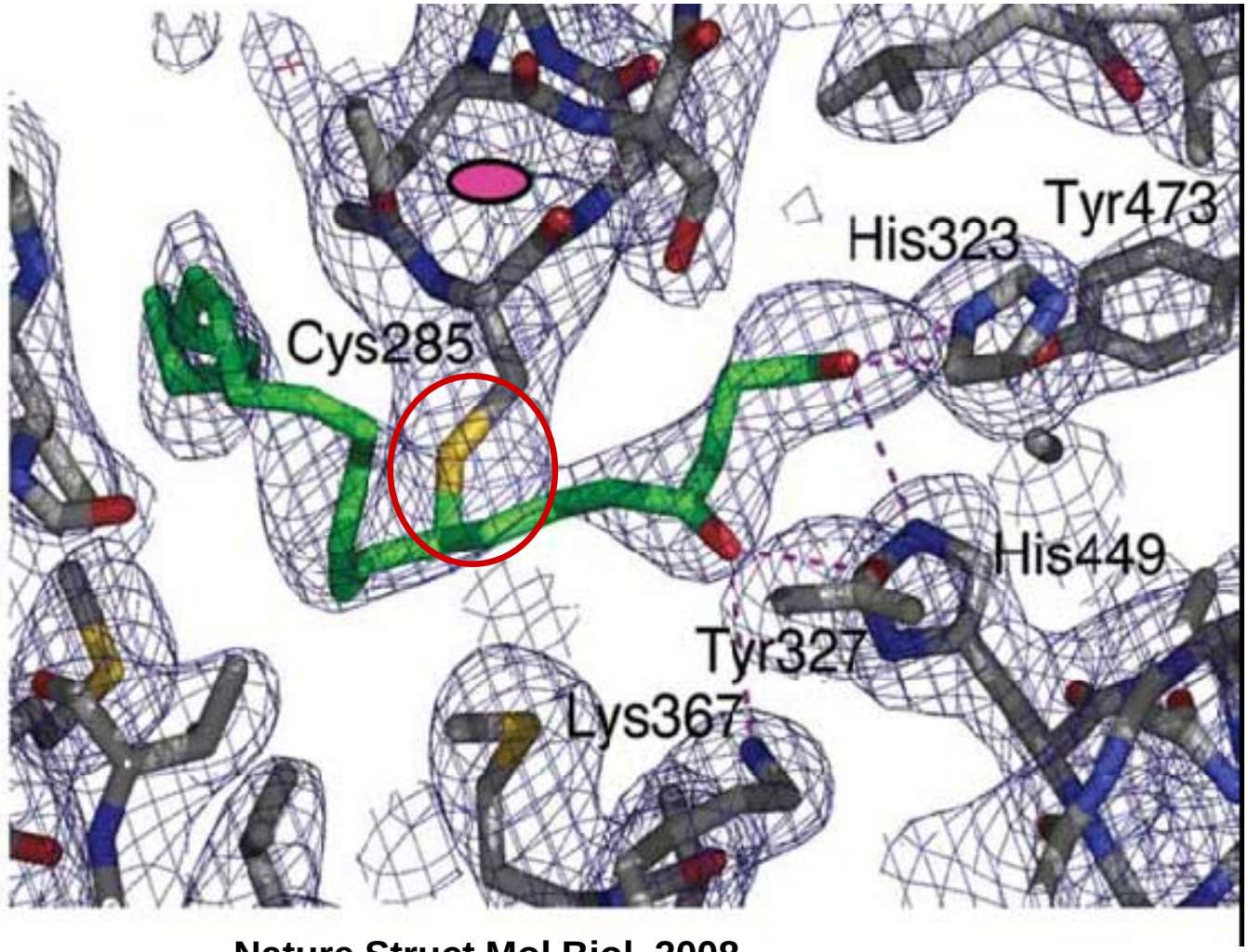
- Ligand alters Helix 12 conformation
- Helix 12 directs ligand-specific co-repressor dissociation, co-activator binding patterns
- Co-regulator protein interactions define nature of transcriptional complex and thus ligand-specific gene expression patterns



Ligand	EC50
Unsaturated Fatty acids (18-22 C, 1-6 C=C)	>25 μM
9 and 13-hydroxy-octadecadienoic acids	>10 μM
Various hydroxy-eicosatetraenoic acids	>10 μM
15-d-PGJ_2, LTB_4	> 1 μM
PAF, lyso-PC	> 1 μM
Rosiglitazone	50 nM
Nitro derivatives of 18:1, 18:2	5-30 nM
Various oxo derivatives of 20:5, 22:6	? μM

PPARγ Helix 12

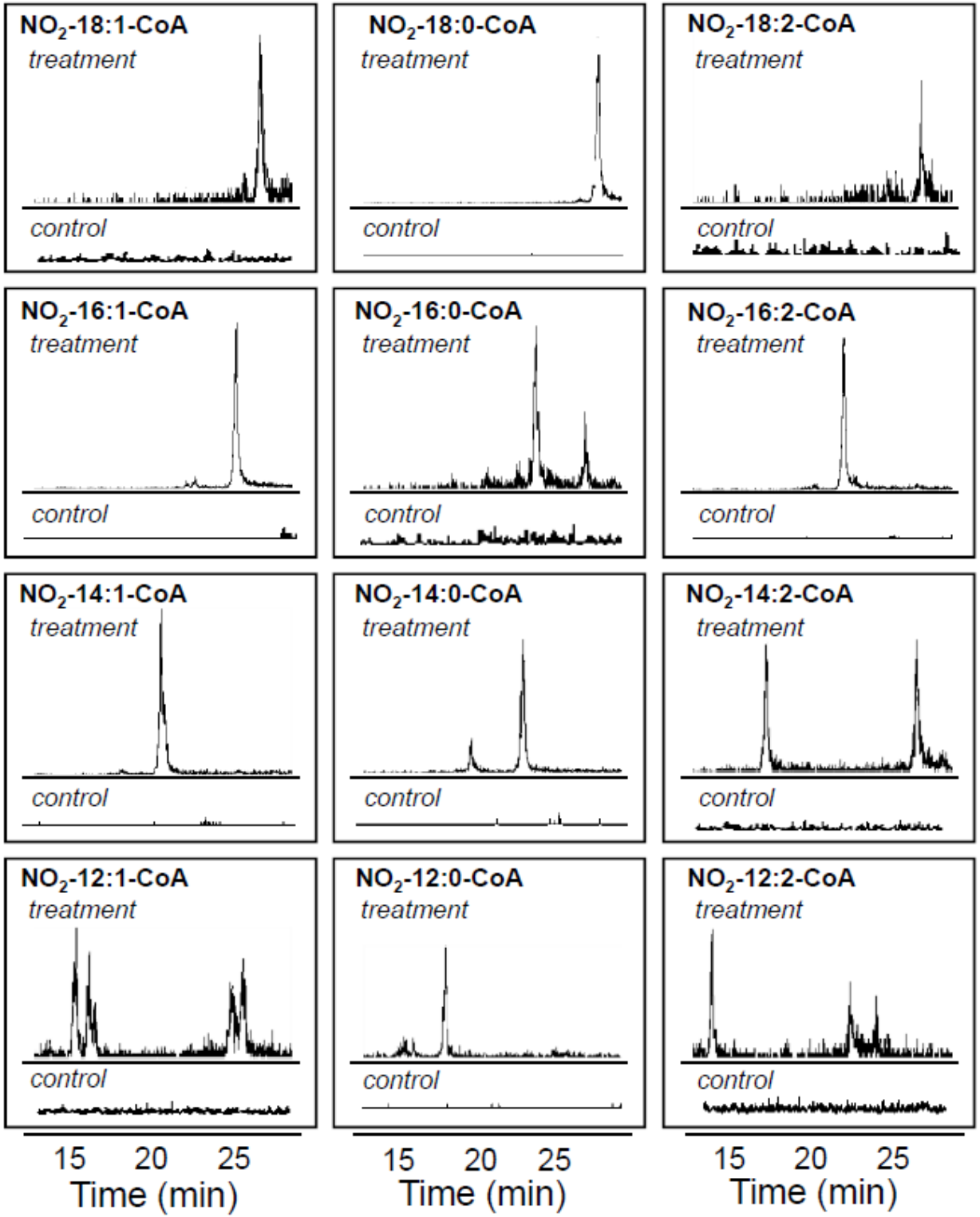
Covalent binding of electrophilic fatty acid ligands (via Michael addition to Cys285) uniquely alters Helix 12 conformation, displays non-traditional binding kinetics and ultimately induces unique gene expression patterns



Nature Struct Mol Biol, 2008

Detection of Lipid Oxidation

- Oxygen consumption
- Loss of unsaturated fatty acids by GC- or HPLC-MS
- Conjugated diene formation by UV detection
- Schiff's base fluorophores
- Malonyldialdehyde (thiobarbituric acid-reactive)
- Ethane (n-3), pentane (n-6)
- Isoprostanes, neuroprostanes, isoketals
- HPLC-MS detection of specific oxidation products


NO2-18:1-CoA
treatment
control
NO2-18:0-CoA
treatment
control
NO2-18:2-CoA
treatment
control
NO2-16:1-CoA
treatment
control
NO2-16:0-CoA
treatment
control
NO2-16:2-CoA
treatment
control
NO2-14:1-CoA
treatment
control
NO2-14:0-CoA
treatment
control
NO2-14:2-CoA
treatment
control
NO2-12:1-CoA
treatment
control
NO2-12:0-CoA
treatment
control
NO2-12:2-CoA
treatment
control
15 20 25
Time (min)
15 20 25
Time (min)
15 20 25
Time (min)

Cool and Useful Lipid Links

http://www.cem.msu.edu/~reusch/VirtualText/lipids.htm

http://dietary-supplements.info.nih.gov/Health_Information/omega_3_fatty_acids.aspx

http://www.nursa.org/

http://www.chem.qmul.ac.uk/iupac/lipid/

http://www.lipidsonline.org/

http://www.lipidmaps.org/

http://www.avantilipids.com/

http://www.lipidlibrary.co.uk/lipids.html

http://www.cyberlipid.org

http://www.en.wikipedia.org/wiki/Lipid

http://*www.jlr.org/*

http://cellbio.utmb.edu/cellbio/membrane_intro.htm