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ENZYMATIC LIPID PEROXIDATION HOMEOSTASIS and pollution chaos multiple parallel chain reaction products **RLS**-reactive lipid species and non enzymatic damages

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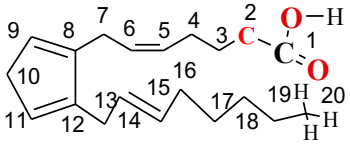
Eicosanoids. Almost all mammalian cells except erythrocytes **enzymatic** produce one or more of **eicosanoids**, 20-carbon compounds (Greek *eikosil* , "twenty"), that include: **prostaglandins** (**PGs**), **prostacyclins** (**PGIs**), **thromboxanes** (**TXs**) and **leukotrienes** (**LTs**).

Prostaglandins PGA_2, PGE_1, PGE_2, PGE_3, $PGF_{2\alpha}$, PGG_2, PGH_2 and Prostacyclin PGI_2.

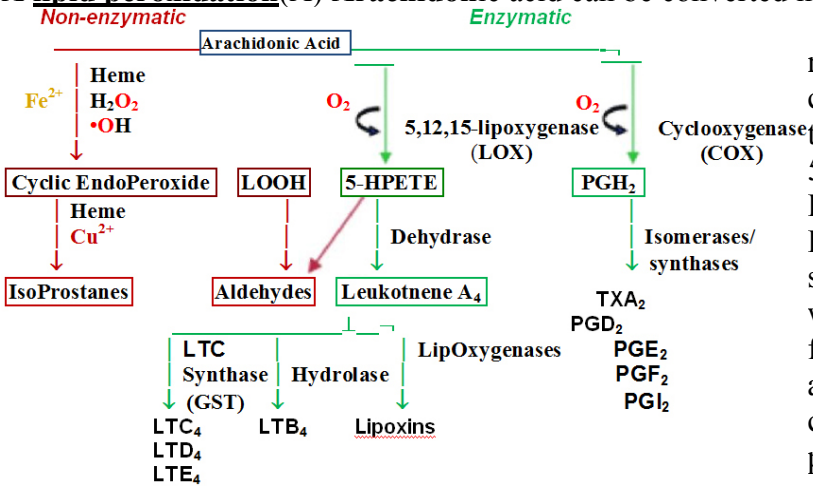
Thromboxanes TXA_2 and TXB_2. Leukotriene LTE_4.



Initial compound EICOSANOID-arachidonic acid **C20:4** with cross-link between C_8 —C_{12} . Unsaturated compound 4 four cis double bonds C=C C=C C=C C=C..

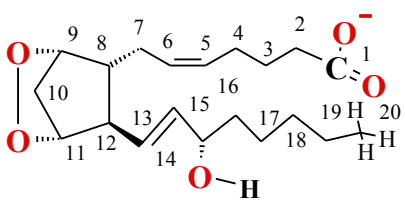
Figure 1 **Formation of lipid electrophiles via non-enzymatic and enzymatic**

A lipid peroxidation(A) **Arachidon**ic acid can be converted into several products through **enzymatic** and **non-enzym**atic **lipid** peroxidation. Both free-radical-catalyzed as well as **enzymatically** controlled oxidation yields a subset of products that are **electrophilic**.

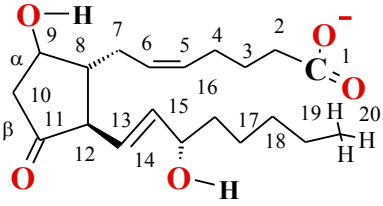


5-HPETE, 5-hydro peroxy eicosatetraenoic acid; L**OOH**, linoleum acid hydro **peroxide**.

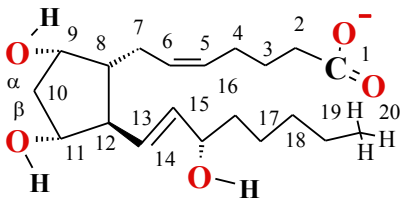
ENZYMATIC LIPID PEROXIDATION Best-studied **enzyme**s is cyclo-oxygenase **COX**, which is responsible for the formation of **PG**s from **arachidon**ic acid (Figure 1). Since **COX** acts predominantly on free fatty acids, in many cases the production of **PG**s is dependent upon phospholipase A_2 [69]. **COX** contains two active sites including a **COX** domain and a peroxidase domain [70]. The **COX** site is responsible for



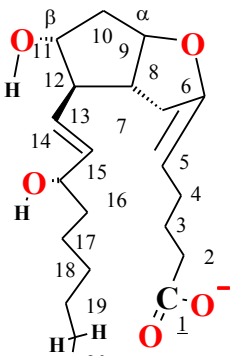
PGH_2



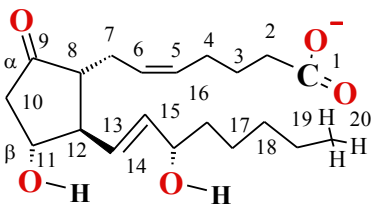
α,β unsaturated ketone PGD_2



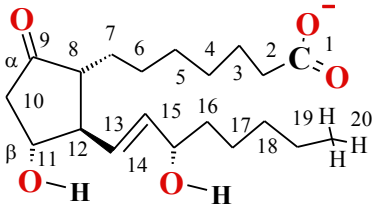
$PGF_{2\alpha}$



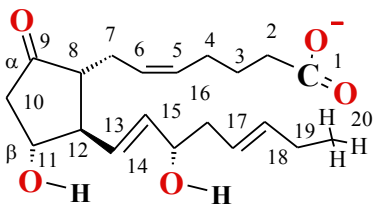
PGI_2



PGE_2

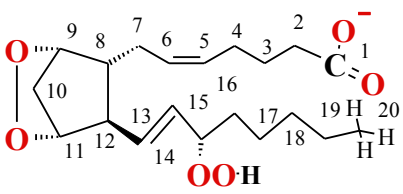


PGE_1

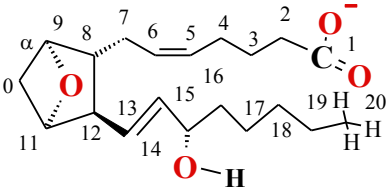


PGE_3

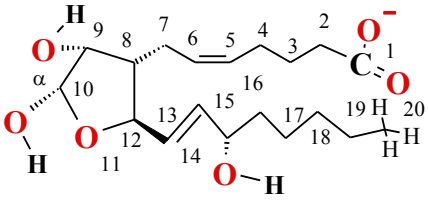
oxygenating **arachidon**ic acid to form hydro **peroxide** PGG_2. The peroxidase site then reduces PGG_2 to the



cyclic end peroxide PGG_2



TXA_2



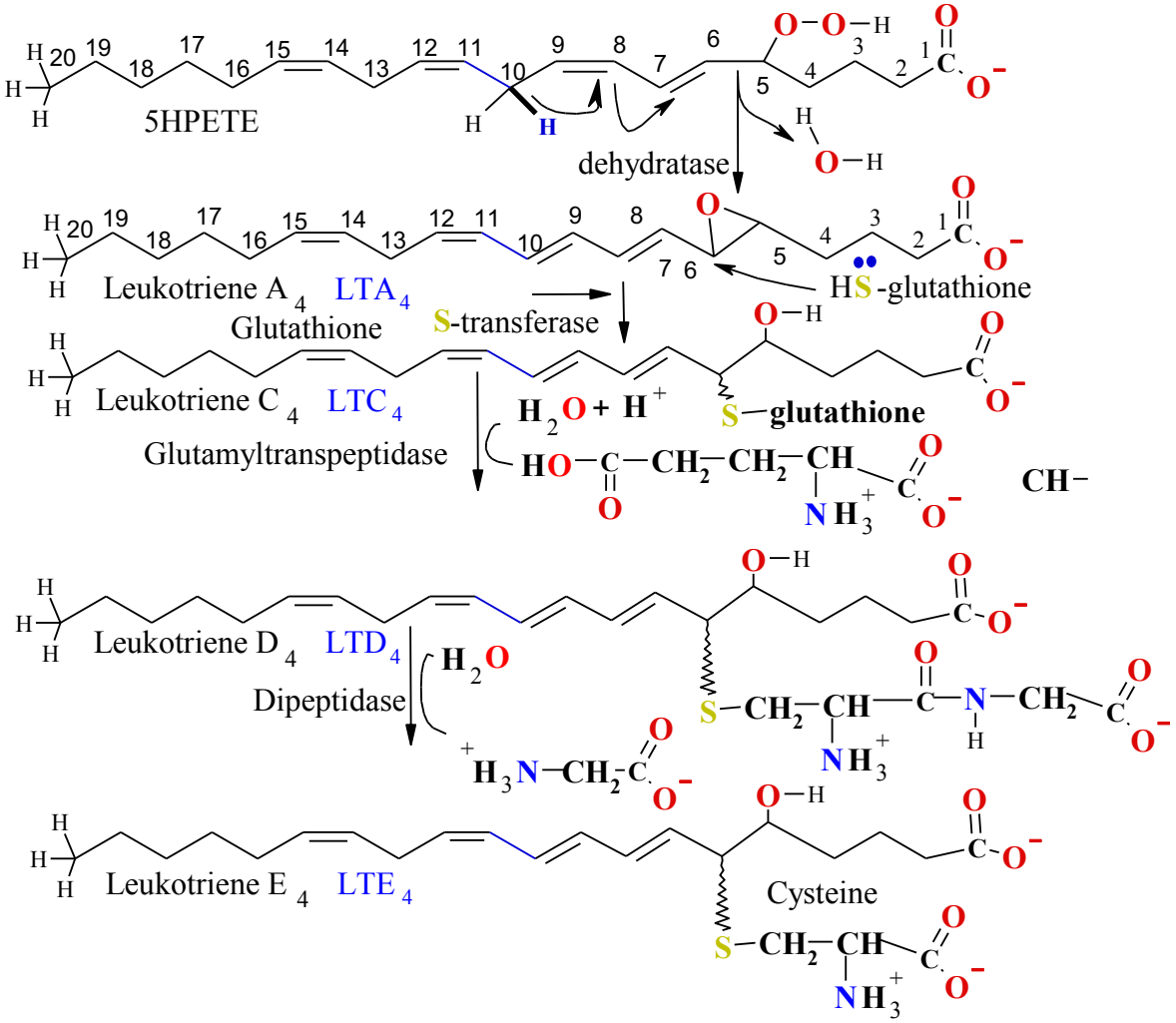
TXB_2

alcohol PGH_2, the final product of **COX**. There are two isoforms of **COX** in the cell [70]. **COX**-1 is constitutively expressed in all **tissue**s; however, **COX**-2 is normally only detected in **tissue**s with active inflammation except kidney and brain where **COX**-2 is constitutively expressed [71]. The protein expression of **COX**-2 is regulated by several transcription factors relevant to inflammation including NF-κB (nuclear factor κB), NF-IL-6 (nuclear factor for interleukin-6 expression) and CREB (**cAMP**-response-element-binding protein) [72,73]. Once expressed, **COX**'s activity can also be regulated in a transcription-independent manner [74,75]. Several **ROS** are known to regulate **COX**-2 activity by regulating the levels of the **lipid peroxide** tone

which is required for activation [75–77]. The major product of both **COX**-1 and **COX**-2 is PGH_2, which can then be **metabolized** to other **PGs** through the action of Prostaglandins **PGD**, **PGE**, **PGF**, and **PGI** Prostacyclin synthases [78–83]. PGA_2, PGJ_2 and 15d-PGJ_2 are examples of **electrophilic PGs**.

The **COX enzymes** generate several anti-inflammatory **electrophilic RLS** from **arachidonic** acid (e.g. cyclopentenones) as well as products of ω−3 fatty acids [e.g. DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid)] [40,84]. The latter products derived from **COX**-2 have been shown to be important anti-inflammatory mediators [84,85]. Interestingly, a subset of these are **electrophilic**, termed the EFOXs (**electrophilic** oxo-derivatives of ω−3 fatty acids) [84]. These **enzymatically** produced **RLS** may be important for the protection afforded by ω−3 supplementation.

Another important source of **enzymatic** **lipid peroxidation products** is through the action of **LOXs**. **LTs** and lipoxins are the products of this pathway and have been extensively studied in the field of immunology [29,86,87]. There are three **LOX** isoforms, with 5-, 12- and 15-**LOX** expressed in leucocytes, platelets and endothelial cells respectively [29,88]. The active site of **LOX** contains a non-heme iron which is critical to the **enzyme**'s activity [89,90]. As with **COX**, **LOX** activity is also modulated by **ROS** through regulation of the **enzyme**'s **peroxide** tone [91,92]. Among the **LOXs**, 5-**LOX** is the most well-studied in the context of cardiovascular disease [68]. It was originally found to contribute to asthma and was targeted with inhibitors developed to minimize airway inflammation [86]. It is now well-established that 5-**LOX** products also contribute to other inflammatory processes including the development of coronary artery disease [51,93]. As shown in Figure 1, following generation of LTA_4 from **LOX**, the product LTB_4 is formed by hydration, whereas the **cysteinyl LTs**, LTC_4, LTD_4 and LTE_4, are produced by a specialized **GST** (**glutathione** transferase) **enzyme**, LTC_4 synthase [94,95]. Aside from the known **receptor**-mediated effects of the **LTs**, one **LT** is known to be capable of **receptor**-independent effects through covalent **modification**. Because LTA_4 is uniquely **electrophilic** owing to its epoxide group, it is capable of adducting to **nucleophilic** amino acids as well as DNA bases [96,97]. The **nucleophilic** attack of 5-**LOX** by LTA_4 leads to the covalent **modification** and inactivation of the **enzyme** [98].



INTRODUCTION The oxidation of **PUFAs** (polyunsaturated fatty acids), such as **arachidonic** acid, generates a broad range of **oxidation products** which historically have been used as markers of **oxidative stress** [1,2].

For example, the unique structural attributes of the non-specific **oxidation product**s known as the iso**prostane**s have allowed for the development of accurate high-throughput **assays** for their measurement in complex biological systems [3]. **Lipid peroxidation product**s have been detected in the **blood**, **plasma**, **urine**, and **tissue** samples of humans and animal models using an array of techniques, and, in many cases, their levels are elevated in pathological conditions [4–7]. The application of these analytical techniques has led to the concept that **RLS** (**reactive lipid species**) are mediators, not simply by-products, of multiple pathophysiological conditions [8–11]. The cell **signalling** mediated by **RLS** has some unique biochemical attributes. Importantly, many **lipid peroxidation product**s are also **electrophilic**, which allows them to form stable covalent adducts with **nucleophilic** residues on proteins [12–14]. This is important since it is now well recognized that the **thio**l **–S-H** groups on **cystein**e residues act as **redox** switches controlling cell **signalling** and **metaboli**sm [15–17]. The **cystein**e **thio**l **–S-H** group is particularly versatile, and the concept has emerged that different **–S-H thio**l-reactive **signalling** molecules can selectively modulate protein function [16]. Specific mechanisms that have been shown to modify **redox** cell **signalling** include **S-nitrosation**, S-**glutathion**ylation and Michael addition with biologically active **electrophil**es [15,18,19]. Other oxidative mechanisms mediated by either **hydrogen peroxide** or **lipid peroxide**s to form sulfenic or sulfinic acids were initially thought to be markers of oxidative damage. However, a previous study suggested that they may also play a role in cell **signalling** [20]. Interestingly, although early studies implied that **lipid** peroxidation always results in damage, a more refined view of this process has evolved and suggests that **oxidized lipid**s can elicit different cellular effects depending on the species present, their concentrations and their reactivity with protein targets [14,21–23].

Oxidized lipids can mediate biological responses through two diverse mechanisms: classic reversible binding and irreversible covalent **modification** of **receptor**s [15,22,24–26]. Some **oxidized lipid**s are ligands for specific **receptor**s [e.g. **PG** (**prostaglandin**) **receptor**s] and mediate biological effects through reversible **receptor**–ligand interactions [27,28]. This is best understood for the **enzym**atically produced **PG**s and **LT**s (**leukotriene**s) [29]. In contrast, some **lipid peroxidation product**s modulate cellular activity through irreversible covalent **modification** of **nucleophilic** amino acid residues on proteins [15,30]. This concept was initially in conflict with the classical paradigms for cell **signalling** since to 'turn-off' the signal, the protein must be selectively degraded. However, **signalling** through the covalent **modification** of proteins is now accepted for a number of well-defined protein–**lipid** interactions, and selective degradation is mediated through the proteasome [31,32]. Interestingly, **signalling** though the covalent **modification** of proteins changes the relationship between the concentration of the ligand, in this case an **oxidized lipid**, and the resultant signal [33]. Since irreversible covalent **modification**s of proteins can accumulate over time and amplify a signal [33], even low levels of **oxidized lipid**s initiate **signalling**. We have termed this concept 'the covalent advantage' [22].

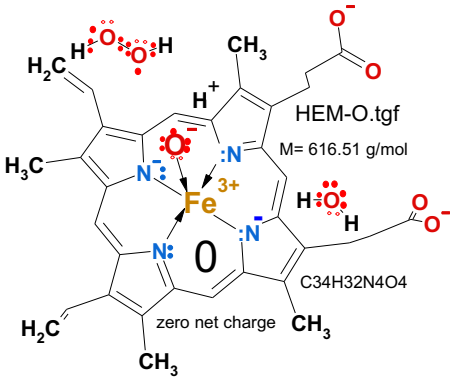
In the present review, we will discuss: (i) the formation of **RLS** through both **non-enzym**atic and **enzym**atic processes; (ii) **oxidized lipid signalling** through classic **receptor**-mediated **pathways** and by covalent **modification** of protein targets; and (iii) susceptibility of **thio**ls **–S-** to **modification** by **RLS**. We will then relate these concepts to the ability of **oxidized lipid**s to trigger adaptive and damaging biological effects with a focus on the role of sub cellular localization. **PUFA**s (polyunsaturated fatty acids)

FORMATION OF RLS Much of the early research into mechanisms of **lipid** peroxidation was performed by scientists in the food industry. It was well appreciated that off odours and flavours could be attributed to **lipid** oxidation, and inhibiting this process results in products with a longer shelf life [34]. As the field evolved, it was recognized that **lipid** peroxidation is endogenous to living organisms and has multiple functions dependent on the site and mechanism of oxidation. For example, as shown in **Figure 1**(A), **enzym**atic sources of **lipid** peroxidation yield important biological mediators of inflammation such as the **PG**s from **COX**s (cyclo-oxygenases), and the **LT**s from **LOX**s (lipoxygenases) [35,36]. Importantly, both **non-enzym**atic and **enzym**atic oxidation of **PUFA**s results in the formation of **RLS** that are **electrophilic** (**Figure 1**B). A major substrate for **lipid** peroxidation is **arachidon**ic acid, and its oxidation results in the formation of several products (**Figure 1**A). Of these **lipid peroxidation product**s (**Figure 1**A), a subset are **electrophilic** in nature, and there are both structurally distinct species derived from either **non-enzym**atic or **enzym**atic **lipid** peroxidation (**Figure 1**B). Examples of **electrophilic** products of **non-enzym**atic **lipid** oxidation include aldehydes such as HNE (4-hydroxynonenal), malondialdehyde and acrolein as well as the J- and A-series iso**prostane**s. Other **RLS** include the isoketals, which result from the rearrangement of end **peroxide** intermediates of the iso**prostane** pathway and have the potential to react with both proteins and **lipid**s [37]. The approach to research with **RLS** has largely focused on defining the reactivity and biological effects of a candidate molecule. For this reason, we know a great deal about the behaviour of HNE, 15d-**PGJ**$_2$ (15-deoxy

prostaglandin J_2) and nitroalkanes [21,38–44]. From these studies, two key facts have emerged: (i) the effects of all the **RLS** are dependent on the amount exposed to the cell with many exhibiting anti-inflammatory or cytoprotective effects over the lower concentration range; and (ii) the biological effects of the **RLS** vary according to the specific **RLS** and target cells [33,45]. The implications of these findings are that each **RLS** reacts with a specific family of proteins which we have called the **electrophil**e-responsive proteome [12,22]. This concept will be explored in more depth throughout the present review.

NON-ENZYMATIC LIPID PEROXIDATION PUFAs, such as **arachidon**ic and linoleic acid, are targets for **lipid** peroxidation. Non-specific **lipid** peroxidation proceeds through a chain reaction composed of three main steps: initiation, propagation and termination. In **enzym**atic **lipid** peroxidation, initiation is controlled and stereo specific and propagation does not occur. The production of specific **lipid** oxidation **signalling** molecules is controlled by **enzyme pathways** and the release of **non-enzym**e-bound radical intermediates is minimized. Due to their unsaturated double bonds, the allylic hydrogen atoms in **PUFA**s are readily abstracted by initiating species such as ferryl radical ($[Fe^{3+}O^{-}\bullet]^{2+}+H^{+}\rightarrow Fe^{3+}+HO\bullet$), peroxy nitrite ($ONOO^{-}$), hydro peroxyl radicals ($HO_2\bullet$) and hydroxyl radical ($\bullet OH$). This results in the formation of **lipid** radicals which react with oxygen if it is available. The products that are formed are diverse and depend on the substrate oxidized (e.g. **arachidon**ic compared with linoleic acid) and the mechanism of oxidation (**non-enzym**atic or **enzym**atic). Once **lipid** peroxidation is initiated, **lipid** alkoxyl ($LO\bullet$) and **lipid** peroxyl ($LOO\bullet$) radicals are capable of abstracting a hydrogen atom from another fatty acid molecule, thus contributing to the propagation of **lipid** peroxidation [46]. In biological membranes, the presence of proteins can result in transfer of the **lipid** radicals to protein side chains and adduct formation [47,48]. In this setting, the proteins become active participants in the propagation of the **lipid** peroxidation reactions. Molecular oxygen (O_2) is required for the propagation phase, and, for this reason, **lipid** peroxidation proceeds at a higher rate when oxygen concentrations are high [46]. **Lipid** peroxidation can be terminated by radical–radical reactions with other **lipid** radical species or with protein radicals. Termination can also occur by radical–radical reaction of a **lipid** radical species with the nitric oxide radical ($NO\bullet$) [49].

Cardiovascular disease is a pathological condition in which predominantly non-specific **lipid** peroxidation occurs *in vivo*. For example, in atherosclerotic lesions, the **lipid peroxidation product**s found are mostly those lacking stereo specificity which is a characteristic of the **non-enzym**atic **pathways** [50]. However, increases in inflammation do lead to production of low levels of stereo specific **enzym**atic **lipid oxidation product**s in atherosclerosis [51,52]. Several factors may promote **lipid** peroxidation through **non-enzym**atic reactions *in vivo* [53]. For example, the production of **ROS** (reactive oxygen species H_2O_2, O_2^{-}) and **RNS** (reactive nitrogen species $NO\bullet$, $\bullet ONOO^{-}$) in inflammation may result in damage to iron- or copper-containing proteins and the release of the metal from a protein environment in which radical reactions can be controlled. This can occur with the proteins myoglobin and hemoglobin [54,55]. Heme proteins then play an important role in **lipid** peroxidation by decomposing **lipid** hydro **peroxid**es and facilitating the propagation phase [56]. However, unlike free heme, heme proteins can also initiate **lipid** peroxidation [57].



Hydrogen peroxide H_2O_2 and **proton** H^{+} interaction with myoglobin or hemoglobin leads to the formation of an activated heme protein with a $[P^{+}-Fe^{3+}\leftarrow:O\bullet^{-}]^{0}+H^{+}\rightarrow[P^{+}-Fe^{3+}]^{+1}+HO\bullet$ [58] **porphyry** cation charged **+1** and **HO•** radical. Both ferryl^{+1} radical species are the initiator of **lipid** peroxidation joining proton in hydroxyl radical **HO•** [57]. Because of the ability of **hydrogen peroxide** to 'activate' these heme proteins to initiating species, myoglobin- and hemoglobin-mediated **lipid** peroxidation may be important for catalyzing **lipid** peroxidation in biological systems where **hydrogen peroxide** is elevated [56,59] look below at **three processes on planet Earth**. 58 Kanner, J. and Harel, S. (1985) Initiation of membranal **lipid** peroxidation by activated myoglobin and hemoglobin. Arch.Biochem.Biophys. 237, 314-321

Peroxy nitrite ($\bullet ONOO^{-}$), formed from the rapid reaction of $\bullet NO$ with su**peroxide** $\bullet O_2^{-}$ has also been shown to promote **lipid** peroxidation [38,60,61], probably due to the reactivity of decomposition products hydroxyl radical ($\bullet OH$) and nitrogen dioxide ($\bullet NO_2$). These radical species are capable of abstracting a hydrogen atom from unsaturated fatty acids and this process is iron-independent [60]. i**NOS** (inducible nitric oxide synthase) and NADPH oxidases are cellular sources of $NO^{\bullet}$ and su**peroxide** $\bullet O_2^{-}$ respectively, and their expression is concomitantly increased in several pathologies and can form $\bullet ONOO^{-}$ [62,63].

The **lipid** peroxidation reactions initiated by $\bullet ONOO^{-}$ produce iso**prostane**s, aldehydes and oxysterols, but unique **RLS** such as nitrated **lipid**s only occur with this mechanism of oxidation [64–66]. The interaction of

lipid radicals with **RNS** such as nitrogen dioxide $\bullet NO_2$, or possibly nitrite $\bullet NO_2^-$, results in a family of **electrophilic RLS** known as the nitroalkanes [38,39,67].

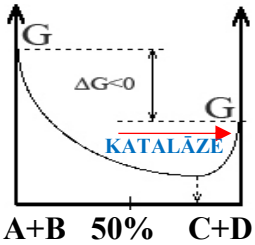
CATALASE indispensable **Life** molecular engine drive unfavored reaction to favored with 100% efficiency

$$\mathbf{Succinat^{2-}}+O_{2aqua} \Rightarrow \mathbf{fumarate^{2-}}+H_2O_{2aqua}\ ;\ pH=7,36$$

$$\Delta G_{Hess}=\Sigma\Delta G°_{products}-\Sigma\Delta G°_{reactants}\ ;\ \Delta G_{Hess}=\Delta G°_{H2O2}+\Delta G°_{fumarat}-\Delta G°_{O2}-\Delta G°_{Succinat}=-165\ {}^{kJ}/_{mol};$$

$$\Delta G_{Hess}=\Delta H_{Hess}-T*\Delta S_{Hess}=-48,16-298,15*0,0458=-61,815\ {}^{kJ}/_{mol}\ \mathbf{exoergic}$$

Essential unsaturated fatty acid elongation C20:4 and ethyl group $-CH_2-CH_2-$ conversion to cis double bond $^{H}>C=C<^{H}$ in peroxisomes occurs exoergic, favored enzymatic conversion with negative free energy change like: $\Delta G_{Hess}=-61,815\ {}^{kJ}/_{mol}$. **CATALASE** in complex reaction sequence favors stabile unsaturated fatty acid product efficiency ● 100% because erasing peroxide H_2O_2: $K_{eq}=10^{8,43}=\dfrac{[Fumarate^{2-}]\cdot[H_2O_2]\swarrow CATALASE}{[Succinate^{2-}]\cdot[O_2]}$,



as consumed to zero $[H_2O_2]^2=0\ {}^{mol}/_{liter}$ and process velocity limits only dehydrogenase. It favors velocity of peroxide **2H-O-O-H** increasing production of life resources O_{2aqua} **+2H₂O +Q** thirty million times $30\bullet10^6$.

Irreversible Catalase reactivity is Prigogine attractor indispensable Brownian molecular engine which drive Life for homeostasis, survival and evolution.

Absence of enzyme CATALASE Absence of CATALYSTS

Hydrogen peroxide H_2O_2 decomposition reaction is negligible slow however spontaneous as is exothermic and entropy growth $\Delta S_{Hess}>0$ so free energy decreases as its change is negative $\Delta H_H-T\bullet\Delta S_H=\Delta G_H<0$.

High activation energy **Ea**=79 ${}^{kJ}/_{mol}$ due to absence of catalyst and low geometric factor **A**=0.01 $M^{-1}s^{-1}$ make the Arrhenius velocity constant expression negligible small:

$$\vec{k}=A\bullet e^{-\frac{Ea}{RT}}=0.01\bullet e^{-\frac{79000}{8,314\bullet298}}=0.01\bullet1.419\bullet10^{-14}=1.419\bullet10^{-16}\ M^{-1}s^{-1}.$$

Velocity of reaction makes very small

$$\vec{v}=\vec{k}\bullet[H_2O_2]^2=1.419\bullet10^{-16}\bullet[H_2O_2]^2\ \ Ms^{-1}\ ;\text{if } [H_2O_2]=1\ M\ ;\ \sqrt{\vec{v}}=\sqrt{\vec{k}}\bullet[H_2O_2]=1.191\bullet10^{-8}$$

each second of $1.191\bullet10^8$ molecules converts to products only one 1 peroxide molecule, that all would be converted we should wait $1.191\bullet10^8$ seconds, 3.78 years. One mol H_2O_2 contains Avogadro number of molecules $N_A=6.021\bullet10^{23}$ particles/mol and expected time complete converting is larger as 3.78 years $6.021\bullet10^{23}/1.191\bullet10^8=5\bullet10^{15}$ times and are $3.78\bullet5\bullet10^{15}=1.9\bullet10^{16}$ years. Universe age is 13.7 billion years, which is million times shorter period.

CATALASE increases reaction velocity thirty million times, so producing life resources O_{2aqua} **+2H₂O +Q**

$$\mathbf{H_2O_2+H_2O_2+CAT}\rightarrow\ \mathbf{H_2O_2\ldots CAT\ldots H_2O_2}\ \rightarrow O_{2aqua}+\mathbf{2H_2O}+\mathbf{Q_{exothermic}}+\mathbf{CAT}$$

toxic transition state active complex **oxygen+water+heat** + CATALASE life resources

$\vec{k}_{CATALASE}=A\bullet e^{-\frac{Ea}{RT}}=0,13\bullet0.988=0,36\ M^{-1}s^{-1}$; $^{CAT}\sqrt{\vec{k}}\ /\sqrt{\vec{k}}=0,36/1,19\bullet10^{-8}=30\bullet10^6$ times greater,

as with CATALASE geometric factor is $A_{CATALASE}=0,13$; activation energy is $E_{aCATALASE}=29$ J/mol.

In comparison with absence of CATALYSTS geometric factor is **A=0,01**; activation energy is **E**=79000 ${}^{J}/_{mol}$.

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Lipid non enzymatic **peroxidation** is a H_2O_2 chain reaction providing a continuous supply of free radicals that **initiate** further **peroxidation**. The whole process (**chain** type reaction) can be depicted as follows:

Let us start from arachidonic acid salt Eicosanoid **C20:4 ω6** essential fatty acid:

(1) **Initiation**: Production of radicals **R•** from precursor by **metal$^{(n)+}$** ion as **Fe^{3+},Mn^{4+},Cu^{2+}**,etc at oxygen **O=O Oxidant** presence of **high energy radiation** (**~hν**) or **Aldehyde OxydoReductase**

ROOH (transfer **H^+** and **e^-** to **Oxidant O=O**) + **metal$^{(n)+}$** → **ROO•** + **metal$^{(n--1)+}$** + **H^+**

Fe^{3+}+ **H_2O_2** (**H:-•O-:-O•-:H**) →[**$Fe^{3+}O^-$•**]$^{2+}$+**H^+**+**HO^-**→**Fe^{3+}**+ **HO•**+ **HO^-** Fenton reaction for free iron ion

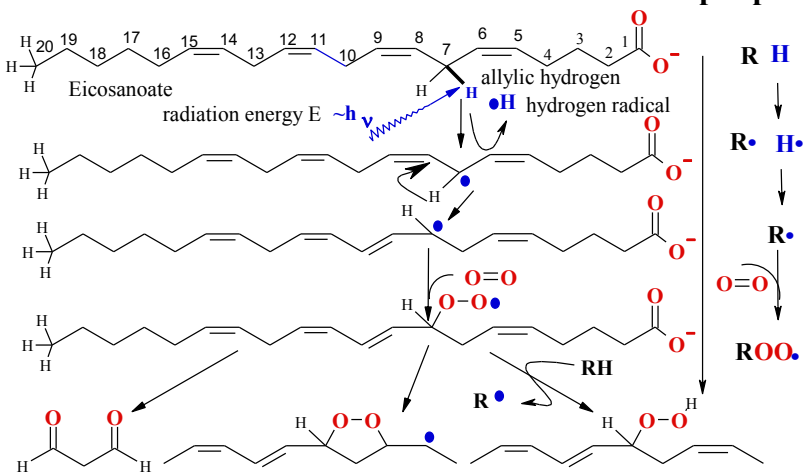
R÷H (transfer **H•** together with **e^-** as **Oxidant**)+ **~hν** → **R•** + **H•**

2**R-C=O-H** + **O=O** (**Aldehyde OxydoReductase**) → 2**RCOO•** + **2H•**

(2) **Propagation**: **ROO• + RH → ROOH + R• ; R• + O_2 → ROO•**, etc.

(3) **Termination-recombination**: **ROO• + ROO• → ROOR + O_2 ; ROO• + R• → ROOR ; R• + R•→ RR**

FIGURE 3. Lipid peroxidation.



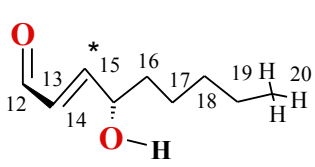
Radical formation **Initiation** as ● unpaired electron

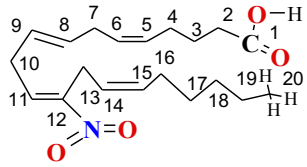
Oxygen **O=O** reactive species peroxide **-O-O●** creation

Relatively stabile products are formed

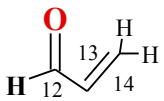
Hydro peroxide ROOH does undergo **oxidation**

Malonil aldehyde **Endoperoxide**





HNE 4-hydroxynonenal 1,2-nitro-arachidonate

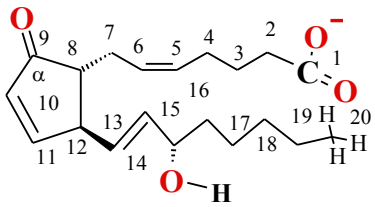


acrolein

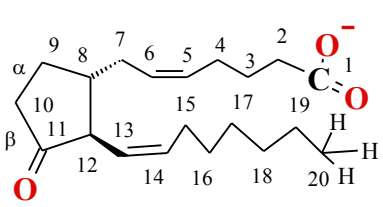
Examples of **RLS** produced from **arachidonic** acid and their structures.

The reaction is **initiated** by radiation (**~hν**), **Aldehyde OxydoReductase** or by metal ions **metal$^{(n)+}$** with **H_2O_2** at presence of oxygen in **water** medium **O_2 + H_2O**.

Malonil aldehyde is only formed by fatty acids with **3** or more **>3** double bonds and is used as measure of lipid **peroxidation** together with ethane from the terminal 2-carbon of **ω3** fatty acids and pentane from the terminal 5-carbon of **ω6** fatty acids.

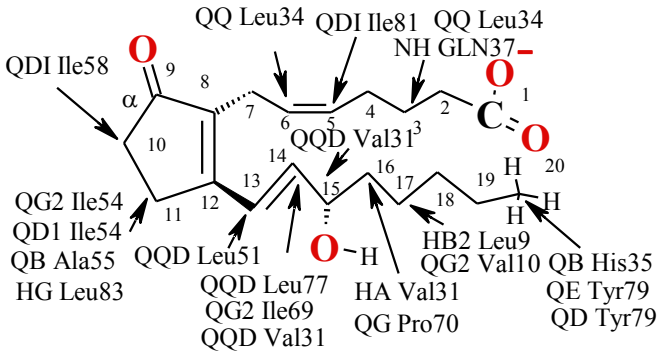


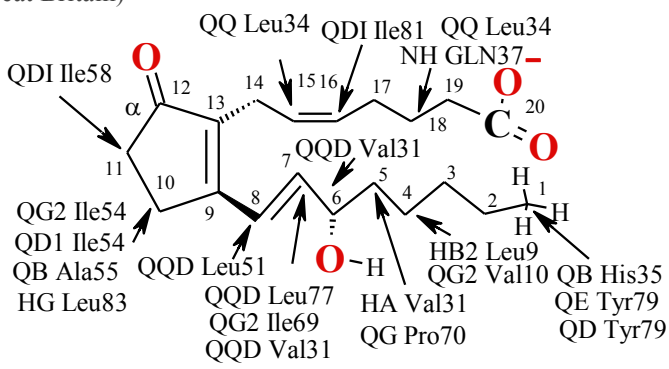
isoprostanoate A_1



soprostanoate J_2

Reference: Biochem. J. (2012) 442 (453–464) (Printed in Great Britain)





Molecular structure of PGB_2 and ligand-protein contacts in the complex wheat ns-LTP/PGB_2. Carbons are numbered from 1 to 20. Conformation of vinyl 7–8 and 15–16 are *trans* and *cis*, respectively. The NOEs detected on the NOESY spectrum at 200 ms between PGB_2 and protein protons in the complex **1CZ2.pdb** are indicated.