Enzymatic oxidation of lipids: mechanisms and functions.

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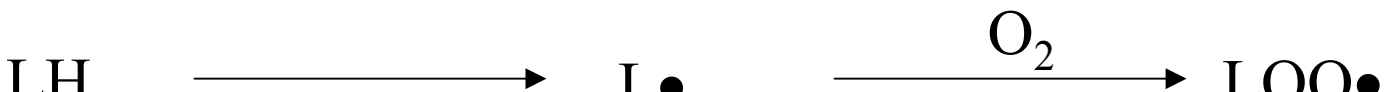
CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

coleg meddygaeth cymru
wales college of medicine

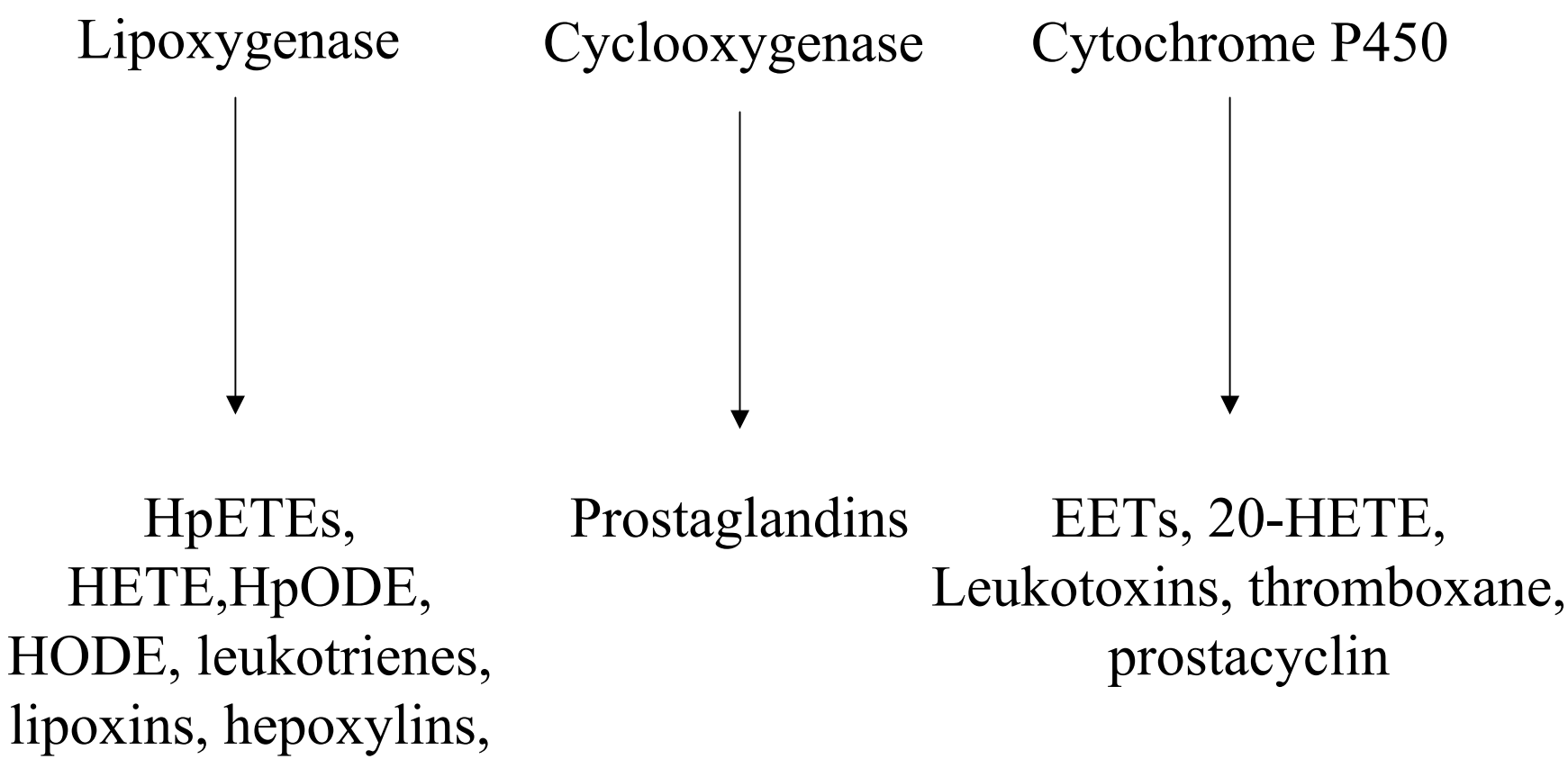
Enzymatic lipid oxidation: involves an enzyme catalyst, and gives very specific stereo- and regiospecific products.

Non-enzymatic: does not form specific products, many stereo- and positional isomers formed.

Initially involves hydrogen abstraction from a carbon, with oxygen insertion forming a lipid peroxyl radical



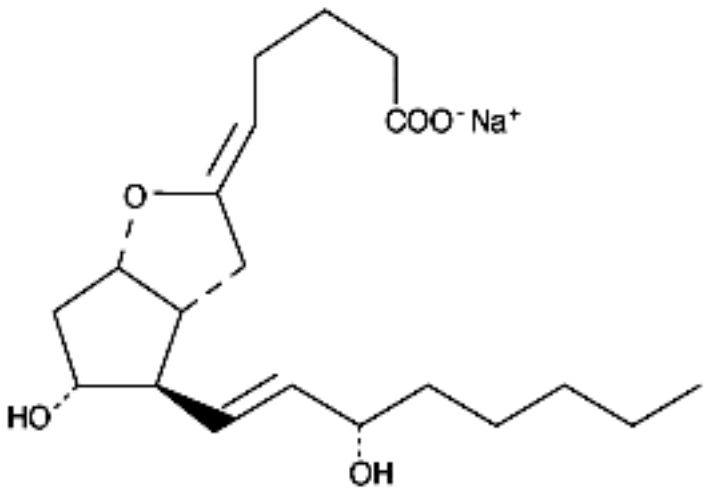
3 main pathways that generate oxidized lipid signaling mediators



Why have enzymes evolved to generate specific oxidized lipids?

Enzyme-generated products mediate specific bioactivities via receptor-dependent pathways that are under tight control. Physiological processes.

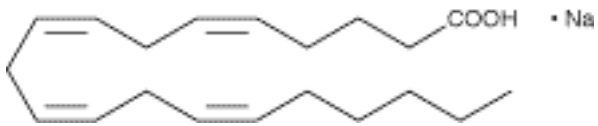
Example: Prostacyclin activates IP (GPCR) in response to bradykinin (etc.) generating cAMP.



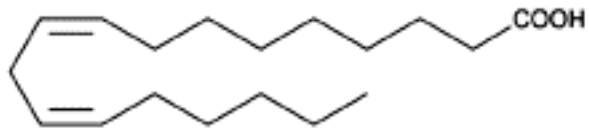
Blocks platelet activation

Smooth muscle relaxation

Substrates: Unsaturated fatty acid from sn2 position of phospholipids: *arachidonate or linoleate, also n3 fatty acids.*

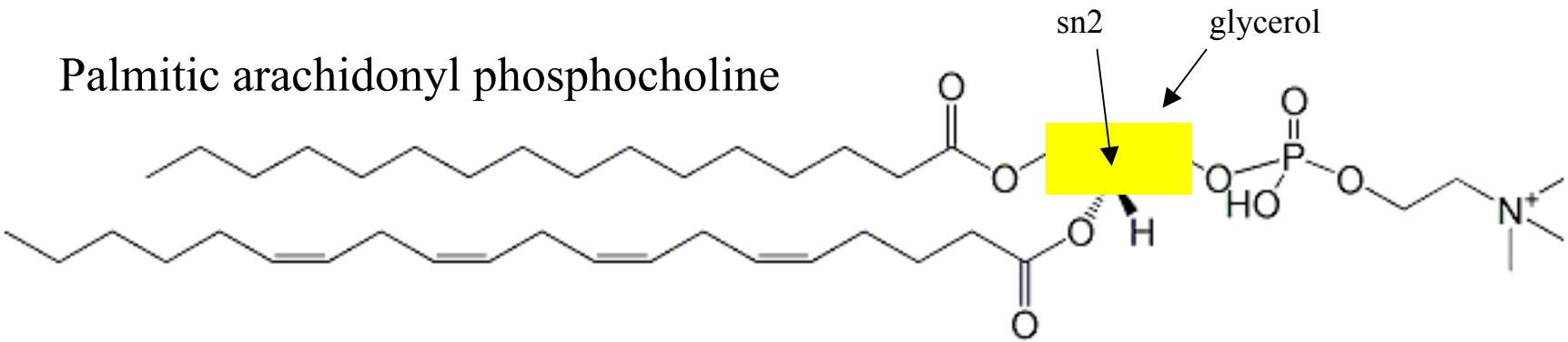


arachidonate



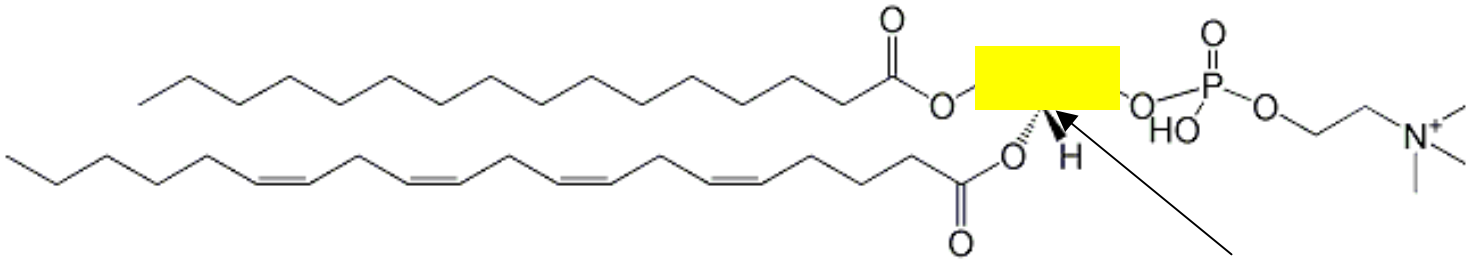
linoleate

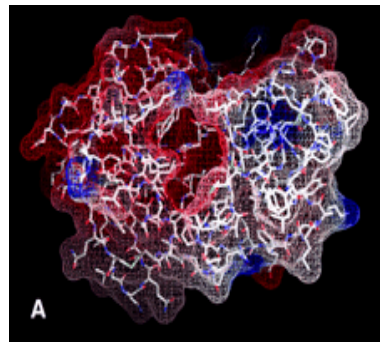
Palmitic arachidonyl phosphocholine



1-hexadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine

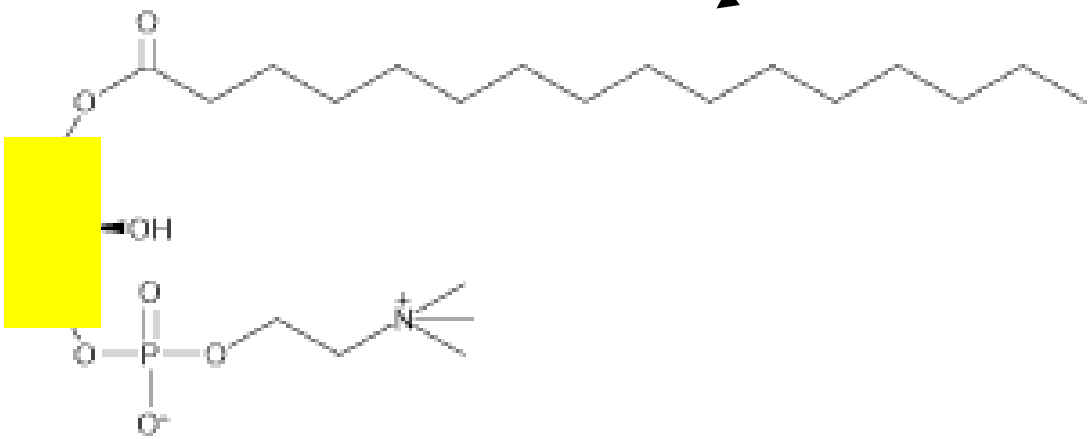
Release of substrate by phospholipase A2



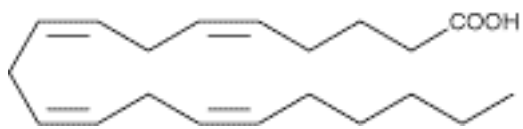


PLA2

Pan et al, JBC 2002



Lyso-PC



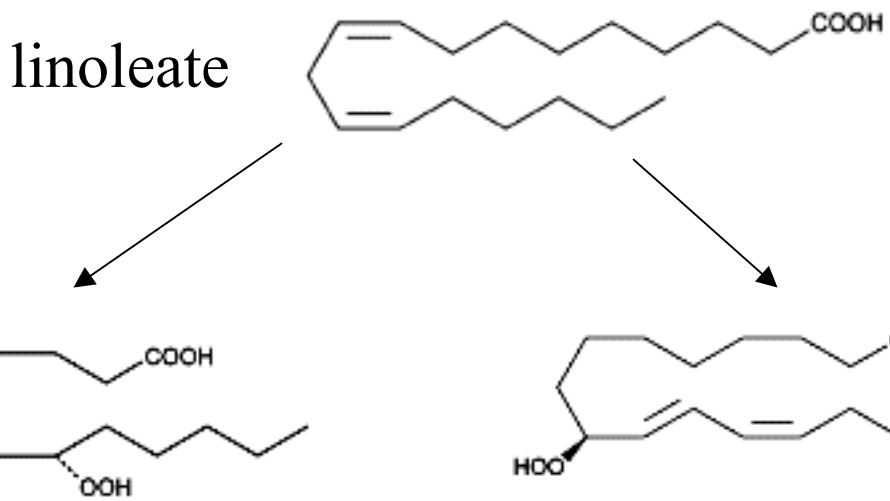
arachidonate

PLA2 action not required for non-enzymatic peroxidation

What do we mean by stereo-, positional-, geometric-isomers, enantiomers, diastereomers?

•There are lots and it's complex!!!

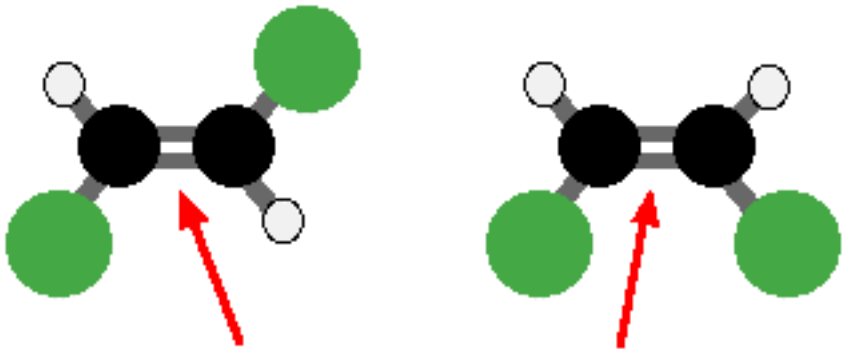
1. Positional: oxygen insertion on different carbons



13-HPODE

9-HPODE

2. Geometric: cis or trans isomers



Trans	Cis
E	*Z*
Entgegen (opposite)	Zusammen (together)

Can have different physical properties, e.g. melting/boiling pt.

http://www.chemguide.co.uk/basicorg/isomerism/geometric.html

3. Enantiomers: non-superimposable mirror images

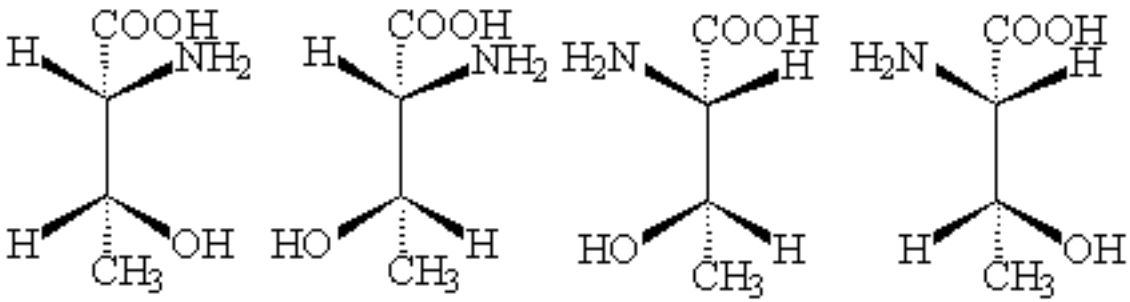
The spatial arrangement of the molecules is different, they contain a chiral center.

Designated S or R depending on the order of the rotation of the groups attached to the chiral center.

http://www.chemguide.co.uk/basicorg/isomerism/geometric.html

4. Diastereomers: have more than one chiral center.

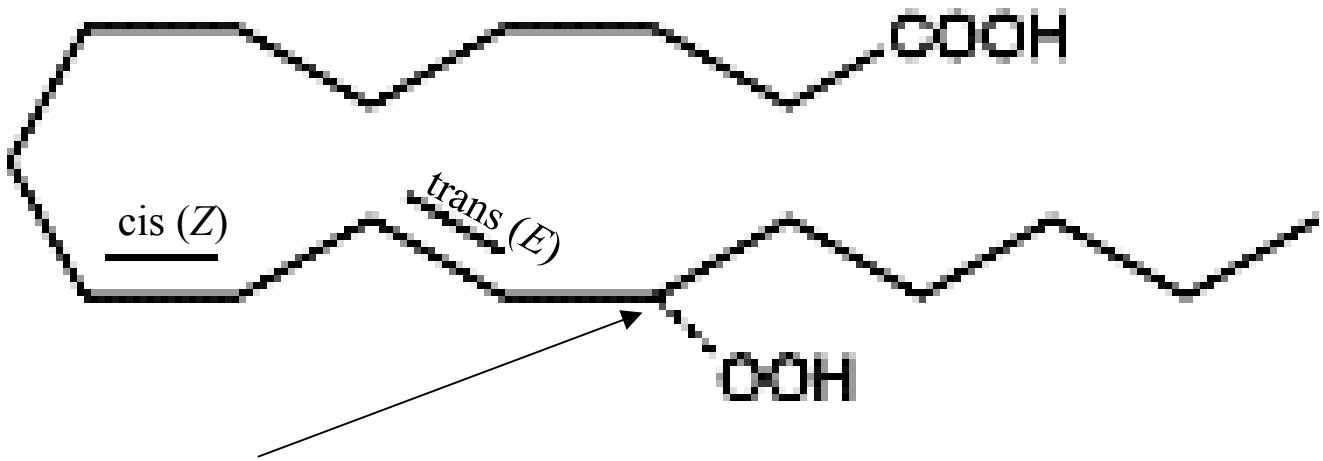
The four diastereomers of threonine



Relevant for oxidized lipids with multiple oxygen additions at different carbons.

How does this work with oxidized fatty acids?

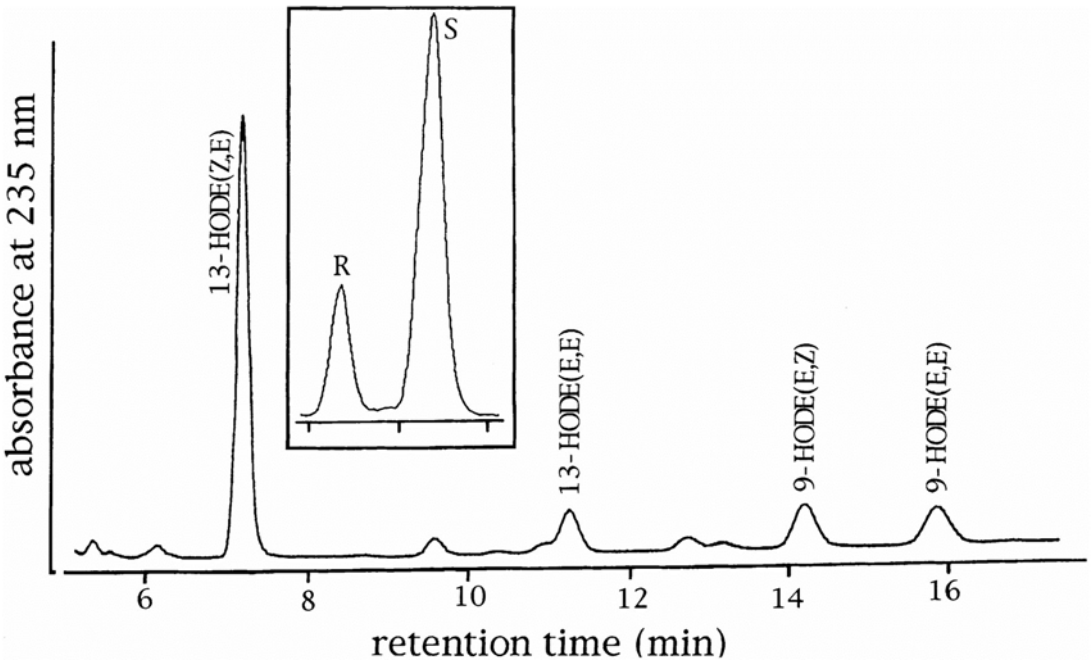
Example: linoleate hydroperoxide made by 15-LOX



Chiral center (S) at C13

13S-hydroperoxy-9Z,11E-octadecadienoic acid

Generation of specific products by an enzyme: 15-LOX generation of 13(S)HpODE (*Z*,*E*)



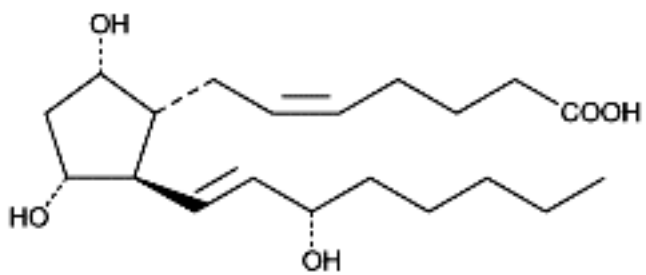
Kühn et al, J. Clin. Invest, 1997

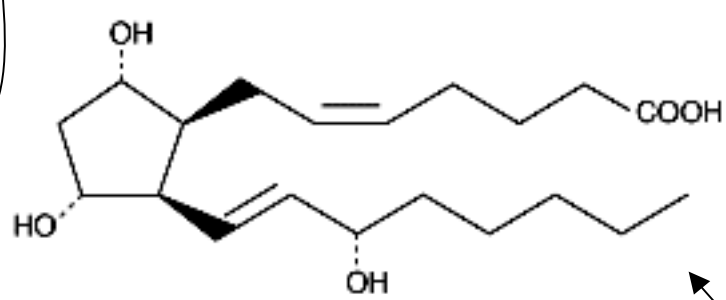
Example:

Prostaglandins and isoprostanes:

Example

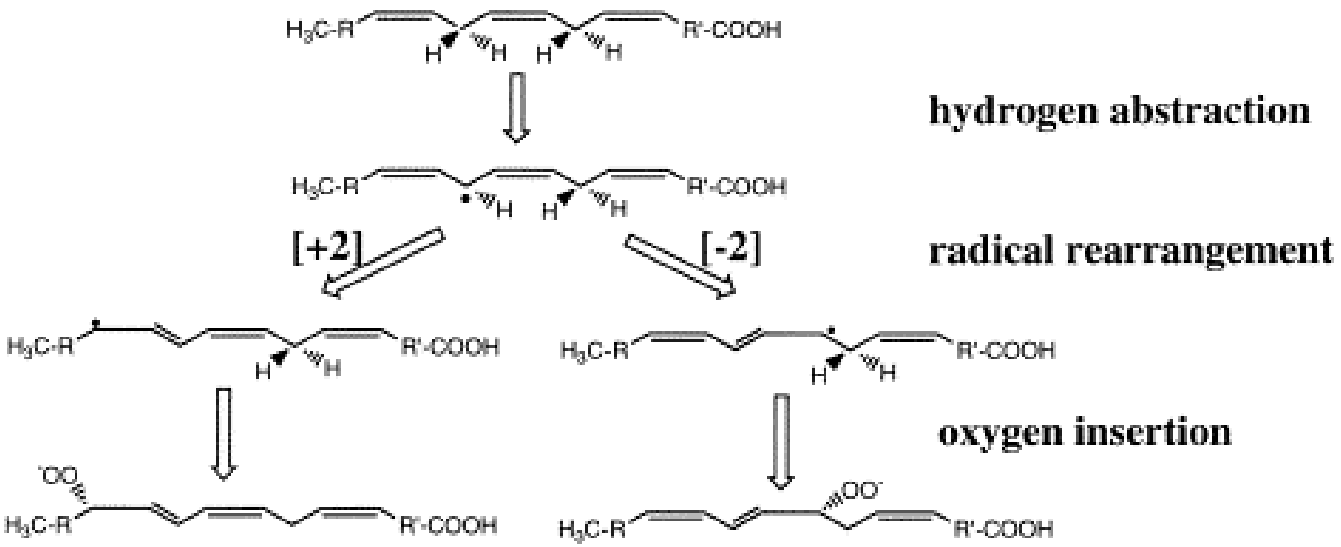
•COX-derived product prostaglandin F2α





•Non-enzymatic oxidation of arachidonate forms many different positional/stereoisomers of isoprostanes, including 8 isoprostane F2α.

How does an enzyme make a specific product?



Kühn et al, FEBS Letts, 1999

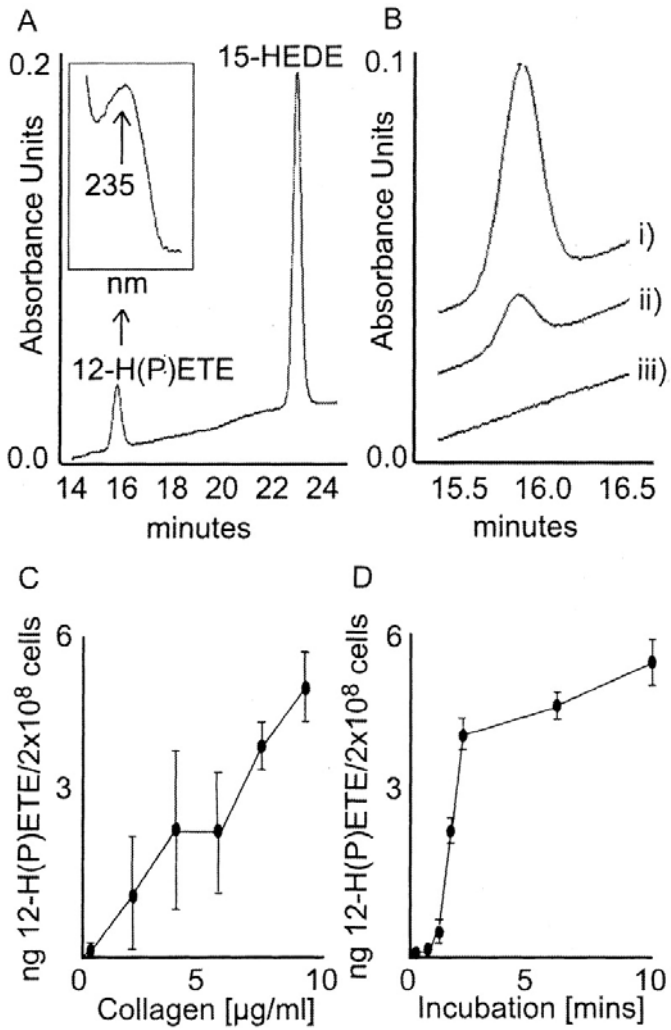
Which predominate in vivo?

Agonist-activated cells generate very specific products: e.g. collagen-activated platelet 12-LOX

Basal levels of isoprostanes versus COX-derived prostaglandins: similar in human urine at ng/ml although isoprostanes may be higher in some diseases.

Disease: In atherosclerosis, early lesions contain more 13(S)HpODE than other isomers, but late lesions show equal mix of racemic products….. *What does this mean?*

Activation of 12-LOX in platelets results in generation of only 12-HPETE with no other positional isomers



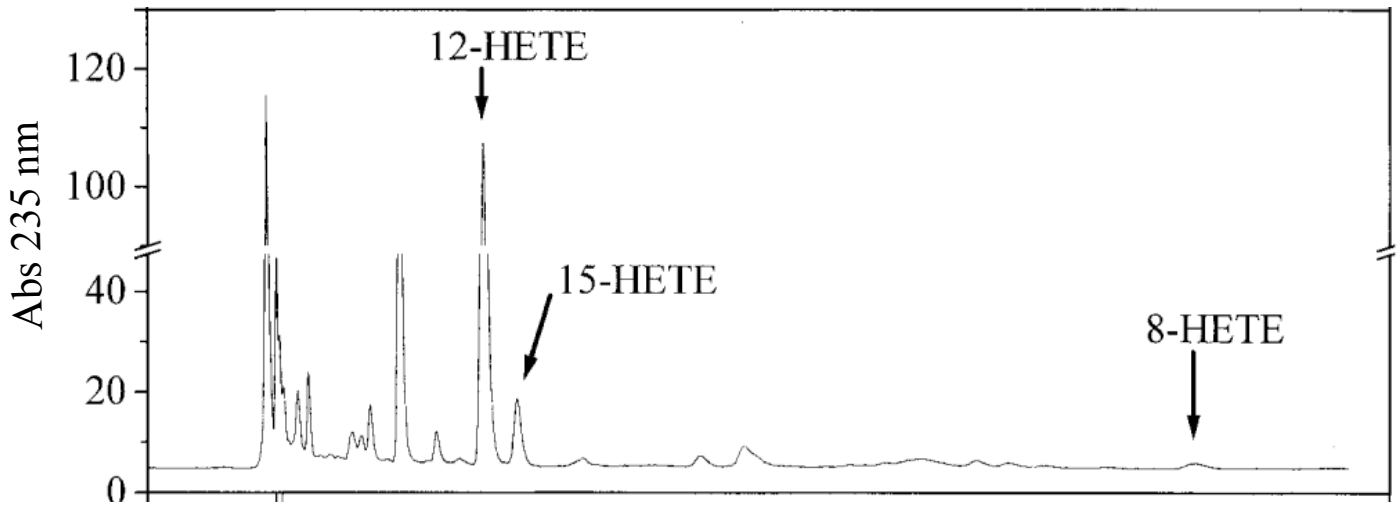
Collagen-related peptide

collagen

control

Coffey et al, Circ Res, 2004

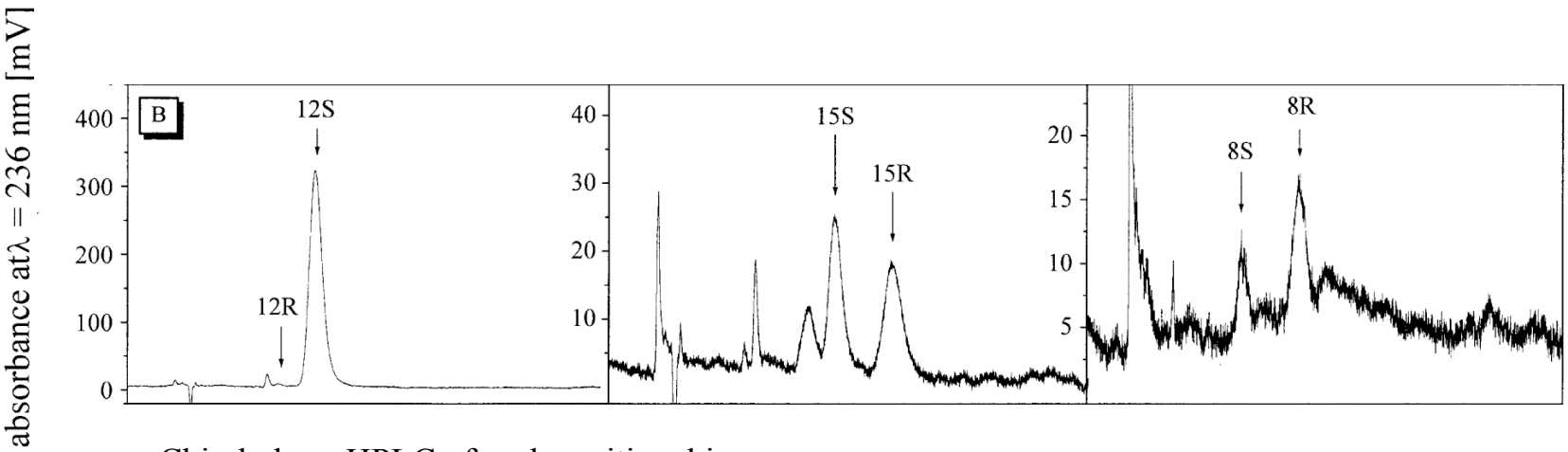
Positional isomers generated by platelet 12-LOX expressed in HEK 293 cells, sonicated, using arachidonate substrate.



Straight phase HPLC of cell extracts

Burger et al, Biochem J. 2000

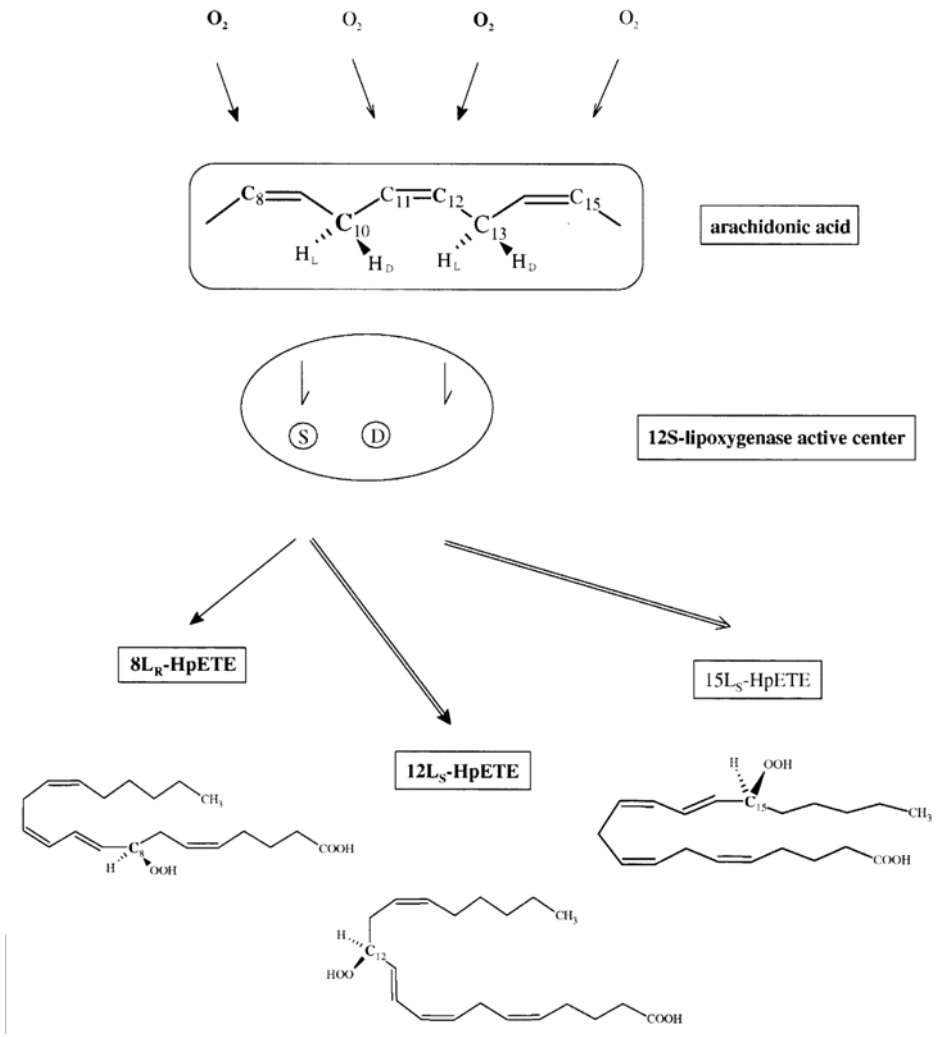
Enantiomers of HETEs generated by platelet 12-LOX expressed in HEK 293 cells, sonicated, using arachidonate substrate.



Chiral phase HPLC of each positional isomer

Burger et al, Biochem J. 2000

Generation of various isomers by platelet 12-LOX.



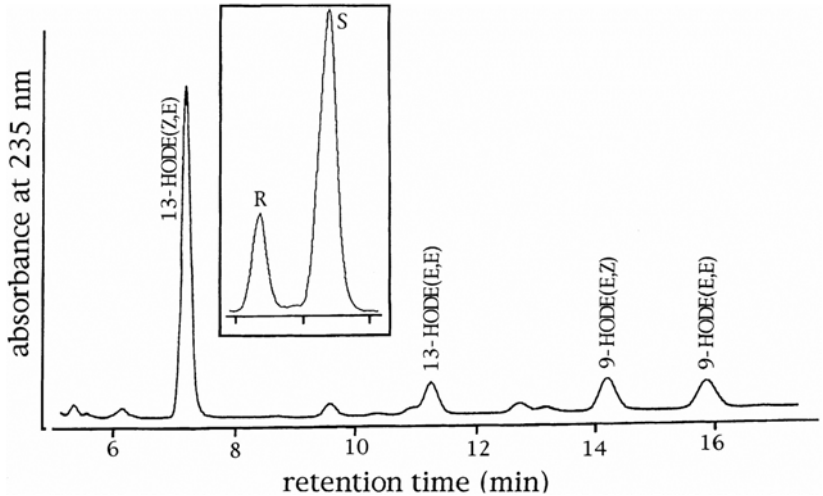
Burger et al, Biochem J. 2000

Comparison of positional, geometric isomers and enantiomers generated by 15-LOX and copper oxidation of LDL.

Table I. Composition of Hydroxy Polyenoic Fatty Acids of In Vitro Oxidized Human LDL

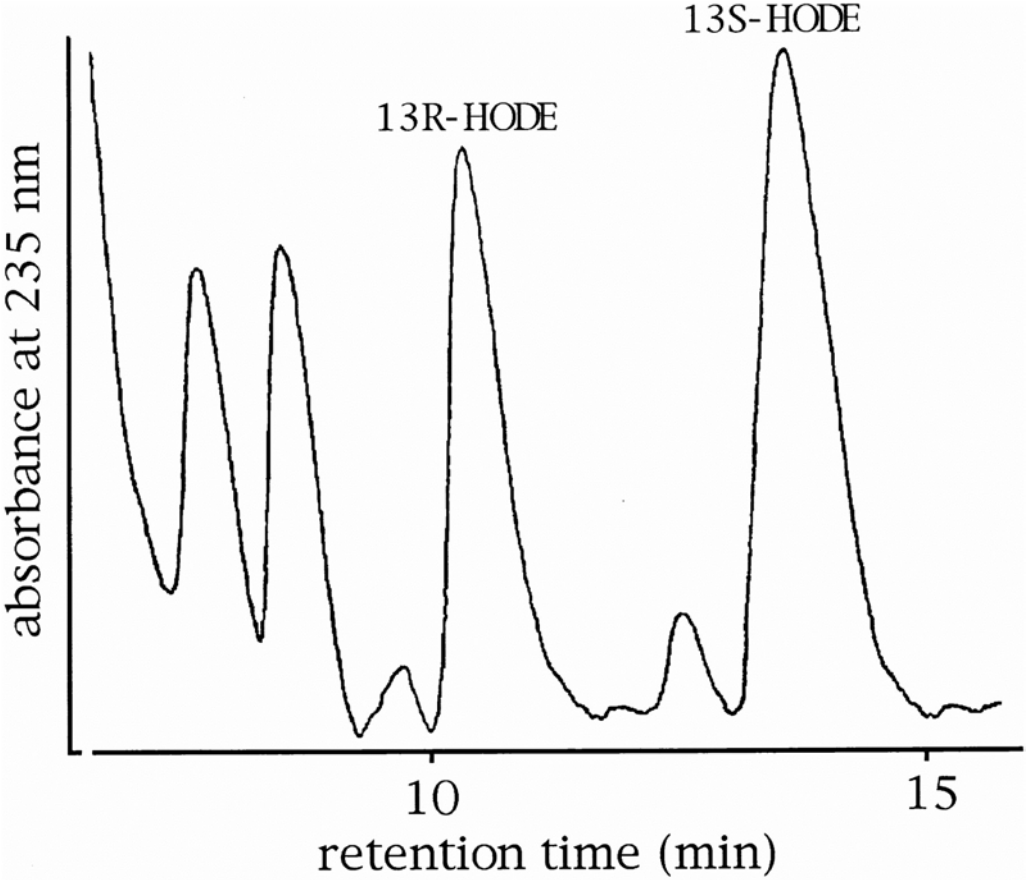
	Share (%)			
Catalyst	13-HODE(Z,E)	13-HODE(E,E)	9-HODE(Z,E)	9-HODE(E,E)
15-LOX	72±12	5±4	15±7	6±3
$n = 11$	(85±5:15±5)		(51±1:49±1)	
$CuSO_4$	33±12	22±8	25±5	20±12
$n = 8$	(51±1:49±1)		(50±1:50±1)	

Lipoxygenase-catalyzed LDL oxidation was carried out as described in the legend to Fig. 1. For copper catalyzed oxidation a 30-fold molar excess of copper over LDL was used. Analysis of the hydroxy linoleate (HODE) isomers was carried out by straight phase-HPLC. The sum of these isomers was set 100%. The enantiomer composition (S/R ratio given in parentheses) was determined by chiral phase HPLC. LOX-lipoxygenase.



Kühn et al, J. Clin. Invest, 1997

Profile of HpODE products in a young human atherosclerotic lesion.



Kühn et al, J. Clin. Invest, 1997

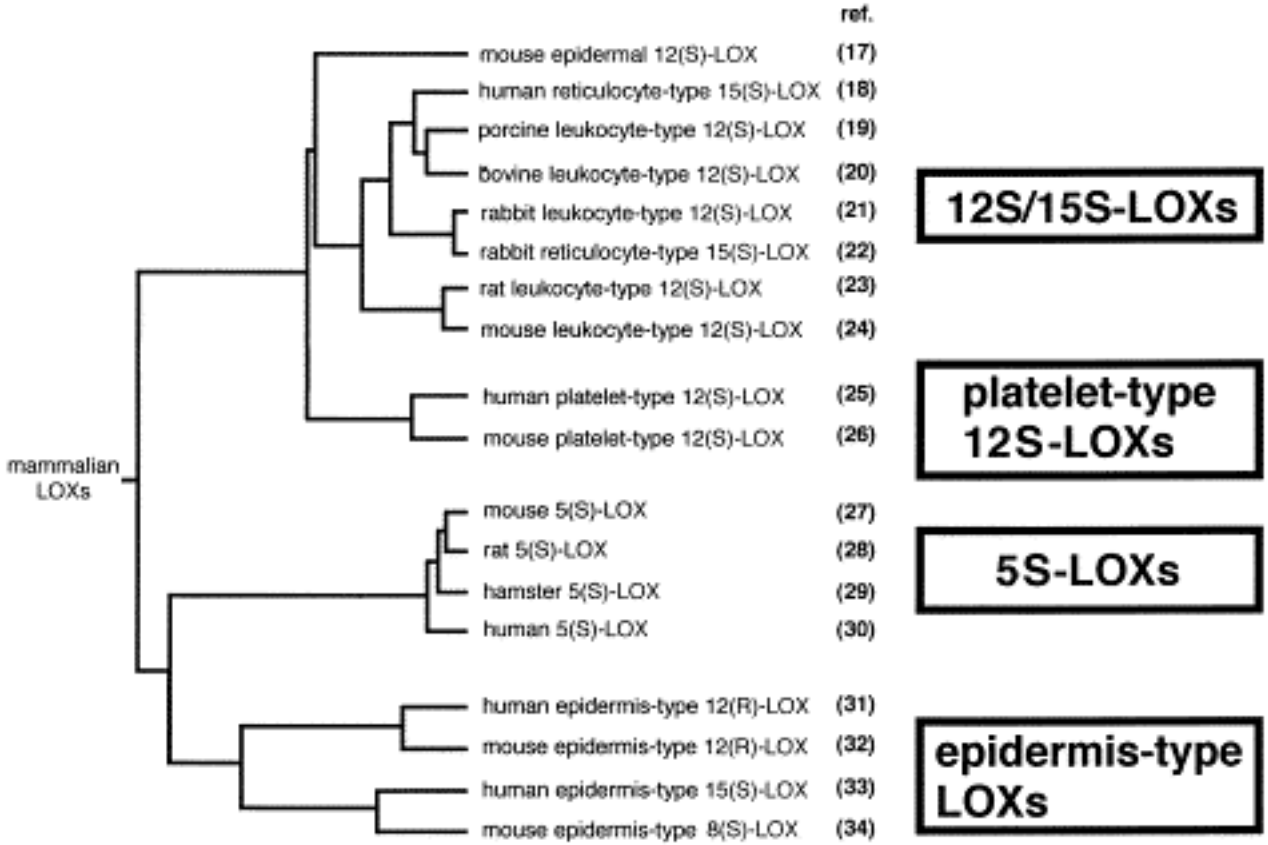
Profile of HODE products in a young human atherosclerotic lesion.

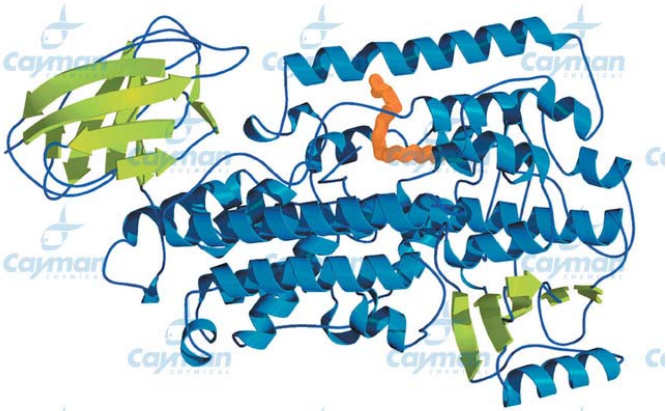
Table II. Enantiomer Composition of 13-Hydroxy Linoleic Acid (13-HODE) Isolated from Human Atherosclerotic Lesions and Oxidized LDL

Sample	*n*	Enantiomer	Share (%) mean±SD	Significance (S vs. R-isomer)	Comparison of the relative shares of S-isomers of 13-HODE significance *P*			
					PDAY			
PDAY	19	13S-HODE	54.0±3.2	< 0.001	–			
		13R-HODE	45.0±3.2					
						Berlin		
Berlin	17	13S-HODE	50.7±3.5	0.260	0.007	–		
		13R-HODE	49.3±3.5					
							15-LOX–treated LDL	
15-LOX–treated LDL	79	13S-HODE	71.1±1.3	< 0.001	< 0.001	< 0.001	–	
		13R-HODE	28.3±1.3					
								Copper-treated LDL
Copper-treated LDL	13	13S-HODE	50.1±1.1	0.470	< 0.001	0.600	< 0.001	–
		13R-HODE	49.9±1.1					

Kühn et al, J. Clin. Invest, 1997

Diversity of mammalian LOX superfamily as of 1999….



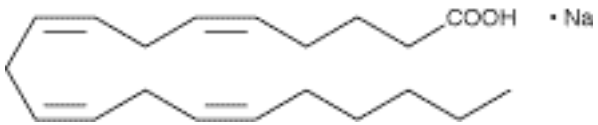


Ribbon diagram of rabbit 15-LOX

S.Gilmore, UCSF, M. Browner, Roche Biosciences

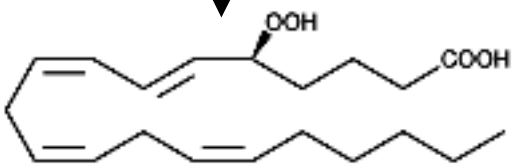
Kühn et al, FEBS Letts, 1999

Formation and functions of 5-LOX products



arachidonate

5-LOX



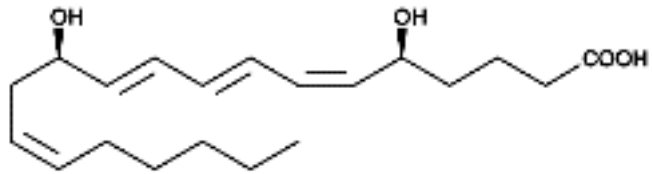
5S-HPETE

5S-HETE

LTA4

Highly unstable

LTA4 hydrolase

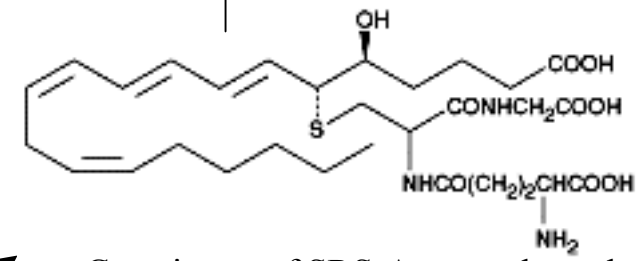


LTB4

Stimulates multiple neutrophil functions at nM and sub uM ranges

LTC4 synthase

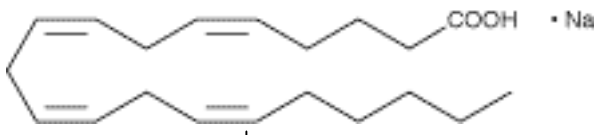
LTD4 → LTE4



LTC4

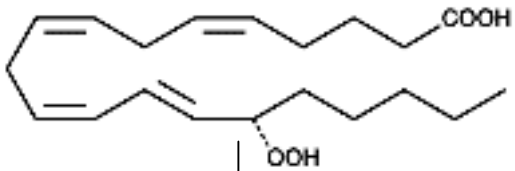
Constituent of SRS-A, vaso- broncho-constrictor

Formation and functions of 15-LOX products



arachidonate

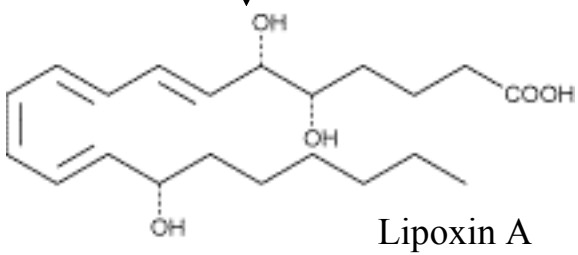
15-LOX



15S-HPETE

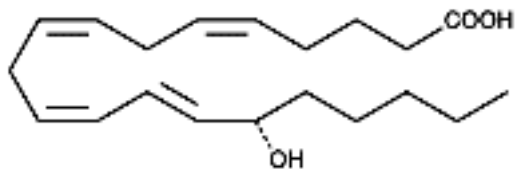
5-LOX

Induction of c-fos, c-jun, activation of AP1

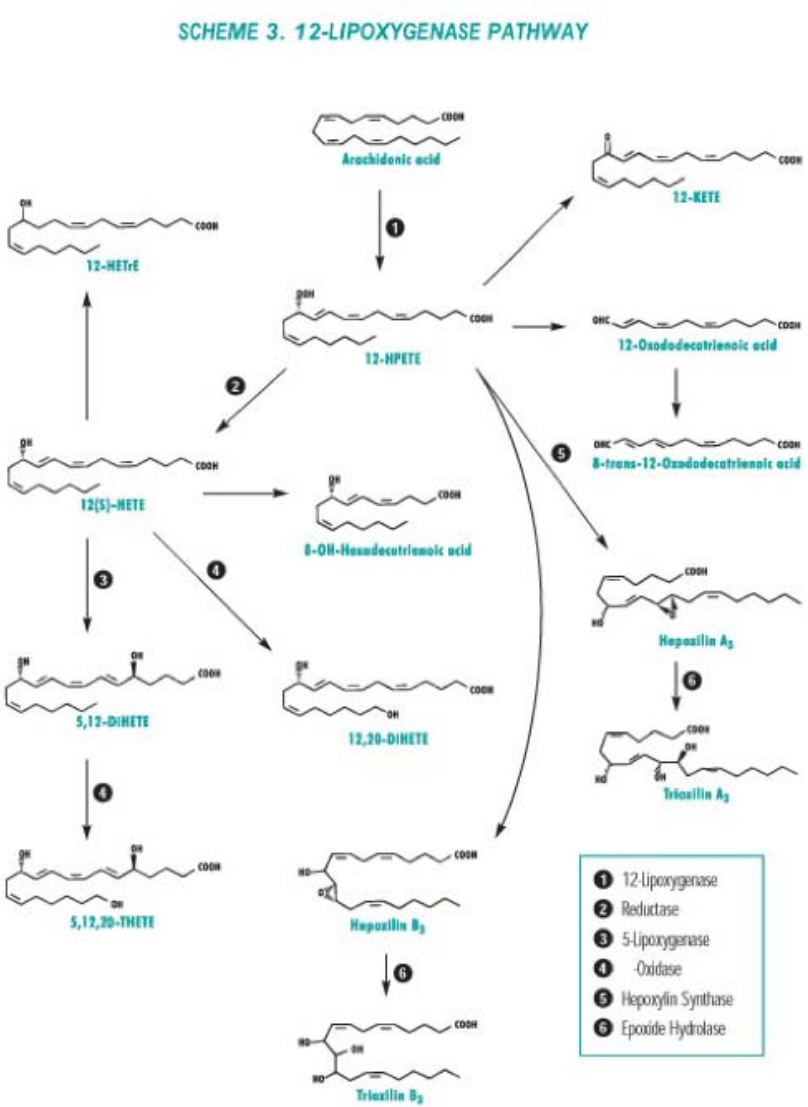


Lipoxin A
Vasoconstrictor
Can also be generated by COX + aspirin
Inflammation resolution, inhibition of neutrophil function, wound healing.

May form endogenous PPARγ ligands but identity of these currently unknown.



15S-HETE

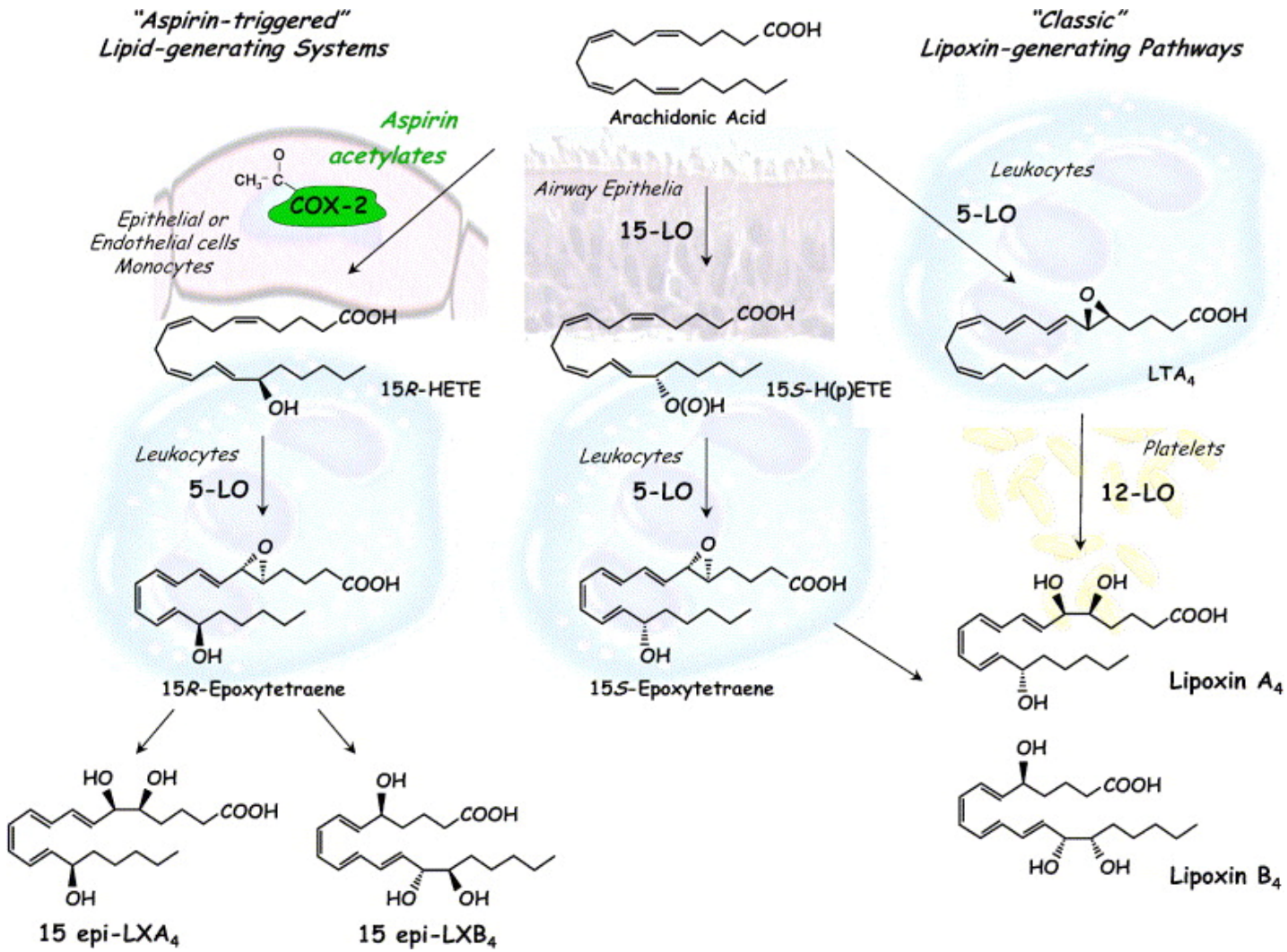


Formation of 12-LOX products

Functions:

12-H(P)ETE: little/no direct effects on platelet function.
Hepoxylins: elevate calcium, induce vascular permeability, neutrophil chemoattractants

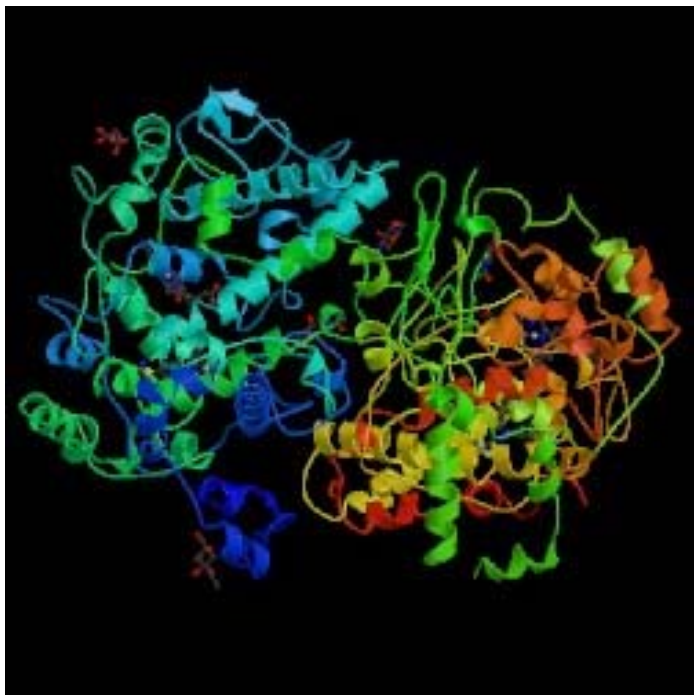
Transcellular formation of lipoxins

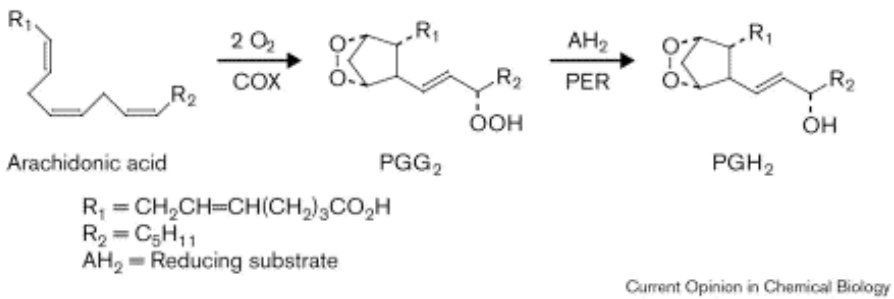


Chiang et al, Prostaglandins Leukot Essent Fatty Acids, 2005

COX isoforms

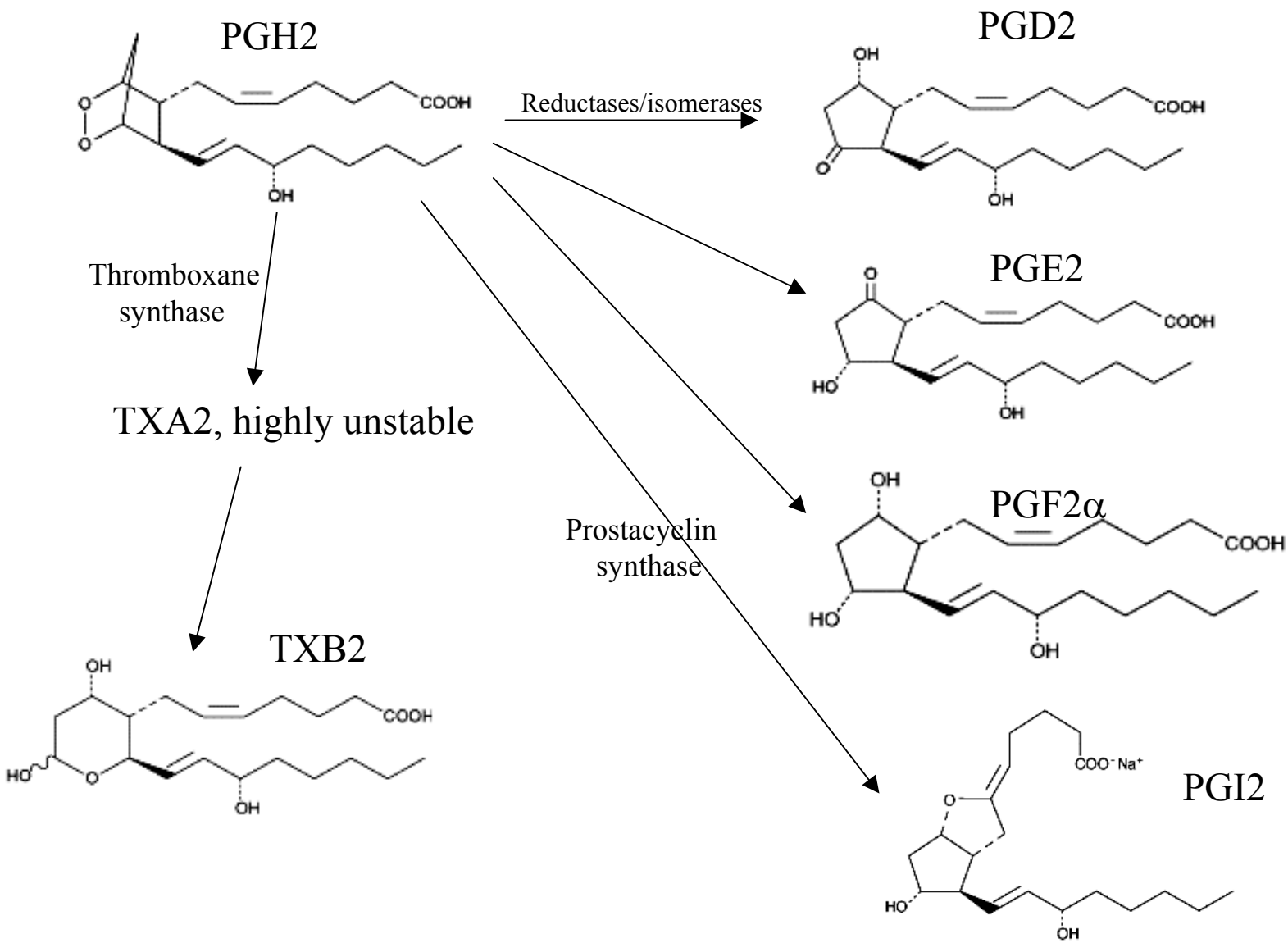
COX-1: platelets, gastric, renal constitutively expressed
COX-2: vessel wall, renal, induced in inflammation and cancer.
COX-3: controversial, thought to be a splice variant.
See Cayman Chemical website for interesting discussions on the current thinking regarding its existence.
http://www.caymanchem.com/app/template/cox3%2CHome.vm/a/z



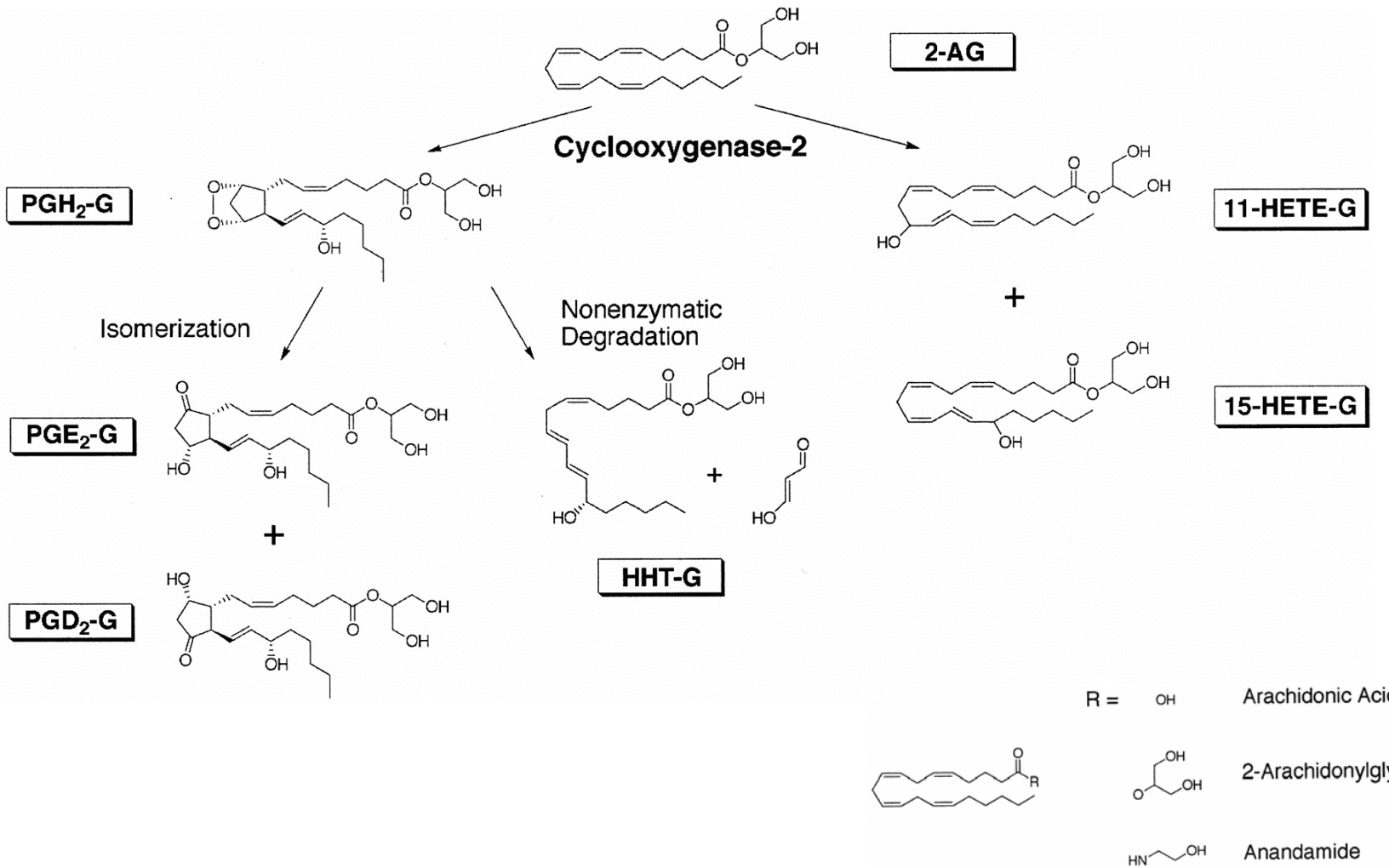


Marnett, Curr Opin Chem Biol, 2000
http://twinstars.office110.co.jp/~ud/bbs/joyful.cgi

Formation of COX products

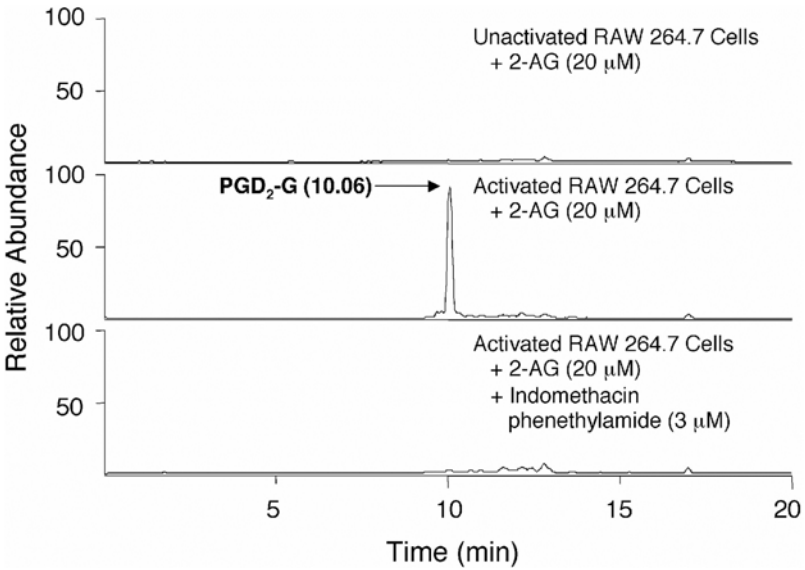


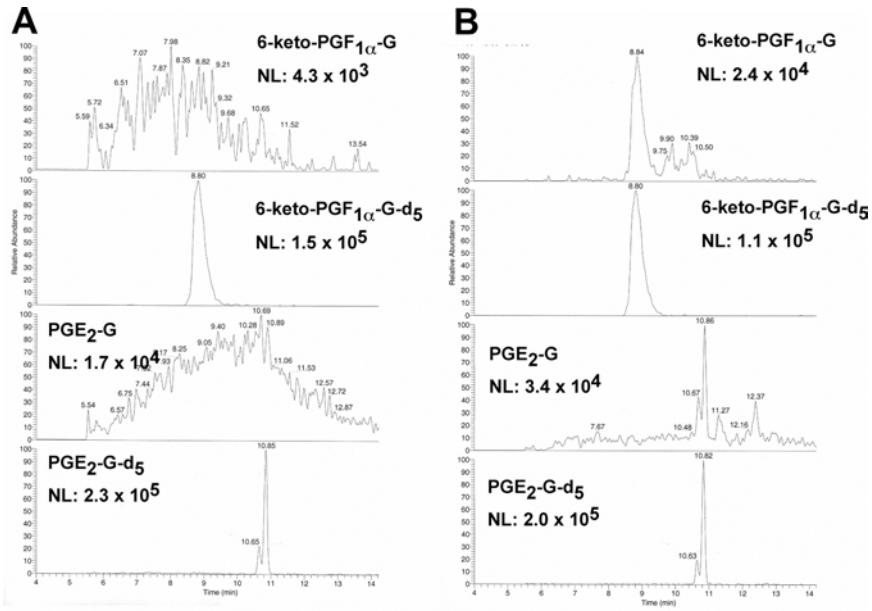
Glyceryl prostaglandins generated by COX-2



Kozak et al, J. Biol Chem, 2000

Glyceryl prostaglandins generated by murine macrophages from exogenous and endogenous substrate.





Control cells Zymosan-activated

Kozak et al, J. Biol Chem, 2000

Rouzer & Marnett et al, J. Biol Chem, 2005

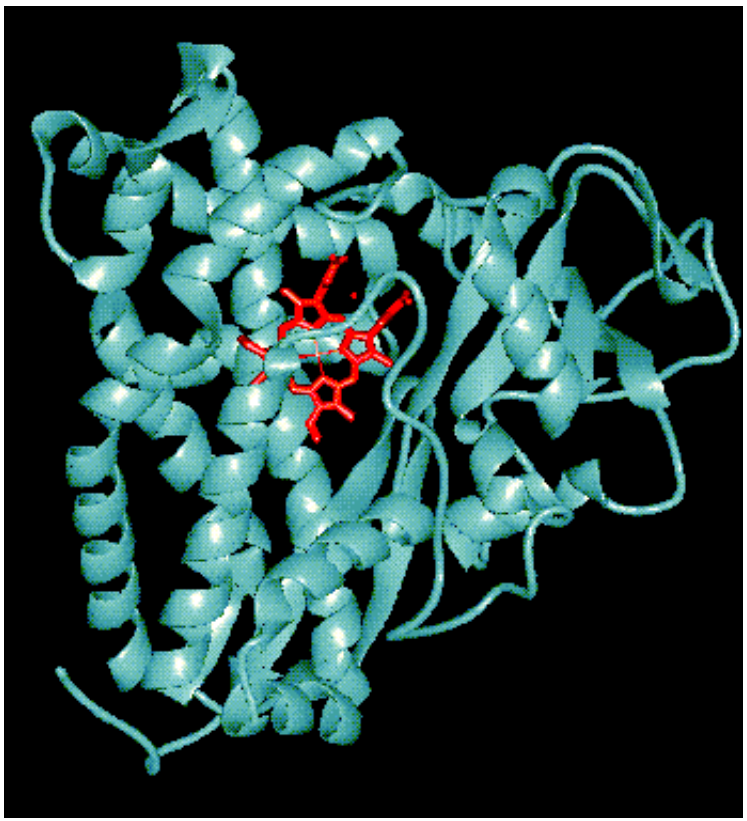
Cytochrome P450.

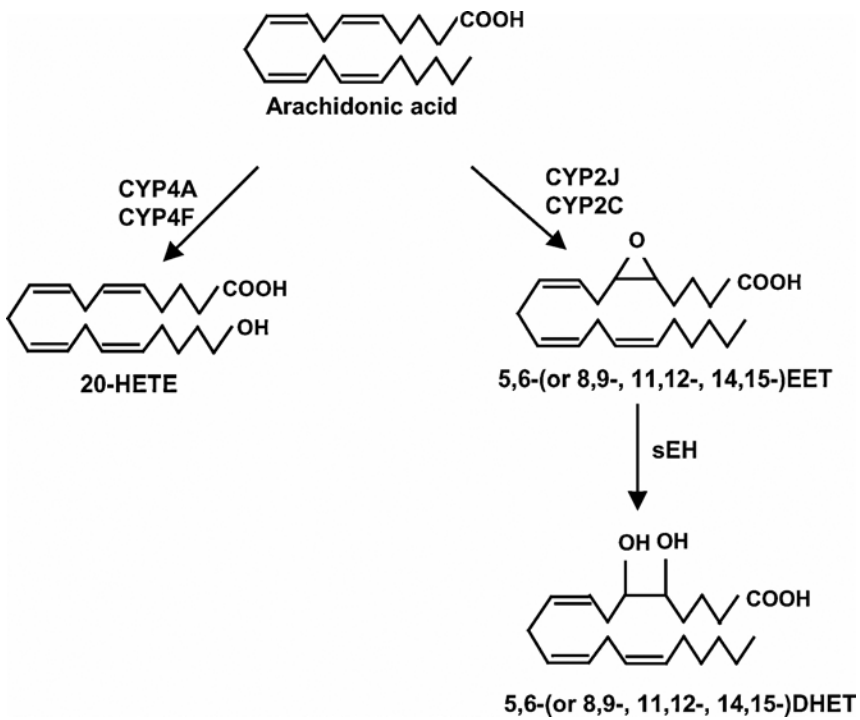
Thromboxane synthase: TXA2

Prostacyclin synthase: PGI2

CYP epoxygenases: EETS formed by CYP2C, 2J in humans

CYP ω-oxidases: ω-terminus hydroxylation by CYP4A, 4F.

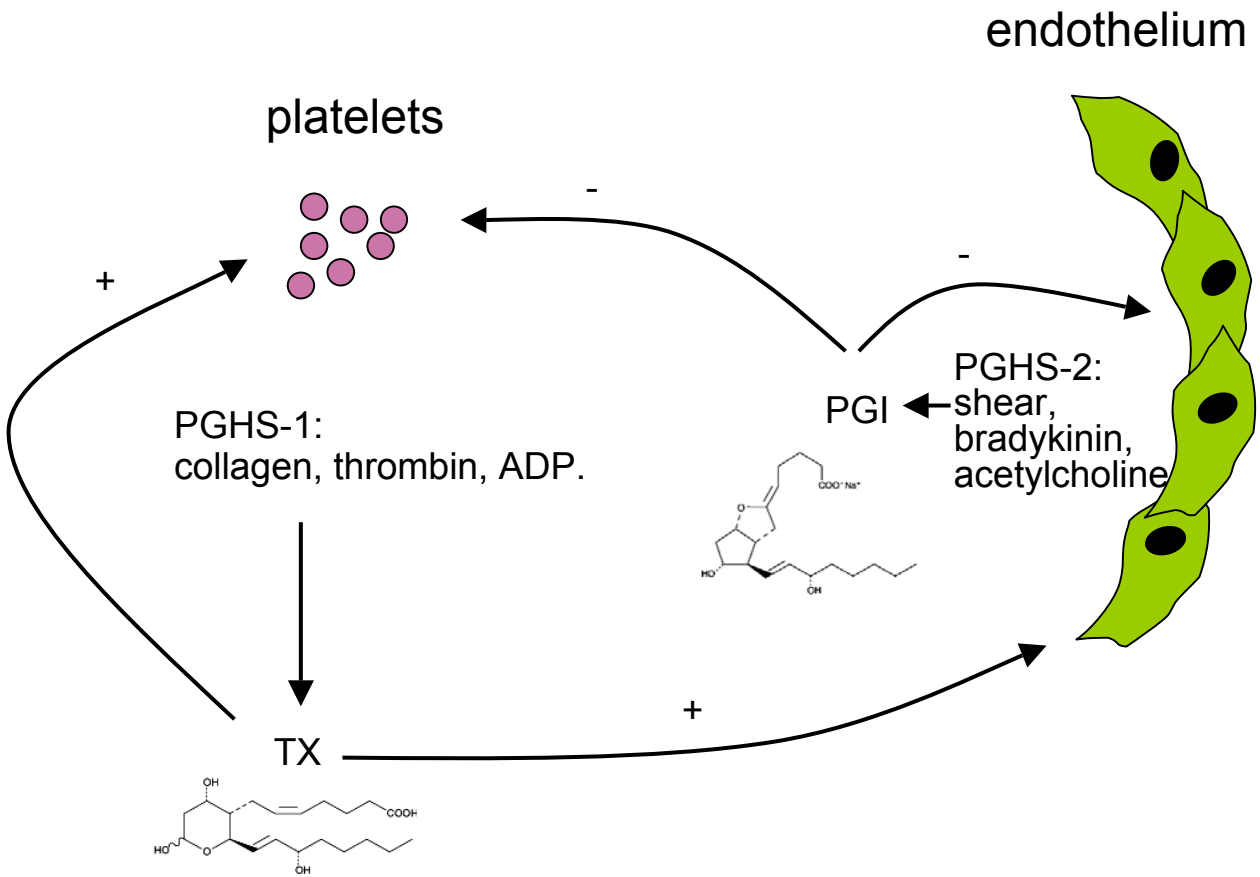




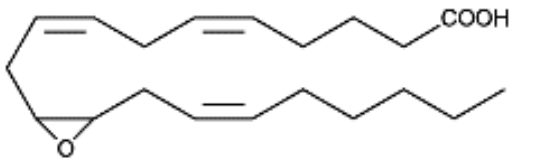
Kroetz & Xu, Ann Rev, Pharmacol & Toxicol, 2005

http://depts.washington.edu/medchem/

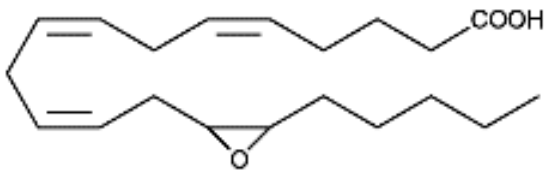
TX and PGI play opposing roles in regulation of vascular function.



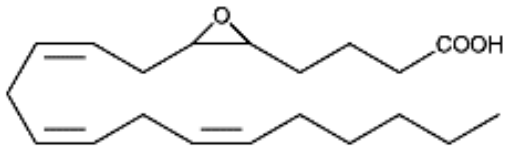
Structures and signaling actions of EETs (EpETrEs): postulated to be endothelium-derived hyperpolarizing factors.



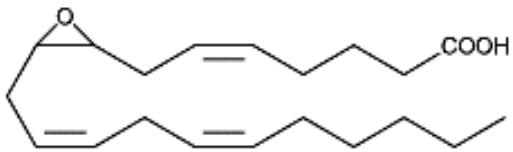
11,12 EpETrE. plays a role in the recovery of depleted Ca2+ pools in cultured smooth muscle cells



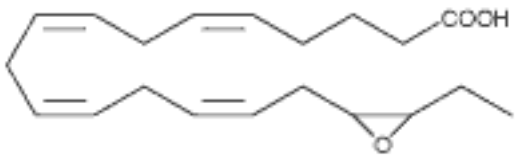
14,15 EpETrE. Made in rat and rabbit liver microsomes.



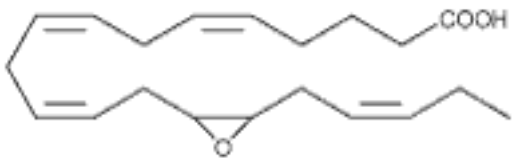
5,6 EpETrE. In neuroendocrine cells, such as the anterior pituitary and pancreatic islet, (±)5(6)-EpETrE has been implicated in the mobilization of Ca2+ and hormone secretion



8,9 EpETrE. reduces GFR through cyclooxygenase-dependent preglomerular vasoconstriction.

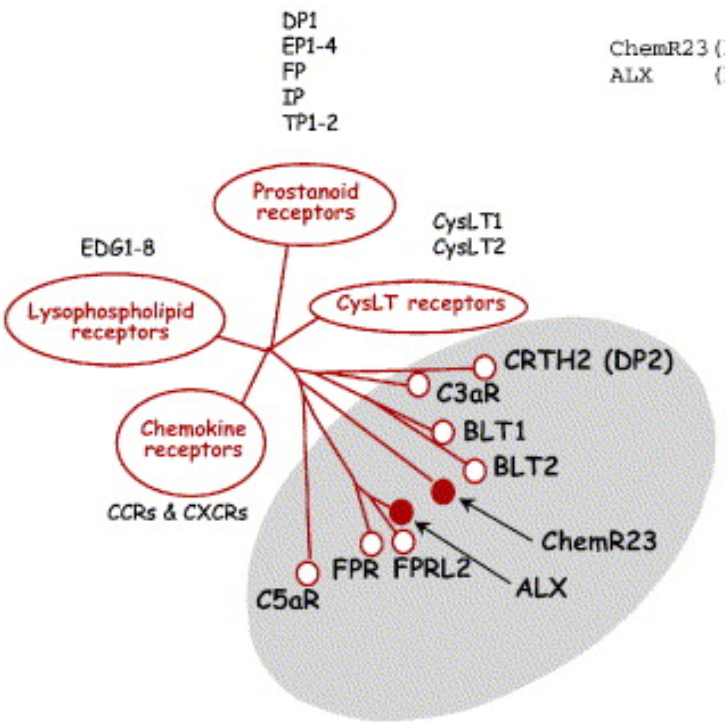


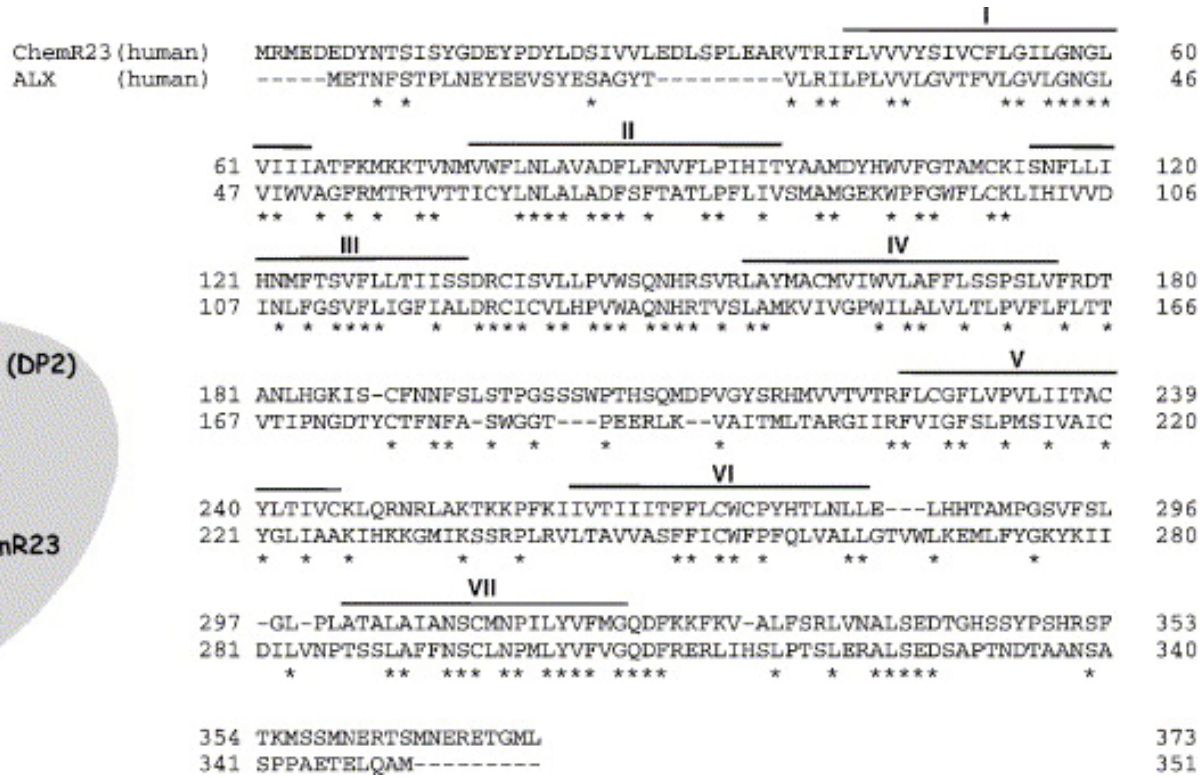
17,18 EpETE. Metabolite of EPA, activator of BK-type calcium activated potassium ion channels in vascular smooth muscle cells



14,15 EpETE. Metabolite of EPA. Activity unknown

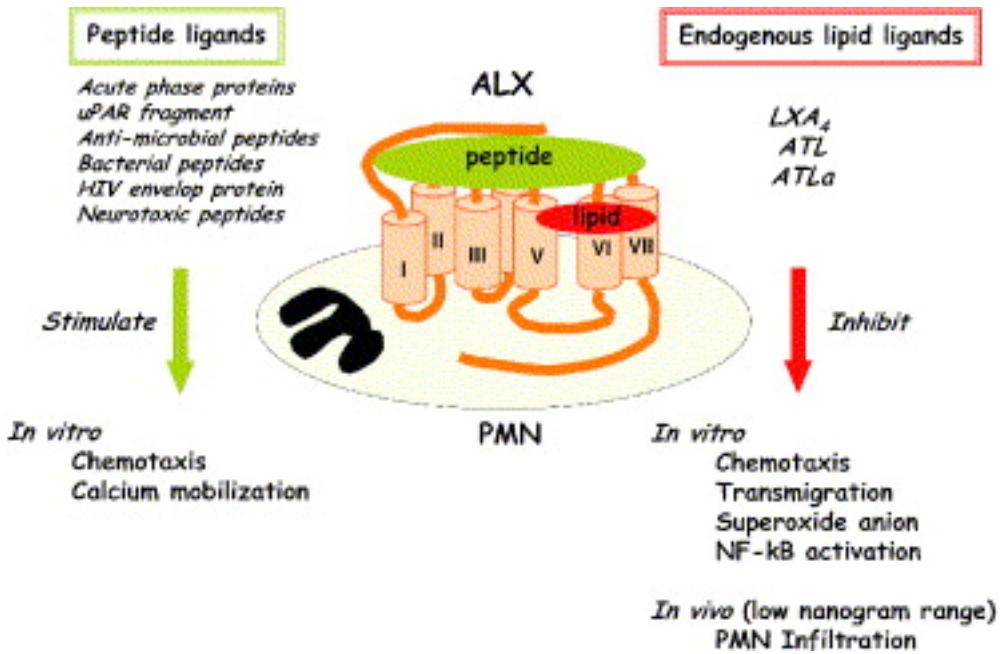
Eicosanoid signaling via 7-transmembrane domain GPCRs.





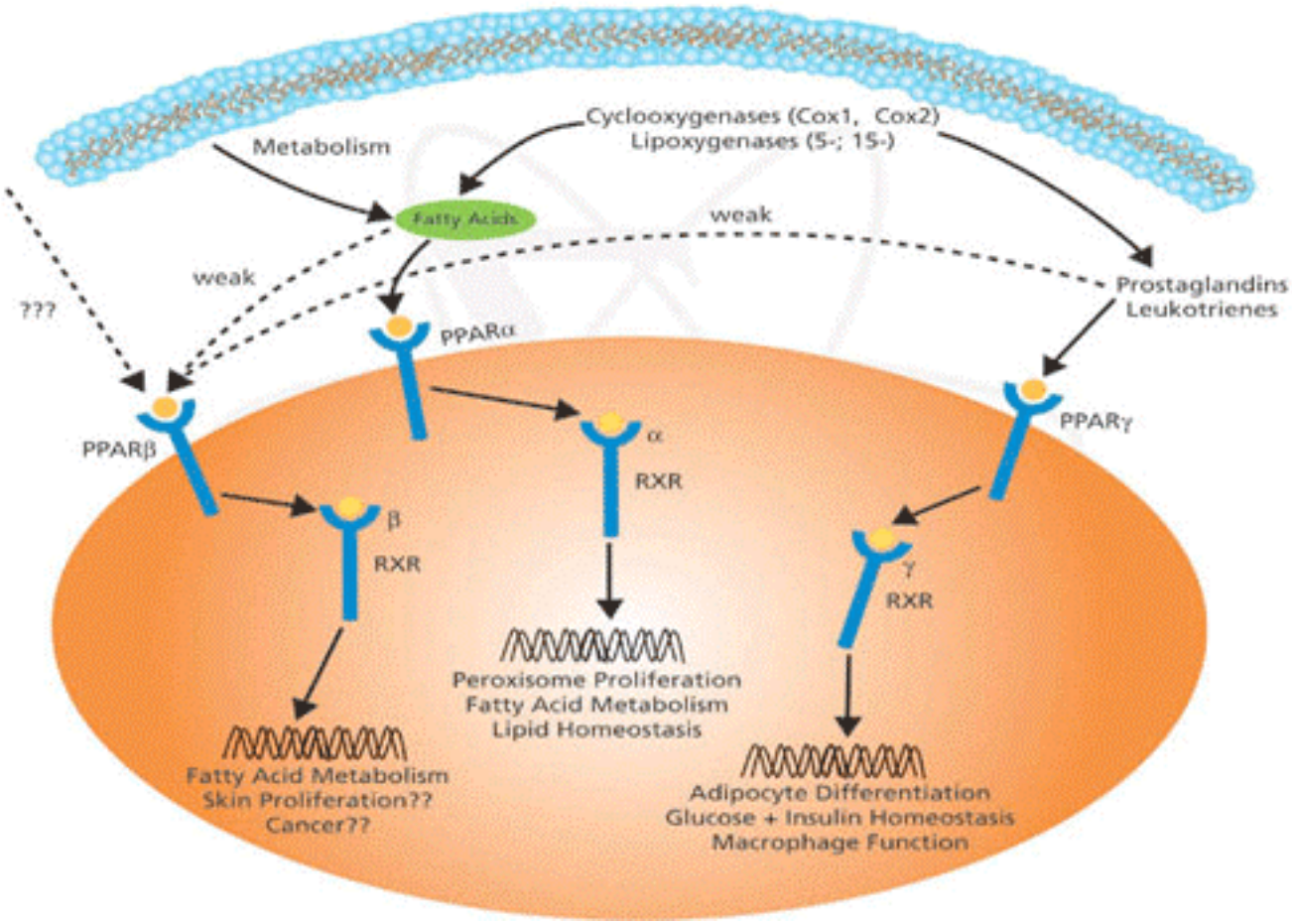
Chiang et al, Prostaglandins Leukot Essent Fatty Acids, 2005

Signaling by oxidised lipids via GPCRs.



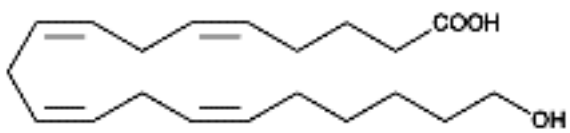
Chiang et al, Prostaglandins Leukot Essent Fatty Acids, 2005

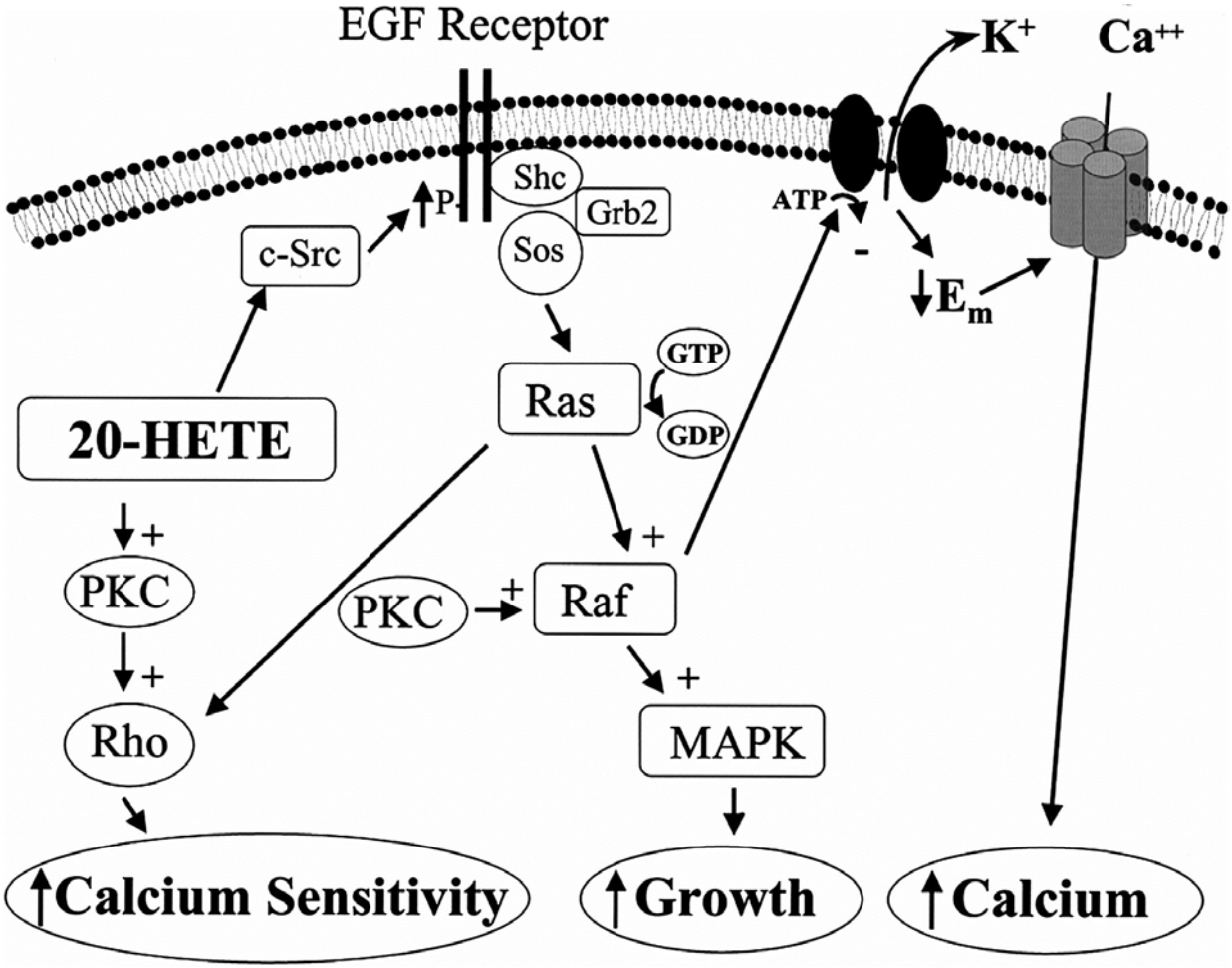
Signaling by oxidised lipids via nuclear receptors.



Sigma Aldrich Catalog

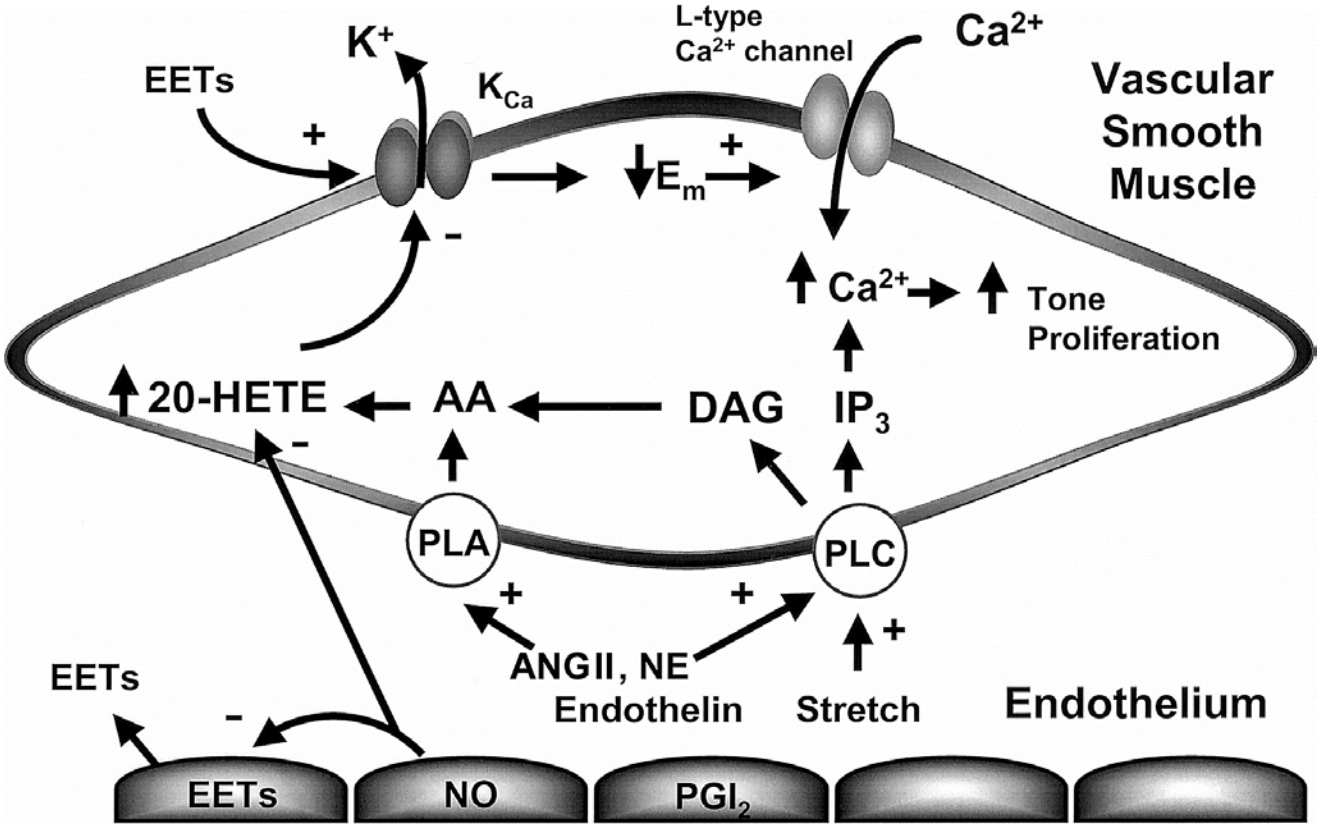
20-HETE, formed by ω-hydroxylation.





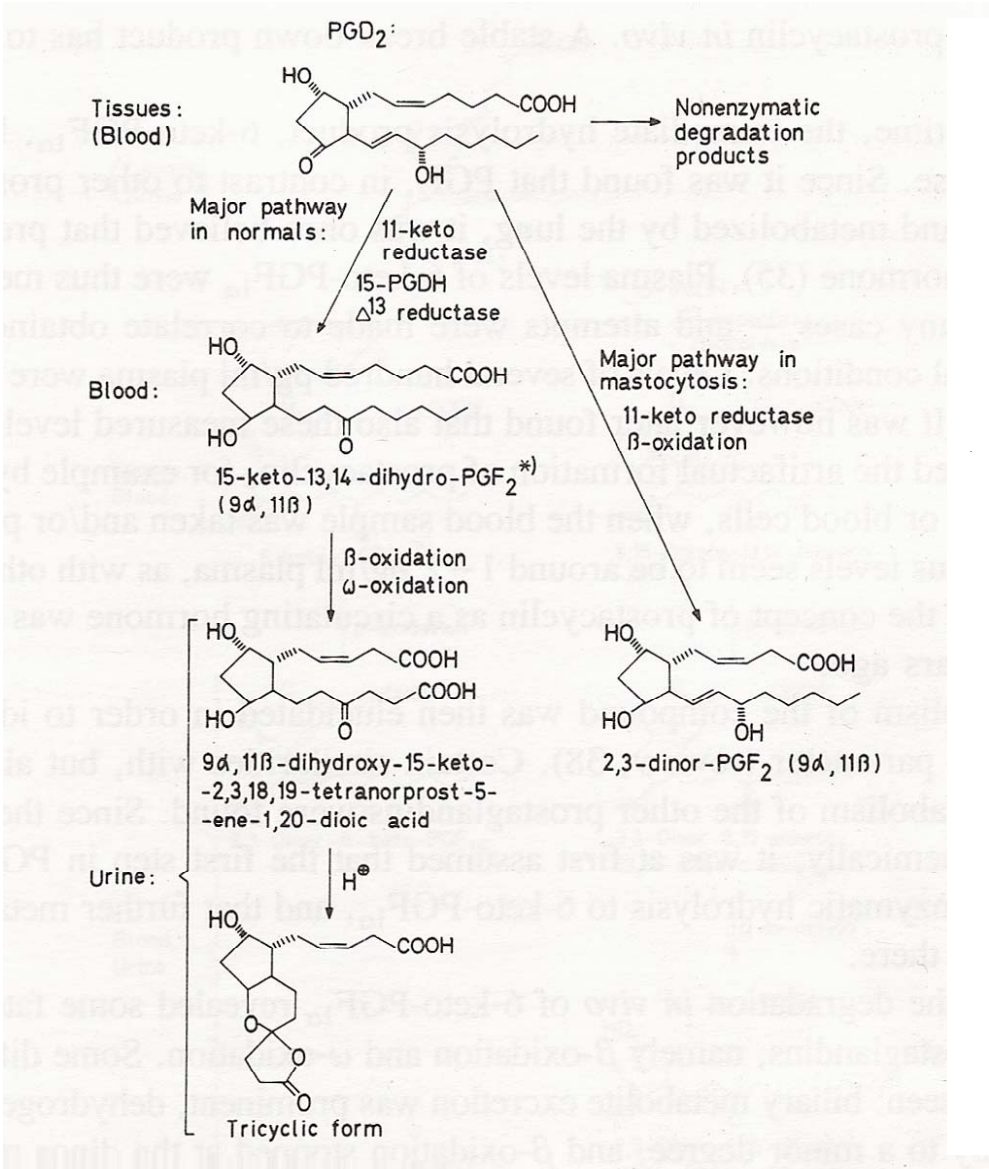
Roman, Physiol. Rev. 82: 131-185, 2002

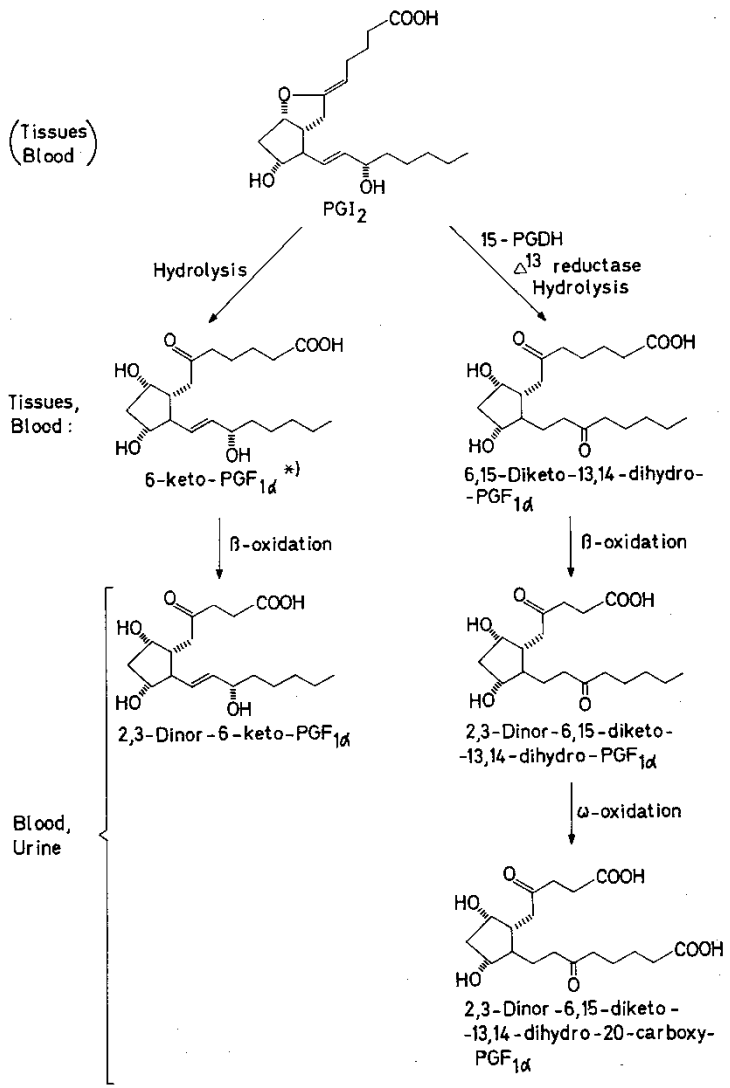
Summary of vascular signaling by 20-HETE and EETs.



Roman, Physiol. Rev. 82: 131-185, 2002
Also: Spector et al, Prog Lipid Res, 2004

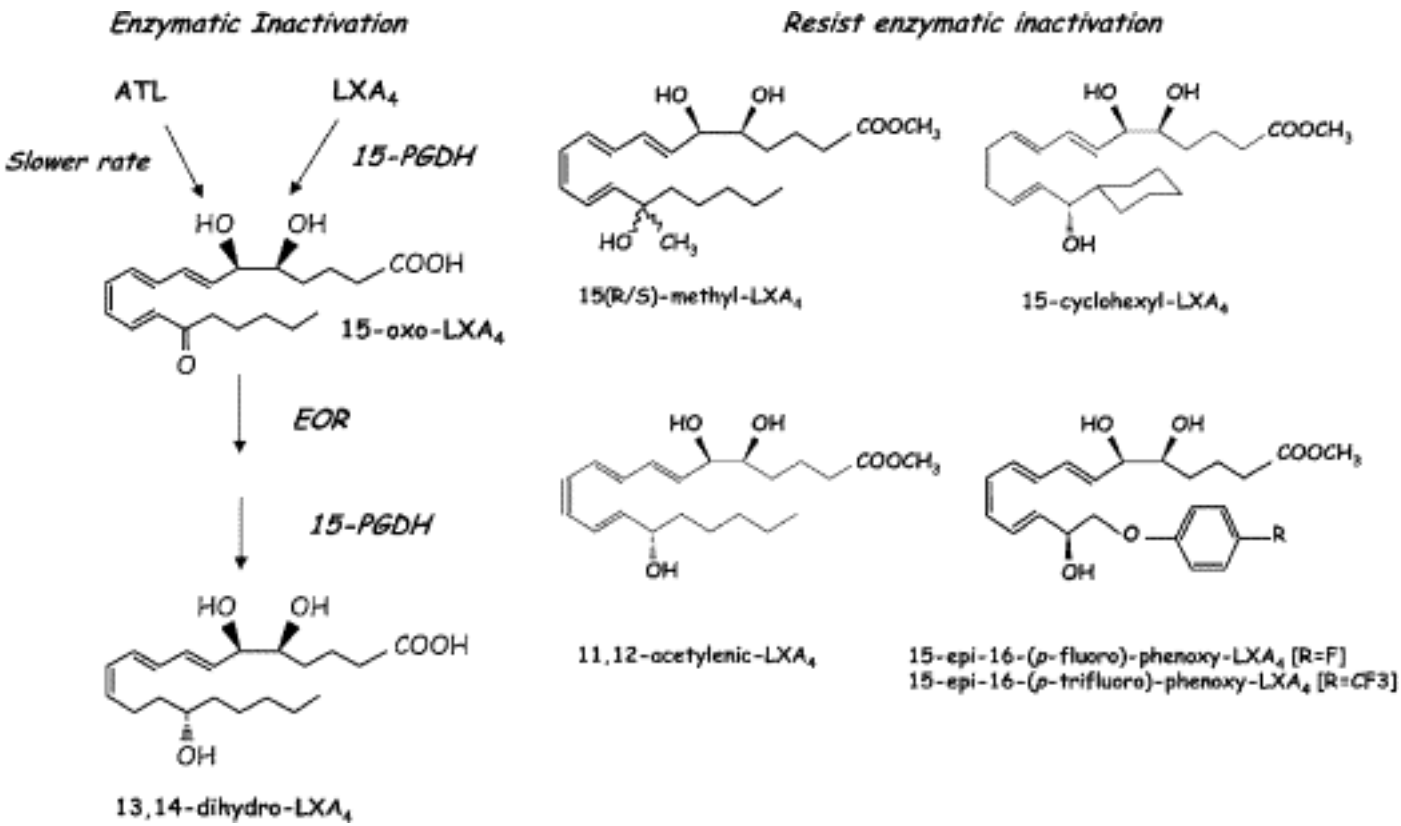
Inactivation of lipid signaling pathways.



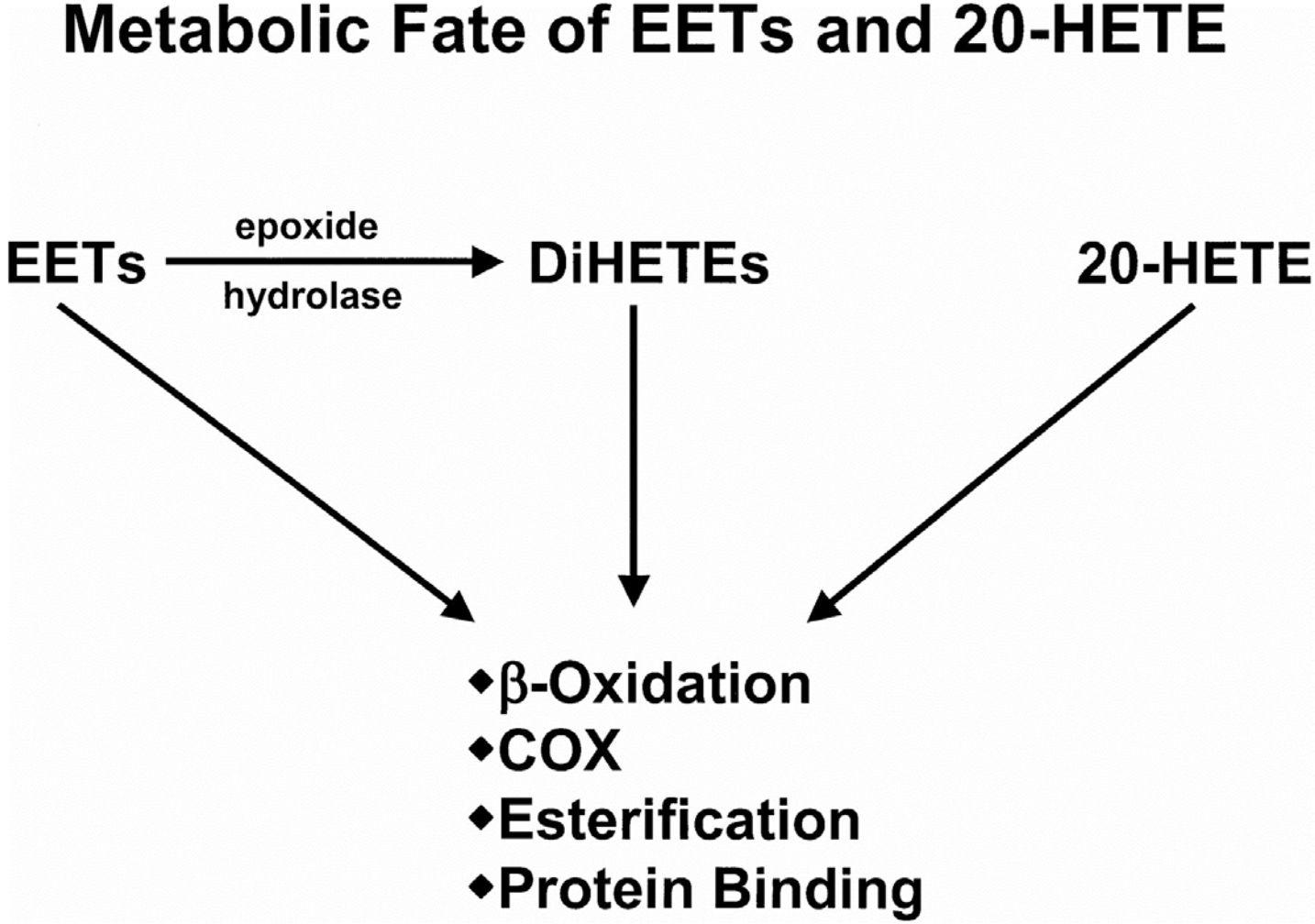


Prostaglandins and related substances, a Practical Approach, IRL Press

Inactivation of lipid signaling pathways.

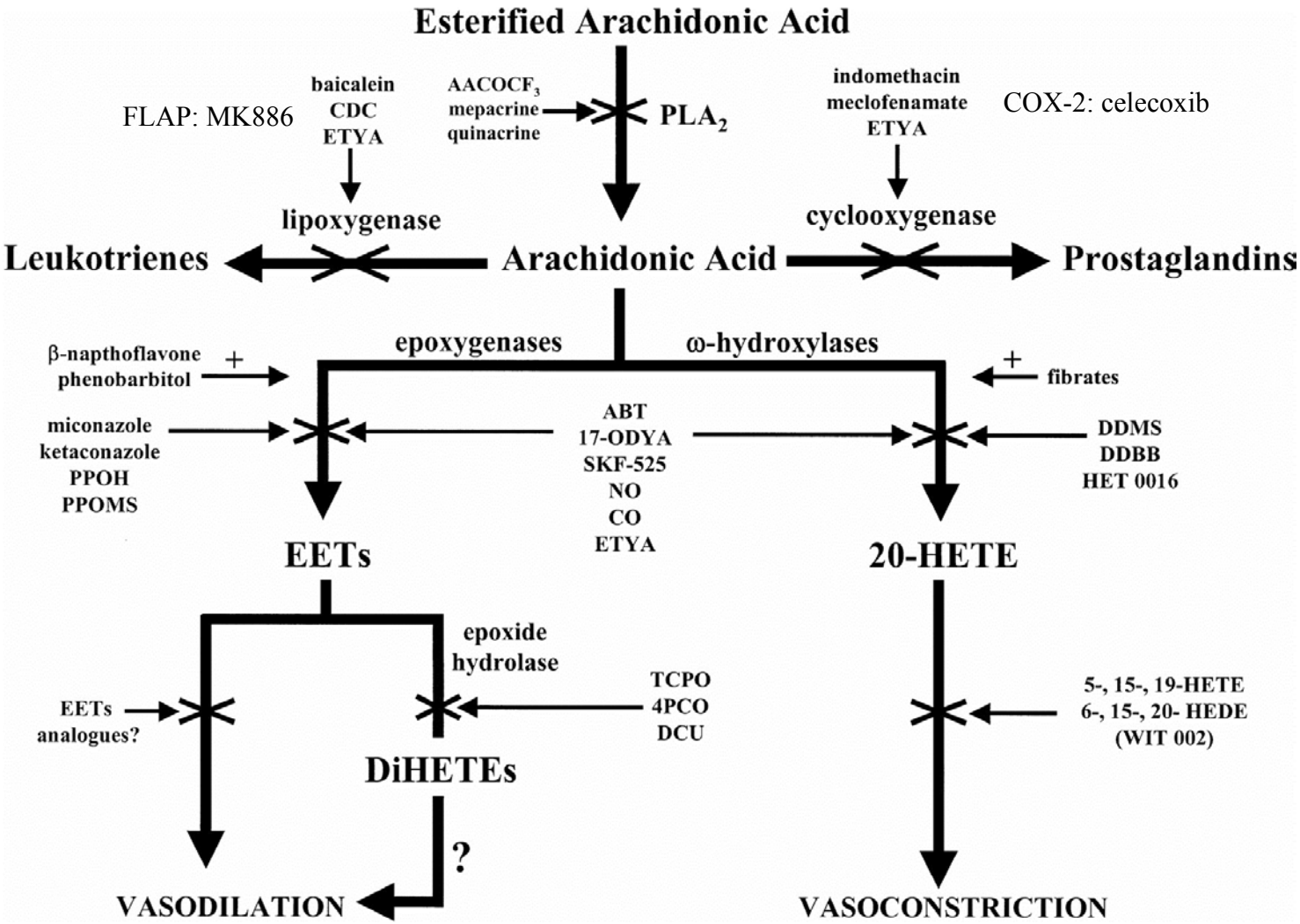


Chiang et al, Prostaglandins Leukot Essent Fatty Acids, 2005



Roman, Physiol. Rev. 82: 131-185, 2002

Pharmacological inhibitors for enzymatic lipid signaling pathways.



Roman, Physiol. Rev. 82: 131-185, 2002