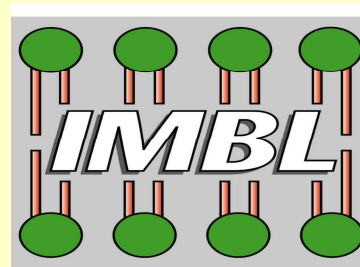


Eicosanoïdes / docosanoïdes et inflammation

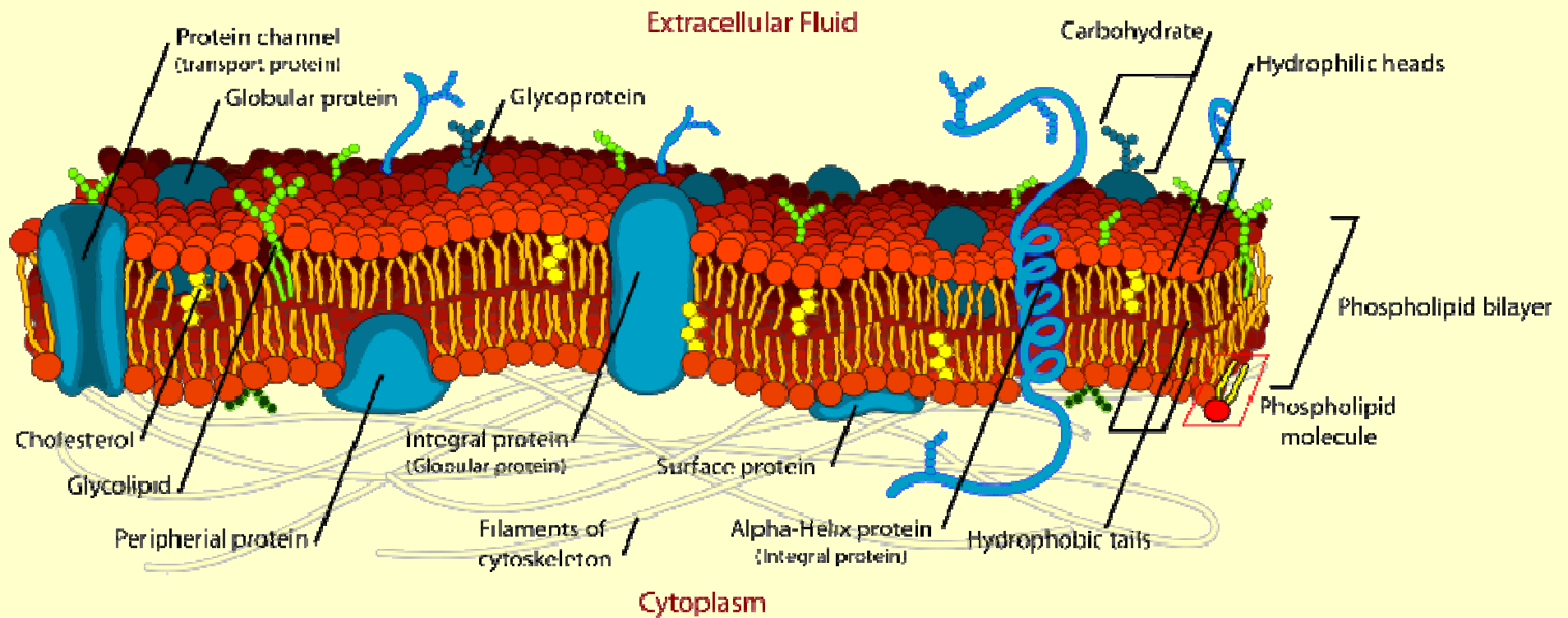
Michel Lagarde

*Université de Lyon,
UMR 870 Inserm/Insa-Lyon,
Inra 1235, Université Claude Bernard, Hospices Civils de Lyon*

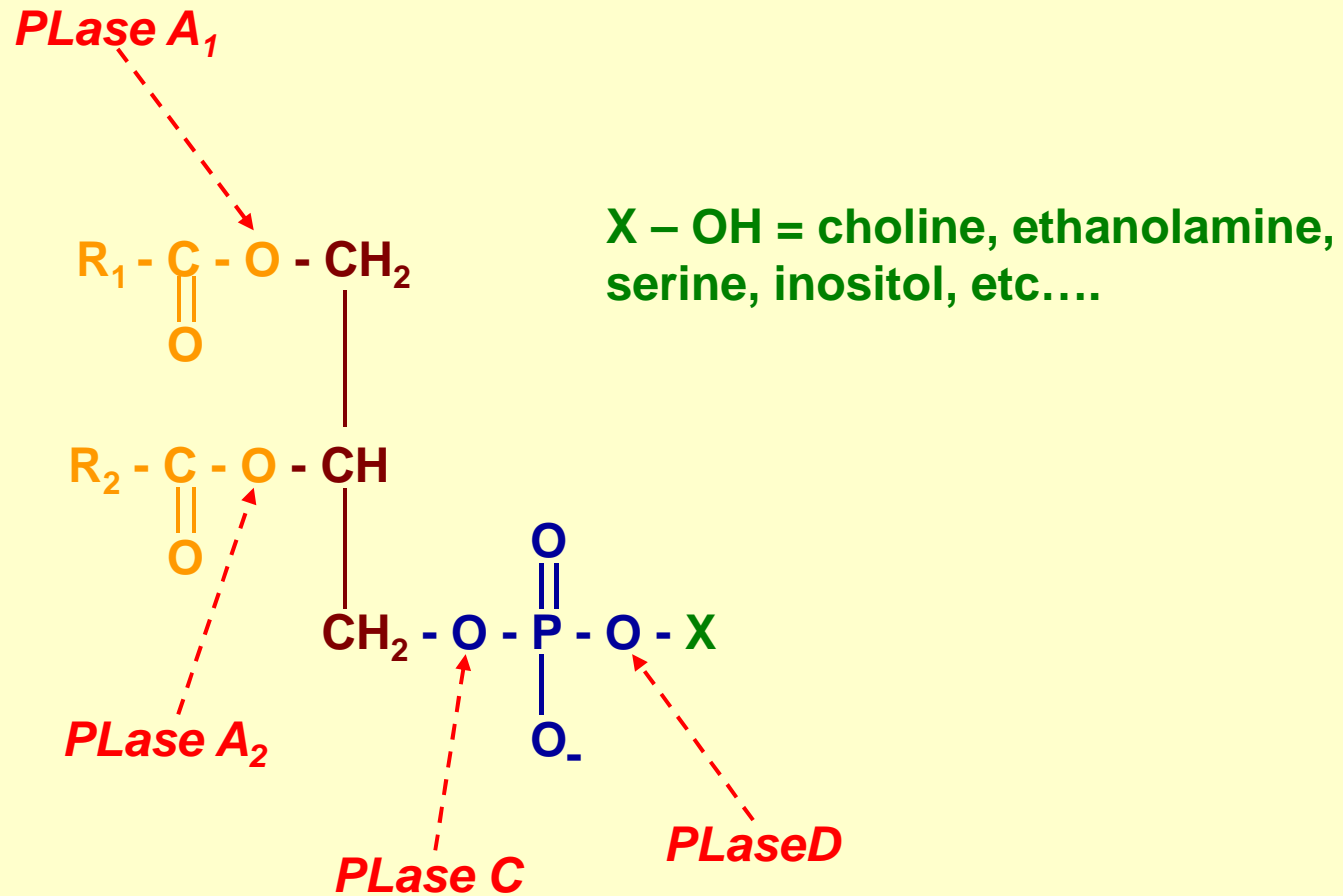
IMBL, Villeurbanne



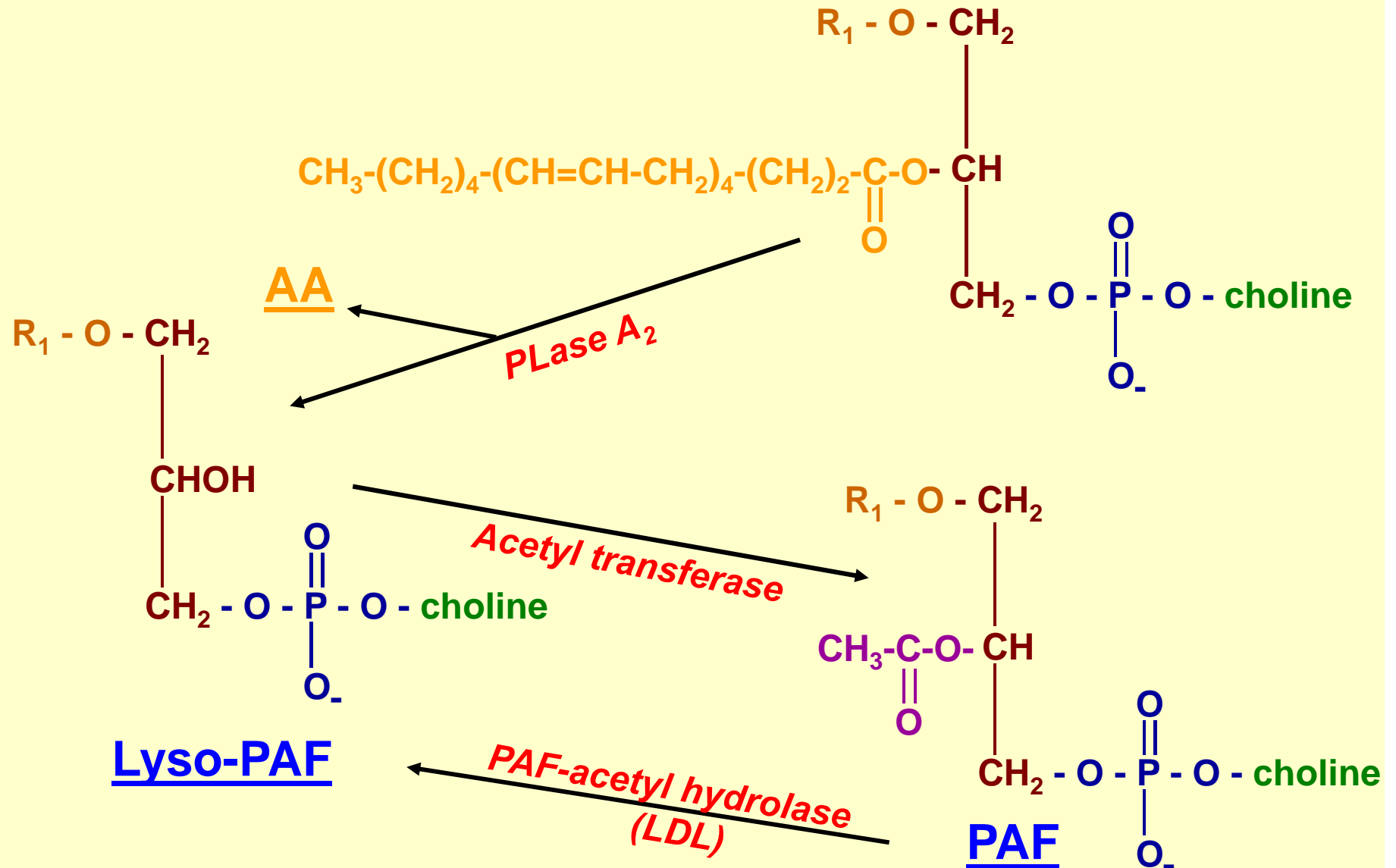
Biological membrane

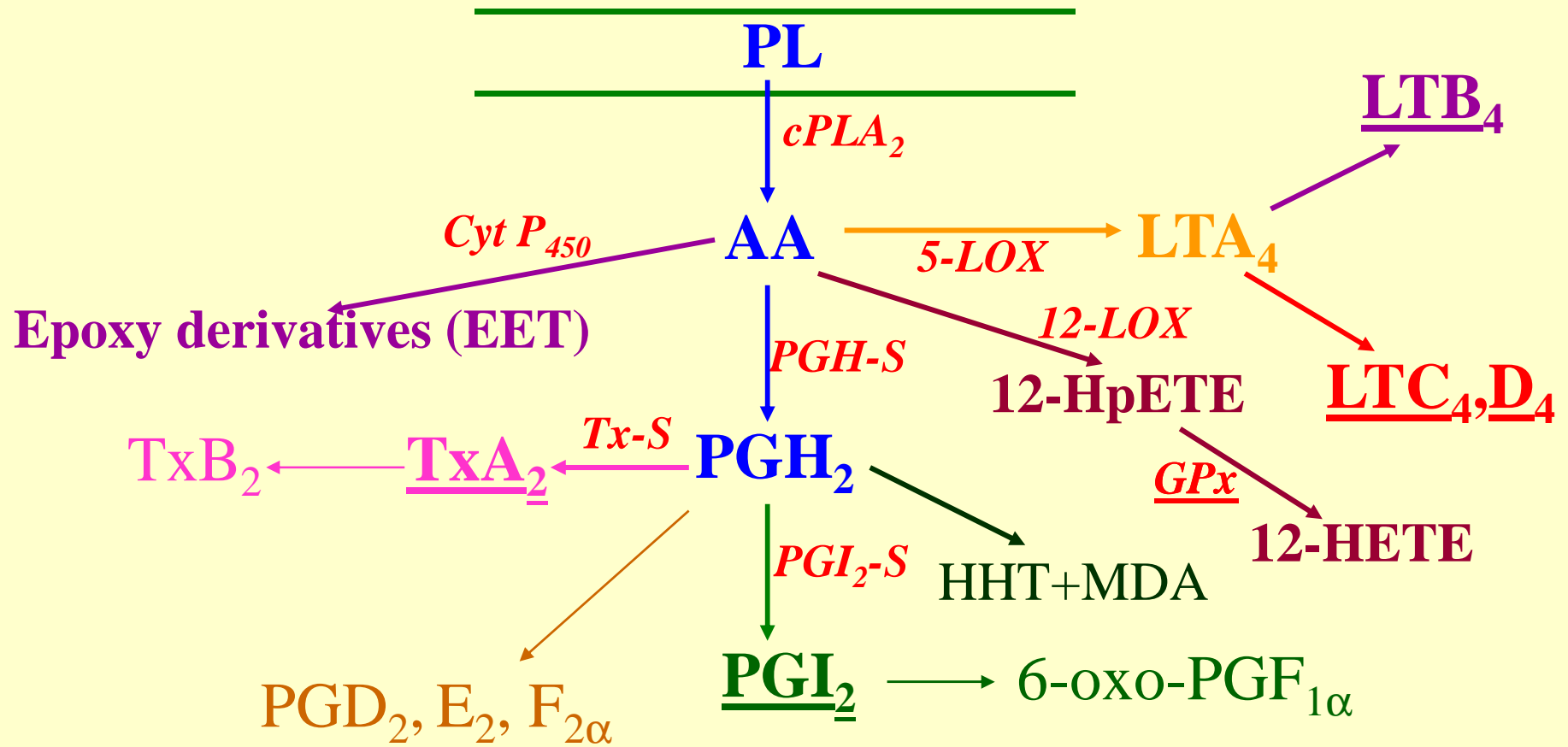


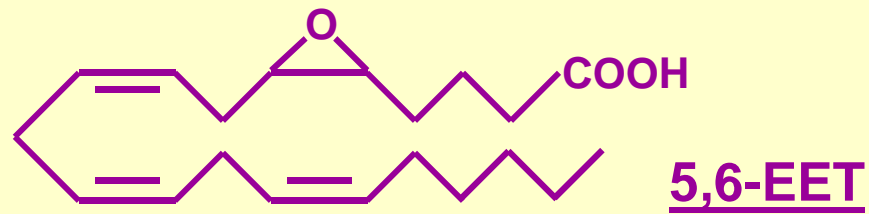
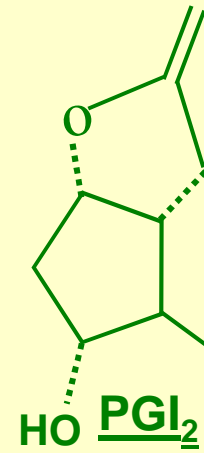
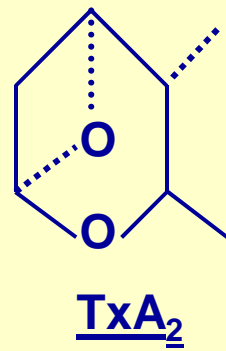
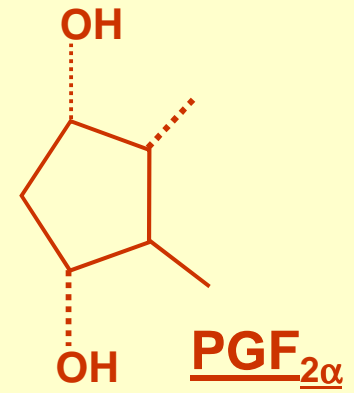
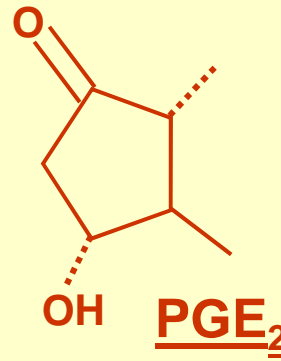
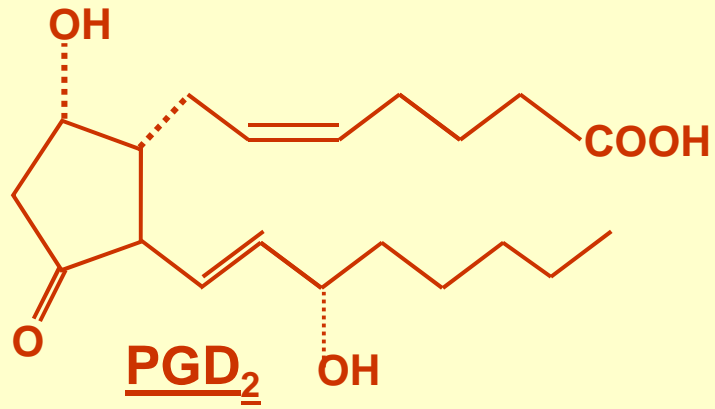
Glycerophospholipids

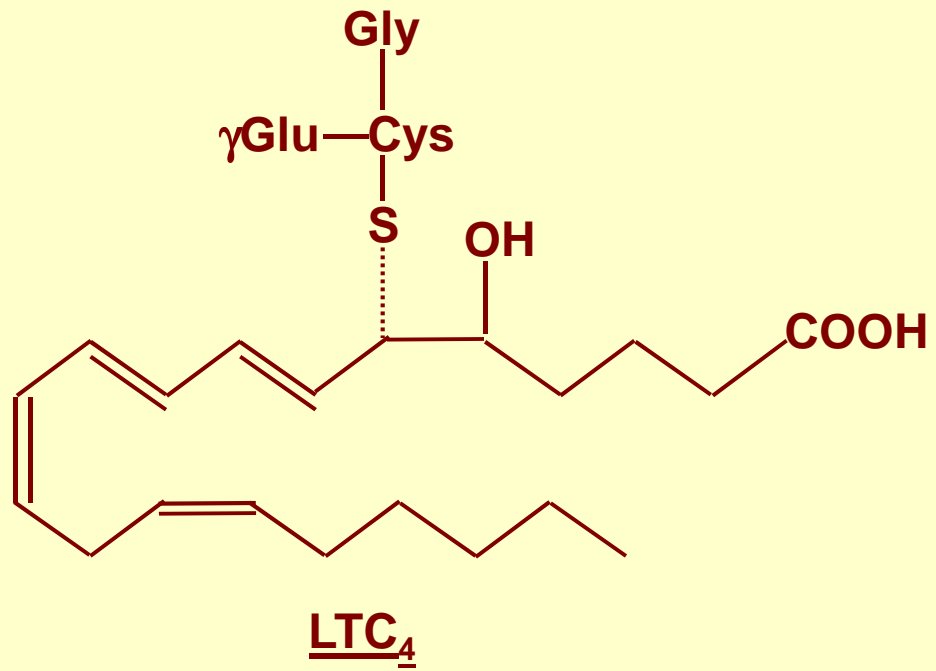
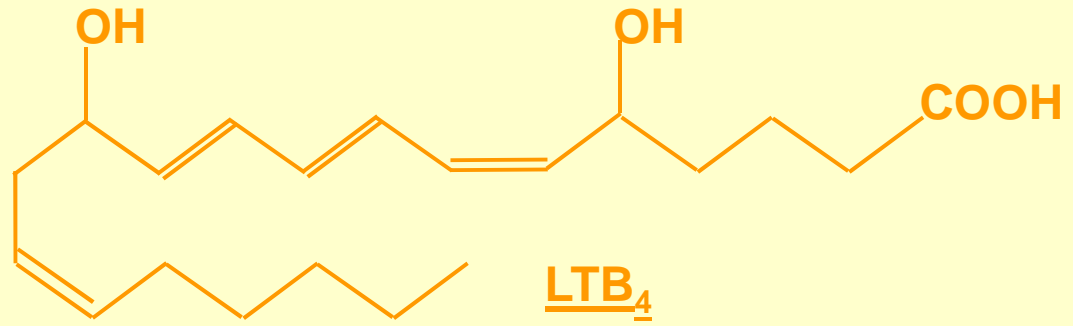


Platelet-Activating Factor









PROSTANOID RECEPTORS

TxA₂ **PGI₂** **PGE₂** **PGF₂α** **PGD₂**
(EP1) (EP2) (EP3) (EP4) (DP1)(CRTH2)

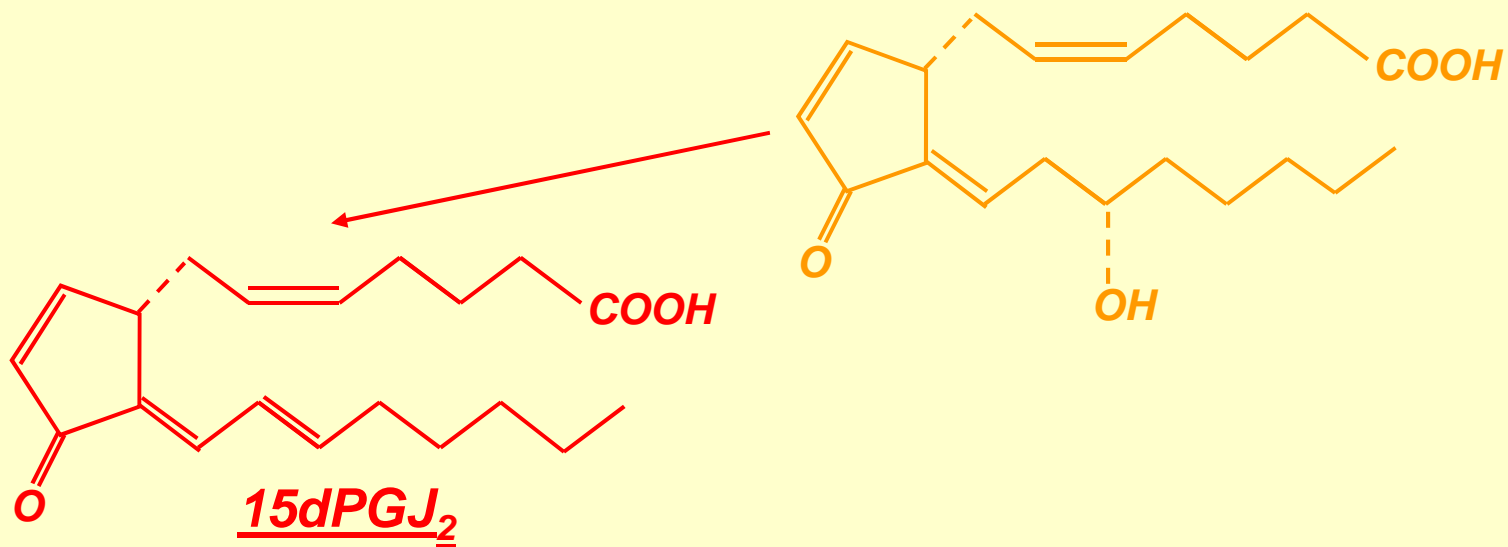
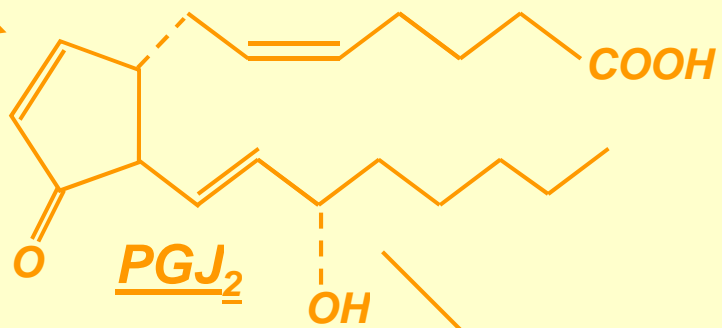
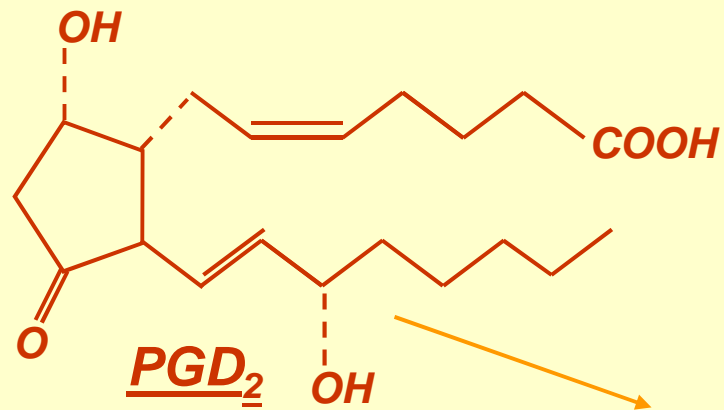
AA residues : 369 386 402 358 390 488 359 366 395

G protein : Gq Gs Gq Gs Gi Gs Gq Gs Gi

LEUKOTRIENE RECEPTORS

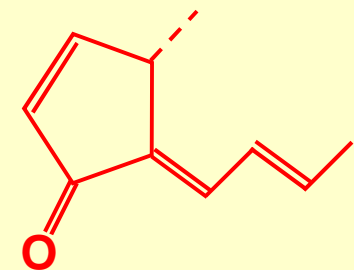
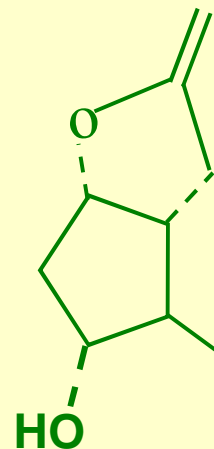
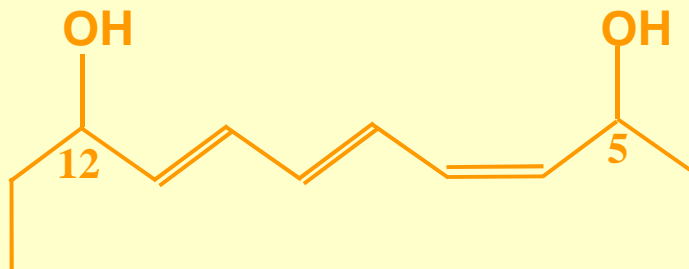
- LTB_4 : two types (BLT_1 & BLT_2)
- LTC_4 and D_4 : two types (CysLT_1 & CysLT_2)

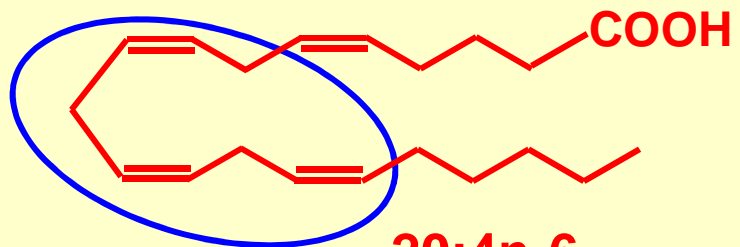
(all receptors coupled with G-proteins,
especially Gq)



Endogenous ligands of PPARs

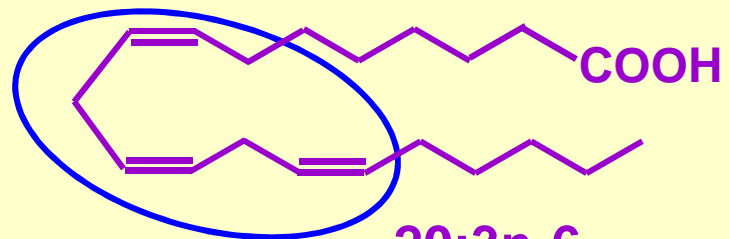
- PPAR α : leukotriene B₄ (LTB₄)
- PPAR β : prostacyclin (PGI₂)
- PPAR γ : 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (15dPGJ₂)





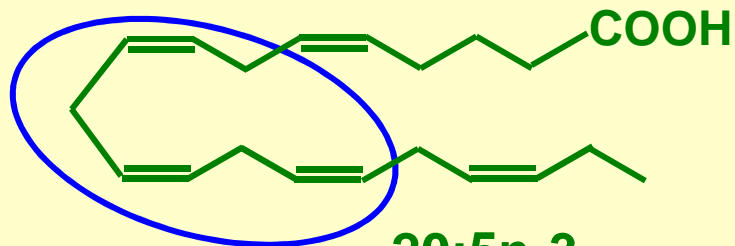
20:4n-6

PG_{2s}



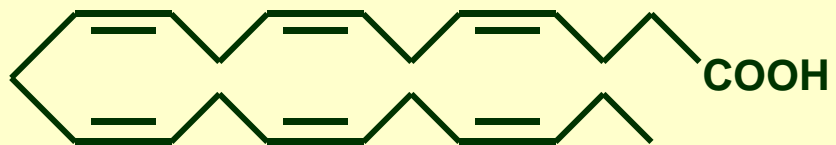
20:3n-6

PG_{1s}

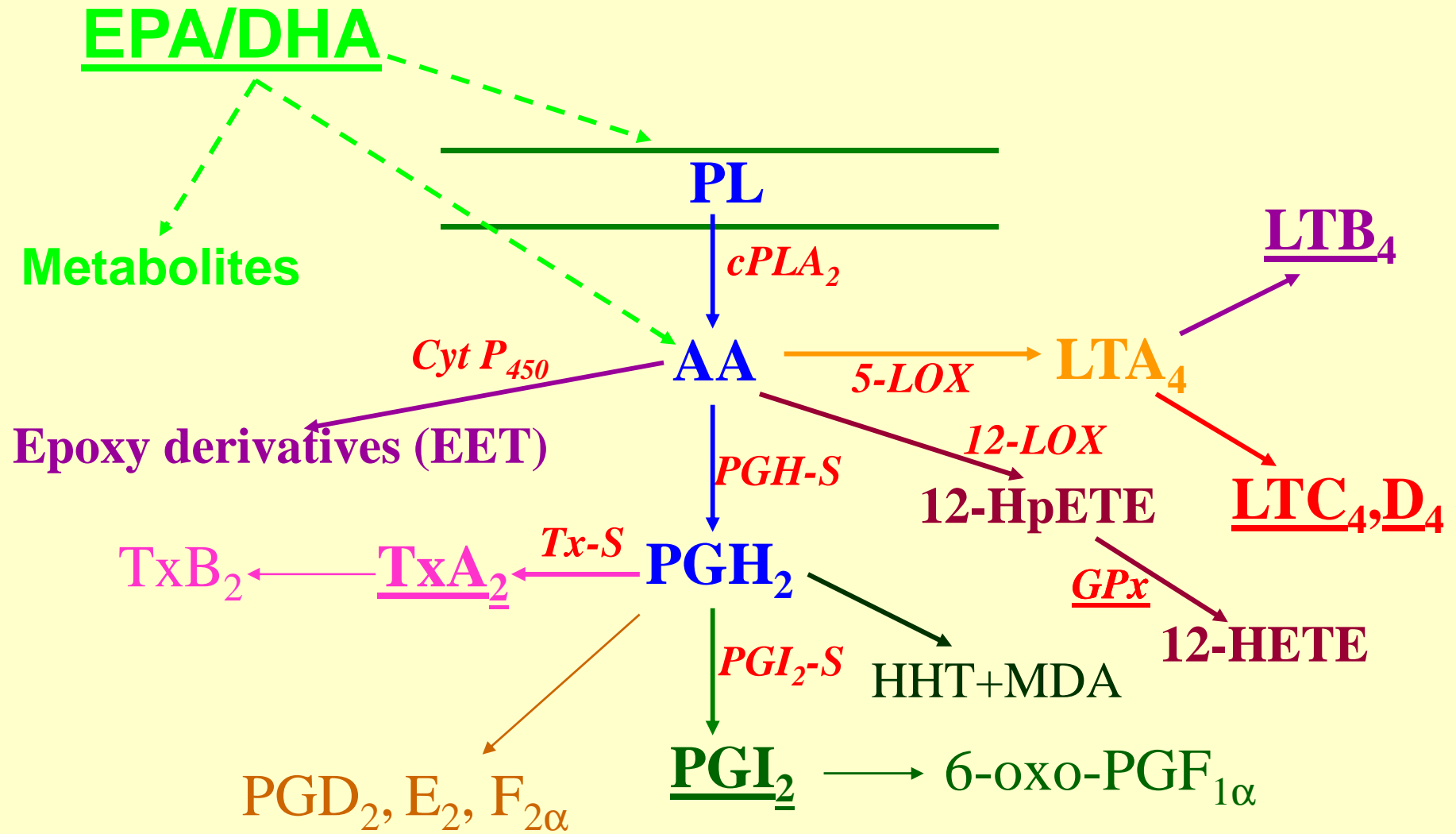


20:5n-3

PG_{3s}



DHA

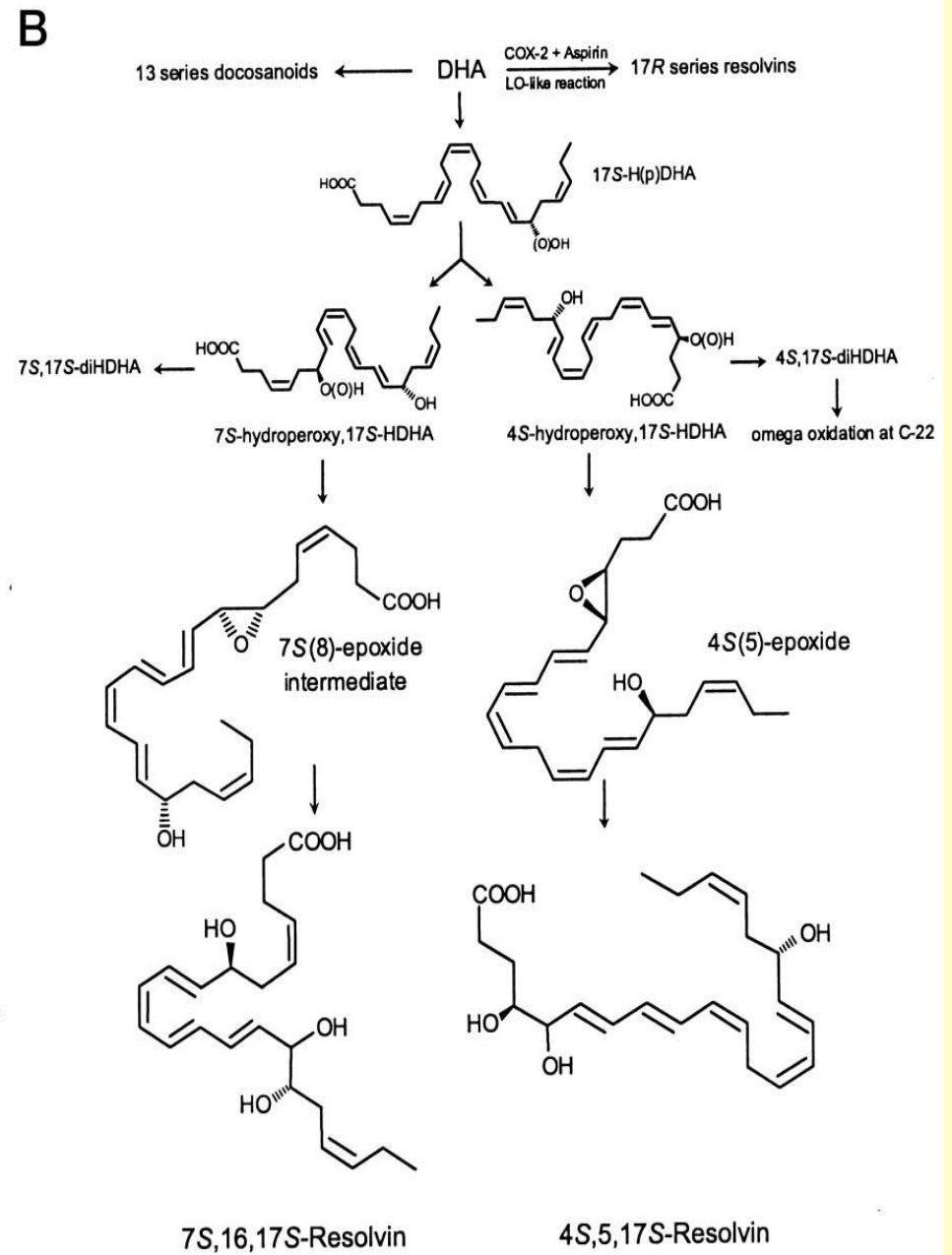
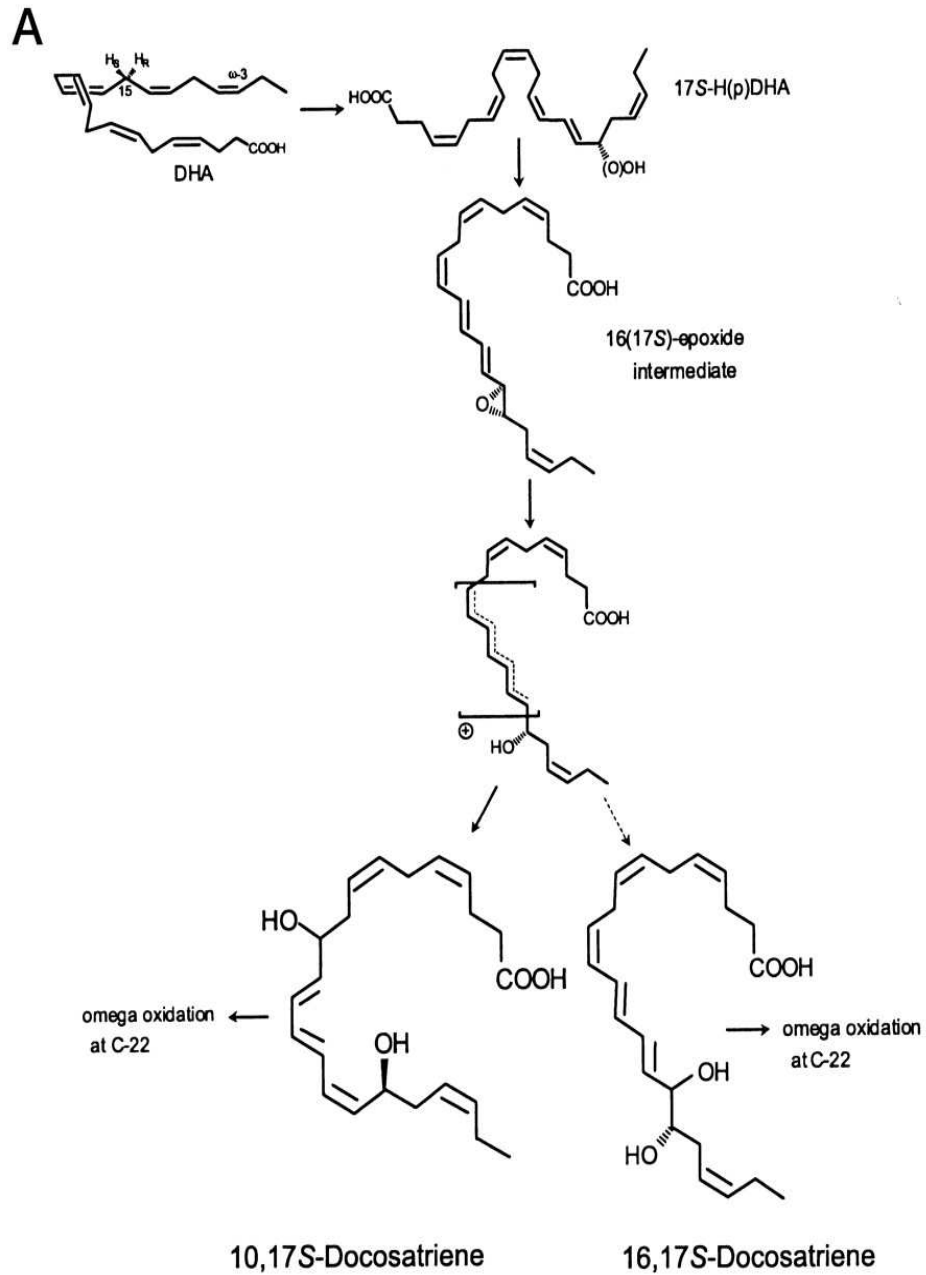


- EPA \longrightarrow PGI₃, TxA₃, PGD₃, LTB₅

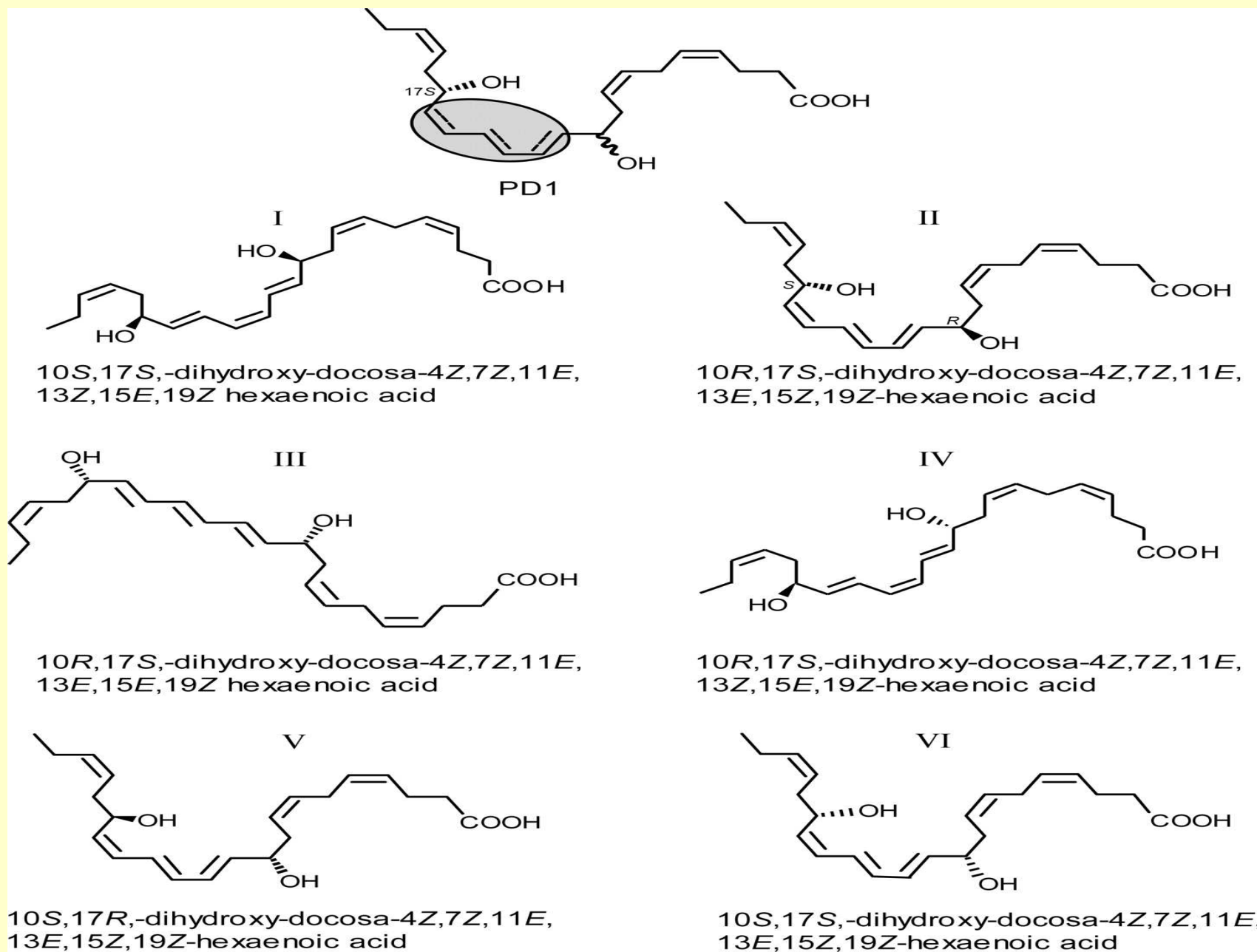
- DHA is a strong inhibitor of cyclooxygenase, and a fairly good substrate of 12-lipoxygenase.

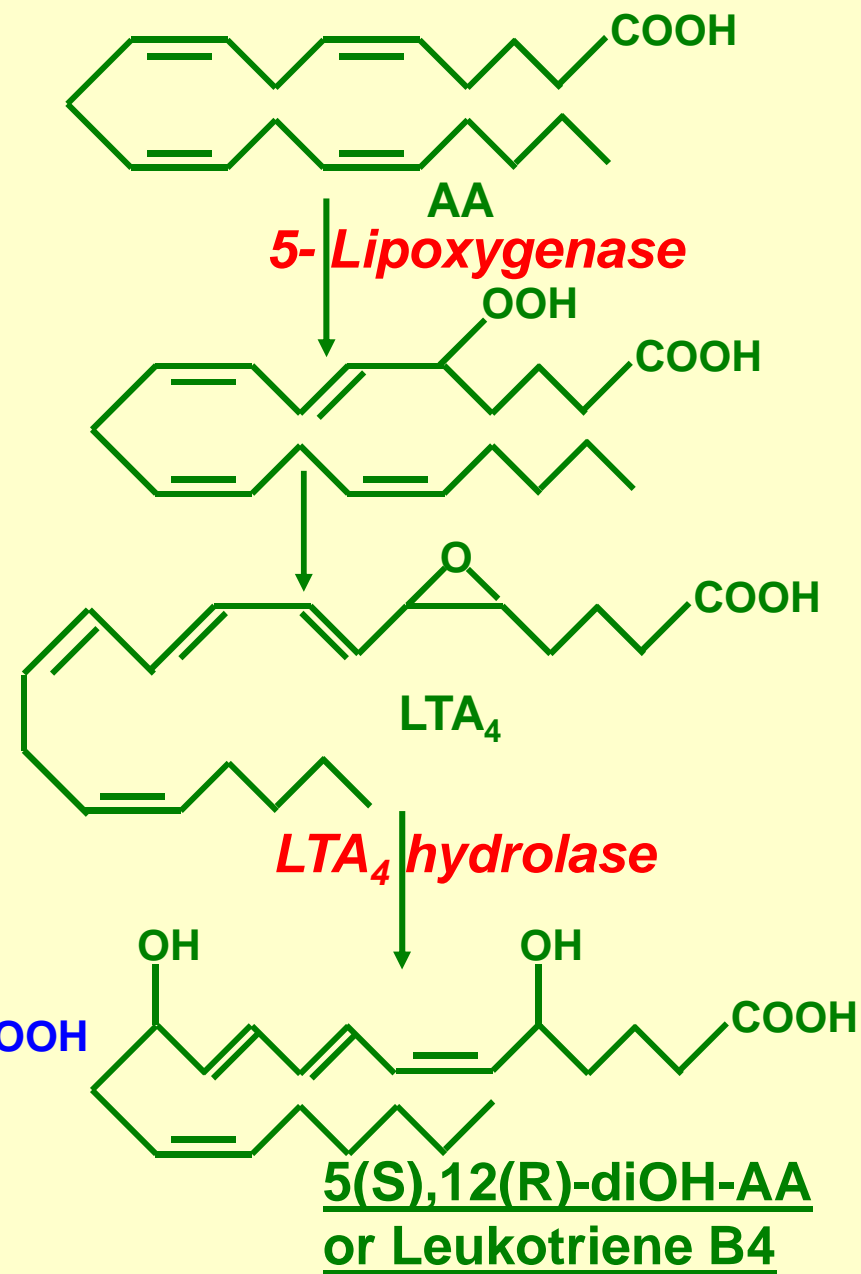
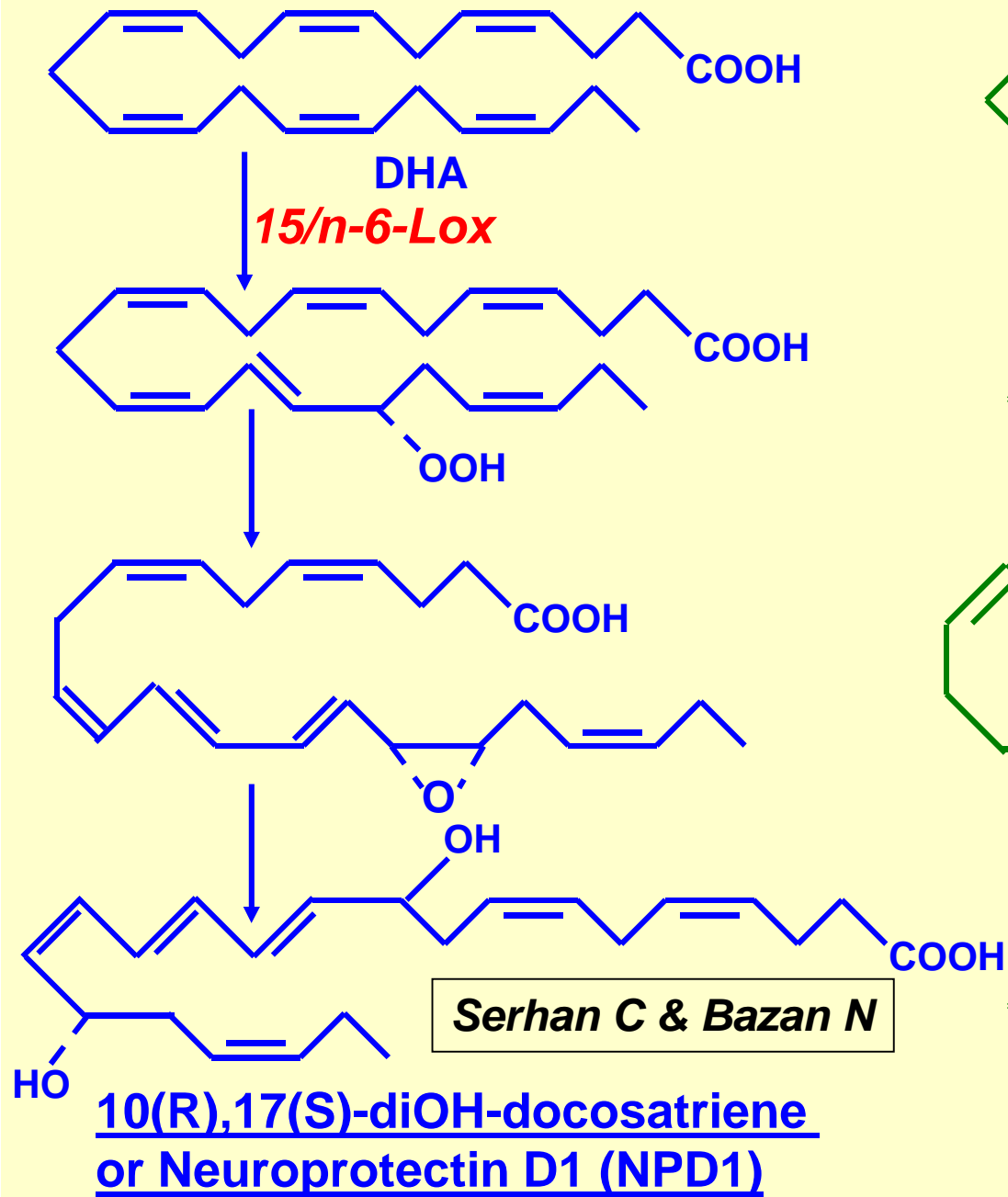
- 14-HO-22:6 has antagonistic effects on TxA₂ receptor site.

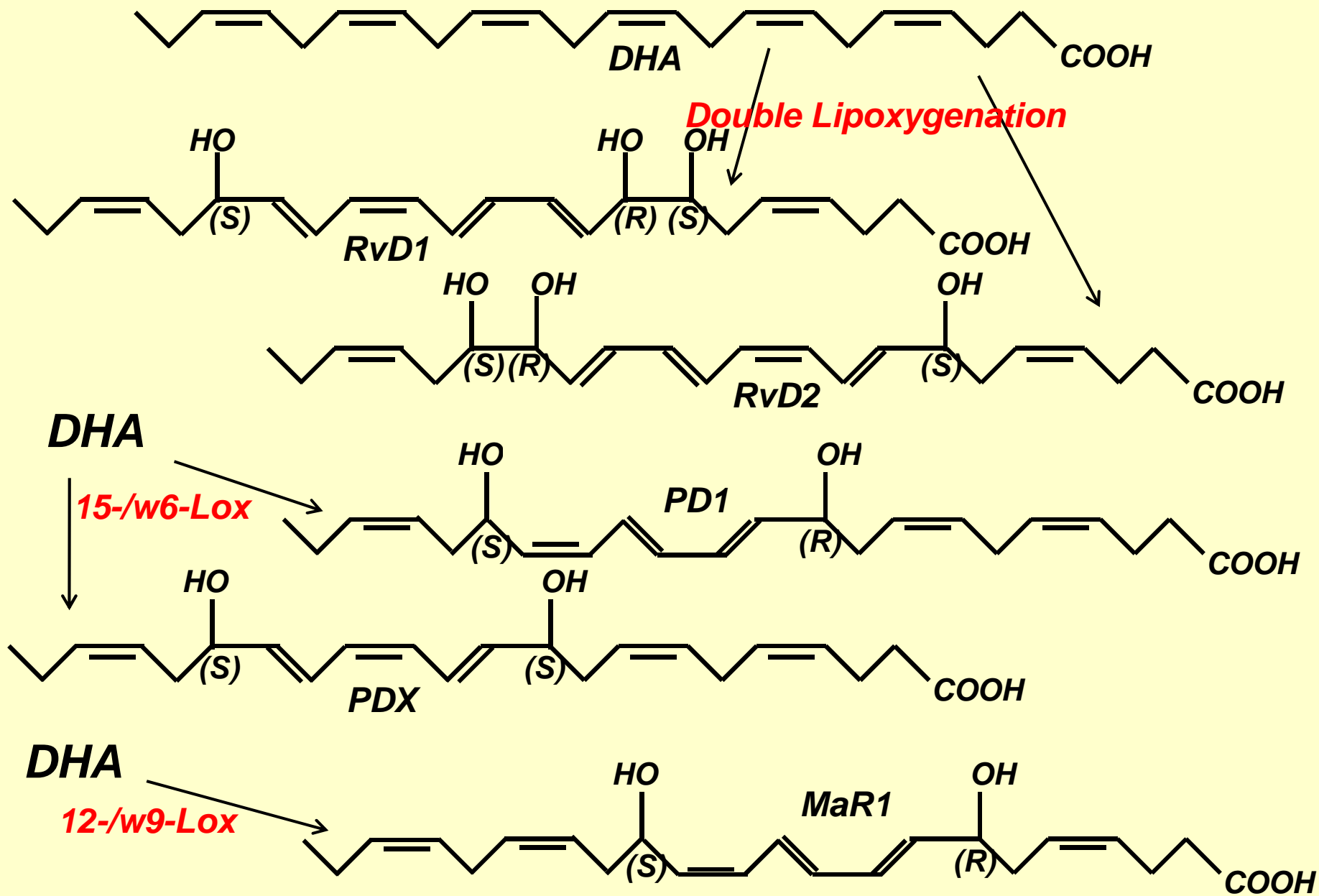
- Production of docosatrienes and docosatetraenes (from DHA), potent inhibitors of inflammation.



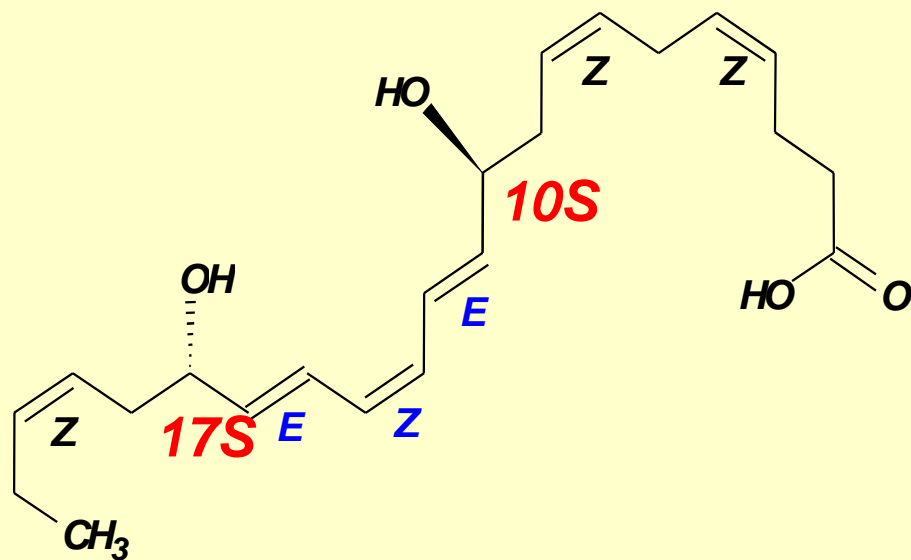
(Hong et al. JBC 2003)







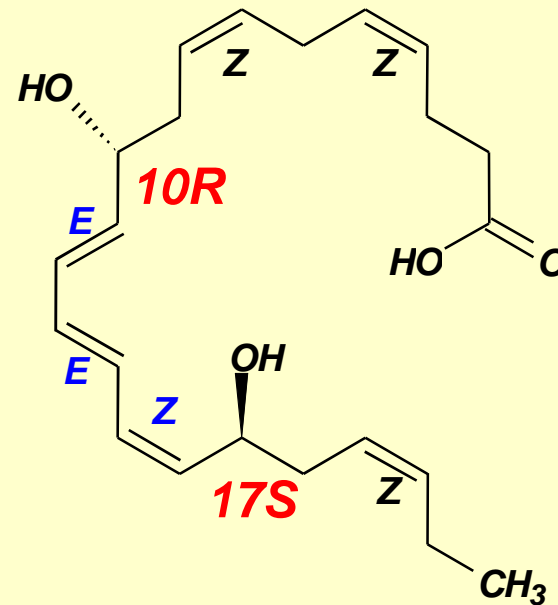
PDX



10(**S**),17(**S**)-dihydroxy-docosa-
4Z,7Z,11E,13Z,15E,19Z-hexaenoic
acid

Chen P. et al. FEBS Lett 2009

(N)PD1 ((Neuro)Protectin D1)

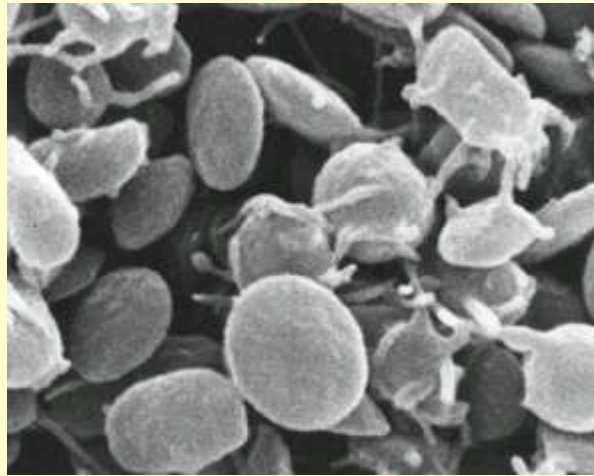


10(**R**),17(**S**)-dihydroxy-docosa-
4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid

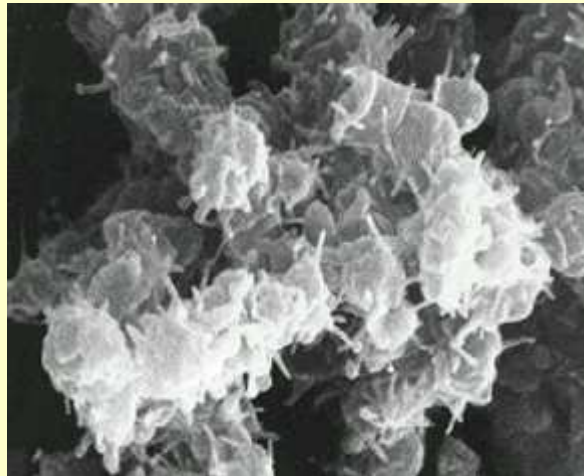
Serhan C.N et al. JBC 2005

Platelet aggregation

Platelet aggregation is part of the sequence leading to the formation of thrombus.



Disk-shaped platelets

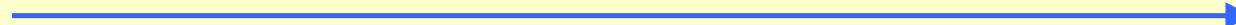


Activated platelets

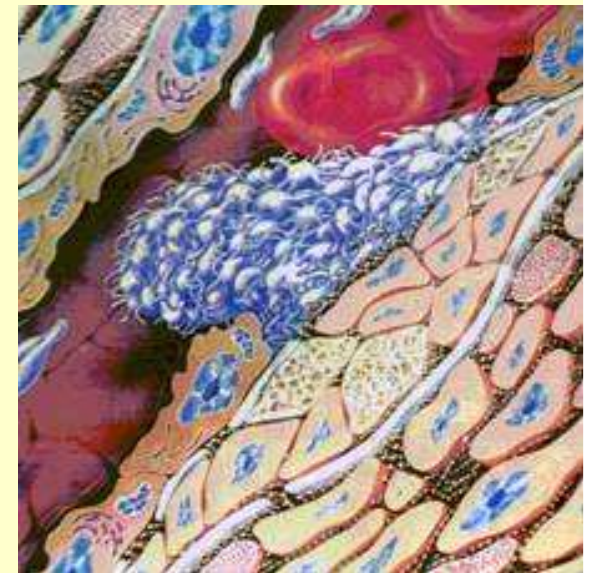
adhesion

activation

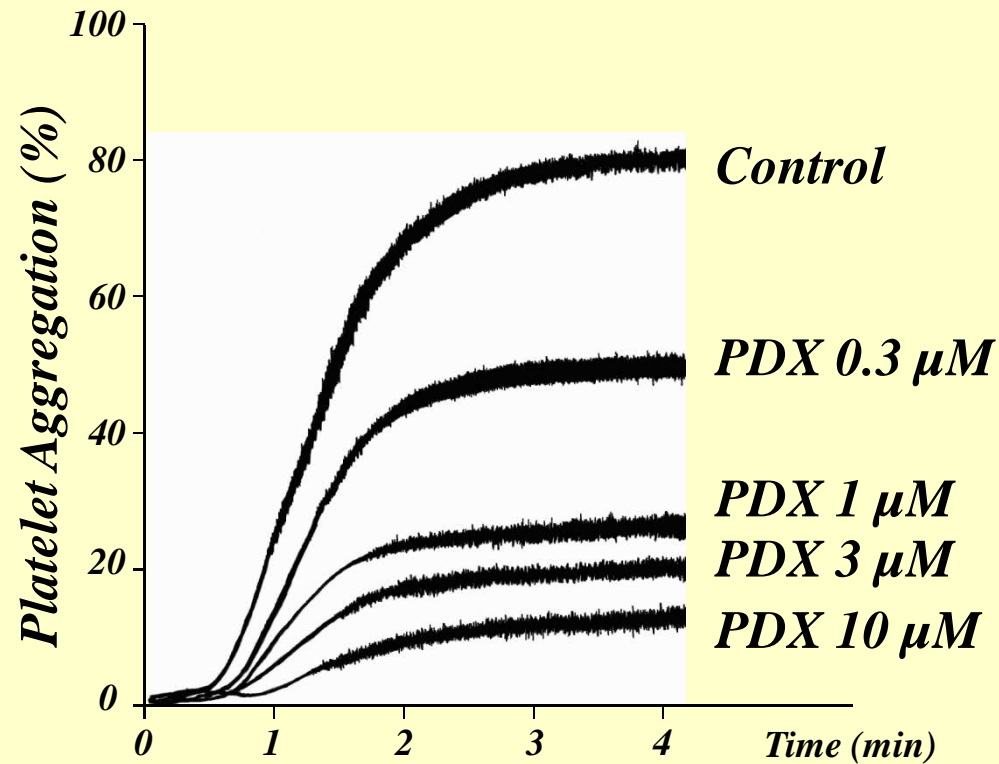
aggregation



Antiplatelet agents inhibit the formation of blood clots by preventing the clumping of platelets.

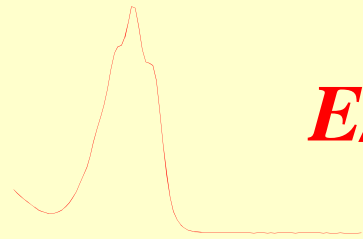


Effect of **PDX** on platelet aggregation triggered by collagen



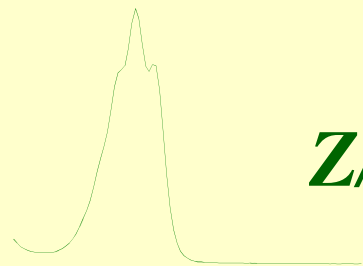
collagen (0.10 ng/μL)

Effect of Compounds with **different conjugated trienes** on platelet aggregation



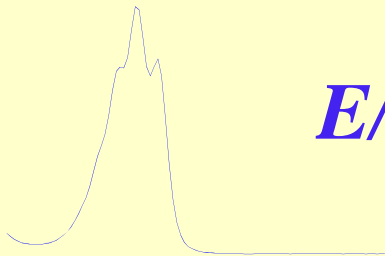
E/Z/E

***PDX; 10(R),17(S)-diOH-DHA;
10(S),17(S)-diOH-22:3;
8(S),15(S)-diHETE; 5(S),12(S)-diHETE;
8(S),15(S)-diOH-20:3; 8(S),15(S)-diOH-20:5.***



Z/E/E

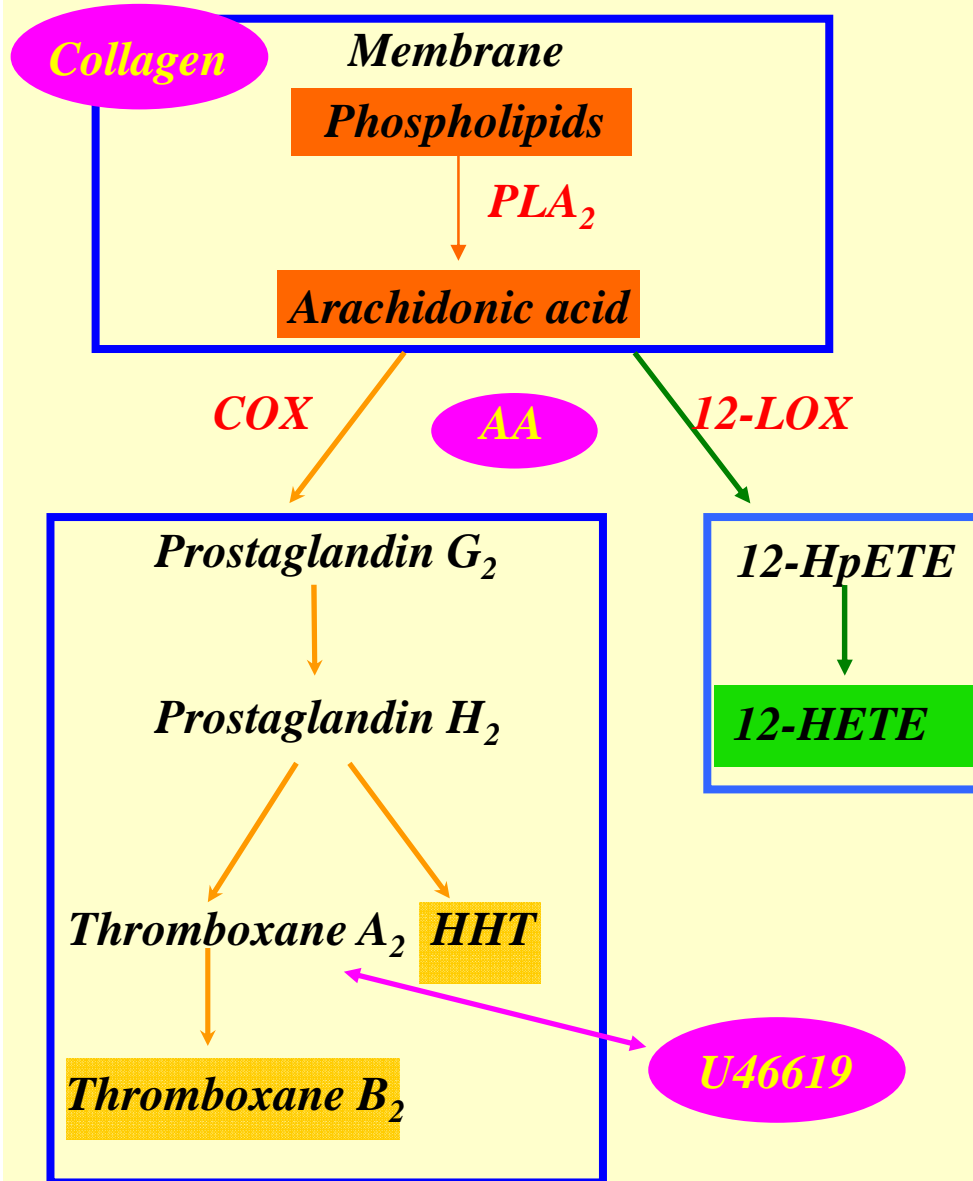
***LTB₄; 12-epi LTB₄;
LTB₃; 12-epi LTB₃.***



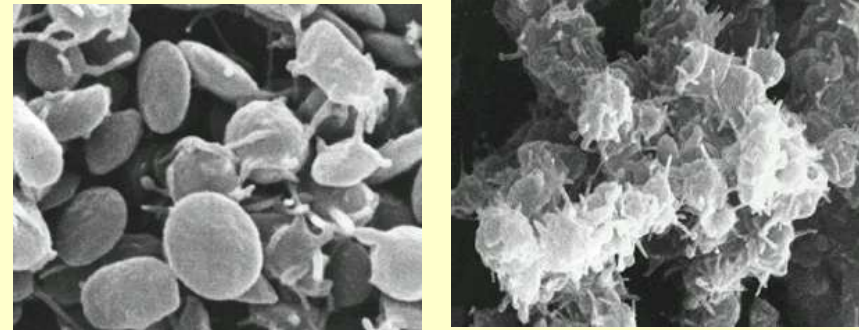
E/E/E

***6-trans LTB₄;
6-trans-12-epi LTB₄.***

Arachidonic acid cascade

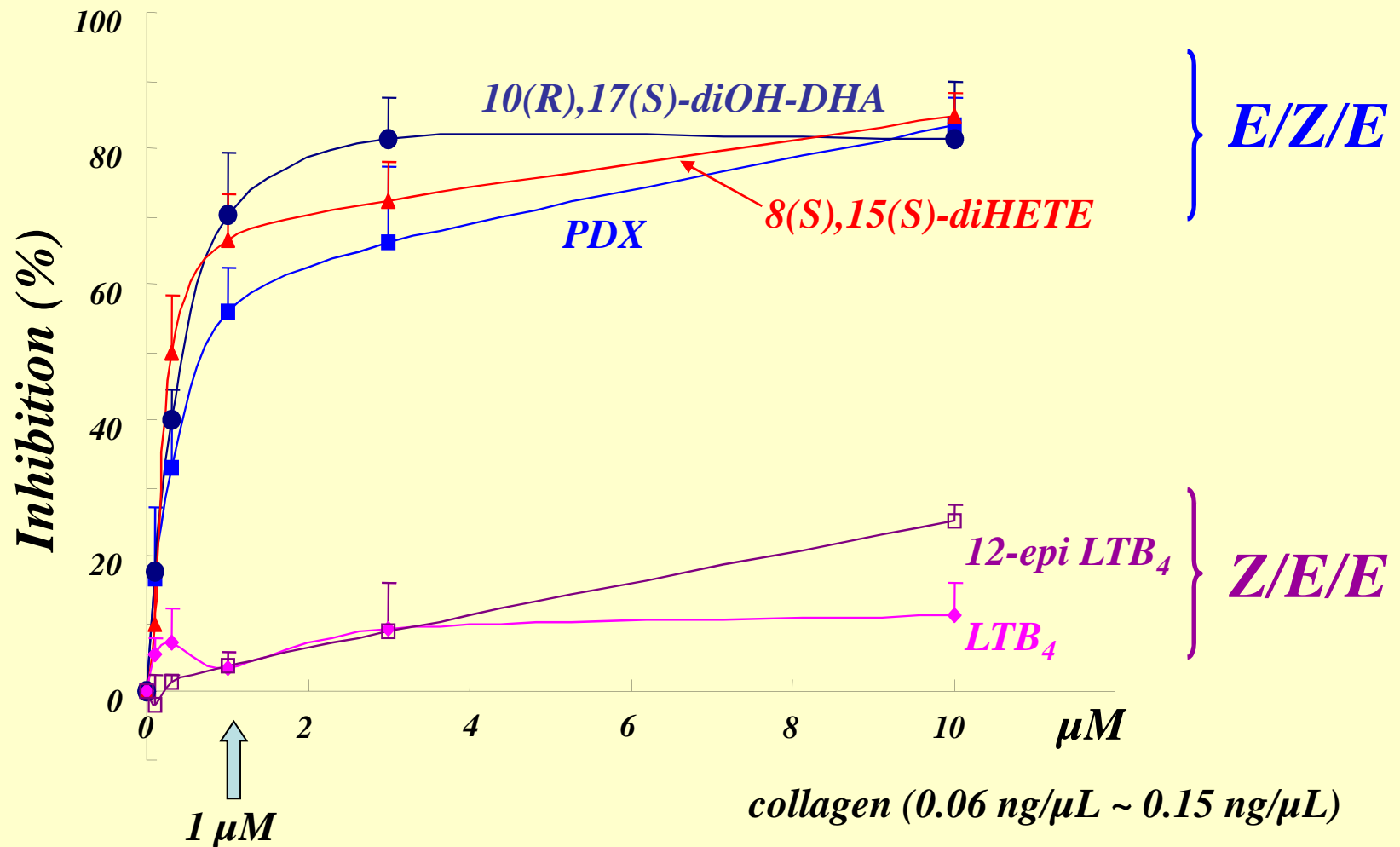


Measurement of platelet aggregation



- ◆ Aggregations were triggered by **Collagen**, **AA**, and **U46619** (thromboxane A₂ receptor agonist)
- ◆ Aggregation curves were recorded for 4 min

Effects of different conjugated trienes on platelet aggregation induced by collagen



Platelet inhibition is only related to the presence of the **E/Z/E** conjugated triene

"poxytrins"

Inhibition (%) of collagen-induced platelet aggregation	<i>E/Z/E</i>							
	PDX	10(R),17(S)-diOH-22:6	10(S),17(S)-diOH-22:3	7(S),14(S)-diOH-22:6	8(S),15(S)-diHETE	5(S),12(S)-diHETE	8(S),15(S)-diOH-20:3	8(S),15(S)-diOH-20:5
1 μ M	56.1\pm6.2**	70.1\pm9.4**	63.8\pm4.6**	48.2\pm7.7**	66.5\pm6.8**	74.9\pm10.7**	70.8\pm5.1**	76.3\pm5.1**

Inhibition (%) of collagen-induced platelet aggregation	<i>Z/E/E</i>				<i>E/E/E</i>	
	LTB₄	12-<i>épi</i> LTB₄	LTB₃	12-<i>épi</i> LTB₃	6-trans LTB₄	6trans-12-<i>épi</i> LTB₄
1 μ M	3.5 \pm 2.5	3.9 \pm 2.1	4.4 \pm 5.4	0.6 \pm 3.7	5.7 \pm 6.4	1.8 \pm 2.8

Mean \pm S.E.M. $n \geq 4$. * $P < 0.05$, ** $P < 0.01$

collagen (0.06 ng/ μ L ~ 0.20 ng/ μ L)

Effects of different conjugated trienes on platelet aggregation triggered by arachidonic acid (AA)

"poxytrins"

Inhibition (%) of AA- induced aggregation	<i>E/Z/E</i>			<i>Z/E/E</i>	
	PDX	10(R),17(S)- diOH-22:6	8(S),15(S)- diHETE	LTB₄	12-<i>epi</i> LTB₄
0.3 μM	25.7 ± 9.2 *	36.4 ± 13.4 *	56.4 ± 9.9 **	5.1 ± 4.6	-2.5 ± 1.6
1 μM	78.9 ± 5.6 **	72.5 ± 9.7 **	61.0 ± 10.5 **	2.1 ± 3.2	-9.8 ± 2.5

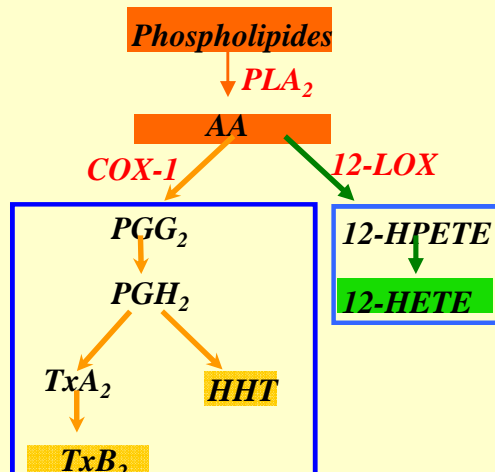
Mean ± S.E.M. n ≥ 4. * P < 0.05, ** P < 0.01

AA (1 μM ~ 2 μM)

Effects of different conjugated trienes (1 μM) on arachidonic acid metabolites

"poxytrins"

% of control	<i>E/Z/E</i>			<i>Z/E/E</i>
	PDX	10(R),17(S)-diOH-DHA	8(S),15(S)-diHETE	LTB ₄
TxB₂	44.7 \pm 7.3 **	51.9 \pm 5.7 *	54.1 \pm 5.5 **	107.4 \pm 9.1
HHT	43.1 \pm 7.7 **	54.8 \pm 4.7 *	63.4 \pm 9.9 **	98.7 \pm 18.5
12-HETE	82.0 \pm 6.4	92.7 \pm 14.4	114.0 \pm 1.9	105.4 \pm 2.9



Mean \pm S.E.M. n \geq 4. *P<0.05, **P<0.01

CONTROL

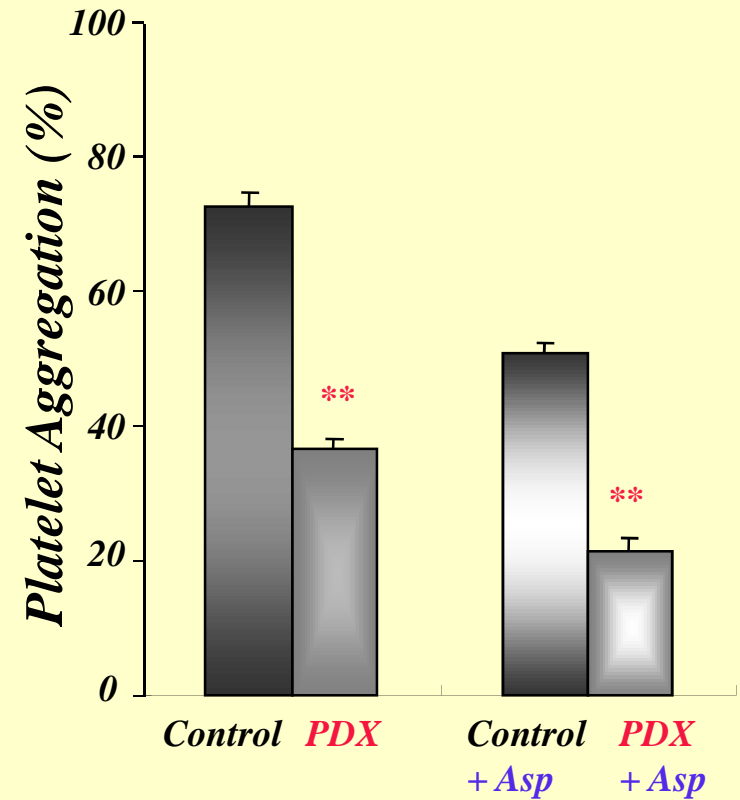
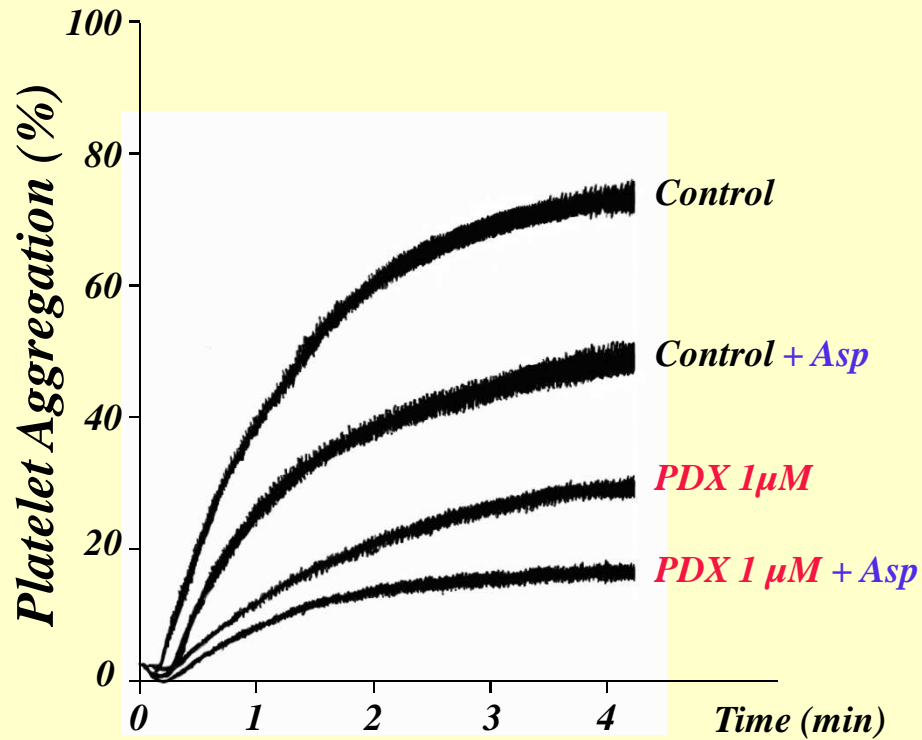
TxB₂: 1.8 \pm 0.2 nmol/10⁹ pl.

HHT: 0.9 \pm 0.2 nmol/10⁹ pl.

12-HETE: 0.8 \pm 0.1 nmol/10⁹ pl.

[1-¹⁴C]AA (1.3 μM ~1.5 μM)

Effect of **PDX** on platelet aggregation triggered by U46619



Mean \pm S.E.M. $n \geq 4$, ** $P < 0.01$.

Aspirin (2×10^{-4} M); U46619 (0.05 ng/ μ L)

SUMMARY

- *PDX inhibits platelet aggregation at submicromolar concentrations.*
- *PDX inhibits the cyclooxygenase pathway (COX-1) but not lipoxygenase pathway.*
- *Moreover, PDX inhibits platelet aggregation at the level of thromboxane A₂ receptor.*
- *“E/Z/E” conjugated triene geometry is required for the inhibition. In contrast, compounds with “Z/E/E” or “E/E/E” conjugated trienes have no inhibitory effect.*
- *The position of the conjugated triene on the chain length seems not important for the inhibition. (8S,15S-diHETE vs 5S,12S-diHETE; 10S,17S-diOH-22:6 vs 7S,14S-diOH-22:6)*
- *The presence of other double bonds does not alter the inhibitory properties. (diOH-22:6 vs diOH-22:3; diOH-20:3 vs diOH-22:4, diOH-20:5)*
- *The stereochemistry of alcoholic carbons does not change the inhibition. (R vs S)*

Acknowledgements

Bernoud-Hubac Nathalie
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Guichardant Michel
Lefils Jennifer
Véricel Evelyne

Inserm
Ministry of Research
ANR

