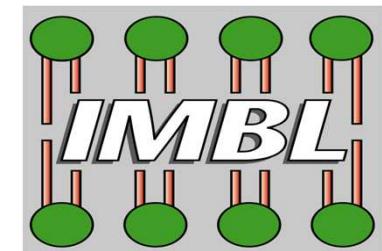




# Principes, outils et perspectives en lipidomique

*Michel Lagarde, Patricia Daira, Patrick Molière,  
Nathalie Bernoud-Hubac, Michel Guichardant*

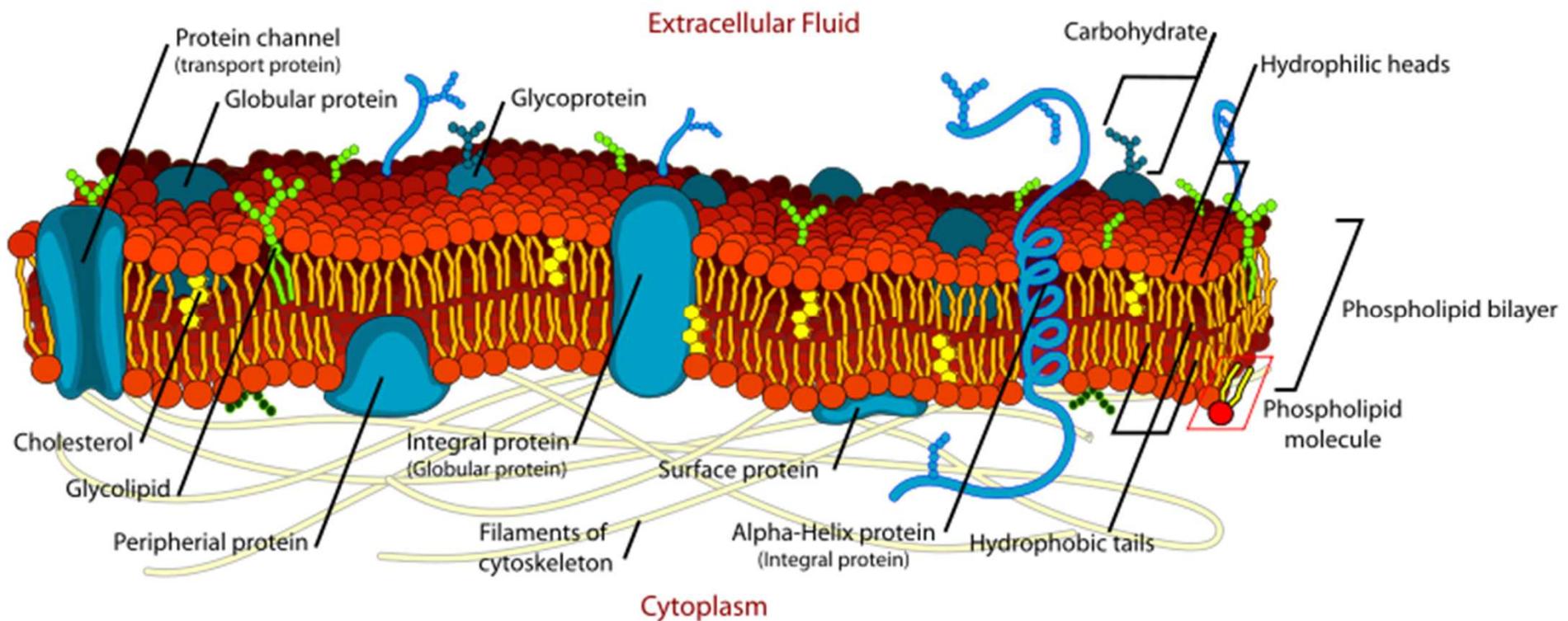
Université de Lyon, UMR 1060 Inserm (CarMeN),  
INSA-Lyon, IMBL, Villeurbanne, France



# Omiques...

- Génomique
- Transcriptomique
- Protéomique
- Métabolomique
  - \* Glycomique
  - \* Lipidomique
  - \* ....

# Biological membrane



## Lipidomique

**Titre contenant « Lipidomics » pour la 1<sup>ère</sup> fois  
en juin 2003 dans J. Lipid Res.**

**296 titres en 10 ans (PubMed)**

**Editorial: Lipidomics is emerging,  
Biochim. Biophys. Acta 2003 Nov 15;1634(3):61.  
Lagarde M, Géloën A, Record M, Vance D, Spener F.**

## **BBA Editorial 2003**

**It can be defined as**

**“the full characterization of lipid molecular species  
and of their biological roles with respect to  
expression of proteins involved in lipid metabolism  
and function, including gene regulation”**

## Subdivisions

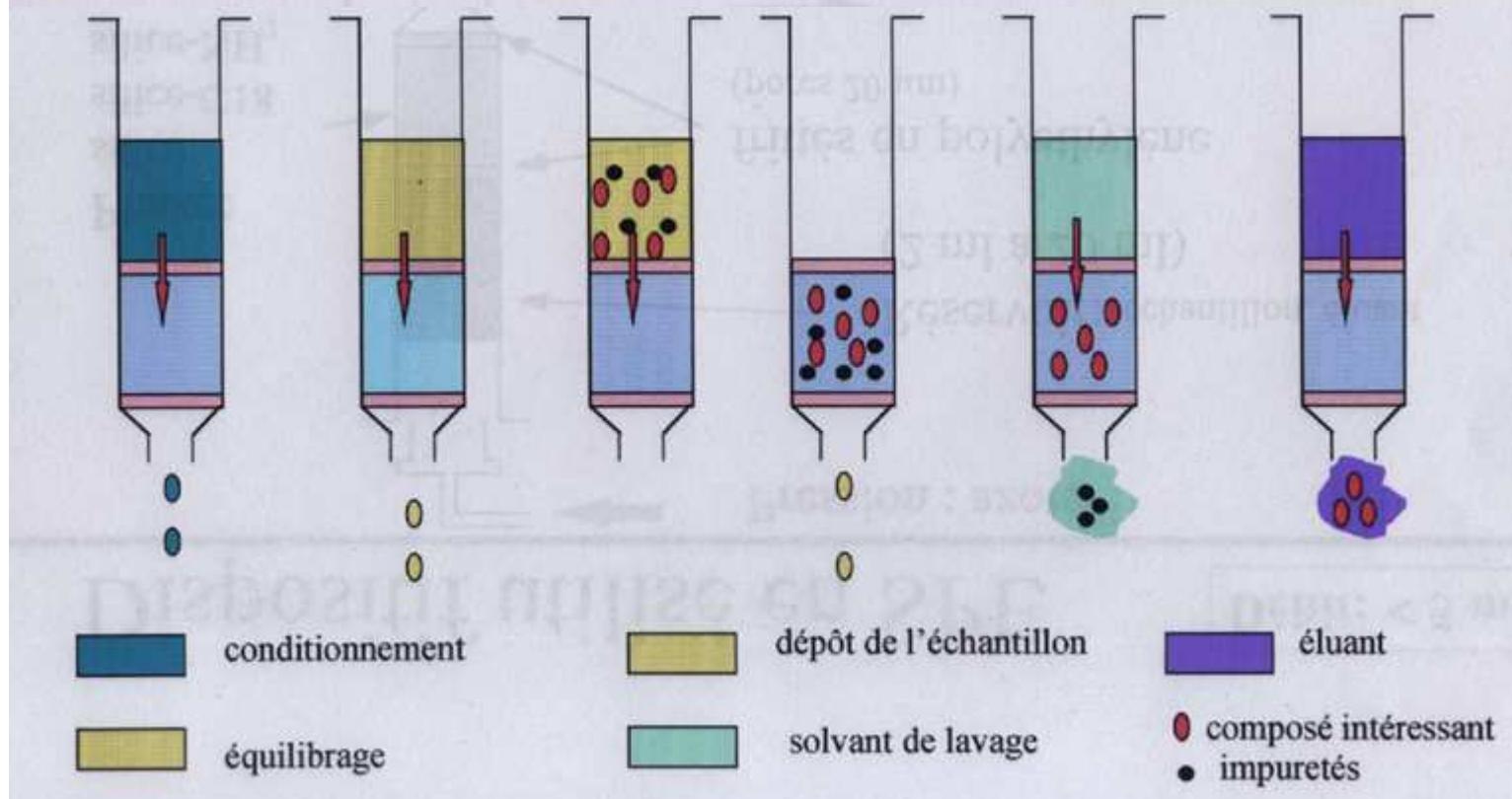
- Structure lipidomics
- Mediator lipidomics
- Targeted lipidomics
- etc.

# Outils analytiques (1)

## Extraction des lipides

- Ajout des standards internes
- Liquide-liquide (phase organique *vs* phase aqueuse)
  - Liquide-solide Sep-pack C<sub>18</sub>  
(Pb de matrices)  
(Exclusion de conteneurs plastiques)
- Concentration de la phase organique (azote ou argon)

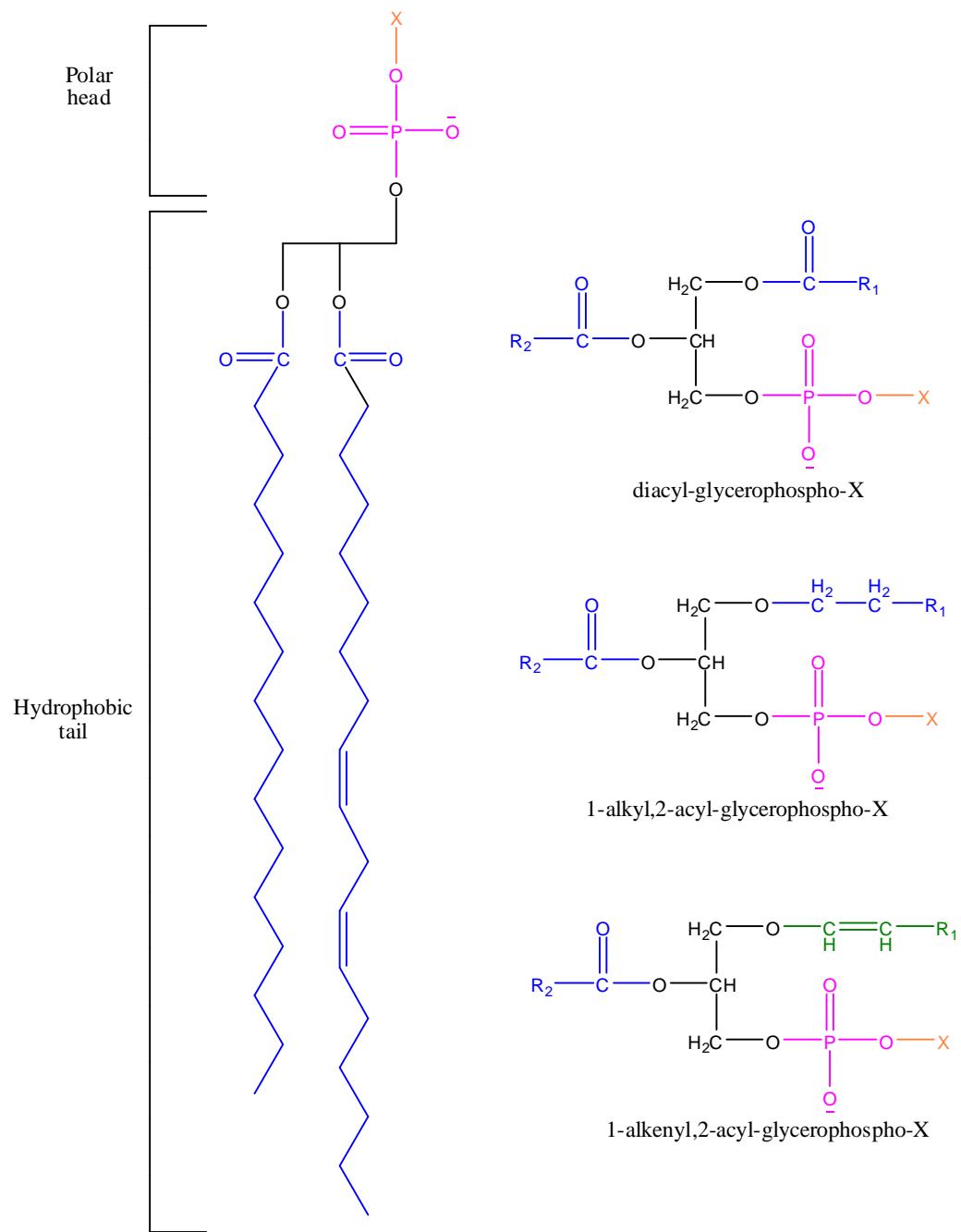
# Purification d'un échantillon par SPE

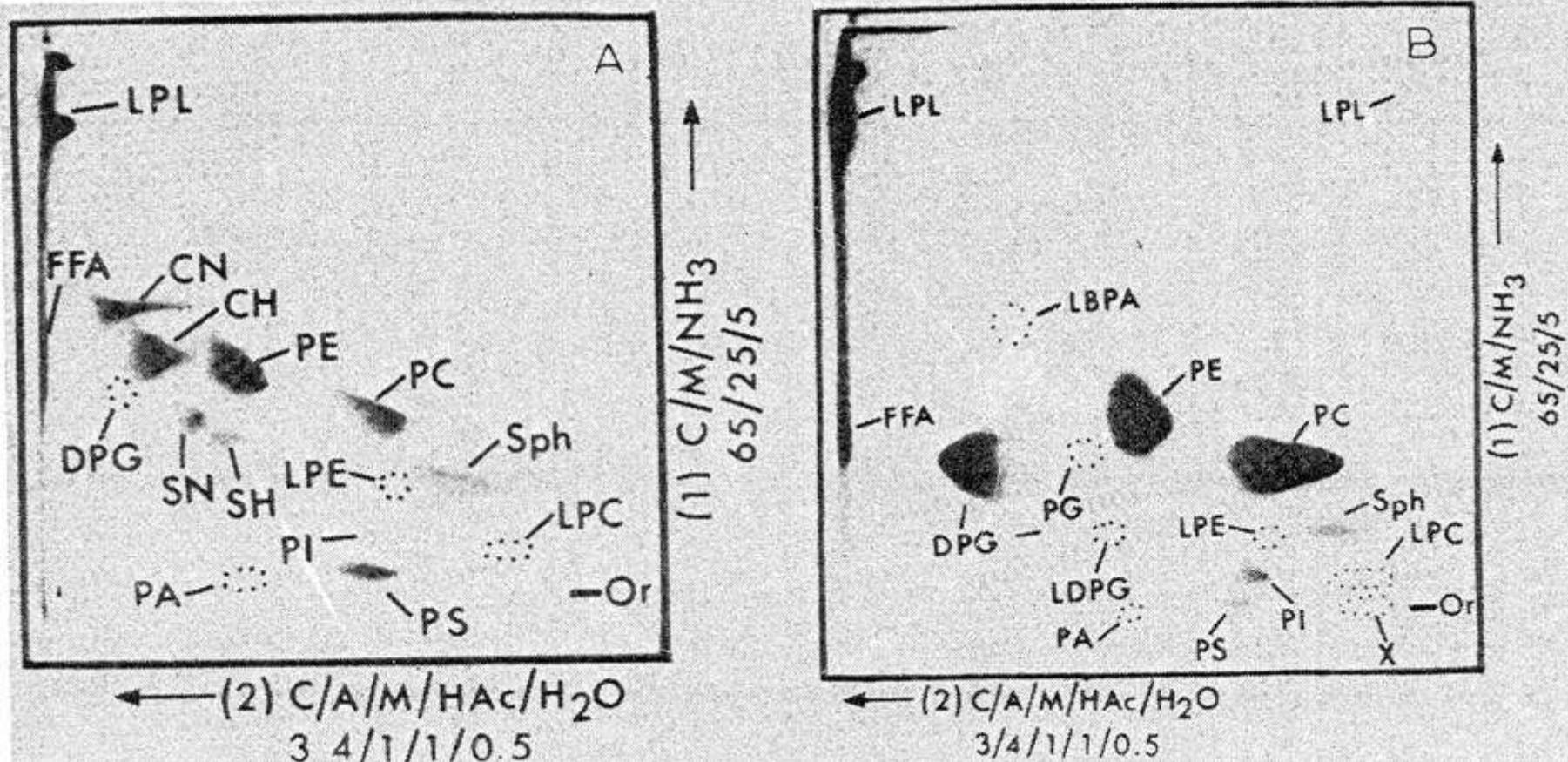


## Outils analytiques (2)

### Séparation et détection des lipides

- Chromatographie couche mince préalable
  - HPLC/UPLC directe si chromophores
  - Dérivation préalable si GC ou GC/MS
- LC-MS vs LC-MS/MS ou GC-MS vs GC-MS/MS

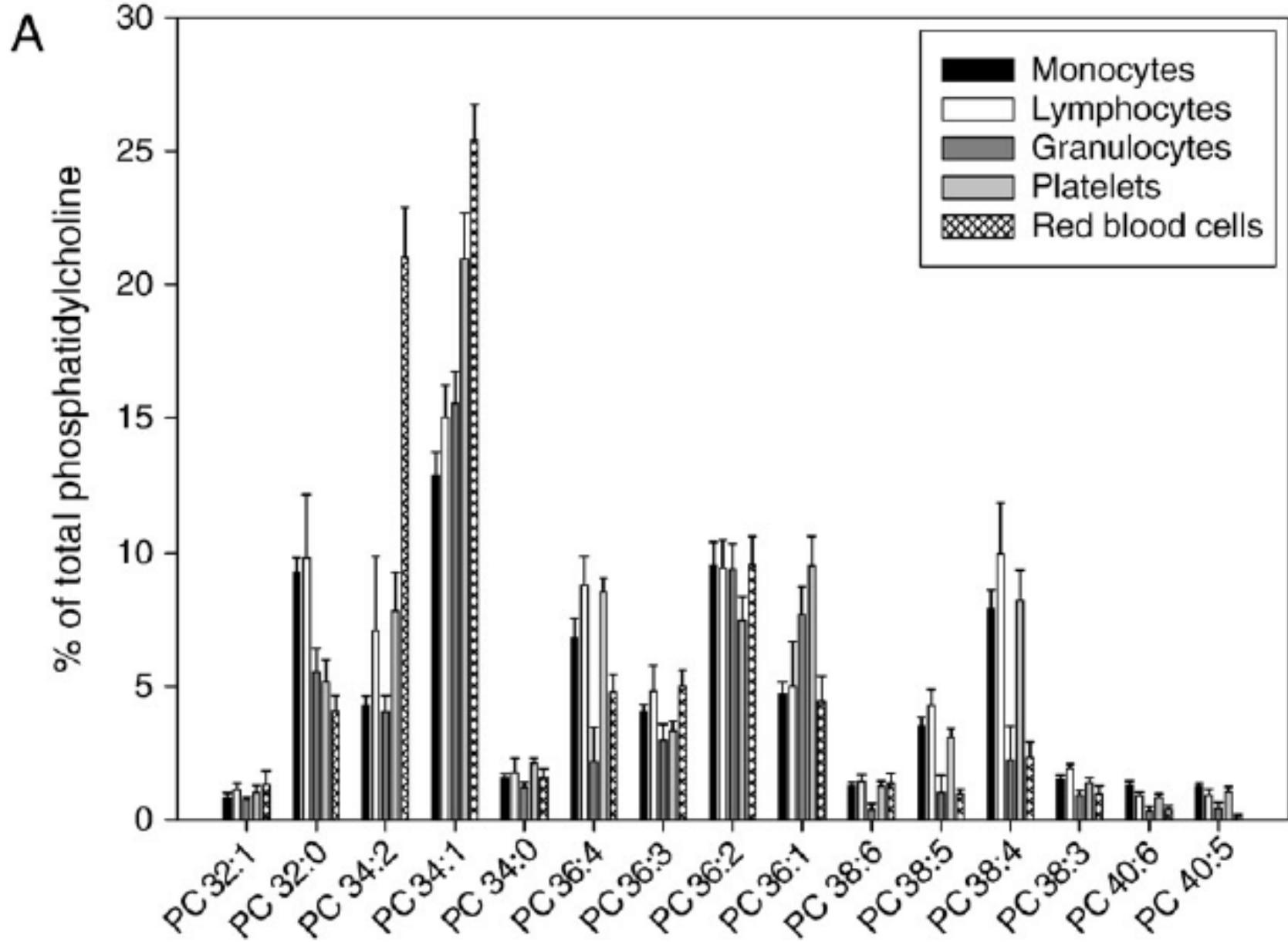




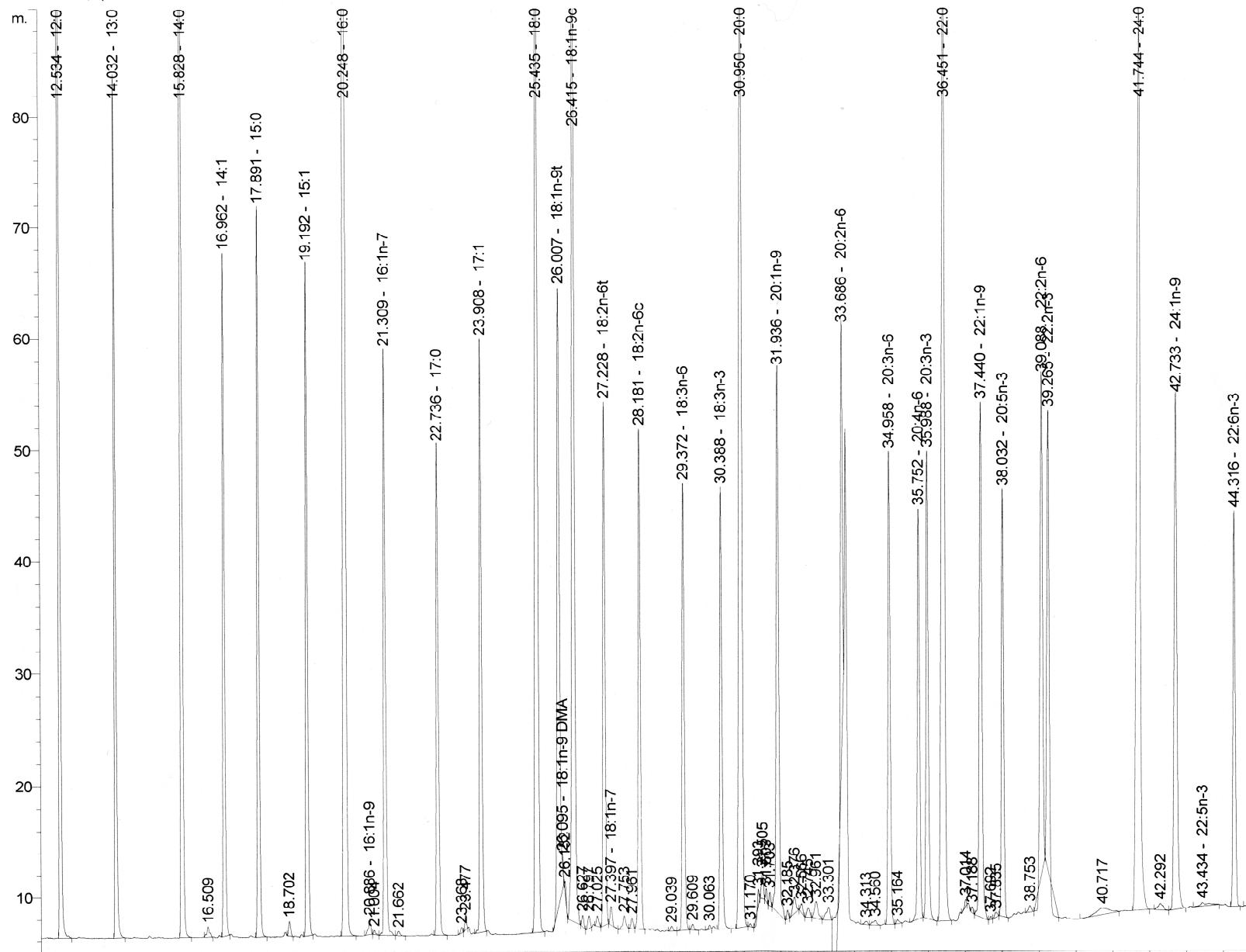
Solvent (1): chloroform/methanol/28%ammonia (65:25:5, v/v)

Solvent (2): chloroform/acetone/methano/acetic acid/lwater (6:8:2:2:1)

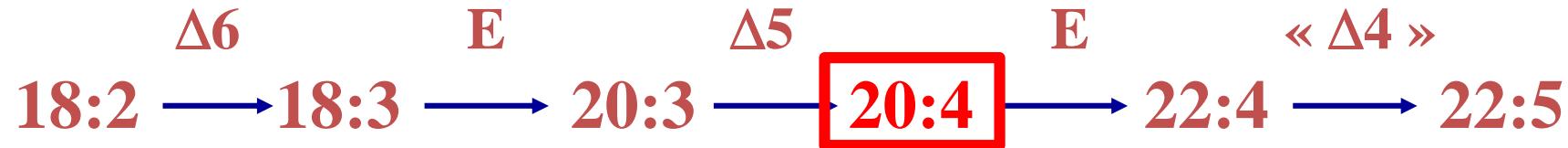
**DPG**, diphosphatidyl glycerol; **LDPG**, lysodiphosphatidyl glycérol; **PA**, phosphatidic acid; **PC**, Phosphatidyl choline; **LPC**, lysophosphatidyl choline; **PE**, phosphatidyl ethanolamine; **LPE**, Lysophosphatidylethanolamine; **PG**, phosphatidyl glycero; **PI**, phosphatidyl inositol; **PS**, Phospatidyl serine; **Sph**, sphingomyelin; **CN**, **CH**, cerebroside with normal and hydroxy fatty acids; **SN,SH**, sulfatides with normal and hydroxy fatty acids; **FFA**, free fatty acids; **LPL**, Less polar lipids-cholesterol, triglycerides,etc; **LBPA** lysobisphosphatidic acid (from Rousset et al 1970)



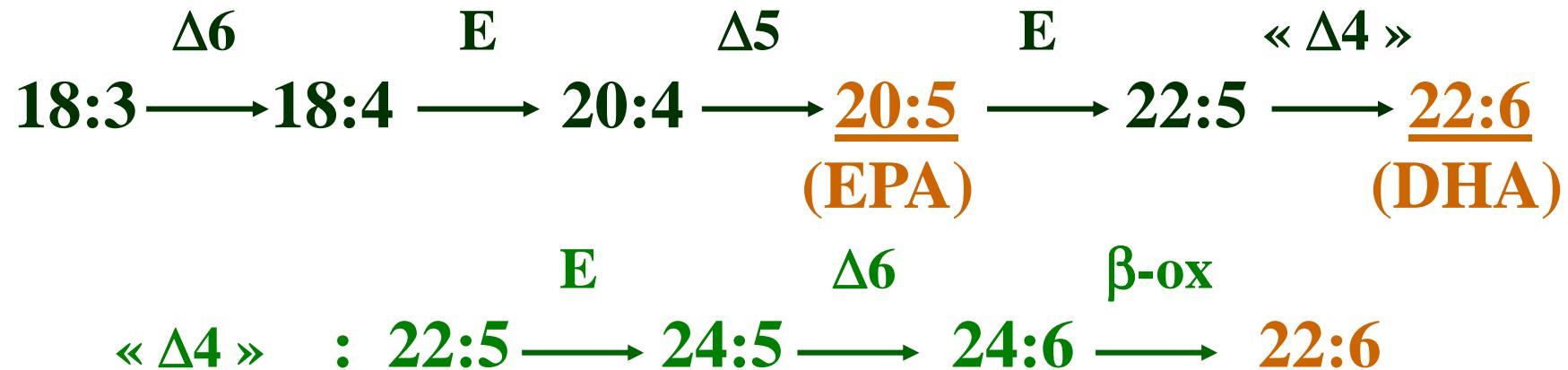
# Fatty acid methyl-ester profile (60m capillary column)

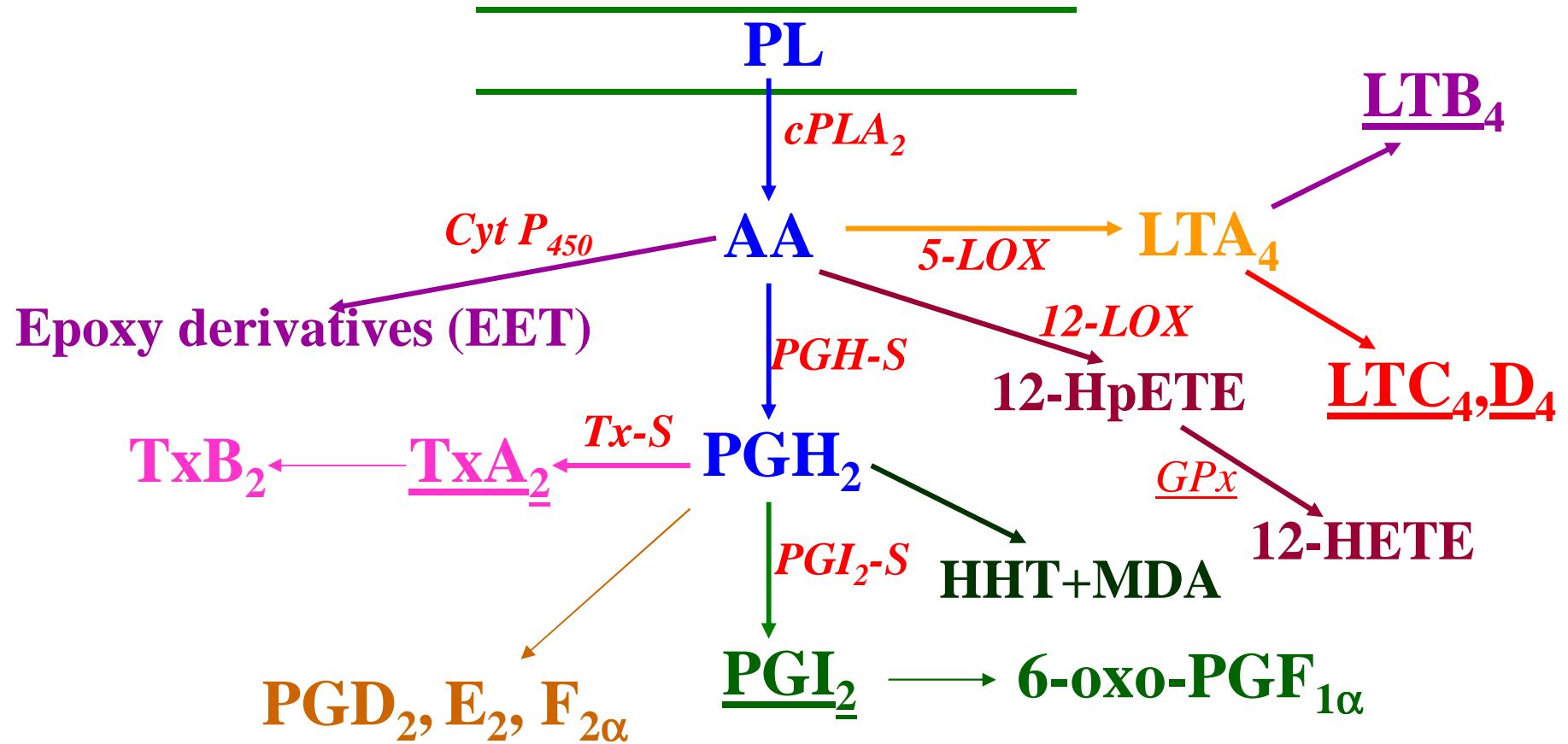


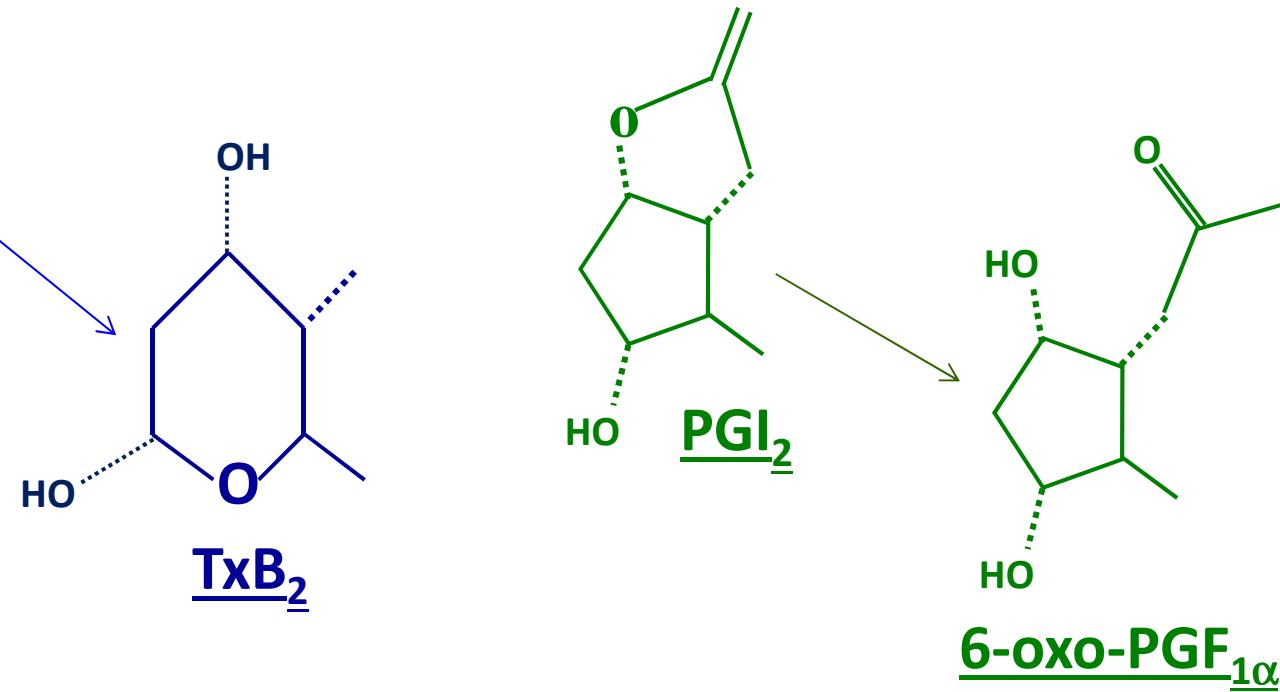
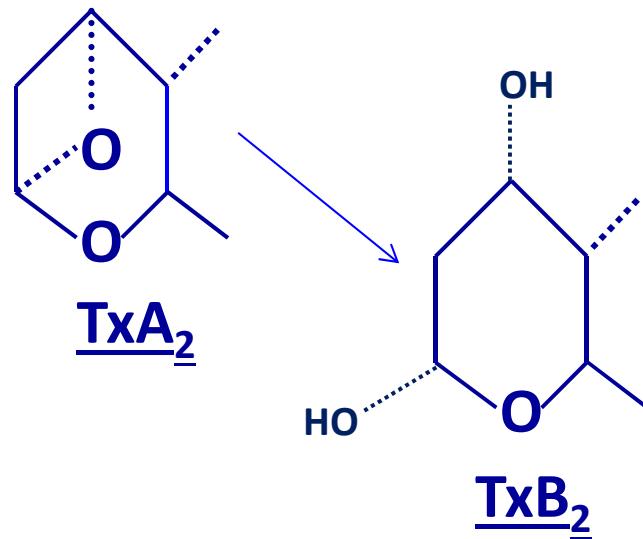
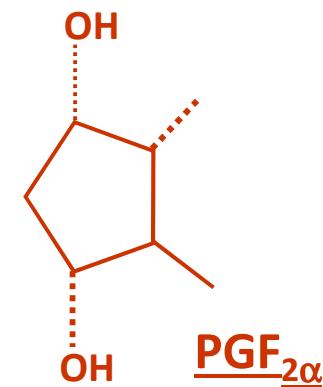
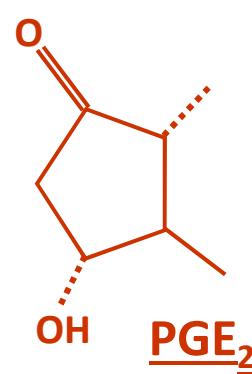
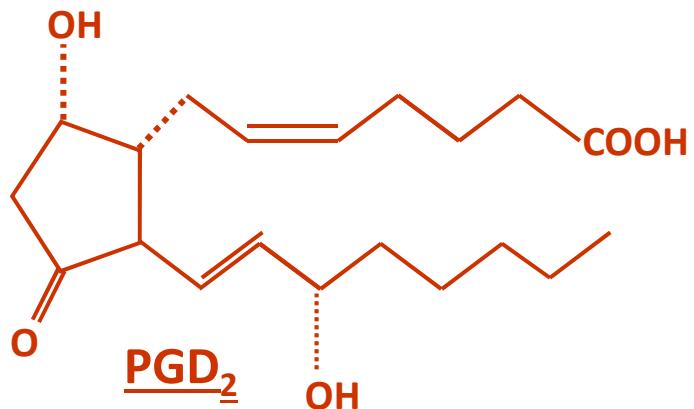
### N-6 series :



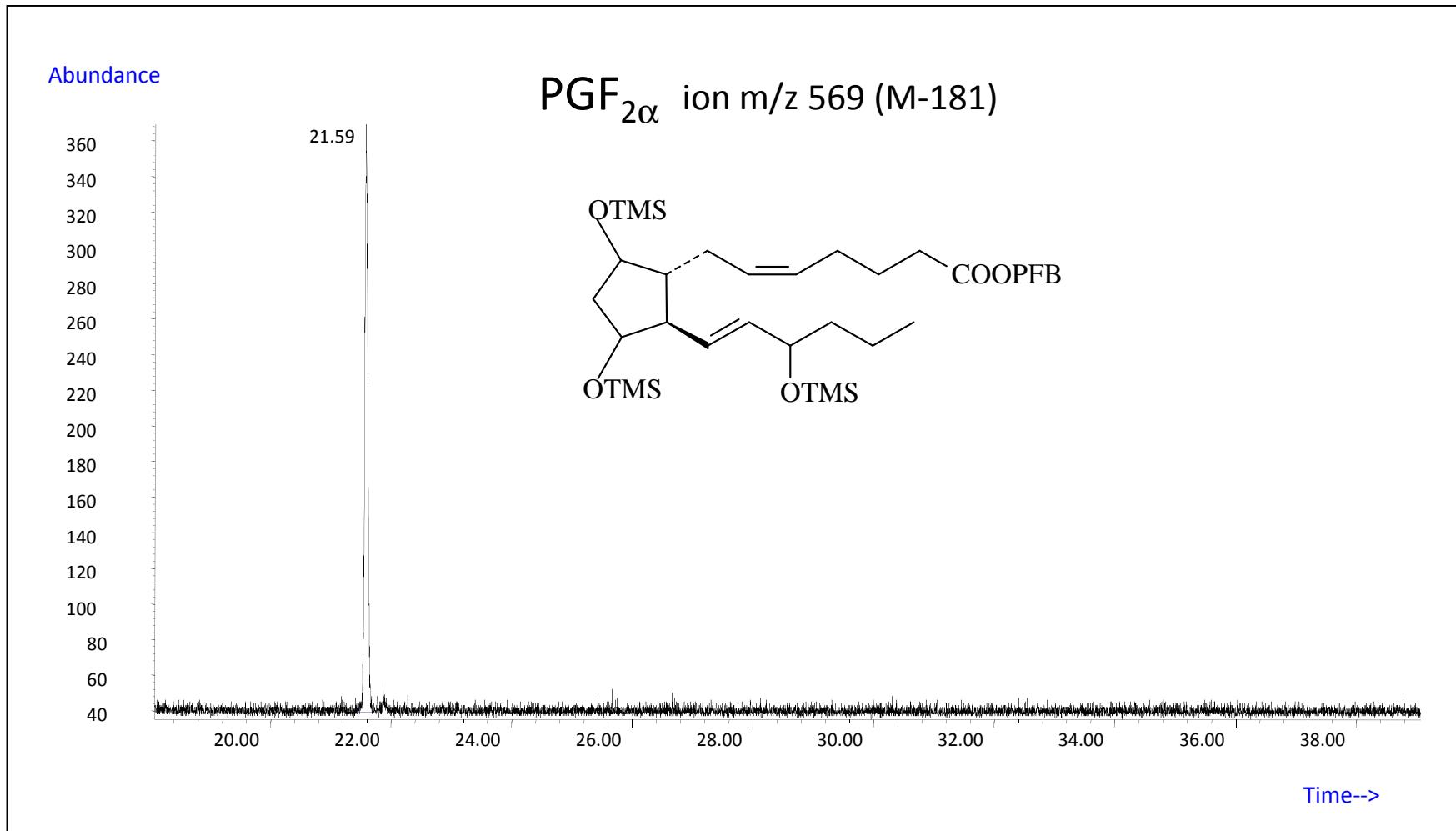
### N-3 series :



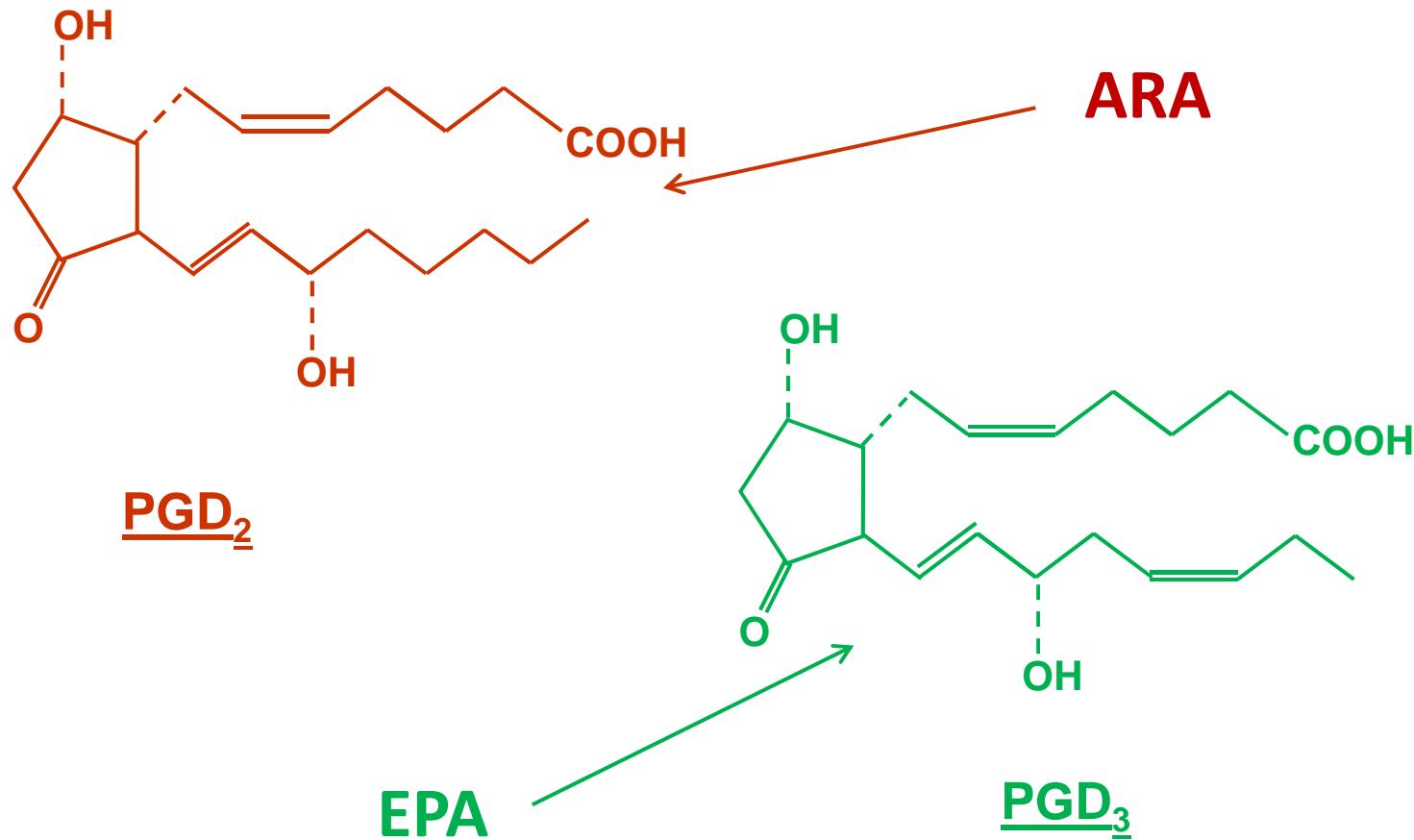


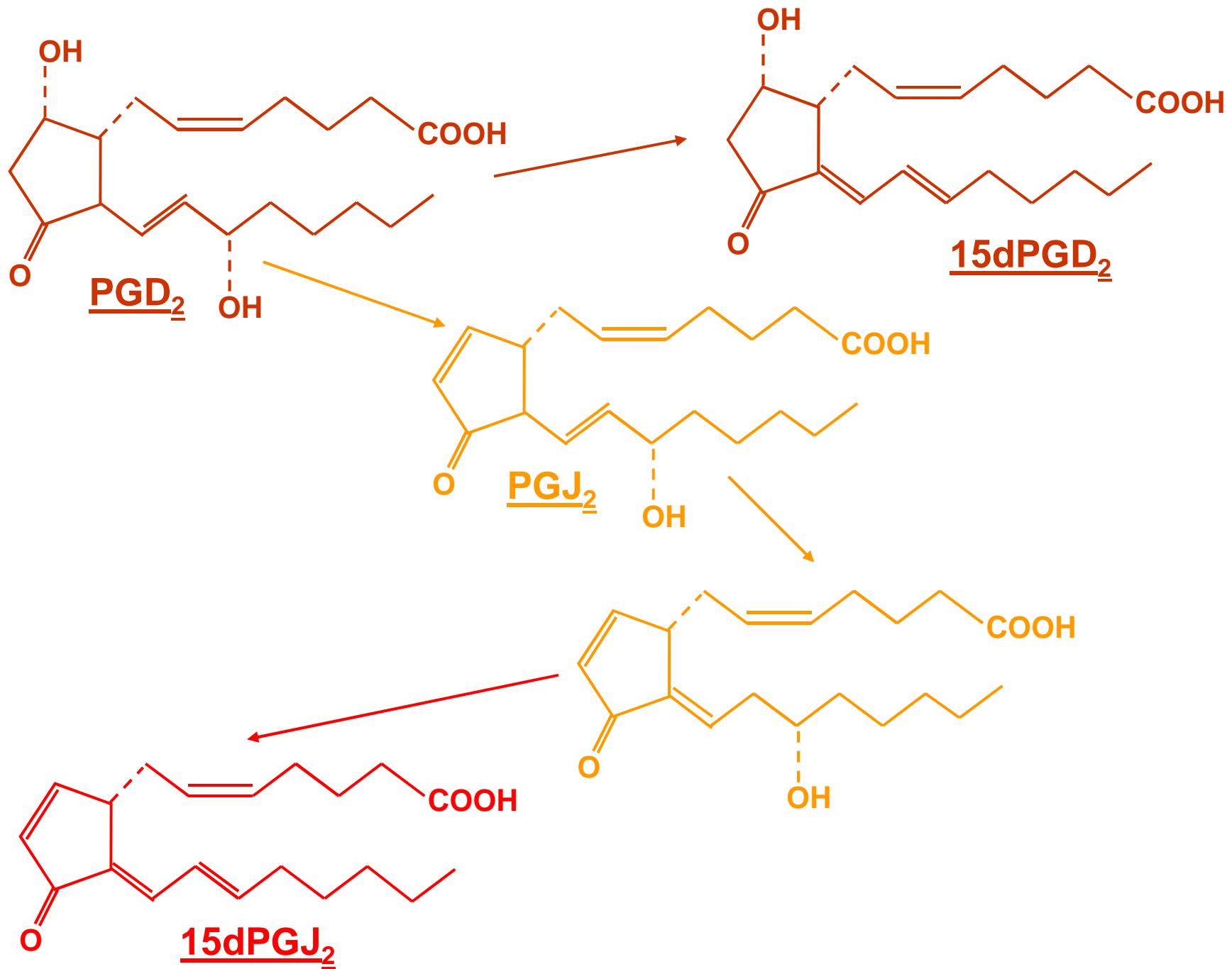


# NICl chromatogram of 1 pg of PGF<sub>2α</sub>

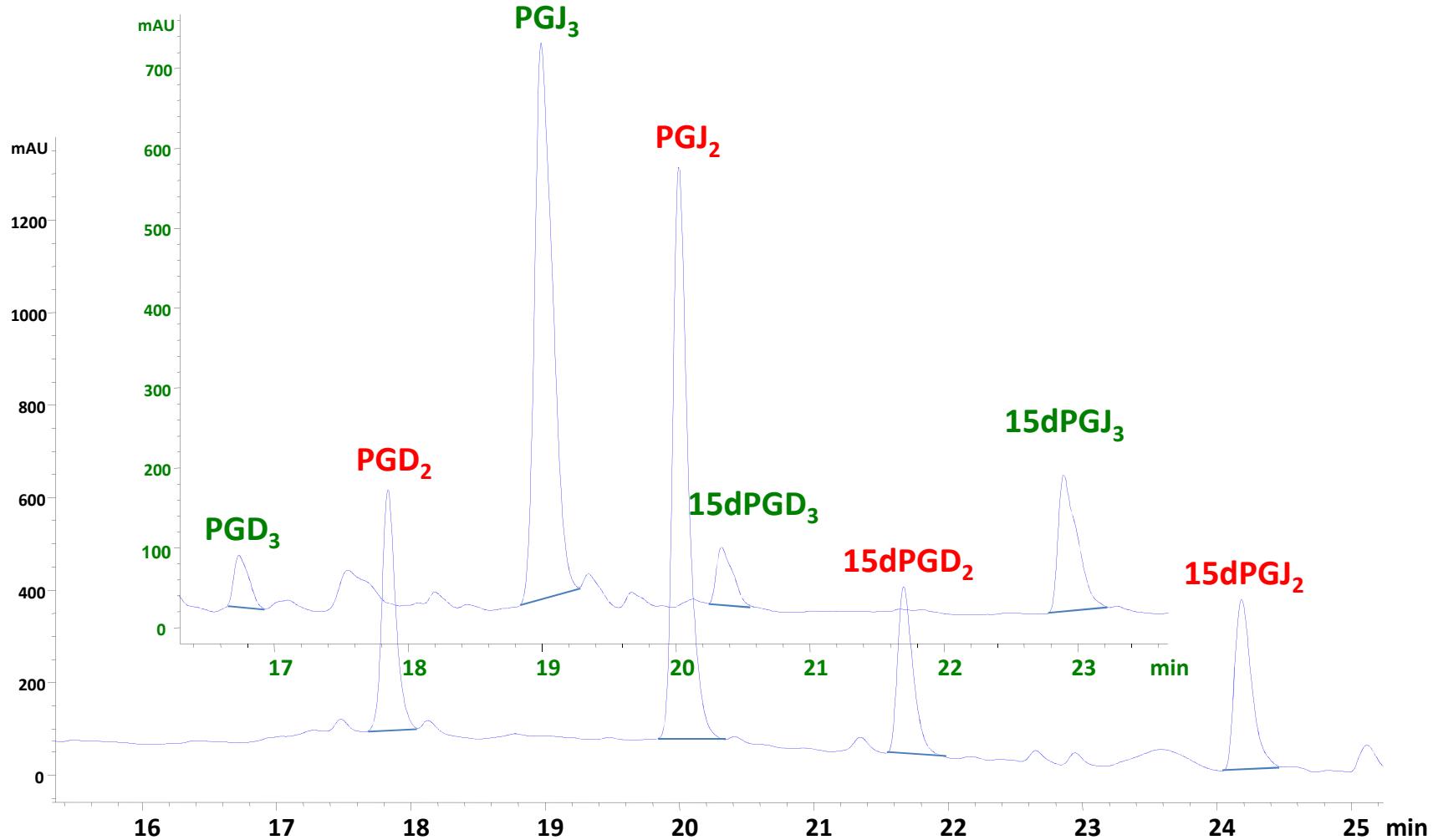


# Prostaglandines D

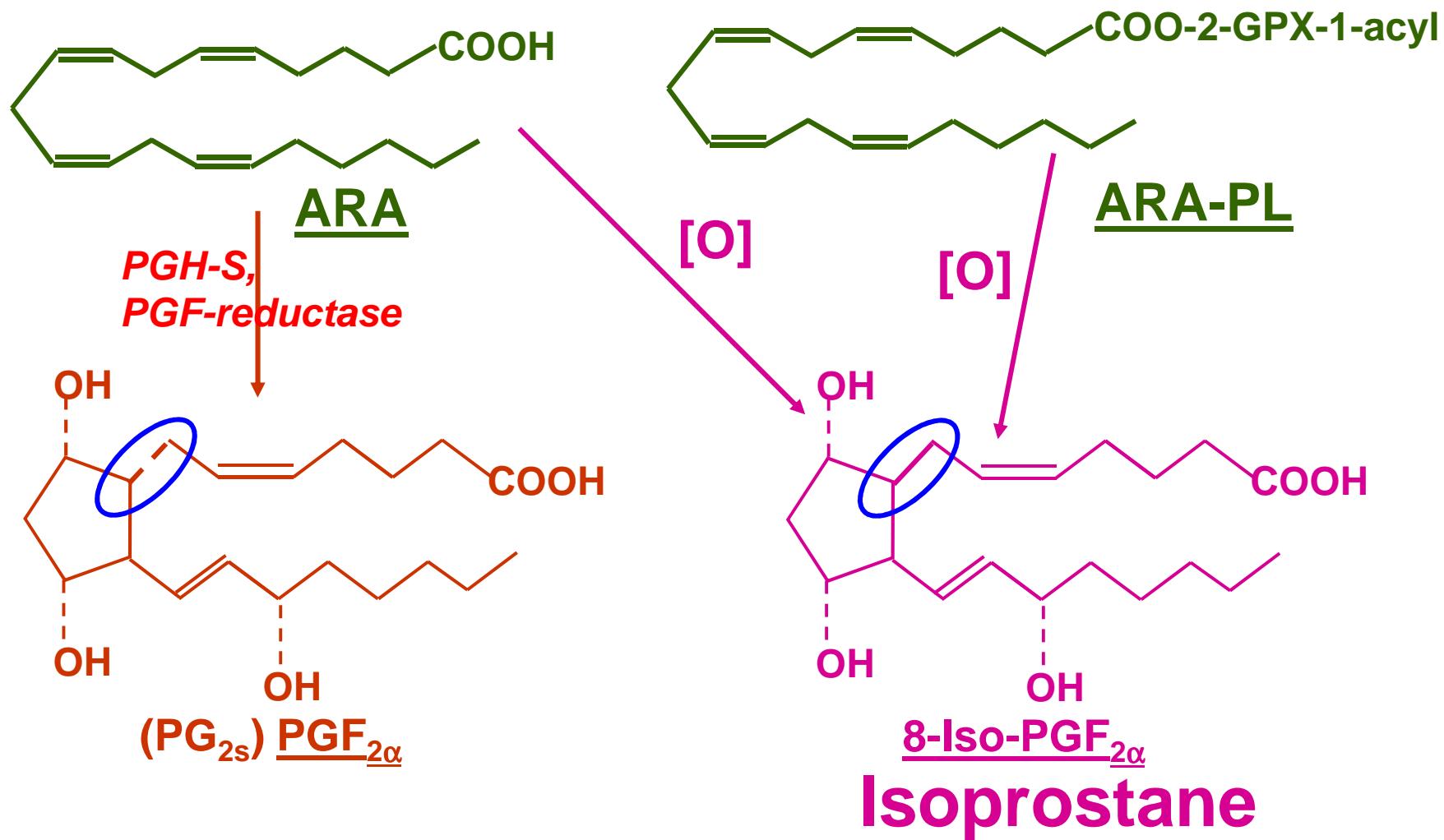




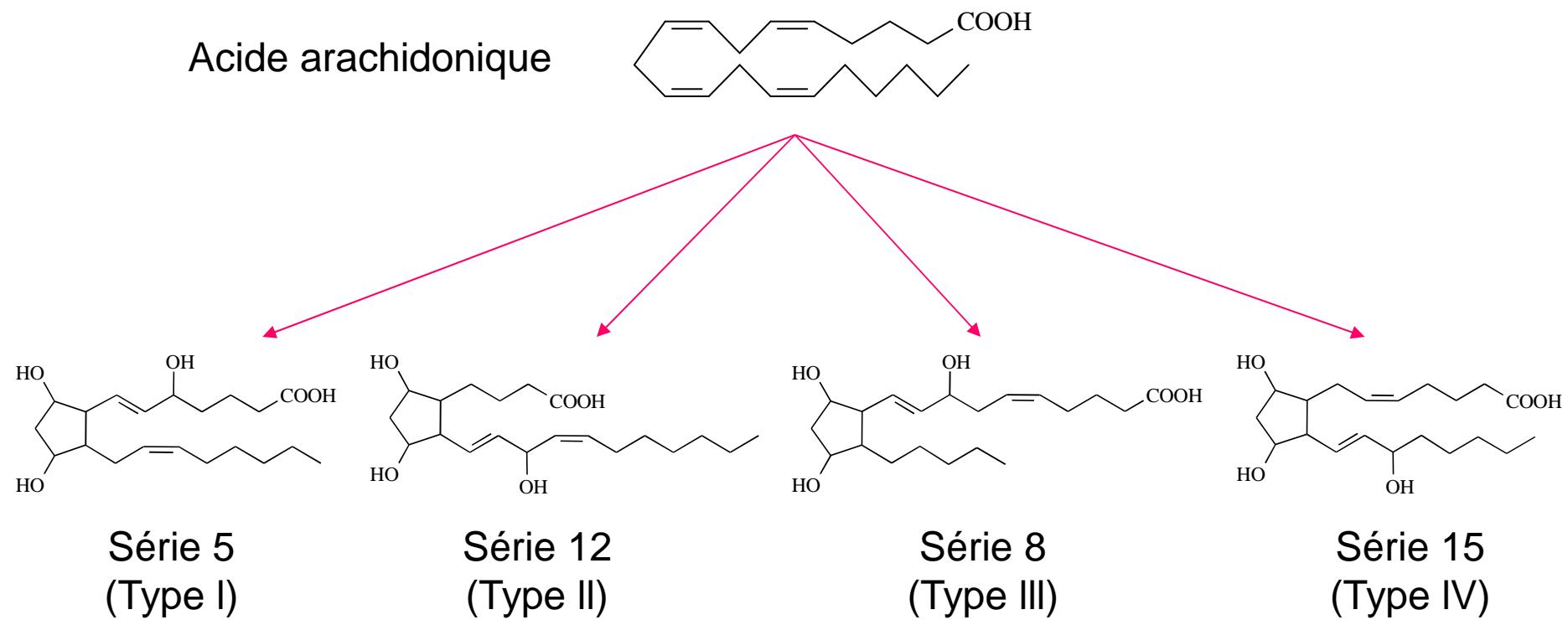
# Generation of 15dPGJ from PGD



# Isoprostanes

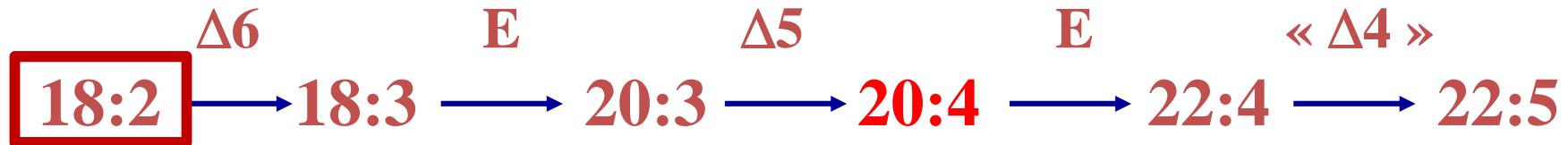


## Différents isoprostanes F<sub>2</sub>

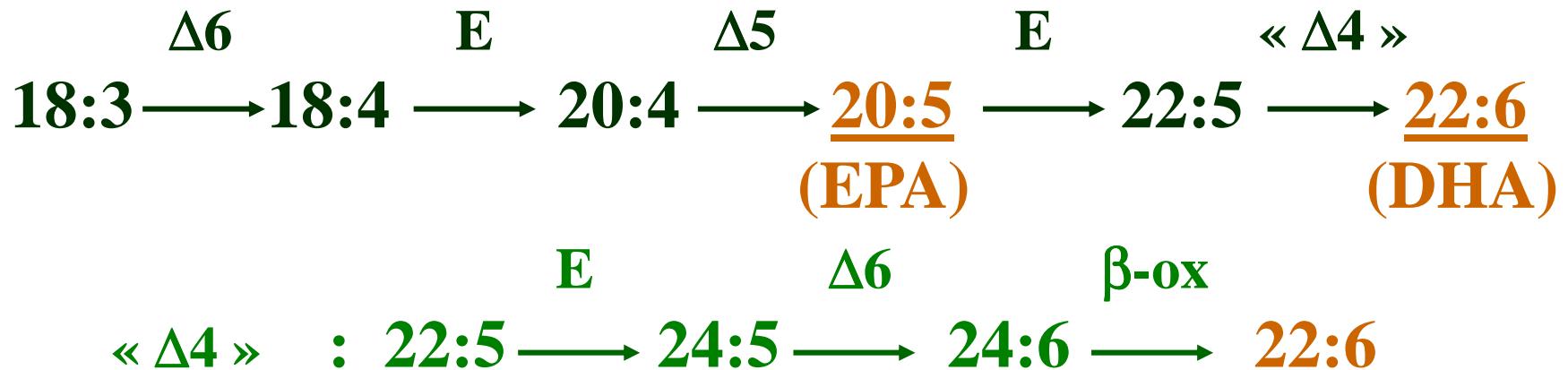


Chaque série comporte 8 diastéréoisomères racémiques

## N-6 series :



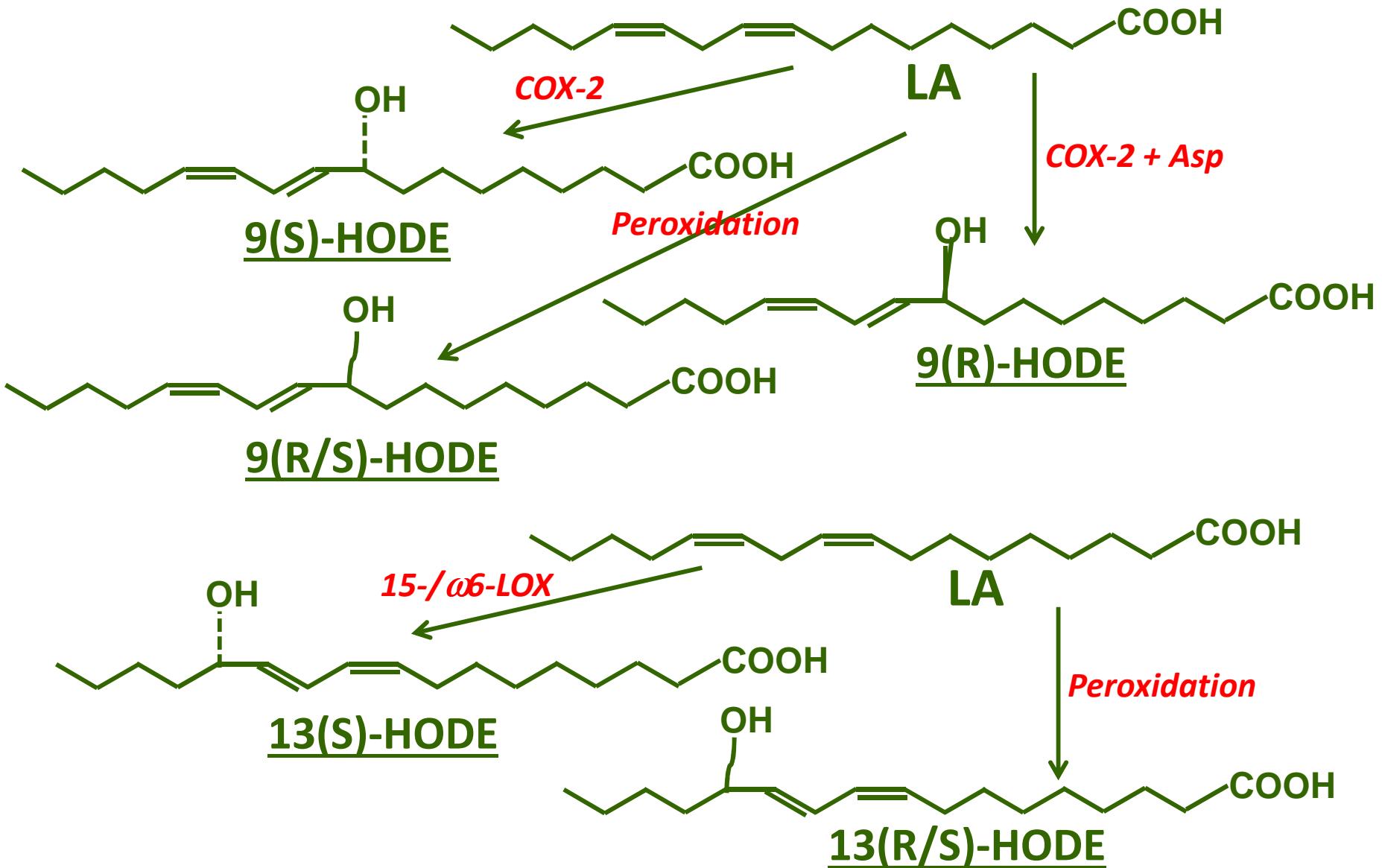
## N-3 series :



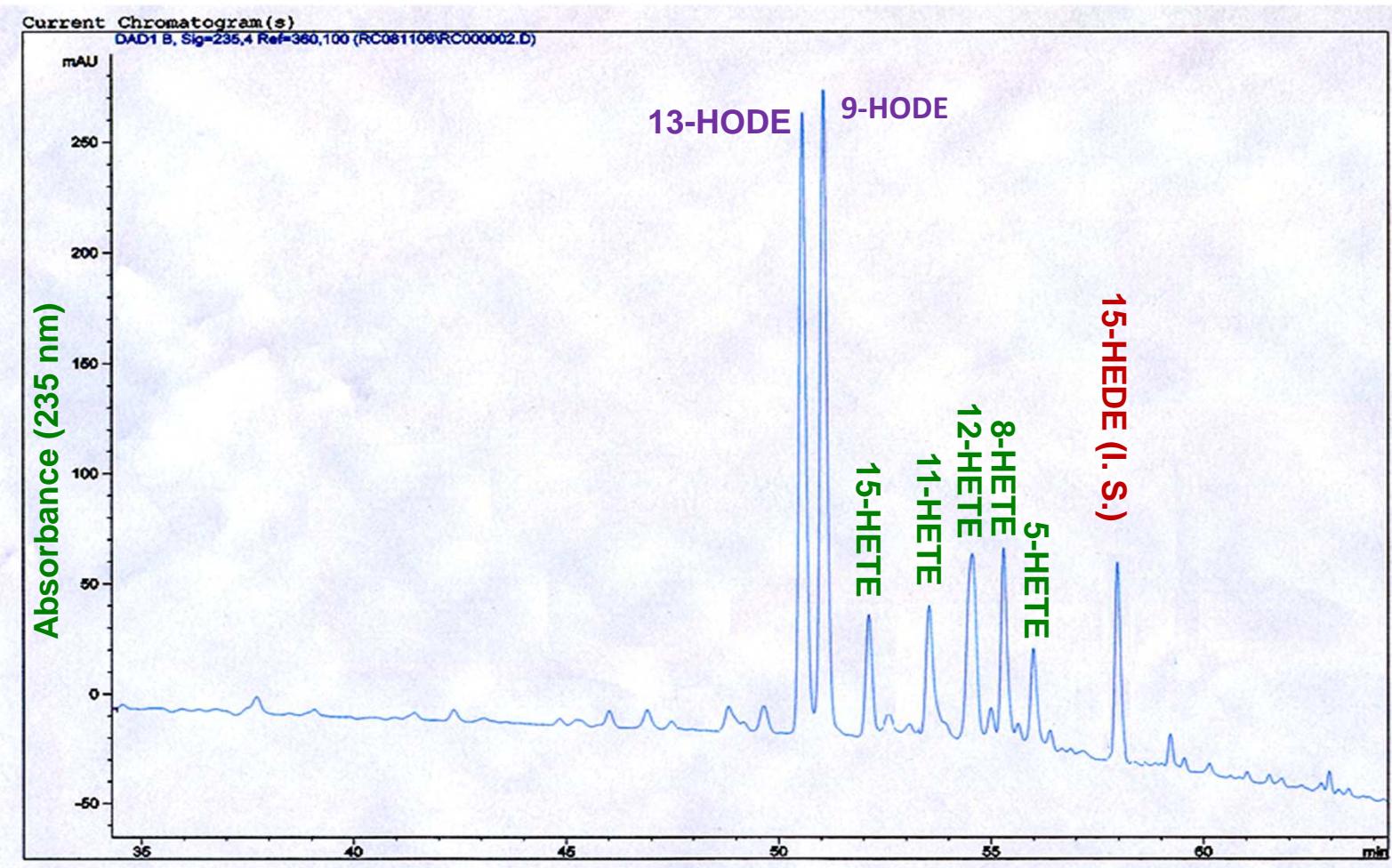
## Oxygénéation du 18:2n-6

**COX-2 et 15-/ω6-LOX + Glutathion peroxydase  
conduisent aux Hydroxy-Octadeca-Dienoates (HODEs)**

# HODE production



## HPLC of HODEs & HETEs (plasma LDL)



*Colas et al. Lipids 2010*

## HODEs & HETEs (LDL) in healthy and T2-DM subjects

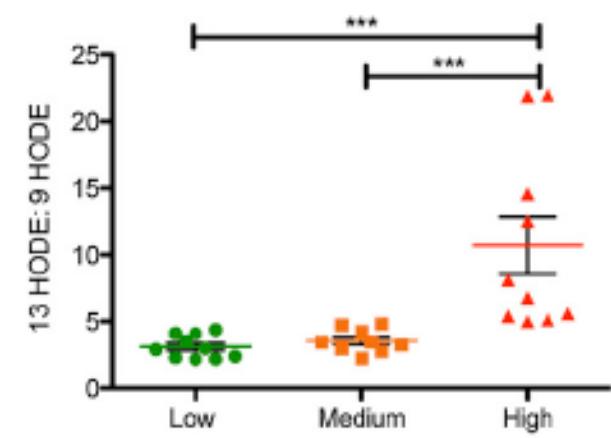
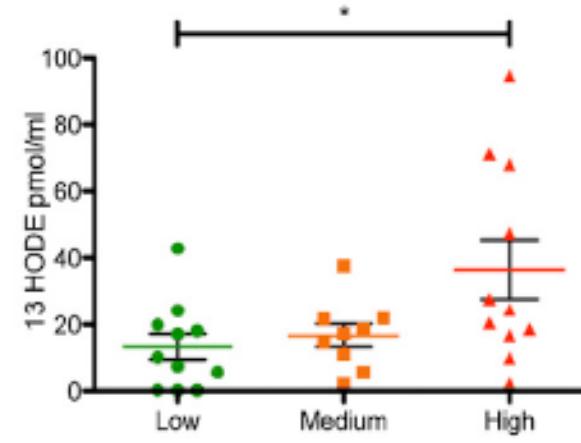
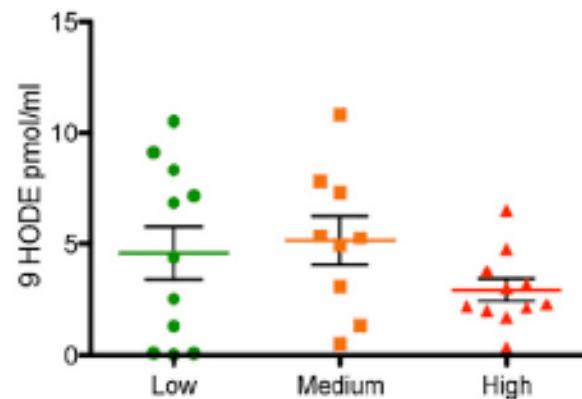
	Healthy	T2-DM
(pmol/mg chol.)		
9-HODE	<b>148 ± 17</b>	<b>235 ± 21*</b>
13-HODE	<b>133 ± 18</b>	<b>205 ± 17*</b>
8-HETE	<b>26 ± 8</b>	<b>44 ± 8</b>
11-HETE	<b>23 ± 9</b>	<b>43 ± 11</b>
12-HETE	<b>13 ± 6</b>	<b>34 ± 7*</b>
15-HETE	<b>29 ± 5</b>	<b>54 ± 8*</b>
(μmol/mol)		
HODEs/18:2n-6	<b>394 ± 39</b>	<b>624 ± 42**</b>
HETEs/20:4n-6	<b>822 ± 188</b>	<b>1222 ± 185</b>

*Colas et al. Lipids 2010*

## Lipidome of nasopharyngeal lavages from human influenza clinical samples

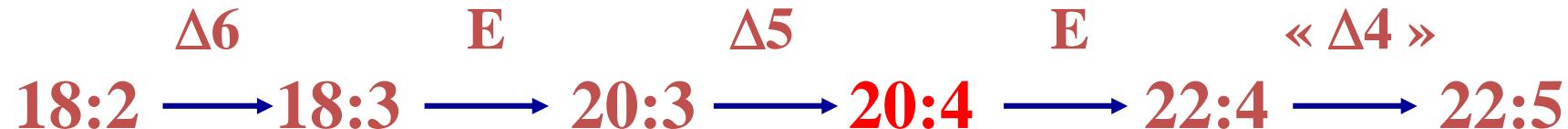
### 13-HODE vs 9-HODE

C

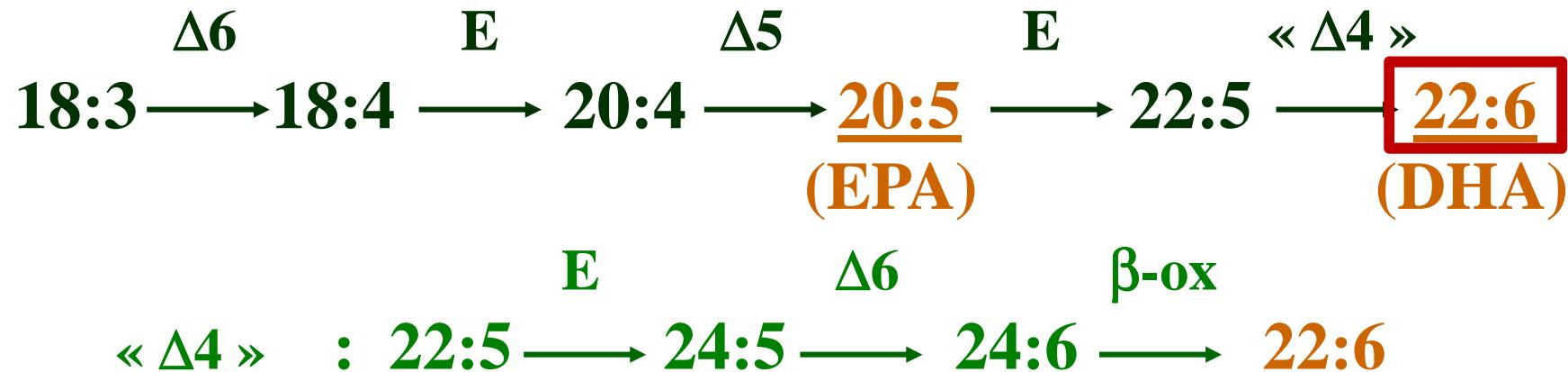


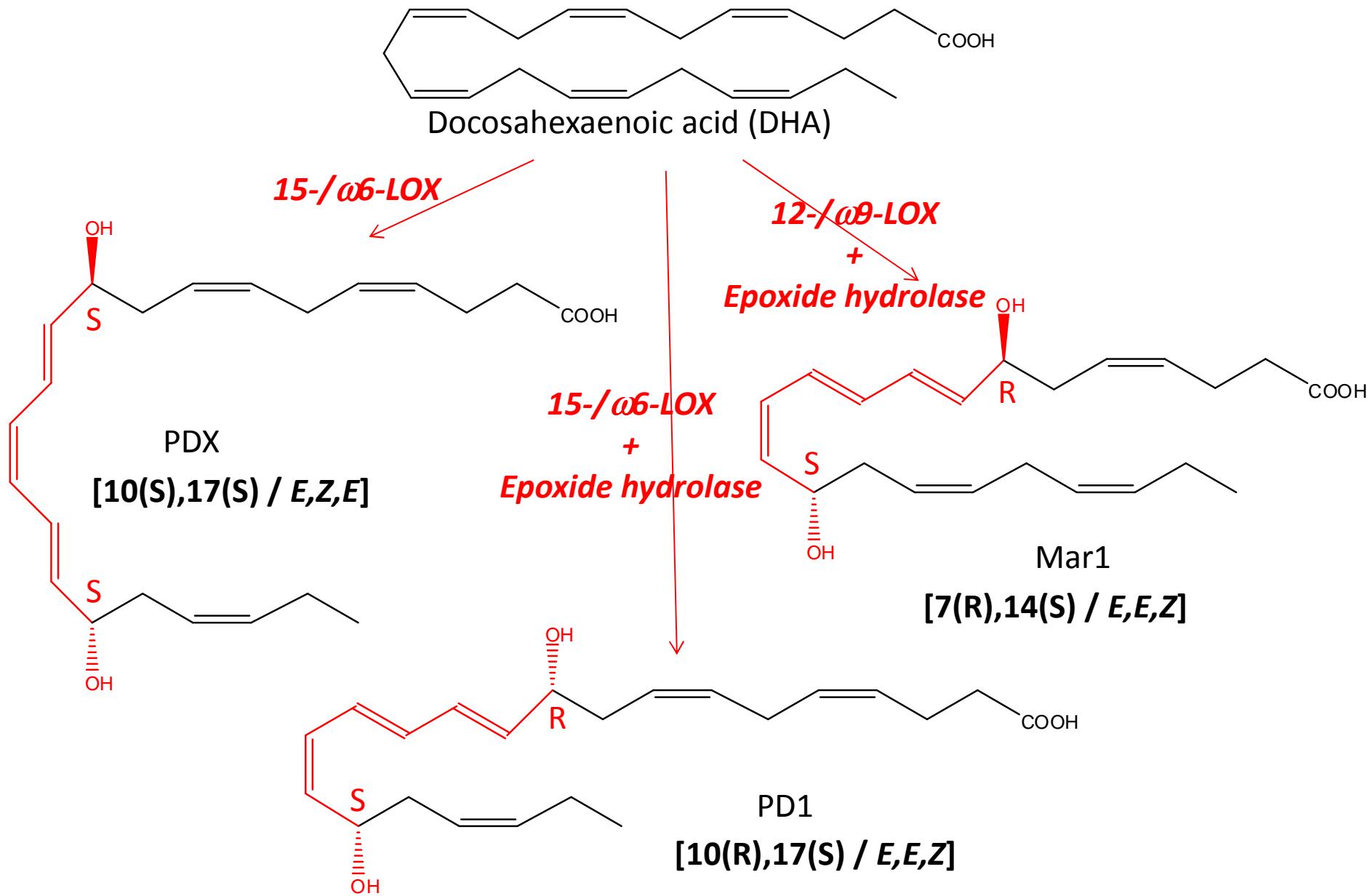
Tam et al. Cell 2013

### N-6 series :

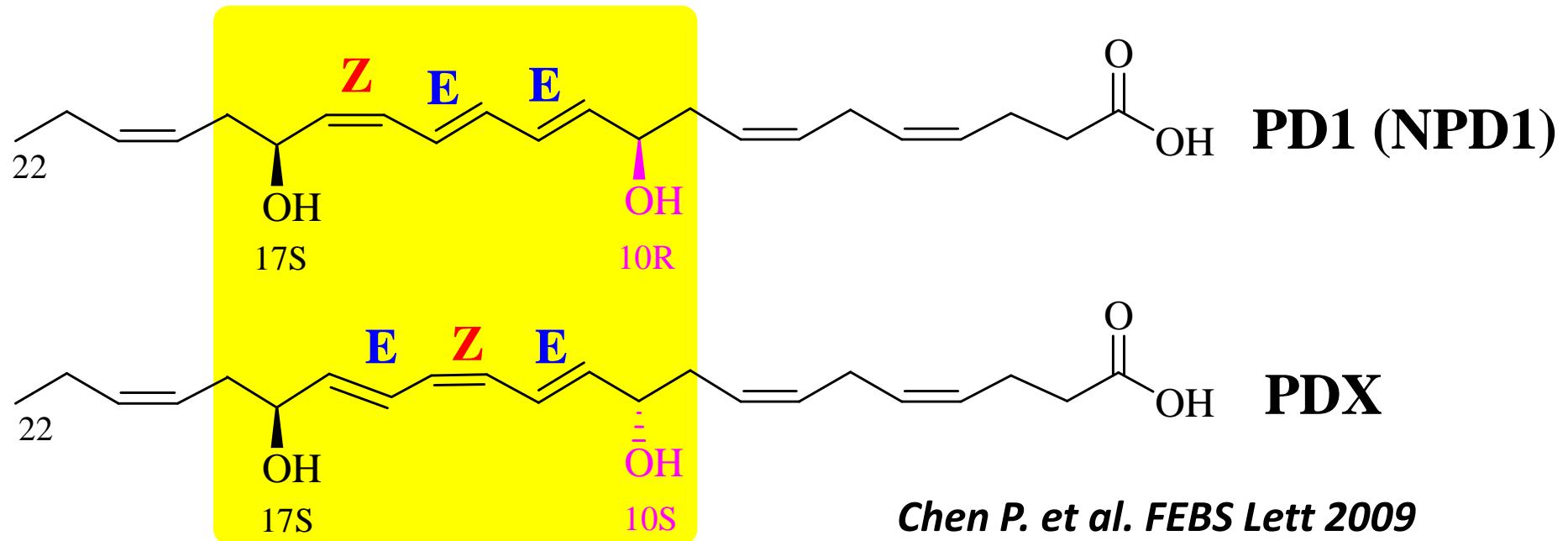


### N-3 series :





*Serhan C.N et al. JBC 2005*



**Morita et al. Cell 2013 : The Lipid Mediator Protectin D1 Inhibits Influenza Virus Replication and Improves Severe Influenza”**

## Perspectives

La lipidomique ciblée sur une classe métabolique ne permet pas toujours d'approcher les phénotypes.

Une lipidomique réalisée en fonction du temps et dans différents compartiments biologiques pertinents permet d'approcher les phénotypes.

Il s'agit d'une véritable fluxolipidomique.  
Ex du suivi des deux acides gras indispensables  
acides linoléique (18:2n-6) et linolénique (18:3n-3).

# Acknowledgements

InFoLiP Team &  
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Baptiste Fourmaux, Michel Guichardant, Patrick Molière  
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ANR, Inserm, Ministry of Research

