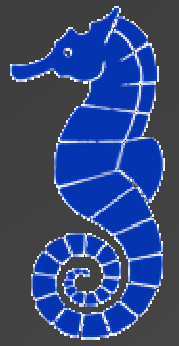
Istituto di Chimica
Biomolecolare



Stazione Zoologica
“A. Dohrn”

Oxylipins in Marine Diatoms

First report of oxylipins in marine diatoms

2-trans-4-trans-7-cis decatrienal

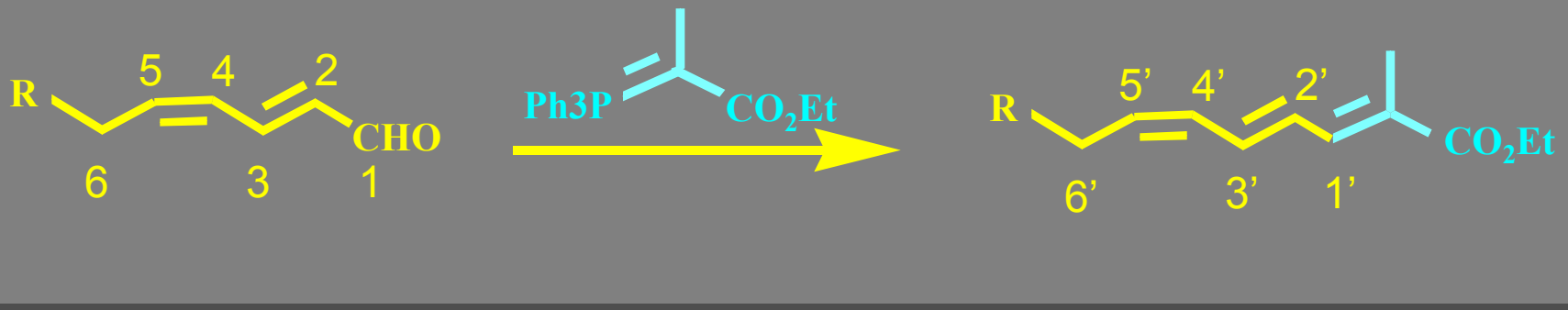
2-trans-4-cis-7-cis decatrienal

2-trans-4-trans decadienal

Miralto et al. *Nature* 1999,402:173-176

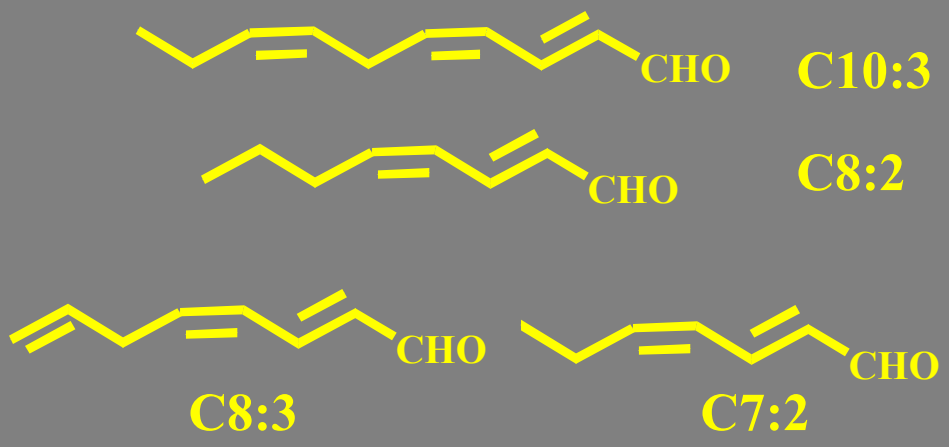
Aldehydes were correlated to the antiproliferative effect of diatoms on copepod reproduction

Detection of Short Chain Aldehydes in Diatom Extracts

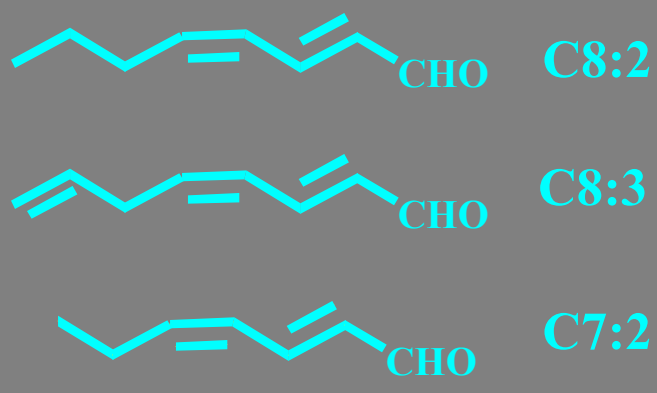


The reaction was directly applied on diatom extracts

Thalassiosira rotula

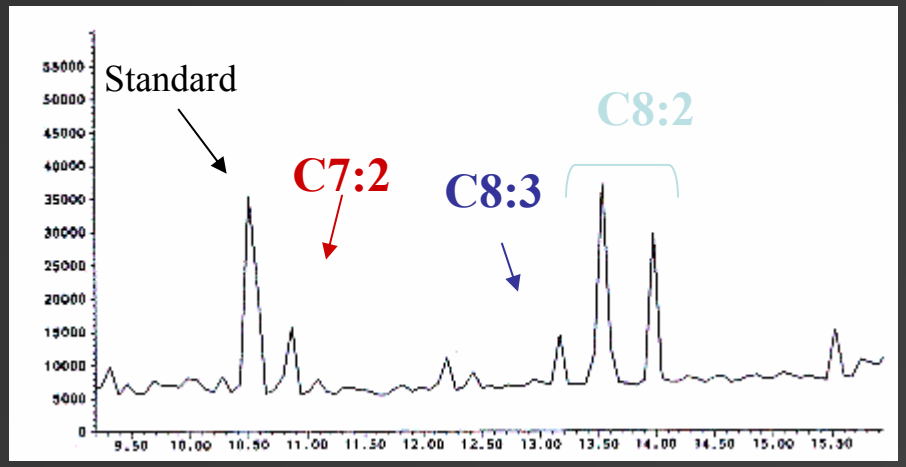


Skeletonema costatum

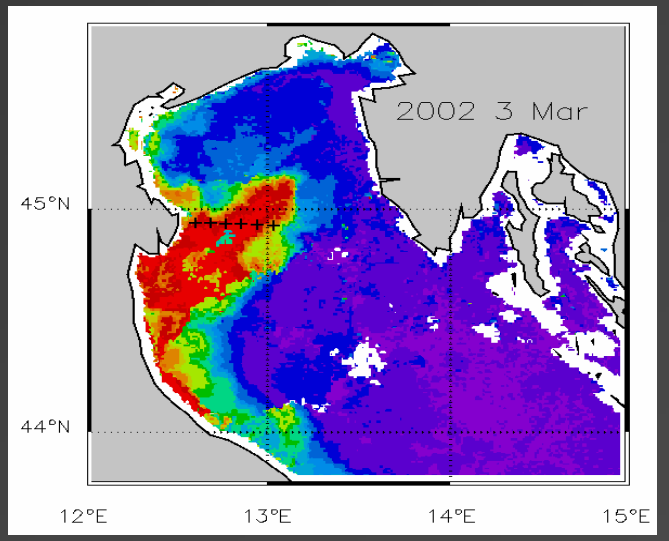


d'Ippolito et al. *Tetrahedron Lett.* 2002, *43: 6137*

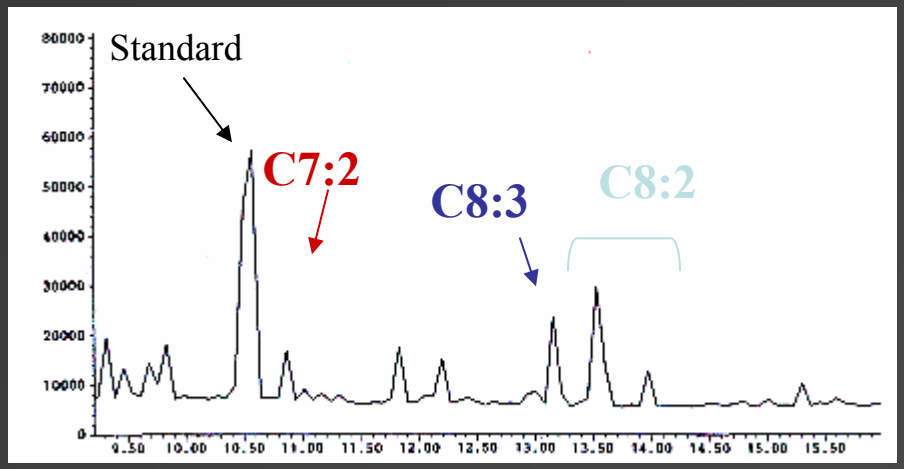
Skeletonema costatum: major diatom blooming species



Aldehydic pool of Adriatic phytoplankton



2001-2005



Aldehydic pool of *Skeletonema costatum*

Chemical data support:
-phytoplankton composition
-production and mortality of copepod eggs

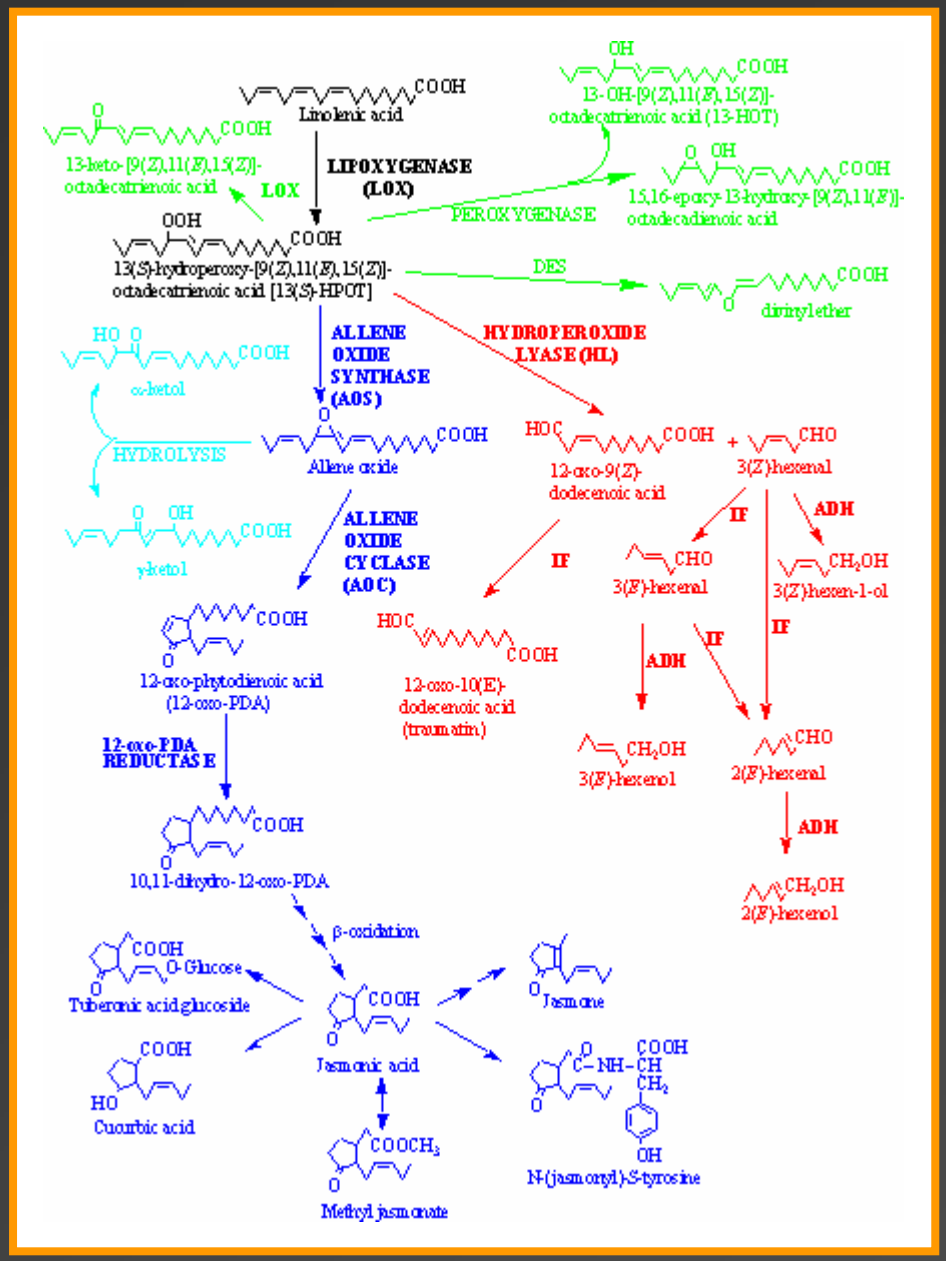
1) Clarification of the biosynthetic pathway leading to aldehydes

- To understand what the regulating factors of the process are
- To develop a molecular approach to study field and lab samples.

2) Distribution and diversity of oxylipin pathway in different diatoms

-To understand if aldehydes are the only molecules involved in diatom-copepod interactions

Oxylipin pathway in plants



"Oxylipins" : oxygenated compounds biosynthesized from fatty acids by oxygenase-catalyzed oxygenation

Aldehydes formed through lipoxygenase/hydroperoxide lyase pathway

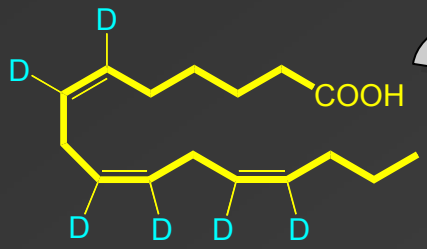
S. costatum

2,4 heptadienal

2,4 octadienal

2,4 octatrienal

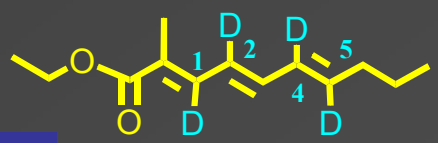
Biosynthesis of octadienal



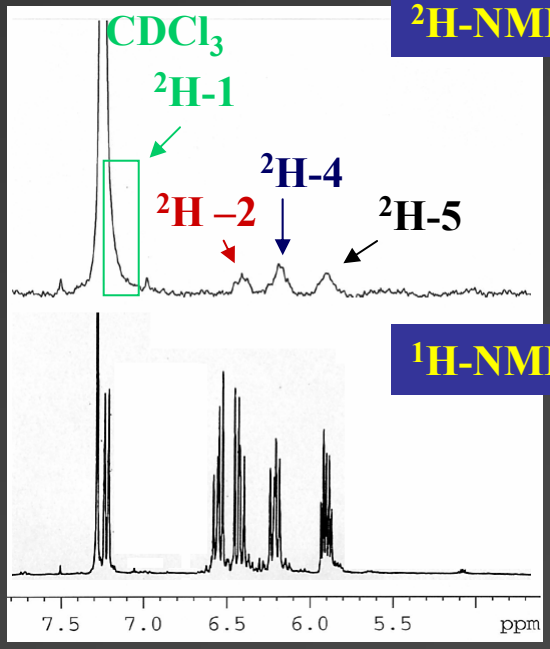
d_6-HDTA (16:3ω4)

Cell-free *S. costatum*

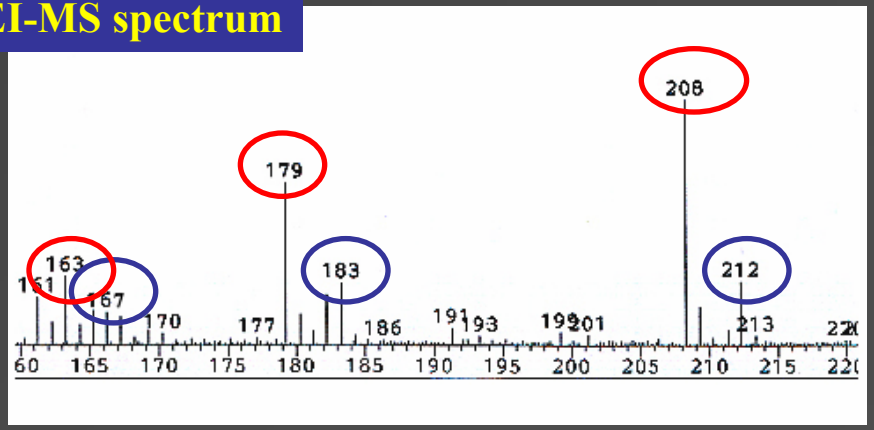
Derivatized aldehydic pool



^{2}H-NMR

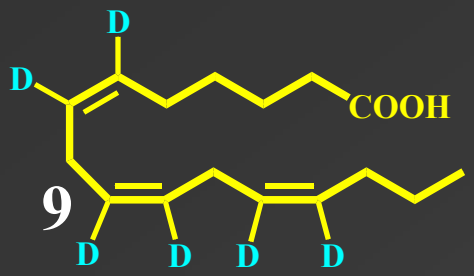


EI-MS spectrum



d'Ippolito et al.,*Org.Lett.* 2003, 5: 885

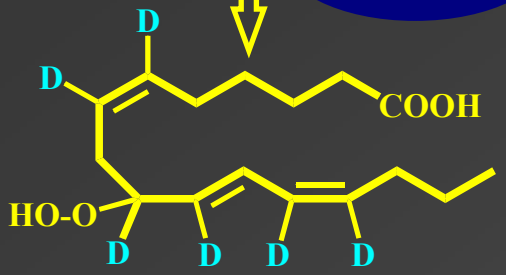
Plausible mechanism of octadienal formation



d_6-HTrA (16:3ω4)

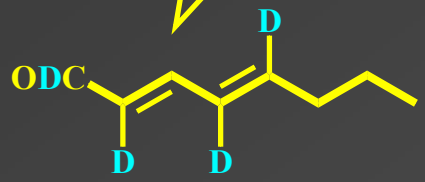
9-LOX

?



d_6-9-HpHTrE

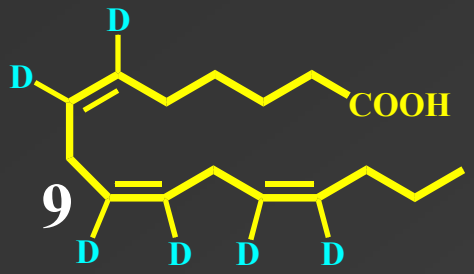
HPL



d_4-octadienal

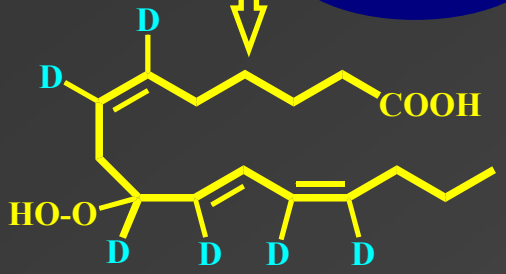
d'Ippolito et al.,*Org.Lett.* 2003, 5: 885

First proof of 9-Lipoxygenase (9-Lox)



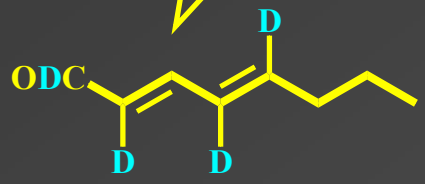
d_6-HTrA (16:3ω4)

9-LOX

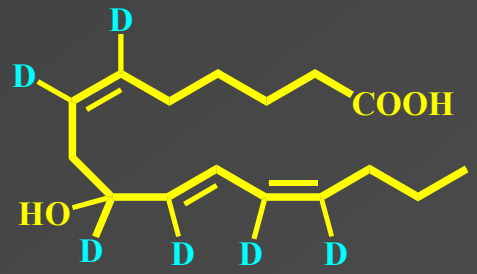


d_6-9-HpHTrE

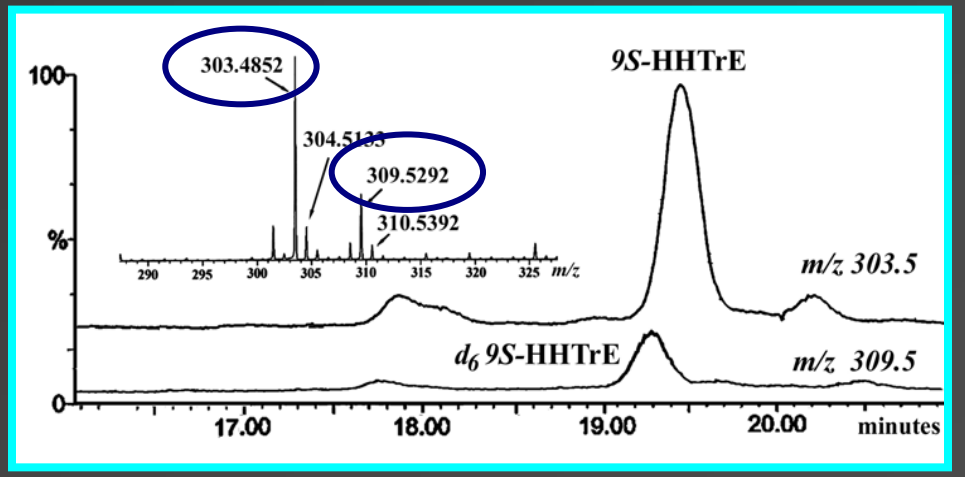
HPL



d_4-octadienal



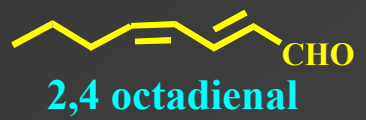
d_6-9-HHTrE



LC-MS of d_6-9-HHTrE

d'Ippolito et al., *Phytochemistry*, submitted

S. costatum



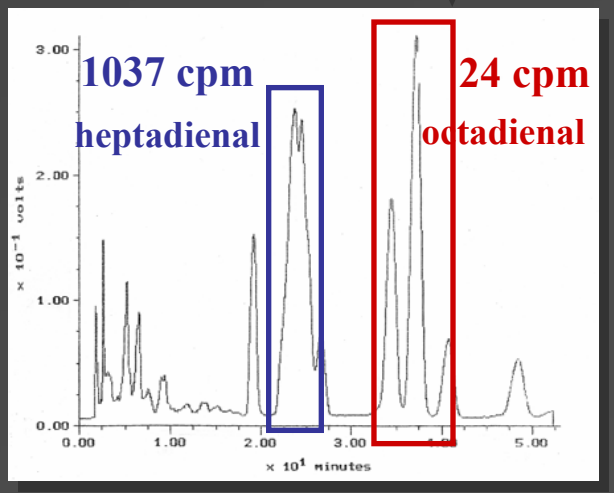
Biosynthesis of heptadienal

- $^{3}H_{10}$-**EPA** (20:5 ω3)
  2 µCi

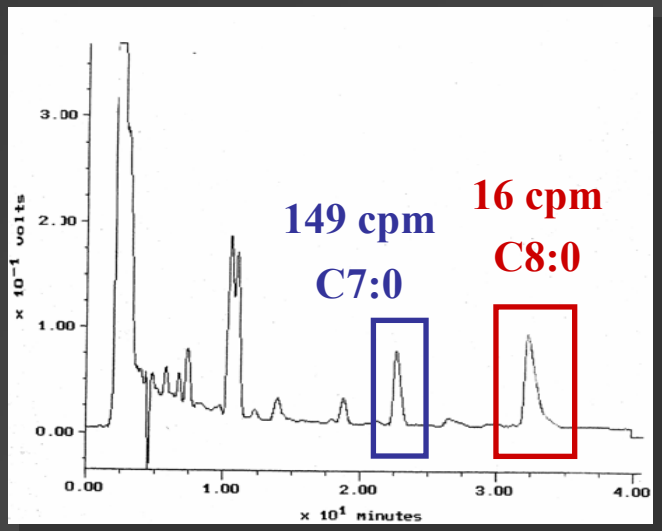
Cell-free
S. costatum

Derivatized
aldehydic pool

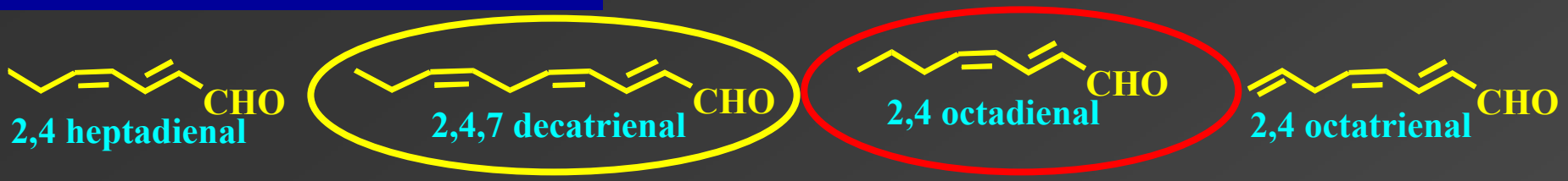
HPLC



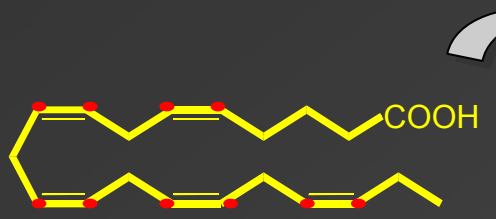
H_2
Pd/C 5%



Thalassiosira rotula



Biosynthesis of decatrienal

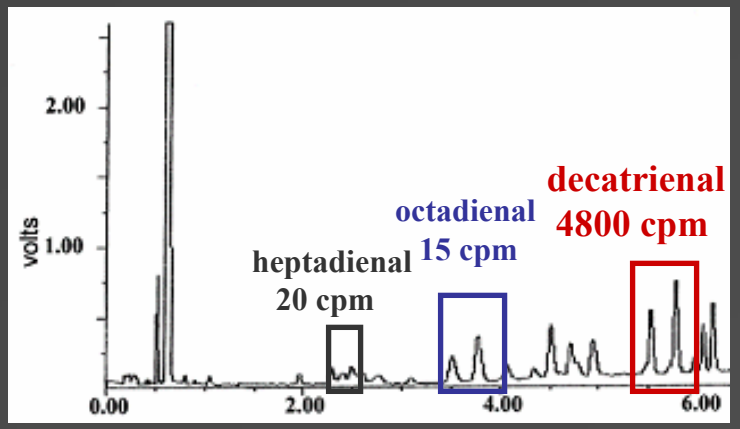


$^{3}H_{10}$-EPA (20:5 ω3)
2 µCi

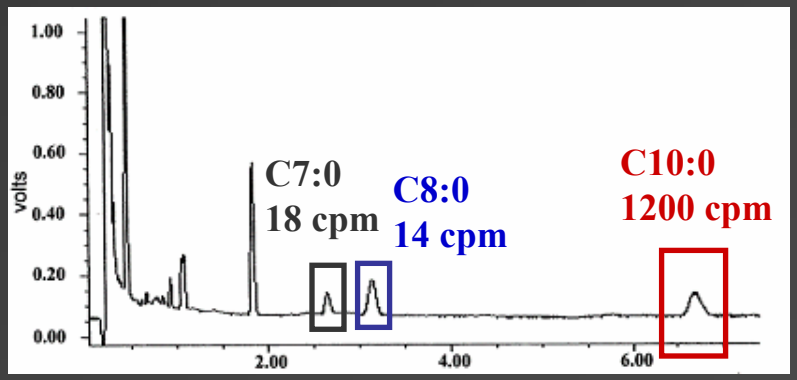
Cell-free
T. rotula

Derivatized
aldehydic pool

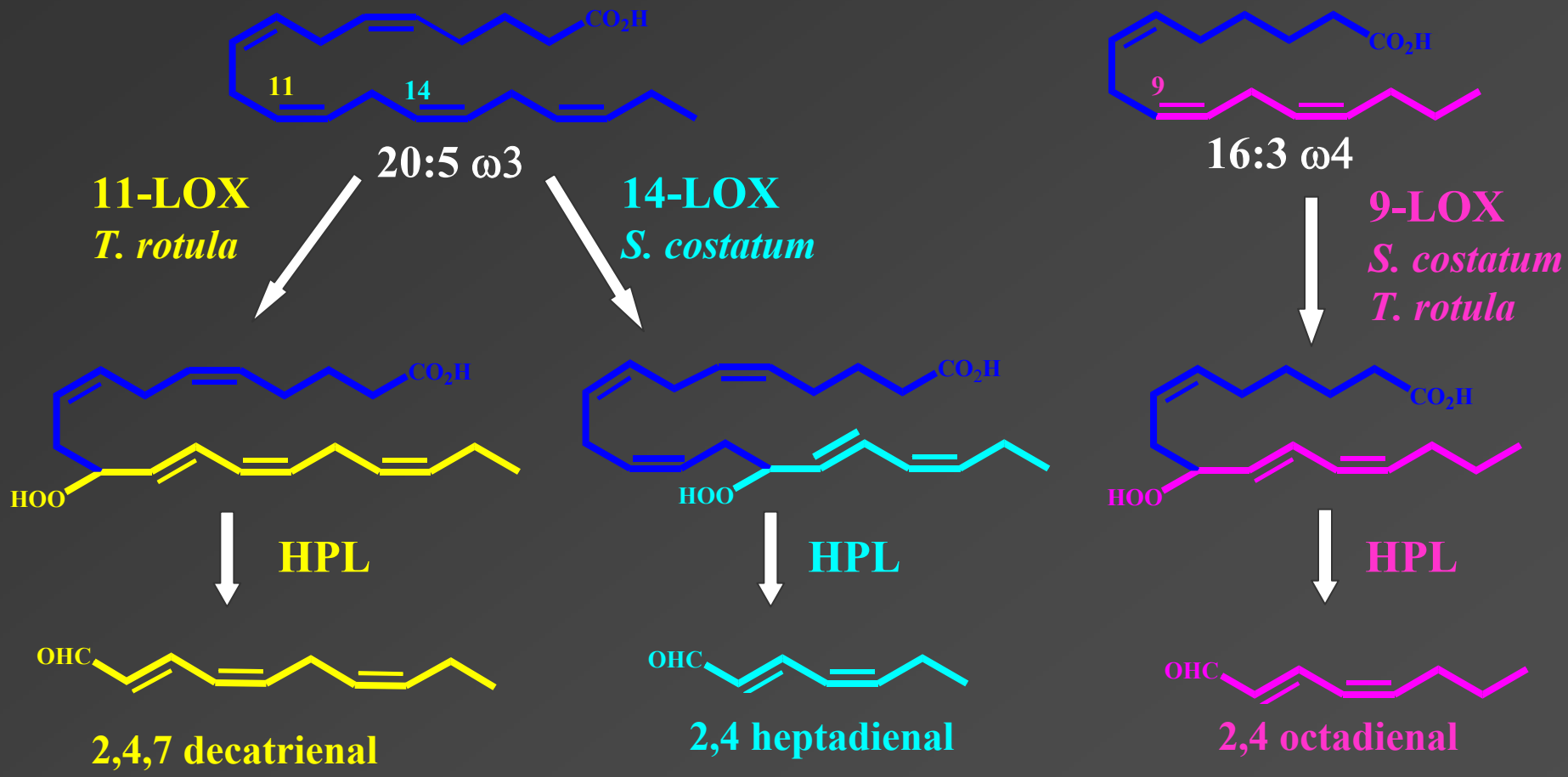
HPLC



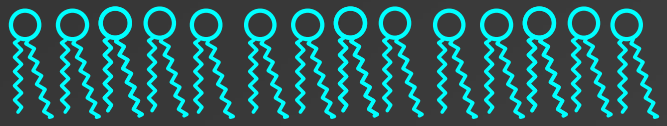
H_2
Pd/C 5%



Lipoxygenase Pathway



LOX= Lipoxygenase HPL=Hydroperoxidelyase



Hydrolytic Actvity

?

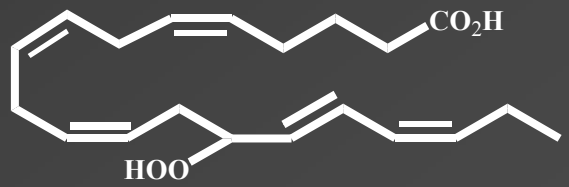
20:5 ω3

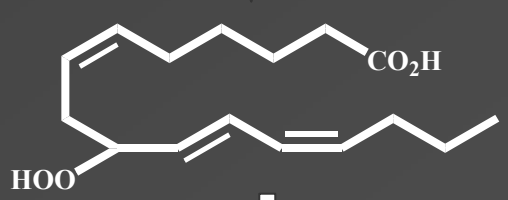
11-LOX
T. rotula

14-LOX
S. costatum

16:3 ω4

9-LOX
S. costatum
T. rotula





HPL

HPL

HPL

2,4,7 decatrienal

2,4 heptadienal

2,4 octadienal

Phospolipase A_2 as key enzyme (Pohnert et al. *Plant Physiology* 2002 129:103)

Distribution of fatty acids in complex lipids of *T. rotula*

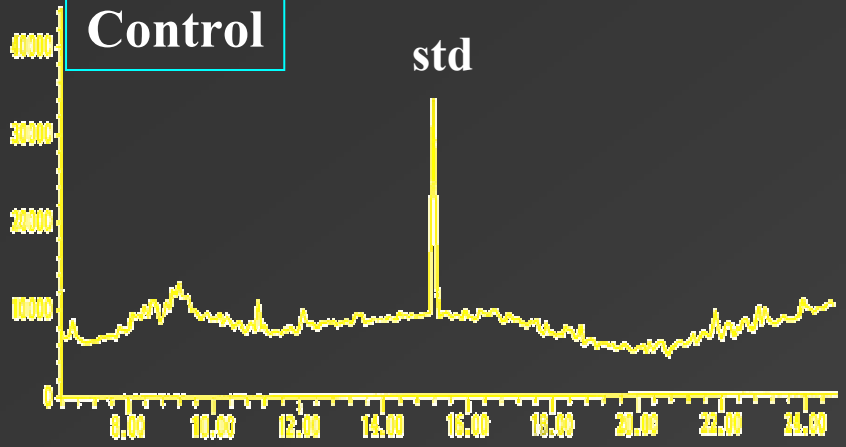
	Glycolipids	Phospholipids	Neutral lipids
C16:0	2,8%	4,8%	11,2%
C16:3ω4	17,1%	–	2,5%
C16:4ω1	17,5%	–	0,73%
C18:4ω3	10,4%	3,6%	8,6%
C20:5ω3	13,7%	30,3%	18,22%

Distribution of fatty acids in complex lipids of *S. costatum*

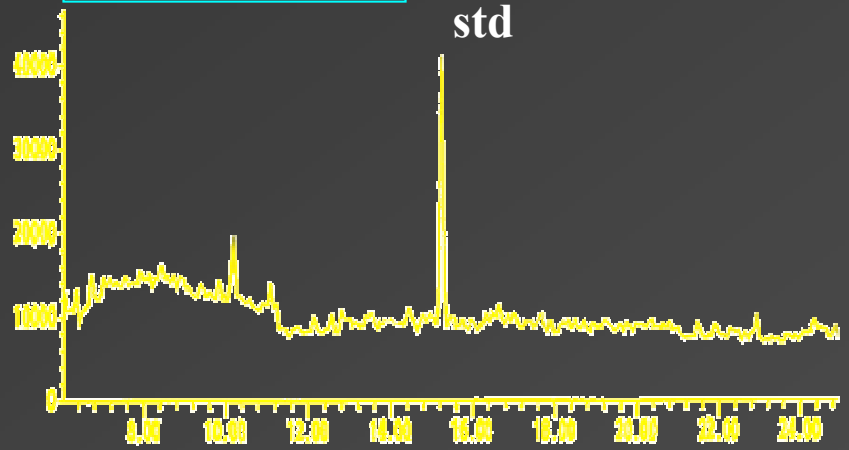
	Glycolipids	Phospholipids	Neutral lipids
C16:0	1,5%	18,6%	90%
C16:3ω4	26,3%	–	–
C16:4ω1	8,4%	–	–
C20:5ω3	16,9%	21,5%	–

Thalassiosira rotula

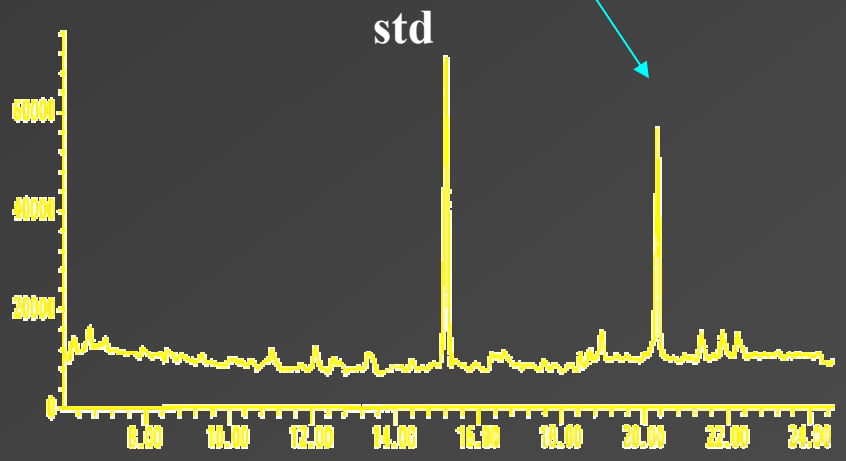
Control



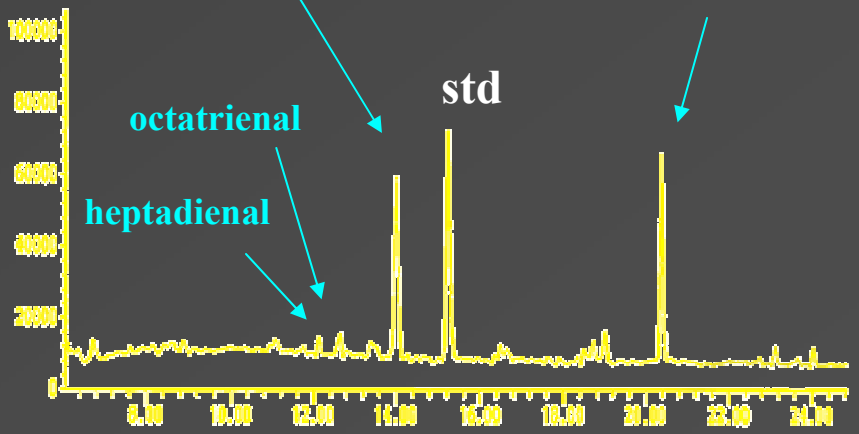
Triglycerides



Phospholipids



Glycolipids



Skeletonema costatum

Labelling complex lipids

$^{3}H_{10}$-EPA

24h incubation

The pelletted cells were traited with boiled methanol

Extraction
Purification

Natural labelled complex lipids

^{3}H-EPA-Glycolipids

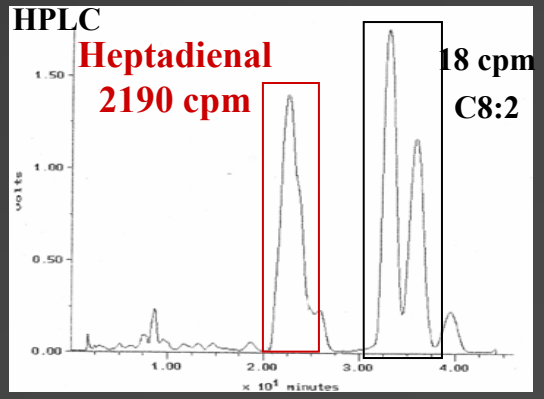
^{3}H-EPA-Phospholipids

S. costatum: incorporation of labelled complex lipids

^{3}H-EPA-Glycolipids

181800 cpm

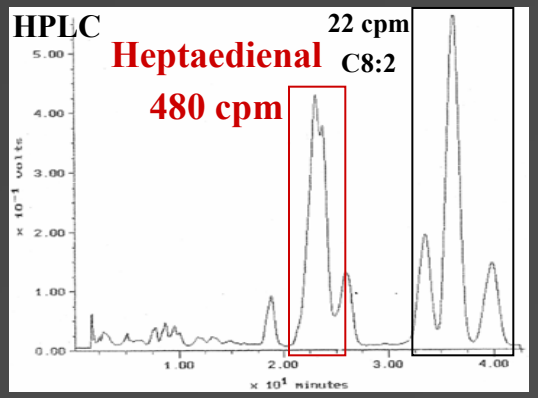
S. costatum



^{3}H-EPA-Phospholipids

933200 cpm

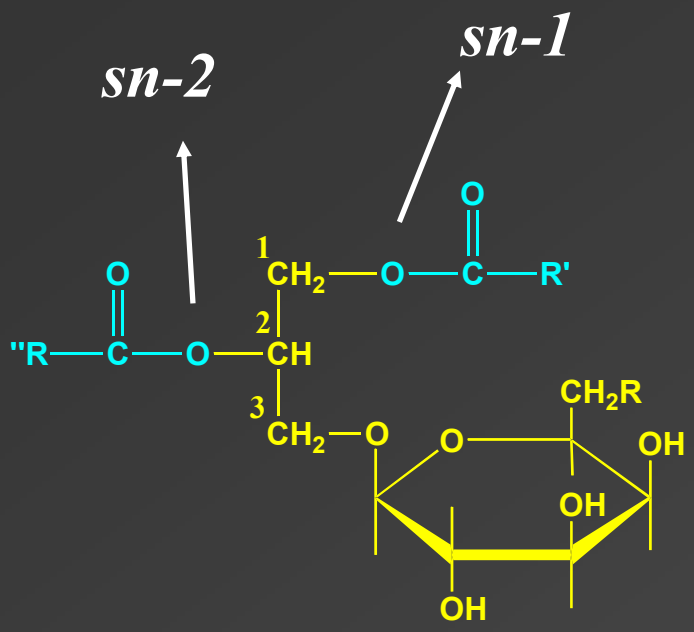
S. costatum

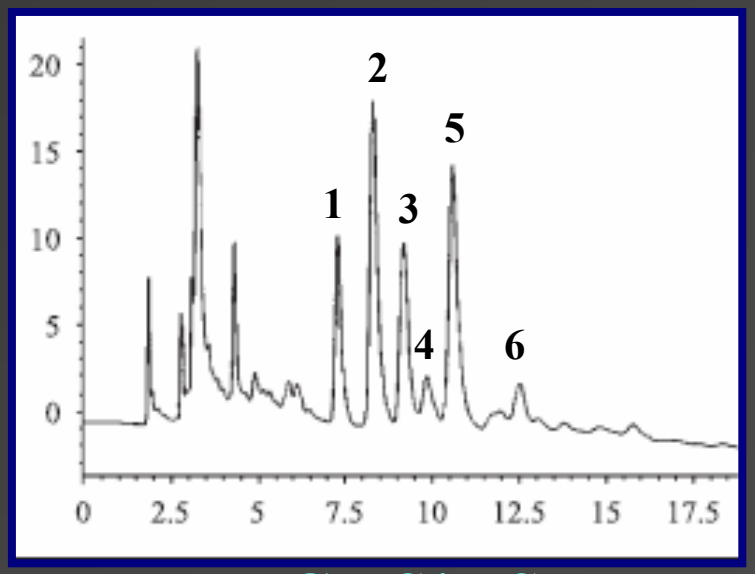


Higher affinity for glycolipids rather than for phospolipids

d'Ippolito et al. *BBA* 2004, 1686, 100-107

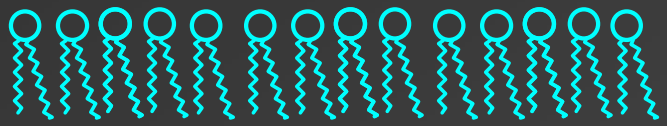
Glycolipids: main pool involved in the release of free fatty acids





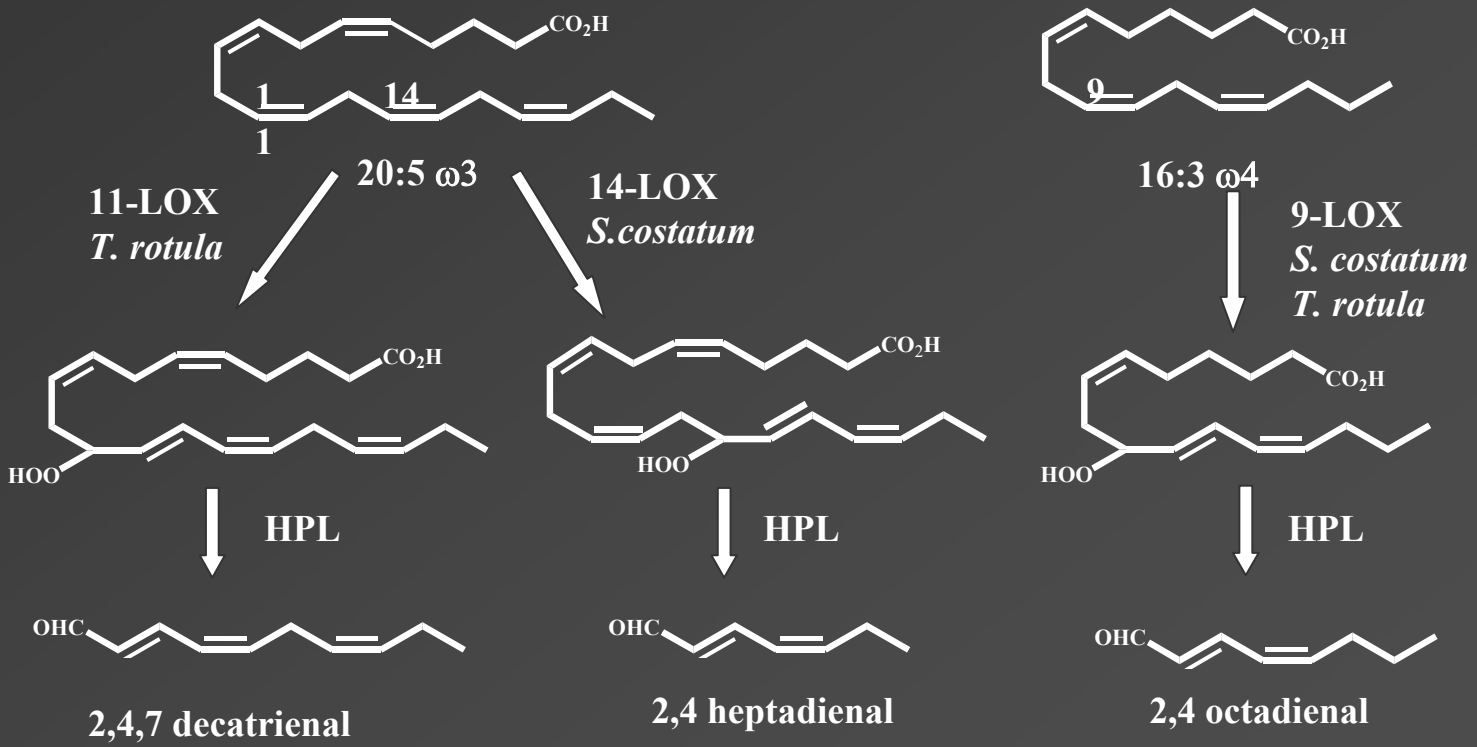
LCMS/MS

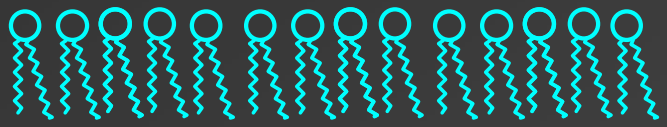
peak	%	*sn*-1	*sn*-2
1	13.2	16:3ω4	16:4ω1
2	25.9	16:3ω4	16:3ω4
3	16.1	20:5ω3	16:4ω1
4	6.5	16:2ω4	16:3ω4
5	26.7	20:5ω3	16:3ω4
6	6.5	20:5ω3	16:2ω4



Hydrolytic Actvity

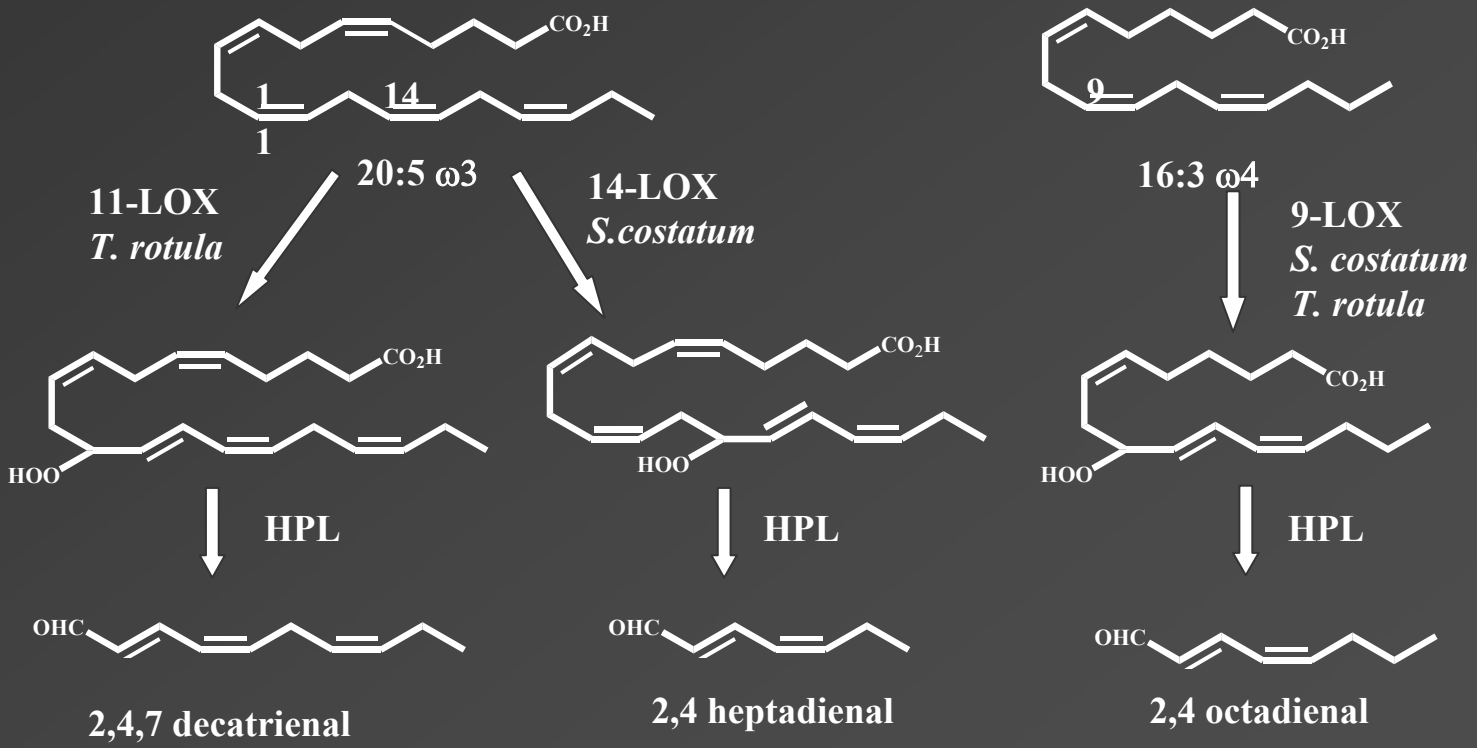
?





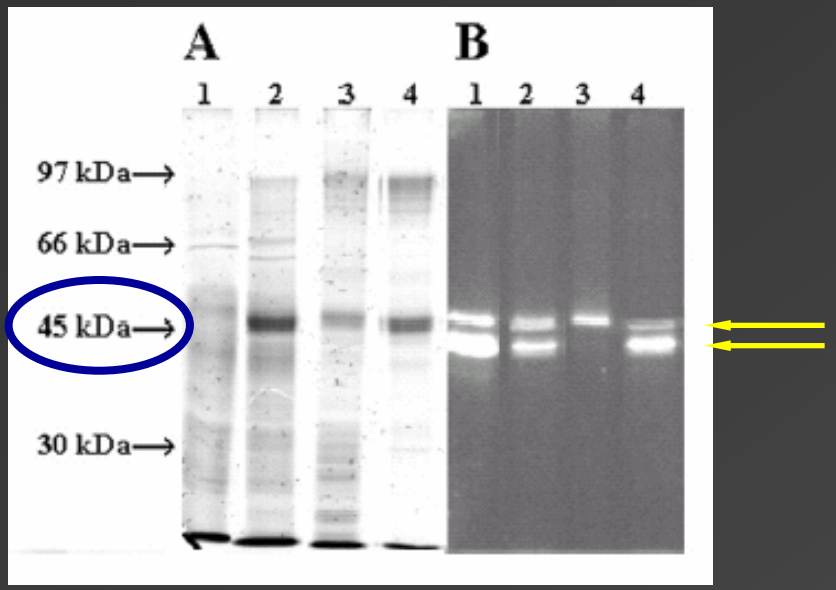
-hydrolysis of glycolipids and phospholipids
-major affinity toward glycolipids
-action on both *sn* positions
-no activity on triglycerides

Lipolytic Acyl Hydrolase (LAH)



Preliminary data on lipase activity

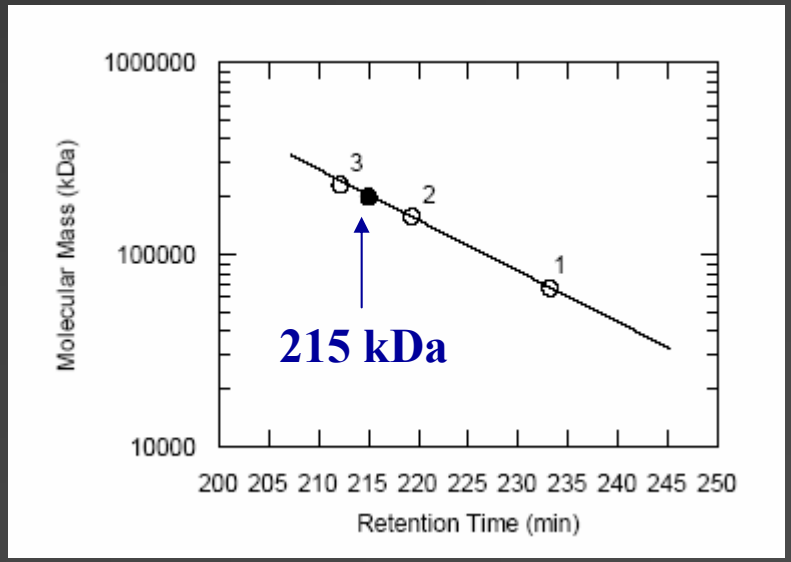
SDS-PAGE



Aspecific (Comassie Blu)

Specific staining

Native molecular mass determination



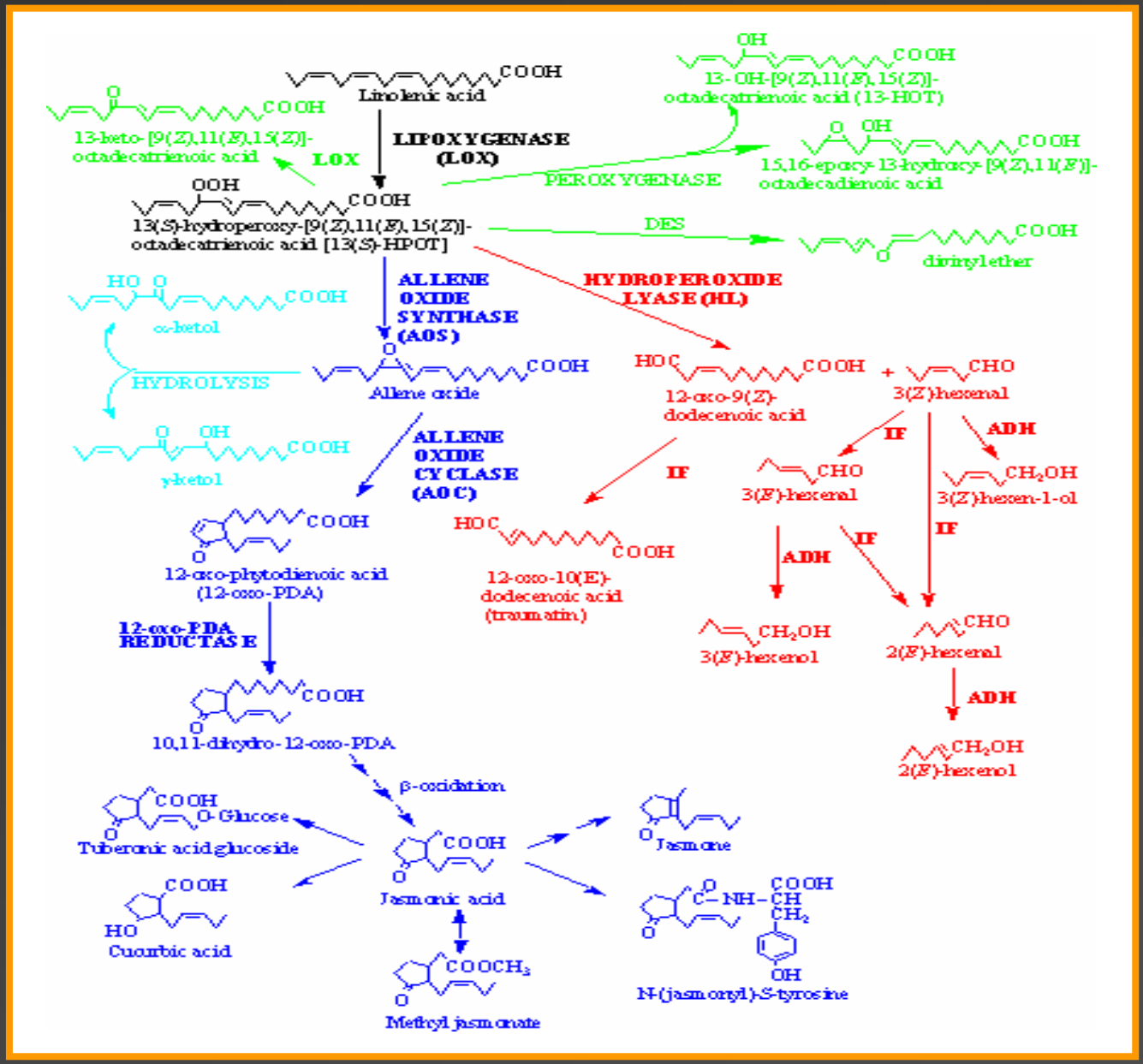
The different mass values between denaturing and native conditions suggested:

-an oligomeric structure

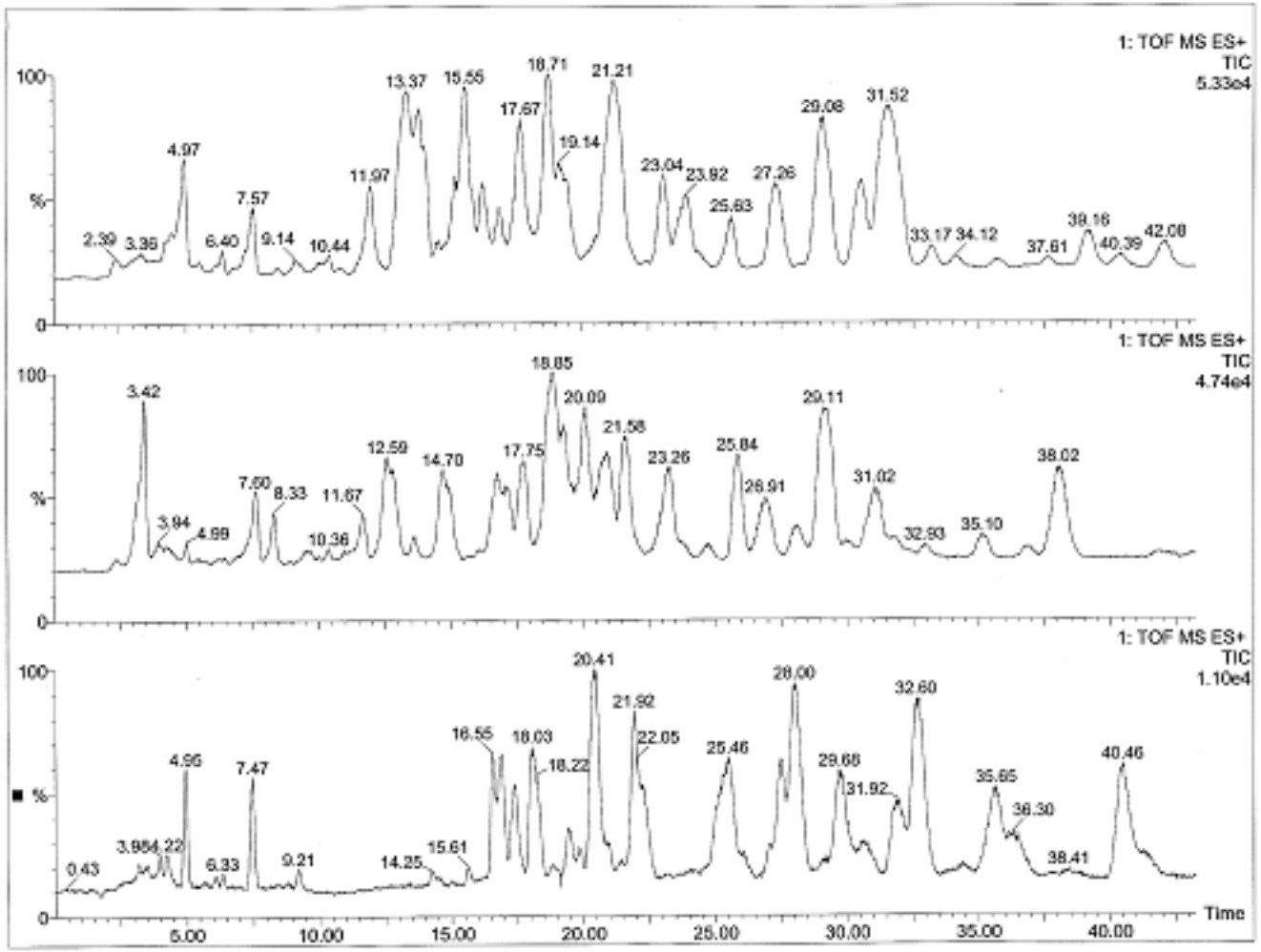
-multi-molecular aggregate

Cutignano et al., *ChemBioChem* (submitted)

Oxylipin pathway in plants

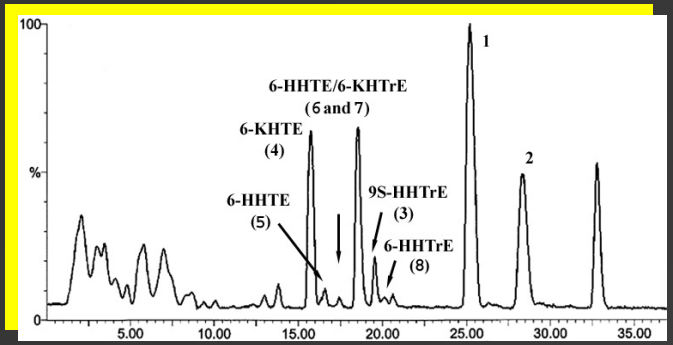


Oxylipin profile in three marine diatoms

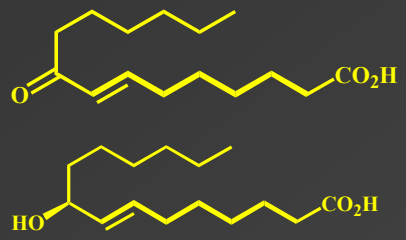


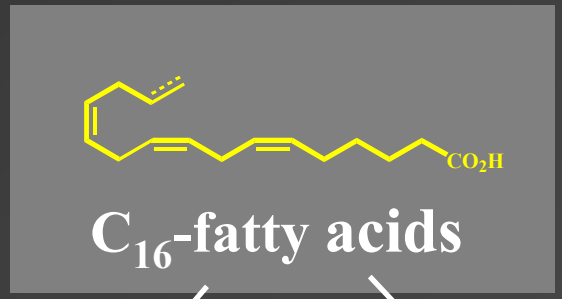
LC-MS chromatogramm

T. rotula: unprecedented C_{16} oxylipin pathway



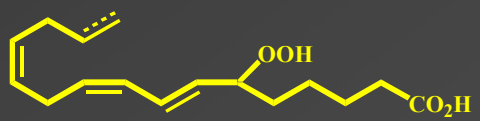
Monounsaturated derivatives



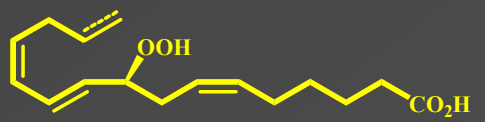


6-LOX

9*S*-LOX



6-HpHTrE



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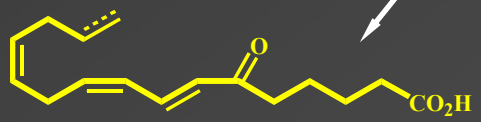

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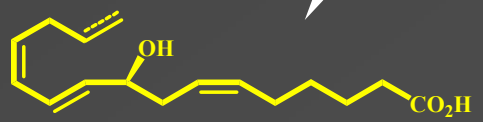

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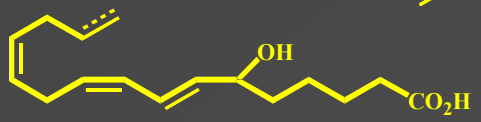

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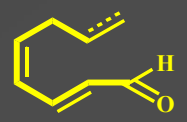

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d'Ippolito et al., *OBC*, 2005 3:4065-4070

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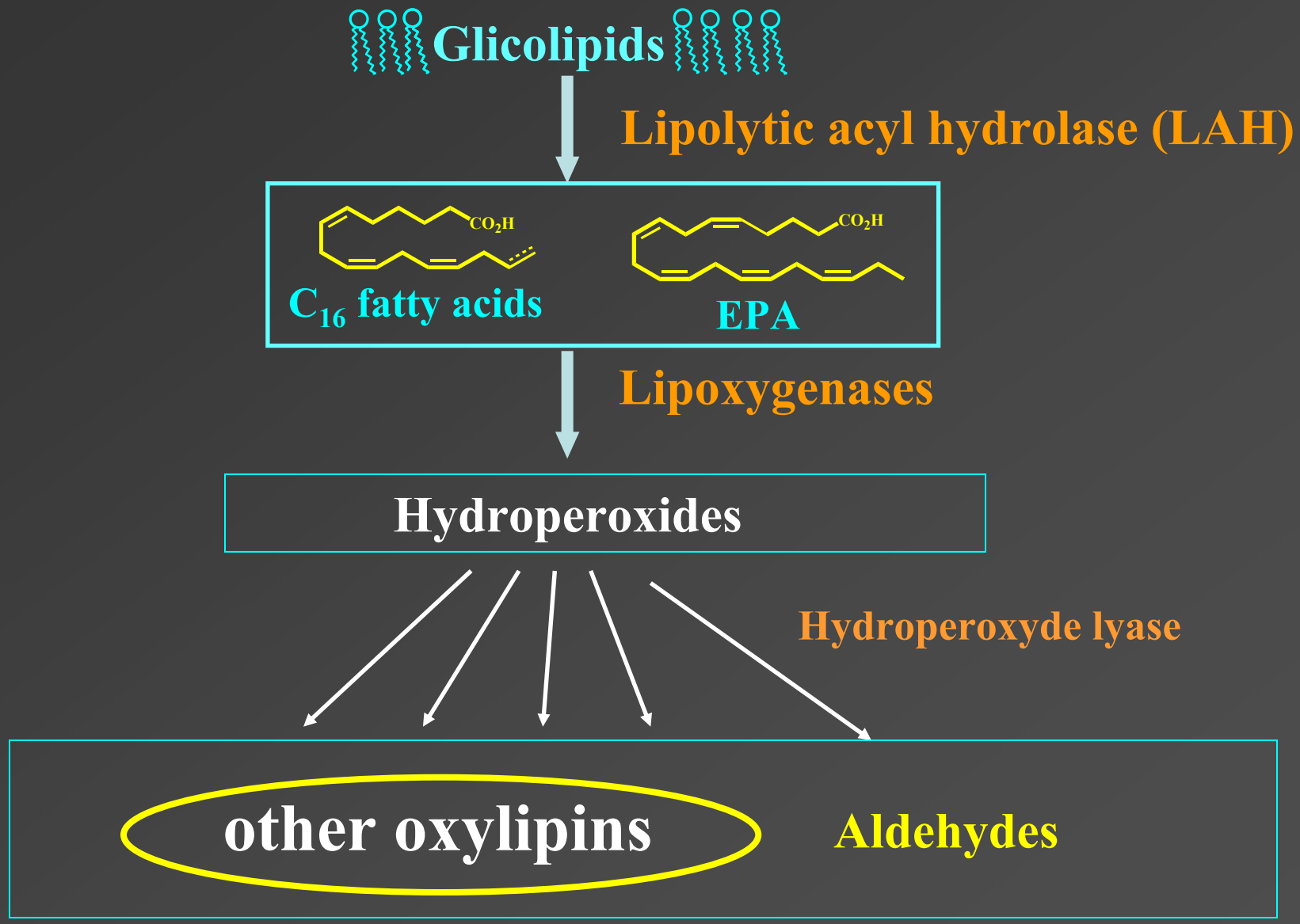

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