Adventures of natural products chemists into "lipidomics world "

UNIVERSITÀ DEGLI STUDI DI TRENTO

Graziano Guella

- *Bioorganic Chemistry Lab-Dept. of Physics*
- *Interdepartmental Center of Integrative Biology (CIBIO)*
- *CNR Institute of Biophysics, Trento*

Scuola delle Sostanze Naturali e
di Chimica Biorganica
"Luigi Minale"

Napoli, 5-9 Giugno 2011

LECTURE OUTLINE

1st

Lipids structural features

Lipids biosynthesis
Membrane structures

2nd

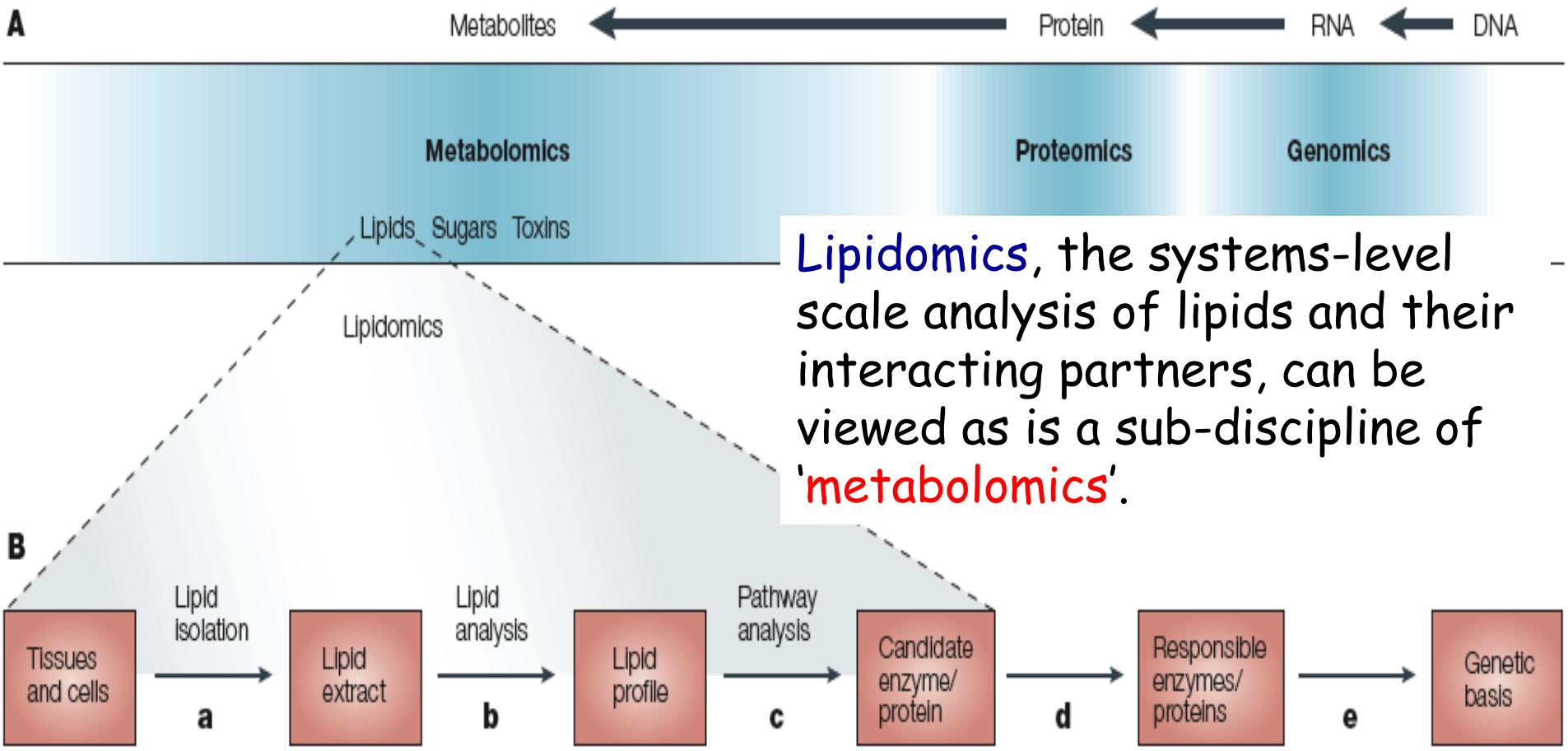
Methodologies in lipid analysis

Examples from our recent research activity

3th

Perspectives & Conclusions

From DNA to lipids and… from lipids to DNA

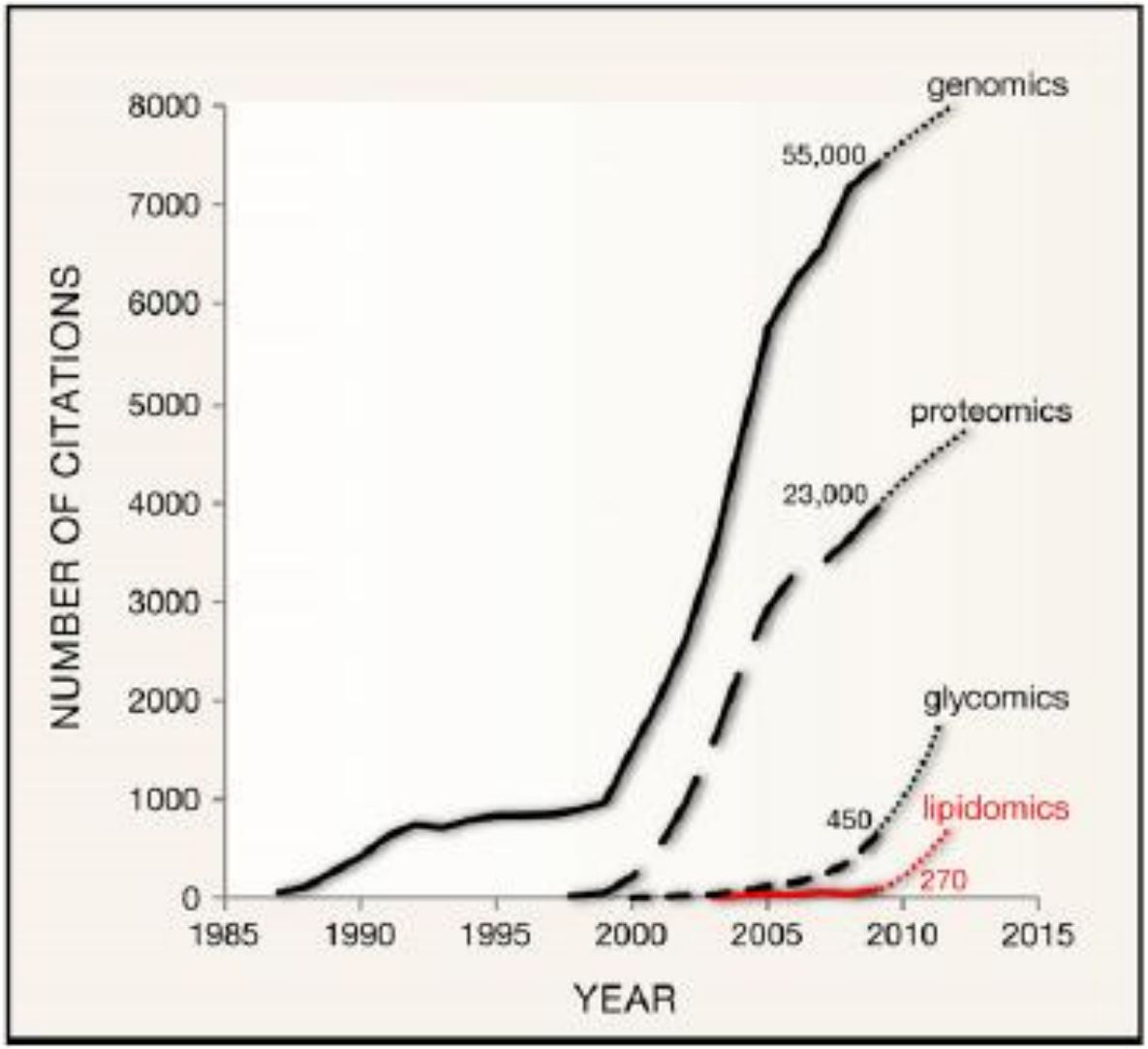


Lipidomics, the systems-level scale analysis of lipids and their interacting partners, can be viewed as is a sub-discipline of 'metabolomics'.

Genes encode proteins that collectively, and together with environmental factors, lead to the metabolite inventory of a cell, tissue or body fluid.

M.R. Wenk, *Nature* **2005**

Lipidomics is an emerging field…



M.R. Wenk, *Lipidomics: New Tools and Applications", Cell* **2010**

A Global Approach to Lipid Analysis in Biological Systems

- Lipids are broadly defined as hydrophobic or amphiphilic small molecules that originate either entirely or in part from two distinct types of building blocks: ketoacyl and isoprene groups.

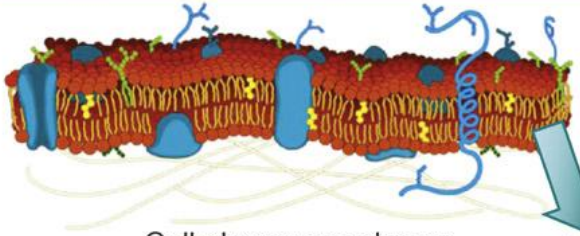
- Lipids are structurally highly diverse owing to the many possible variations of the lipid building blocks and how these blocks are linked. It has conservatively estimated that the theoretical number of lipids covering major lipid classes is close to 200 000.

- Lipids are very abundant in biological systems, constitute 50% of the mass of most animal cell membranes and exhibit an important degree of specialization in specific cellular compartments.

- Maintenance of an appropriate lipid composition in the cellular membranes is required to ensure membrane fluidity, topology of attached proteins, activity of membrane-bound enzymes, degree of exposure of surface proteins, lateral mobility of receptors and activation of specific signaling pathways.

Diverse biological roles of lipids

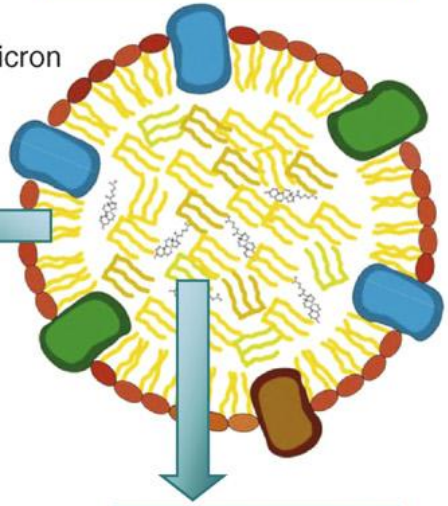
Membrane structure and function



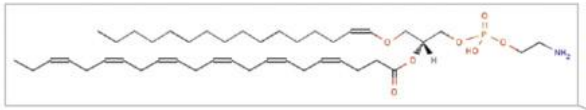
Cell plasma membrane

Energy storage and lipid transport

Chylomicron

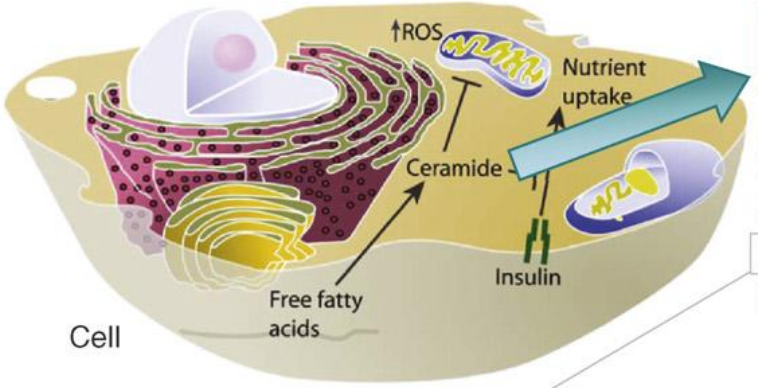


Membrane lipids



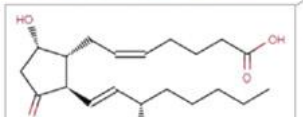
e.g.
PC(16:0/18:1)
PC(18:0/0:0)
PE(O-16:0(1Z)/22:6)
SM(d18:1/24:1)
Cer(d18:1/24:1)

Cell signaling and metabolic homeostasis



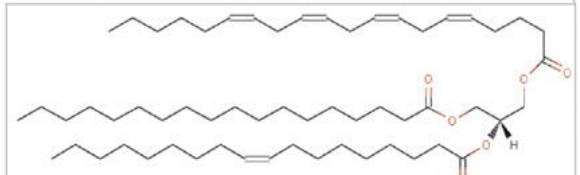
Signaling lipids

e.g.
PC(O-16:0/2:0)
PA(18:1/0:0)
Cer(d18:1/18:0)
PGD2
S1P



Storage lipids

e.g.
TG(14:0/16:0/16:0)
TG(16:0/18:0/18:2)
TG(18:0/18:1/20:4)
DG(16:0/16:0)
CE(20:4)



STRUCTURAL ASPECTS OF LIPIDS

Lipids : definition

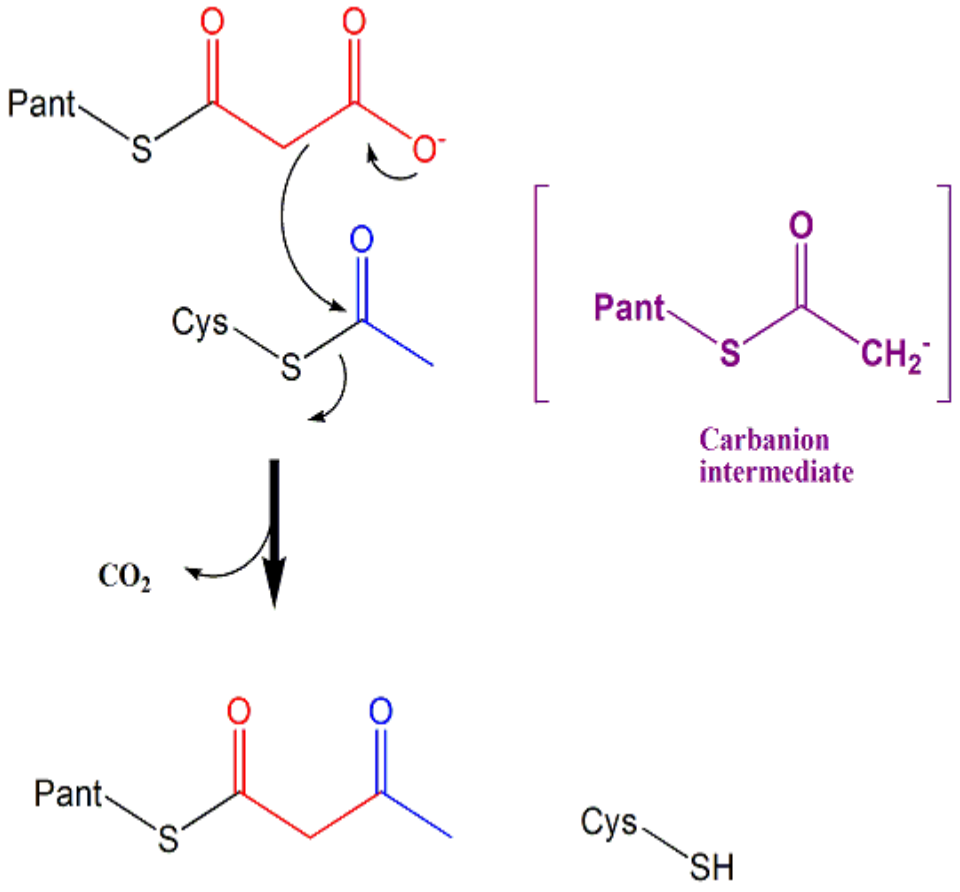
The major difference between lipids and other major components of living tissue is their solubility in organic solvents.
Lipids are defined either by these solubility characteristics or by the presence of long hydrocarbon chains; however, not all lipids satisfy both definitions.

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules that originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups.

Fahy,E. et al, Journal of Lipid Research, 2005, 46, 839

Lipids classification:biosynthetic routes

1: Carbanion-based condensation

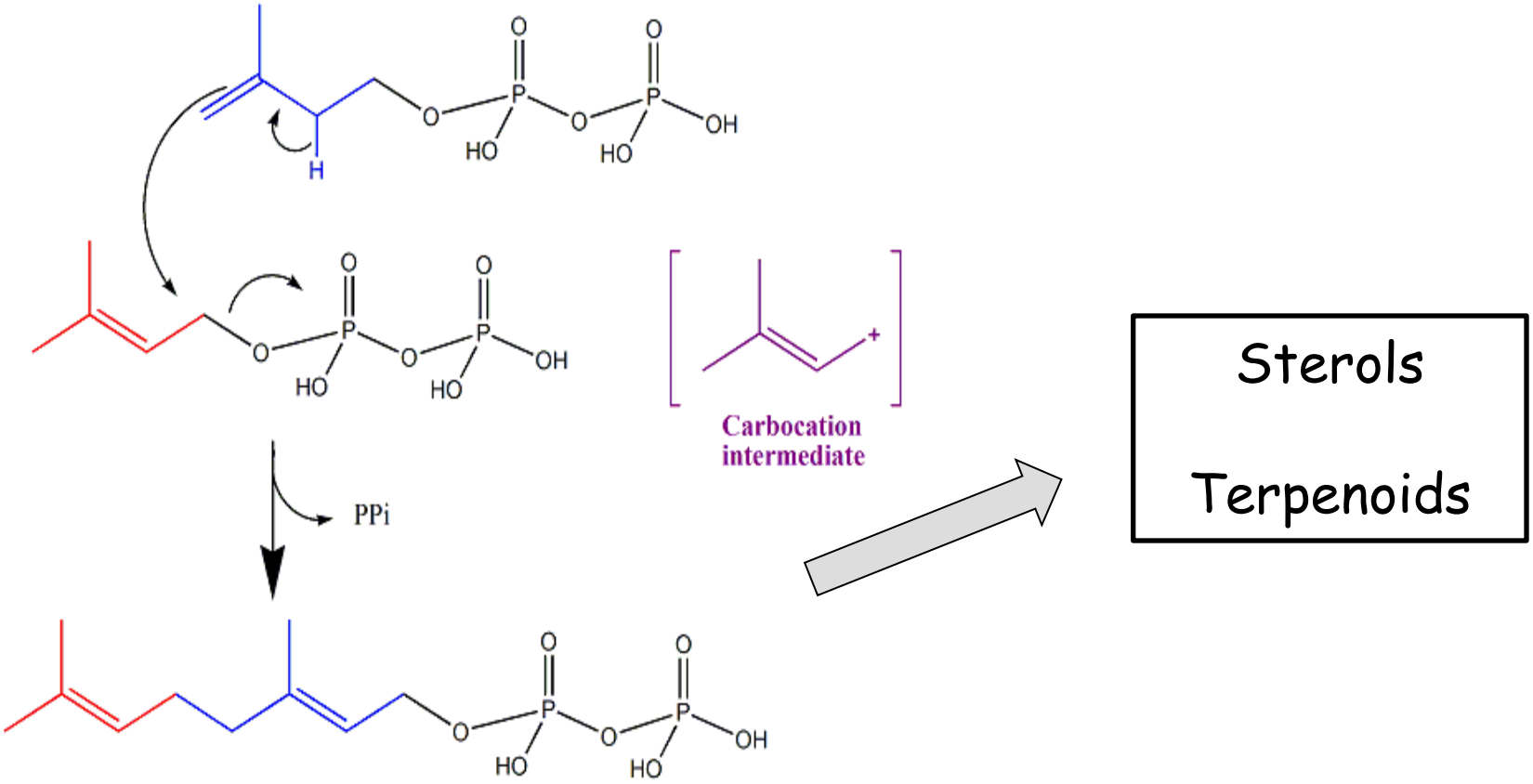


CATEGORIES

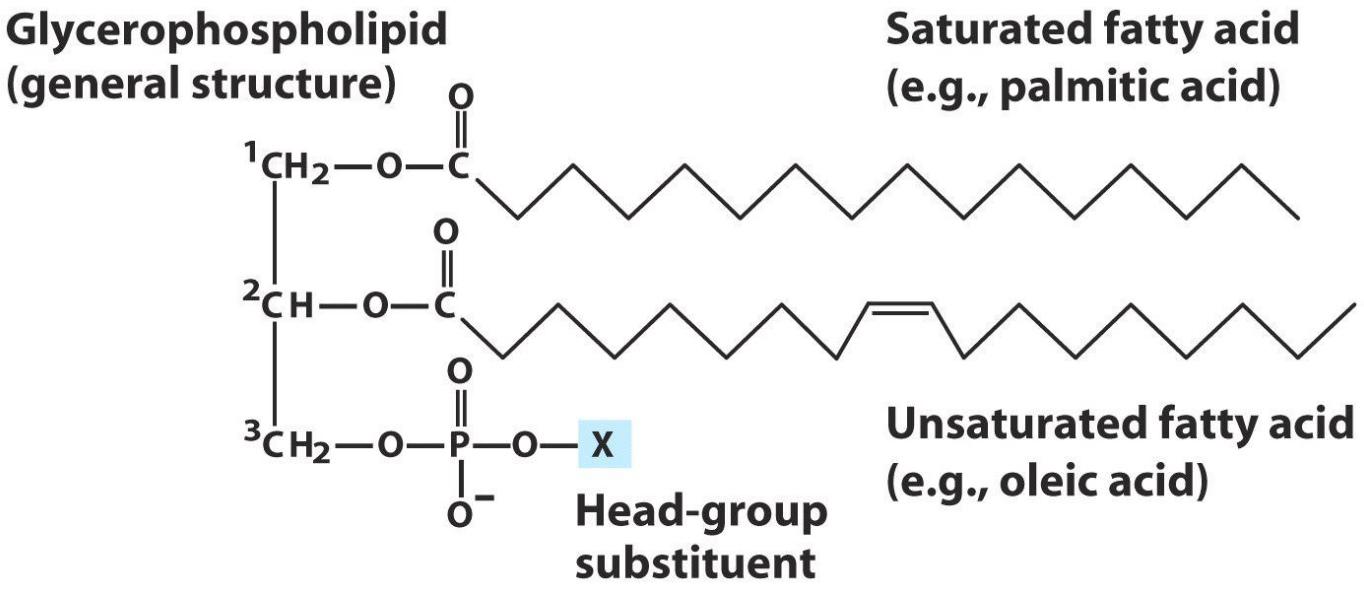
Fatty Acyls
Glycerolipids
Glycerophospholipids
Sphingolipids
Saccharolipids
Polyketides

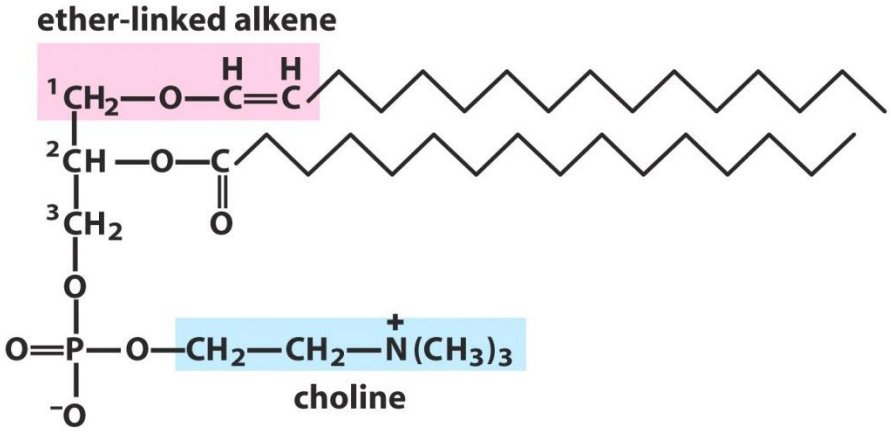
Lipids classification:biosynthetic routes

2: Carbocation-based condensation

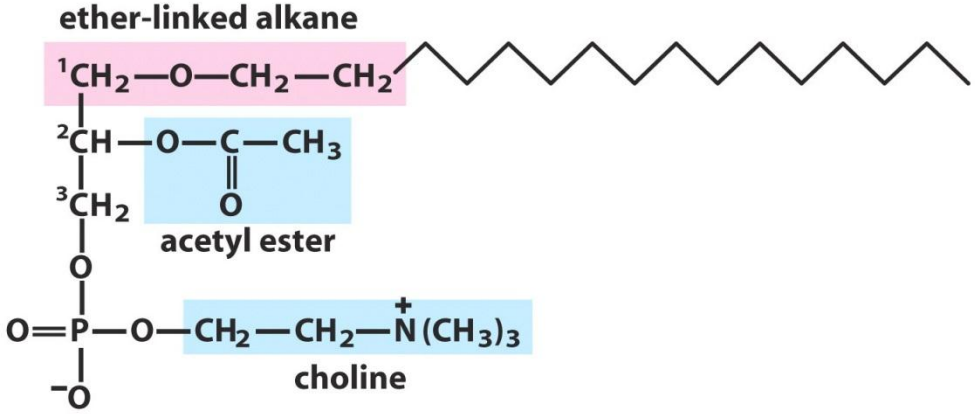


Structural diversity of glycerophospholipids



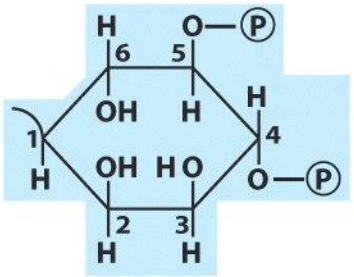
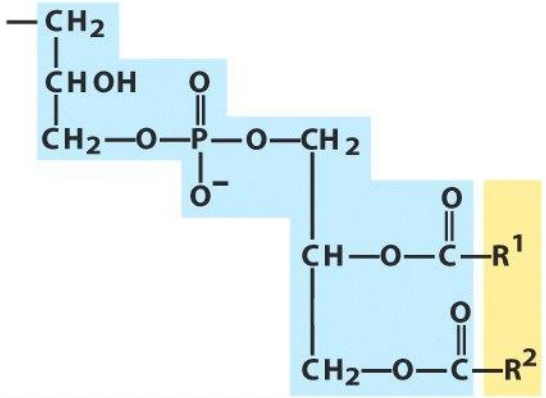


Plasmalogen

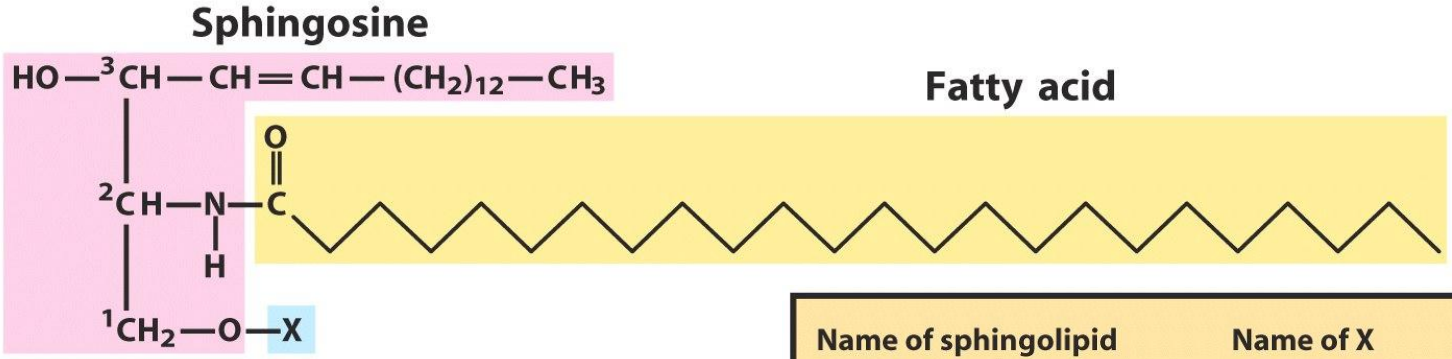


Platelet-activating factor

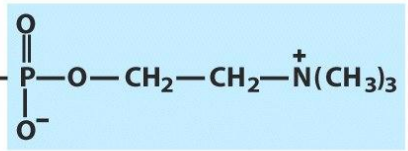
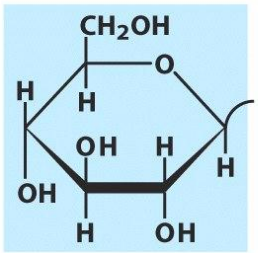
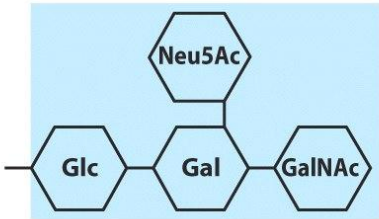
Structural diversity of glycerophospholipids

Name of glycerophospholipid	Name of X	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—	—H	−1
Phosphatidylethanolamine	Ethanolamine	$-CH_2-CH_2-\overset{+}{N}H_3$	0
Phosphatidylcholine	Choline	$-CH_2-CH_2-\overset{+}{N}(CH_3)_3$	0
Phosphatidylserine	Serine	$-CH_2-\underset{\displaystyle COO^-}{\underset{|}{CH}}-\overset{+}{N}H_3$	−1
Phosphatidylglycerol	Glycerol	$-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-OH$	−1
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate		−4
Cardiolipin	Phosphatidyl-glycerol		−2

Structural diversity of sphingolipids

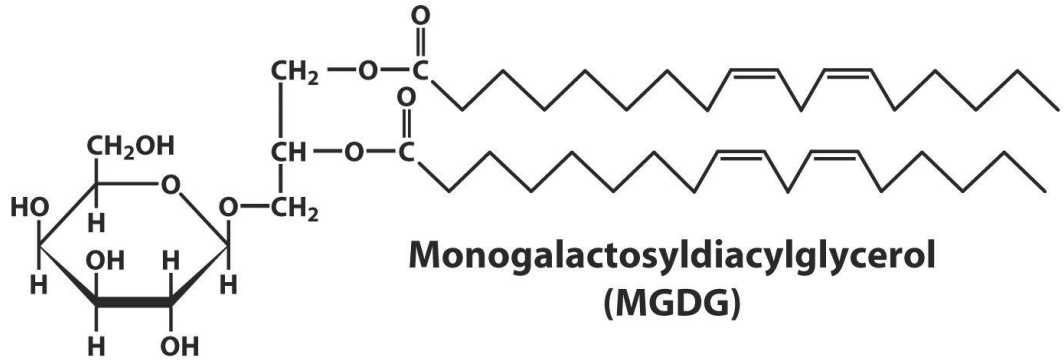


Sphingolipid (general structure)

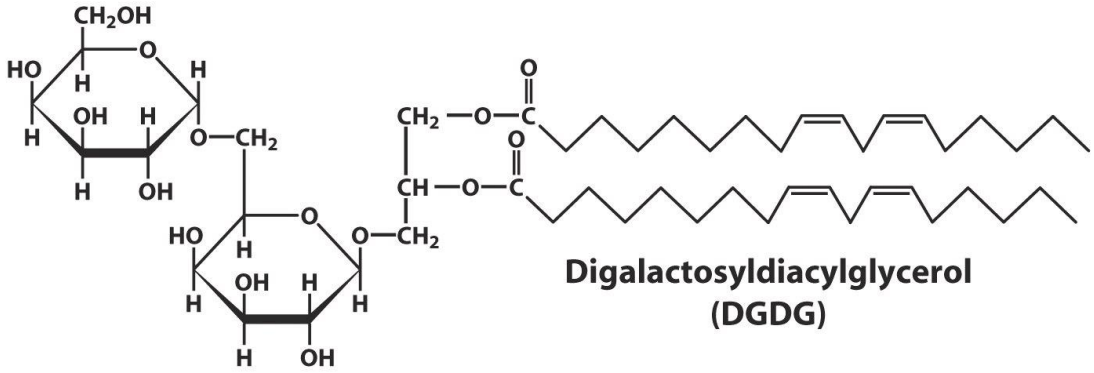
Name of sphingolipid	Name of X	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Galactolipids and Sulfolipids in Chloroplasts

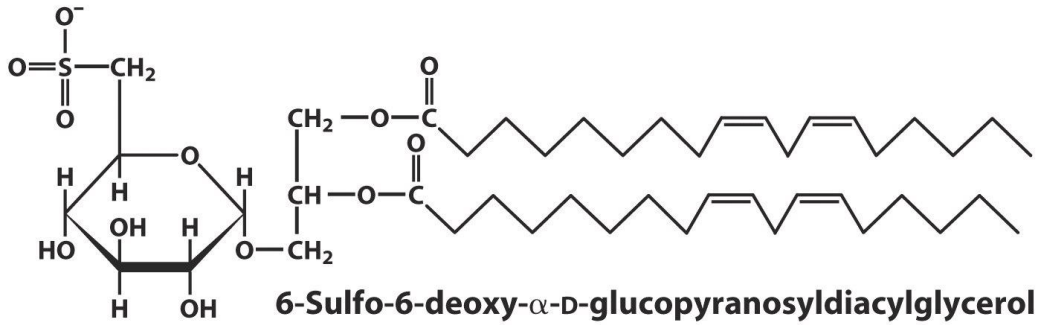
- Galactose (or DiGal or TriGal) attached to C3 of glycerol
- Sulpholipids contain sulphonate on sugar
- Sulphonate charge replaces typical phosphate charge



Monogalactosyldiacylglycerol (MGDG)

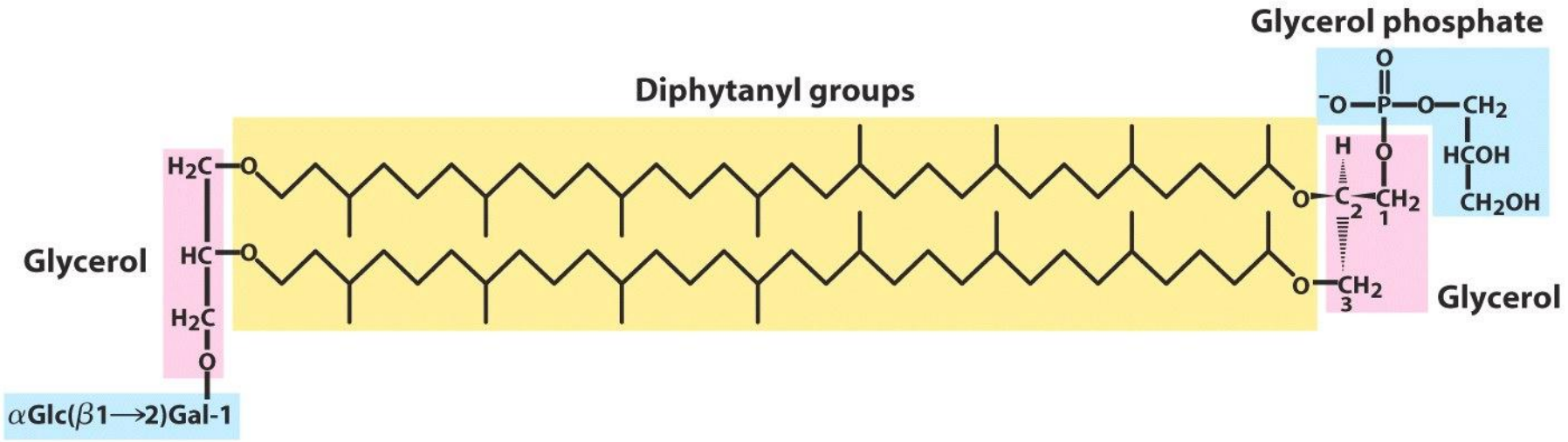


Digalactosyldiacylglycerol (DGDG)



6-Sulfo-6-deoxy-α-D-glucopyranosyldiacylglycerol (a sulfolipid)

Archael "Extremophile" Lipids



- Longer acyl chains and dual head groups can can replace 2 normal phospholipids
  - Replace a bilayer with a monolayer
- Ether linkages
- More stable at high temperatures, acid environments

Lipids chemical diversity

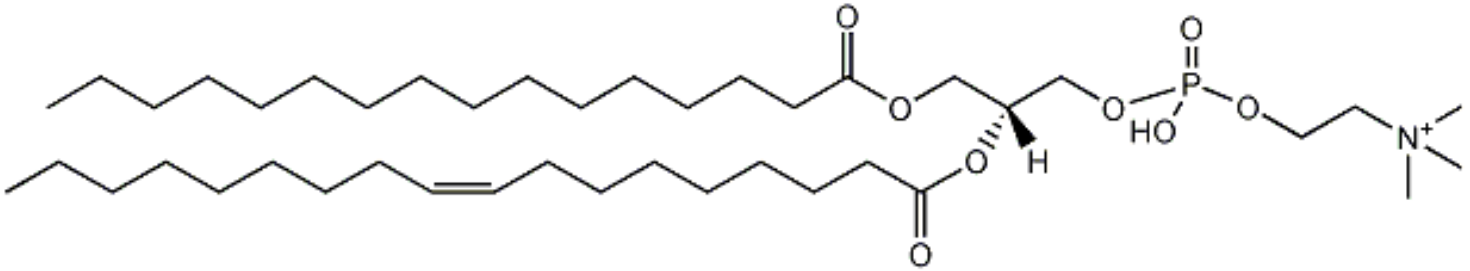
Chemodiversity of lipids given by:

- Backbone type : glycerol or sphingosine
- Head type : Phospho- or glyco
- Acyl, alkyl, vinyl-ether, isoprenyl and oxidized chains
- Number of chains: MAG,DAG, TAG, Lyso PL, PAF etc..
- Chains length and unsaturation index
- Double bonds positions and E/Z stereochemistry: ω-6/ω-3 PUFA
- Regioisomeric distribution of the chains at sn-1 and sn-2
- Phospho-heads types: PC,PE, PG, PS,PI, CL, lyso
- Absolute stereochemistry at sn-2 of glycerol and of oxylipins chiral centers (hydroperoxy- derivatives produced by LOXs)

How to call POPC in the new nomenclature system?

Compound ID : LMGP01010005

http://www.lipidmaps.org/



1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine

Database identifier

-Digits 1-2: Database: LM (LIPID MAPS)

-Digits 3-4: Category: GP (Glycerophospholipids)

-Digits 5-6: Class: 01 (Glycerophosphocholines)

-Digits 7-8: Subclass: 01 (Diacylglycerophosphocholines)

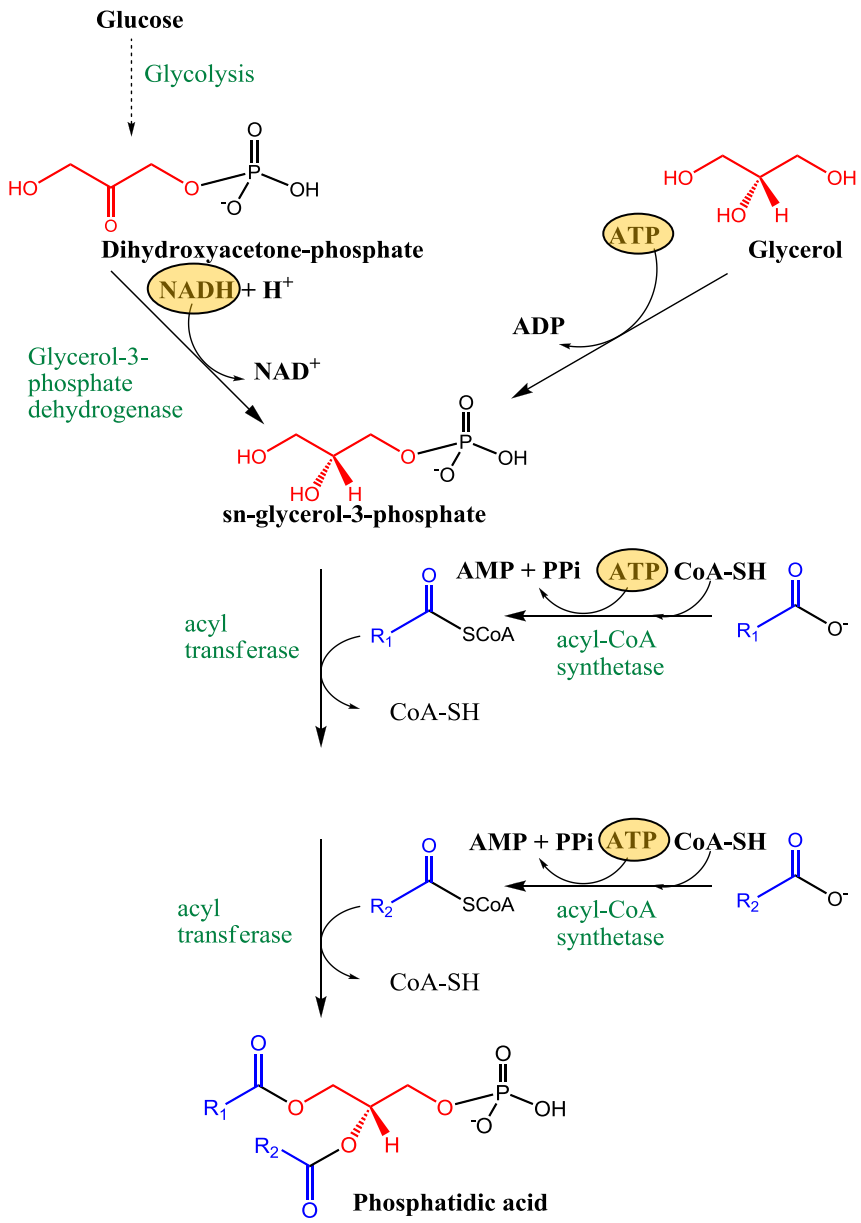
-Digits 9-?: Optional additional class levels (typ. not required)

-Last 4 digits: Unique identifier within subclass: 0005

This system can specify 1.68 million individual lipids.
Advantages of this alphanumeric system of lipid nomenclature for database storage and retrieval (bioinformatics manageability).

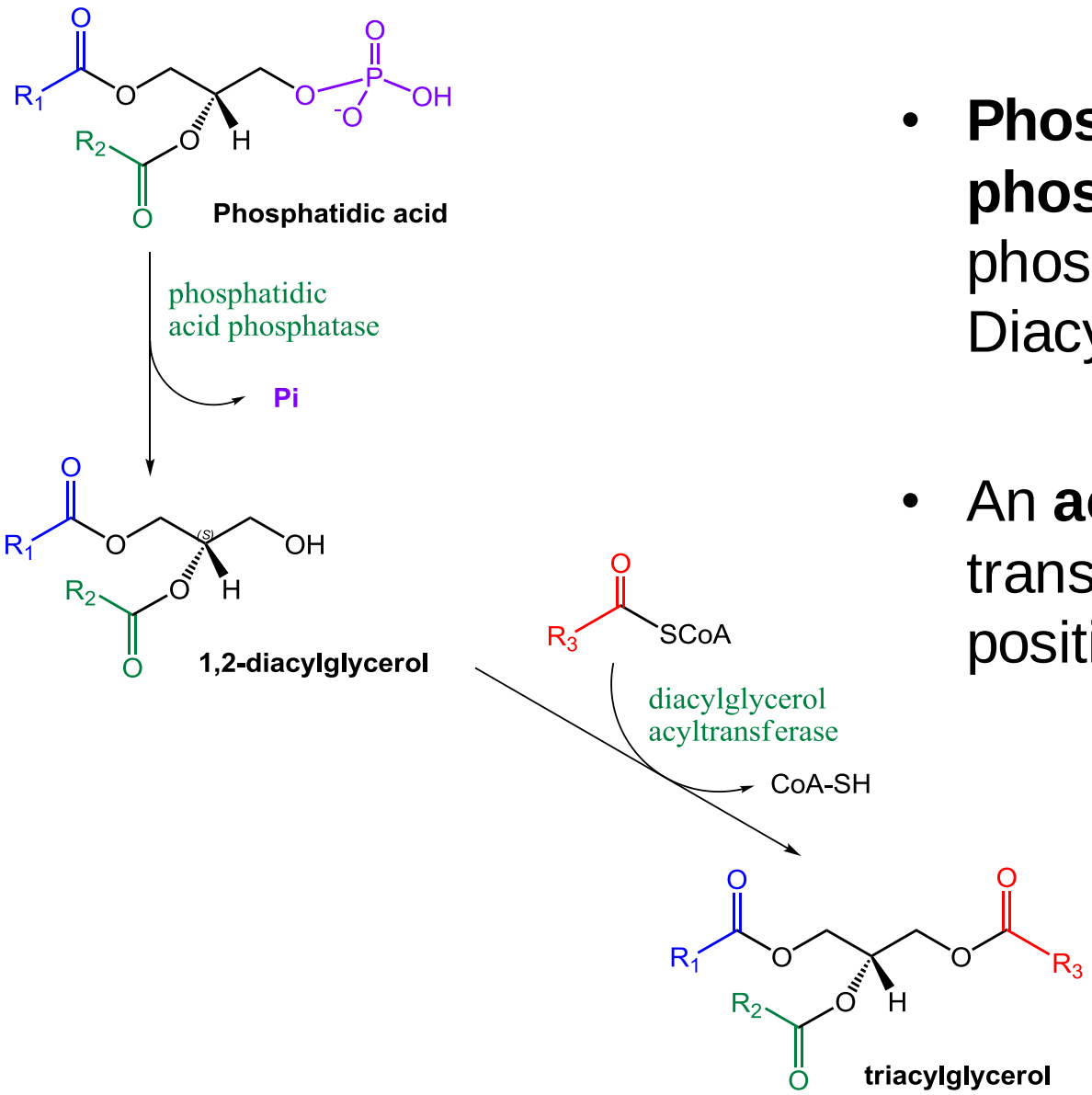
BIOSYNTHETIC PATHS TO PHOSPHOLIPIDS

Biosynthesis of phosphatidic acid



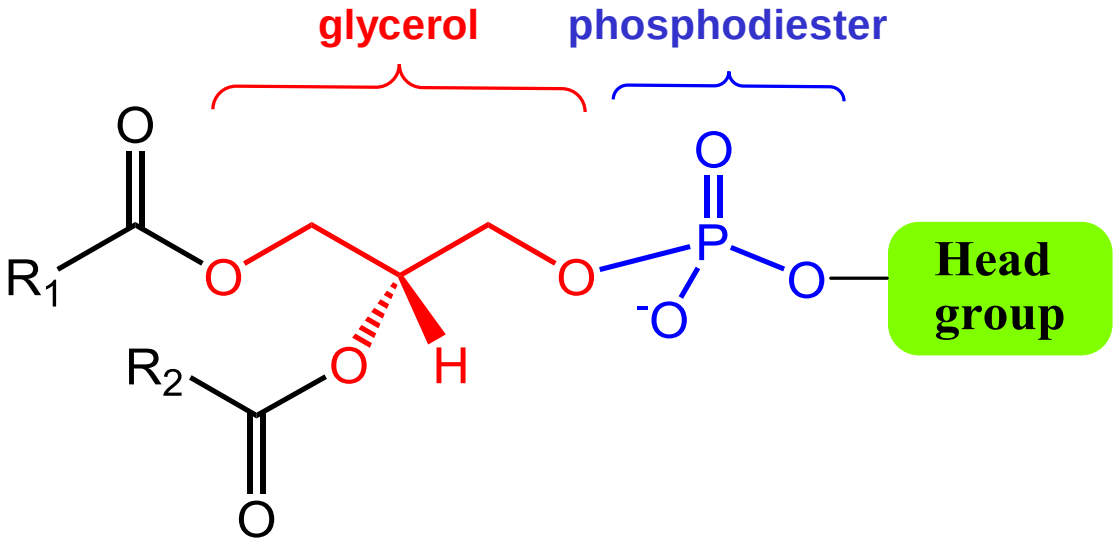
- **Precursors**
  - Fatty acids
  - *sn*-glycerol-3-phosphate
- ***sn*-glycerol-3-phosphate is produced from the**
  - Reduction of DHAP by glycerol phosphate dehydrogenase OR
  - Phosphorylation of glycerol by glycerol kinase and ATP
- **Acyl transferases** perform two successive esterifications with fatty acyl Co A to generate phosphatidic acid

Biosynthesis of triacylglycerols (TAGS)



- **Phosphatidic acid phosphatase** removes the phosphate producing 1,2-Diacylglycerol
- An **acyl transferase** transfers an acyl CoA to position 3.

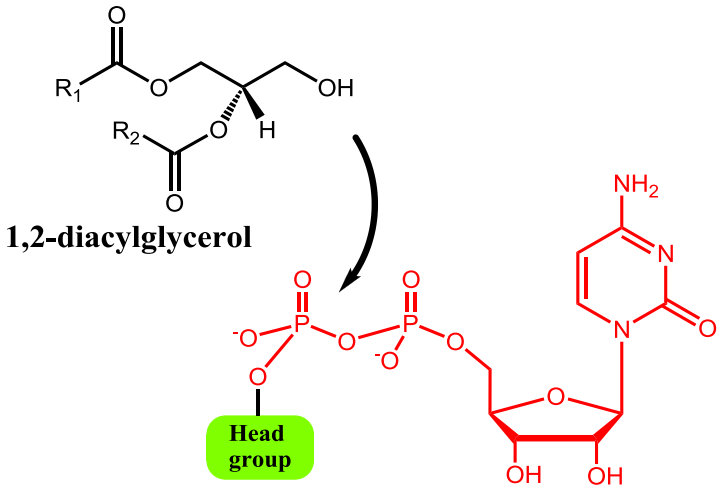
Biosynthesis of glycerophospholipids (GPL)



- Glycerophospholipids (or phospholipids) can be made from
  - Phosphatidic acid OR
  - Diacylglycerol
- There are many different head groups which can be linked to the C3 of glycerol by a phosphodiester bond
- Cytidine triphosphate (CTP) provides the synthetic energy in the synthesis of all PLs

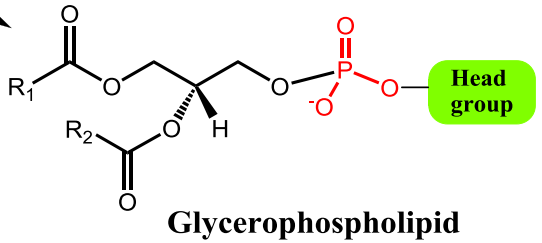
Biosynthesis of glycerophospholipids (GPL)

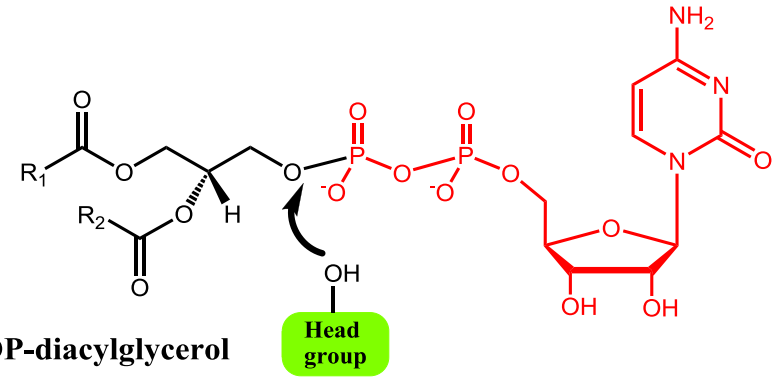
Strategy 1: Headgroup activated with CDP



Strategy 1: The polar head group is activated before being attached to the lipid

– Used during the synthesis of PE and PC



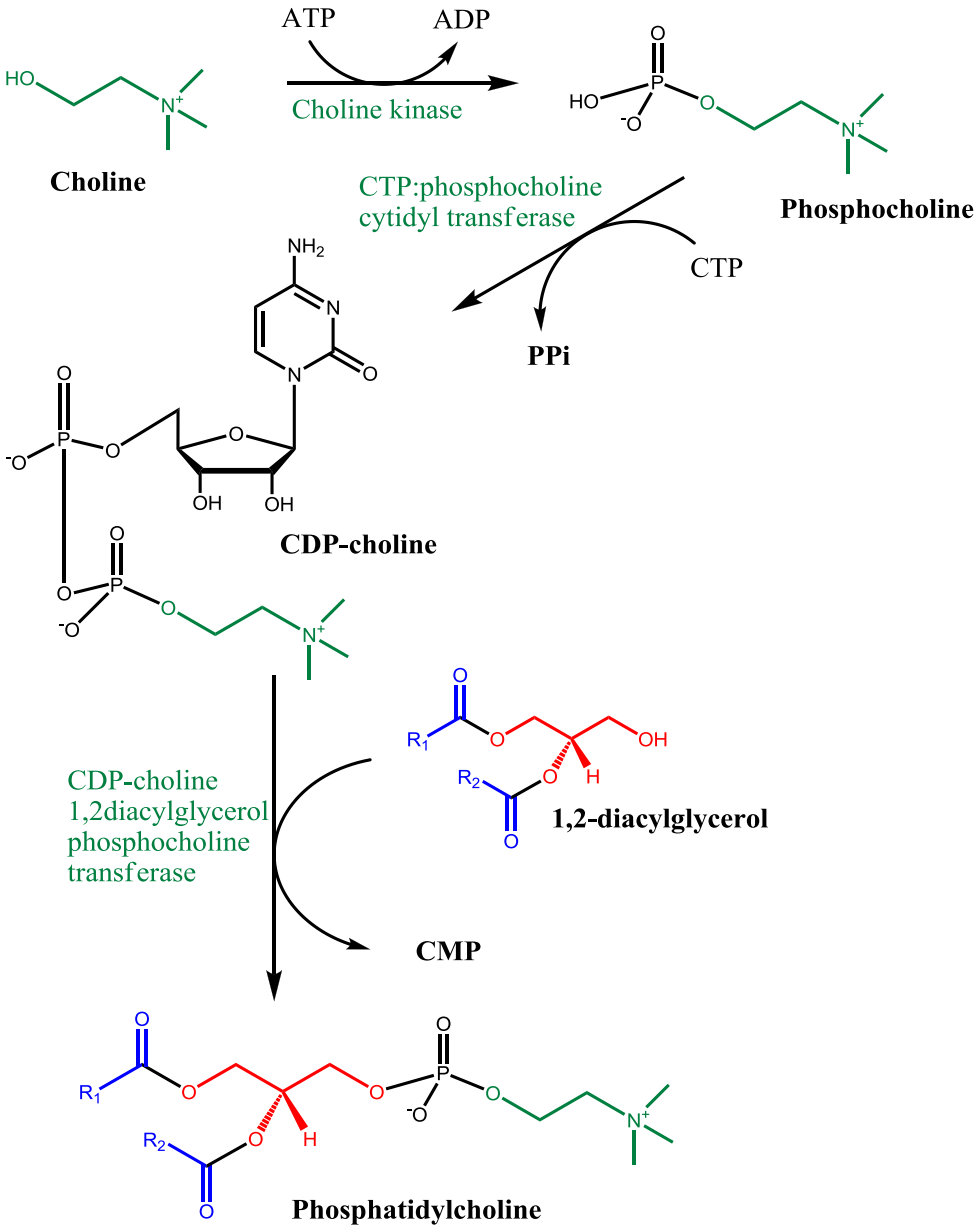


Strategy 2: The hydrophobic tail of diacylglycerol is activated rather than the polar head group

– Used during the synthesis of PI and PG

Strategy 2: Diacylglycerol activated with CDP

De novo synthesis of phosphatidylcholine (PC)



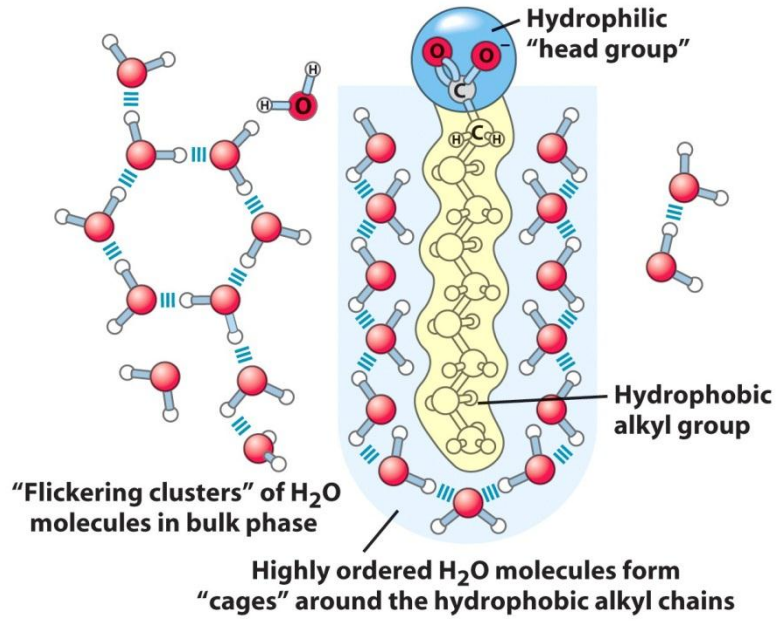
- PC is the most abundant phospholipid in eukaryotic cells
- PC is also known as lecithin

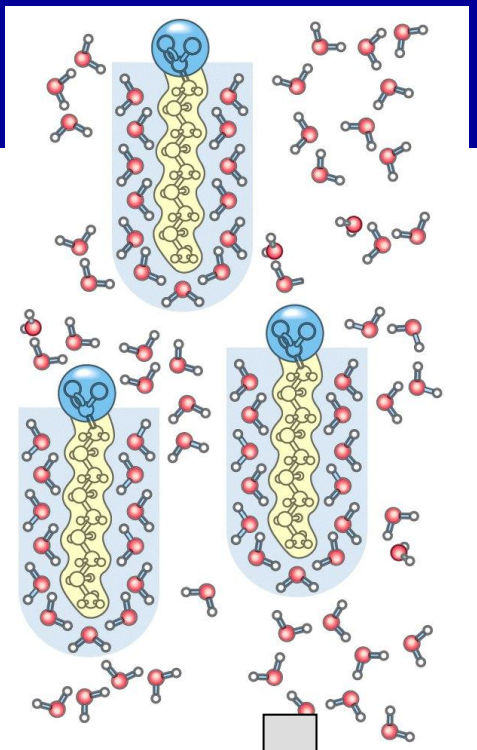
De Novo Synthesis

- Choline is phosphorylated
- Cytidyltransferase makes CDP-choline
- C3 OH groups of DAG attacks the phosphoryl groups of the activated CDP-choline displacing CMP and yielding the glycerophospholipid

BIOPHYSICAL ASPECTS OF LIPIDS

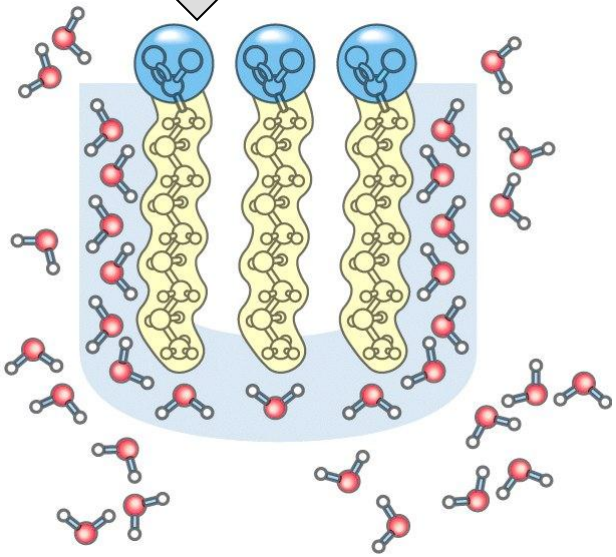
The hydrofobic effect



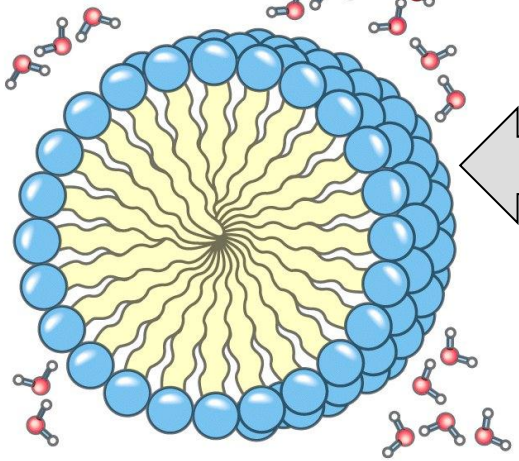


H_2O still highly ordered

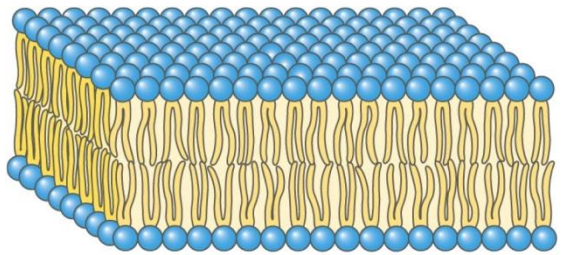
Fewer H_2O are ordered
$\Delta S \uparrow$



Micelles (FA)

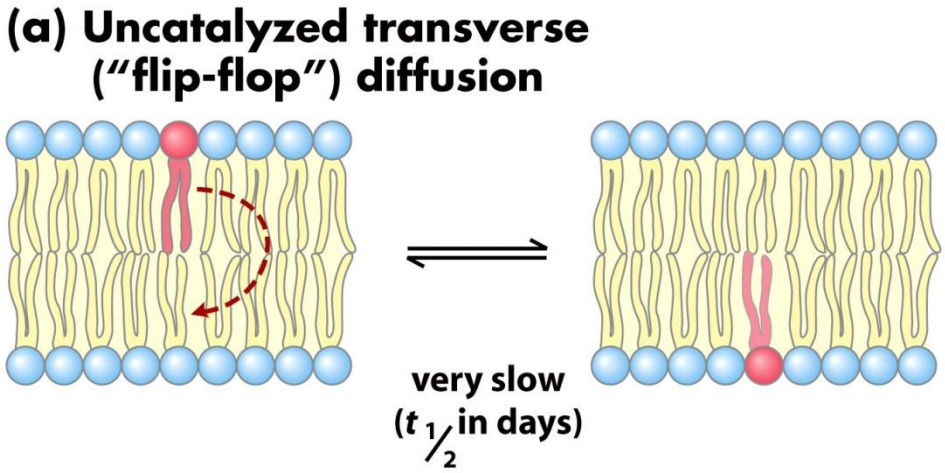


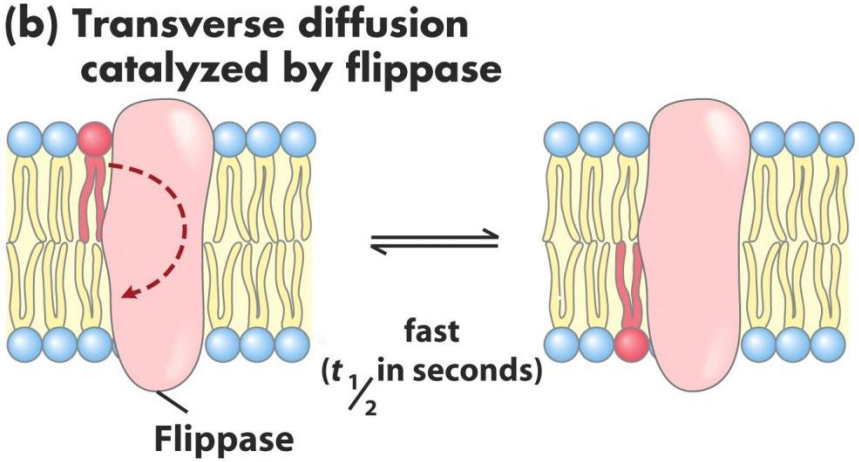
Bilayer (PL)

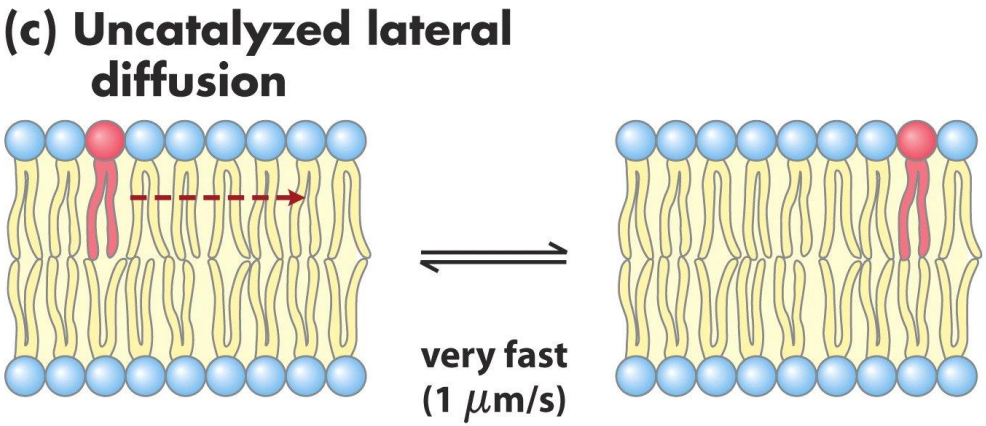


Entropy of water further increased

Lipids dynamics in cell membranes

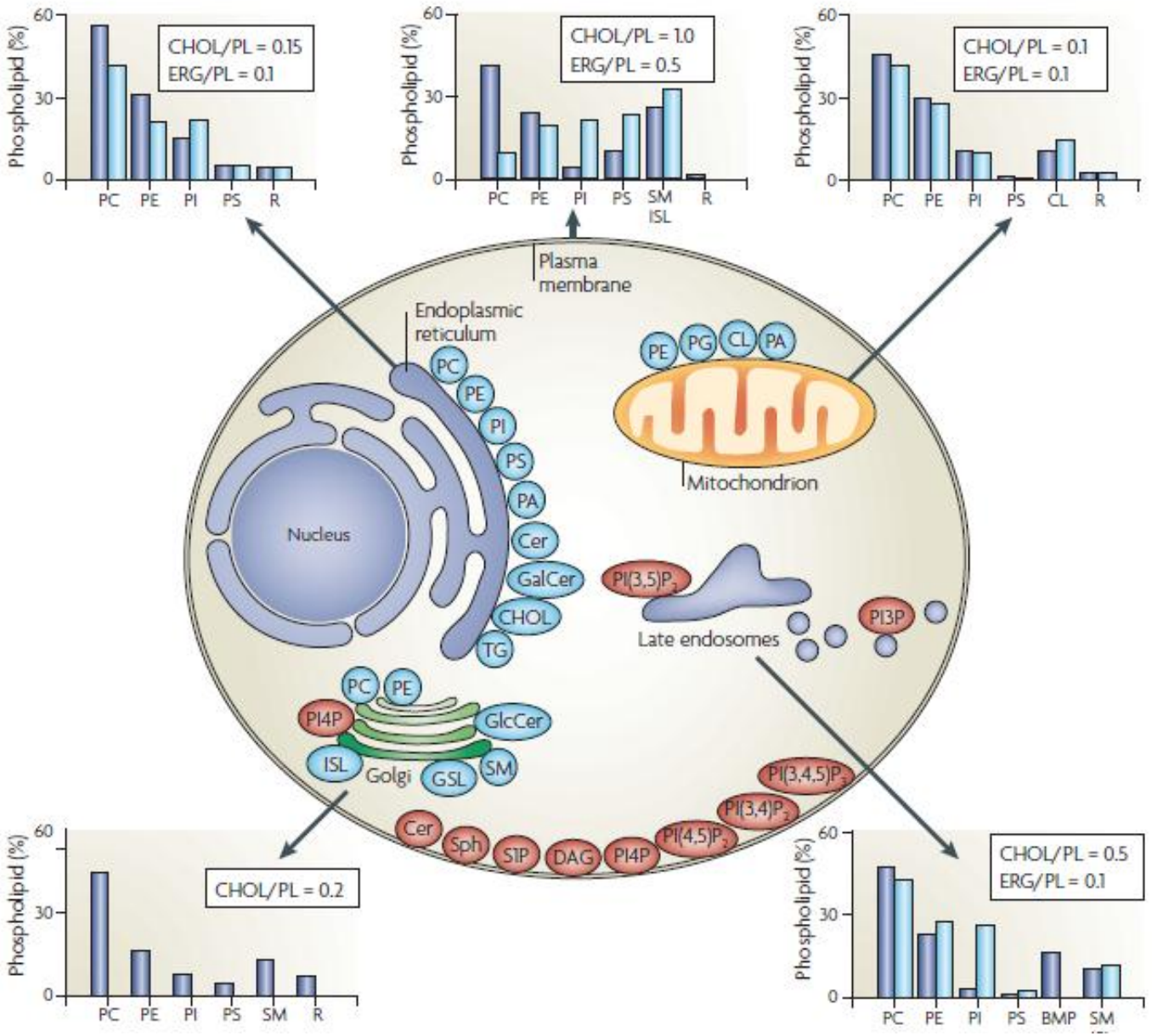




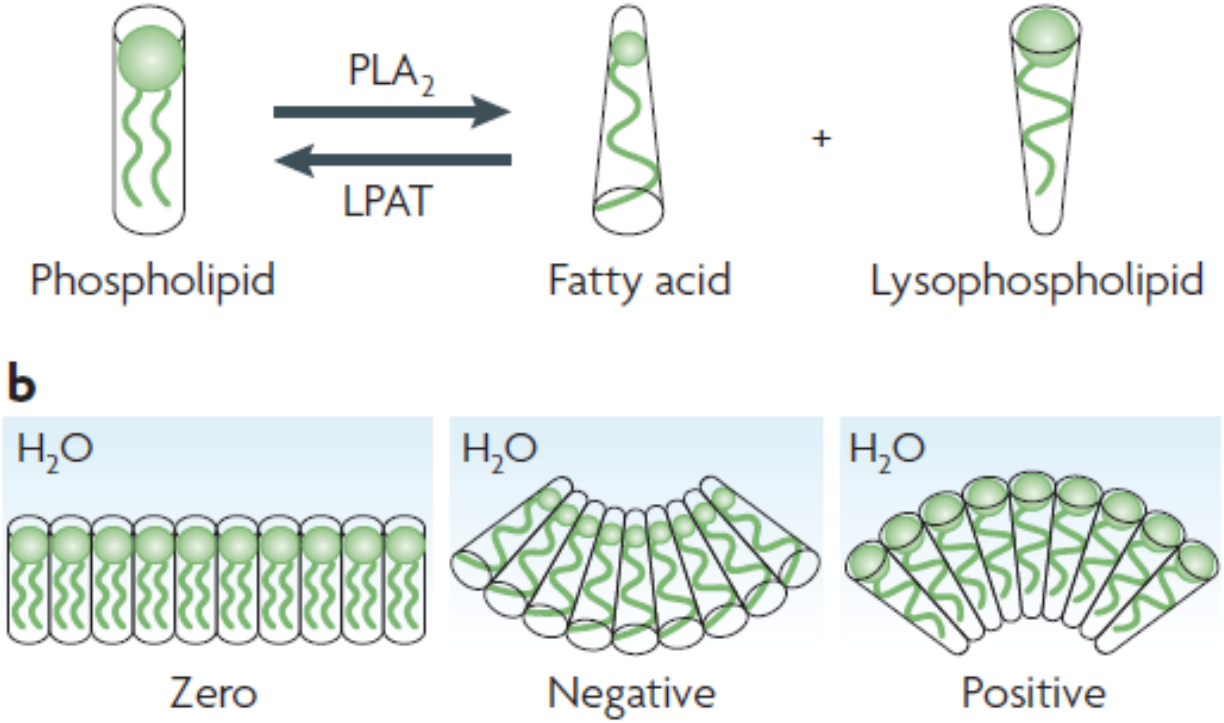


Membrane phospholipid	Percent of total membrane phospholipid	Distribution in membrane (Inner monolayer 100 — 0 — Outer monolayer 100)
Phosphatidyl-ethanolamine	30	
Phosphatidylcholine	27	
Sphingomyelin	23	
Phosphatidylserine	15	
Phosphatidylinositol	5	
Phosphatidylinositol 4-phosphate		
Phosphatidylinositol 4,5-bisphosphate		
Phosphatidic acid		

Lipid composition of cell membranes

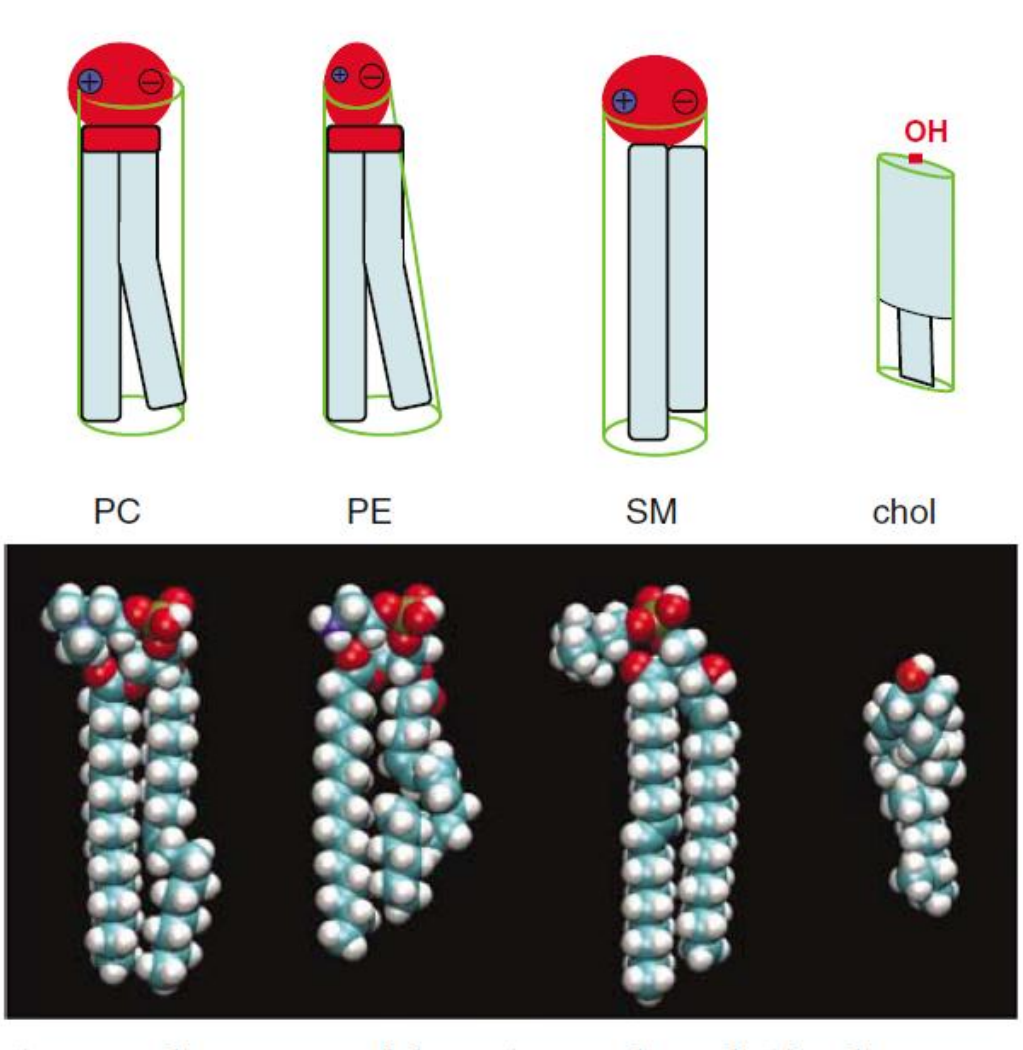


Structure-geometry relationships



Phospholipids in which the polar head group and the fatty acid chains have similar sizes are thought to adopt a cylindrical shape in membranes (filled circles symbolize the polar head groups, wavy lines represent the fatty-acid chains).
In an aqueous environment, cylindrical lipids produce stable planar monolayers, whereas conical and inverted-conical lipids produce monolayers with negative or positive curvature, respectively.

Structure-geometry relationships

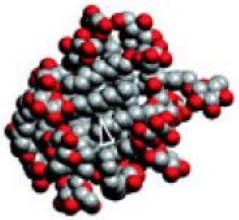
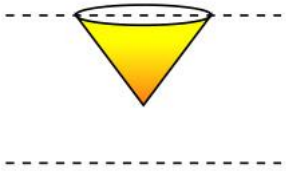
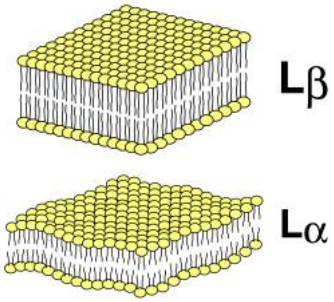
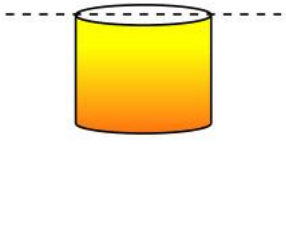
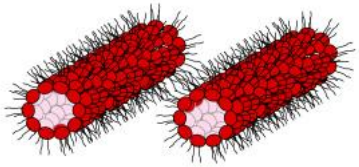
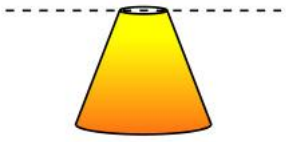


Cellular lipidomics , Van Meer, EMBO Journal 2005, 24, 3159

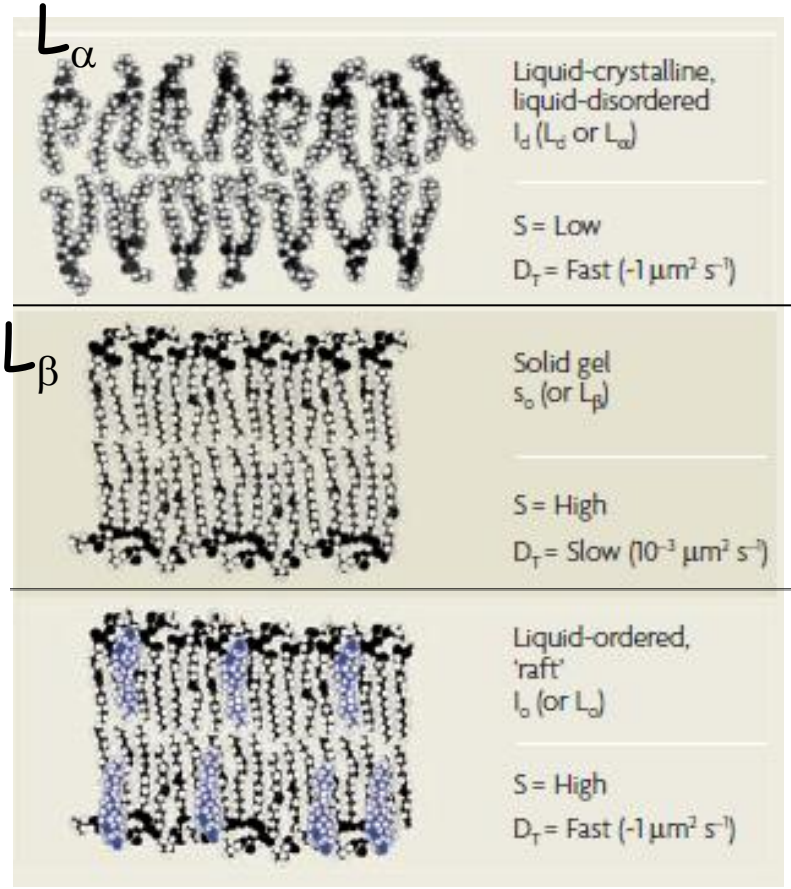
PEs have a small head group relative to its acyl chains and fits better on the inner leaflet of a spherical lipid bilayer. This conical shape creates a stress in the bilayer: the PE-containing monolayer has a tendency to adopt a negative curvature

On the other hand, PC or SM have a larger polar head group and fit better in the outer leaflet (as lysoPLwhich, however form micelles when present in pure liquid form). They tend to order membranes via their straight chains and their high affinity for the flat ring structure of cholesterol (chol).

Structure-geometry relationships

LIPIDS	PHASE	MOLECULAR SHAPE
Lysophospholipids Detergents	 Micellar	 Inverted Cone
Phosphatidylcholine Sphingomyelin Phosphatidylserine Phosphatidylinositol Phosphatidylglycerol Phosphatidic Acid Cardiolipin Digalactosyldiglyceride	 $L\beta$ $L\alpha$ Bilayer	 Cylindrical
Phosphatidylethanolamine Cardiolipin - Ca^{2+} Phosphatidic Acid - Ca^{2+} Phosphatidic Acid (pH<3.0) Phosphatidylserine (pH<4.0) Monogalactosyldiglyceride	 Hexagonal (H_{II})	 Cone

Lipid phases in membranes



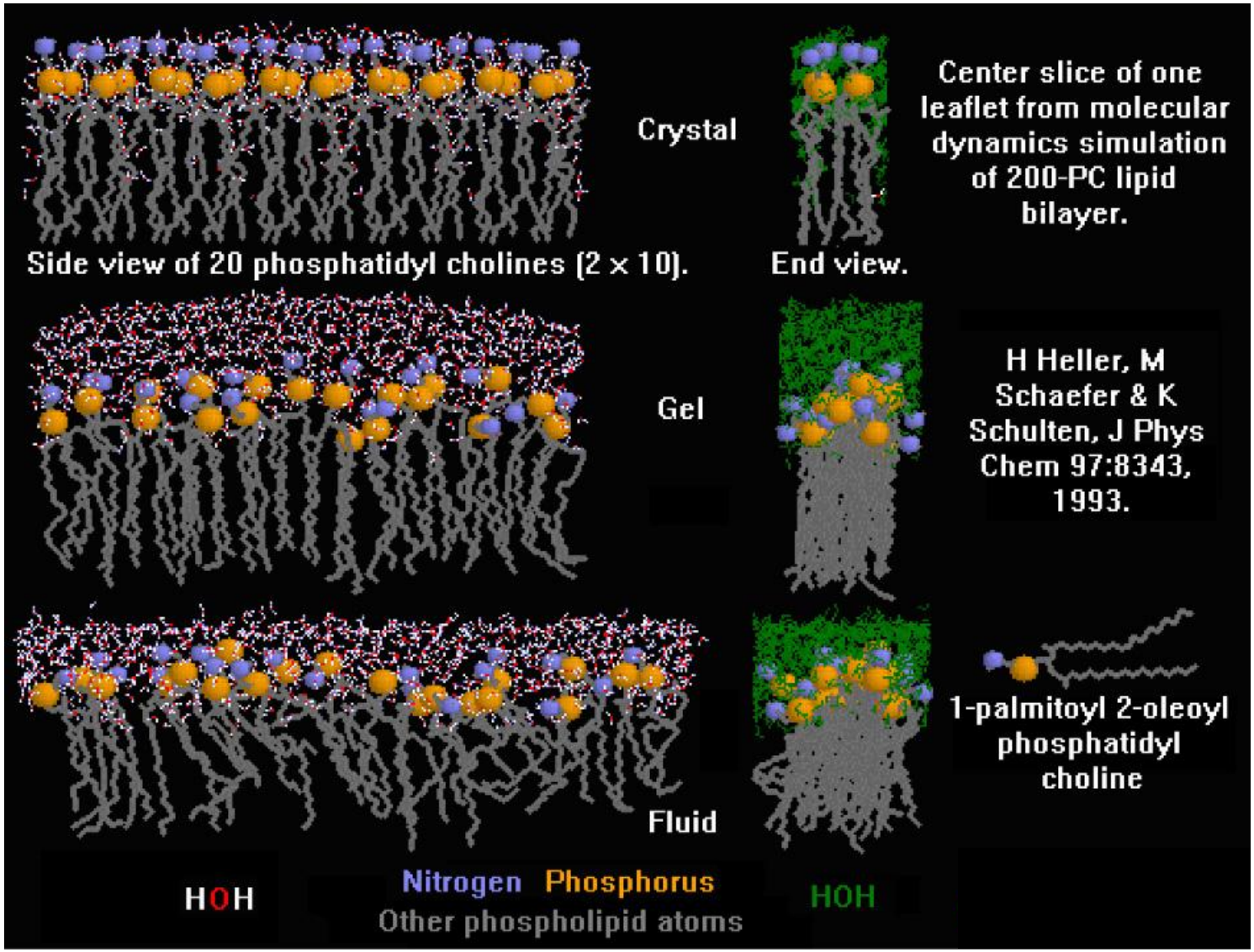
Unsaturated hydrocarbon chains are found in most glycerophospholipids, so these tend to be enriched in liquid, disordered phases

Long, saturated hydrocarbon chains are found in sphingomyelin (SM), so SM-rich mixtures tend to adopt solid-like phases;

Sterols by themselves do not form bilayer phases, but together with a bilayer-forming lipid, the liquid-ordered phase can form.
This remarkable phase has the high order of a solid but the high translational mobility of a liquid.

S = the order parameter of a segment of acyl chain
D_T = the translational diffusion coefficient.

What does a lipid bilayer look like?



http://blanco.biomol.uci.edu/

http://www.umass.edu/microbio/rasmol/slicealt.htm

Summary of factors affecting lipid organization

Temperature and double bonds saturation

- High temperature and unsaturation favors La & HII
- Low temperature and saturation favors Lb

Lipid shape

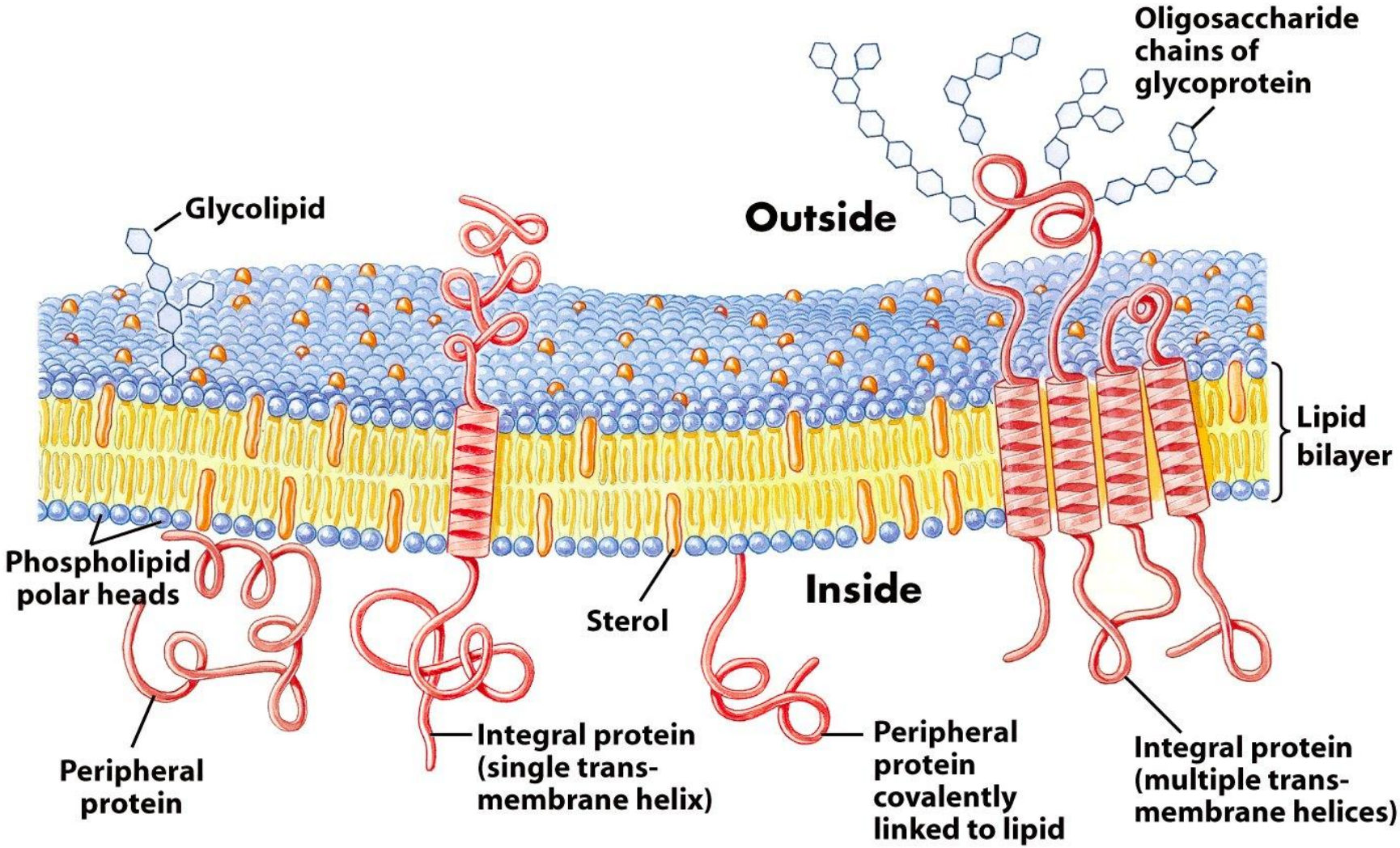
- Predominantly bilayer lipids: PC, PI, DGDAG, PS, PG, CL
- Non-bilayer lipids: PE, CL (Me^{+2}), Plasmalogens, MGDAG.
- High unsaturation or temperature favors non-bilayer

Local pH and ionic strength

Reduction of charge repulsion for PA and CL favors non-bilayer.

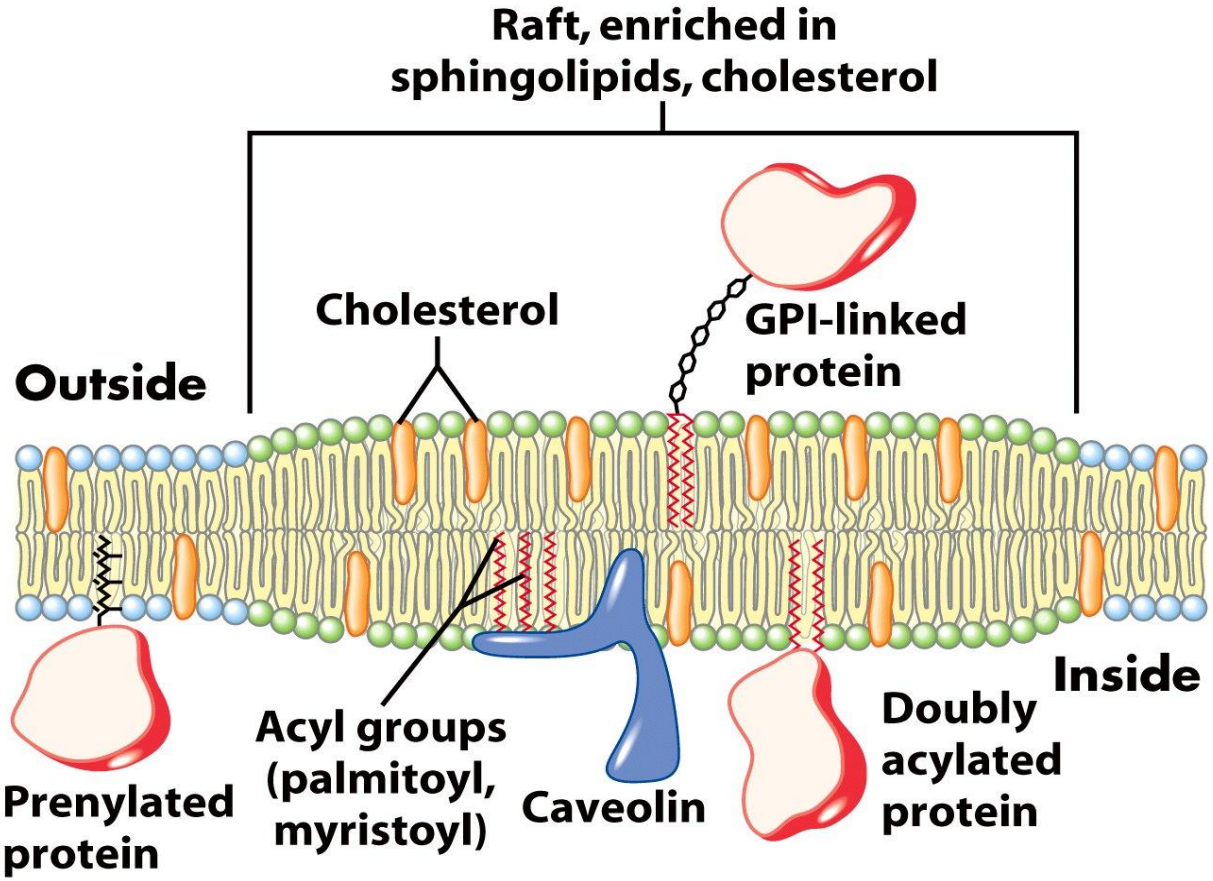
Membranes : fluid mosaic or…

The original description of the "fluid-mosaic" model suggested that membrane proteins were floating in a homogenous bed of excess lipid arranged in a bilayer



Membranes : fluid mosaic or heterogenous?…

The lipid-lipid and lipid-protein interactions appear to be much more dynamic than first appreciated. Lipid microdomains rich in cholesterol, sphingomyelin, and glycolipids, called "lipid rafts", play a role in cell signaling by their relative abundance of (GPI)-anchored proteins as well as receptor and non-receptor kinases.

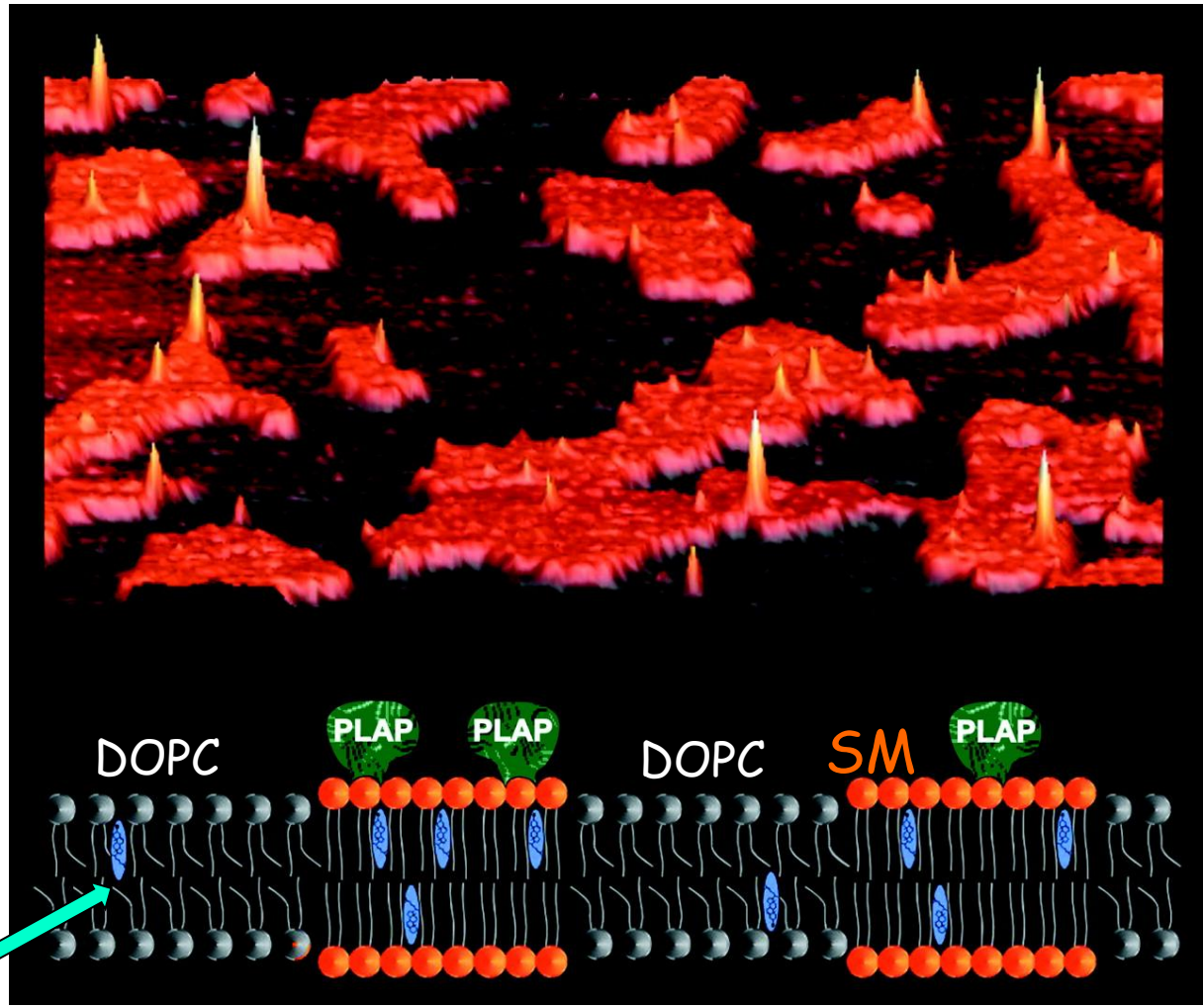


Lipid Rafts: evidences by Atomic Force Microscopy

When added to a preformed sphingolipid/DOPC bilayer, intestinal alkaline phosphatase showed preferential insertion into the sphingomyelin domains

AFM reveals sphingomyelin rafts (orange) protruding from a DOPC background (black) in a mica-supported lipid bilayer.

Placental alkaline phosphatase (PLAP; yellow peaks), a GPI-anchored protein, is shown to be almost exclusively raft associated.



cholesterol

Henderson R M et al. Physiology 2004;19:39-43

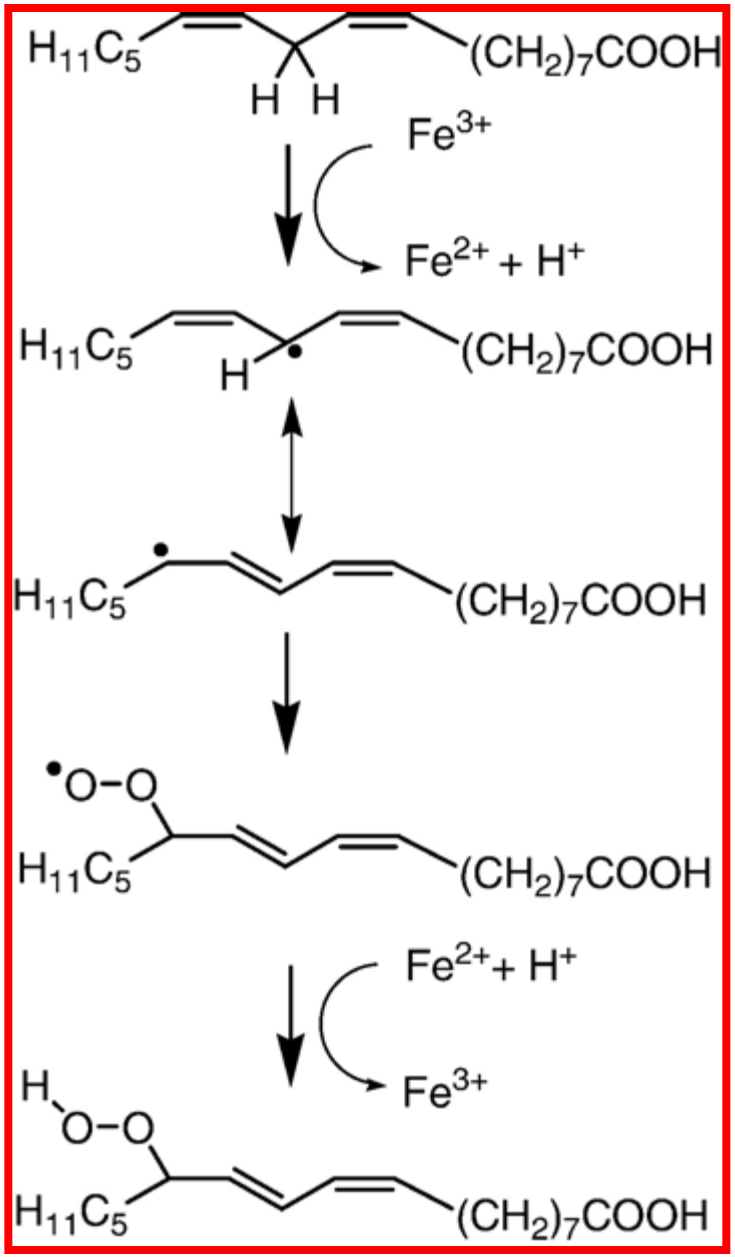
CHEMICAL ASPECTS OF LIPIDS

Lipid Oxidation and Lipids as Markers of Disease

Polyunsaturated fatty chains possess a bis-allylic carbon which is particularly susceptible to oxidation.

These protons can be abstracted, either by enzymes (such as LOX and COX) in the initial step of eicosanoid production or by free radicals leaving a carbon with an unpaired electron.

This carbon-centered free radical undergoes molecular rearrangement to form a conjugated diene and then reacts with oxygen (O_2) to form a peroxyl radical



ANALYTICAL METHODS FOR LIPIDS ANALYSIS

How to analyze entire cellular lipidome?

Identification of all cellular lipids - lipidome

Analytical platforms with high sample throughput

Ability to quantify lipids at a broad dynamic range

Data processing & handling

Pattern-recognition; Data-mining

Integration with other "omics" data

Traditional methods for Lipids analysis: multi-step approaches

- **Extraction of lipids**
  - TAGs, PLs, glycolipids etc
- **Chromatographic separation of lipids**
  - TLC: (followed by derivatisation, scraping off bands, detection, quantification)
  - LC: normal or reversed-phase
- **Separation of PL classes**
  - HPLC: with UV/fluorescence detection (w/wo derivatisation)
  - HPLC: with ELSD detection
- **Fraction collection**
  - Collection of separated PL classes
- **Hydrolysis**
  - Fatty acids
  - Polar head group
- **Derivatisation**
  - Derivatisation of fatty acids
- **Identification of derivatised fatty acids using GC and/or GC-MS**

There are also:

- **enzymatic hydrolysis to determine fatty acid positions**
- **determination of phosphorous content**

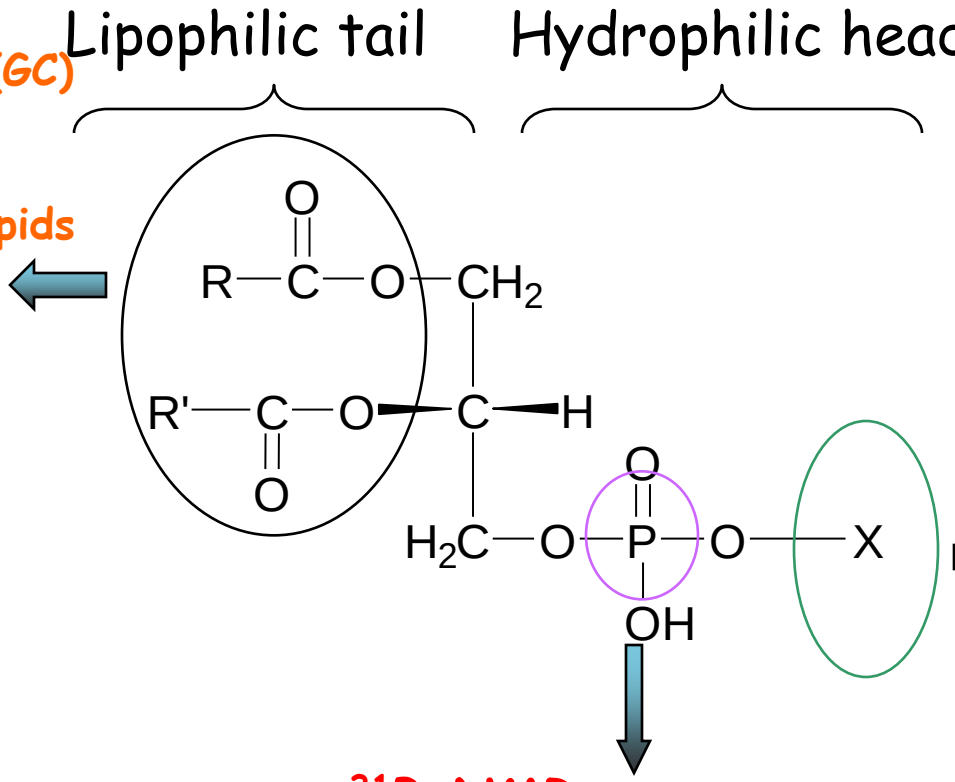
Lack of universal HPLC detector for direct measurement of PLs.

These approaches give a global overview of compositional information regarding PLs

Mass spectrometry	High resolution NMR
• ESI (electron spray ionization) • MALDI (matrix-assisted laser desorption/ionization) • coupling with HPLC or TLC	Most common nuclear probes are ^{1}H and ^{31}P Investigation of the composition, structure and dynamics of biomembranes
Merits • High sensitivity • Direct profiling of mixtures	**Merits** Not destructive Quantitative measurements
Limits • Difficulties in quantitative analysis • Not adequate for structural definition of new lipids	**Limits** Low sensitivity Strong signals overlap even at high magnetic fields

What do actual methods measure?

Gas chromatography (GC)
- OK for fatty acyls
- Not suitable for plasmenyl or sphingolipids chains



Liquid chromatography (LC): information on individual PL classes (PC, PE,PI)

^{31}P-NMR : information on PL classes

Different methods give different information but it is often difficult to put it in a unique "information ensemble"

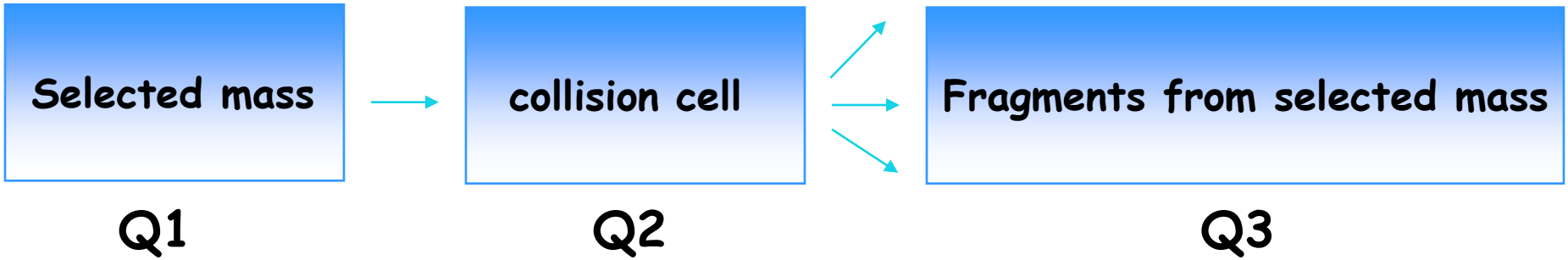
Mass spectrometric approaches

- GC-MS
  - analysis of derivatised fatty acids
- FAB-MS/MS
  - first applications of characterising intact PLs
  - suffered from background suppression effects
- API techniques

ESI-MS is most widely used

  - with chromatographic separation (RP and NP phase)
  - without chromatographic separation (direct infusion experiments)

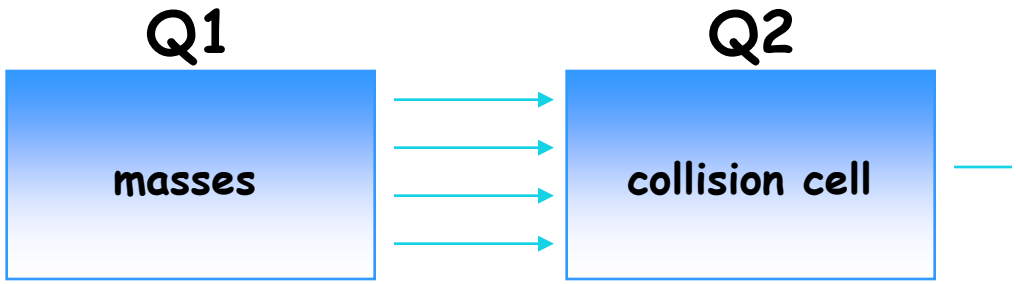
Schematic of MS/MS experiments



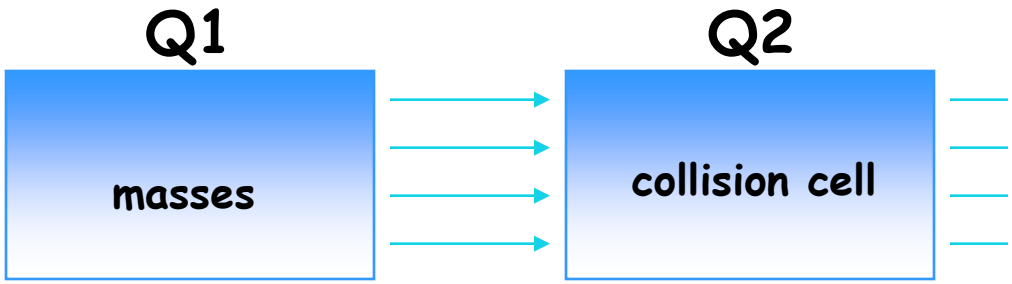
Separation and identification by LC-MS/MS

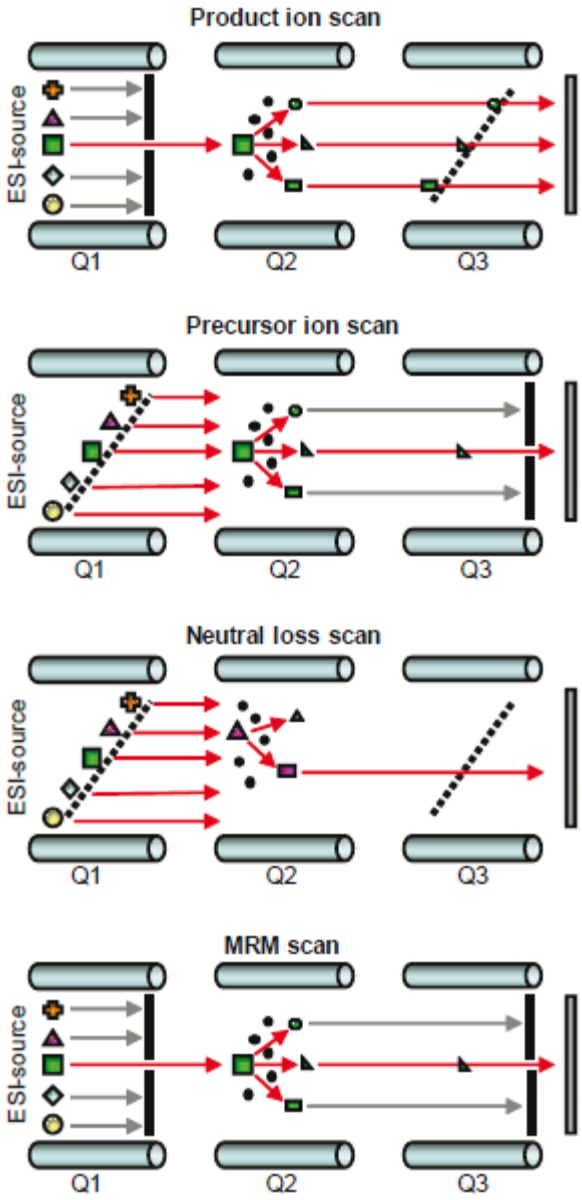
After LC separation, PL species are detect scanning function in a triple quadrupole MS

Parent / precursor ion scan, used for PCs



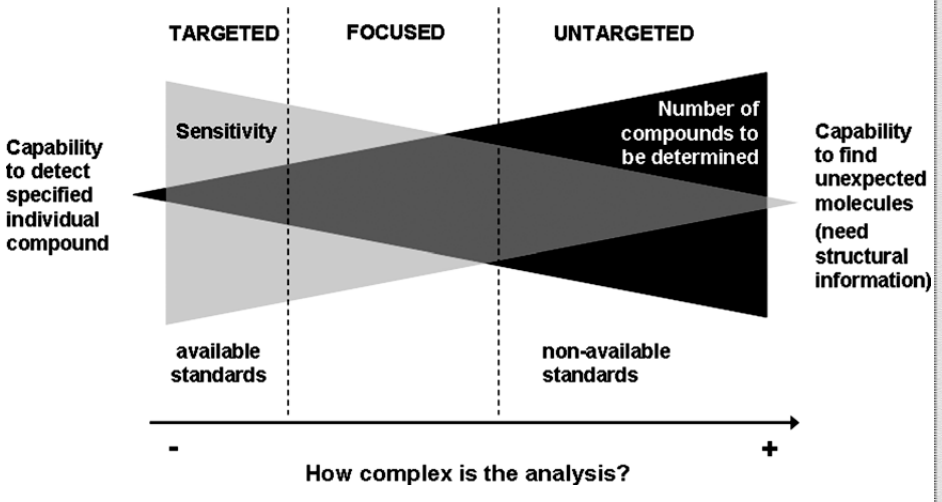
Constant neutral loss scan, used for PEs

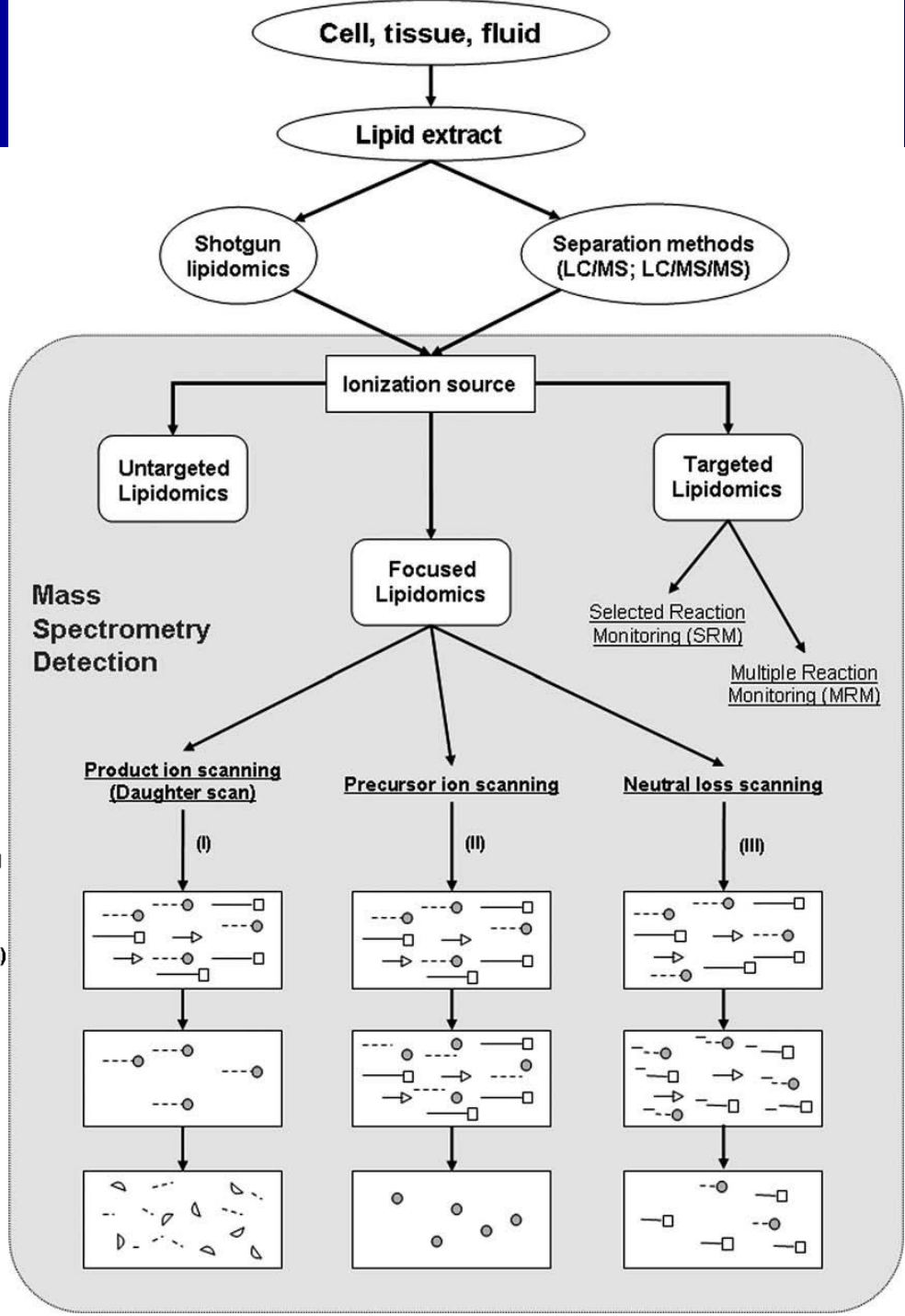




Challenges in lipidomics

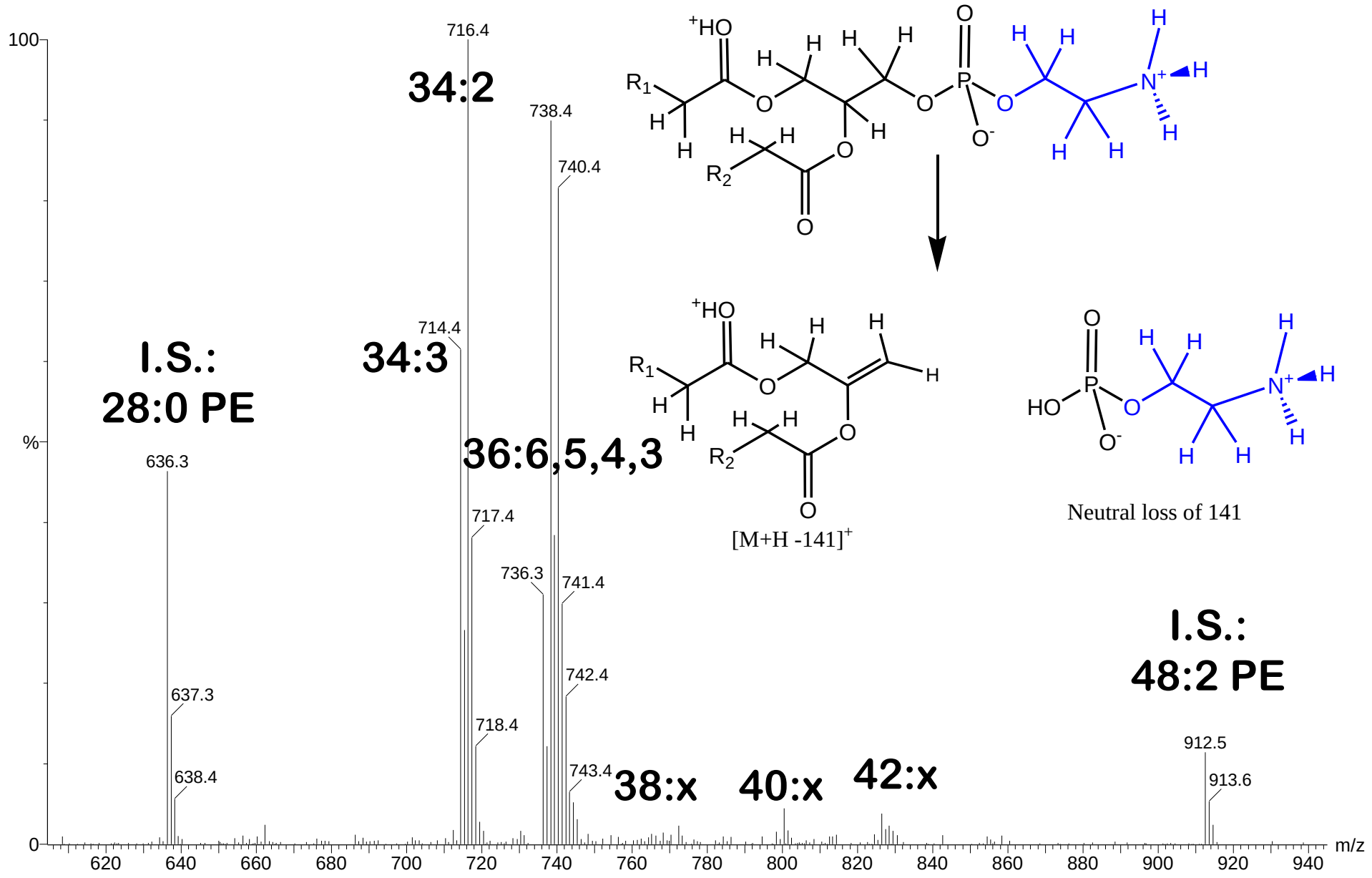
Complexity of the different lipidomics strategies and capability to detect a number of individual molecular compounds





Trends in Analytical Chemistry, 28, 4, **2009**

Phosphatidylethanolamines: Ions that lose a neutral fragment of 141



NMR-based approaches

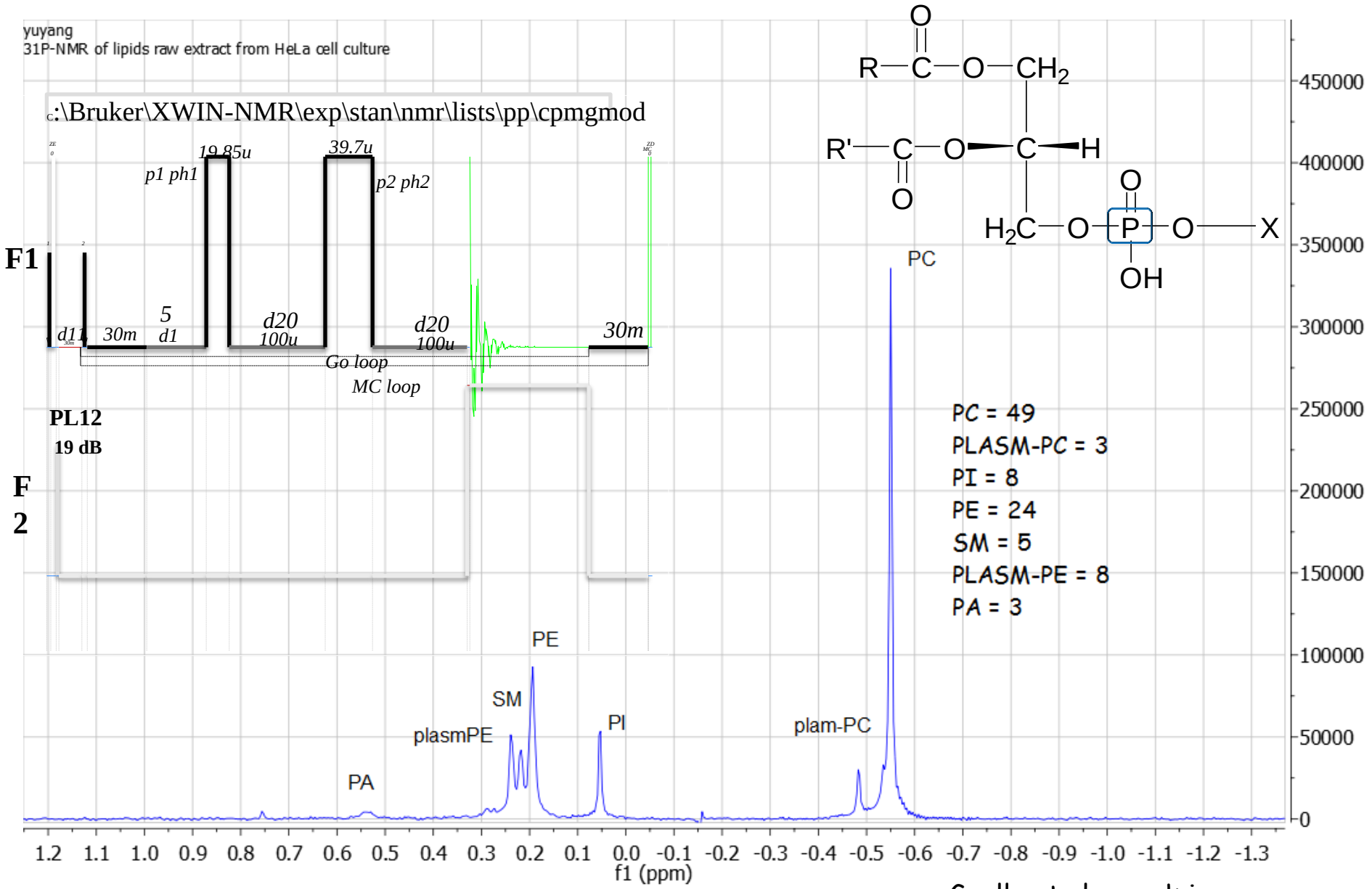
NMR spectroscopy (^{1}H, ^{13}C, and ^{31}P-NMR) is a powerful tool for:

- the elucidation of molecular structures of purified new lipids
- the determination of lipid profile in lipids mixtures
- for investigating the structure and dynamics of lipid membranes

For the analysis of phospholipid mixtures, ^{31}P-NMR is by far the most appropriate approach. The linear response and relatively high speed of ^{31}P-NMR allows for accurate and selective analysis with high sample throughput.

NMR continue to make important contributions, in particular in the characterization of **dynamic protein–lipid interactions** that are important regulatory mechanisms of trans-membrane proteins and ion channels. However, the restricted movement of lipids in macromolecular aggregates such as bilayers or lipoproteins leads **to line broadening** and poor resolution, and therefore *in vivo* measurements are limited.

^{31}P-NMR spectrum : lipids profile of HeLa cells



Guella et al. , work in progress

RESULTS FROM OUR RECENT INVESTIGATIONS

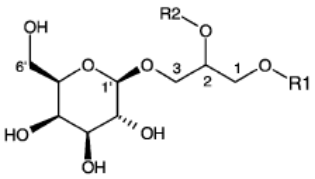
Our first look into microalgal lipidomics

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2003; **17**: 1982–1994
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1142

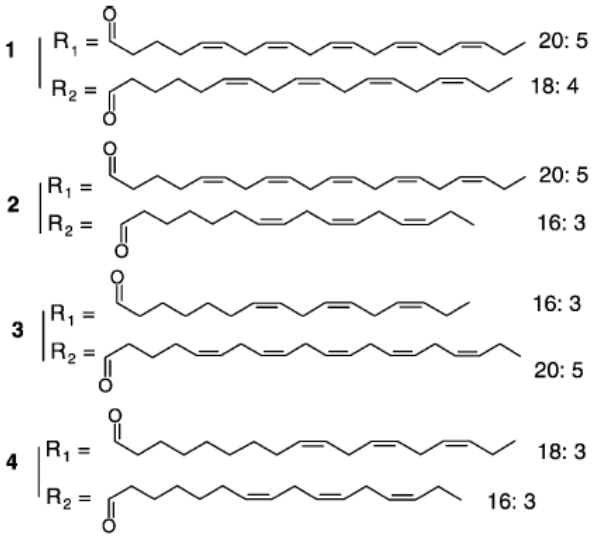
RCM

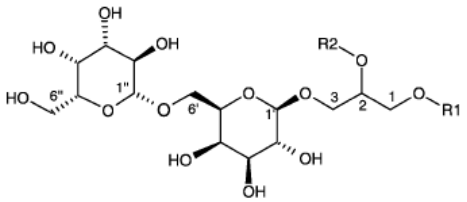
A new solution for an old problem: the regiochemical distribution of the acyl chains in galactolipids can be established by electrospray ionization tandem mass spectrometry

Graziano Guella*, Rita Frassanito and Ines Mancini
Laboratorio di Chimica Bioorganica, Facoltà di Scienze MFN, Università di Trento, 38050 Povo-Trento, Italy



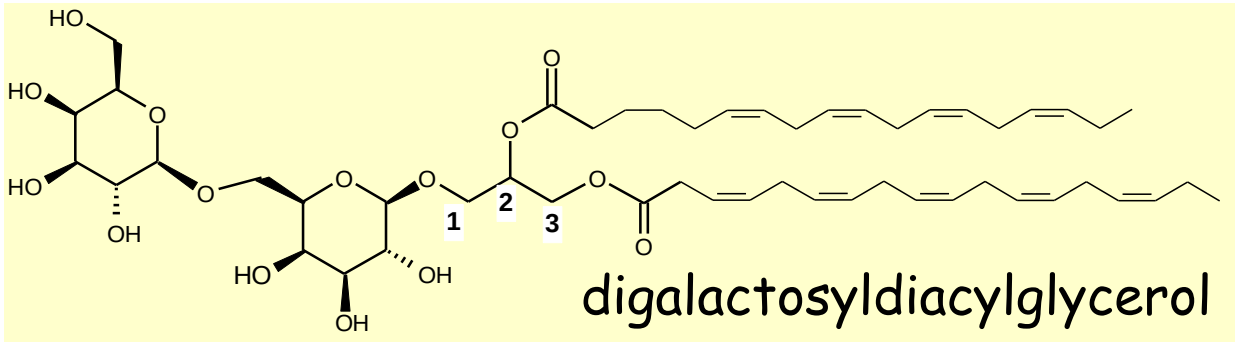
MGDG





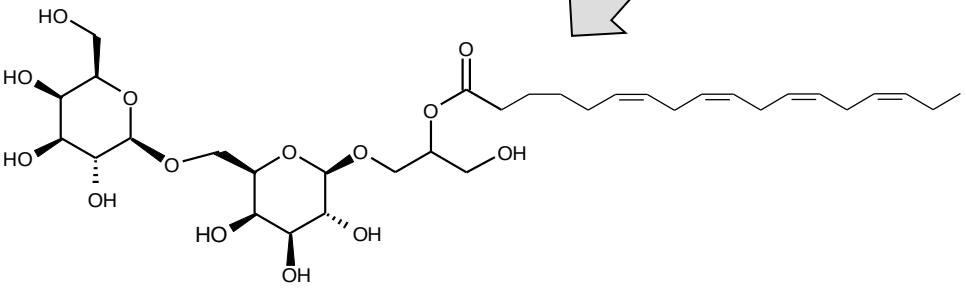
DGDG

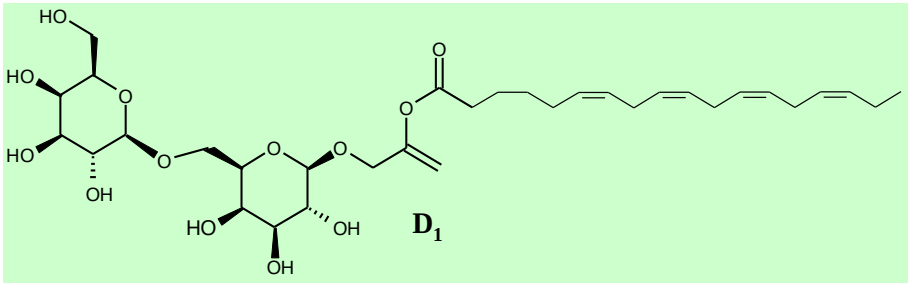
DETERMINATION OF THE REGIOSPECIFICITY OF THE TWO ACYL LINKAGES OF GALACTOLIPIDS



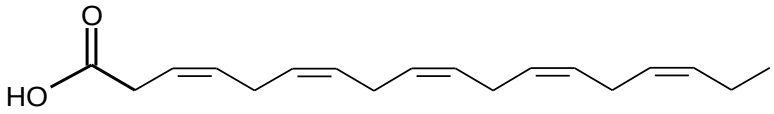
enzymatic hydrolysis by *Rhizopus arrhizus*

Positive ion tandem MS

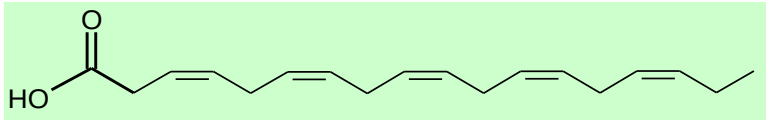




+

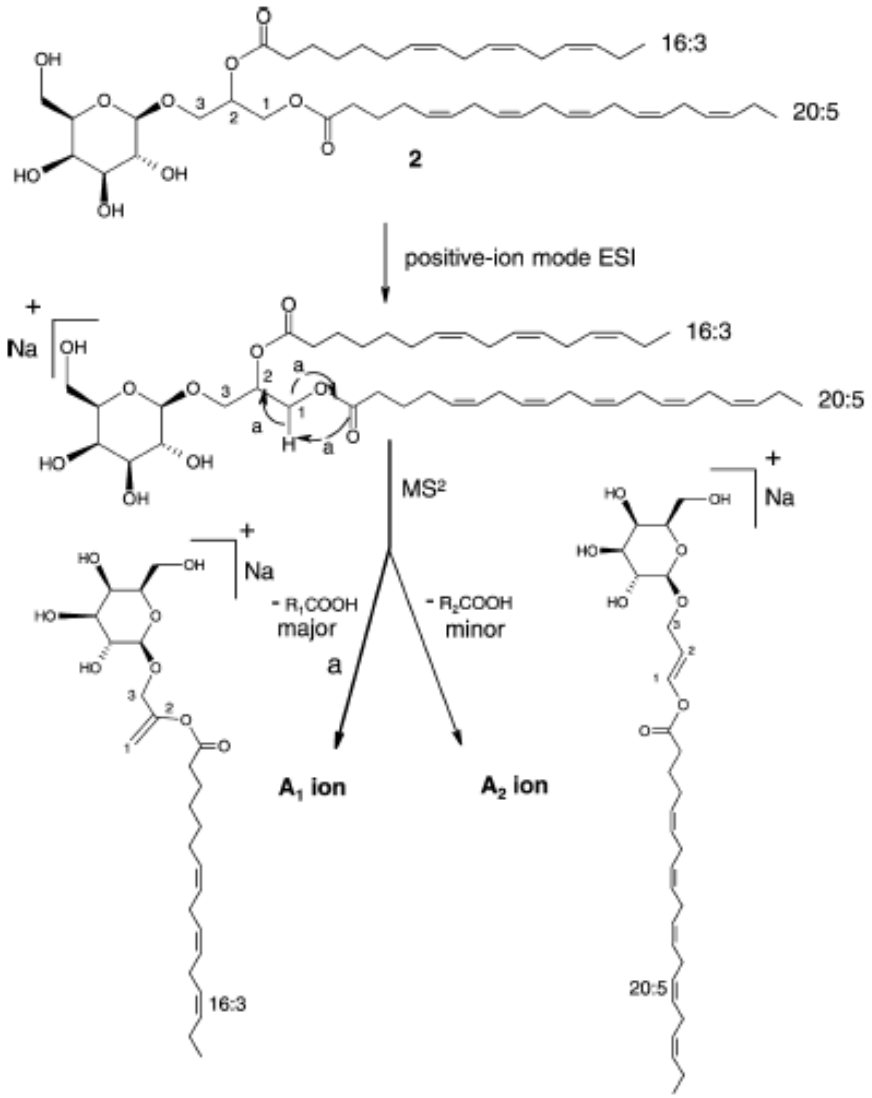


+



Guella et al. RCMS 2003

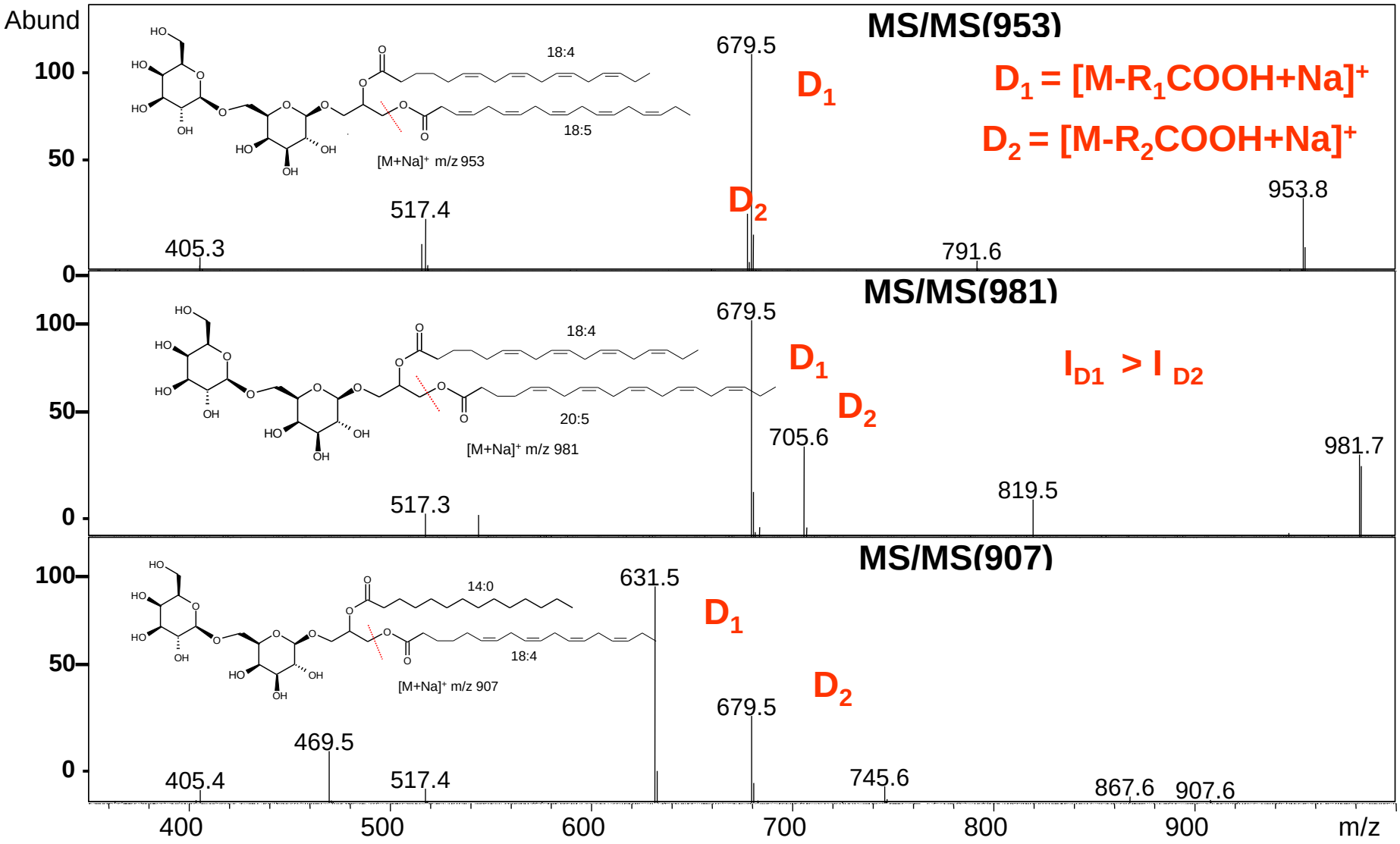
Outcome from ESI (+) tandem MS experiments



The positional distribution of the acyl chains in galactolipids can be established knowing that, in positive-ion mode ESI-MS^2 measurements, the loss of the carboxylic acid linked to the sn-1 glycerol position always produces a more intense peak than that derived from the loss of the sn-2 linked acyl chain.

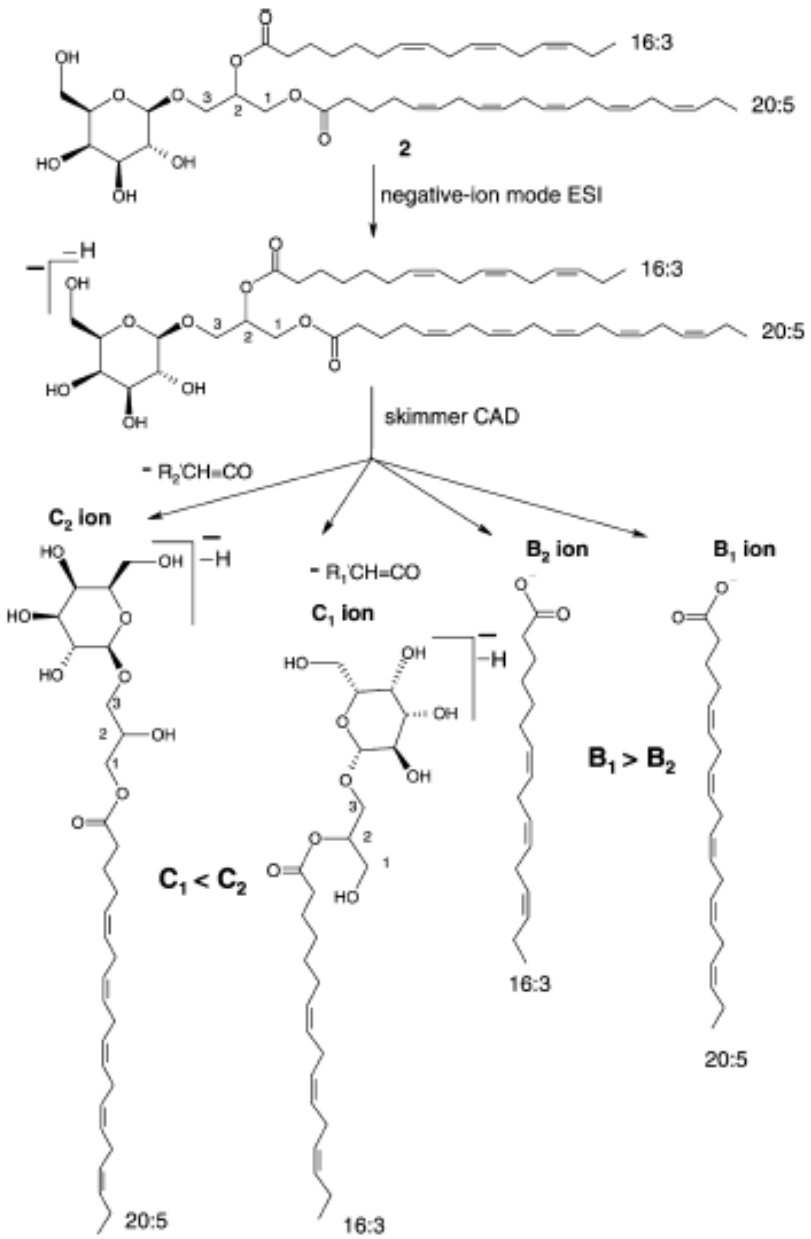
Guella et al. RCMS 2003

Positive ion MS^2 spectra of the $[M+Na]^+$ ions of DGDGs



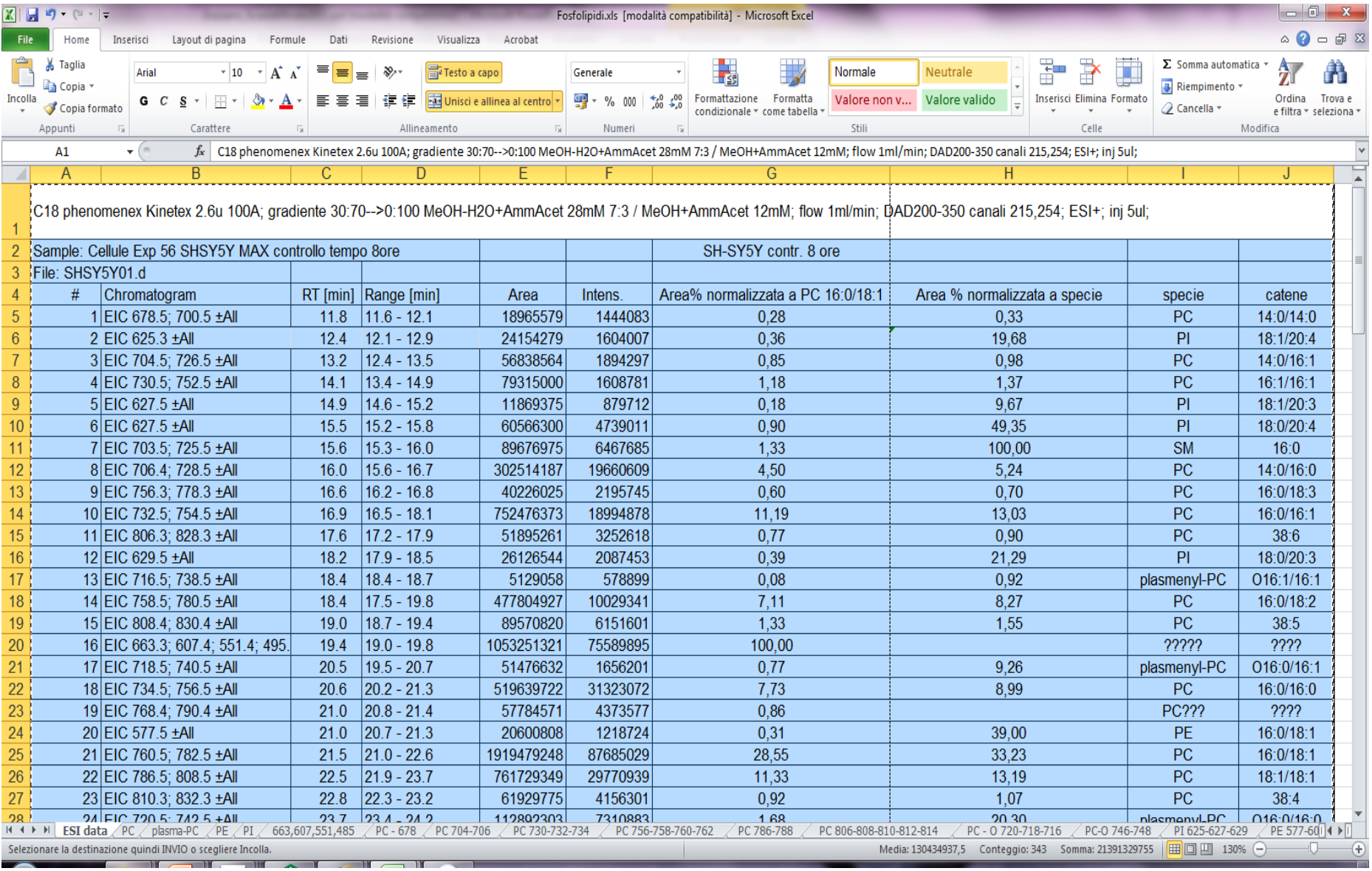
Guella et al. RCMS 2003

Outcome from ESI (-) in source CAD experiments



In the negative-ion mode, the C1/C2 ratio represents a valuable parameter in the determination of galactosyl regiochemistry when acquired by skimmer CAD fragmentations, but this structural information is not reliable when acquired by MS^2 experiments.

Guella et al. RCMS 2003

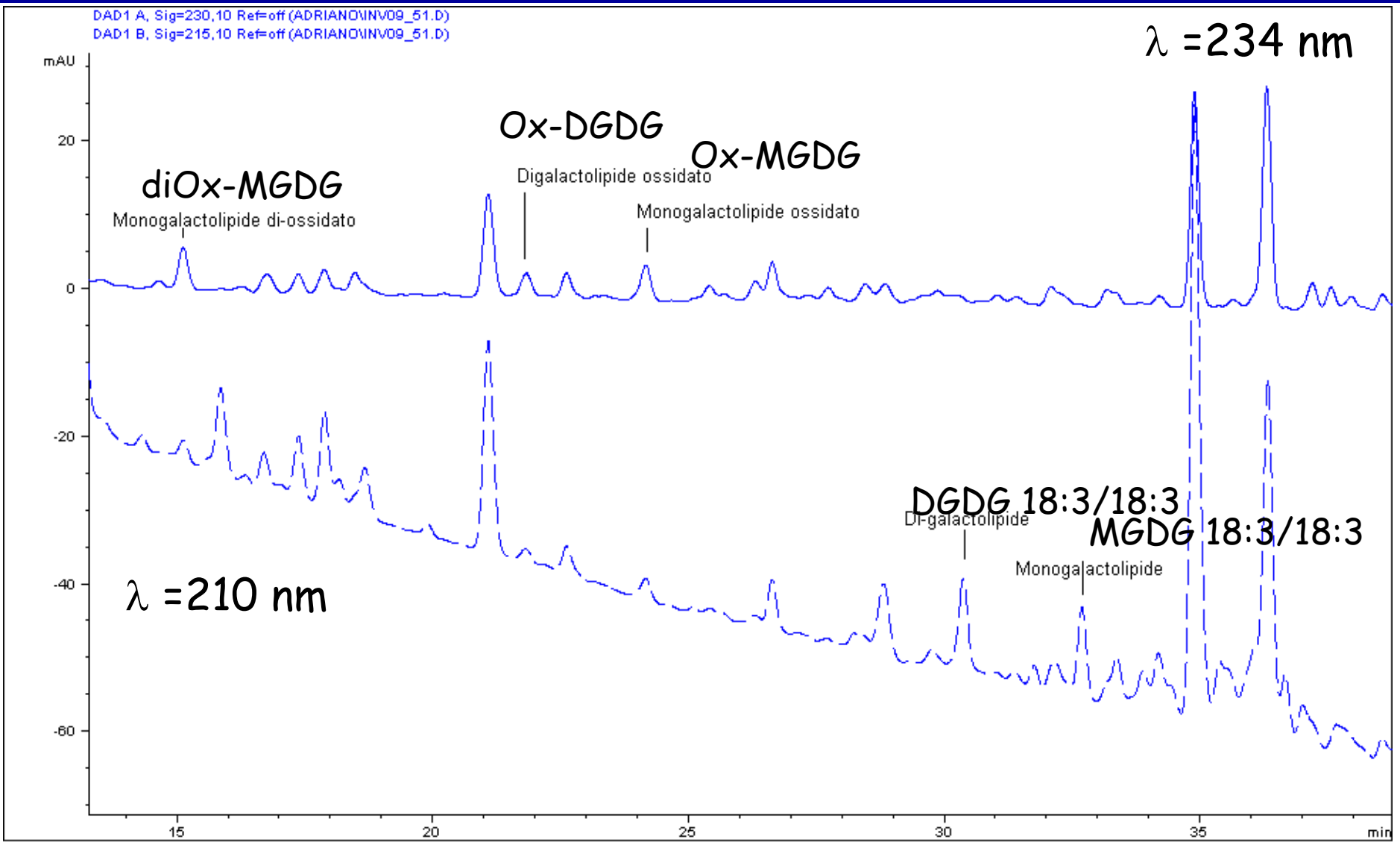


Fosfolipidi.xls [modalità compatibilità] - Microsoft Excel

	A	B	C	D	E	F	G	H	I	J
1	C18 phenomenex Kinetex 2.6u 100A; gradiente 30:70-->0:100 MeOH-H2O+AmmAcet 28mM 7:3 / MeOH+AmmAcet 12mM; flow 1ml/min; DAD200-350 canali 215,254; ESI+; inj 5ul;									
2	Sample: Cellule Exp 56 SHSY5Y MAX controllo tempo 8ore						SH-SY5Y contr. 8 ore			
3	File: SHSY5Y01.d									
4	#	Chromatogram	RT [min]	Range [min]	Area	Intens.	Area% normalizzata a PC 16:0/18:1	Area % normalizzata a specie	specie	catene
5	1	EIC 678.5; 700.5 ±All	11.8	11.6 - 12.1	18965579	1444083	0,28	0,33	PC	14:0/14:0
6	2	EIC 625.3 ±All	12.4	12.1 - 12.9	24154279	1604007	0,36	19,68	PI	18:1/20:4
7	3	EIC 704.5; 726.5 ±All	13.2	12.4 - 13.5	56838564	1894297	0,85	0,98	PC	14:0/16:1
8	4	EIC 730.5; 752.5 ±All	14.1	13.4 - 14.9	79315000	1608781	1,18	1,37	PC	16:1/16:1
9	5	EIC 627.5 ±All	14.9	14.6 - 15.2	11869375	879712	0,18	9,67	PI	18:1/20:3
10	6	EIC 627.5 ±All	15.5	15.2 - 15.8	60566300	4739011	0,90	49,35	PI	18:0/20:4
11	7	EIC 703.5; 725.5 ±All	15.6	15.3 - 16.0	89676975	6467685	1,33	100,00	SM	16:0
12	8	EIC 706.4; 728.5 ±All	16.0	15.6 - 16.7	302514187	19660609	4,50	5,24	PC	14:0/16:0
13	9	EIC 756.3; 778.3 ±All	16.6	16.2 - 16.8	40226025	2195745	0,60	0,70	PC	16:0/18:3
14	10	EIC 732.5; 754.5 ±All	16.9	16.5 - 18.1	752476373	18994878	11,19	13,03	PC	16:0/16:1
15	11	EIC 806.3; 828.3 ±All	17.6	17.2 - 17.9	51895261	3252618	0,77	0,90	PC	38:6
16	12	EIC 629.5 ±All	18.2	17.9 - 18.5	26126544	2087453	0,39	21,29	PI	18:0/20:3
17	13	EIC 716.5; 738.5 ±All	18.4	18.4 - 18.7	5129058	578899	0,08	0,92	plasmenyl-PC	O16:1/16:1
18	14	EIC 758.5; 780.5 ±All	18.4	17.5 - 19.8	477804927	10029341	7,11	8,27	PC	16:0/18:2
19	15	EIC 808.4; 830.4 ±All	19.0	18.7 - 19.4	89570820	6151601	1,33	1,55	PC	38:5
20	16	EIC 663.3; 607.4; 551.4; 495.	19.4	19.0 - 19.8	1053251321	75589895	100,00		?????	????
21	17	EIC 718.5; 740.5 ±All	20.5	19.5 - 20.7	51476632	1656201	0,77	9,26	plasmenyl-PC	O16:0/16:1
22	18	EIC 734.5; 756.5 ±All	20.6	20.2 - 21.3	519639722	31323072	7,73	8,99	PC	16:0/16:0
23	19	EIC 768.4; 790.4 ±All	21.0	20.8 - 21.4	57784571	4373577	0,86		PC???	????
24	20	EIC 577.5 ±All	21.0	20.7 - 21.3	20600808	1218724	0,31	39,00	PE	16:0/18:1
25	21	EIC 760.5; 782.5 ±All	21.5	21.0 - 22.6	1919479248	87685029	28,55	33,23	PC	16:0/18:1
26	22	EIC 786.5; 808.5 ±All	22.5	21.9 - 23.7	761729349	29770939	11,33	13,19	PC	18:1/18:1
27	23	EIC 810.3; 832.3 ±All	22.8	22.3 - 23.2	61929775	4156301	0,92	1,07	PC	38:4
28	24	EIC 720.5; 742.5 ±All	23.7	23.4 - 24.2	112892303	7310883	1,68	20,30	plasmenyl-PC	O16:0/16:0

Guella et al. , work in progress

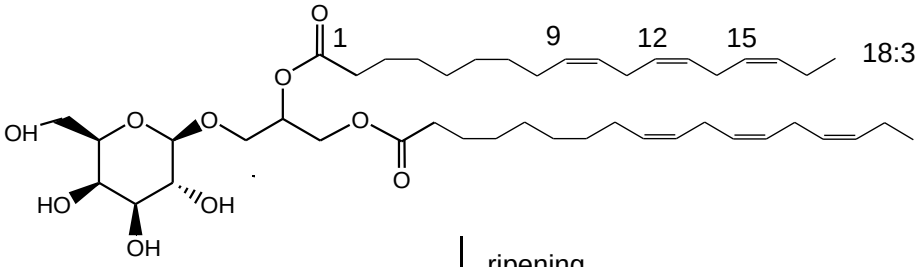
Fruit ripening : an oxidative stress?



LC-MS (ESI+) UV (DAD, 210 & 234nm): grapes ripening during summer 2009; C8 column, MeOH/H_2O (NH_4OAc 28 mM)

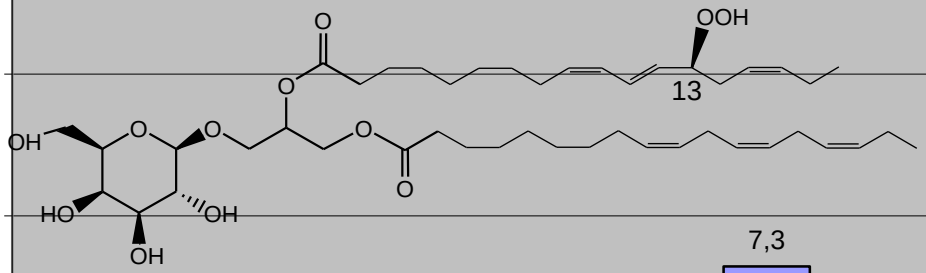
Guella et al. , work in progress

Fruit ripening : an oxidative stress?



Regiochemistry→tandem MS
Stereochemistry → Chiral LC+ CD on FAME derivative

ripening
LOX



Guella et al. , work in progress

Conclusions

- Little is known about complex fatty acids and phospholipids in many taxa (e.g., algae and (cyano)bacteria).
- Profiling/Fingerprinting of large sample sets is possible with current analytical platforms
- Current lipid profiling platforms screen only for common known lipids and lipid classes
- Lipidomics studies have been mainly promoted by researchers from biochemistry, analitycal chemistry, medical or pharmaceutical fields
- Natural products chemists would welcome into this field wherein they can contribute with their "great expertise"' to developing new methods and to resolving structural problems

Lipidomics is waiting for natural products chemists!!