Intact Polar Lipids in the Environment

MOG

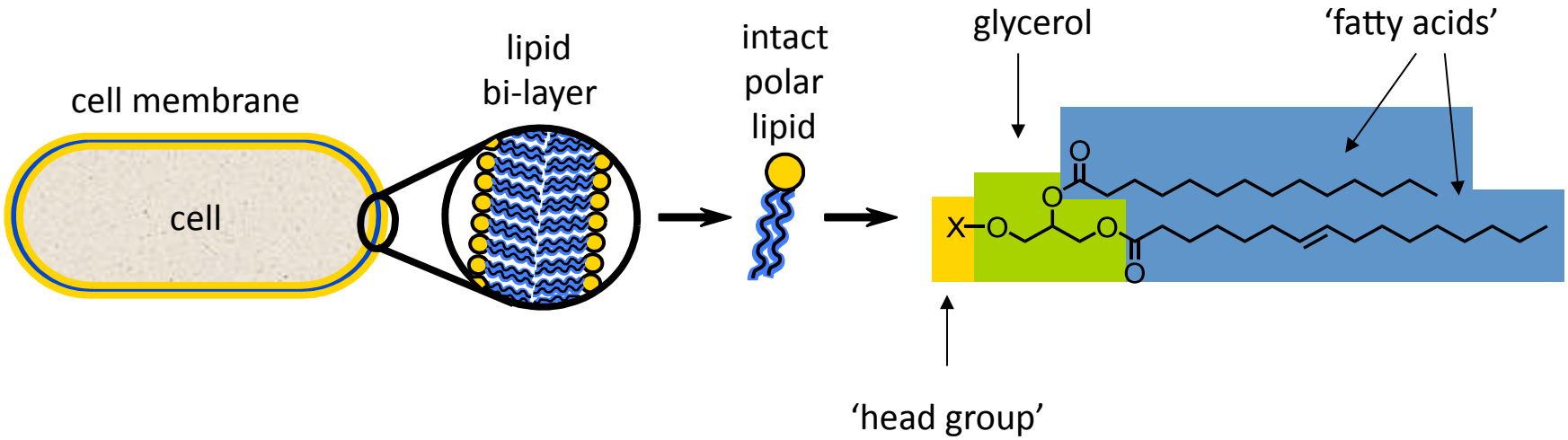
April 21, 2011

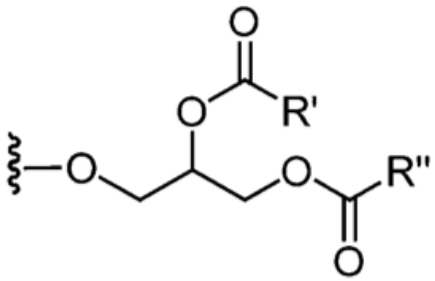
Kim Popendorf

Intact Polar Lipids:

- Structure
- Diversity
- Biogeochemical significance
- Lipid analytical methods
- Studying microbial lipid sources
- Other applications of membrane lipids

Basic structure of Intact Polar Lipids (IPLs)

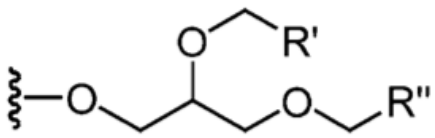




Diacylglycerol

IP-DAG

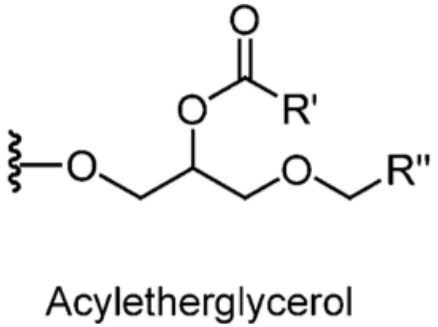
Made by prokaryotes & eukaryotes



Dietherglycerol

IP-DEG

Made by archaea



Acyletherglycerol

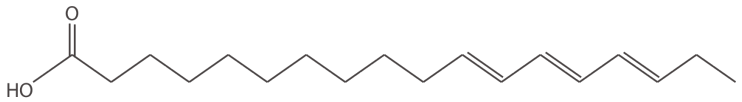
IP-AEG

Structure: Fatty acids

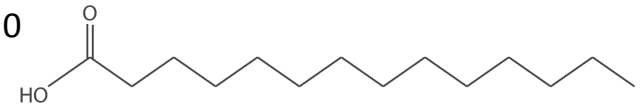
- Major defining features:
  - carbon chain length
  - unsaturations
- Define the fluidity of membrane
  - Length and unsaturation varies with
    - Microbial source
    - Temperature
    - Pressure (ie depth)
- Common range of FAs:
  - C14 to C24
  - Most abundant in ocean are C16 & C18
  - Even c#'s dominate (acetogenic)
  - Odd carbon chains usually from bacteria
- Analysis of FAs by GC-FID, -MS, -IRMS
- Specific FAs used as biomarkers for microbes

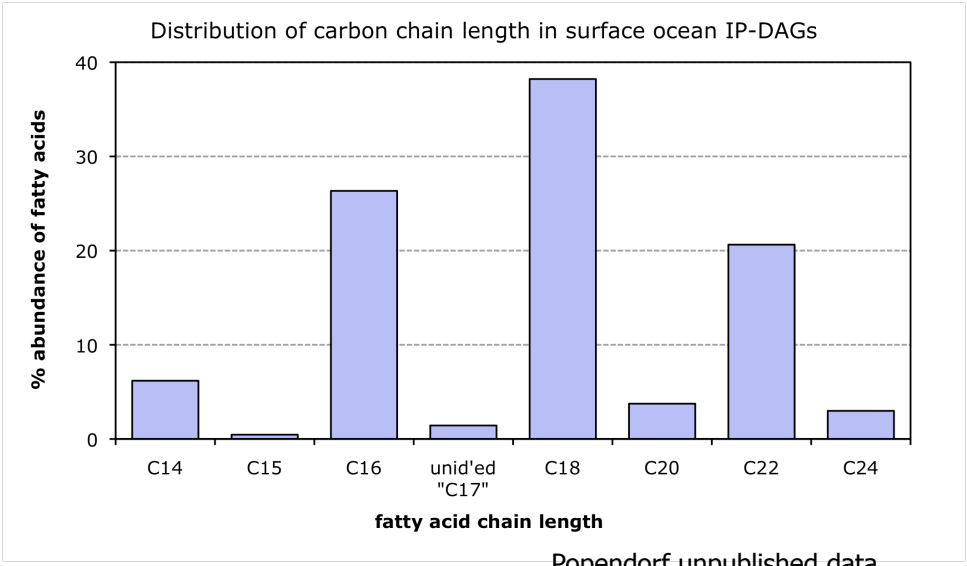
Examples of fatty acids:

C18:3



C14:0

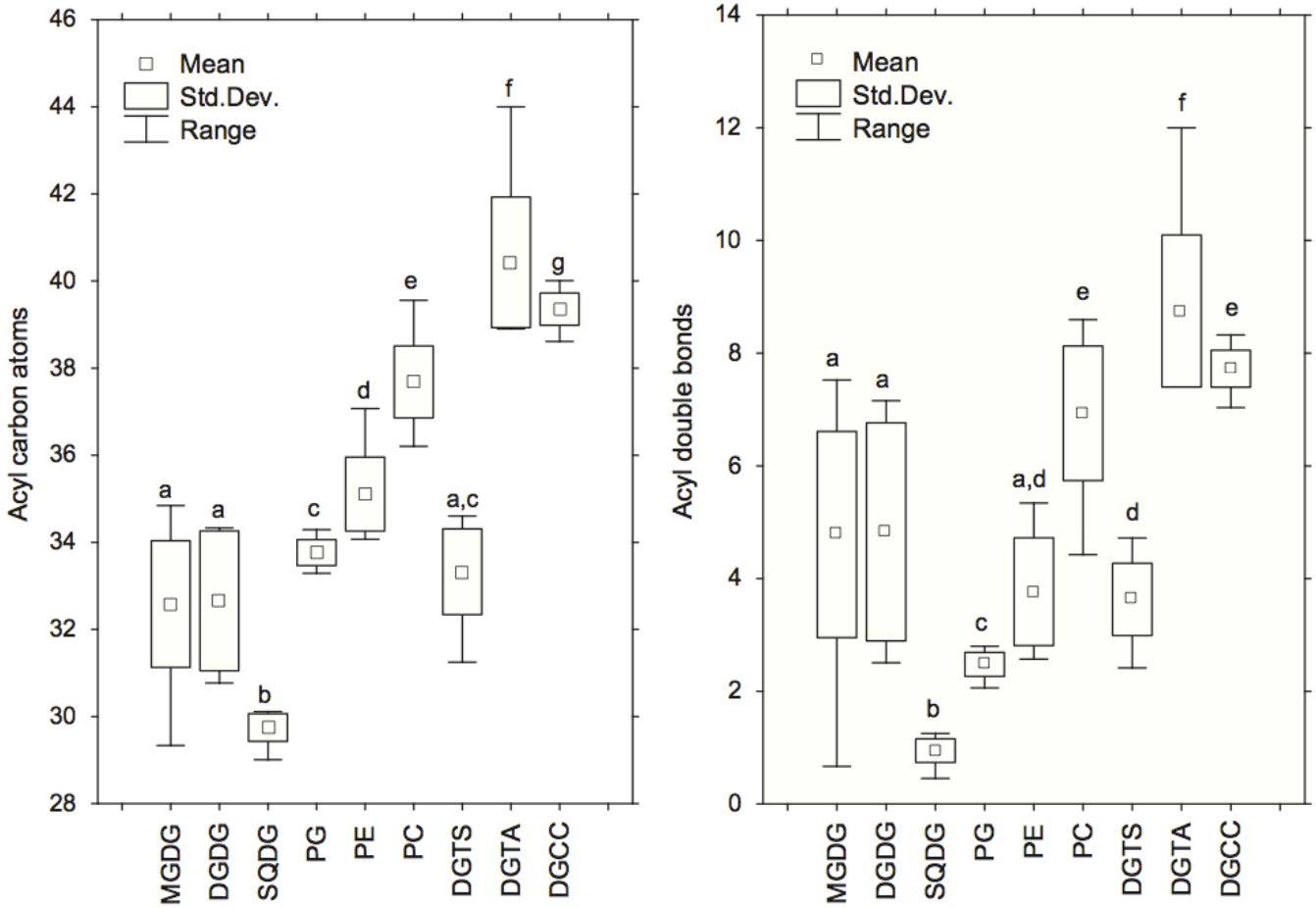




Popendorf unpublished data

Structure: Fatty acids

Diversity of IP-DAGs in the South Pacific euphotic zone

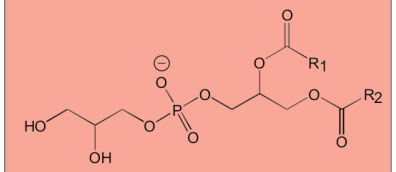


Van Mooy & Fredricks, GCA 2010

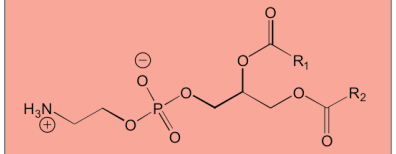
Structure: Headgroups

Major headgroups for marine microbes:

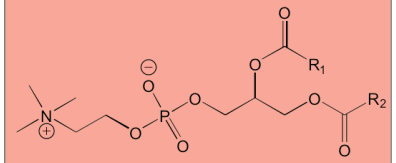
Phospholipids:



PG
phosphotidylglycerol

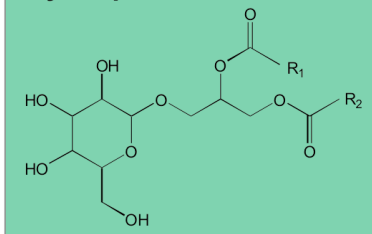


PE
phosphotidylethanolamine

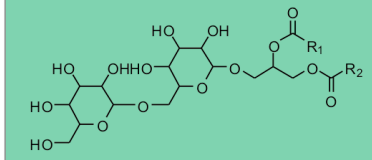


PC
phosphotidylcholine

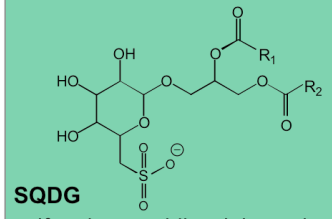
Glycolipids:



MGDG
monoglycosyldiacylglycerol

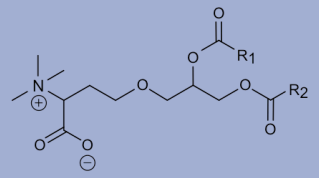


DGDG
diglycosyldiacylglycerol

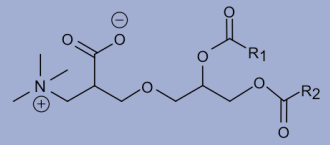


SQDG
sulfoquinovosyldiacylglycerol

Betaine lipids:



DGTS
diacylglyceryl-trimethyl-homoserine



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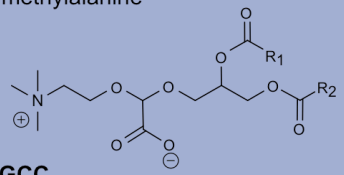
diacylglyceryl-hydroxymethyl-trimethylalanine



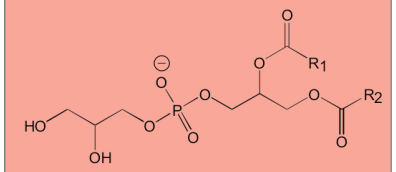
DGCC
diacylglyceryl-carboxyhydroxy-methylcholine

- Intact polar lipids = headgroup+fatty acid
- Headgroup bond to glycerol is labile => IPLs represent live cells
- Analysis of **intact** polar lipids (headgroup+fatty acids) by HPLC-MS

Importance for biogeochemical cycles:

Contains P

Phospholipids:



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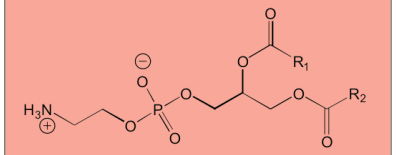

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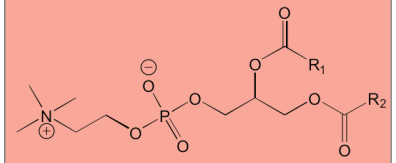
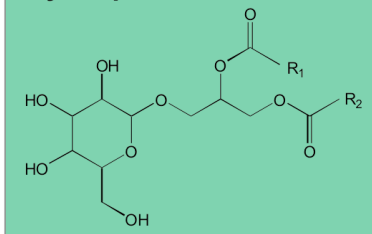


PC
phosphotidylcholine

Contains C

Glycolipids:



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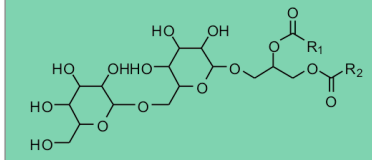

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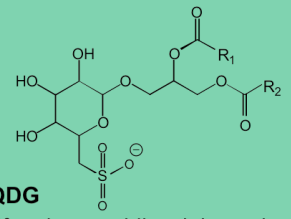
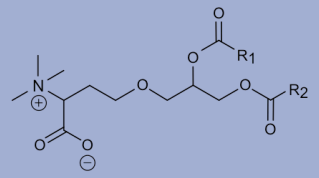


SQDG
sulfoquinovosyldiacylglycerol

Contains N, no P

Betaine lipids:



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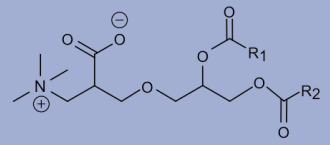

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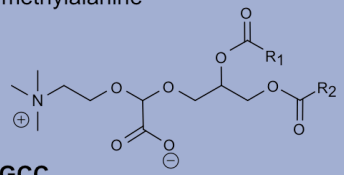
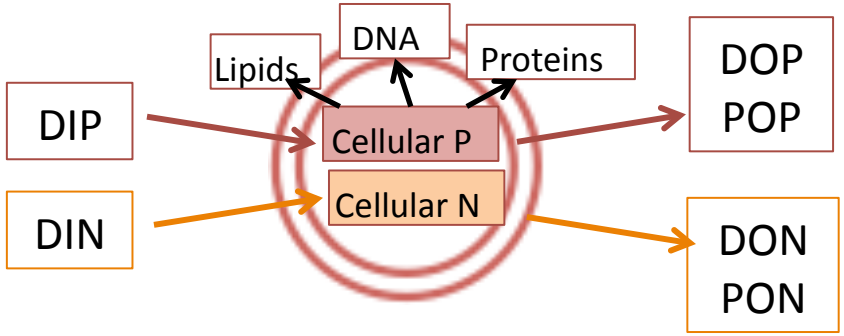


DGCC
diacylglyceryl-carboxyhydroxy-methylcholine

- Membrane lipids compose 11-23% of planktonic carbon (Wakeham et al. DSR 1997)
- Phospholipids can be 1-28% of the cellular phosphate needs (Van Mooy et al. PNAS 2006)
- Substantial and variable cellular nutrient requirement

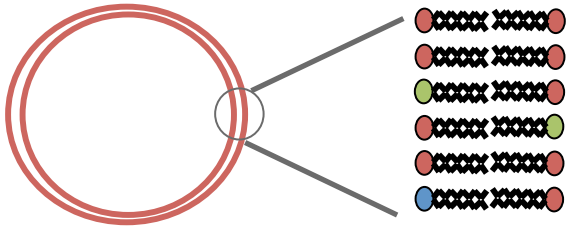
Idea of membrane lipid substitution:

- A lot of cellular demands for nutrients:



- As an adaptation to phosphate stress, microbes can substitute non-phospholipids for phospholipids in their membranes

Phosphate replete:



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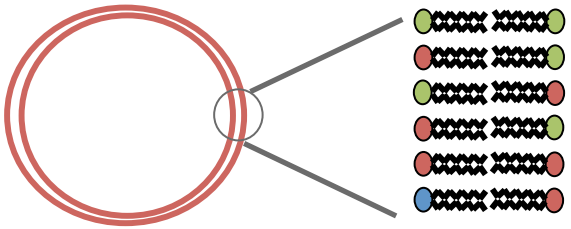
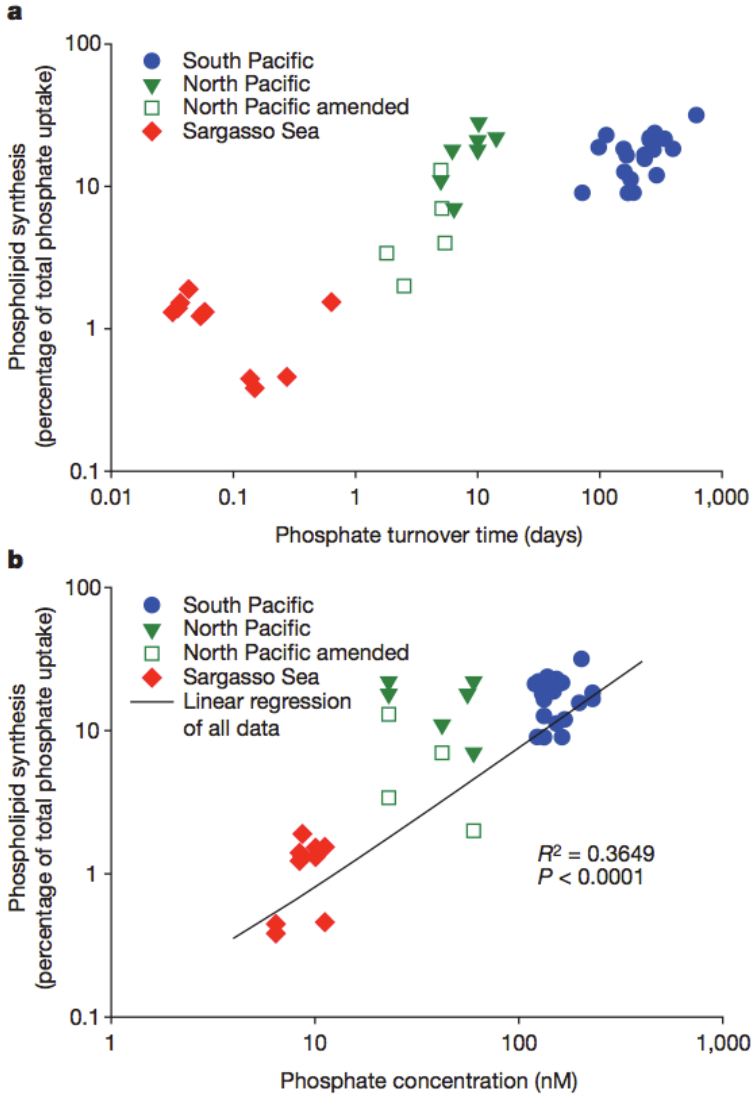


- Proposed in 1990's by Christoph Benning, followed up by studies in the environment & cultures by Ben Van Mooy 2000's

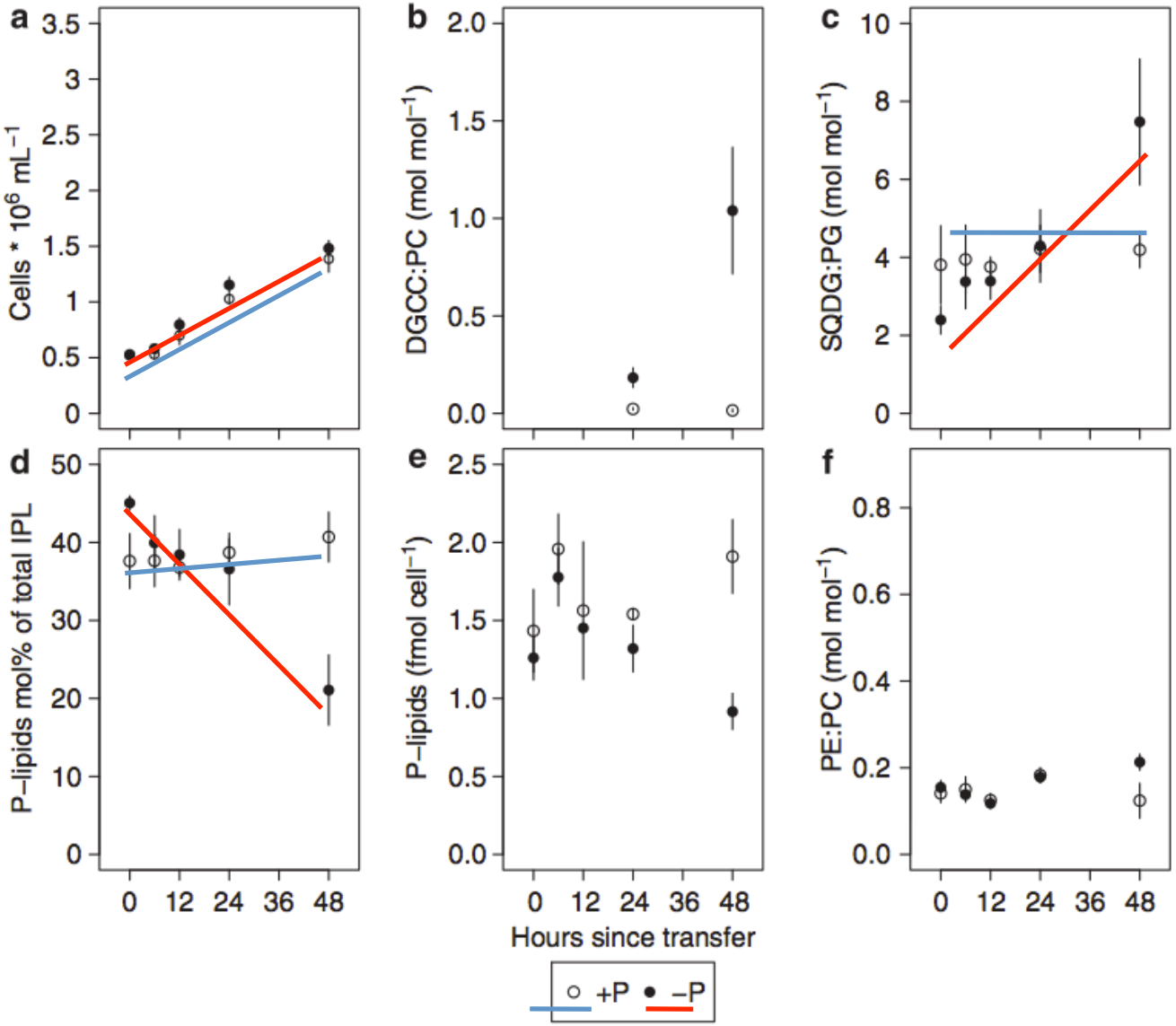
Across different environments, P-lipid synthesis is variable



Van Mooy et al. Nature 2009

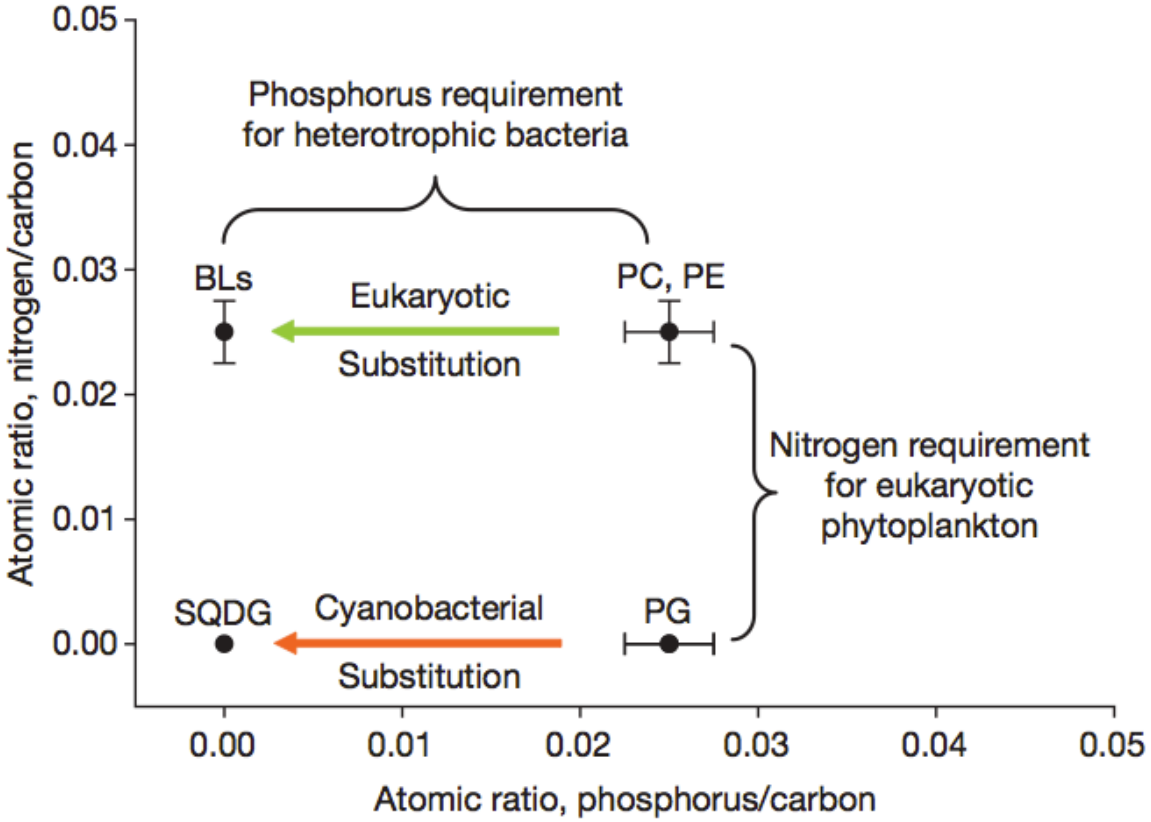
Substitution occurs rapidly in culture

Thalassiosira pseudonana



Martin, Van Mooy, Heithoff, Dyhrman ISME Journal 2010
(modified with colored lines)

When faced with P-stress, adjust membrane composition



Van Mooy et al. Nature 2009

Ability (may be) limited to specific groups of microbes!

What can lipids tell us about what microbes are present?

Phospholipids:
Associated with
prokaryotes & eukaryotes

Glycolipids:
Associated with ***prok.,***
mostly ***phytoplankton***

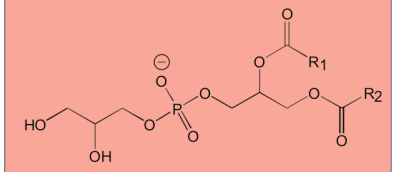
Betaine lipids:
Associated with
eukaryotes

PG:
associated with
prokaryotes,
chloroplast memb.
and ***heterotrophic bacteria***

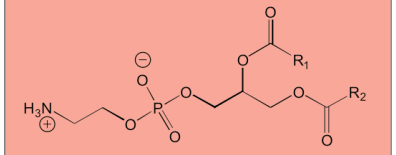
PE:
Associated with
heterotrophic bacteria

PC:
Associated with
eukaryotic phytoplankton
& with het bac

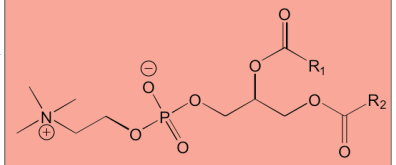
Phospholipids:



PG
phosphotidylglycerol



PE
phosphotidylethanolamine



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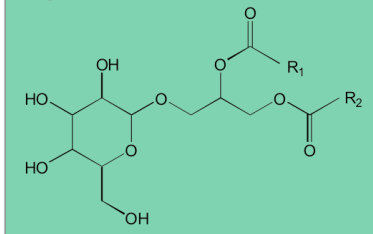

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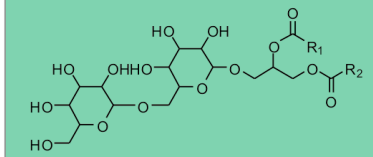

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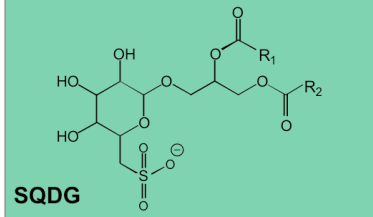

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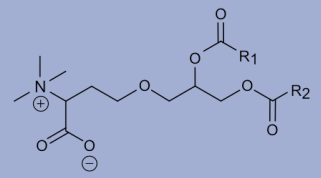

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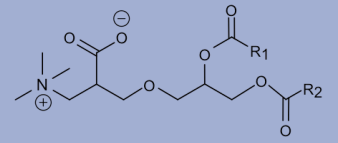

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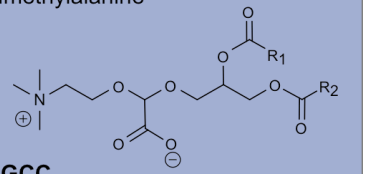


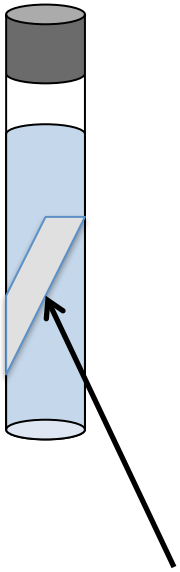
DGCC
diacylglyceryl-carboxyhydroxy-methylcholine

SQDG:
Associated with ***chloroplast membrane***

Lipid extraction

one method: Bligh and Dyer solvent extraction

Extract lipids in dichloromethane or chloroform
Use a change in ratio of solvents to go from one phase to two



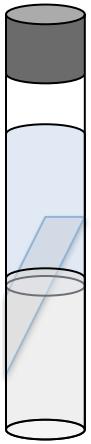
One phase:

Aqueous
(eg water based buffer)
+ organic solvents
(eg methanol & dichloromethane)

Ratio:
Water:MeOH:DCM
0.8:2:1

Filter with particulate matter, or sediment sample, tissue sample, etc

Sonicate, vortex, or otherwise break up cellular material



Separate phases:

Aqueous phase
(water + methanol)

Organic phase
(dichloromethane)
-> Lipids<-

Ratio:
Water:MeOH:DCM
1.8:2:2

Bligh & Dyer, Canadian Journal of Biochemistry and Physiology 1959

Lipid analysis:

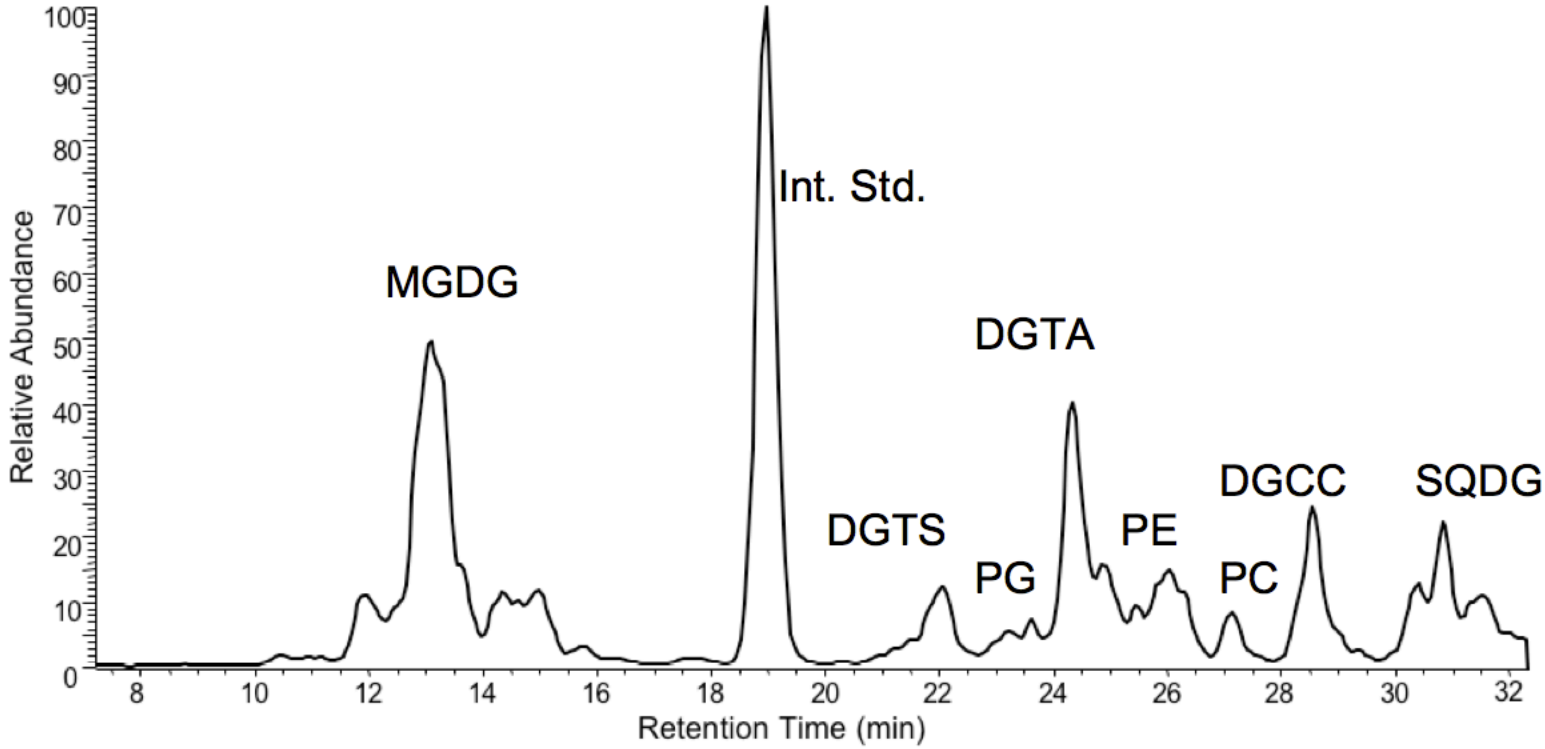
HPLC-
High pressure
Liquid chromatography

ESI –
Electrospray
Ionization

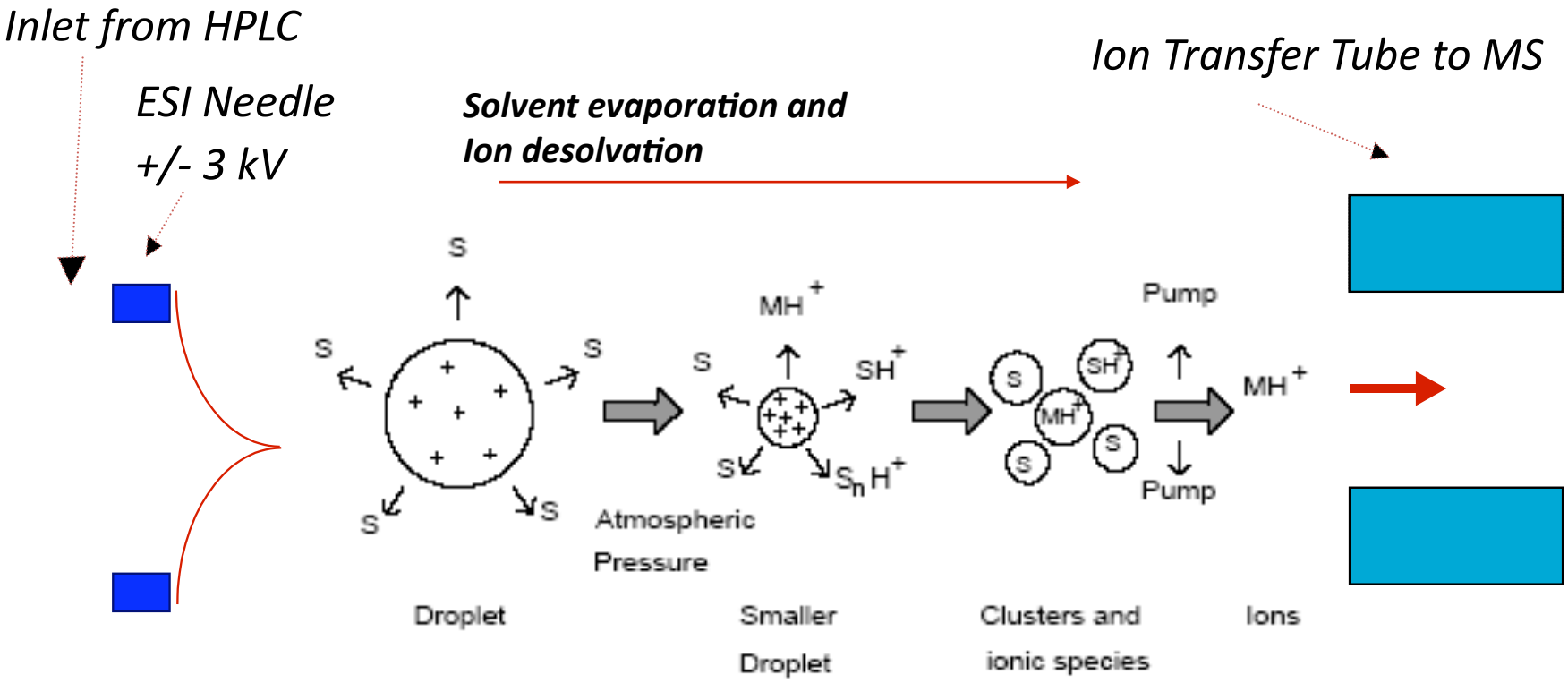
MS
Mass
Spectrometry

HPLC separation by headgroup

Solvent gradient + column selected to separate IPLs by headgroup, roughly less polar to more polar



Electrospray Ionization



ThermoFisher SCIENTIFIC

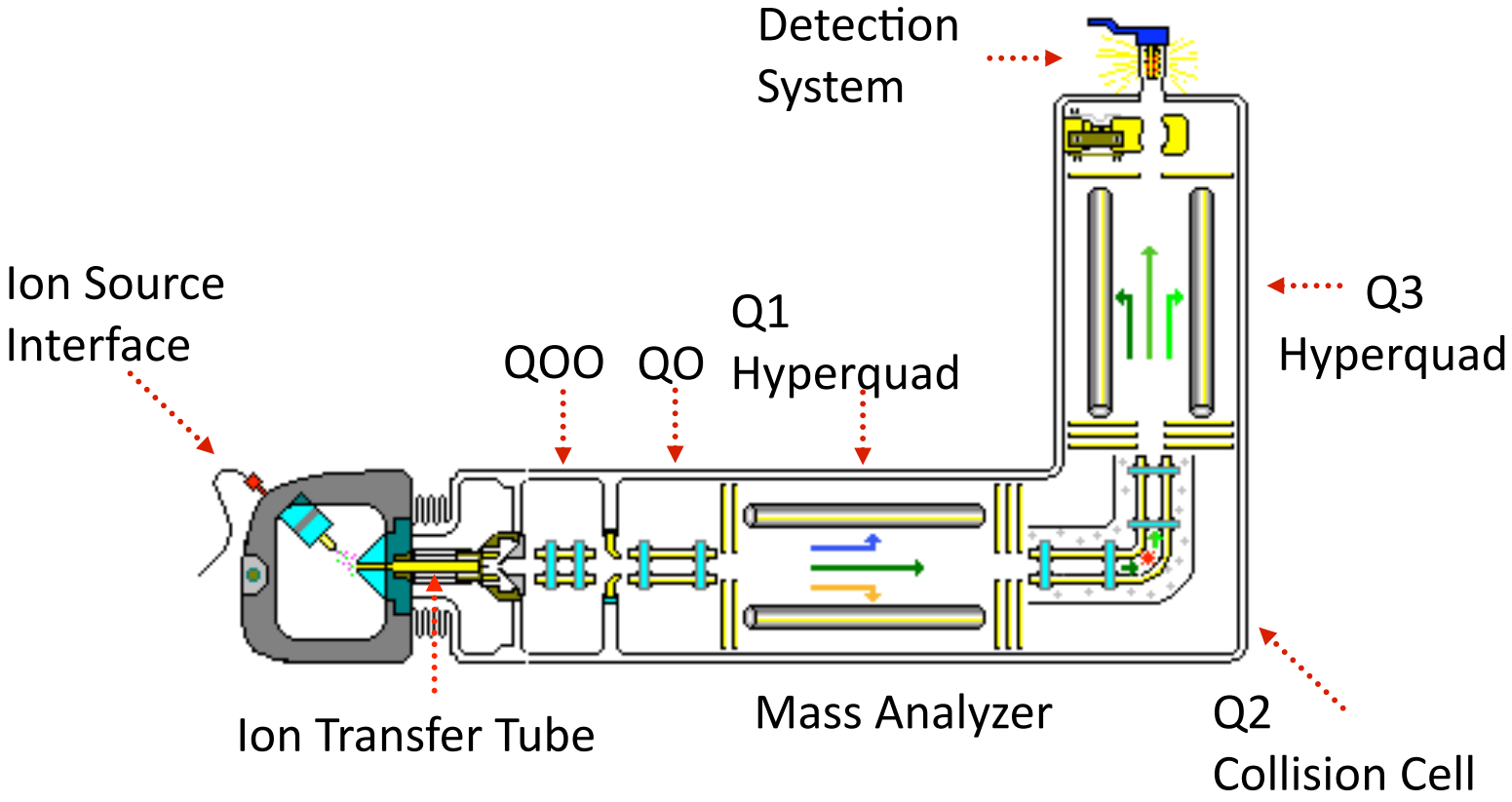
"soft ionization" does not break labile headgroup bond

Does not change ionization of molecules!

For IP-DAGs not naturally ions (MGDG, DGDG, SQDG, PG) use ion adducts in HPLC eluent

Mass Spectrometry

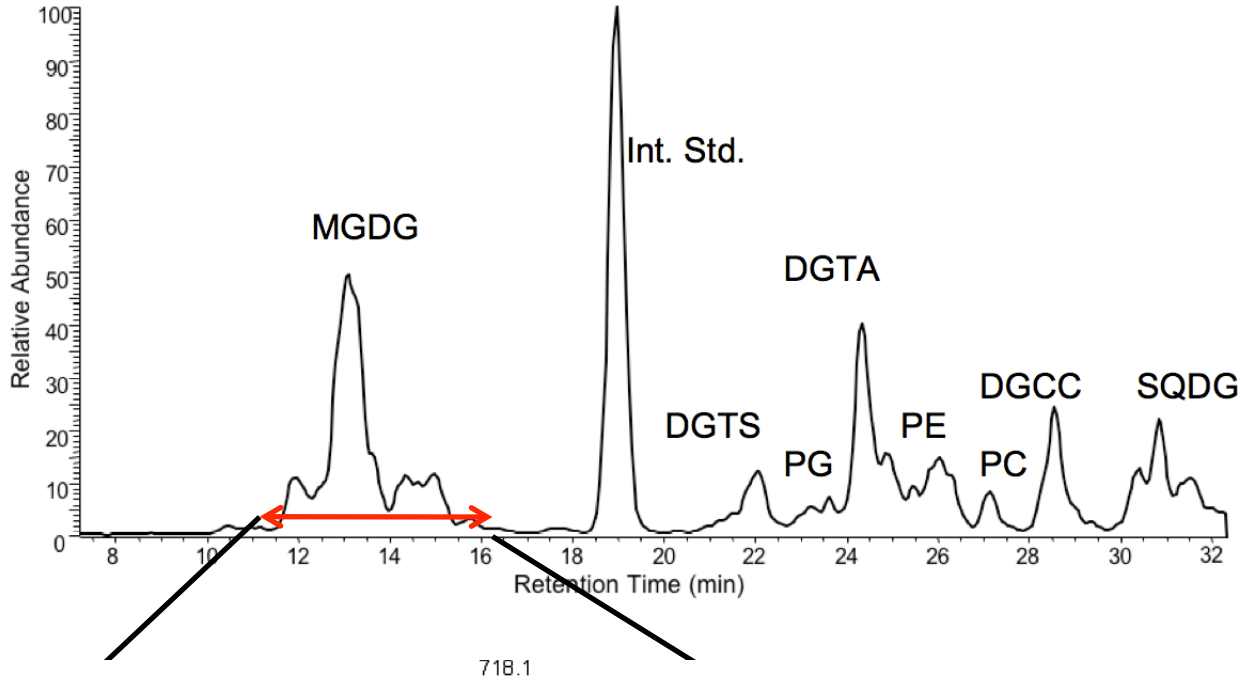
example of triple quadropole system

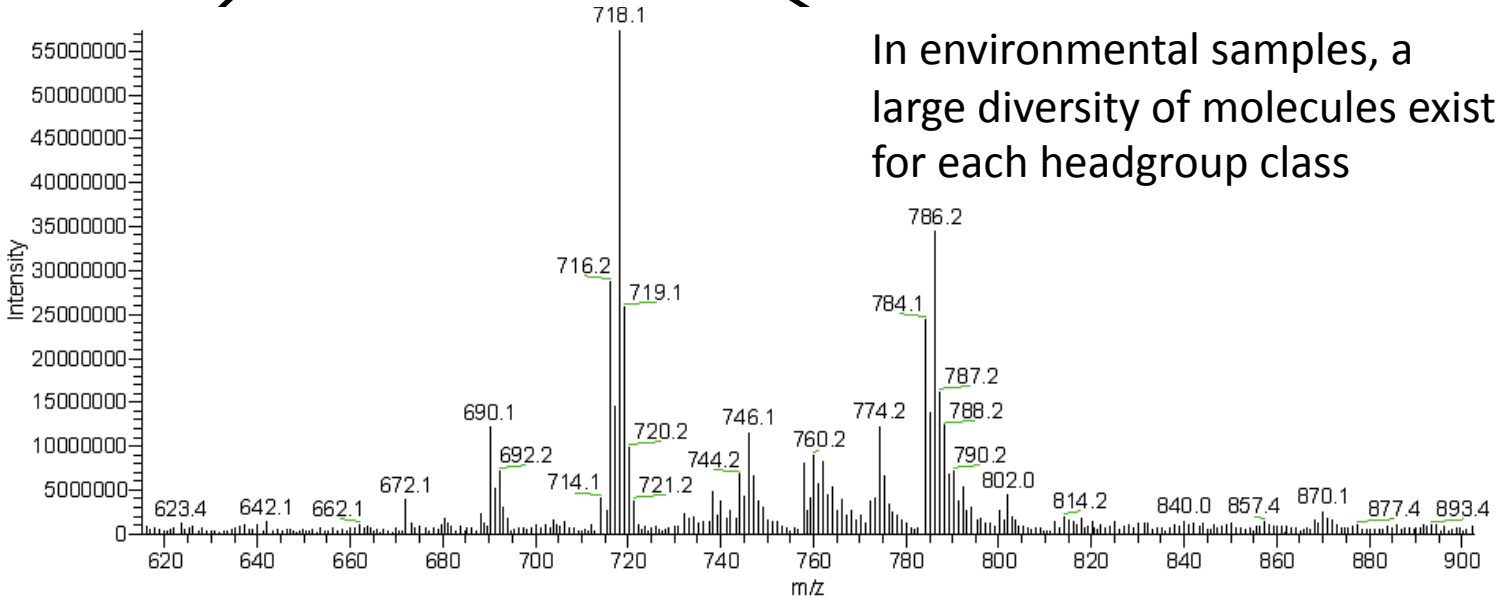


Quadropole 1: detect masses
Quadropole 2: fragment ions
Quadropole 3: detect fragment masses

ThermoFisher SCIENTIFIC

HPLC-MS lipid analysis



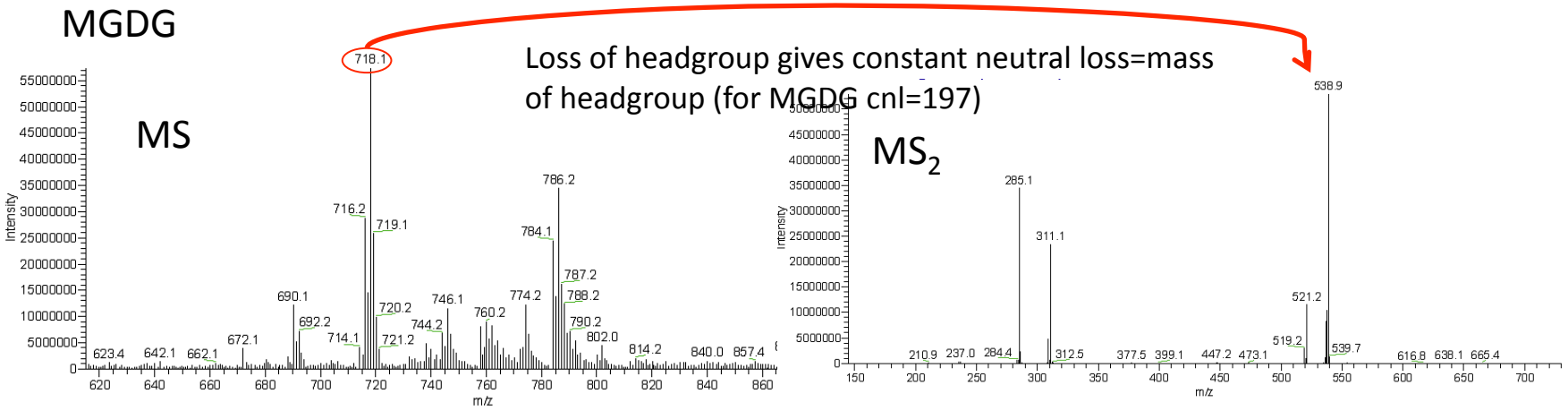


In environmental samples, a large diversity of molecules exist for each headgroup class

Lipid identification:

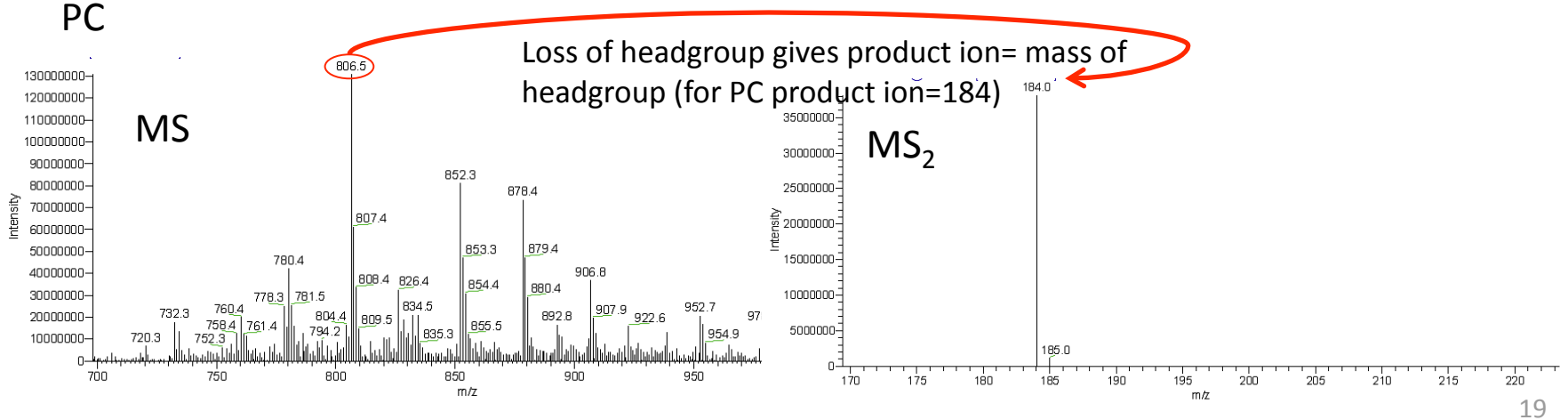
relies on characteristic fragmentation of the headgroup

MGDG

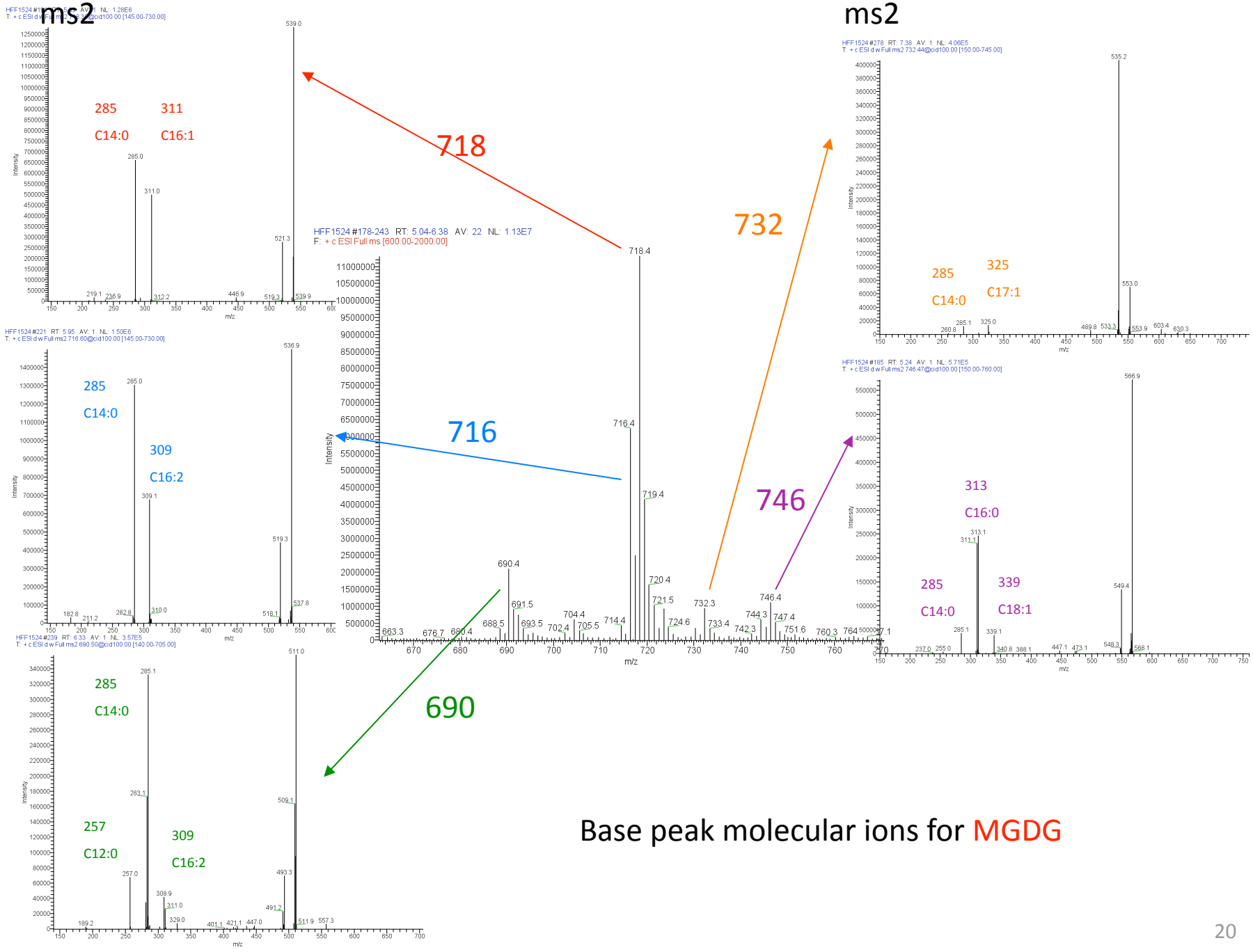


Loss of headgroup gives constant neutral loss=mass of headgroup (for MGDG cnl=197)

PC



Loss of headgroup gives product ion= mass of headgroup (for PC product ion=184)

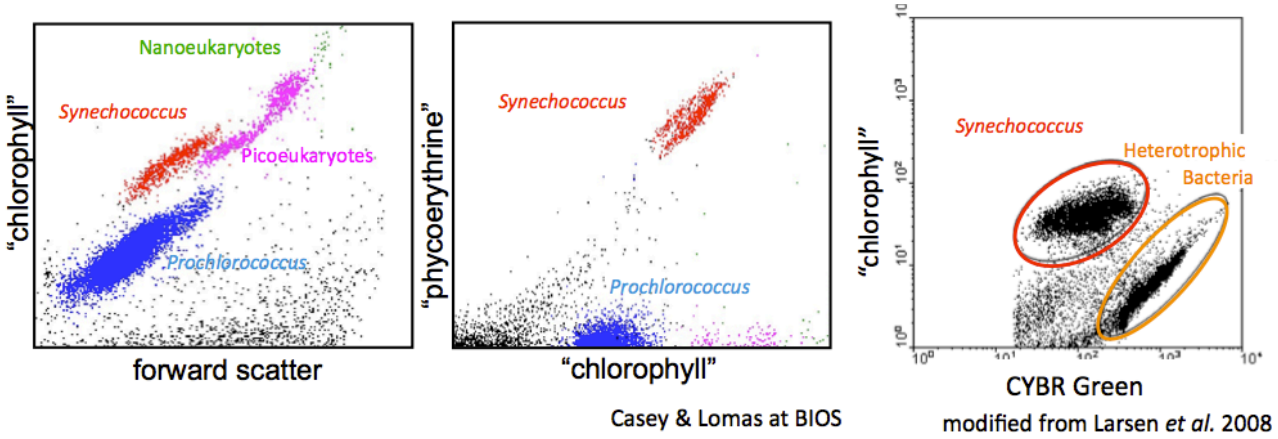


Base peak molecular ions for MGDG

Examples of studies with lipids: In the environment, who makes which lipid?

- Stable isotope tracing

- Cell sorting flow cytometry

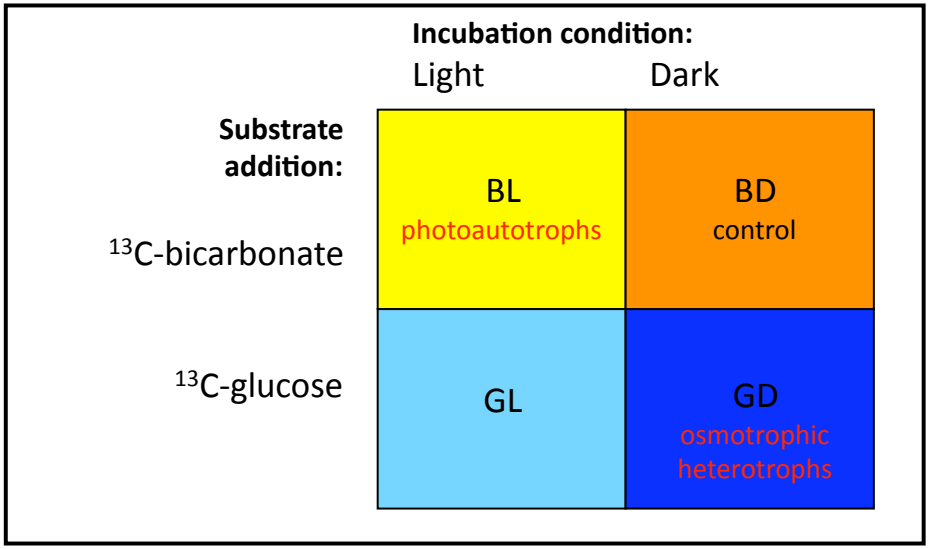


- Targeted incubations

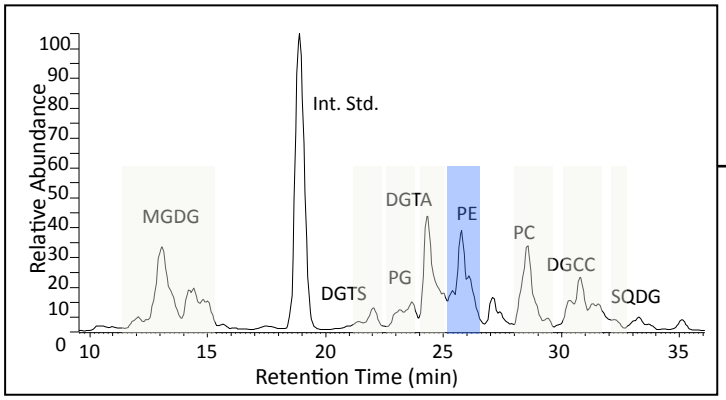
  Filter seawater to remove grazers, incubate in the dark to select for heterotrophs

Examples of studies with lipids:
In the environment, who makes which lipid?
Stable Isotope tracing

Methods:



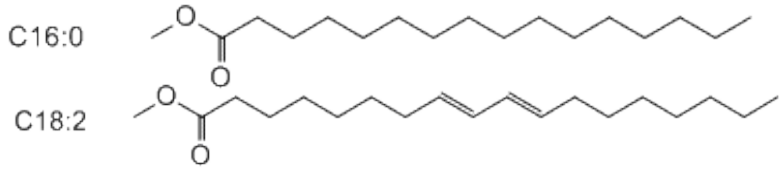
Separate IPLs by prep-HPLC



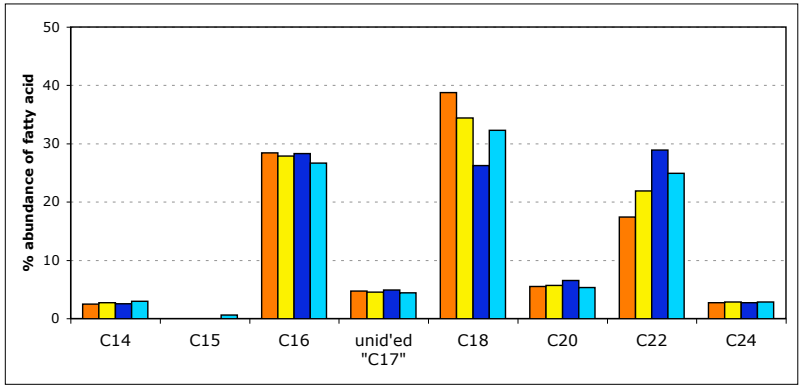
Transesterify to fatty acids

Measure with GC-IRMS

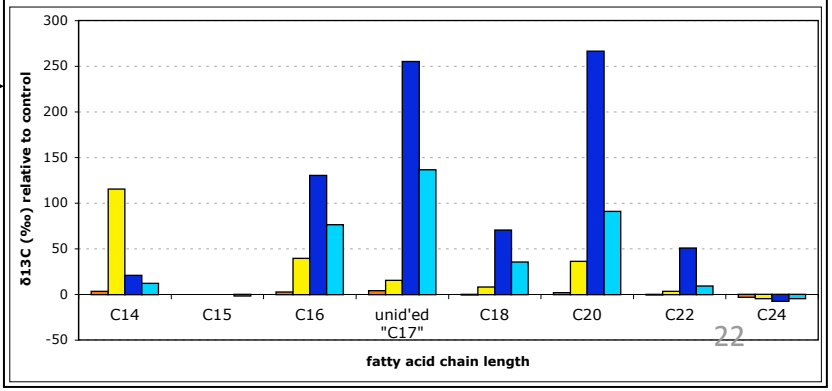
Fatty acid methyl esters



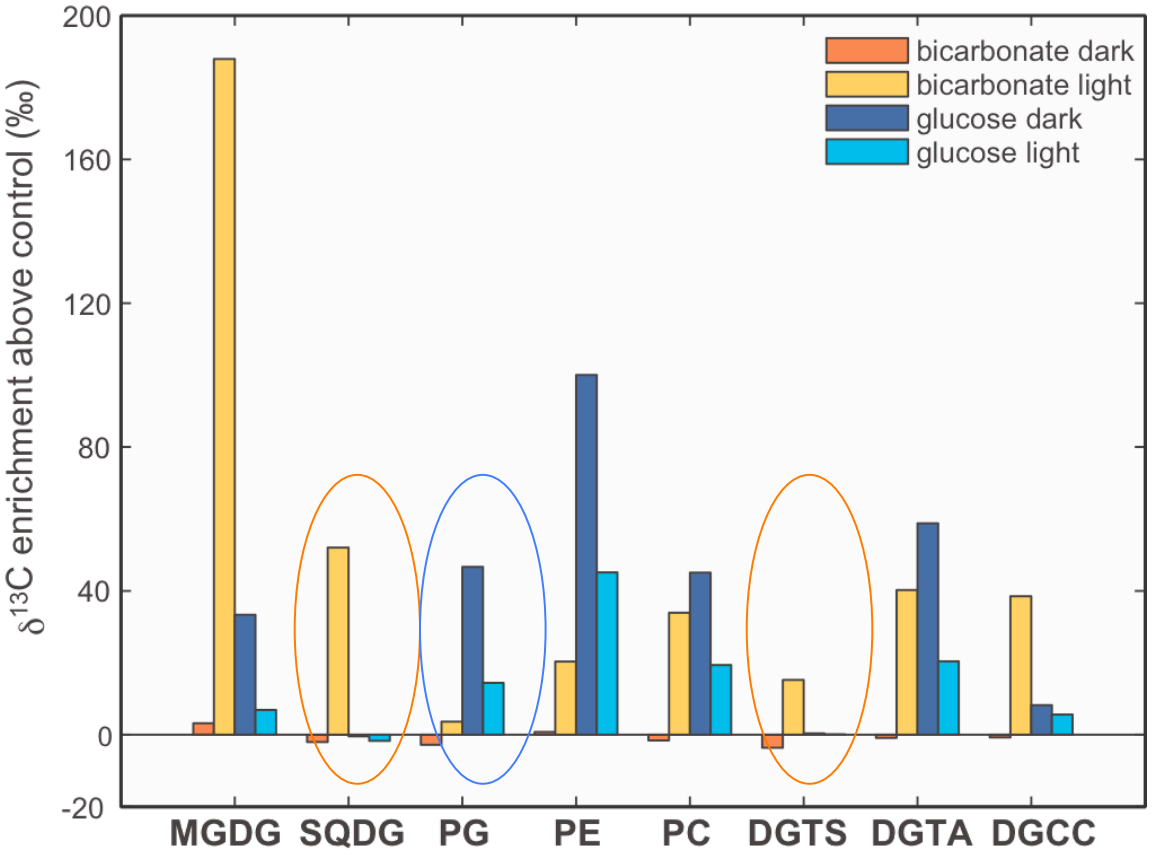
Abundance

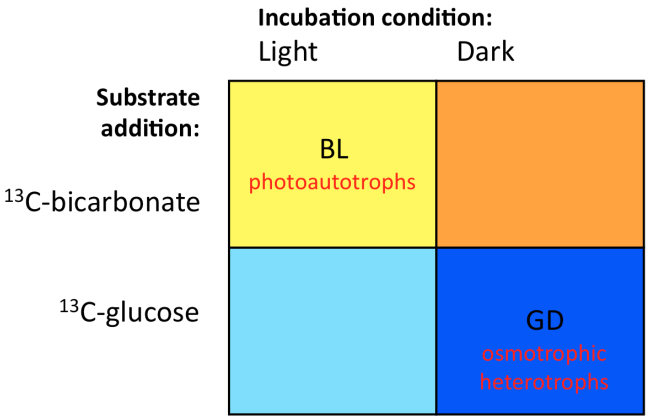


Enrichment



Examples of studies with lipids:
In the environment, who makes which lipid?
Stable Isotope tracing





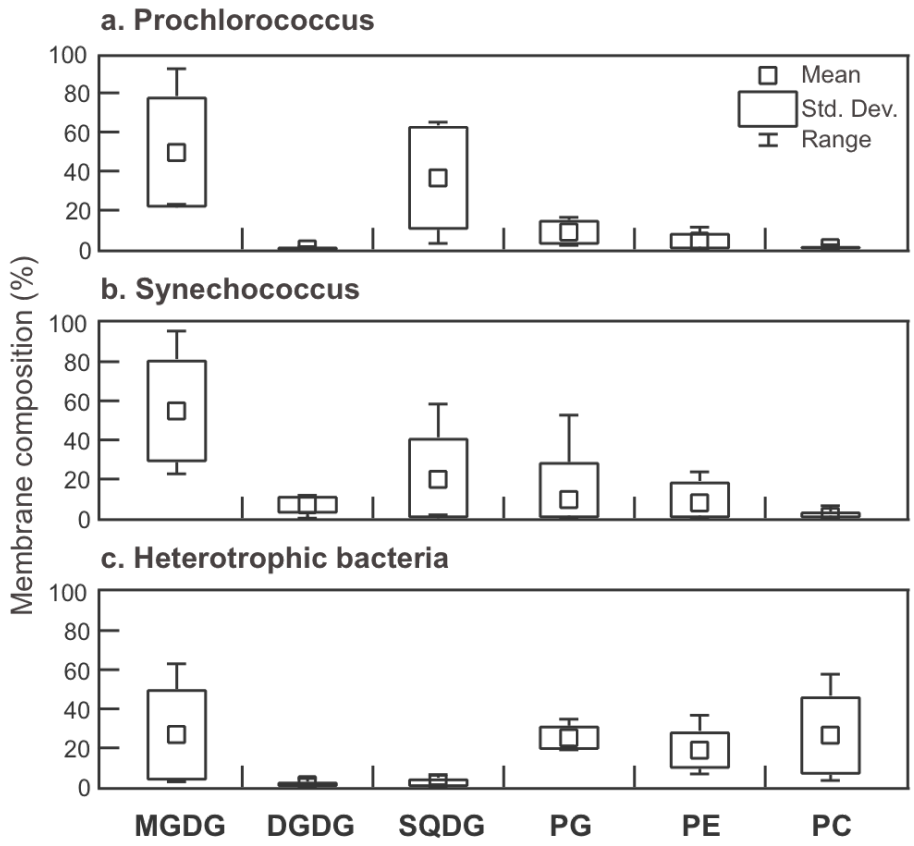
Popendorf et al. Org. Geochem. submitted

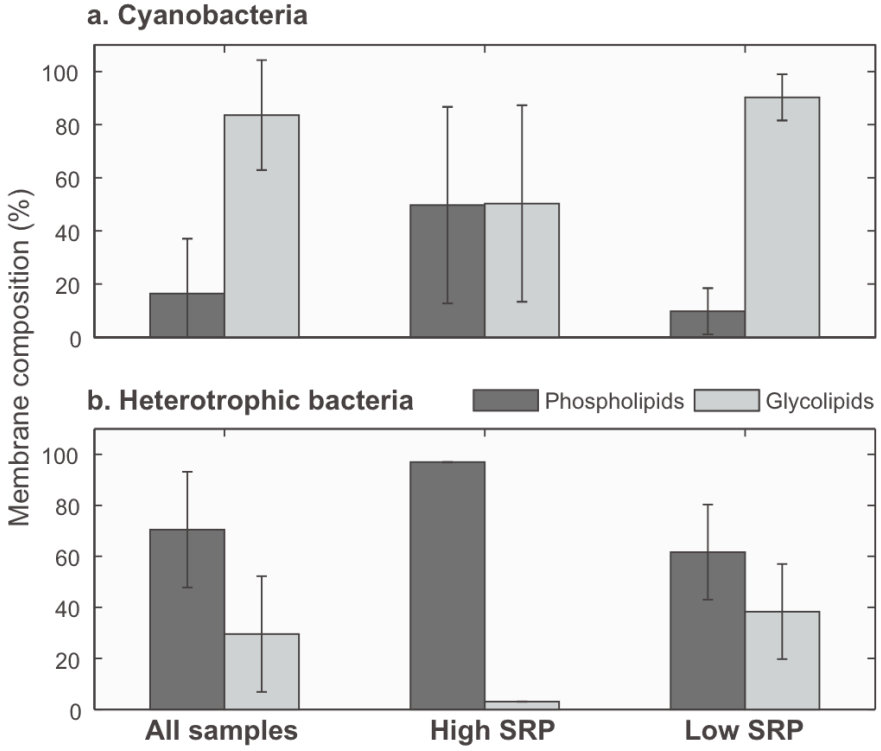
In the Sargasso Sea:

SQDG and DGTS made by photoautotrophs

PG made by heterotrophs

Examples of studies with lipids:
In the environment, who makes which lipid?
Cell sorting flow cytometry in the Sargasso Sea

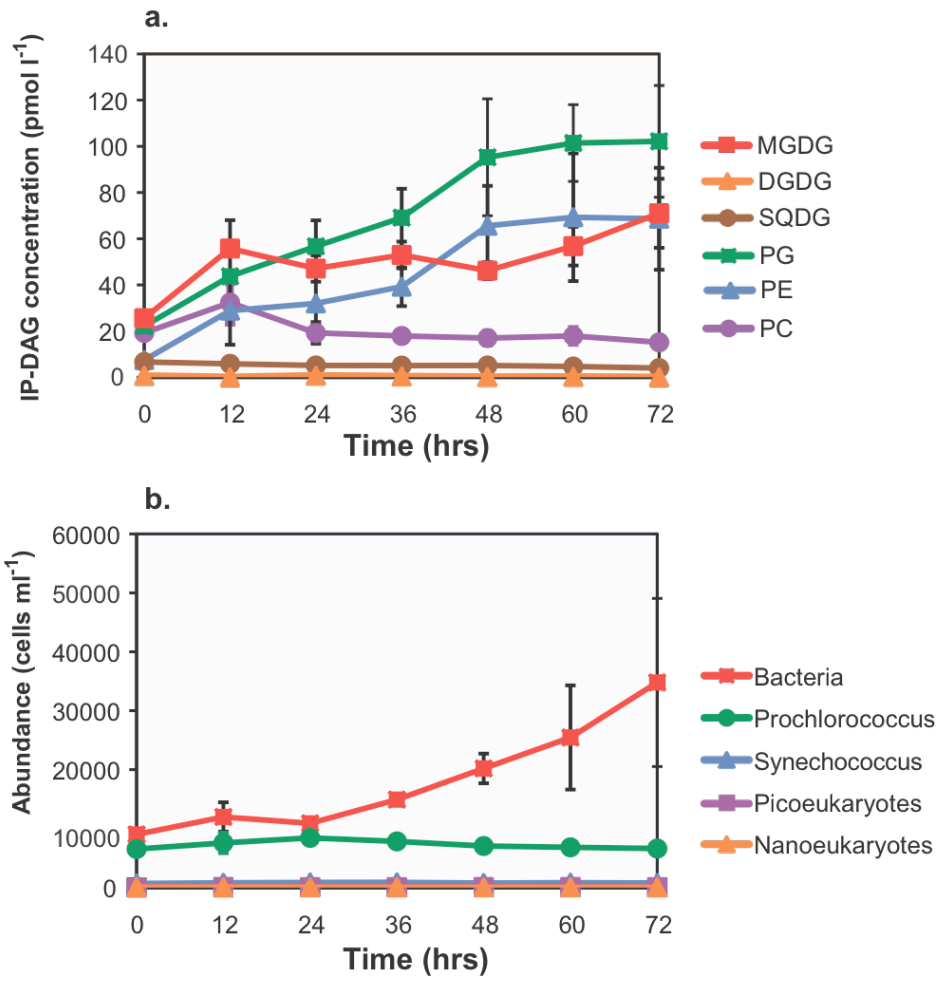




Popendorf et al. Org. Geochem. submitted

Examples of studies with lipids:
In the environment, who makes which lipid?
Targeted incubations

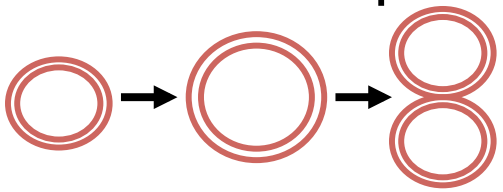
90% filtered seawater, 10% whole seawater, in the dark



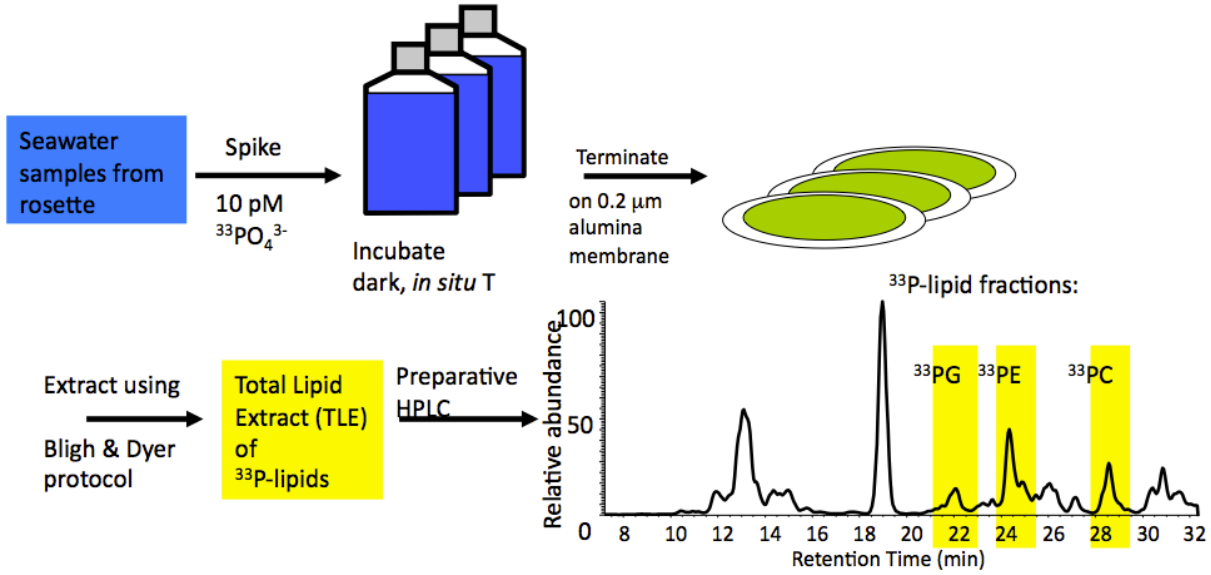
Applications of membrane lipid studies:

Phospholipid production as a measure of biomass production

- Membrane production is obligate with cell growth



- Use addition of radioactive phosphate ($^{33}PO_4$) to trace production of phospholipids in a timed incubation



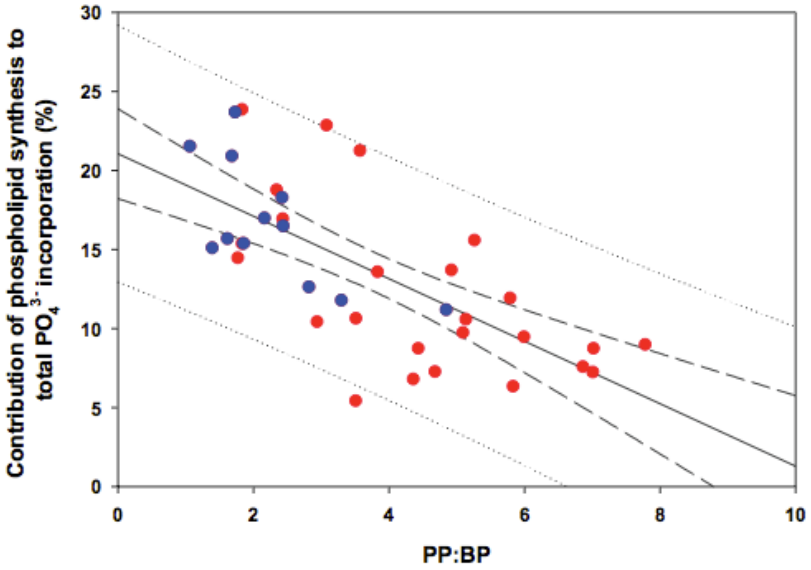
$$\text{P-lipid production rate (mol L}^{-1}\text{ day}^{-1}\text{)} = \left[\frac{^{33}\text{P-lipid dpm}}{\text{liter day}}\right] \times \frac{\left[\text{PO}_4^{3-}\text{ M}\right]}{\left[^{33}\text{P dpm/L}\right]}$$

(^{33}P incorporation into P-lipids; Phosphate concentration in seawater; spike added to sample)

References:

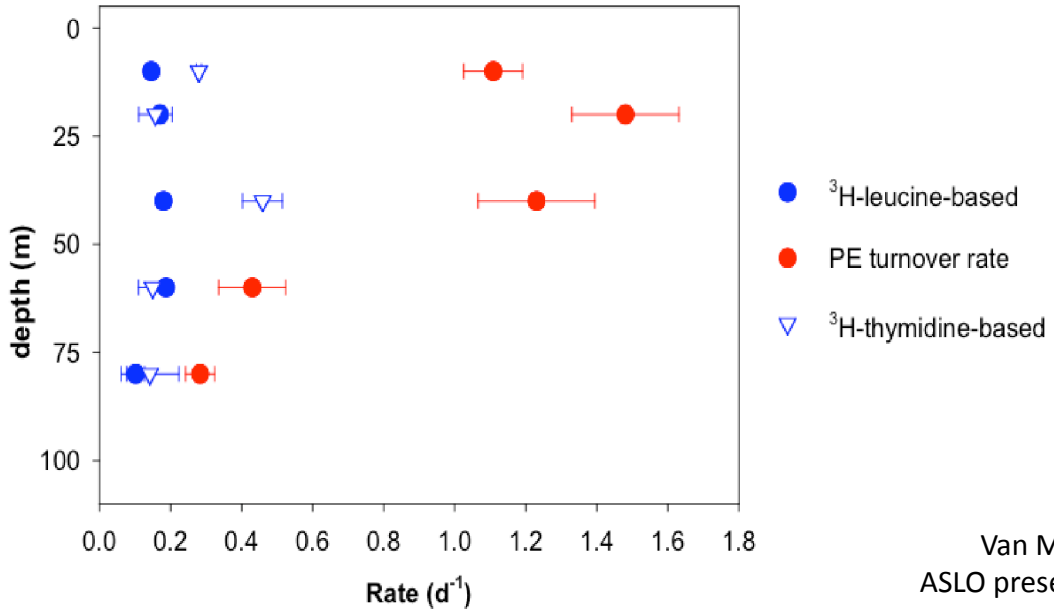
White L&O 1977
Fuhrman & Azam 1982
Van Mooy BGS 2008

Applications of membrane lipid studies: Phospholipid production rate as a measure of biomass production



Van Mooy et al.
Biogeoscienses 2008

Profile in Sargasso Sea.



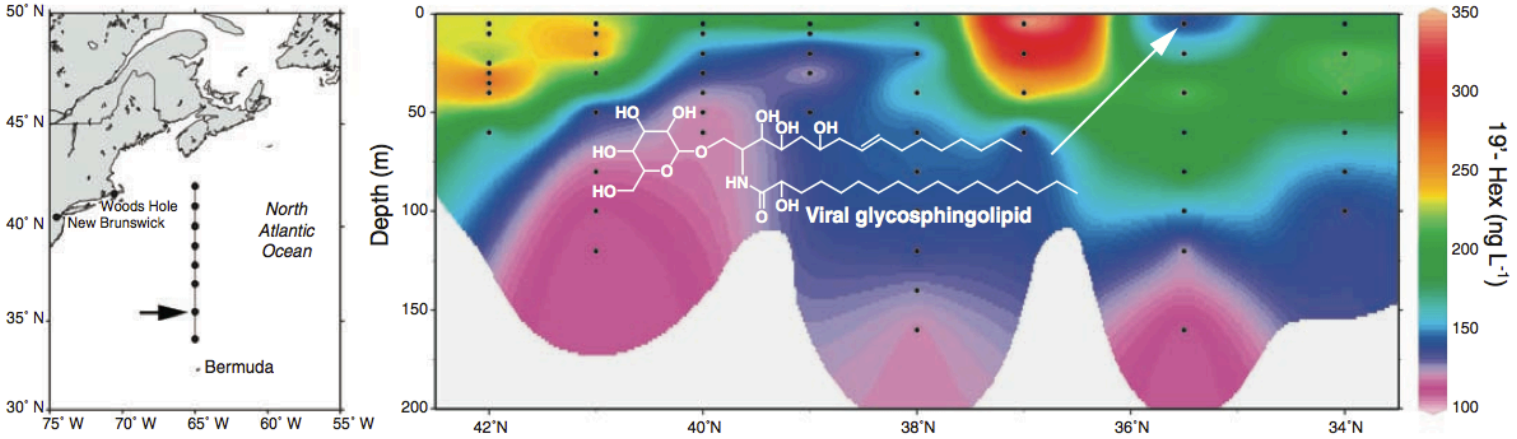
Van Mooy & Rappé
ASLO presentation 2008

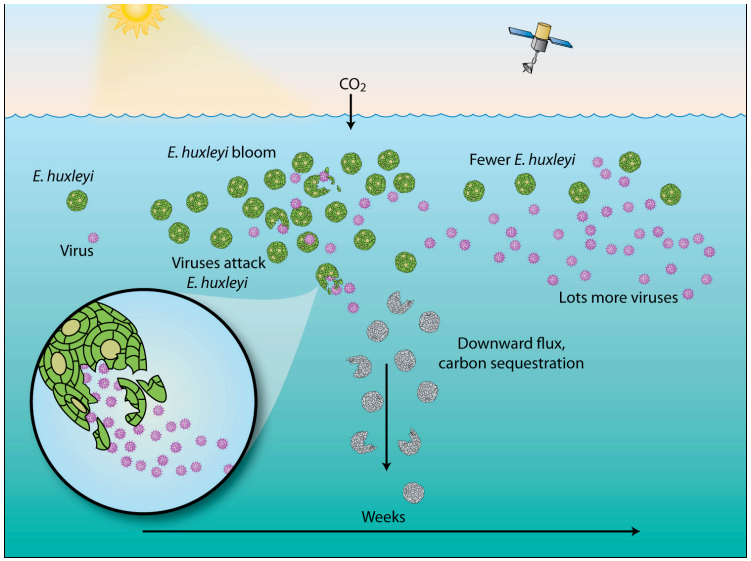
Applications of membrane lipid studies:

Membrane lipids as chemical signals

Vardi et al. Science 2009

A glycosphingolipid can induce programmed cell death in diatoms (E.hux)





Glycosphingolipid production is induced by viruses

-> may play a key role in ending diatom blooms

-> thus a role in carbon flux