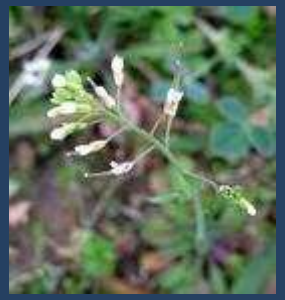


Biodiversity of highly-glycosylated plant phosphosphingolipids: fast screening and structure determination by tandem mass spectrometry

Corinne Buré[1],
Jean-Luc Cacas[2], Sébastien Mongrand[2],
Jean-Marie Schmitter[1]

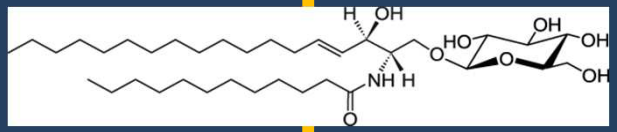
[1] Chimie Biologie des Membranes et Nanoobjets CBMN
[2] Laboratoire de Biogenèse Membranaire LBM

Major sphingolipids

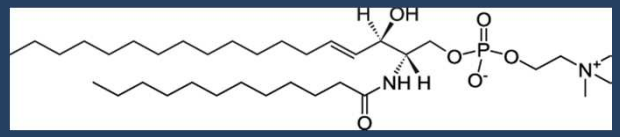
Animal

Plant

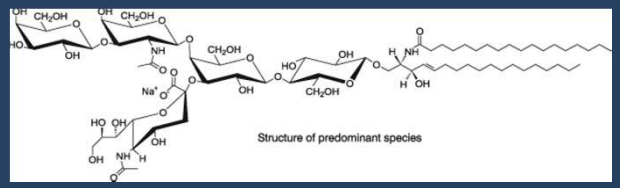
Glucosyl Ceramide



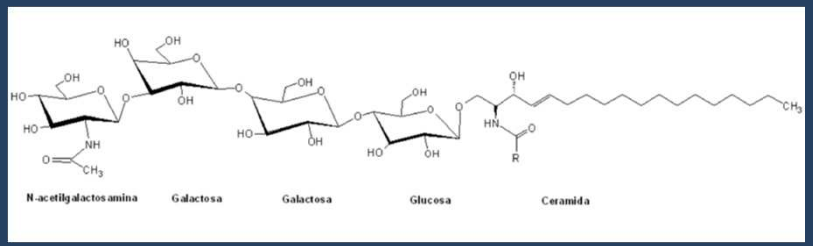
Sphingomyelin



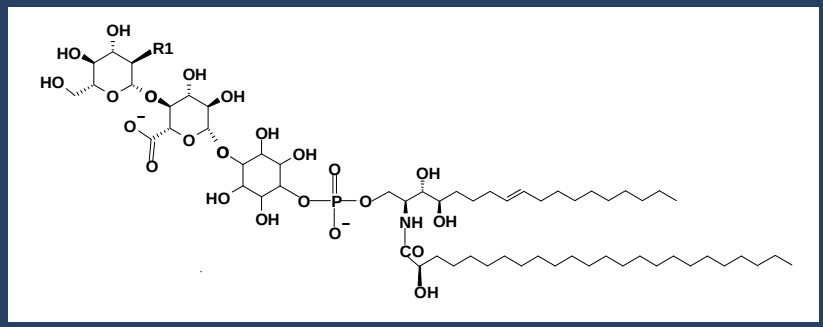
Ganglioside



Globoside



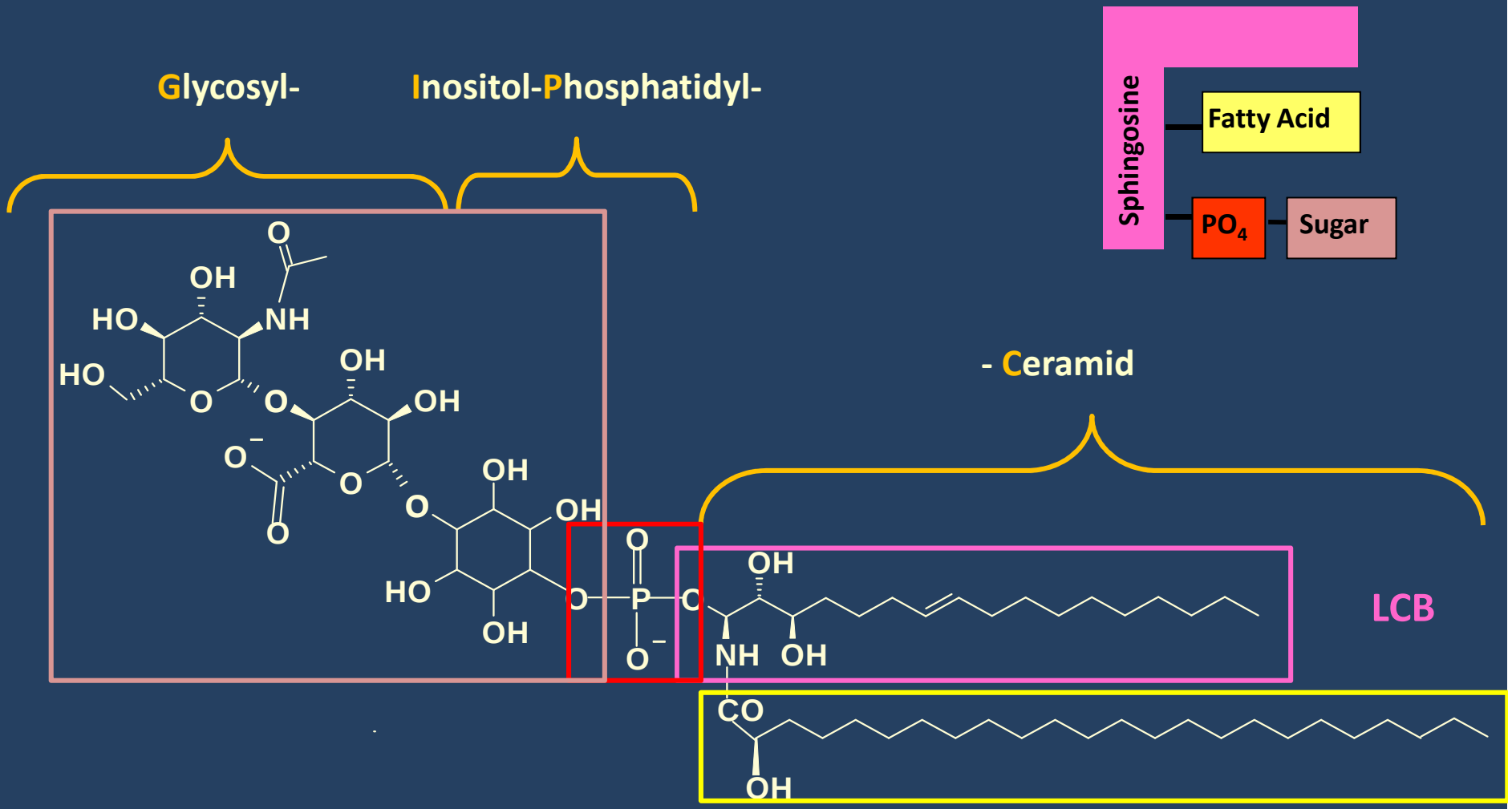
GIPC



A sphingolipid family: GIPC

GIPC : major sphingolipids in plant membranes (64%)

roles in signalling process: plant defence



Markham et al., *RCMS*, **2007**, 21, 1304

Objectives

*** Few existing data on plant GIPC (Glycosyl-Inositol-Phospho-Ceramide):**
tomato, *Arabidopsis thaliana*, soybean, tobacco

*** Fast screening by MALDI-MS of GIPCs in plasmic membranes of 22 plants**

*** Confirmation and/or characterization of GIPC structures**

Choice of plant material:

- **Different taxa: algae, moss, fern, gymnosperm, monocot, dicot**

- **Model organisms:** *Fucus vesiculosus, Arabidopsis thaliana, Nicotiana tabacum, Physcomitrella patens…*

Markham et al., *J. Biol. Chem.*, **2006**, 281, 22684
Markham et al., *RCMS*, **2007**, 21, 1304

1. GIPC extraction/purification

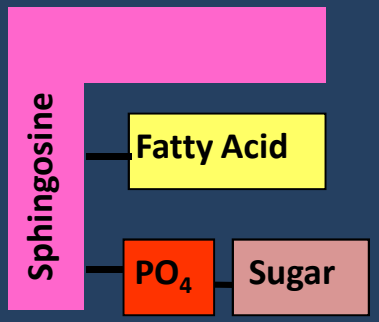
Plant tissue or cell cultures

- **Grinding in cold Acetic Acid, Filtration**
- **70°C for 30 min in 70% ethanol/0.1N HCl**
- **Precipitation at -20°C**
- **Washes**

Total GIPCs: ~ 0.2-1% /dry weight

Adapted from Carter & Koob, *J. Lipid Res.* **1969**, *10*, 363
Buré et al., *RCMS*, **2011**, 25, 3131

2. FA and LCB analysis by GC-MS

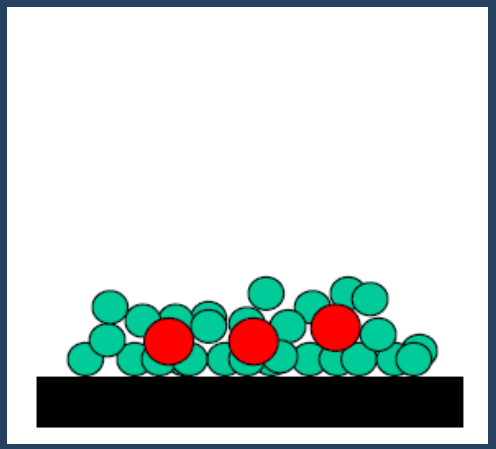


FAs converted into their corresponding Fatty Acid Methyl Esters (acid methanolysis) and silylated (BSTFA) before quantitative GC/MS analysis.

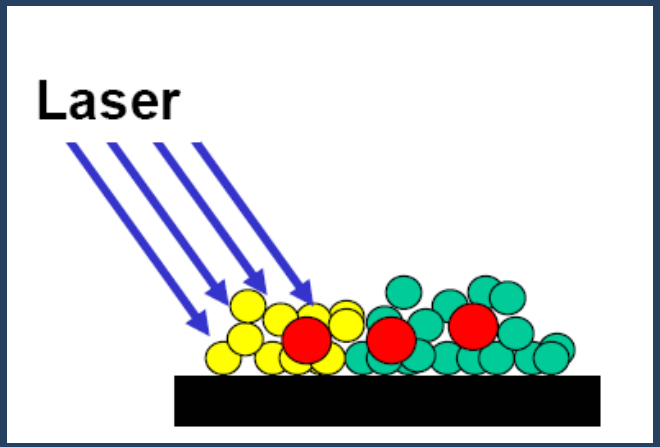
LCBs released from sphingolipids (treatment with $Ba(OH)_2$), converted into their corresponding fatty aldehydes by $NaIO_4$ and quantified by GC-MS.

Cacas et al., *Anal. Bioanal. Chem*, **2012**, 403, 2745

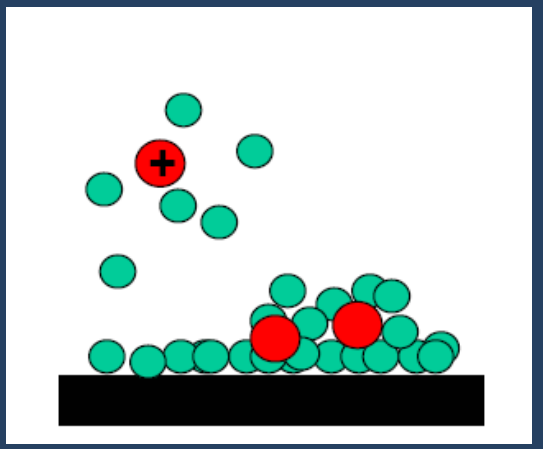
3. MALDI-MS: fast screening



Co-crystallisation of the sample with the matrix



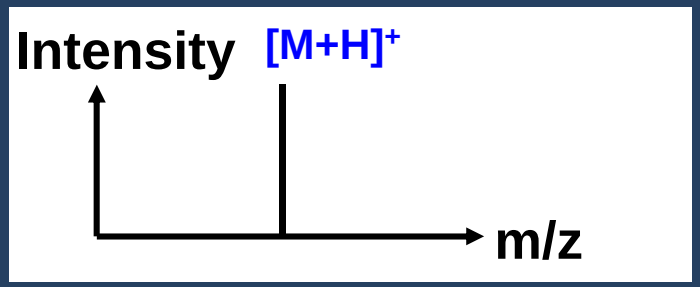
Excitation of matrix molecules by UV laser pulse



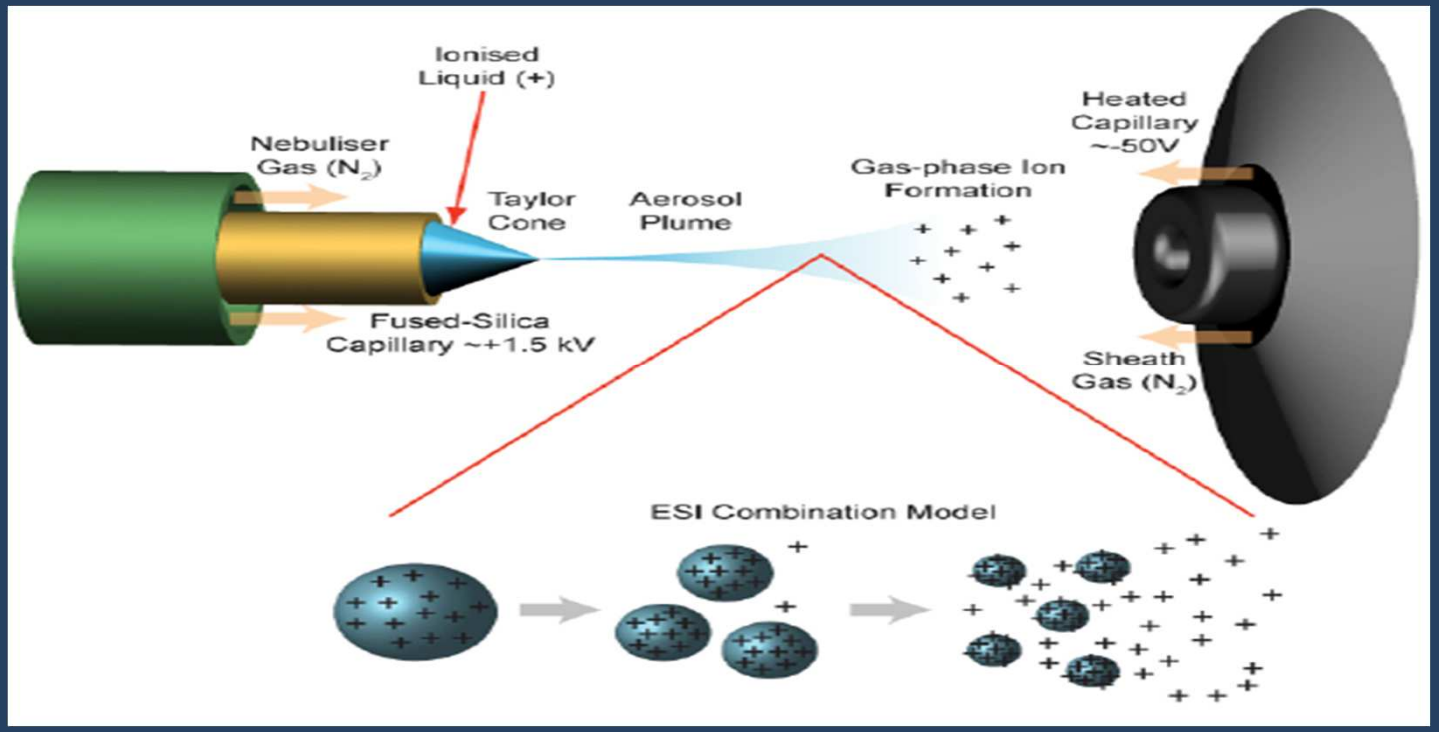
Ionization and desorption of the sample

MALDI-MS:

mono-charged ions

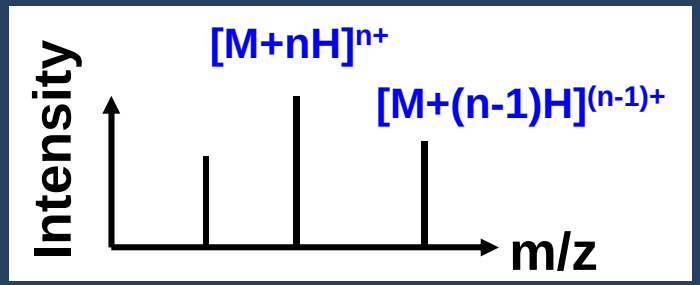


4. ESI-MS/MS: structure analysis



ESI-MS:

multi-charged ions

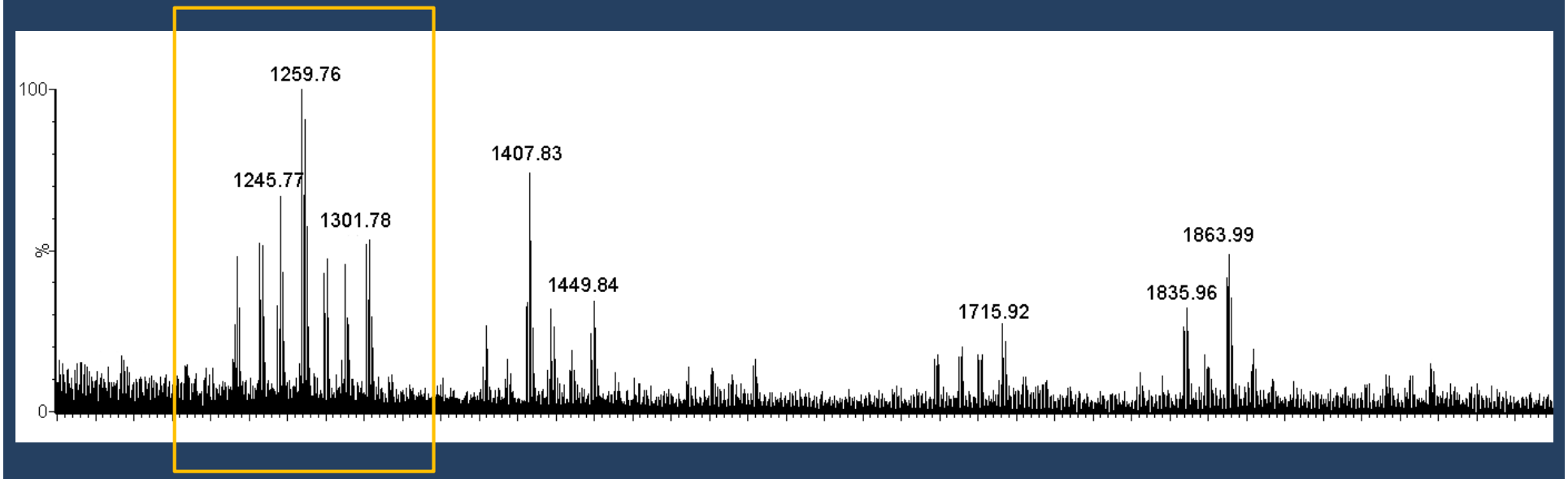


MALDI-MS

Negative ion mode

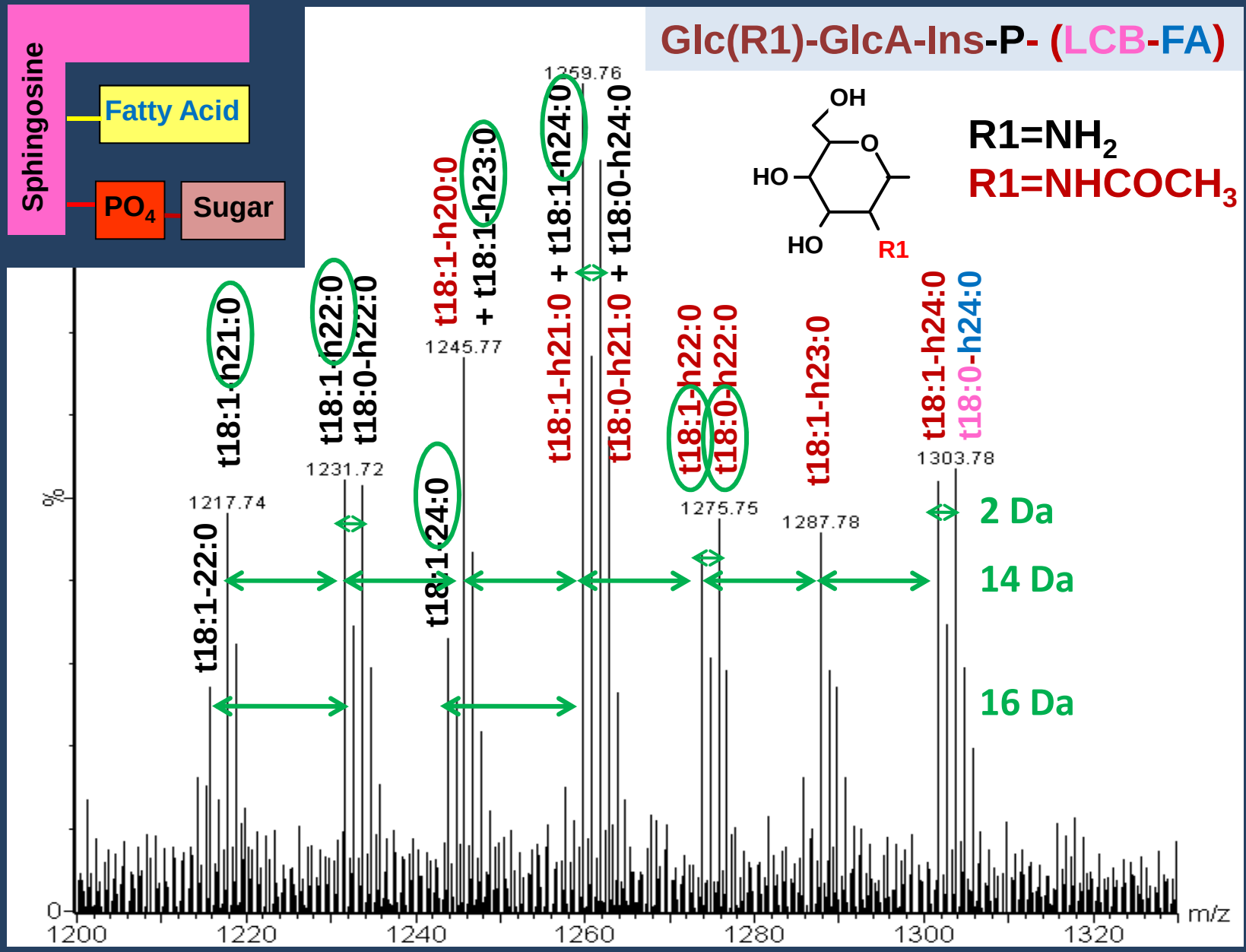
Matrix: 2,6-dihydroxyacetophenone (DHA)

***N. tabacum* cell culture (0.5 mg/mL)**



Buré et al., *RCMS*, **2011**, 25, 3131

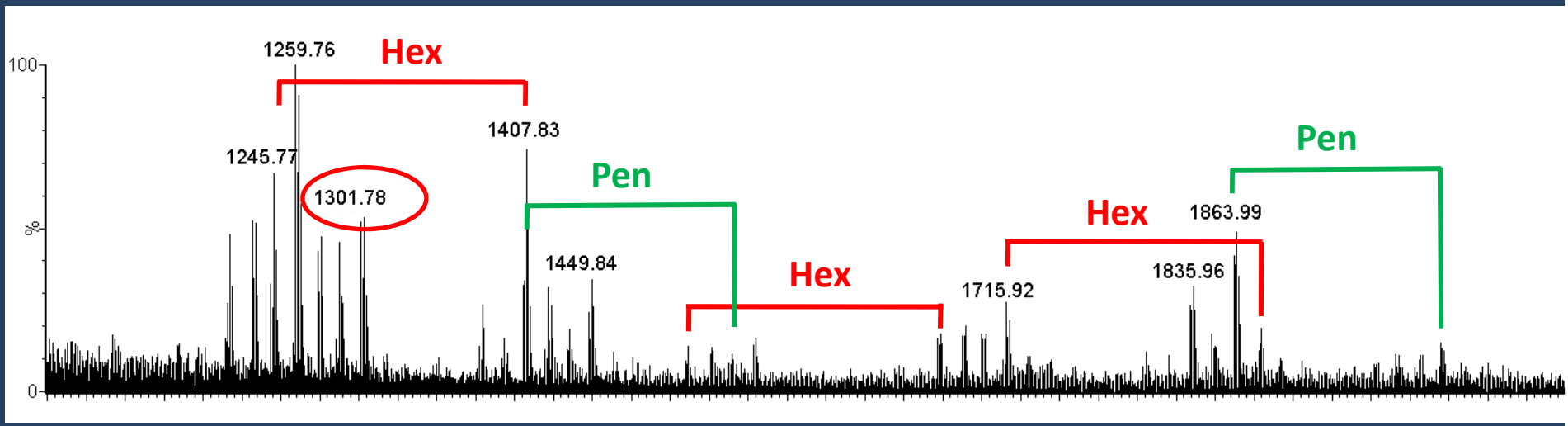
GIPC structure



MALDI-MS

***Sample: N. tabacum* cell culture**

A Series ***2 saccharides*** | **B Series** ***3 saccharides*** | **C Series** ***4 saccharides*** | **D Series** ***5 saccharides*** | **E Series** ***6 saccharides*** | **F Series** ***7 saccharides***



ESI-MS/MS fragmentation

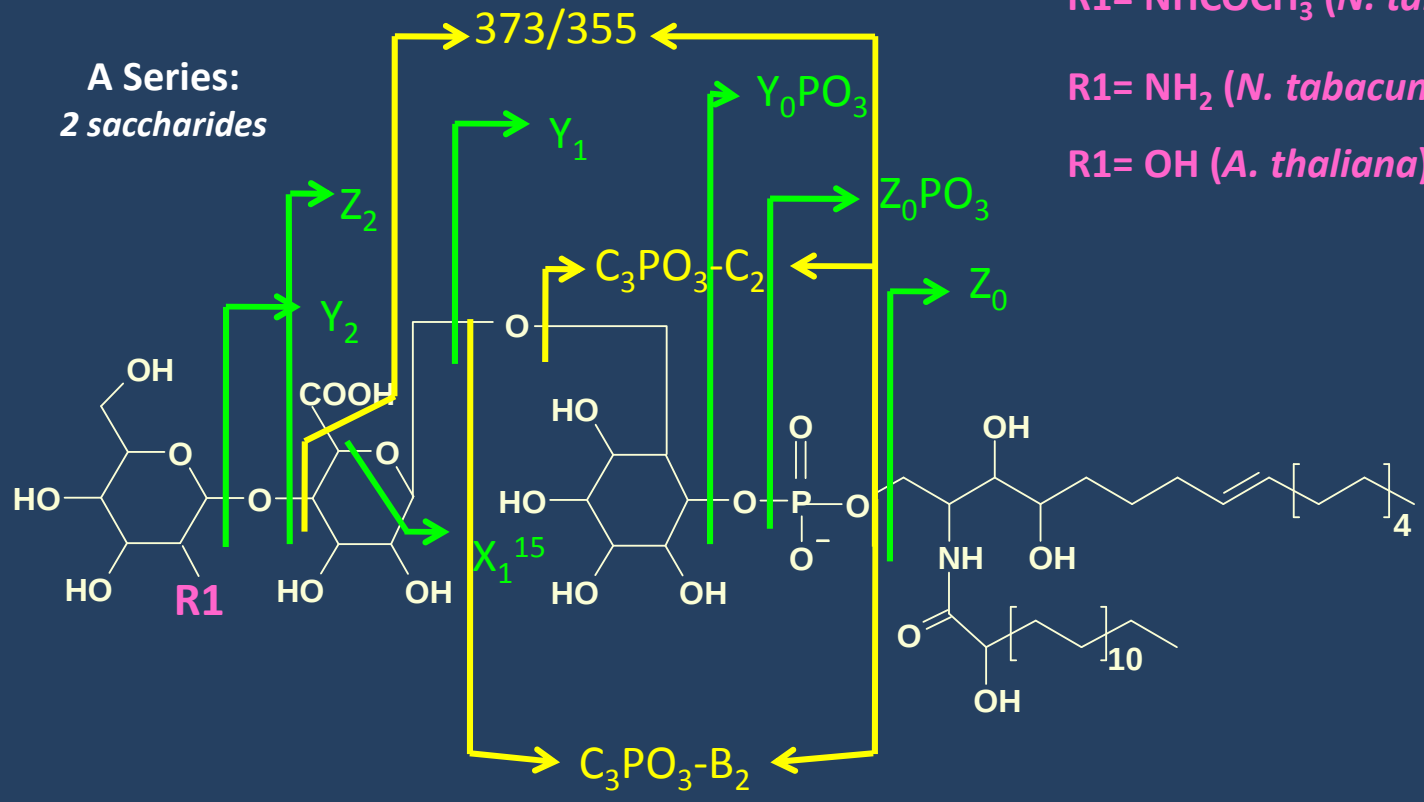
ESI Negative ion mode (QTRAP 5500, AB Sciex), MS/MS on $[M-2H]^{2-}$ ion

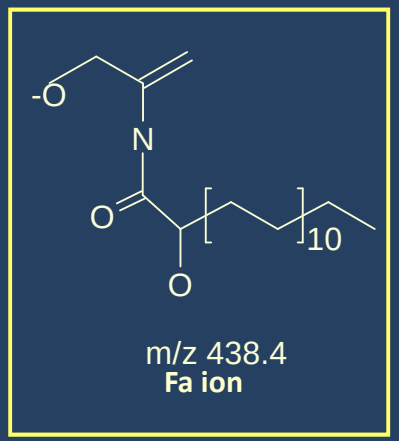
***Sample: N. tabacum* cell culture (0.5 mg/mL),**

R1= $NHCOCH_3$ (*N. tabacum*), (M=1302.8 Da)

R1= NH_2 (*N. tabacum*), (M=1260.7 Da)

R1= OH (*A. thaliana*), (M=1261.7 Da)



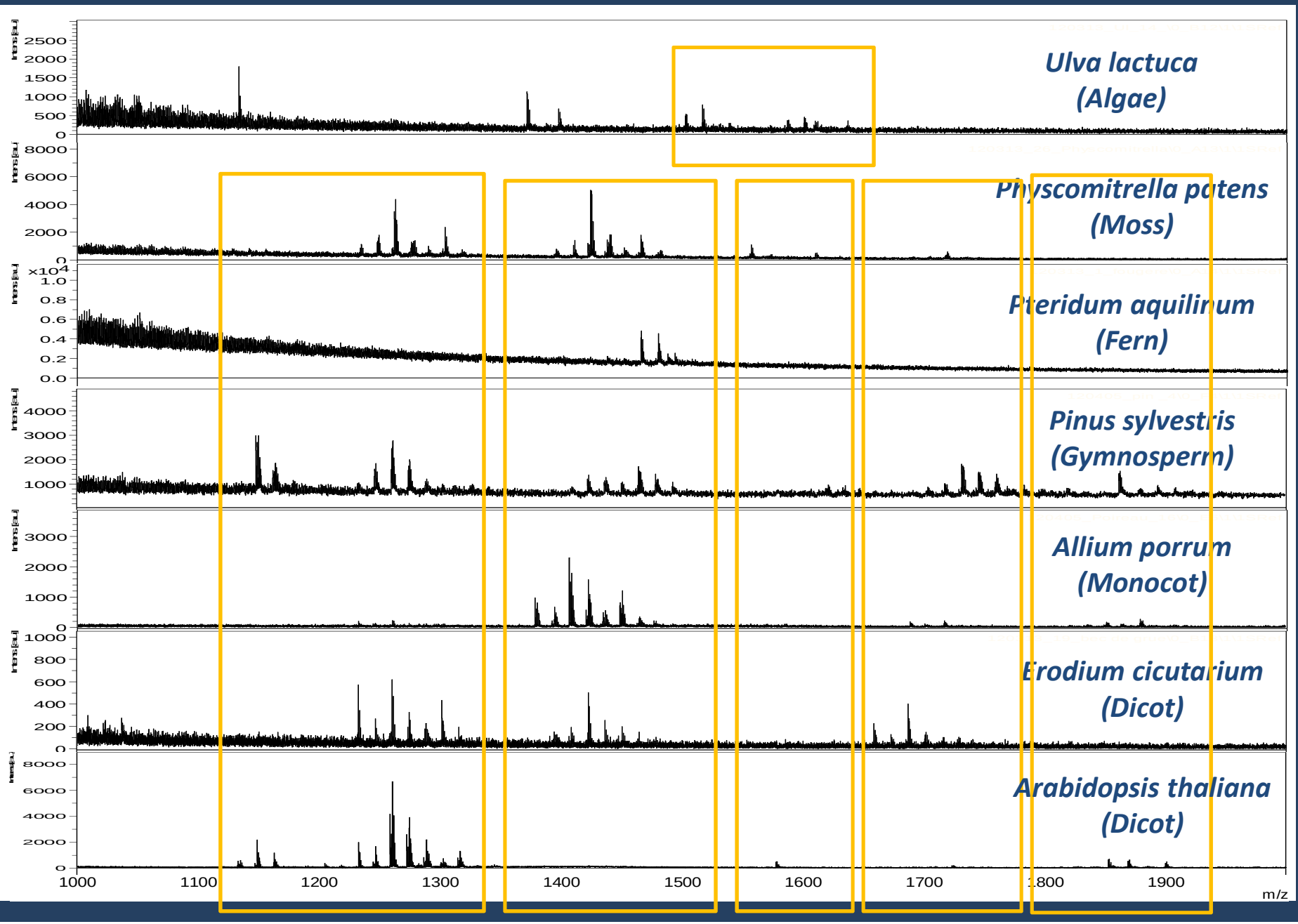


Nomenclature is according to Costello and Vath (1990) and Levery *et al.* (2001)

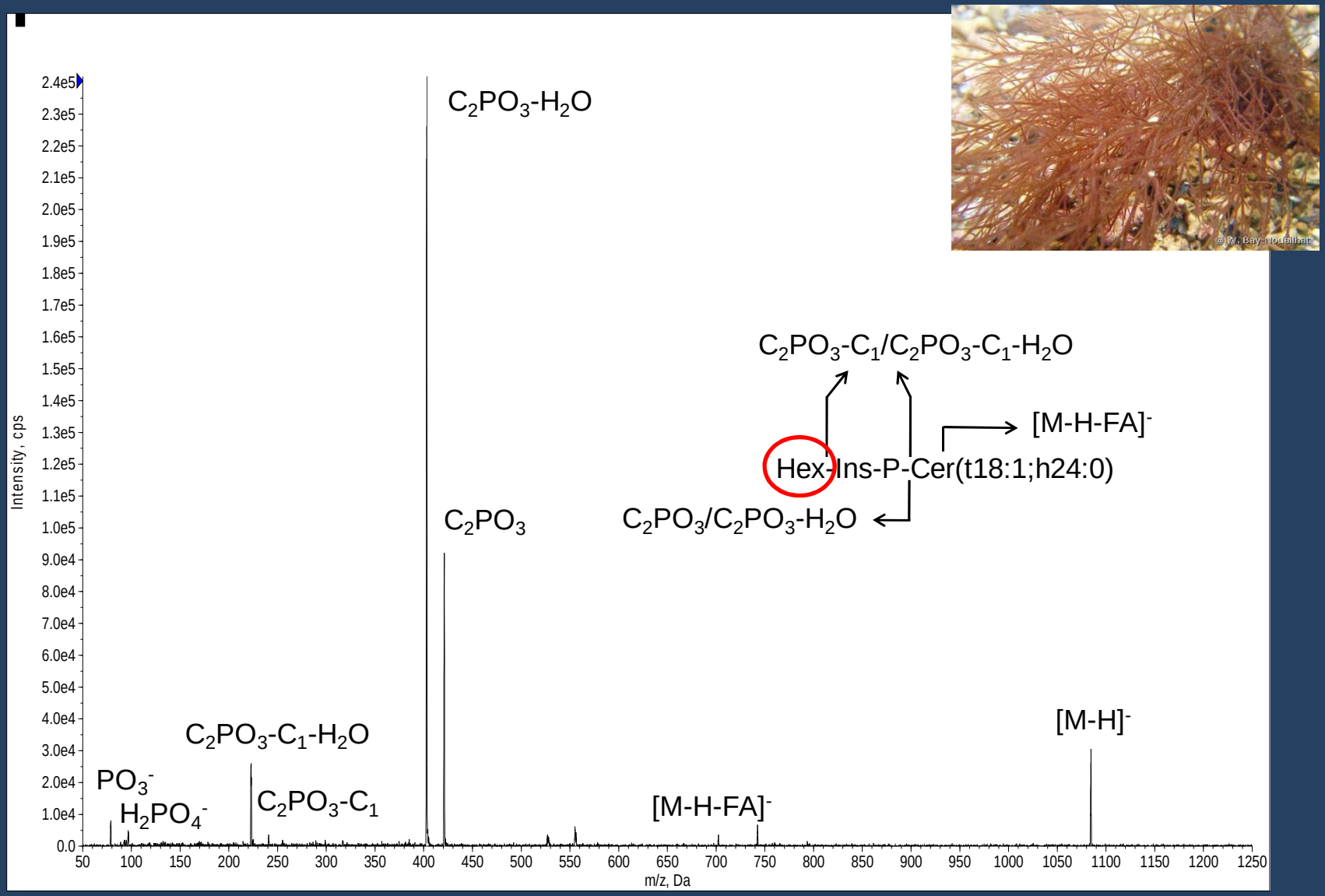
MALDI-MS Screening: biodiversity of plant GIPCs

A Series B Series C Series D Series E Series F Series

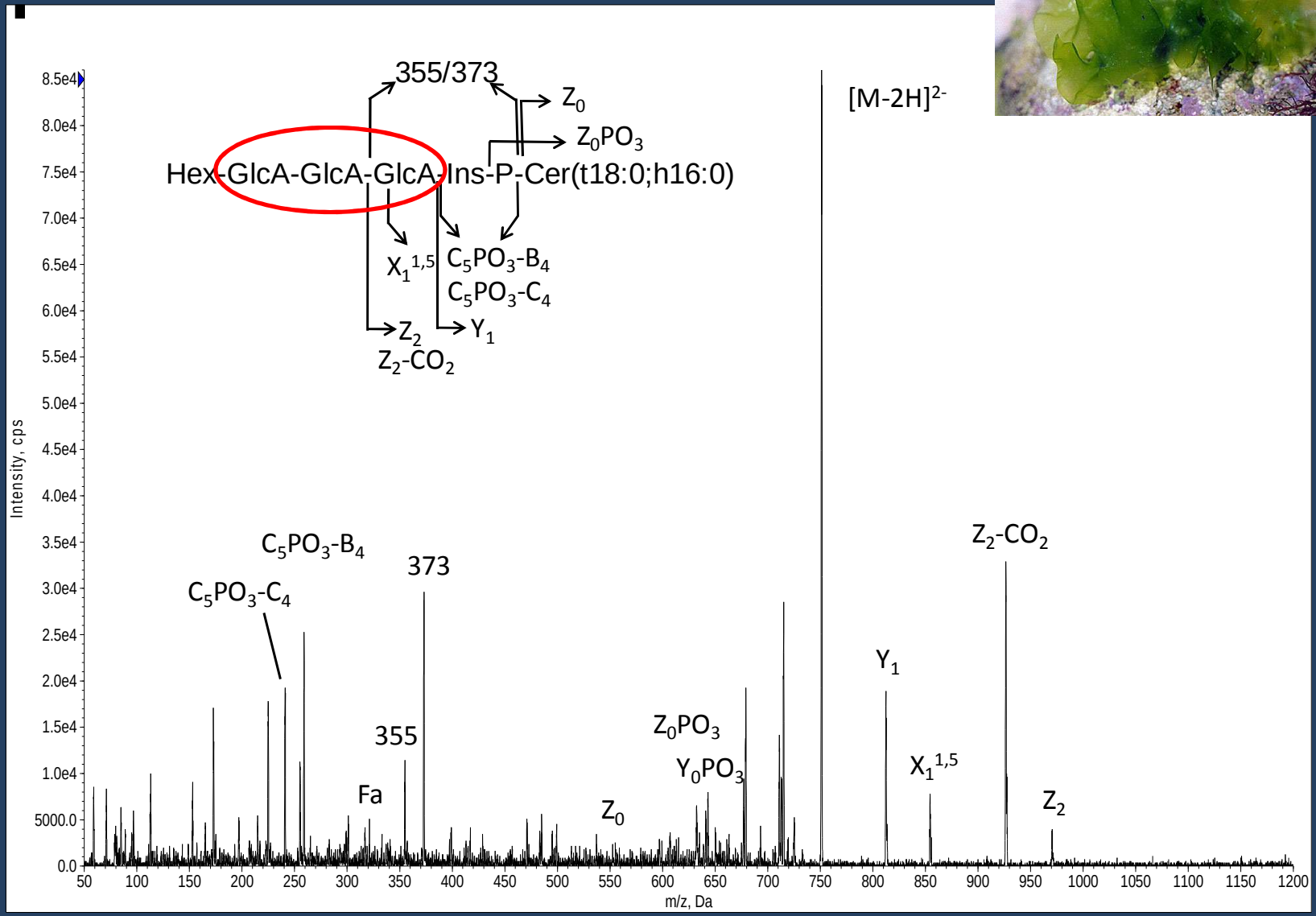
2 saccharides 3 saccharides 4 saccharides 5 saccharides 6 saccharides



New GIPC in *Chondracanthus acicularis* (Algae): only one saccharide



New GIPC in *Ulva lactuca* (Algae): several GlcA



Conclusion

- Extraction/Purification
- MALDI-MS: fast screening of GIPCs from 22 plants
- Great diversity of GIPC structures in the plant kingdom
- ESI-MS/MS: New GIPC polar head structures

Perspectives

- Complete characterization of new GIPC structures (NMR)
- Isolation of reference GIPCs to be used as standards for absolute quantitative analysis

Buré et al. Anal Bioanal Chem, in press
Cacas et al. submitted

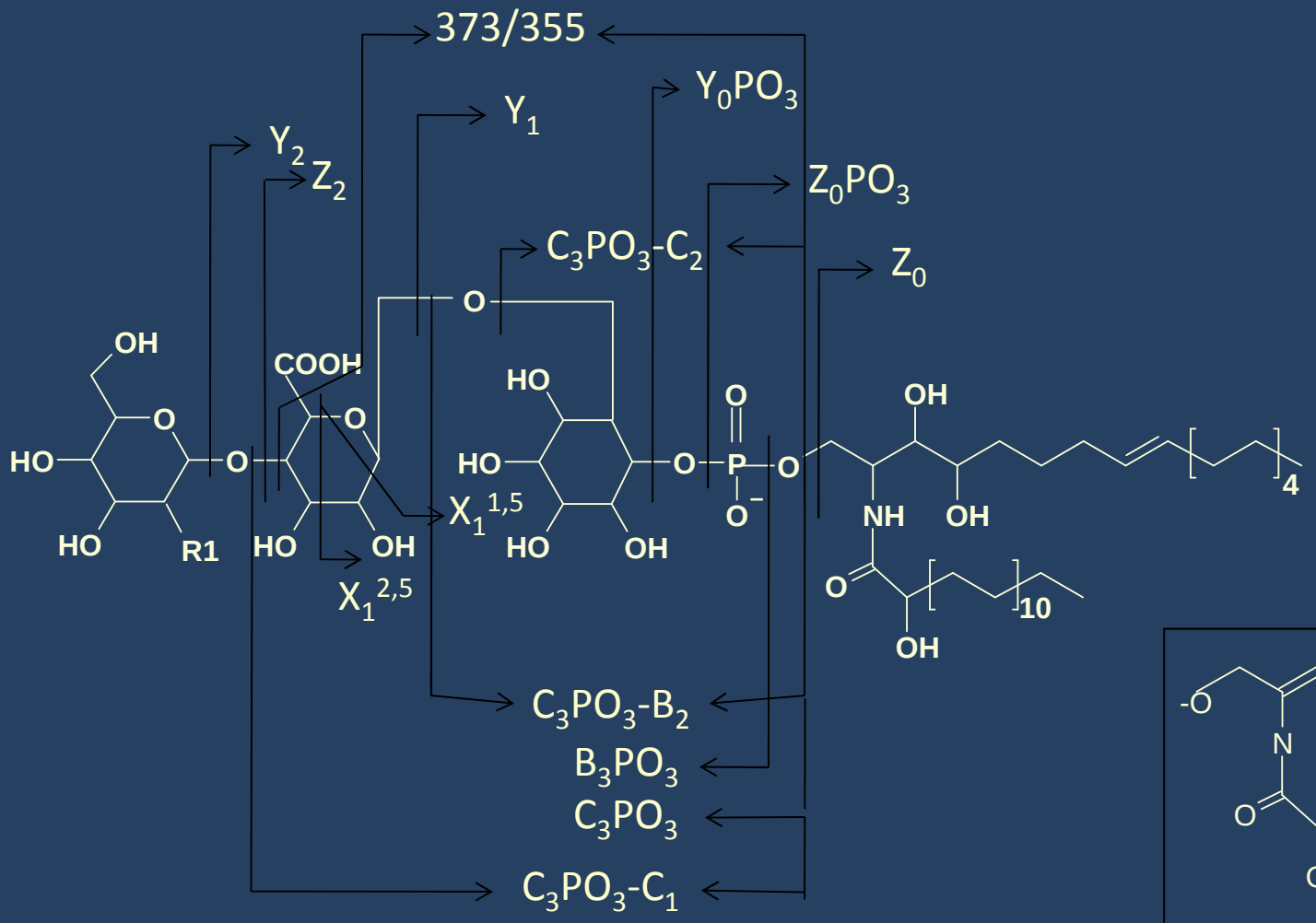
Thanks

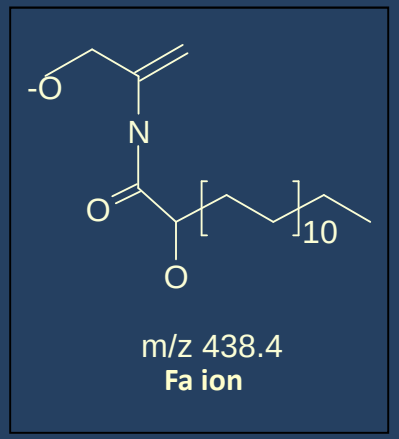
Agence Nationale de la Recherche, programme blanc “PANACEA”

Région Aquitaine

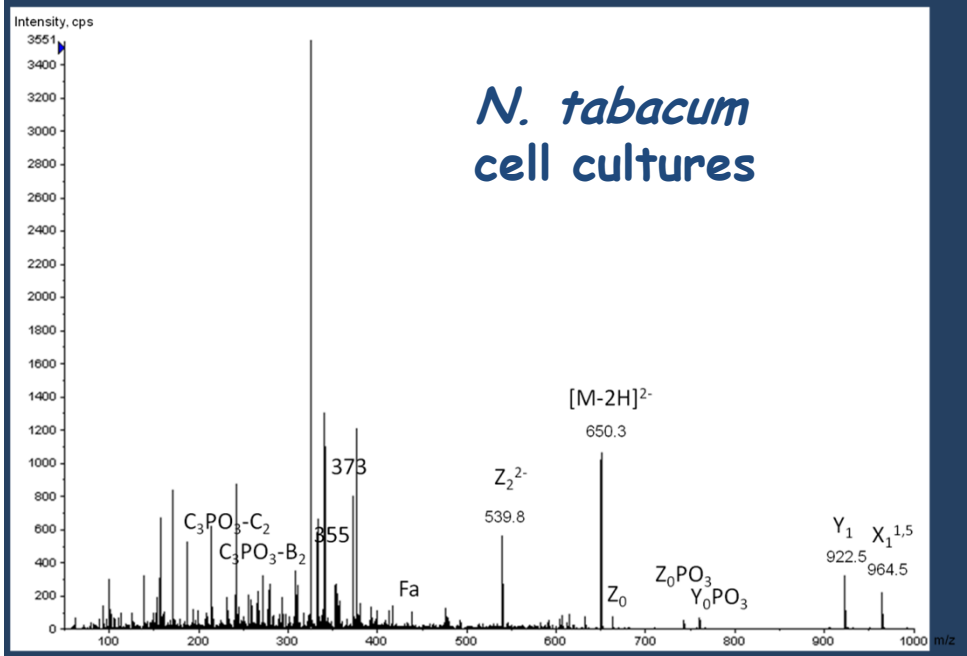
Plateforme Protéome et Plateforme Métabolome-Lipidome-Fluxome

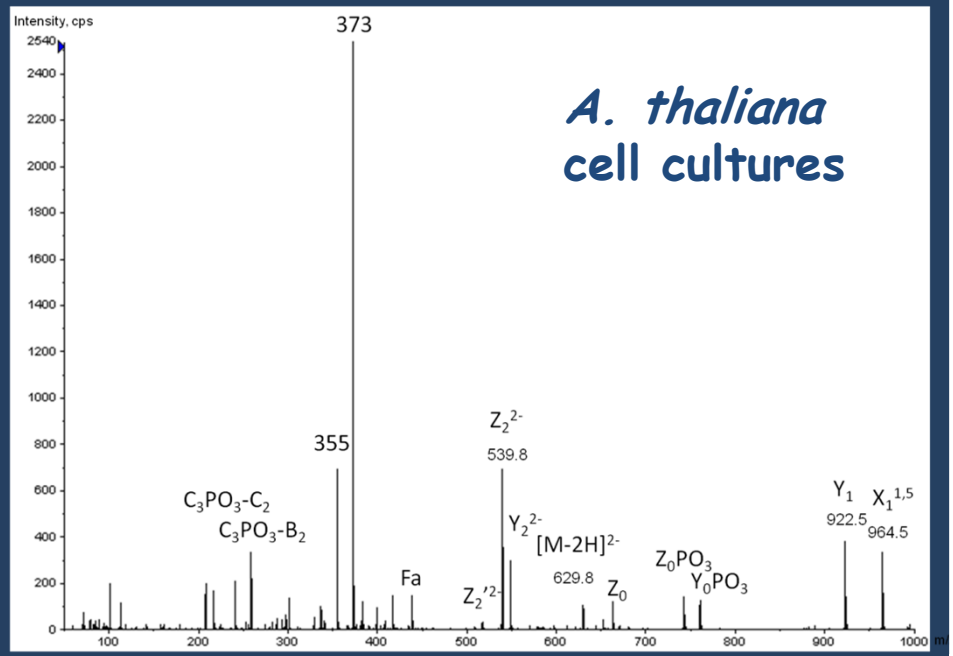
MS/MS fragmentation





ESI-MS/MS


Intensity, cps
3551
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
100
200
300
400
500
600
700
800
900
1000 m/z
N. tabacum
cell cultures
373
355
[M-2H]2-
650.3
Z2 2-
539.8
C3PO3-C2
C3PO3-B2
Fa
Z0
Z0PO3
Y0PO3
Y1
922.5
X1 1,5
964.5


Intensity, cps
2540
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
100
200
300
400
500
600
700
800
900
1000 m/z
A. thaliana
cell cultures
373
355
Z2 2-
539.8
C3PO3-C2
C3PO3-B2
Y2 2-
[M-2H]2-
629.8
Fa
Z2'2-
Z0
Z0PO3
Y0PO3
Y1
922.5
X1 1,5
964.5

ESI-MS/MS

Negative ion mode in ESI-Q-TRAP (QTRAP 5500, AB Sciex)

***Sample: N. tabacum* and *A. thaliana* cell cultures (0.5 mg/mL),**

diluted 16-fold in 65/35 (v/v) isopropanol/water containing 0.03 % ammonium acetate

Infusion of the sample

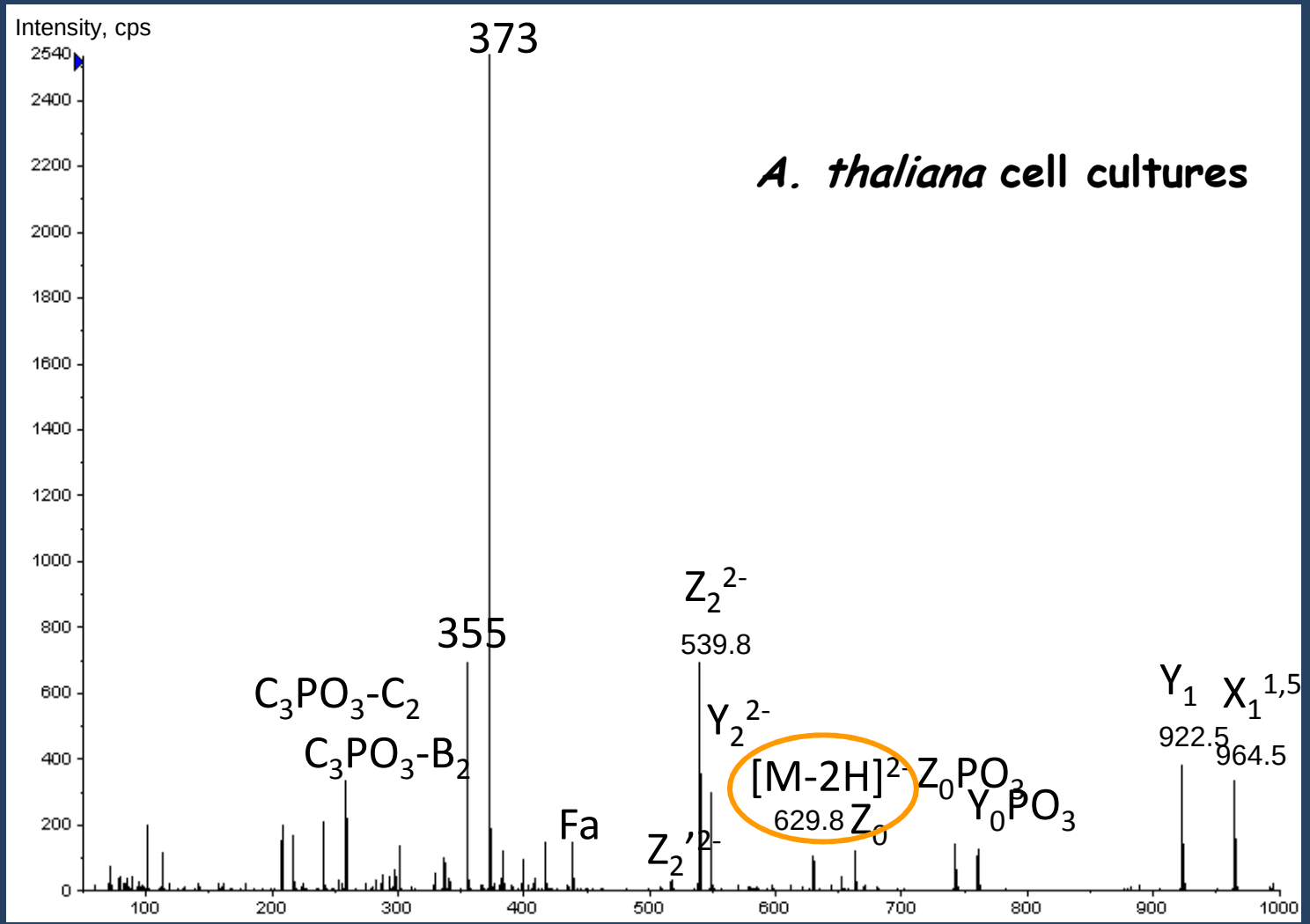
MS/MS on $[M-2H]^{2-}$ ion

Buré et al., *RCMS*, **2011**, 25, 3131

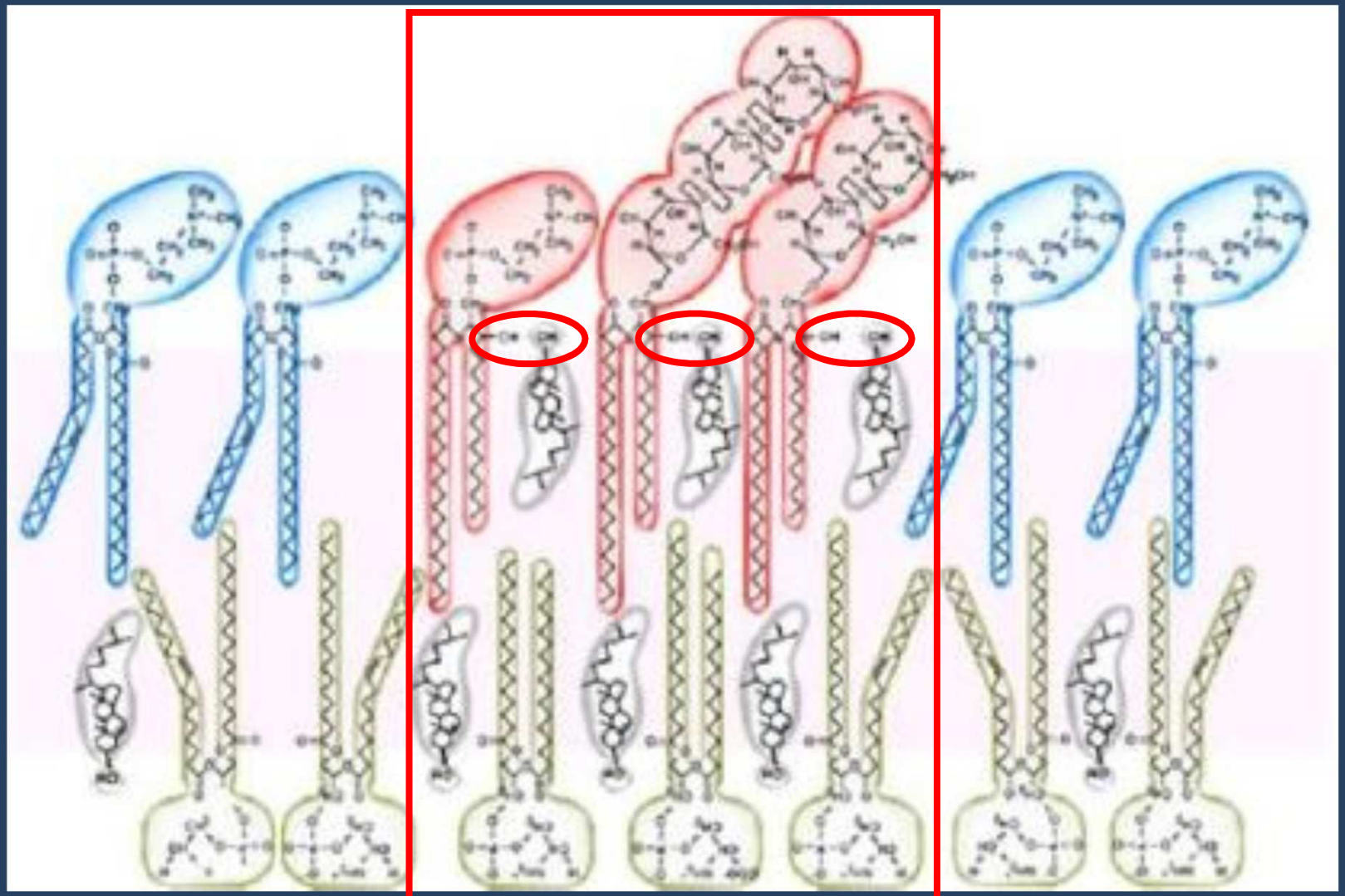
ESI-MS/MS

Glc(R1)-GlcA-Ins-P-Cer(t18:1-h24:0)

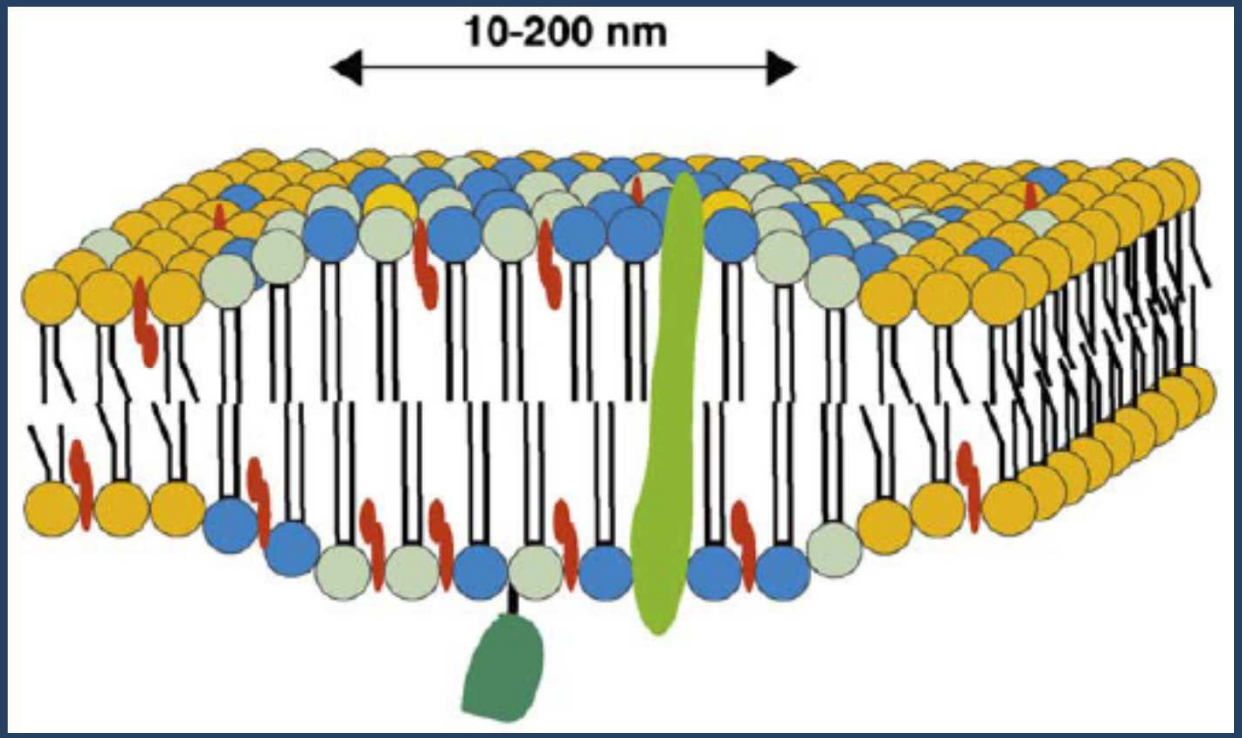
R1 = OH (M= 1261.7 Da)

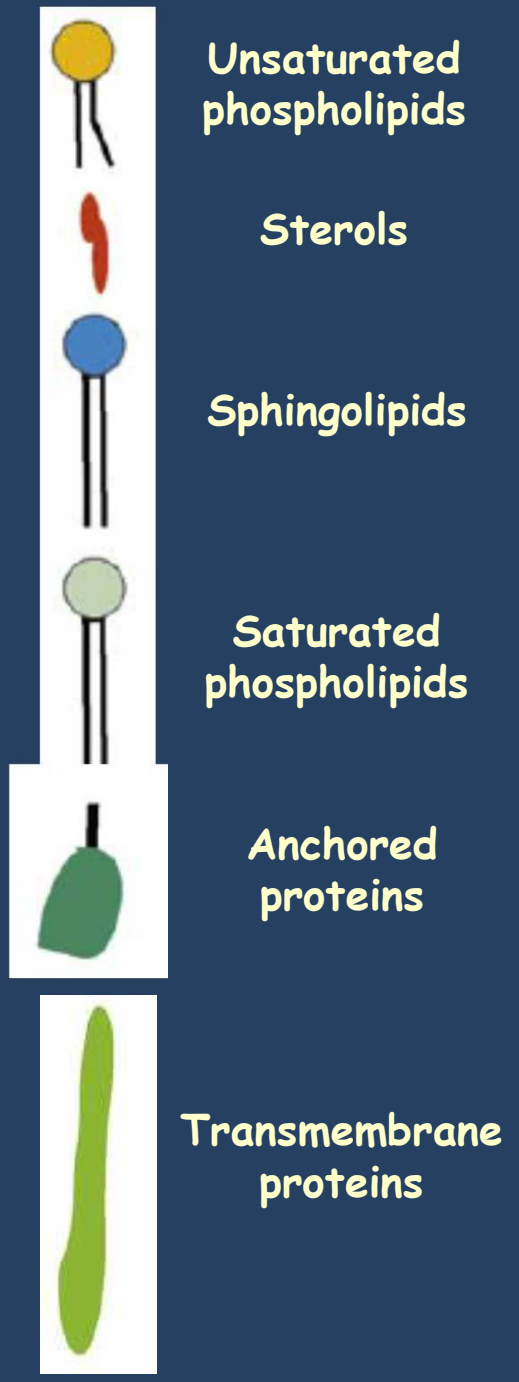


Structural function in lipid raft



Structural function in lipid raft





Unsaturated phospholipids

Sterols

Sphingolipids

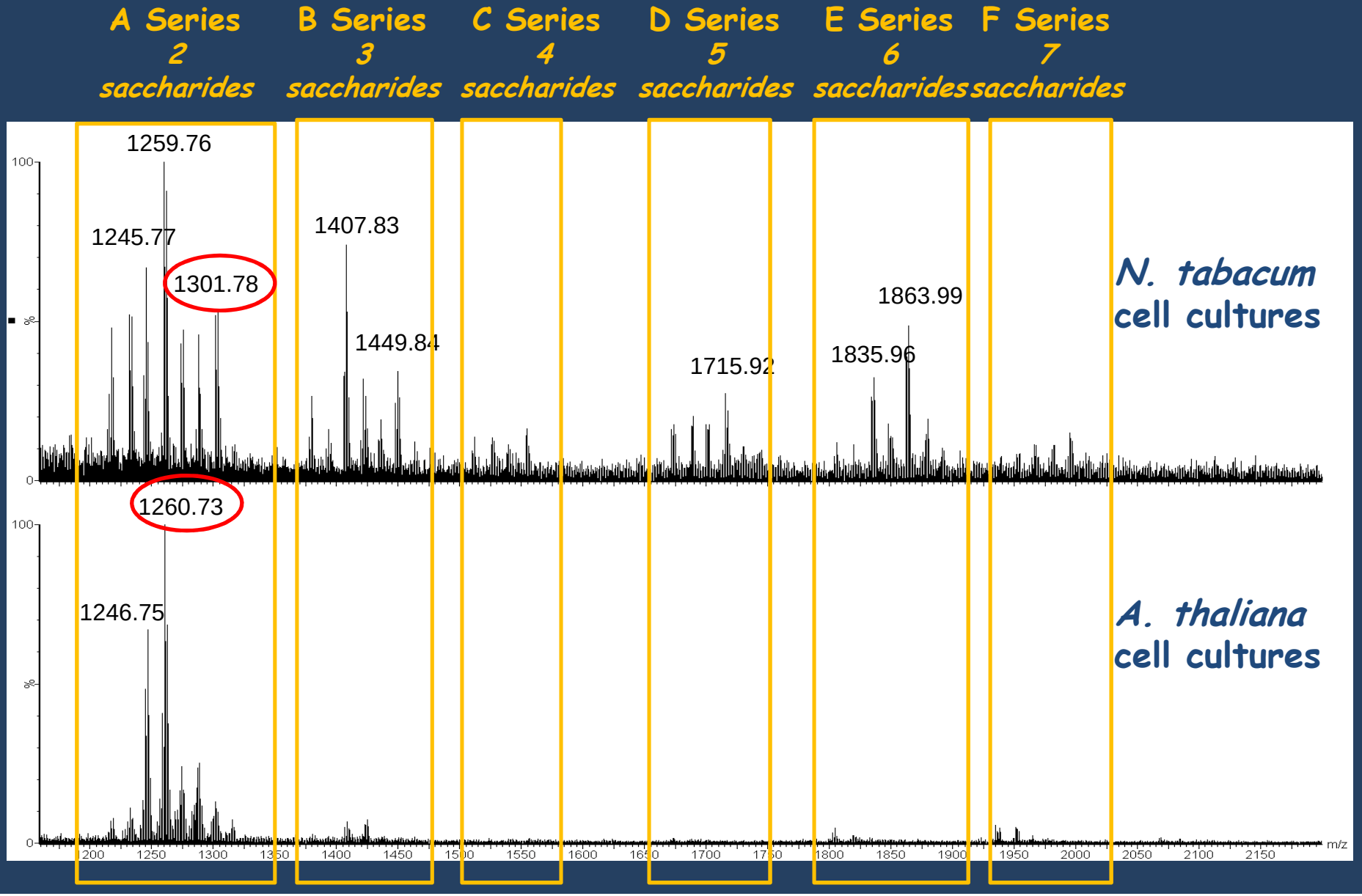
Saturated phospholipids

Anchored proteins

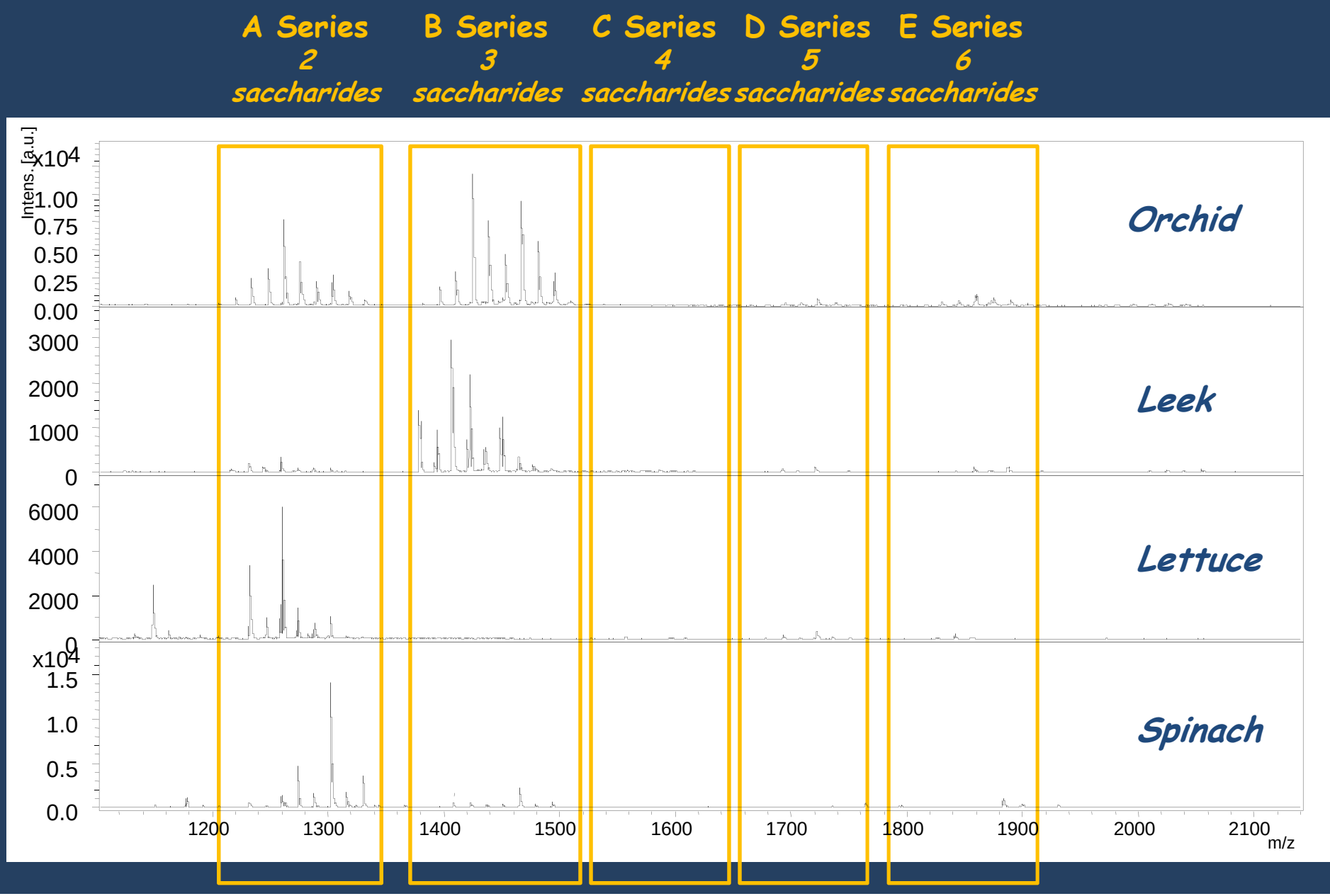
Transmembrane proteins

Bouté & Grebe, *Current Opinion in Plant Biology*. 2009, *12*, 705.

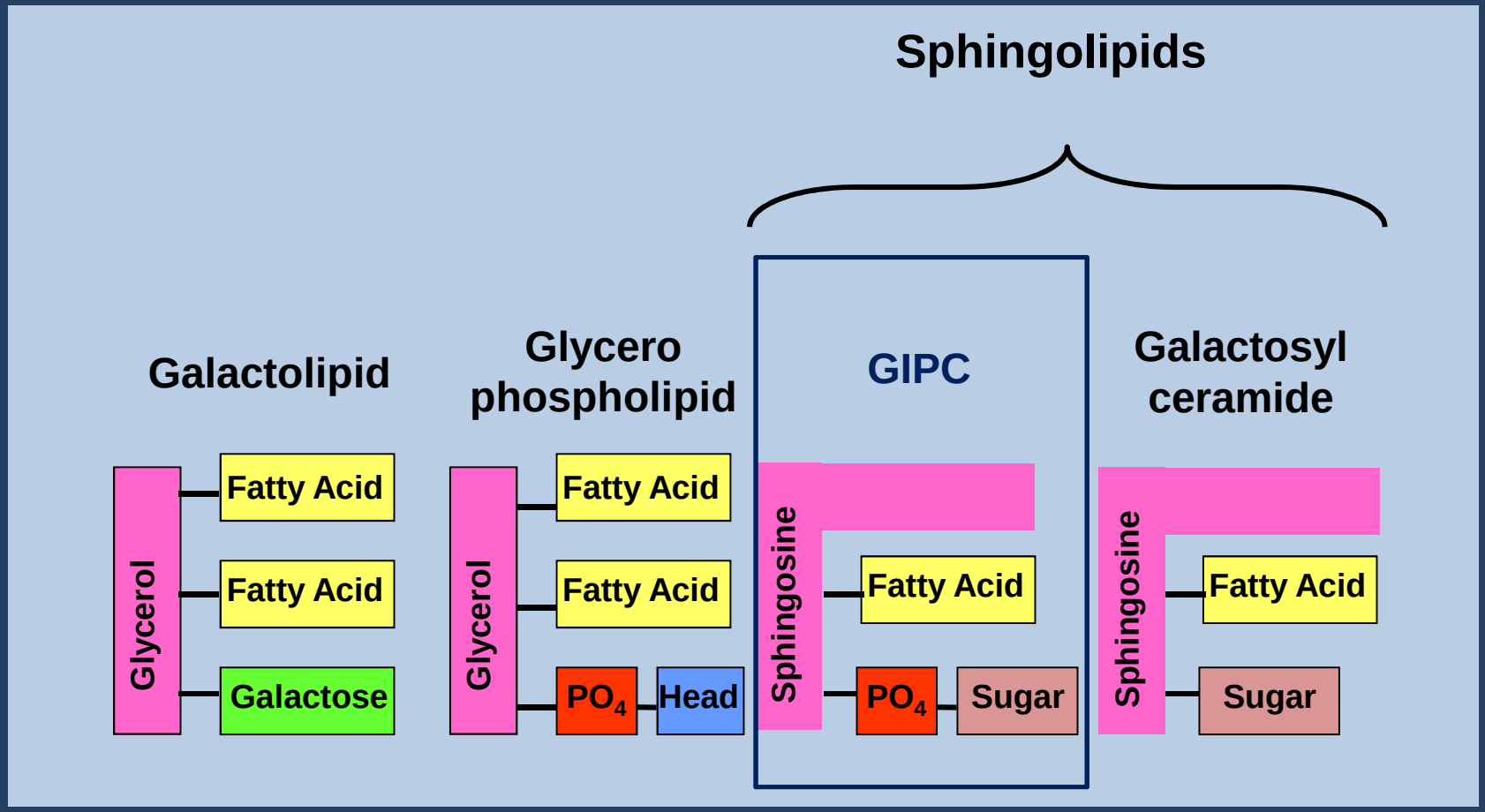
A focus on structure characterisation of GIPCs…



MALDI-MS Screening: biodiversity of plant GIPCs



Membrane Lipids



Sphingolipids: membrane lipids

Table 4. Comparison of methods for detection of plant sphingolipids. Previous methods for plant sphingolipid analysis involved separation of the sphingolipid classes and analysis of the sphingolipid content by hydrolysis and fluorescent derivitization and measurement of the liberated LCBs. These figures were previously published in g/fresh weight,[11] which is approximately one-tenth the amount in g/dw. Figures for LC/MS/MS are the total for each class of the different species shown in Fig. 3

Sphingolipid/method	Separation/LCB analysis	LC/MS/MS
Ceramide	13.7 nmol/g dw	16.3 ± 1.3 nmol/g dw
Hydroxyceramide		12.4 ± 0.8 nmol/g dw
Glucosylceramide	267 nmol/g dw	156 ± 15.3 nmol/g dw
GIPCs	501 nmol/g dw	236 ± 6.7 nmol/g dw
LCB(P)s	nd	3.8 ± 0.9 nmol/g dw
Total	782 nmol/g dw	425 ± 20.4 nmol/g dw

Markham & Jaworski, *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1304.

Céramides	**1.7%**
Glucosylceramides	**34%**
GIPC	**64%**
LCB(P)	**n.d**

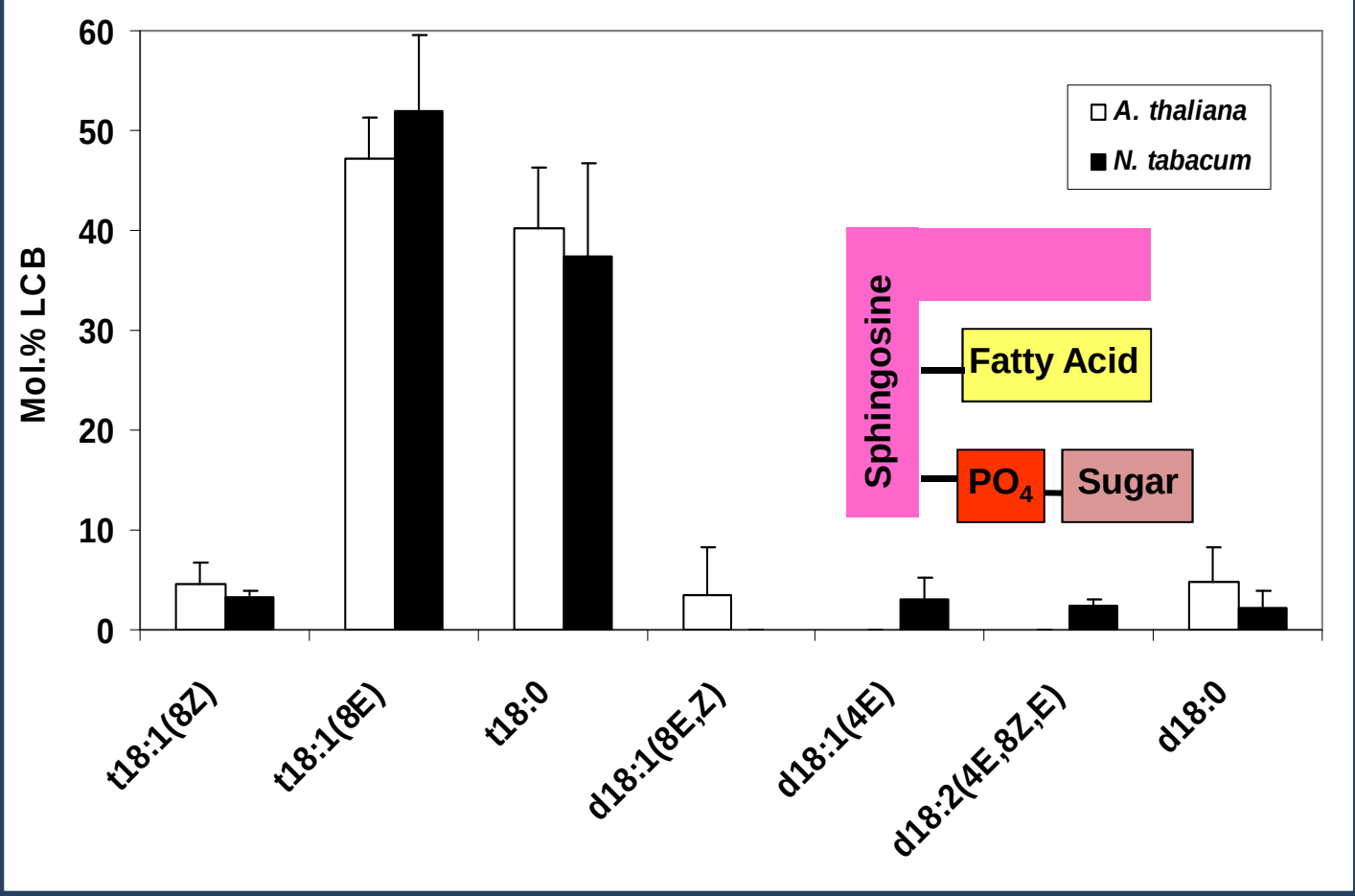
GIPC : major sphingolipids in plant membrane

Sphingolipids roles in signalling process

- Cold tolerance in plants (Glucosylceramides, phosphorylated LCB)
- Dryness tolerance in plants (Glucosylceramides)
- Heavy metals (Al) tolerance in plants (Glucosylceramides)
- Stomate closure (phosphorylated LCB)

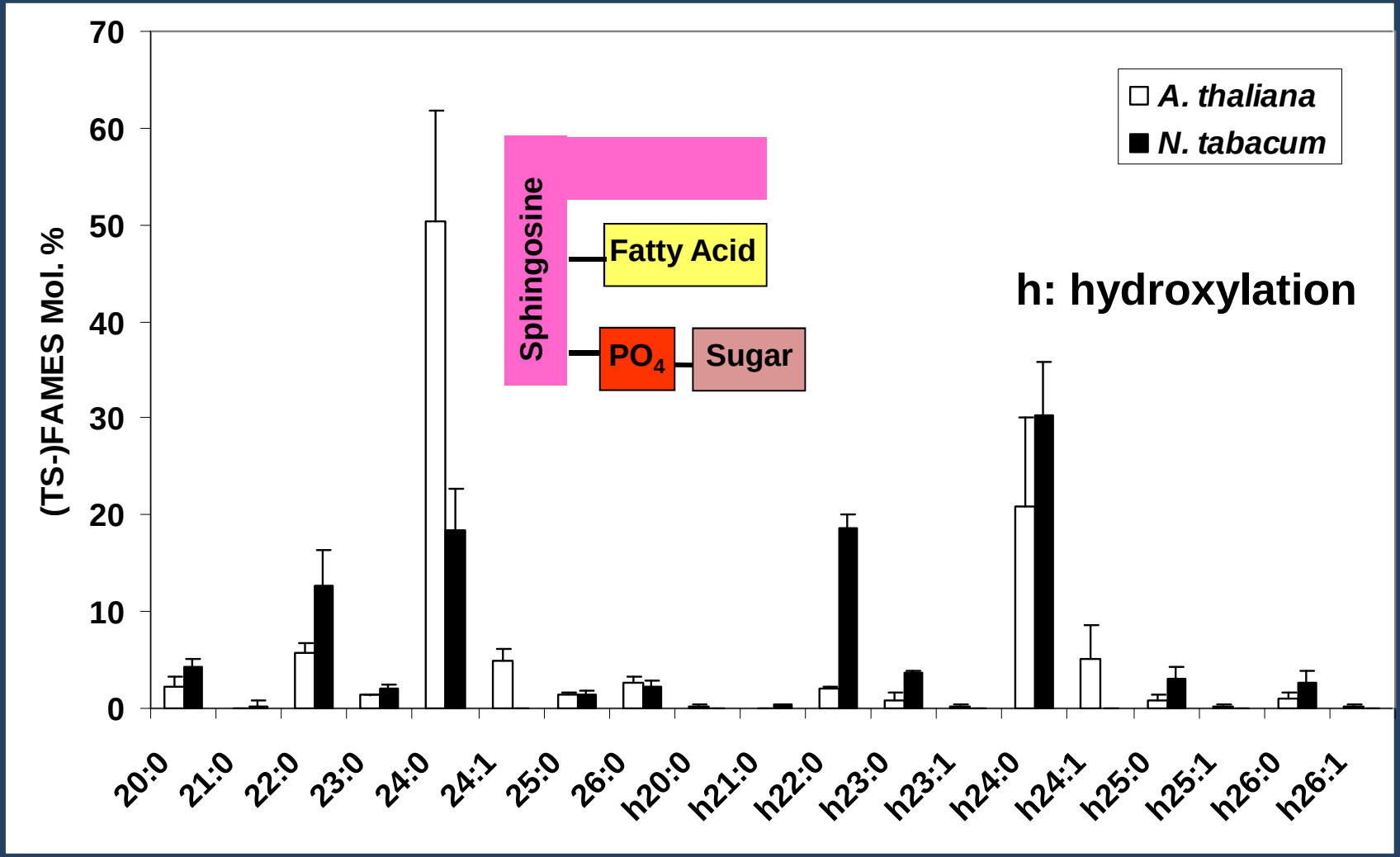
A lot of information concerning plant defence response!

Long Chain Base analyses



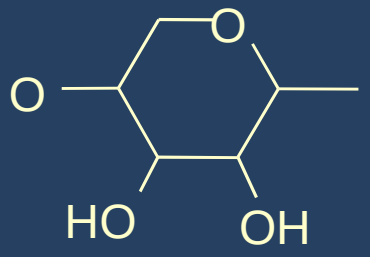
LCBs were released from sphingolipids (treatment with barium hydroxide (10%, w/v) in dioxane at 110℃). LCBs were converted into their corresponding fatty aldehydes by periodate oxidation and quantified by GC-MS (d: dihydroxy; t: trihydroxy).

Fatty Acid analyses

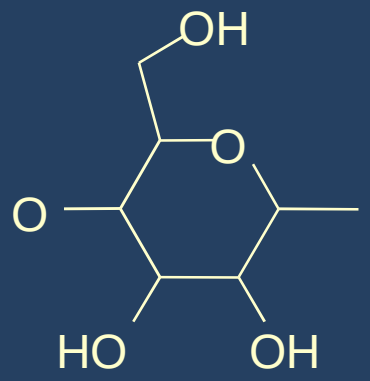


FAs were converted into their corresponding Fatty Acid Methyl Esters (acid methanolysis) and silylated (BSTFA) before GC/MS analysis.

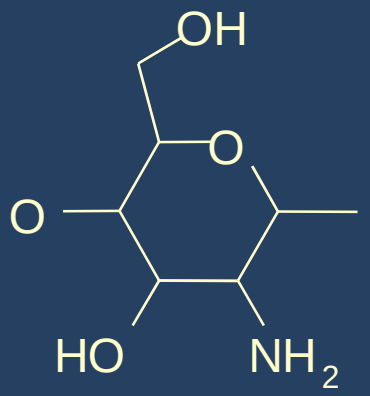
Possible Oses …



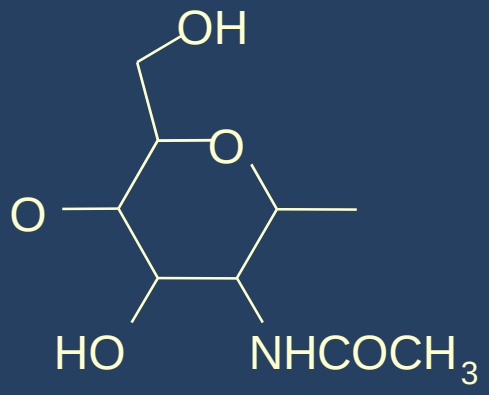
Arabinose (Ara)
ΔM= 132



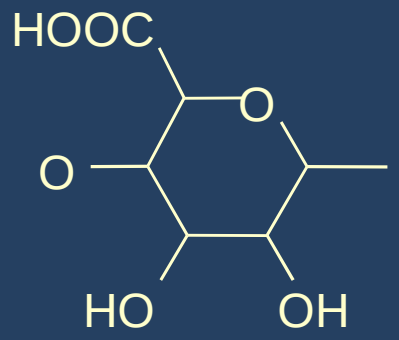
Galactose (Gal)
Glucose (Glc)
ΔM= 162



Glucosamine (GlcN)
ΔM= 161



N-acetylglucosamine (GlcNAc)
ΔM= 203



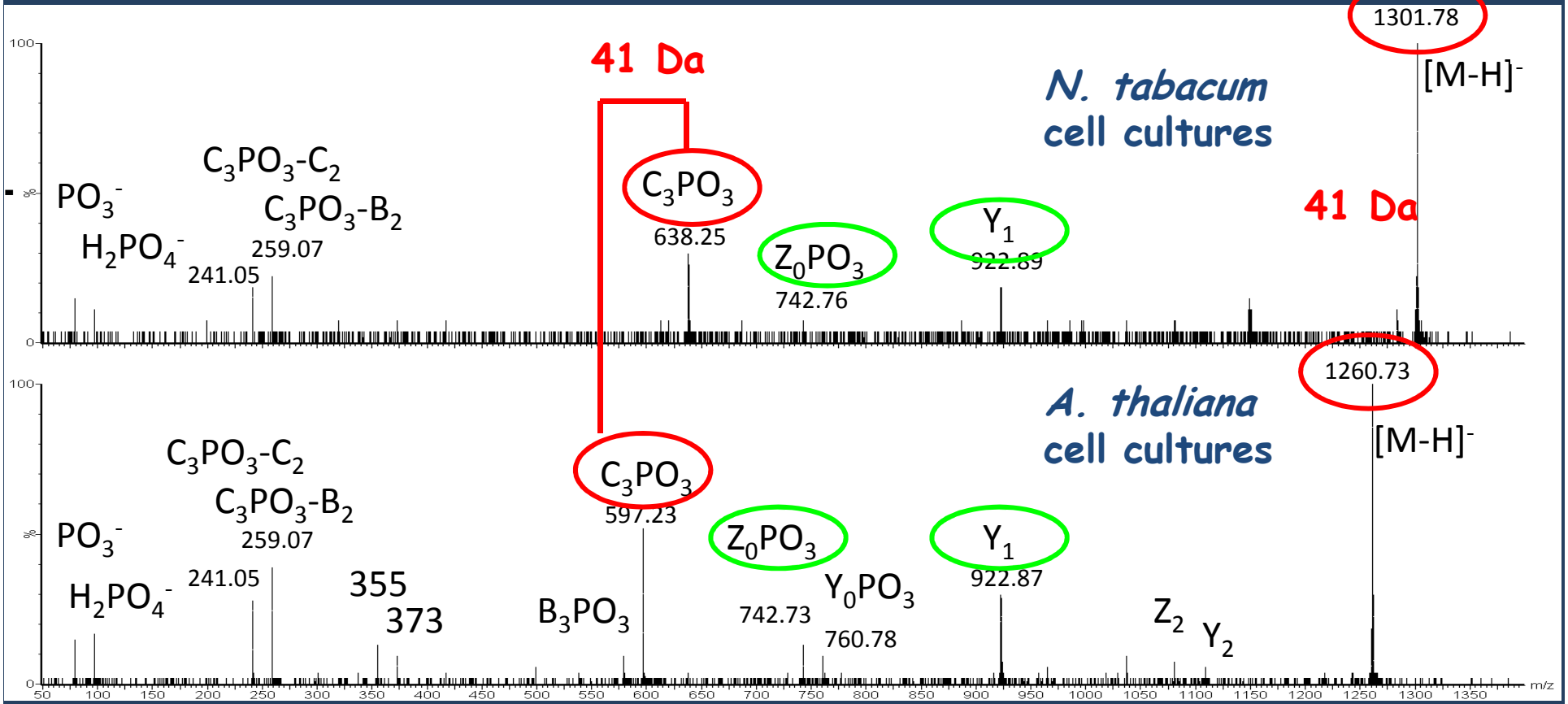
Glucuronic Acid (GlcA)
ΔM= 176

Hsieh *et al.* (1981) and Pata *et al.* (2010)

MALDI-MS/MS

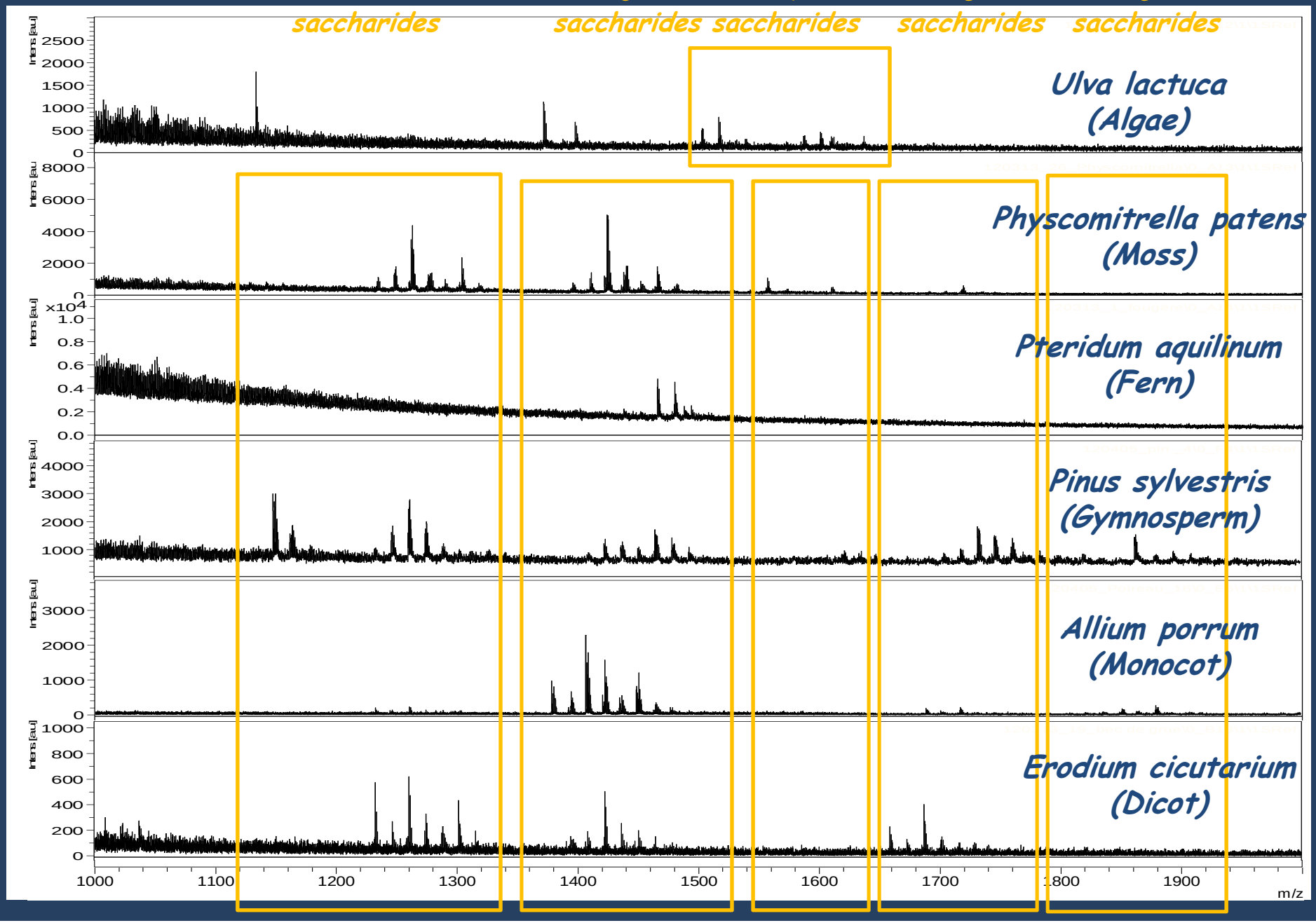
Glc(R1)-GlcA-Ins-P-Cer(t18:1-h24:0)

R1 = $NHCOCH_3$

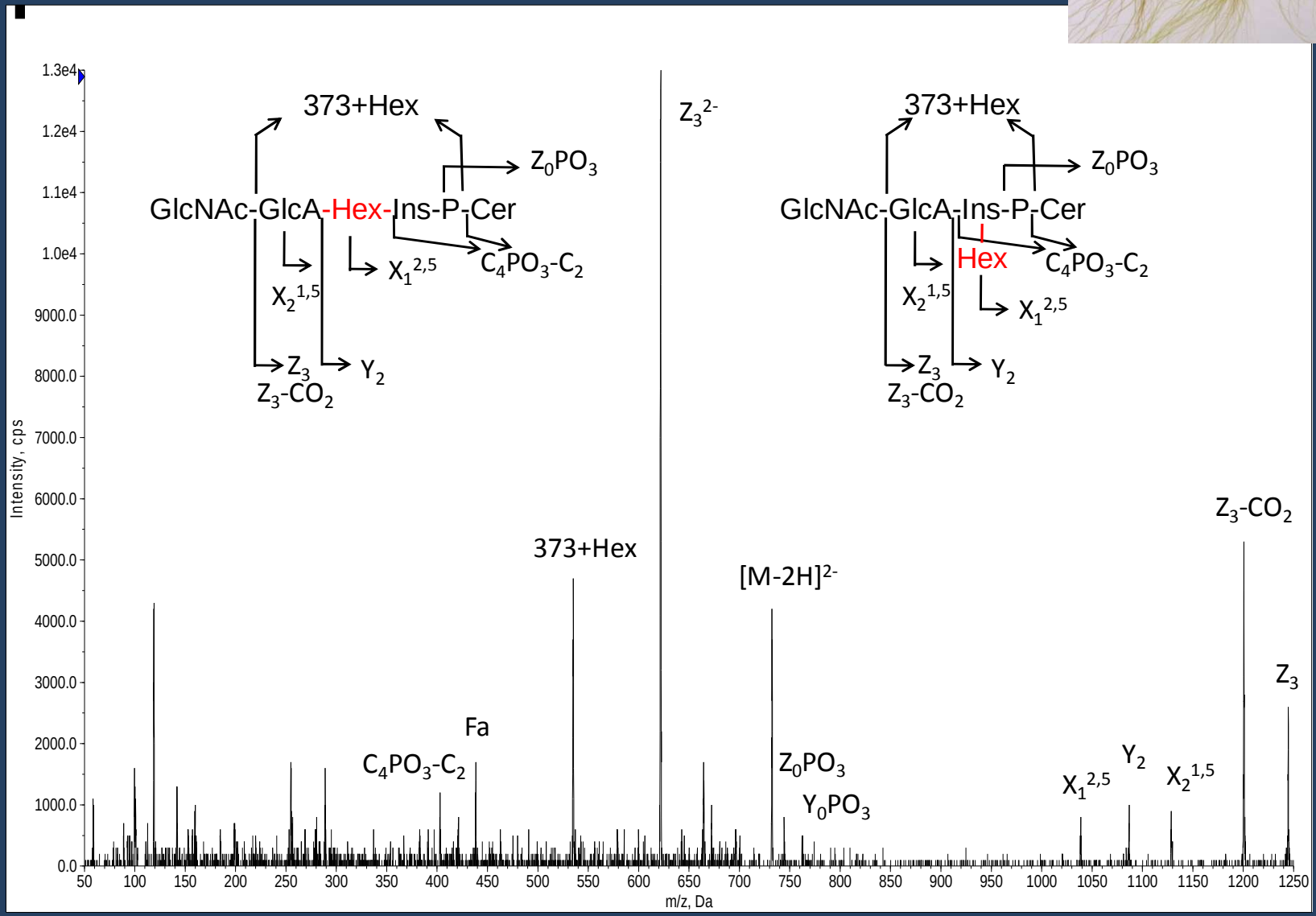


R1 = OH

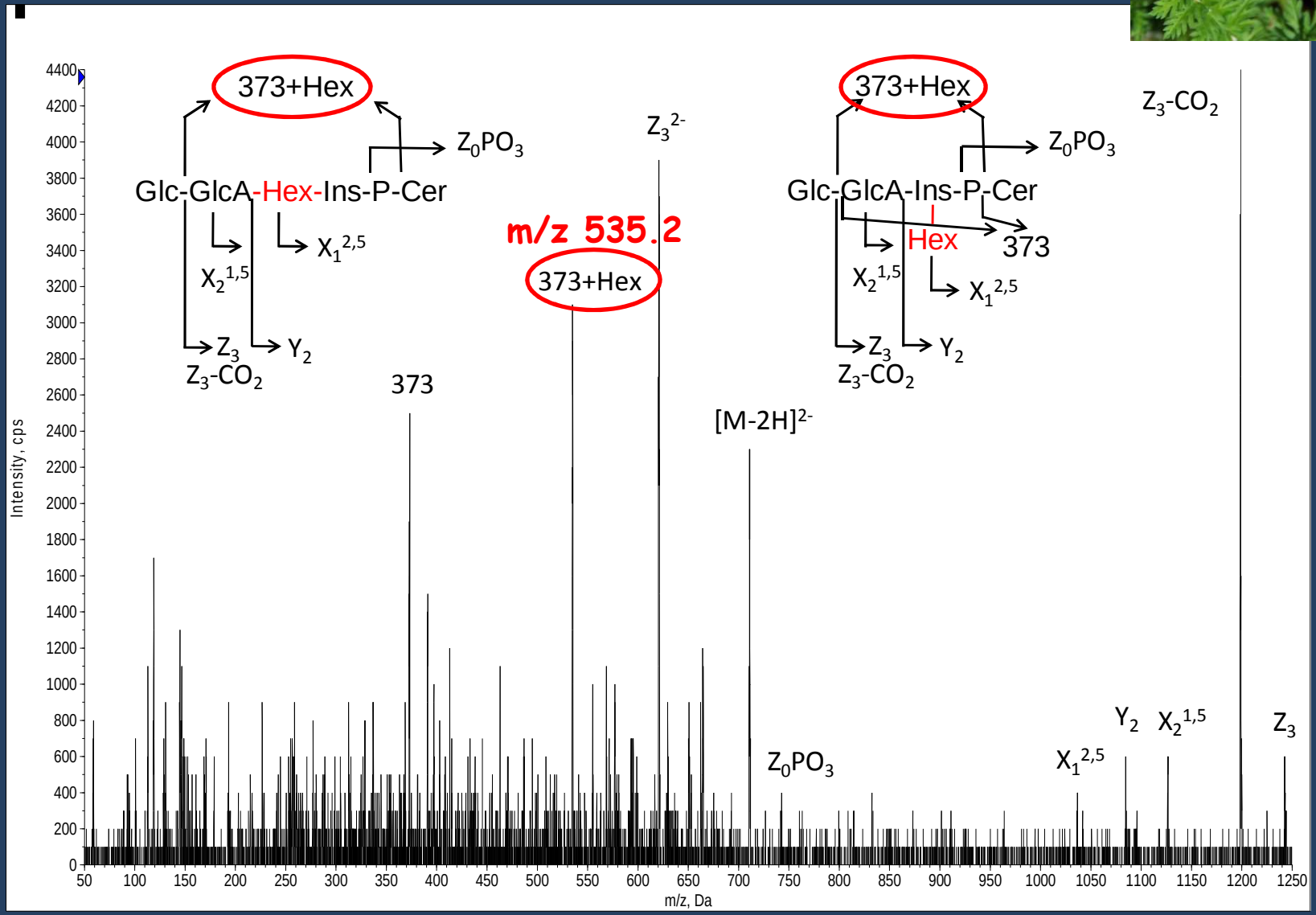
MALDI-MS Screening: biodiversity of plant GIPCs



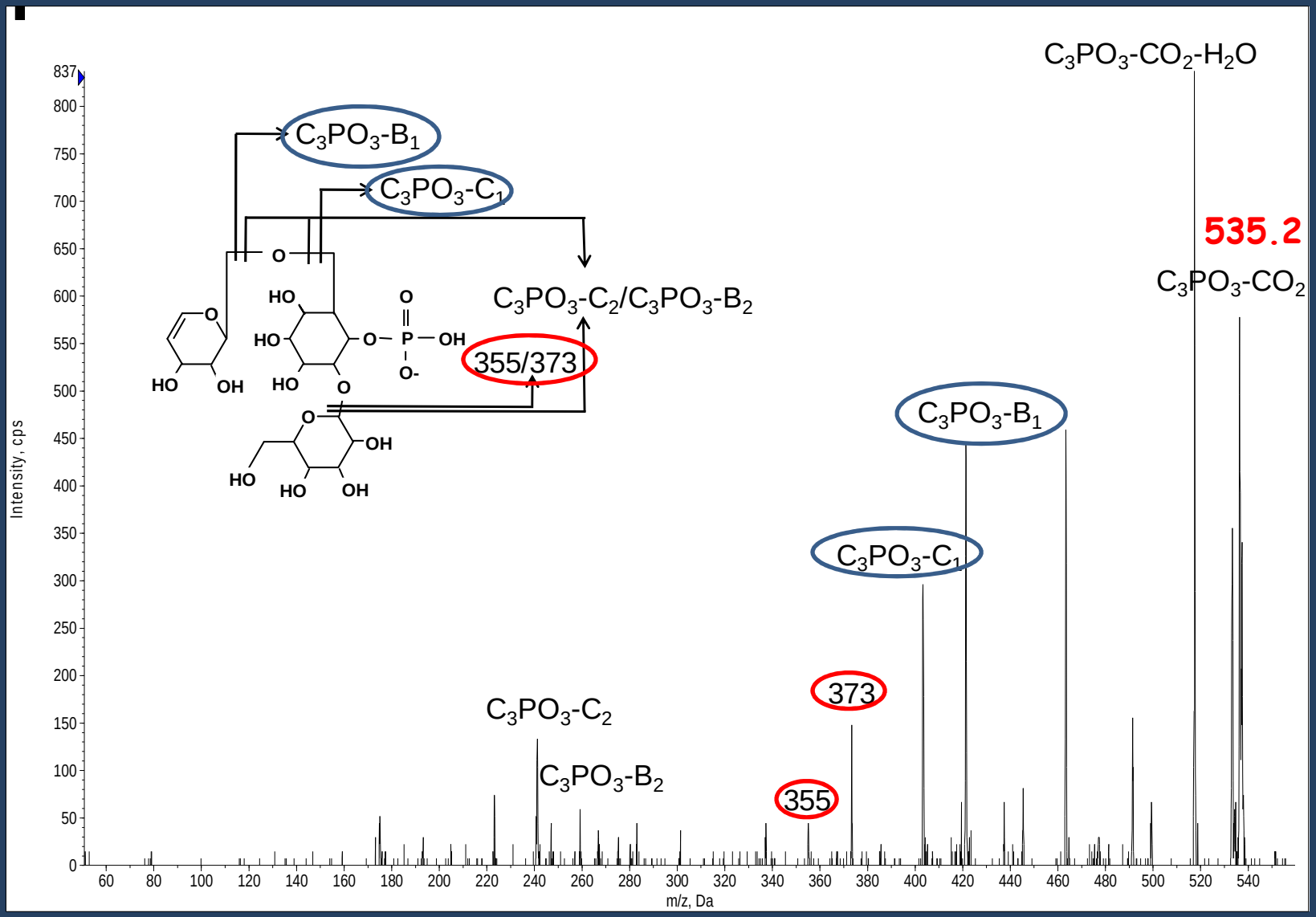
Physcomitrella patens (Moss): ESI-MS/MS



Erodium cicutarium (Dicot): ESI-MS/MS

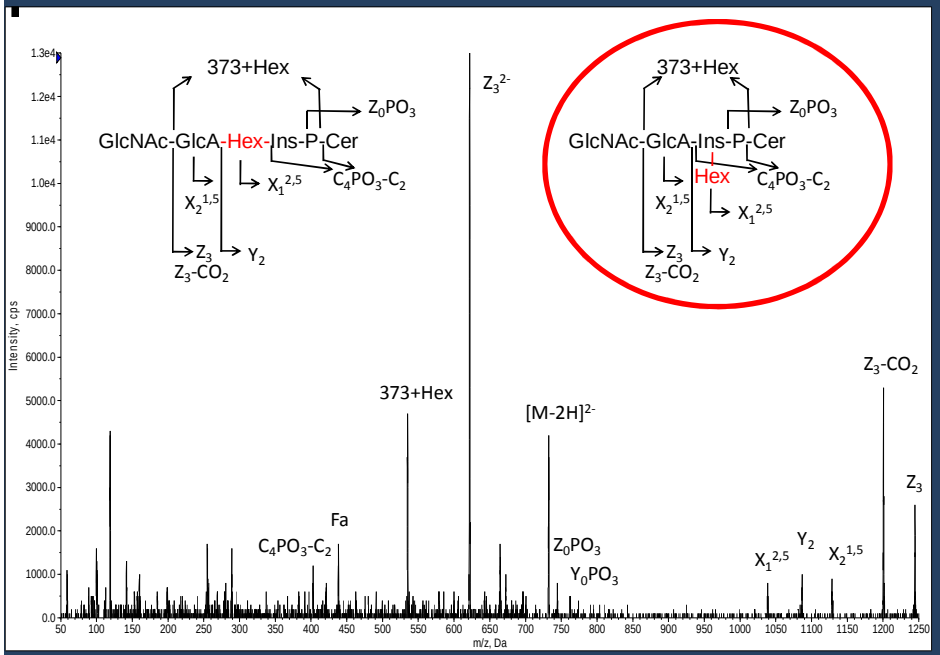


Erodium cicutarium (Dicot): ESI-MS^3 on m/z 535.2

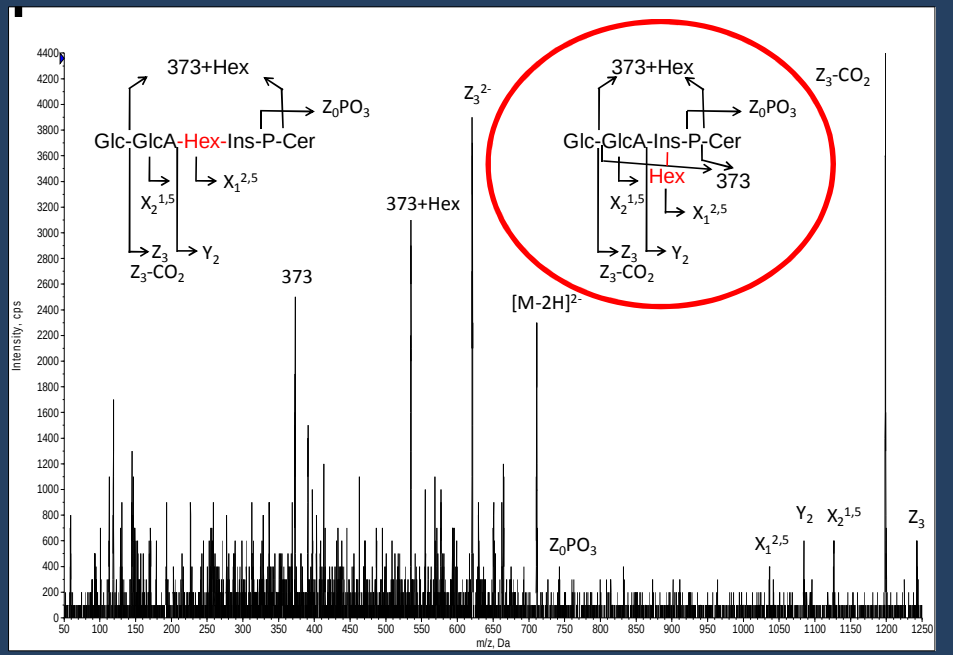


Two saccharides linked to inositol

Physcomitrella patens (Moss)

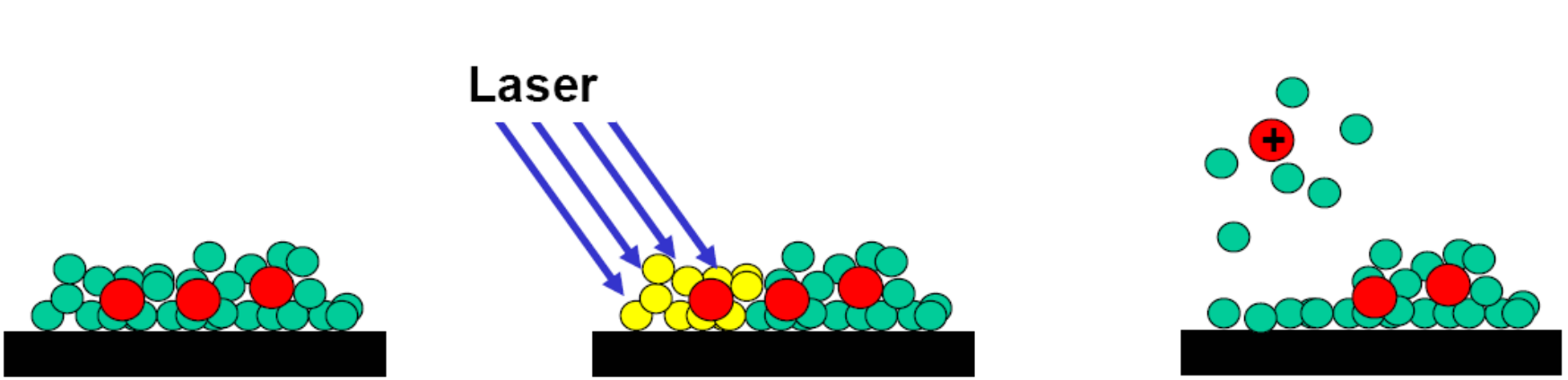


Erodium cicutarium (Dicot)



Hsieh et al., *J. Biol. Chem.*, **1981**, 256, 7747

MALDI
Principe de la désorption de l'échantillon



Co-crystallisation de l'échantillon et de la matrice photosensible

Excitation des molécules de matrices par l'impulsion laser

Ionisation et désorption de l'échantillon

L'impulsion laser provoque localement un échauffement du dépôt et de micro-explosions

L'échauffement entraîne des collisions et le transfert de charges de la matrice à l'échantillon