

Mass Spectrometry of Lipids and Glycoconjugates

Catherine E. Costello
BI 793 Lecture 9

April 3, 2012

Some types of lipids in biological systems

Membrane Lipids: Fatty acids, steroids, glycolipids, lipopolysaccharides (LPS), glycerophospholipids, lipid rafts, caveolae

Protein- and carbohydrate-bound lipids: anchors

Sphingolipids: ceramides, sphingomyelin

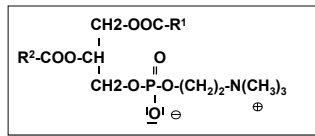
Glycosphingolipids: cerebrosides, gangliosides

Eicosanoids: prostaglandins, leukotrienes, lipoxins, isoprostanes

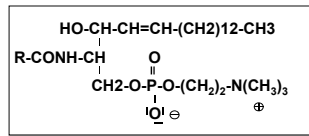
Fat-soluble Vitamins: A, D, E, K

CD1 lipid antigen presentation

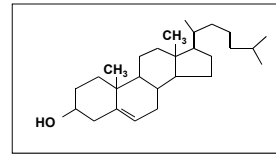
Structures of common lipids



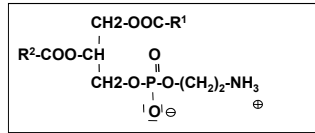
Phosphatidylcholine



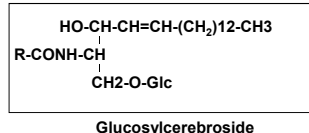
Sphingomyelin



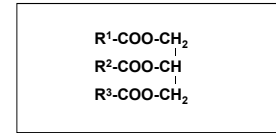
Cholesterol



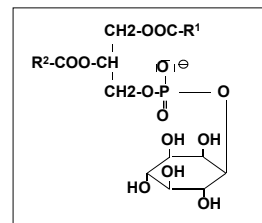
Phosphatidylethanolamine



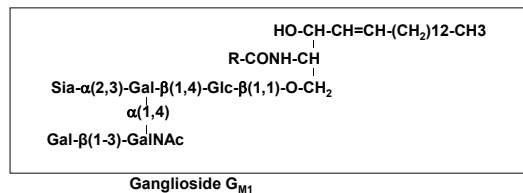
Glucosylcerebroside



Triglyceride

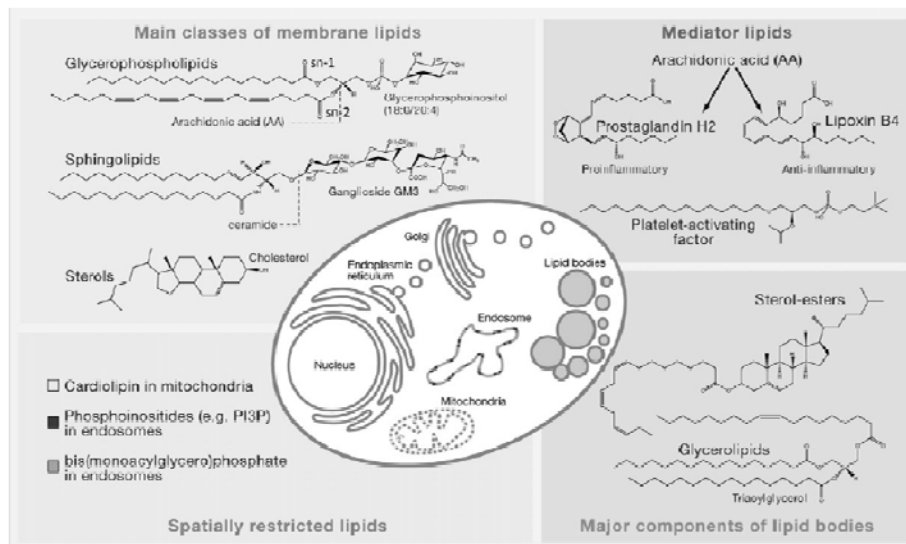


Phosphatidylinositol



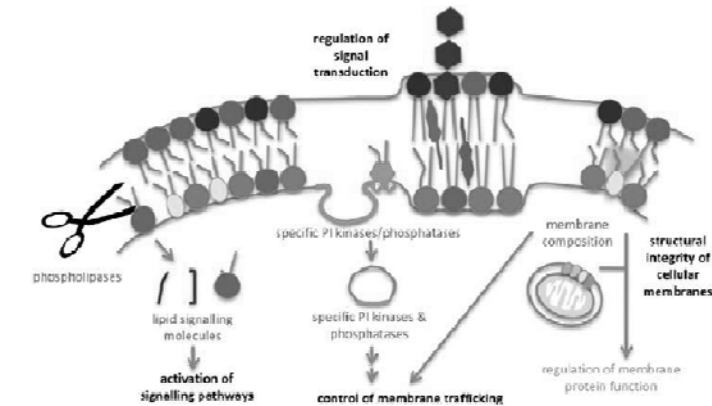
Ganglioside G_{M1}

Lipid locations in biological systems



E Wenk, *Cell*, 2010, 143, 888-895

Biological functions of lipids



Biological functions of lipids. A: Compartmentalization: lipids provide a dynamic and flexible barrier from the extracellular environment, and compartmentalize biological functions within the cell in terms of organelles. B: Membrane trafficking: membrane lipid composition and the combinatorial PI kinase/phosphatase system of organelle-specific lipid/membrane trafficking effector protein recognition mediate membrane trafficking. C: Signal transduction: lipids are involved in signaling (i) by defining specific membrane domains, and (ii) by acting as first and second messengers.

E Wenk, *Cell*, 2010, 143, 888-895

Some roles of lipids

Membrane Lipids: structural components, organization of membrane proteins etc. (lipid rafts), regulation of cell-cell and cell-ligand interactions

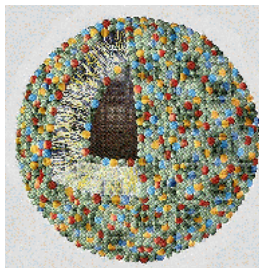
Sphingolipids: structural components, signalling, second messengers, biosynthetic intermediates

Protein and Carbohydrate-bound Lipids: anchors for biopolymers

Prostaglandins, eicosanoids: signalling

Fat soluble vitamins, tissue lipids: free radical traps, response to oxidative stress, energy stores

CD1 lipid antigen presentation: immune system activation



Lipids in Biology

Websites/program to watch:

www.lipidmaps.org

LIPID Metabolites And Pathways Strategy

also:

www.cyberlipid.org

Update of the LIPID MAPS comprehensive classification system for lipids¹

Eoin Fahy,² Shankar Subramaniam,³ Robert C. Murphy,⁴ Masahiro Nishijima,^{5,6} Christian H. H. Rietz,⁷ Takao Shimizu,⁸ Friedrich Spener,^{9,10} Gerrit van Meer,¹¹ Michael J. O. Wakelam,¹² and Edward A. Dennis^{13,14}

San Diego Supercomputer Center,¹ Department of Bioengineering,² and Department of Chemistry and Biochemistry and Department of Pharmacology of the School of Medicine,^{3,14} University of California, San Diego, La Jolla, CA 92037-0505; Department of Pharmacology,⁴ University of Colorado Denver, Aurora, CO 80045-0098; National Institute of Health Sciences,⁵ Setagaya-ku, Tokyo 158-8501, Japan; Department of Biochemistry,⁶ Duke University Medical Center, Durham, NC 27710; Department of Biochemistry and Molecular Biology,⁷ Faculty of Medicine, University of Tokyo, Tokyo 113-0033, Japan; Department of Molecular Biochemistry,⁸ University of Graz, 8010 Graz, Austria; Biopreci Center and Institute of Biochemistry,⁹ Utrecht University, 3584 CH Utrecht, The Netherlands; and The Robert H. Lurie Institute,¹⁰ Northwestern University, Chicago, Illinois 60611, United States

Abstract In 2005, the International Lipid Classification and Nomenclature Committee under the sponsorship of the LIPID MAPS Consortium developed and established a "Comprehensive Classification System for Lipids" based on well-defined chemical and biochemical principles and using an ontology that is extensible, flexible, and scalable. This classification system, which is compatible with contemporary database and bioinformatic needs, has now been accepted internationally and widely adopted. In response to considerable attention and requests from lipid researchers from around the globe and in a variety of fields, the comprehensive classification system has undergone significant revision over the last few years to more fully represent lipid structure from a wider variety of sources and to provide additional levels of detail as necessary. The details of this classification system are reviewed and updated and are presented here, along with revisions to its suggested nomenclature and some new knowledge recommendations for lipids. Fahy, E., S. Subramaniam, R. C. Murphy, M. Nishijima, C. H. H. Rietz, T. Shimizu, F. Spener, G. van Meer, M. J. O. Wakelam, and E. A. Dennis. Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* 2009; 50: S9–S11.

Supplementary key words: lipids • nomenclature • structure • ontology

In an effort to support the growing field of lipidomics and establish the importance of lipids as a major class of biomolecules, the International Lipid Classification and

Nomenclature Committee (ILCNC) developed a "Comprehensive Classification System for Lipids" that was published in 2005 (1). For the purpose of classification, we define lipids as hydrophobic or amphipathic small molecules that may originate entirely or in part by carboxylic acid condensation of diacylglycerols, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides and/or by carbon-chain-based condensation of isoprenoid units (prenyl lipids and sterol lipids). The comprehensive classification system organizes lipids into three well-defined categories (Table 1) that cover eukaryotic and prokaryotic sources. It has been adopted internationally and widely accepted by the lipidomics community. The system is also available online on the LIPID MAPS (2) website (<http://www.lipidmaps.org>). The comprehensive classification system has been under the guidance of the ILCNC, which meets periodically to propose changes and updates to classification, nomenclature, and structural representation.

Abbreviations: ILCNC, International Lipid Classification and Nomenclature Committee; IUPAC/IUBMB, International Union of Pure and Applied Chemistry and the International Union of Biochemistry and Molecular Biology; IUPAC, International Union of Pure and Applied Chemistry; IUPAC/IUBMB, International Union of Pure and Applied Chemistry and the International Union of Biochemistry and Molecular Biology.

¹Current address for the article was Toshi M. Fure, Children's Hospital Oakland Research Institute.

²To whom correspondence should be addressed.

³Current address: University of California, San Diego, La Jolla, CA. The ILCNC currently consists of Dr. Edward A. Dennis, Chair, (US), Dr. Robert C. Murphy (US), Dr. Masahiro Nishijima (Japan), Dr. Christian H. H. Rietz (US), Dr. Takao Shimizu (Japan), Dr. Friedrich Spener (Austria), Dr. Gerrit van Meer (The Netherlands), and Dr. Michael Wakelam (UK). Dr. Shankar Subramaniam serves as Information Advisor, and Dr. Eoin Fahy serves as Treasurer. Meetings were held May 2, 2004 and May 4, 2006 in La Jolla, CA.

Copyright © 2009 by the American Society for Biochemistry and Molecular Biology. All rights reserved. This article is available online at <http://www.lipidmaps.org>.

Journal of Lipid Research, April Supplement, 2009 59

LipidMaps classification system

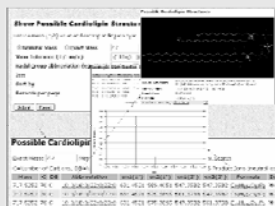
J. Lipid Res., 2009, 50, S9–14

This work was supported by the J. P. Morgan Chase Foundation Grant number 250-00000000 from the National Institute of Health. Manuscript received 10 November 2008 and in revised form 10 December 2008 and is available from 17 December 2008.

DOI: 10.1194/jlr.S900009J2009

Copyright © 2009 by the American Society for Biochemistry and Molecular Biology. All rights reserved. This article is available online at <http://www.lipidmaps.org>.

Mass Spectrometry Tools



Find mass, number of carbons, number of double bonds, abbreviation, MS/MS product ions (neutral loss), formula, and ion based on input criteria (mass, mass tolerance ($\pm$ m/z), radyl group abbreviation, headgroup, and/or ion), with links to structure and isotopic distribution.

Current search categories include:

- **Cardiolipins** (Search by mass, mass tolerance ($\pm$ m/z), radyl group abbreviation, and/or ion.)
- **Glycerophospholipids** (Search by mass, mass tolerance ($\pm$ m/z), radyl group abbreviation, headgroup, and/or ion.)
- **Mono/Di/Triacylglycerols** (Search by mass, mass tolerance ($\pm$ m/z), radyl group abbreviation, and/or ion.)

LipidMaps tools for mass spectrometry

www.lipidmaps.org

Online resources for lipid classification, databases

Resource	URL	Country	Comments
LIPID MAPS	http://www.lipidmaps.org/	U.S.	Lipid classification scheme
LipidBank	http://lipidbank.jp/	Japan	Covers lipids from animals to plant
LIPIDAT	http://www.lipidat.ul.ie/	U.S.	Contains phospholipids and their thermodynamic data
LMSD	http://www.lipidmaps.org/data/structure/index.html	U.S.	Composed of structures and annotations
LMPD	http://www.lipidmaps.org/data/peptoids/index.html	U.S.	proteins and genes associated with lipids
Cyberlipid	http://www.cyberlipid.org/	France	Lipid database that includes isoprenoid-derived molecules.
SphingOMAP	http://sphingoab.biology.gatech.edu/	U.S.	Pathway map for sphingolipid biosynthesis
Lipid Library	http://www.lipidlibrary.co.uk/	U.K.	Composed of information about lipid chemistry, biology and analysis.
KEGG	http://www.genome.jp/kegg/pathway.html	Japan	Manually drawn pathway maps that included fatty acid biosynthesis and degradation, sterol metabolism, and phospholipids pathway.
GOLD	http://gold.uni-graz.at/index.html	Austria	Composed of annotated pathway and curated data set.

MB Khalil, ...D Figeys, *Mass Spectrom Rev*, 2010, 29, 877-929

Software for lipidomics data processing

Commercial

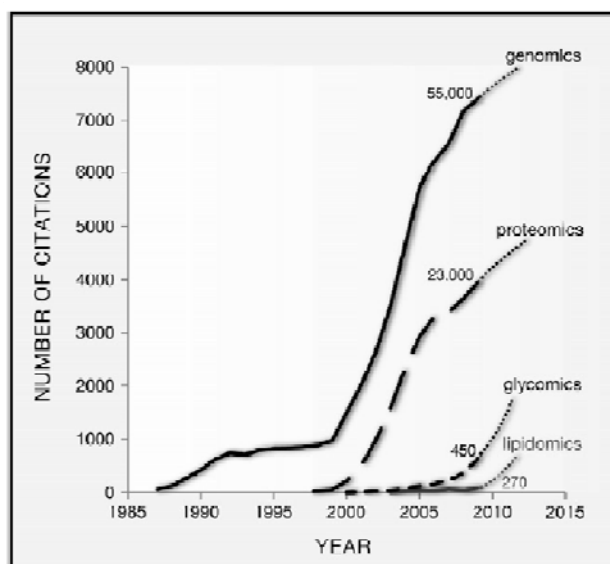
Name	Vendor	Main application field
Lipid Profiler	MDS Sciex	Lipidomics with LC-MS
Bluefuse	BlueGnome	Metabolomics with MS and NMR
MarkerLynx	Waters	Metabolomics with LC-MS
Metabolic Profiler	Bruker BioSpin	Metabolomics with MS
metAlign	Plant Research International	LC-MS and GC-MS
MS Resolver	Pattern Recognition Systems	LC-MS and GC-MS
Profile	Phenomenome Discoveries	Metabolomics with MS
MarkerView	Applied Biosystems	Metabolomics with LC-MS

Free

Name	License	Main application field
MZmine	GNU General Public License	Metabolomics with LC-MS and GC-MS
LIMS	GNU General Public License	Lipidomics with LC-MS and SECD data
SECD	Freely available	Metabolomics with LC-MS
TriglyAPCI	Freely available	Lipidomics with LC-MS
MSPECTRA	Freely available	Lipidomics with LC-MS
XCMS	GNU General Public License	Metabolomics with LC-MS and GC-MS

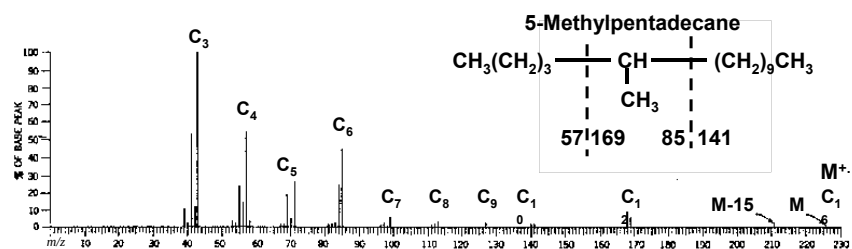
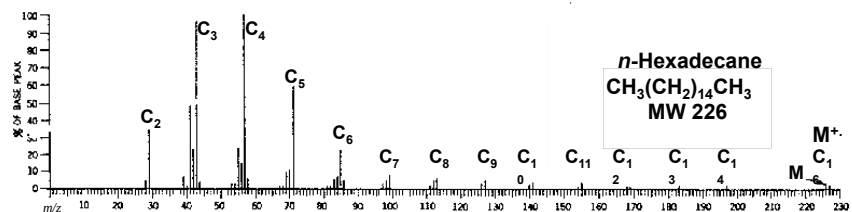
MB Khalil, ...D Figeys, *Mass Spectrom Rev*, **2010**, 29, 877-929

Growth of publications in “omics”



MB Khalil, ...D Figeys, *Mass Spectrom Rev*, **2010**, 29, 877-929

EI MS of isomeric C14 hydrocarbons



EI MS of methyl caprylate

Methyl caprylate

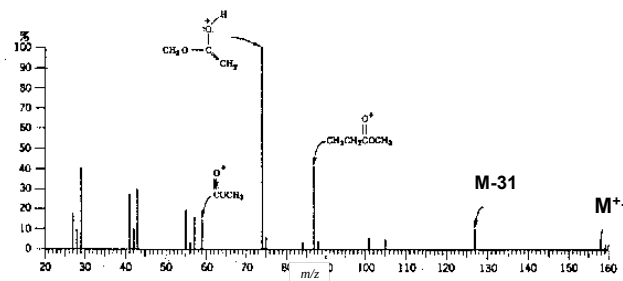
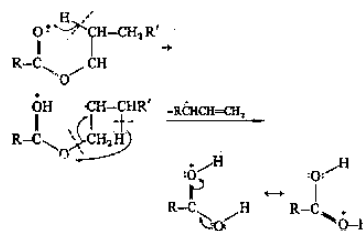
$\text{CH}_3(\text{CH}_2)_6\text{COOCH}_3$

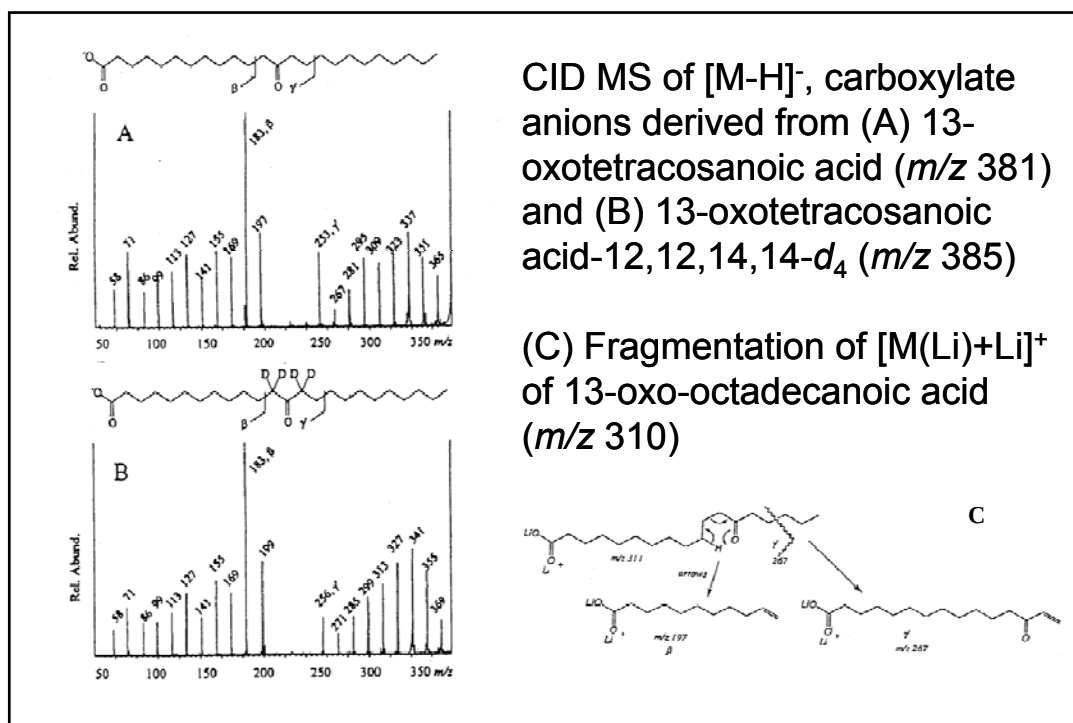
MW 158

m/z 158 (M) 100.00

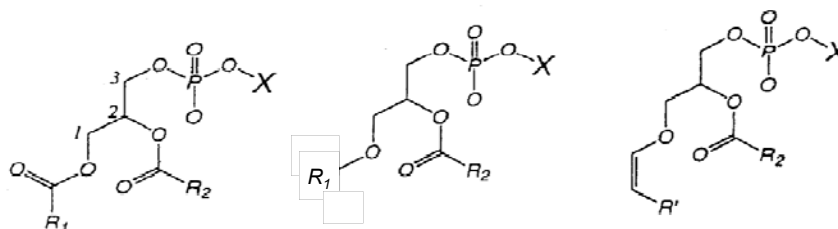
m/z 159 (M+1) 12.90

m/z 160 (M+2) 1.00





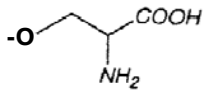
Glycerophospholipids



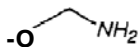
1,2-diacyl 1-O-alkyl,2-acyl plasmalogens
 $R_1, R_2, R' =$ alkyl chain; lysophospholipid 2-OH

Mono-, di- and triacylglycerides

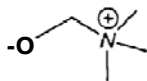
Glycerophospholipid polar head groups (X)



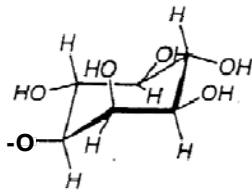
glycerophosphoserine (GPS)



glycerophosphoethanolamine (GPE)



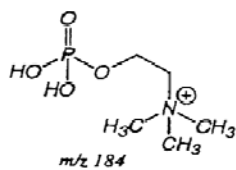
glycerophosphocholine (GPC)



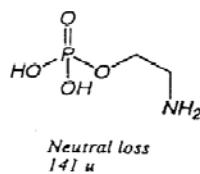
glycerophosphoinositol (GPI)

Characteristic fragment, neutral losses for glycerophospholipid polar head groups (X)

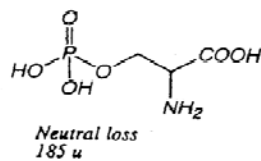
GPC



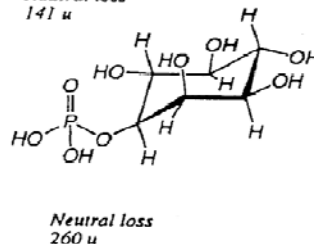
GPE



GPS



GPI



Characterization of a lipid mixture by nanospray MS

specificity	polarity	fragment structure	scan mode	fragment type	optimal collision offset
all [M-H] ⁻ ions of glycerophospholipids	neg.		precursor of 153	glycerol-phosphate -H ₂ O	+50 V
phosphatidylinositol	neg.		precursor of 241	head group H ₂ O	+45 V
phosphatidylserine	neg.		neutral loss of 87	head group -H ₂ PO ₄	+20 V
phosphatidylethanolamine	neg.		precursor of 196	diolysis -H ₂ O	+50 V
sphingomyelin	neg.		sCID + precursor of 188	head group (demethylated)	sCID +65 & +40 V
phosphatidylcholine and sphingomyelin	pos.		precursor of 184	head group	-38 V
phosphatidylethanolamine	pos.		neutral loss of 141	head group	-25 V
phosphatidylserine	pos.		neutral loss of 185	head group	-22 V

Instrument: Finnigan-MAT TSQ 7000 triple quadrupole MS

Nanospray MS

MeOH/CHCl₃ 2:1, in pos. ion mode 1% HOAc

Other useful scans (not from this ref.):

PI pos.: NL of 260

Cholesterols pos.: PC of 369

Fatty acids neg.: PC 255, 279, 281, 303 etc.

Sugars pos.: PC 163, 204, 292 etc.

B. Brügger, G. Erben, R. Sandhoff, F. T. Wieland, and W. D. Lehmann (1997). Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. *Proc.Nat.Acad.Sci.USA* **94**, 2339-2344.

LC-MS of lipids

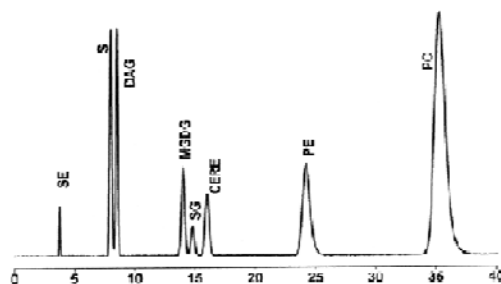
Why LC?

- Quantification of minor components (signal suppression)
- Sample cleanup: getting rid of cluster formers etc.
- Retention time helps to characterize a compound.

Why MS? (instead of light scattering, UV etc)

- Even simple MS allows an immediate impression of the molecular species involved, isomers excepted.

U Sommer, H Herscovitz, FK Welty, and CE Costello, *J Lipid Res*, **2006**, 47, 804-814



SE: sterol esters,
S: sterols,
DAG: diacylglycerols,
MGDG: monogalactosyldiglycerides,
SG: sterol glycosides,
CERE: cerebrosides,
DGDG: digalactosyldiglycerides,
PE: phosphatidylethanolamine,
PI: phosphatidylinositol,
PC: phosphatidylcholine

Column: YMC PVA-Sil (250 x 4.6 mm, 3 μ m from Hichrom),
the phase is prepared by bonding a layer of polymerized vinyl alcohol to silica.
Ternary HPLC pump (40 min @ 1 ml/min, 10 min reequilibration time)
Evaporative light-scattering detector

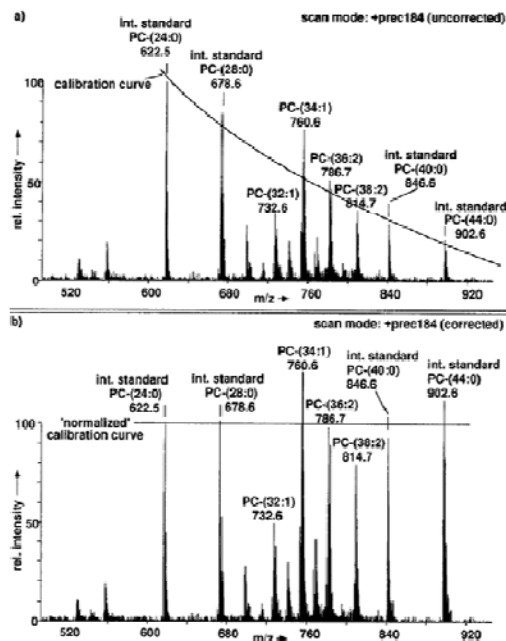
Solvent A: isooctane/methyl tert-butyl ether (98/2, v/v)
Solvent B: isopropanol/acetonitrile/chloroform/acetic acid (84/8/8/0.025, v/v)
Solvent C: isopropanol/water/triethylamine (50/50/0.2, v/v)
Isooctane may be replaced by isohexane (for safety reasons), with similar results.

(from www.cyberlipid.org, after WW Christie *et al*, *J High Resol Chromatogr*, 1995, 18, 97

Lipid Quantification

Ion intensity depends on:

- Ionization efficiency of head groups
- Fatty acid length and saturation
- Suppression
- Solvent composition (small changes)
- Instrument settings (actual settings can vary from day to day!)

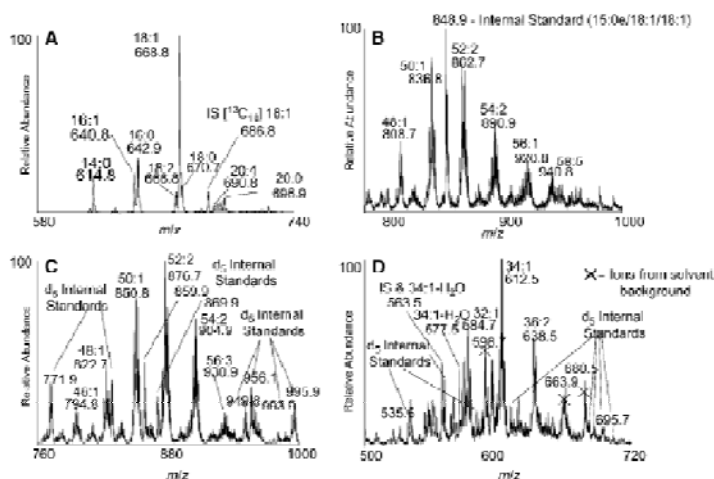


B Brügger, G Erben, R Sandhoff, FT Wieland, WD Lehmann, *PNAS*, 1997, 94, 2339-2344

PC Lipids in B100 (WT) and B67 LDLs

[illegible]

Neutral Lipids in RAW 264.7 cells



Summed positive-ion MS data recorded for precursors of m/z 369.3 or as total ion current of neutral lipids from RAW cells. (A) CE fraction (B) MeDAG fraction (C) TAG fraction (D) DAG fraction. Selected $[M+NH_4]^+$ of samples and internal standards.

(Hutchins *et al.*)

Location of sites of unsaturation in lipids

Ozone-Induced Dissociation: Elucidation of Double Bond Position within Mass-Selected Lipid Ions

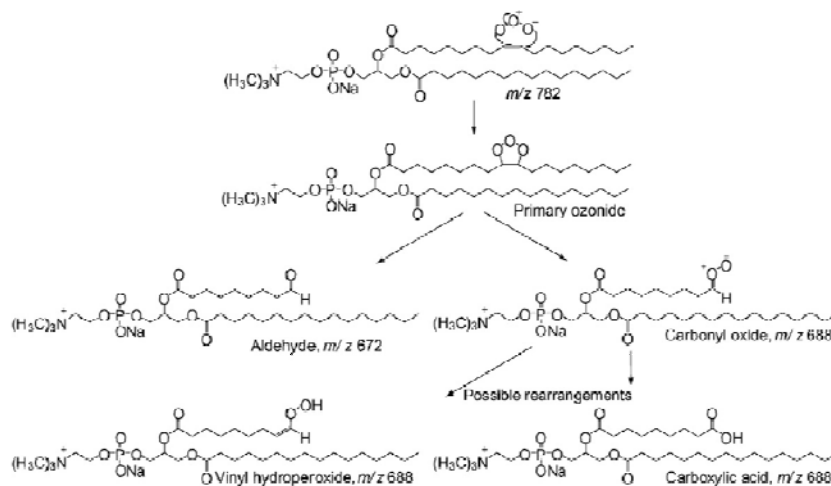
MC Thomas, TW Mitchell, DG Harman, JM Deeley, JR Nealon, SJ Blanksby, *Anal. Chem.*, **2008**, 80, 303–311.

Identification of double bond position in lipids. From GC to OzID.

TW Mitchell, H Pham, MC Thomas, SJ Blanksby, *J Chromatogr B Analyt Technol Biomed Life Sci.* **2009**, 877, 2722–2735.

Online ozonolysis methods for the determination of double bond position in unsaturated lipids. MC Thomas, TW Mitchell, SJ Blanksby. *Methods Mol Biol.* **2009**, 579, 413–441.

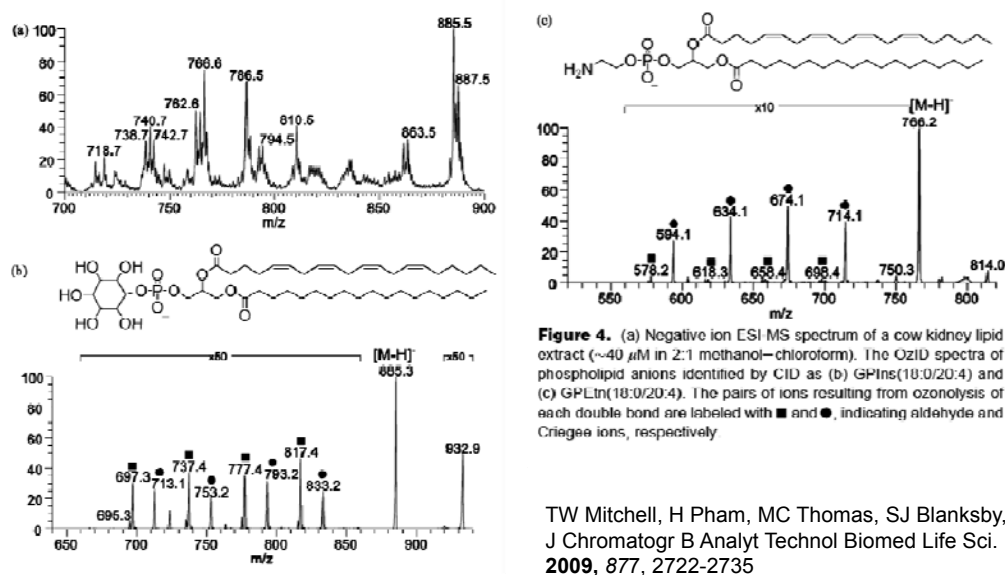
OzID location of sites of unsaturation in lipids



Scheme 3. The proposed mechanism for the gas phase reaction of mass-selected IPC(16:0/18:1(9Z))+NaI⁺ ions (*m/z* 782) with ozone in an ion-trap mass spectrometer.

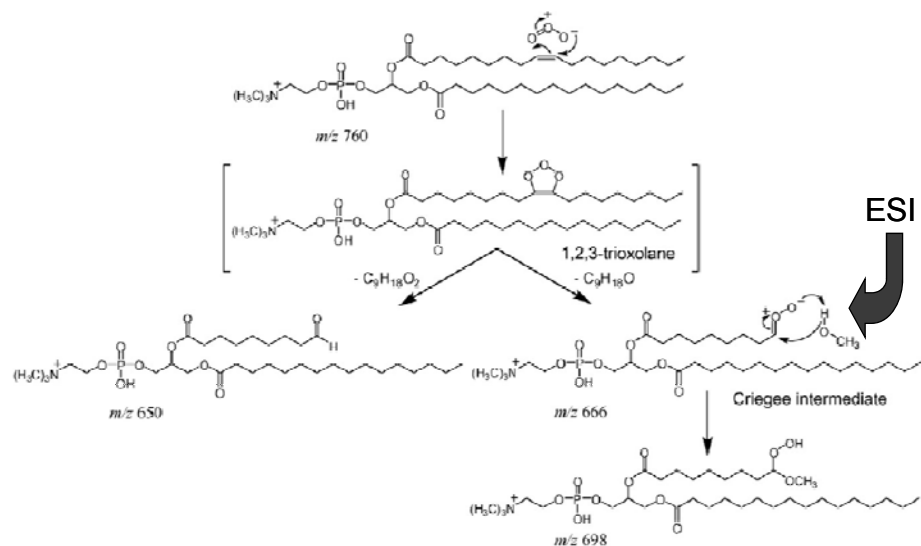
MC Thomas, TW. Mitchell, DG Harman, JM Deeley, JR Nealon, SJ Blanksby, *Anal Chem*, **2008**, *80*, 303–311

Location of sites of unsaturation in lipids



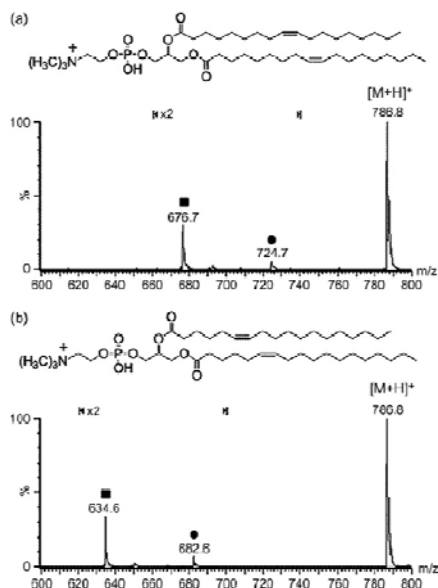
TW Mitchell, H Pham, MC Thomas, SJ Blanksby,
J Chromatogr B Analyt Technol Biomed Life Sci.
2009, 877, 2722-2735

Oz-ESI for location of sites of unsaturation in lipids



TW Mitchell, H Pham, MC Thomas, SJ Blanksby, J Chromatogr B Analyt Technol Biomed Life Sci, **2009**, 877, 2722-2735

Location of sites of unsaturation in lipids



The OzESI-MS spectrum of a 1 M methanolic solution of (a) PC(18:1(9 Z)/18:1(9 Z)) and (b) PC(18:1(6 Z)/18:1(6 Z)). Both spectra are recorded as m/z 184 precursor ion scans and thus show only the $[M+H]^+$ + molecular ion and corresponding chemically induced fragment ions. The symbols ■ and ● identify the ozonolysis product ions as methoxyhydroperoxides and aldehydes, respectively (reproduced from ref. [103] with permission).

TW Mitchell, H Pham, MC Thomas, SJ Blanksby, J Chromatogr B Analyt Technol Biomed Life Sci. **2009**, 877, 2722-2735

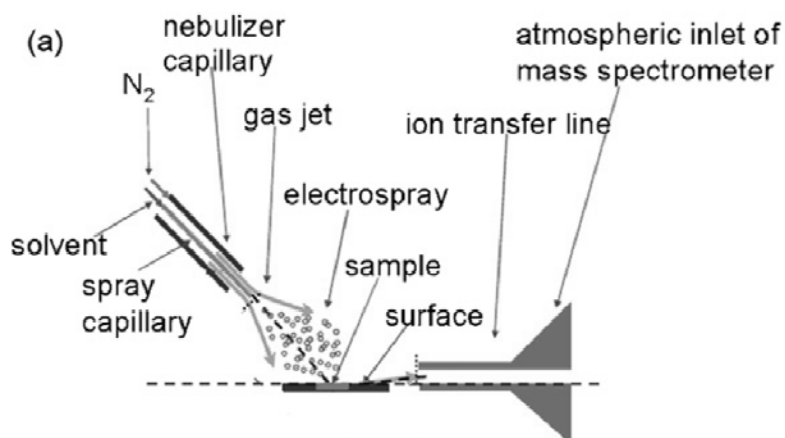
Lipids analyzed by DESI directly from samples

Mass spectrometric imaging of lipids using desorption electrospray ionization.

Dill AL, Ifa DR, Manicke NE, Ouyang Z, Cooks RG. J Chromatogr B Analyt Technol Biomed Life Sci. **2009**, 877, 2883-2889.

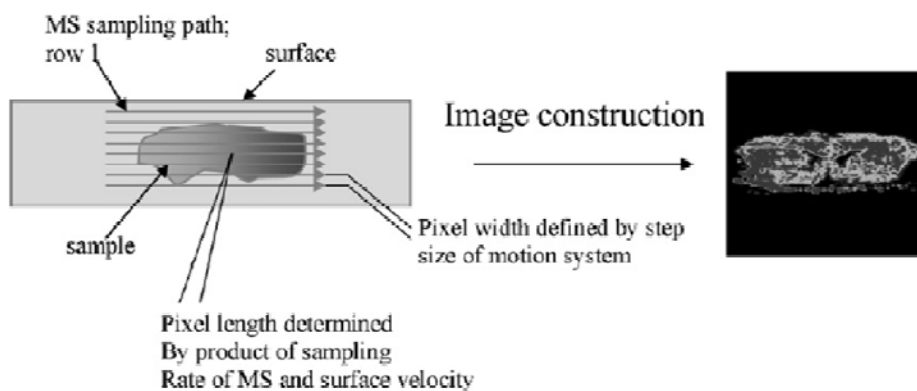
courtesy of RG Cooks, Purdue University

Lipids analyzed by DESI directly from samples



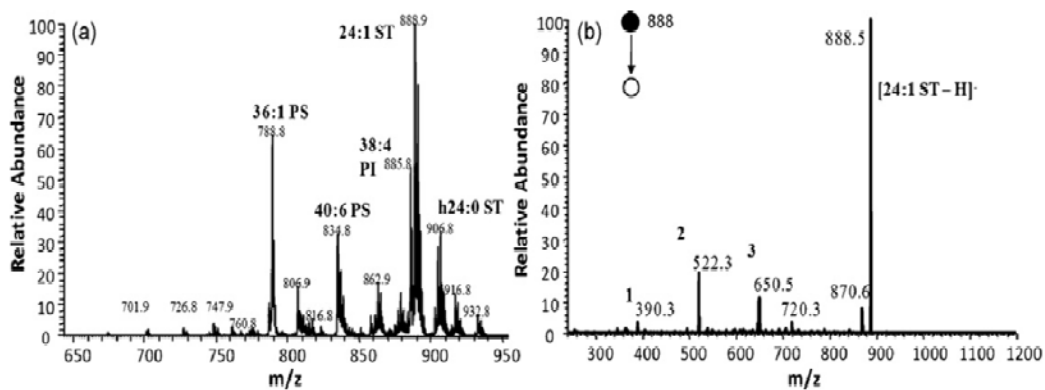
courtesy of RG Cooks, Purdue University

Lipids analyzed by DESI directly from samples



courtesy of RG Cooks, Purdue University

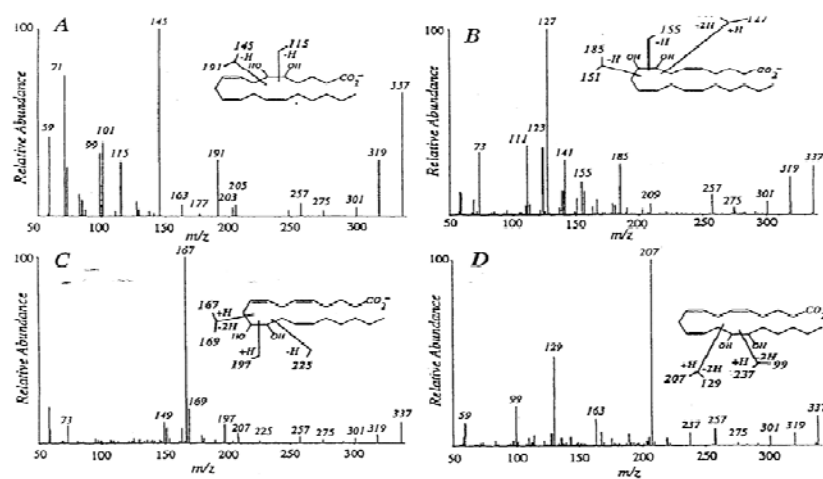
Lipids analyzed by DESI directly from samples



courtesy of RG Cooks, Purdue University

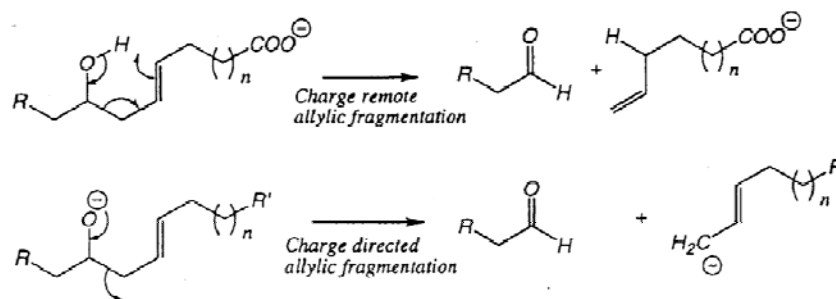
Eicosanoids

ESI-CID MS of anions, m/z 337, from isomeric dihydroxyeicosatetraenoic acids



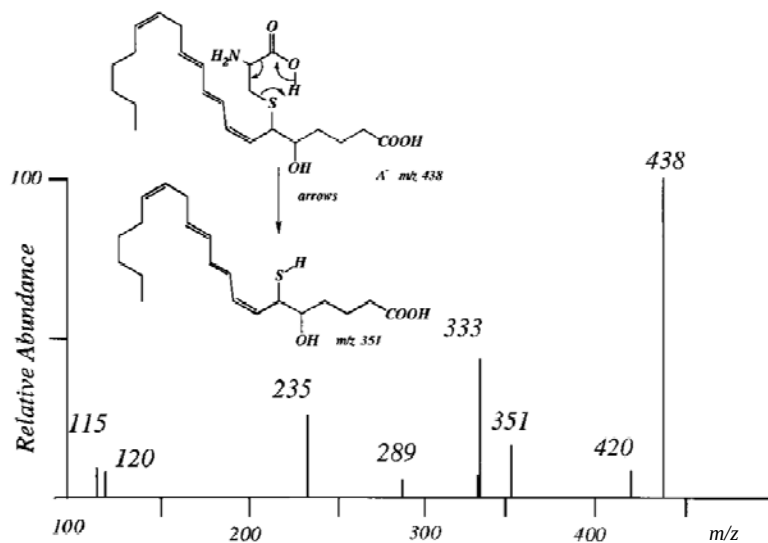
P Wheelan et al, *J Am Soc Mass Spectrom*, 1996, 7, 140

Fragmentation of anions, m/z 337, from isomeric dihydroxyeicosatetraenoic acids

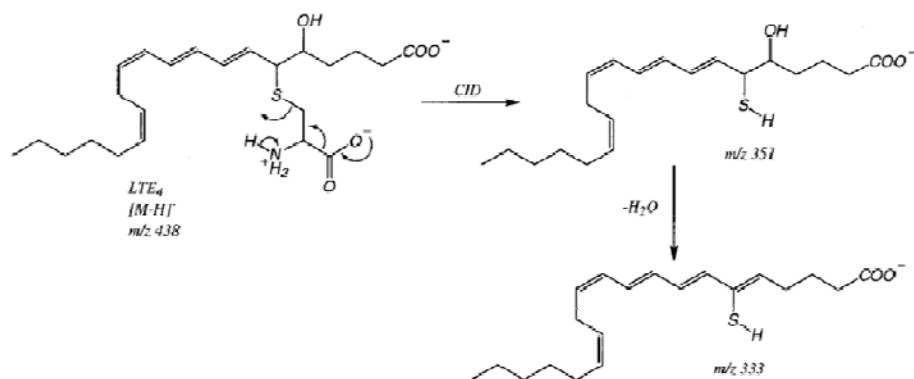


P Wheelan et al, *J Am Soc Mass Spectrom*, **1996**, 7, 140

ESI-CID MS of leukotriene E₄, $[M-H]^-$ m/z 438



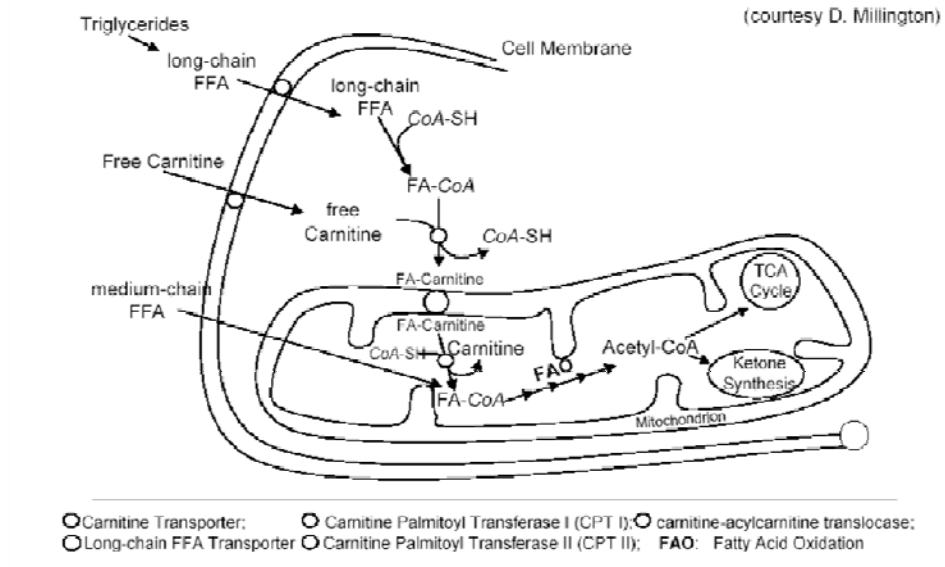
Fragmentation of leukotriene E₄, [M-H]⁻ *m/z* 438



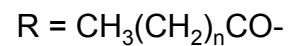
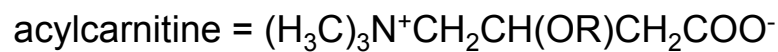
RC Murphy *et al*, *Chem Rev*, **2001**, 101, 479

Lipids and oxidative stress

Mitochondrial fatty acid metabolism



Application of acylcarnitine and amino acid analysis (ca. 50 metabolites)



■ Newborn screening – for ~30 metabolic disorders

D Millington *et al*, Duke Univ Medical Center

Fatty acid metabolism overview

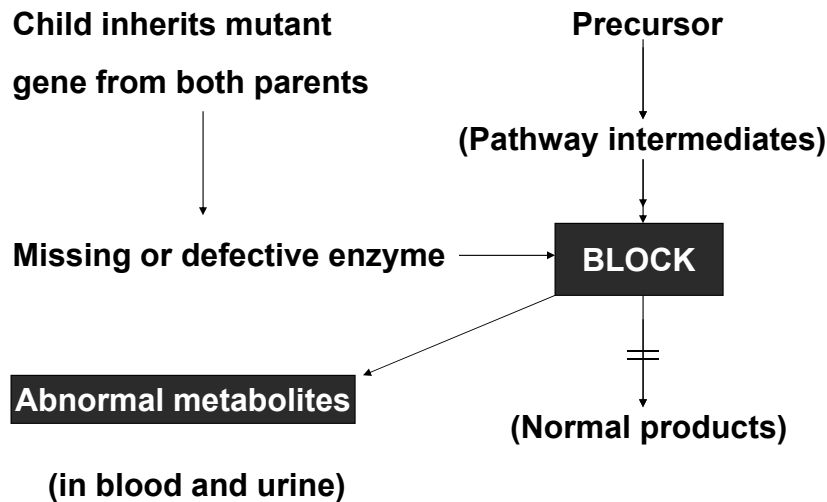
(courtesy D Millington)

- **Mitochondrial fatty acid β -oxidation (FAO) is a physiological response to tissue energy depletion when fasting, during febrile illness, and increased muscular activity**
- **In the liver, FAO fuels synthesis of ketone bodies, used as an alternative energy source for the brain (especially) and other extrahepatic organs when glucose reserves are exhausted (after only a few hours in neonates)**
- **FAO is the primary energy source for the heart muscle at all times**

Fatty acid metabolism overview - continued

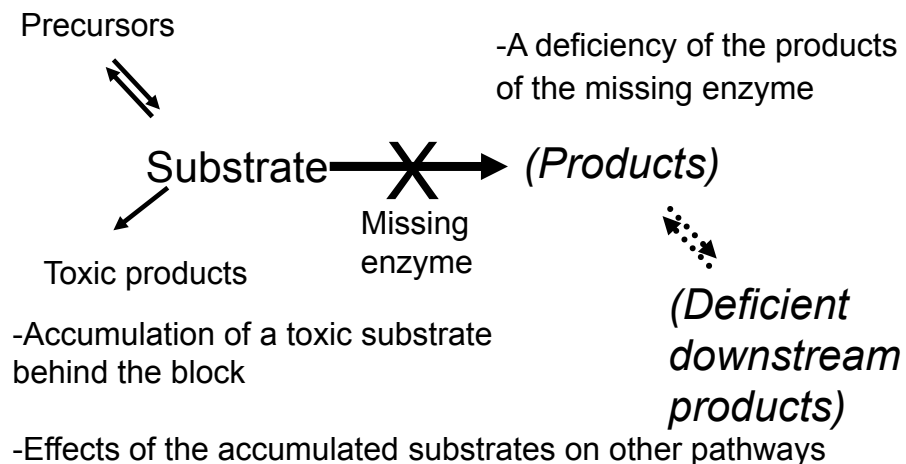
- **More than 20 diseases of the FAO pathway have been described to date – all are of autosomal recessive inheritance; most are treatable.**
- **The clinical manifestations are diverse, and can include hypoglycemia, vomiting, lethargy, coma, encephalopathy (Reyes-like syndrome), sudden unexpected death, liver failure, cardiomyopathy, respiratory distress, skeletal myopathy, myoglobinuria, pregnancy complications.**
- **The symptoms are often episodic.**

Basis of a recessive metabolic disease



Underlying enzyme deficiency blocks a unique biochemical pathway

(courtesy D. Millington)

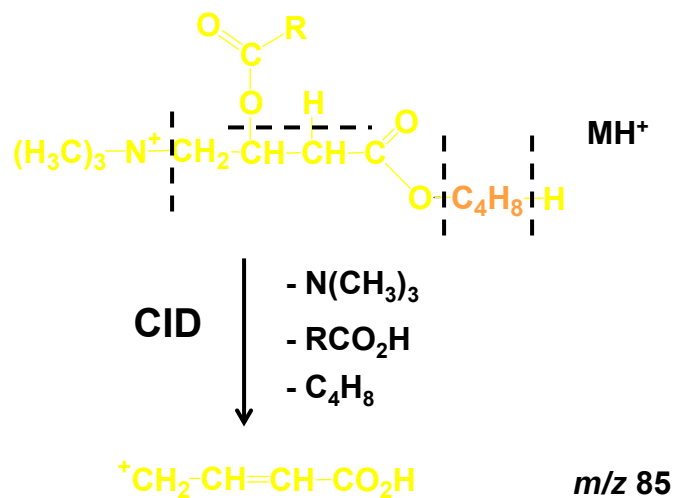


Diagnostic testing for FOA disorders

(courtesy D. Millington)

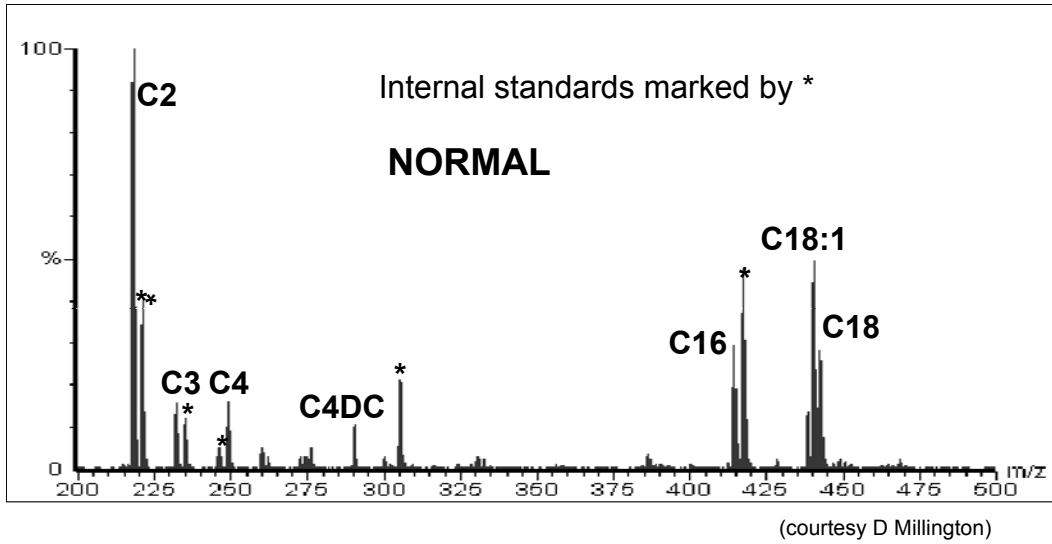
- The most useful test is the acylcarnitine profile (plasma or whole blood).
- Free and total carnitine in plasma should be analyzed at the same time.
- Urine organic acids analysis is helpful in diagnosis of some FAO disorders.
- *In vitro* testing (skin fibroblasts) and/or molecular testing is often necessary for confirmation.

Fragmentation of acylcarnitines in MS-MS (c) as *n*-butyl esters

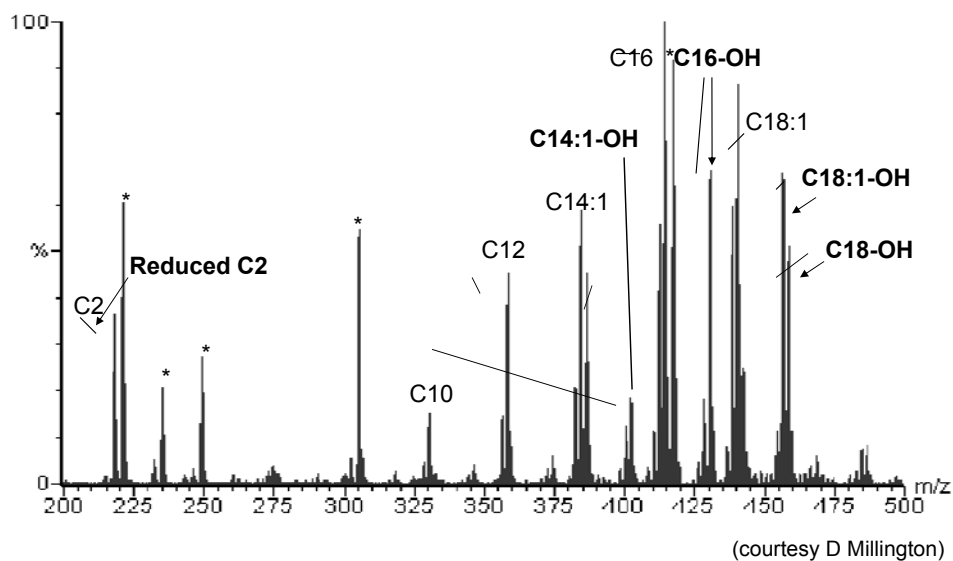


(courtesy D Millington)

Analysis of acylcarnitines by MS/MS in newborn's blood spot



LCHAD or TFP deficiency (metabolic stress)



MS/MS newborn screening in North Carolina: 5 yr Summary

Total screened = 635,168 (7/28/97 to 12/31/02)

Confirmed diagnoses after abnormal screen:

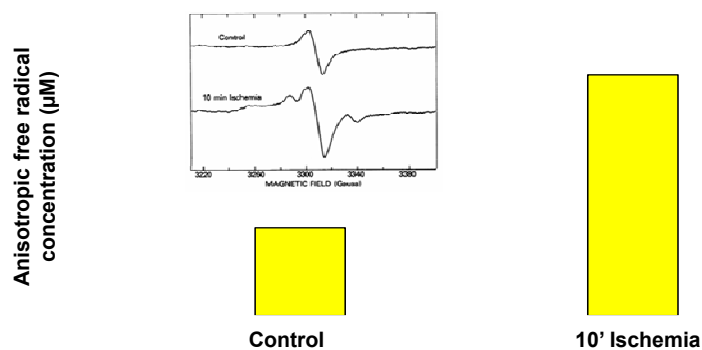
■ Fatty acid oxidation disorders	62
MCADD (48)	
■ Organic acidemias	37
3-methylcrotonylglycinuria (13)	
■ Amino acid disorders	43
PKU/hyperphenylalaninemia (30)	

Total = 142

Overall Incidence: 1:4,473 (D. Frazier, UNC)

(courtesy D Millington)

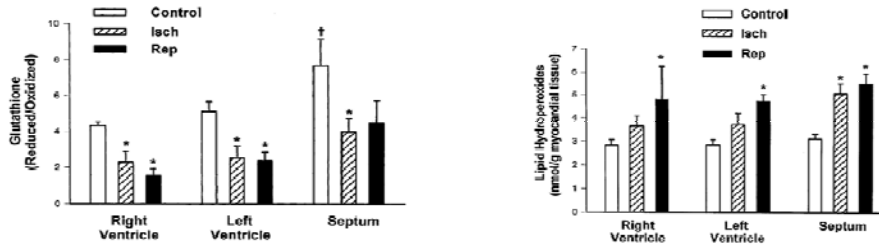
Myocardial ischemia increases free radical generation



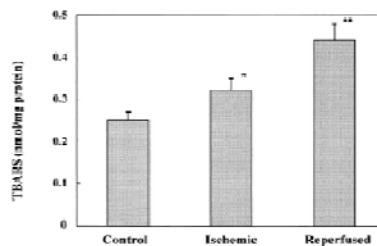
Free radical generation during myocardial ischemia. EPR spectra of control and ischemic heart (inset).

JL Zweier *et al*, *Proc Natl Acad Sci USA* **1987**, 84, 1404-1407

Ischemia and reperfusion cause oxidative stress

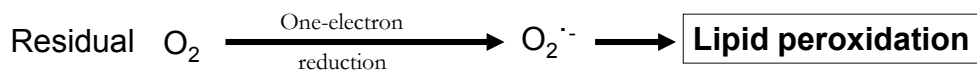
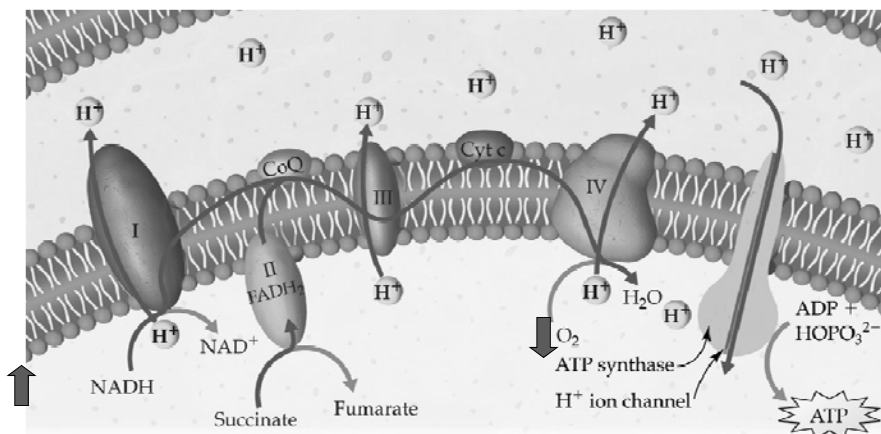


V Palace, *J Mol Cell Cardiol*, **1999**, 31, 193-202



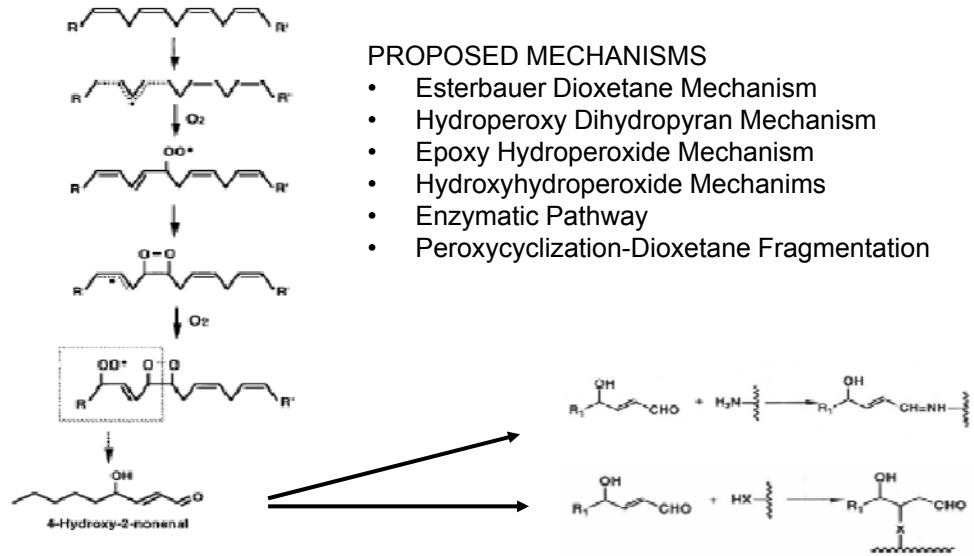
G Paradies, *Free Radic Biol Med*, **1999**, 27, 42-50

ROS are generated in the heart during ischemia

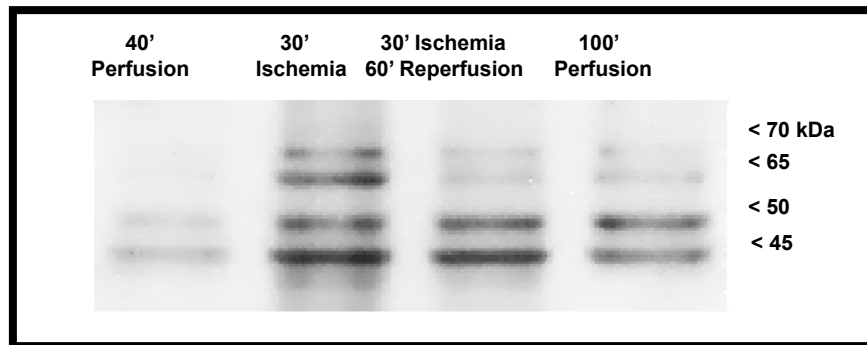


BG Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004

Lipid peroxidation generates aldehydes



Global ischemia induces HNE modification of proteins in the heart



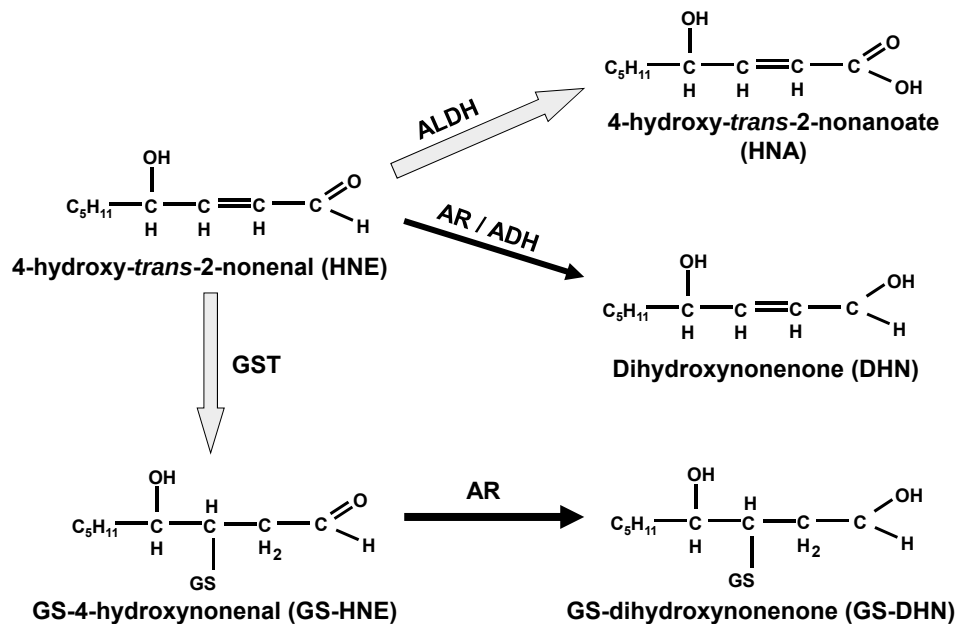
Myocardial proteins were separated by SDS-PAGE followed by Western blot (WB) analysis with anti-protein-HNE antibodies.

Specific proteins are modified to a greater extent during ischemia vs. ischemia-reperfusion.

MALDI-TOF/MS identification of proteins displaying positive immunoreactivity with anti-protein-HNE antibodies

#	Protein	MW (Da)	pI	# Peptides Matched	Coverage	Mowse Score*	Accession # (gi #)
1	ATP synthase β subunit	51171	4.92	12	34%	213	1374715
2	HSP 60	58061	5.35	11	27%	185	1334284
3	MDH2	36089	8.92	6	20%	73	13592145
4	VDAC1	30851	8.62	9	46%	171	6755963
5	LDH B	36874	5.70	8	27%	103	6981146
6	Enolase	47273	7.59	8	20%	98	6978811
7	Creatine kinase	43220	6.58	10	30%	167	6978661

BG Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004



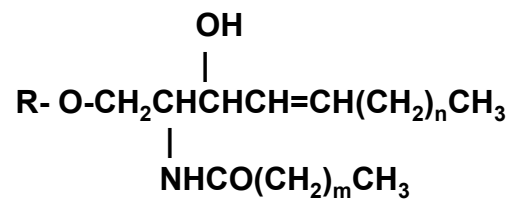
BG Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004

Glycoconjugates

Information available from MS analysis of glycoconjugates

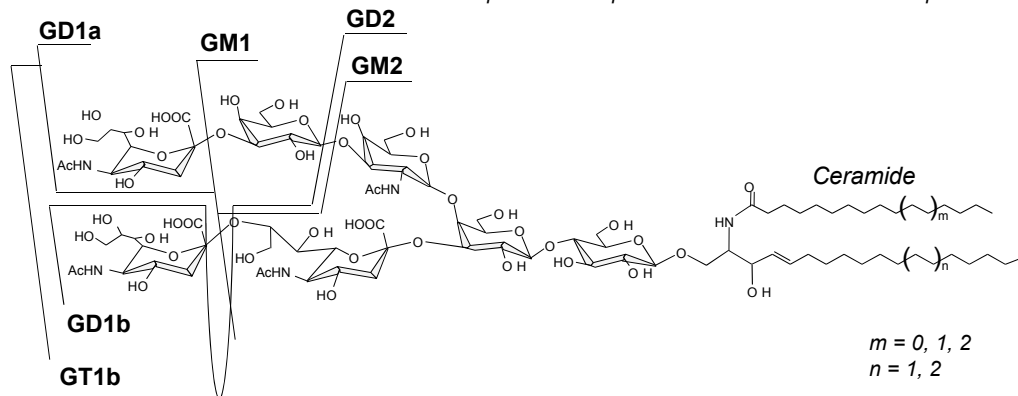
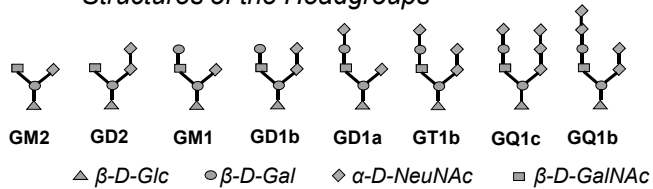
- Molecular weight distribution
- Carbohydrate sequence(s)
- Linkage analysis
- Aglycon structure
- Unusual modifications
- Mixture analysis

Sphingolipids and Glycosphingolipids

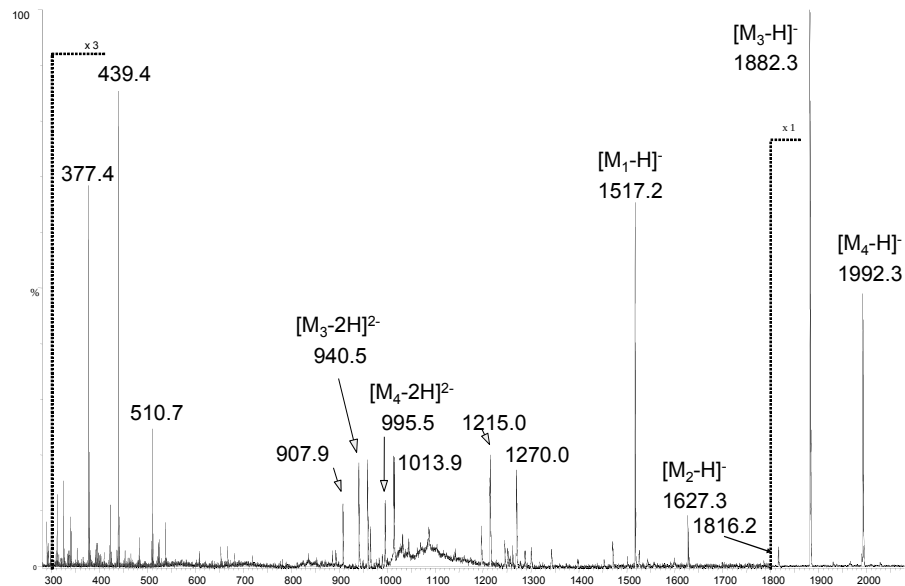


Structures of the gangliosides

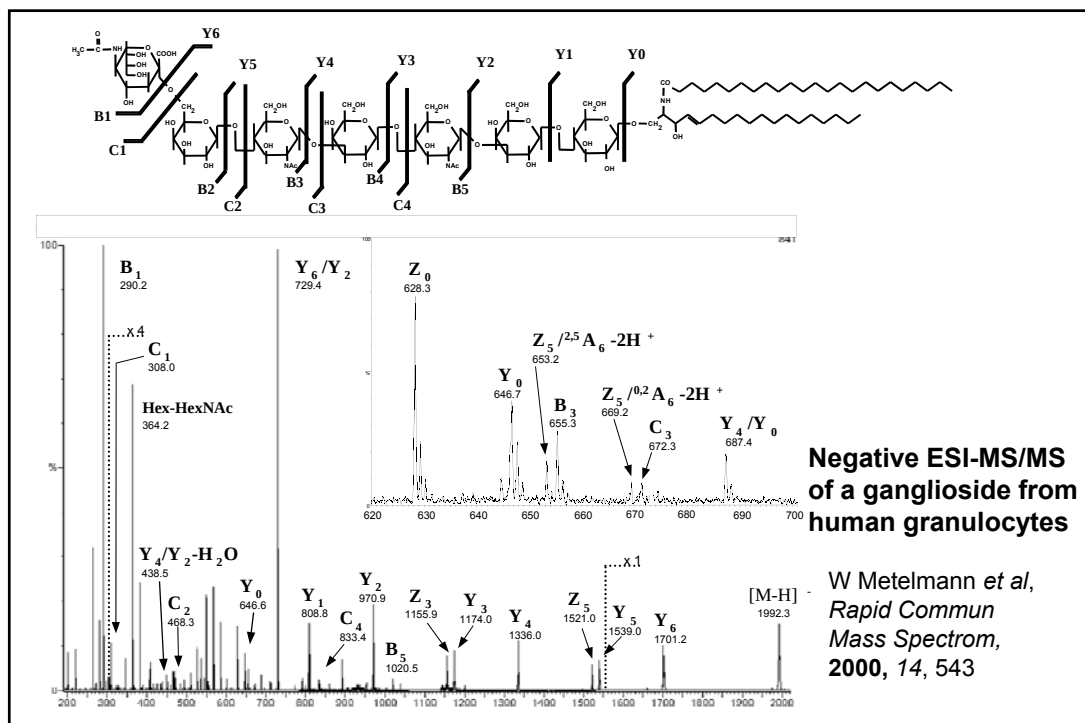
Structures of the Headgroups



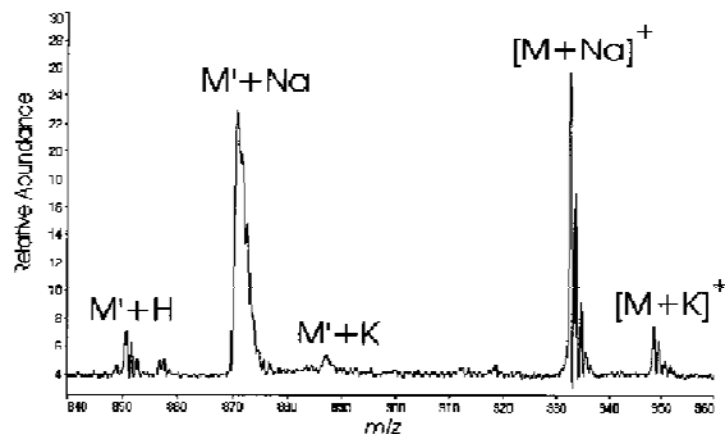
Q-oTOF ESI-MS of gangliosides from human granulocytes



W. Metelmann, J. MÜthing and J. Peter-Katalinic, *RCMS*, **14**, 543 (2000)

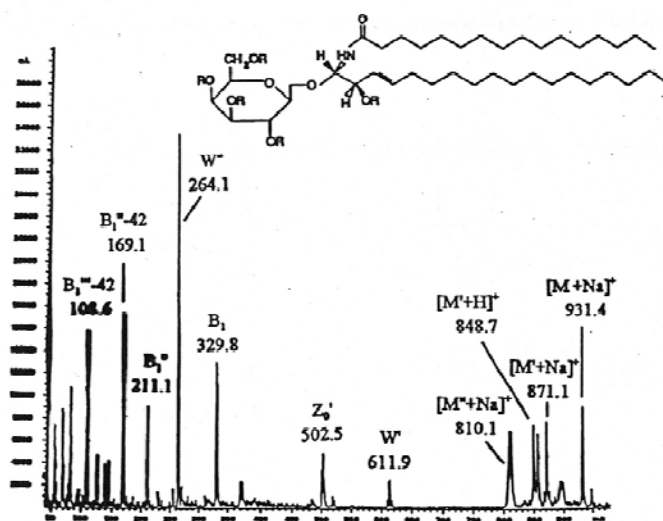


Molecular ion region in the UV-MALDI-TOF MS of peracetylated *N*-palmitoyl galactosyl sphingenene, $[M+Na]^+$ m/z 932.6



M = neutral molecular mass, M' = M-HOAc

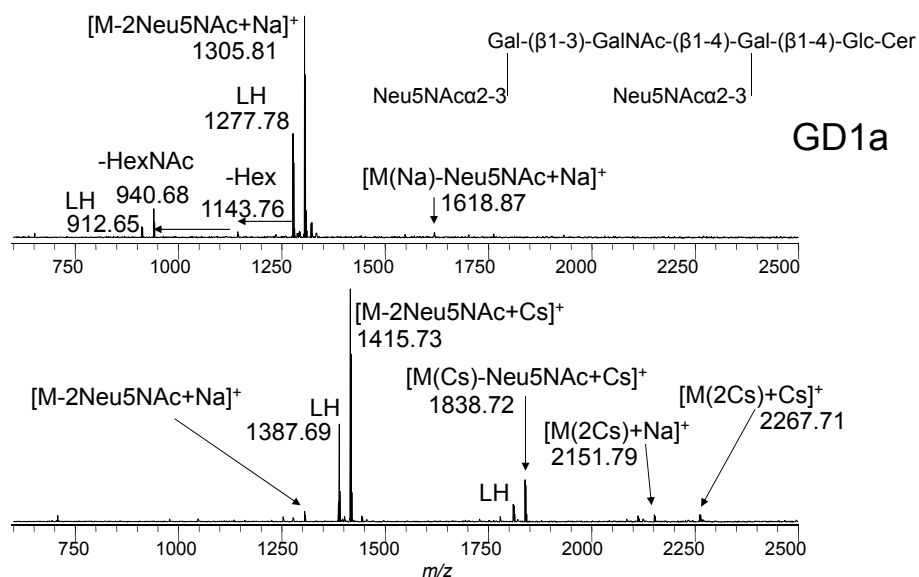
CE Costello, unpublished data



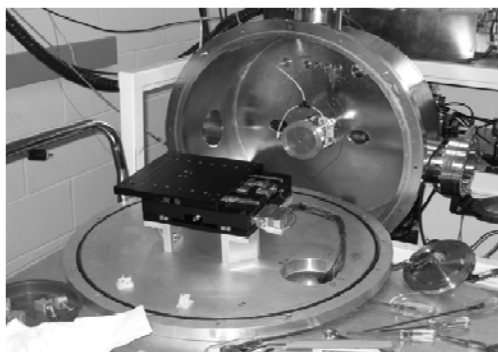
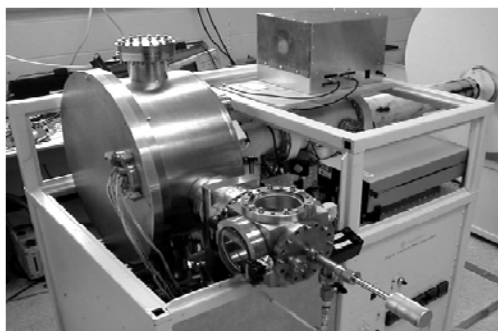
MALDI-PSD mass spectrum of peracetylated *N*-palmitoyl galactosyl sphingenene, $[M+Na]^+$ m/z 932.6

M = neutral molecular mass, M' = M-HOAc

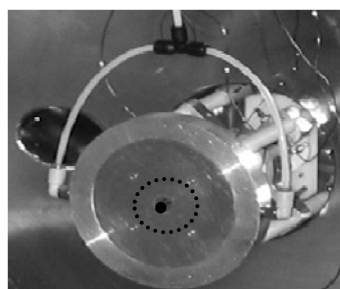
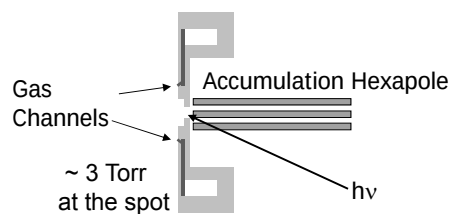
CE Costello and JE Vath unpublished data



Low pressure positive-ion MALDI-FTMS spectra of GD_{1a} and Na, Cs

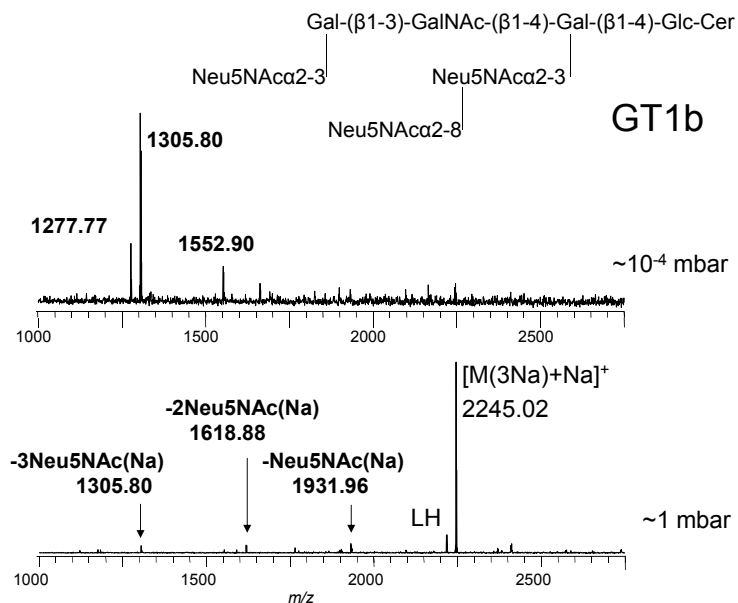


Vibrational Cooling MALDI-FTMS



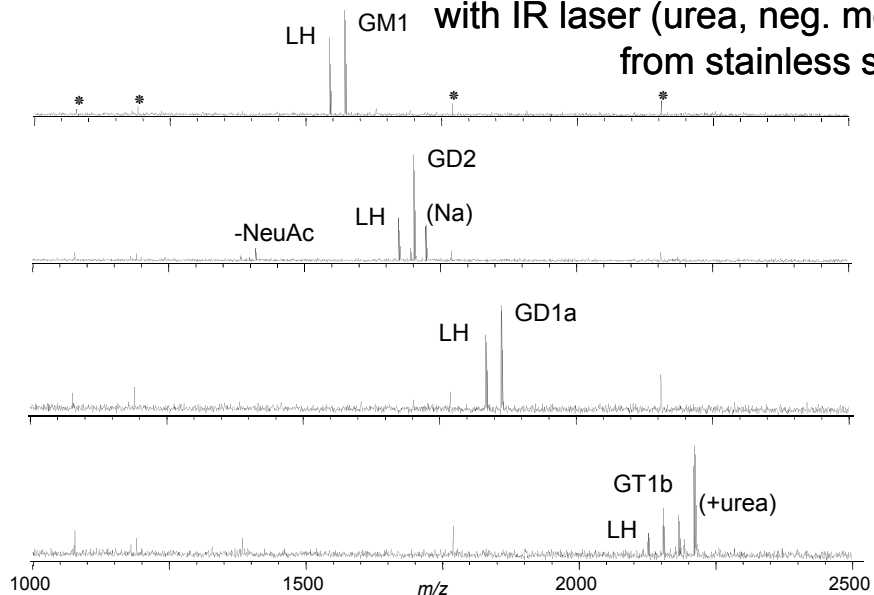
Ganglioside Analysis

VC MALDI FTMS
allows intact
desorption.



PB O'Connor and CE Costello, *RCMS*, **2001**, 15, 1862
PB O'Connor *et al*, *JASMS*, **2002**, 13, 402

Gangliosides desorbed with IR laser (urea, neg. mode) from stainless steel



V Ivleva *et al*, *Anal Chem* **2004**, 76, 6484

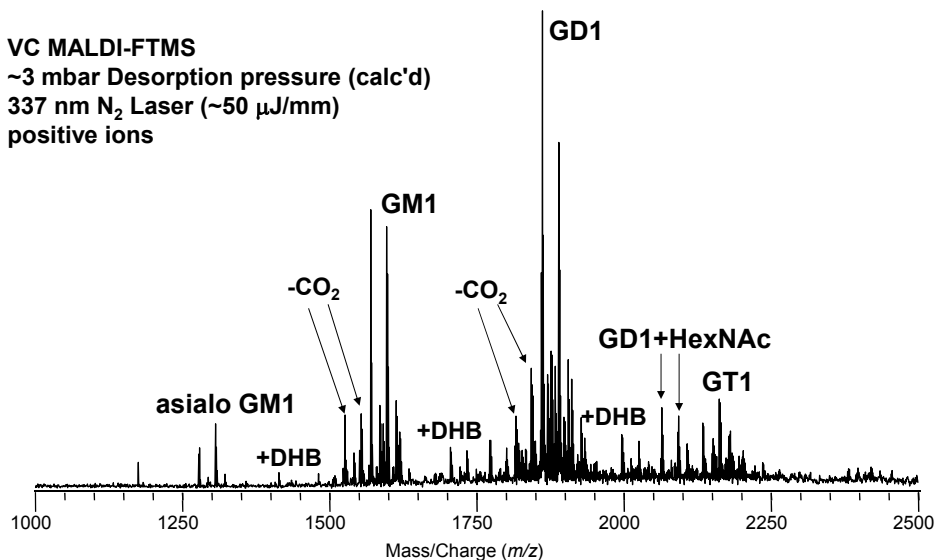
Bovine whole brain gangliosides

VC MALDI-FTMS

~3 mbar Desorption pressure (calc'd)

337 nm N₂ Laser (~50 μ J/mm)

positive ions



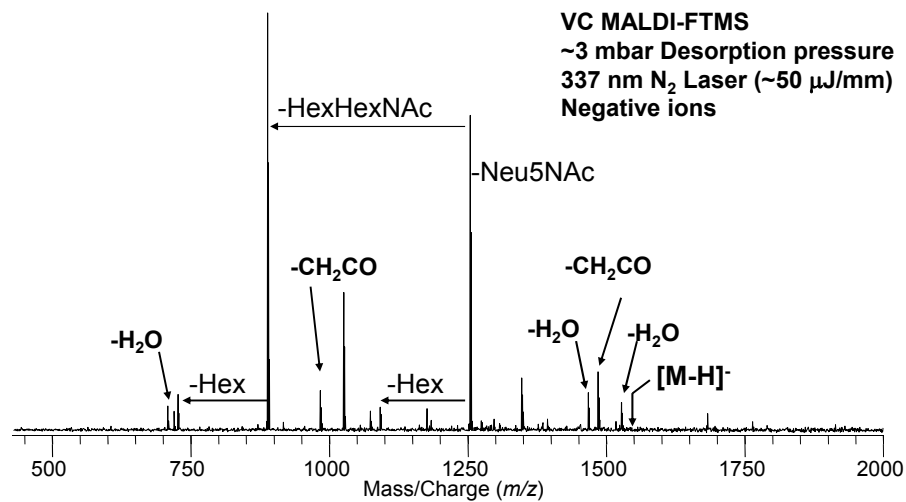
MS/MS of GM1 from WBG mixture

VC MALDI-FTMS

~3 mbar Desorption pressure

337 nm N₂ Laser (~50 μ J/mm)

Negative ions



Challenges of MALDI MS from surfaces

- Determination of sample positions
- Sample location within surface
- Inadequate mixing with matrix
- Inefficient transfer to membrane
- Uneven surface
- Inadequate penetration of laser beam
- Compromised instrument performance
- Metastable decomposition

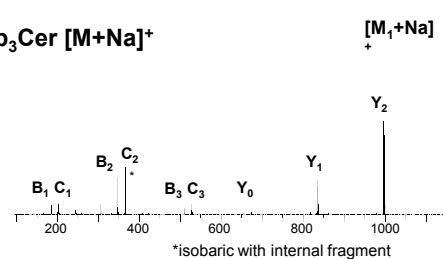
Desorption from polymer membranes

Desorption from steel

⇒ Sodiated Ions

= oligosaccharide cleavages

MS/MS of Gb₃Cer [M+Na]⁺

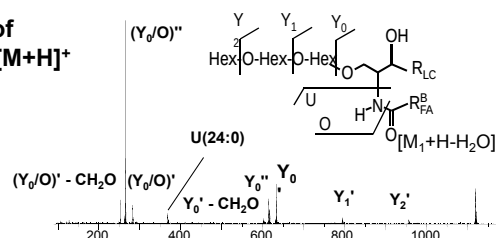


Desorption from Nafion™

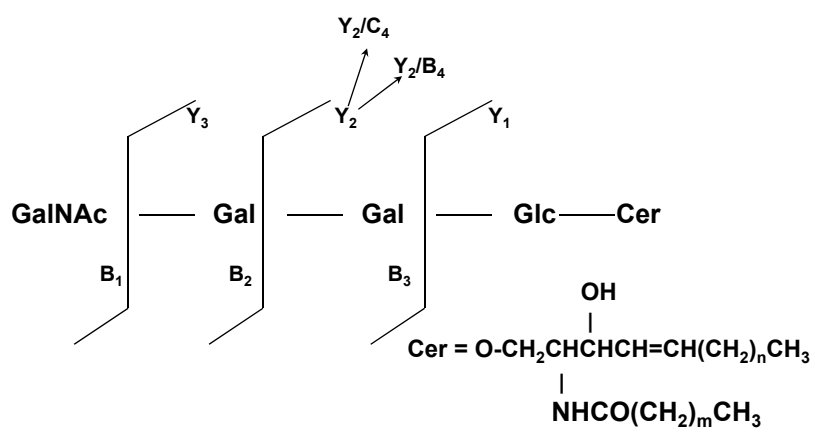
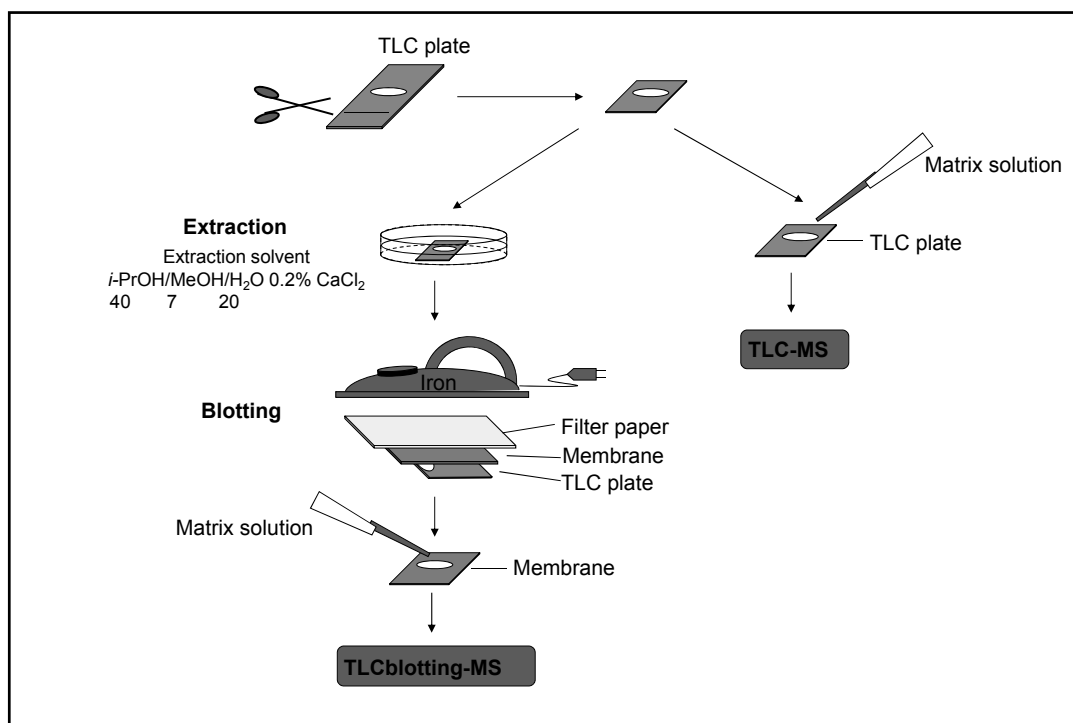
⇒ Protonated Ions

= lipid cleavages

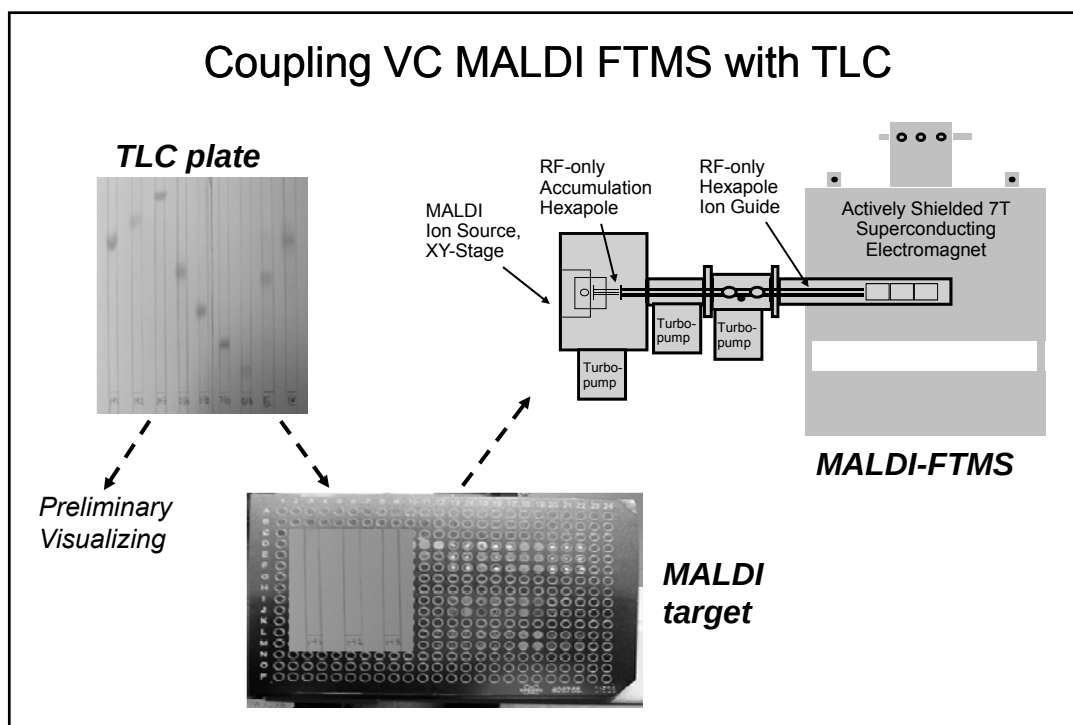
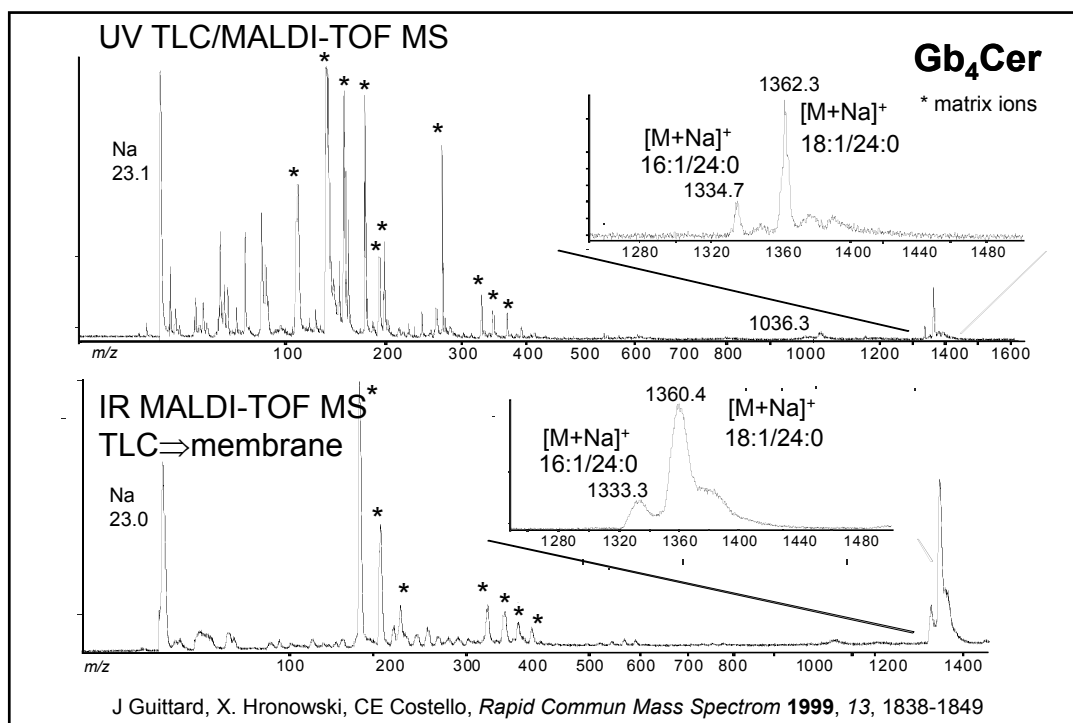
MS/MS of Gb₃Cer [M+H]⁺



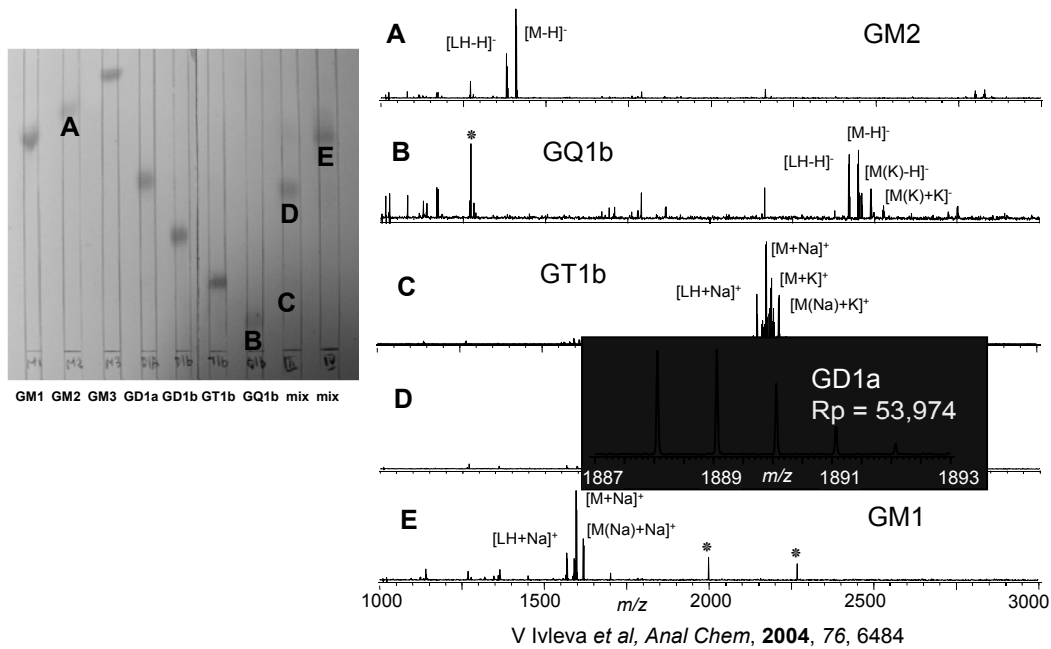
ME McComb *et al*, ASMS 2002



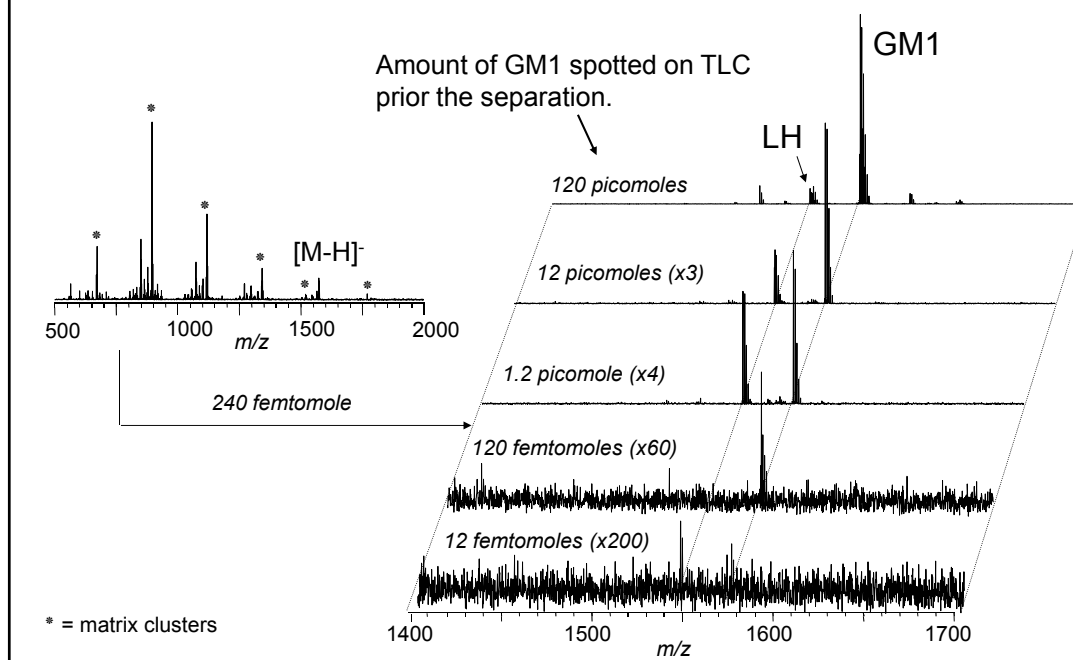
Globotetraosyl ceramide, Gb_4Cer



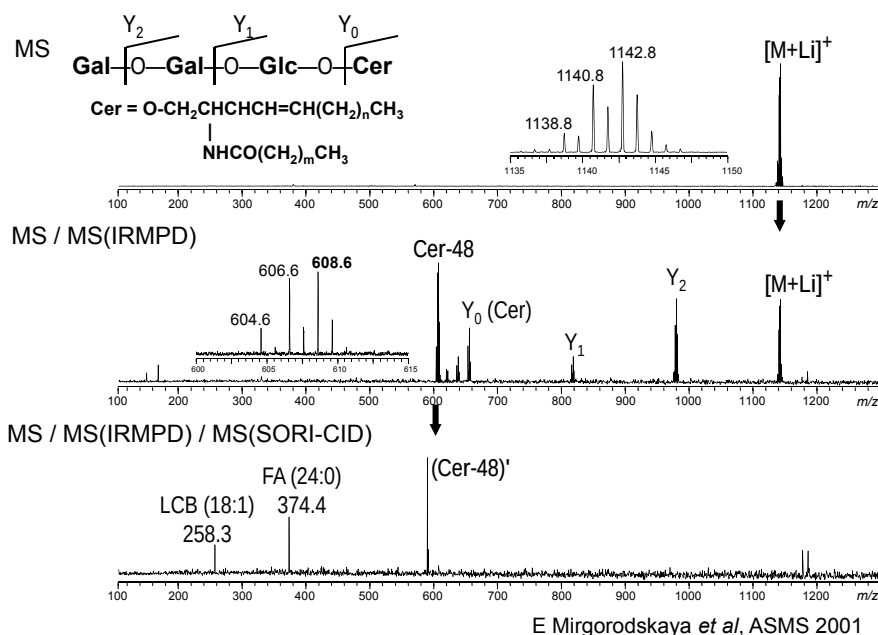
VC MALDI FTMS of TLC-separated gangliosides



Detection limit of TLC-VC MALDI-FTMS



Combination of IRMPD & SORI-CID



Summary of TLC-VC MALDI

- TLC-VC-MALDI-FTMS of glycolipids is simple, accurate.
- External MALDI source avoids deleterious effects of surface irregularities.
- IR & UV VC MALDI both minimize decomposition.
- Broadband resolving power > 50,000; accuracy > 1.5 ppm
- Detection limit is ~100 fmol deposited on plate.
- Tuning ion source pressure allowed isomer differentiation.
- VC-MALDI QoTOF MS shows similar advantages for glycolipids.

Advantages of derivatization before MS analysis of glycoconjugates

- Sensitivity increase
- Mass shifts characteristic of functional groups
- Control over fragmentation pathways
- Location of functional group sites
- Conversion of oligosaccharides to glycolipids

Summary: MS approaches for lipids and glycoconjugates $\Rightarrow$ an increasing range of options $\Rightarrow \Rightarrow$ Lipidomics, Glycomics!

- *Derivatization*
- *On- and offline separations*
-
- ESI-QqQ MS and MS/MS, ESI-QIT MSⁿ
- MALDI-TOF MS and PSD
- ESI and MALDI QoTOF MS and MS/MS
- ESI and VC MALDI FT-ICR MSⁿ
- ESI LTQ-Orbitrap

References

- AL Dill *et al*, *J Chromatogr B Analyt Technol Biomed Life Sci*, **2009**, 877, 2883
- E Fahy E Dennis , *J Lipid Res*, **2009**, 50 (Suppl), S9–14
- X Han, RW Gross, *J Lipid Res*, **2003**, 44, 1071
- PM Hutchins *et al*, *J Lipid Res*, **2008**, 49, 804
- V Ivleva *et al*, *Anal Chem*, **2004**, 76, 6484
- RA Johanson *et al*, *Anal Biochem*, **2007**, 362, 155
- MB Khalil, ...D Figeys, *Mass Spectrom Rev*, **2010**, 29, 877
- W Metelmann *et al*, *Rapid Commun Mass Spectrom*, **2000**, 14, 543
- S Milne *et al*, *Methods*, **2007**, 39, 92
- TW Mitchell *et al*, *J Chromatogr B Analyt Technol Biomed Life Sci*, **2009**, 877, 2722
- RC Murphy, *Chem. Rev.* **2001**, 101, 479
- PB O'Connor, CE Costello, *J Am Soc Mass Spectrom*, **2002**, 13, 402
- M Pulfer , RC Murphy, *Mass Spectrom Rev*, **2003**, 22, 332
- U Sommer *et al*, *J Lipid Res*, **2006**, 47, 804
- MC Thomas *et al*, *Anal Chem*, **2008**, 80, 303
- MR Wenk, *Cell*, **2010**, 143, 888
- P Whelan *et al*, *J Am Soc Mass Spectrom*, **1996**, 7, 140