Mass Spectrometry of Lipids and Glycoconjugates

Catherine E. Costello

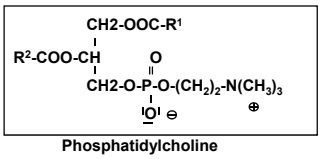
BI 793 Lecture 11

April 13, 2010

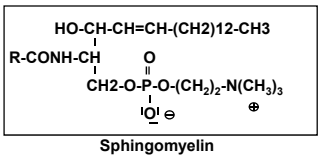
Some types of lipids in biological systems

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

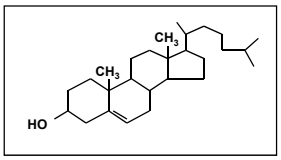
Structures of common lipids



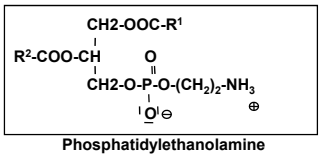
Phosphatidylcholine



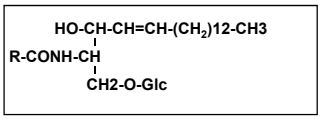
Sphingomyelin



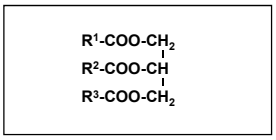
Cholesterol



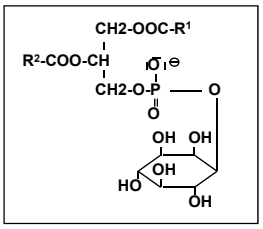
Phosphatidylethanolamine



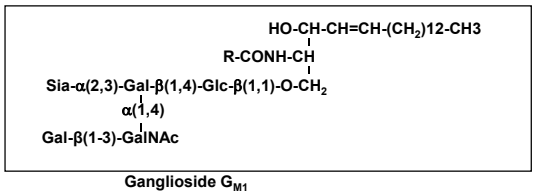
Glucosylcerebroside



Triglyceride



Phosphatidylinositol



Ganglioside G_{M1}

A comprehensive classification system for lipids[1]

Eoin Fahy,* Shankar Subramaniam,† H. Alex Brown,§ Christopher K. Glass, Alfred H. Merrill, Jr.,†† Robert C. Murphy,§§ Christian R. H. Raetz,*** David W. Russell,††† Yousuke Seyama,§§§ Walter Shaw,**** Takao Shimizu,†††† Friedrich Spener,§§§§ Gerrit van Meer,***** Michael S. VanNieuwenhze,†††††† Stephen H. White,§§§§§ Joseph L. Witztum,****** and Edward A. Dennis§,†††††††**

San Diego Supercomputer Center,* University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0505; Department of Bioengineering,† University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0412; Department of Pharmacology,§ Vanderbilt University Medical Center, Nashville, TN 37232-6600; Department of Cellular and Molecular Medicine,** University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0651; School of Biology,†† Georgia Institute of Technology, Atlanta, GA 303332-0230; Department of Pharmacology,§§ University of Colorado Health Sciences Center, Aurora, CO 80045-0508; Department of Biochemistry,*** Duke University Medical Center, Durham, NC 27710; Department of Molecular Genetics,††† University of Texas Southwestern Medical Center, Dallas, TX 75390-9046; Faculty of Human Life and Environmental Sciences,§§§ Ochanomizu University, Tokyo 112-8610, Japan; Avanti Polar Lipids, Inc.,**** Alabaster, AL 35007; Department of Biochemistry and Molecular Biology,†††† Faculty of Medicine, University of Tokyo, Tokyo 113-0033, Japan; Department of Molecular Biosciences,§§§§ University of Graz, 8010 Graz, Austria; Department of Membrane Enzymology,***** Institute of Biomembranes, Utrecht University, 3584 CH Utrecht, The Netherlands; Department of Chemistry and Biochemistry,††††† University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358; Department of Physiology and Biophysics,§§§§§ University of California at Irvine, Irvine, CA 92697-4560; Department of Medicine,****** University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0682; and Department of Chemistry and Biochemistry and Department of Pharmacology,††††††† University of California, San Diego, La Jolla, CA 92093-0601

Abstract **Lipids are produced, transported, and recognized by the concerted actions of numerous enzymes, binding proteins, and receptors. A comprehensive analysis of lipid molecules, "lipidomics," in the context of genomics and proteomics is crucial to understanding cellular physiology and pathology; consequently, lipid biology has become a major research target of the postgenomic revolution and systems biology. To facilitate international communication about lipids, a comprehensive classification of lipids with a common platform that is compatible with informatics requirements has been developed to deal with the massive amounts of data that will be generated by our lipid community. As an initial step in this development, we divide lipids into eight categories (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides) containing distinct classes and subclasses of molecules, devise a common manner of representing the chemical structures of individual lipids and their derivatives, and provide a 12 digit identifier for each unique lipid molecule. The lipid classification scheme is chemically based and driven by the distinct hydrophobic and hydrophilic elements that compose the lipid. This structured vocabulary will facilitate the systematization of lipid biology and enable the cataloging of lipids and their properties in a way that is compatible with other macromolecular databases.—**Fahy, E., S. Subramaniam, H. A. Brown, C. K. Glass, A. H. Merrill, Jr., R. C. Murphy, C. R. H. Raetz, D. W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F. Spener, G. van Meer, M. S. VanNieuwenhze, S. H. White, J. L. Witztum, and E. A. Dennis. **A comprehensive classification system for lipids. *J. Lipid Res.* 2005.** 46: **839–861.**

Supplementary key words lipidomics • informatics • nomenclature • chemical representation • fatty acyls • glycerolipids • glycerophospholipids • sphingolipids • sterol lipids • prenol lipids • saccharolipids • polyketides

The goal of collecting data on lipids using a "systems biology" approach to lipidomics requires the development of a comprehensive classification, nomenclature, and chemical representation system to accommodate the myriad lipids that exist in nature. Lipids have been loosely defined as biological substances that are generally hydrophobic in nature and in many cases soluble in organic solvents (1). These chemical properties cover a broad range of mole-

Manuscript received 22 December 2004 and in revised form 4 February 2005.
Published, JLR Papers in Press, February 16, 2005.
DOI 10.1194/jlr.E400004-JLR200

[1] The evaluation of this manuscript was handled by the former Editor-in-Chief Trudy Forte.

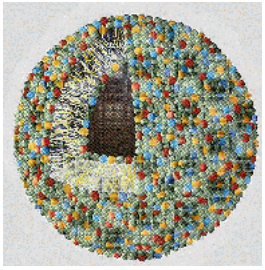
[2] To whom correspondence should be addressed.
e-mail: edennis@ucsd.edu

LipidMaps classification system

J. Lipid Res., **2005**, *46*, 839-872

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

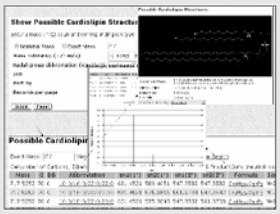
Websites/program to watch:

www.lipidmaps.org
LIPID Metabolites And Pathways Strategy

also:
www.cyberlipid.org

LIPID MAPS *Highlights*

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 (+/- m/z), radyl group abbreviation, headgroup, and/or ion), with links to structure and isotopic distribution.

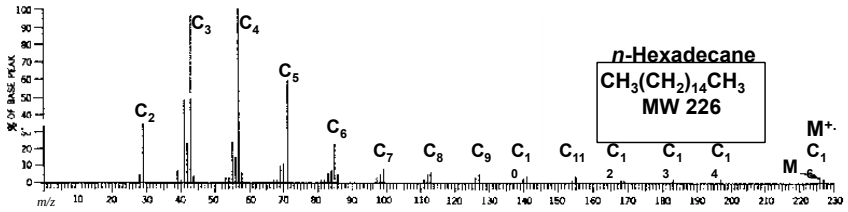
Current search categories include:

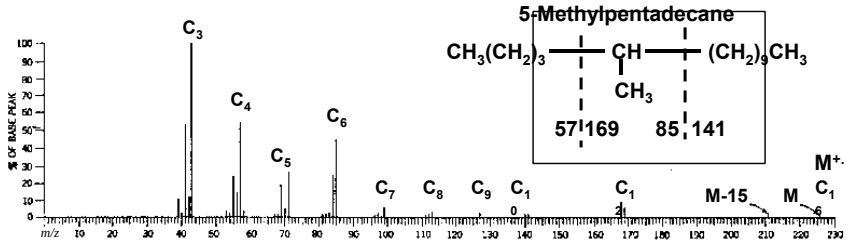
- Cardiolipins (Search by mass, mass tolerance (+/- m/z), radyl group abbreviation, and/or ion.)
- Glycerophospholipids (Search by mass, mass tolerance (+/- m/z), radyl group abbreviation, headgroup, and/or ion.)
- Mono/Di/Triacylglycerols (Search by mass, mass tolerance (+/- m/z), radyl group abbreviation,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

LipidMaps tools for mass spectrometry

www.lipidmaps.org

EI MS of isomeric C14 hydrocarbons





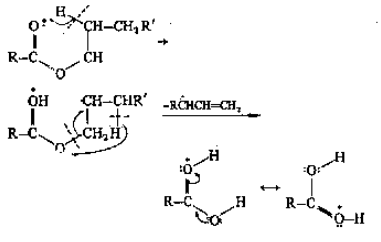
EI MS of methyl caprylate

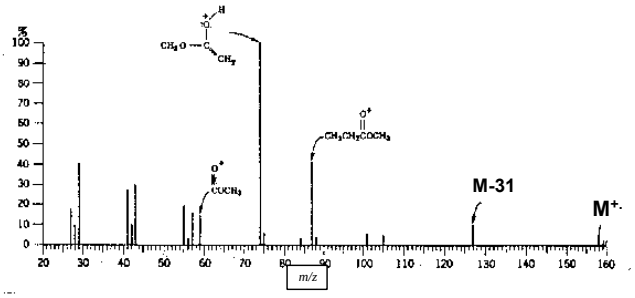
Methyl caprylate

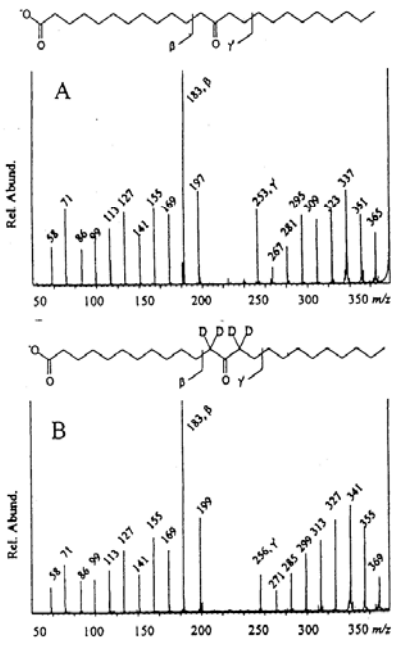
$CH_3(CH_2)_6COOCH_3$

MW 158

***m/z* 158 (M)**	**100.00**
***m/z* 159 (M+1)**	**12.90**
***m/z* 160 (M+2)**	**1.00**

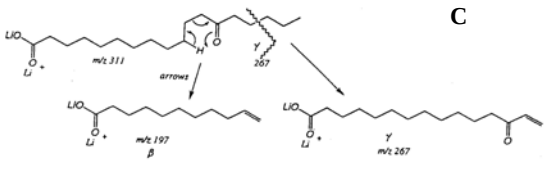


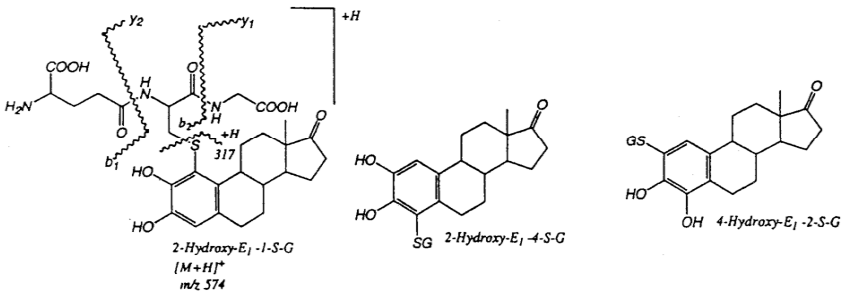




CID MS of $[M-H]^-$, carboxylate anions derived from (A) 13-oxotetracosanoic acid (*m/z* 381) and (B) 13-oxotetracosanoic acid-12,12,14,14-d_4 (*m/z* 385)

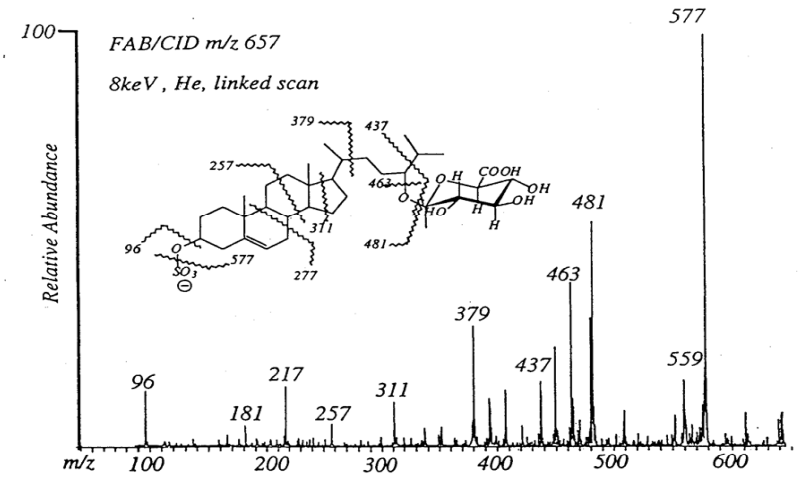
(C) Fragmentation of $[M(Li)+Li]^+$ of 13-oxo-octadecanoic acid (*m/z* 310)





High- and low-energy CID MS fragmentation of the $[M+H]^+$ *m/z* 574 in the positive-ion ESI mass spectra of the glutathione conjugates of estrone and estradiol

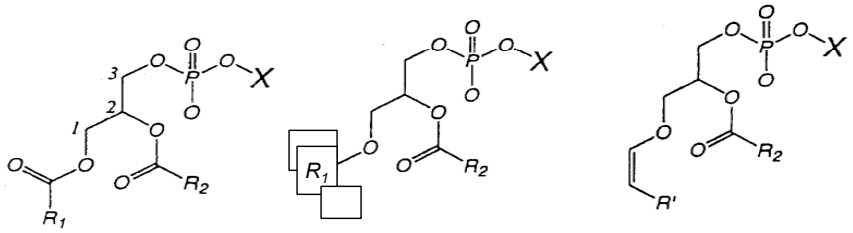
Ramanathan *et al*., *JASMS*, **9**, 612 (1998)



High-energy CID fragmentation of $[M-H]^-$ *m/z* 657 in the negative-ion FAB mass spectrum of sulfonated and glucuronidated double conjugate of 24-hydroxy cholesterol

Meng *et al*., *J Lipid Res.*, **38**, 926 (1997)

Glycerophospholipids

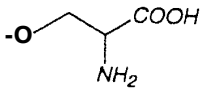


1,2-diacyl 1-*O*-alkyl,2-acyl plasmalogens

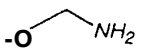
R_1, R_2, R' = alkyl chain; lysophospholipid 2-OH

Mono-, di- and triacylglycerides

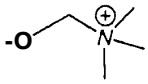
Glycerophospholipid polar head groups (X)



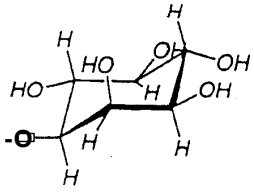
glycerophosphoserine (GPS)



glycerophosphoethanolamine (GPE)



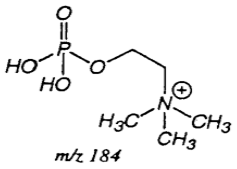
glycerophosphocholine (GPC)



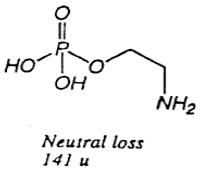
glycerophosphoinositol (GPI)

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

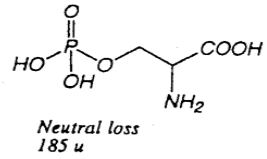
GPC



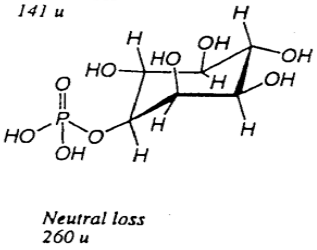
GPE



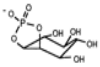
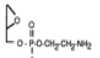
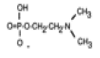
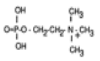
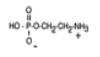
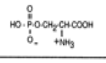
GPS



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 glycerophospho-lipids	neg.		precursor of 153	glycerol-phosphate -H_2O	+50 V
phosphatidyl-inositol	neg.		precursor of 241	head group -H_2O	+45 V
phosphatidyl-serine	neg.		neutral loss of 87	head group -H_3PO_4	+28 V
phosphatidyl-ethanolamine	neg.		precursor of 196	dilyso -H_2O	+50 V
sphingomyelin	neg.		sCID + precursor of 168	head group (demethyl-ated)	sCID +65 & +40 V
phosphatidyl-choline and sphingomyelin	pos.		precursor of 184	head group	-35 V
phosphatidyl-ethanolamine	pos.		neutral loss of 141	head group	-25 V
phosphatidyl-serine	pos.		neutral loss of 185	head group	-22 V

Instrument: Finnigan-MAT TSQ 7000 triple quadrupole MS

Nanospray MS

MeOH/$CHCl_3$ 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

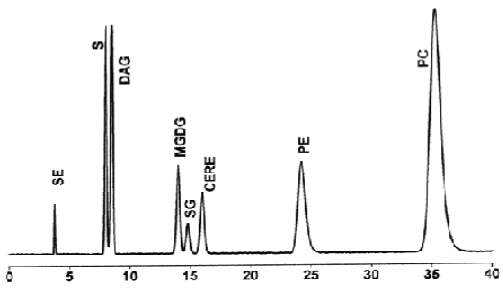
Why LC?

- 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, F. K. Welty, and C. E. 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 µm 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 W.W.Christie *et al.* (1995). *J High Resol Chromatogr* **18**, 97.)

Lipid Quantification

Ion intensity depends on:

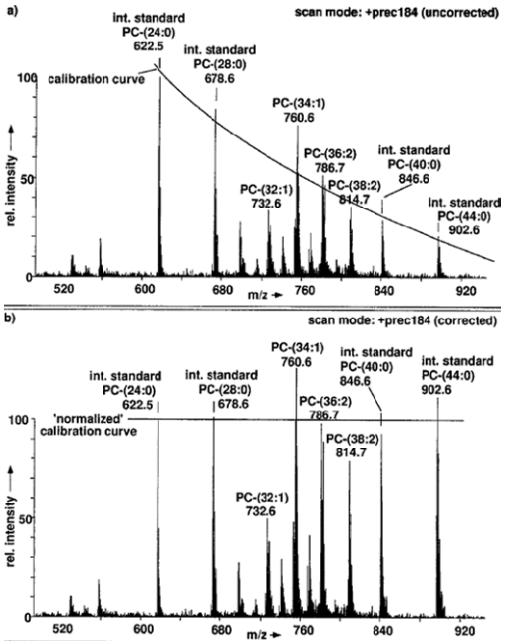
-Ionization efficiency of head groups

-Fatty acid length and saturation

-Suppression

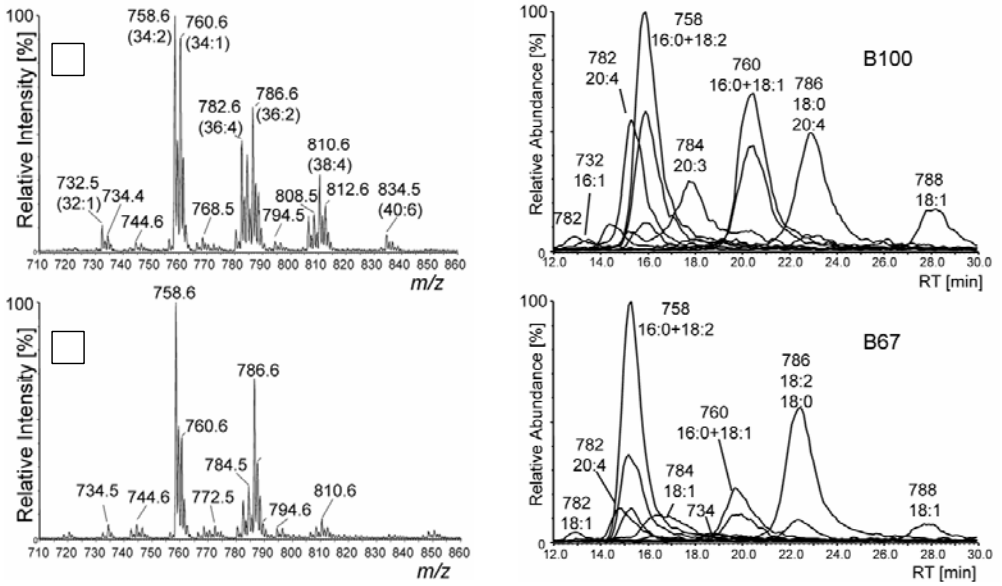
-Solvent composition (small changes)

-Instrument settings (actual settings can vary from day to day!)



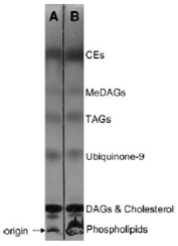
B Brügger, G Erben, R Sandhoff, FT Wieland, WD Lehmann, *PNAS,* **1997***, 94,* 2339-2344

PC Lipids in B100 (WT) and B67 LDLs



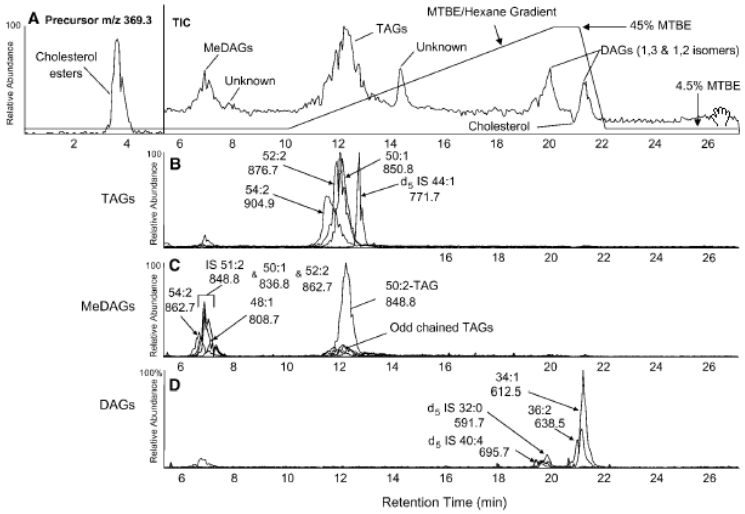
(Sommer *et al.*)

Neutral Lipids in RAW 264.7 cells



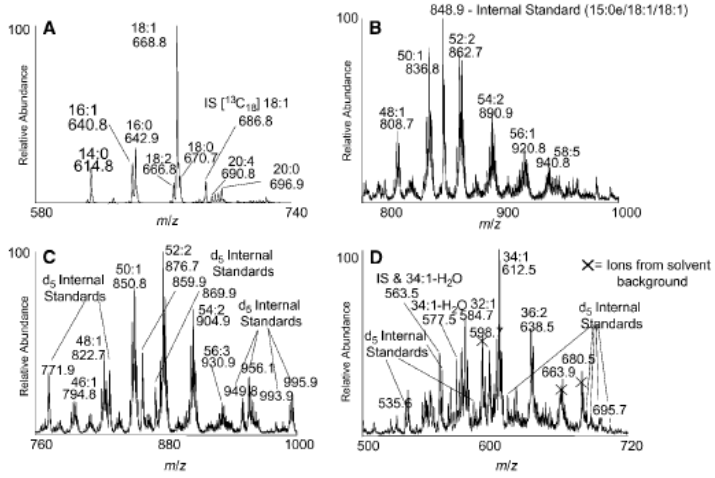
TLC separation of (A) isooctane-EtOAc extract of neutral lipids (B) Bligh-Dyer extract of total lipids

(Hutchins *et al.*)



(A) Normal-phase LC separation and MS detection as precursors of *m/z* 369.3 or as total ion current of neutral lipids from RAW cells. (B)-(D) Overlaid mass chromatograms of selected $[M+NH_4]^+$.

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.*)

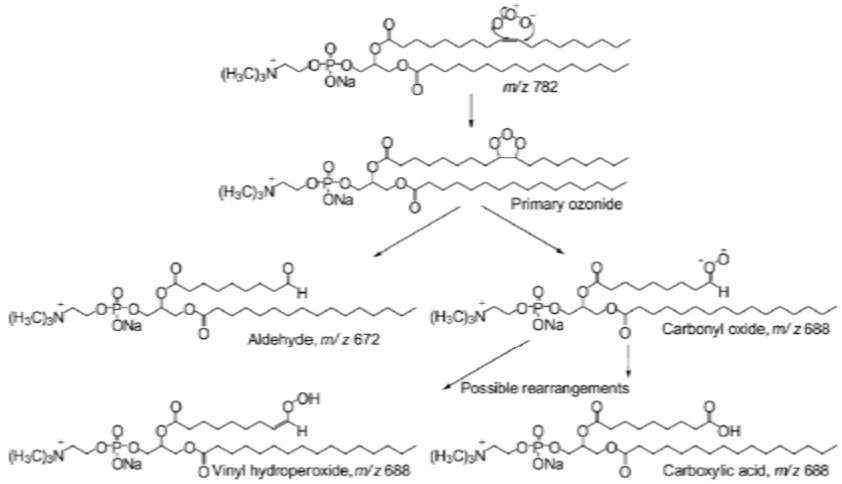
Location of sites of unsaturation in lipids

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 5. The proposed mechanism for the gas phase reaction of mass-selected [PC(16:0/18:1(9Z)) + Na]$^+$ 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

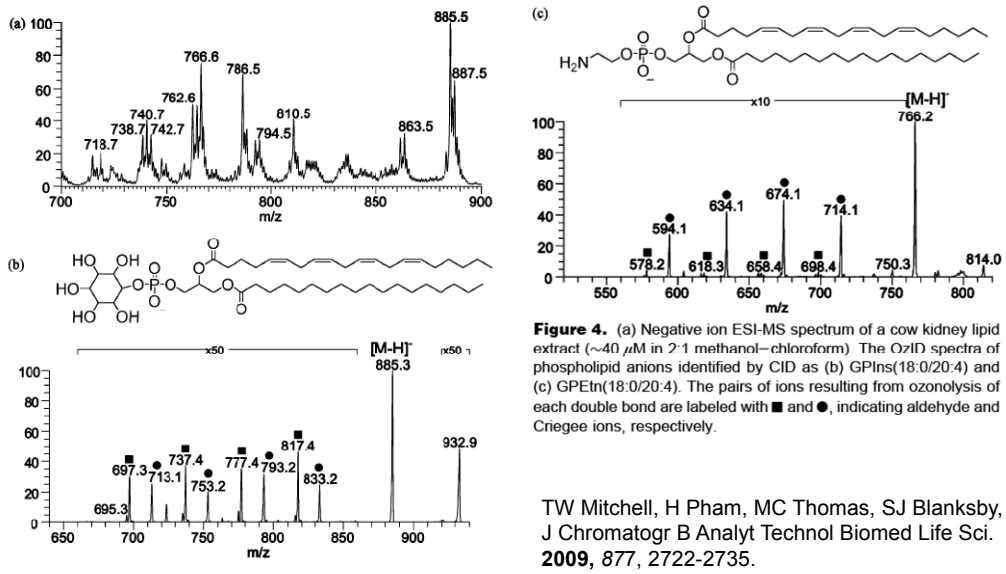
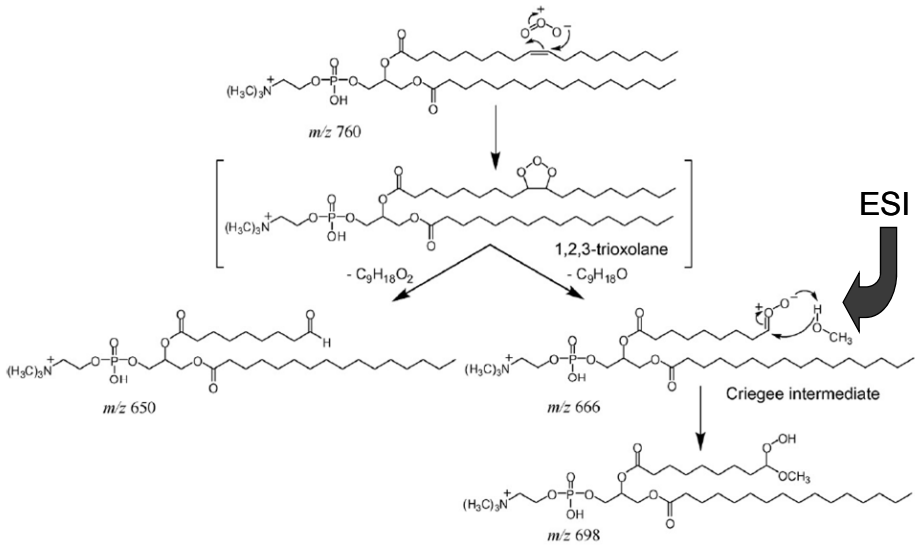


Figure 4. (a) Negative ion ESI-MS spectrum of a cow kidney lipid extract (~40 μM in 2:1 methanol–chloroform). The OzID spectra of phospholipid anions identified by CID as (b) GPIns(18:0/20:4) and (c) GPEtn(18:0/20:4). The pairs of ions resulting from ozonolysis of each double bond are labeled with ■ and ●, indicating aldehyde and Criegee ions, respectively.

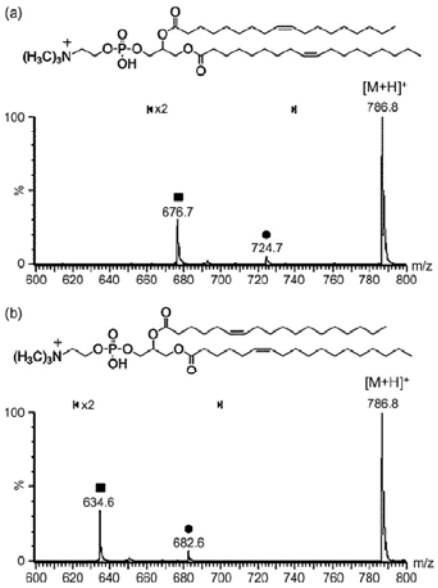
TW Mitchell, H Pham, MC Thomas, SJ Blanksby, J Chromatogr B Analyt Technol Biomed Life Sci. **2009,** *877*, 2722-2735.

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.

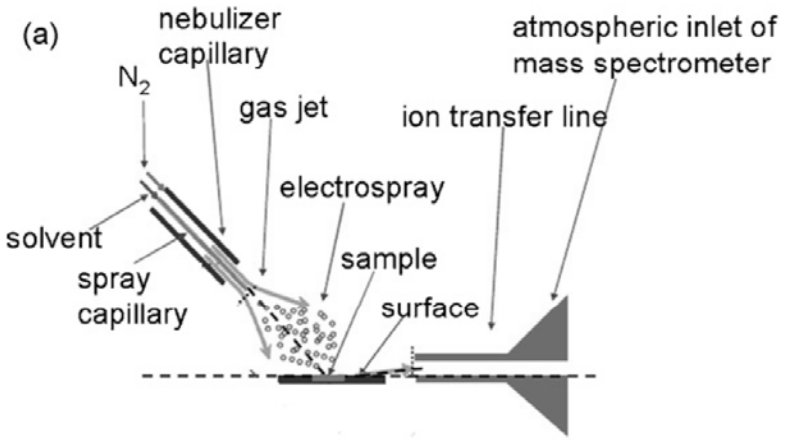
Lipids analyzed by DESI directly from samples

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.

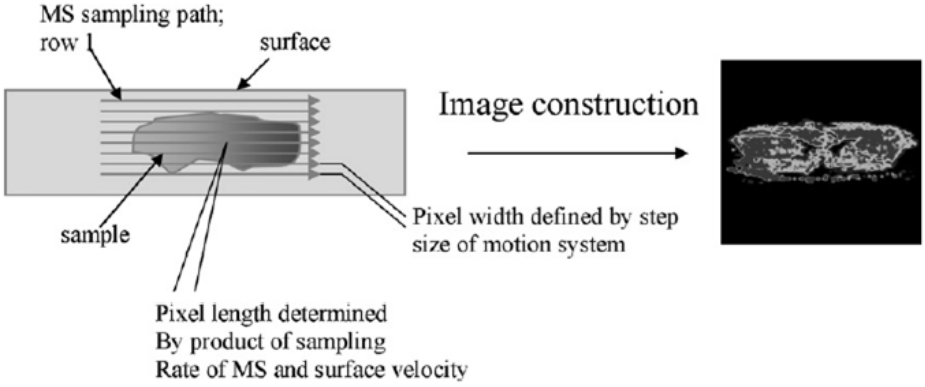
courtesy of R. G. Cooks, Purdue University

Lipids analyzed by DESI directly from samples



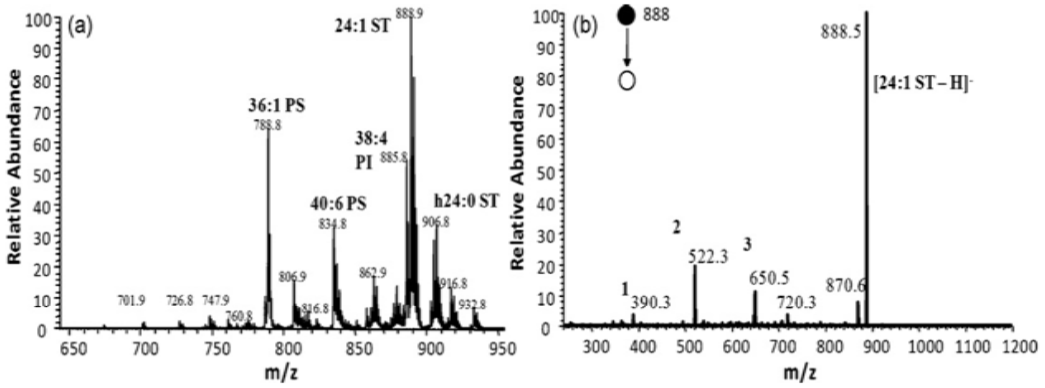
courtesy of R. G. Cooks, Purdue University

Lipids analyzed by DESI directly from samples



courtesy of R. G. Cooks, Purdue University

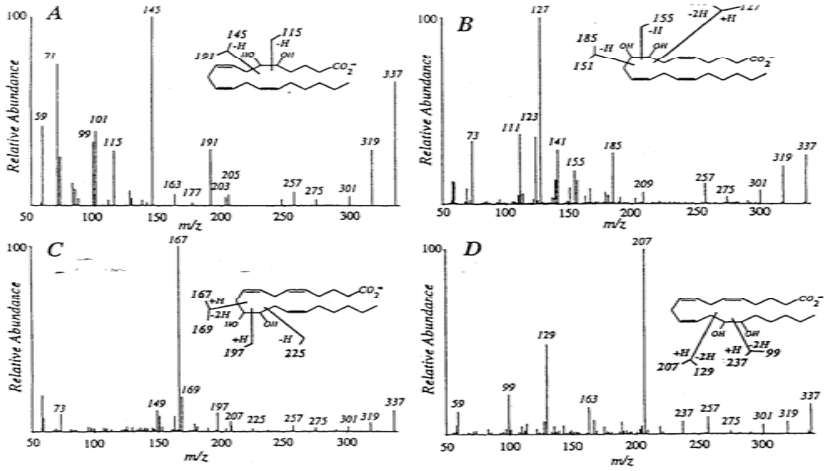
Lipids analyzed by DESI directly from samples



courtesy of R. G. Cooks, Purdue University

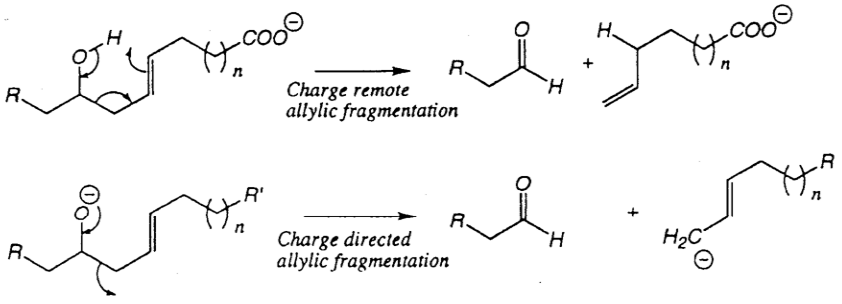
Eicosanoids

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



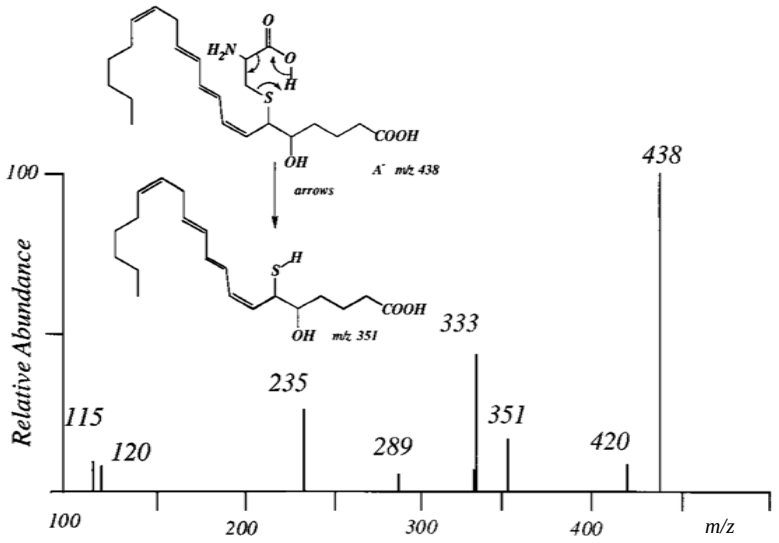
P Wheelan *et al.*, *JASMS*, **1996**, *7*, 140

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



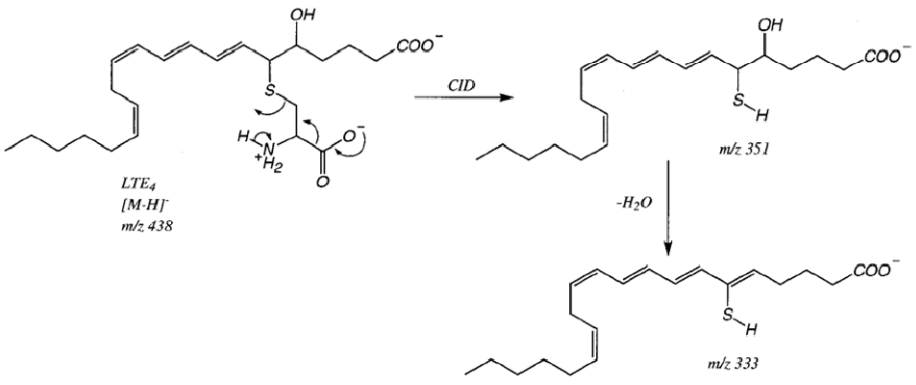
P Wheelan *et al.*, *JASMS*, **1996**, ***7*** 140

ESI-CID MS of leukotriene E_4, $[M-H]^-$ *m/z* 438



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

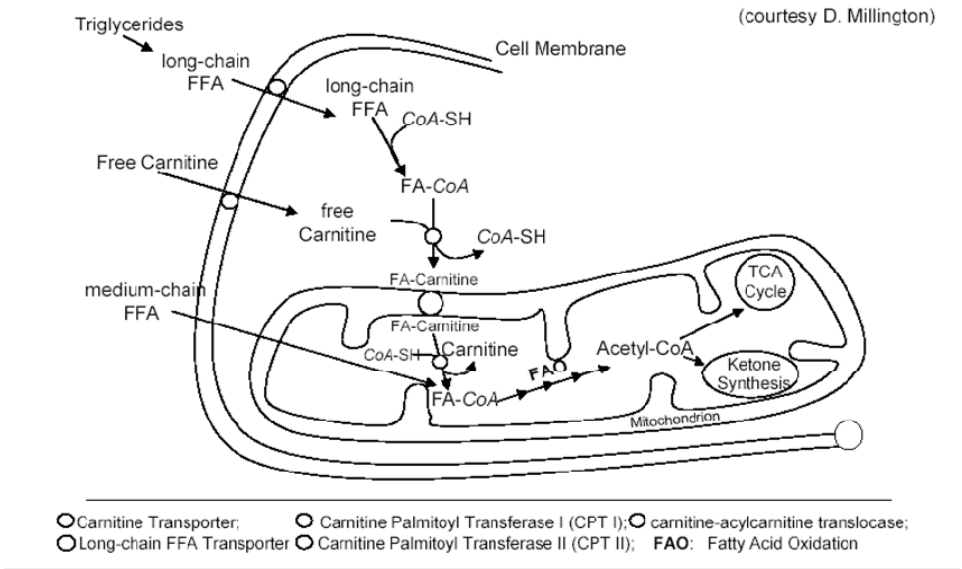
Fragmentation of leukotriene E_4, $[M-H]^-$ *m/z* 438



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)

acylcarnitine = $(H_3C)_3N^+CH_2CH(OR)CH_2COO^-$

$R = CH_3(CH_2)_nCO-$

- **Newborn screening**
  - **for ~30 metabolic disorders**

D. Millington *et al*., Duke Univ. Medical Center

Fatty acid metabolism overview

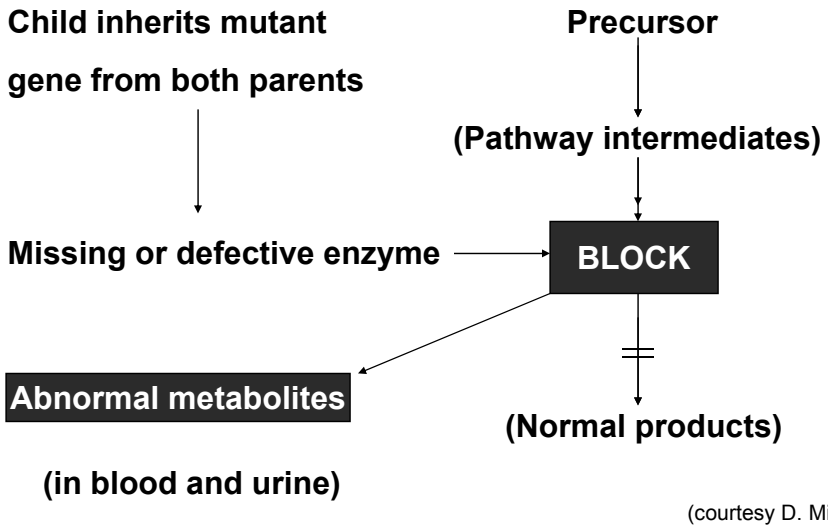
(courtesy D. Millington)

- **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**

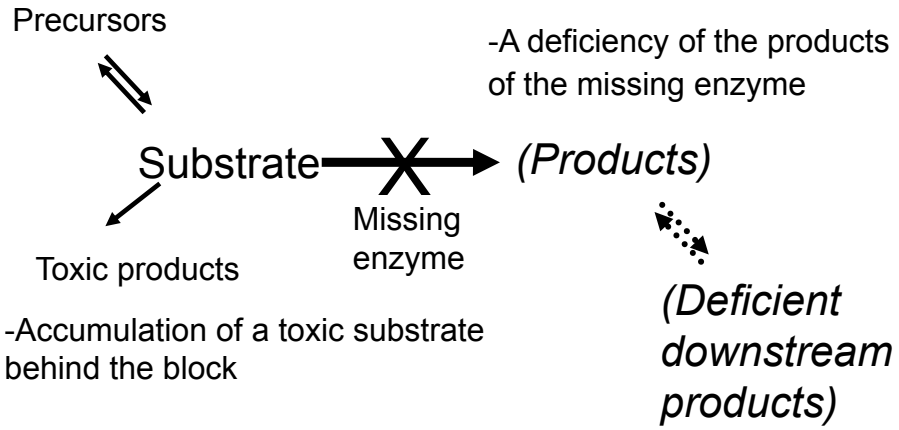
Basis of a recessive metabolic disease



(courtesy D. Millington)

Underlying enzyme deficiency blocks a unique biochemical pathway

(courtesy D. Millington)



-A deficiency of the products of the missing enzyme

-Accumulation of a toxic substrate behind the block

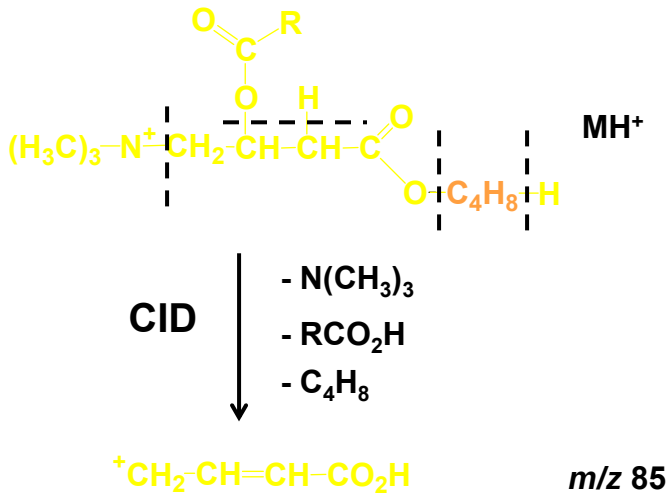
-Effects of the accumulated substrates on other pathways

Diagnostic testing for FOA disorders

(courtesy D. Millington)

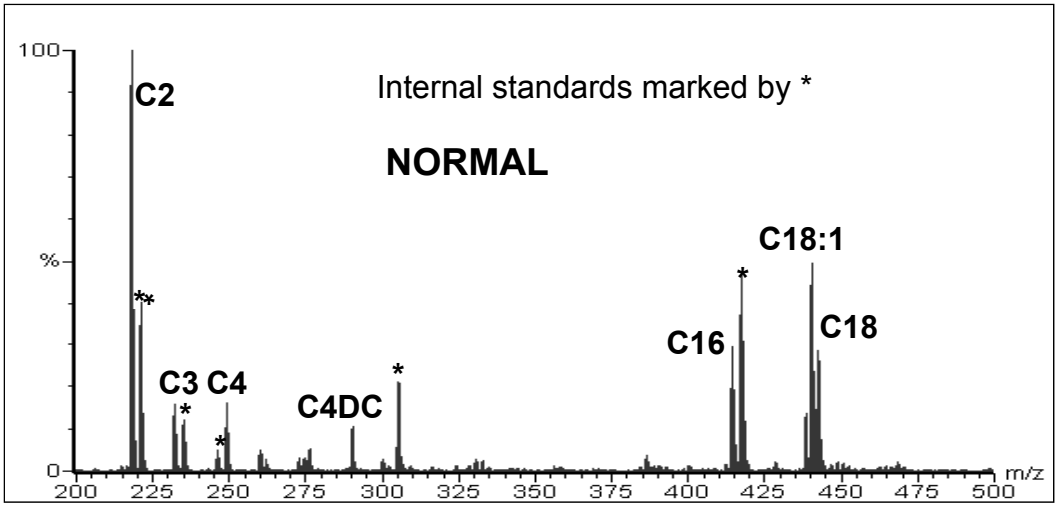
- **The most useful test is the acylcarnitine profile (plasma or whole blood)**
- **Free and total carnitine in plasma should be analyzed at the same time**
- **Urine organic acids analysis is helpful in diagnosis of some FAO disorders**
- ***In vitro* testing (skin fibroblasts) and/or molecular testing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

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



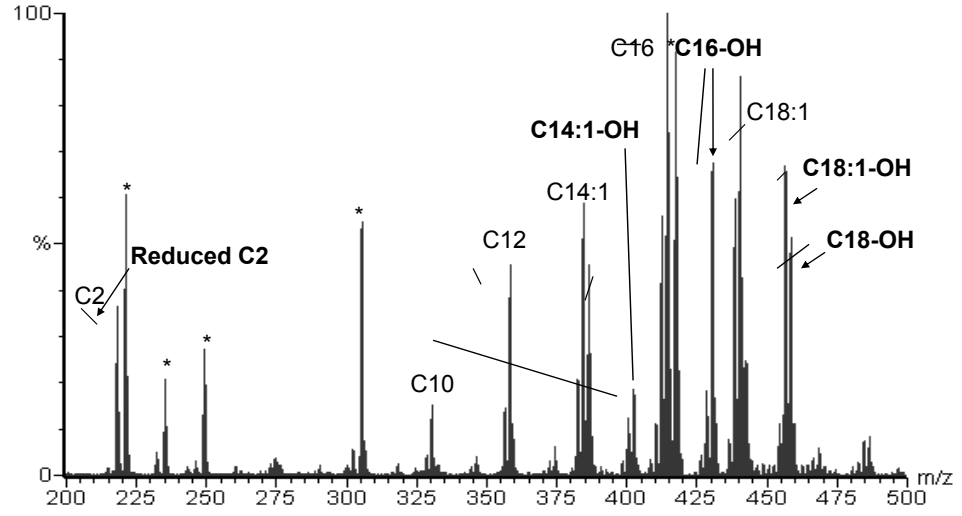
(courtesy D. Millington)

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



(courtesy D. Millington)

LCHAD or TFP deficiency (metabolic stress)



(courtesy D. Millington)

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:

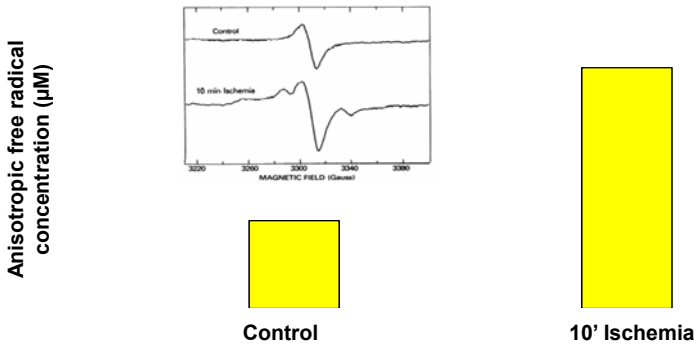
- Fatty acid oxidation disorders 62
  MCADD (48)
- Organic acidemias 37
  3-methylcrotonylglycinuria (13)
- Amino acid disorders 43
  PKU/hyperphenylalaninemia (30)

Total = 142

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

(courtesy D. Millington)

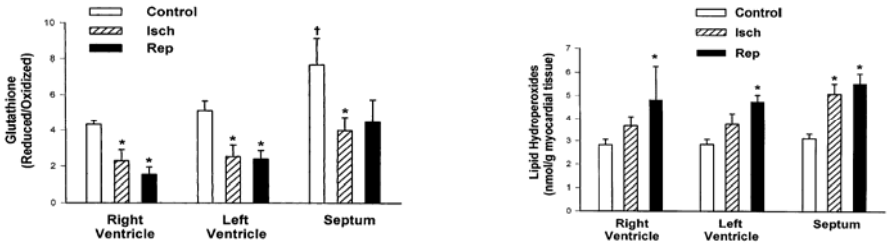
Myocardial ischemia increases free radical generation



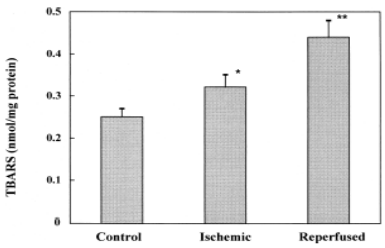
Free radical generation during myocardial ischemia. EPR spectra of control and ischemic heart (inset).

J. L. Zweier *et al*. *Proc Natl Acad Sci USA* **1987**, *84*, 1404-1407

Ischemia and reperfusion cause oxidative stress

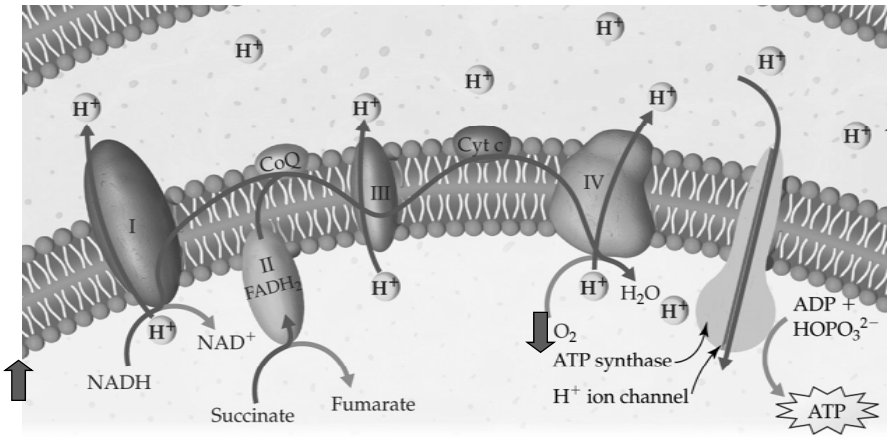


V. Palace, *J Mol Cell Cardiol* 1999 31:193-202



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

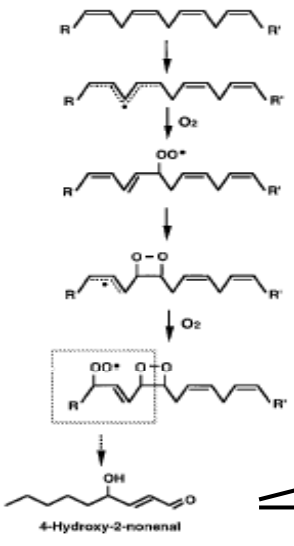
ROS are generated in the heart during ischemia



Residual O_2 —(One-electron reduction)→ $O_2^{\cdot -}$ → **Lipid peroxidation**

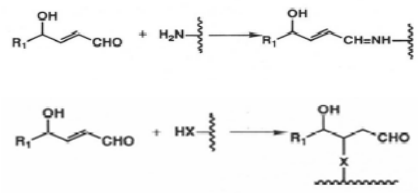
B. G. Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004

Lipid peroxidation generates aldehydes

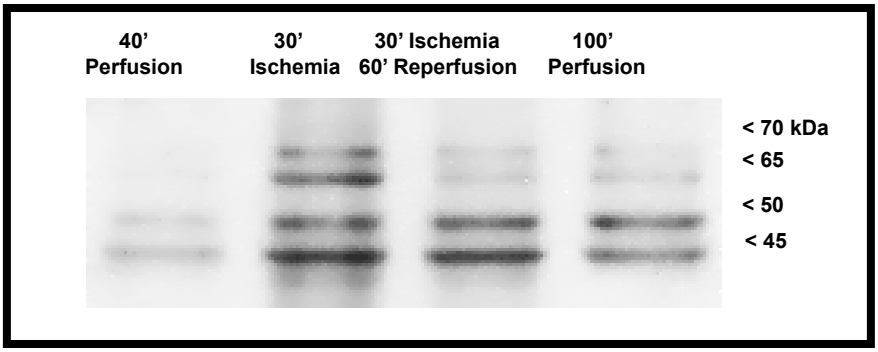


PROPOSED MECHANISMS

- Esterbauer Dioxetane Mechanism
- Hydroperoxy Dihydropyran Mechanism
- Epoxy Hydroperoxide Mechanism
- Hydroxyhydroperoxide Mechanims
- Enzymatic Pathway
- Peroxycyclization-Dioxetane Fragmentation



Global ischemia induces HNE modification of proteins in the heart



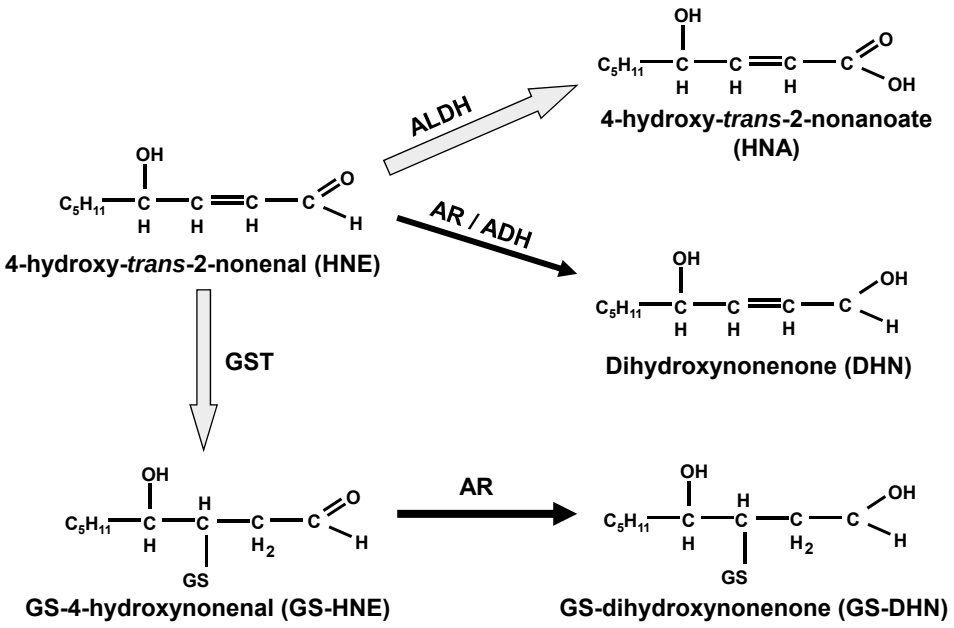
Myocardial proteins were separated by SDS-PAGE followed by Western blot (WB) analysis with anti-protein-HNE antibodies.

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

B. G. Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004

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

B. G. Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004



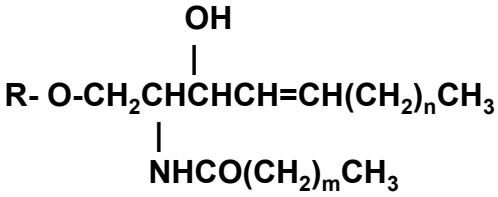
B. G. Hill, BUSM OPTM in Cardiovascular Disease Symposium, October 2004

Glycoconjugates

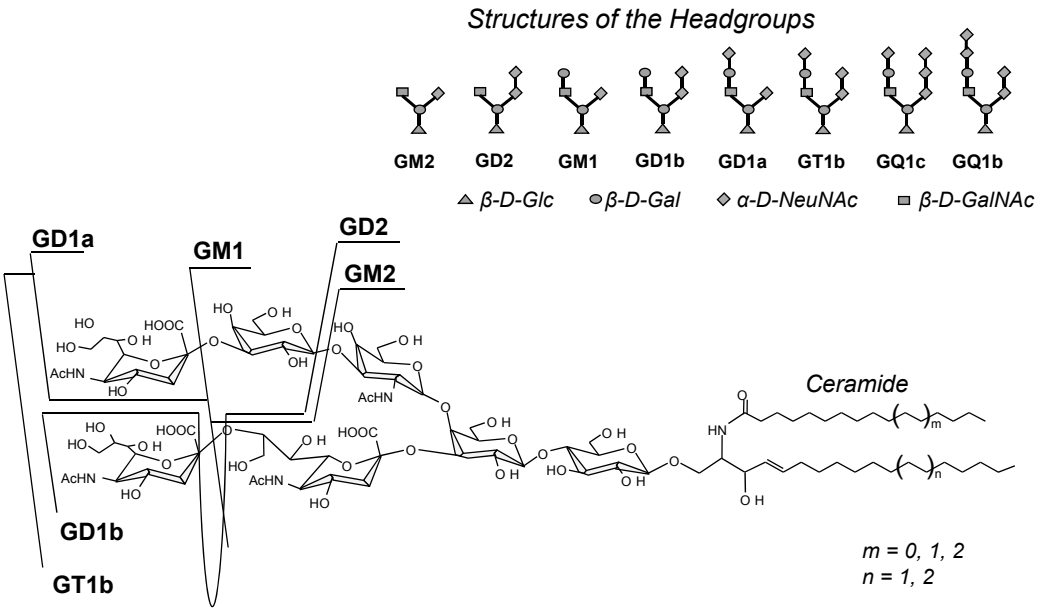
Information available from MS analysis of glycoconjugates

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

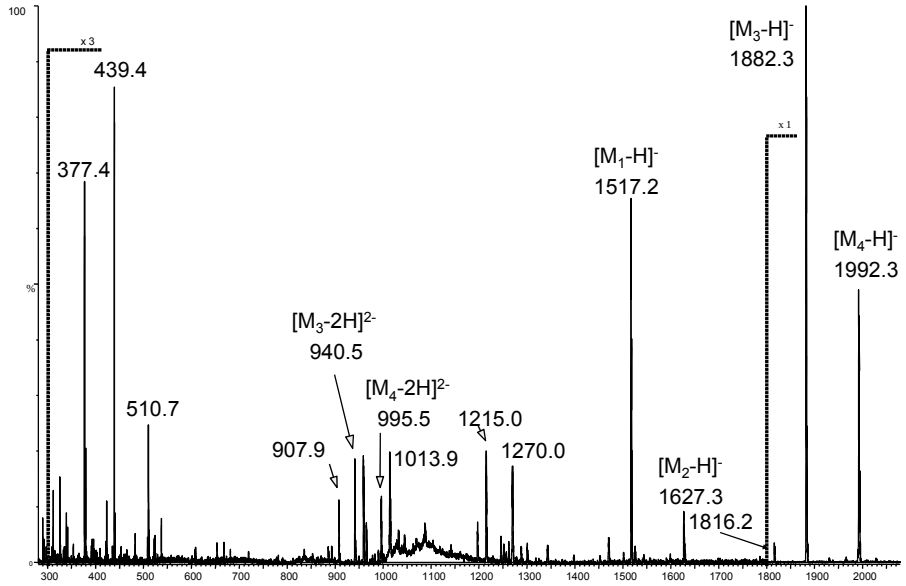
Sphingolipids and Glycosphingolipids



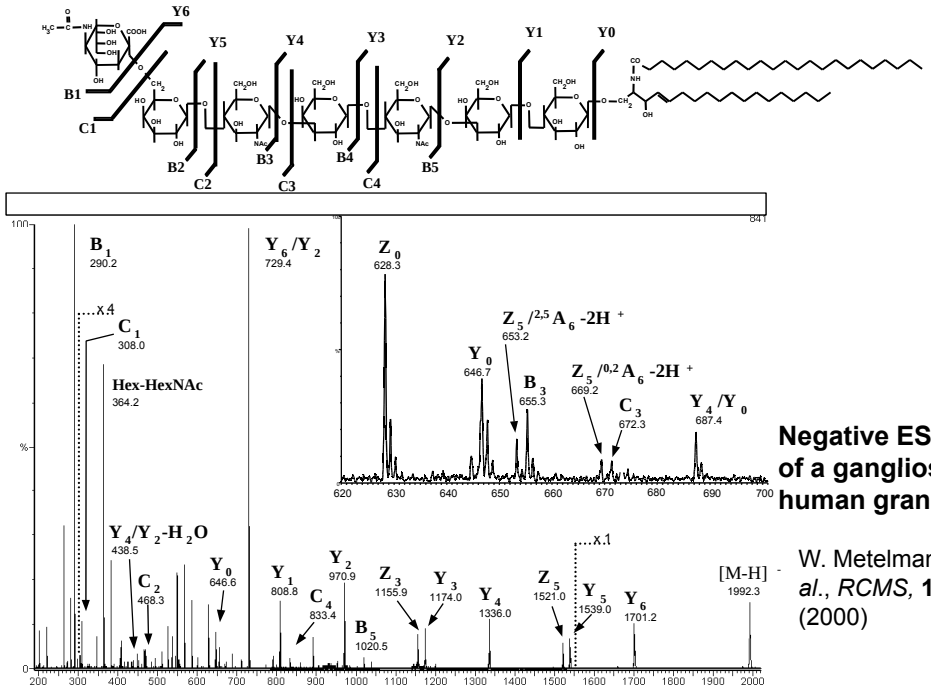
Structures of the gangliosides



Q-oTOF ESI-MS of gangliosides from human granulocytes



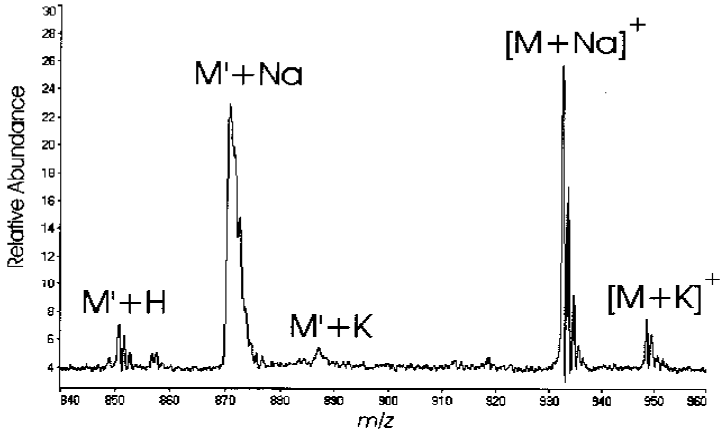
W. Metelmann, J. Müthing and J. Peter-Katalinic, *RCMS,* **14**, 543 (2000)



Negative ESI-MS/MS of a ganglioside from human granulocytes

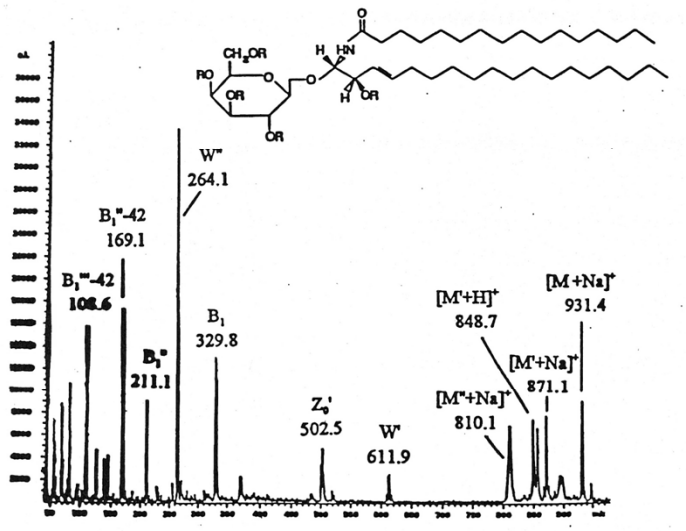
W. Metelmann, *et al*., *RCMS,* **14**, 543 (2000)

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

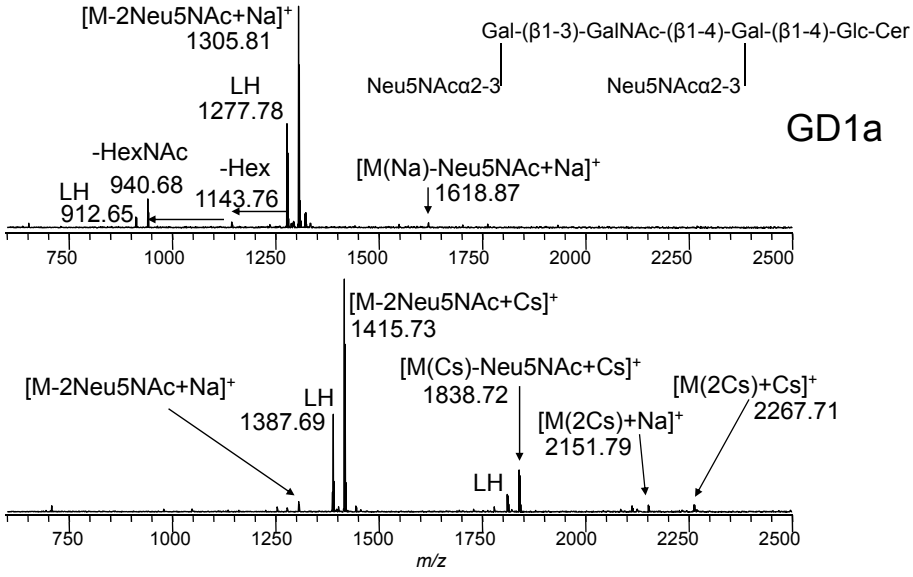
C. E. Costello, unpublished data



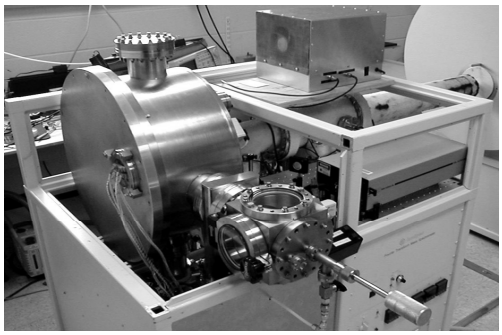
MALDI-PSD mass spectrum of peracetylated *N*-palmitoyl galactosyl sphingenene, $[M+Na]^+$ *m/z* 932.6

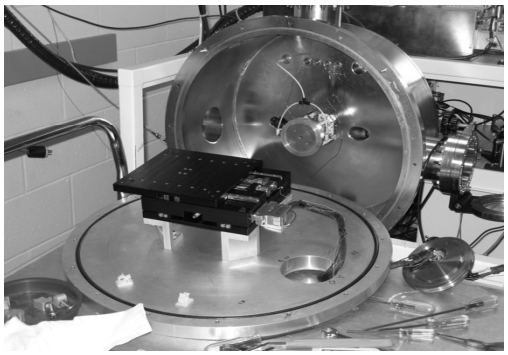
M = neutral molecular mass, M' = M-HOAc

C. E. Costello and J. E. Vath unpublished data

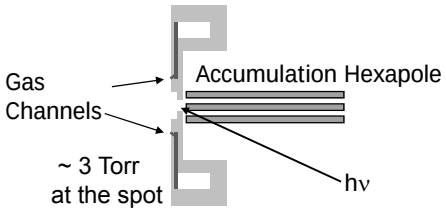


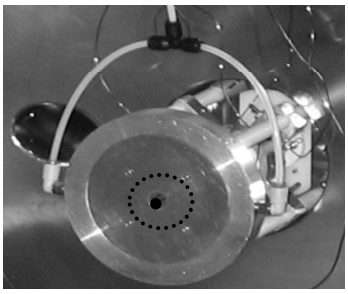
Low pressure positive-ion MALDI-FTMS spectra of GD_{1a} and Na, Cs





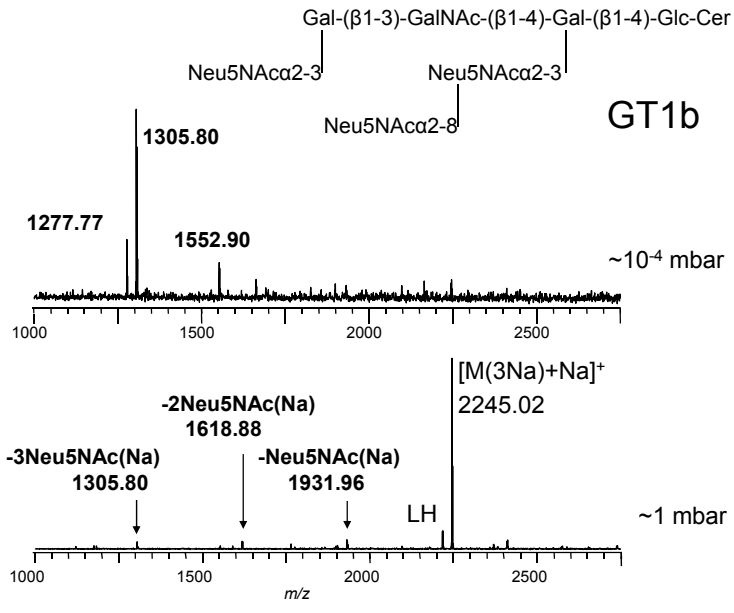
Vibrational Cooling MALDI-FTMS





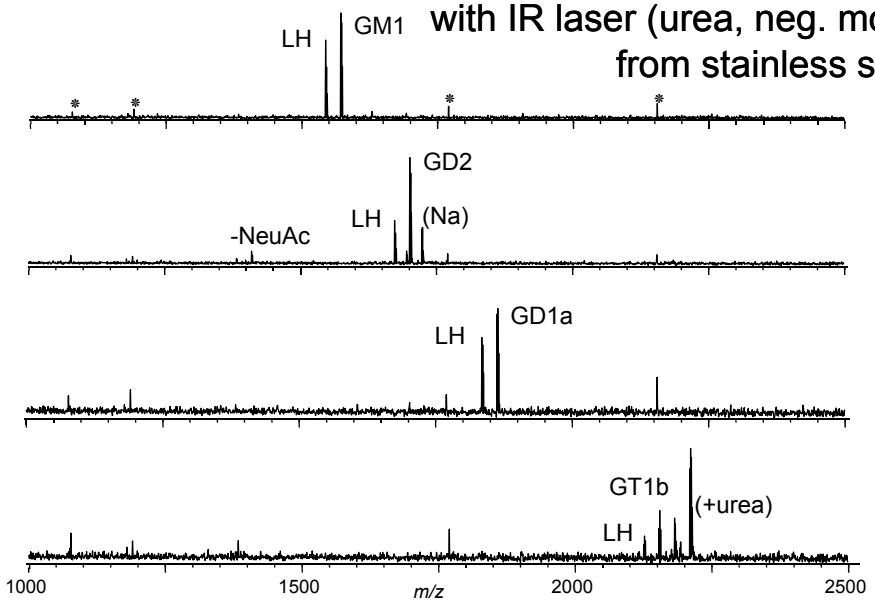
Ganglioside Analysis

VC MALDI FTMS allows intact desorption.



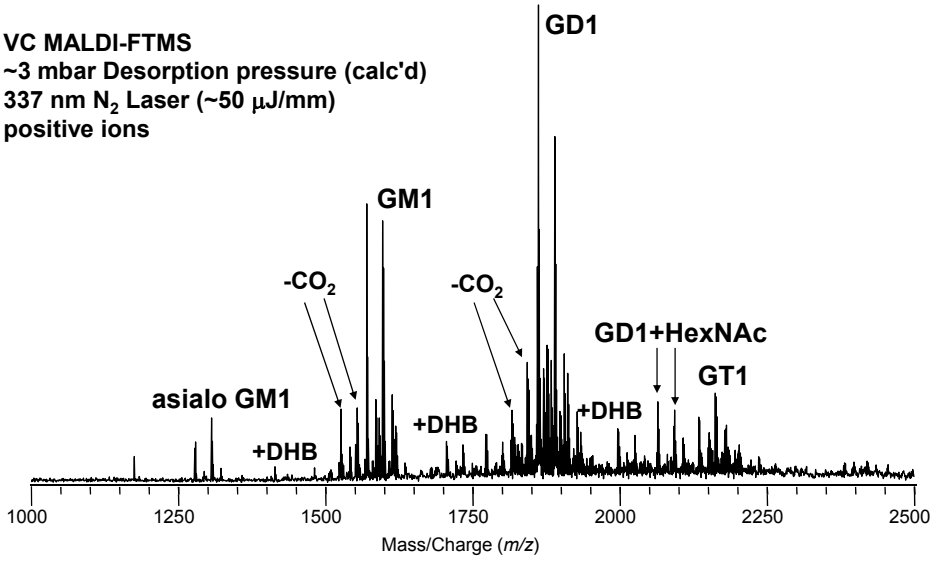
PB O'Connor and CE Costello, *RCMS*, **2001**,*15*, 1862
PB O'Connor *et al*., *JASMS*, **2002**,*13*, 402

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

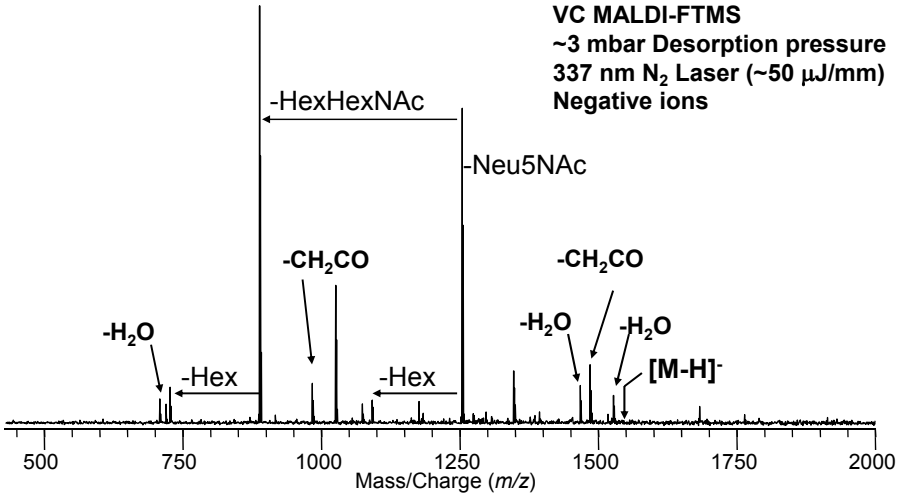


V. Ivleva *et al.*, *Anal. Chem.* **2004**. *76*, 6484.

Bovine whole brain gangliosides



MS/MS of GM1 from WBG mixture



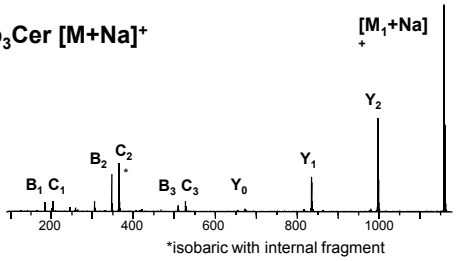
Challenges of MALDI MS from surfaces

- 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

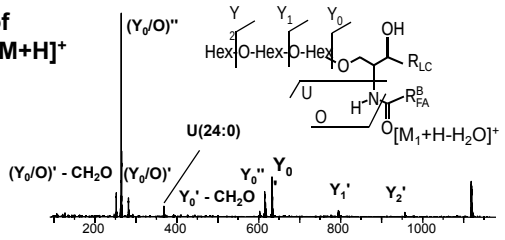
Desorption from steel
⇒ Sodiated Ions
= <u>oligosaccharide</u> cleavages

MS/MS of Gb_3Cer $[M+Na]^+$

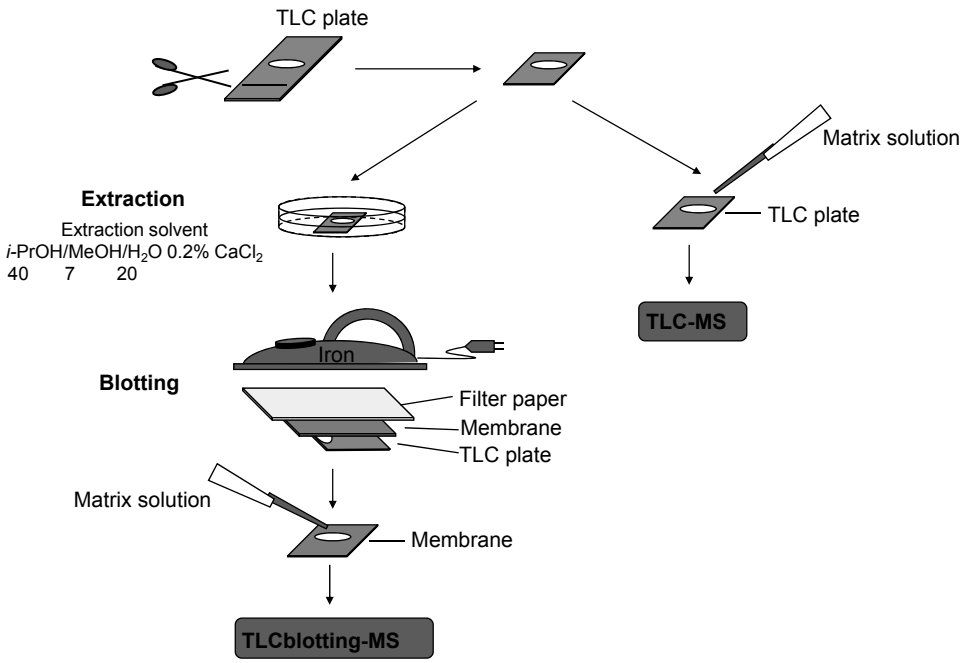


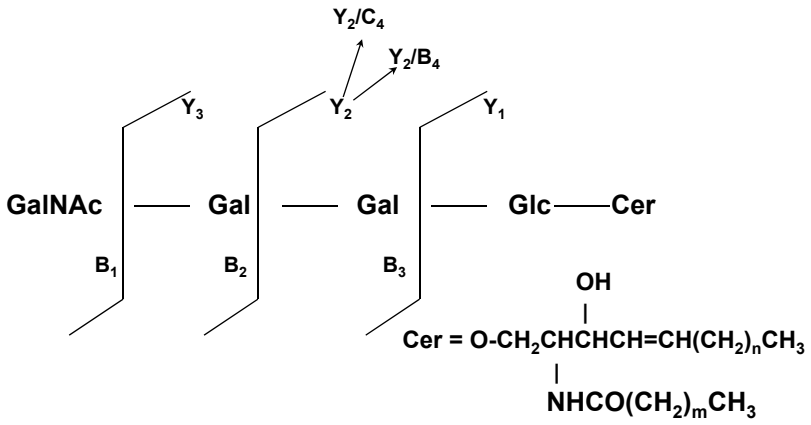
Desorption from Nafion™
⇒ Protonated Ions
= <u>lipid</u> cleavages

MS/MS of Gb_3Cer $[M+H]^+$

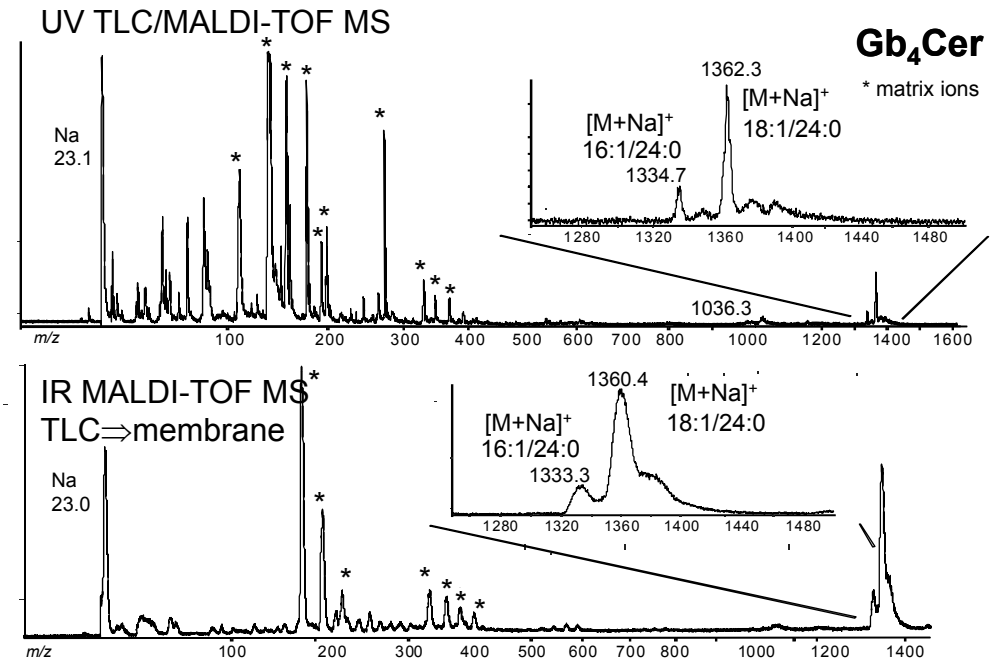


M. E. McComb *et al*., *ASMS* **2002**

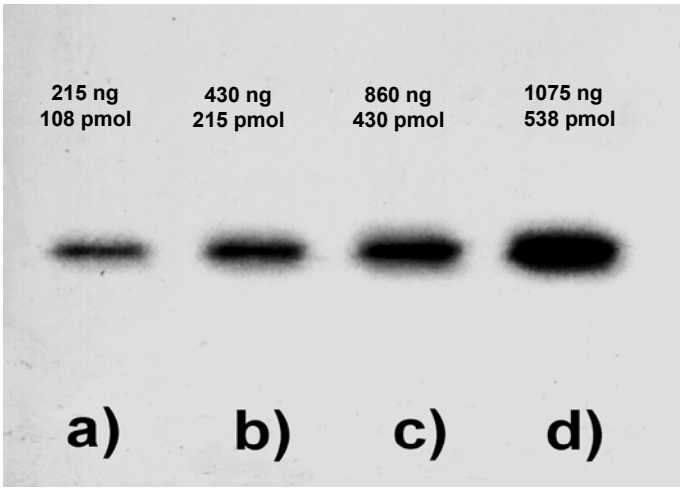




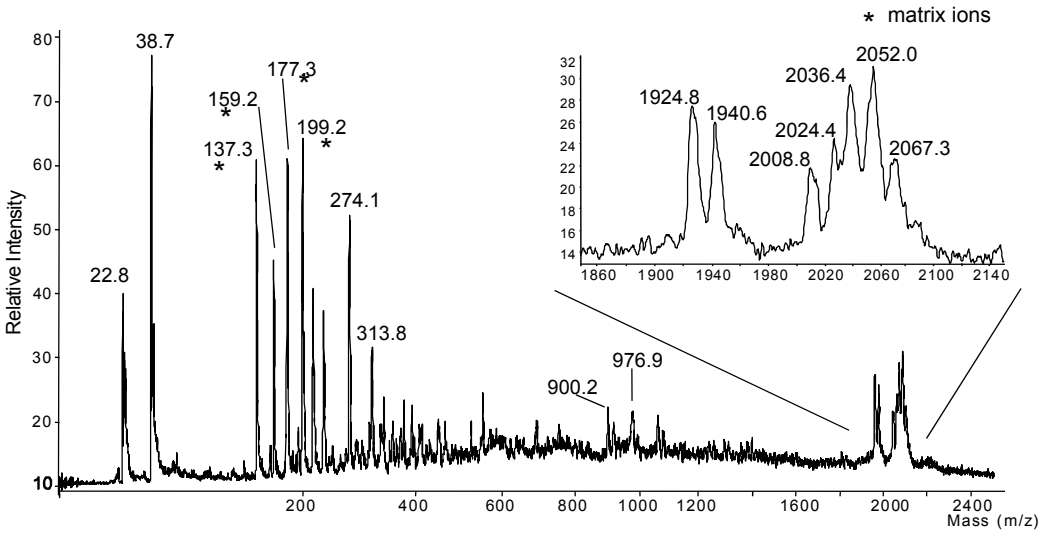
Globotetraosyl ceramide, Gb_4Cer



J. Guittard, X. L. Hronowski and C. E. Costello, *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1838-1849.

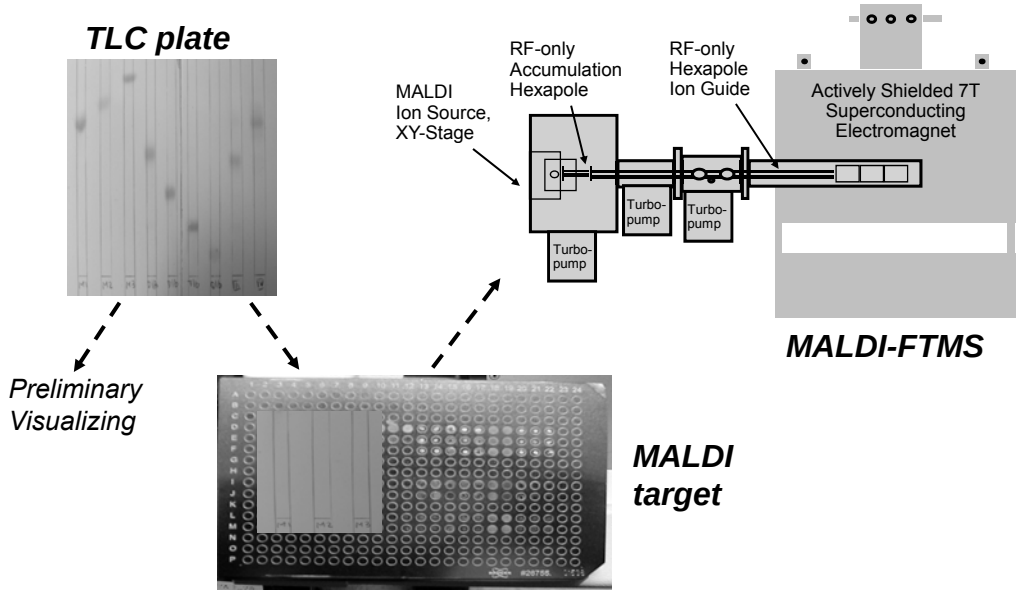


Immunostained TLCs of mouse kidney glycolipids reacted with the 7A antibody (reactive with the Lewis^x determinant)

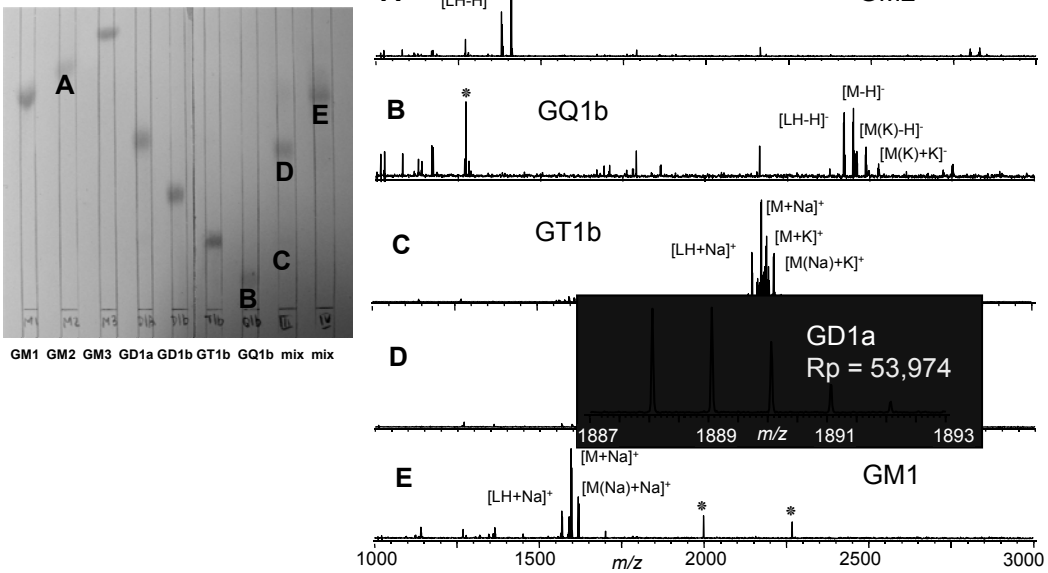


TLCblotting MALDI-TOF mass spectrum (DE, linear) of track with 215 pmol mouse kidney glycolipids; 2,5-DHB matrix, PVDF P membrane

Coupling VC MALDI FTMS with TLC

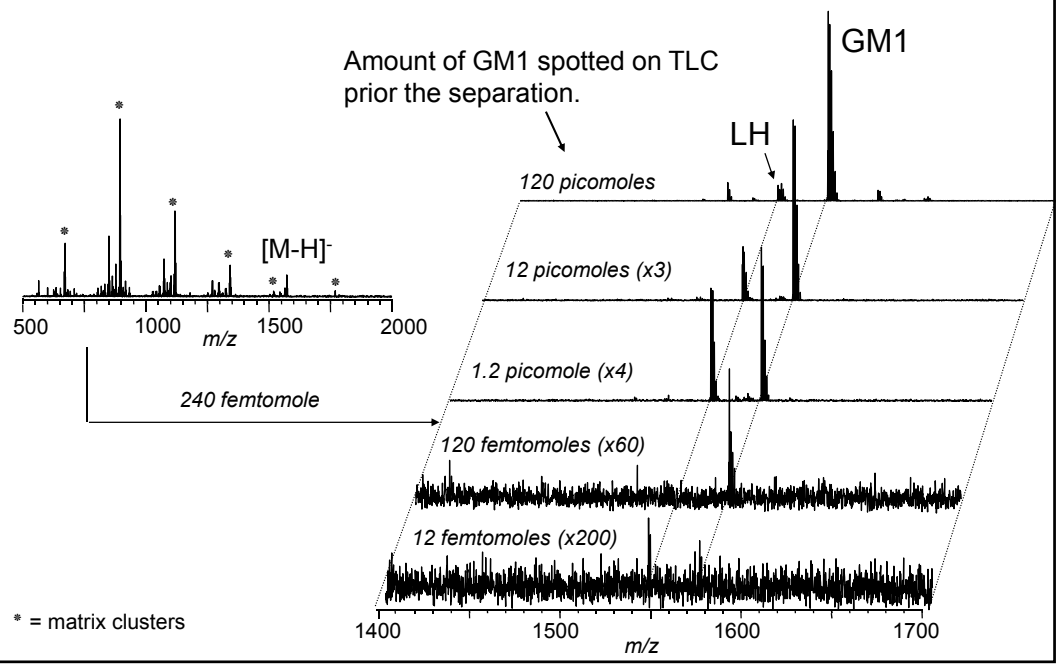


VC MALDI FTMS of TLC-separated gangliosides



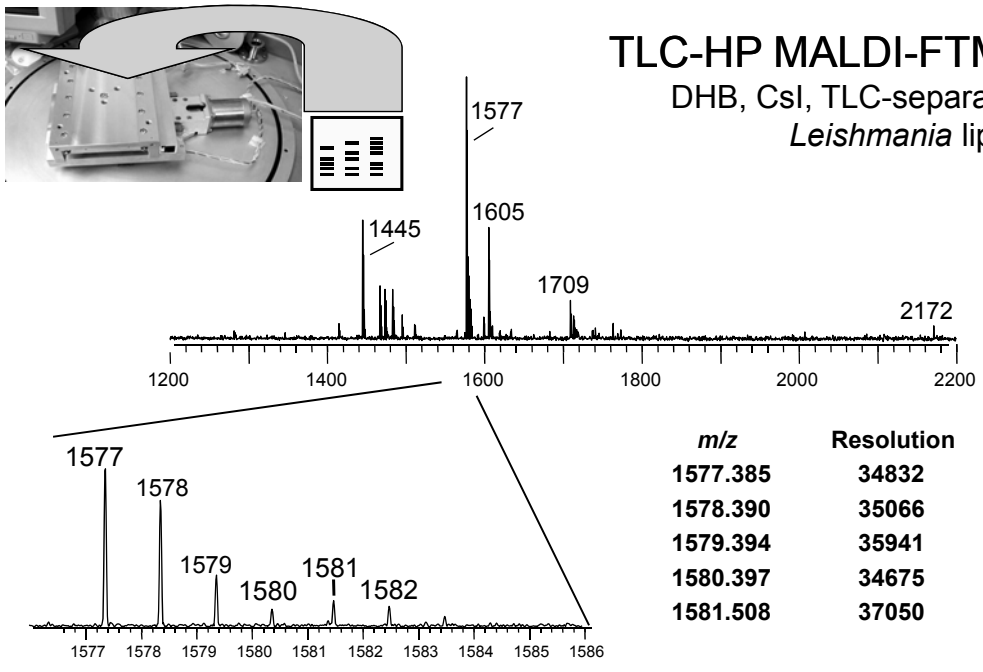
V. Ivleva *et al., Anal. Chem.*, **2004**, *76*, 6484.

Detection limit of TLC-VC MALDI-FTMS



TLC-HP MALDI-FTMS

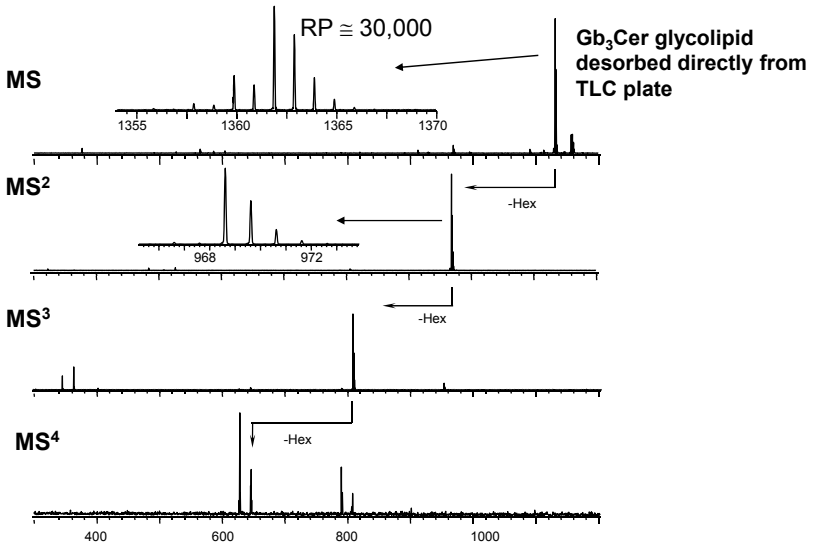
DHB, CsI, TLC-separated
Leishmania lipids



m/z	Resolution
1577.385	**34832**
1578.390	**35066**
1579.394	**35941**
1580.397	**34675**
1581.508	**37050**

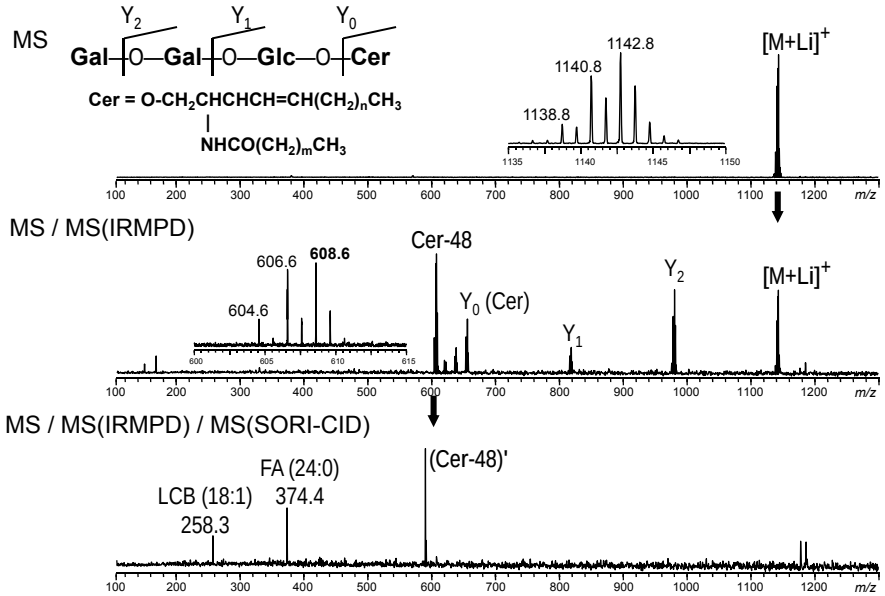
C. E. Costello, V. Ivleva, U. Sommer, D. McMahon-Pratt, P. B. O'Connor, Desorption 2002

MALDI-FTMS (high resolution, MS^n) directly from TLC plates



E. Mirgorodskaya *et al*., ASMS 2001

Combination of IRMPD & SORI-CID



E. Mirgorodskaya *et al*., ASMS 2001

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 ⇒ an increasing range of options ⇒ ⇒ Lipidomics, Glycomics!

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

References

- A. L. Dill *et al., J Chromatogr B Analyt Technol Biomed Life Sci.* 2008 Dec 31. [Epub]
- E. Fahy *et al., J. Lipid Res*., **46**, 839. (2005)
- X. Han and R. W. Gross, *J. Lipid Res*., **44**, 1071 (2003)
- P. M. Hutchins *et al., J. Lipid Res*., **49**, 804 (2008)
- V. Ivleva *et al*., *Anal. Chem*., **76**, 6484 (2004)
- R. A. Johanson *et al*., *Anal. Biochem*. **362**, 155 (2007)
- W. Metelmann *et al*., *Rapid Commun. Mass Spectrom*., **14**, 543 (2000)
- S. Milne *et al*., *Methods*, **39**, 92 (2007)
- T. W. Mitchell *et al,, J Chromatogr B Analyt Technol Biomed Life Sci.* **2009** Jan 21. [Epub]
- R. C. Murphy, *Chem. Rev*. **101**, 479 (2001)
- P. B. O'Connor and C. E. Costello, *J. Am. Soc. Mass Spectrom*., **13**, 402 (2002)
- M. Pulfer and R.C. Murphy, *Mass Spectrom. Rev.,* **22**, 332 (2003)
- U. Sommer *et al*., *J. Lipid Res*., **47**, 804 (2006)
- M. C. Thomas, TW. Mitchell, DG Harman, JM Deeley, JR Nealon, SJ Blanksby, *Anal. Chem.*, **80**, 303 (2008)
- P. Whelan *et al*., *J. Am. Soc. Mass Spectrom*., **7**, 140 (1996)