LC-MS/MS und *Shotgun Lipidomics*:

die Analyse von Lipiden als Beitrag

zur biomedizinischen Forschung

7. Berliner LC-MS/MS Symposium
14. März 2017

Dr. Susanne Brodesser
CECAD Forschungszentrum, Lipidomics Core Facility, Universität Köln

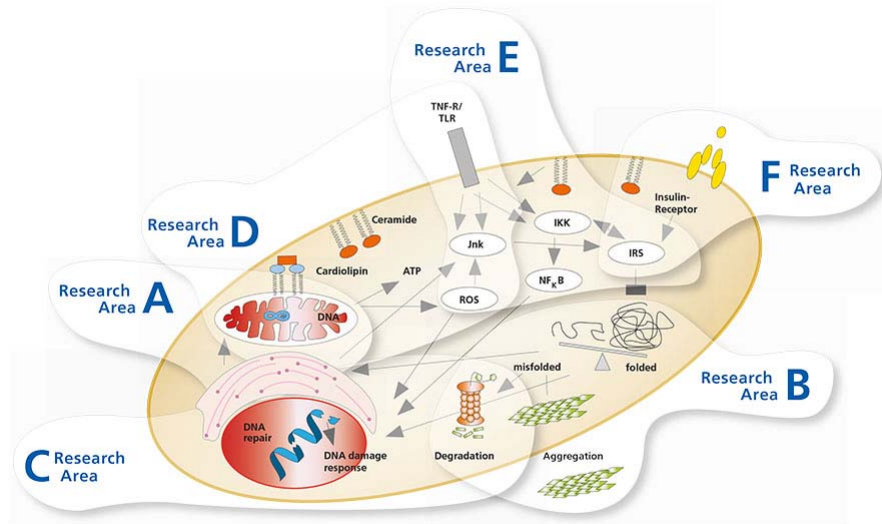Universität zu Köln | UNIKLINIK KÖLN | MAX PLANCK INSTITUTE FOR BIOLOGY OF AGEING | Max Planck Institute for Metabolism Research | CECAD COLOGNE

CECAD Cologne

CECAD: „Cluster of Excellence: Cellular Stress Responses in Aging-Associated Diseases"

Research Areas

A: Mitochondrial dysfunction in aging and neurodegeneration

B: Disruptions in protein metabolism cause aging-associated diseases

C: DNA damage responses in aging-associated diseases

D: The aging of membranes

E: Inflammation in aging-associated diseases

F: Metabolism in aging process, diabetes and obesity

Core Facilities

➢ Proteomics

➢ Lipidomics

➢ Imaging

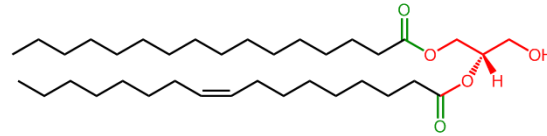➢ Bioinformatics

➢ in vivo Research
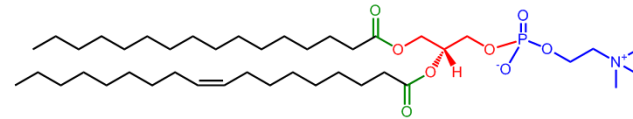
Lipids: structural diversity and functions

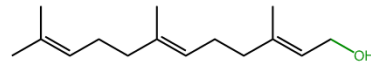**Fatty Acyls (FA)**
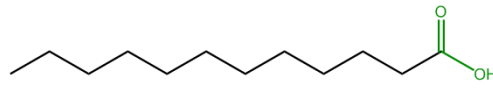
Glycerolipids (GL)

Glycerophospholipids (GP)

Sphingolipids (SP)
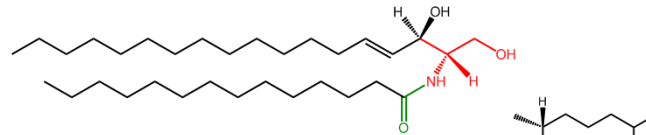
Sterol Lipids (ST)
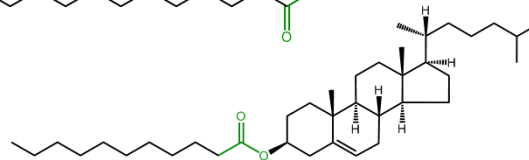
Prenol Lipids (PR)

Saccharolipids (SL)

Polyketides (PK)

E. Fahy 2010 ©

8 lipid classes each comprising 100s of individual molecular species

⇓

10,000s of different structures!

Lipids have numerous cellular functions:

- Cell membrane components
- Energy storage, mobilization
- Signalling molecules
- Cell growth, differentiation
- Precursors of bile acids, steroids
- Myelin sheath components
- Lipid-protein interactions

Impairment of lipid homeostasis contributes to a multitude of diseases:

- Obesity and diabetes
- Cardiovascular disorders
- Inflammation
- Cancer
- Neurodegenerative diseases
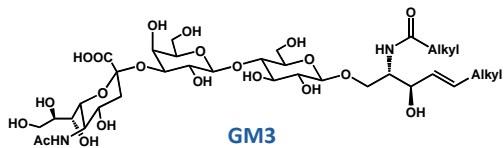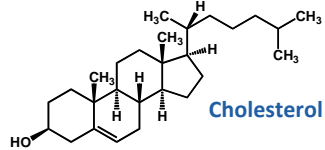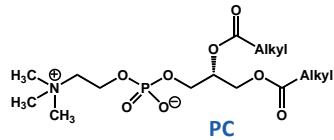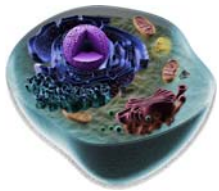- Lysosomal storage disorders

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Fahy *et al.* (2005) J Lipid Res 46(5):839-61
Shevchenko, Simons (2010) Nat Rev Mol Cell Biol 11(8):593-8

Universität zu Köln   UNIKLINIK KÖLN   MAX PLANCK INSTITUTE FOR BIOLOGY OF AGEING   Max Planck Institute for Metabolism Research   CECAD COLOGNE

(Semi-)Targeted lipid quantification: *Shotgun Lipidomics*

| Tissues Cells Organelles | → | Lipid extraction: LLE, SPE | → | Infusion of total lipid extract | → | Mass Spectrometry: (M)PIS, NLS | → | Data analysis: Lipid identification + quantification |

PC

Cholesterol

GM3
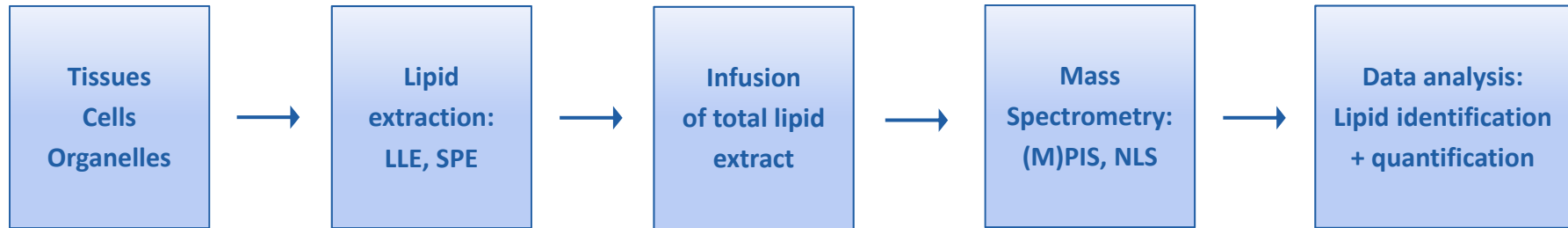
TriVersa NanoMate (Advion) → **QTRAP 6500 (SCIEX)**
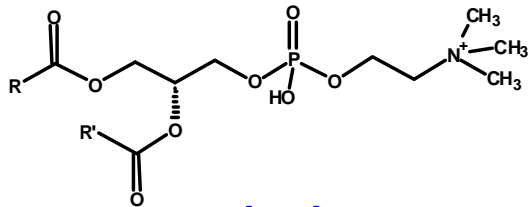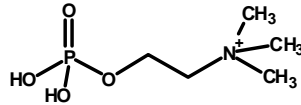
LipidView (SCIEX)

Methodology: Brügger *et al.,* PNAS 1997; Ekroos *et al.,* Anal Chem 2002; Ejsing *et al.,* Anal Chem 2006
Cell picture: Zaldua I, Equisoain JJ, Zabalza A, Gonzalez EM, Marzo A, Public University of Navarre – Own work

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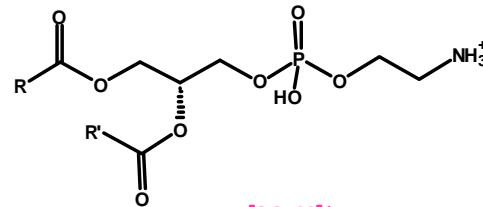# Diagnostic scans for detection of Glycerophospholipid subclasses
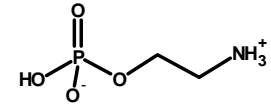
Phosphatidylcholines (PC)

[M+H]$^+$

(+) PIS 184.1
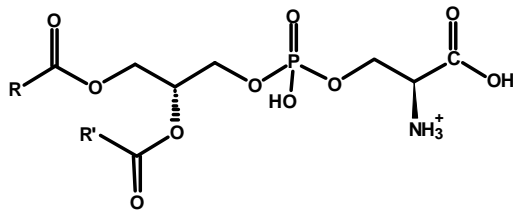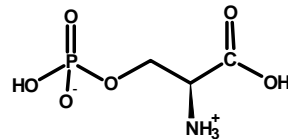
Phosphatidylethanolamines (PE)

[M+H]$^+$
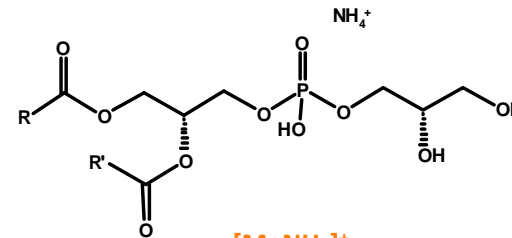
(+) NL 141.0

Phosphatidylserines (PS)
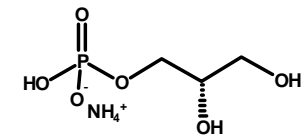
[M+H]$^+$

(+) NL 185.0
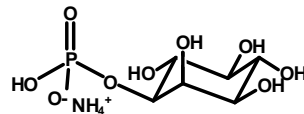
Phosphatidylglycerols (PG)

[M+NH$_4$]$^+$
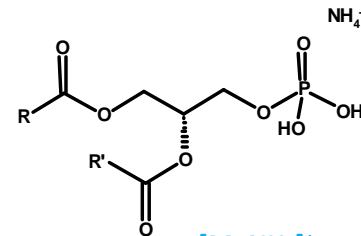
(+) NL 189.0

Phosphatidylinositols (PI)
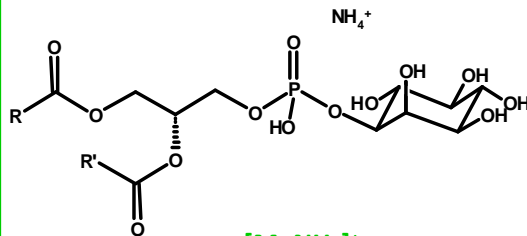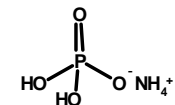
[M+NH$_4$]$^+$

(+) NL 277.0

Phosphatidic acid (PA)

[M+NH$_4$]$^+$

(+) NL 115.0

Multiple Precursor Ion and Neutral Loss Scanning of Glycerophospholipids

GPLs from isolated mitochondria of cultured murine keratinocytes
Samples provided by RG Thomas Magin (University Leipzig) / RG Rudolf Wiesner (CECAD Cologne)

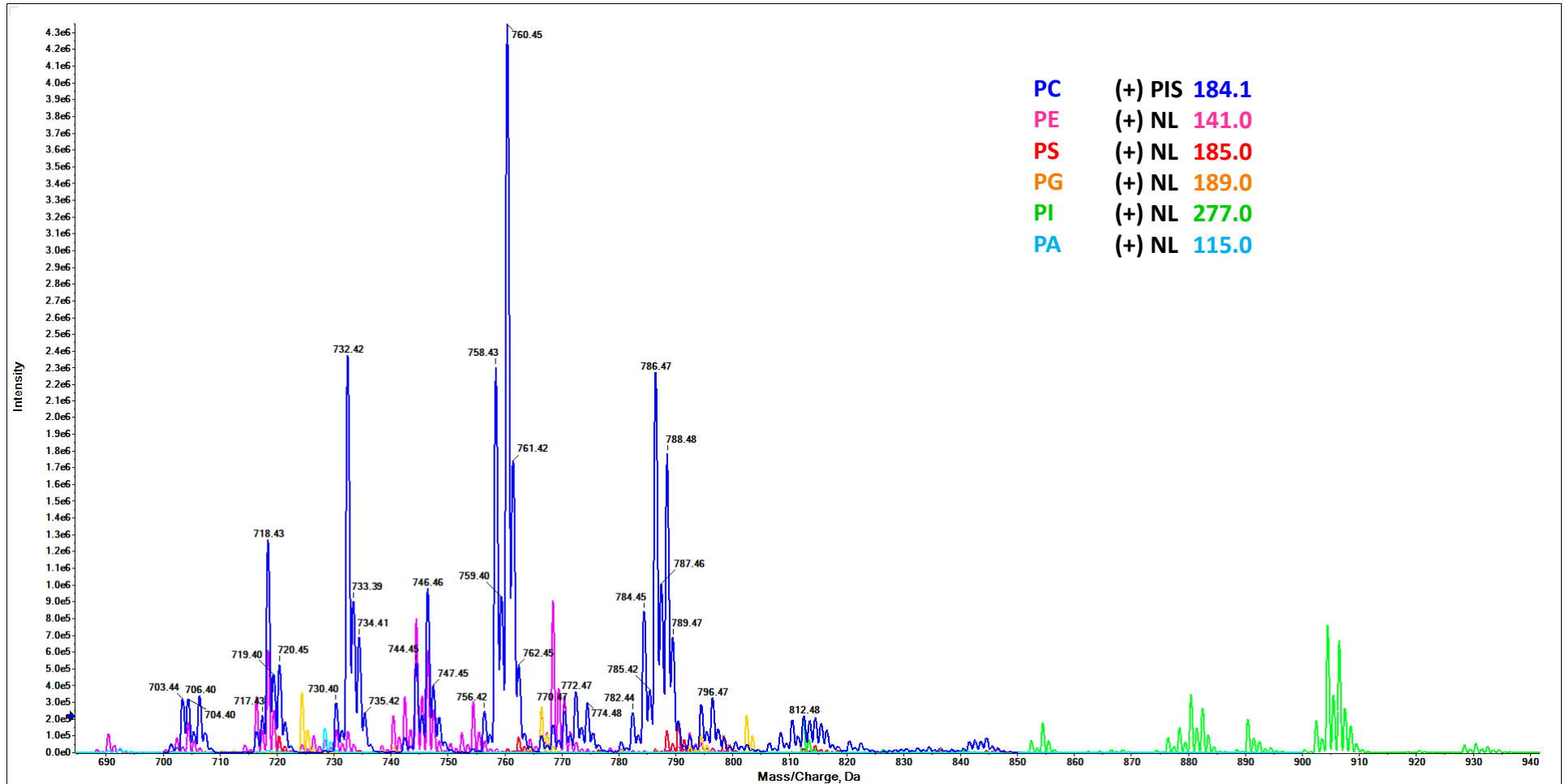|			
PC	(+) PIS	184.1
PE	(+) NL	141.0
PS	(+) NL	185.0
PG	(+) NL	189.0
PI	(+) NL	277.0
PA	(+) NL	115.0

Multiple Precursor Ion and Neutral Loss Scanning of Glycerophospholipids

GPLs from isolated mitochondria of cultured murine keratinocytes
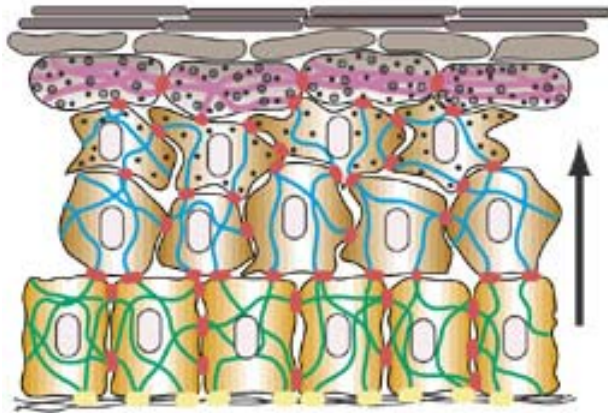**Samples provided by RG Thomas Magin (University Leipzig) / RG Rudolf Wiesner (CECAD Cologne)**
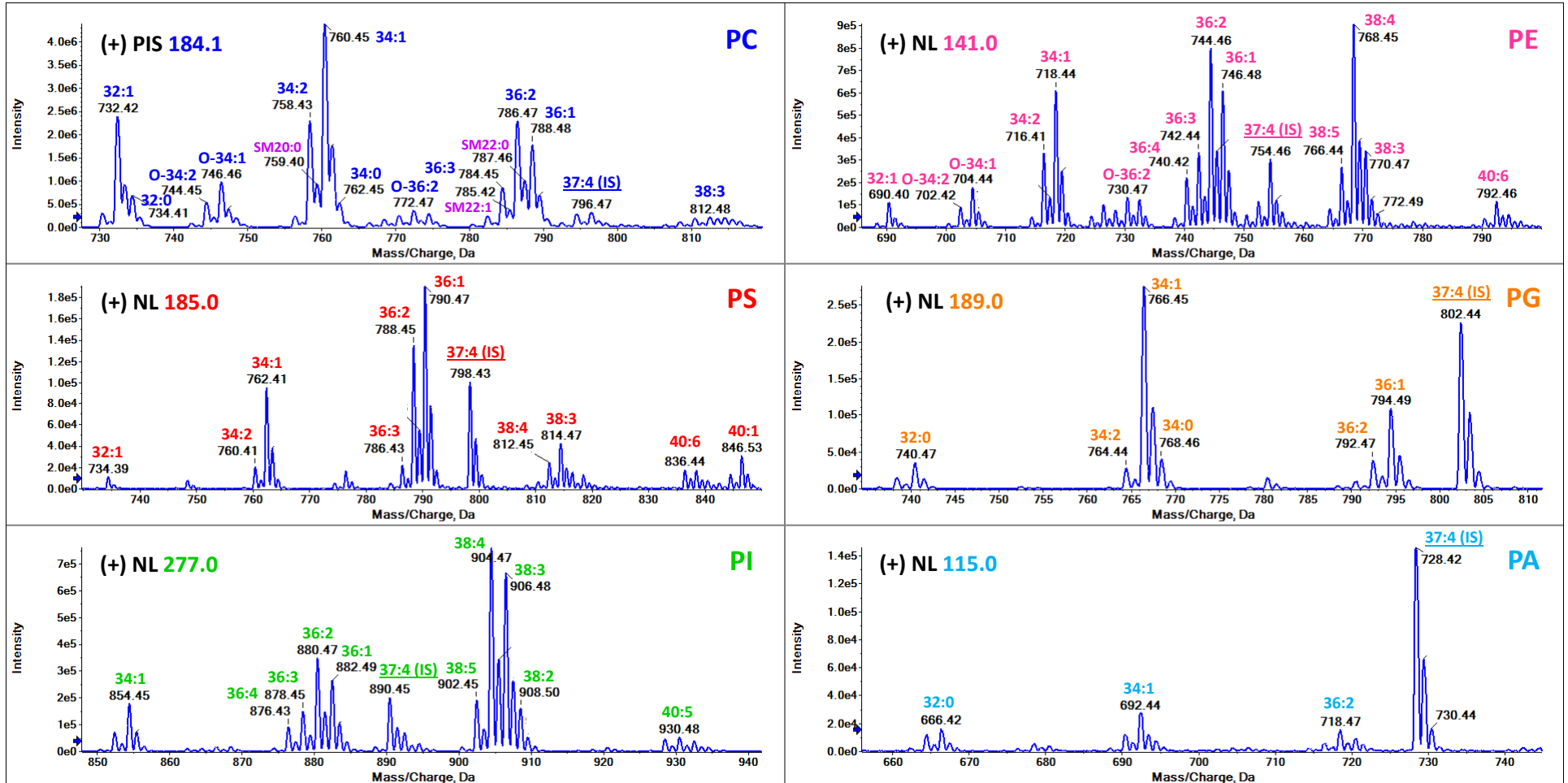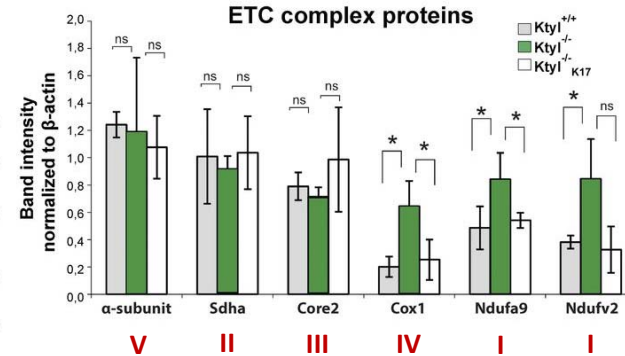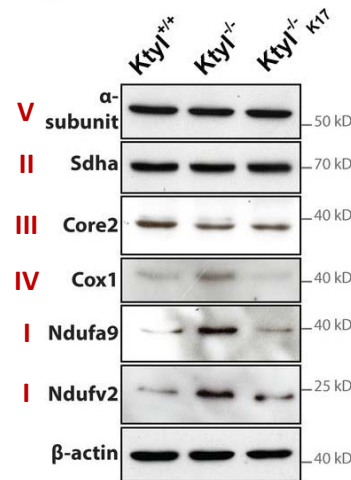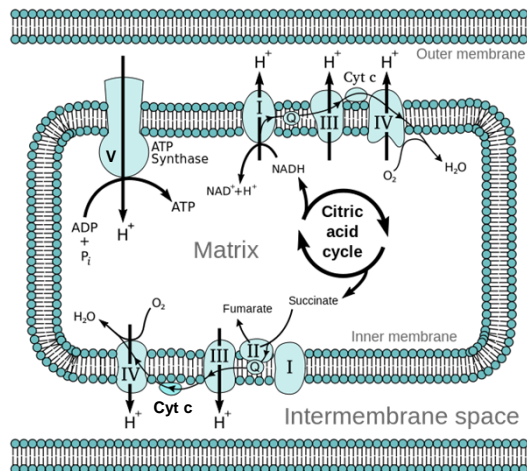
A keratin scaffold regulates epidermal barrier formation, mitochondrial lipid composition, and activity

Epidermis picture: Coulombe and Wong, Nat Cell Biol 2004

Mitochondria from cultured murine keratinocytes isolated by RG Rudolf Wiesner (CECAD Cologne)

Project of RG Thomas Magin (University Leipzig)
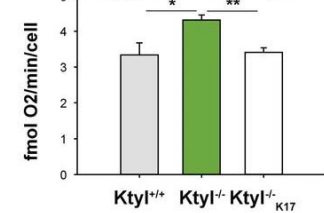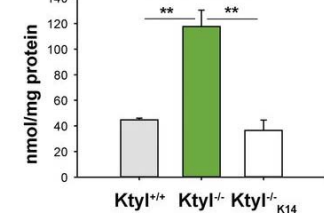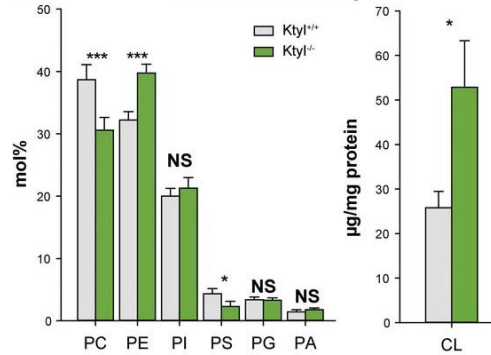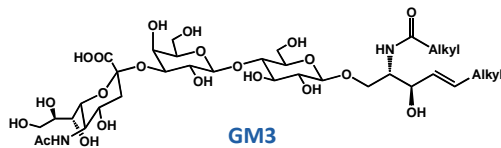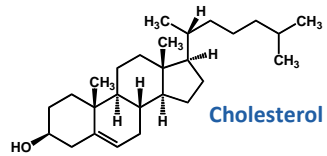

> Mitochondrial activity is increased, probably to supply energy for maintaining barrier resistance in absence of keratins

Targeted lipid quantification: LC-MS/MS

| Tissues Cells Organelles | → | Lipid extraction: LLE, SPE | → | HPLC | → | Mass Spectrometry: MRM | → | Data analysis: Lipid quantification |

PC

Cholesterol

GM3
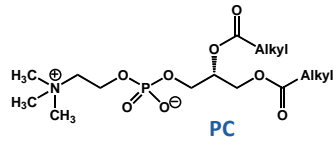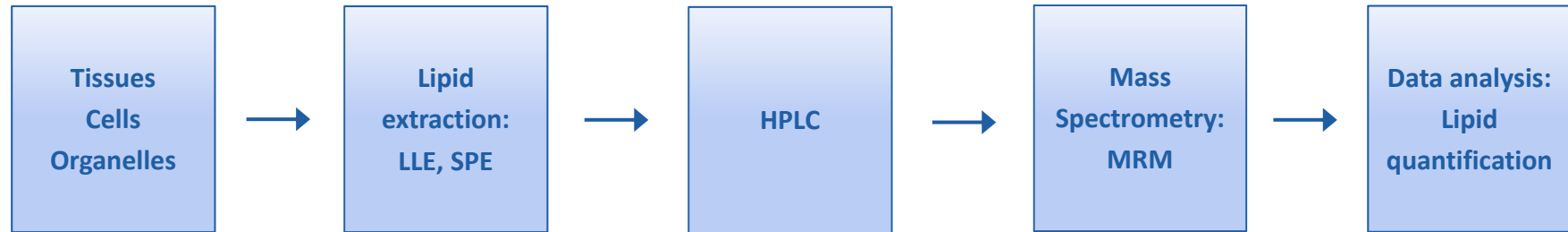
1260 Infinity Binary LC (Agilent)

QTRAP 6500 (SCIEX)

Analyst (SCIEX)

MultiQuant (SCIEX)

Cell picture: Zaldua I, Equisoain JJ, Zabalza A, Gonzalez EM, Marzo A, Public University of Navarre – Own work

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Analysis of sphingolipids by LC-MS/MS (MRM)

Ceramides

Glucosylceramides

Sphingomyelins

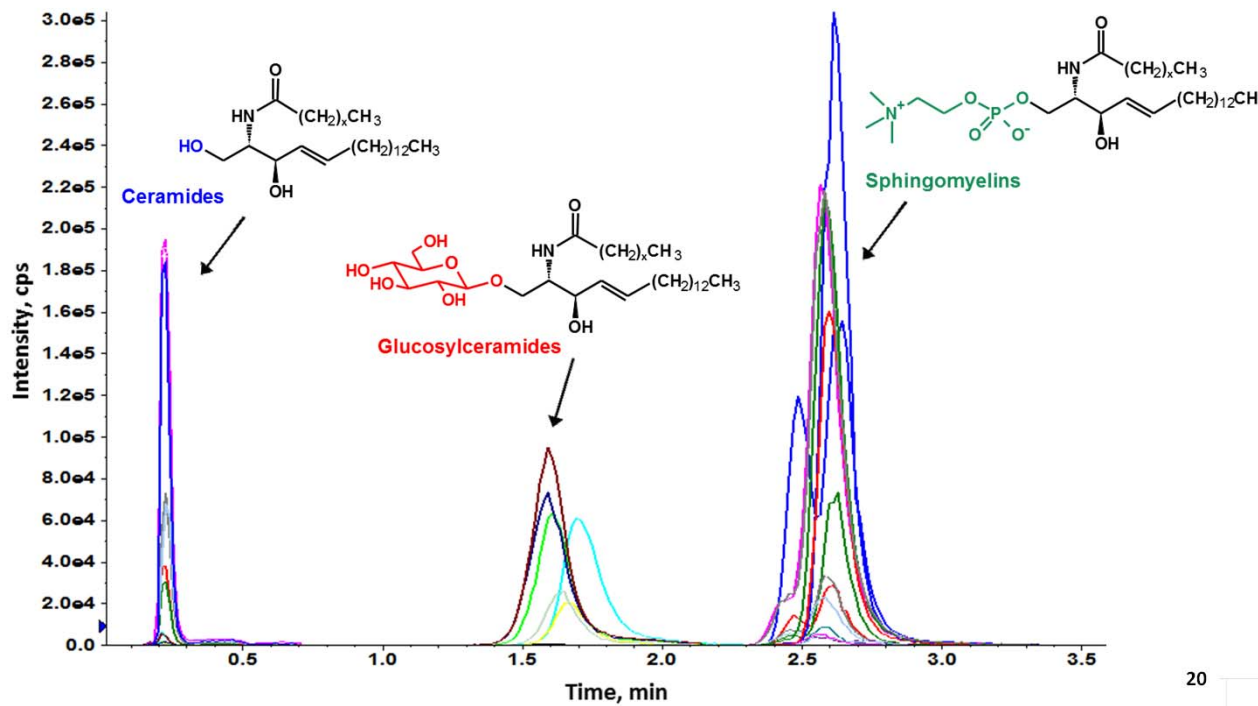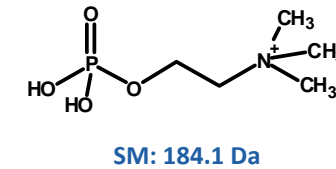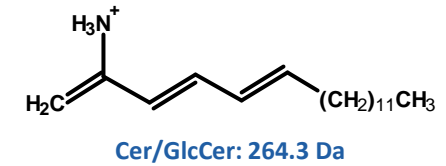**Characteristic product ions selected in Q3**

Cer/GlcCer: 264.3 Da

SM: 184.1 Da

Mouse skeletal muscle samples provided by RG Martin Krönke (Microbiology, University Hospital, Cologne)

Column: Nucleosil NH2 (Aminopropyl-modified silica gel),
50 mm × 2 mm ID, 3 µm, 120 Å, Macherey-Nagel

Method modified from: Shaner *et al.*, J Lipid Res 2009, 50, 1692-707

Peak Area ratio (Cer/IS)

- ◆ Cer16:0
- ■ Cer18:0
- ▲ Cer18:1
- ● Cer20:0
- ■ Cer22:0
- ● Cer24:0
- ✚ Cer24:1

Molar ratio (Cer/IS)

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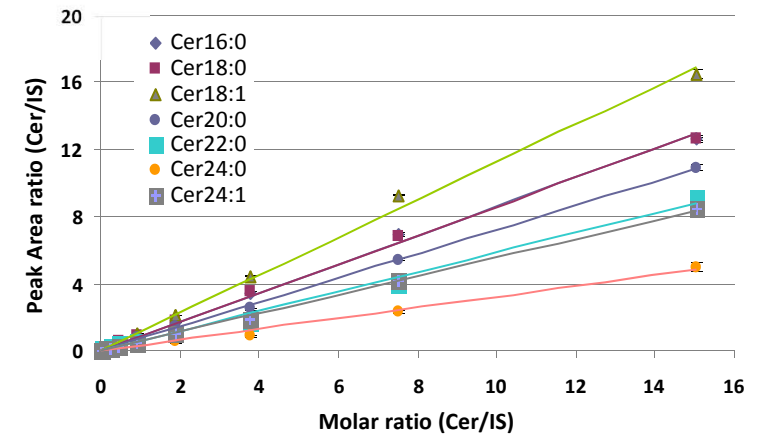
Obesity-Induced CerS6-Dependent C16:0 Ceramide Production Promotes Weight Gain and Glucose Intolerance

Project of RG Jens Brüning (MPI for Metabolism Research, Cologne)
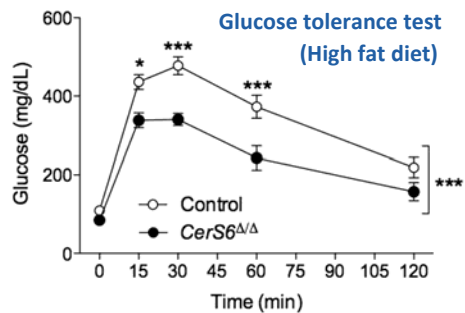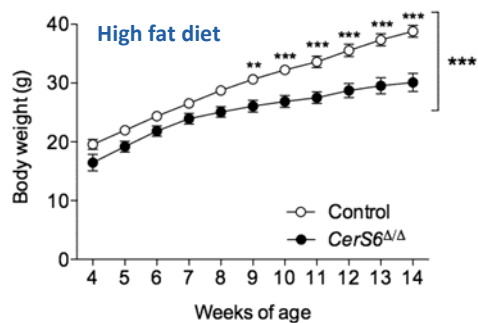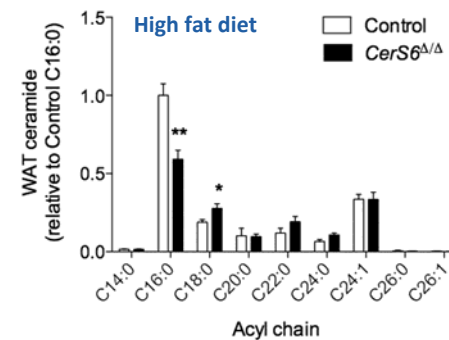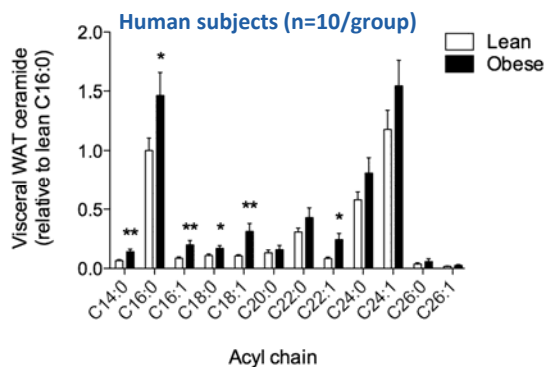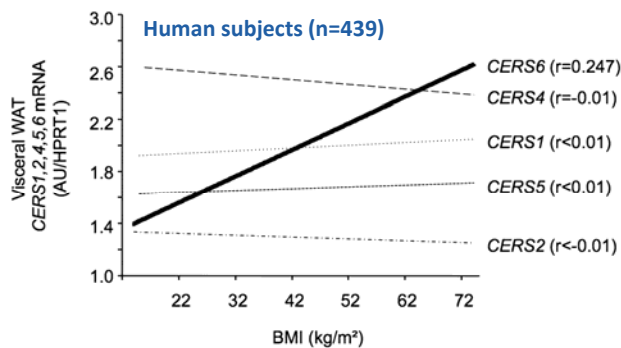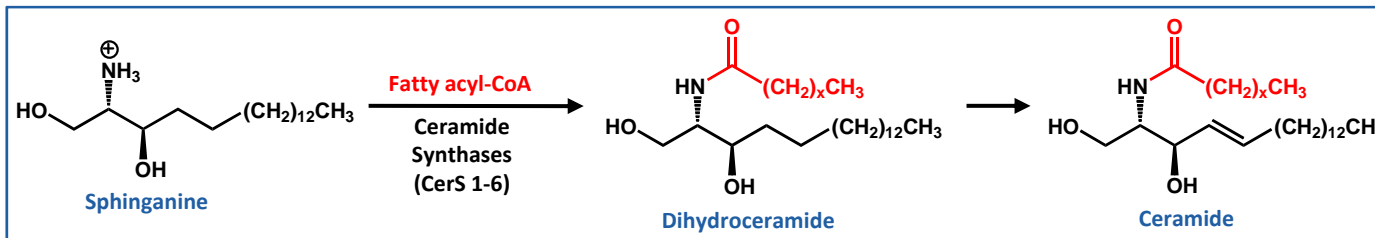
Turpin SM *et al.*, Cell Metab 2014, 20(4):678-86

Non-targeted lipid profiling: MS/MS[ALL]

| Tissues Cells Organelles | → | Lipid extraction: LLE, SPE | → | Infusion of total lipid extract | → | Mass Spectrometry: MS/MS[ALL] | → | Data analysis: Lipid identification + quantification |

PC

Cholesterol

GM3
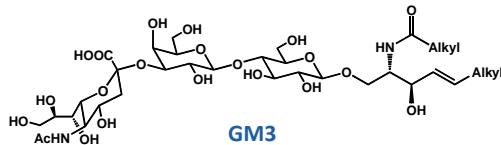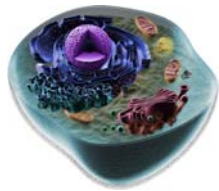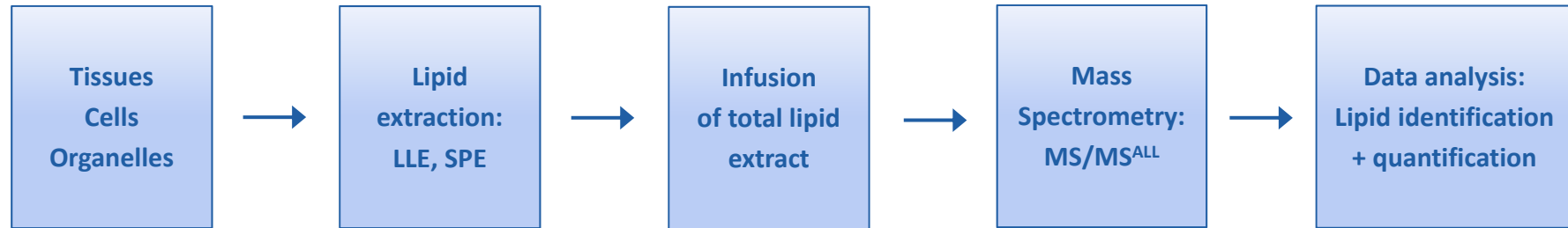
1260 Infinity Binary LC (Agilent)

TripleTOF 6600 (SCIEX)

PeakView (SCIEX)

LipidView (SCIEX)

MarkerView (SCIEX)

MS/MS[ALL]-Methodology: B. Simons *et al.*, Metabolites 2012, 2, 195-213
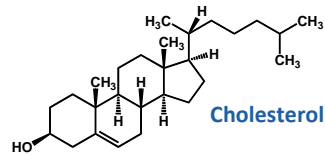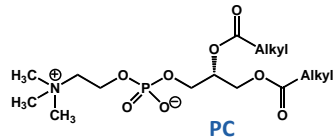Cell picture: Zaldua I, Equisoain JJ, Zabalza A, Gonzalez EM, Marzo A, Public University of Navarre – Own work

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MS/MS[ALL] Data Independent Acquisition: Products of All Precursors

Scan Precursors

Fragmentation

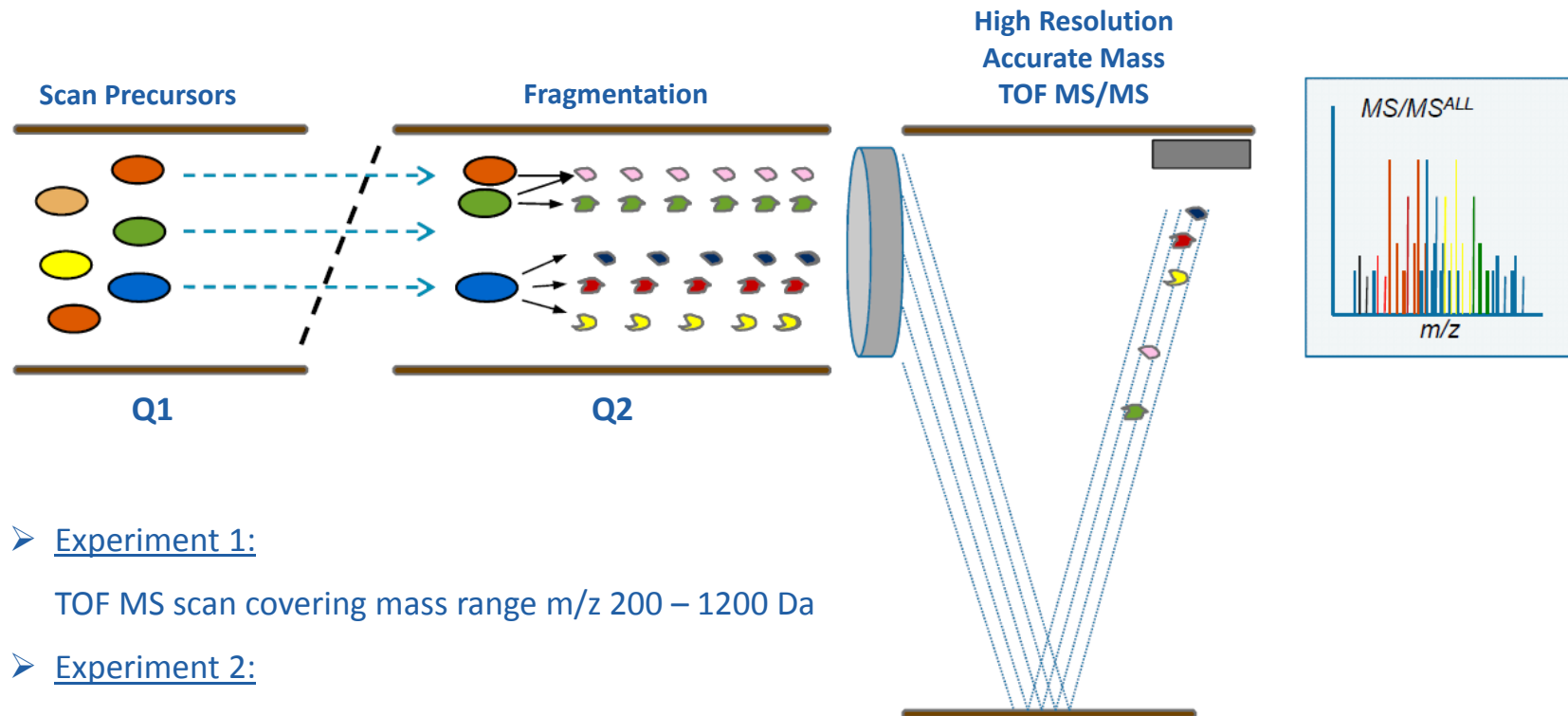**High Resolution Accurate Mass TOF MS/MS**

MS/MS[ALL]

m/z

Q1

Q2

➢ Experiment 1:

   TOF MS scan covering mass range m/z 200 – 1200 Da

➢ Experiment 2:

   Series of 1000 high resolution MS/MS scans

   (Q1 precursor isolation steps of ~ 1 Da)

➢ Acquisition in positive and negative mode

MS/MS[ALL]-Methodology: B. Simons *et al.*, Metabolites 2012, 2, 195-213

Figure: SCIEX

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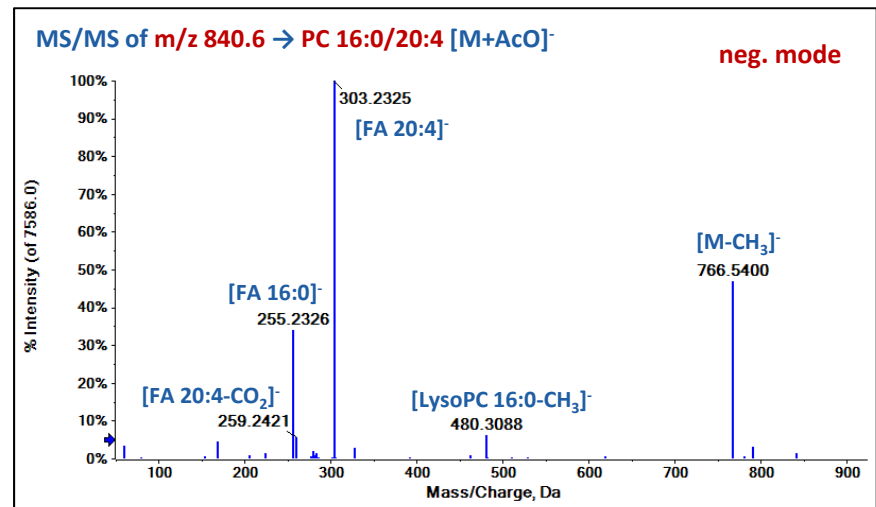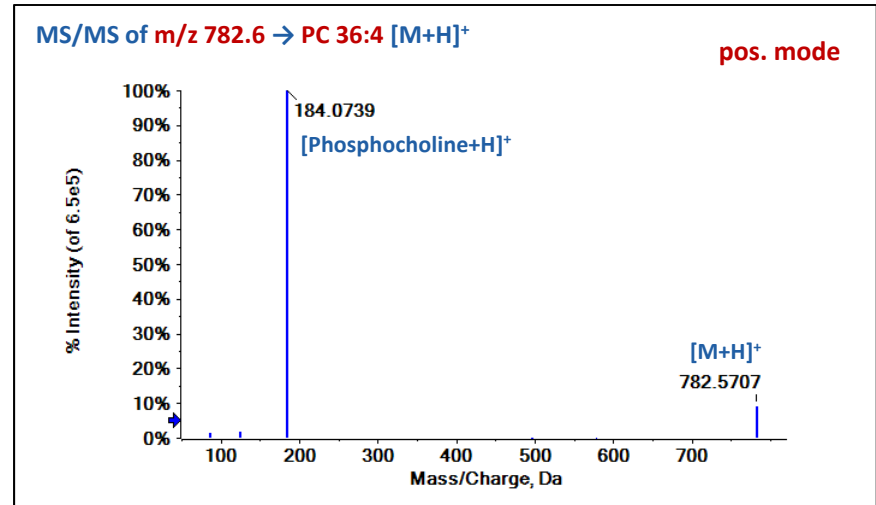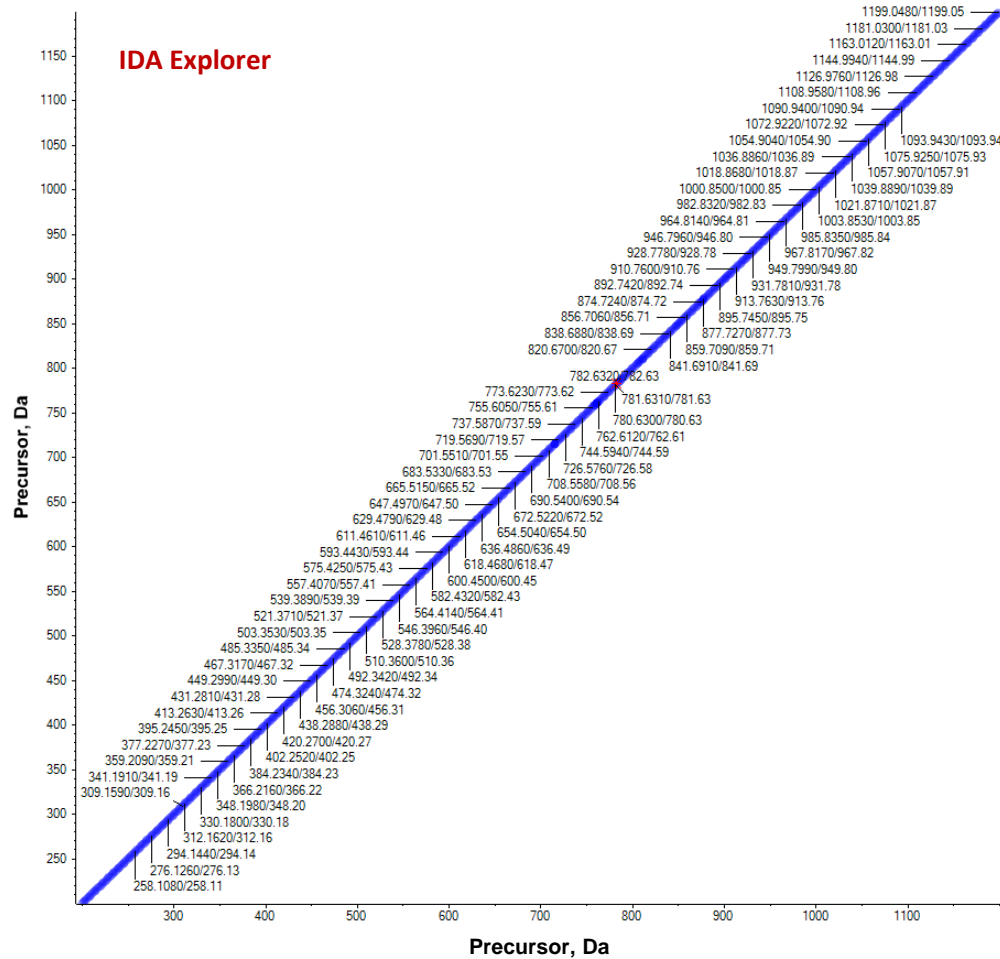# MS/MS^{ALL} Data Independent Acquisition: Products of All Precursors

Lipids from primary mouse hepatocytes
Cells provided by RG Jan Kornfeld (MPI for Metabolism Research, Cologne)
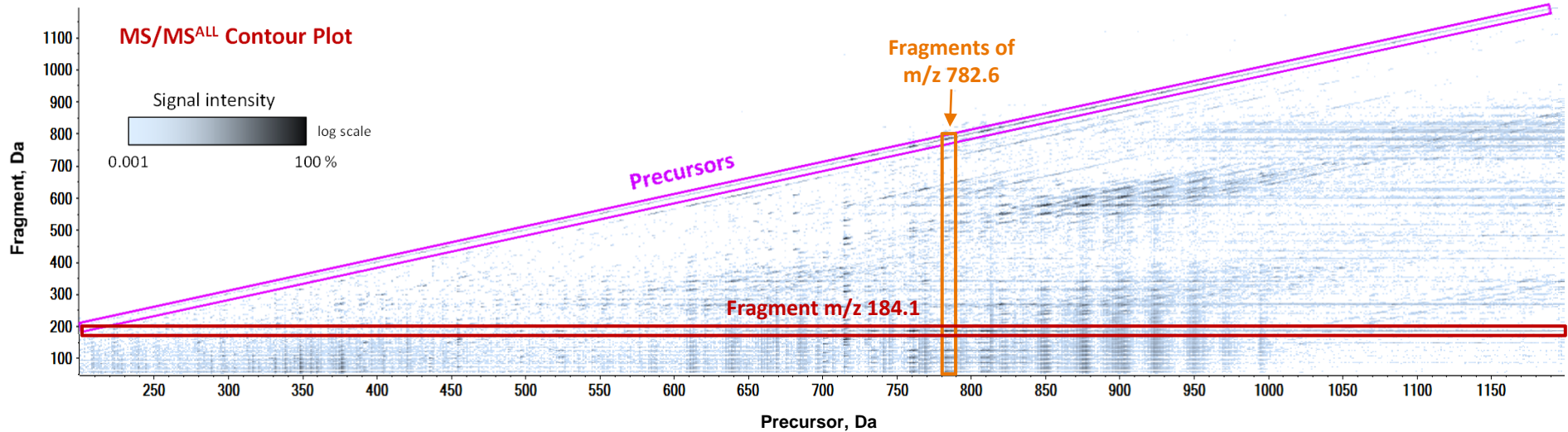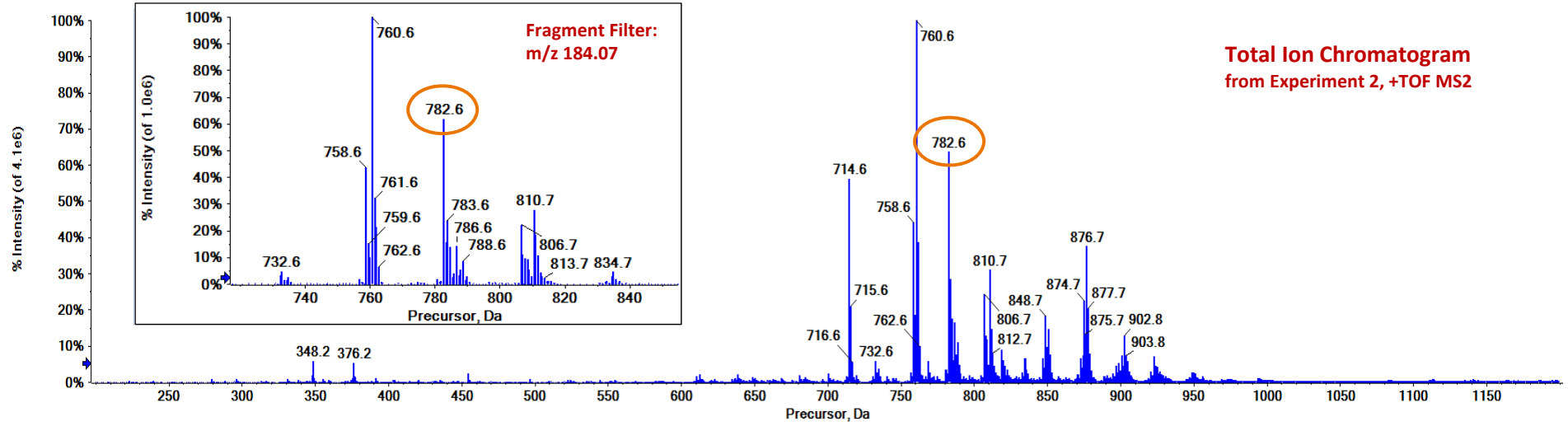
MS/MS^{ALL} Data Independent Acquisition: Products of All Precursors

Lipids from primary mouse hepatocytes
Cells provided by RG Jan Kornfeld (MPI for Metabolism Research, Cologne)

Lipid species identified in primary mouse hepatocytes using MS/MS^{ALL}

Cells provided by RG Jan Kornfeld (MPI for Metabolism Research, Cologne)

Lipid class	# of identified species (positive mode)	# of identified species (negative mode)
PC	79	83
PE	50	57
PS	26	107
PI	0	98
PG	7	75
PA	0	78
LysoPC	10	5
LysoPE	1	5
LysoPS	2	7
LysoPI	0	21
LysoPG	0	3
LysoPA	0	2
TAGs	60	-
Sphingomyelins	30	-
Cholesteryl esters	3	-
Free fatty acids	-	10
Total	268	551