UNIVERSITÄT LEIPZIG

Combined TLC-MALDI Analysis of Lipids

Jürgen Schiller

B. Fuchs, R. Süß, A. Nimptsch

M. Schürenberg*, M. Becker*, D. Suckau*

University of Leipzig, Medical Faculty
Institute of Medical Physics and Biophysics
and *Bruker Daltonics, Bremen

BRUKER

A short Outline of my Talk

1. **Why are Lipids of Interest?**
2. **How does MALDI-TOF Mass Spectromety work?**
3. **The Need to separate a Lipid Mixture prior to MS**
4. **Recording Mass Spectra directly from the TLC Plate**
   ⇒ Simple Identification & Spatial Resolution
5. **Some selected Examples (Egg, Cells, Brain ...)**
6. **Summary: Capabilities and Limitations**

Why are Lipids of Interest? – Nutrition!

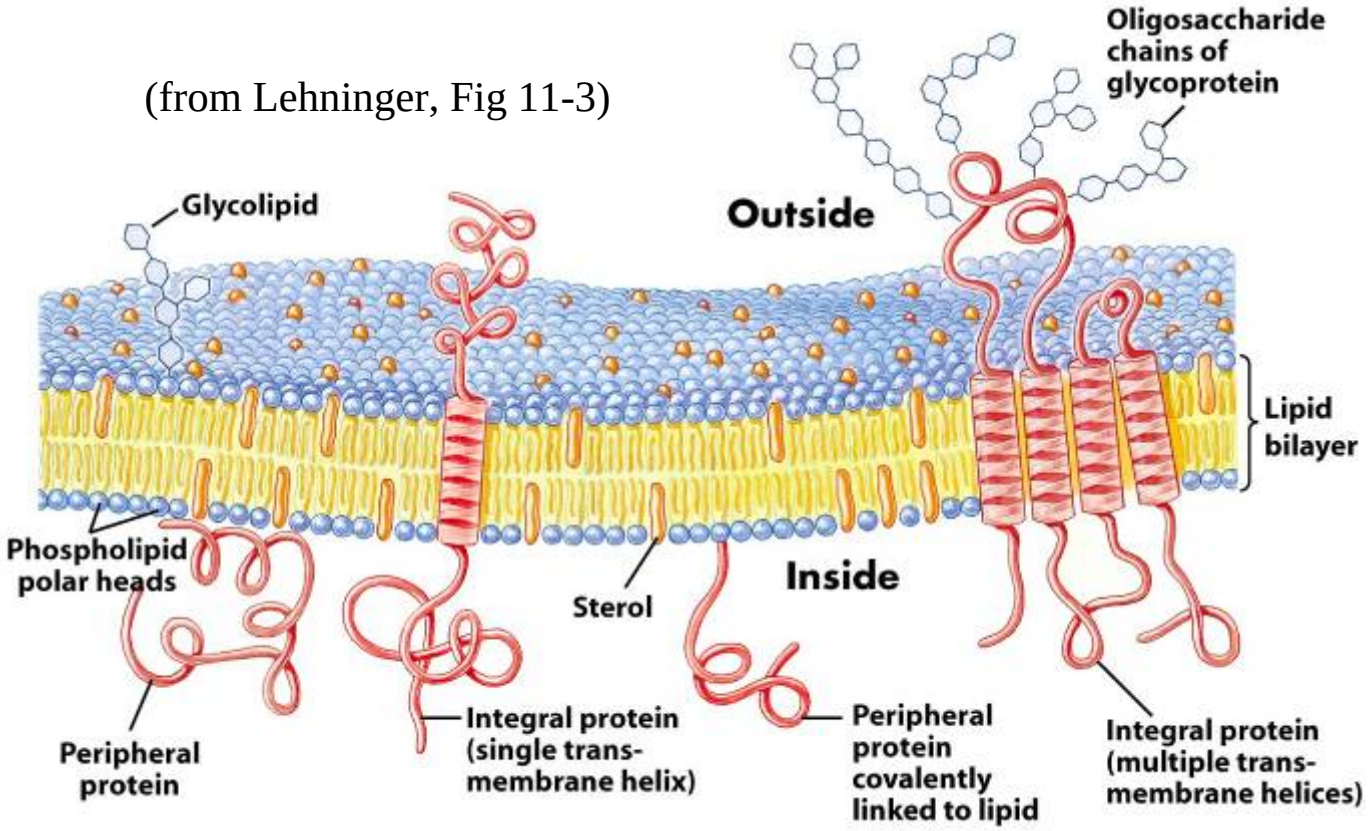
"Wait A Minute!
What About Those Reports
That Worms Are Terribly
High in Cholesterol!"

- **Nutrition**
- **Food Analysis**
- **Nutrition-related Diseases as Atherosclerosis**
- **"Good" and "Bad" Cholesterol of Blood (HDL/LDL)**

NMALDI LRIPID

Why are Lipids of Interest? – Cellular Membranes

(from Lehninger, Fig 11-3)

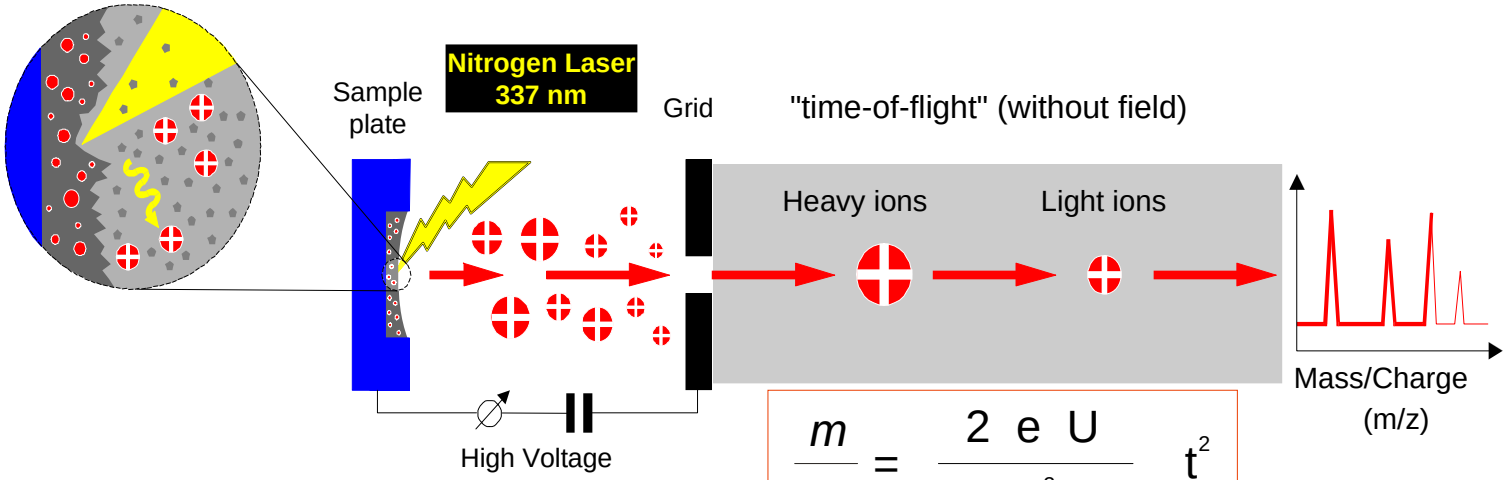


No (Phospho)Lipid ⇒ No Life!

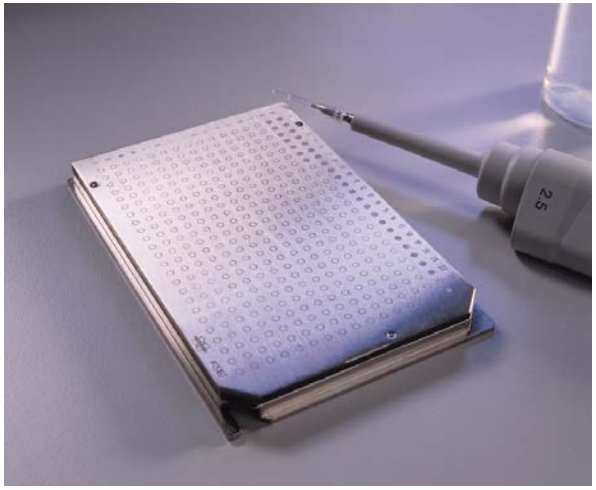
Changes of Membrane Composition

⇒ Changes of the functional State of the Cell!

How does MALDI-TOF MS work?



$$\frac{m}{z} = \frac{2\ e\ U}{L^2}\ t^2$$



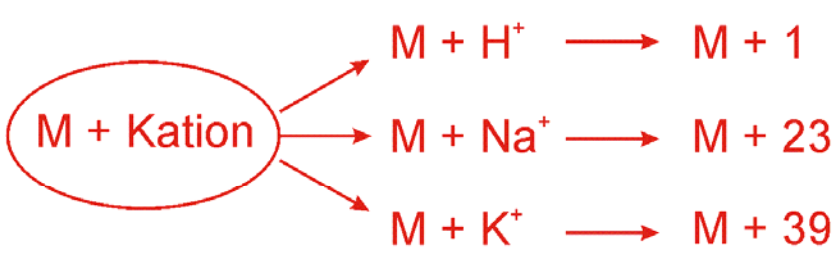
MALDI Sample Plate „Target“

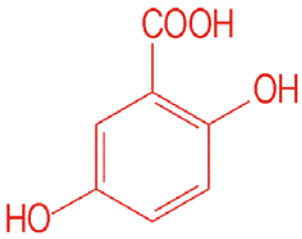
Advantages:

- Simple Mass Spectra!
- High Sensitivity (ng – pg)!
- All Lipid Classes may be analyzed!

"Quasimolecular" Ions are generated

a) Positive Ion Spectra

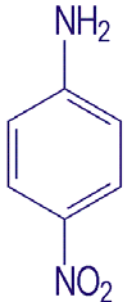




2,5-Dihydroxy-benzoic acid (*DHB*)

For neutral PL (PC etc.)

b) Negative Ion Spectra



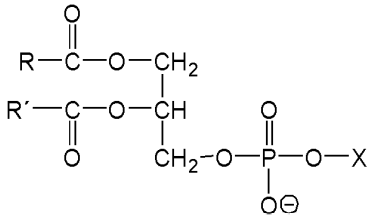
para-Nitroaniline (*PNA*)

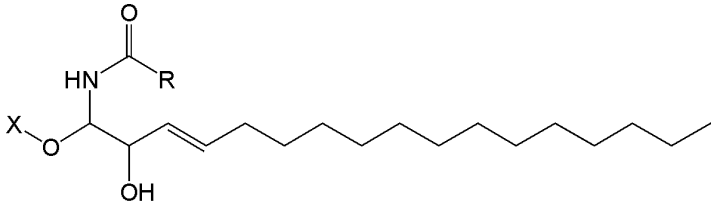
Alkaline!

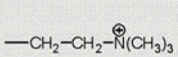
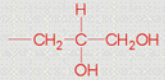
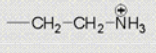
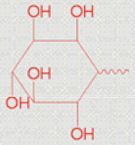
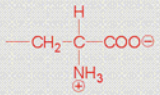
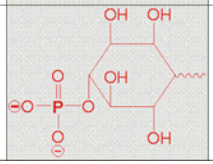
Ideal for acidic PL (PS, PI etc.) and for PE in mixtures!

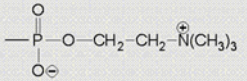
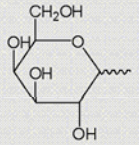
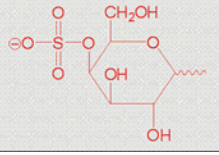
The pK Values of Matrix and Analyte determine the Sensitivity of Detection!

Structures of selected Lipids and Phospholipids





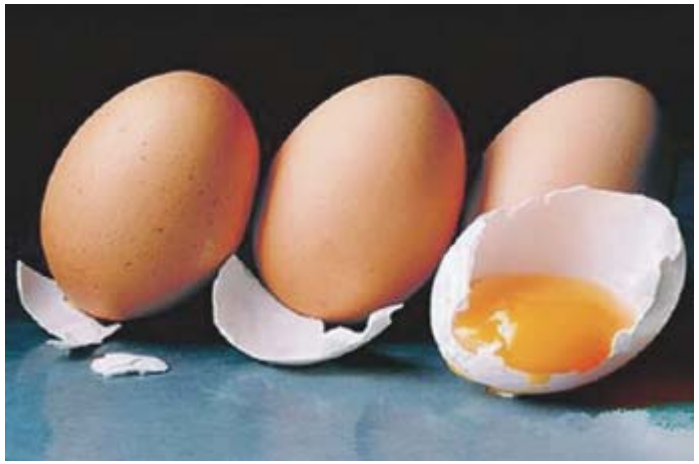
Residue -X	Sub Class	Residue -X	Sub Class
	Phosphatidyl-choline (PC)		Phosphatidyl-glycerol (PG)
	Phosphatidyl-ethanolamine (PE)		Phosphatidyl-inositol (PI)
	Phosphatidyl-serine (PS)		Phosphorylated Phosphatidyl-inositol (PIP)

Residue -X	Sub Class
	Sphingo-myelin (SM)
	Galacto-cerebroside (Cer)
	Sulfo-galacto-cerebroside (ST)

Different Charge States of Lipids

⇒ Different Detectabilities by MALDI MS!

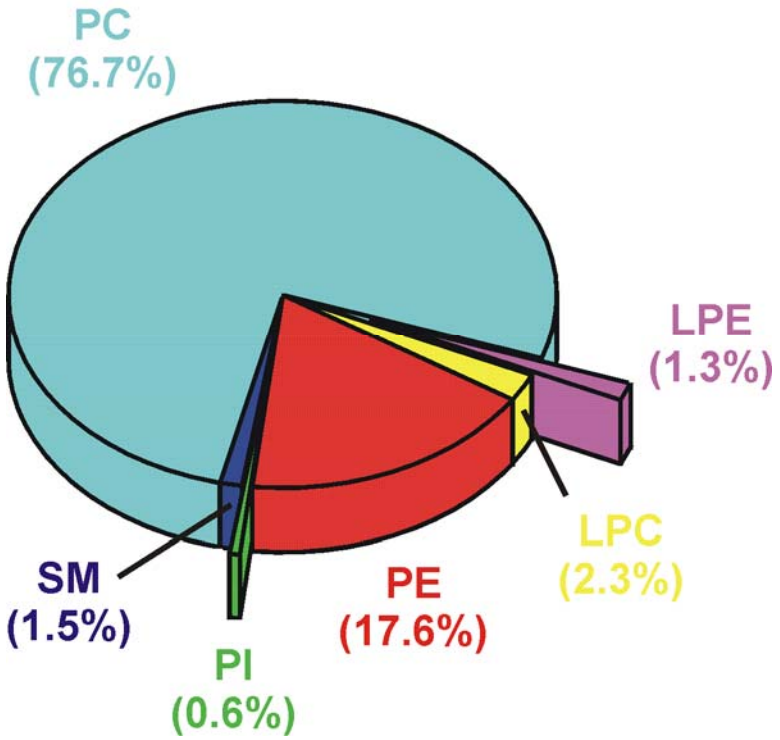
A total Extract from Hen Egg Yolk is a useful Example



Advantages:

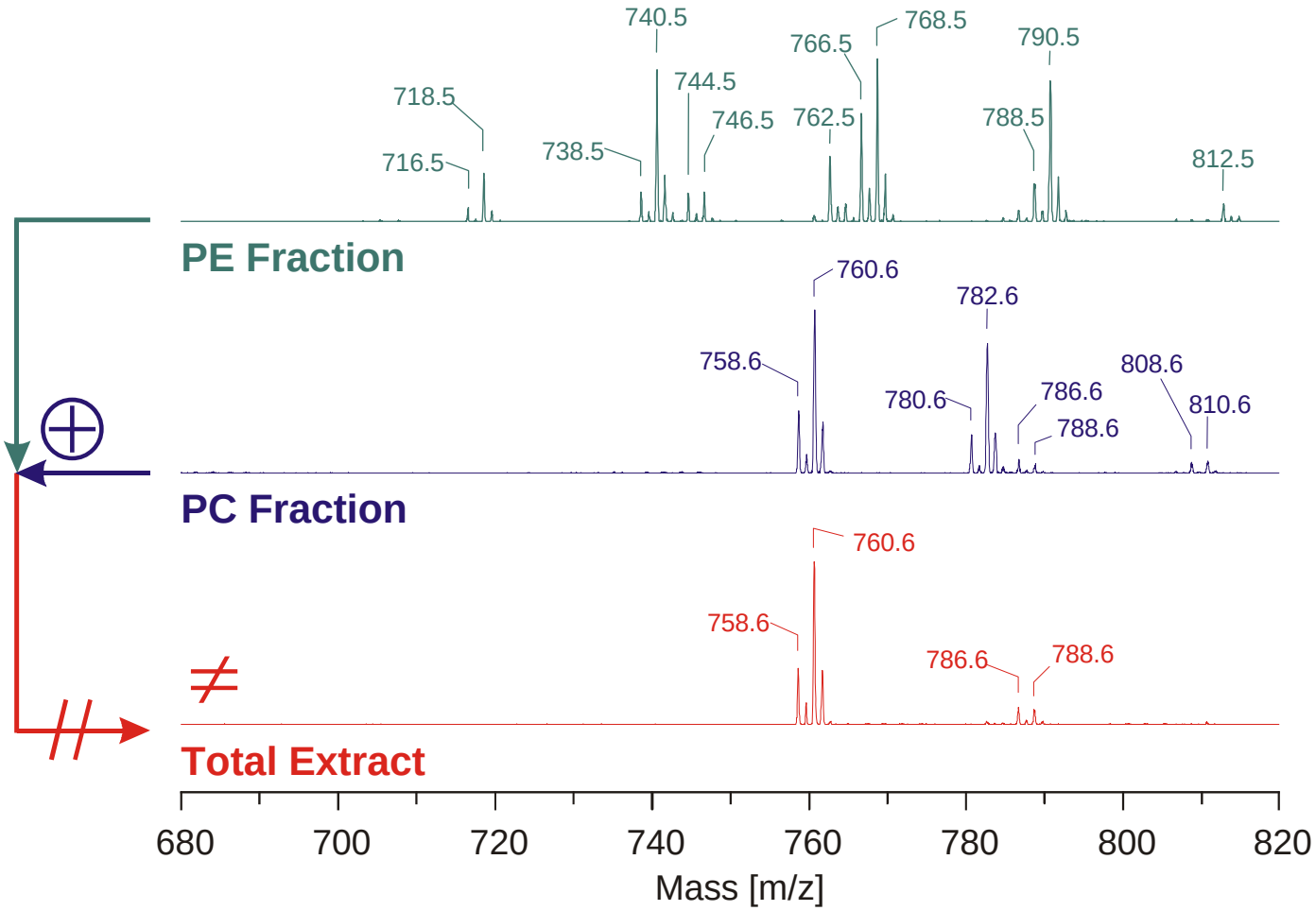
- Can be easily obtained!
- Available in large Amounts!
- Rich Source of many different Phospholipids, but in strongly varying Amounts!

Rough Composition of the Hen Egg Yolk....



Fuchs, B., Schiller, J., Süß, R., Schürenberg, M., Suckau, D.: A direct and simple Coupling Method between Matrix-Assisted Laser Desorption and Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and Thin-Layer Chromatography (TLC). *Anal. Bioanal. Chem.* **389** (2007) 827-834.

Positive Ion MALDI Spectra of the Hen Egg Yolk



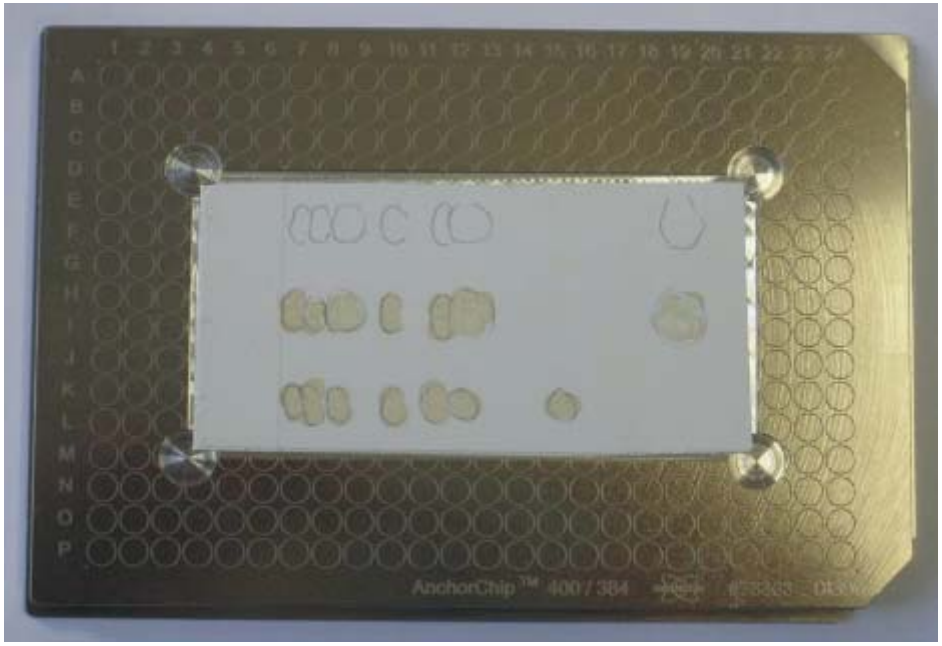
Spectrum of total Extract ≠ Sum of individual Spectra!
Different Lipids ⇒ Different Detection Limits!

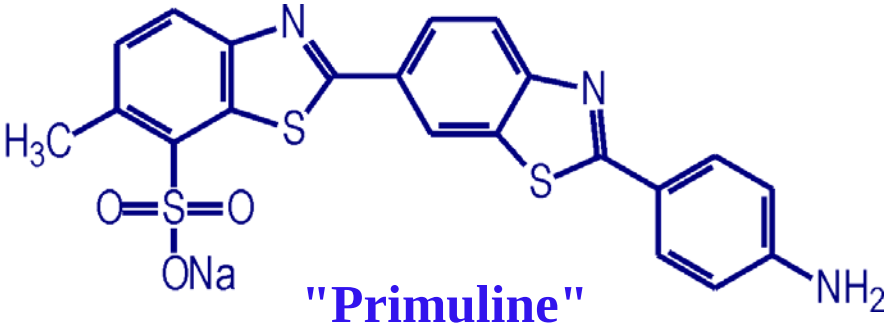
"Take Home Message" – One

The Separation into the individual Lipid Classes is required prior to MALDI-TOF MS Analysis in Order to detect ALL Phospholipids!

MALDI-TOF MS may be easily combined with TLC

Prototype of TLC/MALDI Adapter Target (Bruker)





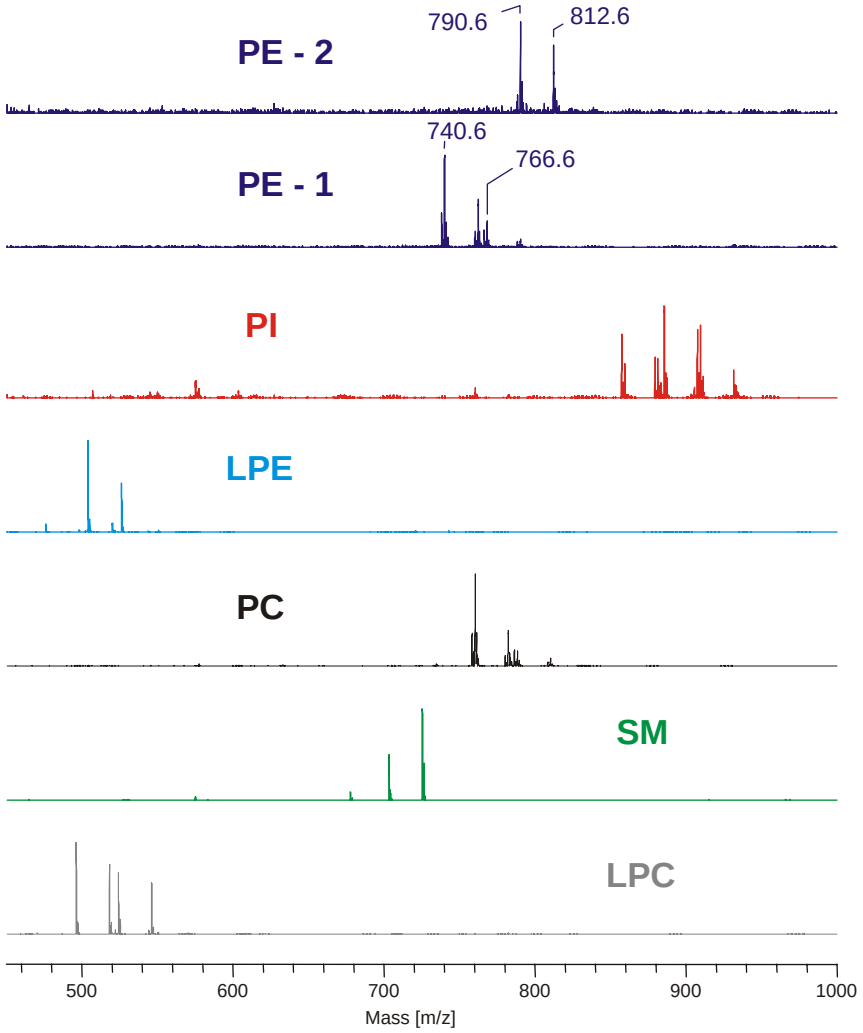
"Primuline"

1. TLC-Separation (Alu Back!)
2. Staining with **Primuline**
3. Densitometry
4. Marking of "Spots"
5. **Application of Matrix (100 mg/ml DHB in 1:1 (v/v) H_2O/CH_3CN)**
6. Attachment of the TLC Plate onto the Target
7. Record MALDI Spectrum **(Outlook: Automated Spectra Acquisition and Matrix Application!)**

The Presence of Primuline does not affect the MALDI Results!

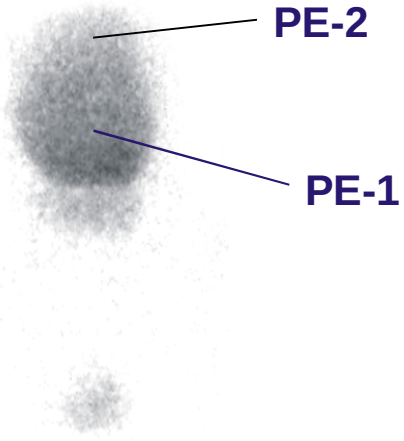
UNIVERSITÄT LEIPZIG

Some selected Results with Egg Yolk



PE
(9 nmol)

PI
(0.2 nmol)



LPE
(1 nmol)

PC
(35 nmol)

SM
(0.8 nmol)

LPC
(1.6 nmol)

PI can be easily detected in the order of a few pmoles!

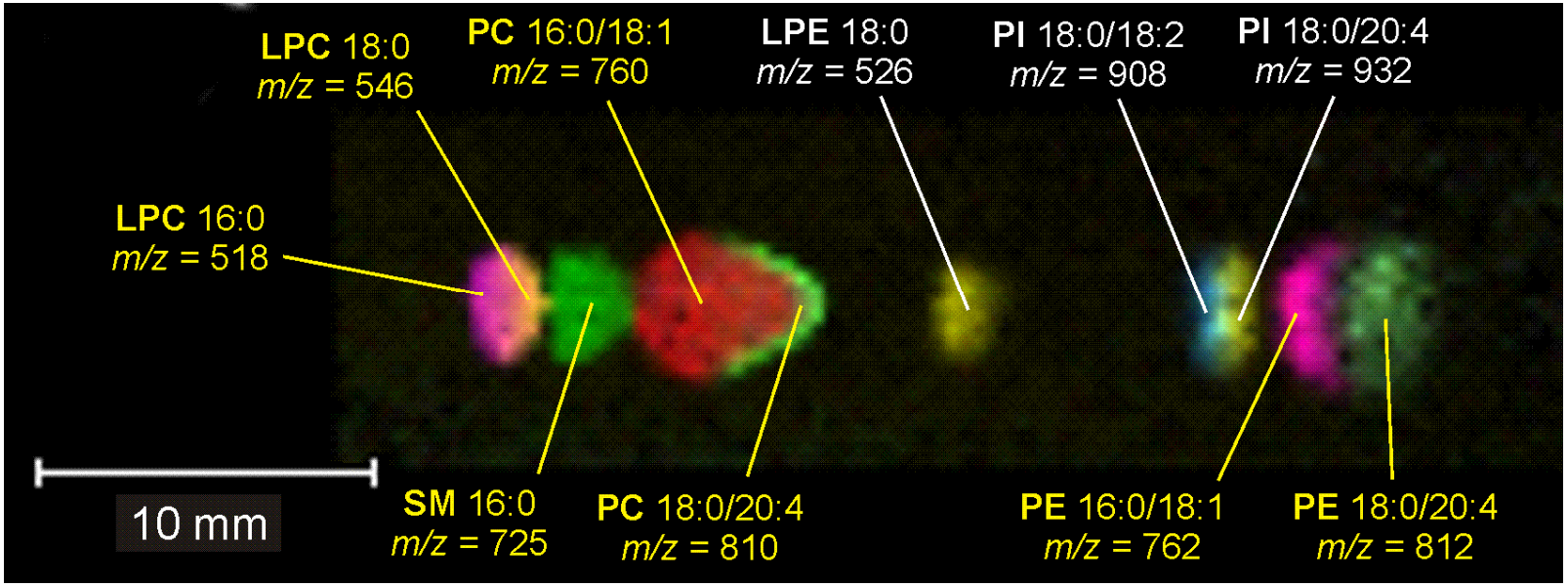
Spatial Resolution ⇒ Important compositional Information!

"Take Home Message" – Two

- **Combining TLC Separation and MALDI Detection is very simple!**
- **"Normal" Phase Separation (Lipid Classes) is extented by Information about Fatty Acyl Compositions!**
- **Only very minor Fragmentation of the Analyte!**
- **Detection of (at least) Picomole Amounts is possible!**

MALDI-"Imaging" is also possible

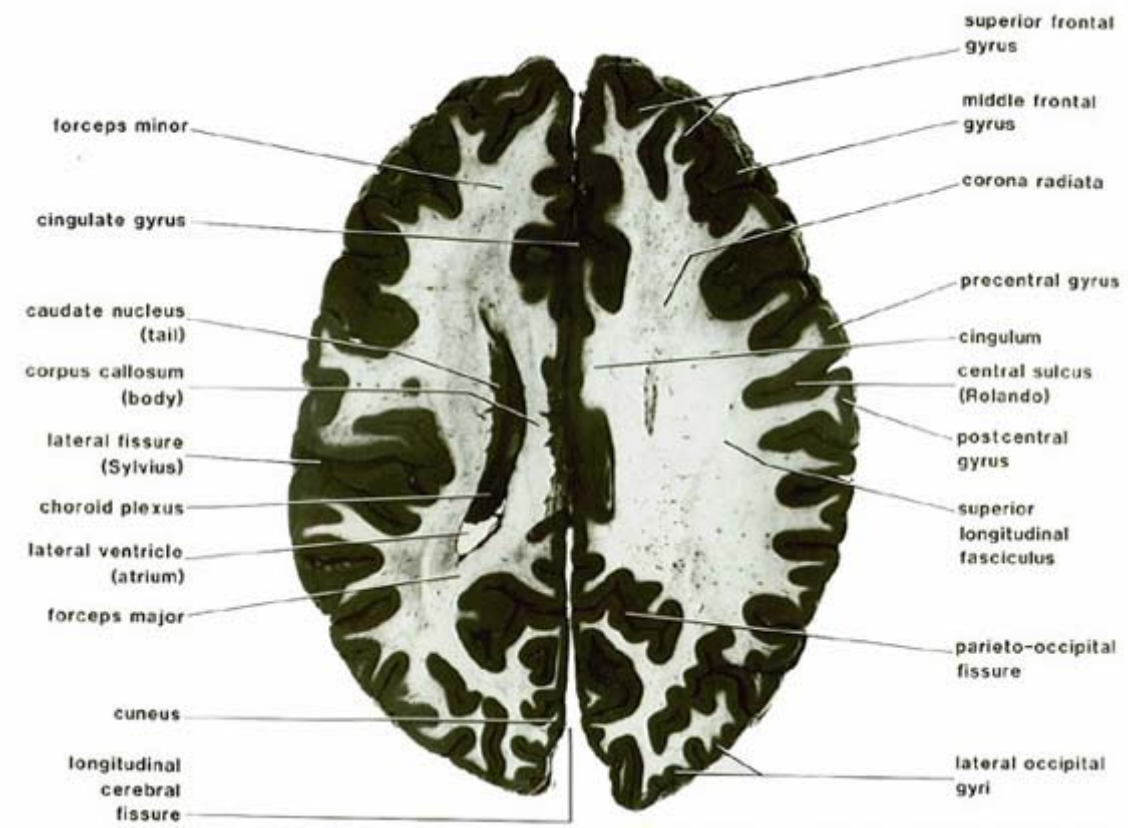
(Automated Application of the Matrix by "Spraying")



- High Resolution (about 100 µm – depending on the Laser Spot)!
- Combination of "Normal Phase" (Lipid Classes) and "Reversed Phase" (Fatty Acyl Residues) Separation!

NMALDI LRP ID

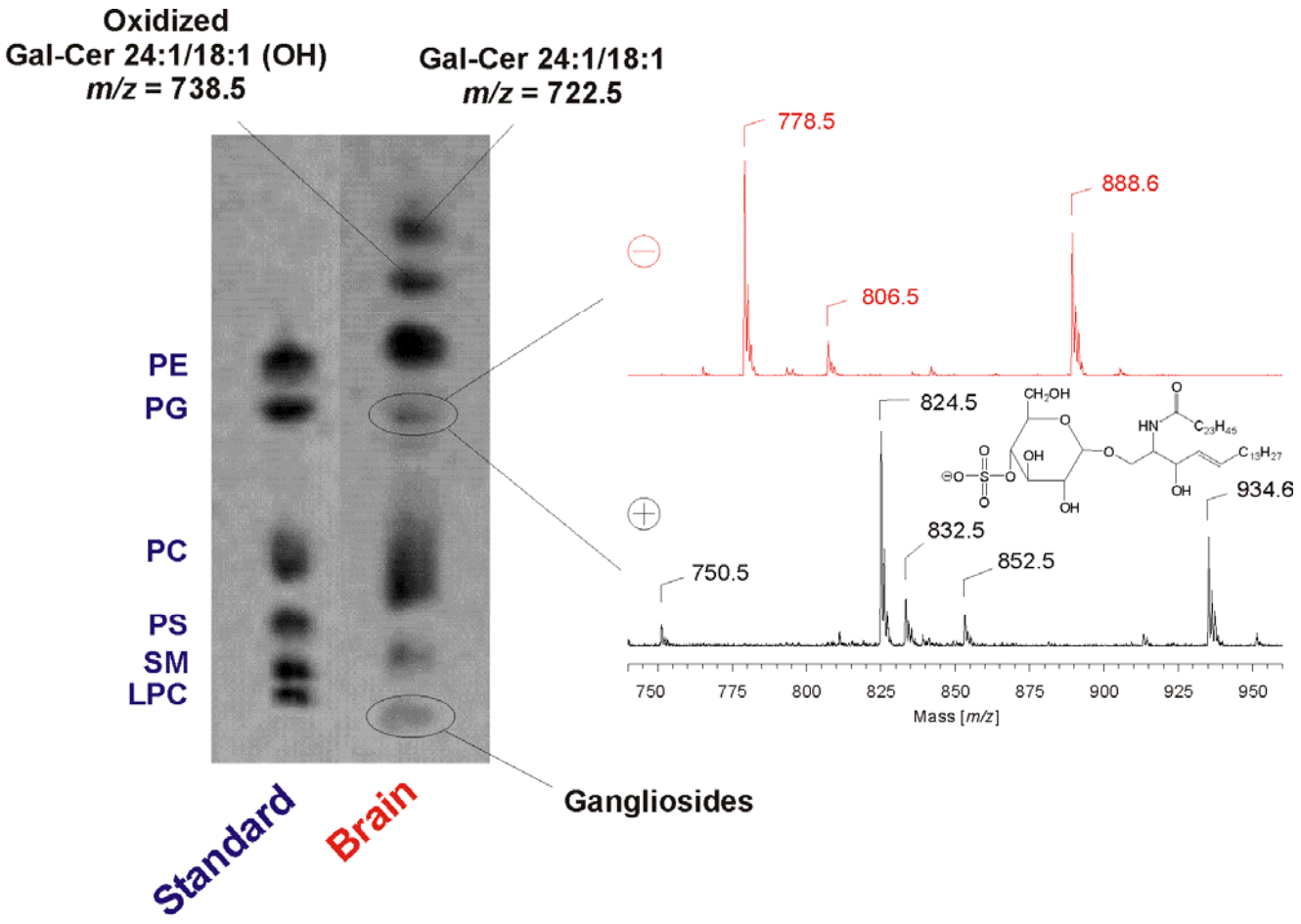
Second Example: Brain Lipids



Complex Tissue ⇒ Complex Lipid Composition
⇒ Different TLC Runs are required!

NMALDI R LIPID

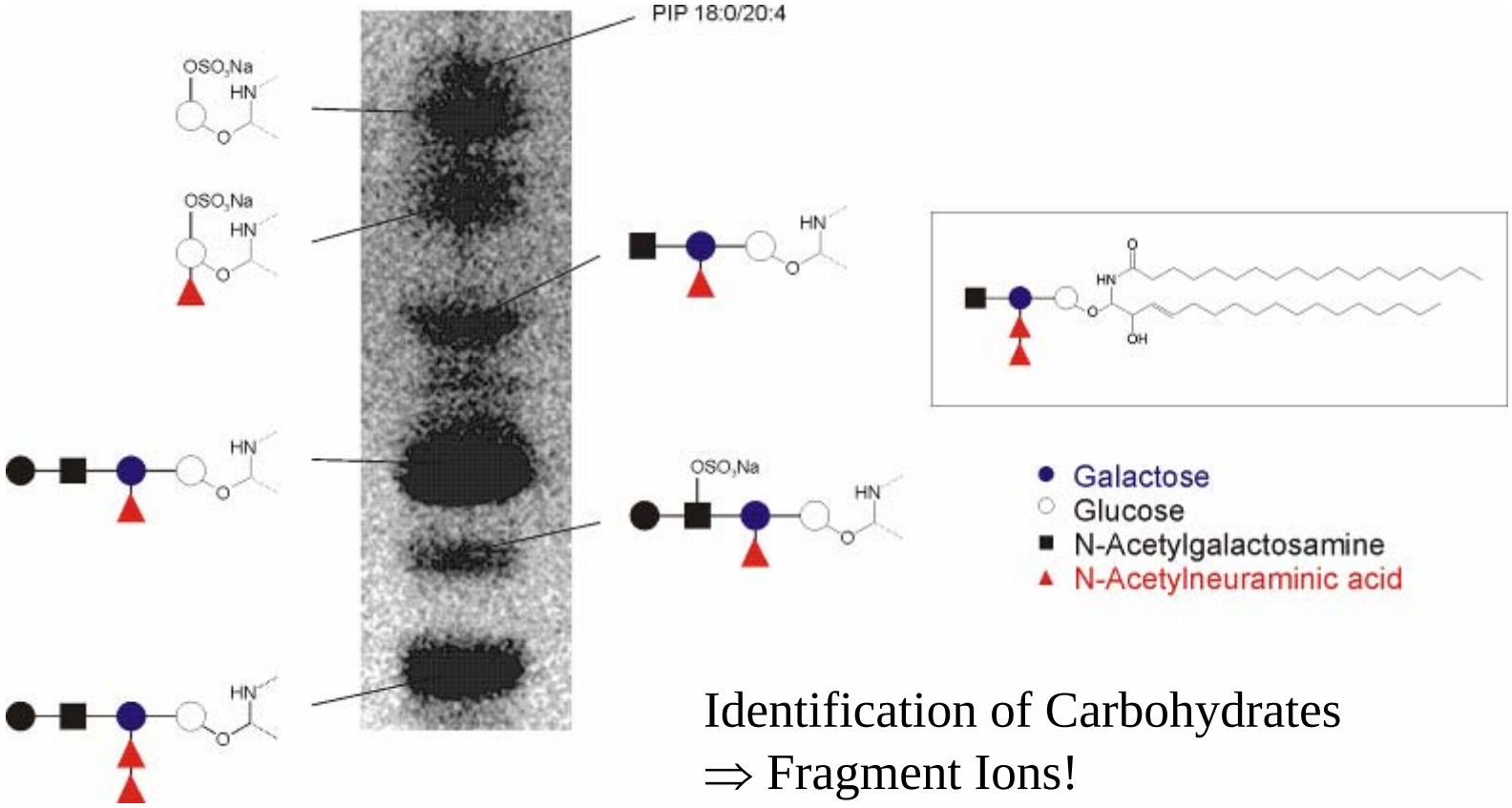
Brain Lipids: Complex Composition!



Alkaline Solvent System ⇒ Standard Lipids!

($CHCl_3$, Ethanol, Water, Triethylamine = 35:35:7:35)

Even complex Brain Lipids may be identified!

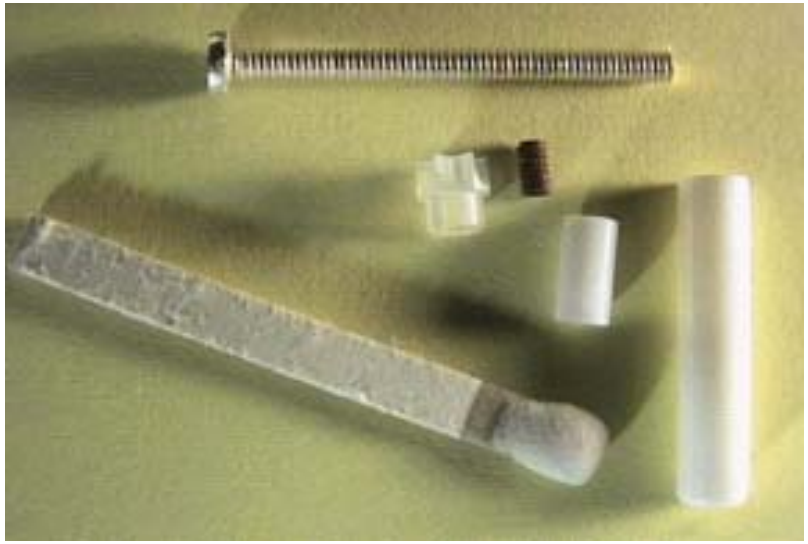


Identification of Carbohydrates
⇒ Fragment Ions!

Acidic Solvent System ⇒ Complex Glycolipids

$CHCl_3$, Acetone, CH_3OH, CH_3COOH, H_2O = 46:17:15:14:8

And if Fragmentation does not work? ⇒ NMR!



Only very small Amounts of the Analytes (at least for ^{1}H) are required!

Problem:
Purity of Solvents and/or complete Removal!

Separate Lipids by TLC as usual

↓

Scratch relevant Fractions from the TLC Plate

↓

Suspend Particles in water (D_2O)

↓

Record NMR Spectrum

NMALDI LRPID

Summary and Conclusions

- **The demonstrated Method is fast and convenient**
- **It can be easily implemented on standard MALDI Devices (axial Geometry; UV Laser)**
- **Resolution and Accuracy: Sufficient to allow the Differentiation of individual Lipid Species (2 amu, one Double Bond)**
- **Very sensitive and reproducible (qualitative!)**
- **Quantitative Data Analysis??**

⇒ Next Time!!

NL MALDI RAPID

UNIVERSITÄT LEIPZIG

Thank you very much!

Beate

Rosi

Ariane

BRUKER DALTONICS

Detlev Suckau
Martin Schürenberg

Deutsche Forschungsgemeinschaft DFG

DFG Schi 476/5-1
DFG Schi 476/7-1

Bundesministerium für Bildung und Forschung

BMBF 0313836