Physics PhD Workshop 2008

Physical techniques on lipidomics

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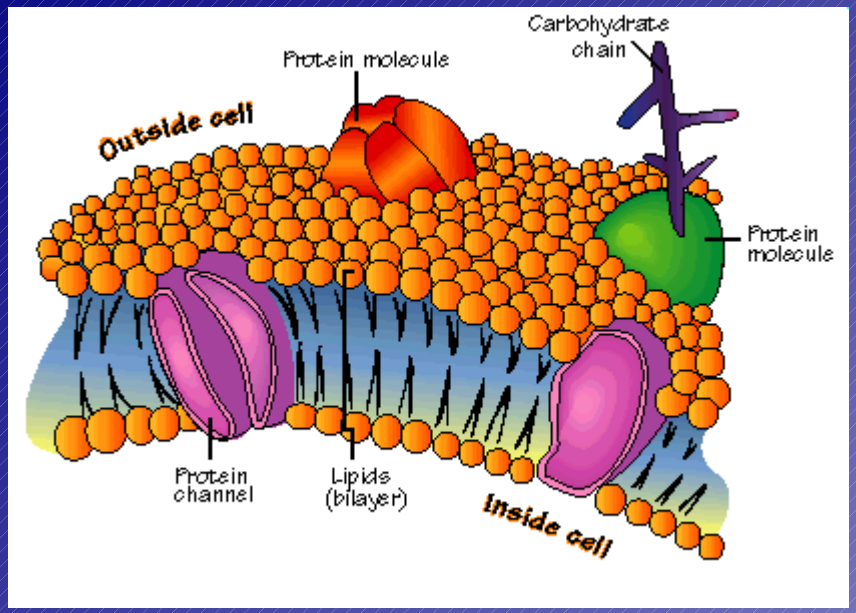

Lipids

Many different forms with many roles

Structural role

Physiological role

Principal component of cell wall

Precursors of signalling molecules

Lipid-protein interactions

Necessity of system-level analysis of lipids and factors interacting with them

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LIPIDOMICS

Promising area of biomedical research with a variety of applications

Poor knowledge due to:

- Complexity of lipids (small differences among molecules)
- Lack of powerful tools for analysis

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Solutions: two main physical techniques

- Mass spectrometry coupled with liquid chromatography
- Nuclear Magnetic Resonance (high resolution and solid state)

Mass spectrometry

Great improvement after the introduction/development of soft ionization methods

- ESI (electron spray ionization)
- APCI (atmospheric pressure chemical ionization)
- MALDI (matrix-assisted laser desorption/ionization)

with tandem mass spectrometry possibility.

Another improvement is the coupling of mass spectrometry techniques with chromatographic methods, particularly HPLC, joining the high capacity of separation of HPLC with the high sensitivity of mass spectrometry.

These methods allow to obtain information about the molecular weight of lipids, the class and the fatty acyl profile of a single phospholipid from the fragmentation pattern.

Advantages:

- High resolution and sensitivity
- Direct profiling of complex lipid mixtures

Disadvantages:

- Difficult absolute quantification
- Different degree of ionization of the phospholipid classes
- Extraction from biological matrix (for ESI)

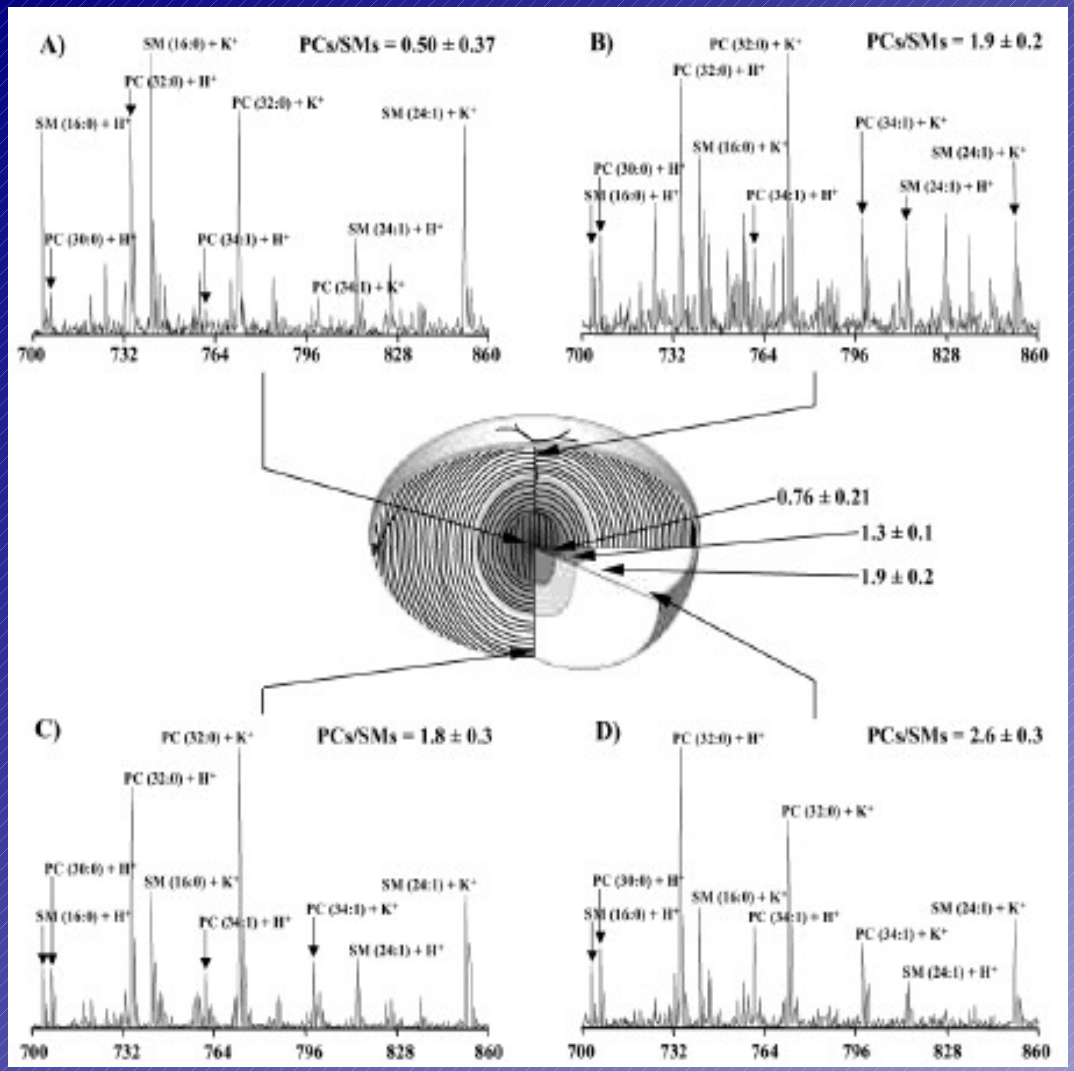


Figure from Rujoi et al., *Anal. Chem.*, **2004**, 76, 1657

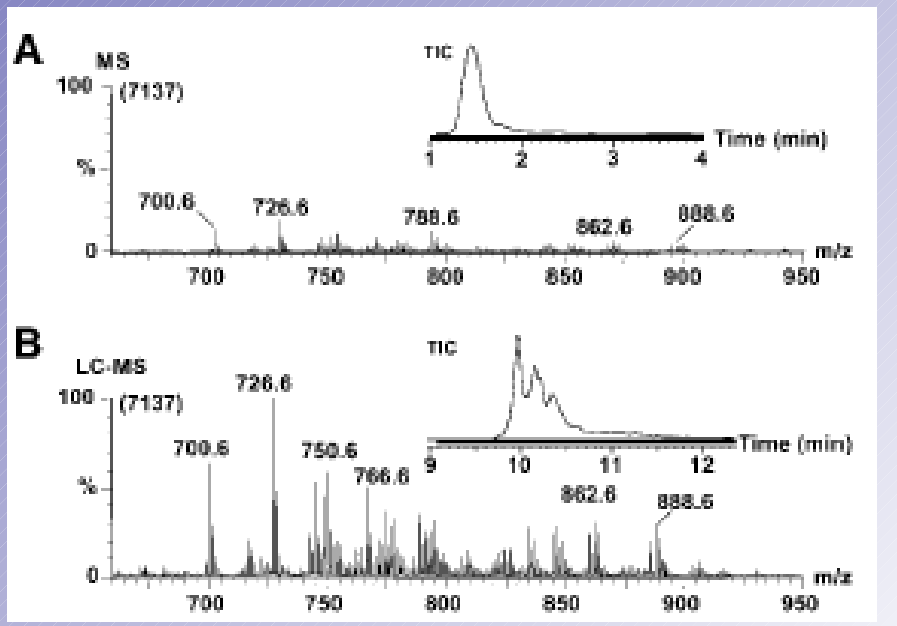
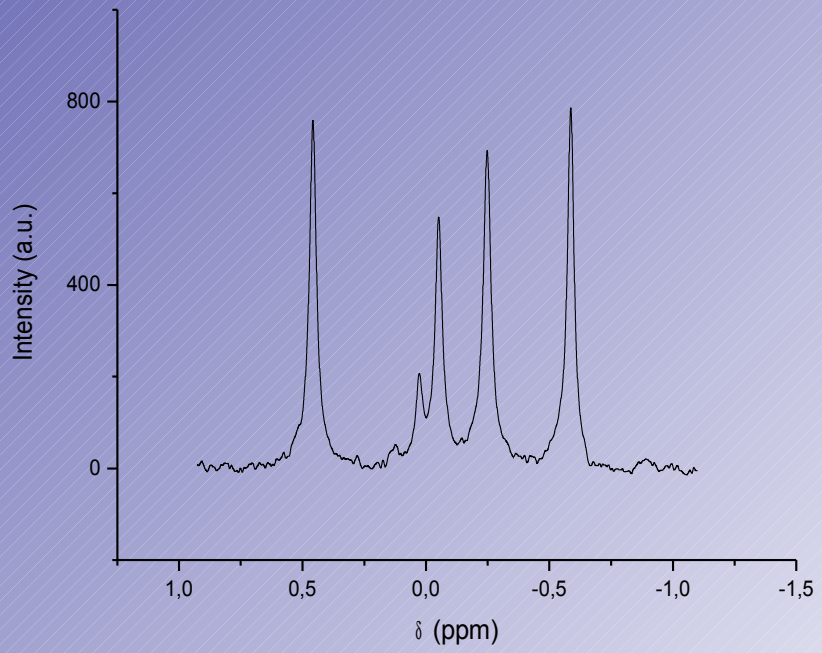


Figure from Shui et al., *J. Lip. Res.*, **2007**, 48, 1976

High resolution NMR Spectroscopy

Powerful tool for investigation of the structure and dynamics of biomembranes and for metabonomic studies

The most common nuclear probe used for analysis of phospholipid mixture is ^{31}P



Advantages:

- Direct measurement, not destructive
- Quantitative measurements

Disadvantages:

- Low sensitivity
- Need of extraction from biological matrix
- Need of a ternary mixture solvent or a detergent to separate all peaks with a difficult recovery of the sample
- Large line broadening in macromolecular aggregates

Solid-state NMR

This techniques allows to do experiments "*ex vivo*" directly on cells and tissues, avoiding extraction from biological matrix and the use of solvent mixture, with a spectral resolution similar to high-resolution NMR

Advantages:

- Direct measurement, not destructive
- Quantitative measurement

Disadvantages:

- Low sensitivity
- Need of a discrete amount of sample (1-10 mg)

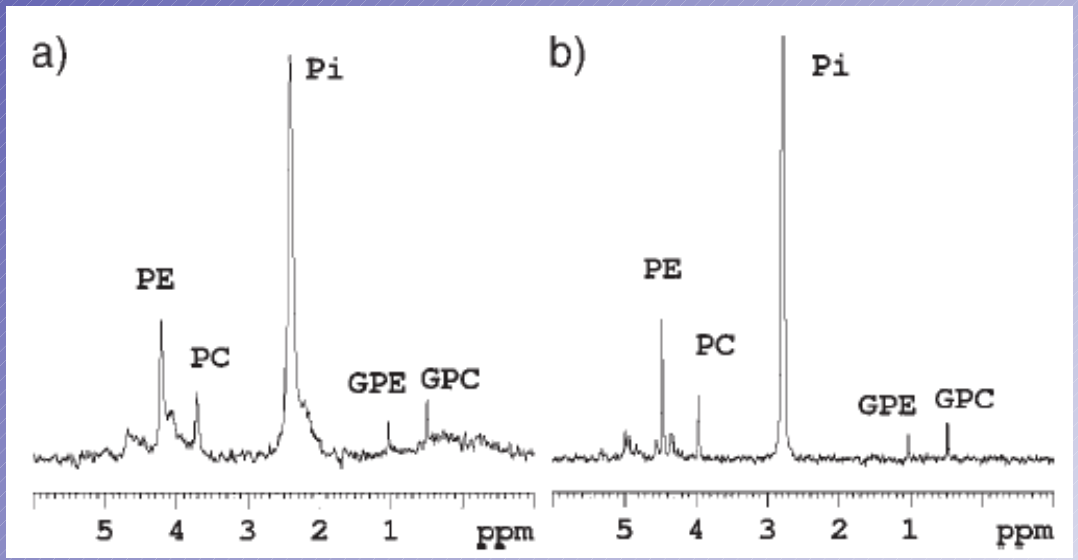


Figure from Payne et al., *NMR Biomed.*, **2006**, 19, 593

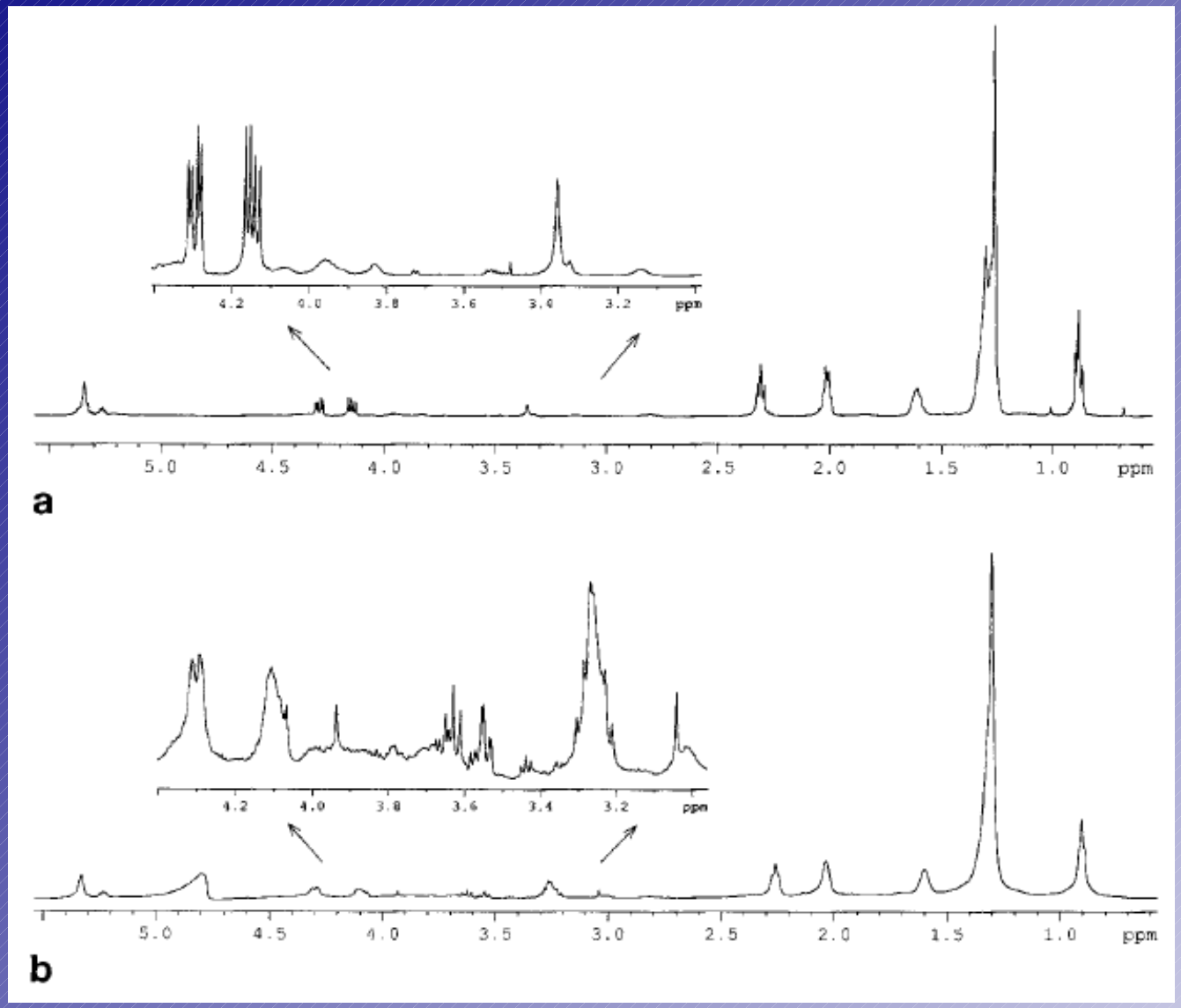


Figure from Weybright et al., *Magn. Res. Med.*, **1998**, 39, 337

We are developing studies on various systems:

- ✔ RAFT: microdomains present in the cellular membrane
- ✔ Lipids involved on Parkinson disease
- ✔ ciliates

The research team

- **Graziano Guella,** ass. prof, **group leader**
- **Ines Mancini,** ass. prof, **organic chemistry**
  (natural products chemistry, organic synthesis)
- **Rita Frassanito,** post-doc, **analytical organic chemistry**
  ("biomolecular mass spectrometry")
- **Tommaso Sandron**, 2st year PhD student in Physics, **physical organic chemistry** ("biomolecular NMR spectroscopy")
- **Mario Rossi,** chemistry-technician
  (chromatography, lab maintenance)
- **Adriano Sterni,** electronic-technician
  (all the technical aspects of scientific instr.)

Andrea Defant, visiting prof., Sec High School TN
(organic and medicinal chemistry)

Intra moenia scientific collaborations

Department of Physics, UNITN

- **Paolo Tosi (FAM)**, Reactivity of ions-neutral mol. in MS ion-sources, Development of new API-MS ionization and detection methods, Gas phase oxidations of nucleobases
- **Gabriele Viliani (OS/SOFT)**, Non-covalent host-guest complexes, Raman spectr. applications on whole eukaryotic cells, experiments and computations in vibrational spectra of nat products.
- **Antonio Miotello (IDEA)**, NMR kinetics measurements in BH_4^- cat. hydrolysis, catalysts development for the chemical production of H_2

Local Research Institutions (FBK,FEM,CNR, MTSN)

- **Mauro Dalla Serra**, B Kessler Foundation/CNR Institute of Biophysics biophysical chemistry, Multinuclear-NMR on natural and artificial membranes (vesicles, bicelles etc), structural characterization of pore forming peptides
- **Fulvio Mattivi/Giovanna Flaim**, Foundation E. Mach, bioanalytical chemistry/microbiology, NMR characterization of natural stilbenes oligomers, environmental analysis
- **Marco Cantonati/Valeria Lencioni**, MTSN Trento, environmental biology, Aquatic ecology, biodiversity and bioprospecting

Extra moenia scientific collaborations

- **Fernando Dini**, Dept of Biology, UNIPI, microbiology Secondary metabolites from cell cultures of marine ciliates, marine ecology, in vivo biotests
- **Davide Cervia,** Dept of Environmental Sciences, UNI Tuscia VT, biochemistry, Cell molecular mechanisms, apoptosis,biotests
- **Alessandro Bagno**, Dept of Chem, UNIPD, phys org chemistry
  Ab initio QM computations of NMR spectra
- **Daniele Fabbri**, InterDept Centre of Environmental Sciences UNIBO, environmental and analytical chemistry. biomass analysis, chemical synthesis,sustainable development
- **Marco Faimali**, CNR-Genova, biotests
- **Angelo Fontana**, CMIB, CNR-Napoli,, nat products chemistry
  Lipids analysis, biosynthetic experiments
- **Tom Turk,** Dept of Biology, Univ of Ljubljana,Slovenia biochemistry, Natural polycharged oligomers, biotests
- **R.H. Scott**, Coll of Med Science, Univ Aberdeen, Scotland, nat products chemistry
- **Danielle Skropeta**, University of Wollongong,Australia,
  nat products chemistry, Antarctic biodiversity, climate change

Research areas

Natural products chemistry: **isolation and structural characterization of new molecules,** total synthesis, micromolecular chemotaxonomy, drug discovery, lipid profiling of natural membranes

Biophysical chemistry: bindings in non-covalent host-guest complexes, supramolecular chemistry, MS and NMR measurements on artificial membranes liposomes, micelles and bicelles, **lipidomics**, structural characterization of pore-forming peptides

Physical Chemistry: mechanistic investigations of ion-molecules processes in MS ion-sources, gas phase oxidations of nucleobases, ab initio computations on small-medium organic mol. or complexes