MASS SPECTROMETRY IN METABOLOMICS

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Stanford Mass Spectrometry Users' Meeting

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Stanford University MASS SPECTROMETRY

Origin of Metabolomics

Proc. Nat. Acad. Sci. USA
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Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

(orthomolecular medicine/vitamins/controlled diet)

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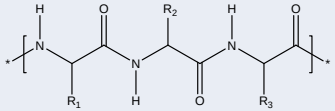
Contributed by Linus Pauling, July 29, 1971

Metabolomics and Proteomics

	Proteomics	Metabolomics
Quantitation	**Under development** SILAC, AQUA, iTRAQ Relative quantitation (x fold)	**Well established** Classical Analytical Chemistry Absolute (nM, pg/mL) or relative (x fold) quantitation
Identification	**Well established** *De novo* sequencing  PTMs still a challenge	**Under development** Huge diversity of structures, NMR often required Moderate success with mass spectral libraries

Types of Experiments in Metabolomics

targeted	non-targeted

quantitative	semi-quantitative	
• Number of analyzed metabolites is limited by the number of available ***standards*** • ***Absolute quantitation*** of metabolites (nM, mg/mL)	•Number of analyzed metabolites is limited by the number of available ***library spectra*** • ***Relative quantitation*** of metabolites (fold)	•Number of analyzed metabolites is limited by capacity of ***analytical instrumentation*** • ***Relative quantitation*** of metabolites (fold)

Applications of Metabolomics

- Studies of biochemical pathways
- Observational studies ("hypothesis generating" studies)
  - Phenotyping
  - Search of diagnostic biomarkers of disease
  - Early detection of toxic effects of drug candidates

GC-MS Analysis of Metabolites: Overview

- **50-600 (300) amu mass range**
  mono- and disaccharides, amino acids, fatty acids (mostly primary metabolites)
- **Derivatization required**
- **Metabolite libraries are available** due to instrument-independent and well understood nature of electron ionization that generates extensive fragmentation and information reach spectra
- Advantageous for flux analysis using ^{13}C labeling

GC-MS Analysis of Metabolites: Workflow

- Sample preparation:

-depletion of abundant metabolites (urine: urease treatment)

-extraction, homogenization, or lyophilization

-oximation (sugars), and silylation

- GC-MS analysis

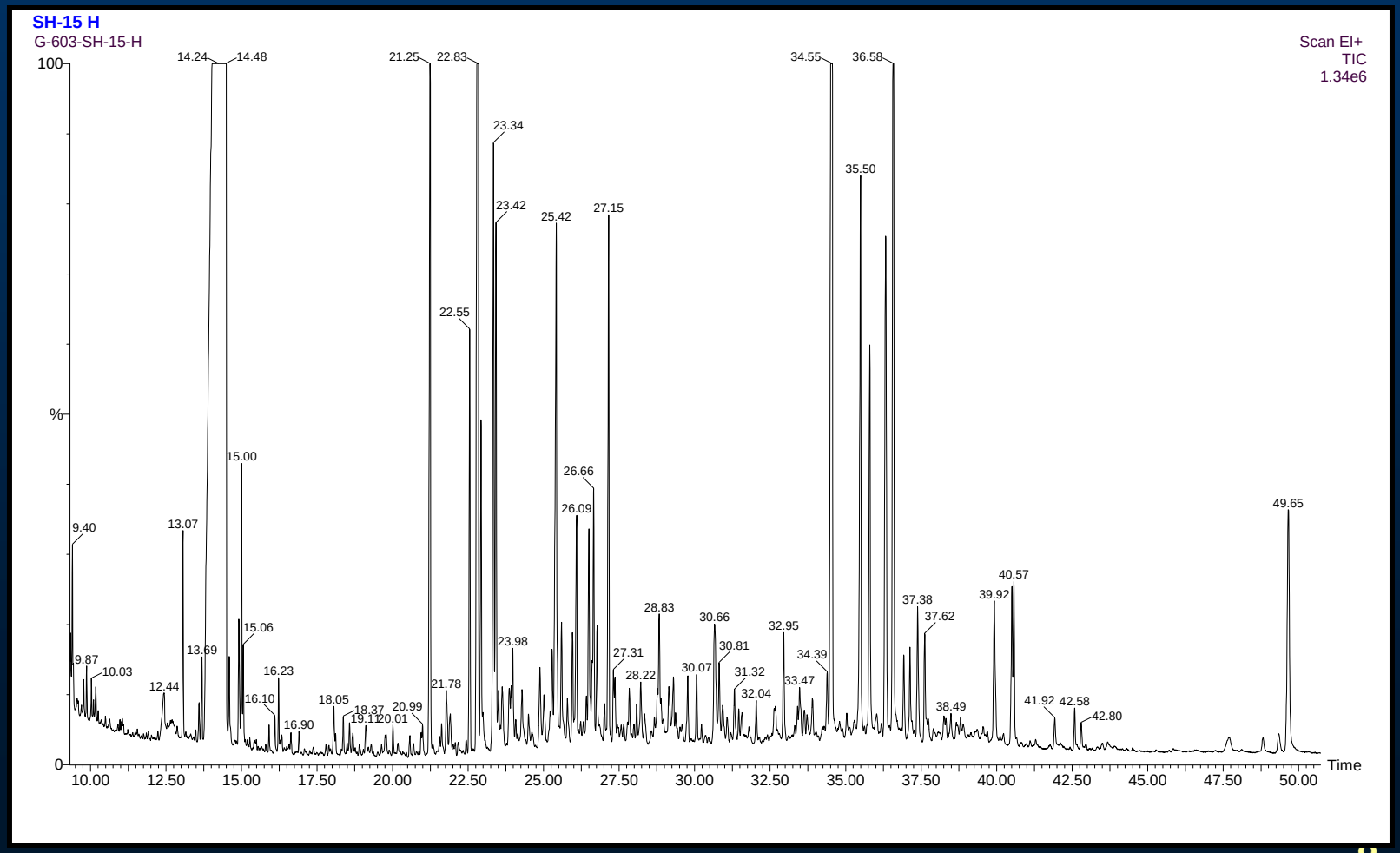
-disposable glass liners are preferred to eliminate carry-over

-retention index (RI) standards can be used to aid identification

- Deconvolution of mass spectra using libraries

-AMDIS (freeware from NIST)

GC-MS Profile of Urine



LC-MS Analysis of Metabolites: Overview

- 100-2000 amu mass range
  sugars, amino acids, peptides, lipids, secondary plant metabolites
- No derivatization required
- Low efficiency of LC
  especially for polar compounds
- Metabolite mass spectral libraries are missing
  instrument-dependent nature of collision induced dissociation, insufficient fragmentation
- Ultra-high resolution MS (FT ICR, Orbitrap, TOF) may aid identification

LC-MS Analysis of Metabolites: Workflow

- Sample preparation:

-depletion of abundant metabolites (urine: urease treatment)

-extraction or lyophilization; automation with online extraction

- LC-MS analysis

-combination of ionization modes is preferred (ESI, APCI, +, -)

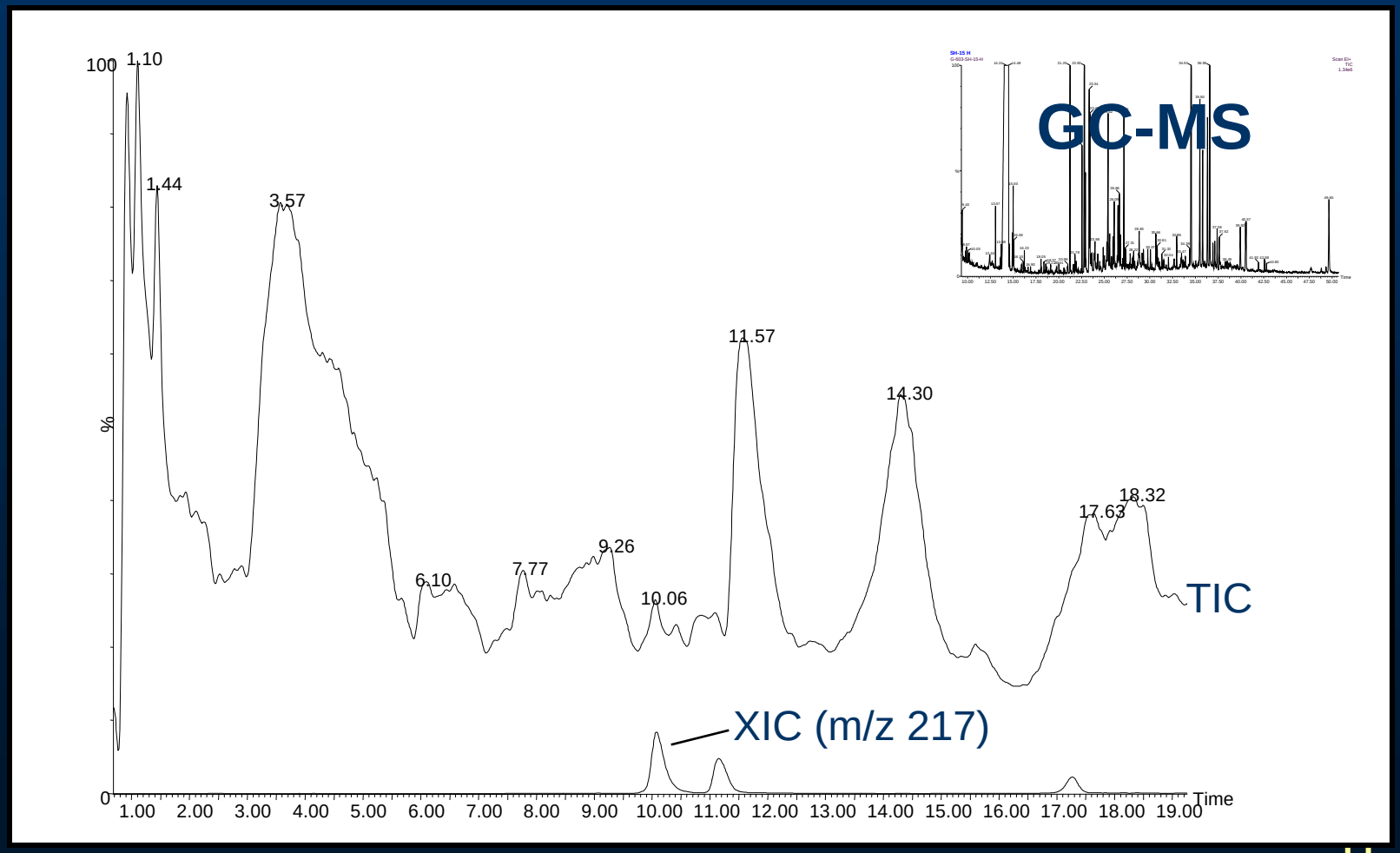
-reverse phase LC for non-polar metabolites and hydrophilic interaction chromatography (HILIC) for polar metabolites

- Detection of spectral “features” (molecular ions) using metabolomics software

-freeware XCMS and MZmine

- Identification based on retention time, accurate mass, and fragmentation (libraries)

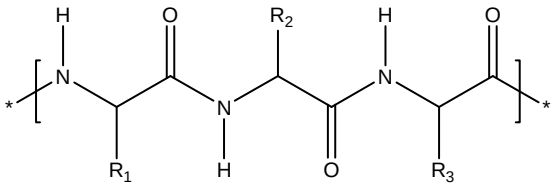
HILIC-MS Profile of Urine



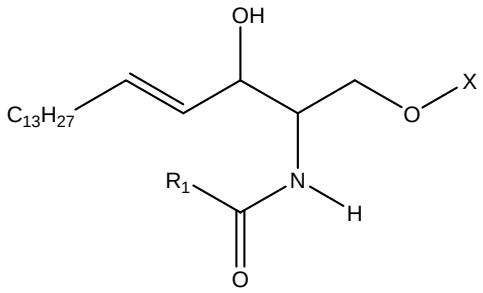
Lipid Analysis by ESI-MS

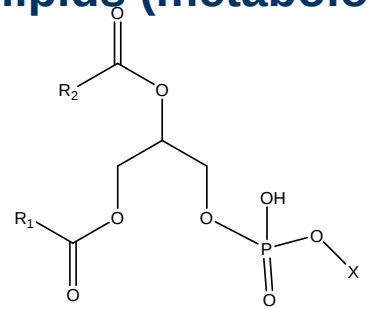
- Many classes of lipids are too heavy and non-volatile for GC separation (> 500 amu)
- Surface activity of polar lipids is advantageous for electrospray ionization (ESI)
- Some lipids have simple structure based on glycerol or sphingoid base scaffold

Peptides (proteomics)

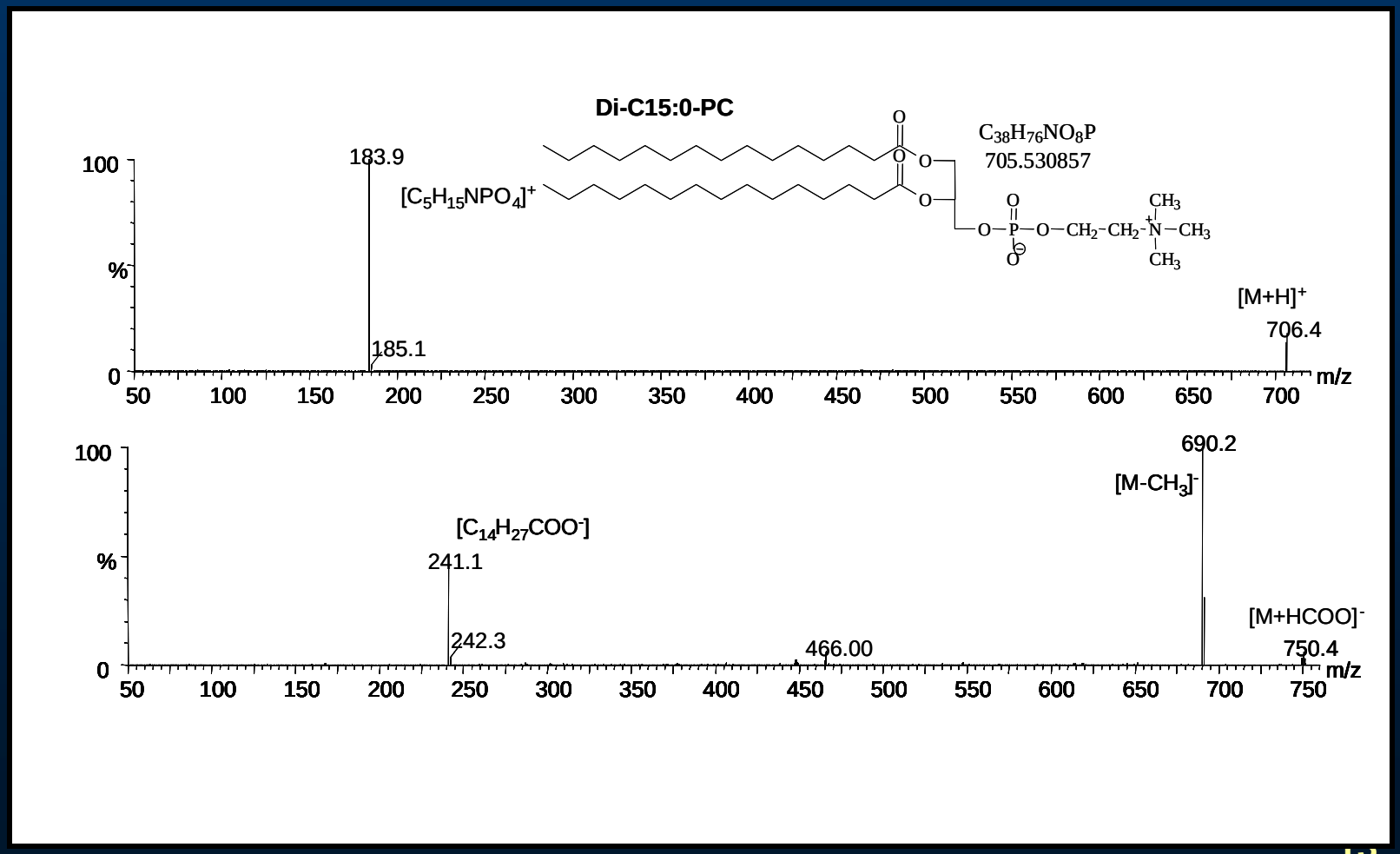


Sphingo- and phospholipids (metabolomics)





Identification of Phospholipids Using ESI-MS



Summary

- Three metabolomics platforms can be provided at SUMS in the future:

1. Profiling of phospholipids and sphingolipids using nanoESI-MS-QTOF
2. Profiling of primary metabolites including ^{13}C flux analysis using GC-MS
3. Profiling of polar (HILIC) and non-polar (C18, C4) metabolites using LC-ESI-MS-QTOF

- #1 and #2 may be automated in the future to provide simple sample drop-in service

References

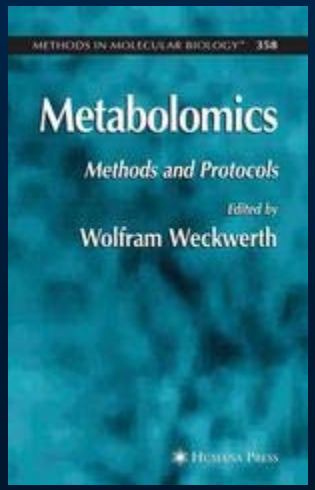
Overview:

http://fiehnlab.ucdavis.edu/

http://masspec.scripps.edu/index.php

Metabolomics: Methods and Protocols

http://www.springerprotocols.com/BookToc/doi/10.1007/978-1-59745-244-1



Metabolomics Webinar

Metabolomics: Analytics, Tools, and Applications (Free!)

Broadcast Date Thursday, August 28, 2008
Time 1:00 - 2:00 pm EDT

Key learning points for this webinar:

- Introduction to and definition of metabolomics
- Examples of metabolomes and where they fit into the larger biological picture
- Analytical techniques for uncovering and exploiting metabolomics—capabilities and limitations
- Implications of metabolomics for biomedicine, drug discovery, and development
- Metabolomics case studies in diabetes and prostate cancer
- Because the metabolome is reflected in small molecules typically generated in low abundance and with a wide dynamic range, analytical science has been scrambling to devise methods for meeting the metabolomic challenge. Two techniques in particular—gas chromatography and high-performance liquid chromatography—in tandem with mass spectrometry have become the de facto platforms for analyzing the metabolome.
- Your hosts for this webinar, **Steven Fischer, Ph.D.** (Agilent Technologies), **Chris Beecher, Ph.D.** (University of Michigan), and **Christopher Newgard, Ph.D.** (Duke University), bring years of combined experience surrounding metabolomics. They will present specific examples of how metabolomics can be applied to translational medicine, diabetes, and prostate cancer. A live Q&A session will follow the presentations, offering you a chance to pose questions to our expert panelists.

Acknowledgements

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