MASS SPECTROMETRY BASED METABOLOMICS

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MASS SPECTROMETRY

Types of Experiments in Metabolomics

targeted	non-targeted
• Number of analyzed metabolites is limited by the number of available ***standards***	•Number of analyzed metabolites is limited by capacity of ***analytical instrumentation***
• ***Absolute quantitation*** of metabolites (nM, mg/mL)	• ***Relative quantitation*** of metabolites (fold)
• Selective MS detectors (quadrupoles, triple quadrupoles)	•Scanning MS detectors (ion trap, TOF, FT)

Separation Techniques

- Direct injection/infusion (primarily lipidomics)
- Capillary Electrophoresis

  (Publications by Tomoyoshi Soga and others)

- **Gas Chromatography**
- **Liquid Chromatography**

GC-MS vs LC-MS

	GC	LC
	-**Derivatization usually required (except VOC)** -**Upper mass limit at ~400-500 amu** -Preferred for small polar metabolites (primary metabolism) -Relatively high peak capacity	-**No derivatization usually required** -**Upper mass is limited by column permeability** -Preferred for bigger molecules (e.g. some lipids, secondary metabolites) -Relatively low peak capacity
MS	-**EI ion source** (extensive fragmentation, reproducible, libraries available -CI ion source (little fragmentation, advantage for accurate mass measurement	-**ESI ion source** (ionic compounds, ion suppression) -APCI ion source (less ion suppression and more amenable for non polar compounds than ESI but usually lower sensitivity)

GC-MS Analysis of Metabolites: Overview

- **50 - 600 (400) amu mass range**
  mono- and disaccharides, amino acids, fatty acids (mostly primary metabolites)
- **Derivatization usually 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)

-homogenization, extraction and lyophilization

-derivatization: oximation (sugars), and silylation

- GC-MS analysis

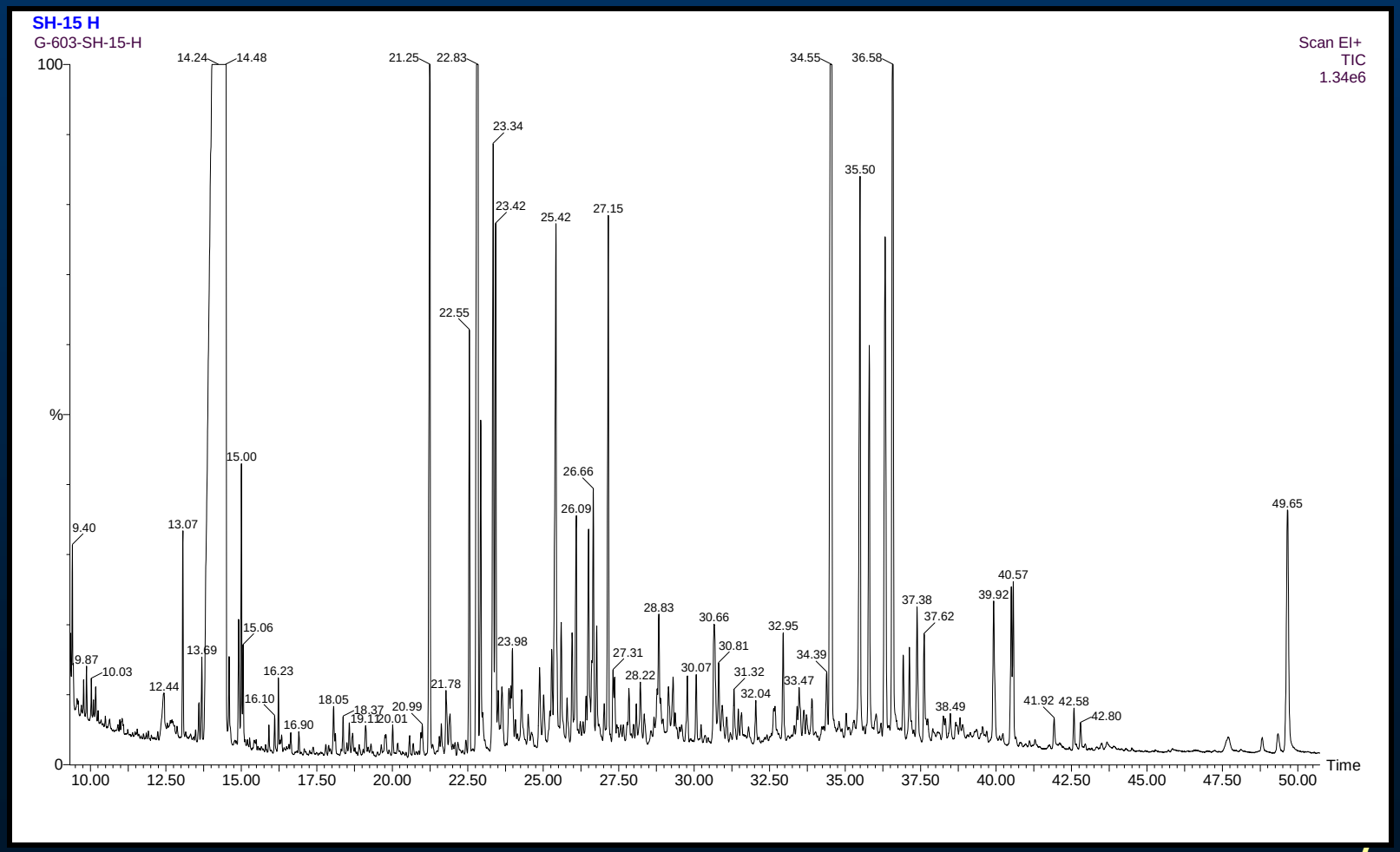
-disposable glass liners are preferred to eliminate carry-over

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

- Deconvolution of mass spectra using libraries

-AMDIS or BinBase (freeware)

GC-MS Profile of Urine



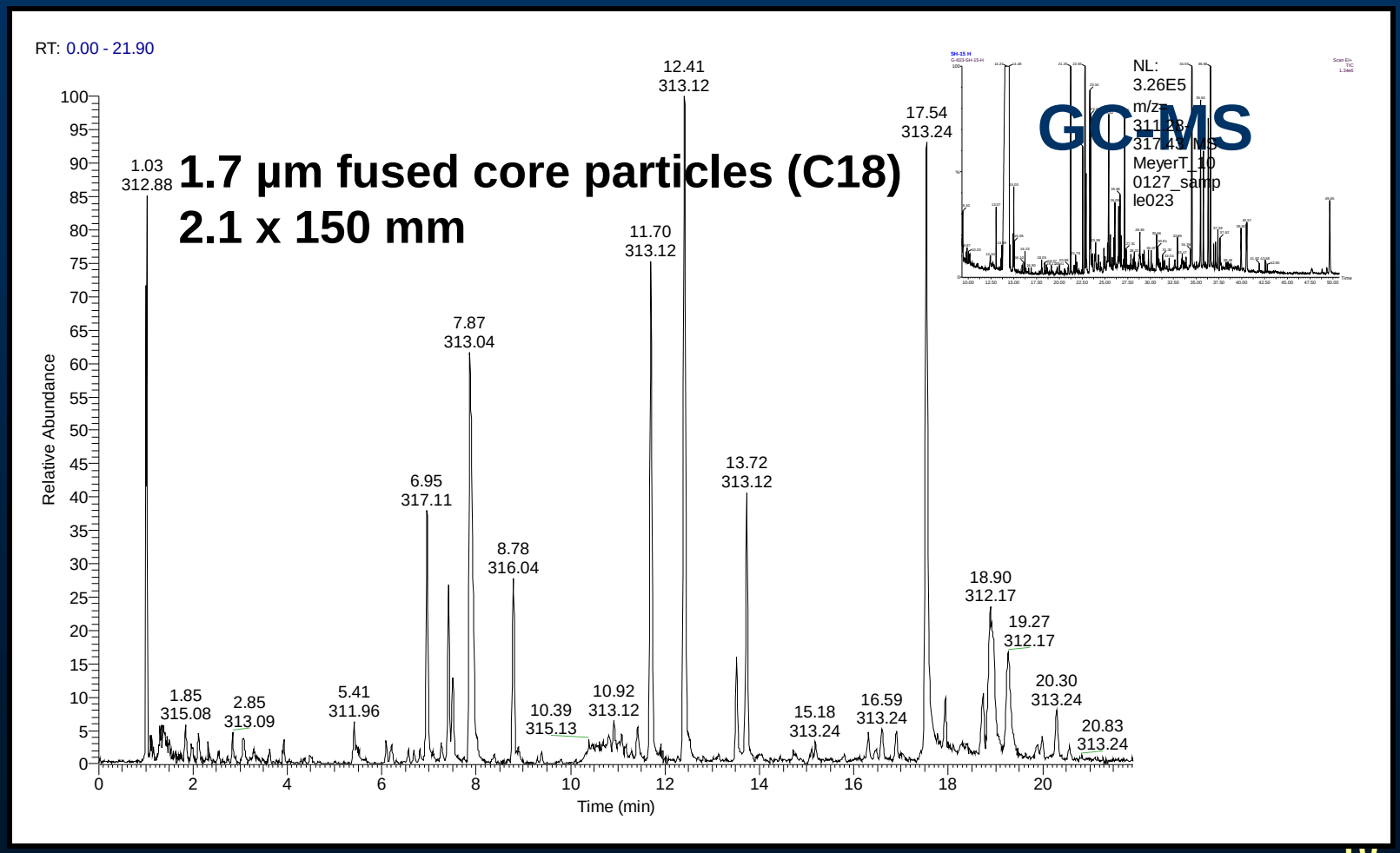
LC-MS Analysis of Metabolites: Overview

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

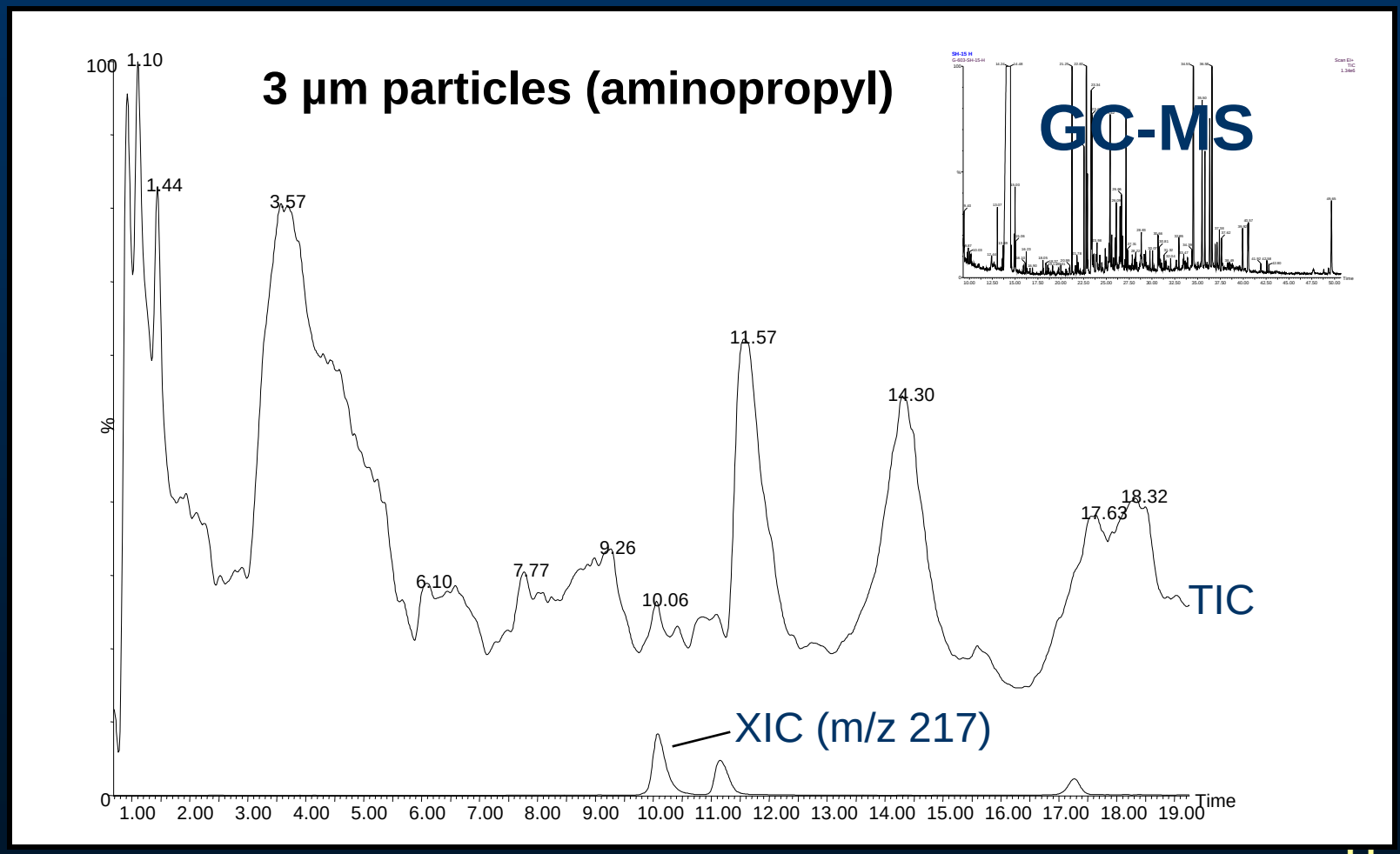
LC-MS Analysis of Metabolites: Workflow

- Sample preparation:
  -extraction or protein precipitation, lyophilization, filtration
- 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” (ions) using metabolomics software
  -freeware XCMS and MZmine
- Identification based on retention time, accurate mass, and fragmentation

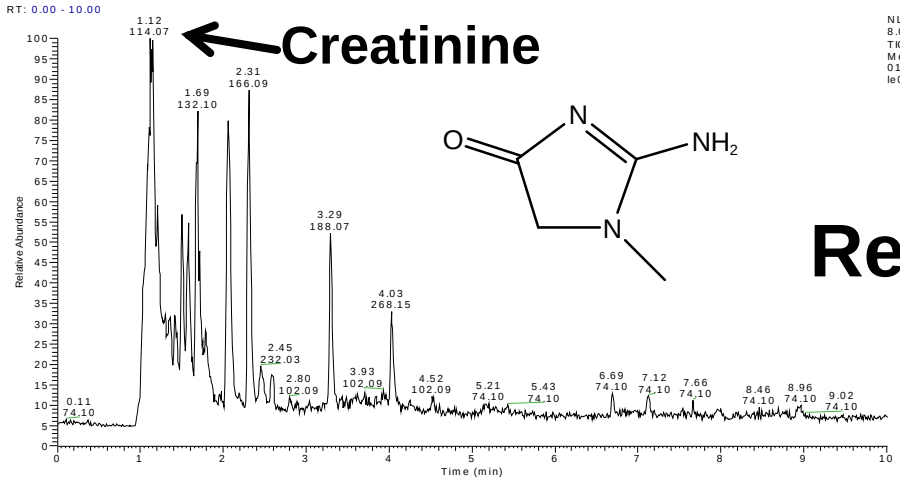
RP-LC-MS Profile of Plasma

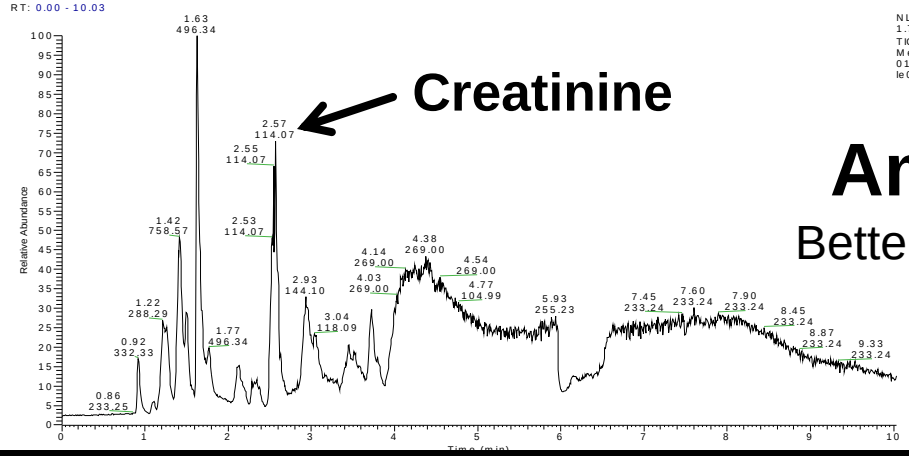


HILIC-LC-MS Profile of Urine



RP and HILIC





Reversed Phase C18

Aminopropyl HILIC

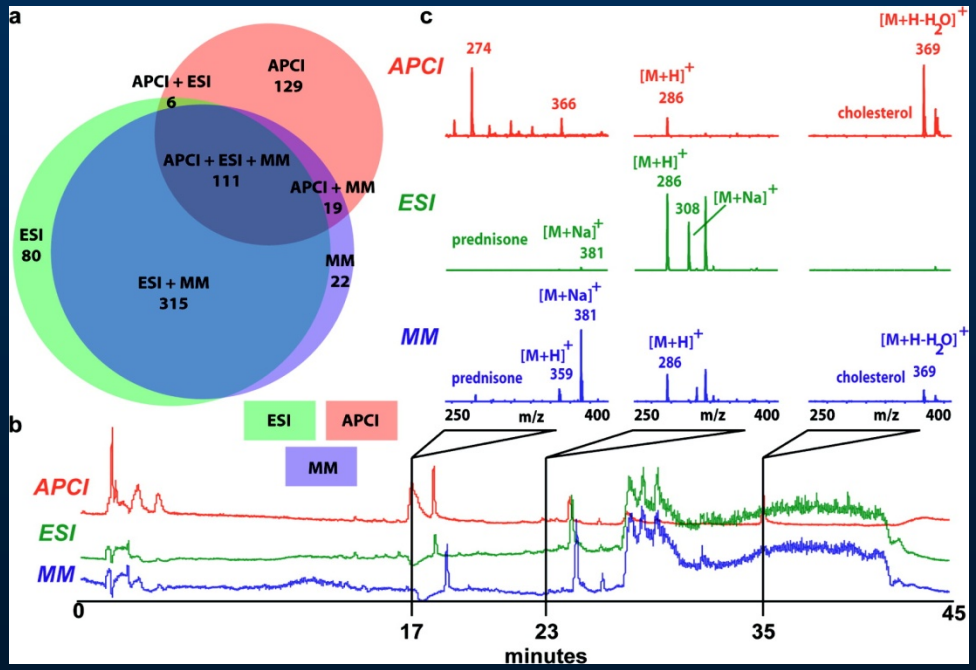
Better retention for polar molecules

Combination of Separation Modes and Ionization Techniques

Separation modes: *Reversed phase and HILIC*

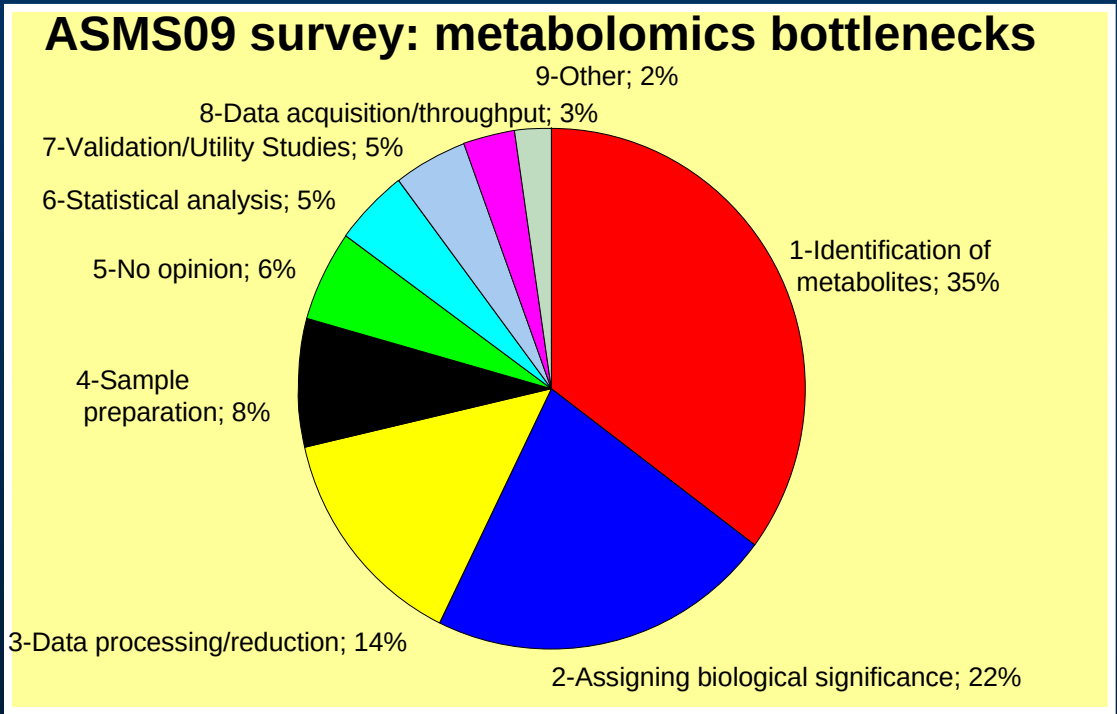
Ionization modes: *ESI and APCI or combined ESI/APCI (MM)*

Ionization polarities: *+ and -*



Nordstrom A. et al, Anal Chem, 2008.

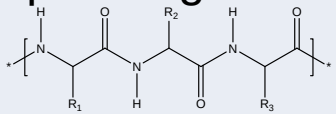
Bottlenecks in Metabolomics



Although modern MS is capable of fast polarity switching and implements combined ion sources, there are always some data quality trade-offs for using universal approaches (less points per peak, lower ionization yield)

throughput (3 %) vs. post-acquisition bottlenecks (5 + 35 + 22 + 14 = 76 %)

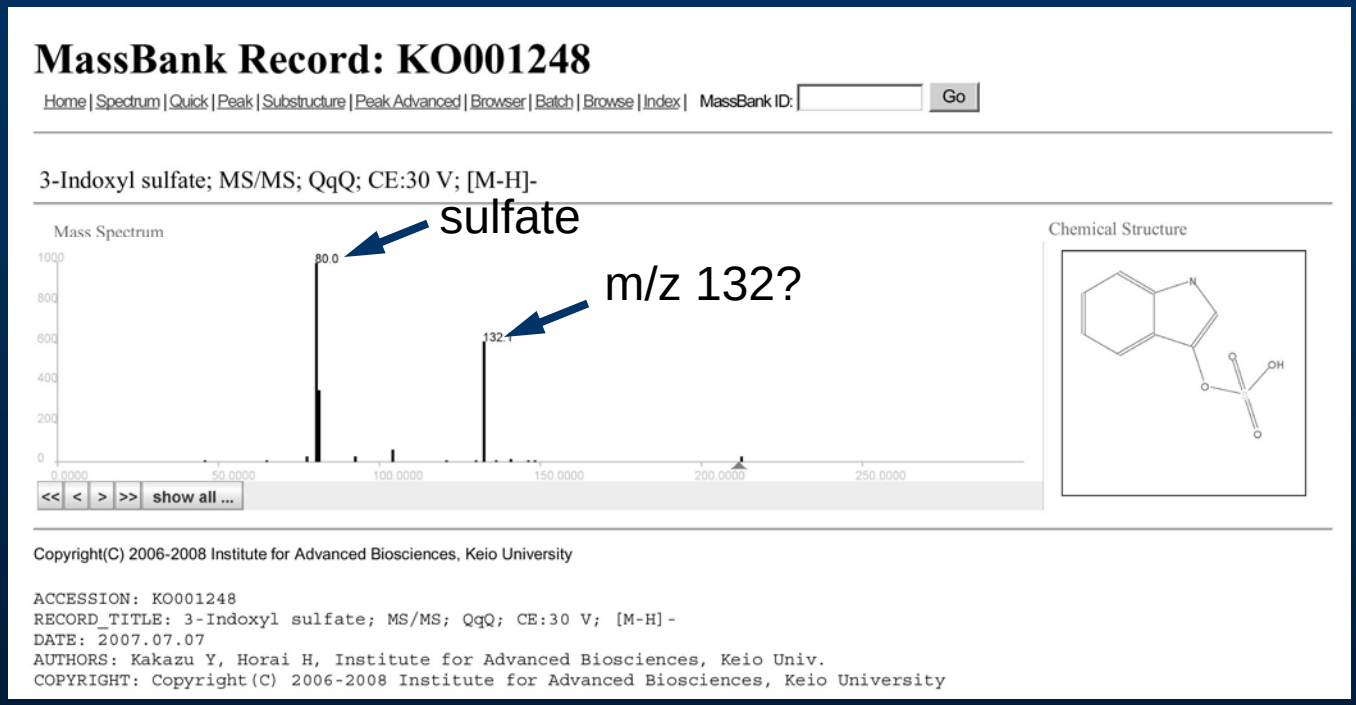
Identification in Metabolomics

	Proteomics	Metabolomics
Identification	**Well established** sequencing  PTMs still a challenge	**Under development** Huge diversity of structures, NMR often required Moderate success with mass spectral libraries

- **Peptide** structure is sequential, MS/MS experiments are usually sufficient (ion traps).

- Typical CID MS/MS does not break all bonds in **metabolites**; *accurate mass measurements* provide more information than MS/MS

Low resolution MS/MS

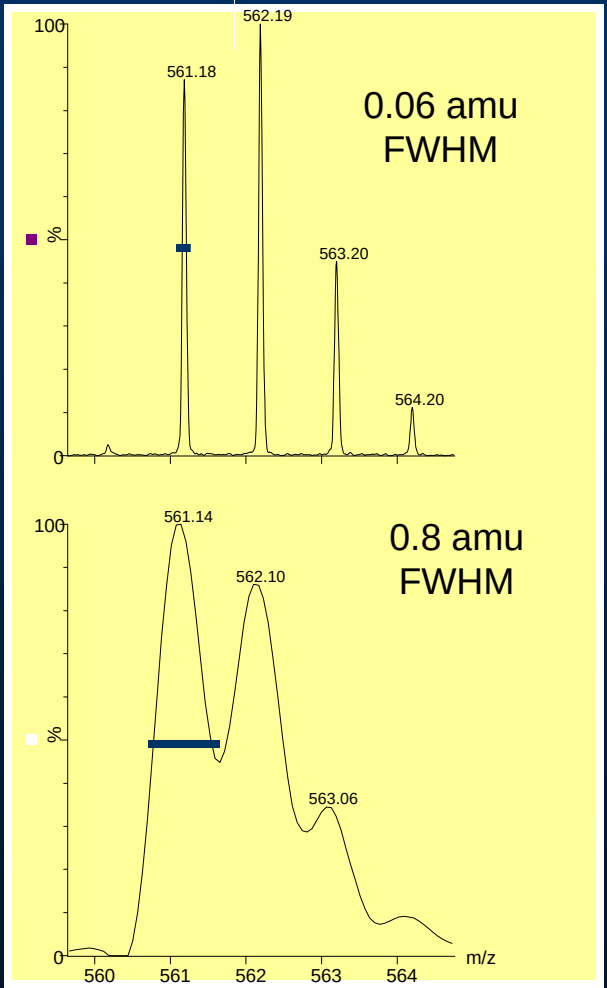


m/z 132

$C_6H_{14}NO_2$
$C_5H_{14}N_3O$
$C_9H_{10}N$
$C_5H_{10}NO_3$
C_8H_6NO
$C_4H_6NO_4$

- Typical CID MS/MS does not break all bonds in **metabolites**; *accurate mass measurements* provide more information than MS/MS

High Resolution



High Resolution: R = 561/0.06 ~ 9,000

TOF: 7,000-50,000
Orbitrap: 10^4-10^5
FT ICR: 10^5-10^6

Nominal Mass Resolution (<1000)
R = 561/0.8 ~ 700

Quadrupoles and ion traps, some TOFs

Determination of Elemental Composition from Accurate Mass

^{1}H	1.0078 u
^{12}C	12.0000 u
^{14}N	14.0031 u
^{16}O	15.9949 u

What is 28 u?

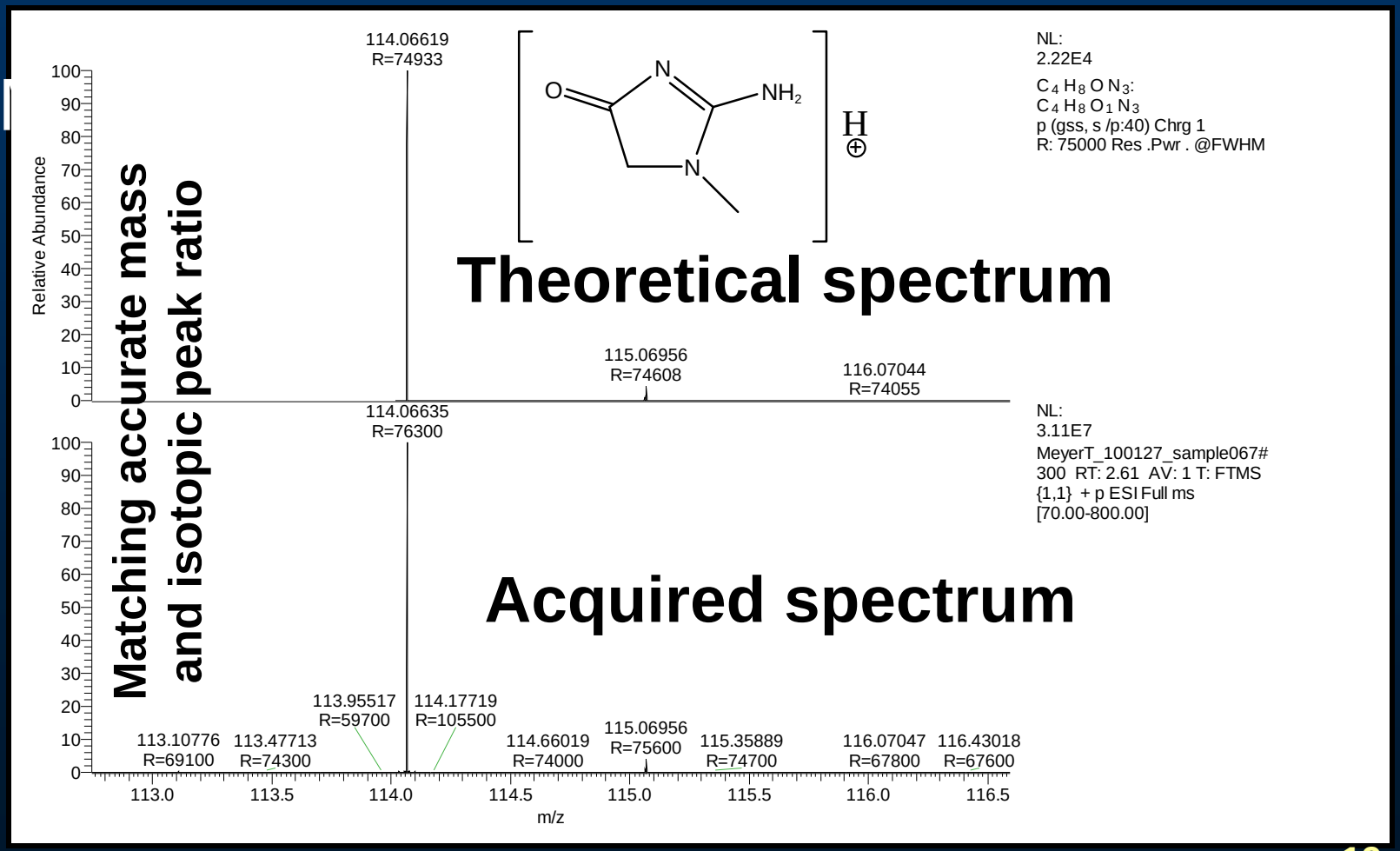
N_2 (2 x 14 u), CO (12 u + 16 u) or C_2H_4 (2 x 12 u + 4 x 1 u)?

What is 28.0313 u? [high accuracy]

C_2H_4 (2 x 12.0000 u + 4 x 1.0078 u)

Kind T. et al, BMC Bioinformatics, 2007

Identification based on accurate mass



Further identification steps

- MS/MS experiments (library search or de novo)
- Chemical derivatization or H/D exchange to map functional groups
- Comparison with pure standards
- Other techniques: NMR and X-ray crystallography (stereochemistry)

Data Processing and Statistics

- Proprietary Software

  Most of MS companies have software packages for metabolomics data analysis

- Freeware Software

  XCMS and METLIN database

  MZmine

Data output: XCMS

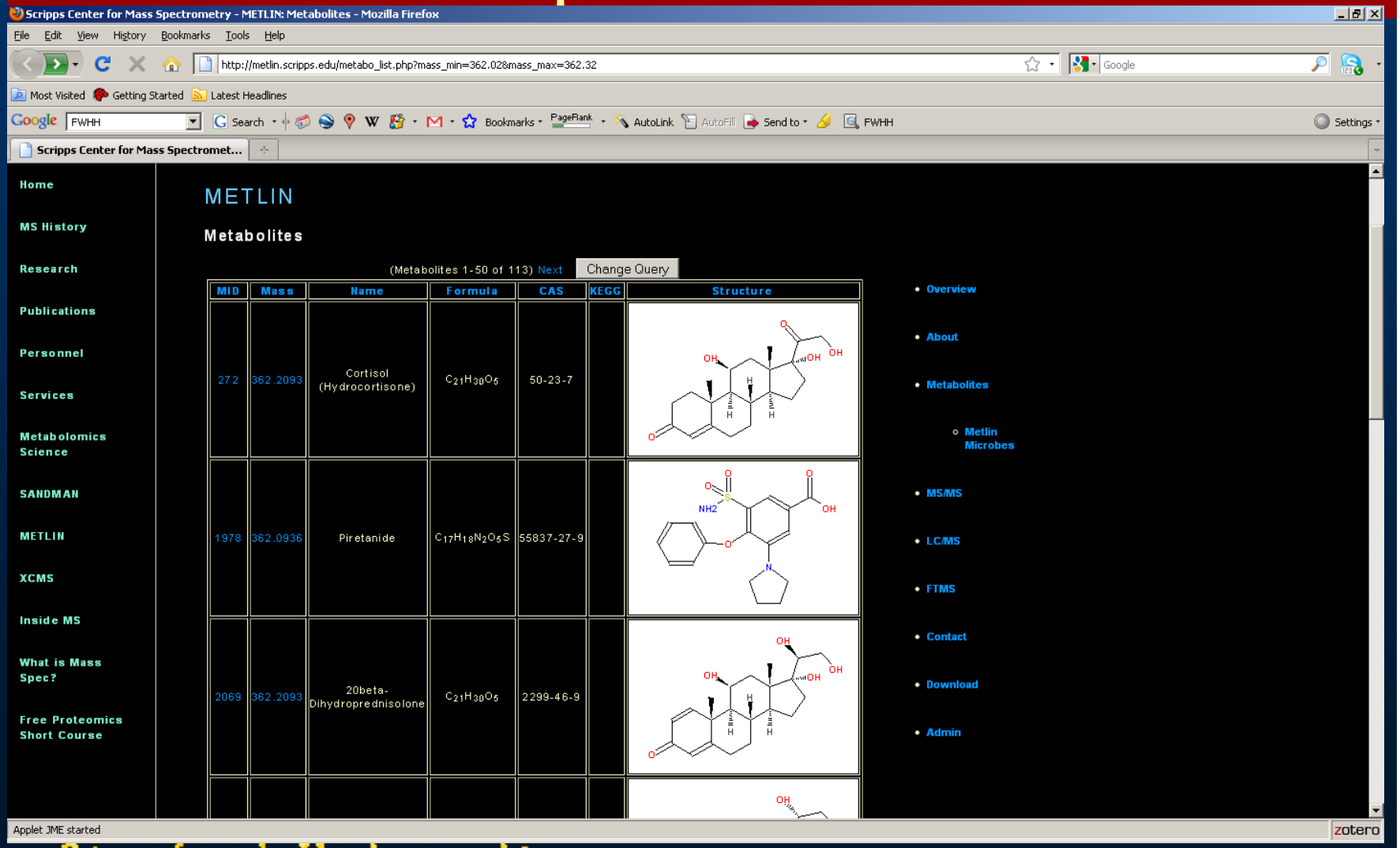
XCMS: http://masspec.scripps.edu/xcms/xcms.php

Support high resolution data and MS/MS data (XCMS^2)

Statistics			Accurate Mass			Retention Time			Groups		
fold	tstat	pvalue	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	npeaks	KO	WT
4.921	9.407	0.000814	363.1651	363.1604	363.1671	1062.014	1061.567	1063.066	3	3	0

http://metlin.scripps.edu/metabo_list.php?mass_min=362.02&mass_max=362.32

Data output: METLIN



References

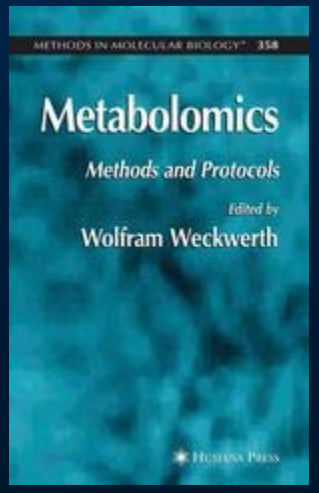
Overview:

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



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