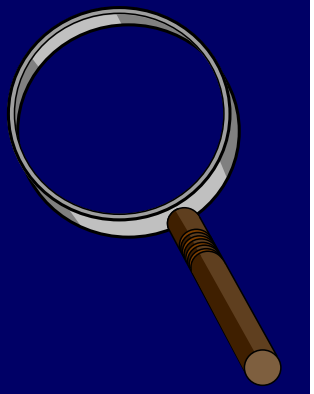
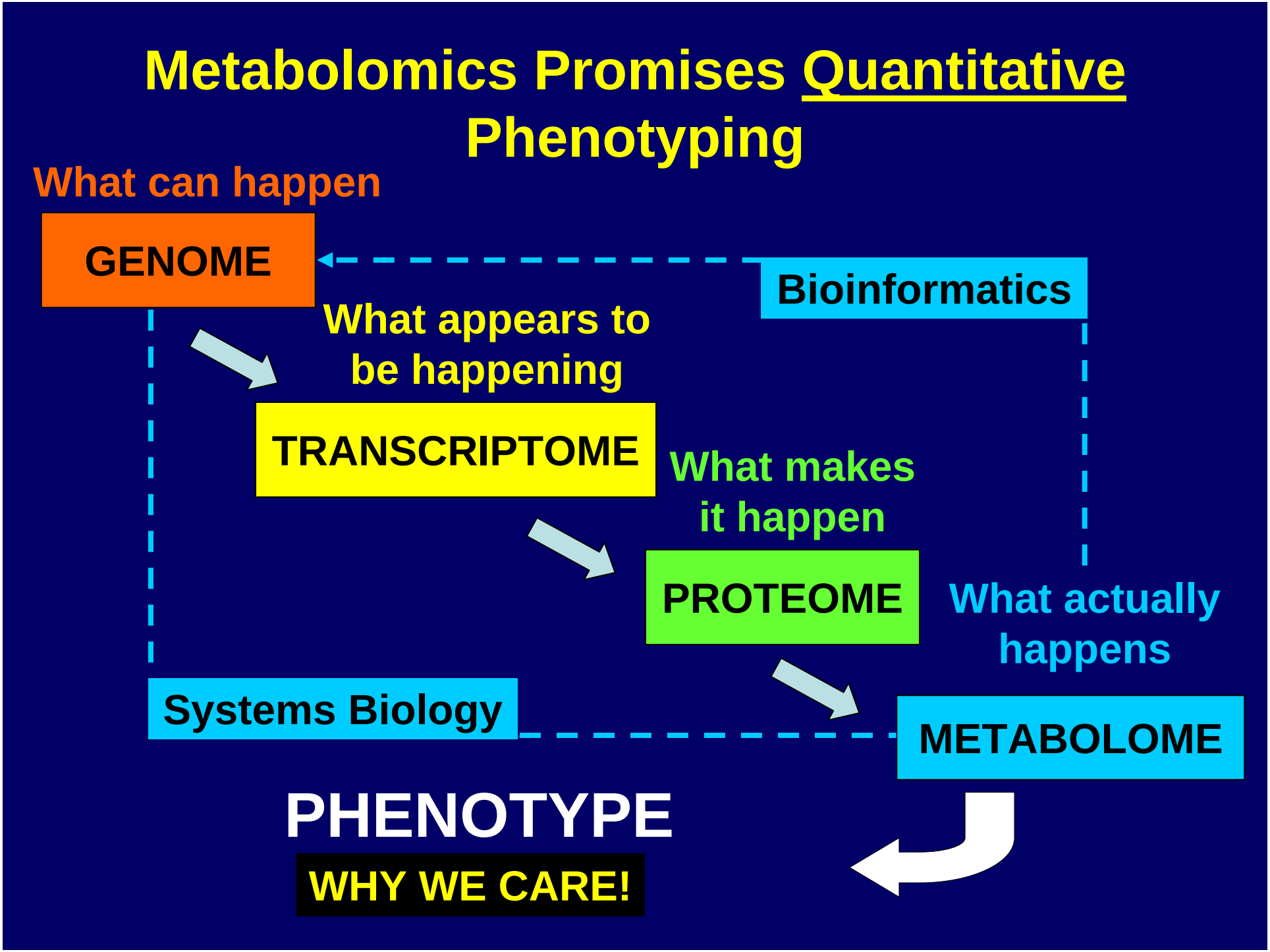
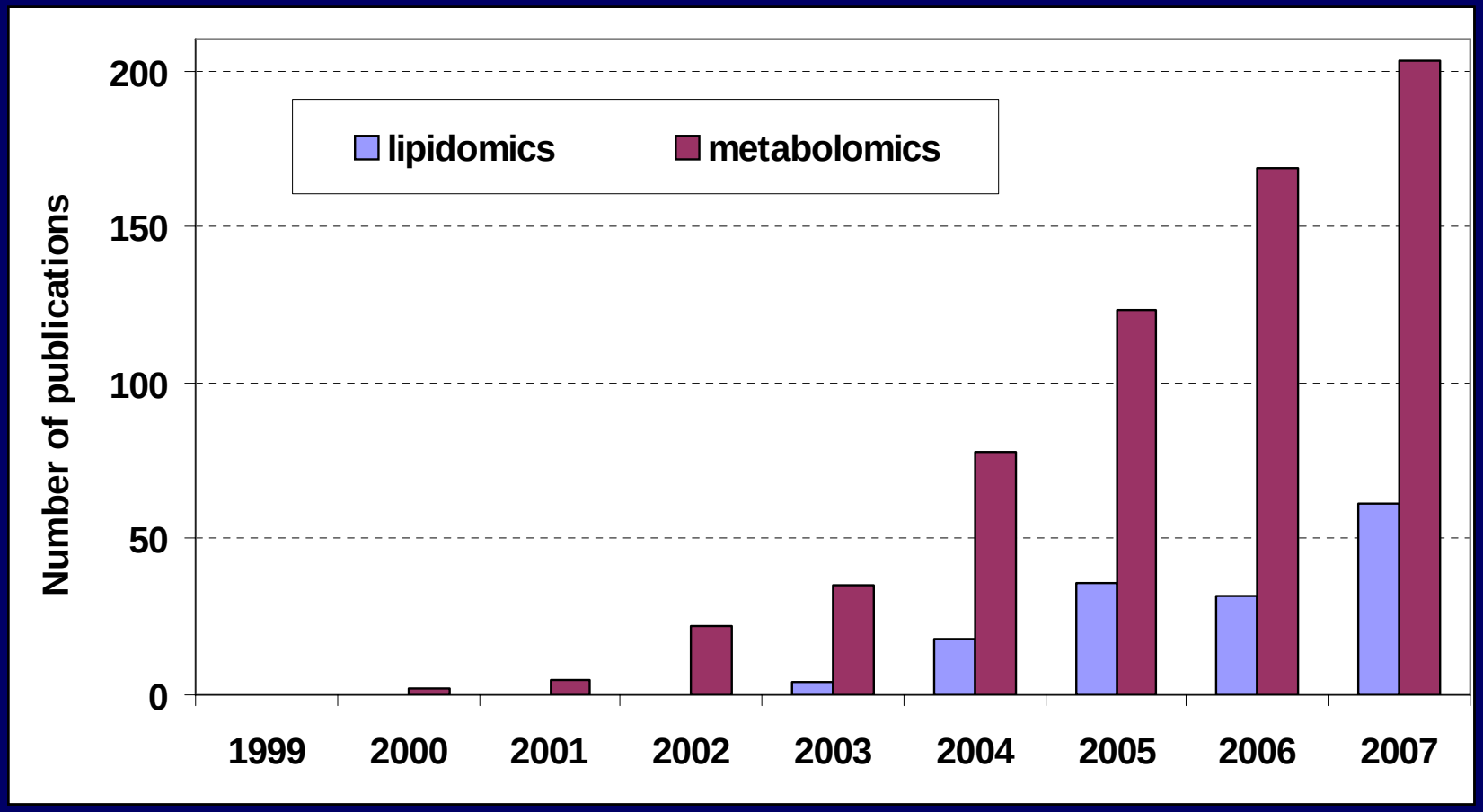
Metabolomics: quantifying the phenotype




Metabolomics Promises Quantitative Phenotyping
What can happen
GENOME
Bioinformatics
What appears to be happening
TRANSCRIPTOME
What makes it happen
PROTEOME
What actually happens
Systems Biology
METABOLOME
PHENOTYPE
WHY WE CARE!

Metabolomics publications in Pubmed



History of metabolomics

Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

(orthomolecular medicine/vitamins/controlled diet)

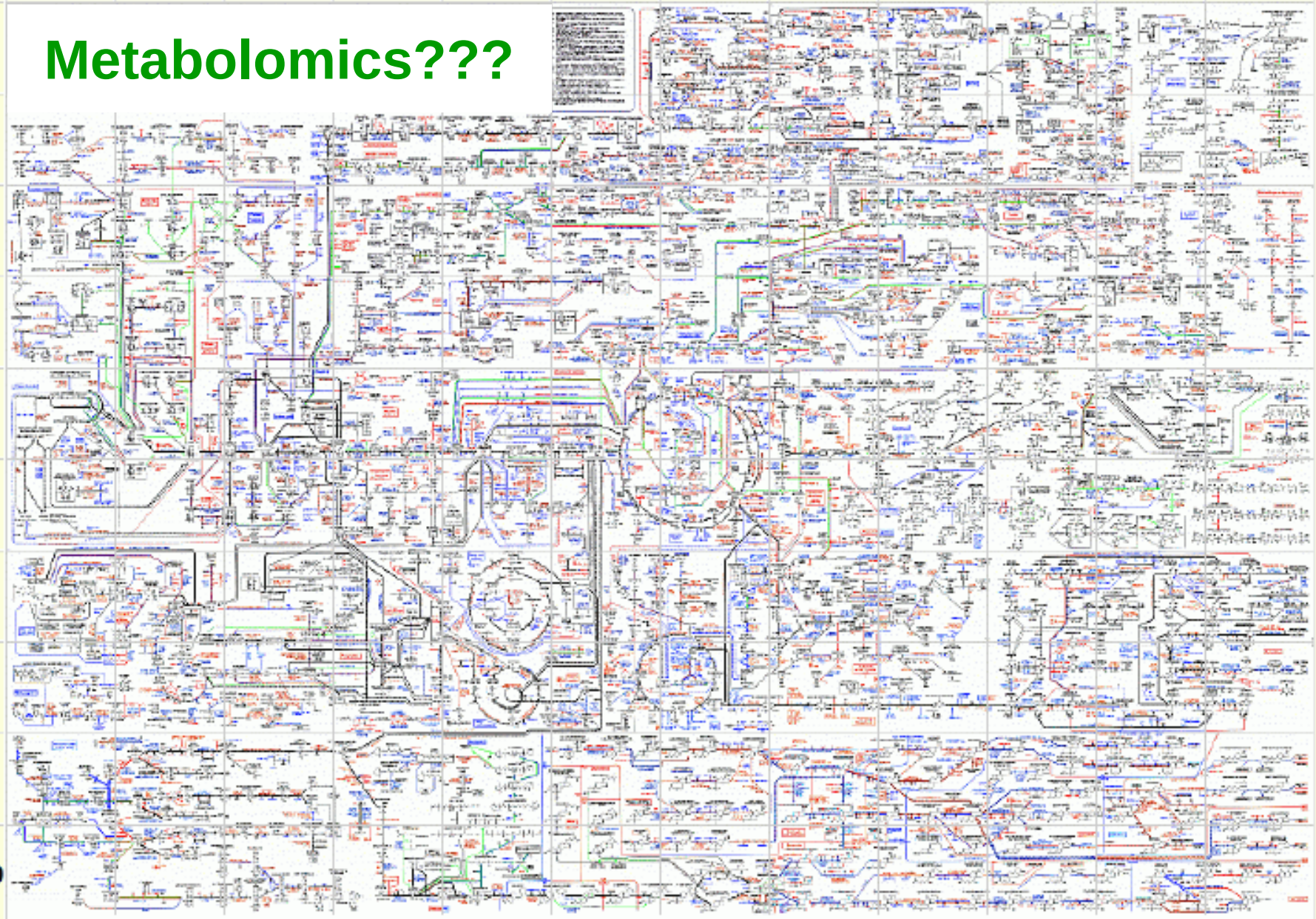
LINUS PAULING*, ARTHUR B. ROBINSON*, ROY TERANISHI†, AND PAUL CARY*

* Department of Chemistry, Stanford University, Stanford, California 94305; and † Western Regional Laboratory, U.S. Department of Agriculture

Contributed by Linus Pauling, July 29, 1971

- **metabolomics developed by Pauling in 1970**
- **the term metabolomics first used in 1998**
  - **Oliver SG et al (1998). Trends Biotechnol 16:373**
- **Metabolomics Society founded 2004**
- **January 23rd, 2007 first draft of the human metabolome “completed”**

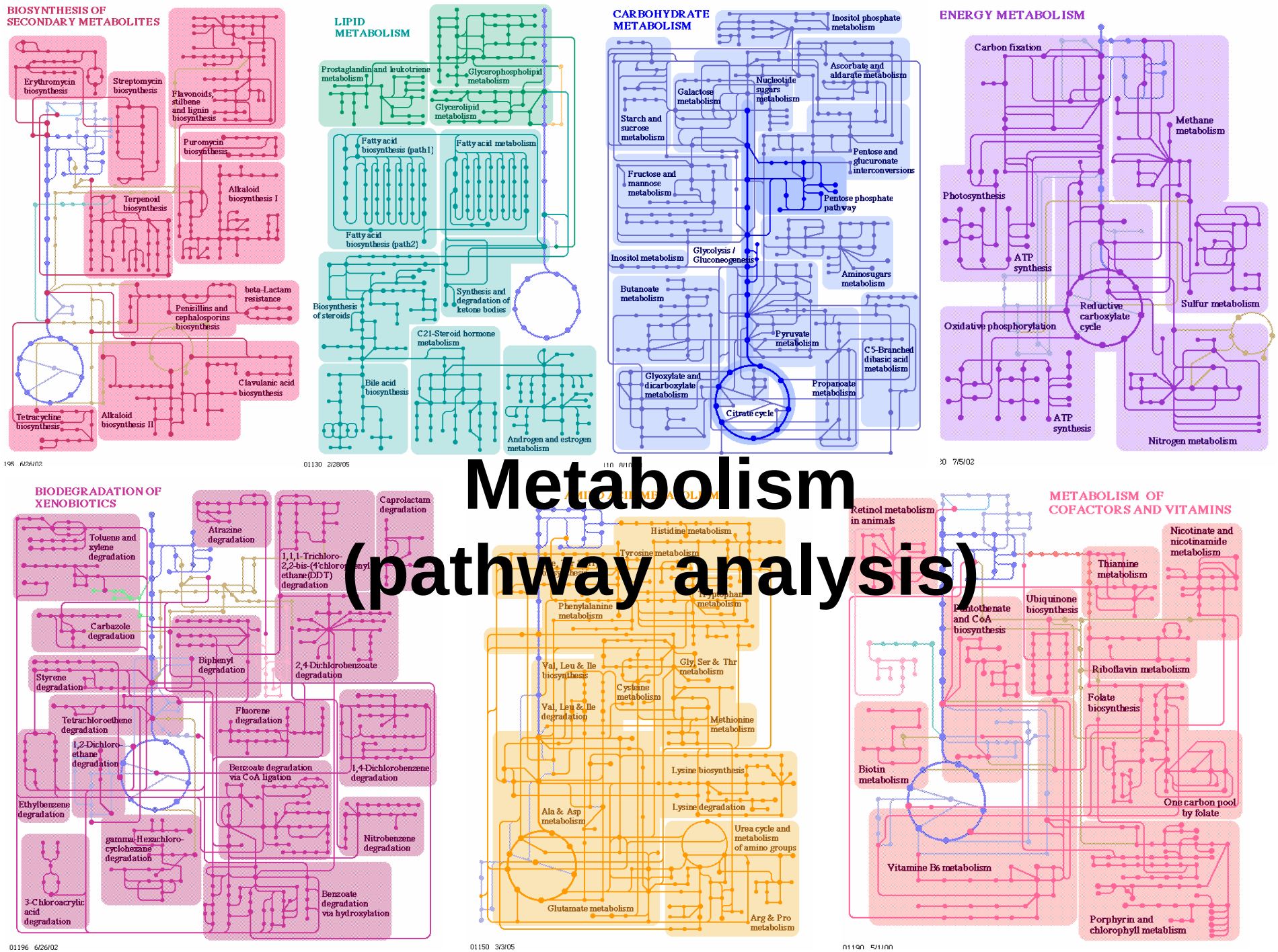
Metabolomics???



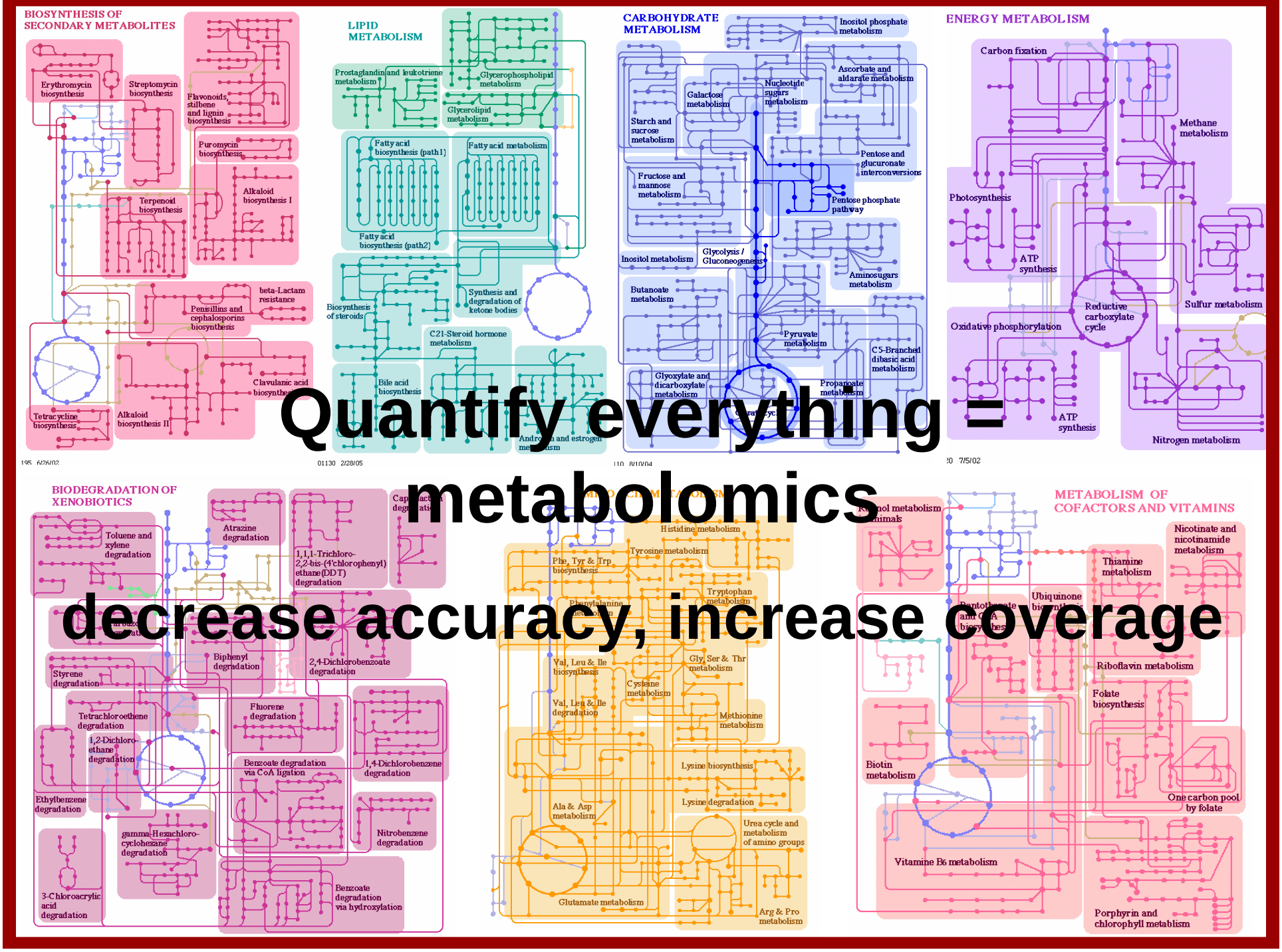
Approaches to Investigating the Metabolome

- **Metabolic Fingerprinting**
  - **Pattern recognition** to classify samples by shifts in "global" metabolite composition.
- **Metabolic Targeting**
  - **Quantification** of a small number of known compounds.
- **Metabolic Profiling**
  - **Quantification** of a group of related compounds or metabolites within a specific metabolic pathway.

- **Metabolomics**
  - **Quantification** of "all" metabolites at a defined time under specific environmental conditions.

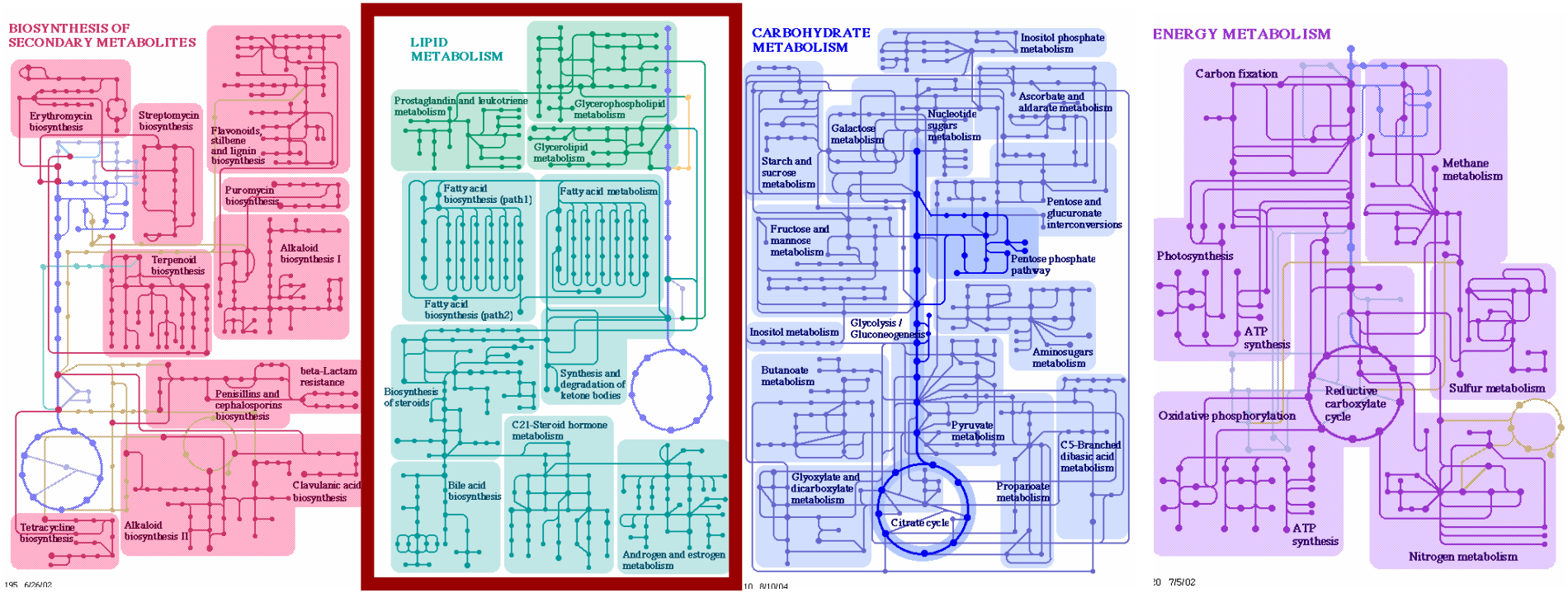


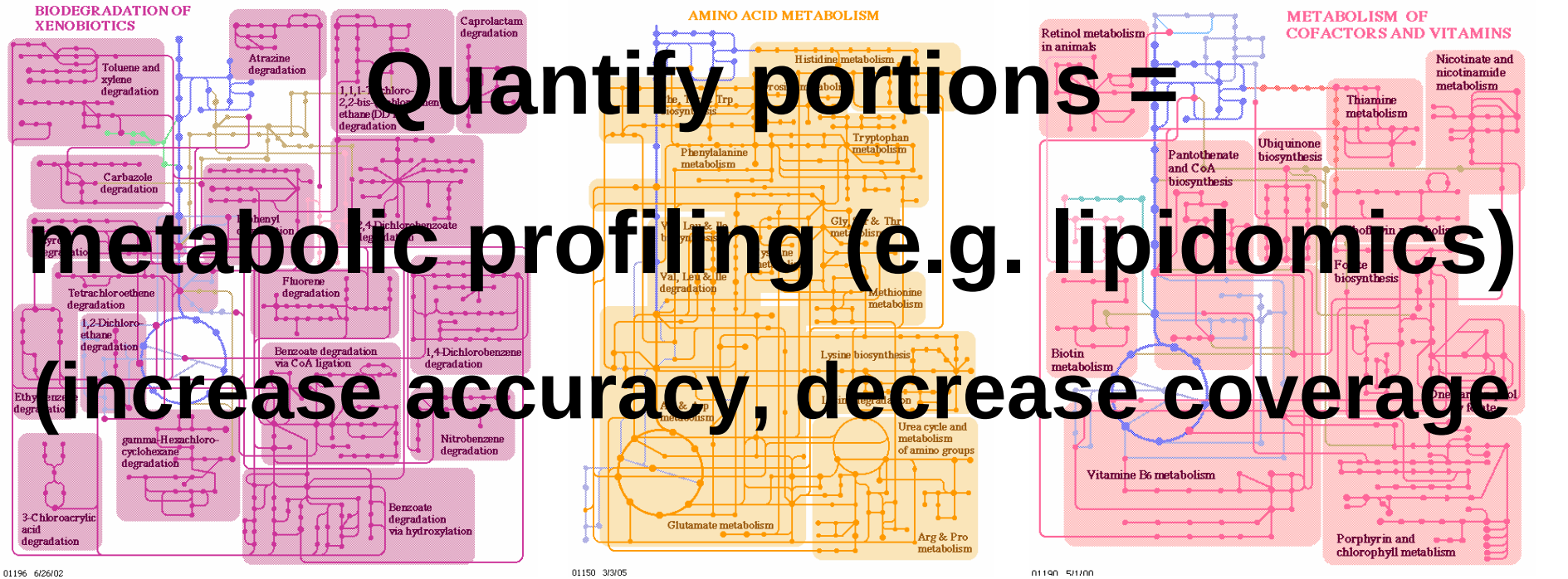
Metabolism (pathway analysis)



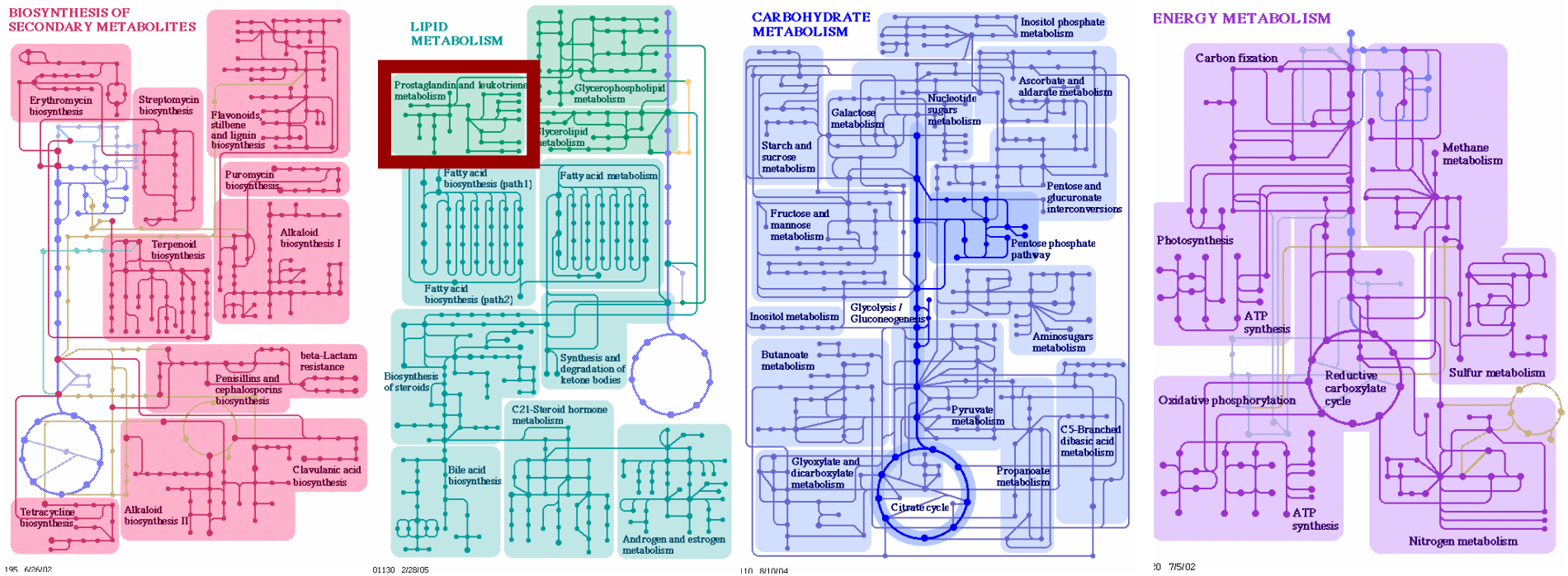
Quantify everything = metabolomics

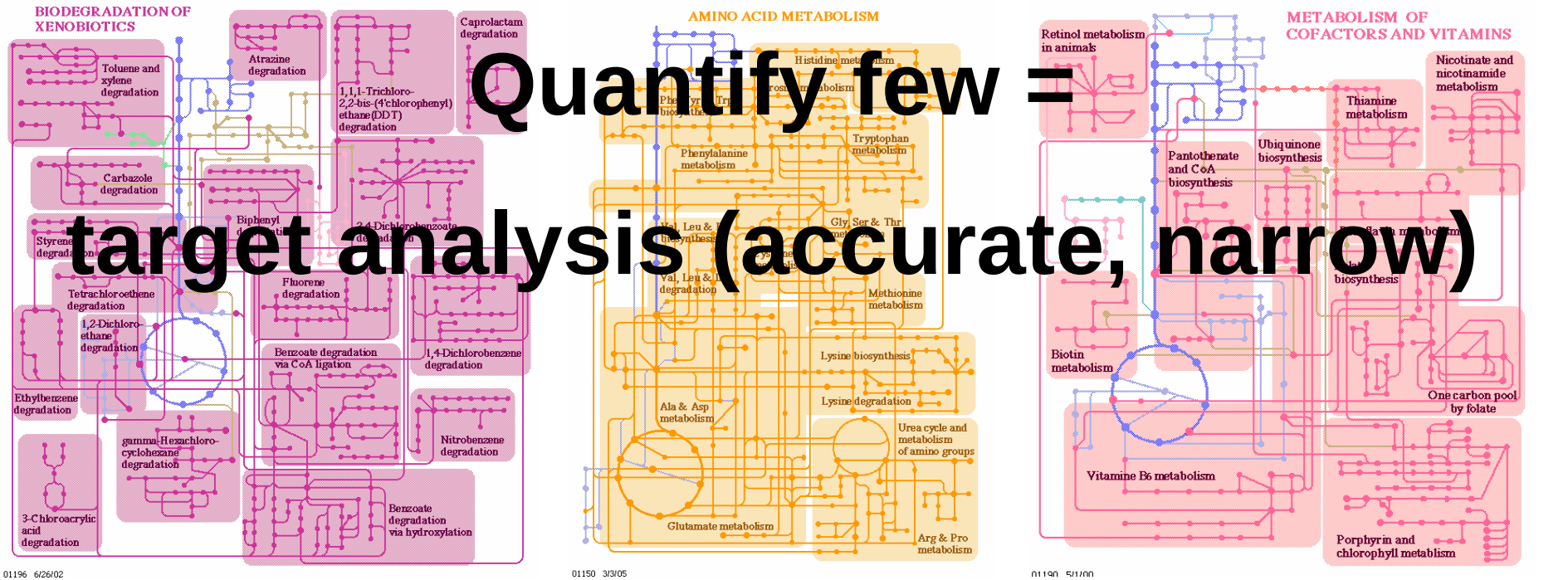
decrease accuracy, increase coverage





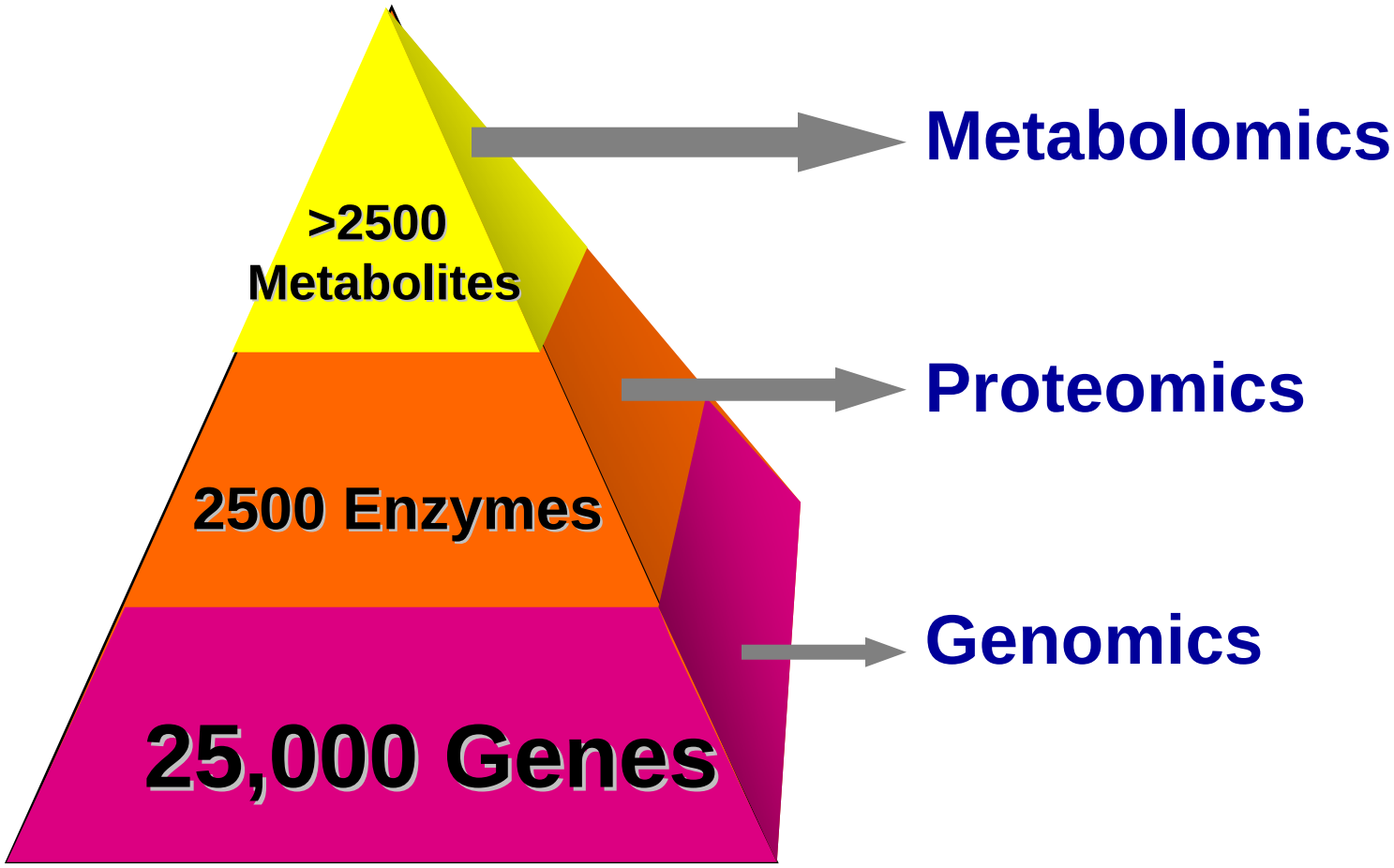
Quantify portions = metabolic profiling (e.g. lipidomics) (increase accuracy, decrease coverage





Quantify few = target analysis (accurate, narrow)

The Pyramid of Life



From the Human Metabolome Project: http://metabolomics.ca/

What is metabolomics?

A comprehensive quantitative analysis of all metabolites in the metabolome under a given set of conditions

What is a metabolite?

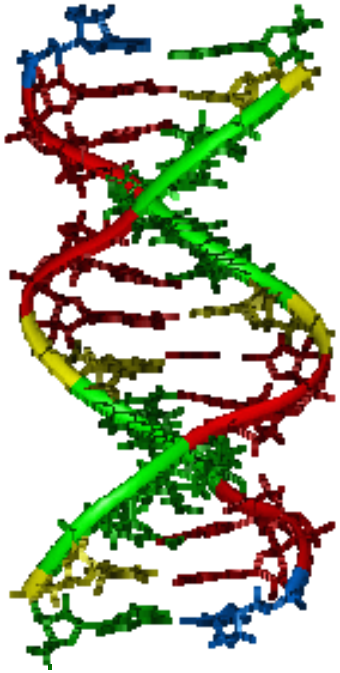
- **Substance involved in metabolism**
- **A by-product of the breakdown of either food or medication by the body**
- **Compound produced from the chemical changes of a drug in the body**
- **Any compound detected in the body <1500 Da**

Substance produced in or by biological processes

What is a metabolite?

- peptides
- oligonucleotides
- sugars
- nucleosides
- organic acids
- ketones
- aldehydes
- amines
- amino acids
- lipids
- steroids
- alkaloids
- drugs
- xenobiotics

Why Are Metabolites Relevant?



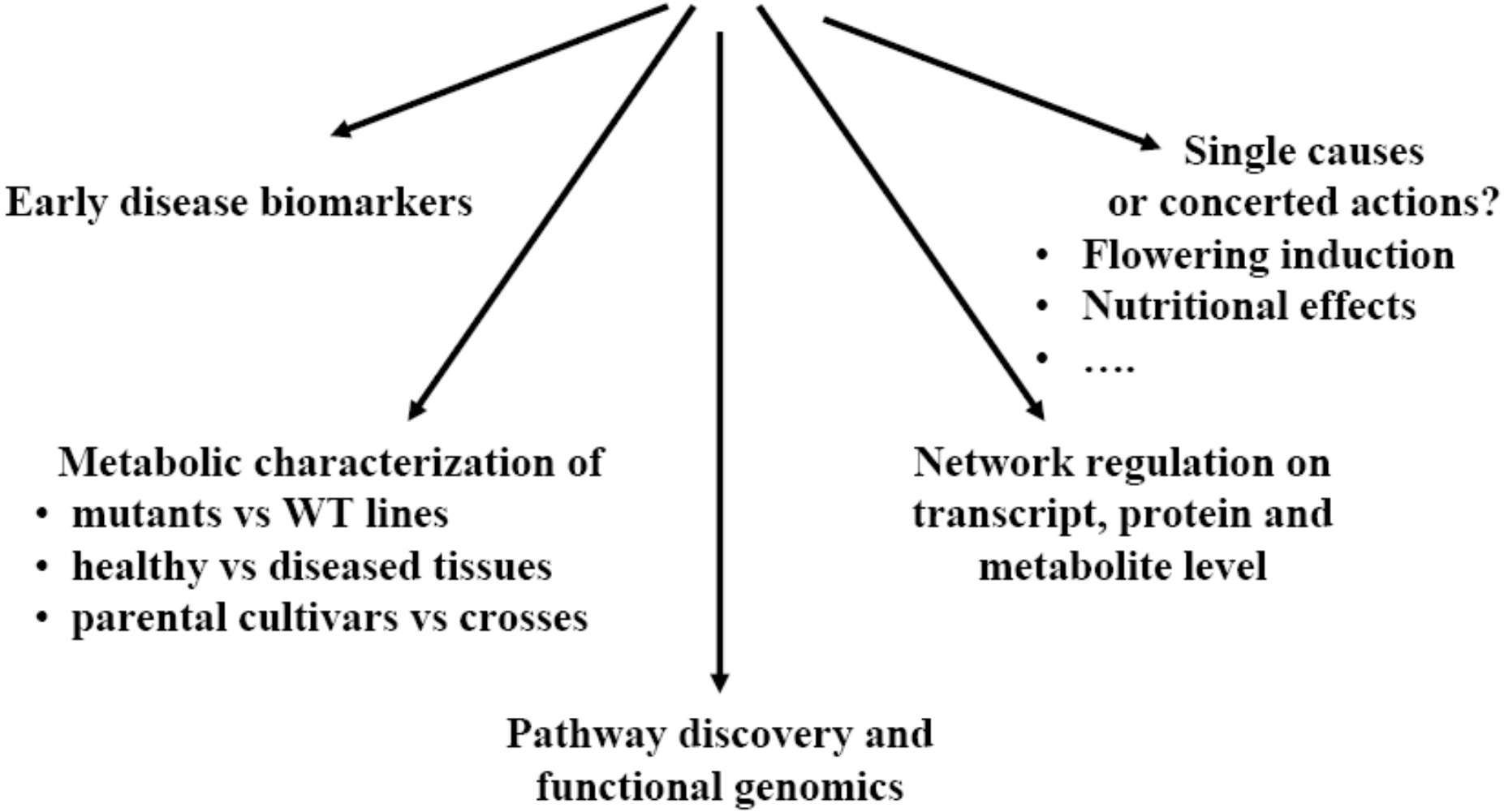
Metabolites are the Canaries of the Genome

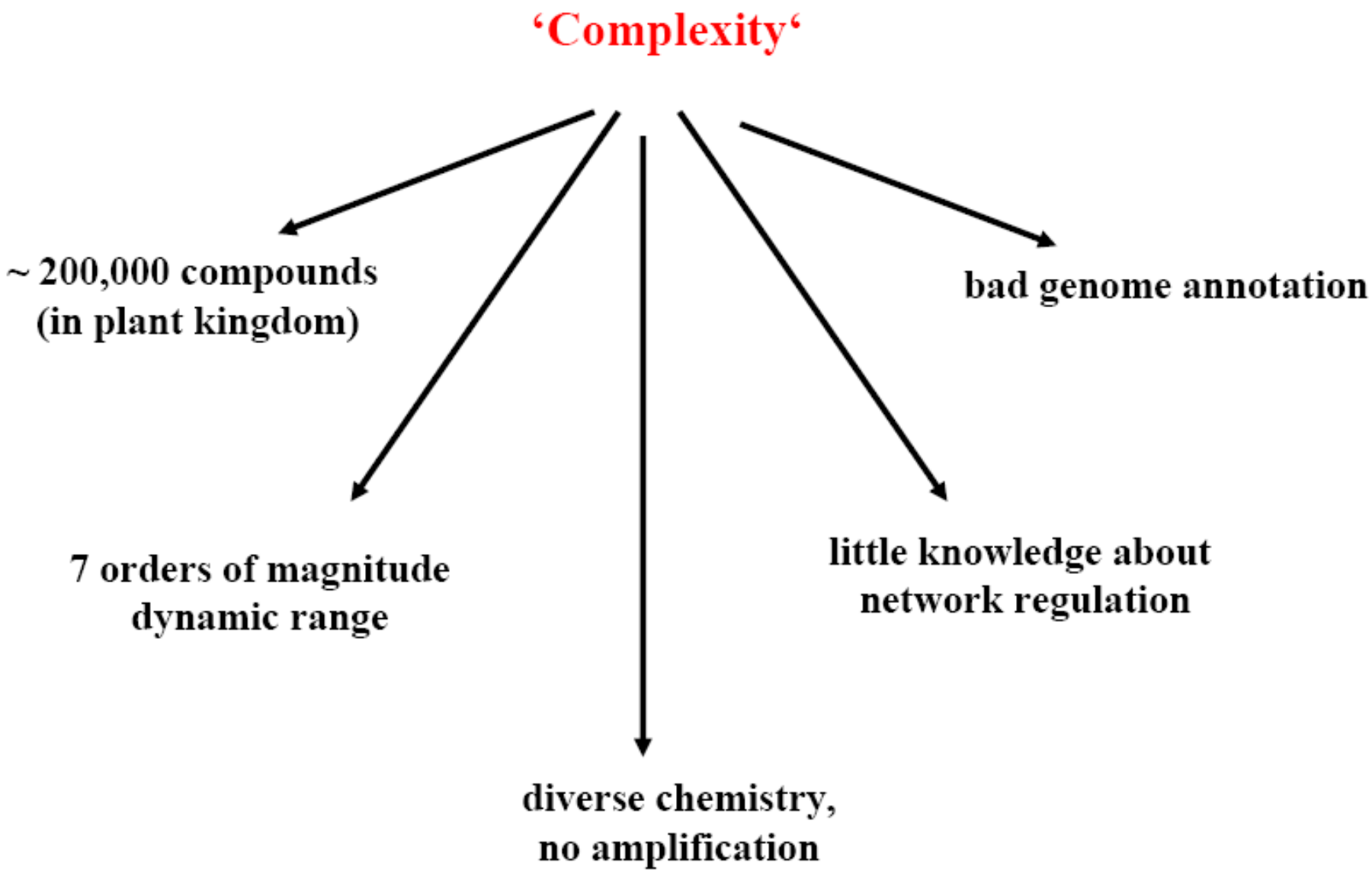
Why measure metabolites?

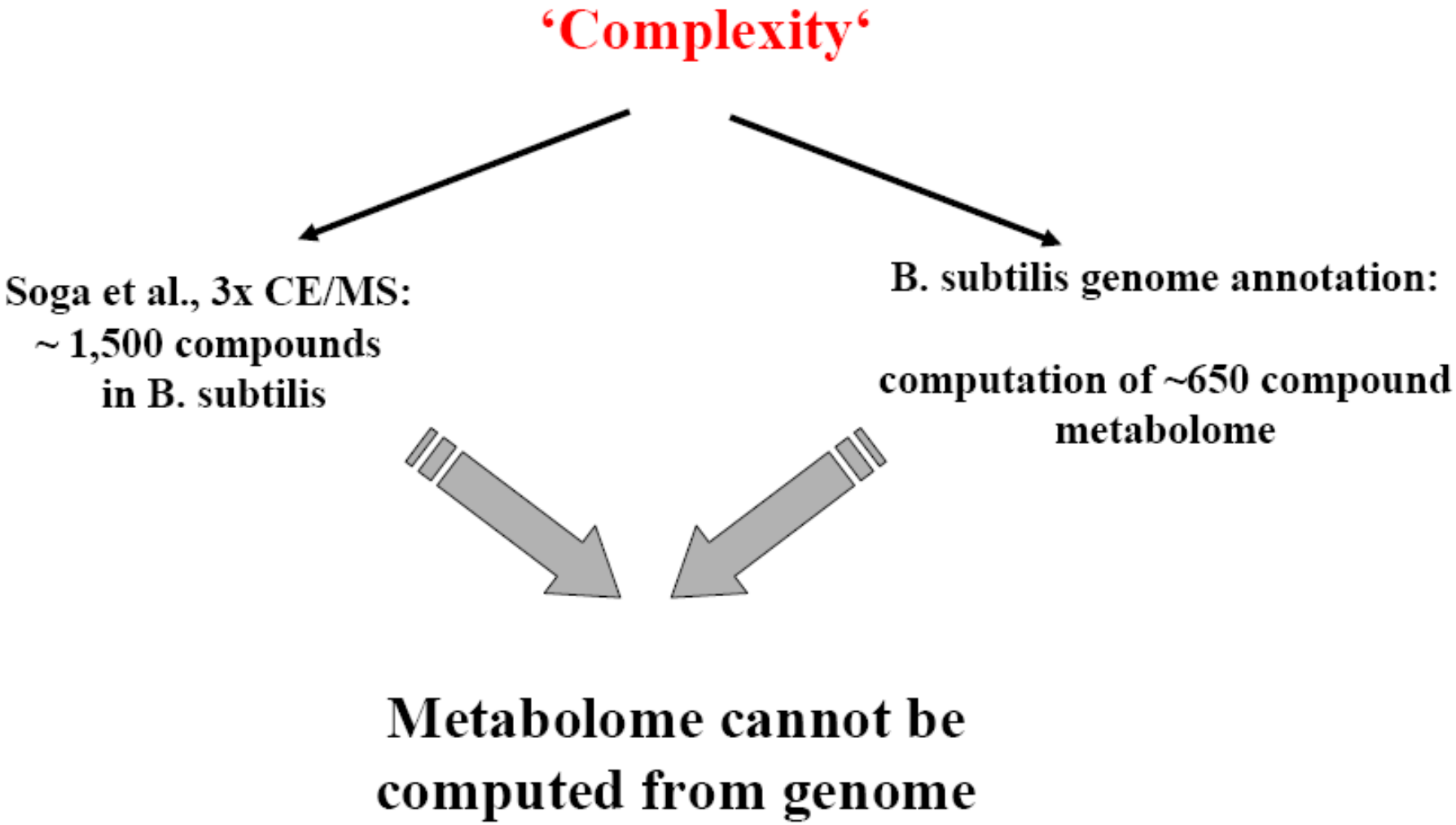
- **Simple answer**
  - **Readout of underlying molecular network**
  - **Infer enzyme activities**
  - **Reflective of any observable phenotype**
  - **Diagnostics, functional genomics**

- **Not so simple answer:**
  - **Not victims, but actors**
  - **A cause somewhere in the network can have effects elsewhere**

Use of metabolomic approaches:
'Dectect the unexpected'




‘Complexity‘
~ 200,000 compounds
(in plant kingdom)
bad genome annotation
7 orders of magnitude
dynamic range
little knowledge about
network regulation
diverse chemistry,
no amplification

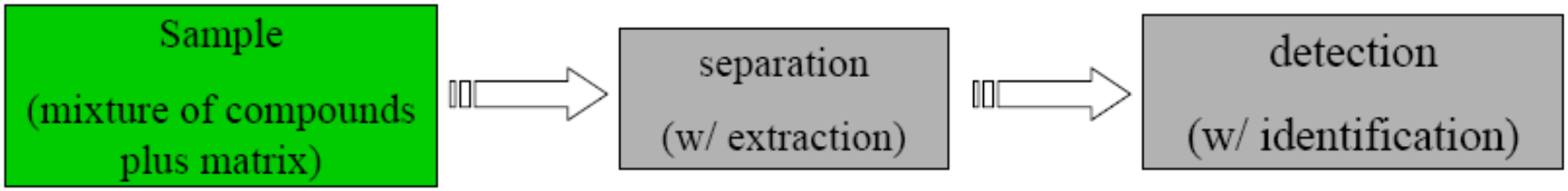

'Complexity'
Soga et al., 3x CE/MS:
~ 1,500 compounds
in B. subtilis
B. subtilis genome annotation:
computation of ~650 compound
metabolome
Metabolome cannot be
computed from genome

Metabolomics is a branch of

Analytical Biochemistry

Analytical biochemistry is the analysis of material samples
to gain an understanding of their biochemical composition and structure.

Qualitative analysis seeks the presence of a given functional group or organic compound in a sample.
Quantitative analysis seeks the amount of a given compound in a sample.



separation types:

(a) physicochemical (b) biochemical properties

(a) electrical charge, lipophilicity, hydrophilicity, volatility, size…
(b) binding, metabolism, precipitation…

detection types:

(a) physicochemical (b) biochemical properties

(a) mass, vibration, rotation, magnetic resonance, radiation absorption/fluorescence/scattering redox potential, thermal conductivity, refractive index…
(b) antibody, protein folding/binding→FRET

Metabolomics experiment

1. **Extraction from biological tissues**
2. **Separation**
   - **chromatography**
3. **Detection**
   - **mass spectrometry**
4. **Identification & quantification**

Metabolomics methods

Separation methods

- **Gas chromatography (GC)**
  - **one of the most widely used and powerful methods**
  - **high chromatographic resolution**
  - **compounds must be volatile (or derivatized)**
- **High performance liquid chromatography (HPLC)**
  - **lower chromatographic resolution**
  - **wider range of analytes can be analyzed (polar)**
- **Capillary electrophoresis (CE)**
  - **higher theoretical separation efficiency than HPLC**
  - **suitable for wider range of metabolites than GC**
  - **most appropriate for charged analytes (electrophoretic technique)**

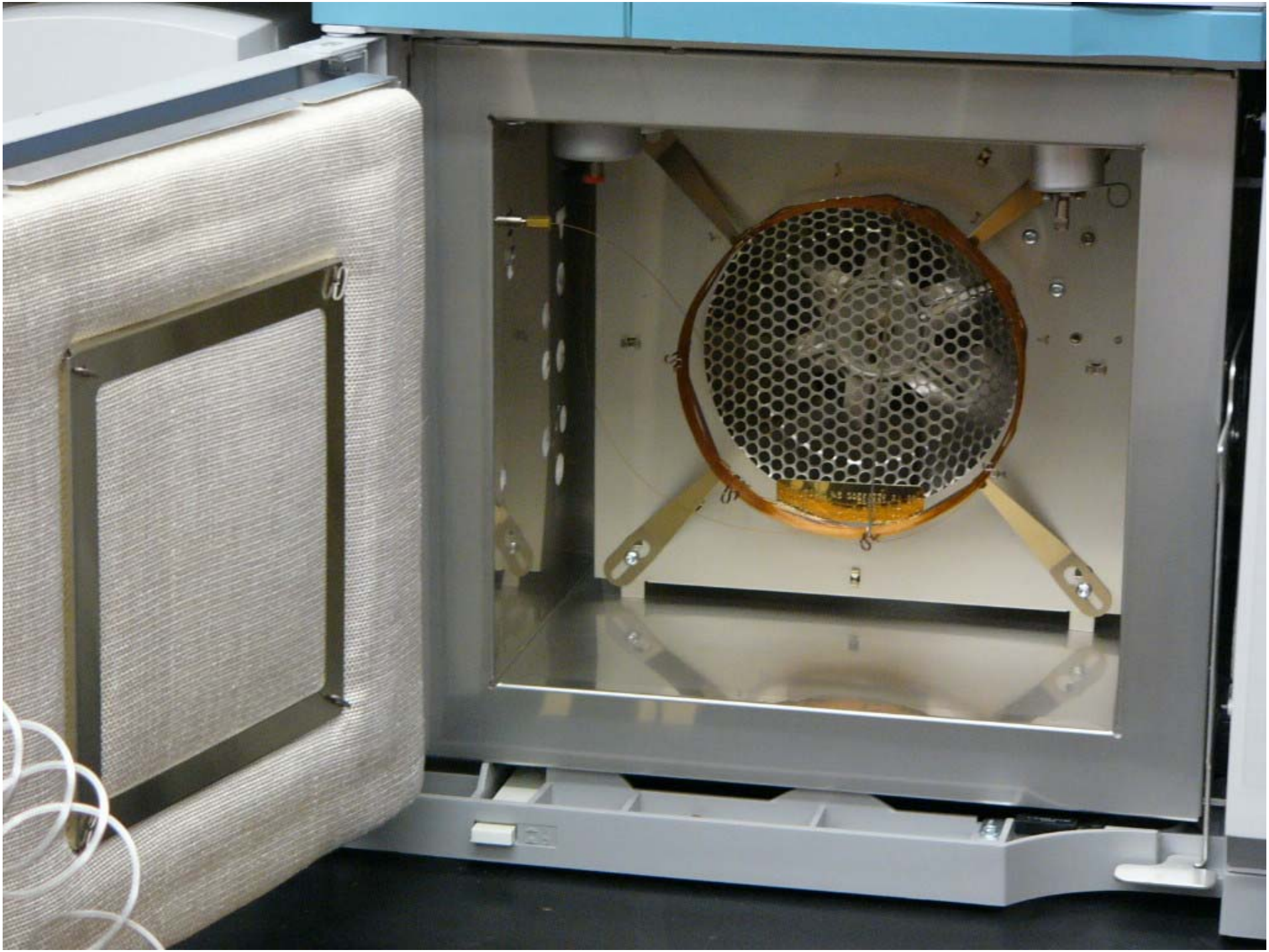
Metabolite detection techniques

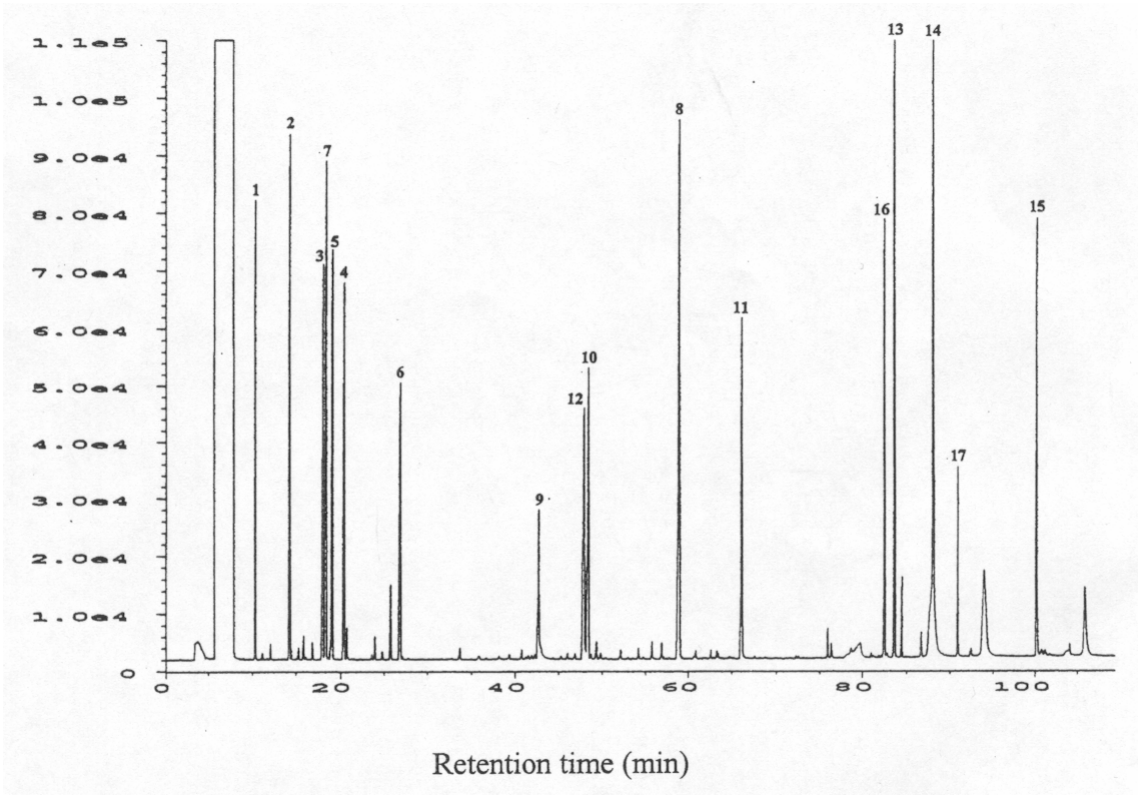
Physical Property	Method
• mass	• mass spectrometry
• rotation, vibration	• infrared spectrometry
• *hv* absorbance	• ultraviolet-visible spectroscopy
• *hv* emittance	• fluorescence spectroscopy
• spin	• nuclear magnetic resonance
• volatility	• gas chromatography
• hydrophobicity	• liquid chromatography
• charge	• capillary electrophoresis
• size	• size-exclusion chromatography

Gas chromatography

- **Advantages**
  - **Very high chromatographic resolving power**
  - **Good selection of stationary phases**
  - **Wide dynamic range**

- **Disadvantages**
  - **Compounds must be sufficiently volatile (derivatized)**
  - **Compounds must be thermally stable**
  - **Limited to nonpolar and slightly polar molecules**




1.1e5
1.0e5
9.0e4
8.0e4
7.0e4
6.0e4
5.0e4
4.0e4
3.0e4
2.0e4
1.0e4
0
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Retention time (min)

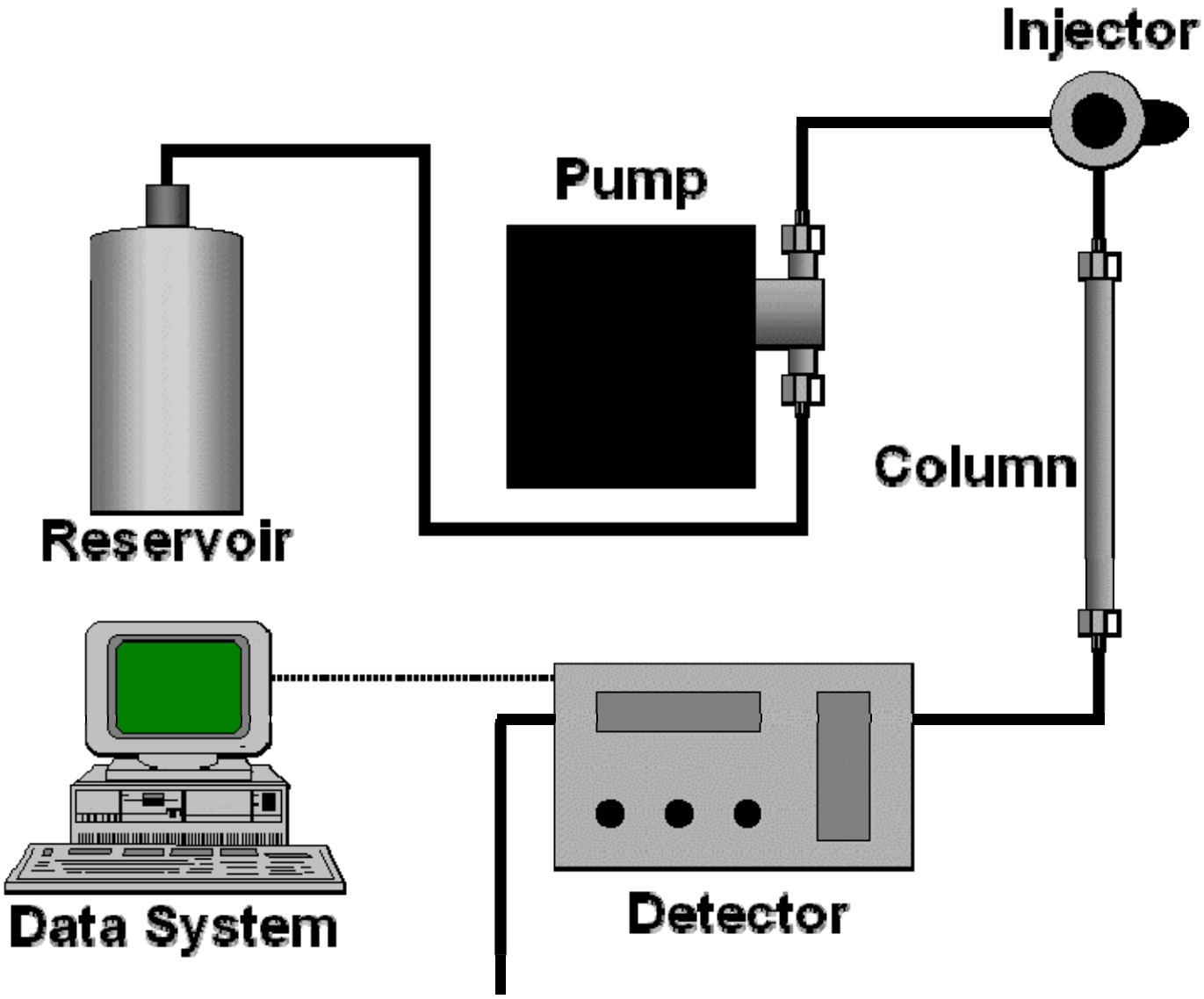
Liquid chromatography

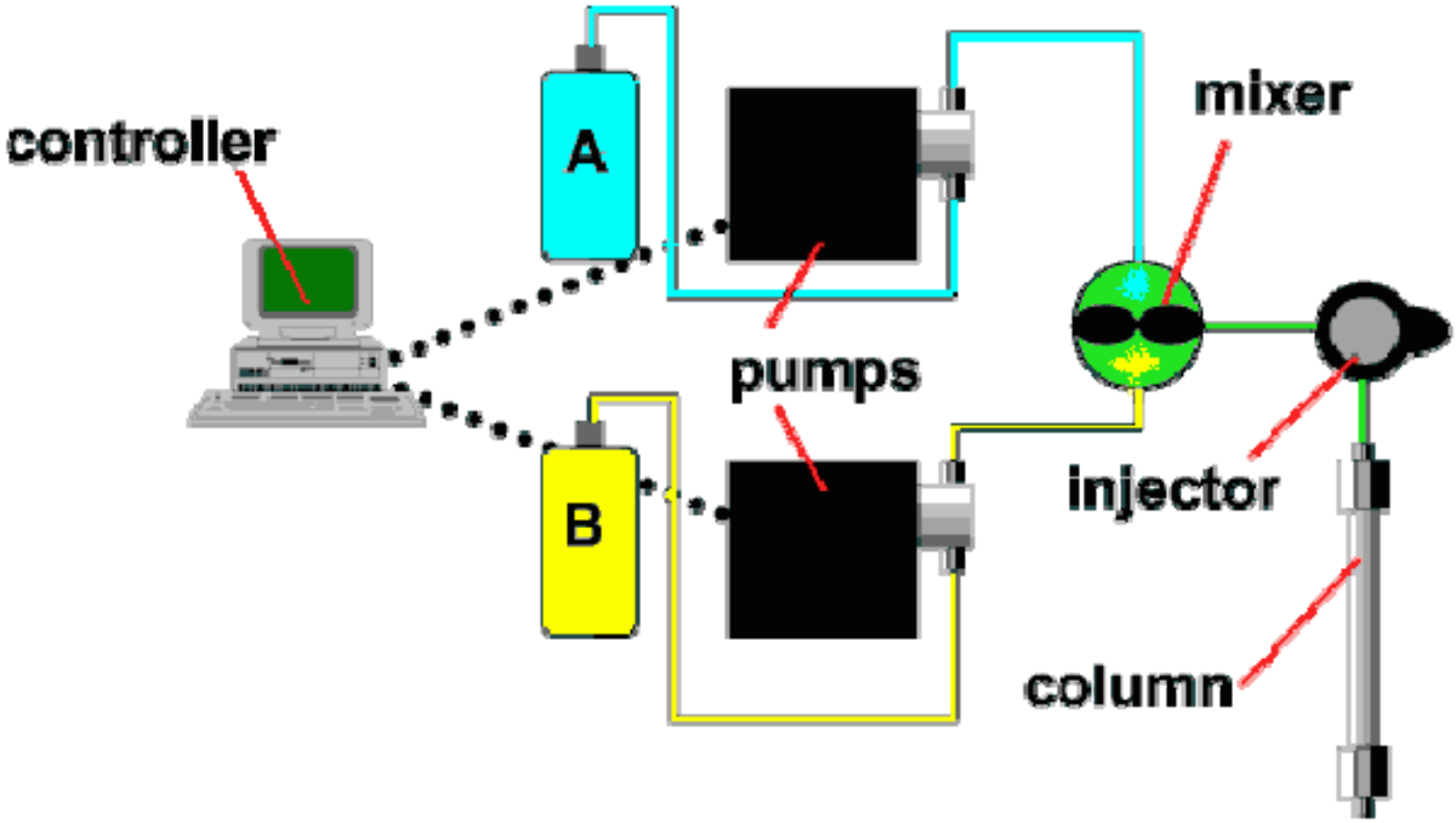
- **Advantages**
  - **Capable of analyze wide range of metabolites**
  - **(thermally labile, polar, high molecular mass)**
  - **Good selection of stationary phases**

- **Disadvantages**
  - **Fragmentation rules not well-established**
  - **No MS fragmentation libraries**
  - **Limited resolution (but UPLC is an improvement)**

HPLC

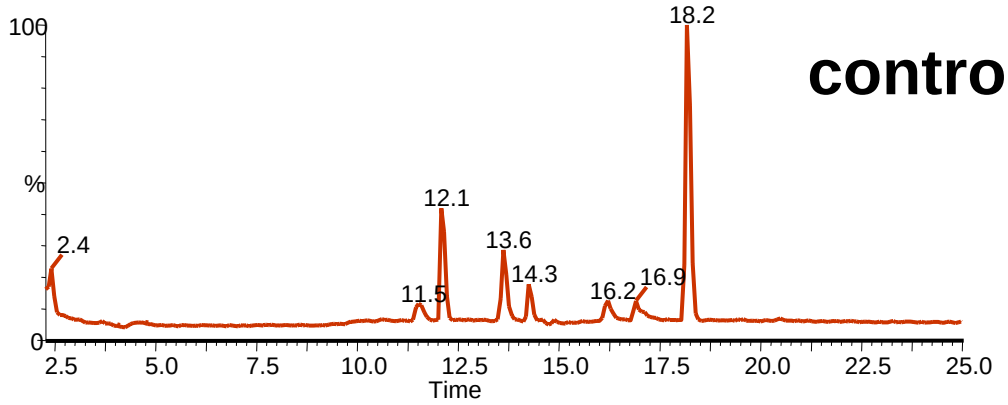
high performance liquid chromatography


Injector
Pump
Reservoir
Column
Data System
Detector


controller
A
B
pumps
mixer
injector
column

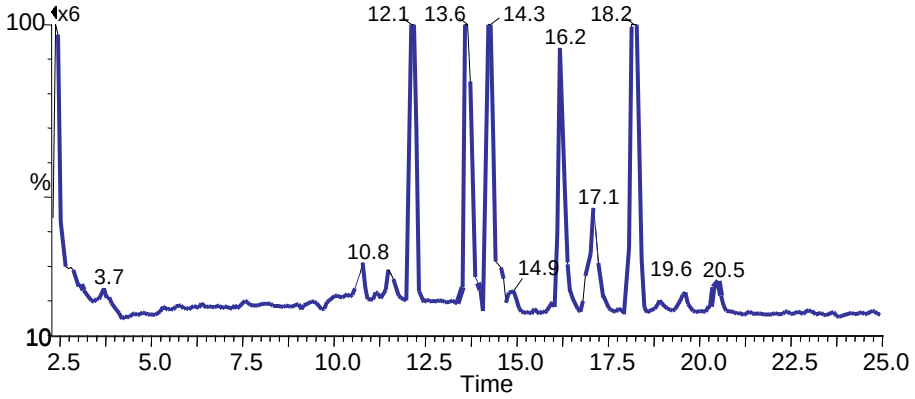

PLASTIPAK
Compact Rocker

Metabolic Fingerprinting – MS Analysis



control

ozone treated

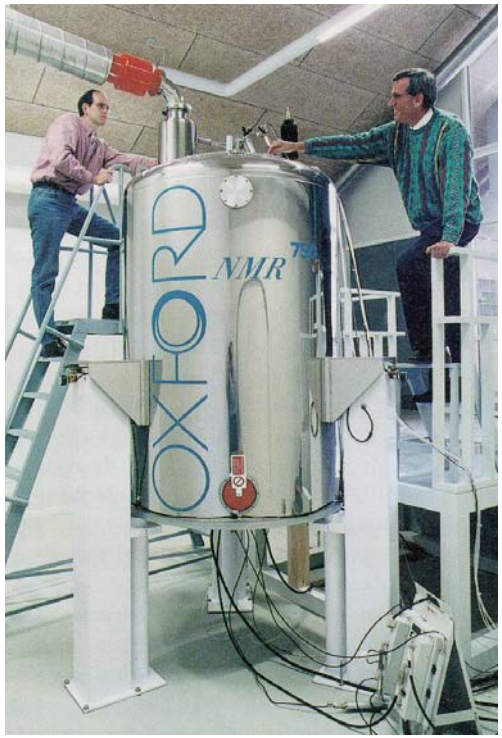


Online SPE-HPLC-TOF-MS analysis of 20 µL bronchiolar alveolar lavage fluid

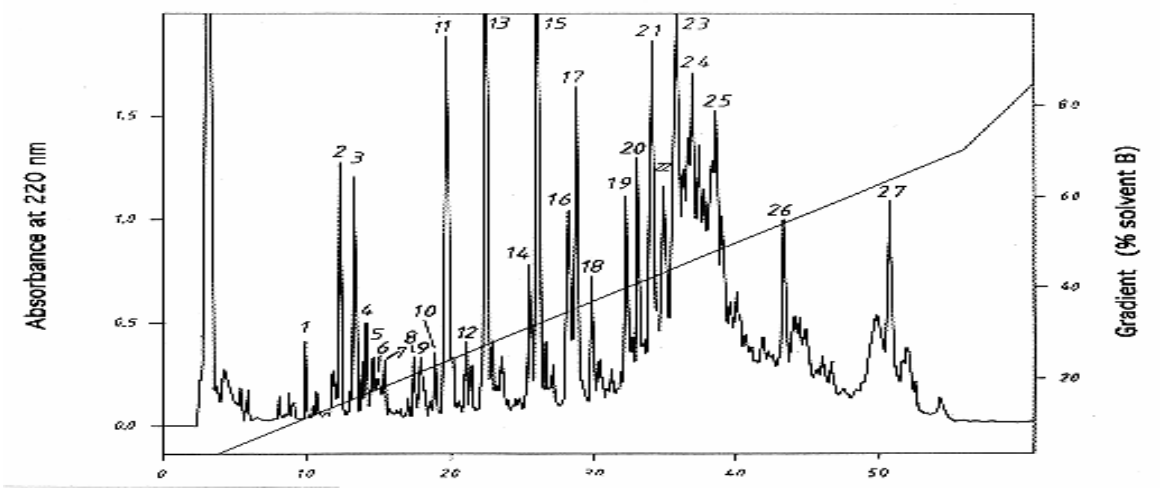
NMR

- **Advantages**
  - **No sample separation necessary**
  - **Essentially universal detector**
  - **Non-destructive**

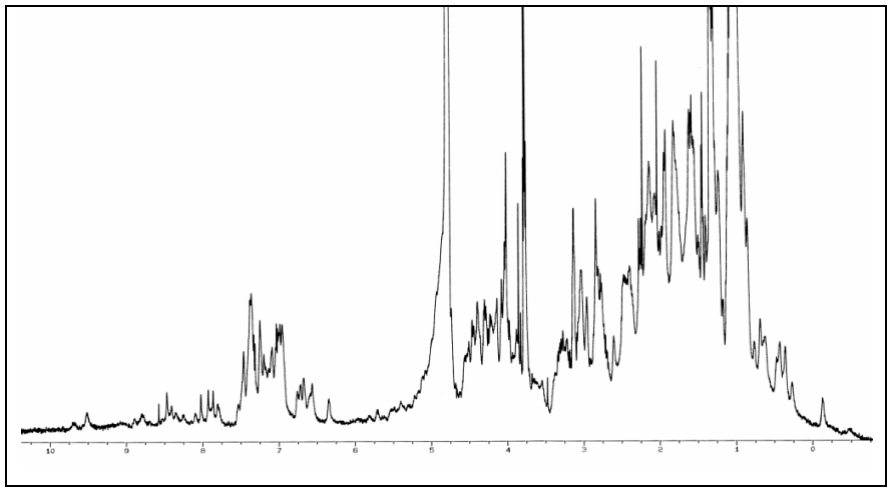
- **Disadvantages**
  - **Low sensitivity**
  - **Results difficult to interpret**
  - **Decreased quantification**
  - **Expensive**



Why NMR?



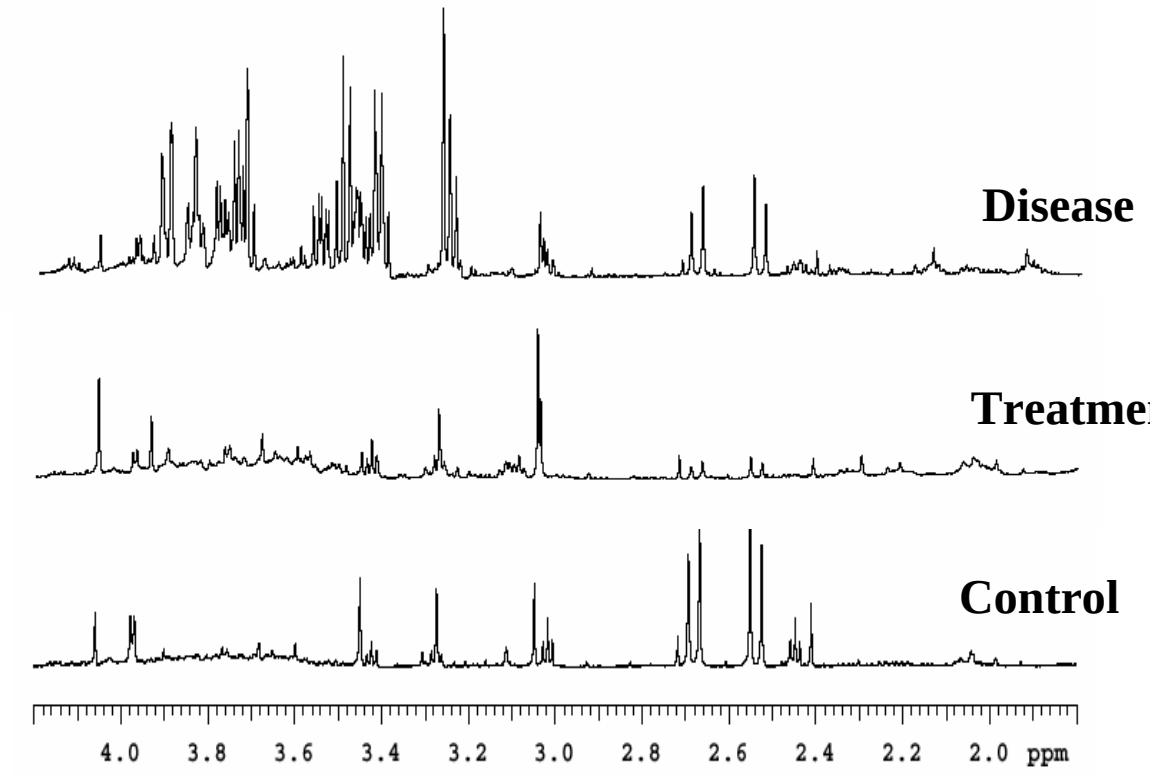
Mixture separation by HPLC (followed by ID via Mass Spec)

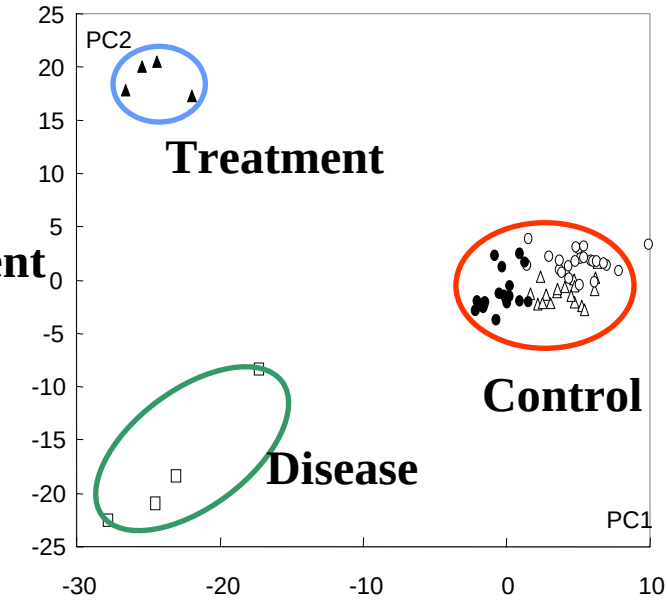


Mixture separation by NMR (simultaneous separation & ID)

Chemical Shift Chromatography

NMR Metabolic Profiling and Drug Toxicology





Principal Component Analysis

Mass spectrometry

- ***chemical analysis technique to measure the mass of molecules by ionizing, separating & detecting ions according to their mass-to-charge ratios.***

- ***involves the study of ionized molecules in the gas phase with the aim of one or more of the following:***

- **Molecular weight determination**
- **Identify unknown compounds**
- **Structural characterization**
- **Qualitative and quantitative analysis of mixtures**
- **Carbon dating**

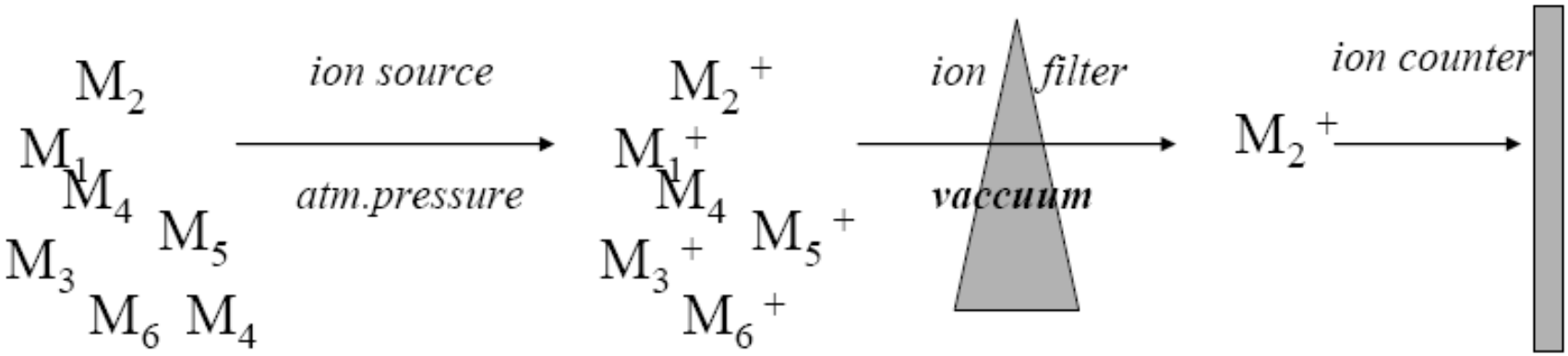
- **What is ionization?**
- **producing an electrically charged molecule from a neutral molecule by adding protons or removing electrons.**

- **Why is ionization of sample required in mass spectrometry?**
- **sample to be analyzed by mass spectrometry must be ionized to separate ions according to their mass-to-charge ratio**

- **What is the "mass-to-charge" ratio of an ion?**
- **The mass of the ion divided by the charge on the ion.**
- **The charges on an ion are always positive integers (1, 2, 3, ...).**
- **Charged ions are produced by ionization.**
- **Ions charged by adding a proton or by removing an electron.**

- **For example:**
  - **molecule C_2H_6 with mass of (2 x 12) + (6 x 1) = 30 *Da*.**
  - **can acquire a charge of 1 unit in 2 ways:**
    - **losing an electron (mass-to-charge ratio = 30/1 = 30)**
    - **accepting a proton (mass-to-charge ratio = (30+1)/1 = 31)**

What is mass spectrometry????



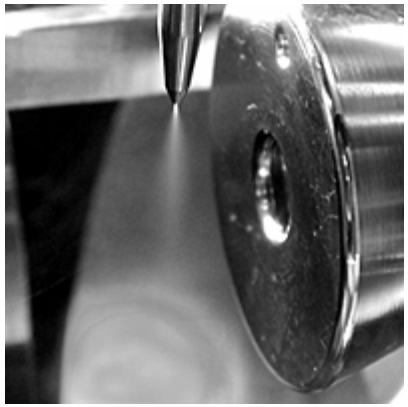
Ion sources used in metabolomics

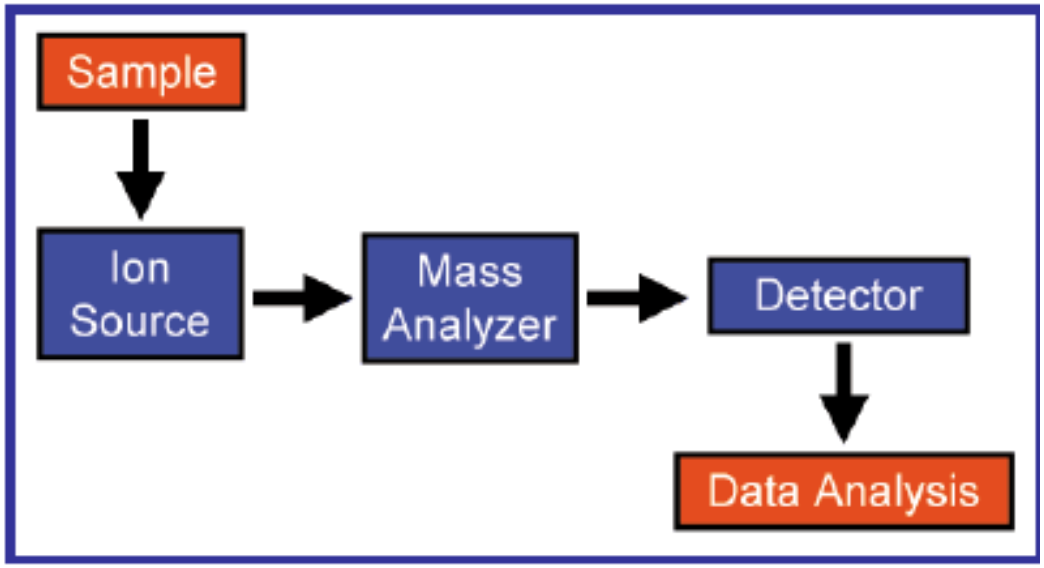
HPLC

ESI – electrospray ionization (Nobel prize in 2002)
API – atmospheric pressure ionization
MALDI – matrix-assisted laser desorption ionization
ICP – inductively coupled plasma

GC

EI – electron impact ionization
CI – chemical ionization

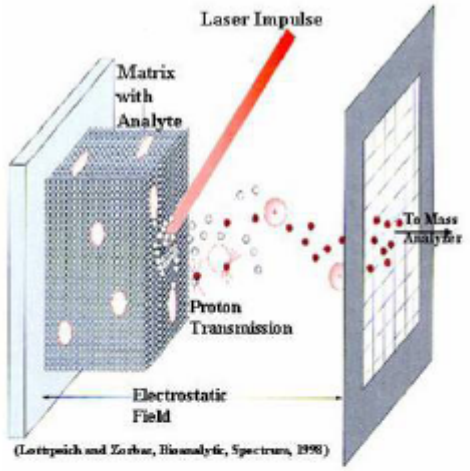




Three most common types of ionization

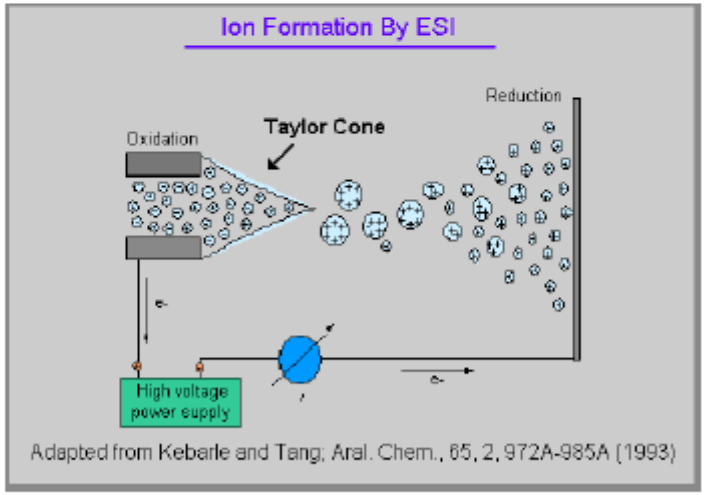
MALDI-MS

Matrix Assisted Laser Desorption Ionization



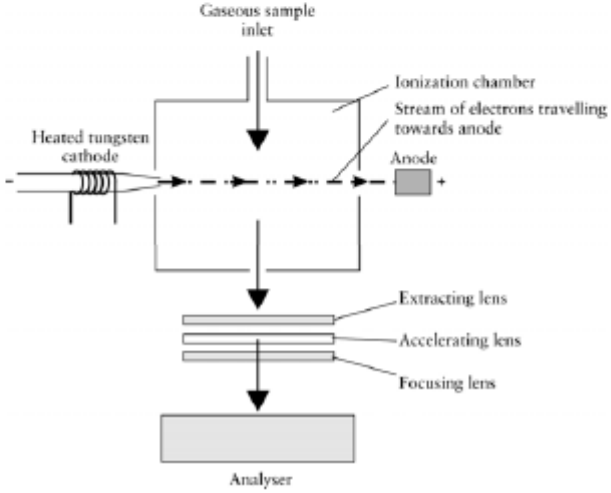
LC/ESI-MS

Electrospray Ionization



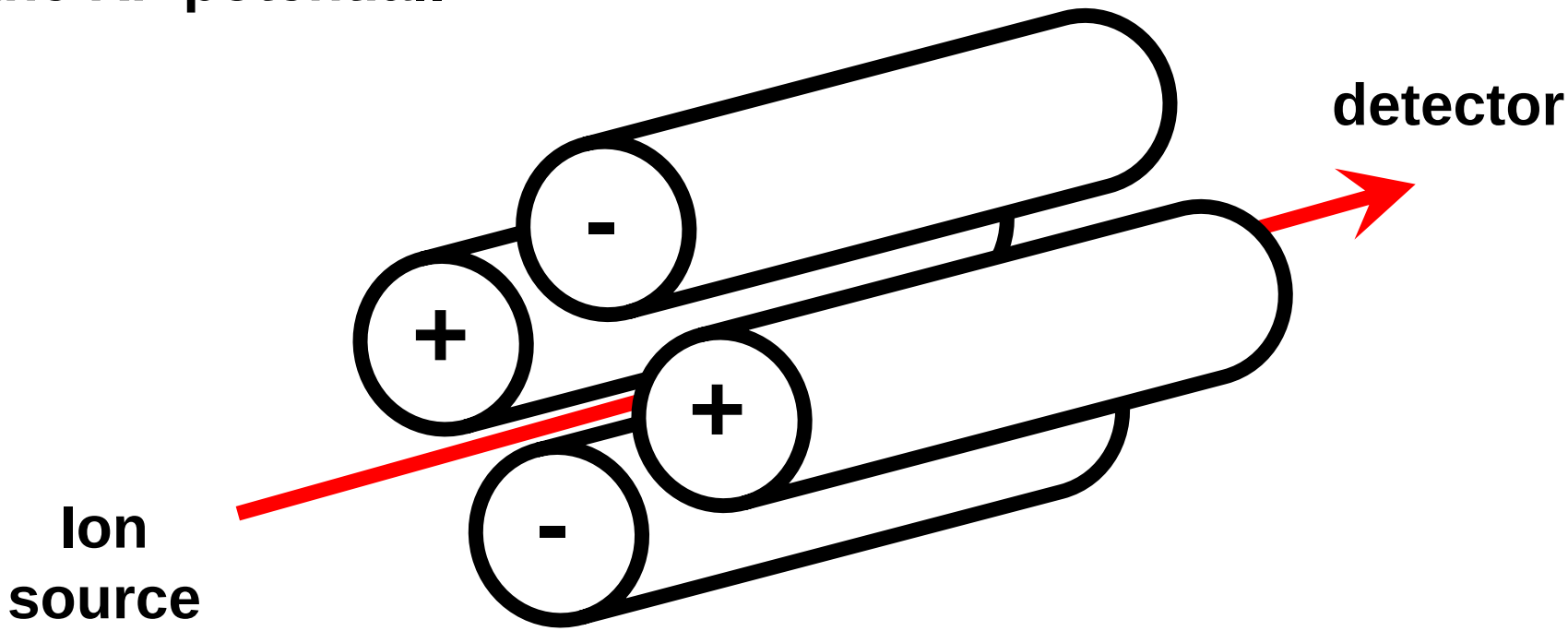
GC/EI-MS

Electron Impact Ionization

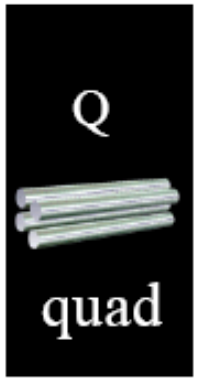


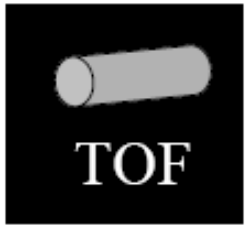
The basic unit of mass filtering is a quadrupole

- **The quadrupoles are 4 parallel rods controlled by DC voltage & an RF potential.**
- **Ions with specific *m/z* ratios separated by controlling the RF potential.**



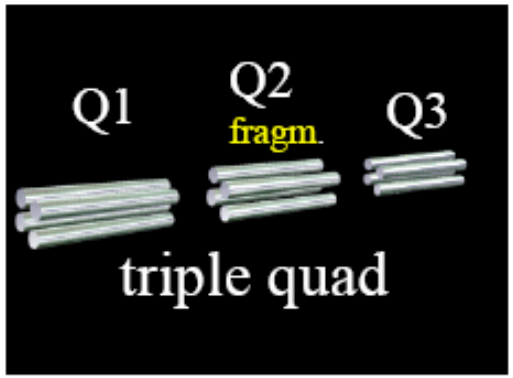
Types of Mass Spectrometers (‘ ion separators ‘)

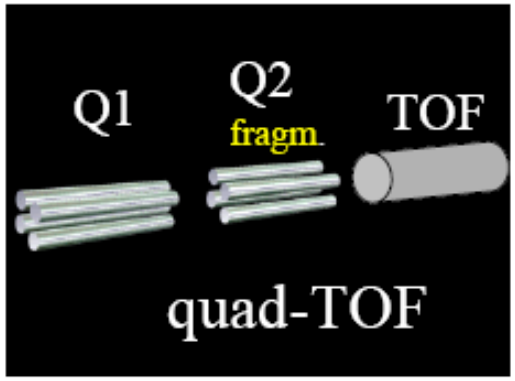


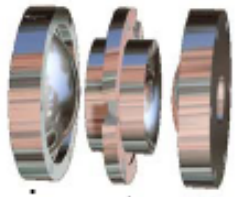


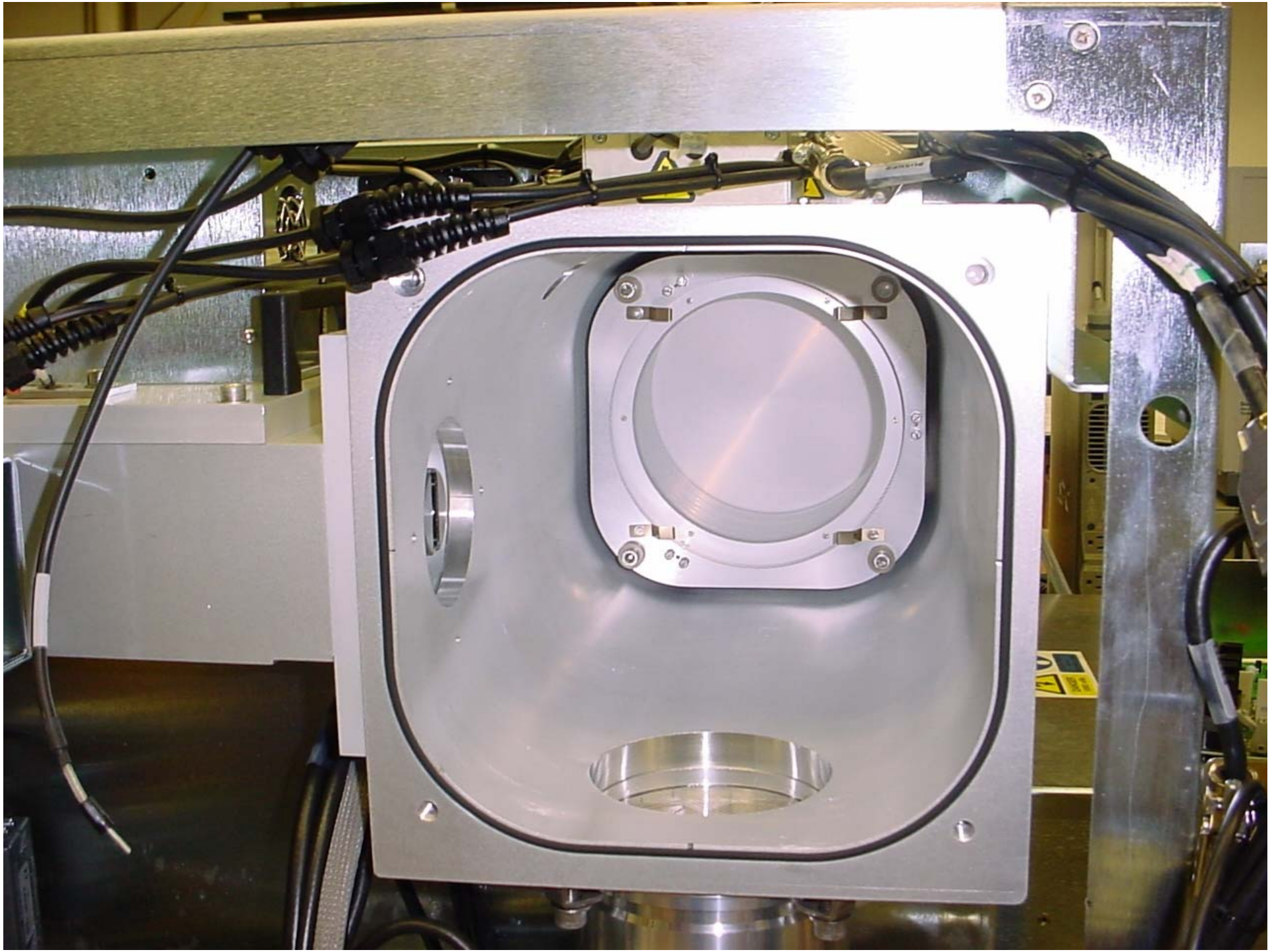
mostly without MS/MS fragmentation

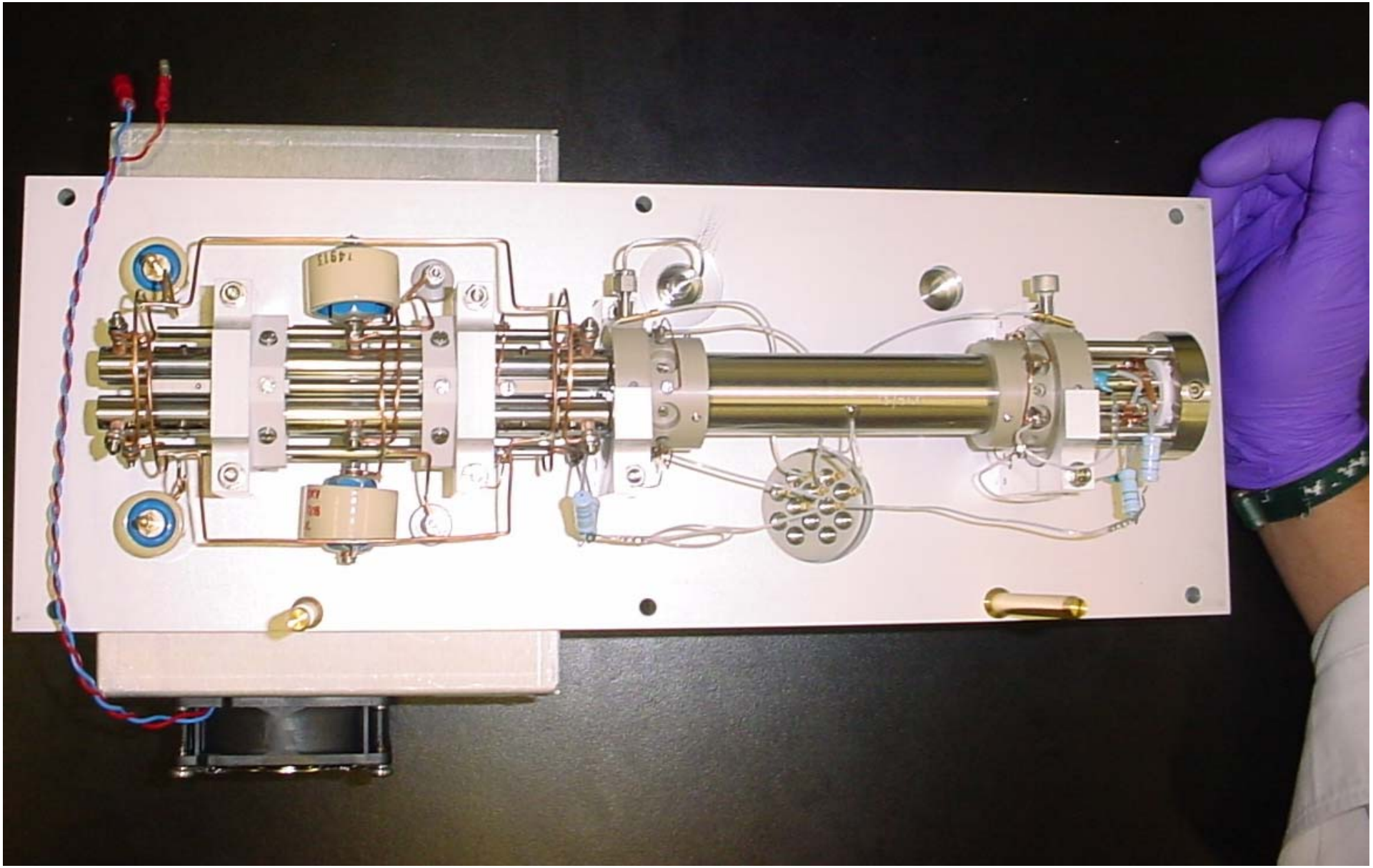
mostly with MS/MS fragmentation






ion trap

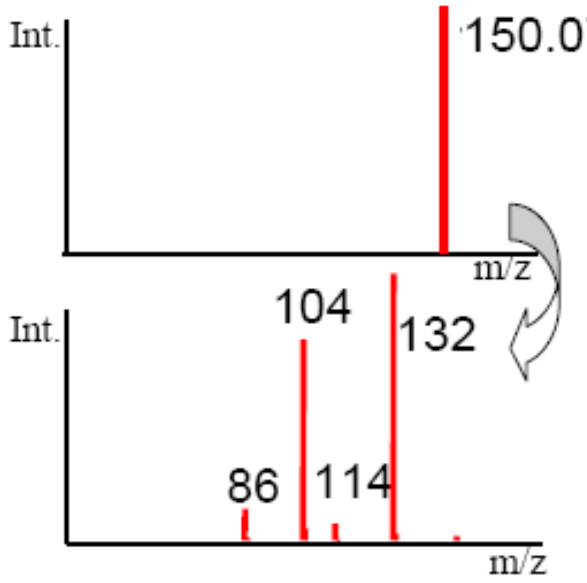


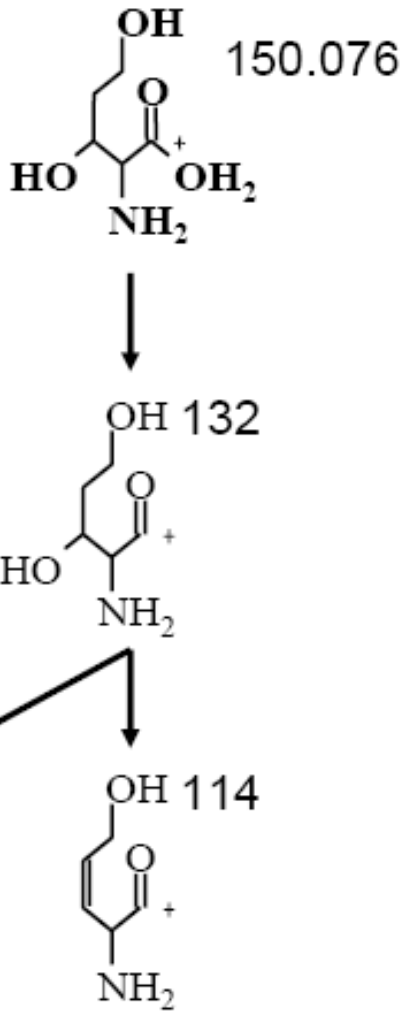


Qualitative analytical biochemistry: metabolite identification in LC/MS

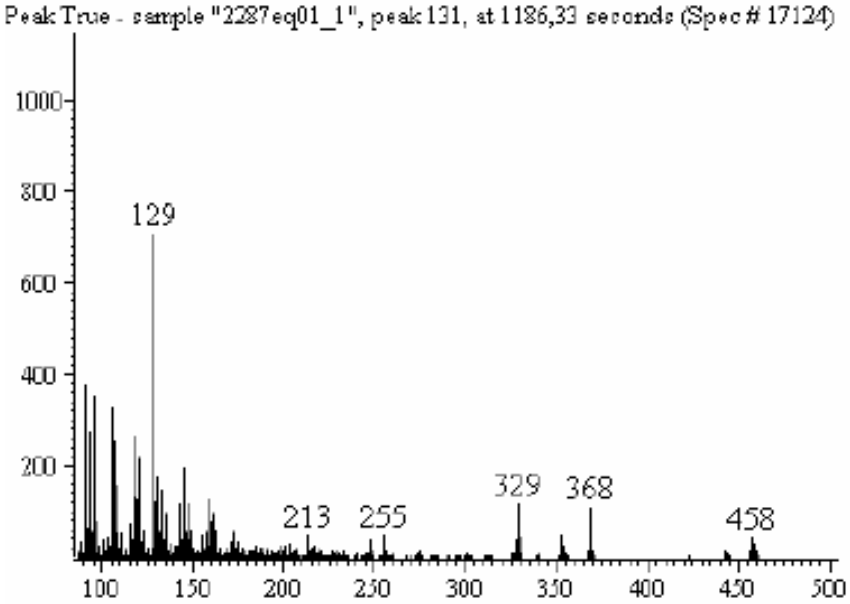
MS/MS

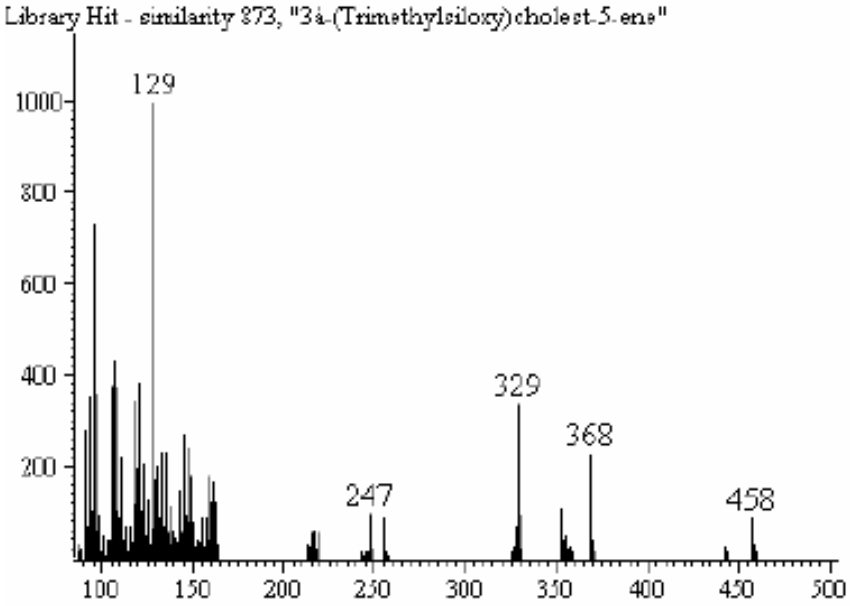
(ion trap, Q3, ICR, QTOF)



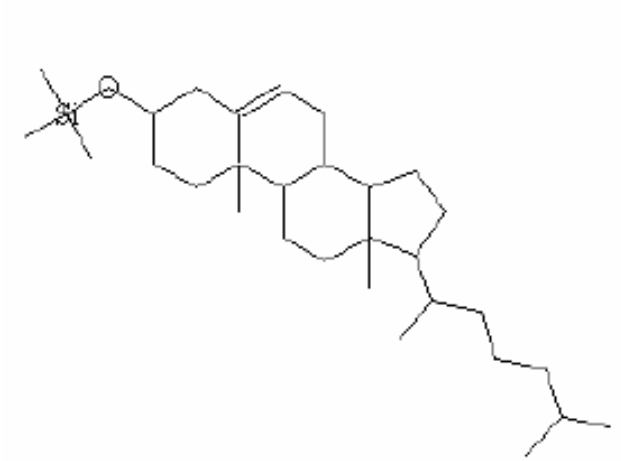


Qualitative analytical biochemistry: metabolite identification in GC/MS





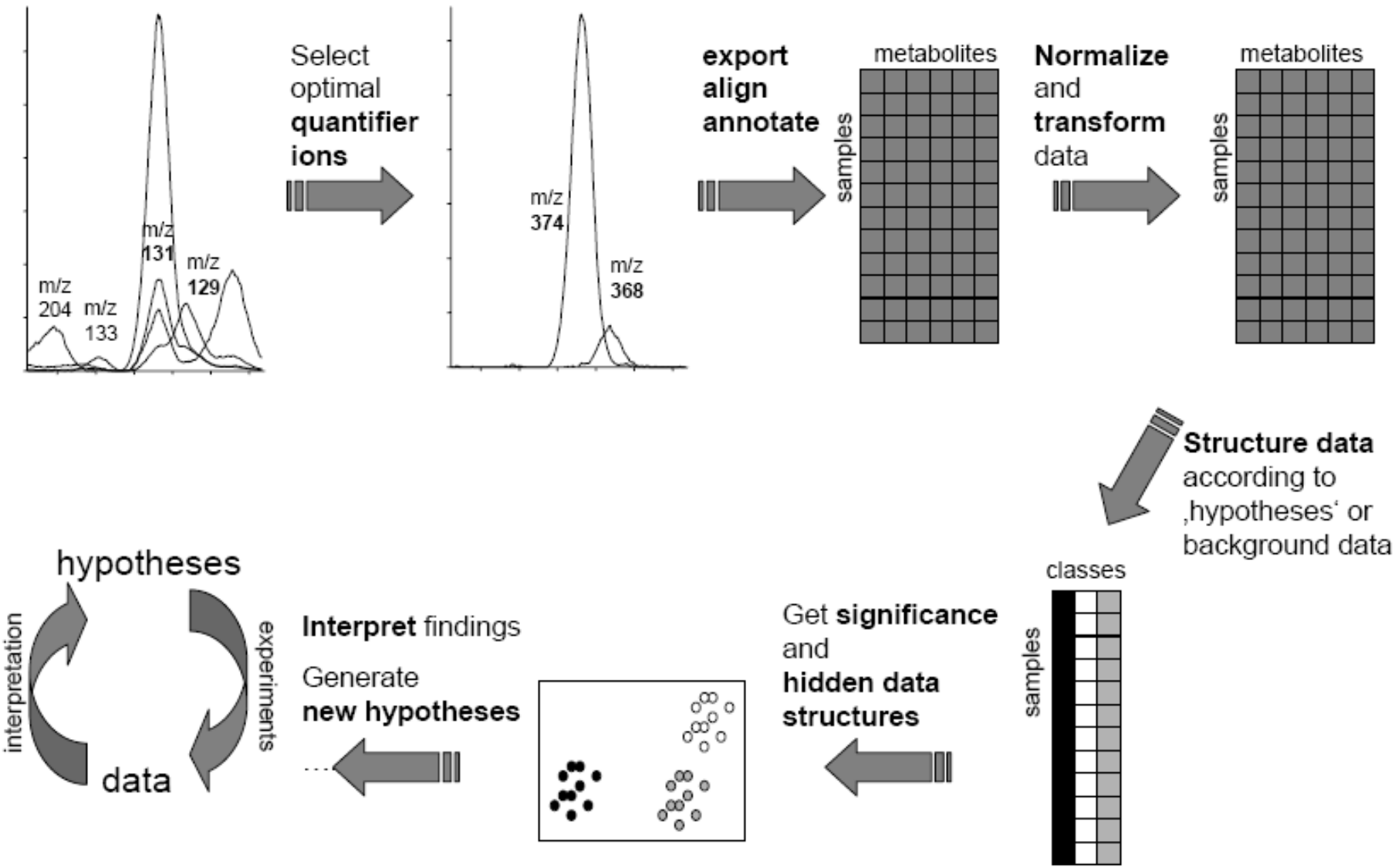
cholesterol TMS
mw=458 Da

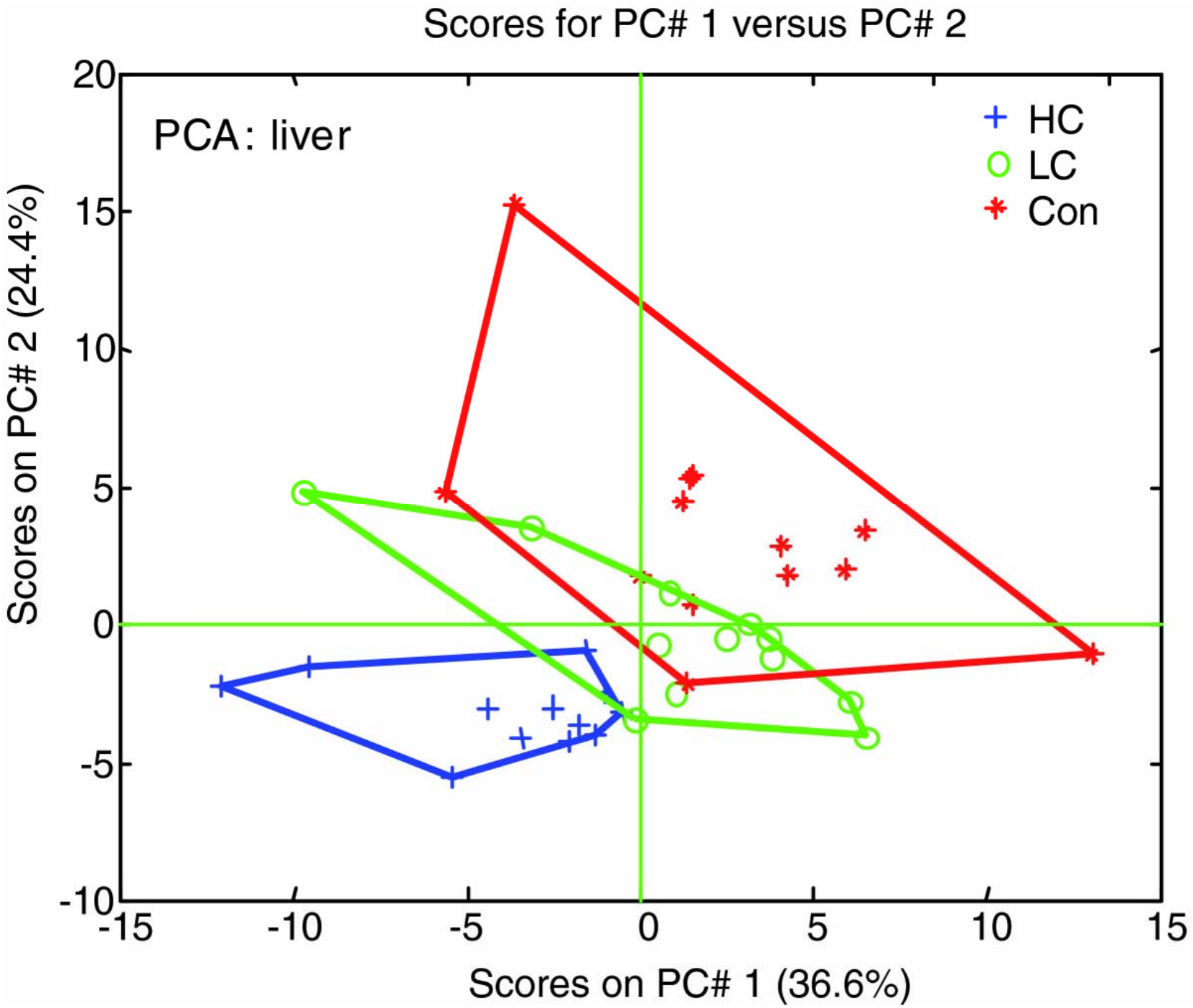


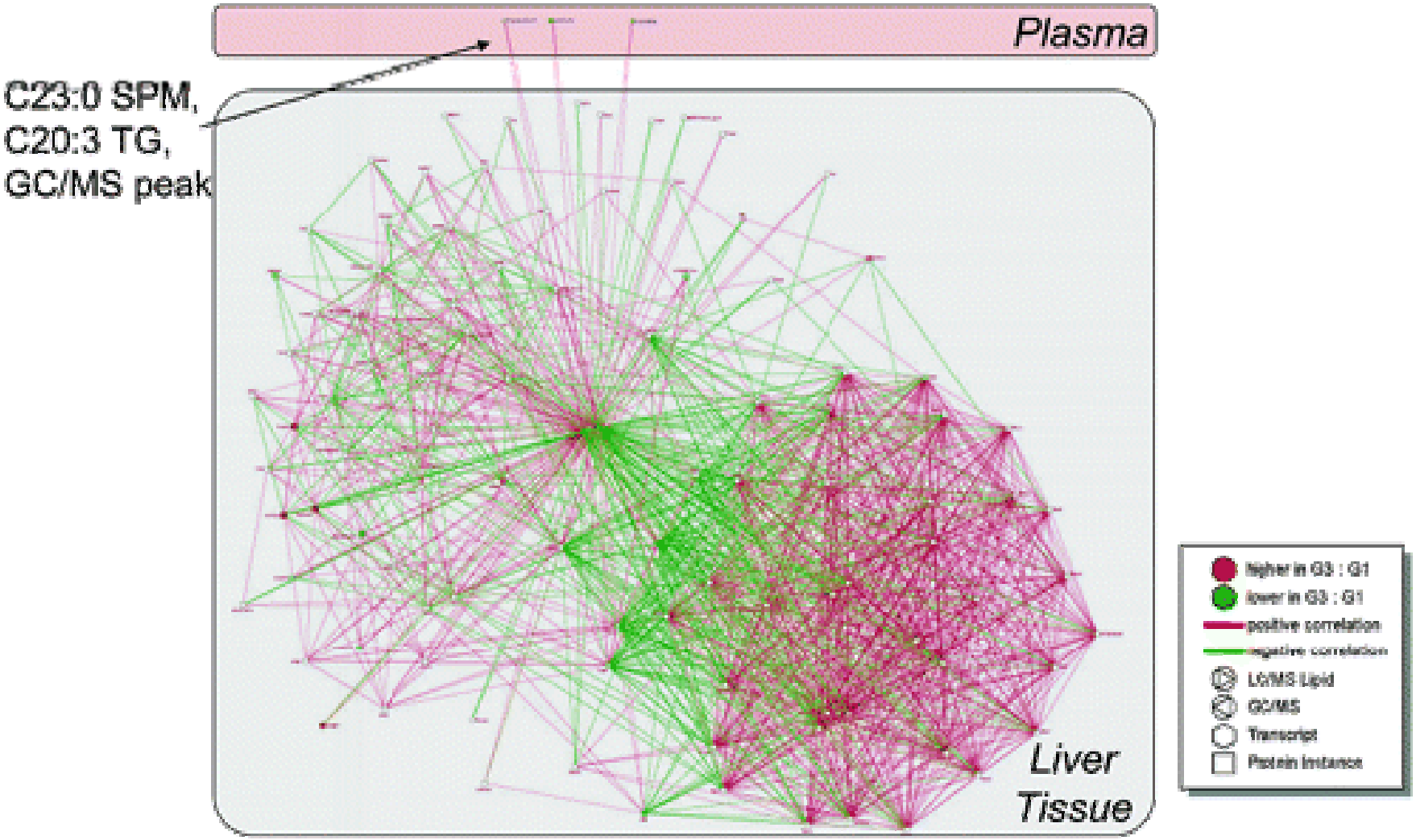
Metabolomics experimental design

- **If classification is most important aim, get as many samples as possible & run fingerprints!**
- **If you have no clue what to expect, combine GC/MS, LC/MS and 2D-NMR data**
- **IF you have valid hypotheses in mind, use metabolite profiling (e.g. lipidomics)**
- **Use more than one statistical tool to be certain**
- **Biological variance has different sources, but is usually larger than analytical error**
- **Metabolomics is suggestive of phenotype**

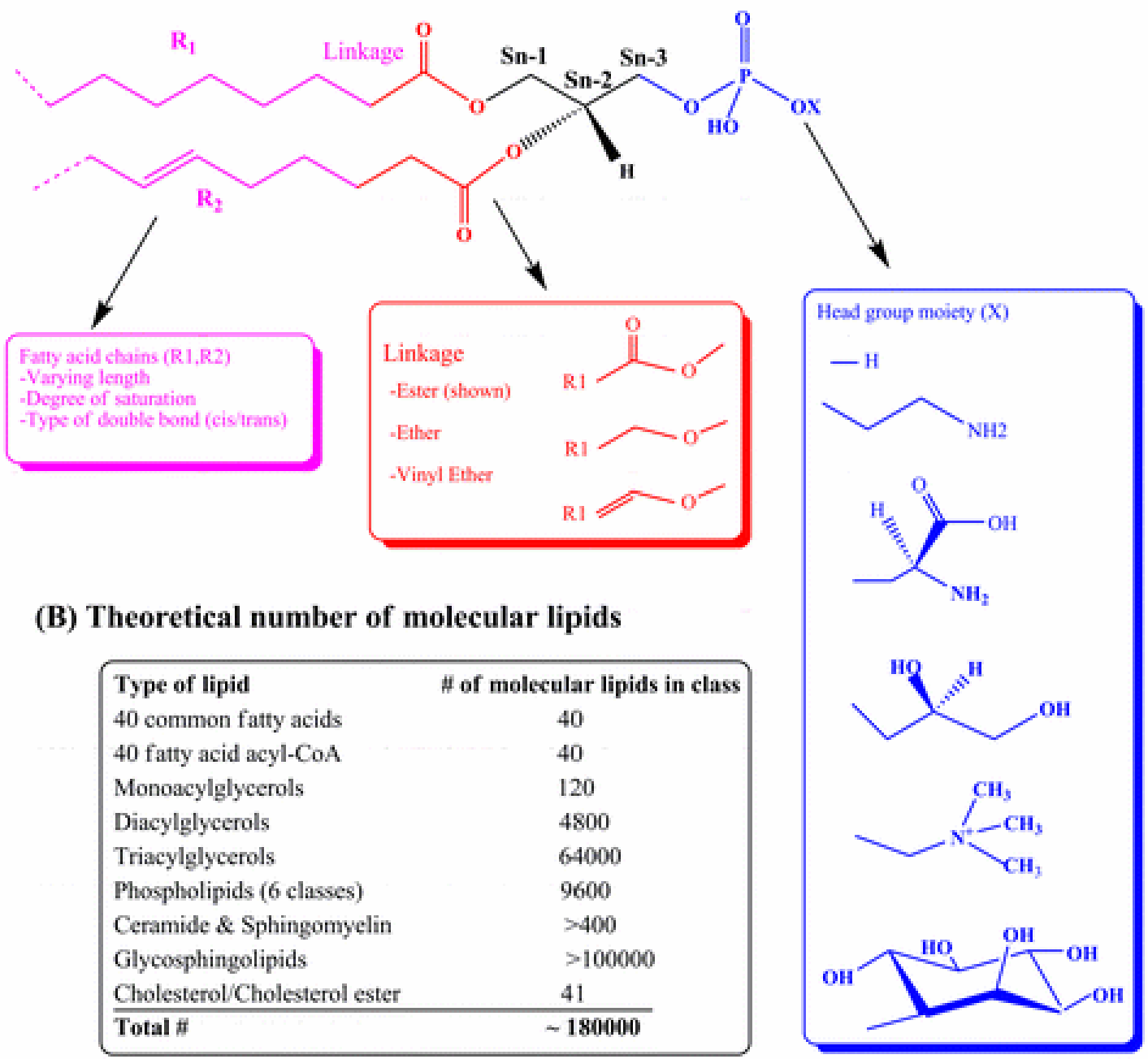
Metabolomic experiment flowchart




Scores for PC# 1 versus PC# 2
PCA: liver
HC
LC
Con
Scores on PC# 2 (24.4%)
Scores on PC# 1 (36.6%)
20
15
10
5
0
-5
-10
-15
-10
-5
0
5
10
15

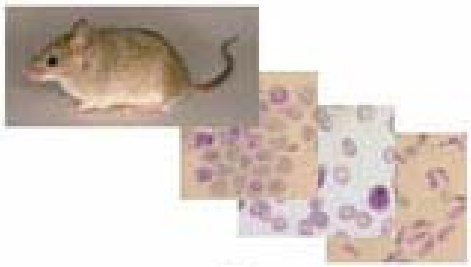

Plasma
C23:0 SPM,
C20:3 TG,
GC/MS peak
Liver
Tissue
higher in G3 : G1
lower in G3 : G1
positive correlation
negative correlation
LC/MS Lipid
GC/MS
Transcript
Pathway Instance

(A) Glycerophospholipid Structure



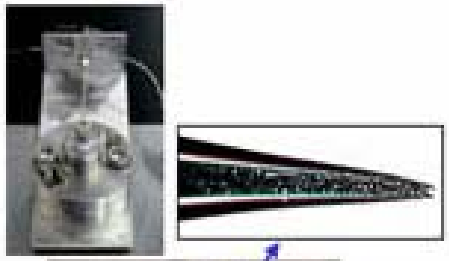
(B) Theoretical number of molecular lipids

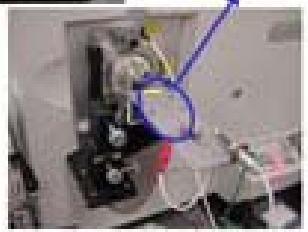
Type of lipid	# of molecular lipids in class
40 common fatty acids	40
40 fatty acid acyl-CoA	40
Monoacylglycerols	120
Diacylglycerols	4800
Triacylglycerols	64000
Phospholipids (6 classes)	9600
Ceramide & Sphingomyelin	>400
Glycosphingolipids	>100000
Cholesterol/Cholesterol ester	41
Total #	**~ 180000**



Extraction of metabolites

Selective enrichment using various StageTips



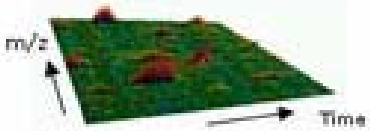


nanoLC-MS with Arch-stone columns

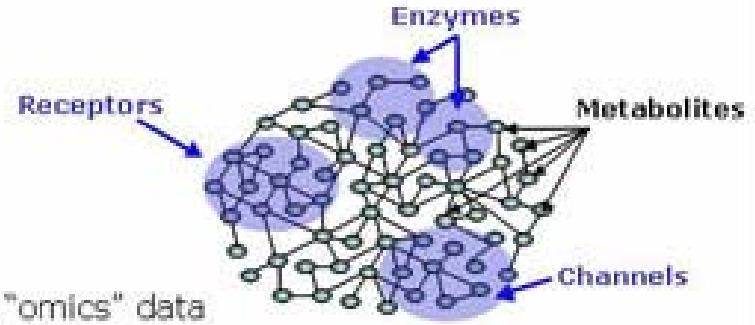
Raw data

Transcriptome

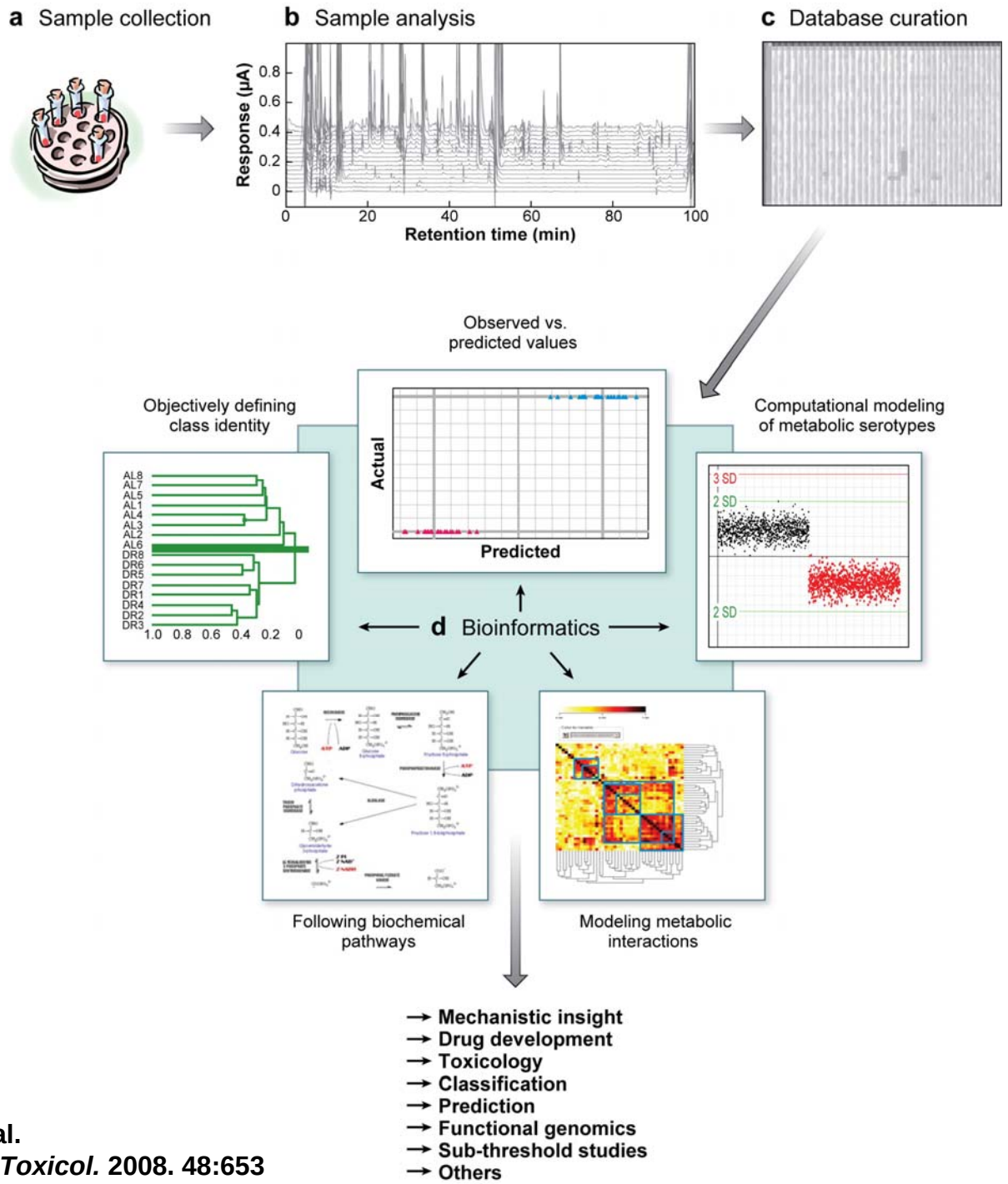
Proteome



Data management system



Mapping all "omics" data

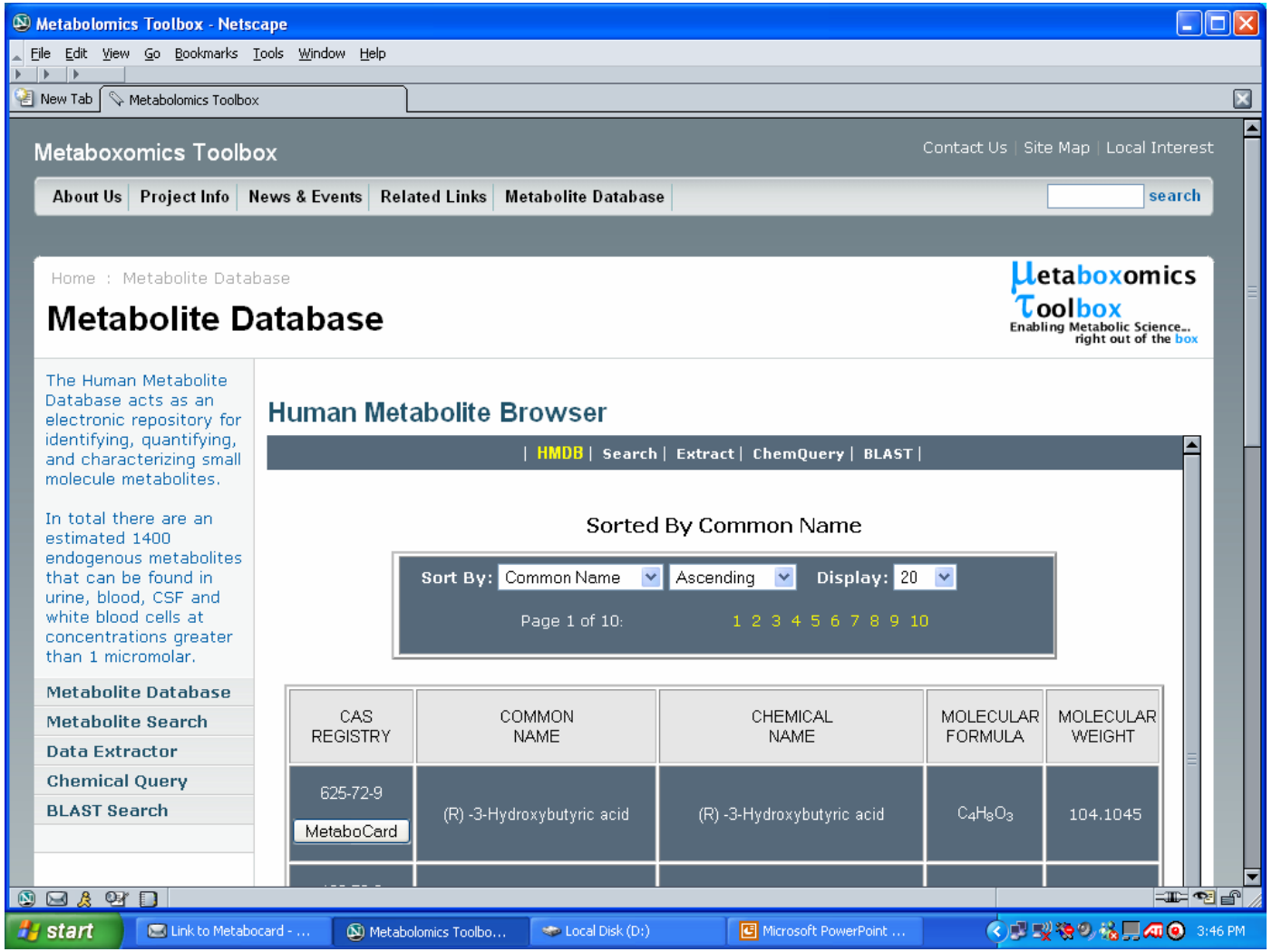


Kaddurah-Daouk et al.
***Annu Rev Pharmcol Toxicol.* 2008. 48:653**

Metabolic Profiling: The Possibilities

- Toxicology Testing
- Clinical Trial Testing
- Fermentation Monitoring
- Food & Beverage Tests
- Nutraceutical Analysis
- Drug Phenotyping
- Water Quality Testing
- Petrochemical Analysis
- Genetic Disease Tests
- Nutritional Analysis
- Clinical Blood Analysis
- Clinical Urinalysis
- Cholesterol Testing
- Drug Compliance
- Dialysis Monitoring
- Forensics

Human Metabolome Database



www.hmdb.ca

More than one method needed for metabolomics

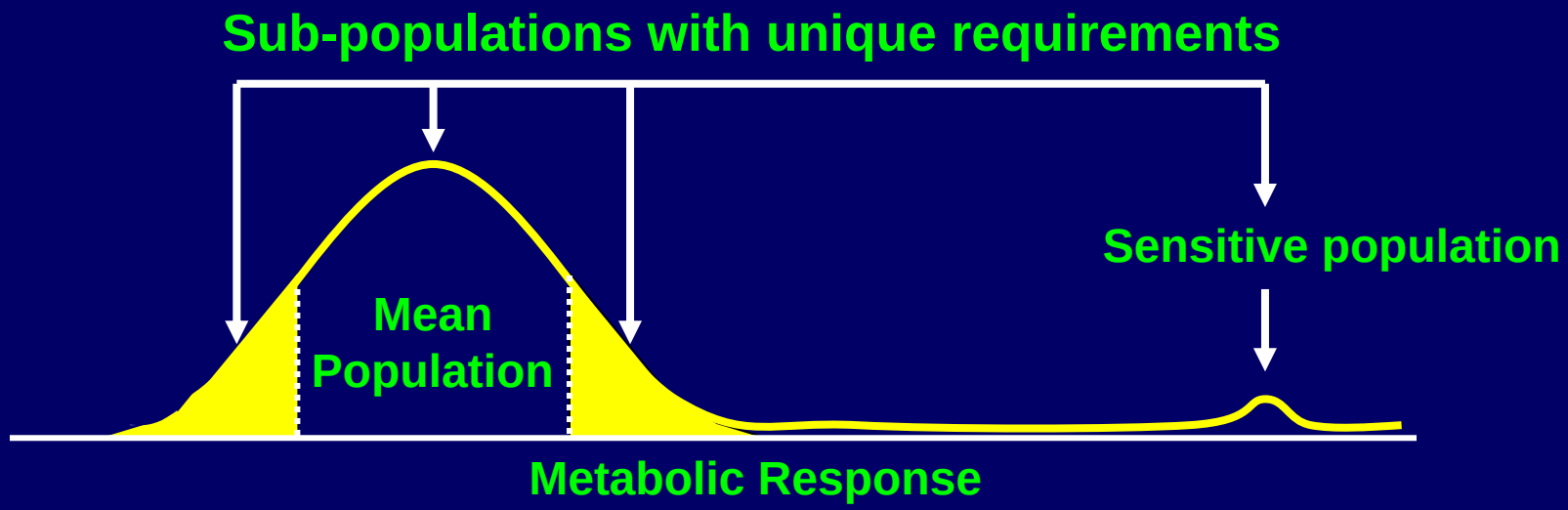
- **NMR or MS fingerprinting**
  - **Very robust, 500-10,000 variables high-throughput 5-50 identified compounds**
- **GC/MS**
  - **Robust, ~500-1000 primary metabolites <550 Da high-throughput 50-200 identified compounds**
- **LC/MS**
  - **Not as robust, ~50-70 secondary metabolites <2500 Da low-throughput 20-100 identified compounds**

- **Exciting methodology that can provide quantitative phenotyping**
- **Important step in the omics cascade**
- **Leads to personalized medicine**

What is the pay off from metabolomics?

Mechanistic Insight into Biological Processes

A Step Towards Personalized Medicine



We are not all the same!

And we do not want to be the same!!!!

Sports Illustrated 2002

Thank you for your attention