MRG: Metabolomics Research Group

William Wikoff : UC Davis (Committee chair)

Pavel Aronov: Stanford University

John Asara: Harvard University

Vladimir Shulaev: University of Texas

Chris Turck: Max Planck (EB liason)

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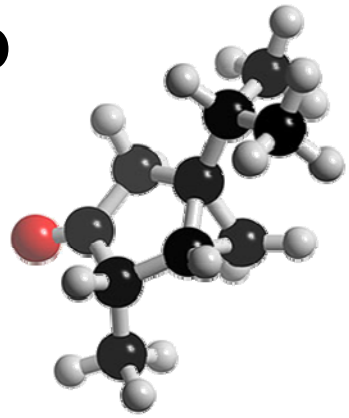
What is Metabolomics ?



"Systems Biology of Small Molecules"

All biomolecules in a system

NOT (protein OR DNA/RNA)

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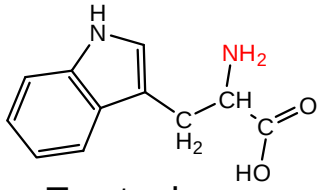
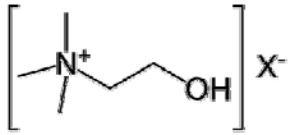
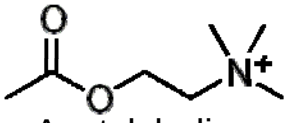
Chemical diversity of the metabolome



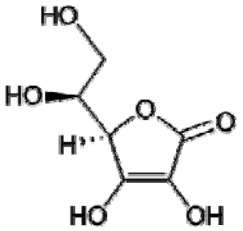
Tryptophan



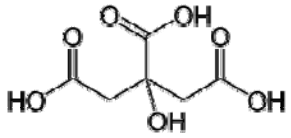
choline



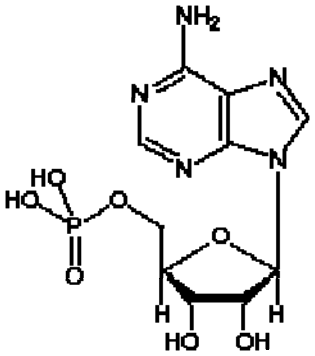
Acetylcholine



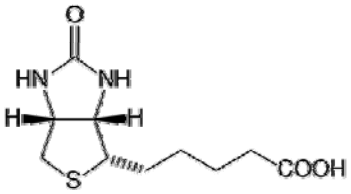
Ascorbic Acid
(Vitamin C)



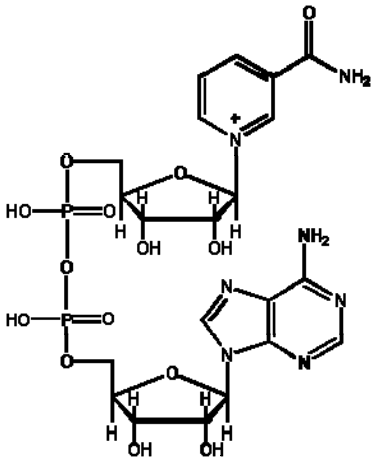
Citric acid



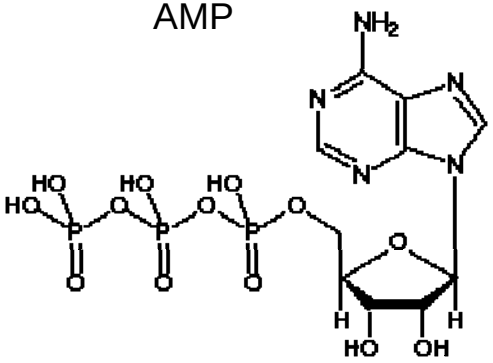
AMP



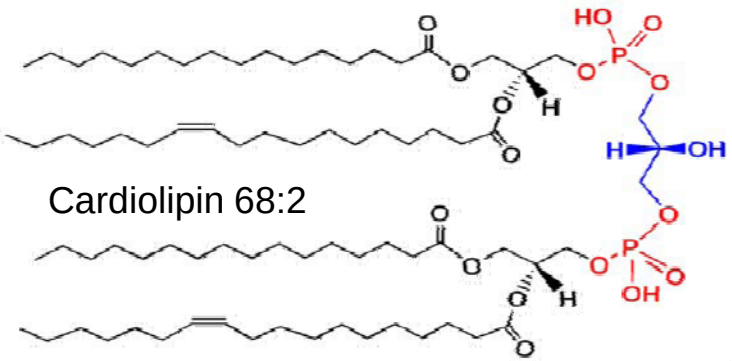
Biotin
(Vitamin H; B7)



NAD



ATP



Cardiolipin 68:2

Workflow for untargeted metabolomics

Experimental Design

Sample Collection

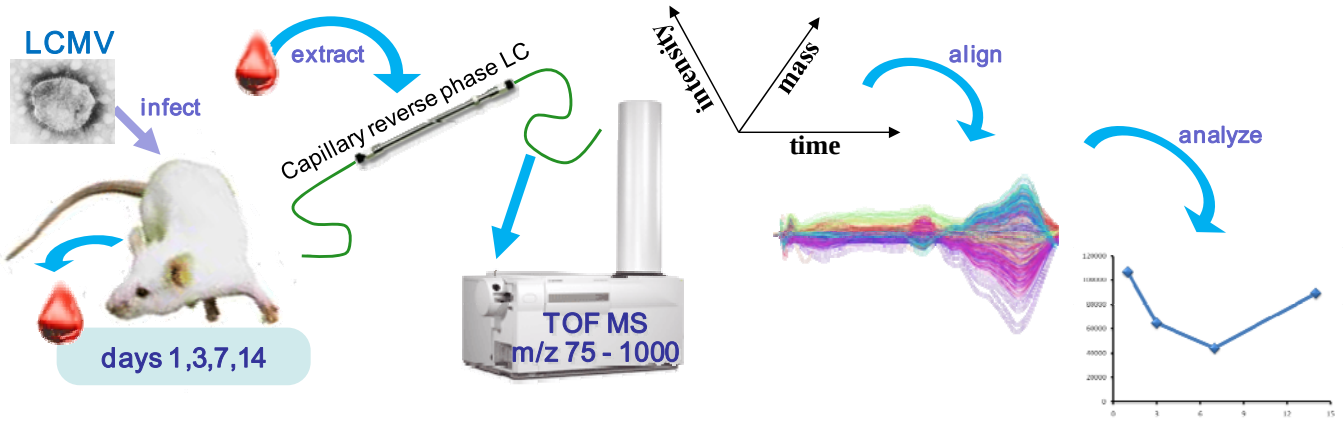
Sample Preparation Extraction

LC/MS Profiling

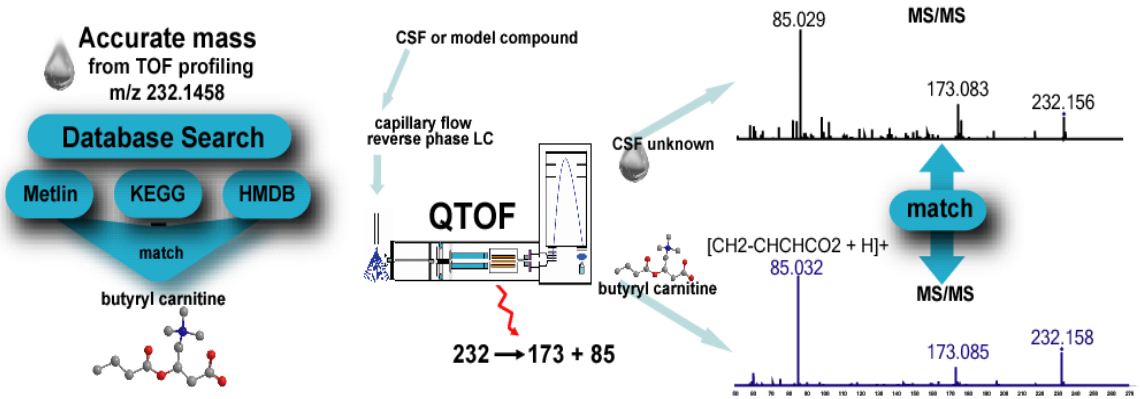
Data Postprocessing

Data Analysis and Statistics

Step 1: Untargeted Metabolomic profiling

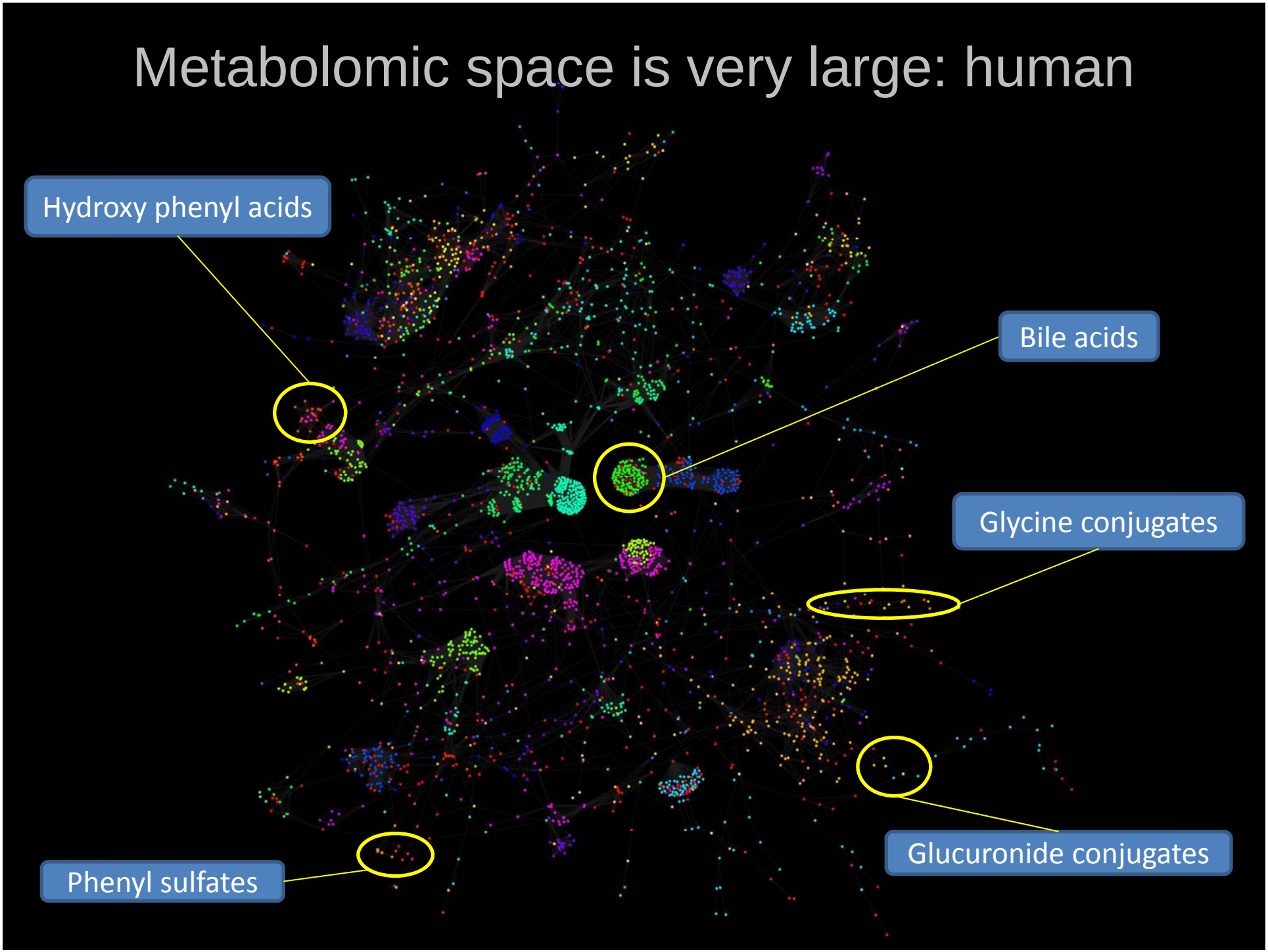


Step 2: Compound Identification

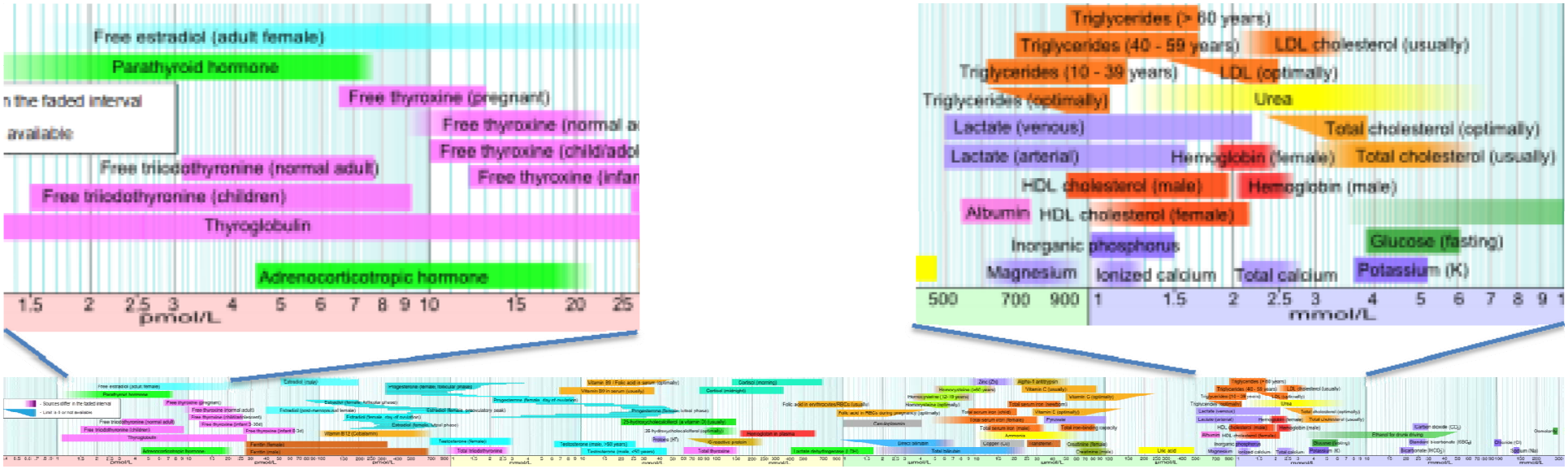


Compound Identification → Biochemical Analysis and pathway mapping

Metabolomic space is very large: human



Incredibly wide concentration range of plasma metabolites



pM → mM

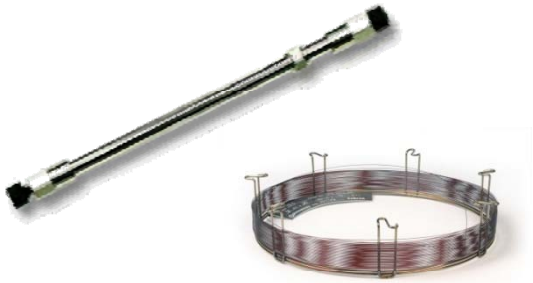
hormones

Range is 10^9 fold

cholesterol

No single method can capture

Multiple analytical approaches to achieve complete metabolome coverage



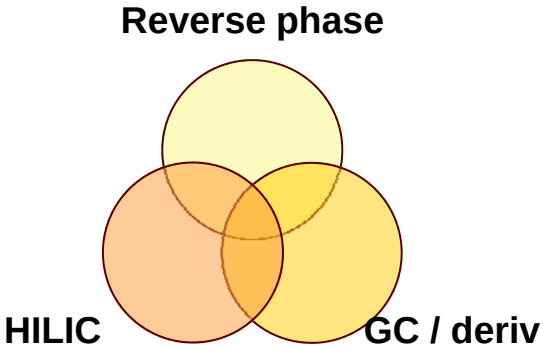
Chromatography

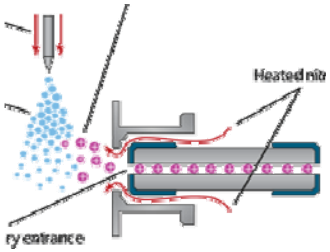
Reverse phase

HILIC

Capillary → higher flow

GC





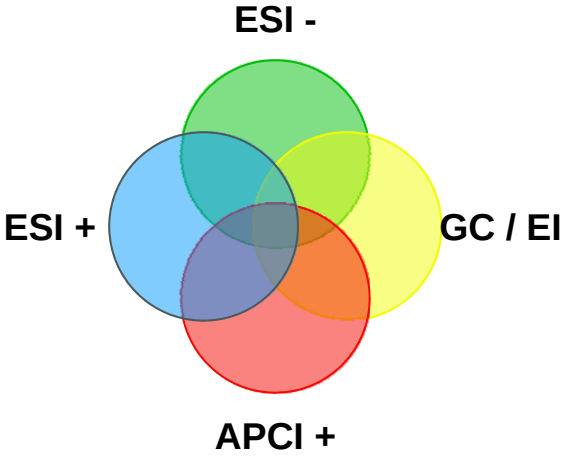
Ionization

ESI (-)

ESI (+)

APCI (+)

EI (+) [GC]



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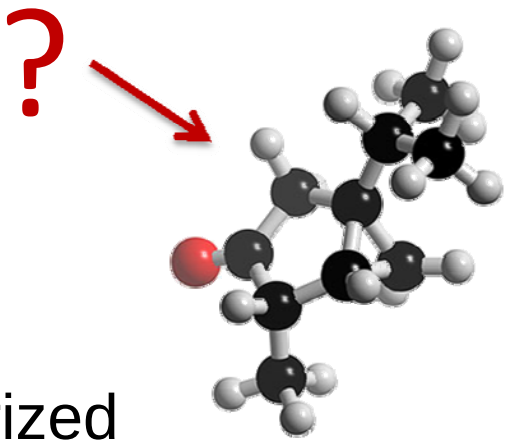
Untargeted Metabolomics: why is compound identification challenging ?



<u>No linear “blueprint”</u>

Playing field is ill-defined

Most metabolites probably uncharacterized

How many metabolites are we looking for ?

<u>MS/MS Fragmentation patterns</u>

Not characterized…no “library” (MS/MS)

Not predictable (as with peptides)

MRG Inter-laboratory metabolomics study : 2011

design a study that resembles a typical metabolomics experiment

Participants will identify differences between groups of samples: compound identification most challenging.

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International character of MRG study respondents

Initial solicitation of interest from metabolomics labs, ABRF members, etc. by email.

Participating Countries

US
Canada
England
Scotland
Ireland
Germany
Spain
Italy
Netherlands
Australia
Japan
South Korea
China
Singapore

~25% USA & Canada
~35% Europe
~25% Asia

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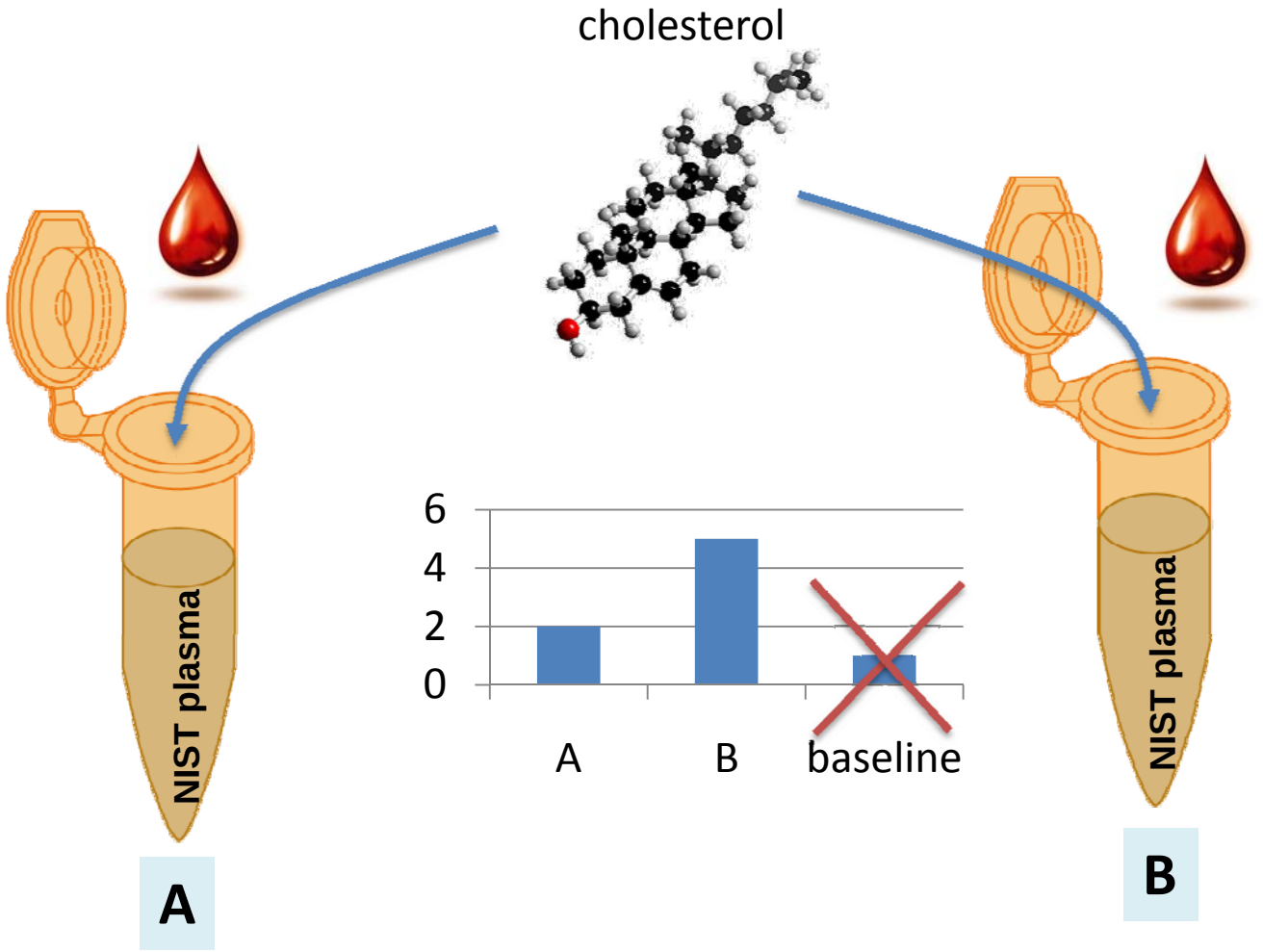
Spike-in experimental design



Testing & validation

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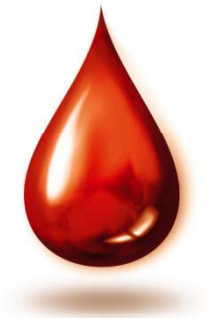
Four principles of compound selection

1. For most of the endogenous plasma compounds, the compounds should be chosen that have already been measured in concentration by NIST.
2. Compounds should be selected such that they are well distributed in terms of ability to analyze by a particular technique. For example, some compounds should be detectable in ESI+ whereas others should be detectable in ESI-, EI or APCI.
3. Compounds should be selected with a range of difficulty of identification, regardless of technique used.
4. High purity compounds should be chosen.

New NIST plasma standard is an ideal matrix for inter-laboratory studies



Analyzed and Validated by multiple analytical platforms

and multiple groups

Can be used for comparisons over long periods of time

NIST has generously donated the plasma that will be used for the MRG study

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Platforms used for characterization & validation

GC-TOF w/ library: Tolstikov

Q-TOF: Wikoff

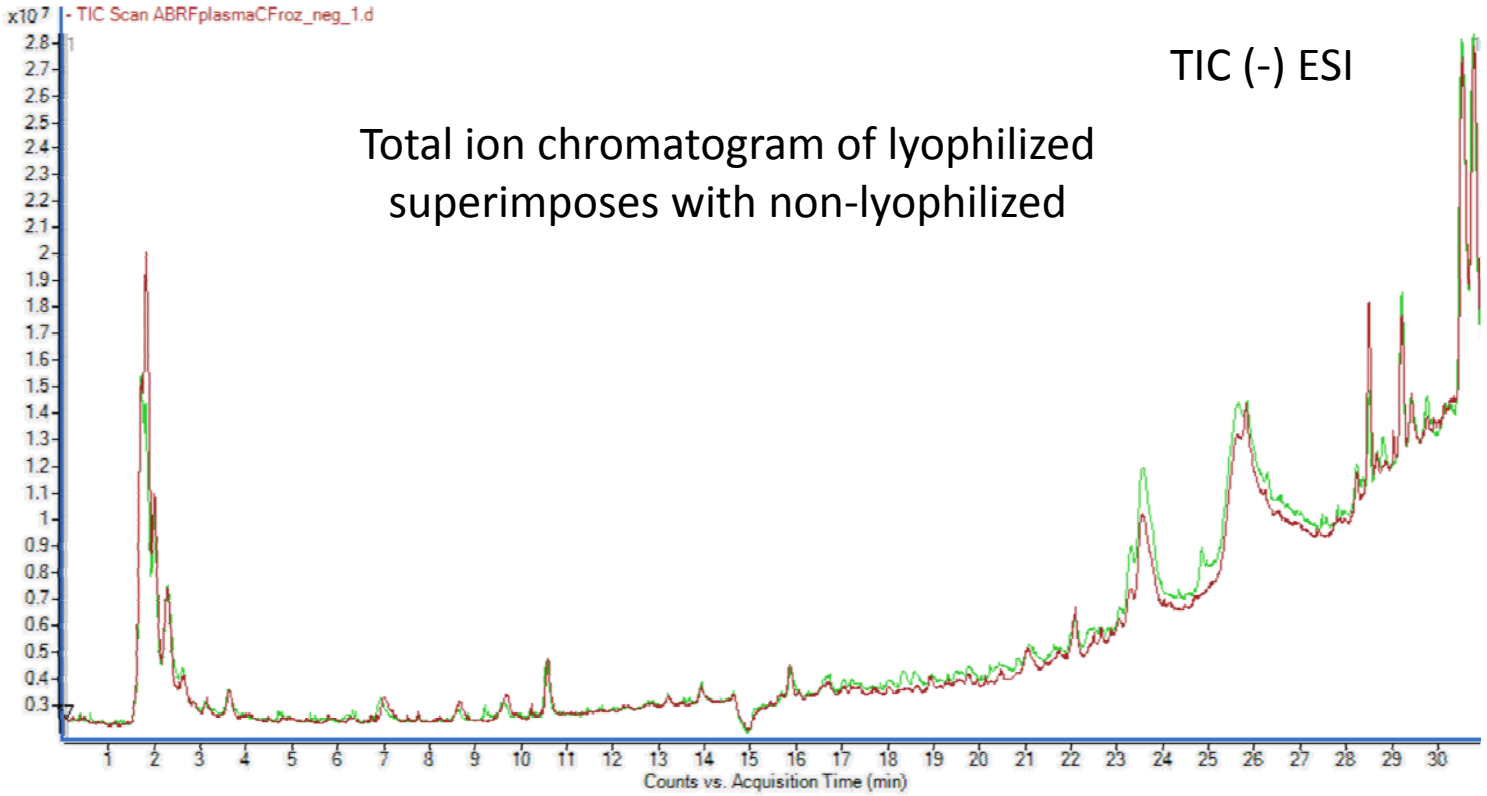
Exactive: Aronov

Triple Quadrupole (2 platforms): Asara & Shulaev

Can we lyophilize samples to simplify & reduce cost of shipping?

Lyophilized material sat for ~ 3 weeks at room temp before reconstitution

Overall Validation of Lyophilization for sample preparation: comparison to frozen sample



TIC (-) ESI

Total ion chromatogram of lyophilized superimposes with non-lyophilized

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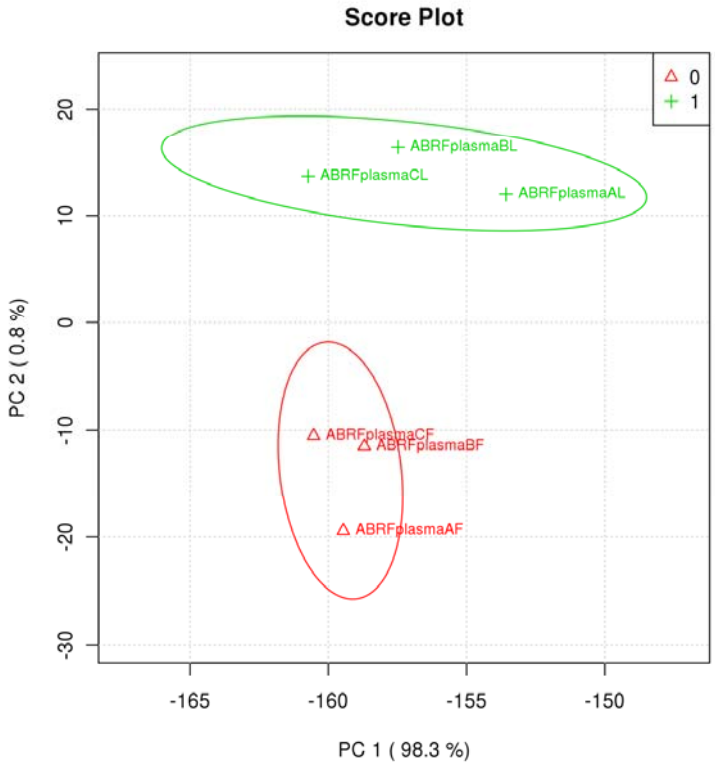
There are differences between lyophilized and frozen plasma: PCA



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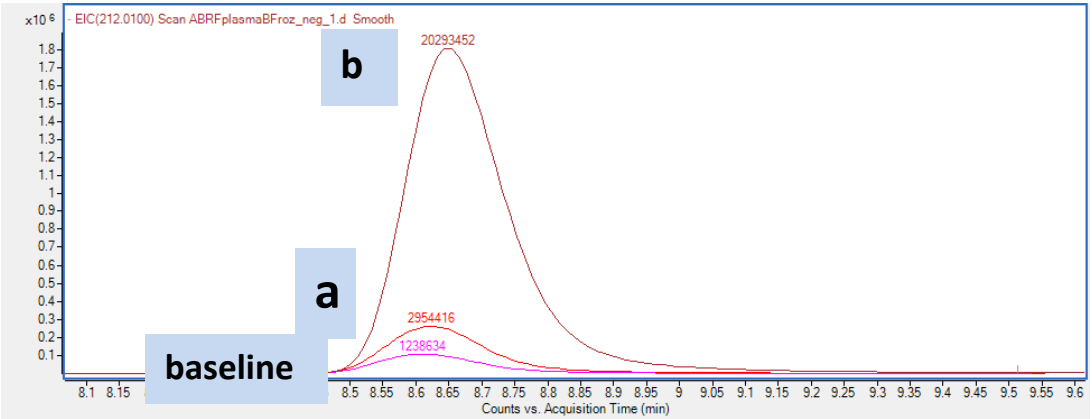
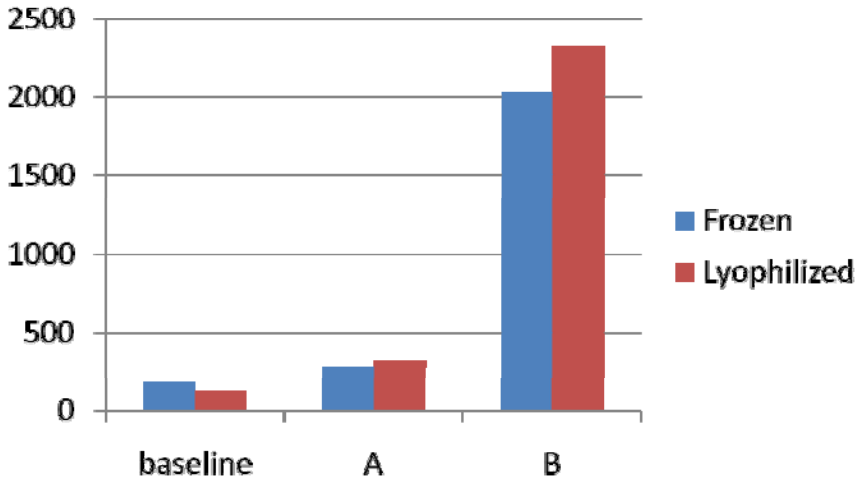
Validation of lyophilization versus frozen sample for compound X





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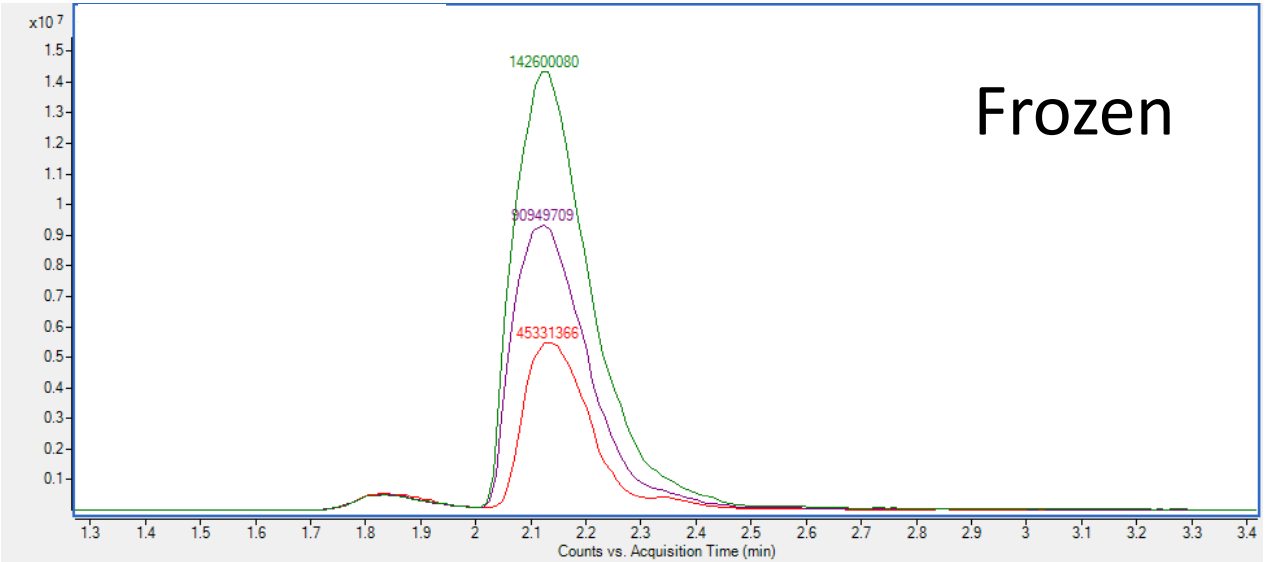
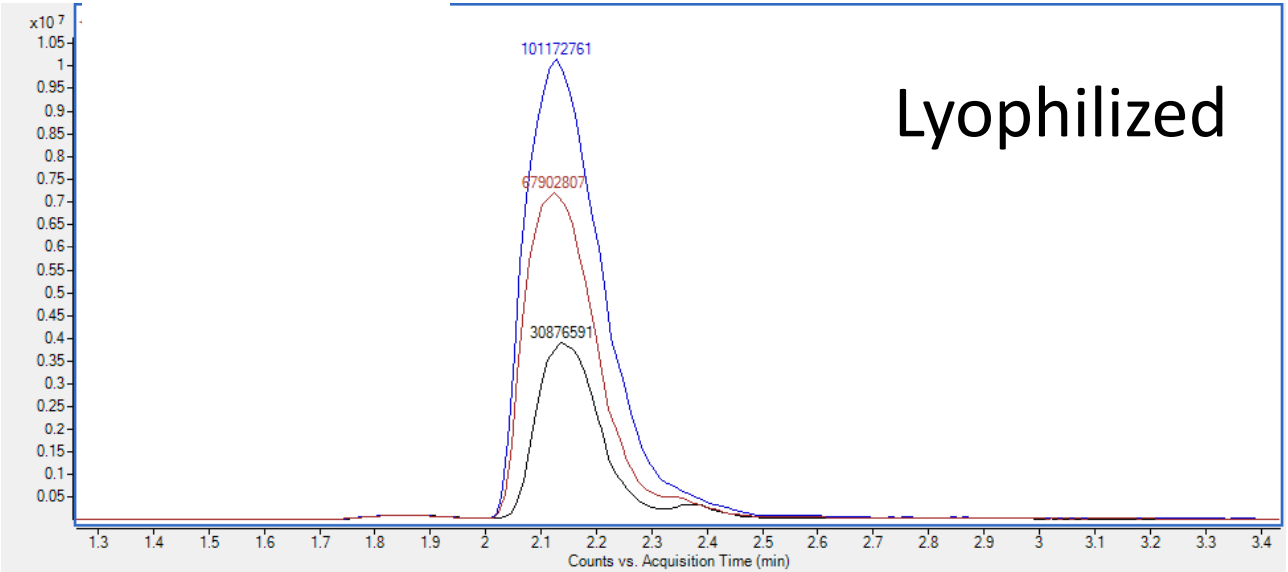
Compound ‘Y’ Frozen vs. Lyophilized



Frozen



Lyophilized

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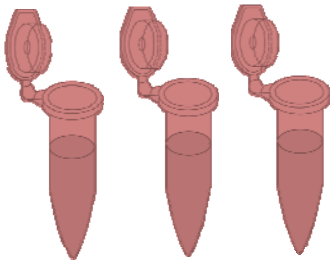
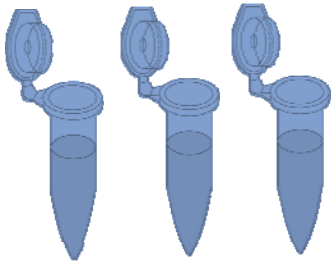
Study Design

NIST plasma matrix
Pure compounds spiked into each tube at different levels

Group A



Group B



~100 µl per tube

Enough material is available to send to approximately 100 individuals.
Limitation is the amount of NIST plasma available.

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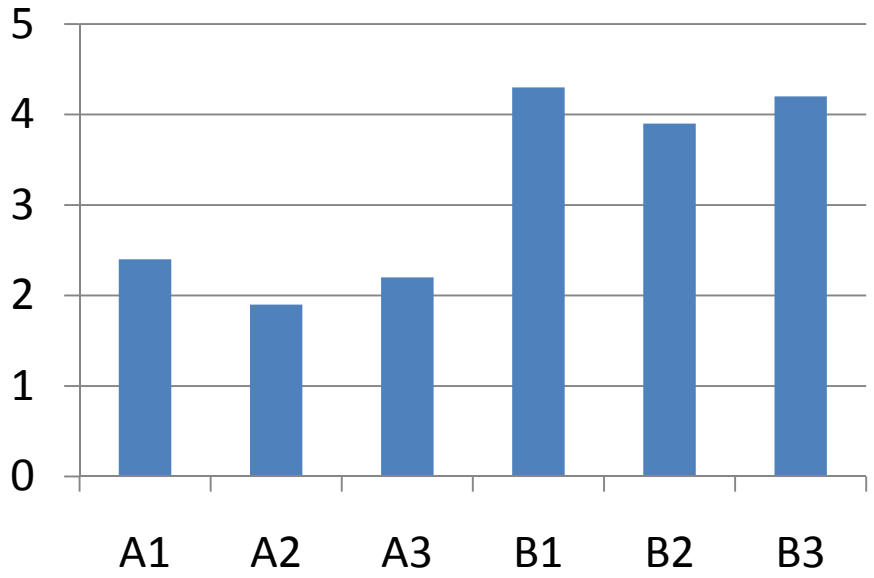
Example data for compound ‘X’

Compound ‘X’
n = 3, two groups



~ 2-fold difference between groups
A and B, with $p < 0.01$

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Result Reporting

For each compound:

m/z, ion mode of each compound (mass specrometry)

Molecular formula (or multiple formulas if amiguous)

Fold-change

Statistical metric for difference

Identity of compound

Next Steps

Additional rounds of compound vetting and validation

Final validation for spiked samples

Send out ~100 samples → August/September 2011

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