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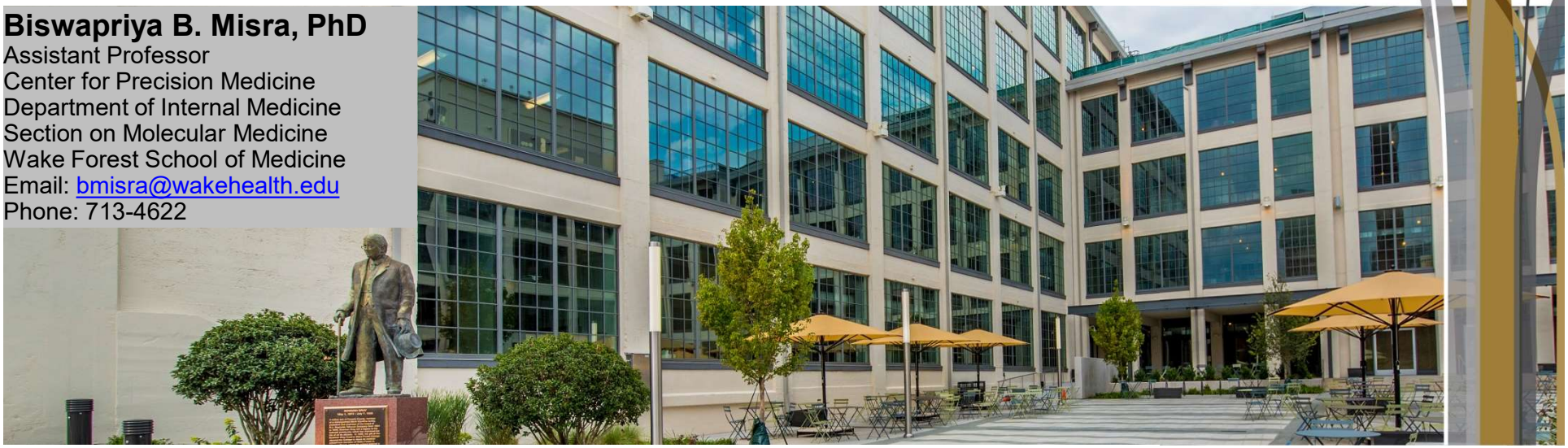
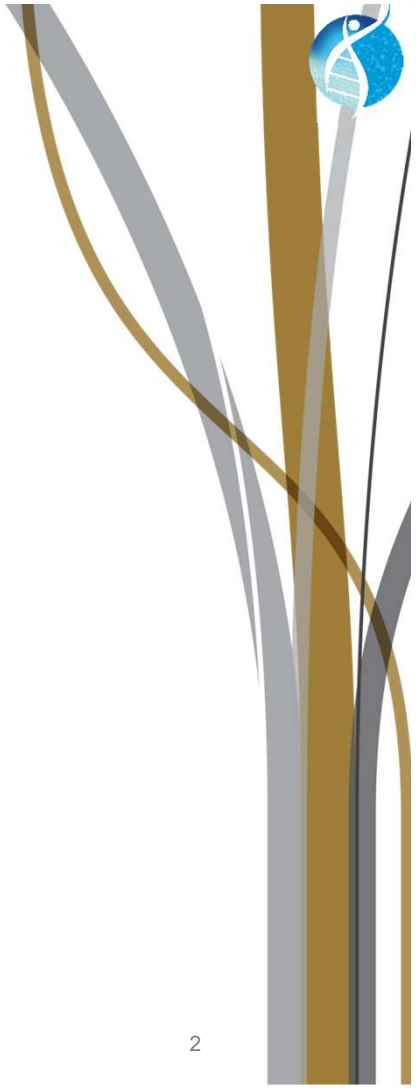
# Metabolomics as a Biomarker Discovery Platform: Opportunities in Alzheimer's Disease Research

**Biswapriya B. Misra, PhD**
Assistant Professor
Center for Precision Medicine
Department of Internal Medicine
Section on Molecular Medicine
Wake Forest School of Medicine
Email: bmisra@wakehealth.edu
Phone: 713-4622

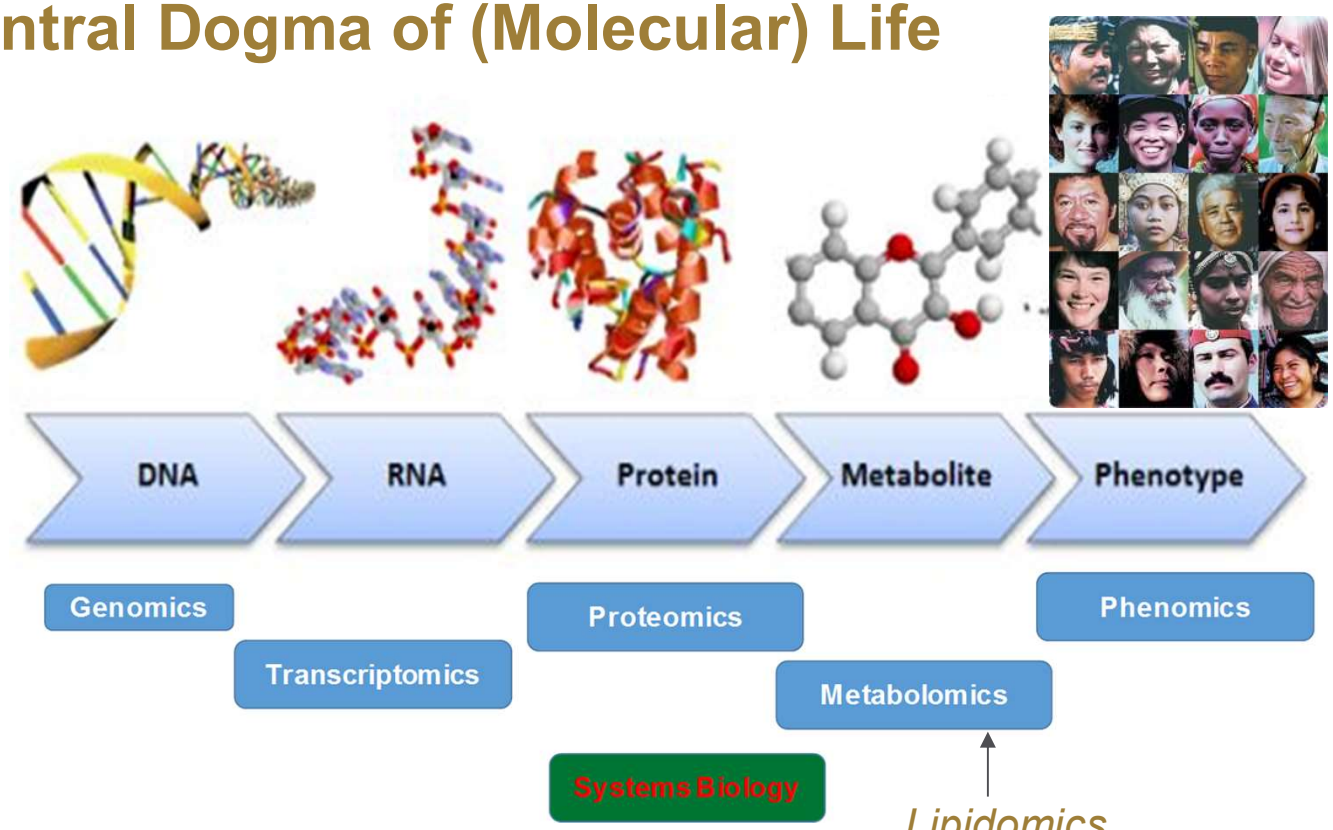


# Outline of the Talk

1. Why Metabolomics- Definitions and Scope
2. Metabolomics Biomarkers: The State of the Art
3. Metabolomics in Alzheimer's Disease
4. Future Scope of Metabolomics with CPM

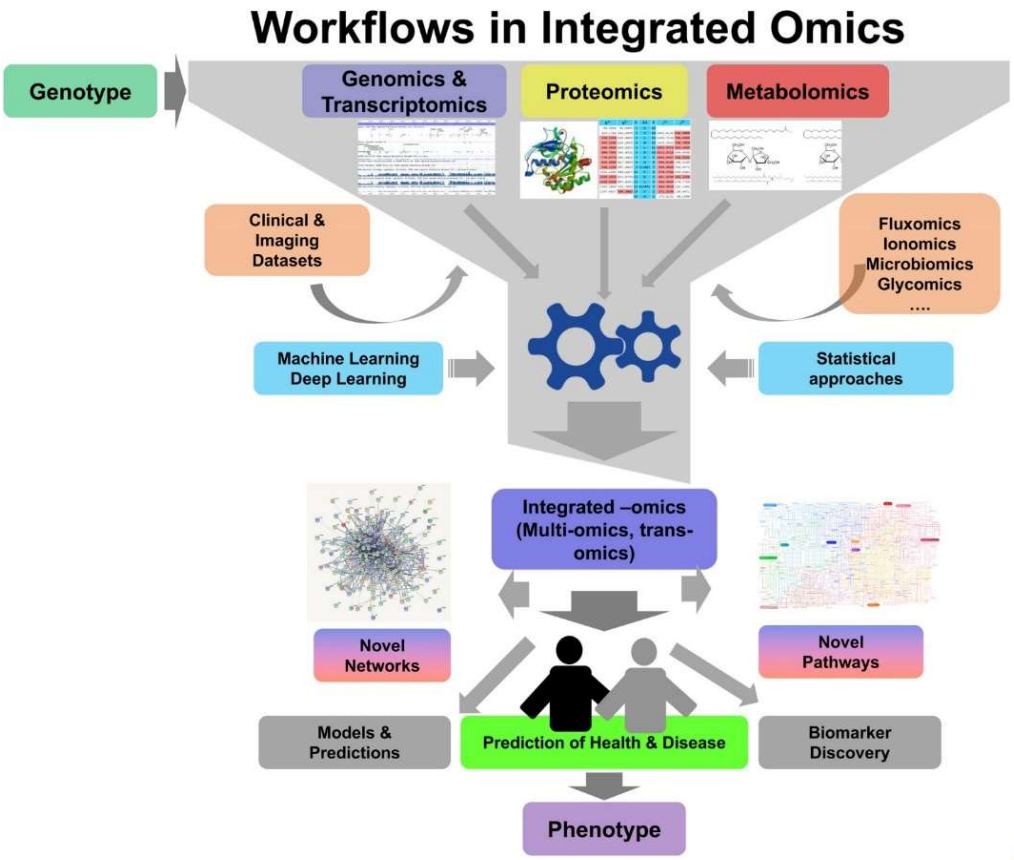


# Central Dogma of (Molecular) Life




Modified from Source: https://www.mdpi.com/1422-0067/14/3/4885

# Current State of Art in Integrated Omics



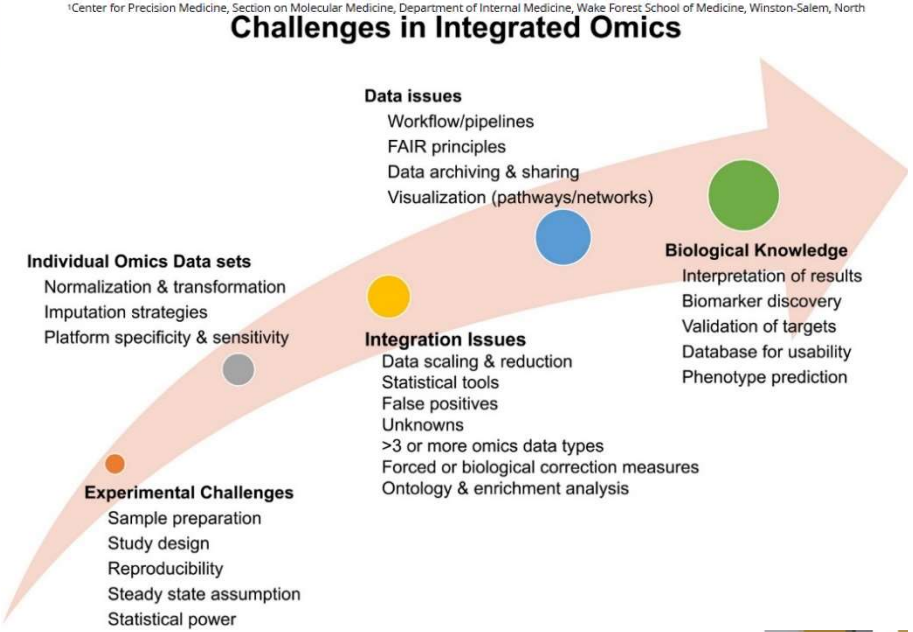

Journal of Molecular Endocrinology | B B Misra *et al.* | Approaches and tools in integrated omics | 62:1 | R21–R45

REVIEW

## Integrated omics: tools, advances and future approaches

**Biswapriya B Misra**[1], **Carl Langefeld**[1,2], **Michael Olivier**[1] and **Laura A Cox**[1,3]

[1]Center for Precision Medicine, Section on Molecular Medicine, Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, North

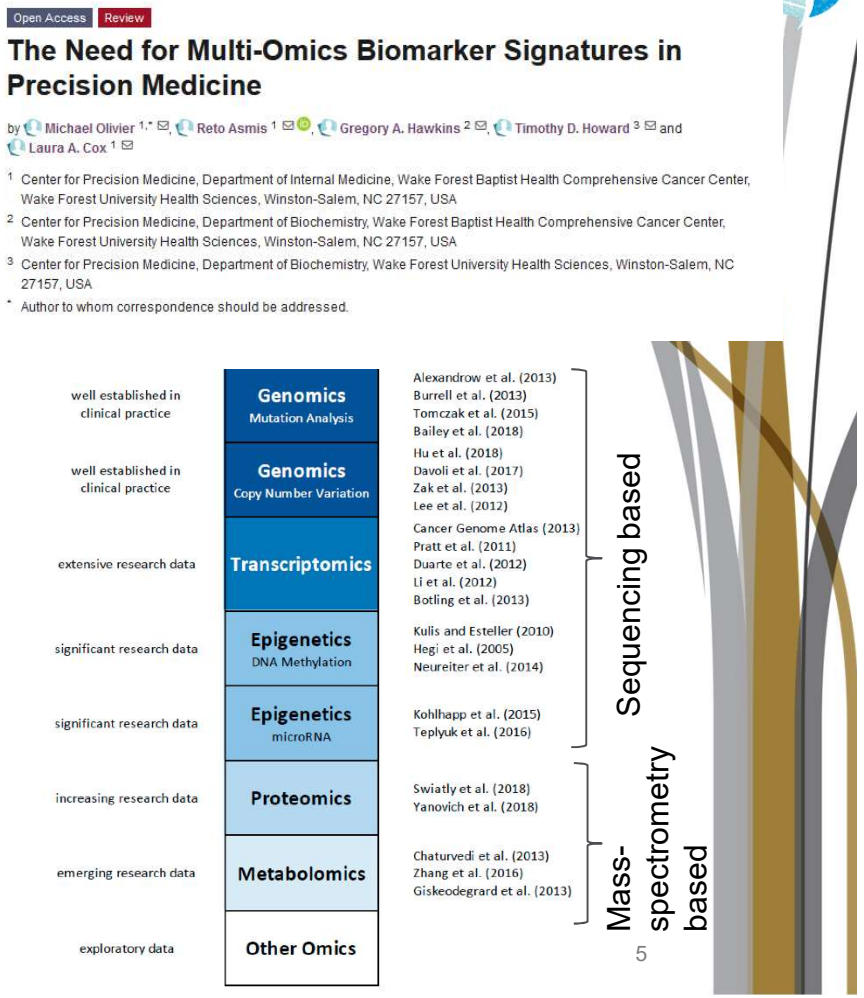


# Omics: Definitions & Importance

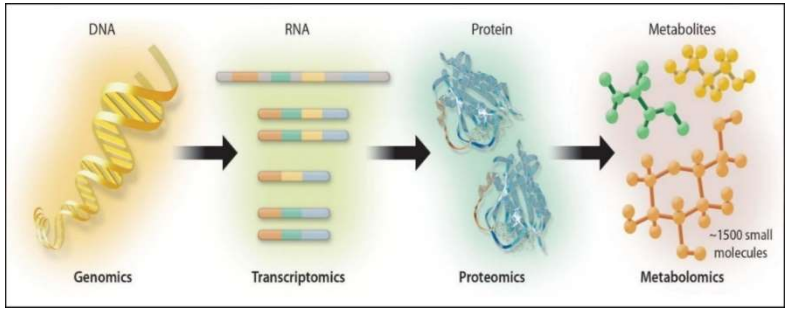
## Omics

The English-language neologism **omics** informally refers to a field of study in biology ending in *-omics*, such as genomics, proteomics or metabolomics.

Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms.



# Metabolomics: Definitions & Concepts




*Patti GJ et al., Nature reviews Molecular cell biology. 2012;13(4):263-9.*

**Metabolite**: Any organic molecule detectable in the body with a MW < 2000 Da (C, H, N, O, P, S)

Includes *sugars, nucleosides, organic acids, ketones, aldehydes, peptides, oligonucleotides, amines, amino acids, lipids, steroids, alkaloids and drugs (xenobiotics)* from humans, plants & microbial products

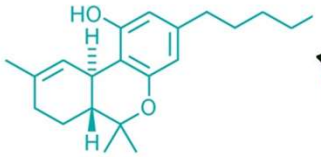
**Metabolomics:** The quantitative measurement of the metabolic profiles of model organisms to characterize their phenotype or phenotypic response to genetic or nutritional perturbations

*NOT:* ~~Metabolite Profiling~~

# Examples of Metabolites (aka. Biochemicals)

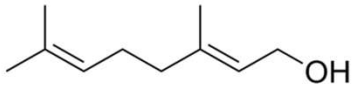
**Cannabis**

**Tetrahydrocannabinol**



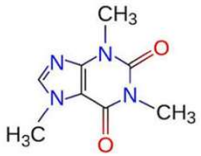

**Rose Scent**

**Geraniol**



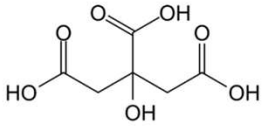

**Coffee & Tea**

**Caffeine**




**Lime juice**

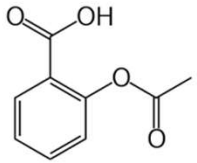
**Citric acid**




**Aspirin**

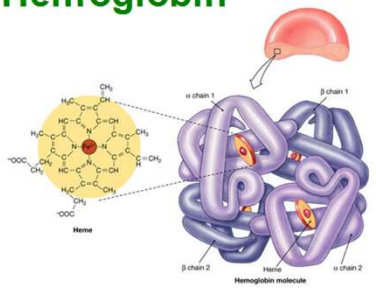



**Acetylsalicylic acid**




**Hemoglobin**

**Heme**




**Rain smell**

**Geosmin**

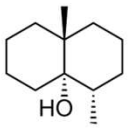
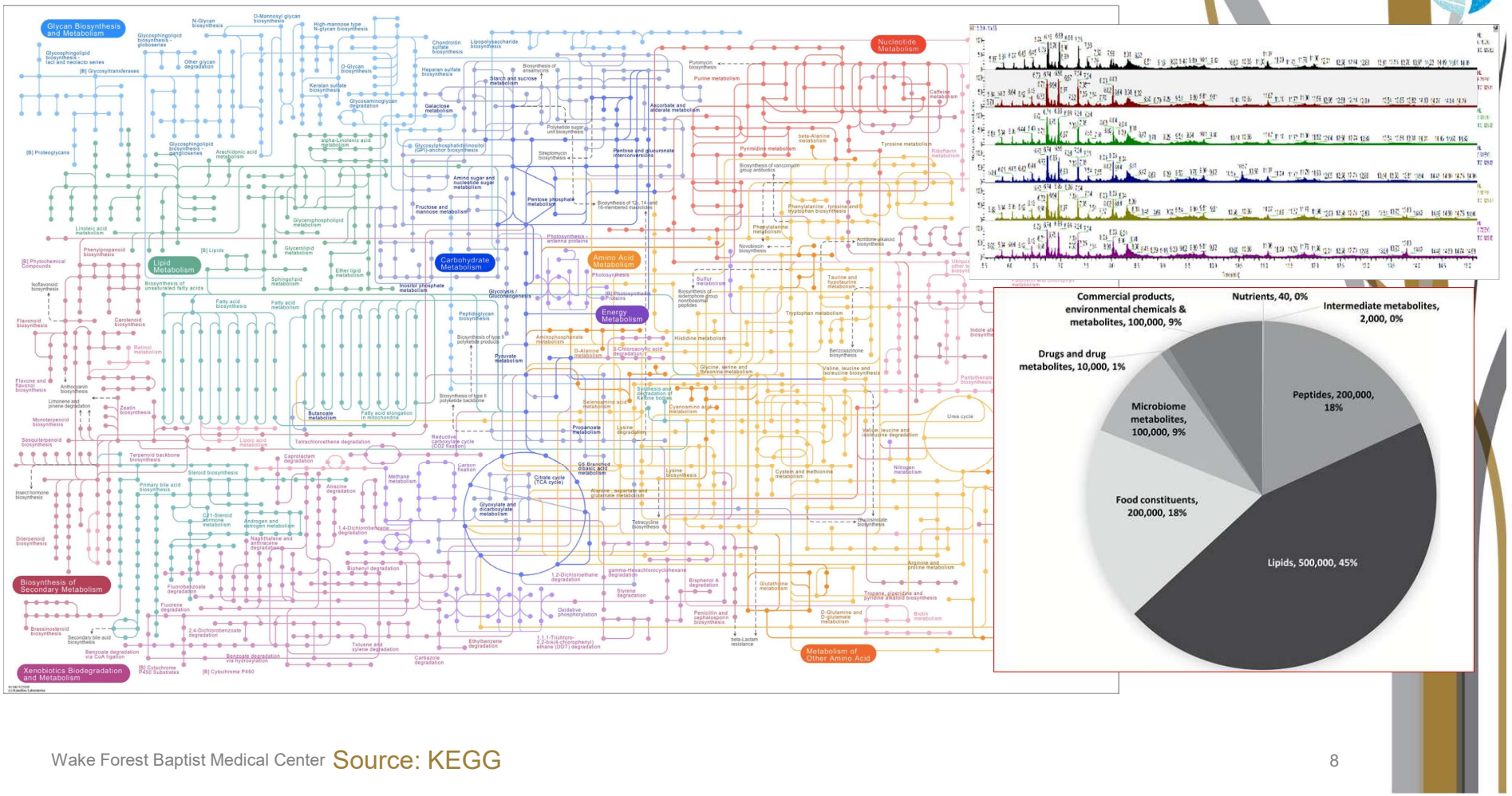





Image Sources: Various, CC0 licensed

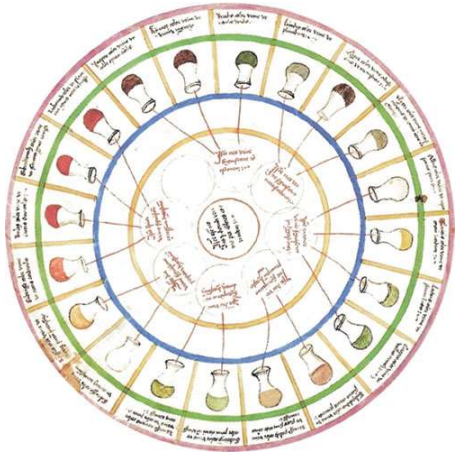
# Cellular Metabolism is very Complex!




 Source: KEGG

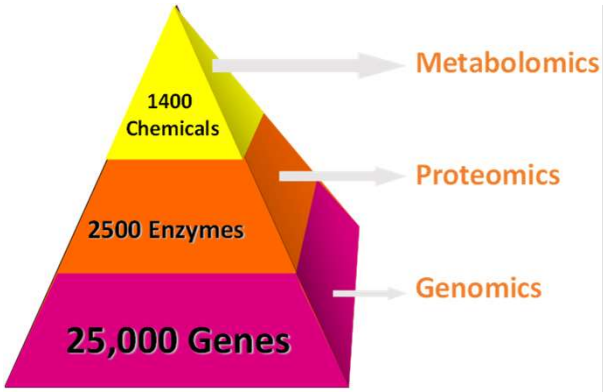
# History and Development of Metabolomics

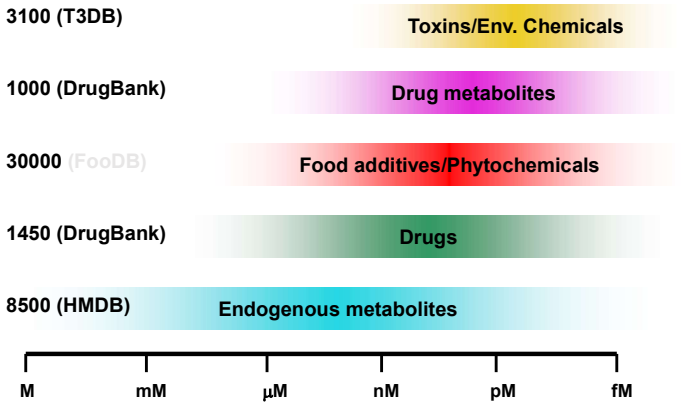
## Urine Wheel



Ullrich Pinder (1506)

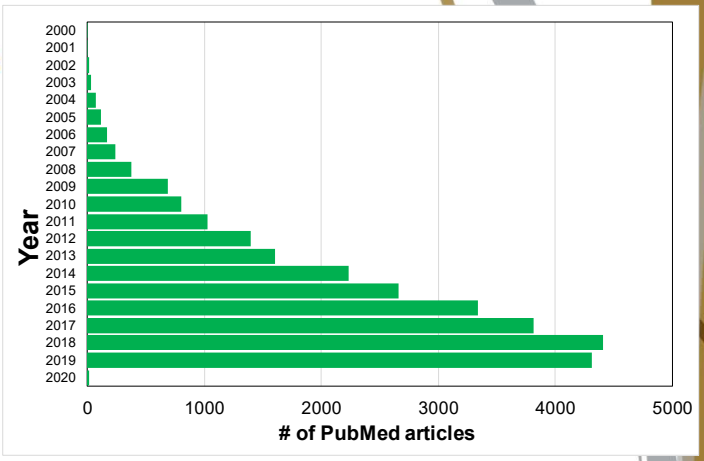
## The Pyramid of Life




Source: David Wishart

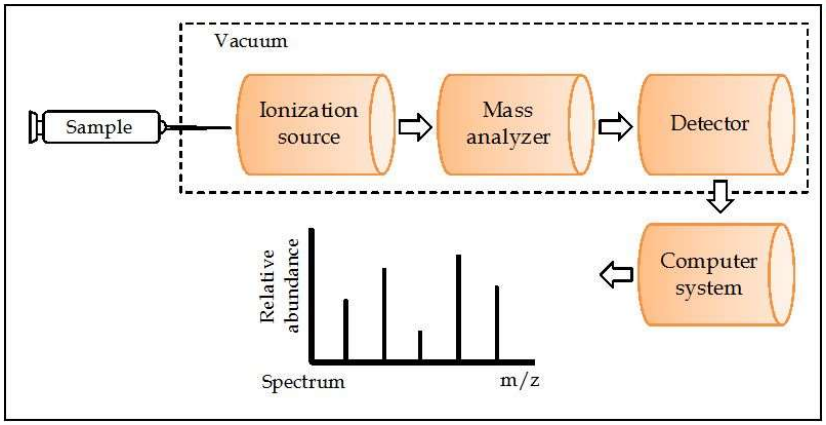
## Metabolomics Is Growing



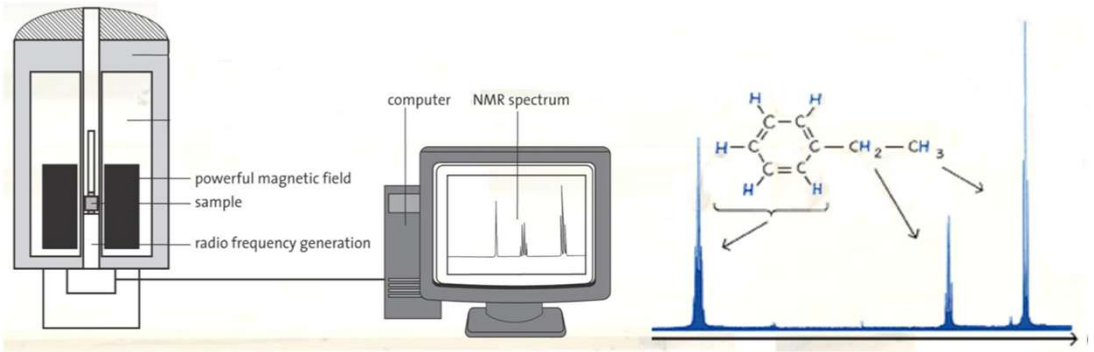
# Metabolomics: Technology/ Platforms

- UPLC, HPLC
- CE/microfluidics
- LC-MS
- GC-MS
- FT-MS
- QqQ-MS
- NMR spectroscopy
- Raman, IR, FTIR
- X-ray crystallography
- LIF detection

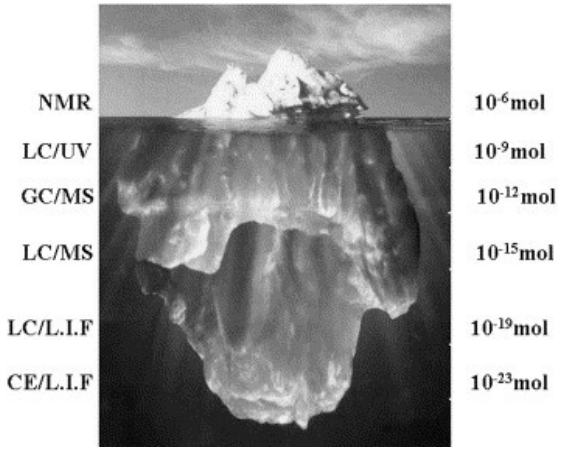
## Mass Spectrometry (MS)

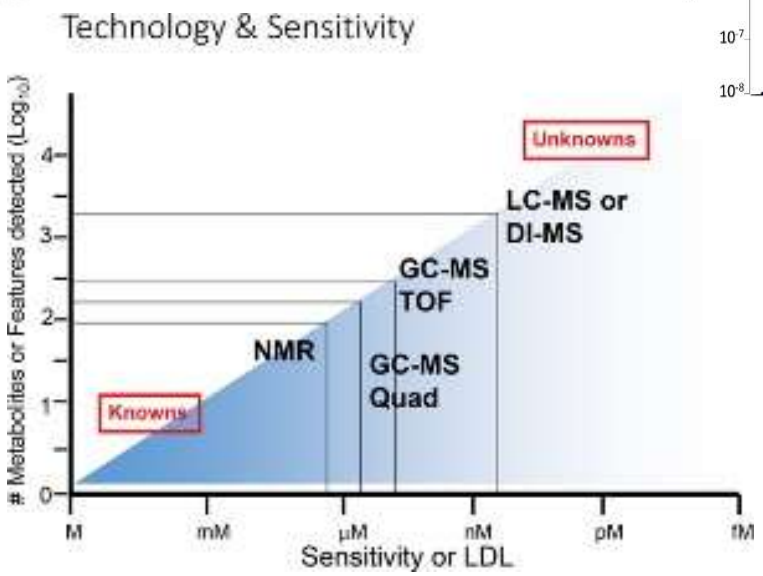


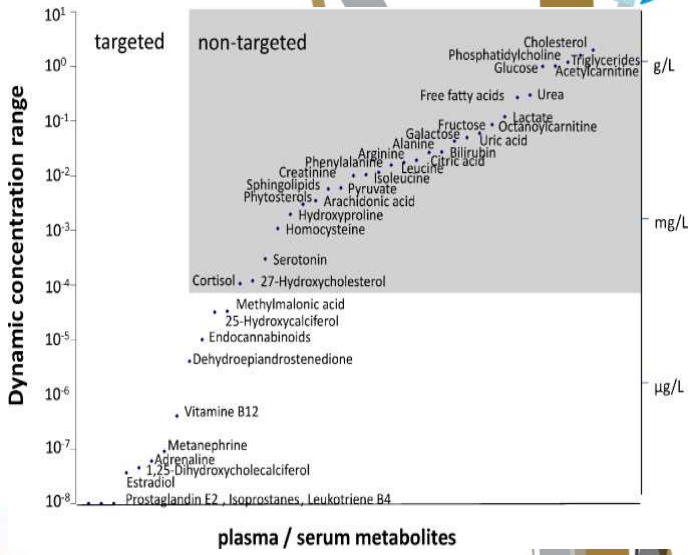

NMR: Nuclear Magnetic Resonance (aka. MRI)



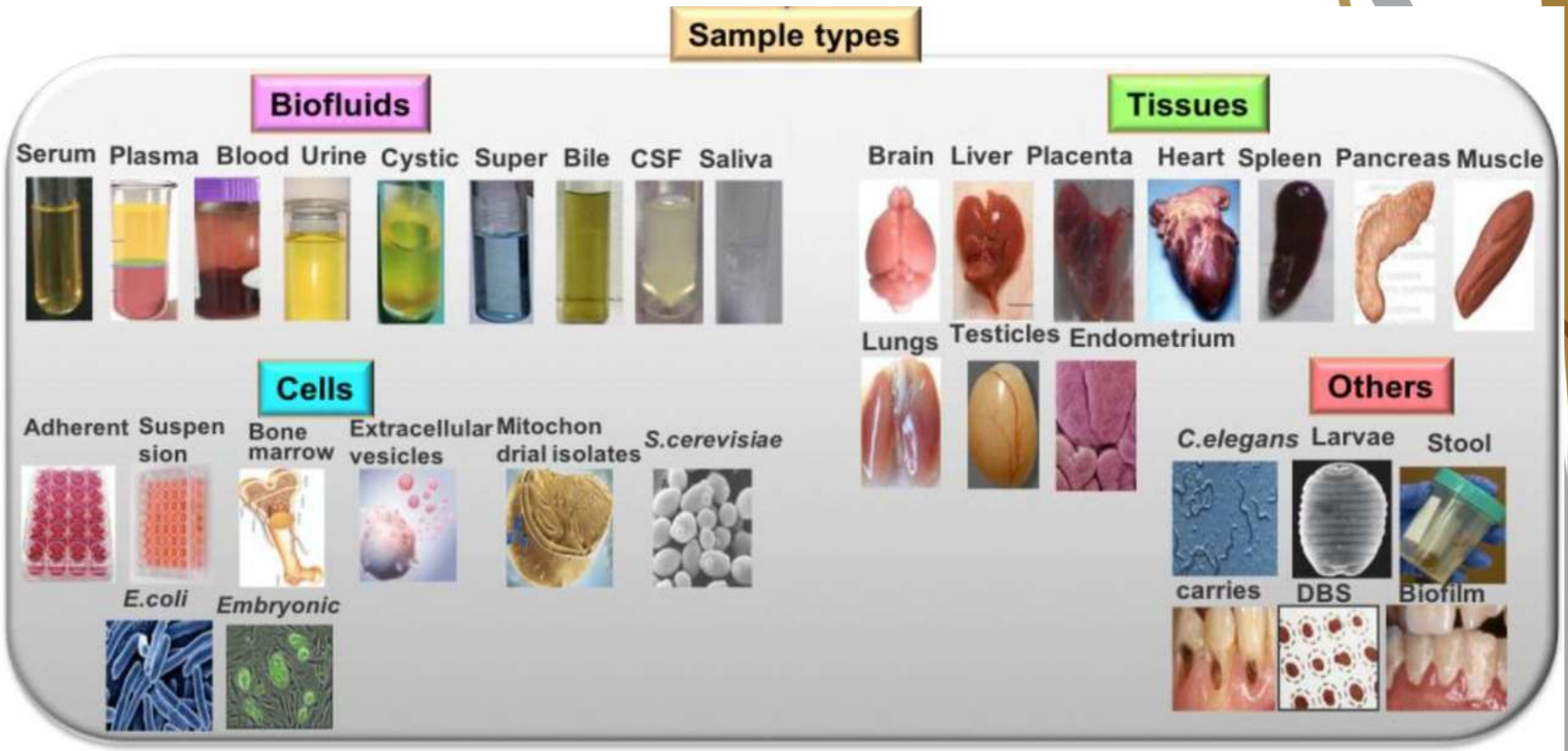
# How to choose the right platform for metabolites?



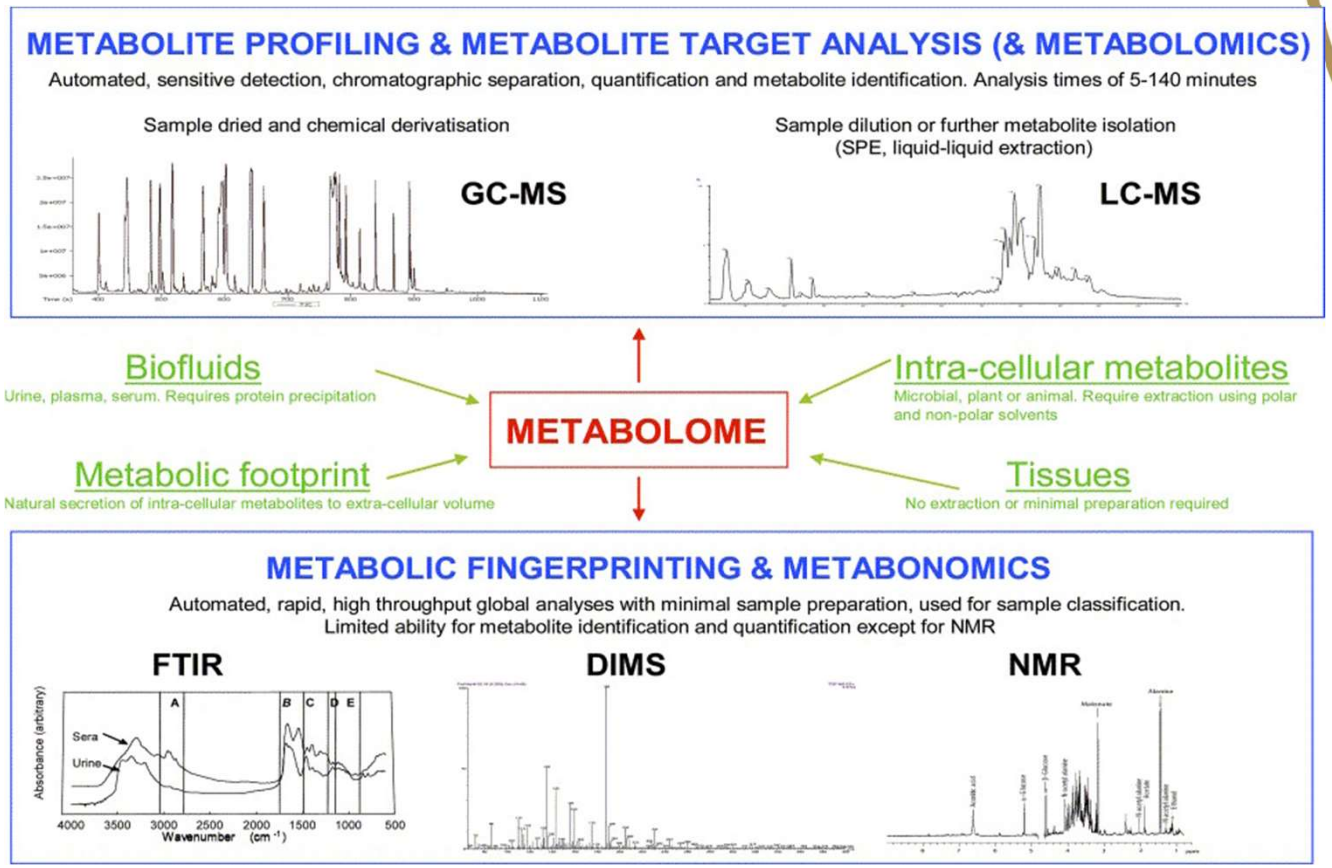



# Where does one find metabolites ?




Nandania et al., 2018, bioRxiv

# Metabolomics Platforms of Choice



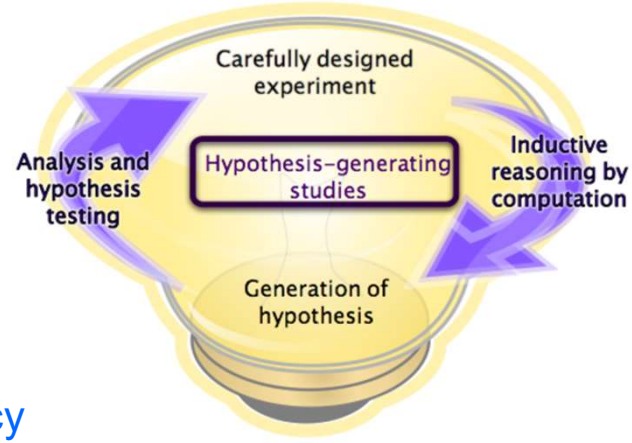
# Targeted Metabolomics vs Untargeted Metabolomics

## Targeted:

"detect the expected"

- ✓ Hypothesis driven
- ✓ Absolute quantification
- ✓ Needs reference standards
- ✓ Not comprehensive
- ✓ More precision/ accuracy
- ✓ Better sensitivity
- ✓ Easy interpretation
- ✓ Pathway mapping

*i.e., do sphingolipids in CSF change in AD?*




## Untargeted:

"detect the unexpected (novel, unknown…)"

- ✓ Hypothesis generating
- ✓ Semi-quantitative
- ✓ No reference standards
- ✓ Fold changes
- ✓ Biomarker discovery
- ✓ Comprehensive, unbiased
- ✓ Lots of unknowns
- ✓ Multivariate, Network analysis

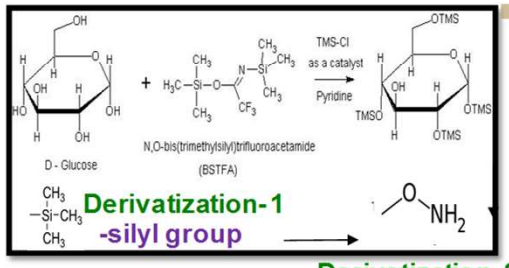
*i.e., what metabolites change in urine in AD ?*

# Metabolomics Workflow:
# Gas chromatography mass-spectrometry (GC-MS)

Samples

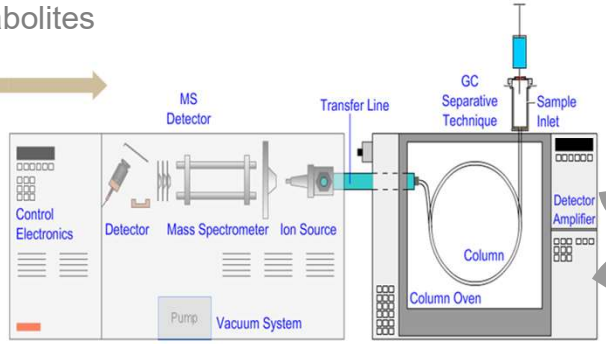
Solvent extraction

Removes proteins,
enriches metabolites

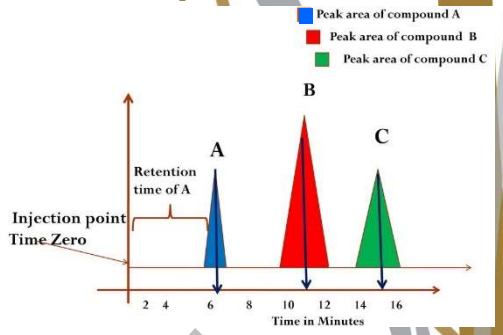



Chemical modification

Derivatization-2
-methoxyamine group

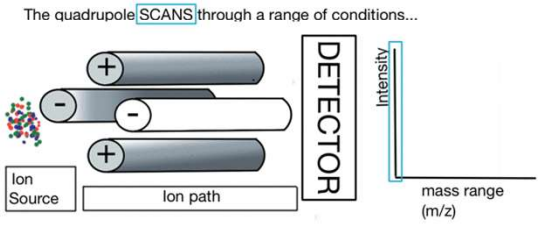


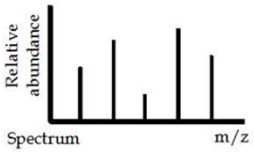

Mass Spectrometer (MS)  Gas Chromatograph (GC)



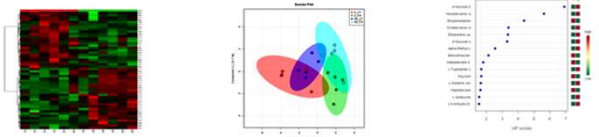

I

II




Spectrum



Statistics and Data Analysis

# What is a Mass Spectrum ? Example

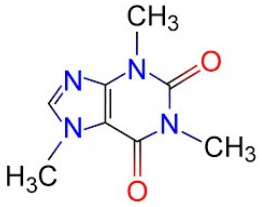

**Caffeine**

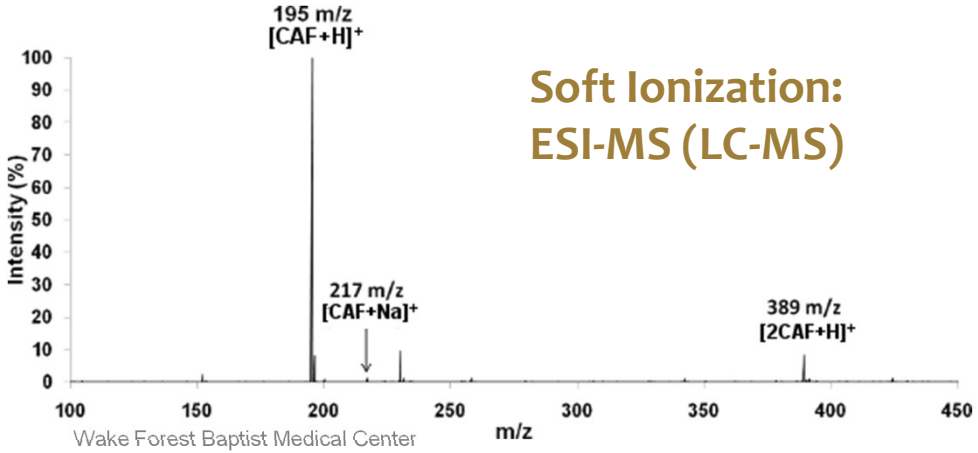
Formula : $C_8H_{10}N_4O_2$

Nominal Mass: 194

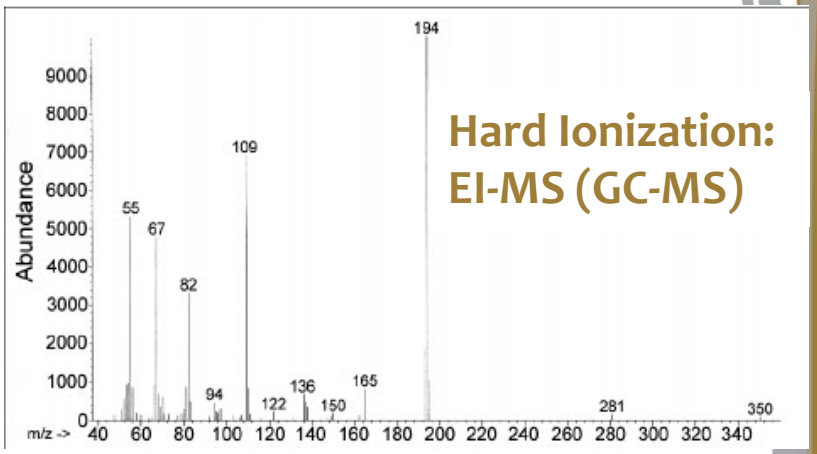
Monoisotopic Mass: 194.0804

Average Mass: 194.1932



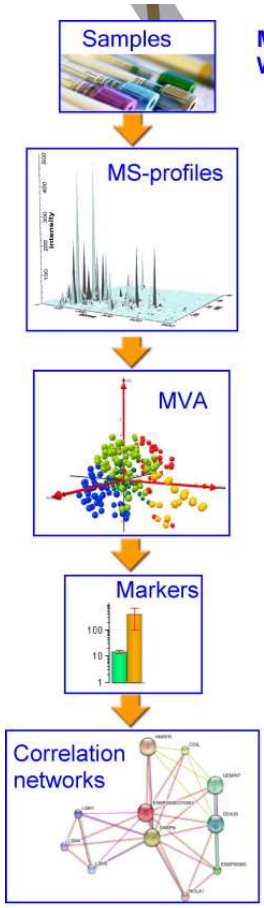

**Soft Ionization:**
**ESI-MS (LC-MS)**




**Hard Ionization:**
**EI-MS (GC-MS)**

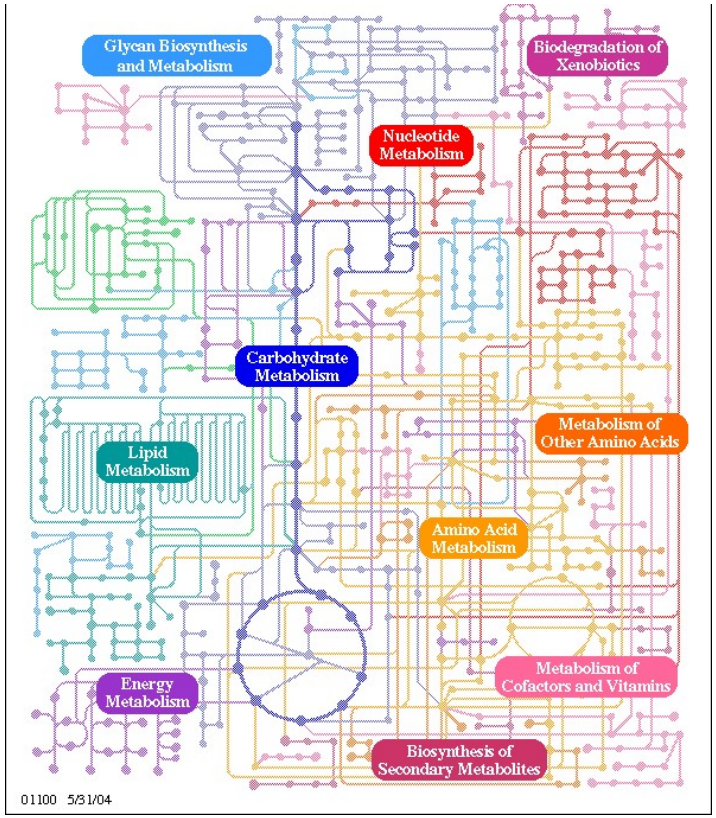
# Steps in a Metabolomics Study

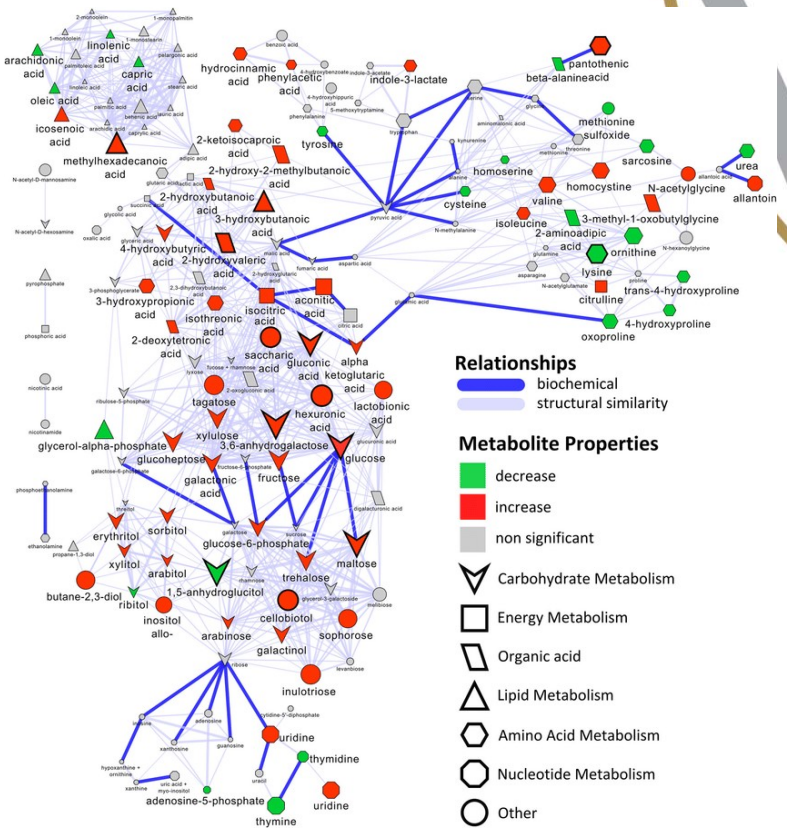
| | |
|---|---|
| *Question-driven* | ➢ Goals? Hypotheses? Questions? |
| *Platform/ Instrument* | ➢ What technology to choose ? Source of metabolites ?<br>➢Targeted vs. Untargeted approaches ? |
| *Experimental design* | ➢ Replication level (biological vs technical replicates)<br>➢What statistics, what analysis software? |
| *Laboratory steps (Sample Preparation)* | ➢ Sample selection, preparation, quenching, storage, extraction,<br>➢ Running of samples, QCs, MS-based Data acquisition |
| *Bioinformatic steps/ Data Analysis*<br>*Data interpretation (Identification/ Pathway)* | ➢ Data transformation and normalization<br>➢ Analysis of differentially accumulated metabolites (multiple testing issue, multivariate statistics, ID-conversion)<br>➢ Pathway mapping/ network viewing<br>➢ Visualization (graphics)<br>➢ Data storage (databases, MSI standards) |
| | Answers? New Hypotheses? Follow-up experiments?<br>➢ Validation? |



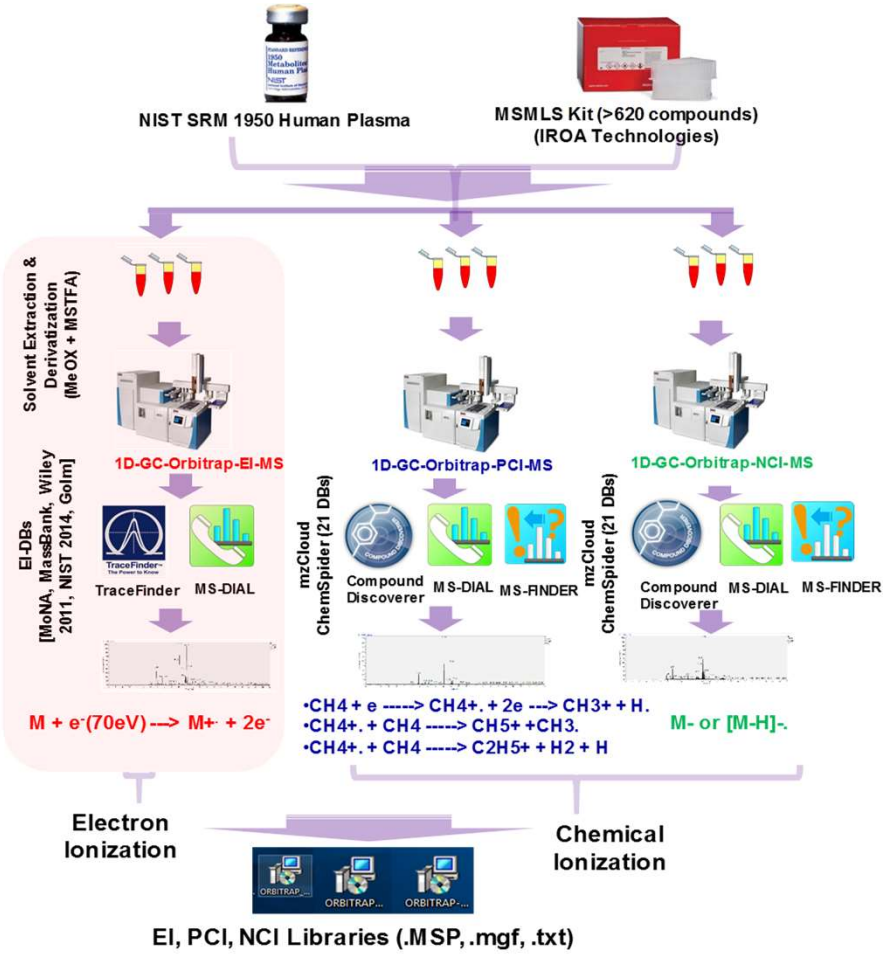

Theodoridis et al., 2012; doi:10.1016/j.aca.2011.09.042

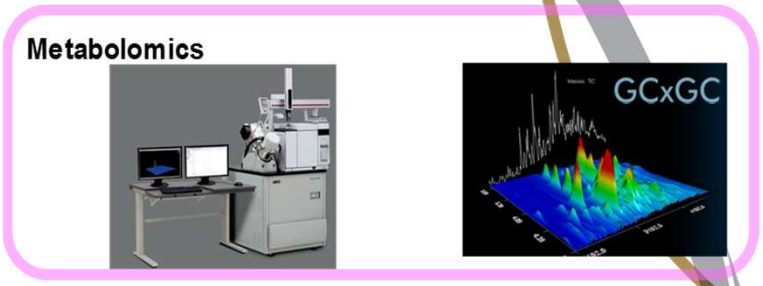
# Metabolic Pathways vs Metabolic Networks

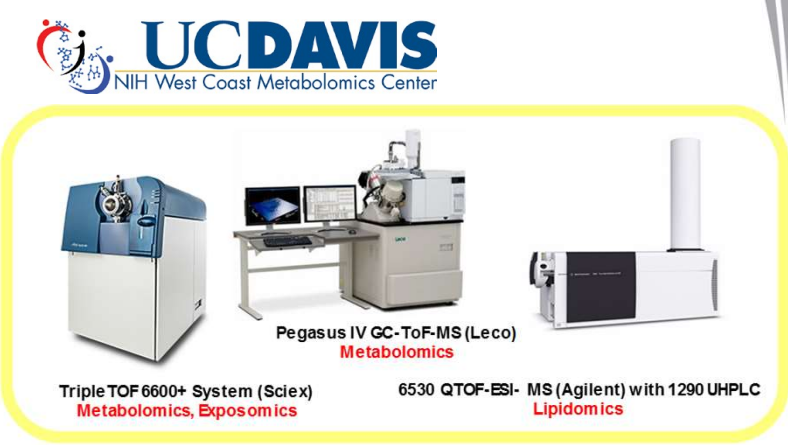


# GC-MS Spectral and RT Libraries *in house*



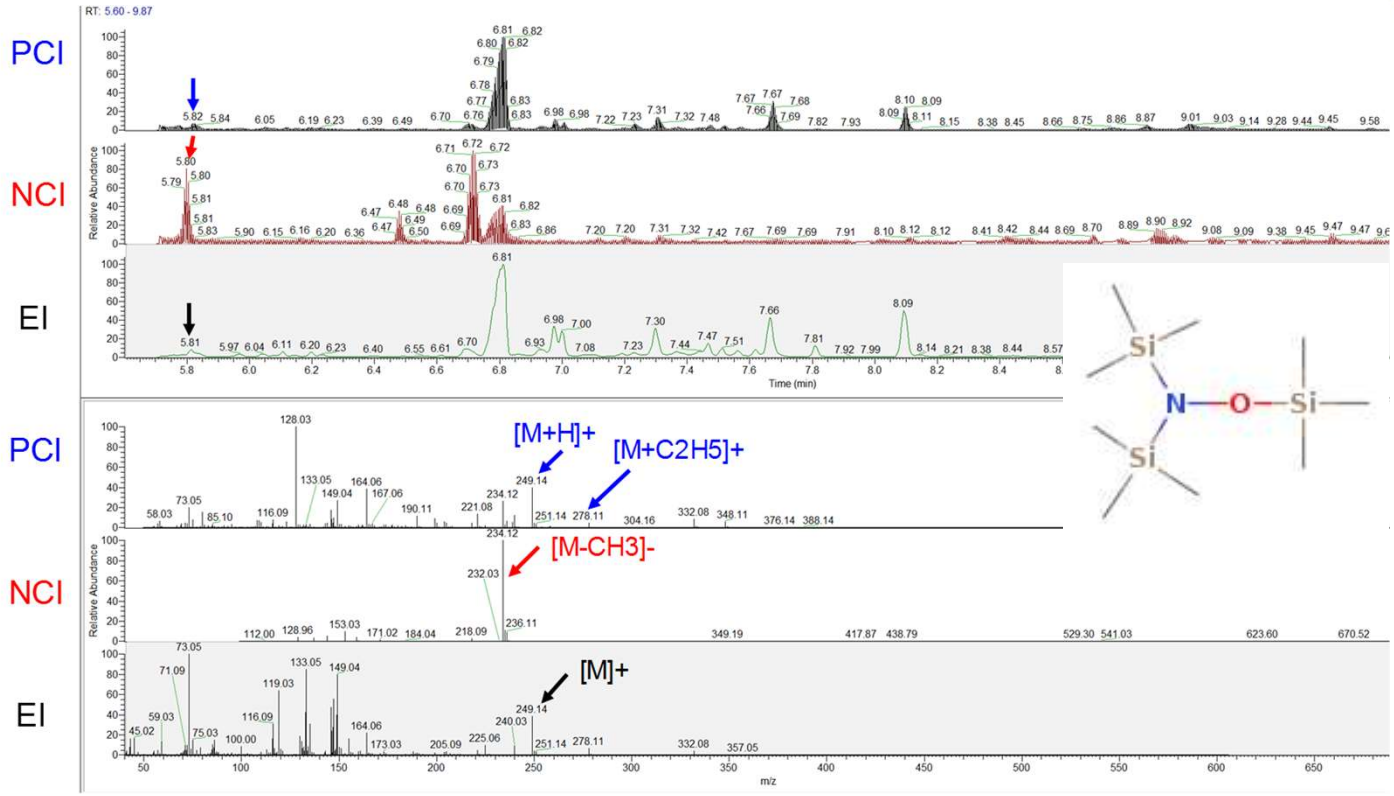



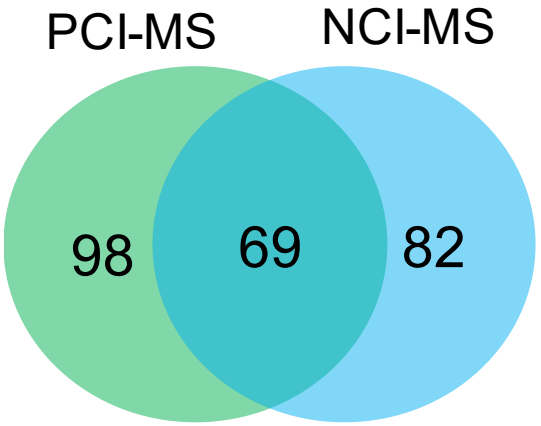
# QEGC-Orbitrap-MS at work for metabolomics at CPM

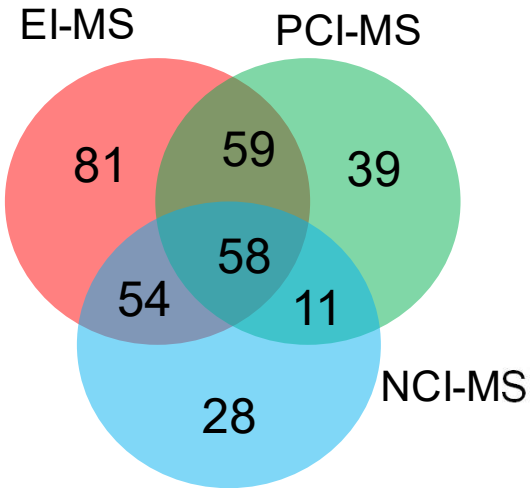
# Example of GC-MS Metabolomics Spectral Acquisition

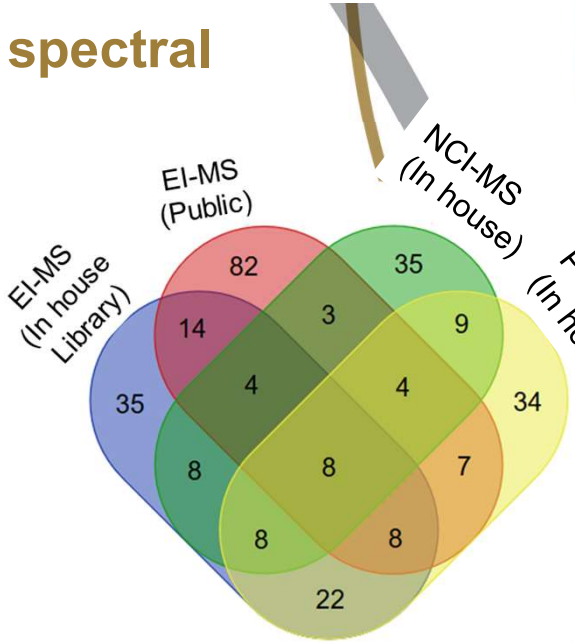
Hydroxylamine 3TMS [RT: 5.81] MW: 249.5733

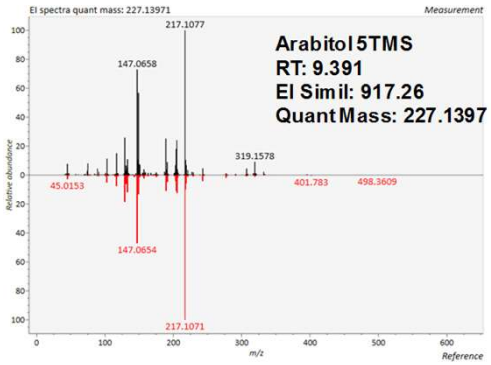


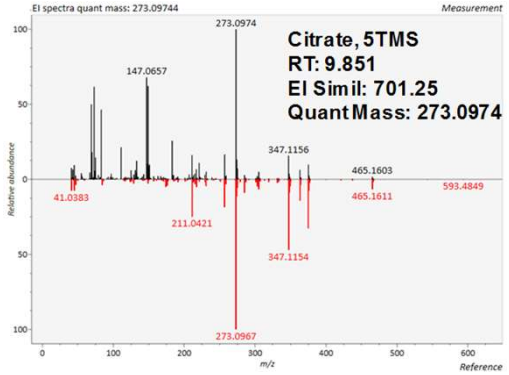
# Validation of in house EI-MS, PCI-MS, NCI-MS spectral library using *NIST SRM 1950 plasma*

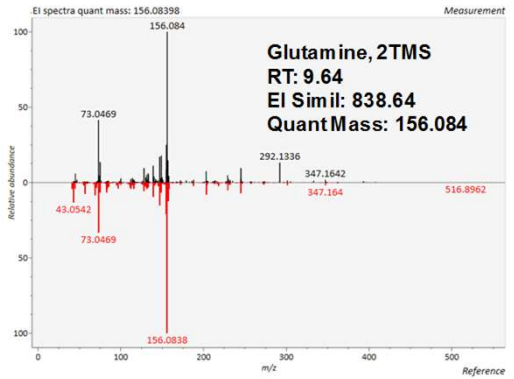


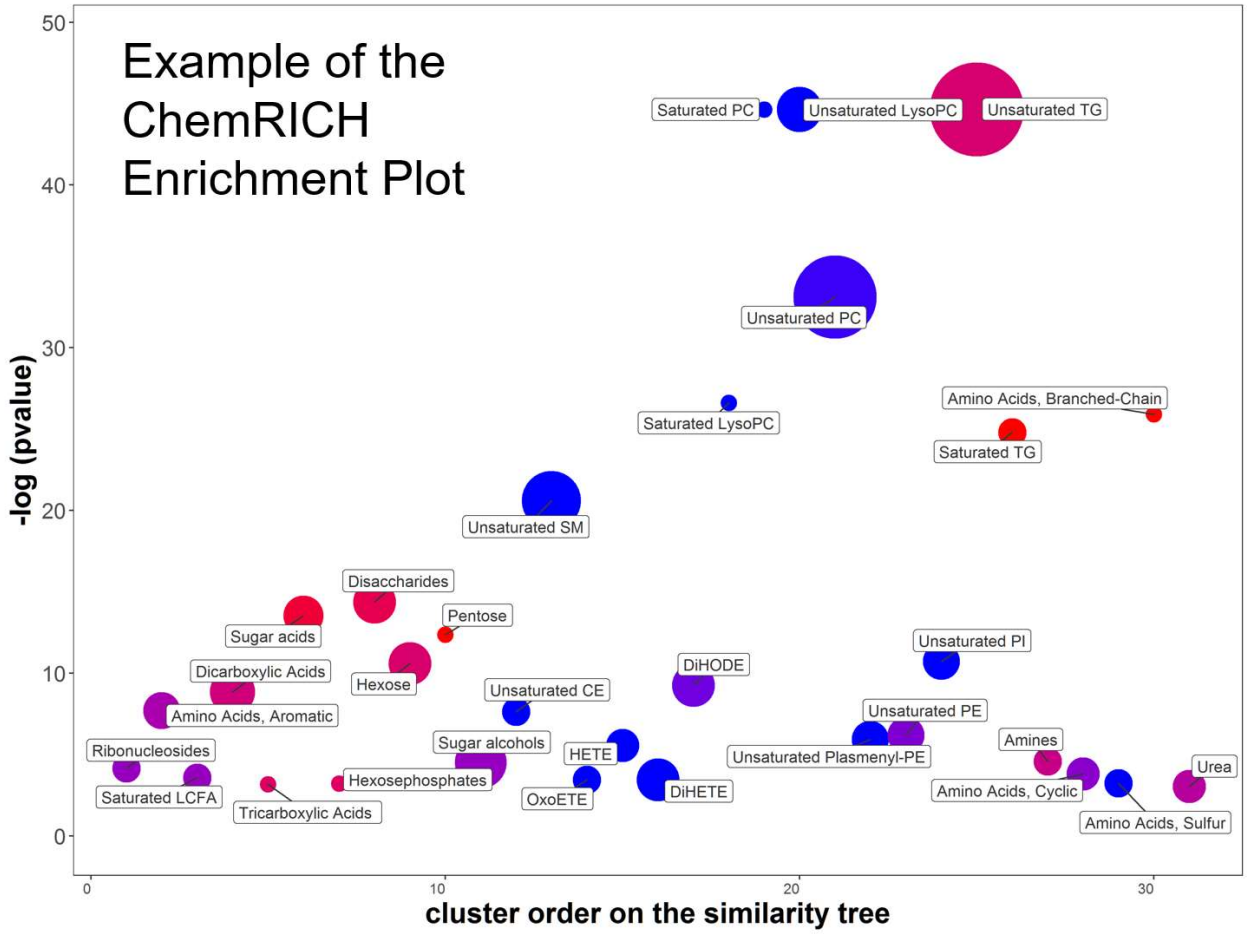



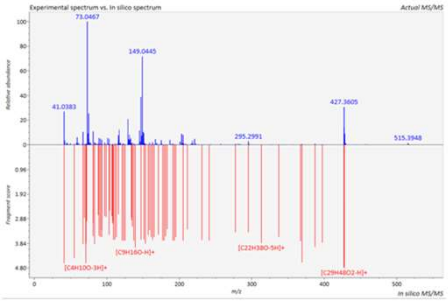



# What metabolite classes do the platforms capture ?

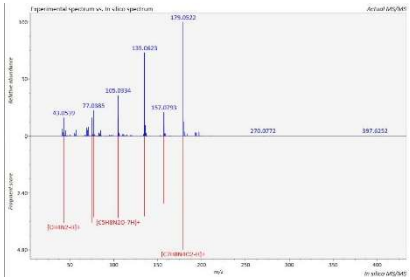


# Expanding on the In House High Resolution Spectral Libraries

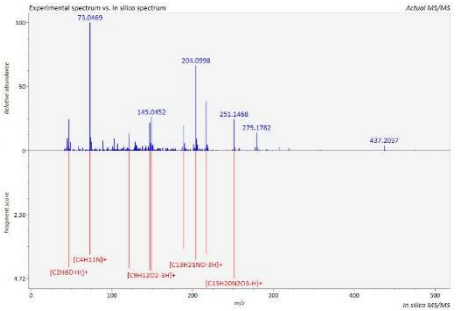
## Exposomal Compounds:



Betulin, Score: 6.91

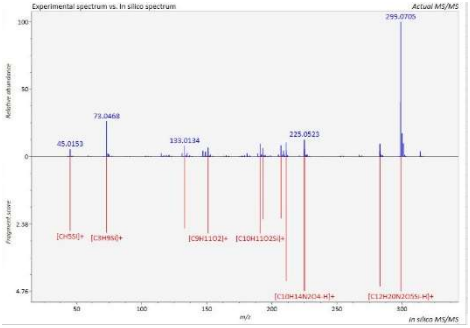


Caffeine, Score: 8.29

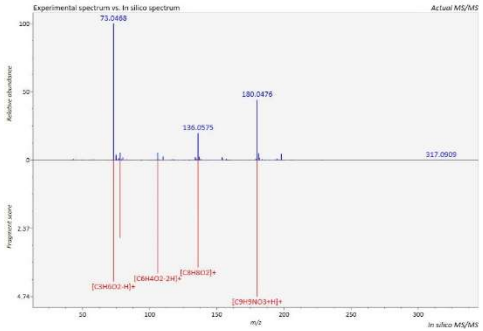


Atenolol, Score: 8.06

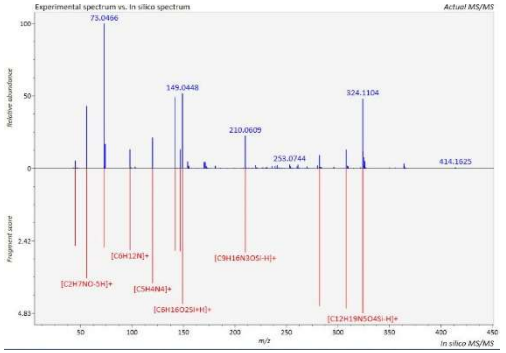
## Endogenous Metabolites:



Thymidine, Score: 9.04



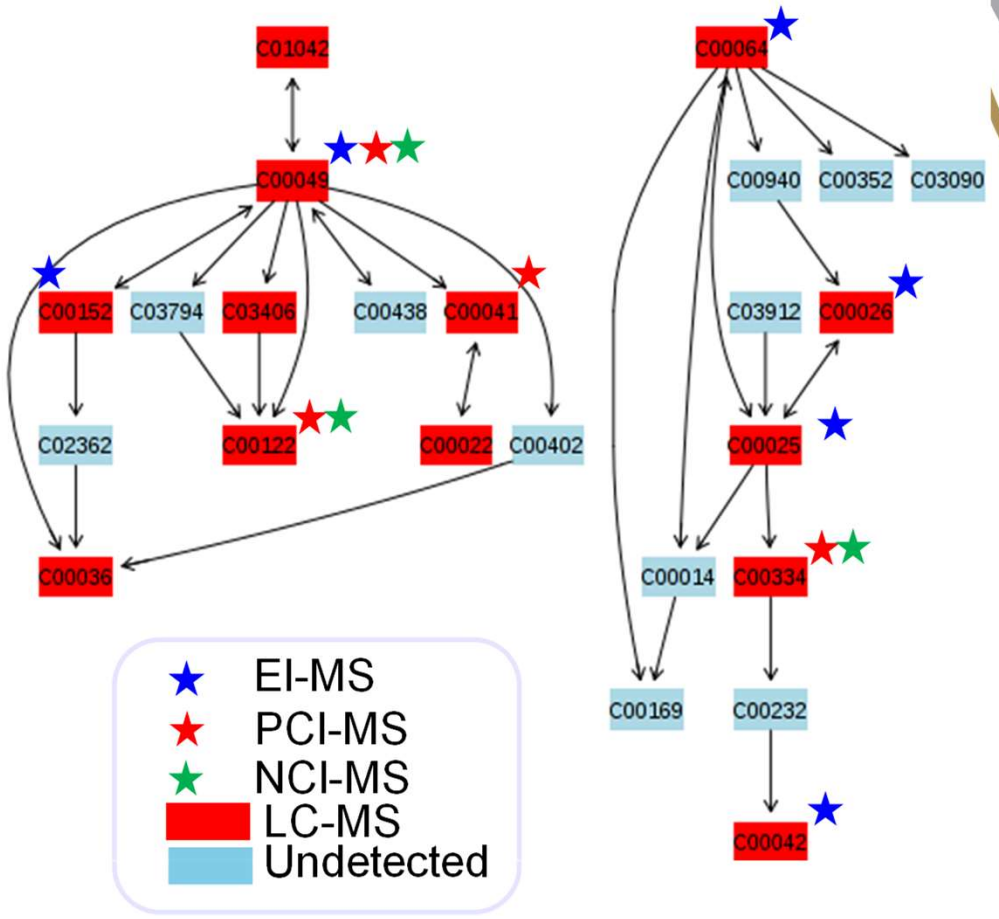
Dopaquinone, Score: 8.36



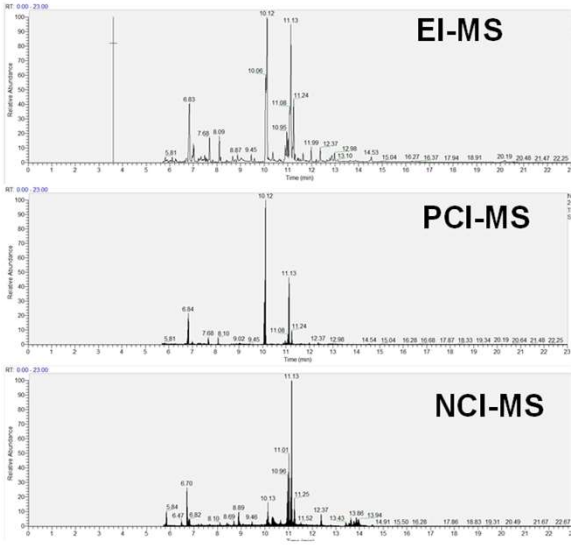
Adenosine, Score: 9.05

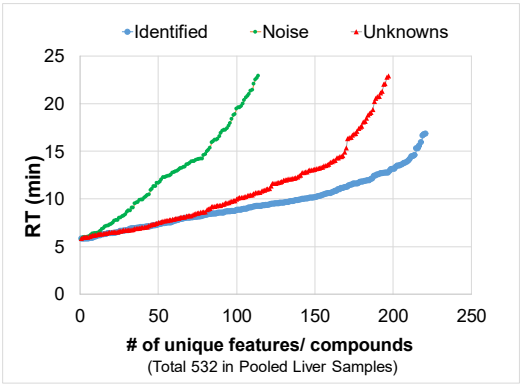
# The 3 Modes and Coverage of Metabolic Pathways

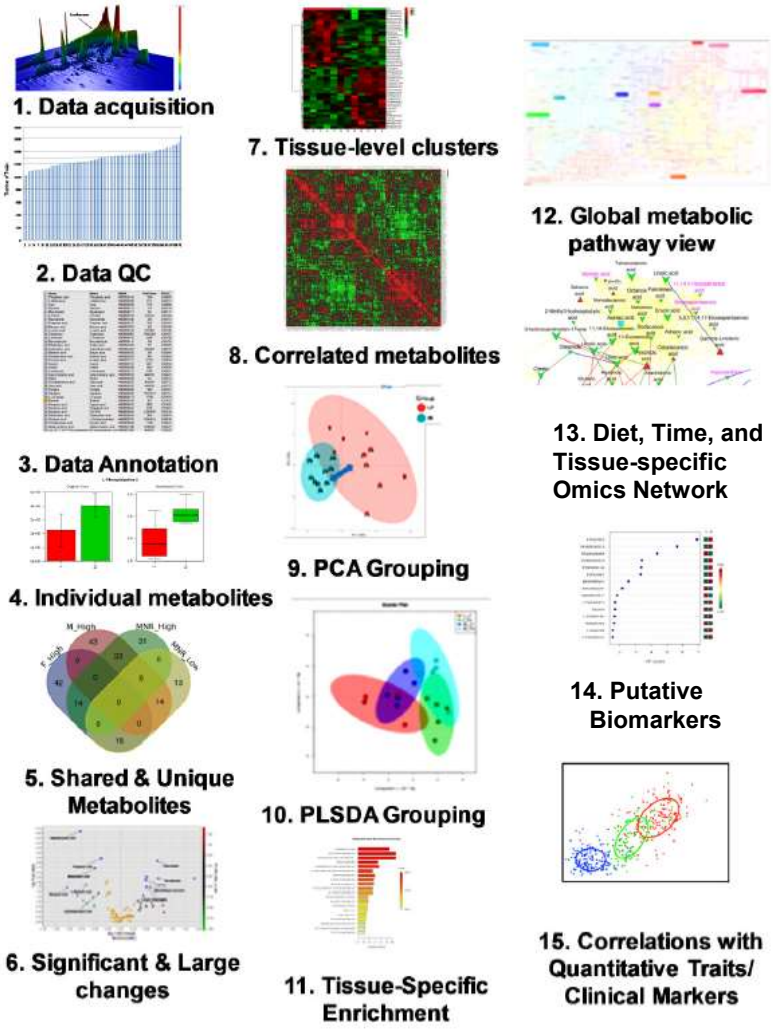
Example: Alanine, aspartate and glutamate metabolism.



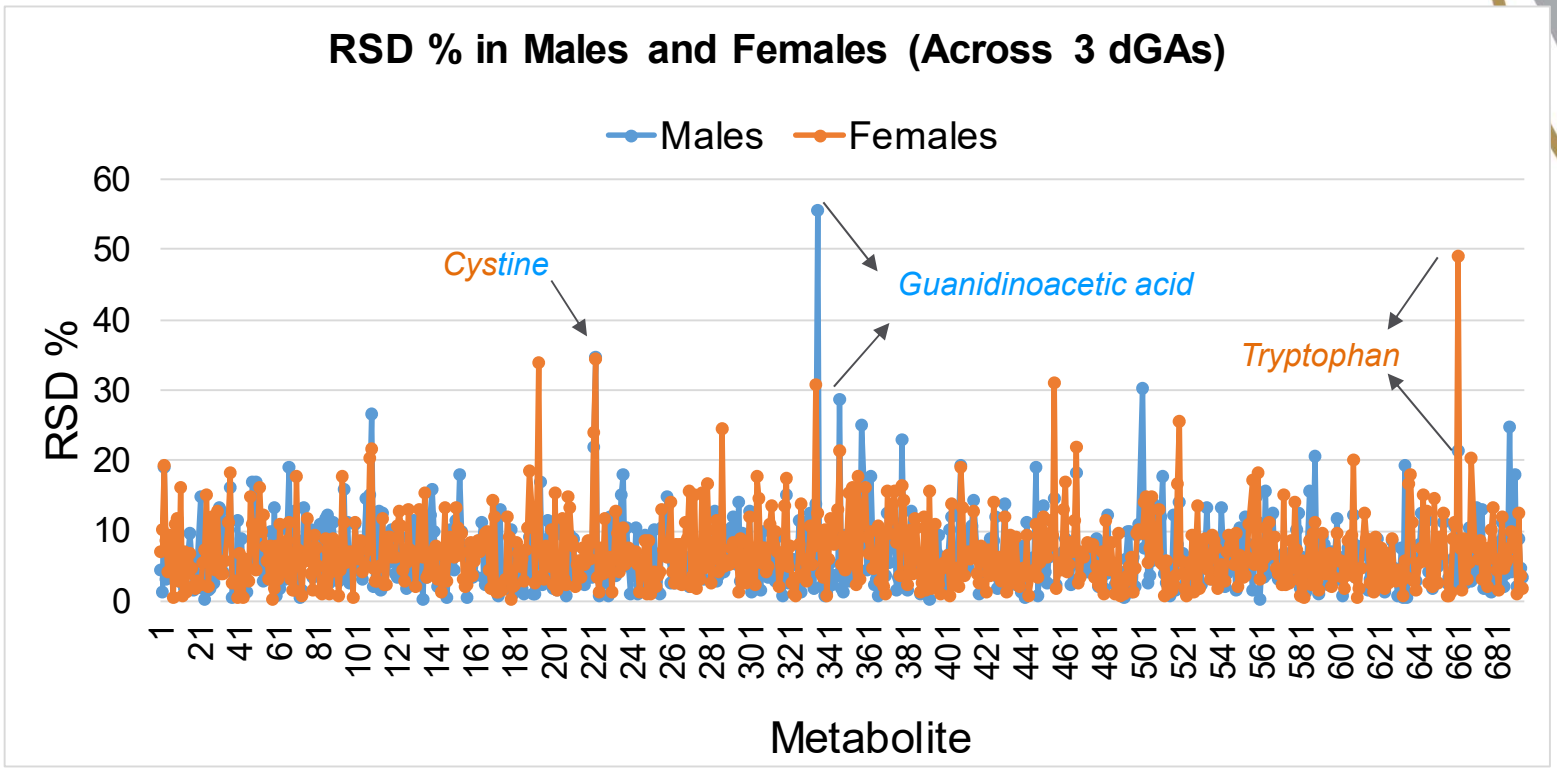
# Data Analysis Workflow



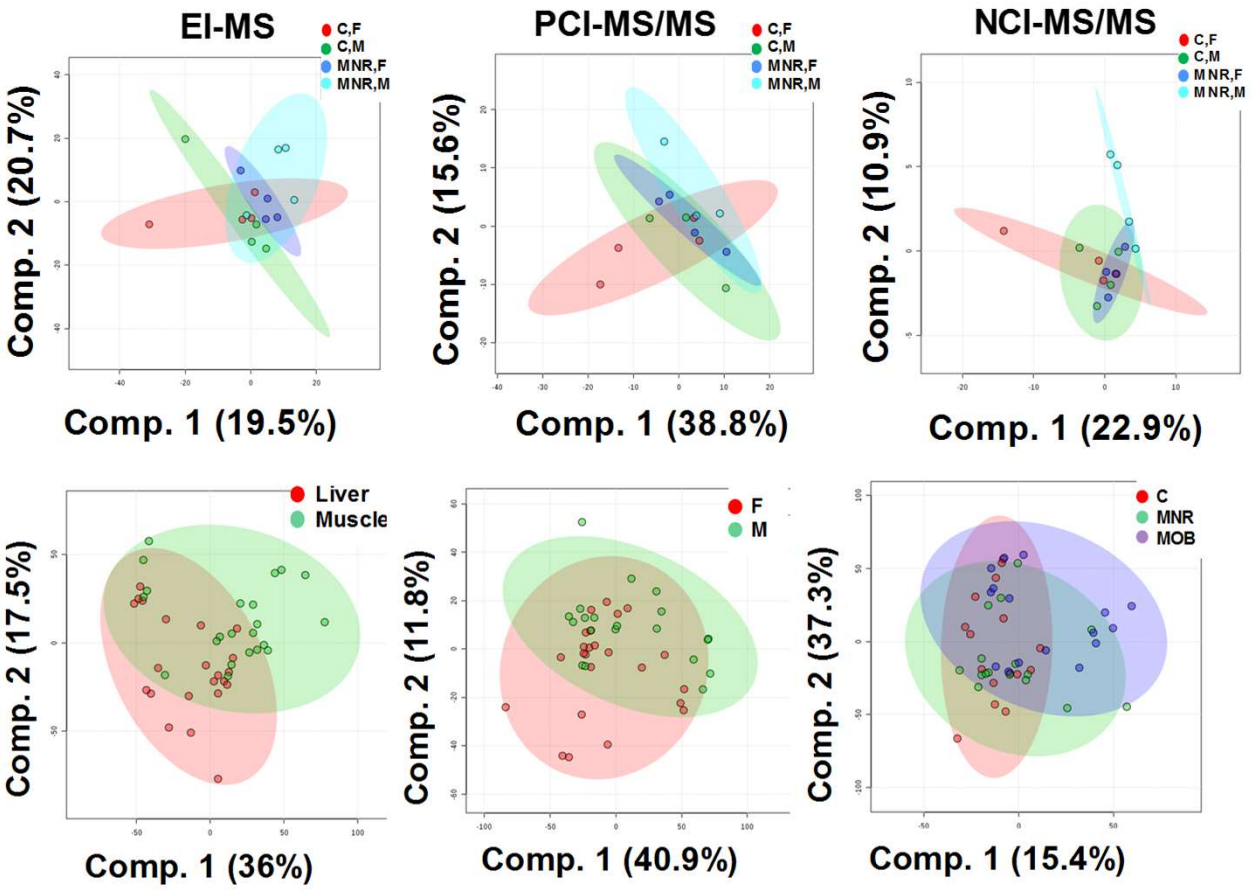



# Sex-specific Changes in Metabolites



# Platform-independent Discrimination of Samples (i.e. metabotypes)



# Metabolomics @ Center for Precision Medicine (2018-2019)

RESEARCH ARTICLE

WILEY Rapid Communications in Mass Spectrometry

## High-resolution gas chromatography/mass spectrometry metabolomics of non-human primate serum

Biswapriya B. Misra[1,2] | Ekong Bassey[3] | Andrew C. Bishop[2] | David T. Kusel[3] | Laura A. Cox[1,2,4] | Michael Olivier[1,2,4]

Metabolomics (2018) 14:75
https://doi.org/10.1007/s11306-018-1373-5

ORIGINAL ARTICLE

## Optimized GC–MS metabolomics for the analysis of kidney tissue metabolites

Biswapriya B. Misra[1,2] · Ram P. Upadhayay[2] · Laura A. Cox[1,2,3] · Michael Olivier[1,2]

RESEARCH ARTICLE

PLOS ONE

## Analysis of serum changes in response to a high fat high cholesterol diet challenge reveals metabolic biomarkers of atherosclerosis

Biswapriya B. Misra[1,2]*, Sobha R. Puppala[1,2], Anthony G. Comuzzie[3], Michael C. Mahaney[4], John L. VandeBerg[4], Michael Olivier[1,2,5], Laura A. Cox[1,2,5]

Journal of Breath Research

PAPER

## Nonhuman primate breath volatile organic compounds associate with developmental programming and cardio-metabolic status

Andrew C Bishop[1], Mark Libardoni[2], Ahsan Choudary[3], Biswapriya Misra[4], Kenneth Lange[5], John Bernal[5], Mark Nijland[7], Cun Li[8], Michael Olivier[4,6], Peter W Nathanielsz[9,8] and Laura A Cox[4,6]

Cold Spring Harbor Laboratory

bioRχiv

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New Results

Comme

## Metabolic Reprogramming: Short-term Western Diet Exposure Induces Sustained Changes in Plasma Metabolites

Biswapriya Biswavas Misra, Ram P Upadhayay, Vicki Mattern, John S. Parks, Laura A Cox, Anthony G Comuzzie, Michael Olivier

Cold Spring Harbor Laboratory

bioRχiv

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New Results

Comment o

## Comparison of a GC-Orbitrap-MS with Parallel GC-FID Capabilities for Metabolomics of Human Serum

Biswapriya B. Misra, Ekong Bassey, Michael Olivier

# Metabolomics in Alzheimer's Disease

## Alzheimer Disease Metabolomics Consortium

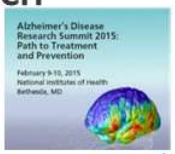
### Part of NIA ECOSYSTEM FOR INTEGRATED ALZHEIMER RESEARCH


GEOFFREY BEENE

US4against Alzheimer's

Alzheimer's Drug Discovery Foundation

alzheimer's association





Rush Columbia | Mayo | Emory | Harvard | Duke

ACCELERATING MEDICINES PARTNERSHIP (AMP)

ALZHEIMER'S DISEASE

MT Sinai

FNIH Foundation for the National Institutes of Health

NIH National Institute on Aging

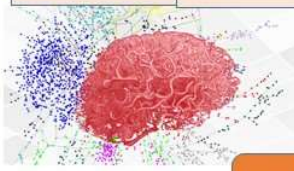
NIH National Institute of Neurological Disorders and Stroke

MODEL AD

Sage

ADNI Alzheimer's Disease Neuroimaging Initiative

Emory | Columbia/ SUNY | Duke | Mt Sinai



M²OVE-AD

Molecular Mechanisms of the Vascular Etiology of Alzheimer's Disease

Harvard

Mayo

Resilience consortium



https://sites.duke.edu/adnimetab/

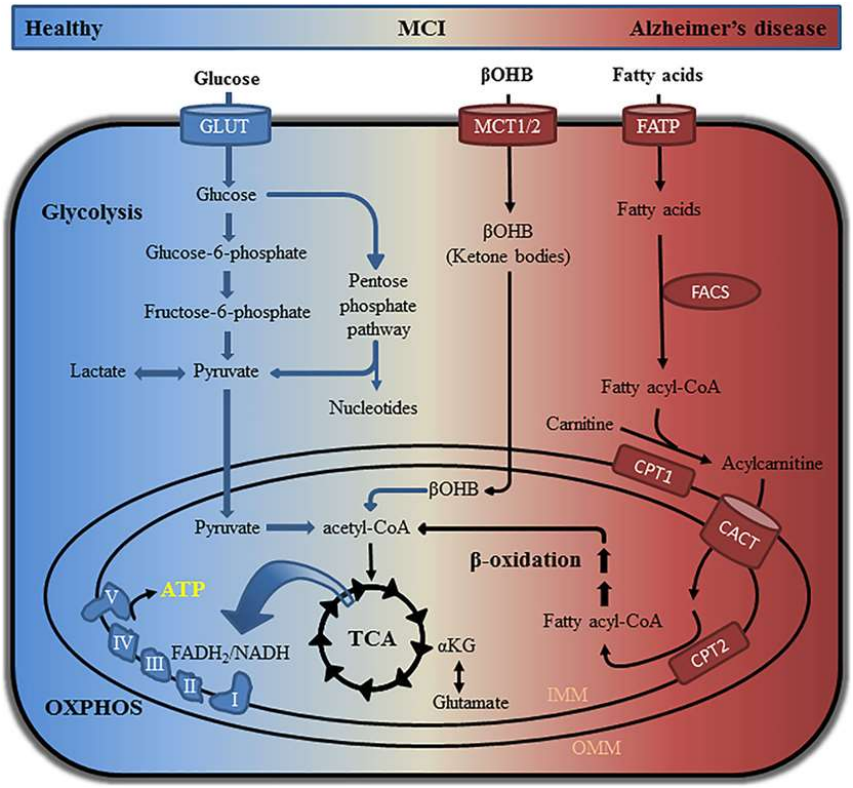
# Metabolites as Biomarkers in AD

| Title of the Manuscript | Authors | N | Platforms | No. of Metabolites | Major Findings | Tissue/ Biofluid | Year | Model |
|---|---|---|---|---|---|---|---|---|
| 1H NMR Metabolomic Si | Toulouse, Ce | 18 | NMR | 30s | Glu, NAA, myo-inositol, creatine, phosphocholin | 5 Brain Regions | 2014 | Mice |
| Graded perturbations of | UK, New Zea | 1 AD, 1C | GC-MS | 100s | total of 55 metabolites that were altered in at leas | 7 Brain Regions [H | 2016 | Human |
| Investigation of the Hum | UK | 15 AD, 15 C | High resolutic | 1264 and 1457 ions | 36 ions responsible for explaining the variation i | Brain | 2013 | Human |
| Metabolic profiling of Alz | Nagoya, Jap | 10 AD, 10 C | UPLC-TOF-M | 300-1100 Compounds | downstream metabolites of ornithine are increas | Brain | 2013 | Human |
| Metabolomic Profiling of | Green et al., | 10 AD, 10 C APP/PS | LC-MS/MS | 22 bile acids | higher brain lithocholic acid (p = 0.05) and lowe | Brain extracts, Pla | 2017 | Mice |
| Metabolomic Signatures | Korea | 10 Alike, 10 C | NMR,HR-MAS | 30s | metabolites involved in energy metabolism, inclu | Brain hippocampus | 2014 | Mice |
| Region-specific metabol | CYSMA, Spa | 60 mice | UPLC-MS, GC | 60 metabolite s+ lipids | associated with abnormal fatty acid composition | Brain regions | 2014 | mice |
| Defects in Mitochondrial | Mayo Clinic | 9 mice | GC-MS | 100 | mitochondrial stress and altered energy metabol | Brain tissue | 2012 | Mouse |
| Metabolic signatures of I | UK | 15 AD, 15 C | NMR | 30s metabolites | elevations in brain alanine (15.4 %) and taurine | Brain tissue | 2013 | Human |
| 1H NMR metabolomics i | Northern Irel | 6 | NMR | 16-20 mets | ascorbate, creatine, γ-aminobutyric acid and *N* | Brain tissue | 2013 | Mice |
| Metabonomic Profiling o | China, PNNL | 16 TASTPM, 5 WT | GC-MS | 75-143 chromatograph | both brain (d-fructose, l-valine, l-serine, l-threoni | Brain, Plasma | 2012 | Mice |
| Alzheimer's disease–like | Green et al., | 8-9 mice/ group C, AF | LC-MS | 187 metabolites | disturbances in essential amino acids, branched | Brain, Plasma | 2016 | Mice |
| Toward a Predictive Moc | Cifuentes, K | 73 | CE-MS | 71 metabolites, unknov | Choline, dimethylarginine, arginine, valine, proli | CSF | 2012 | Human |
| Metabolite Profiling of Al | Roche, Swis | 79 patients, 51 C | GC-MS, LC-M | 343 identified | Increased cortisol levels | CSF | 2012 | Human |
| Comparing metabolomic | Rima, Fiehn | 40 AD, 38 C | GCMS, LC-MS | 299 metbaolites | monopalmitin , Phosphoethanolamine | CSF | 2013 | Human |
| Metabolomic changes in | Rima, Duke | 15 AD + 15 C | LC-MS, target | 30 metabolites | alterations in tyrosine, tryptophan, purine, and to | CSF | 2010 | Human |
| Alterations in metabolic p | Rima | 40 AD, 36 MCI, 38 C | LC-MS-electro | 71 metabolites | methionine (MET), 5-hydroxyindoleacetic acid (5 | CSF | 2013 | Human |
| Deregulation of purine m | Ferrer et al., | 23 +35 Ads, 34 C | LC-MS | 100s? | altered levels of Purine metabolism dGMP, glyci | Entorhinal cortex | 2014 | Human |
| A metabolomic study of | Babraham In | 13 mice | NMR | 30-40s | a decrease in *N*-acetyl-l-aspartate, glutamate, g | Extract from eight brain re | | Mice |
| Metabolic network failure | Rim, Cristina | 199 C, 365 MCI, 175 A | UPLC-MS/MS | 180 metabolites | sphingomyelins and ether-containing phosphati | Fasting Serum | 2017 | Human |

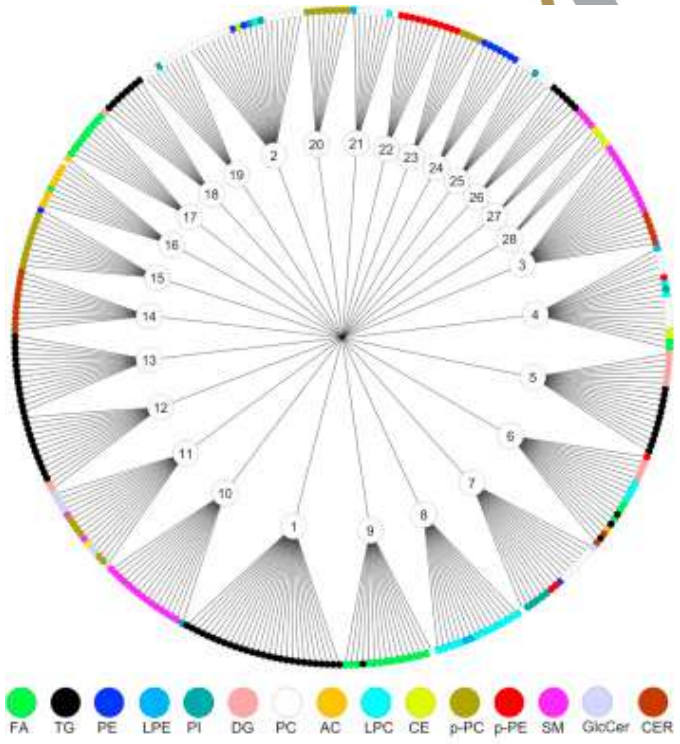
# Metabolites as Biomarkers in AD

| Title of the Manuscript | Authors | N | Platforms | No. of Metabolites | Major Findings | Tissue/ Biofluid | Year | Model |
|---|---|---|---|---|---|---|---|---|
| Plasma Metabolite Profil | China | 57 AD, 58 aMCI, 57 C | UPLC-ToF-MS | 85 + 238 metabolites | plasma metabolites (thymine, arachidonic acid, | Plasma | 2014 | Human |
| A new metabolomic worl | Cifuentes, K | 75 patients | R+HILIC_UPL | 524 high confident mel | , histidines, acylglycines | Plasma | 2013 | Human |
| Identification of a new pl | Eisaid Co, Ja | 10 people | LC-APCI-MS, | 1-50s | Desmosterol | Plasma + CSF | 2011 | Human |
| Identification of Altered I | Mayo Clinic | 30 patients | LC-MS | 352 + 351 metabolites | Cholesterol and sphingolipids transport | Plasma + CSF | 2013 | Human |
| Studies on diagnostic bi | China | AD, C, Therapeutic | UPLC-QTOF-I | ? Metabolites | lysophosphatidylcholine and intermediates of sp | Plasma, Hippocam | 2017 | Rats |
| **Evidence of altered ph** | **Cristina, Kir** | **42 AD, 50 MCI, 49 C** | **NMR, LC-MS** | **100s** | **3 PCs were found to be significantly lower in** | **Plasma?** | **2013** | **Humar** |
| Diagnostic Biomarkers c | USA, Canad | 8 MCI, 9 AD, 12 C | 1H-NMR | 50s, 22 metabolites | identified significant concentration changes in 2 | Saliva | 2017 | Human |
| Metabolomics-based scr | China | 256 AD, 218 C | F-UPLC-MS | ? | sphinganine-1-phosphate, Sphinganine-1-phos | Saliva | 2015 | Human |
| High-throughput metabol | China | AD, MCI, C | UPLC-MS | ? Features? | major contributors were cytidine (P = 0.0003) ar | Salivary | 2016 | Human |
| Discovery of serum meta | China | AD, MCI, C | ?? | ?? | sphinganine-1-phosphate and 7-ketocholesterol | Serum | 2015 | Human |
| Metabolomic profiling of | CYSMA, Spa | 42 AD, 14 MCI, 37 C | CE-MS | 537 features, 20 biom | increase in in levels of choline, creatinine, asyn | Serum | 2014 | Human |
| Metabolomic research o | CYSMA, Spa | 7 Mice | DI-MS | 100s | impaired homeostasis of histamine, altered meta | Serum | 2015 | Mice |
| Metabolomic study of lip | Spain | 22 AD , 18 C | DI-MS | | membrane breakdown, diacylglycerols, oleamide | Serum | 2014 | Human |
| Using direct infusion ma | CYSMA, Spa | 22 AD, 18 C | DIMS-ESI+ve | ?? | high levels of phospholipids containing saturated | Serum | 2014 | Human |
| Metabolomic screening | CYSMA, Spa | 30 mice | DIMS-ESI+ve | ?? | phospholipids, fatty acids, purine and pyrimidine | Serum | 2015 | Mice |
| Combination of metabol | CYSMA, Spa | 17 AD, 19 C | ESI-LC-MS, + | 60> Lipids | phosphatidylcholines, phosphatidylethanolamine | Serum | 2014 | Human |
| Application of metabolon | CYSMA, Spa | 60 mice | FI-APPI-MS, | 46 (TAGs, CE, DAG, F | energy metabolites, amino acids and lipids | Serum | 2015 | Mice |
| Serum fatty acid profiles | China | 46 AD, 39 C | GC-MS | 50-80s | 2 saturated fatty acids (C14:0 and C16:0; p < 0. | Serum | 2012 | Human |
| Metabolite profiling for th | CYSMA, Spa | 23 AD, 21 C | GC-MS | 50-100 metabolites | pyroglutamate, adenosine, impaired metabolism | Serum | 2014 | Serum |
| Targeted lipidomics distri | Italy, USA | 77 MCI, 90 AD, C 51 | LCOrbiTrap-M | ?? | DAGs were increased in the serum of a subset | Serum | 2015 | Human |
| Deciphering metabolic a | CYSMA, Spa | 30 AD, 30 C | UPLC-MS | 100s, LP, SL, P, SL | deficiencies in energy metabolism, altered amin | Serum | 2015 | Mice |
| Discovery of serum meta | China | MCI, AD, C | UPLC-MS | ? | sphinganine-1-phosphate and 7-ketocholesterol | Serum | 2016 | Human |
| Metabolome in progressi | VTT, Finland | 46 C, 143 MCI, 47 AD | | 100s | lowered ether phospholipids, phosphatidylcholin | Serum | 2011 | Human |
| Blood metabolite markers of preclinica | | 93 AD, 99 C | HPLC-MS, tar | 187 metabolite | distinct metabolites associated , phospholipids | Serum- Time cours | 2016 | Human |
| Targeted Metabolomic A | Austria | 90 C, MCI, AD | FIA-MS/MS ta | 163 metabolites | lipid PC aeC40:4 significantly differentiated AD | soluble lysates of p | 2017 | Human |
| Metabolomics reveals sig | CYSMA, Spa | 30 APP/PS1, 30 C | GC-MS and u | 64 metabolites | abnormal metabolism of phospholipids, energy d | Speen and Thymus | 2015 | Mice |
| Urinary Metabolomics R | China | CRND8 mice AC, C | ? | 73 diff. metabolites | perturbations of aromatic amino acid metabolism | Urinary | 2016 | Mice |
| Development of Isotope | Wishart, Car | 24 | Iso-LC-MS | 600 metabolites | methionine, desaminotyrosine, taurine, N1-acety | Urine | 2014 | Mice |
| NMR-based metabolomic | Japan | 3-5 mice | NMR | 30s | 3-hydroxykynurenine, homogentisate and allanto | Urine | 2013 | Mice |
| High-Throughput Metabo | China | 30 AD, 30 C | UPLC-ToF-MS | 24 diff metabolites | pentose and glucuronate interconversions, glyox | Urine | 2017 | Mice |
| Alterations of the volatile | Cleaveland | 10 AD, 12 C | Volatilome, GC | 80s | phenylacetone (increased concentration in APP | Urine | 2016 | Mice |

# Many Interpretations of Metabolic Changes in AD



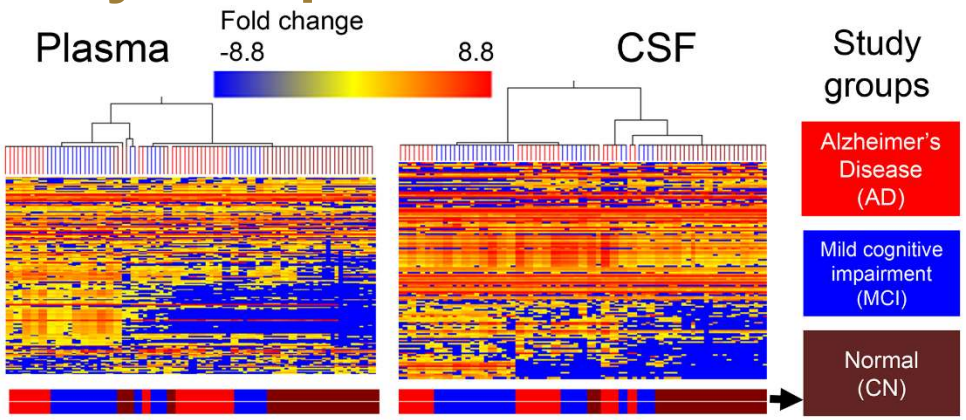

Wilkins & Trushina, 2018

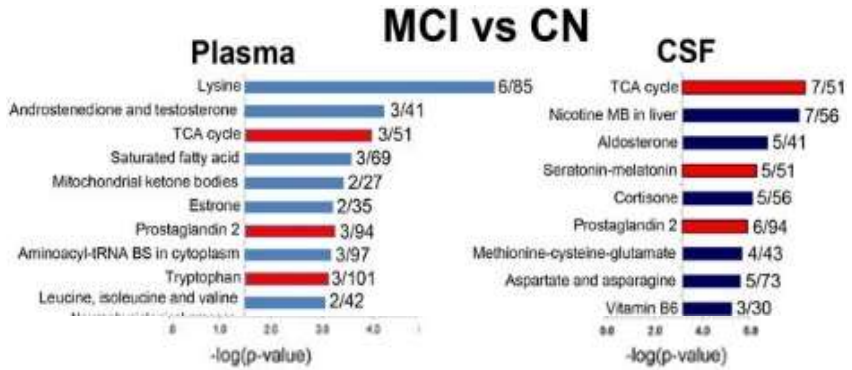


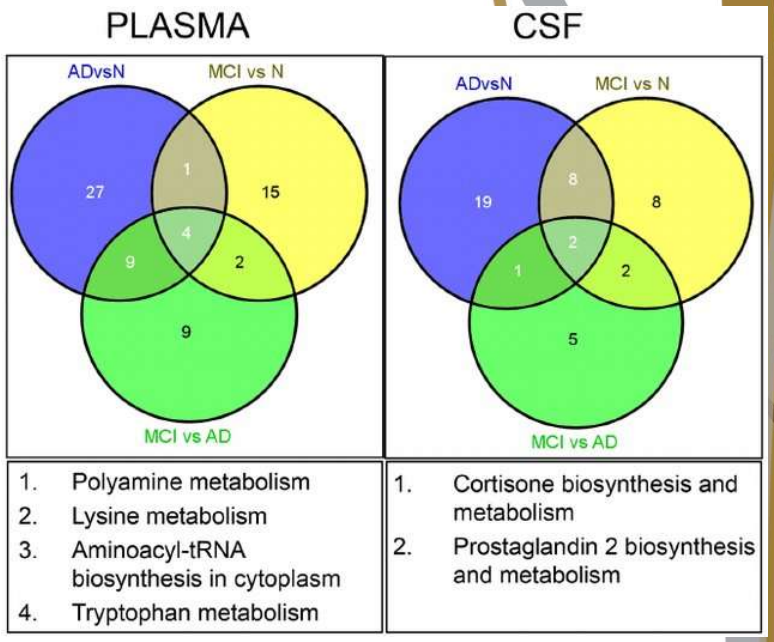

Coregulated sets of serum lipids in the ADNI lipidomics data set.

Barupal et al., 2019

# Many Interpretations of Metabolic Changes in AD




| PLASMA | CSF |
|---|---|
| 1. Polyamine metabolism<br>2. Lysine metabolism<br>3. Aminoacyl-tRNA biosynthesis in cytoplasm<br>4. Tryptophan metabolism | 1. Cortisone biosynthesis and metabolism<br>2. Prostaglandin 2 biosynthesis and metabolism |

*Trushina, E., Dutta, T., Persson, X.M.T., Mielke, M.M. and Petersen, R.C., 2013. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PloS one, 8(5), p.e63644.*

# Summary

- Spectral acquisition and validation of > **330 unique compounds** with EI and CI HR spectra in the EI-MS and CI-MS/MS libraries. And is growing with novel annotation (in silico approaches).
- Quantification of **>500 unique compounds** in muscles and liver in a single sample using 3 runs (~66 mins together) using spectral and RT libraries.
- Ample opportunities and scope to find AD Biomarkers of Metabolism in CSF, Plasma, Urine, Fecal samples and tissues from NHP models, mice etc.
- Complementarity of metabolomics in ongoing/ future ADRC collections and (-omics) studies.

# Acknowledgements

Michael Olivier Lab

Laura Cox Lab

Center for Precision Medicine

Dr. Suzanne Craft

Dr. Tim Hughes

Dr. Samuel Lockhart

WFSOM Resources

Southwest National Primate Research Center

Texas Biomedical Research Institute


Thank you !