Graduate –Omics course

Metabolomics

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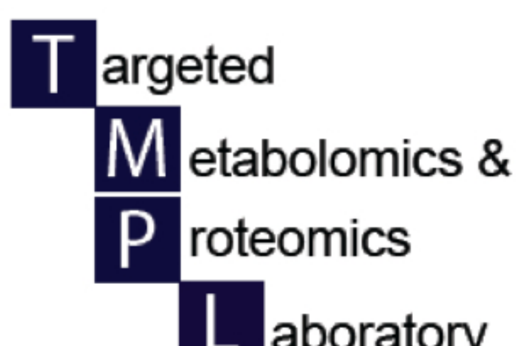
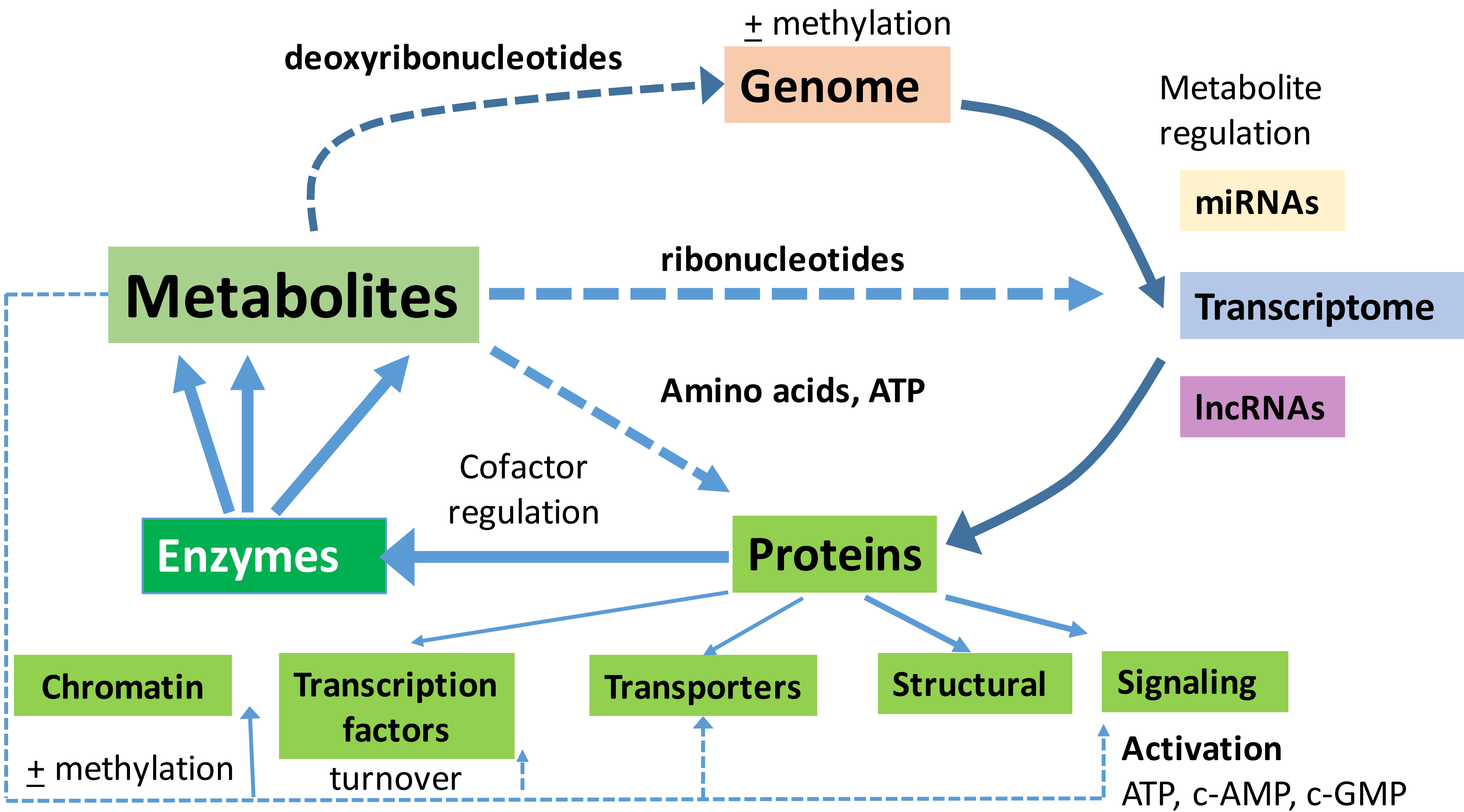


Metabolites are associated with every aspect of cellular events



Metabolomics and NIH Research 1950-2015

Microbiome
Metabolomics

1950s-60s emphasis on determining metabolic pathways – 20+ Nobel prizes

1950s-early 1980s Identification and purification of proteins

Bloch Lynen Krebs

1980-1988 Sequencing of genes – cDNA libraries – *orthogonal research*

1988-2000 Sequencing of the human genome – *period of non-orthogonal research* – where did all the genes go? junk DNA?

2004 Tiling arrays reveal that most of the genome is expressed

2006 First ENCODE project on 1% of the human genome reveals RNAs coming from more than one gene

2012 Human genome ENCODE project reveals the extent of DNA expression and roles for "junk" DNA, as well as intergenic proteins

2014 –"deep" proteomics reveals the presence of 400+ proteins that **are not encoded by the genome**

What are the goals of metabolomics?

- The metabolites are the fuel and messengers in and between cells in an organized system
  - Messengers as distinct from message
- To identify the critical metabolite or combination of metabolites that is(are) associated with a particular phenotype
  - The metabolite(s) may be known, or need to be characterized

Predicting the metabolome

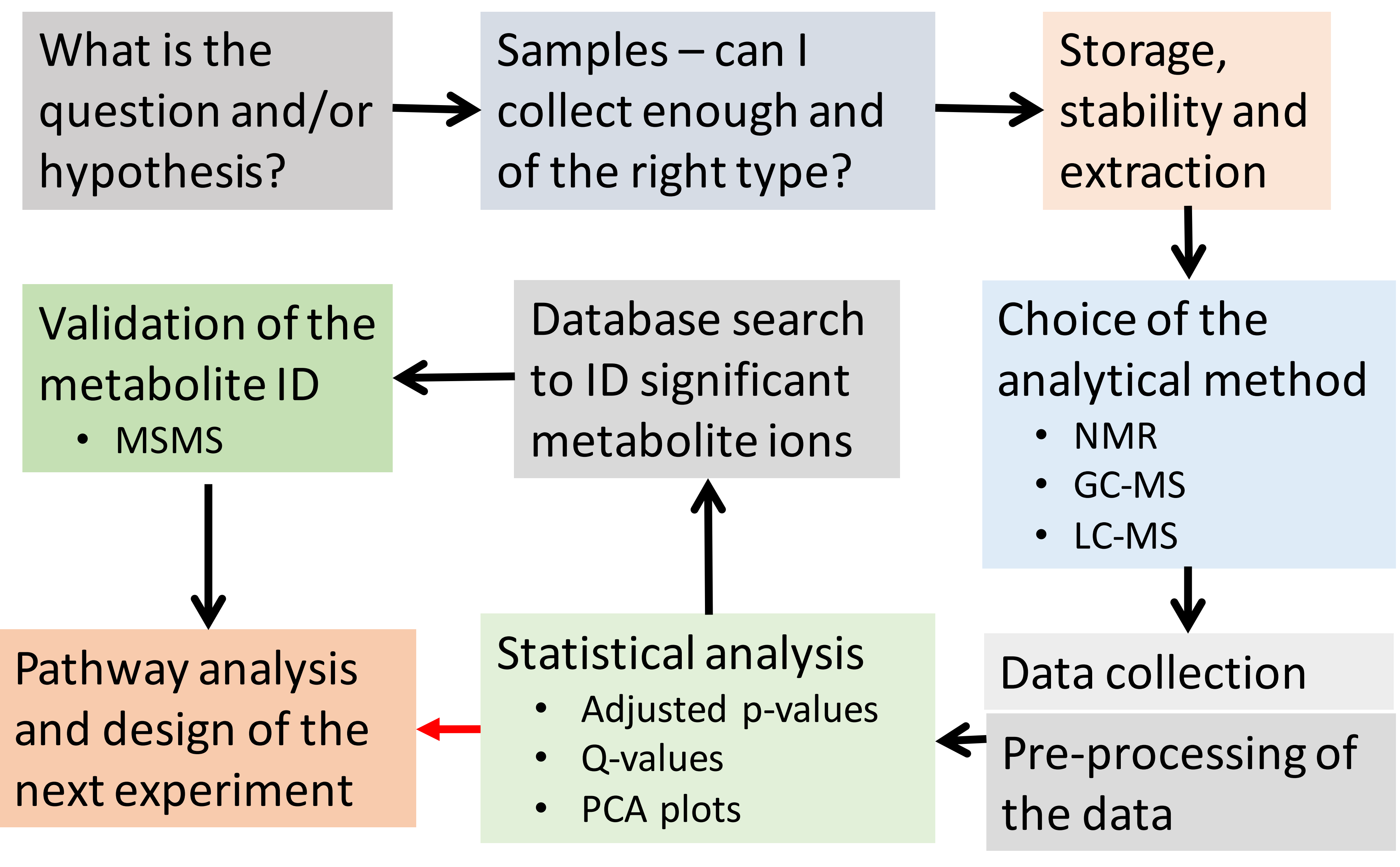
- Predicting the proteome was a logical translation of sequencing the genomes
  - Computers (largely) were able to identify open reading frames
  - Knowing the start sites and codons, the amino acid sequence for known and putative proteins could be interpreted
- At this time, we cannot predict the metabolites made by enzymes
  - Rely on existing pathway information and annotations
  - Metabolomics is re-writing our knowledge of pathways

The metabolome is more than just metabolites

- The *metabolome* is considered to be all molecules with masses up to 1,500 Da
  - These molecules can come from ‘genomes’ other than the model you’re studying
    - Foods, particularly plants, that form the diet
    - Gut microorganisms
    - Environmental contaminants
    - Therapeutics and their metabolites
- *Exposome*
  - The integrated exposure to all metabolomes over your lifetime

The metabolome is very complex!

Metabolomics workflow

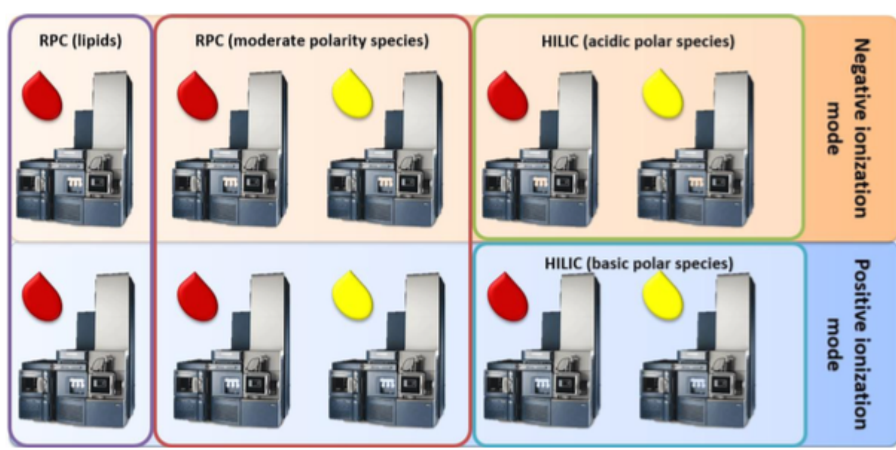


Great challenges in metabolomics

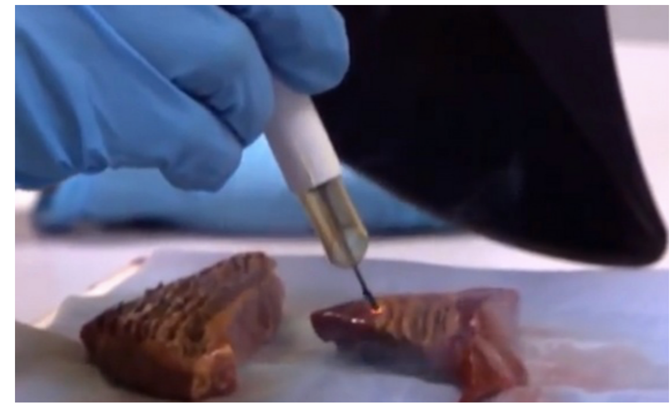
- The extent of the metabolome
  - From gaseous hydrogen to earwax
  - A much wider range of chemistry than the genome, epigenome and transcriptome, and the proteome
- Having complete databases
  - METLIN has 60,000+ metabolite records, but your problem always creates a need to have more
  - Current lack of a substantial MSMS database (but it's coming)
- Storing and processing TBs/PBs of data
- Standards and standard operating procedures
- Being able to do the analyses in "real time"

MRC-NIHR National Phenome Centre

600 MHz NMR instruments in surgical suite



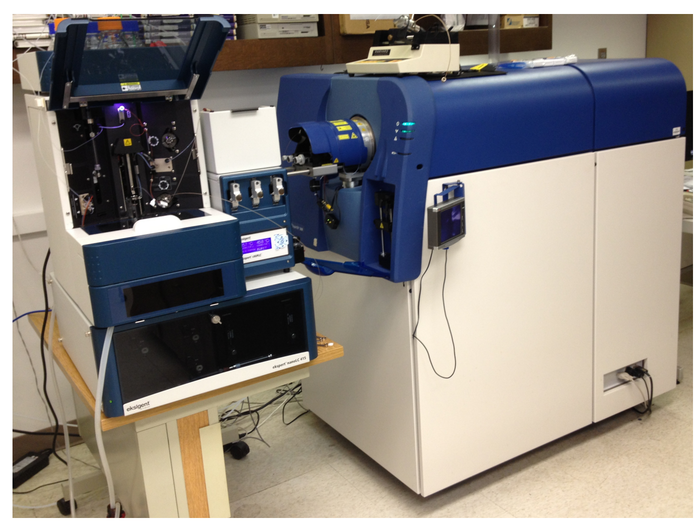
Mass spectrometers (10 Q-TOFs) each dedicated to one assay format



Iknife - revolutionizing surgery

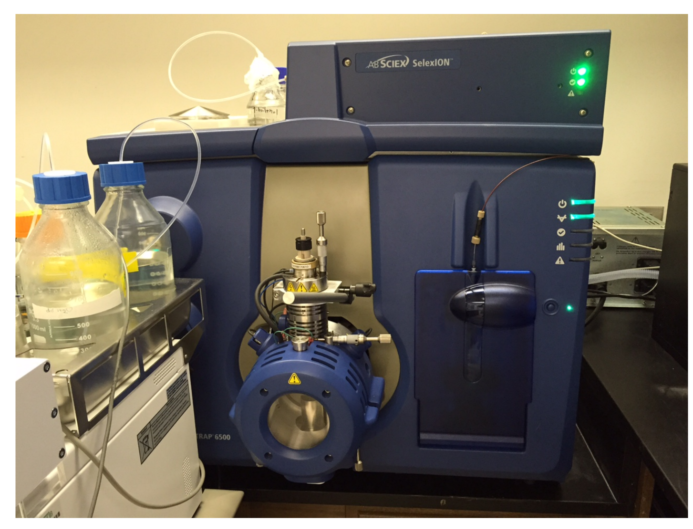
This is Next-GEN precise medicine

UAB capabilities in metabolomics

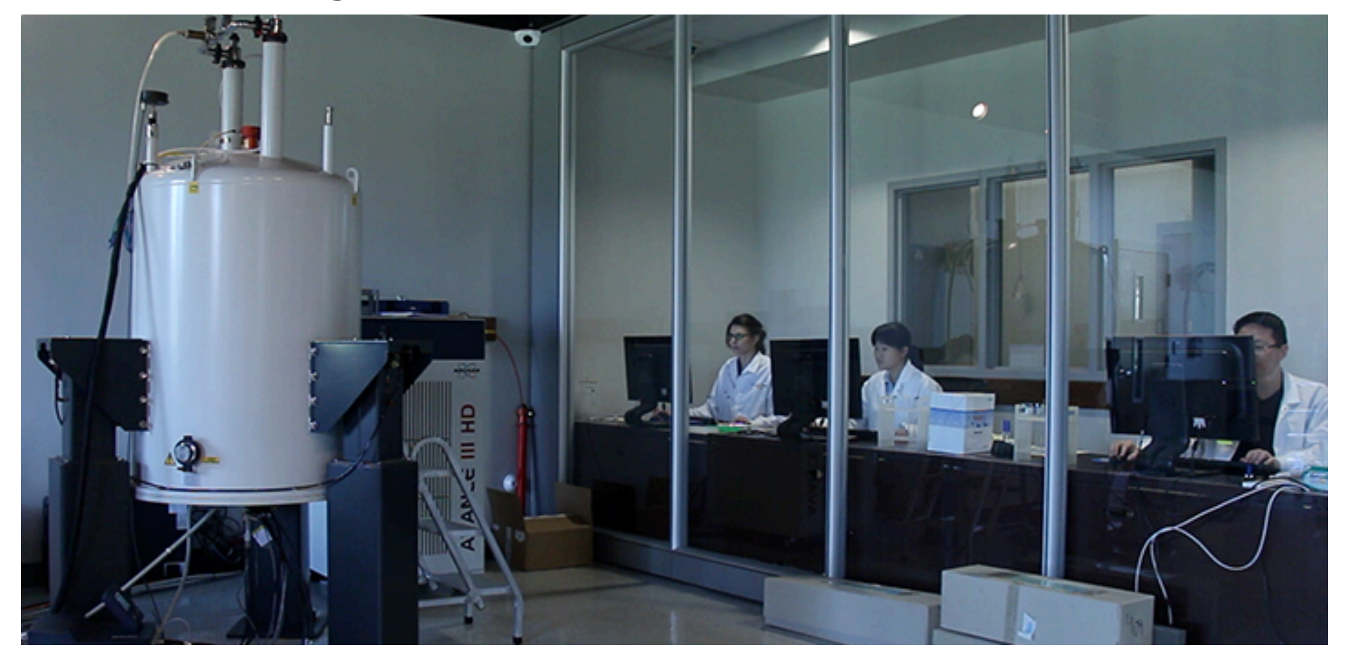


SCIEX 5600 TripleTOF
with Eksigent nanoLC

TMPL mass spec lab
MCLM 459/427
Stephen Barnes, Director
934-7117/3462



SCIEX 6500 Qtrap with SelexION



Central Alabama NMR facility
Chemistry Bdg
N. Rama Krishna, Director
934-5695

Sample selection

- **This is the most important part of a metabolomics experiment**
- **The samples should be collected according to a written, agreed upon protocol**
- **Sample types**
  - **Biofluids (**whole blood, **plasma, serum,** CSF, sputum, follicular fluid, bile, duodenal fluid, **fecal water, lung lavage,** aqueous humor**)**
  - **Tissues (brain,** liver**, heart, kidney,** adrenals, muscle, ovaries, testes, lung**)**
  - **Cells (cancer cells,** cardiomyocytes**, yeast, oral bacteria)**
  - **Food**

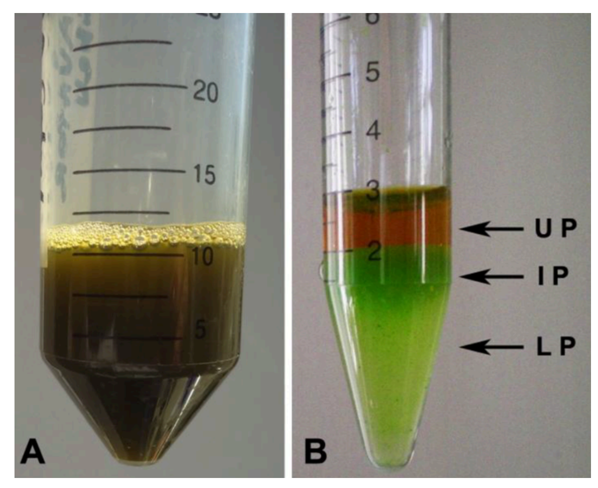
Anesthetics/analgesics

- Prior to sampling of blood, other biological fluids and tissues, it may be necessary to use an anesthetic.
  - The time to anesthetize an animal will alter the metabolome
  - Ideal method is to use a guillotine, fast tissue excision and liquid N_2
- If the IACUC-approved protocol requires an analgesic, it (and its metabolites) will be present
  - Discuss with IACUC possible alternative methods

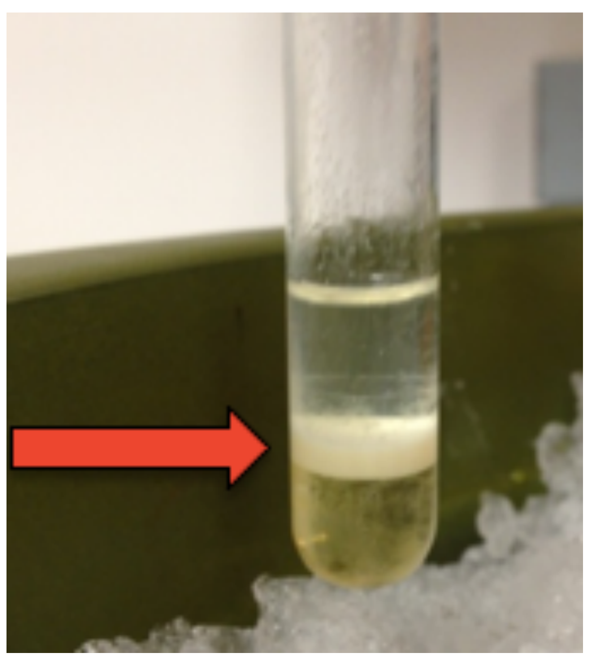
Documenting patient therapeutics

- Most patients in a study are taking additional drugs or dietary supplements that add compounds to the metabolome
- These xenobiotics also undergo metabolism
  - Phase I
  - Phase II
  - Microbiome-based
  - Also regulate the microbiome which in turn may alter the metabolism
- Watch out for patients taking antibiotics

Extracting biofluids



Protein precipitation with acetone or methanol



- Plasma partitioned between chloroform-methanol (lower phase) and water (upper phase)
- Proteins precipitate at the interface
- Lower phase contains lipids
- Upper phase has more hydrophilic metabolites

Internal standards

- Isotopically labeled metabolite standards are essential to monitor recovery during the extraction process
  - Same amount added to all samples
  - ^{13}C is better than ^{2}H, but is more expensive
  - Need to increase the mass by 4 Da compared to the unlabeled biological metabolite to avoid natural abundance ^{13}C
  - A typical set would be $^{13}C_4$-succinate, $^{13}C_{16}$-palmitate and L-$^{13}C_9$-tyrosine

The LC-MS platform

- Metabolites are separated on the basis of their hydrophobicity (using a reverse-phase column) or hydrophilicity (HILIC column) using solvent gradients
- UPLC
  - High resolution chromatography (human samples)
- NanoLC
  - for precious or low volume samples

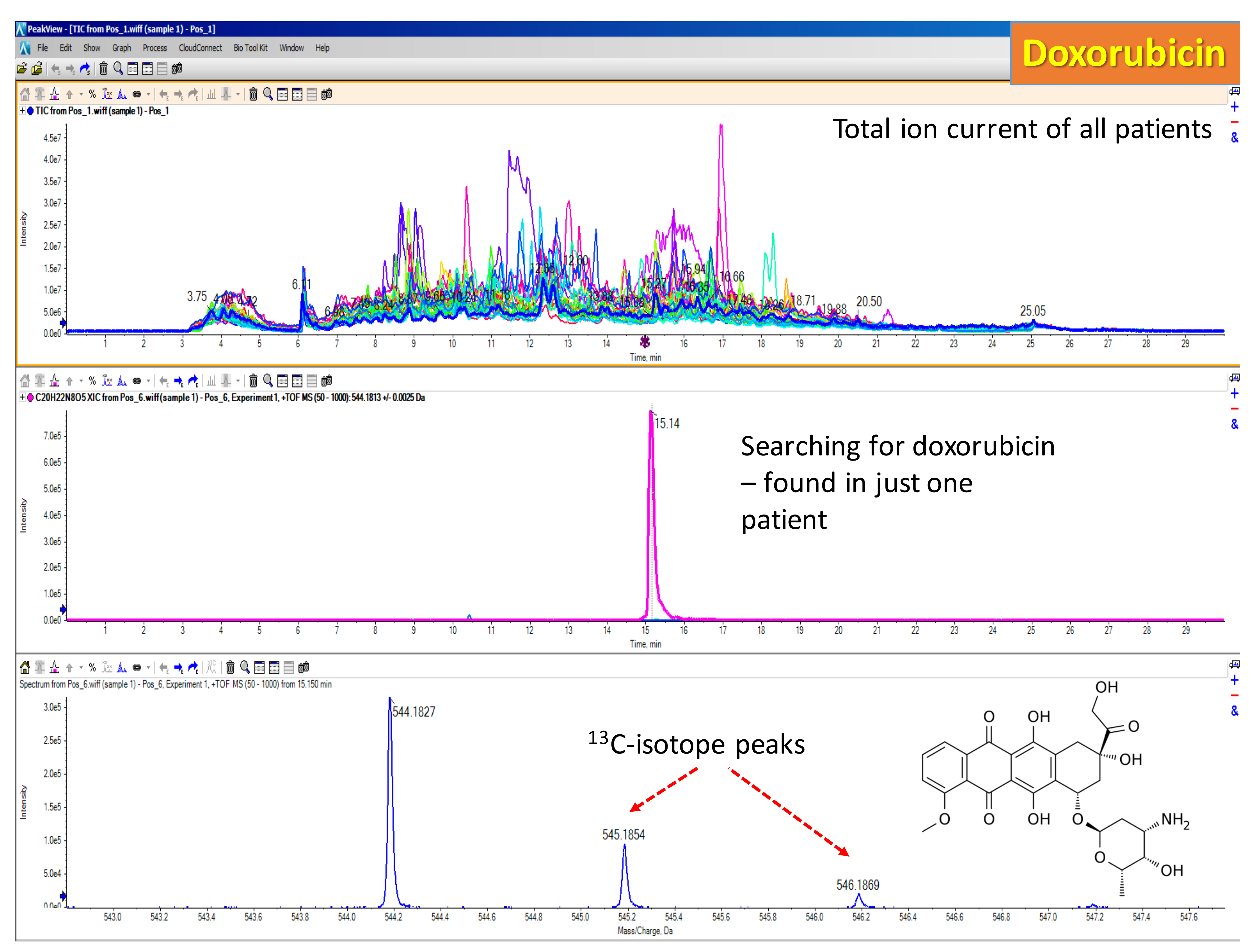
Column etched on a chip

nanoLC placed in a temperature-controlled Nanoflex™

All about reproducibility

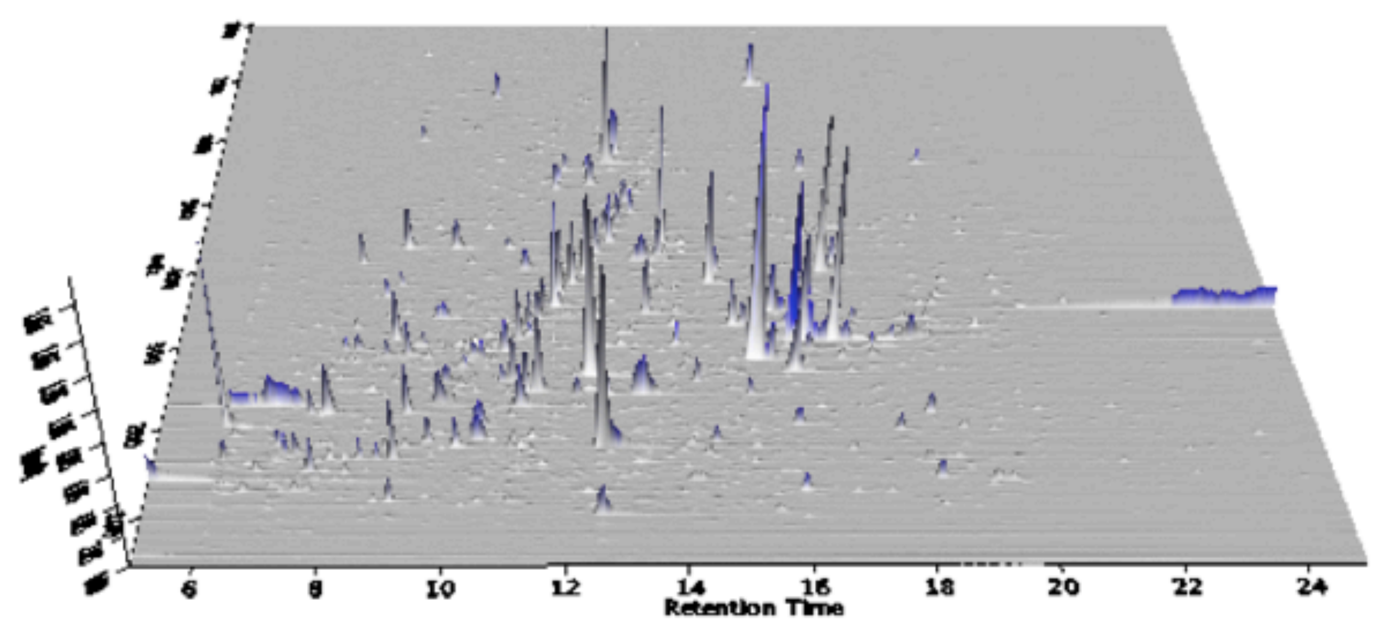
The mass spectrometer

- Ions
  - These can be +ve and –ve (require separate LC runs)
  - Several thousands can be measured
  - Some are adducts of the same metabolite
    - $[M+H]^+$, $[M+Na]^+$, $[M-H]^-$, [M-H.COO]-
- Untargeted LC-MS
  - In this mode need a (very) fast analyzer for MS and MSMS analyses
  - Time-of-flight (TOF) is the best
    - New instrument from Sciex can collect data at 5 msec intervals
  - Orbitrap/FT-MS have better mass accuracy, but not at this speed
    - Used in follow up experiments where more time is available



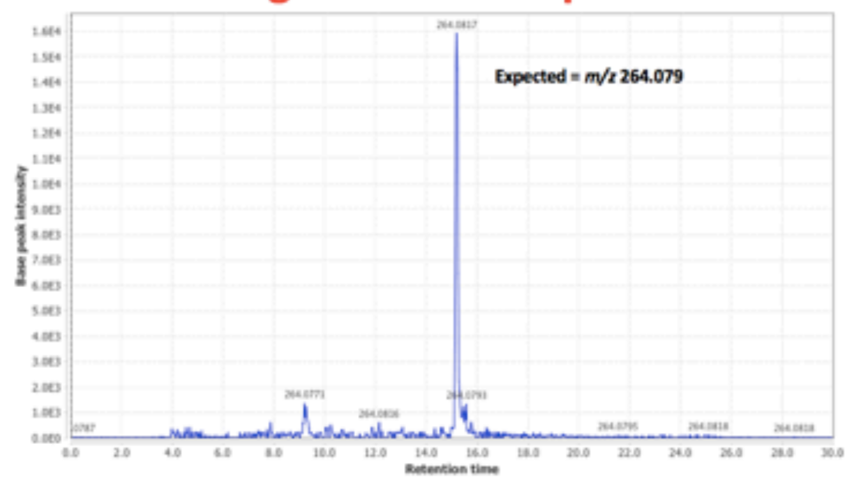
Doxorubicin
Total ion current of all patients
TIC from Pos_1.wiff (sample 1) - Pos_1
Searching for doxorubicin – found in just one patient
C20H22N8O5 XIC from Pos_6.wiff (sample 1) - Pos_6, Experiment 1, +TOF MS (50 - 1000): 544.1813 +/- 0.0025 Da
15.14
Spectrum from Pos_6.wiff (sample 1) - Pos_6, Experiment 1, +TOF MS (50 - 1000) from 15.150 min
544.1827
545.1854
546.1869
^{13}C-isotope peaks
Time, min
Mass/Charge, Da
Intensity

Metabolomics and drugs in patients



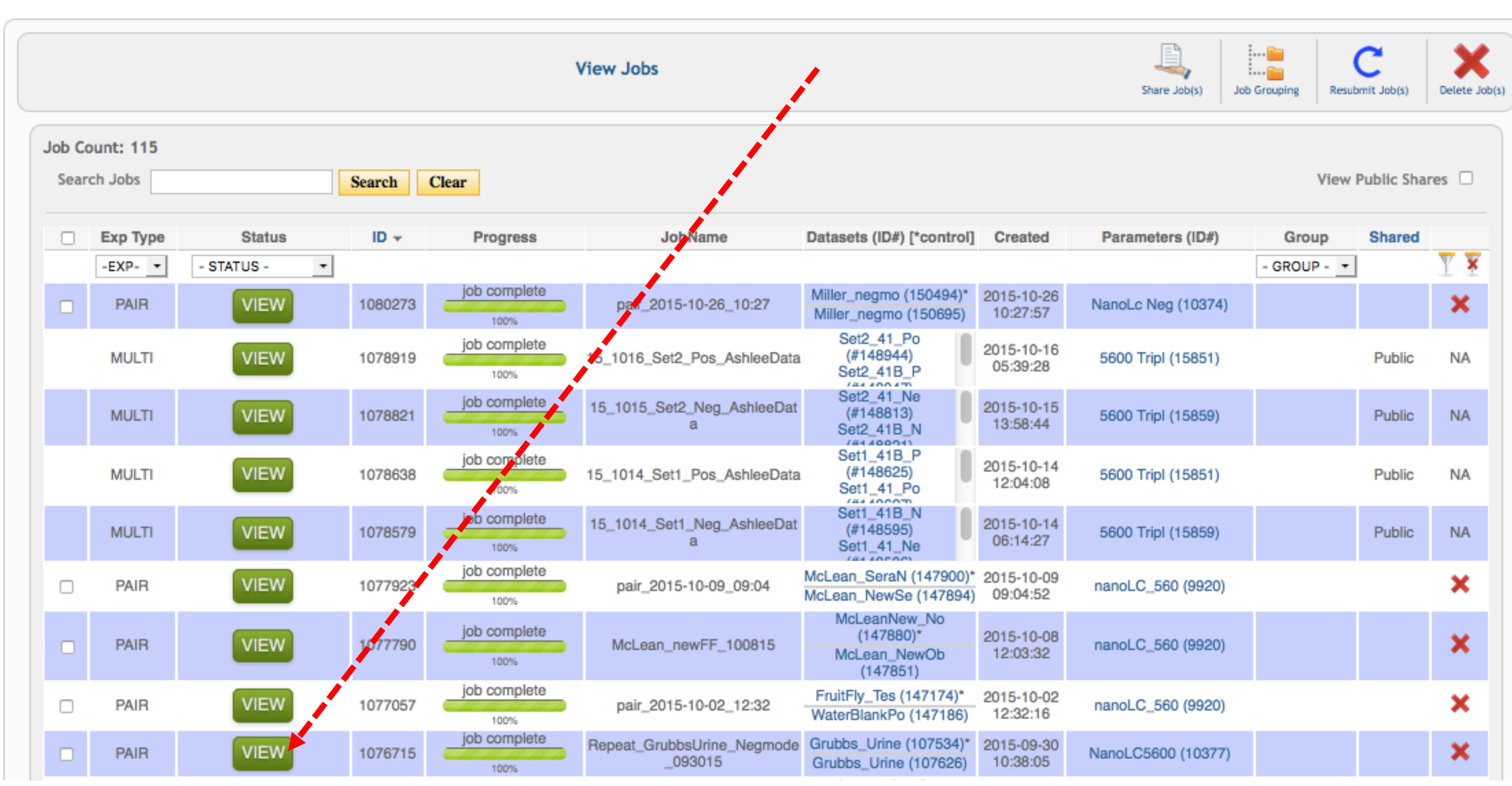
A data library of the urinary metabolome of a BC patient

Analysis with MZmine



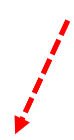
Untargeted metabolomics better defines the patient in a clinical study

XCMSonline



Overlay of all samples

Download the processed Excel file



Pairwise Results Summary: Repeat_GrubbsUrine_Negmode_093015 (#1076715)

Download Results

hash: d9a76940e8d1d7b75e8d6f4becfa2ac9

Submit Date	Finish Date	Paired Samples	Total Aligned Features	Parameter ID#	Log	Shared
2015-09-30 10:38:44	2015-09-30 11:47:18	False	3087	NanoLC5600 TripleTof (10377)	View Log	NOT SHARED

WARNINGS:

2015-09-30 11:35:45 : iHeatMap data prep, memory requires limiting to top 1000 features <0.0758642 p-values

View Results Table

View Interactive Cloud Plot

View Interactive Heatmap

View iPCA

Total Ion Chromatograms (original) — PNG PDF

Retention Time Deviation vs. Retention Time — PNG PDF

Total Ion Chromatograms (corrected) — PNG PDF

Cloud Plot 345 features with p-value ≤ 0.01 , fold change ≥ 1.5 — PNG PDF

Non-Metric Multidimensional Scaling — PNG PDF

PCA Scores Unit Variance scaling Centered — PNG PDF

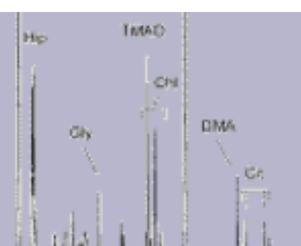
Datasets Used

- Grubbs_UrineGro (107534) *
- Grubbs_UrineGro (107626)

Creating .csv files for each sample

A	B	C
mzmed	rtmed	negmode_10
307.1720	14.09	45623
283.1867	19.33	164991
123.0812	13.24	7324
284.1876	19.36	31102
214.1409	18.94	116750
214.1200	18.67	101854
601.3749	19.89	9011
261.1688	18.43	86490
257.1743	19.38	90202
248.1270	19.49	60049
330.2275	19.93	131465
341.1934	17.39	70079
228.1583	20.52	84973
272.3130	15.99	10642
329.2251	19.94	642272
185.1135	16.87	187571
262.1722	18.44	14198
347.2041	15.69	80983
281.1741	19.11	71836
258.1773	19.41	19191
148.0397	12.35	31319
233.1225	12.88	29802
343.2103	15.96	99157

- Copy the median *m/z* and median Rt values into a new Excel file. Then copy the column of areas from the first sample in Group_1. Save as an Excel .csv file.
  - Note that the file name must not have spaces – use an underscore instead of a space.
- Leave the file open and replace the yellow column with the areas from the next Group_1 sample. Save as a second .csv file.
- Continue until all Group_1 and Group_2 samples have a corresponding .csv file.
- Make a .zip file for MetaboAnalyst



MetaboAnalyst 3.0

– a comprehensive tool suite for metabolomic data analysis

Welcome click here to start

News & Updates

- We are testing our mirror site (mirror.metaboanalyst.ca) on Google Cloud. Traffics will be distributed between the two websites. Let us know if you experience any issue.
- Several feature improvements and bug fixes based on user feedback (*10/16/2015*); NEW
- Added support for logistic regression in ROC Tester (*08/12/2015*); NEW
- Added support for computing compound ratios in biomarker analysis (*08/03/2015*); NEW
- Minor bug fixes and feature enhancements (data IO, PLS-DA, enrichment analysis) to deal with special cases in user inputs (*07/20/2015*);
- Updated Multivariate Biomarker Analysis module with flexible interface and improved capacity for computing on large datasets (*06/05/2015*);
- MetaboAnalyst 3.0 paper is now available on the 2015 NAR web server issue

Read more ......

Please Cite:

Xia, J., Sinelnikov, I., Han, B., and Wishart, D.S. (2015) MetaboAnalyst 3.0 - making metabolomics more meaningful . Nucl. Acids Res. (DOI: 10.1093/nar/gkv380).

Xia, J., Mandal, R., Sinelnikov, I., Broadhurst, D., and Wishart, D.S. (2012) MetaboAnalyst 2.0 - a comprehensive server for metabolomic data analysis . Nucl. Acids Res. 40, W127-W133.

Xia, J., Psychogios, N., Young, N. and Wishart, D.S. (2009) MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucl. Acids Res. 37, W652-660.

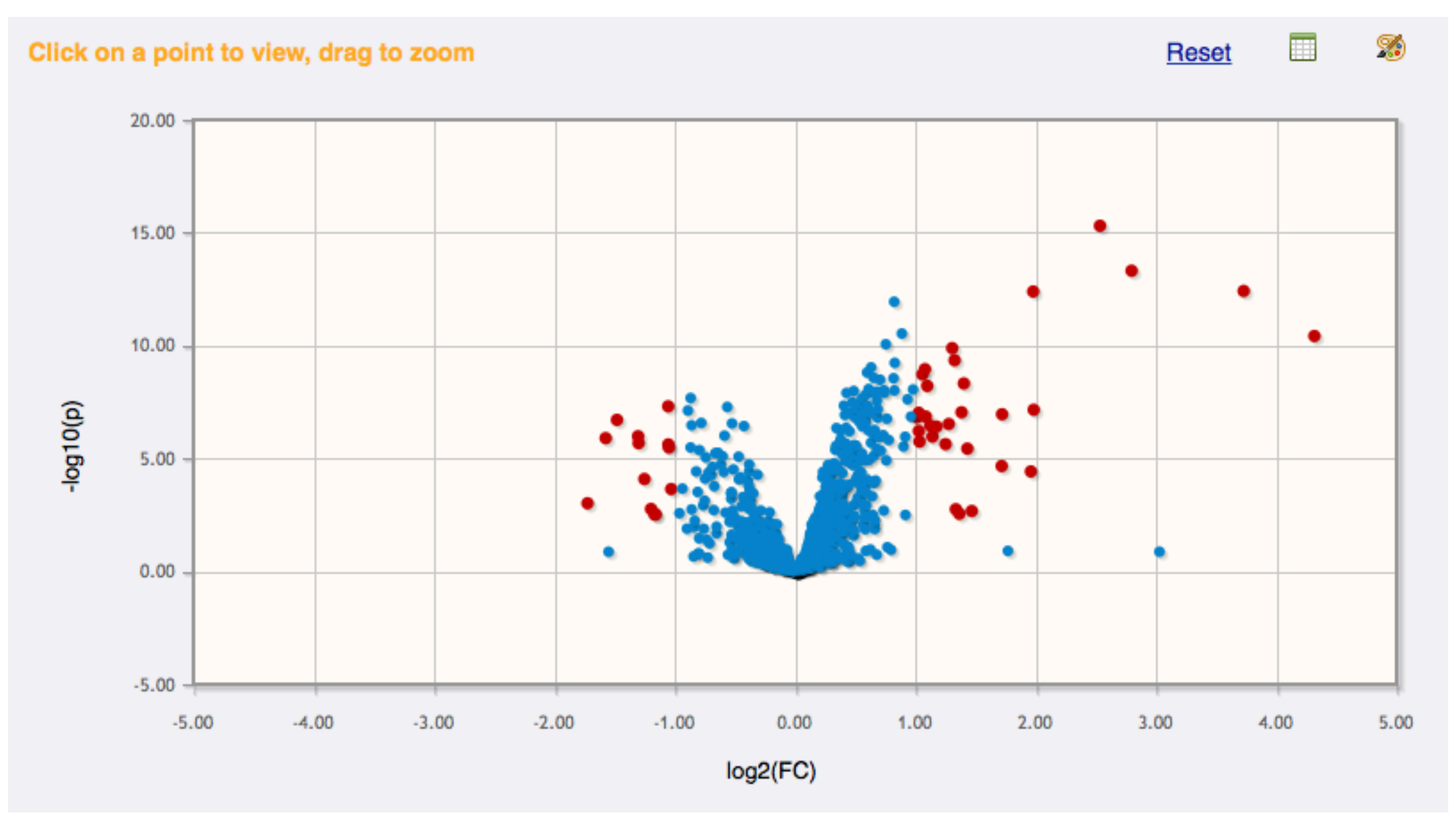
Before Normalization

193.0506/14.
278.1405/20.
203.091/12.7
684.1206/14.
548.3373/16.
182.0822/15.
200.1005/16.
447.2197/19.
216.1068/13.
484.2015/14.
279.129/16.0
262.1722/18.
345.0865/19.
437.0863/12.
303.0776/8.4
462.283/19.9
210.0627/12.
450.2161/15.
190.1093/15.
312.0864/12.
179.05/12.47
405.2614/21.
183.1021/18.
453.12/14.4

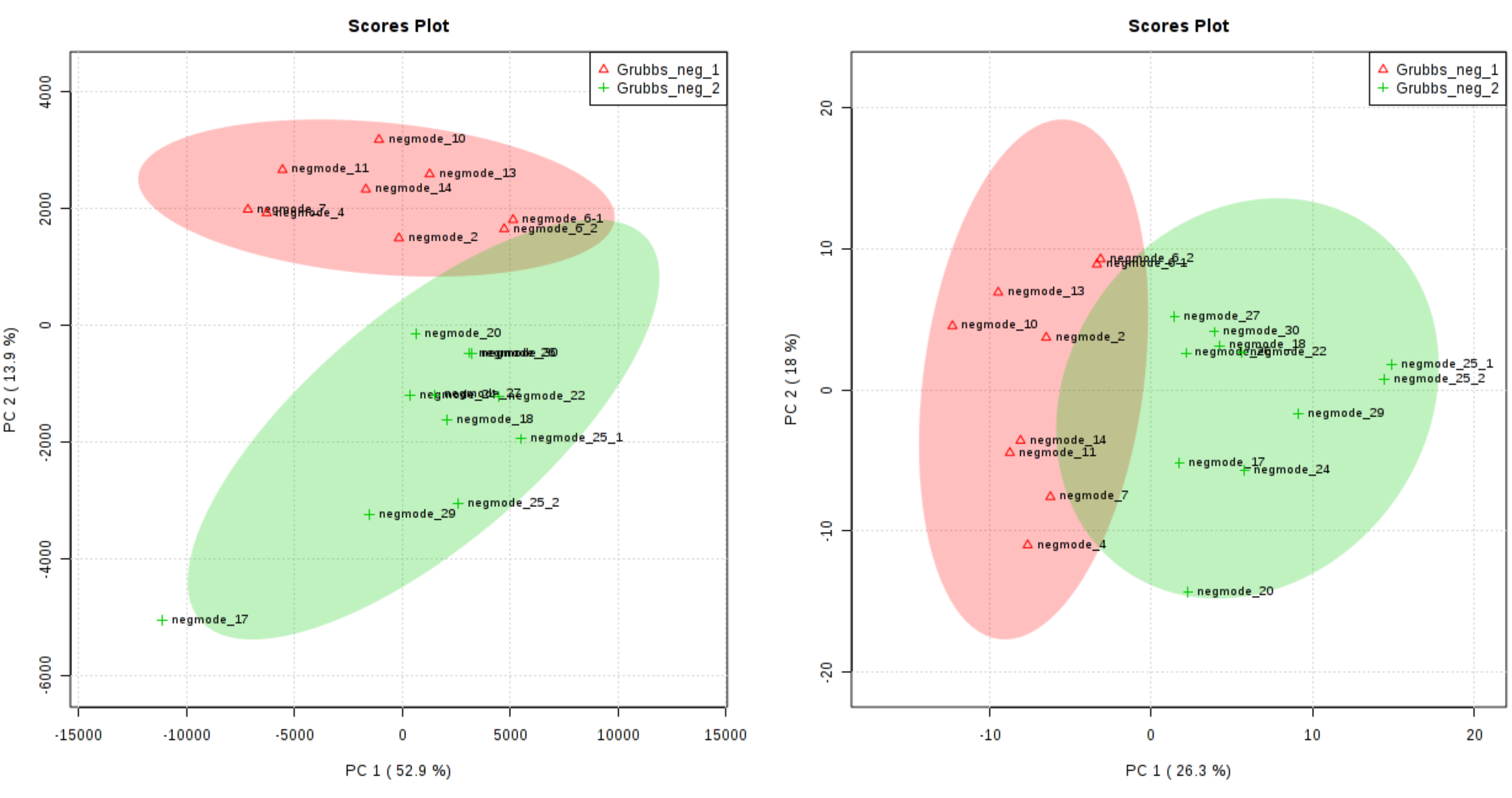
After Normalization

193.0506/14.
278.1405/20.
203.091/12.7
684.1206/14.
548.3373/16.
182.0822/15.
200.1005/16.
447.2197/19.
216.1068/13.
484.2015/14.
279.129/16.0
262.1722/18.
345.0865/19.
437.0863/12.
303.0776/8.4
462.283/19.9
210.0627/12.
450.2161/15.
190.1093/15.
312.0864/12.
179.05/12.47
405.2614/21.
183.1021/18.
453.12/14.4

Volcano plot with fold change=1.5 and p <0.01

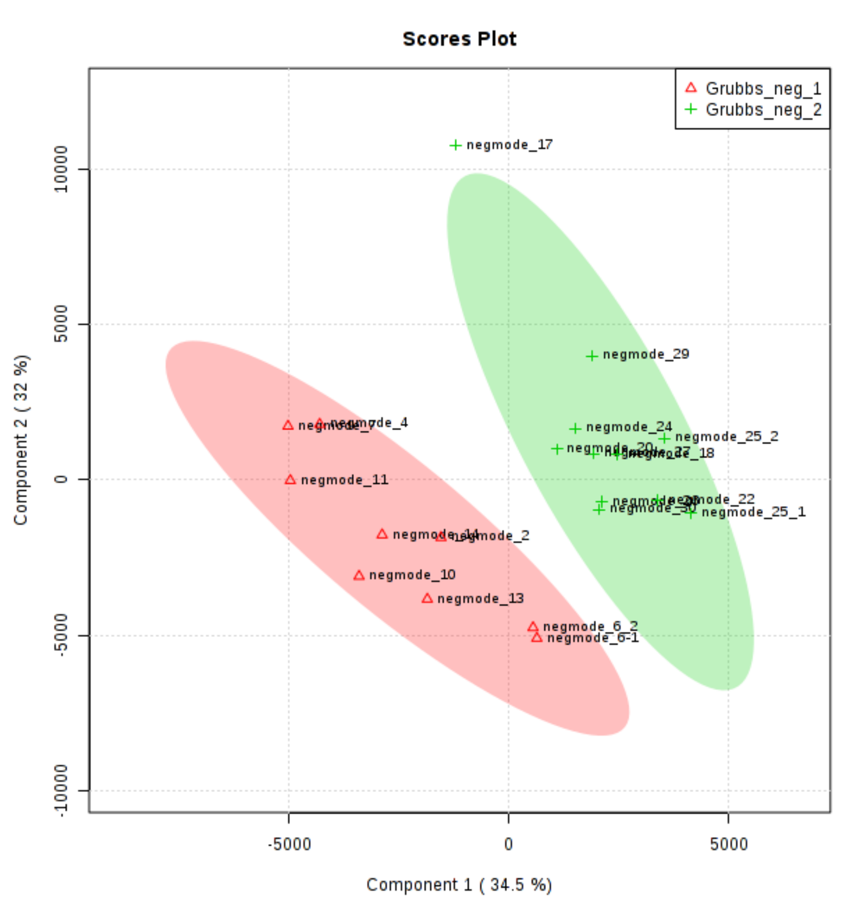


PCA plot shows that there are metabolite differences in urines from animals on irradiated (red) and non-irradiated (green) diets

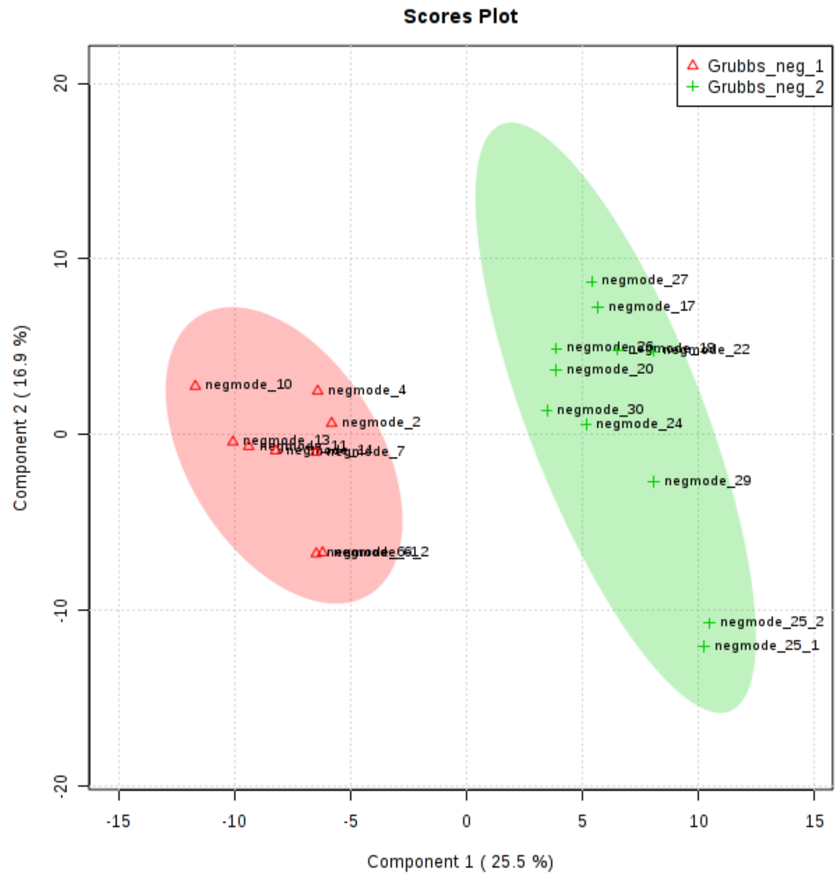


No normalization **With normalization**

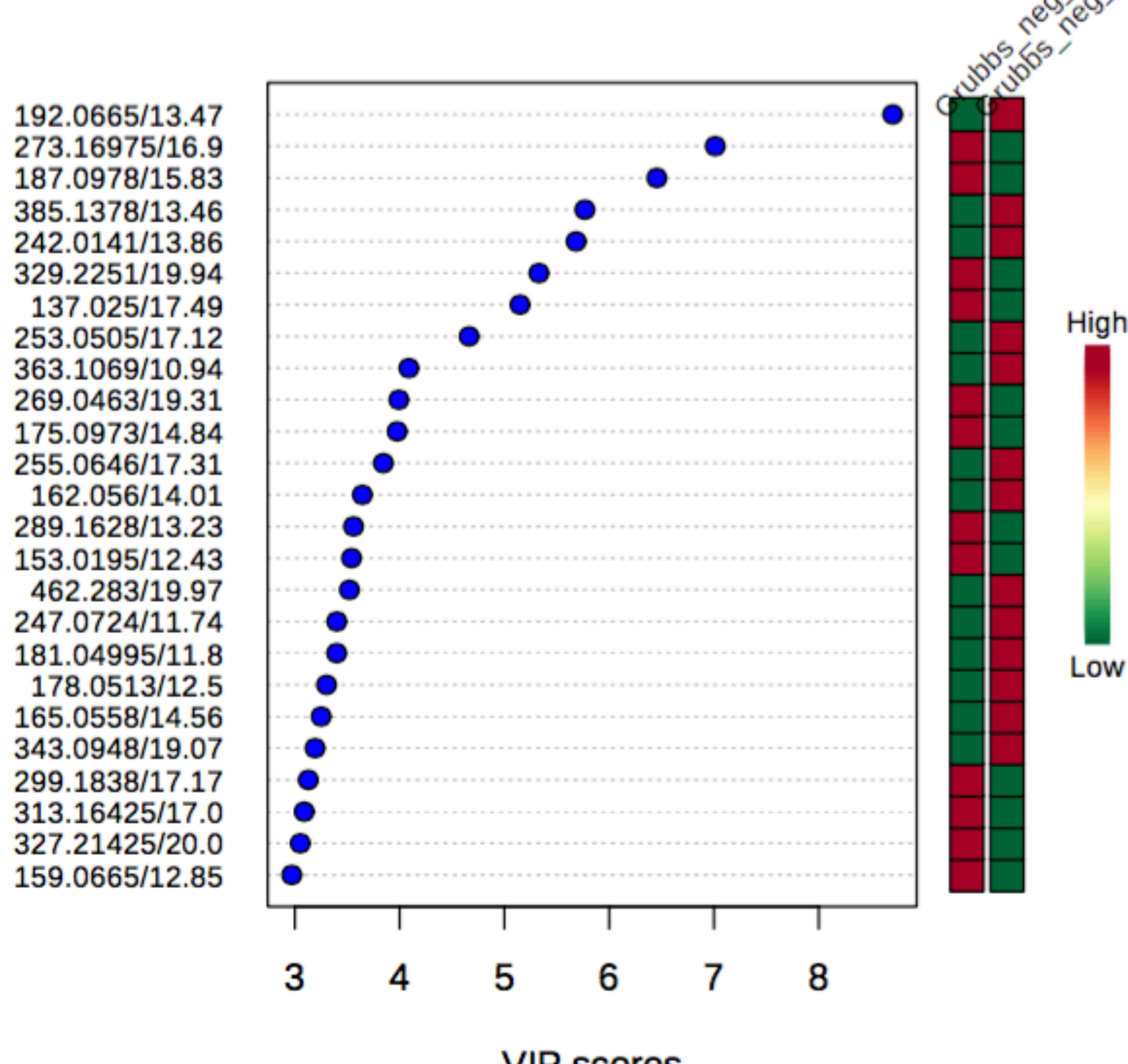
PLS-DA plot shows that there are metabolite differences in urines from animals on irradiated (red) and non-irradiated (green) diets



No normalization



With normalization



192.0665/13.47
273.16975/16.9
187.0978/15.83
385.1378/13.46
242.0141/13.86
329.2251/19.94
137.025/17.49
253.0505/17.12
363.1069/10.94
269.0463/19.31
175.0973/14.84
255.0646/17.31
162.056/14.01
289.1628/13.23
153.0195/12.43
462.283/19.97
247.0724/11.74
181.04995/11.8
178.0513/12.5
165.0558/14.56
343.0948/19.07
299.1838/17.17
313.16425/17.0
327.21425/20.0
159.0665/12.85
3
4
5
6
7
8
VIP scores
Grubbs_neg_1
Grubbs_neg_2
High
Low

The challenge in metabolomics

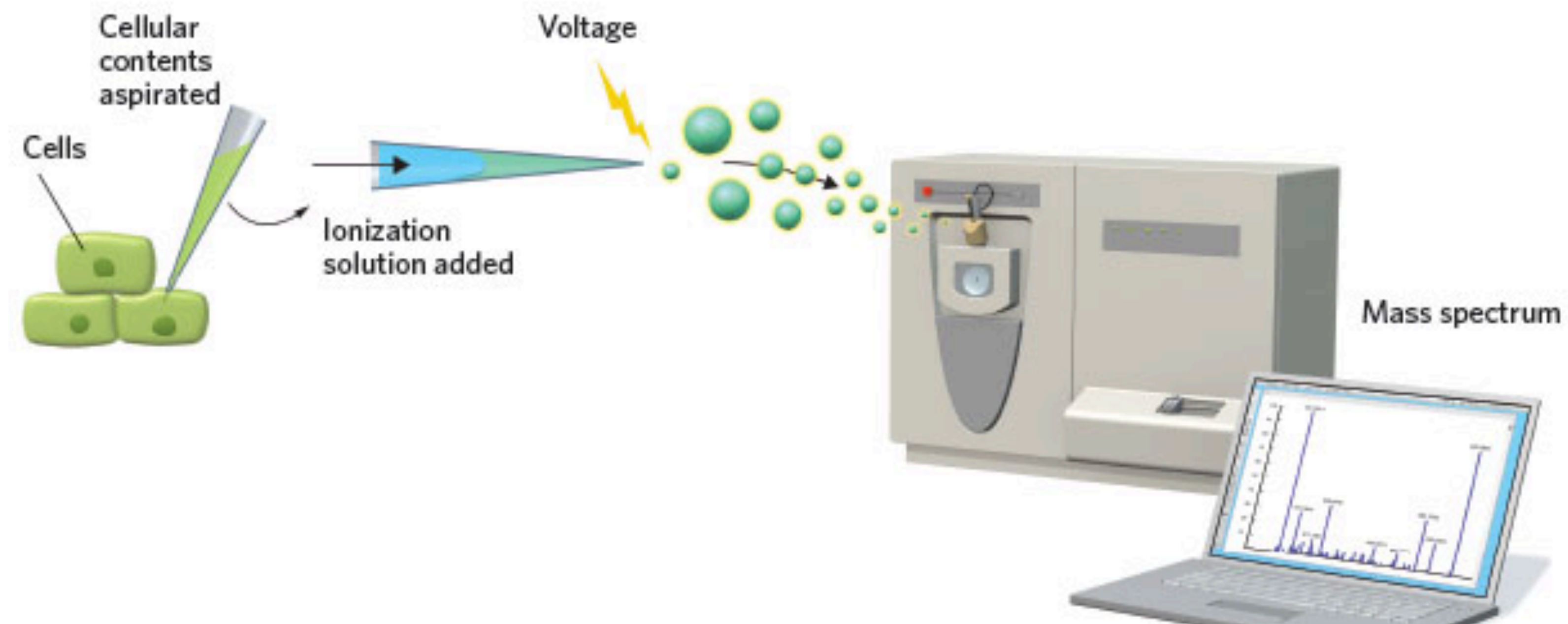
- Modern analytical methods have revealed that most of the metabolome is undocumented metabolites
  - Their identification requires analytical chemical expertise found in those with training in natural products chemistry
- This will rewrite metabolic pathways
  - Existing pathways are monogenomic, whereas you, me and most of our research models are multigenomic

Where we could be if……

Single-Cell Suck-and-Spray

A nanoscopic needle and a mass spectrometer reveal the contents of individual cells.

By Ruth Williams | December 1, 2015

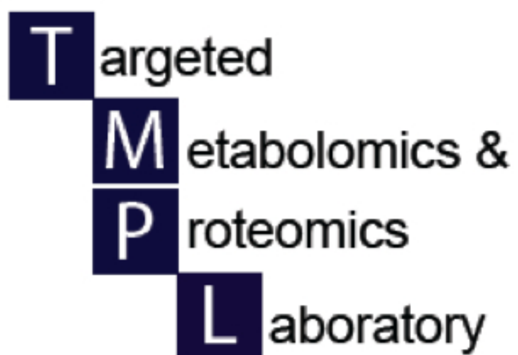


Graduate –Omics course

Nuclear Magnetic Resonance (NMR) Metabolomics

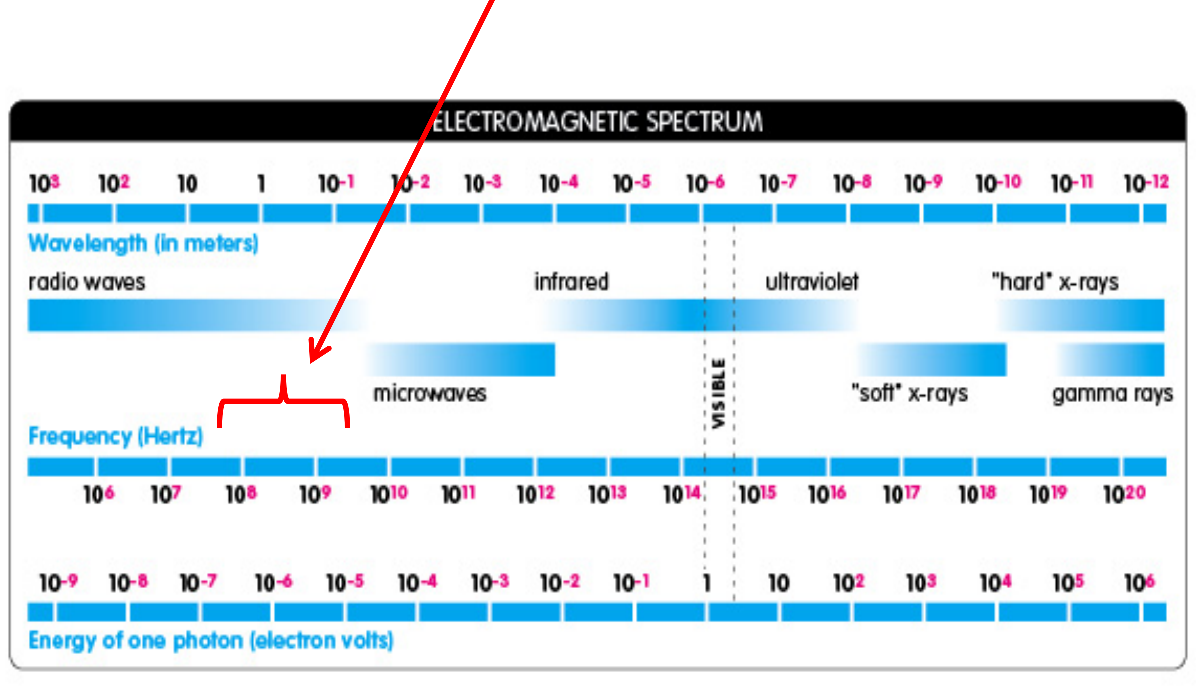
N. Rama Krishna, Ph.D., Director
Central Alabama High-Field NMR Facility

Acknowledgments:
We thank Wimal Pathmasiri and Rodney Snyder of RTI for permission to use some of their slides (identified by the RTI logo).



NMR Spectroscopy

Where is it?



NMR Metabolomics

Advantages

Quantitative estimate of concentration of metabolites

Highly Reproducible

Detects all metabolites simultaneously

Nondestructive. You can recover the sample completely
(and use it for MS Metabolomics)

Minimal sample preparation and no need for derivatiation

Disadvantages:

Sensitivity (micromolar to millimolar concentration range).

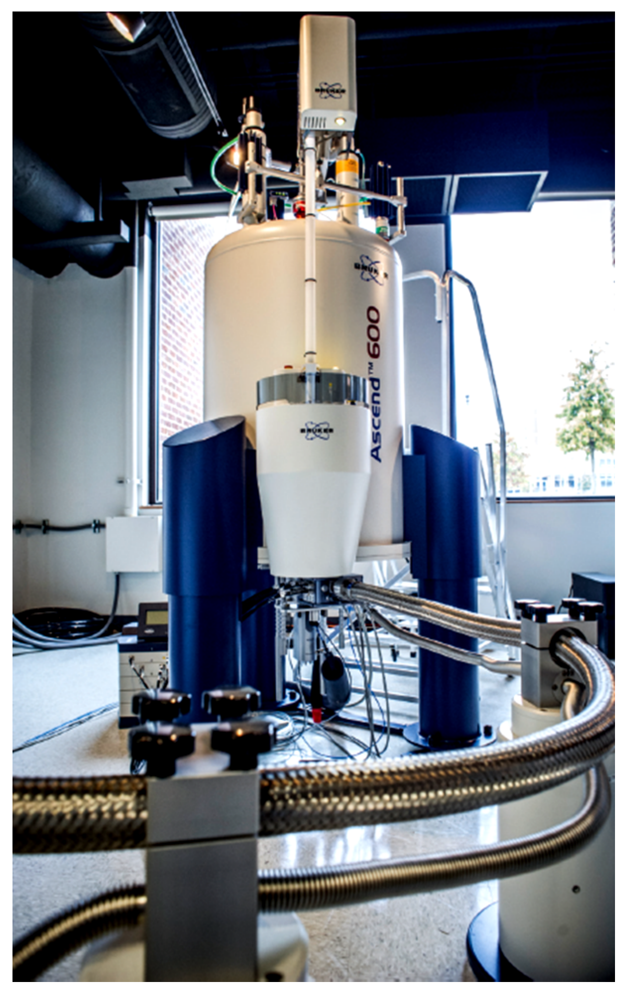
NMR spectra are complex (signals from different metabolites can overlap)

Optimal and Minimal Sample Volumes

	Minimum sample for MS Based Detection	Minimum Sample for NMR- Based Detection	Optimal Sample
Serum	50 ul	100 ul	1 ml
Urine	50 ul	200 ul	1 ml
Feces	20 mg	20 mg	500 mg
Tissue	50 mg	100 mg	500 mg
Cells	$1x10^6$	$1x10^7$	$1x10^7$

Bruker-Biospin Avance III HD
600 MHz NMR Spectrometer with
TCI-CryoProbe and Sample Case

Central Alabama High-Field NMR Facility



Bruker-Biospin Avance III 600 MHz NMR system with
TCI CryoProbe and Sample Case

AT 71000 GAUSS (7.1 TELSLA)

(1T = 10,000G)

W_0(MHz)	0	30	75	121	280	300	320
Nucleus		^{15}N	^{13}C	^{31}P	^{19}F	^{1}H	^{3}H

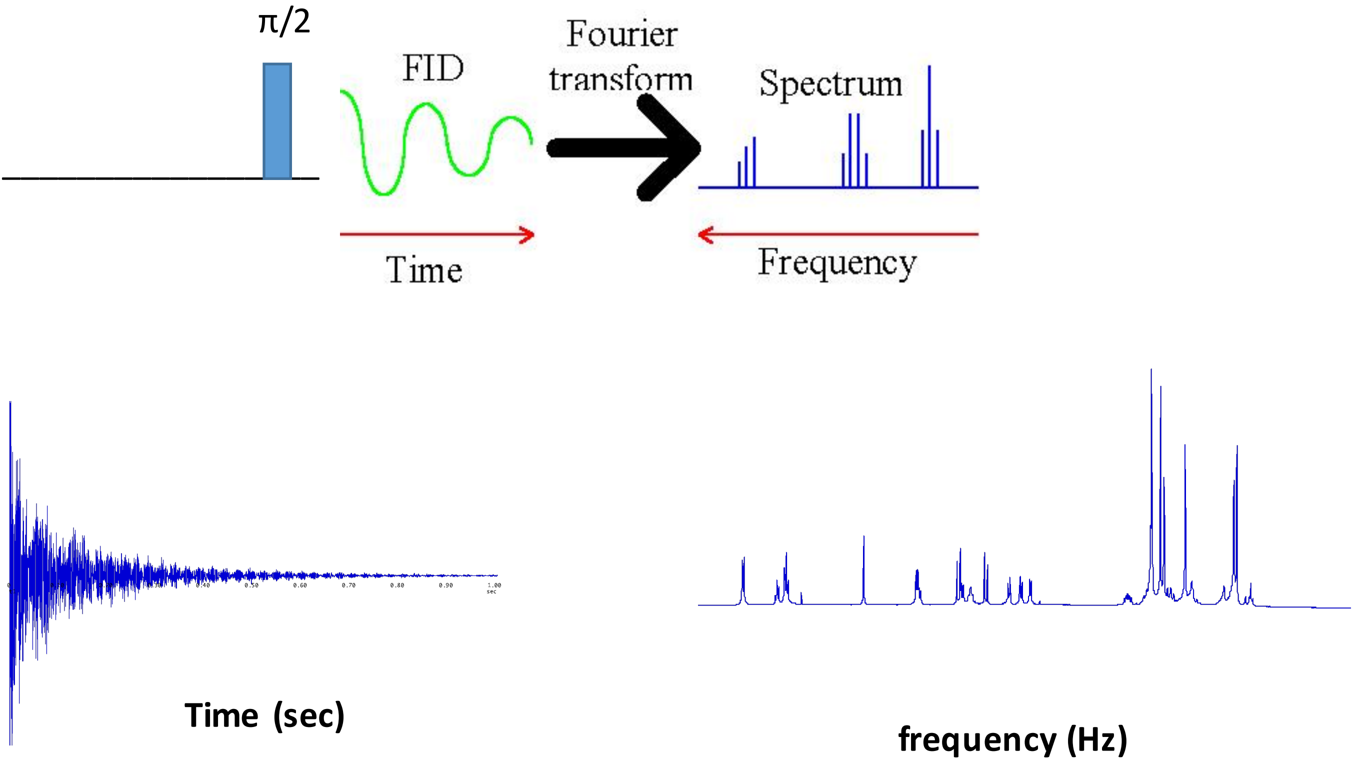
Table 1.1 Nuclei of Major Interest to NMR Spectroscopists

Iostope	Abundance (%)	Z	Spin	μ^2	$\gamma \times 10^{-8b}$	Relative[c] sensitivity	ν_0 at 1T(MHz)	At 7.04T
^{1}H	99.9844	1	1/2	2.7927	2.6752	1.000	42.577	300
^{2}H	0.0156	1	1	0.8574	0.4107	0.00964	6.536	46
^{10}B	18.83	5	3	1.8006	0.2875	0.0199	4.575	
^{11}B	81.17	5	3/2	2.6880	0.8583	0.165	13.660	
^{13}C	1.108	6	1/2	0.7022	0.6726	0.0159	10.705	75.4
^{14}N	99.635	7	1	0.4036	0.1933	0.00101	3.076	
^{15}N	0.365	7	1/2	-0.2830	-0.2711	0.00104	4.315	30.4
^{19}F	100	9	1/2	2.6273	2.5167	0.834	40.055	282.3
^{29}Si	4.70	14	1/2	-0.5548	-0.5316	0.0785	8.460	
^{31}P	100	15	1/2	1.1305	1.0829	0.0664	17.235	121.4

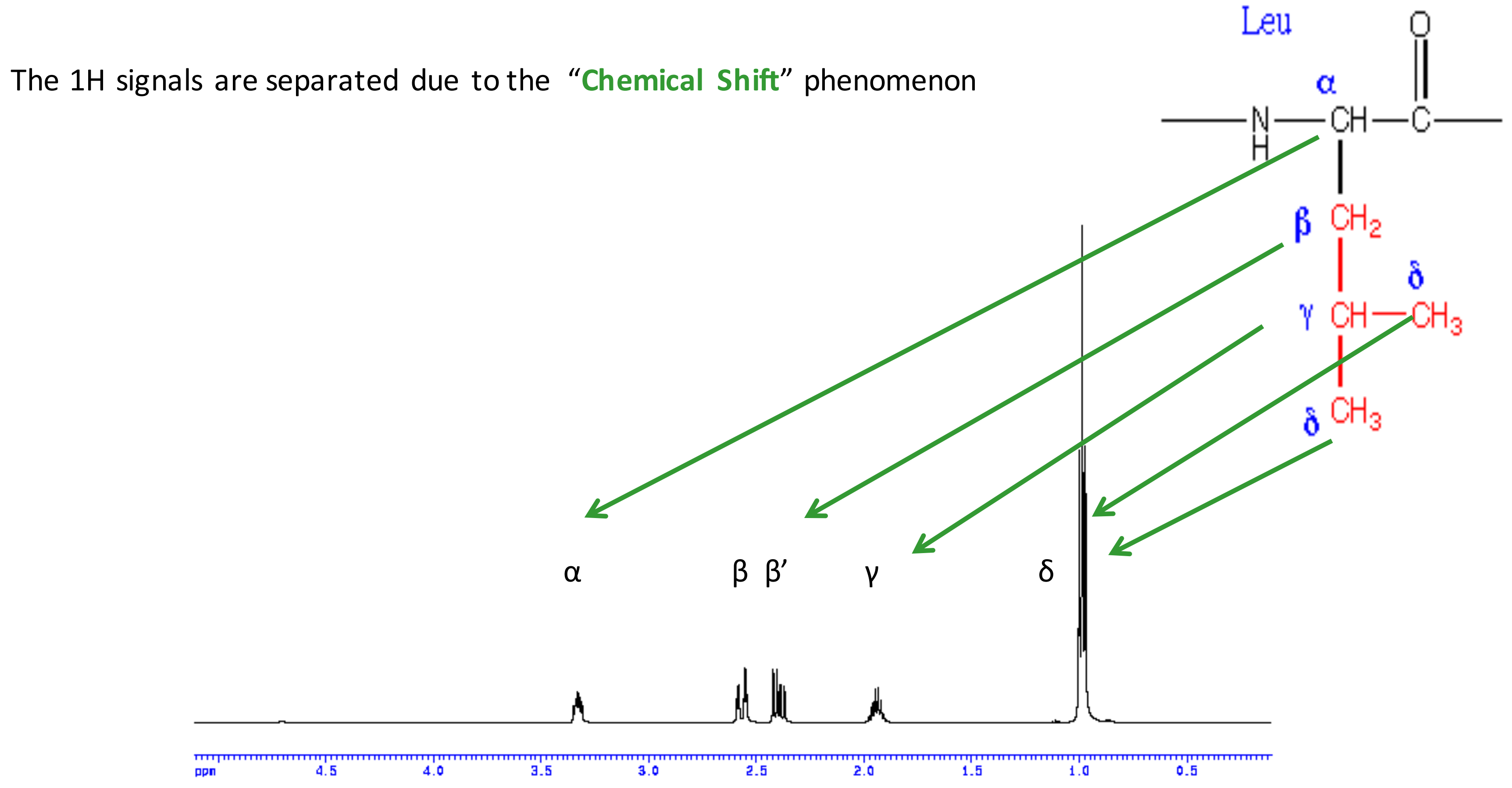
a Magnetic moment in units of the nuclear magneton, $eh/(\ \ M_p c)$
b Magnetogyric ratio in SI units
c For equal numbers of nuclei at constant field

5. NMR Data Processing

- A π/2 rf pulse is applied to cause transitions. The resulting signal (called FID (free induction decay)) is then Fourier transformed to frequency domain to obtain the NMR spectrum for each different nuclei.

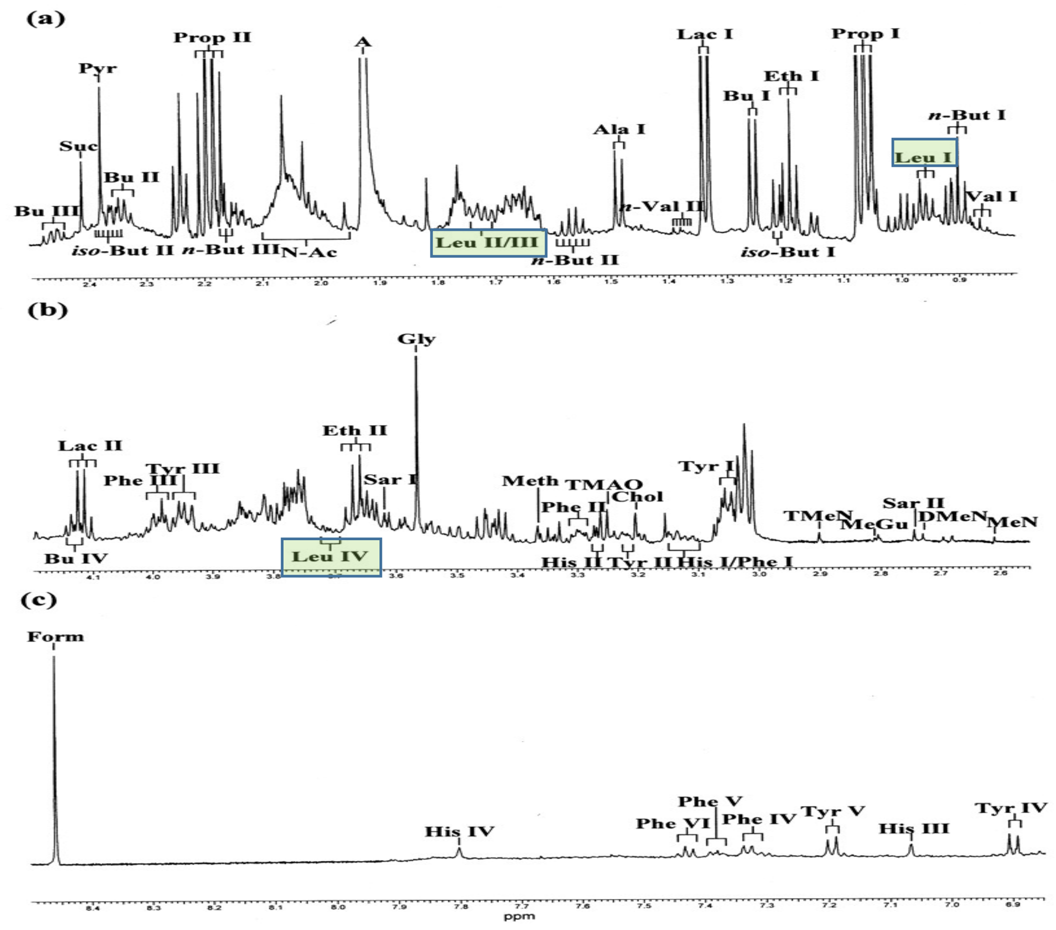


The 1H signals are separated due to the "**Chemical Shift**" phenomenon



1H 1D-NMR spectrum of Leucine/D2O, showing splittings from J-couplings

Figure 1. Single-pulse 1D 1H NMR spectrum of a human salivary supernatant specimen.



Silwood C et al. J DENT RES 2002;81:422-427

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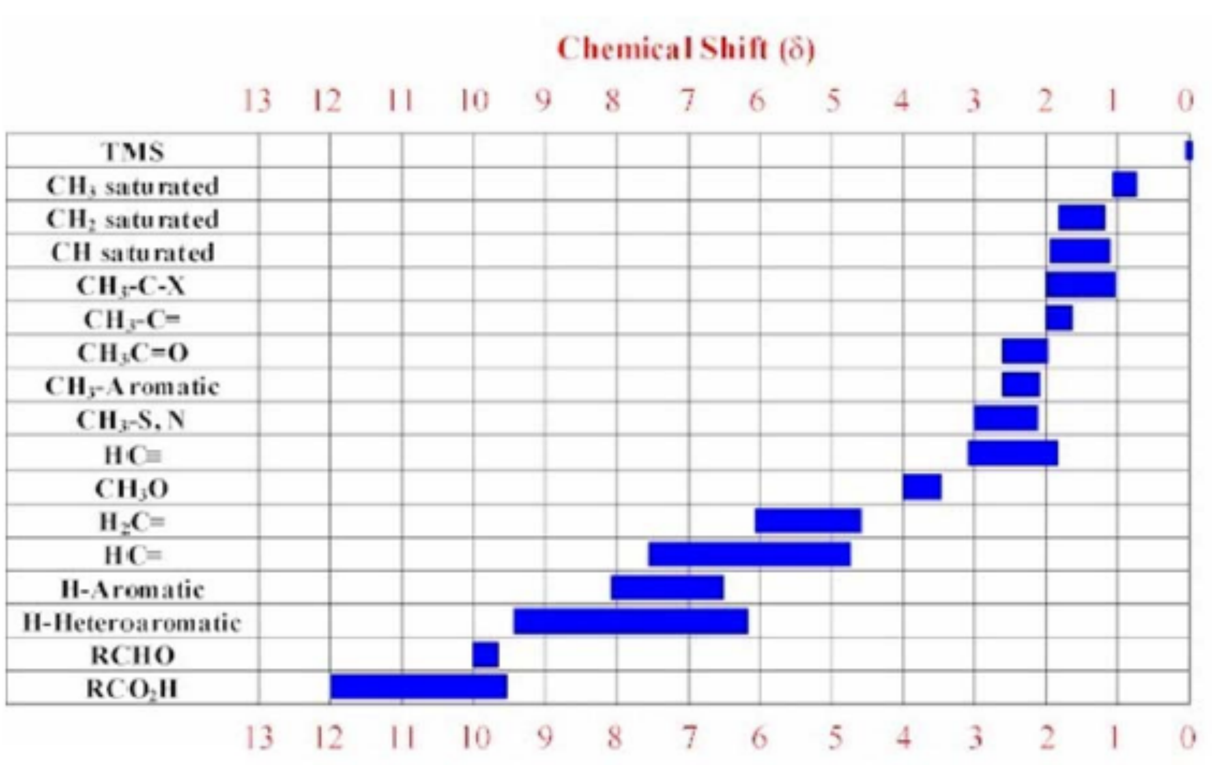
Characteristic Chemical Shifts



Clin Cancer Res January 15, 2009 vol. 15 no. 2 431-440

Clinical Applications of Metabolomics in Oncology: A Review

Table 1.

Biofluid and sample preparation requirements

Biofluid	**Required sampling handling**	
Urine	Add deuterated phosphate buffer to 0.2-0.4 mL urine	
Blood/plasma /serum	For 0.5 mL of heparinized blood product	
		—Add deuterium oxide (to lock)
		—Add acetonitrile (for protein precipitation)
		—Add methanol/chloroform extraction (for lipid extraction)
CSF	Add deuterium oxide to 0.5 mL of CSF	
EPS	Add deuterium oxide to 0.03-0.10 mL of EPS	
Bile	Add deuterium methanol to 0.5 mL of bile	
BALF	Add deuterium oxide to 0.5 mL of BALF	
Tissue	—Add 0.01 mL of deuterium oxide to 3-10 g of tissue in MAS rotor	
		—Add perchloric acid extraction on 20-200 g frozen tissue
		—Add methanol/chloroform extraction to 20-200 g frozen tissue

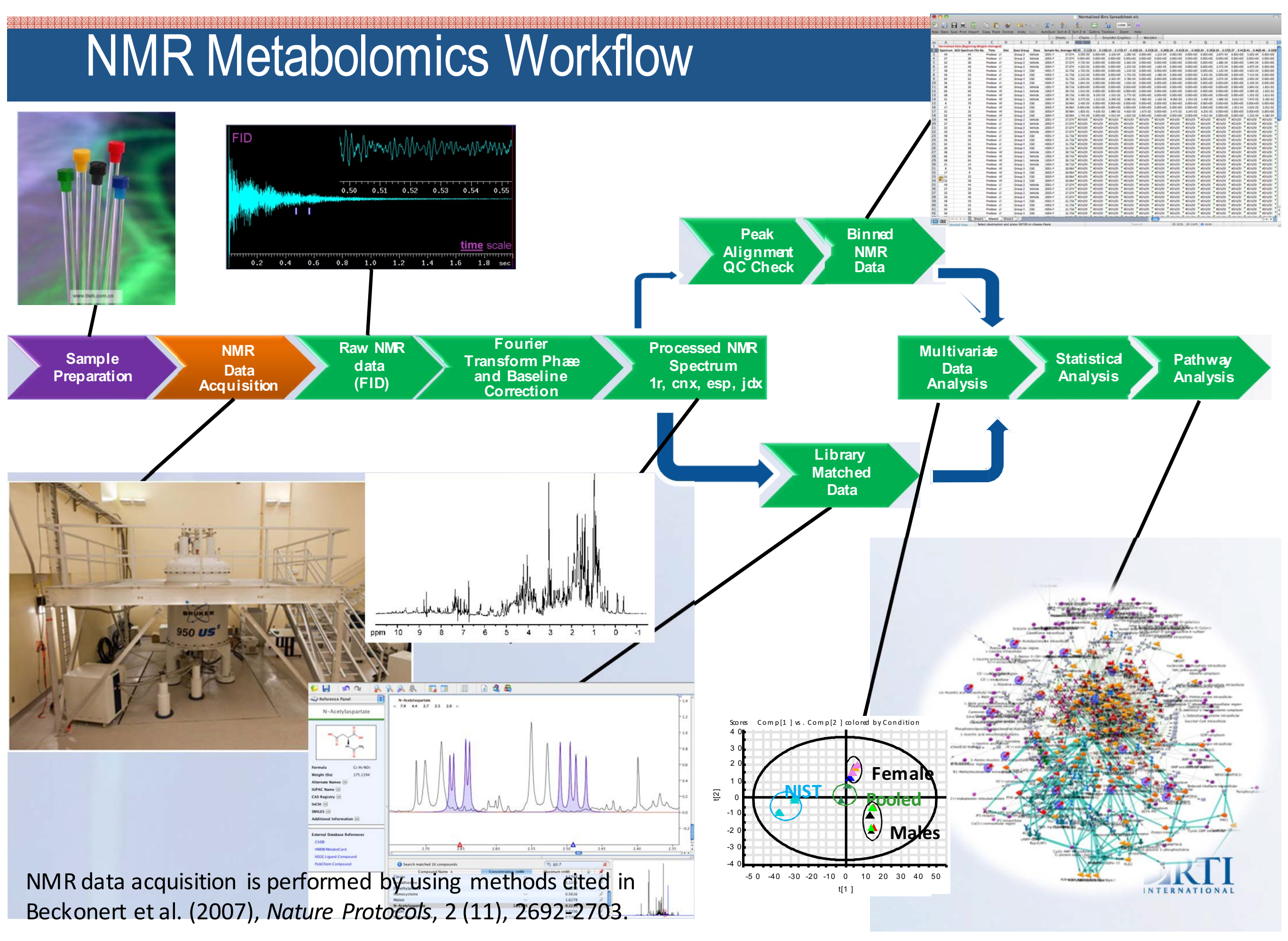
- NOTE: Adapted from ref. 13.

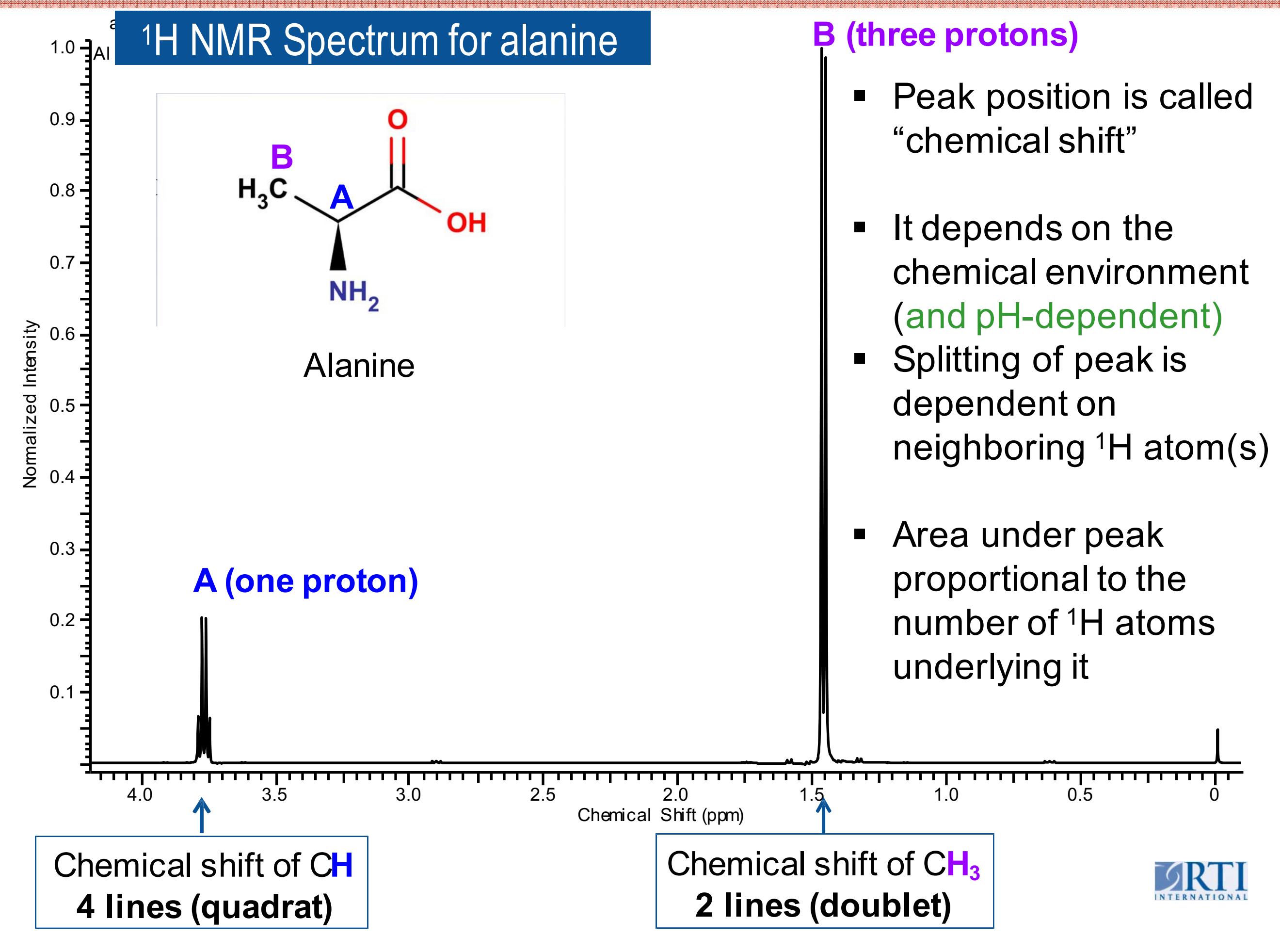
Abbreviations: CSF, cerebrospinal fluid; EPS, expressed prostatic secretions; BALF, bronchoalveolar lavage fluid; MAS, magic angle spinning.

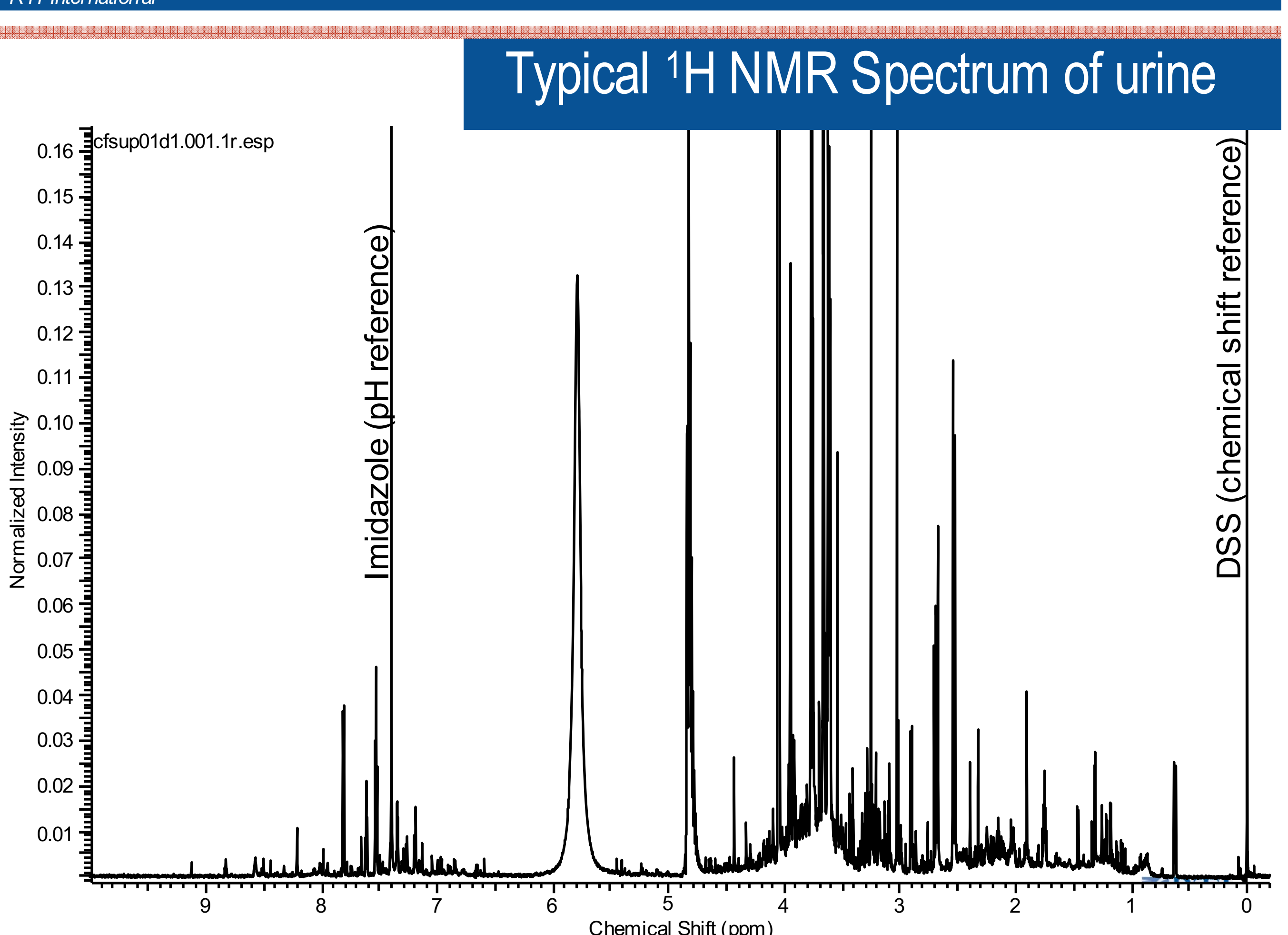
Note:

(a) It is typical to add some deuterated solvent (e.g., 5% D2O) to the solution for Field-frequency lock, to compensate for the slow field drift of the magnet.
Often, extracts of tissues (e.g., PCA extracts) are dissolved in D2O to record CH proton NMR Signals.

(b) Since some metabolites have pH-sensitive chemical shifts, it is critical to record all NMR spectra at same pH (e.g., pH 7).


NMR Metabolomics Workflow
FID
time scale
Sample Preparation
NMR Data Acquisition
Raw NMR data (FID)
Fourier Transform Phase and Baseline Correction
Processed NMR Spectrum 1r, cnx, esp, jdx
Peak Alignment QC Check
Binned NMR Data
Library Matched Data
Multivariate Data Analysis
Statistical Analysis
Pathway Analysis
950 US
Female
Pooled
NIST
Males
RTI INTERNATIONAL
NMR data acquisition is performed by using methods cited in Beckonert et al. (2007), *Nature Protocols*, 2 (11), 2692-2703.


1H NMR Spectrum for alanine
B (three protons)
Peak position is called "chemical shift"
It depends on the chemical environment (and pH-dependent)
Splitting of peak is dependent on neighboring 1H atom(s)
Area under peak proportional to the number of 1H atoms underlying it
B
H3C
A
O
OH
NH2
Alanine
A (one proton)
Normalized Intensity
Chemical Shift (ppm)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical shift of CH
4 lines (quadrat)
Chemical shift of CH3
2 lines (doublet)
RTI INTERNATIONAL



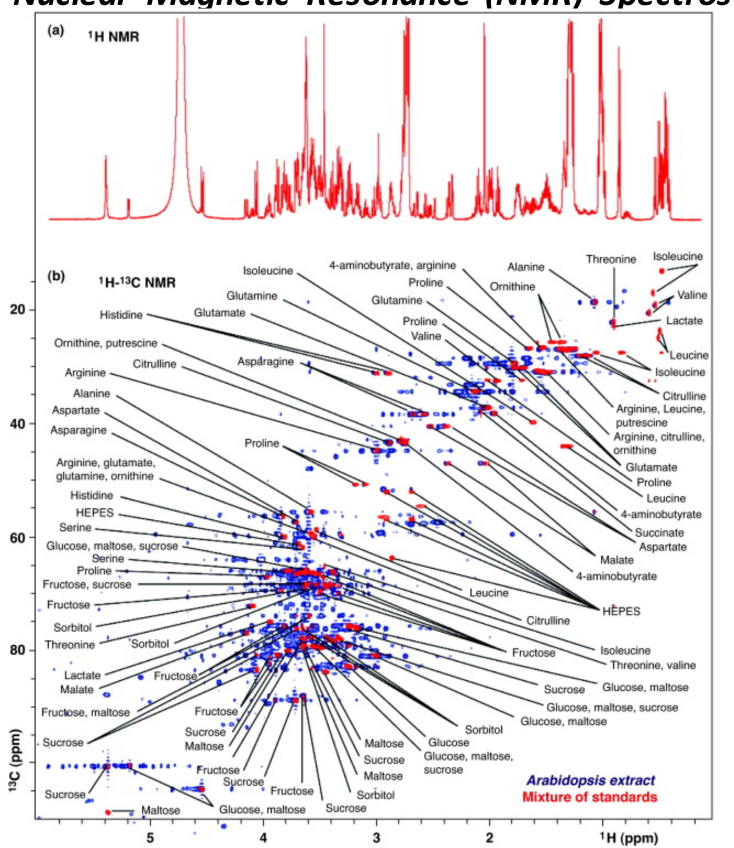
RTI International
Typical 1H NMR Spectrum of urine
cfsup01d1.001.1r.esp
Imidazole (pH reference)
DSS (chemical shift reference)
Normalized Intensity
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

How do you assign the NMR peaks to distinct metabolite signals ?

Metabolic Profiling Methods

Main Analytical Techniques

Nuclear Magnetic Resonance (NMR) Spectroscopy



HSQC used to select for protons directly bonded to ^{13}C.

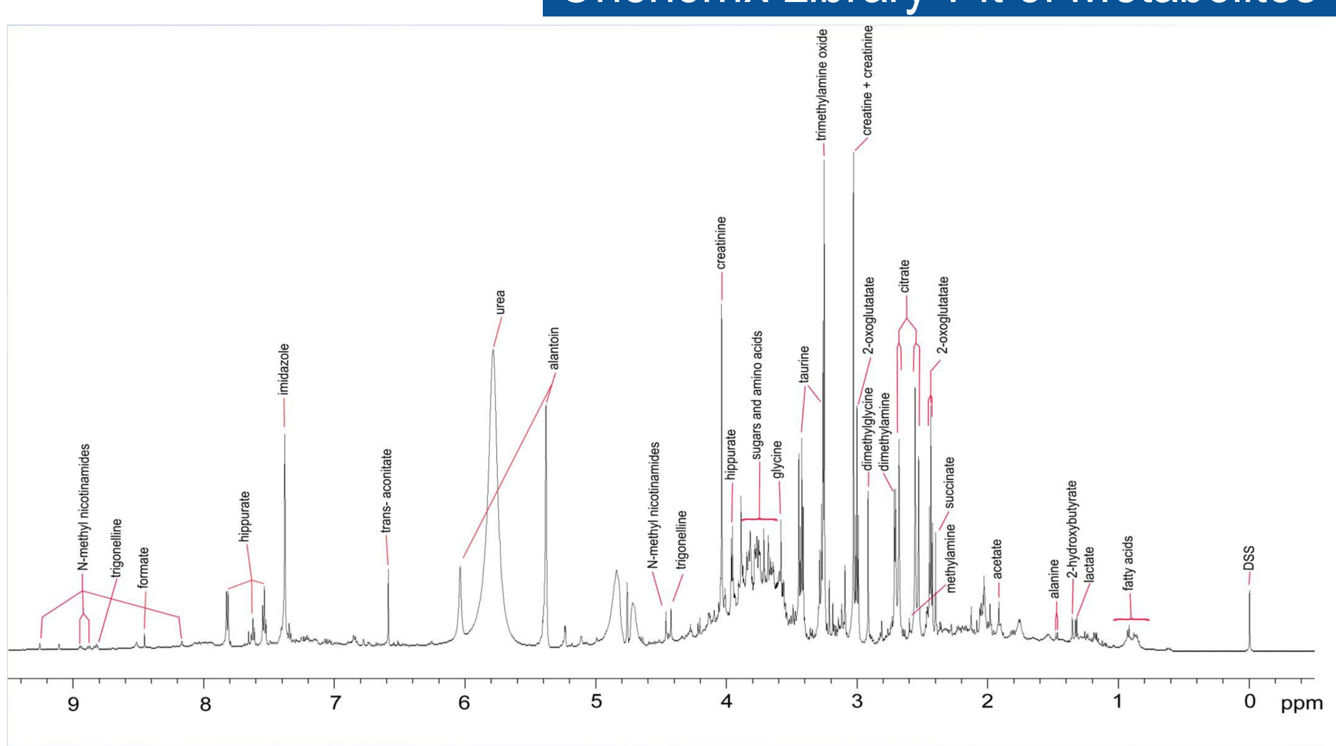
Use of HSQC spectroscopy for analysis of common metabolites. In 1D spectra, overlapped signals hamper identification of individual metabolites, whereas in 2D correlation, spots are easily visible.

(**a**) 1D ^{1}H NMR spectrum of an equimolar mixture of the 26 standards.

(**b**) 2D ^{1}H–^{13}C HSQC NMR spectra of the same synthetic mixture (red) overlaid onto a spectrum of aqueous whole-plant extract from *Arabidopsis* (blue).

PMID: 21435731

NMR Spectrum of Urine with Chenomx Library Fit of Metabolites



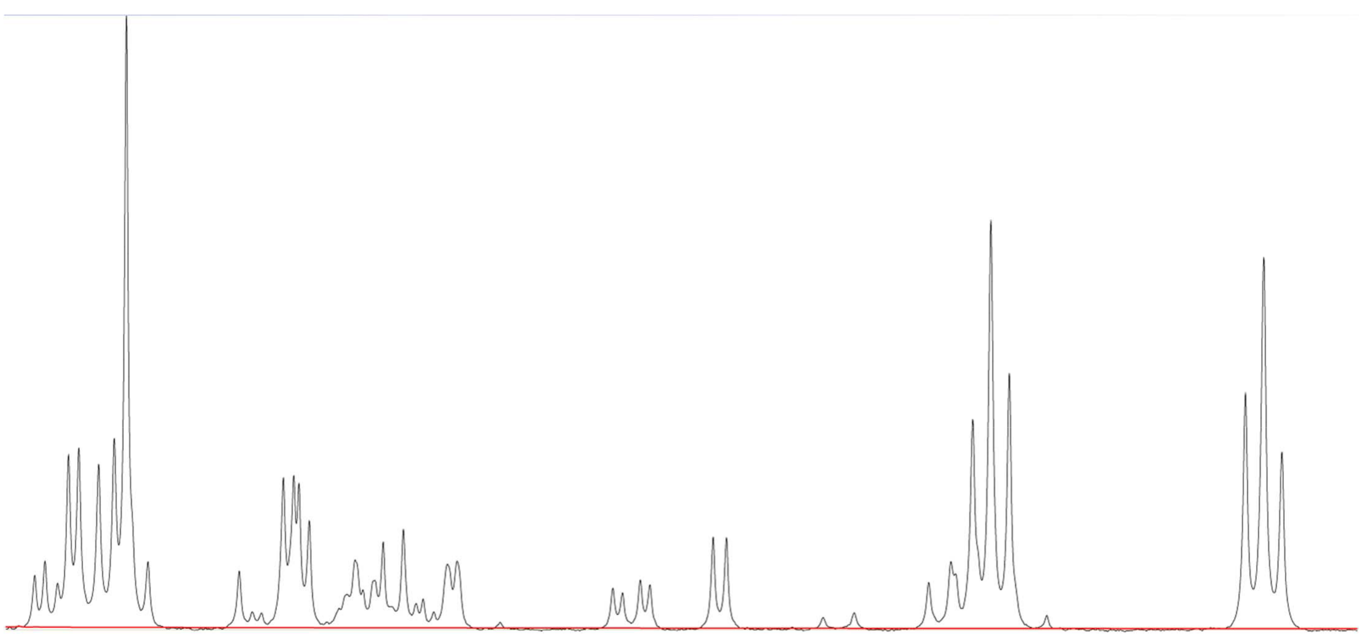
Chenomx Library

- Over 320 metabolites
- pH sensitive library of 1H NMR Spectra
- Customizable

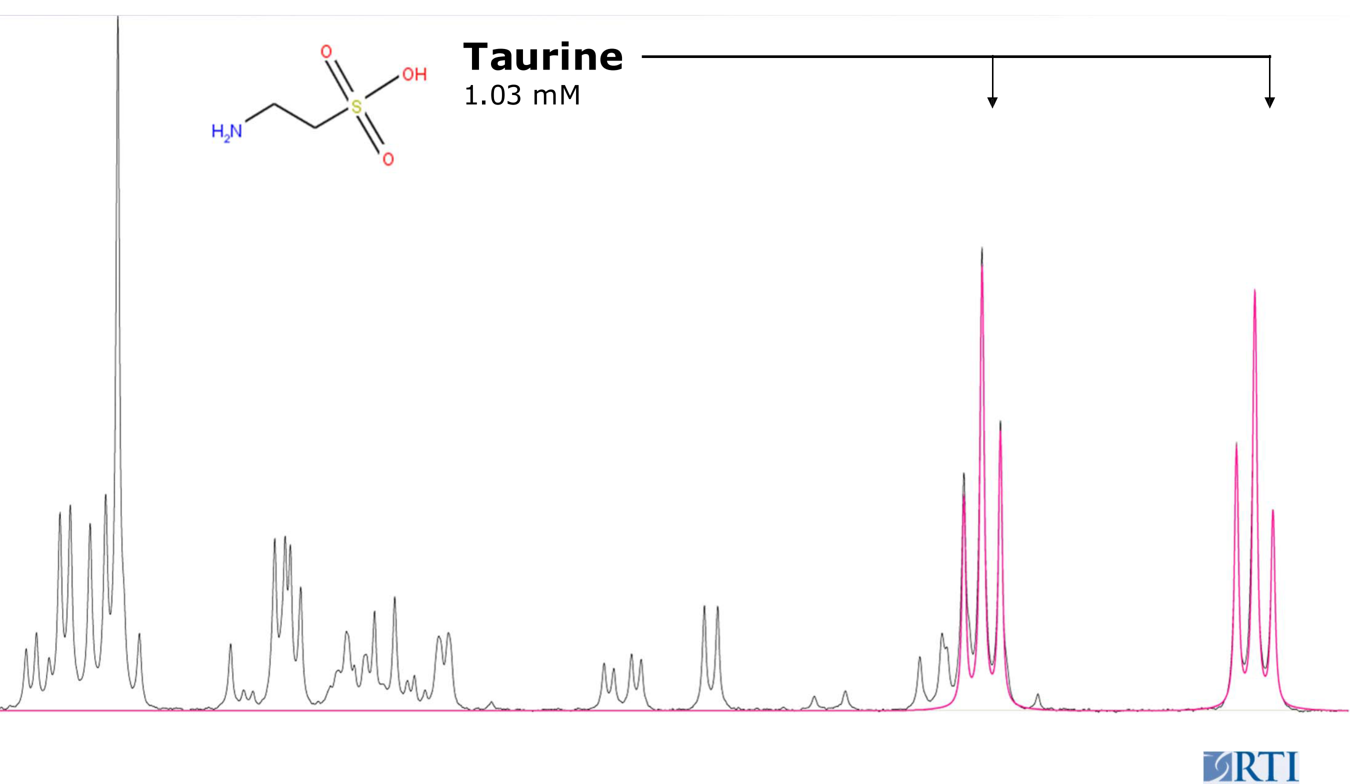
1,3-Dihydroxyacetone, 1,3-Dimethylurate, 1,6-Anhydro-β-D-glucose, 1,7-Dimethylxanthine, 1-Methylnicotinamide, 2'-Deoxyadenosine, 2'-Deoxyguanosine, 2'-Deoxyinosine, 2-Aminoadipate, 2-Aminobutyrate, 2-Ethylacrylate, 2-Furoate, 2-Hydroxy-3-methylvalerate, 2-Hydroxybutyrate, 2-Hydroxyglutarate, 2-Hydroxyisobutyrate, 2-Hydroxyisocaproate, 2-Hydroxyisovalerate, 2-Hydroxyphenylacetate, 2-Hydroxyvalerate, 2-Methylglutarate, 2-Octenoate, 2-Oxobutyrate, 2-Oxocaproate, 2-Oxoglutarate, 2-Oxoisocaproate, 2-Oxovalerate, 2-Phosphoglycerate, 3,4-Dihydroxymandelate, 3,5-Dibromotyrosine, 3-Aminoisobutyrate, 3-Chlorotyrosine, 3-Hydroxy-3-methylglutarate, 3-Hydroxybutyrate, 3-Hydroxyisovalerate, 3-Hydroxymandelate, 3-Hydroxyphenylacetate, 3-Indoxylsulfate, 3-Methyl-2-oxovalerate, 3-Methyladipate, 3-Methylxanthine, 3-Phenyllactate, 3-Phenylpropionate, 4-Aminobutyrate, 4-Aminohippurate, 4-Hydroxy-3-methoxymandelate, 4-Hydroxybenzoate, 4-Hydroxybutyrate, 4-Hydroxyphenylacetate, 4-Hydroxyphenyllactate, 4-Pyridoxate, 5,6-Dihydrothymine, 5,6-Dihydrouracil, 5-Aminolevulinate, 5-Hydroxyindole-3-acetate, 5-Hydroxylysine, 5-Methoxysalicylate, Acetaldehyde, Acetamide, Acetaminophen, Acetate, Acetoacetate, Acetone, Acetylsalicylate, Adenine, Adenosine, Adipate, Alanine, Allantoin, Alloisoleucine, Anserine, Arginine, Argininosuccinate, Asparagine, Aspartate, Benzoate, Betaine, Biotin, Butanone, Butyrate, Caffeine, Caprate, Caprylate, Carnitine, Carnosine, Choline, Cinnamate, Citrate, Citrulline, Creatine, Creatinine, Cysteine, Cystine, Cytidine, Cytosine, DSS (Chemical Shift Indicator), Dimethylamine, Epicatechin, Ethanol, Ethanolamine, Ethylene glycol, Ethylmalonate, Ferulate, Formate, Fructose, Fucose, Fumarate, Galactarate, Galactitol, Galactonate, Galactose, Gentisate, Glucarate, Glucose, Glutamate, Glutamine, Glutarate, Glutaric acid monomethyl ester, Glutathione, Glycerate, Glycerol, Glycine, Glycolate, Glycylproline, Guanidoacetate, Guanine, Hippurate, Histidine, Homocitrulline, Homocystine, Homogentisate, Homoserine, Homovanillate, Hypoxanthine, Ibuprofen, Imidazole, Indole-3-acetate, Inosine, Isobutyrate, Isocaproate, Isocitrate, Isoleucine, Isopropanol, Isovalerate, Kynurenate, Kynurenine, Lactate, Lactose, Leucine, Levulinate, Lysine, Malate, Maleate, Malonate, Mannitol, Mannose, Methanol, Methionine, Methylamine, Methylguanidine, Methylmalonate, Methylsuccinate, N,N-Dimethylformamide, N,N-Dimethylglycine, N-Acetylaspartate, N-Acetylglutamate, N-Acetylglycine, N-Carbamoyl-β-alanine, N-Carbamoylaspartate, N-Isovaleroylglycine, NAD+, Niacinamide, Nicotinate, O-Acetylcarnitine, O-Phosphocholine, O-Phosphoethanolamine, O-Phosphoserine, Ornithine, Oxalacetate, Oxypurinol, Pantothenate, Phenol, Phenylacetate, Phenylacetylglycine, Phenylalanine, Pimelate, Proline, Propionate, Propylene glycol, Protocatechuate, Pyridoxine, Pyroglutamate, Pyruvate, Quinolinate, Riboflavin, Ribose, S-Adenosylhomocysteine, S-Sulfocysteine, Salicylate, Salicylurate, Sarcosine, Serine, Suberate, Succinate, Succinylacetone, Sucrose, Tartrate, Taurine, Theophylline, Threonate, Threonine, Thymine, Thymol, Tiglylglycine, Trigonelline, Trimethylamine, Trimethylamine N-oxide, Tryptophan, Tyramine, Tyrosine, Uracil, Urea, Uridine, Urocanate, Valerate, Valine, Valproate, Vanillate, Xanthine, Xanthosine, Xylose, cis-Aconitate, myo-Inositol, o-Cresol, p-Cresol, trans-4-Hydroxy-L-proline, trans-Aconitate, β-Alanine, π-Methylhistidine, τ-Methylhistidine

RTI INTERNATIONAL

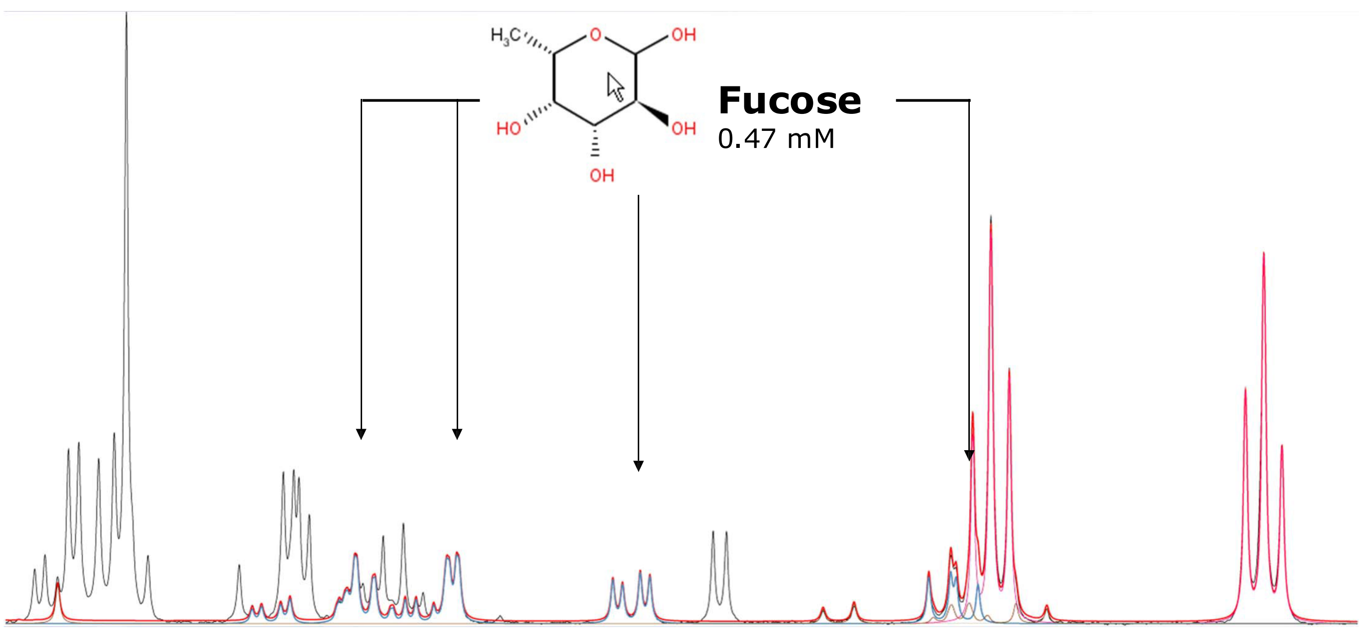
Fitting of metabolites



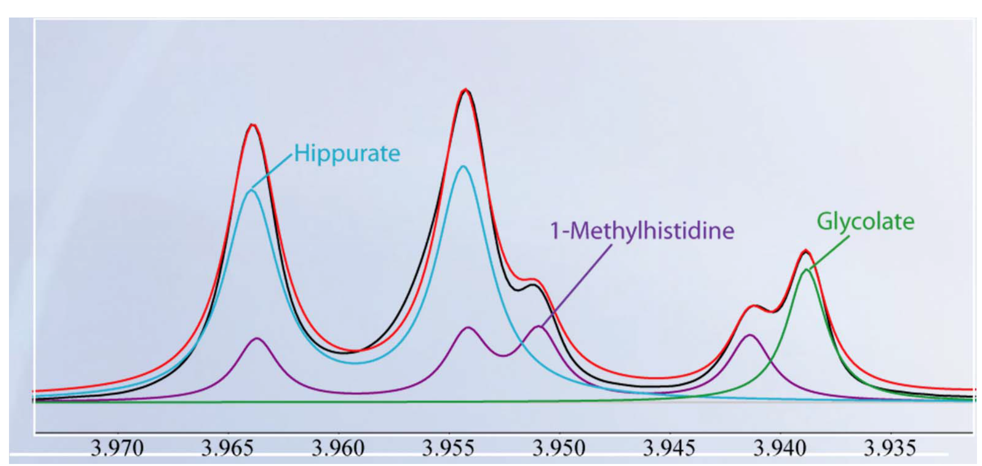
Fitting taurine



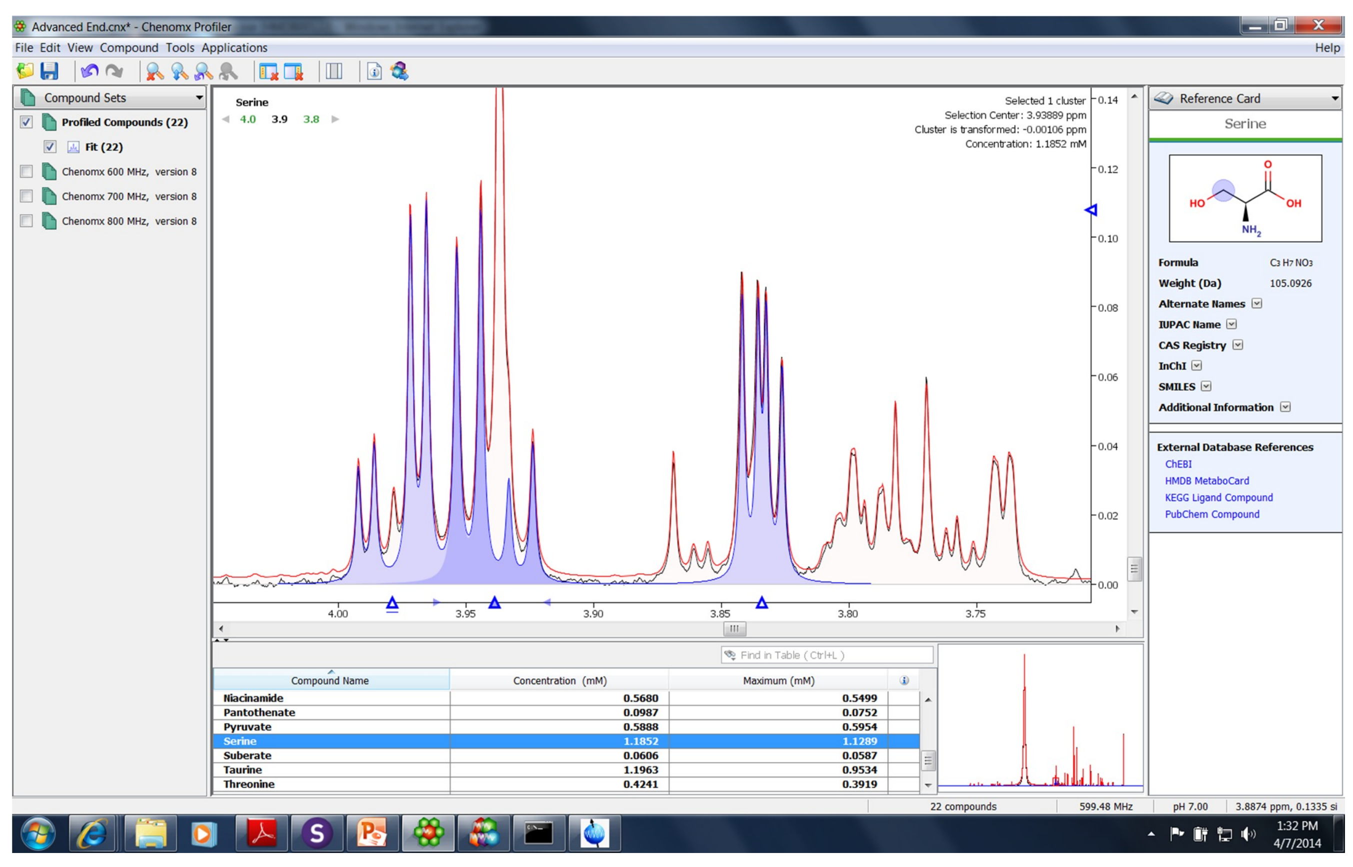
Fitting fucose



Chenomx Helps Resolving Ambiguity in Highly Overlapped Regions



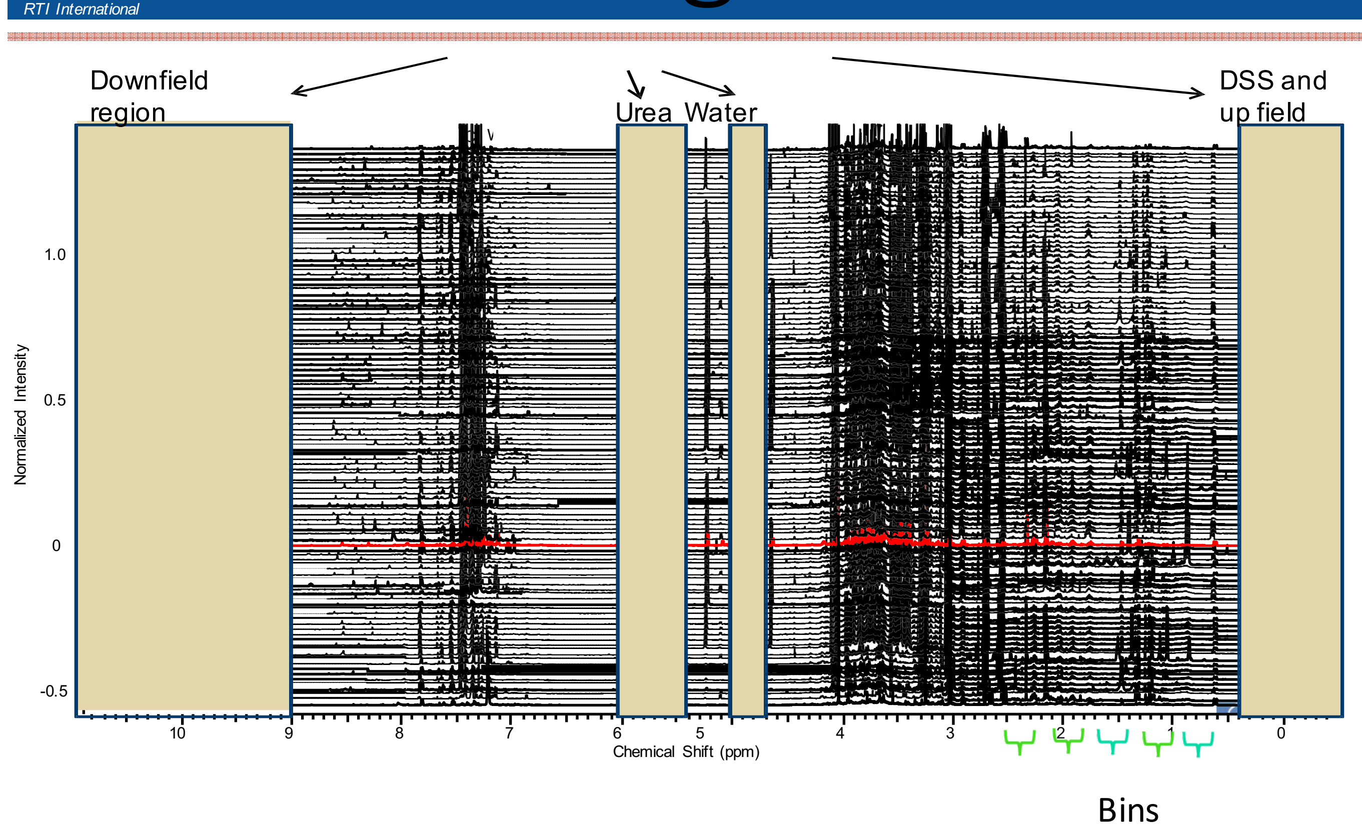
Additive fit



Broad Spectrum (or untargeted) Metabolomics

(and NMR Binning)

Remove regions



Wimal Pathmasiri & Rodney Snyder

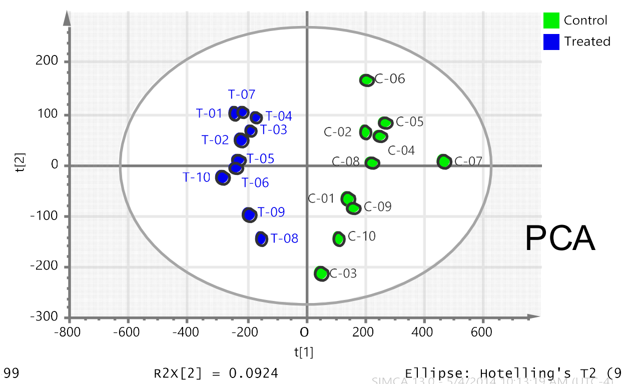
- Integrate bins (0.04 ppm bin size)
- Normalize bins to the total integral of each spectrum
- Merge metadata
- Result is a spreadsheet ready for further multivariate data analysis and other statistical analysis

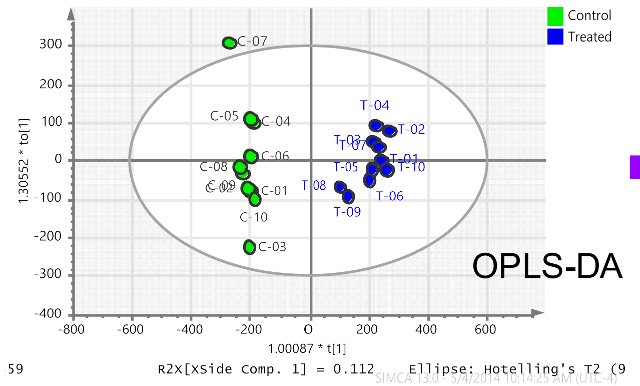
Sample ID	Disease Group	[0.40 .. 0.46]	[0.46 .. 0.52]	[0.52 .. 0.54]	[0.54 .. 0.57]	[0.57 .. 0.60]	[0.60 .. 0.66]	[0.66 .. 0.68]	[0.68 .. 0.71]	[0.71 .. 0.75]
C0559	Cases	7.60E-05	0.00E+00	7.32E-02	8.48E-02	3.20E-02	1.84E+00	1.31E-01	3.60E-01	3.67E-01
C0629	Cases	0.00E+00	1.78E-02	0.00E+00	2.18E-02	0.00E+00	1.08E+01	0.00E+00	0.00E+00	3.02E-02
C0640	Cases	3.44E-04	0.00E+00	1.83E-03	1.86E-04	0.00E+00	4.51E+00	0.00E+00	0.00E+00	0.00E+00
C0835	Cases	6.41E-04	0.00E+00	6.44E-03	0.00E+00	3.96E-03	3.28E+00	0.00E+00	5.12E-03	1.75E-02
D0613	Cases	6.63E-03	0.00E+00	0.00E+00	1.06E-02	0.00E+00	5.79E+00	0.00E+00	6.36E-02	3.02E-01
D0762	Cases	0.00E+00	0.00E+00	1.79E-02	1.98E-02	0.00E+00	9.37E+00	0.00E+00	0.00E+00	1.74E-02
D1113	Cases	3.14E-03	2.42E-03	8.02E-02	1.04E-01	5.32E-03	3.74E+00	0.00E+00	2.02E-02	1.84E-01
D1158	Cases	0.00E+00	3.71E-03	2.35E-02	4.83E-02	0.00E+00	5.02E+00	0.00E+00	1.91E-02	0.00E+00
D2090	Cases	0.00E+00	0.00E+00	2.45E-03	9.98E-04	0.00E+00	5.76E+00	0.00E+00	1.24E-02	1.04E-02
E0004	Cases	1.72E-03	0.00E+00	6.85E-02	3.05E-02	0.00E+00	1.47E+00	6.90E-02	3.61E-01	4.08E-01
E0195	Cases	0.00E+00	1.69E-03	5.57E-02	6.29E-02	0.00E+00	2.77E+00	1.34E-01	2.04E-01	4.56E-01
E0225	Cases	1.25E-03	0.00E+00	4.40E-03	1.69E-02	0.00E+00	9.17E+00	0.00E+00	1.08E-02	2.30E-02
E0309	Cases	4.11E-03	0.00E+00	2.23E-02	7.54E-03	3.08E-03	3.54E+00	0.00E+00	3.28E-02	9.09E-01
E0487	Cases	1.72E-03	0.00E+00	0.00E+00	1.00E-02	0.00E+00	4.00E+00	0.00E+00	1.36E-02	0.00E+00
F0036	Cases	1.66E-02	0.00E+00	0.00E+00	2.06E-02	0.00E+00	1.22E+01	1.04E-02	0.00E+00	5.97E-01
F0108	Cases	0.00E+00	2.31E-03	6.30E-03	1.11E-02	0.00E+00	7.17E+00	0.00E+00	1.65E-02	2.21E-01
A0233	Control	0.00E+00	1.86E-02	0.00E+00	1.82E-02	0.00E+00	1.61E+01	0.00E+00	2.91E-03	0.00E+00
A0490	Control	0.00E+00	0.00E+00	2.99E-03	3.60E-02	0.00E+00	2.97E+00	0.00E+00	4.00E-02	5.46E-01
A2003	Control	0.00E+00	0.00E+00	3.45E-02	2.20E-02	0.00E+00	1.80E+00	0.00E+00	0.00E+00	0.00E+00
C0586	Control	0.00E+00	1.69E-02	0.00E+00	6.64E-03	0.00E+00	1.92E+01	0.00E+00	6.51E-02	0.00E+00
C2177	Control	0.00E+00	0.00E+00	3.02E-02	3.59E-02	0.00E+00	2.35E+00	0.00E+00	3.19E-02	1.49E-01
D0177	Control	9.21E-03	0.00E+00	1.69E-02	1.47E-02	0.00E+00	2.43E+00	0.00E+00	4.46E-02	0.00E+00
D0729	Control	0.00E+00	1.88E-03	5.58E-02	7.87E-02	2.92E-02	3.16E+00	6.59E-02	2.80E-01	4.30E-01
D0909	Control	0.00E+00	1.08E-03	0.00E+00	5.69E-03	0.00E+00	2.49E+00	0.00E+00	1.01E-02	1.87E-01
D0945	Control	0.00E+00	4.79E-04	7.00E-03	0.00E+00	4.19E-03	3.99E+00	0.00E+00	1.11E-03	3.96E-02
D1174	Control	0.00E+00	9.33E-04	0.00E+00	3.43E-03	1.30E-02	7.21E+00	6.53E-03	0.00E+00	1.66E-02
D2054	Control	1.55E-03	0.00E+00	0.00E+00	1.22E-02	0.00E+00	2.07E+00	0.00E+00	1.28E-02	3.90E-01
D2062	Control	2.39E-05	0.00E+00	6.04E-02	2.99E-02	0.00E+00	4.94E+00	0.00E+00	9.95E-03	0.00E+00
D2079	Control	2.73E-02	0.00E+00	1.81E-03	1.17E-02	0.00E+00	3.38E+01	7.87E-02	0.00E+00	5.91E+00

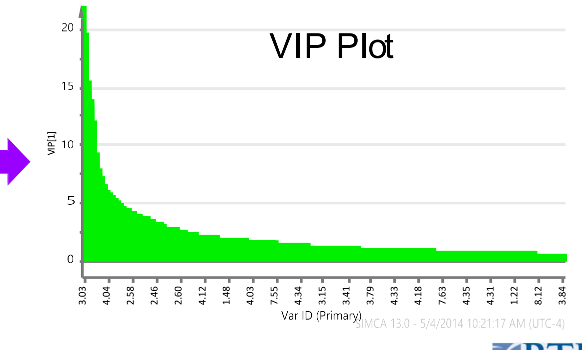
Multivariate Data Analysis & Other Statistical Analysis

Multivariate data analysis and other statistical analyses

- Mean centered and scaled data
- Non-supervised analysis
  - Principal component analysis (PCA)
- Supervised analysis
  - PLS-DA and OPLS-DA
- Loadings plots and VIP Plots to identify discriminatory bins
- p-Value, fold change







Some Software available for NMR Based Metabolomics

COMMERCIAL

- NMR Data-preprocessing
  - ACD Software (ACD Labs, Toronto, Canada)
  - Chenomx
- Multivariate data analysis
  - SIMCA 13
- Other statistical analysis
  - SAS, SPSS
- Library matching and quantification
  - Chenomx
- Pathway analysis
  - GeneGo (MetaCore Module)
  - Ingenuity Pathway Analysis (IPA)

AV III HD 600 AV III HD 850 AV III HD 500

AV III HD 850

Central Alabama
High-Field NMR Facility
(CHEM Bldg, Rm.153)

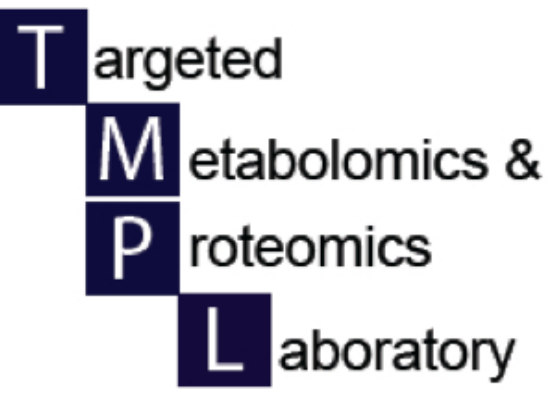
Contact: **Dr. Ronald Shin**
Ext: 4-5696
E-mail: shinr@uab.edu

AV II 700

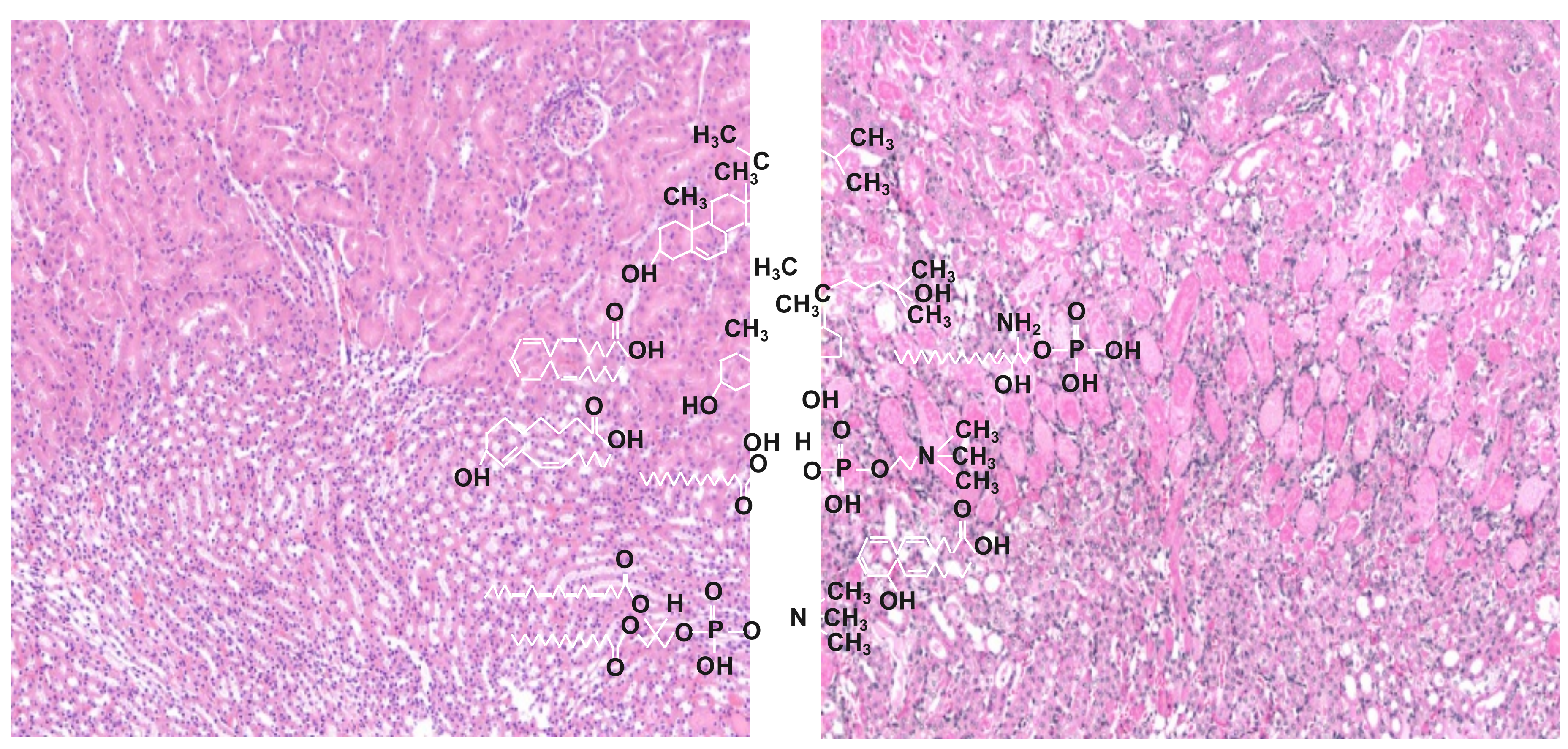
Graduate –Omics course

Imaging metabolomics

Janusz H. Kabarowski, PhD



MALDI-IMS for Spatial Analysis of Metabolites in acute kidney injury



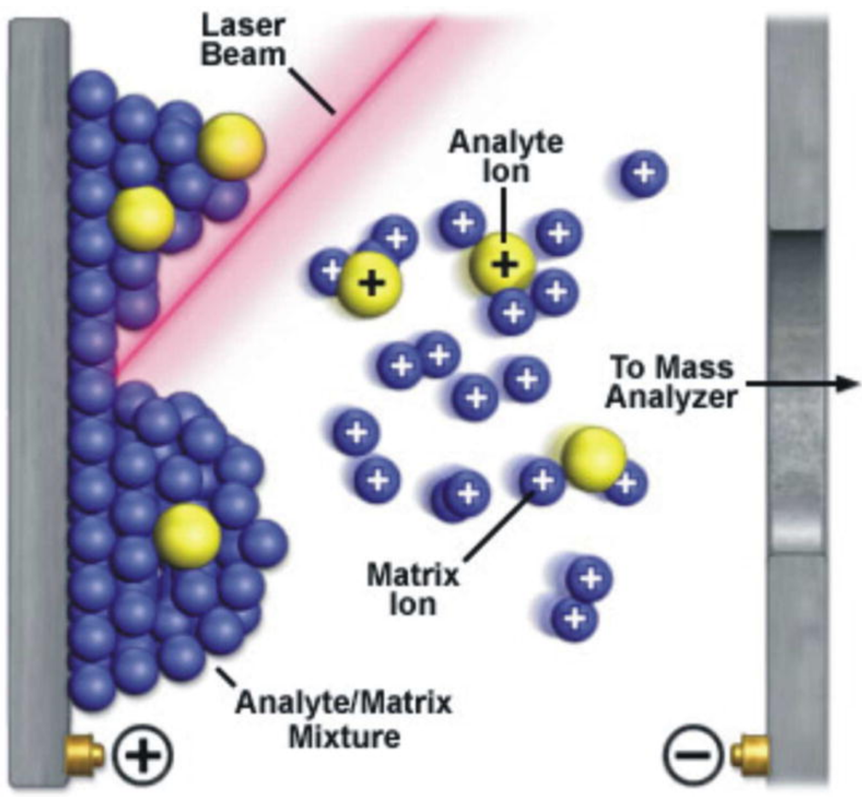
Janusz Kabarowski, Dept. Microbiology, UAB.

Matrix-Assisted Laser Desorption/Ionization (MALDI):

Matrix molecules absorb laser light, enter an excited state, and collide with sample molecules, facilitating charge transfer to create ions.

Conventional MALDI plate

MALDI-TOF instrument



Mass Spectrometric Imaging for biomedical tissue analysis
Kamila Chughtai and Ron M.A. Heeren
Chem Rev. Vol.110(5): pp3237–3277, 2010.

Vacuum sublimation

Used to apply an even microscopically thin uniform layer of matrix compound onto tissue section without the need for solvents

Sublimation: *the transition of a substance from solid to gas phase without an intermediate liquid phase*.

MALDI matrices for lipid imaging:

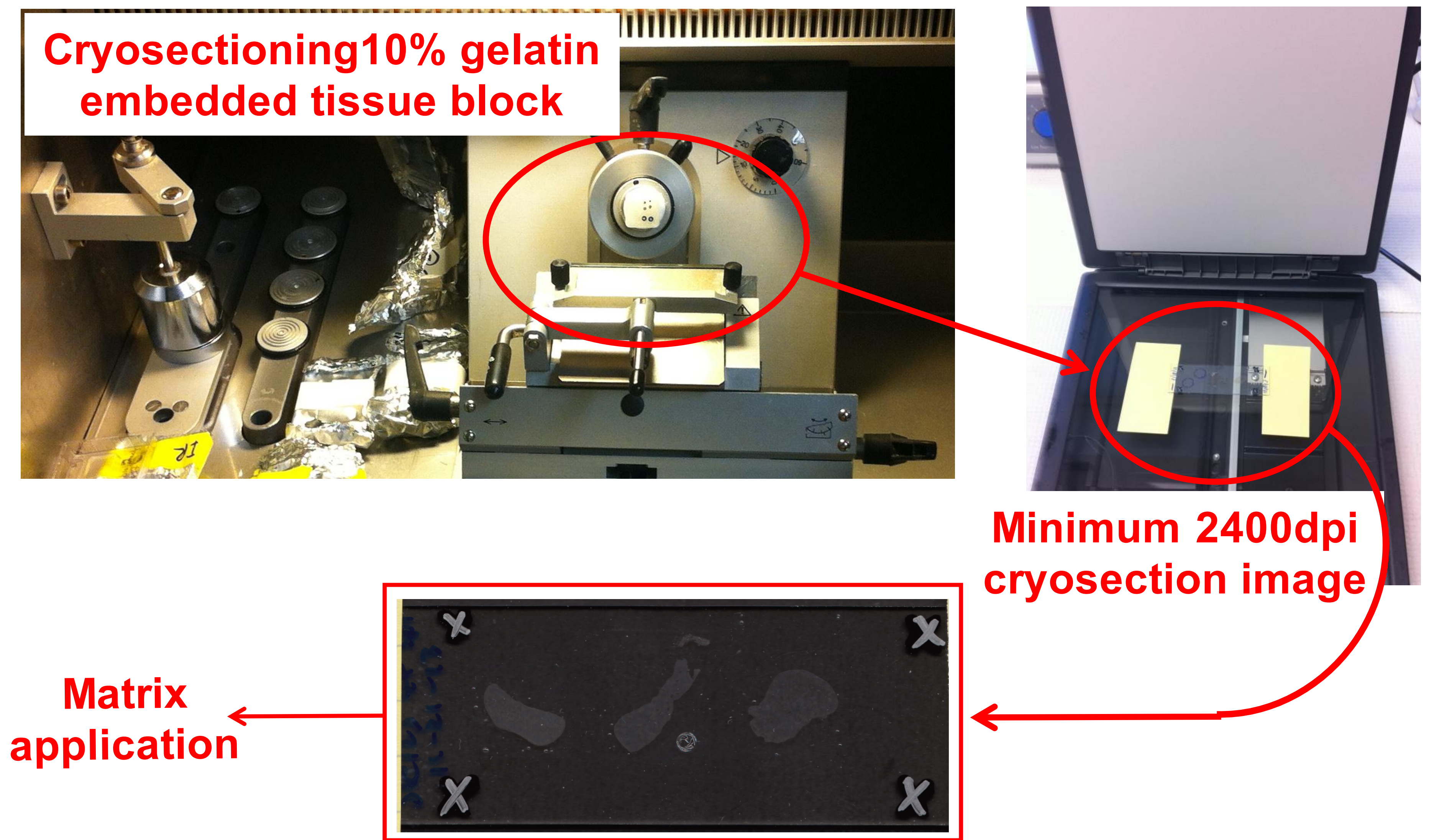
DHB: 2,5-dihydrobenzoic acid (+ve mode)

HO
O
OH
OH

1,5-diaminonapthalene (-ve mode)

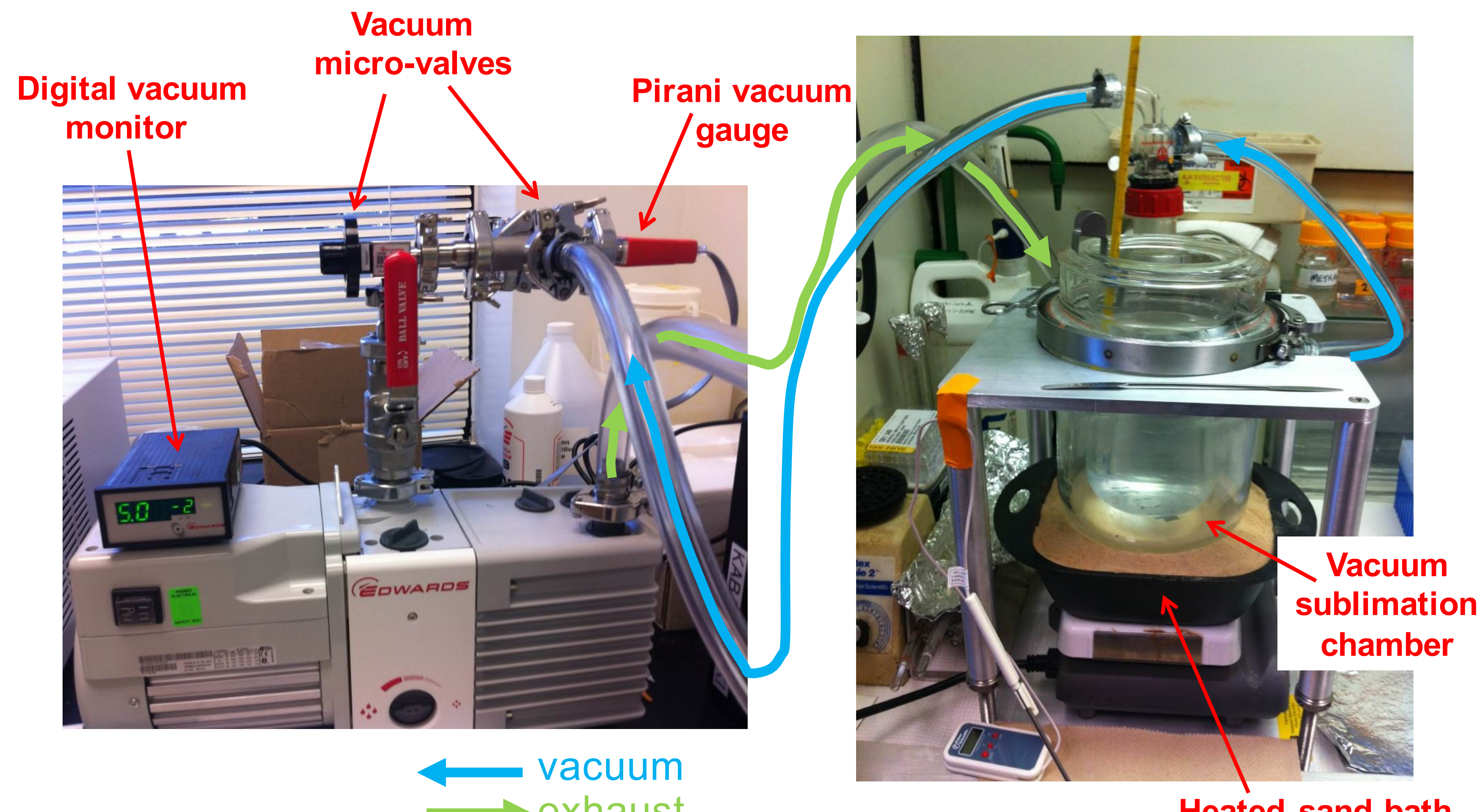
NH_2 NH_2

Cryosectioning onto Indium Tin Oxide (ITO) coated glass slides and scanning digital image of slide for "teaching" FlexControl software on MALDI-TOF.

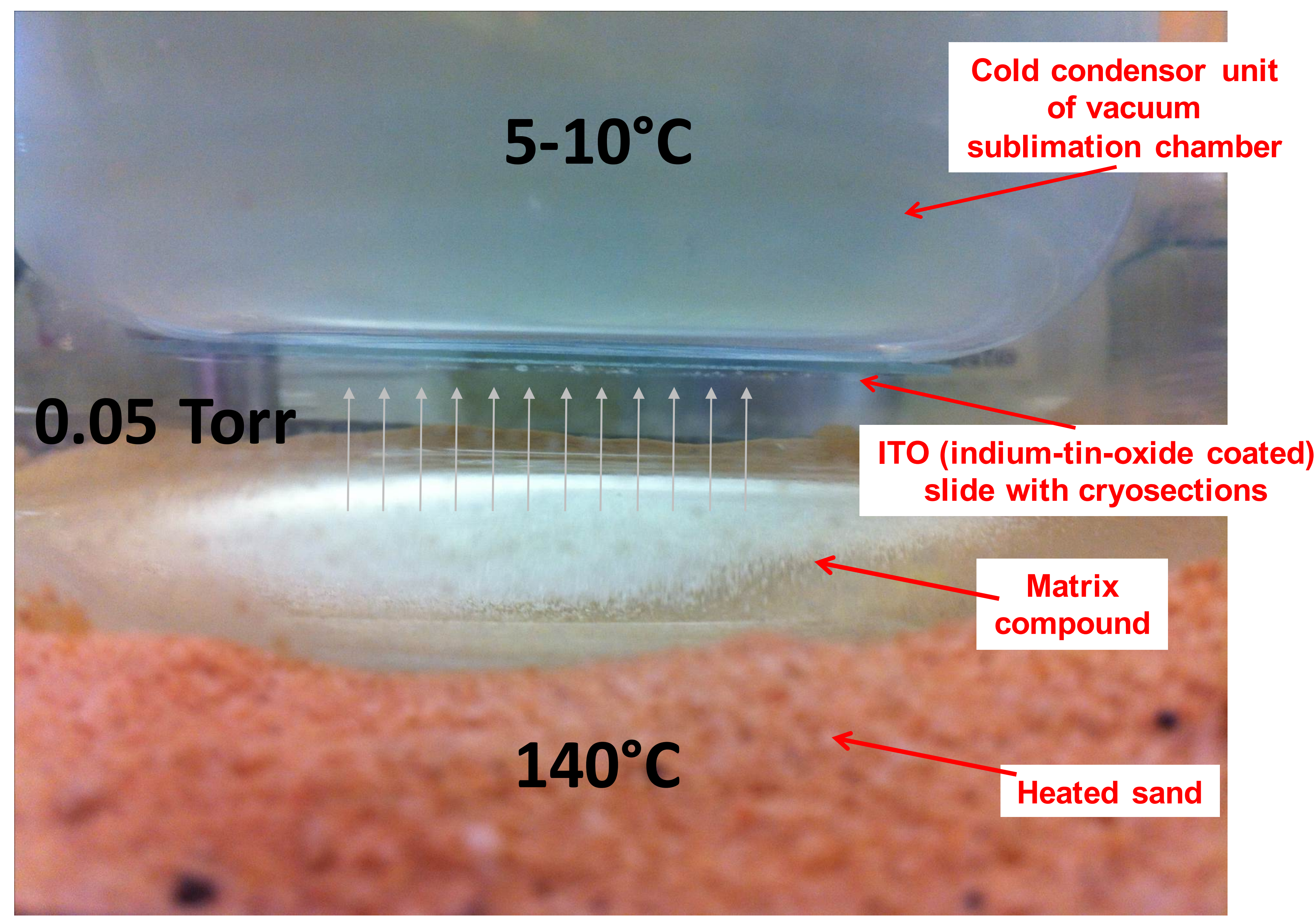


How do we apply matrix for MALDI Imaging?

We built a vacuum sublimation apparatus.



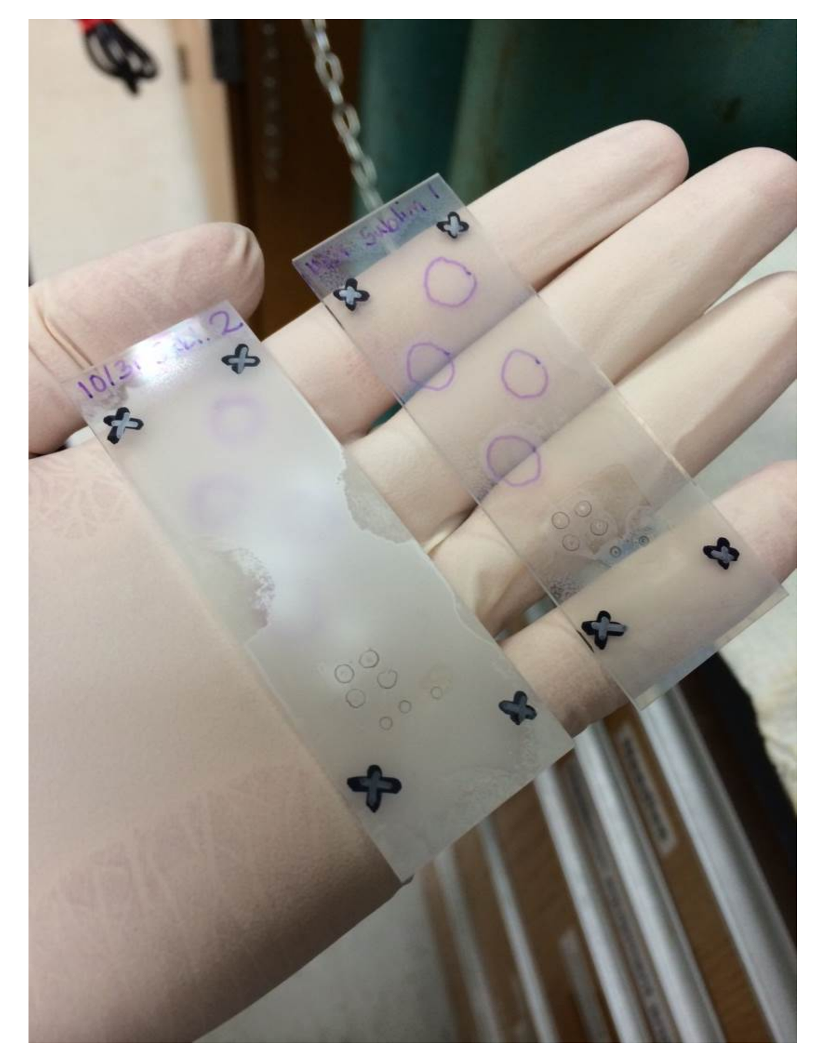
Matrix deposition by vacuum sublimation



Slides with matrix applied by vacuum sublimation

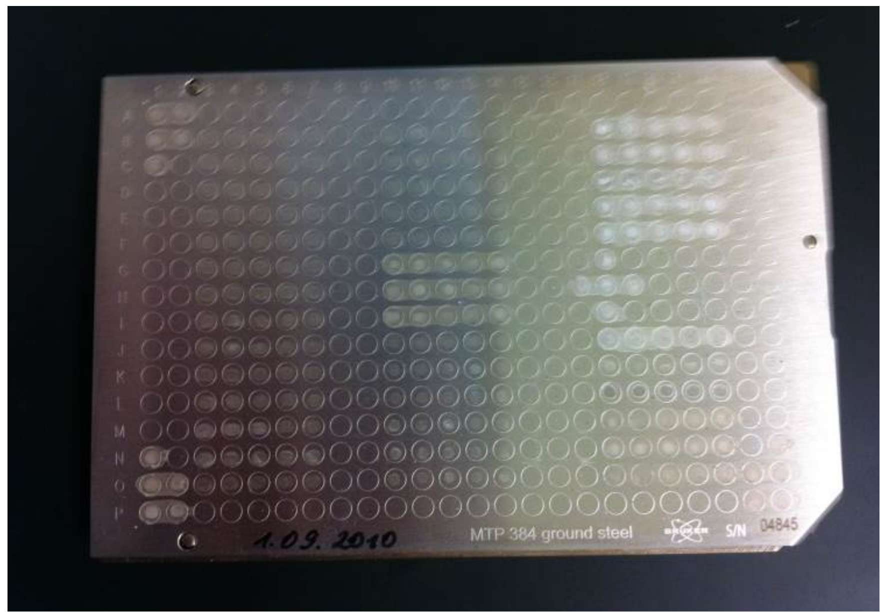
Deposition of the matrix compound is at the molecular level because gaseous molecules recrystallize at the relatively cold surface of the tissue section attached to the cold condenser.

The uniformity of matrix deposition onto the slide attached to the cold condenser surface reflects the random Brownian motion of the released gaseous matrix molecules.

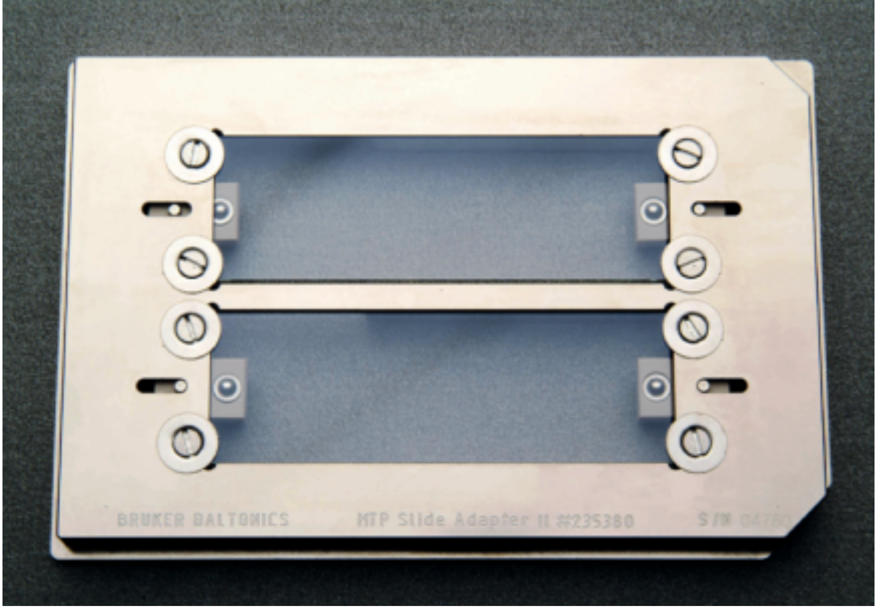


Adapted MALDI plate holds slides for MALDI-imaging MS

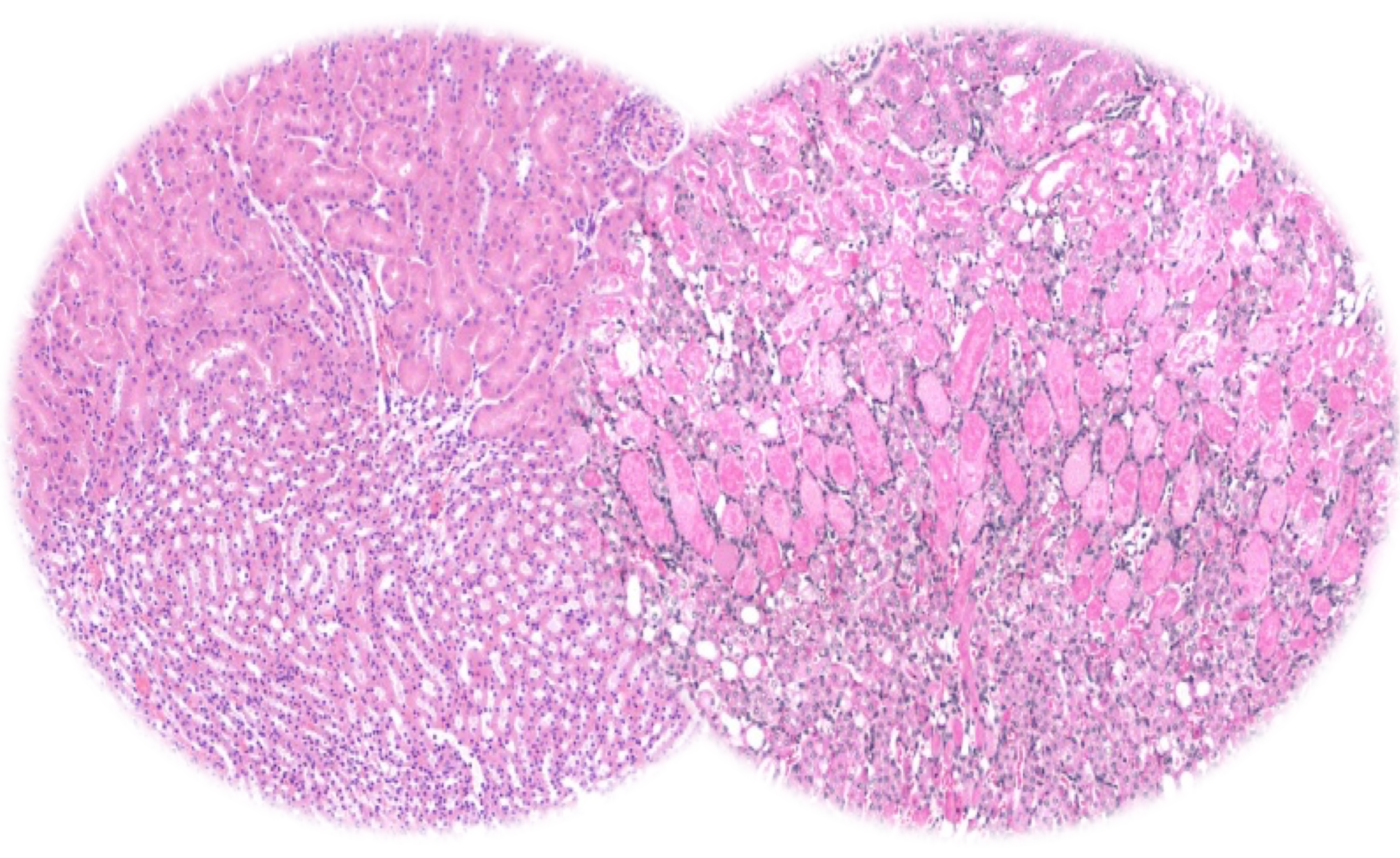
Conventional MALDI plate



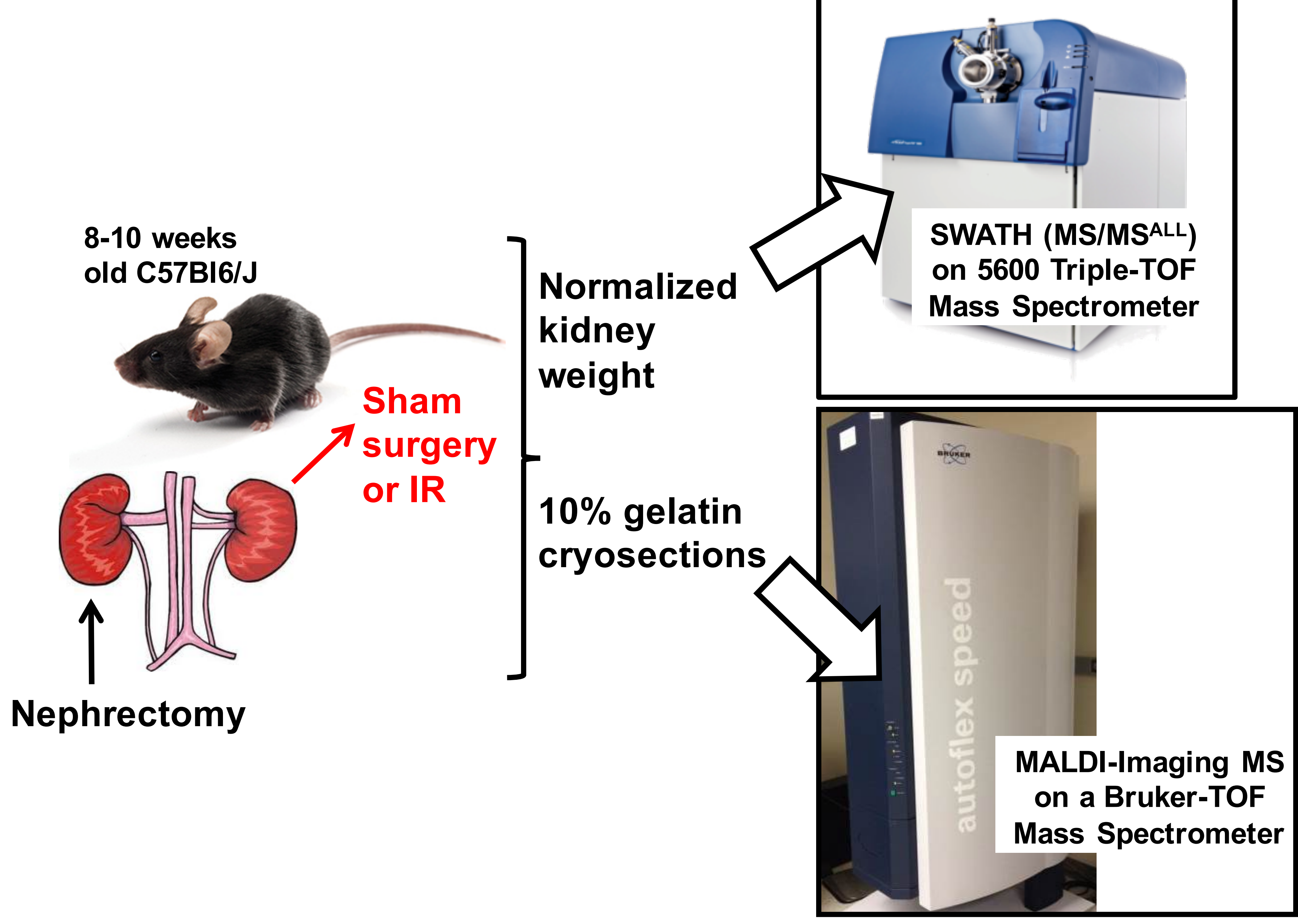
MALDI plate for cryosections

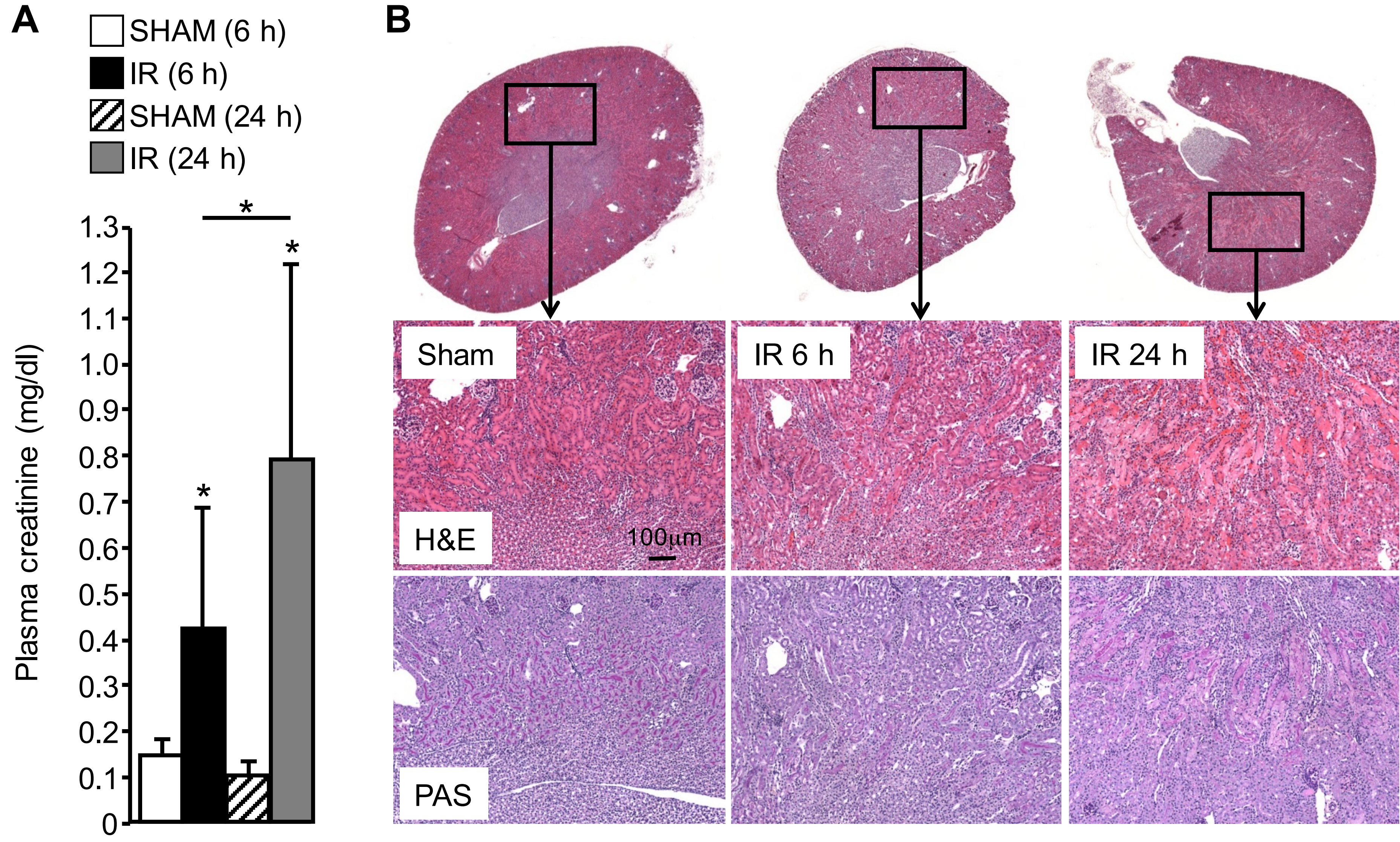


Quantitative and Spatial Analysis of Lipids Involved in Acute Kidney Injury

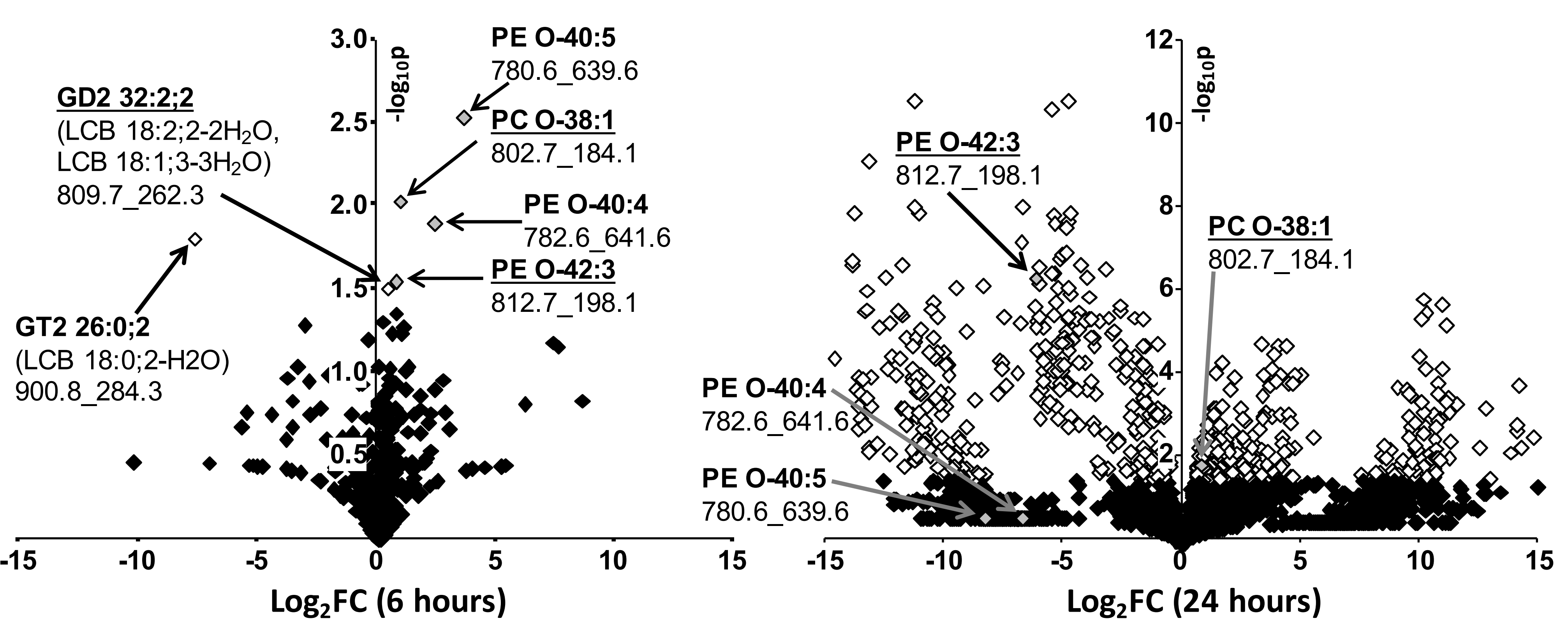


BRUKER
autoflex speed

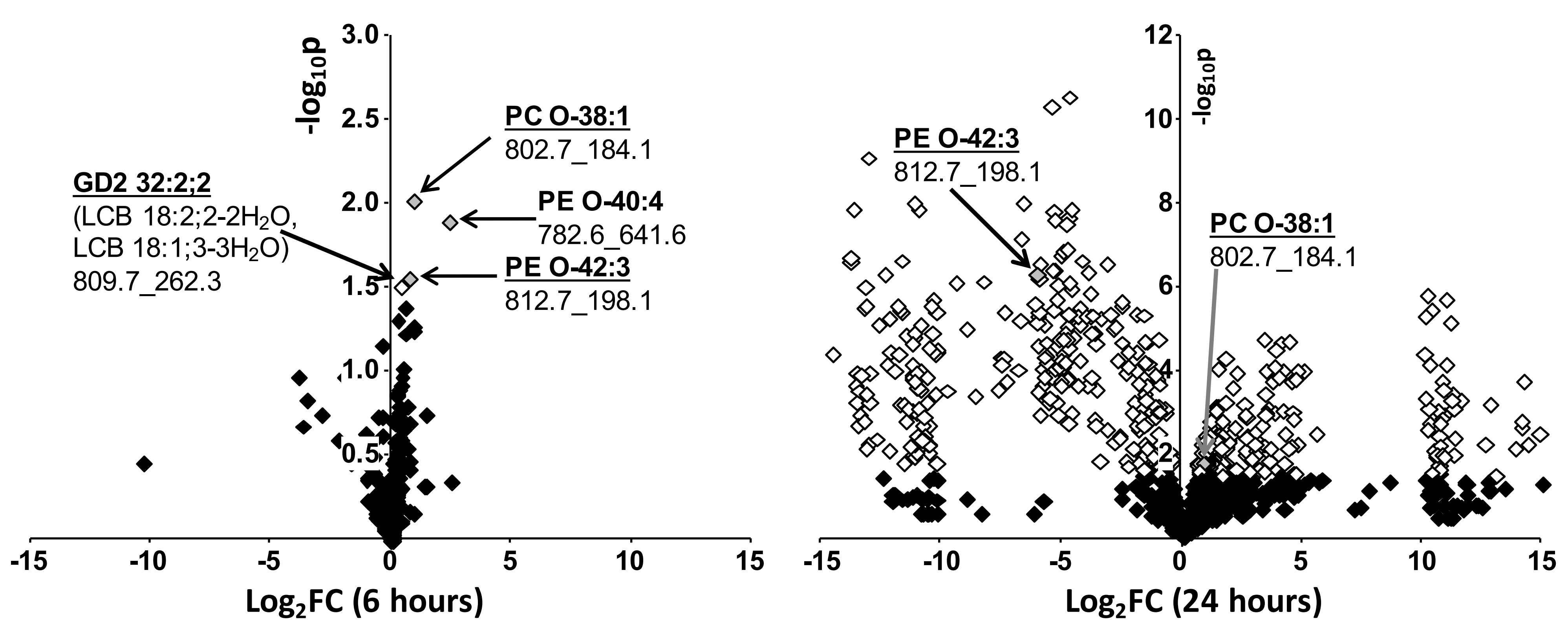

8-10 weeks
old C57Bl6/J
Sham
surgery
or IR
Nephrectomy
Normalized
kidney
weight
10% gelatin
cryosections
SWATH (MS/MS^ALL)
on 5600 Triple-TOF
Mass Spectrometer
autoflex speed
MALDI-Imaging MS
on a Bruker-TOF
Mass Spectrometer
SWATH (Sequential Window Acquisition of all Theoretical Mass Spectra


A
SHAM (6 h)
IR (6 h)
SHAM (24 h)
IR (24 h)
Plasma creatinine (mg/dl)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
*
*
*
B
Sham
IR 6 h
IR 24 h
H&E
100μm
PAS

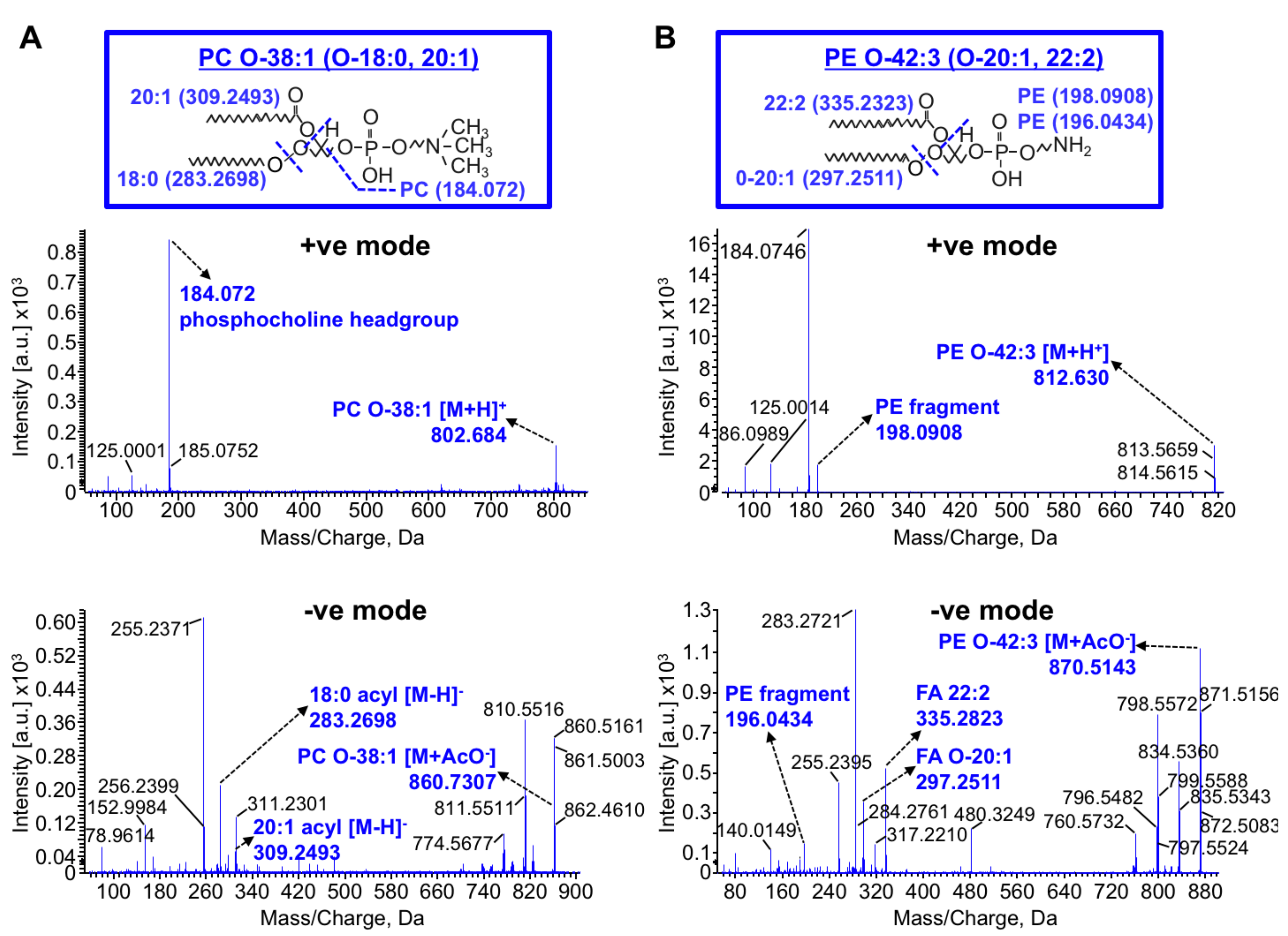
SWATH-MS on renal lipids following ischemia/reperfusion (IR)-induced kidney injury



SWATH-MS on renal lipids following ischemia/reperfusion (IR)-induced kidney injury

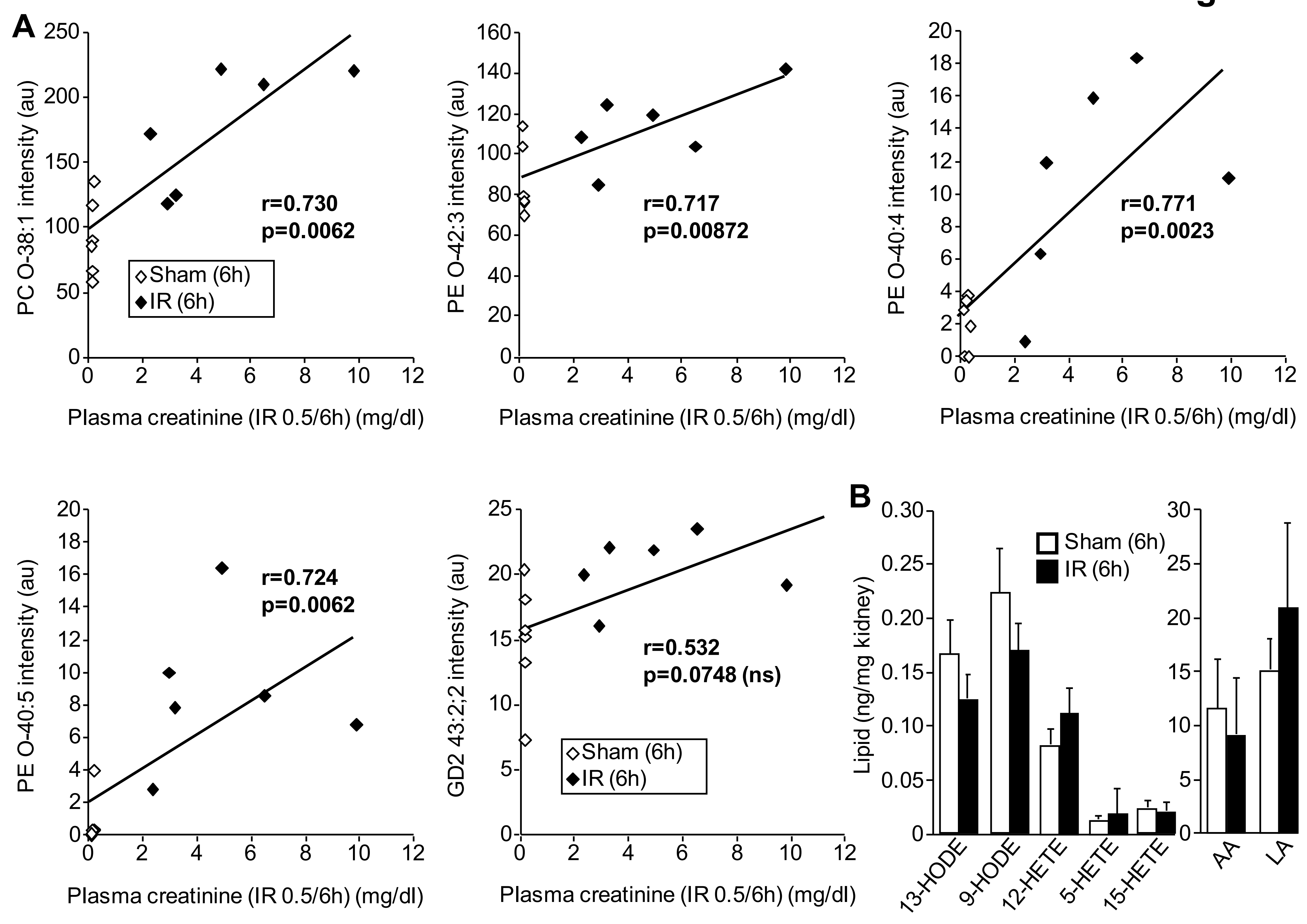


Intensity >10

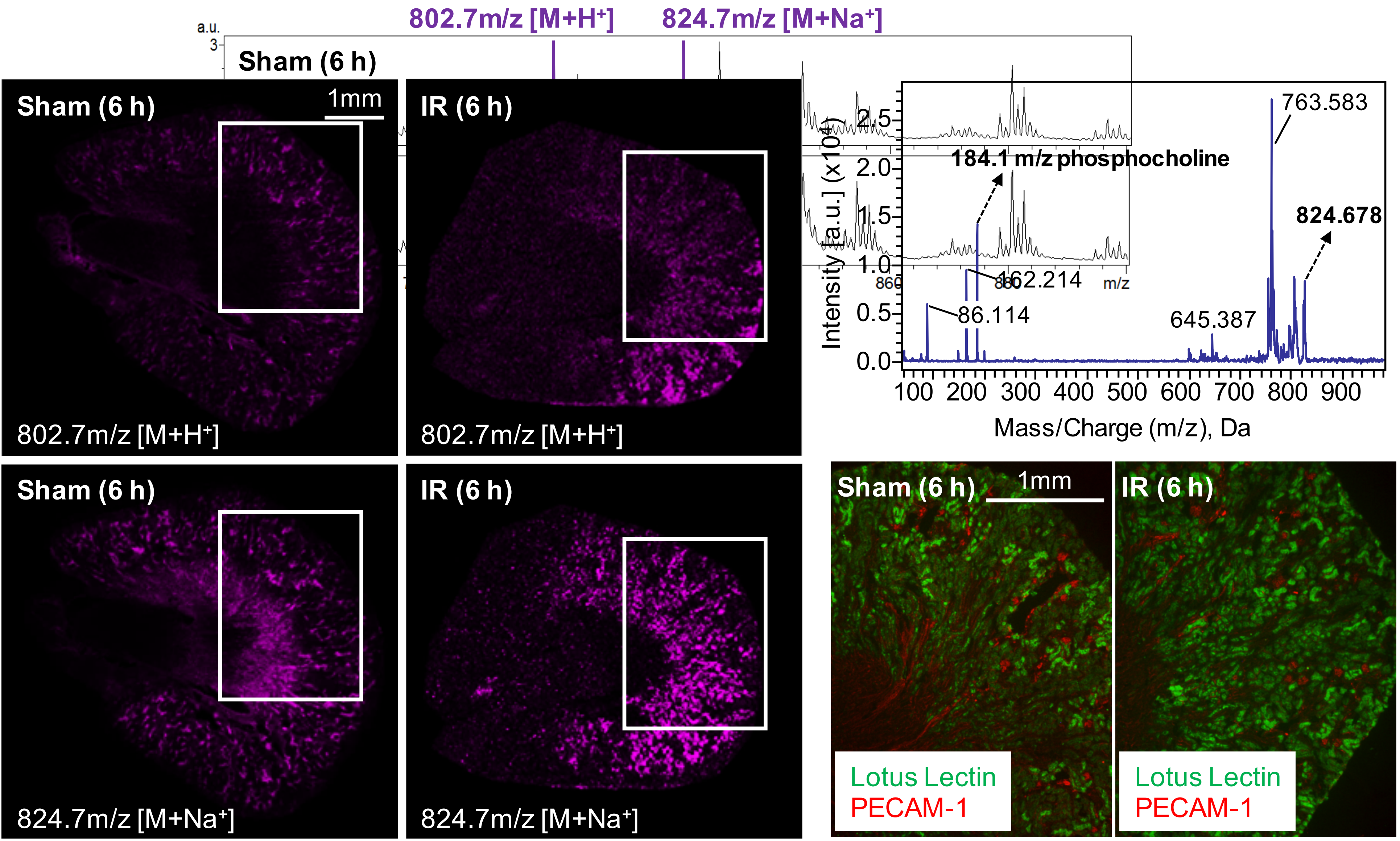


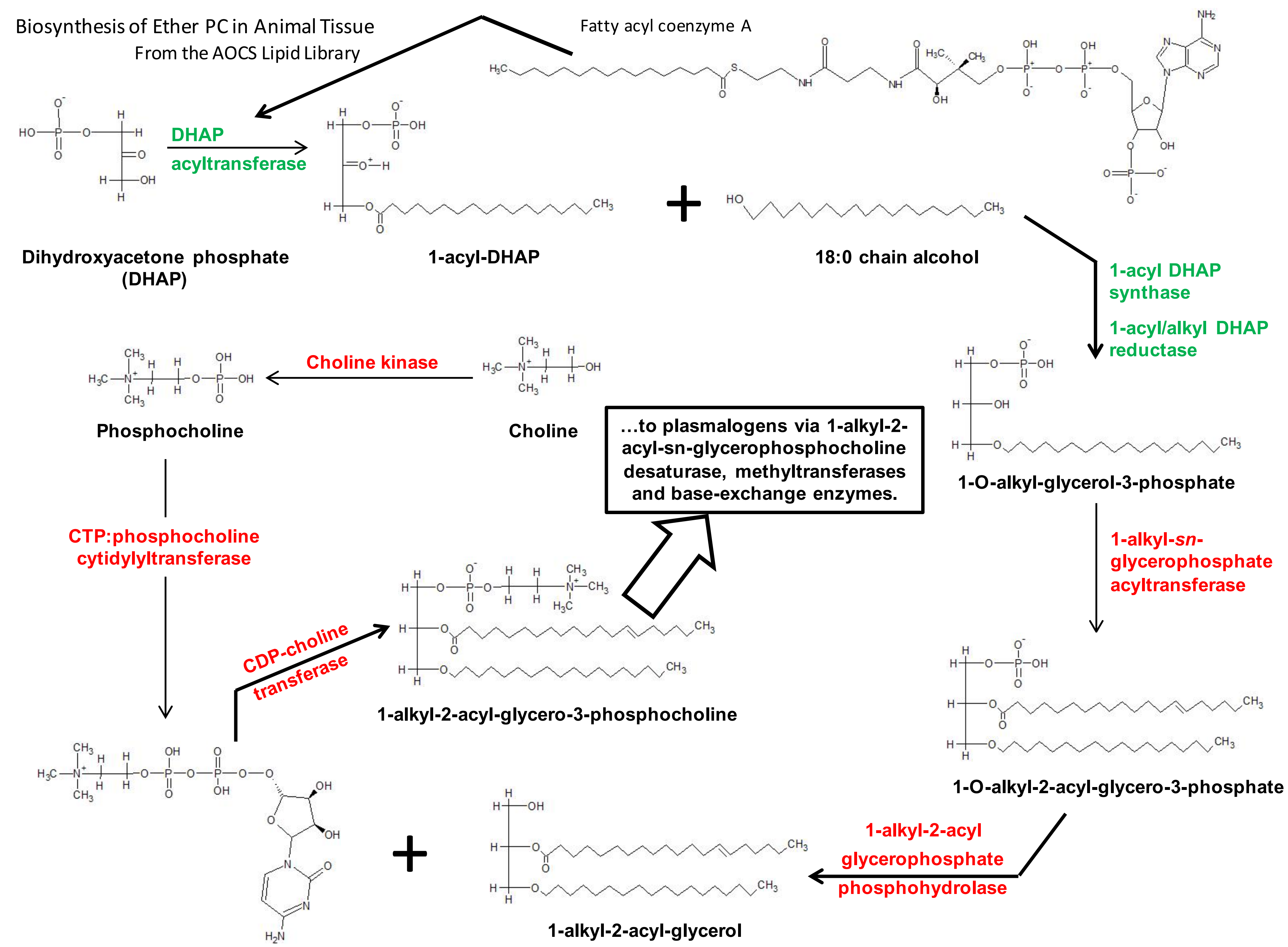
A
PC O-38:1 (O-18:0, 20:1)
20:1 (309.2493)
18:0 (283.2698)
PC (184.072)
+ve mode
184.072
phosphocholine headgroup
PC O-38:1 [M+H]+
802.684
125.0001
185.0752
Intensity [a.u.] x10³
Mass/Charge, Da
-ve mode
255.2371
18:0 acyl [M-H]-
283.2698
PC O-38:1 [M+AcO-]
860.7307
20:1 acyl [M-H]-
309.2493
256.2399
152.9984
78.9614
311.2301
810.5516
860.5161
861.5003
811.5511
862.4610
774.5677
B
PE O-42:3 (O-20:1, 22:2)
22:2 (335.2323)
PE (198.0908)
PE (196.0434)
0-20:1 (297.2511)
184.0746
PE O-42:3 [M+H+]
812.630
125.0014
86.0989
PE fragment
198.0908
813.5659
814.5615
283.2721
PE O-42:3 [M+AcO-]
870.5143
PE fragment
196.0434
FA 22:2
335.2823
FA O-20:1
297.2511
255.2395
284.2761
317.2210
480.3249
140.0149
798.5572
871.5156
834.5360
799.5588
796.5482
835.5343
760.5732
872.5083
797.5524

Figure 4



PC O-18:0/20:1 imaging in sham and IR kidneys





Biosynthesis of Ether PC in Animal Tissue
From the AOCS Lipid Library
Fatty acyl coenzyme A
DHAP
acyltransferase
Dihydroxyacetone phosphate (DHAP)
1-acyl-DHAP
18:0 chain alcohol
1-acyl DHAP synthase
1-acyl/alkyl DHAP reductase
Choline kinase
Phosphocholine
Choline
…to plasmalogens via 1-alkyl-2-acyl-sn-glycerophosphocholine desaturase, methyltransferases and base-exchange enzymes.
1-O-alkyl-glycerol-3-phosphate
CTP:phosphocholine cytidylyltransferase
1-alkyl-sn-glycerophosphate acyltransferase
CDP-choline transferase
1-alkyl-2-acyl-glycero-3-phosphocholine
1-O-alkyl-2-acyl-glycero-3-phosphate
1-alkyl-2-acyl glycerophosphate phosphohydrolase
1-alkyl-2-acyl-glycerol
Rate-limiting peroxisomal enzymes most abundant in kidney proximal tubules.

Summary

- **MALDI-TOF imaging mass spectrometry is a very powerful technique to determine the distribution of small molecules within a tissue**
  - **In this study it was applied to lipid species in the damaged kidney**
  - **It has the potential for application to many other tissues such as brain, heart, liver, lung and the visual system**

Acknowledgments