Pre-Processing of Metabolomics Data (NMR)
Wednesday, July 24, 2013 (9:15-9:55 am)

SOP for Sample Preparation:
- Urine Samples
- Blood Samples
- Tissue and Cell Extractions

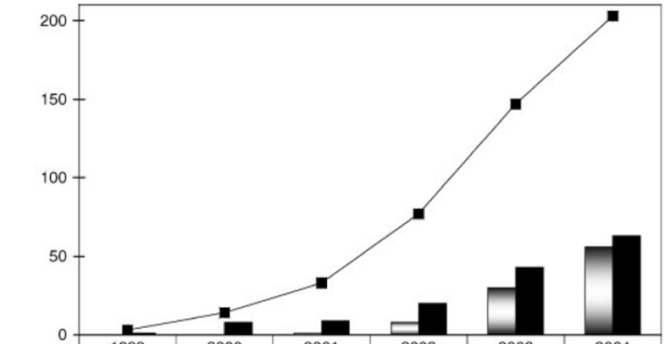
What Will I Need to Run NMR:
- Deuterated Solvents
- External Standards
- NMR Tubes and Rotors

^{1}H-NMR Acquisitions:
- Water Suppression
- Large Molecule Suppression
- Acquisition Parameters

^{31}P/ ^{13}C-Multinuclear NMR:
- High Energy Phosphates
- ^{13}C-Metabolic Fluxes

Two-Dimensional (2D)-NMR:
- COSY, HSQC
- Metabolic Chemical Shift Libraries

Bibliographic Search in PubMed and Chemical Abstracts Plus



	1999	2000	2001	2002	2003	2004
MS	0	0	1	8	30	56
NMR	1	8	9	20	43	63
total	3	14	33	77	147	203

Dettmer, Aronov, Hammock, 2006

Step 1: Sample Collection

- If you have a new study, the recommended study design is

Cell cultures → Animal Tissues → Patient

- All specimens for metabolomics need to be snap frozen!
- Diet, exercise, age, sex, animal strain – artifacts for metabolomics analysis

Step 2: Sample Analysis

NMR-based analysis:

- robust,
- fast,
- minimal sample preparation,
- non-destructive,
- large data base;
- low sensitivity (umol),
- low metabolite resolution,
- only small molecules

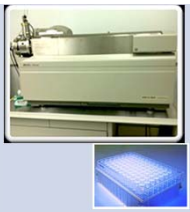
NMR-Analysis

•1H-NMR: global profile of watersoluble and lipid metabolites (amino acids, neurotransmitters, osmolytes, carbohydrates, lipids, lactate – total up to 35 quantitative end-points)

•31P-NMR: (tissues and cells only): high energy phosphates, phospholipiods and their precursors, sugar phosphates (total 10-12 quantitative endpoints)

•13C-NMR: requires addition (incubation) of a 13C-precursor (glucose and fatty acid metabolism, metabolic fluxes, gluocse uptake, lactate production)

^{1}H-NMR of Biological Samples

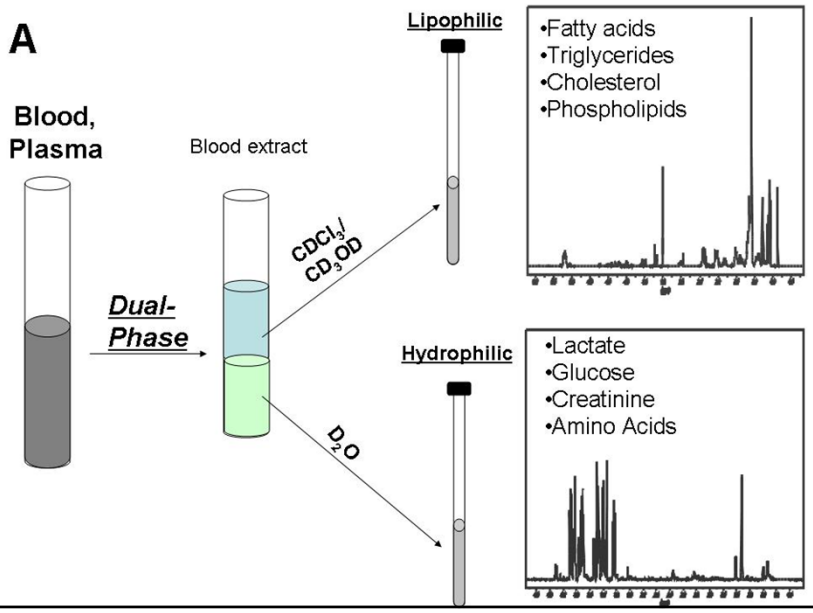
	NMR (with cryo probe)	GC-MS	DI-MS
Techniques			
Metabolites	Water-soluble (amino acids, organic acids, sugars)	mainly water-soluble (some hydrophobic)	Mainly hydrophobic (some water-soluble)
Types of samples	Biofluids, plant, bacterial, animal tissue extracts, Food	Biofluids, plant, bacterial, animal tissue extracts, Food	Mainly biofluids
Sample Volume	0.1-0.5 mL (min)	30-50 µL (min)	10 µL

^{1}H-NMR of Biological Samples

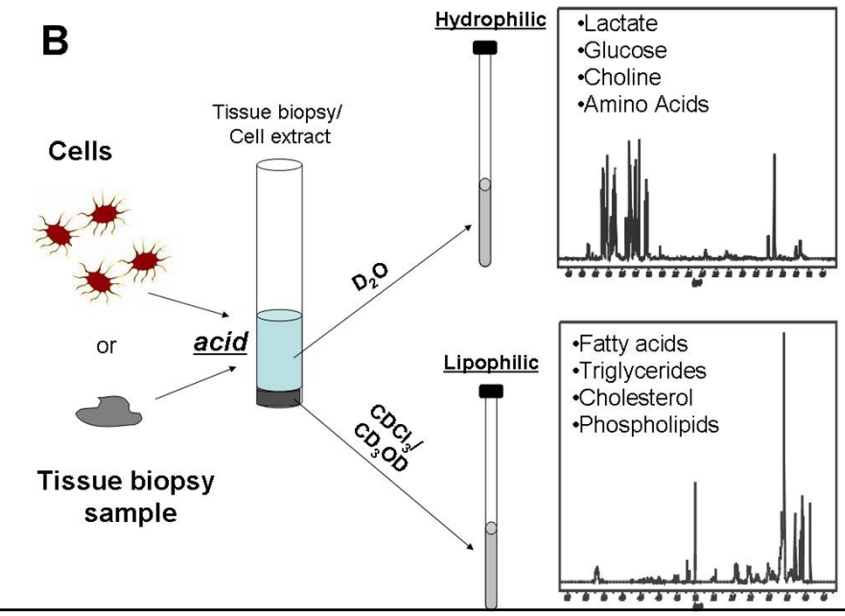
	NMR	GC-MS	DI-MS
Sample prep time	**30 -120 min/20 samples**	**30 -120 min/20 samples**	**3-4 h for 96 samples**
Run time	**20 -90 min/sample**	**30-60 min/sample**	**7 min/sample**
Data Analysis	**30-60 min / sample**	**30-60 min / sample**	**1-2 h for 96 samples**
Limit of Detection	**~ 5 µM**	**~ 100 nM**	**~ 5 nM**
No. of metabolites	**~ 20 - 50**	**~20 -50**	**~ 100-180**
Overlapping Metabolites	**10-15**	**10-15**	**10-15**
Cross-checking	**10-30 %**	**10-30 %**	**10-30 %**

Biofluid	***Required Sample Handling***	***Disease Application***
Urine	Add deuterated phosphate buffer to 0.5 – 3 mL urine	Drug Toxicity/ Efficacy Inborn Metabolic Error Renal Transplantation **Renal/ OBGYN/ GU Cancer?**
Blood **Plasma** **Serum**	For 0.5 mL of heparinzed blood product: -only deuterium oxide addition (lock) -acetonitrile addition (protein precipitation) -methanol/chlorofom extraction (lipid separation)	**Drug Treatment** **Cancer** Transplantation Obesity and Diabetes Cardiovascular Disorders
CSF	Addition of deuterium oxide to 0.5 mL CSF	Neurology Psychiatry
EPS	Add deuterium oxide to 0.03 – 0.10 mL EPS	**Prostatic Cancer**
Bile	Add deuterated methanol to 0.5 mL bile	Liver Transplantation
BALF	Add deuterium oxide to 0.5 mL BALF	Pulmonary Drug Toxicity
Tissue	-add 0.01 mL deuterium oxide to 3-10 g tissue in MAS rotor -perchloric acid extraction on 20-200 g frozen tissue -methanol/chloroform extraction on 20-200 g frozen tissue	**Cancer** **Drug Treatment** Transplantation Obesity Ischemia/ Reperfusion Others

^{1}H-NMR of Processed Blood

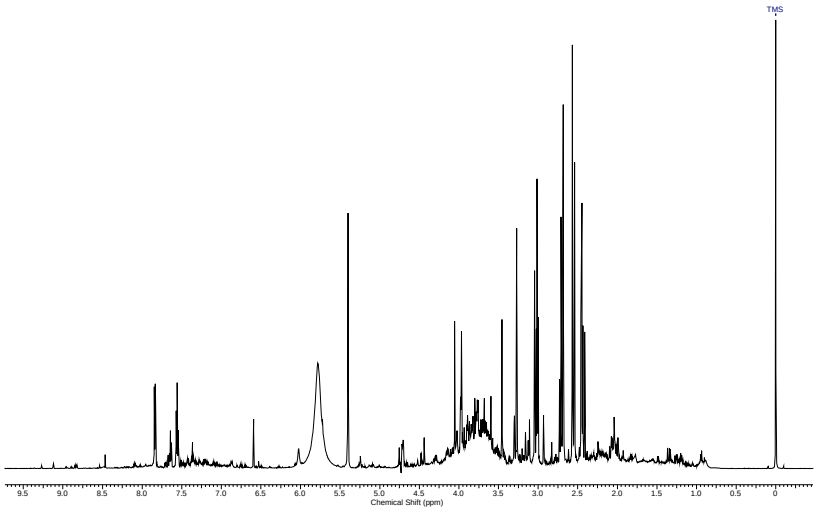


NMR of Tissues/ Cells



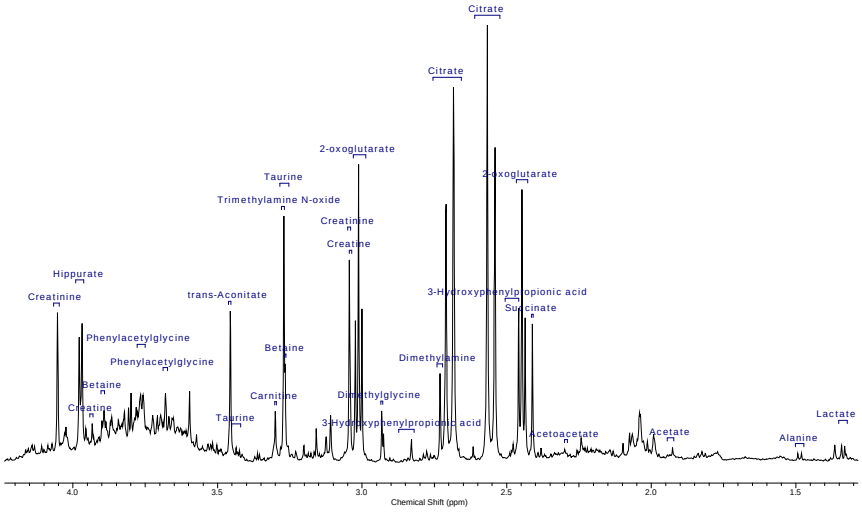
^{1}H-NMR of Body Fluids

Data from NMR Spectroscopy: A Typical Control Rat Urine

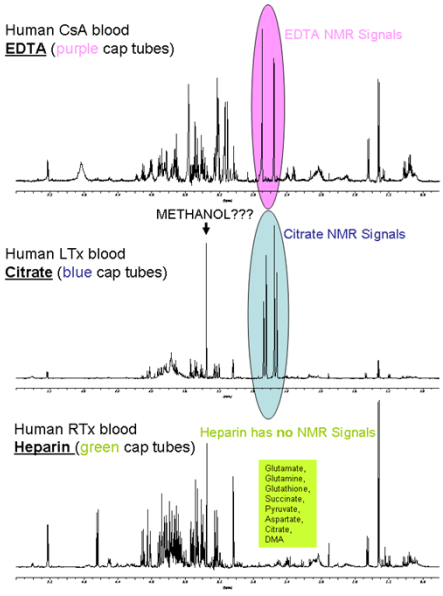


^{1}H-NMR of Body Fluids

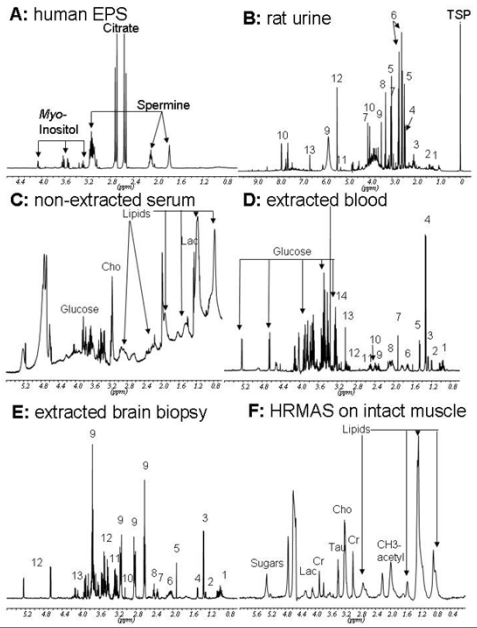
Annotated Rat Urine Spectrum



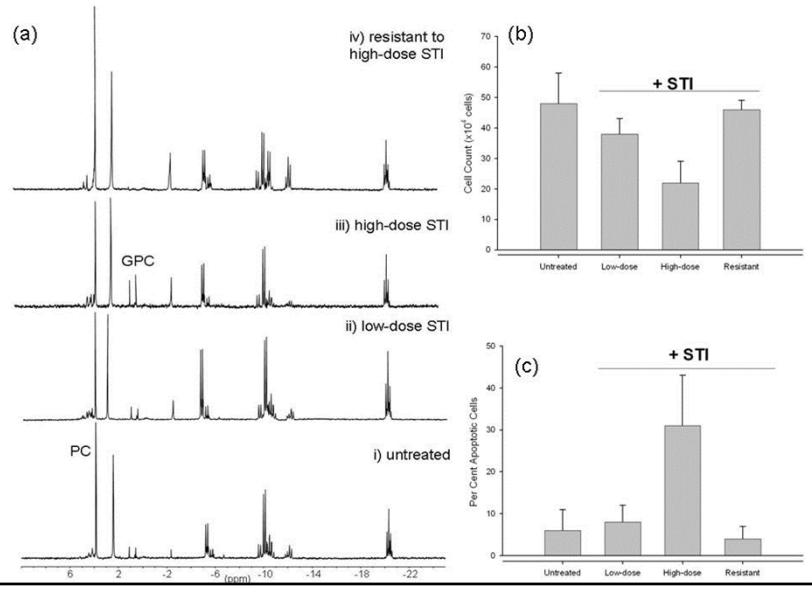
^{1}H-NMR of Body Fluids



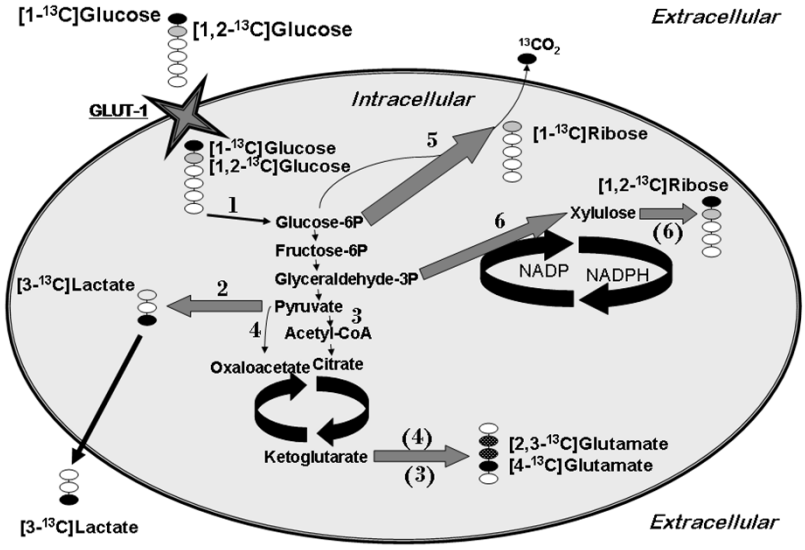
^{1}H-NMR of Body Fluids



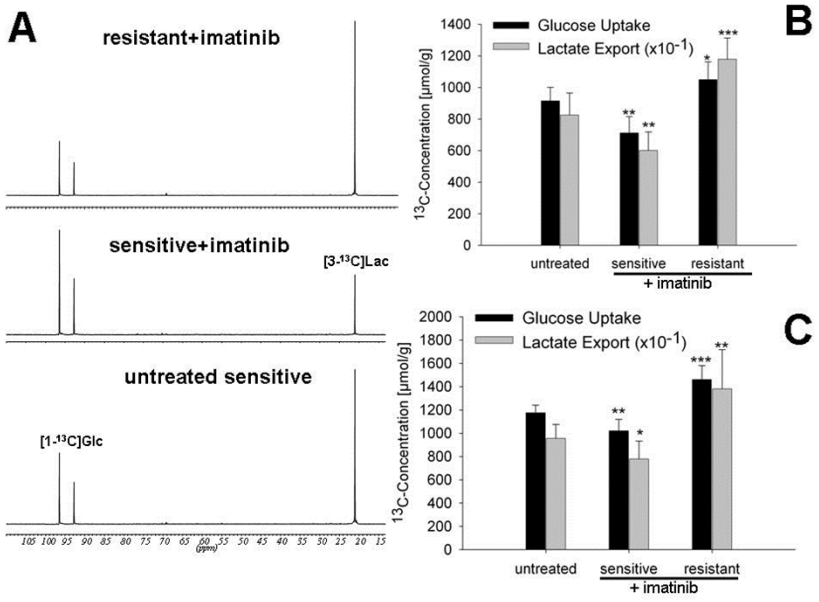
$^{1}H/^{31}P$-NMR of Tissues/ Cells



^{13}C-NMR of Metabolic Fluxes



^{13}C-NMR of Metabolic Fluxes

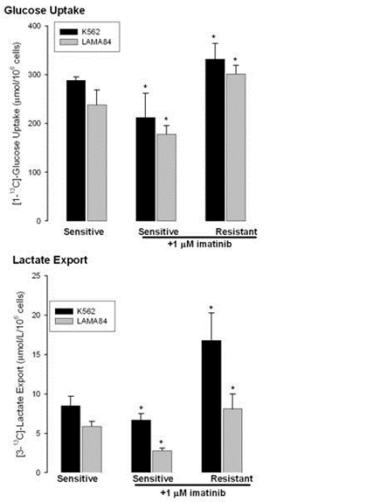


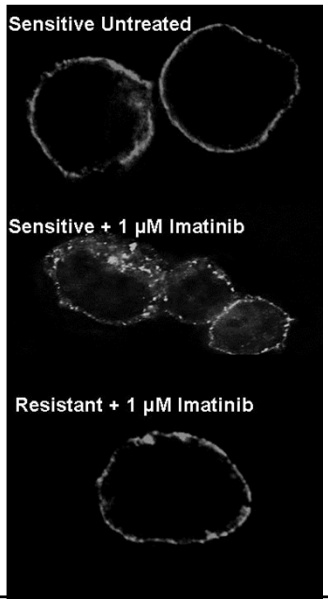
^{13}C-NMR of Metabolic Fluxes

Figure 1:

Intracellular location of GLUT-1 ➔

Glucose uptake and lactate export ↓

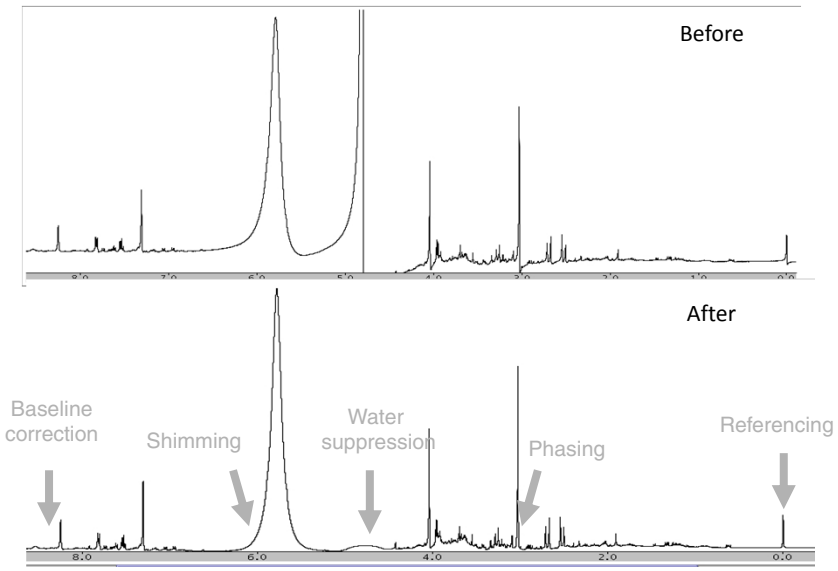




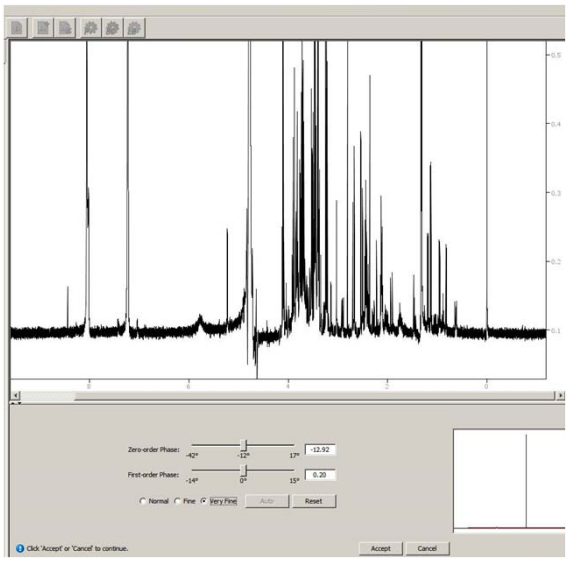
NMR Spectra Acquisition

- Chemical shift referencing (TMS, DSS)
  - Calibrates/normalizes chemical shifts
- Shimming
  - Fixes line shape to look Lorentzian
- Phasing
  - Fixes line shape to look "absorptive"
- Water suppression/removal
  - Removes large water signal
- Baseline correction
  - Makes spectrum look flat – not wobbly

NMR Spectra Acquisition

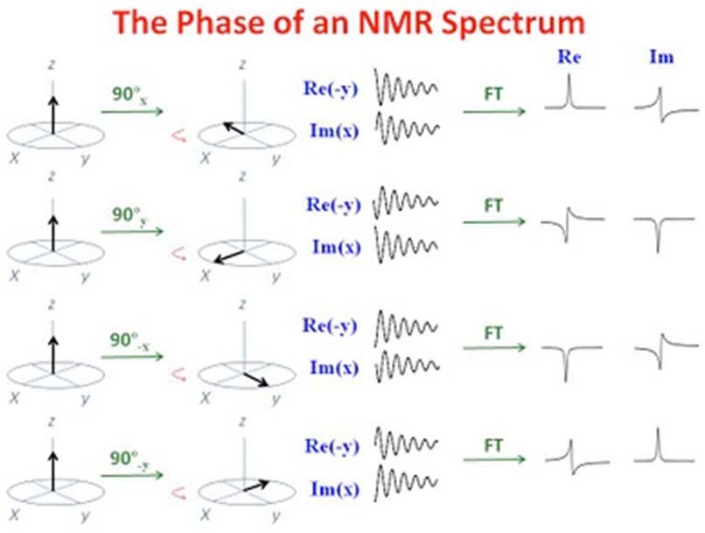


Phase Spectrum

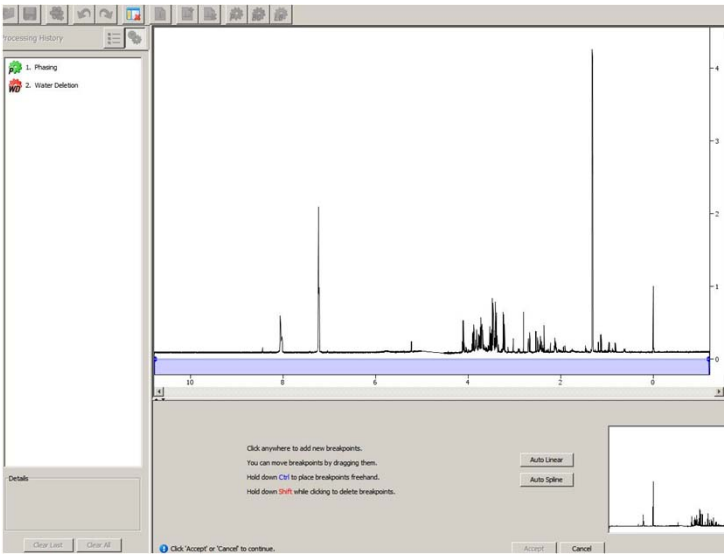


After auto phasing, do manual phasing as necessary

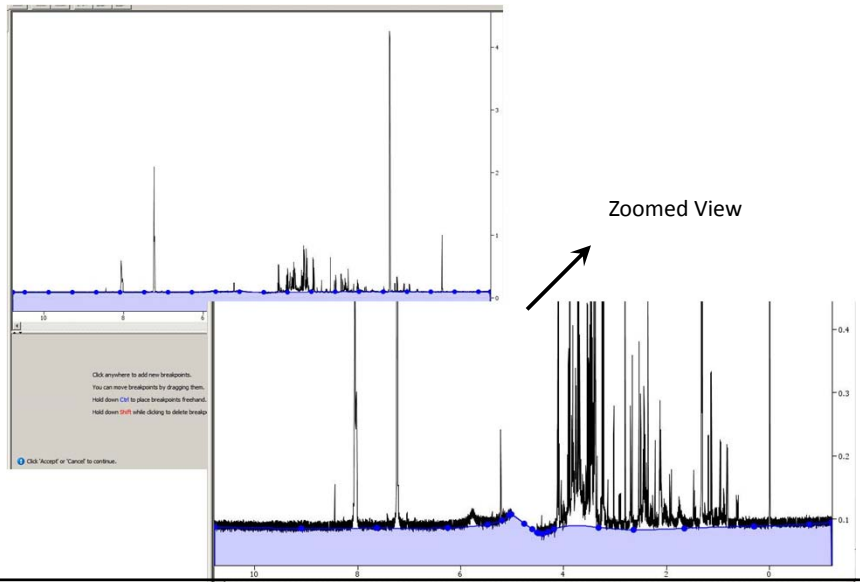
NMR Phasing



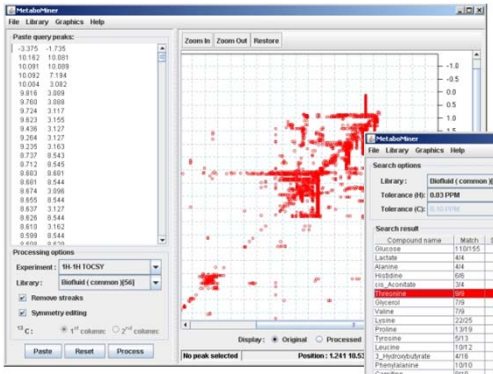
Baseline Correction



Baseline Correction – Auto Spline

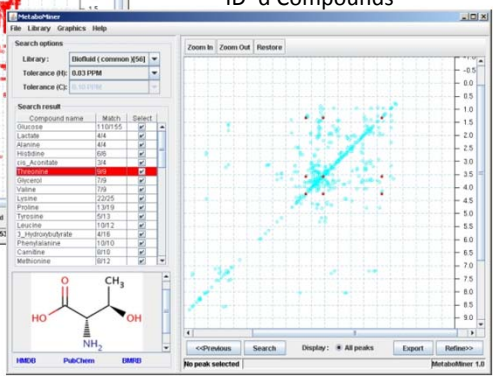


Two-Dimensional NMR



Raw TOCSY Spectrum

ID'd Compounds

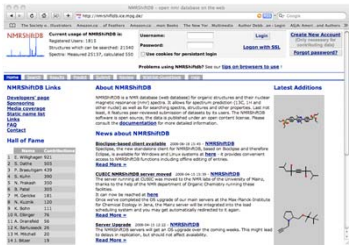


http://wishart.biology.ualberta.ca/metabominer/

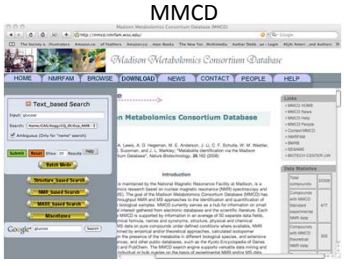
NMR Spectral DBs

SBDS

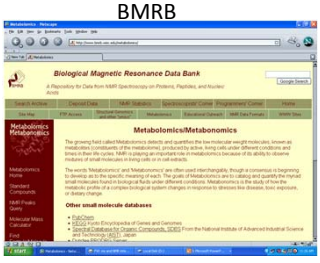
NMRShiftDB



MMCD

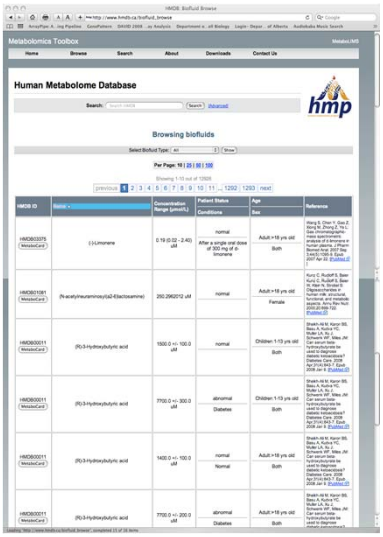


BMRB



The HMDB Biofluid Database

- Reference metabolite concentrations for >450 different diseases & conditions
- Abnormal and normal metabolite concentrations for >15 biofluids and >4500 different metabolites
- Designed for clinical chemists & physicians
- Largest & most complete resource of its kind



David Wishart' Project