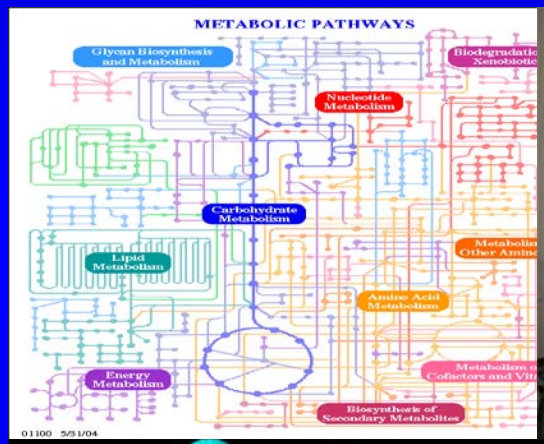
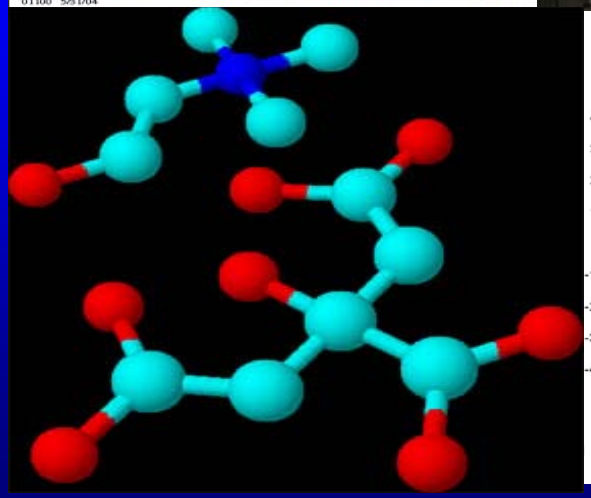
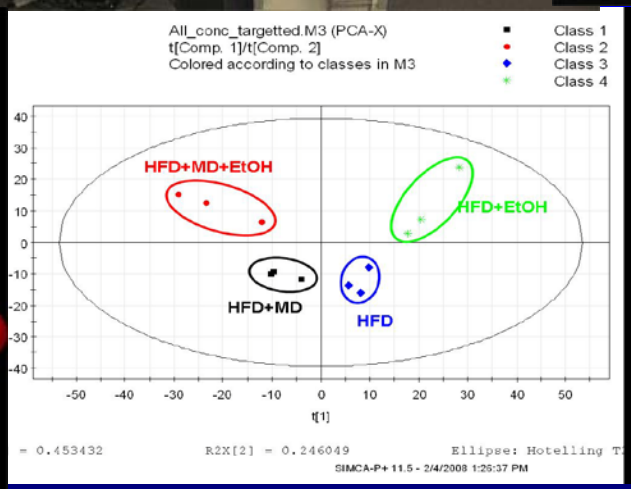
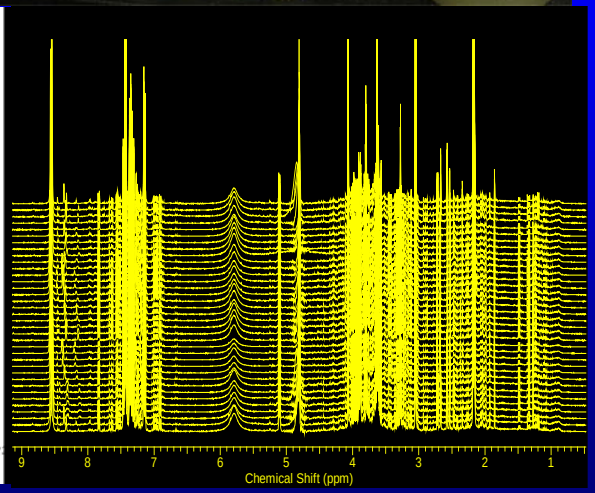
An Introduction to NMR-Based Metabolomics at the UNC Metabolomics Laboratory

Tom O'Connell, Ph.D. Director & Associate Prof. Pharmacotherapy









The Definition of Metabonomics

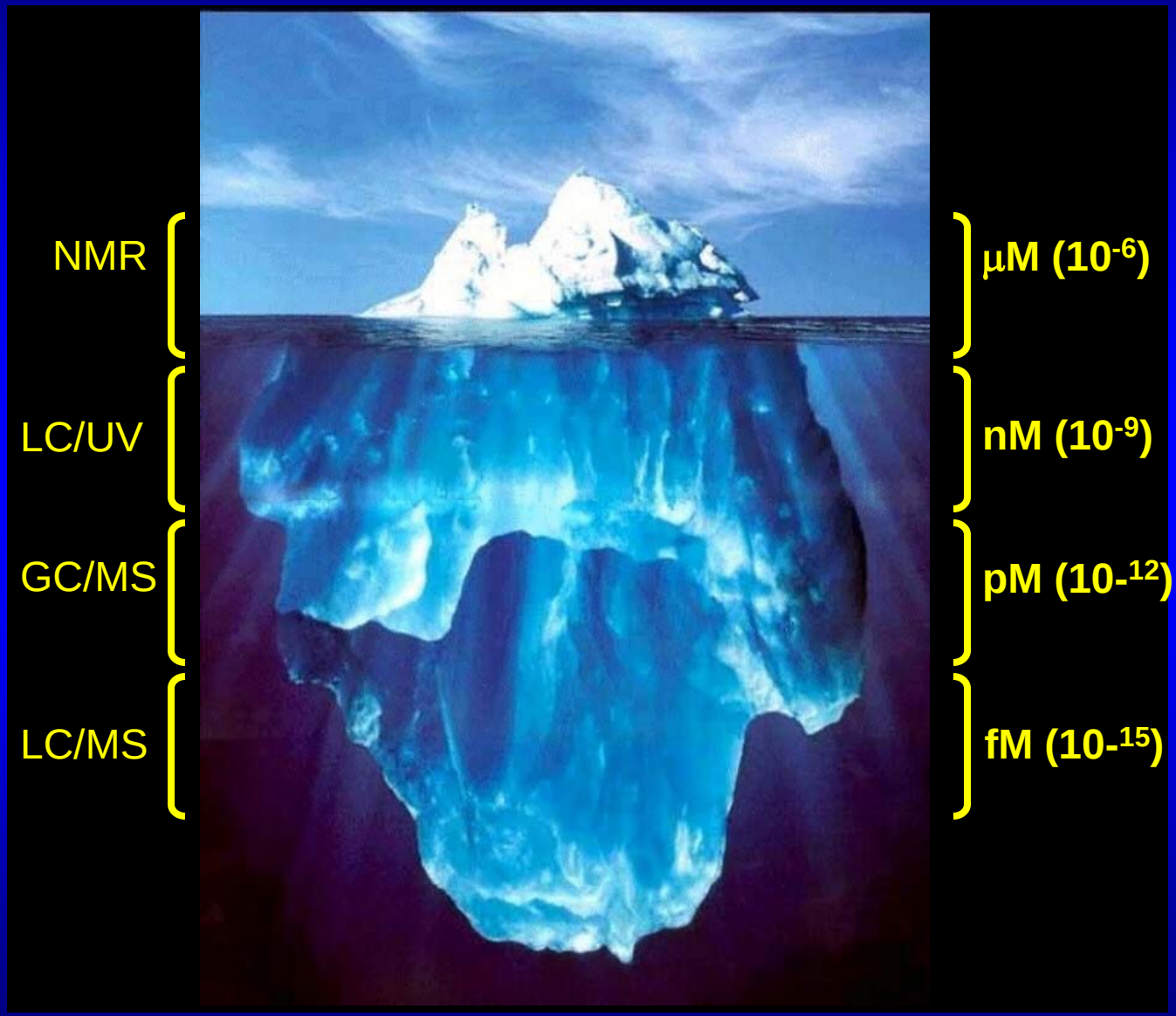
The quantitative measurement of metabolic responses in biological fluids, cells or tissues to pathophysiological stimuli.

Adapted from Nicholson and Wilson, Nature Reviews in Drug Discovery, 2, 668, 2003

Metabolomics vs. Metabonomics?

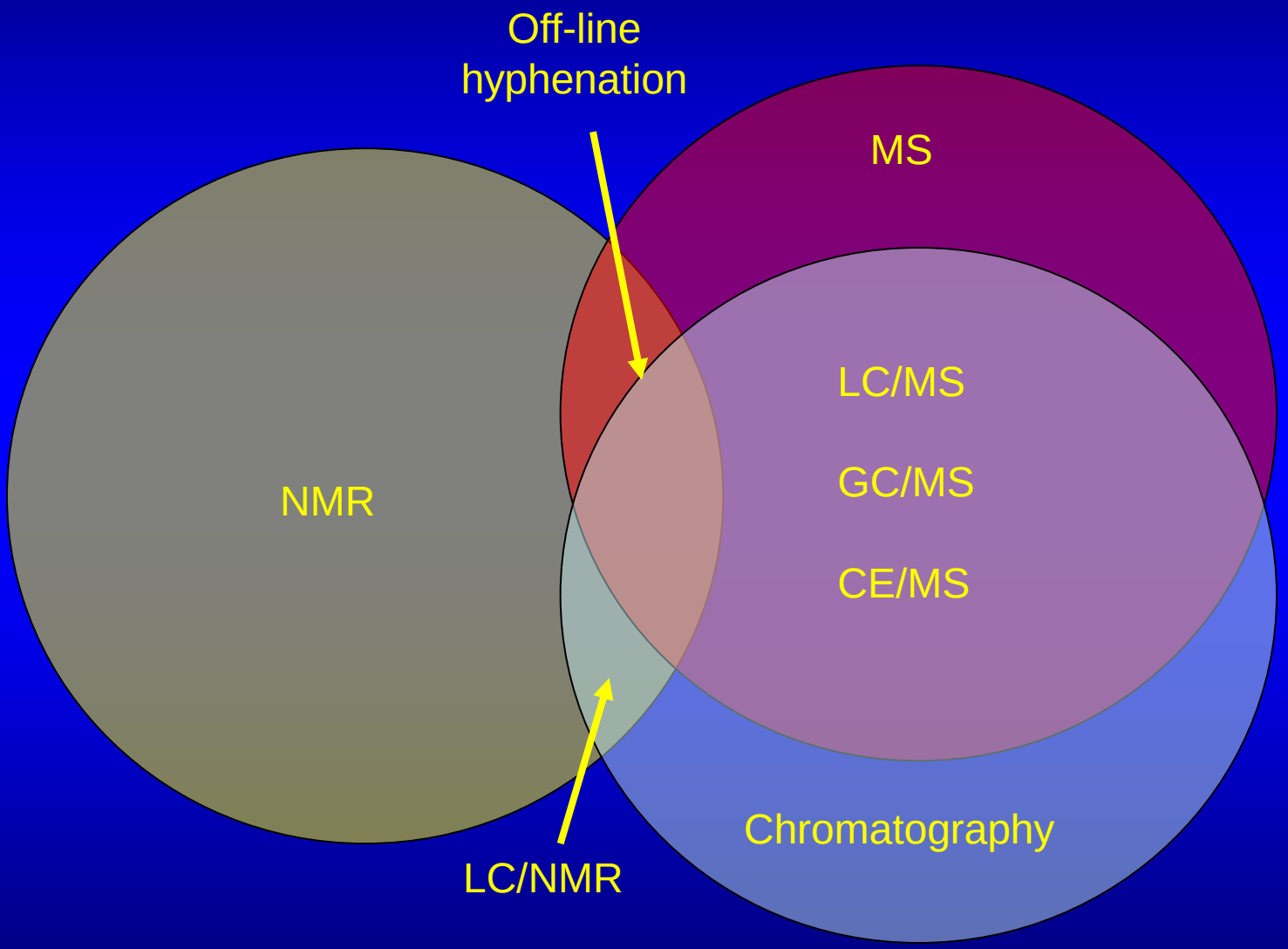
Don’t’ worry about it.

Analytical Coverage of the Metabolome



Adapted from Sumner, LW, et al., Phytochem, 62, 817,2003

Main Analytical Approaches to Metabolomics



Pros & Cons of NMR-Based Metabolomics

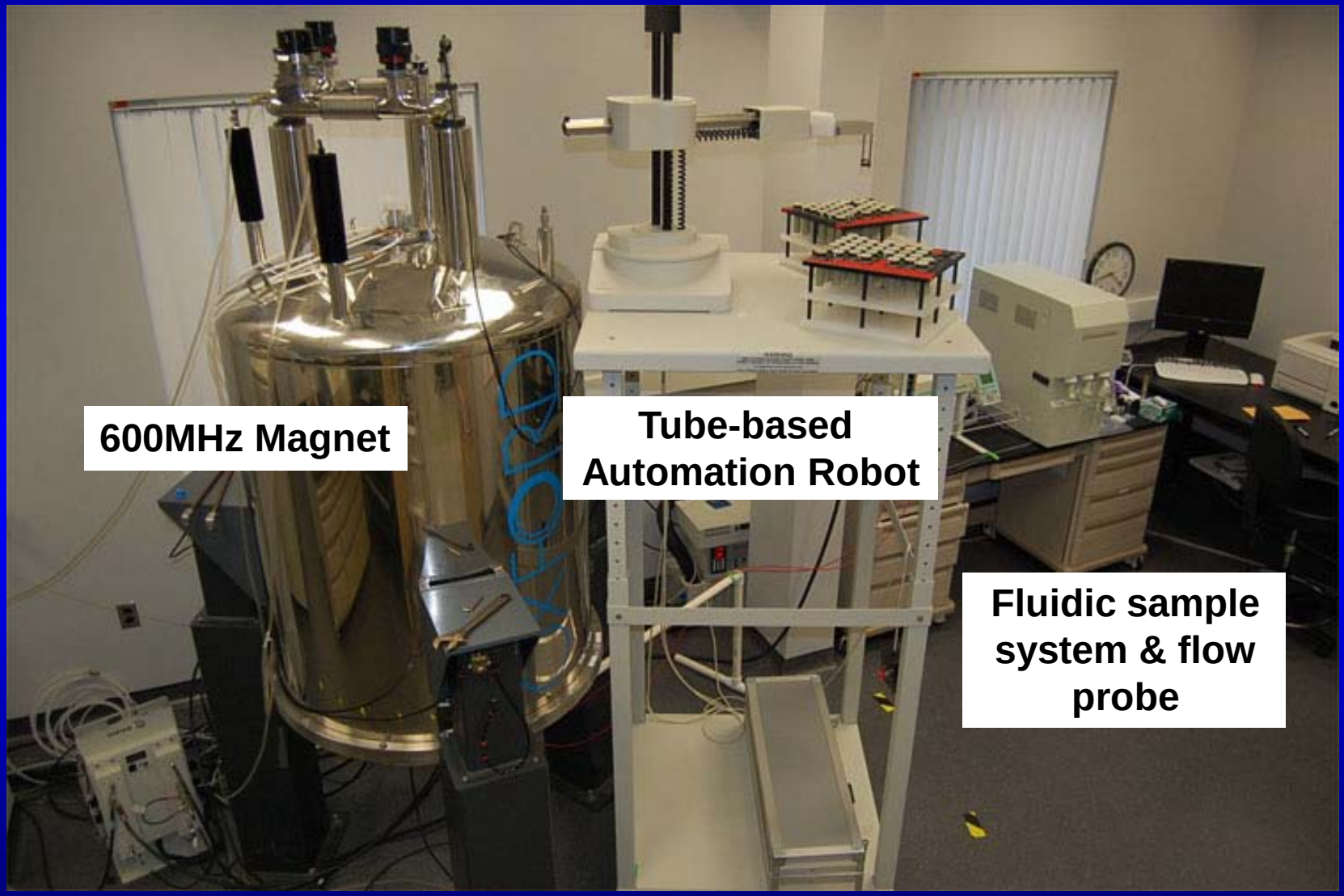
Pros

- Comprehensive profiling by a single non-destructive method
- Inherently quantitative signals
- Minimal sample preparation
- Very high information content for structure elucidation (stereo/regiochemistry)
- Very high long term intra/inter-lab reproducibility

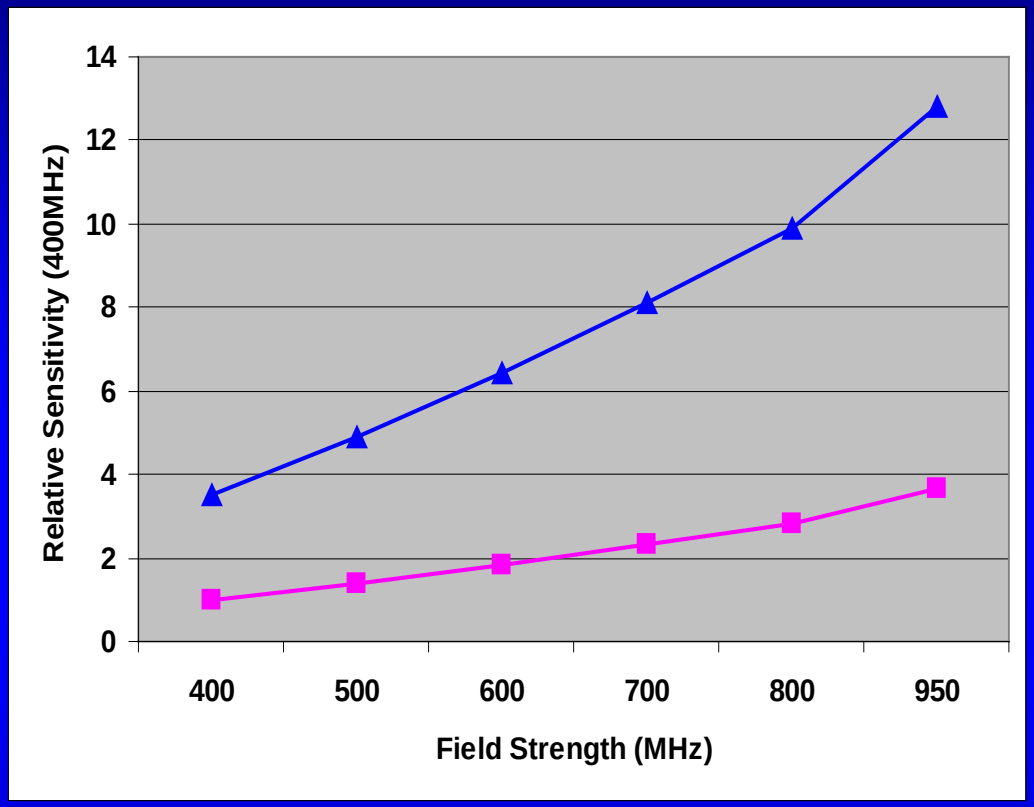
Cons

- Relatively insensitive; concentrations in low μ-mole
- Spectral crowding necessitates advanced methods (2D, 1H-13C) on some samples
- NMR spectral data libraries are growing, but still relatively small

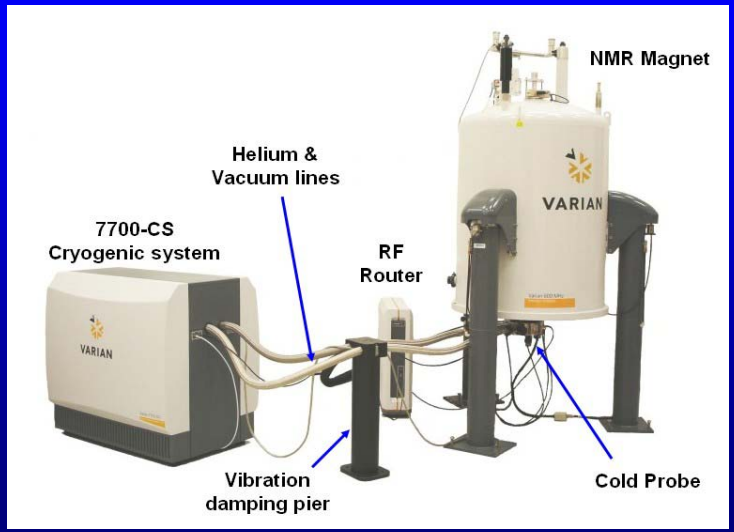
UNC Metabolomic Laboratory



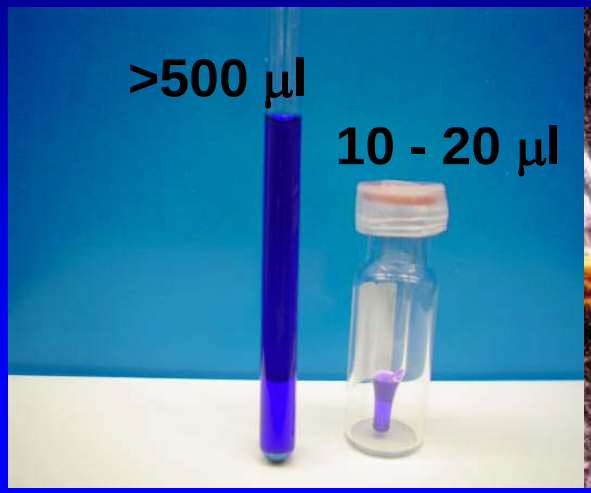
Sensitivity & Resolution



- Sensitivity increases as B3/2
- Resolution increases linearly
- Cold probe adds ~ 3-4 fold sensitivity

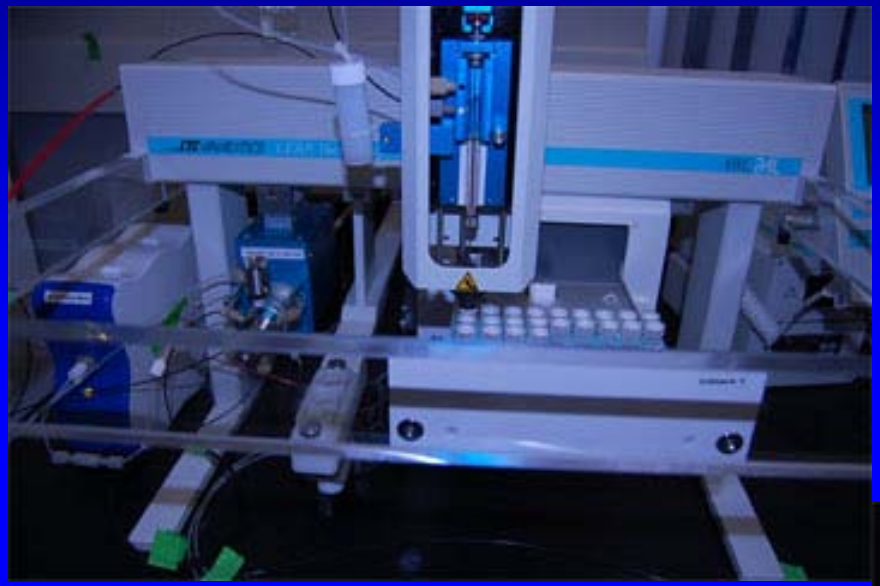


Micro-coil NMR System



- **Sample volumes of 10 µl**
- **Urine & serum analyzed in ~ 25 minutes w/out concentration**
- **Sensitivity gains with concentration**
- **Fully automated data collection**
- **Sample returned to vial/plate after data collection**

Protasis High-throughput Sample System



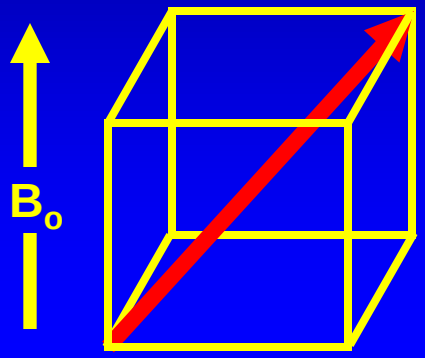
Temperature controlled sample stack for up to 6 plates

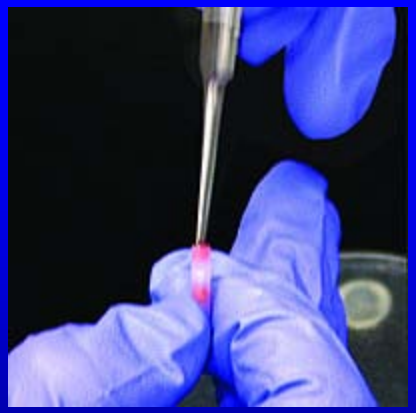
Automated syringe injection from vials

In-line filters and reverse rinse protocols minimize clogs from biofluids

Sampling format is customizable allowing the use of multiple types of vials or microplates

Magic Angle Spinning NMR for Profiling of Intact Tissue





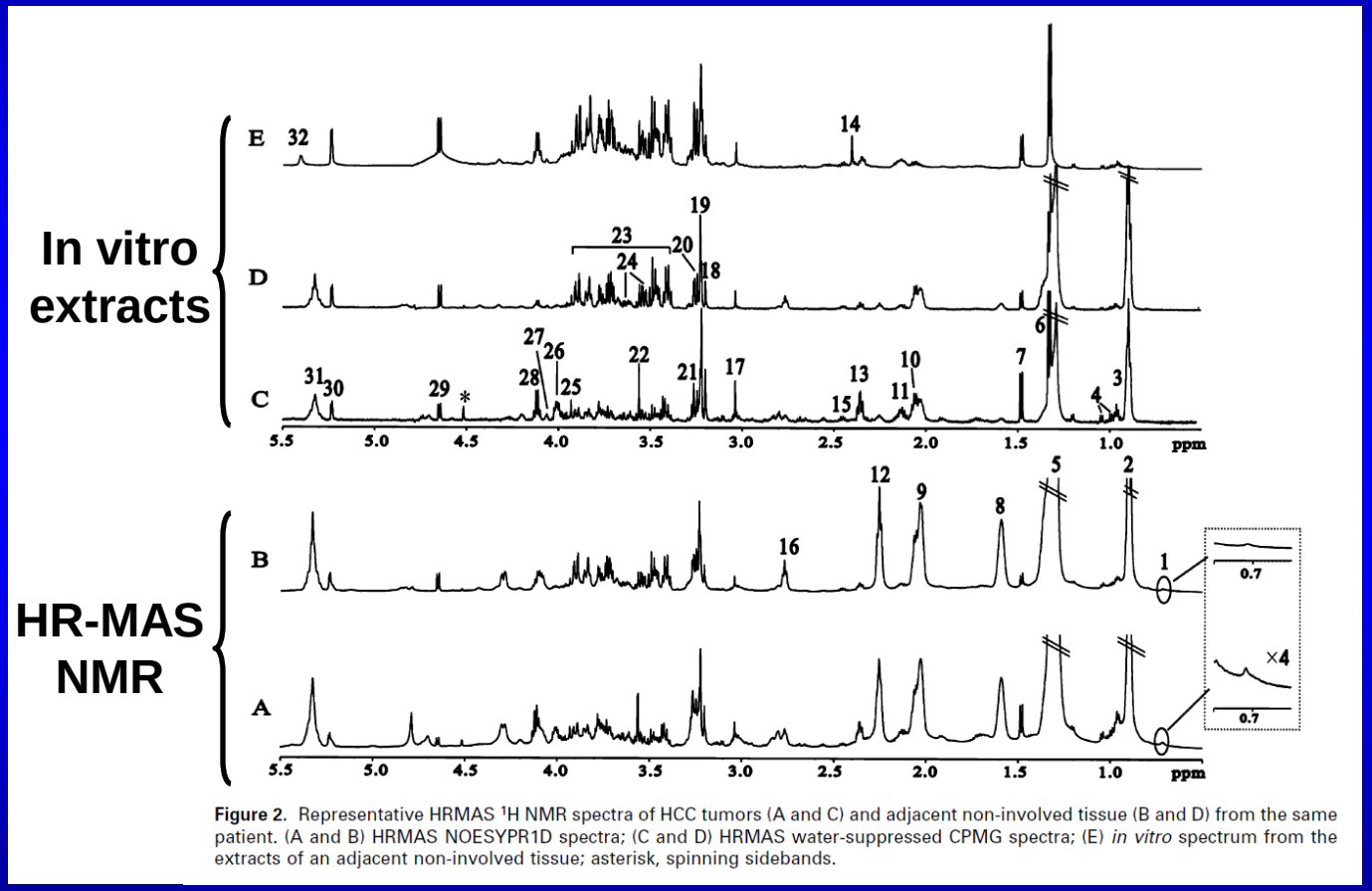
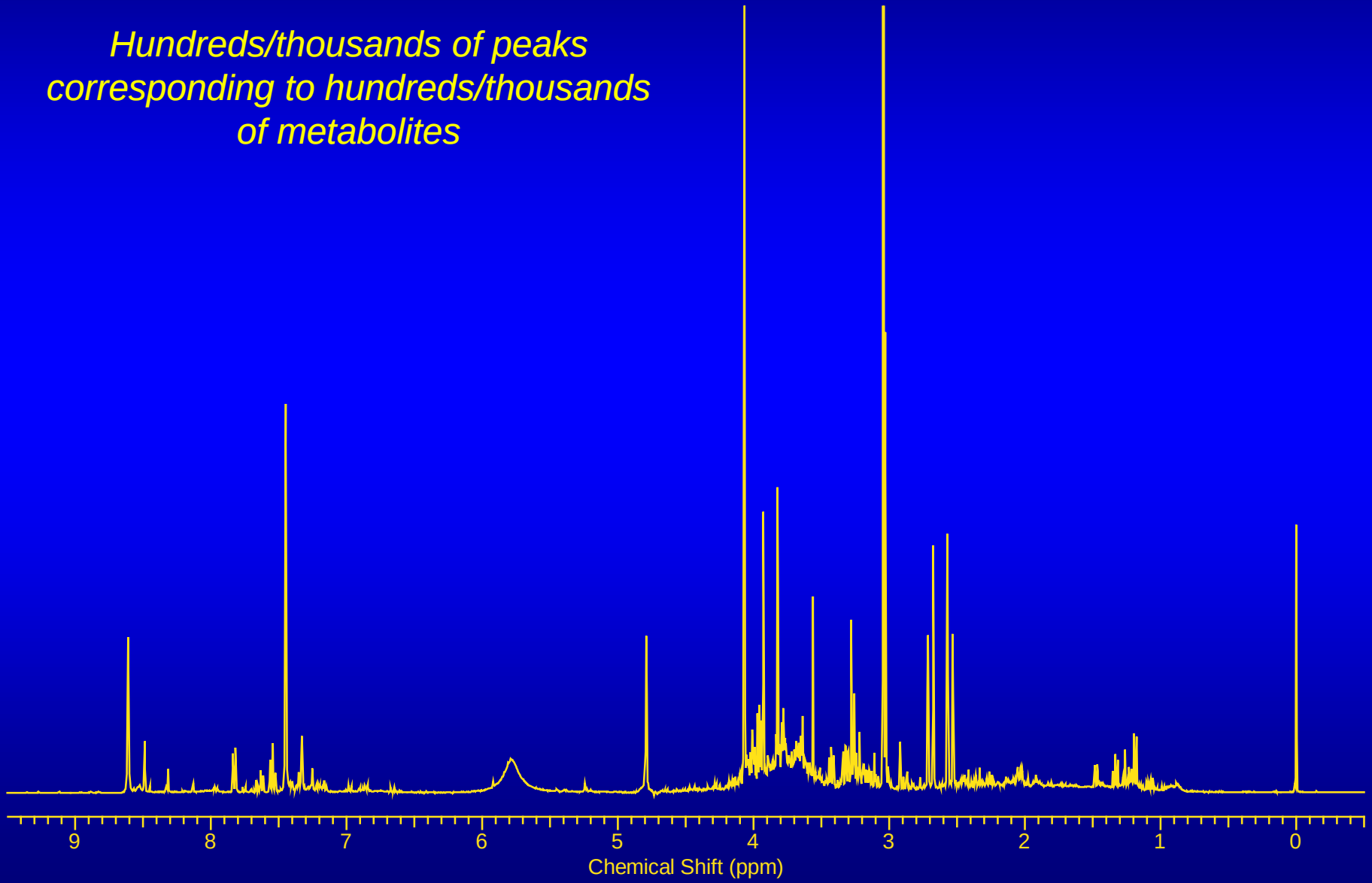


Figure 2. Representative HRMAS ^{1}H NMR spectra of HCC tumors (A and C) and adjacent non-involved tissue (B and D) from the same patient. (A and B) HRMAS NOESYPR1D spectra; (C and D) HRMAS water-suppressed CPMG spectra; (E) *in vitro* spectrum from the extracts of an adjacent non-involved tissue; asterisk, spinning sidebands.

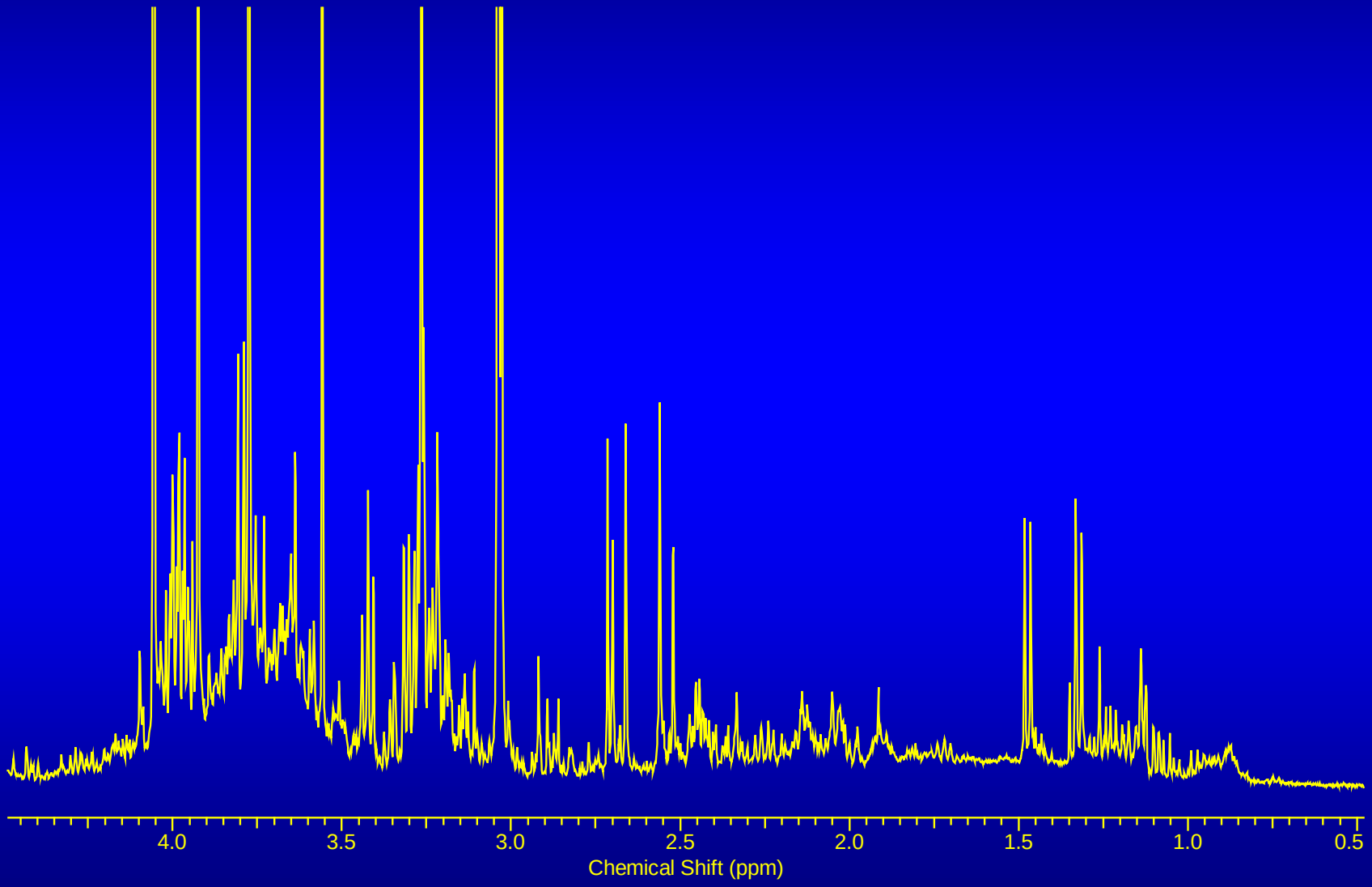
Yang et al., J. Proteome Res. 6, 2605, 2007

Typical Urine NMR Spectrum

Hundreds/thousands of peaks corresponding to hundreds/thousands of metabolites

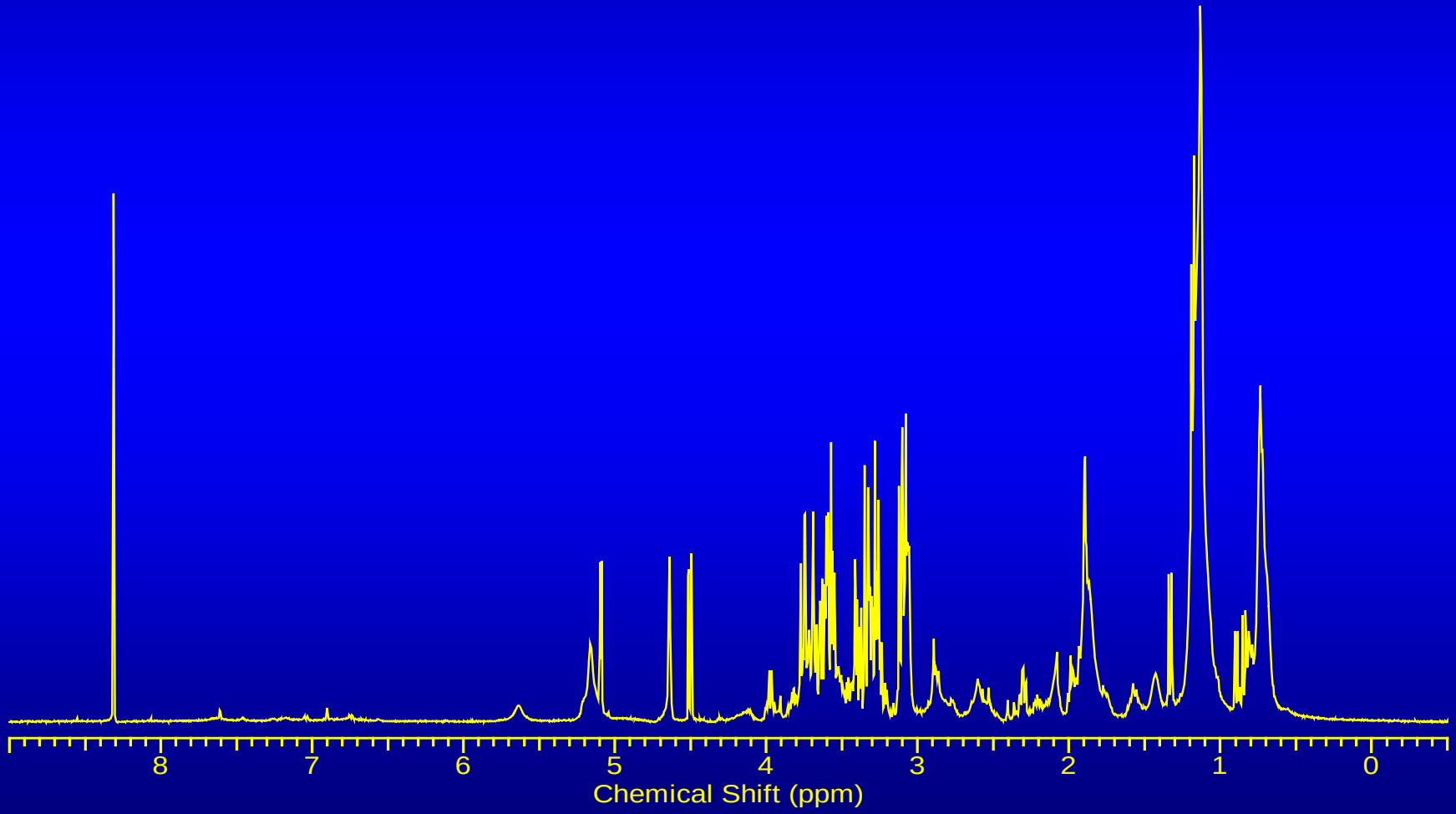


Typical Urine NMR Spectrum



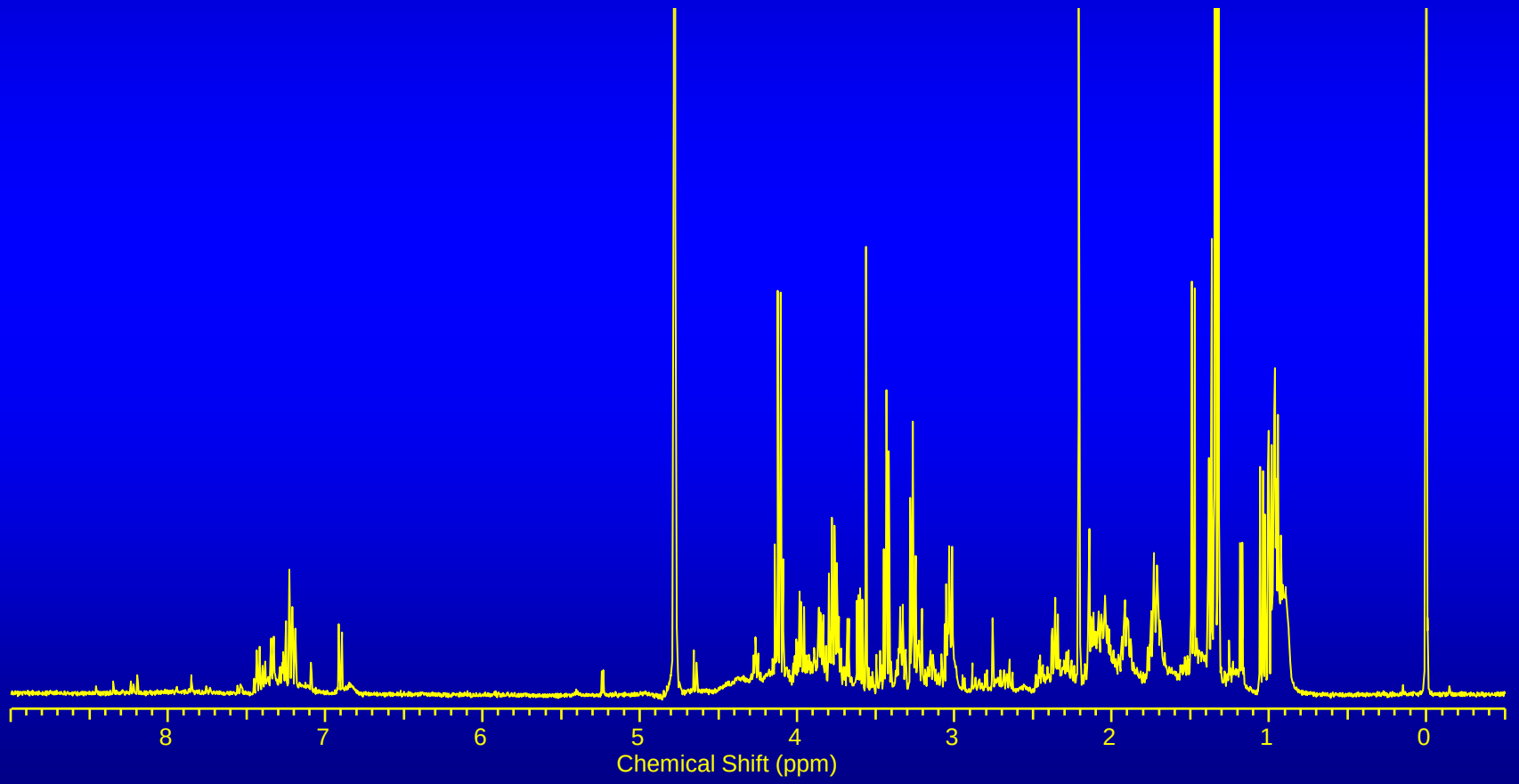
What types of samples can we look at?

Human serum

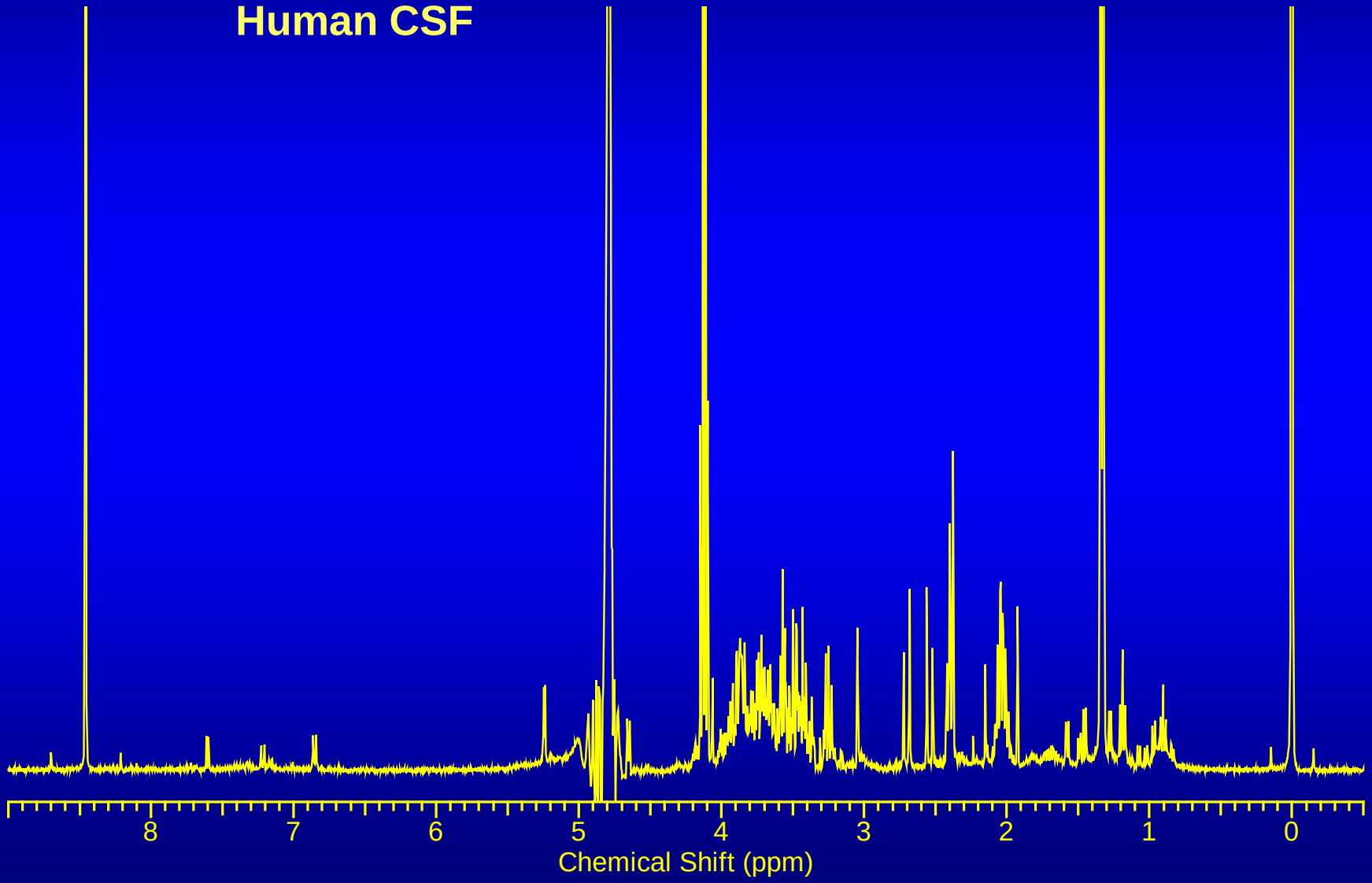


What types of samples can we look at?

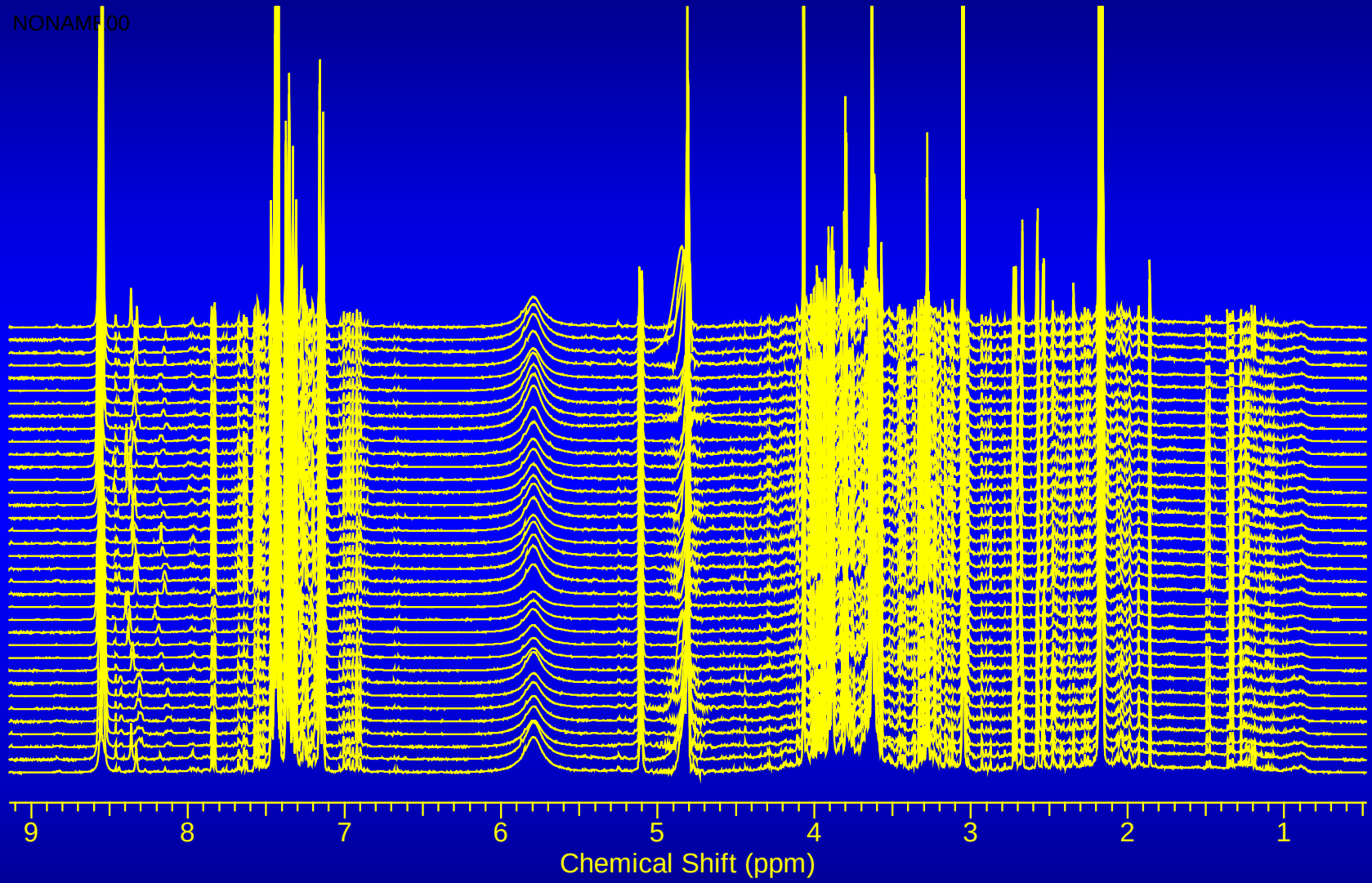
Human bronchoalveolar lavage fluid



What types of samples can we look at?



Metabolomics Involves Many Samples



Looking for subtle differences in many spectra requires some data reduction/simplification

Data Reduction

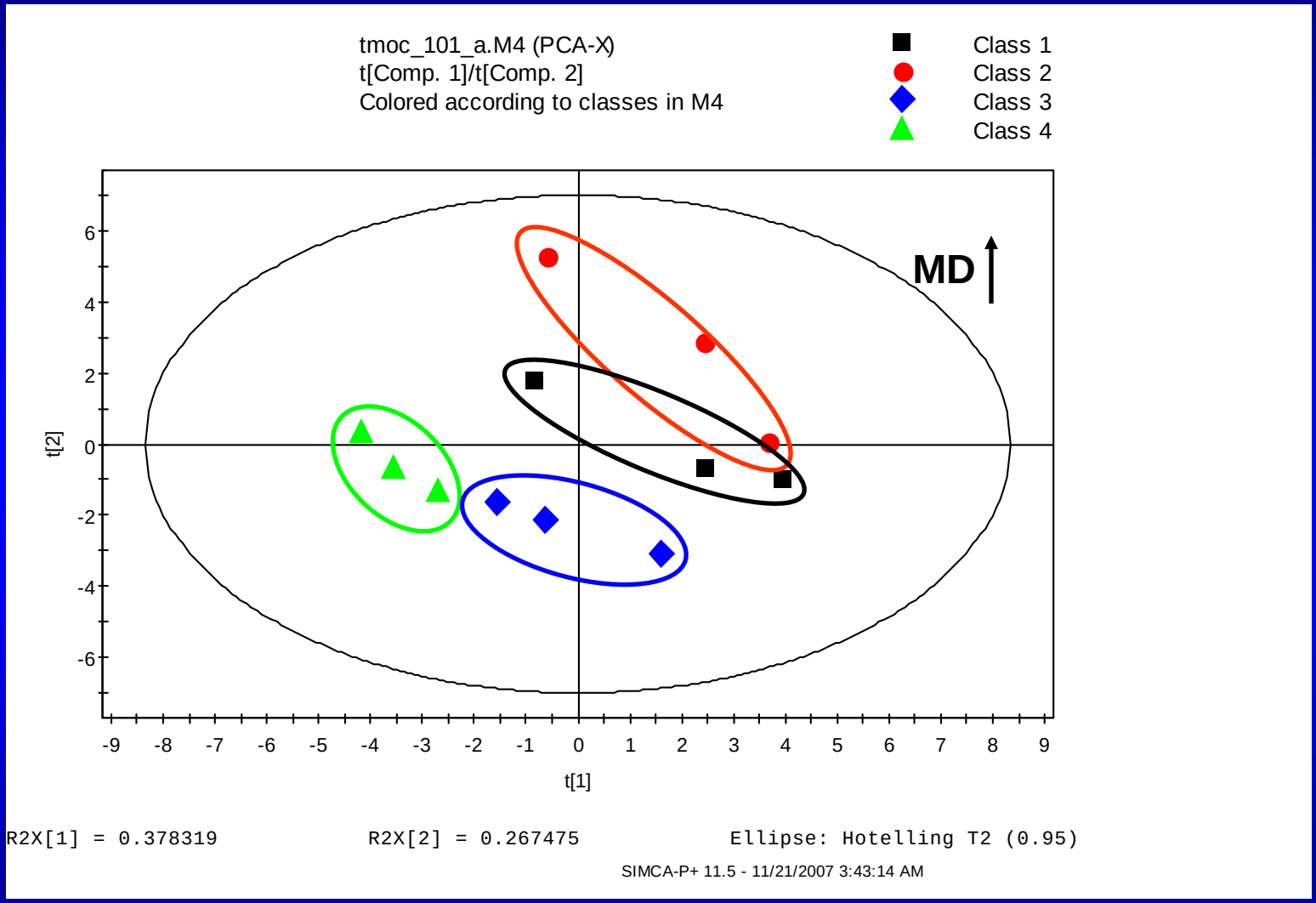
Each sample is described by ~ 200 variables
Reduce the data by capturing the variance with combinations of variables

(ppm)	File Name	[0.50 .. 0.53]	[0.53 .. 0.59]	[0.59 .. 0.61]	[0.61 .. 0.63]	[0.63 .. 0.66]	[0.66 .. 0.69]	[0.69 .. 0.72]	[0.72 .. 0.75]	[0.75 .. 0.79]	[0.79 .. 0.81]	[0.81 .. 0.83]	[0.83 .. 0.85]	[0.85 .. 0.9
3	s01_d01	2.04	4.20	1.88	1.60	2.17	2.08	2.42	2.64	2.83	1.80	1.76	2.01	9.75
4	s01_d03	2.15	4.38	1.95	1.68	2.25	2.19	2.51	2.69	2.81	1.82	1.81	2.00	9.62
5	s01_d05	2.37	4.81	2.18	1.85	2.47	2.38	2.75	2.88	3.05	1.98	1.95	2.10	9.36
6	s01_d07	2.47	4.97	2.26	1.91	2.54	2.45	2.83	3.03	3.18	2.01	1.98	2.13	8.91
7	s02_d01	2.12	4.42	1.94	1.66	2.24	2.15	2.52	2.70	2.85	1.86	1.83	2.15	10.26
8	s02_d03	2.29	4.73	2.15	1.81	2.42	2.35	2.71	2.93	3.04	1.99	2.01	2.25	10.06
9	s02_d05	2.36	4.85	2.19	1.87	2.46	2.37	2.76	2.91	3.04	1.98	1.95	2.11	9.05
10	s02_d07	2.41	4.86	2.20	1.89	2.50	2.40	2.80	2.94	3.07	1.99	1.97	2.14	8.88
11	s03_d01	2.39	4.88	2.22	1.88	2.52	2.48	2.87	3.22	3.56	2.20	2.17	2.58	11.11
12	s03_d03	2.41	4.88	2.22	1.89	2.58	2.48	2.89	3.19	3.45	2.15	2.12	2.47	10.62
13	s03_d05	2.43	4.92	2.24	1.91	2.56	2.47	2.88	3.17	3.38	2.14	2.06	2.32	9.41
14	s03_d07	2.38	4.83	2.20	1.87	2.50	2.43	2.83	3.18	3.41	2.09	2.02	2.26	9.42
15	s04_d01	2.43	5.00	2.21	1.89	2.57	2.51	2.89	3.22	3.49	2.21	2.16	2.54	11.18
16	s04_d03	2.53	5.20	2.35	2.00	2.67	2.57	3.05	3.36	3.61	2.27	2.21	2.51	10.79
17	s04_d05	2.85	5.58	2.51	2.12	2.94	2.84	3.28	3.45	3.63	2.31	2.27	2.43	8.84
18	s04_d07	2.74	5.49	2.45	2.11	2.83	2.79	3.17	3.45	3.63	2.32	2.24	2.46	9.85
19	s05_d01	2.24	4.71	2.14	1.82	2.44	2.43	2.78	3.06	3.48	2.20	2.09	2.52	11.43
20	s05_d03	2.35	4.85	2.22	1.89	2.51	2.57	2.96	3.25	3.56	2.26	2.19	2.52	10.68
21	s05_d05	2.44	4.98	2.27	1.93	2.59	2.52	2.88	3.09	3.28	2.13	2.05	2.26	9.04
22	s05_d07	2.42	4.91	2.21	1.92	2.55	2.49	2.88	3.11	3.35	2.14	2.12	2.19	9.48
23	s06_d01	2.77	5.63	2.51	2.15	2.94	2.86	3.31	3.58	3.73	2.39	2.42	2.77	11.49
24	s06_d03	2.89	5.92	2.66	2.30	3.04	2.98	3.47	3.63	3.82	2.50	2.50	2.71	10.84
25	s06_d05	3.09	6.24	2.80	2.41	3.18	3.12	3.57	3.78	3.97	2.59	2.55	2.76	10.60
26	s06_d07	3.01	6.05	2.71	2.34	3.11	3.01	3.50	3.69	3.89	2.53	2.50	2.75	10.67
27	s07_d01	2.89	5.81	2.58	2.23	3.02	2.94	3.36	3.66	3.85	2.51	2.49	2.75	11.38
28	s07_d03	2.79	5.71	2.53	2.22	2.91	2.83	3.29	3.55	3.78	2.43	2.42	2.70	11.02
29	s07_d05	3.00	6.05	2.71	2.31	3.08	3.01	3.47	3.73	3.91	2.53	2.46	2.70	10.38
30	s07_d07	2.87	5.85	2.62	2.27	2.99	2.89	3.39	3.60	3.73	2.46	2.46	2.57	10.22
31	s08_d01	2.04	4.21	1.86	1.66	2.19	2.13	2.48	2.77	2.99	1.91	1.89	2.16	10.86
32	s08_d03	2.35	4.82	2.19	1.89	2.55	2.49	2.89	3.30	3.54	2.22	2.23	2.48	11.50
33	s08_d05	2.60	5.27	2.41	2.07	2.75	2.68	3.09	3.34	3.51	2.25	2.25	2.40	9.80
34	s08_d07	2.39	4.88	2.24	1.89	2.54	2.48	2.87	3.11	3.35	2.15	2.12	2.27	9.96
39	s10_d01	2.37	4.84	2.18	1.87	2.53	2.41	2.80	2.99	3.14	2.07	2.07	2.22	10.01
40	s10_d03	2.44	5.02	2.30	1.94	2.63	2.55	2.94	3.11	3.26	2.12	2.13	2.21	10.07
41	s10_d05	2.76	5.57	2.55	2.14	2.89	2.78	3.16	3.42	3.54	2.29	2.21	2.33	9.05
42	s10_d07	2.70	5.46	2.49	2.10	2.79	2.73	3.19	3.38	3.50	2.23	2.19	2.33	9.07
43	s11_d01	2.28	4.72	2.10	1.79	2.44	2.40	2.82	3.00	3.19	2.05	2.03	2.39	10.45
44	s11_d03	2.54	5.20	2.39	1.98	2.66	2.62	3.09	3.25	3.44	2.21	2.18	2.46	10.20

Samples

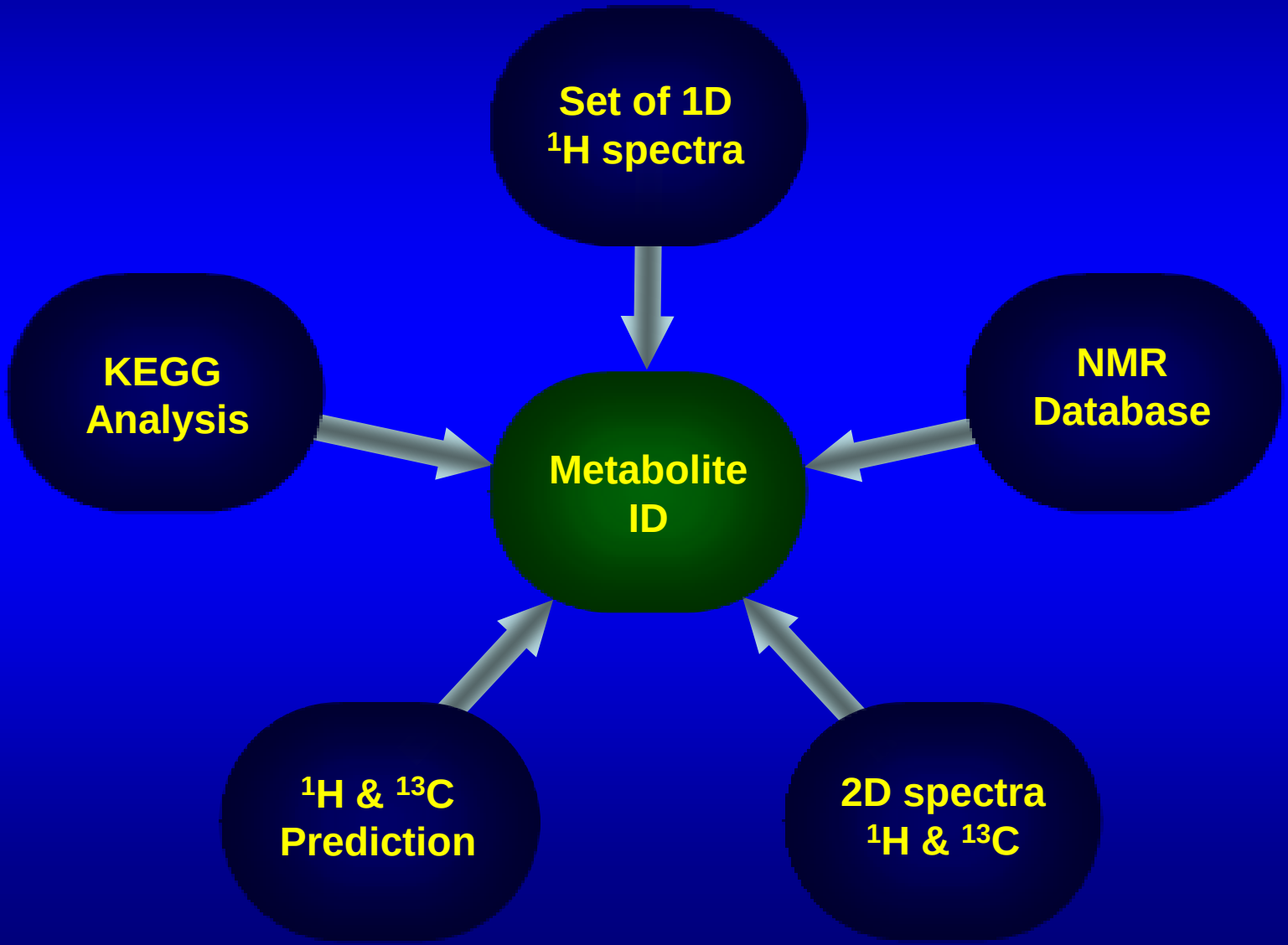
Variables (chemical shift bins)

Serum Metabolomics Analysis from Binned Data

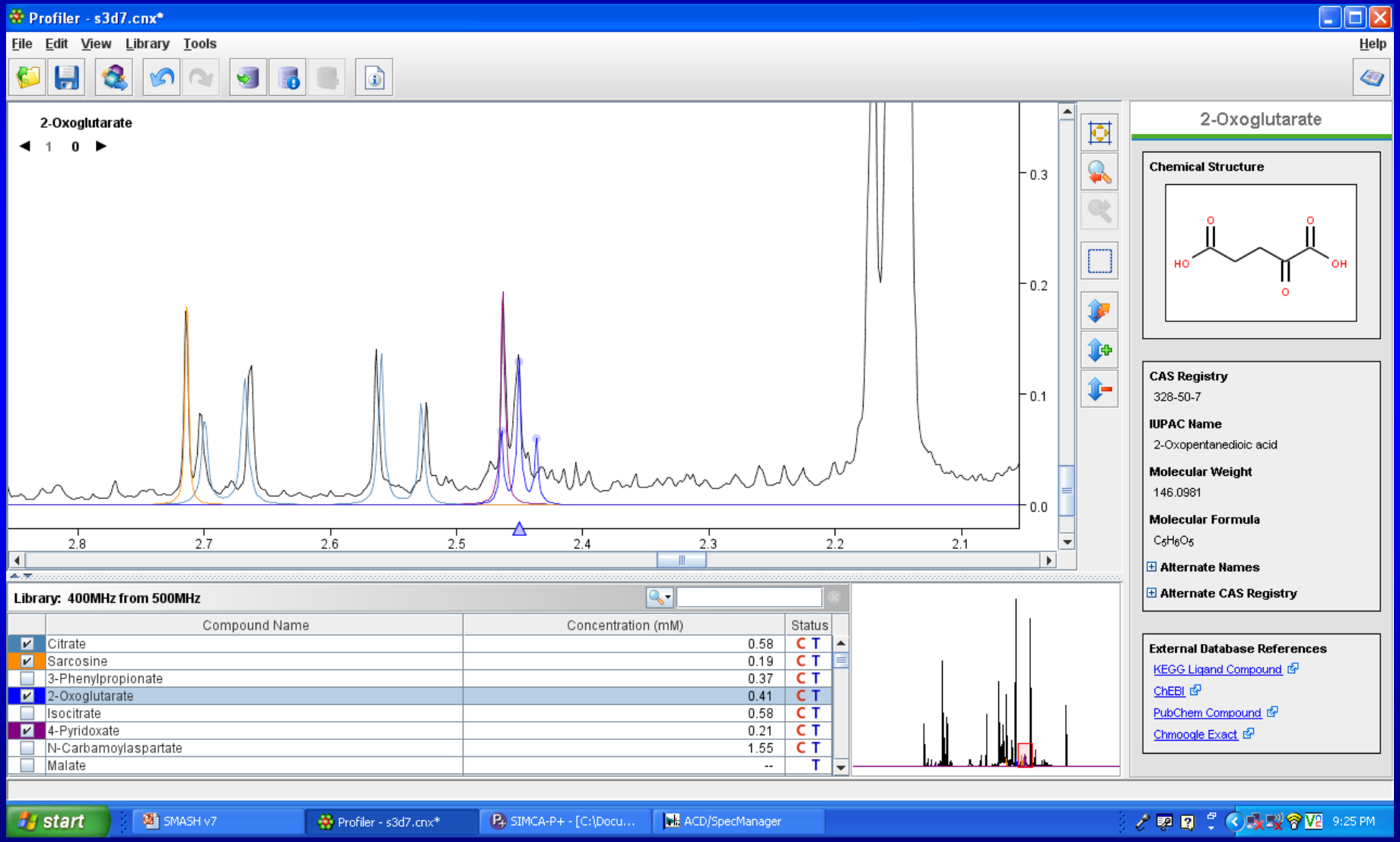


Effects of methyl donor rich diet (choline, betaine, folic acid) on high dose ethanol consuption

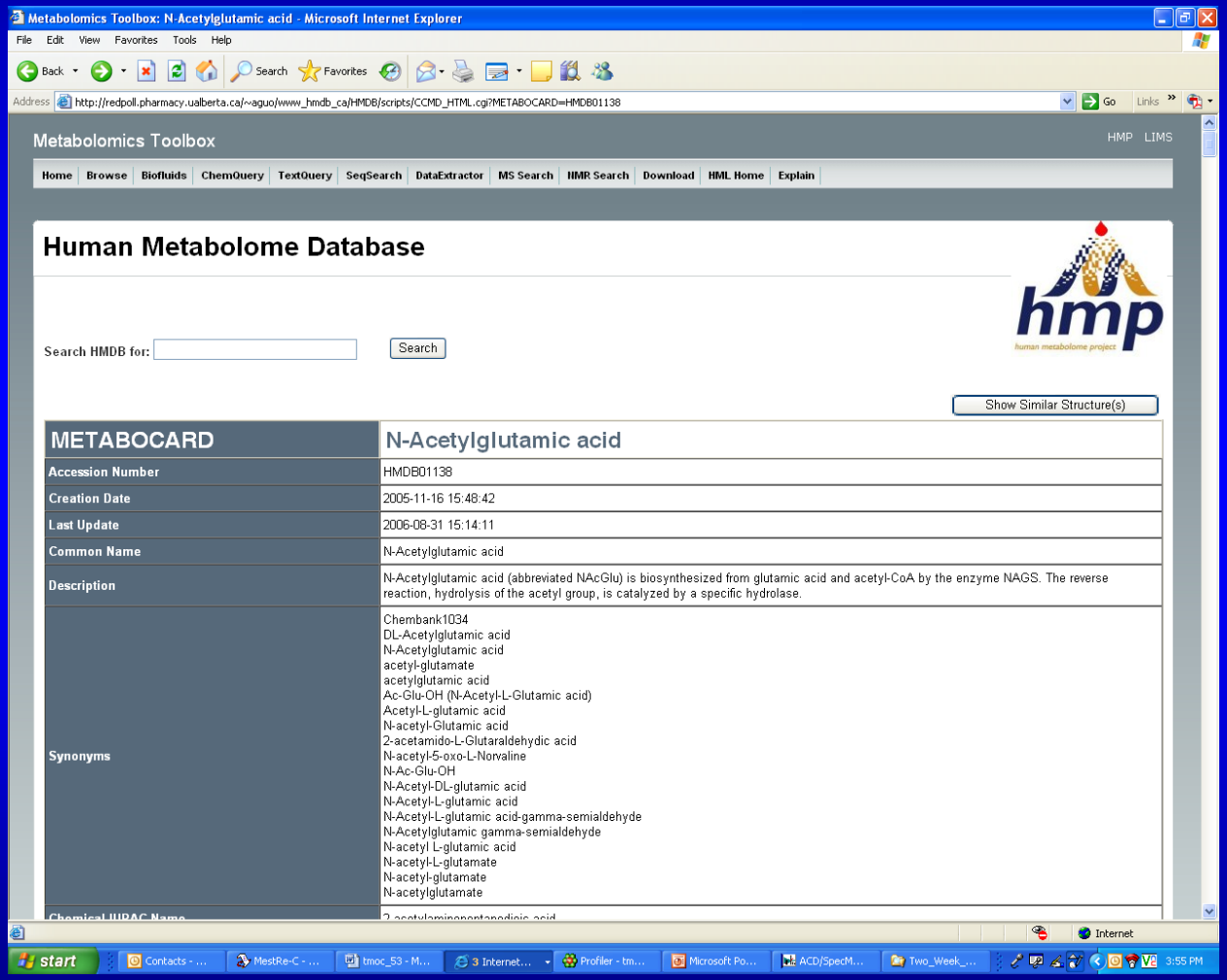
Tools to Identify Biomarkers

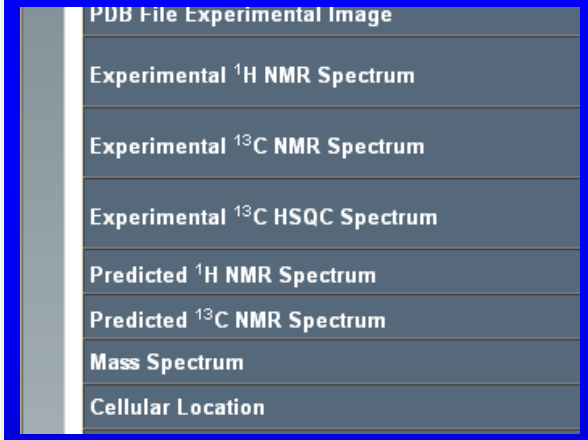


Quantitative Fitting with NMR Database



The Human Metabolome Database

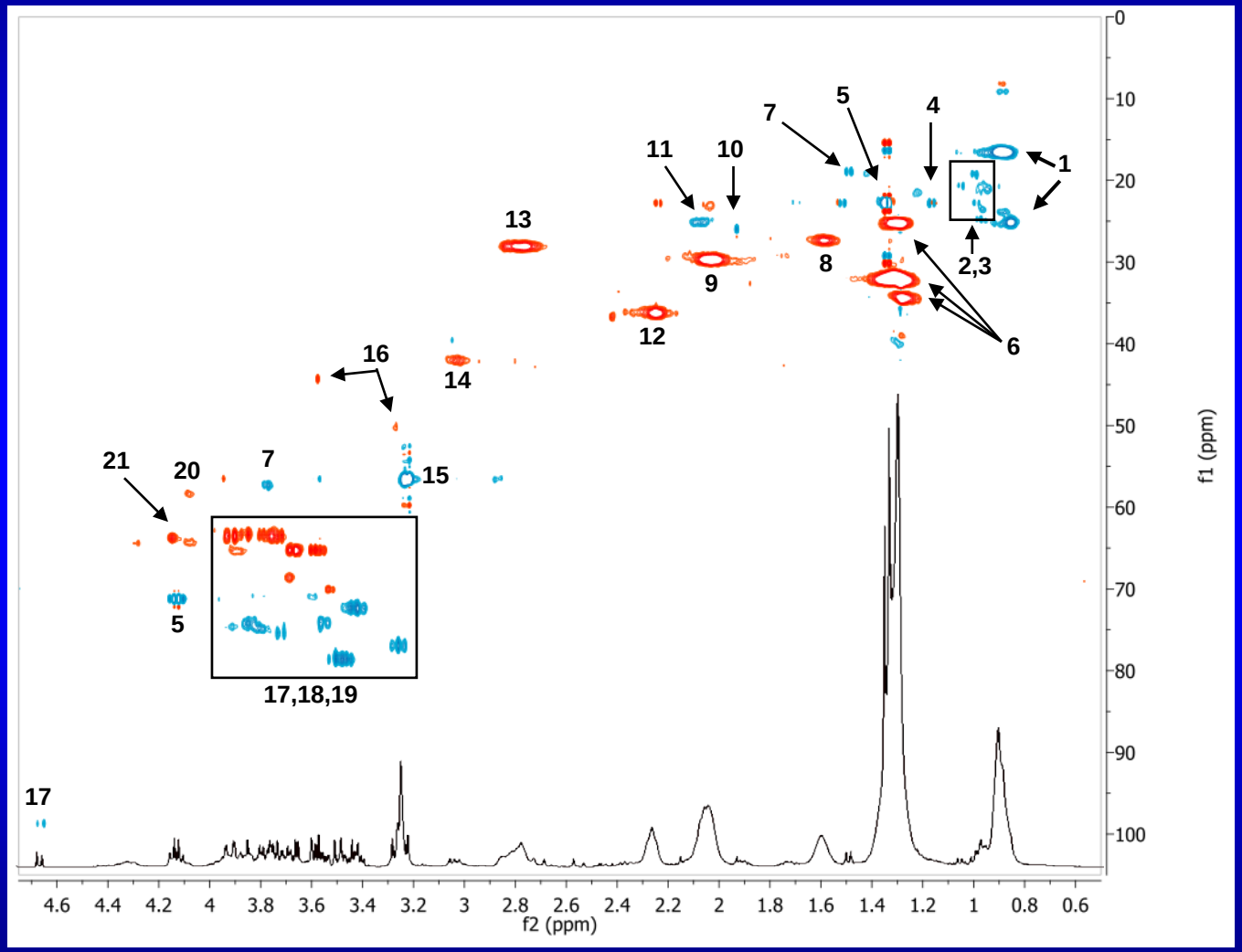




http://www.metabolomics.ca/

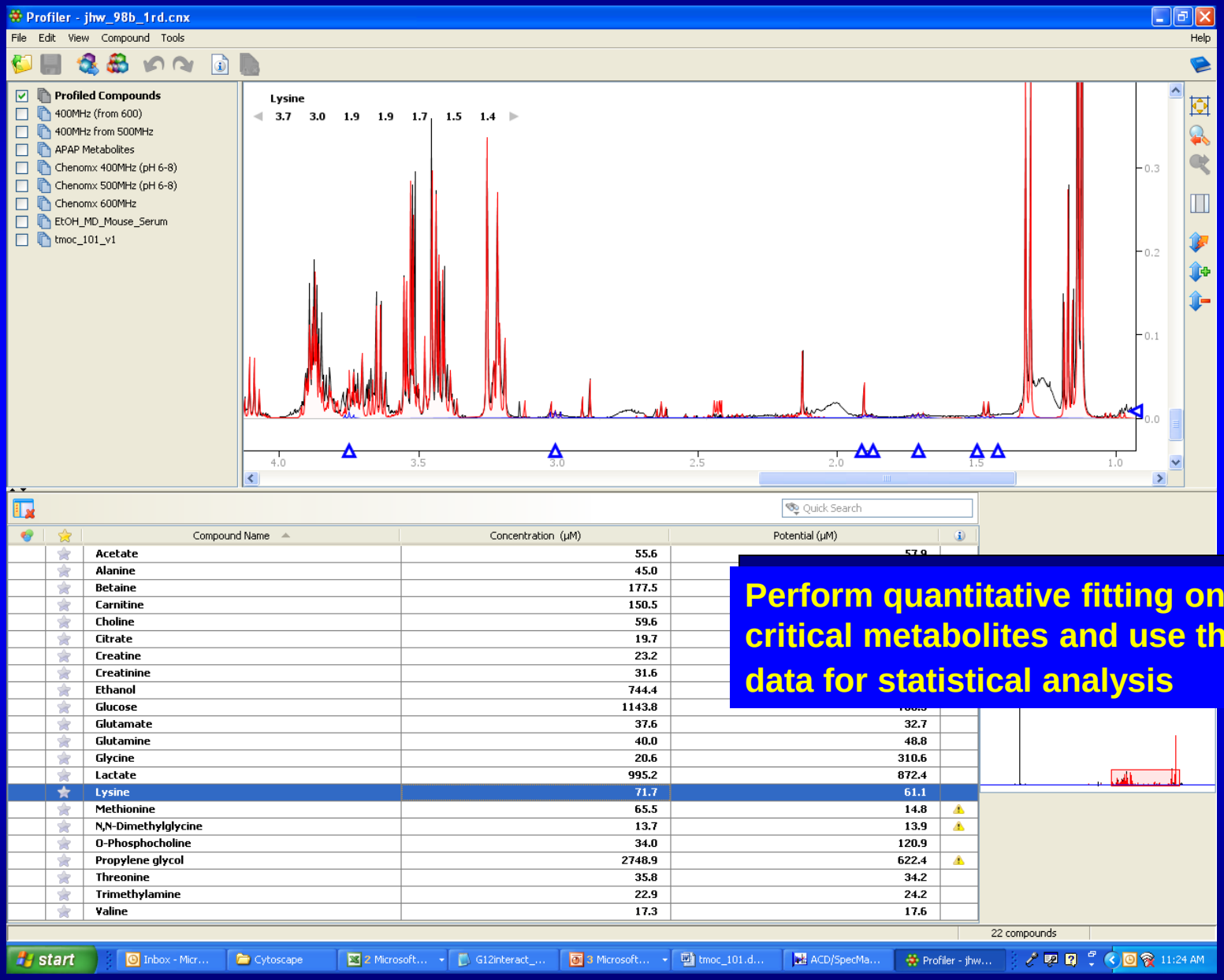
Metabolite ID with 2D Datasets

^{1}H-^{1}H or ^{1}H-^{13}C correlation spectra on selected samples



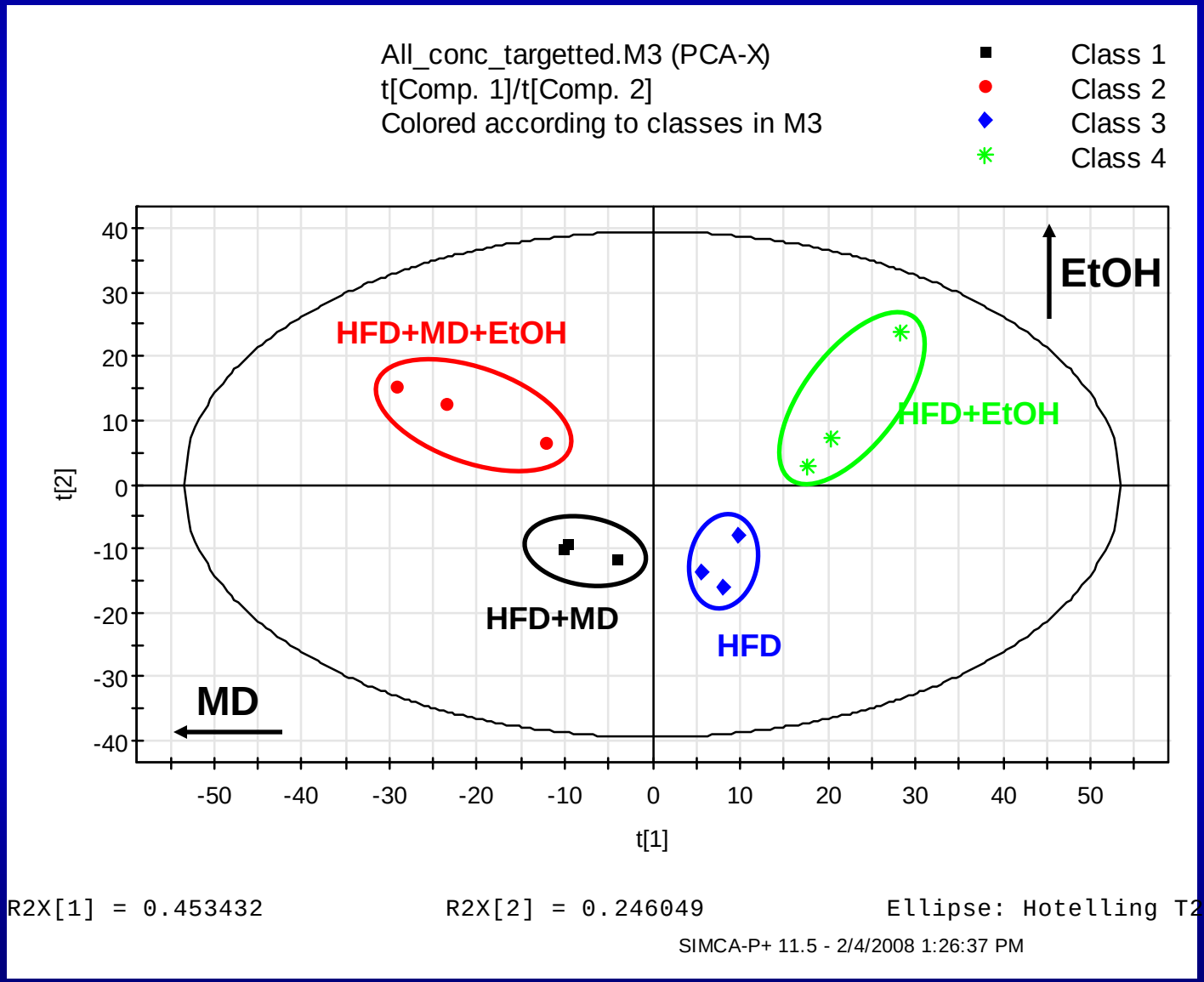
1 = terminal methyl groups of low density (LDL) and very low density lipoproteins (VLDL). 2 = valine. 3 = leucine. 4 = 3-hydroxybutyrate. 5 = lactate. 6 = methylene protons of LDL and VLDL. 7 = alanine. 8 = methylene protons of C3 of VLDL lipoproteins. 9 = allylic methylenes of lipoproteins. 10 = acetate. 11 = N-acetylated glyoproteins. 12 = methylene protons of C2 of VLDL. 13 = methylene protons between olefinic groups of lipoproteins. 14 = albumin lysyl methylene groups. 15 = phospholipid choline headgroups. 16 = taurine. 17 = glucose. 18 = glycerol. 19 = amino acid Ca protons. 20 = choline. 21 = methylene groups of phosphatidylethanolamines.

Targetted Metabolomics



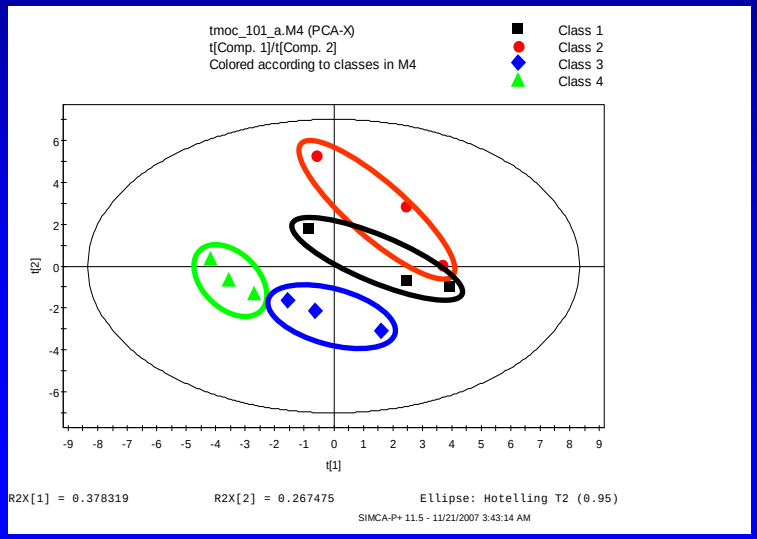
Perform quantitative fitting on all critical metabolites and use this data for statistical analysis

Serum Metabolomics Analysis from Targetted Metabolite Profiles

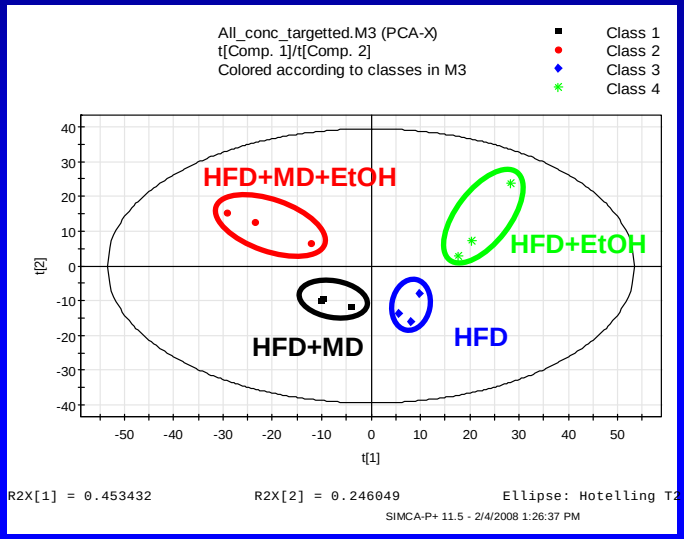


Moving from Global to Targeted Metabolomics

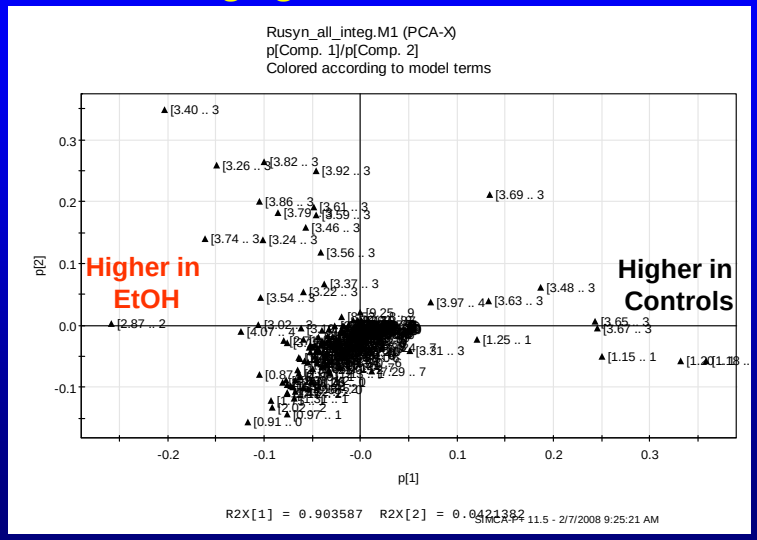
Global models show separation



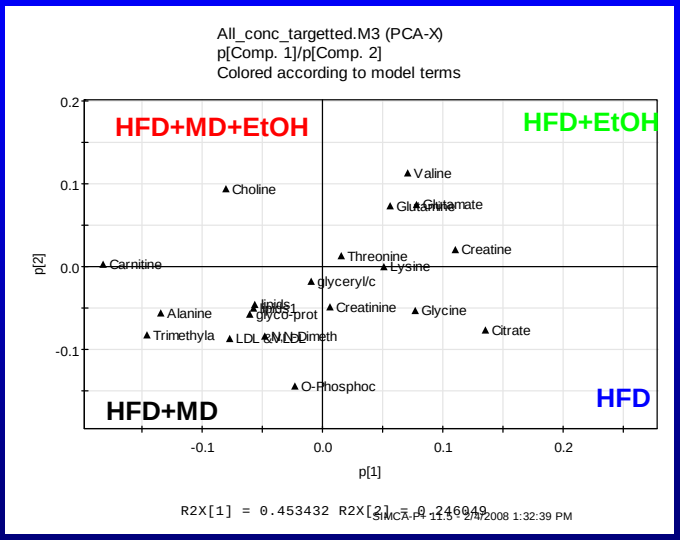
Targeted model improves separation



Loadings guide metabolite ID



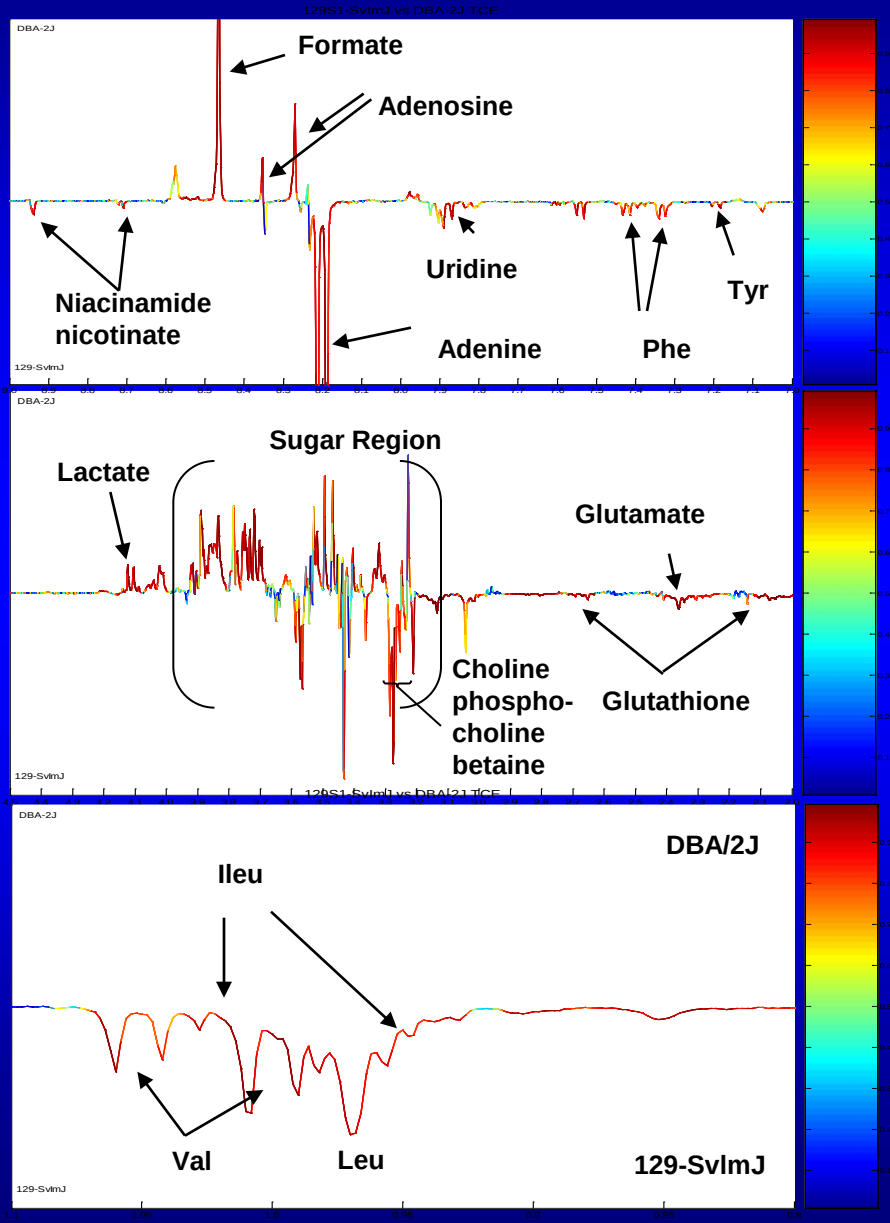
Targeted loadings guide interpretation



OPLS Loadings Plot for Metabolite ID

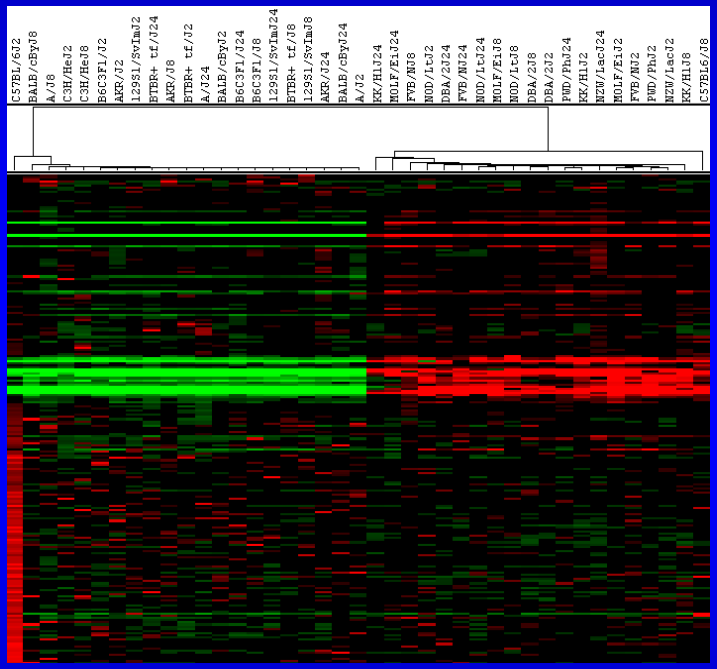
Peak intensity relates to importance in discriminating the groups

Color relates to the confidence in the model

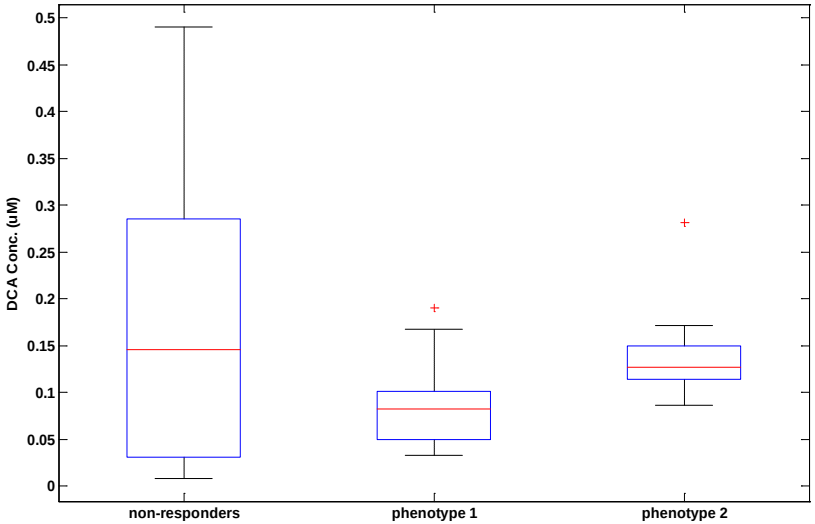


Combining Global Metabolomics with Targeted Metabolite Assays

Heat map of NMR spectra



DCA Levels by Targeted LC/MS

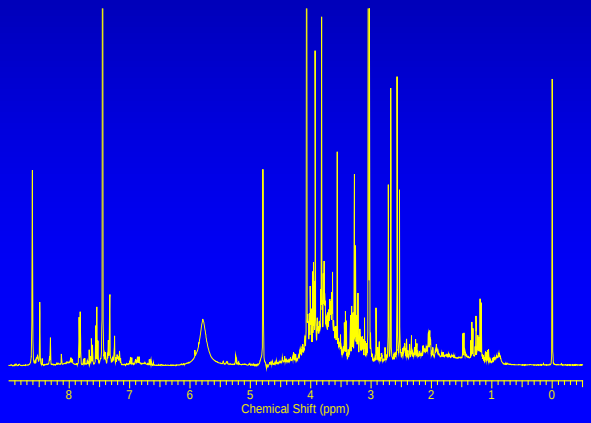


16 strain inbred mouse panel of acute trichloroethylene (TCE) dosing

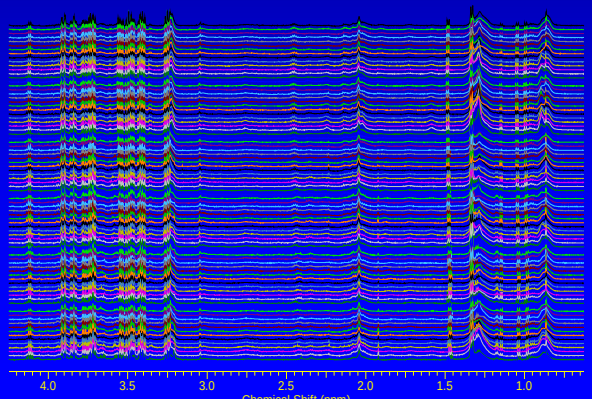
Global metabolomics identifies two responder "metabotypes" (global NMR)

Responder metabotypes have distinct levels of DCA metabolite (targetted MS)

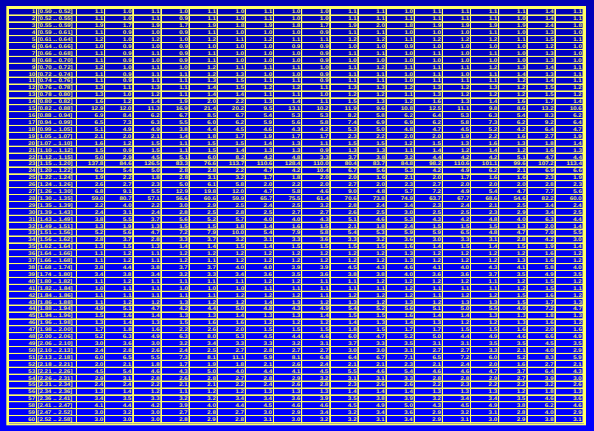
Overall Process of Metabolomics Investigations



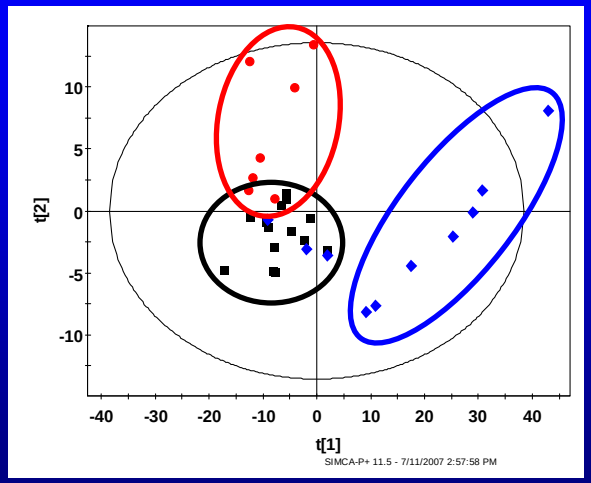
NMR spectra



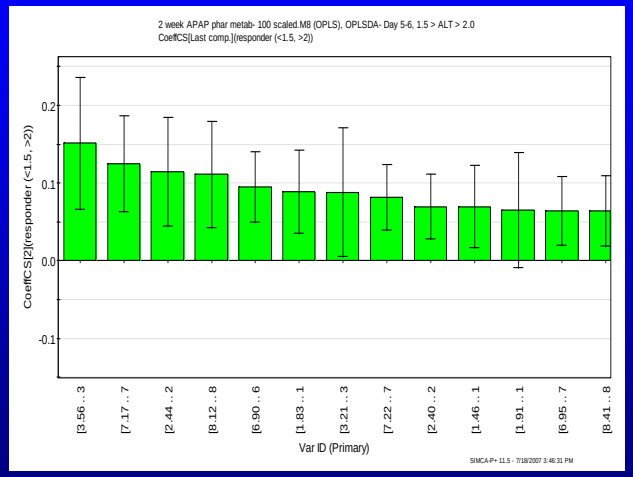
High throughput collection



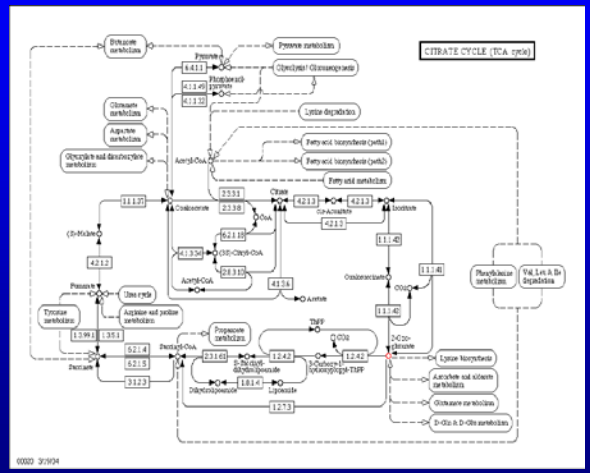
Data Processing/Reduction



Multivariate Statistics



Identify Critical Metabolites



Pathway Analysis

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