

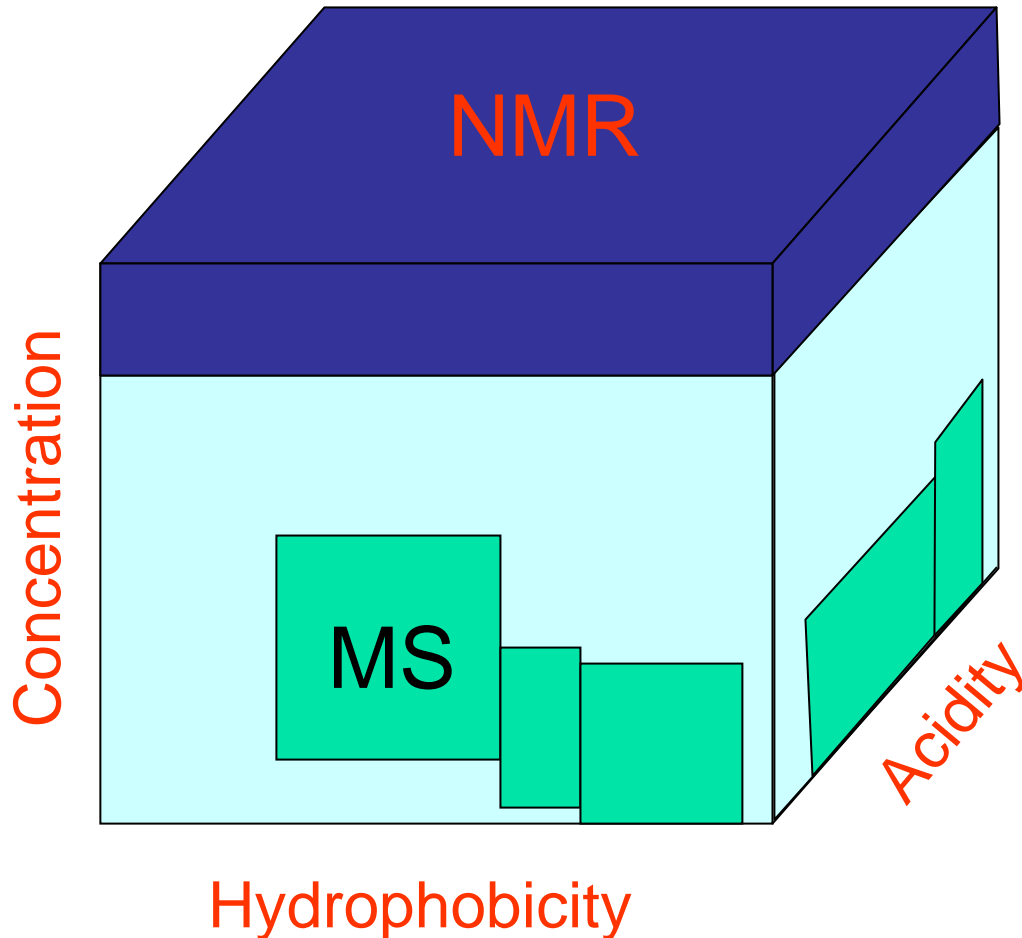
Metabolomics-Based Methods for Biomarker Discovery of Disease

Daniel Raftery
Professor of Chemistry

PURDUE
UNIVERSITY



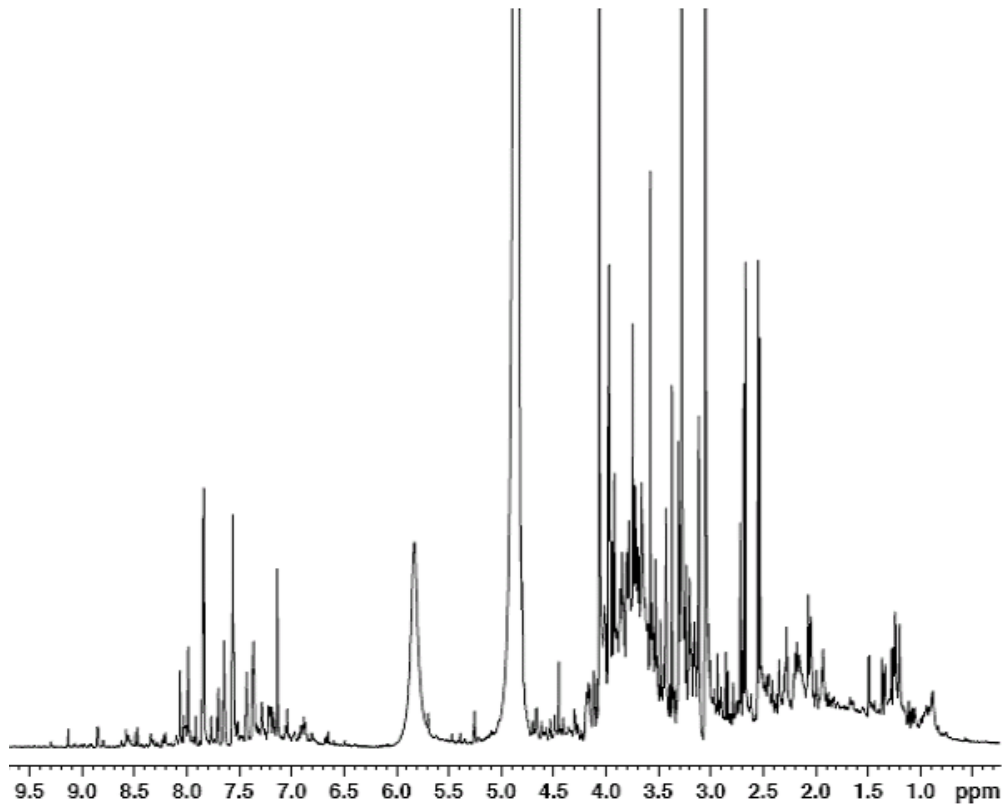
Analytical Methods for Metabolite Profiling



NMR provides broad coverage of metabolites and is quantitative and very reproducible

MS is 10^3 more sensitive, but less quantitative, and more selective.

Challenges



800 MHz urine spectrum from a healthy male volunteer

NMR Sample of Human Urine

NMR:

- Complexity

100's of compounds
1000's of lines

- S/N

Low concentration,
Chemical noise

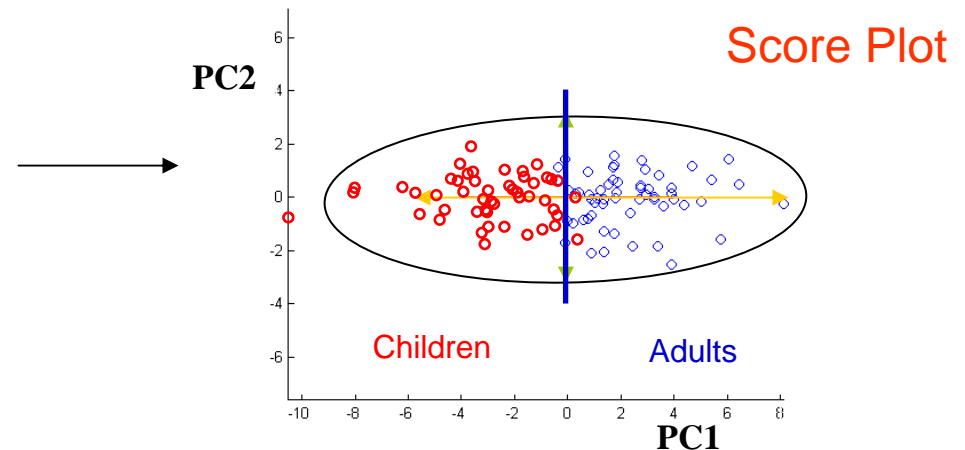
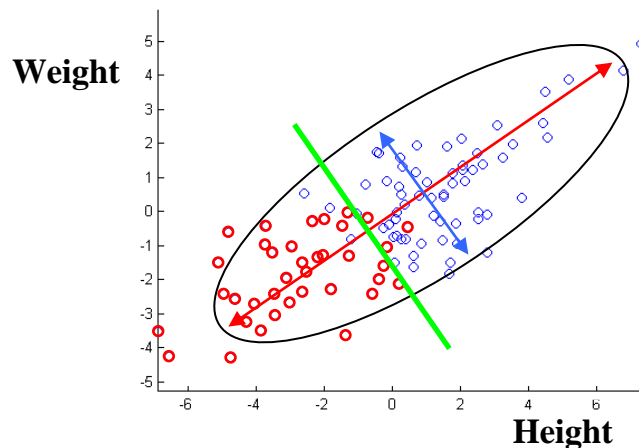
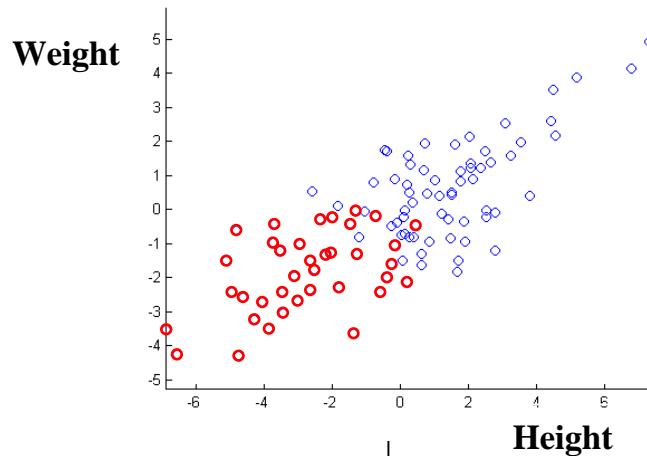
MS:

- Reproducibility

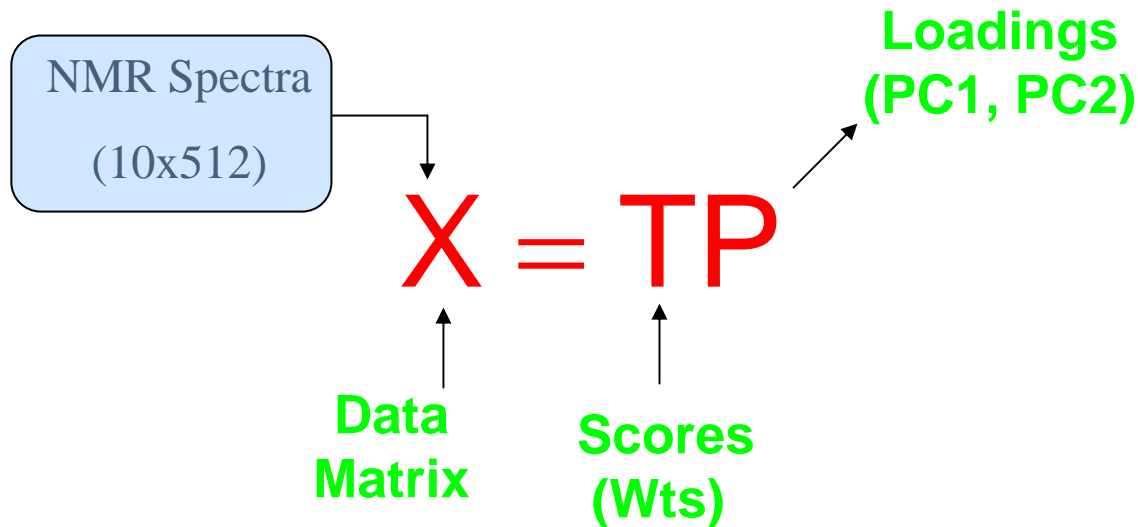
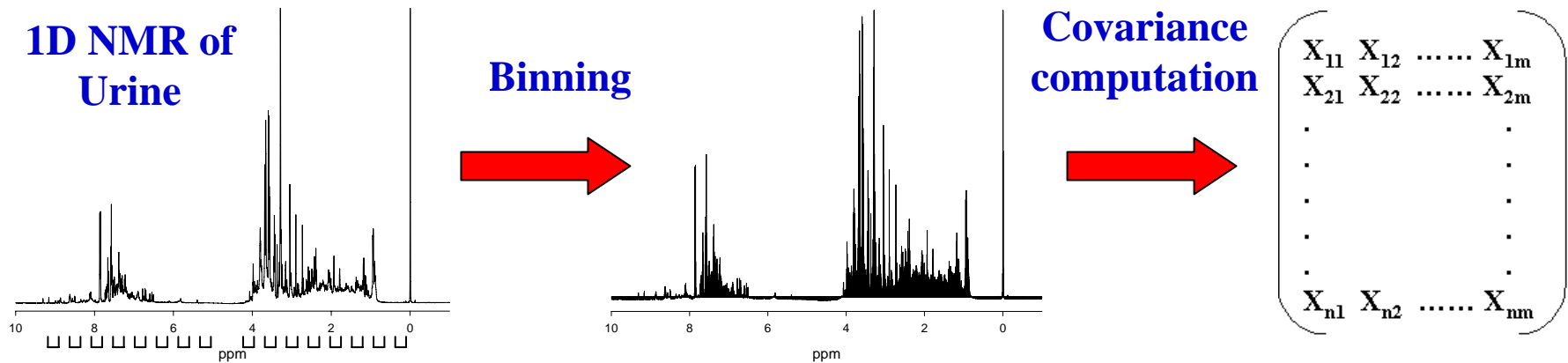
Ion suppression
Chromatography

Principal Component Analysis (PCA)

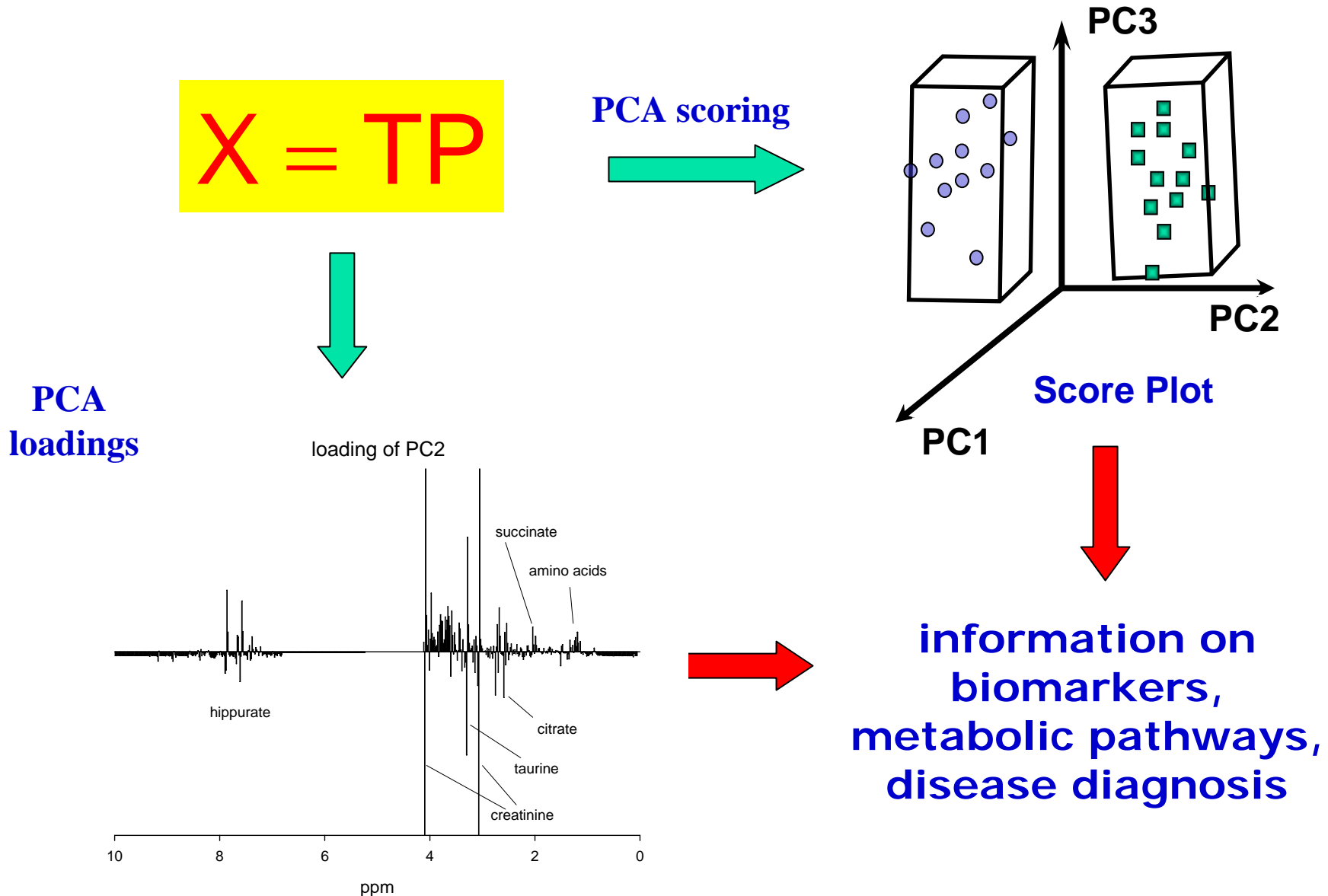
- Starting point in multivariate analysis
- The goal of PCA is to find the direction of the greatest variability
- The **score plots** allow one to classify clusters
- The corresponding **loading plots** show how they are classified



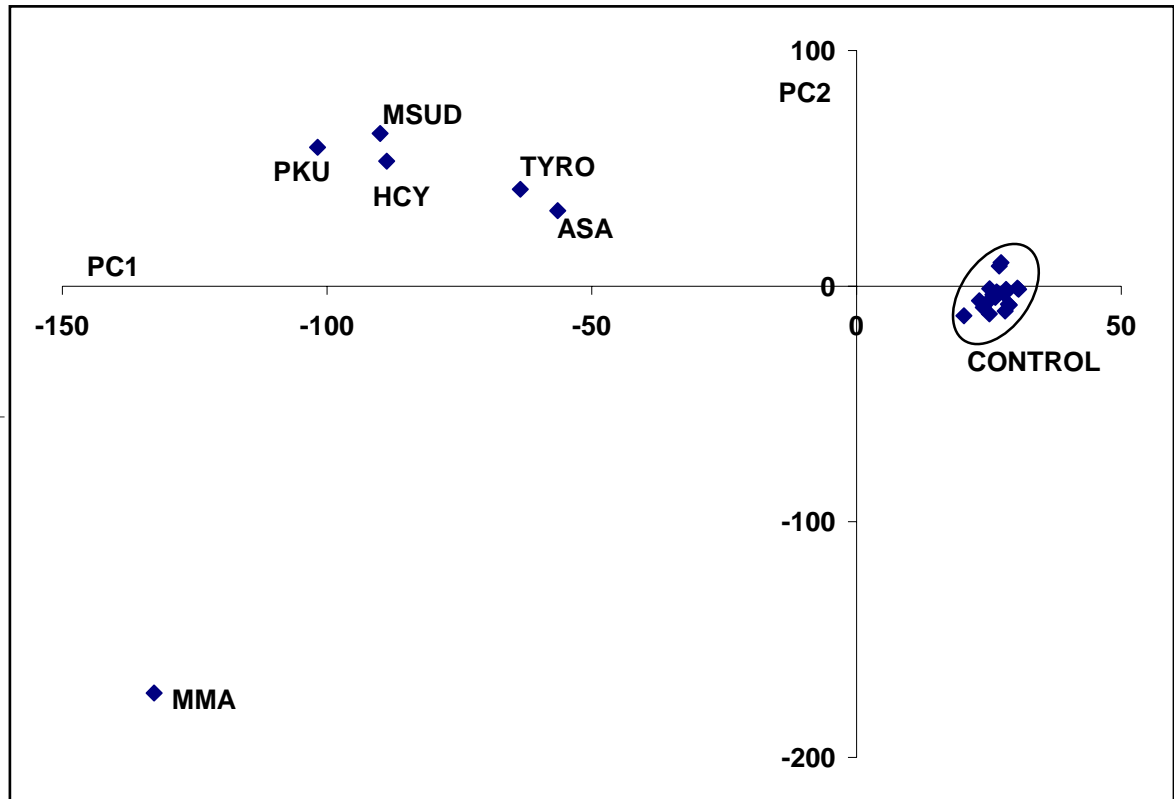
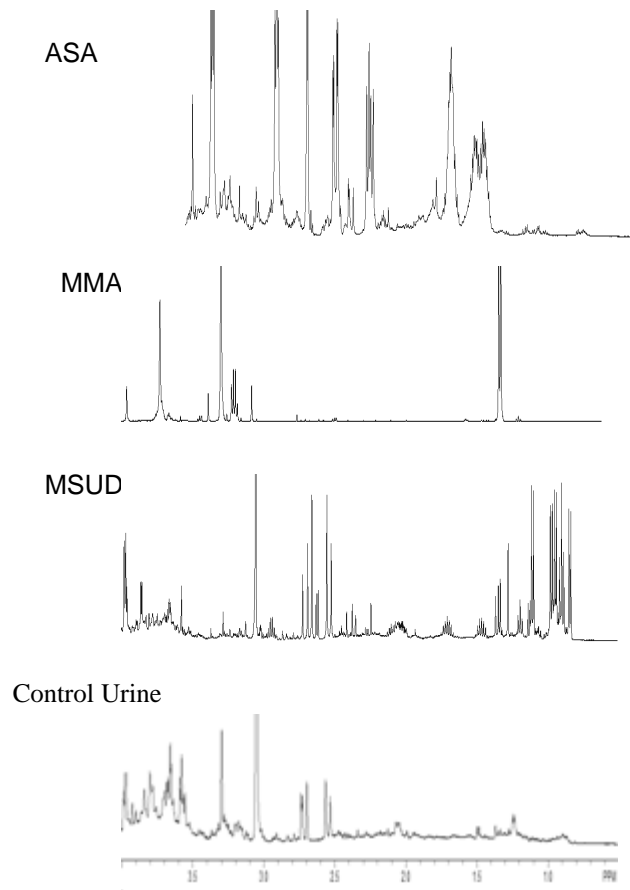
PCA Procedure for Biofluid Samples



PCA Procedure for Biofluid Samples

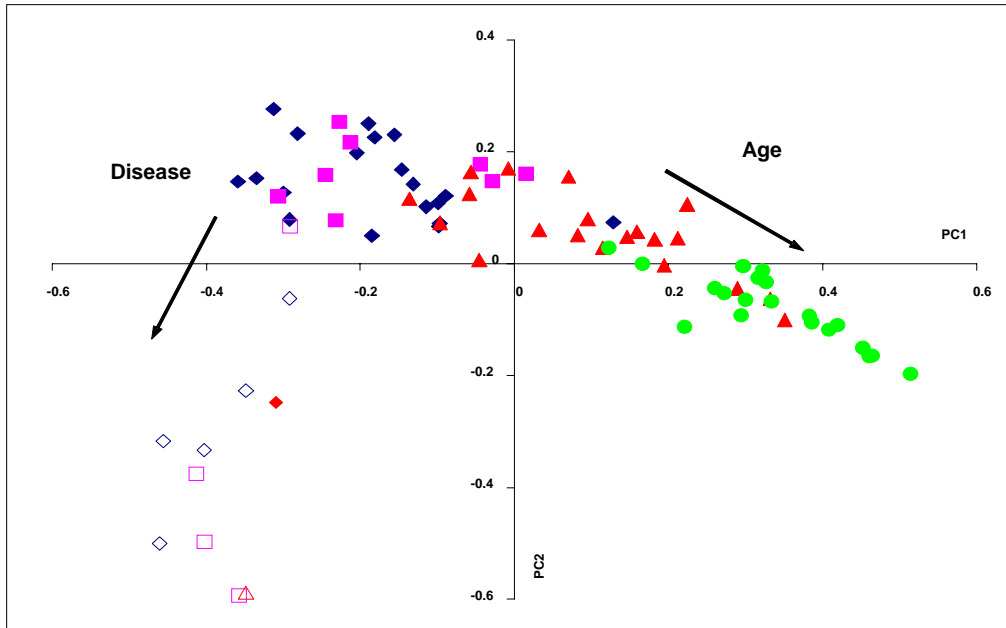


(1) Human Inborn Disease Study



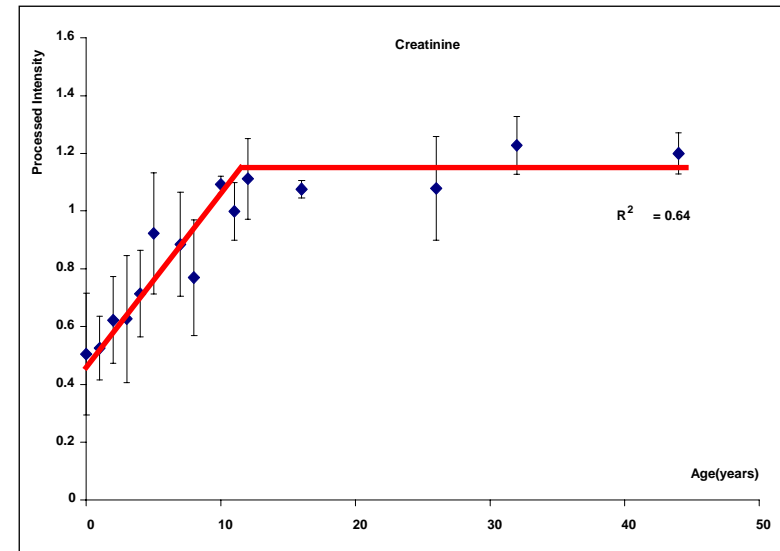
NMR can be used for metabolic profiling of IEMs. Biomarkers for certain (IEMs can be obtained from the loading plots. Metabolic pathways can be analyzed for the purpose of disease detection and screening.

Human Inborn Disease Study



Using a larger number of samples, one can still discriminate disease, but we also see an age effect in the samples.

This can be eliminated, or studied using supervised studies such as OSC-PLS.

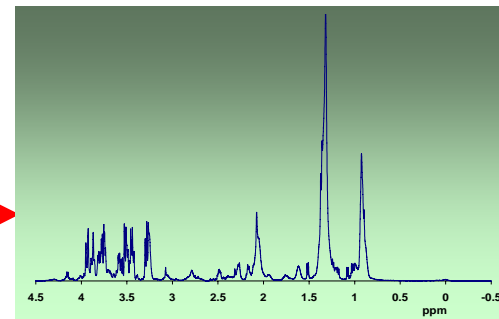


(3) Combining NMR and MS Methods:

Nuclear Magnetic Resonance (NMR) Spectroscopy



High resolution NMR



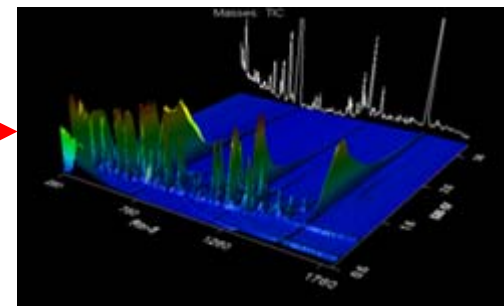
Essentially orthogonal methods can detect hundreds of small molecules in blood or urine.

Sample

Mass Spectrometry (MS)

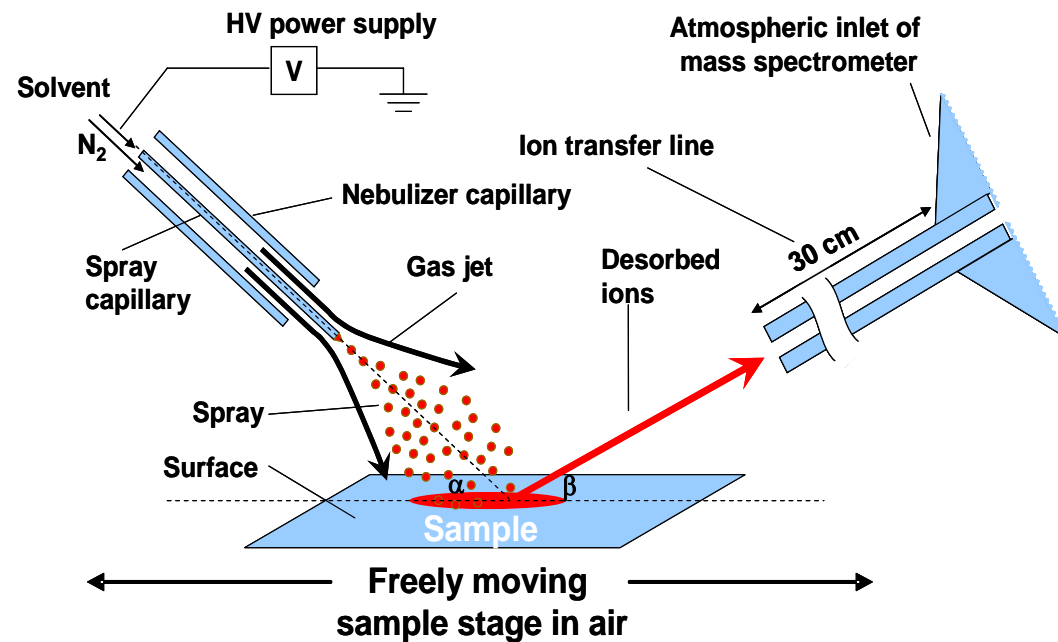


New MS technologies



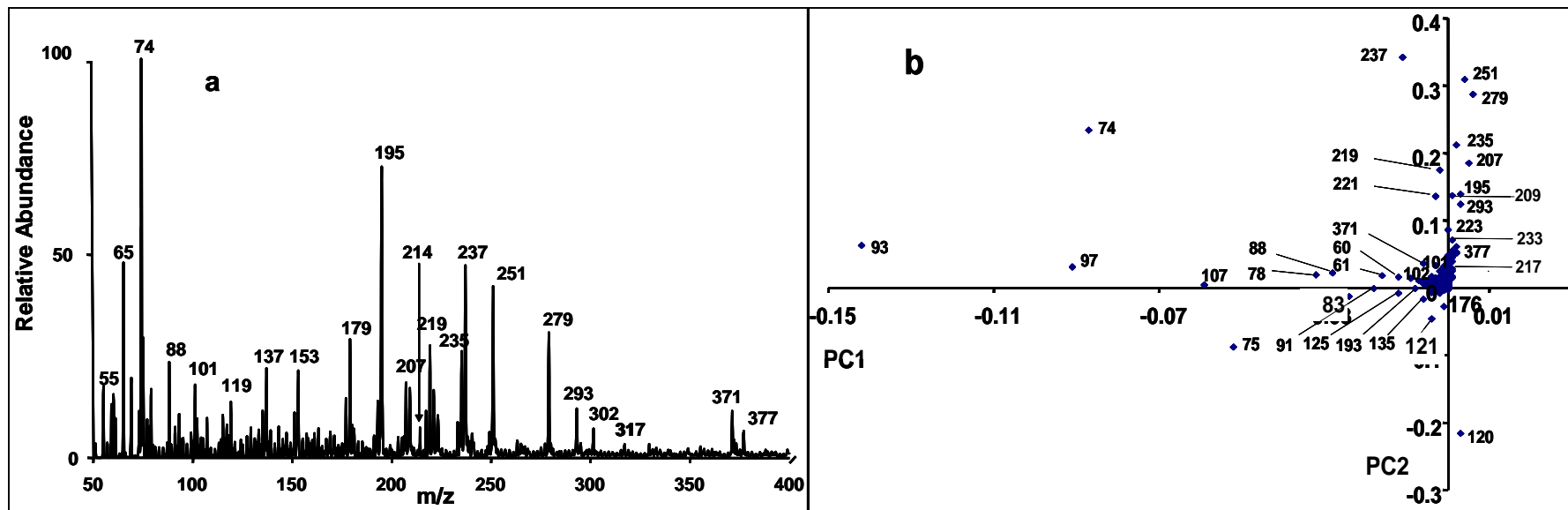
Combined approach improves disease detection, metabolite identification, pathway monitoring, systems biology studies, etc.

DESI MS



Desorption-Ionization Mass Spectrometry allows atmospheric introduction of samples from a surface.

DESI-MS of Mouse Urine

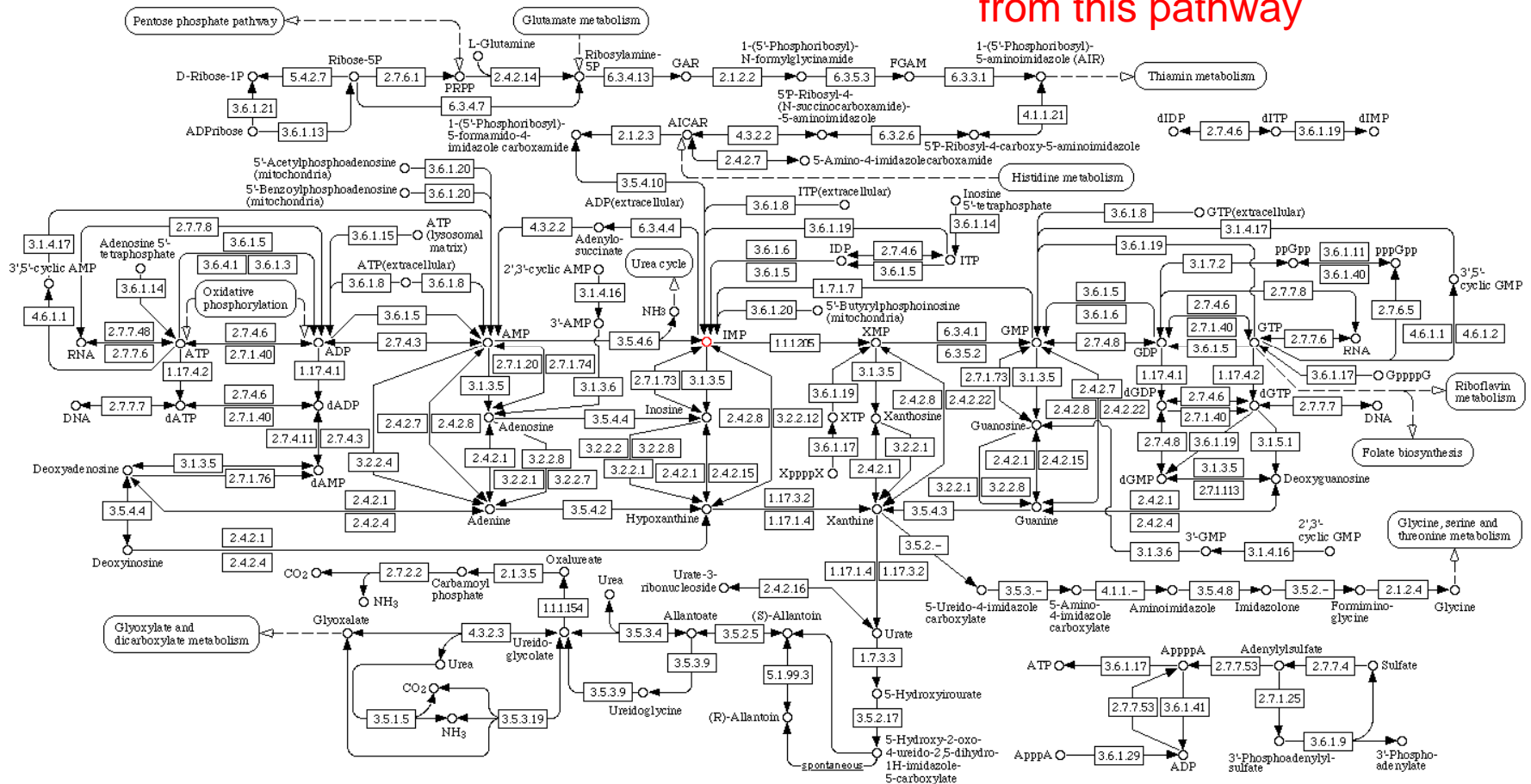


DESI-MS spectrum of mouse urine and corresponding PCA plot from a study of mouse-lung cancer. 80 compounds were identified as differentiating the tumor-bearing and healthy mice.

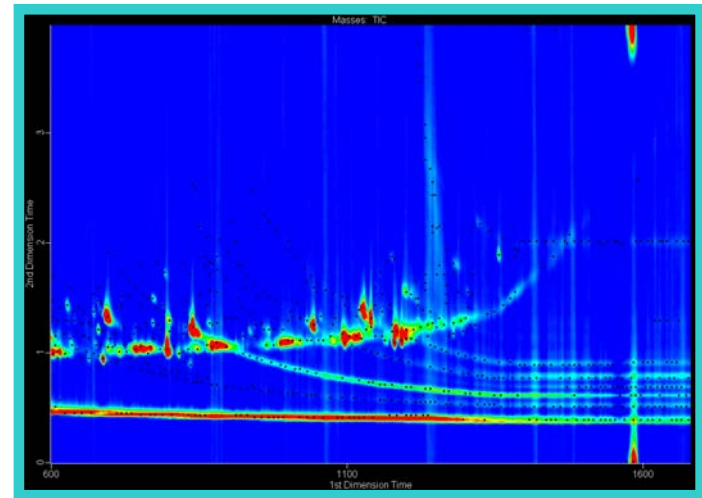
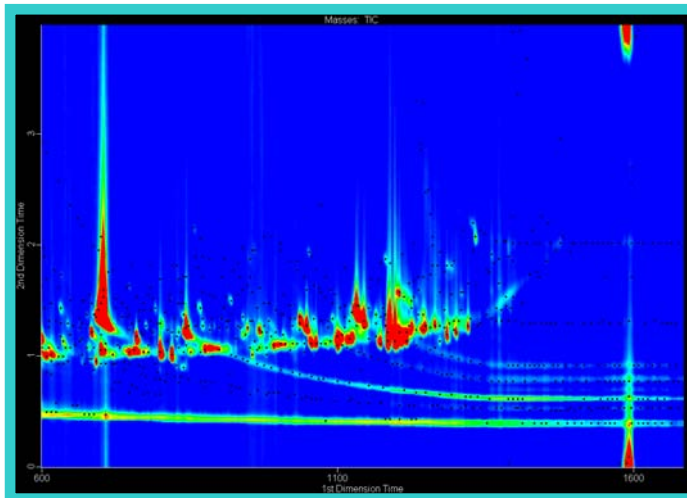
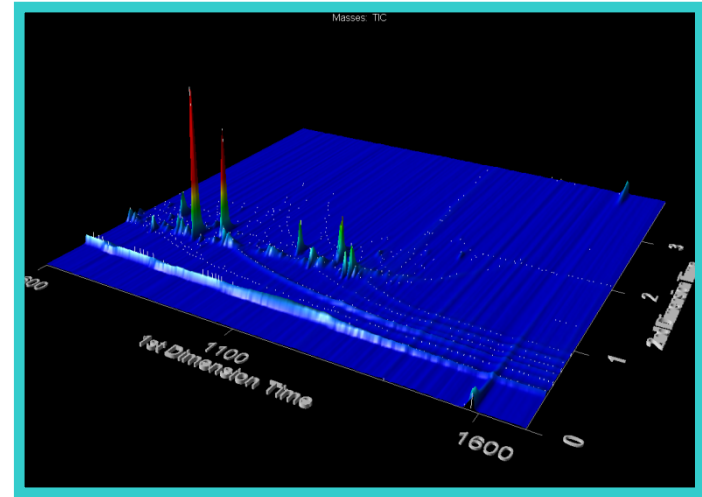
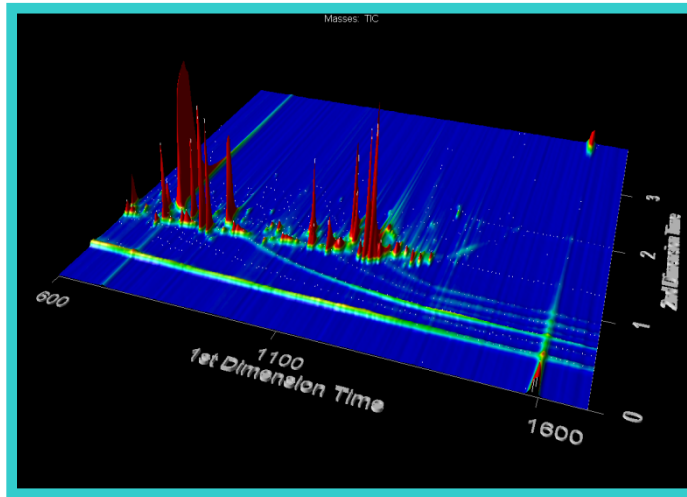
Purine Metabolism Pathway Changes Indicated in Mouse Lung Cancer Study

26 compounds that changed concentration were observed from this pathway

PURINE METABOLISM



GCxGC/MS Spectra of Healthy Control and Cancer Samples

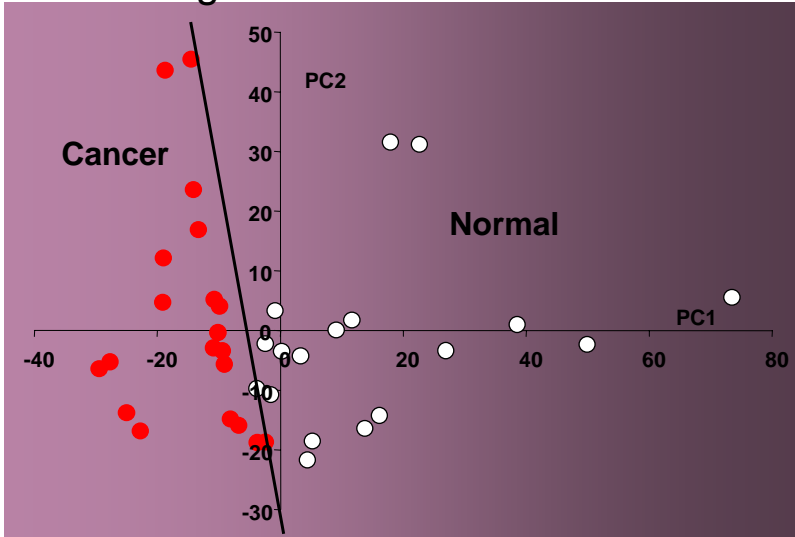


Normal

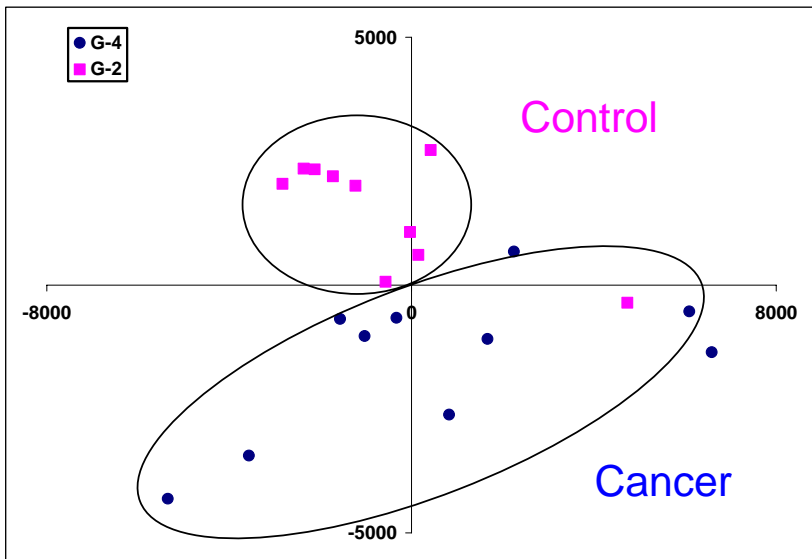
Cancer

Combining MS and NMR

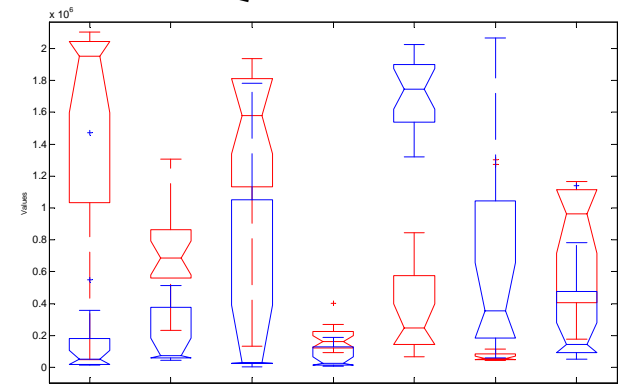
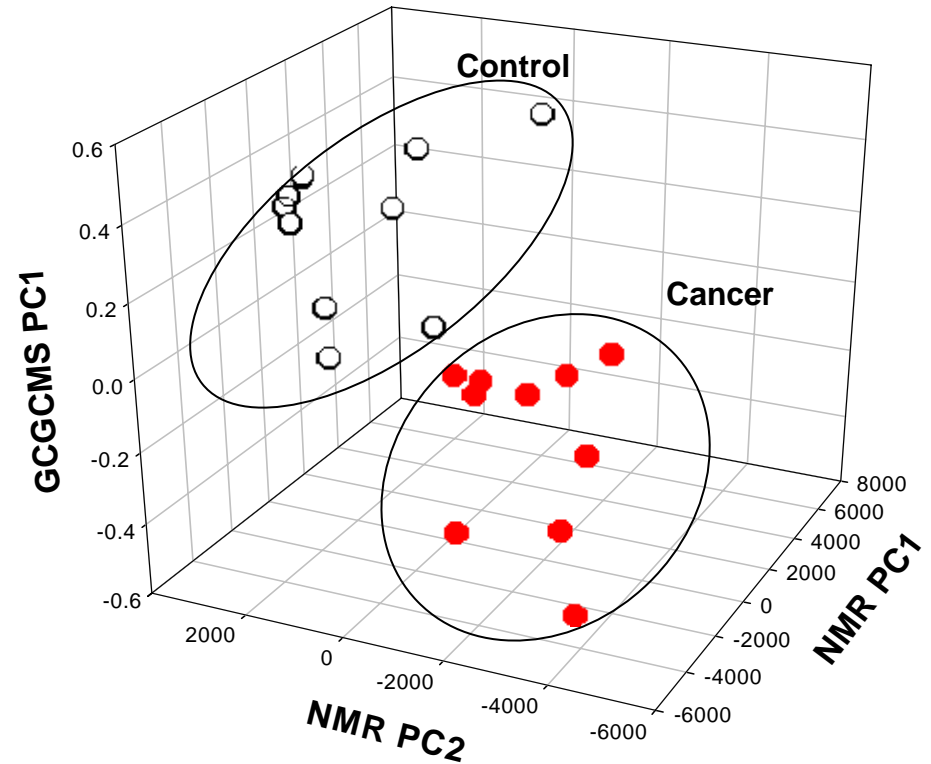
Starting with GC data, we get a pretty good discrimination:



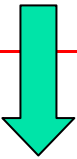
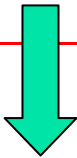
Then add NMR data:



Putting the orthogonal measurements together, we improve the discrimination:



Our Approach: Discovery and Validation

	Un-targeted	Targeted
Discovery	<p>Initial sample set</p> <p>Run NMR, MS</p> <p>Discover “biomarkers”</p>	<p>Literature-based “biomarkers”</p>
		
Validation	<ol style="list-style-type: none">1) Test “biomarkers” against additional sample sets.2) Determine diagnostic accuracy	<ol style="list-style-type: none">1) Test “biomarkers” against several sample sets.2) Determine diagnostic accuracy

Overlaying detected metabolic changes over known pathways improves understanding, provides biological validation, and provides potential targets.

Conclusions

Metabolite profiling provides an important piece to help understand the systems biology puzzle.

Advanced methods in NMR, MS, statistics including correlations are useful in identifying and quantifying selected components in complex mixtures.

Combining NMR and MS provides a powerful approach for improving classification and identifying biomarkers. Correlation with proteomics or genomic data is also possible to improve the biological picture.

Implications for the future include improved, chemical based **early disease detection**, **therapy feedback**, **disease monitoring**, and **personalized therapies**, as well as faster and safer **drug development** and **nutritional** studies.