BSc Pharmacology and Translational Medicine

Module: Principles of Pharmacodynamics and Pharmacokinetics
Lecture: Introduction to Metabonomics
Date: Thursday 1st November 2012

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Introduction to Metabonomics

Learning Objectives

By the end of the lecture and the associated learning activities the student should:

- Be able to give a definition or description of metabolic profiling (metabonomics/metabolomics)

- Describe some of the reasons metabolites are important in understanding health and disease

- Compare a top-down approach to more traditional approaches for investigating biological problems

- Outline the workflow of a metabonomics experiment, including the type of samples that might be used, the type of instrumentation that might be used to characterise them, what the resulting data reflect, and how this data might be analysed.

Introduction to Metabonomics

Lecture Outline

- Background - Biomarkers
  - What are biomarkers?
  - Where do we find them?
- Background - Metabolism
  - Why profile metabolites?
  - Metabolism and systems biology
  - Some definitions and analysis strategy
- Generating ^{1}H NMR Metabolic Profiles
  - Recap on ^{1}H NMR spectroscopy
  - Common Sample Types
    - Biofluids
    - Tissues
- Multivariate Analysis of Spectral Data Sets
  - Pattern recognition
    - Unsupervised analysis
    - Supervised analysis
- Other Analytical Platforms for Metabonomics
- Characterising Biomarkers
  - Statistical correlation tools - STOCSY
- Application Areas of Metabonomics
- Summary

Background - Biomarkers

Background - Biomarkers

What are Biomarkers?

NIH definition:

"*a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention* "

Biomarkers Definitions Working Group (2001).

Where do we find them?

Many sampling source (urine / plasma / tissue / breath / etc.)
Combinations of measurements

Considerations for sampling sources:

Urine	Time-average systemic excretion
Blood	'Snapshot' of system
Tissue	Likely site of injury/concern/action/effect

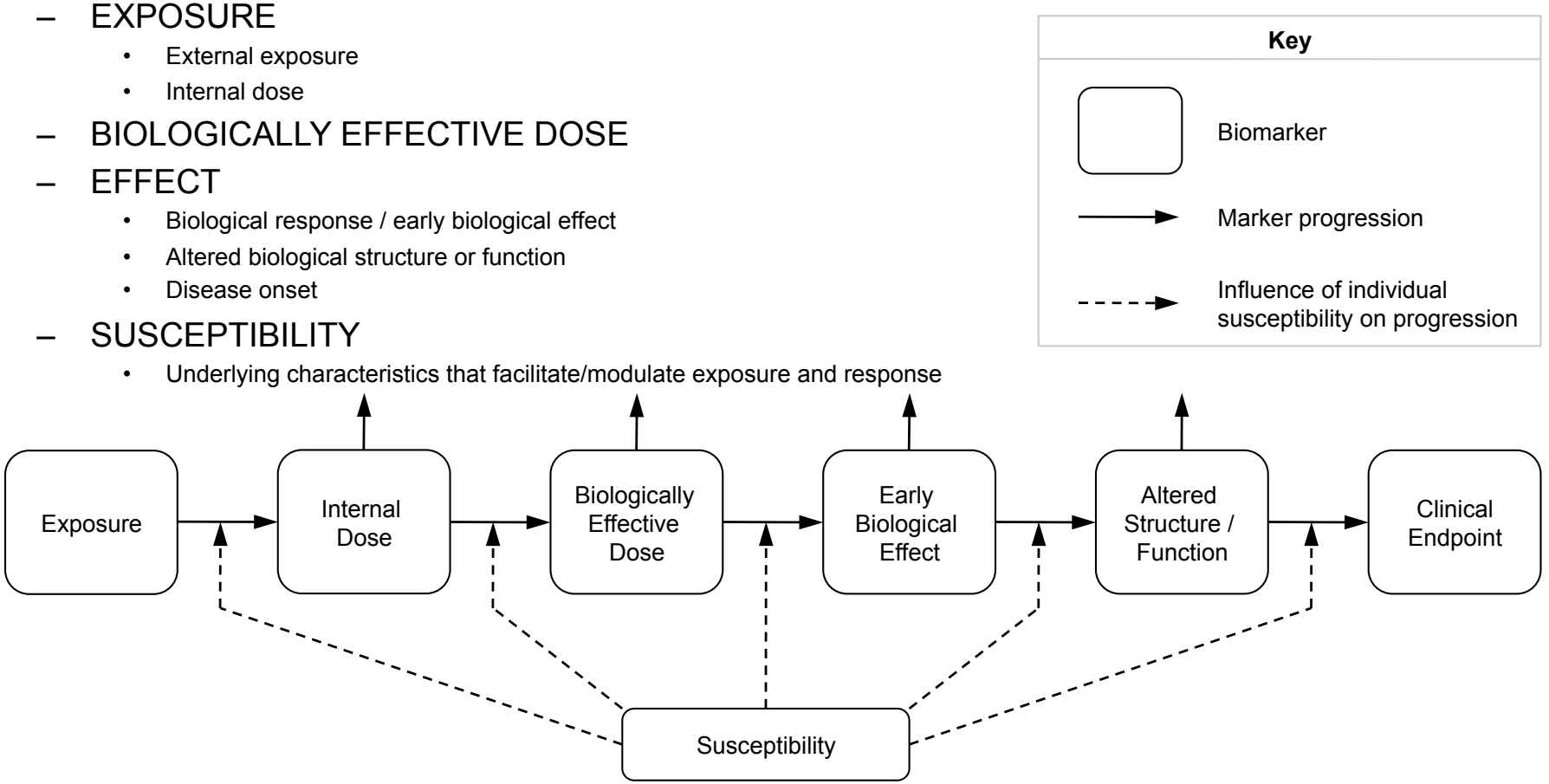
Biomarkers can potentially:

Report *more specifically* on type of exposure
Give a better idea on the exposure that has *actually occurred*
Reflect a variety of stages in the initiation and progression of disease, and efficacy of treatment

Background - Biomarkers

Where do we find them?

- Finding biomarkers
  - EXPOSURE
    - External exposure
    - Internal dose
  - BIOLOGICALLY EFFECTIVE DOSE
  - EFFECT
    - Biological response / early biological effect
    - Altered biological structure or function
    - Disease onset
  - SUSCEPTIBILITY
    - Underlying characteristics that facilitate/modulate exposure and response



Adapted from: Committee on Biological Markers of the National Research Council. Biological markers in environmental health research. *Environ. Health Perspect*. 74: 3-9, 1987.

Background - Metabolism

Background

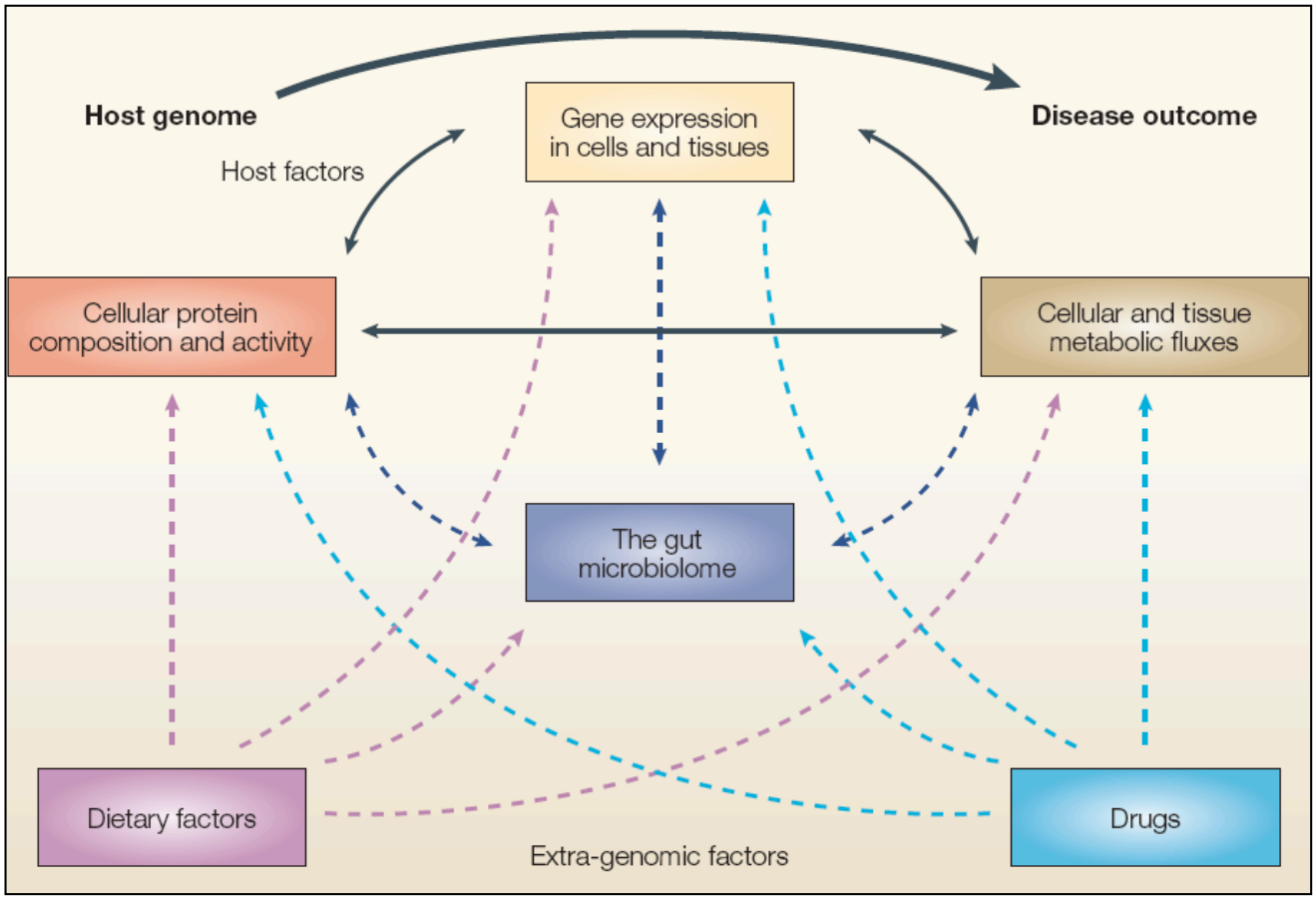
Why Profile Metabolites?

- Defines a metabolic phenotype

- Metabonomics…
  - works at the metabolic interface between a system's biology and its environment
  - can report on early consequences of exposure

- Constant exogenous input to the 'metabolome' mediating environmental interactions

Metabolism in Systems Biology



Complex nature of system interactions

Nicholson *et al.* 2007. Metabonomics: a platform for studying drug toxicity and gene function *Nat. Rev. Drug Disc.* **1** 151-161

Some Definitions

Metabonomics

"*…the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification…"* *- Metabonome*

Nicholson JK, Lindon JC, Holmes E. 1999. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica*. 29:1181-1189.

Metabolomics

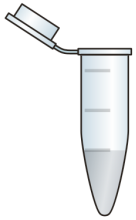
"...the complete set of metabolites / low-molecular-weight intermediates, which are context dependent, varying according to the physiology, developmental or pathological state of the cell, tissue, organ or organism…" *- Metabolome*

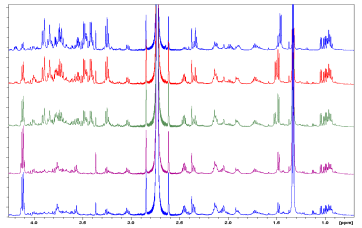
Fiehn O. 2002. Metabolomics – the link between genotypes and phenotypes. *Plant Mol. Biol.* 48(1-2) 155-171.

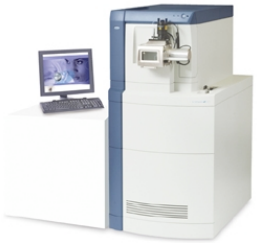
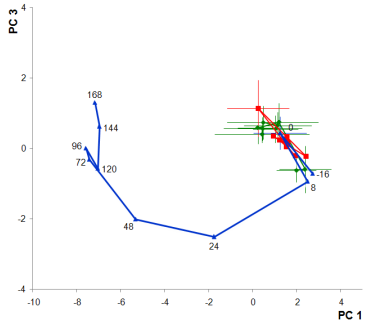
Metabolic Profiling

General term for 'omics' metabolite measurements

Outline of Analysis Strategy

Collection → Preparation → Analysis → Data Analysis

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1. **Generate profiles** that reflect the metabolic state of biological system under study
2. Use multivariate analysis to indicate **profile features related to intervention**/disease
3. Identify **metabolites responsible** for these profile features – putative biomarkers
4. Generate **testable hypotheses** to validate putative biomarkers
5. Use profiles or features **to derive classification models**

Generating ^{1}H NMR Metabolic Profiles

Generating ^{1}H NMR Metabolic Profiles

Recap on NMR Spectroscopy

- Nuclear Magnetic Resonance (NMR) Spectroscopy

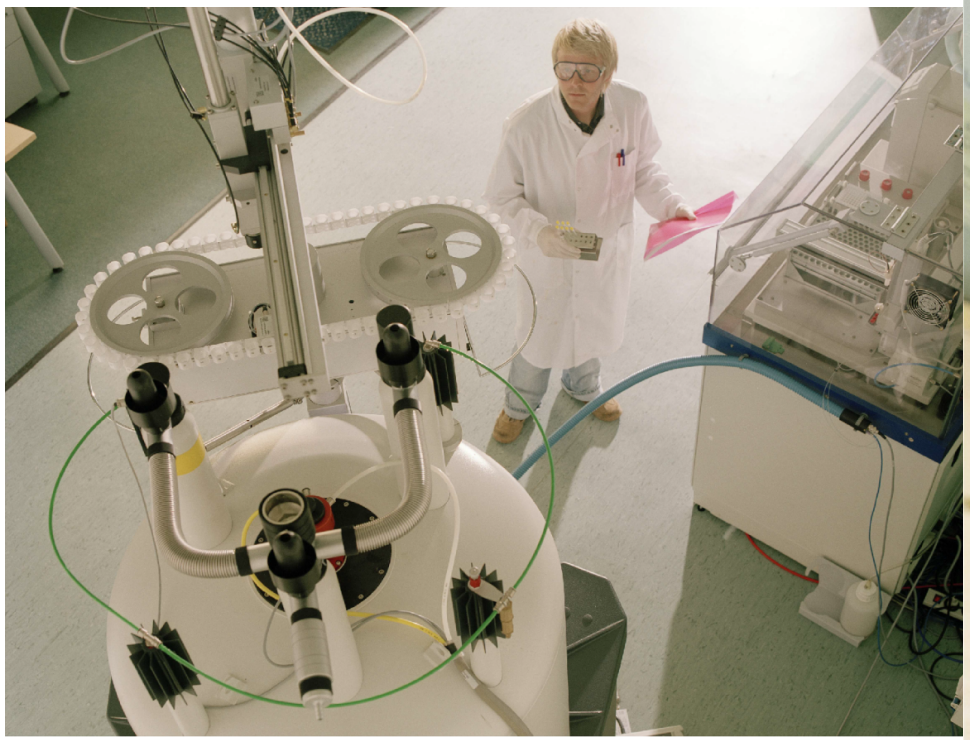
- Generates spectra that describe the chemical environment of nuclei in biofluid molecules
  - Chemical structure
  - Interactions
  - Bulk properties can be studied
  - Diffusion
  - Compartmentation

- Typically concentrate on ^{1}H

- Ubiquitous in biomolecules and drugs

Generating ^{1}H NMR Metabolic Profiles

Recap on NMR Spectroscopy



1. Place sample in a large magnet

2. Pulse with radiofrequency electromagnetic radiation

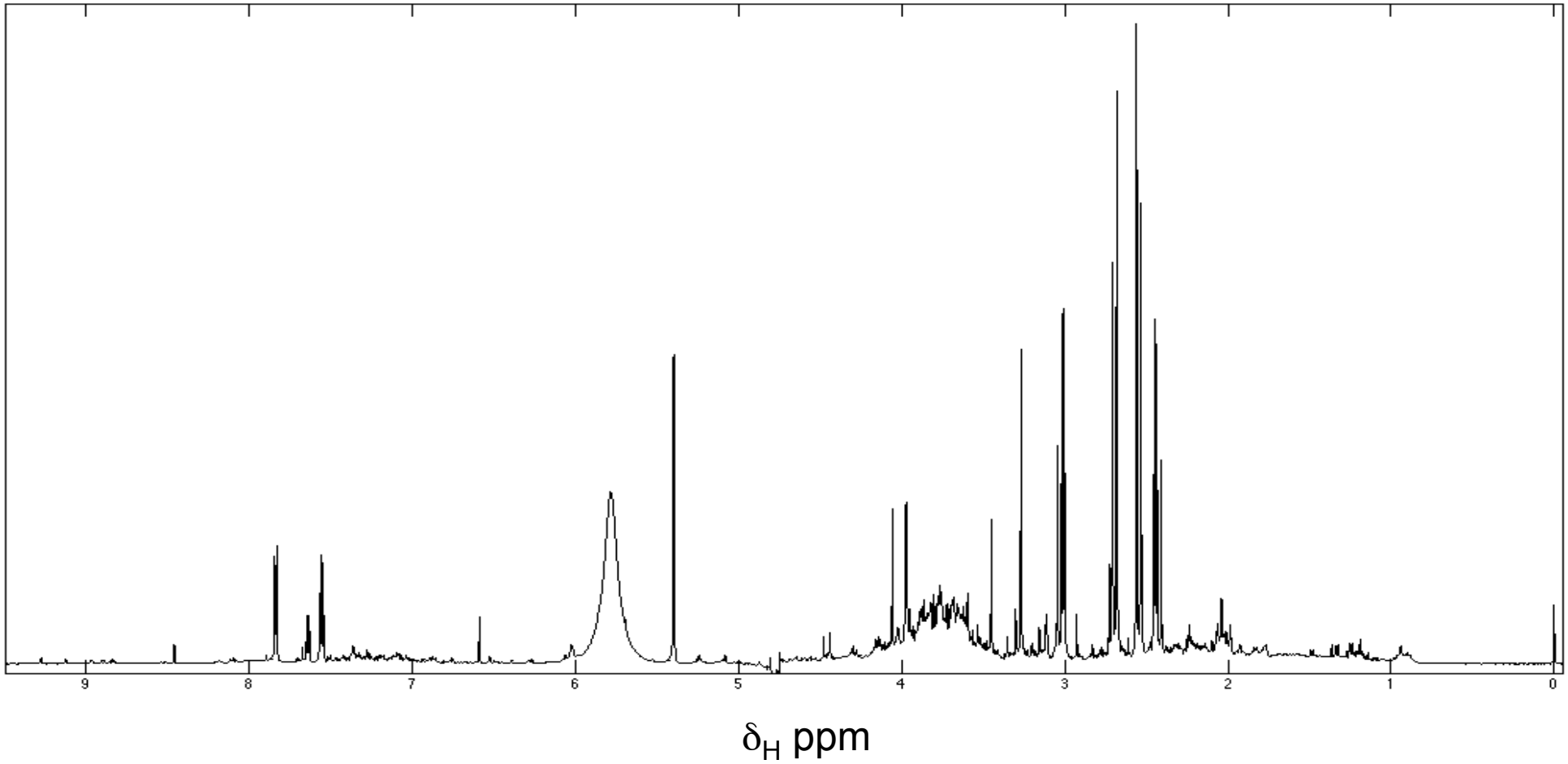
3. Detect resulting signal

Photos: © Metabometrix Ltd., London

Generating ^{1}H NMR Metabolic Profiles

Recap on NMR Spectroscopy

- 600 MHz 1H NMR spectrum of rat urine



Other Analytical Platforms for Metabonomics

Common Sample Types - Biofluids

- Urine

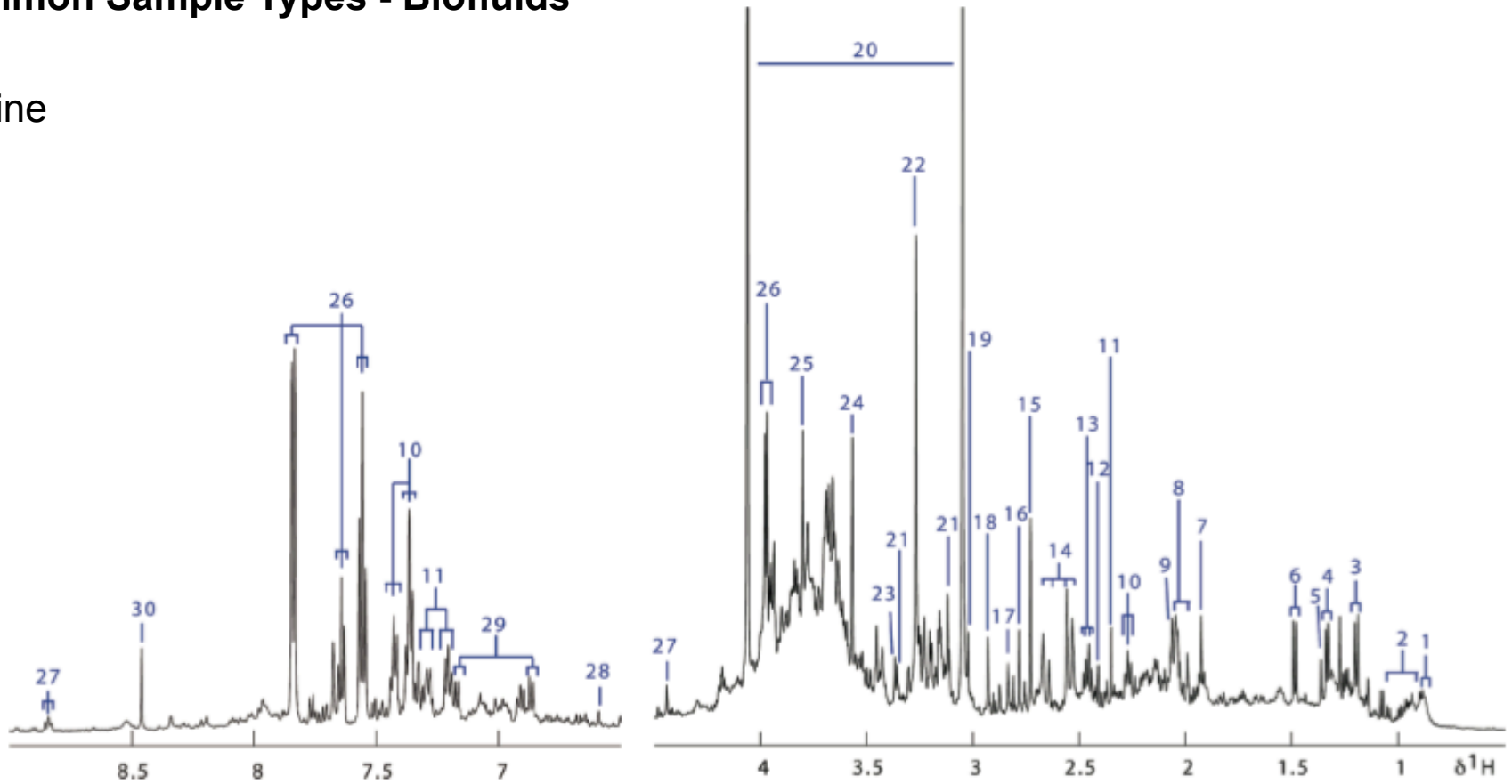


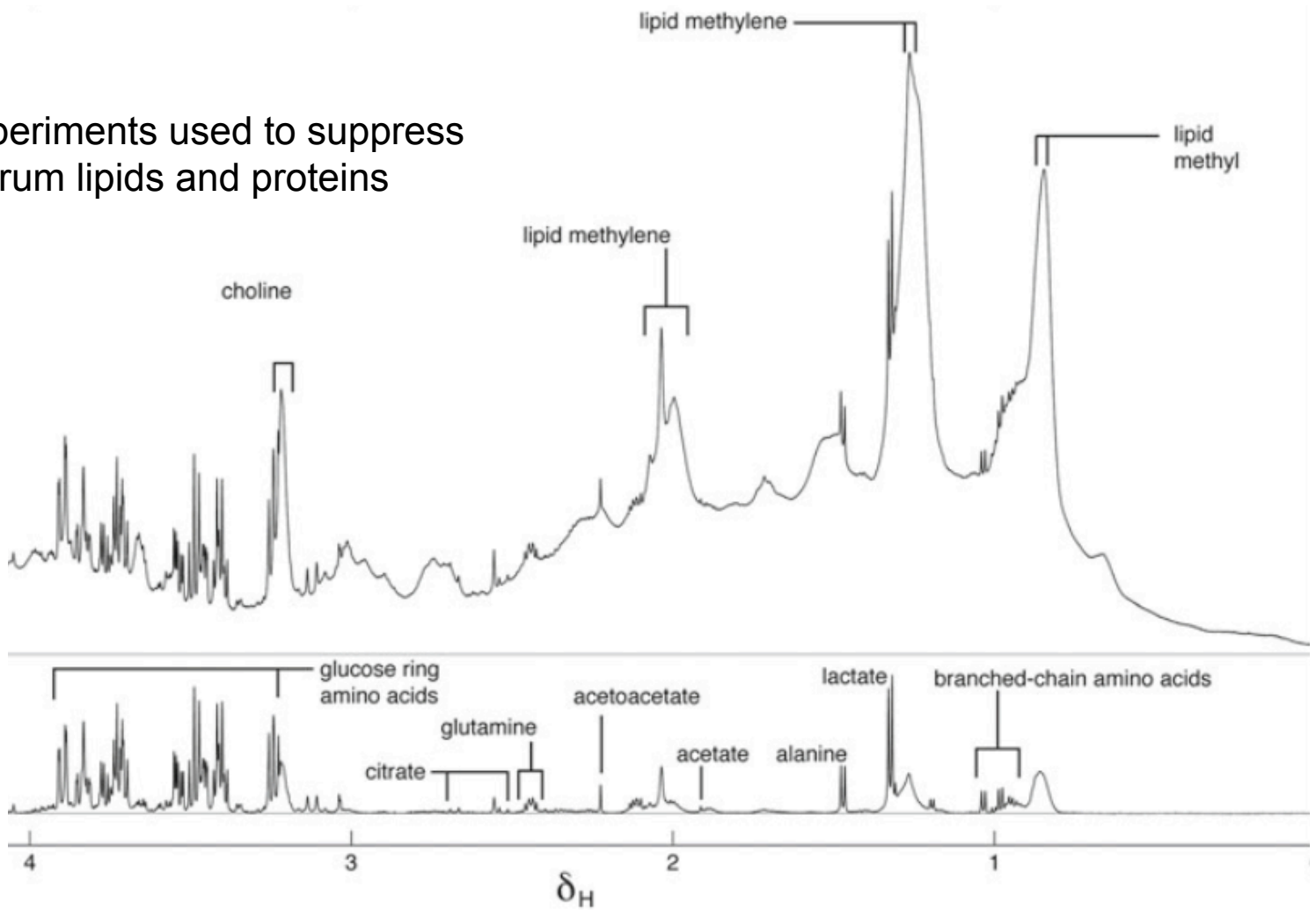
Figure 1. Median urinary ^{1}H NMR spectrum of INTERMAP Chinese population samples, based on the first urine collection ($N = 747$). 1, Pentanoic/heptanoic acid; 2, Branched-chain amino acids (leucine, isoleucine, valine); 3, D-3-hydroxybutyrate; 4, Lactate; 5, 2-hydroxyisobutyrate; 6, Alanine; 7, Acetate; 8, *N*-acetyls of glycoprotein fragments (including uromodulin); 9, *N*-acetyl neuraminic acid; 10, Phenylacetylglutamine; 11, 4-cresyl sulfate; 12, Succinate; 13, Glutamine; 14, Citrate; 15, Dimethylamine; 16, Methylguanidine; 17, Trimethylamine; 18, Dimethylglycine; 19, Creatine; 20, Creatinine; 21, Prolinebetaine; 22, Trimethylamine *N*-oxide; 23, *Scyllo*-inositol; 24, Glycine; 25, Guanidinoacetate; 26, Hippurate; 27, *N*-methyl nicotinic acid; 28, *Trans*-aconitate; 29, Tyrosine; 30, Formate.

Yap *et al.* 2010. J. Proteome Res. 9(12), 6647-6654

Other Analytical Platforms for Metabonomics

Common Sample Types - Biofluids

- Serum
- Special ^{1}H NMR experiments used to suppress broad signals from serum lipids and proteins
- "Spectral editing"
- 1D ^{1}H NMR
- 1D CPMG ^{1}H NMR

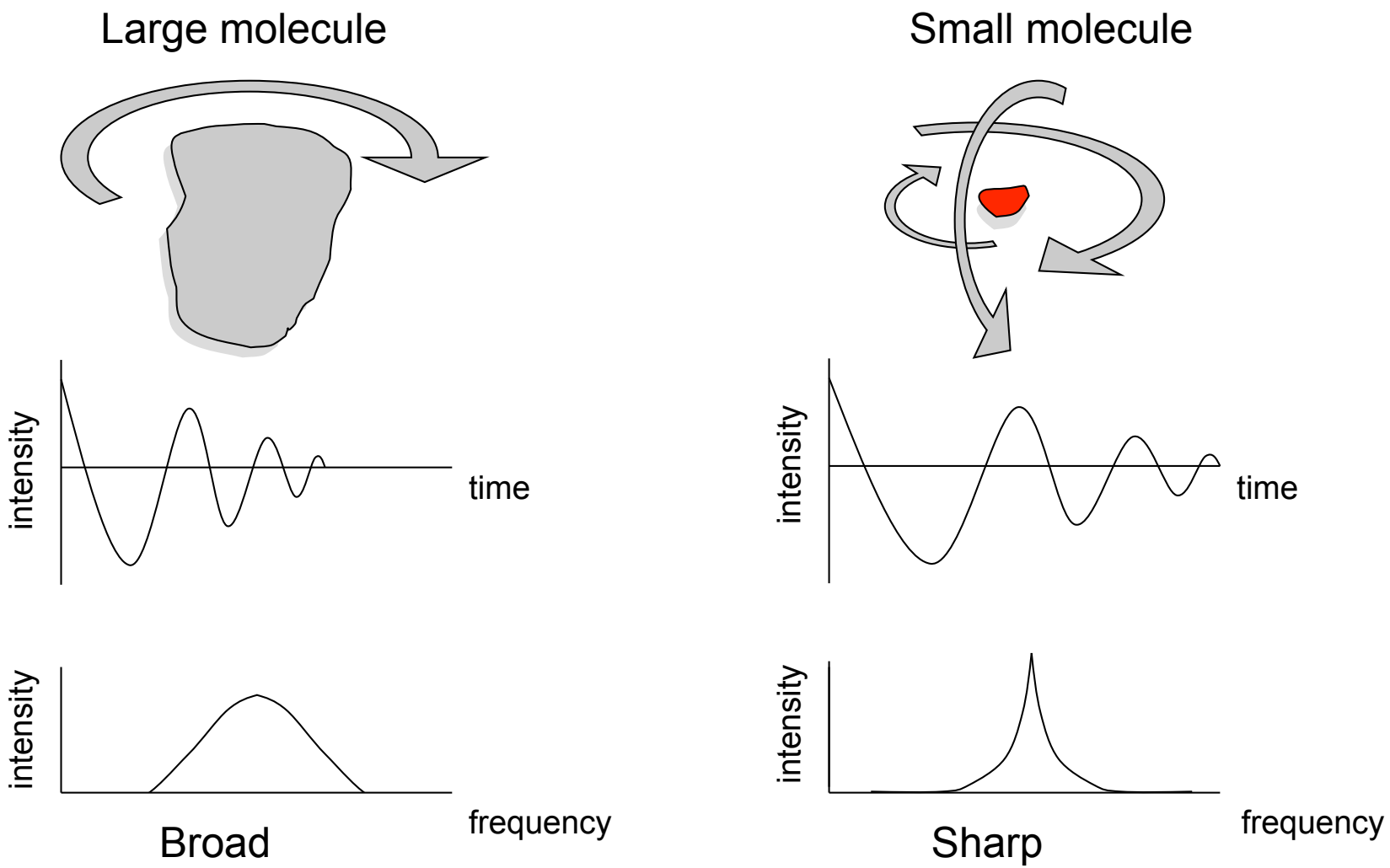


Keun and Athersuch. 2011. Metabolic Profiling: Methods in Molecular Biology 708. 321 – 334.

Other Analytical Platforms for Metabonomics

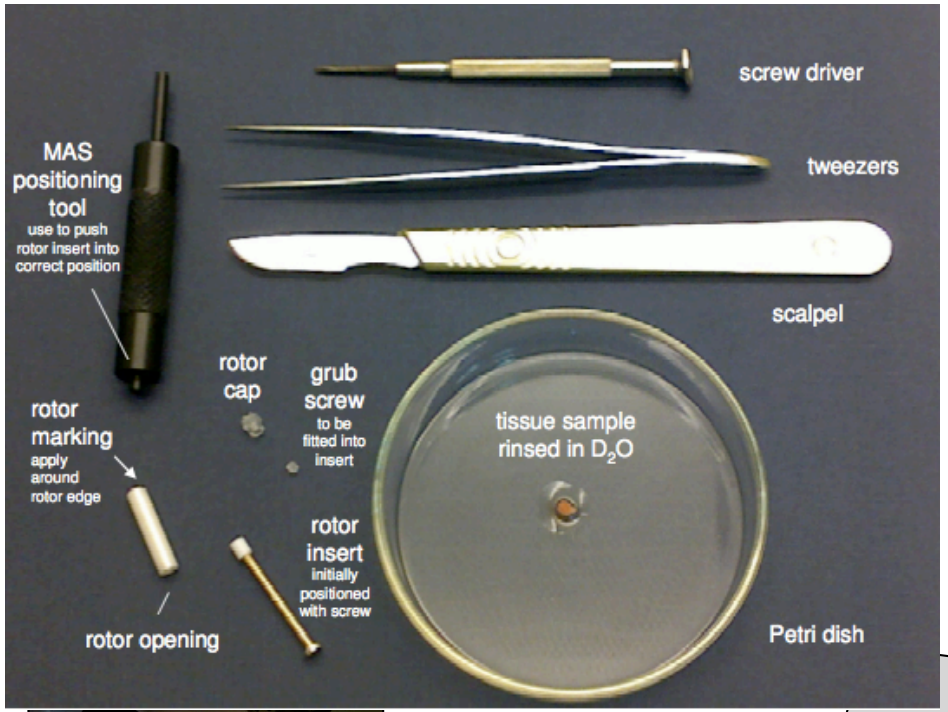
Common Sample Types – Biofluids

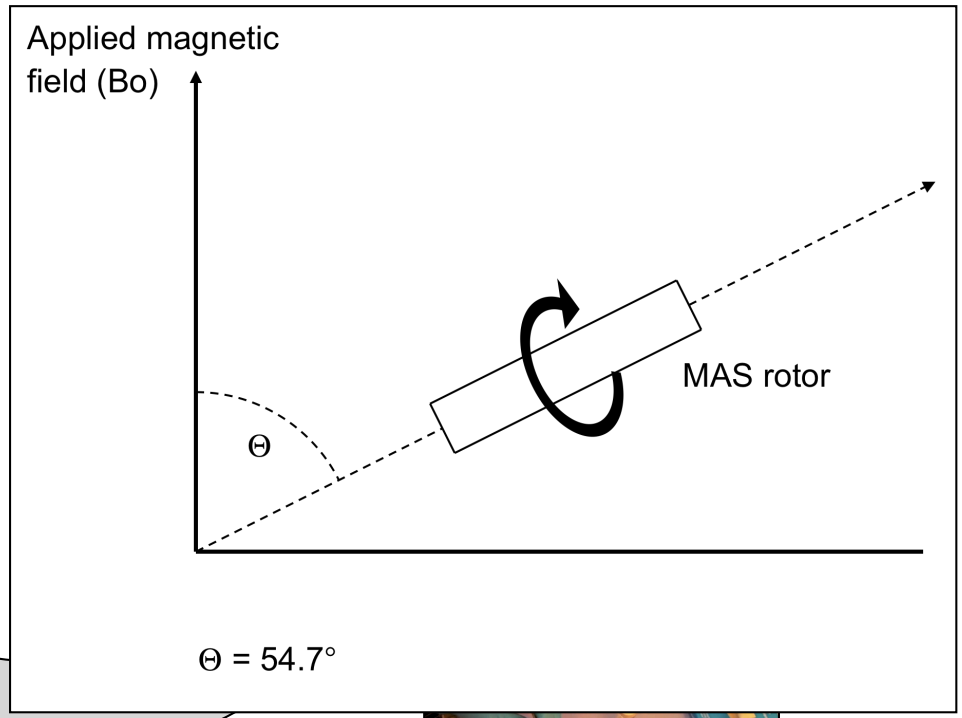
Spectral editing: Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence

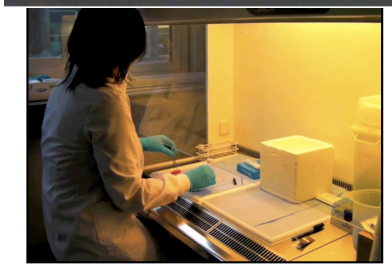


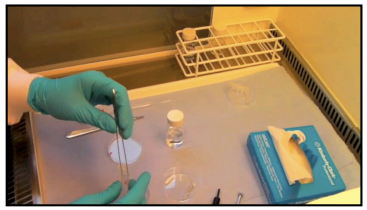
Generating ^{1}H NMR Metabolic Profiles

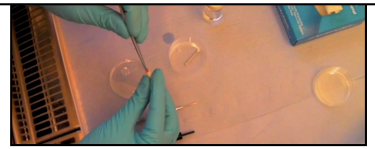
Common Samples Types – Tissue









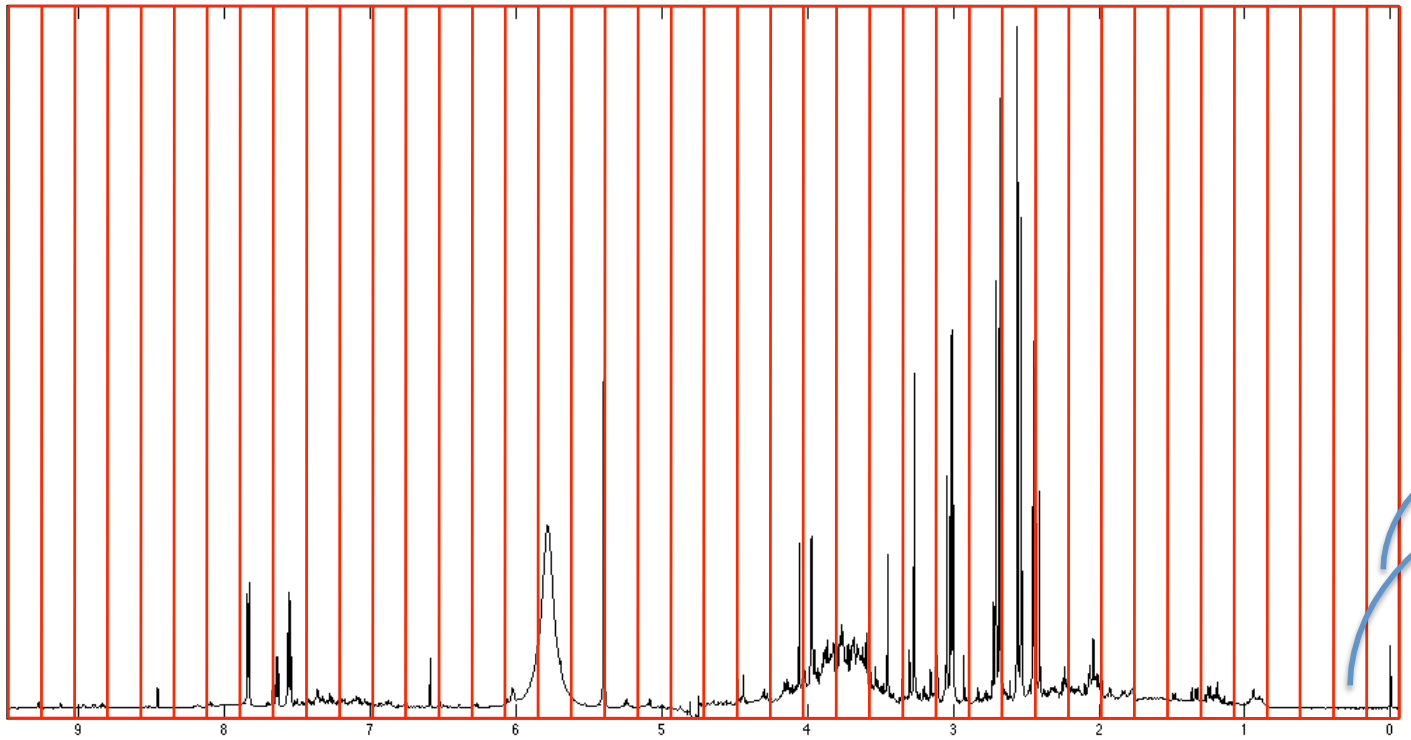


Multivariate Analysis of Spectral Data Sets

Multivariate Analysis of Spectral Data Sets

1D 1H NMR Data Amenable to Analysis

- Full resolution
  - e.g. 32 K points / spectrum
  - high computational load
  - potentially affected by minor chemical shift variation <- - - - >
- Integrated regions
  - arbitrary / targeted
  - ability to incorporate moderate chemical shift variation
  - less specific output from data analysis

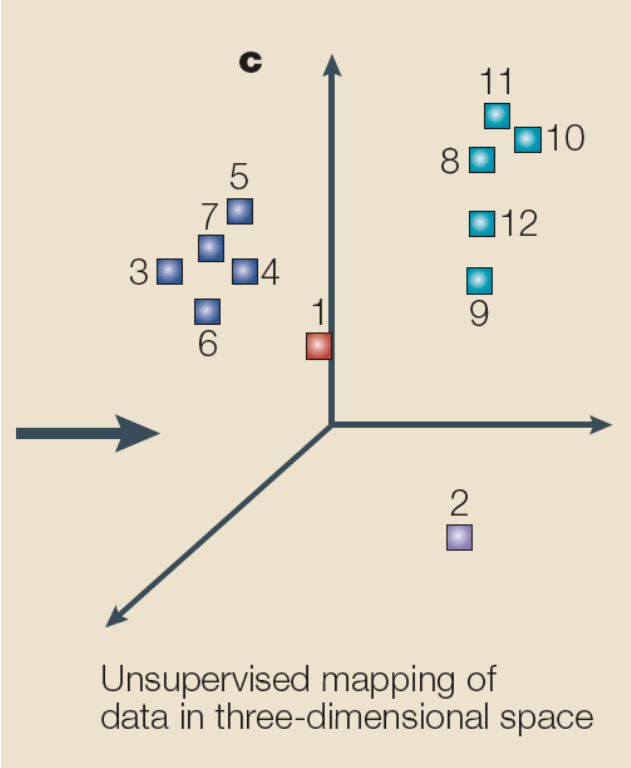


	Region			
	1	2	3	4
Sample 1				
Sample 2				
Sample 3				
...				

Multivariate Analysis of Spectral Data Sets

Pattern Recognition – Unsupervised Analysis

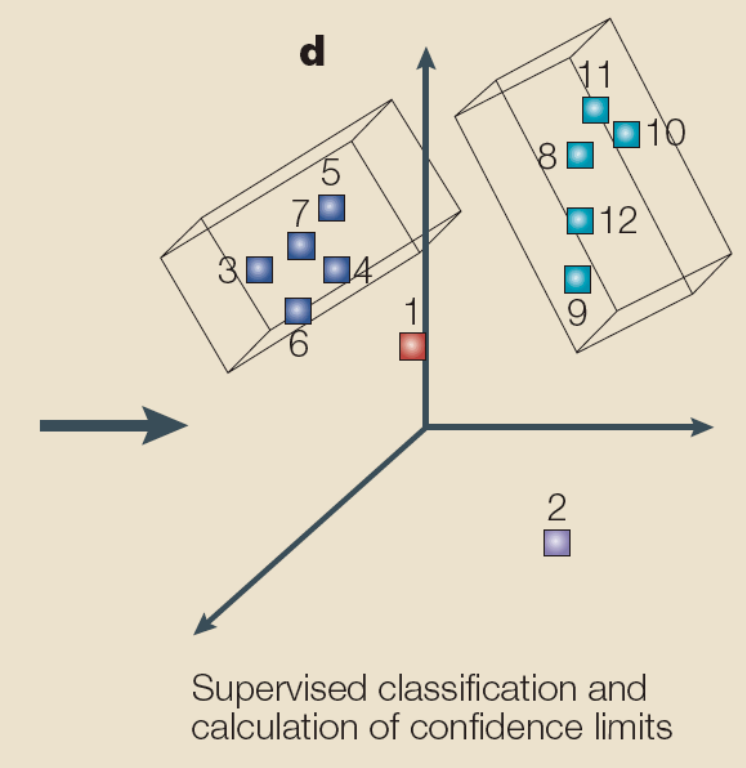
- Use only the information contained in the spectral data (latent information)
  - Multivariate analysis
    - e.g. principal component analysis (PCA), hierarchical cluster analysis (HCA, etc.



Multivariate Analysis of Spectral Data Sets

Pattern Recognition – Supervised Analysis

- Uses information about the samples themselves (e.g. class, timepoint, etc)
  - Multivariate analysis
    - e.g. soft independent modelling by class analogy (SIMCA), partial least squares (PLS)

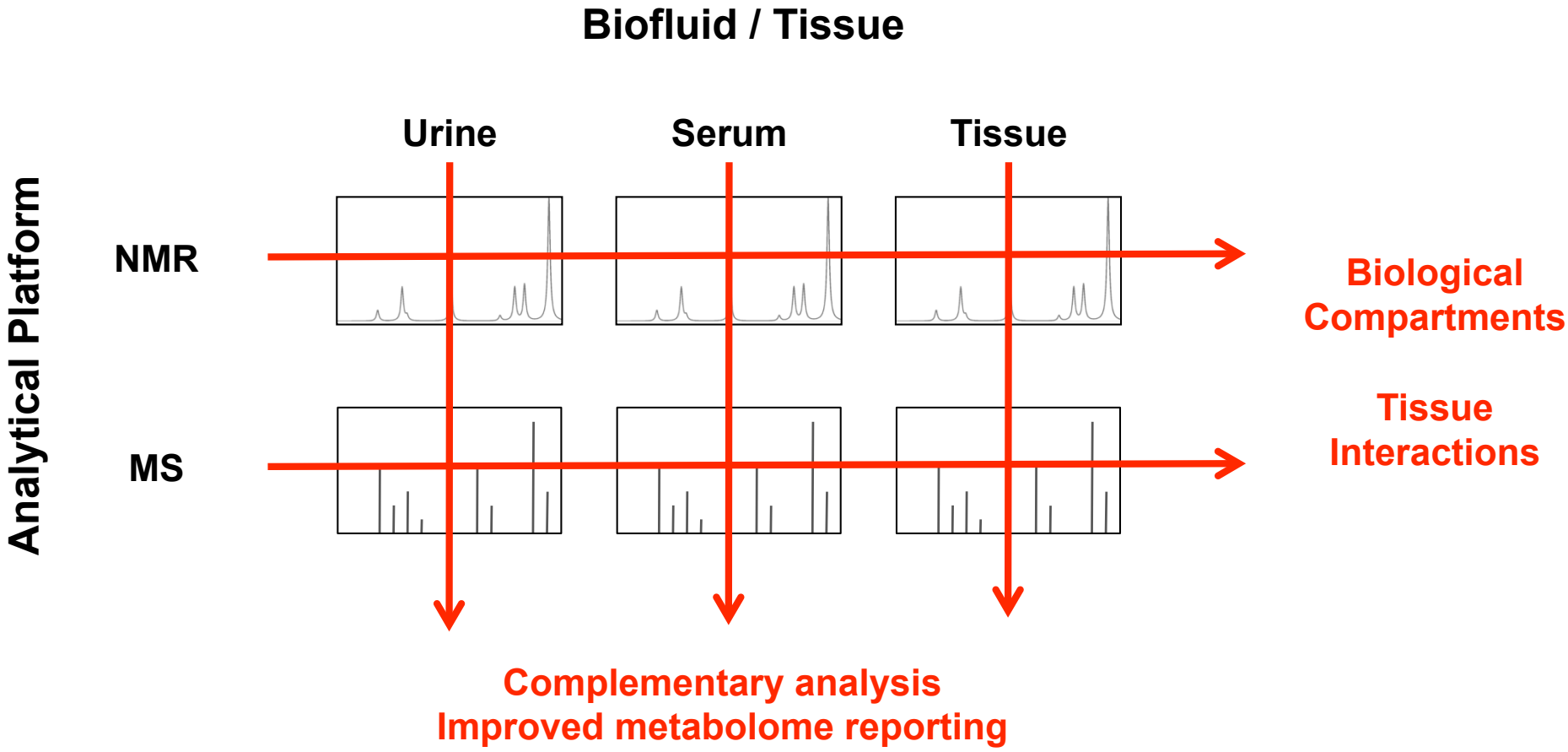


Supervised classification and calculation of confidence limits

Multivariate Analysis of Spectral Data Sets

Integrating Data

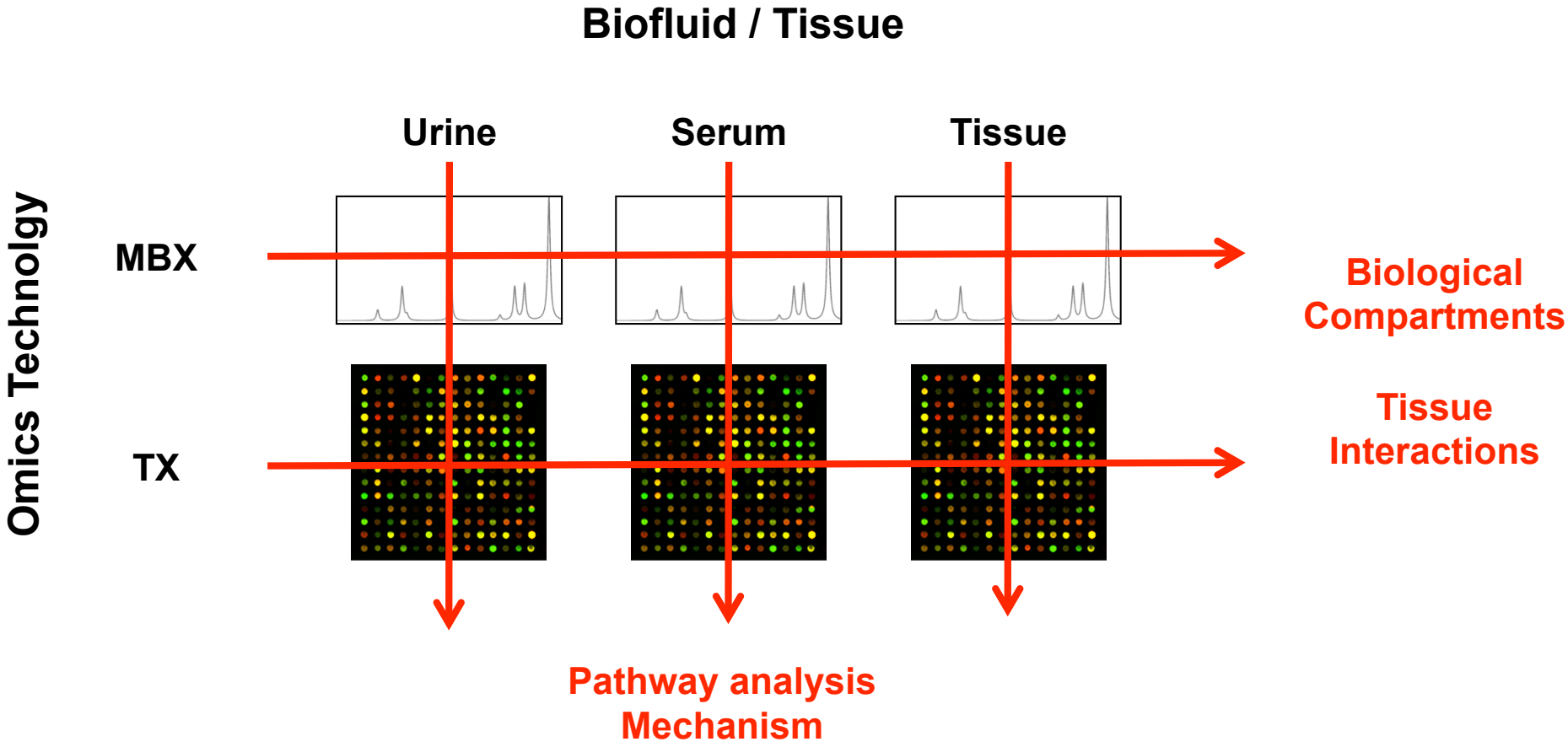
- Intra-omic statistical integration
  - e.g. STOCSY / SHY



Multivariate Analysis of Spectral Data Sets

Integrating Data

- Inter-omic integration
  - e.g. Over-representation pathway analysis



Other Analytical Platforms for Metabonomics

Other Analytical Platforms for Metabonomics

NMR Spectroscopy

1D NMR
Multidimensional NMR
Magic angle spinning NMR
Flow injection NMR
Capillary NMR

Chromatography

Gas chromatography
Capillary electrophoresis
UPLC
HPLC
SPE

Hyphenation

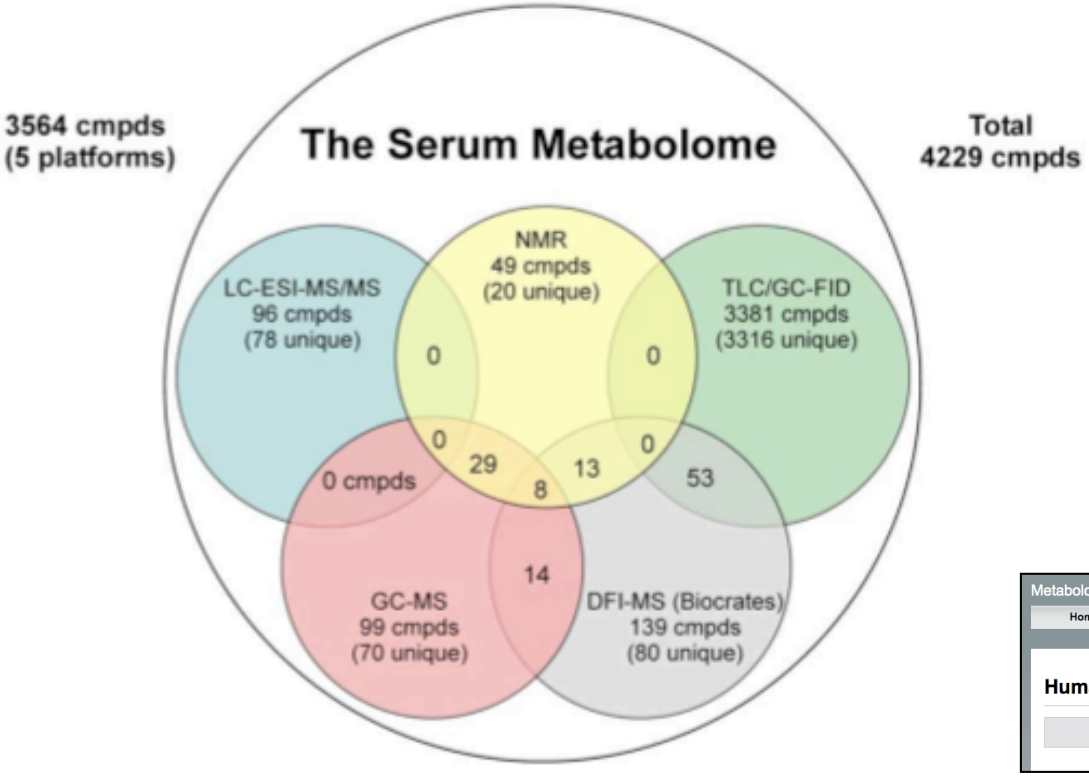
Mass Spectrometry

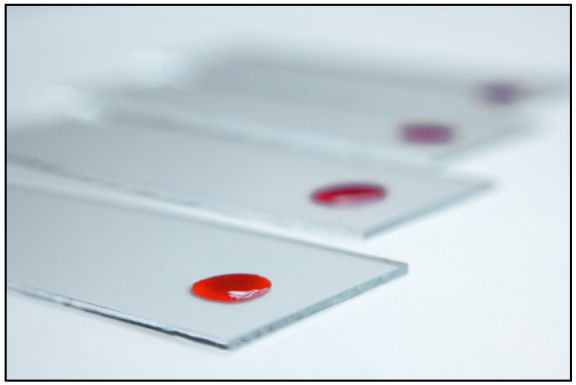
Single quad MS
Triple quad MS
Time-of-flight MS
Ion mobility MS
Ion trap MS
FTMS

High Throughput

Preparation Robotics
Automation
LIMS

Other Analytical Platforms for Metabonomics





Venn diagram showing the overlap of serum metabolites detected by different analytical methods

http://www.hmdb.ca/

Psychogios *et al.* 2011. The Human Serum Metabolome. PLoS One. 6(2) e16597

Characterising Biomarkers

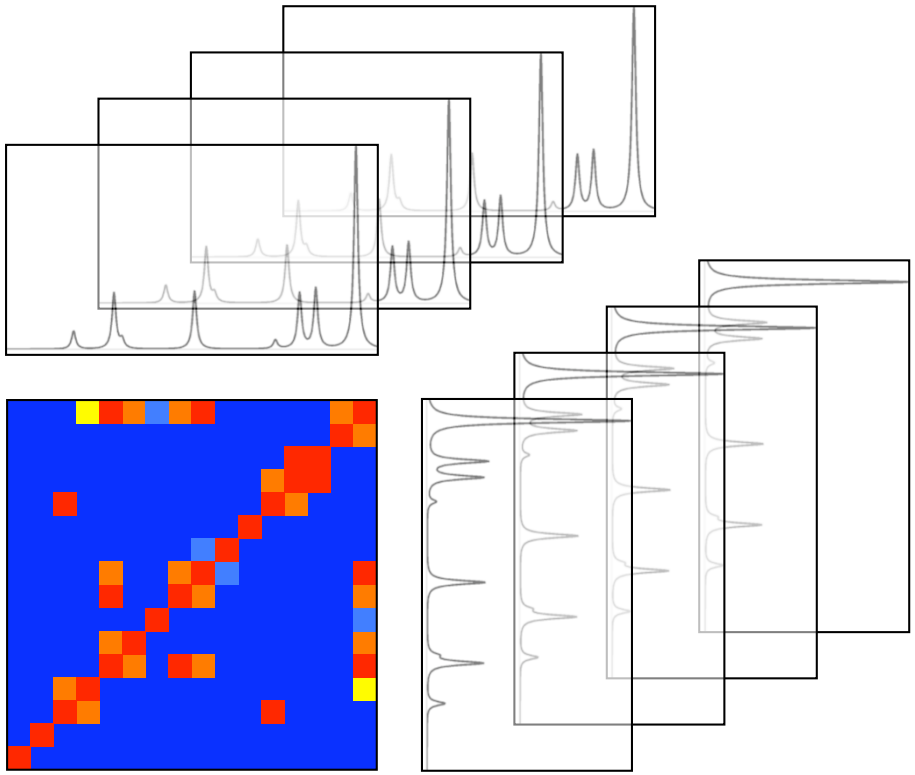
Characterising Biomarkers

Statistical Total Correlation Spectroscopy (STOCSY)

- Statistical equivalent of TOCSY experiment (used to identify molecular connectivity)
- Useful for assignment of peaks in NMR spectra
- e.g. endogenous metabolites in urine
- Calculate correlation matrix (C)
  - between all computer points

$$\mathbf{C} = \frac{1}{n-1}\mathbf{X}_1^t\mathbf{X}_2$$

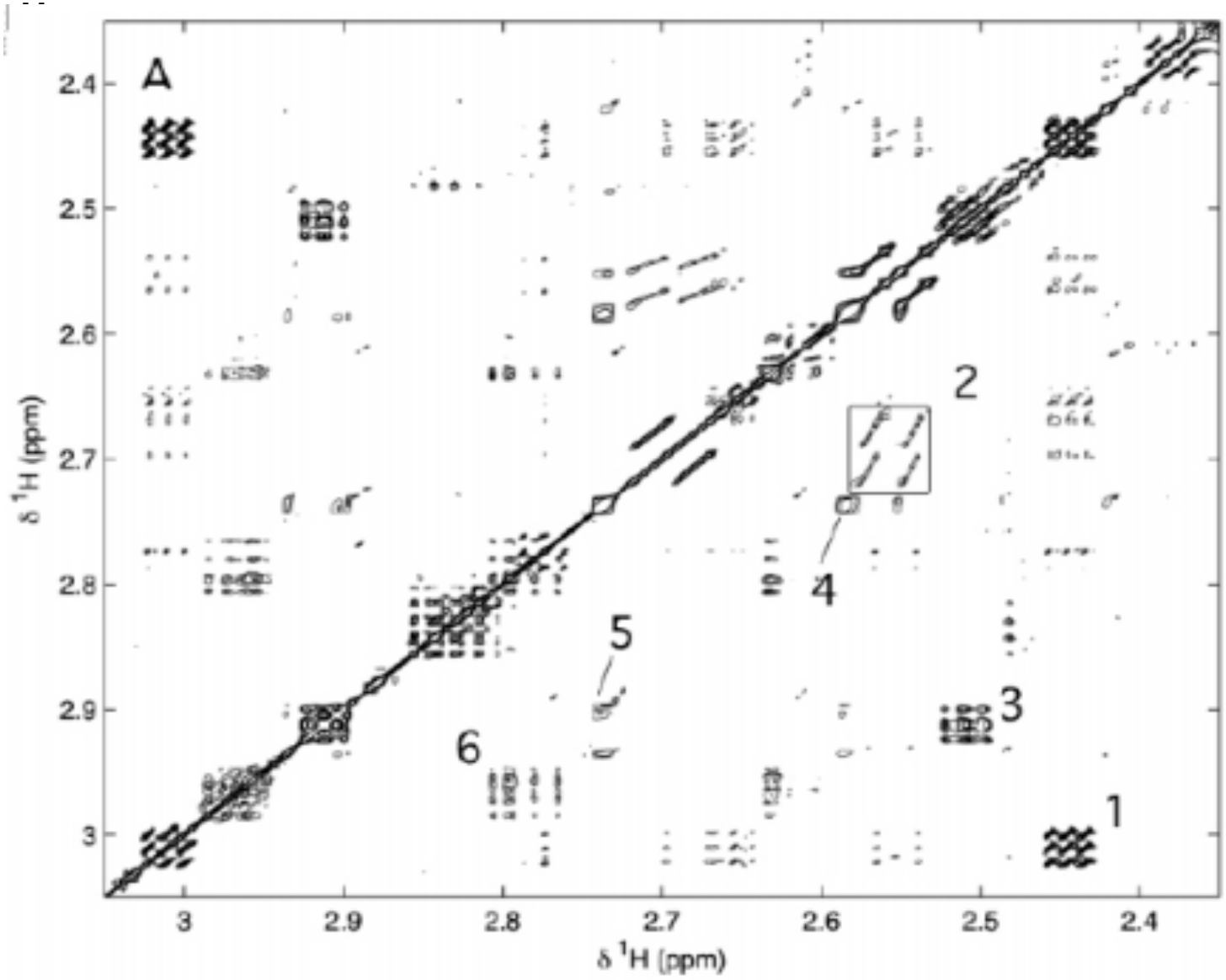
- X1 and X2 auto-scaled experimental matrices
- n number of spectra in each class
- v1 and v2 number of variables in each matrix



Homonuclear
Correlation matrix

Cloarec O, Dumas ME, Craig A, Barton RH, Trygg J, Hudson J, Blancher C, Gauguier D, Lindon JC, Holmes E, Nicholson JK. 2005. Statistical Total Correlation Spectroscopy: An Exploratory Approach for Latent Biomarker Identification from Metabolic ^{1}H NMR Data Sets. *Anal. Chem.* 77 (5) 1282-1289.

Characterising Biomarkers



10 x 1D NMR spectra

Mouse urine

2D ^{1}H-^{1}H STOCSY
Only self molecular correlations where:
$r^2 > 0.9$ plotted

High F1 and F2 Resolution:
64K x 64K

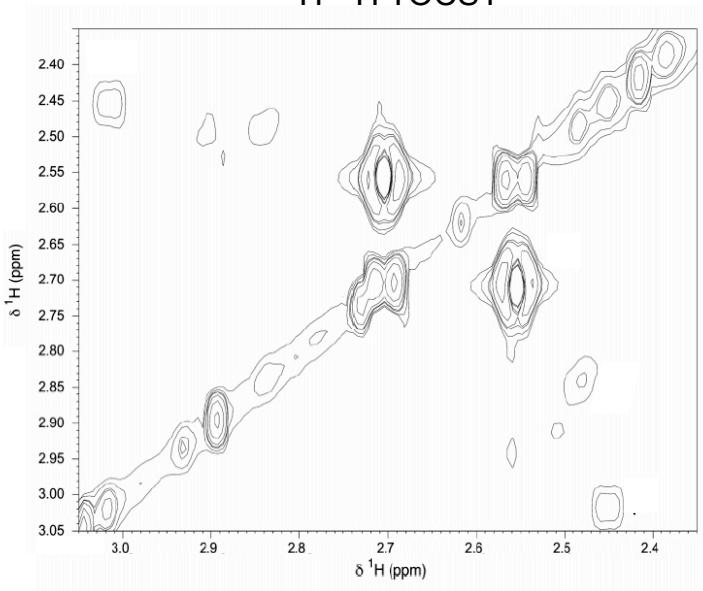
No scalar couplings or n.O.e.'s required

Characterising Biomarkers

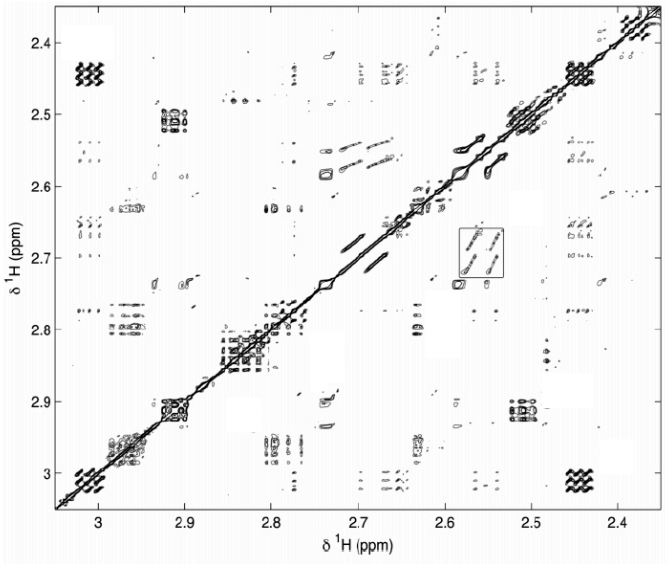
Statistical Total Correlation Spectroscopy (STOCSY)

- Use statistical correlation to infer structural or pathway connectivity
- Unlimited range of correlation - independent of structural connectivity
- High native resolution in all dimensions
- The data required may already have been routinely acquired

^{1}H- ^{1}H TOCSY



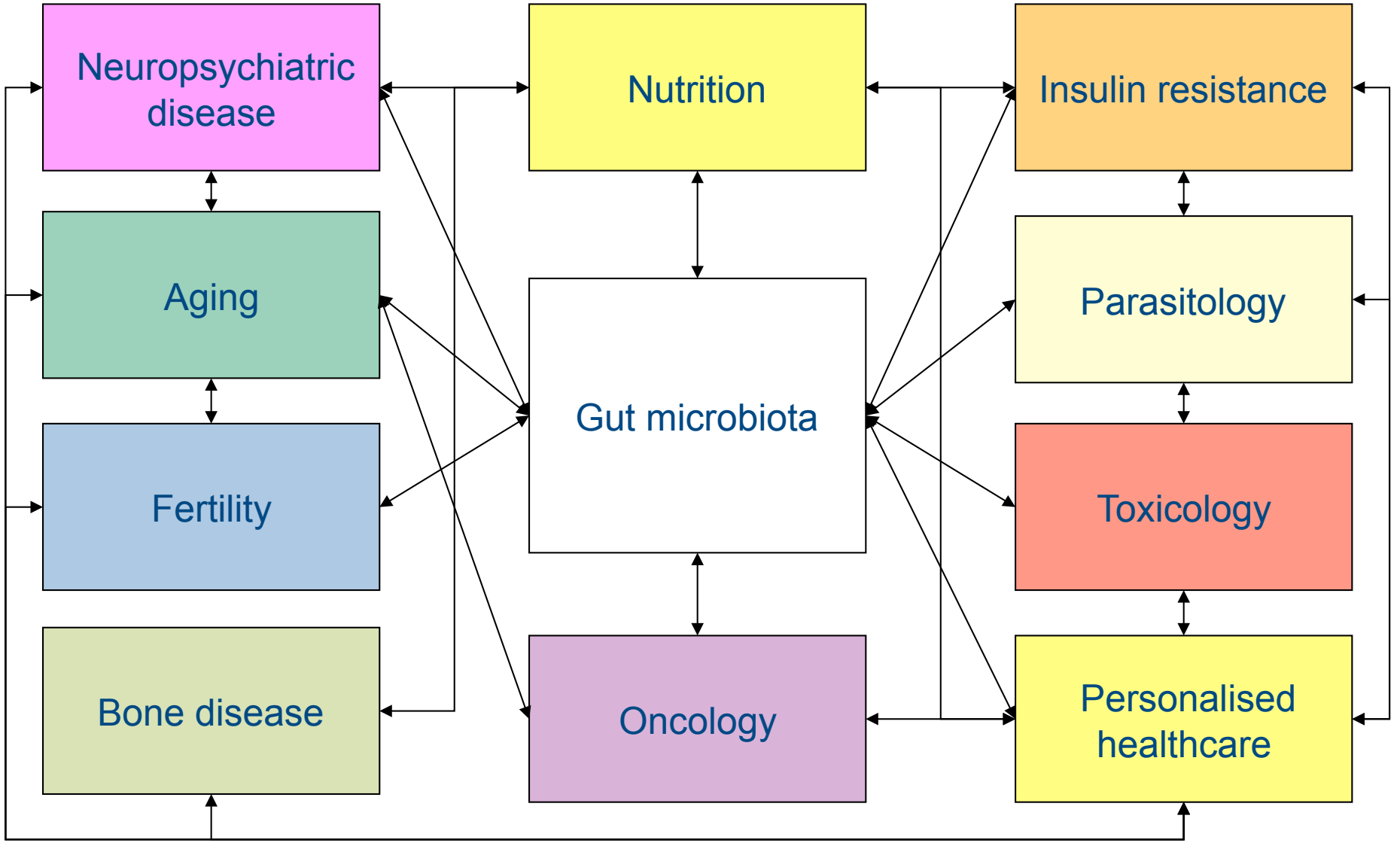
^{1}H- ^{1}H STOCSY



Application Areas of Metabonomics

Application Areas of Metabonomics

A wide range of applications are possible



Introduction to Metabonomics

Summary

- Metabolic profiles reflect a wide range of biological processes
- The sample type will largely determine the information content (e.g. urine vs serum)
- ^{1}H NMR is a powerful analytical tool for generating metabolic profiles in an efficient and untargeted way
- Multivariate analysis can help discover latent information contained in the spectral data
- Statistical tools can help produce classification models and interrogation of the spectra themselves
- Integration with other ‘omics’, or between biological compartments can help understand biological systems