Metabolomica

Analisi di biofluidi
mediante spettroscopia NMR

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Terminology

- **Metabolite: substance produced or used during metabolism such as lipids, sugars and amino acids**

- **Metabolome: the quantitative complement of all the low molecular weight molecules present in cells or biofluids in a particular physiological or developmental state**

- **Metabolomics: a comprehensive analysis of the whole metabolome under a given set of conditions**

Metabo*omics

• **Metabonomics:**

The quantitative measurement of the dynamic multiparametric response of living systems to pathophysiological stimuli or genetic modification (*Nicholson et al., Xenobiotica 1999*)

holistic analysis of biofluids and tissues in order to determine metabolic composition

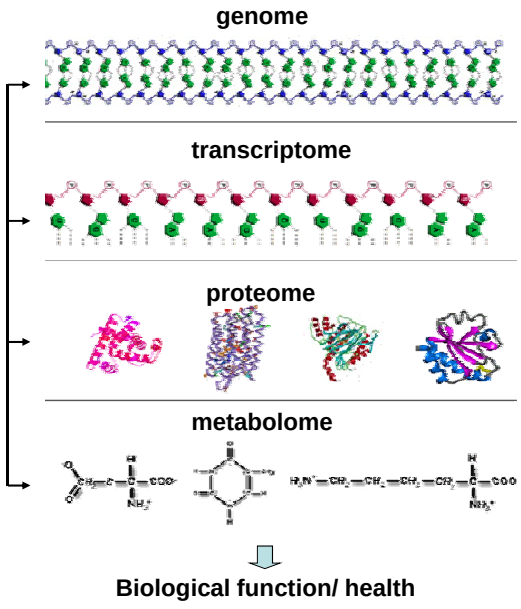
deals with integrated, multicellular, biological systems including communicating extracellular environments in animal and human biochemistry

• **Metabolomics:**

Measurement of metabolite concentrations and fluxes in isolated cell systems (*Nicholson et al., Xenobiotica 1999*)

deals with simple cell systems and mainly intracellular metabolite concentrations in microbial and plant biochemistry

Metabolomics vs genomics and proteomics



Metabolomics vs genomics and proteomics

Genomics and proteomics tell you what might happen, but metabonomics tells you what actually did happen (*Bill Lasley, UC Davis*)

Although changes in the quantities of individual enzymes might be expected to have little effect on metabolic fluxes, they can and do have significant effects on the concentrations of numerous individual metabolites.

The metabolome is further down the line from gene to function and so reflects more closely the activities of the cell at a functional level. Thus, as the 'downstream' result of gene expression, changes in the metabolome are expected to be amplified relative to changes in the transcriptome and the proteome.

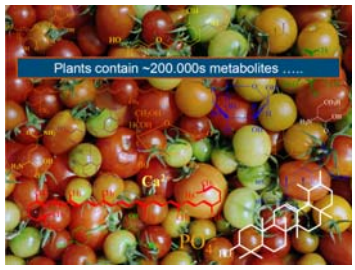
Metabolic fluxes (at least as exemplified by glycolysis in trypanosomes) are not regulated by gene expression alone.

General aplications

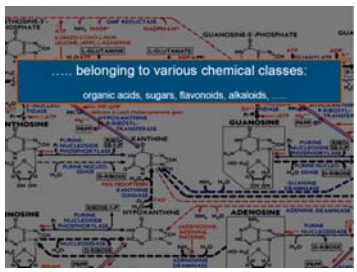
- **Assessing gene function and relationships to phenotypes**
- **Understanding metabolism and predicting novel pathways**
- **To increase metabolite fluxes into valuable biochemical pathways using metabolic engineering**
- **To compare genetically modified organisms**
- **To assess the effect of environmental/stress/temperature changes that lead to changes in gene expression, flux pathways, and extent of carbon and electron flow through them**

Characteristics of the metabolomes

Metabolome size:
- **S. cerevisiae: about 600 metabolites**
- **Plants: estimated 200,000 primary and secondary metabolites**
- **Mammalians: ?**



Metabolite chemical diversity:
- **the metabolome extends over an estimated 7–9 magnitudes of concentration (pmol–mmol)**
- **wide variations in chemical (molecular weight, polarity, solubility) and physical (volatility) properties**



Classification of metabolomics approaches

Metabolomics is the study of metabolic changes. It encompasses metabolomics, metabolite target analysis, metabolite profiling, metabolic fingerprinting, metabolic profiling, and metabonomics – *the Metabolomics Society*

Metabolite target analysis: analysis restricted to metabolites of, for example, a particular enzyme that would be directly affected by abiotic or biotic perturbation

Metabolite profiling: analysis focused on a group of metabolites, for example, a class of compounds such as carbohydrates, amino acids or those associated with a specific pathway

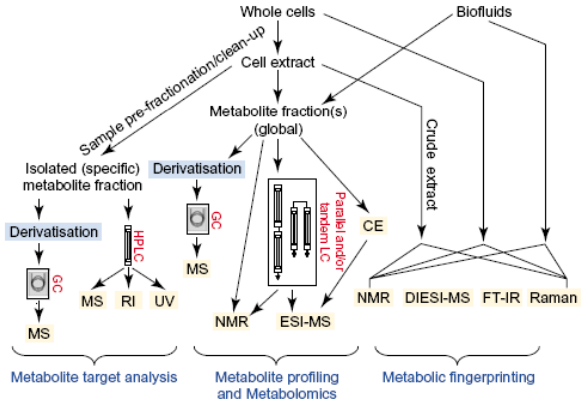
Metabolomics: comprehensive analysis of the whole metabolome under a given set of conditions

Metabolic fingerprinting: classification of samples on the basis of provenance of either their biological relevance or origin

Metabolic profiling: often used interchangeably with 'metabolite profiling'; m.p. is commonly used in clinical and pharmaceutical analysis to trace the fate of a drug or metabolite

Metabonomics: measure the fingerprint of biochemical perturbations caused by disease, drugs and toxins

Technologies for metabolome analysis



General strategies for metabolome analysis.

CE, capillary electrophoresis; DIESI, direct-infusion ESI, which can be linked to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS); NMR, nuclear magnetic resonance; RI, refractive index detection; UV, ultraviolet detection

NMR-based metabolomics

What kind of samples can be analysed by NMR?

•All type of biological liquids (urine, plasma, cerebrospinal fluid, amniotic fluid, sperm, synovial fluid, saliva) or cellular or organ extracts

•All kind of biological samples such as biopsies of organs and cell cultures

Why is NMR competitive?

• NMR offers a direct biochemical window into a living system in a holistic way (no a priori selection)

• NMR is fully quantitative

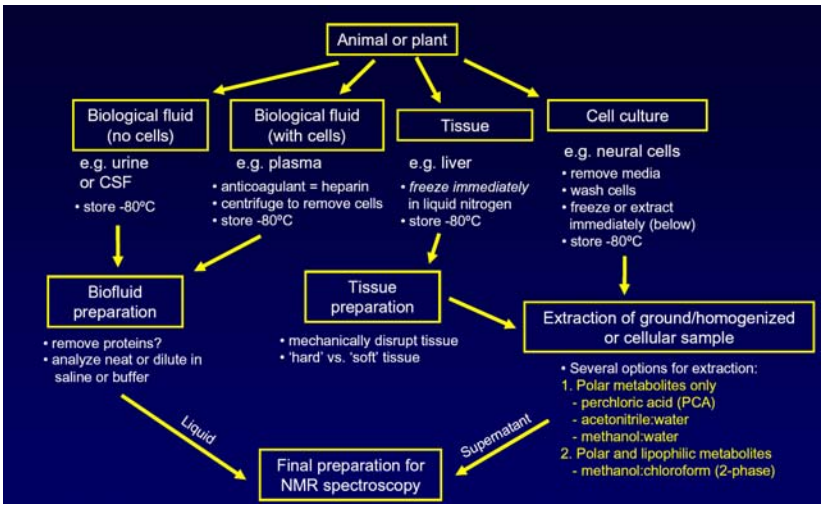
• There is no need for special sample preparation (fractionation, derivatization, …)

• NMR is non-destructive and allows to completely recover the samples

• NMR has emerged into a high throughput analysis system with minimal sample preparation (cost effective)

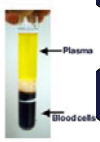
• Nearly all metabolic intermediates have unique NMR signatures

Sample preparation for NMR-based metabolomics



Plasma sample preparation

Blood collection



Plasma separation

Filtration

Sample volume and pH adjustment

Ready for NMR spectroscopy

Final NMR sample preparation

Urine

- Add 10% phosphate buffer in D_2O (pH=7.0)
- Add sodium azide (Antibacterial)
- Add TSP (Reference)
- Centrifugation
- Transfer in NMR tube

Also possible liophylization!

Plasma

- Add 10% D2O
- Add DSS (reference)

Chemical shifts

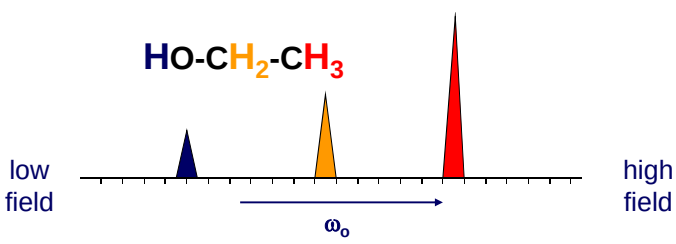
• If each type of nucleus has its characteristic ω_o at a certain magnetic field, why is NMR useful?

• Depending on the ***chemical environment*** we have variations on the magnetic field that the nuclei feels, even for the same type of nuclei. It affects the local magnetic field.

$$B_{eff} = B_o - B_{loc} \quad --- \quad B_{eff} = B_o(1 - \sigma)$$

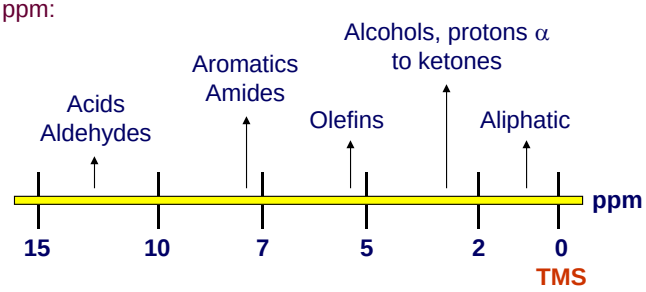
• σ is the ***magnetic shielding*** of the nucleus. Factors that affect it include neighboring atoms, aromatic groups, etc., etc. The polarization of the bonds to the observed nuclei are also important.

• As a crude example, ethanol looks like this:

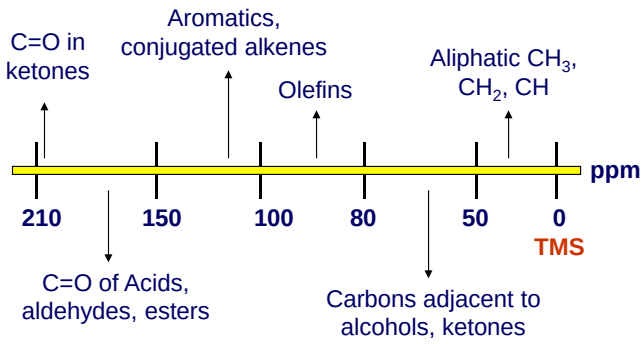


Scales for different nuclei

• For protons, ~ 15 ppm:

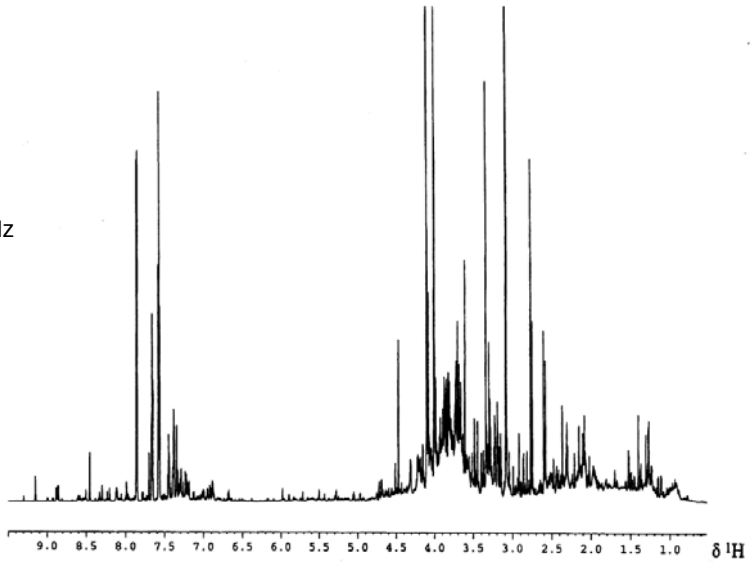


• For carbon, ~ 220 ppm:

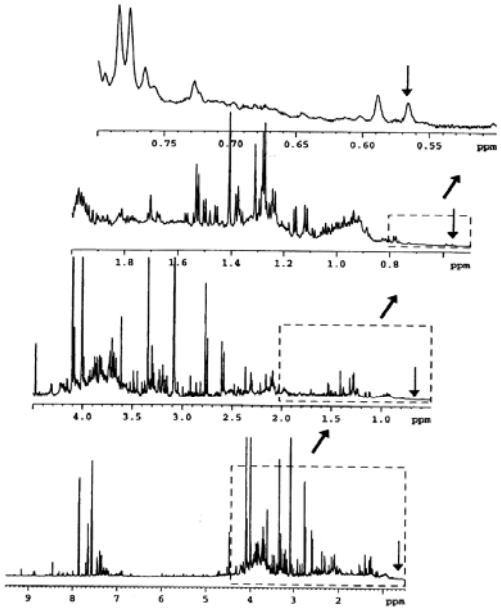


Human urine NMR profile

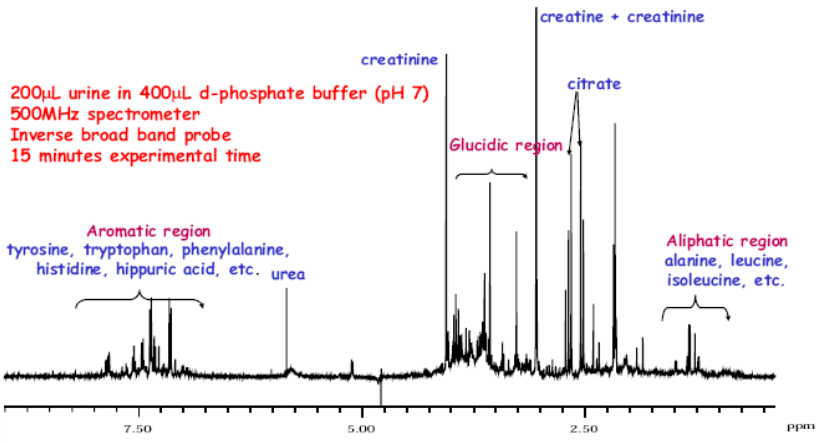
td 64K
d1 4s
ns 64
800 MHz



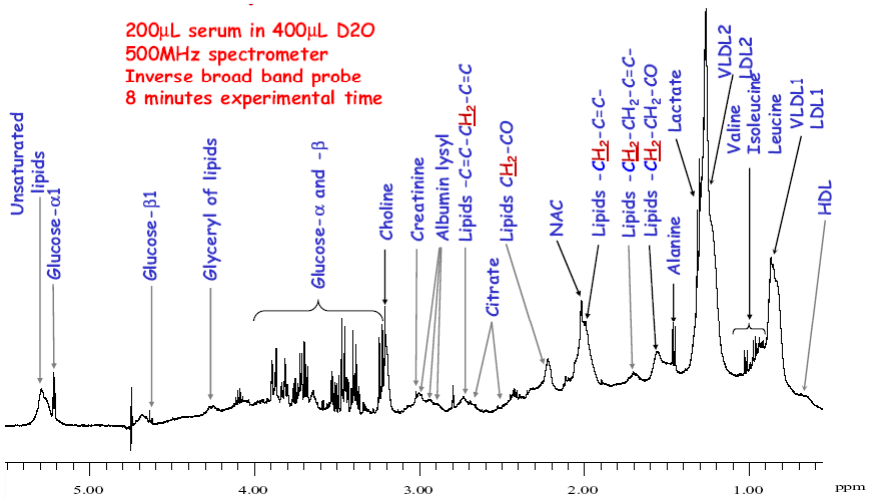
Spectral resolution



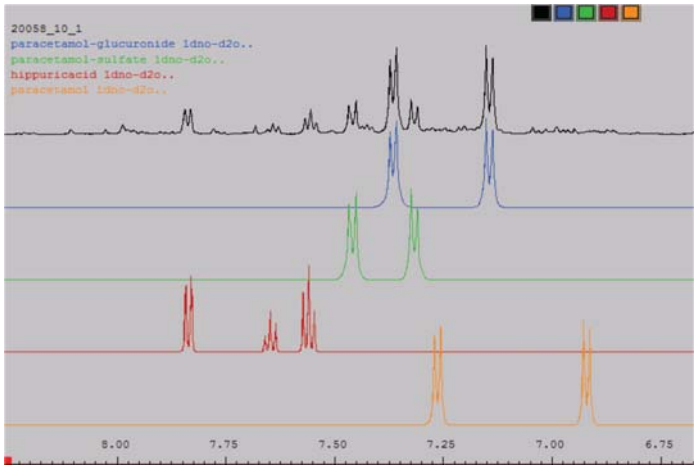
Assignment of major peaks/regions



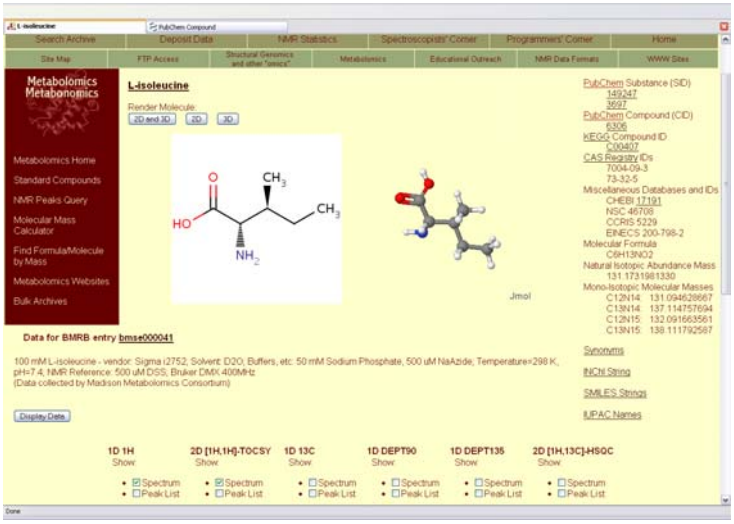
1H NMR of human blood serum



Peak assignment: reference databases



… BMRB: metabolite entries, isoleucine



NMR Metabolomics Experiments

1-D NMR - *for measurement of metabolite fingerprints*

- Single pulse ^{1}H NMR sequence
- CPMG ^{1}H spin-echo sequence

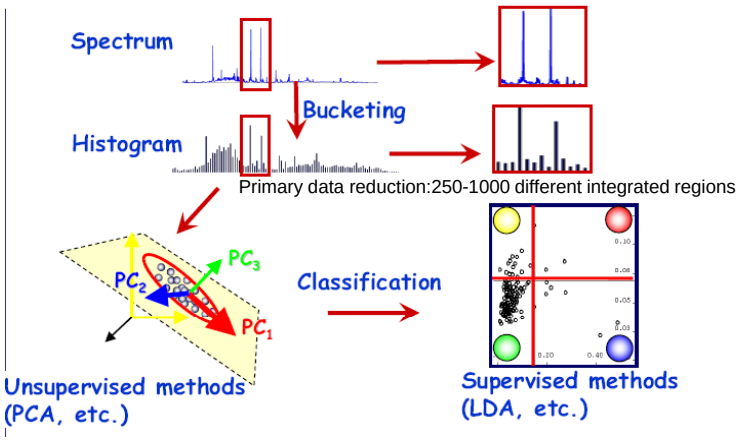
2-D NMR - *for measurement of metabolite fingerprints*

- projections from J-Resolved spectra

2-D NMR - *for confirmation of peak assignments*

- ^{1}H-^{1}H correlation spectroscopy (COSY)
- ^{1}H-^{13}C heteronuclear single quantum coherence

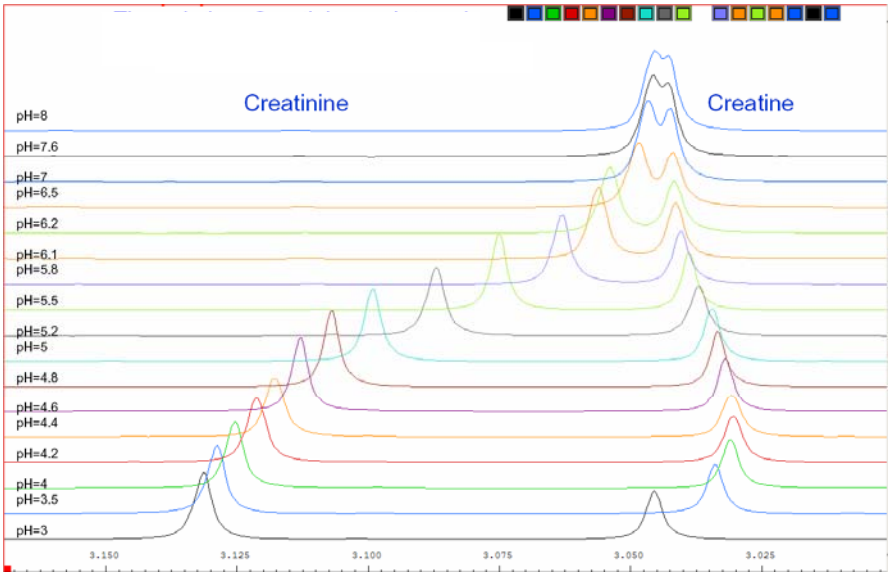
NMR-based metabolomics: the concept



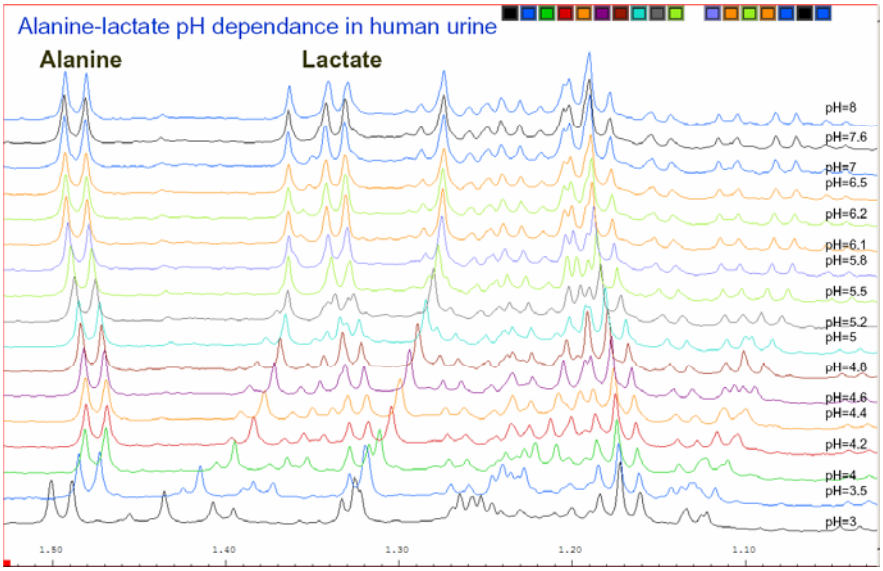
No *a priori* knowledge of the class of samples

Model for the prediction of independent data
Use class information to maximise separation among classes

pH changes



pH changes (…)

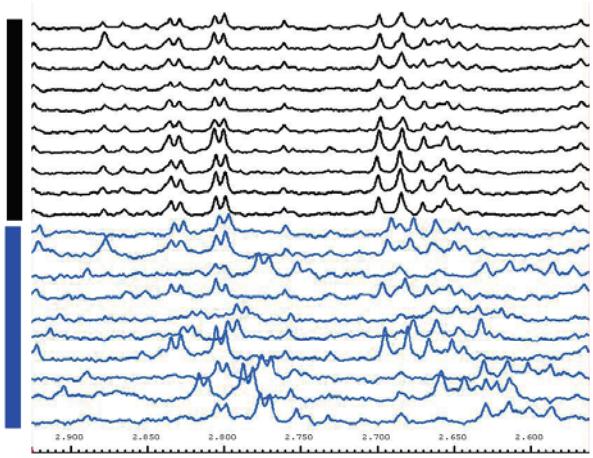


pH adjustment

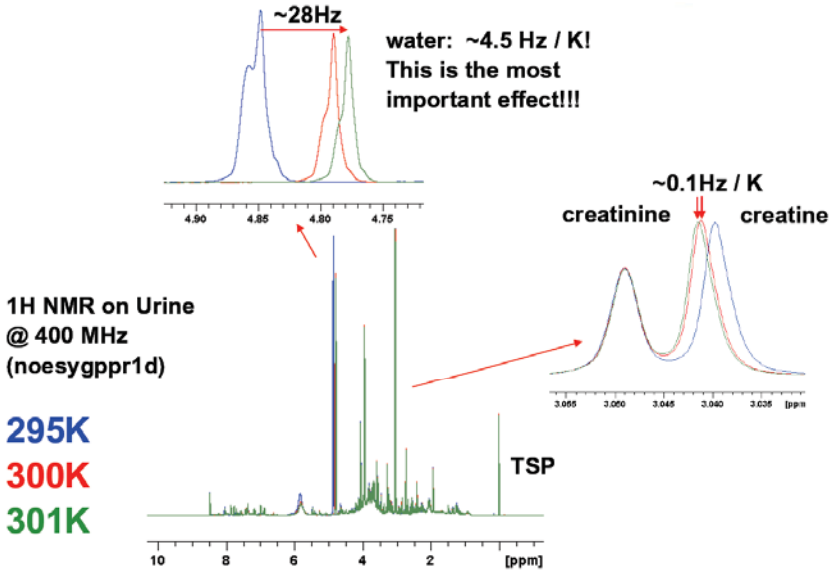
Buffer + pH adjustment (pH = 7)

Buffer:
1.5M phosphat buffer (KH2P04) in D20.
~0.01% NaN3 and
0.1% TSP is added.

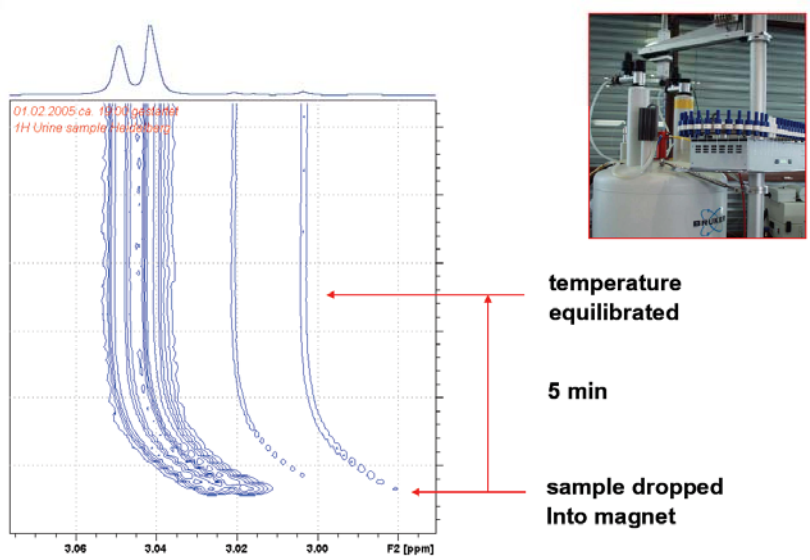
extreme cases most affected region



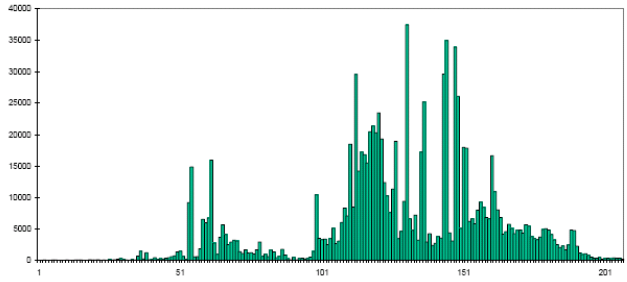
Temperature effects



Temperature equilibration

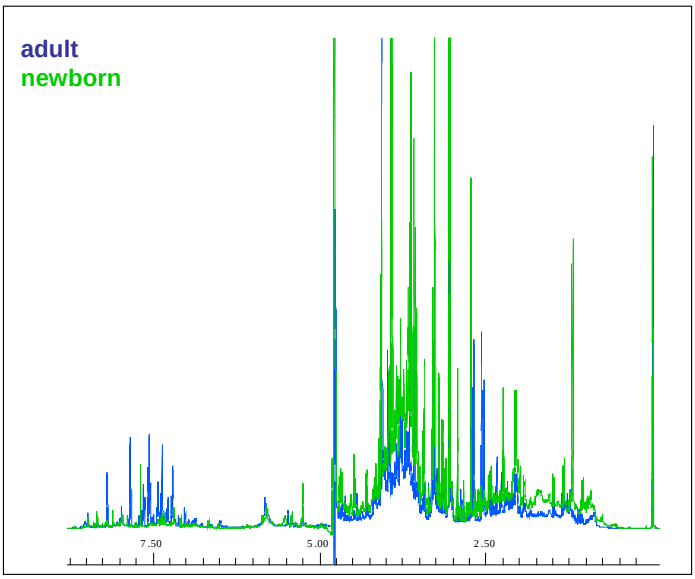


Data pre-processing

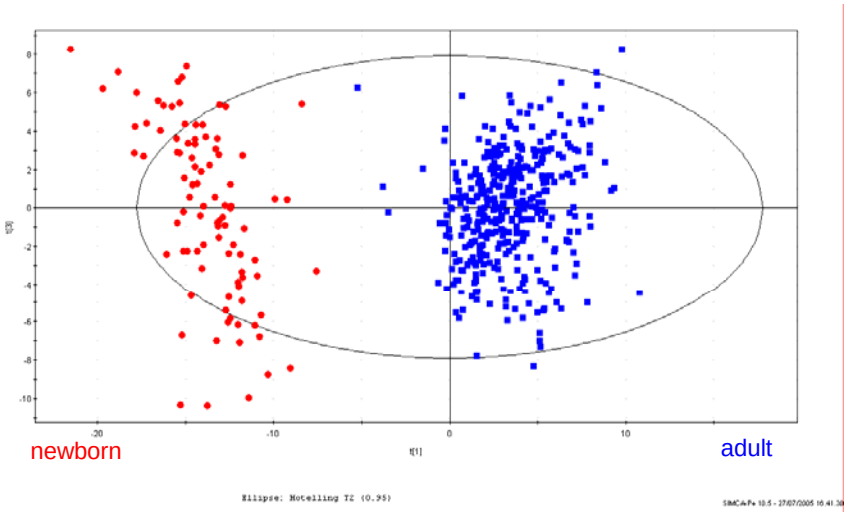


- Discretise x-axis into *n* equal sized bins, height = area under intensity (reduces impact of small variations in chemical shift e.g. due to pH)
- Normalise bars for constant total area (removes effect of differences in concentration across samples)
- Remove insignificant regions (e.g. water and urea resonances in urine spectra)

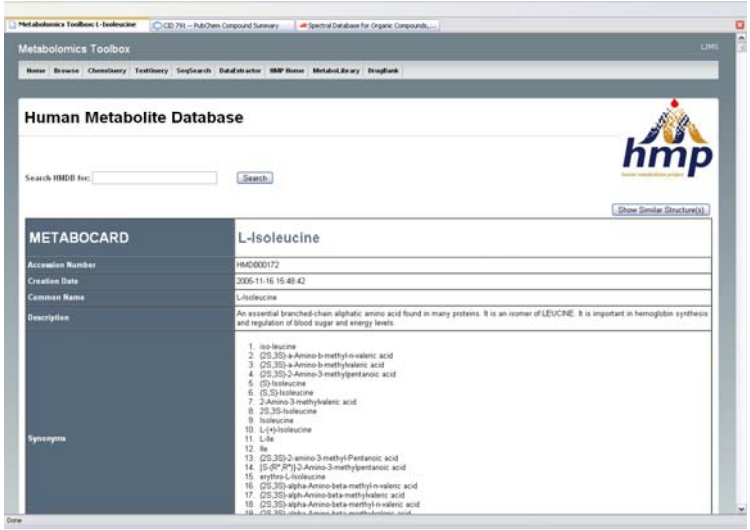
Visualizing age-related differences



PCA newborns vs adults



THE HUMAN METABOLITE DATABASE
(www.hmdb.ca)



The metabolomics society
(www.metabolomicssociety.org)

… KEGG: metabolic pathways