

# 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

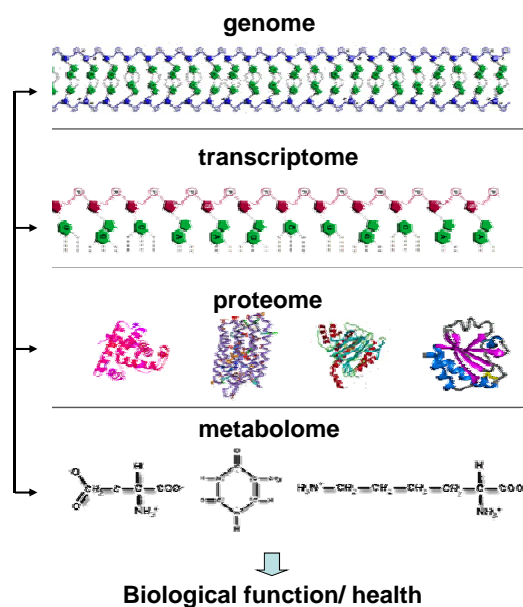
deals with integrated, multicellular, biological systems including communicating extracellular environments in animal and human biochemistry

- **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 metabolomics 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 applications

- 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

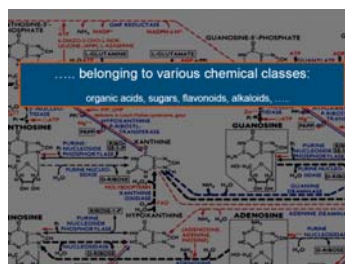
### Metabolome size:

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



### 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

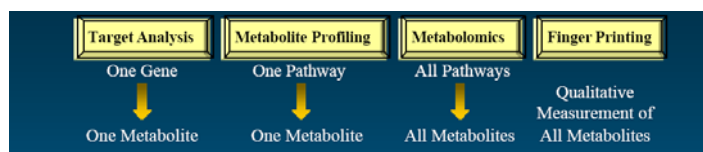
**Metabolite profiling:** analysis focused on a group of metabolites, for example, a class of compounds such as carbohydrates, amino acids or those associated with a specific pathway

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

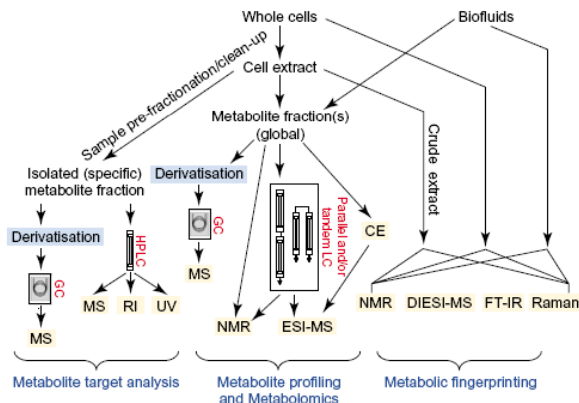
**Metabolic fingerprinting:** classification of samples on the basis of provenance of either their biological relevance or origin

**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

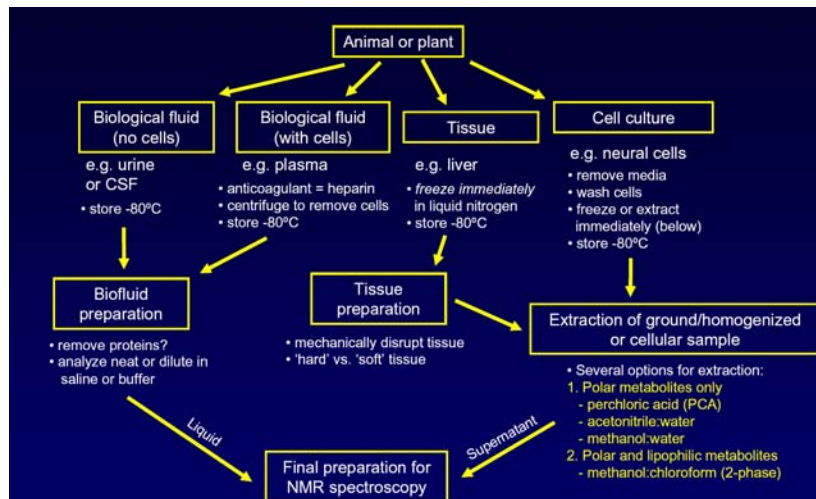
### What kind of samples can be analysed by NMR?

- 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

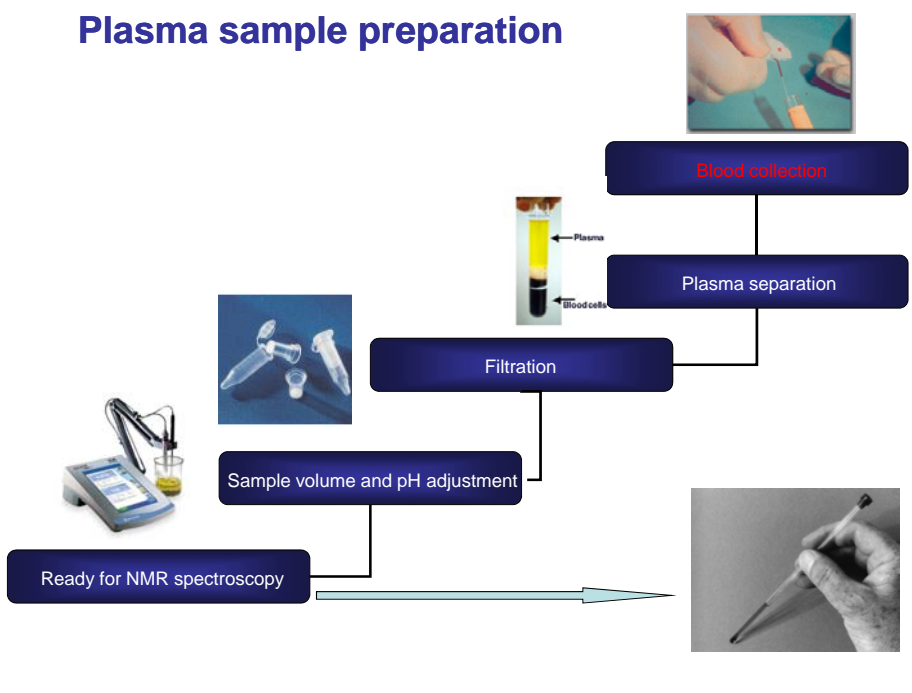
### Why is NMR competitive?

- 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



## Final NMR sample preparation

### Urine

- Add 10% phosphate buffer in D<sub>2</sub>O (pH=7.0)
- Add sodium azide (Antibacterial)
- Add TSP (Reference)
- Centrifugation
- Transfer in NMR tube

### Also possible liophylization!

### Plasma

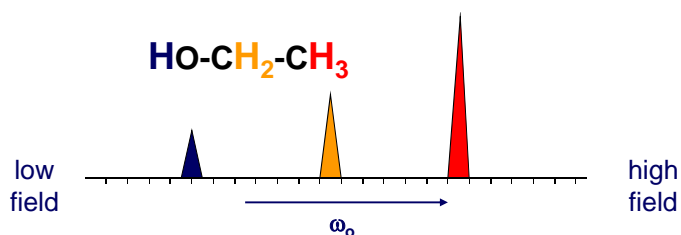
- Add 10% D<sub>2</sub>O
- Add DSS (reference)

## Chemical shifts

- If each type of nucleus has its characteristic  $\omega_0$  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.

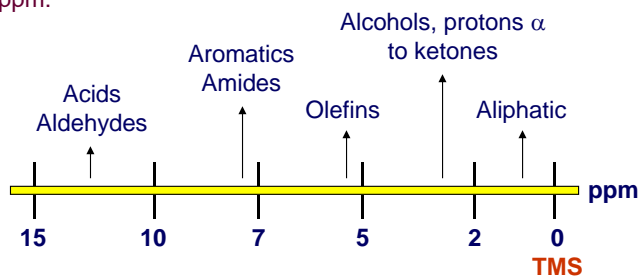
$$B_{\text{eff}} = B_0 - B_{\text{loc}} \quad \text{---} \quad B_{\text{eff}} = B_0(1 - \sigma)$$

- $\sigma$  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:

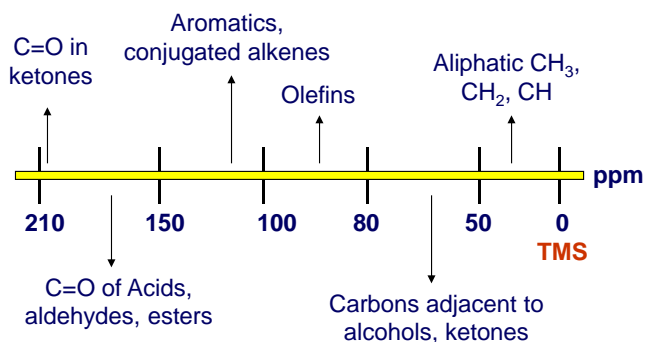


## Scales for different nuclei

- For protons, ~ 15 ppm:

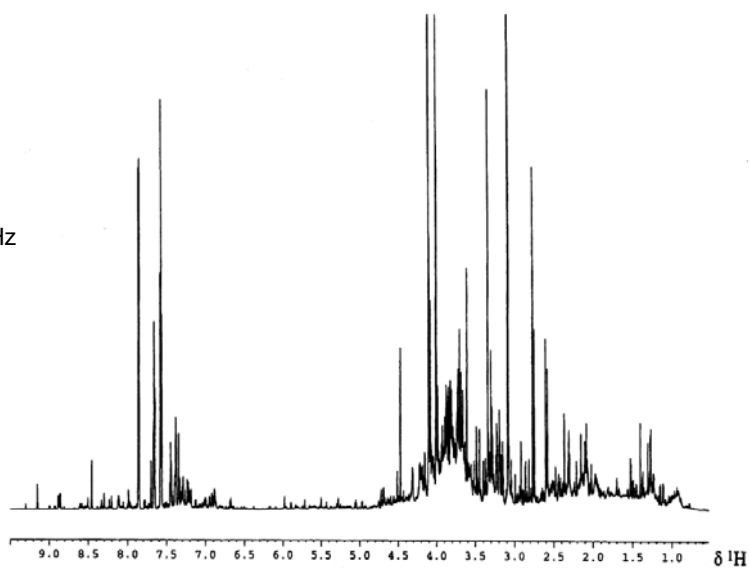


- For carbon, ~ 220 ppm:



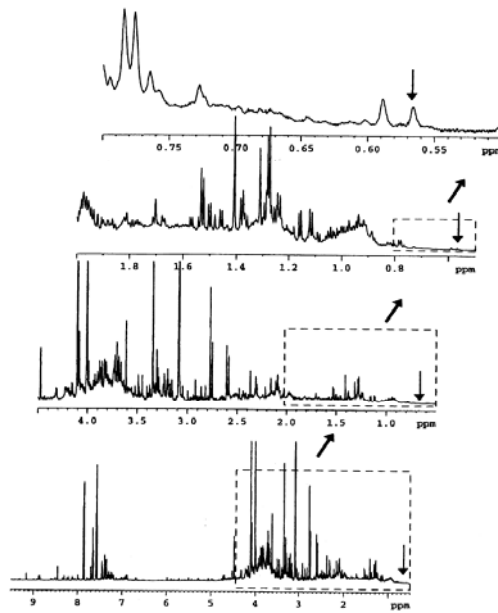
## Human urine NMR profile

td 64K  
d1 4s  
ns 64  
800 MHz

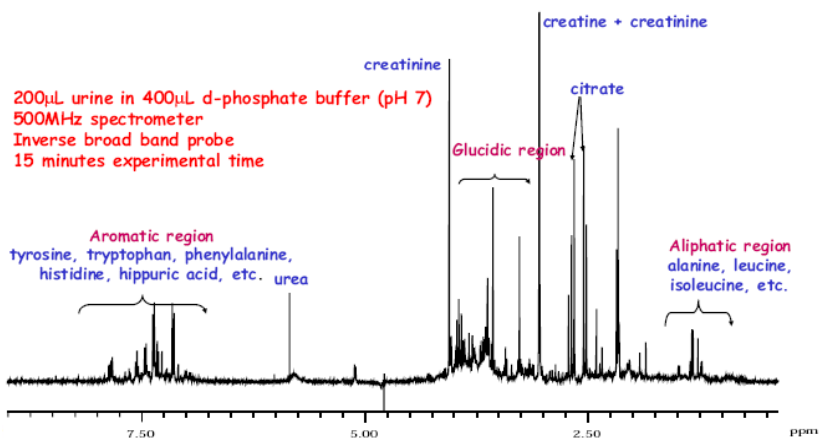




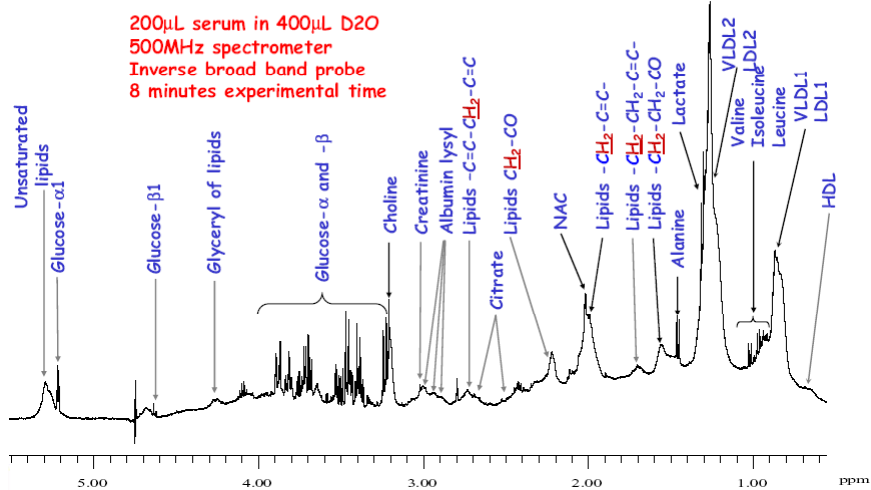
## Spectral resolution



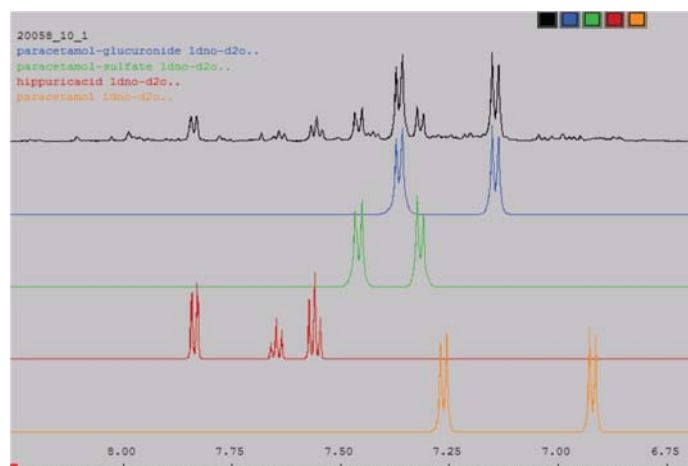
## Assignment of major peaks/regions



## 1H NMR of human blood serum



## Peak assignment: reference databases



## ... BMRB: metabolite entries, isoleucine

**L-isoleucine**

PubChem Substance (SD) 142347  
1327

PubChem Compound (CID) 8308

KEGG Compound ID C00487

CAS Registry No. 7004-09-3

73-32-6

Miscellaneous Databases and IDs  
CHEBI 17191  
NSC 46709  
CCRIS 5229  
EINECS 200-798-2

Molecular Formula C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub>

Natural Isotopic Abundance Mass 131.1731981330

Monoisotopic Molecular Masses  
C12H14 131.094628667  
C13H14 137.147576694  
C13H15 132.091663561  
C13H15 138.111722597

SMILES String  
IUPAC Names

**Data for BMRB entry bmrse000041**

100 mM L-isoleucine - vendor: Sigma (2752, Solvent: D2O, Buffers, etc.: 50 mM Sodium Phosphate, 500 uM NaAzide, Temperature=298 K, pH=7.4, NMR Reference: 500 uM DSS, Bruker DMX 400MHz (Data collected by Madison Metabolomics Consortium)

Display Data

1D 1H	2D [1H,1H]-TOCSY	1D 13C	1D DEPT90	1D DEPT135	2D [1H,13C]-HSQC
Show	Show	Show	Show	Show	Show
<input checked="" type="checkbox"/> Spectrum <input type="checkbox"/> Peak List	<input checked="" type="checkbox"/> Spectrum <input type="checkbox"/> Peak List	<input type="checkbox"/> Spectrum <input type="checkbox"/> Peak List	<input type="checkbox"/> Spectrum <input type="checkbox"/> Peak List	<input type="checkbox"/> Spectrum <input type="checkbox"/> Peak List	<input type="checkbox"/> Spectrum <input type="checkbox"/> Peak List

## NMR Metabolomics Experiments

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

- Single pulse <sup>1</sup>H NMR sequence
- CPMG <sup>1</sup>H spin-echo sequence

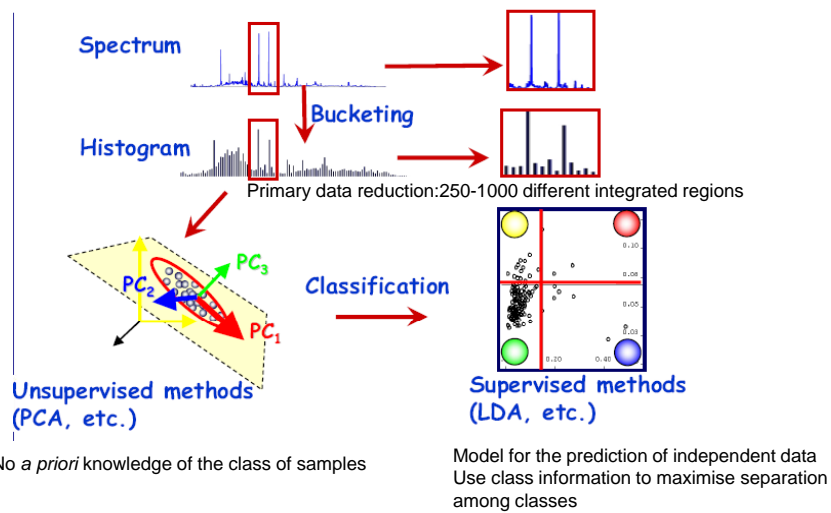
### 2-D NMR - for measurement of metabolite fingerprints

- projections from J-Resolved spectra

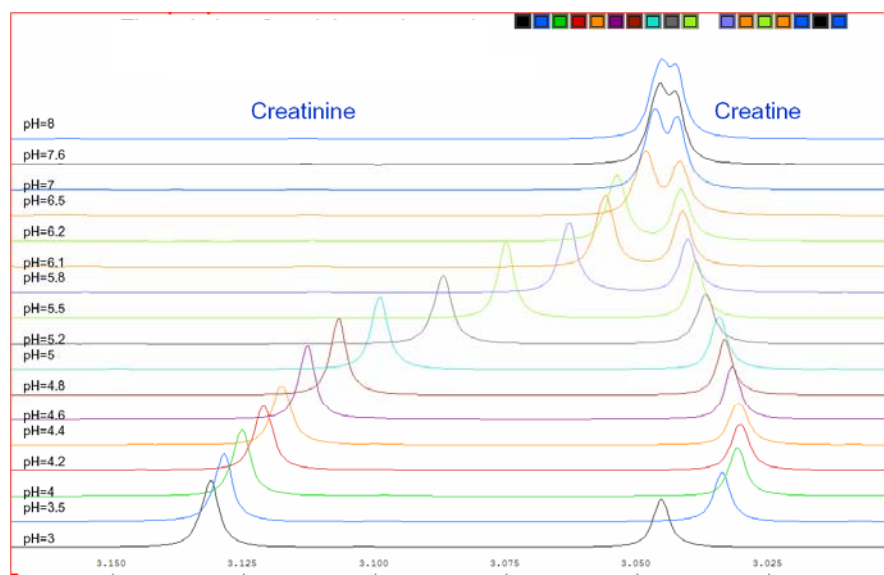
### 2-D NMR - for confirmation of peak assignments

- <sup>1</sup>H-<sup>1</sup>H correlation spectroscopy (COSY)
- <sup>1</sup>H-<sup>13</sup>C heteronuclear single quantum coherence

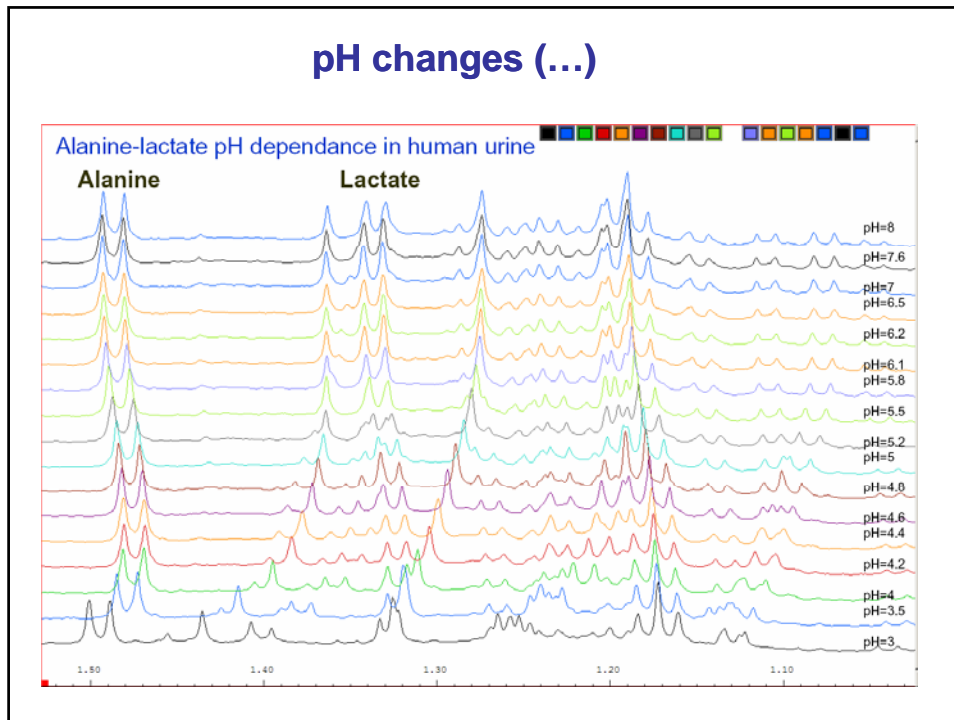
## NMR-based metabolomics: the concept



## pH changes



## pH changes (...)

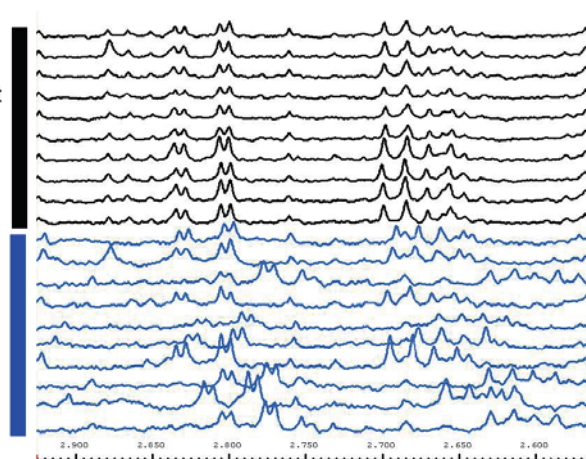


## pH adjustment

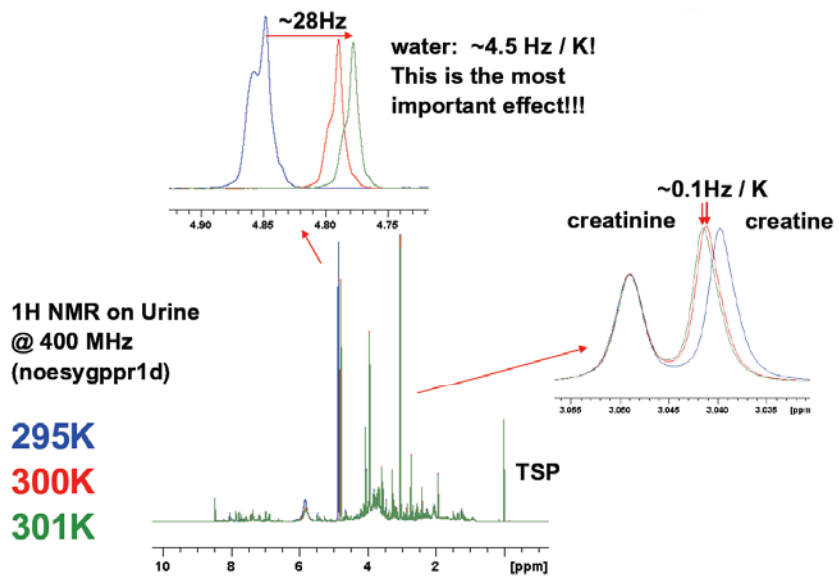
Buffer + pH adjustment  
(pH = 7)

Buffer:  
1.5M phosphat buffer  
(KH<sub>2</sub>P0<sub>4</sub>) in D<sub>2</sub>O.  
~0.01% NaN<sub>3</sub> and  
0.1% TSP is added.

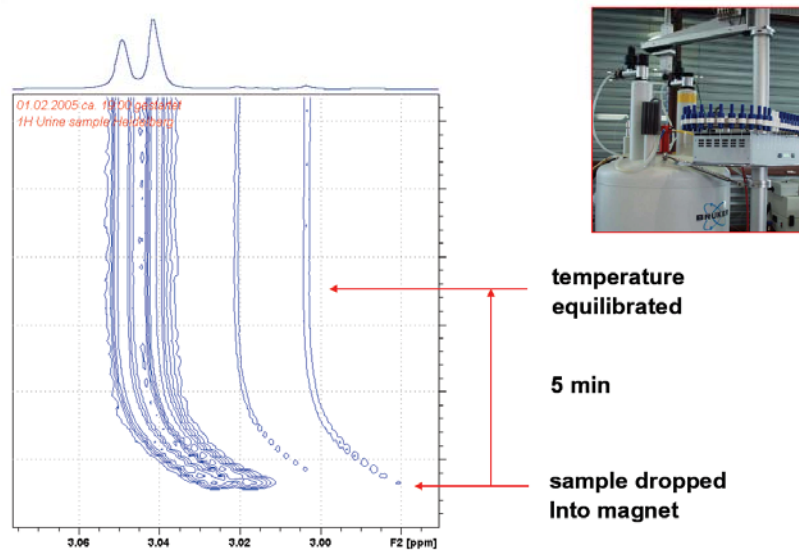
**extreme cases  
most affected  
region**



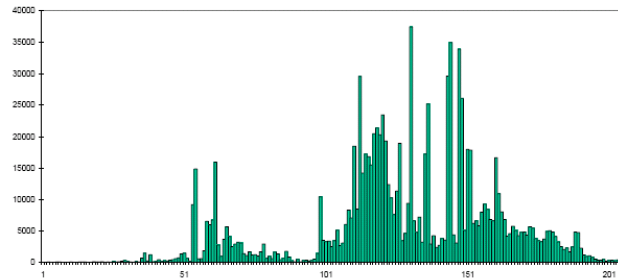
## Temperature effects



## Temperature equilibration

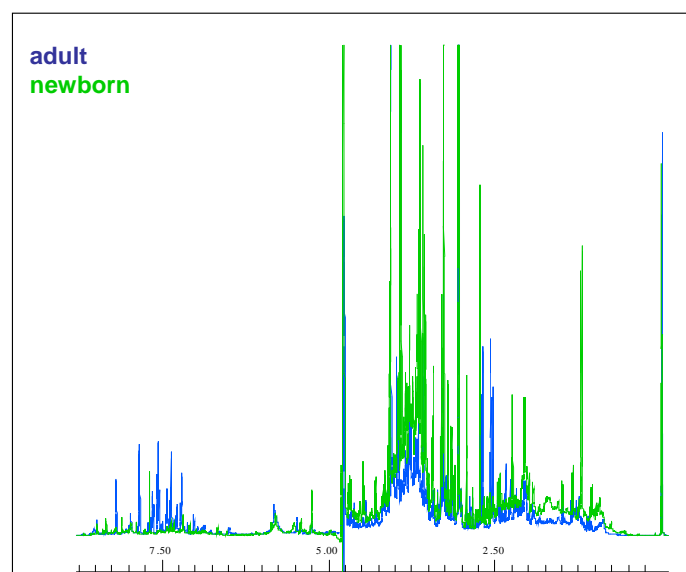


## Data pre-processing

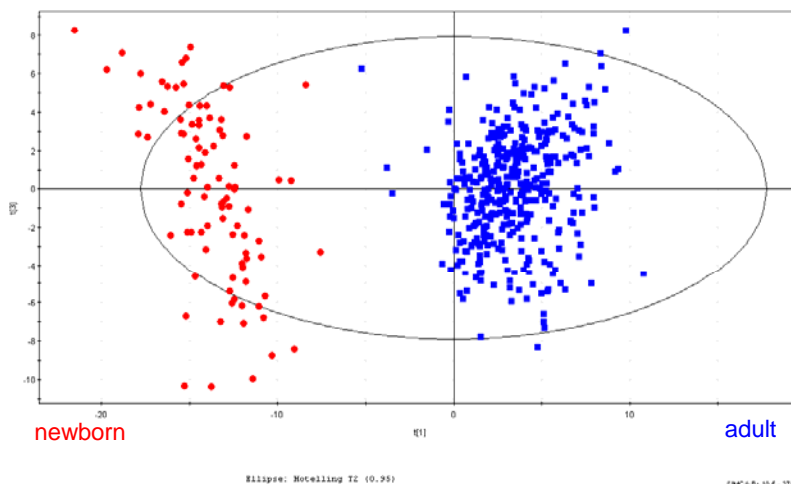


- Discretise x-axis into  $n$  equal sized bins, height = area under intensity (reduces impact of small variations in chemical shift e.g. due to pH)
- Normalise bars for constant total area (removes effect of differences in concentration across samples)
- Remove insignificant regions (e.g. water and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

## Visualizing age-related differences



### PCA newborns vs adults



### THE HUMAN METABOLITE DATABASE (www.hmdb.ca)

Metabolomics Toolbox | L-Isoleucine | PubChem Compound Summary | Spectral Database for Organic Compounds...

Metabolomics Toolbox

Home | Browse | Chemistry | Toxicology | SeqSearch | DataExplorer | SMP Home | MetabolLibrary | DrugBank

#### Human Metabolite Database

Search HMDB for:

[\(Show Similar Structure\)](#)

METABOCARD	L-Isoleucine
Accession Number	HMDB000172
Creation Date	2005-11-16 15:48:42
Common Name	L-Isoleucine
Description	An essential branched-chain aliphatic amino acid found in many proteins. It is an isomer of LEUCINE. It is important in hemoglobin synthesis and regulation of blood sugar and energy levels.
Synonyms	<ol style="list-style-type: none"> <li>1. iso-leucine</li> <li>2. (2S,3S)-<math>\alpha</math>-Amino-<math>\beta</math>-methyl-<math>\gamma</math>-valeric acid</li> <li>3. (2S,3S)-<math>\beta</math>-Amino-<math>\beta</math>-methylvaleric acid</li> <li>4. (2S,3S)-2-Amino-3-methylpentanoic acid</li> <li>5. (2S)-isoleucine</li> <li>6. (S,S)-isoleucine</li> <li>7. 2-Amino-3-methylvaleric acid</li> <li>8. 2S,3S-isoleucine</li> <li>9. isoleucine</li> <li>10. L-Iso-leucine</li> <li>11. L-Ile</li> <li>12. Ile</li> <li>13. (2S,3S)-2-amino-3-methyl-pentanoic acid</li> <li>14. (S,S)-<math>\beta</math>-<math>\beta</math>-<math>\beta</math>-2-Amino-3-methylpentanoic acid</li> <li>15. erythro-<math>\beta</math>-isoleucine</li> <li>16. (2S,3S)-<math>\alpha</math>-Amino-<math>\beta</math>-methyl-<math>\gamma</math>-valeric acid</li> <li>17. (2S,3S)-<math>\alpha</math>-Amino-<math>\beta</math>-methyl-valeric acid</li> <li>18. (2S,3S)-<math>\alpha</math>-Amino-<math>\beta</math>-methyl-<math>\gamma</math>-valeric acid</li> <li>19. (2S,3S)-L-Ileu; (S,S)-Ileu; (S,S)-<math>\beta</math>-<math>\beta</math>-<math>\beta</math>-2-amino-3-methylpentanoic acid</li> </ol>



## The metabolomics society (www.metabolomicsociety.org)

The screenshot shows the website for the Metabolomics Society. At the top, there are browser tabs for 'Metabolomics Society', 'CD 705 - PubChem Compound Summary', and 'Spectral Database for Organic Compounds...'. The main content area includes a navigation menu with links such as 'about us', 'mission statement', 'board members', 'membership', 'sponsorship', 'metabolomics', 'scientific events', 'scientific assistance', 'news and links', and 'contact us'. A central banner reads 'METABOLOMICS 2006 THE WEDDING SCIENTIFIC MEETING OF THE METABOLOMICS SOCIETY'. Below this, there is a section for 'Boston 2006 - Second scientific meeting' and a list of news items, including 'Metabolomics 2006 - NOW AVAILABLE ONLINE!', 'Boston 2006 - Meeting Location - 277 Lewis Palmer Hall - Boston, MA 02115', and 'Changes of meeting address & phone numbers for the meeting'. The footer contains logos for various organizations like Metabolomics Society, ESI/MS, and others.

## ... KEGG: metabolic pathways

