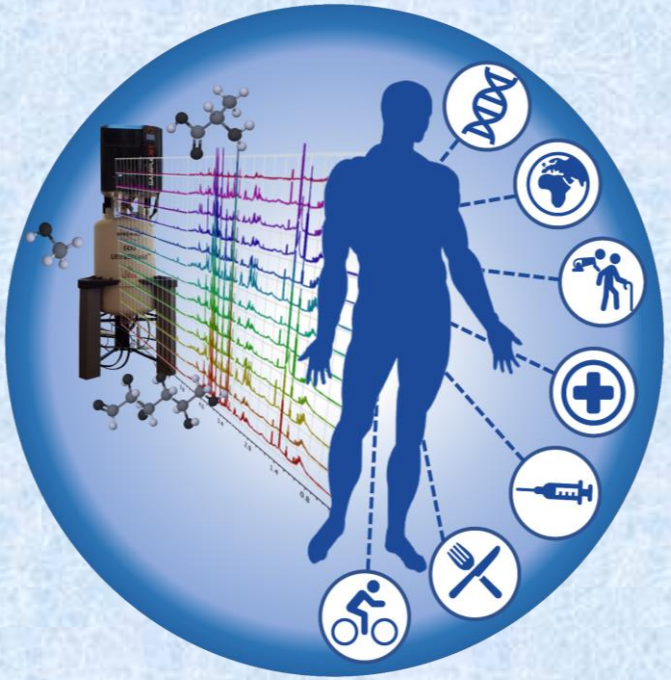
Untargeted NMR metabolomics for disease fingerprinting and biomarker discovery



Leonardo Tenori

CERM/CIRMM and

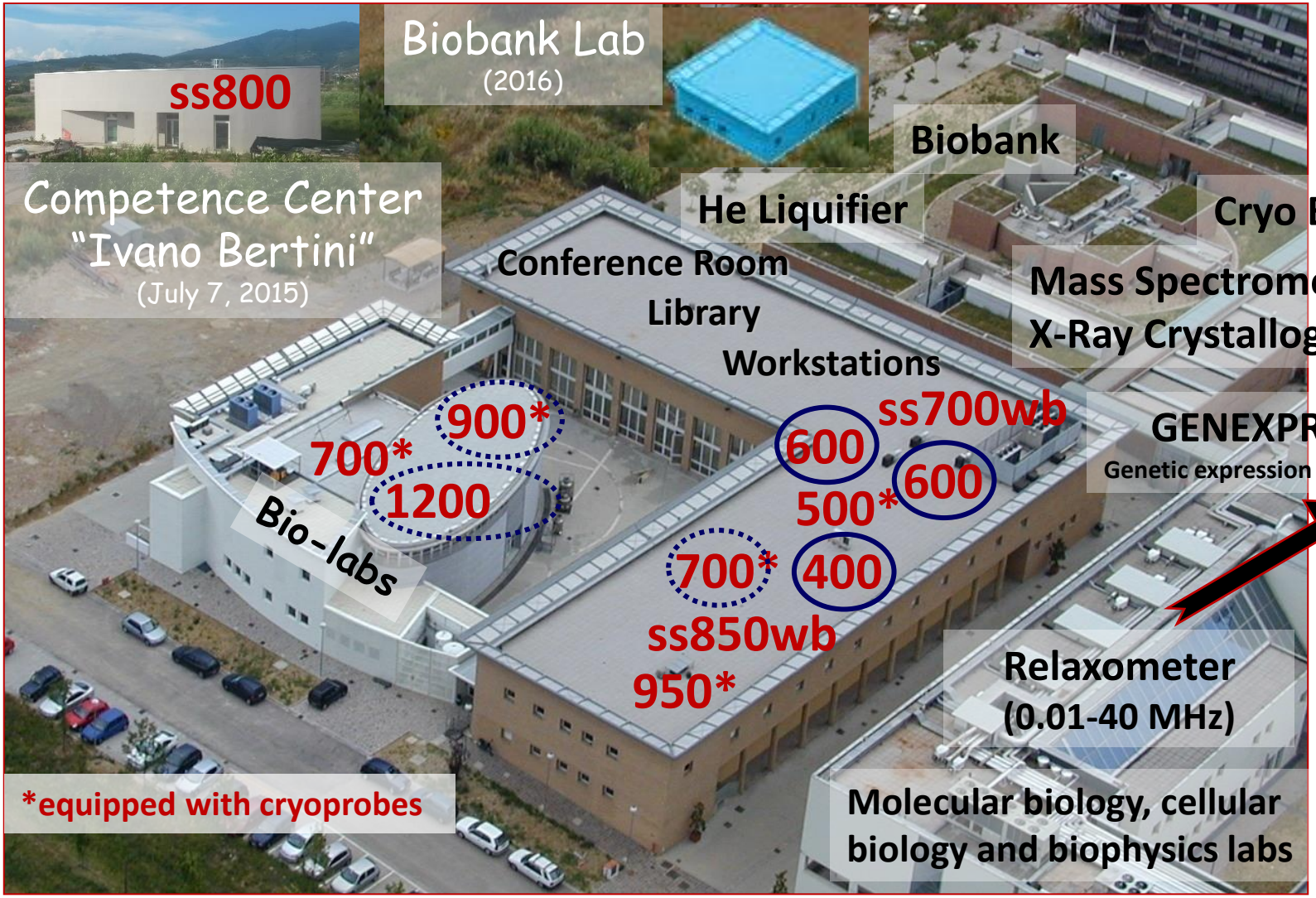
Department of Chemistry

University of Florence

Italy

CERM/CIRMMP Magnetic Resonance Center

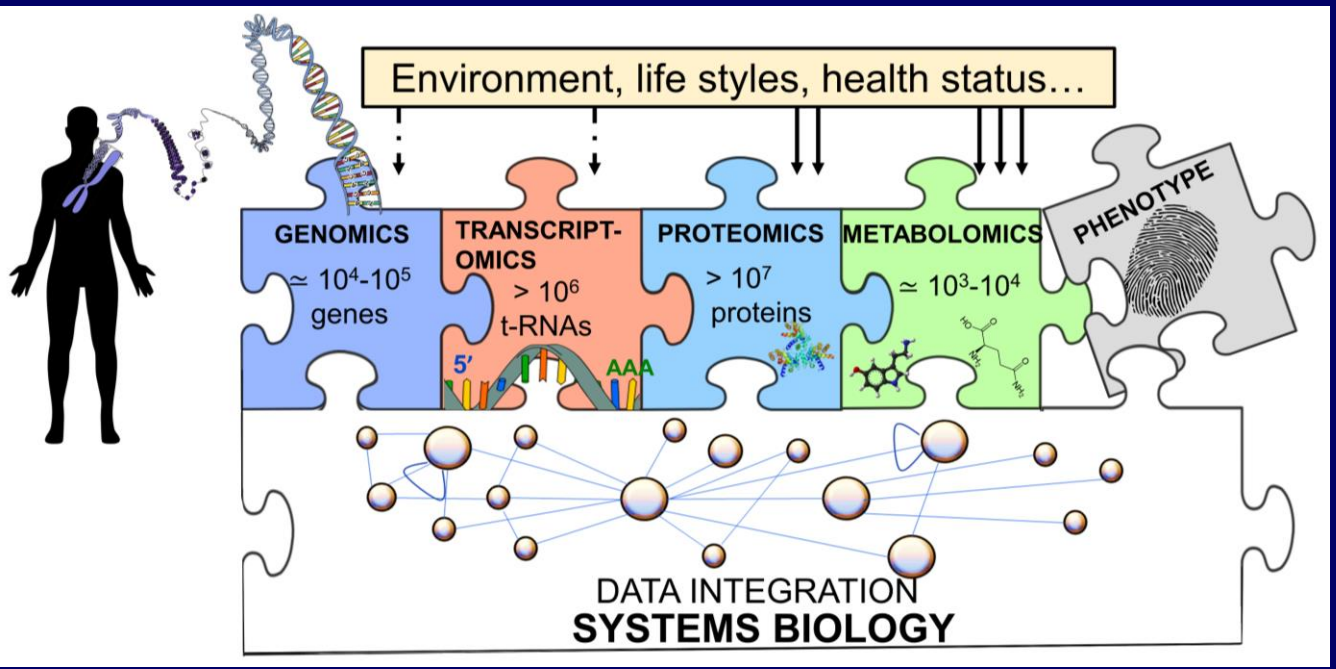
Main node of the Structural Biology Instruct-ERIC infrastructure



Fully ◯ **or partially** ◯ **for metabolomics**

Metabolomics

"Metabolomics is one of the "omic" sciences. It aims at providing a comprehensive analysis of the metabolome, which is the complete set of metabolites in a biological fluid, cell, tissue, organ or organism".



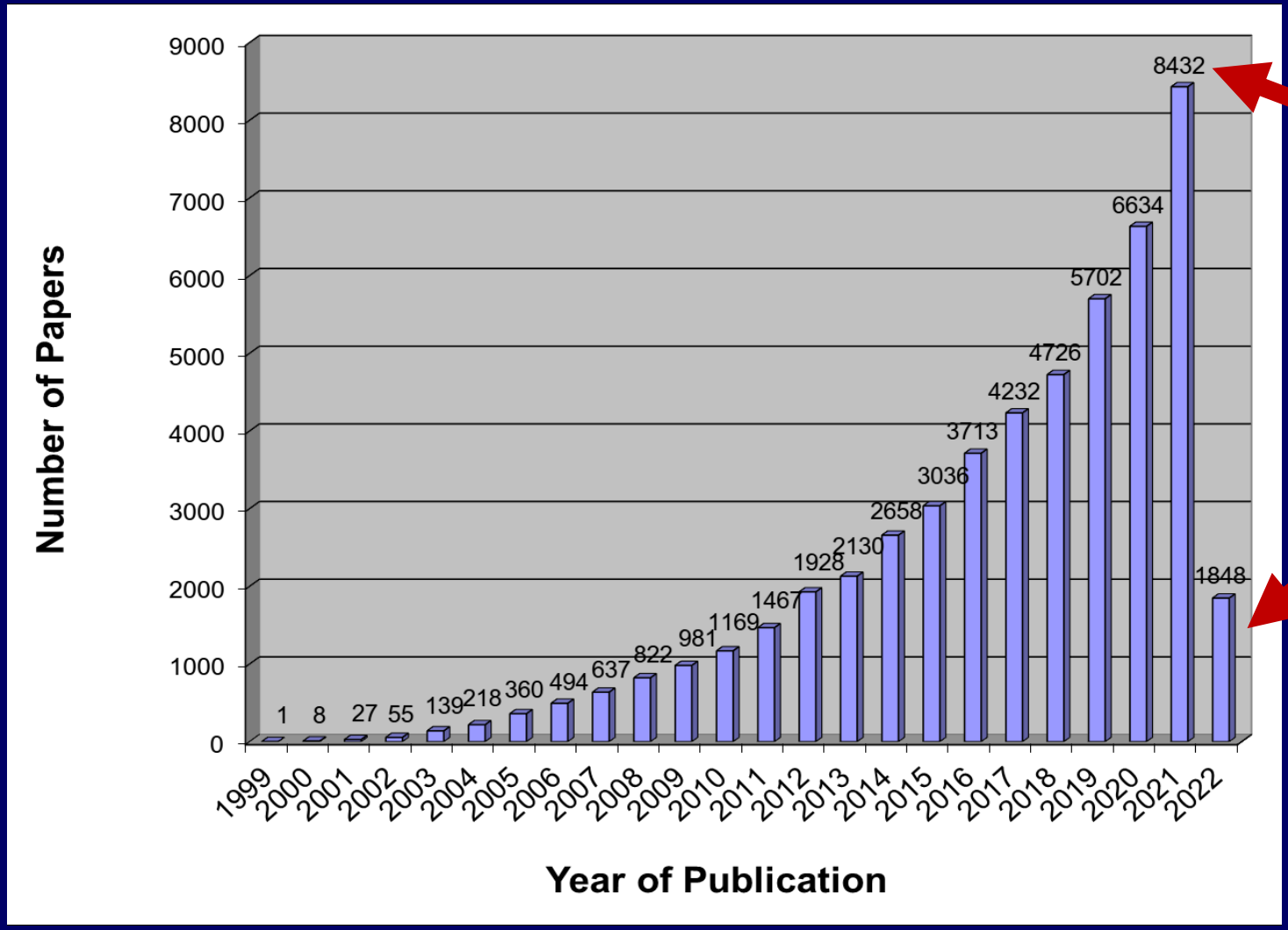
Metabolomics can provide tissue-specific information

Biological fluids such as blood and urine can provide information at the whole-body level

Not negligible external environment influence - Only a few thousand metabolites

Genomics tells you what could happen;
Metabolomics tells you what is happening

Metabolomics is still a growing field

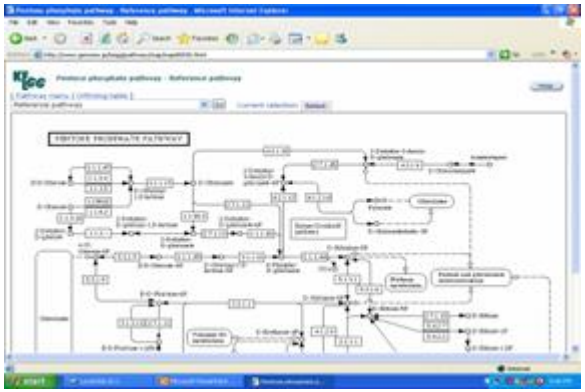


Since the late 1990s, metabolomic studies have undergone an explosive growth and this trend is still continuing, with more than eight thousand* papers published in 2021!

*Clarivate web-of-science search, 2022

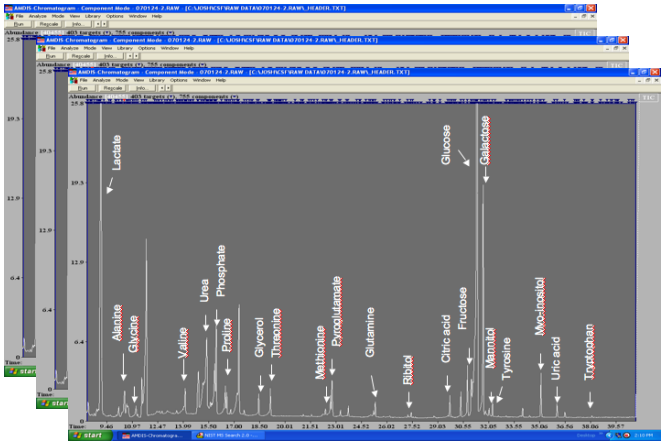
Quantitative (Targeted): preferred MS way

-Very sensitive
-Small sample size needed
-Several different implementations, tailored for specific chemical species



Biological Interpretation

Sample Prep

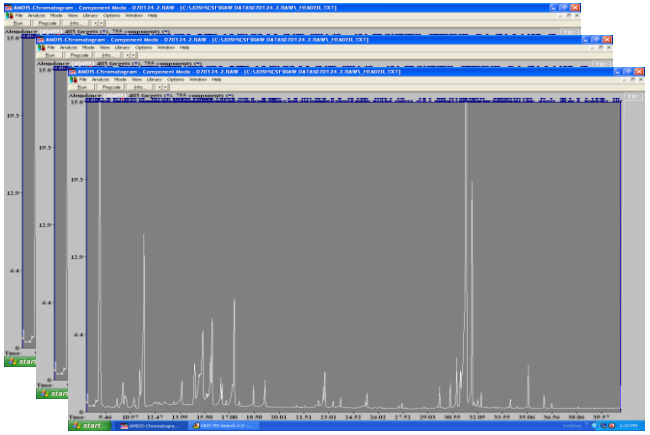


Metabolite Identification & Quantification

Fingerprinting (Untargeted)- preferred NMR way

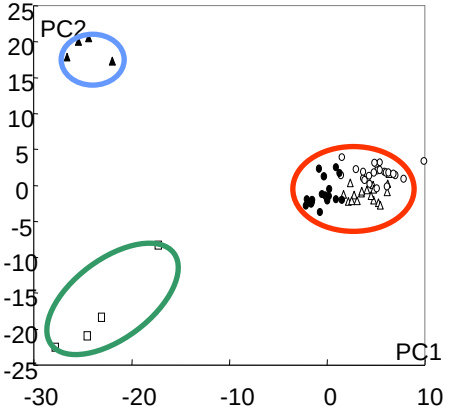
-Fast
-Easy sample preparation
-Non need for analytical separation

Sample Prep



Data Collection

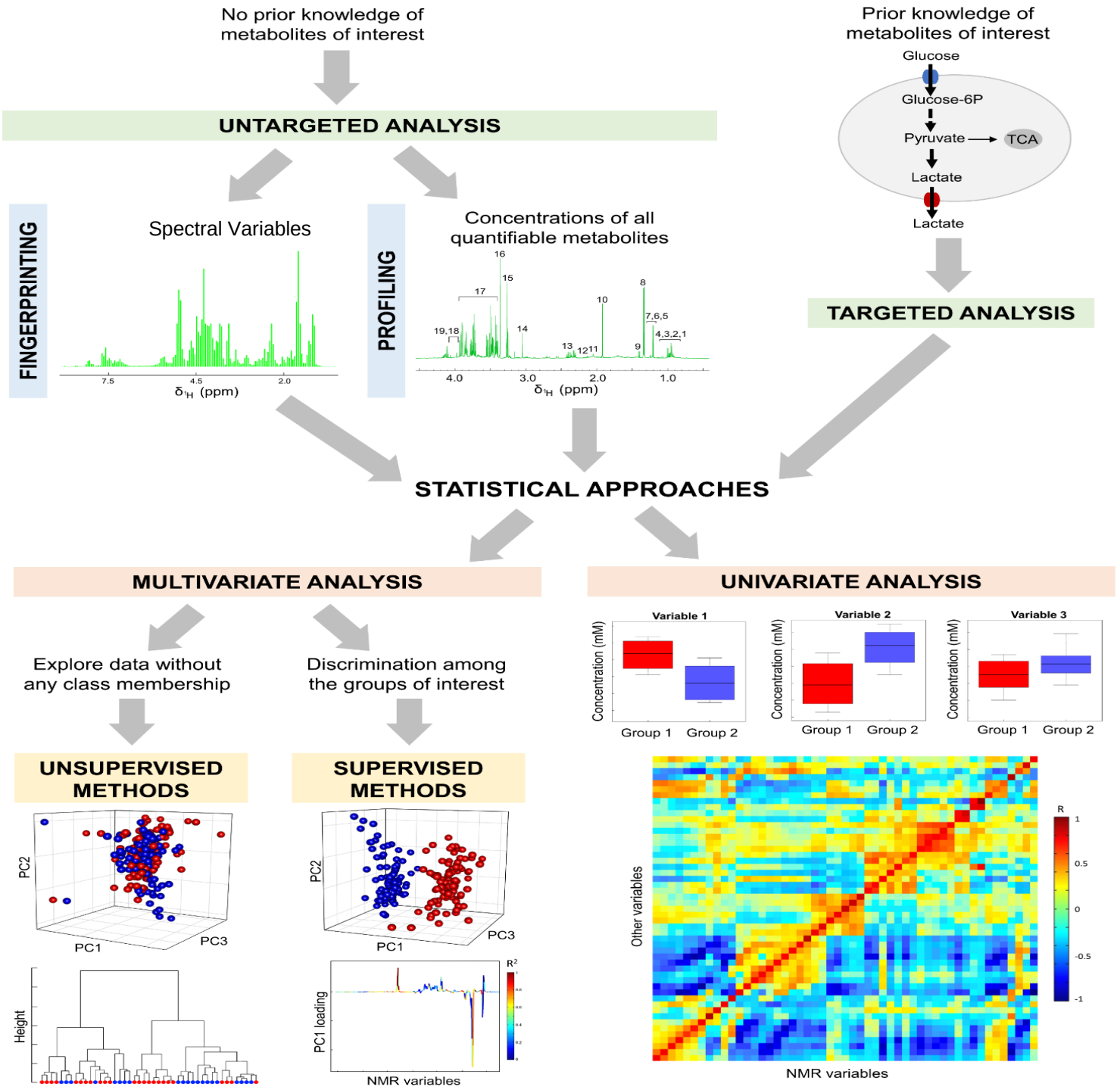
Data Reduction



If NMR identify some abundant metabolites belonging to a specific metabolic cycle, then using MS it is possible to look for target metabolites, not detected by NMR but that are known to be related to that specific biological pathway.

NMR generates hypotheses, MS can confirm them and can go in deeper details

COMPLEMENTARY TECHNIQUES



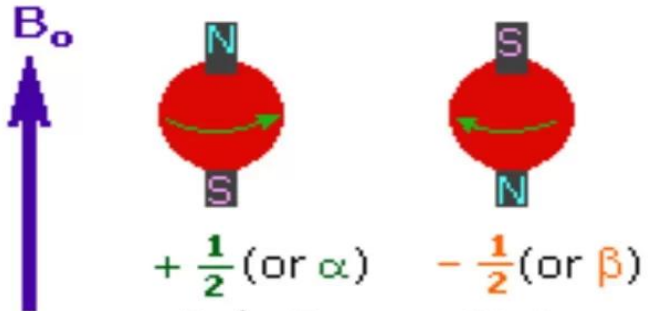
Angew Chem Int Ed Engl. 2018 Jul 12. doi: 10.1002/anie.201804736

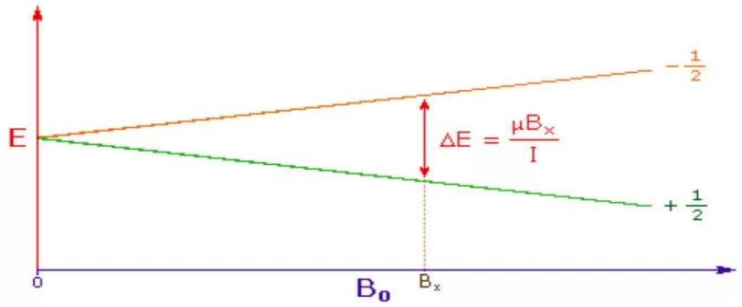
The NMR Spectrometer

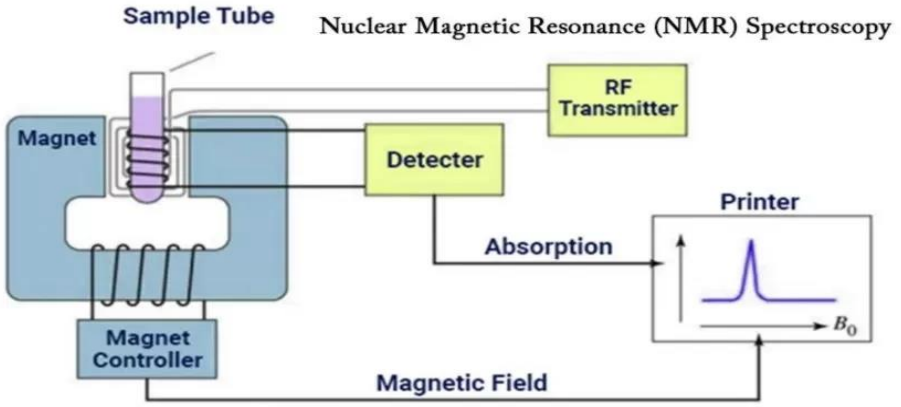
Nuclear Magnetic Resonance (NMR) measures the absorption of electromagnetic radiation in the radio-frequency region (~4-1200 MHz)

-nuclei are involved in absorption process

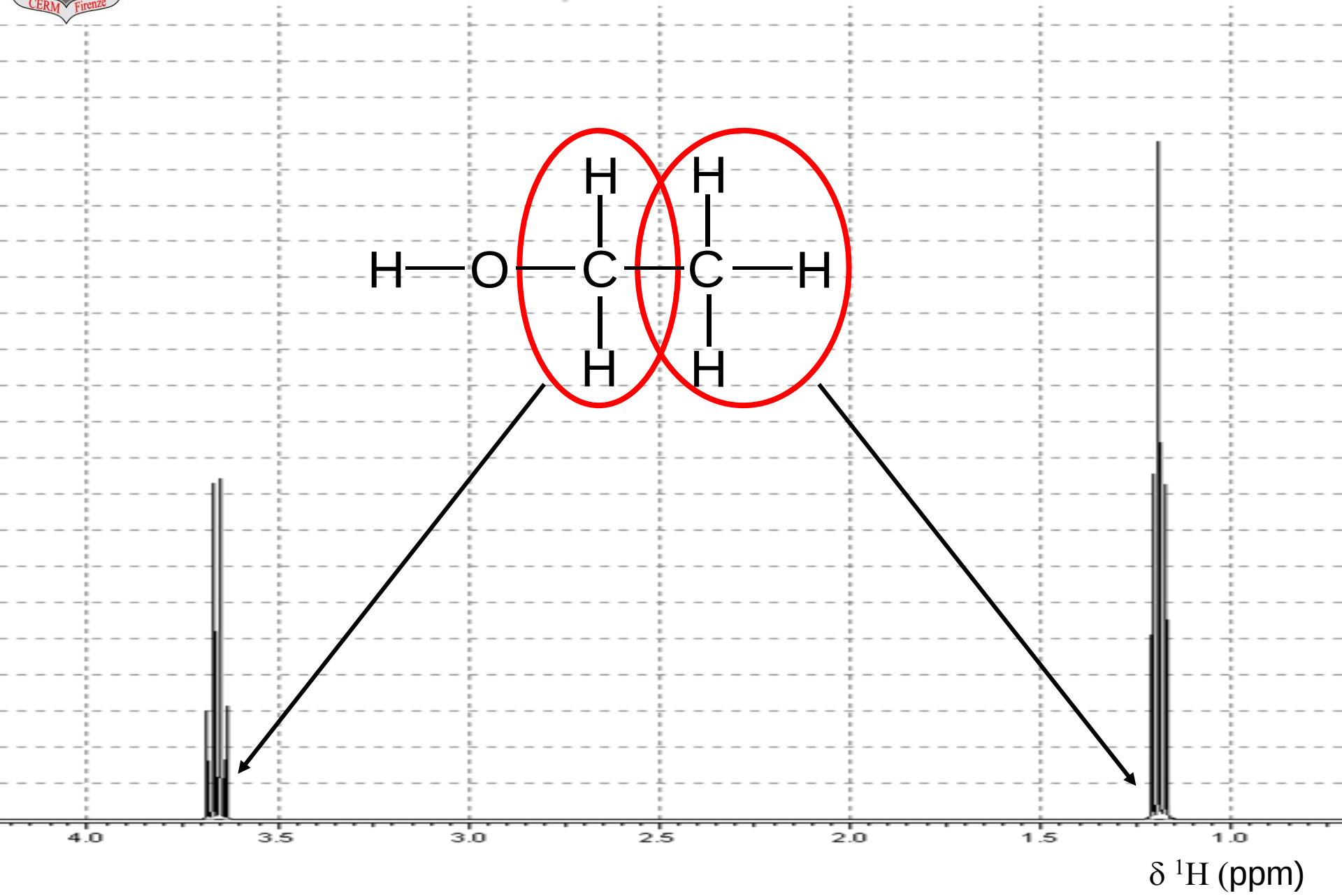
-sample needs to be placed in magnetic field to cause different energy states



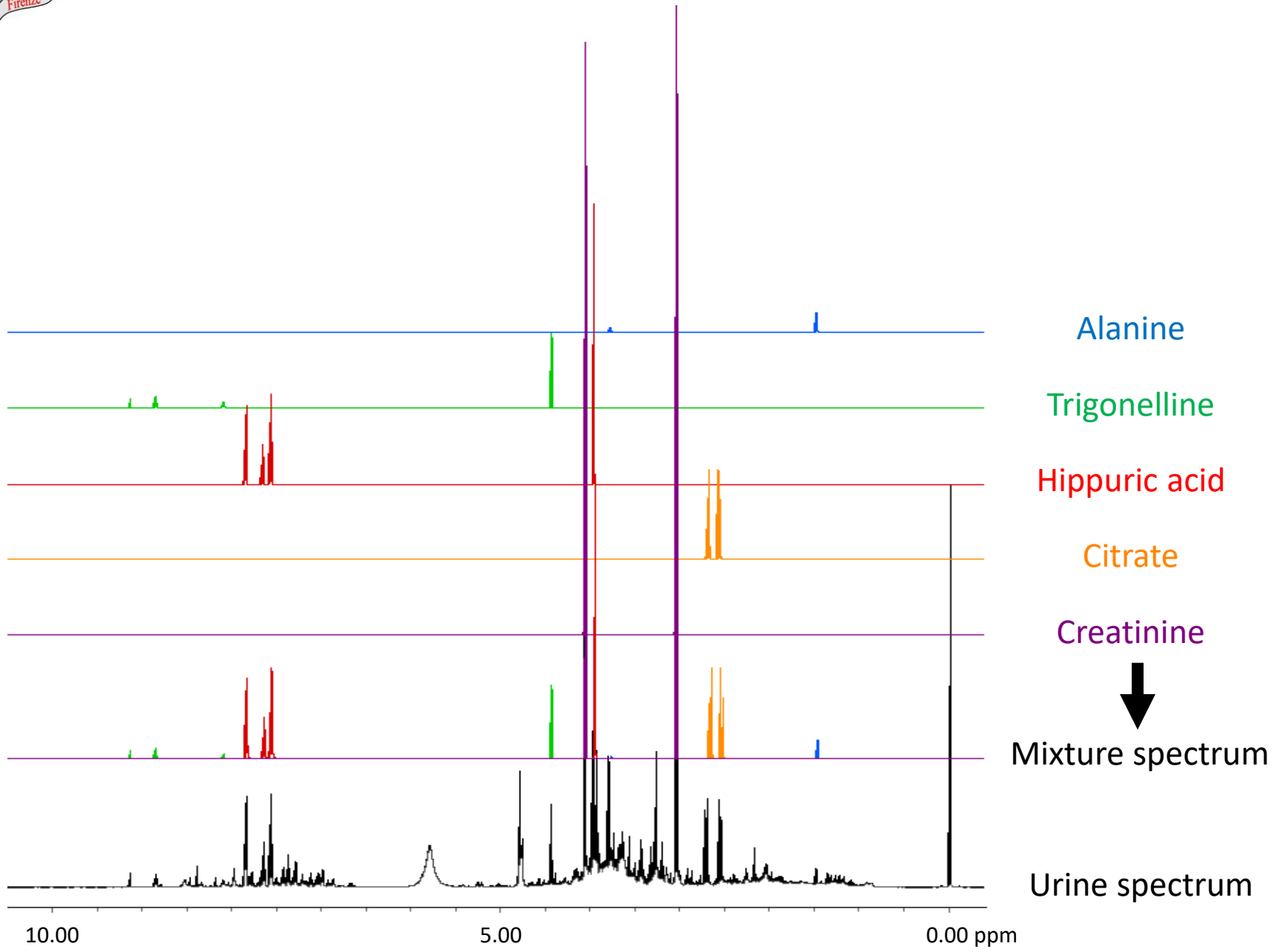




^{1}H NMR spectrum of ethanol

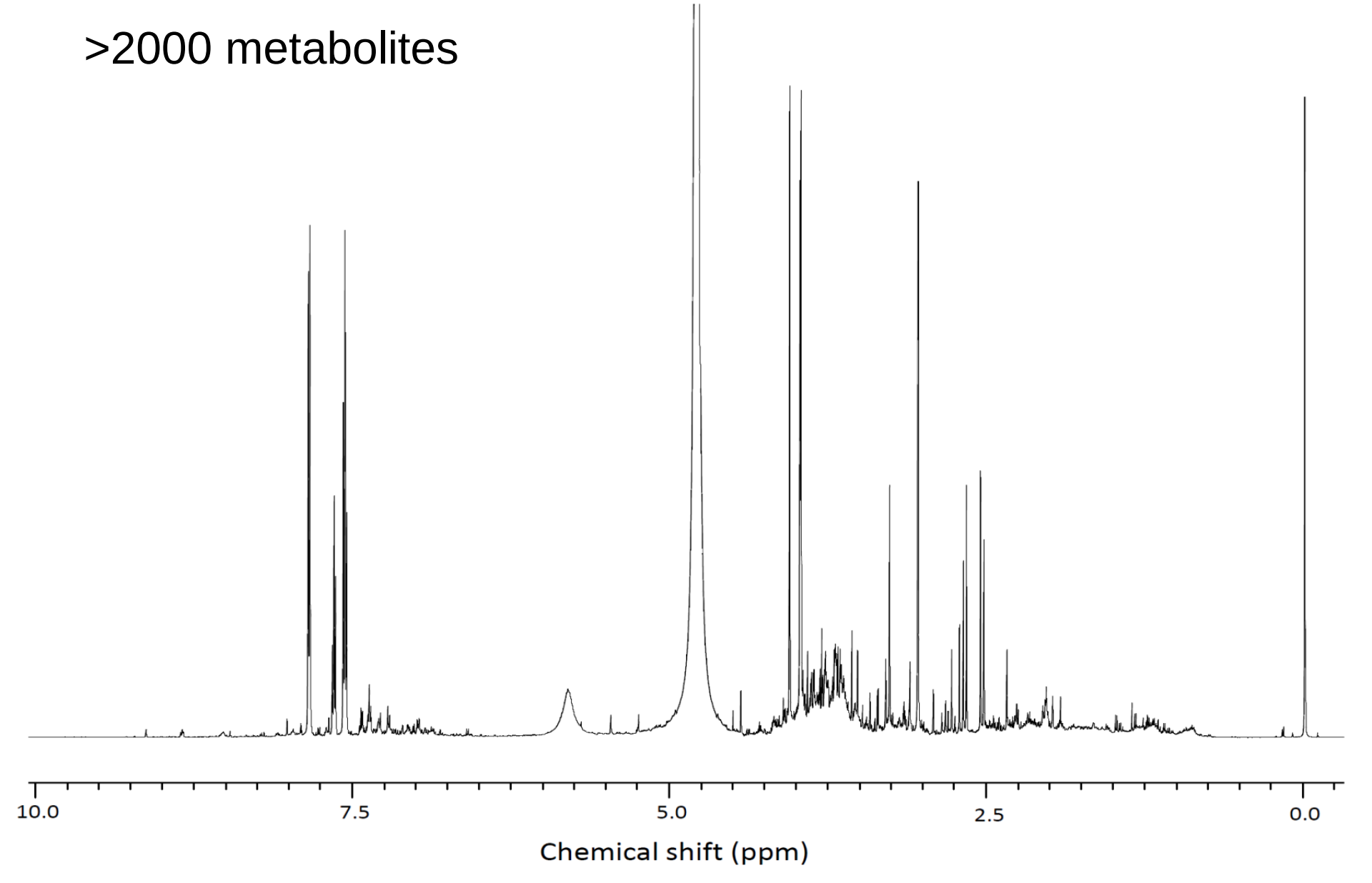


^{1}H NMR spectrum of a urine sample



^{1}H NMR of human urine

>2000 metabolites



Handling and preparation of the samples

Typical biofluids used in NMR metabolomics

- Urine
- Serum/plasma
- Saliva
- Fecal extracts
- Exhaled breath condensate
- Cells/tissues extracts

And also tears, sweat, vaginal fluid, seminal fluid, synovial fluid, bile, cerebrospinal liquid, …

Intact tissues with HRMAS

Acquisition of the NMR spectra

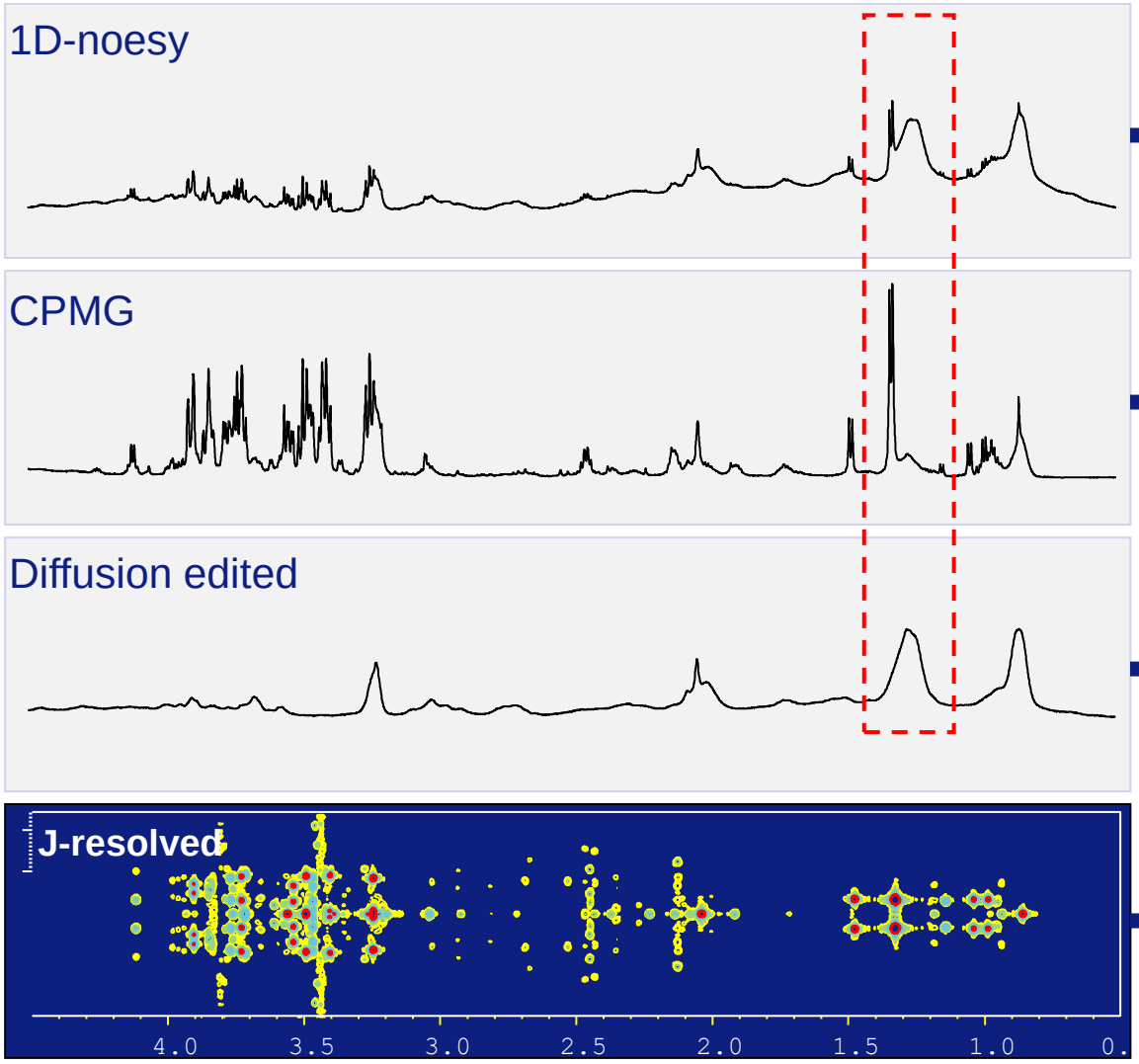
600 MHz standard field
for metabolomics

5 mm PATXI 1H-13C-15N probe including an automatic and refrigerated sample changer (SampleJet, Bruker BioSpin s.r.l.; Rheinstetten, Germany)

The automatic samples charger allow us to work in (moderately) high throughput

~15 min for a urine sample
~30 min for a serum sample (more experiments)

1H NMR analysis of a serum sample

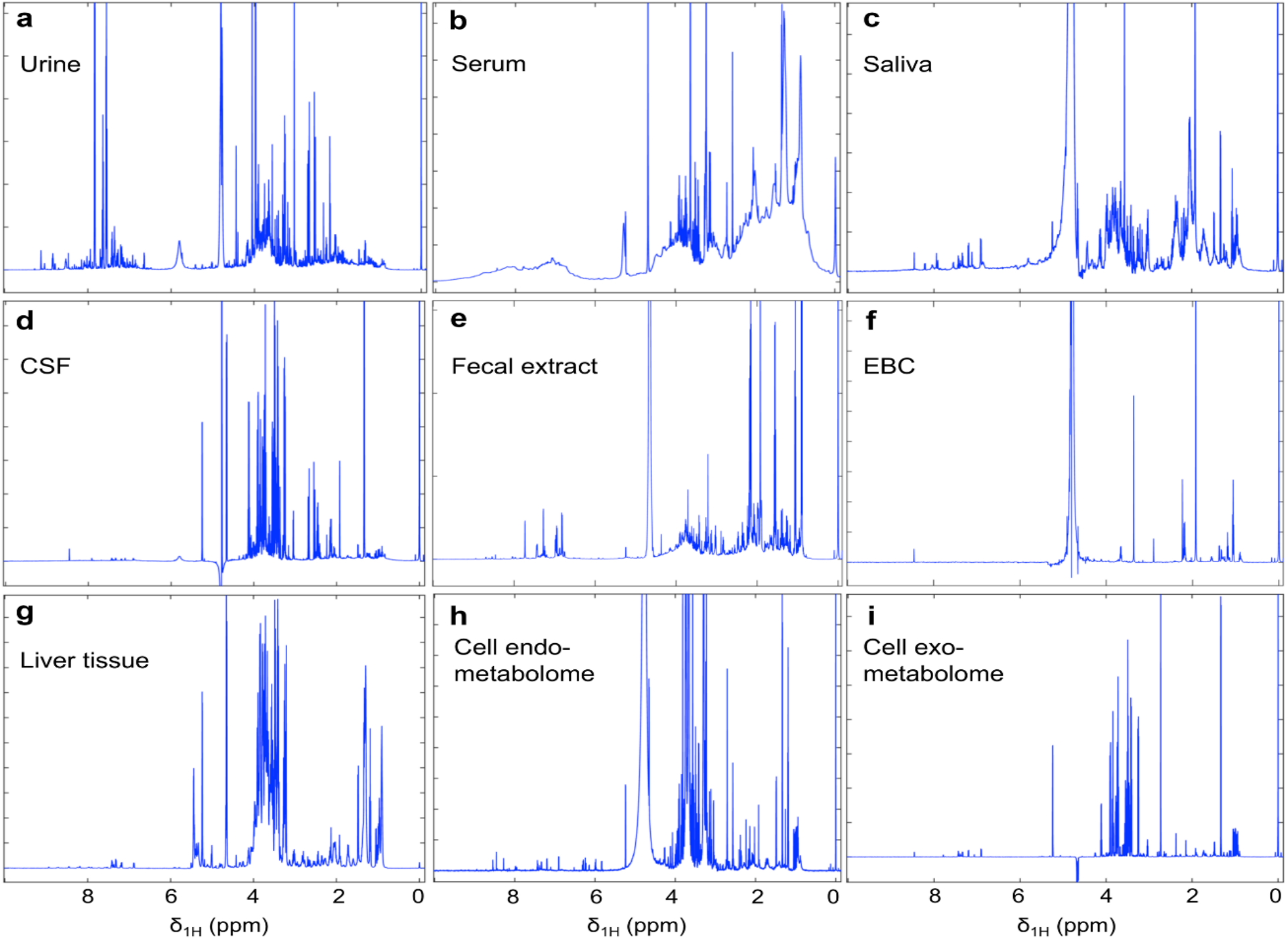


Low and high molecular weight profile

Low molecular weight metabolite profile

High molecular weight metabolite profile

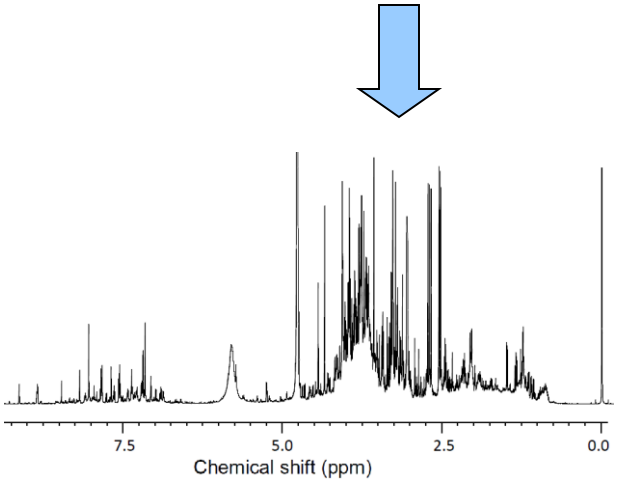
Identification purposes

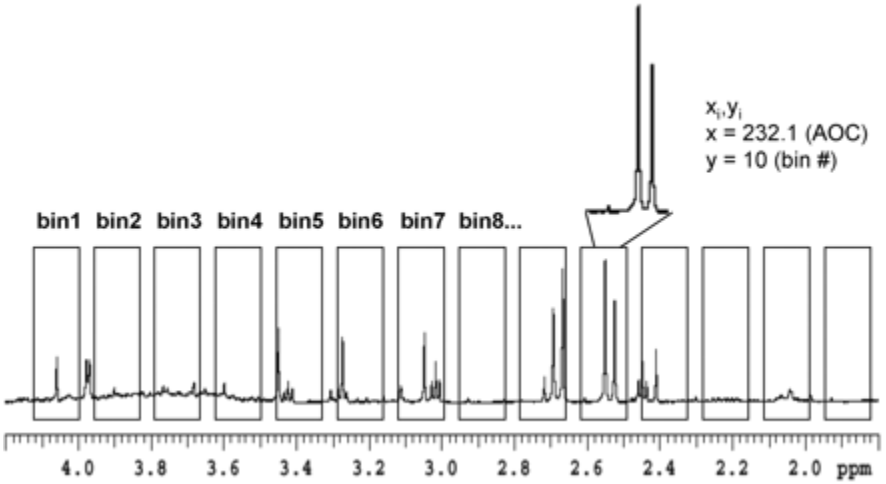


Angew Chem Int Ed Engl. 2018 Jul 12. doi: 10.1002/anie.201804736

Data Preparation for fingerprinting

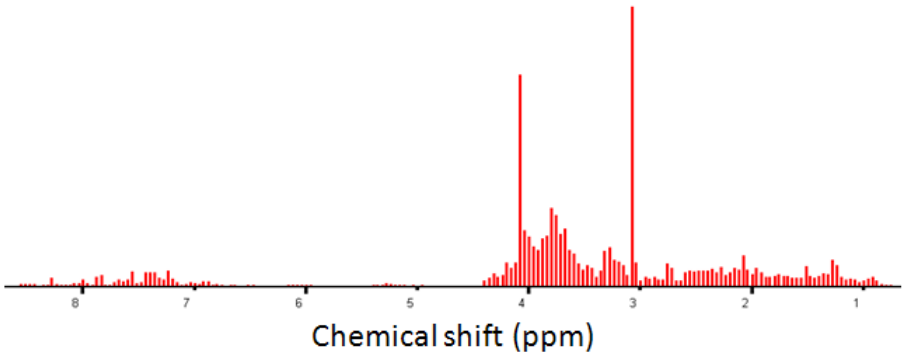
phasing, baseline correction…
NMR spectra with 64 or 128k points



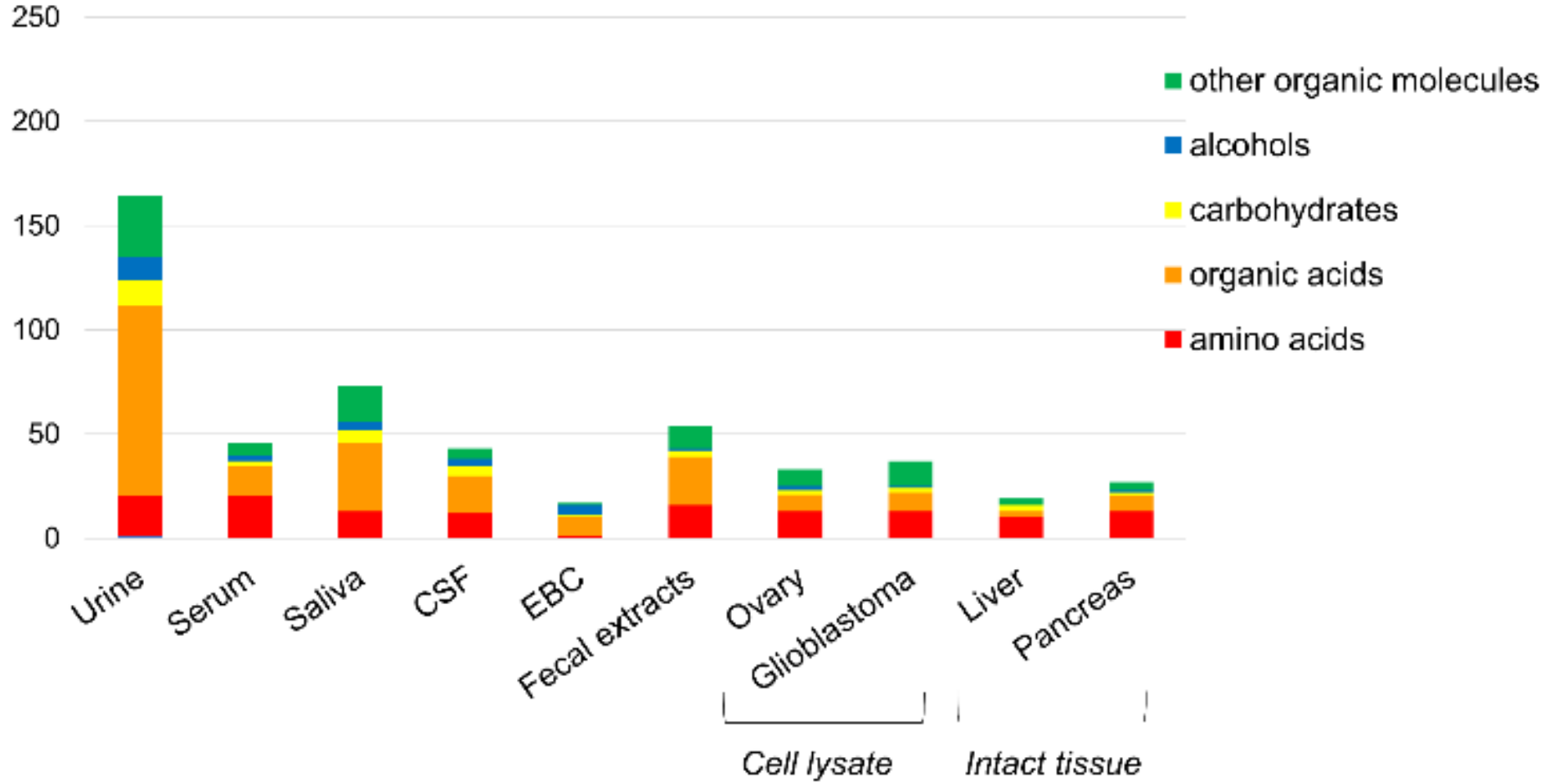


Bucketing is a means to reduce the number of total variables and to compensate for small shifts in the spectra.

Binned spectra, n=~400 bins



Identification and quantification for profiling



Number of detectable and quantifiable metabolites with ≥50% occurrence in the ^{1}H NMR spectra of different biological fluids

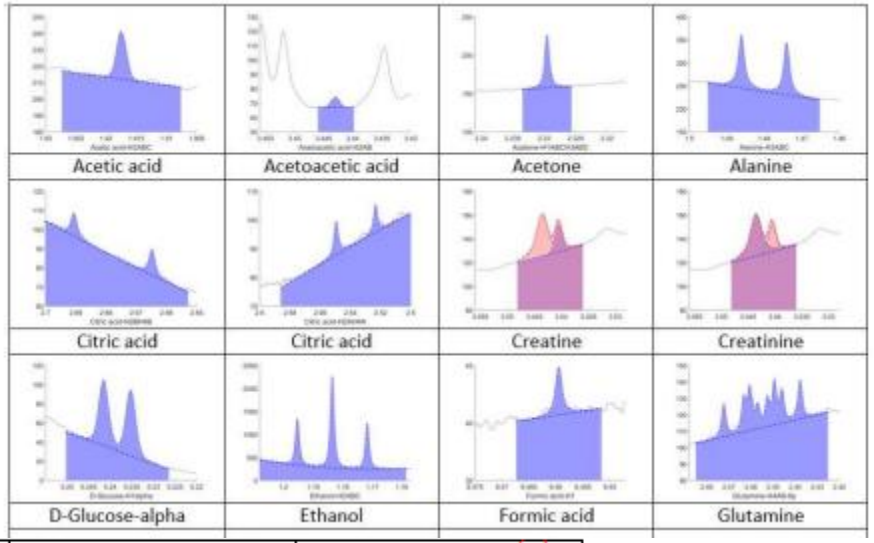
Angew Chem Int Ed Engl. 2018 Jul 12. doi: 10.1002/anie.201804736

Analysis Report

Bruker **I**VDr **Quant**ification in **P**lasma/**S**erum B.I.Quant-PS b™

Sample ID: B_A_172.100000.10r

Measuring Date: 23-Feb-2017 15:45:58
Reporting Date: 03-Oct-2018 17:49:36, 4 page(s), Version 0.0.1
Quantification Method Version: Quant-PS B.0.0.1



Amino acids and derivatives

Compound	Conc. mmol/L	LOD mmol/L	95% Range mmol/L	Graphics (*)
Alanine	0.29	0.02	0.29 - 0.63	
Creatine	0.06	0.01	≤ 0.07	
Glutamic acid	0.11	0.05	≤ 0.25	
Glutamine	0.86	0.02	0.29 - 0.82	
Glycine	0.34	0.10	0.15 - 0.42	
Histidine	0.10	0.02	0.07 - 0.17	
Isoleucine	0.06	0.03	0.03 - 0.11	
Leucine	0.08	0.01	0.06 - 0.20	
Phenylalanine	0.07	0.05	≤ 0.06	
Threonine	0.17	0.06	≤ 0.51	
Tyrosine	0.05	0.03	≤ 0.08	
Valine	0.20	0.03	0.15 - 0.36	

Anal Chem. 2018 Sep 27.
doi:10.1021/acs.analchem.8b02412

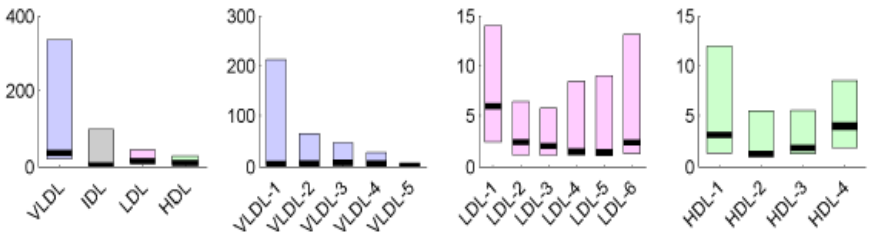
Analysis Report

Bruker IVDr Lipoprotein Subclass Analysis B.I.LISA™

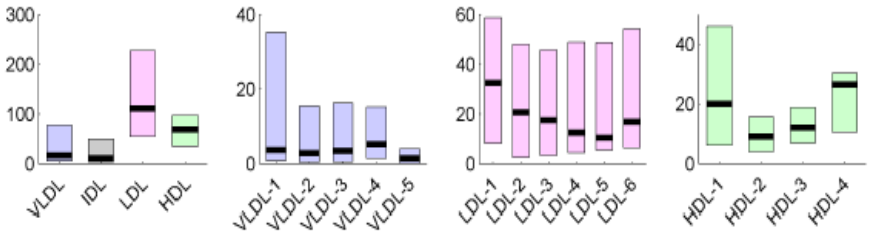
A tool from Bruker, for the calculation of lipoproteins subclass using NMR spectra

Lipid Distribution Overview

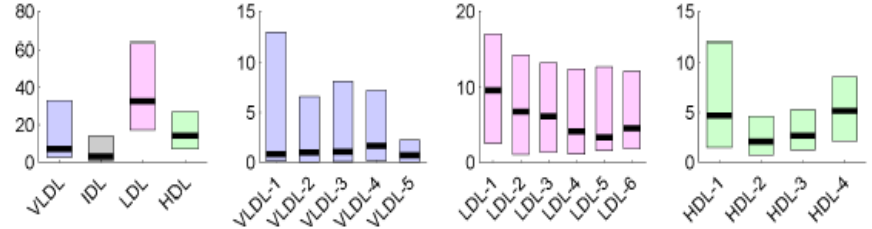
Triglycerides distribution (concentrations in mg/dL together with 95% range of model)

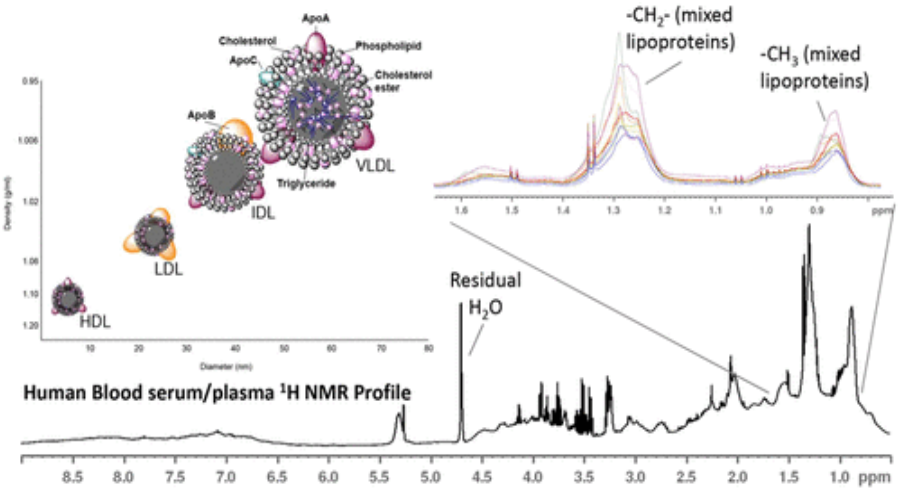


Cholesterol distribution (concentrations in mg/dL together with 95% range of model)



Free Cholesterol distribution (concentrations in mg/dL together with 95% range of model)





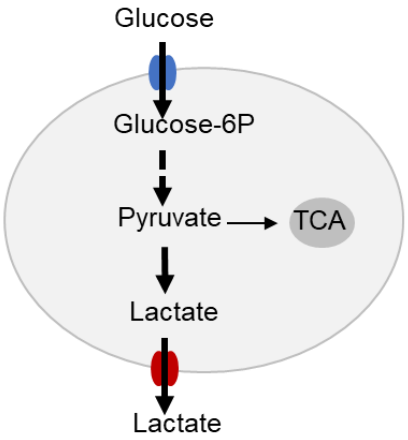
Anal Chem. 2018 Sep 27.
doi:10.1021/acs.analchem.8b02412

Fingerprinting

No prior knowledge of metabolites of interest

NMR DATA

Prior knowledge of metabolites of interest

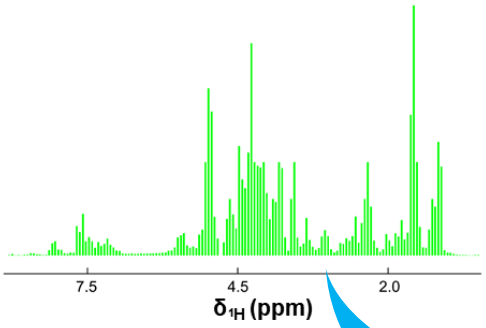


TARGETED ANALYSIS

UNTARGETED ANALYSIS

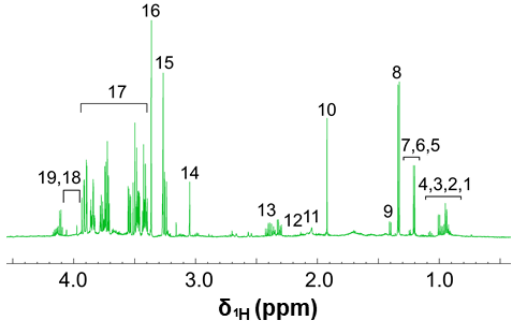
FINGERPRINTING

Binned spectra

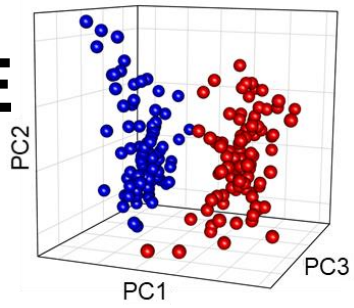


PROFILING

Concentrations of all quantifiable metabolites



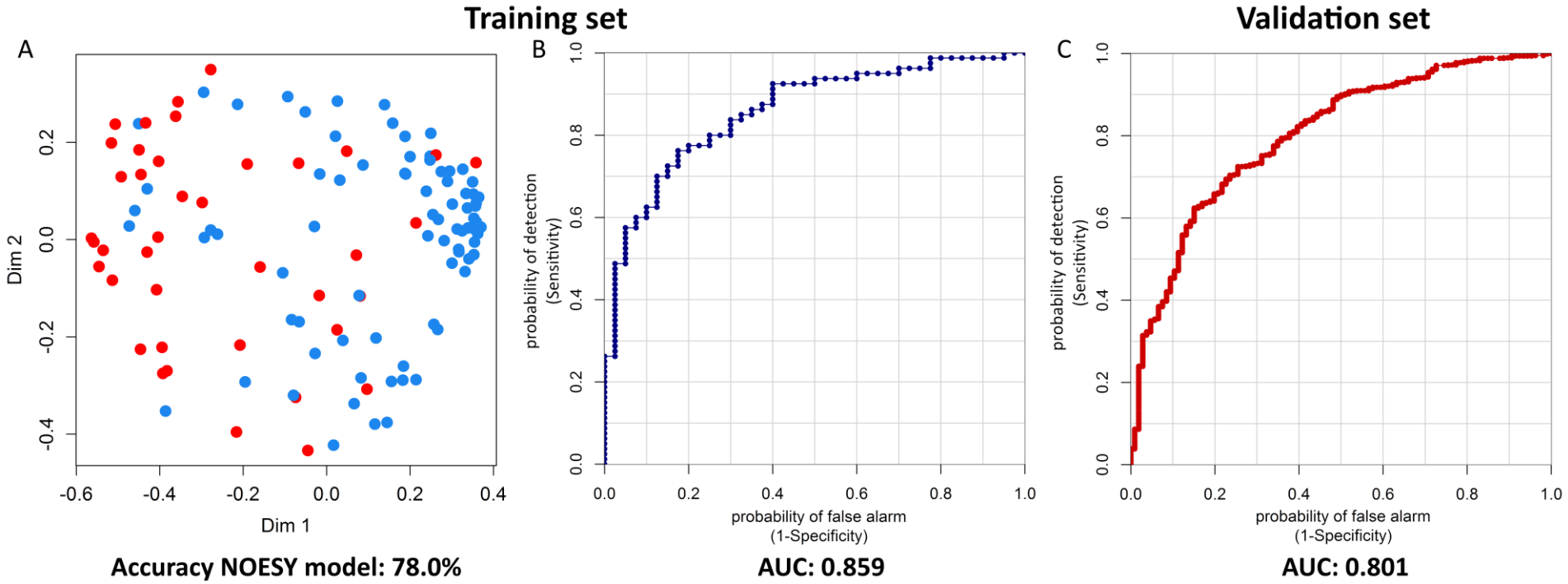
MULTIVARIATE ANALYSIS



A. Vignoli, V. Ghini, G. Meoni, et al. *High-Throughput Metabolomics by 1D NMR*. Angewandte Chemie, 2019, 58(4): 968–994

Amiflorence II study

NMR-based metabolomics was performed in **978 serum samples** to identify **patients at high risk of death** within two years from AMI.



Metabolomic fingerprint of **80 SURVIVOR** and **40 DECEASED** patients after AMI showed significant differential clustering using a Random Forest classifier.

Results were replicated in a validation set with 752 survivor patients and 106 deceased patients

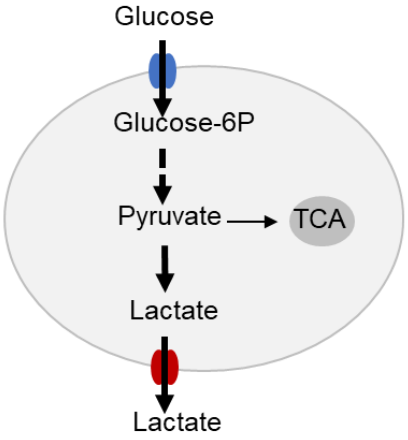
A. Vignoli, L. Tenori, B. Giusti, et al., *NMR-based metabolomics identifies patients at high risk of death within two years after acute myocardial infarction in the AMI-Florence II cohort*, BMC Med. 2019; 7;17(1):3.

NMR Metabolomics

No prior knowledge of metabolites of interest

NMR DATA

Prior knowledge of metabolites of interest

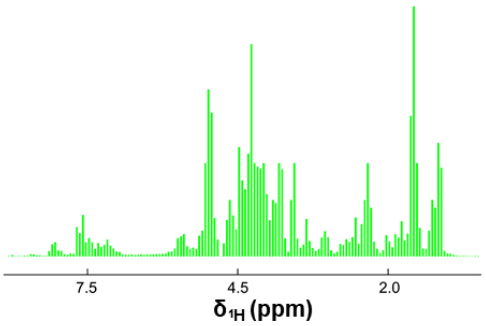


TARGETED ANALYSIS

UNTARGETED ANALYSIS

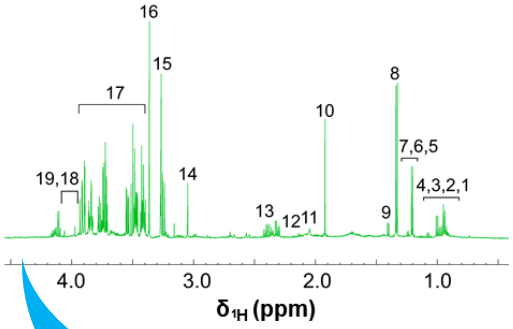
FINGERPRINTING

Binned spectra



PROFILING

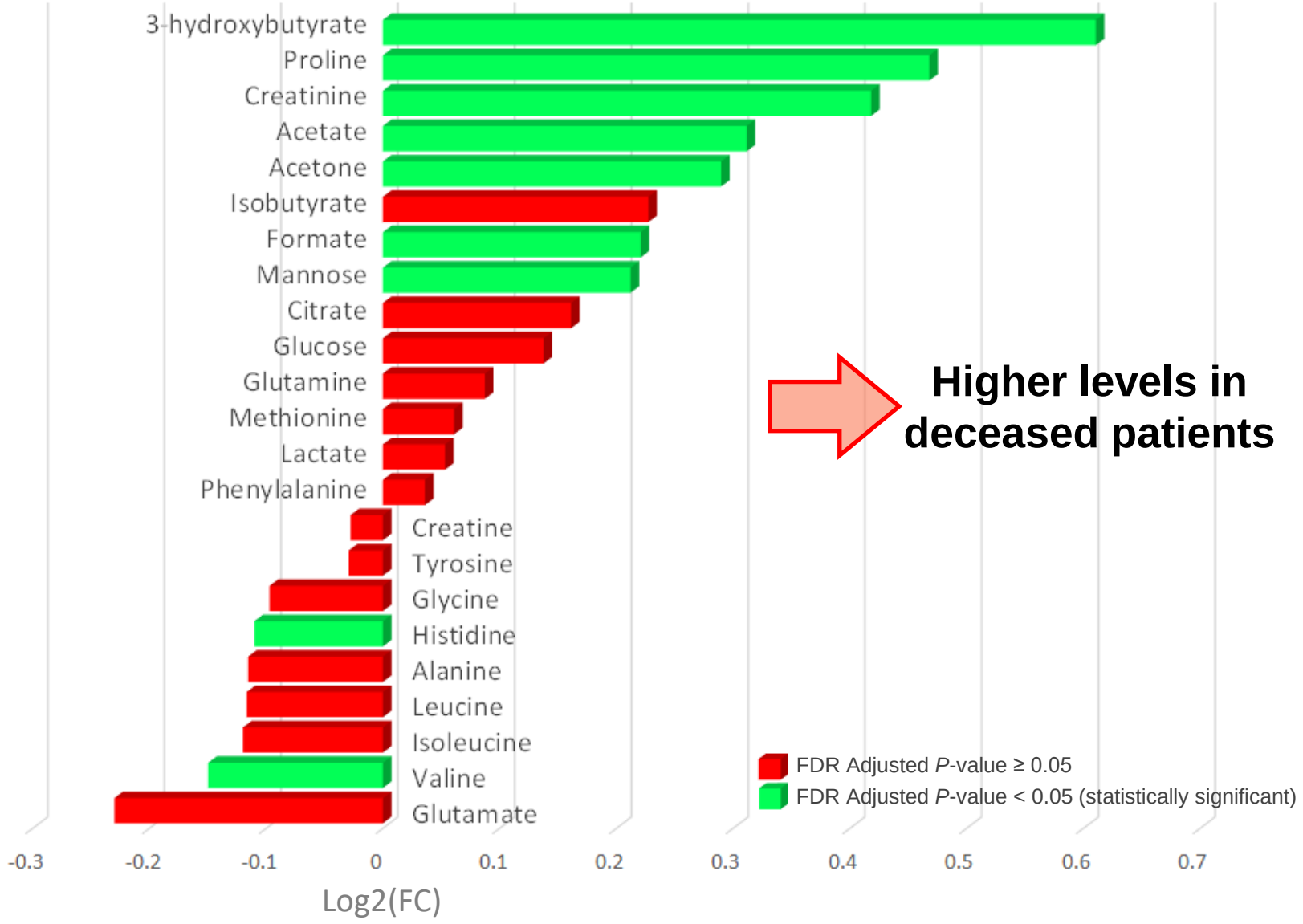
Concentrations of all quantifiable metabolites



UNIVARIATE ANALYSIS

A. Vignoli, V. Ghini, G. Meoni, et al. *High-Throughput Metabolomics by 1D NMR*. Angewandte Chemie, 2019, 58(4): 968–994

Metabolite analysis



A. Vignoli, L. Tenori, B. Giusti, et al., *NMR-based metabolomics identifies patients at high risk of death within two years after acute myocardial infarction in the AMI-Florence II cohort*, BMC Med. 2019; 7;17(1):3.

Thank you for your attention!

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Centro Risonanze Magnetiche
FIRENZE

CIRMMP
FIRENZE

UNIVERSITÀ DEGLI STUDI FIRENZE

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Dr. Veronica Ghini
Dr. Alessia Vignoli
Dr. Gaia Meoni
Francesca Di Cesare

Questions?