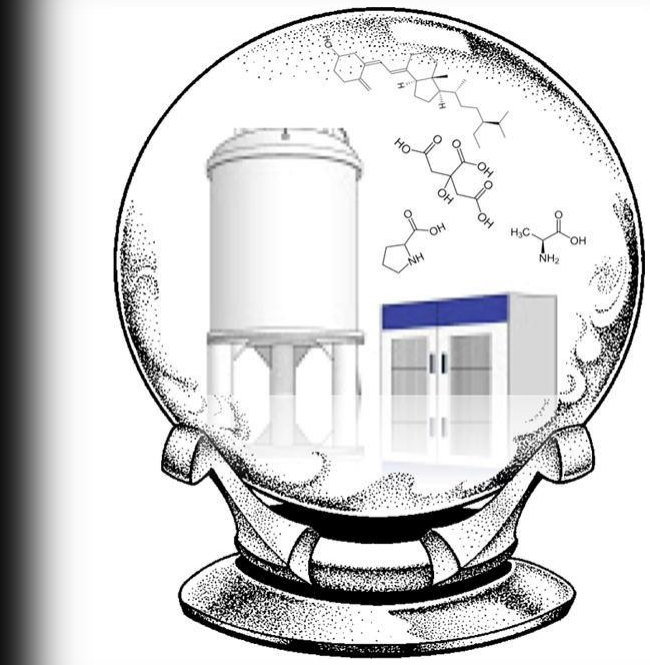


NMR Spectroscopic ‘maps’:

A tool for exploring mixtures

Let’s discuss!

Leesa J. Klau

NMR Club – 02.10.2019

Image source: 1. Wishart, D. S. *J. Magn. Reson.* **2019**, 306:155-161

Applications of NMR spectroscopy to analysing mixtures

Targeted

Aim: identify (and quantify) defined set of metabolites

Metabolomics

Metaboloic profiles of entire contents of cells within the test sample (e.g. biofluids, bacterial cells, plant material…)

Untargeted

Aim: compare entire profiles

Have you had experience with any other applications?

Bingol, K. *High-throughput*. **2018,** 7:9

Applications of NMR spectroscopy to analysing mixtures

Aquatic Toxicology 184 (2017) 123–132

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

^{1}H NMR-based metabolomics reveals sub-lethal toxicity of a mixtu
of diabetic and lipid-regulating pharmaceuticals on amphibian larv

Steven D. Melvin [a,*], Leesa J. Habener [b], Frederic D.L. Leusch [a,b], Anthony R. Carroll [b]

[a] Australian Rivers Institute, Griffith University, Southport, QLD 4222, Australia
[b] Griffith School of Environment, Griffith University, Southport, QLD 4222, Australia

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol

Next-generation metabolomics in lu
treatment and precision medicine: mini

Li Yu[1], Kefeng Li[2] and Xiaoye Zhang[1]

[1]Department of Oncology, Shengjing Hospital, China Medical University, Shen
[2]School of Medicine, University of California San Diego, San Diego, CA, USA

Phytochemistry 71 (2010) 773–784

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Metabolic classification of South American *Ilex* species by NMR-based metabolomics

Hye Kyong Kim [a,1], Saifullah [a,b,1], Saifullah Khan [b], Erica G. Wilson [a], Sergio D. Prat Kricun [c], Axel Meissner [d], Sibel Goraler [d], André M. Deelder [d], Young Hae Choi [a,*], Robert Verpoorte [a]

The Emerging Field of Quantitative Bloo
for Biomarker Discovery in Critical Illnes

Natalie J. Serkova[1-3], Theodore J. Standiford[4], and Kathleen A. Stringer[5]

[1]Department of Anesthesiology, School of Medicine; [2]NMR Metabolomics Core of the Colorado Clinica
[3]University of Colorado Cancer Center, University of Colorado Denver, Aurora, Colorado; [4]Division of P
Department of Internal Medicine, School of Medicine; and [5]Department of Clinical, Social, and Admini
University of Michigan, Ann Arbor, Michigan

Secondary Metabolome Variability and Inducible Chemical Defenses in the Mediterranean Sponge *Aplysina cavernicola*

M. Reverter[1] · T. Perez[2] · A. V. Ereskovsky[2,3] · B. Banaigs[1]

Review

Metabonomics in Toxicology: A Review

Donald G. Robertson[1]

Metabonomics Evaluation Group, Department of World-Wide Safety Sciences, Pfizer Global Research and Development, Ann Arbor, Mich

Received December 2, 2004; accepted January 31, 2005

R A Review of Applications of Metabolomics in Cancer

Richard D. Beger

Metabolomic analysis in food science: a review

Juan M. Cevallos-Cevallos[a], José I. Reyes-De-Corcuera[a,*], Edgardo Etxeberria[a], Michelle D. Danyluk[a] and Gary E. Rodrick[b]

Metabolomic analyses have been generally classified as targeted or untargeted (Fig. 1). Targeted analyses focus on a specific group of intended metabolites with most cases requiring identification and quantification of as many metabolites within the group (Ramautar, Demirci, & Jong, 2006). Targeted analyses are important for assessing the behavior of a specific group of compounds in the sample under determined conditions. Targeted metabolomics typically requires higher level of purification and a selective extraction of metabolites. In contrast, untargeted (a.k.a. comprehensive) metabolomics focuses on the detection of as many groups of metabolites as possible to obtain patterns or fingerprints without necessarily identifying nor quantifying a specific compound(s) (Monton & Soga, 2007). Untar-

Environmental metabolomics: a critical review and future perspectives

Jacob G. Bundy · Matthew P. Davey · Mark R. Viant

NMR metabolomics and drug discover

Robert Powers*

Recent applications of NMR spectroscopy in plant metabolomics

Jane L. Ward, John M. Baker and Michael H. Beale

The National Centre for Plant and Microbial Metabolomics, Rothamsted Research, Harpenden, UK

Metabolomics Reviewed: A New "Omics" Platform Technology f
Biology and Implications for Natural Products Research

Simone Rochfort*

*Environmental Health and Chemistry, Department of Primary Industries, Primary Industries Researc
Centre, Victoria, Australia*

Received July 18, 2005

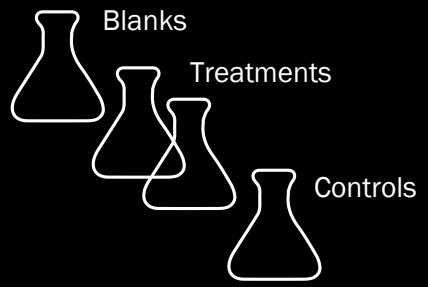
Review on metabolomics for food authentication

Elena Cubero-Leon, Rosa Peñalver, Alain Maquet *

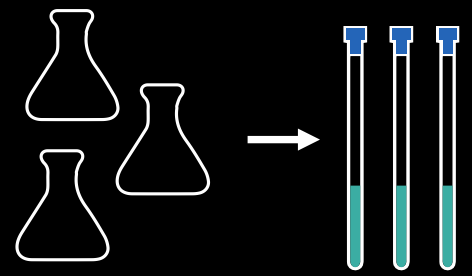
European Commission, Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Retieseweg 111, 2440 Geel, Belgium

Have you had experience with any other applications?

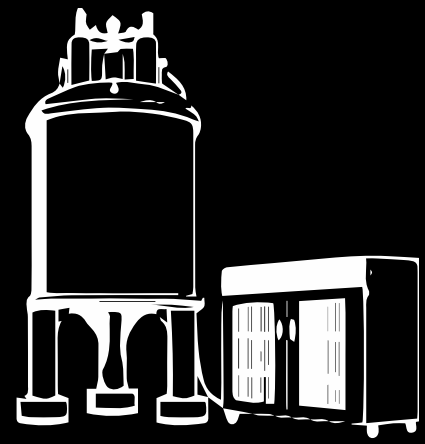
Common Approach/Workflow



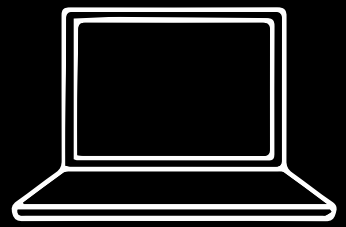
Experimental Design



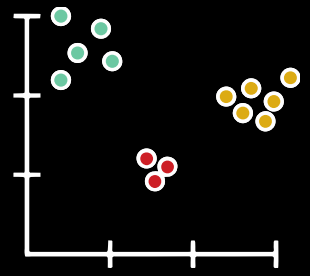
Sample Preparation



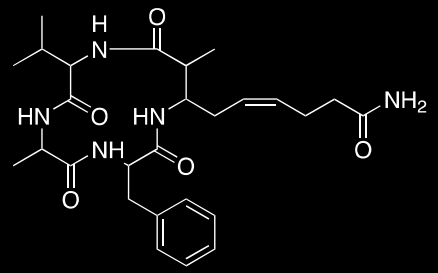
Spectral Acquisition



Processing Spectra



Multivariate Statistical Analysis



Compound Identification

What are things to consider at each step?

Smolinksa, A *et al. Analytica Chimica Acta*. **2012** 750:82-97

1D Proton NMR

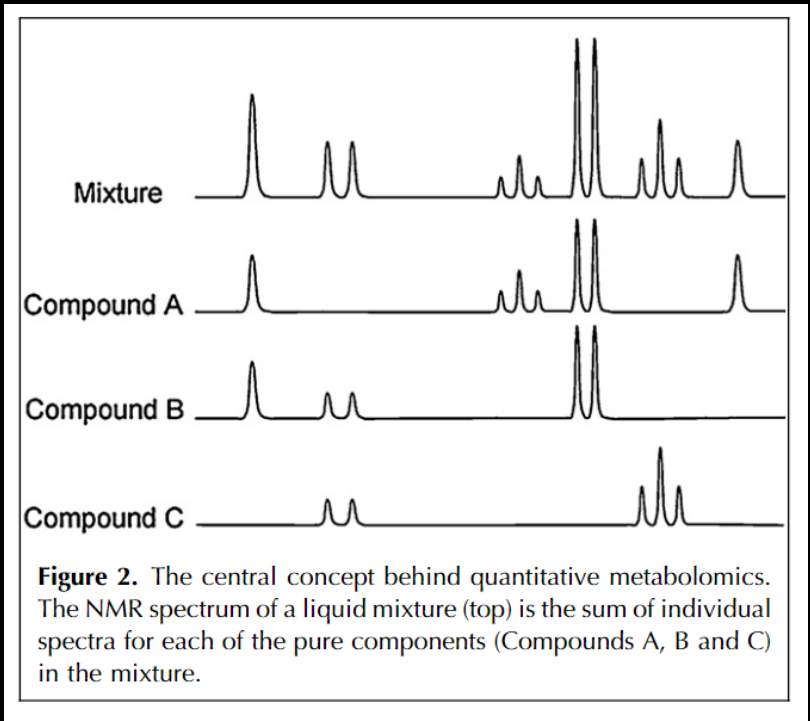


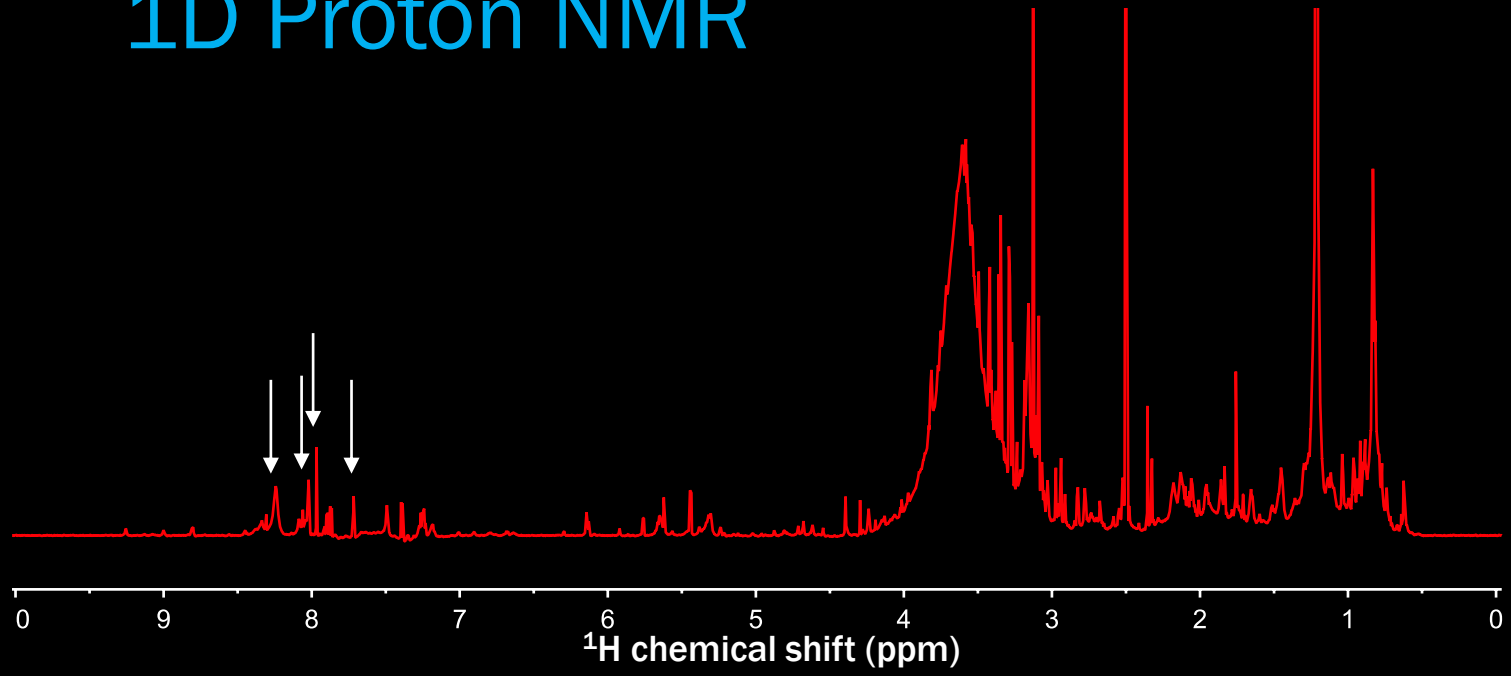
Figure 2. The central concept behind quantitative metabolomics. The NMR spectrum of a liquid mixture (top) is the sum of individual spectra for each of the pure components (Compounds A, B and C) in the mixture.

Wishart, D. S. *TrAC Trends in Analytical Chemistry*. **2008** 27:228-37

1D Proton NMR

How to process the spectra?

Individual peak picking



'Binning'

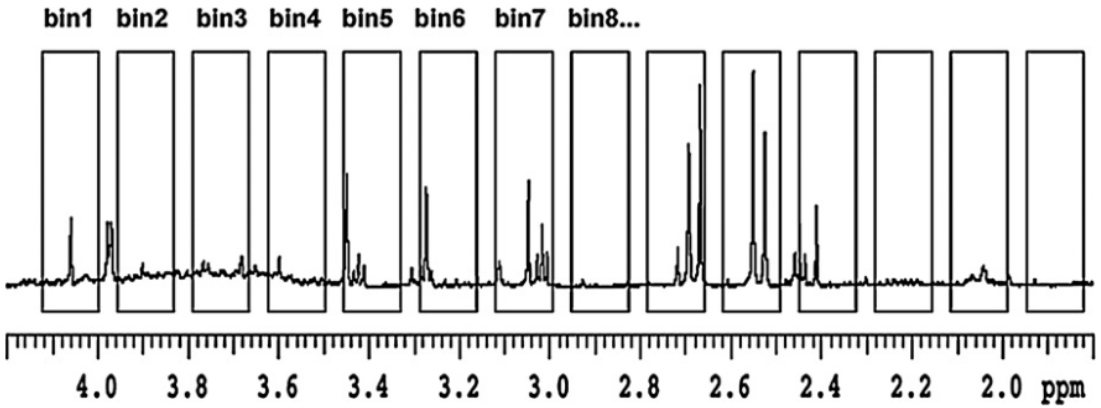


Figure 1. An example of how an NMR spectrum would be binned or partitioned prior to analysis by principal component analysis (PCA) or other chemometric methods.

Wishart, D. S. *TrAC Trends in Analytical Chemistry*. **2008** 27:228-37

1D Proton NMR

Benefits:

- 1D 1H NMR can be quantitative
- Binning enables rapid and reproducible results

Challenges:

- 1D spectra can still result in overlapping signals
- Complex samples results in complex spectra
- Multiple peaks belong to the same compound

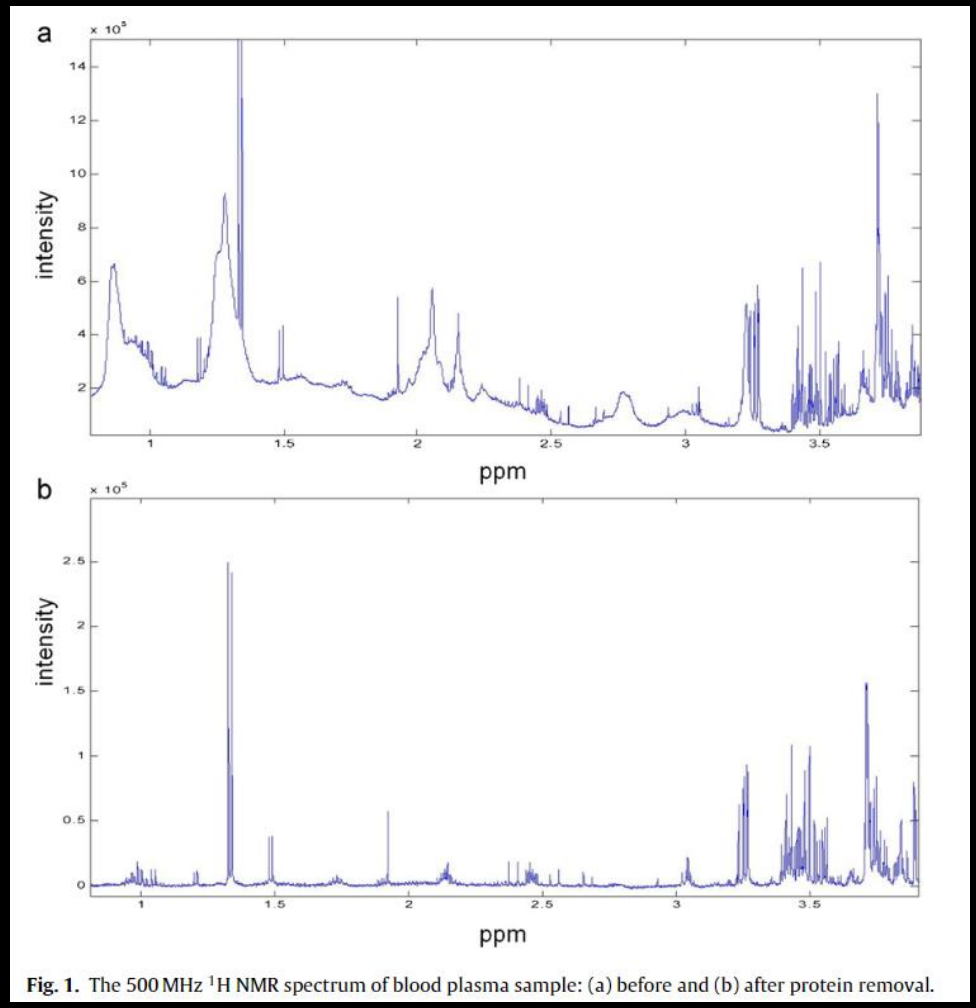


Fig. 1. The 500 MHz ^{1}H NMR spectrum of blood plasma sample: (a) before and (b) after protein removal.

Smolinksa, A *et al. Analytica Chimica Acta*. **2012** 750:82-97

Think of any other possible benefits or challenges?

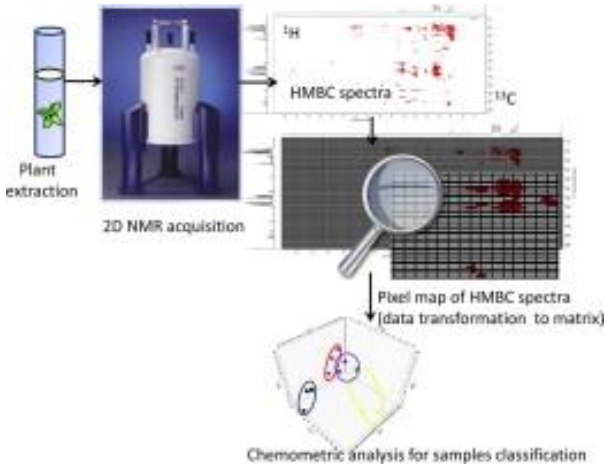
2D NMR Approaches to Metabolomics/Profiling

Benefits:

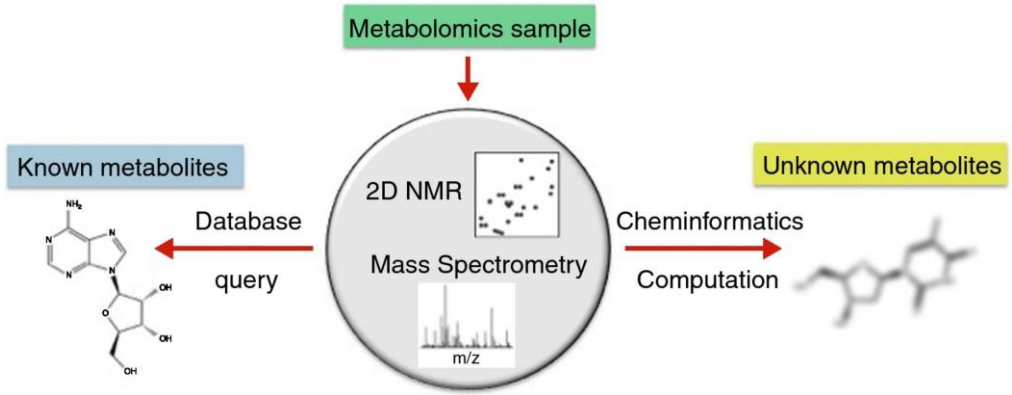
- Separate signals over second dimension
- Range of different 2D experiments available

Challenges:

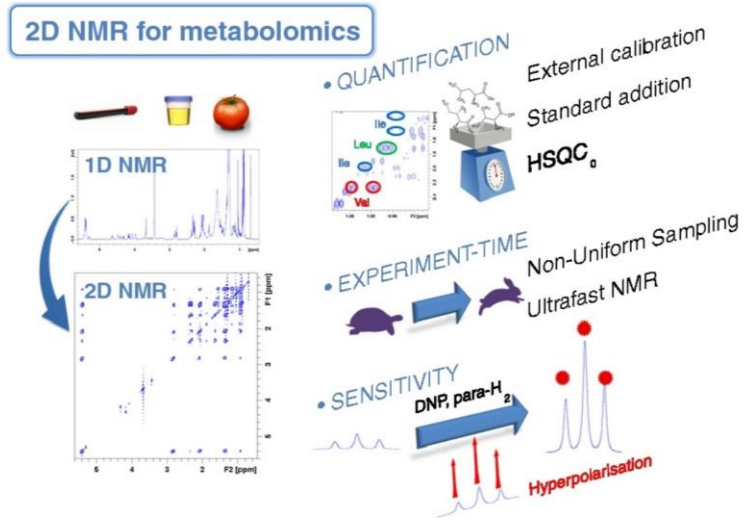
- Multiple peaks belong to the same compound
- Acquisition time
- Choice of experiment



Mahrous, E. A.; Farag, M. A. *Journal of Advanced Research*. **2015** 6:3-15



Bingol, K., Brüschweiler, R. *Current Opinion in Biotechnology*. **2017** 43:17-24



Marchand, J. *et al. Current Opinion in Biotechnology*. **2017** 43:49-55.

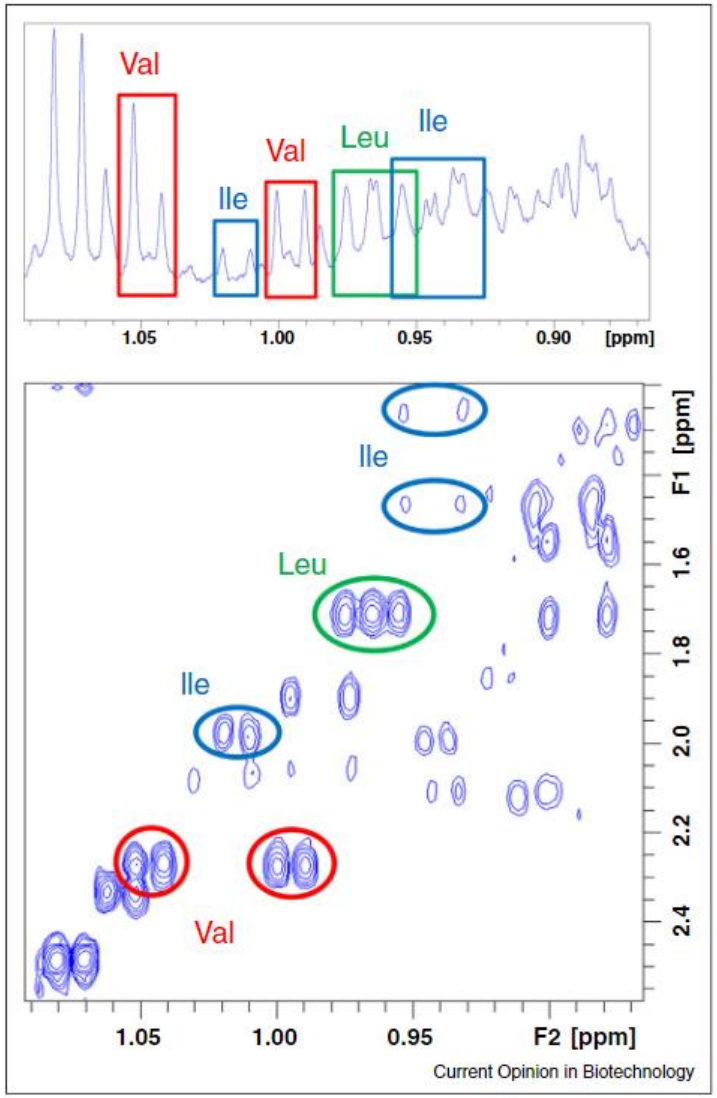


Figure 1

Illustration — in the case of urine — of the separation capabilities of 2D versus 1D NMR for metabolomics. Top: zoom of a ^{1}H 1D NMR spectrum obtained on a human urine sample at 298 K, with presaturation of the water signal through a 1D-NOESY pulse sequence. The spectrum was acquired with 64 transients on a 700 MHz spectrometer equipped with a cryogenic probe. The signals from the identified metabolites are strongly overlapped, thus making their accurate quantification difficult. Bottom: corresponding zoom of a ^{1}H COSY spectrum for the same sample. The 2D spectrum was acquired with four transients and 300 increments in the indirect dimension, with the same hardware configuration. All the metabolites whose signals were overlapped on the 1D spectrum show at least one well-separated signal on the 2D spectrum, making their quantification possible. Val: Valine; Leu: Leucine; Ile: Isoleucine.

Marchand, J. *et al. Current Opinion in Biotechnology*. **2017** 43:49-55.

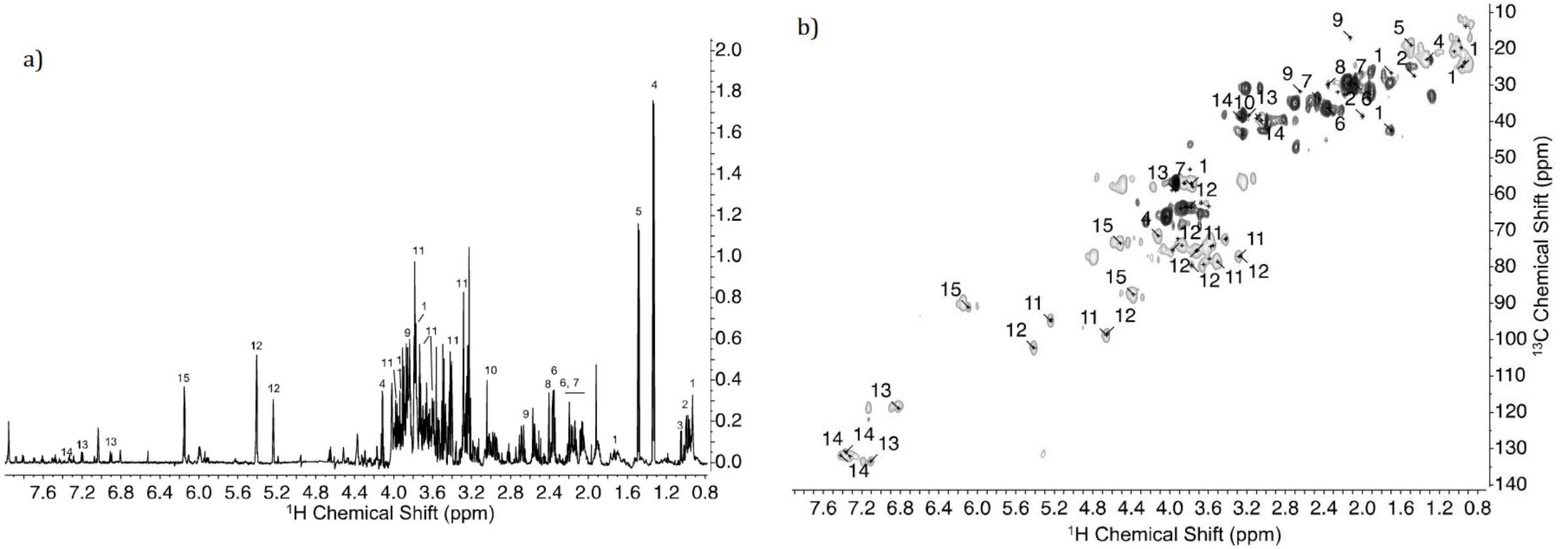


Fig. 5. Typical 800 MHz a) ^{1}H NMR spectra and b) HSQC spectra of liver extracts from striped marsh frog (*Limnodynastes peronii*) tadpoles, with identified metabolites shown. 1 Leucine, 2 Isoleucine, 3 Valine, 4 Lactic acid, 5 Alanine, 6 Glutamate, 7 Glutamine 8 Pyruvic acid, 9 Methionine, 10 Creatine, 11 Glucose, 12 Maltose, 13 Tyrosine, 14 Phenylalanine, 15 Nucleotides.

Discussion

What are the possible ways to extract *meaningful* data?

- Manually select individual peaks/correlations (..the slow human way)
- Convert entire spectrum into data matrix (artefacts/miss some signals)

Any suggestion of other possible ways to analyses this data?

- Image recognition
- Machine learning
- Other bioinformatic techniques..?

References:

1. Wishart, D. S. NMR metabolomics: A look ahead. *Journal of Magnetic Resonance*. **2019,** 306:155-61
2. Bingol K. Recent advances in targeted and untargeted metabolomics by NMR and MS/NMR methods. High-throughput. 2018, 7:9
3. Smolinska, A.; Blanchet, L.; Buydens, L. M.; Wijmenga, S. S. NMR and pattern recognition methods in metabolomics: from data acquisition to biomarker discovery: a review. *Analytica Chimica Acta*. **2012** 750:82-97
4. Wishart, D. S. Quantitative metabolomics using NMR. *TrAC Trends in Analytical Chemistry*. **2008.** 27:228-37
5. Bingol, K.; Brüschweiler, R. Knowns and unknowns in metabolomics identified by multidimensional NMR and hybrid MS/NMR methods. *Current Opinion in Biotechnology*. **2017** 43:17-24
6. Mahrous, E. A.; Farag, M. A. Two dimensional NMR spectroscopic approaches for exploring plant metabolome: A review. *Journal of Advanced Research*. **2015** 6:3-15
7. Marchand, J.; Martineau, E.; Guitton, Y.; Dervilly-Pinel, G.; Giraudeau, P. Multidimensional NMR approaches towards highly resolved, sensitive and high-throughput quantitative metabolomics. *Current Opinion in Biotechnology*. **2017** 43:49-55
8. Melvin, S. D.; Habener, L. J.; Leusch, F. D.; Carroll, A. R. ^{1}H NMR-based metabolomics reveals sub-lethal toxicity of a mixture of diabetic and lipid-regulating pharmaceuticals on amphibian larvae. *Aquatic Toxicology*. **2017** 184:123-32

Other sources/tools that were discussed during session:

- MetaboAnalyst – online tool for statistical, functional, and integrative analysis of metabolomics data
- Chenomx – software for NMR mixture analysis
- Quantitative HSQC
  - Peterson, D. J.; Loening, N. M. QQ-HSQC: a quick, quantitative heteronuclear correlation experiment for NMR spectroscopy. *Magnetic Resonance in Chemistry*. **2007** 45:937-41
  - Hu, K.; Westler, W. M.; Markley, J. L. Simultaneous quantification and identification of individual chemicals in metabolite mixtures by two-dimensional extrapolated time-zero ^{1}H– ^{13}C HSQC ($HSQC_0$). *Journal of the American Chemical Society*. **2011** 133:1662-5