

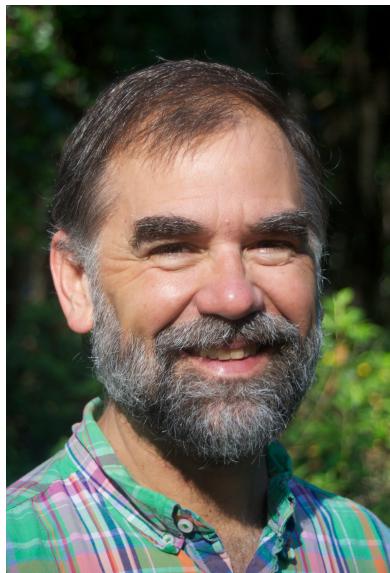


I 7 8 5

The University of Georgia

®

# Introduction to NMR in Metabolomics (1)



Art Edison

Email) [aedison@uga.edu](mailto:aedison@uga.edu)

<http://edison.ccrc.uga.edu/>

# Student Discussion

What is the difference between a  
guitar, violin, trumpet, and piano?

# NMR in Metabolomics

- Students should learn:
  - The information content of a 1D  $^1\text{H}$  NMR spectrum
  - The basics of how an NMR spectrum is obtained
  - Important database resources for NMR metabolomics:
  - BMRB and HMDB
  - The information content of a 2D NMR spectrum
  - Some strategies for the identification and quantification of NMR data
    - STOCSY, COLMAR, OTHER
- Students should already know:
  - Basic NMR from organic chemistry
- Recent review article:
  - Markley, J. L., Bruschweiler, R., Edison, A. S., Eghbalnia, H. R., Powers, R., Raftery, D., and Wishart, D. S. (2017) The future of NMR-based metabolomics, *Curr Opin Biotechnol* 43, 34-40.
- Web Resources:
  - <http://www.bmrb.wisc.edu/>
  - <http://www.hmdb.ca/>
  - <http://www.metabolomicsworkbench.org/>

# NMR Implementation

Very Strong Magnet

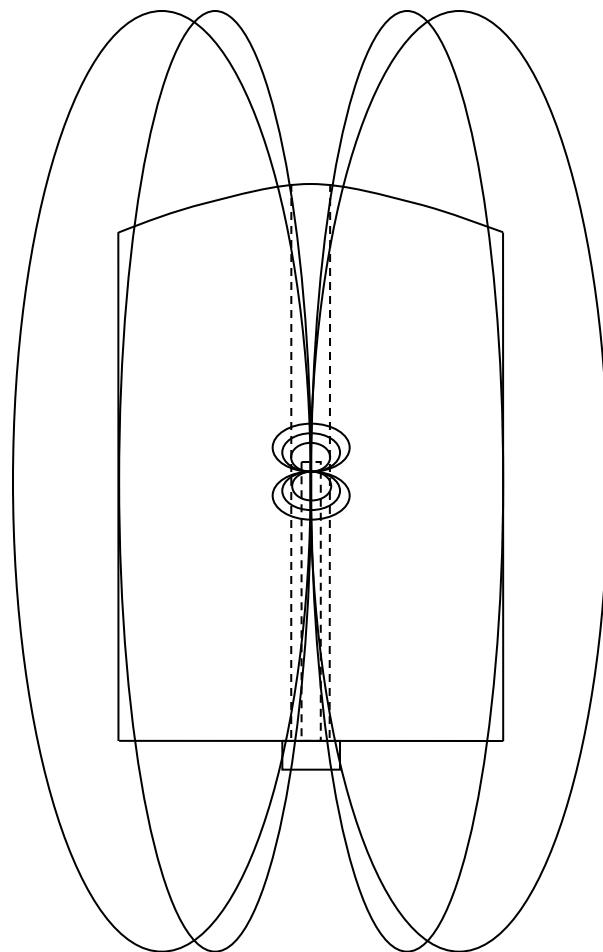


[http://wwwnmr.pharmazie.uni-marburg.de/www/jeo\\_l\\_magnet/fig6.jpg](http://wwwnmr.pharmazie.uni-marburg.de/www/jeo_l_magnet/fig6.jpg)

~400,000x Earth's magnetic field

Up to 14x greater than average MRI magnet

Probe

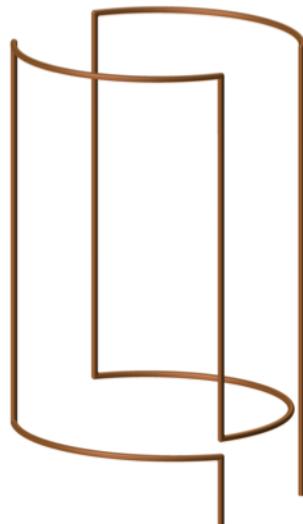


The NMR console is a radio transmitter and receiver

# Common Coils for NMR Probes

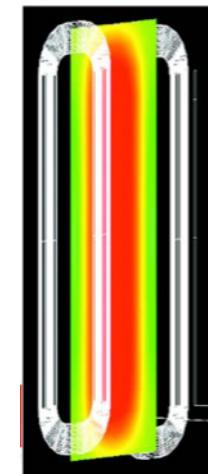
The basic requirements for a probe are that it has an electrical conductor oriented to deliver a magnetic field  $B_1$  that is perpendicular to the static field  $B_0$ .

Standard coil for  $^1\text{H}$



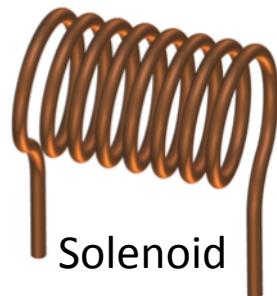
Saddle Coil

Side view of single pair



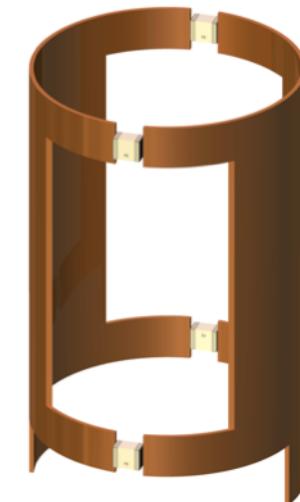
HTS coils

Flow probe: Protasis



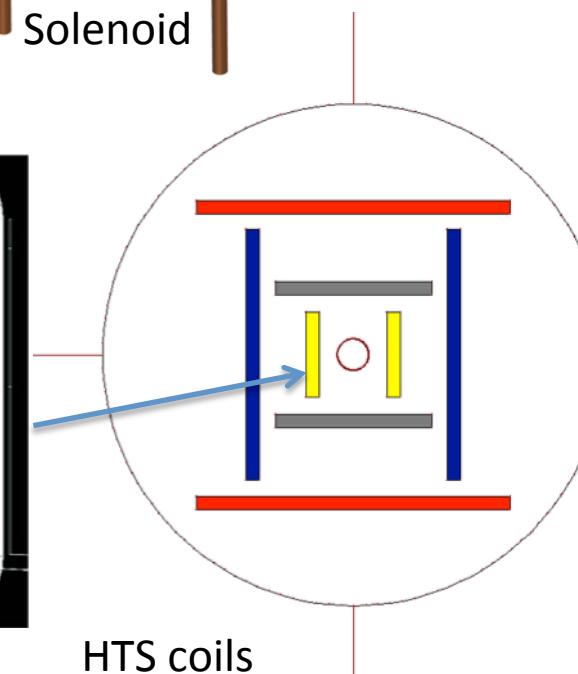
Solenoid

Standard coil for X nuclei

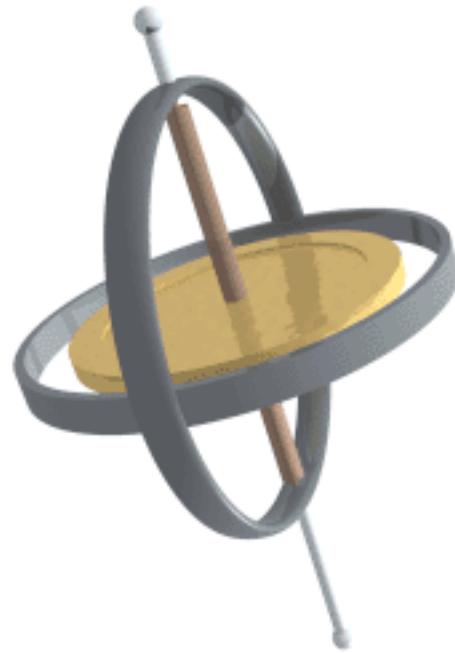


Alderman-Grant coil

Top view of HTS probe layout for 4 nuclei



# In a Magnetic Field, Nuclear Spins Behave Like a Gyroscope in the Earth's Gravitational Field

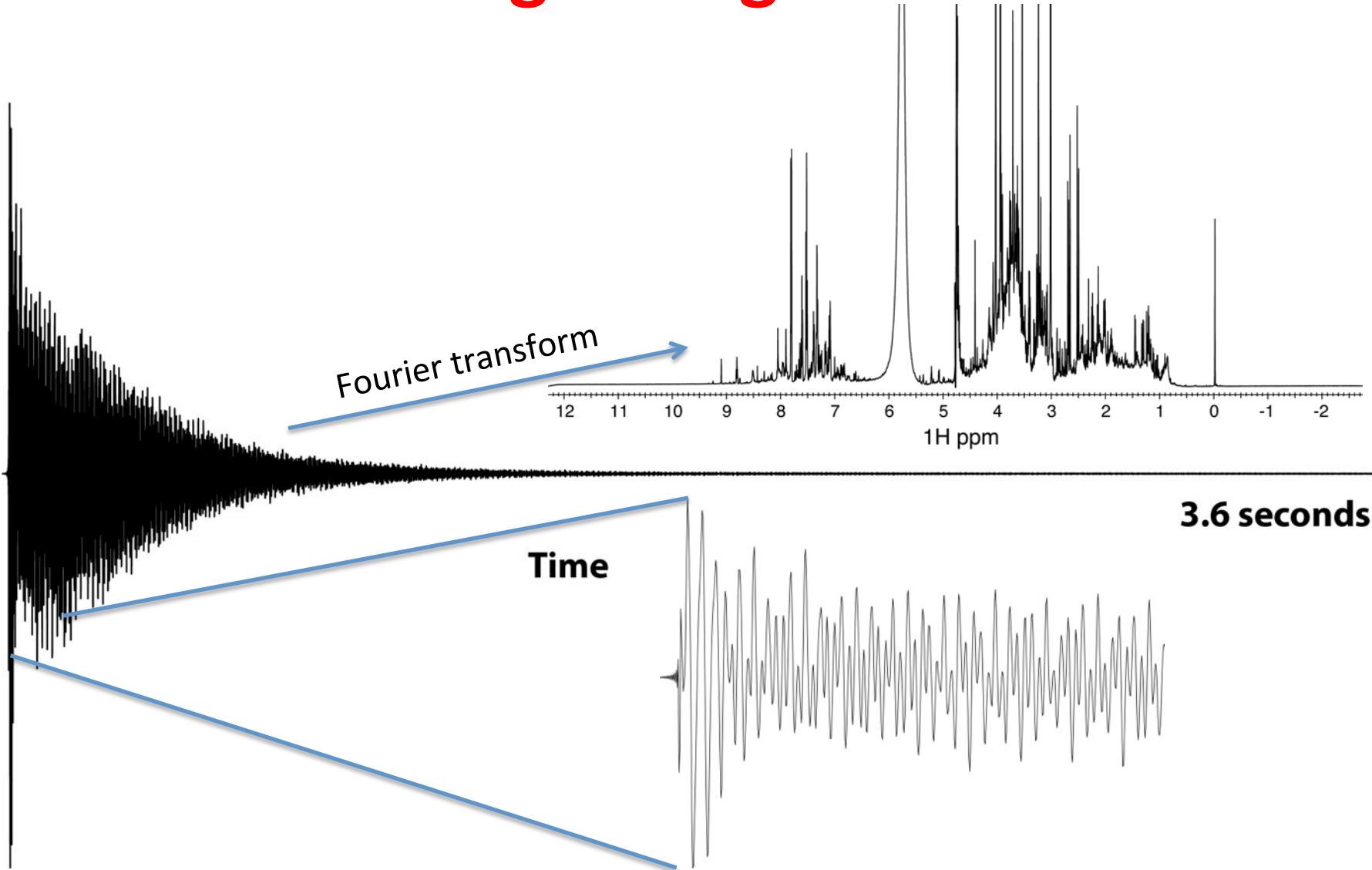


# **NMR spins are excited by a pulse**

This is like a bell.

How many sounds can you hear in a guitar?

# The nuclear spins induce an oscillating voltage in the coil



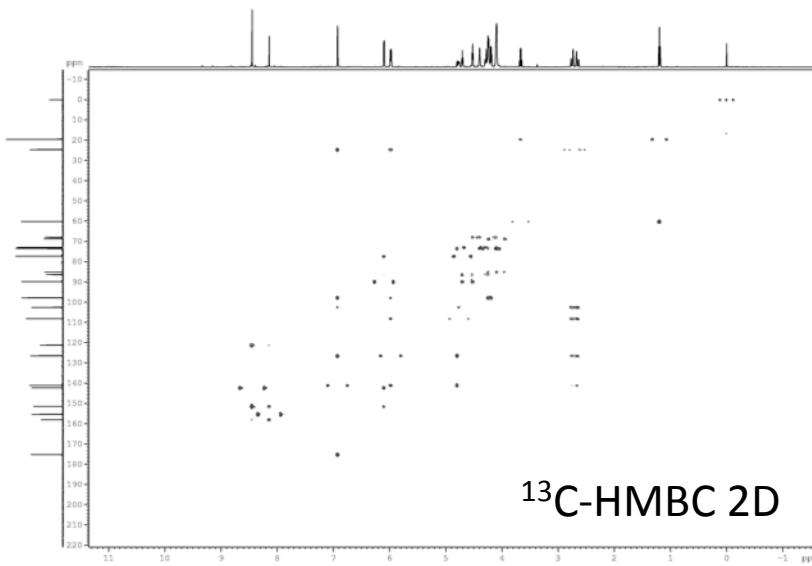
# Just some of the things you get when you search for “NADH” on the BMRB metabolomics site

<http://www.bmrb.wisc.edu/metabolomics/>

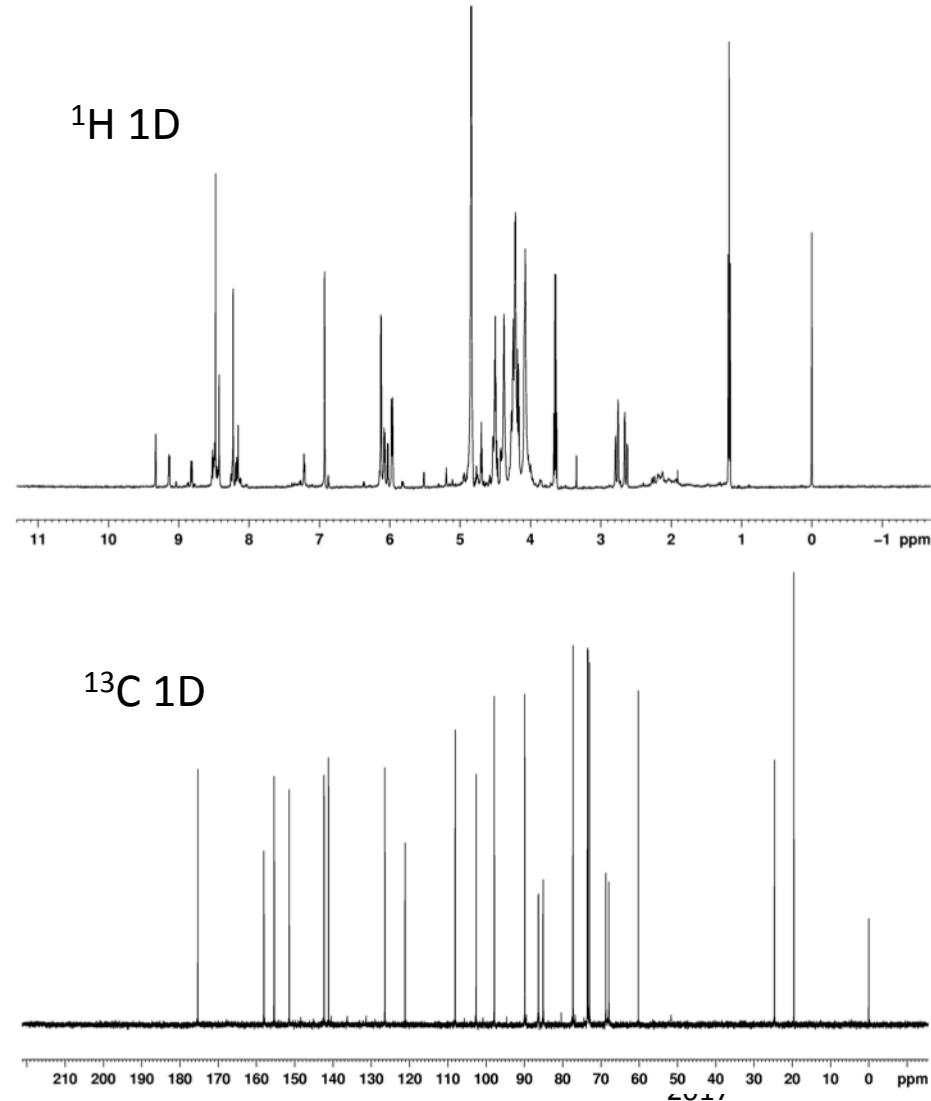


Natural Isotopic Abundance Mass  
665.440982

Also HSQC, DEPT, COSY, TOCSY, etc.  
You can download the real data too!

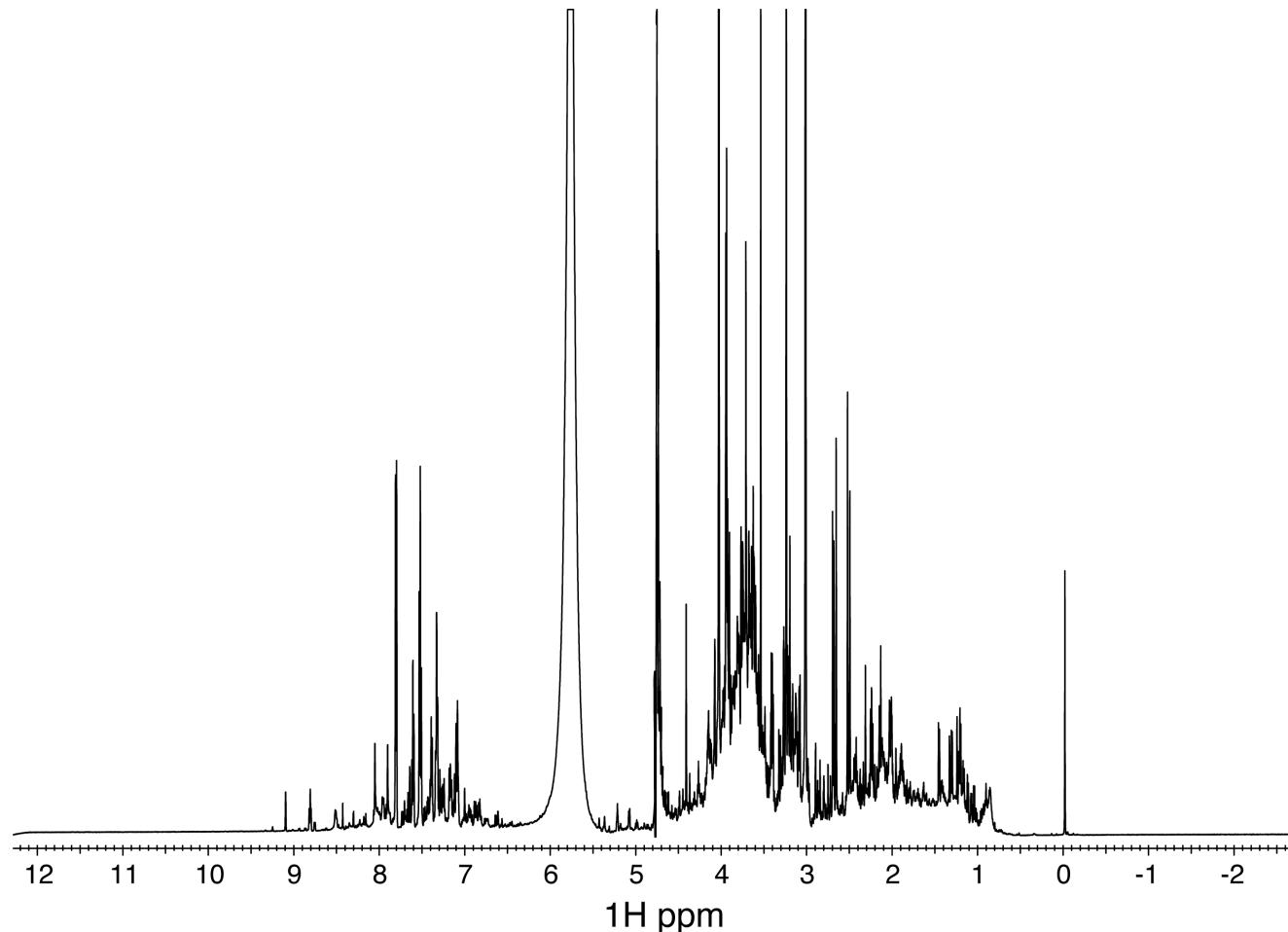


13C-HMBC 2D

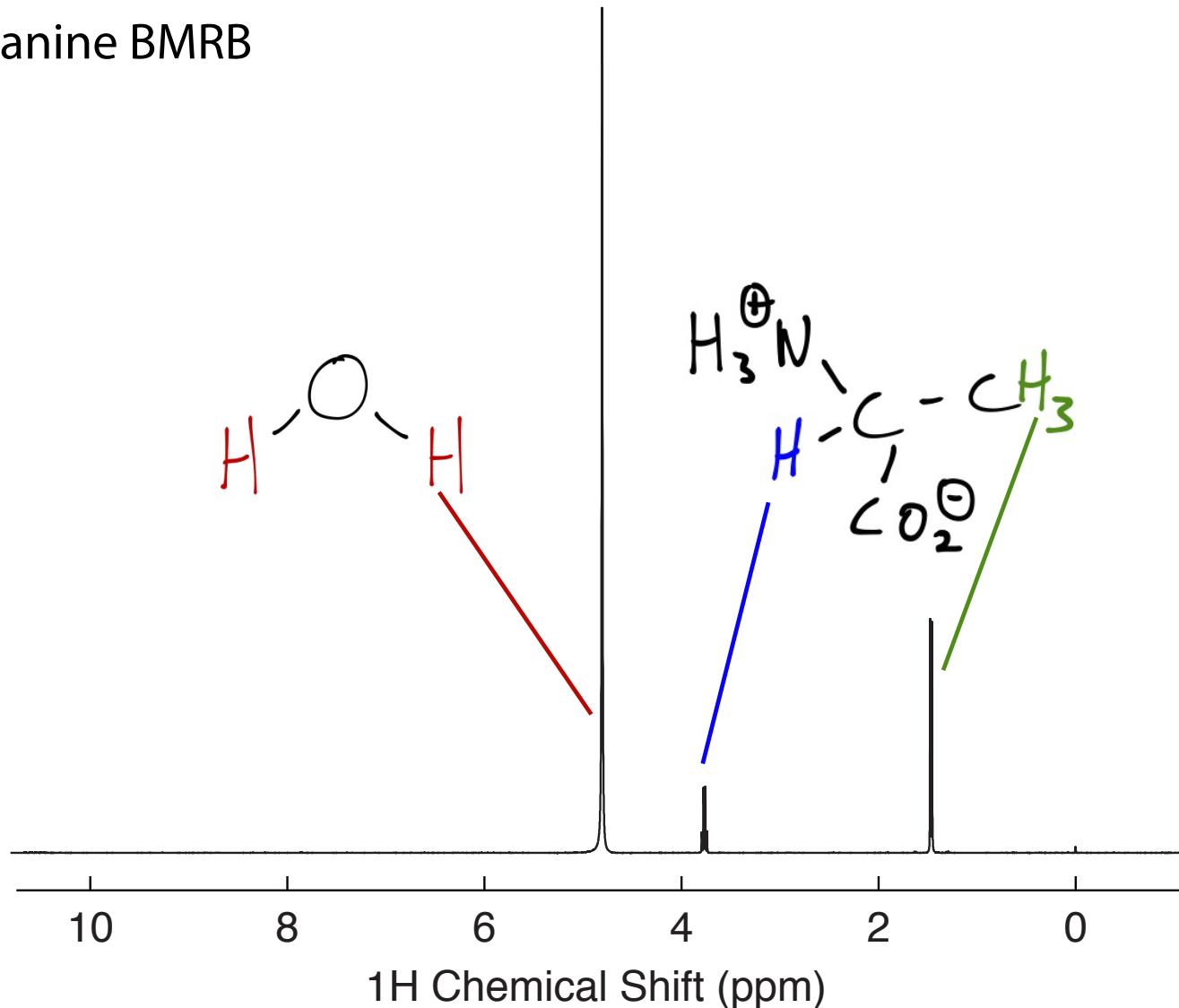


# The anatomy of 1D $^1\text{H}$ NMR

This urine spectrum arises from 10s to several 100s of simpler spectra



## Alanine BMRB

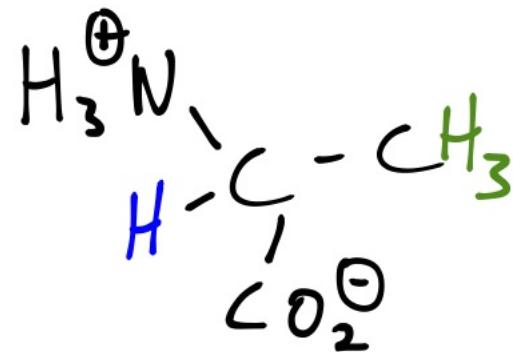


# J-coupling

doublet

For spin  $\frac{1}{2}$ , splitting at one nucleus is  $i+1$  where  $i$  is the number of  $^1\text{H}$ s at coupled nucleus

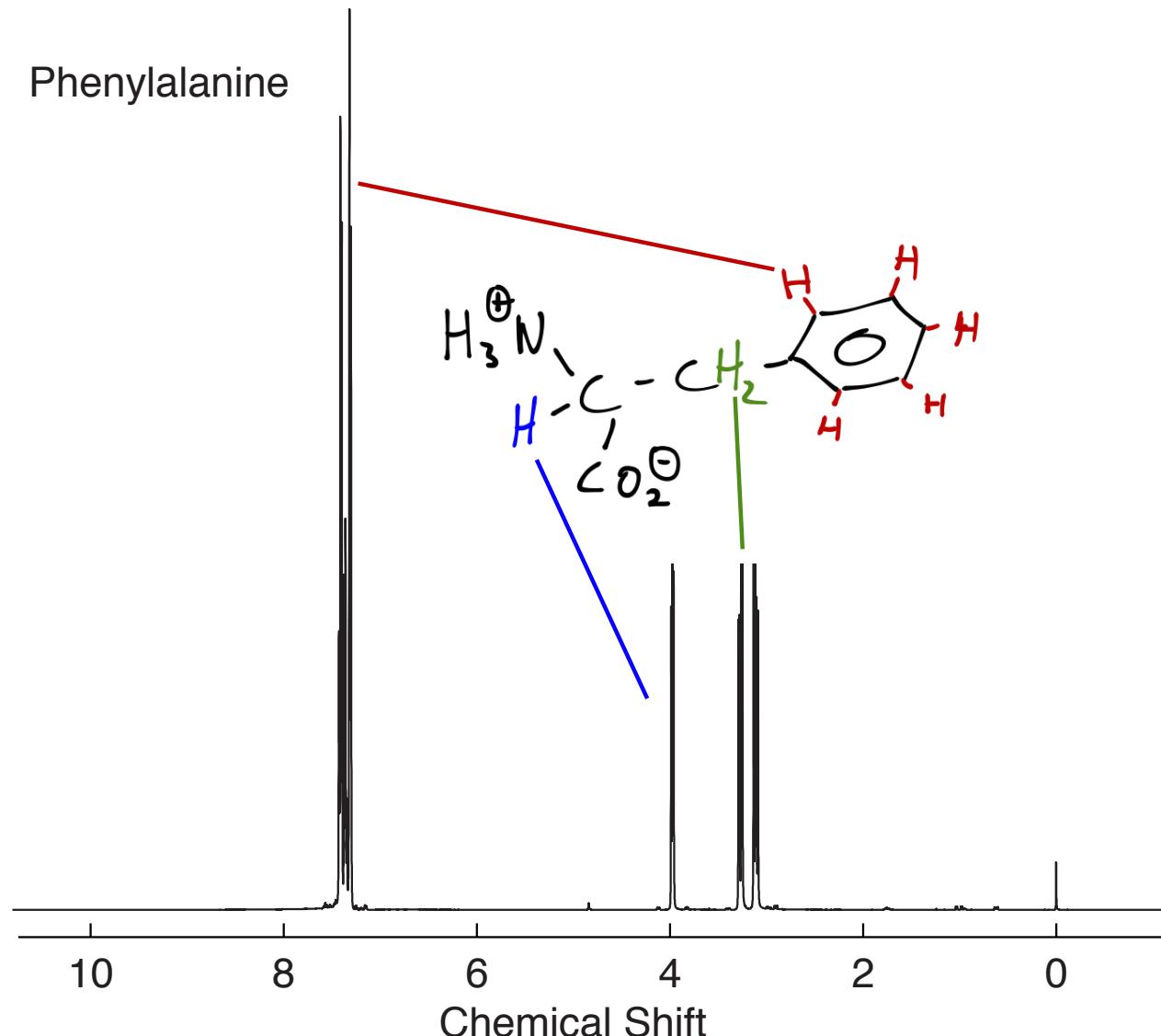
quartet



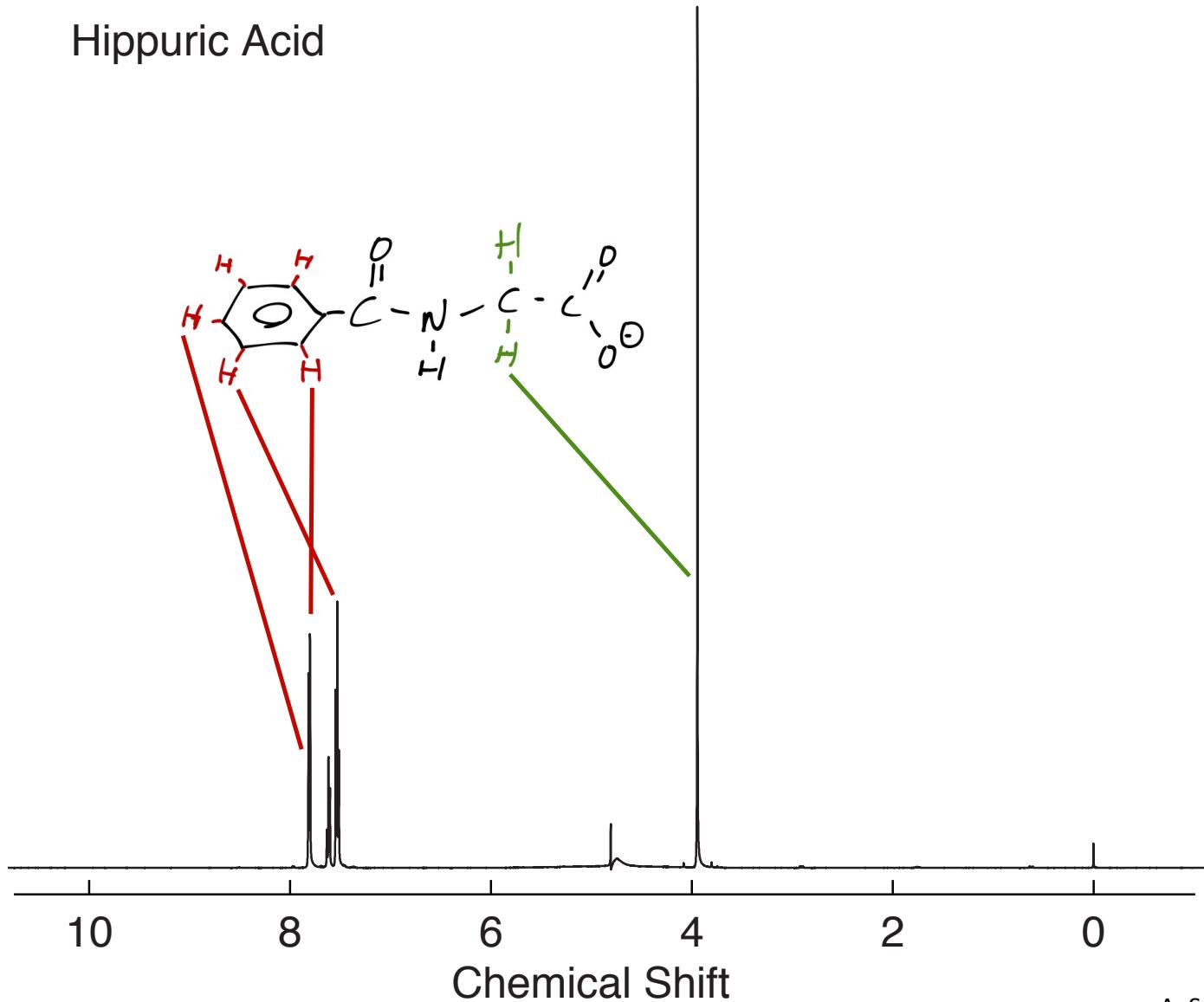
Coupling occurs up to about 3 chemical bonds

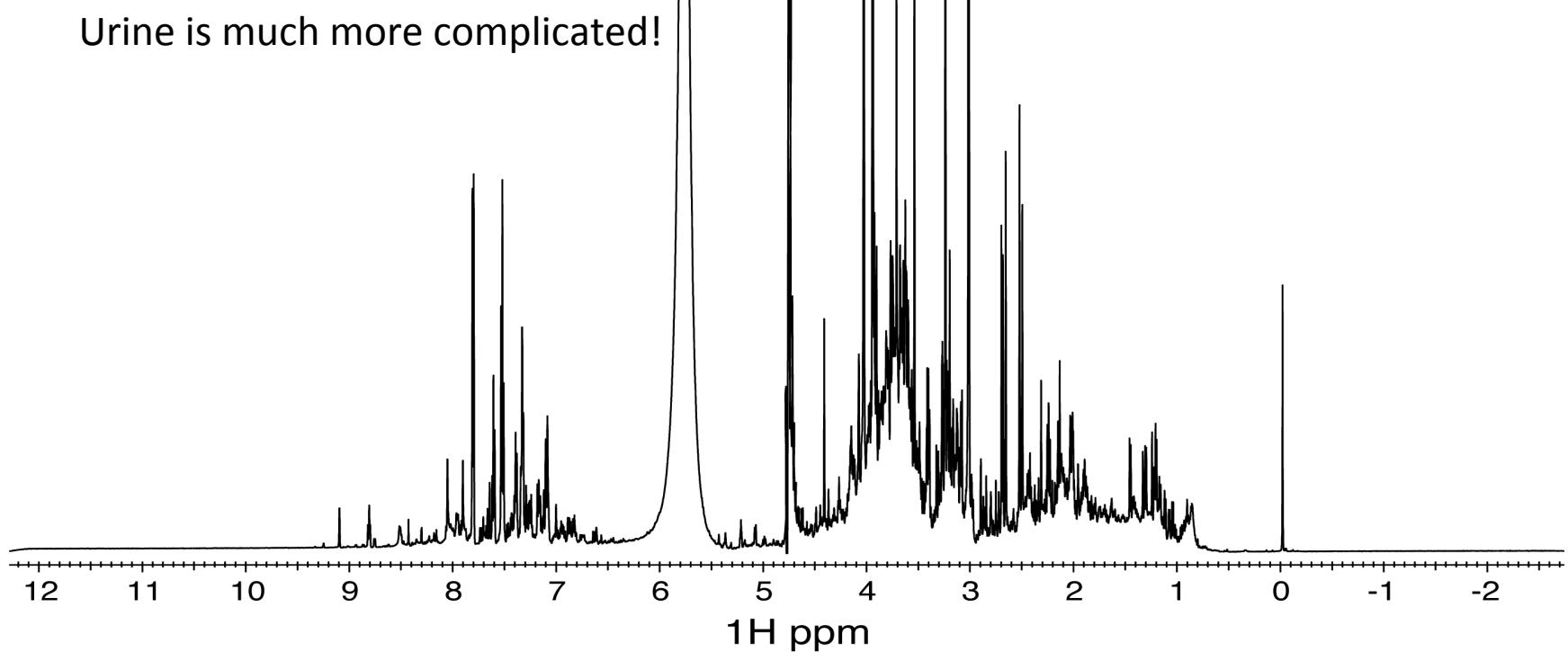


Phenylalanine

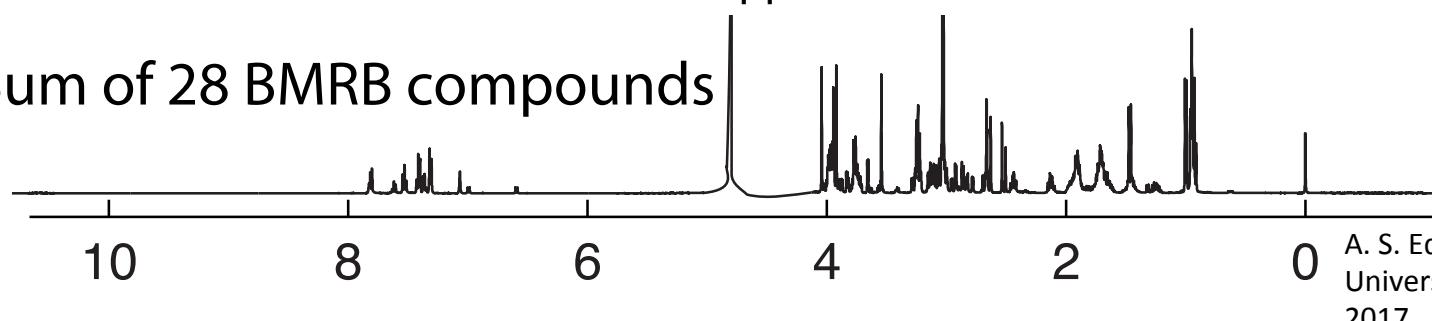


## Hippuric Acid





Sum of 28 BMRB compounds



# **How do we sort through all of the peaks?**

How can we possibly identify and  
quantify compounds from these  
NMR spectra?

# Necessary First Steps...

...for any subsequent analysis

- We need to properly process spectra
  - remove residual water
  - flatten baselines
- Align peaks
  - pH and salt influence the chemical shifts of some but not all NMR peaks
- Normalize and scale according to the needs of the study
- These steps and some of the methods introduced in this and the following lecture will be demonstrated in an interactive lab demonstration that will be available with these lectures.

# There are several methods to ID and quantify NMR metabolomics data, but none are perfect

- Chemonx is a very popular commercial software package that attempts to fit 1D data to built in libraries. This can be effective when peaks are not highly overlapped but a real challenge when they are. It is also manual and subject to interpretation.
- BATMAN is an academic program from Imperial College that attempts to fit 1D data with libraries using Bayesian analysis. This is powerful but requires knowledge of the peaks you want to fit before the analysis.
- BAYESIL: An alternative to BATMAN that uses Bayesian analysis: <http://bayesil.ca>

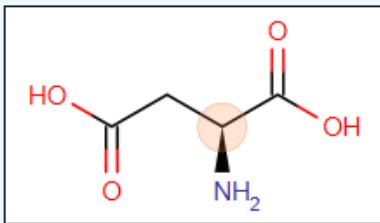


File Edit View Compound Tools Applications Help



Reference Card

### Aspartate



Formula  $\text{C}_4\text{H}_7\text{NO}_4$

Weight (Da) 133.1027

IUPAC Name

Alternate Names

CAS Registry

InChI

SMILES

Additional Information

### External Database References

[HMDB MetaboCard](#)

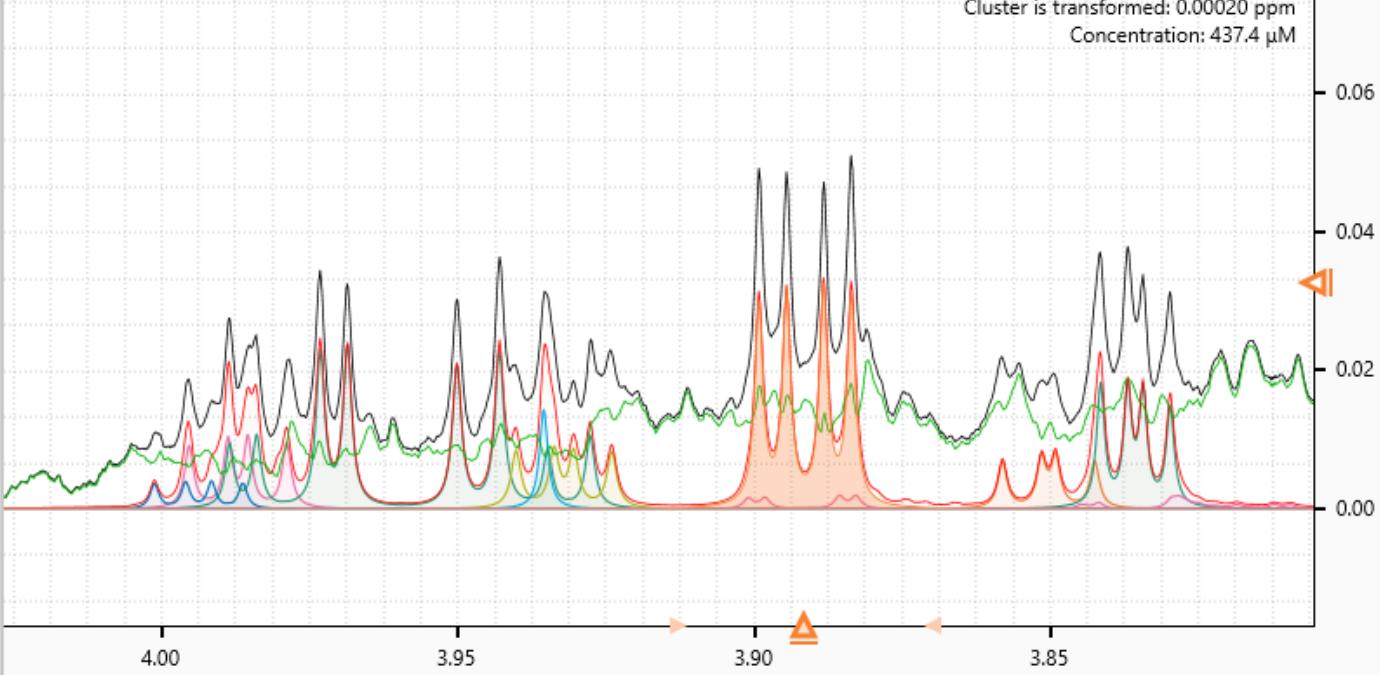
[KEGG Ligand Compound](#)

[PubChem Compound](#)

[ChEBI](#)

### Aspartate

3.9 2.8 2.7



Find in Table (Ctrl+L)

	Compound Name	Concentration ( $\mu\text{M}$ )
Aspartate		437.4
ATP		--
Azelate		--
Benzoate		--
Betaine		--

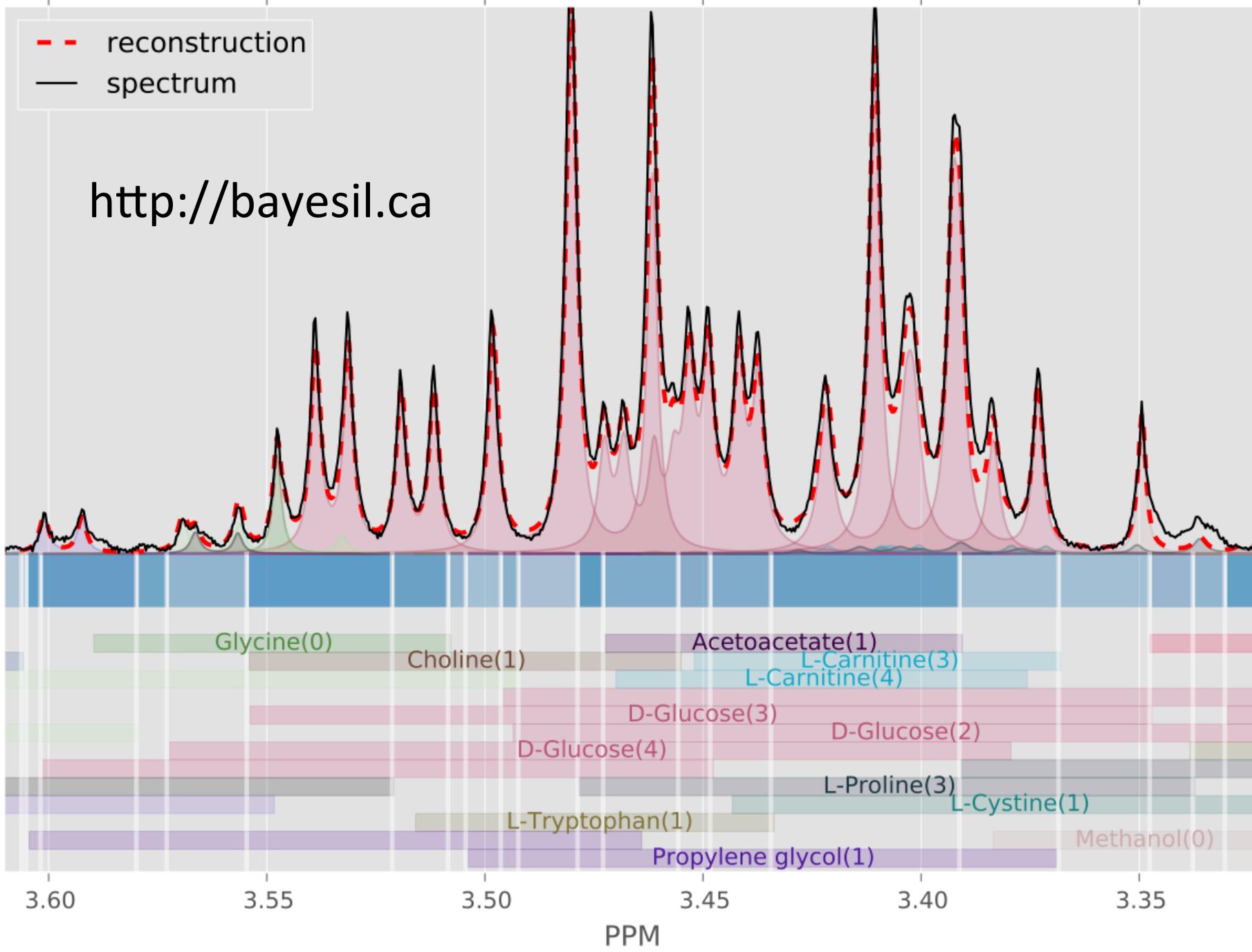
335 compounds

799.80 MHz

pH 7.10

--- reconstruction  
— spectrum

<http://bayesil.ca>

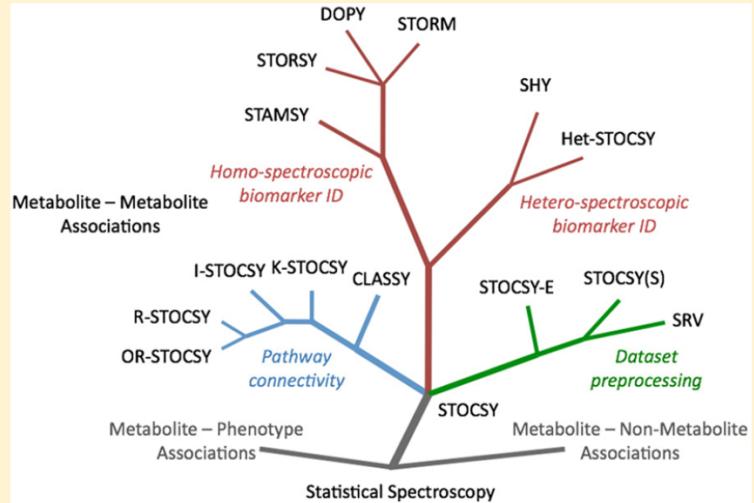


# STOCSY is very helpful

Robinette, S. L.; Lindon, J. C.; Nicholson, J. K. *Anal. Chem.* **2013**, *85*, 5297–5303.

Holmes, E.; Cloarec, O.; Nicholson, J. K. *J Proteome Res* **2006**, *5*, 1313–1320.

**ABSTRACT:** Metabolic profiling based on comparative, statistical analysis of NMR spectroscopic and mass spectrometric data from complex biological samples has contributed to increased understanding of the role of small molecules in affecting and indicating biological processes. To enable this research, the development of statistical spectroscopy has been marked by early beginnings in applying pattern recognition to nuclear magnetic resonance data and the introduction of statistical total correlation spectroscopy (STOCSY) as a tool for biomarker identification in the past decade. Extensions of statistical spectroscopy now compose a family of related tools used for compound identification, data preprocessing, and metabolic pathway analysis. In this Perspective, we review the theory and current state of research in statistical spectroscopy and discuss the growing applications of these tools to medicine and systems biology. We also provide perspectives on how recent institutional initiatives are providing new platforms for the development and application of statistical spectroscopy tools and driving the development of integrated “systems medicine” approaches in which clinical decision making is supported by statistical and computational analysis of metabolic, phenotypic, and physiological data.



# Next Lecture: Introduction to NMR in Metabolomics (2)

- Intro to STOCSY
- 2D in metabolomics
- COLMAR database matching