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The University of Georgia

®

Introduction to NMR in Metabolomics (2)



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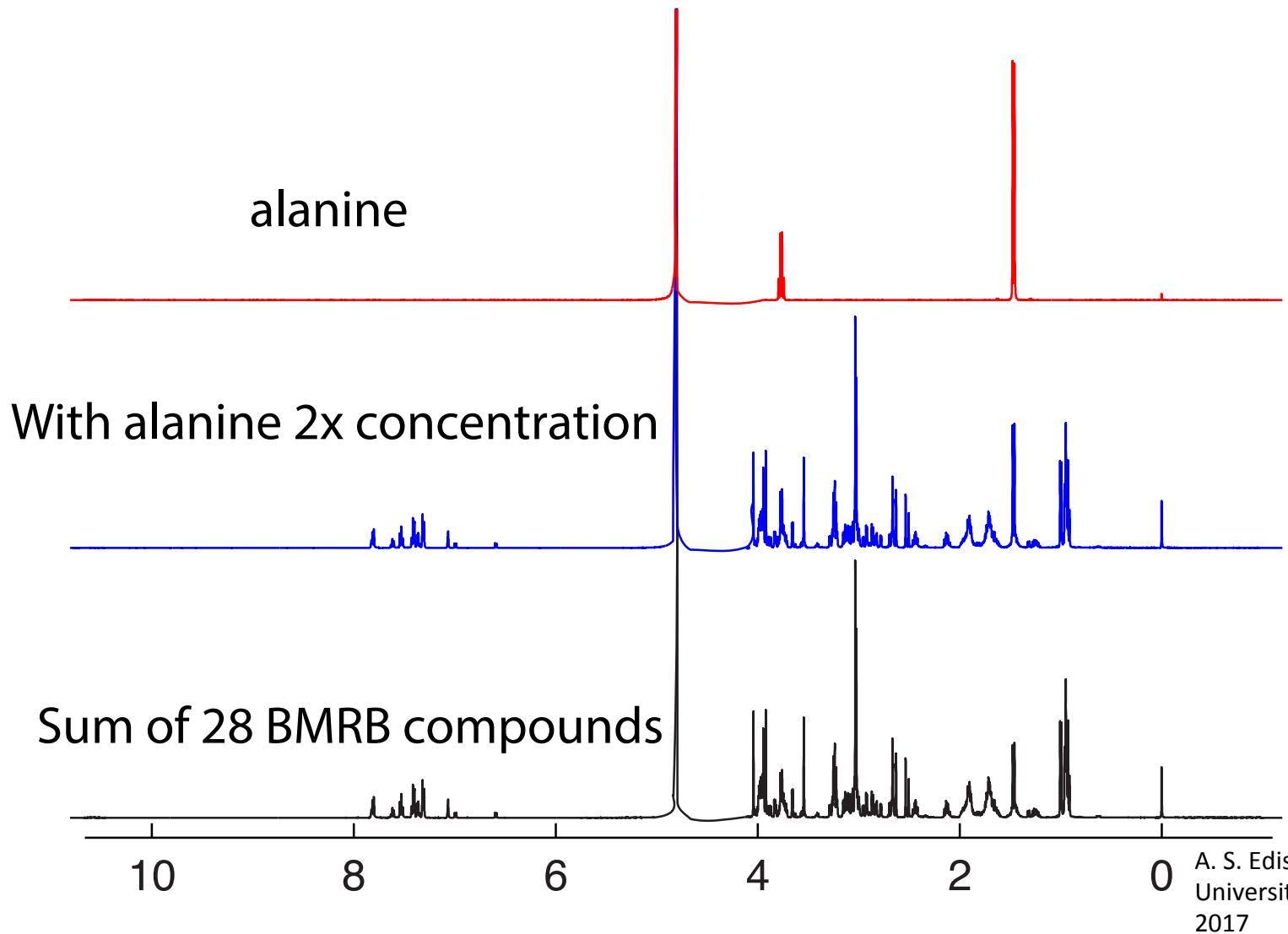
<http://edison.ccrc.uga.edu/>

STOCSY Basics

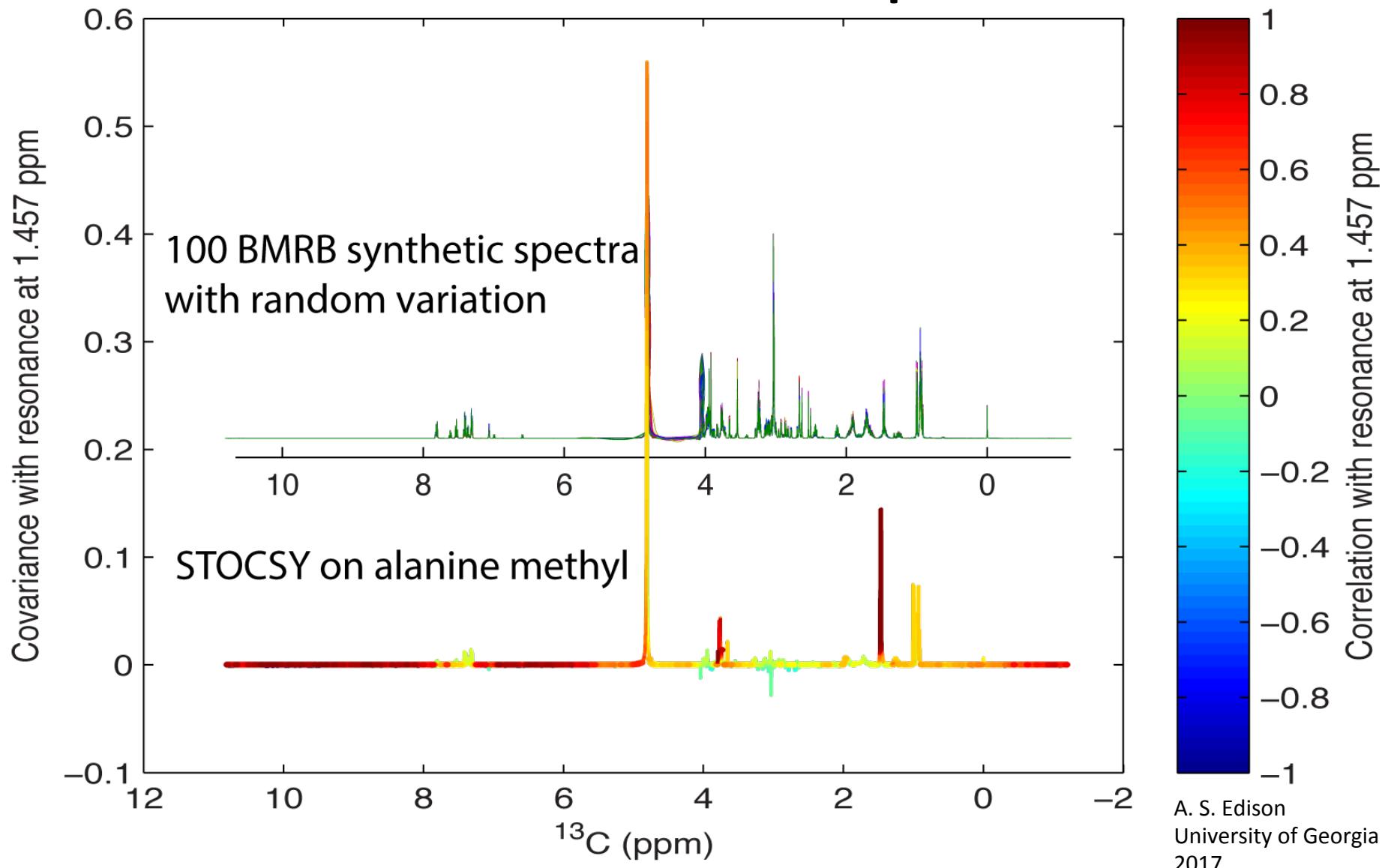
- Multiple 1D NMR datasets are collected
 - Biological replicates
 - Different conditions
- The resonances are correlated against each other
- Ones with strong correlations are either in the same molecule or on the same biosynthetic pathway

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

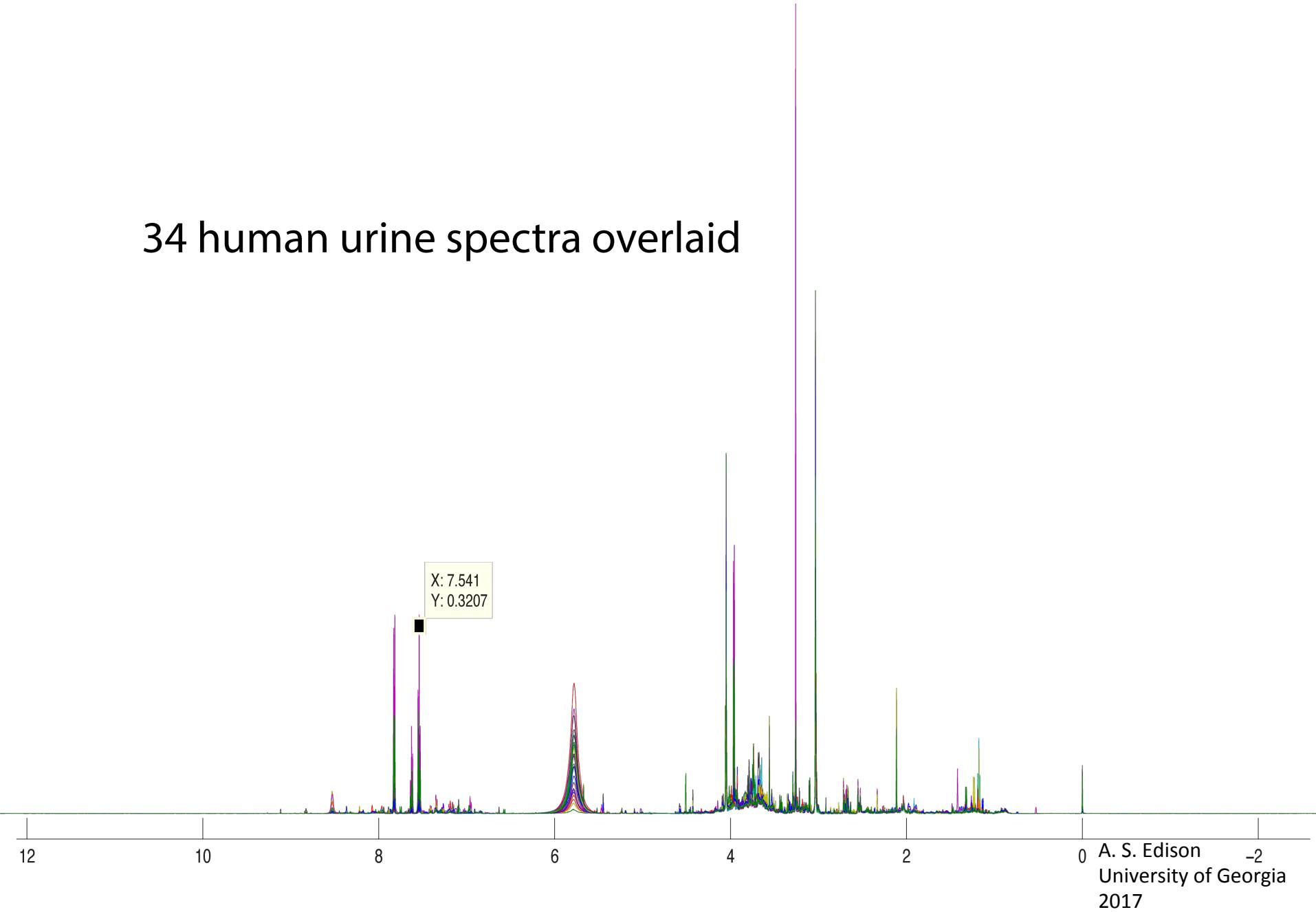
Recall the example from before with different concentrations of alanine. Those peaks would be well correlated.



STOCSY Example

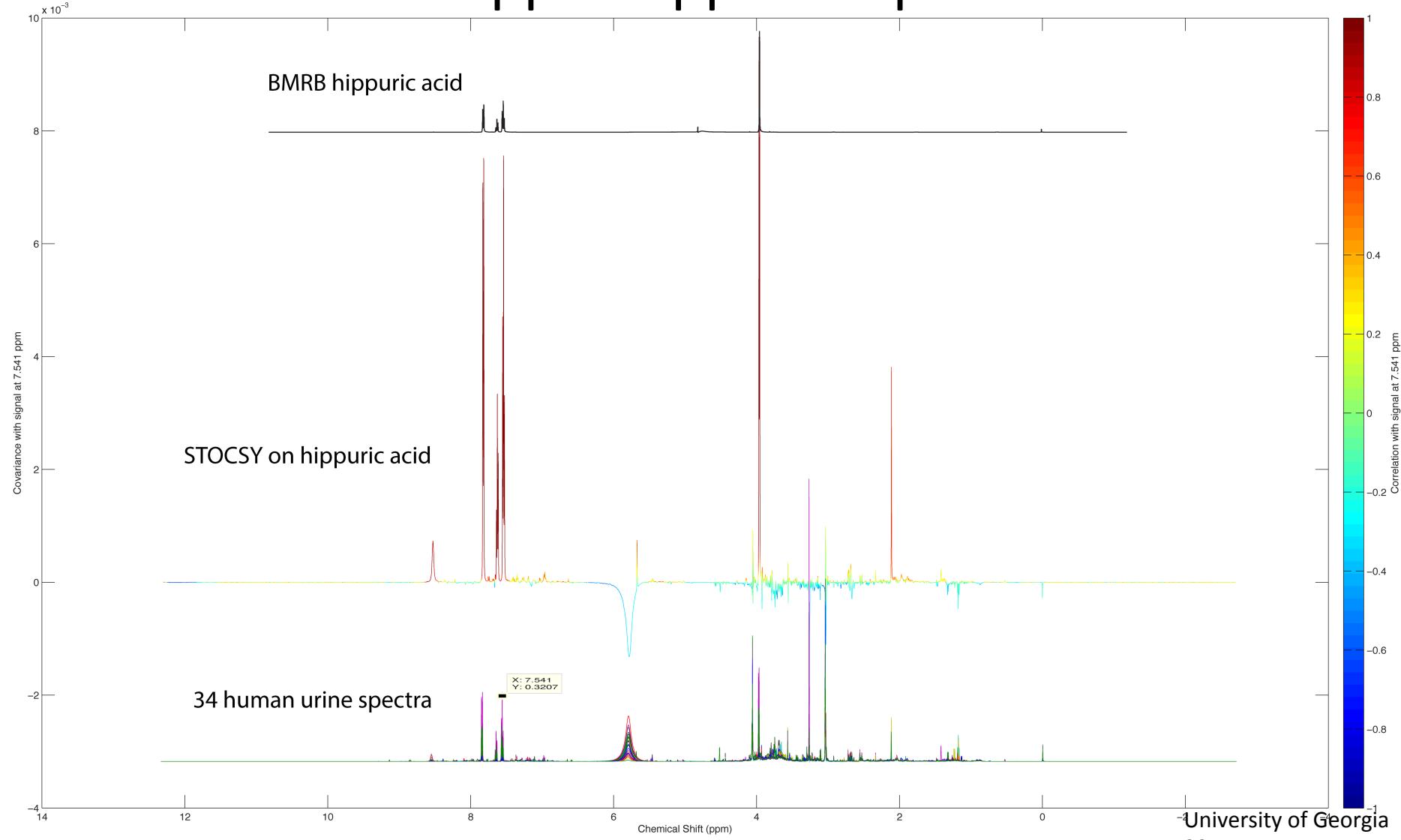


34 human urine spectra overlaid



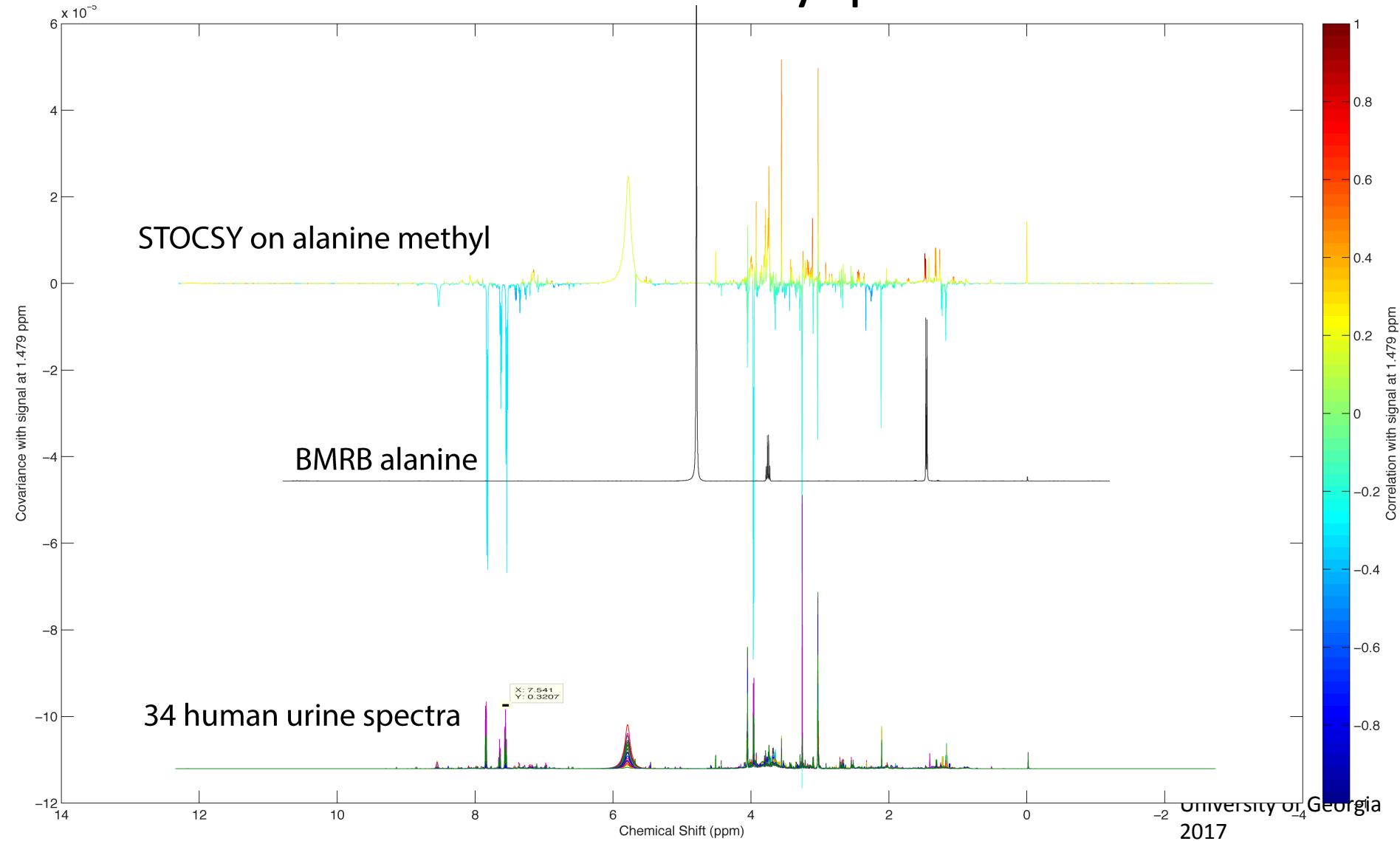
STOCSY works well with resolved peaks

7.5 ppm hippurate peak



STOCSY has trouble with too much overlap

Alanine methyl peak



STOCSY With Other Data

STOCSY can also be used to correlate other datasets with NMR data. This can be accomplished by linking two or more types of quantitative data that were collected from the same sample. For example, NMR and MS data can be linked and statistically correlated. This is not always simple, especially with issues such as normalization.

Crockford, D. J., Holmes, E., Lindon, J. C., Plumb, R. S., Zirah, S., Bruce, S. J., Rainville, P., Stumpf, C. L., and Nicholson, J. K. (2006) Statistical heterospectroscopy, an approach to the integrated analysis of NMR and UPLC-MS data sets: Application in metabonomic toxicology studies, *Analytical Chemistry* 78, 363-371.

Clendinen, C. S., Lee-McMullen, B., Williams, C. M., Stupp, G. S., Vandenborne, K., Hahn, D. A., Walter, G. A., and Edison, A. S. (2014) ^{13}C NMR metabolomics: applications at natural abundance, *Anal Chem* 86, 9242-9250.

2D NMR

Like in protein structural biology, 2D NMR is useful in metabolomics.

- Reduces overlap in complicated spectra
- Provides a lot of additional information through correlations
- Can ID molecules *de novo* using 2D NMR
- Can be used as a basis for database matching:
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Information Content of Common 2D NMR Experiments

COSY (Correlation Spectroscopy): J coupling (generally up to 3 covalent bonds)

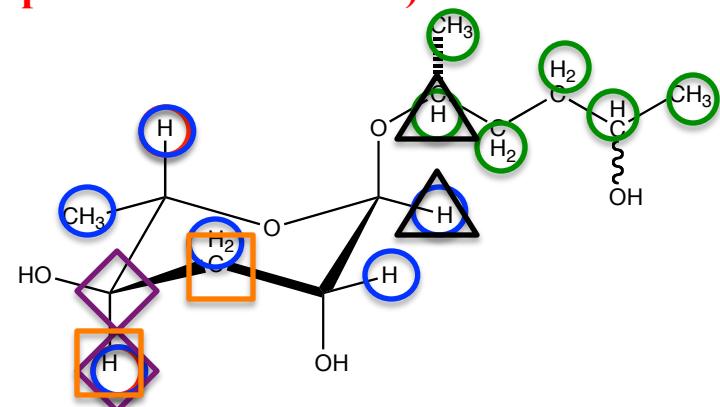
**TOCSY (Total Correlation Spectroscopy):
J coupling along coupled networks**

Blue is one TOCSY network, green is another

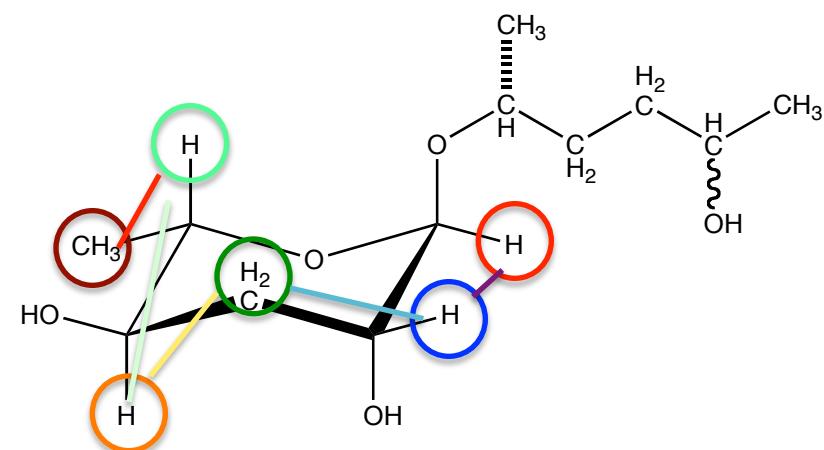
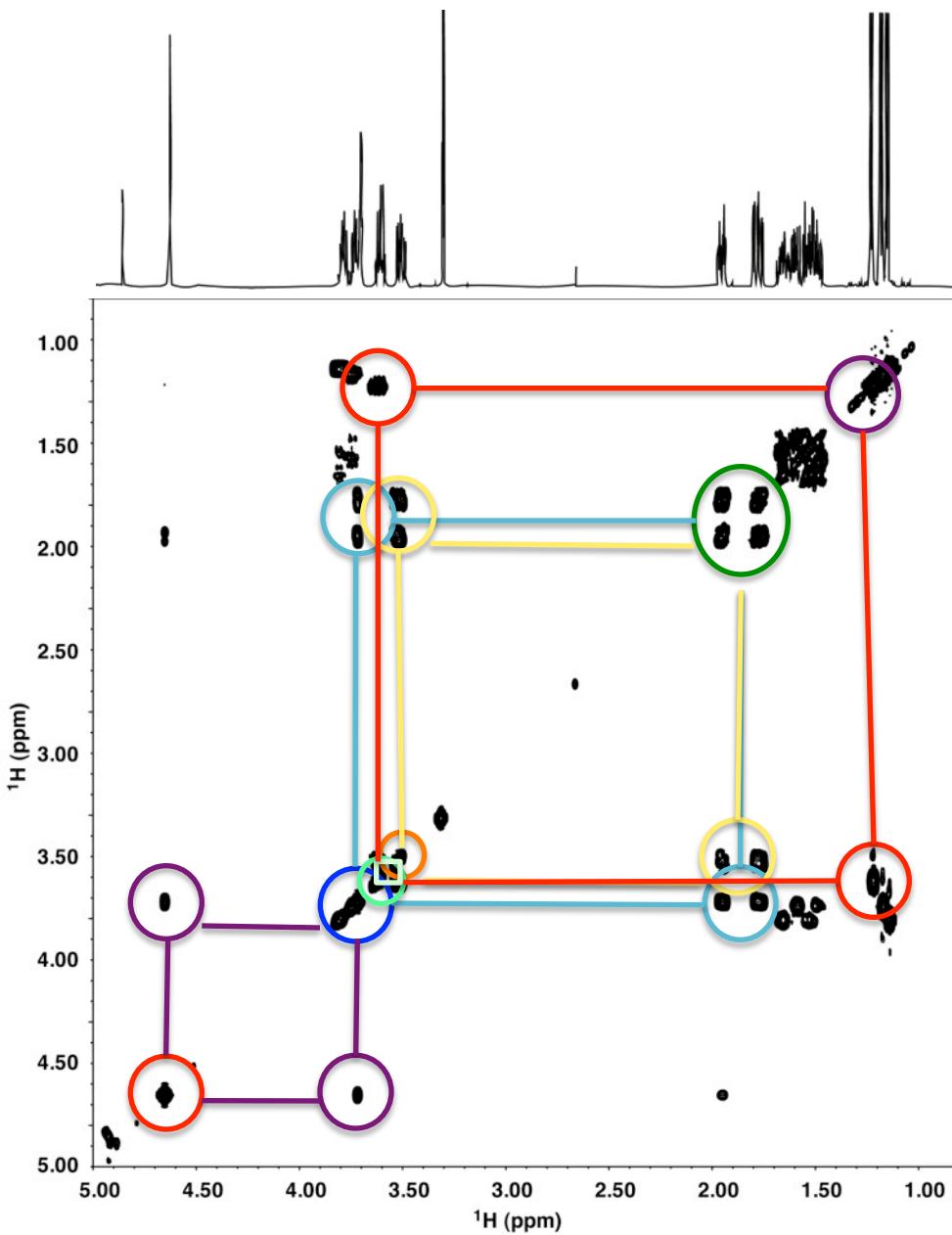
**HSQC (Heteronuclear Single Quantum Correlation):
Directly bonded ^{13}C - ^1H or ^{15}N - ^1H**

**HMBC (Heteronuclear Multiple Bond Correlation):
2 or 3 bond ^{13}C - ^1H or ^{15}N - ^1H**

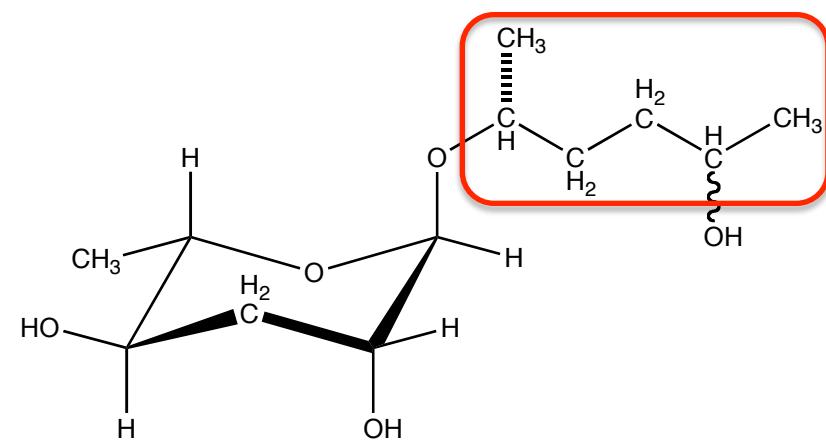
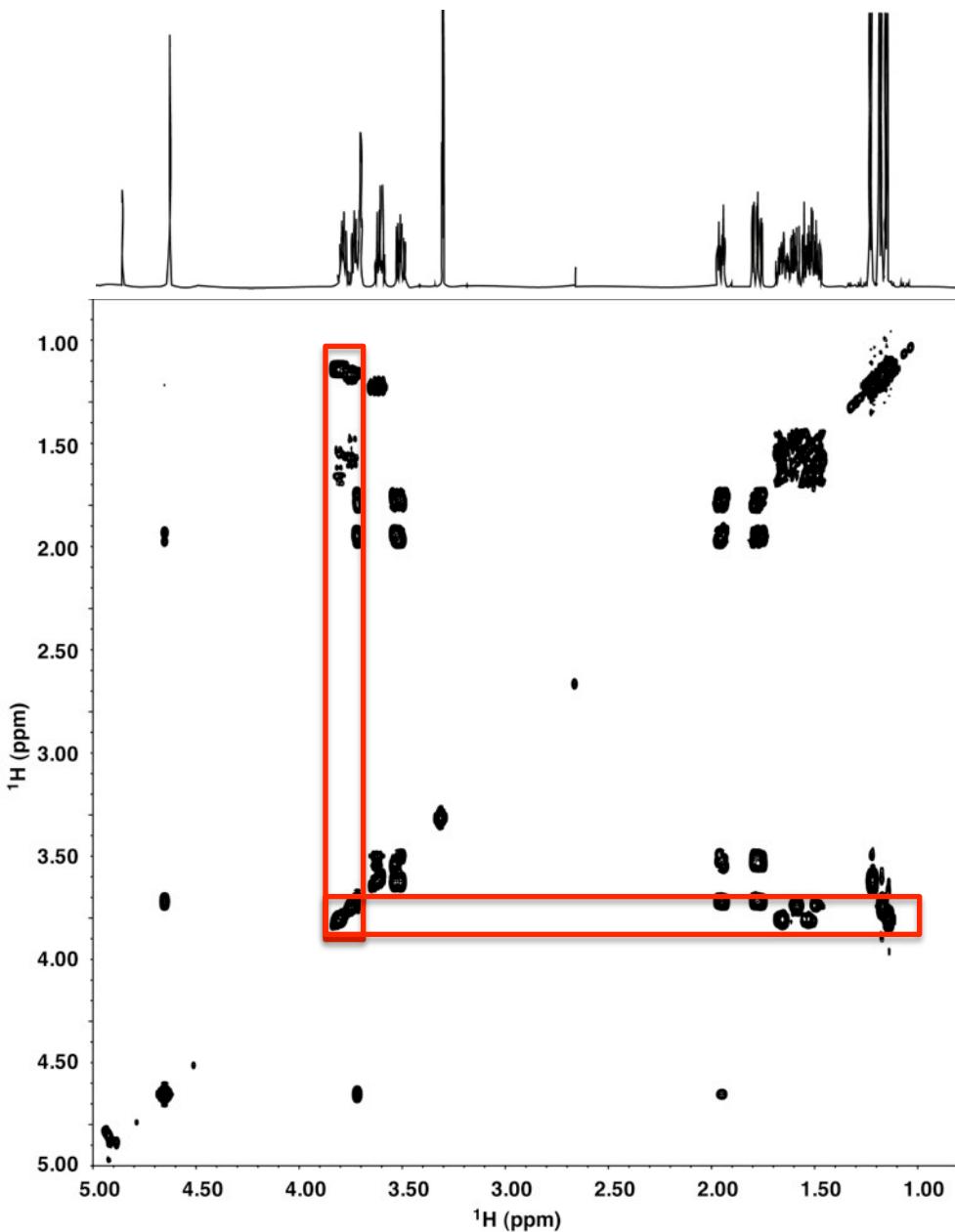
**NOESY (Nuclear Overhauser Effect Spectroscopy)
Or ROESY (Rotating Frame Overhauser Effect Spectroscopy):
 ^1H to ^1H distances up to 5-6 Å**



Using 2D NMR to ID Metabolites

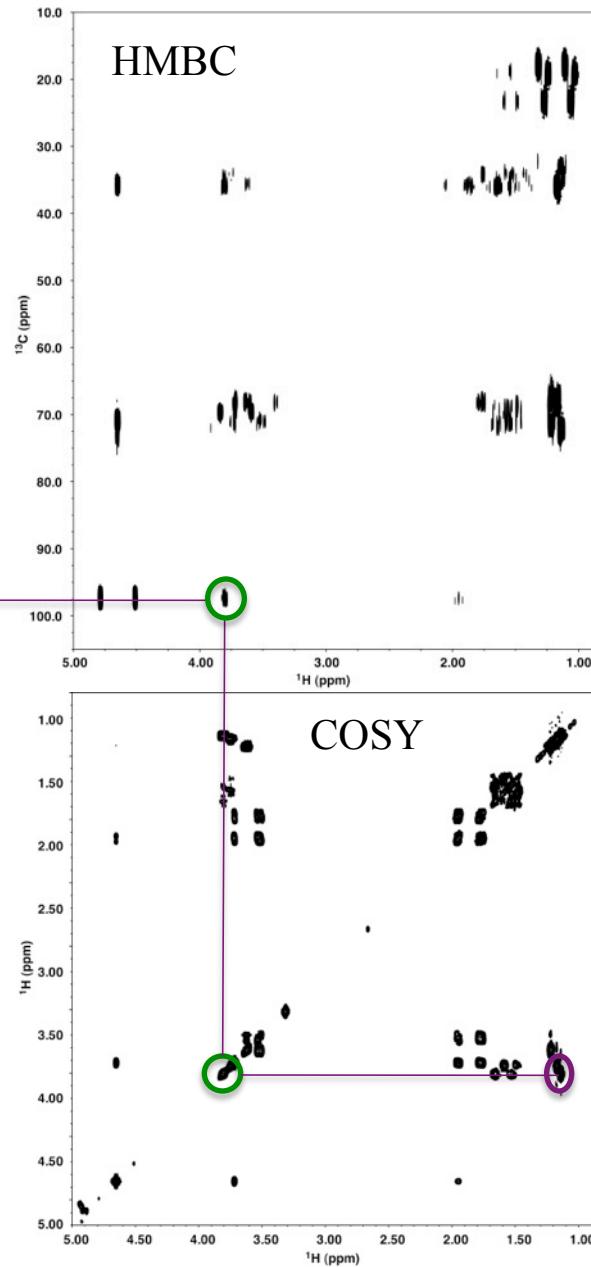
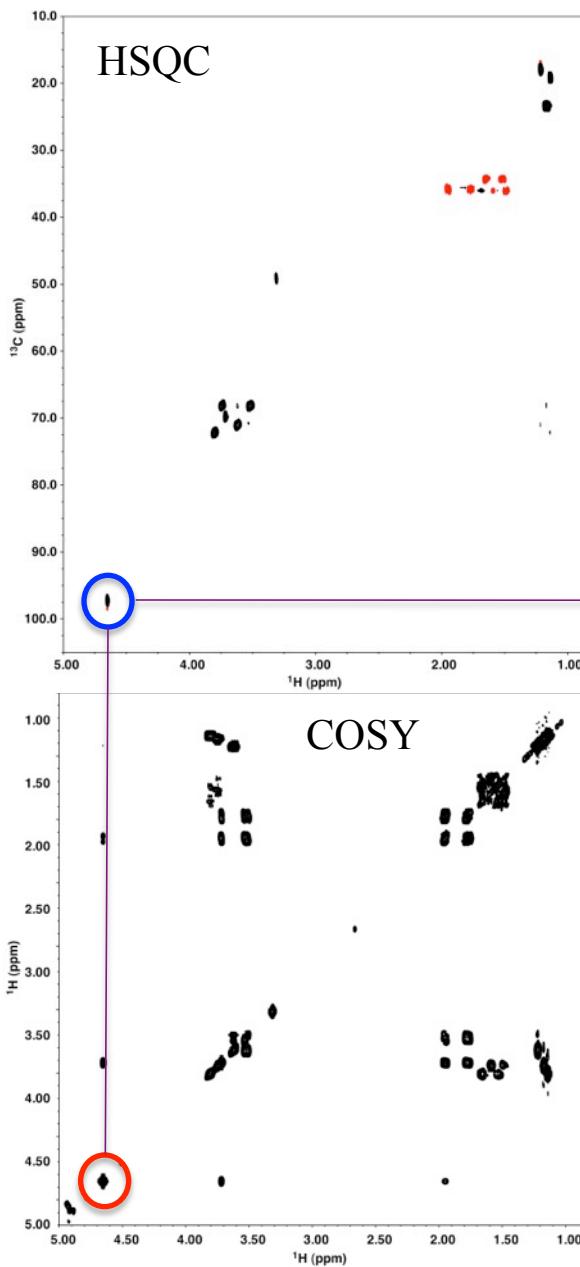


COSY spectrum

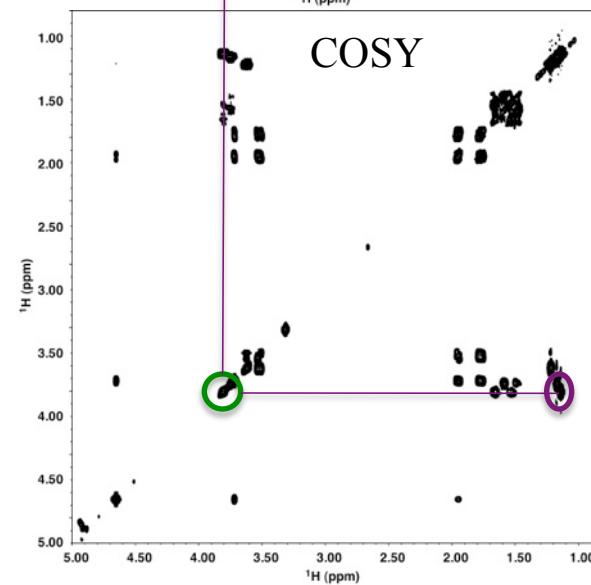
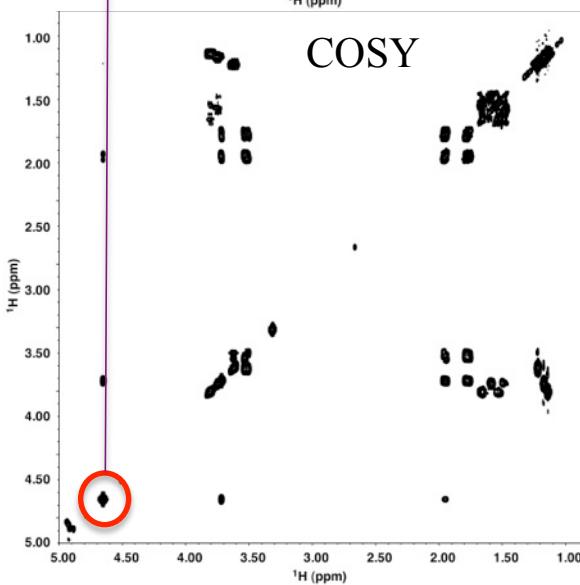
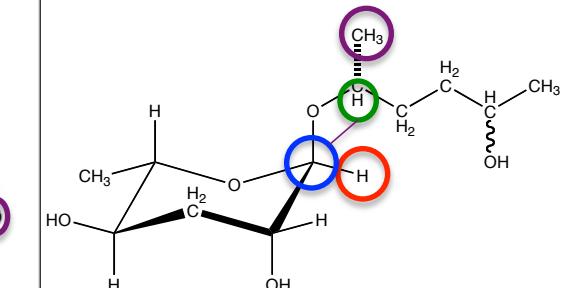


This chain is almost symmetric.

How do we know which end is which?



Working with multiple 2D datasets.



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Complex Mixture Analysis by NMR

<http://spin.ccic.ohio-state.edu/>

COLMAR covariance

Trbovic, Smirnov, Zhang,
Brüschweiler, J. Magn. Reson. 171,
277–283 (2004)

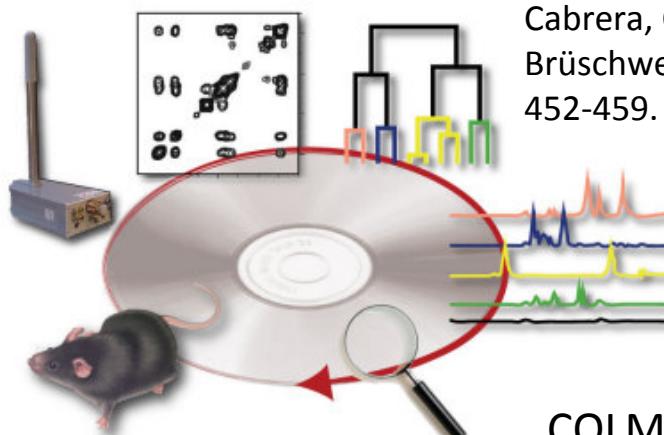
Brüschweiler, Zhang, Chem. Phys.
120, 5253–5260 (2004).

Brüschweiler, J. Chem. Phys. 121,
409–414 (2004).

COLMAR DemixC

Zhang, Dossey, Zachariah, Edison, & Brüschweiler Anal.
Chem. 79, 7748-52 (2007).

Zhang, F., Bruschweiler-Li, L., Robinette, S. L., and
Bruschweiler, R. Anal Chem 80, 7549-7553 (2008).



COLMAR HSQC

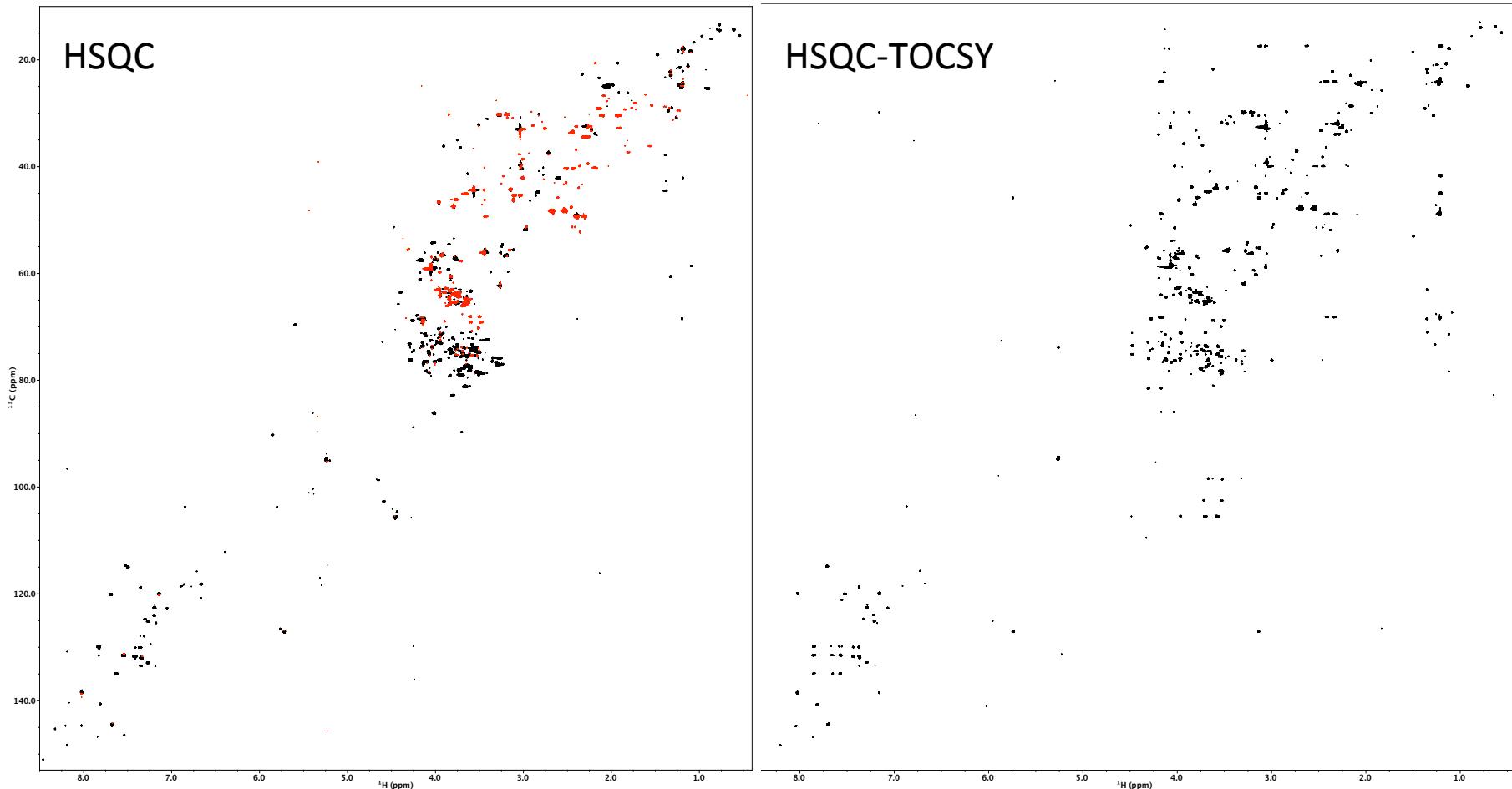
Bingol, K., Li, D.-W., Bruschweiler-Li, L.,
Cabrera, O., Megraw, T., Zhang, F., and
Brüschweiler, R. (2014), ACS Chem Biol 10,
452-459.

COLMAR query

Robinette, Zhang, Brüschweiler, & Brüschweiler Anal.
Chem. 80, 3606–3611 (2008).

Zhang, F., Robinette, S. L., Bruschweiler-Li, L., and
Bruschweiler, R. (2009), Magnetic Resonance in Chemistry
47 Sup 1, S118-122.

2D HSQC and HSQC-TOCSY of human urine



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2017

COLMARM Analysis of Urine HSQC and HSQC-TOCSY Data



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2017

Coming soon: Simple Intro to PCA