A Natural Abundance ^{13}C NMR Approach for Metabolomic Studies of Complex Mixtures

Clendinen, C.S. (UF, Biochemistry and Molecular biology); Lee-Mcmullen, B.A. (UF, Biochemistry and Molecular biology); Stupp, G.S. (UF, Biochemistry and Molecular biology); Williams, C. (UF, Department of Entomology and Nematology); Ramaswamy, V. (FSU, Maglab); Edison, A.S. (UF, Biochemistry and Molecular Biology)

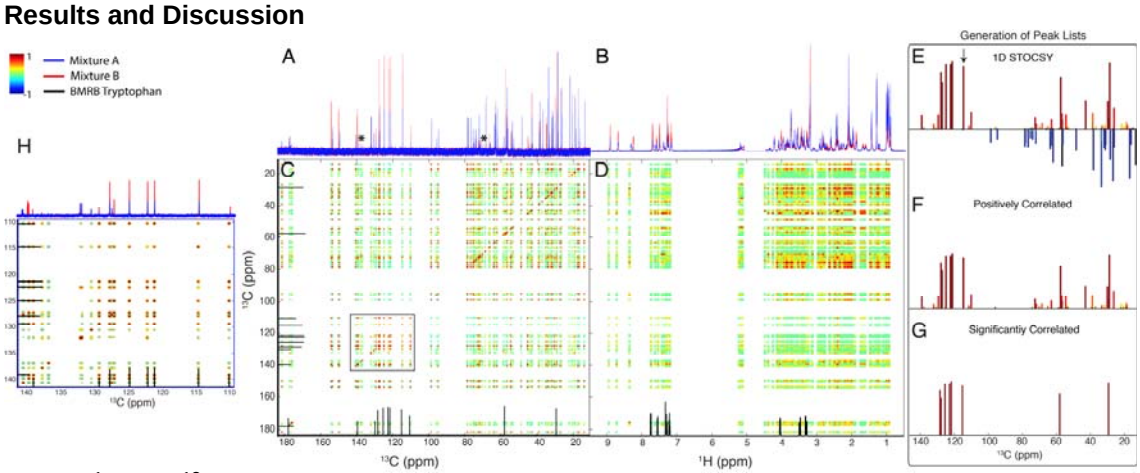
Introduction

Usually with NMR-based metabolomics, 1D and 2D ^{1}H-detected methods are used to analyze the global response of a biological system to perturbation/stress. Though ^{1}H-detected methods are extremely useful, the advantages of ^{13}C NMR techniques over traditional ^{1}H NMR include the carbon spectral width and molecular backbone information. Here, we determine the potential of ^{13}C-detected NMR metabolomics at natural abundance. In order to develop and validate the approach, we first show results from synthetic mixtures of 20 common metabolites. We then applied the overall approach to mice serum and *D. melanogaster* extracts.

Experimental

1D ^{1}H and ^{13}C spectra were collected on an Agilent VNMRS-600 spectrometer using our custom 1.5-mm ^{13}C HTS probe (Ramaswamy, 2013). Synthetic mixture and fly extract ^{1}H data were collected with a simple pulse sequence with preset of residual water with a spectral width of 12 ppm (7183.9 Hz) at an observe frequency of 599.68 MHz. Synthetic mixture and fly ^{13}C spectra were collected on a total time of 2hr 3min, with a 212 ppm (32051.3 MHz) with an observe frequency of 150.79 MHz; 0.10 sec relax delay; 0.800 acquisition time. Both used a 60-degree pulse. All ^{1}H was decoupled at 599.68 MHZ with a power of 37 dB continuously on WALTZ-16

Results and Discussion



1D ^{1}H and ^{13}C NMR spectra were collected for each sample and those spectra for the synthetic compounds are shown in Figure 1A and 1B, respectively. Figure 1C and 1D displays the 2D STOCSY and SHY maps respectively. A closer look at the SHY is displayed in Figure 1H for between 110-150 ppm. Generation of peak lists (Figure 1E-G) were much more useful when using carbon that when proton was used. This workflow was used for the mouse serum and the fly extracts.

Conclusions

The HTS probe allows for minimized time and sample allowing for carbon metabolomics. The synthetic mixtures provided proof of concept that we would be able to identify metabolites within 40-200 nM. Unlike the ^{1}H spectra, the ^{13}C spectra were easily annotated. All results are published in Clendinen, 2014.

Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy. In addition, this work is supported NIH/NIBIB grant R01EB009772-01

References

[1] Clendinen, C., *et al.,* AC, **86**, 9242-9250 (2014)
[2] Ramaswamy,V., *et al*., eMagRes, **2**, 215-228 (2013).