



## Accurate Identification of Unknown and Known Metabolic Mixture Components by Combining 3D NMR with FT-ICR MS/MS

Wang, C. (Ohio State U., Chemistry); He, L. (FSU, Chemistry); Li, D. and Brüscheiler-Li, L. (Ohio State U., Campus Chemical Instrument Center); Marshall, A.G. (FSU, Chemistry; NHMFL, ICR) and Brüscheiler, R. (Ohio State U., Chemistry)

### Introduction

Metabolite identification in metabolomics samples is a key step that critically impacts downstream analysis. We recently introduced the SUMMIT NMR/MS hybrid approach for the identification of the molecular structure of unknown metabolites, based on the combination of NMR, mass spectrometry (MS), and combinatorial cheminformatics.

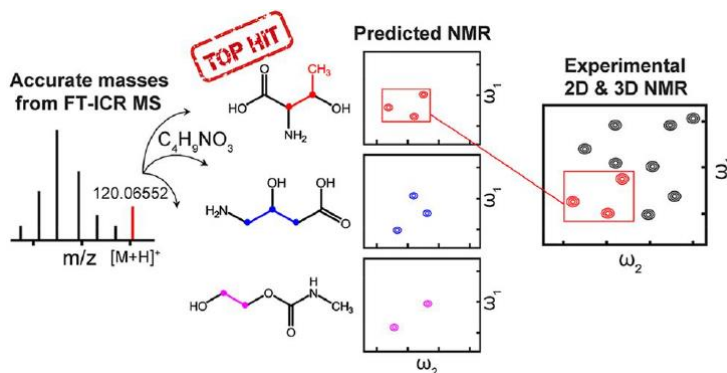
### Experimental

For a mixture of metabolites, NHMFL's 9.4 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) enabled resolution and identification of metabolite chemical formulas (Fig. 1, left). For each chemical formula, the possible molecular structures were generated, and their  $^1\text{H}$  and  $^{13}\text{C}$  NMR shifts calculated. Comparison to experimental two-dimensional  $^1\text{H}/^{13}\text{C}$  chemical shift spectra then enables rank-ordering of the possible structures. (Fig. 1) The final structure may then be confirmed by FT-ICR MS/MS.

### Results and Discussion

Here, we demonstrate the feasibility of the approach for an untargeted analysis of both a model mixture and an *E. coli* cell lysate, based on 2D/3D NMR experiments in combination with FT-ICR MS and MS/MS data. For 19 of the 25 model metabolites SUMMIT yielded complete structures that matched those in the mixture independent of database information. Of those, 7 top-ranked structures matched those in the mixture, and 4 of those were further validated by positive ion MS/MS. For 5 metabolites, not part of the 19 metabolites, correct molecular structural motifs could be identified. For *E. coli*, SUMMIT MS/NMR identified 20 previously known metabolites with 3 or more  $^1\text{H}$  spins independent of database information. Moreover, for 15 unknown metabolites, molecular structural fragments were determined consistent with their spin systems and chemical shifts. By providing structural information for entire metabolites or molecular fragments, SUMMIT MS/NMR greatly assists the targeted or untargeted analysis of complex mixtures of unknown compounds.

**Fig. 1.** Procedure for metabolite identification by combining chemical formula from ultrahigh-resolution FT-ICR MS with comparison of experimental and calculated two-dimensional NMR spectra (see text).



### Acknowledgements

FT-ICR experiments were performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida. All NMR experiments were performed at the CCIC NMR facility at OSU, supported by the National Institutes of Health grant R01 GM 066041, and SECIM (Southeast Center for Integrated Metabolomics) grant U24 DK097209-01A1 to R.B. We thank Felix Hoffmann for performing quantum-chemical chemical shift calculations.

### Reference

[1] He, L., *et al.*, *J. Proteome Res.*, **16**, 3774-3786 (2017).