



# Two elephants in the room: new hybrid nuclear magnetic resonance and mass spectrometry approaches for metabolomics

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## Purpose of review

This review describes some of the advances made over the past year in NMR-based metabolomics for the elucidation of known and unknown compounds, including new ways of how to combine this information with high-resolution mass spectrometry.

## Recent findings

A new method allows the back-calculation of mass spectra from NMR spectra that have been queried against databases improving the accuracy of the identified compounds by validation and consistency analysis. For the de-novo characterization of unknown compounds, an algorithm has been introduced that predicts all viable NMR spectra from accurate masses allowing, by comparison with experimental NMR data, the determination of the structures of new metabolites in complex mixtures.

## Summary

Recent advances in NMR and mass spectrometry-based metabolomics and their synergistic use promises to significantly improve metabolomics sample characterization both in terms of identification and quantitation, and accelerate metabolite discovery.

## Keywords

combined NMR and MS hybrid approaches, complex mixture analysis, mass spectrometry, metabolomics, nuclear magnetic resonance spectroscopy

## INTRODUCTION

The field of metabolomics has become a key discipline to study metabolism of living organisms in a wide range of contexts, including health and disease [1]. Many metabolomics studies seek information about both the identity and the quantity of dozens to hundreds of different metabolites present in a biological sample. The two main analytical techniques in metabolomics are mass spectrometry (MS) and NMR, which both allow the detection of many different metabolites directly in complex mixtures with little or no prior purification [2,3]. Although each of the two techniques has been successful in its own right, only very few applications make synergistic use of the complementary information they provide. This short review describes advances made over the past 12 months in NMR-based and MS-based metabolomics with an emphasis on how these two powerful techniques can be synergistically combined.

MS and NMR have played a dominant role since the early days of metabolomics and their complementary strengths and limitations have been recognized. NMR is able to detect metabolites present

in solution at concentrations larger than 1  $\mu\text{M}$ , with little or no prior knowledge [4]. A hallmark of NMR is that it provides outstanding reproducibility between different NMR spectrometers and users. MS, on the other hand, is bound to the detection of ionizable metabolites that enter the gas phase, which can be detected at significantly lower concentration than NMR (pM – nM) [5]. MS provides superior performance for the analysis of lipids whose NMR spectra tend to have limited resolution

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## KEY POINTS

- Rigorous analysis of metabolomics samples remains a challenge. New hybrid approaches that combine the powers of high-resolution NMR and mass spectrometry show significant potential to push current boundaries.
- Approaches that combine NMR and mass spectrometry are distinguished using five different categories: hardware-based, chemical-modification-based, statistics-based, stable isotope-based, and combinatorial cheminformatics-based approaches.
- Routine metabolomics analysis has been limited mostly to 'known' compounds, i.e. those that are contained in databases. A new combinatorial cheminformatics-based protocol has been introduced, which uses NMR data as an effective filter to identify one of the many metabolite structures belonging to each accurate mass determined by high-resolution mass spectrometry promises complex mixture analysis beyond the reach of current mass spectrometry and NMR databases.

[6]. Sample preparation protocols for both NMR and electrospray ionization (ESI) ion source mass spectrometers either with direct infusion or liquid chromatography-mass spectrometry (LC-MS) are fairly straightforward provided that the metabolic material is dissolved in a suitable solvent. Sample preparation for gas chromatography-mass spectrometry (GC-MS), on the other hand, requires the derivatization of nonvolatile metabolites. Apart from LC-MS and GC-MS, capillary electrophoresis-mass spectrometry [7] and ion mobility-mass spectrometry [8] are other MS approaches that are used for the analysis of solution-state metabolomics samples. MS and NMR are able to work with semi-solid samples, for example for the analysis of intact tissue. Metabolome profiling of semisolid samples is often performed by using high-resolution magic angle-spinning-NMR [9] and MS-imaging [10]. MS and NMR data derived from the same sample provide complementary information. We review recent advances toward this goal with special emphasis given to the approaches that aim to solve common challenges of NMR-based and MS-based metabolomics.

Identification of biomarker metabolites for disease diagnosis is one of the most important applications of metabolomics. To date, a significant number of potential metabolite biomarkers have been discovered by profiling the human metabolome [11,12]. Another critical advancement in metabolomics is the comprehensive analysis of human biofluids, such as serum and urine, by combining NMR, MS (GC-MS, LC-MS, inductively coupled plasma mass spectrometry) and other analytical

techniques (e.g. liquid chromatography-ultraviolet spectroscopy) [13,14]. These efforts resulted in the identities and absolute mean concentrations of hundreds of the most abundant metabolites in these biofluids with similar efforts applied to the metabolomics analysis of tissues, organs, and plants. Such information is highly valuable for modeling human metabolic pathways and fluxes [15]. Moreover, it provides important input for establishing interlaboratory consistency and transferability of metabolomics results. Comprehensive evaluation of major biofluids demonstrated large metabolome diversity between different human biofluids. For instance, unlike human urine, human serum turned out to consist of a variety of different lipids. However, there are still many metabolites in biofluids whose identities and quantities remain unknown. Missing information will continue to be filled in with further advances made in analytical technologies. In the following sections, we review some of the major advancements in NMR and MS in the light of their contribution to further extend the number of identified and quantitated metabolites in biofluids.

In MS-based metabolomics, the conversion of the experimentally detected MS signals into metabolite identities, i.e. compound names, represents a key step. A crucial development has been the availability of mass spectrometers that provide mass accuracy extending into the sub-5 ppm regime, wherein the measured mass of a compound ("accurate mass") often permits the unambiguous determination of its elemental composition, i.e. molecular formula. Metabolite identification is then typically performed by electrospray ionization-mass spectrometry (ESI-MS) through querying the accurate mass against a MS metabolite database, sometimes together with the retention time or with MS/MS fragmentation information [16]. For larger metabolites (>200 Da), mass accuracy becomes increasingly critical for the determination of unique molecular formulas. Ultra high-resolution MS instruments, in particular Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), can reach a resolution in the sub-ppm range [17]. As in NMR, the resolution of an FT-ICR MS is proportional to the magnetic field strength, with the first 21 T instruments commissioned last year at the National High Magnetic Field Laboratory (Tallahassee, FL, USA) and the Pacific Northwest National Laboratory/Environmental Molecular Sciences Laboratory (Richland, WA, USA).

## ADVANCES IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

One-dimensional (1D)  $^1\text{H}$  NMR is the most common approach in NMR-based metabolomics allowing analysis of hundreds to thousands of samples in

a short period. However, the identification of metabolites in complex mixtures solely based on 1D  $^1\text{H}$  NMR is a challenge because of peak overlaps due to the crowdedness of the spectra. Substantially improved spectral resolution can be obtained by going from 1D to two-dimensional (2D) NMR experiments requiring longer experiment times [18]. In 2D experiments, spin magnetization is transferred between different nuclear spins, which can be depicted in the form of 'cross-peaks' by plotting the spectrum against two frequency dimensions. The chance of peak overlap is thereby significantly reduced. Two of the most commonly used 2D NMR experiments in metabolomics are the 2D  $^{13}\text{C}$ - $^1\text{H}$  heteronuclear single quantum coherence spectroscopy (HSQC) experiment providing correlations between chemical shifts of  $^1\text{H}$  spins with their directly attached  $^{13}\text{C}$  spins and the 2D  $^1\text{H}$ - $^1\text{H}$  total correlation spectroscopy (TOCSY) experiment providing chemical shifts between all  $^1\text{H}$  spins within the same molecule or spin system. The TOCSY experiment provides valuable information about which resonances belong to the same molecule, which is hard to obtain from 1D experiments alone and which is beneficial both for the identification of known compounds and for the elucidation of the structure of unknown compounds in complex metabolite mixtures. Many metabolomics groups use 2D NMR in some situations [19], whereas others rely almost exclusively on 2D NMR experiments for the accurate and comprehensive identification of metabolites.

A recent development in NMR-based metabolite identification has been the introduction of customized metabolomics databases for 2D NMR experiments that increased the accuracy of metabolite identification significantly [20]. The  $^1\text{H}$ ( $^{13}\text{C}$ )-TOC-CATA customized database specializes on the querying of  $^1\text{H}$ - $^1\text{H}$  TOCSY and  $^{13}\text{C}$ - $^1\text{H}$  HSQC-TOCSY spectra of complex metabolite mixtures [21<sup>¶</sup>]. This database sorts the TOCSY spectra of metabolites into their individual spin system. This increased the accuracy of metabolite identification by more than 35% and more than 21% over existing 1D  $^1\text{H}$  and 1D  $^{13}\text{C}$  NMR metabolomics databases, respectively. Moreover, a customized NMR metabolomics database for the analysis of  $^{13}\text{C}$ - $^1\text{H}$  HSQC spectra was introduced [22<sup>¶</sup>]. This database, termed COLMAR  $^{13}\text{C}$ - $^1\text{H}$  HSQC, unifies the NMR spectroscopic information of two of the largest public metabolomics databases, namely the Biological Magnetic Resonance Data Bank [23] and The Human Metabolome Database [24]. COLMAR  $^{13}\text{C}$ - $^1\text{H}$  HSQC sorts HSQC spectra of metabolites into their individual isomeric states, which permits improved query in cases where lowly populated isomers are below the HSQC detection limit. Together with an improved

query algorithm, the COLMAR  $^{13}\text{C}$ - $^1\text{H}$  HSQC metabolomics database increases the accuracy of metabolite identification by more than 37% and decreases the false positive identification rates by more than 82% over existing  $^{13}\text{C}$ - $^1\text{H}$  HSQC metabolomics databases.

Another key challenge of NMR-based metabolomics is the accurate quantitation of metabolites. Although, in principle, analysis of a standard 1D  $^1\text{H}$  NMR spectrum provides the simplest and most accurate concentration determination, in practice, peak overlaps prevent this approach for many metabolites. The problem can be reduced by measuring at higher magnetic field strength, for example at 700 MHz and above, which at the same time also improves sensitivity. Another way of resolution enhancement is to eliminate homonuclear proton-proton scalar J-couplings using 'pure shift' methods, which result in narrow peaks without multiplet splittings [25,26]. However, these methods typically come with a significant loss in sensitivity and, hence, are limited to some of the more abundant metabolites. Finally, 2D NMR spectroscopy [27] can be employed providing access to resolved cross-peaks of most or all mixture compounds. However, since magnetization transfers during the 2D J-correlation experiments depend on the precise J-coupling constants and relaxation properties of each compound, the quantitative character of these experiments becomes somewhat distorted. These issues can be largely overcome by using reference 2D NMR spectra for each compound or methods that allow one to subtract the mixing time effects, as is the case in the HSQC<sub>0</sub> approach [28]. Alternatively, one can use experiments that are relatively immune to small variations in J-coupling constants as was shown for  $^{13}\text{C}$ - $^{13}\text{C}$  CT-TOCSY of uniformly  $^{13}\text{C}$ -labeled mixtures [29] provided that the carbon-backbone topology is known, which can be readily extracted from the same 2D NMR experiment [30] or from an Incredible Natural Abundance Double Quantum Transfer Experiment (INADEQUATE)-type experiment [31]. A main limitation of 2D NMR is the need for longer measurement times. Recent advances in nonuniform sampling and reconstruction techniques [32] along the indirect dimension(s) can considerably shorten the NMR time, provided that one is not limited by overall sensitivity. The same applies also to ultrafast NMR methods. These developments start to find applications in NMR-based metabolomics [33]. In order to address the sensitivity limitation, dynamic nuclear polarization techniques are being developed, which can also be used to 'illuminate' and monitor selected metabolites and their conversions in real time along biochemical pathways in living cells and organs [34].

## COMBINING NUCLEAR MAGNETIC RESONANCE WITH MASS SPECTROMETRY

The selection of the instrumental technique for a particular metabolomics study depends on multiple factors, including the accessibility to instrumentation, the specific questions at hand, and the training and background of the scientists involved. Although the use of both NMR and MS methods is becoming increasingly popular, in the majority of these studies the two methods are used essentially independently from each other. Only at the end, the metabolites identified by each method are compared with the goal to increase the total number of detected metabolites in complex mixtures as some metabolites show up only in one of either technique. This strategy is straightforward, but does not fully capitalize on the complementary strengths of these two analytical approaches when the two datasets stem from the same sample. Therefore, metabolomics scientists have started exploring new ways to combine NMR with MS for high-accuracy metabolite identification and quantitation as well as for the accurate analysis of biochemical pathways. Currently, nuclear magnetic resonance/mass spectrometry (NMR/MS) approaches can be classified into five different categories, namely hardware-based, chemical-modification-based, statistics-based, stable isotope-based, and combinatorial cheminformatics-based NMR/MS approaches.

'Hardware-based NMR/MS approaches' physically connect NMR and MS instruments, as is the case for commercially available LC-MS-NMR instruments that are primarily used for structure elucidation of unknown compounds. Chemical-modification-based NMR/MS approaches, in general, introduce chemical agents into samples that interact with certain types of metabolites and make their signals more visible in both NMR and MS spectra. Recent examples are  $^{15}\text{N}$ -labeled agents that selectively and covalently attach to carboxyl [35] and carbonyl [36] group containing metabolites. This makes signals of these metabolites directly visible in 2D  $^{15}\text{N}$ - $^1\text{H}$  HSQC NMR spectra as well as in mass spectra because of the introduction of a permanent charge, thereby linking NMR and MS signals of the modified metabolites. 'Statistics-based approaches' integrate NMR and MS by means of multivariate statistical analysis applied to a large number of samples [37,38]. Recently, it was shown that multivariate statistics of combined NMR and MS datasets of metabolomics samples provide better separation of groups and greater levels of model interpretability as compared with multivariate statistics applied to NMR or MS datasets alone [39]. 'Stable isotope-based methods' are used for the accurate analysis of isotopomer/isotopologue patterns of isotopically

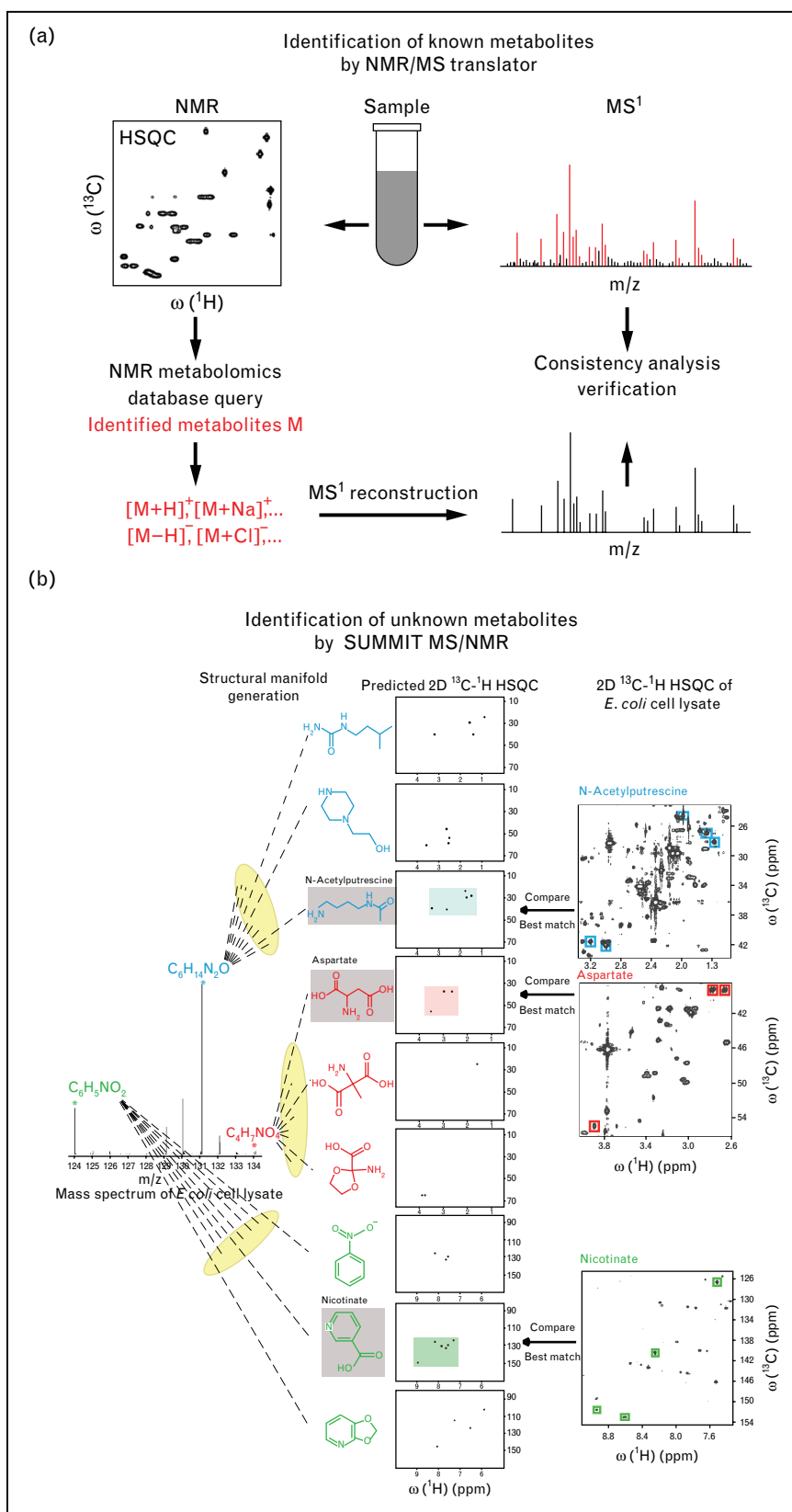
labeled metabolites, which report about the activity of selected biochemical pathways in response to biological perturbations [15].

Most recently, two 'combinatorial cheminformatics-based NMR/MS approaches' have been introduced for the rapid and accurate identification of metabolites, which are the 'NMR/MS Translator' and 'SUMMIT MS/NMR'. The NMR/MS Translator approach (Fig. 1a) combines NMR and accurate MS data of the same metabolomics sample providing fast and highly accurate identification of catalogued, i.e. known metabolites [40]. The SUMMIT MS/NMR approach (Fig. 1b), on the other hand, combines NMR and accurate MS data for high-throughput structure elucidation of unknown metabolites [41]. In the following, we briefly describe both of these approaches.

The NMR/MS Translator first identifies metabolite candidates from 1D or 2D NMR spectra by NMR database query, which is followed by the determination of the masses ( $m/z$ ) of all of their most likely ions, adducts, and characteristic isotope distributions. The expected  $m/z$  ratios are then compared with the  $\text{MS}^1$  spectrum for the direct assignment of those signals of the mass spectrum that correspond to the metabolites identified in the NMR spectra (Fig. 1a). In this way, the MS and NMR spectra can be rapidly assigned in a fully automated manner. Furthermore, since chemical shift and accurate mass data were co-analyzed, it increased the accuracy of metabolite identification as compared with separate studies by NMR and MS alone [40]. The NMR/MS Translator was applied to human urine by combining  $^{13}\text{C}$ - $^1\text{H}$  HSQC with direct infusion ESI-MS spectra. It was able to identify for 88 metabolites consistent signals in both NMR and MS spectra, which compares favorably even to some of the most extensive studies reported on the same type of sample also using both NMR and MS [40].

The NMR/MS Translator synergistically uses the power of NMR and MS to enhance the accuracy and efficiency for the identification of metabolites, but only for those metabolites that have already been compiled in databases. Hence, the NMR/MS Translator is not designed for the discovery of new metabolites. Although excellent progress has been made in expanding databases with more metabolites, a large fraction of metabolites contained in many samples is still unknown. Traditionally, identification of unknown metabolites requires their isolation through time-consuming purification from complex mixtures by using chromatographic separation techniques, followed by extensive characterization by NMR, MS, X-ray, and other techniques. The utility of the traditional approach is limited in the context of high-throughput applications, because





**FIGURE 1.** Schematic representation of recently proposed combined MS/NMR approaches for rapid and accurate identification of known and unknown metabolites in complex metabolite mixtures: (a) the NMR/MS Translator strategy allows rapid identification of catalogued metabolites and (b) the SUMMIT MS/NMR strategy allows rapid identification of unknown metabolites. Reproduced with permission from [40<sup>■</sup>,41<sup>■</sup>]. Copyright 2015 American Chemical Society. MS, mass spectroscopy.

purification of every unknown metabolite from their complex matrix is a challenge. In order to address this challenge, a purification-free metabolite identification strategy, which is termed SUMMIT MS/NMR, has been developed [41<sup>\*</sup>]. The approach, which also combines NMR and MS data, first extracts accurate masses of all detected metabolites from high-resolution mass spectra and generates all structures consistent with the derived chemical formulas. The comparison of the predicted NMR spectra of all candidate structures with the experimental NMR spectra of the same sample permits accurate identification of the structures present in the complex mixture of interest (Fig. 1b). This combined MS/NMR technology was applied to an *Escherichia coli* cell extract, where it correctly identified a wide range of different types of metabolites [41<sup>\*</sup>]. The results suggest that SUMMIT MS/NMR should become suitable for high-throughput applications for the discovery of new metabolites in biological and biomedical mixtures overcoming the need for experimental MS and NMR metabolite databases or extensive metabolite purification for the elucidation of the structures of unknown metabolites.

## CONCLUSION

Metabolomics is a rapidly expanding and highly promising discipline with many powerful applications in clinical and nutritional research. Recent advances to address two of the key challenges in metabolomics research have been reviewed here concerning the accurate identification and quantitation of all metabolites present in a biological system with focus on the combined use of NMR and MS techniques. Easy accessibility to high-end NMR and MS hardware allows co-measurements of samples using both techniques and permits the use of the data in new synergistic ways for the more comprehensive and more accurate identification of metabolites in complex metabolomics mixtures. This is likely to promote discovery and profiling of an ever larger number of unique metabolites and thereby provides a progressively complete picture of metabolism in many different living systems.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Dumas ME, Kinross J, Nicholson JK. Metabolic phenotyping and systems biology approaches to understanding metabolic syndrome and fatty liver disease. *Gastroenterology* 2014; 146:46–62.
2. Wolfender JL, Marti G, Thomas A, Bertrand S. Current approaches and challenges for the metabolite profiling of complex natural extracts. *J Chromatogr A* 2015; 1382:136–164.
3. Bingol K, Bruschweiler R. Multidimensional approaches to NMR-based metabolomics. *Anal Chem* 2014; 86:47–57.
4. Simmler C, Napolitano JG, McAlpine JB, et al. Universal quantitative NMR analysis of complex natural samples. *Curr Opin Biotechnol* 2014; 25:51–59.
5. Scalbert A, Brennan L, Manach C, et al. The food metabolome: a window over dietary exposure. *Am J Clin Nutr* 2014; 99:1286–1308.
6. Cajka T, Fiehn O. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. *Trends Anal Chem* 2014; 61:192–206.
7. Hirayama A, Wakayama M, Soga T. Metabolome analysis based on capillary electrophoresis-mass spectrometry. *TrAC Trend Anal Chem* 2014; 61:215–222.
8. May JC, Goodwin CR, McLean JA. Ion mobility-mass spectrometry strategies for untargeted systems, synthetic, and chemical biology. *Curr Opin Biotechnol* 2015; 31:117–121.
9. Wong A, Li X, Molin L, et al.  $\mu$ High resolution-magic-angle spinning NMR spectroscopy for metabolic phenotyping of *Caenorhabditis elegans*. *Anal Chem* 2014; 86:6064–6070.
10. Tsai YH, Garrett TJ, Carter CS, Yost RA. Metabolomic analysis of oxidative and glycolytic skeletal muscles by matrix-assisted laser desorption/ionization-mass spectrometric imaging (MALDI MSI). *J Am Soc Mass Spectrom* 2015; 26:915–923.
11. Duarte IF, Diaz SO, Gil AM. NMR metabolomics of human blood and urine in disease research. *J Pharm Biomed Anal* 2014; 93:17–26.
12. Gika HG, Theodoridis GA, Plumb RS, Wilson ID. Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics. *J Pharm Biomed Anal* 2014; 87:12–25.
13. Psychogios N, Hau DD, Peng J, et al. The human serum metabolome. *PLoS One* 2011; 6:e16957.
14. Bouatra S, Aziat F, Mandal R, et al. The human urine metabolome. *PLoS One* 2013; 8:e73076.
15. Fan TWM, Lorkiewicz PK, Sellers K, et al. Stable isotope-resolved metabolomics and applications for drug development. *Pharmacol Ther* 2012; 133:366–391.
16. Zhu ZJ, Schultz AW, Wang J, et al. Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database. *Nat Protoc* 2013; 8:451–460.
17. Marshall AG, Chen T. 40 years Fourier transform ion cyclotron resonance mass spectrometry. *Int J Mass Spectrom* 2015; 377:410–420.
18. Ernst R, Bodenhausen G, Wokaun A. Principles of nuclear magnetic resonance in one and two dimensions. Oxford; 1987.
19. Misawa T, Date Y, Kikuchi J. Human metabolic, mineral, and microbiota fluctuations across daily nutritional intake visualized by a data-driven approach. *J Proteome Res* 2015; 14:1526–1534.
20. Bingol K, Zhang F, Bruschweiler-Li L, Bruschweiler R. TOCCATA: a customized carbon total correlation spectroscopy NMR metabolomics database. *Anal Chem* 2012; 84:9395–9401.
21. Bingol K, Bruschweiler-Li L, Li DW, Bruschweiler R. Customized metabolomics database for the analysis of NMR  $^1\text{H}$ - $^1\text{H}$  TOCSY and  $^{13}\text{C}$ - $^1\text{H}$  HSQC-TOCSY spectra of complex mixtures. *Anal Chem* 2014; 86:5494–5501.
- This article presents the first customized 2D  $^1\text{H}$ - $^1\text{H}$  TOCSY and 2D  $^{13}\text{C}$ - $^1\text{H}$  HSQC-TOCSY metabolomics database and query, which significantly improved the accuracy of metabolite identification. The web server is open to the public at <http://spin.ccic.ohio-state.edu/index.php/toccat2/index>.
22. Bingol K, Li DW, Bruschweiler-Li L, et al. Unified and isomer-specific NMR metabolomics database for the accurate analysis of  $^{13}\text{C}$ - $^1\text{H}$  HSQC spectra. *ACS Chem Biol* 2015; 10:452–459.
- This article presents the first customized 2D  $^{13}\text{C}$ - $^1\text{H}$  HSQC metabolomics database and query, which significantly improved the accuracy of metabolite identification. The web server is open to the public at <http://spin.ccic.ohio-state.edu/index.php/hsqc/index>.
23. Ulrich EL, Akutsu H, Doreleijers JF, et al. BioMagResBank. *Nucleic Acids Res* 2008; 36:D402–D408.
24. Wishart DS, Jewison T, Guo AC, et al. HMDB 3.0: the human metabolome database in 2013. *Nucleic Acids Res* 2013; 41:D801–D807.
25. Foroozandeh M, Adams RW, Meharry NJ, et al. Ultrahigh-resolution NMR spectroscopy. *Angew Chem Int Ed* 2014; 53:6990–6992.
26. Zangger K. Pure shift NMR. *Progr NMR Spectr* 2015; 86:87:1–20.
27. Girardeau P. Quantitative 2D liquid-state NMR. *Magn Reson Chem* 2014; 52:259–272.

28. Hu K, Westler WM, Markley JL. Simultaneous quantification and identification of individual chemicals in metabolite mixtures by two-dimensional extrapolated time-zero  $^1\text{H}$ - $^{13}\text{C}$  HSQC (HSQC<sub>0</sub>). *J Am Chem Soc* 2011; 133:1662–1665.
29. Bingol K, Zhang F, Bruscheweiler-Li L, Brüscheweiler R. Quantitative analysis of metabolic mixtures by two-dimensional  $^{13}\text{C}$  constant-time TOCSY NMR spectroscopy. *Anal Chem* 2013; 85:6414–6420.
30. Bingol K, Zhang F, Bruscheweiler-Li L, Brüscheweiler R. Carbon backbone topology of the metabolome of a cell. *J Am Chem Soc* 2012; 134:9006–9011.
31. Clendinen CS, Pasquel C, Ajredini R, Edison AS.  $^{13}\text{C}$  NMR metabolomics: INADEQUATE network analysis. *Anal Chem* 2015; 87:5698–5706.
32. Mobli M, Hoch JC. Nonuniform sampling and non-Fourier signal processing methods in multidimensional NMR. *Progr NMR Spectr* 2014; 83:21–41.
33. Guennec AL, Giraudeau P, Caldarelli S. Evaluation of fast 2D NMR for metabolomics. *Anal Chem* 2014; 86:5946–5954.
34. Lerche MH, Jensen PR, Karlsson M, Meier S. NMR insights into the inner workings of living cells. *Anal Chem* 2015; 87:119–132.
35. Tayyari F, Gowda GAN, Gu H, Raftery D.  $^{15}\text{N}$ -cholamine—a smart isotope tag for combining NMR- and MS-based metabolite profiling. *Anal Chem* 2013; 85:8715–8721.
36. Lane AN, Arumugam S, Lorkiewicz PK, *et al.* Chemoselective detection and discrimination of carbonyl-containing compounds in metabolite mixtures by  $^1\text{H}$ -detected  $^{15}\text{N}$  nuclear magnetic resonance. *Magn Reson Chem* 2015; 53:337–343.
- This article introduces  $^{15}\text{N}$ -labeled aminoxy probe to facilitate the detection of carbonyl group containing metabolites in NMR and mass spectrometry spectra of lung adenocarcinoma cell line.
37. Crockford DJ, Holmes E, Lindon JC, *et al.* Statistical heterospectroscopy, an approach to the integrated analysis of NMR and UPLC-MS data sets: application in metabolomic toxicology studies. *Anal Chem* 2006; 78:363–371.
38. Pan Z, Gu H, Talaty N, *et al.* Principal component analysis of urine metabolites detected by NMR and DESI-MS in patients with inborn errors of metabolism. *Anal Bioanal Chem* 2007; 387:539–549.
39. Marshall DD, Lei S, Worley B, *et al.* Combining DI-ESI-MS and NMR datasets ■ for metabolic profiling. *Metabolomics* 2015; 11:391–402.
- This article combines  $^1\text{H}$  NMR and direct infusion ESI-MS spectra of metabolomics samples by using multiblock-principal component analysis (PCA), which provided better separation of classes as compared with PCA of  $^1\text{H}$  NMR or direct infusion ESI-MS spectral dataset alone.
40. Bingol K, Brüscheweiler R. NMR/MS Translator for the enhanced simultaneous ■ analysis of metabolomics mixtures by NMR spectroscopy and mass spectrometry: application to human urine. *J Proteome Res* 2015; 14:2642–2648.
- This article presents a combined NMR/MS approach that provides rapid and accurate identification of catalogued, i.e. known metabolites detected in both NMR and mass spectrometry spectra of the same metabolomic sample.
41. Bingol K, Bruscheweiler-Li L, Yu C, *et al.* Metabolomics beyond spectroscopic ■ databases: a combined MS/NMR strategy for the rapid identification of new metabolites in complex mixtures. *Anal Chem* 2015; 87:3864–3870.
- This article introduces a combined NMR/MS approach that allows the structure elucidation of unknown metabolites in complex metabolite mixtures. The approach does not require purification of unknown compounds from the matrix, therefore, it provides a platform with the potential for high-throughput discovery of new metabolites.