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NMR metabolomics: A look ahead

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ABSTRACT

NMR has been used to perform metabolic studies, metabolic profiling and metabolomics in biofluids and tissues for more than 40 years. This close connection between metabolic measurements and NMR has flourished because of NMR's many unique strengths for characterizing the chemical composition of complex mixtures. However, a number of other technologies, including mass spectrometry, have appeared in the past few years that are encroaching on NMR's dominance in metabolomics and metabolic studies. In this brief review, some of the current strengths and existing limitations of NMR-based metabolomics are highlighted. Additionally, a number of recent advances in NMR hardware, methodology and software are also described and these advancements are used to speculate about where NMR-based metabolomics is going, what needs to be done to make it more popular and how it will evolve in the next 5–10 years.

1. Introduction

NMR spectroscopy has played a key role in our understanding of metabolism and metabolic processes for almost 50 years. One of the first examples demonstrating how NMR could be used in metabolic studies was published in 1974 [1]. This early paper, by Wilson and Burlingame, described how ^{13}C isotope-tracer analysis could be used in conjunction with NMR spectroscopy to decipher a number of details in mammalian ethanol metabolism. As NMR spectrometers improved and magnet field strengths increased throughout the 1970s and 1980s, this early NMR-based metabolic study started to attract the attention of other scientists. Indeed, the richness of information found in high-resolution NMR spectra, combined with the ability to simultaneously identify multiple metabolites in biofluid mixtures made NMR particularly appealing to many others in the 1970s and 1908s who were interested in looking at various models of drug and animal metabolism [2–6]. These pioneering NMR studies revealed a tremendous amount about unknown or little-understood biochemistry and permitted real-time monitoring of many, previously inaccessible, metabolic processes.

The fact that NMR was being routinely used for "classical" metabolic studies to characterize complex metabolite mixtures, actually made NMR the preferred technology platform to launch the field of metabolomics. While the term "metabolomics" did not officially appear in the literature until 2000 [7], arguably the very first metabolomic studies were conducted in the 1980s using 1D ^1H NMR spectroscopy [8,9]. These early studies analyzed the metabolic composition of human serum and urine. By the late 1980s, NMR-based metabolomic studies (called metabolic profiling at that time) of human plasma led to the identification of several putative biomarkers of cancer and coronary artery disease [10,11]. These efforts were complemented by other NMR-based metabolomic studies focussing on urine to characterize inborn errors of metabolism (IEM) and evaluate drug toxicity [12,13].

Over the past 20 years the field of NMR-based metabolomics has experienced tremendous growth. In 1999 there were less than a half-dozen papers published on the subject, in 2018 there were more than 700. Over the same period of time, NMR has also expanded its reach into other subfields of metabolomics including lipid and lipoprotein protein profiling (called lipidomics) [11,14], metabolic flux analysis or fluxomics [15] and metabolite imaging or magnetic resonance spectroscopy [16]. The diversity of applications, from conventional metabolomics to lipidomics to fluxomics to metabolite imaging highlights the tremendous instrumental flexibility that NMR offers. Indeed, almost no other analytical method today offers the depth and diversity of applications that can be found via NMR spectroscopy – except possibly mass spectrometry (MS).

Interestingly, technological developments in MS over the past 10–15 years have allowed MS to match or even exceed some of the capabilities of NMR. Indeed, the competition between NMR spectroscopy and mass spectrometry, especially in the field of small molecule analysis, has already led to a few corporate casualties (i.e., Varian). Both NMR and MS have their strengths and both have their weaknesses (see Table 1). Right now, at least with

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Table 1
Comparing and Contrasting NMR and MS-based Metabolomics. Strengths are highlighted in **bold** and weaknesses are in *italics.*

NMR	MS
Non-destructive (sample)	*Destructive (sample)*
Robust instrumentation	*Frail instrumentation*
Instrument downtime minimal	*Instrument downtime frequent*
Excellent reproducibility	*Moderate reproducibility*
Simple sample preparation	*Complex sample preparation*
No requirement for chromatography	*Requirement for chromatography*
No need for chemical derivatization	*Frequent need for chemical derivatization*
Spectra are predictable	*Spectra not very predictable*
Allows precise structure determination	*Allows partial structure determination*
Inherently quantitative	*Not inherently quantitative*
Easily automated workflow	*Difficult-to-automate workflow*
Poor to moderate sensitivity (μM)	**Excellent sensitivity (nM)**
Modest metabolite coverage	**Extensive metabolite coverage**
Very expensive instrumentation	**Moderately expensive instrumentation**
Large instrument footprint	**Small instrument footprint**
Requires cryogens	**No required cryogens**
Expensive to maintain	**Moderately expensive to maintain**
Small spectral databases	**Large spectral databases**
Few software resources	**Many software resources**

regard to metabolomics, MS-based techniques appear to hold the edge with nearly 70% of metabolomics papers (published in 2018) mentioning mass spectrometry as the primary analytical technique. Indeed, the tremendous popularity of mass spectrometry and the rapid growth of MS-based metabolomics has led some to question the long-term future of NMR-based metabolomics [17]. However, it is far too early to write obituaries about NMR or to worry about its existential role in metabolomics. Indeed, there are many areas where NMR-based metabolomics is showing tremendous promise and where NMR offers unique strengths not found in any other technology or platform. In this short review I will discuss the ongoing tension between MS and NMR-based metabolomics in some detail. I will also highlight some of the most interesting and potentially transformative developments in NMR and use this information to speculate where NMR-based metabolomics is going and how it will evolve in the next 5–10 years.

2. NMR-based metabolomics versus MS-based metabolomics

The nearly-20-year "love affair" that metabolomics researchers have had with NMR spectroscopy has to do with its robustness, its high level of reproducibility, its superb instrument stability, its simple sample preparation protocols, its strongly quantitative character, its non-destructive nature and its easy amenability to automation. However, as highlighted in Table 1, NMR is not without some limitations. In particular, NMR is relatively insensitive compared to MS-based metabolomics (with MS being ∼100× better in concentration sensitivity [18]), it has a large instrument footprint (often requiring 10–20 m^2 versus 2 m^2 for a mass spectrometer), its very expensive (most NMR instruments are priced at over $1 million compared to MS instruments that are ½–¼ the cost) and it often comes with very high maintenance costs ($25,000–35,000 a year for cryogens and cryoprobe maintenance). These instrumental issues are steadily eating away at NMR's popularity among many metabolomics researchers.

In addition to these inherent instrumental challenges, NMR-based metabolomics is also handicapped by limitations in its data acquisition and data processing tools, as well as its limited collection of databases and software. Despite tremendous advances in NMR pulse sequence design and data acquisition technology over the past 20 years, NMR-based metabolomics is still mostly restricted to using lower-resolution 1D NMR techniques. While inherently fast, the reliance on 1D NMR limits the number of compounds that can be unambiguously identified or quantified via NMR. In contrast to NMR, most MS-based techniques are inherently two-dimensional (capturing retention time vs. m/z) with sample collection times being as fast or even faster than NMR. Indeed, the lack of faster, more quantitative 2D NMR approaches continues to be a major issue for NMR-based metabolomics.

Another major challenge faced by NMR-based metabolomics researchers is the fact that they do not have access to the large spectral libraries commonly found within the MS-based metabolomics community. Referential spectral libraries, consisting of 1D and/or 2D NMR spectra of pure compounds collected at multiple spectrometer frequencies, are key to compound identification and compound quantification in NMR-based metabolomics. The largest publicly available NMR spectral libraries cover fewer than 800 metabolites and only a limited number (500 and/or 600 MHz) of NMR spectrometer frequencies [19,20]. In contrast, MS spectral libraries covering 10,000–20,000 metabolites or more and have been collected for many different LC and GC–MS platforms [20,21].

Still another challenge faced by NMR metabolomics researchers lies in the fact that they do not have the low-cost kits or simple "systems solutions" that are beginning to appear for MS-based metabolomics [22]. Kit or systems-based metabolomics makes metabolite measurement far more accessible and much more reproducible for a wider number of users. If NMR metabolomics is going to grow or wishes to maintain its popularity in the future, it will need to address these hardware and software issues head on. In the following pages I will briefly discuss a number of encouraging advancements in NMR-related hardware, pulse sequence design, kit development and software/databases that should soon make NMR-based metabolomics, better, faster and cheaper.

3. Hardware advances

In order for NMR to compete with mass spectrometry, especially in the small-molecule world, NMR instruments will need to become smaller, cheaper and/or more sensitive. A number of promising developments are occurring in magnet technologies that appear to be addressing these issues. In particular, the development of permanent magnet (neodymium-boron-iron), benchtop NMR instruments with field strengths approaching or exceeding 90 MHz [23] could significantly reduce NMR instrument prices (by a factor of 20), the instrument footprint (by a factor of 10) and maintenance costs (by a factor of 1000). These instruments (produced by vendors such as Spinsolve, pcioSpin, NMReady and Puslar) offer excellent resolution (approaching 1 Hz), fast FT-based acquisition, multiple nuclei detection, very simple user interfaces and a range of 1D and 2D spectral acquisition options for chemical identification and quantification. A recent study in 2018 described how a 60 MHz benchtop instrument could be used in a point-of-care diagnostic setting and demonstrated that more than a dozen metabolites could be detected and quantified in urine and serum, with lower limits of detection of ∼25 μM [24]. Obviously, a 60 MHz NMR instrument is no match for a 600 MHz NMR instrument in terms of spectral resolution and metabolite identification capabilities. However, given the challenges of getting large super-conducting NMR instruments into clinical settings or into labs without large budgets or any specialized NMR expertise, it appears that benchtop NMRs may offer a compelling solution.

Another emerging solution to cheaper and smaller NMR instruments lies in cryogen-free superconducting magnet technology. These systems use cryocoolers or closed cycle helium refrigerators to bring magnet temperatures close to 4 K. They do not require constant liquid He or liquid N_2 refills and they can achieve much higher magnetic fields than permanent magnet benchtop NMR systems. Recently a power-driven high temperature superconducting (HTS) NMR was described that operated at 400 MHz [25]. Developed in New Zealand by HTS-110, this cryogen-free instrument exhibited near identical performance to a standard 400 MHz cryogenically cooled instrument. Given the rising costs of liquid helium and other challenges associated with regular cryogen fills, cryogen-free NMR system could have the same appeal to clinical labs and non-expert NMR labs as benchtop NMRs.

Reducing the cost and size of NMR instruments obviously comes at the expense of reduced sensitivity and resolution. If cost and size are not a problem, the expected appearance of 1.2 GHz NMR instruments in 2019 or 2020 promises to improve the sensitivity and resolution of many NMR-based metabolomics studies [26]. These ultra-high field instruments will allow for high nanomolar detection limits (compared to $\sim$5 μM detection limits with lower field NMR). 1.2 GHz NMR could bring the concentration sensitivity of NMR to within a factor of $\sim$10 of what is routinely achievable for mass spectrometry. For those performing NMR-based metabolomic studies, it will mean that more than 100 metabolites could be detected in serum (compared to 50–60 detectable at lower magnetic fields) and more than 200 metabolites could be detectable in urine (compared to 100–140 at lower fields).

Another emerging route to improving the sensitivity of NMR instruments is spin hyperpolarization. Hyperpolarization exploits the magnetic moment of unpaired electrons to polarize nuclear spins. The electron's magnetic moment is approximately 2800 times larger than that of the ^{13}C nucleus and 6900 times larger than that of ^{15}N nucleus. In other words, sensitivity enhancements of several thousand fold (i.e., metabolite detection limits at the low nanomolar level) are technically possible. Hyperpolarization, such as dynamic nuclear polarization or DNP was initially done to measure low abundance ^{13}C-labeled metabolites [27,28]. More recently, dissolution DNP (D-DNP), assisted by cross polarization has been shown to work with natural abundance ^{13}C cell extracts [29]. Furthermore, the reproducibility of single-scan ^{13}C signal intensity measurements with these natural abundance samples (just 20 mg of material) was within 6% [30]. These are exciting developments and underline the tremendous potential of D-DNP for a wide range of metabolomics studies. However, the cost of the instrumentation and the difficulties associated with the D-DNP protocol have limited its uptake.

On the other hand, the development of the SABRE-SHEATH hyperpolarization technique in 2016 is particularly exciting [31,32]. The SABRE-SHEATH method is a low-cost, remarkably efficient catalytic polarization transfer method for hyperpolarizing nuclear spins at room temperature. SABRE (*signal amplification by reversible exchange*) works *via* the transient binding of *para*-hydrogen to a transition-metal complex to form a network that permits the transfer of spin order from the *para*-hydrogen to the nuclei on the substrate molecule or molecules of interest. The set-up and equipment costs for a SABRE-SHEATH system are typically less than a few hundred dollars, yet enhancements on the order of 10,000 fold are possible and the hyperpolarization state lasts for over an hour. The SABRE-SHEATH approach has recently been extended so that it can work with any functional group containing an exchangeable proton through a technique called the SABRE-Relay [33]. Even more recently, a *para*-hydrogen SABRE method was used to detect flavour components in whisky at low micromolar concentrations [34]. This represents the first application of SABRE methods to metabolomics. If detection limits could be further lowered, SABRE and SABRE-SHEATH could revolutionize the field.

Continuing developments in NMR probe technology are also leading to some exciting possibilities for improving NMR instrument sensitivity. For instance the appearance of a ^{13}C-optimized 1.5-mm cryoprobe that uses high temperature superconductors has enabled ^{13}C NMR studies of small quantities of natural products to be done at natural ^{13}C abundance [35]. Given that many studies in metabolomics are sample-limited with only micrograms of material being available, this sort of probe would obviously be of interest to many metabolomics researchers. This probe development has been complemented with the appearance of a ^{1}H–^{13}C dual-optimized NMR probe that permits 2D ^{1}H–^{13}C HSQC experiments to be collected at natural abundance [36]. Given the utility of HSQC experiments for 2D NMR-based metabolomics and the fact that isotopic labeling in many metabolomic experiments is often not an option, the existence of such a dual-optimized probe could have significant implications for the NMR metabolomics community.

Some of the most exciting developments in probe development have occurred in solid state NMR. Microprobes for magic angle spinning (μMAS) of sub-microgram specimens with high-resolution (<0.01 ppm) capability have recently appeared [37]. These are now permitting the metabolomic characterization of different anatomical regions of plants and tissues [38]. While sample preparation can be difficult, the capabilities of μMAS probes gives NMR spectroscopists the opportunity to systematically explore tissue-specific metabolism with nearly equal precision as imaging mass spectrometry [39]. Given the many different technologies and materials put into probe design is hard to predict future trends in probe development. However, it is reasonable to predict that probe improvements to enhance sensitivity and performance will occur with greater regularity than with magnet design or magnet field improvements.

4. Pulse sequence advances

Almost all NMR-based metabolomic studies are based on using the *metnoesy* 1D NOESY experiment, or a variation of it. However, 1D NMR experiments are not the only choice available to NMR metabolomics researchers. In particular, metabolically complex samples, such as urine, with hundreds of different metabolites should ideally be analyzed using 2D NMR experiments. Indeed, homonuclear 2D experiments such as 2D ^{1}H COSY, 2D ^{1}H INADEQUATE or 2D J-resolved experiments have been widely used in NMR-based metabolomics studies for many years. Heteronuclear 2D experiments such as 2D ^{1}H-^{15}N HSQC and ^{1}H–^{13}C HSQC experiments can further enhance metabolite identification due to the additional chemical shift information captured in the second dimension as well as additional atomic connectivity information displayed in the 2D plot. However standard 2D NMR experiments require significant amounts of time to collect, process and interpret (hours as opposed to minutes). Furthermore, 2D NMR lacks the sensitivity (per unit time) of 1D NMR, with a lower limit of detection about 10X greater than a 1D experiment. Likewise it does not provide sufficiently uniform signals for accurate metabolite quantification.

Developing or implementing faster, more sensitive and more quantitative 2D NMR experiments needs to be a priority for the NMR metabolomics community. One approach to shorten the experimental acquisition time of a 2D experiment is to reduce the interscan delay (i.e., shorten the relaxation time). Experiments with a short interscan delay are known as *band-selective optimized flip angle short transient* (SOFAST) and *band selective excitation short transient* (BEST) methods. However, these techniques require that spin diffusion must be an effective relaxation mechanism, which is often seen for macromolecules or small molecules in viscous solvents. Early versions of the SOFAST and BEST techniques were limited to metabolomic studies involving living cells, which have a viscous cytoplasm [40]. However, improvements in these techniques, including the development of SOFAST-HMQC (with non uniform sampling), ALSOFAST (*alternate SOFAST*) and ASAP (*acceleration by sharing adjacent polarization*) HSQC pulse sequences have permitted the acquisition of 2D heteronuclear spectra of natural abundance urine and serum, along with various ^{13}C-labeled cell/tissue samples, in as little as a few minutes [41–43].

Another approach to shorten 2D experimental time involves non uniform sampling (NUS). In this technique, not all data points in the indirect dimension are recorded. Rather the data points that are collected are randomized and differentially weighted across the indirect dimension(s). After collecting the data, the spectrum is reconstructed by calculating the skipped data points. With the NUS technique, the acquisition time of 2D and 3D experiments can be reduced by up to 75% while still retaining the same spectral resolution as a full-time multidimensional experiment [44].

A third technique for reducing 2D experimental time is ultrafast (UF) 2D NMR. In this approach the physical length of the sample tube is used to generate slices which correspond to different time points in the second dimension (called spatial encoding). With UF-NMR, a 2D spectrum can be acquired in a single scan. In principle, this is the fastest 2D NMR technique available. However, with UF-NMR some compromise between spectral widths, resolution and sensitivity is often necessary. Fortunately, the limited spectral width in UF-NMR can be addressed by spectral folding and the sensitivity can be improved by increasing the number of scans [45,46]. As yet there have been relatively few published reports making use of these rapid 2D techniques or data processing methods in NMR-based metabolomics. Examples include the application of 5-min UF-COSY experiments to quantify metabolites in tomato extracts [47], the collection of a 2 min UF-COSY on a benchtop NMR to analyze edible oils [48] and the application of ultrafast 2D NMR to analyze pig serum samples [49]. Given their many potential benefits, the use of UF-NMR in metabolomics should increase in the very near future.

Because 2D NMR techniques use multiple pulses they tend to be more sensitive to pulse imperfections. This can lead to inconsistencies in terms of peak intensity and peak volume, thereby limiting the reliability of compound quantification. Recently it has been shown that it is possible to generate ^{13}C-HSQC data that are inherently quantitative. This can be done either by extrapolating the time point to zero, which is called the HSQC0 experiment (which requires three separate HSQC experiments) or with quantitative sequences such as Q-HSQC experiment (which requires four times more scans to achieve the same sensitivity as a normal HSQC) or the quicker variant of the Q-HSQC experiment called QQ-HSQC [50]. Still another quantitative heteronuclear experiment that shows good promise for metabolomics is the QUIPU HSQC [51]. These advances in quantitative 2D NMR techniques are encouraging developments for NMR-based metabolomics and it is likely that quantitative 2D NMR will become much more widely used by the NMR metabolomics community in the near future.

5. Metabolomic kits, services and systems

Metabolomics is evolving from a specialized field practiced by a small number of highly trained analytical chemists to a broadly accessible field occupied by biologists, clinicians, nutritionists, environmental scientists and other non-chemists. This trend has required that metabolomic assays (and equipment) become simpler, easier, more robust and more accessible. In the world of MS-based metabolomics this trend has led to the development of MS metabolomics kits (offered by Biocrates and MRM-Proteomics) and MS metabolomic services (provided by various companies and academic core labs). Not to be outdone, NMR-based metabolomics appears to be moving in the same direction.

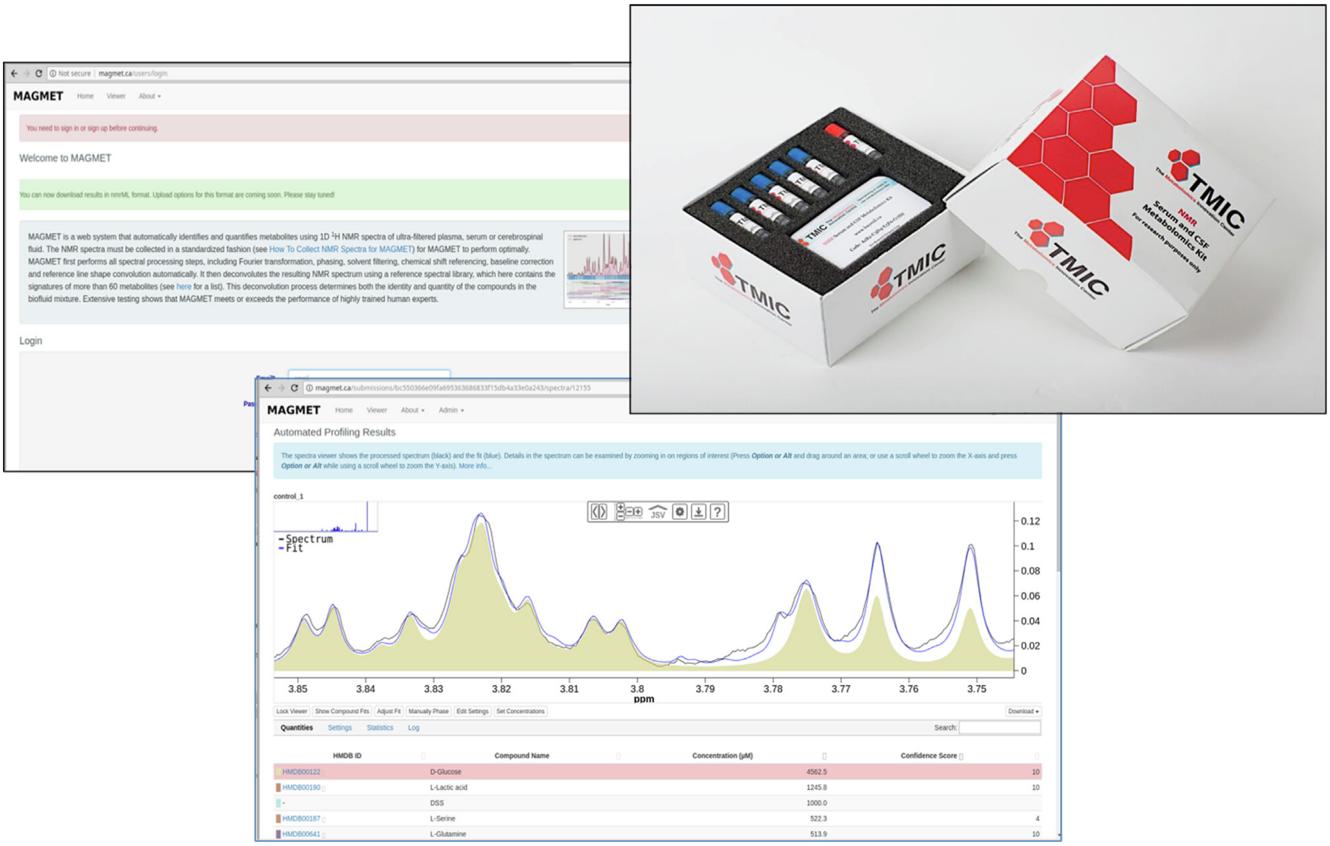
Over the past two years several labs, including my own laboratory, have been developing low-cost (or cost-recovery), vendor-agnostic NMR-based metabolomics kits. These kits typically include protocol booklets, chemical shift and phasing reagents, specially modified ultrafiltration devices and software to analyze serum samples at 500, 600 and 700 MHz (an example of one is shown in Fig. 1). Extensive testing at multiple sites around the world have shown that these kits offer significantly improved consistency over manual spectral deconvolution. Furthermore, they allow more than 55 serum metabolites to be automatically identified/quantified with just a few minutes of computer processing time. Other kinds of NMR kits are being developed to analyze different biofluids (saliva, fecal water, urine) and beverages (milk, wine, beer, juice, etc.). Given their many advantages, it is likely that NMR kits may become more widespread in the metabolomics community thereby ensuring that NMR-based metabolomics can become more consistent and robust from lab to lab and study to study.

Bruker has also moved towards NMR metabolomic kits and systems with its FoodScreener instruments and IVDr software systems. The Foodscreener is a dedicated spectrometer-software system (400 MHz) that comes with separate software modules to analyze wine, juice and/or honey. This dedicated metabolomic platform performs metabolite identification and quantification for up to 30 different metabolites and measures up to 60 different parameters (depending on the food type). It is specifically designed to assist with food and beverage testing, food authentication and the assessment of food adulteration. In addition to the FoodScreener, Bruker has also introduced the LISA-IVDr lipoprotein profiling system. This is a collection of software and protocols designed for 600 MHz NMR instruments that permits the measurement of lipoprotein particles (HDL, LDL, VLDL) in blood. While Bruker's approach to "kit-based" metabolomics is very expensive, the dedicated systems it has developed are robust, easy to use and well validated. Interestingly, Bruker is appears to be positioning NMR-based metabolomics to be used is ISO-certified or clinical environments. This will be an important and necessary step for NMR-based metabolomics if it is going to stay competitive with MS-based metabolomics over the coming decade.

One area where NMR-based metabolomics has far exceeded expectations has been in the provision of low cost metabolomic services, especially with regard to lipidomics or lipoprotein profiling. The current "darling" of the field is Nightingale Health, a Finnish company that offers inexpensive, automated, very high throughput (10,000+ samples) NMR-based lipoprotein profiling to users from around the world. The Nightingale NMR assay measures more than 220 lipid parameters (including 18 non-lipid metabolites) from a single blood sample. Prior to Nightingale, another NMR company, called LipoScience, dominated the lipoprotein profiling field. In fact LipoScience evolved lipoprotein profiling into such a profitable business that it was acquired by LabCorp in 2014. The LipoScience assay was actually the first NMR-based metabolomic assay to achieve clinical and FDA approval. Given the success that NMR has had in this area, it is likely that more services, more open source software and potentially even low cost kits will appear to make lipoprotein profiling even more accessible.

6. Software and database advances

While hardware and methodological advances are important for moving NMR metabolomics forward, so too are software and database advances. As noted earlier, the NMR metabolomics com-



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MAGMET is a web system that automatically identifies and quantifies metabolites using 1D ^{1}H NMR spectra of ultra-filtered plasma, serum or cerebrospinal fluid. The NMR spectra must be collected in a standardized fashion (see How To Collect NMR Spectra for MAGMET) for MAGMET to perform optimally. MAGMET first performs all spectral processing steps, including Fourier transformation, phasing, solvent filtering, chemical shift referencing, baseline correction and reference line shape convolution automatically. It then deconvolutes the resulting NMR spectrum using a reference spectral library, which here contains the signatures of more than 60 metabolites (see here for a list). This deconvolution process determines both the identity and quantity of the compounds in the biofluid mixture. Extensive testing shows that MAGMET meets or exceeds the performance of highly trained human experts.

Automated Profiling Results

The spectra viewer shows the processed spectrum (black) and the fit (blue). Details in the spectrum can be examined by zooming in on regions of interest (Press ***Option or Alt*** and drag around an area; or use a scroll wheel to zoom the X-axis and press ***Option or Alt*** while using a scroll wheel to zoom the Y-axis). More info...

HMDB ID	Compound Name	Concentration (µM)	Confidence Score
HMDB00122	D-Glucose	4562.5	10
HMDB00190	L-Lactic acid	1245.8	10
-	DSS	1000.0	
HMDB00187	L-Serine	522.3	4
HMDB00641	L-Glutamine	513.9	10

Fig. 1. A montage of the software (screenshots) and contents of a low-cost NMR metabolomics kit developed in the author's laboratory. The kit includes a protocol booklet, quality control reagents, chemical shift reference compounds, phasing reference compounds, ultrafiltration filters (not visible) and the software (called MagMet) for spectral deconvolution for 50 samples.

munity is currently struggling with tiny (<800 compound) databases of shareable NMR spectra. This greatly limits the utility of NMR spectral deconvolution software thereby reducing the ability of researchers to identify many compounds in common biofluids. The recent development of nmrML as a universal standard to exchange NMR spectra and NMR assignments may help address the problem of small NMR spectral databases [52]. In particular, nmrML should greatly increase the number of shareable NMR spectra for spectral comparison and deconvolution. This will allow everyone in the NMR metabolomics community to save or send their NMR spectra in a common format. The nmrML mark-up language is based on XML and is similar to the data exchange format now used by mass spectrometry (called mzML). Having common data exchange or data formatting standards will be critical to sustaining NMR-based metabolomics into the future.

An equally exciting development in the field of NMR concerns the recent call by the NIH for proposals to establish a sustainable, open access natural products NMR spectral database. The broad scope of what the NIH considers to be a natural product (i.e. any metabolite from plants, animals or microbes) indicates that when this database is eventually established in 2020 or 2021, it will also serve as a superb resource for the NMR-based metabolomics community. This dedicated NMR database is expected to house hundreds of thousands of referential 1D and 2D NMR spectra (covering many different types of NMR experiments) from 10s of thousands of primary and secondary metabolites. Having so many high quality NMR spectra available from a single, open-access source will fundamentally transform the field of NMR-based metabolomics.

Another area where software developments in metabolomics are likely to have a significant impact over the coming years is in the area of NMR spectral deconvolution. Spectral deconvolution involves the decomposition of mixture spectra into their individual pure compound components to allow individual metabolites to be identified and quantified. Spectral deconvolution has been used for many years in NMR metabolite imaging and classical NMR-based metabolomics [53]. Currently many NMR groups use manual or computer-aided methods that require manual phasing, water removal, baseline correction and manual spectral fitting [54,55]. More recently, new software programs have appeared that perform these steps automatically. These include Bayesil, BATMAN, AQuA, MagMet and rDolphin [56–59]. The development of automated deconvolution methods should make NMR-based metabolomics faster, more consistent and much more user-friendly. Some of these automated software tools are already being incorporated into low-cost NMR kits (Fig. 1). Given the paucity of free or open-source software for NMR-based metabolomics there are clearly plenty of opportunities for enterprising programmers or NMR spectroscopists to develop these much-needed tools. Indeed, many of the rapid advances seen in MS-based metabolomics have been due to advances in software development. The same should be true for NMR-based metabolomics.

7. Conclusion

A brief summary of the key advances in NMR hardware, methodology and software that are affecting, or will soon affect, NMR-based metabolomics is given in Table 2. While this table and its projections are somewhat speculative, it does provide a potential roadmap for the future of NMR-based metabolomics. One significant advance added at the bottom of this table is "Inte-

Table 2
Key NMR advances, their expected timeframes to widespread implementation and their predicted impact on metabolomics.

NMR Advance	Timeframe	Impact
Benchtop magnets for metabolomics	0–3 years	Moderate
Cyrogen-free high-field NMR magnets	2–5 years	Modest
Ultra-high field NMR (1.2 GHz)	2–5 years	Moderate
Natural abundance dissolution DNP	0–3 years	Moderate
Para-hydrogen hyperpolarization	2–5 years	Significant
Probe design improvements	0–10 years	Significant
Pulse sequence improvements	0–10 years	Moderate
Fast 2D NMR for metabolomics	0–3 years	Significant
Quantitative 2D NMR for metabolomics	0–3 years	Moderate
NMR metabolomic kits	0–3 years	Moderate
Dedicated NMR metabolomic systems	0–3 years	Modest
Low-cost NMR metabolomic services	0–3 years	Significant
Common NMR data exchange formats	2–5 years	Moderate
Large NMR spectral databases	2–5 years	Significant
Automated NMR deconvolution software	0–3 years	Significant
Improved NMR metabolomics software	0–10 years	Significant
Integrated NMR/MS techniques	0–5 years	Significant

grated NMR/MS techniques". While not discussed in this short review, these integrated techniques combine both NMR and MS or NMR, MS and HPLC to perform high-throughput metabolomics or metabolite identification [60,61]. Combined NMR/MS techniques build on the respective strengths of NMR and MS and have shown considerable promise in the rapid identification of unknown metabolites [62]. They also highlight the potential benefits of technological cooperation over technological "conflict".

No doubt many other unanticipated innovations in NMR instrumentation, spectral collection or in data analysis will certainly occur over the coming years and these are obviously not listed. Indeed, anyone who has been in the NMR field for more than a few years quickly learns to never underestimate the potential of NMR spectroscopists to come of up with some remarkable, paradigm-changing innovations. It is probably fair to expect that the next 20 years of NMR-based metabolomics will be as interesting and fruitful as the past 20 years.

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