Parallel Nuclear Magnetic Resonance (NMR) Spectroscopy

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Abstract

Nuclear Magnetic Resonance (NMR) spectroscopy is a principal analytical technique used for the structure elucidation of molecules. This Primer covers different approaches to accelerate data acquisition and increase sensitivity of NMR measurements through parallelisation, enabled by hardware design and/or pulse sequence development. Starting with hardware-based methods, we discuss coupling multiple detectors to multiple samples so each detector/sample combination provides unique information. We then cover spatiotemporal encoding, which uses external field gradients and frequency selective manipulations to parallelize multi-dimensional acquisition and compress it into a single-shot. The parallel manipulation of different magnetisation reservoirs within a sample is then considered, yielding new, information-rich pulse schemes using either homo- or multinuclear detection. The Experimentation section describes the setup of parallel NMR techniques. Practical examples revealing improvements in speed and sensitivity offered by the parallel methods are demonstrated in the Results section. Examples of use of parallelization in small molecule analysis are discussed in the Applications section with experimental constraints addressed under the Limitation, Optimisations, and Reproducibility and Data Deposition sections. The most promising future developments are considered in the Outlook section, where the largest gains are expected to emerge once the discussed techniques are combined.

Introduction

The separation of a large task into multiple segments that can be tackled simultaneously has been exploited in many fields of science and technology. For example, parallel genome sequencing has reduced long-term cost and increased throughput by more than three orders of magnitude[3]. Similar developments in magnetic resonance imaging (MRI) have provided great increases in sensitivity and faster image acquisition[4-6] via multiple receiver coils and detector channels. In MRI, an image is formed from the water protons in tissue, with magnetic field gradients being applied to impose a unique combination of precessional **[G]** phase and frequency at each position within the image. By using multiple receive coils, the unique spatial profiles of each of the coils can be used to reduce the requirements for gradient-based spatial encoding **[G]**, enabling under-sampling of reciprocal space, and increasing imaging speed without compromising the image quality. These examples have served as the inspiration for similar techniques in nuclear magnetic resonance (NMR) spectroscopy, which we broadly term parallel NMR spectroscopy.

NMR spectroscopy, in its simplest form, is the measurement of energy differences between spin states of nuclei with nonzero spin induced by external or internal magnetic fields. Due to differences in their surrounding chemical environments, each nucleus in a molecule will have characteristic energy differences, which are often expressed in terms of resonance frequencies or chemical shifts **[G]**. NMR can also detect magnetic interactions between nuclear spins via either through-bond (scalar) or through-space (dipolar) coupling interactions, which can be directly correlated with molecular structure.

Routine NMR applications typically involve spin-½ nuclei commonly found in organic compounds and biomolecules, such as ^{1}H, ^{13}C, ^{15}N, ^{19}F, and ^{31}P. Of these, ^{1}H, ^{19}F, and ^{31}P are particularly important as their high natural abundance and magnetogyric ratio **[G]**, γ provide greater detection sensitivity. One-dimensional (1D) NMR experiments directly measure the chemical shifts of and couplings between these nuclei, which provide direct information about their chemical environments. More intricate multidimensional (*n*D) NMR experiments use separate spectral dimensions **[G],** usually for correlating different nuclei in a molecule. In an *n*D pulse sequence, each spectral dimension corresponds to a time period in which chemical shifts and spin-spin couplings are allowed to evolve. These are interspersed with mixing periods that transfer magnetisation from one nucleus to other adjacent nuclei. Different forms of mixing enable the identification of different forms of molecular connectivity (see BOX 1 for examples). In *n*D spectra, a total of $n - 1$ evolution periods must be independently incremented, leading to long experiment times.

In this Primer, we describe various parallelization techniques that allow the concurrent acquisition of different signals to reduce the time needed for collection of *n*D NMR data. The small energy differences between nuclear spin states make NMR spectroscopy an inherently low-sensitivity method. We show that the parallelization techniques offer new ways of increasing the sensitivity of NMR experiments.

The first parallel NMR technique covered in this Primer is the use of multiple radiofrequency (RF) microcoils in NMR[7]. Microcoils, which have dimensions of a millimeter or less, are typically used for high-sensitivity NMR of mass-limited samples **[G]** such as small proteins[8]. For parallel NMR, multiple microcoils are fitted within the usable volume of the NMR magnet, and many (different) samples are then inserted in the bore of the NMR magnet. The aim is to use one coil per sample and acquire all sample signals simultaneously. Another mode of operation is to use multiple microcoils on a single sample which has been split into different aliquots, a process that can significantly reduce total data acquisition times in nD

NMR spectroscopy. In order to process the data from separate coils, they must first be aligned in time by a suitable phase shift **[G]** and then any slight differences in coil sensitivity corrected by normalization.

This concept of physically separating a sample into sub-samples studied by individual coils has a natural link to the next parallel technique, spatially-encoded ultrafast NMR. In ultrafast NMR experiments, the sample is effectively sectioned by using magnetic field gradients[11-15], which spread the frequencies of the spins in the sample according to their physical positions. When combined with frequency-selective pulses, this allows a 2D NMR (or pseudo-2D NMR; e.g., relaxation, diffusion) experiments to be performed in a single transient **[G]** instead of multiple scans with different time increments. The ultrafast approach to 2D NMR thus endows different spatial "bins" with what amounts to different time evolutions. This "binning" manipulation can be executed in a single shot and is compatible with either homo- or heteronuclear 2D correlation pulse sequences (COSY, TOCSY, HSQC, HMQC, see also BOX 1). Thus ultrafast NMR enables the collection of complete multidimensional NMR spectral sets with orders-of-magnitude shorter timeframes than conventional techniques, and couples well with measurements that are incompatible with prolonged acquisition times, such as experiments involving hyperpolarized nuclear states in dynamic nuclear polarization **[G]**.[16]

The introduction of multiple receivers in NMR spectroscopy, also adopted from MRI, has led to the development of new types of experiments involving simultaneous detection of free induction decays **[G]** (FIDs) from several nuclear species[17]. This allows the design of more efficient pulse schemes and supersequences **[G]**. Furthermore, experiments with simultaneous detection of high abundance, high γ nuclei, such as ^{1}H and ^{19}F, double the amount of detected signal in direct and indirect detection experiments and improve the sensitivity per unit of measurement time[18].

Finally, we describe recent approaches to data acquisition that provide time-efficient 2D NMR data collection by strategically sampling separate pools of magnetization **[G]** available within samples whilst storing others for subsequent collection in the same experiment[19,20]. This tailored sampling of magnetization reservoirs manipulated in parallel yields multiple 2D datasets from a single experiment. One parallelized experiment can thus provide a complete set of through-bond and through-space correlations within molecular structures, which may then be combined to yield complete molecular connectivity and three-dimensional structures. We describe various approaches to parallelized sampling that use the direct acquisition of proton magnetization reservoirs for maximum sensitivity and that can be collected using conventional spectrometer hardware. These include time-shared experiments, experiments that exploit differing magnetization transfer pathways, and the modular NOAH supersequences. The time-efficiency of the methods will be exemplified by the elucidation of complete structures from single multi-FID experiments.

All of the aforementioned examples of NMR parallelization serve to greatly reduce data acquisition times, which can frequently be translated into associated gains in sensitivity per unit time. Along with the fundamental concepts of each of these techniques, we will show selected examples of obtained spectra and consider their application in a variety of contexts where NMR is already used routinely. We end by briefly covering a few key points about experiment reproducibility, limitations and potential avenues for future improvement.

Experimentation

Generally, NMR is considered to be a low sensitivity technique. The two main approaches to tackle this problem are improvements in instrumentation and methodology developments. Parallelization of NMR involves both of these approaches. We therefore start this section by briefly introducing the sensitivity aspects of NMR measurements, focussing on NMR probes. This is followed by instrumentation used in parallel NMR such as probes with multiple microcoils and use of multiple receivers. We then describe experimental setups and essential considerations for parallelization methods based on simultaneous detection of multiple FID-s, spatiotemporal encoding and ultrafast NMR spectroscopy.

NMR Probes and Sensitivity

The sensitivity of routine NMR experiments largely depends on the available probe configurations and the magnetic field strength B_0. Nuclei with high γ and high natural abundance (^{1}H, ^{19}F, ^{31}P) offer the highest sensitivity, while magnetically diluted and low γ spins (^{13}C, ^{15}N, ^{29}Si) are typically observed indirectly, via high γ abundant nuclei (see Table 1). In experiments involving polarization transfer **[G]** between nuclei the signal to noise ratio (*S/N*) can be defined as:

$$S/N \propto n\gamma_e\sqrt{\gamma_d^3 B_0^3 T_{exp}} \qquad (1)$$

where *n* is number of observed spins, T_{exp} is the experiment time, γ_e and γ_d are magnetogyric ratios of the excited and detected spins respectively, and B_0 is the magnetic field strength[21,22]. The highest commercially available magnetic field strength suitable for NMR is currently 28.2 T, corresponding to a ^{1}H resonance frequency of 1.2 GHz. Larger magnetic fields allow greater sensitivity (Eq. 1) as well as improved peak dispersion in NMR spectra. However, due to the large cost of purchasing and installing very high-field spectrometers, routine small molecule experiments are typically conducted on 400 – 700 MHz systems.

Cryogenic probes[23], while still expensive, offer a considerably cheaper way to improve the sensitivity of measurements relative to the cost of increasing B_0. In most commercial NMR probes there are typically two nested RF coils that are tuned to the NMR frequencies of various nuclear species. The sensitivity depends on the probe filling factor (the fraction of the coil's active volume that is filled with sample) and configuration of the RF coils. The inner coil is closer to the sample and therefore offers better sensitivity. It is usually tuned to ^{1}H to maximize the sensitivity in most routine experiments. The less sensitive outer coil is used primarily for indirect detection of other nuclear species (the "hetero nuclei") via pulsing and decoupling. This coil is typically tuneable to either a wide range of frequencies (broadband probes) or a set of two or three fixed resonance frequencies of the most commonly observed nuclei in organic molecules and biomolecules such as ^{13}C, ^{15}N, ^{19}F or ^{31}P. Indirect-detection cryoprobes, for example, are tuned to ^{1}H (inner coil), and ^{13}C and ^{15}N (outer coil). In direct detection probes, the coil configuration is reversed to maximize the sensitivity of experiments involving direct detection of hetero nuclei.

Probes dedicated to detection of a single nuclear species, such as cryoprobes optimized for ^{13}C detection, offer the highest sensitivity. Larger diameter probes accommodating increasing sample volumes increase *n* in Eq. 1 and result in further improvement of S/N, with direct detection probes designed for 10 mm, 8 mm and, most commonly, 5 mm diameter sample tubes. Small diameter probes can significantly reduce the amount of sample necessary for measurements, thereby increasing the sensitivity of mass-limited **[G]** samples [24]. For example, on a 600 MHz instrument equipped with a 1.7 mm inverse detection cryoprobe, 10 µg of substance is sufficient for detecting ^{13}C–^{15}N correlations at the natural abundance of isotopes (ca. one ^{13}C–^{15}N pair in 25000 molecules)[25]. Likewise, the 3D structure of a protein has been determined using a 6 µL sample of 1.4 mM 68-residue protein and a 1 mm microcoil NMR probe[26].

For low-concentration samples, sensitivity is a crucial issue, and typically a large number of transients are required to obtain spectra of acceptable quality. In this sensitivity-limited regime, parallelized experiments are useful only when a gain in sensitivity per unit time can be achieved, as this means that less time is required to reach the S/N threshold. In this respect it is important to note that the sampling of indirect domains in nD NMR experiments involves a sort of signal averaging - a summation which becomes evident upon performing the full Fourier analysis of the data. On the other hand, the primary constraint for more concentrated samples is spectral resolution, particularly in indirect dimensions. In this resolution-limited regime, the time savings afforded by parallelized experiments can be fully exploited, as any moderate decreases in S/N resulting from the shorter experiments are easily tolerated. For mass-limited samples, high detection sensitivity will therefore prove to be advantageous as they allow one to move from the sensitivity-limited to the resolution-limited regime, with probes optimized for proton detection and/or cryogenic probes being the most beneficial.

NMR with multiple microcoils

There are two hardware-enabled approaches to parallel NMR with multiple microcoils: the first uses multiple coils that are connected in parallel, with the combination being impedance matched by a single electronic circuit and fed into a single receiver via a single coaxial cable to the NMR spectrometer (FIG. 1). This has the advantage of electrical simplicity, but since noise for microcoils is coil-dominated, the overall noise contribution is higher than that for a single microcoil. In parallel configuration the signals from each sample are separated through the use of spatially-selective pulse sequences using a frequency-selective pulse[27-30]. The sequence in FIG. 1b used to acquire the data employs a frequency-selective Gaussian pulse: the frequency offset of this pulse selects one of the four samples shown in the inset of FIG. 1a. If a conventional hard pulse is used then the signals from all the samples are acquired at once (FIG. 1c). By selecting only the relevant frequency range (FIG. 1d) the signal from only one of the samples at a time can be acquired (FIG. 1e).

Approaches using magnetic field gradients in combination with frequency-selective pulses for a single sample in a single coil have been extensively reviewed.[31] These techniques have the advantage of requiring no additional hardware to a standard NMR spectrometer, and in cases where S/N is high enough these methods can significantly increase the rate of data acquisition, such as for efficient T_1 measurements,[32] diffusion ordered spectroscopy (DOSY) experiments,[33] and homonuclear broadband decoupling.[34].

The second approach is to separate each of the coils for each to have their own impedance matching circuit, which gives optimal S/N[35-39]. This increases the complexity of the electrical circuitry: although NMR systems may have multiple receive channels, there is typically only one transmit channel for each nucleus, and so pulse transmission must be switched between the coils (FIG. 2). The major challenge with multiple microcoils is to achieve electrical isolation between the coils so that neither signal nor noise is transferred between coils, and to ensure that B_0 is sufficiently homogeneous over each of the samples to achieve high spectral resolution. Provided that the physical separation between coils is sufficiently large compared to the size of the coil, high isolation can be achieved. If this condition is not met, then small shielding conductors can be placed between coils. The B_0 homogeneity is further improved by placing magnetic susceptibility matching fluid around each of the microcoils. This enables to resolve scalar-coupled multiplets and resonances with close chemical shift values.

There are different types of parallel NMR experiments. The simplest is to run several samples at once, with one sample associated with one microcoil, to increase throughput. Alternatively, a single sample can be split into different aliquots and flowed into each of the detector coils and different sequences, or different components of sequences, run simultaneously. For example, a COSY spectrum might be acquired with coil 1, a TOCSY with coil 2, an HSQC with coil 3 etc.; or the first *n* time increments in the indirectly detected domain (t_1 increments) of a TOCSY on coil 1, the second n t_1-increments on coil 2 etc. When the data from all coils is concatenated, it yields a dataset with the total number of required t_1-increments acquired in a time reduced by a factor of the number of coils. Both these types of experiment have been shown using two, four and eight RF coils, for proton high resolution spectroscopy of small molecules and multinuclear spectroscopy of small proteins[35-37].

Single Scan nD NMR

The concept of physically separating a sample into sub-samples studied by individual coils can be notionally extended to sectioning a sample into multiple emitters using magnetic field gradients. This is the idea underlying ultrafast multidimensional NMR, where magnetic field gradients and frequency selective pulses are combined to distinguish different positions within a sample[13,42,43]. This idea is easiest to visualize when considering a 2D NMR acquisition, where $S(t_1,t_2)$ signals are collected within the framework of the Jeener-Ernst classical scheme[44,45]:

$$\text{Relaxation/Preparation – Evolution } (t_1) \text{ – Mixing – Detection } (t_2) \tag{2}$$

Here the preparation and the mixing events remain constant, while signals are collected by changing the parameter t_1 through a series of independent scans. This leads to a collection of signals depending on two time variables t_1 and t_2. Instead of considering a t_1 time evolution parameter that is incremented scan-by-scan to monitor the frequencies $I(\Omega_1)$ making up an indirect-domain spectrum, ultrafast 2D NMR ports the encoding along a spatial direction as illustrated in FIG 3.

FIG.3 (*a*) shows the classical scheme of a 2D experiment, where the duration of t_1 is changed from scan-to-scan over *N1* increments for all spins in the sample, taken as one homogeneous set. FIG 3 (b) shows the spatiotemporally encoded scheme, where different positions along the sample's z-axis B_0 are first endowed with different evolution times as a function of their position within the NMR tube (referred to as the "encoding" process), and then acquired in a spatially-resolved fashion as a function of t_2 (referred to as the " decoding" process). Ultrafast 2D NMR executes both the encoding and the decoding process within a single scan, by using magnetic field gradients. Such fields are used during the evolution to address spins positioned at different z coordinates, and impart an evolution time t_1 that is proportional to their positions. As further detailed in the Supplementary Information, a unique feature of this kind of spatiotemporal (z/t_1) encoding, is the creation of magnetization helices - patterns of magnetization, where the pitch of the helices has encoded the indirect-domain interaction. Ultrafast 2D NMR then uses the echo-planar spectroscopic imaging (EPSI) block [46] over the course of the acquisition, for collecting the 2D NMR data. EPSI views its signal as a function of the direct-domain acquisition time t_2, and as function of a variable k:

$$k = \gamma \int_0^{t_2} G_a(t')dt' \qquad (3)$$

where k describes the evolution imposed by a linear magnetic field gradient. In EPSI, k oscillates back-and-forth by periodically reversing the sign of the acquisition gradient G_a. Fourier analysis vs. t_2 provides the equivalent of a conventional, directly-detected NMR spectrum; for each direct-domain peak, k then maps the frequencies that acted during the initial t_1 evolution. This is done by "unwinding" the aforementioned magnetization helices, leading to indirect-domain peaks that will thus show up as echoes **[G]**. The k-axis then becomes equivalent to the indirect F_1 frequency axis in 2D NMR; as a result of this, there is no need to perform an explicit Fourier transform to retrieve an indirect-domain spectrum that has been spatiotemporally encoded. This type of single-shot 2D acquisitions only requires splicing of the single string of collected EPSI data into a 2D array, rearrangement of this 2D array in a bi-dimensional space according to each data point's $(k/F_1,t_2)$ coordinate, and a final 1D Fourier transform along the directly-detected t_2 axis to transform these time evolutions into frequencies along the second F_2 domain.

This single-scan parallelization of Jeener-Ernst's 2D NMR scheme (*Eq.* 2) does not need to be circumscribed to one indirect domain: multiple gradients can be used to encode multiple independent dimensions; x, y, and z gradients for instance have been used simultaneously to coalesce a four-dimensional NMR experiment into a single scan[43]. Furthermore, as long as samples are spatially homogeneous, the same strategy can be used to encode incoherent processes such as spin-lattice relaxation behaviour or diffusivity[31-33,47-49]. Somewhat related to this topic but outside the bounds of this survey, is the use of gradients and frequency selective pulses for achieving homonuclear broadband decoupling.[34] Last but not least, this spatiotemporal encoding strategy arguably sees its most widespread applications in cases of heterogeneous samples, where it has been used to deliver single-shot multidimensional MRI images and multidimensional spectral images with unprecedented speed and robustness[50-55].

Homo-nuclear Multi-FID detection

Biomolecular NMR studies typically involve low concentration samples (≤ 1 mM) that are routinely enriched in ^{13}C (natural abundance = 1.1%) and ^{15}N (natural abundance = 0.37%) to improve measurement sensitivity.In contrast, in small molecule research, more concentrated samples are usually available and sample enrichment is considered too expensive or impractical for routine applications. In this section, we will focus on small molecule NMR, which is typically performed at natural isotopic abundance.

In a given sample, there typically exist multiple magnetization pools that can be manipulated selectively since they originate from different nuclear spins (FIG. 4a). For example, protons directly bound to ^{13}C can be distinguished from other protons on the basis of their large one-bond C–H coupling using pulse sequence elements such as BIRD pulses[56], TANGO/BANGO elements[57,58] or zz-filters[59,60]. The development of parallelized *n*D experiments relies on the concurrent manipulation of multiple magnetization pools such that the respective signals arising from each pool may be detected without the conventional recovery delay **[G]** (d_1 in FIG. 4) between individual pulse schemes (FIG. 4b). The recovery delay is typically the longest time period in any pulse sequence and significant time can be saved by reducing the number of these delays during data collection. This can be done even on basic desktop spectrometers equipped with only a single receiver and no gradients (see Supplementary Figure 2).

The signals from each magnetization pool can be collected either simultaneously as part of one FID or sequentially in a multiple-FID experiment. Simultaneous acquisition is exemplified by time-shared experiments[61], in which multiple indirect-dimension frequencies (typically ^{15}N and ^{13}C) are encoded and then transferred back to ^{1}H for detection (FIG. 4c). Each resulting FID is a sum of the component signals, and direct Fourier transformation will yield a spectrum containing signals from every magnetization pool sampled. These signals can be separated using nucleus editing **[G]** [62], following which addition and subtraction of the phase-labelled datasets yield spectra containing only one signal.

Sequential acquisition is typified by NOAH methods—experiments in which signals from different magnetisation pools are consecutively sampled without intervening relaxation delays. NOAH supersequences are constructed by direct concatenation of suitably tailored modules which sample only their respective magnetization pools (FIG. 4d)[20,59,60,63-65], with each module designed to return other magnetization pools to equilibrium (+*z*). In these experiments, the FIDs are acquired independently and are initially stored in a file partitioned into multiple memory blocks. These blocks may be separated such that each experiment has its own matrix of raw data, after which processing takes place as usual. These procedures, which can generate multiple datasets from one experiment (up to five in the case of NOAH to date[20],) are typically invisible to the end user as they are executed by automated processing scripts. These scripts also make it possible to run these experiments under automation, which is essential for optimizing throughput.

An alternative strategy for sequential acquisition is polarization sharing, where a magnetisation pool is divided into multiple portions that are independently manipulated and detected. This is most readily achieved by the PEP (Preservation of Equivalent Pathways) scheme[66] employed, for example, in HSQC[67] and TOCSY[68] experiments, where following t_1 evolution there are orthogonal (i.e. cosine- and sine-modulated) components that can be separately manipulated[69-71]. In a TOCSY experiment, these components can be subjected to different durations of isotropic mixing **[G]** , thereby yielding two TOCSY spectra from one experiment displaying a different set of correlations[69]. Likewise, the two HSQC pathways can be used to separately generate HSQC spectra with different F_1 spectral widths[71], both coupled and decoupled HSQCs, or HSQC and HSQC-TOCSY spectra from the respective components[69]. Alternatively, pulse sequence elements such as BANGO can be used to excite only a portion of magnetisation for one module[57,58], retaining the remainder for use in a subsequent module[72]. It should be noted that all such polarization sharing methods suffer from a decreased sensitivity. Nonetheless, the time savings still represent a substantial benefit for samples that are not sensitivity-limited.

Lastly, a magnetisation pool can also be sequentially sampled by subjecting magnetization that has already been detected once to another mixing process before sampling it again. For example, the HMBC **[G]** experiment ordinarily records correlations between ^{13}C and ^{1}H nuclei separated by 2–3 bonds. Appending a COSY mixing period after the HMBC acquisition transfers magnetization from ^{1}H nuclei to other ^{1}H spins coupled to it; this yields a HMBC-COSY spectrum where ^{13}C nuclei are correlated with precisely those coupled partners, which are typically 3–4 bonds away from the original ^{13}C nuclei. Applying such mixing periods more than once opens up novel possibilities such as HMQC **[G]** - or HMBC-COSY relay chains for stepwise detection of through-bond correlations[73]. Such strategies can be further combined with time-sharing principles to create even more information-rich experiments[19].

All of these parallelized experiments share one major feature in common: they enable the collection of multiple 2D spectra with a single recovery delay (compare FIG. 4b–d), thus providing significant reductions in the time needed to obtain all the constituent spectra. Furthermore, as long as the S/N in the parallelized sequence is not excessively compromised, these time savings can also be translated into an overall gain in sensitivity per unit time, as they allow more transients to be collected in the same duration.

Multi-nuclear detection experiments

Basic multi-nuclear detection techniques

Typical NMR systems are designed with multi-nuclear functionality in mind. For instance, most commercial probes are built to allow simultaneous irradiation and detection of up to five nuclei simultaneously. However, until recently commercial NMR consoles were equipped with only a single receiver and for sensitivity reasons most NMR experiments were designed to involve ^{1}H detection. However, sensitivity permitting, direct detection of hetero-nuclei (X) has important advantages such as better resolution[74] and advantageous relaxation properties[75]. Furthermore, cryogenic high temperature superconductor probes optimized for direct detection of hetero-nuclei can potentially increase the sensitivity of X-detected experiments by a factor of more than ten[76,77]. Together with the advent of commercial multiple receiver NMR systems, this has prompted the development and routine use of multi-nuclear detected experiments.

Three basic types of multi-nuclear data acquisition techniques are shown in FIG. 5a-c: parallel, interleaved, and sequential acquisitions, each with their own pulse schemes. The parallel acquisition pulse schemes or PANSY (Parallel Acquisition Nmr SpectroscopY)[17] typically involve polarization transfer from more sensitive nuclei (usually ^{1}H) to less sensitive nuclei (^{13}C, ^{15}N, ^{19}F, ^{31}P and similar). In the conventional COSY experiment, one of the two orthogonal components of magnetization is discarded[78]; however in the PANSY-COSY pulse scheme, it is transferred to other nuclear species for detection, which increases the amount of observed magnetization. Mutual decoupling of two nuclear species during parallel acquisition is not feasible and is mainly applicable in situations where such decoupling is not essential, for instance if the mutual scalar couplings are unresolved and can be neglected. The repetition rate of the PANSY experiments is determined by the recovery time of the high γ nuclei that typically serve as the polarization **[G]** source and usually have the shortest recovery time. However, this is not always the case. For instance, despite lower γ, the ^{19}F nuclei often have shorter recovery times as compared to ^{1}H due to more efficient relaxation mechanisms[79].

Parallel acquisition experiments may not involve polarization transfer at all, for example if there is no scalar coupling between nuclear species of interest or in parallel relaxation measurements, diffusion measurements and similar pulse schemes with no polarization transfer between the nuclei of interest[18]. In such experiments the repetition rate is determined by the slowest relaxing nuclear species.

The interleaved experiments (see FIG. 5b) are easy to design and are constructed simply by placing one of the two (or more) experiments into the recovery delay of the other experiment (see also FIG. 2b)[80,81]. In practice the standard pulse programs are simply concatenated with minor adjustments. Since the interleaved pulse sequences are cyclic, it does not matter which of the experiments is executed first. In this way both recovery periods are used to record additional data. In practice the total duration of the pulse sequence, τ_{pp} and the data acquisition period, τ_{aq} for 2D experiments is considerably shorter (*ca* 100 ms) compared to a typical recovery period, d_1 (> 1 s). If the recovery periods of the two nuclear species are very different, it may be possible to improve the efficiency of the interleaved experiments by placing several experiments involving fast relaxing nuclei in the recovery delay of slow relaxing nuclei[18]. Any disturbance of spins that are recovering must be avoided in order to preserve speed and sensitivity advantages.

For sequential detection experiments (See FIG. 5c) the pulse schemes involving sequential acquisition usually begin with transfer of otherwise unused magnetization from the more sensitive nuclei to the less sensitive nuclear species of interest. In addition to enhancing sensitivity this often also reduces the experiment repetition rate determined by the typically shorter recovery times of the sensitive high γ nuclei. In these pulse schemes, one of the spectra is acquired during a long evolution period within the specially-designed pulse sequence. Following the first acquisition period the second part of the experiment is then completed and the second FID is acquired. For example, the 2D H-C HETCOR **[G]** spectrum can be recorded during the mixing period of the 2D H-H TOCSY experiment[17]. In this experiment the isotropic H-H mixing sequence of the TOCSY pulse scheme works as a composite pulse decoupling for the HETCOR scheme to produce a fully decoupled HETCOR spectrum (see FIG. 5c). Once the HETCOR data are acquired the final part of the TOCSY experiment is executed. Similar techniques have been employed in biomolecular NMR where magnetization of one of the experiments is stored on slowly relaxing nuclei (for example ^{15}N) during the acquisition period of an alternative experiment[82,83].

Combining techniques with direct multi-nuclear detection.

Experiments involving direct detection of multiple FID-s may combine several basic techniques. For example, the COSY/PANSY-COSY scheme shown in FIG. 5d combines parallel and interleaved acquisition methods. This experiment records three 2D spectra (H-H COSY, P-P COSY and P-H COSY) in the time of one conventional 2D COSY experiment. Likewise, experiments of different dimensionality can be combined using the techniques discussed above. For example, the 2D HETCOR / TOCSY pulse scheme has been modified to allow the recording of 1D ^{13}C spectrum of non-protonated carbons in parallel with the 2D H-C HETCOR using the time-shared acquisition technique[84]. Similarly, the sequential 2D HETCOR / TOCSY experiment has been extended to acquire 2D H-H TOCSY and 3D H-C HSQC-TOCSY spectra in a time-shared manner (FIG. 5e)[85,86]. In Fig 5e, a polarization sharing scheme is used to split the ^{1}H-^{13}C magnetization between the HETCOR and HSQC-TOCSY pulse schemes sacrificing some of the sensitivity advantage. Projection spectroscopy is employed to acquire tilted 2D projections of the 3D HSQC-TOCSY experiment and significantly reduces the experiment time[87,88].

The PANACEA **[G]** experiment combines the 2D ^{13}C-detected INADEQUATE **[G]** with ^{1}H-detected

2D HSQC and 2D or 3D *J*-HMBC into a single supersequence[89]. The block diagram of the basic PANACEA pulse scheme is shown in FIG. 5f. It is based on sequential acquisition of ^{13}C and ^{1}H detected spectra. The PANACEA supersequence is built around the 2D C-C INADEQUATE pulse scheme, which provides one bond C-C connectivities[90,91]. While the 2D INADEQUATE experiment is the least sensitive in the PANACEA supersequence because it is designed to detect pairs of directly bound ^{13}C nuclei with naturally low abundance (ca 0.012 %), it is one of the most powerful tools for structure elucidation of small organic molecules as it traces down the skeleton of organic molecules. Usually, 99% of the bulk ^{13}C magnetization from molecules containing a single ^{13}C isotope is destroyed in the conventional INADEQUATE pulse schemes. In the PANACEA experiment, however, this magnetization is recorded in a time-shared manner to produce a 1D ^{13}C spectrum and then further used in a 2D ^{13}C-^{1}H HSQC experiment acquired sequentially that exploits the high sensitivity of ^{1}H detection. Due to the low sensitivity of the INADEQUATE scheme, highly-concentrated samples are needed. Consequently, the HSQC experiment can be recorded with a very few scans. Once the HSQC spectrum is acquired the ^{13}C decoupling is switched off and the HSQC pulse scheme is replaced with the HMBC sequence. The INADEQUATE spectrum typically requires a significant (often >64) number of scans. This can be exploited to record up to three HSQC spectra separating CH, CH_2 and CH_3 resonances. Several HMBC spectra may be recorded to cover a rather large spread of the long-range ^{1}H-^{13}C couplings. Alternatively, a 3D C-H J-HMBC spectrum is acquired. Thus, the sensitivities of individual experiments are balanced by adjusting their acquisition times – less time is used to record the more sensitive spectra. Consequently, in a single measurement the basic PANACEA experiment delivers 1D ^{13}C, 2D C-C INADEQUATE, three multiplicity edited 2D H-C HSQC spectra and several 2D H-C HMBC spectra or a 3D J-HMBC spectrum.

The 1D ^{13}C spectrum can also be used to eliminate spectral distortions caused by environmental instabilities, such as temperature variations and magnetic field fluctuations. This allows recording of the PANACEA spectra in pure liquids and eliminates the need for deuterated solvents[77]. In concentrated samples, such as cholesterol (1 M in $CDCl_3$) the basic PANACEA can be recorded in as little as 20 minutes. Sensitivity permitting, the experiment duration can be further reduced to just 56 seconds by exploiting Hadamard encoding[92]. Together with Hadamard encoding, spectral aliasing[93] has also been used to further reduce the measurement time. The extended version of the PANACEA experiment (FIG. 5g) involves recording of 1D ^{15}N spectrum in parallel with the ^{13}C INADEQUATE followed by time-shared acquisition of the ^{15}N HSQC and HMBC spectra in parallel with the corresponding ^{13}C spectra delivering 11 spectra in a single measurement.

The PANACEA experiment has been modified to replace the ^{13}C-based pulse schemes with their ^{29}Si analogues for studies of silicon oils[94]. Since there are no Si-H bonds in silicone oils, the HMBC module is discarded and the HSQC module is optimized for long range ^{1}H-^{29}Si couplings.

Results

The most significant use of 2D NMR spectra remains the structural verification and elucidation of small molecules via hetero- and homonuclear correlation experiments, such as those summarised in BOX 1. Accelerated data acquisition and the recording of multiple experiments offered by parallel NMR techniques enable a rapid identification of spin correlations within a molecular structure (yielding connectivity) and spatial proximity between nuclei (defining stereochemistry) following conventional data analyses procedures[95,96].

Parallel NMR with multiple microcoils

The electronic setup shown in FIG 2 has been used with an eight coil probehead to acquire high resolution 2D proton spectra from small molecules. Automatic shimming on the central two coils was used as a starting point, and then manual shimming used for fine adjustment to determine a “universal shim setting” that produces linewidths **[G]** in the low-Hz range for each of the samples. With careful construction, the 90° pulse durations for each coil were essentially identical.

COSY, TOCSY and gradient COSY spectra from sucrose, galactose, arginine, chloroquine, cysteine, caffeine, fructose and glycine samples, one of each in each coil, are shown in Table 2. Providing that the data from each coil are stored separately, then standard data processing can be applied, i.e. zero-filling, filtered with an appropriate window function, symmetrized and displayed in either magnitude or phase mode[95].

Homo-nuclear multi-FID detection

Advantages of multi-FID detection.

The duration of a typical NMR sequence T_{exp} is generally given by:

$$T_{\mathrm{exp}} = N(d_1 + \tau_{\mathrm{pp}} + \tau_{\mathrm{aq}}), \tag{4}$$

where τ_{pp} is the duration of the pulse program, τ_{aq} is the data acquisition (FID) time, d_1 is the recovery delay and N is the total number of scans. In nD experiments, the pulse sequence duration τ_{pp} is typically a few milliseconds and usually is the shortest of the three components of Eq. 4, except in NOESY **[G]** , ROESY **[G]** or TOCSY experiments with long mixing periods. The acquisition time, τ_{aq} is the next-shortest component (ca. 100 ms), and the recovery delay, d_1 is the longest pulse sequence element (typically 1–5 s).

In parallel NMR spectroscopy that incorporates M conventional experiments, the time savings provided by multi-FID experiments are defined by the ratio ρ_t:

$$\rho_t = \frac{\sum_{i=1}^{M} T_{\mathrm{exp}}(i)}{T_{\mathrm{exp}}(\mathrm{MF})} = \frac{\sum_{i=1}^{M}[d_1(i) + \tau_{\mathrm{pp}}(i) + \tau_{\mathrm{aq}}(i)]}{T_{\mathrm{exp}}(\mathrm{MF})} \tag{5}$$

where, $T_{\mathrm{exp}}(i)$ is the duration of the i-th conventional experiment, and $T_{\mathrm{exp}}(\mathrm{MF})$ is the duration of the multi-FID experiment. Since the speedup arises from the elimination of recovery delays, the value of ρ_t depends on how significant these delays are as a proportion of total experimental time. In the limit where τ_{pp} and τ_{aq} are negligible compared to d_1, ρ_t will be equal to M, the number of experiments combined into (or number of FIDs acquired in) one sequence.

Parallel NMR spectra provide identical information to those obtained from conventional measurements, but in a reduced time frame as reflected in ρ_t. Given all other parameters in Eq. 1 are equal, the performance of the parallelized experiments can be measured in terms of the relative sensitivity enhancement per unit time, ε_t for each module, as defined by:

$$\varepsilon_t = R_{\mathrm{S}} \cdot \rho_t^{1/2} \tag{6}$$

where R_{S} is a factor indicating the sensitivity losses due to parallelization, i.e. the relative signal intensity from the parallelized experiment with respect to an equivalent conventional experiment acquired with the same parameters.

Practical Examples

Time-shared experiments which detect two different signals have a maximum attainable ε_t value of $2^{1/2} \approx 1.41$. In practice, slightly lower sensitivity improvements are observed due to necessary compromises in delay timings, which result in a reduced R_S factor. For example, the careful optimization of a time-shared sensitivity-enhanced HSQC yielded ^{15}N and ^{13}C ε_t improvements of 1.34 and 1.07 respectively for doubly labelled proteins[97]. Likewise, time-shared HSQC-TOCSY experiments provided ^{15}N and ^{13}C ε_t improvements of 1.36 and 1.21 respectively for natural abundance samples[98].

For NOAH supersequences using sequential acquisition, up to five experiments have been combined[20], yielding concomitant increases in ρ_t. A selection of NOAH experiments is presented in Table 3, along with their values of ρ_t and ε_t. Since all modules in a supersequence share the same recovery delay d_1, the incorporation of additional modules leads to minimal increases in experiment time, which arise only from τ_{pp} and τ_{aq} of the newly added module(s). In general, the gains in sensitivity per unit time, ε_t in the NOAH experiments increase with the number of modules, M as exemplified in Table 3, rows 1–3. The exact value of ρ_t depends on the relative sizes of τ_{pp}, τ_{aq}, and d_1.

Supersequences involving NOESY modules will typically have ρ_t somewhat smaller than the number of modules M, because of the relatively long mixing time (500 ms in these examples) that contributes to τ_{pp}. For example, the NOAH-2 MS supersequence has a ρ_t of 1.90, which is closer to 2 than the SN supersequence (rows 1 and 4 in Table 3). However, COSY experiments can be fully nested within the NOESY pulse sequence using the COCONOSY scheme[99,100], which means that an extra module can be recorded without any increase in experimental time (compare rows 1 and 2 in Table 3). The NOAH-4 MSCN spectra of cyclosporine and the relative sensitivity advantages, ε_t across the NOAH experiments (Table 3, rows 1-3) are illustrated in Supplementary Figure 3. The threefold reduction of experimental time (ρ_t = 3.01) leads to significant sensitivity enhancements per unit time.

As mentioned above, the primary benefit arising from parallelisation is accelerated data collection, with increases in sensitivity per unit time relative to conventional data collection a secondary gain in some instances. To what extent such gains are realised depends strongly on the supersequence employed, molecular properties (nuclear relaxation times), sample conditions (solvent properties, temperature) and experimental parameters (recovery delays and acquisition times). The gains indicated in Table 3 should therefore be viewed as representative values typical for the characterisation of small organic molecules.

Multi-nuclear detected schemes

Basic experiments

Three typical examples of parallel, interleaved and sequential acquisition experiments with multi-nuclear detection involving abundant (^{1}H, ^{19}F and ^{31}P) nuclei and magnetically diluted nuclei (^{13}C) are shown in FIG. 6. One of the simplest and most versatile experiments is the COSY pulse scheme. The dual receiver 2D H-P PANSY-COSY experiment records homo-nuclear (H-H) COSY and heteronuclear (H-P) COSY spectra in the same experiment time as normally is required to acquire a single conventional 2D COSY spectrum (see FIG. 6a). Both spectra share the t_1 evolution period and therefore the F_1 (^{1}H) frequency axis, which ensures peak alignment in the two spectra and facilitates the resonance assignment in complex spectra.

An example of interleaved H-H/F-F COSY experiment is shown in FIG. 6b. In this case, two homonuclear 2D COSY spectra are recorded in the same time it takes to record one conventional COSY experiment involving nuclei with the longest recovery time, in this case ^{1}H. Similar interleaved experiments can involve more complex pulse schemes[18], other abundant nuclei (e.g. ^{31}P)[60] or isotopically enriched samples[101,102].

Spectra recorded using 2D H-C HETCOR / H-H TOCSY pulse scheme that employs sequential acquisition are shown in FIG. 6c. In this example diluted nuclear species (^{13}C) have been chosen.. Generally, the HETCOR spectra contain the same information as the corresponding HSQC spectra, but can be recorded faster and with better resolution. However, this requires relatively high sample concentrations (>20 mM) due to the low ^{13}C sensitivity and low natural abundance (1.1%) but there is no sensitivity advantage in this particular case.

Combined multi-nuclear detection schemes

Various combinations of the basic parallelization techniques have yielded sophisticated, efficient and information rich experiments. For instance, interleaved H-H COSY and P-P/P-H PANSY-COSY spectra recorded using the pulse scheme of FIG. 5d are shown in FIG. 7. The three 2D spectra were recorded in time that is required to acquire a single conventional 2D H-H COSY spectrum not only providing correlations within but also between the homo-nuclear ^{1}H and ^{31}P spin systems. In addition to saving time ($\rho_t = 3$), this experiment detects more magnetization per unit time offering considerable sensitivity improvements ($\varepsilon_t = 1.73$). Similarly, a supersequence that combines the PANSY-COSY module (C^{2}) with the NOAH technique BSCC^{2}, can record five spectra in a single measurement (H-C HMBC, H-C HSQC, H-H COSY, P-P COSY and P-H COSY)[60]. The C^{2} module can easily be appended to many other NOAH supersequences. An example of spectra recorded using the NOAH-4 SCRC^{2} supersequence is shown in Supplementary Figure 4.

The HETCOR/TOCSY/HSQC-TOCSY experiment[85,86] (see FIG. 5e) effectively delivers 3D information in a time of a 2D experiment by simultaneously recording two orthogonal 2D projections – the HETCOR and TOCSY spectra, together with tilted projections of the 3D multiplicity-edited HSQC-TOCSY[87,88]. The increased dimensionality in combination with C-H multiplicity editing and non-uniform sampling provided highly resolved spectra significantly reducing spectral overlap and the related resonance assignment ambiguities.

The PANACEA spectra of quinine recorded using the pulse scheme of FIG. 5f are shown in FIG. 8 that also details small molecule structure elucidation steps from these spectra[89]. First, the number of carbon atoms is obtained from the 1D ^{13}C spectrum (FIG. 8a). The INADEQUATE spectrum reveals how the carbon atoms are interconnected (dotted lines in FIG. 8b) tracing down the carbon skeleton of the molecule (FIG. 8c). The multiplicity edited HSQC spectra (FIG. 8d) reveal the number of hydrogen atoms attached to each carbon site (FIG. 8e). Finally, the HMBC spectra (FIG. 8f) connect molecular fragments separated by heteroatoms (FIG. 8g). The chemical shift information coupled with elemental analysis data reveals the final structure of the molecule (FIG. 8h).

The internal lock (or frequency correction) built into the PANACEA experiment allowed recording spectra in pure peanut oil and neat silicon oil with no deuterium lock and no temperature regulation[77]. This highlights one of the main advantages of parallel NMR – all spectra are recorded under equivalent environmental and magnetic instabilities that can be corrected by post-processing. The latter involves measuring the frequency shifts in PANACEA 1D ^{13}C spectra and applying the correction to all other PANACEA spectra scaled according to their chemical shifts. The highly accurate stereospecific long-range ^{1}H-^{13}C coupling information recorded in a high-resolution PANACEA experiment indicated the presence of intramolecular hydrogen bond[103]. The extended PANACEA pulse scheme of FIG. 5g applied to nitrogen-containing organic molecule (melatonin) yielded 1D ^{15}N, 2D N-H HSQC and 2D N-H HMBC spectra in addition to the basic PANACEA data[89] thus facilitating structure elucidation.

Parallel Ultrafast NMR

Single-scan 2D NMR can be combined with other techniques. One such experiment is nuclear hyperpolarization, as has been demonstrated in the realm of natural products[105]. The 2D single-scan C-H HMBC and HSQC spectra of limonene, α-pinene and camphene (1:1:2 mM) solution were acquired sequentially in a single measurement lasting only a few hundred milliseconds. The 4.4 µL sample was polarized with microwaves at 1.4K in a Hypersense (Oxford Instruments) polarizer and then dissolved in warm methanol-d_4 before being transferred to a 600 MHz Varian Inova NMR spectrometer. The spectral aliasing combined with selective excitation was used to reduce the 150 ppm ^{13}C observation bandwidth and to preserve non-protonated ^{13}C magnetization prior the single scan HSQC pulse scheme for use in the sequential HMBC experiment.

Ultrafast 2D NMR can also be combined with parallel techniques of multi-FID detection discussed in the previous sections. For instance, combining the ultrafast COSY methodology with the parallel acquisition COSY experiment (see FIG. 5a) enables recording of several multi-dimensional spectra in a single scan[104]. The methodology is accordingly named PUFSY (Parallel Ultra-Fast Spectroscopy). Two examples of H-H/H-F PUFSY-COSY spectra of fluorinated compounds recorded on a spectrometer equipped with two receivers are shown in FIG. 9. Each pair of the 2D PUFSY spectra was recorded in about 100 ms. The 2D H-H and H-F COSY spectra recorded in parallel were stored in separate memory locations, each processed and analysed as individual ultrafast spectra (see FIG. 3b). Computer optimized folding was used to reduce the large ^{19}F spectral window and the associated gradient amplitudes. Similar experiments involving ^{31}P were also reported[104]. These experiments serve as a proof-of-principle with potential applications in studies of fast chemical reactions, bio-chemical interactions and in hyperpolarized samples.

Applications

In this section we discuss various applications of parallel NMR techniques. In addition to more efficient NMR spectra acquisition, each of these techniques offers unique ways of obtaining new information. Probes equipped with multiple microcoils allow simultaneous monitoring of multiple samples offering new insights in electrophoretic separations and diagnostic NMR. Ultrafast NMR methodology finds applications in real-time monitoring of chemical reactions and biophysical rearrangements. Multi-FID detection techniques involving one or more receivers elucidate small molecule structures from a single measurement.

Multiple microcoils

Multiple microcoils enable the time-efficiency of studies of mass-limited samples to be increased significantly, up to a factor equal to the number of coils. However, they also enable information to be more easily acquired because of the multiple coils. For example, Raftery devised a difference-probe[30], in which the degree of cancellation of common signals from each probe was 90%, that enabled identification of protein-ligand interactions using glutathione and glutathione S-transferase binding protein. Ciobanu described how multiple-microcoils can be used to increase the temporal sampling frequency of chemical-reactions induced in small sample volumes via micromixers[39]. Wolters *et al*. demonstrated that a dual-probe could be used in interleaved mode to overcome some of the disadvantages of coupling electrophoretic separations with microcoil NMR detection[38]. There have also been applications of multiple microcoils in microscopic MRI[106] as well as diagnostic magnetic resonance (DMR)[107].

FIG. 10 shows a photograph of a dual-coil, multi-frequency, probehead which can be used for protein samples. The small solenoidal coils have an active volume of 15-20 μL, requiring only small quantities of isotopically-labeled protein. Each of the two coils were tuned to both ^{1}H and ^{15}N frequencies, and a single external lock coil was also integrated. The high spectral quality achievable using this probe are highlighted in Fig. 10 where one coil was loaded with 1.25 *mM* ^{15}N-labeled ubiquitin (highly structured, Fig. 10b) and the other contained a sample of 1 *mM* ^{15}N-labeled yeast proteinase A inhibitor (IA-3) (intrinsically unstructured, Fig. 10b) in low-deuterated buffers solutions.

Applications of single-scan nD NMR

Spatiotemporal encoding can provide the ultimate parallelization at the price of reduced sensitivity. When considering potential applications, the question thus arises of when such price is worth paying. One family of experiments where spatiotemporal encoding is a must is in cases of time-dependent samples; where either the compounds' lifetimes are incompatible with conventional multidimensional acquisitions, or where time instabilities become the dominant sources of noise –particularly of t_1 noise. Applications include homonuclear and heteronuclear acquisitions on unstable magnets[108], monitoring of organic reactions as they happen[109-112], and the real-time monitoring of biophysical rearrangements on proteins and nucleic acids (see FIG. 11)[113,114]. Furthermore, the single-shot homonuclear 2D ^{1}H correlations are also well suited for monitoring the flow of metabolites and chemicals –for instance as they elute out from a chromatographic column and through an NMR spectrometer acting as detector[115,116].

As mentioned, a particularly intriguing combination arises from merging high-speed and limited-sensitivity ultrafast 2D NMR, with nuclear hyperpolarization. These methods can have much greater signal intensities than conventional NMR samples, but limited measurement lifetimes. An early example of this was provided by the combination of homonuclear single-shot 2D NMR and **CIDNP** **[G]**, an optical enhancement technique leading to photobleaching after a few scans[117]. Sensitivity and speed enhancements of ca. three orders of magnitude each, were subsequently demonstrated by the combination of *ex situ* dynamic nuclear polarization and heteronuclear single-shot 2D NMR[105,118,119]. Homonuclear single-scan 2D correlations relying on enhancements derived from para-hydrogen have also been implemented[120]. Single-scan 2D spectral-spatial correlations have also been demonstrated in this manner, including in hyperpolarized and thermal preclinical, as well as in clinical human MRI settings[121-125]. As mentioned, the spatial extent of a homogeneous sample can also be used to encode other sorts of information besides chemical shifts of evolving spins. The measurement of relaxation times[47] and of molecular self-diffusivities[48,49], for instance, have also been sped up in this manner. More recent applications of ultrafast NMR can be found elsewhere.[126-129]

Structure elucidation from a single experiment

[H3] The PANACEA experiment. A step by step example of manual structure elucidation of quinine from the PANACEA spectra is shown in FIG. 8. Although computer-assisted structure elucidation can be used, it was not available at the time when the experiment was published. This example is used to demonstrate the power of the INADEQUATE experiment that makes the manual structure elucidation easy. The 1D ^{13}C spectrum of the quinine in CDCl3 recorded in the PANACEA experiment (top panel) provides the number of carbon atoms in the molecule. The 2D INADEQUATE spectrum reveals the connections between the carbon atoms. Some connections are interrupted by heteroatoms leaving several fragments disconnected from the main scaffolding. The HSQC spectrum provides information about the number of protons attached to the corresponding carbon atoms and revealing potential sites for the missing heteroatoms. The HMBC spectra provide long range correlations connecting the fragments and closing the rings. Finally, the nature of heteroatoms is revealed by the chemical shifts in combination with data from elemental analysis. Similar examples of structure elucidation based on the PANACEA experiment can be found elsewhere[77,89,94,103].

While the structure elucidation based on the PANACEA spectra is simple and largely unambiguous, the main disadvantage of this experiment is the need for substantial amounts of sample. Cryoprobes optimized for direct ^{13}C detection can reduce the required sample amount by up to a factor of 10 as compared to room temperature probes[76,77]. ^{1}H detected techniques coupled with CASE[96] (Computer Assisted Structure Elucidation) and small diameter cryoprobes have the potential to reduce the required sample amount even further, often to sub-milligram quantities.

[H3] CASE: from experiment direct to structure.

BSC and BSCN-based NOAH supersequences (where the single-letter codes indicate B=HM**B**C, S=H**S**QC, C=**C**OSY and N=**N**OESY) are capable of providing hetero- and homonuclear correlation information from a single experiment. Although this is possible with conventional sequences, the NOAH experiments achieve the same goal in a far shorter time and, for some combinations, with increased sensitivity. This not only enables optimal use of instrument time but should also prove to be critical for systems which are reacting or are otherwise unstable since it avoids temporal variations between individual experiments. The direct extraction of a molecular structure from NOAH spectra using CASE routines[96,130] has been successfully demonstrated[59,60], and is illustrated here for the pharmaceutical zolmitriptan (FIG. 12). A single NOAH-4 BMSC experiment (M indicating ^{15}N HMQC) provided all necessary correlations for the structural identification and resonance assignment of the compound but required only one-third of the instrument time required by four conventional experiments of equal resolution (ρ_t =

2.98), illustrating the gains afforded through parallelization.

Although not the case for zolmitriptan, structural elucidation can occasionally be hampered by overlap in the proton dimension, which is particularly problematic in COSY spectra. In situations such as these, additional dispersion in a ^{13}C indirect dimension can prove highly useful, such as in 2BOB or H2OBC[131] experiments, which have been incorporated into multiple-FID sequences[60,132].

Applications in metabolomics

Metabolomics involves identification of metabolites - small molecules produced by cell metabolism, in biological mixtures, such as bodily fluids (blood plasma, urine etc.) and tissue[76,86b]. Parallelization of NMR methods potentially can increase sensitivity and throughput in metabolomics studies as well as mitigate the effects of sample degradation and environmental and instrumental instabilities that often complicate the analysis. To date, only one pulse scheme recording several spectra simultaneously, namely the HETCOR/TOCSY/HSQC-TOCSY, has been applied to metabolomics studies designed with this specific application in mind[85,86]. One of the basic steps in analysis of NMR spectra of metabolomics samples is signal assignment usually by 2D NMR techniques[76]. While 3D NMR offers even higher resolution and is widely used in biomolecular studies, such techniques are deemed to be too time-consuming for metabolomics applications to time-sensitive samples. Instead, the HETCOR/TOCSY/HSQC-TOCSY pulse scheme yields 3D information that is acquired in the time frame of a 2D measurement. The experiment achieved a complete resonance assignment in a mixture of 17 amino acids and 4 common metabolites comprising a medium used in human in vitro fertilization (IVF)[86a] resulting in a reduction of experiment time by an order of magnitude. This has opened new avenues for high-throughput metabolomics studies using NMR, even as similar combinations of multi-nuclear detection and projection spectroscopy have been used in biomolecular NMR[133,134]. Recent reports of ultrafast NMR in metabolomics studies[135,136] are indicative of potential applications for parallel ultrafast NMR methods in this field.

Reproducibility and data deposition

The spectra yielded by parallel acquisition techniques provide equivalent data to standard 2D experiments once the requisite processing has been undertaken. This means that spectral information may be reported in the same format as existing methods. For data reproducibility, it generally suffices to specify a subset of acquisition parameters that will have the greatest impact on spectrum appearance and quality. Precise details will vary according to the sequence in use but will most often include parameters that directly influence spectrum resolution, sensitivity and the transfer processes within the mixing period, such as spectral widths for each dimension and FID acquisition times, the recovery delay and number of transients, as well as experiment-specific parameters such as scalar coupling constants or durations of mixing periods. Significant instrumental details include the vendor, field strengths (expressed typically in terms of ^{1}H frequencies), detection probe characteristics (coil configurations and diameter) and sample temperatures. Where possible, the design of parallelized pulse sequences seeks to employ similar parameters to conventional experiments, standardising nomenclature and thus simplifying their implementation. Similarly, as for conventional 2D data sets, parallelized data tends not to be suitable for quantitative measurements, and attempts to use the experiments in this way would require suitable endeavours to establish their viability for such work.

For small organic molecules, metadata from processed spectra such as structural assignments, chemical shifts, coupling constants, and integrals may also be stored in plain-text format according to the recent NMReDATA (extracted data) specification[137]. The file format is an extension of the existing Structure Data Format (SDF), which is compatible with the commonly used molfile format for describing molecular structures. The association of an NMReDATA file with the raw spectroscopic data from which it originates constitutes an NMR record. The NMR-STAR (Self-defining Text Archival and Retrieval) [138-142] standard is particularly relevant for biomolecular NMR and includes the description of experiments, the data generated, and the derived results such as molecular structures, dynamics, and functional properties whilst nmrML (markup language)[143] has been similarly promoted for metabolomics NMR data.

As with experimental parameters, new pulse programmes and any processing scripts should be made available to users in order to ensure reproducibility. Most often these are included in the supplementary material sections of journal articles describing novel techniques and also through author websites. However, having a curated, centralised online "library" of pulse programmes, such as the Bruker User Library, is a desirable alternative that is scalable, robust and more likely to be future-proof. Ideally, instrument vendors may distribute these files together with NMR software releases; for example, a number of NOAH supersequences, as well as the associated scripts, are already distributed by instrument vendors.

Limitations and optimizations

NMR with multiple microcoils

The current limitations to the use of multiple microcoils are the number of coils that can fit into the bore of a standard NMR magnet and the number of receivers. Microfabrication techniques can potentially increase the number of coils, but as the sample volume becomes smaller so does the S/N, leading to samples requiring higher concentrations. Commercial NMR systems are currently limited to four receivers, and the only way to increase the number of separate experiments is to time-domain multiplex **[G]** the signals from more than four coils: there is no intrinsic limitation to the number of channels so we anticipate that this may increase in the future. When considering sample preparation and handling, one of the biggest challenges is producing samples with microvolumes closely matched to that of the microcoil, and delivering the sample to the coil accurately and reproducibly. One possibility is to use microdrop technology or zero-dispersion segmented flow, which is being actively developed [145].

Single-scan 2D NMR

2D—and in general, nD—NMR information originating from conventional and from single-shot acquisitions should be equivalent in terms of peak positions, line shapes, spectral resolution, and intensities[146]. In practice, however, the need to constantly apply magnetic field gradients for defining the encoding and readout spectral characteristics imparts constraints to the single-scan acquisition methods. For instance, gradient-based manipulations, and in particular those relying on strong gradients applied over 10s-100s of ms, will only work if high-quality gradient coils and amplifiers are employed. Such quality is routinely achieved by human MRI scanners as it is essential for their operation; the same is not always the case for high resolution NMR, where gradient quality and agility have not been given the highest priority. This in turn leads to special requirements for phasing the data, setting up suitable spectral widths, and so on[147]. However, the most important limitation of ultrafast nD NMR pertains to sensitivity and the presence of added noise.

In conventional NMR acquisitions, noise will be determined by how much the receiver's bandwidth is opened—noise increasing, in general, as the √bandwidth. This bandwidth will be given by the inverse of the acquisition dwell time (i.e. the time between sampled data points), which in conventional 2D NMR is $1/\Delta t_2$.[22,95] In ultrafast NMR, however, the full F_1 indirect-domain information is contained within the dwell time Δt_2; for acquisitions aiming at defining the F_1 domain with N_1 equidistantly sampled k-domain points, the receiving bandwidth will thus have to be opened up by an additional N_1 factor to capture these changes. This leads to the S/N per scan decreasing by $\sqrt{N_1}$ over its conventional counterpart. This is a sensitivity penalty that will increase as the number of indirect-domains, that are spatiotemporally encoded, increases. This is because the additional sampled domains have to be collected within each direct-domain acquisition dwell time. In cases where sensitivity is not a limiting factor, for example when dealing with sufficiently concentrated samples, this may not be an obstacle. But when dealing with sensitivity-limited samples, this price can be particularly onerous.

Sensitivity can be improved by signal averaging in different forms, be it collecting the same scan over and over, interleaving the data so as to reduce the gradient demands,[12] or performing ≥3D NMR acquisitions employing a mixed of temporal and spatiotemporal encodings[148] (in this later case the temporal encoding provides a sort of signal averaging). Special cases where *a priori* information is known—for instance, an F_1 spectral region which is known to be devoid of resonances and hence in no need of sampling—can be free from the full demands of the aforementioned condition; in such instances, the effective number N_1 of points sampled, can be *de facto* reduced, leading to an improved sensitivityand lead to improved sensitivity. Another way to reduce these sampling-derived demands is with the use of multiple high-quality (micro)coils and receivers, leading the savings in the sampling requirements that are analogous to those enjoyed by parallel receiving in MRI. Such concepts, however, remain to be tested in the domain of high-resolution spectroscopy.

Optimizing multi-FID detection schemes

The same number of FIDs must be acquired for every module in a parallelized multi-FID experiment, meaning the minimum experiment time is necessarily limited by the least sensitive module. In practice, the time savings realized may not be as great, particularly for low-concentration samples that require many transients for the least sensitive module but not for others. It is possible to increase the number of scans only for the least sensitive module with a corresponding reduction in the number of t_1 increments to trade resolution for sensitivity[149]. However, this is limited to situations where high spectral resolution is not required for the least sensitive module, which is most often the case for ^{15}N correlations of small molecules. In cases of high sensitivity, benefits can be realised through the incorporation of non-uniform sampling (NUS) schemes[150], either to afford additional time savings or to increase resolution in indirect dimensions without extending experiment durations.[63]

The use of the same magnetization pool multiple times can also lead to drops in performance. This is most obvious in polarization sharing experiments, where R_S is necessarily lower than 1. However, this can also inadvertently arise in NOAH supersequences involving the HMBC module, which destroys the bulk magnetization required for any subsequent homonuclear module. For example, the NOAH-3 BSC supersequence[59,63] (HMBC/HSQC/COSY), recorded on a sample of the cyclosporine in benzene-d_6 has ρ_t = 2.65 and R_S = 1.0, 0.92, and 0.27 for the three modules respectively, leading to ε_t = 1.63, 1.50, and 0.44 (E. Kupce, J. Yong & T. Claridge, unpublished work). The observed COSY signal in this case derives from magnetization that was excited during the HMBC module but has only partially recovered during the two subsequent FID periods prior to its use in the COSY. Nevertheless, this decrease in R_S is readily tolerated, as the COSY spectrum is naturally far more intense than the HMBC. A more insidious problem arises from the differing relaxation rates of the different protons in the sample, leading to imbalanced crosspeak intensities in the COSY module. This can however be ameliorated by inserting a period of isotropic mixing prior to the COSY[59,151,152], which serves to rebalance the available magnetization across all protons in the manner of the ASAP (acceleration through sharing of adjacent polarization) experiments[153-155].

Balancing the sensitivities in multi-receiver experiments involving insensitive and diluted nuclei such as ^{13}C, ^{15}N, and ^{29}Si is a significant challenge, particularly in low concentration samples. Probes optimized for direct detection of such nuclei are essential in multi-nuclear detected experiments. Instrument software can also impose limits on method development. For example, the commercial Bruker software TopSpin limits the number of memory blocks and hence NOAH modules to 5. This limit has been reached and should be at least doubled to allow design and recording of experiments similar to the extended PANACEA pulse scheme.

Outlook

Although the advantages of parallelization have been realized in many fields of science and technology including MRI[4-6], NMR parallelization has not yet reached its full potential. We have discussed the basic parallelization approaches that have been developed in NMR over the past 20-30 years and their significant gains in speed and sensitivity. Many of these techniques are still under active development, with new methods continually being demonstrated[11,17,20,35,61,89]. However the largest gains are expected to emerge once the techniques discussed in this article are combined to multiply the benefits of individual approaches.

One of the major advances in multiple microcoil NMR that can be envisioned is the use of three-dimensional microfabrication techniques to improve the quality and reproducibility of the detector setup used for data acquisition[156,157]. Most of the studies outlined in this Primer have used RF coils wound by hand, with discrete capacitors used for impedance matching: as such these are very labour-intense to produce, and there is considerable inter-element variability in performance. Microfabrication techniques are much more accurate and reproducible, with the proviso that the conductor thickness needs to be much greater than typically used in this type of manufacturing, otherwise the coil resistance at high frequencies will be very large and the S/N will be correspondingly reduced. In addition, it should be possible to integrate much of the electronic circuits used for signal detection and demodulation very close to the RF coil, which will improve performance. Another potential application is in the field of metabolomics, which studies enormous numbers of samples. The time required could be reduced significantly by, for example, running several samples simultaneously using the multiple microcoil technology.

The existing approaches towards time-shared and multiple-FID experiments lead to significant reductions in experiment time and improved sensitivity per unit time relative to conventional experiments[60]. However, the manipulation of multiple magnetization components typically requires the introduction of additional pulse sequence elements, reducing efficiency due to instrumental imperfections[158]. An immediate challenge is therefore the design of improved pulse sequences that have comparable or enhanced performance relative to conventional experiments, for example through optimal control theory[159-161].

The extension of these techniques to higher-dimensional experiments is an obvious area for future development. Although time-shared 3D and 4D sequences have long been used in biomolecular NMR[162-164], there has been much less work on sequential and multi-nuclear acquisition experiments[82,83,102]. The potential time savings realized through these would be even more substantial than for their 2D counterparts.

The combination of different approaches to parallelized acquisition provides a fertile area for future investigation. For example, one may even envision experiments in which separate magnetization pools can be sampled in an interleaved fashion without any explicit recovery delays. This can be easily done when detecting multiple nuclei, but the homonuclear case is substantially more difficult due to the precise control required over each magnetization pool. Marrying multi-FID schemes with spatial encoding could enable rapid and continuous sampling of magnetization pools for even greater gains in time efficiency and sensitivity per unit time.

In the area of multinuclear, multi-receiver detection, the very different intrinsic sensitivities of diluted nuclei such as ^{13}C, ^{15}N, ^{29}Si and similar versus high-γ nuclei such as ^{1}H and ^{19}F present significant challenges, particularly in low-concentration samples. Probes optimized for direct detection of such nuclei are essential. For instance, the best performance of parallel NMR experiments that are based on direct detection of ^{13}C is achieved with cryoprobes that are designed to maximize the ^{13}C sensitivity. Constructing RF coils from high-temperature superconductors roughly doubles the sensitivity of cryogenic probes. For example, a home-built 1.5 mm (35 μL) high-temperature superconductor probe optimized for ^{13}C detection improved sensitivity by a factor of >20 compared to a commercial RT probe for the same sample mass[76] thus reducing the measurement time for small sample quantities by a factor of >4000. It is expected that future probe developments and advances in hyperpolarization techniques will make ^{13}C detected experiments such as PANACEA more practical. For instance, hyperpolarization allowed the recording of INADEQUATE spectra at the natural abundance of ^{13}C in a single scan[165].

Parallelized experiments could also be employed for monitoring time-dependent processes such as chemical reactions to alleviate the spectral crowding and associated ambiguities in interpreting conventional 1D methods. Furthermore, the use of multi-FID pulse schemes in conjunction with time-resolved non-uniform sampling[166] or ultrafast methodology is extremely promising, but has yet to be investigated. Further development of ultrafast supersequences including direct detection of nuclei other than protons also offers promise in structure characterization and reaction monitoring, with ^{19}F offering applications in fluorine chemistry and drug design. Combining multiple microcoil technology with efficient NMR supersequences would produce unprecedented throughput capability. Likewise, merging the ultrafast methodology with various pulse schemes for structure elucidation from a single measurement such as NOAH and PANACEA, opens amazing opportunities to design experiments yielding organic molecule structures in a fraction of a second.

In conclusion, parallelization in NMR enables several concepts in hardware, data acquisition and data processing to be co-developed in ways in which the total gain is greater than the sum of the parts. There is a strong analogy with MRI, where recent advances in rapid patient imaging using massively undersampled data and the introduction of artificial intelligence into data processing have only been made possible by the availability of parallel data acquisition. We hope that future researchers will look back on the days of a single sample/single experiment mode in the way that we currently remember the early days of NMR where spectra were laboriously collected by sweeping across the entire frequency range one step at a time.

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Author contributions

Introduction (A.W., L.F., E.K., T.C., J.Y.); Experimentation (A.W., L.F., E.K., T.C., J.Y.); Results (A.W., L.F., E.K., T.C., J.Y.); Applications (A.W., L.F., E.K., T.C., J.Y.); Reproducibility and data deposition (T.C., J.Y.); Limitations and optimizations (A.W., L.F., E.K., T.C., J.Y.); Outlook (A.W., L.F., E.K., T.C., J.Y.); Overview of the Primer (A.W., L.F., E.K., T.C., J.Y.).

Competing interests

E.K. declares no competing interests; L.F. declares no competing interests; A.W. declares no competing interests; J.Y. declares no competing interests; T.C. declares no competing interests.

Tables

Table 1. **NMR properties of the most commonly observed nuclei[22]**

Nucleus	γ (MHz/Tesla)	Natural Abundance (%)	Relative Sensitivity
^{1}H	42.58	99.98	1.00
^{13}C	10.71	1.11	1.76×10^{-4}
^{15}N	-4.32	0.37	3.85×10^{-6}
^{19}F	40.08	100.0	0.83
^{29}Si	-8.46	4.7	3.69×10^{-4}
^{31}P	17.25	100.0	6.65×10^{-2}

Table 2 **| NMR spectra acquired with the 8-coil probe and the chemical structures of the compounds used.** Column 1: Each sample (10 *mM* solution in D_2O) was loaded into the coil via the attached teflon tubes. Column 2: chemical structures of sucrose, galactose, arginine, chloroquine, cysteine, caffeine, fructose, and glycine. Column 3: COSY spectra. Data acquisition parameters: data matrix 2048×256, 8 scans, *sw* = *sw1* = 6000 Hz. Data were zero filled in t_1 to 2048 points, processed with shifted sine-bell window functions applied in both dimensions, symmetrized and displayed in magnitude mode. Column 4: Phase sensitive TOCSY spectra, mixing time = 30 *ms*. Data were zero filled in the t_1 dimension to 1024 points and processed with a Gaussian window function applied in both directions. Other parameters as in Column 3. Column 5: Gradient COSY spectra. gradient duration 1.9 ms, gradient strength 10 gauss / cm. Other parameters as in Column 3. Explanation of what the various spectra show is found in BOX 1.

[the table is submitted as a separate figure].

Table 3 | **Selected examples of sensitivity and speed advantages achieved by NOAH supersequences[a]**

Entry	NOAH[b]	*M*	$T_{exp}(MF)$	$\Sigma_i^M T_{exp}(i)$	Speed, ρ_t	Sensitivity advantage, ε_t
1	SN	2	19 min 38 s	33 min 16 s	1.69	1.30(S), 1.30(N)
2	SCN	3	19 min 38 s	47 min 38 s	2.42	1.56(S), 1.39(C), 1.50(N)
3[c]	MSCN	4	20 min 39 s	62 min 5 s	3.01	1.73(M), 1.50(S), 1.38(C), 1.61(N)
4	MS	2	15 min 11 s	28 min 54 s	1.90	1.38(M), 1.20(S)

[a]The sample was cyclosporin (50 mM in C_6D_6). Two scans were acquired for each increment, with 256 t_1 increments per module. A recovery delay (d_1) of 1.5 s was used for all experiments. [b]Single-letter codes for NOAH modules are: M = ^{1}H–^{15}N HMQC, S = ^{1}H–^{13}C HSQC, C = ^{1}H–^{1}H COSY, N = ^{1}H–^{1}H NOESY (mixing time of 500 ms). [c]See Supplementary Figure 3.

Figure Legends

Fig. 1 **| Approach to multiple microcoil NMR using RF coils connected in parallel. a |** In this configuration data from multiple samples are acquired in a single experiment by using a spatially selective pulse – a frequency selective pulse applied simultaneously with a magnetic field gradient in the z-direction - akin to slice selection in MRI. **b |** Spatially selective excitation pulse sequence. **c |** NMR spectrum with no gradient. **d |** NMR spectra from all samples separated with gradient, G and selective excitation of individual coils with transmitter offsets tof1 – tof4. **e |** spectrum recorded with coil 1.

Fig. 2 **| Approach using RF coils which are electrically and magnetically separate from one another. a |**A switch 1-4 is used to rapidly switch between transmitting pulses, Tx 1-4 to each of the eight RF coils. The received signals are fed into separate receive channels, Rx 1-4. Since the probehead contains eight coils, but there are only four receivers on the system, an interleaved mode is used to record separate spectra from all eight samples.

Fig. 3 | **Comparison of data acquisition schemes in conventional vs ultrafast 2D NMR. a |** The conventional 2D NMR experiment; **b |** the ultrafast single-shot 2D NMR experiment. While this schematic compares the two experimental techniques by retaining the same nominal number of N_1 elements, the actual ultrafast experiments are usually performed using continuous spatiotemporal encoding. Processing of the two types of data sets is usually different, even as both approaches lead to the same final spectra. For further details see Supplementary Fig 1.

Fig. 4 | **Exploiting isotope-specific magnetisation. a |** Isotopomers of a typical organic molecule illustrating the different magnetisation pools present, namely ^{13}C-bound protons (blue), remote ^{13}C-coupled protons (purple), ^{15}N-bound protons (orange) and protons not scalar coupled to heteroatoms (green), which contribute to different correlation spectra. **b |** Conventional (separate) acquisition of the ^{1}H–^{13}C and ^{1}H–^{15}N HSQC and ^{1}H–^{1}H COSY spectra. Each experiment requires a recovery delay (d_1) to allow proton magnetisation to relax toward equilibrium before being sampled. **c |** Simultaneous acquisition of both FIDs using the time-shared principle. The final dataset contains two phase encoded (ϕ = +x or -x) sub-sets that are separated at the processing stage (blue and orange). Fourier transformation of each subset gives a spectrum containing peaks from both ^{15}N- and ^{13}C-bound protons, which can be separated via nucleus editing. Only a single recovery delay is required. **d |** Sequential acquisition in a NOAH-style experiment comprising multiple experiment modules indicated using single letter codes; here the NOAH-4 BMSC where B = HMBC, M = HMQC, S = HSQC and C = COSY. All FIDs are stored in a single 2D matrix which has been partitioned into memory blocks. Separation of these blocks and Fourier transformation yield the individual spectra. As with simultaneous acquisition, only a single recovery delay is needed, but far greater flexibility in experiment design is available.

Fig. 5 | **Multi-nuclear acquisition techniques.** H-1, C-13, etc are different RF channels corresponding to the nuclei they detect. Each channel is equipped with a separate receiver. Overlapping FID-s denote time-shared data acquisition. The arrows indicate polarization transfer with color coding that corresponds to the target spectra. **a |** PANSY-COSY with parallel acquisition, **b |** interleaved H-H COSY/F-F COSY, **c |** HETCOR/TOCSY with sequential acquisition, **d |** interleaved COSY /PANSY-COSY, **e |** HETCOR/TOCSY/HSQC-TOCSY with sequential acquisition and time-sharing, **f |** the basic PANACEA experiment and **g |** the extended PANACEA experiment. All experiments have a common recovery period, d_1, except for panels b and d, where nuclei-specific recovery periods are indicated.

Fig. 6 **| Spectra recorded with basic dual receiver pulse schemes. a** | H-H/H-P COSY spectra of $P(Ph4)^+Cl^-$ recorded with parallel acquisition (see FIG. 5a), **b** | H-H/F-F COSY spectra of a mixture of 2,4,5 and 2,3,6- trifluoro-benzoic acids recorded with interleaved experiment (see FIG. 5b), **c** | C-H HETCOR/H-H TOCSY of gibberellic acid recorded with sequential acquisition experiment (see FIG. 5c). Note the joint F_1 frequency axis in (a) and (c) that guarantees perfect peak alignment in the complementary spectra.

Fig. 7 | **Interleaved H-H COSY and P-P/P-H PANSY-COSY spectra of a mixture of ATP and GTP in D_2O.** The top trace shows peak assignment in the 1D proton spectrum. The dotted lines show connectivity of resonances **a |** in H-H COSY spectrum (blue); **b |**in P-P COSY spectrum (red) and **c |** in H-P COSY spectrum (pink) that connects the ^{1}H and ^{31}P spin systems in (a) and (b). The spectra were recorded on a 700 MHz (^{1}H) Bruker NEO spectrometer equipped with the QCIP cryoprobe. Experiment time was 10 min.

Fig. 8 | **Step-by-step manual structure elucidation from the PANACEA spectra. a |** The spectra of quinine were recorded on a Varian NMR system operating at 500 MHz (^{1}H) using the basic PANACEA pulse scheme shown in FIG. 5f and equipped with a cryogenic HTS probe optimized for ^{13}C detection. The trace is a 1D ^{13}C spectrum indicating the number of carbon atoms. **b, c |** The 2D INADEQUATE spectrum (b) provides C-C connectivities as indicated by the dotted lines. This traces down the carbon skeleton of the molecule (c). **d, e |** The multiplicity edited HSQC spectrum (d) reveals the number of attached protons (CH_2 signals are negative and shown in red) as shown in (e). **f, g |** The HMBC spectra (f) connect the molecular fragments (g). **h |** The last step establishes nature and position of heteroatoms as revealed by chemical shifts and elemental analysis. All spectra were recorded in a single measurement[89].

FIG. 9 | Ultrafast H-H and H-F PUFSY-COSY spectra recorded in parallel. a | PUFSY spectra of compound 2; **b |** PUFSY spectra of a mixture of compounds 1-3, all 5 % solutions in DMSO-d_6. In both cases the two spectra share the F_1 (^{1}H) frequency axis. The spectra were recorded on a 600 MHz (^{1}H) Varian DDR spectrometer. The excitation and acquisition gradients were G_e = ±10 G/cm and G_{acq} = 14.9 G/cm; the acquisition times, T_{acq} were 488 μs in 200 acquisition cycles (top spectra) and 490 μs in 150 acquisition cycles (bottom spectra), a pair of 15 ms long chirp pulses were used for encoding the indirect dimension. The total experiment times were 128 ms (top) and 104 ms (bottom).

Fig. 10 | **Parallel studies of protein samples. a |** A photograph of the two-coil double-tuned probe with the external lock coil and electronic circuitry. **b |** A ^{1}H-^{15}N HSQC spectrum of 1.25 mM ^{15}N-labeled ubiquitin in H_2O/D_2O (9:1). Data matrix 1024 x 192 (States), 1 s water presaturation, 32 signal averages. Total data acquisition time 3.5 hours. **c |** A ^{1}H-^{15}N HSQC spectrum of 1 mM ^{15}N-labeled IA-3 in H_20/D_2O (9:1), Identical data acquisition parameters were used. Interleaved data were acquired using an RF switch controlled via TTL signals from the pulse programme.

Fig. 11 | **Single-shot 2D NMR spectra.** ^{15}N-^{1}H HSQC spectrum of Ubiquitin (a) and ^{13}C-^{1}H HMQC spectrum of the methyl region of Protein A (b), acquired in <80 ms.

Fig. 12 **| NOAH data recorded for the pharmaceutical zolmitriptan.** Spectra recorded using the NOAH-4 BMSC sequence illustrated in FIG. 4d. This supersequence yields the four following spectra: **a |** ^{1}H–^{13}C HMBC, **b |** ^{1}H–^{15}N HMQC, **c |** ^{1}H–^{13}C multiplicity-edited HSQC, and **d |** ^{1}H–^{1}H COSY. The inset shows the CASE derived structure and assignments in order of decreasing ^{13}C chemical shift. The duration of the NOAH BMSC was 20 min 12 s, whilst the sum for the four conventional 2D experiments was 60 min 9 s (ρ_t = 2.98). Data were collected on a 50 mM zolmitriptan sample in DMSO using a Bruker AVIII 700 equipped with a TCI H/C/N cryoprobe, with 2 transients, d_1 =

1.5 s, and 256 t_1 increments per module. Parameters were optimized for ${}^1J_{CH}$ = 145 Hz, ${}^nJ_{CH}$ = 8 Hz, ${}^1J_{NH}$ = 90 Hz. The CASE structure was generated using the Bruker CMC-SE software.

Boxes

Box 1 | **Basic 2D NMR Experiments**

2D NMR pulse sequences generally follow a "preparation–evolution–mixing–detection" scheme (see Eq. 2). Processing of the recorded data (Fourier transformation of both frequency domains) leads to crosspeaks in the 2D spectrum at (ω_1, ω_2) that correlate the spin interactions of the two nuclei. Experiments differ according to the nature of the mixing process employed, which is commonly designed to reveal though-bond (scalar) or through-space (dipolar) spin coupling interactions between nuclei. Some examples of classes of 2D experiments are summarized below.

- **COSY**: Correlation Spectroscopy. Correlates mutually scalar coupled (*J*-coupled) ^{1}H spins.
- **NOESY and ROESY**: Identifies ^{1}H spins that are close in space (typically < 5 Å) and hence share dipolar coupling.
- **TOCSY**: Distributes magnetization within a ^{1}H spin system to identify a group of mutually scalar coupled protons.
- **HSQC** and **HMQC**: Heteronuclear experiments which typically link an insensitive nucleus (such as ^{13}C or ^{15}N) with directly attached protons.
- **HMBC:** Heteronuclear experiments that correlate an insensitive nucleus (such as ^{13}C or ^{15}N) with protons that are remote in a molecular structure (typically within 2 or 3 bonds).
- **INADEQUATE:** Correlates scalar coupling between insensitive nuclei (usually ^{13}C).

Related links

https://www.bruker.com/service/information-communication/nmr-pulse-program-lib/bruker-user-library.html

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Glossary

Precessional - The process by which nuclei spins rotate (precess) about an applied magnetic field.

Gradient-based spatial encoding – selective excitation in the presence of magnetic field gradients.

Chemical shifts - The resonant frequency of a nucleus relative to that of a defined chemical group within a reference compound.

Magnetogyric ratio – the ratio of the magnetic moment of a nucleus to its angular momentum.

Spectral dimensions- Frequency dimensions in NMR spectra that will typically reflect chemical shifts and/or coupling constants.

Mass-limited samples: samples of limited amount; the term is used to distinguish from the situation of low concentration due to poor solubility. The sensitivity of NMR measurements of mass-limited samples can be improved by using small diameter probes and higher sample concentrations prompting use of a term ‘mass-sensitivity’ for small diameter probes.

Phase shift: The altering of initial phase of one oscillating signal, such as a free-induction decay, to match that of another.

Transient – The acquisition of a solitary free induction decay, also referred to as a scan.

Dynamic nuclear polarization: a technique that uses unpaired electron spins to boost the NMR signal by as much as 100,000.

Free induction decays (FID) – the observable NMR signal generated by non-equilibrium nuclear spin magnetization precessing about the magnetic field.

Supersequences – a sequence of NMR experiments (pulse schemes) with a common relaxation delay

Pools of magnetization – subsets of nuclear spins, typically defined by their coupling interactions with other NMR-active spins.

NOAH: NMR by Ordered Acquisition using ^{1}H Detection: an experimental scheme for acquiring multiple experiments in one whilst requiring only a single relaxation delay.

Polarization transfer – transfer of nuclear polarization between subsets of nuclear spins.

COSY: Correlation Spectroscopy: a technique for identifying directly scalar coupled (*J*-coupled) nuclei, most often protons.

TOCSY: Total Correlation Spectroscopy; a technique related to COSY that distributes magnetization within a network of mutually scalar coupled protons so as to group them within a structure.

HSQC: Heteronuclear Single Quantum Correlation: an experiment used to correlate an insensitive nucleus (such as ^{13}C or ^{15}N) with its directly attached proton(s) via one-bond scalar coupling.

Magnetization helices: spatially-dependent magnetization patterns, where each chemical site's magnetization subtends a helix whose pitch is linearly proportional to the site's chemical shift

Echoes: signals that peak as a function of the *k*-domain variable, according to their indirect-domain chemical shift

Recovery delay also relaxation delay; a time period in which spins recover their equilibrium populations between scans.

Nucleus editing: recording multiple datasets in which signals from separate pools are phase-labelled relative to one another

Isotropic mixing – transfer of x-, y-, and z-magnetization (hence isotropic) between *J*-coupled spin systems.

HMBC: Heteronuclear Multiple-Bond Correlation: and experiments that correlates an insensitive nucleus (such as ^{13}C or ^{15}N) with protons that are remote in a molecular structure (typically within 2 or 3 bonds) via their long-range scalar coupling.

HMQC: Heteronuclear Multiple Quantum Correlation: an experiment closely related to HSQC and HMBC used to correlate an insensitive nucleus (such as ^{13}C or ^{15}N) with its directly attached proton(s) via one-bond scalar coupling.

Polarization – the degree of alignment of nuclear spins with the applied magnetic field that gives rise to an observable NMR signal.

HETCOR: Heteronuclear Correlation: a technique for correlating an insensitive nucleus (such as ^{13}C) with neighboring proton(s) via scalar coupling whilst using direct detection of the insensitive nucleus.

PANACEA: Parallel Acquisition Nmr, an All-in-one Combination of Experimental Applications: a method that combines three standard pulse sequences (INADEQUATE, HSQC and HMBC) into a single supersequence.

INADEQUATE: Incredible Natural Abundance Double-Quantum Transfer Experiment: a method for correlating adjacent insensitive nuclei (typically ^{13}C) via one-bond scalar coupling.

Linewidth – width of an NMR peak at half height, usually defined in hertz.

NOESY: Nuclear Overhauser Effect Spectrosopy: a technique for identifying nuclei, most often protons, that are close in space (typically < 5 Å) and hence share dipolar coupling.

ROESY: Rotating-frame Overhauser Effect Spectrosopy: a technique related to NOESY that is also used to identify spatial proximity between protons.

Time-domain multiplex: another term for parallelizing NMR experiments.