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New Advances in Fast Methods of 2D NMR Experiments

Abdul-Hamid Emwas, Mawadda Alghrably,

Samah Al-Harthi, Benjamin Gabriel Poulson,

*Kacper Szczepski, Kousik Chandra and Mariusz Jaremko*

Abstract

Although nuclear magnetic resonance spectroscopy is a potent analytical tool for identification, quantification, and structural elucidation, it suffers from inherently low sensitivity limitations. This chapter focuses on recently reported methods that enable quick acquisition of NMR spectra, as well as new methods of faster, efficient, and informative two-dimensional (2D) NMR methods. Fast and efficient data acquisition has risen in response to an increasing need to investigate chemical and biological processes in real time. Several new techniques have been successfully introduced. One example of this is band-selective optimized-flip-angle short-transient (SOFAST) NMR, which has opened the door to studying the kinetics of biological processes such as the phosphorylation of proteins. The fast recording of NMR spectra allows researchers to investigate time sensitive molecules that have limited stability under experimental conditions. The increasing awareness that molecular structures are dynamic, rather than static, has pushed some researchers to find alternatives to standard, time-consuming methods of ^{15}N relaxation observables acquisition.

Keywords: NMR, 2D NMR, ultrafast data processing, SOFAST, relaxation

1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical tool for identifying, quantifying, and elucidating structures of molecules. Moreover, NMR offers several approaches to studying molecular dynamics and to probing the interactions of inert molecules such as protein-protein, protein-DNA, and protein-ligand. The main strength of NMR spectroscopy is its ability to discern or resolve individual resonance frequencies of a wide range of different nuclei (e.g., ^{1}H, ^{13}C, ^{15}N, ^{29}Si, ^{30}Al, ^{31}P to ^{235}U). As the resonating frequency is unique for each type of nuclei, one can envision an NMR for each nucleus as a separate spectroscopy such as ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, ^{235}U NMR spectroscopy (**Figure 1**), etc. More importantly, NMR has the ability to evaluate information about the environment of each atom and their neighbor's nuclei (both through space and through bond), allowing researchers to differentiate the unique magnetic environments of the same nuclei in different positions of a single molecule. Thus, NMR spectroscopy is extensively used for the identification and in the structural elucidation in a wide
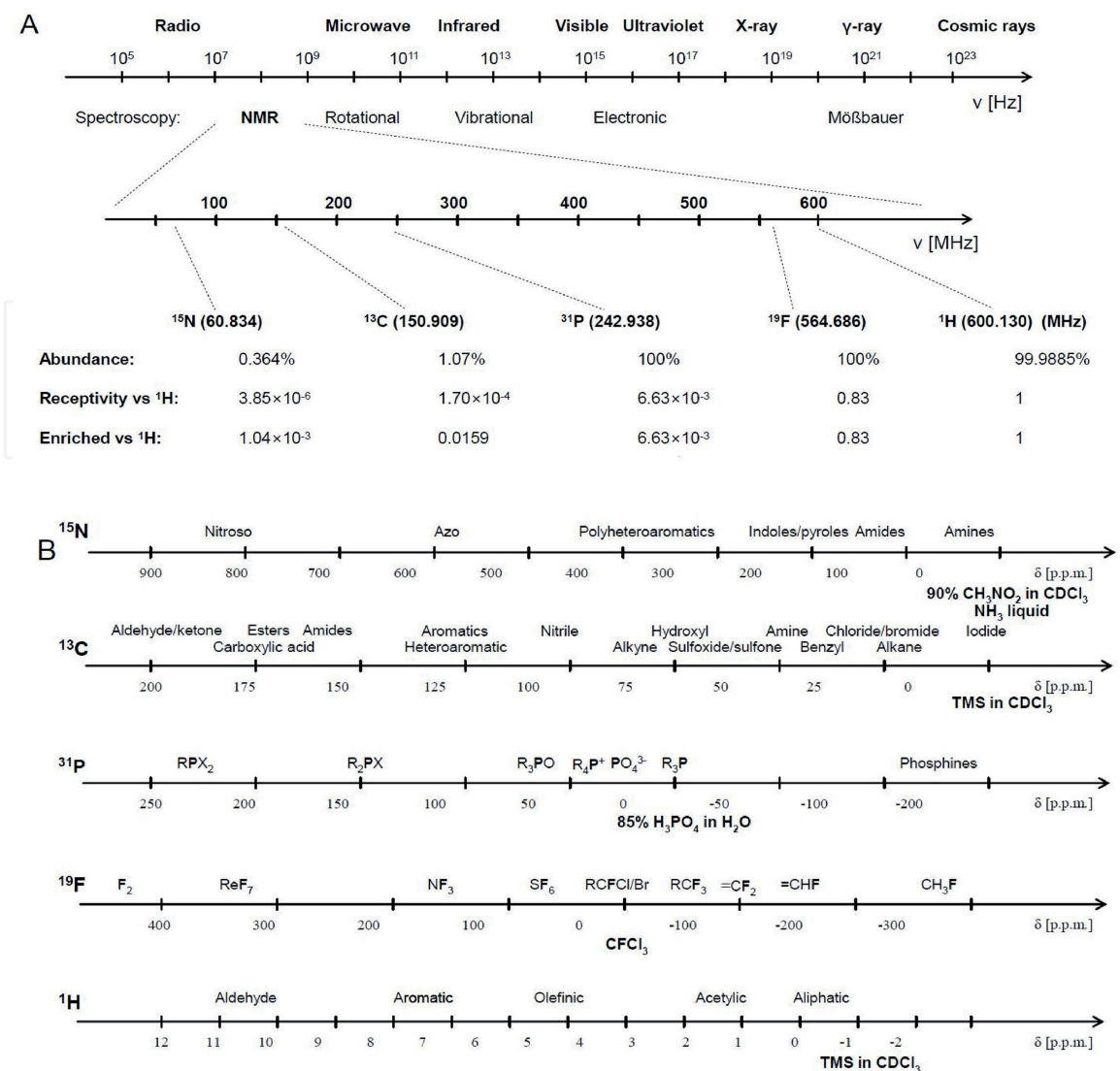
Figure 1.
Frequency scale ranges and types of spectroscopies that correspond to them, respectively. The natural abundances and chemical shifts of the most common (^{15}N, ^{13}C, ^{31}P, ^{19}F, and ^{1}H nuclei) nuclei biomolecules studies with respect to the scale of the 600 MHz proton frequency adapted from [1].

range of applications in gas [2, 3], liquid [4–16], and solid-state samples [17–28]. Nowadays, NMR spectroscopy is one of the most important analytical tools that has been used in several fields. These fields include structural biology [29–38], organic chemistry [39–52], polymer characterization [40, 46, 53–64], inorganic chemistry [65–75], and physics [76–83].

Despite its significant advantages, NMR suffers from some limitations, of which the relatively low sensitivity seems to be the most severe. An NMR sample can be treated as a collection of many nuclear spins of magnetically active nuclei that act as small bar magnets. These nuclear spins have two possible orientations with different energy levels that adapt when placed within the strong magnetic field. The number of nuclear spins occupying each energy level is determined by the Boltzmann distribution equation:

$$N_U/N_L = e^{-\Delta E/kT} = e^{-h\nu/kT}$$

where NU and NL are the nuclei (expressed in numbers) in the upper and lower energy states, respectively, k is the Boltzmann constant, ΔE is the energy gap between two energy states of the spins, higher (NU) and lower (NL) energy, respectively, and T is the temperature expressed in Kelvin (K). Many of the nuclei

are at 298 K, the most common temperature in which biomolecular NMR measurements are performed. At this temperature, there is miniscule excess of the nuclei in the lower energy state that could be excited to the higher energy state by absorbing the energy given by the radio frequency (RF) pulse and later detected successfully during the data acquisition process. Therefore, the relatively low sensitivity of the NMR measurements seems to be the most severe limitation of this method. Another limitation comes from each measured nuclei having a defined range of frequencies at which it resonates (for practical reasons, frequencies are recalculated into the "unit-less" ppm scale in order to make it independent of the applied external magnetic field, B0). This in turn depends on its magnetic neighborhood, and, therefore, the whole range must be covered with good resolution.

In earlier days, most NMR experiments only recorded one-dimensional (1D) spectra of which several were of ^{1}H or ^{13}C nuclei. For complex biomacromolecules, such as proteins, nucleic acids and their complexes, and complex mixtures, this technique has a particularly low spectral range of ^{1}H resonance, what leads to the low resolution of the spectra. This low resolution proves insufficient for analysis.

NMR signals depend on several nuclear properties such as the natural abundance of investigated isotopes, nuclear spin (I), gyromagnetic ratio (γ), quadrupolar moment (Q), and spin relaxation rates. Although most elements have an NMR active isotope, NMR spectroscopy is still not a practical approach for detecting many elements. For example, quadrupolar nuclei with nuclear spin > ½ possess a quadrupolar moment that causes an effective spin relaxation mechanism, causing dramatic line broadening in the NMR spectra. Unfortunately, the nuclear spin for important elements such as carbon ^{12}C isotope and ^{16}O isotope is zero; thus, no NMR signals can be detected for such nuclei. For example, the NMR active isotope of oxygen, ^{17}O has a low natural abundance (0.037%), low receptivity, quadrupolar nuclear spin = 5/2, and very short spin relaxation T1 values. These factors make ^{17}O NMR spectroscopy an unfeasible approach. Thus, nuclei with nuclear spin = ½; high natural abundance similar to those of ^{1}H, ^{31}P, and ^{19}F; and high γ are the most accessible nuclei for NMR studies in organic and biological samples. Even though the 1D ^{1}H NMR spectroscopy is the most common method, it suffers from the peak overlap as the spectra is very narrow (about 10 ppm). To overcome the challenge of spectra overlap, the multidimensional techniques which encode the indirect dimensions were proposed (i.e., Ernst, 2D, Bax 3D 90s, Clore 4D, Michal, etc.). Multidimensional NMR spectra have efficiently tackled the resolution problems for the spectra of large molecules such functional biomolecules and their respective native complexes. Applying two-dimensional (2D) or multidimensional NMR experiments are usually used to resolve overlapping peaks. Nevertheless, 2D experiments require considerably larger amounts of time than the usual 1D experiments do. The coincidence of simultaneous increase in measurement time with increase in dimensionality is directly correlated with the exact mechanism of how exactly the additional dimension is built in NMR. In an nD NMR experiment, n dimensions are created by n independent time increments which encode chemical-shift information. In 1D NMR, chemical shifts are encoded during acquisition time when data is written referred to as direct dimension hereafter. There we have no restriction in putting the number of complex points as it will not increase the measurement time significantly. The scenario changes drastically when moving to multidimensional NMR. In multidimensional NMR, first there is a prepare period and then a mixing period where the magnetization is transferred to another dimension. Here both homonuclear or heteronuclear magnetization can be generated which can be frequency labeled. This frequency labeling is the evolutionary period, and here the number of pints will determine the resolution of this dimension and the total measurement time. Each time increment is equivalent to record one additional

experiment. Hence, for nD experiment, we can construct n dimensions, and in each dimension, some points have to be recorded to achieve the desired resolution in that dimension. This is the main reason the measurement time increases in a multiplicative manner with respect to the number of points in each dimension one needs to acquire to achieve the desired resolution. In the simplest multidimensional NMR, two-dimensional NMR thus uses two frequency axes. These are namely direct as explained earlier and indirect axis which is created by time increments after mixing. Now each time increment encodes one 1D spectra individually, and the second frequency is generated by the second Fourier transformation. Signal intensity is usually presented on the spectra as contours, bearing resemblance to topographic maps, or as an intensity plot. Thus, some studies (such as untargeted metabolomics) do not fully utilize 2D NMR because there are a large number of samples that would require more time to complete. Several approaches have been continuously developed to reduce the experimental time and to enhance the NMR sensitivity. These approaches include hyperpolarization methods such as dynamic nuclear polarization (DNP), cryoprobes, and new ultrahigh magnetic fields. These methods, however, are costly and need substantial hardware additions.

Other methods such as cross-polarization, INEPT, DEPT, and fast methods provide good enhancements without hardware additions and are available in most existing NMR systems. Fast 2D NMR methods and band-selective optimized-flip-angle short-transient (SOFAST) 2D NMR methods provide good NMR spectra with a short experimental time, which in turn enables the researchers to carry out powerful 2D NMR experiments in a few minutes. Increasing the number of spectral dimensions increases the spectra acquisition time dramatically. In order to overcome this issue and reduce the demand for the experimental time needed to perform the spectra measurement, different methods of acquisition of the spectra have been proposed. In this chapter, we introduce the relaxation mechanisms, and then we briefly discuss a few methods of fast methods including SOFAST, ultrafast (UF) 2D NMR, pure shift NMR, and post-processing methods. The pure shift NMR method produces spectra that only contain chemical-shift data and is void of any coupling information. This reduces the spectral complexities to a large extent as all multiplets collapse in singlets. This saves a lot of analysis time. Pure shift as such is not a fast method. However, we have incorporated this as it is indeed an important milestone in achieving high spectral resolution and a very important step towards minimizing the spectral analysis time.

2. Fast 2D NMR approaches

The power of NMR is well realized because it is the only noninvasive technique which has an atomic level resolution and can work well for both solution and solid-state samples. Although one-dimensional NMR in principle contains a wealth of information, the situation becomes severely limited due to signal overlap for small organic molecules onwards. This requires spreading the signals in other dimensions which can be the same nuclei or different NMR active nuclei. Here, concepts of 2D or multidimensional NMR arise, and with the development of 2D, 3D, and even higher-dimensional NMR, the overlap problem is solved even for large biomolecules. The price we pay is an increase in measurement time. Any higher-dimensional spectra require certain time increments to achieve certain spectral resolution, and as a result, the measurement time increases enormously as the number of dimensions increases. Generally, for the same sample moving from 1D NMR to 2D, the measurement time increases by at least two orders of magnitudes. Moving from 2D to 3D the experimental time increases further by more than one

order of magnitude. Similarly, 4D and 5D become so time-consuming that they are hardly used in practice. This is the main reason for steady research in the direction to overcome the time limitation so that speedy acquisition can be feasible. Here we will discuss some of these advancements and will limit the discussion to 2D. The basic building block of the 2D or any multidimensional experiment consists of a preparation period, evolution period that incorporates incremental time delay, mixing period, and one recycle delay which is required for getting longitudinal magnetization back to equilibrium. There have been numerous methodological developments focusing on each aspect of these building blocks. Nonuniform sampling (NUS) reduces the number of incremental delays. FAST or SOFAST methods reduce recycle delay which is on the order of seconds. Ultrafast works on preparation and mixing components, and with the help of slice selection gradient, single-scan experiments are designed. We will go through each aspect separately. Lastly, there is pure shift NMR which focuses on enhancement of resolution.

2.1 SOFAST 2D NMR

We first focus on SOFAST method that aims to reduce the inter-scan delay and eventually manages to lower the 2D acquisition times [84]. This technique is optimized in order to obtain fast and sensitive 2D NMR HMQC with ^1H-^{15}N and ^1H-^{13}C correlation of biomolecules with a different size range [84]. The SOFAST-HMQC experiment makes use of selective ^1H pulses affecting only a portion of proton spins while keeping a large pool of proton spins unperturbed at Z axis. These unperturbed spins enhance the spin-lattice relaxation (T1) rate via dipolar interactions with the perturbed spins and effective recycle delay decreases. Additionally, selective excitation is achieved with Ernst angle excitation which helps in acquiring more sensitivity [85] at lower recycle delay. Therefore, the concerted use of Ernst angle excitation and higher repetition rates of the pulse sequence yield high signal-to-noise ratio compared to conventional experiments. This strongly is recommended in biomolecular NMR, to study protein modifications and real-time molecular kinetics [85] (**Figure 2**). Numerous versions of SOFAST-HMQC experiments have been proposed for different purposes depending on the experimental conditions [86, 87]. As an example, ^{13}C methyl SOFAST experiment has been developed, allowing the recording of high-quality methyl ^1H-^{13}C correlation spectra of protein with high
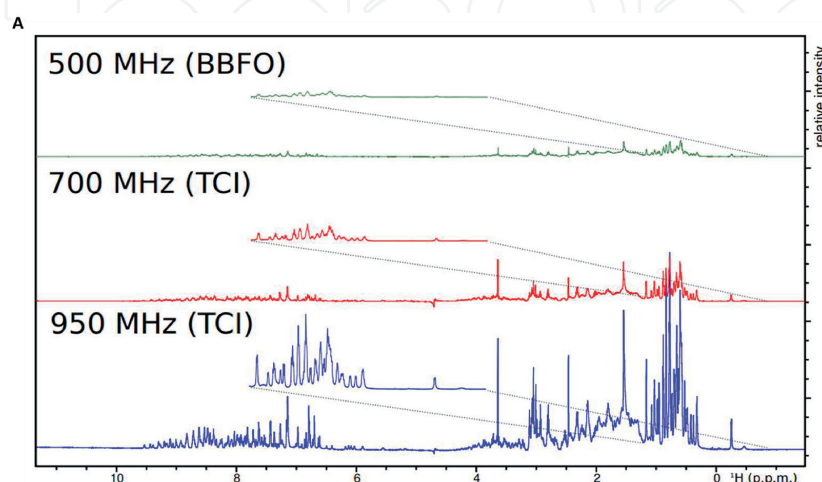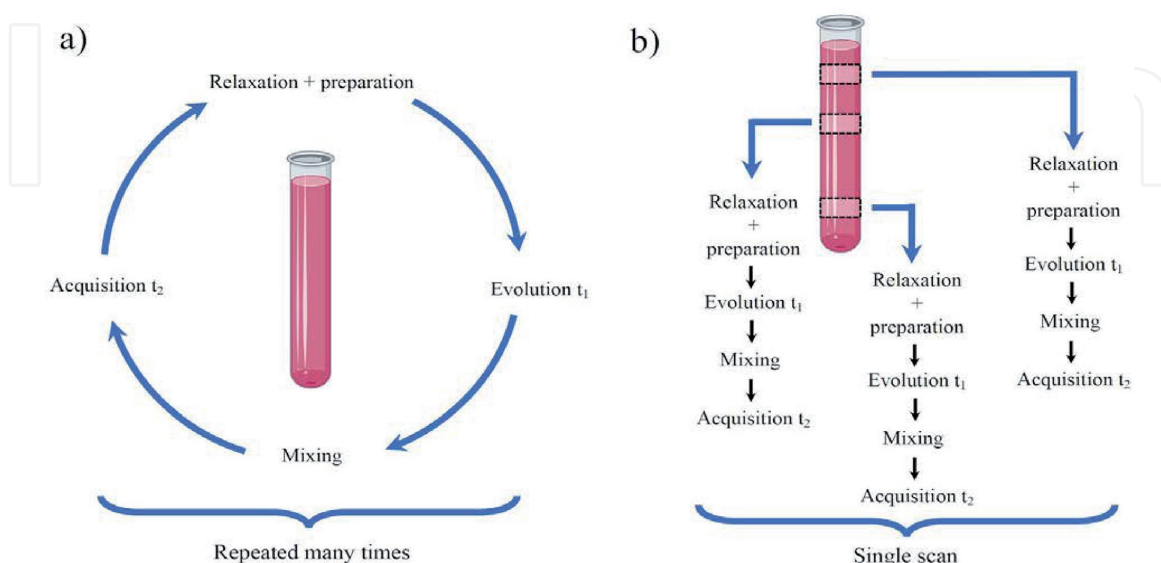

Figure 2.
Profits of the high-field NMR magnetic fields on spectral resolution and sensitivity. High-resolution 1D 1H NMR spectral of a representative small well-folded protein (A) U-15 N human ubiquitin of 1 mM concentration in 20 mM phosphate buffer, 10% D₂O, and 0.02% NaN₃ recorded with the same parameter set at three spectrometers working at 500, 700, and 950 MHz proton frequencies at 24°C.

molecular weight in a few second acquisition time [88]. Methyl groups are used as spectroscopic probes of protein structure, dynamics, and kinetics because they are dispersed naturally throughout regions of folded protein. It has been shown that the use of methyl groups as a probe is very useful, particularly for drug binding and molecular interactions studies [88].

For biomolecular NMR applications, the use of longitudinal relaxation optimized experiments permits the acceleration of 2D NMR data acquisition which provides a tool for high-throughput screening for macromolecules like proteins or nucleic acids. Such experiments allow one to obtain a high signal-to-noise ratio compared to its counterpart, as mentioned previously. Theillet et al. present an example of the use of SOFAST-HMQC experiment in order to monitor protein phosphorylation reactions [89]. In this study, the N-terminal transactivation domain of human p53 ^{15}N-labeled protein was phosphorylated by either recombinant enzyme or endogenous kinases present in cell extracts. The protein phosphorylation reactions then were monitored by SOFAST-HMQC NMR experiments in real time to obtain a high atomic-resolution understating of phosphorylations level of serine and threonine residues in kinase reactions [89].

2.2 Ultrafast 2D NMR

Secondly, we focus on ultrafast 2D NMR that is able to deliver any kind of 2D NMR spectra involving homo- or heteronuclear correlation in a single scan and, therefore, in a fraction of a second. The basis of this approach relies on the concept of revealing the spin interactions measured by spatiotemporal encoding [90]. The strategy is essentially that, instead of measuring N1 successive experiments multiple times on the sample with an independent increase of time, a better way to do it, as in the case of UF spectroscopy, is the one that "divides" the sample into N1 fractions and records all of them simultaneously within one scan (**Figure 3**) [90]. The application of slice-selective gradients encodes different phases in different slices similarly to incremental decay. As a result, the entire spectrum can be built from a single scan. However, the cost is reduced sensitivity.

Figure 3.
Comparison between the conventional and UF 2D NMR approaches. (a) Conventional approach of 2D NMR is done on the sample by measuring N1 successive experiments multiple times with an independent increase of time. (b) UF 2D NMR approach can be conceptualized as "dividing" the sample to fractions and recording all of them simultaneously within one scan [adapted and modified from ref. [90]].

The so-called fast 2D NMR spectroscopy has found multiple applications in the field of metabolomics [86, 91, 92], as well as in the analysis of natural products [92–94]. The ultrafast quantitative 2D NMR spectroscopy is also an appropriate choice for measuring ^{13}C enrichments in a single scan (with ultrafast COSY and zTOCSY experiments). The authors of this study have reported an accuracy of 1–2%, an average precision of 3%, which is believed to be better than those previously achieved with different approaches [95]. This approach is also used in acquiring R1 longitudinal relaxation rates [4].

2.3 Nonuniform sampling and processing

Multidimensional experiments have two significant limitations: sensitivity and low resolution, especially for large molecules. For obtaining good sensitivity, we need a very high number of scans for ^{13}C, ^{15}N-labeled samples, and J-coupling transfer, and dipole-dipole interactions were utilized. For instance, metabolomics research is based on the analysis of the natural abundance of carbon and hydrogen nuclei; therefore, the sensitivity remains the main issue because isotope labeling cannot be applied. Enhancing the sensitivity and reducing the acquisition time may require the concerted use of methods such as SOFAST method and nonuniform sampling while decreasing the number of recorded points for indirect dimensions. Sometimes the single-scan approach can be used [96–98]. The main limitation of single-scan methods, however, is recording only ^{1}H plane, even for 2D experiments [99].

Some studies demonstrated that a combination of fast methods with reducing the number of data points can benefit multidimensional NMR spectra like BEST-Trosy, which provide magnification of sensitivity and resolution for structural and kinetic investigation of biomolecular and complex systems [100].

Normal 2D experiments yield 2D spectra, while during data acquisition in the indirect dimension (marked as t1), the second "vertical" dimension has incremental time delay. This time delay is linear, and the gap between the time points is the same. In contrast, NUS approaches the t1 change in a semi-random way. In NUS, only some of the time increments are recorded, and then the complete spectra are constructed after reconstructing the missing data points. Various algorithms are designed to select which data points to acquire and what will lead to successful reconstruction. The advantage of NUS is that the same level of resolution can be achieved with significantly fewer t1 increments. Thus, overall measurement time decreases. This is practical for NMR spectroscopist because this means one can obtain well-resolved 2D NMR spectra in less time.

There has been extensive research on the optimization of finding suitable combinations for sampling schemes and reconstruction algorithms [98]. NUS sampling conventionally selects random acquisition points in the indirect dimensions. The "randomness" of NUS is restricted by factors such as the transverse relaxation rate. Exponentially weighted random sampling is designed to overcome the T2 decay as more signals decay incrementally in the initial phase. Poisson gap sampling has also been used to yield excellent results as described later. Processing the recorded data can be classified in several ways [101]. For example, the sparse time domain data may be directly transformed with Fourier transform (FT) algorithms, and subsequently the missing data are added by interpolation and then followed by conventional Fourier transform [101] in the second dimension. This can also be reversed for reconstruction, followed by FT in both dimensions.

NUS can be a good tool, especially for triple resonance experiments, which have a low dynamic range. An almost identical intensity and a small number of peaks allow the use of low-density sampling. For a highly dynamic range of signals in experiments like NOESY (which provides information on the restraint through

the space between atoms), reconstruction will be better with less mixing time. The positive results of reconstructed spectra were demonstrated with 20% and more sparse incremental time [102]. NUS methods have also been widely used for resonance backbone and side-chain assignments of macromolecules, determination of cross-correlated relaxation rates (CCR), kinetic processes, posttranslational modifications, and metabolomics [103].

The determinative criteria of spectra quality have a big gap in the random sampling schedule, especially at the beginning and at the end of dataset points. To overcome this limitation, the Poisson distribution [104] was used with sinusoidal variation of average gap length. In particular, sinusoidal variation of gap length keeps a degree of order in the sampling schedule, and it condenses points at the beginning of the time domain data. This provides significant benefits for time domain data with exponential decay [104].

The approach for the reconstruction of traditional uniform sampling is FT [105]. The processing spectra from NUS acquisition experiments, however, demand different types of reconstruction methods because regular discrete FT leads to artifacts as several data points are missing. Among the reconstruction algorithms, multidimensional decomposition (MDD) is the model where the full matrix of different dimensions can be identified as a sum of one-dimensional vectors (components), in which each of the components is coherent to a peak or group of peaks. MDD essentially breaks multidimensional data into sets of one-dimensional data, which are much easier to analyze and solve. The overlap in multidimensional NMR is common, and since MDD is able to resolve the overlapping resonances, MDD is well equipped for resolution enhancement of crowded datasets. MDD typically only needs a subset (20–30%) of the full dataset in order to reconstruct the full NMR signal [106, 107]. **Figure 4** gives a visual demonstration of the sampling of the full dataset to make a smaller dataset and create the spectra. One paper reports a method demonstrated on nonuniformly sampled [16] N-NOESY-HSQC datasets recorded for the 14 kDa protein azurin [108]. MDD typically only needs a subset (20–30%) of the full dataset in order to reconstruct the full NMR signal [106, 107].

Another algorithm is compressed sensing (CS) which is one of the best reconstruction methods available. Maximum entropy method (Max Ent) is another algorithm. CS is very useful in under sampled multidimensional experiments for solid-state NMR, fast metabolomics studies, and chemical-shift imaging [109, 110]. CS was demonstrated to give better reconstruction of weaker peaks, compared to an existing Max Ent implementation [110]. In one paper, two time-equivalent 3D
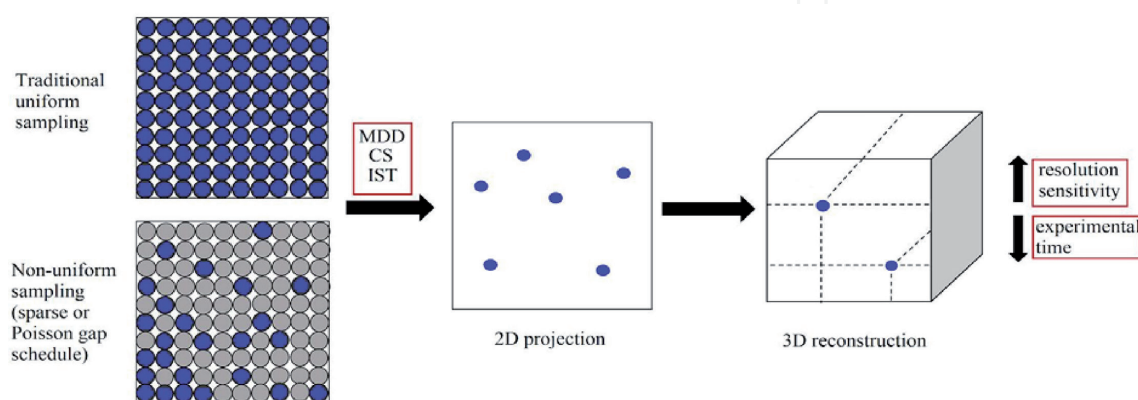
Figure 4.
Visual demonstration of NUS for obtaining NMR spectra. NUS takes a small subset of the total dataset (crowded blue dots) and uses that comparatively small dataset (20–30% of the total dataset) to create an accurate NMR spectra of the molecule of interest.

NUS TROSY-HNCA semi-constant time experiments were recorded, with CS giving better overall peak resolution [111].

Iterative soft threshold (IST) is another type of reconstruction algorithm. Like other methods, IST is able to "clean up" the NMR data by filtering the spectra. It does this by setting a threshold value and then, as its name suggests, runs through a fixed number of iterations until the procedure is complete. The best approach to IST is to use it in sync with an optimized NUS schedule. IST computational methods only take a reasonable amount of time and greatly reduce the analysis time required to obtain NMR spectra [112, 113]. One paper reports successful application of the IST approach to the ubiquitin protein. On a dual core, 2.2GHz CPU, the overall time required to process the sampled NMR dataset took under 60 seconds [114], a significant contribution to decreasing total analysis time.

3. Pure shift NMR

Proton NMR suffers from extensive signals splitting due to the presence of many weak and strong couplings, leading to significant spectral overlap, even for small molecules. The problem is severe at low magnetic fields. Although the coupling constant gives useful structural information, difficulty in obtaining them due to strong overlap often becomes a major roadblock. Ideally, a spectrum, where all multiplets collapse in singlets, gives the most straightforward information which can be achieved by pure shift. Subsequently, the analysis of couplings can be introduced in a systematic manner. The problem is simple for ^{13}C NMR as natural isotopic abundance is low and only singlets are present. However, the situation becomes complex for ^{13}C isotope-enriched samples, especially for large biomolecules. In those cases, spin-state-selective decoupling are most commonly used. Both components of the splitting due to ^{13}C-^{13}C coupling are recorded in a linear combination of inphase mode and antiphase mode. This is known as IPAP (inphase-antiphase) [115] or direct excitation of single-quantum coherences [116]. This method mainly works because of clear ^{13}C signals in different spectral shift regions and different and distinct spin-spin coupling values between those different classes of spins. This is clearly not the case for proton NMR where coupling values spread much wider ranges and also spectral overlap is more severe.

Pure shift NMR focuses on chemical shift and removes homonuclear couplings which lead to complex multiplet structure of individual peaks. This results in severe overlap even for small molecules and particularly for ^{1}H spectra since the chemical-shift dispersion of the proton is normally lower (~10 ppm) compared to other heteronuclei like ^{13}C or ^{15}N. Having the highest gyromagnetic ratio and natural abundance, ^{1}H NMR is the most obvious choice for almost all routine and conventional small and large molecule analysis. However, the spectral overlap due to homonuclear coupling limiting the resolution presents a major limitation of 1D proton NMR spectroscopy. Pure shift NMR was developed to simplify the overlapped proton NMR spectra by keeping only chemical-shift information. There have been great contributions coming first from Zangger [117–120] and subsequently by Morris and others [117, 121–127] in this direction. Some of the recent advances are presented here.

First experimental demonstration of homonuclear broadband decoupling in proton NMR was reported by Ernst and coworkers [128]. The basic pulse program consists of a 90° excitation pulse followed by spin echo. So, in direction dimension, homonuclear coupling is active, and chemical-shift evolution refocuses at the end of t1, while both chemical-shift and homonuclear couplings are active during acquisition. So, the resultant spectrum shows tilted multiplets where the indirect

dimension spreads out the J-coupling. Now, 45° projection of the 2D spectrum yields pure shift spectra where all the scalar couplings have been removed. The main problem of J-resolved spectra is the twisted phase accumulation during t1 which leads to both absorptive and dispersive phase components in the indirect dimension. This results in partial or complete cancelation after taking the projection. To avoid this, normally absolute value mode calculation or power spectrum is taken before the calculation 45° projection. This generates broad peaks with long tails in the final decoupled spectrum.

The most prominent contribution came from Zangger and Sterk [120] who employed selective pulses and gradient encoding simultaneously. In this approach, first a selective 90° pulse was applied, followed by an incremental delay and combination of hard and soft selective 180° pulse with the same incremental delay and subsequent acquisition. The pulses were applied with weak gradient encoding implying spatial resolution in the sample. Hence, the whole sample is now divided in different slices, and the first frequency-selective pulse excites signals of different frequencies in different slices of the sample tube. Most importantly all signals are excited at once, but the price we pay is loss in sensitivity as the effective signal is coming from a single slice. Normally, the first experiment proposed was ~2% sensitive compared to conventional 1D NMR. In each slice, selective signals get excited by the selective first excitation pulse. Subsequently, coupling with all other spins evolves in the incremental delay. Next the selective spins undergo complete 360° rotation, while all other spins undergo 180° rotation initiated by hard 180° pulse. Therefore, after the same incremental delay, the J-coupling between the selected spin with all other spins gets refocused and homonuclear decoupling is achieved. The initially proposed scheme recorded FID with different incremental time delays. Then, from each FID, the first few points (tc/DW) determined by the incremental delay (tc) and the dwell time (DW) were concatenated to generate the final FID. The excitation bandwidth is determined by the relative proximity of the scalar-coupled protons. If coupled protons with very similar chemical shifts need to be resolved, then the selective pulses must be highly selective. This also implies the application of higher gradient strengths to generate more slices to cover the full spectral width which decreases sensitivity immensely as the effective slice thickness reduces. There have been considerable developments to overcome the limitations in the first proposed scheme.

First the two-dimensional mode of data acquisition was omitted, and the homonuclear broadband decoupling was performed during acquisition [119]. This generates a single-scan decoupling sequence like normal 1D NMR. This simplified the whole thing as these signals can be stored as regular 1D or as acquisition dimension of multidimensional spectra. Most importantly, the processing of the data becomes a routine, and there is a huge reduction of total measurement time. In this modified scheme shown in **Figure 5**, the acquisition is interrupted approximately every $1/3(^3JHH)$ to incorporate decoupling block. Additionally the decoupling of the signals should be placed right in the middle of the data chunk. So the first and the last acquisition block length is only half as long of all other blocks sandwiched between two.

Since relaxation is always there, some chunking artifacts arise after Fourier transform as FID gets interrupted in between. Further, to reduce the overall measurement time, this slice-selective excitation was combined with fast pulsing by shifting the frequency of the selective pulse between individual scans [117]. In slice-selective decoupling, in each slice only the excited magnetization is used for the acquisition. So, by shifting the offset of the 90° excitation pulse and also the selective 180° refocusing pulse after every scan, the unused equilibrium magnetization can be used without any recycle delay. The next scan or the subsequent scans with different offset act as recycle delay for the first set of spins which were excited in the first scan. This makes repetition much faster, and overall signal-to-noise ratio
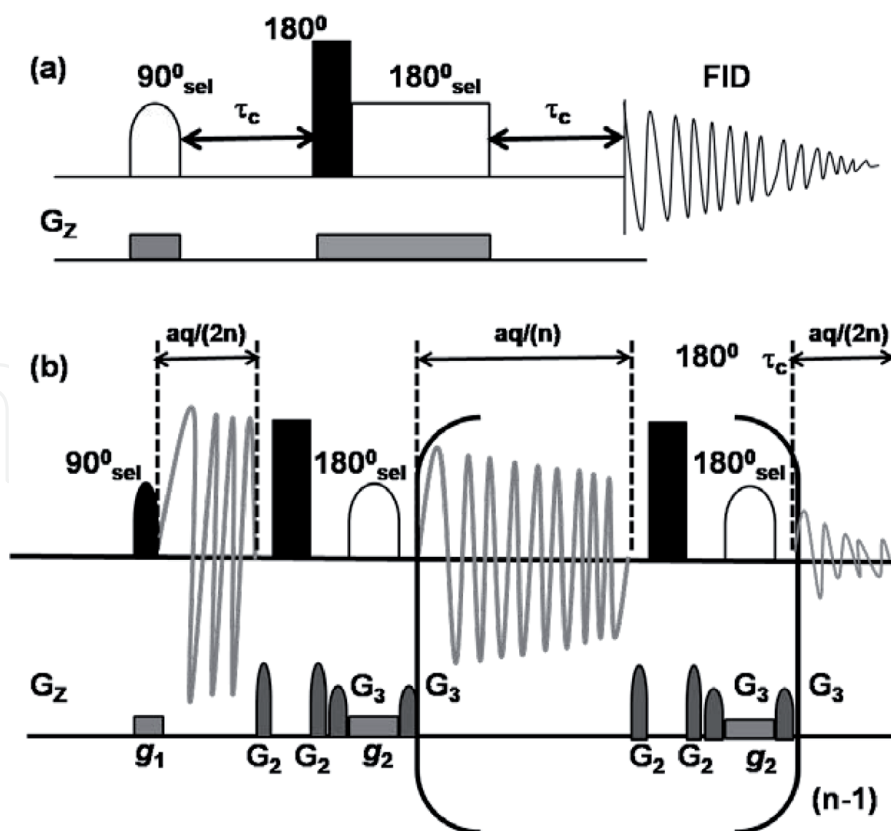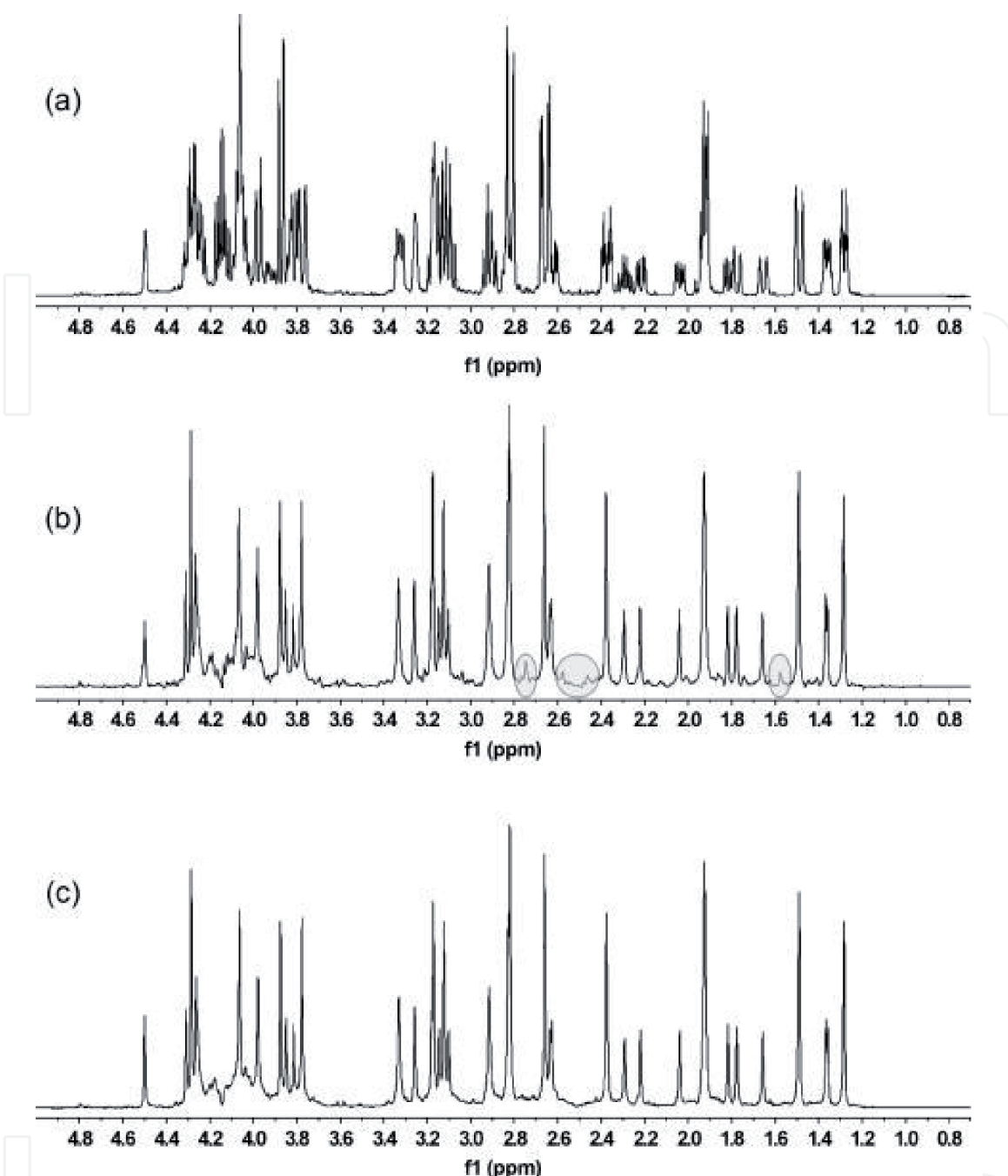
Figure 5.
(a) Pulse sequence of first Zangger-Sterk experiment. The delay τ c was incremented by several milliseconds. The first of each FID was concatenated to produce the final FID as outlined in the text. (b) the real-time slice-selective broadband homodecoupling approach. The data chunking times are variable which is represented by number n. frequency shifting is achieved for all selective pulses in parallel after each transient. The slice-selective gradients are represented by g1 and g2; G2 and G3 clear any imperfection of the refocusing pulse.

increases per unit time as shown in **Figure 6**. Apart from varying offset in between different transients, FID chunk size also varied to suppress the decoupling side-bands. The decoupling sidebands arise due to truncation of FID, and the longer the FID chunk, the more dominant are the artifacts as more antiphase magnetization can build up. On the contrary, if keeping the individual FID blocks, short leads to broad signals as more relaxation losses occur during the more frequent acquisition interruptions. The variation of chunking time between subsequent scans leads to smearing out of the decoupling sidebands which otherwise add up if they are kept the same. The slice-selective decoupling has been employed for two-dimensional homonuclear TOCSY [127, 129] and NOESY [122] spectra. The enhanced resolution in the direct dimension can be propagated to the indirect dimension by applying covariance processing which yield pure shift spectra in both the dimension for TOCSY spectrum. Additionally for heteronuclear HSQC, the slice-selective excita-tion was employed for intrinsically disordered proteins (IDPs) [130] where proton spectral dispersion is severely low. Additionally slice-selective excitation has been used for fast data acquisition [131] and was able to monitor chemical reaction from different spatial location of the sample tube [132].

Recently a new method named as pure shift yielded by chirp excitation (PSYCHE) experiment has been proposed by Morris et al. [125] which is based on anti Z-COSY experiment. This experiment employs two small flip angle chirp pulses which selectively refocus a small proportion of spins, namely, active spins, while leaving the majority ones undisturbed, namely, passive spins. Now this selection of active and passive spins are purely statistical which leads to an obvious advantage over ZS method as there the selection was based on selective pulse. The sequence is closely related to anti Z-COSY except to the fact that instead of using two hard small

Figure 6.
(a) A regular 1D spectrum, (b) pure shift with fixed chunking time of 25 ms, (c) pure shift with variable chunking time with real-time acquired spectrum of a mixture of strychnine with unknown degradation products in CDCl3. Gray circles indicate artifacts resulting from decoupling sidebands. Weak peaks visible in (b) result from additional minor compounds in the mixture, which are way below the level of artifacts in the fixed chunking time spectrum. The figure presented is from [117].

flip angle pulses, it uses two symmetric, low power, frequency-swept chirp pulses along with weak pulsed-field gradient. This gradient employs spatial resolution and suppresses the cross-peak terms as related to anti Z-COSY since they experience different chemical shifts as they are excited at different times during the chirp pulses. The major advantage of PSYCHE is that it is most sensitive among the existing pure shift methods. Several further developments are achieved to obtain ultraclean arti-fact-free spectra and applied in 2D NMR as well [133, 134]. Very recently one such modification named SAPPHIRE-PSYCHE methodology is applied for plant metabo-lomics study. In that report, single-pulse, PSYCHE, and SAPPHIRE-PSYCHE spectra were compared from aqueous extracts of *Physalis peruviana* fruits. It was found using pure shift methodology; many proton NMR signals can be cleanly observed which

were not possible in conventional NMR. These signals belong to amino acids, organic acids, and sugars which are critical components of plant mixture. One example of such metabolite was the identification of glutamic acid from Cape gooseberry which was not possible to isolate due to heavy overlap. Thus, ultra-clean pure shift spectra look very promising and provide new benchmark for metabolomics studies.

4. Summary and future perspectives

High-resolution NMR with high sensitivity is the desired aim for any NMR application. There have been significant hardwire advances in achieving high sensitivity. This includes the development of high magnetic field, cryogenically cooled probes [135], recent advent of high-mass-sensitive probe for small volume [136], etc. A combination of these advances results in an increase of sensitivity by more than an order of magnitude. These advancements fueled the developments of fast methodology. Additionally due to the sample instability, which results in the relatively fast decomposition of the biomolecule being an object of the study, the quick methods of 2D spectra acquisition needed to be developed. In this regard, previously mentioned SOFAST, NUS, etc. methodologies were developed and subsequently applied in many different scenarios [90, 93, 94, 137]. Above that, these fast methods can be also used for studying either chemical or biochemical processes that are characterized by the relatively fast kinetics and also carry some structural information, like hydrogen/deuterium (H/D) exchange [138, 139]. With NUS approach, one problem is the generation of very large file size, especially for 4D and 5D experiments. Artifacts are also produced, which generates "unwanted" signals that do not accurately represent the studied molecule. However, there is considerable effort in the scientific community to reduce the amount of produced artifacts. For example, newly proposed MUNIN uses three-way decomposition and a simplified model for NMR spectra based on generally accepted assumptions. This method achieves high-resolution and good sensitivity while avoiding artifacts [106]. Also, a signal separation algorithm is shown to suppress sampling artifacts in high-resolution four-dimensional NMR spectra [140]. It is likely that NUS-based methods will continue to be used in the future, mainly because they will greatly reduce experimental time, and still provide satisfactory results with increased resolution of the spectra what facilitates their analysis. However, with the increase in magnetic field, resolution has been improved only two times and not more than that. Even with the availability of very high-field magnet such as 1.1 GHz and upwards which is limited, resolution was still a problem. Hence, the development of pure shift methods became necessary, and with the techniques great resolution can be achieved uniformly for all scenarios. In this regard, both the methodologies and developments in the direction of reducing measurement time and increasing resolution remain of utmost importance and have become an area of research itself.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Abdul-Hamid Emwas[1], Mawadda Alghrably[2], Samah Al-Harthi[2],
Benjamin Gabriel Poulson[2], Kacper Szczepski[2], Kousik Chandra[2]
and Mariusz Jaremko[2*]

1 Core Labs, King Abdullah University of Science and Technology (KAUST),
Thuwal, Saudi Arabia

2 Biological and Environmental Science and Engineering (BESE), King Abdullah
University of Science and Technology (KAUST), Thuwal, Saudi Arabia

*Address all correspondence to: mariusz.jaremko@kaust.edu.sa

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References

[1] Emwas AH, Roy R, McKay RT, Tenori L, Saccenti E, Gowda GAN, et al. NMR spectroscopy for metabolomics research. Metabolites. 2019;**9**(7)

[2] Jackowski K. Multinuclear NMR spectroscopy in the gas phase. Journal of Molecular Structure. 2006;**786**(2-3):215-219

[3] Jameson CJ. Gas-phase NMR spectroscopy. Chemical Reviews. 1991;**91**(7):1375-1395

[4] Hajjar D, Kremb S, Sioud S, Emwas AH, Voolstra CR, Ravasi T. Anti-cancer agents in Saudi Arabian herbals revealed by automated high-content imaging. PLoS One. 2017;**12**(6):e0177316

[5] Harvey DJ, Royle L, Radcliffe CM, Rudd PM, Dwek RA. Structural and quantitative analysis of N-linked glycans by matrix-assisted laser desorption ionization and negative ion nanospray mass spectrometry. Analytical Biochemistry. 2008;**376**(1):44-60

[6] Guleria A, Misra DP, Rawat A, Dubey D, Khetrapal CL, Bacon P, et al. NMR-based serum metabolomics discriminates Takayasu arteritis from healthy individuals: A proof-of-principle study. Journal of Proteome Research. 2015;**14**(8):3372-3381

[7] Blindauer CA, Emwas AH, Holy A, Dvorakova H, Sletten E, Sigel H. Complex formation of the antiviral 9-2-(phosphonomethoxy) ethyl adenine (PMEA) and of its N1, N3, and N7 deaza derivatives with copper(II) in aqueous solution. Chemistry—a European Journal. 1997;**3**(9):1526-1536

[8] Cao M, Zhao L, Chen H, Xue W, Lin D. NMR-based metabolomic analysis of human bladder cancer. Analytical Sciences. 2012;**28**(5): 451-456

[9] Ali M, Shaw DR, Zhang L, Haroon MF, Narita Y, Emwas AH, et al. Aggregation ability of three phylogenetically distant anammox bacterial species. Water Research. 2018;**143**:10-18

[10] Asghar S, Shahzadi T, Alazmi M, Gao X, Emwas AH, Saleem RSZ, et al. Iridium-catalyzed regioselective borylation of substituted biaryls. Synthesis-Stuttgart. 2018;**50**(11):2211-2220

[11] Bin Farrukh SU, Javed I, Ather AQ, Emwas AH, Alazmi M, Gao X, et al. Synthesis and identification of novel pyridazinylpyrazolone based diazo compounds as inhibitors of human islet amyloid polypeptide aggregation. Bioorganic Chemistry. 2019;**84**:339-346

[12] Cui GX, Liew YJ, Li Y, Kharbatia N, Zahran NI, Emwas AH, et al. Host-dependent nitrogen recycling as a mechanism of symbiont control in Aiptasia. PLoS Genetics. 2019;**15**(6):e1008189

[13] Gallo A, Farinha ASF, Dinis M, Emwas AH, Santana A, Nielsen RJ, et al. The chemical reactions in electrosprays of water do not always correspond to those at the pristine air-water interface. Chemical Science. 2019;**10**(9):2566-2577

[14] Jameel AGA, Naser N, Emwas AH, Sarathy SM. Surrogate formulation for diesel and jet fuels using the minimalist functional group (MFG) approach. Proceedings of the Combustion Institute. 2019;**37**(4):4663-4671

[15] Jameel AGA, Naser N, Issayev G, Touitou J, Ghosh MK, Emwas AH, et al. A minimalist functional group (MFG) approach for surrogate fuel formulation. Combustion and Flame. 2018;**192**:250-271

[16] Jameel AGA, Van Oudenhoven V, Emwas AH, Sarathy SM. Predicting octane number using nuclear magnetic resonance spectroscopy and artificial neural networks. Energy and Fuels. 2018;**32**(5):6309-6329

[17] Aburabie J, Emwas AH, Peinemann KV. Silane-crosslinked asymmetric polythiosemicarbazide membranes for organic solvent nanofiltration. Macromolecular Materials and Engineering. 2019;**304**(1):1800551

[18] Alahmari F, Davaasuren B, Emwas AH, Costa P, Rothenberger A. Tris(ethylenediamine)nickel(II) thio-hydroxogermanate monohydrate: Synthesis, crystal structure,^{1}H-NMR, EPR, optical and magnetic properties. Inorganica Chimica Acta. 2019;**488**:145-151

[19] Alahmari F, Davaasuren B, Emwas AH, Rothenberger A. Thioaluminogermanate M(AlS$_2$)(GeS$_2$)$_4$ (M = Na, Ag, Cu): Synthesis, crystal structures, characterization, ion-exchange and solid-state Al-27 and Na-23 NMR spectroscopy. Inorganic Chemistry. 2018;**57**(7):3713-3719

[20] Alahmari F, Dey S, Emwas AH, Davaasuren B, Rothenberger A. Layered copper thioaluminate K$_2$Cu$_3$AlS$_4$: Synthesis, crystal structure, characterization and solid-state Al-27 and K-39 NMR studies. Journal of Alloys and Compounds. 2019;**776**:1041-1047

[21] Aljuhani MA, Zhang ZY, Barman S, El Eter M, Failvene L, Ould-Chikh S, et al. Mechanistic study of Hydroamination of alkyne through tantalum-based silica-supported surface species. ACS Catalysis. 2019;**9**(9):8719-8725

[22] Davaasuren B, Emwas AH, Rothenberger A. MAu$_2$GeS$_4$-chalcogel (M = Co, Ni): Heterogeneous intra- and intermolecular hydroamination catalysts. Inorganic Chemistry. 2017;**56**(16):9609-9616

[23] Mallick A, El-Zohry AM, Shekhah O, Yin J, Jia JT, Aggarwal H, et al. Unprecedented ultralow detection limit of amines using a thiadiazole-functionalized Zr(IV)-based metal-organic framework. Journal of the American Chemical Society. 2019;**141**(18):7245-7249

[24] Simoes FRF, Batra NM, Emwas AH, Costa P. Validation of alkaline oxidation as a pre-treatment method for elemental quantification in single-walled carbon nanotubes. Analytical Methods. 2019;**11**(14):1884-1890

[25] Brauckmann JO, Zolfaghari P, Verhoef R, Klop EA, de Wijs GA, Kentgens APM. Structural studies of polyaramid fibers: Solid-state NMR and first-principles modeling. Macromolecules. 2016;**49**(15):5548-5560

[26] Cabrera Y, Cabrera A, Larsen FH, Felby C. Solid-state Si-29 NMR and FTIR analyses of lignin-silica coprecipitates. Holzforschung. 2016;**70**(8):709-718

[27] Elkins MR, Wang T, Nick M, Jo H, Lemmin T, Prusiner SB, et al. Structural polymorphism of Alzheimer's beta-amyloid fibrils as controlled by an E22 switch: A solid-state NMR study. Journal of the American Chemical Society. 2016;**138**(31):9840-9852

[28] Lang S, Benz M, Obenaus U, Himmelmann R, Hunger M. Novel approach for the characterization of Lewis acidic solid catalysts by solid-state NMR spectroscopy. ChemCatChem. 2016;**8**(12):2031-2036

[29] Chu SD, Maltsev S, Emwas AH, Lorigan GA. Solid-state NMR paramagnetic relaxation enhancement immersion depth studies in phospholipid bilayers. Journal of Magnetic Resonance. 2010;**207**(1):89-94

[30] Emwas AHM, Al-Talla ZA, Guo XR, Al-Ghamdi S, Al-Masri HT. Utilizing

NMR and EPR spectroscopy to probe the role of copper in prion diseases. Magnetic Resonance in Chemistry. 2013;**51**(5):255-268

[31] Mayo D, Zhou A, Sahu I, McCarrick R, Walton P, Ring A, et al. Probing the structure of membrane proteins with electron spin echo envelope modulation spectroscopy. Protein Science. 2011;**20**(7):1100-1104

[32] Akbey U, Oschkinat H. Structural biology applications of solid state MAS DNP NMR. Journal of Magnetic Resonance. 2016;**269**:213-224

[33] Caro JA, Wand AJ. Practical aspects of high-pressure NMR spectroscopy and its applications in protein biophysics and structural biology. Methods. 2018;**148**:67-80

[34] Ciambellotti S, Turano P. Structural biology of iron-binding proteins by NMR spectroscopy. European Journal of Inorganic Chemistry. 2019;**5**:569-576

[35] Dass R, Grudziaz K, Ishikawa T, Nowakowski M, Debowska R, Kazimierczuk K. Fast 2D NMR spectroscopy for in vivo monitoring of bacterial metabolism in complex mixtures. Frontiers in Microbiology. 2017;**8**:1306

[36] Malar AA, Smith-Penzel S, Camenisch GM, Wiegand T, Samoson A, Bockmann A, et al. Quantifying proton NMR coherent linewidth in proteins under fast MAS conditions: A second moment approach. Physical Chemistry Chemical Physics. 2019;**21**(35):18850-18865

[37] Opella SJ, Marassi FM. Applications of NMR to membrane proteins. Archives of Biochemistry and Biophysics. 2017;**628**:92-101

[38] Rahman S, Byun Y, Hassan MI, Kim J, Kumar V. Towards understanding cellular structure biology: In-cell

NMR. Biochimica et Biophysica Acta - Proteins and Proteomics. 2017;**1865**(5):547-557

[39] Al-Masri HT, Emwas AHM, Al-Talla ZA, Alkordi MH. Synthesis and characterization of new N-(diphenylphosphino)-naphthylamine chalcogenides: X-ray structures of $(1\text{-}NHC_{10}H_7)P(Se)Ph\text{-}2$ and $Ph_2P(S)OP(S)Ph\text{-}2$. Phosphorus Sulfur Silicon and the Related Elements. 2012;**187**(9):1082-1090

[40] Atiqullah M, Al-Harthi MA, Anantawaraskul S, Emwas AHM. Ethylene homo- and copolymerization chain-transfers: A perspective from supported $(nBuCp)(2)ZrCl_2$ catalyst active Centre distribution. Journal of Chemical Sciences. 2015;**127**(4):717-728

[41] Batool F, Parveen S, Emwas AH, Sioud S, Gao X, Munawar MA, et al. Synthesis of fluoroalkoxy substituted arylboronic esters by iridium-catalyzed aromatic C-H borylation. Organic Letters. 2015;**17**(17):4256-4259

[42] Jameel AGA, Elbaz AM, Emwas AH, Roberts WL, Sarathy SM. Calculation of average molecular parameters, functional groups, and a surrogate molecule for heavy fuel oils using H-1 and C-13 nuclear magnetic resonance spectroscopy. Energy and Fuels. 2016;**30**(5):3894-3905

[43] Jameel AGA, Naser N, Emwas AH, Dooley S, Sarathy SM. Predicting fuel ignition quality using H-1 NMR spectroscopy and multiple linear regression. Energy and Fuels. 2016;**30**(11):9819-9835

[44] Li S, Winters H, Jeong S, Emwas AH, Vigneswaran S, Amy GL. Marine bacterial transparent exopolymer particles (TEP) and TEP precursors: Characterization and RO fouling potential. Desalination. 2016;**379**:68-74

[45] Blumich B, Singh K. Desktop NMR and its applications from materials science to organic chemistry. Angewandte Chemie International Edition. 2018;**57**(24):6996-7010

[46] He TY, Musah RA. Multidimensional high-resolution NMR structural characterization of a carborane cluster derivative: The case of 2-amino-3-(1,7-dicarba-closo-dodecaboranyl-1-thio)propanoic acid. Polyhedron. 2019;**163**:171-177

[47] Saito T, Yamazaki T, Numata M. Development of nuclear magnetic resonance as a tool of quantitative analysis for organic materials. Metrologia. 2019;**56**(5):054002

[48] Yu Y, Rebek J. Reactions of folded molecules in water. Accounts of Chemical Research. 2018;**51**(12):3031-3040

[49] Campbell K, Ooms KJ, Ferguson MJ, Stang PJ, Wasylishen RE, Tykwinski RR. Shape-persistent macrocycles - self-assembly reactions and characterization by hyperpolarized Xe-129 NMR spectroscopy. Canadian Journal of Chemistry. 2011;**89**(10):1264-1276

[50] Figueiredo S, Gomes AC, Neves P, Amarante TR, Paz FAA, Soares R, et al. Synthesis, structural elucidation, and application of a pyrazolylpyridine-molybdenum oxide composite as a heterogeneous catalyst for olefin epoxidation. Inorganic Chemistry. 2012;**51**(15):8629-8635

[51] Primikyri A, Kyriakou E, Charisiadis P, Tsiafoulis C, Stamatis H, Tzakos AG, et al. Fine-tuning of the diffusion dimension of -OH groups for high resolution DOSY NMR applications in crude enzymatic transformations and mixtures of organic compounds. Tetrahedron. 2012;**68**(34):6887-6891

[52] Rudzinska E, Dziedziola G, Berlicki L, Kafarski P. Enantiodifferentiation of alpha-hydroxyalkanephosphonic acids in P-31 NMR with application of alpha-cyclodextrin as chiral discriminating agent. Chirality. 2010;**22**(1):63-68

[53] Atiqullah M, Adamu S, Emwas AHM. UHMW Ziegler-Natta polyethylene: Synthesis, crystallization, and melt behavior. Journal of the Taiwan Institute of Chemical Engineers. 2017;**76**:141-155

[54] Adamu S, Atiqullah M, Malaibari ZO, Al-Harthi MA, Emwas AHM, Ul-Hamid A. Metallocene-catalyzed ethylene-alpha-olefin isomeric copolymerization: A perspective from hydrodynamic boundary layer mass transfer and design of MAO anion. Journal of the Taiwan Institute of Chemical Engineers. 2016;**60**:92-105

[55] Alkordi MH, Haikal RR, Hassan YS, Emwas AH, Belmabkhout Y. Poly-functional porous-organic polymers to access functionality - CO_2 sorption energetic relationships. Journal of Materials Chemistry A. 2015;**3**(45):22584-22590

[56] Atiqullah M, Anantawaraskul S, Emwas AHM, Al-Harthi MA, Hussain I, Ul-Hamid A, et al. Effects of supported ((BuCp)-Bu-n)(2)ZrCl2 catalyst active-center distribution on ethylene-1-hexene copolymer backbone heterogeneity and thermal behaviors. Industrial and Engineering Chemistry Research. 2013;**52**(27):9359-9373

[57] Atiqullah M, Anantawaraskul S, Emwas AHM, Al-Harthi MA, Hussain I, Ul-Hamid A, et al. Silica-supported ((BuCp)-Bu-n)(2)ZrCl$_2$: Effect of catalyst active center distribution on ethylene-1-hexene copoly-merization. Polymer International. 2014;**63**(6):955-972

[58] Atiqullah M, Winston MS, Bercaw JE, Hussain I, Fazal A, Al-Harthi MA, et al. Effects of a

vanadium post-metallocene catalyst-induced polymer backbone inhomogeneity on UV oxidative degradation of the resulting polyethylene film. Polymer Degradation and Stability. 2012;**97**(7):1164-1177

[59] Bahuleyan BK, De SK, Sarath PU, Furquan SA, Masihullah JK, Emwas AH, et al. Effect of aluminium nitride on the properties of polyethylene obtained by In situ polymerization using Ni(II) diimine complex. Macromolecular Research. 2012;**20**(7):772-775

[60] Kamal MS, Bahuleyan BK, Sohail OB, Emwas AHM, Bercaw JE, Al-Harthi MA. Crystallization analysis fractionation of poly(ethylene-co-styrene) produced by metallocene catalysts. Polymer Bulletin. 2013;**70**(9):2645-2656

[61] Arachchilage I, Patel MK, Harmon JP. Synthesizing radiation-hard polymer and copolymers using laccol monomers extracted from lacquer tree toxicodendron succedanea via cationic polymerization. Polymer Engineering and Science. 2019;**59**(8):1611-1623

[62] Arrabal-Campos FM, Aguilera-Saez LM, Fernandez I. Algebraic reconstruction technique for diffusion NMR experiments. Application to the molecular weight prediction of polymers. Journal of Physical Chemistry A. 2019;**123**(4):943-950

[63] Brown SP. Advanced solid-state NMR methods for characterising structure and self-assembly in supramolecular chemistry, polymers and hydrogels. Current Opinion in Colloid and Interface Science. 2018;**33**:86-98

[64] Zare Y, Dabbaghi A, Rahmani S. Increasing the hydrophilicity of star-shaped amphiphilic co-networks by using of PEG and dendritic s-PCL cross-linkers. Polymers for Advanced Technologies. 2019

[65] Bosnjakovic-Pavlovic N, Bajuk-Bogdanovic D, Zakrzewska J, Yan ZY, Holclajtner-Antunovic I, Gillet JM, et al. Reactivity of 12-tungstophosphoric acid and its inhibitor potency toward Na+/K+-ATPase: A combined P-31 NMR study, ab initio calculations and crystallographic analysis. Journal of Inorganic Biochemistry. 2017;**176**:90-99

[66] Brauniger T, Jansen M. Solid-state NMR spectroscopy of quadrupolar nuclei in inorganic chemistry. Zeitschrift für Anorganische und Allgemeine Chemie. 2013;**639**(6):857-879

[67] Ha M, Thiessen AN, Sergeyev IV, Veinot JGC, Michaelis VK. Endogenous dynamic nuclear polarization NMR of hydride-terminated silicon nanoparticles. Solid State Nuclear Magnetic Resonance. 2019;**100**:77-84

[68] Marbella LE, Millstone JE. NMR techniques for noble metal nanoparticles. Chemistry of Materials. 2015;**27**(8):2721-2739

[69] Martin RW, Kelly JE, Collier KA. Spatial reorientation experiments for NMR of solids and partially oriented liquids. Progress in Nuclear Magnetic Resonance Spectroscopy. 2015;**90-91**:92-122

[70] Alezi D, Belmabkhout Y, Suyetin M, Bhatt PM, Weselinski LJ, Solovyeva V, et al. MOF crystal chemistry paving the way to gas storage needs: Aluminum-based soc-MOF for CH_4, O_2, and CO_2 storage. Journal of the American Chemical Society. 2015;**137**(41):13308-13318

[71] Dey R, Samantaray MK, Poater A, Hamieh A, Kavitake S, Abou-Hamad E, et al. Synthesis and characterization of a homogeneous and silica supported homoleptic cationic tungsten(VI) methyl complex: Application in olefin metathesis. Chemical Communications. 2016;**52**(75):11270-11273

[72] Geng GQ, Taylor R, Bae S, Hernandez-Cruz D, Kilcoyne DA, Emwas AH, et al. Atomic and nano-scale characterization of a 50-year-old hydrated C3S paste. Cement and Concrete Research. 2015;**77**:36-46

[73] Kirchheim AP, Dal Molin DC, Fischer P, Emwas AH, Provis JL, Monteiro PJM. Real-time high-resolution X-ray imaging and nuclear magnetic resonance study of the hydration of pure and Na-doped C(3) A in the presence of sulfates. Inorganic Chemistry. 2011;**50**(4):1203-1212

[74] Mroue KH, Emwas AHM, Power WP. Solid-state Al-27 nuclear magnetic resonance investigation of three aluminum-centered dyes. Canadian Journal of Chemistry. 2010;**88**(2):111-123

[75] Saliba D, Ezzeddine A, Emwas AH, Khashab NM, Al-Ghoul M. Dynamics and mechanism of intercalation/de-intercalation of Rhodamine B during the polymorphic transformation of the CdAl layered double hydroxide to the brucite-like cadmium hydroxide. Crystal Growth and Design. 2016;**16**(8):4327-4335

[76] Grigor'ev GY, Nabiev SS. Production and applications of spin-polarized isotopes of noble gases. Russian Journal of Physical Chemistry B. 2018;**12**(3):363-377

[77] Witte C, Kunth M, Dopfert J, Rossella F, Schroder L. Hyperpolarized xenon for NMR and MRI applications. Journal of Visualized Experiments. 2012;**67**:e4268

[78] Auccaise R, Araujo-Ferreira AG, Sarthour RS, Oliveira IS, Bonagamba TJ, Roditi I. Spin squeezing in a quadrupolar nuclei NMR system. Physical Review Letters. 2015;**114**(4)

[79] Glenn DR, Bucher DB, Lee J, Lukin MD, Park H, Walsworth RL. High-resolution magnetic resonance spectroscopy using a solid-state spin sensor. Nature. 2018;**555**(7696):351

[80] Mananga ES, Charpentier T. Revisiting the applications of Floquet-Magnus and Fer expansion approaches in physics and solid-state NMR. International Journal of Modern Physics B. 2018;**32**(22):1850236

[81] Xin T, Wang BX, Li KR, Kong XY, Wei SJ, Wang T, et al. Nuclear magnetic resonance for quantum computing: Techniques and recent achievements. Chinese Physics B. 2018;**27**(2)

[82] Emwas AH, Saunders M, Ludwig C, Gunther UL. Determinants for optimal enhancement in ex situ DNP experiments. Applied Magnetic Resonance. 2008;**34**(3-4):483-494

[83] Mattar SM, Emwas AH, Calhoun LA. Spectroscopic studies of the intermediates in the conversion of 1,4,11,12-tetrahydro-9,10-anthraquinone to 9,10-anthraquinone by reaction with oxygen under basic conditions. Journal of Physical Chemistry A. 2004;**108**(52):11545-11553

[84] Schanda P, Kupče Ē, Brutscher B. SOFAST-HMQC experiments for recording two-dimensional deteronuclear correlation spectra of proteins within a few seconds. Journal of Biomolecular NMR. 2005;**33**(4):199-211

[85] Brutscher B. SOFAST HMQC. In: GCK R, editor. Encyclopedia of Biophysics. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. p. 2407

[86] Ghosh S, Sengupta A, Chandra K. SOFAST-HMQC—An efficient tool for metabolomics. Analytical and Bioanalytical Chemistry. 2017;**409**(29):6731-6738

[87] Theillet F-X, Rose HM, Liokatis S, Binolfi A, Thongwichian R, Stuiver M,

et al. Corrigendum: Site-specific NMR mapping and time-resolved monitoring of serine and threonine phosphorylation in reconstituted kinase reactions and mammalian cell extracts. Nature Protocols. 2016;**11**(1):192

[88] Schanda P. Development and Application of Fast NMR Methods for the Study of Protein Structure and Dynamics. Biological Physics. Université Joseph-Fourier - Grenoble I. 2007. English

[89] Zhan H, Lin X, Wei Z, Ye Q, Cai S, You X, et al. A single-scan inhomogeneity-tolerant NMR method for high-resolution two-dimensional J-resolved spectroscopy. IEEE Transactions on Biomedical Engineering. 2018;**66**(6):1559-1566

[90] Giraudeau P, Frydman L. Ultrafast 2D NMR: An emerging tool in analytical spectroscopy. Annual Review of Analytical Chemistry. 2014;7:129-161

[91] Emwas A-H, Roy R, McKay RT, Tenori L, Saccenti E, Gowda G, et al. NMR spectroscopy for metabolomics research. Metabolites. 2019;**9**(7):123

[92] Ghosh S, Sengupta A, Chandra K. Quantitative metabolic profiling of NMR spectral signatures of branched chain amino acids in blood serum. Amino Acids. 2015;**47**(10):2229-2236

[93] Giraudeau P, Shrot Y, Frydman L. Multiple ultrafast, broadband 2D NMR spectra of hyperpolarized natural products. Journal of the American Chemical Society. 2009;**131**(39):13902-13903

[94] Guennec AL, Giraudeau P, Caldarelli S. Evaluation of fast 2D NMR for metabolomics. Analytical Chemistry. 2014;**86**(12):5946-5954

[95] Giraudeau P, Massou S, Robin Y, Cahoreau E, Portais J-C,

Akoka S. Ultrafast quantitative 2D NMR: An efficient tool for the measurement of specific isotopic enrichments in complex biological mixtures. Analytical Chemistry. 2011;**83**(8):3112-3119

[96] Frydman L, Scherf T, Lupulescu A. The acquisition of multidimensional NMR spectra within a single scan. Proceedings of the National Academy of Sciences. 2002;**99**(25):15858-15862

[97] Mishkovsky M, Frydman L. Progress in hyperpolarized ultrafast 2D NMR spectroscopy. ChemPhysChem. 2008;**9**(16):2340-2348

[98] Barna J, Laue E, Mayger MR, Skilling J, Worrall S. Exponential sampling, an alternative method for sampling in two-dimensional NMR experiments. Journal of Magnetic Resonance. 1987;**73**(1):69-77

[99] Tal A, Frydman L. Single-scan multidimensional magnetic resonance. Progress in Nuclear Magnetic Resonance Spectroscopy. 2010;**57**(3):241-292

[100] Solyom Z, Schwarten M, Geist L, Konrat R, Willbold D, Brutscher B. BEST-TROSY experiments for time-efficient sequential resonance assignment of large disordered proteins. Journal of Biomolecular NMR. 2013;**55**(4):311-321

[101] Billeter M. Non-uniform Sampling in Biomolecular NMR. Journal of Biomolecular NMR. Springer. 1 June 2017;**68**(2):65-66

[102] Rovnyak D, Hoch J, Stern A, Wagner G. Resolution and sensitivity of high field nuclear magnetic resonance spectroscopy. Journal of Biomolecular NMR. 2004;**30**(1):1-10

[103] Zeng J, Zhou P, Donald BR. Protein side-chain resonance assignment and NOE assignment using RDC-defined backbones without TOCSY data. Journal of Biomolecular NMR. 2011;**50**(4):371

[104] Hyberts SG, Takeuchi K, Wagner G. Poisson-gap sampling and forward maximum entropy reconstruction for enhancing the resolution and sensitivity of protein NMR data. Journal of the American Chemical Society. 2010;**132**(7):2145-2147

[105] Ernst RR, Anderson WA. Application of Fourier transform spectroscopy to magnetic resonance. The Review of Scientific Instruments. 1966;**37**(1):93-102

[106] Orekhov VY, Ibraghimov IV, Billeter M. MUNIN: A new approach to multi-dimensional NMR spectra interpretation. Journal of Biomolecular NMR. 2001;**20**(1):49-60

[107] Tugarinov V, Kay LE, Ibraghimov I, Orekhov VY. High-resolution four-dimensional 1H−13C NOE spectroscopy using methyl-TROSY, sparse data acquisition, and multidimensional decomposition. Journal of the American Chemical Society. 2005;**127**(8):2767-2775

[108] Orekhov VY, Ibraghimov I, Billeter M. Optimizing resolution in multidimensional NMR by three-way decomposition. Journal of Biomolecular NMR. 2003;**27**(2):165-173

[109] Kazimierczuk K, Orekhov VY. Accelerated NMR spectroscopy by using compressed sensing. Angewandte Chemie International Edition. 2011;**50**(24):5556-5559

[110] Holland DJ, Bostock MJ, Gladden LF, Nietlispach D. Fast multidimensional NMR spectroscopy using compressed sensing. Angewandte Chemie International Edition. 2011;**50**(29):6548-6551

[111] Bostock M, Nietlispach D. Compressed sensing: Reconstruction of non-uniformly sampled multidimensional NMR data. Concepts in Magnetic Resonance Part A. 2017;**46**(2):e21438

[112] Drori I. Fast minimization by iterative thresholding for multidimensional NMR spectroscopy. EURASIP Journal on Advances in Signal Processing. 2007;**2007**(1):020248

[113] Hyberts SG, Milbradt AG, Wagner AB, Arthanari H, Wagner G. Application of iterative soft thresholding for fast reconstruction of NMR data non-uniformly sampled with multidimensional Poisson gap scheduling. Journal of Biomolecular NMR. 2012;**52**(4):315-327

[114] Shrot Y, Frydman L. Compressed sensing and the reconstruction of ultrafast 2D NMR data: Principles and biomolecular applications. Journal of Magnetic Resonance. 2011;**209**(2):352-358

[115] Duma L, Hediger S, Brutscher B, Böckmann A, Emsley L. Resolution enhancement in multidimensional solid-state NMR spectroscopy of proteins using spin-state selection. Journal of the American Chemical Society. 2003;**125**(39):11816-11817

[116] Meissner A, Sørensen OW. Spin-state-selective excitation. Application for E. COSY-type measurement ofJHHcoupling constants. Journal of Magnetic Resonance. 1997;**128**(1):92-97

[117] Mauhart J, Glanzer S, Sakhaii P, Bermel W, Zangger K. Faster and cleaner real-time pure shift NMR experiments. Journal of Magnetic Resonance. 2015;**259**:207-215

[118] Sakhaii P, Haase B, Bermel W, Kerssebaum R, Wagner GE, Zangger K. Broadband homodecoupled NMR spectroscopy with enhanced sensitivity. Journal of Magnetic Resonance. 2013;**233**:92-95

[119] Meyer NH, Zangger K. Simplifying proton NMR spectra by instant

homonuclear broadband decoupling. Angewandte Chemie International Edition. 2013;**52**(28):7143-7146

[120] Zangger K, Sterk H. Homonuclear broadband-decoupled NMR spectra. Journal of Magnetic Resonance. 1997;**2**(124):486-489

[121] Aguilar JA, Nilsson M, Bodenhausen G, Morris GA. Spin echo NMR spectra without J modulation. Chemical Communications. 2012;**48**(6):811-813

[122] Aguilar JA, Colbourne AA, Cassani J, Nilsson M, Morris GA. Decoupling two-dimensional NMR spectroscopy in both dimensions: Pure shift NOESY and COSY. Angewandte Chemie International Edition. 2012;**51**(26):6460-6463

[123] Aguilar JA, Faulkner S, Nilsson M, Morris GA. Pure shift 1H NMR: A resolution of the resolution problem? Angewandte Chemie International Edition. 2010;**49**(23):3901-3903

[124] Aguilar JA, Nilsson M, Morris GA. Simple proton spectra from complex spin systems: Pure shift NMR spectroscopy using BIRD. Angewandte Chemie International Edition. 2011;**50**(41):9716-9717

[125] Foroozandeh M, Adams RW, Meharry NJ, Jeannerat D, Nilsson M, Morris GA. Ultrahigh-resolution NMR spectroscopy. Angewandte Chemie International Edition. 2014;**53**(27):6990-6992

[126] Foroozandeh M, Adams RW, Nilsson M, Morris GA. Ultrahigh-resolution total correlation NMR spectroscopy. Journal of the American Chemical Society. 2014;**136**(34):11867-11869

[127] Morris GA, Aguilar JA, Evans R, Haiber S, Nilsson M. True chemical shift correlation maps: A TOCSY experiment with pure shifts in both dimensions. Journal of the American Chemical Society. 2010;**132**(37):12770-12772

[128] Aue W, Karhan J, Ernst R. Homonuclear broad band decoupling and two-dimensional J-resolved NMR spectroscopy. The Journal of Chemical Physics. 1976;**64**(10):4226-4227

[129] Koivisto JJ. Zero-quantum filtered pure shift TOCSY. Chemical Communications. 2013;**49**(1):96-98

[130] Meyer NH, Zangger K. Enhancing the resolution of multi-dimensional heteronuclear NMR spectra of intrinsically disordered proteins by homonuclear broadband decoupling. Chemical Communications. 2014;**50**(12):1488-1490

[131] Wagner GE, Sakhaii P, Bermel W, Zangger K. Monitoring fast reactions by spatially-selective and frequency-shifted continuous NMR spectroscopy: Application to rapid-injection protein unfolding. Chemical Communications. 2013;**49**(30):3155-3157

[132] Pöppler AC, Frischkorn S, Stalke D, John M. Toluene and lithium amide diffusion into polystyrene: A slice-selective NMR-spectroscopic study. ChemPhysChem. 2013;**14**(13):3103-3107

[133] Foroozandeh M, Morris GA, Nilsson M. PSYCHE pure shift NMR spectroscopy. Chemistry - A European Journal. 2018;**24**(53):13988-14000

[134] Foroozandeh M, Castañar L, Martins LG, Sinnaeve D, Poggetto GD, Tormena CF, et al. Ultrahigh-resolution diffusion-ordered spectroscopy. Angewandte Chemie. 2016;**128**(50): 15808-15811

[135] Kovacs H, Moskau D, Spraul M. Cryogenically cooled probes—A leap in NMR technology. Progress in Nuclear Magnetic Resonance Spectroscopy. 2005;**46**(2):131-155

[136] Everett JR, Harris RK, Lindon JC, Wilson ID. NMR in Pharmaceutical Science. John Wiley & Sons; 2015

[137] Giraudeau P, Akoka S. A new detection scheme for ultrafast 2D J-resolved spectroscopy. Journal of Magnetic Resonance. 2007;**186**(2):352-357

[138] Schanda P, Brutscher B. Very fast two-dimensional NMR spectroscopy for real-time investigation of dynamic events in proteins on the time scale of seconds. Journal of the American Chemical Society. 2005;**127**(22):8014-8015

[139] Gal M, Kern T, Schanda P, Frydman L, Brutscher B. An improved ultrafast 2D NMR experiment: Towards atom-resolved real-time studies of protein kinetics at multi-Hz rates. Journal of Biomolecular NMR. 2009;**43**(1):1-10

[140] Stanek J, Augustyniak R, Koźmiński W. Suppression of sampling artefacts in high-resolution four-dimensional NMR spectra using signal separation algorithm. Journal of Magnetic Resonance. 2012;**214**:91-102