

Preface: Fast NMR Methods Are Here to Stay

A distinct feature of NMR spectroscopy, the possibility to simultaneously observe hundreds of atoms in complex macromolecules, finds its foundation in the invention of multidimensional experiments almost 40 years ago [1, 2]. The approach, however, has an important caveat: the ultimate resolution obtained in multidimensional experiments comes at a very high price, the long data collection times needed to systematically sample the large multidimensional spectral space. The number of measured data points increases polynomially with the spectrometer field and the desired spectral resolution, and exponentially with the number of dimensions. The problem of lengthy sampling compromises or even prohibits many applications of multidimensional spectroscopy in chemistry and molecular biology. Fortunately, the advent of “fast” NMR spectroscopy offers a number of solutions.

NMR experiments can be dramatically accelerated by reducing the time needed for individual measurements and/or the number of collected data points. Examples of the former include reducing the magnetization recovery time after each scan [3, 4], or spatial encoding of spectral dimensions in single-scan spectroscopy [5, 6]. The contributions to this volume are focused on the latter approach, namely the retrieving of spectral information from a limited number of data points.

The time-consuming systematic sampling of the signal on the entire multidimensional Nyquist grid describing the indirect dimensions is replaced by acquiring FIDs for only a relatively small number of grid points, while preserving all essential information that would be present in the full data set. Two distinct approaches can be traced back to the early years of multidimensional NMR spectroscopy. The former is based on the spectral projection theorem and Fourier Transform [7], and applied for example in the ACCORDION experiment 30 years ago [8]. In the second approach, the positions of the measured points are not constrained and often selected randomly [9]. Both approaches require novel analysis tools and non-standard processing methods, often resulting in significantly increased calculations times, and making them only recently a practical approach.

Deducing three-dimensional information from two-dimensional projections is not a new idea, as pointed out in the first chapter of this volume: the most obvious examples are the three-dimensional descriptions that our brain forms from the two-dimensional images collected by our eyes. The history of projections in high-resolution NMR, from the ACCORDION experiment presented in the early years of multidimensional NMR [8] via reduced dimensionality [10] to GFT [11] and

Projection-Reconstruction [12], has been presented many times, for example [13]. The projection concept for NMR spectroscopy has been implemented in various flavours. In GFT, the following chemical shift combinations for a N -dimensional signal ($\Omega_1, \Omega_2, \dots, \Omega_N$) spectra are recorded: (Ω_1), ($\Omega_1 \pm \Omega_2$), ($\Omega_1 \pm \Omega_2 \pm \Omega_3$), etc. Successive inspection of these spectra yields the chemical shifts in their numbered order. Generalizations of this scheme include the recording of any combination of shifts, e.g. also ($\Omega_1 \pm \Omega_3$), ($\Omega_2 \pm \Omega_3$), or the variation of the proportionality factor between the different shifts; the latter is usually referred to as allowing any projection angle (“45°” would correspond to the same number of time increments in all projected dimensions). The restriction to projection angles of “45°” often simplifies the direct interpretation of the projections, where peak picking or reconstructions are deferred to a later stage; an example is multi-way decomposition with PRODECOMP [14]. Reconstruction of the full-dimensional spectrum, for example with the various back-projection schemes implemented in Projection-Reconstruction (Chap. 1), accepts the most general types of projections. The same holds also when each projection is immediately subjected to peak-picking as in the APSY approach [15] (Chap. 2).

Acquiring spectral projections pertains to measuring linear cross sections in the time domain. This can be considered as a special case of a more general sampling scheme, where data points are sampled at any position of the time domain. The method is known as non-uniform or non-linear sampling (NUS or NLS). A historical perspective of this approach is well presented in Chap. 3. It was introduced almost a quarter of a century ago in a seminal publication by Laue and co-workers [9]. In a typical NUS implementation, a small fraction of the data points that would be collected in the conventional uniform sampling is randomly selected and measured. This provides dramatic savings of measurement time. The spectrum is reconstructed using specialized signal processing algorithms such as Maximum Entropy (ME) [9] (see Chaps. 3 and 5), Multi-Dimensional Decomposition (MDD) [16, 17], Fourier transform (FT) [18, 19] (see Chap. 4), Compressed Sensing (CS) [20, 21], etc. The approach provides maximum flexibility in designing the sampling schedule; thus significant efforts in the field are devoted to sampling optimization, which is based on ideas of matched acquisition [9] or improving the random distribution that are used for selecting points for measuring [22].

Despite the fact that fast sampling techniques were known over a long period of time, their broad use by the NMR community started only recently. The turning point was defined by several factors: (1) As a consequence of higher sensitivity provided by a new generation of high-field spectrometers equipped with cryo-probes, the ever-increasing signal frequency range and spectral dimensionality made sampling the limiting condition for more and more practical applications when traditional uniform sampling is used. (2) The increasing demand for high-throughput and automated analysis of an ever-increasing volume of spectral data can only be met by increased resolution and spectra dimensionality. (3) The dramatically increased performance of modern computers makes even the most computationally demanding signal processing algorithms practical. (4) This resulted in the

development of novel, powerful algorithms for spectra reconstruction and analysis from sparsely collected measurements.

Within only a few years, fast sampling techniques have been established as an indispensable tool in biomolecular NMR. Sparse sampling is routinely used for resonance assignment and structure determination of globular proteins [23, 24], (Chap. 2), including high-throughput applications by the North-East Structural Genomic Consortium (NESGC) [25, 26] and the Joint Center for Structural Genomics (JCSG) [27].

Spectra of denatured proteins and intrinsically disordered proteins show high peak overlap due to very low dispersions of signal frequencies, making sparse sampling methods a prerequisite for successful analysis. Examples are a 60-residue fragment of nucleoprotein N from the paramyxovirus Sendai [28]; the 148-residue outer membrane protein X (OmpX) from *Escherichia coli* [29]; a 115-residue CD3 Z domain [24]; a 81-residue delta-subunit of RNA polymerase from *Bacillus subtilis* [30]; the 441-residue, intrinsically disordered protein Tau [31]; the 70-residue N-terminal domain of SKIP [32].

The fast sampling has been used in studies of large protein systems: the 86 kDa Maltose-binding protein G [33], a 37 kDa fragment of the *E. coli* enterobactin synthetase module EntF [34], the integral membrane protein Volt-dependent Anion Channel [35] in micelles, the 23 kDa catalytically inactive phosphatase Ssu72 [36], and the 22.4 kD protein kRas [37].

Sparse sampling has been demonstrated also in solid-state NMR [38–41] and metabolomics [42–46].

At this turning moment when novel sampling methods have become routine for resonance assignment and structure determination, we also witness the application of these fast methods to new challenges such as short living molecular systems for example with in-cell NMR [47, 48], unstable proteins, and other cases when measurement time is limited by the sample life time. Integration into automated, comprehensive packages for studies of protein structure and interactions will be one of the next steps of many-fold improving efficiency of biomolecular NMR spectroscopy [49].

This volume presents a discussion of some of the most popular sampling schemes used in “fast” approaches to high-dimensional NMR. Novel ideas, regarding both experimental (sampling) schemes and processing algorithms, keep coming up. In particular, the novel sampling approaches are being integrating with automated assignment, structure determination, and beyond. As the above and many other applications show, “fast” NMR is here to stay.

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