# Technical aspects of NMR spectroscopy with biological macromolecules and studies of hydration in solution

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# 1. Introduction

The first nuclear magnetic resonance (NMR) spectrum of a protein was published some 40 years ago [1] and ever since, NMR of biological macromolecules has been a growing field in research and applications [2]. The capability to observe signals from individual atoms in complex biological macromolecules in solution makes possible the measurements of parameters that can be analysed in terms of molecular structure, conformation and dynamics. Complete assignments of signals in a NMR spectrum to individual atoms in the molecule are a prerequisite for such studies; a problem that cannot generally be solved on the basis of one-dimensional (1D) NMR spectra. Only the application of two-dimensional (2D) NMR spectroscopy [3, 4] which spreads signals into two frequency dimensions allowed the development of a general strategy for the assignment of proton signals in protein spectra using two types of 2D spectra [5–7]. In [$^1$H, $^1$H]-COSY spectra [3, 4] protons are correlated which are separated by up to three chemical bonds. In [$^1$H, $^1$H]-NOESY spectra [8, 9] correlations between protons which are closer than 0.5 nm through space are detected. The combination of these two techniques allows the assignment of most proton NMR signals to individual protons in small proteins [6, 10, 11]. In a further step all distances obtainable from NOESY spectra provide the data for the calculation of protein structures [12, 13]. These relatively simple techniques allow the determination of structures of proteins with a molecular weight up to 10 kDa whereas for larger proteins extensive signal overlap and increasing resonance linewidths prevent complete assignments of all signals. This barrier can be overcome with three-dimensional (3D) NMR techniques [14] and uniformly $^{13}$C and $^{15}$N labelled proteins. With these methods, systems with molecular weights up to 30 kDa can be studied. However, not only overlapping signals limit the size of the macromolecules that can be investigated, in addition faster relaxation of the signals with increasing molecular weight leads to a substantial sensitivity loss of experiments. The molecular weight limit can be increased to about 50 kDa using deuteration of the protein to reduce relaxation. Simultaneously with the methodological developments, technical advances revolutionized the design of NMR spectrometers making it possible to implement the complex experimental schemes needed for multidimensional NMR experiments. The increased complexity of the instrumentation has been more and more hidden from the user by a complex software control which allows the selection of all modes of operation from a software interface.

In parallel to the methodological and technical developments NMR has become an accepted tool in structural biology and investigations of structure and dynamics of biological macromolecules by NMR are established techniques. The rapid expansion of NMR techniques for applications to biological macromolecules increases the number of interested users with little technical background in NMR spectroscopy. These newcomers find it increasingly difficult to follow and make use of the myriads of NMR experiments available today. Applications and theoretical foundation of biomolecular NMR are described in many excellent books [*e.g.* 15–27], however, often only a few experimental schemes are discussed and the common features of different pulse sequences are not always made transparent. In addition important technical details of experimental implementations are either not discussed or may be missed in the overwhelming amount of information. This review is intended to address the need for an introduction to general technical and methodological aspects of modern NMR experiments with biological macromolecules to these newcomers. The basis for this presentation are not complete experimental schemes which change rather rapidly, but the underlying basic technical methods and the basic segments from which individual experiments are constructed and which change much more slowly. The understanding

of the basic segments is the basis for clarifying the functioning of existing and newly developed experiments and to assist the reader in making his own adjustments to experiments or even in developing new methods.

This review was written with a reader in mind who is interested in technical and methodological aspects of NMR with macromolecules in solution, who has had first contact with spectra of proteins in one and two dimensions and knows the principles of their analysis. In addition knowledge of the product operator formalism [28] is an advantage since the discussion of the basic segments requires the application of this formalism. This text should help such readers to quickly become familiar with the technicalities of multidimensional NMR experiments.

The main text starts with Section 2 where some theoretical aspects are briefly discussed, followed by an introduction of technical principles starting with radiofrequency pulses and ending with multidimensional NMR and data processing. Not only in this section but throughout the text mathematics is kept to the minimum necessary for the presentation of the technical aspects of NMR spectroscopy. Section 3 introduces those parts of a modern NMR spectrometer which critically influence the performance of NMR experiments. Section 4 concentrates on the basic experimental segments from which most of the vast number of experiments available today are constructed. Section 5 discusses hydration studies with NMR and serves two purposes. Firstly, it gives examples of experiments using the segments introduced in Section 4 and the principles discussed in Section 2. Secondly, it introduces the technical aspects of a very interesting application of NMR which allows detailed studies of individual water molecules in the hydration shell of a protein.