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Direct Measurement of Dihedral Angles with High-Resolution NMR Spectroscopy

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Since the discovery of the nuclear Overhauser effect (NOE)[1] and scalar coupling constants[2] decades ago, NMR-derived structure calculations of biomolecules largely depended on measurement of these two parameters.[3] Therefore, few scientists in this field expected that a new parameter for structure determination could be developed. The group of Griesinger at the University of Frankfurt recently changed this paradigm with the invention of an NMR method which directly measures angles between bond vectors.[4] The new parameter is widely applicable for isotopically labeled molecules and will certainly set a new direction in future design of experiments to determine the structure of biomolecules by NMR spectroscopy. The information extracted from these experiments will significantly improve the resolution of NMR structures and may provide new ways to obtain information on molecular dynamics.

The new parameter, cross-correlated dipolar relaxation (CCDR), can easily be explained in a simplified form. All coherences between nuclear spins that finally give rise to NMR signals relax (decay) with a certain rate and eventually disappear. In dipolar relaxation the relaxation of a spin is mediated by the fluctuating magnetic field caused by adjacent spins. Cross-correlated dipolar relaxation indicates that the dipolar coupled spin pair is not isolated, but experiences fluctuating magnetic fields from other spin pairs, which influence its relaxation rate. The dipolar interaction between these magnetic moments depends on the angle between them [Eq. (1) in which k is defined according to Eq. (2)].[5] θ is the

$$\mathrm{CCDR}_{ijkl} = k(3\cos^2\theta - 1) \quad (1)$$

$$k = \frac{2}{5}\frac{\gamma_i\gamma_j}{r_{ij}^3}\frac{\gamma_k\gamma_l}{r_{kl}^3}\left[\frac{\hbar\mu_0}{4\pi}\right]^2\tau_c \quad (2)$$

angle between the internuclear vectors that link nuclei i and j, and k and l (Figure 1). The internuclear distances r are well-known, and the rotational correlation time τ_c can be measured

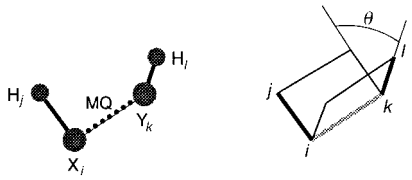

Figure 1. Schematic representation of a pair of bond vectors. The relaxation rate of the multiple quantum coherence (MQ) is dependent on the angle θ between the bond vectors.

independently. All other factors are constants. The angle θ between bond vectors is then the sole unknown in Equation (1) and can be readily and precisely determined by measurement of the cross-correlated dipolar relaxation rate. It should be emphasized that θ is measured *directly*, without the need of experimental calibration as, for example, the Karplus curve for J coupling constants.

Cross-correlated dipolar relaxation can conveniently be measured in novel triple-resonance experiments, that is, experiments that utilize the ^{1}H, ^{13}C, and ^{15}N nuclei in isotopically enriched biomolecules. In triple-resonance NMR experiments designed for that purpose, for example a nondecoupled heteronuclear multiple quantum experiment, each signal has a double doublet fine structure. The splitting of the signal is caused by the large one-bond couplings $^1J_{\mathrm{H,N}}$ and $^1J_{\mathrm{H,C}}$ (Figure 2). The individual lines of this double doublet relax with different rates due to the phenomenon of cross-correlated dipolar relaxation. Thus, the relaxation rate can be directly extracted from the intensity of individual lines [Eq. (3)]. CCDR is the relaxation rate, t is the time the

$$\mathrm{CCDR}_{ijkl} = \frac{1}{4t}\ln\left[\frac{I(\alpha\beta)I(\beta\alpha)}{I(\alpha\alpha)I(\beta\beta)}\right] \quad (3)$$

selected coherences experience relaxation, and I the signal intensities of the spin states $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$, and $\beta\beta$. The relative

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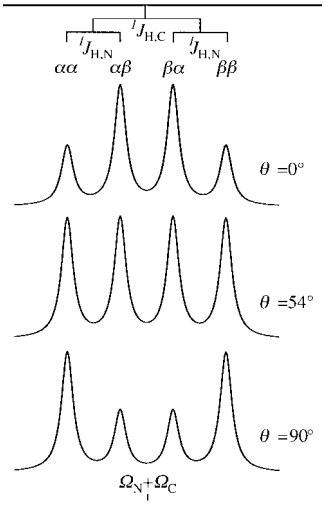

Figure 2. Cross-section through a double doublet signal obtained for each pair of correlated bond vectors. The signals represent double quantum coherence formed between N_i and C^{α}_{i-1} and are split by the $^1J_{H,N}$ and $^1J_{H,C^{\alpha}}$ couplings. The population of the spin states $\alpha\alpha$,$\alpha\beta$,$\beta\alpha$ and $\beta\beta$ is represented by the intensity of the individual peaks and is correlated with the angle θ between the H^N-N and $H-C^{\alpha}$ bond vector. The middle graph shows the magic angle condition where CCDR equals zero (Equation 2).

intensity of the individual lines thus reflects directly the relative orientation of the involved nuclei.

Cross-correlated dipolar relaxation can be measured between a variety of nuclei. The measurement requires two central nuclear spins, each of which is directly attached to a remote nuclear spin (Figure 1). The central spin and its attached remote spin must be connected by a large scalar coupling, and the remote spin must be the primary source of dipolar relaxation for the central spin. The two central spins do not need to be scalarly coupled, although the necessity to create multiple quantum coherence between them requires them to be close together in a scalar or dipolar coupled network. In practice, the central spins will be heteroatoms (for example, ^{13}C or ^{15}N in isotopically enriched biomolecules), and the remote spins will be their directly attached protons.

Reif et al.[4] chose the amide ^{15}N nucleus and the preceeding $^{13}C^{\alpha}$ nucleus as central nuclei, and their directly attached protons as remote nuclei. This choice of atoms allows the measurement of the angle θ between the H^N-N bond vector and the $H^{\alpha}-C^{\alpha}$ bond vector of the preceeding residue in the sequence (Figure 3). Assuming the planarity of the *trans* peptide bond between the two amino acid residues, the angle θ depends only on the protein backbone angle ψ. Measurement of θ is thus an indirect measurement of ψ, which is hard to determine with previous NMR technology.

The pulse sequence is similar to that in the conventional HN(CO)CA experiment,[6] which correlates the amide proton and the ^{15}N atom attached to it with the C^{α} atom of the preceeding amino acid residue. The main difference to the regular HN(CO)CA experiment is that the signal of C^{α} is recorded as double quantum coherence with amide nitrogen atom, and without decoupling of protons. A slice through the resulting 3D spectrum thus shows for every amino acid residue the double doublet of signals schematically shown in Figure 2. From the signal intensities of individual lines, the cross-correlated dipolar relaxation rate according to Equation (2), and the angles θ and ψ according to Equation (1) can be calculated. Reif et al. demonstrate the method with a small protein, rhodniin, for which they could determine a large number of ψ angles.

As with almost all NMR experiments, there is a limitation on the molecular size of biomolecules that can be investigated. The line widths of the individual lines of the double doublet (Figure 2) must be narrow relative to the one-bond scalar coupling of the central spin (^{13}C, ^{15}N) to the remote spin directly attached to it (the proton; 90 or 140 Hz, respectively). More importantly, the sensitivity of the experiment may become an issue for large biomolecules: large molecules tumble slowly in solution, and their coherences relax rapidly. During the (necessarily) long delays in the pulse sequence, a large portion of the magnetization will therefore relax before it is converted into detectable magnetization. This is particularly severe, since this type of (nondecoupled) multiple quantum coherence relaxes very rapidly. However, the cross-correlated relaxation rates grow with molecular weight analogous to the "normal" relaxation rates.

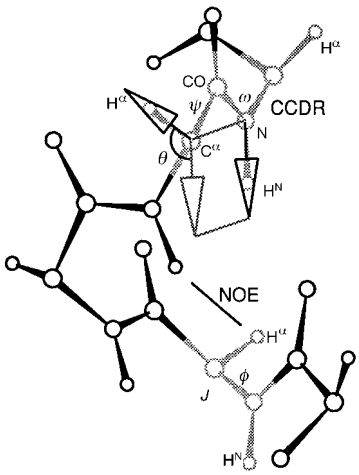

Figure 3. The measurement of cross-correlated dipolar relaxation (CCDR) yields the angles between bond vectors, such as the angle θ between the H^N-N vector and the $H-C^{\alpha}$ vector of the preceeding amino acid in the sequence. It complements information obtained from NOE and *J* coupling, that are schematically shown in the lower part of the figure.

Equation (2) is an approximation based on the assumption of isotropic tumbling of the rigid molecule. Thus, potential artefacts in the angle determination with Equation (1) can arise in the case of anisotropic tumbling, or in the presence of fast internal motions in the molecule.

Apart from its usefulness in biomolecular structure determination, measurement of cross-correlated dipolar relaxation may also help in the investigation of molecular dynamics. Cross-correlated dipolar relaxation involves a time scale that is different from the time scale for scalar coupling. In the case of internal motions, measurement of the three independent parameters, NOE, *J*, and CCDR will thus not necessarily yield identical results. Structures calculated on the basis of data that include experimental constraints from CCDR measurements may therefore be more accurate than structures calculated without CCDR information, but they are not necessarily more precise. The additional information from CCDR may be particularly important in the analysis of multiple-state equilibria, where the number of observables (NOEs and *J* coupling constants) has often not been sufficient, and the problem was consequently underdetermined. In such cases, new independent structural data may contribute to a more detailed picture of biomolecular structure, which includes structural and dynamical aspects.

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Apart from the relaxation mechanism described here, other mechanisms like relaxation involving cross-correlation between dipole – dipole coupling and chemical shift anisotropy can also provide structural information[7]. The extraction of this information from the relaxation rates is, however, less straightforward than the CCDR method, mainly because many of the CSA tensors are not known. They may, however, be accessible from solid-state NMR. This relaxation mechanism is also expected to be explored with the aim of extracting structural information.

At high magnetic fields proteins with an intrinsic magnetic dipole moment experience a preferred orientation relative to the magnetic field. For these cases residual dipolar coupling can be measured, which provides information on the orientation of internuclear vectors relative to the molecular frame[8]. This method is interesting, since it gives structural information on the long-range relationships between parts of the molecules.

Measurement of cross-correlated relaxation has been described earlier for homonuclear cases[9] and is widely used in solid-state NMR.[10] It is the availablility of isotopically labeled biomolecules and their application in solution-state NMR that makes the new method so interesting. Presumably the method will be extended to determine additional structural parameters, such as the angles ϕ or χ_1, or even angles between bond vectors that are close in space but not close in sequence. It can be applied in a more general perspective, and beyond the scope of proteins, for example, in nucleic acids and small organic molecules. The new structural parameter is directly measured, without the use of experimental calibration, as, for example, in the case of coupling constants that need to be interpreted with Karplus-type curves. In addition, it is fortunate that this new parameter can be accessed rather easily without the need to produce new samples or to invest in new spectrometer hardware. The experiment is based on existing triple-resonance experiments, and uses an only slightly modified pulse sequence. It is likely that this method will rapidly gain acceptance in the field of biomolecular NMR and that it will soon be standard methodology for biomolecular structure determination by NMR.

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