Communication

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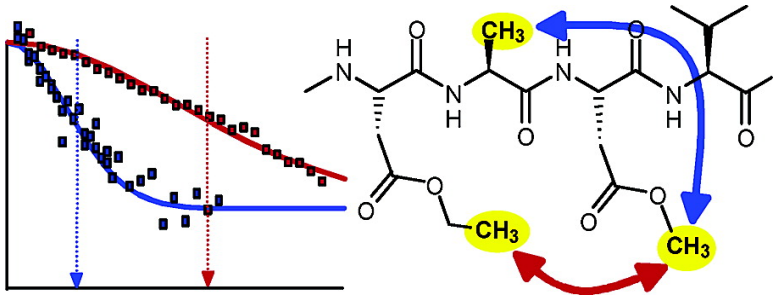

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Determination of Long-Range Distances and Dynamic Order Parameters by Dipolar Recoupling in High-Resolution Magic-Angle Spinning NMR Spectroscopy

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By introducing dipolar recoupling methods to high-resolution magic-angle spinning (HRMAS) NMR spectroscopy, we have developed a class of experiments that allows us to measure residual dipole−dipole couplings of ∼1 Hz in weakly immobilized molecules. Using homonuclear ^{1}H−^{1}H recoupling, distances of up to ∼8 Å can be selectively determined, while heteronuclear ^{1}H−^{13}C recoupling provides access to dynamic order parameters (S) of individual molecular segments on the order of $S \approx 10^{-3}$.

For partially immobilized or heterogeneous systems, NMR lines are often severely broadened by residual anisotropic interactions, in particular by susceptibility effects. To enhance spectral resolution, standard solution-state NMR experiments are nowadays performed under MAS, and the approach has been termed high-resolution MAS.[1,2] The anisotropies, however, in particular residual dipole−dipole couplings, contain valuable information that is not directly accessible in isotropic liquids. In fact, even soluble proteins are deliberately aligned in dilute liquid-crystalline media in order to regain a weak anisotropy and to provide a means of measuring residual dipole−dipole couplings.[3] In HRMAS NMR, it therefore seems promising to apply recoupling techniques in order to restore the information on anisotropic interactions, which is removed by MAS.[4]

In this Communication, we present HRMAS NMR experiments that consist of a selection/transfer step followed by a homonuclear (^{1}H−^{1}H) or heteronuclear (^{1}H−^{13}C) dipolar recoupling procedure (Figure 1). For ^{1}H−^{1}H recoupling (Figure 1a), the rotational resonance (RR) technique is employed,[5] which selectively reintroduces dipole−dipole couplings between protons whose NMR frequency difference exactly matches the MAS frequency. To compensate for relaxation effects, a constant-time approach has been chosen with a DANTE π-pulse ensuring selective inversion of one of the ^{1}H species involved. In addition, reference experiments are conducted, where the MAS frequency is shifted away from the RR condition. In Figure 2a, the situation of two interacting methyl groups is considered. The calculated intensity of a methyl ^{1}H signal is plotted as a function of the time t_1 during which the ^{1}H−^{1}H dipole−dipole couplings *between* the two methyl groups are recoupled by rotational resonance. Effectively, the decay curve depends on the product Dt_1 of the dipolar coupling constant D and the recoupling time t_1. As the systems studied here are not macroscopically aligned but only motionally restricted, a full orientational average is taken into account. When a distribution of couplings or transverse relaxation[5] is included, the oscillations of the curve vanish, and it can be approximated by a Gaussian curve with the half-maximum value at $D_{av}t_1 \approx 0.3$. This relation provides a simple means of extracting an average coupling D_{av} from experimental data obtained for two methyl groups.

^{1}H−^{13}C recoupling is provided by a REREDOR pulse sequence[6] (Figure 1b), which is based on the well-known REDOR technique.[7]

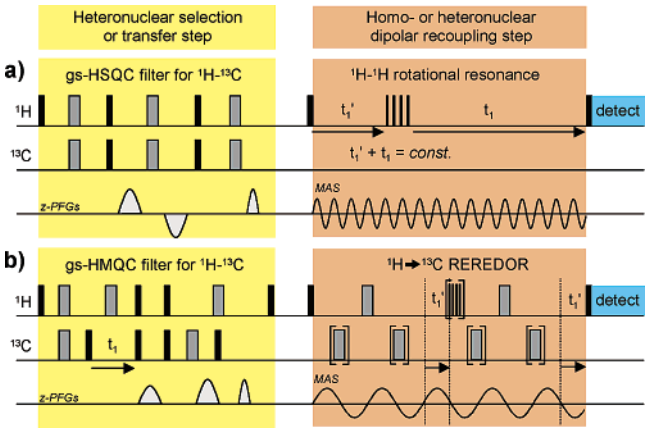

Figure 1. Pulse sequences providing dipolar recoupling under HRMAS after a ^{1}H−^{13}C HSQC/HMQC selection step. (a) Homonuclear ^{1}H−^{1}H recoupling by a constant-time rotational resonance experiment with a DANTE π-pulse for selective ^{1}H inversion. (b) Heteronuclear ^{1}H−^{13}C REREDOR experiments, yielding dipolar sideband patterns in the indirect dimension. A selective π-pulse on ^{1}H removes J-couplings that would lead to line splittings and signal losses.

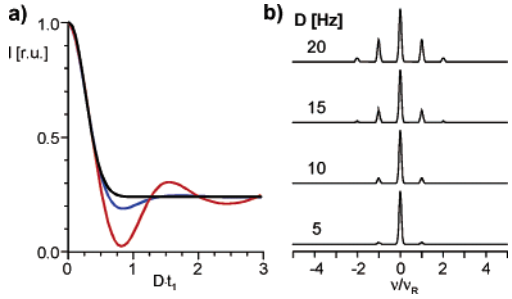

Figure 2. (a) Calculated rotational-resonance (RR) decay curves (experiment in Figure 1a). Red: Signal intensity of methyl protons under RR recoupling to the protons of another methyl group as a function of the coupling constant D and the recoupling time t_1. Blue: Curve for a Gaussian distribution of couplings (width of $2D$). Black: Approximation of the decay by a Gaussian curve with its half-maximum value at $D_{av}t_1 = 0.3$. (b) ^{1}H−^{13}C REREDOR sideband patterns for a methyl group with residual ^{1}H−^{13}C couplings D. The overall recoupling time is 61 ms with MAS at 2 kHz (experiment in Figure 1b).

In the π-pulse train, a short t_1' dimension is inserted, which gives rise to rotor-encoding of the recoupled Hamiltonian. The sideband patterns thus observed in the spectra depend on the underlying ^{1}H−^{13}C couplings.[6,8] In Figure 2b, patterns are calculated for a methyl group with ^{1}H−^{13}C couplings of $D = 5$ ... 20 Hz for 61 ms recoupling under MAS at 2 kHz. A preceding selection step (based on conventional HSQC or HMQC schemes) is required for the ^{1}H−^{13}C experiment to select ^{13}C-sites, while for the ^{1}H−^{1}H experiment, the ^{1}H−^{13}C filtration step is only relevant when the

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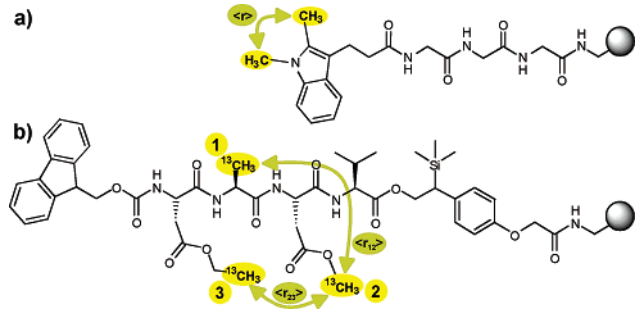

Figure 3. Samples investigated in this study. The peptide derivatives are attached to an amino-functionalized polystyrene resin that is swollen in DMF-d_7 prior to investigation. The investigated methyl groups as well as the methyl−methyl distances are highlighted. (a) Dimethylindole derivative, resin-bound via a Gly−Gly−Gly linker. (b) Tetrapeptide with three methyl-^{13}C labels: Fmoc−(AspOCH$_2$***C**H$_3$)−Ala(***C**H$_3$)−(AspO***C**H$_3$)−Val−, resin-bound via a PTMSEL linker.[9]

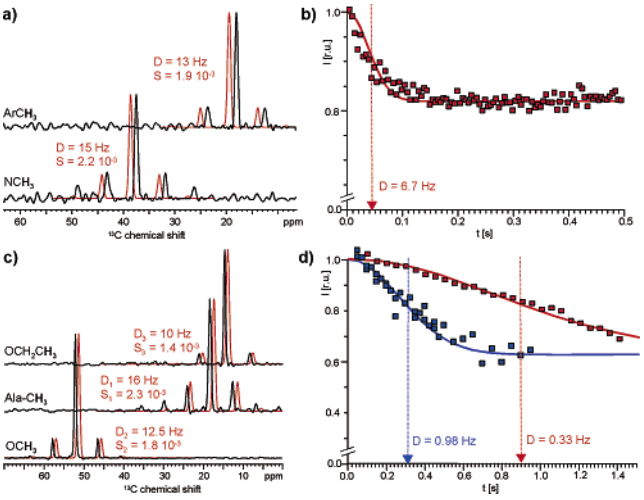

Figure 4. (a) ^{1}H−^{13}C REREDOR sideband patterns for the methyl groups of the indole derivative (Figure 3a), observed for 61 ms recoupling and MAS at 2 kHz (experiment in Figure 1b). Calculated patterns are shown in red, together with the determined residual couplings D and dynamic order parameters S. (b) ^{1}H−^{1}H RR decay curve for the indole derivative (experiment in Figure 1a). A Gaussian curve is fitted into the data, and from the half-maximum value (indicated by the arrow) the coupling D is extracted using $Dt_1 \approx 0.3$. (c) ^{1}H−^{13}C REREDOR sideband patterns for the methyl groups of the tetrapeptide (Figure 3b). (d) ^{1}H−^{1}H RR decay curve for the methyl pairs 1−2 (blue) and 2−3 (red) with Gaussian curves and extracted couplings D.

sites of interest are ^{13}C-enriched and the respective ^{1}H resonances need to be selected from a crowded ^{1}H spectrum.

The experiments are demonstated on two functionalized oligopeptides that are attached to a polymer resin (Figure 3). The dimethyl-indole derivative serves as a model compound with a known methyl−methyl distance r and two methyl−^{1}H resonances of 3.59 and 2.33 ppm, corresponding to a frequency difference of 885 Hz at 700 MHz ^{1}H frequency. As dipole−dipole couplings depend both on the distance r and the orientation of the coupling vector, the motional reduction of the coupling, i.e., the dynamic order parameter S of the respective molecular segment, needs to be determined separately. The latter information can be gained from the REREDOR sideband patterns of the methyl groups, which are shown in Figure 4a. From the ^{1}H−^{13}C couplings (D = 13 ... 15 Hz), $S \approx 2.0 \times 10^{-3}$ is obtained for the indole moiety. To derive S, the measured couplings are divided by the coupling (6.9 kHz) of a rigid methyl group that only rotates about its threefold symmetry axis.[6] The RR decay curve (Figure 4b) yields a ^{1}H−^{1}H coupling of $D_{av} = (6.7 \pm 0.5)$ Hz. This value needs to be divided by S in order to compensate for motional averaging effects, yielding $D_{av}/S = (3.3 \pm 0.6)$ kHz, which corresponds to a distance of $r = (3.3 \pm 0.2)$ Å. Practically, this can be considered as the carbon−carbon distance of the two methyls, and it is in good agreement with $r' = 3.2$ Å, which results from the molecular geometry.

For the three ^{13}C-enriched methyls in the tetrapeptide (Figure 3b), REREDOR sideband patterns yield $S_1 = 2.3 \times 10^{-3}$, $S_2 = 1.8 \times 10^{-3}$, and $S_3 = 1.4 \times 10^{-3}$ (Figure 4c). As expected, the parameter is slightly higher for the alanine in the peptide backbone than for the side chain ends. The ratios $S_2/S_1 = 0.78$ and $S_3/S_1 = 0.61$ reflect the flexibility of the respective chain segments, which are found to be relatively stiff. Obviously, the Asp side chain conformation is quite restricted. From RR decay curves, methyl−methyl couplings can be determined for pairs 1−2 and 2−3, as the respective ^{1}H chemical shift differences (1568 and 1692 Hz) can be matched with an applicable MAS frequency (>500 Hz). Couplings of $D_{12} = (0.98 \pm 0.10)$ Hz and $D_{23} = (0.33 \pm 0.05)$ Hz are measured (depicted in Figure 4d), which have to be divided by the respective order parameters to extract the distance information. For the 1−2 pair, S_2 can be directly used, while the effective parameter of the two side chain ends is approximated by $S_{23} = 1.1 \times 10^{-3}$ (see Supporting Information). From the resulting coupling values, $D_{12}/S_2 = (540 \pm 100)$ Hz and $D_{23}/S_{23} = (300 \pm 60)$ Hz, distances of $r_{12} = (6.1 \pm 0.4)$ Å and $r_{23} = (7.5 \pm 0.5)$ Å are obtained, which are to be viewed as average distances between mobile methyl groups. However, as indicated by the high order parameters within the peptide, the distances characterize a preferential structure, which is also confirmed by computationally optimized static structures.

In conclusion, we show that even very weak residual dipole−dipole couplings can efficiently be recoupled under HRMAS conditions. Robust NMR experiments provide quantitative information on local molecular mobilities and distances in a range of up to ~8 Å. In addition to the methyl groups used in this study, the techniques can readily be extended to other functional units in complex (bio)macromolecules.

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Supporting Information Available: Synthesis of the samples, experimental conditions, discussion of the dynamic order parameters, and additional NMR data confirming the results (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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