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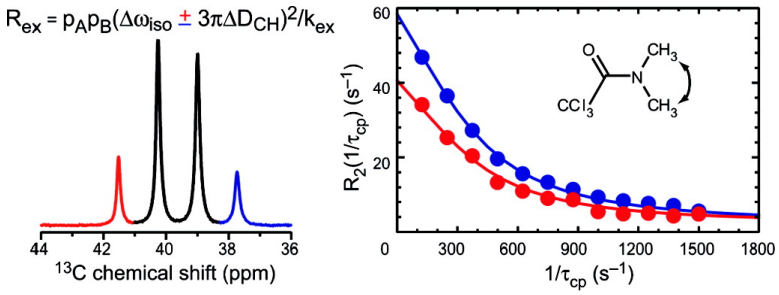

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Characterization of Chemical Exchange Using Residual Dipolar Coupling

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Chemical kinetic processes contribute to NMR transverse relaxation via temporal modulation of eigenfrequencies of nuclear spin states.[1] This relaxation mechanism is referred to as chemical or conformational exchange. Methods for quantification of chemical exchange arising from variation in isotropic chemical shifts[2,3] have enabled powerful applications to functional dynamics of proteins and other biological macromolecules.[4,5] However, such methods have three drawbacks: (i) broadening of nuclear spins with differences in isotropic chemical shifts $\Delta\nu_{iso} \approx 0$ for exchanging states cannot be detected,[6,7] (ii) isotropic shifts for particular nuclei can be indirectly affected by motions of remote moieties,[8,9] and (iii) $\Delta\nu_{iso}$ is not a direct structural constraint and must be interpreted using database analysis or theoretical modeling.[9,10]

The present work demonstrates that anisotropic spin interactions, such as residual dipolar couplings (RDCs), in weakly aligned molecules can be used to detect and quantify chemical exchange processes. These interactions contribute to chemical exchange even if $\Delta\nu_{iso} = 0$, are modulated only by motions of the affected nuclei, and provide direct orientational constraints on molecular structure. RDCs, generated using liquid crystalline media or other means, have been extensively used for structural and dynamical studies of small molecules[11] and proteins.[12,13] ^{1}H-^{1}H RDCs and ^{2}H quadrupolar coupling constants also have been used to characterize chemical exchange in small molecules dissolved in thermotropic and lyotropic liquid crystalline phases.[14−18] The complexity of strongly coupled ^{1}H spectra and the requirement for selective ^{2}H labels limit applications of these methods to fairly small systems. Heteronuclear ^{1}H-^{13}C RDCs are devoid of these limitations and are the primary focus of this work.

The small molecule *N*,*N*-dimethyltrichloroacetamide (DMTCA) was used as a model system for three reasons: (i) chemical exchange kinetics in isotropic solution are well characterized,[19,20] (ii) the transition from slow to fast chemical exchange occurs within an accessible temperature range, and (iii) the chemical structure and hence molecular alignment tensor remain unchanged by the kinetic exchange process. Weakly aligned samples of natural abundance and [$^{13}C_2$-methyl] DMTCA were prepared using poly-γ-benzyl-L-glutamate (PBLG), which forms a lyotropic nematic phase when dissolved in chloroform.

At low temperature, rotation around the amide partial double-bond is slow, and the methyl groups occupying cis and trans positions relative to the carbonyl oxygen resonate at different frequencies. A coupled 125 MHz ^{13}C NMR spectrum of weakly aligned DMTCA shows two resolved quartets with different apparent multiplet splittings resulting from different ^{1}H-^{13}C RDCs for the downfield ($D_{CH,down}$) and upfield ($D_{CH,up}$) methyl groups. (Figure 1A). The frequency differences between pairs of corresponding quartet components are, starting with the most downfield quartet components: $\Delta\nu_{iso}+1.5\Delta D_{CH}$, $\Delta\nu_{iso}+0.5\Delta D_{CH}$, $\Delta\nu_{iso}-0.5\Delta D_{CH}$, and $\Delta\nu_{iso}-1.5\Delta D_{CH}$, where $\Delta D_{CH} = D_{CH,down} - D_{CH,up}$. A slight asymmetry in quartet intensities is caused by non-negligible intermethyl ^{1}H-^{1}H RDCs (Supporting Information, Figure S4) and not by slow conformational dynamics or temperature gradients in the NMR sample (Figure S5). In an isotropic sample, $\Delta D_{CH} = 0$ and the frequency differences are all equal to $\Delta\nu_{iso}$, confirming that differences between J_{CH} scalar coupling constants are negligible.

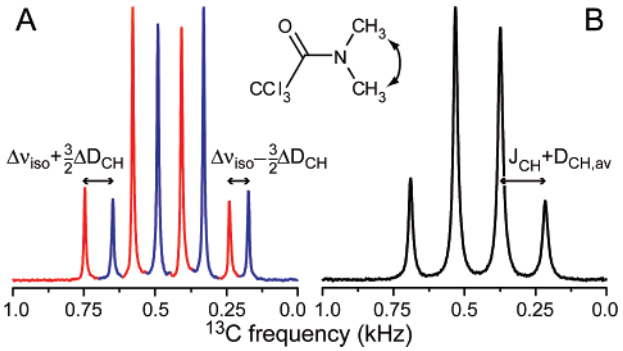

Figure 1. Methyl region of the slow (A) and fast (B) exchange ^{13}C spectra of a weakly aligned [$^{13}C_2$-methyl] DMTCA sample, collected at 281.3 and 325.5 K, respectively. J_{CH} is the ^{13}C-^{1}H scalar-coupling constant, and $D_{CH,av}$ is the motionally averaged ^{13}C-^{1}H RDC. The difference between downfield (red) and upfield (blue) methyl ^{1}H-^{13}C RDCs, ΔD_{CH}, contributes to the frequency differences between the equivalent quartet components in the slow exchange regime (A) and causes differential line-broadening of the quartet components in the fast exchange regime (B).

At high temperature, rotation around the amide bond is fast and a coupled ^{13}C NMR spectrum of weakly aligned DMTCA displays one quartet with population-averaged resonance frequencies (Figure 1B). The contribution of ΔD_{CH} to the frequency differences between quartet components results in differential broadening manifested in the overall asymmetry of the quartet. Control simulations and experiments eliminate temperature gradients, ^{1}H-^{1}H intermethyl RDCs, and relaxation interference as the origins of this asymmetry (Figures S4D and S5).

Linewidths of the quartet components for DMTCA at three temperatures in the fast-exchange regime (Figure S3) were used to calculate $\Delta D_{CH} = k\Delta\nu_{fwhh}/|n|\pi\Delta\nu_{iso}$, in which k is the kinetic rate constant, site populations are set to 0.5, $\Delta\nu_{fwhh}$ is the difference in linewidths for outer (inner) quartet components, and $|n| = 3$ (1) for outer (inner) lines. Values of ΔD_{CH} obtained from inner and outer quartet components are in excellent agreement (Figure S6).

To corroborate the line-shape analysis, ^{13}C-detected relaxation dispersion experiments were performed for aligned and isotropic DMTCA samples at 325.5 K using a constant-time Carr−Purcell−Meiboom−Gill (CPMG) relaxation period[22] incorporating a central S^{3}CT element[23,24] to refocus effects of cross-relaxation. Dispersion curves for quartet components in the aligned sample have the same characteristic decay constant but different amplitudes (Figure 2). Frequency differences obtained from fitting the experimental data with the Carver−Richards equation[21] were used to calculate ΔD_{CH}

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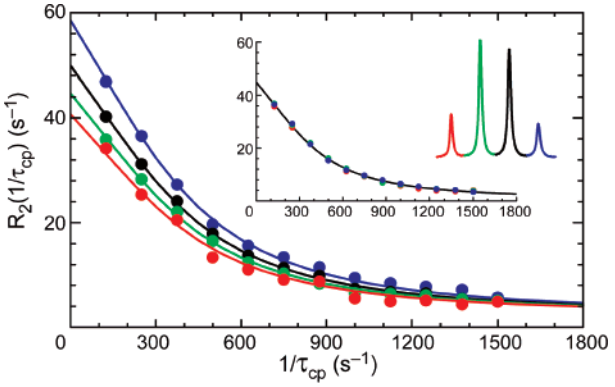

Figure 2. CPMG relaxation dispersion curves for individual quartet components collected using the aligned and (inset) isotropic DMTCA samples at 325.5 K. The delay between 180° pulses in the CPMG sequence is τ_{cp}. Fits shown with solid lines were generated using the Carver−Richards equation.[21] Differences in the dispersion amplitudes (*y*-intercepts) for the quartet components arise for $\Delta D_{CH} \neq 0$. Fitting procedures and data analysis are provided in the Supporting Information.

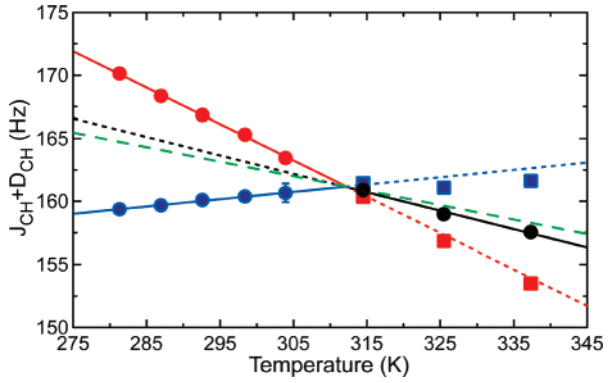

Figure 3. Temperature dependence of $J_{CH} + D_{CH}$ for downfield (red), upfield (blue), and motionally averaged (black) methyl ^{13}C quartets in DMTCA. Experimentally measured values are shown as circles, corresponding linear fits with solid lines, and extrapolated linear fits with dotted lines. The green dashed line is the average of the linear fits for the upfield and downfield quartets in slow exchange. Squares represent data obtained from ΔD_{CH} and $D_{CH,av}$ under fast exchange conditions.

$= -4.7 \pm 0.2$ and -4.2 ± 0.4 Hz from outer and inner quartet components, in excellent agreement with the results of the line-shape analysis at 325.5 K, −4.2 and −4.3 Hz. Identical relaxation dispersion curves were observed for quartet components in the isotropic sample (Figure 2, inset). Parameters for the chemical exchange process are given in Table S2.

The ^{13}C-^{1}H RDCs for DMTCA obtained from slow- and fast-exchange spectra are consistent and show linear behavior for both upfield and downfield methyl quartets over the temperature range from 281 to 337 K (Figure 3). At temperatures >312 K, ΔD_{CH} becomes negative, in agreement with Figures 1B and 2, where the most downfield resonance has the smallest line width and relaxation dispersion amplitude, respectively.

In conclusion, RDCs can be used to fully characterize conformational exchange processes for weakly aligned molecules, even if $\Delta\nu_{iso}$ approaches zero in isotropic solution. The approaches presented above are not limited to ^{1}H-^{13}C RDCs and are equally valid for any weakly coupled spin multiplet. These methods also can be applied to biological macromolecules using multidimensional NMR techniques already established for isotropic measurements.[2,3,25] However, multiple ^{1}H-^{1}H couplings in macromolecules significantly impede ^{1}H spectral resolution, especially if high degrees of alignment are needed to amplify RDC differences between chemical states. Deuteration[26] in combination with ^{1}H homonuclear decoupling[27] may be necessary for ^{1}H-detected experiments or direct ^{13}C detection[28] may be advantageous. Finally, if chemical exchange parameters already have been obtained using an isotropic sample, a single-field relaxation dispersion or line-shape experiment on an aligned sample suffices to obtain RDCs for use as orientational constraints for structural characterization of exchanging chemical states.

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Supporting Information Available: Sample preparation protocols, verification of Arrhenius parameters, temperature series of ^{1}H and ^{13}C spectra, simulations of ^{13}C line shapes, temperature gradient controls, ^{13}C line-shape and relaxation dispersion analysis protocols, and temperature dependence of intramethyl proton RDCs. This material is available free of charge via the Internet at http://pubs.acs.org.

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