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Quantifying conformational dynamics using solid-state $R_{1\rho}$ experiments

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Abstract

We demonstrate the determination of quantitative rates of molecular reorientation in the solid state with rotating frame ($R_{1\rho}$) relaxation measurements. Reorientation of the carbon chemical shift anisotropy (CSA) tensor was used to probe site-specific conformational exchange in a model system, d_6 -dimethyl sulfone (d_6 -DMS). The CSA as a probe of exchange has the advantage that it can still be utilized when there is no dipolar mechanism (i.e. no protons attached to the site of interest). Other works have presented $R_{1\rho}$ measurements as a general indicator of dynamics, but this study extracts quantitative rates of molecular reorientation from the $R_{1\rho}$ values. Some challenges of this technique include precise knowledge of sample temperature and determining the R_2^0 contribution to the observed relaxation rate from interactions other than molecular reorientation, such as residual dipolar couplings or fast timescale dynamics; determination of this term is necessary in order to quantify the exchange rate due to covariance between the 2 terms. Low-temperature experiments measured an R_2^0 value of $1.8 \pm 0.2 \text{ s}^{-1}$. Allowing for an additional relaxation term (R_2^0), which was modeled as both temperature-dependent and temperature-independent, rates of molecular reorientation were extracted from field strength-dependent $R_{1\rho}$ measurements at 4 different temperatures and the activation energy was determined from these exchange rates. The activation energies determined were $74.7 \pm 4.3 \text{ kJ/mol}$ and $71.7 \pm 2.9 \text{ kJ/mol}$ for the temperature-independent and temperature-dependent R_2^0 models respectively, in excellent agreement with literature values. The results of this study suggest important methodological considerations for the application of the method to more complicated systems such as proteins, such as the importance of deuterating samples and the need to make assumptions regarding the R_2^0 contribution to relaxation.

Keywords

Rotating frame relaxation; chemical shift anisotropy; dynamics; molecular reorientation; dimethyl sulfone

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Introduction

As dramatic improvements in resolution and signal-to-noise have been made in recent years, the quantitative study of conformational dynamics using solid state nuclear magnetic resonance (NMR) has become experimentally feasible [1–3]. Methods used to study molecular reorientation in the solid state cover a broad range of timescales from picoseconds to seconds. Slow (millisecond-to-second) timescale methods include 2-dimensional exchange experiments [4] and newer 1-dimensional exchange techniques such as CODEX and dipolar CODEX [5, 6]. Intermediate timescale (microsecond-to-millisecond) experimental methods include rotating frame relaxation [7–9] and lineshape analysis [10, 11]. Fast (nanosecond-to-picosecond) timescale experiments include laboratory-frame relaxation [12] and measurements of order parameters [13, 14].

Rotating frame relaxation is one of the few methods available to observe intermediate timescale dynamics [2]. Signals for sites undergoing intermediate exchange are often not observable in solution due to exchange broadening. The thermodynamics of $R_{1\rho}$ experiments have been described previously [8, 16, 17]. Spin-spin interactions due to static dipolar spin fluctuations of protons and spin-lattice relaxation due to the molecular motions of interest can both contribute to the observed $R_{1\rho}$ value.

$R_{1\rho}$ studies in the solid state have been used as qualitative probes of molecular reorientation. In the solid state, rotating frame relaxation can be studied through 2 different interactions: monitoring reorientation of a dipole vector [8] or reorientation of a chemical shift anisotropy tensor [9]. Using the CSA tensor to observe $R_{1\rho}$ relaxation can be useful as it provides a means for monitoring dynamics when the site of interest does not have a dipolar mechanism (i.e. no attached proton, for example a phosphate group or carbonyl group in a sparsely labeled protein). Furthermore, a difference in isotropic chemical shift between the two exchange sites is not necessary as is required for solution NMR $R_{1\rho}$ measurements [24].

$R_{1\rho}$ studies have been used to gain insight into the general timescales of protein dynamics. Helmus et al used $R_{1\rho}$ relaxation (among other techniques) to investigate the dynamics of the amyloid core residues of Y145 Stop human prion protein [21]. Lewandowski et al measured site-specific $R_{1\rho}$ values and modeled correlation times for GB1 [19]. Krushelnitsky et al compared $R_{1\rho}$ values to order parameters to investigate microsecond timescale dynamics in the SH3 domain of α -spectrin [18].

The development of quantitative methods to interpret relaxation data and extract exchange rates has become increasingly important as site-specific measurements of $R_{1\rho}$ relaxation rates has become an increasing popular technique [18–21]. Quantitative measurements of site-specific protein dynamics, specifically precise rates of molecular reorientation using $R_{1\rho}$ measurements may be an important development to gain insight into protein function.

Analytical approaches to determining exchange rates from $R_{1\rho}$ relaxation for solid-state systems have been developed [7, 22]. These analytical descriptions to calculating $R_{1\rho}$ and/or exchange rates are based upon solving the master equation for the density matrix. While these derivations provide insight into the mechanism of $R_{1\rho}$ relaxation, they have limitations. To our knowledge, little has been done in the way of applying these analytical expressions to experimental data. We chose a numerical approach to solving the problem of extracting exchange rates from $R_{1\rho}$ data. Using Spinevolution [23], allows implementation for a wide range of spin systems and pulse sequences.

For solid state rotating frame relaxation studies, complications arise at the rotary resonance conditions ($\omega_1 = n\omega_R$, $n = 1, 2$). On these conditions, the CSA is recoupled [25, 26], leading to dramatically faster relaxation rates. As shown in the expressions for $R_{1\rho}$ relaxation due to

reorientation of a CSA tensor derived by Fares et al [7] and Kurbanov et al [22], there is also enhanced relaxation due to dynamics when $\omega_1 = n\omega_R$. However, the rotary resonance condition is by far the dominant effect. To allow for quantitative results, the rotary resonance conditions are avoided in this work until we can better quantify the relaxation due to both CSA recoupling and CSA reorientation of a dynamic system on and near these conditions. In order to quantify R_{ex} and k_{ex} , it is necessary to also quantify the R_2^0 contribution to relaxation which is due to interactions other than molecular reorientations. Accurately determining R_2^0 is necessary to fitting k_{ex} because of the covariance between the two parameters as expressed in equation 1. In the solid state, R_2^0 may include fast rotation of methyl groups mentioned above, as well as incomplete decoupling of dipolar interactions. These complications will be further discussed below.

In this paper we will show that rotating-frame relaxation studies using reorientation of the CSA tensor as the probe of dynamics can be used to quantify the rate of molecular reorientation in a model system, d_6 -DMS. To our knowledge, this is the first example of $R_{1\rho}$ experiments being utilized to extract experimental exchange rates using the CSA tensor and the use of this model system serves to validate this new approach. Future extension of this method to biological systems may provide a new method to site-specifically quantify intermediate timescale dynamics with the novel advantages that dipolar interactions or differences in isotropic chemical shift between the two exchange sites are not necessary to still observe relaxation.

Materials and Methods

Protonated DMS was obtained from Fluka and d_6 -DMS was obtained from Cambridge Isotopes. Compounds were stored under vacuum for 24 hours to ensure they were dry, as hydration has been shown to affect observed dynamics in DMS [27]. Power diffraction was done to confirm the space group of the crystals and solution NMR was used to check the purity and % deuteration of the compounds (~99.5% 2H for d_6 -DMS). Approximately 35 mg of each DMS compound was doped with 1–2% KBr and packed into the center third of Varian 4 mm rotors. Comparison with relaxation curves collected with no KBr indicated that this doping had no effect on the measurements.

All data was collected on a Varian Chemagnetics 400 MHz spectrometer spinning at 10000 ± 3 Hz. The variations in the $R_{1\rho}$ pulse sequence used in the different on-resonance experiments were as follows: 1H -DMS: $([90^C(\phi_1)] - [CW \text{ dec}]^H(\phi_2) / SL^C(\phi_3)) - [(TPPM \text{ dec}]^H/acq]$; d_6 -DMS ($R_{1\rho}$ and R_2^0 experiments): $([90^C(\phi_1)] - [SL^C(\phi_3)] - [acq])$; d_6 -DMS (2H decoupling controls): $([90^C(\phi_1)] - [CW \text{ dec}]^D(\phi_2) / SL^C(\phi_3)) - [acq]$. The simple phase cycling scheme was $\phi_1=[x, -x, y, -y]$; $\phi_2=[x]$; $\phi_3=[-y, y, x, -x]$. Typical ^{13}C 90° pulse lengths were 5 μs . On-resonance spin-lock pulses were applied for lengths of 100 μs , 5 ms, 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 35 ms, and 40 ms and at range of field strengths from 25–45 kHz for 40° – $80^\circ C$ experiments. For R_2^0 measurements at $-25^\circ C$ and $-40^\circ C$, the on-resonance spin-lock pulses were applied for 100 μs , 10 ms, 20 ms, 30 ms, 40 ms, 50 ms, 60 ms, 70 ms, 80 ms, 90 ms, and 100 ms. The true field strength of the applied spin-lock was measured for each decay curve collected. Both the protonated and deuterated experiments utilized direct excitation to generate carbon magnetization, as experiments and simulations indicated that some relaxation occurs during cross polarization, resulting in erroneously low relaxation rates for 1H -DMS when excited using cross polarization, inhibiting quantitative interpretation of relaxation rates. 1H decoupling field strengths for 1H -DMS experiments were 93 kHz and 2H decoupling field strengths for d_6 -DMS experiments that used 2H decoupling were 10–20 kHz during the spin-locks.

Data was collected at several different temperatures. For dynamics studies, an accurate measure of temperature is important, so temperature calibrations were performed, using the ^{79}Br chemical shift of the KBr-doped DMS [28]. Briefly, a reference point was acquired by spinning the rotor as slowly as possible (1–3 kHz). Under these conditions, we assume that there is no heating due to magic angle spinning, and that the KBr chemical shift is representative of the true sample temperature. Spinning the sample up to 10 kHz and measuring the change in the ^{79}Br chemical shift allows the measurement of heating due to faster spinning. A 10 kHz MAS rate was found to contribute an additional 7°C ($\pm \sim 2^\circ$) of heating and data analysis took this additional heating into account. Temperatures indicated in figures and discussions are true sample temperatures, based on the calibration data. For crystalline DMS, no heating due to high-power radio frequency irradiation was observed to occur.

Data analysis was performed as follows. The decay in the normalized integrated intensity of the carbon peak as a function of spin-lock length was fit to a single-exponential decay to obtain $R_{1\rho}$. In some cases (particularly at lower spin-lock field strengths), the decay curves were better statistical fits to double-exponential decays. We do not have a model to explain this multi-exponential behavior and for this reason, spin-lock field strengths below 25 kHz were generally avoided. The observed $R_{1\rho}$ and R_2^0 values were plotted as a function of spin-lock field strength to generate the dispersion curves as shown in figures 2–4. It should be noted that fitting the exchange rates as described below was done using raw data and therefore requires no assumption regarding the single- vs. multi-exponential behavior of the decay curves.

Two-site molecular reorientation between two conformations A and B can be modeled by the reaction

in which k_1 is the rate constant for the forward reaction and k_{-1} is the rate constant for the reverse reaction. Measuring relaxation due to molecular reorientation is one means of obtaining information about the exchange process of interest [15].

Rotating frame ($R_{1\rho}$) relaxation experiments measure the rate of decay of magnetization parallel to the effective field in the rotating frame:

$$R_{1\rho} = R_1 \cos^2 \theta + R_2^0 \sin^2 \theta + R_{ex} \sin^2 \theta \quad \text{Eq. 1}$$

where R_1 is longitudinal relaxation, R_2^0 is transverse relaxation in the absence of a dynamic process, R_{ex} is transverse relaxation due to molecular reorientation and θ is the tilt angle between the static magnetic field and the effective field in the rotating frame. $R_{1\rho}$ experiments are sensitive to dynamics on the microsecond-to-millisecond timescale. For intermediate timescale exchange processes, the exchange contribution is distinguished from the transverse and longitudinal relaxation terms by its characteristic field strength dependence. Fast timescale dynamics, such as rotation of a methyl group, also contribute to $R_{1\rho}$. However under the condition that ω_e (the effective spin-lock field) $\ll 1/k$, $R_{1\rho}$ due to fast timescale dynamics is independent of the applied spin-lock field strength [16] and can be viewed as an R_2^0 process. This R_2^0 process can be considered the asymptote of an $R_{1\rho}$ dispersion curve.

To obtain k , the optimization function in Spinevolution [23] was used to minimize the χ^2 difference between the raw decay curves and simulated decay curves with k and a scaling factor as the fitting parameters. In this data-fitting process, Spinevolution generates simulated decay curves (intensity as a function of spin-lock length, corresponding to the spin-lock lengths utilized in the experiments) at the specified spin-lock field strengths (i.e. the spin-lock field strengths used in the actual experiments). The difference between the experimental decay curve and these simulated decay curves was minimized using the NL2SOL non-linear least squares algorithm and the exchange rate k as the fitting parameter, as this is the mechanism of decay in these experiments. Spinevolution performs calculations numerically by solving either the Liouville- von Neumann equation or Master equation using approximations such as the Chebyshev expansion to approximate the propagators of the Hamiltonian. Further detail can be found in reference [23]. The exchange process was modeled as a 2-site hop between 2 carbon atoms with a β angle of 109° . The CSA tensor parameters used were $\delta = -36.77$ ppm and $\eta = 0.0625$ [11]. 538 crystallites were used for powder averaging. An additional R_2^0 relaxation term was included to account for the measured R_2^0 relaxation as shown in Figure 4 and discussed further in the results. All relaxation curves collected at a single temperature (but different spin-lock field strengths) were combined into a single optimization and fit to a single k .

Sample raw data, as well as fits to exponential decays and simulations can be found in the supplementary material.

Results and Discussion

Dimethyl sulfone (Figure 1) undergoes a 2-site hop around the molecule's C_2 symmetry axis, an exchange process that has been extensively studied using solid state NMR [4, 5, 8, 9, 11, 29–36]. The methyl group of the molecule also undergoes fast timescale rotation ($\sim 10^9$) [37]. The 2-site hop is a slow-to-intermediate timescale activated process that R_{ex} is expected to report on. The fast methyl rotation is likely spin-lock field independent and captured in the R_2^0 component of the observed relaxation.

Figure 2 shows dispersion curves collected for protonated and deuterated DMS at 77°C , 10 kHz MAS. These curves have 2 notable features. The first, discussed in our previous work [9], is that $R_{1\rho}$ for both compounds has a dependence on the applied spin-lock field strength, indicating that our method is probing relaxation due to reorientation of the CSA tensor. The second notable feature is that the relaxation rates are significantly higher for protonated DMS than deuterated DMS. We attribute this difference to the strong coupling of the carbon to its methyl protons, which could not be fully eliminated despite applying high-power proton decoupling during the spin-lock. VanderHart and Garraway reported the dominant contribution of static dipolar interactions with protons to the observed carbon rotating-frame relaxation rate [17]. As suggested by our previous work on the static control molecule alanine [9], where the absence of an $R_{1\rho}$ field-dependence suggests the spin-spin contribution is being suppressed [38], the observed relaxation for protonated DMS likely has a spin-lattice contribution from reorientation of the C-H dipolar tensor in addition to relaxation due to reorientation of the CSA tensor. We reported previously that while the application of proton decoupling did lead to slower relaxation rates relative to no decoupling, the strength of the decoupling did not affect the relaxation rates. It may be that the dynamics of the molecule itself contributes to the inefficient decoupling [39]. For the deuterated DMS dispersion curve, control experiments showed that deuterium decoupling had no effect on the observed relaxation, suggesting that C-D dipolar relaxation is not a mechanism that contributes to relaxation in these experiments. While deuterated DMS is a very special case with an extremely limited number of relaxation mechanisms, we have chosen this simple model system to validate our method of extracting exchange rates from

relaxation curves using numerical computations. In the future, we hope to show that this method can be extended to more complicated systems, such as proteins as well as other relaxation mechanisms, including relaxation due to reorientation of dipolar vectors.

A further challenge to obtaining quantitative exchange rates arises from the method of excitation. Both curves shown in Figure 2 were obtained using direct excitation rather than cross polarization. Both simulations and experiments (data not shown) indicate that some relaxation occurs during cross polarization, leading to erroneously low measured relaxation rates for ^1H -DMS when excited using cross polarization. Additional indicators of the complex relaxation behavior in protonated DMS include the failure of global analysis using protonated data sets to reach convergence, and the experimental observation of higher order rotary resonance conditions. Because of the challenges that arise from attempting to account for the multitude of relaxation contributions present in the protonated compound, we have chosen to perform our quantitative work on deuterated DMS where these additional terms are minimized. Fares et al also commented on the challenges of accounting for the residual strengths of existing interactions [7]. It is common practice to use deuteration to simplify relaxation measurements and eliminate undesired cross relaxation terms [18, 40, 41].

Dispersion curves were obtained for d_6 -DMS at 4 different temperatures by measuring the decay of magnetization as a function of spin-lock length for a range of applied spin-lock fields, as shown in Figure 3. The field- and temperature-dependence of these curves are indicative of the exchange process. The multi-exponential behavior at lower spin-lock fields is reflected in the larger error bars when these data sets are fit to single-exponentials.

As shown in equation 1, the relaxation rate R_2^0 is independent of the conformational exchange rate but can contribute to the observed $R_{1\rho}$ relaxation rate through a number of processes. One challenge of this method for quantifying rates of molecular reorientation is determining the R_2^0 contribution to $R_{1\rho}$. The accurate measurement of R_2^0 is critical to obtaining correct exchange rates due to the covariance between R_{ex} and R_2^0 as expressed in equation 1. In order to determine the R_2^0 contribution to the observed relaxation, we determined where the $R_{1\rho}$ dispersion curves shown in Figure 3 reached an asymptotic temperature- and spin-lock independent $R_{1\rho}$ value, indicating that the R_{ex} component has been eliminated. $R_{1\rho}$ experiments were performed at very low temperatures and high spin-lock field strengths where the R_{ex} component to the relaxation (i.e. the intermediate exchange C_2 180° flip of DMS) is suppressed. At temperatures of -40°C and -25°C , we were able to obtain spin-lock and spinning speed independent relaxation rates, suggesting that R_2^0 is the process being observed. At these low temperatures, the exchange rate for the 180° flip is well below the timescale that $R_{1\rho}$ experiments are sensitive to. The average R_2^0 value from these measurements was $1.8 \pm 0.2 \text{ s}^{-1}$, shown in Figure 4.

Two possible interpretations of the measured low-temperature $R_{1\rho}$ value are that this value corresponds to a temperature-dependent process, most likely rotation of the deuterated methyl groups, or temperature-independent dephasing processes, including pulse imperfections, field inhomogeneity, or deuterium spin diffusion. The choice of a temperature-dependent or independent model has a small impact on the resulting exchange rates for the 180° flip of DMS. As we have no concrete reason to reject either model, we have presented both, showing that they agree within the error of the method.

To determine quantitative exchange rates from the observed relaxation, the R_2^0 values were then included in Spinevolution calculations using the program's optimization function to minimize the χ^2 difference between experimental relaxation curves and simulated curves which used the exchange rate k as the sole fitting parameter as described in the Materials and Methods section. The exchange rates determined through this fitting procedure for both

the temperature-dependent and temperature-independent models for R_2^0 are shown in Table 1 and an Arrhenius plot of the exchange rates obtained from this analysis are shown in Figure 5, along with data previously measured through a wide range of other techniques.

The low temperature $R_{1\rho}$ value, attributed to R_2^0 relaxation, may arise from the fast-limit, spin-lock field independent rotation of the deuterated methyl groups, which even at low temperatures is still in the fast limit for $R_{1\rho}$ experiments ($\sim 10^9$) [37, 42, 43]. Assuming an Arrhenius temperature-dependence for the methyl group rotation, with an activation barrier on the order of 20–30 kJ/mol and a pre-exponential factor of 10^{13} – 10^{15} , physically realistic and typical for a methyl group, we can estimate an $R_{1\rho}$ contribution from this fast-limit process over the range of -40°C to $+80^\circ\text{C}$ according to the expressions in reference [16]. Both the small uncertainty in the true sample temperature (5– 10° sample heating due to MAS) and activation barrier lead to a range of possible R_2^0 values for the methyl rotation at each temperature. The contribution to $R_{1\rho}$ would be expected to be relatively higher at -40°C and -25°C , and of the order of our experimentally derived value, 1.8 s^{-1} , and would be expected to be essentially negligible at 80°C . The exchange rates presented in Table 1 represent the middle of the range of sample temperature and activation energy with the errors indicating the exchange rates determined at the highest and lowest values determined in the range of both temperature and activation energy. It is important to note that the error due to the uncertainty in these experimental parameters is greater than the error due to data-fitting in Spinevolution.

Another possible explanation for the observed R_2^0 contribution to relaxation could be due to unidentified temperature-independent dephasing processes such as pulse imperfections, field inhomogeneity, or deuterium spin diffusion. The Spinevolution [23] optimization to determine exchange rates was also done using the experimentally determined R_2^0 value of 1.8 s^{-1} at all temperatures. The exchange rates determined using this temperature-independent R_2^0 model agreed within error with the results using the temperature-dependent model described above, as shown in Table 1. The errors in the fit from Spinevolution is the same order of magnitude as the error introduced by the error in R_2^0 .

It is significant to note that the agreement between the exchange rates calculated using the 2 different models for R_2^0 are in agreement within the error of the method. The possible range of R_2^0 values in each model is the largest source of error expressed in the exchange rate (rather than the data-fitting). Over the range of temperatures utilized in this study, these small R_2^0 contributions have little effect on the exchange rates determined in data-fitting.

The errors on the exchange rates were determined as follows. For the temperature-independent model, simulations were repeated using the maximum and minimum R_2^0 values based on the error in the experimentally determined R_2^0 value, and the exchange rates determined from these calculations were used as the lower and upper limits of the exchange rate error. For the temperature-dependent model, the largest source of error arises from the choice of Arrhenius parameters for the methyl rotation and the uncertainty in the temperature, and the corresponding range of possible R_2^0 values. The upper and lower limits of the exchange rates were determined by determining the R_2^0 values corresponding with activation energies of 20 kJ/mol and 30 kJ/mol for the methyl group rotation, extrapolated to the higher temperature range used (calibrated temperature $\pm \sim 2^\circ\text{C}$) and calculating corresponding exchange rates with Spinevolution.

An Arrhenius analysis of the exchange rates from the temperature-independent R_2^0 model gave an activation energy of $74.7 \pm 4.3\text{ kJ/mol}$ and an $\ln(A)$ value of 34.6 ± 1.6 . Using the temperature-dependent model gave an activation energy of $71.7 \pm 2.9\text{ kJ/mol}$ and an $\ln(A)$ value of 33.6 ± 1.0 . These values are in excellent agreement with the Arrhenius parameters

determined for DMS using other techniques and are presented for comparison in Table 2. The variation in these parameters among the various methods may be attributed to disagreement in the Arrhenius pre-factor A . From the crystal structure of DMS, the $\ln(A)$ value was calculated by Brown et al [29] to be 29.0 ($A \sim 10^{12}$), lower than that determined experimentally by all methods reported in Table 2. A common explanation for inaccurate $\ln(A)$ measurements is that the activation energy is temperature-dependent. This disagreement could also be attributed to error propagation within the measurements such as systematic errors in the determination of R_2^0

or another methodological issue. It has also been noted that Arrhenius plots using NMR data (particularly over a small temperature range) can be intrinsically flawed [29, 31, 44].

It is of interest to note that a plot of $\ln(R_{1\rho})$ as a function of inverse temperature as we previously presented [9] does not provide the same robust results that an Arrhenius analysis of the exchange rate does. It is possible that this failure of the Arrhenius behavior of $R_{1\rho}$ may be due to deviations from fast-limit behavior. Furthermore, other systematic errors such as different behavior at different spin-lock field strengths may limit a straight-forward Arrhenius analysis of $R_{1\rho}$ to extract activation energies. Thus it is advantageous to quantify the exchange rates for a dynamic process and determine the activation energy in the manner presented in this paper.

As an alternative to measuring R_2^0 experimentally, we considered whether this value could be extracted by fitting the dispersion curve to a Lorentzian function, which is the expected functional form of the dispersion curve [7]. Using the experimentally determined exchange rates and R_2^0 values at 77°C for the temperature-independent R_2^0 model ($R_2^0 = 1.8 \text{ s}^{-1}$, $k = 7464 \text{ s}^{-1}$), the simulated dispersion curve shown in blue in Figure 6 was obtained. The points $\pm 1 \text{ kHz}$ of the rotary resonance conditions were eliminated due to the non-dynamic components to the relaxation at these points. The dispersion curve was fit to a double Lorentzian function, fixing the centers of the Lorentzians at 10 kHz ($1\omega_R$) and 20 kHz ($2\omega_R$). From this fitting, we were interested in extracting the offset of the Lorentzian from the baseline, which would be representative of the system's R_2^0 value. Despite simulating the dispersion curve with a broad range of spin-lock field strengths (out to 200 kHz), the R_2^0 value we obtained from fitting the dispersion curve was in error ($R_2^0 = 4.12 \pm 1.98 \text{ s}^{-1}$, compared to the true value of 1.8 s^{-1}). Practically we must restrict the range of spin-lock field strengths further to $\nu_1 < \text{approximately } 100 \text{ kHz}$ due to probe limitations and $\nu_1 > \text{about } 25 \text{ kHz}$ because the $\omega_1 = n\omega_R$ conditions are broad in practice. This indicates that although fitting a dispersion curve to a Lorentzian function can give an order-of-magnitude value for R_2^0 , we believe that the experimental approach used in this paper can give more accurate results.

In conclusion, we have shown that the chemical shift anisotropy tensor can be used as a site-specific, quantitative probe of intermediate timescale conformational dynamics in $R_{1\rho}$ experiments to obtain exchange rates and activation energies. Performing these studies in the solid state is advantageous because in solution NMR, signals for sites undergoing intermediate exchange are often not observable due to exchange broadening. This method has unique advantages in that dipolar couplings and isotropic chemical shift differences between exchange sites are not necessary to observe dynamics. In the future this approach to extracting exchange rates may be applied to other mechanism, such as reorientation of dipolar tensors. Our conclusions suggest important methodological considerations for the application of the method to more complicated systems such as proteins. The challenge of removing the strong dipolar coupling to protons indicates that deuteration is necessary to minimize this interaction. However deuteration of proteins to reduce undesired couplings is already common practice for the measurement of relaxation values [40, 41]. While DMS is

an ideal case where the CSA parameters are well known, in more complicated systems the magnitudes and orientations of tensors may not be known, presenting an additional challenge. However techniques for the determination of CSA magnitudes and tensor orientations in proteins are readily available [45, 46]. When applying $R_{1\rho}$ techniques to quantify exchange rates, direct excitation is optimal to eliminate relaxation occurring during cross polarization to obtain accurate relaxation rates. As in any dynamics study, knowledge of sample temperature is important. Finally, the ability to minimize or quantify R_2^0 is a limiting factor in fitting accurate exchange rates using $R_{1\rho}$ relaxation, and may present an even greater challenge in more complicated systems than DMS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Quantitative exchange rates were obtained with solid state $R_{1\rho}$ relaxation studies
- Reorientation of the CSA tensor in d_6 -DMS was used as the probe of exchange
- Exchange rates and activation energies are in agreement with literature values
- The ability to quantify R_2^0 and temperature limit the accuracy of the method
- Methodological considerations for application to protein systems are given

Figure 1. Structure and motional model of dimethyl sulfone (DMS). DMS undergoes a 180° flip around the molecule's C_2 axis, a motion that has been well-characterized in the solid state NMR literature. In this study we have used both the protonated and deuterated forms of DMS.

Figure 2.

Dispersion curves for $^1\text{H-DMS}$ (■) and $d_6\text{-DMS}$ (●) collected at 77°C , 10 kHz MAS. The field-strength dependence of both dispersion curves indicates that molecular reorientation of the carbon CSA tensor is being observed. The dramatically higher relaxation rates for protonated DMS are due to contributions from C-H dipolar interactions. The complexity of interactions occurring in the protonated compound suggests that deuteration is necessary to obtain quantitative exchange rates. The relative error for both data sets, generated from the error in the fit to a single-exponential decay, is the same, $\sim 5\text{--}10\%$. As is sometimes seen in the literature, a logarithmic plot of this data is shown in the supporting material.

Figure 3.

Dispersion curves collected at 4 different temperatures, (♦) 37°C, (◄) 57°C, (■) 67°C, and (●) 77°C for d_6 -DMS. The greater dependence of $R_{1\rho}$ on spin-lock power at higher temperature indicates a faster exchange rate for the two-site hop. The larger error at low spin-lock field strengths is due to residual multi-exponential behavior near the rotary resonance condition. As is sometimes seen in the literature, a logarithmic plot of this data is shown in the supporting material.

Figure 4.

$R_{1\rho}$ values measured at low temperatures and high spin-lock fields are shown. The average $R_{1\rho}$ determined from these measurements was $1.8 \pm 0.2 s^{-1}$. The observed temperature and spin-lock field independence suggest that the minimum $R_{1\rho}$ value is being measured. Data was collected under the following conditions: (●) $-40^{\circ}C$, 10 kHz MAS; (■) $-40^{\circ}C$, 5 kHz MAS; and (●) $-25^{\circ}C$, 5 kHz MAS.

Figure 5.

Arrhenius plot of exchange rates for the $180^\circ C_2$ flip of DMS measured using a wide range of solid-state NMR techniques highlighting the results for each individual technique. (●) $R_{1\rho}$ relaxation with a temperature-independent R_2^0 model (our data presented herein); (●) $R_{1\rho}$ relaxation with a temperature-dependent R_2^0 model (our data presented herein); (●) quadrupolar echo [29]; (●) on-resonance selective inversion [29]; (●) off-resonance selective inversion [29]; (●) static 2D lineshape analysis [31]; (●) ODESSA [31]; (●) time-reverse ODESSA [31]; (●) exchange-induced sidebands [31]; (●) VACSYS lineshape analysis [32]; (■) 1D static lineshape analysis [32]; (●) 1D MASS lineshape analysis [34]; (●) 2D MAS lineshape analysis [33]; (●) 1D static lineshape analysis [11]. Error bars are plotted when available. The Arrhenius parameters determined from each of these methods are reported in Table 2.

Figure 6.

(—) Simulated dispersion curves using experimentally determined parameters at 77°C, 10 kHz MAS for the temperature-independent model ($k = 7464 \text{ s}^{-1}$, $R_2^0 = 1.8 \text{ s}^{-1}$). (- -) Fit of dispersion curve to a double Lorentzian function. The points $\pm 1 \text{ kHz}$ of the $1\omega_R$, $2\omega_R$ conditions were eliminated from the dispersion curve because of the non-dynamic contributions to R_{1p} at these conditions. The R_2^0 value determined from the fit to a double Lorentzian was $4.12 \pm 1.98 \text{ s}^{-1}$, compared to the true value of $1.8 \pm 0.2 \text{ s}^{-1}$. Although this extrapolation method can determine order-of-magnitude R_2^0 fits, we believe that the experimental approach used in this paper gives a more accurate measure of R_2^0 .

Table 1

Exchange rates determined for the C_2 180° flip in d_6 -DMS using $R_{1\rho}$ relaxation and applying both temperature-independent and temperature-dependent models for the additional R_2^0 relaxation term. The errors in the exchange rates were determined by the error in the experimental R_2^0 measurement for the temperature-independent model and the error that arises from the choice of the methyl rotation activation energy in the range of 20–30 kJ/mol for the temperature-dependent model. However at such small R_2^0 values the differences observed between these two models are small.

Temperature	Temperature-independent Dephasing Model Exchange Rate (s ⁻¹)	R_2^0 Arrhenius Methyl Rotation Model Exchange Rate (s ⁻¹)
37°C	300 ± 30	350 ± 40
57°C	1600 ± 200	1800 ± 300
67°C	4500 ± 400	4500 ± 400
77°C	7500 ± 800	7900 ± 800

Table 2

Arrhenius parameters measured for protonated and deuterated DMS using our method, rotating-frame relaxation, and values obtained from linear fits of exchange rates from other solid-state NMR methods reported in the literature. Our values are in excellent agreement with previous measurements. The data indicates the wide range of Arrhenius parameters determined for this system, possibly due to the challenge that accurately measuring the pre-factor A presents in NMR experiments, particularly over a small temperature range [29, 31, 44].

Method	E_A (kJ/mol)	$\ln(A)$
1D static lineshape analysis [11]	63.2 ± 3.0	30.9 ± 1.2
VACSY lineshape analysis [32]	64.3 ± 0.2	32.0 ± 0.1
1D static lineshape analysis [32]	64.6 ± 0.6	32.1 ± 0.2
2D MAS lineshape analysis [33]	69.2 ± 0.03	33.3 ± 0.01
Time-reverse ODESSA [31]	71.0 ± 7.2	32.7 ± 3.1
$R_{1\rho}$ relaxation (temperature-dependent R_2^0)	71.7 ± 2.9	33.6 ± 1.0
ODESSA [31]	74.0 ± 7.5	34.0 ± 3.2
$R_{1\rho}$ relaxation (temperature-independent R_2^0)	74.7 ± 4.3	34.6 ± 1.6
Quadrupolar echo [29]	75.7 ± 2.7	35.6 ± 0.9
1D MASS lineshape analysis [34]	75.7 ± 9.6	35.7 ± 3.7
Exchange induced sidebands [31]	76.1 ± 5.0	35.0 ± 2.2
Static 2D lineshape analysis [31]	81.3 ± 4.8	37.0 ± 2.0
On-resonance ^2H selective inversion [29]	86.1 ± 4.9	39.0 ± 1.9
Off-resonance ^2H selective inversion [29]	88.6 ± 3.0	40.1 ± 1.1