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Evaluating the Uncertainty in Exchange Parameters Determined from Off-Resonance R1ρ Relaxation Dispersion for Systems in Fast Exchange

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Abstract

Spin relaxation in the rotating frame (R1ρ) is a powerful NMR technique for characterizing fast microsecond timescale exchange processes directed toward short-lived excited states in biomolecules. At the limit of fast exchange, only $k_{ex} = k_1 + k_{-1}$ and $\Phi_{1x} = p_G p_E (\omega)^2$ can be determined from R1ρ data limiting the ability to characterize the structure and energetics of the excited state conformation. Here, we use simulations to examine the uncertainty with which exchange parameters can be determined for two state systems in intermediate-to-fast exchange using off-resonance R1ρ relaxation dispersion. R1ρ data computed by solving the Bloch-McConnell equations reveals small but significant asymmetry with respect to offset (R1ρ(Ω) R1ρ(−Ω)), which is a hallmark of slow-to-intermediate exchange, even under conditions of fast exchange for free precession chemical exchange line broadening ($k_{ex}/\omega > 10$). A grid search analysis combined with bootstrap and Monte-Carlo based statistical approaches for estimating uncertainty in exchange parameters reveals that both the sign and magnitude of ω can be determined at a useful level of uncertainty for systems in fast exchange ($k_{ex}/\omega < 10$) but that this depends on the uncertainty in the R1ρ data and requires a thorough examination of the multidimensional variation of χ^2 as a function of exchange parameters. Results from simulations are complemented by analysis of experimental R1ρ data measured in three nucleic acid systems with exchange processes occurring on the slow ($k_{ex}/\omega = 0.2$; $p_E = \sim 0.7\%$), fast ($k_{ex}/\omega = \sim 10$ –16; $p_E = \sim 13\%$) and very fast ($k_{ex} = 39,000 \text{ s}^{-1}$) chemical shift timescales.

Keywords

Rotating-frame relaxation dispersion; fast exchange; excited state; dynamics; DNA; RNA

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Supporting Information.

Table of spin lock powers and offsets used for synthetic data sets.

Introduction

Relaxation dispersion NMR techniques provide a powerful approach for characterizing transient excursions away from energetically favorable ground state (GS) conformations of biomolecules toward short-lived (lifetimes < milliseconds) and low-populated (populations < 30%) excited states (ES). Such fleeting states have been shown to play important roles in signaling, folding, catalysis, and recognition.[1–7] Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion experiments involve the application of a series of 180° pulses during a relaxation period at varying time intervals to modulate the chemical exchange contribution (R_{ex}) to the observed transverse relaxation rate ($R_{2obs} = R_2 + R_{ex}$). [2, 8] CPMG relaxation dispersion experiments can be used to characterize processes with exchange rates ($k_{ex} = k_1 + k_{-1}$) in the range of $\sim 10 \text{ s}^{-1} < k_{ex} < \sim 6000 \text{ s}^{-1}$. [8, 9] CPMG data is typically measured at multiple magnetic field strengths and combined with additional experiments (e.g. HSQC/HMQC) in order to determine the sign of the excited state chemical shift. [10] A second class of dispersion experiments involves measuring the rotating frame relaxation rate constant ($R1\rho$) upon application of a radiofrequency (RF) spin lock field with varying offset frequency (Ω) and power (ω_1) (Figure 1). [11] The exchange timescale accessible to $R1\rho$ is broader than CPMG ($\sim 60 \text{ s}^{-1} < k_{ex} < \sim 100,000 \text{ s}^{-1}$) [11] and for slow-intermediate exchange, the sign of excited state chemical shift can be deduced at a single magnetic field strength [12]. For processes occurring at even slower timescales ($\sim 20 \text{ s}^{-1} < k_{ex} < \sim 300 \text{ s}^{-1}$) chemical-exchange saturation transfer (CEST) experiments employing weak RF spin lock fields have recently been shown to be a robust approach to characterize lowly populated conformational states in both proteins [13, 14] and nucleic acids [15].

Much effort has been directed towards establishing criteria for obtaining reliable exchange parameters from fitting relaxation dispersion data. [9, 16–21] Statistical analysis of CPMG data has resulted in improved experimental strategies for minimizing data collection times and has helped define the exchange scenarios that can be reliably characterized experimentally. [9, 19] While CPMG relaxation dispersion is widely used in studies of proteins, $R1\rho$ methods are often applied to uniformly $^{13}\text{C}/^{15}\text{N}$ labeled nucleic acids because unwanted carbon-carbon interactions can be more easily suppressed. [15, 22–25] Because higher effective fields can be used in the $R1\rho$ experiment as compared to CPMG, it is also better suited for characterizing faster microsecond exchange processes. [11, 26]

Many useful algebraic equations have been derived to approximate off-resonance $R1\rho$ relaxation dispersion data. [12, 27–29] Studies have also examined the robustness with which exchange parameters can be characterized using $R1\rho$ data for systems in slow exchange ($k_{ex} \sim 20$ milliseconds) with the aid of weak spin lock fields (typically as low as 25–100 Hz). [15, 24, 30, 31] However, the reliability of exchange parameters obtained from $R1\rho$ relaxation dispersion data for systems in fast exchange has not been thoroughly investigated. Given that $R1\rho$ relaxation dispersion is particularly well-suited for characterizing fast exchange, it is of great interest to evaluate and understand the uncertainty with which exchange parameters can be deduced from $R1\rho$ data near the fast exchange limit. Indeed fast exchange ($k_{ex} = \sim 25,000 - 40,000 \text{ s}^{-1}$) has been widely observed in biomolecules, including in proteins [32], DNA [24], and RNA [33].

At the fast exchange limit, $R_{1\rho}$ scales with $\Phi_{ix} = p_G p_E (\omega)^2$, where p_G and p_E are the population of the ground and excited state, respectively, and ω is the difference between the ES and GS chemical shifts ($\omega = \omega_E - \omega_G$). [34] Thus, in the fast exchange limit ($k_{ex} \gg \omega$), it is impossible to independently determine the chemical shift and population of the excited state, and therefore its structure [33, 35–37] and free energy relative to the ground state. However, as noted previously, the conditions that satisfy the fast exchange limit in the $R_{1\rho}$ experiment differ from free precession chemical exchange line broadening and will also depend on the spin lock power and offset. [28] Few studies have examined how the uncertainty in exchange parameters derived from $R_{1\rho}$ varies as one approaches the fast exchange limit.

In this study we use simulations to evaluate the uncertainty in exchange parameters determined using $R_{1\rho}$ relaxation dispersion for systems spanning the slow to fast exchange limits ($k_{ex}/\omega = 0.2 - 16$). We use grid searching of χ^2 as a function of the exchange parameters p_E , ω , k_{ex} (“ χ^2 surface”) to examine the behavior of exchange parameter fitting and implement bootstrap and Monte-Carlo based statistical approaches [38], employed previously in analyzing CPMG data [9, 19], to estimate the uncertainty in exchange parameters determined using $R_{1\rho}$ data. We find that both the sign and magnitude of ω can be determined at a useful level of uncertainty near conditions of fast exchange ($k_{ex}/\omega < 10$) but that this depends on the uncertainty in the $R_{1\rho}$ data and requires a thorough examination of the multidimensional χ^2 surface. The results from simulations are complemented by analysis of $R_{1\rho}$ relaxation dispersion data measured on three nucleic acid systems exhibiting exchange processes on the slow ($k_{ex}/\omega = 0.2$; $p_E = \sim 0.7\%$), fast ($k_{ex}/\omega = 10-16$; $p_E = \sim 13\%$) and very fast ($k_{ex} = 39,000 \text{ s}^{-1}$) chemical shift timescales.

Methods

Theory

The $R_{1\rho}$ relaxation dispersion experiment measures the rotating frame relaxation rate constant, $R_{1\rho}$, as a function of the frequency (ω_{rf}) and amplitude (ω_1) of a constant radiofrequency (RF) spin lock field. [11] $R_{1\rho}$ is determined by measuring the exponential decay of the projection of the magnetization about the effective magnetic field (ω_{eff}) (Figure 1) as a function of time. An expression for $R_{1\rho}$ is given by,

$$R_{1\rho} = R_1 \cos^2 \theta + R_2 \sin^2 \theta \quad (1)$$

$$\theta = \tan^{-1} \left(\frac{\omega_1}{\Omega - \omega_{rf}} \right)$$

where ω_{rf} (in units of s^{-1}) is the frequency of the applied spin lock field, ω_1 (in units of s^{-1}) is the amplitude of the applied spin lock field and Ω is the resonance frequency; R_1 and R_2 (in units s^{-1}) are the longitudinal and transverse relaxation rate constants, respectively.

In the presence of conformational exchange, the Larmor frequency of a given nucleus changes stochastically during the spin lock period. As a result, the magnetization

stochastically precesses about either the effective field of the excited (ω_E) or ground (ω_G) state, leading to dephasing of the magnetization and a contribution, R_{ex} , to $R_1\rho$ that is given by,

$$R_{1\rho} = R_1 \cos^2 \theta + (R_2 + R_{ex}) \sin^2 \theta \quad (2)$$

$$\theta = \tan^{-1} \left(\frac{\omega_1}{\Delta\Omega} \right),$$

$$\Delta\Omega = \bar{\Omega} - \omega_{rf},$$

$$\bar{\Omega} = p_G \Omega_G + p_E \Omega_E,$$

p_G and p_E are the populations of the ground and excited states, respectively; Ω_G and Ω_E are the resonance frequencies of the ground and excited states, respectively; Ω is the resonance offset, and $\bar{\Omega}$ is the average resonance frequency.

The evolution of the magnetization under the influence of a spin lock in the presence of chemical exchange can be described by the Bloch-McConnell equations (Equation 3). [12, 39, 40] This set of differential equations describes the time evolution of the ground (M_{Gx} , M_{Gy} , M_{Gz}) and excited state (M_{Ex} , M_{Ey} , M_{Ez}) magnetization components as a function of time,

$$\begin{aligned} \frac{d}{dt} \begin{pmatrix} M_{Gx} \\ M_{Ex} \\ M_{Gy} \\ M_{Ey} \\ M_{Gz} \\ M_{Ez} \end{pmatrix} &= \begin{pmatrix} -k_{GE} - R_2 & k_{EG} & -\delta_G & 0 & 0 & 0 \\ k_{GE} & -k_{EG} - R_2 & 0 & -\delta_E & 0 & 0 \\ \delta_G & 0 & -k_{GE} - R_2 & k_{EG} & -\omega_1 & 0 \\ 0 & \delta_E & k_{GE} & -k_{EG} - R_2 & 0 & -\omega_1 \\ 0 & 0 & \omega_1 & 0 & -k_{GE} - R_1 & k_{EG} \\ 0 & 0 & 0 & \omega_1 & k_{GE} & -k_{EG} - R_1 \end{pmatrix} \begin{pmatrix} M_{Gx} \\ M_{Ex} \\ M_{Gy} \\ M_{Ey} \\ M_{Gz} \\ M_{Ez} \end{pmatrix} \\ &+ R_1 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ M_{G0} \\ M_{E0} \end{pmatrix} \end{aligned} \quad (3)$$

$$\delta_G = \Omega_G - \omega_{rf},$$

$$\delta_E = \Omega_E - \omega_{rf},$$

k_{GE} and k_{EG} (in units of s^{-1}) are the forward and backward rate constants for ground to excited state transitions, ω_1 and ω_{rf} (in units of s^{-1}) are the amplitude and frequency of the applied RF spin lock field, respectively. Here we assume that R_1 and R_2 are population-weighted averages for the ground and excited states. This assumption is valid for small differences in ground and excited state R_1 and R_2 values.[28] However, exceptions have been shown to occur when the excited state conformation has a significantly different R_2 . [15, 41]

Multiple algebraic equations which are more user friendly and less computationally taxing for fitting experimental data have been derived that accurately calculate $R_{1\rho}$ in terms of exchange parameters under certain exchange conditions.[11] An expression for $R_{1\rho}$ for systems in fast exchange is given by[34],

$$R_{1\rho} = R_1 \cos^2 \theta + \left(R_2 + \frac{\Phi_{ex} k_{ex}}{k_{ex}^2 + \omega_{eff}^2} \right) \sin^2 \theta, \quad (4)$$

$$\Phi_{ex} = p_G p_E \Delta \omega^2,$$

where $k_{ex} = k_{GE} + k_{EG}$. For systems in fast exchange, the excited state population, p_E , is coupled to ω making it impossible (in the absence of any other information) to determine the sign and magnitude of ω . Palmer and coworkers have derived a number of algebraic expressions that are accurate for exchange scenarios in the intermediate and slow exchange regimes ($k_{ex} \approx \omega$). [12, 27–29] One of the most versatile equations was derived by determining the characteristic sixth-order polynomial of the Bloch-McConnell equations and then using Laguerre's method for polynomial root finding[29],

$$R_{1\rho} = R_1 \cos^2 \theta + R_2 \sin^2 \theta + \frac{\sin^2 \theta p_G p_E \Delta \omega^2 k_{ex}}{\omega_G^2 \omega_E^2 / \omega_{eff}^2 + k_{ex}^2 - \sin^2 \theta p_G p_E \Delta \omega^2 \left(1 + \frac{2k_{ex}^2 (p_G \omega_G^2 + p_E \omega_E^2)}{\omega_G^2 \omega_E^2 + \omega_{eff}^2 k_{ex}^2} \right)} \quad (5)$$

$$\Delta \omega = \omega_E - \omega_G,$$

$$\omega_{eff}^2 = \Delta \Omega^2 + \omega_1^2,$$

$$\omega_G^2 = (\Omega_G - \omega_{rf})^2 + \omega_1^2,$$

$$\omega_E^2 = (\Omega_E - \omega_{rf})^2 + \omega_1^2.$$

Equation 5, which we will refer to as the “Laguerre equation” was used to fit all of the synthetic and experimental R1ρ data reported in this study. We also verified that the R1ρ values predicted using the Laguerre equation match those predicted using the Bloch-McConnell equations[42] with any notable deviations being restricted to conditions previously outlined by Palmer and co-workers[29] involving large populations $p_E > 5\%$, slow-intermediate exchange ($k_{ex}/\omega < 2$), and low spin lock fields (< 400 Hz). As noted later, these deviations did not significantly influence the fitted exchange parameters when accounting for error corruption. Together, our results further support the validity of the Laguerre expression under the conditions described by Palmer and co-workers.[29]

Computing R1ρ data

All of the simulations carried out in this study assume two site exchange between a GS and ES. Synthetic carbon R1ρ relaxation data at 14.1 T magnetic field strength consisting of 150 data points with varying resonance offset (Ω) and power (ω_1) (listed in Table S1) reflecting values typically used in our nucleic acid studies[33, 37] were computed by solving the Bloch-McConnell equations (Equation 3) numerically using Mathematica 8.0 (Wolfram Research). To be consistent with the R1ρ experimental data analyzed in this study, we generated the synthetic Bloch-McConnell R1ρ data assuming the ^{13}C selective 1D R1ρ experiment by Hansen *et. al.*[24, 31] In this experiment an equilibration period exists prior to alignment with the effective field (ω_G or ω_{eff} for exchange on the slow and intermediate-fast timescale, respectively) for the spin lock period (Figure 1). The equilibration period allows for the build up of excited state magnetization that ends up aligned via a hard pulse with the effective field (ω_G or ω_{eff} , Figure 1). Simulations of the spin lock period with the Bloch-McConnell equations showed that this introduces negligible deviations relative to the conventional scheme for the asymmetric populations and intermediate/fast exchange rates examined here and in our previous dispersion studies of nucleic acids.[24, 33, 37] However, for relatively large $p_E > 15\%$ and slow exchange ($k_{ex}/\omega < 0.5$) the above scheme can result in oscillations due to precision of the initial ES magnetization that can be mitigated by simple modification of the pulse sequence as described by Kay[31] and more recently by Zhang[15]. R1ρ was computed by fitting the resultant projection of the net magnetization of the ground and excited states following the relaxation period along $\omega_{eff} = (\Delta\Omega^2 + \omega_1^2)^{1/2}$ as a function of time to a monoexponential decay. For fast exchange ($k_{ex}/\omega > 2$), R1ρ was calculated by initially aligning both the GS and ES magnetization along the average effective field (ω_{eff}) and by projecting the resultant GS and ES magnetization following the spin lock period about the average effective field (ω_{eff}) (Figure 1). For slow exchange ($k_{ex}/\omega < 1$), R1ρ was calculated by initially aligning both the GS and ES magnetization along the GS effective field (ω_G) and by projecting the resultant GS and ES magnetization following the spin lock period about the average effective field (ω_{eff}) (Figure 1). We note

that for systems in slow exchange, and when using the initial magnetization preparation scheme that aligns both the ES and GS along the GS effective field, the actual observed projection would be about the ground state effective field (ω_G). [24] Simulations using the Bloch-McConnell equations show that this can lead to small deviations relative to projections about the average effective field for a small subset of conditions examined here involving slow exchange, low spin lock fields of $\omega_1 < 1000$ Hz and for $p_E > 15\%$. However, these deviations have negligible effects on the extracted exchange parameters. Similar exchange parameters are obtained when fitting 5% noise corrupted R1 ρ data computed assuming projections along either the average (ω_{eff}) or ground state (ω_G) effective fields (data not shown). We also note that we carried out simulations for scenarios fast on the chemical shift timescale ($k_{\text{ex}}/\omega \gg 2$) in which we calculated R1 ρ by initially aligning both the GS and ES magnetization along GS effective field (ω_G) and obtained nearly identical uncertainties to those reported in Table 1 when fitting 5% noise corrupted data.

The synthetic R1 ρ data was uniformly noise corrupted assuming 5% uncertainty by randomly selecting a value from a normal distribution centered at the R1 ρ value with a standard deviation set equal to 5%. Standard simulations assumed $\omega = 2.12$ ppm (2,000 s⁻¹), p_E of varying asymmetry (5%, 15%, 30%), exchange rates (k_{ex}) ranging between 1,000 s⁻¹ and 30,000 s⁻¹ spanning k_{ex}/ω values between 0.5 and 15. The R_2 and R_1 relaxation rate constants were equal to 11.0 s⁻¹ and 1.5 s⁻¹, respectively to be consistent with prior theoretical studies examining R1 ρ and its associated algebraic expressions. [12, 27, 29, 43] As shown below, increasing the value of R_2 but assuming a constant signal to noise ratio results in larger uncertainty in the extracted exchange parameters (p_E , ω , k_{ex}) due to the increase in the relative contribution of R_2 to transverse relaxation as compared to R_{ex} .

Grid Search

We carried out grid searching by comparing a 5% noise corrupted input R1 ρ data set obtained for a given exchange regime with values computed on a multidimensional grid composed of varying exchange parameters (p_E , ω , k_{ex}) that define the exchange (R_{ex}) contribution to R1 ρ (Equation 2). For each point on the grid, χ^2 was calculated between the input R1 ρ data set and the corresponding theoretical R1 ρ data. The multidimensional grid consisted of ~100,000 distinct R1 ρ data sets for p_E ranging between 0 and 0.5 in increments of 0.005 and ω between -3.0 and 3.0 ppm in increments of 0.1 ppm. The range and increment of k_{ex} was varied depending on the chemical shift timescale to best capture the global minimum along the χ^2 surface. For example, fast exchange scenarios have a broad global χ^2 minimum so it was necessary to sample a broader range of k_{ex} values. All of the calculations were carried out using Mathematica 8.0 (Wolfram Research). The code was written to accommodate data collected at multiple magnetic fields and takes advantage of the parallel computing packages included in Mathematica 8.0. The code is available from the authors upon request.

Bootstrap Analysis

To estimate the robustness of data fitting and uncertainty in exchange parameters, we carried out a bootstrap analysis of the R1 ρ data using an approach similar to that described previously. [9, 38] For a given parent R1 ρ data set containing 150 R1 ρ values obtained at

different spin lock frequency offsets and power levels, 1000 child bootstrap data sets each containing 150 data points were generated by randomly selecting 150 values from the parent data set. In this manner, the bootstrap child data sets were of the same size as the parent data set and each data point from the parent data set can be represented multiple times or be completely excluded. The parent data sets used for bootstrapping were composed of the same spin lock powers and offsets as the grid search data (150 total points for synthetic data).

The bootstrap child data sets were each fitted to the Laguerre equation (Equation 5) using the “Minimize” function built into Mathematica 8.0 (Wolfram Research) to obtain the five exchange parameters R_1 , R_2 , p_E , k_{ex} , and ω . In order to diminish bias in curve fitting, the following constraints were used for the exchange parameters: $0.001 < p_E < 0.999$, $-7 \text{ ppm} < \omega < 7 \text{ ppm}$, $100 \text{ s}^{-1} < k_{ex} < 50,000 \text{ s}^{-1}$, $0.5 \text{ s}^{-1} < R_1 < 4 \text{ s}^{-1}$, and $R_{2\text{actual}} - 10 \text{ s}^{-1} < R_2 < R_{2\text{actual}} + 10 \text{ s}^{-1}$. Here $R_{2\text{actual}}$ was 11 s^{-1} for synthetic data sets unless the R_2 is noted otherwise. These broad fitting parameter ranges were used to assure that the fitting algorithm achieved the global χ^2 minimum when fitting. We found that the “NMinimize” and “Minimize” algorithms failed when using more restricted ranges, particularly for the p_E parameter. In post processing when fits resulted such that $p_E > 0.5$ (making the fitting result reflect the major state) the fitted population was adjusted to be $(1-p_E)$ and the sign of ω from the fits was inverted to reflect the excited state parameters. Errors from bootstrap fits were determined by calculating the standard deviation between the parameters determined for the parent data set and those for 1,000 child data sets. The Mathematica code for the bootstrap fitting was written to accommodate data collected at multiple fields and is available from the authors upon request.

Monte-Carlo Analysis

To complement the bootstrap analysis, we also carried out a Monte-Carlo based analysis for estimating the robustness of fitted parameters.[19, 38, 44] For a given parent $R1\rho$ data set consisting of 150 $R1\rho$ values obtained at different spin lock frequency offsets and power levels, 1000 child data sets for Monte-Carlo analysis were generated in the following manner. First, the parent $R1\rho$ data set was fitted to the Laguerre equation (Equation 5) to determine the best-fit exchange parameters (p_E , k_{ex} , ω , R_2 , R_1). The best-fitted exchange parameters were then used together with the Laguerre equation to generate 1000 synthetic $R1\rho$ data sets for the same spin lock frequency offsets and power levels as the parent data set. Each child data set was then noise corrupted according to the uncertainty from the parent data set. Here the percent error from each parent data point was used to generate a normal distribution centered at the Monte-Carlo generated $R1\rho$ and a random value was selected from the distribution for each child data point. Each noise-corrupted Monte-Carlo data set was fit using the Laguerre equation (Equation 5) as described for bootstrap. Errors from Monte-Carlo fits were determined by calculating the standard deviation between the parameters determined for the parent data set and those for 1,000 child data sets. In general, very similar uncertainties in exchange parameters were deduced using the bootstrap and Monte-Carlo approach.

Bootstrap and Monte-Carlo analysis was carried out three times for each synthetic R1 ρ data set. The synthetic data sets were independently noise corrupted prior to bootstrap and Monte-Carlo analysis. Finally, we note that we carried out the identical bootstrap and Monte-carlo analysis reported in this paper with data sets generated with the Laguerre equation and obtained nearly identical results establishing its validity over the exchange regimes examined here.[29]

Results and Discussion

Examining conditions of the Fast-Exchange Limit in the R1 ρ Experiment

At the limit of fast exchange, R1 ρ scales with $\Phi_{IX} = p_E p_E(\omega)^2$, and it becomes impossible to resolve ω from p_E , or to deduce its sign even if p_E is known.[11, 34] We used the Bloch-McConnell equations to examine when this condition of fast exchange is satisfied in the R1 ρ experiment. As described by Palmer, in slow-intermediate exchange, the broadening due to exchange is maximal when placing the carrier on resonance with the excited state, rather than the ground or average state as in the fast exchange limit.[12] This asymmetry has been exploited by Kay and co-workers to determine the sign of the excited state chemical shift for systems that fall within slow-to-intermediate chemical shift timescales accessible to CPMG.[45–47] However, in the fast exchange limit (Equation 4), we expect R1 ρ to be symmetric with respect to the resonance offset i.e. $R1\rho(\Omega) = R1\rho(-\Omega)$.

In Figure 2, we compare the difference between $R1\rho(\Omega)$ and $R1\rho(-\Omega)$ obtained by solving the Bloch-McConnell equations for increasing exchange rates ($k_{ex} = 4,000$ to $20,000$ s⁻¹) assuming an asymmetric population $p_E = 5\%$, $\omega = 2.12$ ppm for ¹³C at 14.1 T for the spin lock powers and offsets used in all synthetic data sets (Table S1). For intermediate-fast chemical shift timescales ($k_{ex}/\omega = 4$), we observe clear deviations between $R1\rho(\Omega)$ and $R1\rho(-\Omega)$ (Figure 2) and the asymmetry is shifted toward the direction of the ES chemical shift (in this case $-\Omega$). This asymmetry in R1 ρ as a function of Ω implies slow-to-intermediate exchange in which it should be possible to deduce the sign and magnitude of ω . While the difference between $R1\rho(\Omega)$ and $R1\rho(-\Omega)$ diminishes as the exchange rate increases to $k_{ex}/\omega = 10$, in part due to a general reduction in R_{ex} , a difference persists in favor of the ES chemical shift, even for much faster exchange rates ($k_{ex}/\omega > 10$, data not shown) (Figure 2). This implies that in principle, in the absence of experimental uncertainty, one can always determine the sign and magnitude of ω . This asymmetry grows with the population of excited state (data not shown) implying that it should be possible determine the sign and magnitude of ω for even fast exchange processes with increasing excited state populations. The asymmetry varies depending on the spin lock power, offset, as well as the exchange parameters. This underscores the importance of collecting data over a broad range of spin lock powers and offsets. As we describe in what follows, this systematic deviation across multiple R1 ρ data points spanning different spin lock powers and offsets makes it possible to decipher the sign of ω at useful level of confidence, even though the individual deviations between R1 ρ for $+\omega$ and $-\omega$ can be comparable to or smaller than the R1 ρ uncertainty. Together, these data show that the definition of the fast exchange limit intimately depends on the uncertainty in R1 ρ measurements (which includes total number of

data points acquired) with lower uncertainty pushing the experiment outside the fast exchange-limit.

Exploration of the $R_{1\rho}$ χ^2 Surface Via Grid Searching

Data fitting for $R_{1\rho}$ relaxation dispersion data is typically carried out by minimizing a χ^2 function,

$$\chi^2 = \sum_{i=1}^N \left(\frac{R_{1\rho(i)}^{Calc} - R_{1\rho(i)}^{Exp}}{\sigma_{Exp(i)}} \right)^2 \quad (6)$$

where σ is the experimental uncertainty, $R_{1\rho(i)}^{Calc}$ is the value of $R_{1\rho}$ predicted by a model, $R_{1\rho(i)}^{Exp}$ is the experimental $R_{1\rho}$, and N is the total number of data points. Software packages such as Origin (OriginLab Corporation) are typically used to fit experimental data where minimization algorithms such as the Levenberg-Marquardt method are employed to vary model parameters in an effort to minimize χ^2 . One pitfall of these algorithms is that the search can get caught in local minima along the χ^2 surface resulting in a bad fit, where the “correct” global minimum is missed. While more computationally costly, unbiased grid searches reveal all minima of the χ^2 surface allowing for a definitive hunt for the global minimum and more complete exploration of the uncertainty in derived exchange parameters by revealing other competing minima.

As a first step in assessing the robustness and uncertainty with which exchange parameters can be determined for systems near the fast exchange limit, we investigated the χ^2 surface for synthetic $R_{1\rho}$ data generated using the Laguerre Equation (Equation 5) that is noise corrupted assuming an uncertainty of 5% over a range of exchange scenarios. Of particular interest is the ability to deduce the sign and magnitude of the chemical shift difference, ω , which is used to characterize the excited state conformation since at the limit of fast exchange it is impossible to resolve the sign and magnitude of ω . As expected, for slow-intermediate chemical shift timescales ($k_{ex}/\omega = 1$) with $p_E = 5\%$ and $\omega = 2000 \text{ s}^{-1}$, we observe a well-defined global $\chi^2 \sim 0.8$ minimum and the $R_{1\rho}$ data correctly determines all three exchange parameters (Figure 3a). In particular, the sign of ω is clearly defined and a significantly higher $\chi^2 = 65.0$ value is observed for the opposite $-\omega$ value. Increasing the exchange rate to $k_{ex}/\omega = 2$ while keeping all other exchange parameters constant leads to a χ^2 surface that increasingly assumes features characteristic of fast exchange. Local minima begin to appear for $k_{ex}/\omega = 2$ at the opposite sign of ω ($\chi^2 = 16.5$ compared to $\chi^2 = 1.07$ for correct sign) and a correlation emerges between ω and p_E (Figure 3b). The global χ^2 minimum becomes broader and there is generally greater uncertainty in all three-exchange parameters. However, even for fast exchange ($k_{ex}/\omega = 10$), where the χ^2 surface is clearly broad and nearly symmetric with respect to $+\omega$ and $-\omega$, a degree of asymmetry is still apparent over a broad region of the surface that is consistently in favor of the correct ω sign (Figure 3c). Within each ω sign, the value of ω is still confined within a total range of ~ 2 ppm. In other words, there are restrictions on both the sign and magnitude of ω . Larger values of ω with correspondingly smaller p_E values are ruled out because this would effectively push the system toward slow-intermediate exchange (since k_{ex} is fixed)

and result in asymmetry in $R1\rho$ with respect to Ω that is inconsistent with the measured data. Conversely, much smaller values of ω with larger p_E values are ruled out because $p_E > 0.5$, and because the term $\Phi_{ix} = p_G p_E (\omega)^2$ depends quadratically on ω . Therefore, increasing p_E by x can be compensated for by a smaller $x^{1/2}$ -fold reduction in ω . In general, increasing p_E from 5% to 30% leads to better defined global minima.

The above data suggests that it should be possible to obtain important constraints on both the sign and magnitude of ω even for systems that are expected to be in fast exchange in so far as free precession line broadening is concerned. However, the data also highlights pitfalls that could occur when trying to fit $R1\rho$ data for systems in fast exchange on the chemical shift timescale. Depending on the type and quality of search algorithms used and initial model parameters, data fitting might result in solutions that are trapped in local minima. This necessitates the collection of data of very high quality in order to assure that the correct model parameters are determined and as requires the application of grid searching in order to broadly explore the exchange parameter landscape and identify all possible local minima in a given system.

Bootstrap and Monte-Carlo Analysis of Dispersion Data

With an understanding of χ^2 surfaces as a function of exchange regime in hand, we sought to quantify the uncertainty in exchange parameters derived from $R1\rho$ data for systems in fast exchange. Not much work has focused on determining the limits of $R1\rho$ relaxation dispersion as it applies to fast chemical shift timescales; rather, previous studies have primarily explored the limits of CPMG relaxation dispersion. For example, a recent study by Vallurupalli *et al.* characterized the limits for CPMG dispersion for systems with asymmetrically populated excited states ($p_E < 5\%$) and found that when k_{ex}/ω exceeded ~ 2 (11.7, 18.8 T multiple field CPMG data), data fitting did not converge to a common solution when using CPMG data alone.[9] Here the authors employed a bootstrap analysis to determine the uncertainty in the parameters resulting from fitting experimental data.

We performed a similar analysis with synthetic and experimental $R1\rho$ data sets to evaluate the uncertainty in exchange parameters for various exchange scenarios assuming 5% uncertainty in the $R1\rho$ data (see Methods). We systematically carried out bootstrap and Monte-Carlo analyses on synthetic $R1\rho$ data reflecting a broad range of chemical shift timescales ($k_{ex}/\omega = 0.5 - 15$) and populations ($p_E = 5\%, 15\%, 30\%$). In Table 1 we report the uncertainty in derived exchange parameters determined from bootstrap and Monte-Carlo approaches (see Methods). The uncertainty was calculated by determining the standard deviation of each fit ensemble from the correct p_E , k_{ex} , and ω values. However, we note that for some cases the χ^2 surface does not have a Gaussian shaped global minimum, particularly for fast exchange scenarios. Thus, even though there might be a small error based on the standard deviation, it is important to consider the entire χ^2 surface when interpreting the data. The results from bootstrap and Monte-Carlo analysis are shown in Figure 4 and Table 1. Overall, the uncertainty calculated from the bootstrap and Monte-Carlo simulations (Table 1, Figure 4) were very similar and mirrored the results obtained using the grid search (Figure 3).

Generally, the uncertainty in ω , p_E , and k_{ex} increases with increasing timescale of exchange. Here we focus on uncertainties from the bootstrap analysis, but note that nearly identical uncertainties are obtained from Monte-Carlo analysis (Table 1, Figure 4). For $p_E = 5\%$, the uncertainty in ω is 0.03, 0.13, 0.80 ppm for exchange timescales $k_{ex}/\omega = 0.5, 2$, and 10 respectively (Figure 4 and 5). The corresponding uncertainty in p_E is 0.2, 0.6, 4.8 %, respectively. The uncertainty generally decreases with increasing excited state population; for $p_E = 30\%$ the corresponding uncertainties in ω are 0.05, 0.03, 0.31 ppm, respectively and for p_E are 0.8, 1.3, 6.7 %, respectively (Figure 4). These results suggest that for $p_E > 5\%$, both the sign and magnitude of ω can be determined with reasonable uncertainty (< 1 ppm for ω and $< 10\%$ for p_E) even for relatively fast exchange conditions $k_{ex}/\omega < 12$.

As expected, for systems in fast exchange, the uncertainty in exchange parameters, and the confidence with which the sign of ω can be determined are dependent upon the uncertainty in $R_{1\rho}$ and/or magnitude of R_2 . The limit at which the excited state population and chemical shift can be determined from only $R_{1\rho}$ dispersion shifts towards slower timescales upon increasing R_2 (Table 2). For example, in a fast exchange scenario ($k_{ex}/\omega = 8$) larger noise corruption (10%) or increasing R_2 in the range of (11–50 s^{-1}) resulted in significant increases in the uncertainty of p_E and ω (up to $\sim 6\%$ and ~ 3.5 ppm, respectively) relative to a scenario with a small R_2 (11 s^{-1}) and 5% noise corruption. Slower intermediate-fast exchange scenarios ($k_{ex}/\omega = 2$ and 4) were found to be less sensitive to increases in noise or intrinsic R_2 (Table 2). In general, the uncertainty in exchange parameters increases with the uncertainty of $R_{1\rho}$ and increasing R_2 , which effectively diminishes the relative contribution of R_{ex} to $R_{1\rho}$. We also examined scenarios in which the chemical shift timescale (k_{ex}/ω) of the exchange process is held constant while co-varying k_{ex} and ω . The uncertainty calculated from these simulations were very similar to those reported in Table 1. Thus, the uncertainty in extracted parameters is largely determined by the chemical shift timescale (i.e. the k_{ex}/ω) and is largely insensitive to the absolute magnitudes of k_{ex} and ω over the range examined in this study.

One has to be careful not to over interpret the standard deviation derived from bootstrap and Monte-Carlo analyses. As mentioned above, solutions deviate from a Gaussian distribution and although weighted less significantly, do include different signs for ω . It is therefore important to perform a grid search to more fully characterize the multi-dimensional exchange parameter landscape. Nevertheless, notwithstanding the above limitations, the overall uncertainty in exchange parameters derived using bootstrap and Monte-Carlo analyses near conditions of fast exchange ($k_{ex}/\omega \sim 10$) remain small enough to allow useful inferences to be made about excited states. For example, for nucleic acids the uncertainty of $\omega \sim 1$ ppm for ^{13}C nuclei remains smaller than the entire range of possible chemical shifts (e.g. 11.6 and 7.9 ppm for sugar C1' and purine base-C8 carbons in nucleic acids respectively).[48] It makes it possible to discriminate sugar pucker as well as *syn* versus *anti* base conformations that often differ by > 3 ppm. The uncertainty of 1 ppm is also comparable to the uncertainties in structure-based prediction of nucleic acid carbon chemical shifts (~ 1 ppm).[49] In addition, a common strategy to characterize transient states is to introduce mutations and chemical shift modifications designed to favor or trap the transient state.[33, 35, 37, 50] The availability of multiple excited state chemical shifts provides a

strong test for any proposed mutant model for the excited state, even if the uncertainty in each resonance is ~ 1 ppm.

Fitting Experimental R1 ρ Dispersion Data

We used the bootstrap and Monte-Carlo approaches to analyze previously published experimental ^{13}C R1 ρ dispersion data measured in three nucleic acid systems that exhibit exchange across a wide range of timescales and involving distinct populations for the ES. [24, 33, 37] The first system is a DNA duplex where it was shown that ground state Watson-Crick base-pairs are in slow exchange ($k_{\text{ex}} = \sim 750 \text{ s}^{-1}$ and $k_{\text{ex}}/\omega = \sim 0.2$) with excited state Hoogsteen base-pairs that have a p_{E} of $\sim 1\%$ (Figure 6). [37] The second system is the HIV-1 TAR apical loop which was shown to be in fast exchange ($k_{\text{ex}} = 25,000 \text{ s}^{-1}$ and $k_{\text{ex}}/\omega = 10\text{--}30$) with an excited state that features distinct base-pairing with $p_{\text{E}} = \sim 13\%$ (Figure 7). [33] In both cases, the nucleic acid excited state conformation was deduced based on the comparison of excited state chemical shifts with those obtained for constructs containing mutations or chemical modifications designed to stabilize a candidate excited state structure. The ability to infer the excited state structure using this chemical shift fingerprinting approach hinges on being able to determine excited state chemical shifts with sufficiently good uncertainty in this case ~ 1 ppm, which is the difference expected between the excited state and an ES trapped mutant. [33, 37] A third system involves a damaged adenine base in a DNA duplex in very fast exchange ($k_{\text{ex}} = \sim 39,000 \text{ s}^{-1}$), for which data was measured at natural abundance with significant uncertainty and for which the chemical shift could not be deduced. [24]

As an example of slow-intermediate exchange, we applied the bootstrap and Monte-Carlo fitting procedure to G10-C1' and G10-C8 R1 ρ data measured in a duplex DNA (Figure 6a). [37] Similar results were obtained for other residues that exhibit exchange (data not shown). The R1 ρ data sets were collected with spin lock powers ranging between 100 Hz and 3500 Hz and consisted of 156 and 162 data points, respectively. Grid searching of the χ^2 surface for each nucleus in slow exchange revealed a clear global minimum as observed for the slow exchange synthetic data sets (Figure 6c). Consistent with a clear global minimum in the χ^2 surface, the bootstrap and Monte-Carlo fitting distributions were narrow for both G10-C1' and G10-C8 (Table 3) resulting in small uncertainties for ω (~ 0.06 and ~ 0.03 ppm for bootstrap and Monte-Carlo analysis, respectively) and p_{E} (~ 0.05 and ~ 0.03 for bootstrap and Monte-Carlo analysis, respectively). Combining the data in a global fit as was done in the original study is expected to help further define the global minimum in the χ^2 surface.

Next, we applied the same methodology to the more challenging case of the fast exchanging HIV-1 TAR RNA apical loop with $k_{\text{ex}} \sim 25,000 \text{ s}^{-1}$ and k_{ex}/ω ranging from $\sim 10\text{--}30$ depending on the resonance (Figure 7) but with a large population of $\sim 13\%$. [33] The R1 ρ data sets were collected with spin lock powers ranging between 150 Hz and 3000 Hz and consisted of 98–158 data points. [33] We recently proposed that the excited state conformations sampled during the exchange process involve base-pairing rearrangements in the TAR hexanucleotide apical loop (Figure 7b). We grid searched the experimental data for residues in which conformational exchange was detected and we observed a broader χ^2 surface similar to the fast exchange synthetic data sets (Figure 7c). Bootstrap and Monte-

Carlo analysis of R1 ρ data resulted in fitting distributions similar to those observed for the synthetic data sets with similar populations and chemical shift timescales (Figure 4). In this case, the computed uncertainties are larger given the faster exchange timescales; for ω they range between 0.3 ppm and 0.9 ppm and for p_E are between 3.6 and 12.0 (Table 4). While less well defined, the sign of ω remains skewed in favor of one solution (Figure 7c). In the original study the dispersion data sets were fit globally given that the timescale of exchange obtained from individual fits of each residue was similar ($\sim 22,500$ k_{ex} $\sim 27,500$). The global fit with shared k_{ex} and p_E values gave a k_{ex} of $\sim 25,000$ and a p_E of 13%. The global fitted parameters (p_E , ω , k_{ex}) for each residue fall within the uncertainties obtained by our bootstrap and Monte-Carlo analysis of individual fits. Combining the data in a global fit as was done in the original study involving shared p_E and k_{ex} values is expected to lead to lower uncertainty in all derived chemical shift values, because the nuclei with the smaller k_{ex}/ω values aid in the definition of p_E for all residues and therefore their excited state chemical shift. Indeed, all of the signs of ω deduced from analysis of R1 ρ data were in excellent agreement with the corresponding signs of ω obtained by trapping the ES using mutations, chemical modifications, and by changing pH.[33]

Lastly, we also applied our bootstrap and Monte-Carlo analysis to a DNA bearing a damaged 1,N6-ethenoadenine lesion that undergoes a conformational exchange process very fast on the chemical shift timescale ($k_{ex} \sim 39,000$ s $^{-1}$) for which it was not possible to obtain the excited state chemical shift value.[24] The R1 ρ data sets were collected with spin lock powers ranging between 200 Hz and 3500 Hz and consisted of 77 data points.[24] Bootstrap and Monte-Carlo methods failed to converge to a common solution when fitting to the Laguerre equation. The failure of convergence can be attributed to both the fast timescale of the process and the very large R_2 (53 s $^{-1}$) of the residue undergoing exchange. This large R_2 can be partially attributed to the use of a high DNA concentration given that data on this modified base had to be acquired at natural abundance. It is reassuring that the analysis breaks down when the uncertainty in the data is not sufficiently good to define the exchange parameters. Rather, such scenarios in fast exchange are better suited to be fit with the fast exchange expression (Equation 4).

Conclusion

We have combined a grid search with bootstrap and Monte-Carlo based statistical analyses to gain insight into the reliability with which exchange parameters can be determined using R1 ρ relaxation dispersion for various fast exchange scenarios. The grid search shows that the χ^2 surface has a distinct global minimum for systems undergoing exchange on slow-to-intermediate timescales ($k_{ex}/\omega \sim 1$). As the exchange timescale approaches the fast exchange regime ($k_{ex}/\omega \gg 1$), the χ^2 surface flattens out and many local minima appear making it increasingly difficult to accurately determine the population and chemical shift of the excited state. Bootstrap and Monte-Carlo simulations revealed that there is not a specific timescale limit (k_{ex}/ω) for robust parameter determination. Rather, the ability to reliably determine exchange parameters is dependent on many factors including the population of the excited state, the intrinsic R_2 , and the uncertainty in the data. In general, as excited states become more populated, there is an increase in the window of timescales for which excited state chemical shifts and populations can be accurately quantified. Furthermore, as

molecular size and R_2 correspondingly increases, the timescale window for $R_1\rho$ dispersion narrows where excited state populations and chemical shifts become difficult to determine when in fast exchange.

In general, reliable parameters could be determined for all scenarios investigated in which $k_{ex}/\omega \sim 2$. Exchange parameters at a useful level of uncertainty (i.e. uncertainty in ^{13}C $\omega < 1$ ppm) could also be obtained for $k_{ex}/\omega \sim 10$ but this requires a thorough examination of the multidimensional χ^2 surface to assess the various local minima that can exist under these conditions. For systems in fast exchange ($k_{ex}/\omega \sim 2$) careful analysis using bootstrap and Monte-Carlo procedures should be carried out to assess the robustness of the fitted exchange parameters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- p_E , k_{ex} , ω can be determined within useful uncertainty for systems in fast exchange.
- Applied bootstrap and Monte-Carlo methods for estimating uncertainty in p_E , k_{ex} , ω .
- Applied analysis to experimental data for fast and slow-exchanging nucleic acids.

Figure 1.
Resonance offsets and effective fields in R1 ρ relaxation dispersion.

Figure 2.

Difference in for $R_{1\rho}$ between positive and negative offsets for the spin lock powers and offsets listed in Table S1. Each data point was sorted by decreasing difference in $R_{1\rho}$ between a matching spin lock power and its positive-negative offsets. Data is shown for the following exchange scenarios: black, $p_E = 5\%$, $k_{ex} = 4,000 \text{ s}^{-1}$; blue, $p_E = 5\%$, $k_{ex} = 8,000 \text{ s}^{-1}$; cyan, $p_E = 5\%$, $k_{ex} = 12,000 \text{ s}^{-1}$; green, $p_E = 5\%$, $k_{ex} = 16,000 \text{ s}^{-1}$; red, $p_E = 5\%$, $k_{ex} = 20,000 \text{ s}^{-1}$. The ω used to generate the data sets was 2.12 ppm (^{13}C at 14.1 T).

Figure 3.

Grid search analysis of R1ρ relaxation dispersion data. Shown is the value of $e^{(-\chi^2/2\chi_{\min}^2)}$

where $\chi^2 = \sum_{i=1}^N \left(\frac{R_{1\rho(i)}^{Calc} - R_{1\rho(i)}^{Exp}}{\sigma_{Exp(i)}} \right)^2$ as a function of exchange parameter. The ω used to generate the data sets was 2.12 ppm (^{13}C at 14.1 T). Data is shown for different exchange scenarios a) $p_E = 5\%$, $k_{ex} = 1,000 \text{ s}^{-1}$, and $k_{ex}/\omega = 0.5$ b) $p_E = 5\%$, $k_{ex} = 4,000 \text{ s}^{-1}$, and $k_{ex}/\omega = 2$ c) $p_E = 5\%$, $k_{ex} = 20,000 \text{ s}^{-1}$, and $k_{ex}/\omega = 10$.

Figure 4.

Evaluating uncertainty in exchange parameters derived for R1ρ data using bootstrap and Monte-Carlo approaches for various exchange scenarios. Error bars represent the standard deviation between 3 independent runs. The ω used to generate the data sets was 2.12 ppm (^{13}C at 14.1 T). Note that although the absolute uncertainty in population generally increases with input p_E , the fractional error decreases or stays constant.

Figure 5.

Example bootstrap fits for various exchange scenarios. Shown are density plots of exchange parameters obtained upon fitting 1,000 bootstrap R1ρ data sets with 5% noise corruption.

The ω used to generate the data sets was 2.12 ppm (^{13}C at 14.1 T). Results are shown for various exchange scenarios a) $p_E = 5\%$, $k_{\text{ex}} = 4,000 \text{ s}^{-1}$, and $k_{\text{ex}}/\omega = 2$ b) $p_E = 5\%$, $k_{\text{ex}} = 20,000 \text{ s}^{-1}$, and $k_{\text{ex}}/\omega = 10$.

Figure 6.

Grid search analysis of exchange parameters for experimental $R_{1\rho}$ data describing a slow process ($k_{\text{ex}}/\omega = 0.2$) involving Watson-Crick to Hoogsteen transitions in DNA.[37]

Shown is representative example data for G10-C1' and G10-C8. Similar results were obtained from analysis of other dispersion $R_{1\rho}$ data in DNA describing Watson-Crick to Hoogsteen transitions (data not shown). a) The DNA duplex studied by NMR with G10 highlighted in red. b) The Watson-Crick to Hoogsteen transition. c) Shown is value of

$e^{(-\chi^2/2\chi_{\text{min}}^2)}$ where $\chi^2 = \sum_{i=1}^N \left(\frac{R_{1\rho(i)}^{\text{Calc}} - R_{1\rho(i)}^{\text{Exp}}}{\sigma_{\text{Exp}(i)}} \right)^2$ as a function of exchange parameter. The extracted exchange parameters and uncertainties are listed in Table 3.

Figure 7.

Grid search analysis of exchange parameters for experimental $R1\rho$ data describing a fast transition ($k_{ex}/\omega \sim 10$) involving localized changes in base-pairing with the HIV-1 TAR RNA apical loop.[33] a) The TAR sequence studied by NMR with exchanging residues highlighted in red b) The proposed ground to excited state transition with exchanging residues highlighted in red. c) Shown is value of $e^{(-\chi^2/2\chi_{min}^2)}$ where

$$\chi^2 = \sum_{i=1}^N \left(\frac{R_{1\rho(i)}^{Calc} - R_{1\rho(i)}^{Exp}}{\sigma_{Exp(i)}} \right)^2 \text{ as a function of exchange parameter for three representative residues. The extracted exchange parameters and uncertainties are listed in Table 4.}$$

Table 1

Uncertainty (σ) in exchange parameters deduced from bootstrap and Monte Carlo analysis of synthetic R1 ρ data for various exchange scenarios. The uncertainty is obtained from the standard deviation in exchange parameters from three independent fits of R1 ρ data representing either distinct child data sets (bootstrap - BS) or entire datasets with different noise corruption (Monte Carlo - MC). The ω used to generate the data sets was 2.12 ppm (^{13}C at 14.1 T).

k_{ex}/ω	p_E (%)	k_{ex} (s $^{-1}$)	σ ω (ppm)-BS	σ ω (ppm)-MC	σp_E (%) -BS	σp_E (%) -MC	σk_{ex} (s $^{-1}$)-BS	σk_{ex} (s $^{-1}$)-MC
0.5	5	1000	0.03	0.03	0.2	0.1	51	53
1.0	5	2000	0.06	0.06	0.2	0.2	60	61
2.0	5	4000	0.13	0.14	0.6	0.6	101	111
4.0	5	8000	0.19	0.20	1.1	1.1	390	396
6.0	5	12000	0.31	0.33	2.2	2.4	592	587
8.0	5	16000	0.55	0.57	2.7	3.1	1084	1076
10.0	5	20000	0.80	0.83	4.8	4.9	2458	2427
12.0	5	24000	1.57	1.57	13.0	12.7	4372	4420
14.0	5	28000	1.93	1.90	9.8	9.7	6940	7044
15.0	5	30000	1.80	1.65	11.1	10.5	6548	6446
0.5	15	1000	0.02	0.02	0.4	0.3	39	39
1.0	15	2000	0.03	0.03	0.5	0.4	38	39
2.0	15	4000	0.06	0.05	0.9	0.8	72	73
4.0	15	8000	0.12	0.11	2.0	1.9	162	161
6.0	15	12000	0.24	0.32	4.3	4.3	341	379
8.0	15	16000	0.26	0.24	6.4	6.0	621	611
10.0	15	20000	0.48	0.47	5.6	5.7	1478	1466
12.0	15	24000	0.59	0.63	8.4	8.4	1717	1715
14.0	15	28000	0.61	0.61	7.5	7.6	3333	3374
15.0	15	30000	0.71	0.73	8.3	8.8	3154	3351
0.5	30	1000	0.05	0.04	0.8	0.7	87	83
1.0	30	2000	0.04	0.04	2.3	2.3	64	54
2.0	30	4000	0.03	0.03	1.3	1.4	65	68
4.0	30	8000	0.05	0.06	2.6	2.7	131	125

k_{ex}/ω	p_E (%)	k_{ex} (s ⁻¹)	$\sigma \omega$ (ppm)-BS	$\sigma \omega$ (ppm)-MC	σp_E (%) -BS	σp_E (%) -MC	σk_{ex} (s ⁻¹)-BS	σk_{ex} (s ⁻¹)-MC
6.0	30	12000	0.10	0.07	5.2	4.4	406	490
8.0	30	16000	0.12	0.12	5.1	5.2	514	530
10.0	30	20000	0.31	0.55	6.7	7.8	1149	782
12.0	30	24000	0.37	0.40	7.1	7.3	1498	1107
14.0	30	28000	0.47	0.53	10.1	10.3	1593	1741
15.0	30	30000	0.62	0.71	9.6	9.9	2367	2270

Table 2

Uncertainty in exchange parameters derived from bootstrap analysis of synthetic R1ρ data with varied levels of noise corruption and R2. The ω used to generate the data sets was 2.12 ppm (13C at 14.1 T).

k _{ex} / ω	p _E (%)	k _{ex} (s ⁻¹)	σ ω (ppm)-BS	σp _E (%) -BS	σk _{ex} (s ⁻¹)-BS	R ₂ (s ⁻¹)
2.0	30	4000	0.03	1.3	65	11
2.0	30	4000*	0.07	2.7	102	11*
2.0	30	4000	0.07	3.0	151	20
2.0	30	4000	0.09	3.2	146	30
2.0	30	4000	0.11	3.7	185	50
4.0	30	8000	0.05	2.6	131	11
4.0	30	8000*	0.12	4.5	319	11*
4.0	30	8000	0.45	8.0	371	20
4.0	30	8000	0.85	9.3	675	30
4.0	30	8000	1.61	10.9	447	50
8.0	30	16000	0.12	5.1	514	11
8.0	30	16000*	2.04	11.2	1189	11*
8.0	30	16000	1.06	10.1	1544	20
8.0	30	16000	0.96	12.1	1640	30
8.0	30	16000	3.57	11.1	2113	50

* Noise corrupted 10% instead of 5%

Uncertainty in exchange parameters derived using Bootstrap (BS) and Monte-Carlo (MC) analysis of experimental R1ρ data measured in H-DNA (Figure 6).

Table 3

k_{ex}/ω	ω (ppm)	p_E (%)	k_{ex} (s ⁻¹)	σ ω (ppm)-BS	σ ω (ppm)-MC	σp_E (%) -BS	σp_E (%) -MC	σk_{ex} (s ⁻¹) -BS	σk_{ex} (s ⁻¹) -MC	Residue
0.2	3.10	0.68	609	0.05	0.02	0.03	0.03	294	195	G10C8
0.2	3.83	0.60	748	0.06	0.03	0.06	0.02	181	164	G10C1'

Table 4

Uncertainty in exchange parameters derived using bootstrap (BS) and Monte-Carlo (MC) analysis of experimental R1ρ data measured in HIV-1 TAR (Figure 7).

k_{ex}/ω	ω (ppm)	p_E (%)	k_{ex} (s ⁻¹)	σ ω (ppm)-BS	σ ω (ppm)-MC	σp_E (%) -BS	σp_E (%) -MC	σk_{ex} (s ⁻¹) -BS	σk_{ex} (s ⁻¹) -MC	Residue
10.2	2.74	11.47	26353	0.33	0.49	3.6	6.4	1423	2818	G34C8
12.1	1.96	14.10	22343	0.26	0.93	5.0	12.0	1658	2444	G34C1'
15.8	1.86	22.69	27582	0.57	0.90	10.4	10.8	3801	3792	C30C1'
9.5	3.01	10.70	26946	0.50	0.84	3.6	9.4	2024	3810	A35C1'
11.2	2.38	9.15	25005	0.45	0.84	5.9	10.1	2006	3237	U31C1'