Development, simulation, and validation of NMR relaxation-based exchange measurements

R. D. Dortch,^{1,2} R. A. Horch,¹ and M. D. Does^{1,2,3,a)}

¹*Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee 37232-2310, USA and Vanderbilt University Institute of Imaging Science (VUISS), Vanderbilt University, Nashville, Tennessee 37232-2310, USA*

²*Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, Tennessee 37232-2310, USA*

³*Department of Electrical Engineering, Vanderbilt University, Nashville, Tennessee 37232-2310, USA*

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Two-dimensional (2D) nuclear magnetic resonance correlation experiments have recently been proposed as a means for studying exchange in porous media. Most notable of these is the T_2 – T_2 relaxation exchange spectroscopy (REXSY) experiment. Unfortunately, quantifying exchange with this method requires a relatively long, three-dimensional acquisition. To reduce acquisition times, novel 2D methods for quantifying exchange were developed. For each method, model equations were derived (for an arbitrary N -pool system), tested via simulation studies, and validated via experimental studies in an aqueous urea model system. Results indicate that the novel methods outperform REXSY—in terms of uncertainty per unit time for the fitted exchange rate—for certain model systems. The relative merits of each method are discussed in the text. © 2009 American Institute of Physics. [doi:10.1063/1.3245866]

I. INTRODUCTION

Porous media and biological tissue are both characterized by heterogeneity at the microscopic scale, resulting in an observed (i.e., macroscopic) water proton nuclear magnetic resonance (^1H NMR) signal that is a summation of these spatially varying characteristics. Previous work^{1,2} has shown that multiple component characterization of spin-spin relaxation time (T_2) data—via numerical inverse Laplace transform (ILT) methods^{3,4}—can be used to extract information about the microscopic pools that give rise to this heterogeneity. For porous media, these extracted T_2 components are thought to represent pores with characteristic surface-to-volume ratios;⁵ while in biological tissue, these extracted T_2 components are thought to represent unique microanatomical tissue compartments (e.g., intra/extracellular water).^{2,6} Regardless of the system, the extent to which the extracted T_2 s and pool sizes reflect the underlying intrinsic values is dependent on the magnitude of the exchange rate relative to the difference in relaxation rates between pools.⁷ Therefore, quantification of exchange is essential to fully characterize each system, especially when exchange is outside the slow-exchange regime. Additionally, the study of water exchange in these systems provides insight into the dynamic interactions between pools, novel information that could prove valuable in a number of studies (e.g., determining the permeability of barriers between pools).

Two-dimensional (2D) correlation experiments have recently been proposed^{8–12} as a means for studying exchange

in porous media. Most notable of these is the T_2 – T_2 relaxation exchange spectroscopy (REXSY) sequence first proposed by Lee *et al.*¹³ The REXSY sequence consists of two Carr–Purcell–Meiboom–Gill (CPMG) pulse trains separated by a mixing time during which the magnetization is stored on the longitudinal axis. Spins that reside in the same pool during each CPMG train will exhibit the same T_2 and will appear as diagonal peaks in the resultant T_2 – T_2 spectrum (from 2D ILT^{3,14,15} of T_2 – T_2 decay data), while spins that exchange during the mixing time will exhibit different T_2 during each CPMG train and will appear as off-diagonal peaks in the resultant T_2 – T_2 spectrum. Unfortunately, this experiment must be repeated over a range of mixing times in order to quantify exchange rates, resulting in a relatively long, three-dimensional (3D) experiment.

Herein, we propose two novel methods that allow one to extract exchange rates from a much shorter, 2D experiment. Specifically, the goals of these studies are (1) to develop a theoretical framework for an arbitrary N -pool system as well as acquisition and data analysis methods for quantifying exchange between pools of different T_2 , (2) to compare these developed methods in terms of their sensitivity to experimental noise, and (3) to validate these methods experimentally in a model system. To compare approaches, Monte Carlo simulations were performed. To validate each approach, NMR measurements were performed in an aqueous urea $[(\text{NH}_2)_2\text{CO}]$ model system. Aqueous urea contains two chemically distinct proton pools that are chemically shifted by approximately 1.1 ppm: (1) water protons (two per water molecule) and (2) urea protons (four per urea molecule). In the past, this system has been used to study chemical exchange.^{13,16,17} It was chosen for this study because (1) aqueous urea is biexponential (urea protons have a shorter T_2

^{a)}Author to whom correspondence should be addressed. Present address: 1161 21st Avenue South, AA 1105 MCN, Nashville, Tennessee 37232-2310. Tel.: 615-322-8352. FAX: 615-322-0734. Electronic mail: mark.does@vanderbilt.edu.

FIG. 1. Two-pool model (pools *a* and *b*). Equilibrium magnetizations and relaxation rates are defined for each pool. Exchange in each direction is defined via the exchange rates k_{ab} and k_{ba} .

than water protons because they are scalar coupled to fast relaxing quadrupolar ^{14}N spins¹⁶, (2) urea has a high solubility in water (allowing one to create solutions where 30% or more of the total proton signal arises from urea protons), (3) urea and water proton relaxation rates can be individually manipulated with contrast reagents,¹⁸ (4) proton exchange rates can be manipulated by altering pH and temperature,¹⁶ and (5) the system is fully invertible from T_2 data alone because the pool sizes are known from the stoichiometry of the solution. The last of these is especially important as it serves as a “gold standard” against which each method can be compared.

II. THEORY

Consider N exchanging pools with the same chemical shift. Define unique equilibrium magnetizations M_0^i , spin-lattice relaxation rates $R_1^i = 1/T_1^i$, and spin-spin relaxation rates $R_2^i = 1/T_2^i$ for each pool i . The rate of change in magnetization (during free precession) for this system can be expressed as a set of coupled ordinary differential equations, which are commonly referred to as the Bloch–McConnell equations.^{19,20} Using notation similar to that of Kimmich,²¹ these equations can be expressed as

$$\frac{d\mathbf{M}_\perp(t)}{dt} = \mathbf{L}_2 \mathbf{M}_\perp(t), \quad (1)$$

$$\frac{d\mathbf{M}_z(t)}{dt} = \mathbf{L}_1 [\mathbf{M}_z(t) - \mathbf{M}_0], \quad (2)$$

where $\mathbf{M}_\perp$, $\mathbf{M}_z$, and $\mathbf{M}_0$ are vectors ($N \times 1$) containing the transverse, longitudinal, and equilibrium magnetizations of each pool, respectively. Here, $\mathbf{L}_1$ and $\mathbf{L}_2$ are matrices ($N \times N$) defined as

$$\mathbf{L}_{1,2} = -\mathbf{R}_{1,2} + \mathbf{K}, \quad (3)$$

where $\mathbf{R}_1$ and $\mathbf{R}_2$ are diagonal matrices ($N \times N$) containing the relaxation rates for each pool and $\mathbf{K}$ is a matrix ($N \times N$) of pseudo-first-order exchange rates

$$\mathbf{K} = \begin{pmatrix} -\sum_{i \neq 1} k_{1i} & \cdots & k_{N1} \\ \vdots & \ddots & \vdots \\ k_{1N} & \cdots & -\sum_{i \neq N} k_{Ni} \end{pmatrix}. \quad (4)$$

Using this notation, k_{ij} represents exchange from pool i to pool j . Assuming the system is in equilibrium, the forward and backward exchange rates between any two pools can be related using the principle of detailed balance $k_{ij}M_0^i = k_{ji}M_0^j$. The general solutions to these equations are

FIG. 2. Pulse sequence diagrams for the three sequences used to quantify exchange: (a) REXSY, (b) IR-CPMG, and (c) IR-REXSY. (a) esp =echo spacing, t_m =mixing time, n_I =number of refocusing pulses in first CPMG encoding dimension, n_{II} =number of refocusing pulses in second CPMG encoding (or readout) dimension, G_c =crusher gradient; (b) t_i =inversion time, n =number of acquired echoes; (c) t_{e1} =echo time prior to mixing period, $G_{x,y}$ =crusher gradients applied along x or y direction.

$$\mathbf{M}_\perp(t) = \exp(\mathbf{L}_2 t) \mathbf{M}_\perp(0), \quad (5)$$

$$\mathbf{M}_z(t) = \mathbf{M}_0 - \exp(\mathbf{L}_1 t) [\mathbf{M}_0 - \mathbf{M}_z(0)], \quad (6)$$

which can be expanded in terms of the eigenvalues and eigenvectors of $\mathbf{L}_1$ and $\mathbf{L}_2$,

$$\mathbf{M}_\perp(t) = \mathbf{U}_{L_2} \begin{pmatrix} \exp(-\lambda_{L_2}^{(i)} t) & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \exp(-\lambda_{L_2}^{(N)} t) \end{pmatrix} \mathbf{U}_{L_2}^{-1} \mathbf{M}_\perp(0), \quad (7)$$

$$\mathbf{M}_z(t) = \mathbf{M}_0 - \mathbf{U}_{L_1} \begin{pmatrix} \exp(-\lambda_{L_1}^{(i)} t) & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \exp(-\lambda_{L_1}^{(N)} t) \end{pmatrix} \times \mathbf{U}_{L_1}^{-1} [\mathbf{M}_0 - \mathbf{M}_z(0)], \quad (8)$$

where $\lambda_{L_1}^{(i)}$ is the i th negative eigenvalue of $\mathbf{L}_1$ and $\mathbf{U}_{L_1}$ is a matrix whose columns are the corresponding eigenvectors. Similar definitions apply for $\lambda_{L_2}^{(i)}$ and $\mathbf{U}_{L_2}$ with respect to $\mathbf{L}_2$.

From these solutions, it can be seen that the magnetization from each pool evolves as a sum of N exponentials whose rate constants are the negative eigenvalues of $\mathbf{L}_1$ and $\mathbf{L}_2$. From this point forward, we will refer these as the ap-

FIG. 3. Two-pool REXSY data analysis demonstration using the model parameters in the text. (a) T_2-T_2 spectrum from 2D ILT of T_2-T_2 decay data at a single mixing time. (b) To extract exchange rates, this process is repeated over a range of mixing times and the resultant integrated peak amplitudes $\mathbf{P}$ are fitted with the model described by Eq. (12) (solid lines).

parent relaxation rates $\tilde{R}_1^i = 1/\tilde{T}_1^i$ and $\tilde{R}_2^i = 1/\tilde{T}_2^i$. The observed NMR signal is the sum of magnetizations from the individual pools

$$M_{\text{obs}}(t) = \sum_{i=1}^N M_{\perp}^i(t), \quad (9)$$

therefore, the bulk signal also evolves as a sum of N exponentials according to the same apparent relaxation rates. To separate the observed NMR signal into components that represent the underlying pools, the ILT can be applied to T_2 decay data.

Based upon the above general solutions, one can derive an expression for a given pulse sequence. Fitting data from the appropriate sequence to these expressions may allow one to invert the system and extract model parameters such as the exchange rates. In the following sections, the REXSY approach (Sec. II A) along with our two novel approaches (Secs. II B and II C) for extracting exchange rates will be outlined. The two-pool system shown in Fig. 1 will be used to demonstrate each approach. Unless otherwise specified, the following model parameters will be used for these demonstrations: $R_1^a = 1.25 \text{ s}^{-1}$, $R_1^a/R_1^b = 2$, $R_2^a = 50 \text{ s}^{-1}$, $R_2^b = 12.5 \text{ s}^{-1}$, $M_0^a = M_0^b = 0.5$, and $k_{ab} = k_{ba} = 2 \text{ s}^{-1}$.

A. Relaxation exchange spectroscopy

As shown in Fig. 2(a), the REXSY sequence consists of two CPMG pulse trains separated by a mixing time during which the magnetization is stored on the longitudinal axis. For a given mixing time, the number of refocusing pulses in the first CPMG period is arrayed, resulting in a 2D data set that decays according to

$$M_{\text{obs}}(t_{e1}, t_{e2}) = \int \int s(\tilde{T}_2^{(I)}, \tilde{T}_2^{(II)}) \exp(-t_{e1}/\tilde{T}_2^{(I)}) \times \exp(-t_{e2}/\tilde{T}_2^{(II)}) d\tilde{T}_2^{(I)} d\tilde{T}_2^{(II)}, \quad (10)$$

where $s(\tilde{T}_2^{(I)}, \tilde{T}_2^{(II)})$ is the weight of each exponential term, or T_2-T_2 spectrum, and the Roman numerals denote the first and second CPMG encoding dimensions. Inversion of Eq.

(10) into a 2D T_2-T_2 spectrum can be achieved via 2D ILT methods.^{3,14,15} Spins that reside in the same pool during each CPMG train will exhibit the same $\tilde{T}_2$ and will appear as diagonal peaks in the resultant T_2-T_2 spectrum, while spins that exchange during the mixing time will exhibit different $\tilde{T}_2$ during each CPMG train and will appear as off-diagonal peaks in the resultant T_2-T_2 spectrum. For a single mixing time, the T_2-T_2 spectrum allows one to qualitatively observe the exchange of spins between pools of different $\tilde{T}_2$. In order to quantify the exchange rates, the experiment can be repeated over a range of mixing times, fitting the amplitude of the observed diagonal and off-diagonal spectral peaks to the appropriate model (see Fig. 3), which can be derived using the general solutions to the Bloch-McConnell equations above.

Assuming the two-part phase cycle shown in Fig. 2(a)—this subtracts magnetization that is stored on the $-z$ axis during the mixing time from magnetization that is stored on the $+z$ axis—the observed REXSY signal $\mathbf{S}_{\text{REXSY}}$ for each pool under the condition of thermal equilibrium can be derived from Eqs. (5) and (6),

$$\mathbf{S}_{\text{REXSY}}(t_{e1}, t_m, t_{e2}) = 2 \exp(\mathbf{L}_2 t_{e1}) \exp(\mathbf{L}_1 t_m) \exp(\mathbf{L}_2 t_{e2}) \mathbf{M}_0, \quad (11)$$

where t_m is the mixing time, $t_{e1} = \text{esp}^* n_I$ is the T_2 -weighting duration in the first CPMG encoding dimension, and $t_{e2} = \text{esp}^* n_{II}$ is the echo time in the second CPMG encoding (or readout) dimension. Applying the eigenexpansion in Eq. (7) to the terms containing $\mathbf{L}_2$ and factoring with respect to each resulting exponential term, a matrix expression for the amplitude of each exponential term can be derived,

$$\mathbf{P}_{\text{REXSY}}(t_m) = 2[\mathbf{U}_{L_2}^{-1} \exp(\mathbf{L}_1 t_m) \mathbf{U}_{L_2}] \circ (\mathbf{U}_{L_2}^{-1} \mathbf{M}_0 \mathbf{1}_{1 \times N} \mathbf{U}_{L_2})^T, \quad (12)$$

where $\mathbf{1}_{i \times j}$ is a matrix or vector ($i \times j$) of ones, $\circ$ represents the elementwise (or Hadamard) matrix multiplication operation, and the superscript T represents the matrix transpose operation. In this expression, the diagonal elements of $\mathbf{P}_{\text{REXSY}}(N \times N)$ contain the integrated peak amplitudes of the

FIG. 4. Two-pool IR-CPMG data analysis demonstration using the model parameters in the text. (a) T_2 spectra from 1D ILT of T_2 decay data at three inversion times (after subtracting data from thermal equilibrium). (b) To extract exchange rates, this process is repeated over a range of inversion times and the resultant integrated peak amplitudes $\mathbf{P}$ are fitted with the model described by Eq. (15) (solid lines). To demonstrate the deviation from monoexponential decay for each component as a result of exchange, monoexponential fits are also shown (dashed lines).

diagonal peaks in $s(\tilde{T}_2^{(I)}, \tilde{T}_2^{(II)})$ and the off-diagonal elements contain the integrated peak amplitudes of the off-diagonal peaks in $s(\tilde{T}_2^{(I)}, \tilde{T}_2^{(II)})$. In other words, $\mathbf{P}_{\text{REXSY}}$ is an expression for the integrated peak amplitudes derived from 2D ILT of the REXSY data. This N -pool expression reduces to those given in Refs. 11 and 12 for a two-pool system.

B. Inversion-recovery prepared CPMG

Extracting exchange rates from REXSY data requires a relatively long, 3D experiment ($n_I \times n_{II} \times \text{number of } t_m$). Another potential method for extracting exchange rates is based upon the inversion-recovery prepared CPMG (IR-CPMG) pulse sequence shown in Fig. 2(b). The main advantage of this approach is that it potentially allows one to extract exchange rates from a 2D experiment ($n \times \text{number of } t_i$), reducing acquisition time relative to the REXSY approach.

This sequence is most commonly used to measure correlated $T_1 - T_2$,^{14,22,23} however, it was recently proposed as a method for extracting exchange information.⁸ It can be shown that, like REXSY data, 2D ILT of $T_1 - T_2$ data results in spectra with off-diagonal peaks, which reflect exchange between pools of different $\tilde{T}_2$. Unfortunately, the cross peak amplitudes are not all positive, which violates the non-negativity constraint typically employed to robustly perform the ILT operation. To get around this, we have developed a method based upon one-dimensional (1D) ILT^{3,4} of the CPMG data (at each inversion time) that does not violate this constraint (see Fig. 4).

For a given inversion time, the IR-CPMG signal decays according to

$$M_{\text{obs}}(t_e) = \int s(\tilde{T}_2) \exp(-t_e/\tilde{T}_2) d\tilde{T}_2, \quad (13)$$

where t_e is the echo time for the CPMG readout. Subtracting data from thermal equilibrium—this ensures that the non-negative constraint of the 1D ILT is met—the observed IR-CPMG signal $\mathbf{S}_{\text{IR-CPMG}}$ for each pool under the condition of thermal equilibrium can be derived from Eqs. (5) and (6),

$$\mathbf{S}_{\text{IR-CPMG}}(t_i, t_e) = 2 \exp(\mathbf{L}_2 t_e) \exp(\mathbf{L}_1 t_i) \mathbf{M}_0 \quad (14)$$

where t_i is the inversion time. Applying the eigenexpansion in Eq. (7) to the term containing $\mathbf{L}_2$ and factoring with respect to each resulting exponential term, one can derive an expression for the peak amplitudes $\mathbf{P}_{\text{IR-CPMG}}$ ($N \times 1$) as a function of inversion time

$$\mathbf{P}_{\text{IR-CPMG}}(t_i) = 2 \{ \mathbf{U}_{L_2}^{-1} \exp(\mathbf{L}_1 t_i) \mathbf{M}_0 \} \circ (\mathbf{1}_{1 \times N} \mathbf{U}_{L_2})^T. \quad (15)$$

For a two-pool system, it can be shown that one of the peak amplitudes evolves as the *difference* between two decaying exponentials as a function of inversion time, while the other peak evolves as the *sum* of two decaying exponentials in the presence of exchange. Unfortunately, the exchange-related component [short- T_1 component in Fig. 4(b)] is typically much smaller than other component(s), especially for slowly exchange systems. This results in high signal-to-noise ratio (SNR) demands to robustly fit the model.^{24,25} As a result, we have developed an additional approach, which we call inversion-recovery prepared REXSY (IR-REXSY).

C. Inversion-recovery prepared REXSY

The IR-REXSY sequence shown in Fig. 2(c) adds an inversion recovery preparation to the REXSY sequence in order to null one of the components based upon differences in R_1 . During the mixing time, this nulled component will grow due to exchange with the non-nulled component(s). Although this inversion pulse suppresses signal from the non-nulled pool (dependent on the relative R_1 of each pool and the exchange rate), the resultant data may exhibit decreased SNR demands relative to the IR-CPMG approach. This is because much of the nonexchange-related signal is filtered out using this approach.

The data analysis for the IR-REXSY sequence (see Fig. 5) is similar to that of the IR-CPMG sequence. For a given mixing time, the IR-REXSY signal decays according to Eq. (13), which again can be inverted into a T_2 spectrum using 1D ILT methods. In the resultant T_2 spectrum, the peak representing spins from the nulled component will grow as a function of mixing time due to exchange with the other non-

FIG. 5. Two-pool IR-REXS data analysis demonstration using the model parameters in the text. (a) T_2 spectra from 1D ILT of T_2 decay data at two mixing times (pool b has been nulled by the inversion recovery preparation). Note that the shift in the pool b T_2 component is an artifact of regularizing the fit of noiseless exponential decay and has negligible effect on the analysis. (b) To extract exchange rates, this process is repeated over a range of mixing times and the resultant integrated peak amplitudes P are fitted with the model described by Eq. (17) (solid lines).

nulled component(s). Thus, by fitting the amplitude of the observed spectral peaks as a function of mixing time to the appropriate model, one can extract exchange rates from IR-REXS data.

Using the same two-part phase cycle [see Fig. 2(c)] as the REXSY sequence, the observed IR-REXS signal $S_{\text{IR-REXS}}$ for each pool under the condition of thermal equilibrium can be derived from Eqs. (5) and (6),

$$\begin{aligned} S_{\text{IR-REXS}}(t_i, t_{e1}, t_m, t_{e2}) \\ = 2 \exp(\mathbf{L}_2 t_{e2}) \exp(\mathbf{L}_1 t_m) \exp(\mathbf{L}_2 t_{e1}) \\ \times [\mathbf{M}_0 - 2 \exp(\mathbf{L}_1 t_i) \mathbf{M}_0]. \end{aligned} \quad (16)$$

Note that this two-part phase cycle is particularly important for the IR-REXS sequence. This is because it ensures that the observed signal arises solely from spins that are excited by the first $\pi/2$ pulse—spins that are in the transverse plane during the “excite” period in Fig. 2(c)—and converts the T_1 recovery during the mixing time to a decay. This is important here, as any growth in the nulled component can be attributed directly to exchange. Using the previously described approach, we can rewrite this expression as a sum of exponential terms of amplitude $\mathbf{P}_{\text{IR-REXS}}$ ($N \times 1$),

$$\begin{aligned} \mathbf{P}_{\text{IR-REXS}}(t_m, t_i) = 2 \{ \mathbf{U}_{L_2}^{-1} \exp(\mathbf{L}_1 t_m) \exp(\mathbf{L}_2 t_{e1}) \\ \times [\mathbf{M}_0 - 2 \exp(\mathbf{L}_1 t_i) \mathbf{M}_0] \} \circ (\mathbf{1}_{1 \times N} \mathbf{U}_{L_2})^T. \end{aligned} \quad (17)$$

D. Model simplification: Fast-exchange regime with respect to R_1

For a two-pool system in the fast-exchange regime with respect to R_1 ($k_{ab} + k_{ba} \gg |R_1^a - R_1^b|$), one can simplify the models describing the REXSY, IR-CPMG, and IR-REXS peak amplitudes. In this regime, it can be shown that the summed spectral amplitude $P_{\text{tot}} = \sum_i P_i$ will decay monoexponentially as a function of mixing (REXS and IR-REXS) or inversion (IR-CPMG) time according to the rate constant $\lambda_{L_1}^{(2)}$ (assuming $\lambda_{L_1}^{(2)} < \lambda_{L_1}^{(1)}$). As a result, the normalized peak ampli-

tudes P_{norm} —the off-diagonal REXSY peak amplitudes divided by P_{tot} , the nulled component IR-REXS peak amplitude divided by P_{tot} , or the larger of the two IR-CPMG peak amplitudes divided by P_{tot} —will evolve as a monoexponential recovery according to the rate constant $\lambda_{L_1}^{(1)} - \lambda_{L_1}^{(2)}$. This simplification allows one to estimate $\lambda_{L_1}^{(1)}$ and $\lambda_{L_1}^{(2)}$ from monoexponential fits to P_{tot} and P_{norm} , which can be related to the exchange rate from

$$k_x = (\lambda_{L_1}^{(1)} + \lambda_{L_1}^{(2)}) - (R_1^a + R_1^b), \quad (18)$$

where $k_x = k_{ab} + k_{ba}$ (assuming $M_0^a + M_0^b = 1$).²⁶ Note that k_x must be weighted by the pool sizes in each direction to obtain the pseudo-first-order exchange rates (e.g., $k_{ab} = k_x M_0^b$). Unfortunately, one typically does not have independent knowledge of the R_1 of each pool. One can, however, estimate the correlated T_1 – T_2 from 2D ILT of IR-CPMG data, which yields reasonable estimates of $R_1^a + R_1^b$ in some cases. Note that this is similar to the method previously described by Washburn and Callaghan⁹ for REXSY data.

III. MATERIALS AND METHODS

A. Simulations

A two-pool system, as shown in Fig. 1, was defined for all simulations. Each set of simulations required definition of seven independent model parameters: $R_1^a, R_1^b, R_2^a, R_2^b, M_0^a, M_0^b$, and k_x . These model parameters were varied using combinations of the following: $R_1^a = 1.25 \text{ s}^{-1}$, $R_1^b/R_1^a = \{2, 1.5, 1.25\}$, $R_2^a = 50 \text{ s}^{-1}$, $R_2^b = 12.5 \text{ s}^{-1}$, $M_0^a = \{0.2, 0.5, 0.8\}$, $M_0^b = \{0.2, 0.5, 0.8\}$, and $k_x = \{0.5, 1, 2, 4, 8\} \text{ s}^{-1}$.

For each combination of model parameters, data were generated for (1) equilibrium CPMG, (2) REXSY, (3) IR-REXS, and (4) IR-CPMG pulse sequences. The REXSY, IR-REXS, and IR-CPMG data were generated to compare different methods for measuring exchange, while the equilibrium CPMG data were used to constrain the fitting algorithm (see Sec. III C for details). Equilibrium CPMG data were generated using Eq. (5), generating 500 echoes (n) at an echo spacing (esp) of 2 ms. These parameters were also used for

the CPMG trains of other sequences. REXSY data were generated using Eq. (11) at 32 t_m values linearly arrayed in 60 ms increments and at 24 T_2 -weighting periods (t_{e1}) pseudologarithmically arrayed from 2 to 500 ms. IR-REXSY data were generated using Eq. (16) at the same t_m values with $t_{e1}=2$ ms. Two sets of IR-REXSY data were generated, one in which pool a was nulled and one in which pool b was nulled. The t_i required to null each component was determined numerically using Eq. (17). IR-CPMG data were generated using Eq. (14) at 32 t_i values linearly arrayed in 60 ms increments. The simulated magnetization for the individual pools was summed [according to Eq. (9)] for all approaches prior to subsequent analysis.

For each set of simulated data, 1000 Monte Carlo trials were performed. Gaussian noise was added to the simulated data at each trial with a SNR of 2000—SNR is defined as the sum of the equilibrium magnetization divided by the standard deviation of the noise. Note that the SNR is defined relative to the equilibrium magnetization; therefore, the effective SNR of IR-REXSY will be much lower than the REXSY or IR-CPMG data due to the inversion recovery preparation at the beginning of the IR-REXSY sequence. Simulated noisy data were then fitted with the appropriate model (as described in Sec. III C) to extract model parameters. The SNR for each fitted parameter—defined as the expected parameter value divided by the standard deviation in the fitted value across trials—was then used to compare the three methods.

B. Urea phantom studies

1. Phantom preparation

A 7 m urea stock solution was prepared, yielding a ratio of 20/80% for urea/water protons. Urea and water proton relaxation rates were adjusted by addition of approximately 0.2 mM Gd-DTPA (Magnevist®; Berlex, Inc.) and 1 $\mu g/mL$ FeO_{1.44} (Ferodex®; Berlex, Inc.),¹⁸ resulting in a model with relaxation rates similar to values observed in biological tissue ($R_1/R_2 \approx 1/10$). The solution was then buffered with approximately 10 mM phosphate buffer, titrated to a pH of 8 with NaOH, and transferred (50 μL) to 5 mm NMR tubes.

2. NMR

NMR measurements were made at bore temperature ($\approx 20^\circ C$) using a 300 MHz, 16 cm bore Varian Inova (Varian, Inc., Palo Alto, CA) spectrometer equipped with imaging gradients capable of generating 27 G/cm with switching times to full amplitude of 100 μs . An in-house-built loop-gap resonator (10 mm in diameter, 20 mm long) was used for RF transmission and reception.

Data were collected for (1) equilibrium CPMG, (2) REXSY, (3) IR-REXSY, and (4) IR-CPMG pulse sequences. CPMG data were collected using a 1 ms esp, 1024 n , a 15 s predelay to ensure thermal equilibrium before each inversion pulse, and four averaged excitations (NEX). The same CPMG readout parameters and predelay values were used for all other experiments. REXSY data were collected at 30 t_m values linearly arrayed in 50 ms increments and 24 T_2 -weighting periods (t_{e1}) pseudologarithmically arrayed

from 2 to 512 ms. IR-REXSY data were collected using the same t_m values with $t_{e1}=2$ ms. The t_i was experimentally determined—from IR-CPMG data using a previously described method²⁷—to null water proton signal. REXSY and IR-REXSY sequences were repeated twice (NEX=2) to accompany the two-part phase cycle previously described. In order to correct for phase instabilities in our system, signed magnitude data (with the sign determined from the relative phase of the data) were combined instead of the raw complex data. This only had an effect on data acquired at longer mixing times, where the difference in magnitude for data stored on the $\pm z$ -axis is small. IR-CPMG data were collected at 24 t_i values pseudologarithmically arrayed from 50 ms to 3.5 s and NEX=4.

C. Data analysis

1. Apparent relaxation times and pool fractions

For equilibrium CPMG data (both simulated and experimental), the apparent relaxation times $\tilde{T}_2$ and pool sizes $\tilde{M}_0^i$ for each pool i were first determined by fitting the equilibrium CPMG data with the biexponential model

$$M_{\text{obs}}(t_e) = \tilde{M}_0^a \exp(-t_e/\tilde{T}_2^a) + \tilde{M}_0^b \exp(-t_e/\tilde{T}_2^b) \quad (19)$$

in a nonlinear least-squares sense using the Levenberg–Marquardt²⁸ algorithm. This nonlinear approach was chosen over linear ILT methods because the smoothing commonly employed to regularize the ILT operation can bias the resultant apparent relaxation times and pool sizes when the T_2 spectrum is better defined by two delta functions. These apparent values were then used to constrain subsequent fitting as described in Secs. III C 2–III C 4.

2. Inverting CPMG data in urea phantom

As previously stated, it is possible to invert the model from the apparent T_2 s and pool sizes in urea because the relative intrinsic pool sizes (20/80%) are known from the stoichiometry of the solution. To do this, numerically generated apparent T_2 s and pool sizes were fitted with the CPMG-derived experimental values in a nonlinear least-squares sense using the Levenberg–Marquardt algorithm. The apparent T_2 s were generated by calculating the eigenvalues of $\mathbf{L}_2$ [Eq. (3) for a two-pool system] and apparent pool sizes were generated from

$$\tilde{\mathbf{M}}_0 = (\mathbf{U}_{L_2}^{-1} \mathbf{M}_0) \circ (\mathbf{1}_{1 \times N} \mathbf{U}_{L_2})^T, \quad (20)$$

where $\tilde{\mathbf{M}}_0$ is a vector ($N \times 1$) of apparent pool sizes. Given that M_0^a and M_0^b are known, this results in a system with only three independent unknowns (R_2^a , R_2^b , and k_x) and four equations (two expressions each for the apparent pool sizes and T_2 s).

3. Inverting full two-pool model

The following data fitting procedures apply for both simulated and experimental data. REXSY data were 2D inverse Laplace transformed^{3,14,15} at each mixing time, yielding T_2-T_2 spectra as a function of mixing time. A sparse 2×2 grid of exponential terms with relaxation times $\tilde{T}_2^a$ and $\tilde{T}_2^b$

FIG. 6. Flowchart demonstrating the procedure used to invert the model. A trust-region method was used to minimize the least-squares misfit of measured and model peak amplitudes, yielding the exchange rate and spin-lattice relaxation rates for each site. During each iteration of this procedure, the current iteration's exchange rate was used in conjunction with the CPMG-derived apparent pool sizes and spin-spin relaxation rates to numerically determine the remaining model parameters.

(derived from equilibrium CPMG data) was used for these fits. This eliminated the need to integrate the area under the peak amplitudes, which has previously been shown¹¹ to be a major source of uncertainty in this type of analysis. IR-REXSY decay data (at each mixing time) were fitted with Eq. (19) in a nonlinear least-squares sense using the Levenberg–Marquardt algorithm. IR-CPMG data were subtracted from thermal equilibrium (to convert the T_1 recovery into a decay), and T_2 decay data at each inversion time were fitted with Eq. (19) using the same approach. The apparent relaxation times $\tilde{T}_2^a$ and $\tilde{T}_2^b$ derived from the equilibrium CPMG data were used for these fits, resulting in a two-parameter fit ($\tilde{M}_0^a$ and $\tilde{M}_0^b$).

The resultant spectral amplitudes were then fitted with the appropriate model—Eq. (12), Eq. (15), or Eq. (17)—in a nonlinear least-squares sense using a subspace trust-region method.²⁹ This method was chosen here because it was less sensitive to initial parameter guesses than more traditional approaches (e.g., Levenberg–Marquardt). During each iteration of the optimization procedure, the current iteration's k_x was used in conjunction with the equilibrium CPMG-derived apparent T_2 s and pool sizes to numerically determine the current iteration's intrinsic T_2 s and pool sizes (using an analogous approach as described in Sec. III C 2). This effectively constrained these four parameters (R_2^a , R_2^b , M_0^a , and M_0^b), reducing the number of free parameters to three (R_1^a , R_1^b , and k_x). This fitting procedure is summarized in Fig. 6.

4. Inverting fast-exchange two-pool model

The following data fitting procedures apply for both simulated and experimental data. REXSY, IR-CPMG, and IR-REXSY peak amplitudes were calculated as described in Sec. II and were, in turn, used to calculate P_{tot} and P_{norm} (see Sec. II D). P_{tot} and P_{norm} were then fitted with a monoexpo-

ponential decay and recovery, respectively, in a nonlinear least-squares sense using the Levenberg–Marquardt algorithm. The exchange rate k_x was then estimated from Eq. (18) using an IR-CPMG-derived T_1 – T_2 spectrum to estimate the R_1 of each pool. The remaining model parameters were determined numerically from CPMG-derived apparent pool sizes and T_2 s as previously described.

IV. RESULTS

A. Simulations

Results from the Monte Carlo simulations—specifically the fitted exchange rate SNR [$\text{SNR}(k_x)$] as calculated from inverting the full model—are summarized in Fig. 7 for various combinations of k_x , R_1^a/R_1^b , and pool sizes. Results from an additional set of simulations with $M_0^a=0.8$ and $M_0^b=0.2$ are not shown as they were nearly identical to those given for $M_0^a=0.2$ and $M_0^b=0.8$. In absolute terms, $\text{SNR}(k_x)$ was found to be greatest when the pool sizes were similar ($M_0^a=0.5$ and $M_0^b=0.5$), which is consistent with previous analyses of multiexponential fitting.^{24,25} In relative terms, results for the three approaches were independent of the pool sizes. Therefore, the following discussion should apply to any two-pool system.

For the IR-REXSY sequence, results were similar when component a or b was nulled; therefore, the choice of which pool to null is arbitrary for this system. Also, $\text{SNR}(k_x)$ was found to decrease (for the most part) as a function of k_x and R_1^a/R_1^b , which can be attributed to increased signal suppression by the inversion recovery preparation. Interestingly, although the IR-REXSY signal has a lower effective SNR than the IR-CPMG signal, the resultant fitted parameters exhibit a higher relative SNR in many cases ($k_x \leq 4 \text{ s}^{-1}$). This can be attributed to that fact that the inversion recovery preparation in the IR-REXSY sequence filters out much the nonexchange-related signal, allowing one to directly fit the signal related to exchange (much like fitting the off-diagonal peaks in REXSY-derived T_2 – T_2 spectra).

For the REXSY sequence, $\text{SNR}(k_x)$ increased (for the most part) as a function of k_x , which can be attributed to an increase in the relative off-diagonal amplitude with increasing k_x . REXSY outperformed the other techniques for nearly all combinations of model parameters; however, the acquisition time for the REXSY sequence is approximately n_1 times longer than the acquisition time for the IR-REXSY and IR-CPMG sequences. To compare these sequences in terms of SNR efficiency, plots of the $\text{SNR}/\sqrt{n_1}$ were also generated for REXSY data ($\text{REXSY}_{\text{norm}}$). When comparing these sequences in these terms, REXSY outperformed the other techniques only when exchange is relatively fast and when the ratio of R_1^a/R_1^b is near unity. That is, under these circumstances, for the same scan time, one can get higher SNR estimates of k_x with IR-CPMG or IR-REXSY as compared with REXSY. Also, when SNR is not the limiting factor, the minimum REXSY scan time remains fixed by the total number of T_2 preparation and mixing periods required to invert the model, allowing much faster acquisitions with the 2D methods.

FIG. 7. SNR of fitted k_x values from Monte Carlo simulations. IR-REXSY_A=IR-REXSY acquisition with pool a nulled; IR-REXSY_B=IR-REXSY acquisition with pool b nulled; REXSY_{norm}=SNR(k_x)/ $\sqrt{n_I}$.

Regardless of the relative performance of these three approaches, both 2D approaches (IR-REXSY and IR-CPMG) perform quite well over most combinations of model parameters. In fact, for relatively slow to intermediate exchange rates ($k_x \leq 4$ s⁻¹), these results indicate that we can fit the exchange rate between two pools with an uncertainty of less than 10% (when the data SNR is ≥ 2000). In contrast to the full model fits, which exhibited no bias for any of the fitted model parameters, the fast-exchange model fits exhibited significant bias for certain exchange regimes. In order to quantify this, the percent bias in the fitted k_x as a function of k_x and R_1^a/R_1^b was calculated for fast-exchange model fits, the results of which are summarized in Fig. 8. The remaining model parameters for these simulations were $R_1^a = 1.25$ s⁻¹, $R_2^a = 50$ s⁻¹, $R_2^b = 12.5$ s⁻¹, $M_0^a = 0.2$, and $M_0^b = 0.8$. For all three methods, a substantial bias was observed for relatively slow exchange. This can be attributed to deviation from the

monoexponential model used to describe P_{tot} and P_{norm} in this regime. Moving into the fast exchange regime—increasing k_x and/or decreasing R_1^a/R_1^b —this bias decreased substantially. This is in spite of the fact that the apparent R_1 s, as derived from T_1 – T_2 spectra, are increasingly biased in this regime. In fact, one can estimate the exchange rate with a bias of less than 10% for a number of model systems using this fast-exchange model.

B. Urea phantom studies

The SNR for the experimental urea data was $>10^4$. This was more than sufficient to reliably invert each model. Sample full model fits acquired in aqueous urea are given in Fig. 9 for each approach and the corresponding fitted parameter values are given in Table I. Recall that inversion of CPMG data using the known solution stoichiometry (20/80%)

FIG. 8. Bias in fitted exchange rate as a function of actual exchange rate under the fast-exchange assumption (with respect to R_1) for each of the three methods.

FIG. 9. Urea data model fits for each of the three methods (full model inversion). Note that the first two points for the IR-REXSY data fit are not shown for display purposes (the peak amplitude of the nulled component was negative at these mixing times).

for urea/water protons) is the gold standard against which all approaches were compared. In order to obtain a measure of uncertainty, CPMG measurements were repeated four times over the course of the experiment, and the standard deviation in fitted parameter values across these acquisitions was computed. In order to obtain a measure of uncertainty for the other approaches, the standard deviation of each fitted parameter was estimated from the diagonal elements of a numerically estimated parameter covariance matrix.³⁰

The exchange rates derived from the full model inversion of each approach were in good agreement and were within 9%, 7%, and 6% of the exchange rates derived from the CPMG data and solution stoichiometry for the REXSY, IR-REXSY, and IR-CPMG approaches, respectively. The derived pool fractions and relaxation rates from each approach were also in good agreement, further validating each approach. Also, the general trend of experimental uncertainties in the fitted exchange rates—from lowest uncertainty to highest: REXSY, IR-REXSY, and IR-CPMG—was in agreement with the simulated data [see Fig. 7(c), $k_x=1\text{ s}^{-1}$]. The exchange rates obtained from the fast-exchange model inver-

sion were within 18%, 12%, and 5% of the exchange rates derived from the full model inversion for the REXSY, IR-REXSY, and IR-CPMG approaches, respectively. Thus, this simplified fitting approach yields reasonable estimates of exchange in this model system.

V. DISCUSSION

The REXSY pulse sequence was recently proposed by Washburn and Callaghan⁹ as a means of studying exchange in porous media. Two more recent studies have sought to develop¹² and validate¹¹ the theoretical framework for REXSY data in a two-pool system. The purpose of the work herein was to extend this theoretical framework to an arbitrary N -pool system and to develop methods for robustly fitting REXSY data. One drawback of the REXSY approach is that it requires a relatively long, 3D experiment to quantify exchange rates. As a result, we developed two novel approaches (IR-CPMG and IR-REXSY) that allow one to quantify exchange rates from a much shorter 2D experiment. These approaches were (1) compared in terms of their sen-

TABLE I. Fitted two-pool model parameters for aqueous urea at pH 8.0 (a =urea, b =water protons). The uncertainty in CPMG-derived parameters is the standard deviation across multiple acquisitions. Standard deviations in full model fitted parameters were estimated from the diagonal elements of the parameter covariance matrix. Uncertainties are not given for the fast-exchange model parameters as they rely on an independent estimate of uncertainty for R_1 (obtained from 2D ILT of T_1 - T_2 data), which was not computed. Note that $M_0^b=1-M_0^a$.

	k_x (s^{-1})	R_1^a (s^{-1})	R_1^b (s^{-1})	R_2^a (s^{-1})	R_2^b (s^{-1})	M_0^a
Gold standard						
CPMG	1.03 ± 0.02	...	...	17.04 ± 0.05	5.00 ± 0.01	0.2^a
Full model						
REXSY	0.94 ± 0.01	1.44 ± 0.01	1.24 ± 0.01	17.11 ± 0.01	5.01 ± 0.01	0.20 ± 0.01
IR-REXSY	1.10 ± 0.03	1.35 ± 0.01	1.24 ± 0.01	17.00 ± 0.01	4.99 ± 0.01	0.20 ± 0.01
IR-CPMG	0.98 ± 0.04	1.45 ± 0.01	1.24 ± 0.01	17.08 ± 0.01	5.01 ± 0.01	0.20 ± 0.01
Fast-exchange model						
REXSY	1.11	1.38^b	1.25^b	16.98	4.98	0.20
IR-REXSY	0.95	1.38^b	1.25^b	17.11	5.01	0.20
IR-CPMG	1.03	1.38^b	1.25^b	17.04	5.00	0.20

^aKnown from urea solution stoichiometry.

^bEstimated from T_1 - T_2 spectrum.

sitivity to experimental noise via Monte Carlo simulations and (2) experimentally validated in an aqueous urea model system. The findings presented herein indicate that all three methods allow one to accurately and robustly extract exchange rates between pools of different T_2 s. The relative merits of each are discussed in Sec. V A in more detail.

A. Comparison of methods

The relative performance of each approach (in terms of sensitivity to experimental noise) was previously discussed over a range of model parameters. Regardless of these relative performances, all three methods allow one to quantify the exchange rate between two pools with an uncertainty of less than 10% for a wide range of model systems (Fig. 8). This is likely only true when the data SNR is adequate ($\approx 10^3$). In cases where one does not have adequate SNR, the fast-exchange fitting approach may be used as it relies on monoexponential fitting, which is less sensitive to experimental noise. When using this approach, however, one needs to be aware that this can significantly bias results when the system is in the slow- to intermediate-exchange regime (Fig. 9).

Several additional factors need to be considered in choosing the optimal approach. When choosing between the 3D (REXSY) and 2D (IR-CPMG and IR-REXSY) approaches, total acquisition time is likely the determining factor. In choosing between the 2D approaches (IR-REXSY and IR-CPMG), one must consider a couple of additional factors. First, the IR-REXSY experiment requires time to experimentally determine the optimal timings to null one of the components. As a result, the total experimental time needed for the IR-CPMG approach is expected to be less than for IR-REXSY. Second, when applying the fast-exchange model fitting approach, the IR-CPMG allows one to estimate all of the terms needed— R_1 for each pool and eigenvalues of $\mathbf{L}_1$ [Eq. (18)]—from a single acquisition.

One potential application for the IR-REXSY approach is higher-dimension experiments. Recently, Washburn and Callaghan³¹ proposed adding an additional dimension to the REXSY sequence, which they refer to as the propagator dimension. Essentially, this approach adds diffusion gradients on both sides of the mixing period, which are applied over a range of magnitudes. A q -space analysis³² is then applied to the resultant REXSY data to determine the characteristic length scale between exchanging pores. Adding this dimension to the IR-REXSY sequence could potentially allow one to collect these data using a 3D sequence, resulting in an approach with more manageable acquisition times compared with a four-dimensional REXSY sequence. The IR-CPMG approach is not amenable to this extension as it does not contain a storage period about which diffusion weighting could be added.

B. Urea as an exchange model system

In this work, a urea model system was used to experimentally validate the models developed for each approach. The relative merits of this model system include its flexibility in terms of the relative pool sizes, relaxation rates, and

exchange rates between pools. In addition, the urea model system has a distinct advantage over previous validation studies in borosilicate and soda lime glass spheres¹¹ because the relative pool sizes are known *a priori* from the solution stoichiometry. This allows one to invert the model from CPMG data, yielding a gold standard against which each of our methods could be compared.

One potential issue with the aqueous urea model is pH instability, which arises due to decomposition of urea into ammonium and cyanate ions.³³ Previous work by our group¹⁸ noted a significant pH drift in unbuffered solutions at non-neutral pH values. Exchange rates are very slow at neutral pH ;¹⁶ therefore, non-neutral pH preparations are likely of more interest for most model systems. In this study, we chose to buffer the solution to minimize this issue. Phosphate buffer was chosen because it has been shown to greatly reduce cyanate production in urea solutions.³³ A relatively low concentration of buffer (10 mM) was chosen because the addition of buffer can significantly increase exchange. This preparation resulted in a pH drift of less than 0.1 pH unit per 24 h period. The addition of phosphate buffer makes it difficult, if not impossible, to compare the exchange rates derived herein with these previous studies. However, this model does provide a relatively stable and flexible—in terms of the relative relaxation rates and pool sizes—system that is ideal for testing novel approaches as well as routine calibration and quality control.

VI. CONCLUSIONS

In this work, model equations describing exchange between pools of different T_2 were developed for an existing (REXSY) and two novel (IR-CPMG and IR-REXSY) methods. Simulation and experimental studies were used to test and validate these methods. Future work includes accessing the effect of magnetization transfer on these data and applying these methods in biological tissue (e.g., optic and sciatic nerve).

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