Methods for Measuring Intercompartmental Exchange: Aqueous Urea as a Model System

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Introduction: The NMR signal has been shown to exhibit multiexponential T_2 in a number of tissues (e.g., white matter [1]) due to microanatomical water compartition. Inverting T_2 decay data into a relaxation distribution — *via* inverse Laplace transform methods [2] — allows one to decompose bulk NMR signal into components that represent underlying microanatomical tissue compartments. Intercompartmental exchange is often ignored in this analysis, as it is not possible to fully invert the system from T_2 data alone when exchange is included; however, exchange can have a significant effect on the extracted pool sizes and T_2s in some cases.

In this study, a novel approach for measuring intercompartmental exchange is presented and compared to T_2–T_2 relaxation exchange spectroscopy (REXSY; Fig. 1) [3,4]. Extracting exchange rates from REXSY data requires a relatively long, 3D experiment (n x m x number of τ_m). Our novel approach adds an inversion pulse at the beginning of the sequence (IR-REXSY) to null one the components based upon difference in compartmental T_1s. The evolution of the perturbed components amplitudes as a function of mixing time can then be fitted to the appropriate model to extract exchange rates from a 2D experiment (n x number of τ_m, m = 0), resulting in a significant reduction in scan time compared to REXSY.

In the current study, exchange measurements were performed in an aqueous urea model. This model system was chosen because: 1) aqueous urea is biexponential (urea protons have a shorter T_2 than water protons), 2) urea has a high solubility in water, 3) urea and water proton relaxation rates can be individually manipulated with contrast agents [5], 4) proton exchange rates can be manipulated by altering pH and/or temperature, 5) and the system is fully invertible from T_2 data alone because the pool sizes are known from the stoichiometry of the solution.

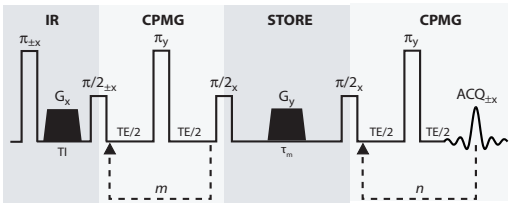

Fig. 1. Pulse sequence diagram for CPMG-store-CPMG (REXSY) sequence with optional inversion recovery period (IR-REXSY). TE = echo time; TI = inversion time; τ_m = mixing time; $G_{x,y}$ = spoiler gradients.

Theory: Consider pools of urea, u, and water, w, protons that are exchanging according to the pseudo first-order exchange rates, k_{uw} and k_{wu} (Fig. 2). Defining equilibrium magnetizations (M_0) and relaxation rates (R_1, R_2) for each pool, the rate change of bulk magnetization can be expressed as [6]

$$\begin{bmatrix} d\tilde{M}^{u}_{z,\perp}/dt \\ d\tilde{M}^{w}_{z,\perp}/dt \end{bmatrix} = \begin{bmatrix} -R^{u}_{1,2} - k_{uw} & k_{wu} \\ k_{uw} & -R^{w}_{1,2} - k_{wu} \end{bmatrix} \begin{bmatrix} \tilde{M}^{u}_{z,\perp} \\ \tilde{M}^{w}_{z,\perp} \end{bmatrix} \quad (1)$$

$$\tilde{M}^{u,w}_{z} = M^{u,w}_{z} - M^{u,w}_{0},\ \tilde{M}^{u,w}_{\perp} = M^{u,w}_{\perp},\ k_{uw}M^{u}_{0} = k_{wu}M^{w}_{0}.$$

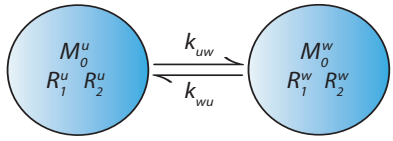

Fig 2. Compartmental model for aqueous urea, which consists of two proton pools (u = urea, w = water) in chemical exchange.

From solution to Eq. (1), Monteilhet *et al* [4] developed expressions describing the amplitude of the observed diagonal and off-diagonal T_2–T_2 distribution peaks (see Fig. 3a) as a function of mixing time for REXSY data. A similar approach was used herein to derive expressions (not shown) for the observed T_2 distribution peaks (see Fig. 3b) as a function of mixing time for IR-REXSY data.

Methods: A 7-molal 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 (Magnvist®; Berlex, Inc.) and 1 µg/mL $FeO_{1.44}$ (Ferodex®; Berlex, Inc.) [5], resulting in a model with relaxation rates similar to values observed in tissue ($R_1/R_2 \approx 1/10$ s^{-1}). 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.

NMR measurements were made at bore temperature (≈ 20 ºC) using a 7.0-T, 16-cm bore Varian Inova spectrometer and a 10-mm diameter single-turn RF coil. Exchange was measured by: 1) inverting CPMG data (n = 1024, TE = 1 ms, predelay (pd) = 15 s, NEX = 4) using the known stoichiometry of the solution, 2) fitting REXSY data (m arrayed logarithmically between 10 and 512 in 24 steps, τ_m arrayed linearly between 0.05 and 1.5 s in 30 steps, n = 1024, TE = 1 ms, pd = 15 s, NEX = 2) to the model derived in [4], and 3) fitting IR-REXSY data (TI = 555 ms to null the water component, τ_m arrayed linearly between 0.05 and 1.5 s in 30 steps, n = 1024, TE = 1 ms, pd = 15 s, NEX = 2) to the model derived herein.

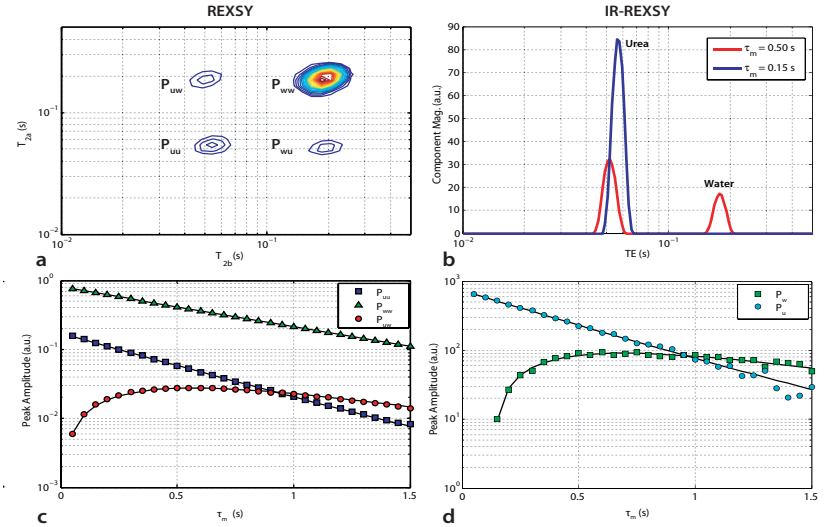

Fig 3. (a) Sample REXSY T_2–T_2 distribution (τ_m = 1 s), showing the diagonal (P_{uu}, P_{ww} represent stationary spins) and off-diagonal (P_{uw} = P_{wu} represent exchanging spins). (b) Sample IR-REXSY T_2 distribution (TI to null water peak). (c and d) Peaks amplitudes and model fits for each sequence. Note the growth then decay of the off-diagonal peak in (c) (and water peak in (d)) with increasing τ_m due to exchange and T_1, respectively.

Results and Discussion: Fig. 3 shows sample relaxation distributions for REXSY and IR-REXSY approaches as well as the model fits for each. The exchange rates derived from these model fits were within 10% of the rate derived from the CPMG data and the known stoichiometry (k_{uw} = 0.84 s^{-1}) for both approaches (REXSY: k_{uw}= 0.76 s^{-1}; IR-REXSY: k_{uw} = 0.81 s^{-1}). The derived pool fractions (REXSY: M_o^u = 0.197, M_o^w = 0.803; IR-REXSY: M_o^u = 0.199, M_o^w = 0.801), and relaxation rates (REXSY: R_1^u = 1.44 s^{-1}, R_1^w = 1.23 s^{-1}, R_2^u = 17.12 s^{-1}, R_2^w = 5.02 s^{-1}; IR-REXSY: R_1^u = 1.50 s^{-1}, R_1^w = 1.16 s^{-1}, R_2^u = 17.01 s^{-1}, R_2^w = 5.00 s^{-1}) from both approaches were also in good agreement, further validating our novel approach. Future work includes performing these measurements at different exchange rates (by altering pH) and extension of this approach to tissue (e.g., optic nerve).

References: [1] Stewart WA. *MRM* **21**:767 (1993). [2] Whittall KP. *JMR* **84**: 134 (1974). [3] Lee J. *J Am Chem Phys* **115**: 7761 (1993). [4] Monteilhet L. *Phys Rev E* **74,** 061404 (2006). [5] Horch RA. *MAGMA* **20**: 51 (2007). [6] McConnell HM. *J Chem Phys* **28**: 430 (1958).

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