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Synopsis

The dipolar order relaxation time (T_{1D}) is a probe of membrane dynamics and microstructure and could serve to further understand the relationship between the myelin membrane integrity and its biological function. In this work, the ability of quantitative inhomogeneous Magnetization Transfer (qihMT) to estimate the several T_{1D} components of a synthetic lipid membrane system, a surrogate for the myelin membrane, was evaluated by comparison with the gold standard method for T_{1D} quantification, the NMR Jeener-Broekaert (JB) sequence.

Introduction

Slow collective membrane motional processes (1 to 10^7 Hz) are the main driving mechanisms of dipolar relaxation[1]. The dipolar relaxation time (T_{1D}) could thus serve as a probe to further understand the myelin membrane dynamics and the relationship with its biological function. Inhomogeneous Magnetization Transfer (ihMT)[2] data are amenable to quantitative analyses (qihMT) and provide access to various parameters of interest[3–5]. In particular, variations of the switching time (Δt) between the application of RF pulses at positive and negative offset frequencies in the dual saturation were previously used to estimate T_{1D} in clinical[4] and preclinical studies[6] in healthy Central Nervous System tissues. However, T_{1D} values derived from ihMT have yet to be validated. In this study, we propose to compare the T_{1D} values estimated with qihMT and the gold-standard NMR Jeener-Broekaert (JB) sequence[7] on a synthetic model of lipid membrane mimicking myelin.

Methods

Synthetic membrane : Three types of lipids were weighted to obtain a molar ratio of 40%/40%/20% (POPC/Cholesterol/Cerebrosides) for a total mass of 25 mg. The powder blend was dissolved in an organic solvent to assure a homogeneous mixture. After removal of the solvent, the resultant dry lipid film was hydrated in 100 µL of D_2O for the NMR experiments or in 100 µL of H_2O for the MRI experiments. After three liposome formation cycles (vortex/freeze/thaw) the multilamellar vesicle suspension was transferred into test tubes for experimentation.

Data acquisition and processing : All experiments were carried out at 500 MHz (NMR: Bruker Avance III spectrometer equipped with a ^1H-X CP/MAS probe for 4-mm rotors; MRI: Bruker Avance, 89-mm wide bore vertical imager) with a sample's temperature maintained at 298 K.

NMR: Jeener-Broekaert experiments were performed at various mixing time values (t_m; Table 1). The signal decay, normalized to that of the first t_m, follows a multi-exponential decay:

$$S_{\mathrm{JB}}(t_m) = \sum_{i=1}^{N} \alpha_i e^{-t_m/T_{1D,i}}$$

A Non-Negative Least-Square algorithm was used to decompose the decay curve into multiple T_{1D}s from a distribution of 1280 logarithmically spaced T_{1D} values ranging from 1.0 µs to 40.0 s. For each detected lobe within the estimated distribution, the associated geometric mean and weight (area under the curve) are reported.

MRI: IhMT experiments were performed using an ihMT-prepared RARE sequence[8]. The experimental design included the variation of saturation parameters across three experimental dimensions: frequency offset Δf, pulse power B_{1rms}, and switching time Δt (Table 1), for a total of 170 experimental data points. A quantitative multi-T_{1D} ihMT model[6] was used for the estimation of qihMT parameters. All macromolecular pools associated with various T_{1D}s were assumed to share the same exchange rate (R) and transverse relaxation time (T_{2b}). R_{1b}, the longitudinal relaxation rate of the Zeeman macromolecular pools was fixed to 1 s^{-1} and T_{2f}, the transverse relaxation time of the water pool, was fixed to 100 ms.

Processing: To evaluate the feasibility of estimating multiple T_{1D} components with ihMT, Cramér-Rao Lower Bounds (CRLB)[9] for a multi-compartment qihMT framework were estimated from the experimental design (Table 1), using the T_{1D} values and associated weights from the JB spectrum.

Results

Figure 1 presents the T_{1D} spectrum estimated from the JB experiments. A total of seven components were estimated (T_{1D} values of 40.0 µs, 208.0 µs, 1.2 ms, 8.4 ms, 678.3 ms for weights>8%). Figure 2 shows representative ihMT ratio images along the Δt and Δf dimensions at B_{1rms}=20 µT. The qihMT fitting curves for a bi-T_{1D} model applied to experimental data points are illustrated in Figure 3. The estimated T_{1D}s were 500.6±102.0 µs and 13.3±0.8 ms (associated M_{0b}s 14.7±8.3% and 10.3±5.3%, respectively). Including more T_{1D} components in the qihMT model failed to estimate parameters with reasonable standard errors (not shown). As such, a CRLB analysis was performed in our experimental framework, using the seven T_{1D}s and associated weights estimated from the JB analysis and assuming RM_{0b}/R_{1f}=1.5, T_{2b}=9.5 µs (Figure 3). Figure 4 shows the normalized CRLB standard deviation of each T_{1D} component as a function of the signal-to-noise ratio (SNR). These results demonstrate that the experimental design does not allow to resolve more than two T_{1D} components (T_{1D} of 1.2 ms and 8.4 ms from the JB spectrum) with a suitable error (<10%) given our experimental SNR of 2000.

Discussion and Conclusions

Of the seven components revealed by JB and putatively characterizing the synthetic membrane, qihMT and the proposed experimental design

revealed up to two T_{1D}s in the range of the expected ones. The SNR is one identified cause of not being able to quantify the remaining components given the current acquisition protocol. A one-to-one comparison between T_{1D}s estimated by JB and qihMT is not straightforward. While JB experiments directly interrogate the semisolid pool, the ihMT signal relies on the exchange between the semisolid and the free water pools which may not occur for all components. As such, some of the components estimated by JB might not exchange and the estimated spectrum using MRI would contain less T_{1D}s. Exchange can also impact the underlying T_{1D}s, thus comparing T_{1D}s measured in D_2O by JB to those with ihMT in H_2O may be an error source. These interesting results call for a more optimized experimental design, which will be performed in future investigations using CRLB.

Acknowledgements

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Figures

	Off-resonance saturation parameters					
(a) MRI - ihMT	pw	N_p	τ_{sat}	Δf	B_{1rms}	Δt
	0.5 ms	3200	2720 ms	8; 10; 12; 15; 20 kHz	10; 20 µT	0.05; 1.7; 3.4; 4.25; 6.0; 8.5; 13.6; 21.25; 27.2; 34; 68; 85; 136; 170; 340; 680; 1360 ms
	Readout parameters					
	TR	TE	TA	Resolution	FOV	Slice thickness
	23 s	2.4 ms	4 min 36 s	156x156 µm²	20x20 mm	8 mm
(b) NMR - JB	t_m					
	64 mixing times spanning from 1.0 µs to 10.0 s					

Table 1: (a) Off-resonance saturation and readout parameters for the MRI-ihMT protocols: a total of N_p Hann-shaped pulses of a duration of pw were played at different root-mean-square powers (B_{1rms}) calculated over the saturation time (τ_{sat}), frequency offsets (Δf) and switching times for the dual-frequency saturation offset (Δt). (b) The interval of mixing times (t_m) for the Jeener-Broekaert acquisitions.

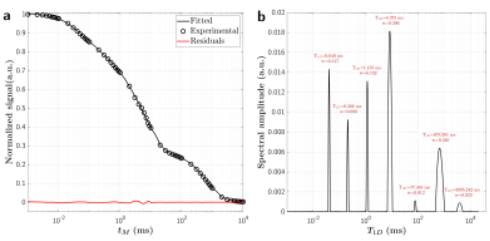

Figure 1: (a) Jeener-Broekaert decay curve along with the corresponding fitting residuals and (b) the estimated regularized T_{1D} spectrum. The geometric mean on each

lobe (T_{1D}) is reported along with the weight of each T_{1D} component corresponding to the area under the lobe (w).

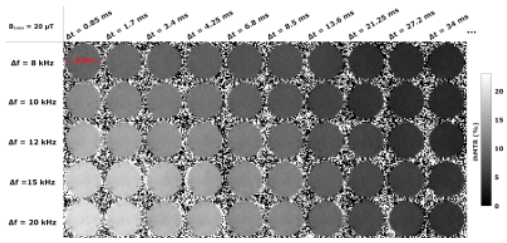

Figure 2: Example of ihMT ratio (ihMTR) maps of the synthetic lipid membrane system for various offset frequencies (Δf) and switching time (Δt) values. Images correspond to a B_{1rms} value of 20 µT. Experimental SNR evaluated in the ihMT image corresponding to Δf = 10 kHz and Δt = 0.85 ms was higher than 2000.

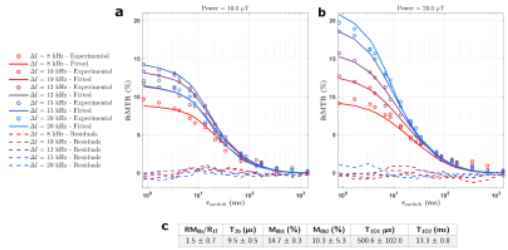

Figure 3: Bi-T_{1D} model fit of ihMTR on all the experimental data points (r^2 = 0.99). Fit curves and residuals for (a) B_{1rms} of 10 µT and (b) B_{1rms} of 20 µT. (c) The estimated qihMT model parameters and the associated errors.

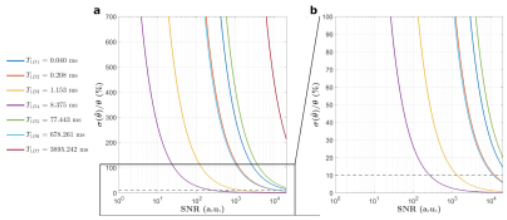

Figure 4: (a) Cramér-Rao lower bound analysis on the seven T_{1D} components estimated by JB as a function of SNR. (b) zoom on the 0% to 100% interval of the Y-axis.