Supporting Information for

Size-induced enhancement of chemical exchange saturation transfer (CEST) contrast in liposomes

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1. DLS measurements of liposome sizes

The hydrodynamic size distributions of the liposomes were measured using dynamic light scattering (DLS). DLS measures the scattered laser intensity fluctuations from liposomes and calculates the distribution of liposome sizes from the autocorrelation function $G(\tau)$ of the fluctuations. The z-average diameter and polydispersity index are commonly used to parameterize the size distribution and can be calculated from the exponential-sum part, $g_1(\tau)^2$, of $G(\tau)$. The cumulant expansion of $g_1(\tau)^2$ is

$$\ln[g_1(\tau)^2] = a + b\tau + c\tau^2 \qquad \text{(S1)}$$

where b is the z-average diffusion constant, from which the z-average diameter can be computed from the Stokes-Einstein equation, and $2c/b^2$ is the polydispersity index, which is a measurement of the width of the size distribution. The manufacture's software was used to calculate all the DLS parameters. The samples were prepared by diluting 20-50 μL of liposomes in 1 mL of PBS. Each measurement is an average of 12-15 subruns and each sample was measured three times (Table S1). The z-average diameters are highly reproducible for consecutive measurements.

Table S1. Z-average diameters of the liposome sizes (nm) from three consecutive DLS measurements.

Sample	Measurement 1	Measurement 2	Measurement 3
99nm	98.6	99.1	99.4
199nm	199	199	196
536nm	525	536	537

2. Optimization of TmDOTA concentrations

Table S2. Effect of initial TmDOTA concentrations on intraliposomal water shift (ω_m). The bulk water is centered at 0 ppm. The corresponding MTR_{asy} vs. frequency plots are shown in Fig. 1 in the paper.

$[TmDOTA]_0$, mM	Size, nm	[liposome], nM	ω_m, ppm
100	80	126	0.56
150	80	175	0.81
200	79	106	1.0
300	94	192	1.25
400	80	311	1.5

3. Proton NMR spectra

High resolution proton spectra were acquired with a single $\pi/2$ pulse followed by detection. At 11.7T, radiation damping has a large effect on the lineshape of the spectra. Typical full widths at half maximum of water were approximately 20 Hz for both PBS and liposome samples after the probe was detuned to minimize radiation damping. These linewidths broadened to about 40 Hz due to radiation damping after the probe was tuned to resonance. Fig. S1 shows the proton spectra for the three liposome samples and PBS with a detuned probe.

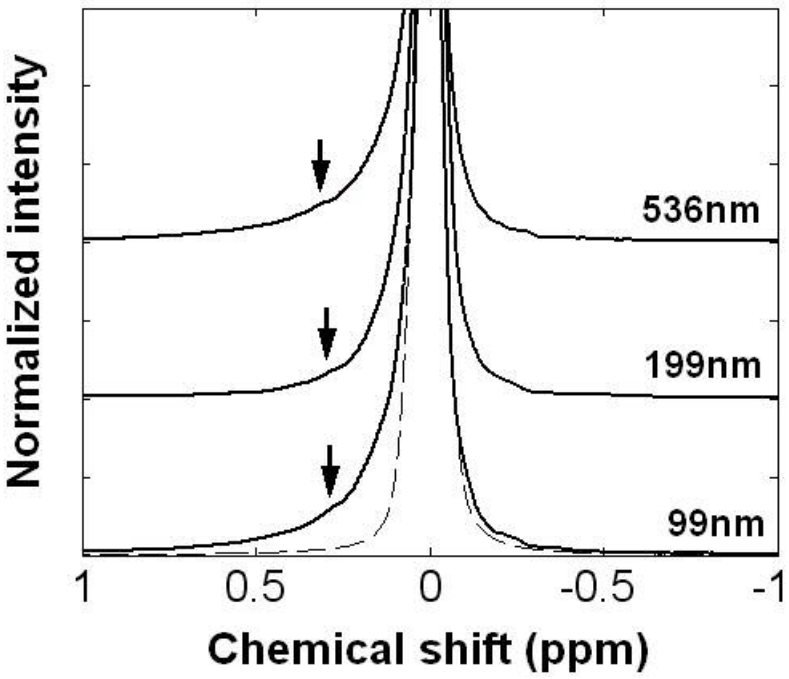

Figure S1. High resolution proton NMR spectra of PBS buffer (dashed line) and three liposome samples (dark solid lines) of different size each containing 200 mM TmDOTA. The arrows mark the positions of small shoulders corresponding to the intraliposomal water.

4. Error analysis of the measured permeability

There are several factors that contribute to the accuracy of the measured permeability P_l. P_l was determined from the linear fit of exchange rates (k_{wl}) vs. surface-to-volume ratio (ΣS/V). The major sources of error in k_{wl} come from spectral centering and the fitting of MTR_{asy} as a function of T_{sat}.

Centering of the MTR_{asy} spectrum is crucial for the asymmetry analysis in Eq. 10. A poor centering results in underestimating of MTR_{asy} and k_{wl}. Spectral centering is relatively straightforward for *in vitro* experiments in the current work, but becomes more challenging for *in vivo* applications. Here we performed all experiments on resonance and the bulk water frequency, which was locked with D_2O, should not vary much over the homogeneous sample.

The 95% confidence intervals of the exchange rate $\bar{k}_{lw}$ of method (iii) are 221±13, 93±3, 13±1 for the 99 nm, 199 nm, 536 nm samples, respectively. So the errors in $\bar{k}_{lw}$ due to the fitting of MTR_{asy} contribute to about 3-8% errors in P_l.

Additionally, errors in determining the total surface-to-volume ratio (ΣS/V) propagate into errors in P_l. The source of error in ΣS/V comes from calculating the particle size distribution using the dynamic light scattering (DLS). Raleigh scattering implies that scattered intensity goes with diameter to the 6th power, thus DLS is much more sensitive at detecting larger particles than small ones. Table S1 shows that the z-average diameters of the particles sizes of the consecutive DLS measurements on each sample are highly reproducible. To estimate the accuracy of the DLS diameter measurements, three nanosphere standards (Duke Scientific, Fremont, CA) that are traceable by National Institute of Standard and Technology (NIST) were used with hydrodynamic size ranges: 100-109 nm, 297-310 nm, 591-602 nm. The corresponding DLS z-average diameters are: 106±1 nm, 315±3 nm, 634±8 nm, with corresponding accuracies of 1%, 4%, 6%. Thus the DLS determination of the particle sizes contributes to approximately 1-6% systematic error in P_l.

To determine the reproducibility of measured permeability values for liposome batches prepared on different days, we observed the following average values using method (iii): batch #1 (without PEG-PE), P_l = 2.3±1.4 μm/s; batch #2, P_l = 1.8±0.4 μm/s; batch #3, P_l = 3.1±0.7 μm/s; batch #4 (presented this paper), P_l = 1.11±0.14 μm/s, where the quoted errors are the standard deviations from the three samples in a given batch. The average permeability from batch #2 to #4 (all with PEG-PE) is 2±1 μm/s. These batch-to-batch variations are attributed to the slight differences in the sample preparation conditions and uncertainties in the DLS and MR measurements discussed above.