Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary information for

Oxidation-Responsive $Eu^{2+/3+}$–Liposomal Contrast Agent for Dual-Mode Magnetic Resonance Imaging

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Experimental Procedures

Commercially available chemicals were of reagent-grade purity or better and were used without further purification unless otherwise noted. Water was purified using a PURELAB Ultra Mk2 water purification system (ELGA) and degassed prior to use. NMR spectroscopy and inductively coupled plasma optical emission spectroscopy (ICP–OES) analyses were performed at the Lumigen Instrument Center in the Department of Chemistry at Wayne State University and in the Department of Chemistry at Oakland University. In vitro phantom imaging was performed at Henry Ford Hospital.

Inversion-recovery T_1 measurements were obtained using a Varian VNMRS 500 (499.48 MHz, 11.7 T) spectrometer before air exposure or after 24 h of air exposure. Deuterium oxide (300 mOsm NaCl) was added to make liposome suspensions 5% D_2O (v/v) for the purpose of locking and shimming.

MRI scans were performed with a 7 T Varian small animal MRI scanner (299.44 MHz, 7.0 T) equipped with a 12 cm bore magnet and a 38 mm diameter homemade transmit/receive quadrature birdcage coil. Samples included liposomes that were not exposed to air, liposomes that were exposed to air for 24 h, and water. The T_1-weighted images were acquired at ambient temperature (echo time: 11 ms; repetition time: 320 ms; seven image slices at 1 mm thickness; 24 × 24 mm field of view; and four averages). The liposome-encapsulated Eu^{3+} (chemical exchange saturation transfer, CEST) effects were measured at ambient temperature under the same parameters used in a previous CEST MRI study.[1] A RARE MRI pulse sequence with a RARE factor of 8 (repetition time/echo time, 4.0 s/11 ms) was applied with a 17 μT saturation power for 2 s. A total of 64 s was required to acquire a single MR image with 128 × 128 pixels that covered a 24 × 24 mm field of view, a single slice with a thickness of 1 mm, and a single average. The water signal was measured for each phantom when saturation was applied between

5 and –5 ppm in 0.2 ppm increments to measure the CEST effect of liposomes, and Figure 4 in the manuscript was acquired at 1.2 ppm ($S^{\Delta\omega}$) and –1.2 ppm ($S^{-\Delta\omega}$).

Varian flexible data format (FDF) files were converted to tagged image file format (TIFF) files with a MATLAB code.[2] TIFF files were processed to produce chemical exchange saturation transfer (CEST) spectra by measuring pixel intensities with ImageJ 1.47.[3] Percent CEST (%CEST) was calculated using **eq 1**.[4]

eq 1. $$\%\mathrm{CEST} = \left(1 - \frac{M_Z}{M_0}\right) 100$$

In **eq 1**, M_Z and M_0 are the average signal intensities (calculated with ImageJ) of the same phantom tube slice at 360 Hz (1.2 ppm) and –360 Hz (–1.2 ppm), respectively. The CEST image was created by subtracting the TIFF slice at 360 Hz (1.2 ppm) from the identical slice at –360 Hz (–1.2 ppm) and the difference was divided by the slice at 360 Hz (–1.2 ppm). The %CEST scale bar was created by calibrating the pixel range of the CEST image to the maximum %CEST value obtained from **eq 1** using a linear fit.

Dynamic light scattering data were obtained using a Malvern Zetasizer Nano-ZS instrument (ZEN3600) operating with a 633 nm wavelength laser. Dust was removed from samples by filtering through 0.2 μm hydrophilic filters (Millex–LG, SLLGR04NL). Liposome samples were prepared for light scattering experiments by diluting purified liposome suspensions in iso-osmolar phosphate-buffered saline (PBS, 1:10, 29 mM Na_2HPO_4, 46 mM NaH_2PO_4, 57 mM NaCl, and 2.1 mM KCl). For liposome size measurements with no air exposure, air-tight cuvettes were filled in a glovebox under an atmosphere of Ar.

ICP–OES measurements were acquired on a Jobin Yvon Horiba Ultima or PerkinElmer Optima 7000 DV spectrometer. All samples were diluted with 2% HNO_3, which was also used for blank samples during calibration. The calibration curves were created using the Eu emission

intensity at 381.965 nm for a 1–60 ppm concentration range (diluted from Alfa Aesar Specpure AAS standard solution, Eu_2O_3 in 5% HNO_3, 1000 µg/mL) and the Sr emission intensity at 407.771 nm for a 0.5–5 ppm concentration range (diluted from Fluka Analytical Sr ICP standard, 1000 mg/L), and all samples were diluted to fall within the concentration range of standards for the respective element.

Preparation of Hydration Solution

The hydration solution was prepared by stirring an aqueous solution of $EuCl_2$ and 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (cryptand) for 12 h under an atmosphere of Ar followed by a phosphate-buffer-workup.[5] To account for loss of phosphate during the precipitation step of this experiment, a PBS stock solution was prepared with a high concentration of phosphate (1 M). The purpose of the high phosphate concentration was to ensure PBS buffer capacity was not lost upon phosphate precipitation in the presence of uncomplexed Eu^{3+} in the oxygen-exposed samples and to maintain physiological osmolality (300 mOsm). This PBS solution was prepared in a glovebox under an atmosphere of Ar by dissolving anhydrous dibasic sodium phosphate (42.6 g, 0.300 mol), monobasic sodium phosphate monohydrate (27.6 g, 0.200 mol), sodium chloride (22.3 g, 0.381 mol), and potassium chloride (1.01 g, 13.6 mmol) in H_2O (500 mL). The pH of the resulting solution was brought to 7.0 with the addition of solid sodium hydroxide (3.87 g, 96.8 mmol).

To a 4 mL glass vial equipped with a magnetic stir bar was added aqueous $EuCl_2$ or $SrCl_2$ and aqueous cryptand under an atmosphere of Ar. The resulting clear, colorless solution was stirred for 12 h before addition of the PBS solution described above (390 mM Na_2HPO_4, 610 mM NaH_2PO_4, 762 mM NaCl, 27.2 mM KCl, pH 7.0) and water to bring the osmolality of the solution to 300 mOsm. Upon addition of PBS, a slightly turbid suspension formed that was

stirred for 1 h and then filtered through a 0.2 μm hydrophilic filter. The final concentrations of Eu (13, 24, 40 and 45 mM) or Sr (28 mM) of the clear, colorless filtrates were determined by ICP–OES. This filtrate was used for liposome preparation. The hydration solution used to prepare blank liposomes consisted of iso-osmolar (300 mOsm) PBS prepared by dilution of the PBS solution described above.

Preparation of Liposomes

Liposomes were prepared via the thin-film hydration technique.[6] To a 4 mL vial was added 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (22.0 mg, 2.89 μmol, 1.4 equiv), cholesterol (8.0 mg, 2.1 μmol, 1 equiv), and chloroform (1 mL) to produce a clear, colorless solution. Solvent was removed under reduced pressure to afford a visible film on the bottom of the vial. Under an atmosphere of Ar, the hydration solution (1.15 mL) and vial containing the lipid thin film were placed in a water bath at 55 °C for 30 min, and then the hydration solution was added to the vial containing the thin film. The resulting white suspension was stirred at 55 °C for 1 h. Extrusion of the suspension was accomplished using a mini-extruder and heating block (Avanti Polar Lipids, Alabaster, AL, USA) heated to 55 °C (4 passes through a 0.2 μm polycarbonate filter followed by 15 passes through a 0.1 μm polycarbonate filter). After extrusion, the suspension was allowed to cool to ambient temperature within the Ar-filled glovebox for 1 h.

Non-encapsulated M^{2+}-containing cryptate (M = Eu, Sr) was removed from the liposome suspension in an Ar-filled glovebox via spin filtering (Amicon Ultra regenerated cellulose 3,000 molecular weight cut off). The liposome suspension was filtered in aliquots because the volume of the suspension exceeded the volume of the spin filter. When the volume of suspension in the filter reached 0.3 mL after spinning, the volume was brought to 0.5 mL with the addition of iso-

osmolar (300 mOsm) PBS prepared by dilution of the PBS solution described above. Spin-filtered fractions were collected until Eu or Sr was not detectable by ICP–OES (17 fractions).

CEST Imaging Data

In vitro phantom image intensities were plotted as a function of frequency offset of presaturation for blank liposomes (Figure S1a), liposomes containing 28 mM $Sr(2.2.2)^{2+}$ (Figure S1b), liposomes containing 13 mM $Eu(2.2.2)^{2+}$ (Figure S1c), liposomes containing 24 mM $Eu(2.2.2)^{2+}$ (Figure S1d), liposomes containing 40 mM $Eu(2.2.2)^{2+}$ (Figure S1e), and liposomes containing 45 mM $Eu(2.2.2)^{2+}$ (Figure S1f). This data was used for Lorentzian curve fitting.

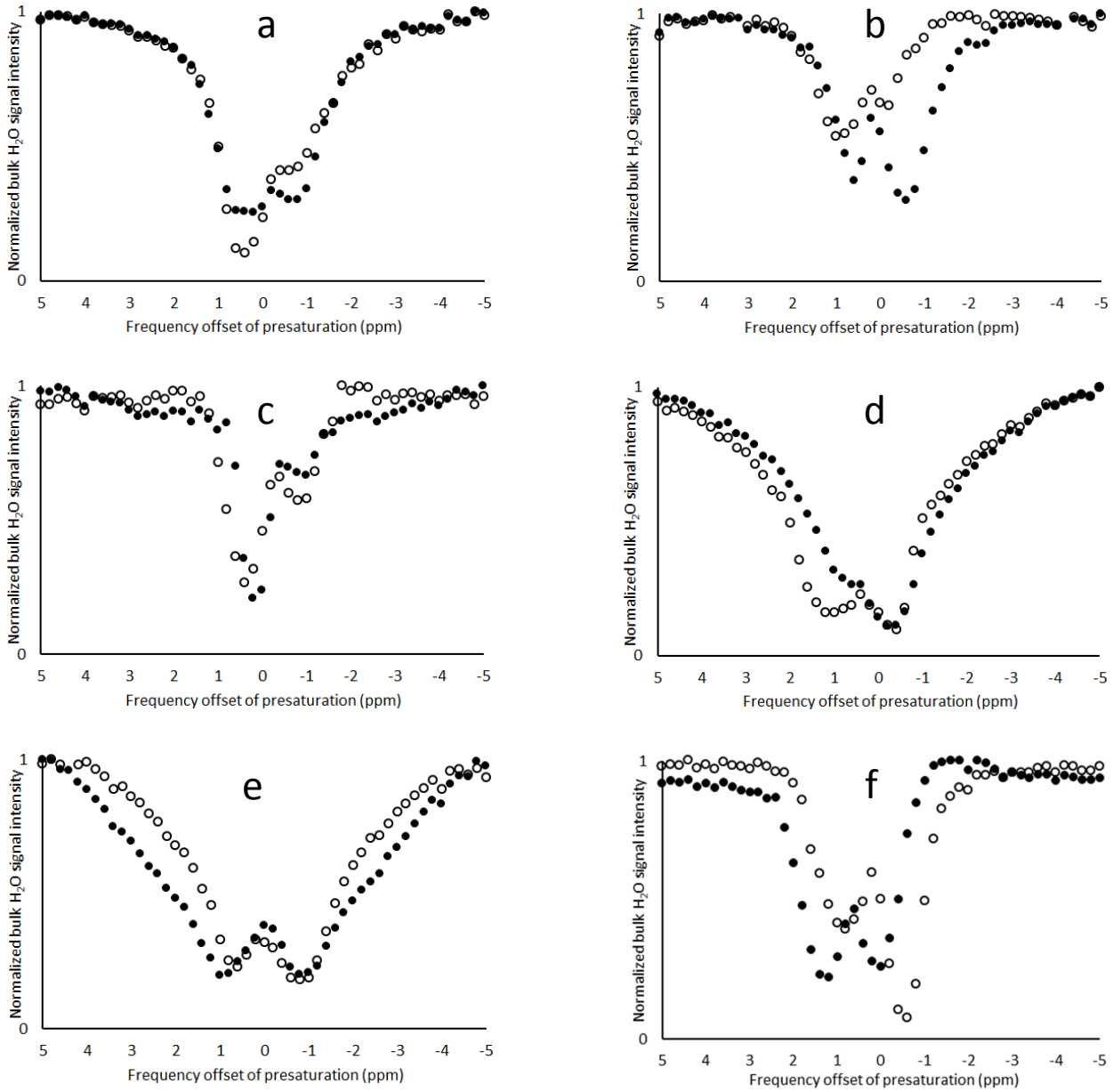


Figure S1. CEST spectra (7 T, ambient temperature) of raw image intensity data before (hollow circles) and after (solid circles) air exposure of (a) blank liposomes, (b) liposomes containing 28 mM $Sr(2.2.2)^{2+}$, (c) liposomes containing 13 mM $Eu(2.2.2)^{2+}$, (d) liposomes containing 24 mM $Eu(2.2.2)^{2+}$, (e) liposomes containing 40 mM $Eu(2.2.2)^{2+}$, and (f) liposomes containing 45 mM $Eu(2.2.2)^{2+}$.

Lorentzian Function Fitting

The raw CEST imaging data before and after air exposure was modeled with Mathematica 9.0 using a sum of two Lorentzian functions (**eq. 2**) optimized with least squares fitting.

eq 2. $y = \frac{a_1 k_1}{{k_1}^2+(x-x_1)^2} + \frac{a_2 k_2}{{k_2}^2+(x-x_2)^2}$

In **eq 2**, a_1, a_2, k_1, k_2, x_1, and x_2 are the fitting variables optimized with least squares to produce a Lorentzian function for imaging data. The CEST data for liposomes before and after air exposure were modeled with Mathematica commands in Figure S2. These commands included a calculation and plot of relative error per data point, which was plotted with the fitted function for blank liposomes (Figure S3).

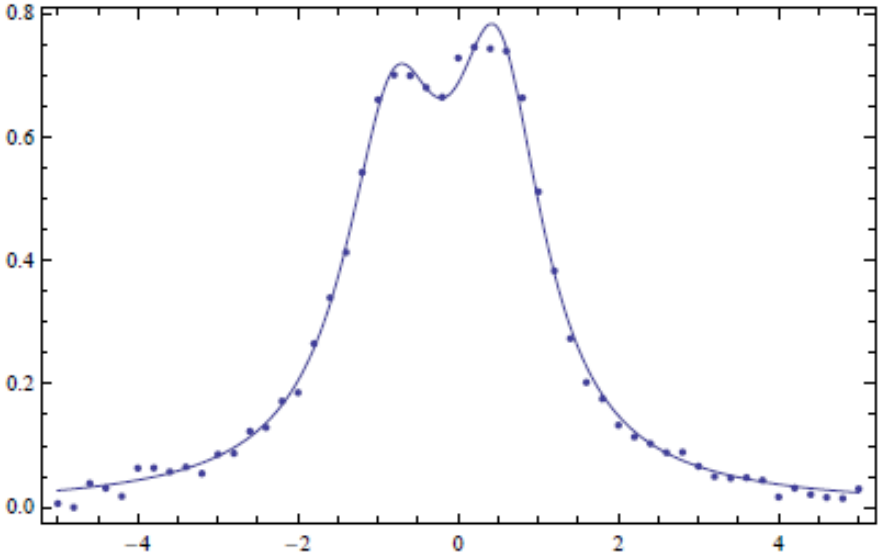
```
fitdata = tempdata;
fitfunc = a1 * k1 / ((x - x1) ^ 2 + k1 ^ 2) + a2 * k2 / ((x - x2) ^ 2 + k2 ^ 2)
sumdiff = Sum[
    ((fitfunc /. x → fitdata[[j, 1]]) - fitdata[[j, 2]]) ^ 2, {j, 1, Length[fitdata]}];
fitpara = NMinimize[{sumdiff, -2 < x1 < 1, -1 < x2 < 3}, {k1, k2, x1, x2, a1, a2}]
Show[Plot[fitfunc /. fitpara[[2]], {x, -5, 5}, PlotRange → All],
 ListPlot[tempdata], PlotRange → All, Frame → True, Axes → False]
diff = Table[{fitdata[[j, 1]],
     (fitdata[[j, 2]] - (fitfunc /. fitpara[[2]] /. x → fitdata[[j, 1]])) /
      (fitfunc /. fitpara[[2]] /. x → fitdata[[j, 1]])}, {j, 1, Length[fitdata]}];
ListPlot[diff, Frame → True, Axes → {True, False}, PlotRange → {-5, 5}]
```

Figure S2. Mathematica commands for Lorentzian curve fitting CEST imaging data and relative error per data point for blank liposomes.

a

```
{0.0112693, {k1 → 0.745301, k2 → -0.695292,
  x1 → -0.806634, x2 → 0.487788, a1 → 0.422894, a2 → -0.442135}}
```

b



c

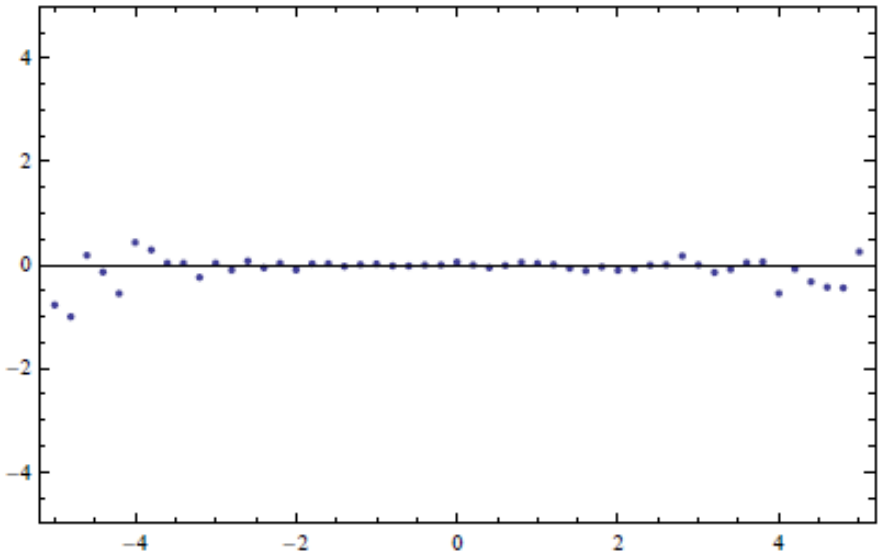


Figure S3. Mathematica output for blank liposomes of (a) fitted variables, (b) Lorentzian function plotted with raw CEST data, and (c) relative error calculated for each data point.

The Lorentzian function reported in the manuscript (Figure 3) was generated using the fitted variables defined in **eq 2**, but plotted in the form of **eq 3.**

eq 3. $$y = 1 - \left(\frac{a_1 k_1}{{k_1}^2 + (x - x_1)^2} + \frac{a_2 k_2}{{k_2}^2 + (x - x_2)^2}\right)$$

Dynamic Light Scattering Data

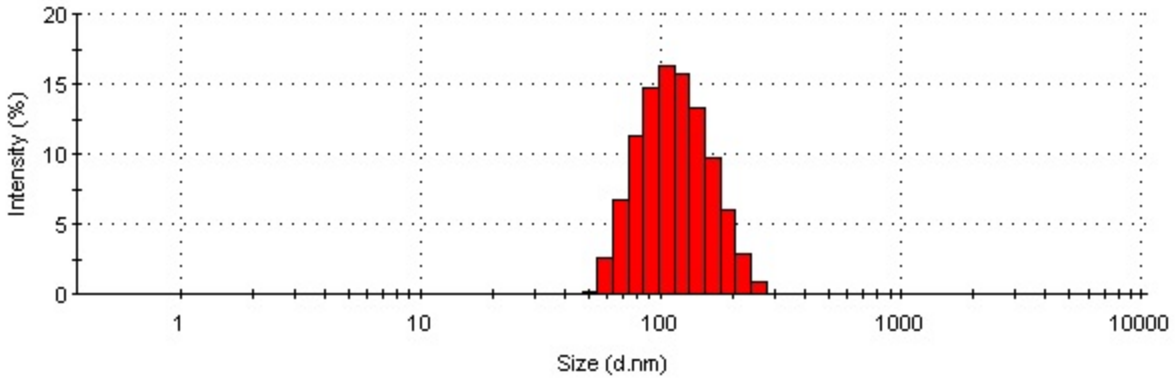


Figure S4. Intensity percent vs size of liposomes containing 45 mM Eu(2.2.2)$^{2+}$ used for in vitro CEST imaging prior to air exposure.

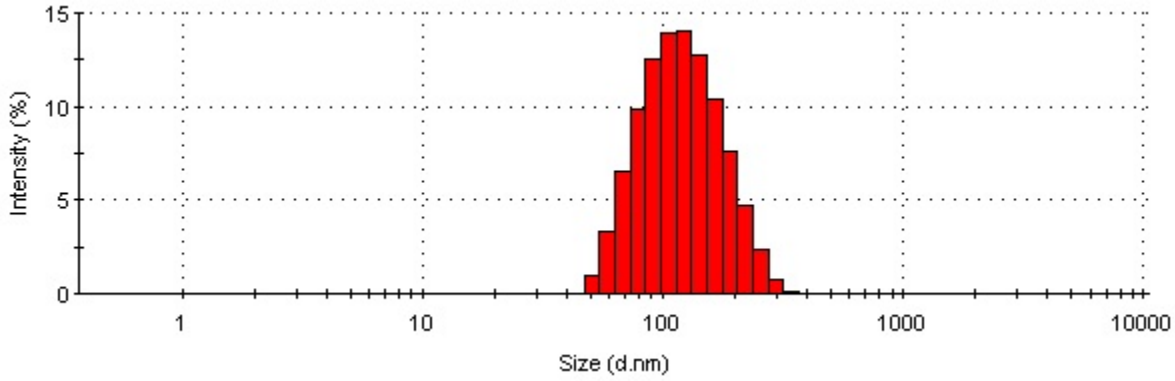


Figure S5. Intensity percent vs size of liposomes containing 45 mM Eu(2.2.2)$^{2+}$ used for in vitro CEST imaging after 24 h of air exposure.

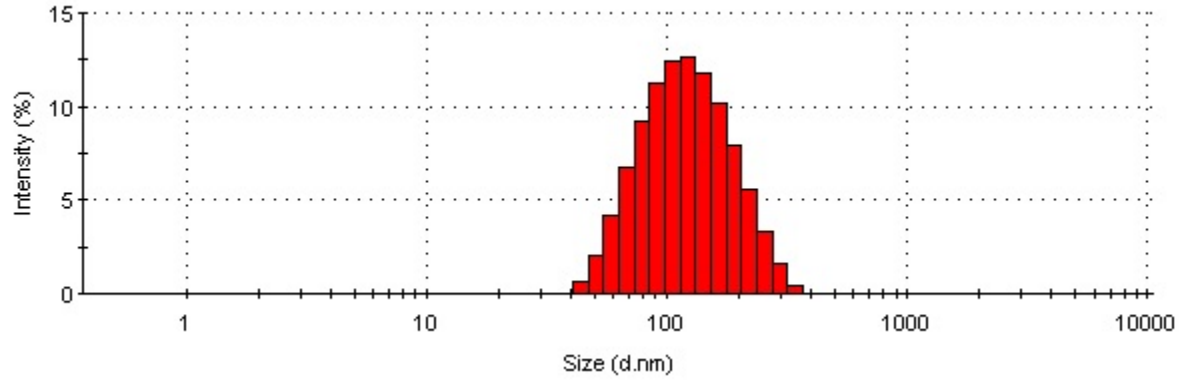


Figure S6. Intensity percent vs size of liposomes containing 45 mM Eu(2.2.2)$^{2+}$ used for in vitro T_1-weighted imaging prior to air exposure.

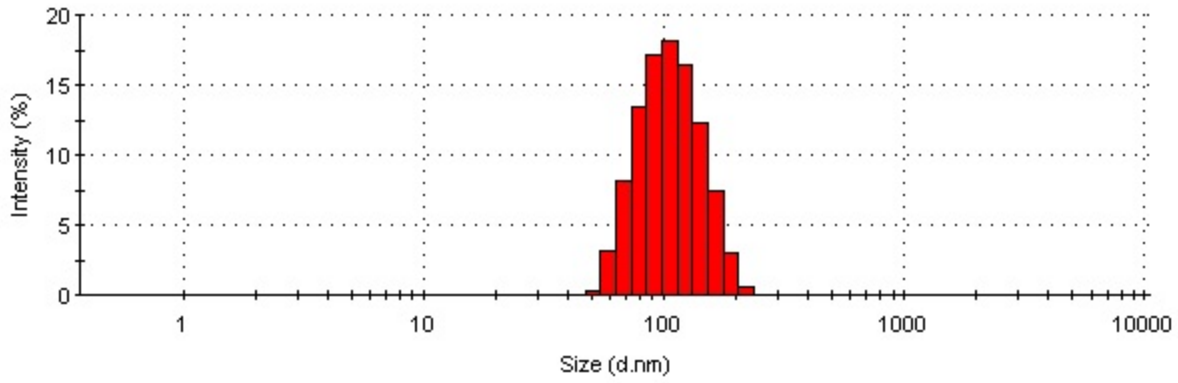


Figure S7. Intensity percent vs size of liposomes containing 45 mM $Eu(2.2.2)^{2+}$ used for in vitro T_1-weighted imaging after 24 h of air exposure.

References

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