A Temperature-Sensitive Liposomal ^{1}H CEST and ^{19}F Contrast Agent for MR Image-Guided Drug Delivery

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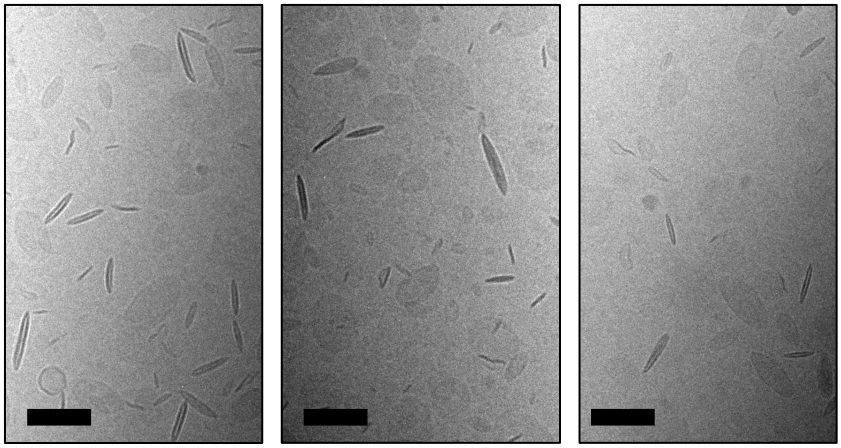

Figure S1. Cryo-TEM images of non-spherical liposomes (MPPC/DPPC/DPPE-PEG2000, 10:90:4) containing NH_4PF_6 and [Tm(hpdo3a)(H_2O)] (magnification: 7100:1). The scale bar represents 200 nm.

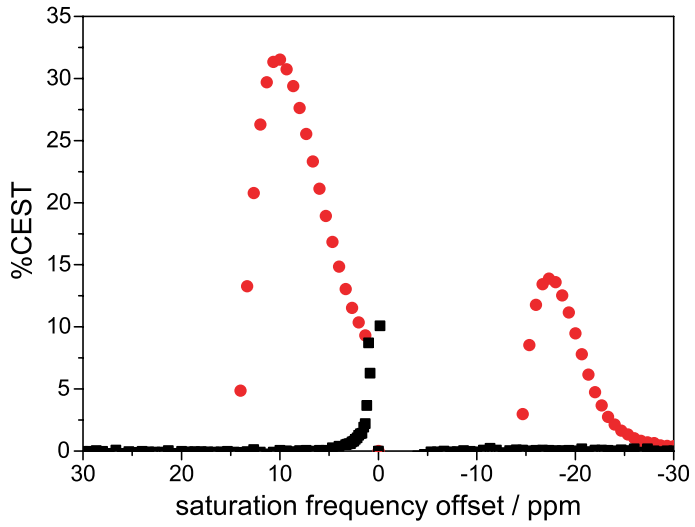

Figure S2. The CEST effect of liposomes (MPPC/DPPC/DPPE-PEG2000, 10:90:4) containing NH_4PF_6 and [Tm(hpdo3a)(H_2O)] at 7 T and 298 K (red dots) and 315 K (black squares). The CEST effect was calculated according to %CEST = $(M_{-S} - M_S) / M_{-S} \times 100\%$, in which M_S is the magnitude of the bulk water signal during saturation on resonance, and M_{-S} is the intensity of the bulk water signal at the opposite saturation frequency offset.

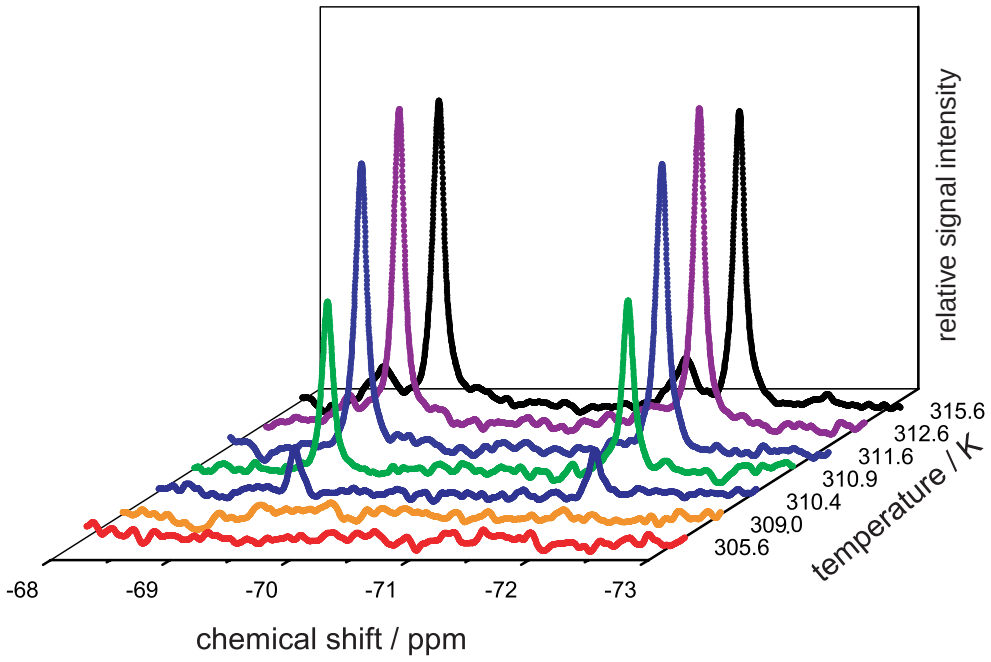

Figure S3. The ^{19}F NMR spectrum of liposomes (MPPC/DPPC/DPPE-PEG2000, 10:90:4) containing NH_4PF_6 and [Tm(hpdo3a)(H_2O)] at different temperatures at 7 T.

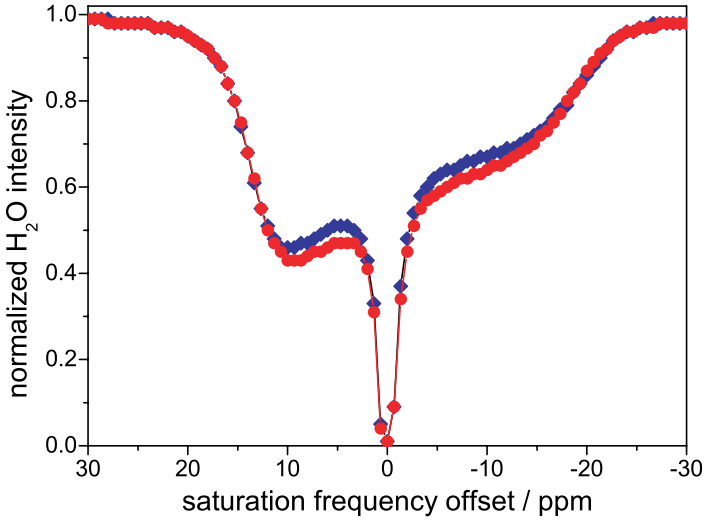

Figure S4. The Z-spectra (B_1 = 4.5 μT) of two independently prepared liposome solutions (MPPC/DPPC/DPPE-PEG2000, 10:90:4) containing [Tm(hpdo3a)(H_2O)] and NH_4PF_6 at 298 K (B_0 = 7 T). The red data points are as reported in Figures 1 and S2. The blue data points correspond to a control experiment performed using the same protocol.

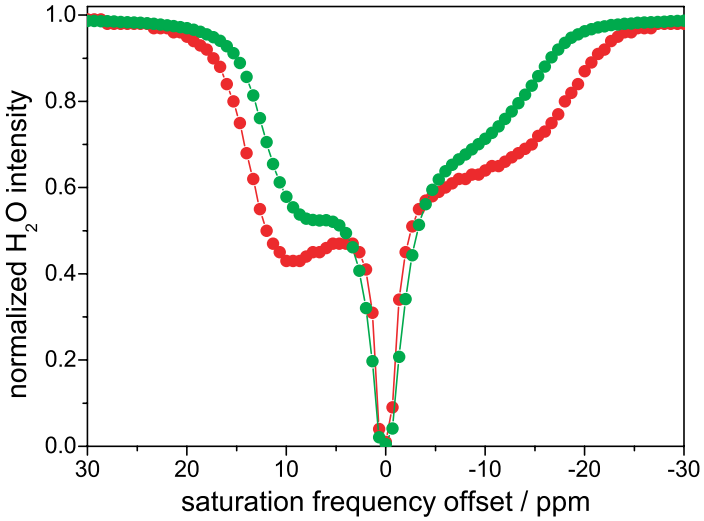

Figure S5. The Z-spectra ($B_1 = 4.5$ µT) of liposome solutions (MPPC/DPPC/DPPE-PEG2000, 10:90:4) containing [Tm(hpdo3a)(H_2O)] and NH_4PF_6 (red dots) and of liposome solutions containing only [Tm(hpdo3a)(H_2O)] (green dots) at 298 K ($B_0 = 7$ T). The red data points are as reported in Figures 1 and S2.

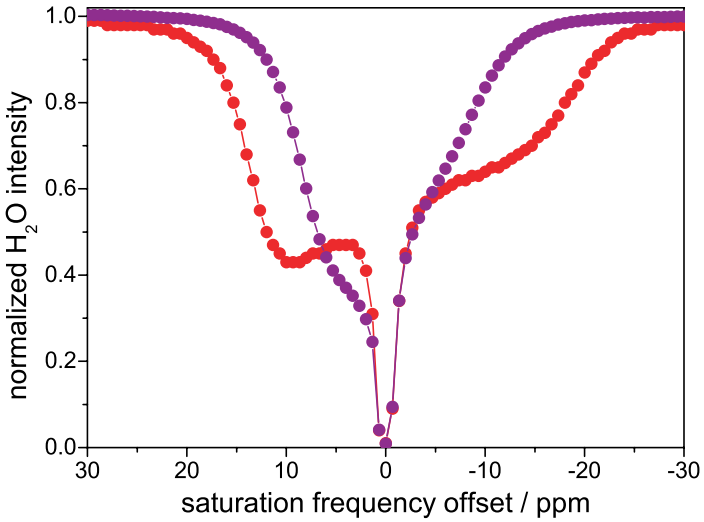

Figure S6. The Z-spectra ($B_1 = 4.5$ µT) of temperature-sensitive liposomes containing [Tm(hpdo3a)(H_2O)] and NH_4PF_6 at 298 K ($B_0 = 7$ T). The red data points are liposomes (MPPC/DPPC/DPPE-PEG2000, 10:90:4) as reported in Figures 1 and S2. The purple data points correspond to thermo-sensitive liposomes (HSPC/DPPC/DPPE-PEG2000/cholesterol, 50:100:6:30) with a higher melting temperature of the lipid membrane ($T_m = 314$ K).

Preparation of the liposomal contrast agent

DPPC (75.3 mg, 103 µmol), MPPC (5.68 mg, 11.5 µmol), and DPPE-PEG2000 (12.5 mg, 4.6 µmol) were dissolved in chloroform/EtOH (4:1 *v/v,* 12 mL). The solvent was evaporated under reduced pressure at T = 50ºC. The obtained lipid film was hydrated at 60°C using a solution of [Tm(hpdo3a)(H_2O)] (115 mg, 195 µmol) and NH_4PF_6 (24.5 mg, 150 µmol) in 3 mL HEPES buffer (20 mM, pH 7.4). The obtained vesicles were successively extruded through a polycarbonate filter with pore sizes of 400 nm (3 times), 200 nm (5 times), and 100 nm (5 times). The extrusion of the liposomes was performed under a nitrogen pressure in a LIPEX thermo-barrel extruder at 60ºC. The liposomes were dialyzed (Spectra/Por 7 membrane, MWCO 50 kDa) against a hypertonic HEPES buffer (20 mM HEPES, 300 mM NaCl, pH 7.40). The average hydrodynamic diameter of the liposomes was 118 nm with a polydispersity index PDI ≈ 0.15 as determined by dynamic light scattering ($\alpha = 90°$). The concentration of Tm for the dialyzed sample was 3.5 ± 0.2 mM as determined by inductively coupled plasma – atomic emission spectrometry (ICP-AES).

NMR experiments

NMR data were recorded on a Bruker Avance NMR spectrometer equipped with an Oxford wide-bore 7 T superconducting magnet. Samples consisted of 450 µL of the liposomal contrast agent in 5 mm NMR tubes containing a coaxial glass capillary insert filled with deuterated tetrachloroethane for the purpose of frequency-locking. The ^{19}F-NMR spectra were acquired at 282 MHz using 128k complex data points and a dwell time of 5 µs. Prior to Fourier transform, the raw data were multiplied with an exponential window function (2 Hz line broadening). For the CEST measurements, a series of 64 to 128 smoothed CHIRP [J. J. Dunand, J. Delayre (1976) US Patent 3975675] pulses (60 ms duration; 4.5 µs) interleaved with magnetic-field gradient pulses (500 µs; 41 mT/m) was used for frequency-selective saturation ending 500 µs before reading out the bulk water MR signal. For the frequency-dependent CEST data in Figure 1 (Z-spectrum) and Figure S2 (CEST spectrum), the bulk water MR signal was measured for 103 different values of the presaturation frequency offset (0, +200 000, -200 000, +200, -200, +400, -400, …, +10 000, and –10 000 Hz with respect to the bulk water frequency). The CEST spectrum was calculated from the Z-spectrum using the following equation: $\%CEST = (M_{-S} - M_S) / M_{-S} \times 100\%$, in which M_S is the magnitude of the bulk water signal during saturation on resonance, and M_{-S} is the intensity of the bulk water signal at the opposite frequency offset. For the temperature-dependent CEST data in Figure 2, the bulk water MR signal was measured 256 times during a period of 39.3 minutes in which the temperature was raised from 298 to 337 K at a constant rate of 1.0 K/min. After each measurement, the saturation frequency offset of the selective saturation pulse train was switched from +3200 Hz (the intraliposomal water frequency, M_S) to -3200 Hz (M_{-S}) or *vice versa*. In this way the CEST effect (corrected for nonselective saturation) could be measured at a rate of 3.3 min^{-1}.