Supporting Information

Release Rates of Liposomal Contents are Controlled by Kosmotropes and Chaotropes

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Synthetic Materials and General Methods. The Q-DOPE was prepared as previously described.[1] Large unilamellar vesicles (LUVs) were made using a modified protocol as described by Hope *et al*.[2] Dry lipid films of Q-DOPE were hydrated at room temperature with 0.050 M phosphate buffer possessing Hofmeister salt concentrations of 0.075 M or 0.500 M. Hydrated Q-DOPE suspensions were subjected to six freeze-thaw cycles, followed by 19 cycles of extrusion through a 100-nm pore Whatman Nuclepore polycarbonate track-etched membrane using a hand-held Mini-Extruder (Avanti Polar Lipids, Alabaster, AL). The Q-DOPE LUVs were roughly 120 nm in diameter, as judged by light scattering.[1] Liposome concentrations were adjusted to 1×10^{-4} M by dilution, with the concentrations being determined from UV-vis absorbance measurements using $\varepsilon_{265\ nm} = 5500\ M^{-1}\ cm^{-1}$ for the quinone head group. 4.0×10^{-2} M calcein was encapsulated within the liposomes, and the non-encapsulated excess removed by column chromatography using Sephadex G-75. Fluorescence release traces were measured at 25 °C under argon. Reduction of the trimethyl-locked quinone head group was achieved by the addition of various molar ratios of $Na_2S_2O_4$ in 0.050 M phosphate buffer. Differential scanning

calorimetry measurements were performed on 1.88×10^{-2} M DOPE using pH 7.40, 0.050 M phosphate buffer, with the appropriate concentration of added salt. DOPE samples were prepared below the reported T_H of 10 °C in pure water and subjected to six freeze/thaw cycles using liquid nitrogen and a cold-water bath at 1 °C. The DOPE suspensions were maintained below 10 °C and loaded into the calorimeter at 1.5 °C. DSC experiments were performed on a VP-DSC (Microcal, Piscataway, NJ) with a scan rate of 40 °C h^{-1}.

Static light scattering experiments of reduced ~120-nm diameter Q-DOPE liposomes were performed using an LS55 fluorescence spectrometer from Perkin Elmer equipped to record scattered light at an angle of 90°, as described in the literature.[3] A xenon discharge lamp was used as the light source. The incident light was 600 nm (slit width 10 nm); the scattered light was recorded at 610 nm (slit width 10 nm). Measurements were performed in 0.050 M phosphate buffer with either 0.075 M KSCN or KCl, pH 7.40. Lipid concentration was 1×10^{-4} M, and the liposomes did not contain calcein. Q-DOPE liposomes that had not been reduced did not show any significant change in light scattering for periods up to a day (the maximum measured).

Zeta potentials of ~100-nm diameter Q-DOPE liposomes that had been reduced were measured using a Zetasizer Nano from Malvern. Liposomes were prepared as described above but did not contain calcein. Liposomes were anaerobically reduced by their incubation with a 5-fold molar excess of $Na_2S_2O_4$, at which point they were transferred to the measurement cell of the Zetasizer. Experiments were carried out in 0.050 M phosphate buffer containing either 0.075 M KSCN or KCl, pH 7.40; the lipid concentration was 1×10^{-4} M. For Q-DOPE liposomes that had not been reduced, there was no observed change of zeta potential for periods up to 3 h (the maximum measured).

Lamellar to Inverted Hexagonal Phase Transition Upon Dithionite Reduction of Q-DOPE as Judged by ^{31}P NMR Spectroscopy. Giant unilamellar vesicles, GUVs, were prepared by a modified procedure described by Hub *et al.*[4] Briefly, 25 mg of lipid were dissolved in 10 mL of $CHCl_3$ which was evaporated into a homogenous lipid film inside of a round bottom flask using a rotary evaporator. The resulting film was placed under high vacuum for 1 h. The lipid was hydrated with 500 mL of 0.140 M sucrose and 0.010 M TES (*N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid) buffer, pH 7.40 without agitation for ~6 h in a water bath (70 ºC) under N_2. The solution was centrifuged at 1000 x g, and the supernatant was decanted. The resulting pellet consisting of the lipid GUVs was then resuspended in a minimal amount (~200 μL) of 0.075 M KCl and 0.010 M TES buffer pD 7.40 prepared with D_2O and transferred into a Shigemi 5-mm advanced NMR microtube matched for D_2O. The GUV-containing solution was visually inspected using a wide-field optical microscope from Nikon (Diaphot 300) with a 100x oil immersion objective. The resulting diameter of the Q-DOPE vesicles ranged from 1−20 μm. As noted in Figure S-1, the ^{31}P NMR spectrum of Q-DOPE GUVs prior to reduction (A) has the characteristics expected for a lamellar liquid crystalline phase (L_α), as noted by the intense upfield peak and less intense downfield shoulder.[5] The line shape of Q-DOPE after reduction (B) is consistent with lipids in the inverted hexagonal micelle phase (H_{II}).[5] The superimposed isotropic peak centered at roughly 1 ppm is due to rapid tumbling and diffusion of small lipid particles <<1 μm in diameter that evidently result from the reduction process.

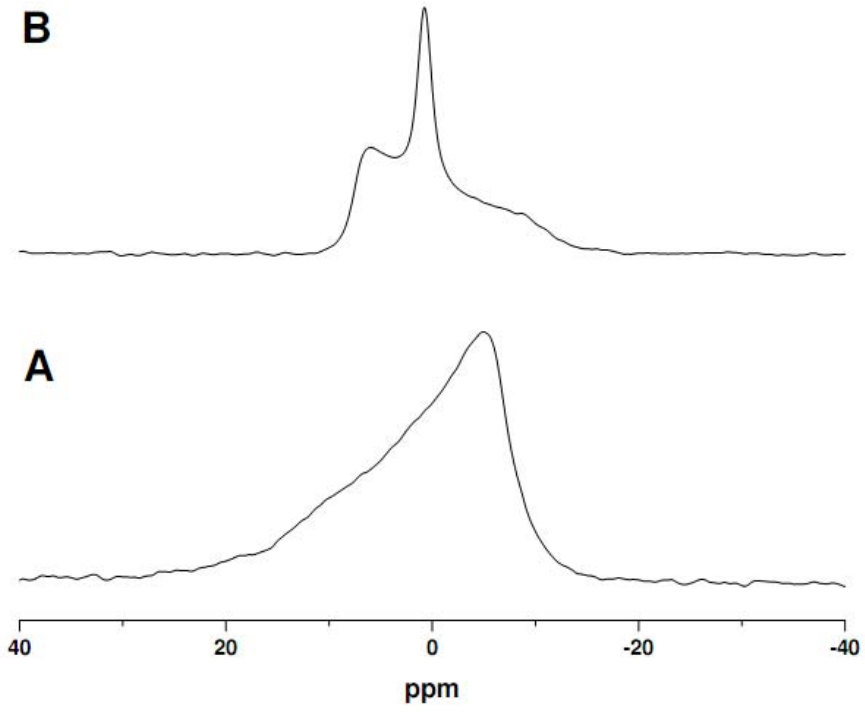

Figure S-1. ^{31}P NMR spectra of ~0.100 M Q-DOPE giant unilamellar vesicles, GUVs, in pD 7.40, 0.075 M KCl and 0.010 M TES buffer (D_2O) before (A) and after (B) the addition of dithionite (2:1 mol/mol). Both spectra were generated from 40960 transients of a proton-decoupled spin echo pulse sequence on a Bruker DPX-400 spectrometer operating at 161.975 MHz.

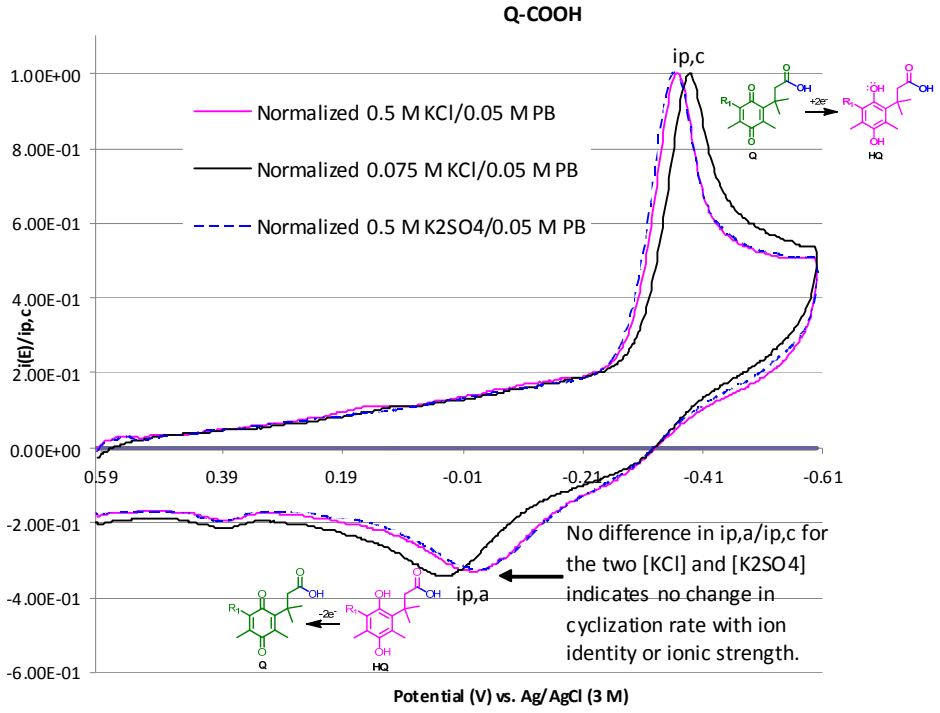

Figure S-2. Cyclic voltammetry of quinone propionic acid (Q-COOH; $R_1 = CH_3$) in KCl and K_2SO_4 showing no change in cyclization rate (cyclized lactone formation) with anion identity or concentration. A glassy carbon working electrode was used to examine ~1×10^{-3} M solutions of the Q-DOPE cleavable head group, Q-COOH, in pH 7.10, 0.50 M phosphate buffer.

Cyclizative Removal of Reduced Q Head Group is Unchanged in Presence of Different Anions and Anion Concentration. The influence of anion identity and concentration on the cyclization reaction that leads to production of DOPE in the outer leaflet of the Q-DOPE liposomes was examined by measuring the peak current ratio associated with the 2-electron reduction of the quinone propionic acid in pH 7.10 aqueous solutions at a glassy carbon electrode.[1] If anion identity or changes in anion concentration were to accelerate the cyclization

reaction, the ratio of the anodic peak current ($i_{p,a}$ - oxidation of the hydroquinone to the quinone) to that of the cathodic peak current ($i_{p,c}$ - reduction of quinone to hydroquinone) should be less, due to the nature of the electrochemical-chemical (EC) reaction;[6] this is not found for changing the concentration of Cl^- from 0.075 M to 0.500 M or changing the anion from Cl^- to SO_4^{2-}, Figure S-2. Thus, there is no significant influence of anion identity or its concentration on the cyclization reaction.

References Cited

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