Supplementary

Materials and methods

All chemical reagents for synthesis were of analytical grade, obtained from Sigma-Aldrich and used without further purification unless otherwise noted. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids. 5,6-Carboxyfluorescein was obtained from Molekula. Milli-Q™ water with a resistivity of 18 MΩ cm^{-1} was used in the preparation of all solutions. Tris buffer solution (20 mmol L^{-1}) was prepared with Tris-HCl adjusted to biological pH with NaOH and degassed under vacuum. Polycarbonate membranes and mini-extruder were obtained from Avanti Polar Lipids.

Microanalyses were performed at the Campbell Microanalytical Laboratory, University of Otago, NZ.

^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 MHz and 100 MHz, respectively on a Varian 400 MR spectrometer. The signals are reported in ppm relative to TMS as an internal standard and $CDCl_3$ as a solvent referenced to $CHCl_3$. All chemical shifts have an uncertainty of ± 0.1 ppm, with coupling constants (J) rounded to the nearest 0.1 Hz. Multiplicities are reported by the convention s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), qn (quintet), m (multiplet), and b (broad). Resonances were assigned according to the convention: chemical shift (number of protons, multiplicity, coupling constant(s)).

IR spectra were recorded on a Bruker Alpha-P diamond anvil spectrometer using OPUS 6.5 software using the notation s (strong), b (broad), m (medium), and w (weak).

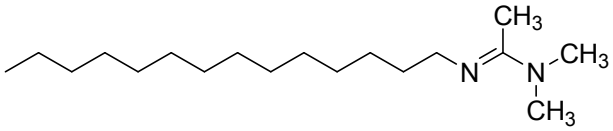
Melting points were recorded on a Mettler Toledo FP62 Melting Block to an accuracy of ± 1 °C.

Fluorescence measurements were made on a Perkin-Elmer LS50B luminescence spectrometer.

Dynamic light scattering measurements were performed on a Malvern Zetasizer Nano ZS.

Synthesis

N'-tetradecyl-N,N-dimethylacetamidine (1)

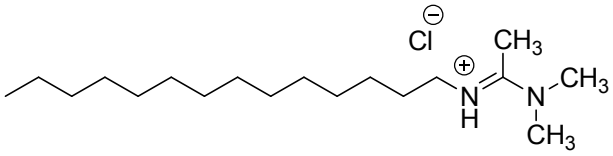


Molecular Weight: 282.51

N'-Tetradecyl-N,N-dimethylacetamidine (**1**) was synthesised using a method adapted from Harjani *et al.* (2011).[1] Tetradecylamine (1.0 g, 241.46 $gmol^{-1}$, 4.14 x 10^{-3} mol) was dissolved in THF. To this N,N-dimethylacetamide dimethyl acetal (0.55 g, 133.19 $gmol^{-1}$) was added dropwise over 5 min, stirred for 10 min and then left for 18 h. The solvent and methanol byproduct was removed *in vacuo*. To remove the imidate ester impurity the crude oil was mixed with ether, two drops of water and CO_2 was bubbled through the solution. The bicarbonate salt (**2.HCO_3**) was filtered, dissolved in water (50 mL) and basified with NaOH and extracted into ether (3 x 50 mL). The ether was removed *in vacuo* to give yellow oil in a 74.6 % yield. This was then stored in the freezer until further used.

^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 7.0 Hz), 1.25-1.29 (m, 22H), 1.49 (qn, 2H, *J* = 7.3, 7.2 Hz), 1.87 (s, 3H), 2.87 (s, 6H), 3.17 (t, 2H, *J* = 7.4). ^{13}C NMR ($CDCl_3$) δ 12.51, 14.22, 22.81, 27.72, 29.49, 29.7-29.9, 32.05, 32.53, 38.13, 50.31, 158.83; IR (Diamond anvil) ν: 2954 (s), 2851 (s), 1627 (s), 1466 (m), 1341 (m) cm^{-1}; elemental analysis calculated for $C_{18}H_{36}N_2$ (282.58) C 76.53, H 13.56, N 9.92; found C 76.56, H 13.62, N 9.89

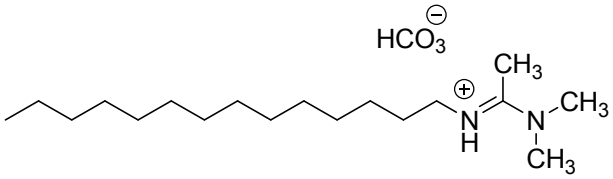
N'-tetradecyl-N,N-dimethylacetamidium chloride (2.Cl)



Molecular Weight: 318.97

HCl gas was generated by adding conc. HCl to magnesium sulfate, which was then bubbled through a solution of 0.5 g of crude compound **1** in 20 mL of diethyl ether. The white solid was recovered by filtration to give N'-tetradecyl-N,N-dimethylacetamidium chloride (**2.Cl**) in a 88% yield. ^{1}H NMR (D_2O) δ 0.90 (t, 3H, *J* = 6.9 Hz), 1.31-1.35 (m, 22H), 1.63 (qn, 2H, *J* = 5.4, 6.3 Hz), 2.32 (s, 3H), 3.22 (s, 6H), 3.43 (t, 3H, *J* = 7.5). ^{13}C NMR 14.27, 14.99, 22.84, 26.86, 29.39, 29.50, 29.67, 29.71, 29.77, 29.80, 29.83, 30.50, 32.07, 45.36, 163.38. IR (Diamond Anvil) ν: 3457 (m), 3427 (m), 3170 (w), 2946 (s), 2847 (s), 1638 (s) cm^{-1}. Elemental analysis calculated for $C_{18}H_{39}N_2Cl.H_2O$ (337.06) C 64.16, H 12.26, N 8.31, Cl 10.52; found C 64.30, H 12.39, N 8.38, Cl 10.42. M.P. 126 – 128 °C.

N'-tetradecyl-N,N-dimethylacetamidinium bicarbonate (2.HCO_3)



Molecular Weight: 344.53

Crude compound **1** was dissolved in ether with two drops of water and CO_2 was bubbled through the solution until a precipitate formed (≈ 10 min). The precipitate was filtered to give **2.HCO_3** in 81% yield. ^{1}H NMR (D_2O) δ 0.90 (t, 3H, *J* = 6.9 Hz), 1.31-1.35 (m, 22H), 1.63 (qn, 2H, *J* = 5.4, 6.3 Hz), 2.32 (s, 3H), 3.22 (s, 6H), 3.43 (t, 3H, *J* = 7.5). ^{13}C NMR 14.27, 14.99, 22.84, 26.86, 29.39, 29.50, 29.67, 29.71, 29.77, 29.80, 29.83, 30.50, 32.07, 45.36, 163.38. IR (Diamond Anvil) ν: 3457 (m), 3427 (m), 3170 (w), 2946 (s), 2847 (s), 1638 (s) cm^{-1}. Elemental analysis calculated for $C_{19}H_{40}N_2O_3$ (344.61) C 66.24 H 11.70 N 8.13; Found C 66.29 H 11.74 N 8.24. M.P. 64 – 66 °C.

CMC determination and extent of counterion binding

The CMCs of the carbonate and chloride salts of **2** was determined conductometrically with treatment of the data by the sigmoidal Boltzmann method.[2] Conductivity measurements were obtained, in triplicate, using a Suntex SC-170 conductivity meter (referenced to 0.100 mol L^{-1} KCl), a water-bath and a jacketed beaker thermostated at 25 °C. The initial solutions were prepared at a concentration higher than the CMC (7 mM) in 5 mL of Milli-Q™ water. Stepwise dilution of the initial

QNS solutions was undertaken by the addition of water, to achieve data points sufficient to give two linear plots whose intercept approximates the CMC (Figure 1).

The rate of change of the conductivity (*dK*) was divided by the rate of change of the concentration (*dC*) and plotted against concentration to give a sigmoidal Boltzmann distribution. A line of best fit was generated through the data using Origin Pro (Version 8.5 (2010)) with the inflection point (X_o) indicating the greatest rate of change of the conductivity and, hence the CMC (2.83 ± 0.05 mmol L^{-1} (**2.Cl**), 3.53 ± 0.13 mmol L^{-1} (**2.HCO_3**).

The degree of the counterion dissociation of the micelles (α) is assumed to be the ratio between the slopes of the postmicellar region (A_2) to that of the premicellar region (A_1) (α = A_2/A_1). The α was determined to be 0.39 ± 0.02 and 0.57 ± 0.04 for **2.Cl** and **2.HCO_3**, respectively.

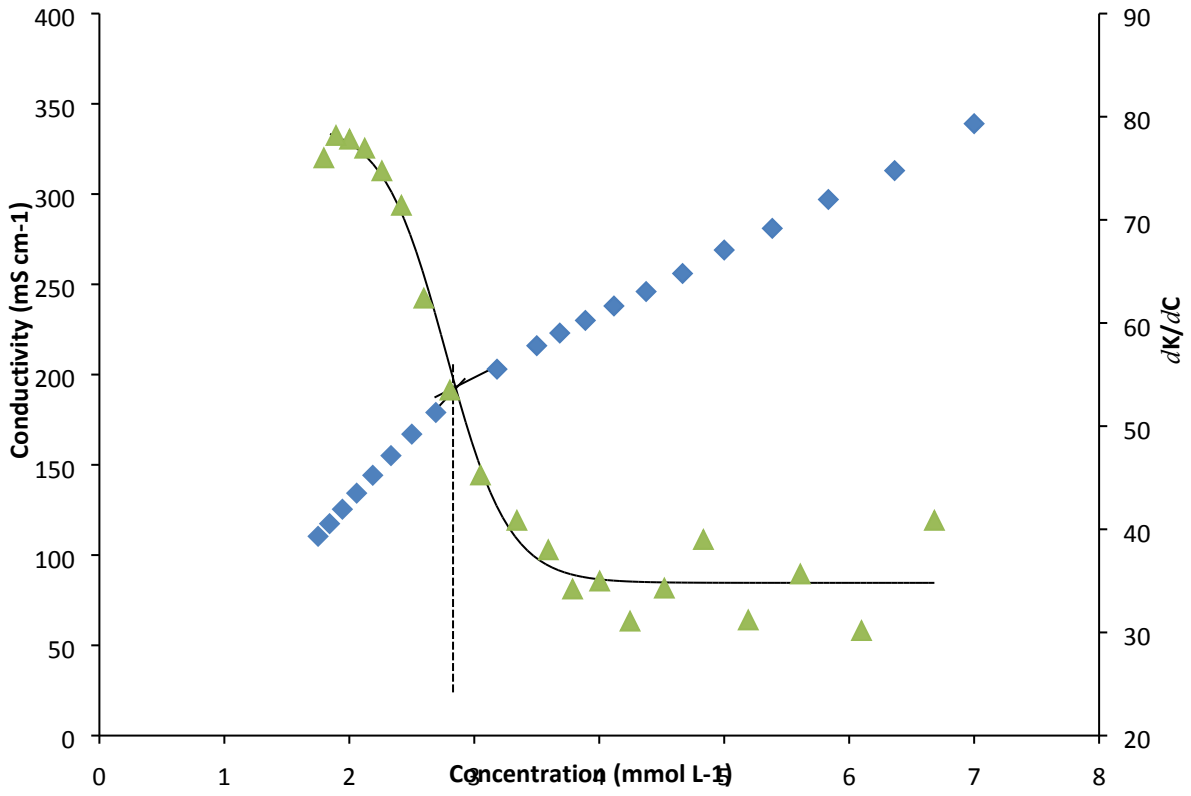


Figure 1. Conductivity versus concentration plot for **2.Cl** (diamonds) with the intercept of the two linear trends indicating the CMC. The *d*K/*d*C vs concentration plot (triangles) gave a sigmoidal Boltzmann distribution with the black line indicating best fit. The midpoint of the sigmoid (X_0) indicates the CMC.

General Procedure to Formulate Liposomes

Liposomes incorporating prosurfactant were prepared by adding chloroform solutions of DPPC (16 mg mL^{-1}, 276 µL), cholesterol (2 mg mL^{-1}, 58 µL) and the desired quantity of prosurfactant/additive (12 mmol L^{-1}). The solvent was removed *in vacuo* over one hour to produce a dry thin lipid film on the surface of round bottom flask. This was rehydrated by adding 1 ml of CF solution (100 mmol L^{-1}, pH 12.4, Tris buffer 20 mmol L^{-1}) at the required concentration. This resulted in a final DPPC

concentration of 6 mmol L^{-1} with 5 % cholesterol. To ensure complete rehydration, whilst avoiding saponification, solutions was heated for short periods (>1 min) to between 45 and 50 °C, above the gel to liquid crystal phase transition of DPPC (41 °C) and vortexed until absence of the lipid film on the glass was observed. To obtain a unilamellar liposome solution of defined size and high homogeneity, the solution was first extruded 11 times through an Avanti® Mini-Extruder, heated to 50 °C, fitted with a 1000 nm polycarbonate membrane, and subsequently a further 11 times through a 400 nm membrane.

The unentrapped solute was removed by size exclusion chromatography using Sephadex G-100 gel. The liposome solution was collected and the phospholipid concentration was determined using the Stewart Assay[3] whereupon it was adjusted to a concentration of 1 mmol L^{-1}. To confirm that liposomes were formed with minimal polydispersity and at the correct diameter, the suspension was measured using dynamic light scattering (DLS). To ensure consistent results the final suspension was then subjected to any further measurements within four hours of being collected.

General Procedure to Record CF Release

Fluorescence measurements were performed at an emission of 465 nm, an excitation of 520 nm, slit widths of 2.5 nm and an integration time of 0.5 s. The fluorescence intensity measurements were recorded in quartz fluorescence cuvettes at a scattering angle of 90°. The quartz cuvette was filled with 1.9 mL of buffered solution consisting of the desired property to induce switching. To this was added 100 µL of a 1 mmol L^{-1} CF loaded liposome suspension to give a final lipid concentration of 0.05 mmo L^{-1}. The resulting solution was thoroughly mixed for 1 second by aspirating and expelling with a pipette. The fluorescence was recorded over 250 s, after which the total fluorescence was determined by adding 100 µL of a 10% T X-100 solution, allowing equilibration for 50 s, and then recording the resulting fluorescence for 20 s. The raw fluorescence data was first converted to CF concentration using the appropriate standard curve (for pH 7.4 and 12.4) then converted to % CF release using the following equation;

$$\% \text{ CF release} = ((C_t - C_0)/(C_{t\,x\text{-}100} - C_0)) \times 100$$

where, C_0 and C_t are the CF concentrations initially and at time t respectively, whereas $C_{T\,X\text{-}100}$ is the average fluorescence over 20 s after the addition of T X-100. All samples were measured in triplicate and the standard error was calculated at final release which was recorded as an average of the last five measurements in order to increase the signal to noise ratio of the data.

CF Release from Liposomes with Variation of pH

To measure the CF release profile from liposomes acidified to give a final pH of 7.4, 100uL of the pH 12.4 stock liposomes were added to 1.9 mL of Tris buffered water at pH 7.3. To determine the CF release with varying pH, the stock liposome solution was added to unbuffered milli-Q™ water at different pHs to give a final pH of 7.6, 8.0, 8.5 and 12.4, respectively. CF release over 250 s was determined as described above.

Reversible Switching Experiment

The initial switch from pH 12.4 to 7.4 was undertaken as described as above except the CF release was measured until equilibration was achieved (400 s). To switch from pH 7.4 back to 12.4, a volume

of 1 M NaOH was added (~75 μL) and the resultant CF release was determined over 200 s. To switch back to pH 7.4, a volume of 1 M HCl was added (~50 μL) and CF release was measured over 200 s.

CO_2 Induced pH Switching of CF Encapsulated Liposomes

CO_2 switching was performed by bubbling CO_2 through 6 mL suspensions of liposomes (0.05 mM) incorporating either 0% or 10% **1** for around 1 minute at a flow rate of 0.5 L min^{-1}. The pH was monitored and the flow of CO_2 was stopped when the pH reached 7.4. The suspensions were allowed to equilibrate for 250 s after which the amount of CF released was determined as outlined in the general procedure to record CF release.

Free Surfactant Induced Switching of CF Encapsulated Liposomes

To a suspension of CF encapsulated DPPC liposomes (0.05 mmol L^{-1}, 20 mmol L^{-1} Tris, pH 7.4), either 0, 50, 100 or 200 μL of **2.Cl** or **2.HCO_3** (0.1 mmol L^{-1}) was added to give respectively, 0, 5, 10 or 20% surfactant relative to DPPC with a final concentration of 2 mL. The percentage CF released was determined as described in the General Procedure to Record CF Release.

CF Release at pH 12.4

The effect of PS on the integrity of the liposome membrane was investigated. C_{14}DMA was incorporated into DPPC liposomes at 0, 5, 10 and 20% and 100 uL of the solution (pH 12.4) was diluted with 2.9 mL of phosphate buffer and the CF release was monitored over 150 seconds (Figure 2).

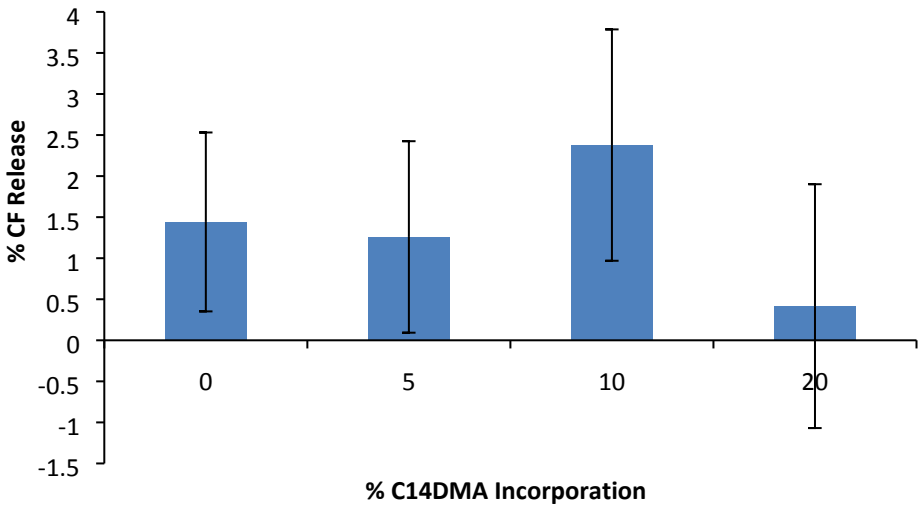


Figure 2. CF release after 150 seconds from 0, 5, 10 and 20% C_{14}DMA liposomes (100 uL, pH 12.4) diluted into pH 12.4 buffer (2.9 mL).

Dynamic light scattering

The hydrodynamic diameter (HD) of the liposome solutions were determined before fluorescent measurement using DLS. 2 ml of the 1 mmol L^{-1} DPPC liposome solution was placed in a plastic disposable sizing cuvette. Each sample was measured in triplicate at 25 ^{o}C, with an equilibration time of 120 s, at a backscatter angle of 173^{o}. The data was processed using Malvern Zetasizer software (Version 6.30 (2011)).

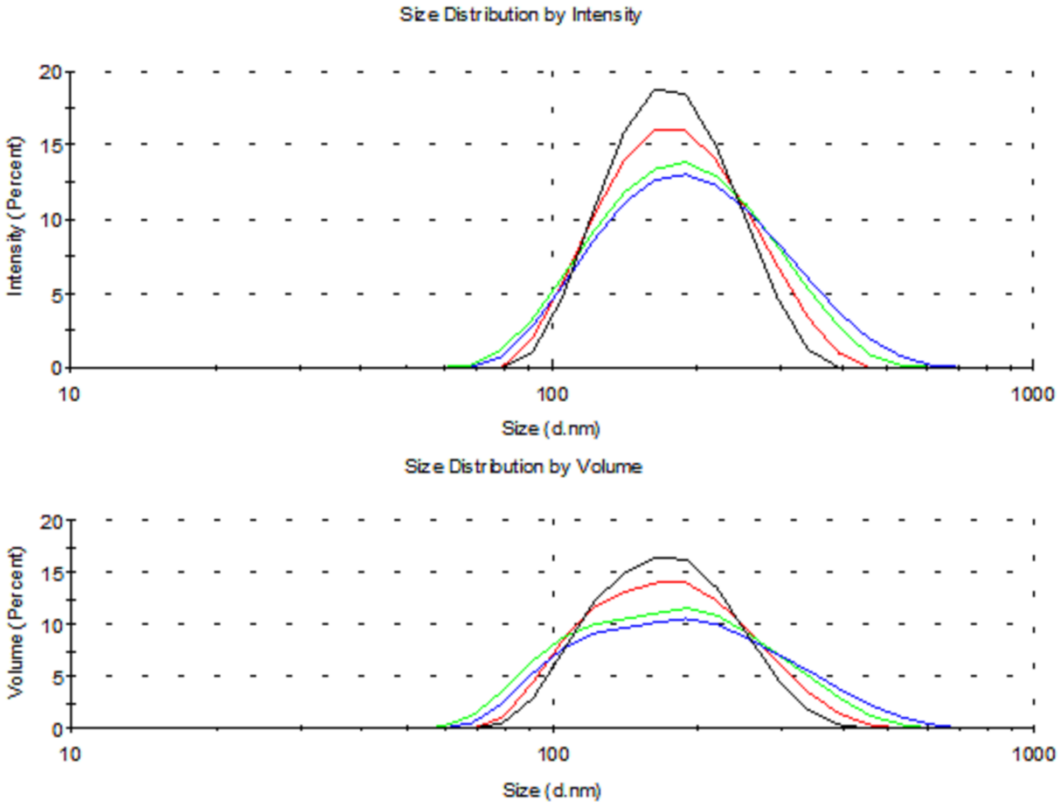


Figure 2. DLS measurements of DPPC liposomes incorporating 10% **1**, extruded by 200 nm polycarbonate membranes, before (pH 12.4, red) and after (pH 7.4, black) the addition of HCl and DLS measurements of DPPC liposomes extruded by 200 nm polycarbonate membranes, before (pH 12.4, green) and after (pH 7.4, blue) the addition of HCl.

The traces in Figure 2 show that the incorporation of **1** into the bilayer does not significantly alter the size of the liposomes formed. It also indicates that there is little difference in the liposome size after protonation of **1** by the addition of HCl.

References

1. J. R. Harjani, C. Liang and P. G. Jessop, *The Journal of Organic Chemistry*, 2011, **76**, 1683.
2. P. Carpena, J. Aguiar, P. Bernaola-Galván and C. Carnero Ruiz, *Langmuir*, 2002, **18**, 6054.
3. J. C. M. Stewart, *Analytical Biochemistry*, 1980, **104**, 10.