Membrane Composition Determines the Fate of Aggregated Vesicles

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Supplementary information

Lipid **1** was made using standard synthetic procedures. Poly-L-histidine hydrochloride (DP 39, MW 6,700 $mol^{-1}g$) was purchased from Sigma-Aldrich Co. Ltd and used as received.

Analytical data for Lipid 1.

(Found: C, 53.63; H, 4.96; N, 1.60. $C_{32}H_{37}NO_9.2(CF_3CO_2H)$ requires C, 53.53; H, 4.87; N, 1.73%); ν_{max}(Nujol)/cm^{-1} 1735 (C=O), 1676 (C=O), 1192 (C-N), 1138, 850, 714; δ_H(300 MHz, $CDCl_3$, 25°C) 2.15 (2H, m, $ArCH_2CH_2CH_2$), 2.46 (2H, t, ^3J (H,H)=5.8 Hz, $Ar(CH_2)_2CH_2CO_2$), 3.35 (2H, t, ^3J (H,H)=7.35 Hz, $ArCH_2CH_2$), 3.44-3.66 (16H, m, RCH_2O, NCH_2CH_2O), 4.23 (4H, s, NCH_2CO_2), 7.83 (1H, d, ^3J (H,H)=7.54 Hz, CH_{Ar}), 7.94-8.15 (7H, m, CH_{Ar}), 8.27 (1H, d, J=9.04 Hz, CH_{Ar}); δ_C(75 MHz, $CDCl_3$, 25°C): δ 27.1 ($CH_2CH_2CH_2$), 33.1 ($ArCH_2CH_2$), 34.1 ($CH_2CH_2CO_2$), 48.2 (NCH_2CH_2O), 52.6 (NCH_2CO_2), 63.7 (OCH_2), 69.7 (3 × OCH_2), 70.5 (3 × OCH_2), 123.7 (CH_{Ar}), 125.2 (2 × CH_{Ar}), 125.3 (C_{Ar} + CH_{Ar}), 126.3 (2 × CH_{Ar}), 127.1 (CH_{Ar}), 127.8 (CH_{Ar}), 128.9 (CH_{Ar}), 129.0 (C_{Ar}), 130.3 (C_{Ar}), 131.2 (C_{Ar}), 131.7 (C_{Ar}), 136.1 (2 × C_{Ar}), 169.9 ($NCH_2CO_2^-$), 173.8 ($RCO_2CH_2CH_2O$); *m/z* (ES) 580.2546 ($[M+H]^+$. $C_{32}H_{38}NO_9^+$ requires 580.2547), 602.4 ($[M+Na]^+$), 618.4 ($[M+K]^+$).

Preparation of Vesicles

Unilamellar vesicles were prepared by dissolving the appropriate phosphatidylcholine (16 mg, 20 μmol) and the required amount (1 μmol, 5 mol % of synthetic lipid relative to phospholipid) of synthetic lipid in spectroscopic grade ethanol-free chloroform (5 mL), followed by removal of the solvent to give a thin film of phospholipid on the interior of a round-bottomed flask. The appropriate buffer (20 mM MOPS, 100 mM NaCl, pH 7.4 at room temperature, 1 mL) was added to the flask, and the thin film detached by vortex mixing to give a suspension of multilamellar vesicles. These were extruded through a single 800 nm polycarbonate membrane at a temperature above the lipid T_m using an Avestin Liposofast extrusion apparatus to give unilamellar vesicles. The final concentration of phospholipid is 20 mM, that of the synthetic lipid 1 mM.

Changes in absorbance were monitored after each addition of reagent by taking multiple wavelength scans using a Cary 400 Scan UV spectrophotometer. The parent vesicle solution was diluted 1 in 10, added to a cuvette, and left to equilibrate for 5 min at 25°C or 60°C before any external reagents were added. All turbidity measurements were repeated at least once. Changes in fluorescence were monitored after each addition of reagent by taking full emission scan (excitation 346 nm) using a Perkin-Elmer LS55 luminescence spectrometer. The parent vesicle solution was diluted 1 in 1000, added to a fluorescence cell, and left to equilibrate for 5 min at 25°C before any external reagents were added. A Zeiss LSM 510 confocal fluorescence microscope and a Nikon Eclipse E600 fluorescence microscope were used to image vesicles and vesicle aggregates containing lipid **1**.

Analysis of the incorporation of Lipid 1 into DSPC vesicles

Fluorescence measurements

A solution of 5 % lipid **1** in DSPC vesicles (1 mM lipid, 0.8 μm diameter) was passed through a 0.2 μM disposable syringe tip filter (Gelman Sciences Acrodisc) with minimum pressure, to remove vesicles from the solution. The sample was diluted to 10.5 μL in 3.5 mL and the fluorescence spectrum recorded. The intensity of the fluorescence emission from lipid **1** (377 nm) prior to filtration was 954, after filtration 84. The same procedure was repeated for DSPC vesicles with a

total lipid concentration of 3 μM. The intensity of the fluorescence emission from lipid **1** (377 nm) prior to filtration was 817, after filtration 35. Thus the incorporation of lipid **1** into DSPC vesicles is between 90 and 95 %.

Differential Scanning Calorimetry (DSC) measurements

Vesicle suspensions of DSPC and 5 mol % lipid **1** in DSPC were prepared as described in the experimental details. Each suspension (50 mg) was introduced into a stainless steel pan and submitted to a cycle of heating and cooling from 10°C to 90°C at 5°C/min. On incorporation of lipid **1** into a suspension of DSPC vesicles, no fusion peak was observed for free lipid **1**, whilst the endotherm resulting from the DSPC gel to liquid phase transition underwent a small shift to 53°C from 55°C. The lack of an endotherm for lipid **1** and the small change in the DSPC endotherm supports incorporation of the lipid into the DSPC bilayers.

Supplementary fluorescence microscopy pictures

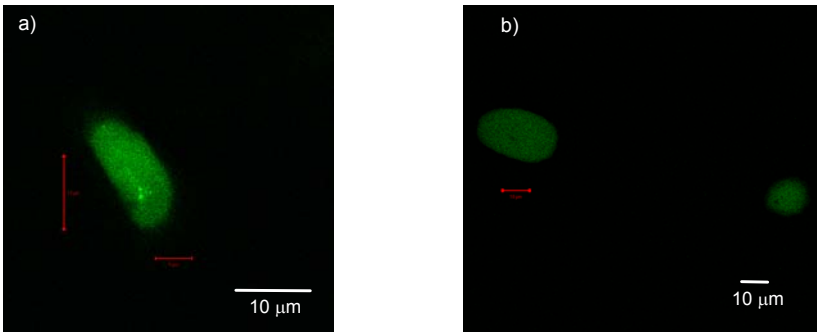


Figure S1. Confocal fluorescence micrographs of EYPC (2 mM) vesicles containing 5% lipid **1** (0.1 mM) at 25°C a) After the addition of copper(II) (1 eq.) b) After the addition of copper(II) (1 eq.) and polyhistidine (5 eq. histidine residues).

Fluorimetric data

μL 10 μM Cu^{2+} added	Intensity of fluorescence emission at 377 nm from lipid **1** in EYPC vesicles
0	936
2	915
4	878
6	859
8	807
10	712
12	667
14	642
16	612
18	578
20	546

Table S1. The change in the pyrene fluorescence emission at 377 nm caused by adding copper(II) (10 μM) to EYPC (2 μM lipid) vesicles containing 5% lipid **1** (0.1 μM) at 25°C.

μL 10 μM Cu^{2+} added	Intensity of fluorescence emission at 377 nm from lipid **1** in DSPC vesicles	Intensity of fluorescence emission at 377 nm from lipid **1** in DSPC vesicles with 1% wt/wt Triton X-100
0	751	822
2	526	589
4	442	380
6	388	289
8	353	262
10	309	247
12	270	229
14	248	224
16	225	219
18	208	219
20	187	211
25	173	198
30	156	193
35	144	188
40	135	183
50	126	172
60	118	171
70	116	164
80	114	167
90	111	165
100	107	157

Table S2. The change in the pyrene fluorescence emission at 377 nm caused by adding copper(II) (10 μM) to DSPC or DSPC with 1 wt % Triton X-100 (2 μM lipid) vesicles containing 5% lipid **1** (0.1 μM) at 25°C.

Time /min	Intensity of fluorescence emission at 377 nm from lipid **1** in DSPC vesicles
0	107
1	113
3	111
6	113
9	114
12	115
15	114
18	115
21	115
24	110
27	115

Table S3. Change in the pyrene fluorescence emission at 377 nm caused by adding 1 wt % Triton-X100 to DSPC (2 μM) vesicles containing 5% lipid **1** (0.1 μM) and copper(II) (5 eq, 0.5 μM) at 25°C.

Vesicle 5/6-carboxyfluorescein leakage assays

Unilamellar vesicles encapsulating 5/6-carboxyfluorescein were prepared by dissolving the appropriate phosphatidylcholine (16 mg, 20 μmol) and the required amount (1 μmol, 5% mol of synthetic lipid to phospholipid) of synthetic lipid in spectroscopic grade ethanol-free chloroform (5 mL), followed by removal of the solvent to give a thin film of phospholipid on the interior of a round-bottomed flask. The buffer containing 5/6-carboxyfluorescein (0.1 M 5/6 CF, 20 mM MOPS, 100 mM NaCl, pH 7.4 at room temperature, 1 mL) was added to the flask, and the thin film detached by vortex mixing to give a suspension of multilamellar vesicles. These were extruded through a single 800 nm polycarbonate membrane at a temperature above the lipid T_m using an Avestin Liposofast extrusion apparatus to give unilamellar vesicles. The final concentration of phospholipid is 20 mM, that of the synthetic lipid 1 mM. The parent solutions were diluted 1 in 10, then the appropriate combination of a) copper(II) solution (10 mM) or b) poly-L-histidine solution (0.5 mM, 20 mM in histidine residues) added.

	Lipid	Vesicles only	+ **2** (5 eq. His residues)	+ Cu^{2+} (1 eq)	+ Cu^{2+} (1 eq) + **2** (5 eq. His residues)	+ excess Triton-X100
Fluorescence intensity (% release)	EYPC	272 (31%)	170 (20%)	40 (5%)	728 (85%)	859 (100%)
	DSPC	58 (18%)	55 (17%)	56 (18%)	87 (28%)	315 (100%)

Table S4. Increase in the fluorescence at 540 nm at 25°C due to the release of encapsulated 5/6 CF from DSPC and EYPC vesicles (each 5 mol % lipid **1**), caused by addition of copper(II) and polyhistidine. The EYPC vesicles were analysed after 20 minutes and the DSPC vesicles after 3 days. The concentration of phospholipid in each case was 1×10^{-9} M.

Turbidimetric data

μL 10 mM Cu^{2+} added	Absorbance at 700 nm from DSPC vesicles at 25°C containing 5 mol % lipid **1**	Absorbance at 700 nm from EYPC vesicles at 25°C containing 5 mol % lipid **1**	Absorbance at 700 nm from DSPC vesicles at 60°C containing 5 mol % lipid **1**	Absorbance at 700 nm from POPC/ DSPC vesicles at 25°C containing 5 mol % lipid **1**
0	1.4860	0.60600	0.67000	0.35300
2	1.3982	0.60399	0.67300	0.35200
4	1.3690	0.60436	0.71400	0.34950
6	1.3796	0.63791	0.70800	0.35750
8	1.4070	0.98390	0.72600	0.37000
10	1.3989	1.1446	0.69900	0.40150
12	1.4119	1.1520	0.69700	0.41350
14	1.4179	1.1551	0.69600	0.41750
16	1.4472	1.1557	0.69500	0.41450
18	1.4570	1.1591	0.69800	0.41300
20	1.4826	1.2031	0.69500	0.41500

Table S5. The change in the absorbance at 700 nm caused by adding copper(II) (10 mM) to DSPC (2 mM) or EYPC (2 mM) vesicles containing 5% lipid **1** (0.1 mM).

μL 0.5 mM poly-L-histidine added	Absorbance at 700 nm from DSPC vesicles at 25°C containing 5 mol % lipid Cu(**1**)	Absorbance at 700 nm from EYPC vesicles at 25°C containing 5 mol % lipid Cu(**1**)	Absorbance at 700 nm from DSPC vesicles at 60°C containing 5 mol % lipid Cu(**1**)	Absorbance at 700 nm from POPC/ DSPC vesicles at 25°C containing 5 mol % lipid Cu(**1**)
0	1.4826	1.2031	0.6950	0.4150
2	1.5179	1.1963	0.7090	0.4245
4	1.5141	1.1877	0.7230	0.4310
6	1.5396	1.1839	0.7390	0.4395
8	1.5698	1.1910	0.7560	0.4480
10	1.5970	1.1885	0.7590	0.4540
12	1.6261	1.1906	0.7700	0.4640
14	1.6551	1.1938	0.7690	0.4775
16	1.6816	1.2023	0.8000	0.4920
18	1.7011	1.2063	0.7920	0.4995
20	1.7242	1.2075	0.8170	0.5050
25	1.7632	1.2129	0.8190	0.5185
30	1.7798	1.2121	0.8470	0.5250
35	1.7963	1.2165	0.8450	0.5320
40	1.8180	1.2158	0.8540	0.5440
45	1.8233	1.2123	0.8630	0.5455
50	1.8320	1.2213	0.8710	0.5495

Table S6. The change in the absorbance at 700 nm caused by adding polyhistidine (0.5 mM, 20 mM histidine residues) to DSPC (2 mM) or EYPC (2 mM) vesicles containing 5% lipid Cu(**1**) (0.1 mM).

Time /min	0	5	10	15	20	25	30	After polyhistidine
Absorbance	1.0870	1.0928	1.0974	1.1011	1.1048	1.1076	1.1109	1.2862

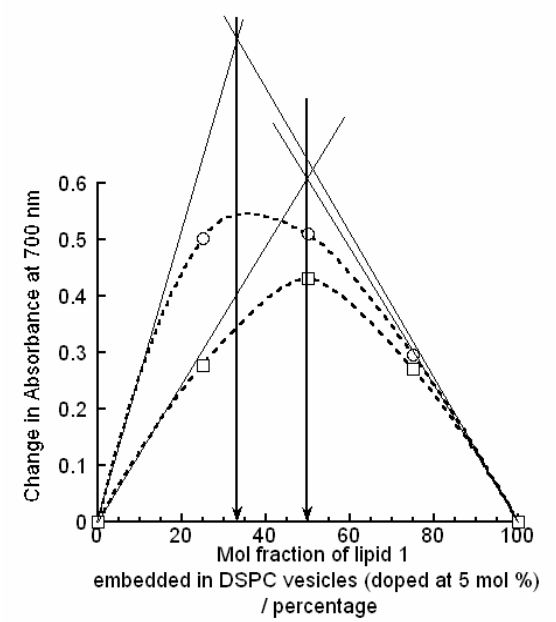
Table S7. The change in the absorbance at 700 nm caused by incubating DSPC (2 mM) vesicles containing 5% lipid **1** (0.1 mM) and 1 equivalent of copper(II) (0.1 mM) at 25°C. After aggregation caused by addition of polyhistidine (5 eq. histidine residues) A = 1.2862.

Time /min	0	5	10	15	20	25	30
Absorbance	1.2862	1.2753	1.2762	1.2773	1.2783	1.2792	1.2800

Table S8. The change in the absorbance at 700 nm caused by incubating DSPC (2 mM) vesicles containing 5% lipid **1** (0.1 mM), copper(II) (1 eq, 0.1 mM) and polyhistidine (5 eq. histidine residues) at 25°C.

Turbidimetric Job Plot

The aggregation of vesicles resulting from the recognition between polyhistidine and Cu(**1**) doped vesicles was monitored by measuring the turbidity change at 700 nm using UV-visible spectrophotometer in a 2 mL cuvette kept at 25°C. For Job Plot **A**, dispersions of 800 nm diameter Cu(**1**) vesicles (5% Cu(**1**) 0.1 mM, 95% DSPC 2 mM) and polyhistidine (0.1 mM in histidine residues) were mixed to give the ratios 1:0, 3:1, 1:1, 1:3, 0:1 in a total volume of 2 mL. For Job Plot **B**, dispersions of 800 nm diameter Cu(**1**) vesicles (5% Cu(**1**) 0.1 mM, 95% DSPC 2 mM) and polyhistidine (0.2 mM in histidine residues) were mixed to give the ratios 1:0, 3:2, 1:2, 1:6, 0:1 in a total volume of 2 mL.



Graph S1. Job Plots using different concentrations of polyhistidine:
Job Plot **A** (□), DSPC vesicles containing Cu(**1**) (0.1 mM Cu(**1**), 2 mM DSPC, 800 nm) mixed with polyhistidine solution (0.1 mM in histidine residues).
Job Plot **B** (○), DSPC vesicles containing Cu(**1**) (0.1 mM Cu(**1**), 2 mM DSPC, 800 nm) mixed with polyhistidine solution (0.2 mM in histidine residues).