Supplementary Material (ESI) for Soft Matter

Electronic Supplementary Information

Cell-Sorting of Robust Self-Reproducing Giant Vesicles Tolerant to Highly Ionic Medium

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Experimental Methods

Phase Contrast and Fluorescence Microscopy

Phase contrast and fluorescent micrographs were recorded using an Olympus IX71 inverted microscope equipped with a 3CCD camera (Toshiba Corp., JT-TU52H). The fluorescent micrographs were obtained using WIB filter units (Olympus; λex: 460-490 nm, λem: >515 nm) and a high pressure mercury lamp.

Flow Cytometer and Cell Sorter

EPICS ALTRA (Beckman Coulter, BA) was equipped with a water-cooled argon ion laser emitting at 488 nm for flow cytometry. As shown in fig. S1, the intensities of forward light scattering (FS) and fluorescence (FL) at 525 nm from each object were collected. As the highly ionic solution for the cell-sorting technique, IsoFlow® (Beckman Coulter, BA) was used for the sheath flow. The giant vesicles sorted were counted 50000 at the cell-sorting. As the size calibration of flow cytometric dot plots, we analyzed the dot plots of giant vesicles the sizes of which were modulated by filtration through 12-, 3-, and 1.2-μm pore filters [see also *Langmuir* **2008**, *24*, 3037-3044. Supporting Information p.9-10]. The flow cytometric data were analyzed by EXPO32 analyzing software (Beckman Coulter) and win MDI (written by J. Trotter).

Data Assembly on Flow Cytometry Histograms

Histogram data obtained by EPICS ALTRA is put in 1024 channels through an A/D converter since the analogue data of the photomultiplier tube detecting forward light scatter or fluorescence of giant vesicles is ready to be calculated as digital data. Then the 1024 values were assembled to 32 channels by being averaged in each set of 32 values. The each value in 32 channels was normalized by the maximum value which was determined neglecting the values in the vicinity of FS = 1. Fig. 3a and 3b thus demonstrate the assembled data with deviation bars (2σ). Error bars in Fig. 3c represent the width of FS or FL histogram at 90 % of each peak value. At fig. 5a in the main text, the histograms of the sorted GVs (GV-A and GV-B) were assembled as the aforementioned way and, in

addition, were multiplied by arbitrary factors, showing the contrast of the peak difference.

Materials

Synthesis of membrane molecule (V)

4-(12-bromododecyroxy) benzaldehyde

Sodium (460 mg, 20 mmol) was dissolved in dry ethanol (50 mL), and 4-hydroxybenzaldehyde (2.44 g, 20mmol) was added to the solution. Then 1,12-dibromododecane (19.7 g, 60mmol) was added to the above solution, and the mixture was refluxed for 40 hours. The solution was washed with water, and the organic phase was dried over magnesium sulfate. The solvent was removed by rotary evaporation, and the crude product was purified with silica column chromatography (ethyl acetate/hexane = 1/5). The target product was obtained as a white solid (5.75 g, 75.5%).^{1}H-NMR (270 MHz, $CDCl_3$):δ9.34 (1H, s), 7.84 (2H, d, *J* = 8.8 Hz), 7.09 (2H, d, *J* = 8.8 Hz), 4.06 (2H, t, *J* = 6.5 Hz), 3.50 (2H, t, *J* = 6.8 Hz), 1.79-1.67 (4H, m), 1.32-1.24 (16H, m).

N, *N*-dimethyl-*N*-[12((4'-formyl) phenoxy) dodecyl]-*N*-dodecyl-ammonium bromide (V)

4-(12-bromododecyroxy) benzaldehyde (2.83 g, 7.66 mmol) and *N*, *N*-dimethyl dodecyl amine (3.16 mL, 11.5 mmol) were dissolved in 10 mL of dry methanol, and refluxed for 14 hours. After the reaction completed, the solution was cooled to room temperature under ambient conditions. The solvent was removed by rotary evaporation, and residue was dissolved in a small amount of CH_2Cl_2. After reprecipitation with diethyl ether, the desired compound was obtained (2.21 g, 49.5%). ^{1}H-NMR (270 MHz, $CDCl_3$):δ9.36 (1H, s), 7.85 (2H, d, *J* = 8.8 Hz), 7.11 (2H, d, *J* = 8.8 Hz), 4.08 (2H, t, *J* = 6.5 Hz), 3.24-3.18 (4H, m), 2.98 (6H, s), 1.68-1.62 (6H, m), 1.27-1.25 (34H, m), 0.85 (3H, t, *J* = 7.0 Hz). FABMS: m/z: 502.5 [M^+].

Synthesis of membrane precursor (V)*

N, *N*-dimethyl-*N*-[12-[4'-[[4'-2'''-*N*', *N*', *N*'-trimethyl ammonio] ethoxy] phenyl] imino] methyl] phenoxy] dodecyl]-N-dodecyl ammonium bromide (V*)

N, *N*-dimethyl-*N*-[12-((4'-formyl) phenoxy) dodecyl]-*N*-dodecyl-ammonium bromide (2.03 g, 3.5 mmol) and [2-(4'-aminophenoxy) ethyl] trimethyl ammonium bromide (1.15 g, 4.2 mmol) was dissolved in 5 mL of dry ethanol and a few drops of acetic acid was added to the solution. After the solution was refluxed for 42 hours, the solvent was evaporated and the concentrated product was added to 30 mL of acetone. The solution was filtered under reduced pressure and the residue was dissolved in diethyl ether. The precipitates were filtered to afford a white solid compound (920 mg, 31.4%). ^{1}H-NMR (270 MHz, d_6-DMSO):δ8.56 (1H, s), 7.67-7.55 (4H, m), 6.99-6.96 (2H, m),

6.72-6.67 (2H, m), 4.10-4.01 (2H, m), 3.82-3.76 (2H, m), 3.42-3.13 (17H, m), 1.25-1.12 (44H, m), 3.82 (3H, t, J = 6.2Hz).

Synthesis of non-fluorescent catalyst (C_N)

N-octadecylimidazole

Imidazole (2.0 g, 29 mmol) was dissolved in 22 mL of acetonitrile, and potassium hydroxide (3.3 g, 59 mmol) and 1-chlorooctadecane (10 mL, 30 mmol) was added to the solution. After the solution was refluxed for 4 hours, the solution was cooled to room temperature. The oil phase was extracted with hexane, washed with water, and dried over anhydrous magnesium sulfate. After purified via silica gel column chromatography (ethyl acetate/methanol = 25/1), a purified compound was obtained (6.9 g, 75%). ^{1}H-NMR (270 MHz, $CDCl_3$):δ7.45 (1H, bs), 7.04 (1H, bs), 6.98 (1H, bs), 3.91 (2H, t, J = 7.2 Hz), 1.30-1.19 (32H, m), 0.86 (3H, t, J = 6.3 Hz).

N-octadecylimidazole chloride (C_N)

N-octadecylimidazole (5 g, 14 mmol) was dissolved in 20 mL of ethyl acetate and then hydrochloric gas was bubbled into the solution. White precipitate were filtered under reduced pressure and dissolved in a small amount of methyl chloride. A white solid was reprecipitated by the addition of 100 mL of ethyl acetate. The product was filtered under reduced pressure and dried in a vacuum. ^{1}H-NMR (270 MHz, $CDCl_3$):δ9.30 (1H, bs), 7.40 (1H, bs), 7.11 (1H, bs), 4.33-4.24 (2H, m), 1.34-1.24 (32H, m), 0.88 (3H, m). ESIMS: m/z: 321.1 $[M+H]^+$.

Synthesis of fluorescent catalyst (C_F)

2-octadecanoylpyrrole

A solution of pyrrole (4.02 g, 60 mmol) in 120 mL of anhydrous toluene was added dropwise over 5h periods to a stirred solution of stearoyl chloride (6.06 g, 20 mmol) in 120 mL of dry toluene maintained in a argon atmosphere. The solution was heated at reflux temperature for an additional 5 h and then the solvent was removed by rotary evaporation. The residue was subjected to silica gel column chromatography (hexane/ethyl acetate = 10/1) and then solid compound was obtained (5.93 g, 88.9%). ^{1}H-NMR (270 MHz, $CDCl_3$): δ9.52 (1H, bs), 7.03-7.01 (1H, bs), 6.92-1.90 (1H, bs), 6.29-6.26 (1H, bs), 2.75 (2H, t, J = 7.7 Hz), 1.30-1.21 (30H, m), 0.88 (3H, t, J = 6.6 Hz).

2-octadecylpyrrole

Lithium aluminum hydride (2.39 g, 63 mmol) was added by portions to the solution of 2-octadecanolylpyrrole (6.00 g, 18 mmol) in 50 mL of dry tetrahydrofuran under ice-cooling. After

the reaction, the solution was refluxed for 19 hours. The product was extracted into ethyl acetate, and the extract was washed with saline, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was subjected to silica gel column chromatography (hexane/ethyl acetate = 7/3) to elute the product (5.30 g, 92.2%). ^{1}H-NMR (270 MHz, $CDCl_3$):δ7.90 (1H, br), 6.67-6.65(1H, m), 6.13 (1H, dd, *J* = 3.0 Hz, 8.6 Hz), 5.92-5.89 (1H, m), 2.59 (2H, t, *J* = 7.7 Hz), 1.30-1.21 (32H, m), 0.88 (3H, t, *J* = 7.0 Hz).

4'-(2''-bromoethoxy) phenyl-1, 9-dioctadecyldipyrromethene

The solution of 2-octadecylpyrrole (1.28 g, 4mmol) and 4-(2-bromoethocy)-benzaldehyde (458 mg, 2 mmol) in 120 mL of dry toluene purged with argon was stirred for 40 min. Catalyst quantity of trifluoroacetic acid was added to the solution, and the mixture was stirred for 22 hours at room temperature under argon atmosphere. To the solution was added 2, 3-dichloro-5, 6-dicyano-*p*-benzoquinone (500 mg, 2.20 mmol), and the mixture was stirred for 4 hours at room temperature. The product was extracted, washed, and dried over anhydrous sodium sulfate, The solvent was evaporated, and the crude product was purified by alumina column chromatography (hexane/ethyl acetate = 10/1) to afford green crystals (1.42 g, 84%). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.42 (2H, d, *J* = 8.9 Hz), 6.94 (2H, d, *J* = 8.6 Hz), 6.50 (2H, d, *J* = 4.1 Hz), 6.18 (2H, d, *J* = 4.3 Hz), 4.34 (2H, t, *J* = 6.2 Hz), 3.67 (2H, t, *J* = 6.2 Hz), 3.67 (2H, t, *J* = 6.2 Hz), 1.33-1.23 (64H, m), 0.90 (6H, t, *J* = 6.8 Hz).

3, 5-dioctyl-4, 4-difluoro-4-bora-8-[4'-(2''-bromoethoxy) phenyl]-3a, 4a, diaza-*s*-indacene

4'-(2''-bromoethoxy) phenyl-1, 9-dioctadecyldipyrromethene (848 mg, 1 mmol) was dissolved in 50 mL of chloroform dehydrated purged with nitrogen. Under argon atmosphere, BF_3-OEt_2 (2.1 mL, 11.4 mmol) and triethylamine (1.5 mL, 20.4 mmol) was added dropwise through a syringe, and the mixture was stirred for 11 hours at room temperature. A deep green fluorescence was observed in the mixture. The reaction mixture was extracted, washed and dried with magnesium sulfate. The solvent was evaporated, and the crude product was purified by alumina column chromatography (hexane/ethyl acetate = 5/1) to afford green powder (880 mg, 98.3%). ^{1}H-NMR (270 MHz, $CDCl_3$):δ 7.41 (2H, d, *J* = 8.6 Hz), 6.97 (2H, d, *J* = 8.6 Hz), 6.71 (2H, d, *J* = 4.1 Hz), 6.31 (2H, d, *J* = 3.8 Hz), 4.33 (2H, t, *J* = 6.3 Hz), 3.65 (2H, t, *J* = 6.1 Hz), 3.03 (4H, t, *J* = 7.7 Hz), 1.32-1.22 (64H, m), 0.88 (6H, t, *J* = 6.3 Hz).

1-[2'-[4''-(3''', 5'''-dioctadecyl-4''', 4'''-difluoro-4'''-bora-3a''', 4a'''-diaza-*s*-indacene-8''') phenoxy] ethyl] imidazole

Imidazole (400 mg, 5.89 mmol) and 3, 5-dioctyl-4, 4-difluoro-4-bora-8-[4'-(2''-bromoethoxy) phenyl]-3a, 4a, diaza-*s*-indacene (526 mg, 0.59 mmol) was dissolved in 0.5 mL of ethanol

dehydrated ,and then the solution was refluxed for 17 hours. After the reaction, the product was extracted into diethyl ether and washed with 1M aqueous of sodium hydride, dried over sodium sulfate. The solvent was evaporated to afford red crystals (330 mg, 63.6%). ^{1}H-NMR (270 MHz, $CDCl_3$):δ 7.64 (1H, s), 7.43 (2H, d, *J* = 8.4 Hz), 7.08 (2H, d, *J* = 6.2 Hz), 6.95 (2H, d, *J* = 8.6 Hz), 6.71 (2H, d, *J* = 4.1 Hz), 6.32 (2H, d, *J* = 3.8 Hz), 4.39 (2H, t, *J* = 4.5 Hz), 4.29 (2H, t, *J* = 4.2 Hz), 3.03 (4H, t, *J* = 7.3 Hz), 1.29-1.22 (64H, m), 0.88 (6H, t, *J* = 6.8 Hz).

1-[2'-[4''-(3''', 5'''-dioctadecyl-4''', 4'''-difluoro-4'''-bora-3a''', 4a'''-diaza-*s*-indacene-8'''-yl) phenoxy] ethyl] imidazole chloride (C_F)

1-[2'-[4''-(3''', 5'''-dioctadecyl-4''', 4'''-difluoro-4'''-bora-3a''', 4a'''-diaza-*s*-indacene-8''') phenoxy] ethyl] imidazole (330mg, 0.37 mmol) was dissolved in a small amount of diethyl ether, and then 50 mL of hydrochloric acid was added dropwise to the solution. The solvent was azeotroped with ethanol to afford the dark red product, which was dried under reduced pressure to obtain the dark red crystals (331 mg, 97.1%). ^{1}H-NMR (270 MHz, $CDCl_3$):δ 9.77 (1H, bs), 7.39-7.34 (3H, m), 6.95 (2H, d, *J* = 6.2 Hz), 6.67 (2H, d, *J* = 8.6 Hz), 6.61 (2H, d, *J* = 4.1 Hz), 6.24 (2H, d, *J* = 4.9 Hz), 4.23 (2H, t, *J* = 6.2 Hz), 2.94 (2H, t, *J* = 4.2 Hz), 1.66-1.63 (4H,m), 1.22-1.01 (59H, m), 0.80-0.77 (9H, m). FABMS: m/z: 913.7 [M^+].

Preparation of Giant Vesicle Dispersion and Precursor Solution

Evaporation of a chloroform solution (10 mM, 100 μL) containing **V**, $\mathbf{C_N}$ and $\mathbf{C_F}$ ($\mathbf{V/C_N/C_F}$ = 90/10/1, molar ratio) under nitrogen gas flow afforded thin films on the bottom of the flask. After the remaining solvent was removed under reduced pressure for 5 h, an NaCl solution (150 mM) was added to the films. The resulting dispersion of giant vesicles ([**V**] = 5 mM) was stirred for at least 5 h at room temperature. Using optical microscopy and FCM, we confirmed that the population of GVs reached a stable state during stirring. Immediately before being mixed with the GV dispersion, a solution (5 mM) of precursor **V*** was prepared in Milli-Q water and then filtered through a 3-μm polycarbonate filter (Millipore).

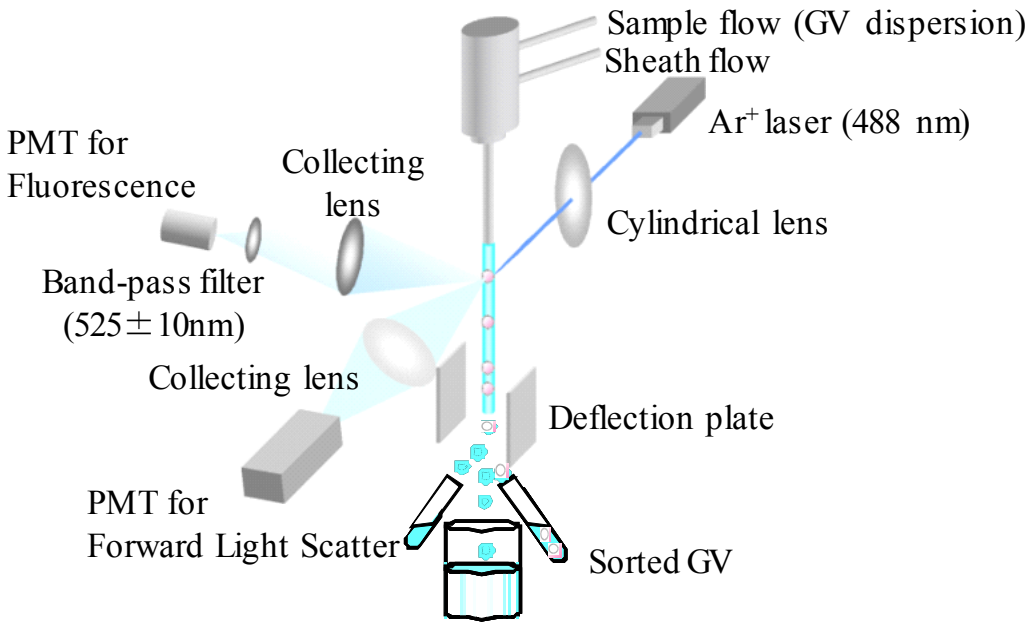

Fig. S1 Schematic illustration of the flow cytometric experiment with cell-sorting technique: PMT, Photomultiplier tube.

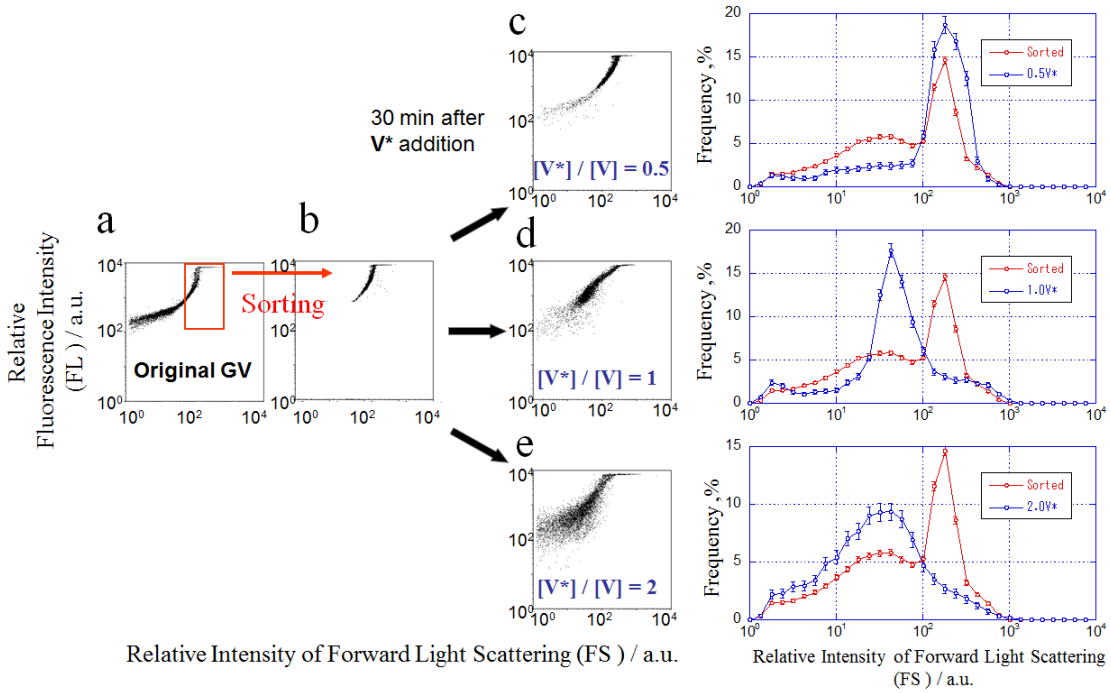

Fig. S2 Difference of the dot plots of the GVs sorted from the original group (a), 30 min after the addition of **V*** in terms of the ratio of [**V***] to [**V**]. The dot-plot of the sorted GVs before the addition of **V*** is shown in (b). The dot-plots and FS histograms of the GVs 30 min after the addition of **V*** are shown in (c), (d) and (e) at the [**V***]/[**V**] = 0.5, 1 and 2, respectively. At [**V***]/[**V**]=0.5, the self-reproducing dynamics was scarcely induced. At [**V***]/[**V**]=2, the population of giant vesicles spread widely along FS axis and small GVs were produced. On the other hand, the appropriate population shift of the self-reproduced GVs was observed at [**V***]/[**V**]=1.