Supplementary Material (ESI) for Chemical Communications

Supporting Information

Target-Selective Vesicle Fusion Induced by Molecular Recognition on Lipid Bilayer

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Contents:

Experimental details

Synthesis of the boronic acid derivatives *1* and *2*.

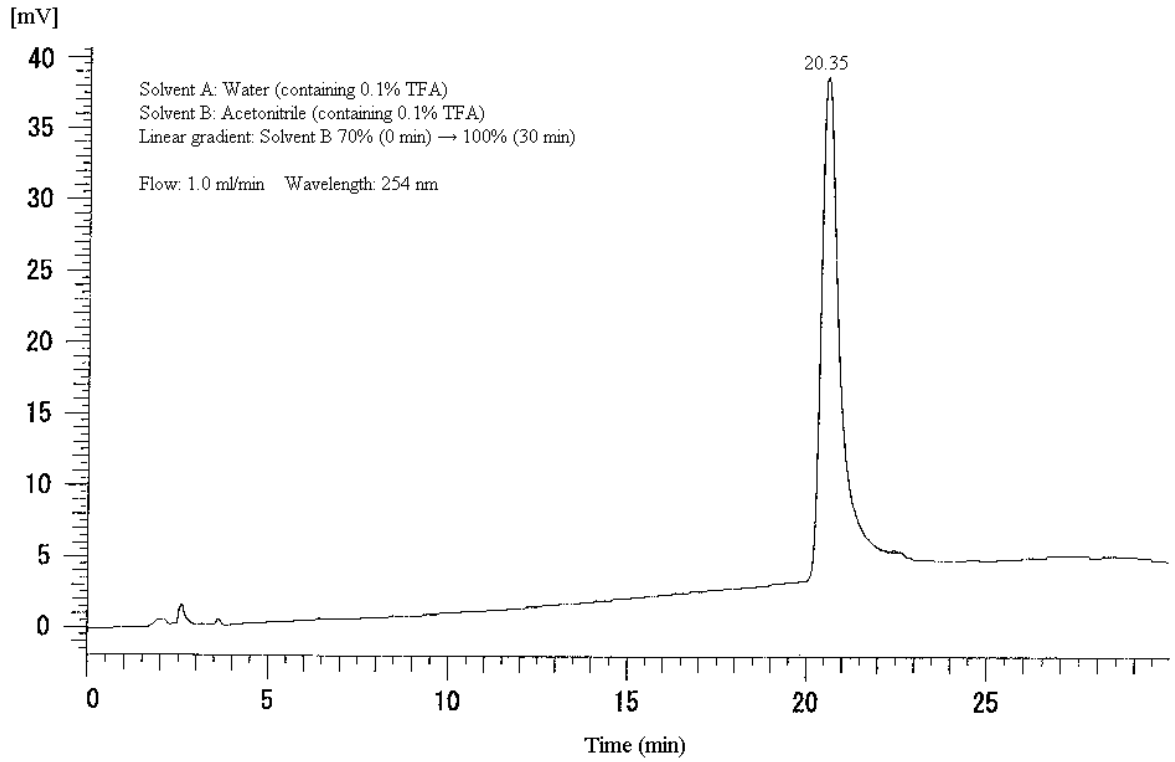
General. FAB-MS analyses were performed on a JEOL MS600H mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer in DMSO-d_6. Tetramethylsilane (TMS) served as the internal standard (0 ppm) for ^{1}HNMR, and DMSO-d_6 served as the internal standard (40.0 ppm) for ^{13}CNMR.

Method. The boronic acid derivatives were synthesized by the solid-phase synthesis method on Wang resin. Fmoc-Lys(Mtt) was attached to Wang resin by 2,6-dichlorobenzoyl chloride activation.[1] The side-chain Mtt group was removed by 1% TFA in DMF for 30 min.[2] After confirmation of removal of Mtt group by ninhydrin test, stealic acid was condensed to the side chain amino group of Lys using HBTU/HOBt. The reaction was confirmed by ninhydrin test. And then, Fmoc-NH-$(PEG)_5$-COOH and *p*-Carboxyphenylboronic Acid was coupled by standard Fmoc strategy. Deprotection and cleavage were performed by treatment with TFA / H_2O (95/5, v/v) for 2 hr. Purification was carried out by a Hitachi L-7000 HPLC System on a Inertsil ODS-3 column (10 mm i.d. × 250 mm, 5 μm, GL-science, Japan) eluted at 5 cm^3/min with linear acetonitrile/water gradients containing 0.1% (v/v) TFA over the course of 30 min. Compound *1* and *2* were afforded 92% and 78% yields, respectively.

***Compound 1**:* FAB-MS spectroscopy for MH^+: 561.44 (calcd. 561.57). ^1H NMR (400 MHz, DMSO-d_6) : δ = 8.50 (d, 1H), 8.32 (s, 2H), 7.84-7.85 (d, 2H), 7.79 (d, 1H), 4.29 (q, 1H), 3.01(d, 1H), 2.50-2.51 (m, 8H), 2.00 (t, 1H), 1.20-1.22 (m, 32H), 0.85 (t, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) : δ = 174.43, 172.57, 167.20, 135.73, 134.41, 126.86, 53.15, 38.60, 35.99, 31.85, 30.73, 29.59, 29.56, 29.48, 29.34, 29.30, 29.26, 29.20, 25.87, 23.82, 22.65, 14.51 ppm.[3]

***Compound 2**:* FAB-MS spectroscopy for MH^+: 896.79 (calcd. 896.97). ^1H NMR (400 MHz, DMSO-d_6) : δ = 8.33 (s, 2H), 8.06 (d, 1H), 7.84 (d, 1H), 7.78-7.80 (d, 2H), 4.13 (q, 1H), 3.35-3.52 (m, 28H), 2.99 (t, 1H), 2.50-2.51 (m, 8H), 2.34 (d, 1H), 2.02 (t, 1H), 1.23 (m, 32H), 0.84 (t, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) : δ = 174.52, 172.51, 170.62, 166.95, 136.13, 134.44, 126.57, 79.71, 70.28, 70.20, 70.17, 70.04, 69.38, 67.29, 38.70, 36.37, 35.97, 31.84, 31.50, 31.25, 29.58, 29.49, 29.33, 29.25, 29.23, 25.86, 23.34, 22.65, 14.51 ppm.[3]

HPLC trace of *Compound 1* (after purification)



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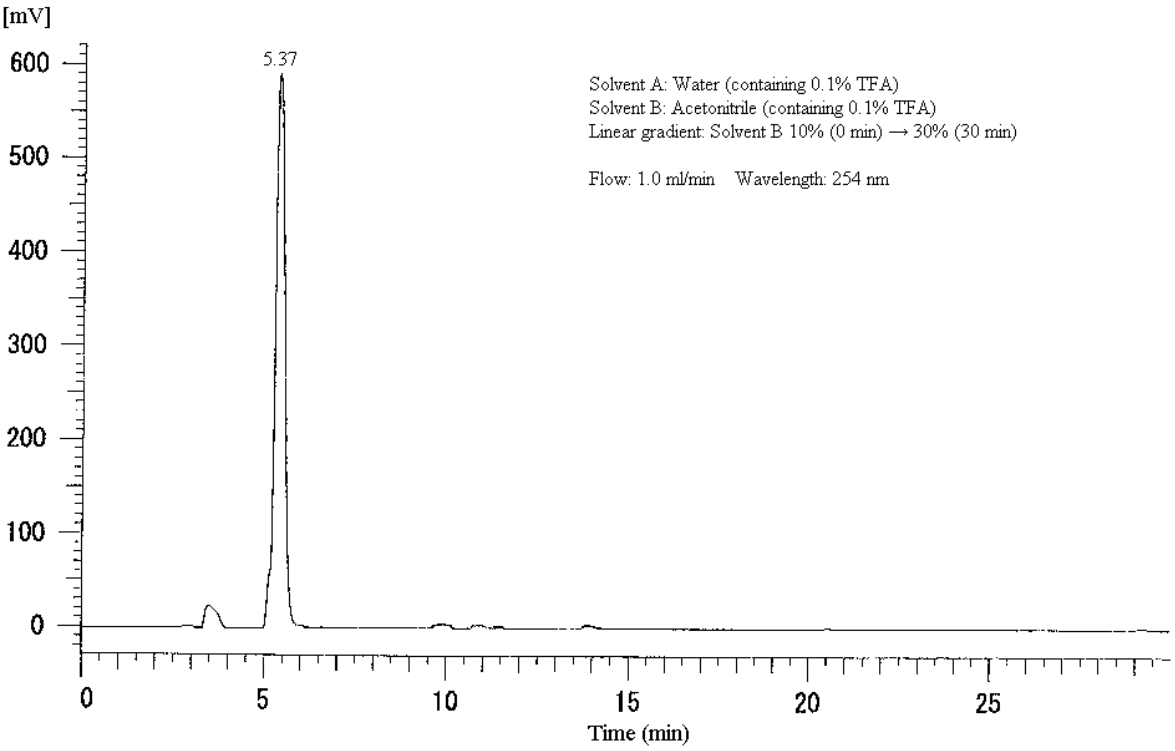
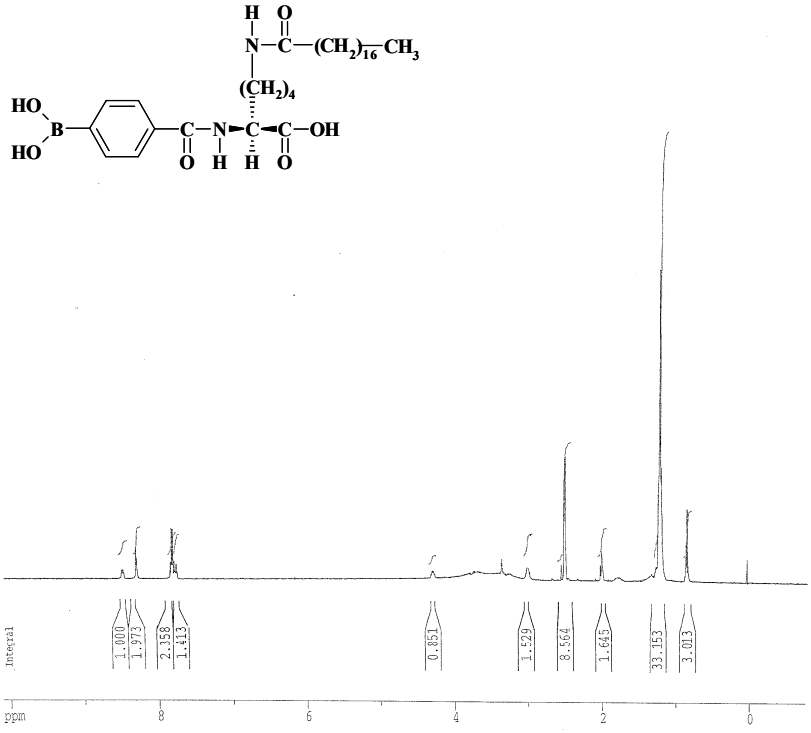


^{1}H and ^{13}C NMR charts of *Compound 1*

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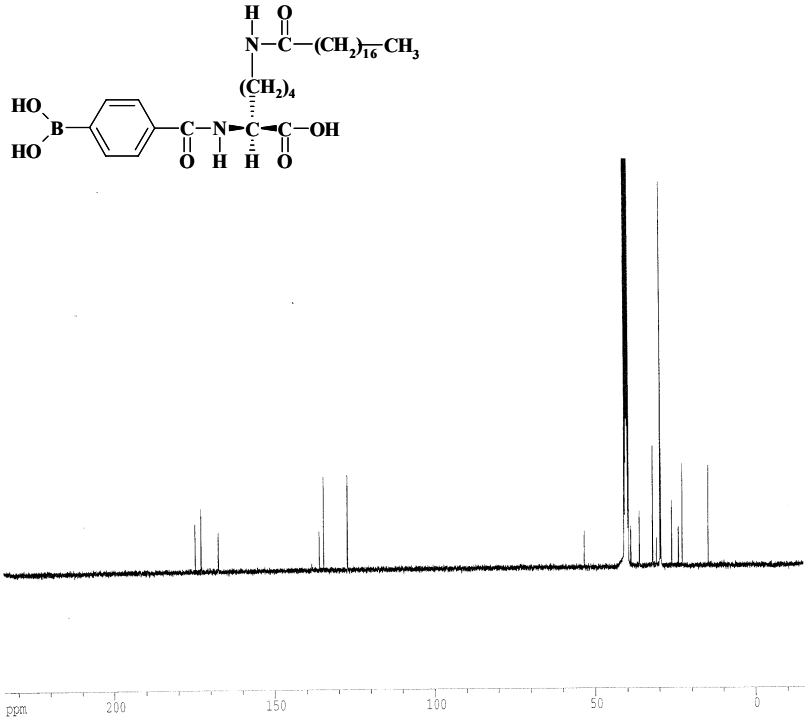


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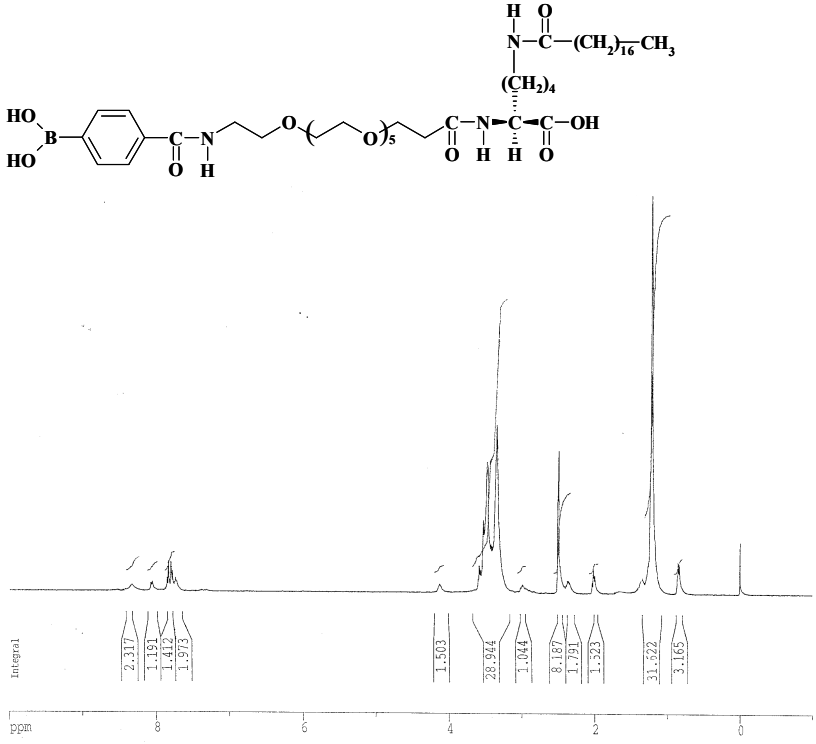
^{1}H and ^{13}C NMR charts of *Compound 2*

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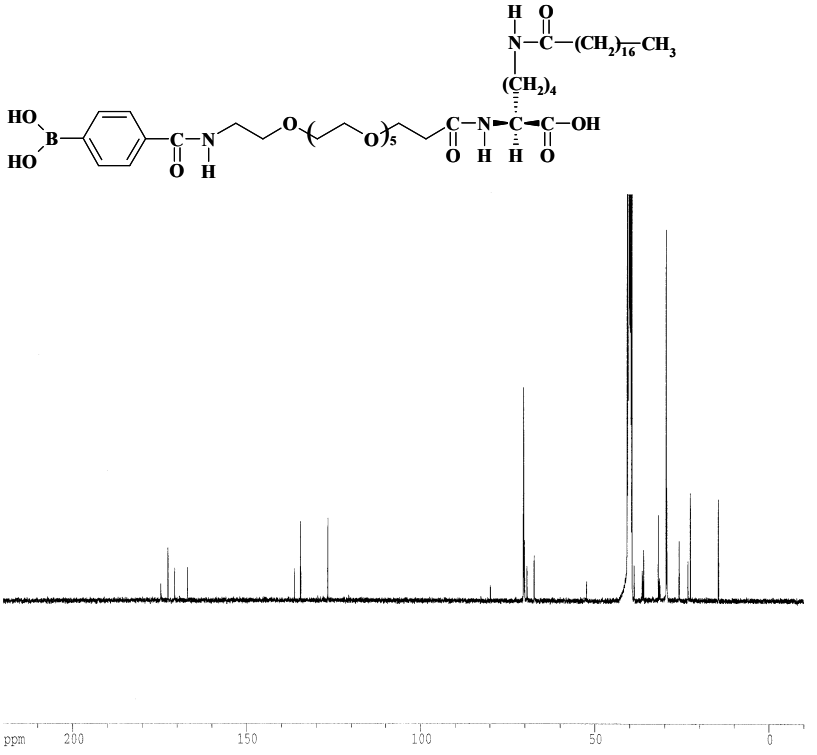


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Vesicle preparation

Vesicles of following lipid compositions were prepared. The *donor* vesicle: EggPC/Compound ***1*** or ***2*** (95:5, molar latio) and the *target* vesicle: EggPC/PI (95:5). Small unilamellar vesicles (SUVs) were prepared by evaporation of chrloroform solution of lipid mixture in a round bottom flask, followed by hydration in 10 mM sodium carbonate buffer (containing 100 mM NaCl, pH 10.5). The suspension was submitted to five freeze-thaw cycles for equilibration. Then, SUVs for this experiment were prepared by extrusion of the suspension across 100 nm polycarbonate unipore membranes (Whatman) 10 times using a Mini-Extruder Set (Avanti). The final total lipid concentration was 2.5 mM.

Liposome size distribution determination

Dynamic light scattering (DLS) of liposome suspensions was studied on an N5 Plus autocorrelator (Beckman-Coulter), equipped with a 632.8 nm He-Ne laser light source. Single scans with 2 minute averaging time were performed on the sample with a 90.0° angle. Particle size distributions were calculated from autocorrelation data. All buffer solutions used were filtered with a 0.22 μm filter just before liposome preparation. The collection times for the autocorrelation data were 1~4 min.

Lipid mixing assays

The *target* vesicles encapsulated NBD-PE and Rh-PE was prepared by the same procedures as described above. The concentrations of the NBD-PE and Rh-PE were 0.5 mol% against the lipid

mixture. The mixing of phospholipids was followed by the fluorescence resonance energy transfer (FRET) method. In this work, we used equal concentrations of the unlabeled (*donor*) and labeled (*target*) vesicles diluted into a buffer solution at the appropriate pH, and monitored the fluorescence of 531 nm from NBD and 590 nm of Rh. The degree of fusion was estimated by the lipid mixing defined by $I = (I_t - I_0) / (I_{max} - I_0) \times 100$, where I_0 and I_t are the fluorescence intensity at 531 nm at time 0 and a defined time (t), respectively, and I_{max} is the fluorescence (at 531 nm) after disruption of the vesicles in 0.5% (w/v) Triton X-100. Experimental data were acquired by the use of a HITACHI F-2500 spectrofluorometer at the excitation wavelength of 470 nm.

Inner leaflet mixing assays

Reduction of NBD-PE and Rh-PE labeled vesicles was carried out as follows. A 1:1 mixture of NBD-PE/Rh-PE labeled vesicles (2.0 mM) and 100 mM sodium dithionite (in 10 mM sodium carbonate buffer containing 100 mM NaCl, pH 10.5) were incubated at 4 °C for 1 h. Free sodium dithionite was removed by gel filtration using Sephadex G-25 fine columns. The inner leaflet mixing assay was identical with the lipid mixing assay described above.

Fusion assay by content mixing

The DPA (0.2 mM) encapsulated *donor* vesicles and the Tb^{3+} (0.05 mM) encapsulated *target* vesicles were prepared by the same procedures as described above. Vesicle fusion results in formation of the fluorescent Tb^{3+}/DPA chelates. Fluorescence of the chelates is detected at 493 nm

with excitation at 276 nm. The fluorescence measurements for content mixing experiments were carried out by a HITACHI F-2500 spectrofluorometer.

References and Note

[1] P. Sieber, *Tetrahedron Lett.* 1987, **28**, 6147.

[2] L. Bourel, O. Carion, H. Gras-Masse and O. Melnyk, *J. Pep. Sci.* 2000, **6**, 264.

[3] In the ^{13}CNMR spectra of Compounds *1* and *2*, one of the aromatic carbon signals is silent. The resonance of the aromatic carbon bound to the boron atom around 139 ppm is considered to be very weak in DMSO-d_6 based on the ^{13}CNMR spectrum of *p*-Carboxylphenylboronic Acid (Commercial-grade reagent purchased from Wako).

Supporting figures

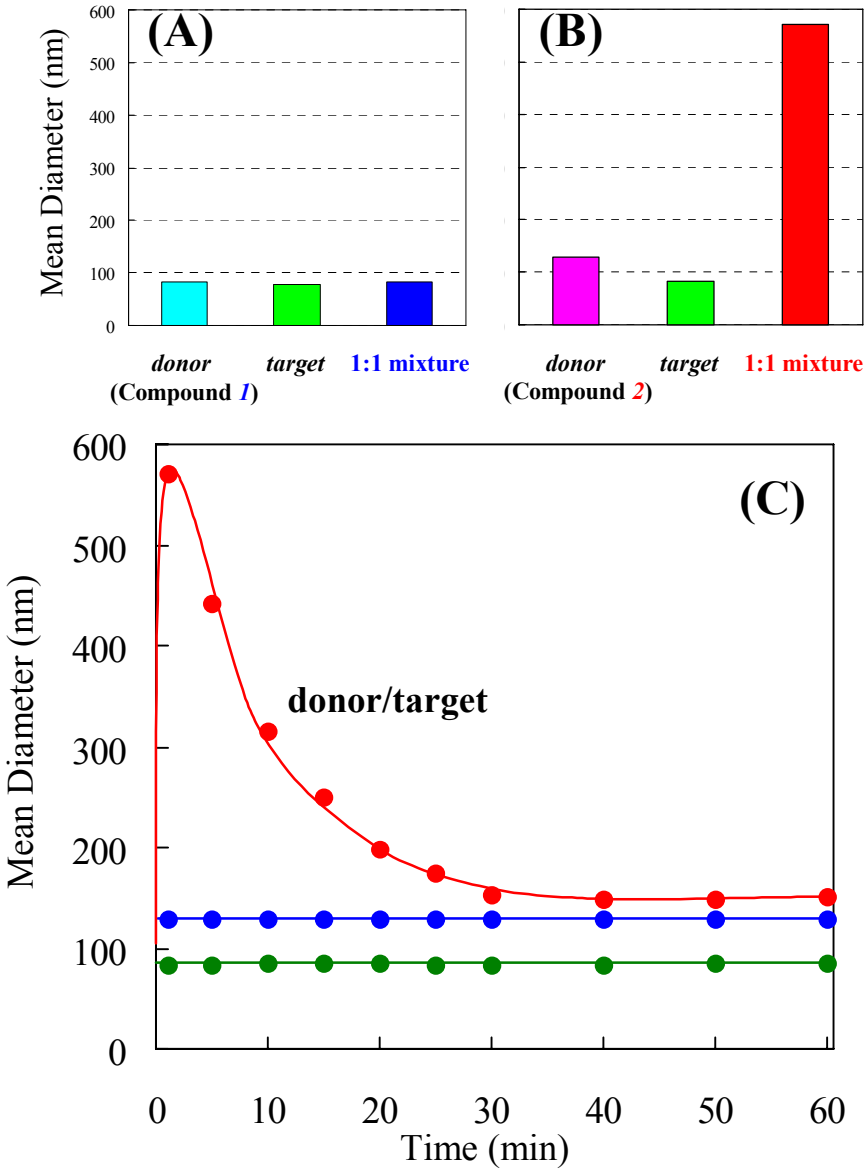

Fig. S1 Mean diameter from DLS measurements of the donor vesicles composed of 5 mol% of ***1*** (A) or ***2*** (B) in EggPC, the target vesicles composed of 5 mol % of PI in EggPC, and mixture of both vesicles. For the vesicle mixture, measurements were taken immediately after mixing. (C) Time course for the variation in the size of the donor vesicles composed of 5 mol% of ***2*** in EggPC (blue circle), the target vesicles composed of 5 mol % of PI in EggPC (green circle), and mixture of both vesicles (red circle). The measurements were performed in 10 mM sodium carbonate buffer (containing 100 mM NaCl, pH 10.5) at 30 °C.

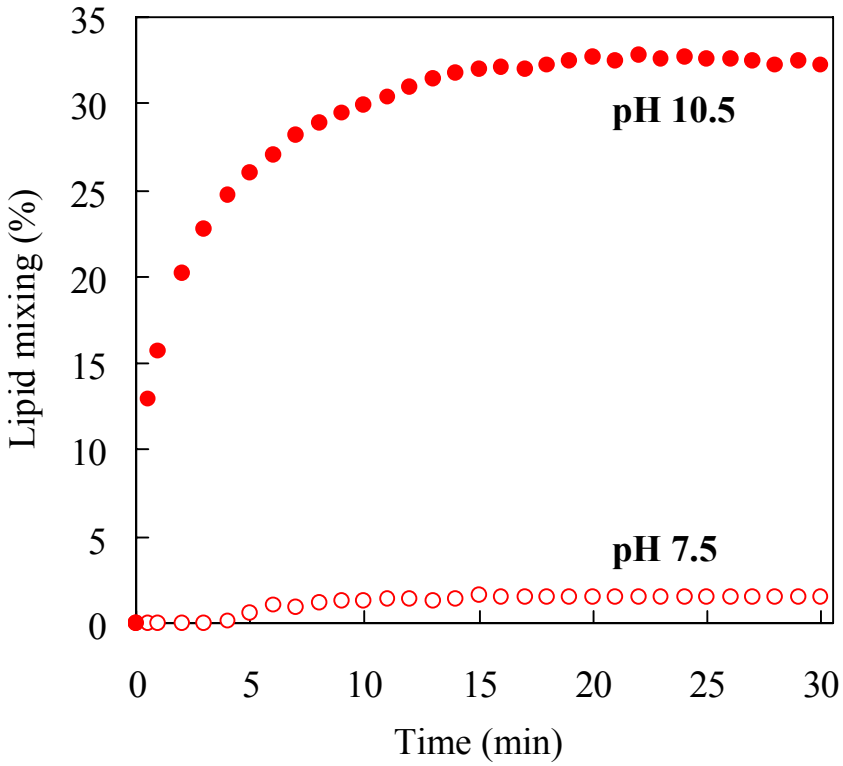

Fig. S2 pH dependence upon lipid mixing. The fluorophores containing *target* vesicles were mixed with the *donor* vesicles displaying ***2*** at pH 10.5 (closed circle) and pH 7.5 (open circle). The measurements were performed in 10 mM sodium carbonate buffer (containing 100 mM NaCl, pH 10.5) or 10 mM tricine buffer (containing 100 mM NaCl, pH 7.5) at 30 °C.