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Autocatalytic Membrane-amplification on a Pre-existing Vesicular Surface

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Experimental Section

General method

All commercially available reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd., Kanto Chemical Co., Inc., Tokyo Chemical Industry Co., Ltd., Avanti Polar Lipids, Inc., or Sigma Aldrich Japan Co., Ltd. and were used without further purification. To prepare 1/15 M (67 mM) phosphate buffer, KH_2PO_4 and Na_2HPO_4 was used. ^{1}H- and ^{13}C-NMR spectra and ^{31}P-NMR spectra were recorded on a JEOL GSX-270 spectrometer and a JEOL α-500 spectrometer, respectively. Tetramethylsilane was used as an internal reference for ^{1}H and ^{13}C-NMR measurements, and phosphoric acid was used as an external reference for ^{31}P-NMR. Mass spectra was recorded on a JEOL JMS-700 spectrometer. For fluorescent microscopy, an Olympus IX-71 microscope equipped with WIG filter set (Olympus U-MWIG3: λex = 530–550 nm; λem > 575 nm) and 100× objective lens (N. A. = 1.35) was used. For differential interference contrast microscopy, an Olympus BX-51 microscope equipped with 100× objective lens (N. A. = 1.35) was used. An EPICS ALTRA (Beckman Coulter) system equipped with an air-cooled 15-mW argon ion laser and a 575 nm bandpass filter for fluorescent measurement was used for flow cytometry. Before being measured by the flow cytometer, each sample suspension was filtered off by ϕ = 40 μm filter. For the trace of the reaction, a Shimazu UV-3150 UV-Vis spectrometer and a Shimazu SCL10A HPLC system equipped with a reversed phase column (TOSOH Octyl-80Ts) and a UV-detector (detected by λ = 254 nm light) were used. The eluent for HPLC was a mixed solution of MeOH (60%(v/v)), acetonitrile (30%(v/v)) and 20 mM phosphate buffer (pH 6.8, 10%(v/v)). The ζ-potential of $\mathbf{V}^-$-vesicular suspension was measured using a ζ-potential measurement system (OTSUKA ELECTRONICS ELS-6000). The diameters of assemblies were determined using a dynamic light scattering apparatus (NIKKISO MicrotracUPA150). The turbidity was measured by a HANNA Instruments HI93703 microprocessor turbidity meter.

Synthesis of potassium 10-(*p*-formanylphenoxydecanyl)-dodecyl-phosphate (K^+V^-)

A dichloromethane solution of 4-(10-hydroxydecanoxy)benzaldehyde (279 mg, 1.0 mmol) and Et_3N (202 mg, 2.0 mmol) was added to a dichloromethane solution of 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (236 mg, 1.0 mmol). After stirring for 1 h at room temperature, the reaction mixture was washed with $NaHCO_3$ aq, and dried over Na_2SO_4. After removal of dichloromethane under reduced pressure, a mixture of THF and acetonitrile (20 mL, v/v = 1/1) of dodecylalcohol (268 mg, 1.0 mmol) and tetrazole (280 mg, 4.0 mmol) was added to the residue in a dropwise manner, and the mixture was stirred for 1 h at room temperature. To the reaction mixture, a solution of THF and water (10 mL, v/v = 2/1) of iodine (305 mg, 1.2 mmol) and a few drops of pyridine were added in a dropwise manner, and the solution was stirred for 15 min at room temperature. The crude products were afforded by extraction using chloroform and water, the organic solvent was evaporated *in vacuo*, and the residue was purified by gel permeation liquid chromatography using chloroform as eluent to give 272 mg (41%) of 2-cyanoethyl-10-(*p*-formanylphenoxydecanyl)-dodecylphosphate (**1**) as colorless oil. An aqueous solution (10 mL) of potassium carbonate (79 mg, 0.57 mmol) was added to a EtOH solution (10 mL) of **1** (125 mg, 0.57 mmol), and the mixture was stirred for 1 h. An organic layer was extracted with chloroform and concentrated *in vacuo*. The reaction mixture was dissolved into dichloromethane, and hexane was added to the solution to form precipitates of K^+V^- (174 mg, 63 %): ^{1}H NMR (270 MHz, $CDCl_3$): δ = 9.86 (1H, s), 7.80 (2H, d, J = 9.0 Hz), 6.95 (2H, d, J = 9.0 Hz), 4.00 (2H, t, J = 4.5 Hz), 3.77–3.79 (4H, m), 1.78 (2H, m), 1.57 (4H, m), 1.44 (4H, m), 1.23–1.27 (28H, m), 0.85 (3H, t, J = 5.0 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 190.5, 164.0, 131.8, 129.6, 114.6, 68.3, 65.7, 32.0, 31.1, 31.0, 30.0, 29.9, 29.8, 29.6, 29.5, 29.2, 26.1, 22.8, 14.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 0.01; MS (EI) *m/z* = 527 ($M-K^+ + 2H^+$).

Synthesis of

4-(4-(2-*N*,*N*,*N*-trimethylammoniumethoxy)-phenylimino)-phenoxydecyl-dodecyl-phosphate (V*)

The mixture of (2-[4-aminophenoxy]ethyl)trimethylammonium bromide ($\mathbf{E}^+Br^-$) (137 mg, 0.5 mmol), and $K^+\mathbf{V}^-$ (282 mg, 0.5 mmol) was added to EtOH (10 mL). After addition of 2–3 droplets of acetic acid, the mixture was refluxed for 24 h. After removal of EtOH under reduced pressure, the residue was purified by an ion-exchange column chromatography (amberlite 400J OH, MeOH as eluent) and a reversed phase chromatography (ODS, MeOH as eluent). The purified product was dissolved into EtOH, and ether was added to the solution to form precipitate of 144 mg (58%) of **V***: ^{1}H NMR (270 MHz, CD_3OD): δ = 8.46 (1H, s), 7.83 (2H, d, *J* = 8.9 Hz), 7.26 (2H, d, *J* = 8.9 Hz), 7.05 (2H, d, *J* = 8.9 Hz), 6.99(2H, d, *J* = 8.9 Hz), 4.50 (2H, brs), 4.01 (2H, t, *J* = 6.5 Hz), 3.78–3.98 (6H, m), 3.28 (9H, s), 1.72–1.79 (2H, m), 1.55–1.62 (4H, m), 1.26–1.32 (30H, m), 0.87 (3H, t, *J* = 6.5 Hz); ^{13}C NMR (67.5 MHz, CD_3OD): δ = 163.2, 160.9, 157.1, 147.0, 131.5, 129.8, 123.3, 116.3, 115.7, 69.2, 66.5, 66.4, 66.3, 63.4, 54.8, 54.7, 54.6, 33.1, 32.0, 31.8, 30.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 27.2, 27.0, 23.8, 14.6; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 0.01; MS (EI) *m/z* = 702 (M^+).

Typical method for vesicular self-reproduction reaction

Thin film composed of $K^+\mathbf{V}^-$ (2 μmol) and cholesterol (0.6 μmol) was afforded by the completely evaporation of the solvent from their chloroformic solution by flowing N_2 gas and incubating under reduced pressure. Aqueous solvent (e.g. phosphate buffered water containing 15%(v/v) MeOH, 1 mL) was added to the vial, and the solution was mixed by a vortex mixer to afford $\mathbf{V}^-$-vesicular suspension. To the suspension, aqueous solution of **V*** (10 mM, 1 mL) that was prepared from mixing of methanolic solution of **V*** and phosphate buffered water and filtered off using polycarbonate filter (ϕ = 1.5 μm) was added. To prevent the hydrolysis of **V*** before mixing two solutions, the operation for preparing the aqueous **V*** solution was done quickly.

Preparation of vesicular suspension for fluorescent microscopic study and flow cytometric study

Thin film composed of $K^+\mathbf{V}^-$ (2 μmol), cholesterol (0.6 μmol), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) ammonium salt (Rho-DOPE, 0.02 μmol) was formed in a vial by evaporation of the solvent from a chloroformic solution by blowing N_2 gas and by incubating the residue under reduced pressure. To the vial was added 1 mL of an aqueous buffering solvent (67 mM phosphate buffered water containing 15%(v/v) MeOH), and the resulted solution was mixed by a vortex mixer to afford a suspension of $\mathbf{V}^-$-vesicles labeled with Rho-DOPE. To a 100 μL of the suspension, 100 μL of a buffering solvent was added (for control) or 100 μL of aqueous solution of **V*** (10 mM, 100 μL) was added (for sample). Then, both of the suspensions were incubated for 180 min at 25 ˚C, and the suspension were observed by fluorescent microscopy using a WIG filter set. And the dispersions were subjected to be measured by flow cytometry after diluted by nine times using a buffered solution before analysis.

SI 1. The size distribution of assemblies composed of **V***.

Bolaamphiphilic precursor **V*** formed aggregates in water. The sizes of the aggregates were measured to be smaller than 10 nm by a dynamic light scattering experiment as shown in ***Figure S1***,

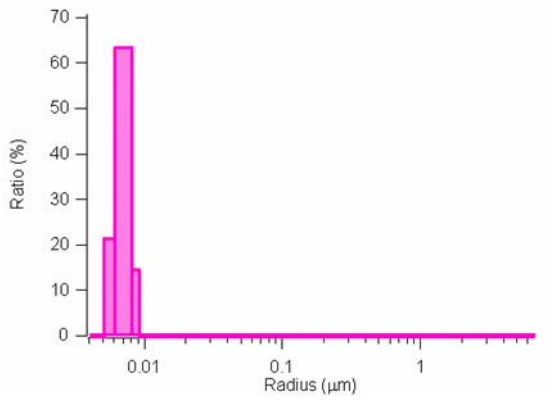

Figure S1. The size distribution of assemblies in the solution of **V*** (0.1 mM) at pH 7.1 in a phosphate buffered solution containing 15%(v/v) MeOH measured by dynamic light scattering.

SI 2. Trace of hydrolysis of **V*** in a dispersion of $\mathbf{V}^-$-vesicles in water and in phosphate-buffer by UV-Vis absorption spectroscopy.

The hydrolysis of **V*** was traced by the decrease of the peak at 330 nm assignable to the diphenylazomethine moiety in a dispersion of deionized water (***Figure S2***) and in phosphate buffered solution (pH 5.8) (***Figure S3***). ***Figure S4*** shows time courses of the hydrolysis without preformed vesicles in phosphate buffer (pH 7.2) and in deionized water.

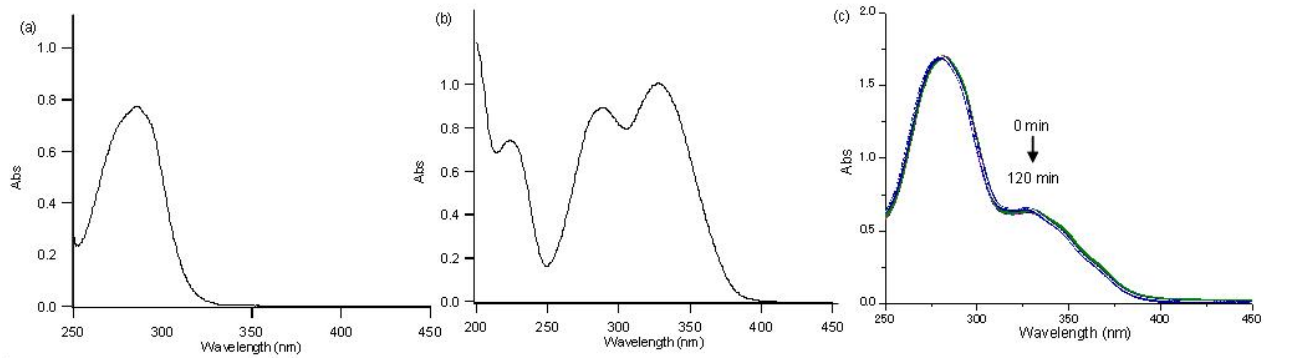

Figure S2. UV-Vis absorption spectra of **V*** and time course of the spectral change of **V***: (a) absorption spectrum of $\mathbf{V}^-$ (0.05 mM) in MeOH; (b) absorption spectrum of **V*** (0.05 mM) in MeOH; (c) time course (at 12 min interval) of the spectral change of **V*** (0.1 mM) in the presence of $\mathbf{V}^-$ (0.1 mM) vesicular suspension in deionized water containing 15%(v/v) MeOH.

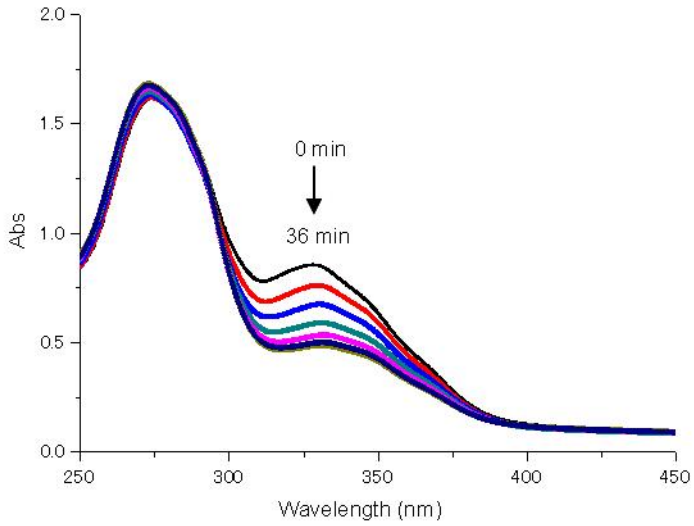

Figure S3. Time course (at 6 min interval) of UV-Vis spectral change of **V*** (0.1 mM) in the presence of $\mathbf{V}^-$ (0.1 mM) vesicular suspension in 67 mM phosphate buffered water (pH 5.8) containing 15%(v/v) MeOH.

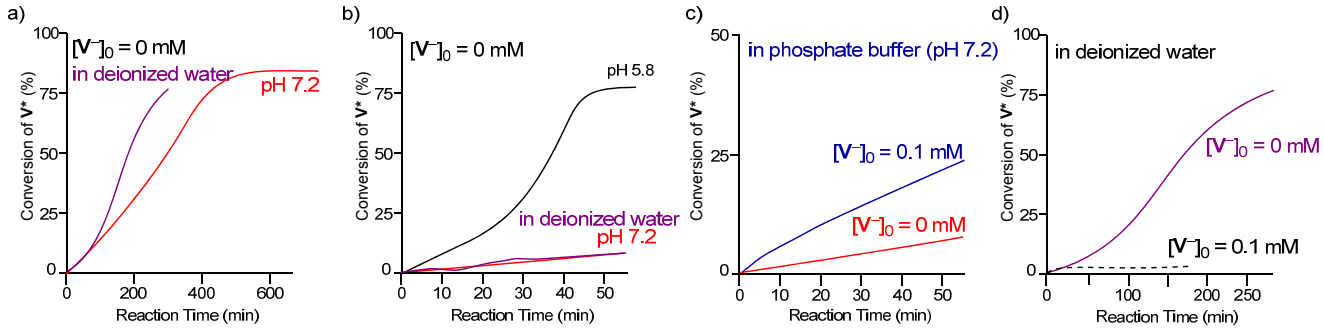

Figure S4. (a) Time course for conversion of **V*** (0.1 mM) in the absence of preformed vesicle: (red) in pH 7.2 phosphate buffer, (purple) in deionized water. (b), (c) and (d) Comparing data of (a), shown in Figure 1 in the text.

SI 3. The time courses of hydrolysis of **V*** in various initial concentrations of $\mathbf{V}^-$ measured by HPLC.

The hydrolysis rates of **V*** became larger as the $\mathbf{V}^-$ concentration increased, accompanied with the decrease of the induction periods in the sigmoidal delay curves. This tendency indicates the significance of catalytic function of both of pre-existed and generated $\mathbf{V}^-$-vesicles.

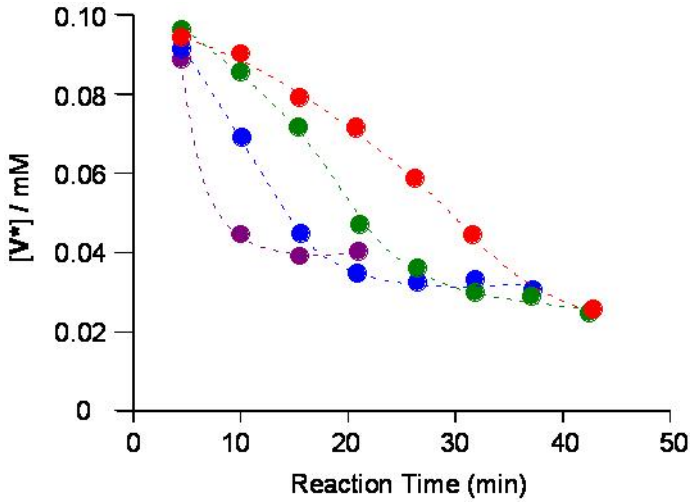

Figure S5. The time courses of concentration of **V*** (initial: 0.1 mM) in a 67 mM phosphate buffer containing 15%(v/v) MeOH measured by HPLC: $[\mathbf{V}^-]_0$ /mM = 0 (red), 0.01 (green), 0.03 (blue), and 0.1 (purple).

SI 4. Hydrolysis of **V*** in several concentrations of buffered solutions.

We noticed that the hydrolysis of **V*** in deionized water (in the absence of $\mathbf{V}^{-}$) was very slow (half life-time > 150 min) but it showed a sigmoidal rise of $\mathbf{V}^{-}$ as shown by the green plot in ***Figure S6***. This phenomenon strongly suggests that the hydrolytic reaction is accelerated by the accumulation of the product ($\mathbf{V}^{-}$). If a **V***-micelle serves as a catalyst efficiently, the reaction must be accelerated from the beginning. However this is not the current case. Therefore we could conclude that $\mathbf{V}^{-}$**C**Vs serve as a catalyst much more efficiently than **V***-micelles.

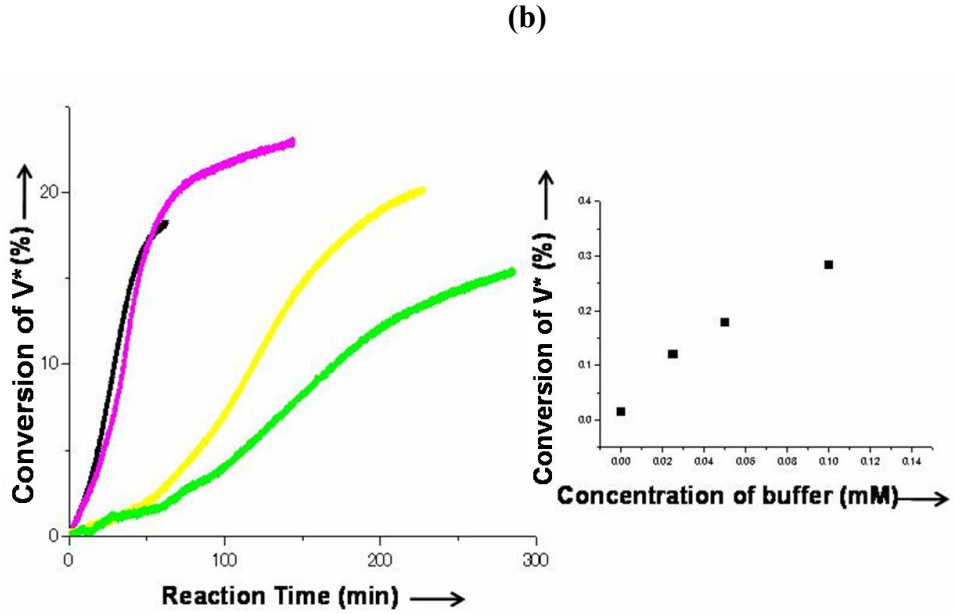

Figure S6. (a) The rate of hydrolysis of **V*** in several concentrations of the phosphate buffer (pH = 5.8) without initial $\mathbf{V}^{-}$**C**V: (green) without phosphate buffer, (yellow) 0.025 mM phosphate buffer, (magenta) 0.05 mM phosphate buffer, (black) 0.1 mM phosphate buffer. (b) The dependence of buffer-concentration on the conversion of **V*** during 1 min reaction.

SI 5. Change of vesicular size associated with hydrolysis of **V***.

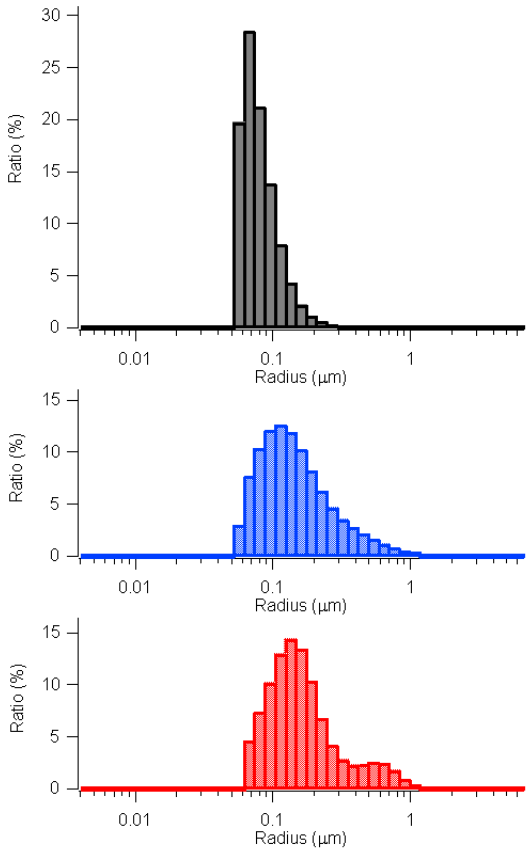

Figure S7. Change in size of $\mathbf{V}^-$-vesicles under the vesicular amplifying reaction ($[\mathbf{V}^-]_0$ = 0.1 mM, [**V***] = 0.1 mM, 67 mM phosphate buffer (pH 5.8) containing 15%(v/v) MeOH) measured by dynamic light scattering: (black) after 10 min; (blue) after 20 min; (red) after 50 min.

SI 6. Change of turbidity of a $\mathbf{V}^{-}$-vesicular suspension.

The turbidity of the suspension of $\mathbf{V}^{-}$-vesicles increases after the addition of **V*** (***Figure S8***). It is independent evidence for the increase in the number of vesicles.

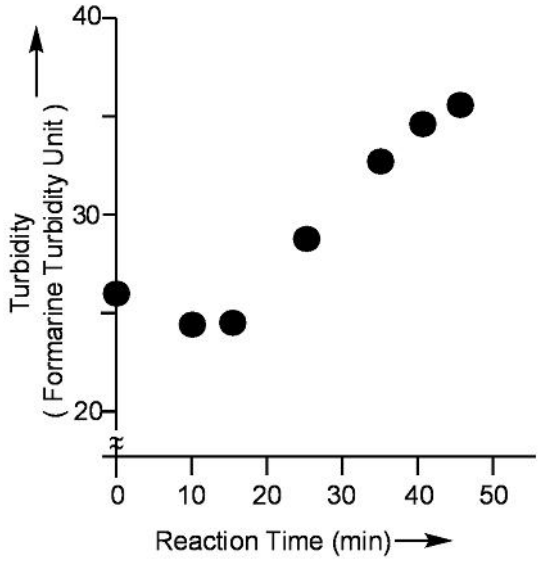

Figure S8. Change of turbidity of a $\mathbf{V}^{-}$-vesicular suspension (1 mM with 30 mol% cholesterol in 1 mL of pH = 5.8 phosphate buffer) caused by the addition of a phosphate buffered solution of **V*** (0.3 mM, 10 mL, pH 5.8).

SI 7. Microscopic images of the morphological change of $\mathbf{V}^-$-vesicles.

The number of $\mathbf{V}^-$-vesicles increased as a result of the auto-catalytic membrane formation (***Figure S9a*** and ***S9b***). When **V*** is hydrolyzed on the protic surface of a vesicle, the generated membrane molecules dissolve into the outer leaflet of the bimolecular membrane, which induces birthing dynamics according to the ADE (area-difference elasticity) model. We observed the vesicular morphological change shown in ***Figure S9c***. First, a new membrane peeled off from the original vesicle, and then this membrane is gradually transformed into a new vesicle.

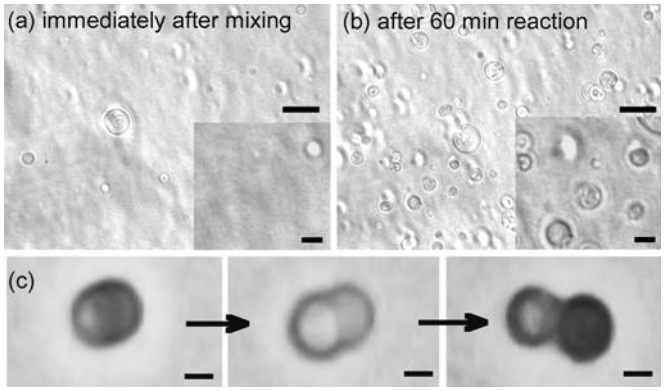

Figure S9. Microscopic image of the morphological change of giant vesicles under the membrane formation ($[\mathbf{V}^-]_0$ = 1 mM, [**V***] = 3 mM, [cholesterol] = 0.3 mM, 67 mM phosphate buffer (pH 5.8) containing 15%(v/v) MeOH): (a) immediately after mixing **V*** and $\mathbf{V}^-$; (b) 60 min after mixing **V*** and $\mathbf{V}^-$; (c) morphological change of giant vesicles (larger one) in the vesicular suspension (all of the vesicles are not necessarily divide likewise). The pictures of (a) and (b) were captured by phase contrast microscope (bar = 5 μm), and the picture of (c) was captured by differential interference microscopy (bar = 2 μm).

SI 8. Microscopic images of the morphological change of $\mathbf{V}^-$-vesicles with Rho-DOPE.

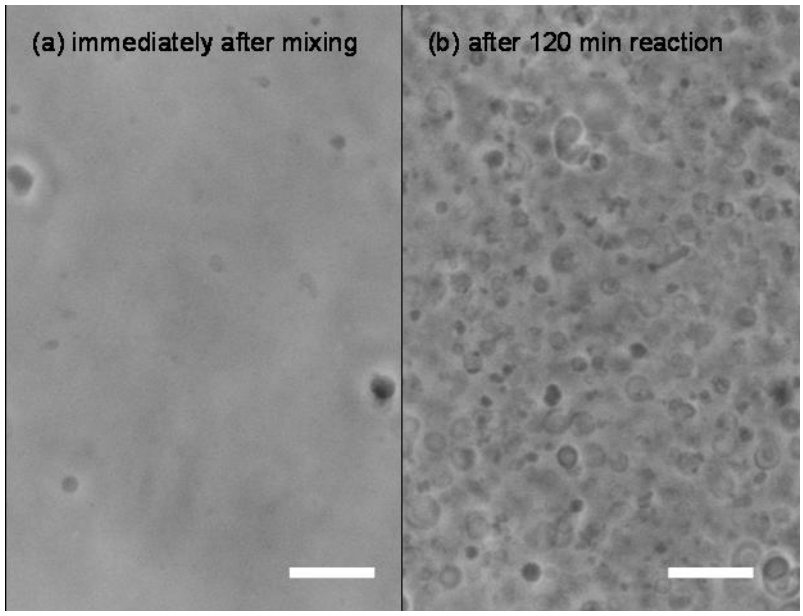

Figure S10. Phase contrast micrographs of vesicles (a) before and (b) 120 min after addition of 5 mM **V*** to Rho-DOPE labeled $\mathbf{V}^-$**C**Vs ($[\mathbf{V}^-]_0$ = 1 mM, [Cholesterol] = 0.3 mM, and [Rho-DOPE] = 10 μM) (bar = 10 μm) in phosphate buffered solution (pH = 5.8).