Supporting Information

Quantitative analysis of the lamellarity of giant liposomes prepared by the inverted emulsion method

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Supporting Information Guide

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1. Supporting Figures and Legends S1 to S8

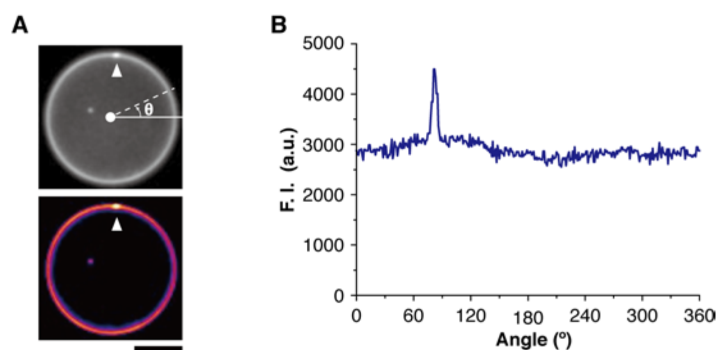

Figure S1 Example of the liposome that had lipid aggregates on the membrane. (*A*) Epi-fluorescence image of the liposome at the equatorial plane (top), and the pseudo-color image (bottom). The liposome had a lipid aggregate on the membrane (white arrowheads). Scale bar: 5 μm. (*B*) Fluorescence intensity profile along circumference of the membrane at the equatorial plane. The intensity profile had a sharp peak due to the lipid aggregate. Such liposomes were not used for the lamellarity analysis.

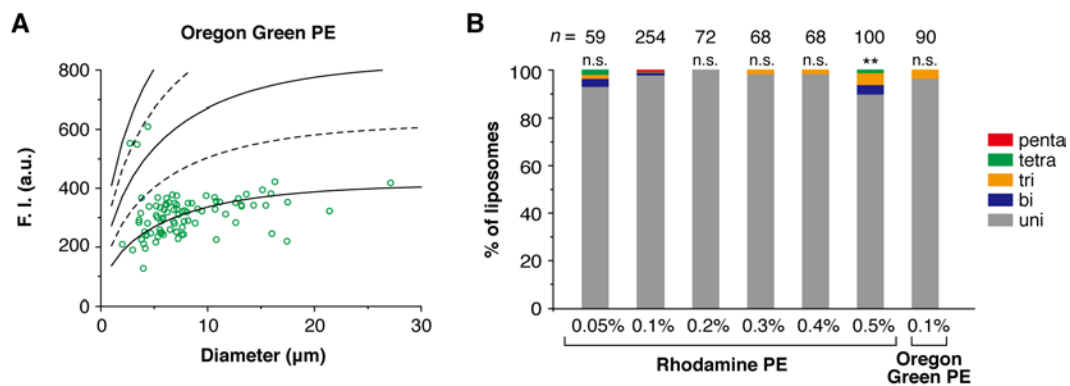

Figure S2 Effect of fluorescently-labeled phospholipids on the lamellarity distribution. (*A*) Plots of the membrane fluorescence intensities of individual liposomes prepared by using 0.1% (mol/mol) Oregon Green PE instead of rhodamine PE. (*B*) Lamellarity distributions of the liposomes at various fluorescently-labeled lipid conditions. The proportions of unilamellar liposomes at each condition were compared with that of egg PC containing 0.1% (mol/mol) rhodamine PE by Z-test and Fisher's exact test. n.s.: not significant ($p > 0.05$), **: $p < 0.01$.

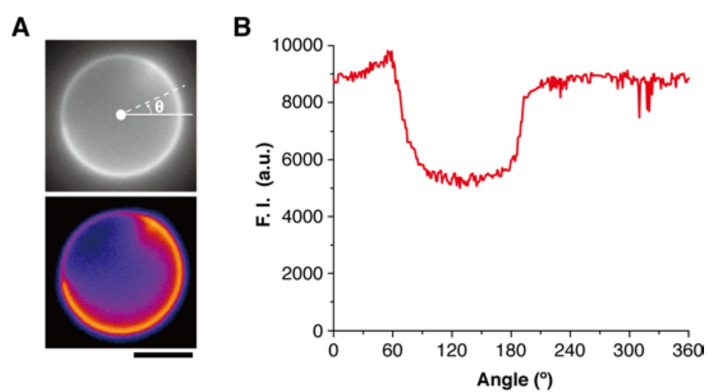

Figure S3 Example of the partially multilamellar liposome. (*A*) Epi-fluorescence image of the partially bi-lamellar liposome at the equatorial plane (top), and the pseudo-color image (bottom). Scale bar: 5 μm. (*B*) Fluorescence intensity profile along circumference of the membrane at the equatorial plane. The fluorescence intensity changed stepwise, indicating that the membrane was partially bi-lamellar. 5 mM Egg PC containing 0.1% (mol/mol) rhodamine PE was used for liposome preparation.

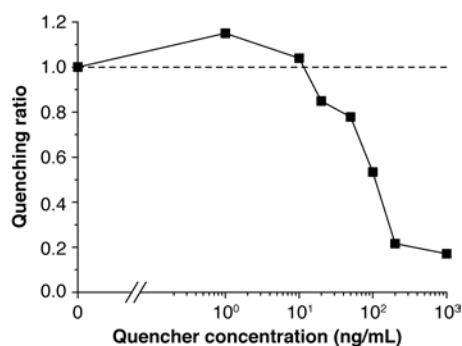

Figure S4 Relationship between the quencher concentration and the membrane fluorescence intensity. The membrane fluorescence quenching ratios at various quencher concentrations were plotted. The quenching ratio was defined by the fluorescence intensity of the unilamellar membrane per unit surface area I_0 in the presence of quencher divided by the original I_0 (in the absence of quencher). For the quencher, QSY7 was used. The quenching ratio decreased monotonically and no plateau phases were observed around the quenching ratio = 0.5, indicating that the quencher QSY7 molecules quenched the fluorescence signal of rhodamine PE not only in the outer leaflet but also in the inner leaflet of the liposomal membrane.

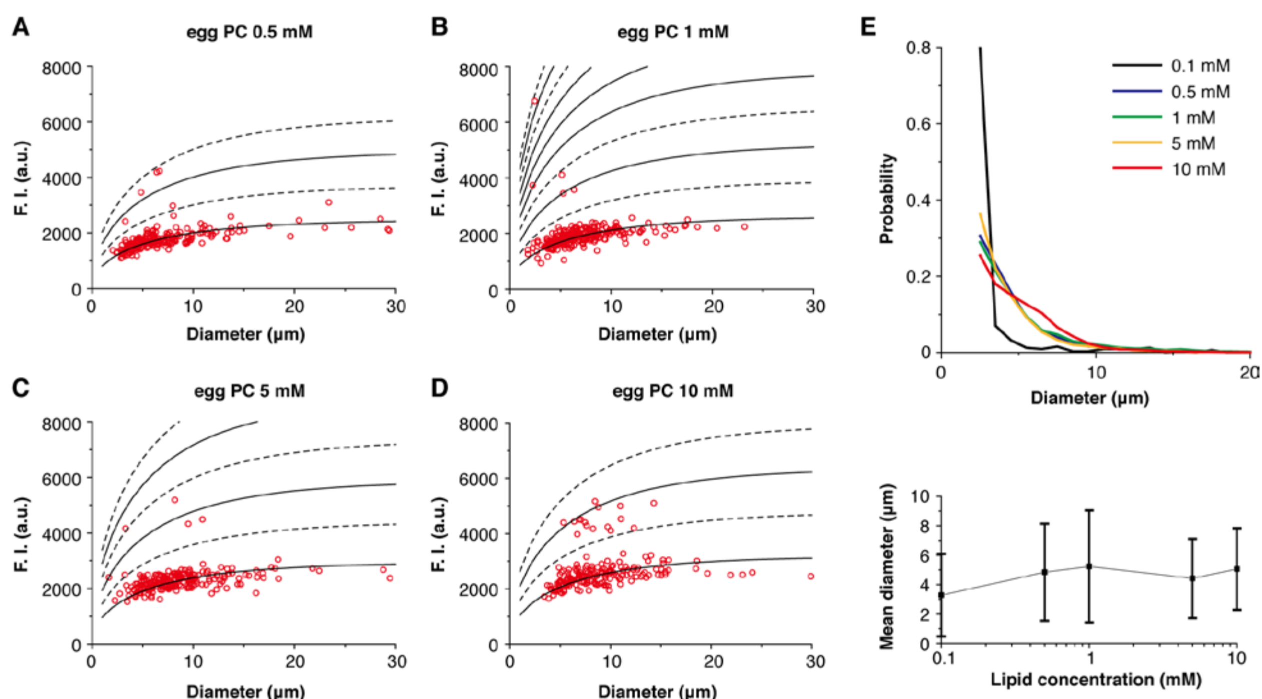

Figure S5 Effects of lipid concentrations on the lamellarity and size of liposomes. (*A–D*) Membrane fluorescence intensities of individual liposomes at various concentrations of egg PC in mineral oil were plotted against the diameter of the liposomes. (*E*) Size distributions of the liposomes at various lipid concentrations (top), and the mean diameter (bottom). Error bars indicate the SD The size distribution and the mean diameter of the liposomes prepared by 0.5 mM or higher than 0.5 mM egg PC showed no significant differences, suggesting that the vesicle size was almost independent of the lipid concentrations dissolved in oil.

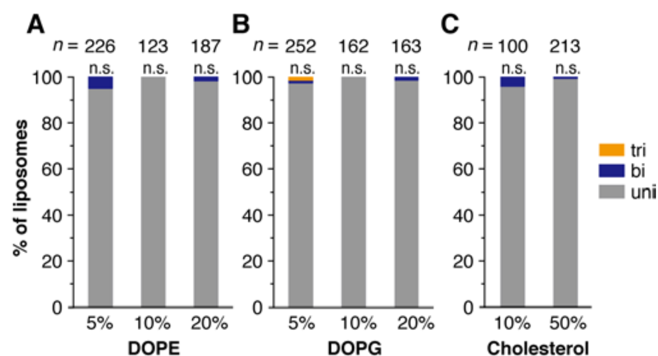

Figure S6 Effects of lipid compositions on the lamellarity of liposomes. Lamellarity distributions of the liposomes (*A*) made of egg PC and 5%, 10%, or 20% (mol/mol) DOPE, (*B*) made of egg PC and 5%, 10%, or 20% (mol/mol) DOPG, and (*C*) made of egg PC and 10 or 50% (mol/mol) cholesterol. In all experiments, the total lipid concentration in oil was fixed at 1 mM and 0.1% (mol/mol) rhodamine PE was added. The proportions of unilamellar liposomes at each lipid composition were compared with 1 mM egg PC by Z-test and Fisher's exact test. In all conditions, the proportions showed no significant differences (n.s.: $p > 0.05$).

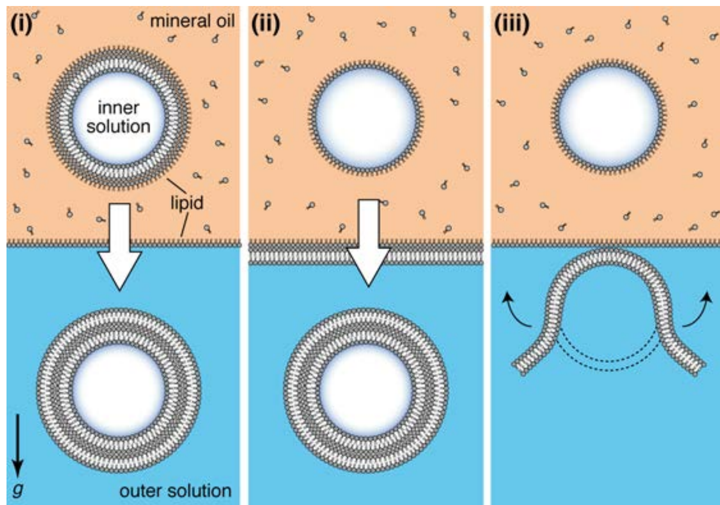

Figure S7 Possible mechanisms of the formation of multilamellar liposomes. Although further experiments are needed to clarify the mechanism of the formation of multilamellar liposomes, the following three possibilities could be expected. (i) Lipid bilayer is occasionally assembled on the lipid monolayer inside the water-in-oil droplet. That is, the water droplet is surrounded by triple lipid layer (mono-layer plus bi-layer). This droplet is transformed into a bilamellar liposome by passing through the oil/outer buffer interface. (ii) Lipid bilayer is occasionally assembled just beneath the lipid monolayer at the oil/outer buffer interface. The droplet passing through this interface is transformed into a bilamellar liposome. (iii) The mechanism of (ii) may be attributable to the following one: Just after the water-in-oil droplet is transformed into a liposome, the liposome may be broken, and the lipid bilayer is spread out on the oil/outer buffer interface. This results in the formation of triple lipid layer at the interface, and thus the droplet passing through this interface is transformed into a bilayer liposome.

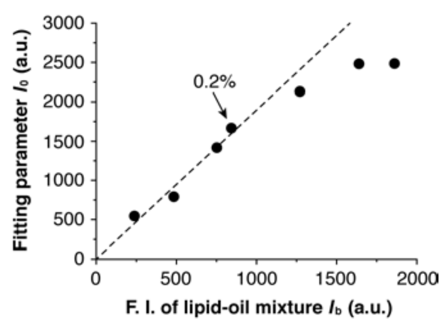

Figure S8 Relationship between I_0 and I_b The fluorescence intensity of the unilamellar membrane per unit surface area I_0 showed linear correlation with the bulk fluorescence intensity of the lipid-oil mixture I_b at low rhodamine PE concentrations (up to ~0.2%). In all experiments, 1 mM egg PC was used and the additive amount of Rhodanime PE was varied.

2. Supporting Movie Legend S1

Movie S1

Time-lapse series of the actin-encapsulated liposome showing spontaneous formation of actin bundles inside the vesicle. The time interval between the frames is 5 min. Every frame shows the maximum projection image. High salt buffer containing α-hemolysin was added to the external solution at 0 min. Actin: 10 μM, Scale bar: 5 μm.

3. Supporting Table Legend S1

Table S1

Lamellarity distributions of liposomes at various lipid concentrations, compositions, and vesicle inclusions are listed. In addition, the proportion of unilamellar liposomes at each condition is compared with that of 1 mM egg PC containing 0.1% (mol/mol) rhodamine PE by Z-test and Fisher's exact test, and the *p*-values obtained are listed.