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Supplemental Information

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Supporting Material

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Figure S1

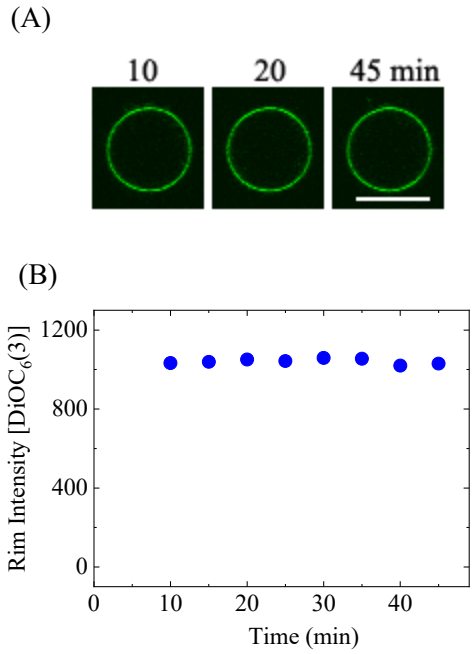

Figure S1: Stability of the rim intensity due to $DiOC_6(3)$.Time course of rim intensity due to $DiOC_6(3)$ in single PG/PC (2/8)-GUVs interacting with 2 nM $DiOC_6(3)$ at $\varphi_m = -59$ mV. (A) CLSM images of the GUVs interacting with 2 nM $DiOC_6(3)$. The numbers above each image show the time in min after the $DiOC_6(3)$ solution was mixed with a GUV suspension. The bar corresponds to 20 μm. (B) The detailed time course of rim intensity shown in Panel (A).

S.1. Effects of negative membrane potential on the entry of CF-TP10 into single PG/PC (2/8)-GUVs containing small GUVs

Figure S2A and S2B indicate that interaction of 0.40 μM CF-TP10 with PG/PC (2/8)-GUVs containing small GUVs at φ_m = – 118 mV and – 28 mV. Figure S2A (1) shows that the FI in the GUV lumen due to AF647 remained essentially constant over 6 min during the interaction of CF-TP10 with the GUV, indicating that AF 647 did not leak during the interaction, i.e., pore formation did not occur in the GUV membrane. Figures S2A (2) and S2C show that for – 118 mV the rim intensity due to CF-TP10 increased gradually to reach a steady value at 210 s. In contrast, initially no fluorescence was observed in the mother GUV lumen, but at 340 s fluorescence stated to emit at small GUVs in the mother GUV lumen (Fig. S2A (2)). P_{entry} (6 min) = 1.0 (n = 15). In contrast, for – 28 mV no entry of CF-TP10 occurred during 6 min (i.e., P_{entry} (6 min) = 0 (n = 10)) (Fig. S2B).

Figure S2

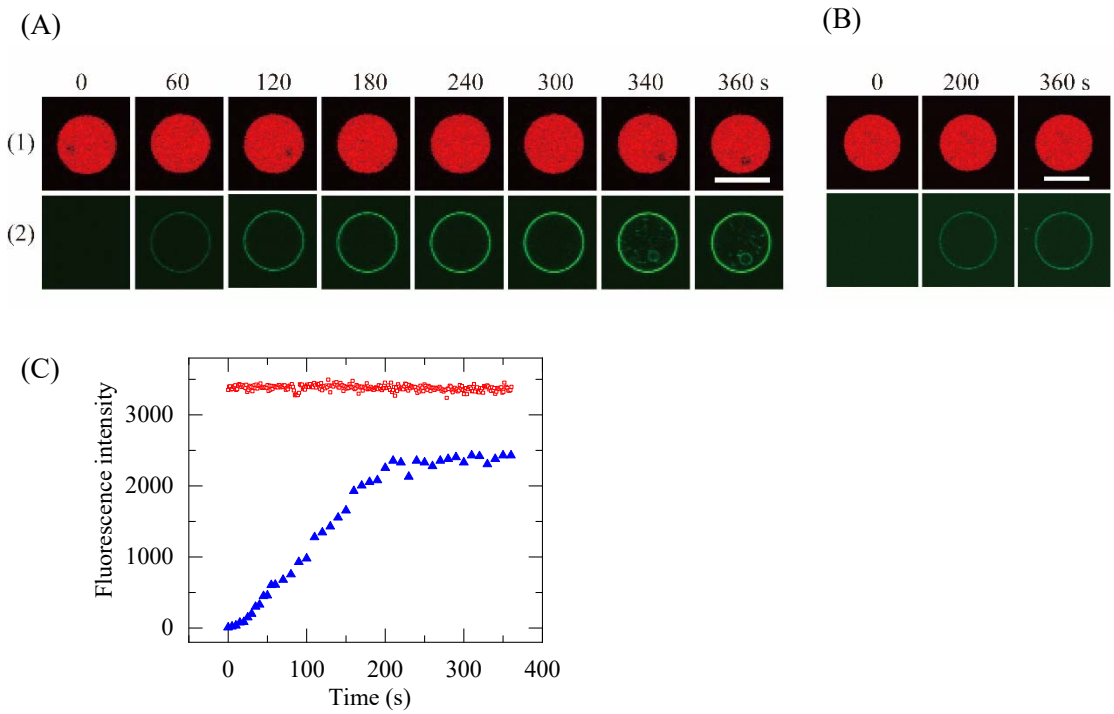

Figure S2: Entry of CF-TP10 into single PG/PC-GUVs containing small GUVs. (A) (B) CLSM images of (1) AF647, and (2) CF-TP10 in a PG/PC (2/8)-GUV for the interaction of 0.40 μM CF-TP10 with the GUV under φ_m = – 118 mV (A) and –28 mV (B). The numbers above each image show the time in seconds after the addition of CF-TP10 was started. The bar corresponds to 20 μm. (C) Time course of the change in the FI of the GUV shown in (A). Red squares and blue triangles correspond to the FI of the GUV lumen due to AF647 and that of the GUV rim due to CF-TP10, respectively.

S.2. Effects of negative membrane potential on the entry of CF-TP10 into single PG/PC (2/8)-GUVs containing LUVs

Figure S3A indicate that interaction of 0.40 μM CF-TP10 with PG/PC (2/8)-GUVs containing LUVs at – 118 mV. Figure S3A (1) shows that the FI in the GUV lumen due to AF647 remained essentially constant over 6 min during the interaction of CF-TP10 with the GUV, indicating that pore formation did not occur in the GUV membrane. On the other hand, the rim intensity due to CF-TP10 increased gradually to reach a steady value at 250 s. In contrast, initially the FI of the GUV lumen due to CF-TP10 was zero, but after 300 s, it gradually grew with time without pore formation (Fig. S3A (2) and S3C). These results indicate the CF-TP10 entered the GUV lumen. In contrast, at – 28 mV, the FI of the GUV lumen due to CF-TP10 did not increase up to 6 min (Fig. S3B (2)), indicating no entry of CF-TP10 into the GUV lumen.

Figure S3

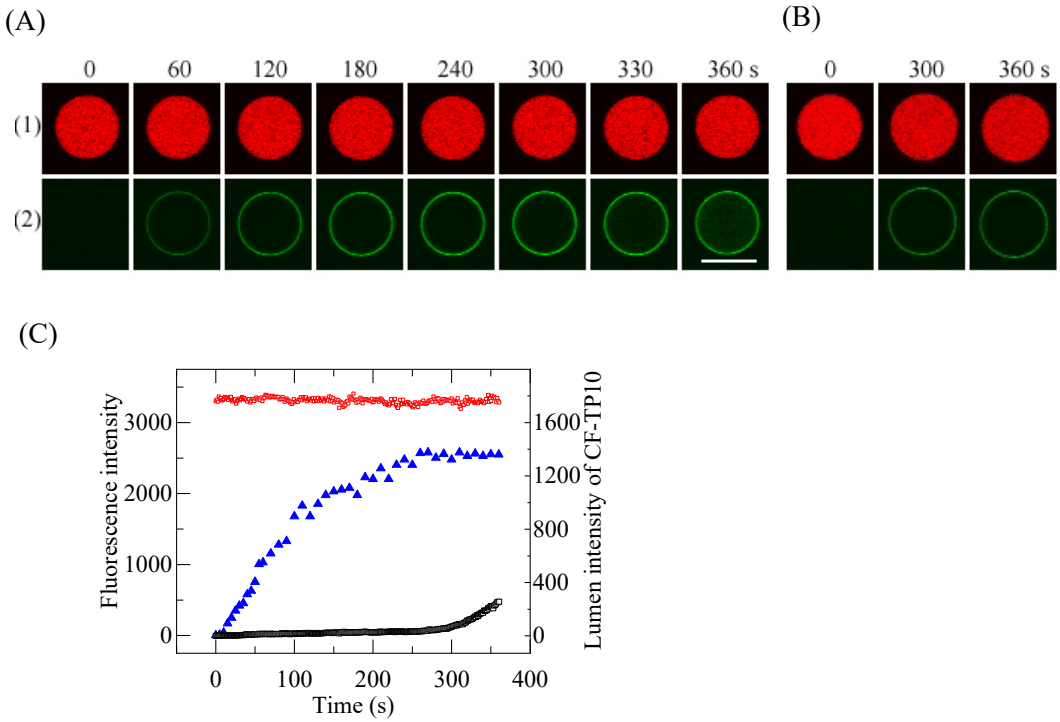

Figure S3: Entry of CF-TP10 into single PG/PC-GUVs containing LUVs. (A) (B) CLSM images of (1) AF647 and (2) CF-TP10 in a PG/PC (2/8)-GUV for the interaction of 0.40 μM CF-TP10 with the GUV under φ_m = – 118 mV (A) and –28 mV (B). The numbers above each image show the time in seconds after the addition of CF-TP10 was started. The bar corresponds to 20 μm. (C) Time course of the change in the FI of the GUV shown in (A). Red squares correspond to the FI of the GUV lumen due to AF647. Blue triangles and black squares correspond to the FI of the GUV rim and the GUV lumen due to CF-TP10, respectively.

Figure S4

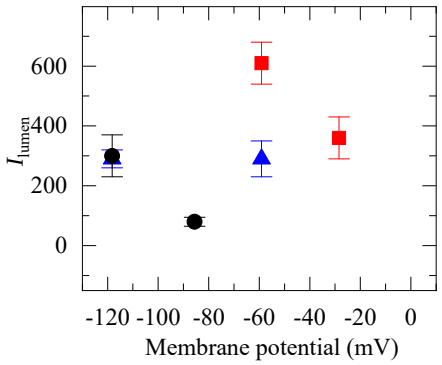

Figure S4: Effect of membrane potential on I_{lumen} (6 min) without pore formation. (red ■) 0.50 μM, (blue ▲) 0.40 μM, (●) 0.30 μM CF-TP10. For each condition, the mean values and SEs of I_{lumen} (6 min) of only the GUVs which CF-TP10 entered.

Figure S5

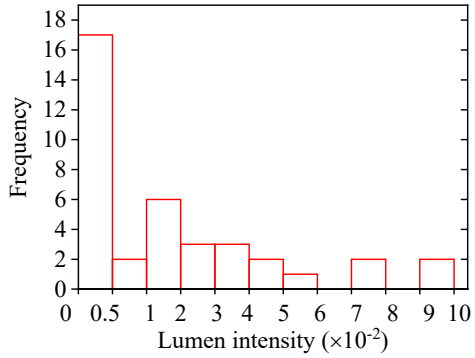

Figure S5: The histogram of the frequency of I_{lumen} (6 min) for 0.50 μM CF-TP10 with the GUV under φ_m = – 28 mV.