Supporting Information (SI)

Effects of Mechanical Properties of Lipid Bilayers on Entry of Cell-Penetrating Peptides into Single Vesicles

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S1. Relationship between the rim intensity and the CF-TP10 concentration in a GUV membrane.

In the analysis of the time course of the rim intensity (i.e., the fluorescence intensity of a GUV membrane), we used the assumption that the CF-TP10 concentration in a GUV membrane, $C_M(t)$, is proportional to the rim intensity. Recently, it is reported that self-quenching of fluorescent probe-labeled CPPs in the membrane occurred in a highly charged membrane (i.e., 100% negatively charged DOPG membrane) based on the experiments using the large unilamellar vesicle (LUV) suspension method.[1] However, the self-quenching of fluorescent probes greatly depends on the distance between the probes, r (according to the Förster theory,[2] the rate of energy transfer, k_T, is proportional to $(1/r)^6$), and thereby the concentration, C_M, of the probe in a lipid membrane. Since $C_M \propto (1/\langle r \rangle)^2$ where $\langle r \rangle$ is the average distance between the probes, we can roughly estimate that k_T increases greatly with an increase in C_M. C_M in 100% DOPG membrane is larger than C_M in DOPG/DOPC (2/8) membrane with lower surface charge density in our case, and hence k_T in 100% DOPG membrane is much larger than k_T in DOPG/DOPC (2/8) membrane. Therefore, we can consider that self-quenching of fluorescent probe-labeled CPPs in DOPG/DOPC (2/8) membrane is much smaller than that in 100% DOPG membrane. Moreover, we try to examine experimentally the relationship between $C_M(t)$ is and the rim intensity in DOPG/DOPC (2/8) membrane used in our experiments. We can approximate that $C_M(t)$ is proportional to the CF-TP10 concentration in aqueous solution near a GUV, C_{out}^{eq}, for only low concentrations of C_{out}^{eq}, and for high concentrations, $C_M(t)$ values are lower than the expected values from the linearity due to the decrease in the binding site in the membrane (Langmuir adsorption). Therefore, for the above purpose we can examine the relationship between the rim intensity and C_{out}^{eq}. Figure S1 shows one example of the relationship between the rim intensity and C_{out}^{eq} for the data of Figure 2 C (σ = 0.2 mN/m). Based on this data, we can approximate that

the rim intensity is proportional to $C_{\mathrm{out}}^{\mathrm{eq}}$, although there is some deviations due to the decrease in the binding sites. Therefore, we can approximate that $C_{\mathrm{M}}(t)$ is proportional to the rim intensity under our experimental conditions.

Figure S1

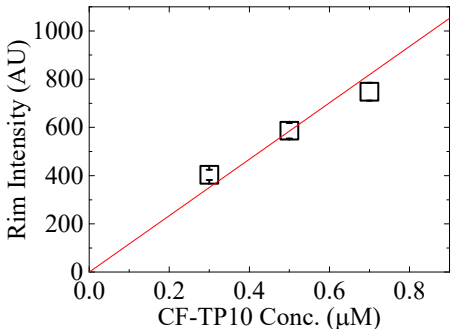

Figure S1: Relationship between the rim intensity and the CF-TP10 concentration in aqueous solution near a GUV, $C_{\mathrm{out}}^{\mathrm{eq}}$. The rim intensities of the steady state of the GUVs used for the analysis of Figure 2C (σ = 0.2 mN/m) were obtained, and their mean values and standard errors at each $C_{\mathrm{out}}^{\mathrm{eq}}$ were determined from the data of 20–26 GUVs.

Figure S2

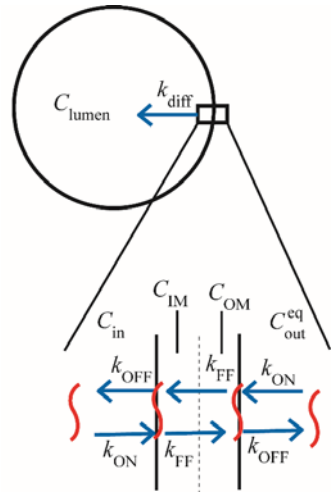

Figure S2: A schematic diagram for the elementary processes for the entry of CF-TP10. C_{lumen}, C_{in}, and $C_{\mathrm{out}}^{\mathrm{eq}}$ are respectively the CF-TP10 concentration in the GUV bulk lumen, in the GUV lumen adjacent to the membrane, and in the aqueous solution outside the GUV adjacent to the membrane. C_{OM} and C_{IM} are the CF-TP10 concentration in the external and internal monolayer of the GUV, respectively. k_{ON}, k_{OFF}, and k_{diff} are the rate constants for the binding of CF-TP10 to the monolayer of a GUV from aqueous solution, for the unbinding of CF-TP10 from the monolayer and release into the aqueous solution adjacent to the membrane, and for the diffusion from the GUV lumen adjacent to the membrane and into the bulk lumen (i.e., the central region of the GUV). k_{FF} is the rate constant for the translocation of CF-TP10 from one monolayer to the other. This figure is reprinted from ref. 4 with permission from the American Chemical Society.

S2. Time course of CF-TP10 concentration in a DOPG/DOPC/chol-GUV membrane

To analyze the rim intensity more accurately, we investigated the interaction of CF-TP10 with single DOPG/DOPC/chol (2/6/4)-GUVs not containing small vesicles. Figure S3A shows the effect of the interaction of 4.0 μM CF-TP10 with single GUVs. During the addition of the 4.0 μM solution of CF-TP10, the fluorescence intensity inside the GUV remained essentially constant over the first 246 s, after which it gradually decreased (Figure S3A (1) & red data points in Figure S3B), indicating that pore formation occurred at 246 s. On the other hand, Figure S3A(2) shows that the rim intensity rapidly increased to a steady value, I_1, at around 45 s, then remained constant until 230 s (green square in Figure S3B). This result indicates that the steady binding of CF-TP10 between the aqueous phase and the GUV membrane was attained at ~45 s and that the surface concentration of CF-TP10 remained constant for ~180 s. At 230 s (16 s before the start of membrane permeation), the rim intensity restarted to increase and after 21 s reached another steady value, I_2.

As we described in our previous paper,[3] CF-TP10 can translocate from the outer monolayer to the inner monolayer of DOPG/DOPC (2/8)-GUVs gradually and continuously (i.e., the rate constant of transfer of CF-TP10 between two monolayers, k_{FF}, is large), and enter the GUV lumen before pore formation. The scheme and the mechanism of the translocation of CF-TP10 in the membranes containing high concentration of cholesterol are greatly different from those in the membranes without cholesterol. The results of Figure 4 and Figure 5 show the two-step increase in the rim intensity, and the result of Figure S3B indicates the ratio of the intensities of two stationary states (I_2/I_1) is almost 2.0. Therefore we can reasonably conclude that high concentration of cholesterol essentially inhibited the translocation of CF-TP10 from the outer to the inner monolayer. This is the same as in the interaction of CF-R_9 with DOPG/DOPC/chol (2/6/4)-GUVs.[4] At the state corresponding I_1, CF-TP10 exists only in the outer monolayer and the intensity of I_1 corresponds to the equilibrium concentration of the outer monolayer. The time t_1 when the rim intensity started to increase from I_1 to I_2 corresponds to the time when the transfer of CF-TP10 from the outer to the inner monolayer started. Therefore, we observed the entry of CF-TP10 into a GUV after t_1, because CF-TP10 entered the GUV from the inner monolayer. According to this scenario, CF-TP10 concentration in the GUV membrane, $C_M(t)$ [M], is approximately equal to the CF-TP10 concentration in the outer monolayer, C_{OM} [M]. In our previous paper, we derived the equation of $C_M(t)$ in this situation;[4]

$$C_M(t) = A\,[1 - \exp(-k_{app}t)] \quad \text{(S1)}$$

$$\text{where} \quad k_{app} = k_{ON}C_{out}^{eq} + k_{OFF} \quad \text{(S2)}$$

The time course of the fluorescence intensity of the rim of the GUV (Figure S3B) was fit well by eq. S1, which gave a value for the rate constant k_{app} of 5.3×10^{-2} s^{-1}. We also obtained the dependence of k_{app} on CF-TP10 concentration. Figure S3C shows that k_{app} increased linearly with an increase in CF-TP10 concentration, C_{out}^{eq}, which agrees with eq. S2. Fitting the data to eq. S2 in Figure S3C provided values $k_{ON} = (1.3 \pm 0.1) \times 10^4$ $M^{-1}s^{-1}$ and $k_{OFF} = (1.0 \pm 1.5) \times 10^{-3}$ s^{-1}. Within experimental errors, the value of k_{ON} for the membrane containing cholesterol were almost half of that for the membranes without cholesterol (for DOPG/DOPC (2/8), $k_{ON} = (2.2 \pm 0.3) \times 10^4$ $M^{-1}s^{-1}$).[3] Since $C_M(t) = (C_{OM} + C_{IM})/2$ in the case of DOPG/DOPC (2/8)-GUVs, if we consider $C_{IM} = 0$ we can explain reasonably the above result. Therefore this result supports that CF-TP10 exists only in the outer monolayer of GUVs containing cholesterol before starting the increment of the rim intensity from I_1 to I_2, indicating $k_{FF} \approx 0$. This is the same result as the interaction of CF-R_9 with DOPG/DOPC/chol (2/6/4)-GUVs.[4]

Figure S3

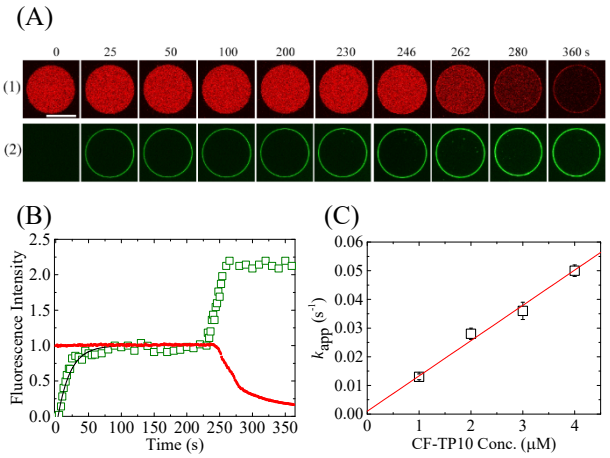

Figure S3: Time course of the change in rim intensity due to CF-TP10 in single DOPG/DOPC/chol (2/6/4)-GUVs not containing small vesicles induced by CF-TP10. (A) CLSM images of (1) AF647 and (2) CF-TP10. The numbers above each image show the time in seconds after 4.0 μM CF-TP10 addition was started. The bar corresponds to 20 μm. (B) Time course of the change in normalized fluorescence intensity of the GUV shown in (A). Red data points and green squares correspond to the fluorescence intensity of AF647 in the GUV lumen and of CF-TP10 at the rim of the GUV, respectively. The solid black line represents the best fit curve using eq. S1. (C) Dependence of k_{app} on CF-TP10 concentration in aqueous solution outside the GUV. The time course of the change in the fluorescence intensity of 14-17 GUVs with a diameter of 20-30 μm was measured. The mean values and SEs of k_{app} are shown. The solid red line represents the best fit curve using eq. S2.

Figure S4

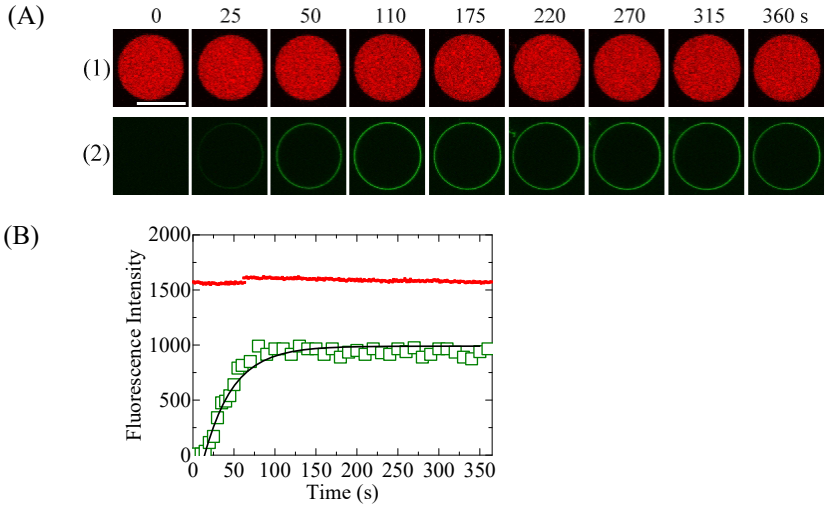

Figure S4: Time course of the change in rim intensity due to CF-TP10 in single DOPG/DOPC/chol (2/6/4)-GUVs not containing small vesicles induced by CF-TP10. (A) CLSM images of (1) AF647 and (2) CF-TP10. The numbers above each image show the time in seconds after 2.0 μM CF-TP10 addition was started. The bar corresponds to 30 μm. (B) Time course of the change in fluorescence intensity of the GUV shown in (A). Red data points and green squares correspond to the fluorescence intensity of AF647 in the GUV lumen and of CF-TP10 at the rim of the GUV, respectively. The solid black line represents the best fit curve using eq. S1.

Figure S5

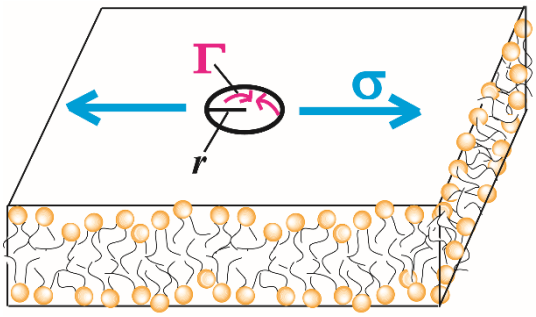

Figure S5: A scheme of a prepore in a lipid bilayer. σ is lateral tension, Γ is line tension at the edge of a prepore with radius r.

References

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