Supporting Information

Entry of a Six-Residue Antimicrobial Peptide Derived from Lactoferricin B into Single Vesicles and *Escherichia coli* cells without Damaging their Membranes

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S.1. Interaction of SYTOX green with *E.coli* cells adsorbed on the glass surface in buffer A

To examine whether *E. coli* cells adsorbed on the glass surface in buffer A were live or dead cells, we investigated the interaction of SYTOX green with these *E. coli* cells, because the SYTOX green staining is the standard method to distinguish dead cells from live cells.[1,2] Using the method described in the Materials and Methods section, *E. coli* cells in buffer A were settled down onto the glass surface in a microchamber set in a confocal laser scanning microscope (CLSM). For CLSM measurements, fluorescence images of SYTOX green (473 nm laser) were obtained using a 60×objective (UPLSAPO060X0, Olympus) (NA = 1.35). For the interaction of SYTOX green solution with single cells of *E. coli,* 5.0 μM SYTOX green solution in buffer A prepared according to the Materials and Methods section was added continuously to the neighborhood of single cells of *E. coli* through a 20-μm-diameter glass micropipette positioned by a micromanipulator. Figure S1 (A) shows a typical result of the interaction of SYTOX green with an adsorbed *E. coli* cell on the glass surface in buffer A. No fluorescence increase was detected in the cell, indicating that the cell was live one. We made the same experiments using 10 cells of *E. coli* under the same situations, and obtained the same results. On the other hand, as a control experiment, we investigated the interaction of 5.0 μM SYTOX green solution in buffer A with single cells of *E. coli,* which were treated with 70% 2-propanol according to the Materials and Methods section and then resuspended in buffer A, which is considered dead cells. Figure S1 (B) shows a typical result of the interaction of SYTOX green with the 2-propanol-treated *E. coli* cell on the glass surface in buffer A. Fluorescence intensity inside the cell rapidly increased within a short time (Figure S1 (C)), showing that SYTOX green entered into the cytoplasm and bound with DNA molecules, indicating that the cell was dead one. We made the same experiments using 5 cells of *E. coli* under the same situations, and obtained the same results.

Figure S1

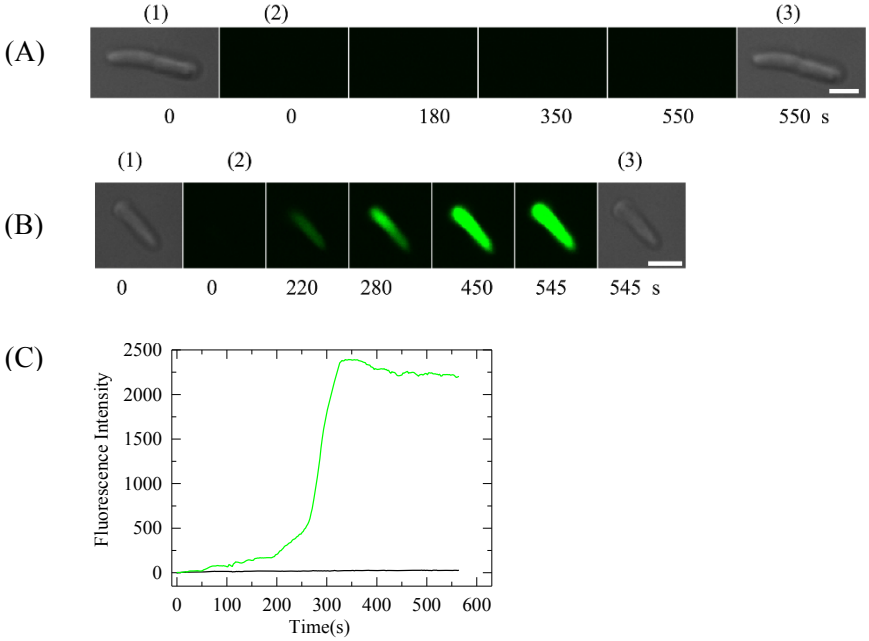

Figure S1: Interaction of SYTOX green with *E. coli* cells. (A) An adsorbed *E. coli* cell on the glass surface in buffer A, (B) an adsorbed *E. coli* cell, which was treated with 70% 2-propanol, on the glass surface in buffer A. (A) (B) CLSM images of (1) (3) DIC and (2) STTOX green. The numbers below each image show the time in seconds after the addition of SYTOX green solution was started. The bar is 2.0 μm. (C) Time course of fluorescence intensity of *E. coli* cells shown in (A) and (B) are represented by a black line and a green line, respectively.

Figure S2

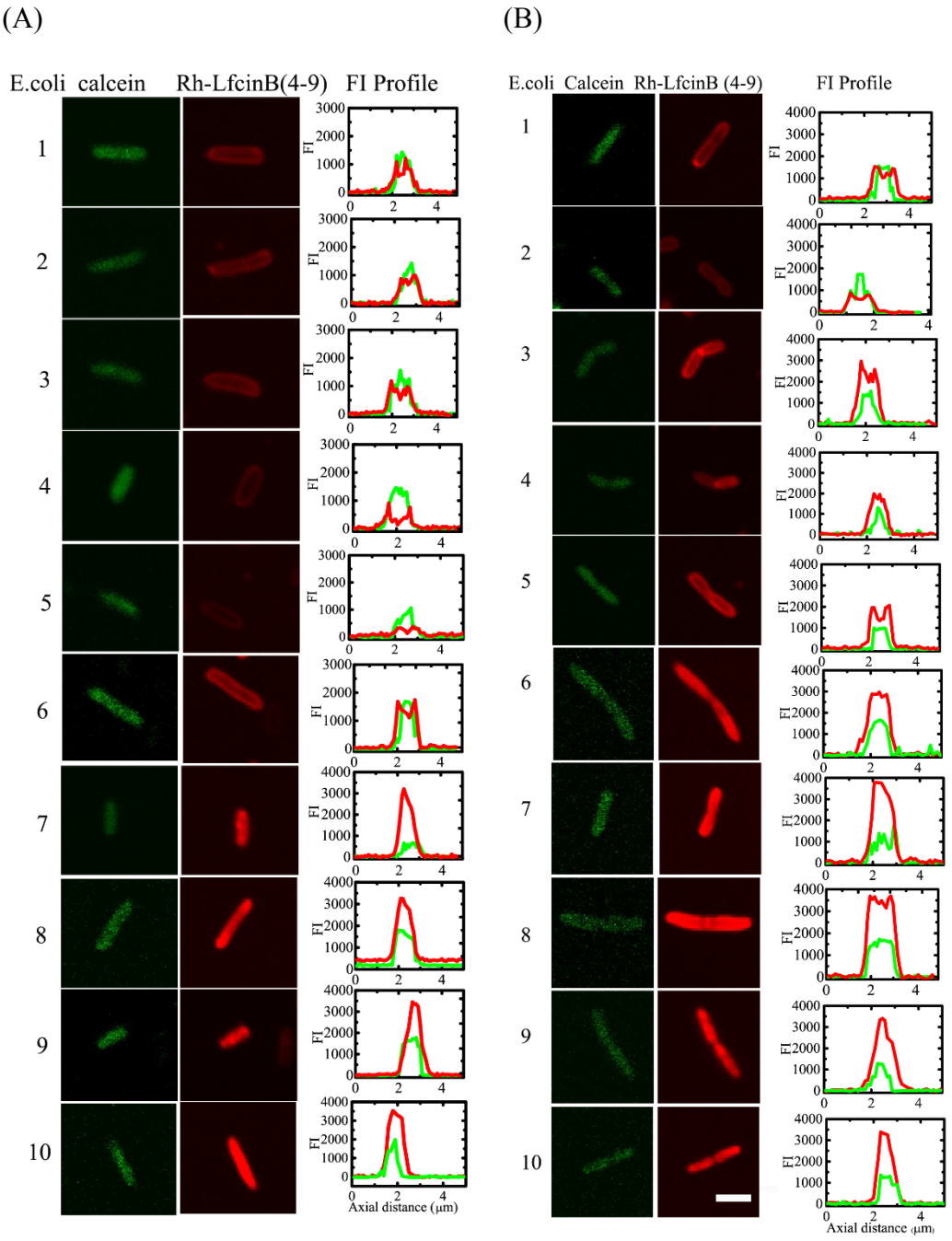

Figure S2: Interactions of 5.0 μM Rh-LfcinB (4-9) with single cells of *E. coli* containing calcein. (A) Non-septating cells, (B) Septating cells. The images of all the examined *E. coli* cells and FI profiles along a line perpendicular to the long axis of the cells after the interaction with Rh-LfcinB (4-9) for 10 min. CLSM images due to calcein (left images with green color) and Rh-LfcinB (4-9) (right images with red color). The bar is 2.0 μm. The figure on the right side of each CLSM image shows the FI profile along a line perpendicular to the long axis of the cells, where green and red curves correspond to FI of calcein and of Rh-LfcinB (4-9), respectively.

Figure S3

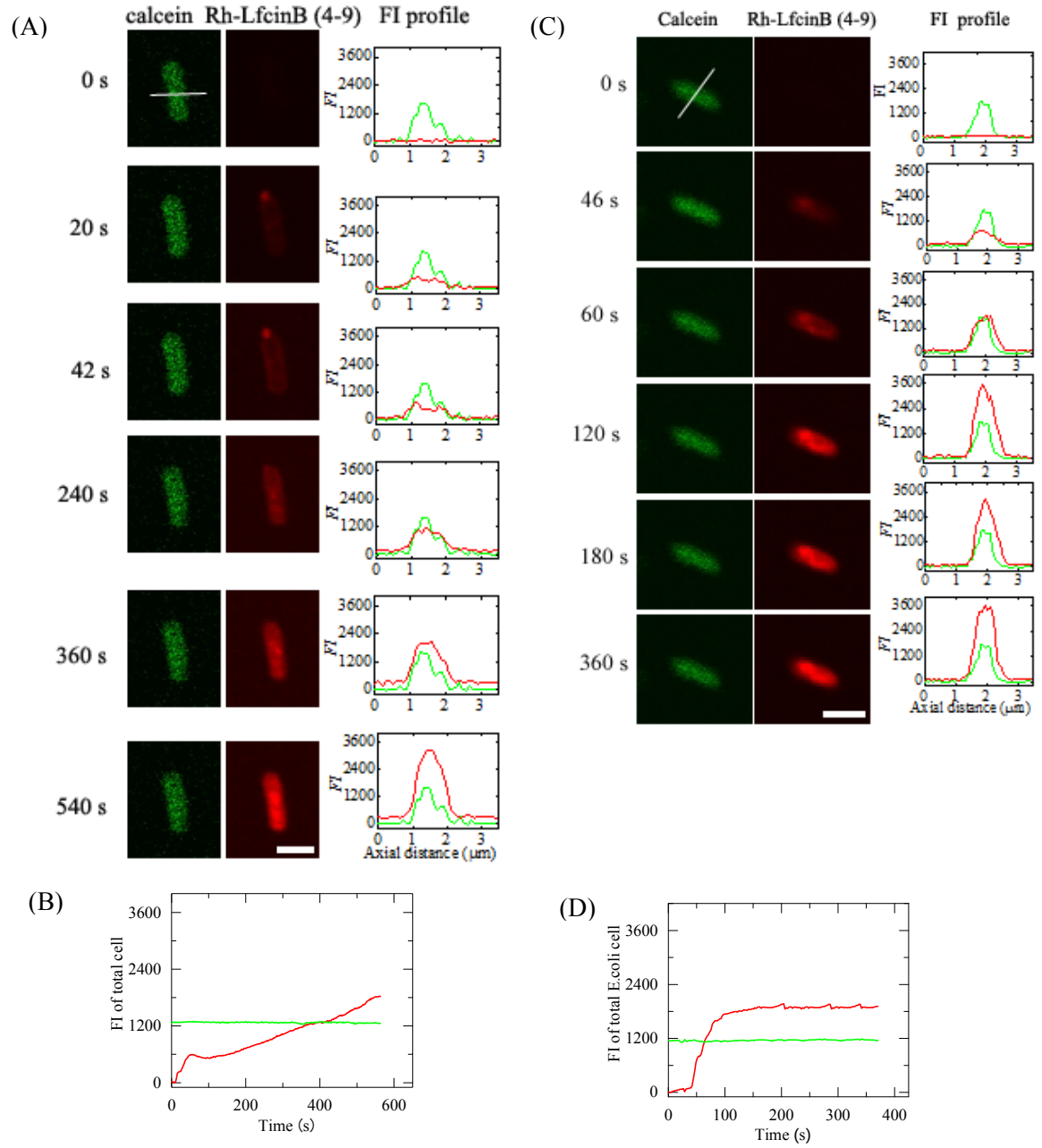

Figure S3: Interactions of 9.0 μM Rh-LfcinB (4-9) with single non-septating cells of *E. coli* containing calcein. (A) (C) CLSM images due to calcein (left images with green color) and Rh-LfcinB (4-9) (right images with red color). The number on the left side of each image shows the time in seconds after the addition of 9.0 μM Rh-LfcinB (4-9) was started. The bar is 2.0 μm. The figure on the right side of each CLSM image shows the FI profile along the white line in the calcein image at 0 s, where green and red curves correspond to FI of calcein and of Rh-LfcinB (4-9), respectively. (B) and (D) show change in the FI of the total cell of *E. coli* during the interaction of Rh-LfcinB (4-9) over time shown in (A) and (C), respectively. Green and red lines correspond to the FI of calcein and of Rh-LfcinB (4-9) in the cell of *E. coli*, respectively.

Figure S4

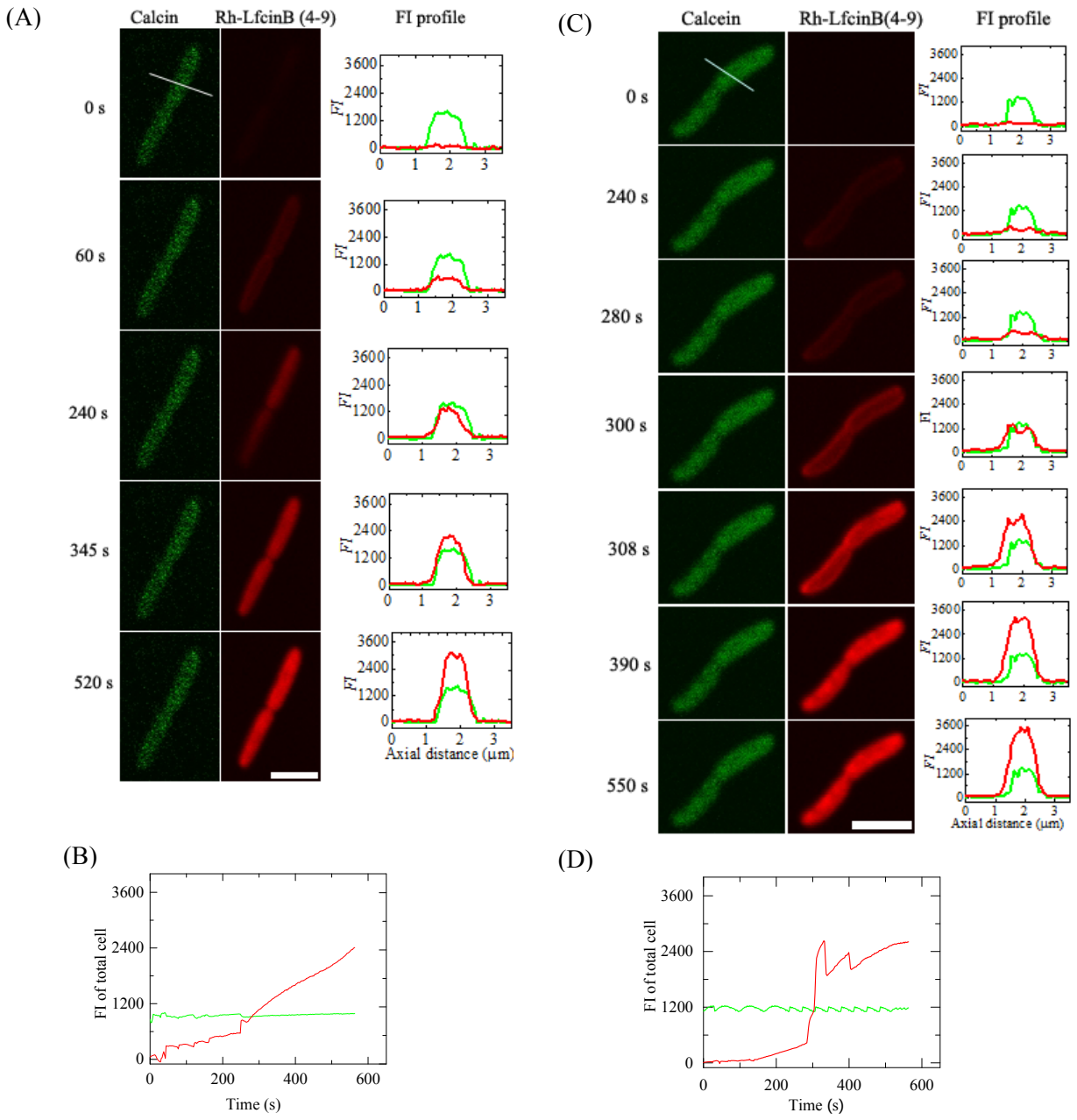

Figure S4: Interactions of 9.0 μM Rh-LfcinB (4-9) with single septating cells of *E. coli* containing calcein. (A) (C) CLSM images due to calcein (left images with green color) and Rh-LfcinB (4-9) (right images with red color). The number on the left side of each image shows the time in seconds after the addition of 9.0 μM Rh-LfcinB (4-9) was started. The bar is 2.0 μm. The figure on the right side of each CLSM image shows the FI profile along the white line in the calcein image at 0 s, where green and red curves correspond to FI of calcein and of Rh-LfcinB (4-9), respectively. (B) and (D) show change in the FI of the total cell of *E. coli* during the interaction of Rh-LfcinB (4-9) over time shown in (A) and (C), respectively. Green and red lines correspond to the FI of calcein and of Rh-LfcinB (4-9) in the cell of *E. coli*, respectively.

Figure S5

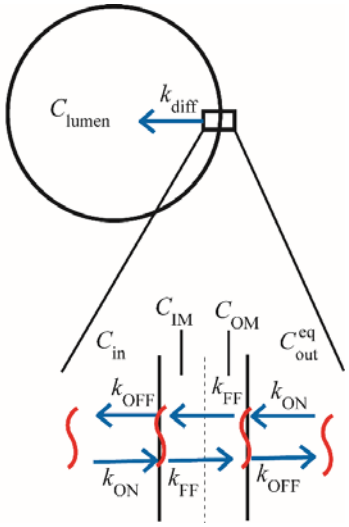

Figure S5: Scheme of the elementary processes for the entry of Rh-LfcinB (4-9). C_{lumen}, C_{in}, and C_{out}^{eq} are respectively the Rh-LfcinB (4-9) concentration in the GUV bulk lumen (i.e., the central region of the GUV), in the GUV lumen adjacent to the membrane, and in the aqueous solution outside the GUV adjacent to the membrane. C_{OM} and C_{IM} are the Rh-LfcinB (4-9) concentration in the external and the internal monolayer of the GUV, respectively. k_{ON}, k_{OFF}, and k_{diff} are the rate constants for the binding of Rh-LfcinB (4-9) to the monolayer of a GUV from aqueous solution, for the unbinding of Rh-LfcinB (4-9) 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. k_{FF} is the rate constant for the translocation of Rh-LfcinB (4-9) from one monolayer to the other. This figure is reprinted from ref. 3 with permission from the American Chemical Society.

Figure S6

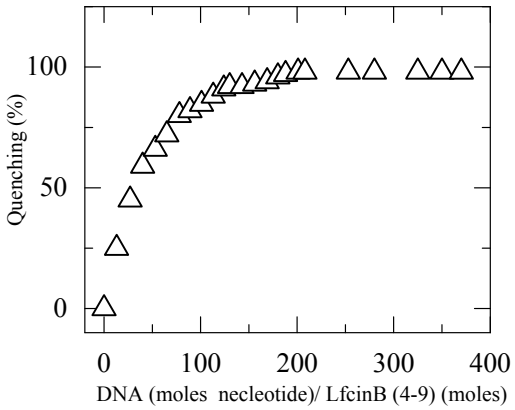

Figure S6: Quenching of fluorescence of LfcinB (4-9) by DNA. DNA solution was added to LfcinB (4-9) solution. The amount of quenching expressed by percentage is the normalized fluorescence change from the initial LfcinB (4-9) solution; $(FI_0 - FI) / FI_0$, where FI_0 is the FI of LfcinB (4-9) in the absence of DNA and FI is that in the presence of DNA. The quenching is plotted as a function of the molar ratio of nucleotides of DNA to LfcinB (4-9), which was obtained by replotting Figure 6D.

References:

(1) Roth, B. L., Poot, M., Yue, S. T., and Millard, P. J. (1997) Bacterial viability and antibiotic susceptibility testing with SYTOX green nucleic acid stain. *App. Env. Microbiol*. *63*, 2421-2431.

(2) Mukherjee, S., Zheng, H., Derebe, M. G., Callenberg, K. M., Patch, C. L., Rollins, D., Propheter, D., Rizo, J., Grabe, M., Jiang, Q.-X., and Hooper, L. V. (2014) Antibacterial membrane attack by a pore-forming intestinal C-type lectin, *Nature 305*, 103-107.

(3) Sharmin, S., Islam, M. Z., Karal, M. A. S., Shibly, S. U. A., Dohra, H., and Yamazaki, M. (2016) Effects of lipid composition on the entry of cell-penetrating peptide oligoarginine into single vesicles. *Biochemistry*, *55*, 4154-4165.