Supporting Information

Membrane potential is vital for rapid permeabilization of plasma membranes and lipid bilayers by the antimicrobial peptide lactoferricin B

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Figure S1

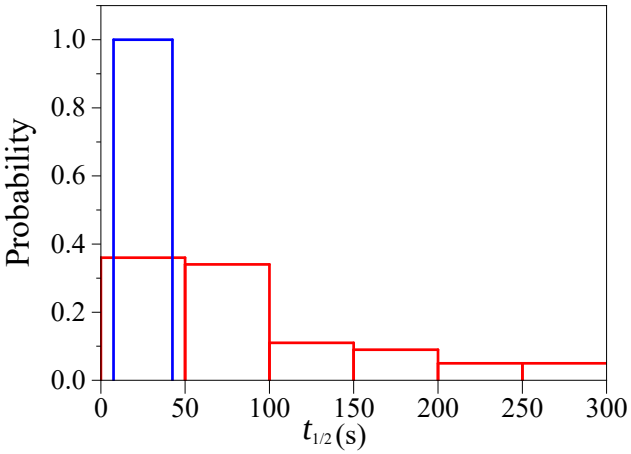

Figure S1. The rate of LfcinB-induced leakage of calcein from single cells interacting with 3.0 μM LfcinB. The distribution of half-time (i.e., $t_{1/2}$) of leakage of calcein in all the examined cells is shown using a histogram. Blue bar and red bar indicate the probability of each range of $t_{1/2}$ in septating cells and in non-septating cells, respectively.

1. Preparation methods of spheroplasts of *E. coli* cells

An *E. coli* suspension was subcultured on nutrient agar plates at 37 °C for overnight to get single colonies. A single colony of bacteria was then grown in Nutrient Broth medium for 10 to 12 h at 37 °C in an incubator shaker (MBR-032P. TAITEC, Koshigaya, Japan) at 200 rpm. After diluting this culture 10 times (final volume: 5 mL), 60 μL of 5 mg/mL cephalexin was added to this culture (final cephalexin concentration; 60 μg/mL). Then, the culture was shaken at 42 °C for 10–12 h, producing single-cell filaments with 50–150 μm length, which were confirmed using CLSM. We obtained these filaments as a pellet by centrifugation of the above suspension at 1500×g for 5 min, then rinsed the pellet (without resuspension) by gentle addition of 1 mL of 0.8 M sucrose, and incubated it at room temperature for 1 min. The supernatant was removed using a Pasteur pipette and resuspended in 2.5 mL of 0.8 M sucrose. We added the following reagents to this suspension in order; 150 μL of 1 M Tris-HCl (pH 7.8), 120 μL of 5 mg/mL lysozyme, 30 μL of 5 mg/mL DNase I, and 120 μL of 0.125 M EDTA-Na (pH 8.0), and then incubated it at room temperature for 8–10 min. To stop the reaction, we added 1 mL of a solution containing 20 mM $MgCl_2$, 0.7 M sucrose, and 10 mM Tris-HCl (pH 7.8) over 1 min while stirring, and incubated it at room temperature for 4 min. We layered 2 mL of this suspension over 4 mL buffer (10 mM Tris-HCl (pH 7.8) containing 1.5 mM KCl, 48.5 mM NaCl, and 0.73 M sucrose (or 10 mM Tris-HCl (pH 7.8) containing 10 mM $MgCl_2$ and 0.8 M sucrose (1,2)), and then concentrated it using Vivaspin 6 (100 kDa MWCO, GE Healthcare, Buckinghamshire, UK) by centrifugation at 6000×g for 20 min to obtain ~1.0 mL suspension of the spheroplasts. We repeated this purification procedure. 4 mL of a new buffer (10 mM Tris-HCl (pH 7.8) containing 1.5 mM KCl, 48.5 mM NaCl, and 0.73 M sucrose (or 10 mM 10 mM Tris-HCl (pH 7.8) containing 10 mM $MgCl_2$ and 0.8 M sucrose (1,2)) was added to this ~1.0 mL suspension and mixed, and then concentrated it using Vivaspin 6 under the same condition to obtain 1 mL suspension of the spheroplasts.

References

1. Martinac, B., Buechner, M., Delcour, A. H., Adler, J., and Kung, C. (1987) Pressure-sensitive ion channel in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 84, 2297-2301.
2. Wei, L., LaBouyer, M. A., Darling, L. E. O., and Elmore, D. E. (2016) Bacterial spheroplasts as a model for visualizing membrane translocation of antimicrobial peptides. *Antimicrobial Agents Chemo*. 60, 6350-6352.

Figure S2

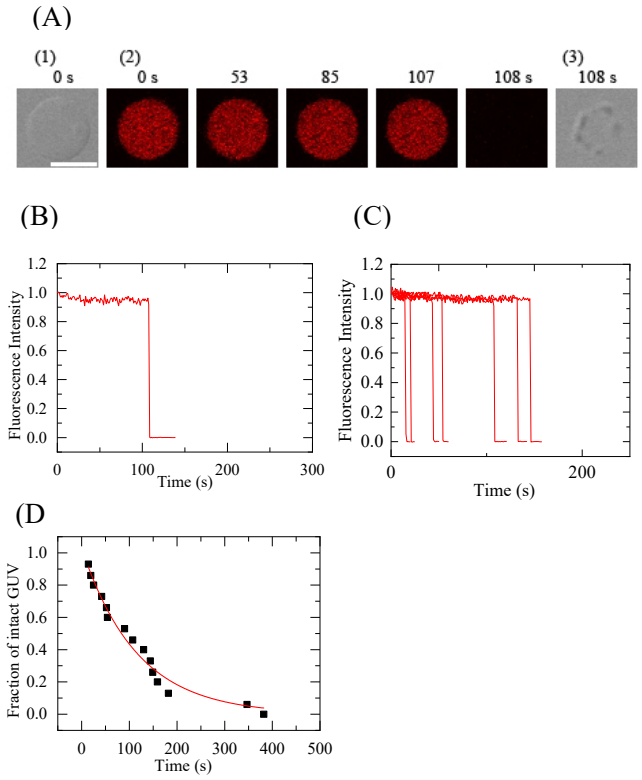

Figure S2. LfcinB-induced leakage of AF647 from single *E. coli*-lipid-GUVs with $\Delta\varphi$ = – 86 mV. (A) Leakage of AF647 from single *E. coli*-lipid-GUVs induced by 20 μM LfcinB in buffer at 25 °C. CLSM images (2) show that the AF647 concentration inside the GUV rapidly decreased after some lag time of the addition of LfcinB. The numbers above each image show the time in seconds after the LfcinB addition was started. Also shown are DIC images of the GUV at time 0 (1) and 108 s (3). The bar corresponds to 20 μm. (B) Time course of the change in the normalized FI of the GUV shown in (A). We obtained the normalized FI of a GUV as the ratio of the FI at time *t* to that before the addition of LfcinB. (C) Other examples of the time course of the change in the normalized FI of several "single GUVs" under the same conditions as in (A). Each curve corresponds to the time course of each GUV. (D) Time course of fraction of intact GUV, P_{intact}, among all examined GUVs. The solid line represents the best fit curve of eq. 1.