Supporting Information

Interaction of Daptomycin with Lipid Bilayers: A Lipid Extracting Effect

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Movies S1 and S2  The movies of Fig. 2 show the effect of daptomycin on DOPC/DOPG 7:3 GUVs (including 1% Rh-PE) in the presence of Ca$^{2+}$ ions, imaged by the rhodamine fluorescence. The time frame of the movies is 6 times faster than the real time. Movie S1 shows aggregates exuded from the GUV surface and mostly staying on the GUV surface. This is typical of most GUV movies. Movie S2 shows aggregates coming off and leaving the GUV surface. The movements of the aggregates leaving the GUV surface tend to be in the same direction. After viewing many GUV movies, we believed that in this case there was a flow of solution in the sample chamber.

Movie S3  The movie of Fig. 4 shows the lipid extracting effect when the inside and outside solutions of the GUV are almost the same.
Figure S1  Control experiments without daptomycin. At $t = 0$, DOPC/DOPG 7:3 GUVs containing 199 mM sucrose and 1 mM Tris at pH 7 were introduced into an observation chamber containing ~190 mM glucose, 10 mM Tris at pH 7, or 190 mM glucose, 10 mM Tris at pH 7 and 1 mM CaCl$_2$. The inside and outside solutions of GUV had equal osmolality. The GUVs did not show detectable changes with time. Scale bar = 10 µm.

Figure S2  (Left) Another run of the molecular leakage experiment as shown in Fig. 5. (Right) For comparison, a run of a GUV encapsulating TRsc exposed to a melittin solution is reproduced from $^1$. The pore formation by melittin caused a rapid decrease of TRsc content (red circles) in the GUV [see the interpretations for the melittin binding (green circles) and the GUV protrusion length change (diamond) in ref $^1$].
Note S1. Lipid extracting effect vs. detergent effect
We believe that the lipid extracting effect is different from detergent (or surfactant) effect. In our previous studies, we found detergent (Triton X100) monomers participated into a lipid bilayer. Detergent effects on lipid vesicles were observed only at surfactant concentrations higher than their critical micelle concentrations (CMC). The CMC values for most surfactants are in the millimolar range. At such high concentrations, the detergent effects on lipid vesicles are rather drastic as described in. This is very different from the lipid extracting effect where the membrane-inserted daptomycin somehow aggregates and exits from the lipid bilayer as described in the text. The lipid extracting effect occurs at daptomycin concentrations in the micromolar range. We did not detect a lipid extracting effect by Triton X100 at its sub-CMC concentrations.

Note S2. Calcein leakage experiment
The only calcein leakage experiment that showed significant leakage used daptomycin concentrations at least 10 times higher than the daptomycin MIC and it also inexplicably required pre-incubation of daptomycin with Ca\(^{2+}\). It is very difficult to be certain of the integrity of the submicron-sized lipid vesicles used in the experiments. In contrast, the integrity of a GUV is directly observable. We detected no leakage of TRsc (MW 625) in our GUV leakage experiments (Fig. 5 and S2) at 1 \(\mu\)M daptomycin. Also, in all of our GUV experiments with daptomycin \(\leq 5\) \(\mu\)M, we detected no loss of phase contract between the interior sucrose (MW 340) solution and the exterior glucose (MW 180) solution.

References for SI
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