Supporting Information

Sochacki et al. 10.1073/pnas.1101130108

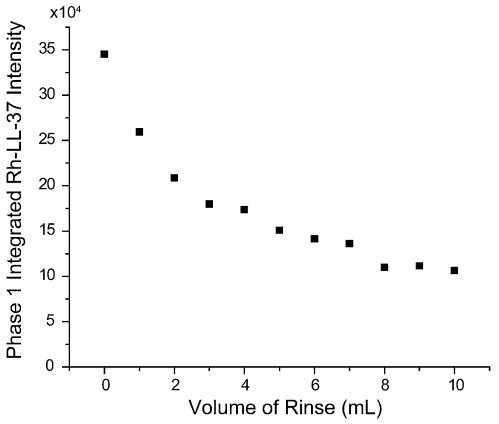


Fig. S1. Some 70% of Rh-LL-37 phase 1 binding can be rinsed away. Rh-LL-37 was injected over WT MG1655 *Escherichia coli*. Several minutes into phase 1, a 10-frame movie was taken. Cells were then rinsed with 1 mL EZRDM lacking Rh-LL-37. The 10-frame movie and rinsing steps were then repeated until there was little change in intensity associated with the cell. Each data point is the intensity of Rh-LL-37 associated with a single cell (with background subtracted) in the last frame of a movie, and it is adjusted to account for the loss of intensity because of photobleaching.

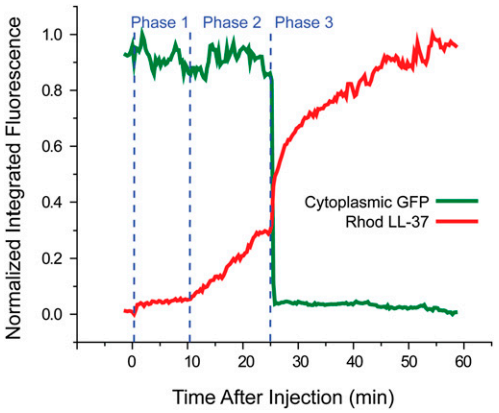


Fig. S2. Cytoplasmic GFP exits the cytoplasm at the onset of phase 3 at the same time that Sytox Green enters the cytoplasm in other experiments. The normalized integrated intensity for a single cell containing cytoplasmic GFP is shown in green. The normalized integrated intensity of Rh-LL-37 (injected at 8 μM) associated with the same cell is shown in red.

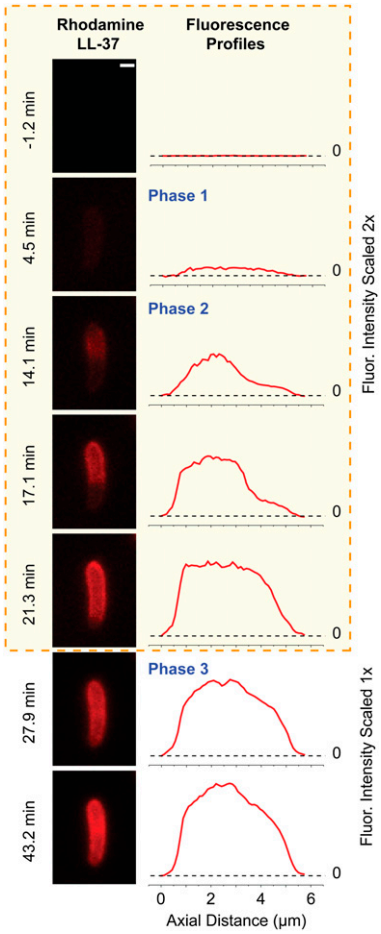


Fig. S3. A cell exposed to 8 μM Rh-LL-37 and exhibiting phase 2 with no visible septation. Phase 2 begins diffusely and is not centered along the cell. Growth halts at the beginning of phase 2, and cytoplasmic membrane (CM) permeabilization occurs at the beginning of phase 3.

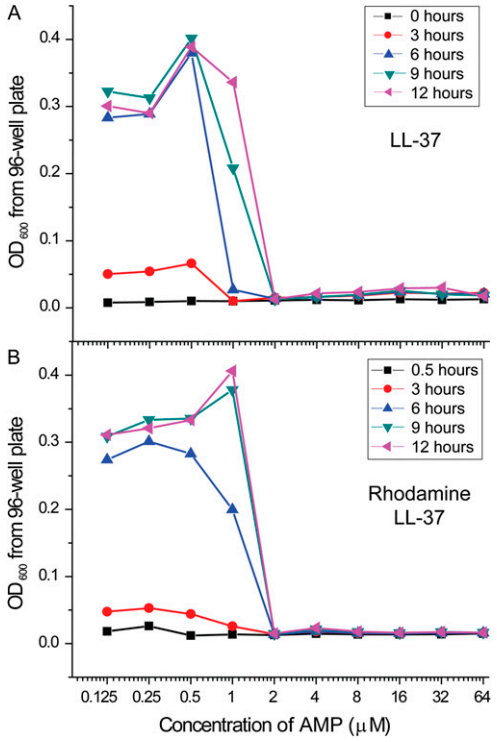


Fig. S4. A bulk minimum inhibitory concentration (MIC) assay was performed for both (*A*) unlabeled LL-37 (with C-terminal amine) and (*B*) Rh-LL-37 (no C-terminal amine). Cell growth was measured by OD in each well at several different time points. From a single measurement at 12 h, the MIC for both LL-37 and Rh-LL-37 is 2 μM. However, the MIC vs. time of LL-37 reproducibly lags behind that of Rh-LL-37. This may indicate that LL-37 likely has slightly higher activity than Rh-LL-37. Unlabeled LL-37 lacking a C-terminal amine and the *D* stereoisomer of LL-37 with a C-terminal amine both also exhibited an MIC of 2 μM (at 12 h).

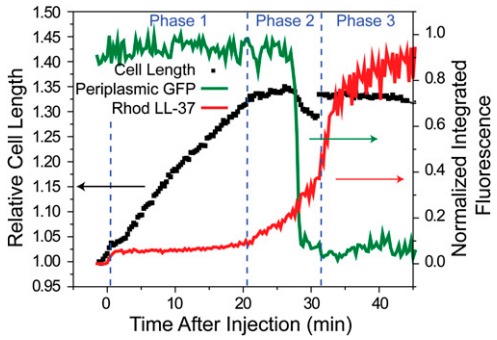
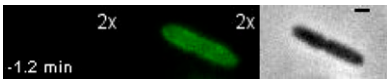
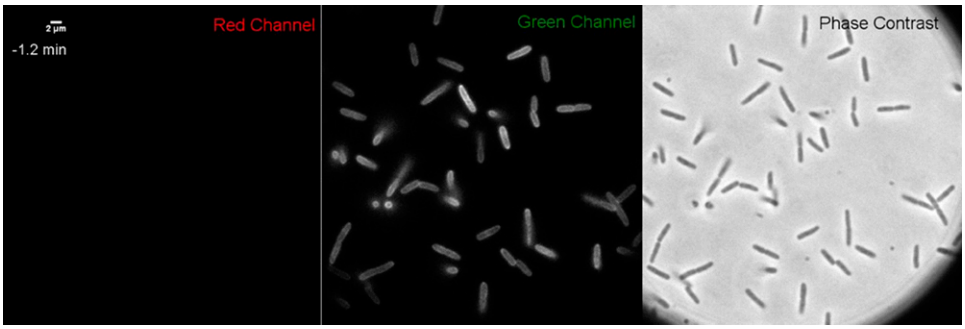


Fig. S5. Total intensity vs. time for a single cell expressing periplasmic GFP (green channel) and exposed to 8 μM Rh-LL-37 (red channel). Relative cell length is measured by phase contrast. No change in the timeline of events was observed when Sytox was absent.



Movie S1. Forty minutes of time-lapse imaging of an *E. coli* MG1655 cell that is septating at $t = 0$. The cell contains periplasmic GFP and is in the presence of 8 μM Rh-LL-37 and 5 nM Sytox Green. The red channel showing Rh-LL-37 fluorescence is shown on the left. The green channel showing the halo fluorescence pattern of periplasmic GFP (−1.2 to 9.3 min) and then, the nucleoid pattern of Sytox Green (starting at 16.5 min) are shown in the middle. Both the red and green channels are shown at two times intensity for the first 58 frames and shown at one time intensity for the remainder of the movie. Phase contrast is shown on the right-hand side. (Scale bar, 1 μm.) Injection of Rh-LL-37 and Sytox Green occurs at $t = 0$ min.

Movie S1



Movie S2. One hour of time-lapse imaging of a field of cells during a typical experiment of *E. coli* MG1655 containing periplasmic GFP in the presence of 8 μM Rh-LL-37 and 5 nM Sytox Green. The red channel showing Rh-LL-37 fluorescence is shown on the left. The green channel showing the halo fluorescence pattern of periplasmic GFP and then, the DNA binding pattern of Sytox Green are shown in the middle. Phase contrast is shown on the right. (Scale bar, 2 μm.) Injection of Rh-LL-37 and Sytox Green occurs at $t = 0$ min. Cells that are not lying flat and are out of focus are not analyzed. The intensity is scaled to enable visual observation of both periplasmic GFP in the green channel and phases 1 and 2 in the red channel. This causes Phase 3 in the red channel and the Sytox Green intensity in the green channel to appear saturated.

Movie S2