

Supporting Information

Abou Alaiwa et al. 10.1073/pnas.1422091112

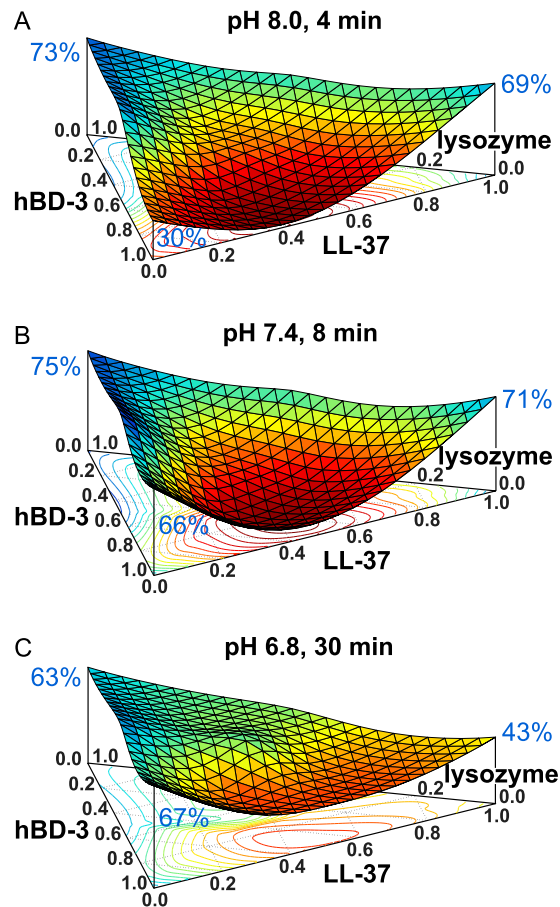


Fig. S1. Ternary surface plot of the effect of pH antimicrobial activity of the triple combination of lysozyme, hBD-3, and LL-37. Data are like those in Fig. 7 except that the concentrations of antimicrobials and the duration of incubation are different. Maximal concentrations (1.0) were 4 $\mu\text{g}/\text{mL}$ for HBD-3, 200 $\mu\text{g}/\text{mL}$ for LL-37, and 10 mg/mL for lysozyme. (A) pH 8.0 after 4-min incubation. (B) pH 7.4 after 8-min incubation. (C) pH 6.8 after 30-min incubation. Results are from a single experiment in triplicate. Experiment was repeated three times.

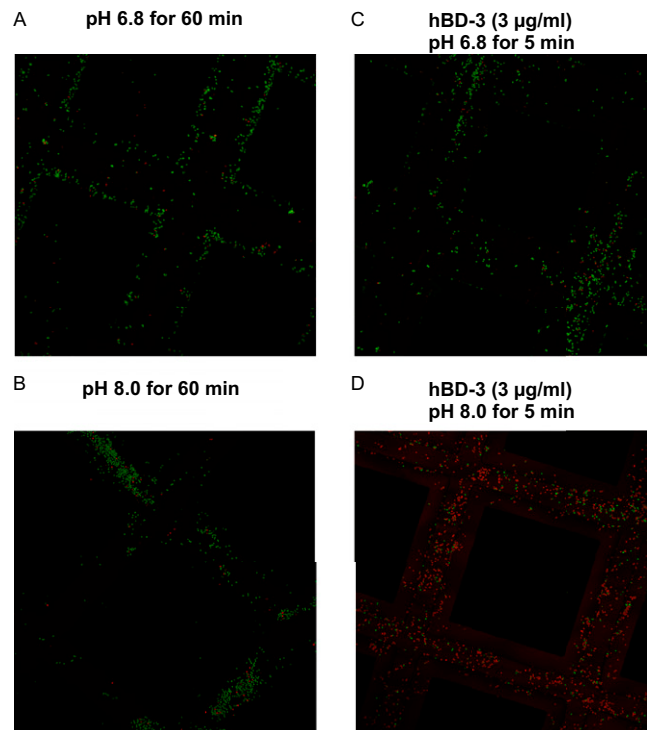


Fig. S2. Effect of pH on *S. aureus* viability. Data are *S. aureus* (Xen-29) attached to gold grids and exposed to phosphate buffer (10 mM potassium phosphate, 1% TSB, 100 mM NaCl) in the presence and absence of hBD-3. (A) pH 6.8 for 60 min. (C) pH 8.0 for 60 min. (B) pH 6.8 with 3 µg/mL hBD-3 for 5 min. (D) pH 8.0 with 3 µg/mL hBD-3 for 5 min. Red and green represent dead and live bacteria, respectively. Bacteria-coated grids were prepared by functionalizing gold electron microscopy grids (200 mesh) and coating them with Neutravidin (Thermo Fisher Scientific). Bacteria *S. aureus* (Xen-29) were cultured to log-phase growth and mixed with EZ-link NHS-LC-Biotin [succinimidyl-6-(biotinamido)hexanoate] (Pierce Biotechnology). Neutravidin-coated grids were then incubated with biotinylated bacteria and rinsed in PBS. Bacteria-coated grids were placed in phosphate buffer with or without hBD-3 for the indicated period, removed, immediately rinsed with PBS, and then immersed in NaCl containing the fluorescent indicators SYTO 9 and propidium iodide (Live/Dead Bacterial Viability Assay, Life Technologies) to assess viability. After 15 min, grids were rinsed with PBS and imaged with a laser-scanning confocal microscope (Olympus) (1).

1. Pezzulo AA, et al. (2012) Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. *Nature* 487(7405):109–113.