SUPPORTING INFORMATION

Bacterial strains, growth conditions, and materials

The background strain is MG1655 in all cases (doubling time 50 min in EZRDM at 30°C). For experiments on periplasmic GFP, TorA-GFP was expressed from a plasmid pJW1 as previously described (1). The bulk doubling time is 51 min. APEX2 was amplified with CGTA**GAATTC**ATGGACTACAAGGA TGACGAC and CGTA**GGATCC**TTAGTCCAGGGTCAGGCG and inserted into a pASK-IBA3plus vector using EcoRI and BamHI, yielding strain ZY01 (doubling time 53 min). APEX2 was expressed using tetracycline in same manner as TorA-GFP. The strain with *parS*-ParB-GFP labeling of the DNA locus called "Right2" was received from Boccard lab (doubling time 47 min) (2).

Bulk cultures were grown in EZ rich defined medium (EZRDM) (3), which contains a MOPS-buffered solution with supplemented metal ions (M2130; Teknova), glucose (2 mg/mL), supplemental amino acids and vitamins (M2104; Teknova), nitrogenous bases (M2103; Teknova), 1.32 mM K_2HPO_4, and 76 mM NaCl. Cultures were grown from glycerol frozen stock to stationary phase overnight at 30°C. Subcultures were grown to exponential phase (OD = 0.2-0.6 at 600 nm) before sampling for the microscopy experiments at 30°C, unless otherwise specified.

CM15 is a hybrid of the N-terminus of cecropin A and the N-terminus of melittin, designed to maximize antimicrobial action while minimizing hemolysis of eukaryotic cells (4). We received *L*-CM15 with C-terminal amidation from Dr. Jimmy Feix (Wisconsin Medical College). The sequence is: KWKLFKKIGAVLKVL-NH_2. The net charge is +5 at neutral pH. The oxidation sensitive dye CellROX Green (Stock Item No. C10444) was purchased from

Invitrogen. Flavin adenine dinucleotide (FAD) (F6625), protocatechuic acid (PCA) (37580), protocatechuate 3,4-dioxygenase (PCD) (P8279), 2,2'-dipyridyl (D216305), superoxide dismutase (S5639), xanthine (X0626), and xanthine oxidase (X1875) were purchased from Sigma Aldrich. Amplex Red was purchased from Invitrogen (A22188).

MIC measurements in aerobic and anaerobic conditions

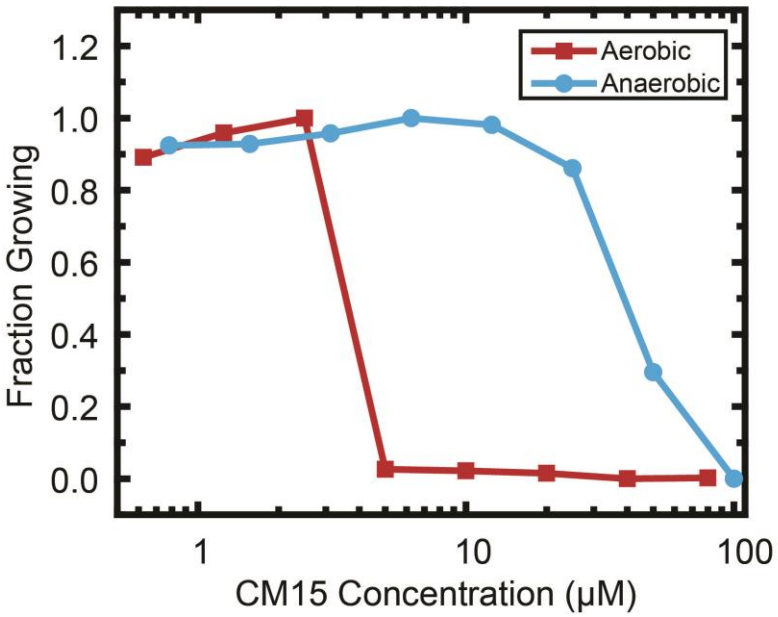

Figure S1. Bulk minimum inhibitory concentration (MIC) assay was performed for CM15 on K12 *E. coli* at 30ºC in anaerobic (blue) and aerobic (red) conditions. Cell growth was measured by optical density (OD) in each well at the 6-hr time point. The data are plotted as *Fraction Growing* = (OD – minOD)/(maxOD – minOD) to enable direct comparison between aerobic and anaerobic conditions. Here OD is the measurement at a given CM15 concentration, minOD is the minimum OD obtained at large CM15 concentration, and maxOD is the maximum OD obtained at zero CM15 concentration. The MICs of CM15 in aerobic and anaerobic conditions were determined to be 5 μM and 100 μM, respectively.

In vitro tests of CellROX oxidation

The structure of CellROX Green is proprietary. CellROX Green oxidation was tested for the reactive oxygen species superoxide, hydroxyl radical, and hydrogen peroxide. The first test used a mixture buffered at pH = 7.8 and containing 50 mM K_2HPO_4, 100 μM EDTA, 50 mM xanthine, 2.5 μM CellROX, and 40 μg/mL λ DNA. To suppress possible Fenton chemistry, EDTA was added to chelate any iron contaminant in the solution. DNA was added so that oxidized CellROX* can bind to λ DNA and become fluoresce. The CellROX* fluorescence was measured in 96-well plate containing xanthine oxidase (XO, 0.05 unit/mL), superoxide dismutase (SOD, 10 unit/mL), or both. XO uses xanthine as a substrate to produce either hydrogen peroxide or superoxide, depending on the oxidation state of the flavin cofactor. As shown in Fig. S2, XO alone produces a large CellROX* signal. This signal presumably results from superoxide, as the signal is fivefold smaller on inclusion of SOD. Neither SOD alone nor horseradish peroxidase (HRP) alone is able to oxidize CellROX Green. Evidently CellROX is not oxidized by heme Fe^{2+}. Hydrogen peroxide (1 mM) does not oxidize the dye. Instead, it evidently quenches the background fluorescence from the mixture.

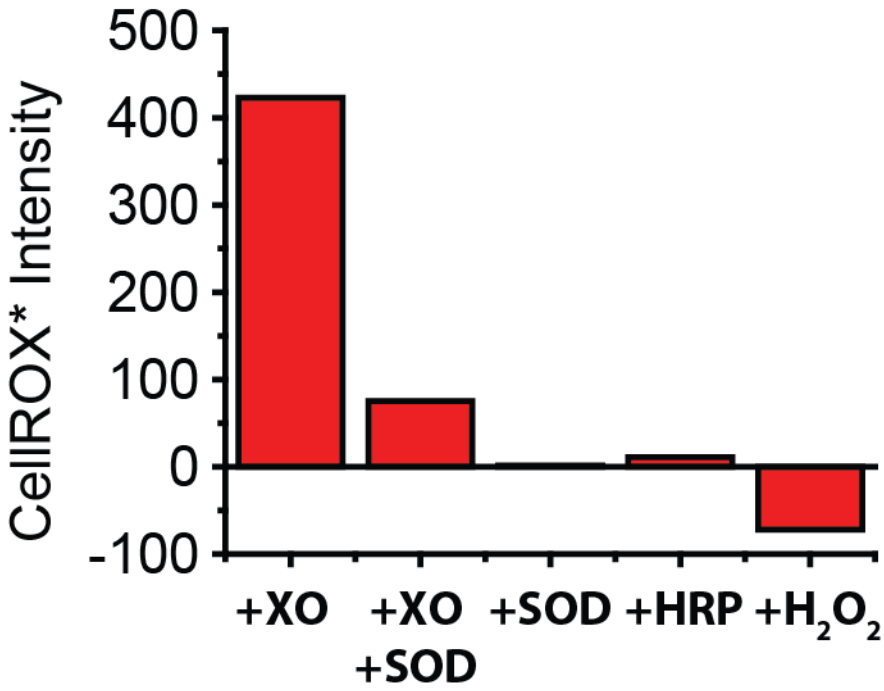

Figure S2. *In vitro* assay for CellROX* fluorescence in 96-well plate reader at 25°C. In all cases the buffered mixture contains 50 mM K_2HPO_4, 100 µM EDTA, 50 mM xanthine, 2.5 µM CellROX Green, and 40 µg/mL λ DNA. Each bar shows total CellROX* fluorescence on inclusion of the reagents shown, measured 5 min after mixing. All data are corrected by subtracting the 50 units of background fluorescence observed for the mixture alone. The concentrations of xanthine oxidase (XO) and superoxide dismutase (SOD) are 0.05 units/mL and 10 units/mL respectively. The concentrations of horseradish peroxidase (HRP) and hydrogen peroxide are 10 units/mL and 1 mM, respectively.

External addition of 10 μM H_2O_2 does not oxidize CellROX Green

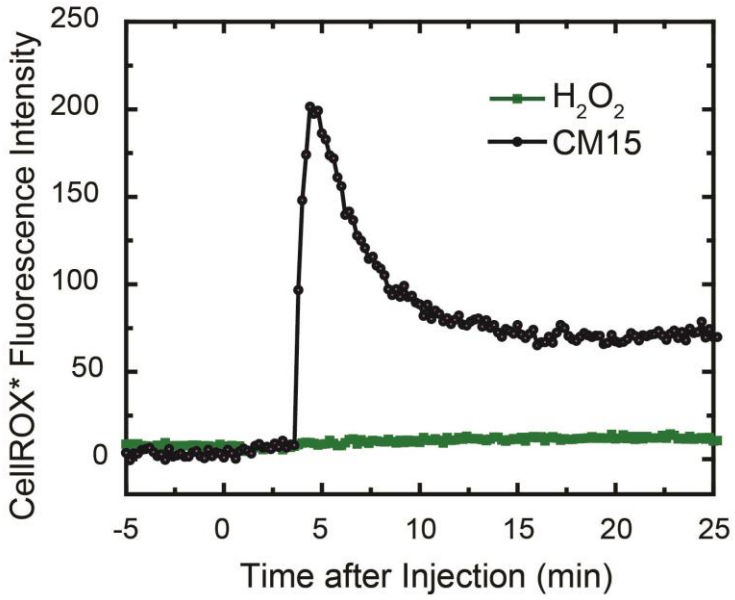

Figure S3. Single-cell CellROX* fluorescence intensity vs time after injection of 10 μM H_2O_2 plus CellROX Green (2.5 μM). CellROX* signal following CM15 addition in aerobic conditions was plotted as a comparison.

Permeabilization of cell membrane by Triton-X does not induce CellROX* fluorescence

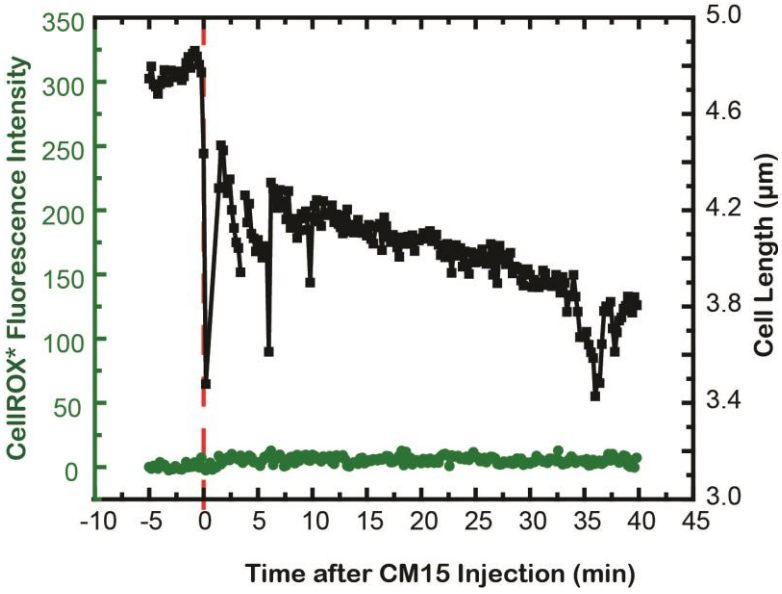

Figure S4. Single-cell length and total CellROX* fluorescence intensity vs time after injection of 2% Triton-X 100 plus CellROX Green at 2.5 µM. The detergent was dissolved in PBS. The dashed line indicates the time of injection.

CM15 effects in anaerobic conditions on cells expressing periplasmic GFP

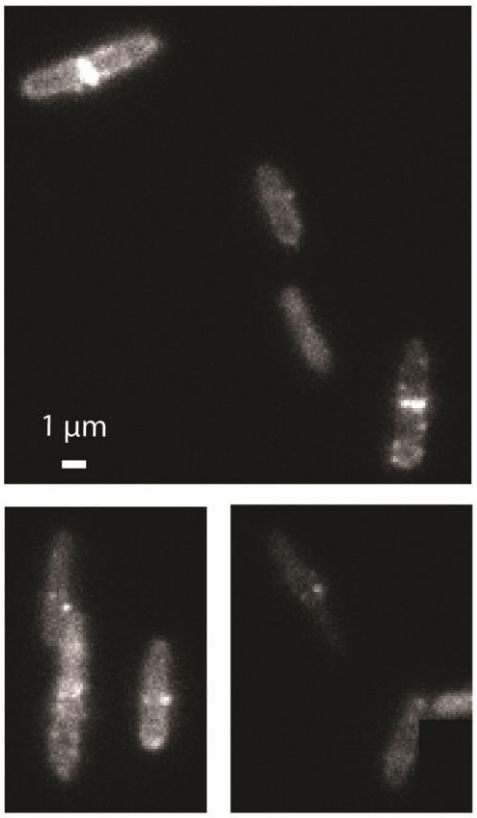

Figure S5. Heterogeneity of CM15 effects on *E. coli* expressing periplasmic GFP under anaerobic conditions. Images taken 1 min after abrupt cell shrinkage.

External Fe^{2+} is not involved in CellROX* formation by CM15

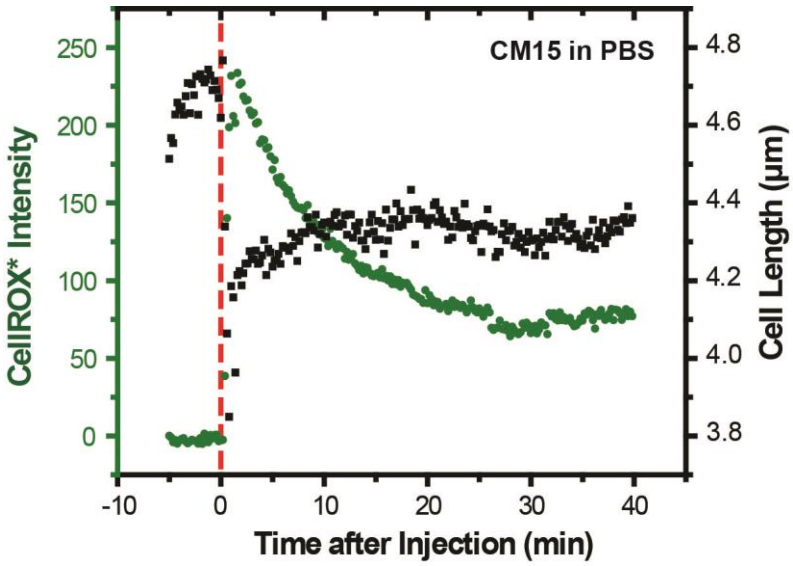

Figure S6. Single-cell length vs time and CellROX* fluorescence intensity vs time after injection of 10 µM CM15 with CellROX Green at 2.5 µM in PBS. The dashed line indicates the time of injection.

Oxygen scavenging substrate PCA alone does not affect growth or quench CellROX* fluorescence

Separate tests showed that the protocatechuic acid/protocatechuate 3,4-dioxygenase (PCA /PCD) additive that removes O_2 did not perturb cell growth in anaerobic conditions. PCA is itself a permeable antioxidant that scavenges free radicals. It seemed possible that in our anaerobic conditions, PCA (rather than the absence of O_2) was preventing oxidation of CellROX on addition of CM15. In aerobic conditions, we tested the effects of PCA by itself (without the enzyme PCD) on the CellROX response to CM15. In fact, PCA *enhanced* the peak amplitude of the CellROX fluorescence burst by about a factor of two and also slowed its decay time by about a factor of two (Fig. S7). If further oxidation of the fluorescent form CellROX* causes the decay of fluorescence, then PCA may partially protect the CellROX*.

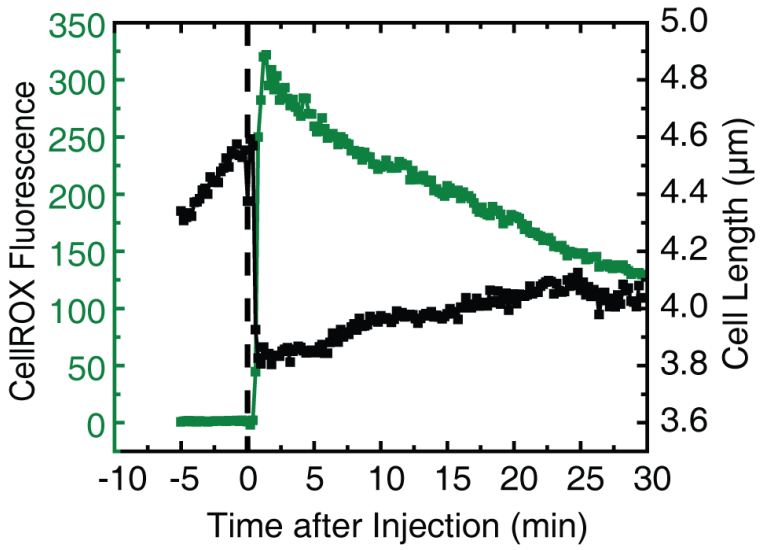

Figure S7. Single MG1655 cell length and CellROX Green fluorescence vs time after injection of 10 µM CM15, 2.5 µM CellROX Green and 5 mM PCA.

Assay for jiggling of DNA locus and diffusive motion of RNA polymerase

We monitored the movement of the chromosomal locus Right2 fluorescently labeled by a *parS*–ParB-GFP construct and of RNA polymerase (RNAP) labeled by expression of β'-YFP from the chromosome. In normal conditions, the Right2 locus exhibits sub-diffusive motion (jiggling in place). Within 12 s of cell shrinkage by CM15, the jiggling motion as judged by eye halted completely (Movie S4). When CM15 was added to cells pre-treated with 2,2'-dipyridyl (chelating Fe^{2+} and preventing Fenton chemistry), abrupt halting of Right2 jiggling again occurred. Treatment of cells with NaN_3 alone (no CM15) did not halt Right2 jiggling.

We quantify the relative motion of two foci as the mean-square displacement of the distance between them at fixed lag time τ = 1 frame = 12 s:

$$\delta r_{rel}^2(t) = |\boldsymbol{r}_1(t+\tau) - \boldsymbol{r}_2(t+\tau) + \boldsymbol{r}_1(t) - \boldsymbol{r}_2(t)|^2 \qquad \text{(S1)}$$

Here $\boldsymbol{r}_1$ and $\boldsymbol{r}_2$ are position vectors of two foci within the same cell at times t and $t + \tau$.
We prefer this relative squared displacement over a single-particle measure of displacement because the entire microfluidics chamber abruptly moves several microns relative to the camera on injection of CM15. The two-particle quantity $\delta r_{rel}^2(\tau)$ is fairly insensitive to this motion. Plots of $\delta r_{rel}^2(t)$ for normal cells and for cells before and after CM15 treatment are provided in Fig. S8.

Normal RNAP motion on the 12-s timescale is heterogeneous. Copies searching for transcription initiation sites undergo fast diffusion (non-specific binding plus 3D "hopping" between DNA strands) while copies actively transcribing jiggle in place. Shortly after cell shrinkage induced by CM15, RNAP motion halts essentially completely (Movie S5).

These results suggest that CM15 induces DNA damage. We speculate that the mechanism may involve inter-strand crosslinking of DNA with itself and protein-DNA crosslinking.

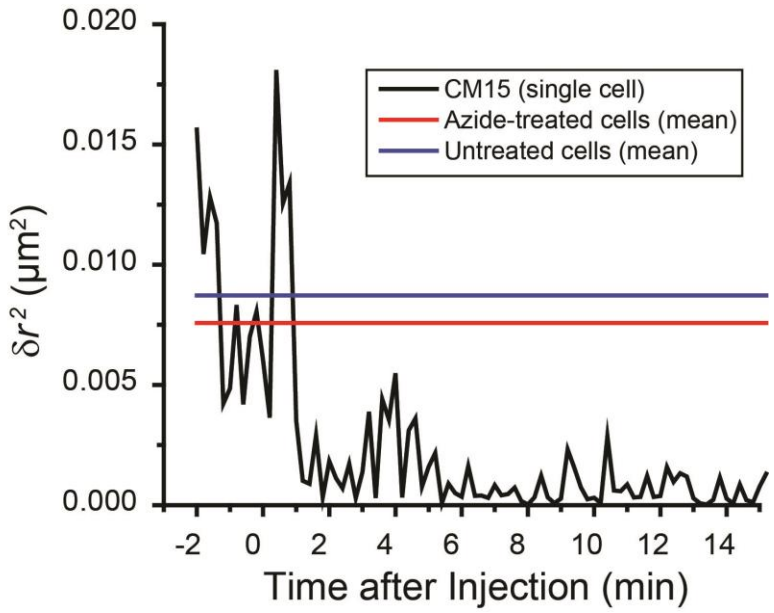

Figure S8. Plot of δr^2 between two particles vs time after injection of 10 μM CM15. The average δr^2 for several untreated cells (blue line) and for several cells treated with azide alone (red line) are shown for comparison.

CellROX* assay at different CM15 concentrations

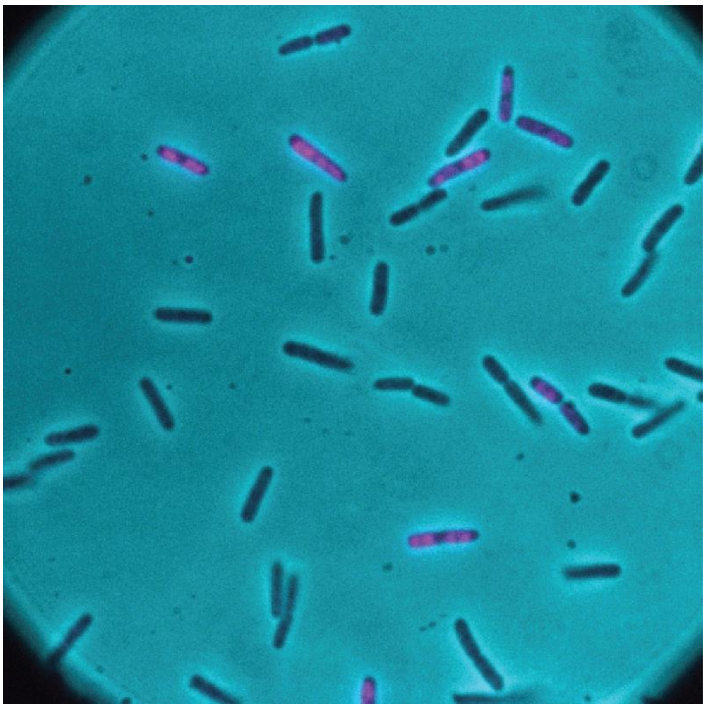

Figure S9. Image of CellROX* fluorescence 30 min after treatment of cells growing aerobically with 2.5 μM of CM15 (half the MIC). The fluorescence image is superimposed on a phase contrast image. Only a small fraction of the cells exhibit CellROX* fluorescence; the remainder continue to grow normally.

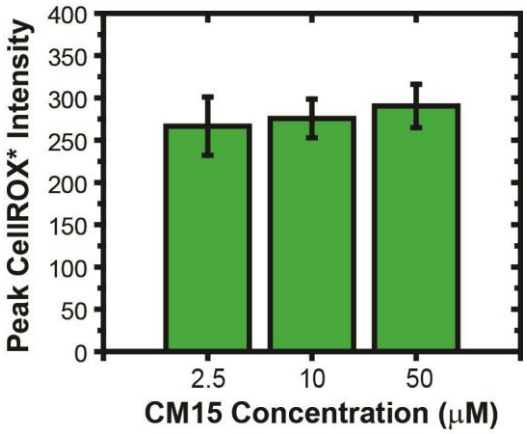

Figure S10. Comparison of mean peak amplitudes of CellROX* fluorescence from ten *E. coli* cells each after treatment with 2.5 μM (half the MIC), 10 μM (twice the MIC), and 50 μM (ten times the MIC) of CM15. At 2.5 μM, only cells that exhibited CellROX* fluorescence are included in the mean. The response is the same within measurement error at all concentrations.

CellROX* assay with addition of the natural antimicrobial peptide LL-37

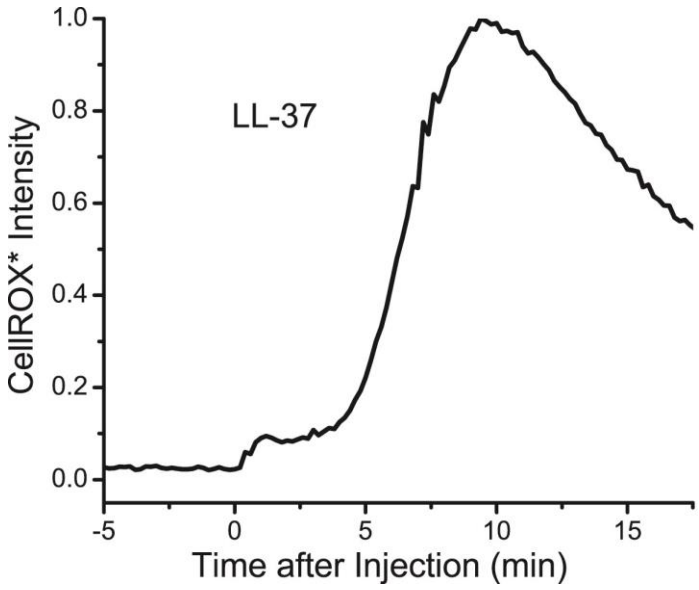

Figure S11. CellROX* fluorescence intensity vs time for a single *E. coli* growing aerobically and treated with 4 μM of LL-37 (1X the MIC) at $t = 0$. All cells in the field of view exhibited similar fluorescence intensity. The cause of the two phases of the rise in intensity is under investigation.

Supporting Movies

Movie S1. Phase contrast (left panel) and periplasmic GFP (right panel) movies upon treatment with 10 μM CM15 at $t = 0$. GFP moves into the cytoplasm at the time of abrupt cell shrinkage and leaves the cell much later, at $t = 40$ min.

Movie S2. Phase contrast (left panel) and CellROX fluorescence (right panel) images of a cell upon treatment with 10 μM CM15. The injection of CM15 with CellROX at 2.5 μM occurs at $t = 0$. Fluorescence images were acquired at a rate of 1 frame per 12 s with exposure time of 50 ms for a total of 40 min. Phase contrast images were interleaved at the same rate. The lag between phase contrast and fluorescence images is 6 s.

Movie S3. Phase contrast (left panel) and Resorufin fluorescence (right panel) images of a cell upon treatment with 10 μM CM15. The injection of CM15 with Amplex Red at 10 μM occurs at $t = 0$. Fluorescence images were acquired at a rate of 1 frame per 12 s with exposure time of 50 ms for a total of 40 min. Phase contrast images were interleaved at the same rate. The lag between phase contrast and fluorescence images is 6 s.

Movie S4. Phase contrast (left panel) ParB-GFP fluorescence (right panel) images upon treatment CM15 with 10 μM CM15. The plated cell is expressing ParB-GFP. ParB-GFP is located at parS site near Right2. Fluorescence images were acquired at a rate of 1 frame per 12 s with exposure time of 50 ms for a total of 40 min. Phase contrast images were interleaved at the same rate. The lag between phase contrast and fluorescence images is 6 s.

Movie S5. Fluorescence images of YFP-labeled RNA polymerase motion upon CM15 treatment. The plated cell is expressing the subunit β'-yGFP from its chromosome. The injection of CM15 occurs at $t = 0$. Images were acquired at a rate of 1 frame every 6 s with exposure time of 50 ms for 60 min. Apparent motion of RNAP decreases abruptly shortly after CM15 injection.

REFERENCES FOR SUPPORTING INFORMATION

1. Sochacki KA, Shkel IA, Record MT, & Weisshaar JC (2011) Protein diffusion in the periplasm of *E. coli* under osmotic stress. *Biophys J* 100(1):22-31.

2. Thiel A, Valens M, Vallet-Gely I, Espeli O, & Boccard F (2012) Long-range chromosome organization in *E. coli*: A site-specific system isolates the ter macrodomain. *PLoS Genetics* 8(4):564-575.

3. Neidhardt FC, Bloch PL, & Smith DF (1974) Culture medium for enterobacteria. *J Bacteriol* 119(3):736-747.

4. Andreu D, *et al.* (1992) Shortened cecropin-A melittin hybrids--significant size reduction retains potent antibiotic activity *FEBS Lett.* 296(2):190-194.