SUPPORTING INFORMATION FOR

DCAP: A broad-spectrum antibiotic that targets the cytoplasmic membrane of bacteria

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Materials and Methods

In vitro ATPase screen with purified recombinant MipZ. We purified recombinant MipZ for in vitro screens as described previously[1]. We used two ATPase assays to screen three small molecule libraries (a total of 43,400 compounds) at the University of Wisconsin Carbone Cancer Center. One assay utilized pyruvate kinase and lactic dehydrogenase as coupling enzymes, and phosphoenolpyruvate and NADH as their substrates, respectively. A solution of coupling enzymes, their substrates, Triton X-100 and MipZ were aliquoted (22.3 μL per well) in 384-well black plates using Biomek FX liquid handler (Beckman Colter). Plates were briefly centrifuged to pull liquids to the bottom of the wells. We used pin tools to deliver 0.2 μL of a stock solution of unique small molecules (10 mM in DMSO) from chemical libraries to each well. The first two columns of each plate were reserved for controls and did not receive compounds from the libraries. We then added a solution of ATP (2.5 μL per well) using a Biotek Fill instrument to all wells except the first column for each plate to initiate ATP hydrolysis. Final concentrations of assay components are: 0.01% Triton X-100, 1 mM phosphoenolpyruvate, 0.3 mM NADH, 3 U/mL pyruvate kinase, 3 U/mL lactic dehydrogenase, 7.5 μM MipZ, and 1 mM ATP in a buffered solution of 50 mM Tris-HCl, 50 mM KCl, and 10 mM $MgCl_2$. We gently vortexed the plates to mix the solution and incubated for 3 h at 30 °C. After incubation, we measured the fluorescence emission from NADH using a Tecan Safire II plate reader (λ_{ex} = 340/35 nm; λ_{em} = 460/10 nm).

Measurements of the fluorescence intensity from control wells were used to calculate the Z-factor; the minimum Z-factor for all plates was 0.7. We used the coupling enzyme assay to screen compounds from Maybridge and Life Chemicals libraries. Compounds that inhibited ≥ 60% of ATP hydrolysis compared to the positive control were identified as hits and were screened using a secondary assay to eliminate compounds that target coupling enzymes. The secondary assay consisted of the same reaction components as the primary assay, except for the omission of MipZ and ATP. A solution of ADP was added instead. The fluorescence emission of compounds that did not inhibit coupling enzymes was measured at the specified wavelengths used for NADH, and the compounds were retested for their inhibitory effect on MipZ activity in vitro.

In addition to the coupling enzyme assay, we used a fluorescence polarization (FP) assay to monitor the ATPase activity of MipZ in vitro. Reaction conditions and component concentrations were same as the coupling enzyme assay unless noted otherwise. The FP assay utilized anti-ADP antibodies and Alexa633-labeled ADP. We purchased a Transcreener ADP² FP assay kit from BellBrook Labs (Madison, WI). A solution of MipZ was aliquoted (10 μL per well) into plates, and addition of ATP (1 μL of 5 mM stock solution per well) initiated the reaction. After 3 h, we added 10 μL of ADP detection mix (541 μg/mL of antibody) to each well, and incubated the plates for 1 h at 25 °C. We used the following wavelengths for FP measurements: 635 nm for excitation and 670/20 nm for emission. The Z-factor for the FP assay was ≥ 0.7. The FP

assay was used for screening compounds from the Life Chemicals library and the Spectrum Collection. Hits from the FP assay were tested for inhibition of the anti-ADP antibody by repeating the assay in the absence of MipZ, and checked for their intrinsic fluorescence at the specified wavelengths used for the Alexa633 probe.

In vivo screen with a Caulobacter crecentus *strain that expresses MipZ-YFP.* Hits from in vitro screens were tested for activity in vivo. We used a *C. crescentus* strain (MT97) that expresses *mipZ-yfp* from the native *mipZ* promoter. An overnight culture of MT97 was diluted to an absorbance (λ=600 nm) of ~0.1; the diluted culture was grown further for at least 1 h prior to compound treatment. Compounds were mixed with a solution of 1% agarose in M2G media[2] to achieve a final concentration of 20 μM. We transferred cells (1 μL per pad) on top of the compound-containing agarose pad, and imaged the cell morphology and localization of MipZ-YFP for a period of 24 h using epifluorescence microscopy. In between microscopic observations, we incubated the inoculated pads at 30 °C to promote growth.

Nuclear magnetic resonance (NMR) spectroscopy. DCAP used in all experiments described in this manuscript was purchased from Ryan Scientific (catalog number F3255-0148, Mt. Pleasant, SC). We used a Varian MercuryPlus 300 MHz instrument (Magnetic Resonance Facility in the Chemistry Department of the University of Wisconsin-

Madison) to obtain 1D ^{1}H and ^{13}C NMR spectra for DCAP at 25 °C. We also used a Bruker Avance III 500 MHz instrument (National Magnetic Resonance Facility at Madison) to collect 2D ^{13}C-HMBC, ^{15}N-HMBC, HSQC, COSY, and TOCSY spectra for DCAP structure verification. The solvent used was deuterated dimethyl sulfoxide. We analyzed the data using Sparky (T. D. Goddard and D. G. Kneller, University of California, San Francisco) and NUTS (Acorn NMR).

High resolution electrospray ionization. We analyzed DCAP using high resolution electrospray ionization mass spectrometry in positive ion mode to determine the exact mass of the compound (Mass Spectrometry Facility, Department of Chemistry at the University of Wisconsin-Madison).

Bacterial strains and growth conditions. Organisms and strains used in this study, their genotype, and relevant references are summarized in Table S2. We used Luria-Bertani (LB) media (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract, pH 7.2) to grow *B. subtilis* 168, *S. aureus* FRI100, *P. aeruginosa* K1115, *S. typhimurium, V. cholera, S. boydii, K. pneumonia, E. aerogenes, A. baumannii, E. tarda*, and *M. morganii* strains. For *P. aeruginosa* PAO1 and K1119 strains and two *E. coli* BW25113 strains, we used M8 minimal media (241 mg/L $MgSO_4$, 4 mg/L glucose, 5 mg/L casamino acids, 12.8 g/L $Na_2HPO_4 \cdot 7H_2O$, 3 g/L KH_2PO_4, and 0.5 g/L NaCl). PYE media (2 g/L peptone, 1 g/L yeast extract, 0.8 mM

$MgSO_4$, and 0.5 mM $CaCl_2$) was used for culturing *C. crescentus* cells at 30 °C. All cultures were grown at 37 °C, except for *C. crescentus* (30 °C). All cultures were grown while shaking at 200 rpm with the exception of clinical pathogens which were statically incubated.

Determination of the minimum inhibitory concentration (MIC) of bacterial growth. We determined the MIC of *E. coli, P. aeruginosa, B. subtilis*, and *C. crescentus* strains in liquid media using the macrodilution method according to the NCCLS guidelines[3]. For clinical pathogenic organisms including *S. aureus*, *S. typhimurium, V. cholera, S. boydii, K. pneumonia, E. aerogenes, A. baumannii, E. tarda*, and *M. morganii* strains, we used 96-well microplates (100 μL/well) and the microdilution method from the NCCLS guidelines[3].

Determination of the minimum stationary-bactericidal concentration. We grew cultures of *C. crescentus* (24 h incubation) and *S. aureus* cells (5 d incubation) from single colonies. Cells were collected by centrifugation (5800 g for 2.5 min) and re-suspended in M2 salt solution (for *C. crescentus*; 1.74 g/L Na_2HPO_4, 1.06 g/L KH_2PO_4, and 0.5 g/L NH_4Cl) or phosphate-buffered saline (for *S. aureus*; PBS, Fisher Scientific). The centrifugation and re-suspension was repeated two more times. After the repeated washing steps, we diluted the cell suspension 10-fold to achieve ~10^8 cells/mL. We transferred aliquots of the diluted cell suspension into wells of a 96-well plate (100 μL/well), and performed 2-

fold serial dilutions to test a range of antibiotic concentrations. We sealed the plate with parafilm and incubated for 24 h at room temperature in the dark. After incubation, we took 50 μL from each well to spread on nutrient agar plates (1.5 % agar in PYE for *C. crescentus*, and LB for *S. aureus*). We counted colonies on each plate after growing the cells for 1-2 days. For determining the kinetics of bactericidal activity of DCAP on stationary cultures, we diluted the washed cells by 100-fold in appropriate solutions and tested a single concentration of the compound while including a DMSO control sample. Cell suspensions were kept in closed microcentrifuge tubes instead of the wells of 96-well plates. We removed 100 μL of the suspensions for plating at each time point. All MSC experiments were performed in static conditions.

Determination of the minimum biofilm inhibitory concentration of growth (bMIC) and biofilm eradication concentration (MBEC). We grew overnight cultures of *C. crescentus* and *S. aureus*, and diluted them 100-fold into appropriate nutrient media. We transferred aliquots of the diluted suspension into wells of 96-well plates (150 μL/well). We performed 2-fold serial dilutions for compound-containing wells. For every experiment, we included three replicates for each compound concentration tested. After transferring aliquots of the suspension, we closed the plate with specialized lids that have protruding pins (Nunc STP System), and sealed the plates using parafilm. We incubated plates in a static incubator at 30 °C (*C. crescentus*) or 37 °C (*S. aureus*) for 24 h. After cells

had formed biofilms on the surface of the pins, we rinsed the pins by dipping them in M2 (*C. crescentus*) or PBS (*S. aureus*) solutions. To rinse the pins, we used clean, sterile 96-well plates (aliquots of 200 μL/well); each rinse lasted 10 sec. After washing away planktonic cells loosely bound to biofilms, we inserted the pins into a 96-well plate that was pre-aliquoted with nutrient media containing antibiotics (2-fold dilutions, a final volume of 150 μL/well). We sealed the plates with parafilm, incubated them for 17 h, and measured the bMIC at the end of the incubation by visual inspection. After the bMIC run, we repeated the rinse steps to remove planktonic cells, and inserted the pins into a 96-well plate that was pre-aliquoted with drug-free nutrient media (aliquots of 150 μL/well). We sealed the plates with parafilm, incubated for 24 h, and measured the MBEC at the end of the incubation by visual inspection.

Rabbit red blood cell (RBC) hemolysis assay. We used rabbit RBCs from Lampire Biological Laboratories. Prior to preparing the RBCs, we serially diluted compound-containing PBS solutions into a 96-well plate (a final volume of 100 μL/well). We included the RBC lysis solution (Epicentre Biotechnology) as a positive control for hemolysis. For each assay, we removed 1 mL of the RBC suspension from the stock bottle and centrifuged the cells for 2 min at 2000 rpm. We resuspended pelleted cells in sterile PBS solution and centrifuged again. We resuspended the cells in PBS and diluted them 5-fold into the same solution. We added 100 μL aliquots of RBCs into wells of a 96-well plate that

contained an equal volume of a solution of compound in PBS. We incubated the plates for varying amounts of time depending on the microbial assay conditions that we wanted to emulate. For MIC-like conditions, we incubated plates for at least 17 h at 30 °C or 37 °C. For MSC-like conditions, the incubation time was either 2 h or 6 h at 30 °C or 37 °C. During the incubation, un-lysed RBCs settled at the bottom of the wells. At the end of the incubation, we transferred 90 μL of the supernatant into the wells of a fresh 96-well plate, and measured the absorbance of the heme at λ=405 nm.

Measurement of membrane potential using fluorescence microscopy and flow cytometry. We used the fluorescent probe, 3,3′-diethyloxacarbocyanine iodide ($DiOC_2$) to measure $\Delta\Psi$ in DMSO and in compound-treated cells. To eliminate the possibility of an interaction between the probe and compounds, we measured the fluorescence intensity of solutions of DiOC2 in the presence and absence of the compounds, as described in Foss et al.,[4]. For experiments with *B. subtilis* cells, we used the identical procedure described by Foss et al, to prepare and label cells[4]. We measured the fluorescence of these cells using a BD LSR II flow cytometer. We used the following instrument settings for detection: low flow rate, 488 nm excitation laser, 530/30 nm emission filter for green fluorescence, and 575/26 nm emission filter for red fluorescence. We used FlowJo software to analyze flow cytometry data. First we drew a gate around the region where cells were detected in the forward versus side scatter area plot. Gating enabled us to exclude any non-cellular

materials in our sample that the instrument detected. We calculated the ratio of red-to-green fluorescence for each particle within the gate using the software and exported the data to GraphPad Prism. We used this graphing software to create box plots shown in Figure 2 and calculated the statistical parameters using one-way analysis of variation (GraphPad InStat).

For experiments with *C. crescentus* cells, we prepared the cells by diluting the overnight culture 10-fold into fresh PYE medium. The diluted culture was grown for 1 h at 200 rpm and 30 °C. We treated the cells with compounds for 10 min at room temperature, added $DiOC_2$ dye at a final concentration of 30 μM, and incubated for 10 min. After labeling, we transferred small aliquots (1-2 μL) of the suspensions of cells on 1% agarose pads for fluorescence microscopy. We used a Nikon Eclipse TE2000E inverted microscope with an Andor DU-895 EMCCD camera, a Perfect Focus system, and an encoded z-stage for phase contrast and epifluorescence microscopy. For detecting green and red fluorescence of the dye, we used the following wavelengths ($\lambda_{ex}/\lambda_{em}$): 484/520 nm and 555/620 nm, respectively. We analyzed acquired images using the MATLAB-based script MicrobeTracker[5]. Using this software, we segmented the area of individual cells in the phase contrast images. The segmentation was applied to the fluorescence images to calculate the fluorescence intensity for individual cells. We wrote a separate MATLAB script to process the results from MicrobeTracker, in which we calculated the cell-area normalized ratio of red to green fluorescence for each cell.

This data was exported to GraphPad Prism and GraphPad InStat for creating box plots and performing statistical analysis (one-way analysis of variation), respectively. All scripts we created for image analysis are available on our laboratory website (https://www.biochem.wisc.edu/faculty/weibel/lab/gallery/default.aspx).

Measurement of membrane permeability using fluorescence microscopy and flow cytometry. We used the DNA-binding probe, propidium iodide (PI) to measure the relative membrane permeability between DMSO, ethanol (50 % v/v for *C. crescentus* and 70 % v/v for *B. subtilis*) and compound-treated cells. To eliminate the possibility of an interaction between the fluorophore and small molecules, we measured the fluorescence intensity of solutions of PI in the presence and absence of the compounds, as described[4]. For experiments with *B. subtilis* cells, we used the procedure previously described[4] to prepare and label the cells. We detected the fluorescence of *B. subtilis* cells using a BD LSR II flow cytometer. We used the following instrument settings for detection: low flow rate, 488 nm excitation laser, 610/20 nm emission filter. When using FlowJo to analyze the data, we excluded auto-fluorescence from cells from PI fluorescence. Other details for the analysis are identical to the description in the previous section.

For experiments with *C. crescentus* cells, we prepared the cells by diluting the overnight culture 10-fold into fresh PYE medium. The diluted culture was grown for 1-3 h at 200 rpm and 30 °C. We treated the cells with compounds for 10 min at room

temperature, added PI at a final concentration of 20 μM, and incubated for 10 min. We imaged the cells and analyzed the data as described in the previous section.

Measurement of protein localization using fluorescence microscopy and image analysis. For experiments with *C. crescentus* expressing MipZ-YFP, we diluted overnight cultures by 10-fold and incubated at least 1 h prior to treatment with compounds. After adding compounds, we incubated cells at 200 rpm and 30 °C for 20 min. We imaged the cells as described in the previous section. Following cell segmentation and fluorescence signal calculation in MicrobeTracker[5], we used a separate MATLAB script to detect signal peaks within an individual cell. We catalogued the number of total peaks and peak locations (i.e. poles or mid-cell) for each cell. Poles were defined as 1-25% and 75-100% along the normalized cell length (1-100 %). We classified cells as 'wildtype' if its catalogued information agreed with one of the following criteria: 1) MipZ-YFP was unipolar, meaning there was one peak and the peak resides within a pole region; and 2) MipZ-YFP was bipolar, meaning there were two peaks and both peaks are within a polar region. After this classification, we created a contingency table with the total number of cells analyzed, and the number of cells with the 'wildtype' localization. Using InStat program, we applied the Fisher's exact test to calculate two-sided p-values between DMSO and compound-treated cells.

For experiments with *C. crescentus* expressing Venus-FtsA, we used a synchronized population of cells. First we grew an overnight culture with kanamycin (5 μg/mL) and glucose (0.02 % w/v). The presence of glucose suppressed the transcription of *venus-ftsA* from its xylose-inducible promoter. We diluted 2 mL of this culture into 25 mL of fresh PYE with the antibiotic and glucose, and incubated further to achieve an absorbance of ~0.6 (λ=600 nm). Once the cells reached mid-exponential phase, we centrifuged the culture for 10 min at 5400 g and 4 °C. We resuspended the cell pellet in ice-cold M2 (a final volume of ~ 1000 μL) and added 750 μL of this suspension to an equal-volume of sterile Percol (Sigma Aldrich). After thoroughly mixing the solution, we centrifuged it for 20 min at 9800 g and 4 °C. Upon centrifugation, we took the bottom band (swarmer cells) and washed the cells in ice-chilled M2 solution (1 mL). The washed cell pellet was suspended in fresh PYE containing kanamycin (no glucose, 6 mL). We incubated the cell suspension for 20 min and then added xylose (0.03 % w/v) to induce the expression of *venus-ftsA*. Cells were grown for another 20 min and subsequently treated with compounds. We incubated cells for 20 min again in the presence of the compounds prior to imaging. Thus, the total time of xylose induction was 40 min, and the total time of compound treatment was 20 min. At this time point (60 min post synchrony), the majority of the cells were at the beginning of cell division. Imaging conditions and data analysis were identical to the conditions described for MipZ-YFP, except for a set of criteria used for defining 'wildtype' protein localization.

We defined 'wildtype' as 1) Venus-FtsA is unipolar, meaning there was one peak, and the peak resided within a polar region; and 2) Venus-FtsA was at the mid-cell, meaning there was one peak and it was within the mid-cell region (40-60 % of the normalized cell length).

We performed protein localization experiments with *B. subtilis* expressing GFP-MinD, as described previously[4]. Imaging conditions and data analysis were identical to those described for MipZ-YFP, except for the definition of poles, and a set of criteria used for defining 'wildtype' protein localization. We defined 'wildtype' as 1) GFP-MinD was bipolar, meaning there were two peaks, and both peaks were at the poles (1-20% and 80-100% of the normalized cell length); 2) GFP-MinD was at the mid-cell in addition to the bipolar localization, meaning there were three peaks with two of them at the poles and one at mid-cell; and 3) GFP-MinD was at an quaternary position (20-40% and 60-80% of the normalized cell length) in addition to the bipolar and mid-cell localization (the total number of peaks was 4 if there is one quaternary peak, and the total was 5 if there are two quaternary peaks). Because *B. subtilis* cells can initiate division prior to finishing an earlier division and the completion of septation, we found that these normal cells have peaks at quaternary positions. Any cells that did not fit these criteria were categorized as non-wildtype.

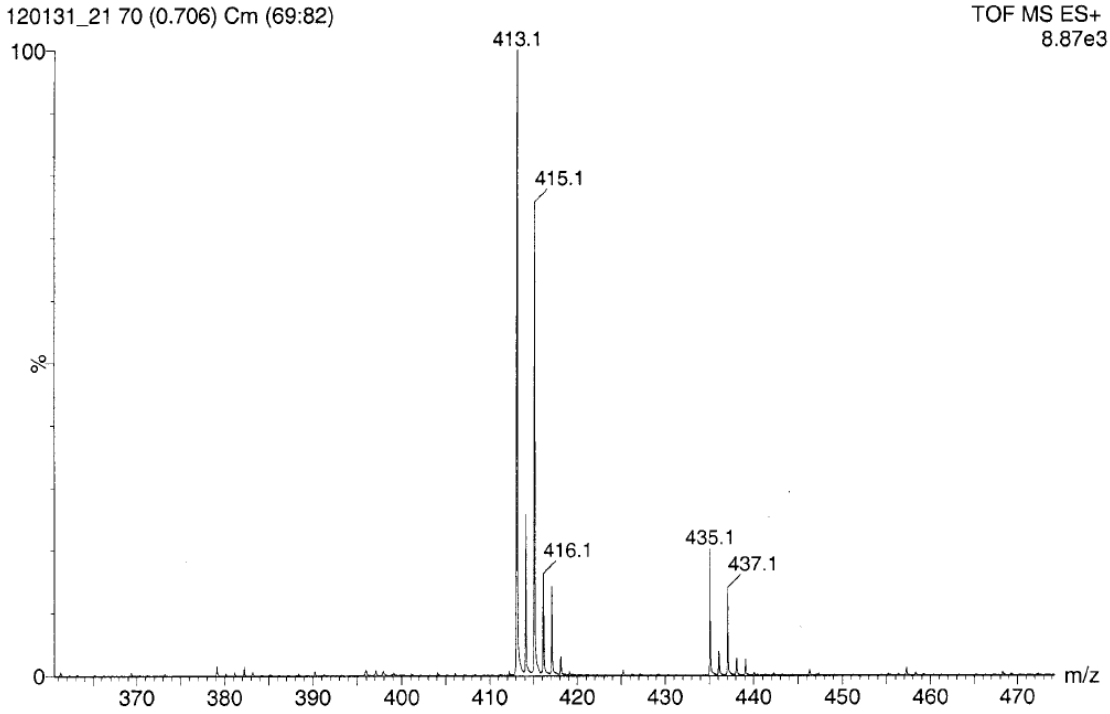
Mammalian cell viability assay. We measured the cytotoxicity of small molecules on human epithelial kidney (HEK) cells by using the CellTiter-Glo Luminescent Cell Viability Assay from Promega (Madison, WI). The HEK cells (293T/17; ATCC number CRL-11268) were grown in 10% heat-inactivated fetal bovine serum in Dulbecco's modified Eagle medium, supplemented with penicillin and streptomycin. Cultures were incubated in an atmosphere of 5% CO_2 at 37°C. To seed cells into 96-well microplates, we suspended the HEK cells in media (5×10^4 cells/mL) and aliquoted 200 μL of the suspension to each well (1×10^4 cells/well). For each condition tested, we included three replicates. Cells were incubated in the wells of plates (white, flat-bottom, sterile, and tissue-culture treated from Nunc) for 24-30 hrs prior to treatment with small molecules. For 2-hr compound treatments, the final concentration of DMSO was 0.5 % v/v or less. For longer incubations of cells with compounds, the concentration of DMSO was 0.16% v/v or less to minimize cell death in the DMSO solvent control samples. Stock solutions of compounds in DMSO were diluted in fresh growth media to their final concentrations and then added to microplate wells (100 μL/well). Since the growth media containing small molecules contained different amounts of DMSO, we created a separate DMSO solvent control for each compound concentration that we tested. We later used data from these DMSO controls to normalize the values from compound-treated cells. Control wells without any small molecules or solvent received fresh medium so that all wells contained fresh media dosed with or without small molecules.

During the treatment of cells with compounds, cells were kept at 37 °C except the last 30 min of the incubation. Plates and CellTiter-Glo reagent were left out on the bench during this 30-min period to equilibrate to 25 °C. At the end of compound treatment, we added CellTiter-Glo solution to wells (100 μL of reagent/well; final volume in each well was 200 μL), and mixed the contents by pipetting. To ensure complete cell lysis, plates were shaken for 2 min using the orbital and linear shaking programs (6 mm amplitude; 1 min of each program) in a Tecan M1000 microplate reader. We incubated the plates for 10 min at 25 °C and measured the luminescence using the microplate reader.

Measurement of mitochondrial ΔΨ in mammalian cells. We measured the mitochondrial ΔΨ in HEK cells in the presence of small molecules. We prepared HEK cells using an identical procedure described earlier, except black 96-well microplates with clear bottoms were used in this assay. At the end of compound treatment, we aliquoted a solution of $DiOC_2$ in growth media (50 nM) into each well, and incubated the plates for 30 min at 37 °C. After the incubation, we washed the cells once with 1x PBS solution. We measured the fluorescence of $DiOC_2$ using a Tecan M1000 microplate reader with the following instrument settings: for green fluorescence, λ=488±5 and 530±5 nm for excitation and emission, respectively, with a gain of 200. For red fluorescence, λ= 590±5 and 615±5 nm for excitation and emission, respectively, with a gain of 255. Other

settings include 50 flashes at 400 Hz with an integration time of 20 μs and the z-position of 20,000 μm. After acquiring the fluorescence intensities, we calculated the ratio of green-to-red fluorescence for each sample. Ratio values from compound-treated samples were normalized against data from DMSO-treated controls.

Figure S1. Low-resolution electrospray ionization mass spectra. High-resolution data: expected mass was $(M+H)^+$ = 413.0952 and $(M+Na)^+$ = 435.0849; observed mass was 435.0832.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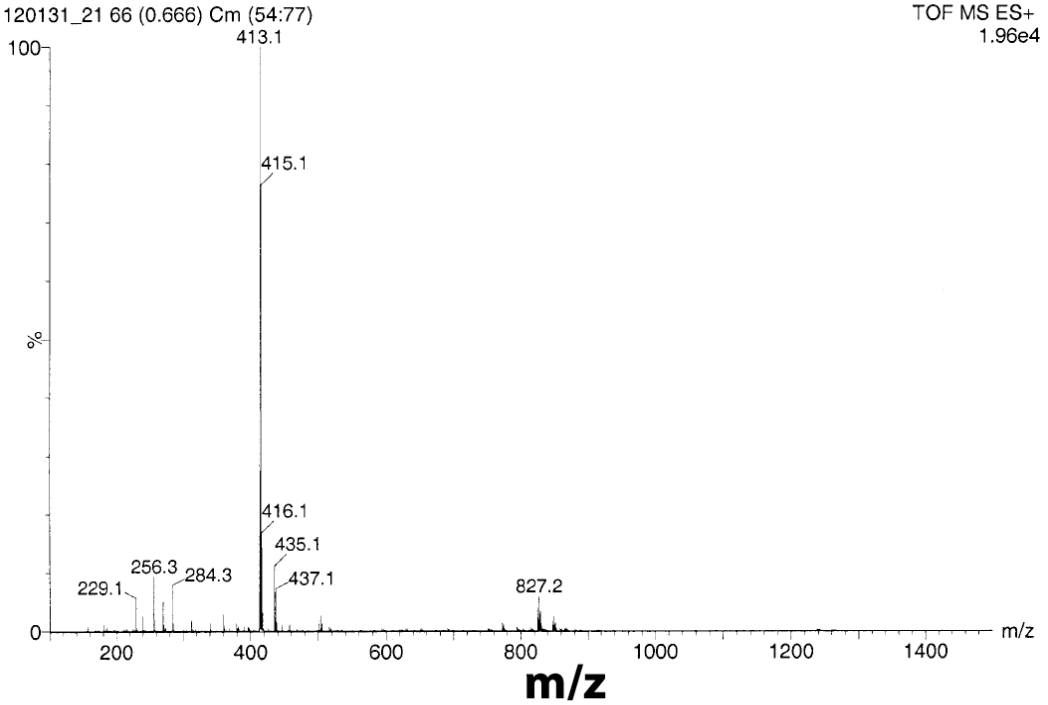
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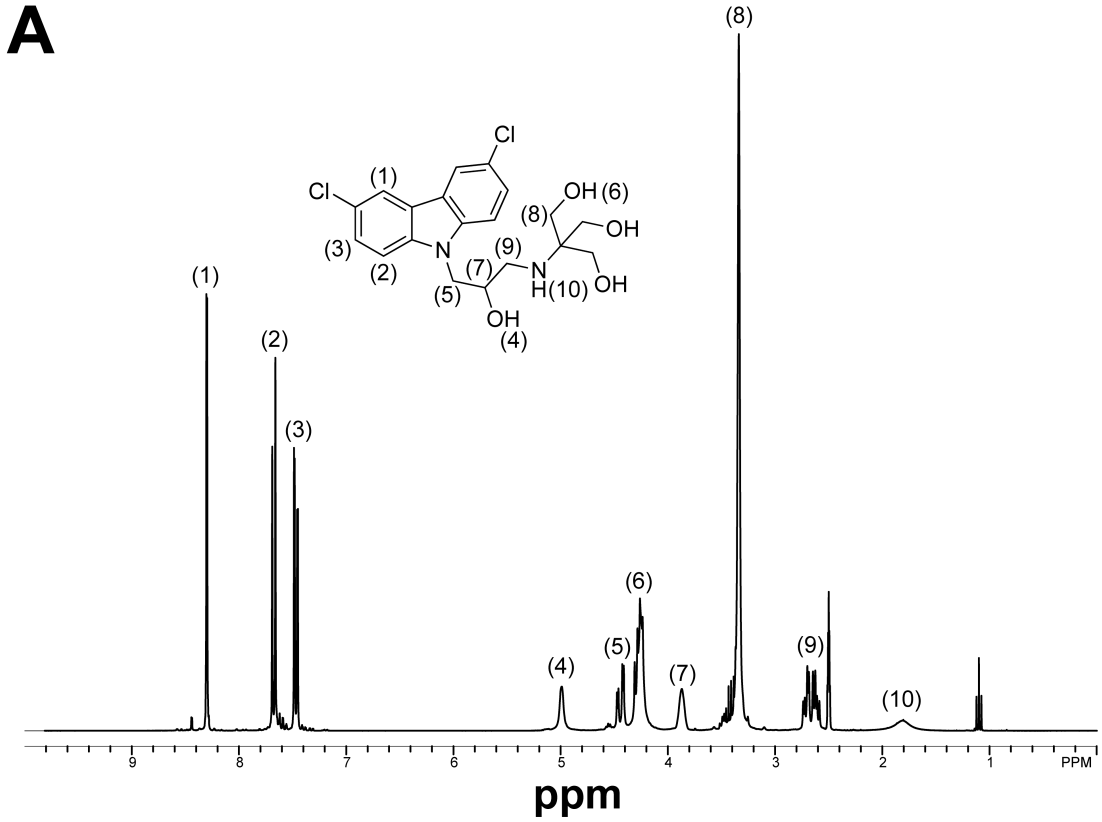
Figure S2. NMR spectra of DCAP. (A) ^{1}H NMR spectrum. ^{1}H NMR (299.7 MHz, DMSO) δ 8.30 (d, J = 2.1 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.47 (dd, J = 8.8, 2.2 Hz, 2H), 4.99 (s, 1H), 4.44 (dd, J = 14.8, 4.0 Hz, 2H), 4.27 (m, 3H), 3.87 (s, 1H), 3.34 (s, 6H), 2.67 (m, 2H), 1.81 (s, 1H). (B) ^{13}C NMR spectrum. ^{13}C NMR (75.4 MHz, DMSO) δ 139.6, 126.0, 123.4, 122.4, 120.1, 111.9, 70.0, 61.2, 59.4, 47.6, 45.1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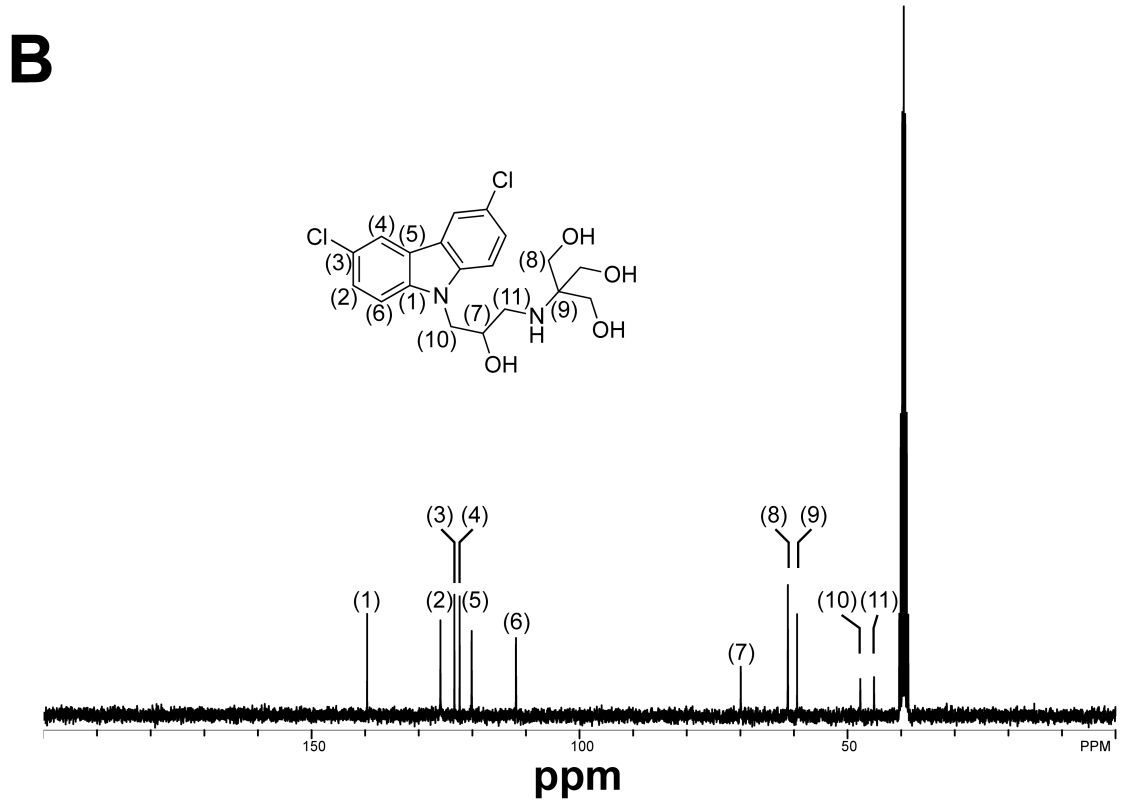
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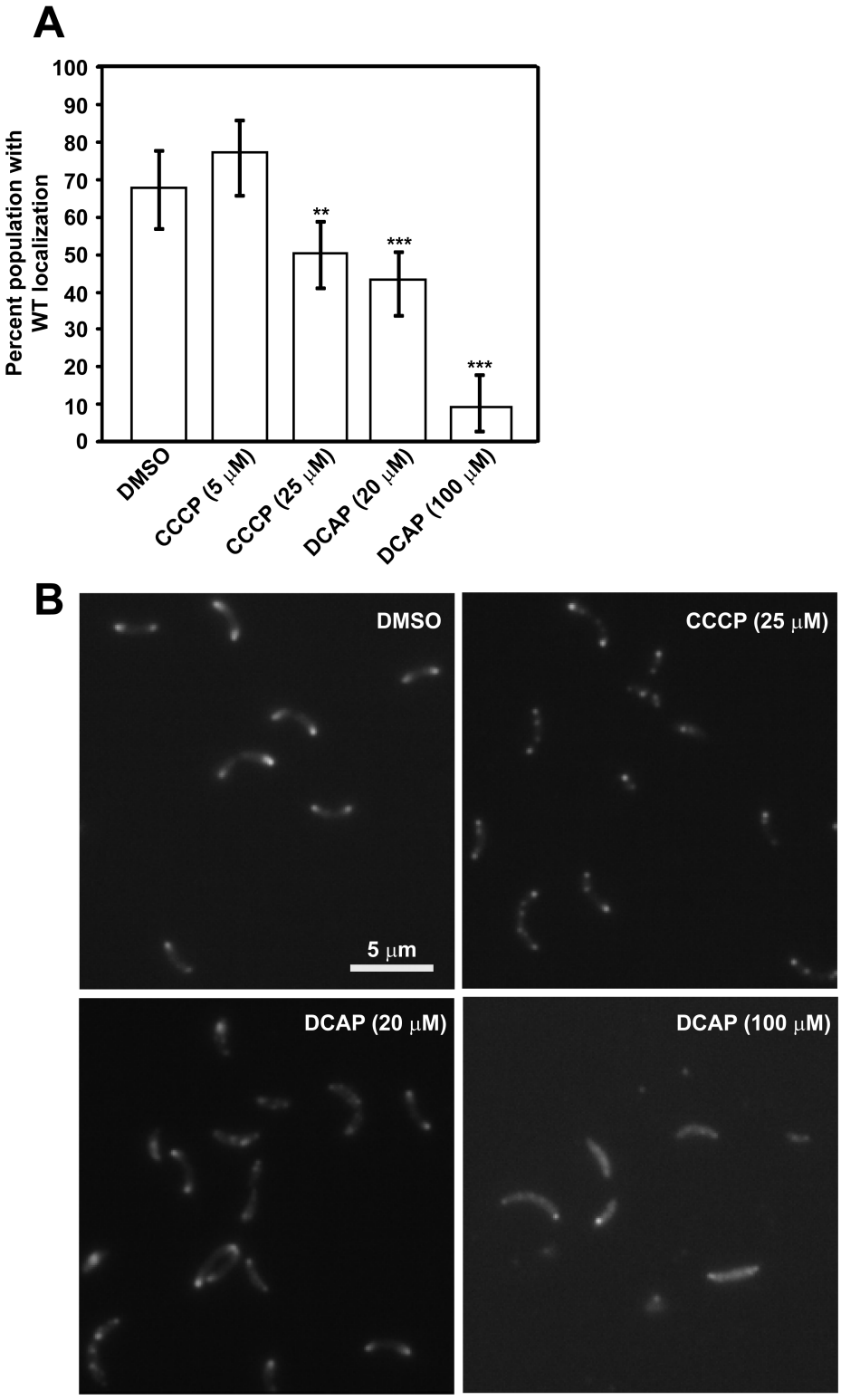
Figure S3. Analysis of MipZ-YFP localization. (A) We defined 'wildtype' (WT) localization as 1) unipolar (a single peak of fluorescence signal at a pole) and 2) bipolar (two peaks at poles). All images were acquired 20 min after treating cells with compounds. At least 69 cells were analyzed for each sample. We calculated two-sided p-values using Fisher's exact test in comparison to DMSO: $p = 0.0075$ (**) for 25 μM of CCCP, $p = 0.0002$ (***) for 20 μM of DCAP, and $p < 0.0001$ (***) for 100 μM of DCAP. (B) Representative fluorescence images of *C. crescentus* cells expressing MipZ-YFP.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80
70
60
50
40
30
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10
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CCCP (5 μM)
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DCAP (20 μM)
DCAP (100 μM)
B
DMSO
CCCP (25 μM)
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DCAP (20 μM)
DCAP (100 μM)

Figure S3.

Figure S4. *C. crescentus* cells 20-min after treatment with DCAP (100 μM). We observed cell lysis in the population (cells with red arrows). The lysed cells look fainter in phase contrast images, compared to cells that did not lyse (e.g. a cell in the white circle). Scale bar, 5 μm.

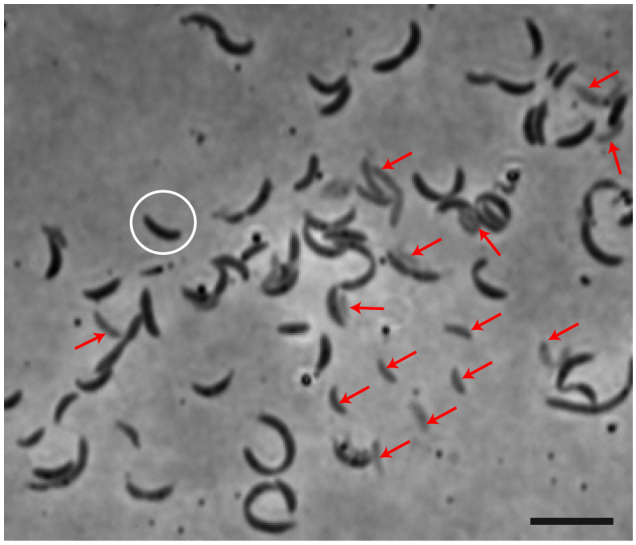

Figure S5. Representative fluorescence images of cells treated with DMSO, CCCP, or DCAP for 20 min. To the right of images, cartoons of bacterial cells depict different wildtype (WT) localizations for MinD and FtsA. (A) *B. subtilis* cells expressing GFP-MinD. The DCAP image shows cells with a few peaks mis-localized to random locations in the cell (orange arrows), cells that contain a large number of fluorescent peaks (white arrows), and cells with diffused fluorescence signal throughout the cell and no polar localizations (blue arrrows). (B) *C. crescentus* cells expressing Venus-FtsA.

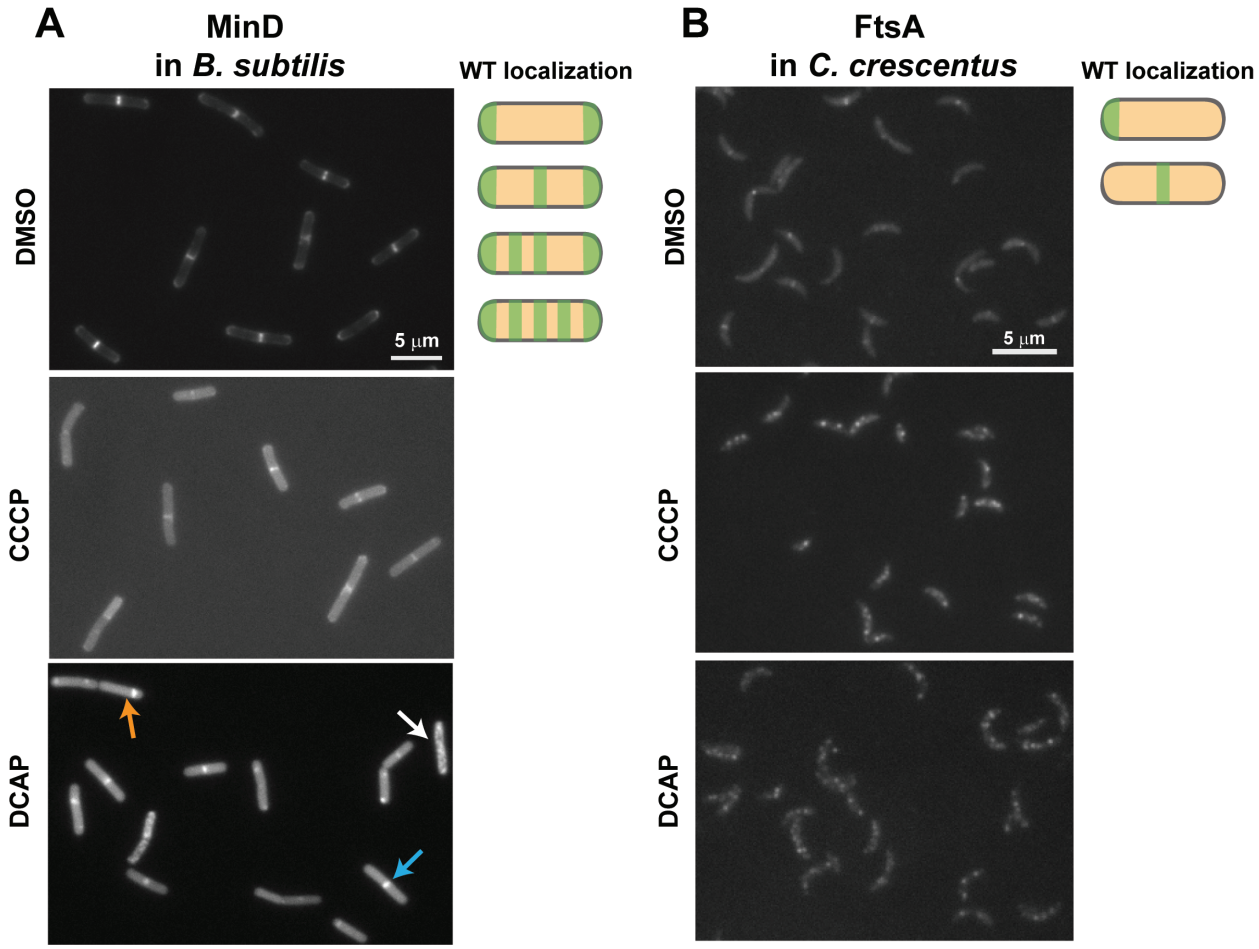

Figure S6. Transmembrane potential (ΔΨ) of mitochondria in HEK cells treated with small molecules. We measured ΔΨ over the course of four time points: 20 min, 6 h, 24 h, and 48 h. Mean values of four independent experiments are plotted. Error bars indicate the standard error of the mean. The percent ΔΨ values plotted are relative to the values obtained from DMSO controls for each compound.

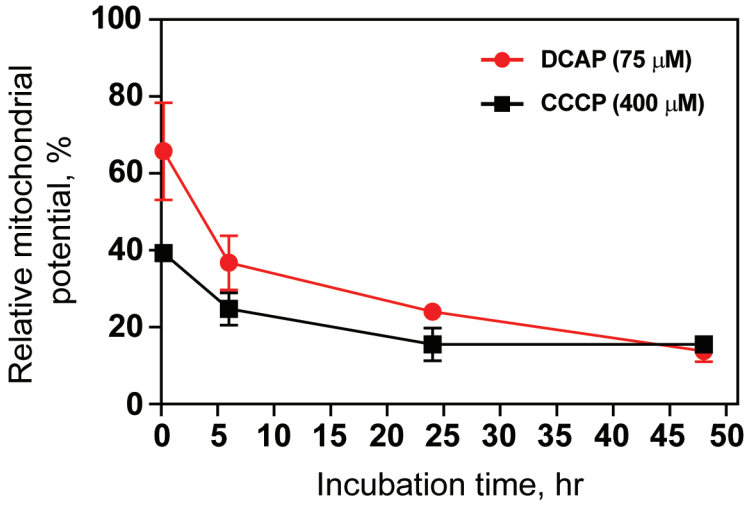

Figure S7. Cell viability of human epithelial kidney cells in the presence of membrane-active compounds. Mean values of four independent experiments are plotted. Error bars indicate the standard error of the mean. The percent viability values are relative to the values obtained from DMSO controls for each compound concentration. The duration of compound treatment is indicated above the bars.

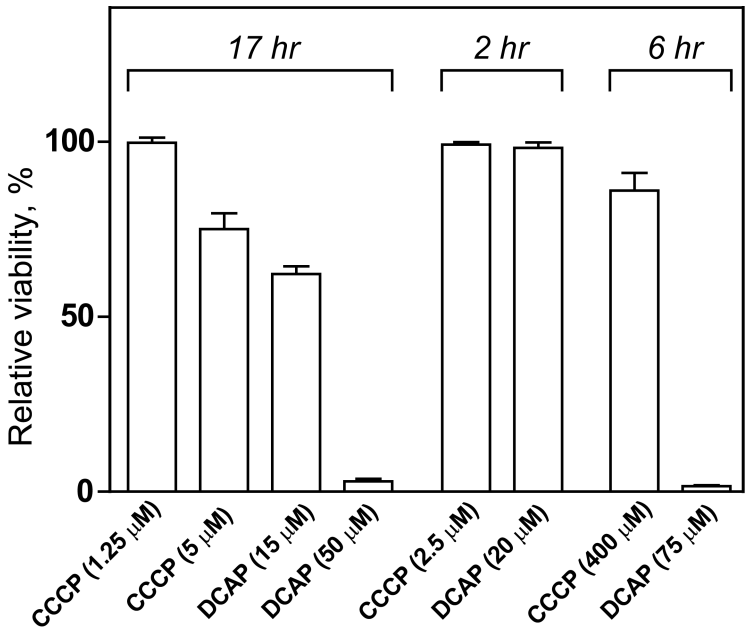

Table S1. List of strains used in this study

Organism/Strain	*Genotype/Description*	*Reference*
C. crescentus CB15N	synchronizable derivative of wild-type CB15	1
C. crescentus MT97	CB15N *mipZ-yfp*	1
C. crescentus AM138	*Pxyl-venus-ftsA, kan^R*	6
S. aureus FRI 100	*sea$^+$ (Tmm^s Hem^{+a} Em^s)*	7
E. coli BW25113	*∆(araD-araB)567 ∆lacZ4787*(::rrnB-3) lambda$^-$ *rph-1 ∆(rhaD-rhaB)568 hsdR514*	8
E. coli BW25113 *ΔtolC*	BW25113 *tolC::kan^R*	8
P. aeruginosa PAO1	Prototroph	9
P. aeruginosa K1115	*ilv-220 thr-9001 leu-9001 met-9011 pur-67 aphA ΔmexCD-oprJ ΔmexAB-oprM*	10
P. aeruginosa K1119	PAO1 *ΔmexAB-oprM*	10
B. subtilis 168	*trpC2*	9
B. subtilis DS4294	*amyE::Pxyl-gfp-minD, cat^R*	4
Salmonella typhimurium	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11
Vibrio cholera	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11
Shigella boydii	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11
Klebsiella pneumonia	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	12
Enterobacter aerogenes	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11
Acinetobacter baumannii	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11
Edwardsiella tarda	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11
Morganella morganii	Clinical isolates from the Department of Medical Microbiology and Immunology (MMI) at the University of Wisconsin–Madison	11

Table S2. Minimum inhibitory concentrations (MIC) for DCAP against various pathogenic strains of bacteria.

Organism/Strain	DCAP (μM)
E. coli BW25113†	80
E. coli BW25113 ΔtolC†	20
P. aeruginosa PAO1†	160
P. aeruginosa K1115§	80
P. aeruginosa K1119†	160
B. subtilis 168§	32
Salmonella typhimurium §	50
Vibrio cholera §	100
Shigella boydii §	100
Klebsiella pneumonia §	100
Enterobacter aerogenes §	200
Acinetobacter baumannii §	200
Edwardsiella tarda §	200
Morganella morganii §	200

*Cultures grown in PYE media at 30 °C. §Grown in LB media at 37 °C. †Grown in M8 media at 37 °C.

References

(1) Thanbichler, M.; Shapiro, L. *Cell* **2006**, *126*, 147.

(2) Ely, B. *Methods Enzymol* **1991**, *204*, 372.

(3) *Methods for Dilution Antimicrobial Susceptiblility Tests for Bacteria That Grow Aerobically*; Fifth Edition ed.; National Committe for Clinical Laboratory Standards: Wayne, PA, 2000.

(4) Foss, M. H.; Eun, Y.-J.; Grove, C. I.; Pauw, D. A.; Rensvold, J. W.; Pagliarini, D. J.; Shaw, J. T.; Weibel, D. B. *Manuscript submitted* **2012**.

(5) Sliusarenko, O.; Heinritz, J.; Emonet, T.; Jacobs-Wagner, C. *Mol Microbiol* **2011**, *80*, 612.

(6) Moll, A.; Thanbichler, M. *Mol Microbiol* **2009**, *72*, 1037.

(7) Foss, M. H.; Weibel, D. B. *Antimicrob Agents Chemother* **2010**, *54*, 3988.

(8) Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H. *Mol Syst Biol* **2006**, *2*, 2006 0008.

(9) Eun, Y. J.; Weibel, D. B. *Langmuir* **2009**, *25*, 4643.

(10) Li, X. Z.; Zhang, L.; Srikumar, R.; Poole, K. *Antimicrob Agents Chemother* **1998**, *42*, 399.

(11) Ho, J. Y. Cira, N. J.; Crooks, J. A.; Baeza, J.; and Weibel, D. B. *Manuscript submitted* **2012**.

(12) Cira, N. J.; Ho, J. Y.; Dueck, M. E.; Weibel, D. B. *Lab Chip* **2012**, *12*, 1052.

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(1) Bush, K.; Courvalin, P.; Dantas, G.; Davies, J.; Eisenstein, B.; Huovinen, P.; Jacoby, G. A.; Kishony, R.; Kreiswirth, B. N.; Kutter, E.; Lerner, S. A.; Levy, S.; Lewis, K.; Lomovskaya, O.; Miller, J. H.; Mobashery, S.; Piddock, L. J.; Projan, S.; Thomas, C. M.; Tomasz, A.; Tulkens, P. M.; Walsh, T. R.; Watson, J. D.; Witkowski, J.; Witte, W.; Wright, G.; Yeh, P.; Zgurskaya, H. I. *Nat. Rev. Microbiol.* **2011**, *9*, 894.