Supporting Material

Guiding Bacteria with Small Molecules and RNA

Shana Topp and Justin P. Gallivan*

Department of Chemistry and Center for Fundamental and Applied Molecular Evolution, Emory University, 1515 Dickey Drive, Atlanta, GA 30322

*To whom correspondence should be addressed.
email: justin.gallivan@emory.edu, phone: 404-712-2171, fax: 404-727-6586

Experimental

General Considerations. Synthetic oligonucleotides were purchased from Integrated DNA Technologies. Culture media was obtained from EMD Bioscience. Amino acids were purchased from Sigma. Ampicillin was purchased from Fisher. DNA polymerase, restriction enzymes, and the pUC18 cloning vector were purchased from New England Biolabs. The pGFPuv and pDsRed-Express vectors were purchased from Clontech. Purifications of plasmid DNA, PCR products, and enzymatic digestions were performed using kits from Qiagen. Plasmid manipulations were performed using *E. coli* TOP10F' cells (Invitrogen) that were transformed by electroporation. All new constructs were verified by DNA sequencing at the NSF-supported Center for Fundamental and Applied Molecular Evolution at Emory University. Experiments were performed with *E. coli* strains RP437 and RP1616, both of which were kindly provided by J. S. Parkinson.

Plasmid Construction. The *cheZ* gene was cloned from *E. coli* TOP10F' cells using PCR. A cassette containing the *tac* promoter, a theophylline-sensitive riboswitch[S1], and the *cheZ* gene was assembled using PCR and was subcloned into the *Bam*HI and *Sac*I sites of SKD1248, a derivative of pUC18 (Ap^R), to create the riboswitch-containing plasmid. A fluorescent reporter gene, GFPuv, flanked by a constitutively active promoter and a transcriptional terminator was

[S1] Lynch, S. A.; Desai, S. K.; Sajja, H. K.; Gallivan, J. P. *Chem. Biol.* **2007**, *14*, 173-84.

amplified from the plasmid pGFPuv and subcloned into the *Sap*I site of the riboswitch-containing plasmid.

Dose-Dependent Migration on Semi-Solid Media. To perform the migration experiments shown in Figure 3, selective media (tryptone broth with 0.25% agar, 50 μg/mL ampicillin, and various concentrations of theophylline) was prepared in Petri dishes (85 mm dia.). Diluted cell suspensions from mid-log-phase cultures (1.5 μL, ~2×10^5 cells/μL) were applied to the center of the plates, which were dried in air for 15 min, and incubated at 37 °C for 10 h. The migration radii were determined by measuring the diameter of the outermost ring of growth, dividing by two, and subtracting the radius of migration of non-motile RP1616 cells grown under the same conditions. The motility of the parent strain (RP437) was unaffected by concentrations of caffeine or theophylline below 2 mM; above this concentration, motility was decreased as a result of cell death.

Gradient Response to Theophylline on Semi-Solid Media. Macroscopic motility experiments using a gradient of theophylline were performed as above, with the following modifications: Media was prepared in 100 mm square Petri dishes. Layers (15 mL) of selective 0.25% agar containing theophylline (1 mM, 0.25 mM, 0 mM) were poured in the pattern shown in Figure 4. Each layer was allowed to solidify for 50 min before applying the following layer. After all layers were applied, the media was allowed to equilibrate at room temperature for 3.5 h, after which diluted cell suspensions from mid-log-phase cultures (3 μL, ~2×10^5 cells/μL) were applied to the locations shown in Figure 4. The plates were dried in air for 15 min, and the cells were grown at 37 °C for 13.5 h.

Gradient Response to Aspartate on Semi-Solid Minimal Media. Square Petri dishes (100 mm) were filled with 50 mL of M9 minimal media containing 0.25% agar, 2% (v/v) glycerol, 50 μg/mL ampicillin, and 1 mM each of methionine, leucine, histidine, and threonine. Plates also contained a static concentration of theophylline (0 mM or 2 mM). After two hours, 40 μL of a 10 mM solution of L-aspartate dissolved in M9 salts was evenly distributed along a thin line in the center of each plate, as indicated in Figure 5. The plates were left at 21 °C for 3.5 h to permit diffusion of aspartate. Cells were grown to mid-log-phase in selective tryptone

broth (50 μg/mL ampicillin). Cells were pelleted at 4,000 rcf and resuspended with M9 salts. After two washes with M9 salts, diluted suspensions of cells (2.0 μL, ~2×10^5 cells/μL) were applied approximately 13 mm from the center of the plates. For each plate, the reprogrammed cells were spotted to the left of center, while wild-type cells were spotted to the right of center. Plates were dried in air for 10 min and incubated at 30 °C for 16 h.

Microscopic Behavior on a Glass Surface. To track the behavior of individual cells, cells were grown to mid-log-phase (OD_{600} = 0.55) in selective tryptone broth containing various concentrations of theophylline. A square (~1 cm/side) was drawn on a glass microscope slide using a wax pencil, the cell suspension (3 μL) was placed at the center of the square, and a glass coverslip was placed on top of the wax to seal the chamber. Samples were imaged in two dimensions using differential interference contrast microscopy (DIC) with an inverted microscope (Leica) equipped with a C.C.D. camera and a 40× objective (N.A. = 1.4). A red filter was used to improve DIC images and to protect cells from high-intensity blue light. Digital images at the surfaces of the coverslip and the slide were captured at 30 Hz and the positions of individual cells were identified using macros within the Interactive Data Language (IDL)[30]. Cells that were visibly stuck to the glass surface or were tracked for less than 1 s were discarded from further data analysis. To differentiate motile cells from non-motile cells within the complex populations, the mean-square displacement (MSD) of each cell was determined at lag times of 0.2 s and 0.6 s. A cell was considered motile if the following three conditions were met: MSD ≥ 4.7 μm at 0.2 s; MSD ≥ 9.4 μm at 0.6 s; MSD at 0.6 s ≥ 2.5× MSD at 0.2 s. These values were determined empirically by examining the behavior of wild-type (RP437, motile) and Δ*cheZ* (RP1616, non-motile) cells.

Determining the fraction of cells that contribute to the overall motility of the population is complicated by the fact that motile cells appear on both the top and bottom of the chamber, while dead and non-motile cells tend to collect on the bottom of the chamber. At low theophylline concentrations, most cells on the bottom of the chamber are non-motile, while at high concentrations, a large fraction of these cells are dead. While these cells do not move in either case, their existence impacts the motility of the population as a whole. To account for this contribution, data were collected from locations on both the top and bottom of the chamber for

equal lengths of time, and the fraction of motile cells in the combined population was determined as a function of theophylline concentration for wild-type and reprogrammed cells (Figure 6a).

To determine the behavior of motile cells, data were collected on the top of the chamber, where cell motion was generally not obstructed by dead or non-motile cells. To minimize tracking errors, the x and y coordinates of each track were independently filtered by calculating the mean of the 3 median points of a 5 point moving window as previously described [28]. Using this filtered data, the maximum run speed of each track was calculated by averaging the top 10% instantaneous velocities in the track. A cell was considered to be tumbling when its instantaneous velocity was less than 30% of its maximum run speed, and its rate of change of direction (RCD) exceeded 5.9 rad/s for at least 2 consecutive frames. The tumbling frequency of the motile population was determined by dividing the total number of tumbling events by the total tracking duration summed over all tracks. The run speeds and tumbling frequencies are shown as a function of theophylline concentration for wild-type and reprogrammed cells (Figure 4).

Supporting Video. Supporting videos of reprogrammed cells in the presence of theophylline are available: 0.05 mM theophylline: SW_0_05mM.mov; 0.25 mM theophylline: SW_0_25mM.mov; 2.0 mM theophylline: SW_2_00mM.mov.

Spatial Localization on Semi-Solid Media. To perform the spatial localization experiments shown in Figure 7, selective media (tryptone broth with 0.25% agar, 50 μg/mL ampicillin) was prepared in Petri dishes (85 mm dia.). After solidification of the media, solutions of caffeine or theophylline (10 μM in tryptone broth), or tryptone broth alone, were applied in the pattern shown in Figure 7a by spotting with a micropipet (1 μL/mm), and the plates were air-dried for 90 min. Diluted cell suspensions from mid-log-phase cultures (2 μL, ~2×10^5 cells/μL) were applied at the location shown in Figure 7a, the plates were dried in air for 15 min, and incubated at 37 °C for 10 h. Plates were imaged on a Kodak transilluminator with NEN filters #25 (red) or #61 (green). False color (red or green) was applied using ImageJ; adjustments to brightness and contrast were performed to the whole image using Adobe Photoshop 8.0.