Supporting Information

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SI Materials and Methods

Strains and Culture Conditions. All strains used in this study are indicated with their sources in Table S1. For all adhesion assays, *Escherichia coli* strains were inoculated from Luria Bertani (LB) agar plates into LB broth and incubated at 37 °C overnight with agitation. The overnight cultures were diluted 1:100 in M63 salts plus 0.5% (wt/vol) casamino acids and 0.2% (wt/vol) glucose (M63+). Polydimethylsiloxane (PDMS) coupons (patterned or flat) were placed at the bottoms of 6- or 12-well tissue culture plates; the overnight cultures diluted in M63+ were used to cover these coupons, and the plates were incubated at 37 °C under static conditions for the times indicated in each experiment.

In experiments requiring force-wetting of the HEX substrates, ethanol was used to reduce surface tension and infiltrate the surface microstructures. Ethanol was then displaced with culture medium, which underwent two additional exchanges before culture to remove traces of ethanol. When fixation was required, the medium was aspirated at the appropriate time point and the PDMS coupons were rinsed twice in PBS. Then, cells were fixed in a modified Karnovsky's fixative [2% (vol/vol) glutaraldehyde, 2.5% (vol/vol) paraformaldehyde in 0.08 M Sorenson's phosphate buffer] for 15 min and washed twice in PBS before imaging. When needed, antibiotics were used at the following concentrations: kanamycin, 75 μg/mL; chloramphenicol, 25 μg/mL.

Atomic Force Microscopy. Overnight cultures of wild-type *E. coli* were diluted 1:100 in M63+ and grown to exponential phase (~4 h; OD_{600} = 0.4). A drop of culture was placed on a flat PDMS coupon and cells were allowed to adhere for 2–4 min. Coupons were then rinsed twice in PBS and examined under a light microscope to ensure a density appropriate for atomic force microscopy (AFM) measurements. Cells were fixed as described above for 1 min and rinsed twice again. Cells were imaged in liquid contact mode on an Asylum MFP-3D AFM using a silicon nitride cantilever with a spring constant k = 0.06 N/m. The z-sensor channel was used to determine bacterial diameters.

Substrate Fabrication. To fabricate the HEX substrate, we used methods described previously (1, 2). A negative structure was defined with a photomask/photoresist and etched in a Si wafer using the Bosch process. We deposited a nonstick layer of (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane onto the Si master and directly molded the PDMS substrates from the coated Si wafers. To produce intermediately-spaced structures, we generated negatives in photocurable epoxy resin (UVO114; Epotek), using the PDMS samples as molds. These served as masters for structural transformation by electrodeposition on patterned substrates (STEPS), described previously (2, 3). Briefly, epoxy negatives were sputter coated with Au to make the surfaces conductive. We immersed the substrates in a bath of 0.1 M sodium dodecyl benzene sulfonate and 0.1 M purified pyrrole and applied a voltage (~600 mV), using the conductive substrate as the working electrode in a standard three-electrode electrodeposition configuration. Samples were slowly withdrawn using a syringe pump, as polypyrrole was conformally electrodeposited. This generated a negative with a gradient of feature sizes. The STEPS-modified gradient samples were then molded in PDMS to obtain substrates with variable spacing/diameter.

Construction of Mutants. To generate deletion mutants, we obtained knockout BW25113 *E. coli* strains for the genes of interest from the Keio collection (Coli Genetic Stock Center; Table S1; ref. 4). These mutations were transferred to the ZK2686 background strain via P1*vir* phage transduction (5). Strains were selected for kanamycin resistance and transductants were confirmed by PCR using primers internal to the kanamycin gene and upstream of the disrupted gene.

Motility assays were used to confirm the loss of motility in Δ*motB* (deletion of the motB flagellar motor protein), Δ*flhD* (deletion of the master regulator of flagella synthesis), and Δ*fliC* (deletion of the gene encoding flagellar filament proteins) strains. Cells were stabbed into the center of LB, tryptone broth, and M63+ plates with 0.3% agar. After overnight growth at 30 and 37 °C, plates were examined for loss of motility compared with wild type. Additionally, exponential phase cells were examined by microscopy for swimming behavior.

Scanning Electron Microscopy. Cells were cultured on PDMS coupons as described above. After fixation, coupons were rinsed two times in PBS and then dehydrated in an ethanol series of 25%, 50%, 75%, 95%, 100%, 100%, 100% (vol/vol) ethanol (dilutions were in deionized water) for 10 min each. Samples were then critical point-dried, mounted on aluminum stubs with carbon tape, and sputter coated with gold. Conductive paths were painted with colloidal silver. Micrographs were obtained on a Zeiss Supra 55VP FE-SEM using a secondary electron Everhart–Thornley or in-lens detector.

Quantification of Biofilms with Confocal Microscopy. Biofilms grown on PDMS coupons were rinsed and fixed as described above. Cells were permeabilized with 0.1% Triton X in PBS for 15 min. Samples were then rinsed with PBS and stained with 5 μg/mL 4′,6-diamidino-2-phenylindole, dihydrochloride for 15 min. The stain was removed, and samples were rinsed in PBS. Biofilms were imaged in PBS using a water-immersion lens mounted on a Zeiss LSM 710 upright confocal microscope. We obtained ≥5 z stacks per sample from random locations on the surface.

Biovolume was quantified using a MATLAB script modified from Comstat (6), in which a Canny edge detector was used to locate edges of biofilms in the stack slices and generate a binary image of edges. These edges were dilated and eroded, and the binary images were then despeckled, followed by thickening of the edges. This image was used as a mask to determine the median pixel intensity for the areas occupied by bacteria. The slice with the maximum median value was used and averaged with its two adjacent slice medians to determine the threshold intensity value for the z stack. Z stacks were then thresholded and voxels were counted and scaled to determine total volume of bacteria within the stack. These volumes were normalized to projected surface area to give average thicknesses for the stacks.

Measurement of Liquid Surface Tension and Surface Contact Angles. To obtain conditioned medium for surface tension measurements, liquid was aspirated from 16-h cultures of *E. coli* biofilms and centrifuged at 2,000 × g, preserving the supernatant. This was passed through a 0.2-μm filter and loaded into a Hamilton syringe. The syringe was inverted and droplets of ~10 μL were imaged with a contact angle goniometer at 20 °C. Surface tension was determined using the pendant drop method (7).

To measure contact angles of PDMS substrates, *E. coli* cultures were grown on HEX substrates as described above. At the time points indicated (Fig. 3*D*), PDMS substrates were removed, rinsed in deionized water, and then sonicated for 10 min in deionized water to remove adherent bacteria (bacterial detachment

was confirmed by crystal violet staining and microscopic examination after contact angle measurements were completed). PDMS coupons were used for contact angle measurements after drying under N_2 gas. Advancing and receding contact angles were measured at 20 °C using a contact angle goniometer to image deionized water on the substrate surfaces. Receding angles lower than 10° or those that were too small to be measured were assumed to be 10°, a conservative lower limit for the goniometer. The difference in these angles was the contact angle hysteresis.

Live Imaging of Flagella. To observe the movement and orientation of flagella, live cells were fluorescently stained with Alexa Fluor carboxylic acid succinimidyl esters as described previously (8). We used both Alexa 488 and Alexa 594 for this application. Briefly, exponential-phase cells grown in M63+ were centrifuged at 2,000 × *g* for 10 min and medium was removed. The pellet was resuspended gently in a wash buffer of 0.01 M KPO_4, 0.067 M NaCl, 10^{-4} M EDTA, pH-adjusted to 7.0 with HCl, and then spun down. After two additional rinses, cells were incubated with 0.5 mg/mL Alexa Fluor carboxylic acid succinimidyl ester for 1 h at room temperature, gently rocking. After staining, cells were washed twice, as above, to remove residual dye, and cells were resuspended in M63+ for imaging.

Stained cells were imaged using fluorescence microscopy. After ~3 h of incubation of bacteria on substrates, images were taken to visualize flagellar orientation. To measure orientation, lines were drawn manually on images along the main axis of flagellar orientation. The angles of these lines with the horizontal were measured and used to generate a histogram (Fig. 5*C*). For videos, the samples were prepared as above and the dynamic adhesion process was recorded at 15–20 frames/s using a QImaging Exi Blue monochrome CCD camera.

Statistical Analysis. Values are reported in the text as value ± SD. For statistical comparisons between groups with normal distributions, Student's two-tailed *t* test was used. Error bars in figures are either SD or standard error of the mean, as indicated in the legends.

1. Pokroy B, Epstein AK, Persson-Gulda MCM, Aizenberg J (2009) Fabrication of bioinspired actuated nanostructures with arbitrary geometry and stiffness. *Adv Mater* 21(4):463–469.
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5. Thomason LC, Costantino N, Court DL (2007) *E. coli* genome manipulation by P1 transduction. *Curr Protoc Mol Biol* Chapter 1:Unit 1.17.
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8. Turner L, Ryu WS, Berg HC (2000) Real-time imaging of fluorescent flagellar filaments. *J Bacteriol* 182(10):2793–2801.

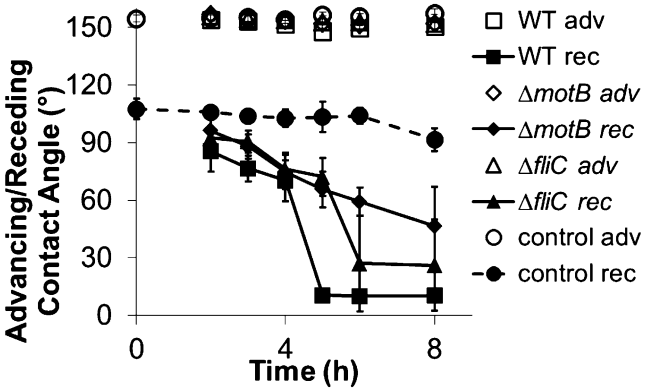

Fig. S1. Advancing and receding contact angles on HEX substrates. HEX substrates were exposed to WT, Δ*motB*, or Δ*fliC E. coli* cultures, or M63+ medium only (control) and measured at the time points indicated. Advancing (open symbols) and receding (closed symbols) angles of droplets of distilled water were measured on these substrates after sonication of surfaces to remove adherent bacteria.

Table S1. Strain names, genotypes, and sources for all *E. coli* strains used in this study

Strain	Description	Reference or source
ZK2686	W3110, Δ(*arg*F-*lac*) U169	Ref. 1
ZK2687	ZK2686, Δ*wca*F31::cam	Ref. 1
JW5665-1	BW25113, F-, Δ(*ara*D-*ara*B)567, Δ*lac*Z4787(::*rrn*B-3), λ-, Δ*bcsA787*::kan, rph-1, Δ(*rha*D-*rha*B)568, *hsd*R514	CGSC
JW4277-1	BW25113, F-, Δ(*ara*D-*ara*B)567, Δ*lac*Z4787(::*rrn*B-3), λ-, Δ*fimA782*::kan, rph-1, Δ(*rha*D-*rha*B)568, *hsd*R514	CGSC
JW1025-1	BW25113, F-, Δ(*ara*D-*ara*B)567, Δ*lac*Z4787(::*rrn*B-3), λ-, Δ*csgA784*::kan, rph-1, Δ(*rha*D-*rha*B)568, *hsd*R514	CGSC
JW1881-1	BW25113, F-, Δ(*ara*D-*ara*B)567, Δ*lac*Z4787(::*rrn*B-3), λ-, Δ*flhD745*::kan, rph-1, Δ(*rha*D-*rha*B)568, *hsd*R514	CGSC
JW1908-1	BW25113, F-, Δ(*ara*D-*ara*B)567, Δ*lac*Z4787(::*rrn*B-3), λ-, Δ*fliC769*::kan, rph-1, Δ(*rha*D-*rha*B)568, *hsd*R514	CGSC
JW1878-4	BW25113, F-, Δ(*ara*D-*ara*B)567, Δ*lac*Z4787(::*rrn*B-3), λ-, Δ*motB742*::kan, rph-1, Δ(*rha*D-*rha*B)568, *hsd*R514	CGSC
RSF31	ZK2686, Δ*bcsA787*::kan	This study
RSF30	ZK2686, Δ*fimA782*::kan	This study
RSF28	ZK2686, Δ*csgA784*::kan	This study
RSF29	ZK2686, Δ*flhD745*::kan	This study
RSF43	ZK2686, Δ*fliC769*::kan	This study
RSF41	ZK2686, Δ*motB742*::kan	This study

cam, chloramphenicol resistance gene; CGSC, Coli Genetic Stock Center (Yale); kan, kanamycin resistance gene.

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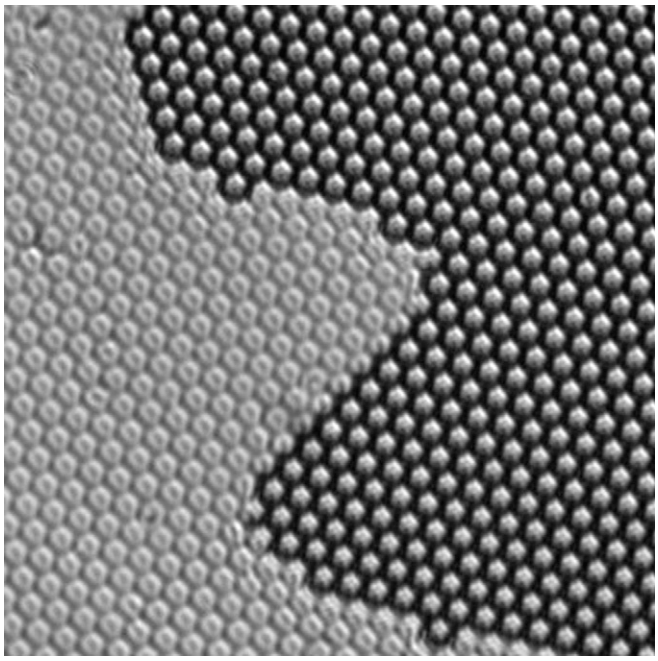
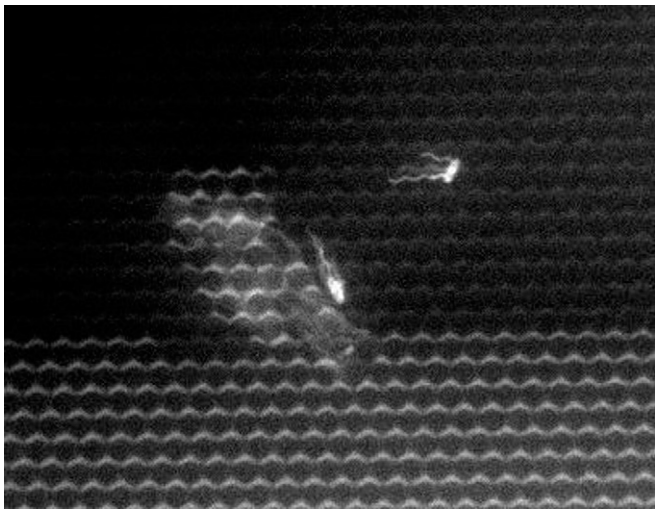


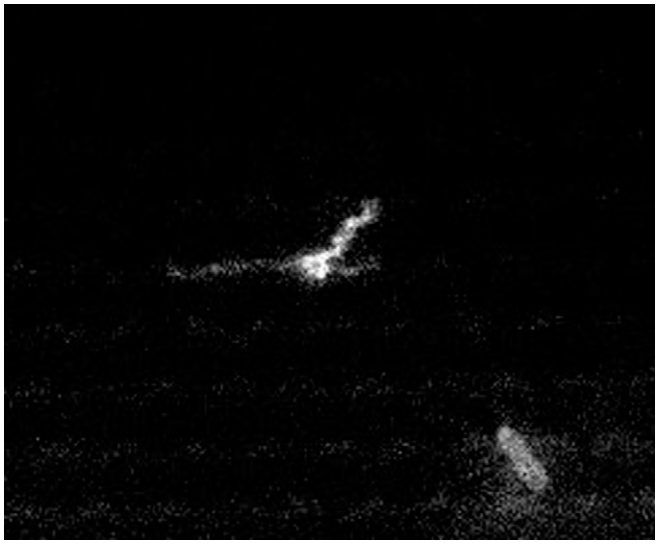
Movie S1. Progression of wetting front after 4 h of culture, shown using phase contrast imaging. Note faintly visible bacteria swimming over substrate. Movie is at 160× actual speed. See also Fig. 3.

Movie S1



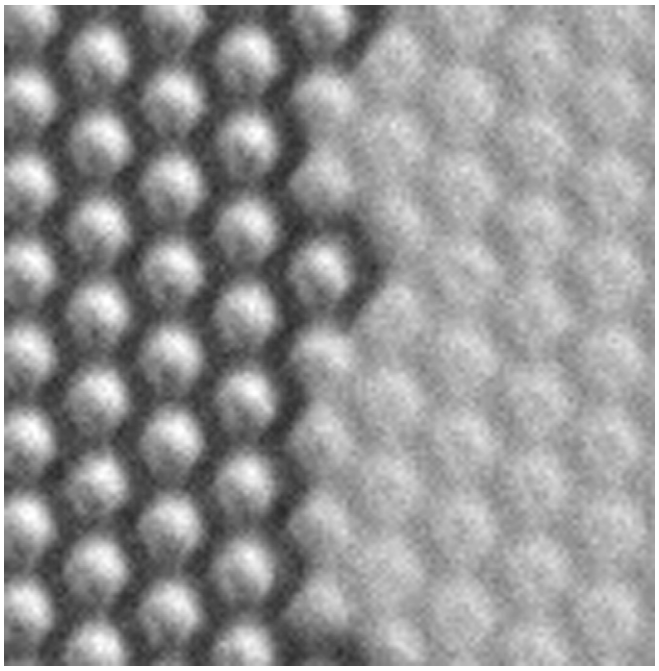
Movie S2. Attachment and settling of *E. coli* using flagella to penetrate surface trenches. Movie is in real time. See also Fig. 5.

Movie S2



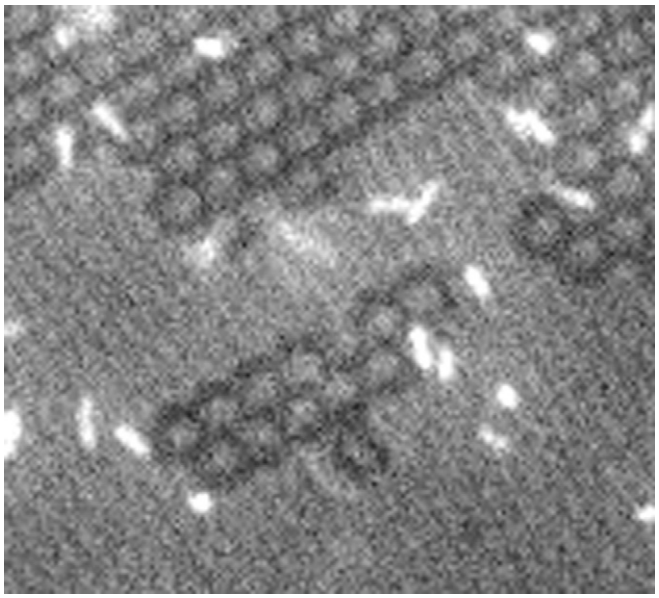
Movie S3. Surface attachment of *E. coli* and alignment of flagellar filaments along the crystallographic axes of the substrate. Movie is in real time.

Movie S3



Movie S4. Motion of bacteria and wetting front at 6 h into culture, shown using phase contrast imaging. Bacteria can be faintly observed moving back and forth over the substrate. Note the advancement of the meniscus and local motion of *E. coli*. Movie is in real time.

Movie S4



Movie S5. Motion of bacteria and wetting front at 6 h into culture, shown using bright-field and fluorescence imaging. Bacteria are observed actively moving over the substrate and providing agitation at the wetting fronts. Note the advancement of the meniscus and local motion of *E. coli* at the bottom-left interface, 7 s into the movie. Movie is in real time.

Movie S5