Protocol for “Bacterial motility patterns adapt smoothly in response to spatial confinement and disorder”, Zhang, Wetherington, Ko, FitzGerald, & Nirody.

Experimental Protocol

Strains and growth conditions:
Experiments were performed using wild-type strains of *E. coli* (HCB 33) labeled with red fluorescent proteins (mScarlet-I). Single colonies were grown from -80C glycerol stocks on solid LB agar plates (LB Broth EZMix, Sigma-Aldrich + 1.5% Bacto Agar, MOLAR Chemicals) and subsequently inoculated in 3mL Lysogeny Broth medium and antibiotics (LB Broth EZMix, Sigma-Aldrich, 50μg/mL Ampicillin and Streptomycin) for 16h +/- 30min overnight at 30C, 250rpm. Overnight cultures were back-diluted 1:10,000 in 3mL LB medium + antibiotics and grown to an optical density at 600nm (OD600) of 0.5. A volume of 10μL of this culture was then gently mixed with 990μL of fresh LB medium + antibiotics for a final OD600 = 0.005.

Microfluidic device fabrication and preparation:
Pillar device design was created using Klayout. A direct write of the pillar device design was performed on a 5” Cr mask plate (Heidelberg Mask Writer - DWL2000) and subsequently developed (Hamatech Mask Chrome Etch 1) and then placed 2x10 minutes in a hot strip bath.
A new Si wafer was primed by spin coating (3000rpm/1000rps/30s) a thin layer of MicroPrime MP-P20 (ShinEtsu MicroSi, Inc.) helping to promote adhesion for photoresist. Next, Shipley 1813 photoresist was spin coated onto the wafer (same protocol) before immediately being placed onto a 115C hotplate for 60s. Surface analysis was performed (Filmetrics) to check for surface irregularities. The outer (~1cm) of photoresist was removed from the edge of the wafer with an Edge Bead Removal System. Using ABM’s High Resolution Mask Aligner the wafer was then exposed for 3s with Near-UV (405 nm) through our developed mask. The wafer was subsequently developed using MIF 726 (Hamatech-Steag wafer processor). After inspection under a microscope, the wafer went through a cycle of plasma descum in order to remove residual layers of photoresist (Anatech Resist Strip). Next, we performed a Bosch fluorine process (Unaxis 770 Deep Silicon Etcher) for a final etch depth of 5 microns, confirmed using the P-7 profilometer. The wafer was then stripped of all resist using 2x10 minute wet bath soaks. Finally, the MVD-100 molecular vapor deposition system was used with the anti-stiction coating (1H,1H,2H,2H-Perfluorooctyl)Trichlorosilane (FOTS).
At this point the wafer was ready to be used as a master mold of the pillar device. To make the chips used for experiments, Polydimethylsiloxane (PDMS, Sylgard 184 Silicone Elastomer Kit, Dow Corning) was mixed in a ratio of 1:10, degassed in a desiccator and poured onto the master mold and baked at 60C for 2h. Devices were cut out from the wafer and 1.5mm diameter holes were hole-punched at both inlets.

Microfluidic experiments:
Prior to inoculation into the device, LB + antibiotics was used to wetten the device using a syringe. This was performed immediately after the post-bake upon plasma binding the device to a clean glass bottom petri-dish (See Microfluidic device fabrication and preparation). Once wetted, the device was inoculated with 1μL of the OD600 = 0.005 suspension in one inlet using a pipette tip. Once inoculated, both inlets were then sealed using High-Vacuum Grease (Dow Corning). The sealed device was then submerged in Milli-Q water (preheated to 30C) to prevent the device from drying out and placed onto the microscope stage ready for imaging. A waiting

period of ~2h was needed for sufficient cell density (10-50 cells in the field of view) to accumulate into the main arena of the device. During the time-lapse microscopy experiment, the microscope set-up was equipped with an incubator controlling the temperature of the environment to 30C.

Image acquisition and data analysis:

Each device contained 3 levels of confinement for obstacle regions and 4 levels of disorder for each level of confinement, amounting to a total of 12 unique pillar regions. Video recordings were taken by a Nikon Eclipse Ti-E microscope, equipped with a 20x Plan Fluor objective, mCherry fluorescence (49008, Chroma Inc.), and an Andor Neo sCMOS camera. The videos were 40s (20 fps, 800 frames) for each of the 12 regions along with one unconfined region without pillars. This process was repeated for a total of 7 times over the course of 30 minutes to ensure sufficient cell trajectories.