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ONLINE METHODS

Strains used

The genotypes and sources of all *E. coli* strains used in this study are given in **Supplementary Table 1**. RP437 is wild type for chemotaxis[31]. CR20 and CR33 are 'runner' (run only) and 'tumbler' (no runs) mutants, due to *cheY* and *cheZ* deletions, respectively. PS2001 is a strain where environmental signals are decoupled from the behavior of flagellar motors[21,24]. Tumbling bias in this strain can be adjusted by expressing CheYD13K[32] from a plasmid (pMS164), under an inducible promoter. As opposed to the wild-type CheY, CheYD13K does not require phosphorylation to be active[24]. Thus, all CheYD13K proteins expressed in the cell are able to bias flagellar motors for longer clockwise rotation. Clockwise rotation of flagellar motors tends to cause the swimming cell to tumble[1].

Cell growth and media

E. coli cells were harvested from a single colony on an agar plate and grown overnight in 1 ml tryptone broth (1% (wt/vol) Bacto tryptone, 0.8% (wt/vol) NaCl)[33]. The overnight culture was diluted 100-fold into 1 ml tryptone broth in a 14-ml round bottom Falcon tube and grown for 4.5 hours to mid-log phase (OD_{600} ~0.5). The PS2001-pMS164 strain was grown with antibiotics chloramphenicol (34 $\mu g\ ml^{-1}$),

kanamysin (40 μg ml^{-1}), and various levels (1-100 μM) of the inducer isopropyl-β-D-galactopyranoside (IPTG)[21]. All bacterial growth in this study was done at 30 ^{o}C with 265 rpm rotation[34]. Cells were washed from growth medium by centrifugation (2,000 g, 10 min) followed by gentle resuspension in trapping medium. Resuspension by pipetting was minimized, as it can cause the flagella to break due to shear forces.

Trapping medium contained tryptone broth (TB) supplemented with 2% (wt/vol) glucose, 100 mM Tris-Cl (pH7.5), and an oxygen scavenging system (20 μg ml^{-1} glucose oxidase and 4 μg ml^{-1} catalase, Sigma; adapted from[35]) in order to reduce trap-mediated oxidative damage to the cells[36]. The oxygen scavenging system was added 2 hours before use in order to reach a steady oxygen level. Resazurin (Sigma) was added (0.0001% (wt/vol)) as an oxygen indicator. Glucose acts as a substrate for the oxygen scavenging system and provides energy for the cells swimming in anaerobic condition[37]. Tryptone broth is an appropriate growth medium for obtaining reproducible cell motility[38]. However, it is not suitable for chemotaxis studies, since it contains amino acids that can act as chemoattractants. To demonstrate the future feasibility of chemotaxis measurements using our method, we also examined cell behavior in a "motility buffer" containing 70 mM NaCl[14], supplemented by 100 mM Tris-Cl, 2% (wt/vol) glucose and the oxygen scavenging system. Trapped cells under these conditions display the same behavior as those described in the main text: they remain motile for long durations, exhibit similar flagellar and body rotation rates, and tumble at similar frequencies (see **Supplementary Table 2**). A high buffering capacity is necessary to prevent acidification of the medium by gluconic acid, a side product of the oxygen scavenging reactions[39]. Though a 100 mM Tris-Cl buffer is preferable for long-term stability of our trapping medium, a lower molarity is acceptable for shorter time periods (~1 hr) if ionic strength is a concern. Cells resuspended in a buffer containing 70 mM NaCl, 10 mM Tris-Cl, 2% glucose (wt/vol) and the oxygen scavenging system are well-behaved in our trap (see **Supplementary Table 2**).

Fluorescent labeling of cells.

Wild-type cells were labeled with Cy3 monofunctional NHS ester (GE Healthcare) following a previously reported protocol[14]. Cells were grown to mid-log phase in tryptone broth and washed twice (1000 g, 10 min) with the final resuspension concentrating cells 20-fold in a buffer containing 10 mM KPO_4 (pH 7.0), 70 mM NaCl and 0.1 mM EDTA. Cy3 dye suspended in 25 μl of 1 M $NaHCO_3$ was added to 500 μl of the culture. The labeling reaction was incubated with slow rotation (~10 rpm) at room temperature in the dark for 90 minutes. The labeled culture was washed once and diluted 100-fold in 1 ml of modified

trapping medium. The modified trapping medium contained 50 mM ascorbic acid (Sigma) in place of the oxygen scavenging system. Ascorbic acid is known to quench oxygen radicals and reduce photobleaching[40].

Construction of chemotaxis mutants.

The *cheY* and *cheZ* deletion strains were both made using the method of Datsenko and Wanner[41]. In the case of the *cheY* deletion strain, the primers Y_F and Y_R (see **Supplementary Table 3**) were used to PCR amplify the chloramphenicol resistance cassette from pKD3 with 40-nt extensions homologous to the flanking regions of the gene in order to generate an in-frame deletion. Similarly, the primers Z_F and Z_R (see **Supplementary Table 3**) were used to generate the *cheZ* deletion strain. In the case of the *cheY* deletion, the FRT-cm-FRT cassette was removed by passing pCP20 through the strain.

Combined optical tweezers and fluorescence microscope.

A detailed description of the optical tweezers design can be found elsewhere[10]. Briefly, the optical tweezers component consisted of two orthogonally polarized beams from a single 5-W, 1064-nm diode-pumped solid-state laser (BL-106C, Spectra-Physics). Both beams were tightly focused to generate optical traps by a 60X, water-immersion (1.2 NA) microscope objective (Nikon). The separation between the two traps was controlled by a piezo-actuated mirror stage (Nano-MTA, Mad City Labs). An identical objective lens collected transmitted light for position detection and bright-field imaging. A custom flow cell (see detailed description below) positioned between the two objective lenses served as the experimental chamber, and could be displaced relative to the two traps in all directions by a motorized three-axis translational stage (ESP300, Newport). Cell motion was detected directly by the optical traps themselves, using the standard optical techniques known as back-focal plane interferometry, in which trap light scattered by an object relays the object's position relative to the trap in all three directions. Epifluorescence excitation in wide-field configuration was provided by a 30-mW, 532-nm diode laser (TECGL-30, World Star Tech). Emitted fluorescence light passed through a dichroic mirror (Di01-R532-25x36, single-edge 532-nm laser dichroic, Semrock), a 5X beam expander (for additional image magnification), and an emission filter (FES0900, band-pass 540-870 nm, Thorlabs) before being imaged onto an intensified charge-coupled device (I-PENTAMAX, Princeton Instruments). When displaying the fluorescence images, which were taken at 10 frames per second, the contrast was adjusted to maximize the contrast between the flagella and the background and opening/closing morphological operations were applied to smooth-out the noise. Opening first erodes and then dilates the image using a structure

element, which in our case was a disk with a 3-pixel radius. Erosion takes darker pixels and expands them to the neighboring pixels using the structure element. Dilation does the same with lighter pixels. Closing performs a dilation followed by an erosion. For more information on these morphological operations see[42]. Each frame in **Figure 2a** and **Supplementary Figure 5** is an average of three such images.

Construction of the flow cell.

Glass coverslips (Fisher, No. 1) were sonicated in dry acetone for 5 minutes, and rinsed with deionized water. Flow channel patterns were cut out from Nescofilm (Karlan) and placed in between two coverslips, one of which had custom-drilled holes (0.05-inch diameter) for inlets and outlets. A short piece of glass capillary (100 μm outer diameter) was positioned near the experimental region as a spacer and a point of reference. The Nescofilm flow channel pattern was bonded to coverslips by melting on a hot plate for 4 minutes. The completed flow cell was inserted into a custom metal frame where inlet and outlet tubing were screwed on for a tight seal (see **Supplementary Fig. 1**).

Optical trapping assay.

E. coli cells suspended in trapping medium at a low density (OD_{600} ~0.01) were injected into the top "antechamber", and flowed through an inlet into the bottom channel containing blank trapping medium. Cells were initially trapped by a single beam along the beam axis, and the second beam was repeatedly brought near the first and pulled away until the two ends of the rod-shaped cell were held by each trap. The separation between the two traps was adjusted until it appeared roughly equal to the cell length. 50 mW of trapping power at the sample plane was used in each trap, sufficient to stably trap and manipulate the cells, yet low enough to minimize photodamage. Before data acquisition, the trapped cell was moved sufficiently far away from the connecting region between the top and the bottom channels in order to prevent possible interruptions by other cells in the flow cell. In addition, the use of water immersion objectives to form our traps allowed us to position cells far from the sample chamber surfaces (50 μm), minimizing potential cell-surface interactions.

Two-dimensional swimming assay.

A small drop (~5 μl) of trapping medium containing *E. coli* cells at OD_{600} ~0.1 was placed on a coverlip (Fisher, No. 1.5) and spread evenly by gently covering with a 22 mm x 22 mm coverslip (Fisher, No. 1) from the top[21]. Care was taken to prevent formation of air bubbles. Coverslips were used directly out of

the box[20]. To prevent drift due to evaporation, open sides were sealed with molten wax. 2-D swimming of *E. coli* cells confined in the resulting ~10 μm-thick fluid chamber was observed in phase contrast with a 10X objective (Nikon Eclipse TE2000-E). 30-second video images were taken at 30 frames per second at various locations on each slide[21].

Run-tumble analysis of optical trap data.

All routines for analyzing optical trap data were written in Matlab (Mathworks). Raw data obtained at 1000 Hz sampling frequency were low-pass filtered to 100 Hz, and the amplitude was normalized in non-overlapping 1-second windows. Two separate sets of *y* and *z* signals obtained from the two ends of the cell body were combined by taking the difference for enhancement in signal-to-noise ratio[43]. Motion of the cell in the *x* direction was also detected by the PSD, but was more inconsistent. We believe this is because the cell trajectory is predominantly in the orthogonal *y*-*z* plane. Thus, signals along the *x* direction were not used in our analysis. Using the *y* and *z* signals, the peak frequency component (Ω) at each time point was obtained from a continuous wavelet transform. This method is preferable to a Fourier transform over a running time window. Whereas the Fourier transform is limited by the trade-off between temporal and spectral resolution for a given window size (a larger time window results in high frequency resolution but low time resolution, and vice versa), the continuous wavelet transform does not require a characteristic window size. Instead, this transform makes use of a "mother wavelet" that can be scaled and shifted in order to find the best match for the data trace at each time point. The scale can then be converted to a corresponding frequency. Our wavelet analysis was performed using the complex Morlet mother wavelet in a linearly-scaled frequency range of 2-40 Hz[22]. A typical result is shown in **Supplementary Figure 3**. Runs and tumbles were distinguished by applying a single threshold value to the Ω time trace. The threshold was determined by examining the distribution of Ω and finding the local minimum between peaks corresponding to run and tumble (**Supplementary Fig. 3b**). For cases where a clear local minimum could not be found, an arbitrary threshold of 4 Hz was applied. Detected runs and tumbles that were shorter than 100 ms were removed, since our detection limit was expected to be one cycle in the sinusoidal pattern of the running cell (10 Hz body roll frequency is taken as an arbitrary standard). We obtained two separate binary traces from the signals in *y* and *z* directions from each cell. The same threshold value was used for both *y* and *z* directions, and the two resulting binary traces were combined using an AND-gate to produce a single binary trace for the cell. Various swimming parameters of individual cells were estimated from the resulting binary trace. For instance,

the mean run and tumble durations were determined by fitting the run and tumble duration distributions with exponential distribution functions.

Analysis of higher-order features in trap data.

In addition to the peak frequency component (Ω), the corresponding phase (ϕ) at each time point was obtained from the continuous wavelet transform of the swimming signals in *y* and *z* directions (see **Supplementary Fig. 6b-d**). 2-D histograms of Ω and $\Delta\phi$ (phase difference between the *y* and *z* directions) were manually examined to define different swimming states as follows: 1) A threshold in Ω that divides the tumble state from the run states was determined from the local minimum in the Ω histogram. 2) A threshold in $\Delta\phi$ that divides the run states into runs in two opposite directions was determined as the mid-point between the two peaks in the $\Delta\phi$ histogram. 3) If multiple run states were clearly visible from the 2-D histogram in either of the run directions, a threshold dividing those states was determined as the mid-point between the center points in the 2-D histogram. A typical result of these procedures is shown in **Supplementary Figure 6a**. Each time point in the data trace was then assigned to a particular state according to Ω and $\Delta\phi$ values (**Supplementary Fig. 6e**). Detected events shorter than 100 ms were removed for the same reason as discussed above. Unlike the case of two-state analysis, we did not obtain two separate multi-state traces from the *y* and *z* directions. Instead, the average Ω of the *y* and *z* directions was used.

Analysis of two-dimensional swimming videos.

The field of view of video images was 512 x 512 pixels, covering approximately 320 μm x 320 μm. Images were analyzed using a custom routine written in Matlab, loosely based on previously reported algorithms[21,44]. Images were adjusted for contrast, and a threshold was applied to discriminate cells as black objects against a white background. Contiguous black objects between 3-30 pixels in size were identified as cells, and their centroid and long-axis orientation were determined. Trajectories connecting the cell positions in successive image frames were tracked by matching each cell with one cell in the next frame that was within 5 pixels. A trajectory was terminated if there were no cells or multiple cells connected in the next frame. Trajectories shorter than 3 μm in contour length or 1.33 s in duration were discarded. The remaining trajectories were filtered using a modified median filter following[21]. The filtered trajectories were then analyzed to determine run-tumble statistics, distributions of angle changes, run speed, and other parameters of potential interest. Of particular interest were run and tumble durations. Tumbles were identified by setting thresholds in both linear velocity and angular

velocities. First, the "average speed" of a cell was defined by sorting the linear velocity values for each cell, excluding the top and bottom 10%, and taking the mean of the remaining velocities. The tumble threshold was then defined as any drop in linear velocity below half the 'average speed' of the cell, and an increase in angular velocity to three times the average angular velocity (similar to the algorithm used in Amsler[44]). The end of each tumble was determined by comparing the speed after a tumble to the 'running speed', which was defined as the mean of the fastest 10% of speeds in a trajectory[21]. After initiation of a tumble a standard student's t-test was applied to compare a moving 3-point window of speeds to the running speed. When the t-test had a p-value higher than 0.05, the ends of tumbles were scored.

Supplementary Figure 1	Microfluidic chamber.
Supplementary Figure 2	Optically trapped cell grows and divides.
Supplementary Figure 3	Run-tumble analysis of optical trap data.
Supplementary Figure 4	Distributions of tumble duration as detected in the optical trap assay.
Supplementary Figure 5	Optically trapped cell undergoing reversal of swimming direction.
Supplementary Figure 6	Analysis of optical trap data for multi-state swimming behavior.
Supplementary Figure 7	Detection of swimming direction reversal in the 2-D swimming assay.
Supplementary Table 1	Strains and plasmids used.
Supplementary Table 2	Comparison of trapping media.
Supplementary Table 3	Primer sequences.
Supplementary Note 1	Limitations of current assays for cell motility.
Supplementary Note 2	The relation between trap oscillatory frequencies and cell motility.
Supplementary Note 3	Difference in tumble duration between trapped and free-swimming cells.
Supplementary Note 4	The possible role of reversals.
Supplementary Note 5	Changes in swimming speed.
Supplementary Note 6	Directional bias of cell swimming.
Supplementary Note 7	Future experimental directions.

AOP

Optically trapping an individual *E. coli* cell allows the long-term quantitation of bacterial swimming phenotype: the stochastic transitions between 'running' and 'tumbling' as well as changes in swimming speed and direction.

Issue

Optically trapping an individual *E. coli* cell allows the long-term quantitation of bacterial swimming phenotype: the stochastic transitions between ‘running’ and ‘tumbling’ as well as changes in swimming speed and direction.