Osmotaxis in *Escherichia coli* through changes in motor speed

Jerko Rosko, Vincent A. Martinez, Wilson C. K. Poon, and Teuta Pilizota

*Centre for Synthetic and Systems Biology, Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3FF, United Kingdom; and *Scottish Universities Physics Alliance and School of Physics & Astronomy, University of Edinburgh, Edinburgh EH9 3FD, United Kingdom

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Bacterial motility, and in particular repulsion or attraction toward specific chemicals, has been a subject of investigation for over 100 years, resulting in detailed understanding of bacterial chemotaxis and the corresponding sensory network in many bacterial species. For *Escherichia coli* most of the current understanding comes from the experiments with low levels of chemotactically active ligands. However, chemotactically inactive chemical species at concentrations found in the human gastrointestinal tract produce significant changes in *E. coli*’s osmotic pressure and have been shown to lead to taxis. To understand how these nonspecific physical signals influence motility, we look at the response of individual bacterial flagellar motors under stepwise changes in external osmolality. We combine these measurements with a population swimming assay under the same conditions. Unlike for chemotactic response, a long-term increase in swimming/motor speeds is observed, and in the motor rotational bias, both of which scale with the osmotic shock magnitude. We discuss how the speed changes we observe can lead to steady-state bacterial accumulation.

Many bacterial species are able to not only self-propel (exhibit motility), but also direct their motion toward more favorable environments. This behavior, called taxis (1, 2), has long been a subject of scientific investigation, as it serves a variety of purposes: seeking out nutrients and avoiding toxic substances (3, 4) and identifying thermal (5) and oxygen (6) gradients, as well as aiding pathogenic species in infecting their hosts (7, 8). The understanding of bacterial taxis is not only important when it comes to bacterial motility and accumulation; it also serves as a model for biological signal processing. Of particular interest are the precision (9, 10), sensitivity (11), and robustness (12, 13) that can be achieved with biological networks and potentially used for human design purposes (14, 15). Specifically, bacterial chemotaxis, motion toward or away from specific chemicals (3), has been the focus of scientific investigation for over 100 years, serving as a model of both biological sensory transduction and self-propulsion. Consequently, chemotaxis of *Escherichia coli* is one of the best-understood biological networks. Nevertheless, the exact roles of taxis and motility in *E. coli*’s life cycle, particularly in host invasion, remain unknown, partly because of the complexity of its natural habitat. By looking at the response of both individual bacterial motors and a swimming population, we investigate *E. coli*’s response to changes in external osmolalities similar to those found in the human gastrointestinal tract. We find that, unlike chemotaxis, osmotic response changes the motor speed and discuss how the observation can lead to previously observed osmotaxis.

**Significance**

Bacterial taxis has been a subject of active investigation for over 100 years, serving as a model of both biological sensory transduction and self-propulsion. Consequently, chemotaxis of *Escherichia coli* is one of the best-understood biological networks. Nevertheless, the exact roles of taxis and motility in *E. coli*’s life cycle, particularly in host invasion, remain unknown, partly because of the complexity of its natural habitat. By looking at the response of both individual bacterial motors and a swimming population, we investigate *E. coli*’s response to changes in external osmolalities similar to those found in the human gastrointestinal tract. We find that, unlike chemotaxis, osmotic response changes the motor speed and discuss how the observation can lead to previously observed osmotaxis.


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1 To whom correspondence should be addressed. Email: teuta.pilizota@ed.ac.uk.

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negative osmotaxis is less known (35, 36, 39, 40), and contradictory observations have been reported. Osmotic stimuli can send a signal down the network through mechanical stimulation of chemoreceptors (41); yet, free-swimming cells were observed to tumble in response to an osmotic stimulus even when completely devoid of all components of the chemotactic network (35, 40). Intriguingly, this tumbling was later attributed to CCW rotation of flagella that normally results in cell runs (40). The finding that osmotaxis on agar plates requires the presence of at least some chemosensors and all of the chemotactic signaling proteins further complicates the picture (35). Open questions thus remain about the nature of this motile behavior that drives spatial accumulation and its similarities, differences, and possible interactions with taxis toward chemicals.

To clarify the exact nature of the osmotic response, we study the phenomenon on both single-cell and population levels, observing the rotation of individual flagellar motors under stepwise increases in osmolality and measuring the swimming speeds of a population of ~10,000 bacteria after exposure to an osmotic shock. The shock magnitudes we administer are motivated by those encountered in the human gastrointestinal tract (28, 31). We find that a stepwise increase in osmolality results in an elevated, long-term CW bias. In addition, we observe osmokinesis after osmotic shock, i.e., significant changes in the motor and, consequently, swimming speeds of bacteria. Finally, for higher shock magnitudes, we observe a loss of motor speed immediately after shock, followed by a speed recovery. We discuss how the observed nonadaptive response and osmokinesis can lead to taxis.

Results

Single-Motor Response to an Osmotic Shock Is Complex. We begin with the reasonable assumption that at least one component of the chemotactic network responds to an osmotic stimulus to generate previously observed osmotactic behavior (35). Then, the response should be evident in the output of the chemotactic network, the CW bias of a single BFM. CW bias is defined as the fraction of time the BFM spends rotating in the clockwise direction (42).

$$\text{CW Bias} = \frac{N_{cw}}{N_{tot}},$$  

where $N_{cw}$ is the number of data points corresponding to CW rotation and $N_{tot}$ is the total number of data points in a given time interval.

To compute the CW bias, we measured the speed of an individual BFM when exposed to a stepwise increase in the external osmolality. SI Appendix, Fig. S1 gives a schematic of the bead assay used for measuring the motor speed (25, 42). Briefly, we attach cells to the coverslip and attach a polystyrene bead, 0.5 µm in diameter, to a short filament stub (25, 42). The rotation of the BFM-driven bead is recorded using back-focal-plane interferometry at a 10-kHz sampling rate (43–45). A representative single-motor rotation trace so obtained is shown in Fig. 1A. The shaded light-blue interval shows the motor speed and rotational direction before osmotic stimulus. Positive motor speed $\sim 120 \mu \text{m/s}$ (47), resulting in a shock magnitude of 488 mOsmol/kg. (B, Top) A histogram of CW bias computed by binning the trace in $A$ into 30-s bins and dividing the time spent rotating CW by the bin length (Eq. 1 and Materials and Methods). B, Bottom shows the same histogram condensed into a color map, with an intensity scale to the right. (C) Single-motor speed trace of a $\Delta \text{CheY}$ mutant exposed to the same osmotic upshock as in $A$.

Postrecovery phase, which we define as the period after motor switching has resumed, is characterized by an increased switching frequency. Consequently, the CW bias is also elevated (to 0.16) and is maintained over at least 20 min, suggesting that, unlike chemotaxis, the osmotic response does not exhibit perfect adaptation (10, 24). The color map in Fig. 1B, Bottom is a compact representation of the histogram in Fig. 1B, Top.

To determine whether CheY-P is necessary for the osmotic response observed in Fig. 1A and B, we performed single-motor measurements on a strain lacking CheY [$\Delta \text{CheY}$ mutant (48, 49)]. A representative trace is shown in Fig. 1C, and several more traces are given in SI Appendix, Fig. S2. The recovery phase of the $\Delta \text{CheY}$ mutant is the same as observed in Fig. 1A for the chemotactic wild type. However, the postrecovery phase of the mutant shows no switching events, indicating that CheY-P is necessary for the elevated bias observed in Fig. 1A and B.

Osmotic Response Does Not Exhibit Perfect Adaptation. We analyzed 69 cells exposed to three upshock magnitudes to confirm the absence of perfect adaptation observed in postrecovery phase (Fig. 1A and B). Upshocks were delivered as in Fig. 1, by exchanging volume recovery (VR) buffer (Materials and Methods) with the same buffer supplemented with 100 mM, 200 mM, and 400 mM sucrose, corresponding to osmotic shocks of 111 mOsmol/kg, 230 mOsmol/kg, and 488 mOsmol/kg [similar to osmolalities found in the human intestine (28, 31); see also SI Appendix, Table 1 for osmolality measurements of all our buffers]. Fig. 24 shows color map histograms of each individual single-motor bias trace, where darker color represents higher CW bias.

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**Fig. 1.** (A) An example 30-min speed trace obtained from a single BFM. The cell was initially in VR buffer (Materials and Methods), indicated in light-blue shading, and exposed to an osmotic upshock of 488 mOsmol/kg at $t = 5$ min. The shock was delivered as a step increase by flowing in VR containing an additional 400 mM sucrose, resulting in a shock magnitude of 488 mOsmol/kg. (B, Top) A histogram of CW bias computed by binning the trace in $A$ into 30-s bins and dividing the time spent rotating CW by the bin length (Eq. 1 and Materials and Methods). B, Bottom shows the same histogram condensed into a color map, with an intensity scale to the right. (C) Single-motor speed trace of a $\Delta \text{CheY}$ mutant exposed to the same osmotic upshock as in $A$. 
Evidence for this assertion is shown in the CW bias histograms in Fig. 2B, which shows a distribution computed from 3-min recordings in VR buffer before shock, and Fig. 2C, which shows a distribution computed from 3-min intervals taken at various time points, 12 or more minutes after shock with 200 mM sucrose. A total of 120 single-motor recordings were used for the preshock condition and 96 recordings for after it. The median value of the population CW bias shifts from 0.01 preshock to 0.06 after addition of 200 mM sucrose. Fig. 2D shows the median CW bias in time for each of the three different shocks, calculated from the raw speeds of individual cells presented in Fig. 2A, using a 60-s-wide moving window. Here, only medians are shown for clarity and the means, together with the interquartile range for each shock magnitude, are plotted in SI Appendix, Fig. S3 B–D. While the CW bias shows some recovery in time, in particular for 111-mOsmol/kg and 230-mOsmol/kg shocks, it proceeds on a slow timescale and the median values at the end of our measurement time remain elevated with respect to the initial value.

A further corroboration of long-term increase in CW bias after osmotic shock comes from separating the CW bias values of Fig. 2B according to the time point at which they were measured, relative to the administration of the osmotic shock (t = 0). This is displayed in Fig. 2C, Inset and shows that the elevated CW bias persists over a timescale as long as 1 h.

Osmotic Response Shows Osmokinesis, That Is, Changes in Motor Speed. The response to an osmotic upshock includes not only CW bias dynamics, but also changes in motor and free-swimming speed. Fig. 3A shows a color map plot of normalized single-motor speeds of the same 69 cells presented in Fig. 2A. All cells were originally in VR buffer and subsequently exposed to an osmotic upshock, using 100 mM, 200 mM, or 400 mM sucrose, corresponding to upshifts of 111 mOsmol/kg, 230 mOsmol/kg, and 488 mOsmol/kg. BFM speeds were normalized with respect to the initial speed of each motor, i.e., to the average value of the first 15 s of the recording.

Following the osmotic upshock, motors show two kinds of behavior. If a shock is of a large magnitude, such as 488 mOsmol/kg, the speed drops sharply and significantly, with a period of speed recovery that follows. As can be seen from Fig. 3A and B, the speed recovery (T_{rec,CW}) lasts ~4.4 min on average (5.2 min median). This is of similar magnitude to T_{rec,ω} (5.4 min mean, 5.0 min median; SI Appendix, Fig. S4) and likely corresponds to the time necessary to recover volume after an osmotic shock (50); however, T_{rec,ω} and T_{rec,CW} for individual motors do not occur simultaneously (SI Appendix, Fig. S4). After recovery, the speed increase continues, leading to elevated levels compared with the preshock values (Fig. 3C). Weaker upshocks, 111 mOsmol/kg and 230 mOsmol/kg, are characterized by an increase in motor speed without a significant speed drop, as seen in Fig. 3A–C. Elevated speed levels we observed are reversible. SI Appendix, Fig. S5 shows motor speeds of cells exposed to 100 mM sucrose upshock, which have been allowed to recover before transferring them back to VR buffer (VRB). Upon recovery to the downshock, motor speeds return to approximately the same initial levels.

To explore the population-level significance of our observation of osmokinesis in single cells, we next performed differential dynamic microscopy (DDM) (Materials and Methods) (52, 53). DDM is a fast, high-throughput method for measuring the distribution of swimming speeds in populations of a range of different self-propelled particles, averaging up to ~10^4 particles at
Fig. 3. (A) Stacked single-motor (also single-cell) speed histograms, where the motor speed for each BFM is normalized to the average value of the first 15 s. Bin widths are 15 s and the color represents the bin height. Results are grouped by upshock magnitude, as indicated at Left. The white hatched column represents the point where an osmotic shock was performed by exchanging VRB for VRB + sucrose and the flow lasted for 10–15 s. Twenty-two, 24, and 23 cells are given for the 111-mOsm/kg, 230-mOsm/kg, and 488-mOsm/kg conditions, respectively. A, Top shows the color map scale. (B) Time necessary to recover the average value of preshock speed. Red horizontal bars are mean, and yellow triangles are median values, and the graph contains 18, 22, 23, and 20 single-motor data points for the 0-mOsm/kg, 111-mOsm/kg, 230-mOsm/kg, and 488-mOsm/kg upshocks. One value for the 230-mOsm/kg condition and three for the 488-mOsm/kg condition have been excluded from the graph as these motors do not recover average initial speed in the course of recording. The 0-mOsm/kg condition is a buffer-to-buffer control flush, where we used the same shocking protocol as for osmotic upshocks. (C) Single-motor speeds calculated as 3-min averages corresponding to a section between $t = 17$ min and $t = 20$ min in A. The graph contains 12, 22, 24, and 23 single-motor data points for the 0-mOsm/kg, 111-mOsm/kg, 230-mOsm/kg, and 488-mOsm/kg upshocks. The 0-mOsm/kg condition contains 12 of 18 control flushes that were at least 20 min long. (D) DDM measurement of swimming speeds following an osmotic shock. Cells were shocked in microfuge tubes and brought into a microscope within 2 min. The key shows shock magnitudes and the mean speed is the average of swimming speeds obtained for each time point in a range of different length scales (Materials and Methods). The systematic error of our measurements is then calculated as the 5D of the mean and falls within ~5% of the mean value. Here it was not plotted for clarity.

The increase in BFM speed observed in single cells, Fig. 3A, translates to an increase in population swimming speed with increasing upshock magnitudes, Fig. 3D. Similarly, in agreement with Fig. 3A, for high shock magnitudes, in particular for 473-mOsm/kg and 785-mOsm/kg sucrose upshocks, a sharp speed drop is observed immediately upon upshock. We also see a period of speed recovery, with duration increasing with the shock magnitude.

To demonstrate that, on the timescales of our experiments, the speed increase is set by the external osmolality, in SI Appendix, Fig. S7, as well as administering a sharp step increase, we upshocked the cells to VRB with 200 mM sucrose in a series of 10 steps, each 12 s apart, lasting 2 min in total. The DDM measurements commenced ~2 min after completion of the slower shock, and the slower shock shows identical speed behavior to that of the step increase.

Increasing sucrose concentrations results in the increased viscosity of the media, consequently increasing the drag coefficient on the motor (measured values of viscosity of our solutions are given in SI Appendix, Table 2). In Fig. 3 we show BFM speeds and cell swimming speeds uncorrected for this effect, as the actual speed of the cell will be relevant for taxis. The viscosity-corrected BFM speeds, calculated under the assumption that the motor torque does not change with the increasing viscosity, are shown in SI Appendix, Fig. S8.

Furthermore, we check for the presence of steps during single-motor speed recovery. To that end, in SI Appendix, Fig. S9 we show examples of BFM speeds during the recovery phase after 488-mOsm/kg upshock, starting just after the osmotic shock.
was administered. The majority of the traces do not show obvious steps during the BFM speed recovery period.

Discussion

Origins of Osmotaxis. With no external stimuli, E. coli swims in an almost straight line and reorients every so often in a nearly random fashion, performing a random walk with an effective diffusion constant $D \sim v^2/\alpha$ (56, 57), where $v$ is the swimming speed and $\alpha$ the tumbling rate given by

$$\alpha = \frac{3(1 - \cos \phi_0)(\tau_{\text{run}} + \tau_t)}{\tau_{\text{run}}}$$  \hspace{1cm} [2]

with $\tau_{\text{run}}$ mean run time, $\phi_0 \sim 71^\circ$ mean reorientation angle following a tumble, and $\tau_t$ the duration of the tumble event (56, 58).

Upon sensing a sudden increase in attractant concentration, E. coli sharply elevates the CCW bias for $\sim 1$ s and then slightly lowers it for $\sim 3$ s, returning to the prestimulus level in $\sim 4$ s (24). It is this characteristic impulse response of modulating the tumbling rate that allows E. coli to navigate toward a favorable environment. The detailed chemotactic performance and its origins have often been studied (56, 59–64).

The observation that a chemotactic mutant of E. coli (CheR/CheB) lacking normal methylation and demethylation enzymes does not show chemotactic accumulation, but does respond to raised attractant concentrations by lowering the tumbling rate without adaptation (10, 24), led researchers to calculate the consequences of spatially varying $\alpha(x)$ and $v(x)$ on steady-state bacterial density (56, 63). Similar calculations were performed for particles with $\alpha$ and $v$ dependent on the local density (57) or when studying diffusion of bacteria in porous media (65). These calculations offer possible explanations for previously observed negative osmotaxis, given the characteristic speed and bias response we here observed.

If we assume the simplest scenario, where there is no directional dependence of $\alpha(x)$ and $v(x)$, the steady-state E. coli density will be inversely proportional to its swimming speed (57):

$$\rho(x) \sim \frac{1}{v(x)}.$$  \hspace{1cm} [3]

In Fig. 3C we show motor speeds, $\omega$, and in Fig. 4 mean CCW motor interval ($\tau_{\text{CCW}}$) and tumbling rate ($\alpha$) at a given osmolarity. The $\omega$ and $\tau_{\text{CCW}}$ were obtained from the last 3 min of measurements presented in Fig. 3A, which represent the long-term osmolality-dependent changes. We then calculated $\alpha$ from $\tau_{\text{CCW}}$ and $\tau_{\text{CW}}$ (Fig. 4 and SI Appendix, Fig. S11), using Eq. 2. The assumption was made that swimming speed $v$ and the mean run time $\tau_{\text{run}}$ are proportional to the motor speed $\omega$ and the mean CCW interval $\tau_{\text{CCW}}$, in agreement with previous studies (66) and our own DDM and motor measurements given in Fig. 3.

Thus, based on experimental results we present here and previous theoretical calculations (Eq. 3 and refs. 56 and 57) we would expect accumulation at lower osmolalities, equivalent to negative taxis previously observed (35). The changes in tumbling rates suggest differences in the time-dependent approach to steady state, but not the steady-state density distribution itself (56, 63).

Theoretical calculations we refer to make the assumption that spatial gradients of $v(x)$ and $\alpha(x)$ are small (56, 57), such that we do not expect local gradients in steady-state bacterial densities. For steeper osmotic gradients we expect a more complex osmotic response, in particular since the recovery phases shown in Figs. 2 and 3 will no longer be vanishingly small, as we assumed above, which could explain some previous contradictory osmotic observations (35, 40).

Our assumption that $\alpha(x)$ and $v(x)$ lack directional dependence in the case of osmotaxis could be satisfied if the observed changes in $\alpha$ are not due to signaling within the chemotactic network, but purely due to changes in the interaction of CheY-P with the rotor units of the motor. For example, previous observations indicate that at higher external osmolarities, osmotic pressure is kept the same (50). Thus, the crowding in an already crowded cytoplasm (67) increases, which can affect cytoplasmic interactions (68, 69) and the binding of CheY-P to the BFM, perhaps in a similar fashion to that observed at higher hydrostatic pressures (70).

However, it is also possible that the observed changes in $\tau_{\text{CCW}}$ and $\omega$ are a consequence of signal processing by the chemotactic network. In particular, Vaknin and Berg (41) reported that osmotically induced changes in cell volume can perturb the chemoreceptors and that the signal could travel down some of the network components. In such a scenario, $\alpha(x)$ and $v(x)$ would be directionally dependent (as in chemotactic response) and steady-state cell density would need to be calculated, taking into account the directional dependency as well as the characteristic signal response we observed. Future work needs to investigate strains lacking specific parts of the network to determine the contribution of signaling and different components of the network to the osmotic response.

Origins of Osmokinesis. The large increase in motor speed after osmotic shock could be due to increases in PMF, possibly through alterations in cell metabolism, or, as an alternative explanation, due to the increase in number of stator units through mechanosensation (71, 72) or adaptive motor remodeling (12). The BFM has been shown to act as a mechanosensor, increasing the number of stator units in response to higher loads (71, 72). As the viscosity of the medium rises with addition of sucrose, the speed increase we observed could be due to the incorporation of additional stator units. In fact, at higher loads, an increase in the mean CW interval has been reported as well (71, 73). At our load, a 0.5-μm bead attached to the motor via a short filament stub, the motor is still expected to operate in the high-load, “plateau” region of the torque-speed curve (74, 75) with the estimated full number of stator units (75). In addition, we observe an average long-term 30-Hz increase in motor

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speeds even at our lowest osmotic shocks where the viscosity of the solution hardly changes (it increases by 1.057 times). Furthermore, free-swimming cells with the motor operating in the linear-torque regime, where motor remodeling due to increased load is more likely, do not increase the swimming speed at viscosities well above the highest viscosity used in our experiments, as measured using Ficoll (76). Therefore, it is less likely that additional stator unit incorporation or adaptive motor remodeling is the sole explanation for the speed increases we observe.

SI Appendix. Fig. S9 shows the motor speeds during the speed recovery phase for all of the BFMIs recorded in the +488-mOsmol/kg condition. In the majority of the traces no obvious steps are observed, suggesting that the increase in motor speed could be due to the increase in PMF with the full set of stator units present. Here we note that the engagement of stator units with the rotor has been reported as torque dependent (72), where in the absence of torque stator units disengage from the rotor. We would then expect stator resurrection during the recovery phase shown in SI Appendix, Fig. S9. Absence of obvious stepwise increases indicates that volume shrinkage caused by the osmotic shock could affect motor dynamics and perhaps prevent, or slow down, stator disengagement.

The length of the motor speed recovery period observed in Fig. 3 is ∼5–10 min, in line with the volume recovery timescales observed previously after osmotic upshocks (33, 50). The timescales of speed recovery (at similar shock magnitudes) observed from DDM data in Fig. 3D are longer, with recovery lasting ∼40 min for the 473-mOsmol/kg upshock. Some variation in these timescales can be due to the difference between individual motor speed recovery and subsequent bundle formation. However, based on Fig. 3D, we suspect that greater contribution to the difference comes from alterations in oxygen consumption rate, which in turn shifts the time it takes to reach the maximum swimming speeds.

By looking at individual BFMIs and population swimming speeds together, we reveal the main characteristics of E. coli’s motility response to step increases in external osmolarity. The response consists of long-term CW bias and motor rotation/cell swimming-speed increase. This is a unique observation of osmoskinesis in E. coli. We discuss how such observed speed increases can lead to negative taxis previously reported. Our study underlines the importance of manipulating bacterial motility in environments that are closer to natural habitats, in the effort to understand the role and evolutionary advantage swimming offers to bacterial cells (77–79).

Materials and Methods

E. coli Strains and Plasmids. E. coli strains KAF84 and KAF95 (49) were used for BFM speed and bias and a highly motile MG1655 with an insertion of a single motility element inserted in the fliC operon regulatory region, which is the master operon for the expression of flagellar genes (80, 81), for DDM experiments. Both KAF84 and KAF95 carry the Δflic26 allele (produce nonflagellate phenotypes) and contain a plasmid carrying an ampicillin-resistance and a flicCin65 gene (produces flagellar filaments that stick readily to surfaces). Additionally, KAF84 is a chemotactic wild type and KAF95 is a ΔCheY strain and therefore cannot perform chemotaxis, producing a smooth-swimmer phenotype. MG1655 is a K-12 strain and a chemotactic wild type.

E. coli Growth and Culturing. KAF95, KAF84, and MG1655 cells were grown in Tryptone Broth (1% Bacto tryptone, 0.5% NaCl) at 30 °C while being shaken at 200 rpm (42, 52). KAF95 and KAF84 were supplemented with 100 µg/mL of ampicillin and grown to OD_{600} = 0.8–1.0 (Spectronic 200E Spectrophotometer; Thermo Scientific) and MG1655 to OD_{600} = 0.6. We have calibrated the single scattering range of our spectrophotometer (SI Appendix, Fig. S10) and ensured we stay within that range during OD_{600} readings by appropriately diluting the culture when needed (82). In SI Appendix, Fig. S12 we give the growth curves of the three strains we used for the experiments and indicate the OD_{600} at the point of culture harvest. After growth cells were washed in VRB composed of modified motility buffer (MMB), which is 10 mM sodium phosphate buffer, pH 7.1 (an aqueous solution with 6.1 mM of Na_{2}HPO_{4}, 3.9 mM of NaH_{2}PO_{4}, and 0.01 mM of EDTA, with added glycine betaine, potassium chloride, and choline chloride to final concentrations of 20 mM, and 10 mM, respectively. These compounds act as osmo-protectants and allow the cell to recover volume after an osmotic shock. MMB is a variant of the motility buffer, commonly used in flagellar motor and chemotaxis experiments (25, 42), with sodium phosphates substituted for potassium phosphates. After washing, all of the experiments were performed in VRB. KAF95 and KAF84 cells were washed by centrifuging them into a pellet and exchanging solution, while MG1655 cells were washed by gentle filtration to preserve filaments (55) and experiments were performed in VRB as for motor speed measurements.

Sample Preparation and Osmotic Shock. For BFM experiments flagellar filament were truncated by passing a bacterial suspension through two syringes with narrow-gauge needles (26 gauge) connected with a plastic tube (“shearing device” (25, 42, 83)).

Subsequently, cells with truncated filaments were washed by centrifugation. Slides for BFM experiments were prepared as before (42, 83) by layering two parallel strips of double-sided sticky tape onto a microscope slide and covering them with a cover glass, forming a tunnel slide (SI Appendix, Fig. S1) of approximate volume of ∼8 μL. One percent poly-L-lysine was added into the tunnel and extensively washed out after keeping it in for ∼40 min to allow glass coating. Cells with truncated filaments were loaded into the tunnel and incubated for 10 min in a humid environment to prevent evaporation. Subsequently, nonattached cells were washed out. Next, 0.5-μm beads in diameter (Polysciences) were added and incubated for 10 min to allow sticking to the filaments and excess beads were washed out postincubation. Osmotic shocks were performed while the slide was in the microscope by adding 24 μL of the shocking solution to one end and immediately bringing a piece of tissue paper to the other, resulting in flow and exchange of media. The flow duration was ∼10–15 s and the local flow velocity next to the attached bacterium was 60 μm/s (47). The tunnel was sealed after the shock to prevent evaporation and thus potential further increase in osmolality throughout the course of the experiment. For DDM experiments an ∼400-μm-deep flat capillary (Vitrocom) glass sample was filled with ∼150 μL of bacterial suspension immediately after upshock and subsequently sealed to prevent evaporation during the experiment. Osmolalities of the solutions were measured with a freezing-point depression osmometer (Camlab) and are listed in SI Appendix, Table 1.

Microscopy and Data Collection. For BFM experiments back-focal plane interferometry (43, 44) was performed using a custom-built microscope and an 855-nm laser (Blue Sky Research) which formed a weak optical trap. The rotating bead attached to a flagellar stub was brought into the focus of the laser and the back-focal plane of the condenser was imaged onto a position-sensitive detector (PSD Model 2931; New Focus). The voltage signal from the PSD was passed through an analog anti-aliasing filter (low-pass, Bessel-type filter with a cutoff frequency of 2.5 kHz; Krohn-Hite Corporation) and sampled at 5000 Hz (Data Translation DT3351 DAC, National Instruments). For DDM experiments imaging within the glass capillary was performed at 100 μm away from the bottom of the capillary to avoid any interaction with the glass wall. The imaging began within ∼2 min postupshock and consisted of a time series of phase-contrast images (Nikon TE300 Eclipse fitted with a Nikon Plan Fluor 10Ph1 objective, N.A. = 0.3, Ph1 phase-contrast illumination). Imaging was performed at a 100-Hz sampling rate (Mikrotron high-speed camera (MC 1362) and frame grabber (Inspecta 5, 1-GB memory)).

Data Analysis. Data collected during BFM experiments were analyzed in the following way. X and Y signals (obtained from voltages from the PSD) were passed through a moving-window discrete Fourier transform, where the window moves point by point and the window size was 1.684 s. Time traces of single motor speed obtained in such a way were processed to calculate the CW bias, using Eq. 1. CW biases were calculated for fixed interval sizes (30 s in Fig. 2A, 3 min in Fig. 2C, and 5 min in Fig. 2B) and using a 60-s-long moving window in Fig. 2D and SI Appendix, Fig. S3 B–E. To calculate \( \tau_{cw} \) we first take a mean of the \( \tau_{cw} \) intervals belonging to each individual BFM rotation trace. We then pool so-obtained mean cell intervals into a distribution and obtain its mean (the same was done for \( \tau_{cw} \)). We include only bounded CCW and CW intervals (SI Appendix, Fig. S11). In DDM experiments, the mean swimming speed was calculated from DDM movies as an
average over $\sim 10^3$ cells/mL. Details of the image processing and data analysis were as before (S2, 52, 53, 76). DDM allows the measurement of an adhesive speed, simultaneously, over a range of spatial-frequency $q$, where each $q$ defines a wavelength $\lambda = 2\pi/q$ (for more details, see refs. S2 and 53). A mean swimming speed is extracted by averaging over $q$. Data plotted in Fig. 3D were obtained by averaging over the range $0.5 < q < 2.2 \mu m^{-1}$, corresponding to a range in length-scale $3 \lesssim L \lesssim 13 \mu m$

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Supporting Information

Growth Media Osmolarities. Growth media osmolalities were measured with an osmometer (Micro-Digital Osmometer MOD200 Plus, Camlab, Cambridge, UK) and are given in Table S1.

Motility Media Viscosities. Motility media viscosities were measured with a rheometer (TA Instruments AR2000, cone-plate geometry, 60 cm, 0.5°) and are given in Table S2.

Supporting Figures. Supporting Figures to the main text are given with the captions.

Table 1. Media osmolalities

<table>
<thead>
<tr>
<th>Media</th>
<th>Osmolarity (mOsmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRB</td>
<td>92</td>
</tr>
<tr>
<td>VRB+40 mM sucrose</td>
<td>135</td>
</tr>
<tr>
<td>VRB+60 mM sucrose</td>
<td>157</td>
</tr>
<tr>
<td>VRB+100 mM sucrose</td>
<td>203</td>
</tr>
<tr>
<td>VRB+200 mM sucrose</td>
<td>320</td>
</tr>
<tr>
<td>VRB+400 mM sucrose</td>
<td>580</td>
</tr>
<tr>
<td>VRB+600 mM sucrose</td>
<td>877</td>
</tr>
</tbody>
</table>

Table 2. Measured media viscosities (in agreement with listed in [1])

<table>
<thead>
<tr>
<th>Media</th>
<th>Viscosity (Pa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility Media</td>
<td>0.00112 ± 0.00001</td>
</tr>
<tr>
<td>Motility Media + 300 mM sucrose</td>
<td>0.00146 ± 0.00001</td>
</tr>
<tr>
<td>Motility Media + 600 mM sucrose</td>
<td>0.00192 ± 0.00002</td>
</tr>
<tr>
<td>Motility Media + 900 mM sucrose</td>
<td>0.00290 ± 0.00007</td>
</tr>
<tr>
<td>Motility Media + 1850 mM sucrose</td>
<td>0.0172 ± 0.0001</td>
</tr>
</tbody>
</table>

Fig. S 1. Schematic representation of the bead assay and tunnel slide used for measurements. (A) Genetically modified bacterial flagellar filament (FliCSticky [2]) stub was attached to a latex bead of 0.5 μm diameter. The bead was placed in a heavily attenuated optical trap and the bead rotation recorded via back focal plane interferometry (Methods). (B) A tunnel slide, formed by a double sided sticky tape separating a microscope slide and a glass cover slip, was used to deliver osmotic shocks. The slide was sealed with vaseline upon the osmotic shock to prevent evaporation.
Fig. S 2. Four representative traces of motor speed against time for the $\Delta$CheY strain KAF95. The blue shaded, vertical span marks the delivery of the osmotic shock. The top two traces have been recorded in the $+111 \text{ mOsmol/kg}$ condition, while the bottom two have been recorded in the $+488 \text{ mOsmol/kg}$ condition (base buffer is, as in all other experiments, VRB). We observe no switching before the shock, as expected from a $\Delta$CheY strain that is smooth swimming, and also none after the shock. Yet, the speed dynamics are similar to KAF84 (Main text Fig. 3).
Fig. S 3. (A) Recovery Phase duration ($T_{rec,CW}$), calculated as the time needed for the post-shock median population bias to recover to the median VR Buffer bias (CW Bias = 0.01, histogram in main text Fig. 2B). $T_{rec,CW}$ was calculated for each of the three shock conditions, whose biases are presented in panels C-D. The points represent times at which the post shock median reaches 0.01, relative to the time of shock delivery. The error bars give the populational variability in terms of recovery times for the 25th and the 75th percentile of population bias. (B) Buffer to buffer control flush CW Bias during the course of the experiment is given. Biases were calculated on a moving window of 60 s length and the VR Buffer was flown into the tunnel slide in the same way and duration as for the osmotic shock experiments. 12 cells in total are presented, the solid black line is the median, dashed is the mean and the shading represents the area between the 25th and 75th percentile. (C) Same as B but for the 111 mOsmol/kg osmotic upshift and the plot represents the median of the 22 single cell bias traces (thick red line), the mean (dashed black line) and the red shading is the area between the 25th and the 75th percentile. (D) and (E) are the same as C, only for the 230 mOsmol/kg (24 cells) and the 488 mOsmol/kg (23 cells) osmotic upshifts, respectively.
Fig. S 4. $T_{rec,\omega}$, the time necessary to recover the average value of single motor speed following an osmotic shock, is given against $T_{rec,cw}^{sc}$, the time between the administration of the shock and the first detected switch event (post shock). Values are plotted for every single cell in the +488 mOsmol/kg condition, showing that the recovery of speed and switching in individual motors do not occur simultaneously. +111 and +230 mOsmol/kg conditions show no correlation between the two; the speed recovery there is instantaneous or shorter than the switching recovery time.
Fig. S 5. Osmolality induced speed increases are reversible. Box plot of motor speeds is shown against the final media cells are kept in. The box denotes the interquartile range and the whiskers the 10th and the 90th percentile. The Buffer (VRB) box contains the entire VRB speed distribution shown in SI Appendix Fig. S6. The 111 mOsmol/kg box contains speeds from osmotic upshock measurements 12 or more minutes after shock with 100 mM sucrose. The 'Back to Buffer' box contains measurements of motor speeds where cells were first exposed to 111 mOsmol/kg upshock in a tube, and after ∼20 min recovery were transferred back to VRB. The measurements commenced ∼20 min after return to VRB. Cells that have been washed back into VRB from VRB+111 mOsmol/kg have similar speed levels to the pre-shock VRB condition. The boxes contain 118, 45 and 27 cells respectively, with population average speeds of 116 ± 2 Hz, 156 ± 5 and 97 ± 2 Hz.
Fig. S 6. Histogram of single-motor speeds for *E. coli* KAF84 in the Volume Recovery Buffer (VRB). Each motor comes from a different single cell. Each speed is computed by taking the first 15 s of one of various recordings made in VRB, selecting the CCW speed from the trace and averaging it. The histogram contains 118 cells and the mean value of the distribution is 116±2 Hz.
Fig. S 7. DDM measurement of swimming speeds of osmotically shocked cells, where the magnitude of the shock stays the same, but the shock is delivered over a different time interval. A single step bringing the cells up to 235 mOsmol/kg from VRB is given in orange. The same shock performed in 10 steps prior to DDM measurement is given in red (dashed line). The inset shows the change in osmolality cells experience over time with the two methods.
Fig. S 8. (A) Version of the main Fig. 3 where the motor and swimming speeds have been corrected for sucrose viscosity, under the assumption that no additional stators are incorporated at this increased load (see Discussion in the main text). Viscosity correction for 100, 200 and 400 mM sucrose used are a multiplicative factor of 1.057, 1.195 and 1.444, respectively (measured and listed in [1]). Each line represents a single motor speed trace binned into 15 s intervals where the color represents the height of the bin, normalized to the first bin. Results are grouped by upshock magnitude, as indicated on the left hand edge. The white hatched column represents the point where an osmotic shock was administered by exchanging VR Buffer for VR Buffer + sucrose and the flow lasted for 10-15 s. There are 22, 24 and 23 cells for the 111, 230 and 488 mOsmol/kg condition, respectively. The color map scale is given at the top of the figure. (B) DDM measurement of swimming speeds following an osmotic shock. Cells were shocked in microfuge tubes and brought into a microscope within 2 min. The legend shows shock magnitudes and the mean speed is the average of swimming speeds obtained for each time point in a range of different length scales (Methods). The systematic error of our measurements is then calculated as the standard deviation of the mean, and falls within ~5% of the mean value (here not plotted for clarity). The traces shown have been viscosity corrected as well, as free-swimming cells with the motor operating in linear-torque regime do not increase the swimming speeds with viscosities we used in our experiments [4]. (C) Viscosity corrected single motors speeds calculated as 3 min averages corresponding to a section between t=17 and t=20 min in A. The 0 mOsmol/kg condition is sampled from a set of buffer to buffer control flushes which were at least 20 minutes long (12 out of 18 control flushes). Red horizontal bars represent mean and yellow triangles median values.
Fig. S 9. Traces of motor rotation after addition of 400 mM sucrose (all cells given in Fig. 2A and Fig. 3A of the main text are given). We do not plot the initial motor speed, and start at the point of speed drop due to an osmotic shock. Motor speeds were obtained as described in Methods.
**Fig. S 10.** Top: Mean CW interval distribution ($\tau_{CW}$) for cells in Volume Recovery Buffer. Motor rotation of 102 cells was sampled for 5 min and intervals counted as illustrated in SI Appendix Fig. S11. Bottom: Mean CW interval distribution for motors where the sampling interval (3 min) begins 12 or more minutes after an osmotic shock with 200 mM sucrose in VRB (+230 mOsmol/kg). The inset contains data from the histogram plotted against the start time of the sampling interval and t=0 corresponds to the osmotic shock.
Fig. S 11. A portion of a single-motor speed trace illustrating the counting of interval lengths within a given sampling window. Positive speeds correspond to CCW rotation and negative to CW rotation. Intervals $i_1$ and $i_7$ are not included when calculating the mean CCW interval due to uncertainty in their beginning or end. Only intervals bounded by two CW intervals are counted. Similar holds true when calculating mean CW interval, only those bounded by two CCW intervals were included.

Fig. S 12. Growth curves of all strains used in this work are given in (A) and (B). (A) Optical density measured at 600 nm is given against time for growth of KAF84 (black) and KAF95 (orange) strain inoculated from an overnight frozen stock. (B) As in (A) but for MG1655 strain. (C) Calibration curve of our spectrophotometer. Various concentrations of cells were prepared to determine the range where OD is linear with the concentration. In (A) and (B) sample was diluted so that OD never surpassed 0.25 and ensuring we always measure within the linear range of our spectrophotometer. Points in (A) and (B) are taken from several different flasks, showing that growth from frozen samples is highly reproducible as all points collapse onto a single curve.