Chemotactic adaptation kinetics of individual *Escherichia coli* cells

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*Escherichia coli* chemotaxis serves as a paradigm for the way living cells respond and adapt to changes in their environment. The chemotactic response has been characterized at the level of individual flagellar motors and in populations of swimming cells. However, it has not been previously possible to quantify accurately the adaptive response of a single, multiflagellated cell. Here, we use our recently developed optical trapping technique to characterize the swimming behavior of individual bacteria as they respond to sudden changes in the chemical environment. We follow the adaptation kinetics of *E. coli* to varying magnitudes of step-up and step-down changes in concentration of chemotactant. We quantify two features of adaptation and how they vary with stimulus strength: abruptness (the degree to which return to prestimulus behavior occurs within a small number of run/tumble events) and overshoot (the degree of excessive response before the return to prestimulus behavior). We also characterize the asymmetry between step-up and step-down responses, observed at the single-cell level. Our findings provide clues to an improved understanding of chemotactic adaptation.

Many species of bacteria swim by rotating helical filaments (flagella) driven by bidirectional rotary motors (1). In the peritrichously flagellated *Escherichia coli*, counterclockwise (CCW) rotation induces formation of a flagellar bundle that propels the cell forward in a “run.” Clockwise (CW) rotating flagella break from the bundle and cause the cell to change swimming direction abruptly in a “tumble” (2–4). A swimming cell alternates stochastically between running and tumbling, exploring its surroundings in a random walk (5). The flagellar motor’s rotational bias, and thus the cell’s swimming behavior, is modulated by the chemicals in the surrounding environment. Exposure to attractants causes cells to tumble less frequently whereas depletion of attractant causes them to tumble more, leading to net migration toward favorable environments (6). This chemotactic response is governed by a protein network that is well-characterized (7, 8) (SI Discussion). One hallmark of this network is its ability to adapt to a wide range of chemical environments. When cells are exposed to a sudden change in environment, they respond by temporarily changing their swimming behavior (tumbling more or less frequently), but then return over time to their prestimulus swimming state (9, 10). Chemotactic adaptation is believed to be exact (11), allowing cells to maintain a high sensitivity to their environment over a wide range of background chemoeffector concentrations.

Various techniques have been used to study the chemotactic response and adaptation in *E. coli*. By following populations of swimming cells, the relation between adaptation time and stimulus strength, as well as the robustness of exact adaptation, have been studied (10–13). Detailed features of the flagellar motor’s response to chemical stimuli of various forms have been characterized by tethering individual cells to the surface of a microscope slide and monitoring the motor’s rotational direction (9, 14–16). More recently, a Förster resonance energy transfer (FRET) reporter system was used to probe the activity of the essential kinase (CheA) in the chemotactic protein network from a population of cells (17–19). Despite providing invaluable information, the approaches above suffer significant limitations. Population measurements average over cell-to-cell variability, potentially masking important features of the single-cell response (12). At the other end, single-motor measurements may not accurately reflect the swimming behavior of the cell because this behavior arises from the collective state of multiple flagellar motors (3, 4).

In this study, we utilize our recently developed optical tweezers technique (20) to apply controlled step-up and step-down chemical stimuli to individual swimming *E. coli* cells and to monitor their chemotactic adaptation. The acquisition of trajectories from many individual cells, for a long duration (>10 min) at high temporal resolution (approximately 100 Hz) allowed us to characterize adaptation kinetics at an unprecedented level of detail. In particular, we quantified two features of adaptation: (i) abruptness (the degree to which return to prestimulus behavior occurs within a small number of run/tumble events) and (ii) overshoot (the degree of excessive response before the return to prestimulus behavior). Though abruptness and overshoot have been previously reported in the literature (9, 16, 21–23), they have not yet been characterized in detail. Here, we quantify both features in response to both step-up and step-down stimuli across a broad range of stimulus strengths. We also characterize the striking asymmetry in the cell’s response to step-up and step-down stimuli. We suggest how our findings provide clues to an improved understanding of chemotactic adaptation.

Results

Optical Trapping Enables Following Adaptation Kinetics in Individual Swimming Cells. To enable precise long-term measurement of a single cell’s swimming behavior, we used our optical trapping assay (20) (Fig. 1). Briefly, two optical traps were used to hold a single cell by the two ends of its body and to orient it horizontally in the imaging plane (the xy plane in Fig. 1A). Movement of the trapped cell was monitored by imaging the trap light scattered by the cell onto position-sensitive photodiodes. For a freely swimming cell, rotation of the flagellar bundle causes the body to counter-rotate about its long axis during a run (24). During a tumble, the cell body moves erratically as it changes direction (3). For an optically trapped cell, both flagellar bundle and cell body are free to rotate even though the cell is immobilized in space. Thus, runs and tumbles manifest themselves in the trap as oscillatory and erratic signals from rotation and random motion of the...
and Fig. S1 occurred within approximately 300 flow (the change from 10% to 90% of the maximum concentration laminar with minimal mixing, creating well-defined boundaries in the inlet) and the flow speed (m). The cross-section of the flow chamber (5, 11, 14) reflected by the measured run/tumble statistics (20).

Because the trapped cells were moved at a speed of 5-min measurement (Fig. 1D) of a trapped cell undergoing a step down in attractant concentration, where switching between oscillatory and erratic signals resumed. The trap signal was converted to a binary time series of runs and tumbles (Fig. 1D) as described previously (20) (SI Materials and Methods).

To deliver chemical stimuli to the optically trapped cells, we created a chemical attractant concentration profile in a laminar flow chamber. In this chamber, three separate streams containing different solutions merged into a central channel: a “cell-injection” stream, a “blank” stream, and an “attractant” stream (Fig. 1B). The cross-section of the flow chamber (100 μm × 1000 μm per inlet) and the flow speed (70 μm/s) ensured that fluid flow was laminar with minimal mixing, creating well-defined boundaries in the chemical profile along the direction perpendicular to the flow (the change from 10% to 90% of the maximum concentration occurred within approximately 300 μm) (SI Materials and Methods and Fig. S1 A–C). In a typical experiment, a swimming cell was captured from the cell-injection stream of the flow chamber containing many cells and oriented along the flow direction using the optical traps. By moving the flow chamber using a motorized translation stage, the trapped cell was then positioned into the blank stream containing trap motility buffer (TMB) (see Materials and Methods). Following measurement of the steady-state swimming behavior for up to 5 min, the trapped cell was moved rapidly to the attractant stream containing the chemotactic agent and monitored for at least an additional 7 min. Because the trapped cells were moved at a speed of 100 μm/s, they experienced a chemical stimulus in the form of a step up in attractant concentration over a span of approximately 3 s (Fig. S1A). A unique aspect of our technique is the ability to apply step-down stimuli simply by moving cells in the reverse manner. Step down is difficult to achieve in free-swimming assays, where flow cannot be used to remove chemoeffectors without flushing cells away. Fig. 1C shows three short segments out of an approximately 5-min measurement (Fig. 1D) of a trapped cell undergoing a step up in attractant concentration, from 0 μM to 100 μM. In the first segment the cell was in its steady-state and exhibited alternating periods of oscillatory and erratic signals corresponding to runs and tumbles. In the next segment the cell underwent a prolonged oscillation (a long run) in response to the applied chemical stimulus. The last segment shows the cell after it adapted to the new level of attractant concentration, where switching between oscillatory and erratic signals resumed. The trap signal was converted to a binary time series of runs and tumbles (Fig. 1D) as described previously (20) (SI Materials and Methods).

Using the methods described above, we characterized the adaptation of individual cells (E. coli strain RP437, wild type for chemotaxis) (26) in response to a step up in L-aspartate concentration of varying strength (0 μM to 1–1,000 μM) (Fig. 2). From each single-cell binary time series, we determined the adaptation response by calculating the tumble bias in a running 10-s time window (Fig. S2). When the adaptation response was averaged over many individual cells, the average response curve showed a gradual adaptation time course (Fig. 2A), similar to that observed in previous studies (11, 14, 17, 19, 25). For each stimulus, we also determined the average adaptation time, defined as the time elapsed between the application of the stimulus and the recovery of tumble bias to 50% of its prestimulus average value. The dependence of adaptation time on attractant concentration (Fig. 2C) exhibits the Michaelis–Menten-like behavior reported in earlier studies (9, 10). The curve is also in quantitative agreement with...
a recent theoretical model for the chemotactic network (27). In the long term, after the transient response to the step-up stimulus, cells achieved a steady-state tumble bias. For each stimulus, we determined the exactness in adaptation, defined as the ratio of post- to prestimulus steady-state tumble biases. For the majority of stimulus strengths assayed, adaptation was exact within our experimental error (Fig. S3), as expected from previous studies (11, 16, 19). [For the highest step up in l-aspartate concentration (1000 µM), we did observe a decrease in steady-state tumble bias poststimulus, though we attribute this to limited observation time]. This agreement with previous experimental and theoretical results on chemotactic adaptation, taken together with our previous report regarding the free-swimming behavior of trapped cells (20), demonstrates that optically trapped cells exhibit a normal behavior in all aspects of motility, including chemotactic response.

### Individual Cells Exhibit a Stimulus-Dependent Abruptness of Adaptation

The average response curves in Fig. 2A, though useful when comparing our results to previous studies, mask important features of adaptation kinetics at the single-cell level. As seen from the binary time traces (Fig. S4), individual cells exhibit large cell-to-cell variations in adaptation times because of the stochastic nature of the underlying network reactions as well as variability in the chemotaxis network protein numbers (12). Thus, the population-averaged traces in Fig. 2A smooth over cell-to-cell differences in adaptation kinetics. In order to elucidate the “typical” behavior of the individual cell, we analyzed our data using a recently introduced scheme (16) in which individual traces are indexed by “events”—run and tumble pairs—rather than time (Materials and Methods and Fig. S5). Fig. 3A displays the result of averaging individual traces according to run/tumble event number, aligned relative to the delivery of the stimulus (i.e., run/tumble events were enumerated from the time the stimulus was applied). The ordinate represents the mean tumble bias, and the abscissa, the mean duration of the i-th run/tumble pair averaged across the cell population. This averaging scheme is not subject to stochastic variability in run or tumble duration, and thus better captures the typical adaptation kinetics of individual cells (16).

In comparison to the population-averaged response curves in Fig. 2A, the corresponding event-averaged curves in Fig. 3A reveal the abruptness with which individual cells adapt. The predominant adaptive response to a step-increase in attractant consisted of a single, long run/tumble event (specifically, a single long run; tumble duration did not change significantly) (Fig. S6A and B), after which the cell’s swimming returned to its prestimulus behavior. Abrupt adaptation kinetics at the level of individual motors were reported many years ago for the case of saturating stimulus (13) and Fig. 3A corresponds to the time when cells were moved along the chemical gradient and data was not recorded. The same raw data as in Fig. 2A were used in this analysis. (B) Histograms of the number of run/tumble pair ETA from individual cells. Black line is a fit to an exponential. (C) Histograms of adaptation time from individual cells. Black line is a fit to a Gaussian. (D) Same as A, for a step-down stimulus. The same raw data as in Fig. 2B were used in this analysis. (E) The average number of ETA as a function of stimulus strength for step up (black solid circles, values obtained from Q and step down (open gray circles). Error bars designate standard error of the mean. The solid black line is a fit to a sigmoidal function. The dashed gray line is the mean of the three step-down data points. See SI Materials and Methods for details of the event-based analysis.

![Graph](image-url)
stimulus strength. The average ETA exhibits an almost stepwise increase from one to approximately six as the stimulus level exceeds 50 μM. ETAs obtained from the population-averaged event-based adaptation curves in Fig. 3A exhibit similar behavior (Fig. S8). Below we discuss possible explanations for the stimulus-dependent abruptness of adaptation exhibited by individual cells.

Individual Cells Exhibit an Overshoot Response. After the application of a step-up stimulus and the resulting long run/tumble event(s) discussed above, many cell traces exhibited an overshoot, during which the tumble bias exceeded the prestimulus steady-state. The tumble bias eventually returned to the prestimulus value. This feature was observed in population-averaged traces (Fig. 2A) and event-averaged traces (Fig. 3A) alike, and quantified at the single-cell level (Fig. S7 and SI Materials and Methods). Fig. 4A displays the average amplitude of the overshoot—defined as the fractional excess tumble bias over the poststimulus steady-state—for different stimulus strengths. Interestingly, the overshoot amplitude exhibited a nonmonotonic dependence on stimulus strength, negligible at our lowest (1 μM) and highest (1 mM) stimulus strengths but peaking to a value of approximately 20% at intermediate (5–50 μM) strengths. An overshoot response of individual motors was reported many years ago (9) but is absent from later studies of chemotactic adaptation (11, 14, 19) (see Discussion). Fig. 4B displays the corresponding single-cell histograms of the overshoot.

Adaptation Kinetics Show Asymmetry in Response to a Step Up Versus a Step Down. In addition to the above measurements, we also quantified the response of individual cells to a step down in L-aspartate concentration. In agreement with recent reports (19), the chemotactic response was not merely a mirror image of that seen for a step-up stimulus; distinctly different adaptation kinetics were observed in the two cases (compare Fig. 2A and B). We characterized this asymmetry between step-up and step-down responses in detail. Cells adapted to step-down stimuli in much shorter times (6). Whereas adaptation times for the step-up stimuli ranged from approximately 15 s to over 4 min in the range of concentration jumps tested, adaptation times for the step-down stimuli saturated at approximately 15 s and showed little variation over two orders of magnitude change in the step-down concentration jumps (Fig. 2C).

Analysis of individual cell traces (Fig. 3D) revealed additional differences. In contrast to adaptation to step-up stimuli, the average number of run/tumble event pairs before adaptation to a step down was consistently high (approximately seven) and was largely independent of stimulus strength (Fig. 3E). This behavior is explained by the fact that run/tumble events were significantly shorter during step-down stimuli compared to events during a step up (Fig. S6 C and D) and the adaptation time was uniform (approximately 15 s) across the range of stimulus strengths. Finally, adaptation traces exhibited significant overshoot (approximately 20%) at all stimulus strengths tested (Fig. 4A and C). Overshoot was noticeable in the population-averaged traces as well (Fig. 2B). Below we discuss possible explanations for the observed asymmetry between step-up and step-down responses in individual cells.

Discussion

Our ability to perturb the chemotaxis network through step-up and step-down changes in chemosensory concentration and to measure the response of the individual cell reveals features of the adaptation kinetics that are masked in population-averaged measurements. Specifically, the precise, long-term characterization of chemotactic adaptation allowed us to quantify two features of adaptation kinetics in individual cells, and how they vary as a function of the stimulus strength.

A first such feature is the abrupt adaptation to a step up in attractant concentration. Upon stimulation, swimming cells entered a run of extended duration, but returned to their steady-state behavior after a small number of run/tumble event pairs (Fig. 3A). As seen from the histograms of ETA values in Fig. 3B, the most common response shown by cells at all stimulus levels was a single, prolonged run. The average number of events until adaptation ranged between one and six, tending to increase with stimulus strength (Fig. 3E). In contrast, adaptation to a step down in attractant concentration involved a larger number of run/tumble event pairs, exhibiting no clear dependence on the stimulus strength (Fig. 3E).

In the literature, chemotactic adaptation is typically described as a gradual process (6, 11, 14, 17, 19, 25). It is important to note, however, that these studies all involved averaging over multiple cells. As noted above, such averaging masks important features of single-cell adaptation kinetics because of the asynchrony in adaption between different cells (compare Figs. 2A and 3A). In studies where the response of individual flagellar motors was examined, the motors were described to undergo abrupt switches in behavior during the course of adaptation (9, 16), but this abruptness was not characterized in detail. Our measurements extend these findings by quantifying the level of abruptness as a function of stimulus strength, and by moving from the level of single flagellar motors to the (physiologically relevant) whole-cell swimming behavior.

What is the source of abrupt adaptation, and what makes the abruptness stimulus-dependent? As noted above, abruptness is already observed at the single-motor level (16). Therefore, the source of abruptness cannot be in the transition from the indivi-

![Fig. 4. Overshoot response to step-up and step-down stimuli. (A) The overshoot amplitude, normalized by the steady-state tumble bias and averaged over individual cells, is plotted as a function of the step up (solid black circles) and step down (gray open circles) stimulus. Error bars designate standard error of the mean. (B) Histograms of single-cell overshoot amplitudes in response to varying magnitudes of step-up stimuli. (C) Same as B, for step-down stimuli. Black lines are fits to a Gaussian. Color notations and sample sizes at each stimulus level are the same as in Fig. 3. See SI Materials and Methods for details of the overshoot calculation.](https://www.pnas.org/doi/10.1073/pnas.1120218109)
dual motors to the whole-cell behavior. Rather, the source must lie upstream in the cascade of interactions. Prima facie, a natural candidate to consider is the switch-like dependence of the flagellar rotational state on CheY-P level (the signaling molecule that controls the rotational bias of the motor) (28, 29). However, a simple theoretical argument shows that the cooperativity exhibited by the motor (30) has little to no effect on the abruptness of adaptation (Fig. S9A and SI Discussion). This claim is further supported by numerical simulations (Fig. S10 and SI Materials and Methods). Instead, we believe the evidence points towards a role for strongly interacting receptor clusters in creating the stimulus-dependent adaptation abruptness observed in our experiments. Several experiments on receptor interaction and dynamics in addition to our own theoretical analysis of the chemotactic network support this view (SI Discussion). An alternate mechanism for abruptness may be the recently discovered remodeling of FliM, a component of the flagellar motor to which CheY-P binds, during adaptation (31). Initial theoretical analysis based on this new result demonstrates that remodeling may lead to more abrupt adaptation, but it is unclear how it would explain the dependence of abruptness on stimulus strength (SI Discussion and Figs. S9B and S10D).

A second feature quantified in this work is the degree of overshoot in chemotactic response to step-up and step-down stimuli. Overshoot was evident at intermediate values of step-up stimulus (5–50 μM), and at all values of step-down stimulus (Fig. 4A). To the best of our knowledge, there is only one previous experimental account of chemotactic overshoot, in tethered cells (9). Curiously, other studies of chemotactic adaptation did not report an overshoot, instead describing a monotonous temporal response (6, 11, 14, 17, 19, 25). We speculate that, at least in some of these studies, the reason for failing to detect an overshoot was the insufficient duration of the experiments or an insufficient temporal resolution. The specific choice of stimulus level is also critical for the degree of overshoot (Fig. 4A), making this feature quite easy to miss. The potential mechanisms for an overshoot may provide additional arguments as to why it has not been widely observed. In general, an overshoot response may occur whenever different components of the network adapt at different rates. A recent theoretical model of the chemotaxis network (23) postulates that the overshoot response is caused by the differences in methylation kinetics between different types of receptors. Interestingly, one of the studies mentioned above (19) was conducted using a mutant strain that only expressed a single receptor type, potentially explaining why an overshoot was not reported. FliM remodeling (31) may be another potential candidate mechanism for overshoot, if the remodeling kinetics differ from those of CheA adaptation (SI Discussion and Fig. S10D). This mechanism would account for a lack of overshoot observed in studies probing the chemotaxis network upstream of the flagellar motor (e.g., at the level of CheA). Further work will be necessary to determine the source of the overshoot.

Lastly, we observed several differences in the adaptation response to step-down stimuli compared to the response to a step up. First, we observed that cells adapted much more rapidly to step-down stimuli (Fig. 2B), in agreement with the existing literature (6, 9, 19). It is generally believed that this difference arises from up-regulation of CheB activity by CheA during the step-down response (19, 32). Second, we observed that adaptation times for step-down stimuli were independent of stimulus strength. This result conforms to measurements of swimming and tethered cells, in which short adaptation times (<15 s) were consistently observed for high step-down stimulus strength (6, 9). However, it is in marked contrast with FRET measurements of CheA activity, which revealed longer CheA adaptation times that increased with higher step-down stimulus strength (19). The discrepancy between the FRET study and our own suggests that the source for stimulus independence may lie downstream from CheA in the chemotaxis network, possibly at the level of the flagellar motor or further downstream, at the mapping between individual motors and the whole-cell swimming behavior. Third, the step-down response exhibited a clear overshoot at all stimulus strengths tested, in contrast to step up (Fig. 4A). Because the overshoot arises from two components of the network adapting at widely different rates, we hypothesize that the overshoot may persist over a wider range of stimuli (because CheB kinetics are consistently faster for step down compared to those of CheR for step up). Finally, at all stimulus levels tested, the ETAs for step-down adaptation were consistently larger than for step up (Fig. 4D). In contrast, a prior single-motor study (9) reported only one prolonged CW event prior to adaptation to a step-down stimulus. We speculate that this discrepancy may reflect the differences between single-motor and whole-cell swimming behavior. As the CW bias increases, it is known that CW intervals in single motors increase in duration, whereas tumble durations in swimming cells remain relatively constant (29). Our data is consistent with this picture: Tumble durations remain short throughout the course of step-down adaptation (Fig. S6). Further measurements will be necessary to resolve these many interesting questions.

This last point motivates the need for investigating chemotaxis at the whole-cell level. The cell's swimming phenotype is the complex outcome of multiple flagellar motors acting together. On one hand, individual motors are independent entities that switch stochastically between CCW and CW rotation (33–35). On the other hand, motors exhibit some synchrony because of common control via diffusive CheY-P fields within the cell (36) and, potentially, because of hydrodynamic interactions outside the cell (37). The mapping that connects the conformational state of individual flagella to the cell swimming state (3, 4) has remained elusive, largely due to the lack of quantitative experimental results. Recent theoretical studies predict that cells with different numbers of flagella should exhibit different swimming behavior (38, 39). Our data may provide important clues as to the way multiple motors collectively produce the swimming behavior of a cell. As discussed above, certain features of adaptation kinetics observed at the whole-cell level are reproduced at the single-motor level (and upstream in the network) while others are not. These discrepancies may reflect differences in the way the cell enters into the tumbling state, when the flagellar bundle is disrupted, and exits from that state, when the bundle reforms.

In summary, our ability to elucidate the mechanisms governing bacterial chemotaxis is driven first and foremost by our experimental ability to characterize the system’s behavior with increasing resolution and precision (17, 20, 30, 40). Here, by following the chemotactic response of individual multiflagellated cells in unprecedented detail, we were able to quantitatively assess the fine kinetic features of adaptation, providing unique insights into the workings of the chemotactic network. Further technological enhancements to our ability to quantify cellular parameters during the chemotactic response will provide additional findings and allow us to further refine our understanding of the chemotaxis system. A promising future direction is the addition of a high spatial resolution fluorescence imaging module to our trapping device, which will allow us to follow in real time the spatiotemporal dynamics of key cellular players—such as flagella, membrane receptors, and intracellular proteins—as the chemotactic response is taking place.

Materials and Methods

An overnight culture of E. coli strain RPl37 (wild type for chemotaxis) (26) was diluted 100-fold into 1 ml tryptone broth and grown at 30 °C for 4.5 h (OD600 approximately 0.5). Chemotaxis experiments were conducted in “trap motility buffer” (TMB). TMB contained 70 mM NaCl, 0.1 mM methionine, 100 mM Tris-Cl, 2% (wt/vol) glucose, and an oxygen-scavenging system (80 μM ml−1 glucose oxidase and 13 μg ml−1 catalase). A detailed description of the optical tweezers and flow cell design can be found elsewhere (20).
of the experimental setup and the data-analysis methods are provided in SI Materials and Methods.

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**Supporting Information**

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**SI Text**

**SI Materials and Methods. Microbiology.** *Escherichia coli* cells [strain RP437, wild type for chemotaxis(1)] were collected from a single colony on an agar plate and grown overnight in 1 ml tryptone broth [1% (wt/vol) Bacto tryptone and 0.8% (wt/vol) NaCl] (2). The overnight culture was diluted 100-fold into 1 ml tryptone broth and grown for 4.5 h to mid-log phase (OD₆₀₀ ~ 0.5). Cells were grown at 30 °C with 265 rpm rotation in 15-mL round bottom Falcon tubes. The final culture was diluted 10-fold into “trap motility buffer” (TMB). TMB contained 70 mM NaCl, 0.1 mM methionine, 100 mM Tris-Cl, 2% (wt/vol) glucose, and an oxygen-scavenging system (80 μg ml⁻¹ glucose oxidase and 13 μg ml⁻¹ catalase; EMD Chemicals 345386 and 219001, respectively) to reduce oxidative damage to the cells by the infrared trapping light (3, 4). The oxygen-scavenging system was added immediately before the beginning of the experiment. Under anaerobic conditions, trapped cells grew and divided at the expected rate, and exhibited run and tumble durations within the range reported in the literature (4). We performed control chemotaxis experiments using a 2-D swimming assay, following the protocol from Alon et al. (5). We observed similar adaptation times with (160 ± 30 s) and without (200 ± 30 s) the oxygen-scavenging system in the medium. Methionine provides the methyl groups necessary for chemotactic adaptation to occur (6). Glucose acts as a substrate for the oxygen-scavenging system and provides energy for cell swimming under anaerobic conditions (7). Various concentrations of L-aspartate (1–1,000 mM) were added as a chemical stimulus.

**Optical trap setup.** A detailed description of the optical tweezers design can be found elsewhere (4, 8). Briefly, the optical tweezers component consisted of two orthogonally polarized beams from a single S-W, 1,064-nm diode-pumped solid-state laser (YLIR-5-1064-LP; IPG Photonics). Both beams were tightly focused to generate optical traps by a 60 ×, water-immersion (1.2 numerical aperture) 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. The flow chamber was positioned between the two objective lenses and was 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 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 (9). Epifluorescence excitation in widefield configuration was provided by a 30-mW, 532-nm diode laser (TECGL-30; World Star Tech) to excite rhodamine B for gradient calibration. Emitted fluorescence light passed through a dichroic mirror (Di01-R532-25 × 36, single-edge 532-nm laser dichroic; Semrock) and an emission filter (HQ600/100 m-2p, band-pass 550–650 nm; Chroma) before being imaged onto an intensified charge-coupled device (I-PENTA-MAX; Princeton Instruments).

**Fluidics.** Glass coverslips (Fisher 12-545-M, 24 × 60 – 1) were sonicated in dry acetone for 5 min and rinsed with deionized water. Remaining water was spun off by centrifuging at 1,000 rpm for 3 min. The flow channel pattern was cut out from Nescofilm (Karlan) and placed between two coverslips, one of which had custom-drilled holes (0.05-inch diameter) for inlets and outlets. The Nescofilm flow channel pattern was bonded to coverslips by melting on a hot plate for 4 min. The completed flow chamber was inserted into a custom metal frame where inlet and outlet tubings (Tygon) were screwed on for a tight seal (Fig. S1A). The three channels of the flow chamber were continuously injected with appropriate buffers using a syringe pump (PHD2000; Harvard Apparatus) at a linear speed of 70 μm/s. We performed control experiments to test the effect of flow on cell swimming parameters. Individual trapped cells monitored in 70 μm/s flow exhibited an approximately 10% decrease in run duration and an approximately 20% decrease in tumble duration, compared to no-flow conditions. The tumble bias remained relatively constant over the same range of flow rates (N = 8 cells). In step-up stimulus experiments, the top channel was injected with TMB, 1-aspartate, and rhodamine B. The middle channel contained TMB only, and the bottom channel contained cells in TMB. In step-down stimulus experiments, the top channel contained TMB and rhodamine B, the middle channel contained TMB and 1-aspartate, and the bottom channel contained TMB, 1-aspartate, and cells. Rhodamine B (Sigma R6626) (100 mM) was used to quantify the gradient profile under the fluorescent illumination of the optical trap setup (Fig. S1 B and C) prior to each experiment.

**Post-processing of the optical trap data.** All routines for analyzing optical trap data were written in MATLAB (MathWorks). Raw data obtained at 1,000-Hz sampling frequency were low-pass filtered to 100 Hz, and the amplitude was normalized in non-overlapping 1-s windows (4). Two separate sets of y-direction (Fig. L4) signals obtained from the two ends of the cell body were combined by taking the difference for enhancement in signal-to-noise ratio. Using the combined y-direction signal, the peak frequency component at each time point was obtained from a continuous wavelet transform. Our wavelet analysis was performed using the complex Morlet mother wavelet in a linearly scaled frequency range of 2–40 Hz. Runs and tumbles were distinguished by applying a single threshold value to the peak frequency time trace. The threshold was determined by examining the distribution of peak frequencies and finding the local minimum between peaks corresponding to run and tumble. For cases in which 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, as 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) (4).

**Quantifying adaptation features at the population-average level.** The run/tumble binary time traces were subsequently analyzed using two different methods. In one method, tumble bias was determined from a 10-s moving window by calculating the fraction of time the cell spent tumbling within the window, and moving the window one data point at a time (Fig. S2). Because individual tumble bias traces were noisy (σ/μ = 0.53 ± 0.16 at steady-state, n = 186 cells), they were averaged across the population (Fig. 2 A and B). From the population-averaged tumble bias traces, adaptation time and overshoot were quantified by fitting to analytical expressions. For step-up stimuli (Fig. 2A), the poststimulus portion of the data was fit to the expression $B(t) = 1/(1 + a \exp(ln(b) \exp(-c(t - \tau_{adapt}))))$ using the nonlinear fitting function in MATLAB with $a$ and $b$ as constants and $c$ and $\tau_{adapt}$ (the adaptation time) as fitting parameters. The constants were defined as $a = (1 - B_\infty)/B_\infty$ and $b = 1 + 1/(1 - B_\infty)$, where $B_\infty$ is the steady-state tumble bias (mean of the last 50 s of data), $c$ is the steady-state tumble bias (mean of the last 50 s of data), $B_\infty$ is the steady-state tumble bias (mean of the last 50 s of data), and $\tau_{adapt}$ is the adaptation time.
which ensured that \( B(t) = B_{\text{up}}/2 \) when \( t = t_{\text{adapt}} \). This expression is an approximate analytical solution for \( B(t) \) following the chemotaxis model of Tu et al. (10) (see Simulations) and the approximations outlined in the SI Text. Because \( B(t) \) above does not have an overshoot feature, we fit the residual using the phenom-

enological form \( R(t) = (\text{data} - B(t)) / R(t) = a e^{b t} \exp(c t) \),

where \( a, b, c \) and \( d \) are fitting parameters. Normalized overshoot amplitude was determined as \( (B_{\text{max}} - B_{\text{up}}) / B_{\text{up}} \), where \( B_{\text{max}} \) is the maximum of \( B(t) + R(t) \). For step-down stimuli (Fig. 2B), the poststimulus portion of the data was fit with a phenomenological expression \( B(t) = B_{\text{up}} + a \exp(-b t) + c \exp(-d t) / d \) using

the nonlinear fitting function in MATLAB with \( a, b, c, d \) as fitting parameters. From this fit, the adaptation time was \( t_{\text{adapt}} = \ln b \) and the normalized overshoot amplitude was \( (B_{\text{max}} - B_{\text{up}}) / B_{\text{up}} \), where \( B_{\text{min}} \) is the minimum of \( B(t) \).

**Quantifying adaptation features at the single-cell level.** Alternatively, the run/tumble binary time traces were analyzed in the “event domain” (11). In this method, the run/tumble binary time traces were analyzed by pairing each run with its subsequent tumble (11). For each run/tumble pair event we determined a corre-

sponding tumble bias value [tumble duration / (run duration + tumble duration)] and a duration (run duration + tumble dura-

tion) (Fig. S5). Population-averaged event traces (Fig. 3A and D) were also constructed event by event, where the tumble bias and duration were averaged across the population for each run/tum-

ble event, enumerated relative to the application of the stimulus. Quantification of abruptness, adaptation time, and overshoot from individual cells was performed in the event domain. For abruptness, we determined the number of “events to adaptation” (ETA) using the Mann–Whitney \( U \) test, a nonparametric statisti-

tical test for assessing the null hypothesis that two independently obtained samples are equal in magnitude (12). The reference sample consisted of 65 events prestimulus, normalized by the mean of the last 20 events poststimulus to compensate for the possibility of nonexact adaptation. The test sample consisted of a moving five-event window immediately following the application of stimulus. The five-event window was moved one event at a time until adaptation was scored when the \( U \) statistic of the test sample approached the expected \( U \) within 50% of the standard deviation in \( U \). ETA was the number of events that led to adapta-

tion, and adaptation time was the total duration of the events that led to adaptation. We found that our statistical analysis was more robust when using parameters that increase in response to stimuli and then fall back down as the cell adapts (i.e., run duration for step up, tumble bias for step down). Normalized overshoot ampu-

litude was calculated using the formula (Bias middle—Bias after) / Bias after, where Bias middle was the mean tumble bias of sixth through 15th events following adaptation as deter-

mined above, and Bias after was the mean tumble bias of the final 20 events poststimulus. ETA, adaptation time, and normal-

ized overshoot amplitude of population-average event traces were determined in the same way as the individual event traces (Fig. S8).

**Numerical simulations. Simulating chemotactic adaptation.** In order to explore the effect of varying the receptor cluster size and motor switching cooperativity on the abruptness of adaptation (Fig. S10B), we performed stochastic simulations to generate a population of run/tumble binary time traces for each parameter condition. All simulations were implemented in MATLAB. We followed closely the model by Tu et al. (10). The kinase activity of CheA, \( A \), was determined as a function of ligand concentration \( (|L|) \) and methylation level \( (m) \) according to \( A(|L|, m) = 1/(1 + \exp(N f_L |L| + f_m m)) \), where \( N \) is the receptor cluster size, and \( f_L = \ln(1 + |L| / K_{\text{off}}) - \ln(1 + |L| / K_{\text{on}}) \) and \( f_m = 2(0.5 - m) \) are the free energy functions that depend only on \( |L| \) and \( m \), respectively (10). Free energies were in units of \( k_B T \). Note that the value of \( A \) ranges from 0 to 1. \( A \) can be inter-

preted as the probability that each CheA is in its active (phos-

phorylating) state. \( K_{\text{off}} (5 \mu M) \) and \( K_{\text{on}} (254 \mu M) \) are ligand-

binding constants for receptors associated with CheA in inactive and active states, respectively. The values for \( K_{\text{off}} \) and \( K_{\text{on}} \) were obtained from fitting the expression for \( f_L \) to our adaptation time data (Fig. 2C). The exact values of \( K_{\text{off}} \) and \( K_{\text{on}} \) affect only the adaptation time and not the abruptness of adaptation. At each time step (\( \Delta t = 0.01 \) sec, chosen to match the data rate; shorter time steps did not change the results of simulation), the average methylation level of the receptor-CheA complex was adjusted ac-

cording to \( dm/dt = V_R (1 - A) - V_B A \) (13). \( V_R (0.01 \text{ s}^{-1}) \) and \( V_B (0.02 \text{ s}^{-1}) \) are methylation and demethylation rates, respec-

tively (14). As with \( K_{\text{off}} \) and \( K_{\text{on}} \), the exact values of \( V_R \) and \( V_B \) only affect the adaptation time. The CheA activity, \( A \), was then converted to the flagellar motor bias, \( B \), via a highly cooperative relation \( B(A) = A^B (A^H + K_{\text{d}H}) \) (15). Note that, as with \( A \), \( B \) ranges from 0 to 1. \( K_d \) is the value of \( A \) at which \( B = 1/2 \). Although \( B \) is technically a function of CheY-P concentration and not CheA activity, it is commonly assumed that CheY-P concentration and CheA activity are proportional because CheY-P levels equilibrate faster than other processes (16). Because a general model on how the motor bias gets converted to the cell’s tumble bias is lacking, we assumed they are proportional. At the first time point, every simulated cell started from the tumble state. At each subsequent time point, the cell had a constant probability of switching to the run state. When the cell was in the run state, on the other hand, its probability of switching to the tumble state depended on the tumble bias. As a result, the average tumble duration did not depend on tumble bias but the average run dura-

tion did. This was consistent with a previous study and our own data (17) (Fig. S6). For each combination of \( N \) and \( H \) values, 100 runs of the binary time traces were generated. The simulated traces were analyzed in the same way as the experimental data as described above.

**Simulating the effect of variations in protein expression level.** In order to reproduce the observed population variation in adapta-

tion time (Fig. 3C), we simulated a population of cells with stoc-

astic variation in CheR and CheB expression levels. Expression levels of CheR and CheB were varied in a concerted manner be-

cause they are expressed from the same operon (18). Simulations were run in the same manner as above, except that values of \( V_R \) and \( V_B \) were selected from a Gaussian distribution with 10% or 20% standard deviation. We note that this degree of protein num-

ber fluctuations is in good agreement with typical values found in the literature (19). This fluctuation level is also close to the theoretical expectation based on the known CheR and CheB average copy numbers in the cell (20), and the assumption that proteins are produced in bursts of 5–10 per mRNA (21, 22).

Mean \( V_R \) and \( V_B \) values were the same as above. One thousand cells were simulated for a given level of variation in CheR and CheB expression and stimulus strength. We analyzed the simula-

ted data traces using the same analysis routine used for experi-

mental data to obtain the adaptation time. The coefficient of variation in adaptation time for the simulated adaptation times and the experimentally measured adaptation times (Fig. 3C) are shown in Fig. S4B.

**Simulating the effect of flagellar motor remodeling.** We performed simulations to investigate the effect of flagellar motor remodeling (23) on adaptation kinetics (see SI Discussion). Following Yuan et al. (23), we used a modified expression relating the motor CW bias \( B \) to CheA activity \( A \): \( B(A) = (1 + A/K_A) \) / \( (1 + A/K_A) \) \( + P/(1 + A/K_C) \), where \( \text{Flim} \) is the number of Flim units in the c-ring of the flagellar motor, \( P \) is the ratio of the probability that the motor is in the CCW state to the prob-
ability that it is in the CW state in the absence of CheY-P (A = 0), and C is the ratio of CheY-P dissociation constants for the CCW and CW states, respectively (23). The time-dependent \( \text{FilM}(t) \) was modeled according to \( d\text{FilM}/dt = V_{\text{on}}(\text{FilM}_{\text{max}} - \text{FilM}) - V_{\text{off}}(A)\text{FilM} \). Here, \( \text{FilM}_{\text{max}} \) (assumed to be 45 in our simulations) is the maximum number of FilM units that can be in the c-ring, and \( V_{\text{on}} = 0.02 \text{ s}^{-1} \) and \( V_{\text{off}} = 0.0194 \text{ s}^{-1} \) are association and dissociation rates of FilM to filM. \( V_{\text{on}} \) and \( V_{\text{off}} \) satisfy the steady-state condition \( V_{\text{on}}(\text{FilM}_{\text{max}} - \text{FilM}_{\text{w}}) = V_{\text{off}}(A)\text{FilM}_{\text{w}}, \) where \( A_{\text{w}} = 1/3 \) and \( \text{FilM}_{\text{w}} = 34 \) (14, 23–25) are steady-state CheA activity and number of FilM units, respectively. The flagellar motor remodeling was incorporated in the simulations by varying the value of FilM according to the CheA activity, A, at each time point. The results of these simulations are shown in Fig. S10D.

**SI Discussion. Review of the E. coli chemotaxis network.** A cell’s swimming state is controlled by a cascade of interactions (26, 27) (Fig. S10A). Chemical input signals from the environment are sensed by transmembrane receptors that are coupled to the intracellular kinase CheA. Commonly, CheA activity is parameterized by the quantity \( A \), the probability (ranging from 0 to 1) that the kinase is in its active (phosphorylating) state. The activity is a function of the receptor ligand concentration (28); an increase in chemotactrant leads to a decrease in activity and vice versa. In addition to ligand binding, methylation of the receptors also modulates kinase activity; the higher the methylation, the higher the activity. Thus,

\[
A = A([L], m),
\]  

where \([L]\) is the ligand concentration and \( m \) is the number of methylated receptor sites (ranging from 0 to 8). Notably, \( A \) is an increasing function of the methylation \( m \). Receptors are also known to interact and cooperatively modulate CheA kinase activity (16, 29). Ligand binding to one receptor affects the CheA activity in a neighborhood of \( N \) interacting receptors (29) (Fig. S10A). The net consequence of this interaction is to multiply the effect of methylation \( m \) on \( A \) by the interacting cluster size \( N \) (29, 30).

At the same time, the methylation rate of the receptors is also a function of CheA activity. Methylation and demethylation are controlled by the proteins CheR and CheB, respectively, whose activity depends on \( A \) (31). Thus,

\[
\frac{dm}{dt} = F(A),
\]  

Though the detailed functional form of \( F(A) \) depends on the model used (10, 32), for our purposes it is sufficient to state that \( F(A) \) is a decreasing function of \( A \); as CheA activity increases, the rate of methylation decreases. Eqs. S1 and S2 describe the negative feedback loop that is responsible for adaptation. As CheA activity is perturbed from its steady-state (by a step up or step down in cheemoattractant concentration, for example), this feedback loop ensures its eventual return to the same steady-state (5, 31, 33, 34).

The next link in this chain of interactions is the phosphorylation of the signaling protein CheY by CheA. In its active form, CheY-P binds to the flagellar motors and induces a conformational switch from CCW to CW rotation (17, 18). This interaction is known to be highly cooperative, and described by a sigmoidal function (15) (Fig. S10A). At saturating conditions, the concentration of CheY-P is proportional to CheA activity, and the CW bias of the motor \( B \) (defined as the fraction of time spent in the CW state, a number ranging from 0 to 1) can be written as a Hill function

\[
B = \frac{A^H}{A^H + K_A^2},
\]  

where \( H \approx 10 \) (depicted in Fig. S10A). Importantly, measurements have shown that the steady-state CheA activity sits in the steepest part of this function: \( A_{\text{ss}} = 0.33, B_{\text{ss}} = 0.35 \) (where the subscript \( \text{ss} \) denotes the steady-state), and \( K_A = 0.35 \) (15, 32).

The last component of the cascade of interactions occurs between the flagellar motors and the whole cell. As individual motors that comprise the flagellar bundle undergo a conformational switch from CCW to CW rotation, the cell swimming state switches from a run to a tumble (36). It is important to note that the individual motors in the cell are not perfectly synchronized (37–39), and that the manner in which the collective CCW/CW state of the motors dictates the run/tumble state of the whole cell remains poorly understood (40, 41).

Adaptation abruptness does not reflect the switch-like behavior of the flagellar motor. As discussed in the main text, it is natural to assume that the switch-like manner in which the flagellar rotational state depends on CheY-P level plays a role in abruptness of adaptation. However, we found that this cooperativity, parameterized by the large Hill coefficient \((H \approx 10)\) (15), has little to no effect on the abruptness of adaptation (Fig. S9A). Instead, we believe the evidence points towards alternate mechanisms further discussed below. The derivation that follows explains this in the context of accepted mathematical model of chemotaxis network.

The strong nonlinearity in the Hill equation Eq. S3 and the fact that the CheA steady-state level is in the steepest portion of the curve mean that the bias \( B \) is only sensitive to changes in \( A \) near its steady-state level \( A_{\text{ss}} \). In the context of adaptation, the temporal response in \( B \) is almost solely determined by \( A \) in its approach to the steady-state. For example, in a chemoattractant step-up experiment, 75% of the amplitude of the adaptation response in bias \( B \) (as it increases from 0 to \( B_{\text{ss}} \)) comes from the last approximately 15% of the amplitude of the response in \( A \) as it approaches \( A_{\text{ss}} \). To quantify this effect, we consider the temporal response in \( B(t) \) and \( A(t) \) at a reference time point \( t_{\text{adapt}} \), the adaptation time. This was defined in the main text as the time elapsed between the stimulus and when the bias returned to half of its steady-state value. Expanding \( A(t) \) in a Taylor series about this time yields

\[
A(t_{\text{adapt}}) \approx A_{\text{ss}} + \frac{dA}{dB} \bigg|_{B_{\text{ss}}} (B(t_{\text{adapt}}) - B_{\text{ss}}) = A_{\text{ss}} - \frac{dA}{dB} \bigg|_{B_{\text{ss}}} B_{\text{ss}} \frac{1}{2}.
\]  

The second term depends inversely on the slope of the Hill function, \( dB/dA \), which is proportional to \( H \). Using previously determined values (15), the second term is approximately \( 0.25/H = 0.025 \ll A_{\text{ss}} \) (i.e., small when \( H \) is large). Thus, we may write \( A(t) = A_{\text{ss}} - \Delta A(t) \), valid for times \( t \gg t_{\text{adapt}} \), where \( \Delta A \) is small \((<< A_{\text{ss}})\) and proportional to \( 1/H \).

Based on this observation, we can determine the temporal response of \( A(t) \) for \( t \gg t_{\text{adapt}} \) (i.e., how \( A \) approaches \( A_{\text{ss}} \) in time). Taking Eq. S2 and Taylor expanding \( F(A) \) near the steady-state,

\[
\frac{dA}{dt} \approx F(A_{\text{ss}}) - \frac{dF}{dA} |_{A_{\text{ss}}} \Delta A(t) = -\frac{dF}{dA} |_{A_{\text{ss}}} \Delta A(t),
\]  

where, by definition, \( F(A_{\text{ss}}) = dm/dt = 0 \) at the steady-state. Using the chain rule, we can further write the rate of change for CheA activity in terms of the rate of change in methylation:

\[
\frac{dA}{dt} \frac{dm}{dt} = -\frac{d\Delta A}{dt}.
\]
Substituting Eq. S5 into Eq. S6, we obtain

\[
\frac{d\Delta A}{dt} \approx \left. \frac{dA}{dm} \frac{df}{dA} \right|_{A_o} \Delta A. \tag{S7}
\]

The first factor on the right-hand side of the equation represents how CheA activity is amplified by methylation; this factor is positive, and also proportional to \(N\), the cluster size for interacting receptors. The second factor represents how the methylation rate depends on CheA activity at the steady-state; this factor is negative. These two factors combine to define the time constant with which CheA activity approaches its steady-state:

\[
\frac{d\Delta A}{dt} \approx -\frac{N}{T} \Delta A. \tag{S8}
\]

where we have made the dependence on cluster size \(N\) explicit. Thus, CheA approaches its steady-state exponentially according to \(\Delta A(t) = \Delta A_0 \exp(-Nt/T)\) (depicted in Fig. S9A).

Based on the above, we now use the Hill function, Eq. S3, to estimate the temporal response in bias \(B(t)\), given \(A(t)\) near the steady-state. For our purposes, we specifically determine the rate of change in \(B(t)\) at the adaptation time; this will provide an approximate measure for the abruptness of the adaptation response (Fig. S9A), and its dependence on network parameters. Based on Eq. S4 and S8, the rate of change in CheA activity at the adaptation time is

\[
\left. \frac{dA}{dt} \right|_{t_{\text{adapt}}} \approx \frac{N}{T} \Delta A(t_{\text{adapt}}) = N \left. \frac{B_{\text{eq}}}{dB} \frac{dA}{dB} \right|_{B_{\text{eq}}} \propto \frac{1}{H}. \tag{S9}
\]

proportional to \(1/H\). Using the chain rule, the rate of change in \(B(t)\) at the adaptation time is then

\[
\left. \frac{dB}{dt} \right|_{t_{\text{adapt}}} = \frac{dB}{dA} \left. \frac{dA}{dt} \right|_{t_{\text{adapt}}} \propto \frac{1}{H} \cdot \frac{N}{T} \cdot \frac{N}{T} \tag{S10}
\]

which is independent of \(H\), given that the slope of the Hill function \(dB/dA\) at the adaptation time is proportional to \(H\). Thus, the abruptness in the adaptation response for motor bias \(B\) does not depend on the sigmoidicity of the Hill function, Eq. S3, provided that \(H\) is sufficiently large. This is depicted schematically in Fig. S9B. While cooperativity amplifies the motor’s response to changes in CheA activity (by a factor of \(H\)), it also makes the motor sensitive to small changes in CheA activity only near its steady-state. As CheA activity exponentially approaches steady-state during adaptation, its rate of change becomes smaller. Thus, the larger \(H\) is, the smaller the rate of change in CheA activity that engenders the motor response. These compensating effects result in a flagellar motor temporal adaptation response whose abruptness is largely independent of \(H\) (Fig. S9A). [Note that \(H\) does affect the adaptation time, but to a good approximation the shape of the response \(B(t)\) remains unchanged.] Importantly, however, the dependence on cluster size \(N\) remains.

**Possible role of dynamic receptor clustering in creating a stimulus-dependent adaptation abruptness.** Clusters of chemotaxis receptors are known to be localized predominantly at the cell poles (42, 43), where the number of receptors in each cluster can range from tens to over 1,000 in the case of the Tar receptor (44). As mentioned above, experimental evidence indicates that receptors interact together to amplify their effect on CheA activity in response to external chemical inputs (29). The mathematical model (above) for the chemotaxis network (10, 32) further predicts that larger numbers of interacting receptors will result in a more abrupt response in CheA activity. Thus, abruptness in adaptation response likely originates from the clusters of interacting receptors that cooperatively modulate the activity of the kinase CheA. The abruptness at the level of CheA activity then propagates through the network to the level of whole-cell swimming behavior. Experimentally, CheA activity has only been measured in cell populations (13, 16, 45). We believe this may explain why abrupt adaptation kinetics in CheA has not yet been observed.

Experimental evidence also indicates that receptor clustering may be dynamic; when *E. coli* and *Bacillus subtilis* cells are stimulated with saturating amounts of attractant, the polar clusters disintegrate upon stimulation and reappear after the cells have had enough time to adapt (46, 47). In another study, Borrok et al. (48) found through chemical cross-linking studies that attractants destabilize receptor clusters and repellents stabilize them. These studies support a model in which the degree of chemoreceptor clustering changes dynamically depending on ligand binding. Given that the degree of receptor clustering decreases at higher attractant concentrations, it would be expected that their cooperative effect on CheA activity—and thus the abruptness in adaptation—would decrease. We note, however, that a number of studies found that receptor methylation level increases the degree of receptor clustering and hence the response cooperativity (30). Because methylation should increase with stimulus, this would predict that abruptness would decrease with stimulus, counter to the experimentally observed trend. We speculate that methylation level is low under our experimental conditions, and that this effect may be negligible. Unfortunately, experimental estimates of the methylation level exist only for mutant strains, lacking native receptors (30). Furthermore, theoretical estimates for the parameters that determine the steady-state methylation level vary greatly between different studies (10, 32). In our own simulations (which reproduce the experimental adaptation times), methylation saturates at about 2.5 out of the eight available sites at the highest stimulus level. It is also conceivable that the changes in receptor cluster size caused by methylation are small compared to those produced by ligand binding. More studies are needed to quantify the importance of these competing trends.

To investigate the possible role of receptor clusters, we performed simulations of chemotactic adaptation generalized to allow for strongly interacting receptor clusters of size \(N\). Numerically solving the response to step up and step down in attractant concentration, we found that the simulated ETAs vary inversely with cluster size \(N\) (Fig. S10B). Specifically, the experimentally observed stimulus-dependent abruptness (Fig. 3E) can be reproduced by assuming that \(N\) varies between approximately 18 and 3, decreasing with stimulus strength (Fig. S10C). This range of values is consistent with numbers cited in the literature for wild-type (16, 30, 49) and mutant strains (30), and follows the expected trend with stimulus level. Thus, our simulations support the notion of dynamic receptor clustering as the source for adaptation abruptness and stimulus dependence. We also note that simulations corroborate our view that ETA is unaffected by the cooperativity \(H\) exhibited by CheY-P (Fig. S10B, Inset).

**Possible role of motor remodeling in adaptation abruptness.** Recent experiments (23) have uncovered another dynamic process during adaptation: flagellar motor remodeling. FliM, the component of the flagellar motor c-ring and the protein to which CheY-P binds to engender the conformational switch from CCW to CW rotation, is rapidly exchanged with free FliM in the cytosol. New evidence indicates that CheY-P binding increases the rate of dissociation of FliM from the c-ring, destabilizing it. As a result, changes in CheY-P levels in the cell during adaptation lead to changes in the flagellar motor structure. The net effect is that the dependence of the motor CW bias \(B\) on CheA activity \(A\) is dynamic. According to new models, the Hill function Eq. S3 is modified to
\[
B(A) = \frac{(1 + A/K_A)^{FliM} + P(1 + A/(K_A C))^{FliM}}{(1 + A/K_A)^{FliM} + P(1 + A/(K_A C))^{FliM}}.
\]

where \( P \) is the ratio of the probability that the motor is in the CCW state to the probability that it is in the CW state in the absence of CheY-P, \( A \) is the ratio of CheY-P dissociation constants for the CCW and CW states (23). The number of FliM proteins in the c-ring, FliM, is time-dependent, following

\[
\frac{d}{dt} \text{FliM} = V_{on}(\text{FliM}_{\text{MAX}} - \text{FliM}) - V_{off}(A)\text{FliM} \tag{S12}
\]

where \( V_{on} \) and \( V_{off} \) are the binding (and CheA-dependent) dissociation rates for FliM, and \( \text{FliM}_{\text{MAX}} \) is the maximum number of FliM accommodated in the c-ring. The Hill function Eq. S3 and the new model Eq. S11 coincide with the following parameter values: \( \text{FliM} = 34 \), \( P = 10^{-2} \) (50), and \( C = 4.1 \) (15, 51).

In principle, this recently discovered motor-remodeling mechanism can reproduce several features of adaptation kinetics that we observe at the whole-cell level. During the initial response to a step up in attractant, CheA activity drops, increasing the number of FliM in the flagellar motor. As shown schematically in Fig. S9B and observed directly in experiments (23), this increase in FliM shifts the sigmoidal CW bias vs. CheA activity curve \( B(A) \) to the left. As a result, the steepest part of this curve no longer coincides with the steady-state CheA activity \( A_{\text{ss}} \). Provided the shift persists as CheA activity \( A \) increases toward the steady-state, the time dependence of the CW bias \( B(t) \) will be determined by \( A(t) \) at a time point that precedes its slow, exponential approach to the steady-state \( A_{\text{ss}} \), where its rate of change is far more rapid. This effect can lead to more-abrupt adaptation kinetics at the motor level (shown schematically in Fig. S9B). In addition, an overshoot response may be obtained if the remodeling shift persists at times long enough that \( A \) has approached its steady-state value. Whether these features are reproduced by FliM remodeling depends ultimately on how the time scales for motor remodeling compare to those for adaptation at the CheA level of the network.

We performed simulations incorporating flagellar motor remodeling (see SI Materials and Methods). As shown in Fig. S10D, we could reproduce an overshoot response for a reasonable choice of parameters.

Fig. S1. Laminar flow chamber. (A) Photo of the laminar flow chamber used in this study. Food dyes of different colors were injected into different streams for illustration. (B) Fluorescence microscopy image of a typical gradient established in the flow chamber. The pixelated appearance of the image comes from the montage of multiple fields of view. Green dots indicate the pre- and poststimulus measurement locations, and the green arrow indicates the translocation direction of the trapped cell when stimuli are applied. (C) Concentration profile measured under the experimental conditions (linear flow speed = 70 μm/s, 500 μm downstream from where two streams merge). Fluorescence intensity of rhodamine B was measured at various points along the perpendicular direction (blue circles). Red and black smooth lines are theoretical concentration gradient curves with diffusion coefficient $D = 320 \mu m^2/s$ (rhodamine B) and $D = 1000 \mu m^2/s$ (small molecules), respectively. Green arrows indicate the pre- and poststimulus measurement locations. Theoretical concentrations at these locations are <1 and >99% of the maximum concentration at the low and the high ends, respectively. Taking the liberal estimation of $D = 1000 \mu m^2/s$ for aspartate, cells that are moved along the concentration gradient at a speed of 100 μm/s experience the change from 10 to 90% of the maximum concentration over a span of about 3 s.

Fig. S2. Conversion from binary trace to tumble bias time trace. (A) A 400-s segment of a binary series from a single cell that underwent a 100-μM aspartate step-up stimulus at $t = 0$. (B) The tumble bias at each time point ($\Delta t = 0.01 s$) is determined from a 10-s moving window (red box) over the binary series.
Fig. S3. Exactness of adaptation to step-up and step-down stimuli. (A) Exactness of adaptation determined from the individual cell event traces (blue triangles, mean ± standard error of the mean), population-average event traces (black circles), and population-average time traces (red squares). (B) Same as A, for step-down stimuli. (C) Histograms of single-cell adaptation exactness in response to varying magnitudes of step-up stimuli. (D) Same as C, for step-down stimuli. Black lines are fits to a Gaussian. Color notations and sample sizes at each stimulus level are the same as in Figs. 3 and 4. From event traces, exactness was defined as $\text{Bias}_{\text{after}} / \text{Bias}_{\text{before}}$, where $\text{Bias}_{\text{after}}$ was the mean tumble bias of the final 20 events poststimulus, and $\text{Bias}_{\text{before}}$ was the mean prestimulus tumble bias. From population-average time traces, exactness was defined as the ratio of mean tumble bias of last 50 s after stimulus to mean tumble bias of 200 s before stimulus.
Cell-to-cell variation in adaptation time. (A) Individual binary traces for 39 cells that underwent a 100-μM L-aspartate step-up stimulus (at $t = 0$). All cells initially respond to the stimulus by entering a prolonged run. The subsequent return to run/tumble switching occurred at different times for different cells. Individual cell traces are colored randomly. (B) The coefficient of variation (standard deviation divided by the mean) of adaptation time as a function of the average adaptation time. Blue circles, experimental data binned according to the applied stimulus strengths. Gray squares, numerical simulations of the chemotactic response at various stimulus strengths. For each stimulus strength, a population of 1,000 cells was simulated. Variation in adaptation times was obtained by selecting the methylation and demethylation rates by CheR and CheB for each cell from a Gaussian distribution with 10% (light gray squares) and 20% (dark gray squares) standard deviation. The ratio of methylation and demethylation rates was held constant. In all plots, error bars denote standard error, obtained by repeated resampling of the dataset (bootstrapping). Solid lines are guides to the eye. For details of the numerical simulations see SI Materials and Methods.
Fig. S5. Conversion from binary trace to tumble bias event trace. (A) A 600-s segment of a binary series from a single cell that underwent a 10-μM aspartate step-up stimulus at \( t = 0 \). (B) The tumble bias is calculated for each event pair consisting of a run and a tumble. Events are enumerated starting from the first event following the stimulus. (C) In addition to the tumble bias value, each event is aligned in time to the binary trace for visualization purposes.

Fig. S6. Changes in average run and tumble durations following stimuli. (A) Population-averaged run durations of the first 10 events following step-up stimuli. Different stimulus strengths are color-coded as in the main figures. (B) Same as A, for tumble durations. (C) Same as A, for step-down stimuli. (D) Same as B, for step-down stimuli. Error bars designate standard error of the mean.
Fig. S7. Quantifying the adaptation parameters of an individual cell. (A) An 800-s segment of a tumble bias event trace from a single cell that underwent a 10-μM aspartate step-up stimulus at $t = 0$. Prestimulus events are in brown and poststimulus events are in blue. The four events that lead up to the cell’s adaptation are highlighted in green (see SI Materials and Methods for determination of the number of events to adaptation). The 10 events following adaptation and the last 20 poststimulus events used to determine overshoot are highlighted in black and red, respectively (SI Materials and Methods). Exactness is determined from the mean tumble bias of the last 20 poststimulus events (red horizontal line), and the mean prestimulus tumble bias (brown horizontal line). (B) Zoomed-in view of the four events that lead up to adaptation.

Fig. S8. Quantification of adaptation parameters at the single-cell and the population-average levels. (A) Adaptation parameters in response to step-up stimuli. From top to bottom, adaptation time, number of ETA, and normalized overshoot amplitude quantified from the individual cell event traces (blue triangles, mean ± standard error of the mean), population-average event traces (black circles), and population-average time traces (red squares). Schematics representing the step-up stimulus and each of the three behavioral parameters are shown on the left. ETA values were not obtained from population-average time traces. (B) Same as A, for step-down stimuli.
Abruptness and its dependence on cooperative switching and FliM remodeling of the flagellar motors. (A) The effect of motor cooperativity. The schematic describes how the time course in CheA activity during adaptation, $A(t)$, (gray trace, Bottom; CheY-P level is proportional to CheA activity) is mapped into a corresponding motor bias time course, $B(t)$, (blue and red traces, Right) through the sigmoidal motor bias vs. CheA activity function $B(A)$ (red and blue traces, Center). Two different Hill functions are shown, with high Hill coefficient $H$ (blue) and low $H$ (red). At the adaptation time $t_{\text{adapt}}$, the high-$H$ function amplifies changes in $A(t)$ near its steady-state, where its rate of change (represented by the shaded blue area) is small. Conversely, for the low-$H$ function, the rate of change in $A(t)$ at $t_{\text{adapt}}$ is larger (shaded red area), but amplified less. These compensating effects lead to a motor bias time course whose rate of change at $t_{\text{adapt}}$ (its abruptness) is largely independent of $H$. See SI Discussion for more details. (B) The effect of FliM remodeling. The schematic describes how the time course in CheA activity during adaptation, $A(t)$, (gray trace, Bottom; CheY-P level is proportional to CheA activity) is mapped into a corresponding motor bias time course, $B(t)$, (blue and green traces, Right) through the sigmoidal motor bias vs. CheA activity function $B(A)$ (blue and green traces, Center). Two different motor curves are shown, which correspond to the flagellar motor in its steady-state configuration (blue) and remodeled configuration (green). The steady-state motor curve corresponds to the flagellar motor with equilibrium number of FliM units (low FliM), whereas the remodeled motor curve corresponds to the flagellar motor with transiently increased number of FliM units (high FliM). At the adaptation time $t_{\text{adapt}}$, the low-FliM curve amplifies changes in $A(t)$ near its steady-state where its rate of change (represented by the shaded blue area) is small. On the other hand, for the high-FliM curve, the rate of change in $A(t)$ at $t_{\text{adapt}}$ is larger (shaded green area), and hence amplified more. As a result, the motor bias undergoes a more abrupt change in the case of the high-FliM curve. See SI Discussion for more details.
Fig. S10. Possible mechanisms giving rise to the observed adaptation abruptness and overshoot. (A) A schematic of the chemotaxis signaling network. Receptor clusters of size \( N \) modulate the CheA kinase activity in a cooperative manner, influenced by ligand binding and methylation/demethylation by CheR/CheB. CheA phosphorylates CheY into CheY-P, which then diffuses and binds the flagellar motors. The switching of the flagellar motors' rotational direction caused by the binding/unbinding of CheY-P to the FliM units on the motor occurs with cooperativity \( H \). Binding of CheY-P to FliM, at the same time, lowers FliM's affinity to the rest of the flagellar motor. As a result, the number of FliM units on a motor increase over time in the absence of CheY-P. (B) The number of ETA as a function of the receptor cluster size \( N \). Chemotactic response of individual cells to a 100-\( \mu \)M step up in L-aspartate concentration was simulated by numerically solving a stochastic model of the chemotaxis response, and the mean ETA was obtained by fitting exponential functions to the histogram of individual ETA values from 100 cells. Error bars denote the fitting uncertainty. The cooperativity of the flagellar motor switching behavior, \( H \), was set at 10. Black line is a model fit in the form of \( a/N + b \). (Inset) The effect of varying \( H \) on the ETA. The receptor cluster size, \( N \), was set at six. (C) The estimated receptor cluster size \( N \) as a function of the step-up stimulus. \( N \) was estimated from the experimentally measured ETA (Fig. 3E), using the theoretical relation between ETA and \( N \) (as shown in B). Error bars designate the experimental standard error. (D) Numerical simulation of the effect of FliM remodeling on adaptation kinetics. (Top) A step-up stimulus of 100 \( \mu \)M L-aspartate was given \( t = 0 \). (Middle) With FliM remodeling incorporated into the simulation, the number of FliM units changes upon application of the stimulus (red solid line). The black dashed line shows the case of no FliM remodeling. (Bottom) Motor bias displays a more abrupt adaptation, and an overshoot, in the presence of FliM remodeling (red solid line) compared to the absence of FliM remodeling (black dashed line). For details of the numerical simulations see SI Materials and Methods.