Enhancement of Swimming Speed Leads to a More-Efficient Chemotactic Response to Repellent

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Negative chemotaxis refers to the motion of microorganisms away from regions with high concentrations of chemorepellents. In this study, we set controlled gradients of NiCl₂, a chemorepellent, in microchannels to quantify the motion of Escherichia coli over a broad range of concentrations. The experimental technique measured the motion of the bacteria in space and time and further related the motion to the local concentration profile of the repellent. Results show that the swimming speed of bacteria increases with an increasing concentration of repellent, which in turn enhances the drift velocity. The contribution of the increased swimming speed to the total drift velocity was in the range of 20 to 40%, with the remaining contribution coming from the modulation of the tumble frequency. A simple model that incorporates receptor dynamics, including adaptation, intracellular signaling, and swimming speed variation, was able to qualitatively capture the observed trend in drift velocity.

Rod-shaped bacteria, such as Escherichia coli, have evolved to move away from unfavorable chemicals (chemorepellents) and toward regions containing favorable chemicals (chemoattractants). This strategy of movement, or taxis, is known as chemotaxis. The motion is executed with the help of helically shaped locomotive organelles present on the cell surface known as flagella. E. coli responds to the changing environmental conditions by alternating the rotational direction of their flagella. Counterclockwise (CCW) rotation (as viewed from the tip of the flagellum toward the cell body) results in a motion called a run, whereas clockwise (CW) rotation leads to tumbling of the bacteria. By modulating the duration of runs and tumbles, the bacteria achieves a net motion toward chemoattractants or away from chemorepellents. Bacterial transmembrane chemoreceptors help in detection of chemical stimuli in the environment and subsequent transduction of the signal to the cytoplasmic signal transduction system. The probability of CW rotation is controlled by the phosphorylation state of a chemotactic protein, CheY. When phosphorylated, CheY binds to the switch proteins at the flagellar motor in the cytoplasm, leading to increased tumbling. The phosphorylation of CheY is catalyzed by a kinase, CheA, whose activity in turn is regulated by the chemoreceptors. When an attractant binds to the chemoreceptors, the activity of CheA is reduced, leading to long runs. In contrast, the activity is enhanced in case of a repellent, thereby increasing the number of tumbles (1).

In this work, we focused on the response of the bacteria to the chemorepellent NiCl₂. Tso and Adler (2) were the first to quantify the response of E. coli to various chemorepellents. Using both the agarose gel assay and the microchannel assay, they determined the threshold concentration necessary for negative chemotaxis for a range of chemicals with different chemical groups. They showed that the chemotactic response was dependent on the existence of a suitable receptor; in the absence of such receptors, the bacteria showed no response, even at high repellent concentrations. Similar studies on repellents by others have been reported for various chemicals (3). Mao et al. (4) fabricated a sensitive microfluidic device to detect the chemotactic response to both chemoattractants and repellents, such as nickel sulfate and 1-leucine. The device was demonstrated to be sensitive and robust. A microfluidic device based on similar principles capable of maintaining stable gradients was shown to be an alternative experimental protocol to characterize chemotaxis to both attractants and repellents (5). Here, the cells are introduced at one end of the microfluidic channel with cells convected by the background flow along the channel. A stable chemical gradient is set up across the channel, and the extent of chemotaxis is measured in terms of a chemotaxis partition coefficient and a chemotaxis migration coefficient. While the former quantifies the direction of migration of cells, the latter quantifies the number of cells that migrate across the channel. Khan and Trentham (6) analyzed repellent signal processing in E. coli by flash photorelease of leucine and found that the response amplitudes of free-swimming cell populations increased with a step change in leucine concentration. Further, they showed that the motor response time of individual cells correlated with rotational bias but not the cell size. It has been shown that the transmembrane receptor, Tar, is required for chemotaxis to nickel (7). Eisenbach et al. (8) showed that the response to the repellent nickel sulfate is not influenced by changing the cytoplasmic membrane fluidity. Further, they showed that the response did not change for periplasm-void cells, confirming that the receptors for nickel ions are on the cytoplasmic membrane. This was recently reconfirmed by Englert et al. (9), who showed that the Ni²⁺ ion binds specifically to the periplasmic domain of the Tar receptor but not to the periplasmic domain of Tsr. However, the
exact mechanism involved in the sensing of Ni\(^{2+}\) ions is yet to be elucidated.

While most of the studies on chemotaxis that relate cell motility to the intracellular signaling pathway have focused on attractants by relating quantities such as rotational bias and drift velocity to the attractant concentration and its gradients via the known receptor binding characteristics and signaling pathway (10–19), similar studies have not been performed for repellents. Consequently, very little is known about how repellents interact with receptors and what signals they induce in the intracellular pathway (20). Further, the phenotypic response to repellents, i.e., the influence of repellent concentration and gradient on the details of cell motility, including swimming speed and drift velocity, are unknown. In this study, we focused on the latter by establishing controlled gradients of NiCl\(_2\) in microchannels to quantify the chemotactic response in terms of drift velocity and swimming speed over a wide range of repellent concentrations. While the cells migrate away from regions of high concentration of repellents to regions of lower concentrations, detailed analysis revealed that the swimming speed of bacteria increases with increasing concentration of repellent, which in turn enhances the drift velocity. The increased swimming speed was found to contribute about 20 to 40\% to the drift velocity, with the remaining contribution coming from modulation of tumble frequency. Based on the existing mathematical model for attractants, we propose a simple two-state model to describe the receptor dynamics and adaptation, including the variation in the swimming speed in response to repellents. This model successfully predicts the measured drift velocities and demonstrates that the increased swimming speeds play an important role in migration.

**MATERIALS AND METHODS**

**Strains.** *Escherichia coli* wild-type strain RP437 and the Tar deletion mutant (RP2361, referred to here as RP437Δtor), both gifts from J. S. Parkison (University of Utah, USA), were used in experiments.

**Media.** Tryptone (casein enzymic hydrolysate, 66 wt%; sodium chloride 34 wt%) was obtained from Merck. The chemicals KH\(_2\)PO\(_4\), K\(_2\)HPO\(_4\), MgSO\(_4\).7H\(_2\)O, EDTA, and polyvinylpyrrolidone (PVP) were obtained from Sigma-Aldrich. The motility buffer (MB) contained 11.2 g KH\(_2\)PO\(_4\), 4.8 g K\(_2\)HPO\(_4\), 2 g (NH\(_4\))\(_2\)SO\(_4\), 0.25 g MgSO\(_4\).7H\(_2\)O, 1 g PVP, and 0.029 g EDTA in one liter of distilled water. Tryptone medium contained 15 g tryptone in one liter of distilled water. The repellent NiCl\(_2\).6H\(_2\)O (ultrapure) was obtained from Loba Chemie (Mumbai, India) and was added to the motility buffer to obtain various concentrations of repellent.

**Growth conditions.** A loopful of *E. coli* was inoculated into 50 ml of tryptone broth and was allowed to grow for 9 h at 30°C and 200 rpm. Once the bacterial culture reached mid-exponential phase (optical density [OD] \(\sim 0.8\) to 1.0), 5 to 10\% of the inoculum was transferred to the same medium and grown for 3 h to ensure adaptability to the medium. The culture was then centrifuged at 4,000 rpm for 10 min, and the resulting cell pellet was washed gently twice with motility buffer. About 0.02 \(\mu\)l of the cell pellet was used in the capillary experiments.

**Experiment protocol. (i) Growth and death experiments.** Growth experiments were performed in tryptone medium with various concentrations of NiCl\(_2\). The OD was measured using UV spectroscopy at 600 nm at various times, and the specific growth rate (\(\mu\)) was obtained from the slope of logarithm of the OD-versus-time curve. Experiments were also performed to determine the death rate constant. Here, the cells grown in tryptone medium were resuspended in 100 ml of phosphate buffer solution along with various concentrations of the repellent. The viable cell count at various time points was obtained using the methylene blue reduction assay (21). The slope of the logarithm of the viable cell count versus time yielded the death rate constant (\(K\)).

(ii) Measurement of cell motion. Glass microchannels (RT5010), 5 cm long by 1,000 \(\mu\)m wide by 100 \(\mu\)m high (Arte Glass Associates Co., Ltd., Japan), were used for the chemotaxis experiments. An inverted microscope (IX71; Olympus, Japan) fitted with a monochrome camera (Evolution \(\text{VE}^2\) cooled camera; Media Cybernetics, Japan) was used to image the cells. Imaging was performed using dark-field mode of illumination with a 40× objective lens (numerical aperture [NA], 0.75).

To observe the response of *E. coli* to different NiCl\(_2\) gradients, a 4.5-cm-long liquid plug of low repellent concentration or motility buffer was drawn into the microchannel, followed by a 0.5-cm-long liquid plug of a higher concentration of repellent. Cells were introduced into the microchannel by contacting a cell pellet with the mouth of the microchannel at the higher repellent concentration end (Fig. 1). The ends of the microchannel were sealed with wax. The duration of the experiments varied from 15 to 40 min (the latter in plain motility buffer), which ensured that the motility was robust throughout the experiments. Four different repellent gradients, namely, 10 to 0, 50 to 0, 100 to 0, and 100 to 90 were established in the microchannel, where the first number refers to the higher repellent concentration (in micromolar units) in the 0.5-cm liquid plug and the second refers to the lower concentration in the 4.5-cm liquid plug. Experiments were also performed in plain motility buffer and with a uniform concentration of 100 \(\mu\)M NiCl\(_2\). Each experiment was repeated three times on different days to capture the variability.

The movement of the bacteria was recorded at distances of 500, 1,000, 1,500, and 2,000 \(\mu\)m from the edge of the pellet. The images were taken at
frame rates of 21 frames per second (fps). The trajectories of the cells were obtained using a commercial software, Image Pro Plus, and the data were further processed to obtain the swimming speed, drift velocity, and cell orientation from more than 2,000 cells for each condition. The software parameters were set such that only cells within 1 μm of the focal plane were considered. Further, only those tracks were considered where the cell spent at least 0.5 s in the focal plane, thereby ensuring that all out-of-plane motions were ignored by the analysis. A tumble event was identified when the swimming speed of the cell was below half the mean swimming speed and the change in the turn angle was greater than 4° between successive frames (at 21 fps). These conditions were obtained by visual inspection of run and tumble events and are similar to those reported by Alon et al. (22).

The measured average swimming speed of 18.2 ± 7.9 μm/s (average ± standard deviation) and an average turn angle of 71° for RP437 cells dispersed uniformly in a microchannel containing plain motility buffer are close to those observed for the same strain by Saragosti et al. (23), who reported values of 18.8 ± 8.2 μm/s and 69°, respectively. These results confirm the correctness of the method used in the study. For further details on image analysis, see reference 10.

The head rotation speed was determined using a 10× objective (0.30 NA) in conjunction with a dark-field condenser. High-speed imaging of the cells (400 fps using a Hamamatsu ORCA Flash 4.0 V2 camera) revealed light intensity fluctuation over time, caused by its rotating head. Images at a resolution of 512 by 512 pixels were recorded for approximately 12 s over an area of 332 μm by 332 μm containing around 500 cells. Experiments for each condition were repeated over 10 times over three different days to obtain the average power spectrum from about 5,000 cells. Following the method of Martinez et al. (24), we divided the image into equal-sized bins so that each bin contained approximately one cell and determined the power spectrum of the intensity fluctuation of each bin. The power spectrum was then averaged over all bins to reveal the power spectrum for the entire population. Finally, the power spectrum was normalized by the square of the frequency to eliminate contribution of the spectrum will be unaffected by these factors and is expected to give the dominant head rotation speed of the population.

(iii) Measurement of concentration profile. The concentration profile of repellent was determined using a fluorescent technique using a 4× objective (0.13 NA) in the epifluorescence mode. In order to calibrate the intensity of various NiCl₂ concentrations, a 5-cm-long liquid plug of NiCl₂ along with 1 g/liter of fluorescent indicator, Newport Green DCF dipotassium salt (Invitrogen Inc.), was drawn into the microchannel, and the ends were sealed with wax. The fluorescent intensity (505/535 nm) of the indicator gives a quantitative measure of the concentration of Ni²⁺ ions. The fluorescent intensity was obtained for concentrations up to 500 μM (not shown). To obtain the concentration profile in gradients of repellent, a 4.5-cm-long liquid plug of motility buffer was drawn into a microchannel, followed by a 0.5-cm-long-liquid plug of 500 μM repellent. Both the liquid plugs also contained the fluorescent indicator. The evolving concentration profile was measured and it compared well with that predicted by equation 1 (discussed below). Note that the measurements started 2 min after introduction of the liquid plugs and the initial concentration measured at 2 min was taken as the initial condition for solving equation 1.

RESULTS

Growth and death experiments were performed in order to determine the range of repellent concentrations over which the viability is not adversely affected. Figure 2 shows the normalized specific growth rate and the death rate constants at various concentrations of NiCl₂. The specific growth rate shows a typical sigmoidal drop which can be characterized using the Hill equation. The strain RP437 demonstrated a Hill coefficient of 0.44 and half-saturation constants of 0.3 mM. The normalized death rate constant could be characterized by a power law equation (A c^n), where C is the concentration of the repellent. The exponent (n) was close to 0.38 (dimensionless). The crossover point of profiles of the specific growth and death rates gives a quantitative measure of the harmfulness of the repellent. We find that concentrations less than 2.5 mM are conducive to growth with negligible death rates, while higher concentrations are detrimental to the bacteria’s existence, with low growth and high death rates. These results demonstrate that growth and death rates remain unaffected for concentrations less than about 300 μM. Therefore, we performed experiments with a maximum concentration of 100 μM to ensure good motility of cells.

The chemotactic response of E. coli was characterized in terms of drift velocity, swimming speed (or run speed), and angular orientation as a function of the repellent concentration and concentration gradient in the microchannel. In order to characterize the local repellent concentration and gradient, separate experiments were conducted using a fluorescent indicator for nickel ion concentration. The gradient was set up using the same procedure as that for the bacterial experiments (Fig. 1), wherein a liquid plug 0.5 cm long containing repellent was brought into contact with a 4.5-cm-long liquid plug containing motility buffer inside the microchannel. The mixing of the repellent with the motility buffer resulted in a stable gradient. The fluorescence intensity was quantified in time and space under the microscope (see Materials and Methods). Figure 3A and B present the measured intensity 2 and 25 min, respectively, after the gradients were set up. The measured concentration profiles were compared to the predictions obtained from the unsteady-state diffusion equation for the transport of the repellent.
the swimming speed to higher values in the presence of the repellent. Cases was 10 to 14 m/s. The mean swimming speeds for the 100-to-0 gradient were 40 m/s and 37.4 m/s. The standard deviation in all cases was 10 to 14 m/s. The above results clearly indicate a shift in the swimming speed to higher values in the presence of the repellent. The significance of the difference in the average swimming speeds measured in motility buffer on one hand and the repellent on the other can be quantified by the t-test, which yielded a P value of less than 0.0001, indicating that the differences are extremely statistically significant. Along with the standard t-test, we also performed statistical analysis using the Kruskal-Wallis rank sum test in order to determine if there are differences among the population’s median swimming velocities measured in motility buffer and gradients of 10 to 0, 50 to 0, and 100 to 0. Since the null hypothesis that all populations are identical was rejected, with P being much less than 0.01, a Bonferroni-Dunn post hoc test was performed to compare individual groups. The latter is a multiple-comparison test designed to provide an upper bound on the probability that any comparison will be incorrectly found to be significant. The pairwise differences were found to be significant at the 0.05 level. The aforementioned statistical tests are identical to those performed in the past to determine differences in swimming behavior in bacterial populations (27). Figure 4B presents the average swimming speed as a function of cell orientation for the motility buffer and for the three gradients at 500 μm. In all cases, the swimming speed is independent of the orientation with a mean identical to that obtained from the distribution in Fig. 4A. The mean swimming speeds for various concentrations of the repellent are plotted in Fig. 4C and were obtained from the gradient experiments at all four locations (500, 1,000, 1,500, and 2,000 μm). The swimming speeds increase monotonically from 22.1 μm/s to 38.8 μm/s. These results show that the swimming speeds vary in response to NiCl₂ concentration reaching a maximum speed that is 70% higher than that observed in motility buffer. The figure also includes the swimming speed in plain motility buffer, which is similar to that observed for the lowest gradient. Figure 4D presents the angular orientation of the cells for motility buffer and the 50-to-0 gradient at 500 μm. Clearly, the fraction of cells oriented down the gradient is higher in the gradient than in motility buffer. The cell’s orientation is symmetric about the longitudinal axis of the channel, since no gradient exists perpendicular to the length of the channel.

Control experiments were performed with a mutant strain of RP437 lacking the Tar receptor (RP437Δtar) to show that the Tar receptor is involved in the increase in swimming speed when cells are exposed to the repellent NiCl₂. Figure 5 presents the swimming distribution for the mutant strain in motility buffer and in the presence of a repellent gradient (100 to 0), both measured at 500 μm from the cell pellet. Clearly, the two distributions overlap, suggesting that sensing plays a role in the modulation of the swimming speed. The mean swimming speed was about 24 μm/s (also included in Fig. 4C) and was unaffected by the repellent concentration, as expected in the absence of sensing. Note that the average swimming speeds for the mutant strain at all three spatial locations for both motility buffer and repellent gradient (100 to 0) are identical and higher by about 20% than that obtained for strain RP437 in motility buffer. This is expected, since the mutant strain does not sense the repellent. However, the reasons for the slight increase in the swimming speed merely due to the deletion of Tar receptors are not clear, although the control experiments clearly demonstrate the role of sensing in swimming speed modulation.

The increased swimming speed points to a higher rotational speed of the flagellar motor. The motor performance can be determined via either the tethering technique or the bead assay (28).

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (1)
\]

where the repellent concentration, \(C(x,t)\), is a function of the distance from edge of the microchannel (x) and time (t) and where \(D\) is the diffusivity of the repellent. The above equation was solved subject to the measured initial condition at 2 min and zero flux boundary conditions at both ends of the microchannel, as follows:

\[
\frac{\partial C}{\partial x}\bigg|_{x=0} = 0 = \frac{\partial C}{\partial x}\bigg|_{x=L}
\]

where the length of the channel (L) is 5 cm. The repellent concentration profiles at various times were obtained numerically. A diffusivity value of 12.5 × 10⁻⁶ cm²/s was taken for the calculations (25). Figure 3A and B also show, respectively, the initial profile and the predicted profile at 25 min obtained by solving the unsteady-state diffusion equation. The model equation was able to capture accurately the measured concentration profile, thereby demonstrating the suitability of the model predictions in estimating the concentration and concentration gradient prevailing in the microchannel. It should be noted that the variation in the measured gradient is less than 6%, suggesting that the gradient is stable for the entire duration of the experiment (about 25 min). Further, the observed variation in our microchannel is similar to those observed in flow-based microfluidic devices, wherein the difference in the gradient at the inlet and the outlet of the microfluidic channel is less than 10% (26).

Figure 4A presents the comparison of the swimming speed distributions plotted as a fraction of run events when cells were exposed to plain motility buffer and NiCl₂ gradients of 10 to 0, 50 to 0, and 100 to 0 (see Materials and Methods for definition of gradients). The swimming speed presented in the figure was measured at 500 μm from the edge of the bacterial pellet. A run event is counted when a cell is in run mode between two consecutive frames. The distribution in the presence of the motility buffer is narrow and peaks at about 15 μm/s, with a mean value of 20.8 μm/s. While the observed distribution is similar to that of the motility buffer for the lowest gradient, both the peak and the average swimming speed increase for higher gradients. The peak and the mean swimming speeds for the 100-to-0 gradient were 40 μm/s and 37.4 μm/s, respectively. The standard deviation in all cases was 10 to 14 μm/s. The above results clearly indicate a shift in the swimming speed to higher values in the presence of the repellent. The observed variation in our microchannel is similar to those observed in flow-based microfluidic devices, wherein the difference in the gradient at the inlet and the outlet of the microfluidic channel is less than 10% (26).
Since the aforementioned techniques apply to single cells, they are inadequate in assessing the motor performance of a population of cells. In this respect, Martinez et al. (24) demonstrate a high-throughput method involving dark-field flicker microscopy wherein the average power spectrum of the flickering dark-field image of thousands of swimmers yields the average angular speed of the cell head of the population. They further showed that the head rotation speed is linearly related to the flagellar motor performance, so that any change in the flagellar motor speed is directly reflected in the rotation speed of the head. We employed the same technique to determine the head rotation speed for the wild-type strain and the mutant strain lacking the Tar receptor using the dark-field flicker microscopy technique for a large population of cells.

**FIG 4** (A) Distribution of swimming speeds for plain motility buffer (i.e., in the absence of repellent) (*) and for gradients of 10 to 0 (■), 50 to 0 (○), and 100 to 0 (▲) measured at 500 μm from the edge of the pellet. A run event occurs when a cell is in run mode between two subsequent frames (0.047 s apart). The plot is obtained from more than 10,000 run events for each condition. (B) Angular distribution of the swimming speeds. The numbers along the circumference are angles (in degrees), while the numbers 10, 20, 30, and 40 in the radial direction are the swimming speeds (in micrometers per second). The swimming speed is isotropic in all cases, suggesting that the swimming speed is not a function of the swimming direction. The symbols are as described for panel A. (C) Mean swimming speed as a function of the repellent concentration. The mean standard error is 0.1 μm/s, which corresponds to a standard deviation of about 10 to 14 μm/s. Note that the swimming speed for the lowest gradient (10 to 0) is close to that in motility buffer (star symbol on the ordinate). The symbols are as described for panel A. The mean speeds measured in motility buffer and in a gradient of 100 to 0 for RP437Δtar (□) are also shown. (D) Angular distribution of the swimming direction for motility buffer (left) and a repellent gradient of 50 to 0 (right), both measured at 500 μm from the edge of the pellet. Note that a larger fraction of cells swim down the gradient than in motility buffer.

**FIG 5** Distribution of swimming speeds for the mutant strain (RP437Δtar) in motility buffer (■) and in a gradient of 100 to 0 (▲), both measured at 500 μm from the edge of the pellet. A run event occurs when a cell is in run mode between two consecutive frames (0.047 s apart). The plot was obtained from more than 10,000 run events for each condition.
the motility buffer. Figure 6B presents data for the mutant strain where the peak value and the profile overlap for MB and the gradient. This result implies that in the mutant strain, the motor speed is unaffected in the presence of NiCl$_2$. The above results clearly suggest that the Tar sensor plays a role in enhancing the motor speed. Further, the increase in head rotation speed correlates with a concomitant increase in the swimming speed.

Experiments were also performed at high concentrations of NiCl$_2$ but for uniform concentrations (100-100) and very low gradients (100 to 90). Figure 7A presents the percentage increase in swimming speed and drift velocity at different locations for 100-100 (uniform concentration) relative to motility buffer. The swimming speeds for the 100-100 case were much higher than those in motility buffer at both locations, reconfirming the influence of repellent on swimming speed. The increase in the drift velocity was of similar magnitude, suggesting that the increase is

![Figure 6](image-url)

**FIG 6** The power spectrum for the wild type (RP437) (A) and the mutant strain (RP437Δtar) (B) for plug of cells exposed to MB and in a gradient of 100 to 0 of repellent. In both cases, the measurements were made at 500 μm from the cell pellet. The lines are obtained from a log-normal fit to the averaged data from at least 10 different experiments. The arrows indicate the location of the peak in the power spectrum, and the numbers (with standard deviations) indicate the peak head rotation speed. The standard deviation was obtained by determining the peak value for each of the 10 or so experiments and then determining the standard deviation among the peak values. The inset (A) shows the averaged data for RP437 in MB and the corresponding log-normal fit (black line).

![Figure 7](image-url)

**FIG 7** Enhancement in swimming speed (gray bars) and drift velocity (white bars) in a 100-100 (A), 100-to-90 (B), 10-to-0 (C), and 100-to-0 (D) repellent gradient relative to the swimming speed and drift velocity in motility buffer. The observed swimming speeds in the presence of motility buffer at 500 μm and 1,500 μm were 20.8 and 18.7 μm/s, respectively, while the corresponding drift velocities were 1.7 and 0.6 μm/s. The measured values of the swimming speed and the drift velocity for each case are included above the corresponding bar. The latter has not been subtracted from the corresponding values measured in MB. The percent change was obtained from the ratio of change in swimming speed or drift velocity (with respect to that in MB) to the corresponding value in MB.
mainly due to the increase in swimming speed. However, in the presence of a low gradient (but at a high concentration, 100 to 90), the percentage increase in drift velocity relative to that in motility buffer is much larger than the increase in swimming speed, indicating that the observed high drift velocities are due to both increase in swimming speed and modulation of tumble frequency (Fig. 7B). Thus, even a small gradient leads to a significant increase in the drift velocity, with the contribution of the increased swimming speed to the total increase in drift velocity being about 20 to 25%, indicating that the main contribution to the drift velocity is through modulation of tumble frequency. A similar analysis for gradients at lower concentrations, namely, 10 to 0 (Fig. 7C) and 100 to 0 (Fig. 7D), showed a larger increase in drift velocity than swimming speed, which is in line with the previous result. However, the contribution of the increase in swimming speed toward the increase in drift velocity is in the range of 20 to 40%.

**Model description.** While the sensing and adaptation mechanisms have been well characterized for attractants, not much is known about repellents. Therefore, we adopted a simplified form of the two-state model proposed by Inoue and Kaneko (29) to describe the receptor dynamics. The model considers a receptor to be in two functional states, namely, active and inactive, where the former exhibits kinase activity. The activated receptor transmits the signal to the cytoplasmic proteins of which CheA, CheB, and CheY are considered in the model. CheA autophosphorylates, which in turn phosphorylates two other regulator proteins, CheY and CheB. The phosphorylated CheY (CheY-P) activates the flagellar motor switch protein FlIM, causing the motor to turn clockwise, thereby producing a tumble. It is known that the methylation of receptor by CheR and demethylation by CheB controls adaptation behavior of the cell in response to an attractant. However, the adaptive mechanism is not clearly understood in case of repellents. Therefore, a simple phenomenological model equation is used to capture adaptation to repellents. Equations 2 and 3 capture the dynamics of the concentration of the active receptor (T$_A$),

$$\frac{dT_A}{dt} = \frac{kC^n}{k_{Ni} + C^n} - \beta v T_A - \alpha T_A$$

(2)

$$\frac{dv}{dt} = \beta v T_A - \gamma v$$

(3)

where $k$, $k_{Ni}$, $\alpha$, $\beta$, $\gamma$, and $n$ are the relevant parameters that represent the dynamics of the receptors. The active receptor concentration increases in response to repellent concentration ($C$), while the second and the third terms of equation 2 reduce the activity. The feedback for adaptation is provided by equation 3, wherein the dynamics of the variable $v$ is equivalent to the demethylation process. At steady state, the concentration of the active receptors is a constant ($T_A = \gamma/B$), which ensures perfect adaptation to perturbations in repellent concentration.

The rate of increase of CheA-P increases with $T_A$ and decreases with CheY and CheB concentrations (equation 4). Further, CheA-P phosphorylates CheY and CheB with a first-order dephosphorylation (equations 5 and 6, respectively).

$$\frac{dA_p}{dt} = k_1 T_A - 10 (A_p) Y - 10 (A_p) B$$

(4)

$$\frac{dY}{dt} = 100 (A_p) Y - 30 (Y_p)$$

(5)

Here, $A$, $A_p$, $Y$, $Y_p$, $B$, and $B_p$ represent the concentrations of CheA, phosphorylated CheA, CheY, phosphorylated CheY, CheB, and phosphorylated CheB, respectively. Li and Hazelbauer (30) measured the intracellular total concentrations of chemotaxis protein for wild type E. coli, as follows: $A + A_p = 5.3 \mu M; B + B_p = 0.28 \mu M; Y + Y_p = 9.7 \mu M$. Note that setting the left-hand side of equations 2 to 6 to zero yields the steady-state values of all variables.

In order to predict the drift velocity of the cells in gradients of repellent, the CW bias was related to CheY-P concentration via a Hill equation (see Equation S1 in the supplemental material), similar to that used by Yuan and Berg (31). The model incorporates the variation of swimming speed, since the swimming speed varied with gradient (see Table S1 in the supplemental material). Note that in the case of MeAsp gradients, the swimming speed had remained unchanged (10). The rotational diffusivity ($D_r$) calculated by Berg (32) for a sphere, 0.062 rad$^2$/s, was used in the calculation. The distribution of tumble angles was observed to follow a gamma distribution, in agreement with previous studies (see Fig. S1 in the supplemental material) (33). The model also incorporates directional persistence wherein the tumble angle was higher for cells moving down the gradient than for cells moving in the opposite direction (23). This was achieved by fitting the gamma distribution function to the measured tumble angle distribution and using the same for the model calculations (see Table S2 in the supplemental material). The measured tumble angle distribution of cells moving up and down the gradient were used in the model. The details of the swimming speed variation (see Table S1) and tumble angle distribution (see Table S2) along with model equations and numerical implementation are presented in the supplemental material.

To explain the observed chemotactic response, we use the above-described two-state model to predict the drift velocity for different repellent gradients (see Fig. S2 in the supplemental material for the algorithm). Although the swimming speed is a function of the repellent concentration ($C$), the swimming speed variation at the three spatial locations (500, 1,000, and 1,500 $\mu M$) for a fixed gradient was less than 10%. Thus, the measured average swimming speeds of 22.5, 33.9, and 38.8 m/s at 500 $\mu M$; 45.2, 51.2, and 53.3 m/s at 1,000 $\mu M$; and 66.6, 71.2, and 73.2 m/s at 1,500 $\mu M$ were used to predict the drift velocity for gradients of $-0.001 (10 to 0), -0.005 (50 to 0)$, and $-0.01 (100 to 0)$ $\mu M/\mu M$ (see Table S1 in the supplemental material). Further, the measured drift velocity in plain motility buffer and attributed to the cell diffusion and oxygen gradient (1.7 $\mu M$/m) for a fixed material for the algorithm). Although the swimming speed is a function of the repellent concentration ($C$), the swimming speed variation at the three spatial locations (500, 1,000, and 1,500 $\mu M$) for a fixed gradient was less than 10%. Thus, the measured average swimming speeds of 22.5, 33.9, and 38.8 m/s at 500 $\mu M$; 45.2, 51.2, and 53.3 m/s at 1,000 $\mu M$; and 66.6, 71.2, and 73.2 m/s at 1,500 $\mu M$ were used to predict the drift velocity for gradients of $-0.001 (10 to 0), -0.005 (50 to 0)$, and $-0.01 (100 to 0)$ $\mu M/\mu M$ (see Table S1 in the supplemental material). Further, the measured drift velocity in plain motility buffer and attributed to the cell diffusion and oxygen gradient (1.7 $\mu M$/m) were used to predict the drift velocity for gradients of $-0.001 (10 to 0), -0.005 (50 to 0)$, and $-0.01 (100 to 0)$ $\mu M/\mu M$ (see Table S1 in the supplemental material).
The mathematical model incorporates the observed increase in swimming speed to predict the drift velocities. Figure 8B presents the predicted drift velocity in both the presence and absence of swimming speed enhancement. It is clear that in the absence of the increased swimming speed, the predicted drift velocities are much lower than the observed drift velocity, confirming the role of swimming speed variation in the enhancement of drift velocity. Finally, the drift velocity for the mutant strain at the three locations for the 100-to-0 gradient was identical to that in MB for the same mutant strain, with values of 2.5, 2.1, and 1.7 μm/s at distances of 500, 1,000, and 1,500 μm, respectively, from the cell pellet (data not shown). Note that the observed drift velocity for the mutant strain is due only to the cell diffusion and oxygen gradient, since the sensing mechanism is absent.

DISCUSSION
The pioneering work of Tso and Adler (2) established for the first time negative chemotaxis in response to various chemicals, including metal ions. Using different experimental protocols, they determined the threshold concentration needed for the response. The response in turn was due to the presence of chemoreceptors which sense those particular chemicals. They thus concluded that the harmfulness of a chemical is not responsible for negative chemotaxis. Our work indicates that while the motility of the cells is robust up to 100 μM, the viability of the cells decreases drastically at much higher concentrations. These results are in agreement with those of Englert et al. (5), who found that concentrations lower than 300 μM NiSO₄ had little effect on the aerobic growth of RP437 cells. Tso and Adler (2) reported that the threshold concentration for detecting the Ni²⁺ ions is about 10 μM, which is consistent with our observations, since the measured maximum drift velocity for the lowest gradient (10 to 0) was marginally higher (2 μm/s) than that observed for the motility buffer (1.7 μm/s). Note that the baseline drift velocity observed in motility buffer is due to the cell diffusivity and oxygen effects (10, 11). More recently, Mao et al. (4) used a sophisticated microfluidic setup to quantify the response of RP437 to various concentrations of Ni²⁺ ions, and they too did not observe a response below 10 μM.

The current understanding of chemotaxis in E. coli has been obtained via detailed studies of cell motion in response to α-methyl aspartate (MeAsp), a nonmetabolizable analogue of aspartate, and serine, which are sensed by the Tar and the Tsr receptors, respectively (34, 35). Most studies have reported constant swimming speeds in gradients of MeAsp (10, 33), though recent experiments by Ahmed and Stocker (36) in steep gradients of MeAsp have shown that the swimming speeds can increase by as much as 30%. Similar responses have also been observed in gradients of serine (11, 33). Recently, we demonstrated that the Trg receptor, which senses glucose, plays a role in the variation of swimming speed even in uniform concentrations (zero gradient) of 2-deoxy-d-glucose (2Dg), a nonmetabolizable analogue of glucose (37). The current study demonstrates that swimming speed variation can also occur in the presence of repellents, suggesting that as in the case of 2Dg, the increase is due to sensing alone. As was demonstrated in case of the Trg chemoreceptor, in the current study we demonstrated that even the Tar receptor is capable of modulating the swimming speed. A mutant strain devoid of the Tar receptor did not show this effect, confirming the role of the sensor.

The increased swimming speed can be due to a higher rotational speed of the flagellar motor, which would increase the thrust of the motor, leading to higher swimming speeds. The increased speeds may in addition lead to changes in the flagellar bundle geometry, such as a better alignment of the bundle along the cell head, leading to smoother runs (38). One may therefore speculate that sensing of a repellent leads to a higher flagellar motor speed. To this end, we employed the flickering dark-field microscopy technique to measure the rotation speed of the head, which is a linear function of the flagellar bundle rotation speed (24). In the presence of the repellent gradient, the cell increases its flagellar motor speed, leading to much higher swimming speeds than in MB. These changes in either the swimming speed or the head rotation speed were not observed in the mutant strain lacking the Tar receptor. The above results clearly demonstrate, for the first time, a link between sensing and the motor speed. Recently, Demir and Salman (39) showed that the chemotactic receptor plays a key role in modulating the intracellular pH, leading to a variation in swimming speed. It is possible that a change in the intracellular pH and/or the membrane potential may also occur in response to the repellent to bring about the
observed change in swimming speeds, though more work is required to confirm this.

A simple model that incorporates receptor dynamics, including adaptation, intracellular signaling, and swimming speed variation, was able to qualitatively capture the observed trend in drift velocity. In the absence of the swimming speed variation, the model predicts drift velocities lower than those observed, thereby highlighting the relevance of the swimming speed variation. Our studies indicate that the increased swimming speed leads to an enhancement of about 20 to 40% in the drift velocity and over and above that due to modulation of tumbling frequency. In conclusion, our studies show not only that E. coli modulates the tumble frequency to achieve chemotaxis but also that the above-described process may be accompanied by variations in swimming speed, leading to an overall enhancement in the drift velocity.

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