

Speed-dependent chemotactic precision in marine bacteria

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Edited by Victor Sourjik, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, and accepted by Editorial Board Member Herbert Levine June 10, 2016 (received for review February 10, 2016)

Chemotaxis underpins important ecological processes in marine bacteria, from the association with primary producers to the colonization of particles and hosts. Marine bacteria often swim with a single flagellum at high speeds, alternating "runs" with either 180° reversals or ~90° "flicks," the latter resulting from a buckling instability of the flagellum. These adaptations diverge from Escherichia coli's classic run-and-tumble motility, yet how they relate to the strong and rapid chemotaxis characteristic of marine bacteria has remained unknown. We investigated the relationship between swimming speed, run-reverse-flick motility, and high-performance chemotaxis by tracking thousands of Vibrio alginolyticus cells in microfluidic gradients. At odds with current chemotaxis models, we found that chemotactic precision-the strength of accumulation of cells at the peak of a gradient—is swimming-speed dependent in V. alginolyticus. Faster cells accumulate twofold more tightly by chemotaxis compared with slower cells, attaining an advantage in the exploitation of a resource additional to that of faster gradient climbing. Trajectory analysis and an agent-based mathematical model revealed that this unexpected advantage originates from a speed dependence of reorientation frequency and flicking, which were higher for faster cells, and was compounded by chemokinesis, an increase in speed with resource concentration. The absence of any one of these adaptations led to a 65-70% reduction in the populationlevel resource exposure. These findings indicate that, contrary to what occurs in E. coli, swimming speed can be a fundamental determinant of the gradient-seeking capabilities of marine bacteria, and suggest a new model of bacterial chemotaxis.

ocean | motility | run-reverse-flick | chemotaxis | chemokinesis

otility is an essential component of chemotaxis (1), the ability of organisms to sense chemical gradients and swim toward more favorable conditions, for example, to find dissolved or particulate nutrients, colonize and infect hosts, or evade noxious substances (2). Most of our knowledge of bacterial chemotaxis comes from the study of Escherichia coli, a bacterium that inhabits the lower intestine of warm-blooded animals and swims using multiple (4-10) flagella (2). Counterclockwise (CCW) rotation of all motors causes the flagella to bundle and to propel the cell into a nearly straight "run" at 10-30 µm/s (2). A change in swimming direction occurs when one or more motors switch to clockwise (CW) rotation, disrupting the flagellar bundle and leading to a nearly random reorientation or "tumble" (2). The key to success in E. coli's chemotaxis strategy is the bacterium's ability to control the switching frequency between CCW and CW flagellar rotation, giving rise to the well-known run-and-tumble swimming pattern (2). In this process, the swimming speed remains largely unchanged, and despite the bacterium's ability to sense mechanical stimuli (3), it is generally held that its chemotaxis depends only on the sensing of chemical stimuli. Consequently, the swimming speed has not been considered to affect the ability of cells to retain position in favorable regions of a gradient and does not enter into classic models of E. coli's chemotaxis, beyond simply allowing proportionately faster climbing of a gradient (4).

Marine bacteria often exhibit higher chemotactic speed and precision than E. coli, showing faster gradient climbing and better steady-state accumulation at resource peaks (5, 6). These characteristics make marine bacteria a valuable model system to understand the limits of chemotaxis in microorganisms (7). For example, the marine bacterium Pseudoalteromonas haloplanktis was observed to respond up to 10-fold more rapidly than E. coli to resource pulses (5). The coastal marine bacterium Vibrio alginolyticus was found to accumulate threefold faster and sevenfold more tightly than E. coli toward serine (6). These high chemotactic performances are certainly caused at least in part by high swimming speeds, a common adaptation in marine bacteria (5, 8). However, a quantitative analysis has shown that simply rescaling E. coli's speed to that of marine bacteria is not sufficient to explain the differences in their chemotactic performance (7), pointing at the need to better understand how the latter depends on the swimming pattern overall.

Analysis of a collection of ~600 motile species of marine bacteria has shown that the majority (>90%) have a single, polar flagellum (9). The swimming patterns of marine bacteria have long been known to differ from *E. coli*'s run-and-tumble swimming (6, 10) by not displaying classic tumbles (8). However, only recently has a clearer picture emerged for the motility pattern of marine bacteria (6) and the underlying biomechanics (10), based on

Significance

Our understanding of bacterial chemotaxis, a fundamental nutrient-seeking strategy in the microbial world, mainly derives from *Escherichia coli*. However, it has become clear that marine bacteria evolved fundamentally different chemotaxis adaptations, often allowing them to accumulate at resource peaks more tightly and rapidly than *E. coli*. We studied the origin of this high chemotactic precision and found that it lies in an unexpected dependence of chemotaxis on swimming speed: faster cells have substantially higher precision, counter to all known models of chemotaxis. We elucidate this finding through a combination of single-cell tracking of thousands of marine bacteria in microfluidic gradients and a mathematical model of chemotaxis that explicitly accounts for swimming speed in the chemotaxis pathway.

Author contributions: K.S., F.M., and R.S. designed research; K.S. performed research; K.S. and F.M. contributed new reagents/analytic tools; K.S. analyzed data; and K.S., F.M., and R.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. V.S. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10. 1073/pnas.1602307113/-/DCSupplemental.

detailed observations of *V. alginolyticus. V. alginolyticus* alternates between forward and backward runs, and reorients by 180° reversals or 90° (on average) "flicks" (6). The flick arises from a large, off-axis deformation of the flagellum (6) caused by the buckling of the hook (10), a ~100-nm-long, flexible structure that connects the flagellar filament to the rotary motor. The probability of a flick occurring during each run–reverse–flick cycle, $P_{\rm F}$, is swimming-speed dependent, because the compressive load that causes buckling increases with speed (10), resulting in a sharp transition of the motility pattern from "run–reverse" to "run–reverse–flick" with increasing swimming speed (10). Thus, in contrast to *E. coli*, the motility pattern of *V. alginolyticus* is swimming-speed dependent. However, how this dependence on speed affects chemotactic performance has remained unknown.

A second important adaptation of several species of marine bacteria is chemokinesis (11, 12), the ability to modulate swimming speed in response to the concentration of a chemical (*SI Text*). The origin of chemokinesis remains largely unknown, and *E. coli* is generally believed to not display chemokinesis (13) [although some recent findings have questioned this model (14)]. Chemokinesis can enhance chemotaxis by increasing the speed of the cells, and thus the rate at which they climb chemical gradients (11), and also shifting the distribution of speeds within a population into a regime where flicks are predominant.

By focusing on these two speed-dependent motility adaptations in marine bacteria—flicking and chemokinesis—we study the chemotactic performance of *V. alginolyticus* as a function of the cells' swimming speed. In contrast to chemotaxis in *E. coli* and to current mathematical predictions, we find that the steadystate chemotactic accumulation of *V. alginolyticus* in a chemical gradient is speed dependent. A mathematical model of chemotaxis in marine bacteria that captures these observations suggests that swimming speed is an important parameter in their chemotaxis pathway, and helps explain the fast swimming speeds often observed among bacteria from the ocean.

Results

Dependence of Chemotaxis on Swimming Speed. A steady linear concentration profile ("linear gradient") of the amino acid serine, a chemoattractant for *V. alginolyticus* (6), was generated in a purposely engineered microfluidic device (Figs. S1 and S2, and *Materials and Methods*). The serine concentration in the microchannel varied from 100 to 400 nM (Fig. S1), corresponding to conditions representative of dissolved free amino acids in the ocean (15), and resulting in a steady linear gradient of 0.5 nM/µm. We identified and tracked individual cells via video microscopy and determined the chemotactic response of the population by quantifying the steady-state chemotactic distribution of cells along the serine gradient (Fig. 1). This revealed a strong accumulation of cells in the direction of increasing serine concentration, with 60% of the cells accumulated in the 100-µm region at the top of the 600-µm-wide gradient (Fig. 1*C*, black).

Single-cell tracking allowed us to quantify the natural variation of swimming speed within the population (Fig. 1A) and revealed that the distribution of speeds is well approximated by a gamma function (shape parameter, 8.8; scale parameter, 4.3). The average swimming speed V of each cell was computed by averaging its instantaneous speed over the duration of its trajectory. This approach is justified because the magnitudes of swimming speed fluctuations in a trajectory relative to the mean are moderate (on average, $39 \pm$ 6%). We could thus bin cells in the analysis based on their swimming speed and therefore separately consider the chemotactic distribution of cells having different speeds (Fig. 1 A and B). We further increased the dynamic range of the swimming speed by using a range of sodium concentrations in the solution, $[Na^+] =$ 3–600 mM, exploiting the fact that the motor of V. alginolyticus is driven by transmembrane sodium gradients (10), so that the cells' swimming speed (averaged over the population) increases



Fig. 1. The chemotactic precision of V. alginolyticus increases with swimming speed. (A) Distribution of swimming speeds, p(V), within a V. alginolyticus population in the presence of a serine gradient (defined in Fig. S1). The swimming-speed distribution (markers) is well fitted by a gamma distribution (line). The shaded boxes denote two speed bins: V = 8.4 \pm 1.2 μ m/s (teal) and V = 38.9 \pm 1.3 μ m/s (magenta) (B and C). (B) Single-cell trajectories at steadystate accumulation for cells belonging to the two speed bins identified in A. Note the considerably tighter accumulation of the faster cells (magenta) in the region of high serine concentration (toward x = 0). (C) Steady-state bacterial concentration profiles, B(x), for cells belonging to different speed bins (see Inset for speed color coding) in the same serine gradient as in B. Data were collected over a range of sodium concentrations (3-600 mM) and trajectories were binned into 12 speed bins based on the average swimming speed. V. Each of the 12 speed bins is 2.5-6.1 µm/s wide, except for the highest speed bin (40.5 µm/s wide), and contains the same number of trajectories (4,643). The bacterial distribution B(x) for each speed bin was normalized to a mean of 1. The black line denotes the overall bacterial concentration profile before speed binning. (C, Inset) Bacterial distributions are well fitted by an exponential function, as illustrated for B(x) pertaining to the two speed bins shown in B. (C. second Inset) The exponential fit is good for all speed bins, as demonstrated by R^2 values consistently close to 1. (D) Chemotactic precision length scale, L, obtained as the decay length scale of the exponential fits to B(x) for each of the 12 speed bins shown in C. A low value of L corresponds to a tight accumulation, hence to high chemotactic precision. (E) Chemotactic migration coefficient, CMC, for each of the 12 speed bins shown in C. A high value of the CMC corresponds to a tight accumulation, hence to high chemotactic precision. Grav symbols represent the same data, where speed binning was performed separately for each sodium concentration, [Na⁺]. The CMC values for *E. coli* responding to a gradient of α -methylaspartate (from 20 to 80 μ M) in the same microfluidic device (Fig. S1) are shown for comparison, divided into five speed bins (black open triangles). For C-E, horizontal error bars denote SDs of each speed bin. Where not visible, horizontal error bars are smaller than symbols.

with the sodium concentration, according to $V = V_0$ [Na⁺]/(14.9 + [Na⁺]), where $V_0 = 47.5 \ \mu m/s$ (10). Overall, we quantified the chemotaxis of 55,718 individual cells: in the analysis, each cell was assigned to 1 of 12 speed bins, ranging from 8.4 ± 1.2 to $54.1 \pm 5.9 \ \mu m/s$, based on its swimming speed. In the following, we refer to this process as speed-based binning and to different ranges of speed as speed bins, for brevity. Different speed bins had different widths so that each bin contained the same number of cell trajectories, for statistical robustness in the computation of swimming kinematics.

This speed-based binning revealed a stark difference in the steady-state chemotactic accumulation of cells swimming at different speeds (Fig. 1*B*). Faster cells accumulated more tightly than slower cells, as seen by comparing a slow-speed bin, $V = 8.4 \pm 1.2 \mu$ m/s, and a fast-speed bin, $V = 38.9 \pm 1.3 \mu$ m/s (Fig. 1*B*). To quantitatively determine the dependence of the strength of accumulation on the swimming speed, we computed the bacterial

distribution along the chemoattractant gradient, B(x), separately for each speed bin (Fig. 1*C*). The distributions B(x) corresponding to the 12 speed bins clearly demonstrate that faster cells have higher chemotactic precision, accumulating more tightly in the high-serine region than slower cells.

Two metrics were used to quantify the speed dependence of chemotaxis in V. alginolyticus. First, the cell distribution profile B(x) for each speed bin was fitted by an exponential, $B(x) = B_0$ $\exp(-x/L)$, where x is the direction along the chemoattractant gradient, L is the exponential decay length, and B_0 is a normalization constant (Fig. 1C and Inset). The exponential distribution is the steady-state solution of classic formulations of the bacterial transport equation (13) (Keller-Segel's formulation). We found that an exponential distribution is a very good fit for all 12 speed bins ($R^2 >$ 0.95; Fig. 1C, inner Inset). Thus, the exponential decay length scale, L, provides a robust metric for the chemotactic precision: the smaller L, the tighter the accumulation. For V. alginolyticus, L decreased from 187 μ m at $V = 8.4 \pm 1.2 \mu$ m/s to 54 μ m at $V = 27.8 \pm 0.95 \mu$ m/s, indicating a tighter accumulation with increasing speed in this range, and remained nearly constant for higher speeds (Fig. 1D). For comparison, the full population (Fig. 1C, black) had $L = 88 \,\mu\text{m}$.

As a second metric of the speed dependence of chemotaxis, we computed the chemotactic migration coefficient (16), CMC = $(\langle x \rangle - W/2)/(W/2)$, a widely used parameter in the chemotaxis literature (16) that measures the displacement of a population's center of mass, $\langle x \rangle = \int x B(x) dx$, from the central point in the gradient (x = W/2), with $W = 600 \mu m$ being the spatial extent of the gradient (here, the microchannel's width). CMC = 0 signifies no chemotactic response, CMC = 1 is maximum attraction, and CMC = -1 is maximum repulsion. We found that the CMC increased from 0.44 at $V = 8.4 \pm 1.2 \ \mu m/s$ to 0.74 at $V = 27.8 \pm$ $0.95 \mu m/s$, and saturated for higher speeds (Fig. 1*E*), confirming the trend revealed by the chemotactic precision length scale, L (Fig. 1D) (for the full population, the CMC was 0.59). We could rule out the possibility that the change in accumulation strength with speed was due to physiological changes arising from different sodium concentrations, as the same speed dependence of the CMC was obtained when cells were binned by speed separately for each sodium concentration (Fig. 1E, gray symbols). Based on these results, in the subsequent analysis we consider a low-speed regime comprising speed bins below $V = 30 \,\mu$ m/s and having lower chemotactic performance, and a high-speed regime comprising speed bins above $V = 30 \mu m/s$ and having higher chemotactic performance.

Whereas it is intuitive that chemotactic cells obtain a benefit from swimming faster-because they can climb resource gradients more rapidly (Fig. S3)-the tighter steady-state accumulation of faster cells at the peak of a resource gradient is unexpected and represents an additional benefit of enhanced speed. This speed dependence is absent in E. coli, as we confirmed by repeating experiments with this bacterium exposed to a gradient of 20–80 μ M α -methylaspartate (Fig. 1*E*, black symbols), a commonly used chemoattractant for E. coli, in a range of concentrations known to elicit strong chemotaxis (17). Although E. coli displayed a smaller dynamic range of swimming speeds ($V = 11.9 \pm 2.1$ to $28.8 \pm 3.2 \,\mu$ m/s), this range was sufficiently wide to consider five speed bins and establish that the chemotactic precision was independent of swimming speed (CMC = 0.42 ± 0.02). Furthermore, the fact that the maximum CMC of E. coli responding to optimal concentrations of one of its strongest attractants was on the lower end of the CMC of V. alginolyticus responding to nanomolar serine concentrations underscores the strong chemotactic capabilities of marine bacteria reported in literature (5, 6).

Strong Chemotaxis at High Swimming Speeds Results from Reduced Randomness. To understand the origin of the observed speed dependence of chemotaxis, we first quantified population-level swimming statistics—the random motility D and the chemotactic velocity $V_{\rm C}$ —as a function of swimming speed. The chemotactic

velocity measures the net speed at which cells move up the gradient, and the translational diffusivity (also called "random motility") measures the intrinsic randomness of the swimming pattern and limits the level of accumulation a population can achieve. The precision with which a population of microorganisms accumulates at the peak of a resource gradient is determined by the competition between these two properties, with higher $V_{\rm C}$ enhancing chemotactic precision and higher D reducing it. As predicted by an advection-diffusion model of bacterial transport (13), in a linear chemoattractant gradient the steady-state bacterial distribution is exponential, $B(x) = B_0 \exp(-x/L)$, with the length scale $L = D/V_{\rm C}$ (thus, our exponential fit to observed distribution profiles in Fig. 1C). To gain insights into the observed speed dependence of the chemotactic precision length scale L (Fig. 1D), we thus separately consider how D and $V_{\rm C}$ vary with speed (Fig. 2).

We computed the random component of motility, D, for each speed bin based on a theoretical formulation recently derived for run-reverse-flick motility (18), yielding $D = (V^2/6) [(f + 4D_R)/(f + 2D_R)^2]$. The reorientation frequency f was quantified from individual bacterial trajectories (Fig. 3) and the rotational diffusivity D_R (measuring the gradual change in orientation during runs due to Brownian rotation and off-axis propulsion) was obtained from a resistive force model (11) (*SI Materials and Methods*). Two features stand out from the computed values of the random motility. First, D increases quadratically with speed, $D \sim V^2$, in the high-speed regime ($V > 30 \mu m/s$), but linearly in the low-speed regime, where $D \sim V$ (Fig. 24, *Inset*, and Table S1). Thus, at high swimming speeds, the effect of random motility in hindering tight accumulation is not as strong. This feature, as will be seen below, is at the heart of the high chemotactic precision of fast *V. alginolyticus* cells.

Second, run-reverse-flick swimming is characterized by a low value of the random component of motility. This can be seen by comparing D for V. alginolyticus and E. coli, where the latter was calculated as $D = V^2/(3f(1 - \alpha))$ (2), with f = 1/s being *E. coli's* tumbling frequency and $\alpha = 0.33$ being persistence, defined as the mean of the cosine of the reorientation angle between runs. When the comparison is performed at natural swimming speeds [47.5 µm/s for V. alginolyticus (10); 19.7 µm/s for E. coli (Fig. 1E) (2)], the random motility of V. alginolyticus ($D = 160.8 \ \mu m^2/s$ for the speed bin $V = 47.5 \pm 1.4 \,\mu\text{m/s}$) was 17% smaller than that of *E. coli* ($D = 193.1 \,\mu\text{m}^2/\text{s}$), despite the 2.4-fold higher speed. When the comparison is performed at E. coli's natural swimming speed (using the speed bin $V = 19.2 \pm 2.2 \,\mu\text{m/s}$ for V. alginolyticus), the random motility of V. alginolyticus ($D = 44.5 \ \mu m^2/s$; Fig. 2A) was 77% smaller than that of E. coli ($D = 193.1 \ \mu m^2/s$). This comparison confirms the prediction of a recent model (19) that runreverse-flick motility-owing to the presence of reversals-has lower randomness than run-and-tumble motility, and the reduction in the value of D determined here (77%) is quantitatively even



Fig. 2. The random and directional components of swimming in *V. alginolyticus* in a serine gradient (Fig. 51). (*A*) The random motility coefficient, *D*, and (*B*) the chemotactic velocity, V_{c} , as a function of swimming speed, *V*. Data were collected over a range of sodium concentrations (3–600 mM) and binned by speed. *Insets* show the same data in log-log format, with black lines denoting the *V* and V^2 slopes for reference (Table S1). For both panels, horizontal error bars denote SDs of each speed bin.



Fig. 3. The swimming kinematics of *V. alginolyticus* reveal a low-speed ($V < 30 \mu m/s$) and a high-speed ($V > 30 \mu m/s$) regime. CMC (black circles), probability of flicking, P_F (blue upright triangles), reorientation frequency, f (red squares), and flicking frequency, f_F (brown inverted triangle), as a function of speed *V* in a serine gradient (as in Fig. S1). The green dashed line marks the threshold, $V = 30 \mu m/s$, between the low-speed and the high-speed regimes. Horizontal error bars denote SDs of each speed bin.

larger than that predicted theoretically (19) (50%). This reduced diffusivity contributes to explain the much higher chemotactic precision of *V. alginolyticus* compared with *E. coli*, both at natural swimming speeds (CMC = 0.79 for *V. alginolyticus* swimming at 47.5 μ m/s; CMC = 0.42 for *E. coli* swimming at 19.7 μ m/s) and for the same swimming speed (CMC = 0.66 for *V. alginolyticus* swimming at 19.7 μ m/s, obtained by interpolation of the CMC values as a function of swimming speed) (Fig. 1*E*).

The chemotactic velocity, $V_{\rm C}$, was computed as $V_{\rm C} = D/L$ using the value of the length scale L determined from the exponential fit to the population accumulation profile (13) (Fig. 1D). This revealed a quadratic dependence of $V_{\rm C}$ on the swimming speed, $V_{\rm C} \sim V^2$, over the entire range of swimming speeds (Fig. 2B, Inset and Table S1). This result is in line with predictions from recent theoretical models for E. coli and V. alginolyticus (18, 20) and, to the best of our knowledge, represents the first experimental quantification of the dependence of the chemotactic velocity on the swimming speed. The steady-state chemotactic velocities ranged from $V_{\rm C} = 0.2-3.5 \ \mu \text{m/s}$ (Fig. 2B), corresponding to a relative chemotactic velocity V_C/V of <10%. The ratio V_C/V is a measure of the directionality of the response: less than 10% of the swimming speed of V. alginolyticus is on average directed up the gradient at steady state. This level of directionality is on par with that of *E. coli*, which often is in the range of 5-10% (21). Although the relative chemotactic velocity can be higher for particular gradients and types of chemoattractants [up to 35% (21)], our observations indicate that V. alginolyticus' higher performance in accumulating at resource peaks was not due to a higher chemotactic velocity but rather to a lower random motility (Fig. 2A). This conclusion is also in line with a recent theoretical model, which predicts that run-reverse-flick swimmers have lower diffusivity but the same chemotactic velocity as run-and-tumble swimmers, for the same swimming speed (19).

Reduced Randomness at High Speeds Is Caused by an Enhanced Reorientation Frequency. To understand the changeover between a quadratic and a linear dependence of random motility on swimming speed, which is at the origin of the speed-dependent chemotactic precision of marine bacteria, we quantified singlecell-level swimming kinematics—the reorientation frequency fand the probability of flicking $P_{\rm F}$ —as a function of swimming speed (Fig. 3). The reorientation frequency, f, was computed by identifying both the reversals and the flicks from trajectories (*SI Materials and Methods*). A modulation of the reorientation frequency is typically at the origin of the cells' response to chemical gradients (2, 4). Here, however, we also quantified f in the absence of gradients to focus on its dependence on swimming speed, V (Fig. S4). We found that f increased linearly with V in the low-speed regime ($V < 30 \,\mu$ m/s) and was independent of V in the high-speed regime ($V > 30 \mu m/s$) (Fig. S4*B*), and that the same trend occurred in the presence of a serine gradient (Fig. S4*A*), signifying that *V. alginolyticus* modulates its reorientation frequency as a function of swimming speed.

This result explains the observed speed dependence of the random motility. This can be seen by considering the theoretically predicted random motility for run-reverse-flick swimming, $D = (V^2/6) [(f + 4D_R)/(f + 2D_R)^2]$ (ref. 18; described above), which in the limit of negligible rotational diffusion $(D_R << f)$ becomes $D = (V^2/6f)$. This relation predicts $D \sim V$ in the low-speed regime, where $f \sim V$, and $D \sim V^2$ in the high-speed regime, where f is independent of V: both predictions successfully describe the observed dependence of D on V (Fig. 24, Inset, and Table S1).

Not only the frequency of all reorientations, f, depended on swimming speed, but also and even more so the frequency of flicks, $f_{\rm F}$, which displayed an approximately eightfold increase from $f_{\rm F} = 0.095$ flicks per s at $V = 13.3 \pm 1.7$ µm/s to $f_{\rm F} = 0.78$ flicks per s at $V = 47.5 \pm 1.4 \,\mu\text{m/s}$ (Fig. 3, brown). The frequency of flicks is given by $f_{\rm F} = f/2 \times P_{\rm F}$, where $P_{\rm F}$ is the probability of flicking (the probability that the onset of a forward run is immediately followed by a flick; SI Materials and Methods). The factor of 2 arises because flicks can occur at most every other reorientation (i.e., only after the start of a forward run) (10). The eightfold increase in $f_{\rm F}$ with speed was due in part to a twofold increase (from 1.1/s to 2.3/s) in the overall reorientation frequency, f, and mostly to a fourfold increase in $P_{\rm F}$ (from 18% to 68%) (Fig. 3). The increase of $P_{\rm F}$ with V is in line with previous results in the absence of chemical gradients (10) and was here also observed in the presence of a gradient (Fig. 3, blue). The functional dependence of both $P_{\rm F}$ and $f_{\rm F}$ on V mirrors that of the reorientation frequency, f, and the chemotactic migration coefficient, CMC (Fig. 3, black), further highlighting the connection between the rate and nature of reorientations and the chemotactic precision.

Our data suggest that the dependence of reorientation frequency f, probability of flicking $P_{\rm F}$, and consequently flicking frequency $f_{\rm F}$ on the swimming speed V is largely independent of gradient sensing. For $P_{\rm F}$, this conclusion is supported by the biomechanics of flicking, which results from a buckling instability that is independent of chemical gradients, as well as by a comparison of the dependence of $P_{\rm F}$ on V in the presence (Fig. 3) and absence (10) of a serine gradient. For f, the conclusion is also supported by comparing observations in the presence (Fig. S4A) and absence (Fig. S4B) of a serine gradient: even in homogeneous conditions, f depends strongly on V (Fig. S4B), and, small differences in absolute values notwithstanding, this dependency is similar with and without a gradient. Binning the data by sodium concentration allowed us to exclude the potential for this observation to originate from physiological effects of different sodium concentrations (10) (Fig. S4; gray symbols). This observation supports the conclusion that swimming speed itself is an important determinant of the probability of flicking and the reorientation frequency, irrespective of the presence or absence of a chemical gradient. Because the modulation of the reorientation frequency (in E. coli, the "tumbling rate") is the basis of bacterial chemotaxis (2, 4), this finding suggests that swimming speed plays a key role in the chemotaxis of V. alginolyticus and possibly of other marine bacteria, extending the current paradigm of chemotaxis based on chemical information alone.

Chemokinesis Shifts the Swimming Speed of a Population into the High-Speed Regime. The motility repertoire of V. alginolyticus includes a further, important adaptation—chemokinesis—that is intimately intertwined with the observed speed dependence of swimming kinematics (Fig. 3) and chemotactic precision (Fig. 1 C and E). The quantification of the distribution of speeds within a population of V. alginolyticus from single-cell trajectories revealed strong and rapid chemokinesis, in the form of an overall shift toward higher speeds (Fig. 4, Figs. S5 and S6, and SI Text).



Fig. 4. Chemokinesis shifts the population's swimming speed toward the high-speed regime. (A) Distribution of swimming speeds, p(V), within a *V. alginolyticus* population in the absence (cyan) and presence (blue) of a serine gradient (as in Fig. S1). (B) Temporal evolution of the swimming-speed distribution after the uniform addition of 5 μ M glucose (*SI Materials and Methods*). Different shades of purple and magenta correspond to different times after glucose addition (see *Inset* for time color coding). (B, *Inset*) Population-averaged increase in swimming speed following 5 μ M glucose addition, expressed as a percentage of the speed at time 0. Horizontal error bars denote the width of each time window. For both A and B, the green dashed line marks the speed threshold $V = 30 \mu$ m/s, experiments were performed at a sodium concentration of 600 mM (typical of natural ocean conditions), and each curve is based on at least 2,300 trajectories.

Beyond the obvious effect of chemokinesis in enhancing chemotactic speed by accelerating gradient climbing (11), we hypothesized that chemokinesis in *V. alginolyticus* can significantly impact chemotaxis because a change in swimming speed affects all of the swimming kinematics, including the reorientation frequency and the probability of flicking (Fig. 3), and thus the cells' random motility (Fig. 2*A*). Indeed, the observed chemokinetic speed increases are sufficient to push a sizeable fraction of the population from the lowspeed regime ($V < 30 \,\mu$ m/s) into the high-speed regime ($V > 30 \,\mu$ m/s) (Figs. 3 and 4, green dashed lines). For example, the addition of 0.5 μ M serine increased the fraction of cells in the population swimming at $V > 30 \,\mu$ m/s from 39% to 70% (Fig. 44). Chemokinesis is thus implicated in the strong chemotactic response of *V. alginolyticus*, because faster swimming results in higher chemotactic precision.

Speed-Dependent Chemotaxis Considerably Enhances Resource Exposure. To understand how the different motility adaptations of V. alginolyticus affect its chemotactic performance, we developed a mathematical model of chemotaxis that, in contrast to E. coli's (Fig. 5A), explicitly accounts for swimming speed, V, in the chemotaxis pathway (Fig. 5B). The model is agent-based (Materials and Methods) and considers variable reorientation frequency ("block R"), chemokinesis ("block C"), and flicking ("block F"), each of which depends on V (Fig. 5B). The model successfully captured the observed chemotactic precision in a linear chemoattractant field (Figs. S7A and S8B). We then applied it to Gaussian-shaped chemoattractant fields (Fig. S8 C and D) that mimic those occurring in the dissolved organic matter field in the ocean in the wake of sinking marine snow particles or around phytoplankton cells. We determined the relative contribution of each motility adaptation to the population-level resource exposure, by running the model for "in silico knockout mutants" (each lacking one of the chemotaxis adaptations) and comparing with the in silico wild-type cells having full chemotactic functionality (Fig. 5C and Fig. S8 and SI Text).

The absence of individual adaptations resulted in both a slower migration up the gradient (Fig. S84) and a lower precision at steady state (Fig. 5C and Fig. S8 *B–D*). For the linear gradient used in the experiments, inhibiting chemokinesis (Δ C), flicking (Δ F), or reorientation frequency modulation (Δ R) led to 5.9–38.4% decrease in the resource exposure compared with in silico wild-type cells (Fig. 5C). The percent reduction for the Δ C knockout (5.9%) was comparable to the experimental value (6.8%; *SI Text*). The impact of speed-dependent chemotaxis increases considerably in more realistic, Gaussian-shaped resource

landscapes. For a 1D Gaussian field, the three mutants had a 31.7-37.2% decrease in resource exposure compared with wildtype cells (Fig. 5*C*). For a 2D Gaussian field, the decrease was even larger: 35.0-58.0% when chemokinesis was caused by serine and 64.8-69.1% when it was caused by glucose (Fig. 5*C*). Comparison of the 1D and 2D scenarios suggests that the difference will be even larger in a 3D patch—for example, a phytoplankton phycosphere (22). Overall, these results show that the enhanced chemotactic precision can make a substantial difference in the ability of cells to exploit resource hot spots.

Discussion

Our results show that, contrary to what happens in the enteric bacterium E. coli, swimming speed and its modulation are important determinants of the chemotactic response in the marine bacterium V. alginolyticus. We identified two chemotactic regimes based on speed. At low speeds ($V < 30 \mu m/s$), the bacteria's diffusivity D scaled linearly with V and their chemotactic velocity $V_{\rm C}$ scaled quadratically with V, so that the chemotactic precision length scale $L = D/V_{\rm C} \sim 1/V$ decreased with increasing speed (Fig. 1D and Table S1). In this regime, cells displayed constant run lengths due to the increase in the reorientation frequency with speed (Fig. 3, red) and their chemotactic precision increased with speed, as measured by the increase in CMC with V (Fig. 1*E*). In contrast, at high speeds $(V > 30 \text{ }\mu\text{m/s})$, both D and $V_{\rm C}$ scaled quadratically with V, so that $L = D/V_{\rm C}$ remained constant with speed (Fig. 1D and Table S1). In this high-chemotactic-performance regime, run lengths increased with speed, whereas the reorientation frequency was speed independent (Fig. 3, red) (2), and the cells' chemotactic precision saturated, as indicated by the constancy of the CMC with V (Fig. 1E). In summary, fast cells have in principle a large random motility $(D \sim V^2)$ and should thus disperse more around a chemoattractant peak. However, fast V. alginolyticus cells offset this increase in random motility by increasing their reorientation frequency ($D \sim$ 1/f), and thereby effectively reduce the dispersion and increasing their chemotactic precision.

Intriguingly, the distribution of swimming speeds in a *V. alginolyticus* population under natural sodium conditions and in the absence of chemoattractant gradients sits astride of the $V = 30 \mu m/s$ speed threshold separating the low- from the high-speed regimes (Fig. 4*A*, cyan). This observation suggests that the speed distribution and its modulation in the presence of chemical gradients result from a trade-off between the nutrient uptake benefits from chemotaxis and the energetic cost of locomotion, a cost–benefit framework previously suggested for chemotaxis in turbulent flows (23). Because uptake will increase with residence time in resource-rich regions, by



Fig. 5. A model of speed-dependent chemotaxis predicts a considerable increase in resource exposure. (*A* and *B*) System view of chemotaxis shown as block diagrams for (*A*) *E. coli* and (*B*) *V. alginolyticus.* In *E. coli*'s classic pathway, the reorientation frequency *f*—whose modulation enables gradient climbing—is determined solely by the sensed ligand concentration L_C (block R). In contrast, our observations suggest the presence of additional feedbacks in *V. alginolyticus*, including the ligand-dependent modulation of the speed *V* (chemokinesis; block C), the speed-dependent load on the flagellar hook that governs the probability of flicking P_F (block F), and the speed-dependent modulation of the reorientation frequency (block R). All of these elements involve the swimming speed *V*. (*C*) Population-averaged resource exposure of in silico mutants (color-coded as in *B*), expressed as a percentage of the resource exposure of in silico wild-type cells (black bar). For each case, four scenarios were evaluated, as described by labels over the bars.

being able to localize with greater precision at the resource peak, fast cells will have a fitness benefit over slow cells. At the same time, the energetic cost of swimming increases quadratically with the swimming speed (23), making fast swimming very costly relative to the potential benefits of chemotaxis in the often resource-poor marine environment (23). In this context, the cells' active ability to increase speed in favorable resource conditions—i.e., chemokinesis (Fig. 4)—represents a desirable strategy to explore the environment with reduced motility cost in the absence of nutrients, while activating the speed enhancement that yields the strongest chemotactic performance when a favorable chemical environment is sensed.

Our observation supports this cost-benefit hypothesis, for two reasons. First, the observed chemokinetic speed enhancement shifts a considerable fraction of the population from below to above the speed threshold (Fig. 4), resulting not only in a faster transient response to gradients (11) but also in a tighter steady-state accumulation of cells at the resource peak (Fig. 1 *C-E*). Second, the observed speed modulation is rapid, with one-half of the chemokinetic speed enhancement of a population occurring over 2–4 min (Fig. 4B and Fig. S5). Although some nutrient hot spots in the ocean are briefer than this, many last in the order of 10 min or more (5, 22, 23), indicating that chemokinesis can be advantageous also at the single resource patch.

Our data on the speed dependence of the swimming kinematics raise the question of whether and how bacteria sense their swimming speed (*SI Text*). Irrespectively of the origin of speed sensing, we propose that *V. alginolyticus* integrates information on its speed in its chemotaxis pathway as an additional system input to regulate reorientation frequency (Fig. 5B). The success of our model in capturing the experimental observations (Fig. S7A) lends support to the hypothesis that speed is both a system input and output of the chemotaxis pathway (Fig. 5B), and highlights the need to better understand, at the molecular level, both gradient sensing and speed sensing in *Vibrios*.

The results presented here reshape our understanding of bacterial motility in the ocean by demonstrating the role of swimming speed, its effect on reorientation frequency, and its modulation through chemokinesis, in determining the precision and speed of chemotaxis. The role of these motility adaptations on the ability of bacteria to exploit gradients has received only limited attention, largely owing to their absence in *E. coli*. The frequent occurrence of these chemotaxis elements among marine bacteria then suggests that speed-dependent chemotaxis may not be

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limited to *V. alginolyticus* but might be prevalent among sea microbes (11). Together with the large advantage in resource exposure afforded by the increase in chemotactic precision (Fig. 5*C*), this evidence suggests that this augmented form of chemotaxis, in which cellular decision-making is based on both chemical information as well as the cell's own speed, might be pervasive among marine bacteria.

We surmise that the observed speed dependence of gradient utilization among marine bacteria is related to the defining features of the marine resource landscape at the microscale, which is characterized by small, often ephemeral patches, pulses, and gradients of chemicals (8), as well as ubiquitous fluid flow that both stirs chemical resources (23) and influences bacterial motility (8). A quantitative link between the specific behavioral adaptations reported here and the features of the marine resource landscape remains to be established, and points more in general at the need for the development of an optimal foraging theory for bacteria. The observation that marine bacteria use a form of chemotaxis that is speed dependent demonstrates a previously unidentified, potentially widespread element of bacterial chemotaxis, highlights the rich adaptations in the spatial behaviors of marine bacteria, and calls for a better understanding of the ecosystem consequences of these behaviors.

Materials and Methods

Hydrogel-Based Microchannel and Cell Tracking. Both the chemotaxis and the chemokinesis experiments were performed in a hydrogel-based microfluidic device (Fig. S1) (*SI Materials and Methods*). The hydrogel agarose was used at 2% (wt/vol) concentration in milliQ water to create diffusion-permeable walls between adjacent microfluidic channels (Fig. S1). Bacteria were imaged at 22 frames per second by phase contrast microscopy (Nikon Ti-E; 20x, 0.45 N.A.).

Computational Model of Chemotaxis. An agent-based model was used to integrate in a general model of chemotaxis (24) the experimentally observed, speed-dependent motility adaptations of *V. alginolyticus.* Each agent swims in a 2D landscape made of a 1D linear, 1D Gaussian, or 2D Gaussian chemoattractant field (*SI Materials and Methods*). The population-level resource exposure was computed by weighting the chemotactic response for each speed bin with the percentage of cells in that speed bin.

ACKNOWLEDGMENTS. We thank Jeffrey Guasto for discussions. This work was supported by a Samsung Scholarship (to K.S.), NIH Grant 1R01GM100473 (to R.S.), a Wellcome Trust-University of Edinburgh Institutional Strategic Support Fund (to F.M.), and Gordon and Betty Moore Microbial Initiative Investigator Award 3783 (to R.S.).

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Supporting Information

Son et al. 10.1073/pnas.1602307113

SI Materials and Methods

Experiments and Analysis.

Cell culturing. Vibrio alginolyticus YM4 strain was cultured overnight in VC medium [0.5% (wt/vol) polypeptone, 0.5% yeast extract, 0.4% K₂HPO₄, 3% (wt/vol) NaCl, 0.2% glucose], diluted 1:100 into VPG medium [1% polypeptone, 0.4% K₂HPO₄, 3% (wt/vol) NaCl, 0.5% glycerol] (6), and grown to late-exponential phase $(OD_{600} = 0.5)$. Cells were then washed and resuspended in TMN motility medium [50 mM Tris·HCl (pH 7.5), 5 mM MgCl₂, 5 mM glucose, 300 mM NaCl plus KCl] to change the final sodium concentration, [Na⁺], which for different experiments was in the range of 3-600 mM. This was done to exploit the sodiumdependent swimming speed of V. alginolyticus, whose flagellar motor is driven by sodium gradients (10). The difference in $[Na^+]$ was replaced with potassium to maintain osmolarity, a common approach found to be devoid of other physiological consequences (10). PDMS microchannel fabrication and hydrogel injection. Both the chemotaxis and the chemokinesis experiments were performed in a hydrogel-based microfluidic device (Fig. S1), which was fabricated in two steps: (i) polydimethylsiloxane (PDMS) microchannel fabrication, and (ii) hydrogel injection.

Microchannels were fabricated using standard soft lithography techniques with a mold prepared by depositing SU-8 photoresist (MicroChem Corporation) on silicon wafers and patterning channel reliefs via photolithography. The mold was silanized with trichloromethylsilane (Sigma-Aldrich) to prevent PDMS (Sylgard 184 Silicone Elastomer Kit; Dow Corning) from adhering to the master, especially between the hydrogel-trapping pillars (Fig. S1), and to ease the demolding process. PDMS prepolymer (10:1 mixture of base and curing agent of Sylgard by Dow Corning) was cured by baking at 65 °C for >1 h. PDMS microchannels were then cut and bonded to a clean glass slide via plasma treatment and incubated on a hot plate at 100 °C for 1 h.

The hydrogel agarose was used at 2% (wt/vol) concentration in milliQ water to create diffusion-permeable walls between adjacent microfluidic channels (Fig. S1). Liquid agarose was injected between arrays of trapping pillars made of PDMS through channel C (Fig. S1). Two features fabricated downstream of the inlet for the hydrogel (inlet C1)—a serpentine and a reservoirserved as visual indicators to guide the agarose injection process. Agarose was slowly injected through the inlet port C1 using a syringe pump at a constant flow rate (2-5 µL/min) and the injection process was continuously monitored using an inverted microscope (Nikon Ti-E microscope) by following the front of the meniscus, to ensure accurate creation of the hydrogel wall. Hydrogel injections were conducted on a temperature-controlled stage to prevent liquid agarose from solidifying too rapidly during the sequential injection of liquid agarose in the serpentine, reservoir, branching channels, and hydrogel walls. After completing the injection step, milliQ water was injected into channels A, B, and D before experiments to prevent trapping of air pockets in between the PDMS pillars.

Gradient creation. For all of the chemotaxis experiments, the source channel (A) carried 500 nM serine and the sink channel (B) carried buffer (0 nM serine), both flown at a flow rate of 5 μ L/min (Fig. S1). This resulted in the formation of a steady linear serine concentration profile, *C*(*x*), increasing from 100 to 400 nM across the width of the test channel (D) where bacteria were located (Fig. S1).

For the steady-state chemokinesis quantifications, the source channel (A) carried 50, 100, or 500 nM serine (Fig. S6) and the sink channel (B) carried buffer (0 nM serine). For the transient-phase chemokinesis experiments (Fig. 4*B* and Fig. S5), 5 μ M

serine or glucose were uniformly added in the source and sink channels.

Cell imaging and tracking. Swimming bacteria were imaged at channel middepth at 22 frames per s by phase contrast microscopy (Nikon Ti-E microscope; 20×, 0.45 N.A. objective) using a digital camera (Andor Zyla; 6.5μ m/pixel). All analyses were performed in Matlab (The Mathworks) using in-house, automated software to track cells, and reconstructed trajectories were smoothed using a second-order Savitztky–Golay filter (window size, 182 ms). Based on these trajectories, we binned trajectories by their mean speed, as described before (10).

Identification of reversals and flicks. Reorientations were identified as sharp changes in direction or "kinks" in the trajectories. *V. alginolyticus* alternates between forward and backward swimming, in a "run–reverse–flick" motility pattern (6). Every second reorientation is a "reversal," a change in swimming direction narrowly distributed around 180°, which results from the reversal in the direction of swimming. Every other reorientation may also be a reversal, but has a certain probability $P_{\rm F}$ of instead being a "flick" (10), a change in swimming direction distributed around a mean of 90° (6).

Two simultaneous criteria were used to identify reorientations, as described previously (10): (i) a high rate of change of swimming direction and (ii) a low instantaneous swimming speed. First, to identify changes in the swimming direction, at every time point along a trajectory we calculated the dot product of the swimming velocities before and after that point (directional cosine). We then identified as reorientation events all local minima in the directional cosine (equivalent to local maxima in the instantaneous angular speed) that additionally fell below a threshold value of 0.985, corresponding to a directional change of at least 10°. Second, to identify reorientation events having a change in angle below the 10° detection threshold, we used swimming-speed information, because any reorientation is expected to be accompanied by a brief reduction in swimming speed. Based on this second criterion, we identified all local minima in the instantaneous swimming speed that are below 50% of the mean speed of the trajectory. The absolute reorientation angle, $\Delta \theta$, defined as the angle between the swimming velocities before and after a reorientation, and the reorientation frequency, f, were then quantified from all trajectories containing at least one reorientation (to compute $\Delta \theta$) or at least two reorientations (to compute f). After classifying reorientations as either the flicks or reversals, the probability of flicking was quantified as the relative occurrence of flicks among all of the reorientations, multiplied by 2 to account for the flicks that can only occur after a backward run.

Rotational diffusion. The trajectories of swimming bacteria are affected by rotational diffusion. The value of the rotational diffusion coefficient during runs, $D_{\rm R} = 0.035 \text{ rad}^2/\text{s}$, was calculated based on a resistive force model (11) that accounted for both the cell body (3.2 µm long and 1.2 µm wide) and the helical flagellum (contour length, 4.6 µm; pitch, 1.5 µm) of the same strain of *V. alginolyticus* previously reported (10).

Resource exposure. To quantify the consequences of different levels of chemotactic precision on potential nutrient uptake ("resource exposure"), we used the CMC values (Fig. 1*E*).

The population-averaged resource exposure, $N_T = \int_0^{V_{\text{max}}} CMC(V) \cdot P(V)dV$, was computed as the weighted average of the CMC values over all speed bins (with weights proportional to the number of cells in each speed bin), both with and without the chemokinetic speed enhancement (Fig. 4A). Here, P(V) is

the swimming-speed distribution and V_{max} is the maximum swimming speed observed in the experiments (Fig. 4A).

Computational Model of Chemotaxis. To capture the chemotactic behavior of V. alginolyticus, we used a modeling framework based on cellular automata (or individual based model). In the model, each cell is referred to as an "agent." In the absence of more detailed information about the molecular mechanisms of signaling events governing chemotaxis in V. alginolyticus, we adapted the model of Jackson (24). We modeled the individual agents to implement the block schemes illustrated in Fig. 5B and to proceed in one of two modes: either a "forward run" or "backward run," representing in a real cell a CCW and CW rotation of the polar flagellum (as seen from behind), respectively. The agent runs in a straight trajectory in each of these modes, onto which random noise in orientation is added to mimic rotational diffusion ($D_{\rm R} = 0.035 \text{ rad}^2/\text{s}$). For simplicity, we further assumed that reorientations in between runs are instantaneous (both reversals and flicks), that reversals are perfect inversions of swimming direction (180°), and that all flicks are 90° reorientations (6). Based on these considerations, in our modification of Jackson's model, we modeled V. alginolyticus' forward and backward swimming as Escherichia coli's runs and V. alginolyticus' reorientations (either reversals or flicks) as E. coli's tumbles.

As in the study by Jackson, we set the probability that at a generic time *t* an agent will experience a reorientation within a time interval Δt as follows:

$$P_t = \frac{\Delta t}{\tau},$$
 [S1]

where the mean run time can be expressed as follows:

$$\tau = \tau_0 e^{\alpha_C \frac{\overline{dP_b}}{dt}},$$
 [S2]

with

$$\overline{\frac{dP_b}{dt}} = \frac{1}{T_m} \int_{-\infty}^t \frac{dP_b}{dt'} e^{\frac{t'-t}{T_m}} dt'$$
[S3]

and

$$\frac{dP_b}{dt} = \frac{K_{\rm D}}{\left(K_{\rm D} + C\right)^2} \frac{dC}{dt},$$
[S4]

where *C* is the chemoattractant concentration in the extracellular environment, T_m is a time constant of the bacterial system (taken to be 0.1 s), τ_0 is the mean run time in the absence of concentration gradients, that is, the average unbiased run time, α_C is a constant of the system (taken to be 30 s), P_b is the fraction of a cellular protein surface receptor bound by the substrate, dP_b/dt is the weighted time rate of change of P_b , and K_D is the half-saturation constant of the surface receptor binding to the chemoattractant (here, serine).

Parameter identification. The chemotaxis parameters for *V. alginolyticus* responding to serine remain unknown; therefore, we had to identify them using other approaches, described in the following.

Reorientation frequency. For the reorientation frequency, f, we have direct observational data as a function of speed from our analysis. In the model, we implemented the speed-dependent reorientation frequency (Fig. 3) by fitting a phenomenological model of the following form:

$$f(v) = \left(\frac{\eta}{1 + e^{\zeta(v - v_t)}} + \theta\right)^{-1},$$
 [S5]

to the observations, where parameters of best fit were found to be $\eta = -0.3942$ s/µm, $\zeta = -0.2019$ s/µm, $v_t = 18.88$ µm/s, and $\theta = 0.8452$ s/µm.

Flicks. In the model, flicks are implemented as instantaneous reorientations [neglecting the ~10-ms interval between the reversal and the flick (10)] with a reorientation angle of $\pm 90^{\circ}$ (with equal probability). Consistent with prior observations, flicks could occur in the model only after a backward-to-forward transition (6, 10). To determine whether an agent flicked or reversed in that case, a uniformly distributed random variable was compared with the speed-dependent probability of flicking $P_{\rm F}$, which is approximated by a logistic function, $P_{\rm F} = 0.055 + 0.72/[1 + e^{-0.25(v-36)}]$, determined previously (10). This approach summarizes the contribution of block F to the chemotaxis pathway in Fig. 5*B*.

Chemokinesis. Chemokinesis was implemented in the model as an instantaneous speed enhancement of 30% as a result of increased chemoattractant availability (Fig. S6), in line with a previous mathematical model (11). Therefore, each agent has, at each time point, a speed v(x,y,t) that depends on the local concentration of chemoattractant c(x,y) according to the following rule:

$$v(x,y,t) = \begin{cases} v_i \cdot 1.3 & \text{if } c(x,y) \ge c_{\mathrm{T}} \\ v_i & \text{if } c(x,y) < c_{\mathrm{T}} \end{cases}$$

where $c_{\rm T}$ is the concentration of chemoattractant above which we observed a chemokinetic speed enhancement in our experiments (50 nM; Fig. S6), and v_i is the speed each agent is initialized to at the beginning of the simulation (to represent the different speed bins observed experimentally). This approach reflects the experimental observations both in the presence and absence of a serine gradient (Fig. 4*A*).

Sensing. The parameters of the sensing model determine the dynamics of the block R (Fig. 5B). Here, our ability to determine T_m, K_D , and α_C is limited by the lack of information on the molecular mechanism governing chemotaxis in V. alginolyticus. To overcome this limitation, we identified the values of these parameters using a combination of information from the literature and parameter fitting. In particular, based on previous results for marine bacteria (25), we constrained T_m to the range 0.1–1 s and $K_{\rm D}$ to the range 1–10 μ M. Within these two constrains, a value of T_m (0.1 s) was chosen based on the experimentally observed run time, which is typically in the order of 0.1 s (Fig. 3), and a value of K_D (10 µM) was chosen based on a recent model of Vibrio corallilyticus (11). After fixing these two sensing parameters, we heuristically determined the remaining parameter, α_C (30 s), so as to minimize the discrepancy between the predicted CMC (Fig. S7A) and the experimentally measured CMC (Fig. 1E). Importantly, although the specific value of CMC predicted by the model depends as expected on the specific choice of parameters, the predicted speed dependence of the CMC was robust to moderate changes in the parameters over realistic ranges.

Simulation of the trajectories of individual agents. With the chosen parameters, the model numerically integrated the trajectories of individual agents swimming in (i) a linear, steady chemoattractant gradient, directly mimicking the microfluidic setup used in the experiments (Fig. S1); (ii) a 1D Gaussian chemoattractant field; and (iii) a 2D Gaussian chemoattractant field. For each case, 3,000 agents were initially distributed uniformly with random orientations in (i) a 600-µm-wide channel for the 1D linear gradient case, with the chemoattractant field directly replicating

the one established in the microfluidic setup used for experiments (from 100 to 400 nM; Fig. S1); (ii) a 3,000-µm-wide channel for the 1D Gaussian case, with a maximum chemoattractant concentration of 266 nM; and (iii) a $3,000 \times 3,000$ -µm square domain for the 2D Gaussian case, with the same maximum chemoattractant concentration of 266 nM. The mean and the SD were 1,500 and 450 µm, respectively, for the 1D and 2D Gaussian profiles. The initial swimming speed of each agent was chosen randomly from the range of speeds observed (Fig. 4A, cyan). Agents in the simulations did not interact with each other and they reflected in a random direction upon hitting hard boundaries on the left and right sides of the domain in the 1D simulations and on all four boundaries in the 2D simulations. Agents were constantly affected by rotational diffusion, responsible for a random reorientation component in the swimming trajectories (as described above).

Simulation data analysis. The analysis of the simulations was done as follows.

The 1D linear chemoattractant field. Trajectories were integrated for 7,000 s, a time found to be sufficient for agents to reach a steady spatial distribution, B(x) (Fig. S7B). Then the analysis followed the same steps as in the experimental data: we binned the bacterial distributions along the gradient by swimming speed, using the same upper and lower bounds of speed as in the experiments, and for each distribution B(x), we computed the CMC (Fig. S7A). In addition to in silico wild-type cells with full chemotactic functionality, we also simulated in silico knockout agents, for which one or more of the functions in Fig. 5B were removed in the model. Relative comparisons of the CMC values between in silico wild-type cells and in silico knockout mutants (Fig. S8B) were made using the same set of parameters identified above. All of the simulations reached steady state within 7,000 s, as for the in silico wild-type cells.

From the model runs, normalized steady-state bacterial distributions, B(x), and the corresponding CMC values of agents swimming at 10 different speeds (300 trajectories each) were plotted (Figs. S7A and S8B) by averaging over the last 35 s in the 7,000-s-long simulations (Fig. S7B). The population-level resource exposure (Fig. 5C) was computed as for the experimental results (*SI Text*). Furthermore, in the simulations, we made direct comparisons between in silico wild-type cells and in silico knockout mutants based on the CMC values (Fig. S8B), which were weighted by the speed distribution P(V) to obtain a population-level resource exposure, as $N_T = \int_0^{V_{max}} CMC(V) \cdot P(V) dV$ (Fig. 4).

For the simulations, we further computed the accumulation time, $T_A(V)$, based on the full time course of the transient response (e.g., Fig. S7B). $T_A(V)$ is defined as the time taken by the CMC to reach one-half of the maximum value of the CMC of in silico wild-type cells having the same swimming speed but full chemotactic functionality (Fig. S8A). The population-averaged accumulation time, $\langle T_A \rangle$, was then computed by weighting $T_A(V)$ by the speed distribution, P(V), as $\langle T_A \rangle = \int_0^{V_{max}} T_A(V) \cdot P(V) dV$.

The 1D and 2D Gaussian chemoattractant fields. Having obtained good agreement between the model and the experimental observations (Fig. 1 C and E, and Fig. S7A) for the case of a 1D, linear chemoattractant field, we applied the model to 1D and 2D Gaussian chemoattractant fields, which more realistically capture the main features of resource hotspots in marine environments (22). Trajectories were integrated for 1,000 s. This timescale was chosen based on a recent study showing that the chemotactic accumulation of bacteria in the microenvironment surrounding individual phytoplankton cells enriched in dissolved organic matter lasts in the order of 10–20 min (22). The CMC, used in the linear gradient case, is not applicable as a metric to quantify the magnitude of the chemotactic response in Gaussian chemical profiles. Instead, we used the chemotactic index, $I_{\rm C}$, following previous literature (11). $I_{\rm C}$ measures the enhancement in the cell concentration within the central region of the Gaussian (a 500- μ mwide band for the 1D, Gaussian case; a 500- μ m-diameter circle for the 2D Gaussian case), relative to the cell concentration outside that area, minus 1. $I_{\rm C} = 0$ thus corresponds to a uniform cell distribution (that is, no chemotaxis).

The analysis then followed largely the same steps as described above for in silico wild-type cells and in silico knockout mutants. From the model runs, the I_C values of agents swimming at eight different speeds (375 trajectories each) were plotted by averaging over the last 100 s in each simulation and compared between in silico wild-type cells and in silico knockout mutants (Fig. S8 *C* and *D*). The population-level resource exposure (Fig. 5*C*) was computed by weighting the chemotactic response for each speed bin (Fig. S8 *C* and *D*) with the percentage of cells in that speed bin from the experimentally measured speed distribution P(V)(Fig. 4), as $N_T = \int_0^{V_{max}} I_C(V) \cdot P(V) dV$.

SI Text

Chemokinesis. Several species of marine bacteria exhibit chemokinesis. *Pseudoalteromonas haloplanktis* was found to increase its speed by 23% in response to algal exudates (26), the coral pathogen *V. coralliilyticus* (11) increases its speed by up to 48% when exposed to coral mucus, and *Shewanella putrefaciens*, *Deleya marina*, as well as an enriched assemblage of marine bacteria increased their speed by ~20% in response to various amino acids (12). Chemokinesis can enhance chemotaxis by increasing the swimming speed of the cells and thus the rate at which they climb chemical gradients (11), as demonstrated by a mathematical model based on observations of *V. coralliilyticus* (11).

In the presence of a serine gradient ($0.5 \text{ nM/}\mu\text{m}$; Fig. S1), we observed a 33% increase in the population-averaged speed with respect to a population swimming in the absence of serine (Fig. 4*A*). Similar and sometimes higher speed increases were observed for a range of serine gradients and for different sodium concentrations (Fig. S6). Chemokinesis was also observed in the absence of a chemical gradient, with the spatially uniform addition of 5 μ M serine causing a 20% increase (Fig. S5), whereas up to a 82.3% increase was observed for other attractants such as glucose (Fig. 4*B*). The level of chemokinetic speed enhancement observed in this study (Fig. S6) is comparable to those reported in other marine bacteria (11, 26).

The population-averaged resource exposure (*SI Materials and Methods*), defined here as the mean serine concentration experienced by the population and a determinant of nutrient uptake, indicates that, by reducing the chemotactic precision, the absence of chemokinesis (Fig. 4A) resulted in a 6.8% reduction in the resource exposure of the population.

We also found that the chemokinetic speed increase was rapid. This is shown for example by the temporal dynamics of the distribution of speeds after a uniform addition of 5 μ M serine (Fig. S5), where a 17% increase in the population-averaged swimming speed occurred within 127 s (and a 20% increase overall), or a 5 μ M glucose addition (Fig. 4*B*), where a 58% increase in speed occurred within 226 s (and an 82% increase overall). Therefore, chemokinesis not only boosts chemotaxis because of the enhanced chemotactic speed from the quadratic dependence of the chemotactic velocity $V_{\rm C}$ on the swimming speed V (Fig. 2*B*), as previously found for example in *V. corallilyticus* (11), but also lifts a considerable fraction of *V. alginolyticus* cells from the low-speed to the high-speed regime and thereby improves their chemotactic precision (Fig. 1 *C–E*), owing to the dependence of the reorientation kinematics on the swimming speed (Fig. 3).

A Model of Speed-Dependent Chemotaxis. To understand how the different elements of *V. alginolyticus*' motility combine to determine its chemotactic response, we developed an agent-based model that incorporates all our experimental observations (Figs. 3 and 4) as inputs (*SI Materials and Methods*). In the absence of

species-specific molecular information on gradient sensing in *V. alginolyticus*, we used a general model of chemotaxis (24) as our starting point, in which each bacterium swims in a 2D land-scape containing a spatially heterogeneous chemoattractant concentration. In classic models of chemotaxis, mainly developed for *E. coli*, the ligand concentration $L_{\rm C}$ measured over time is the only control parameter of the chemotaxis pathway (4) and is processed by what we call a "reorientation frequency block" (R) that yields the reorientation (or, in *E. coli*, "tumbling") frequency, *f*, as the pathway's output (Fig. 5*A*: $L_{\rm C} \rightarrow {\rm R} \rightarrow f$).

Our experimental observations of V. alginolyticus led us to augment this model by including the swimming speed, V, as both an input and an output in the chemotaxis model (Fig. 5B and SI Materials and Methods). The model accounts for (i) the chemokinetic behavior, by including a modulation of the swimming speed based on the resource concentration (the "chemokinesis block," C, in Fig. 5B: $L_C \rightarrow C \rightarrow V$; (ii) the variable reorientation frequency, which depends not only on the sensed ligand concentration $L_{\rm C}$ (Fig. 5B: $L_{\rm C} \rightarrow {\rm R} \rightarrow f$), as in the classic pathway (4), but also on the swimming speed (through the "reorientation frequency block," R, in Fig. 5B: $V \rightarrow R \rightarrow f$; and (iii) the linear dependence on swimming speed of the propulsive load, which determines the buckling of the flagellar hook (10) and thus the second output of the system, the probability of flicking (the "flick block," F, in Fig. 5B: $V \rightarrow F \rightarrow P_F$). The model thus implements the run-reverseflick motility pattern, when one also imposes that all reorientations that are not flicks are reversals, as observed experimentally (6, 10). We ran the model for 3,000 bacteria swimming in a linear chemoattractant field (SI Materials and Methods), directly mimicking the microfluidic setup used in experiments (Fig. S1) and confirmed that it successfully captured the experimentally observed speeddependent chemotactic precision of V. alginolyticus (Fig. S7). Furthermore, the predicted distributions B(x) were accurately fitted by exponentials, as in the experiments (Fig. S7A, Insets).

In Silico Knockout Mutants. How do chemokinesis, flicking, and reorientation individually contribute to shaping the chemotactic response we observe in V. alginolyticus? In the absence of a convenient way to genetically manipulate our model organism and address this question studying mutant strains, we used the model to assess the impact of "in silico knockouts" on V. alginolyticus' chemotaxis. We assessed how chemokinesis, flicking, and reorientation individually contribute to shaping the chemotactic response in V. alginolyticus by selectively inactivating individual functions in our model (i.e., blocks C, F, or R in Fig. 5B). In silico mutants lacking chemokinesis (ΔC) swim at a constant speed, in silico mutants lacking flicks (ΔF) only reverse, and in silico mutants lacking the ability to modulate their reorientation frequency with increases in speed (ΔR) had a reorientation frequency of 1/s, in line with that of the slower cells in the population (Fig. 3) and, incidentally, of E. coli (2). We quantified how three metrics of chemotaxis-chemotactic speed (Fig. S8A), chemotactic precision (Fig. S8 B-D), and resource exposure (Fig. 5C)-were affected by each in silico mutation.

Chemotactic speed. The chemotactic speed during the transient part of the chemotactic response was quantified in terms of the accumulation time, T_A (SI Materials and Methods and Fig. S8A), for the linear gradient case, mimicking the experimental setup (Fig. S1). T_A is speed dependent (Fig. S8A) and the lack of chemokinesis (ΔC), flicking (ΔF), or variable reorientation frequency (ΔR) considerably increased the population-averaged accumulation time compared with in silico wild-type cells with full functionality ($\langle T_A \rangle = 96$ s), by a factor of 4.3 ($\langle T_A \rangle = 413$ s), 2.7 ($< T_A > = 259$ s), and 3.0 ($< T_A > = 288$ s), respectively (Fig. S8A, Inset). All three adaptations were important during the transient phase of chemotaxis (Fig. S8A). Chemokinesis favors chemotaxis during the transient phase by accelerating the gradient climb, flicking ensures more effective space exploration and gradient sampling over reversals alone, and modulating the reorientation frequency with speed enables faster cells to offset their random motility by more frequent trajectory corrections. Chemotactic precision. The CMC and $I_{\rm C}$ values in the absence of chemokinesis (ΔC) lie on top of the steady-state curves at slower speed bins (Fig. S8 B-D, compare blue and black). Removing flicks (ΔF) influenced the chemotactic performance differently for different swimming speeds (Fig. S8 B-D, compare green and black). When flicks were inhibited and all reorientations were reversals, the CMC and $I_{\rm C}$ values decreased mainly in the highspeed regime ($V > 30 \mu m/s$) where flicks were originally dominant (Fig. S8 B-D and Fig. 3). Removing the ability to modulate reorientation frequency with speed (ΔR) resulted in a considerable decrease of the CMC and $I_{\rm C}$ values, except for the low-speed bins (Fig. S8 B-D, compare red and black). The population-level resource exposure for three different resource landscapes was computed for each of these in silico mutants and compared with agents with in silico wild-type cells, as shown in the main text (Fig. 5C).

As shown, the absence of individual functions resulted in a reduced chemotactic performance, in terms of both a slower migration speed up the gradient during the transient phase of chemotaxis (Fig. S84) and a lower level of precision at steady state (Fig. S8 *B–D*). This effect becomes more dramatic for cells swimming in more realistic chemical gradients, more closely mimicking the shape of gradients occurring at the microscale in the marine environment (Fig. S8 *C* and *D*). These results illustrate that all of the motility adaptations described in the chemotaxis pathway of *V. alginolyticus* are important components of the speed-dependent chemotactic precision of this bacterium.

Mechanosensing. One potential mechanism of swimming-speedsensing mechanism in bacteria is mechanosensing, the ability to sense mechanical signals such as forces and torques. It has been recently shown that the mechanical load on the motor, sensed at the level of the stator, can contribute to regulate the tumbling rate in *E. coli* (3). Mechanosensing has also been reported in *Vibrios* (27), where inhibition of the rotation of the polar flagellum due to increased viscous resistance—for example, near surfaces or for increased fluid viscosity—leads to the formation of myriad of lateral flagella for swarming motility through induction of the lateral flagellar gene (*laf*) expression. An alternative to sensing force is sensing swimming speed directly, because speed is linearly proportional to motor rotation rate.



Fig. 51. Hydrogel-based microfluidic device used to quantify the chemotactic response of *V. alginolyticus* in a steady, linear concentration profile of serine. Channel D is the test channel (width $W = 600 \mu$ m), where bacteria reside and are observed by video microscopy. Inlets are marked by a "1," outlets by a "2." Channel A is the source channel, carrying 500 nM serine, and channel B is the sink channel, carrying buffer (0 nM serine). Channel C is used before the experiments, for the fabrication of two 200-µm-wide hydrogel walls by injection of liquid agarose between arrays of PDMS pillars used for containment (*Materials and Methods*). Once agarose solidifies, it results in two hydrogel walls separating the test channel from the source and sink channels (*Inset*), which are permeable to diffusion of serine and thus enable the formation of a linear serine concentration profile, *C*(*x*) (from 100 to 400 nM), across the test channel (*x* direction; *Inset* and Fig. S2). All channels are 100 µm deep. During an experiment, the inlet D1 and the outlet D2 are sealed with glass coverslips to create a flow-free condition in the test channel. The imaging window covers the whole width *W* (600 µm) of the test channel and cells were imaged at 15 locations along the length of the test channel.



Fig. S2. Steady-state serine concentration field (color map) inside the microfluidic channel modeled numerically (Fig. S1). The zoomed-in view shows the concentration contour lines near the hydrogel-trapping PDMS pillars (white rectangles), illustrating that the concentration field inside the test channel becomes homogeneous in the along-channel direction within 20 μ m from the pillars.



Fig. S3. Experimentally observed temporal evolution of the CMC during the transient phase (<350 s) and at steady state (>500 s) for *V. alginolyticus* cells swimming at different speeds in the same serine gradient as in Fig. S1.



Fig. S4. (*A* and *B*) The reorientation frequency, *f*, as a function of the swimming speed in the (*A*) presence and (*B*) absence of a serine gradient. Red symbols include data acquired over all sodium concentrations (3–600 mM), and gray symbols refer to data for individual sodium concentrations (see legend in *B*). For all panels, horizontal error bars denote SDs of each speed bin. Where not visible, horizontal error bars are smaller than symbols.



Fig. S5. Temporal evolution of the swimming speed distribution, p(V), plotted at different times after the uniform addition of 5 μ M serine (*SI Materials and Methods*). Different shades of blue correspond to different times after serine addition (see *Inset* for time color coding). The green dashed line marks the speed threshold $V = 30 \mu$ m/s. Experiments were performed at a sodium concentration of 600 mM. Each curve contains information from at least 570 trajectories. (*Inset*) Population-averaged increase in speed following the serine addition, expressed as a percentage of the speed at time 0. Horizontal error bars denote the width of each time window.



Fig. S6. Mean chemokinetic speed enhancement at steady state as a percentage of the swimming speed before chemical addition, for various chemoattractants (different symbol colors) and different sodium concentrations (different symbol shapes). Shown on the *Left* (blue background) is a case in which a gradient of serine was present for the steady-state chemokinesis experiments. The source channel (A in Fig. S1) carried 50, 100, or 500 nM serine, and the sink channel (B in Fig. S1) carried buffer (0 nM serine) (*SI Materials and Methods*). The concentration plotted on the *x* axis for these cases is the concentration in the source channel, and the speed increase was computed over the entire population, over the entire gradient. Shown on the *Right* (green background) is a case in which serine, glucose, or their nonmetabolizable analogs were added uniformly in both the source and sink channels at a concentration of 5 μ M at natural sodium conditions (600 mM). The speed increase was computed again over the entire population, over the entire channel width.



Fig. 57. (*A*) Model prediction of the steady-state chemotactic migration coefficient (CMC) as a function of swimming speed (compare with Fig. 1 *C* and *E*). Horizontal and vertical error bars denote SDs of each speed bin and the CMC, respectively. Where not visible, error bars are smaller than symbols. (*Inset*) Steady-state bacterial distribution, B(x), predicted by the model for agents swimming at 10 different speeds (*SI Materials and Methods*). For all speeds, B(x) was well fitted by an exponential distribution (see R^2 values in the second *Inset*), as in the experiment (Fig. 1*C*). B(x) was normalized to a mean of 1. (*B*) Temporal evolution of the CMC for agents swimming at 10 different speeds (300 agents for each speeds) from the mathematical model (*SI Materials and Methods*). Agents from the slowest speed fraction required >6,000 s to reach steady state.



Fig. S8. Each of the individual functions comprising the proposed chemotactic pathway of *V. alginolyticus* is an essential component of its high chemotactic precision and speed. (A) Model prediction of the accumulation time, T_{A} , in a linear gradient for the same in silico mutants (*SI Materials and Methods*) lacking the ability to modulate reorientation frequency (block R in Fig. 5*B*; Δ R; red), to have chemokinesis (block C in Fig. 5*B*; Δ C; blue), or to flick (block F in Fig. 5*B*; Δ F; green). The black circles represent agents having full chemotactic functionality (in silico wild-type cells; same data as in Fig. S7*A*). (*A, Inset*) Relative time delay of in silico mutants, computed with respect to the accumulation time of in silico wild-type cells (black). (*B*) Model prediction of the chemotactic ingration coefficient (CMC) for an in silico knockout mutant (*SI Materials and Methods* and *SI Text*) lacking the ability to perform chemokinesis (block C in Fig. 5*B*; Δ C; blue), to flick (block F in Fig. 5*B*; Δ F; green), or to modulate their reorientation frequency based on swimming speed (block R in Fig. 5*B*; Δ R; red), for a population swimming in (C) a 1D Gaussian chemo-attractant field and (*D*) a 2D Gaussian chemoattractant field (*Materials and Methods*).

| Table S1. | Dependence of population-level swimming statistics and single-cell-level swimming kinematics on the |
|-----------|---|
| swimming | speed, V, in the low-speed regime (V < 30 μ m/s) and the high-speed regime (V > 30 μ m/s) |

| Parameter | Symbol | Units | Scaling at low-speed regime | Scaling at high-speed regime |
|------------------------------|--------|-------|-----------------------------|------------------------------|
| Chemotactic precision length | L | μm | ~ <i>V</i> ⁻¹ | ~Constant |
| Chemotactic velocity | Vc | μm/s | $\sim V^2$ | $\sim V^2$ |
| Random motility | D | μm²/s | $\sim V$ | $\sim V^2$ |
| Reorientation frequency | f | 1/s | ~V | ~Constant |

The different functional dependence of D and V_c on the swimming speed V (Fig. 2 A and B, Insets) is at the origin of the speed dependence of the chemotactic precision of V. alginolyticus (Fig. 1). In the high-speed regime, both D and V_c scale quadratically with V, and thus the precision of the chemotactic accumulation, measured by $L = D/V_c$ (Fig. 1D), is speed independent. In contrast, in the low-speed regime, V_c again scales quadratically with V but D scales linearly with V; hence, $L = D/V_c \sim 1/V$ decreases with increasing speed (Fig. 1D), resulting in higher chemotactic precision with increasing swimming speed.