Barrier Crossing in Escherichia coli Chemotaxis

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We study cell navigation in spatiotemporally complex environments by developing a microfluidic racetrack device that creates a traveling wave with multiple peaks and a tunable wave speed. We find that while the population-averaged chemotaxis drift speed increases with wave speed for low wave speed, it decreases sharply for high wave speed. This reversed dependence of population-averaged chemotaxis drift speed on wave speed is caused by a “barrier-crossing” phenomenon, where a cell hops backwards from one peak attractant location to the peak behind by crossing an unfavorable (barrier) region with low attractant concentrations. By using a coarse-grained model of chemotaxis, we map bacterial motility in an attractant field to the random motion of an overdamped particle in an effective potential. The observed barrier-crossing phenomenon of living cells and its dependence on the spatiotemporal profile of attractant concentration are explained quantitatively by Kramers reaction rate theory.

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Sensing and responding to changes in external environments are critical for the survival of organisms. One of the well-studied model systems is bacterial chemotaxis. Bacteria use their transmembrane chemoreceptors to sense their environments and control their motion in search of places with more favorable conditions [1–3]. In a homogeneous environment, an E. coli cell performs the run-and-tumble random walk allowing it to explore its environment [4]. In the presence of an attractant gradient, E. coli cells bias their random walk towards the preferred direction by lengthening (shortening) the run time in the “correct” (“wrong”) direction. The intracellular biochemical circuit that allows an E. coli cell to compute a gradient has been studied extensively in the past decades [5–9]. Predictive models have been developed based on knowledge of the bacterial signaling pathway and quantitative molecular and cellular experiments [10–13]. A modeling framework based on the intracellular signaling dynamics and the motor response has also been developed to study cellular and population behaviors [14–17].

However, cells live in heterogeneous environments together with other cells. There can be multiple favorable locations that are separated by unfavorable regions. Cells can also emit chemical signals and act as moving sources of attractants. Can a cell find its way out of a local optimum location to explore the environment globally? Can a cell track a moving attractant source? Here, we investigate these questions by developing a microfluidic device to create environments with multiple attractant peaks that move with a tunable speed. In particular, we created a traveling wave of attractant concentration in an annulus (racetrack) channel as shown in Fig. 1. Both population level behaviors and individual cell trajectories were measured for traveling wave attractant profiles with different wave speeds ($v_w$). Our measurements showed that the population-averaged chemotaxis drift speed $v_d$ increases with $v_w$ for small $v_w$. Surprisingly, we observed a critical wave speed, beyond which $v_d$ decreases sharply with $v_w$ instead of reaching a saturating value. Our individual cell trajectory data revealed that cells can hop from one peak attractant position to another by crossing a barrier region with lower attractant concentrations, and the backward hopping probability increases with $v_w$.

To explain the experimental observations quantitatively, we studied a theoretical model of chemotaxis motion based on the intracellular signaling dynamics. Our model analysis showed that E. coli chemotactic behavior can be mapped to a thermally activated motion in an effective energy landscape, with the cell’s random motion acting as the source of thermal fluctuation and an effective potential determined by the ligand concentration profile. The effective potential barrier height for backward hopping is lowered by $v_w$, which results in a backward drift speed that depends exponentially on $v_w$. This exponential backward drift speed leads to the sharp drop in $v_d$ at high $v_w$. Finally, the barrier-crossing phenomenon is confirmed by a “double well” experiment with different barrier heights.

We first describe our microfluidic device shown in Fig. 1(a). The device is composed of concentration modulating parts, an annular observation channel, two agarose adding channels, and a cell loading channel. Attractant and buffer solutions, after being well mixed in the modulating...
Bacterial motion in the observation channel were imaged by using a dark field lens. *E. coli* (wild type RP437) swimming in the traveling attractant wave [α-methyl-DL-aspartate (MeAsp)] with different wave speeds ($v_w$) of 0.67, 1, 2, 4, 8, 10, and 13.3 $\mu$m/s, are tracked and analyzed (see the Supplemental Material [18] for details of the experiments). The spatiotemporal cell density profiles for different wave speeds are shown in Figs. 2(a)–2(c). The bacterial chemotactic behavior depends strongly on $v_w$. For smaller $v_w \leq 4$ $\mu$m/s, most bacteria form a cluster following the crest of the attractant wave [Figs. 2(a) and 2(b)]. Occasionally, a cell escapes from the cluster and moves in a backward direction opposite to the attractant wave as indicated by the red arrow in Fig. 2(b). However, for higher $v_w > 4$ $\mu$m/s, such backward motion becomes more frequent leading to a more diffused cell distribution [Fig. 2(c)].

To characterize the bacterial population dynamics at different $v_w$ quantitatively, we calculated the bacterial drift velocity $v_d$ by averaging the velocities of all cell trajectories within a period (see the Supplemental Material [18] for details for the trajectory extraction). As shown in Fig. 2(d), for small wave speed $v_w \leq 2$ $\mu$m/s, we have $v_d \approx v_w$ as cells can follow the attractant wave. For intermediate

parts, are pumped into the modulating parts with time varying injection speed. Details of the observation channel are presented in Fig. 1(b). The connection channels between the observation channel and source channels are filled with agarose gel and serve as control points. The hydrogel added from the agarose inlets is used to prevent bacteria escaping and to avoid net flow that affects bacterial motility. The attractant concentration profile in the observation channel is determined by diffusion through the four control points. The same oscillation of attractant concentration (amplitude and period) was introduced in the four source channels with a phase delay of $\pi/2$ between two adjacent control points. As a result, the attractant molecules diffuse into the annular channel, forming a traveling-wave concentration field. The wave speed $v_w$ is determined by the driving attractant period at the four control points. We test the attractant concentration in the observation channel by adding fluorescein in the attractant solution and the spatiotemporal concentration profile is shown in Fig. 1(c) for $v_w = 1$ $\mu$m/s. The cell loading channel and observation channel are linked by a narrow pass. Because the attractant concentration in the observation channel is always higher than that in the loading channel during the experiments, cells that are loaded to the cell loading channel can chemotax to the observation channel through the narrow pass and seldom escape out of it. (See the Supplemental Material [18] for details of the chip layout and fabrication.)

![FIG. 1. Experiment setup. (a) Panorama of the PDMS chip. (b) Enlarged picture of the observation channel. The circumference of the observation channel is $\lambda = 800$ $\mu$m. (c) Spatiotemporal profile of the attractant (MeAsp) concentration in the observation channel for $v_w = 1$ $\mu$m/s. $T$ represents the period of the concentration wave and $P_1$–$P_4$ are the corresponding control points shown in (b). Attractant concentrations at the four source channels oscillate between 0 and 1 mM with a phase delay of $\pi/2$ in every two adjacent control points. The ligand concentration is measured by adding a small amount of fluorescein into attractant stocks.](image)

![FIG. 2. Dynamics of bacterial population. The spatiotemporal cell density profiles for different wave speeds of 1 $\mu$m/s (a), 4 $\mu$m/s (b), and 10 $\mu$m/s (c). The normalized cell density and attractant concentration is represented by the color and gray scales, respectively. The gray scale stripe on the left side of each panel shows the normalized ligand concentrations at $x = 0$. The red arrows indicate the “backward” bacterial flux moving in the opposite direction to the attractant wave. (d) The bacterial drift velocity $v_d$ averaged over a period versus $v_w$ from experiments (black), the SPECS simulations (red), and fitting with Eqs. (4) and (S26) [18] (blue). The error bars denote standard deviation in nine independent experiments.](image)
8 > v_w > 2 μm/s, v_d starts to deviate from v_w but still increases with v_w albeit sublinearly. This slowing down is likely caused by the effect of a finite adaptation time of E. coli in tracking or computing the attractant gradient [15,16]. However, the most surprising observation is that v_d decrease sharply with v_w instead of saturating to a constant value when v_w ≥ 8 μm/s. In the rest of this Letter, we try to understand the observed nonmonotonic dependence of v_d on v_w, in particular the sharp decrease in v_d for large v_w.

To characterize the relative motion of individual cells with respect to the traveling wave, we define the phase shift of a cell for a given period as Δφ_T ≡ 2πΔx/(v_w T), where Δx is the net displacement along the direction of the attractant wave in a period T. If a cell follows the wave exactly, we have Δφ_T = 2π. If a cell hops backwards to the peak behind the current one during time T, we have zero net displacement, Δx = 0, and Δφ_T = 0. Two representative trajectories for Δφ_T ≈ 2π and zero are shown in Fig. 3(a). Individual cell behaviors are analyzed by manually tracking their trajectories over a complete period. Figure 3(b) shows the probability distributions of Δφ_T for v_w = 2, 8, and 13.3 μm/s. For v_w = 2 μm/s, the Δφ_T distribution peaks around a large Δφ_T ~ 1.5π. However, for v_w = 8 μm/s, an additional peak appears in the Δφ_T distribution near Δφ_T ~ 0, indicating the significance of backward hopping, which is responsible for the significant reduction in v_d for large v_w. For the high wave speed v_w = 13.3 μm/s, the distribution is centered around Δφ_T = 0, which means backward hops dominate and the mean drift speed vanishes.

To understand both the population-level and individual cell behaviors quantitatively, we used the signaling pathway-based E. coli chemotaxis simulator (SPECS) [25] instead of the filter-function based phenomenological models [26–28]. The advantage of the SPECS is that it incorporates the internal signaling pathway dynamics with the movements of individual cells (see the Supplemental Material [18] for details of the SPECS simulation). The dependence of v_d on v_w from the SPECS agrees with our experimental data [Fig. 2(d)]. We also studied the statistics of Δφ_T of individual cells for different traveling attractant wave speeds in the SPECS. As shown in Fig. 4, the Δφ_T distributions exhibit multimodality with peaks centered around 2π and zero, and the proportions of bacteria distributed around different peaks change significant with v_w. For v_w = 2 μm/s, a large proportion of cells have Δφ_T around 2π and only about < 20% of the population distributes near zero. As v_w increases, the proportion of the cells with Δφ_T ~ 0 increases, and eventually dominates at high v_w, which agrees with the experiments [Fig. 3(b)].

In our previous work, a mean field theory based on intracellular signaling dynamics was developed for studying population level bacterial chemotaxis behaviors [15,17]. Briefly, the tumbling rate z_t = τ^{-1}(a/a_0)^H is modulated by the chemoreceptor activity a, where τ and a_0 are the average run time and activity of the chemoreceptors at steady state; H (≈ 10) is the Hill coefficient [29]. The total frequency of a cell changing its direction is the sum of the rotational diffusion coefficient z_0 and the tumbling rate z_t: z(a) = z_t + z_0. Thus, the average run time is z_t^{-1} ~ z^{-1}(a=a_0) with a the average activity of all cells at position x, and the average run distance is v z_t^{-1} with v the run speed. The dynamics of the receptor activity is governed by the local attractant concentration and the receptor methylation level, which has a slow dynamics

![Image](3.png)

**FIG. 3.** Single cell dynamics. (a) Two representative single-cell trajectories with Δφ_T ≈ 2π (left panel) and Δφ_T ≈ 0 (right panel) from experiment with v_w = 8 μm/s. The arrow shows the direction of the attractant wave. (b) The statistics of Δφ_T for v_w = 2 μm/s (47 cells), v_w = 8 μm/s (50 cells), and v_w = 13.3 μm/s (51 cells).

![Image](4.png)

**FIG. 4.** The statistics of Δφ_T for different wave speeds from the SPECS. The multimodality of the density probability is caused by the barrier crossing between the neighboring local well. The effective potentials for v_w = 0 and v_w > 0, and the attractant concentration profile are shown in the inset.
and essentially carries a memory of the cell’s environment in the past. Therefore, when cells move in a chemical gradient, the average activity of the left-moving cells at position $x$ is different from the right-moving cells at the same position, as these two populations carry different receptor methylation levels. The activity difference leads to a difference in the tumbling frequency $\Delta\zeta$, which eventually drives the bacterial chemotactic drift.

In our experiments with traveling attractant waves, it is convenient to study the system in a comoving frame with the attractant by using the transformation $x' = x - v_w t$. The dynamics of the bacterial density $\rho(x', t)$ in the moving frame is given by

$$\frac{\partial \rho}{\partial t} = \frac{\partial}{\partial x'} \left( D(x') U'(x') \rho \right) + \frac{\partial}{\partial x'} \left( D(x') \frac{\partial \rho}{\partial x'} \right),$$

(1)

which describes bacterial chemotaxis motility as the thermal motion of particles moving in an external potential [30]. The attractant field gives rise to the external potential $U(x') = \lambda r(x')$, for the stationary wave. The constants $r_0$ is the base attempt rate, and $\alpha$ is the hopping rate for the stationary wave. The constants $\beta_1, \beta_2$ are independent of $v_w$ (see the Supplemental Material [18] for detailed derivations). The discrete hopping events from one well to another explain the multimodality of the probability distribution of $\Delta\rho_T$ [Figs. 3(b) and 4]. As $v_w$ increases, the backward hopping rate $r_b$ increases and the percentage of cells with $\Delta\rho_T = 0$ increases. Put together, $v_d$ is given by

$$v_d = v_w - \lambda (r_b - r_f) \approx v_w - \alpha \lambda (e^{\beta_1 v_w} - e^{\beta_2 v_w}),$$

(4)

where $\lambda = 800 \mu m$ is the peak-to-peak distance of the traveling wave. For small $v_w$, the hopping rates are small, so $v_d \approx v_w$. However, due to the exponential dependence of $r_b$ on $v_w$, there exists a critical wave speed $v_c$ where $dv_d/dv_w = 0$, and the drift velocity is dominated by the backward hopping term $r_b$ and decreases sharply for $v_w > v_c$. In our experiments, the attractant wave amplitude had a weak dependence on $v_w$, which leads to $\alpha = a_0 (v_w/v)^d$, where $d \approx 1.34$ is determined from experiments, and $a_0$ is a $v_w$-independent constant (see the Supplemental Material [18] for details). Quantitatively, Eq. (4) fits the experiments and the SPECS simulations well with $a_0 = 0.0141/s$, $\beta_1 = 0.0713 s/\mu m$, and $\beta_2 = 0.4915 s/\mu m$ as shown in Fig. 2(d). Note that for a high wave speed $v_w = 13.3 \mu m/s$ Eq. (4) needs to be modified to include higher order terms [see Eq. (S26) in the Supplemental Material [18]].

To verify the barrier crossing effect, we studied chemotaxis in a “double well potential.” The double well potential is achieved in the same device by giving a static high concentration at control points $P_1$ and $P_3$, and a static low concentration at $P_2$ and $P_4$. Cells were concentrated initially near $P_1$ by lowering the concentration at $P_3$. Once a majority cell population is established around $P_1$, the concentration at $P_3$ is raised to form the double well potential [Fig. 5(a)]. The cells hop probabilistically between $P_1$ and $P_3$, causing the cell density in $P_1$ to decay

![Fig. 5. Bacterial motion in a double well potential.](image-url)
exponentially to 0.5 over time [Fig. 5(b)]. When we increase the potential barrier by decreasing the concentration at $P_2$ and $P_4$, the decay rate decreases as expected from the transition state theory [Fig. 5(b) inset].

In summary, by developing a "racetrack" microfluidic device we investigate bacterial chemotaxis behaviors in response to traveling attractant waves. The underlying mechanism is understood by a computational model based on realistic signaling pathway dynamics. We believe that this combined approach, i.e., quantitative microfluidics experiments together with predictive models based on realistic signaling dynamics, can be extended to study other interesting biological phenomena, such as collective behaviors due to cell-cell communication through chemical signaling [32–35].

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