

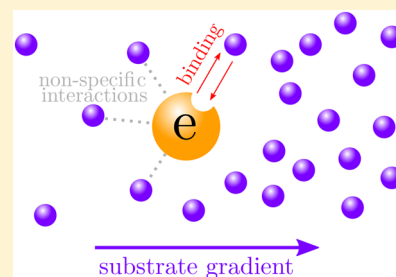
Phoresis and Enhanced Diffusion Compete in Enzyme Chemotaxis

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Supporting Information

ABSTRACT: Chemotaxis of enzymes in response to gradients in the concentration of their substrate has been widely reported in recent experiments, but a basic understanding of the process is still lacking. Here, we develop a microscopic theory for chemotaxis that is valid for enzymes and other small molecules. Our theory includes both nonspecific interactions between enzyme and substrate as well as complex formation through specific binding between the enzyme and the substrate. We find that two distinct mechanisms contribute to enzyme chemotaxis: a diffusio-phoretic mechanism due to the nonspecific interactions and a new type of mechanism due to binding-induced changes in the diffusion coefficient of the enzyme. The latter chemotactic mechanism points toward lower substrate concentration if the substrate enhances enzyme diffusion and toward higher substrate concentration if the substrate inhibits enzyme diffusion. For a typical enzyme, attractive phoresis and binding-induced enhanced diffusion will compete against each other. We find that phoresis dominates above a critical substrate concentration, whereas binding-induced enhanced diffusion dominates for low substrate concentration. Our results resolve an apparent contradiction regarding the direction of urease chemotaxis observed in experiments and, in general, clarify the relation between the enhanced diffusion and the chemotaxis of enzymes. Finally, we show that the competition between the two distinct chemotactic mechanisms may be used to engineer nanomachines that move toward or away from regions with a specific substrate concentration.

KEYWORDS: Chemotaxis, diffusion, phoresis, enzymes



Because of their biocompatibility and ubiquity, enzymes have been extensively studied in recent years as ideal models for nanomachines.¹ A subject of particular interest has been that of the enhanced diffusion of enzymes in the presence of their substrate: with increasing concentration of substrate, enhancements of up to ~50% in the diffusion coefficient of the enzyme have been measured for a wide variety of enzymes.^{2–7} Early hypotheses addressing enhanced diffusion of enzymes relied on nonequilibrium mechanisms such as stochastic swimming^{8–10} and exothermicity of the catalytic reaction.^{6,9,11} However, it was observed recently that even the slow and endothermic enzyme aldolase undergoes enhanced diffusion.⁷ To explain this observation, an equilibrium mechanism for the enhanced diffusion of enzymes has been proposed, which relies on the conformational changes of the enzyme due to binding and unbinding with the substrate.^{7,12}

More recently, a number of experimental studies have reported on the directed motion of enzymes in the presence of concentration gradients of their respective substrate, or chemotaxis. The majority of studies so far have observed chemotaxis of the enzyme toward their substrate; this includes RNA polymerase,² catalase and urease,^{4,13} and DNA polymerase⁵ as well as hexokinase and aldolase.¹⁴ Moreover, in all of these studies, enhanced diffusion of the enzyme in the presence of uniform substrate concentrations was reported, and it was

hypothesized (albeit without a clear connection) that enhanced diffusion may be responsible for the observed chemotaxis.^{2,4,5} Recently, however, Jee et al.¹⁵ reported chemotaxis of urease and acetylcholinesterase away from their respective substrate while still observing enhanced diffusion for the two. The latter results appear to be in conflict with the previous observations for two reasons: first, the trend of enhanced diffusion being concomitant with chemotaxis toward the substrate is not followed; second, the observation of urease chemotaxis away from urea contradicts the earlier observation by Sengupta et al.⁴ of urease chemotaxis toward urea. In a somewhat different, nonenzymatic system, Guha et al.¹⁶ recently observed the chemotaxis of small molecular dyes toward higher concentrations of a large polymer to which they bind while also observing inhibited diffusion of the dye in the presence of the polymer. Whether there is a relation between chemotaxis and enhanced diffusion, and what exactly determines whether an enzyme or small molecule will chemotax toward or away from its substrate, are important questions that need to be addressed.

In this Letter, we present a microscopically detailed theory for enzyme chemotaxis. Our theory is a natural extension of our

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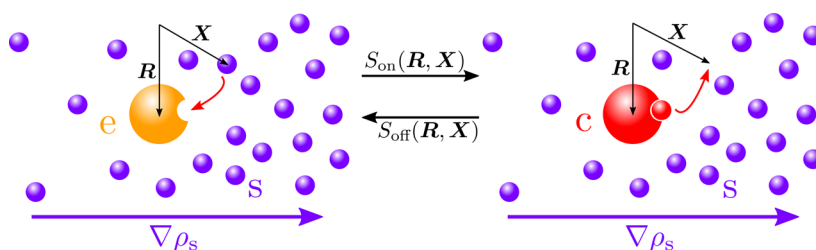


Figure 1. Free enzyme (yellow) in a gradient $\nabla\rho_s$ of substrate molecules (purple). An enzyme–substrate complex (red) can form with probability $S_{\text{on}}(\mathbf{R}, \mathbf{X})$ and decompose with probability $S_{\text{off}}(\mathbf{R}, \mathbf{X})$.

recently proposed equilibrium theory for the enhanced diffusion of enzymes^{7,12} and does not rely on the catalytic activity (i.e., the nonequilibrium reaction step) of the enzyme. We take into account both nonspecific interactions between enzyme and substrate, as well as complex formation through specific binding between the enzyme and the substrate. Here, and in the following, we use the term “nonspecific interactions” to refer to the forces to which all of the substrate molecules that are anywhere near the enzyme are subject. These nonspecific interactions can, in principle, be attractive or repulsive and include van der Waals, electrostatic, steric interactions, etc. In contrast, we use the term “specific binding” to refer to the attractive, short-ranged interactions that occur between a single substrate molecule and the enzyme at a well-defined binding pocket. These typically involve hydrogen bonding, hydrophobic interactions and temporary covalent bonds and are often associated with a conformational change of the enzyme. We find that, for a typical enzyme, chemotaxis is dictated by a competition between two distinct mechanisms. The first one corresponds to diffusiophoresis, which arises from the nonspecific interactions between enzyme and substrate, while the second one is due to the binding-induced changes in the diffusion coefficient of the enzyme. The contribution to chemotaxis due to binding-induced changes in diffusion points away from the substrate in the case of enhanced diffusion, and toward the substrate in the case of inhibited diffusion. We find a critical substrate concentration above which the diffusiophoretic mechanism dominates and below which the mechanism arising from binding-induced changes in diffusion dominates. This competition can explain the conflicting experimental observations for urease and is consistent with the rest of the available experimental evidence. Finally, we show that the competition between phoresis and binding-induced changes in diffusion can be used to engineer nanomachines that move toward or away from regions with a specific substrate concentration.

We begin by considering a single enzyme in a bath of N substrate molecules, as depicted in Figure 1. The enzyme is located at \mathbf{R} , while the substrate molecules are at $\mathbf{X}_1, \dots, \mathbf{X}_N$. An enzyme located at \mathbf{R} can bind to a substrate molecule located at \mathbf{X} with a probability of $S_{\text{on}}(\mathbf{R}, \mathbf{X})$, to form a complex located at \mathbf{R} .¹⁷ In turn, the complex located at \mathbf{R} can decompose, leaving the free enzyme at \mathbf{R} and the substrate molecule at \mathbf{X} , with a probability of $S_{\text{off}}(\mathbf{R}, \mathbf{X})$. The state in which the enzyme is free is described by the $(N + 1)$ -particle probability density $\rho_f(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N; t)$. There are N distinct states in which a complex coexists with $N - 1$ free substrate molecules, each of these states being described by an N -particle probability density $\rho_{b,i}(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_{i-1}, \mathbf{R}, \mathbf{X}_{i+1}, \dots, \mathbf{X}_N; t)$ for $1 \leq i \leq N$. The nonspecific interactions between the enzyme and the substrate molecules are taken into account via an interaction potential $\phi^e(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N)$ in the free state and $\phi^{c,i}(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_{i-1}, \mathbf{R},$

$\mathbf{X}_{i+1}, \dots, \mathbf{X}_N)$ in the bound state. All particles may also interact with each other through hydrodynamic interactions. We can then write the following coupled set of Smoluchowski equations obeyed by the probability distributions, characterizing each of the $N + 1$ distinct states:

$$\begin{aligned} \partial_t \rho_f(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N) &= \nabla_{\mathbf{R}} \cdot \boldsymbol{\mu}^{ee} \cdot [k_B T \nabla_{\mathbf{R}} \rho_f + (\nabla_{\mathbf{R}} \phi^e) \rho_f] \\ &+ \sum_{i=1}^N \{ \nabla_{\mathbf{R}} \cdot \boldsymbol{\mu}^{es} \cdot [k_B T \nabla_{\mathbf{X}_i} \rho_f + (\nabla_{\mathbf{X}_i} \phi^e) \rho_f] \\ &+ \nabla_{\mathbf{X}_i} \cdot \boldsymbol{\mu}^{se} \cdot [k_B T \nabla_{\mathbf{R}} \rho_f + (\nabla_{\mathbf{R}} \phi^e) \rho_f] \\ &+ \nabla_{\mathbf{X}_i} \cdot \boldsymbol{\mu}^{ss} \cdot [k_B T \nabla_{\mathbf{X}_i} \rho_f + (\nabla_{\mathbf{X}_i} \phi^e) \rho_f] \} \\ &- \sum_{i=1}^N S_{\text{on}}(\mathbf{R}, \mathbf{X}_i) \rho_f(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N) \\ &+ \sum_{i=1}^N S_{\text{off}}(\mathbf{R}, \mathbf{X}_i) \rho_{b,i}(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_{i-1}, \mathbf{R}, \mathbf{X}_{i+1}, \dots, \mathbf{X}_N) \end{aligned} \quad (1)$$

$$\begin{aligned} \partial_t \rho_{b,i}(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_{i-1}, \mathbf{R}, \mathbf{X}_{i+1}, \dots, \mathbf{X}_N) &= \nabla_{\mathbf{R}} \cdot \boldsymbol{\mu}^{cc} \cdot [k_B T \nabla_{\mathbf{R}} \rho_{b,i} + (\nabla_{\mathbf{R}} \phi^{c,i}) \rho_{b,i}] \\ &+ \sum_{\substack{j=1 \\ (j \neq i)}}^N \{ \nabla_{\mathbf{R}} \cdot \boldsymbol{\mu}^{cs} \cdot [k_B T \nabla_{\mathbf{X}_j} \rho_{b,i} + (\nabla_{\mathbf{X}_j} \phi^{c,j}) \rho_{b,i}] \\ &+ \nabla_{\mathbf{X}_j} \cdot \boldsymbol{\mu}^{sc} \cdot [k_B T \nabla_{\mathbf{R}} \rho_{b,i} + (\nabla_{\mathbf{R}} \phi^{c,j}) \rho_{b,i}] \\ &+ \nabla_{\mathbf{X}_j} \cdot \boldsymbol{\mu}^{ss} \cdot [k_B T \nabla_{\mathbf{X}_j} \rho_{b,i} + (\nabla_{\mathbf{X}_j} \phi^{c,j}) \rho_{b,i}] \} \\ &+ \int d\mathbf{X}_i S_{\text{on}}(\mathbf{R}, \mathbf{X}_i) \rho_f(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N) \\ &- \rho_{b,i}(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_{i-1}, \mathbf{R}, \mathbf{X}_{i+1}, \dots, \mathbf{X}_N) \\ &\int d\mathbf{X}_i S_{\text{off}}(\mathbf{R}, \mathbf{X}_i) \end{aligned} \quad (2)$$

with $1 \leq i \leq N$ and $\boldsymbol{\mu}^{ab}$ corresponding to the hydrodynamic mobility tensors.

We now seek to obtain evolution equations for the one-particle probability densities $\rho_e(\mathbf{R})$ and $\rho_c(\mathbf{R})$ representing the free enzyme and the complex. These result from integrating out the positional degrees of freedom corresponding to the substrate molecules, such that:

$$\rho_e(\mathbf{R}) = \int d\mathbf{X}_1 \dots d\mathbf{X}_N \rho_f(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N) \quad (3)$$

$$\rho_c(\mathbf{R}) = \sum_{i=1}^N \rho_{c,i}(\mathbf{R}) \quad (4)$$

$$\rho_{c,i}(\mathbf{R}) = \int d\mathbf{X}_1 \dots d\mathbf{X}_{i-1} d\mathbf{X}_{i+1} \dots d\mathbf{X}_N \rho_{b,i}(\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_{i-1}, \mathbf{R}, \mathbf{X}_{i+1}, \dots, \mathbf{X}_N) \quad (5)$$

In general, the mobility tensors $\mu^{\alpha\beta}$ as well as the interaction potentials ϕ^α may depend on the coordinates $\mathbf{R}, \mathbf{X}_1, \dots, \mathbf{X}_N$ of all particles. We will, however, focus on the case of sufficiently dilute solutions and small substrate molecules so that the self-mobilities μ^{ii} can be considered to be constant, and the cross-mobilities μ^{ij} (with $i \neq j$) include only the pair contribution. The interaction potentials are a sum of pairwise interactions, with $\phi^e = \sum_{i=1}^N \phi^{es}(\mathbf{R}, \mathbf{X}_i)$ and $\phi^{c,i} = \sum_{j=1(j \neq i)}^N \phi^{cs}(\mathbf{R}, \mathbf{X}_j)$. For the simplest description of binding and unbinding, we may use $S_{\text{on}}(\mathbf{R}, \mathbf{X}) = k_{\text{on}} \delta(\mathbf{R} - \mathbf{X})$ and $S_{\text{off}}(\mathbf{R}, \mathbf{X}) = k_{\text{off}} \delta(\mathbf{R} - \mathbf{X})$, representing the fact that binding and unbinding are due to very short-ranged interactions and occur with rates k_{on} and k_{off} .

Using these approximations, we can integrate eqs 1 and 2 to obtain the two coupled equations for the free enzyme and complex probability distributions (see the [Supporting Information](#) for details of the derivation):

$$\partial_t \rho_e(\mathbf{R}; t) = \nabla_{\mathbf{R}} \cdot [D_e \nabla_{\mathbf{R}} \rho_e - \mathbf{v}_e(\mathbf{R}) \rho_e] - k_{\text{on}} \rho_e \rho_s + k_{\text{off}} \rho_c \quad (6)$$

$$\partial_t \rho_c(\mathbf{R}; t) = \nabla_{\mathbf{R}} \cdot [D_c \nabla_{\mathbf{R}} \rho_c - \mathbf{v}_c(\mathbf{R}) \rho_c] + k_{\text{on}} \rho_e \rho_s - k_{\text{off}} \rho_c \quad (7)$$

where $\rho_s(\mathbf{R})$ is the concentration of substrate molecules. Eqs 6 and 7 capture the effects of three important physical mechanisms. First, the free enzyme can turn into a complex and vice versa through the binding and unbinding of a substrate molecule with rates k_{on} and k_{off} . Second, the free enzyme and complex diffuse with diffusion coefficients respectively given by $D_e = k_B T \mu^{ee}$ and $D_c = k_B T \mu^{cc}$. Finally, the combination of nonspecific and hydrodynamic interactions between the free enzyme or complex and the substrate molecules leads to a diffusiophoretic drift of the free enzyme and complex with velocities respectively given by:

$$\mathbf{v}_e(\mathbf{R}) \approx \frac{k_B T}{\eta} \left[\int_0^\infty dh h (e^{-\phi^{es}(h)/k_B T} - 1) \right] \nabla_{\mathbf{R}} \rho_s \equiv \frac{k_B T}{\eta} \lambda_e^2 \nabla_{\mathbf{R}} \rho_s \quad (8)$$

$$\mathbf{v}_c(\mathbf{R}) \approx \frac{k_B T}{\eta} \left[\int_0^\infty dh h (e^{-\phi^{cs}(h)/k_B T} - 1) \right] \nabla_{\mathbf{R}} \rho_s \equiv \frac{k_B T}{\eta} \lambda_c^2 \nabla_{\mathbf{R}} \rho_s \quad (9)$$

where η is the viscosity of the fluid, and h is the distance between the surface of the enzyme and the center of the substrate molecule. Eqs 8 and 9 are approximate forms of the diffusiophoretic velocity valid for the typical case in which the range of the nonspecific interactions is shorter than the size of the enzyme/complex (in the general case, there are corrections to these velocities; see the [Supporting Information](#)). We have defined here the Derjaguin length, λ_w , a material parameter that captures the nonspecific interactions between the enzyme or complex and the substrate.^{18,19} Typical values of the Derjaguin length are of a few angstroms.^{19,20} We note that, in this convention, λ_α^2 may be positive or negative, with positive (negative) values corresponding to overall attractive (repulsive) interactions that lead to an enrichment (depletion) of substrate molecules in the proximity of the enzyme. Nonspecific interactions that are always present for every enzyme–substrate pair include repulsive steric interactions and attractive van der Waals interactions. In the [Supporting Information](#), we show

that even rather weak and short-ranged attractive interactions are sufficient to compensate for steric repulsion and induce a positive $\lambda_\alpha^2 > 0$ for a typical enzyme, in which case the phoretic velocity will be directed toward higher concentrations of the substrate.

We note that, in the description above, we have neglected the catalytic step of the enzymatic reaction by which substrate molecules can be turned into product molecules. This catalytic step has two main consequences. First, it represents an alternative pathway by which a complex can dissociate and give back a free enzyme. However, because the rate k_{cat} of the catalytic step is typically much slower than the unbinding rate ($k_{\text{cat}} \ll k_{\text{off}}$) the effect of the catalytic step is therefore often negligible in this regard. Second, when catalysis is taken into account, the number of substrate molecules is no longer conserved and decreases in time. Nevertheless, as long as the concentration of enzyme in solution is much lower than the concentration of substrate, and the experiments are performed in a sufficiently short time, the decrease in substrate concentration due to catalysis may be neglected.

Eqs 6 and 7 already contain all the ingredients necessary to describe enzyme–substrate interactions. Nevertheless, to describe chemotaxis, we are actually interested in the total concentration of enzyme, both free and bound, given by:

$$\rho_e^{\text{tot}}(\mathbf{R}; t) = \rho_e(\mathbf{R}; t) + \rho_c(\mathbf{R}; t) \quad (10)$$

which corresponds to what is actually measured in experiments with fluorescently tagged enzymes (free enzyme and complex cannot be distinguished). Furthermore, the typical time scale of diffusion and phoretic drift is much longer than the typical time scale of binding and unbinding. We can therefore assume that the enzyme is locally and instantaneously at binding equilibrium with the substrate, such that at any position \mathbf{R} , we will have:

$$k_{\text{on}} \rho_e(\mathbf{R}; t) \rho_s(\mathbf{R}; t) \approx k_{\text{off}} \rho_c(\mathbf{R}; t) \quad (11)$$

at time t . Combining eqs 10 and 11, we find the typical Michaelis–Menten kinetics for the free enzyme and the complex:

$$\rho_e = \frac{K}{K + \rho_s} \rho_e^{\text{tot}} \quad \text{and} \quad \rho_c = \frac{\rho_s}{K + \rho_s} \rho_e^{\text{tot}} \quad (12)$$

where we have defined the Michaelis constant $K \equiv k_{\text{off}}/k_{\text{on}}$.

Adding together eqs 6 and 7 and using 12, we finally obtain an expression for the time evolution of the total enzyme concentration:

$$\partial_t \rho_e^{\text{tot}}(\mathbf{R}; t) = \nabla_{\mathbf{R}} \cdot \{D(\mathbf{R}) \cdot \nabla_{\mathbf{R}} \rho_e^{\text{tot}} - [\mathbf{V}_{\text{ph}}(\mathbf{R}) + \mathbf{V}_{\text{bi}}(\mathbf{R})] \rho_e^{\text{tot}}\} \quad (13)$$

with the space-dependent diffusion coefficient:

$$D(\mathbf{R}) = D_e + (D_c - D_e) \frac{\rho_s(\mathbf{R})}{K + \rho_s(\mathbf{R})} \quad (14)$$

a drift velocity arising from phoretic effects:

$$\mathbf{V}_{\text{ph}}(\mathbf{R}) = \mathbf{v}_e(\mathbf{R}) + [\mathbf{v}_c(\mathbf{R}) - \mathbf{v}_e(\mathbf{R})] \frac{\rho_s(\mathbf{R})}{K + \rho_s(\mathbf{R})} \quad (15)$$

as well as a drift velocity arising from the changes in diffusion coefficient due to substrate binding and unbinding:

$$V_{\text{bi}}(\mathbf{R}) = -(D_c - D_e) \nabla_{\mathbf{R}} \left(\frac{\rho_s(\mathbf{R})}{K + \rho_s(\mathbf{R})} \right) \quad (16)$$

Eq 13 constitutes one of the main results of this work and presents a number of interesting features. First, we notice that the diffusion coefficient $D(\mathbf{R})$ corresponds to the “average” of the diffusion coefficients of the free enzyme and the complex. In the absence of substrate $\rho_s = 0$, the diffusion coefficient is that of the free enzyme. With increasing substrate concentration, the diffusion coefficient approaches that of the complex, saturating to D_c for $\rho_s \gg K$. This Michaelis–Menten-like dependence of the diffusion coefficient of the enzyme with the substrate concentration has been observed experimentally and is consistent with the equilibrium theory for enhanced diffusion introduced in refs 7 and 12, which can explain the enhanced diffusion of slow, endothermic enzymes such as aldolase,⁷ in contrast to other mechanisms proposed in the literature,^{6,9,15} which rely on the exothermicity of the catalytic reaction. In refs 7 and 12, several mechanisms were identified by which binding can induce changes in the diffusion coefficient of an enzyme of the order of a few tens of percent. These include binding-induced changes in the hydrodynamic radius of the enzyme or changes in the fluctuations of the internal translational and rotational degrees of freedom of individual subunits of a modular enzyme. These changes were predicted to lead typically to $D_c > D_e$, corresponding to enhanced diffusion. The case with $D_c < D_e$, in contrast, would correspond to inhibited diffusion. At any rate, the theory presented here is independent of the underlying mechanism that may cause D_e and D_c to be different from each other and only depends on their actual values. In practice, the values of D_e and D_c can be extracted from the available experimental data, as they correspond to the measured diffusion coefficients of the enzyme in the absence of substrate and in saturating substrate concentration, respectively.

The drift velocity arising from diffusiophoresis $V_{\text{ph}}(\mathbf{R})$ also corresponds to an “average” of the phoretic velocities of the free enzyme and the complex. With increasing substrate concentration, we again find a smooth Michaelis–Menten-like crossover between the velocity of the free enzyme, v_e , and that of the complex, v_c . In principle, this velocity may be directed toward or away from higher concentrations of substrate, depending on the details of the nonspecific interactions. Nevertheless, as mentioned previously, because enzyme–substrate interactions are generally attractive, we expect that the typical phoretic velocity for enzymes will be directed toward higher substrate concentrations.

Finally, the drift velocity $V_{\text{bi}}(\mathbf{R})$ is a direct consequence of the changes in the diffusion coefficient of the enzyme due to binding and unbinding of the substrate. This drift velocity is directed toward higher concentrations of substrate in the case of inhibited diffusion with $D_c < D_e$ and toward lower concentrations of substrate in the case of enhanced diffusion with $D_c > D_e$. This tendency can be made even more apparent when we notice that $V_{\text{bi}}(\mathbf{R})$ can alternatively be written as $V_{\text{bi}}(\mathbf{R}) = -\nabla_{\mathbf{R}} D(\mathbf{R})$. In the absence of phoresis with $V_{\text{ph}}(\mathbf{R}) = 0$, eq 13 can then be written as $\partial_t \rho_e^{\text{tot}}(\mathbf{R}; t) = \nabla_{\mathbf{R}}^2 [D(\mathbf{R}) \rho_e^{\text{tot}}]$, and we would thus expect $\rho_e^{\text{tot}}(\mathbf{R}) \propto 1/D(\mathbf{R})$ in the steady state, i.e., the enzyme tends to concentrate in regions where its diffusion is slowest. This type of behavior was recently reported experimentally in ref 15 for urease and acetylcholinesterase, and was explored theoretically in ref 21. However, in these two

works the equation $\partial_t \rho_e^{\text{tot}}(\mathbf{R}; t) = \nabla_{\mathbf{R}}^2 [D(\mathbf{R}) \rho_e^{\text{tot}}]$ was simply postulated with no justification, even if it is an established fact that knowledge of the position-dependence of the diffusion coefficient is not sufficient to obtain an evolution equation for the system: due to what is known as multiplicative noise, different evolution equations, such as, e.g., $\partial_t \rho_e^{\text{tot}}(\mathbf{R}; t) = \nabla_{\mathbf{R}} [D(\mathbf{R}) \nabla_{\mathbf{R}} \rho_e^{\text{tot}}]$, may arise from different microscopic mechanisms underlying the position dependence of the diffusion coefficient.^{22,23} Here, we have provided for the first time a microscopic mechanism that can lead to a steady state with $\rho_e^{\text{tot}}(\mathbf{R}) \propto 1/D(\mathbf{R})$.

The results from ref 15 just described, in which urease was observed to chemotax away from higher concentrations of urea, are in apparent conflict with older results in the literature⁴ in which urease was observed to chemotax toward higher concentrations of urea. The existence of two distinct mechanisms for chemotaxis, namely phoresis and binding-induced changes in diffusion as just described, may explain the seemingly contradictory observations. For simplicity, let us consider the typical case in which the phoretic response of the free enzyme and the complex is similar, with $\lambda_e = \lambda_c$. This is expected if the binding-unbinding process does not significantly affect the surface chemistry of the enzyme, such that the nonspecific interactions with the substrate are identical for the free enzyme and the complex. In this case, the phoresis-induced velocity (eq 15) is simply:

$$V_{\text{ph}} = \frac{k_B T}{\eta} \lambda_e^2 \nabla_{\mathbf{R}} \rho_s = 6\pi D_c R_e \lambda_e^2 \nabla_{\mathbf{R}} \rho_s \quad (17)$$

where we have used the Stokes–Einstein relation to express $k_B T/\eta$ in terms of the diffusion coefficient D_e and hydrodynamic radius R_e of the enzyme. In turn, the velocity (eq 16) due to binding-induced changes in diffusion can be written equivalently as:

$$V_{\text{bi}} = -\alpha D_e \frac{K}{(K + \rho_s)^2} \nabla_{\mathbf{R}} \rho_s \quad (18)$$

where $\alpha \equiv (D_c - D_e)/D_e$ represents the dimensionless change of diffusion coefficient between the free enzyme and the complex, which is positive and negative for enhanced and inhibited diffusion, respectively. Typical values observed experimentally for enhanced diffusion correspond to $\alpha = 0.1$ – 0.5 . Now we observe that while both the phoretic and the binding-induced velocity are proportional to the substrate concentration gradient, $\nabla_{\mathbf{R}} \rho_s$, the former is independent of the actual substrate concentration, ρ_s , whereas the magnitude of the latter decreases with increasing substrate concentration. Both contributions will have the same magnitude whenever $|V_{\text{ph}}| = |V_{\text{bi}}|$ or equivalently when:

$$\rho_s = K \left(\sqrt{\frac{|\alpha|}{6\pi R_e \lambda_e^2 |K}} - 1 \right) \equiv \rho_s^* \quad (19)$$

which defines a critical substrate concentration ρ_s^* , above which phoresis dominates over binding-induced changes in diffusion and below which the latter dominate over the former. For any given enzyme–substrate pair with their corresponding enzyme radius R_e , Derjaguin length λ_e , Michaelis constant K , and diffusion change α , there will be a different critical substrate concentration. We also note that phoresis may dominate at all concentrations if the binding-induced changes in diffusion are too small, with $|\alpha| \leq 6\pi R_e \lambda_e^2 |K|$.

In the urease experiments of Jee et al.,¹⁵ in which chemotaxis away from urea was observed, the urea concentration used was 1 mM. In those of Sengupta et al.,⁴ in which chemotaxis toward urea was observed, the urea concentration was 1 M. It is therefore possible that, if the critical substrate concentration for this system lies between these two values, the former experiments are dominated by the binding mechanism, which necessarily points away from urea because urease shows enhanced diffusion with $\alpha > 0$, whereas the latter experiments are dominated by phoresis, which may be directed toward urea as long as $\lambda_c^2 > 0$, which is expected for typical enzymes. Let us now test the plausibility of the proposed hypothesis. The hydrodynamic radius of urease is $R_e = 7$ nm,³ the Michaelis constant of urea–urease is $K = 3$ mM,²⁴ and the diffusion enhancement is $\alpha = 0.3$.³ Using these values and eq 19, we calculate that the critical substrate concentration ρ_s^* will be between 1 M and 1 mM as long as the Derjaguin length λ_e of urease–urea is between 0.034 and 8.4 Å, precisely within the typical range expected for a Derjaguin length.^{19,20} In practice, for the Jee et al. and the Sengupta et al. experiments to be well within the regimes of dominance of the binding and the phoresis mechanisms, respectively, we would expect the Derjaguin length to be somewhere far enough from the two extreme values of the range, say, between 0.1 and 3 Å. As an example, for $\lambda_e = 1$ Å we find the critical urea concentration $\rho_s^* \simeq 30$ mM, which is simultaneously much larger than 1 mM and much smaller than 1 M. As described in the [Supporting Information](#), even rather weak ($\sim 1 k_B T$) and short-ranged (~ 0.75 Å) attractive interactions, consistent with, e.g., van der Waals forces, are sufficient to induce attractive phoresis with $\lambda_e = 1$ Å.

The same type of consistency check can be applied to other studies of chemotaxis of enzymes that show enhanced diffusion. In ref 4, catalase was observed to chemotax toward its substrate, hydrogen peroxide, so we would expect the experiment to be dominated by phoresis. Using the radius $R_e = 4$ nm for catalase,⁴ the diffusion enhancement observed of $\alpha = 0.45$,⁴ the Michaelis constant of $K = 93$ mM,²⁵ and the substrate concentration used in the experiments of $\rho_s = 10$ mM,⁴ we find that those experiments could be dominated by phoresis if $\lambda_e > 2.9$ Å, a somewhat high value but still within the plausible range. In ref 14, hexokinase was observed to chemotax toward its substrate, D-glucose, again implying dominance of phoresis. Using the corresponding values¹⁴ ($R_e = 3.5$ nm, $\alpha = 0.38$, $K = 0.12$ mM, and $\rho_s = 10$ mM), we expect the experiments to be dominated by phoresis if $\lambda_e > 1$ Å, well within reasonable range. In ref 15, acetylcholinesterase was observed to chemotax away from acetylcholine, implying dominance of binding-induced enhanced diffusion in this case. Indeed, using the corresponding values¹⁵ ($R_e = 11$ nm, $\alpha = 0.2$, $K = 0.2$ mM,^{26,27} and $\rho_s = 0.2$ mM), we find that phoresis could dominate only if $\lambda_e > 14$ Å, which is an unreasonably high value.

For completeness, it is worth noting that because the theory presented above does not depend on catalysis in any way, it may be applied not only to enzymes but also to the chemotaxis of small molecules that bind to other molecules. In ref 16, chemotaxis of the molecular dye Rhodamine 6G toward higher concentrations of the polymer Ficoll 400 K was observed. Unfortunately, in this particular system our theoretical description of diffusiophoresis is not expected to apply, given that the polymer (which represents the “substrate”) is much larger than the dye (the “enzyme”), in violation of the assumptions of our derivation (see the [Supporting Informa-](#)

[tion](#)). The chemotactic contribution of binding-induced changes in diffusion, however, should still be applicable. Indeed, in this case binding of the dye to the polymer is expected to inhibit the dye diffusion: because the large polymer diffuses much more slowly than the small dye, we can expect the diffusion coefficient of the complex D_c to be that of the polymer, i.e., $D_c \approx D_{\text{pol}} \ll D_{\text{dye}}$. The change in diffusion coefficient is thus negative with $\alpha \approx (D_{\text{pol}} - D_{\text{dye}})/D_{\text{dye}} \approx -1$, and the binding-induced velocity (eq 18) therefore induces chemotaxis toward higher concentrations of the polymer, as observed experimentally.

In the discussion above, we focused for simplicity on the (presumably most common) case in which the nonspecific interactions with the substrate are similar for the free enzyme and the complex, leading to similar values of the Derjaguin length $\lambda_e \approx \lambda_c$ for the two. Generally, however, λ_e and λ_c may be different from each other: in this case, as described by our full eq 15, the magnitude of the phoretic velocity is affected by substrate binding and, as a consequence, also depends on substrate concentration. Nevertheless, even in this general case, it is easy to show that there is a critical substrate concentration above and below which phoresis and changes in diffusion dominate, respectively. Two particularly interesting limiting cases correspond to those in which the nonspecific interactions with the substrate are negligible either in the free or in the bound state of the enzyme, as would occur if the binding-induced conformational changes of the enzyme significantly affect its surface properties (e.g., surface charges). If the free enzyme is non-interacting ($\lambda_e = 0$ and $\lambda_c \neq 0$), the phoretic velocity is given by $V_{\text{ph}} = \frac{k_B T}{\eta} \lambda_c^2 \frac{\rho_s}{K + \rho_s} \nabla_{\mathbf{R}} \rho_s$ and increases in magnitude with increasing substrate concentration. The critical substrate concentration above which phoresis dominates is then

$$\rho_s^* \equiv \frac{K}{2} \left(\sqrt{1 + \frac{2|\alpha|}{3\pi R_e |\lambda_c|^2 K}} - 1 \right).$$

Alternatively, if the complex is noninteracting ($\lambda_e \neq 0$ and $\lambda_c = 0$), the phoretic velocity is given by $V_{\text{ph}} = \frac{k_B T}{\eta} \lambda_e^2 \frac{K}{K + \rho_s} \nabla_{\mathbf{R}} \rho_s$ and decreases in magnitude with increasing substrate concentration, although this decrease is less pronounced than that of the velocity due to binding-induced changes in the diffusion coefficient (eq 18). For this reason, there will still be a critical substrate concentration above which phoresis dominates, now given by $\rho_s^* \equiv K \left(\frac{|\alpha|}{6\pi R_e |\lambda_e|^2 K} - 1 \right)$.

The competition between phoresis and binding-induced changes in diffusion described in this Letter has some general consequences that may be useful in the design and engineering of intelligent nanomachines. When discussing enzymes, we have focused on the case of enhanced diffusion ($\alpha > 0$) competing against attractive diffusiophoresis ($\lambda^2 > 0$). In this case, for concentrations of substrate above the critical value ρ_s^* , phoresis will dominate, and the nanomachine will drift toward higher concentrations of the substrate. For concentrations below the critical value, binding-induced enhanced diffusion will dominate, and the nanomachine will drift toward lower concentrations of the substrate. This implies that, in a steady-state gradient of substrate, the nanomachine will effectively be repelled from regions with the critical substrate concentration ρ_s^* ; see [Figure 2a](#). Alternatively, it may be possible to engineer nanomachines for which binding of the substrate inhibits diffusion ($\alpha < 0$), while nonspecific interactions with the substrate lead to repulsive diffusiophoresis ($\lambda^2 < 0$). Now, for ρ_s

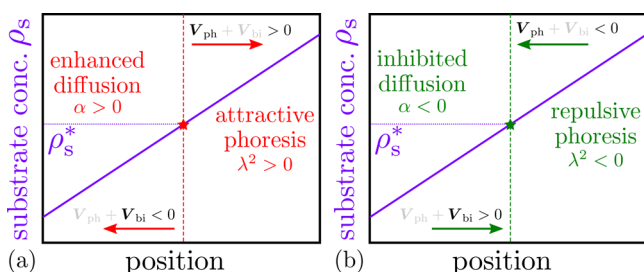


Figure 2. Competition between phoresis and binding-induced changes in diffusion in an externally imposed substrate gradient. (a) A particle subject to binding-induced enhanced diffusion and attractive phoresis will be repelled from regions where the substrate concentration is equal to the critical concentration ρ_s^* . (b) A particle subject to binding-induced inhibited diffusion and repulsive phoresis will be attracted to regions where the substrate concentration is equal to the critical concentration ρ_s^* .

$> \rho_s^*$, phoresis will dominate and the nanomachine will drift toward lower concentrations of the substrate, whereas for $\rho_s < \rho_s^*$, binding-induced inhibited diffusion will dominate and the nanomachine will drift toward higher substrate concentrations. Therefore, in this case the nanomachine will be effectively attracted to regions with the critical substrate concentration ρ_s^* ; see Figure 2b. Exploiting the competition between phoresis and binding-induced changes in diffusion is therefore a promising avenue to achieve finely tuned self-organization at the nanoscale. However, we note that such complex behavior is not always expected to occur: in systems that display enhanced diffusion ($\alpha > 0$) and repulsive diffusiophoresis ($\lambda^2 < 0$), or alternatively inhibited diffusion ($\alpha < 0$) and attractive diffusiophoresis ($\lambda^2 > 0$), the two mechanisms will collaborate instead of competing, and the direction of chemotaxis will not change with substrate concentration.

In summary, we have developed a microscopically detailed theory for the chemotaxis of enzymes (and other small molecules) in the presence of gradients of their substrate. We take into account both the nonspecific interactions between enzyme and substrate and complex formation through the specific binding of the enzyme to the substrate. The experimentally observed Michaelis–Menten-like dependence of the enzyme diffusion coefficient on substrate concentration arises naturally in the theory, and we find a novel contribution to chemotaxis due to binding-induced changes in the diffusion coefficient of the enzyme, which points away from or toward higher substrate concentration depending on whether the substrate enhances or inhibits the diffusion of the enzyme, respectively. In typical cases, the binding-induced contribution to the chemotactic velocity will compete with the diffusiophoretic contribution that arises from the nonspecific enzyme–substrate interactions. Because the two contributions depend differently on the substrate concentration, phoresis will dominate at high substrate concentration, whereas binding-induced changes in diffusion will dominate at low substrate concentration. In this way, we could resolve an apparent contradiction regarding the experimentally observed direction of urease chemotaxis in the presence of urea. We have further checked that our theory is consistent with the available experimental evidence for other enzymes and small molecules. Moreover, the theory can be experimentally tested in a straightforward way by studying the chemotaxis of enzymes in the presence of varying concentrations of their substrate. The competition between phoresis and binding-induced changes in

diffusion described here may be harnessed to engineer nanomachines that are directed toward or away from regions with a specific concentration of a substrate. As a closing remark, it is worth noting that, although we have studied here the effects of substrate concentration, the collective behavior that may arise at high enzyme concentrations^{28,29} remains to be explored both experimentally and theoretically.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.8b00717.

Details of the derivation of the one-particle distribution functions, diffusiophoretic velocities, and the estimation of values of the Derjaguin length for attractive potentials. (PDF)

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The authors declare no competing financial interest.

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■ REFERENCES

- (1) Dey, K. K.; Sen, A. *J. Am. Chem. Soc.* **2017**, *139*, 7666–7676.
- (2) Yu, H.; Jo, K.; Kounovsky, K. L.; Pablo, J. J. D.; Schwartz, D. C. *J. Am. Chem. Soc.* **2009**, *131*, 5722–5723.
- (3) Muddana, H. S.; Sengupta, S.; Mallouk, T. E.; Sen, A.; Butler, P. J. *J. Am. Chem. Soc.* **2010**, *132*, 2110–2111.
- (4) Sengupta, S.; Dey, K. K.; Muddana, H. S.; Tabouillot, T.; Ibele, M. E.; Butler, P. J.; Sen, A. *J. Am. Chem. Soc.* **2013**, *135*, 1406–1414.
- (5) Sengupta, S.; Spiering, M. M.; Dey, K. K.; Duan, W.; Patra, D.; Butler, P. J.; Astumian, R. D.; Benkovic, S. J.; Sen, A. *ACS Nano* **2014**, *8*, 2410–2418.
- (6) Riedel, C.; Gabizon, R.; Wilson, C. A. M.; Hamadani, K.; Tsekouras, K.; Marqusee, S.; Pressé, S.; Bustamante, C. *Nature* **2015**, *517*, 227–30.
- (7) Illien, P.; Zhao, X.; Dey, K. K.; Butler, P. J.; Sen, A.; Golestanian, R. *Nano Lett.* **2017**, *17*, 4415–4420.
- (8) Golestanian, R. *Phys. Rev. Lett.* **2010**, *105*, 018103.
- (9) Golestanian, R. *Phys. Rev. Lett.* **2015**, *115*, 108102.
- (10) Bai, X.; Wolynes, P. G. *J. Chem. Phys.* **2015**, *143*, 165101.
- (11) Hwang, W.; Hyeon, C. *J. Phys. Chem. Lett.* **2017**, *8*, 250–256.
- (12) Illien, P.; Adeleke-Larodo, T.; Golestanian, R. *EPL* **2017**, *119*, 40002.
- (13) Dey, K. K.; Das, S.; Poyton, M. F.; Sengupta, S.; Butler, P. J.; Cremer, P. S.; Sen, A. *ACS Nano* **2014**, *8*, 11941–11949.
- (14) Zhao, X.; Palacci, H.; Yadav, V.; Spiering, M. M.; Gilson, M. K.; Butler, P. J.; Hess, H.; Benkovic, S. J.; Sen, A. *Nat. Chem.* **2017**, *10*, 311–317.
- (15) Jee, A.-Y.; Dutta, S.; Cho, Y.-K.; Tlusty, T.; Granick, S. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 14–18.
- (16) Guha, R.; Mohajerani, F.; Collins, M.; Ghosh, S.; Sen, A.; Velegol, D. *J. Am. Chem. Soc.* **2017**, *139*, 15588–15591.

- (17) Lee, S.; Karplus, M. *J. Chem. Phys.* **1987**, *86*, 1883–1903.
- (18) Derjaguin, B. V.; Sidorenkov, G. P.; Zubashchenko, E. A.; Kiseleva, E. V. *Kolloidn. Zh* **1947**, *9*, 335–47.
- (19) Ebbens, S.; Tu, M. H.; Howse, J. R.; Golestanian, R. *Phys. Rev. E* **2012**, *85*, 020401.
- (20) Anderson, J. L. *Annu. Rev. Fluid Mech.* **1989**, *21*, 61–99.
- (21) Weistuch, C.; Pressé, S. *J. Phys. Chem. B* **2017**, DOI: [10.1021/acs.jpcc.7b06868](https://doi.org/10.1021/acs.jpcc.7b06868).
- (22) Schnitzer, M. J. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **1993**, *48*, 2553–2568.
- (23) Lau, A. W. C.; Lubensky, T. C. *Phys. Rev. E* **2007**, *76*, 011123.
- (24) Krajewska, B. *J. Mol. Catal. B: Enzym.* **2009**, *59*, 9–21.
- (25) Switala, J.; Loewen, P. C. *Arch. Biochem. Biophys.* **2002**, *401*, 145–154.
- (26) Hu, L.; Jiang, G.; Xu, S.; Pan, C.; Zou, H. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1616–1619.
- (27) Pohanka, M.; Hrabínova, M.; Kuca, K.; Simonato, J. P. *Int. J. Mol. Sci.* **2011**, *12*, 2631–2640.
- (28) Saha, S.; Golestanian, R.; Ramaswamy, S. *Phys. Rev. E* **2014**, *89*, 062316.
- (29) Mikhailov, A. S.; Kapral, R. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, E3639–3644.