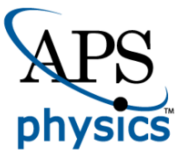

Computational and experimental study of chemotaxis of an ensemble of bacteria attached to a microbead

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Computational and Experimental Study of Chemotaxis of an Ensemble of Bacteria Attached to a Micro-bead

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Abstract

Micro-objects propelled by whole cell actuators, such as flagellated bacteria, are being increasingly studied and considered for a wide variety of applications. In this work, we present theoretical and experimental investigations of chemotactic motility of a 10 μm diameter micro-bead propelled by an ensemble of attached flagellated bacteria. The stochastic model presented here encompasses the behavior of each individual bacterium attached to the micro-bead in a spatiotemporally varying chemo-attractant field. The computational model shows that in a chemotactic environment, the ensemble of bacteria, although constrained, propel the bead in a chemotactic manner with a 67% enhancement in displacement to distance ratio (defined as directionality) compared to non-chemotactic propulsion. The simulation results are validated experimentally. Close agreement between theory and experiments demonstrates the possibility of using the presented model as a predictive tool for other similar bio-hybrid systems.

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I. INTRODUCTION

Eukaryotic cells and unicellular prokaryotic microorganisms have been utilized as whole cell actuators for the controlled propulsion of objects of micro/nano-scale characteristic dimensions [1, 2]. Flagellated bacteria are well known for their superb swimming capabilities at speeds of up to 50 body lengths per second and their viability in a wide range of temperatures and pH. As such, they can be interfaced with microscale structures and be used as actuators [3–5]. These biotic/abiotic engineered systems, also known as bio-hybrid microrobots, are envisioned to be employed in large numbers for applications such as biosensing, transport and delivery of cargo, and minimally invasive treatment of diseases. Although the motility and taxis behavior of unicellular organisms have been extensively studied and modeled, the existing work is not readily applicable to bio-hybrid systems as very often in these systems a large number of microorganisms are configured and constrained in a specific manner. In this work, we have developed a computational stochastic model to investigate the emergent behavior of an ensemble of bacteria attached to a 10 μm spherical micro-bead in presence of a transient chemo-attractant gradient field. The stochastic model presented here encompasses the behavior of each individual bacterium attached to the micro-bead in a spatiotemporally varying chemo-attractant field. The run and tumble rate of each bacterium is adjusted according to their location and the overall force exerted on the micro-bead is calculated at every time step using matrix transformation. It is demonstrated that the constrained population of bacteria exhibits a collective chemotactic behavior evident by a 67% increase in the directionality of the micro-bead's motion in a chemo-attractant gradient field. The proposed model is experimentally validated to demonstrate that a chemo-attractant gradient can be used to autonomously control bio-hybrid microrobots effectively and at a very low cost.

II. MODELING

The comprehensive model for the stochastic motion of the bacteria-propelled micro-bead presented here consists of the following modules:

A. Chemical Concentration Field Model

The model concentration field used in this work is based on a classic chemotaxis assay initially developed by Pfeffer and later modified by J. Adler[6]; a schematic of the setup can be seen in Figure 1. It comprises of a cylindrical capillary that contains a chemo-attractant and is placed at the entrance of an enclosure containing the bacteria-propelled micro-beads. The spatiotemporally varying chemical concentration field that is generated by diffusion of the chemo-attractant from the capillary with initial chemical attractant concentration C_0 is given by [7]:

$$C\left(r,t\right)=\frac{C_0 r_c^2}{2r\sqrt{\pi Dt}}\left[\exp\left(\frac{-r^2}{4Dt}\right)/\left(1+\frac{3r_c r}{4Dt}\right)\right] \tag{1}$$

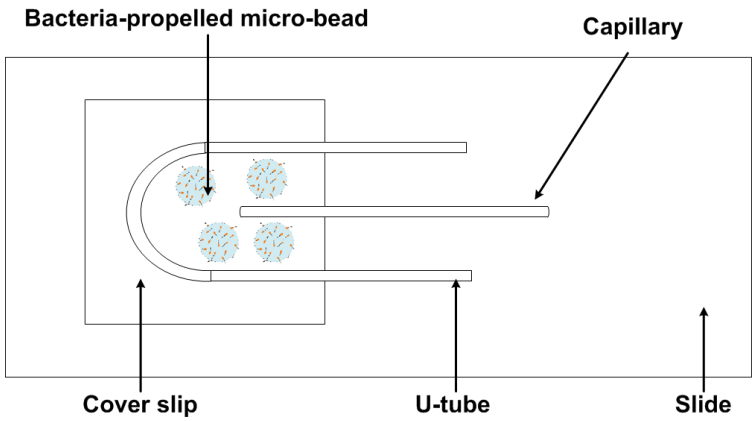

FIG. 1. "(color online)"A capillary-based chemotaxis assay was used to investigate the chemotactic behavior of bacteria-propelled micro-beads. Capillary contains the chemo-attractant solution(1% Casamino acid). Bacteria-propelled micro-beads are enclosed within the experiment area.

where C_0 is the chemo-attractant concentration in the capillary, D is the diffusion coefficient of the chemo-attractant, t is the time from the start of the simulation, r_c is the radius of the capillary, and r is the distance from the capillary to the point of interest in the experiment area. The diffusion coefficient of the chemo-attractant (1% Casamino acid) $D = 8.5 \times 10^{-10}$ m^2/s is determined according to the method described in [8].

B. Stochastic Model of a Bacterium

Flagellated bacteria such as *Escherichia coli* (*E. coli*) and *Serratia marcescens* (*S. marcescens*) possess between four and ten propulsive organelles known as flagella that are 20 nm in diameter and 10 μm long. The motility of bacteria comprises of two distinct states: run and tumble. During the run state, the flagellar motors rotate counterclockwise causing the flagella to coalesce and form a bundle which then produces a propulsion force and causes the bacterium to move forward at constant speed. Each bacterium's run is followed by a tumble. Tumble occurs when one or more of the bacterium's flagellar motors rotate in the clockwise direction causing the disruption of the bundle. During a tumble, the bacterium changes its heading direction randomly to begin a new run cycle. This leads to the stochastic motion of bacteria in 3D and can be modeled as a two-state Markov chain (as shown in Figure 2) with state duration distributions occurring based on an exponential distribution [9]. Therefore, the run and tumble durations can be sampled through the following exponential distribution:

$$f\left(t, \lambda_i\right) = \lambda_i e^{-\lambda_i t} \tag{2}$$

where λ_i is the average rate parameter of the exponential distribution.

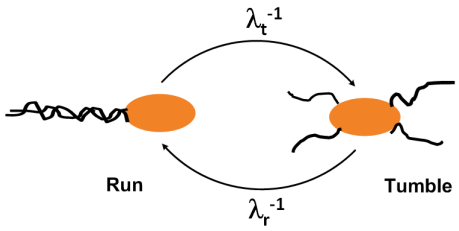

FIG. 2. Two-state continuous Markov chain model for a single bacterium where λ_t and λ_r are the transition rates for a tumble and a run, respectively.

The average run and tumble durations are taken to be respectively $\lambda_r \cong 0.9$ s and $\lambda_t \cong 0.1$ s in an isotropic media (no chemical gradient)[10, 11]. However, in the presence of a positive chemo-attractant gradient, bacteria tend to extend the duration of their runs which leads to a decrease in their tumbling probability. The tumbling probability of a bacterium in a chemical attractant gradient depends on the chemical concentration at the location of the

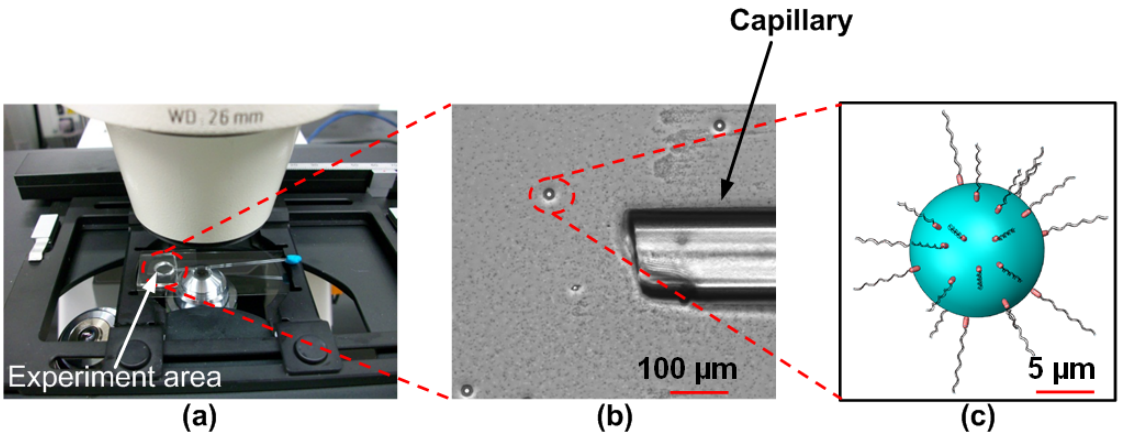

FIG. 3. "(color online)"(a) Image of the chemotaxis assay experimental setup. (b) A microscopy image of the chemotaxis assay experiment. The small particles are micro-beads propelled by an ensemble of bacteria. Chemo-attractant diffuses out from the capillary and the resulting chemo-attractant gradient field affects the dynamics of bacteria-propelled micro-beads. (c) Bacteria attach to the micro-bead at attachment densities of 1 bacterium/7μm^2 and 1 bacterium/11μm^2.

bacterium and the change in chemical concentration the bacterium is subjected to from the start to the end of a run. The tumbling probability can be computed using the following equation [12]:

$$P_t = P_0 \exp(-\sigma[\frac{\partial}{\partial t}(\frac{N_T C}{K_d + C}) + v\nabla(\frac{N_T C}{K_d + C})]) \tag{3}$$

where P_0 is the tumbling probability in an isotropic medium and is equal to 0.1, σ is the chemotactic sensitivity and is equal to 75,000 μm^2/s [13], N_T is the number of homogeneous receptors and is equal to 6, K_d is the dissociation constant which is equal to 0.00014 moles, v is the local speed of the bacterium, and C is the concentration sensed by the bacterium at any particular point in time.

At each time step, the location of each bacterium on the micro-bead with respect to a fixed reference frame is determined and subsequently, the chemical concentration sensed by each bacterium is calculated using Eq. (1). A bacterium is set to increase its running time and therefore decrease its tumbling probability, according to Eq. (3), when the chemical concentration it senses exceeds 5 nanomoles [14]. When in presence of a negative chemical gradient or in an isotropic medium, the bacterium maintains a constant tumbling probability of 0.1.

C. Stochastic model of the micro-bead

The location and orientation of the bacteria attachment on the bead will serve as basis for the development of the stochastic model for dynamics of the 10 μm bead propulsion by an ensemble of attached bacteria. In this model, bacteria were assumed to be uniformly distributed over the surface of the micro-bead. The attachment density was experimentally determined to be about 1 bacterium/$7\mu m^2$ and 1 bacterium/$11\mu m^2$ in the two sets of experiments conducted. Figure 3c illustrates the bacteria attachment configuration.

At the start of each simulation, bacteria are randomly assigned a state of 1 (run) or 0 (tumble). A bacterium with a state of 1 will exert a force at the attachment point that equates to 0.48 pN [1]. The initial direction of the force exerted by each bacterium at the point of attachment on the surface of the bead is randomly chosen. The dynamics of the system is assumed to be dominated by viscous effects and the inertial effects are neglected as the Reynolds number is of order of magnitude of 10^{-4}. Therefore, the overall propulsion force which results from the contributions of all the attached bacteria must equate the translational drag $\vec{F_D}$. Similarly, the overall moment the micro-bead is subjected to is equal to the rotational drag $\vec{M_D}$. This model does not take into consideration the Brownian motion, as its effect on the dynamics of the micro-bead motion is negligible when compared to the effect of bacterial propulsion. Hence, the equations of motion governing the dynamics of the micro-bead are:

$$\vec{F_D} = 6\pi\eta R\vec{V} = \sum_b \vec{F_b} s_b \tag{4}$$

$$\vec{M_D} = 8\pi\eta R^3\vec{\Omega} = \sum_b \vec{r_b} \times \vec{F_b} s_b \tag{5}$$

where $\vec{V}$ is the velocity of the propelled micro-bead, $\eta = 8.9 \times 10^{-16}\ N.s/\mu m^2$ is the dynamic viscosity of the aqueous medium, R is the radius of the micro-bead, and $\vec{\Omega}$ is the angular velocity of the micro-bead. $\vec{r_b}$ and $\vec{F_b}$ are the position vector and propulsion force of each bacterium, respectively. s_b represents the state of each bacterium, its value is 1 when running or 0 when tumbling. Dynamics of the micro-bead were determined by taking into account the effects of all bacterial forces on the translational and rotational displacement for every time step of the simulation. The initial tumble and run time durations are assigned based on the distribution function illustrated in Eq. 2. At the end of every time step, the change in position of each bacterium with respect to a fixed reference frame is determined. According

to the change in the chemical concentration sensed by each bacterium from the start to the end of a run or tumble period, bacteria will individually sample a new run or tumble time duration. In the case of an increase of chemical concentration sensed, the bacterium will sample a new run time from the exponential distribution shown in Eq. 2 with a higher average rate parameter λ_i. The tumble average rate parameter does not change when the chemical gradient is either null or negative.

The emergent parameters utilized to effectively characterize and compare the motion of micro-beads propelled by bacteria in both chemotactic and non-chemotactic settings are the mean velocity of the bead ($\vec{V}$), the total distance traveled by the bead (dist), the overall displacement value (disp), and the directionality. The mean velocity of every simulation run was computed by averaging the ratio of the distance traveled by the center of the bead during a time step to the duration of the time step. The mean velocity values are then averaged to determine a mean velocity value ($\vec{V}$) for the number of times the simulation was run. The total distance (dist) traveled by the bead is a measure of the total length traveled by the centroid of the bead from the start to the end of a single simulation, expressed as: $dist = \sum_{i=0}^{N} \Delta r_{bead,i}$. These values are then averaged over the total number of simulations to obtain an average overall distance. The overall displacement (disp) is a measure of the length of the vector from the start to the end of a single simulation run, expressed as: $disp = \parallel \vec{r_{end}} - \vec{r_{start}} \parallel$. Its final value is obtained by averaging the displacement values over the number of times the simulation was run. The trajectories of bacteria-propelled micro-beads are of stochastic nature, therefore this parameter has not been utilized for comparison between theoretical and experimental results. In order to compare the propulsive behavior in chemotactic and non-chemotactic cases, all numerical simulations were carried out for time durations significantly shorter than the randomization time of the micro-bead. The randomization time is the minimum time required for a system to exhibit its random walk properties and can be obtained from $\tau_R^{-1} = k_B T / 8\pi\eta R^3$, where k_B is the Boltzmann's constant, T is the absolute temperature, η is the dynamic viscosity and R is the radius of the bead [15]. For a 10 μm micro-bead, the randomization time is $\tau_R \cong 11$ minutes. Simulations with time durations of 5 seconds and longer time durations such as 30 seconds were run to observe the effect of simulation durations on the selected emergent parameters. The obtained results in both cases (5 seconds and 30 seconds) showed that the duration of the simulation has no notable effect on the emergent parameters. Significantly shorter time

duration of 5 seconds was then chosen for the simulations mainly to compare simulations with experimental results. Most videos recorded from experiments have micro-beads in the same focal plane (minor changes in the z-direction) for short time periods that range from 5 to 10 seconds.

Both chemotactic and non-chemotactic simulations were run 400 times to identify the average stochastic behavior of the system. The 400 runs were determined to suffice as there were no major differences in the results for a larger numbers of simulation runs. The capillary radius was taken to be 100 μm and the chemical concentration in the capillary was set to C_0=0 and C_0=0.01 moles, respectively for the non-chemotactic and the chemotactic runs. It was assumed that the chemo-attractant concentration within the capillary remains constant throughout the simulation.

III. RESULTS AND DISCUSSIONS

A. Materials and Methods

Wild-type *S. marcescens* (ATCC 274) was grown on L-broth (1% tryptone, 0.5% yeast extract and 0.5% sodium chloride.) culture plates containing 0.65% agar (Difco Bacto agar) and 5 *g/L* glucose. 10 μm polystyrene micro-beads (Fisher Scientific for the 1 bacterium/7μm^2 attachment density and Sigma-Aldrich for the 1 bacterium/11μm^2 attachment density) were washed by repetitive centrifugation in DI water and were finally suspended in motility medium (0.01 M of potassium phosphate, 0.0067 M of sodium chloride, 10^{-4} M of EDTA, 0.01 M of glucose, and 0.002% of Tween-20, pH=7.0). A 10 μL aliquot of 1% (w/v) bead suspension was pipetted behind the edge of the bacteria swarm on the plate and left at room temperature for about 5 minutes to allow bacteria to randomly interact with and adhere to the micro-beads. At the end of the 5 minutes, the bacteria and bead suspension mixture was aspirated and subsequently pipetted in 1 mL of motility medium. A volume of about 200 μL was transferred in the experiment area as shown in Figure 3a. A one-end sealed capillary filled with a 1% casamino acid solution, a commonly used chemo-attractant is then placed at the center of the opening of the experiment area. Figure 3b, depicts a microscope image of the experiment area and the tip of the chemo-attractant capillary.

The motion of the micro-beads was captured using a Zeiss AxioObserver Z1 inverted microscope equipped with an AxioCam HSm camera at 20 frames per second. The images were analyzed using a two-dimensional (2D) particle tracking algorithm developed in MATLAB (The MathWorks, Natick, MA). Briefly, using cell segmentation and image restoration the artifacts existing in most of the captured imaged were removed. This was followed by noise removal and cell boundary recognition using a border following algorithm. Lastly, the nearest-neighbor method was used to link segmented cells in successive frames and to determine the bacteria-propelled beads trajectories.

B. Experimental Validation of Stochastic Model

The simulation results for both chemotactic and non-chemotactic cases are presented in Table I. All four characteristic parameters are affected by the presence of the chemo-attractant concentration field. The micro-beads' mean velocity increased by about 12% from the non-chemotactic to the chemotactic environment. Over the simulation duration of 5 seconds, the directionality of the propelled micro-bead saw an increase of 67% when the micro-bead was in a chemotactic environment with an initial capillary concentration C_0=0.01 mole. A higher directionality value indicates a more directed motion of the bacteria-propelled micro-bead. The increase in the characteristic parameters is attributed to the fact that the chemical gradient sensed by the bacteria affects their tumbling probability P_t. A reduction in the tumbling probability implies an extension in the run period for each bacterium propelling the micro-bead, which will result in not only a larger overall distance but also a more directional path for the motion of the micro-bead.

In order to validate the stochastic model presented here, a chemotaxis assay for bacteria-propelled micro-beads was conducted and the experimental data was compared with the computational results.

The trajectory information was used to determine the average speed, distance, displacement and directionality over the 5 second duration of the experiments. Experimental results are presented in Table I and Table II. Each data point represents an average of at least ten experiments. The trajectories of the bead do vary between experiments because of the stochastic nature of bacteria motion. Representative examples of the bead trajectories are

TABLE I. Summary of results comparing simulations and experiments in chemotactic and non-chemotactic environments for a bacteria attachment density of 1 bacterium/$7\mu m^2$.

	Model		Experiment	
$\mathbf{C_0(mole)}$	**0**	**0.01**	**0**	**0.01**
$\mathbf{V}\,(\mu\mathbf{m/s})$	8.6 ± 0.8	9.6 ± 1.2	8.5 ± 1.7	9.2 ± 1.3
$\mathbf{disp}\,(\mu\mathbf{m})$	11.3 ± 4.4	19.3 ± 3.9	13.4 ± 6.2	21.9 ± 6.4
$\mathbf{dist}\ (\mu\mathbf{m})$	37.2 ± 2.7	41.8 ± 4.9	42.7 ± 8.3	46.1 ± 6.8
disp/dist	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1

TABLE II. Summary of results comparing simulations and experiments in chemotactic and non-chemotactic environments for a bacteria attachment density of 1 bacterium/$11\mu m^2$.

	Model		Experiment	
$\mathbf{C_0(mole)}$	**0**	**0.01**	**0**	**0.01**
$\mathbf{V}\,(\mu\mathbf{m/s})$	8.0 ± 0.6	8.8 ± 0.8	6.4 ± 1.2	7.5 ± 1.5
$\mathbf{disp}\,(\mu\mathbf{m})$	7.4 ± 3.3	18.6 ± 7.5	7.1 ± 4.6	22.2 ± 7.6
$\mathbf{dist}\ (\mu\mathbf{m})$	34.2 ± 2.9	37.9 ± 3.7	38.9 ± 6.0	44.9 ± 9.4
disp/dist	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.5 ± 0.1

shown in Figure 4. Addition of chemo-attractant in the environment contributes to an increase in the directionality value as well as the overall speed of the micro-bead. This can be explained by prolonged force exertion by those attached bacteria which sense an increasing chemical attractant concentration. This will result in an overall extension of the displacement of the micro-bead in a directional manner. The experimental results obtained in both chemotactic and non-chemotactic cases closely match the results obtained from the computational model. The small difference between the computational and experimental results suggest that this computational model can be used as an effective prediction tool in both chemotactic and non-chemotactic environments.

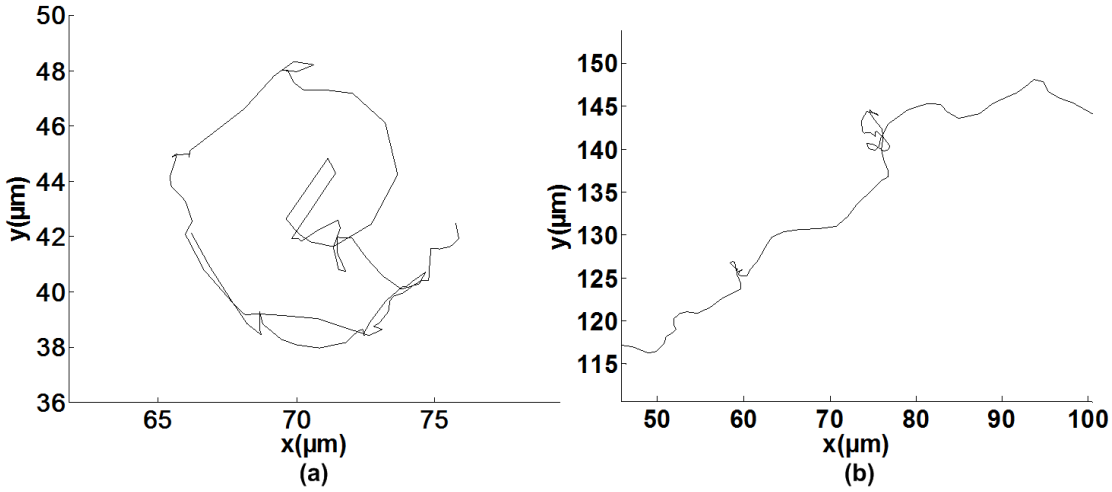

FIG. 4. Sample representative bead trajectory in (a) absence of chemo-attractant, and (b) in a chemo-attractant gradient environment with $C_0 = 0.01$ mole.

C. Effect of Bacteria Attachment Density on Micro-bead Motion

The effect of the density of bacteria attachment was also explored experimentally by constructing bacteria-propelled beads at two attachment densities of: 1 bacterium/$7\mu m^2$ and 1 bacterium/$11\mu m^2$. Table I and Table II show the results for both bacteria attachment densities.

Reducing the bacteria attachment density results in fewer bacteria attached to the micro-bead. In the case of fewer bacteria attached to the micro-bead (no significant non-uniformity in bacteria attachment), the overall force is expected to remain unchanged or slightly reduce (based on degree of variation in attachment density). Indeed, we observe only slight variations in speed and directionality values when the bacteria attachment density is changed from 1 bacterium/$7\mu m^2$ to 1 bacterium/$11\mu m^2$. More than the number of bacteria attached to the micro-bead, the location of attachment is expected to affect the overall behavior. If the attachment density becomes significantly non-uniform, we expect to see a change in the average net resultant force and consequently observe a change in overall speed and displacement to distance ratio. In a prior work, the effect of bacteria attachment site on overall speed in an isotropic (non-chemotactic) environment was investigated [16].

D. Effect of Fluid Viscosity and Particle Size on Micro-bead Motion

The viscosity of the fluid will affect the speed, the displacement and the distance traveled by the micro-bead. However, the directionality will stay unchanged as long as all other parameters are kept same. This is due to the fact that the speed of the micro-bead is proportional to the net force it is subjected to. An increase in the viscosity of the fluid will result in an increase in the drag force and a decrease in the net propulsion force. This will in turn affect the distance traveled for a given period of time. Similarly, the displacement will change with the same rate the distance varies by. Therefore, when the viscosity of the media is changed, the directionality of the micro-bead stays the same while the speed decreases.

The size of the micro-bead should not have a significant effect on the displacement to distance ratio from a non-chemotactic to a chemotactic case assuming that the bacteria attachment density is kept constant. It has previously been demonstrated that for unpatterned particles the net propulsion force is linearly proportional to the radius [1, 3]. According to the Stokes equation, the drag force is also linearly proportional to the radius. Therefore, for the same attachment density the drag and propulsion forces change as a function of radius and the net force should not change significantly. This assertion is supported by the simulation code, which shows that the speed and the ratio of displacement to distance vary slightly when the radius of the bead is changed and the bacteria attachment density is kept intact.

IV. CONCLUSION

In summary, a stochastic model for chemotactic propulsion of micro-beads is presented in this study. The model encompasses key parameters including orientation and location of the attached bacteria, spatiotemporal variations in chemo-attractant concentration field and its effect on run and tumble rates of each of the tens of the attached bacteria. This numerical model was validated experimentally and it was shown that it can effectively describe the emergent dynamics of the motion of a 10 μm micro-bead propelled by an ensemble of flagellated bacteria homogeneously attached, in both chemotactic and non-chemotactic environments. The description of the motion of the micro-bead was based on four emergent parameters of average speed, displacement, distance and directionality. It was determined

that all four parameters increase from the non-chemotactic to the chemotactic case. Most notably, the results show that the presence of a chemo-attractant gradient results in 67% larger directionality values. This proves the feasibility of directed autonomous movement of bio-hybrid micro-robots through the use of chemotaxis. This model can be easily expanded to serve as a predictive tool for other bio-hybrid systems with different whole cell actuators, non-spherical geometries, heterogeneous whole-cell actuator attachment configurations, and different spatiotemporally varying chemical concentration gradients.

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