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CO<sub>2</sub>-driven diffusiophoresis for maintaining a bacteria-free surface<sup>†</sup>Suin Shim,<sup>\*a</sup> Sepideh Khodaparast,<sup>b</sup> Ching-Yao Lai,<sup>c</sup> Jing Yan,<sup>d</sup> Jesse T. Ault,<sup>e</sup> Bhargav Rallabandi,<sup>f</sup> Orest Shardt,<sup>g</sup> and Howard A. Stone<sup>‡a</sup>Received Date  
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Dissolution and dissociation of CO<sub>2</sub> in an aqueous phase induce diffusiophoretic motion of suspended particles with a nonzero surface charge. We report CO<sub>2</sub>-driven diffusiophoresis of colloidal particles and bacterial cells in a circular Hele-Shaw geometry. Combining experiments and model calculations, we identify the characteristic length and time scales of CO<sub>2</sub>-driven diffusiophoresis in relation to system dimensions and CO<sub>2</sub> diffusivity. The motion of colloidal particles driven by a CO<sub>2</sub> gradient is characterized by measuring the average velocities of particles as a function of distance from the CO<sub>2</sub> sources. In the same geometrical configurations, we demonstrate that the directional migration of wild-type *V. cholerae* and a mutant lacking flagella, as well as *S. aureus* and *P. aeruginosa*, near a dissolving CO<sub>2</sub> source is diffusiophoresis, not chemotaxis. Such a directional response of the cells to CO<sub>2</sub> (or an ion) concentration gradient shows that diffusiophoresis of bacteria is achieved independent of cell shape, motility and the Gram stain (cell surface structure). Long-time experiments suggest potential applications for bacterial diffusiophoresis to cleaning systems or anti-biofouling surfaces, by reducing the population of the cells near CO<sub>2</sub> sources.

## Introduction

The manipulation of colloidal particles and materials can be accomplished using fluid shear and application of external fields (gravity, electric, etc.)<sup>1–6</sup>. Chemical gradients enable one variant of this particulate-level control, which is generally referred to as diffusiophoresis<sup>7–12</sup>. Experiments and simulations have been reported for many systems, including different electrolytes, geometries, and particle size and type<sup>13–19</sup>. In particular, CO<sub>2</sub>-driven diffusiophoresis in an aqueous phase is achieved by dissolution and dissociation of CO<sub>2</sub> and H<sub>2</sub>CO<sub>3</sub> in water<sup>7,11</sup>. Due to the large difference in the diffusivity of the two ions (H<sup>+</sup> and HCO<sub>3</sub><sup>–</sup>), a large diffusion potential is created and diffusiophoresis of charged particles is achieved with a dominant electrophoretic contribution. One important potential role for a chemical gradient is the controlled migration of bacteria, as the bacterial cells

have a negative surface charge<sup>20,21</sup>. We investigate this topic, show that CO<sub>2</sub>-driven diffusiophoresis can produce a bacteria-free zone near a surface exposed to dissolved carbon dioxide, and so suggest new routes for reducing bacterial density, and potentially biofilm formation, at surfaces.

Here we use a circular Hele-Shaw geometry with either a CO<sub>2</sub> bubble<sup>22–24</sup> or a CO<sub>2</sub>-pressurized chamber to investigate the transport of polystyrene particles and bacterial cells. Combining experiments and model calculations, we understand the characteristic length and time scales of CO<sub>2</sub>-driven diffusiophoresis in relation to the system dimensions and CO<sub>2</sub> diffusivity. We then study motion of wild-type *Vibrio cholerae* and a mutant lacking flagella ( $\Delta$ *flaA*) near a dissolving CO<sub>2</sub> source, and show that the directional motion is *diffusiophoresis*, not CO<sub>2</sub>-driven *chemotaxis*. Also, our experiments with *Staphylococcus aureus* and *Pseudomonas aeruginosa* show that diffusiophoresis driven by CO<sub>2</sub> dissolution can be achieved for both Gram-positive and Gram-negative bacteria, independent of cell shape. Diffusiophoresis of motile bacterial cells is not identical to that of colloidal particles or immotile cells. We report that the motile bacteria maintain their characteristic swimming speed and show a slow drift in the radially-outward direction due to diffusiophoresis, with an estimate for the Péclet number  $Pe \approx 10^{-3}$ – $10^{-2}$ . Finally, we demonstrate that diffusiophoretic migration of *S. aureus* away from the CO<sub>2</sub> source reduces cell adhesion to a surface, and that the exclusion of *P. aeruginosa* lasts  $\geq 11$  hr after CO<sub>2</sub> is turned off. Thus CO<sub>2</sub>-driven diffusiophoresis can prevent surface contamination or infection by reducing the population of cells approaching an in-

\* sshim@princeton.edu, ‡ hastone@princeton.edu

<sup>a</sup> Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544, USA<sup>b</sup> School of Mechanical Engineering, University of Leeds, Leeds LS2 9JT, UK<sup>c</sup> Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, USA<sup>d</sup> Department of Molecular, Cellular and Developmental Biology, Quantitative Biology Institute, Yale University, New Haven, CT, 06511, USA<sup>e</sup> School of Engineering, Brown University, Providence, Rhode Island 02912, USA<sup>f</sup> Department of Mechanical Engineering, University of California, Riverside, California 92521, USA<sup>g</sup> Bernal Institute and School of Engineering, University of Limerick, Castletroy, Limerick, V94 T9PX, Ireland<sup>†</sup> Electronic Supplementary Information (ESI) at DOI: 10.1039/cXsm00000x/

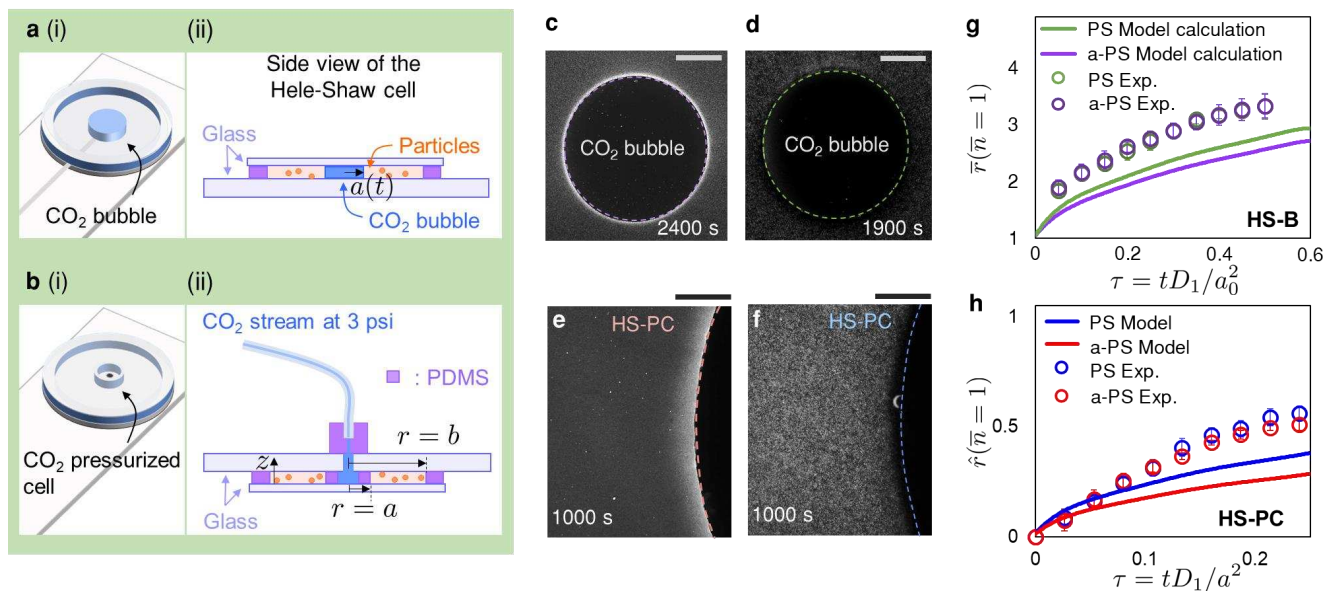


Fig. 1 CO<sub>2</sub>-driven diffusiophoresis of colloidal particles. (a,b) Schematics of experimental setup for (a) HS-B and (b) HS-PC. See SI for details. (c,d) Charged particles near a dissolving CO<sub>2</sub> bubble (HS-B). Distribution of (c) amine-modified polystyrene (a-PS) particles and (d) polystyrene (PS) particles show, respectively, local accumulation and exclusion of charged particles by diffusiophoresis. Bright dots indicate particles. (e,f) Charged particles near the CO<sub>2</sub> source in HS-PC. Distribution of (e) a-PS and (f) PS particles near the fixed CO<sub>2</sub> source show local accumulation and exclusion. (g,h) Comparison between experimental measurements and model calculations (SI) of the macroscopic growth of the accumulation and exclusion zones. (g) Measured and calculated values of  $\bar{r}(\bar{n}=1)$  are plotted versus  $\tau$  for HS-B. (h) Measured and calculated values of  $\hat{r}(\bar{n}=1)$  are plotted versus  $\tau$  for HS-PC. (g,h) No fitting parameter is used. (c-f) Scale bars are 500  $\mu\text{m}$ .

terface, and the CO<sub>2</sub>-enabled reduction of bacterial cell density near a surface can be applied to liquid cleaning systems and anti-biofouling surfaces.

## Results

When an aqueous suspension of charged colloidal particles is exposed to dissolving CO<sub>2</sub>, positively (negatively) charged particles migrate toward (away from) the CO<sub>2</sub> source by diffusiophoresis<sup>7,11</sup>; the fast diffusing H<sup>+</sup> relative to HCO<sub>3</sub><sup>-</sup> from the dissociation of H<sub>2</sub>CO<sub>3</sub> drives the transport. The diffusivity of CO<sub>2</sub> molecules and the ambipolar diffusivity of the ions are, respectively,  $D_1 = 1.91 \times 10^{-9} \text{ m}^2/\text{s}$  and  $D_A = 2.1 \times 10^{-9} \text{ m}^2/\text{s}$ <sup>25</sup>. We investigate the phenomenon using a Hele-Shaw geometry (Fig. 1; a circular cell with radius  $b = 11 \text{ mm}$  and height  $h = 500 \mu\text{m}$ ). Here, diffusiophoresis near a CO<sub>2</sub> source is documented experimentally and calculated (Supplementary Information; SI) for two configurations – a dissolving CO<sub>2</sub> bubble (Fig. 1(a)); we call this system HS-B) and a CO<sub>2</sub>-pressurized chamber (Fig. 1(b); HS-PC) – to examine both moving and fixed boundaries. We provide scaling arguments in derivation of the model (SI) and show that we can assume local chemical equilibrium everywhere in our system. Such condition allows decoupling of the CO<sub>2</sub> diffusion and reaction. Therefore, even though it is the concentration gradient of dissociated ions that drives diffusiophoresis, we choose to use the diffusivity of CO<sub>2</sub> to define the time and velocity scales. The local chemical equilibrium assumption is reasonable as CO<sub>2</sub> dissolves rapidly in water and creates a weak acid; most of the dissolved molecules present in the system are CO<sub>2</sub> (SI).

In HS-B, a CO<sub>2</sub> bubble with radius  $a(t)$  and an initial radius  $a_0$  dissolves at a typical speed  $\frac{da}{dt} \approx \frac{D_1}{a_0} \approx O(0.1-1) \mu\text{m}/\text{s}$  until

the gas exchange reaches steady state<sup>7,22–24</sup>, where  $D_1$  is the diffusivity of CO<sub>2</sub> in water and  $a_0 = 2.5-3 \text{ mm}$  in our system. A bubble reaches its steady state within a nondimensional time  $\tau = \frac{t}{a_0^2/D_1} \approx 1$  ( $\approx 1 \text{ hr}$ ; SI). The typical diffusiophoretic velocity of nearby suspended particles  $u_p = \Gamma_p \nabla \ln c_i$  scales as  $u_p \approx \frac{\Gamma_p}{a_0} \approx O(0.1-1) \mu\text{m}/\text{s}$ , where  $\Gamma_p$  and  $c_i$  are the diffusiophoretic mobility of particles and concentration of ions, respectively (SI, Video 1). The relative motion of particles and the interfaces creates a charge-dependent particle distribution for both HS-B and HS-PC (Fig. 1(c-f)). The radius of the inner chamber (where CO<sub>2</sub> is pressurized) in HS-PC is  $a = 3 \text{ mm}$ , and we use this as the characteristic length scale for HS-PC experiments.

Locally, in the vicinity of the interface, amine-modified

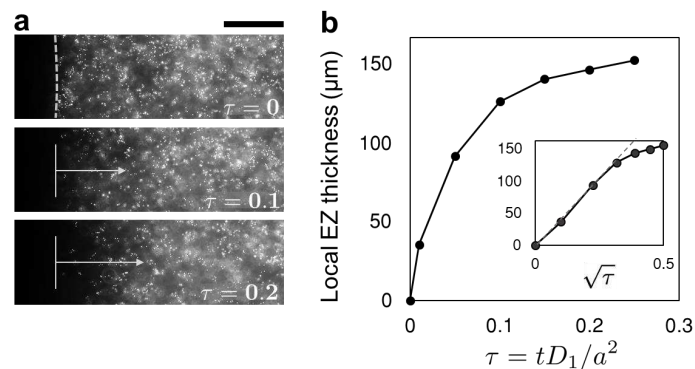


Fig. 2 Growth of local exclusion zone (EZ) in HS-PC system. (a) Images showing exclusion of PS particles near the PDMS wall. Scale bar is 100  $\mu\text{m}$ . (b) Growth of EZ plotted versus nondimensional time. Inset: Growth of EZ plotted versus  $\sqrt{\tau}$  showing early dependence on  $\sqrt{\tau}$ .

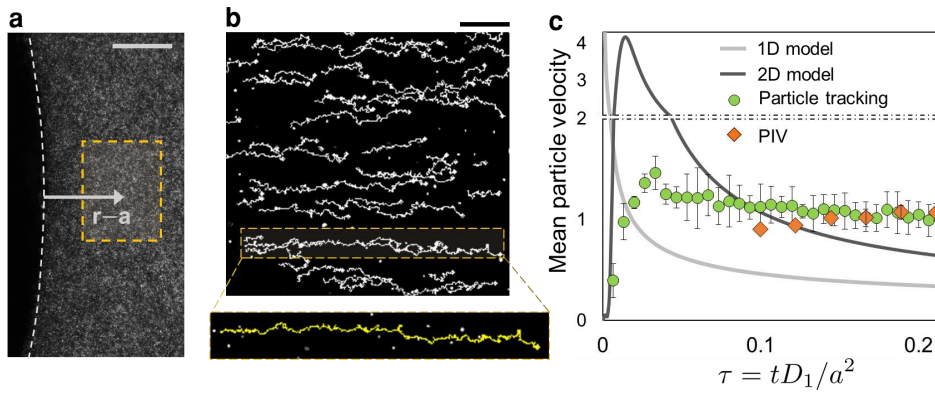


Fig. 3 Velocity measurements for the PS particles. (a) Image showing the fixed boundary and a measurement window for PIV and particle tracking in HS-PC. (b) Images visualizing particle trajectories that are longer than 60 frames (1 min). Bright dots are PS particles. (c) Mean particle (radial) velocities from the PIV and particle tracking are plotted versus nondimensional time and compared with the model calculations. All velocities are nondimensionalized with  $D_1/a$ . Scale bars are, (a)  $300 \mu\text{m}$  and (b)  $50 \mu\text{m}$ .

polystyrene (a-PS, positively charged, diameter =  $1 \mu\text{m}$ ) particles accumulate and form a high particle-density region, whereas polystyrene (PS, negatively charged, diameter =  $1 \mu\text{m}$ ) particles create an exclusion zone (EZ; Fig. 1(c-f)), where the particle concentration is small. Growth of the local EZ in HS-PC (Fig. 2 and SI) is proportional to  $\sqrt{t}$ , similar to EZ formation near an ion-exchange membrane<sup>10</sup>.

Particle accumulation and exclusion also occur on the length scale  $\ell \approx a_0$  (or  $\ell \approx a$ ) in both systems (Videos 2,3). In the one-dimensional model we define the boundaries of such macroscopic accumulation and exclusion as the radial distance where the nondimensional particle concentration  $\bar{n} = n/n_0 = 1$  ( $n(r,t)$  is the particle concentration and  $n_0 = n(r,0)$ ; SI). The nondimensional radial positions are defined as  $\bar{r} = r/a_0$  for HS-B, and  $\hat{r} = \frac{r-a}{b-a}$  for HS-PC. Such boundaries are determined analogously in the experiments (SI) and plotted versus  $\tau$  in Fig. 1(g,h). Both systems show consistent trends in the growth of macroscopic boundaries (Fig. 1(g,h)). In HS-B, the measured boundaries are larger than in the **one-dimensional** model from the beginning due to the initial rapid generation of the bubble, which is not included in the model calculation. Bubble generation introduces fast interface growth, which enhances  $\text{CO}_2$  dissolution at early times and causes faster diffusiophoresis.

The macroscopic boundaries increase up to almost half of the radius of the Hele-Shaw cell ( $\approx 0.5b$ ) within  $\tau = 0.2$  in HS-PC (Fig. 1(h)). In HS-B, as noted from the time evolution of the radius (SI), there is a velocity contribution from the shrinking bubble ( $da/dt$ ) that affects the particle distribution, and this effect lasts up to  $\tau \approx 1$ .

The diffusiophoretic velocities of PS particles in HS-PC can be analyzed further. We selected a measurement window of width =  $400 \mu\text{m}$  and height =  $500 \mu\text{m}$  at a fixed position (Fig. 3(a);  $200 \mu\text{m}$  away from the wall. **All images are obtained at  $z = h/2 = 250 \mu\text{m}$  of the Hele-Shaw cell**). Then we obtained the average velocities of PS particles using particle tracking and particle image velocimetry (PIV) (see Methods for details). Particle trajectories were analyzed with a set of fluorescent images taken every 1 s, and the 1-minute-average of the radial velocities are plotted versus time (Fig. 3(b,c)). The radial velocities are also calculated from one- and two-dimensional models, and plotted versus time for comparison (see SI for details); all velocities are nondimensionalized with  $D_1/a$ . The one-dimensional (1D)

model approximates the Hele-Shaw cell as a disk and solves for radial diffusiophoresis near a dissolving  $\text{CO}_2$  bubble or a fixed  $\text{CO}_2$  chamber. The two-dimensional (2D) model considers a side-view of the Hele-Shaw cell and solves the radial motion of particles under the influence of the diffusiophoretic slip at the top and bottom walls. In the 1D model, we use a constant  $\text{CO}_2$  concentration as the boundary condition, and the diffusiophoretic velocity ( $= \Gamma_p \nabla \ln c_i$ ) is largest at  $t = 0$  due to the sharp concentration gradient. In the 2D model, we used the time-dependent boundary condition for  $\text{CO}_2$  concentration (SI), to include the diffusion of  $\text{CO}_2$  through the PDMS wall. As a result, we obtain an increase then decrease of particle velocities at early time (Fig. 3(c)). A similar trend is observed in the particle tracking analysis, where the nondimensional radial velocity of particles increases up to  $\approx 1.5$  (Fig. 3(c)), then slowly decreases to  $\approx 1$ . As the diffusiophoretic motion of particles develops over time, spatiotemporal variation of the velocities is expected to decrease in the measurement window and the velocimetry can be done for the collective migration, employing PIV.

We denote the distance of the far side of the measurement window from the edge of the  $\text{CO}_2$ -pressurized chamber by  $r - a = \ell_w$  ( $= 600 \mu\text{m}$ ). Then, we note that the characteristic dimensionless time  $\tau = 0.1 = 2.5\tau_{w1}$ , where  $\tau_{w1} = \frac{\ell_w^2/D_1}{a^2/D_1}$ , which is the time needed for  $\text{CO}_2$  to diffuse  $\ell_w$ . Also, the experimental measurements show that the average particle velocity in the measurement window is  $u_w \approx D_1/a \approx 0.7 \mu\text{m/s}$  (Fig. 3(c)). For the particles, it takes them  $\tau_{w2} = \frac{\ell_w/u_w}{a^2/D_1}$ , so  $\tau \approx 0.2$  to travel the distance  $\ell_w$ . From these scaling estimates, we can conclude that  $\tau \approx 0.1$  is the time required for the ion concentration field and the radial diffusiophoretic motion of particles to develop in the region of analysis. Beyond this early time ( $\tau \gtrsim 0.1$ ), three-dimensional complexity near the  $\text{CO}_2$  source is expected to decrease (SI), and we can stably measure the radial velocity of PS particles using PIV. We performed particle image velocimetry (PIV) with the image sequence taken every 10 s, and the 100-second-average of the velocity vectors are plotted versus time (Fig. 3(c)). For further experiments with bacteria, we report spatially resolved PIV measurements obtained for  $\tau > 0.1$  to characterize the diffusiophoresis.

Our understanding of the typical length, time and velocity scales of  $\text{CO}_2$ -driven diffusiophoresis in a Hele-Shaw cell motivated us to extend our investigations to a broader range of particles. Past studies have reported on the use of diffusiophoresis to



achieve migration of living cells<sup>26,27</sup>. For example, the goals of particle manipulation can be to clean a region of liquid, achieve antifouling surfaces, or prevent infection in biological systems. Two previous studies report EZ formation in bacterial suspensions in contact with an ion-exchange membrane (Nafion)<sup>28,29</sup> and discuss possible cleaning applications.

### CO<sub>2</sub>-driven diffusiophoresis of bacteria

As an initial step for demonstrating and investigating diffusiophoresis of bacterial cells by CO<sub>2</sub> dissolution, we chose two types of *V. cholerae* cells – wild-type (WT) and a mutant lacking flagella ( $\Delta flaA$ ), both of which are tagged by mKO (monomeric Kusabira Orange), a bright fluorescent protein<sup>30</sup>. We first confirm the diffusiophoretic contribution to the cell transport in the presence of a dissolving CO<sub>2</sub> source in a Hele-Shaw geometry. Then, using PIV, we measure the velocities of the bacterial cells that move along the ion concentration gradient.

*V. cholerae* is Gram-negative, comma-shaped (length  $\approx 2\text{--}3\ \mu\text{m}$ , diameter  $\approx 1\ \mu\text{m}$ ), and single flagellated. The net surface charge of *V. cholerae* (as well as other bacteria) is negative<sup>31,32</sup>, so the cells are expected to migrate away from a CO<sub>2</sub> source by diffusiophoresis (Video 4). We prepared a bacterial solution by diluting the growth suspension (Methods) to 10% M9 minimal salt solution. No nutrient is provided so no growth and division occur on the time scale of the experiment. Using low salt concentration helps to exclude the effects of coupled ion fluxes on the diffusiophoresis of bacteria<sup>33</sup>. Similar to the particle experiments, we fill the Hele-Shaw cell with bacterial suspension, and introduce either a CO<sub>2</sub> bubble or pressurize CO<sub>2</sub> in the inner chamber. Fluorescence intensities near the CO<sub>2</sub> source for both HS-B and HS-PC systems are measured (SI Fig. S13), and the intensity change shows that the cell number near the dissolving CO<sub>2</sub> source decreased significantly over time.

Similar to the PS particles, the use of PIV near the fixed boundary allows us to measure the diffusiophoretic velocity of the cells by a dissolving CO<sub>2</sub> source. We plotted the velocity vectors versus position in Fig. 4(a,b), where the origin of the  $y$ -axis is at the bottom left corner of the measurement window. After the CO<sub>2</sub> valve is opened at  $\tau = 0$ , both strains of *V. cholerae* migrate radially outward (Fig. 4(a,b)). The radial alignment of the velocity vectors confirms that both motile and immotile *V. cholerae* cells move along the CO<sub>2</sub>-generated ion concentration gradient. In Fig. 4(c), nondimensional velocities ( $\bar{u}_{\text{cell}} = u_{\text{cell}}/(D_1/a)$ ;  $u_{\text{cell}}$  is the  $y$ -average of the measured velocity) of the cells at  $\tau = 0.15$  with and without dissolving CO<sub>2</sub> are plotted versus radial position. Our observation that both motile and immotile cells exhibit directional movement with similar velocities shows that the motion is not a chemotactic effect. **If the motion is CO<sub>2</sub>-driven chemotaxis, the  $\Delta flaA$  strain should not migrate under the concentration gradient.** We also compare the typical velocity scales of the cells and the PS particles in Fig. 4(d). The diffusiophoretic velocity of the bacterial cells is smaller than that of the PS particles, and as a first rationalization, this is due to the smaller diffusiophoretic mobility of the cells. Our comparison suggests that the *V. cholerae* cells have three to four times smaller mobility compared to the PS particles, since the diffusiophoretic velocity scales as  $u_p \approx \frac{\Gamma_p}{a}$ .

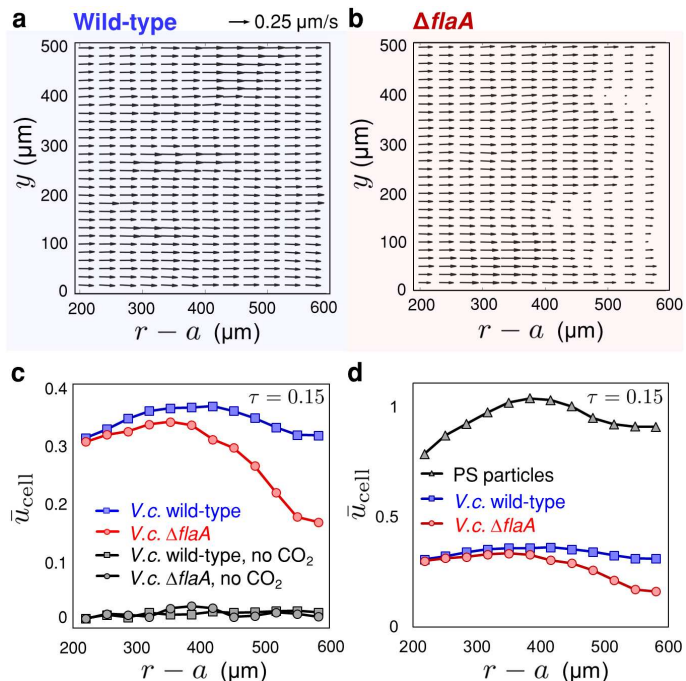


Fig. 4 Velocity measurements for CO<sub>2</sub>-driven diffusiophoresis of *V. cholerae*. (a,b) PIV for *V. cholerae* cells in the HS-PC experiments. Velocity vectors plotted versus position ( $r - a$ ,  $y$ ). Motion of (a) wild-type and (b)  $\Delta flaA$  cells at  $t \approx 10$  minutes. The directional motion of cells is described by aligned velocity vectors in the radially outward direction. (c) Nondimensional  $y$ -averaged velocities obtained from (a,b) and control experiments without CO<sub>2</sub> at  $\tau = 0.15$  ( $\approx 10$  minutes) plotted versus  $r - a$ . (d) Nondimensional  $y$ -averaged velocities of PS particles, WT and  $\Delta flaA$  cells obtained at  $\tau = 0.15$  plotted versus  $r - a$ .

For both wild-type and immotile *V. cholerae*, we use PIV to measure the diffusiophoretic velocities  $\approx 0.3\ \mu\text{m/s}$  (Fig. 4(a,b)) at  $\tau = 0.15$ . This value is much smaller than the typical swimming speed of motile *V. cholerae* ( $\approx 30\ \mu\text{m/s}$ ; SI). Our observations of diffusiophoresis of motile bacteria are that the cells maintain their swimming behavior randomly in all directions, with a slow (radial) drift in the Hele-Shaw cell, following the ion concentration gradient. We interpret the difference in the PIV profiles between the two strains as due to the motility of the wild type cells. Detailed discussions including the effective diffusivity of the motile bacteria are presented in the last section.

To highlight the generality of the phenomenon, two more bacteria were examined – *S. aureus* (mKO labeled, Gram-positive, spherical and immotile)<sup>47</sup> and *P. aeruginosa* (mCherry labeled, Gram-negative, rod shaped and motile)<sup>48</sup> for their diffusiophoretic response to dissolving CO<sub>2</sub> (Fig. 5). For the HS-PC system, the measured velocities ( $\bar{u}_{\text{cell}}$ ) at  $\tau = 0.3$  are plotted versus  $r - a$  in Fig. 5(a). We note that *S. aureus* is slower compared to the other two bacteria. Both *S. aureus* and *P. aeruginosa* have surface zeta potentials  $\zeta \approx -30\ \text{mV}$ <sup>36–38</sup>, so the velocity difference is unexpected given that electrophoresis makes the dominant contribution to CO<sub>2</sub>-driven diffusiophoresis, where  $\Gamma_p$  is a function of  $\zeta$ <sup>9</sup> (SI). One feature of *S. aureus* is that surface adhesive proteins<sup>39</sup> make the cells easily form clusters, which may contribute to the change in the diffusiophoretic velocity and larger clusters are con-

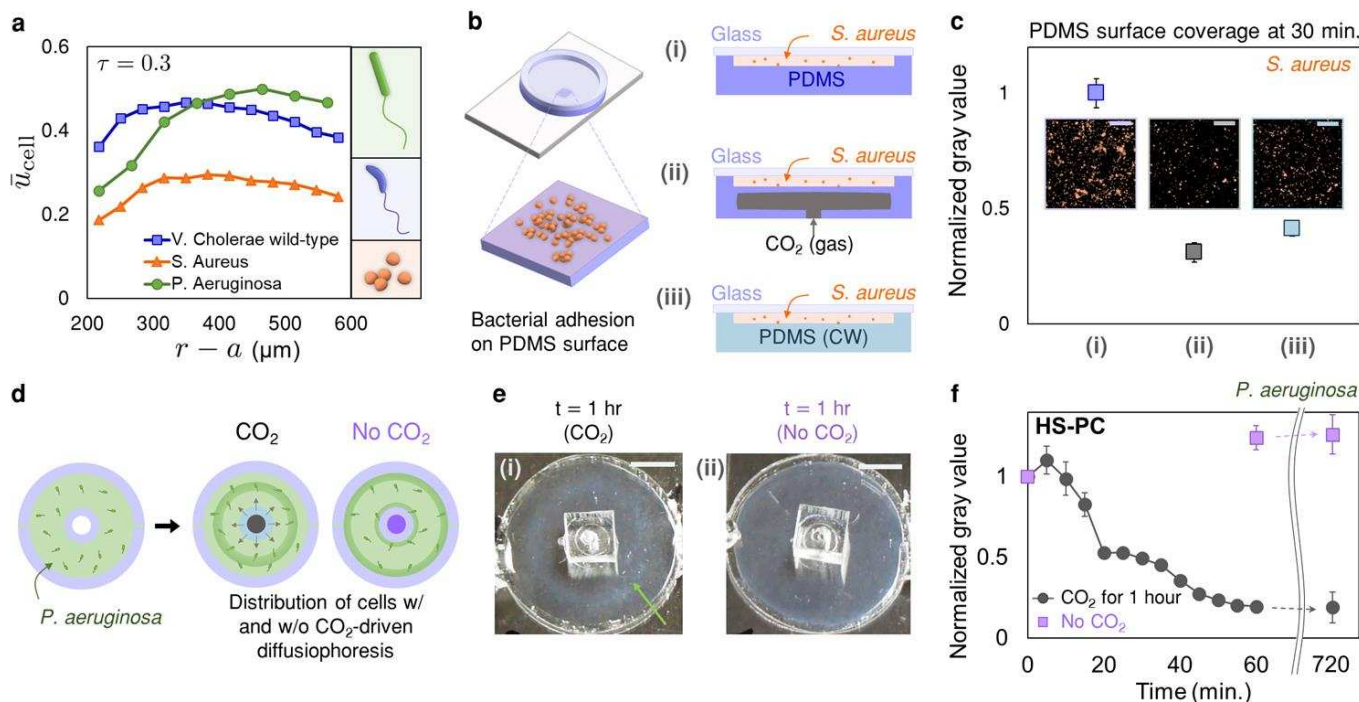


Fig. 5 CO<sub>2</sub>-driven diffusiophoresis of *S. aureus* and *P. aeruginosa*. (a) Velocity measurements of bacterial cells near the CO<sub>2</sub> source (fixed boundary configuration) at  $\tau = 0.3$  ( $t \approx 20$  min). Right panels: schematics showing cell shape. (b) Schematics of surface coverage experiments for *S. aureus* cells on PDMS surfaces. We tested three scenarios: (b-i) plain PDMS substrate in ambient air, (b-ii) pressurized CO<sub>2</sub> gas under the PDMS substrate, and (b-iii) PDMS substrate that is saturated with carbonated water (CW). (c) Intensity measurements for cell coverage on PDMS substrate at  $t = 30$  min. The gray values are normalized by that of a corresponding no-CO<sub>2</sub> experiment. Inset images show surface coverage of *S. aureus* cells on three different PDMS surfaces. (d-f) Long time effect of diffusiophoresis: diffusiophoresis of *P. aeruginosa* cells in the fixed boundary system. (d) Schematic of two control experiments in the fixed boundary configuration. In the presence of a finite-time CO<sub>2</sub> source, cells move radially outward and form an accumulation front (ring structure) in the chamber. On the other hand, when the CO<sub>2</sub> source is replaced by an air source, cells gradually concentrate toward both PDMS walls. (e-i,ii) Images showing the Hele-Shaw chamber at  $t = 1$  hr. (f) Fluorescent intensity measurements near the CO<sub>2</sub> source for two experiments in (d,e). Accumulation and exclusion of bacterial cells near the inner PDMS wall is maintained up to 12 hours, proving long-term effect of diffusiophoresis. Scale bars are (c) 50  $\mu\text{m}$ , and (e) 5 mm.

sistent with the slower diffusiophoretic speeds<sup>15,40</sup>. Also, different diffusiophoretic velocities may arise from different shapes of the cells (Fig. 5(a)). *S. aureus* is approximately spherical with a diameter  $\approx 1$   $\mu\text{m}$ , while *P. aeruginosa* is  $\approx 1\text{-}5$   $\mu\text{m}$  long and  $\approx 0.5\text{-}1$   $\mu\text{m}$  in diameter. Assuming similar  $\zeta$  for the three bacteria and the largest aspect ratio for *P. aeruginosa*, we obtain an aspect-ratio dependence of the diffusiophoretic velocities of the cells<sup>41</sup>. Our results also show that the surface zeta potential is not the only parameter for determining the diffusiophoretic velocity of bacterial cells.

In order to move toward applications for diffusiophoretic bacterial removal using CO<sub>2</sub>, we first quantify the surface coverage of *S. aureus* cells to surfaces under different CO<sub>2</sub> dissolution conditions. Fig. 5(b) illustrates three conditions of PDMS substrates (b-i) without and (b-ii,iii) with CO<sub>2</sub> sources. CO<sub>2</sub> is introduced either by pressurizing CO<sub>2</sub> below a PDMS membrane or by saturating PDMS with carbonated water (CW; Methods). Then the surface coverage 30 minutes after injection of a *S. aureus* suspension into the chamber above the PDMS substrate was measured by the fluorescent intensity (Fig. 5(c)). We observe that the surface coverage of *S. aureus* is significantly decreased in the presence of CO<sub>2</sub>, and this is evidence that the CO<sub>2</sub>-generated ion concentration gradient removed the cells from the vicinity of the substrate,

resulting in reduced surface contamination. Below, with a set of experiments with *P. aeruginosa*, we demonstrate that the bacterial removal lasts  $\geq 11$  hours.

In many discussions of diffusiophoresis, the focus is often on boosting migration of micron-sized particles. This is a clear advantage of the phenomenon, owing to  $\Gamma_p \gg D_p$  ( $D_p \approx 10^{-13}$  m<sup>2</sup>/s is the Stokes-Einstein diffusivity of a micron-sized particle). However, smaller particle diffusivity compared to the diffusiophoretic mobility, can also mean that, after eliminating the gradient, the time required for particles to recover their original distribution is long ( $\approx 1000$  hr for 1  $\mu\text{m}$  particles to move 1 mm by diffusion; SI).

*P. aeruginosa* is known for surviving in dilute media<sup>42</sup> for more than 10 days so it is suitable for long-time diffusiophoretic experiments. We performed HS-PC experiments with and without 1 hr of CO<sub>2</sub> dissolution in *P. aeruginosa* suspension (Fig. 5(d,e)). We predict and observe that, by CO<sub>2</sub>-driven diffusiophoresis, bacterial cells move away from the inner wall, whereas without any CO<sub>2</sub> source, the cells concentrate near both inner and outer PDMS walls where there is an air source. The CO<sub>2</sub> valve was open only for 1 hr, but the result of diffusiophoresis lasted longer than 12 hours (Fig. 5(f)). The distribution of the cells at  $t = 12$  hr are presented in the SI.

Finally, we discuss the diffusiophoresis of *motile* bacteria since it is not identical to that of polystyrene particles or immotile cells of similar size. Both *V. cholerae* and *P. aeruginosa* are single flagellated organisms and exhibit run-reverse patterns<sup>43</sup>. The effective diffusivity of motile bacteria with typical translational speed  $v_t$  and reverse time  $t_r$  can be estimated as  $D_{\text{eff}} \approx v_t^2 t_r \approx O(100) \mu\text{m}^2/\text{s}$  (SI). It is observed (Video 5) that the flow of cells under an ion concentration gradient is a slow advection with an estimated Péclet number  $Pe = \frac{u_p \ell_{\text{cell}}}{D_{\text{eff}}} \approx 10^{-3} - 10^{-2}$ , where  $\ell_{\text{cell}} \approx 1 \mu\text{m}$  is the characteristic length of bacterial cells. Cells are observed to swim randomly with their characteristic velocity  $\approx 30\text{-}50 \mu\text{m}/\text{s}$  (SI), with a slow drift (radially outward) due to the diffusiophoretic contribution (Video 5).

In this article, we demonstrate directed diffusiophoretic migration of colloidal particles and different types of bacteria under a concentration gradient of  $\text{CO}_2$ , and discuss possible applications of  $\text{CO}_2$ -driven diffusiophoresis to prevent contamination. For example, delaying biofilm formation can improve the anti-biofouling properties of surfaces. Currently we are working to realize the mechanism at various salt concentrations to broaden the understanding to physiological or higher salinity conditions. Moreover, understanding the characteristic scales and flow structure near the  $\text{CO}_2$  source is crucial for the next steps of  $\text{CO}_2$ -driven diffusiophoresis for mitigating bacterial growth on, or bacterial removal from, surfaces.

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## Contributions

S.S. and H.A.S. conceived the project. S.S. designed and performed all experiments. S.K. conducted PIV. S.S., C.Y.L., J.T.A. conducted numerical calculations. J.Y. constructed the *V. cholerae* strains. S.S., B.R., O.S., and H.A.S. set up the theoretical model. All authors contributed to data analysis and writing the paper.

## Conflicts of interest

The authors declare no competing financial interests. The corresponding authors have filed a provisional patent application related to this work.

## Materials and Methods

### 1. Materials and design

The Hele-Shaw cell is made by placing a PDMS spacer between a slide glass and a cover glass (Fig. S1). A  $500 \mu\text{m}$ -thick sheet of PDMS was made by standard soft lithography, then cut into circular pieces that have the outer and inner diameters, respectively, 25 mm and 22 mm. For injecting liquid and a  $\text{CO}_2$  bubble into

the Hele-Shaw cell, 1-mm-wide inlet and outlet were made  $180^\circ$  apart from each other. For the Hele-Shaw cell with an inner cell, a circular spacer (PDMS) of 6-mm outer diameter and 4-mm inner diameter was placed concentrically to the outer wall. Then, through the hole on the slide glass,  $\text{CO}_2$  is pressurized in the inner cell at a constant pressure ( $p_1 = 3$  psi).

## 2. $\text{CO}_2$ -driven diffusiophoresis of polystyrene particles

### A. Particle suspension

Amine-modified polystyrene particles (Sigma Aldrich, diameter =  $1 \mu\text{m}$ , batches MKBX6372V and MKCF6014) were diluted in deionized (DI) water at 0.05 vol%. Polystyrene particles (Thermo Fisher Scientific, diameter =  $1 \mu\text{m}$ ) were diluted in DI water at 0.03 vol%.

### B. Charged particles near a dissolving $\text{CO}_2$ bubble

In the Hele-Shaw cell shown in Fig. S1(e), a  $\text{CO}_2$  bubble is injected to obtain an initial diameter  $d_0 \approx 5\text{-}6$  mm. Then with an inverted microscope (Leica DMI4000B), fluorescent images of particles near the moving boundary were taken every 2 seconds.

### C. Charged particles near a $\text{CO}_2$ -pressurized cell

After the particles were loaded in the chamber between the inner cell and the outer wall (Fig. S1(f)), gaseous  $\text{CO}_2$  was filled in the inner cell and maintained pressurized at  $p_1 = 3$  psig. We fix the starting time of the experiments to be the time point when the valve of  $\text{CO}_2$  stream is open. Then with an inverted microscope, fluorescent images of particles near the fixed boundary were taken every 2 seconds.

### D. Long-time experiments with a $\text{CO}_2$ bubble

A square Hele-Shaw cell ( $2 \text{ cm} \times 2 \text{ cm}$ ) was first filled with the particle suspension (a-PS and PS), then a  $\text{CO}_2$  bubble was injected. After  $\approx 20$  minutes, the inlet and outlet were sealed with epoxy adhesive to prevent evaporation-driven particle flow. There are always small air bubbles trapped between the liquid phase and the epoxy (in the inlet and outlet), and thus the epoxy was not in contact with the solutions. Photos of the chambers were taken up to 16 hours (SI Section V).

## 3. $\text{CO}_2$ -driven diffusiophoresis of *V. cholerae* cells

### A. Bacterial suspension

All *V. cholerae* strains used in this study are derivatives of the wild-type *Vibrio cholerae* O1 biovar El Tor strain C6706<sup>44</sup>. Additional mutations were engineered into this *V. cholerae* strain using *Escherichia coli* S17- $\lambda$ pir carrying pKAS32<sup>45</sup>. All *V. cholerae* strains (see SI for strain information) were grown overnight at  $37^\circ \text{C}$  in liquid LB with shaking. After 20 hours of growth in LB, the sample was centrifuged at  $\approx 500 \times g$  for 7-8 minutes. After removing supernatant, the pellet was resuspended in 5 mL M9 minimal salt solution and allowed to regrow for an additional 2 hours (at  $37^\circ \text{C}$ , with shaking) to achieve exponential phase ( $\text{OD} \approx 1$ ). Subsequently, for the  $\text{CO}_2$ -driven diffusiophoresis experiments, the suspension was centrifuged at  $\approx 500 \times g$  for 7-8 minutes, and resuspended into 5 mL 10 % M9 minimal salt solution to achieve

an  $OD_{600}=0.23$  (Beckman Coulter DU730). A detailed strain list is provided in Supplementary Table 1.

#### B. *V. cholerae* near a dissolving $CO_2$ bubble

Similar to the particle experiments, the bacterial suspension was first introduced into the Hele-Shaw cell (Fig. S1(e)), then a  $CO_2$  bubble ( $a_0 = 5-6$  mm) was injected. Fluorescent images were taken near the bubble interface every 30 minutes.

#### C. *V. cholerae* near a $CO_2$ -pressurized cell

The Hele-Shaw cell (Fig. S1(f)) was filled with the *V. cholerae* suspension, and the  $CO_2$  valve was opened. For intensity measurements and PIV, fluorescent images were taken every 5 minutes and every 10 s, respectively.

#### 4. Particle image velocimetry and particle tracking

Micro Particle Image Velocimetry (PIV) was applied to the digital images of fluorescent bacterial cells and PS particles, which were captured every 10 s, with spatial resolution of  $0.5 \mu\text{m}$  per pixel, using the Leica DFC360 FX (mounted on Leica DMI4000B) and a 20x objective. An ensemble cross-correlation scheme was performed using the open source software, JPIV ([www.jpiv.vennemann-online.de](http://www.jpiv.vennemann-online.de)). For data presented here, square interrogation windows of  $128 \times 128$  pixels with 50% overlap were used to obtain a final vector spacing of  $32 \mu\text{m}$  in the calculated velocity field. Wider interrogation windows were used for analyzing the *P. aeruginosa* strain leading to radial vector spacing of  $49 \mu\text{m}$ .

For individual particle tracking, the fluorescent images obtained every 1 second are used. Particle trajectories are obtained with Mosaic Particle Tracker<sup>46</sup> (ImageJ). Trajectories that are longer than 60 frames are chosen for velocity measurements. 1-minute-average of radial velocities are plotted for analysis.

#### 5. $CO_2$ -driven diffusiophoresis of *S. aureus* cells

##### A. Bacterial suspension

*S. aureus* strains<sup>47</sup> were grown overnight at  $37^\circ\text{C}$  in liquid LB with shaking. After 16-17 hours of growth in LB, the sample was centrifuged at  $\approx 500 \times g$  for 7-8 minutes. After removing supernatant, the pellet was resuspended in 5 mL M9 minimal salt solution and allowed to regrow for an additional 2 hours (at  $37^\circ\text{C}$ , with shaking). Then for the  $CO_2$ -driven diffusiophoresis experiments, the suspension was centrifuged at  $\approx 500 \times g$  for 7-8 minutes, and resuspended into 10 % M9 minimal salt solution (after removing supernatant) to achieve an  $OD_{600}=0.3$ .

##### B. *S. aureus* near a $CO_2$ -pressurized cell

The Hele-Shaw cell (Fig. S1(f)) was filled with the *S. aureus* suspension, and  $CO_2$  valve was open. For PIV, fluorescent images were taken every 10 seconds.

##### C. *S. aureus* adhesion to a PDMS substrate

The test chamber was made similar to the Hele-Shaw cell used in the main experiments with a  $500 \mu\text{m}$  PDMS spacer (Fig. S1(e)), but instead of the slide glass, a 4-mm thick PDMS block was bonded as the bottom substrate (Main text Fig. 3(b-i)). For the

scenario in Fig. 3(b-ii), a 1.5-2 mm thick PDMS sheet was used for the bottom substrate, and it was bonded to another circular chamber (diameter  $\approx 1$  cm, height  $\approx 100 \mu\text{m}$ ), which is for pressurizing  $CO_2$ . For the third case (Fig. 3(b-iii)), a 4-mm thick, carbonated-water (CW)-saturated (for 24 hours in total, the CW was replaced after 20 hours) PDMS block was bonded as the bottom substrate. Fluorescent images were taken every 10 seconds.

#### 6. $CO_2$ -driven diffusiophoresis of *P. aeruginosa* cells

##### A. Bacterial suspension

*P. aeruginosa* strains<sup>48</sup> were grown overnight at  $37^\circ\text{C}$  in liquid LB with shaking. After 24-25 hours of growth in LB, the sample was centrifuged at  $\approx 500 \times g$  for 7-8 minutes. After removing supernatant, the pellet was resuspended in M9 minimal salt solution and allowed to regrow for an additional 2 hours (at  $37^\circ\text{C}$ , with shaking). Then for the  $CO_2$ -driven diffusiophoresis experiments, the suspension was centrifuged at  $\approx 500 \times g$  for 7-8 minutes, and resuspended into 10 % M9 minimal salt solution (after removing supernatant) to achieve an  $OD_{600}=0.3$ .

##### B. *P. aeruginosa* near a $CO_2$ -pressurized cell

The Hele-Shaw cell (Fig. S1(f)) was filled with the *P. aeruginosa* suspension, and  $CO_2$  valve was opened. For PIV, fluorescent images were taken every 10 seconds.

For the long-time experiment, the  $CO_2$  valve was kept open for 1 hour, and fluorescent images were obtained every 5 minutes up to 1 hour. Then the fluorescent images were taken at  $t = 12$  hr (11 hours after the  $CO_2$  stream was turned off). For the control experiments without  $CO_2$ , fluorescent images were taken at  $t = 0, 1, \text{ and } 12$  hr.

##### C. *P. aeruginosa* near a dissolving $CO_2$ bubble

The bacterial suspension was introduced to a circular Hele-Shaw cell, then a  $CO_2$  bubble was injected. Then macroscopic images and brightfield microscope images (5 fps) were obtained at  $t = 5$  and 20 minutes.

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