Dynamic states of swimming bacteria in a nematic liquid crystal cell with homeotropic alignment

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Keywords: living liquid crystals, swimming bacteria, Bacillus subtilis, lyotropic chromonic liquid crystals, bacterial tumbling, control of bacterial swimming regimes, active matter

Supplementary material for this article is available online

Abstract
Flagellated bacteria such as Escherichia coli and Bacillus subtilis exhibit effective mechanisms for swimming in fluids and exploring the surrounding environment. In isotropic fluids such as water, the bacteria change swimming direction through the run-and-tumble process. Lyotropic chromonic liquid crystals (LCLCs) have been introduced recently as an anisotropic environment in which the direction of preferred orientation, the director, guides the bacterial trajectories. In this work, we describe the behavior of bacteria B. subtilis in a homeotropic LCLC geometry, in which the director is perpendicular to the bounding plates of a shallow cell. We demonstrate that the bacteria are capable of overcoming the stabilizing elastic forces of the LCLC and swim perpendicularly to the imposed director (and parallel to the bounding plates). The effect is explained by a finite surface anchoring of the director at the bacterial body; the role of surface anchoring is analyzed by numerical simulations of a rod realigning in an otherwise uniform director field. Shear flows produced by a swimming bacterium cause director distortions around its body, as evidenced by experiments and numerical simulations. These distortions contribute to a repulsive force that keeps the swimming bacterium at a distance of a few micrometers away from the bounding plates. The homeotropic alignment of the director imposes two different scenarios of bacterial tumbling: one with an 180° reversal of the horizontal velocity and the other with the realignment of the bacterium by two consecutive 90° turns. In the second case, the angle between the bacterial body and the imposed director changes from 90° to 0° and then back to 90°; the new direction of swimming does not correlate with the previous swimming direction.

1. Introduction
Swimming bacteria have developed efficient mechanisms of propulsion in isotropic environments at low Reynolds numbers that employ rotation of helicoidal flagella [1–3]. Liquid suspensions of swimming bacteria represent an interesting system with out-of-equilibrium dynamics that can be used to power micromachines [4, 5]. Remarkably, the bacterial suspensions are not limited by the constraints of equilibrium thermodynamics. Recently, it was proposed [6–10] to use a lyotropic chromonic liquid crystal (LCLC) [11, 12] as an anisotropic medium to host the swimming bacteria. An LCLC represents a water dispersion of plank-like organic molecules
with a relatively flat rigid polyaromatic core and polar groups at the periphery [11–13]. In water, these molecules aggregate face-to-face in order to minimize the areas of unfavorable contact with water. Unlike their surfactant-based micellar and thermotropic counterparts, the LCLCs are not toxic to biological organisms [14].

Recent experiments demonstrate that the prevailing direction of swimming is parallel to the director \( \mathbf{n} \), i.e. to the average direction of LCLC orientation \( \langle \mathbf{n} \rangle \equiv -\mathbf{n} \), \( |\langle \mathbf{n} \rangle| = 1 \) [7, 8]. The orientational order of the LCLC environment can be controlled by temperature, the concentration of liquid crystal organic molecules, external electromagnetic fields and by surface alignment of the director [9, 11, 12]. The dispersion of swimming bacteria in LCLC, also called a living liquid crystal [8], offers new opportunities to control the dynamic behavior of the bacteria.

The studies of swimming bacteria in LCLCs have been performed mostly for sandwich-type cells, in which the LCLC is confined between two glass plates, with the director being uniformly aligned along a certain direction in the plane of the cell (planar alignment). It has been shown that rod-like flagellated bacteria prefer to swim along the director [7, 8, 15, 16]. It is assumed that the LCLC imposes a strong elastic torque on the bacterial body that keeps it parallel to the director [7, 8, 15–17].

In this work, we present experimental and computational studies of rod-like bacteria *Bacillus subtilis* swimming in a homeotropic nematic cell in which the director is perpendicular to the plates of a sandwich cell. The cell thickness \( d \) is larger than the bacterial body length \( l \). At first sight, such an alignment of the LCLC must prohibit in-plane swimming, as the director coerces the bacteria to align and move perpendicularly to the plates. In fact, our experiments demonstrate that besides this ‘trapped’ vertical spinning state (VSP), there is also a state of horizontal swimming (HSW) in the plane of the cell, perpendicular to the imposed director and about 1–3 \( \mu \text{m} \) away from the substrates. The choice between the vertical entrapment and HSW can be controlled by changing the thickness of the cell and by the swimming motility of the bacteria that depend on the amount of oxygen available to them. A higher oxygen concentration and thus a higher activity favor swimming perpendicularly to the director.

Anisotropy of the nematic LCLC also controls how the bacteria change their swimming direction. Namely, we observe two scenarios: (1) backtracking, i.e. a 180° reversal of the horizontal velocity, and (2) a sequence of two turns by 90° in the vertical plane. In the second scenario, the angle between the bacterial body and the imposed alignment changes from 90° to 0° and then back to 90°; there is no correlation in the swimming directions before and after the vertical tumble.

Two different dynamic states and the transitions between them demonstrate an important role of surface anchoring and elastic response of the LCLC to the shear flows generated by the swimming bacteria. Swimming bacteria distort the surrounding director field, thus creating corridors in their wakes for other bacteria to follow. At high concentrations, these tubular corridors develop into membrane-like attractors of swimming bacteria.

We conducted a numerical study of a single bacterium in a nematic environment for two different model settings. In the first model, we consider the bacterium as a non-motile rod that re-orients away from the imposed homeotropic director. This model allows us to evaluate the relative importance of the nematic elasticity and surface anchoring of the director at the bacterial body but neglects the effects of LCLC flows. The analysis demonstrates that the ability of bacteria to realign away from the substrates-imposed director should be attributed to the finite strength of director anchoring at the bacterial body. In the second model, the bacterium, still represented as a rod, is assumed to swim perpendicularly to the externally imposed director in order to evaluate the director deformations and velocity patterns caused by the shear flows. The director deformations in the second model are of quadrupolar type, markedly different from the antisymmetric director around a realigning non-motile rod, but matching the symmetry of the director field observed experimentally around a swimming bacterium. This director configuration stabilizes the horizontal alignment of the rod-like bacterial body as it corresponds to the local minimum of the elastic energy and thus supports HSW.

### 2. Experimental methods

#### 2.1. Liquid crystal medium

We used an LCLC based on aqueous solutions of disodium cromoglycate (DSCG, Spectrum Chemical, 98% purity) that forms a nematic phase at room temperatures for concentrations of 0.3–0.45 mol kg\(^{-1}\), or 12.5–18 wt\% [12]. Upon heating, the DSCG/water composition experiences a phase transition from the nematic (N) to the nematic/isotropic coexistence (N + I), then to the isotropic (I) phase [18]. To support bacterial activity, we dissolved DSCG in the water-based terrific broth (TB) medium (Sigma T5574) at 13 wt\%. The transition temperature from N to N + I, \( T_{N-N+I}^{13\%TB} \approx 28.5\ ^\circ\text{C} \), is higher than that of 13% DSCG in pure water, \( T_{N-N+I}^{13\%\text{H}_{2}O} \approx 25\ ^\circ\text{C} \), due to the presence of additional salts in the TB solution [19, 20], and is similar to that of 15% DSCG/water solution. To estimate the viscoelastic properties of 13% DSCG/TB LCLC, we used values for 15% DSCG/water LCLC extrapolated from experimental measurements [21], and obtained the following...
parameters of the nematic environment surrounding the bacteria: splay constant $K_1 \approx 7.5 \text{ pN}$, twist constant $K_2 \approx 0.6 \text{ pN}$, bend constant $K_3 \approx 21 \text{ pN}$; splay viscosity $\eta_{\text{splay}} \approx 7 \text{ kg m}^{-1}\text{s}^{-1}$, twist viscosity $\eta_{\text{twist}} \approx 6.3 \text{ kg m}^{-1}\text{s}^{-1}$ and bend viscosity $\eta_{\text{bend}} \approx 0.01 \text{ kg m}^{-1}\text{s}^{-1}$.

2.2. Bacteria preparation
We used the strain 1085 of B. subtilis to conduct all the experiments. B. subtilis has a rod-like body of length $l \approx (3-10) \mu\text{m}$ with a bundle of attached flagella; the total length $L$ is typically 15 $\mu\text{m}$ [8, 15]. The bacteria were first grown on LB agar plates, then transferred into TB to grow until an early stationary stage when the bacterial concentration reached $c_0 \approx 8 \times 10^{14} \text{ m}^{-3}$. B. subtilis bacteria were then separated by centrifuge and mixed into the LCLC to achieve the desired concentration. In the studies of isolated bacteria behavior in LCLCs, we used bacterial concentrations smaller than $c_0$; to study their collective behavior, we used $c \approx (3-6)c_0$. To increase their resistance to oxygen starvation, the bacteria were grown in sealed vials under microaerobic conditions [22].

2.3. Homeotropic cells
Glass substrates were first soaked in Piranha solution (98% sulfuric acid: 30% H$_2$O$_2$ solution = 3:1 by volume) at 80 °C for at least 1 h to thoroughly remove organic residuals. Then the substrates were rinsed with deionized water (DI water, resistivity 18.2 $\Omega\text{m}$) and dried with clean nitrogen gas. The substrates were then soaked in water solution containing 0.4 wt% N, N-dimethyl-N-octadecyl-3-aminopropyl trimethoxysilyl chloride (DMOAP, Sigma-Aldrich) and 0.1 wt% H$_2$SO$_4$ for 1 h [23]. After being rinsed with DI water and dried with nitrogen gas, the substrates were left in 110 °C nitrogen environment overnight. Two treated glass substrates were bonded face-to-face by NOA65 UV glue mixed with a glass spacer to achieve the desired cell gap $d$. Except in the experiments with an external control of oxygen concentration, the open edges of the cell were sealed with epoxy glue after filling in LCLC to prevent water evaporation and a change of DSCG concentration. The deposited DMOAP layer yielded a homeotropic alignment of the LCLC that was stable for several hours. After that, an anchoring transition occurred and the surface alignment became planar [23]. Our experiments were performed within 30 min of the cell preparation while the homeotropic alignment remained intact.

2.4. Oxygen concentration and temperature control
We used a Linkman hot-stage LTS-120/PE94 to control the temperature with 0.1 °C accuracy. The hot-stage provides a well-sealed chamber so that the local environment can be switched between oxygen and nitrogen through gas regulators and a homemade LabView program. The gas flux rate was kept low (1.5–150 cm$^3$ s$^{-1}$) to minimize temperature fluctuation and evaporation. The change of DCSG concentration was less than 1%, estimated by comparing the phase transition temperatures before and after the experiments.

2.5. Optical imaging and data analysis
We used an inverted microscope Olympus IX71 of phase contrast mode with a motorized stage, mounted on a piezoelectric isolation platform Herzan TS-150 and HS2000C camera (Emergent Vision) to study the motion of bacteria in thin cells. A polarizing microscope (Nikon E600) equipped with the Cambridge Research Incorporation (CRI) Abrio LC-PolScope package, which uses an electrically controlled optical retarder to map the local values of optical retardance $\Gamma$ and orientation of the optic axis (the director in our case) of birefringent samples [24, 25], was used to examine the optical retardance induced by swimming bacteria. We also used a fluorescent polarizing confocal microscope (FCPM) [26, 27] to scan vertical cross-sections of the LCLC cell, and also to image horizontal optical slices at different depths, with the steps of $0.2 \mu\text{m}$ along the normal to the cell. The image analysis was performed with a custom made MATLAB script (using the tracking algorithm developed by Crocker and Grier [28]).

3. Results
The cell represents a slab of an LCLC with suspended bacteria confined between two glass plates located at $z = \pm d/2$. The cell thickness $d$ varied in the range 4.6–25 $\mu\text{m}$. We define the laboratory Cartesian coordinates $(x, y, z)$, where the $xy$-plane is parallel to the bounding substrates, and the background LC director $\hat{n}_0 = (0, 0, 1)$, set by the homeotropic surface treatment of the substrates. We first describe the results for a low concentration of bacteria, $c \sim 0.01c_0 \sim 10^{13} \text{ m}^{-3}$. At this concentration, bacterial interactions and collective effects can be neglected. Previous studies of diluted suspensions of flagellated bacteria have established that an individual bacterium swims along the director if the LCLC is aligned in a planar fashion [7, 8, 15, 29, 30]. Immobilized rod-like bacteria also align parallel to the director [17]. The effect is attributed to the anisotropic and elastic nature of the orientational order that minimizes the energy of elastic distortions. As demonstrated below, B. subtilis are capable of overcoming the elastic constraints and swimming perpendicularly to the
imposed director. We find two different dynamic states of an individual bacterium in a homeotropic cell. We call them vertical spinning (VSP) and horizontal swimming (HSW), as previously defined. We also find two different tumbling regimes by which the bacteria change their swimming direction.

3.1. VSP and HSW states
In the VSP state, the bacterium placed in a relatively thick cell, \( d > l \), is parallel to the substrates-imposed director \( \hat{n}_0 = (0, 0, 1) \), with negligible lateral \( xy \)-displacements (figure 1(a)). LC PolScope measurements yield a very small optical retardance near a VSP bacterium, \( \Gamma \approx (1–2) \text{ nm} \) (figure 1(b)) indicating that the director does not deviate much from the \( z \)-axis. The deviation angle \( \theta \) of the LC director can be estimated as \( \theta \approx \sqrt{\Gamma/(d \Delta n)} \), which yields \( 3^\circ–5^\circ \) if one uses the independently measured birefringence \( \Delta n = 0.016 \) of the studied nematic DSCG. The bacterially spins with an average frequency \( f \approx 2 \text{ Hz} \) (see movie 1 of the supplementary information (SI) available online at stacks.iop.org/NJP/19/055006/mmedia), which is close to the frequency measured for bacteria swimming in planar cells [8]. Optical imaging of the vertical cross-sections of the cells by the FCPM reveals that the spinning bacteria are located close to the glass substrates (see figure 2(a)). The probability of finding a bacterium in the middle of the cell is low, but not zero (see figure 2(c)); the VSP bacteria are likely to swim back and forth along \( \hat{n}_0 = (0, 0, 1) \).

The spinning \( B. \text{ subtilis} \) bacteria placed in cells with \( d > l \) are able to escape the trap imposed by the homeotropic director and to swim in the plane of the cell. Under a phase contrast microscope, the HSW bacterium appears as a dark rod with a bright trace behind its body (figure 1(a), SI movie 2). The bright trace visualizes director distortions caused by the rotating flagella [8] (see figure 1(a)). Horizontal FCPM scans at different \( z \)-depths reveal that the probability of finding swimming bacteria peaks at about 1–3 \( \mu \text{m} \) away from the glass plate, and practically vanishes in the middle of the cell (see figures 2(b), (c), SI movies 3, 4). The bacterial trajectories are not necessarily parallel to the glass plates; with the microscope focused at a certain value of \( z \), we observe that an individual bacterium can swim away from the focus and then re-emerge in the focal plane.

The bacteria intermittently change their states between HSW and VSP. The probability of the HSW state in planar cells with \( d < l \) is always in the HSW state, similarly to the case of anomalously long \( P. \text{ mirabilis} \) studied by Mushenheim et al [30]. Figure 3 presents the state diagram as a correlation between the swimming ability (measured as the maximum speed of the bacterium tracked in the experiment) and the length \( l \) of the tracked bacterium’s body.

The average speed of \( B. \text{ subtilis} \) in the homeotropic cells is \( v_{av} \approx 7 \text{ \mu m/s} \). It is not sensitive to the cell gap \( d \) when the latter is in the range 4.6–25 \( \mu \text{m} \) (see figure 4(a)). The swimming speed is about half as fast as the speed in planar cells [15]. Qualitatively, this decrease correlates with the observation [31] that the viscous resistance of the LCLC DSCG to a spherical particle diffusing perpendicularly to the director is by a factor 1.5 higher than the viscous resistance for displacements parallel to the director. The swimming bacterium tilts the director away from the \( z \)-axis, as revealed by the optical retardance \( \Gamma = (10–20) \text{ nm} \) around it (see figures 1(c), 4(b)). The bacterial body itself produces less than 1 nm of optical retardance when viewed in the isotropic phase of the LCLC. Therefore, the nonzero \( \Gamma \) must be attributed to the director tilt. Figure 1(c) suggests that the director tilts in the plane that is parallel to the axis of a swimming bacterium. Twist deformations are also possible; LC PolScope does not detect weak twists because of the so-called Mauguin regime, i.e. guiding the polarization of light by a weakly twisted optic axis [25].
To quantify the director distortions in the HSW state, we use the following population averaged quantities:

(i) maximum optical retardance $\Gamma_{\text{max}}$ that characterizes the maximum tilt of the director from the vertical axis;
(ii) average retardance $\Gamma_{\text{ave}}$ that characterizes an average director tilt; (iii) length $L_{xy}'$ and the width $w$ of the birefringent zone in the $xy$-plane formed by the distorted director. As the cell gap $d$ increases, $\Gamma_{\text{max}}$, $\Gamma_{\text{ave}}$ and $L_{xy}'$ increase, while $w$ remains almost the same (see figures 4(b), (c)).
3.2. Spinning-to-swimming transition

To better understand the dynamics of VSP-to-HSW transition, we placed the experimental cell into a chamber with an adjustable nitrogen/oxygen ratio to control bacterial motility. The glass cell was not completely sealed in order to allow the gas to diffuse into the LCLC bulk. Bacteria deactivated by filling the chamber with nitrogen do not spin, do not swim, and align along the homeotropic director. As oxygen is turned on, the bacteria start to order to allow the gas to diffuse into the LCLC bulk. Bacteria deactivated by filling the chamber with nitrogen do not swim, and then some of them switch into the HSW mode (see SI movie 5). This VSP-to-HSW transition occurs in two steps. First, the bacterial body turns away from the z-axis, thus increasing its projection length $l_{xy}$ in the xy-plane (see figures 5(a,0–1.2s), (b), (d), (e)). At this stage, there is practically no in-plane displacement of the bacterium (figure 5(b)). During the second stage, the flagella bundle also realigns towards the horizontal plane, as displayed by the increased length of the bright wake seen under the phase contrast microscope, $l_{w}$ (figures 5(a), 1.2s and beyond, (b)). After the appearance of the bright wake, the bacterium starts to swim away (figures 5(b), (f)).

To determine the symmetry of the director field in the HSW state, we measure the optical retardance induced by swimming bacteria in a cell that is tilted by different angles $\Omega$ away from the optical axis of the microscope (see figure 6). The tilt plane contains the bacterial body and its swimming direction, normal to the cell, and the optic axis of the microscope. We find that opposite directions of the tilt, $\Omega$ and $-\Omega$, produce the same population-average optical retardance $\Gamma_{\text{max}}(\Omega) \approx \Gamma_{\text{max}}(-\Omega)$, suggesting that the splay-bend distortions around the HSW bacterium are roughly of a quadrupolar type, with mirror symmetry with respect to two planes, one horizontal that contains the bacterial body and another that is perpendicular to the bacterial body (see figures 5(f) and 6(a)). The mirror symmetries should be understood only in relation to the splay-bend distortions, since there might also be some twist which breaks these symmetries. The quadrupolar director pattern is very different from the antisymmetric bend that forms around an inactive rod reorienting in a uniform nematic (for illustrations, see [32–35] and figures 12(a), (c) below). The antisymmetric deformations should show the retardance $\Gamma_{\text{max}}(\Omega)$ being either larger or smaller than $\Gamma_{\text{max}}(-\Omega)$ depending on the direction of tilt. Numerical simulations presented in section 4.3 show that the quadrupolar pattern is caused by the flows generated by a swimming bacterium.

3.3. Change of swimming directions through backtracking

Rod-like peritrichous bacteria (i.e. bacteria with multiple flagella located randomly on their body) change their direction of swimming in an isotropic liquid environment through a ‘run and tumble’ mechanism (see, for example, [3]). During a run, all flagella are gathered into a helical bundle at the rear end of the bacterium that rotates CCW (when viewed from behind). To tumble, one or more of the flagellar motors reverse their sense of circular motion, thus disassembling the bundle; the filaments separated from the bundle cause realignment of
the bacterial body along a new direction. Reassembly of the bundle and reorientation of the bacterium complete the tumble event. A nematic environment suppresses tumbling: the available literature does not report a single example of tumbling in an LCLC. In planar LCLC cells with a low concentration of bacteria, they swim along the director (6–8, 36). In homeotropic cells, bacteria _P. mirabilis_ that are longer than the cell thickness were also

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**Figure 5.** Switching from spinning to swimming in a _d_ ≈ 10 μm homeotropic cell. (a) Sequence of images illustrating switching dynamics. Scale bar 10 μm. (b) Bacterium body length projected on the _xy_-plane, _l_ _xy_, wake length, _l_ _w_, and the displacement of the bacterium as a function of time. There is an approximately 1 s delay between the increase of _l_ _xy_ and _l_ _w_. Schematics of a bacterium in (c) VSP, (d)–(e) switching, and (f) HSW state. θ is the polar angle of the local director that depends on the vertical coordinate _z_.

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**Figure 6.** LC-PolScope measurement of the histogram of the maximum optical retardance induced by each swimming bacterium in the _d_ = (10 ± 0.1) μm cell, at different observation angles Ω defined in (a). (b) Retardance for zero tilt; (c)–(f) Γ _max_ increases as |Ω| increases, due to the increased optical path at an oblique incidence of light. The average optical retardance is the same for the positive and negative tilt angles, suggesting that the director field is of a quadrupolar peak-mode shown in (a).
observed to swim without tumbling [30]. The behavior of *B. subtilis* in homeotropic cells with a thickness larger than the bacterium length is dramatically different, exhibiting two scenarios by which the swimming direction changes; namely, (i) reversal of the horizontal velocity through backtracking and (ii) through HSW–VSP–HSW transitions reminiscent of a full somersault, which we call ‘vertical tumbling’ for brevity. This behavior is different from the backtracking demonstrated by uni-flagellated bacteria *Vibrio alginolyticus* with the turning mechanism rooted in the buckling instability of the flagellar hook [37]. In the forward runs of *V. alginolyticus*, the flagellum pushes the head, and in the backward runs, the flagellum pulls the head. In scenario (i) exhibited by *B. subtilis* in the homeotropic LCLC cells, the flagellum bundle disassembles at one end of the bacterial body and then reassembles at the opposite end, thus the bundle always pushes the body forward. Below we discuss the details.

The 180° reversal of bacterial velocity (see figure 7, SI movie 6) is especially frequent in the case of long (*l* > 6 μm) and fast (*v* ≈ 10 μm s⁻¹) bacteria. At the transition from forward to reversed swimming, the bacterium appears to be immobile for (0.2–0.4) s, with its velocity close to zero. During this period, the body remains horizontal as its projected length, *l*<sub>xy</sub>, remains constant, (see figures 7(a), (b)). What changes is the location of the flagella bundle. Prior to the tumble, the bundle is located at the rear end of the HSW bacterium (see figures 1(a), 5(a), 7(a)). When the bacterium stops, the image of the flagella bundle disappears, suggesting its disassembly (figure 7(a)). The white wake then reappears at the opposite end of the body (figure 7(a–II)). Since the white trace always follows the swimming body, the experiment eliminates the possibility of swimming with the bundle leading the way. Rotation of the reassembled bundle behind the body causes a new run that is reversed with respect to the old run (figure 7(c)). The switching of the flagella bundle must have happened during the ‘immobile’ step that takes (0.2–0.4) s. The swimming speed at the beginning of the new run is always

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Figure 7. Bacteria backtracking. (a) A bacterium reverses its forward swimming (I, trajectory of the bacterial body’s center is marked with red dots) to the opposite direction (II, green dots) through backtracking. Black arrows mark the position of the bacterial body’s center of current image. Scale bar 10 μm. (b) After the backtracking, the swimming direction of the bacterium in the xy plane changes by 180°, Δφ<sub>v</sub> ≈ 180°, while the bacterial body length projected on the plane, *l*<sub>xy</sub>, remains constant. The amplitude of the bacteria velocity, |*v*<sub>xy</sub>|, rapidly drops to zero, and rises to a high value at the beginning stage of reversed swimming (II). However, the wake’s length *l*<sub>w</sub> remains finite at the end of the forward motion, decreases to zero at about 0.3 s after the bacterium reverses the swimming direction, and increases to a value comparable to forward swimming. (c) The tumbling angle, defined as the change of swimming direction before and after the tumbling events, distributes narrowly around 180° for backtracking.
somewhat higher than the average speed of a smooth run (figure 7(b)). One of the possible reasons might be the residual director distortions in the wake of the old run that facilitate the new run.

3.4. Change of swimming directions through vertical tumbling

The second scenario is the sequence of HSW–VSP and VSP–HSW transitions (see figure 8, SI movie 7) usually seen for short ($l < 4 \mu$m) and slow ($v \approx 5 \mu$m s$^{-1}$) bacteria. It starts with a reversal and backtracking motion similar to the scenario described above in figures 7(a), (b) (see stages I and II in figure 8(b)). However, instead of continuing to swim horizontally, the bacterium realigns parallel to the $z$-axis (stage III in figure 8(b)). The polar angle $\alpha$ and the horizontal velocity $|v_{xy}|$ of the bacterium both drop to zero (figure 8(b)). Note that $\alpha$ is estimated as $\alpha = \sin^{-1} \frac{I_{xy}}{l} = \pi - \overline{p}$; here $l \approx l_{xy}^{\max}$ is approximated by the maximum projected length, $\overline{p}$ is the average width of the bacterium. The bacterium gets trapped in this VSP state for up to a few seconds. Then it realigns towards the horizontal plane and swims along a new direction (stage IV in figure 8(b)). The new and the old runs are not correlated as the angle between them is random (see figure 8(c)). The process is reminiscent of a full somersault as the bacterium realigns in the vertical plane.

3.5. Linear collaborative propulsion

When the bacteria concentration is increased, the chance of finding them in close proximity to each other increases. As a result, the bacteria show a propensity to collective behavior. For concentrations about $3c_0$, motile bacteria in cells thicker than 10 $\mu$m form temporary train-like groups with end-to-end coordination.
The train-forming bacteria swim along the same trajectory, similar to ants following the same trail. The guiding direction is provided by the director distortions created by the leading bacterium. The most frequent scenario is when a bacterium finds itself in the wake of a leading bacterium and follows the leader (see figures 9(a)–(e), SI movie 8). This process can be cascaded, with a group of up to four bacteria swimming one after another. Such a chaining does not appear to alter the speed of individual bacteria significantly. For example, if the leader is too slow, the follower might overtake it and move to the head of the train. The slower former leader is then left behind and eventually loses its connection to the fast new leader. When the gap between the bacteria increases so that the follower’s body starts to separate from the bright image of the leader’s wake, the train disassembles, and the follower bacterium chooses its own new path. It implies that the trains are kept together by the director distortions created by the leading bacterium that help the follower to move; once the director has enough time to relax to the original vertical orientation, the connectivity is lost. Parts of the train can also drop out through collisions with other objects (such as immobile particles, spinning or swimming bacteria) along the trajectory.

3.6. High concentration: two-dimensional collaborative propulsion
When the concentration of bacteria is increased to about 6c0, we observe the formation of transient ‘membrane’ regions of director distortions, formed by bacteria swimming in the plane of the cell and exhibiting some degree of parallel local alignment with the neighbors of both end-to-end and side-by-side types (see figure 10, SI movie 9). The optical phase retardation \( \Gamma \) of these membrane regions is on the order of 80 nm, much higher than the retardation of a single HSW bacterium (10–20 nm). The bacterial trajectories within the membranes show different scenarios, with bacteria swimming in ‘trains’ with end-to-tail arrangements, in a side-by-side manner, and often without visible parallelism (figure 10(b)). The transition from a homeotropic to a collectively distorted state is reversible. Once the oxygen is turned off and nitrogen is flushed into the cell, bacteria lose their activity (figure 10(c), SI movie 10). The birefringent membranes shrink and condense the bacteria into clusters of high density; see the blue box in figure 10(c). In some cases, small membrane areas remain birefringent for several minutes after the bacteria stop swimming. These membranes are stabilized by their topology, with the director realigning from the homeotropic state at the bounding plates to the horizontal orientation in the center. The 180° reorientation of the director across the cell can be transformed into a uniform homeotropic state only through nucleation and propagation of disclinations [38]. The latter might be pinned at impurities, which explains the metastable character of the membranes.

4. Discussion

4.1. VSP and HSW states
When placed in an LCLC, elongated flagellated bacteria, such as Escherichia coli [6], P. mirabilis [7, 30, 36], and B. subtilis [8, 15], all preserve their ability to swim [15, 36], being propelled by the rotation of flagella bundles. The CCW rotation of the flagella is counterbalanced by a CW rotation of the bacterial body. The propulsion is
force-free (as the thrust force of the flagella is compensated by a viscous drag force) and torque free. If the LCLC is aligned in the planar fashion, the bacteria swim along the director, provided their concentration is low \[8, 15, 29, 30, 36\]. When the alignment is homeotropic, \(\mathbf{n}_0 = (0, 0, 1)\), previous experiments with \(P.\ mirabilis\) in thick cells (thicker than the bacterial length) showed that the bacteria remain parallel to \(\mathbf{n}_0\) and thus get stuck at the bounding plates. This regime is reminiscent of the VSP state observed in our work (see figures 1, 2, 5, 7). HSW perpendicular to the imposed director was observed only for anomalously long \(P.\ mirabilis\) with a length larger than the homeotropic cell thickness so that they simply cannot fit the gap \[30\].

The dynamic of swimming in homeotropic cells observed in this work is very different from the previously reported scenarios. As shown in figures 1, 5, 7, \(B.\ subtilis\) of short length \(l < d\) is capable of escaping the VSP state and swimming along the horizontal direction, moving perpendicularly to the imposed director. Before analyzing it in the next section, we discuss briefly the experimentally determined times of director relaxation in the wake of a swimming bacterium; these experimental results suggest that the bacterium induces director deformations of all three bulk modes, i.e. bend, splay, and twist.

From the measured average velocity and the total length of the visible birefringent imprint of the swimming bacterium onto the horizontal plane, \(L'_{xy}\), we can estimate the relaxation time \(t\) over which the perturbed director in the wake of a bacterium relaxes back into the homeotropic state (figures 4(d), 5(f)). For a horizontal swimmer, \(L'_{xy} = L + vt\), where \(L\) is the total length of the body along with the attached flagellum. Since the 24 nm-thick flagella are not visible directly, we estimate \(L\) by a linear extrapolation of \(L'_{xy}\) to \(d = 0\), to obtain \(L = L'_{xy,d=0} \approx 15 \mu m\) (figure 3(c)) assuming \(t \rightarrow 0\) when \(d \rightarrow 0\). The latter estimate is in good agreement with the anticipated length of \(B.\ subtilis\) \[8\]. The relationship \(t = (L'_{xy} - L)/v\) yields \(t\) in the range (0.5–2) s, depending on the cell thickness \(d\) (figure 4(d)). The dependence \(t(d)\) is close to being linear. In order to
understand why this dependence is indeed linear, we consider the mechanisms influencing the rate of director relaxation into the equilibrium homeotropic state.

Propagation of the director distortions through a cell of thickness \( d \) requires a time 
\[
  t \approx \frac{\eta d^2}{\pi K} \left( 1 + \frac{K}{W} + \frac{K/W_p}{W} \right),
\]
where \( \eta \) is the effective viscosity and \( K \) is the average elastic Frank constant of the LCLC. The estimate is valid when the surface orientation of the director is fixed by an infinitely strong anchoring [38]. In what follows, we consider a bacterium as being one of the plates of the nematic cell, separated from the other plate by \( d \). This is a rough approximation, as the true distance is somewhat smaller than \( d \) as the bacteria swim at some small distance from the boundary (figures 2(b), (c)). The relaxation time increases if the surface anchoring, i.e. the work needed to deviate the director away from the orientation imposed by the surfaces, is finite [38]:

\[
  t \approx \frac{\eta d^2}{\pi K} \left( 1 + \frac{K}{W} + \frac{K/W_p}{W} \right),
\]
where \( W_p \) is the anchoring energy coefficient at the bounding plate and \( W \) is the anchoring strength at the bacterial body. The DMOAP-coated glass plates used in the experiments are known to produce only weak homeotropic anchoring for DSCG, the anchoring strength at the bacterial body. The DMOAP-coated glass plates used in the experiments are regardless of whether they are immobile or swimming bacteria, the latter assumption is unacceptable since the director fields around a non-motile rod and an actual swimming rod-like bacterium are different [39]. This modification \( d \rightarrow d + K/W_p + W \) explains qualitatively why the dependence of \( \tau(d) \) is not strictly quadratic (see figure 4(d)).

The measured \( \tau \) should also depend on the viscous coefficient \( \eta \), the value of which in LCLC depends on the type of deformations [21]. In the relaxation of splay and twist, the viscosities are large, \( \eta_{\text{play}} \approx \eta_{\text{twist}} \approx 7 \text{ kg m}^{-1} \text{s}^{-1} \), while for bend, the viscosity is much lower, \( \eta_{\text{bend}} \approx 0.01 \text{ kg m}^{-1} \text{s}^{-1} \) [21]. If the bacterium-permutated director relaxes through bend (which is the case in slight deviations from \( \tilde{n}_b = (0, 0, 1) \)), then the relaxation time is 
\[
  \tau_{\text{bend}} = \frac{\eta_{\text{bend}}}{\eta_{\text{twist}}} \left( d + K/W + K/W_p^2/\pi K_{\text{bend}} \right) \approx 0.03 \text{ s} \text{ for typical } d = (4.6 - 25) \mu m, K/W_p \approx 10 \mu m, K_{\text{bend}} \approx 21 \text{ pN and } \eta_{\text{bend}} \approx 0.01 \text{ kg m}^{-1} \text{s}^{-1}.
\]
The estimated time is significantly shorter than the experimental one, \( \tau \approx (0.3 - 2) \text{ s} \). The relaxation time of splay can be estimated as 
\[
  \tau_{\text{play}} = \frac{\eta_{\text{play}}}{\eta_{\text{twist}}} \left( d + K/W + K/W_p^2/\pi K_{\text{play}} \right) \approx 60 \text{ to } 150 \text{ s for } d = (4.6 - 20) \mu m, \text{ since } \eta_{\text{play}} \approx 7 \text{ kg m}^{-1} \text{s}^{-1} \text{ and } K_{\text{play}} \approx 7.5 \text{ pN} \text{ [21]. For twist, the characteristic times are even longer, on the order of minutes, as } \eta_{\text{twist}} \approx \eta_{\text{play}} \text{ but } K_{\text{twist}} \approx 0.1 \text{ K}_{\text{play}} \text{ [21]. In other words, the experimental value } \tau \approx (0.3 - 2) \text{ s} \text{ (figure 4(d)) suggests that the bacterium-induced deformations cannot only bend but also some splay and potentially twist. We now proceed to the discussion of bacterial realignment from the VSP to the HSW state.}

4.2. Spinning-to-swimming transition and finite surface anchoring

The transition starts with the tilt of the bacterium. The realignment is powered by the thrust force \( f_{\text{th}} \) generated by the rotating bundle of flagella that is responsible for the bacterial propulsion. The thrust force measured for various bacterial species with peritrichous flagella varies broadly, depending on the species and the work load of the rotary motor. Under a light load, such as free swimming in water, for E. coli, \( f_{\text{th}} \approx 0.5 \text{ pN} \) [40–42], while under a heavy load, i.e. when the bacterium is stalled by obstacles, one expects \( f_{\text{th}} \approx 5 \text{ pN} \) [43–45]. The thrust is balanced by a viscous drag force, so that a swimming cell represents a ‘force dipole’ of length \( l_{\text{th}} < L \), estimated for E. coli to be about 2 \( \mu m \) [40]. The bacterium is thus capable of producing a realigning torque \( \tau_{\text{th}} \approx f_{\text{th}} l_{\text{th}} \), where \( \alpha \) is the angle between the bacterial body axis and the normal to the substrates \( \tilde{n}_b \). The product \( f_{\text{th}} l_{\text{th}} \) is in the range \( \tau_{\text{th}}/\alpha \approx (1-10) \times 10^{-18} \text{ N m} \).

Previous experiments demonstrated that rod-like bacteria in planar LCLC cells are parallel to the director, regardless of whether they are immobile [17] or swimming [6–8, 29, 30]. This observation is usually explained by the models developed by Brochard and de Gennes [46] and by Smith and Denniston [33], of a thin rod tilting in an otherwise uniform nematic. The crucial assumption is that the rod imposes an infinitely strong unidirectional surface anchoring of the director parallel to the rod’s axis. Another obvious feature of the models [33, 46] is that the rod is non-motile. For swimming bacteria, the latter assumption is unacceptable since the director fields around a non-motile rod and an actual swimming rod-like bacterium are different (see figure 6); we return to this issue in section 4.3. To understand the balance of elasticity and surface anchoring and how it affects the realignment from the VSP into the HSW state, we neglect this difference and treat the bacterium as a non-motile rod for a moment.

A rod of length \( L \) and radius \( r \) with an infinitely strong axial director anchoring at its surface, realigned by an angle \( \alpha \) away from \( \tilde{n}_b \), experiences a restoring elastic torque \( \tau_e = \alpha C K L \), where \( C = 4\pi/\ln(2l/r), \) as calculated by Smith and Denniston [33]. For the typical \( K = 10 \text{ pN}, l = 8 \mu m, r = 0.4 \mu m, \) one finds \( \tau_e/\alpha \approx 3 \times 10^{-16} \text{ N m, a value prohibitively high to allow the bacterium to realign, as } \tau_e/\tau_{\text{th}} \approx 30 - 300. \) The model, if directly applied to a bacterium, overestimates the stabilizing torque and cannot explain the observed VSP–HSW transition and swimming perpendicularly to the director.
The stabilizing torque of the LCLC acting on a realigning rod is significantly reduced if the surface anchoring is finite rather than infinitely strong. Assume that the director prefers to be parallel to the axis of a rod, but the surface energy penalty for a tilt by \( \alpha \) from this orientation is finite, \( \frac{1}{2} W \alpha^2 \) per unit area, where \( W \) is the anchoring strength coefficient, usually on the order of \( W \sim 10^{-6} \text{ Jm}^{-2} \) for LCLCs [23, 47]. The stabilizing anchoring torque is then only \( \tau = -\nabla W \alpha^2 \) comparable to the bacterial realigning torque, \( \tau_{\text{bact}} \). As demonstrated by Tasinkevych et al [35], the stabilizing torque becomes even smaller if the alignment at the surface is tangential, i.e. degenerate in the local plane of the interface. Even if the surface in contact with an LCLC is unidirectionally treated (say, rubbed), the azimuthal anchoring energy is very weak, only about \( W \sim 0.3 \times 10^{-6} \text{ Jm}^{-2} \) [48].

To account for both the elastic and surface anchoring torques resisting realignment of a swimming bacterium, we performed numerical simulations within the framework of the Landau–de Gennes theory of the nematic state. The total energy of the system

\[
U = \int (E_b + E_d) \, dV + \oint E_s \, d\Sigma
\]

is determined by the condensation energy density

\[
E_b = -\frac{A}{2} \text{Tr}Q^2 + \frac{B}{3} \text{Tr}Q^3 + \frac{C}{4} (\text{Tr}Q^2)^2,
\]

the elastic energy density

\[
E_d = \frac{1}{2} K |\nabla Q|^2,
\]

and the anisotropic surface energy density \( E_s \). The condensation energy is minimized in the nematic by a uniaxial tensor \( Q = S_0 \left( \hat{n} \otimes \hat{n} - \frac{1}{3} I \right) \) with \( S_0 = \frac{1}{4C} (-B + \sqrt{B^2 + 24AC}) \). We set \( C = -B = 3A \) so that \( S_0 = 1 \).
Typically, \( A \approx 10^5 \text{ J m}^{-3} \). In our studies, \( A \) is significantly smaller for computational reasons; however, it is still large enough to correspond to a small coherence length in a nondimensional model.

The explicit form of the surface energy density is determined by the type of anchoring. In the case of alignment along the bacterium axis, but with a finite anchoring coefficient \( W \),

\[
E_s^a = \frac{1}{2} W (Q_{ij} - Q_{ij}^0)^2,
\]

where the summation with respect to the indices \( i, j \) is implicit. The tensor \( Q^0 \) is defined as \( Q^0 = \hat{\xi} \otimes \hat{\xi} - \frac{1}{3} I \) with \( \hat{\xi} \) being a unit length vector pointing along the meridional direction at the bacterium body. Degenerate tangential anchoring is modeled by the quadratic term of the Fourier–Galatola potential \[ 49 \]

\[
E_t^d = \frac{1}{2} W (\tilde{Q}_{ij} - \tilde{Q}_{ij}^0)^2.
\]

Here \( \tilde{Q} = Q + \frac{S_0}{3} I \) and \( \tilde{Q}^+ = PQP \), where \( P = \mathbf{1} - \hat{\nu} \otimes \hat{\nu} \) and \( \hat{\nu} \) is normal to the bacterium surface. The system of Euler–Lagrange equations corresponding to equation (1) was solved in FEniCS—a software for the automated solving of differential equations \[ 50 \], see figure 11.

The dependence of the total energy \( U \) on the angle \( \alpha \) between the normal to the glass plates and the bacterial axis is depicted in figure 11. As expected, the stabilizing torque \( \tau = -dU/d\alpha \) shown in figure 12 depends strongly on the strength and type of surface anchoring. For strong polar anchoring, \( W = 10^4 \text{ J m}^{-2} \) (figure 12(a)), the torque is too high for the bacterium to overcome, \( \tau_{\text{axial}} / \alpha \sim \tau_{\text{tang}} / \alpha \sim (3-5) \times 10^{-11} \text{ N m} \). However, if the surface anchoring is weak, \( W = 10^2 \text{ J m}^{-2} \), close to what is expected for LCLCs \[ 19, 23, 39 \], then the maximum stabilizing torques are \( \tau_{\text{axial}} / \alpha \sim 0.4 \text{ kJ m}^{-3} \) for the axial anchoring and \( \tau_{\text{tang}} / \alpha \sim 0.2 \text{ kJ m}^{-3} \) for the degenerate tangential anchoring (figure 12(b)). These values are close in the order of magnitude to the bacterial torques, \( \tau_{\text{bac}} / \alpha \sim (1-10) \times 10^{-13} \text{ N m} \). The stabilizing torque reaches a maximum around \( \alpha \approx \pi/4 \) for the axial anchoring and \( \alpha \approx \pi/6 \) for the degenerate tangential anchoring, and then falls off back to 0 (figure 12(b)). Once the rod is oriented along \( \alpha = \pi/2 \), the torque required to maintain that orientation is small. We conclude that the finite strength of surface anchoring, on the order of \( W = 10^2 \text{ J m}^{-2} \) or less, mitigates the resistance of the LCLC to the realignment of the bacteria, and thus qualitatively explains the observed VSP-to-HSW transition.

In the conclusion of this subsection it is important to stress that the numerical simulations presented above for the non-swimming rod tilting away from the initial vertical orientation always produce antisymmetric director distortions around the rod (figure 13). The quadrupolar symmetric director field around an HSW bacterium is very different from this antisymmetric pattern—compare figures 6 and 13. In section 4.3 below, we
demonstrate that the symmetric director field can be explained by the bacterium-generated quadrupolar pattern of the shear flow characteristic of microswimmers of a pusher type [1–3]. In its turn, the mirror symmetry of the director distortions stabilizes the HSW state with $\alpha = \pi/2$, as discussed in section 4.4.

4.3. Shear-flows and director distortions around swimming bacterium

The exact director field around the swimming bacterium is not known, but the experiments suggest that the director deviates from the $z$-axis rather significantly, as evidenced by optical retardation on the order of tens of nanometers, $\Gamma_{\text{max}} = (10–20)$ nm (figure 1(a)). One can argue that the director tilts at the bounding plates rather than at the bacterial body. Such a tilt is certainly possible, but the corresponding optical retardation would be significantly higher than the observed (10–20) nm, as it would accumulate across the entire thickness of the cell. It is of interest to estimate the maximum angle $\theta_{\text{max}}$ of the director tilt away from the $z$-axis caused by a horizontally swimming bacterium. Assuming the director profile to be linear below and above the horizontal bacterium swimming in the plane $z = d/2$, [38], i.e. $\theta(z) = \theta_{\text{max}}(1 - 2z/d)$, one finds

$$\Gamma_{\text{max}} = \Delta n \int_{-d/2}^{d/2} \theta^2(z) \, dz = \Delta n \theta_{\text{max}}^2 / 3.$$  

With $\Gamma_{\text{max}} = (10–20)$ nm, $d = 10 \mu$m, and $\Delta n = 0.016$, one arrives at $\theta_{\text{max}} = (20–30)^\circ$, i.e. a significant tilt. This value is underestimated, as the director is not in equilibrium.

Numerical simulations (figures 13(b), (d)) suggest that the director around a rod with a weak anchoring is practically perpendicular to its body. The experimental retardance, $\Gamma_{\text{max}} = (10–20)$ nm (figure 1(A)), and the tilt $\theta_{\text{max}} = (20–30)^\circ$, are thus likely caused by a shear flow around a swimming bacterium. In liquid crystals, shear flows are coupled to the director. The Ericksen number $Er = \frac{\eta L v}{K}$ expresses the relative importance of viscous and elastic torques. For splay and twist viscosities, $\eta_{\text{play}} \sim \eta_{\text{twist}} \sim 7$ kg m$^{-1}$ s$^{-1}$, and for the typical bacterial length and speed $v$, $Er_{\text{play}} \approx Er_{\text{twist}} \approx (10–100)$; thus one should expect the director deviations caused by the flow.

Below we consider the hydrodynamic effects using the minimal version of the generalized Ericksen–Leslie equations that only incorporates rotational $\zeta_1 \approx \eta_{\text{twist}}$ and isotropic $\zeta_9$ nematic viscosities [51]

$$\begin{align*}
\frac{\partial E}{\partial Q} - \text{div} \left[ \frac{\partial E}{\partial (\nabla Q)} \right] + \zeta_9 Q &= 0, \\
\rho \dot{\psi} + \text{div} [-T^\text{el} + p I - T^\text{v}] &= 0, \\
\text{div} v &= 0.
\end{align*}$$

(6)

Here, $E = E_b + E_d$ with $E_b$ and $E_d$ as defined in (2) and (3), respectively, while $\rho$ and $p$ are the liquid crystal density and pressure, respectively. The superimposed dot stands for the total (material) time derivative,

$$T^\text{el} = -\frac{\partial E}{\partial (\partial_i Q_{mn})} (\partial_j Q_{mn})$$

(7)

denotes the elastic stress, and

$$T^\text{v} = \zeta_1 (Q Q - Q Q) + \zeta_9 A$$

(8)

is the viscous stress. In addition, $A_{ij} = \frac{1}{2} (\partial_i v_j + \partial_j v_i)$ and $W_{ij} = \frac{1}{2} (\partial_i v_j - \partial_j v_i)$ represent the symmetric and antisymmetric parts of the velocity gradient, respectively, and $\dot{Q}_{ij} = \dot{Q}_{ij} - W_{ik} Q_{kj} - W_{kj} Q_{ik}$ gives the rate of change of the $Q$-tensor relative to the flow vorticity.

A distinctive feature of LCLCs is that a rotational viscosity $\zeta_1$ and consequently the Ericksen number are large; we assume that $\zeta_1/\zeta_9 \sim 100–1000$. Since the results above indicate that weak anchoring deduced from experiments leaves the director field around the bacteria unaffected, in simulations of (6) we assume $W = 0$. To simplify the computational problem, we also assume that the bacterium has a straight thin flagella bundle that generates a flow with a prescribed velocity in the direction opposite to the direction of swimming; the swimming speed is assumed to be constant. The importance of flagella flexibility in swimming dynamics is well established (see, for example, [52]), but here we assume that the flagella bundle is rigid. We are thus left with a need to solve a stationary problem corresponding to equation (6). This problem was solved using the commercial finite-elements software COMSOL [53].

Assuming that the nematic is at rest at infinity, we prescribe the flow velocity on the flagella and determine the propagation velocity of the bacterium by demanding that the total stress on the bacterium body vanishes. We solve a nondimensional version of the system in equation (6), where the velocity is scaled by a typical speed of a bacterium $5 \mu$m s$^{-1}$. Setting the velocity of the nematic on the flagella to $-2$ (or $-10 \mu$m s$^{-1}$ in dimensional units), we find that the bacterium propagates with the velocity $\sim 0.6$ (or $3 \mu$m s$^{-1}$ in dimensional units), producing the director and velocity profiles shown in figure 14. The problem is solved by iterating the bacterium
velocity, finding a numerical solution of the stationary version of equation (6), determining the force on the bacterium body by integrating surface traction, and comparing the resulting force to zero. To evaluate the force accurately, we need a large number of elements on the bacterium surface, especially given that the diameter of the tail is small. To make the computations more tractable, in this section we consider a bacterium with a body that has a larger radius-to-length aspect ratio than a more realistic value considered above for the rod representing a static bacterium (see figure 12).

Numerical solutions to equation (6) are in qualitative agreement with the experimental data, in particular, with the symmetry of optical phase retardation in cells with a different polarity of tilts, \( \Gamma_{\text{max}}(\Omega) \approx \Gamma_{\text{max}}(-\Omega) \), illustrated in figure 6. Namely, the director distortions are of a quadrupolar type with mirror symmetry with respect to the horizontal plane passing through the bacterial body (figure 14(a)), and, to a lesser degree, with respect to the vertical plane perpendicular to the bacterium. The director tilts significantly, by tens of degrees, around the body of a swimming bacterium. Note a stark difference of the director pattern in the horizontal plane passing through the bacterial body with respect to the vertical plane perpendicular to the bacterium. The director tilts significantly, by tens of degrees, around the body of a swimming bacterium. Note a stark difference of the director pattern in the horizontal plane passing through the bacterial body with respect to the vertical plane perpendicular to the bacterium.

**4.4. Quadrupolar elastic distortions supporting the HWS state and repulsion from walls**

The symmetric nature of the director distortions shown in figures 5(l), 6, 14(a) helps the HSW bacterium to maintain its horizontal orientation with \( \alpha = \pi/2 \), since it corresponds to the local minimum of the elastic energy of director distortions, as demonstrated below.

Consider the director field to be of a hybrid type, with \( \hat{n}_b = (0, 0, 1) \) at the bounding plates and parallel to the axis of the bacterium at the LCLC–bacterium interface. The bacterium swims in the middle of the cell and its body represents a parallelepiped of length \( l \) and width \( 2r \). Let the bacterium tilt away from the horizontal direction by a small angle \( \beta = \pi/2 - \alpha \). The elastic energy density of the distortions above and below a short element \( \lambda \) of the bacterial body writes \[ E_{\text{sw}} = \frac{\beta \lambda}{2d} \left( 4\beta + \frac{\pi^2}{\beta} \right) K \arctan \frac{\beta}{2d} \]

which reduces to \[ E_{\text{sw}} = 2Kr \left[ \frac{(\pi/2 - \beta)^2}{d} + \frac{\pi^4}{4d^2} \right] \] for small \( \beta \). This elastic energy is clearly minimized by \( \beta = 0 \), i.e. \( \alpha = \pi/2 \). We conclude that the local minimum of the elastic energy (9) of the symmetric director pattern shown in figures 5(l), 6 and 14(a) helps to keep the bacteria in the state of HSW.

In contrast, the antisymmetric director field tilted rigid rod shown in figures 5(d), (e) and 13 requires realignment towards the vertical axis, \( \beta \rightarrow \pi/2 \). For the antisymmetric director, the energy density \[ E_{\text{sw}} = \frac{8K}{\beta} \left( \frac{\pi}{2} - \beta \right) ^2 \arctan \frac{\beta}{2d} \] for
which the minimum (zero) is achieved at $\beta = \pi/2$, i.e. for the vertical alignment parallel to the imposed director.

The distorted director also helps to repel the bacteria away from the substrates, in a manner similar to elastic levitation described by Pishnyak et al [54] for colloids in an LC. It is known that in water, flagellated bacteria often swim very close to the substrates, leaving a gap of only about 100 nm [55, 56]. In our experiments (see figures 2 (b), (c)), the gap is larger, $h \approx (1–3) \mu$m. Qualitatively, the elasticity of LCCLC keeps the bacteria ‘levitating’ in the bulk, as conflicting boundary conditions, homeotropic at the plates and tangential at the bacterium, imply that the director gradients and thus the elastic energy increase as the separation between the bacterium and the wall decreases. To calculate the elastic contribution to the repulsive force, we consider the angular difference $\theta$ in the director alignment at the bounding plates, $\hat{n}_0 = (0, 0, 1)$, and at the body of a swimming bacterium (figure 5(f)). Surface anchoring sets the director parallel to the bacterium, $\theta = \pi/2$, but the actual angle $\theta$ is smaller than $\pi/2$ because the anchoring strength $W_p$ at the walls and $W$ at the bacterial body are finite. The bacterium is horizontal and interacts only with the closest glass plate located at a distance $\chi \ll d$. The elastic energy caused by the hybrid director alignment is [38]

$$E_{\text{ew}} = Klr \frac{\theta^2}{\chi + K/W_p + K/W},$$

(10)

The elastic force pushing the bacterium away from the plate is then

$$\frac{\partial E_{\text{ew}}}{\partial \chi} = \frac{Klr\theta^2}{(\chi + K/W_p + K/W)^2},$$

increasing as the bacterium gets closer to the boundary. With $W_p \approx W \approx 10^{-6}$ J m$^{-2}$, $\theta \approx 1$, $\chi = 2 \mu$m, the estimated repulsive force is $-\partial E_{\text{ew}}/\partial \chi \approx 0.1$ pN. The estimate is very rough, as the full analysis should consider both hydrodynamic and elastic responses in a self-consistent matter; some progress in this direction is outlined in [57].

4.5. Tumbling scenarios: backtracking and vertical tumbling

The backtracking scenario suggests that the flagella bundle disassembles at one end of the body and then reassembles at the opposite end. The difference between bacteria tumbling in isotropic media and the reversal motion in nematic media is rooted in the anisotropy of the LCCLC. Before the reversal, a forward-swimming bacterium induces in-plane components of the director field around its body that relaxes back to homeotropic on the order of $t \approx 1$ s (figure 4(d)). The typical ‘immobility state’ of the tumbling bacterium, during which the flagella re-bundle at the opposite end, lasts for $(0.2–0.4)$ s $< t$. Thus, before the director relaxes to the homeotropic state, the bacterium starts to move back into the partially planar area it has just created. The distorted director with a nonzero horizontal component facilitates swimming with a higher velocity. In the vertical tumbling case, characteristic for short and slow bacteria, director relaxation is strong enough to reorient the bacterium body and trap it into the VSP state (see figure 3). While the bacterium is in the trap, the surrounding director field relaxes back into the homeotropic state. When the bacterium starts to swim again, the new direction is random since there is no memory left to bias the motion. The LCCLC does not prevent the unbundling/re-bundling of the flagella filaments, possibly because of their small diameter ($\sim 24$ nm) and weak surface anchoring.

4.6. Collective effects

The experiments demonstrate that bacterial interactions in concentrated dispersions result in the formation of linear and even membrane-like regions of director distortions. The main mechanism is rooted in the finite time of director relaxation in the wake of a swimming bacterium that leads to a temporary ‘trail’ that attracts other bacteria. This ‘elastotaxis’ effect is reminiscent of the ‘chemotaxis’ of some motile micro-organisms which secret chemical trails to direct the self-assembly of microcolonies (see, for example, [38]). If the measured relaxation time of the elastically distorted trail, $t \approx (0.3–2)$ s, is smaller than the time $t_{\text{rel}}$ of the new bacterium arrival, the director trail will remain deformed. Bacteria swimming in a similar direction can form linear chains (see figure 9). As the concentration increases and $t_{\text{rel}}$ decreases below $t$, the deformations created by multiple bacteria become stronger, as evidenced by an increase of retardance (see section 3.6). These fully developed deformations relax to the uniform state even slower than the deformations in the wake of an isolated bacterium. As discussed in section 4.1, the relaxation time of a fully developed splay is $t_{\text{sp}} = \eta_{\text{sp}} (d + K/W + K/W_p)^3/(\pi^2 K_{\text{sp}})$ which yields $t = (1–3)$ min. If the membrane develops into a 180° wall-soliton, the relaxation time would be further extended; as such a wall can be eliminated only through nucleation and the propagation of disclinations [38], as discussed in section 3.6.

The director distortions created by swimming bacteria are generally three-dimensional, containing splay, bend, and twist. As shown theoretically [59, 60] for active nematics, mixed splay-bend causes flows for arbitrary small levels of activity (swimming speed and concentration of bacteria in our case). Green et al [60] suggested an
expression for the active force driving these flows, $f \propto \hat{n} \nabla \cdot \hat{n} - \hat{n} \times (\nabla \times \hat{n})$. Recent experiments with bacteria swimming in an LCLC with the mixed splay-bend do indeed show rectified active flows with bacteria swimming in unipolar fashion. In addition, the bacteria show preferences to gather in regions with one type of distortion over the others \cite{5}. The dynamic behavior of bacteria in homeotropic cells presents yet another example of the subtle interplay between the flows and director distortions, with the difference that the director distortions are produced by the bacteria rather than by the boundary conditions; much more needs to be explored before this interplay can be adequately described.

5. Conclusion

We demonstrate that rod-like \textit{B. subtilis} confined in a homeotropic nematic LCLC cell can adopt one of two states: (a) spinning along their long axis and remaining parallel to the imposed director, but not moving in the plane of the cell; (b) swimming perpendicularly to the imposed director about 1–3 $\mu$m away from the substrates. The ability of the bacteria to overcome the stabilizing torque of the nematic environment and swim suggests that the surface anchoring at the bounding substrates is finite. A swimming bacterium produces a symmetric quadrupolar pattern of director distortions around itself, which differs dramatically from the antisymmetric director around a tilted non-motile rod. The elastic energy of this director pattern exhibits a local minimum for a horizontal orientation of the bacterium, thus supporting the state of HSW. The bacteria also show a remarkable ability to change their swimming direction, either through a reversal of the swimming velocity and backtracking, or through a vertical flip-flop with an intermediate VSP state. At higher concentrations, swimming bacteria create large areas of strong director distortions that attract other bacteria. Depending on the concentration, these regions are tube-like or membrane-like. It is important to stress that the director distortions produced by swimming bacteria in homeotropic cells involve mixed splay and bend (and probably some twist that is difficult to identify by optical means) and thus are expected to provide a feedback, controlling the collective dynamics of the bacteria; much more needs to be understood in this regard. The results presented in this work will help in future explorations of how bacterial behavior can be controlled by the anisotropic environment.

Acknowledgments

S Z and O D L were supported by the Petroleum Research grant PRF #56046-ND7 administered by the American Chemical Society and by NSF DMS-1434185; D G and O T were supported by the NSF grant DMS-1434969. A S and I S A were supported by the US Department of Energy (DOE), Office of Science, Basic Energy Sciences (BES), Materials Science and Engineering Division.

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