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Cell orientation of swimming bacteria: From theoretical simulation to experimental evaluation

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The motion of small bacteria consists of two phases: relatively long runs alternate with intermittent stops, back-ups, or tumbles, depending on the species. In polar monotrichous bacteria, the flagellum is anchored at the cell pole inherited from the parent generation (old pole) and is surrounded by a chemoreceptor cluster. During forward swimming, the leading pole is always the pole recently formed in cell division (new pole). The flagella of the peritrichous bacterium *Escherichia coli* often form a bundle behind the old pole. Its cell orientation and receptor positioning during runs generally mimic that of monotrichous bacteria. When encountering a solid surface, peritrichous bacteria exhibit a circular motion with the leading pole dipping downward. Some polar monotrichous bacteria also perform circular motion near solid boundaries, but during back-ups. In this case, the leading pole points upward. Very little is known about behavior near milieu-air interfaces. Biophysical simulations have revealed some of the mechanisms underlying these phenomena, but leave many questions unanswered. Combining biophysics with molecular techniques will certainly advance our understanding of bacterial locomotion.

bacterial motion, cell orientation, hydrodynamic interaction, Brownian motion, wall effect

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There is substantial evidence that bacteria actively respond to external stimuli, including light, chemicals, oxygen, and others [1]. Their directional movement along a chemical gradient, termed chemotaxis, has been well-studied. The migration strategies and signaling pathways of chemotaxis have been elegantly reviewed [1–4]. Motility probably evolved before chemotaxis [5]. The principles of bacterial motion are largely understood, principally from studies of chemotaxis. However, little experimental or theoretical evidence is available concerning the mechanism of orientation of bacterial cell bodies during swimming, or the reason for adopting a particular orientation. This will be discussed here.

The mechanics of the motion of microbes relative to the surrounding fluid belongs to the branch of physics called hydrodynamics. Because of the small size of their cell bodies, which are typically around 2 μm in length, small bacteria generate moving forces through viscous shear, while macroscopic organisms rely on inertial resistance. Different physics models of bacterial locomotion have been reviewed and summarized by Ramia *et al.* [6] and the references therein. Many bacteria move by rotating a single helical flagellum, or a flagellar bundle, approximately 10 μm long. In a viscous milieu, the ratio of the forces required to accelerate the mass (inertial forces) to the forces that generate shear (viscous forces) is called the Reynolds number. For *Escherichia coli* swimming at full speed in water (ca. 25 μm s^{-1}), Reynolds number is ca. 10^{-5} [1]. The effect of inertia is negligible.

The motion of small bacteria is subject to the disturbance of Brownian motion, i.e., the random migration of particles due to thermal energy [7]. Bacteria are unable to maintain

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any particular swimming trajectory for even a few seconds because Brownian motion constantly drives them into new directions [3]. The effect of Brownian motion decreases as cell size increases [8]. At 32°C, translational and rotational diffusion causes the trajectory of an *E. coli* cell to deviate from its original course by an average of 27° in 1 s [9]. A similar phenomenon was observed, but to a less extent, in the lophotrichous bacterium *Chromatium minus*, which is ca. 20 times larger than *E. coli* [10]. As Brownian rotational diffusion scales inversely with the cube of cell size [11], their mean path curved only 8.4° over 1 s at 23°C [12].

To allow the bacteria to frequently reset their course, the swimming of the peritrichous bacteria is composed of two alternating modes: relatively long smooth runs lasting ca. 1 s, and shorter (ca. 0.1 s) tumbles (Figure 1). The helical flagella form a left-handed bundle during the run. When viewed from behind the cell, the bundle rotates counterclockwise at ca. 100 Hz, and the cell body rotates clockwise at ca. 10 Hz [13]. Reversal of rotation causes the flagella to transform into a right-handed helix and, either the whole bundle flies apart [14], or one or a few flagella separate from the bundle [13]. The unbundled flagella generate thrust which drives the cell in a new direction.

Polar monotrichous bacteria that live in aquatic environments do not tumble and swim faster than peritrichous bacteria (Table 1). The flagellum can be either right-handed or left-handed. If right-handed, it rotates clockwise to propel the cell forward; if left-handed, it rotates counterclockwise. When the rotation of the flagellum is reversed, the cell backs up. Backing up is functionally equivalent to the tumble of peritrichous bacteria but the polar flagellum probably does not change its helical form during reversal [15]. Without external disturbance, the backward-swimming cell would follow its original forward-swimming trajectory. However, Brownian motion causes small monotrichous bacteria to swim in a zigzag path by changing the trajectory of the run and, more severely, cell orientation during back-up [15,16]. Recent observation indicates that *Vibrio alginolyticus* also flicks its flagellum upon resuming the run after back-up, which further randomizes its cell body orientation [17]. It has been proposed that the back-up swimming strategy is an adaptation to the high-shear environment because small persistence (average cosine of reorientation angle) is less susceptible to shear [18]. The flicking behavior might be a tradeoff with chemotaxis in quiescent and moving milieu.

The photosynthetic bacterium *Rhodobacter sphaeroides* swims faster than *E. coli*, at an average speed of 35 μm s^{-1}. It has a single flagellum that is a right-handed flat coil at rest and is located medially on the cell body. The flagellum extends and rotates clockwise to force the cell to translate along its short axis. When rotation stops, the relaxed flagellum contracts to the cell body, allowing Brownian motion to change the cell orientation more efficiently [19]. In addition, the coiled flagellum rotates slowly during this time and this also contributes to the reorientation [20].

Whichever swimming strategy a bacterium adopts, the trajectories are essentially random walks [1,16,19]. However, by modulating the length of forward swims, i.e., the runs, locomotion is prolonged in favorable directions and shortened in unfavorable directions. Thus, bacteria are able to migrate in a net favorable direction. Vladimirov *et al*. recently showed that, by controlling the number of reversing flagella, peritrichous bacteria are able to decrease tumbling angles by 3° on average when cells swim up a favorable gradient compared with when cells swim down the gradient, which also facilitates migration in a favorable direction [21].

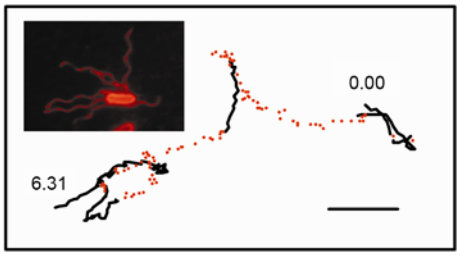

Figure 1 Trajectory of the center of an *E. coli* strain AW405 cell swimming in LB medium. The images were taken every 25 ms with a fast recording camera. Scale bar, 10 μm. The bacterium translated at ca. 32 μm s^{-1} in runs. Red dots indicate that the cell swam out of the focal plane. The real time movie is available as Supplementary Movie S1 in the electronic version. Inset is a fluorescence stained cell of strain AW405 showing the helical flagella.

1 Cell orientation in forward swimming

According to the biophysical model proposed by Berg and Purcell in their classical paper [22], capturing signal molecules in the milieu is most efficient if chemoreceptors are distributed widely over 0.1% of the surface of an idealized spherical cell. However, it was later observed that receptors

Table 1 Swimming behaviors of some polar monotrichous bacteria[a)]

Species	Cell body		Flagellum		Free-swimming speed (μm s^{-1})		Trajectory near a wall		Reference
	Shape	Twist	Helix	Rotation[1]	Run	Back-up	Run	Back-up	
V. alginolyticus[2]	Vibrioid	SC	LH	CCW	37.3	52.9	SC	CCW	[15,23–25]
C. crescentus[3]	Vibrioid	LH	RH	CW	41.7	Same	NR	CW	[26–29]
P. citronellolis	Rod	N.R.	LH[4]	CCW	55.8[5]	Slower	CW[4]	N.R.	[16,30,31]

a) N.R., not reported; SC, slightly curved; LH, left-handed; RH, Right-handed; CW, clockwise; CCW, counterclockwise. 1, Forward swimming, and viewed from behind the cells; 2, a mutant lacking peritrichous flagella; 3, a mutant lacking the adhesive pili; 4, inferred conclusion, see the main text; 5, data of *P. aeruginosa*. That of *P. citronellolis* is not available.

cluster at the flagellated poles of the vibrioid swarmer cells of *Caulobacter crescentus* and at both poles in the rod-shaped *E. coli* [32,33]. One of the two bacterial cell poles is formed in the previous generation (old pole); the other derives from the septum when the cell divides (new pole). Receptors accumulate linearly over time at the poles of *E. coli* cells [34]. A new pole exists, by definition, for only one generation. At each subsequent division that pole (now an old pole) accumulates an additional 83%±4% more Tsr (serine) receptors until, 3.5 generations later, it becomes saturated [34]. In a random population, the old pole contains, on average, 2.6 times more Tsr receptors than the new pole. In addition, small clusters and solitary receptors have been observed to distribute along the cell body [34–36]. It has been proposed that this can flatten the signaling gradient within the cytoplasm or ensure the inheritance of receptors by progeny cells [35].

The need for receptor clustering at the poles, and the mechanism of clustering, remain largely unexplained [4,28]. A typical polar cluster in an *E. coli* cell contains more than 7500 receptor dimers [37] despite the fact that the basic structural unit required for signal transduction is only a trimer of dimers [38]. Small bacteria like *E. coli* are too small to make spatial measurements of concentration differences along the cell body. To obtain a two-fold difference in signal capture between the leading pole and the lagging pole of a spherical cell, the bacterial swimming speed would have to increase 100 times [22].

Chemotaxis is based on comparing current concentrations with those detected several seconds ago [39]. It has been proposed that allosteric interaction between receptor molecules in a cluster enhances signal transduction [40,41] but the optimal number of receptors in a functional cluster is remarkably small (5–25 receptor dimers) as predicted by different mathematical models [42–44]. If the formation of the large cluster at the pole is just a biological spandrel, as suggested by the stochastic nucleation model proposed by Greenfield *et al.* [36], the functionality of the inherited receptors at the old pole is unimportant. But if the old receptors are active, would the old pole be superior to the new pole in signal detection? The question can only be answered by experimentally switching off or removing the old receptors in the cell through genetic or molecular manipulation, e.g., receptors could be blocked by fusion to a masking protein. When the blocking moiety is cleaved by an inducible protease, the receptor would become fully functional. The receptors synthesized after induction could then be compared with the receptors inherited from parent generations.

Tumbles potentially randomize the location of the pole clusters by actively flipping the cell body [45]. Interestingly, on ca. 75% free-swimming cells in an exponential-phase culture of *E. coli*, the old pole trails behind the new pole, a consequence of surplus flagella located adjacent to the old poles [46]. During the reversal of the flagella in tumble, the asymmetric flagellar distribution biases the cell body such that the new pole lies more often in front, which coincides with the observation that the average reorientation angle of *E. coli* in tumble is 68° [7].

The observation that surplus flagella are located adjacent to the old pole and, thus, to the large receptor cluster in peritrichous bacteria is, to some extent, reminiscent of the old-pole localization of receptors and flagellum in polar monotrichous bacteria [32]. Considering the positioning of the flagellar bundle and the receptor clusters in forward-swimming *E. coli* cells, this peritrichous bacterium lies within a continuum, between the polarly flagellated *Caulobacter* and the equatorially flagellated *Rhodobacter* (Figure 2A). Although the polar receptor clusters in *R. sphaeroides* show an asymmetric distribution similar to those of *E. coli* [47], the distances between the two clusters and the flagellum are equal when the cell translates along its short axis. A bipolar flagellated marine vibrioid bacterium also translate along its short axis [48]. Thus, the two polar oxygen sensor regions are able to independently modulate the rotation rate of their adjacent polar flagellar bundles to control the swimming direction. It is not known whether their chemoreceptors also form different-sized clusters on the old and new poles.

A bundle of several flagella produces little more torque than a single flagellum does at low Reynolds number [13]. However, considering the relative locations of the propeller

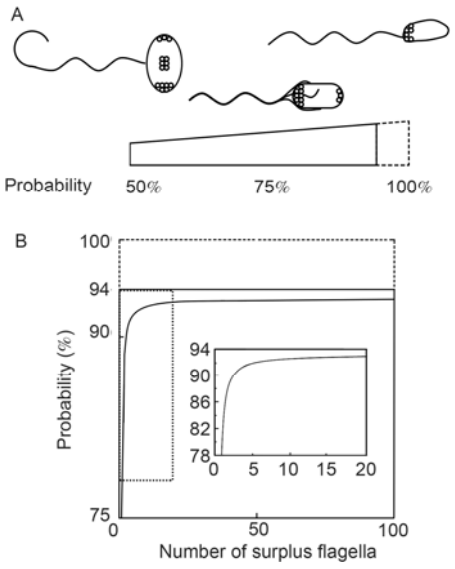

Figure 2 The probability of positioning an old pole at the back of forward-swimming cells. The probability also represents potential efficiency of receptor-flagella coupling. Broken boxes show probability not attainable by peritrichous bacteria. A, The probability of different bacteria. The equatorial flagellated *Rhodobacter* (left) is assigned 50%; the polar flagellated *Caulobacter* (right) 100%; *E. coli* (middle) 75% on average and variable due to the location and number of asymmetric flagella. Small circles represent the chemoreceptors. It is worth noting that *Rhodobacter* also contains cytoplasmic receptor clusters and this does not influence the assignment of 50% probability to the bacterium. B, A plot of the probability against the number of surplus flagella adjacent to the old pole in perichichous bacteria. Inset is an enlarged view of the dotted box.

and the detector, more asymmetric flagella cause peritrichous bacteria to swim more like polar monotrichous bacteria (Figure 2B). The localization of the large receptor cluster close to the surplus flagella in peritrichous bacteria potentially ensures a tight receptor-flagella coupling [46], as proposed for the polar monotrichous *C. crescentus* [45], where the receptors cluster around the base of the flagellum [32]. Because of their proximity to the surplus flagella, the receptors at the old pole could trigger the reversal of surplus flagella more efficiently than the receptors at the new pole. *E. coli* cells with multiple asymmetric flagella indeed migrate towards a chemical gradient more efficiently [46].

2 Head-down orientation near a solid boundary

Motile bacteria encounter boundaries at many critical points in their lives, e.g., when water-borne pathogens invade host tissues, when planktonic aquatic bacteria settle, as marine bacteria approach nutrient-rich algal surfaces, or during colonization of plant roots by rhizosphere bacteria. Near boundaries, bacteria swim markedly differently. The physical influence exerted by a rigid boundary (wall) is called the 'wall effect'.

When *E. coli* swim in a laminar flow, provided the shear is not strong enough to disturb normal motion, they tend to align the long cell axes of their cell bodies with the flow, with the leading pole dipping slightly downwards [49]. Shear flow would naturally orients a translating cell body to face upstream [49]. However, shear flow is not required for the head-down orientation [50]. Without shear flow, the bacteria would perform a circular motion (Figure 3A), which will be discussed in the next section.

Hydrodynamic theory predicts that non-spherical cells (modeled as prolate spheroids without flagella) swimming close to a rigid (nonslip) surface experience drag force resulting from both the wall and from the upcoming fluid [51]. The differential drag from the wall at different points along the cell body causes the cell to tilt, while that of the upcoming flow causes it the to remain parallel to the surface. The equilibrium of the competing torques results in a critical angle of the cell body with respect to the surface, the head-down orientation (Figure 3B). This tilting angle is a weak function of the distance to the wall and of the ratio of the cell's length to its diameter. A more detailed mathematical investigation of various cell shapes and flagellar configurations revealed that cell geometries play an important role in determining the trajectory of near-surface swimming [52]. Some bacteria may deflect and leave the wall, while others run into it or stabilize at a constant distance.

According to simulations, spherical cells without flagella experience only wall drag and would therefore roll forward along the surface [50]. If a flagellum is present, rolling would not take place [52]. This hypothesis could be tested using natural motile cocci, such as the peritrichous *Vagococcus* species, or artificial spherical cells in an isotonic solution, which can be generated by deleting the cytoskeleton MreB and other cell shape proteins [53,54]. Another interesting species is the bilophotrichous magnetotactic coccus MC-1, which has two adjacent flagellar bundles [55]. How the two bundles are coordinated to propel the spherical cell is a very interesting question.

In a test tube, non-chemotactic smooth-swimming cells sediment faster than wild type cells because they cannot counteract the effect of gravity by swimming upward after a tumble. It has been inferred that smooth-swimming mutant

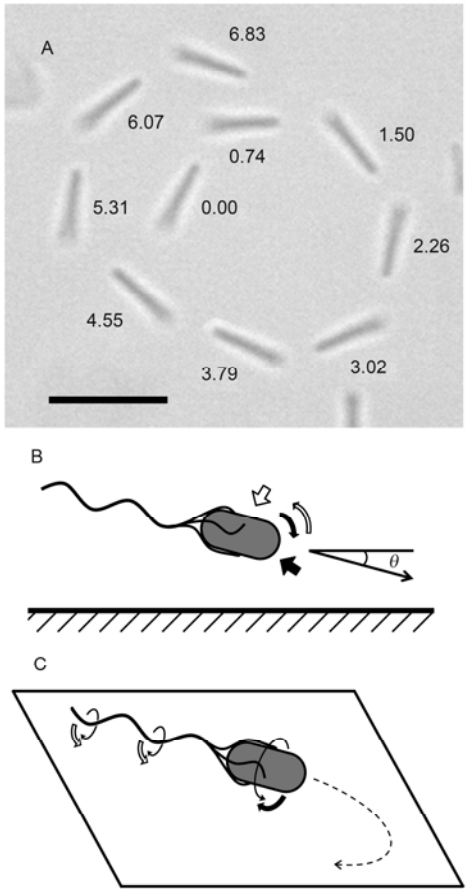

Figure 3 Swimming behavior of *E. coli* near a solid bottom. A, Snap shots of a smooth swimming *E. coli* strain HCB437 doing circular motion on a glass side surface. The recording times are shown beside the cells. Scale bar, 10 μm. The cell tilts ca. 14°, and translates at ca. 12 μm s^{-1}. To show the tilting cell body, an elongated cell was chosen and the microscope was adjusted to a position so that the back end of the cell was out of the focal plane. The original movie taken at 25 μs/frame is available as Supplementary Movie S2 in the electronic version. B, Hydrodynamic explanation for the tilting angle (θ). The angle is generated when two competing torques (curved arrows) reach an equilibrium. One torque is created by the unevenly distributed viscous drag along the cell body (black arrows) due to wall effect. Another is created by the drag from the up-coming fluid (open arrows). At this critical angle, the cell undergoes a torque-free translation parallel to the wall. C, Hydrodynamic explanation for the circular motion. The circular trajectory (broken arrow) results from the cooperation of a right-handed torque, created by the drag counteracting the cell body rotation (black arrow), and a left-handed torque, created by the drag counteracting the lower portion of the rotating flagellar bundle (open arrow).

cells tend to swim downward with the cell tilting towards the bottom [56]. This behavior was attributed to gravity because the sedimentation rate of the head is greater than that of the tail. This passive sedimentation, if it exists, perhaps operates when bacteria swim far from the bottom, where wall effect is negligible. However, such tilting would change the centre of gravity of the cell and this would need to be taken into account.

Hydrodynamic interaction prevents bacterial cells from colliding with the bottom, and causes them to translate parallel to the surface [6]. Due to the wall effect, the approach to the surface would result in an asymptotic decrease in velocity, eventually trapping the bacteria at a certain distance [57]. It has been observed that *E. coli* usually swim about 40 nm above a clean quartz surface [50] and that their swimming speed drops to ca. 16 μm s^{-1} as they approach the surface [58]. Computer simulation confirms that the separation distance would be at the nanometer level, and that elongated cells tend to swim further from the wall than more oblate cells [52], as shown in Supplementary Movie S2 in the electronic version.

3 Circular motion near boundaries

In a static milieu, *E. coli* cells swim circularly when approaching a rigid surface, and often leave the surface after a tumble [58]. Hydrodynamic simulation predicts that the higher viscous drag acting on the lower part of the rotating cell body and flagellar bundle, relative to the upper region, is responsible for the circular motion [6,57]. As the cell body rotates clockwise, wall drag counteracting the rotation creates a torque that pushes the cell body sideways; the lower portion of the helical flagellar bundle, which rotates counterclockwise, experiences a viscous drag in the opposite direction (Figure 3C). Consequently, when viewed from above, the cells appear to be rolling to the right. When the cell approaches an upper surface, it will turn to the left [59].

Maeda *et al*. observed that the radius of swimming trajectory can vary from 10 to 50 μm, which they attributed to temperature variation [60]. Theoretically, the radius of the circular path is equal to the quotient of the swimming velocity divided by the out-of plane rotation rate [57], which depends on the physical dimensions of the organism and the distance to the wall [6]. Bacterial cell shape also influences the radius, as longer cells generally swim in larger circles [52]. Although the chemotactic signaling network in the cytoplasm is robust to temperature change [61], the proton-motive force driving flagellar rotation is generated by cell metabolism, and is temperature dependent [62]. Besides physical dimensions and temperature, the physiological status of individual cells also partially accounts for the variation of swimming curvatures (for example, see [57]) because bacteria can fine tune their flagellar motor rotation rate through the second messenger cyclic di-GMP [63,64].

When *E. coli* were confined in a shallow chamber a few micrometers high with an agar bottom and oxidized poly(dimethylsiloxane) (PDMS) as the sidewall, they swam along the right-hand side of the chamber [59]. This is because *E. coli* prefers to swim near the porous agar surface and avoids the PDMS surface. They turn to the right because the difference in dynamical behaviors near different walls breaks symmetry. As expected, when the channel is larger than 10 μm, the right-hand preference is diminished. The hydrodynamic interaction is significant only when the relevant separation distances are approximately equal to or smaller than the physical dimensions of the organism [6]. The wall effect also leads to a faster chemotaxis rate of *E. coli* in a 10 μm capillary tube than in a 50 μm tube. In 50 μm capillaries, the bacteria are delayed by engaging in circular motion [65]. It would be interesting to investigate whether cell swimming in a thin capillary tube follows a helical path because the sidewall torque is constantly present in small capillaries. The pitch of a swimming trajectory should increase as the diameter of the tube decreases.

Monotrichously flagellated cells also perform circular motion when they swim close to a wall (Table 1). The phenomenon is clearly visible when *V. alginolyticus* swims within 10 μm of a nonslip surface [66]. However, the circular motion is only apparent during back-up (Figure 4). The path of their forward motion is almost straight, although swimming speed decreases as the cell approaches the wall [15,57]. When approaching surfaces, *V. alginolyticus* and *C. crescentus* turn in opposite directions because of the different directions of rotation of their flagella (Table 1). The cells of *Pseudomonas citronellolis* and *Pseudomonas aeruginosa* also perform circular motion near rigid surfaces [16,31]; in the case of *P. aeruginosa*, the direction was not specified but *P. citronellolis* was believed undergo clock-

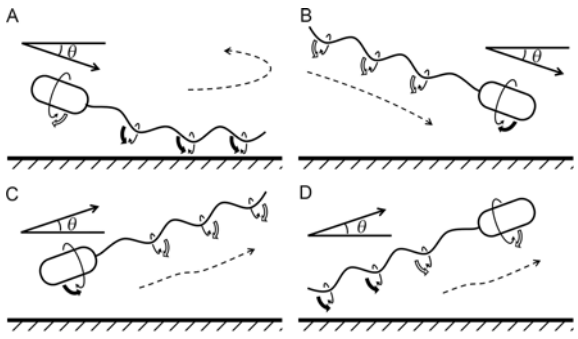

Figure 4 The swimming behavior of a *V. alginolyticus* cell approaching a rigid surface. The angles between cell axes and the surfaces are labeled with "θ". The potential trajectories are represented by broken arrows. Thin black arrows indicate the rotational directions of the cell body and the flagellum. Open arrows indicate the counteracting wall drag. Thick black arrows indicate the combined force generated by wall drag and DLVO interaction. A, A cell translating along the surface with head-up orientation during back-up. B, A cell translating along the surface with head-down orientation in forward swimming. C, A cell translating along the surface with head-down orientation during back-up. D, A cell translating along the surface with head-up orientation in forward swimming.

wise rotation, like the forward swimming of *E. coli* [16]. However, this inference is inconsistent with observations on other monotrichous bacteria. If the cells were actually backing up, would *Pseudomonas* swim in the same manner as does *Caulobacter*?

It is worth noting that in above-discussed simulations, the cell was often modeled as a rigid helix attached to a perfect sphere swimming parallel to the surface [6,57]. Only one kind of spherical cell has been studied briefly so far, that of the spontaneously arisen round cells of *R. sphaeroides* [50]. These cells indeed perform circular motion. Since *R. sphaeroides* does not back up, it must do so in forward swimming. Analysis of the localization of receptors of other motile cocci and their motion near solid boundaries will provide more clues to the importance of the wall effect on bacterial chemotaxis.

A rod-shaped bacterium translating with a tilting angle requires more sophisticated modeling. Frymier *et al*. [58] have proposed that the theory of colloid stability formulated by Derjaguin, Landan, Verwey, and Overbeek (DLVO) could potentially explain the circular motion. According to the DLVO theory, the interaction of a repulsive electrostatic force and an attractive van der Waals force, between the biological surface and the wall, creates a secondary Gibbs energy minimum ca. 4.5 nm from the surface [67]. This secondary energy minimum potentially traps the swimming cell at certain distance from the surface [58].

Combining the theories of wall torque and DLVO interaction could potentially explain the circular swimming behavior of monotrichous bacteria (Figure 4). When the bacteria back up with a head-up orientation, the DLVO interaction is weak on the cell body, but strong on the flagellum (Figure 4A). The wall torque on the cell body cooperates with the combined force generated by the wall drag and the electrostatic force on the flagellum to push the cell into circular motion. When cells swim with other body orientations, either the electrostatic force operates mainly on the cell body and drives the entire cell off-track (Figure 4B and C), or it transiently operates on the flagellum and accelerates the departure of the cell from the surface (Figure 4D). *Caulobacter* has been observed swimming in circle a few hundred nanometers away from the surface [29], near where the secondary Gibbs energy minimum operates.

When the cell backs up with the flagellum downward, the distal portion of the flagellum would be trapped in the secondary Gibbs energy minimum. It has been observed that the flagellum bends in the circular motion of *V. alginolyticus* [68]. The torque generated by DLVO interaction would accelerate the swim. The back-up speed of *V. alginolyticus* was demonstrated to be 1.5 times faster in the presence of a wall than during free swimming, while forward swimming was not affected [69].

DLVO interaction possibly plays a less significant role in the motion of *E. coli*. Increasing the ionic strength of the milieu enhances the DLVO interaction, but failed to influence the motion of *E. coli* near a surface [70]. The flagella of *E. coli* are naked protein filaments, whereas the flagellum of a monotrichous bacterium is sheathed. The sheath is an extension of the bacterial outer membrane [71], which is more favorable to the DLVO interactions than an unsheathed peritrichous flagellum. Although modifying the hydrophobicity of the slide surface did not influence the swimming behavior of *V. alginolyticus* [66], changing the ionic strength of the medium might have a profound effect.

Microbes experience zero-stress boundary conditions during aerotaxis and phototaxis at the interfaces between the milieu and air (free boundary). It has been suggested that change of the direction of the image system for a point force there leads to counterclockwise circular motion [57]. Counterclockwise circular motion was indeed observed with *E. coli* swimming in a buffer containing 0.002% Tween-20 [65]. An almost linear correlation between cell length and radius of the curvature was also detected under this condition, where the surface was covered with surfactant patches. In another experiment with *E. coli* swimming in a medium containing glycerol, both clockwise and counterclockwise motions near the air surface were observed [73]. *V. alginolyticus* cells also exhibited both clockwise and counterclockwise trajectories near the interface between air and medium containing HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and they did so in both forward and backward swimming [69]. Novel experimental designs are required for further understanding bacterial behaviors near real free boundaries. The use of a sample supporter with an appropriate hydrophilic surface to lower the contact angle of the liquid without a surfactant, and observation with a photoacoustic microscope [74], which is able to view much deeper than conventional microscopes, provide a promising solution.

4 Perspectives

Although the phenomena discussed here are largely studied by physicists, they are of interest in the context of bacterial evolution for the selection of their particular sizes, shapes, and flagellation. This review focuses on small bacteria, simply because few experiments and biophysical simulations have considered large bacteria. Large bacteria live in different niches and behave differently from small bacteria. For example, the bipolar flagellated marine vibrio (6 μm long) translate along their short axis like *Rhodobacter* (2.5 μm long) but they are able to detect the oxygen gradient along their cell body [19,48]. The helical *Spirillum volutans* can grow up to 140 μm long [75]. They synchronously rotate tufts of about 25 flagella on each pole. These tufts have the appearance of a blurred cone and they swim in both directions equally well [1]. The small bipolar flagellated *Magnetospirillum magneticum* AMB-1 (ca. 3 μm long) also swim in both directions [76,77]. These two species may

provide a good system for the study of the influence of cell size on motility.

Another interesting question concerns the evolutionary advantages of different types of flagellation. Peritrichously flagellated bacteria are able to perform a type of locomotion, called 'swarming', through semisolid milieu or over wet surfaces, whereas, except for *P. aeruginosa*, bacteria with a signal polar flagellum are unable to swarm [71,78]. Furthermore, the back-and-forth strategy of monotrichous flagellation enables bacteria to remain in favorable locations under high shear, where the run-and-tumble peritrichous bacteria simply behave like non-motile bacteria [79]. Bacteria with two flagellar systems [71] provide a good opportunity to compare monotrichous and peritrichous flagellation. Isogenic strains can be created through gene silencing or recombination and assayed under identical conditions.

There is certainly a correlation between bacterial cell shape and motility. Although most biophysical simulations were performed with spherical cells, and about 10% of the motile forms among 218 free-living bacterial genera are coccoid [80], the motility of neither natural motile cocci nor artificial spherical cells has been carefully examined. Other aspects of the cell shape, such as the handedness of vibrio (Table 1), has largely escaped the attention of biological and biophysical analysis. Through genetic manipulation, it is easy to change *Vibrio* to a straight rod [26], or to change a rod to a spiral [81]. The development of new technologies such as microfabrication also provide convenient ways to analyze single cell motility under well-defined conditions [11]. It is foreseeable that combining molecular biology and biophysics will greatly advance our knowledge of bacterial motility.

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