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Physics of bacterial near-surface motility using flagella and type IV pili: implications for biofilm formation

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Abstract

We review physically-motivated studies of bacterial near-surface motility driven by flagella and type IV pili (TfP) in the context of biofilm formation. We describe the motility mechanisms that individual bacteria deploying flagella and TfP use to move on and near surfaces, and discuss how the interactions of motility appendages with fluid and surfaces promote motility, attachment and dispersal of bacteria on surfaces prior to biofilm formation.

Keywords: Bacterial motility; Type IV pili; Flagella; Biofilm formation

1. Introduction

Over 99% of bacteria live in biofilms, multicellular surface-associated communities surrounded by a protective matrix of extracellular polymeric substances (Hall-Stoodley et al., 2004). The biofilm matrix surrounds and protects the enclosed bacteria, thereby imparting to them increased resistance to host and environmental stresses. As a result, biofilms are difficult to eradicate and cause widespread problems in health and industry. For example, biofilms exacerbate outbreaks of waterborne disease, from which five million people die annually (Hunter et al., 2001); aid attachment of barnacles to ship hulls, reducing the speed of ships and increasing fuel costs (Schultz and Swain, 2000); and cause up to 80% of hospital-acquired infections (Donlan, 2001). A critical complement to chemical and biological strategies toward reducing the prevalence of biofilms in these settings is to identify the physical processes that initiate biofilm formation on surfaces.

Studies of model organisms such as *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, yield insight into the early stages of biofilm formation. Individual *P. aeruginosa* bacteria suspended in liquid first approach the surface and transiently attach; subsequently, bacteria spread and finally attach irreversibly (Klausen et al., 2006; Sauer et al., 2002). This process requires that bacteria switch their motility phenotypes from free swimming to surface-motile, which may entail changes in how *P. aeruginosa* generates motion. Prior to biofilm formation, *P. aeruginosa* uses two motility appendages to move near surfaces (O'Toole and Kolter, 1998): a single polar flagellum, which acts as a helical propeller, and multiple type IV pili (TfP), which act as linear actuators. Both flagella and TfP can also act as adhesins that stick bacteria to surfaces and to one another (Geoghegan et al., 2008; Petrova and Sauer, 2012). The dual role of flagella and TfP suggest that motility and adhesion are related: indeed, mutants of *P. aeruginosa* lacking motility appendages or appendage function attached to surfaces at slower rates than the motile wild-type (Tran et al., 2011). Furthermore, motility on and near surfaces driven by these appendages in turn shapes the biofilm, as demonstrated by the

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cap-shaped structures in *P. aeruginosa* biofilms that require both flagellar motility and TfP to form (Barken et al., 2008; Klausen et al., 2003a,b). Motility and adhesion are also connected in other model biofilm-forming bacteria such as *Escherichia coli*, in which biofilms of flagella-deficient mutants cover less surface area than those of wild-type strains (Wood et al., 2006). Changes in motility coupled to biofilm formation indicate that surface proximity modifies use of appendages by bacteria. Physical interactions between bacterial appendages and the liquids in which they move, arising both from flow and from hydrodynamic interactions with nearby surfaces, may additionally influence bacterial near-surface motility and hence biofilm formation. Fundamental understanding of the ways in which bacteria alter appendage deployment near surfaces may lead to targeted strategies to prevent surface colonization.

In this short review, we highlight physical studies of bacterial near-surface motility driven by flagella and TfP in the context of biofilm formation. To complement recent reviews of collective motility modes such as flagella-driven swarming (Wu et al., 2011) and TfP-driven twitching (Burrows, 2012), we concentrate on near-surface motility mechanisms employed by individual bacteria deploying flagella and TfP. Specifically, we explore how appendage interactions with fluid and surfaces promote motility, attachment and dispersal of bacteria on surfaces prior to biofilm formation, and describe avenues for further physically-motivated studies of these processes.

2. Near-surface swimming with flagella

Micron-sized bacteria are small enough to be deflected by the force of impact from individual liquid molecules and thus bacteria can undergo Brownian motion. The effect of Brownian diffusion on bacterial motion can be measured directly from the trajectories of individual bacteria through the two-dimensional mean-squared displacement as a function of the lag time τ, $(\Delta r^2(\tau)) = ((r(t+\tau) - r(t))^2) = 4D\tau$, where the brackets indicate an average over all starting times t and D is the coefficient of diffusion. Because microscale bacteria move in viscous liquids such as water, mucus, and blood, viscous frictional forces between the liquid and bacteria also affect their motion. The dimensionless Reynolds number measures the relative importance of viscous forces compared to inertial forces that resist changes in motion: $\mathrm{Re} = \rho VL/\eta$, where ρ is the density of the fluid (for water, $\rho = 10^3$ kg/m^3), η is its viscosity (for water, $\eta = 10^{-3}$ Pa s), and V and L are the typical velocity and length of the bacterium. For a one-micron bacterium swimming in water at a speed $V = 30$ μm/s, a typical Reynolds number is $\mathrm{Re} = 3 \times 10^{-5}$, indicating that viscous forces dominate over inertial forces as described in the classic article by Purcell (1977).

In the counterintuitive physical limit of low Reynolds number, the Navier–Stokes equations of fluid mechanics are independent of time for Newtonian fluids (in which the shear stress is proportional to the shear rate) such as water. This result, known as the "scallop theorem", suggests that bacteria must exploit mechanisms that are non-reversible in time for forward propulsion (Purcell, 1977). The most-studied non-reciprocal mechanism for bacterial motility is the flagellum, a filamentous helical propeller connected by a hook to a rotary motor. Flagellated swimming bacteria include *Caulobacter crescentus*, which rotates its single polar flagellum clockwise for swimming and changes course by switching the direction of the motor, and *Rhodobacter sphaeroides*, which stops rotating its single lateral flagellum to change course. The most-studied flagellated swimmer, *E. coli*, possesses on average six flagella of length 10 μm and diameter 20 nm distributed about its body (Berg, 2008). When *E. coli* rotates all its flagella counterclockwise, they form a single helical bundle that pushes the bacterium forward and roughly straight in a "run"; to change direction (via a "tumble"), the bacterium unbundles its flagella by turning at least one motor clockwise (Turner et al., 2000). To create the force- and torque-free swimming required by low Reynolds number in the absence of external forces (Lauga and Powers, 2009), the *E. coli* body rotates clockwise (at ∼25 Hz), whereas the bundle rotates counterclockwise (at ∼100 Hz) (Berg, 2008). Using flagella, bacteria swim at average velocities ranging from 30 μm/s for *E. coli* (Darnton et al., 2007; Turner et al., 2000) to 100 μm/s for the Gram-negative marine bacterium *Vibrio alginolyticus* (Magariyama et al., 1995).

The hydrodynamics of swimming bacteria have been extensively described in two recent reviews (Guasto et al., 2012; Lauga and Powers, 2009), and so here we summarize only the mechanism by which flagella generate motion of the cell body. In the limit of low Reynolds number, only forces arising from interactions of the bacteria and its appendages with the fluid can generate motion in the absence of external flow (Lauga and Powers, 2009). The flagellar motor generates force for forward propulsion via the hydrodynamic drag of the fluid, which opposes rotation of the flagellar filament (Berg and Anderson, 1973). Axial force (thrust) and rotation (torque) arise from the difference in viscous drag felt by the filament as it moves sideways or lengthwise (Berg, 2008). A surprising consequence of this physical mechanism is that swimming bacteria can drift across streamlines of an external flow, which is not expected in low Reynolds number environments in which the flows are laminar (Marcos et al., 2012). Experiments on *Bacillus subtilis* show that in bulk fluids far from surfaces, hydrodynamic stresses on helical flagella produce a net lift force that is balanced by viscous drag (Marcos et al., 2009), whereas the cell body feels only drag. The combined forces on bundle and body induce a torque that rotates the cell body against the direction of flow, allowing the bacterium to swim upstream against the gradient in shear stress in rheotaxis as shown in Fig. 1a (Marcos et al., 2012).

As bacteria swim near solid surfaces, as required for biofilm formation, hydrodynamic interactions with the surface modify the physics of flagellated swimming. Bacteria swimming near a solid surface also exhibit rheotaxis, rapidly and continuously swimming against the direction of moderate flow rates (corresponding to shear rates of up to 6.4 s^{-1}) as shown in Fig. 1b (Kaya and Koser, 2012). In addition to flow,

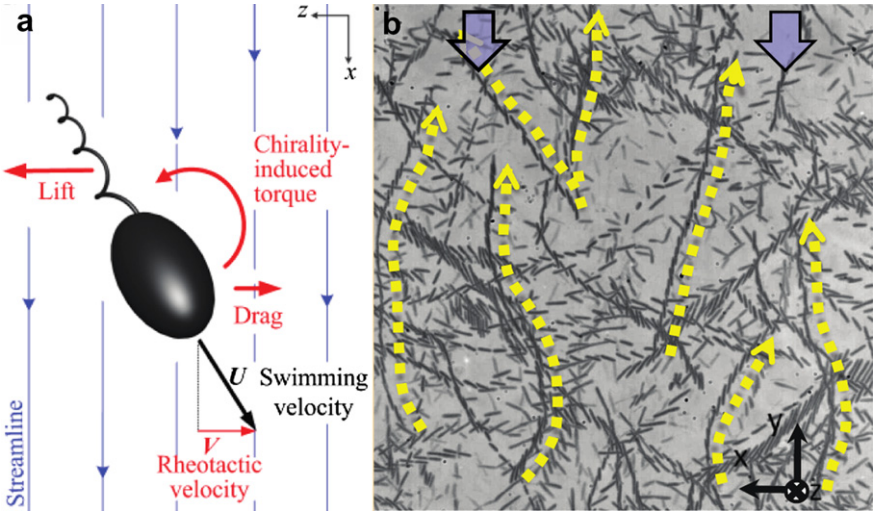

Fig. 1. Swimming bacteria undergo rheotaxis in bulk fluids and near walls. (a) Schematic for rheotaxis in bulk fluids: the chirality of a left-handed flagellum leads to a lift force (in the $+z$ direction as indicated on the figure) that is opposed by the drag on the cell body, thereby producing a torque on the cell that rotates the bacterium so that it can partially swim against the flow direction ($-z$). Figure reproduced with permission from Marcos et al. (2012). (b) Near a solid surface, *E. coli* (K12) exhibit rheotaxis against flow (arrows) when the shear rate is less than 6.4 s^{-1}. Figure reproduced with permission from Kaya and Koser (2012). Copyright 2012 Cell Press.

however, hydrodynamic interactions with the nearby surface can also reorient bacteria: specifically, hydrodynamic drag due to the surface on the back of the cell, downstream, rotates the cell body to point directly upstream and thereby allows the bacterium to swim against the direction of flow (Kaya and Koser, 2009). Physical interactions between appendages, bacteria, and surfaces leading to rheotaxis may thus allow biofilm-forming bacteria to rapidly spread on surfaces against adverse flow.

Changes in the shape of trajectories of swimming bacteria provide a second example of hydrodynamic interactions modifying swimming motility. Strikingly, instead of moving in straight runs separated by tumbles, *E. coli* bacteria swim near solid surfaces in right-handed circles as shown in Fig. 2a (DiLuzio et al., 2005; Frymier et al., 1995). While first observed in multiply-flagellated *E. coli*, singly-flagellated bacteria also swim toward the right (e.g. *V. alginolyticus* (Kudo et al., 2005; Magariyama et al., 2008) and *C. crescentus*, shown in Fig. 2b (Li et al., 2008)). The change from straight to curved trajectories in *E. coli* can be explained via the hydrodynamic influence of solid walls in a force-free model (Lauga et al., 2006): the drag coefficient on an elongated filament decreases as the distance from the wall is increased. The parts of a helical flagellum or bundle nearest the wall thus feel the largest local viscous forces, leading to a net force on the helix. Similarly, the counter-rotating cell body also experiences a viscous force near the surface, and the forces on bundle and body must be equal and opposite in force-free swimming. The spatial distribution of these forces, however, leads to a negative torque on the bacterium, causing it to swim to the right (DiLuzio et al., 2005). The hydrodynamic argument holds in the absence of flow or when the flows

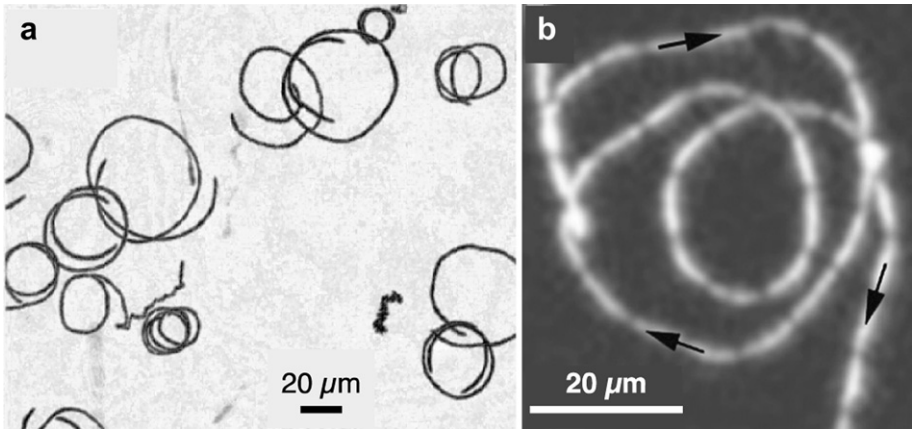

Fig. 2. Flagellated bacteria swim in curved trajectories near surfaces. Trajectories of swimming (a) multiply-flagellated *E. coli* (HCB437) and (b) singly-flagellated *C. crescentus* (YB375, pili-deficient) near a solid surface. Figure (a) reprinted with permission from Lauga et al. (2006). Copyright 2006 Cell Press. Figure (b) reprinted with permission from Li et al. (2008). Copyright 2008 National Academy of Sciences, USA.

are weak. Strong shear flows, however, can additionally modify the direction in which bacteria move by reorienting the bacterial body. Near the bottom surface of a microfluidic device with a fast fluid flow (with a typical shear rate of 100 s^{-1}), flow can reorient the body of the bacterium with respect to the surface and hence modify the direction of torque, allowing the cells to point upstream and swim toward the left (Hill et al., 2007). Hydrodynamic interactions promote bacterial adhesion on surfaces, as bacteria swimming in circular trajectories spend an increased length of time near the surface compared to bacteria swimming in straight trajectories (Lauga et al., 2006). Circular trajectories therefore increase the likelihood that bacteria adhere to the surface and thereby enhance cell deposition and surface coverage (de Kerchove and Elimelech, 2008).

Hydrodynamic interactions can also help bacteria to accumulate near surfaces prior to biofilm formation. For example, the density of swimming *E. coli* increases near surfaces (Berke et al., 2008). One model to explain near-surface accumulation attributes increases in density to hydrodynamic interactions between flagella and solid surfaces. When the separation between the cell and the surface is somewhat larger than the length of the cell body ($L > 10$ microns), the time scale for hydrodynamic reorientation ($\tau \sim L/V$, where V is the velocity of the bacterium; for *E. coli*, this time scale is of the order of seconds) is much faster than the time scale for rotational diffusion of a rod (of the order of 100 s) (Berke et al., 2008). At these separations, the effective flow field around a flagellated swimmer can be approximated as a force dipole (Hernandez-Ortiz et al., 2005). The flagella of *E. coli* push the bacterium forward, repelling fluid along the long axis of the body and drawing fluid in at the sides, so that the force dipole is positive. The attraction between a positive ("pushing") force dipole and the solid surface thus drives *E. coli* to align with the surface (Berke et al., 2008).

Very close to the surface, however, Brownian diffusion can also strongly affect bacterial motility. When the singly-flagellated swimmer *C. crescentus* is less than a micron from a solid surface, small changes in the separation between the bacterium and the surface induced by Brownian fluctuations modify the radius of its circular trajectory (Fig. 2b) (Li et al., 2008) and increase the density of cells near the surface (Li et al., 2003). In an alternate model for bacterial accumulation that includes Brownian fluctuations and neglects hydrodynamic interactions, cells tend to swim parallel to a surface after colliding with it (Li and Tang, 2009; Li et al., 2011). In this model, rotational Brownian motion is fast enough to reorient bacteria that are very close to the surface, and the surfaces are assumed to screen the long-range hydrodynamic interactions between bacteria that drive alignment (Hernandez-Ortiz et al., 2009). Brownian fluctuations play a complicated role for biofilm formation: fluctuations increase the average distance of cells from the surface, but may also bring cells momentarily closer to the surface and thereby increase the likelihood of sticking (Li and Tang, 2009).

These studies show that hydrodynamic interactions and Brownian motion modify how flagellated bacteria swim near surfaces. As a consequence, the length of time that bacteria spend near surfaces and the cell density near the surface increase, with both factors promoting accumulation of bacteria on surfaces. Once attached to the surface, bacteria can no longer freely swim using flagella. Nonetheless, by rotating the flagellar motor, bacteria can still "spin" rapidly while attached to the surface, as shown by *E. coli* cells that attach via adhesins on a flagellum (Chen and Berg, 2000; Neuman et al., 1999). Similar spinning motility has been observed for *P. aeruginosa* bacteria (Conrad et al., 2011; Toutain et al., 2007; Tran et al., 2011), in which the body of the cell rotates at a typical rate of ~5 Hz as shown in Fig. 3 (Conrad et al., 2011). Spinning motility, however, may be transient and not universal: *C. crescentus* bacteria spin for a short time (5 min) after adhering to the surface, but cease rotating after producing the holdfast adhesin (Li et al., 2012). Together, these studies suggest that spinning may help bacteria to detach from surfaces, allowing them to leave microenvironments that do not favor adhesion or that contain few nearby bacteria.

3. Bacteria crawl, slingshot and walk with type IV pili (TfP)

TfP are thin protein filaments of diameter 5–8 nm and length of up to several microns found in some Gram-negative bacteria, including *P. aeruginosa*, the causative agent for gonorrhea *Neisseria gonorrhoeae* (Merz et al., 2000), the predatory soil bacterium *Myxococcus xanthus* (Sun et al., 2000) and the plant pathogens *Acidovorax citrulli* (Bahar

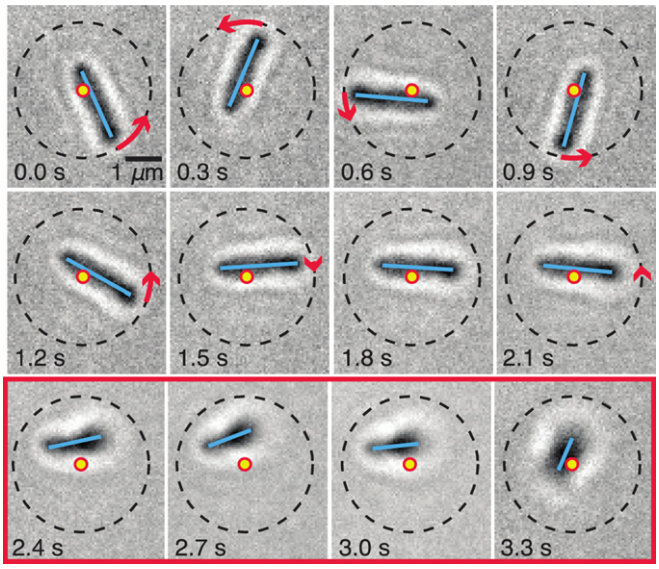

Fig. 3. Bacteria spin on a surface using flagella to detach. In a representative image series of a spinning wild-type *P. aeruginosa* bacterium (ATCC strain 15692), dots indicate the center of rotation, dashed lines indicate the initial radius of the trajectory, solid blue lines indicate the backbone of the bacterium and arrows indicate the direction and magnitude of rotation between consecutive images (with timestamps). The bacterium rotates using its flagellum, slows, tilts away from the surface using TfP (images in the red box) and finally detaches. Reproduced with permission from Conrad et al. (2011). Copyright 2011 Cell Press.

et al., 2009; Bahar et al., 2010) and *Xylella fastidiosa* (De La Fuente et al., 2007, 2008). The distal tip of TfP bears an adhesin that can stick to a variety of materials, including both glass slides and the surfaces of mammalian and plant cells. As a result, TfP play a critical role in the development of microcolonies early in biofilm formation. Structurally, TfP are semiflexible polymers of the protein pilin with a persistence length of ∼5 μm (Skerker and Berg, 2001). Bacteria can polymerize and depolymerize TfP through the cell wall, giving TfP the unique ability, among filamentous appendages, to be dynamically extended and retracted (Merz et al., 2000; Skerker and Berg, 2001; Sun et al., 2000). TfP retract at typical rates of ∼0.5 μm/s for *P. aeruginosa* (Skerker and Berg, 2001) and ∼1 μm/s for *N. gonorrhoeae* (Merz et al., 2000).

Bacteria exert force on surfaces and crawl by retracting TfP adhering to the surface. A single pilus can exert a maximum force of ∼110 pN in *N. gonorrhoeae* (Maier et al., 2002) and ∼150 pN in *M. xanthus* (Clausen et al., 2009a,b), as measured using optical tweezers, and a bundle of TfP can exert stronger forces of up to ∼1 nN, as measured by the bending of microfabricated nanopillars (Biais et al., 2008). To "crawl" lengthwise along a surface, bacteria oriented horizontally (parallel to the plane of the surface) retract TfP adhering to the surface, thereby pulling the cell body forward. Surprisingly, the average velocity at which *P. aeruginosa* crawl (∼0.3 μm/s) is less than the velocity at which their TfP retract (∼0.5 μm/s) (Skerker and Berg, 2001).

Recent microscopy studies, facilitated by advances in automated imaging and tracking of bacteria, provide new physical insight into how TfP retraction actuates crawling. Simultaneous measurements of the velocity of *N. gonorrhoeae* cells moving with TfP and the force exerted by TfP suggest a model to explain why the velocity of crawling cells (1.6 μm/s) differs from that of retracting TfP (2 μm/s) (Holz et al., 2010). The correlation time over which bacteria move locally "straight" increases with the number of TfP per cell as shown in Fig. 4a, leading to larger displacements over time as measured by the mean-squared displacement (Holz et al., 2010). Because the velocity at which TfP retract decreases as the cell bears increasing load (Clausen et al., 2009a,b), both the difference in velocities and the increase in correlation times are consistent with a model in which TfP bear load during crawling and thus retract at lower velocities as shown in Fig. 4b. This "tug-of-war" scenario arises if a single pilus at the rear end of the cell (relative to the direction of motion) pulls against multiple pili at the front end, thereby biasing the cell to move in the forward direction (Clausen et al., 2009a,b).

Interactions between multiple TfP also generate distinct features in the trajectories of crawling *P. aeruginosa* bacteria.

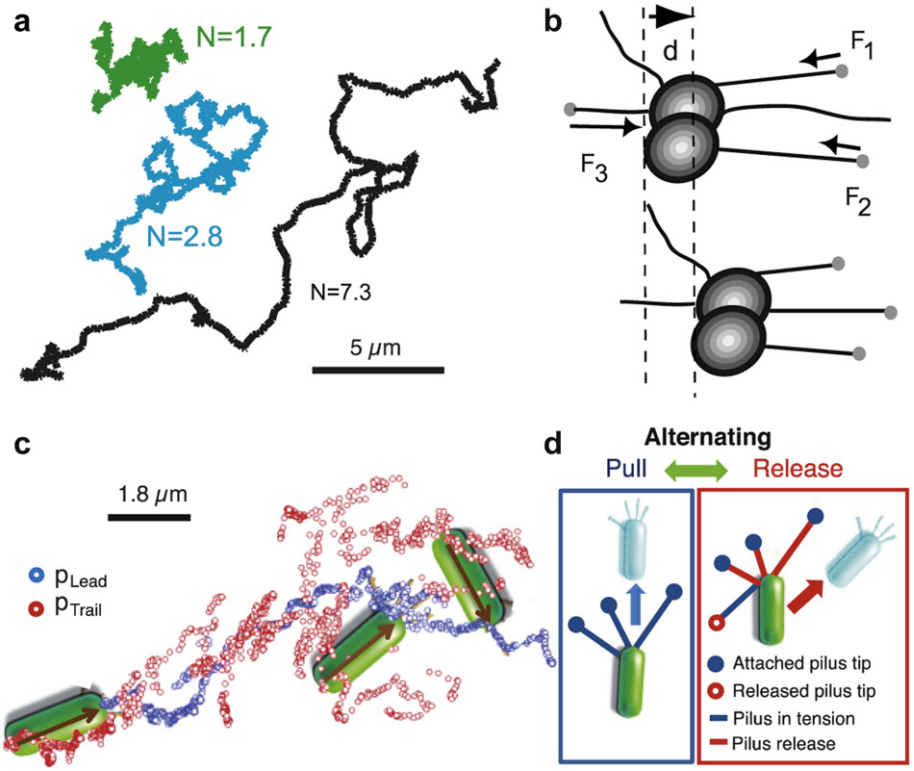

Fig. 4. Attached TfP exert force to modify the trajectories of crawling bacteria. (a) The persistence length over which *N. gonorrhoeae* bacteria move straight increases with the average number of TfP per bacteria. (b) *N. gonorrhoeae* uses its TfP in a tug-of-war to pull against an attached pilus at the rear of the bacterium. Figures (a) and (b) reprinted with permission from Holz et al. (2010). Copyright 2010 by the American Physical Society. (c) The leading (p_{Lead}) and trailing (p_{Trail}) poles of a flagella-deficient *P. aeruginosa* mutant (Δ*fliM* mutant of ATCC strain 15692) move in distinct trajectories leading to rotation. (d) Rotational "slingshot" motion arises when an attached pilus detaches, causing the bacterium to rapidly reorient along the vector sum of the force from the remaining attached TfP. Figures (c) and (d) adapted with permission from Jin et al. (2011).

Highly spatially- and temporally-resolved analyses show that flagella-deficient bacteria do not steadily crawl forward on glass coverslips; instead, these bacteria alternate a slow (average velocity ∼0.03 μm/s) linear translation of long duration (0.3–20 s) with a fast (average velocity ∼1 μm/s) combined rotation–translation of short duration (<0.1 s), as shown in a representative trajectory in Fig. 4c (Jin et al., 2011). This alternating pattern of movements is consistent with a model in which coordinated pulling by multiple TfP generates slow linear translation, whereas the release of a single pilus allows the bacterium to "slingshot" and rapidly rotate-translate as shown in Fig. 4d (Jin et al., 2011). The rapid velocity of slingshot motion may allow bacteria to efficiently travel through shear-thinning fluids, such as the extracellular polymeric substances that bacteria deposit on surfaces during biofilm formation (Jin et al., 2011).

Single-bacterium tracking techniques also allow new mechanisms of motility used by individual bacteria to be identified. Isolated cells of *P. aeruginosa* can use TfP to "walk" while oriented perpendicular to glass substrates as shown in Fig. 5a (Conrad et al., 2011; Gibiansky et al., 2010). In contrast to crawling (Fig. 5b), in which horizontally oriented bacteria persistently move along their body axis (as measured by the directional persistence length $L_p \sim 6$ μm), vertically oriented walking bacteria deploy TfP to move while making only short excursions in any particular direction ($L_p \sim 2$ μm). The short persistence lengths and jagged trajectories of walking bacteria suggest that the TfP of walking bacteria pull in different and uncorrelated directions (Gibiansky et al., 2010). Examining the growth of the mean-square displacement with time for walking and crawling, characterized by the power-law exponent β (i.e. ($\Delta r^2(\tau) \propto \tau^{\beta}$)), yields insight into how bacteria may use these distinct ways of moving to explore surfaces, as exponents of 1.0 and 2.0 indicate random diffusion and ballistic motion, respectively. Walking bacteria exhibit nearly diffusive behavior ($\Delta r^2(\tau) \propto \tau^{1.1}$), which suggests that walking allows bacteria to efficiently explore area. By contrast, crawling bacteria exhibit superdiffusive behavior ($\Delta r^2(\tau) \propto \tau^{1.4}$), which suggests that crawling allows bacteria to efficiently traverse linear distances (Conrad et al., 2011). Walking and crawling are not exclusive phenotypes, and indeed over 1 h, most bacteria switch between these mechanisms (Conrad et al., 2011), further indicating that these mechanisms fulfill distinct functions in surface exploration during biofilm formation.

Walking has only been observed in *P. aeruginosa*, and whether other species of bacteria walk is an open question. Vertical *X. fastidiosa* bacteria also move in jagged trajectories and thus may also walk (De La Fuente et al., 2007). *M. xanthus*, however, uses TfP to slowly jiggle vertically before switching to horizontal crawling in a process that is much slower than walking (Sun et al., 2000). Further studies of these and other TfP-driven processes over a range of bacterial species are needed to identify and characterize the distinct ways in which individual bacteria deploy TfP.

Forces from flowing fluids modify how bacteria use TfP to move on surfaces. Both *X. fastidiosa* (Meng et al., 2005) and *P. aeruginosa* (Shen et al., 2012) use TfP to crawl upstream against flow while oriented parallel to the surface. For *P. aeruginosa*, neither flagella nor chemoreceptors are required for bacteria to migrate upstream, suggesting that TfP drive rheotaxis on a surface. Simple physical arguments on the distribution of TfP on the cell body suggest a mechanism by which TfP enable upstream crawling. TfP that are preferentially located at the poles of bacteria (Cowles and Gitai, 2010) provide an asymmetric tether to the surface. Flow rotates bacteria around the tethered pole and thereby aligns the cell bodies along the direction of flow as shown in Fig. 6a (Shen et al., 2012). To crawl upstream, bacteria retract the TfP at the tethered pole as shown in Fig. 6b (Shen et al., 2012). These studies indicate that flow and TfP interact to align cells against the direction of flow, allowing surface-motile cells to migrate upstream.

4. TfP and flagella cooperate for detaching and dividing

Studies of near-surface motility typically focus on mechanisms driven by a single appendage. However, wild-type bacteria that possess both TfP and flagella may deploy these appendages simultaneously during motion. Tethered rotation in *P. aeruginosa* is one such example: bacteria lacking TfP

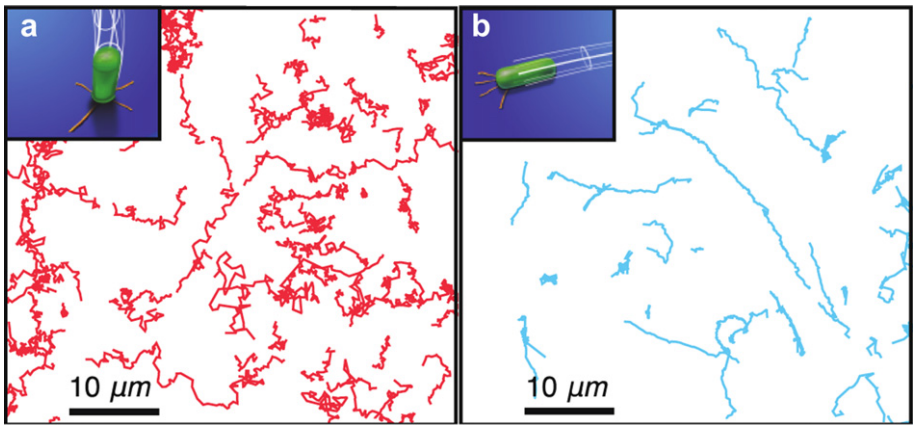

Fig. 5. Bacteria use TfP to walk and crawl. TfP mediate distinct (a) walking and (b) crawling motions in *P. aeruginosa* (Δ*fliM* mutant of ATCC 15692) that can be distinguished by the shapes of the trajectories. Adapted with permission from Gibiansky et al. (2010). Copyright 2010 AAAS.

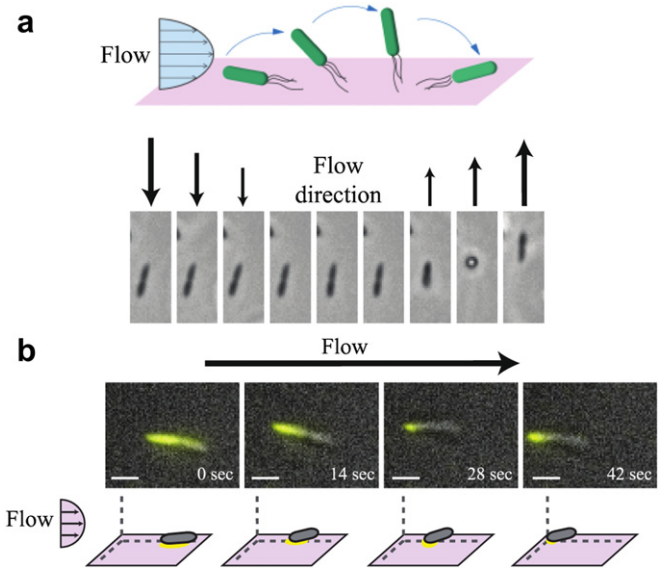

Fig. 6. Bacteria use TfP for rheotaxis on a surface. (a) Schematic (top) and images (bottom) showing a *P. aeruginosa* cell that stands up and flips about its upstream pole after the reversal of the direction of flow. Arrows indicate the direction and magnitude of flow. (b) A cell migrating upstream adheres to the surface at its upstream pole. Yellow highlights indicate the portion of the cell that adheres to the surface. Figures (a) and (b) reproduced with permission from Shen et al. (2012). Copyright 2012 Cell Press.

(Conrad et al., 2011) or TfP function (Tran et al., 2011) are more likely to tether to the surface and rotate using the flagellum than wild-type bacteria. Wild-type bacteria, however, rotate at a greater angle with respect to the surface (70°) than the TfP-deficient mutants (30°), confirming that TfP interact with flagella in this motility phenotype (Conrad et al., 2011).

Two additional examples from life-cycle events in *P. aeruginosa* indicate that bacteria cooperatively deploy these two appendages (Conrad et al., 2011). First, both TfP and flagella affect motion after cell division as shown in Fig. 7: no TfP-deficient (Δ*pilA*) mutants move after division and more flagellum-deficient (Δ*fliM*) than wild-type bacteria move. At the time of separation, one daughter cell bears an underdeveloped flagellum (Amako and Umeda, 1982) and thus TfP are required for this daughter cell to move after division. Second, spinning wild-type bacteria tilt away from the surface using TfP and then detach in a characteristic "launch" sequence shown in Fig. 3 (Conrad et al., 2011). Mutants lacking flagella or TfP do not detach from the surface via this sequence of motility phenotypes, indicating that this sequence requires both appendages. As wild-type bacteria detach at a higher rate from the surface than either appendage-deficient mutant, the launch sequence and other mechanisms of appendage cooperation may enhance the ability of bacteria to redistribute on surfaces and thereby affect the morphology of biofilms as they form. Some support for this idea is found in flow-cell experiments: TfP-deficient bacteria, which lack the launch sequence and cannot easily detach, form large clusters at the surface sites at which they originally attach, whereas wild-type bacteria that can freely detach and redistribute form a uniform and thin layer of cells (Gibiansky et al., 2010). In addition, *P. aeruginosa* bacteria that have flagella and TfP exhibit a higher probability of detaching from surfaces at short times when exposed to shear flow than appendage-deficient mutants (Lecuyer et al., 2011). Further studies correlating use of motility appendages to bacterial life-cycle processes may uncover additional examples of interactions between appendages that influence biofilm formation.

5. Outlook: appendage use during biofilm formation

In this review, we discuss physical constraints that influence how bacteria move on and near surfaces with two motility appendages, flagella and TfP, in the context of biofilm formation. Hydrodynamic interactions and Brownian motion change the shape of trajectories of bacteria swimming with flagella near surfaces, increasing both the time that bacteria spend near the surface and the probability that they attach. When bacteria self-tether to the surface by a flagellum, rotation of the motor drives spinning and detaching. Bacteria using TfP can crawl in nearly straight trajectories or randomly walk on surfaces, thereby covering area or distance as needed for efficient surface dispersal. Walking and the jagged slingshot or tug-of-war motions found in crawling arise when multiple TfP pull and release, allowing bacteria to efficiently travel through shear-thinning fluids. Forces exerted by flowing fluid affect both flagella and TfP-driven motility by reorienting the bodies of bacteria to swim or crawl against flows in rheotaxis, allowing bacteria to spread and disperse upstream. While these simple arguments arising from idealized experiments suggest intriguing connections between physical properties of motility and biofilm formation, full understanding requires investigations in more complex conditions encountered during biofilm formation. Below, we summarize three avenues for further studies of flagella and TfP use during biofilm formation.

First, the simple physical arguments presented here largely neglect the properties of the surface near which bacteria move. Most microbiological assays for motility use solid glass or semisolid agar surfaces, yet bacteria can colonize surfaces and porous media of widely varying chemistry, elasticity and roughness. Advances in microfabrication techniques (Weibel et al., 2007) enable new studies of the effects of surfaces on bacterial adhesion and motility. For example, nanoscale surface patterns can align both *Pseudomonas fluorescens* bacteria (Díaz et al., 2007, 2009) and their flagella (Díaz et al., 2011); similarly, microscale arrays of vertical pillars align *P. aeruginosa* and *B. subtilis* bacteria (Hochbaum and Aizenberg, 2010). Very recent experiments demonstrate that surface properties modify TfP-driven motility. Surface grooves of depth 1 μm confine both crawling *N. gonorrhoeae* and *M. xanthus* bacteria over long times, whereas slightly shorter grooves of depth 0.6 μm reduce the dwell time for *N. gonorrhoeae* (Meel et al., 2012). *N. gonorrhoeae* bacteria crawling on supported lipid membranes, which serve as models for eukaryotic cell membranes, move increasingly slowly as the fluidity of the membrane is increased, suggesting that retraction of TfP bound to lipids is less effective for actuating

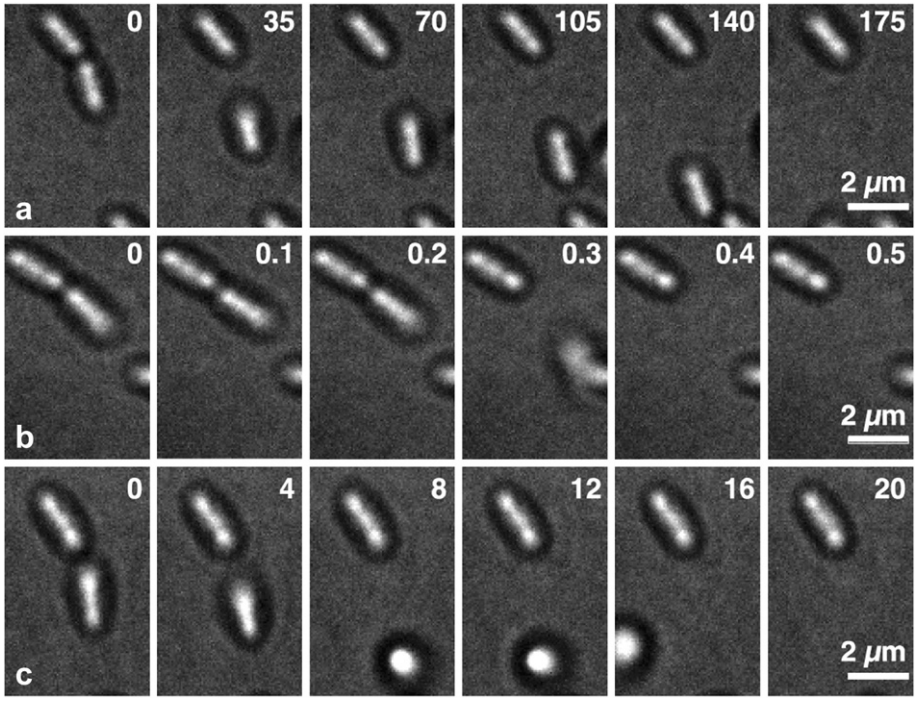

Fig. 7. Bacteria cooperatively use TfP and flagella to move after cell division. Representative time series micrographs of wild-type *P. aeruginosa* (ATCC 15692) show daughter cells (a) crawling, (b) detaching or (c) walking, with timestamps. Reproduced with permission from Conrad et al. (2011). Copyright 2011 Cell Press.

bacterial motion (Holz et al., 2009). These experiments confirm that surface properties affect the interactions between appendages and surfaces and hence also affect bacterial motility. Bacteria may also be confined in thin liquid films or in complex three-dimensional geometries during biofilm formation. Here, microfluidic and microfabrication techniques to create well-controlled model 3-d porous media allow motility to be studied in confinement. For example, both *E. coli* and *B. subtilis* can swim through channels that are marginally smaller than their diameters (Maennik et al., 2009). Studies relating appendage-driven motility to other surface properties, such as charge, elasticity, porosity or roughness, may provide further insight into mechanisms that bacteria use to move on and colonize these varied surfaces prior to biofilm formation.

Second, bacteria can themselves modify the physical properties of a surface by excreting extracellular polymeric substances (EPS). For example, EPS produced by *P. aeruginosa* bacteria modify the properties of the surface and affect the rate at which bacteria adhere to the surface (Gomez-Suarez et al., 2002). Model experiments showing that polymers drive phase separation of bacteria suggest that EPS may induce a depletion attraction between bacteria and surfaces and thereby promote adhesion (Schwarz-Linek et al., 2010a,b). By contrast, how EPS production affects the appendage-driven motion of bacteria on surfaces is less understood. EPS, which contain both polysaccharides and DNA, constitute a non-Newtonian fluid in which the stress depends non-linearly on the shear rate. Swimmers in non-Newtonian fluids such as viscoelastic polymeric solutions (Fu et al., 2009) do not have to obey the scallop theorem (Lauga, 2011). Bacteria therefore may differently deploy their motility appendages to move on or near EPS-covered surfaces, for example by using TfP to rapidly slingshot through shear-thinning fluids (Jin et al., 2011). Advanced experimental techniques, such as super-resolution microscopy imaging of EPS near cells (Berk et al., 2012), coupled with high-throughput tracking of both bacteria and their appendages may provide additional insight into the role of EPS on near-surface motility.

Finally, biofilm formation typically requires bacteria to transition from individual to collective modes of motility on surfaces. For example, many bacteria swarm using flagella (Wu et al., 2011) or twitch using TfP (Burrows, 2012) prior to biofilm formation. As the density of bacteria is increased, hydrodynamic interactions between neighboring cells induce correlations in their velocity and orientation. For example, clusters of swarming *B. subtilis* bacteria on a surface move in regions of correlated velocity and alignment (Zhang et al., 2010) whose typical size is approximately 30% of the cluster size (Chen et al., 2012), and *P. aeruginosa* bacteria also align during swarming (Du et al., 2012). How bacteria deploy appendages in these collective motility modes is still poorly understood, although results on flagellated swarmers suggest that neighboring bacteria may coordinate flagella. *E. coli* bacteria at the edge of a swarming colony collectively orient their flagella out of the swarm (Copeland et al., 2010), pumping fluid outward so that the bacteria can more easily spread (Turner et al., 2010). The interactions observed between flagella of neighboring *E. coli* during swarming may allow bacteria to align for more rapid collective motion (Copeland et al., 2010). Finally, *B. subtilis* bacteria confined in

a thin fluid layer arrange their flagella as a dipole, thereby causing flow to circulate around the body of the bacteria (Cisneros et al., 2008). Further studies of interactions between the flagella and TfP of neighboring cells during collective motility may give new insight into physical processes driving these modes; in addition, whether and how bacteria coordinate TfP is an interesting open question.

Techniques from physical scientists and engineers yield new ways to analyze patterns of bacterial near-surface motility, thereby offering new routes to study interactions of individual and collectively motile bacteria with surfaces and with extracellular polymeric substances. Coupling these multidisciplinary efforts with microbiological analyses, such as fluorescent reporters for genetic pathways implicated in biofilm formation, promises new understanding of how bacteria move and interact in distinct surface microenvironments during biofilm formation (Monds and O'Toole, 2009).

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