A pili-driven bacterial turbine

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2 ABSTRACT

3 Work generated by self-propelled bacteria can be harnessed with the help of microdevices.
4 Such nanofabricated microdevices, immersed in a bacterial bath, may exhibit unidirectional
5 rotational or translational motion. Swimming bacteria that propel with the help of actively rotating
6 flagella are a prototypical example of active agents that can power such microdevices.

7 In this work, we propose a computational model of a micron-sized turbine powered by bacteria
8 that rely on active type IV pili appendages for surface-associated motility. We find that the turbine
9 can rotate persistently over a time scale that significantly exceeds the characteristic times of the
10 single cell motility. The persistent rotation is explained by the collective dynamics of multiple pili
11 of groups of cells attaching to and pulling on turbine. Furthermore, we show that the turbine can
12 rotate permanently in the same direction by altering the pili binding to the turbine surface in an
13 asymmetric fashion. We thus can show that by changing the adhesive properties of the turbine
14 while keeping its symmetric geometry, we can still break the symmetry of its rotation.

15 Altogether, this study widely expands the range of bacteria that can be used to power
16 nanofabricated microdevices, and, due to high pili forces generated by pili retraction, promises to
17 push the harnessed work by several orders of magnitude.

18 **Keywords:** bacterial turbine, bacterial motility, microdevice, type IV pili, active motion

1 INTRODUCTION

19 The last years have seen significant advances in nanofabrication, permitting the invention of a wide
20 range of micron-sized artificial devices. A fascinating question is how such devices can be powered by
21 actively moving biological matter, typically consisting of bacteria. Examples are beads that move due to
22 collision or attachment of cells to its surface [1, 2], swimming devices due to bacterial carpets attached
23 to their surface [3], and geometrically asymmetric devices immersed in a bath of actively moving cells
24 that rotate due to random collisions with the cells [4, 5, 6, 7]. Usually, bacteria that swim with the help
25 of rotating flagella were employed. In this study, we propose to consider twitching bacteria that exhibit
26 surface-associated locomotion mediated by type IV pili [8, 9]. Pili are microns long active polymeric
27 appendages protruding from the cell membrane. They undergo cycles of protrusion and retraction and can
28 bind to a substrate and the pili of other cells. The combination of these two processes leads to aggregation
29 of cells [10, 11, 12] and twitching motility on a substrate [13, 14, 15, 16] by a mechanism reminiscent of a
30 grappling hook (see Fig. 1A).

Here, we investigate how bacteria that can attach type IV pili to a micron-sized turbine can drive its rotation. This system is especially interesting since multiple pili belonging to an individual bacterium can generate total forces in the range of nano-Newtons [17]. In contrast to that, bacteria that swim with the help of flagella typically have thrust forces in the order of 1-10 pN [18]. The molecular motor involved in the disassembly and hence, in the retraction of an individual pilus, called pilT, can generate forces in the range of 100 – 180 pN [19]. This makes pilT the strongest molecular motor known in nature, with forces 2-20 times larger than those generated by kinesins or polymerases [17]. Hence, we are asking if cells with type IV pili that can create so large forces might be more attractive candidates to power microdevices.

In this work, by means of a computational model, we study the dynamics of a rotatable turbine immersed in a bath of twitching bacteria. We investigate how the turbine rotation is affected by the binding and unbinding of the bacterial pili. We find that, due to the adhesion of multiple pili to the turbine over time scales that can strongly exceed the characteristic time scales of the individual pili attachment, the turbine can persistently rotate in one direction over extensive durations. While these persistent rotations have a limited lifetime, one can engineer a system where they become permanently unidirectional by introducing asymmetric binding of the pili to the turbine.

2 MATERIALS AND METHODS

First, we introduce the computational model of twitching bacteria and their interactions with a turbine. A related version of this computational model was considered previously to describe bacterial surface motility [16] and bacterial aggregates [20, 21, 22].

While we consider the bacterium *Neisseria gonorrhoeae* as the primary biological example, the computational model can be easily adapted to account for other bacteria that use type IV pili, e.g. *Neisseria meningitidis* [11] or *Pseudomonas aeruginosa* [23]. In the following, we focus on the regime of low cell density. This allows us not to consider cell-cell interactions and the formation of bacterial aggregates mediated by the binding of the pili of different cells. As a result, we do not expect three dimensional aggregates to form [20, 21]. This also enables us to only consider a simpler two-dimensional system.

2.1 Geometry of the cells, pili and turbine

The experimental system we are mimicking are bacterial cells confined in a box with the turbine at its center (see Fig. 1B). The cells can move over the substrate via pili binding and unbinding and additionally interact with the turbine by pili attachment and excluded volume effects.

While *Neisseria gonorrhoeae* cells typically have a diplococcus shape [24], we simplify the *in silico* cell shape of bacteria as two-dimensional circular disks with a radius R . Please note, however, that a diplococcus shape can be also considered [16, 20, 21, 22]. Each cell possesses exactly N_p pili that are homogeneously distributed on the cell outline (see Fig. 1A). We approximate pili as straight lines connecting two points: their start point (also called anchor point), located at the circular cell surface, and their end point. The distance between these two points is called the contour length l_c of the pilus.

The turbine is described by $N_{\text{turb}} \times 2$ disks (with radius R) arranged in a double row array (see Fig. 1B). Since the only way how the turbine can move is by rotation, the relative locations of the turbine disks towards each other are fixed. The orientation of the turbine is described by the angle α with respect to the y -axis and the turbine center is located at the position $\mathbf{r}^{(\text{turb})}$ (see Fig. 1B).

Figure 1. Overview of the computational model of bacteria driving a rotatable turbine by attachment of type IV pili. **(A)** Sketch of how pili binding and retraction can lead to a force acting on a cell, a process reminiscent of a grappling hook. **(B)** Overview of the system. Cells are located in a box together with the rotatable turbine. The cells possess pili which they use for the motility on the substrate and for attachment to the turbine. The turbine center is located at $\mathbf{r}^{(turb)}$ and has an orientation defined by the angle α . **(C)** Summary of all forces acting on the cells and turbine. Friction forces with the environment (surrounding liquid and substrate) are not shown.

69 2.2 Pili dynamics and binding properties

70 Initially, a pilus protrudes with the velocity v_p in a direction perpendicular to the cell surface. When a
 71 pilus reaches a specific length, drawn from an exponential length distribution with mean length l_p [15, 14],
 72 it starts to retract with a velocity $v_r^{(0)}$. This retraction continues until the pilus has a contour length $l_c = 0$.
 73 In that case, the pilus is removed and immediately, a new pilus protrudes from the cell from the same
 74 position.

75 A pilus binds stochastically to the substrate or the turbine disks, independently whether it is protruding
 76 or retracting. In both cases, a pilus can only bind with its tip and it can bind only to either the substrate
 77 or the turbine disks. After binding, a pilus immediately starts to retract [25]. The binding is modeled as a
 78 Poisson process with the binding rate $\kappa_{att}^{(sub)}$ to the substrate and $\kappa_{att}^{(turb)}$ to any of the turbine disks. Since

the model is two-dimensional, we ignore that, for pili to bind to a substrate, they first need to be long enough to reach the substrate with their tips. In 3D, this leads to a delay in the initial binding event of a newly protruding pilus, but is less relevant for following attachments of pili. Hence, initially, the binding rate might be smaller to allow a pilus to protrude to reach the substrate. To account for this process, pili that bind to the substrate the first time do so with a rate $\kappa_{\text{att}}^{(\text{sub},0)} = v_p/l_p$. Again, the binding is modelled as a Poisson process. The rate $\kappa_{\text{att}}^{(\text{sub},0)}$ corresponds to the binding of a pilus that protrude with the velocity v_p until it reaches a length determined by an exponential distribution with mean length l_p , in which case it binds. On average, this takes the time $1/\kappa_{\text{att}}^{(\text{sub},0)}$.

An attached pilus will generate a pulling force that acts on the cell and, if attached to a turbine disk, also on the turbine. Each pilus is modeled as a Hookean spring with the spring constant k_p . After attachment, a pilus is stretched due to its retraction and hence, mediates a pulling force. This force is proportional to the difference between the contour length l_c and the length of the pilus that it would have if it was not attached, here called the free length l_f . Here, we consider the case where a pilus can only generate a pulling force and no pushing force, thus the force is zero if $l_c \leq l_f$.

Experimentally, it has been shown that the pilus force affects the retraction velocity by

$$v_r = \max \left[0, v_r^{(0)} \left(1 - \frac{F}{F_s} \right) \right], \quad (1)$$

with the stalling force F_s [19]. The force of a pilus also affects the unbinding from the substrate. Pilus detachment is modelled by a Poisson process with the rate

$$\kappa_{\text{det}}^{(\text{sub})}(F) = \frac{1}{\tau_{\text{det}}^{(\text{sub},1)} \exp \left(-\frac{F}{F_{\text{det}}^{(\text{sub},1)}} \right) + \tau_{\text{det}}^{(\text{sub},2)} \exp \left(-\frac{F}{F_{\text{det}}^{(\text{sub},2)}} \right)}, \quad (2)$$

for detachment from the substrate, as motivated by [15]. Here, we introduce the detachment times $\tau_{\text{det}}^{(\text{sub},1)}$ and $\tau_{\text{det}}^{(\text{sub},2)}$ and the detachment forces $F_{\text{det}}^{(\text{sub},1)}$ and $F_{\text{det}}^{(\text{sub},2)}$. For detachment from a turbine disk, we describe the rate by a simpler relation

$$\kappa_{\text{det}}^{(\text{turb})} = \frac{1}{\tau_{\text{det}}^{(\text{turb})} \exp \left(\frac{F}{F_{\text{det}}^{(\text{turb})}} \right)}, \quad (3)$$

with the detachment time $\tau_{\text{det}}^{(\text{turb})}$ and detachment force $F_{\text{det}}^{(\text{turb})}$.

2.3 Cell forces and motility

We model pili as Hookean springs with the spring constant k_p . For a cell i at location $\mathbf{r}_i^{(\text{cell})}$, an attached pilus j causes a force $\mathbf{F}_i^{(\text{pilus } j)}$ at the pilus anchor point $\mathbf{r}_i^{(\text{anchor } j)}$ on the surface of the cell in the direction of the point $\mathbf{r}_i^{(\text{att } j)}$ where the pilus tip is attached (see Fig. 1C).

Next to the active pili force, passive excluded volume forces are acting on the cells. Cells are located in a two-dimensional box with size $L \times L$ and a cell i that overlaps with the boundary wall is exposed to a repulsive force $\mathbf{F}_i^{(\text{wall})}$ (see Fig. 1C). This force is modelled as a harmonic interaction with spring constant $k^{(\text{wall})}$ and acts on the cell center in the normal direction of the boundary wall if the overlap is

smaller than the cell radius R . Additionally, intersections of two cells i and k lead to a repulsive force $\mathbf{F}_{ik}^{(c-c)}$ of the centers of both cells with the spring constant $k^{(c-c)}$ (see Fig. 1C). A similar type of repulsive force $\mathbf{F}_i^{(\text{turb } m)}$ is acting between the cells and the turbine disks m with the spring constant $k^{(\text{turb})}$ (see Fig. 1C).

The total force of the cell i is given by

$$\mathbf{F}_i^{(\text{tot})} = \sum_{\text{attached pili } j} \mathbf{F}_i^{(\text{pilus } j)} + \mathbf{F}_i^{(\text{wall})} + \sum_{\text{cells } k} \mathbf{F}_{ik}^{(c-c)} + \sum_{\text{turbine disks } m} \mathbf{F}_i^{(\text{turb } m)}. \quad (4)$$

Additionally, the total torque acting on a cell is

$$\mathbf{T}_i^{(\text{tot})} = \sum_{\text{attached pili } j} \left(\mathbf{r}_i^{(\text{anchor } j)} - \mathbf{r}_i^{(\text{cell})} \right) \times \mathbf{F}_i^{(\text{pilus } j)} \quad (5)$$

In the overdamped limit [26], a force mediates a translational motion of the cell with the velocity

$$\frac{d\mathbf{r}_i^{(\text{cell})}}{dt} = \mu_t \mathbf{F}_i^{(\text{tot})} \quad (6)$$

and the torque leads to a rotation with the angular velocity vector

$$\boldsymbol{\omega}_i^{(\text{cell})} = \mu_r \mathbf{T}_i^{(\text{tot})} \quad (7)$$

Here, we introduce the translational mobility μ_t and the rotational mobility μ_r . The same forces and torques cause an equivalent displacement of the pili anchor points $\mathbf{r}_i^{(\text{anchor } j)}$ and attachment points $\mathbf{r}_i^{(\text{att } j)}$. The mobilities introduced here are a result of friction with the viscous solvent and the substrate on which the cells are moving.

2.4 Turbine torque and rotation

The turbine is only able to undergo rotational motion. The total torque acting on the turbine is given by

$$\begin{aligned} \mathbf{T}^{(\text{turb})} = & \sum_{\text{attached pili } j} \left(\mathbf{r}_i^{(\text{att } j)} - \mathbf{r}^{(\text{turb})} \right) \times \mathbf{F}_i^{(\text{pilus } j)} \\ & + \sum_{\text{cells } i} \sum_{\text{turbine disks } m} \left(\mathbf{r}_i^{(\text{cell})} - \mathbf{r}^{(\text{turb})} \right) \times \left(-\mathbf{F}_i^{(\text{turb } m)} \right). \end{aligned} \quad (8)$$

The rotation of the turbine is modelled in the overdamped limit with a mobility μ_{turb} . In that case, the turbine angular velocity vector is

$$\boldsymbol{\omega}^{(\text{turb})} = \mu_{\text{turb}} \mathbf{T}^{(\text{turb})}. \quad (9)$$

2.5 Parameters and details of numerical solution

The simulations were performed on the local computing cluster of the Max Planck Institute for the Physics of Complex Systems (Dresden, Germany), consisting of x86-64 GNU/Linux systems. The code was written in C++. We use an Euler algorithm to solve the equations of motion with a time step δt . While this is one of the most simple numerical schemes to solve the equations of our computational model, it

often leads to numerical errors and instabilities when modelling molecular dynamics systems for long times [27]. We do not expect that this is a problem in our system due to the stochastic nature of the pili binding and unbinding, which basically represents our system as a series of many short time events, continuously interrupted by rearrangements in the pili network. Hence, we do not expect any differences in the qualitative outcome of the simulations.

If not stated otherwise, we use the of parameters provided in Table 1. Most parameters we use are based on previous studies. The excluded volume spring constants $k^{(c-c)}$, $k^{(\text{turb})}$ and $k^{(\text{wall})}$ have no effect on the simulation outcome as long as they are chosen large enough to be able to compete with the pili forces. For the remaining parameters, e.g. the turbine mobility, we do not expect a qualitative difference in the results of the simulation.

We initialize the simulation by randomly distributing the cells in the box and only analyse the turbine rotation after an initialization period of 1000 s. To calculate the angular velocity of the turbine rotation, we compute

$$\omega_{\alpha}(t) = \frac{\alpha(t + \Delta t) - \alpha(t)}{\Delta t}, \quad (10)$$

with $\Delta t = 0.5$ s and the turbine orientation α (see Fig. 1B).

3 RESULTS

In the following, we first demonstrate that bacteria binding to the turbine with the help of pili can cause a persistent turbine rotation over time scales that exceeds the pili detachment times significantly. Next, we study how the persistent turbine rotation depends on the binding properties of the pili to the turbine and the number of cells in the system. This allows us to unravel the underlying mechanism that causes the persistent rotation of the turbine. Finally, we propose a system where an asymmetric binding of the pili to the turbine causes a permanent unidirectional rotation of the turbine.

3.1 Adhesion of motile bacteria drives turbine rotation

To investigate how the adhesion of cells to a turbine affects the turbine rotation, we first simulate the computational model for three different cases: (1) pili detach with detachment times of $\tau_{\text{det}}^{(\text{turb})} = 50$ s (see Fig. 2A and Movie S1), motivated by binding times in the order of a few minutes inferred from cell trajectories on plastic surfaces [14], (2) pili detach with $\tau_{\text{det}}^{(\text{turb})} = 2$ s (see Movie S2), motivated by considerably smaller binding times experimentally measured for BSA coated beads [15] and (3) pili not bind to the turbine at all (see Movie S3) and the only way how cells interact with the turbine is by excluded volume forces. In Fig. 2B, we show the trajectories of the turbine angle α . We find that for the largest detachment time, $\tau_{\text{det}}^{(\text{turb})} = 50$ s, the rotation is the strongest. If pili are not permitted to attach to the turbine, the rotation appears to be the weakest. Additionally, the distribution of velocities shows that if pili cannot bind to the turbine, the turbine will move with smaller angular velocities ω , while the distributions seem to not depend on the detachment rate $\tau_{\text{det}}^{(\text{turb})}$, as long as pili are capable of binding to the turbine. To quantify how strong the rotation is, we compute the angular mean squared displacement (angular MSD), given by

$$\delta_{\alpha}(\Delta t) = \langle [\alpha(t + \Delta t) - \alpha(t)]^2 \rangle_t, \quad (11)$$

(see Fig. 2C). Indeed, the angular MSD is the highest for $\tau_{\text{det}}^{(\text{turb})} = 50$ s and the lowest for the case where pili do not attach to turbine disks. For $\tau_{\text{det}}^{(\text{turb})} = 2$ s, the angular MSD is identical to the case

Figure 2. Analysis of rotary motion of a turbine in a bath of twitching bacteria. **(A)** Snapshots of the simulations of a turbine in a bath of bacteria with pili detachment rate $\tau_{\text{det}}^{(\text{turb})} = 50$ s. Blue pili are not attached, orange pili are attached to the turbine and red pili are attached to the substrate. The green arrow indicates the direction of the turbine rotation. **(B)** Turbine angle α as a function of the time t (see Fig. 1B.). The inset figure shows the probability density function of the turbine angular velocity ω . **(C)** Angular mean squared displacement (angular MSD) of the turbine angle, see Eq. (11). **(D)** Normalised angular velocity autocorrelation function of the turbine angle, see Eq. (12).

165 $\tau_{\text{det}}^{(\text{turb})} = 50$ s for small time differences (Δt). In both cases, $\tau_{\text{det}}^{(\text{turb})} = 2$ s and non-attaching pili, the
 166 angular MSD follows a linear scaling, $\delta_\alpha \propto \Delta t$, corresponding to a diffusive regime. For $\tau_{\text{det}}^{(\text{turb})} = 50$ s,
 167 we find a superdiffusive regime for intermediate time differences $\Delta t \approx 10 - 10^3$ s. For particles undergoing
 168 translational motion, such superdiffusive regimes usually emerge from ballistic motion. For the rotatable
 169 turbine, this correspond to a regime where the turbine moves persistently in one direction. Indeed, such
 170 a behaviour is observed in Fig. 2B where trajectories of the angle α move persistently in one direction
 171 over periods of hundreds to thousands of seconds before the rotation turns towards the opposite direction.
 172 For larger time differences, the angular MSD becomes again diffusive, implying that while the rotation is
 173 persistent over a certain time scale, for larger time intervals it becomes random again.

174 To learn more about how persistent the turbine rotation is, we compute the angular velocity autocorrelation
 175 function (angular velocity ACF), given by

$$\nu(\Delta t) = \langle \omega_\alpha(t + \Delta t) \omega_\alpha(t) \rangle_t. \quad (12)$$

Independently of whether pili can bind to the turbine or not, we find that the correlation function $\nu(\Delta t)$ is decaying with time (see Fig. 2D). This confirms our previous observation for the angular MSD where we found that for very large time differences the turbine rotation is diffusive and no longer shows signs of persistence. We also find that for $\tau_{\text{det}}^{(\text{turb})} = 50$ s, the normalised angular velocity ACF $\nu(\Delta t)/\nu(0)$ has the slowest decay. This behaviour correlates with the persistent rotation for time differences between $\Delta t \approx 10 - 10^3$. Surprisingly, we find that the decay of the angular velocity ACF is faster for $\tau_{\text{det}}^{(\text{turb})} = 2$ s than for the case where pili do not bind to the turbine. We will provide an explanation for this behaviour in section 3.3. Before doing that, we will have a closer look at how the turbine rotation depends on the cell adhesion to the turbine disks and the number of cells in the system.

3.2 Turbine rotation is controlled by bacterial adhesion strength and cell number

We begin with a systematic analysis of how the angular MSD $\delta_\alpha(\Delta t)$ depends on the detachment time of pili from the turbine disks $\tau_{\text{det}}^{(\text{turb})}$, shown in Fig. 3A. For small time differences $\Delta t < 10$ s, we find that δ_α is independent of $\tau_{\text{det}}^{(\text{turb})}$ and exhibits a linear scaling, $\delta_\alpha \propto \Delta t$, corresponding to a diffusive regime. For high enough values of the detachment time $\tau_{\text{det}}^{(\text{turb})}$ and $\Delta t > 10$ s, the angular MSD becomes superdiffusive, $\delta_\alpha \propto \Delta t^2$. The duration of this superdiffusive regime increases with increasing $\tau_{\text{det}}^{(\text{turb})}$ and is barely observable for detachment times $\tau_{\text{det}}^{(\text{turb})} < 10$ s. For even longer time differences Δt , the MSD becomes diffusive again, $\delta_\alpha \propto \Delta t$.

Next, we investigate the normalised angular velocity ACF $\nu(\Delta t)/\nu(0)$ (see Fig. 3B) and find that for $\Delta t < 2$ s, it rapidly decreases. For $\Delta t \geq 2$ s, it exponentially decreases with the Δt . By fitting a function of the form $\nu(\Delta t) \propto \exp(-\Delta t/\tau)$ to this later regime, we identify the characteristic time τ of the exponential decay of $\nu(\Delta t)$ and can investigate its dependence on $\tau_{\text{det}}^{(\text{turb})}$. We find that it is increasing with increasing detachment times $\tau_{\text{det}}^{(\text{turb})}$. This is in accordance with the increasing duration of the superdiffusive regime of the angular MSD. The stronger pili of a cell bind to the turbine, the more persistently the turbine rotates in one direction before it inverts its direction.

Additionally, we investigated how the rotation of the turbine is affected by the density ρ of cells in the system. We find that with increasing density, the angular MSD δ_α becomes smaller (see Fig. 3C). While we observe the diffusive regime of $\delta_\alpha(\Delta t)$ for $\Delta t < 10$ s, a pronounced superdiffusive regime for larger Δt vanishes if the cell density becomes too large. For the normalised angular velocity ACF $\nu(\Delta t)/\nu(0)$ (see Fig. 3D), the characteristic decay time τ is initially increasing with cell density ρ and then decreases when ρ increases further.

In the following section, we provide qualitative arguments that explain the observed behaviours.

3.3 Unbinding dynamics of attached bacteria explains characteristic time of turbine rotation

To understand how the persistent rotation of the turbine in an otherwise symmetric system can emerge, we first consider how strongly cells bind to the turbine disks with the help of their pili and how pili binding affects substrate attachment of the remaining pili. Our hypothesis is the following: if cells stay attached to the same position on the turbine surface for an extended time, which is considerably larger than average attachment times of individual pili, the cells will continuously pull the turbine in the same direction. This mechanism is dramatically different to the previously reported rotary microdevices driven by swimming

Dependence on detachment time

Dependence on cell density

Figure 3. Dependence of the turbine rotation on pili detachment time $\tau_{\text{det}}^{(\text{turb})}$ and cell density ρ . **(A and C)** Angular mean squared displacement (angular MSD) $\delta_{\alpha}(\Delta t)$ of the turbine angle α as a function of the pili-turbine detachment time $\tau_{\text{det}}^{(\text{turb})}$ (with the cell density at $\rho = 0.1 \mu\text{m}^2$) or cell density ρ (with the detachment time $\tau_{\text{det}}^{(\text{turb})} = 50 \text{ s}$). **(B and D)** Normalised angular velocity autocorrelation function (angular velocity ACF) $\nu(\Delta t)/\nu(0)$ of the turbine angle α as a function of the pili-turbine detachment time $\tau_{\text{det}}^{(\text{turb})}$ or cell density ρ . The inset figure shows the characteristic time scale of $\nu(\Delta t)$, resulting from a fit $\nu(\Delta t) \propto \exp(-\Delta t/\tau)$. To account for the initial drop of the angular velocity ACF, we only do the fitting for $\Delta t > 2 \text{ s}$. An example fit is shown in **(B)** (black dashed line). Please note that for the investigation of the cell density dependence of the turbine rotation, we only consider a smaller range of Δt to avoid too high numerical cost. As a result, we do not show the diffusive regime in the MSD for large values of Δt since the angular velocity ACF is decaying with increasing Δt .

215 bacteria [4, 5], where cells collide with the microdevice and hence, push it. Instead, in our simulations,
 216 cells pull on the turbine.

217 In Fig. 4A, we show sketch of a cell which is attached to the turbine disk with some of its pili, while the
 218 other pili bind or unbind from the substrate. Only pili that protrude towards the turbine disks can attach,
 219 while pili protruding away from the turbine can only attach to the substrate. Thus, cells preferentially
 220 bind to the substrate in the direction away from the turbine. Since the cell is also attached with some of

Figure 4. Overview of the mechanism driving the persistent rotation of the turbine. **(A)** A cell (light blue circle) with $n = 3$ or $n = 0$ pili attached to a turbine (dark blue circles). **(B)** Stochastic model of n pili attached to a turbine with pili binding (with constant rate k_{att}) and unbinding (with constant rate k_{det}). **(C)** Mean unbinding time to reach for the first time the state $n = 0$ in the stochastic model. Here, we chose $N_0 = 5$, $n(t = 0) = 5$, $k_{\text{att}} = 2 \text{ Hz}$, $k_{\text{det}} = (\exp 1)/\tau_{\text{off}}$. **(D)** Sketch of how turbine rotation is affected by multiple cells binding to the turbine surface. The turbine rotates in the direction in which more cells are attached to its surface (turbine is pulled by cells). **(E)** Sketch of how turbine rotation leads to a pickup and binding of cells on the turbine surface.

its pili to the turbine disks, it is pulling the turbine along the direction pointing from the turbine towards the cell. Thus, the cell pulls on the turbine, leading to a rotation of the turbine in the direction of the attached cell. This process is enhanced by the cooperation of multiple pili of a cell. To clarify this, we investigate the durations of cells attaching to the turbine disk before they detach again. To this aim, we solve a stochastic model of pili binding and unbinding, where a cell, possessing in total N_0 pili that are all growing in the direction of the turbine, has n pili attached to the turbine. With a rate $k_{\text{att}}(N_0 - n)$ a non-attached pilus attaches to the turbine, while with the rate $k_{\text{det}}n$ a pilus detaches from the turbine (see Fig. 4B). By describing the attachment and detachment as Poisson processes, we can numerically solve this system with the help of a Gillespie algorithm and investigate the mean time for a complete unbinding of all pili of a cell from the turbine (see Fig. 4C). We find that the mean unbinding time is increasing rapidly with the pili detachment time $\tau_{\text{off}} = \exp(F_s/F_{\text{det}}^{(\text{turb})})/k_{\text{det}}$, reaching around 10 s for $\tau_{\text{off}} = 2$ s and 10^7 s for $\tau_{\text{off}} = 50$ s. For simplicity, we assume that all pili pull with their stalling force and that the detachment force is identical to the stalling force, $F_s = F_{\text{det}}^{(\text{turb})}$. The duration of how long cells bind to the turbine corresponds to the duration of superdiffusive behaviour in Fig. 3A and also exhibits the same qualitative behaviour as the decay time of the angular velocity autocorrelation (see Fig. 3B). We do not expect a perfect quantitative agreement between the two time scales (Fig. 3B and Fig. 4C) as the simplified model of pili binding and unbinding of a cell to the turbine provided here ignores that cells can indeed move even if its pili are bound to the turbine, e.g. parallel to the turbine surface. Additionally, when multiple cells bind to the same region of the turbine, they will interact via excluded volume effects. This will lead to additional forces acting on the involved pili and thus, might enhance their detachment.

We can now provide an explanation of the three time regimes observed in the angular MSD in Fig. 2C and angular velocity ACF in Fig 2D. For short times, $\Delta t < 10$ s, individual pili stochastically bind and unbind to the substrate, pulling on the turbine, and additionally, cells randomly collide with the turbine. This causes small displacements of the turbine angle α in one direction, also explaining the very sudden drop of the angular velocity ACF for short times since the life time of such displacements is very small. This leads to random fluctuations of the turbine, and as a result, to a diffusive scaling. For larger times, $10 \text{ s} < \Delta t < 10^3 \text{ s}$, asymmetries in the distribution of cells on the surface of the turbine lead to a unidirectional rotation of the turbine and as a result, to a superdiffusive regime. The persistence in the turbine rotation also explains the positive angular velocity ACF. The duration of this regime depends on how strong pili bind to the turbine disk (see Fig. 4C). Finally, for $\Delta t > 10^3 \text{ s}$, the distribution of cells on the turbine surface is re-arranged up to such a degree that the turbine can change its direction of motion. This will then lead to a diffusive regime again. This time also corresponds to the characteristic time of the angular velocity ACF decay, confirming that for too large times, the turbine forgets its initial direction of motion.

Next, we consider the origin of the cell density dependent rotation of the turbine, see Fig. 3C and Fig. 3D. If the cell density is very small, often no cell will bind to the turbine most of the time and thus, the angular MSD is initially increasing with cell density. For moderate cell densities the difference between cells attaching to different sides of the turbine (see Fig. 4D) will be significant and due to the random asymmetry, the turbine will rotate persistently in one direction until cells randomly unbind from the turbine. If the cell density gets too large, more and more cells are pulling in the opposite direction of the turbine rotation, reducing its persistence.

There are additional processes that can have a significant impact on the turbine rotation: (1) Due to the rotation of the turbine, it will constantly "pick up" cells it collides with. For the traditional rotary microdevices that are driven by swimming bacteria that collide with the device and push it [4, 5, 6], this

would lead to a torque that acts in the opposite direction of the original rotation. When cells pull on the turbine instead, the persistent rotation is being enhanced since asymmetric distribution of cells bound to the turbine disks is getting even stronger (see Fig. 4E). (2) In section 3.1, we found that the angular velocity ACF seems to decay faster for the case with pili attachment and $\tau_{\text{det}}^{(\text{turb})} = 2$ s, than for the case without pili attachment (see Fig. 2D). A possible explanation for this is that even without pili-turbine attachment, cells can still rotate the turbine due to collisions and resulting excluded volume forces. That way, we reproduce a system where cells push the turbine, instead of pulling on it. This process possesses its own characteristic time scales that are linked to the substrate motility of the cells.

To summarise, the persistent rotation of the turbine originates from persistent attachment of cells to the turbine over times scales much longer than the characteristic detachment times of individual pili. While the resulting rotation is indeed persistent and, depending on the binding properties, can go on over multiple revolutions, it is not permanently unidirectional. There is no asymmetry in the initial rotational direction of the turbine and even though the turbine can move in one direction over an extended time, this rotation will at some point reverse direction. Next, we will provide an example of how cells using type IV pili can drive a permanent unidirectional rotation of a turbine.

3.4 Permanent unidirectional rotation due to asymmetric cell-turbine attachment

In order to produce a permanent unidirectional rotation of the turbine, an asymmetry of the turbine is required. Typically, such asymmetries are created by altering the turbine geometry [9, 5, 6], but here we have a chance to exploit asymmetries in the adhesion of cells to the turbine instead. In Fig. 5A, Fig. 5B and Movie S4, we provide an example of how altered pili binding properties on one side of the turbine wings can lead to a unidirectional rotation. Here, we consider different cases: (i) pili bind stronger to the manipulated turbine disks, corresponding to a larger detachment time $\tau_{\text{det}}^{(\text{turb})}$ or (ii) pili bind weaker to the manipulated turbine disks, with a lower value of $\tau_{\text{det}}^{(\text{turb})}$. Depending on how pili bind to these regions, the direction of the turbine is affected as, on average, cells are more or less strongly adhered to the manipulated region and thus, more or less cells are bound to these sides of the turbine and mediate the turbine rotation. In case (i), the turbine rotates in the direction of manipulated region because more cells are attached there, for (ii) less cells are attached in the manipulated region and the turbine rotates in the opposite direction.

We also estimate the average angular velocity ω of the turbine and find values around $2 - 4^\circ/\text{s}$. For the used rotational mobility of the turbine μ_{turb} (see Table 1), this corresponds to a turbine torque around $10 \text{ pN } \mu\text{m}$, comparable to previously published values for rotary devices in bacterial baths of hundreds of swimming cells [5]. Here, however, the system consists of 40 cells only. Note that this is only the lower limit of the turbine torque and the same turbine might be able to generate larger torques if it experiences an opposing torque. In the absence of a counteracting torque, the rotation speed of the turbine is limited by the retraction velocity of pili, around $2 \mu\text{m/s}$ [19].

We see evidence that the rotation is permanently unidirectional in the angular MSD (see Fig. 5C) which is superdiffusive (ballistic) for arbitrary time differences Δt , equivalent to a rotation in one direction. Additionally, the normalised angular velocity ACF (see Fig. 5D) is no longer decaying with time Δt , suggesting a constant rotation in the same direction.

In experiments, such manipulated regions on the surface of the turbine could be generated by coating it with a chemical such as BSA, which has been shown to alter the detachment time of the pili [28, 14, 15].

Figure 5. Unidirectional persistent turbine rotation is caused by asymmetric pili binding to the turbine. **(A)** Overview of the different types of turbine disks considered here. **(B)** Angle α of the turbine as a function of time t for different combinations of the manipulated turbine disks. We also provide the average angular velocity $\langle \omega \rangle$ and its standard error for each condition. **(C)** The angular mean squared displacement exhibits superdiffusive (ballistic) scaling, $\delta_{\alpha} \propto \Delta t^2$. **(D)** The normalised angular velocity auto correlation function is no longer decaying with increasing time differences Δt , corresponding to the unidirectional rotation.

4 DISCUSSION

In this study, we investigated how bacteria that use type IV pili for surface motility may drive the rotation of a micron-sized turbine. We found that due to spatial asymmetries in the amount of cells that are bound to the turbine, a persistent rotation is observed over time scales that can strongly exceed the characteristic time scales of the pili. The persistent rotation is enhanced when pili bind more strongly to the turbine and weaker when the density of cells becomes larger. The observed persistent rotation of a symmetric turbine has a characteristic time scale. For larger times, the rotation direction is reversed stochastically and there is no preferred direction of rotation. A persistent and unidirectional rotation can be generated by altering the binding properties of the type IV pili on parts of the turbine in an asymmetric manner.

Here, we have shown that microdevices immersed in a bath of bacteria that use pili, such as *Neisseria gonorrhoeae* or *Pseudomonas aeruginosa*, can efficiently harness the power of the type IV pilus machinery. This system is particularly superior to previously reported microdevices driven by swimming bacteria [4, 5, 6], since the involved molecular motor pilT is the strongest known molecular motor [19] and a single cell is capable of generating forces in the nano-Newton range.

In the future, it will be interesting to investigate the interplay of asymmetries in the geometrical and adhesive properties of the turbine. Additionally, we expect that the binding of pili with the substrate will also have an effect on the turbine rotation. Higher values of the pili-substrate detachment times $\tau_{\text{det}}^{(\text{sub},1)}$ and $\tau_{\text{det}}^{(\text{sub},2)}$ will lead to an increase in the torque exerted by attached cells on the turbine and will likely exceed the observed lower limit of the torque of around 10 pN μm . This will enable the turbine to more efficiently harness the work generated by type IV pili. Importantly, one needs to consider that if pili bind too strongly to the substrate, their substrate mobility will be weakened [16] and hence, it will be less likely that cells will be in the vicinity of the turbine. Furthermore, cells pulling in the direction opposite to the turbine rotation will exert larger forces opposing the rotation. In the future, it will be interesting to investigate the optimal substrate binding properties to maximize the turbine torque. Additionally, bacteria use type IV pili to form aggregates consisting of hundreds to thousands of cells [12, 20, 21, 29, 30]. The impact of this aggregation process on the turbine rotation remains unclear.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

WP and VZ designed the research. WP carried out all numerical solutions. WP carried out analytical calculations. WP analyzed the data. WP and VZ wrote the article.

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SUPPLEMENTAL DATA

DATA AVAILABILITY STATEMENT

The source code of the computational model can be accessed via <https://github.com/wolframponisch/Pili-Driven-Bacterial-Turbine.git>. The Matlab scripts used to analyse the data and generate the figures are available upon request.

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Name		Value	Reference
Cell and turbine disk radius	R	$0.5 \mu\text{m}$	[14]
Cell number	N	40	
Cell density	ρ	$0.1 \mu\text{m}^{-2}$	
Pili number per cell	N_p	15	[13, 14]
Number of turbine disk pairs	N_{turb}	12	
Pili protrusion velocity	v_p	$2 \mu\text{m/s}$	[19]
Pili mean length	l_p	$1.5 \mu\text{m}$	[15, 14]
Pili retraction velocity	$v_r^{(0)}$	$2 \mu\text{m/s}$	[19]
Pili-substrate attachment rate	$\kappa_{\text{att}}^{(\text{sub},0)}$	1.33333 Hz	[19]
	$\kappa_{\text{att}}^{(\text{sub})}$	10 Hz	[15]
Pili-turbine attachment rate	$\kappa_{\text{att}}^{(\text{turb})}$	2 Hz	
Pili spring constant	k_p	$2000 \text{ pN}/\mu\text{m}$	[31]
Stalling force	F_s	180 pN	[19]
Pili detachment times	$\tau_{\text{det}}^{(\text{sub},1)}$	0.85 s	[15]
	$\tau_{\text{det}}^{(\text{sub},2)}$	0.04 s	[15]
	$\tau_{\text{det}}^{(\text{turb})}$	50 s	
Pili detachment forces	$F_{\text{det}}^{(\text{sub},1)}$	1.24 pN	[15]
	$F_{\text{det}}^{(\text{sub},2)}$	33.8 pN	[15]
	$F_{\text{det}}^{(\text{turb})}$	180 pN	
Box size	L	$20 \mu\text{m}$	
Wall spring constant	$k^{(\text{wall})}$	$5000 \text{ pN}/\mu\text{m}$	
Cell-cell spring constant	$k^{(\text{c-c})}$	$5000 \text{ pN}/\mu\text{m}$	
Cell-turbine spring constant	$k^{(\text{turb})}$	$5000 \text{ pN}/\mu\text{m}$	
Cell translational mobility	μ_t	$2 \mu\text{m}/(\text{s pN})$	[20]
Cell rotational mobility	μ_r	$720/\pi^\circ/(\mu\text{m pN s})$	[20]
Turbine rotational mobility	μ_{turb}	$0.9/\pi^\circ/(\mu\text{m pN s})$	
Time step	δt	10^{-5} s	
Simulation time	T	$10^4 - 10^5 \text{ s}$	
Simulation No. per parameter set	N_{sim}	80 – 100	

Table 1. List of parameters used in this study.