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# Dynamics of a bacterial flagellum under reverse rotation

Tapan Chandra Adhyapak\* and Holger Stark

To initiate tumbling of an *E. coli*, one of the helical flagella reverses its sense of rotation. It then transforms from its normal form first to the transient semicoiled state and subsequently to the curly-I state. The dynamics of polymorphism is effectively modeled by describing flagellar elasticity through an extended Kirchhoff free energy. However, the complete landscape of the free energy remains undetermined because the ground state energies of the polymorphic forms are not known. We investigate how variations in these ground state energies affect the dynamics of a reversely rotated flagellum of a swimming bacterium. We find that the flagellum exhibits a number of distinct dynamical states and comprehensively summarize them in a state diagram. As a result, we conclude that tuning the landscape of the extended Kirchhoff free energy alone cannot generate the intermediate full-length semicoiled state. However, our model suggests an *ad hoc* method to realize the sequence of polymorphic states as observed for a real bacterium. Since the elastic properties of bacterial flagella are similar, our findings can easily be extended to other peritrichous bacteria.

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## 1 Introduction

Although much research has been performed on the mechanical properties of a prokaryotic or bacterial flagellum and how these properties are related to the overall bacterial dynamics,<sup>1–16</sup> still our understanding of the complex aspects of bacterial locomotion, such as tumbling of an *E. coli*, remains incomplete.<sup>14,17–19</sup> The locomotion of bacteria involves rich and complex physics.<sup>20–29</sup> In addition, experiments on the collective behavior of bacteria have opened new directions of physics *per se*, demonstrating ground-breaking phenomena such as turbulence and superfluidity in living systems.<sup>30–33</sup> A comprehensive knowledge of bacterial locomotion is also necessary for a complete understanding of these novel phenomena.

One main challenge in dealing with the mechanics of the bacterial flagellum is still to gain a full theoretical understanding of the polymorphic transformations shown by the flagellum during tumbling.<sup>2,17</sup> The flagellum can exist in different stable polymorphic forms, transitions among which are induced mechanically, either by the reverse rotation of the flagellum,<sup>14,17,18</sup> by the application of stretching forces,<sup>3,12</sup> or by external flows.<sup>7,34</sup> Rotation induced polymorphic transitions occur during the locomotion of the bacterium and affect the overall bacterial dynamics.<sup>17</sup> An *E. coli* flagellum, for example, usually stays in the normal form; but under reverse rotation, it transforms first into the semicoiled state, and then fully assumes the curly-I state.<sup>35</sup>

A number of approaches using the Kirchhoff free energy density and extended versions of it, were proposed to deal with the flagellar multistability.<sup>6–8,12</sup> In ref. 14 an extended Kirchhoff free energy successfully generates the rotation-induced polymorphic transformations during the locomotion of the bacterium.<sup>14</sup> However, the landscape of this free energy with the fixed positions of the local minima and the harmonic shape in their neighborhood was not fully explored. Using a linear increase in the ground state energies of the local minima, the authors were able to demonstrate an *E. coli* flagellum transforming from the normal to the curly-I form. However, the transient semicoiled form in between was not observed. In our study here, we explore the flagellar dynamics in the full free energy landscape by studying the impact of the ground state energies. Changes in their values affect the heights of the transition barriers between the local minima,<sup>6,8,12</sup> which the flagellum has to pass to locally transform from one polymorphic state to the other. Thus the full landscape of the free energy determines the flagellar dynamics during reverse rotation including possible transient and final steady states.

In this paper we present a systematic study of how variations in the unknown local ground state energies affect the dynamics of a reversely rotated flagellum attached to a cell body. We study the nature of transitions and the dynamic stability of polymorphic forms as a function of the barrier heights and arrive at a comprehensive state diagram that classifies different scenarios of flagellar dynamics in the parameter space. In particular, we show that at the level of the present formulation of flagellar mechanics the experimentally observed transient

Institut für Theoretische Physik, Technische Universität Berlin, Hardenbergstrasse 36, 10623 Berlin, Germany. E-mail: [tapan.c.adhyapak@tu-berlin.de](mailto:tapan.c.adhyapak@tu-berlin.de)

semicoiled state, intermediate in the sequence of polymorphic states during tumbling, cannot be reproduced. However, we suggest an alternative way within our model to realize the desired sequence of transitions.

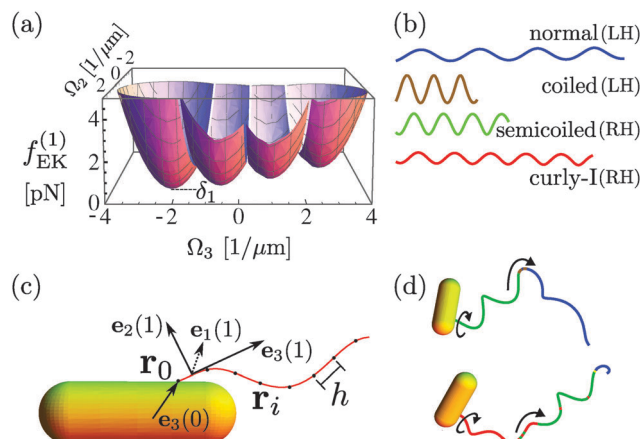
In the following we first outline in Section 2 the observations on a reversely rotated flagellum and introduce the extended Kirchhoff elastic free energy. Section 3 summarizes the equations of motion of the bacterium and their numerical implementation. Results are then presented in Section 4, followed by a discussion and conclusions in Sections 5 and 6, respectively.

## 2 Flagellar conformations during reversal and appropriate modeling

The reversal of rotating flagella during locomotion has been most elaborately studied for *E. coli*.<sup>2,18,35</sup> So, in our study we take *E. coli* as the prototype for flagellated bacteria. However, since prokaryotic flagella of peritrichous bacteria have a common molecular structure, which ultimately determines their elasticity,<sup>36,37</sup> we expect our results to be valid for a wide range of bacteria.

An *E. coli* has several flagella, which act as propelling units for the bacterium.<sup>35</sup> The flagellum is a passive helical filament, connected at one end to a rotary motor embedded in the cell wall. Most of the time the motor rotates the flagellum in the counterclockwise sense (as viewed along the flagellum from its free end toward the cell body) generating the thrust force for propelling the bacterium. At this stage the flagellum assumes the normal form, a left-handed helix of known radius and pitch. It forms a bundle with other flagella and thereby defines a unique direction for the net propulsive force. Frequently, however, the motor reverses and rotates the flagellum clockwise. This forces the bundle to disrupt and the cell body to tumble. At the same time, a sequence of polymorphic transitions in the flagellum is observed.<sup>2,18</sup> In each case these transitions start at the cell body and proceed towards the free flagellar end. The whole flagellum thus changes from the normal state, first to the right-handed semicoiled form, and then finally to the right-handed curly-I form [see Fig. 1(b) and (d)].

We try to give an intuitive picture of the observed transition before becoming more quantitative. The elastic free energy per unit length of the flagellum, which we quantify below and which is illustrated in Fig. 1(a), has a number of local minima separated by transition regions. Each of these minima corresponds to a particular equilibrium polymorphic form of the flagellum. Under counterclockwise rotation the flagellum remains in the left-handed normal form. When the motor rotates clockwise, the sense of rotation of the proximal end of the flagellum attached to the hook is reversed. But, because of the surrounding fluid friction, the rest of the flagellum cannot immediately follow the motion of the proximal end. The torsional stress created thereby inverts the local torsion of the filament and thus causes a local transformation to the right-handed semicoiled and curly-I forms [see local minima with positive  $\Omega_3$  in Fig. 1(a)]. The process then continues until the



**Fig. 1** (a) The first term of the extended Kirchhoff free energy density,  $f_{EK}^{(1)}$ , plotted against  $\Omega_2$  and  $\Omega_3$  for  $\Omega_1 = 0$ . The local minimum of the normal form with ground state energy  $\delta_1$  is shown. The true local minima for the coiled, semicoiled, and curly-I forms are situated at  $\Omega_1 \neq 0$ . (b) Planar projections of four helical polymorphic forms of a filament with a fixed contour length: normal (blue), coiled (brown), semicoiled (green), and curly-I (red). (c) A schematic of the model bacterium used in our simulations. The cell body and part of the discrete flagellum with a discretization length  $h$  (drawn out of proportion) are shown. Other components are as described in the text. (d) Two snapshots from our simulations with a CW rotated flagellum: (top) a growing semicoiled domain (green) evades the normal state (blue) and (bottom) a growing curly-I domain (red) evades the semicoiled state (green).

whole flagellum transforms to a new polymorphic state. For sufficiently strong torque, the polymorphic form with the largest torsion  $\sim \Omega_3$ , the curly-I form, is reached.

An understanding of the polymorphic states was developed based on the molecular structure of the flagellum.<sup>37–39</sup> Following this bottom-up approach, several models describe the dynamics of polymorphism by coarse-graining over the molecular scale.<sup>40–42</sup> However, an alternative approach, where Kirchhoff's continuum theory of an elastic rod was extended to incorporate multistability, turns out to be simpler and more effective.<sup>6,7,43</sup> One example of such an extended Kirchhoff free energy reproduces stretching induced polymorphism most accurately as demonstrated in ref. 12 and is also appropriate for modeling rotation-induced polymorphism in a moving bacterium.<sup>14</sup>

The model uses a harmonic expansion of the elastic free energy density about its local minima. Although, the harmonic approximation is not exact at the transition regions between two minima, we can provide a number of justifications that our model accurately describes polymorphic transitions. The study in ref. 12 involves the transition between two successive minima under the application of stretching forces. In that study the model clearly reproduced experimentally observed force–extension curves and was, in that respect, much better than a general fourth-order potential function with substantial deviation from the harmonic approximation near the transition region. Furthermore, the basic phenomenology during a rotation-induced polymorphic transition was also successfully captured by the model as shown in ref. 14. Finally, a similar model was successful in describing the dynamics of multistable

helical filaments.<sup>43</sup> So, we can assume that the model is a good approximation for studying flagellar reversal in bacterial locomotion. We now describe the model in detail.

## 2.1 Flagellar elasticity: extended Kirchhoff elastic free energy

We treat the flagellum as a slender body and parameterize its centerline  $\mathbf{r}(s)$  by the contour length  $s$ .<sup>12</sup> The conformation of a distorted flagellum including twist deformations is characterized by orthonormal material tripods  $\{\mathbf{e}_1(s), \mathbf{e}_2(s), \mathbf{e}_3(s)\}$  at each point on the centerline, where  $\mathbf{e}_3$  is the local tangent to the centerline at  $s$  and unit vectors  $\mathbf{e}_1$  and  $\mathbf{e}_2$  point along the principal axes of the flagellar cross section. The rotational strain vector  $\mathbf{\Omega}$  in

$$\partial_s \mathbf{e}_\nu = \mathbf{\Omega} \times \mathbf{e}_\nu, \quad (1)$$

with  $\nu = 1, 2, 3$ , transports the material tripod along the centerline. Therefore, together with the position  $\mathbf{r}(s=0)$  of one flagellar end,  $\mathbf{\Omega}(s,t)$  carries the complete information for the conformation of the flagellum at time  $t$ . Conversely, given the conformation in terms of the centerline  $\mathbf{r}(s)$  and the material tripods at each  $s$ , the rotational strain vector  $\mathbf{\Omega}$  can be worked out from eqn (1). Introducing the angle  $\alpha$  between  $\mathbf{e}_1$  and the local normal to the centerline,  $\mathbf{n} \propto \partial_s^2 \mathbf{r}(s)$ , one can relate the components  $\Omega_1, \Omega_2$  and  $\Omega_3$  of  $\mathbf{\Omega}$  with respect to the local material frame to the curvature  $\kappa$  and torsion  $\tau$  of the flagellum:<sup>12</sup>

$$\Omega_1 = \kappa \sin \alpha, \quad \Omega_2 = \kappa \cos \alpha, \quad \Omega_3 = \tau + \partial_s \alpha. \quad (2)$$

In harmonic approximation, the energy required per unit length of the flagellum to induce small deformations  $d\mathbf{\Omega} = \mathbf{\Omega} - \mathbf{\Omega}^{(n)}$  from the stable ground state  $\mathbf{\Omega}^{(n)}$ , is the Kirchhoff elastic free energy density,<sup>44</sup>

$$f_K(\mathbf{\Omega}, \mathbf{\Omega}^{(n)}) = \frac{A}{2} [(d\Omega_1)^2 + (d\Omega_2)^2] + \frac{C}{2} (d\Omega_3)^2, \quad (3)$$

where  $A$  and  $C$  are the respective bending and twist rigidities of the flagellum, assuming a circular cross section. Assigning  $f_K(\mathbf{\Omega}, \mathbf{\Omega}^{(n)})$  and a ground state energy  $\delta_n$  to each polymorphic form  $\mathbf{\Omega}^{(n)}$ , an extended version of the Kirchhoff free energy density, capable of describing the multistability of a flagellum, is formulated as<sup>14</sup>

$$f_{EK} = \min_{\mathbf{\Omega}^{(n)}} \left[ f_K(\mathbf{\Omega}, \mathbf{\Omega}^{(n)}) + \delta_n \right] + \frac{A}{2} \xi^2 (\partial_s \mathbf{\Omega})^2. \quad (4)$$

Here,  $\mathbf{\Omega}^{(n)}$ ,  $n = 1-4$ , correspond, respectively, to the normal, coiled, semicoiled, and curly-I forms of an *E. coli* flagellum.<sup>14</sup> We include the experimentally unobserved left-handed coiled form that is intermediate in  $\kappa$  and  $\tau$  between the normal and semicoiled forms and thus also in  $\Omega$ -space.<sup>39</sup> The first term in eqn (4) implies that for any rotational strain  $\mathbf{\Omega}(s)$  at a given point  $s$  on the flagellum, the elastic free energy density is chosen to be that of the polymorphic form with the lowest energy. The second term in eqn (4) enforces a smooth transition region of width  $\xi$  between two polymorphic domains.

For an *E. coli* flagellum, we use  $A = 5.5 \text{ pN } \mu\text{m}^2$  and  $C = 3.5 \text{ pN } \mu\text{m}^2$ .<sup>3,18</sup> The stable ground states  $\mathbf{\Omega}^{(n)} \equiv \{\Omega_1^{(n)}, \Omega_2^{(n)}, \Omega_3^{(n)}\}$  for the normal, coiled, semicoiled, and curly-I forms are, respectively

**Table 1** Mechanical properties of the flagellum and the hook of an *E. coli* as inferred from experiments

Parameter	Value	Ref.
$A$ [pN $\mu\text{m}^2$ ]	3.5 to $\sim 5.5$	3 and 18
$C$ [pN $\mu\text{m}^2$ ]	3.5	3 and 18
$A_h$ [pN $\mu\text{m}^2$ ]	$10^{-5b}$	49
$C_h$ [pN $\mu\text{m}^2$ ]	$0.2^{a,b}$	52

<sup>a</sup> Considering a uniform cross section of radius  $\sim 10 \text{ nm}$  for the hook.<sup>4</sup>

<sup>b</sup> In the simulations the detailed deformation of the hook is not resolved. The effective values of  $A_h$  and  $C_h$  are chosen to generate the universal joint feature of the hook correctly within the model.

(in  $\mu\text{m}^{-1}$ ):  $\{0.00, 1.30, -2.11\}$ ,  $\{-0.51, 1.74, -0.56\}$ ,  $\{-1.18, 1.84, 0.98\}$ , and  $\{-1.80, 1.56, 2.53\}$ .<sup>14</sup> For comparison, the values of the mechanical properties of an *E. coli* flagellum as inferred from experiments are listed in Table 1.

The ground state energies  $\delta_n$  in eqn (4) are not known yet. Their relative values determine both the height and shape of transition barriers between consecutive minima [Fig. 1(a)]. The positions  $\mathbf{\Omega}^{(n)}$  of the minima of  $f_{EK}$  are already fixed, as are the parabolic shapes in the neighborhood of those minima. Hence, the full landscape of the extended Kirchhoff energy density is known once the ground state energies  $\delta_n$  or equivalently the transition barriers are specified.

## 3 Equations of motion and numerical methods

### 3.1 Dynamics of the flagella

**3.1.1 Equations of motion.** We consider the dynamics of an elongated cell body with a flagellum emanating from an arbitrary point on its surface and moving in an unbounded fluid of viscosity  $\eta$ . The equations of motion of the flagellum are given by the Langevin equations for the dynamics of the centerline  $\mathbf{r}(s,t)$  and the twist angle  $\phi(s,t)$  about the centerline:<sup>45</sup>

$$\partial_t \mathbf{r} = \mu_t (\mathbf{F}_{el} + \mathbf{F}_s + \mathbf{F}_{th}) + \mathbf{v}_h, \quad (5)$$

$$\partial_t \phi = \mu_r (T_{el} + T_{th}). \quad (6)$$

Here the  $\mathbf{F}$ 's and  $T$ 's are, respectively, the local forces and torques acting on the flagellum due to elastic deformations, steric interactions, and thermal noise. We will describe them below. They are connected to the linear ( $\partial_t \mathbf{r}$ ) and angular ( $\partial_t \phi$ ) velocities by the respective self-mobilities  $\mu_t = \mathbf{e}_3 \otimes \mathbf{e}_3 / \gamma_{\parallel} + (\mathbf{I} - \mathbf{e}_3 \otimes \mathbf{e}_3) / \gamma_{\perp}$  and  $\mu_r = 1 / \gamma_R$ , where  $\gamma_{\parallel} = 1.6 \times 10^{-3} \text{ pN s } \mu\text{m}^{-2}$ ,  $\gamma_{\perp} = 2.8 \times 10^{-3} \text{ pN s } \mu\text{m}^{-2}$  and  $\gamma_R = 1.26 \times 10^{-6} \text{ pN s}$  are the anisotropic friction coefficients per unit length for the flagellum of an *E. coli*.<sup>13</sup> Finally,  $\mathbf{v}_h$  describes hydrodynamic interactions between different parts of the flagellum as detailed below.

The elastic forces and torques follow from the total elastic free energy  $\mathcal{F}[\mathbf{r}(s), \phi(s)]$  as:

$$\mathbf{F}_{el} = -\frac{\delta \mathcal{F}}{\delta \mathbf{r}} \quad \text{and} \quad T_{el} = -\frac{\delta \mathcal{F}}{\delta \phi}. \quad (7)$$

Here  $\mathcal{F}[\mathbf{r}(s), \phi(s)] = \int ds (f_{EK} + f_{st})$ , where  $f_{EK}$  is the Kirchhoff elastic free energy density described in the previous section and

$f_{\text{st}} = K(\partial_s \mathbf{r})^2/2$  with  $K = 10^3$  pN is a stretching free energy density introduced to prevent local stretching of the flagellum.<sup>14</sup>

**3.1.2 Discretization procedure, thermal noise, steric and hydrodynamic interactions.** Before we address the other force and torque contributions in eqn (5) and (6), we discuss the numerical scheme to update the flagellar configuration in time. In order to discretize the Langevin equations, we consider discrete positions  $\mathbf{r}_i \equiv \mathbf{r}(s_i)$  along the flagellum and assign  $\{\mathbf{e}_1(i), \mathbf{e}_2(i), \mathbf{e}_3(i)\}$  to the straight segment of length  $h$  between  $\mathbf{r}_{i-1}$  and  $\mathbf{r}_i$  [see Fig. 1(c)]. The forces now act on the discrete points, while the torques are applied to the straight segments. To find the discretized versions of  $\mathbf{F}_{\text{el}}$  and  $T_{\text{el}}$ , we discretize the derivatives in eqn (7) and write  $\mathcal{F} = \int ds(f_K + f_{\text{st}})$  as a sum over the segments.

The thermal forces  $\mathbf{F}_{\text{th}}$  and torques  $T_{\text{th}}$  in eqn (5) and (6), respectively, are negligible for the forward propulsion of bacteria<sup>46</sup> but play an important role in the polymorphic transformations during reversal of the flagellum.<sup>13</sup> We, therefore, include them here. As usual, they are Gaussian random numbers with zero mean and variances  $\langle (F_{\text{th}}^{\parallel/\perp})^2 \rangle = 2k_{\text{B}}T\gamma_{\parallel/\perp}/\Delta t$ , where  $\Delta t$  is the discrete time step used in the simulation, and  $\parallel$  and  $\perp$  denote the directions along and normal to the local tangent of the flagellum, respectively. Similarly,  $\langle T_{\text{th}}^2 \rangle = 2k_{\text{B}}T\gamma_{\text{R}}/\Delta t$ .

The steric force  $\mathbf{F}_{\text{s}}$  enforces excluded-volume interactions among different parts of the flagellum. This is needed in case distant flagellar parts try to go through each other, *e.g.*, during a strongly buckled state. We model the steric forces following ref. 45.

In order to model hydrodynamic interactions between different flagellar parts, we treat each discrete point  $\mathbf{r}_i$  as a sphere of diameter equal to the thickness of the flagellum. Thus, we write  $\mathbf{v}_{\text{h}}(\mathbf{r}_i) = \sum_{j \neq i} \mu_{ij} \mathbf{F}(\mathbf{r}_j)$ , where the summation runs over all points of the flagellum. Here  $\mu_{ij}$  is the Rotne–Prager mobility matrix<sup>47</sup> for spheres at  $\mathbf{r}_i$  and  $\mathbf{r}_j$  and  $\mathbf{F}(\mathbf{r}_j)$  is the force acting at  $\mathbf{r}_j$ . To be consistent with the picture that the spheres are parts of the discretized flagellum, we neglect the hydrodynamic influence of their rotation.<sup>48</sup> Furthermore, to avoid huge computational expenses, we also neglect any correlations between thermal forces  $\mathbf{F}_{\text{th}}$  acting on different points, which occur due to hydrodynamic interactions.

### 3.2 The cell body, rotary motor, and flagellar hook

The elongated cell body is modeled as a spherocylinder of length  $L_{\text{b}} = 2.5 \mu\text{m}$  and width  $d_{\text{b}} = 0.8 \mu\text{m}$ <sup>35,45</sup> [see Fig. 1(c)]. A flagellum with total contour length  $L$  is attached to the point  $\mathbf{r}_0$  on the surface of the cell body. To represent a typical flagellum undergoing a reverse rotation, we choose  $\mathbf{r}_0$  to be on the cylindrical surface. Flagella of an *E. coli* are distributed randomly over the entire cell body.<sup>35</sup> This implies that an arbitrarily chosen flagellum is more likely to be found on the cylindrical surface that has a larger area compared to that of the spherical ends of the cell body.

A motor tripod  $\{\mathbf{e}_1(0), \mathbf{e}_2(0), \mathbf{e}_3(0)\}$  is introduced at  $\mathbf{r}_0$ , where  $\mathbf{e}_3(0)$  coincides with the shaft of the rotary motor driving the flagellum. A motor torque  $\mathbf{T}_{\text{m}} = T_{\text{m}}\mathbf{e}_3(0)$  drives the flagellum by

rotating this tripod. The main part of the flagellum is coupled to the motor tripod through the Kirchhoff elastic free energy density  $f_K$  with a bending rigidity  $A \rightarrow A_{\text{h}} = 10^{-3}$  pN  $\mu\text{m}^2$ , and a twist rigidity  $C \rightarrow C_{\text{h}} = 2.0$  pN  $\mu\text{m}^2$ .<sup>45,49</sup> Thus, the flagellum is connected to the motor shaft through a ‘hook’ that acts like a universal joint with low bending and high twist rigidities<sup>50</sup> and allows the first flagellar segment along  $\mathbf{e}_3(1)$  to be at any angle to the motor shaft and yet efficiently transferring the driving torque to the flagellum.

The cell body translates and rotates with velocities given, respectively, by

$$\mathbf{v}_{\text{b}} = \mu_{\text{b}}^{\text{f}} \mathbf{F}_{\text{b}} \quad \text{and} \quad \omega_{\text{b}} = \mu_{\text{b}}^{\text{r}} (\mathbf{T}_{\text{b}} + \mathbf{T}_{\text{m}}), \quad (8)$$

where  $\mathbf{F}_{\text{b}}$  and  $\mathbf{T}_{\text{b}}$  are the net force and torque acting on the center of mass of the cell body. They result from the force  $\mathbf{F}_{\text{el}} + \mathbf{F}_{\text{s}}$  that acts on the flagellar anchoring point. For simplicity, the mobilities  $\mu_{\text{b}}^{\text{f}}$  and  $\mu_{\text{b}}^{\text{r}}$  are assigned the analytically available values for a prolate spheroid of aspect ratio  $L_{\text{b}}/d_{\text{b}}$ .<sup>51</sup> As in ref. 45 the angle between  $\mathbf{e}_3(0)$  and the long axis of the cell body is set to  $55^\circ$  to tune the ratio for the bundle-to-body rotation rates during forward propulsion, to the experimentally observed range.<sup>18</sup>

Finally, the excluded volume interaction between the cell body and the flagellum is again modeled as in ref. 45.

## 4 Results

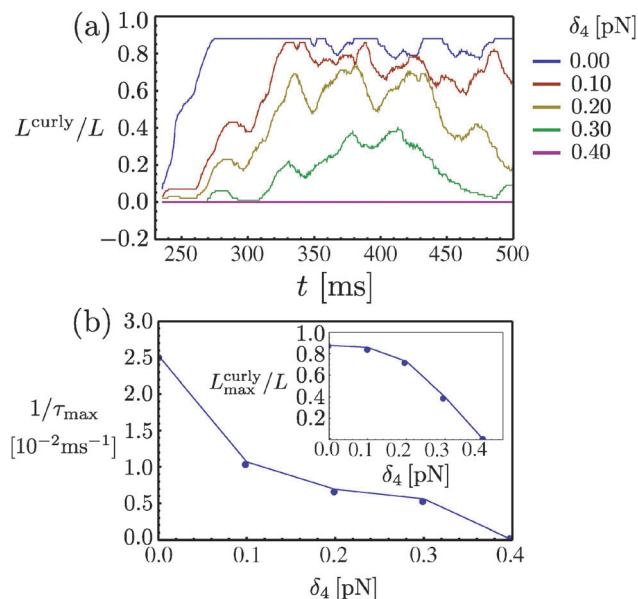
### 4.1 Effect of barrier heights: transition from semicoiled state

As pointed out earlier, since the positions of the minima of our model elastic free energy are already fixed by the polymorphic states of the flagellum, the full energy landscape is determined once the transition barriers between consecutive minima are specified. Within our model, the barriers are determined by the differences in the ground-state energies  $\{\delta_1, \delta_2, \delta_3, \delta_4\}$ , which we vary in the following, in order to test how the free energy landscape affects the flagellar dynamics. Since only the relative heights of the minima are important, we set  $\delta_1 = 0$ .

As a starting point for a systematic study, we investigate a single transition barrier connecting two consecutive minima. In *E. coli* a typical transition between two neighboring polymorphic forms occurs between the semicoiled and curly-I state. So, we take  $\delta_2 = \delta_3 = 0$  and vary  $\delta_4$ . We start with the flagellum entirely in the semicoiled form (minimum 3) and apply a motor torque in the CW sense (as viewed from outside the cell) for a duration comparable to the average tumbling time of  $\sim 1$  s. The magnitude of the torque is fixed to a constant value of  $T_{\text{m}} = -3.0$  pN  $\mu\text{m}$ , in agreement with experimental values.<sup>35</sup> A typical snapshot from our simulations for this case is shown in Fig. 1(d) (bottom).

Our observations on the nature of the semicoiled-to-curly-I transition and the stability of the curly-I form are summarized in Fig. 2. In graph (a) we plot the fraction of the flagellum in the curly-I state,  $L^{\text{curly}}/L$ , versus time  $t$ . Below  $\delta_4 = 0.4$  pN the flagellum always transforms to the curly-I form, since a nonzero  $L^{\text{curly}}$  eventually appears as time progresses. Stability of the





**Fig. 2** Transition from semicoiled to curly-I form studied for different ground-state energies  $\delta_4$  (in pN) and  $\delta_1 = \delta_2 = \delta_3 = 0.0$  pN. (a) Time evolution of the fraction of the flagellum in the curly-I state,  $L^{\text{curly}}/L$ . (b) Transition rate  $1/\tau_{\text{max}}$  plotted versus  $\delta_4$ , where  $\tau_{\text{max}}$  is the time to achieve maximum length  $L_{\text{max}}^{\text{curly}}$  in the curly-I state. Inset:  $L_{\text{max}}^{\text{curly}}/L$  as a function of  $\delta_4$ .

curly-I state increases with decreasing  $\delta_4$  and, ultimately, for  $\delta_4 < 0.1$  pN, after an initial rapid build-up,  $L^{\text{curly}}$  remains close to its maximum value as long as the flagellum is reversely rotated. However, for  $\delta_4 > 0.1$  pN (and  $< 0.4$  pN) fluctuations in  $L^{\text{curly}}$  are huge.

These fluctuations are due to flagellar portions in the curly-I form transforming back to the semicoiled state. As  $\delta_4$  increases, the barrier height for the return jump from curly-I to semicoiled decreases and local elastic stresses built up on the curly-I portion are sufficient to induce the return transition. We find the localized elastic stresses to be due to the enhanced buckling of the curly-I form. The clockwise rotated curly-I state with its right-handed helical structure generates a thrust force towards the tumbling cell body, which is hardly translating. Thus the highly flexible curly-I form buckles more easily and produces localized elastic stresses.

Varying  $\delta_4$  also affects the maximum length  $L_{\text{max}}^{\text{curly}}$  of curly-I form, attained along the flagellum during each run, and the transition time  $\tau_{\text{max}}$  to reach  $L_{\text{max}}^{\text{curly}}$ . A continuous increase in the length  $L_{\text{max}}^{\text{curly}}$  is observed, when  $\delta_4$  decreases below the threshold value 0.4 pN [inset, Fig. 2(b)]. This resembles the behavior of an order parameter characterizing a continuous phase transition. Furthermore, the transition time  $\tau_{\text{max}}$  increases or the transition rate  $1/\tau_{\text{max}}$  decreases with growing  $\delta_4$ , indicating a slowed-down transition to the curly-I form with maximum length [Fig. 2(b)].

## 4.2 Effect of barrier heights: transition from normal state

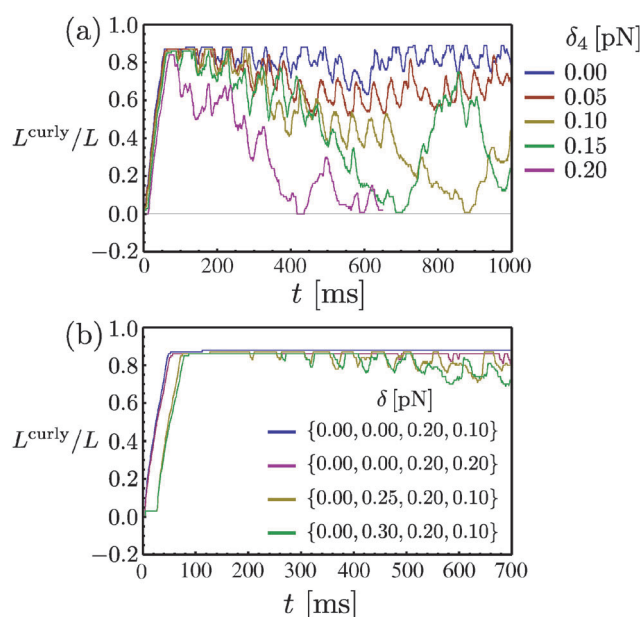
Having established the importance of changing the height of one transition barrier, we now turn to the more complex problem, which is to test the dynamics of the flagellum under

reverse rotation starting from the normal state. Now all three transition barriers, *i.e.*, all three ground state energies  $\delta_2$ ,  $\delta_3$ , and  $\delta_4$  become relevant. We vary them systematically as explained below. We start with a flagellum entirely in the normal form and apply a motor torque,  $T_m = -3.0$  pN  $\mu\text{m}$ , in the CW sense for a time similar to the average tumbling duration. In the following, we first summarize the nature of the transition and the stability of different states and then discuss distinct dynamic phases that emerge out of our observation.

**4.2.1 Nature of transition and stability.** First we set  $\delta_2 = \delta_3 = 0$  pN and observe the time evolution of  $L^{\text{curly}}/L$  as we vary  $\delta_4$  [Fig. 3(a)]. We find that  $L^{\text{curly}}$  begins to increase from  $t = 0$  ms and reaches its maximum very early for each  $\delta_4$ . This implies that the flagellum directly transforms into the curly-I state without residing in a full-length semicoiled state as observed for real bacteria. Moreover, the final curly-I state is highly unstable against fluctuations for any non-zero  $\delta_4$ .

We find that these fluctuations in  $L^{\text{curly}}$  can be greatly reduced, when  $\delta_3$  is also shifted up [blue and violet curve, Fig. 3(b)]. This becomes evident when comparing the corresponding curves of Fig. 3(a) and (b) for the same values of  $\delta_4$  but with  $\delta_3 = 0$  pN and  $\delta_3 = 0.2$  pN, respectively. As explained in the previous section, an increase in  $\delta_4$  reduces the barrier height for leaving the curly-I state. A simultaneous increase in  $\delta_3$ , however, restores the barrier height and thereby stabilizes the final curly-I form.

All curves in Fig. 3(a) and (b) (with  $\delta_2 = 0$ ) attain approximately the same maximum value at nearly the same time. Moreover, for  $\delta_3$  or  $\delta_4 > 0.35$  pN a transition to the curly-I state does not occur at all [see Fig. 5(b)]. This implies that  $L_{\text{max}}^{\text{curly}}$  jumps from zero to a non-zero value at the threshold  $\delta_3 \approx \delta_4 > 0.35$  pN.



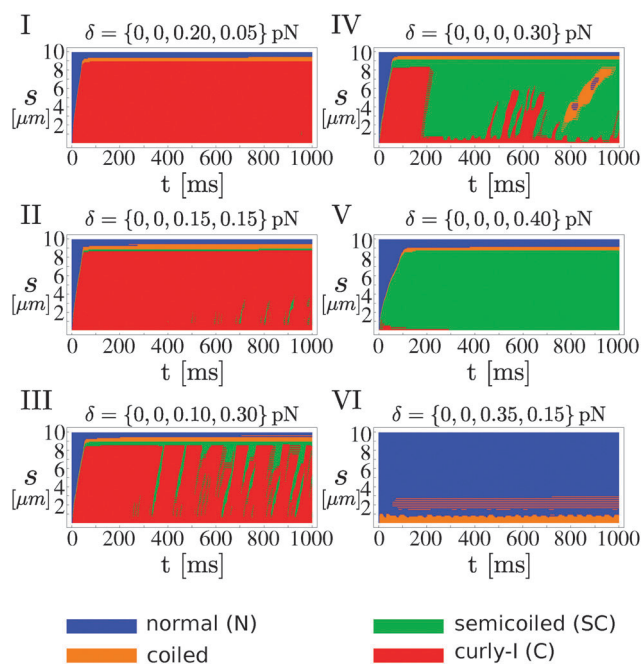
**Fig. 3** Transition from the normal form under reverse rotation. (a) Time evolution of the fraction of the flagellum in the curly-I state,  $L^{\text{curly}}/L$ , for various values of  $\delta_4$  (in pN) and  $\delta_1 = \delta_2 = \delta_3 = 0.0$  pN. (b)  $L^{\text{curly}}/L$  plotted against time for selected values of  $\delta_2$ ,  $\delta_3$  and  $\delta_4$ .

Similarly,  $\tau_{\max}$  also remains nearly constant during the transition from the normal to the curly-I state. This behavior is unlike the transition from the semicoiled to the curly-I state, where both  $L_{\max}^{\text{curly}}$  and  $\tau_{\max}^{-1}$  continuously decrease to zero when increasing  $\delta_4$  to 0.4 pN [see Fig. 2(b)]. Note while the semicoiled and curly-I states are separated by a single barrier, the flagellum has to pass three barriers during the transition from the normal to the curly-I state. This might explain the discontinuous transition.

Next, we examine the effect of non-zero values of  $\delta_2$ . A comparison of the time evolution of  $L^{\text{curly}}/L$  for the same set of  $\delta_3$ ,  $\delta_4$  values but with different values of  $\delta_2$  is shown in Fig. 3(b) (yellow and green curves). It is clear that a non-zero but small value of  $\delta_2$  does not affect the above results qualitatively. The nature of the transition from the normal form and the stability of the curly-I state remain the same as before as long as  $\delta_2$  is small.

This shows that in our quest of identifying the influence of the free energy landscape on the polymorphic transformation, the most important parameters to study are  $\delta_3$  and  $\delta_4$ . In the following we therefore ignore any variation in  $\delta_2$ .

**4.2.2 Dynamic states exhibited by a flagellum under reverse rotation.** According to our findings, we take  $\delta_2 = \delta_1 = 0$  and explore the dynamic behavior of the flagellum as we vary  $\delta_3$  and  $\delta_4$ . After a thorough examination, six distinct dynamic states emerged, which are listed in Fig. 4 in panels I–VI, respectively.



**Fig. 4** Distinct dynamical states exhibited by a flagellum under reverse rotation starting from the normal state. Panels I–VI show how the polymorphic forms indicated by different colors evolve in time along the flagellum. Arclength  $s$  gives the position on the flagellum. For  $\delta_1 = \delta_2 = 0$ , typically observed dynamic states are: (I) dynamically stable C state, (II) stable C state with transient SC regions, (III) repeated emergence of large transient SC regions, (IV) reverse transition from curly-I to a full-length, relatively stable SC state, (V) stable SC state, and (VI) stable N state. See text for a detailed description.

Each panel plots for specific values of  $\delta_3$  and  $\delta_4$  the color-coded polymorphic forms spread along the flagellum as the flagellum evolves in time  $t$ . The distinct dynamic states are characterized as follows.

(I) Direct transition from normal to curly-I state, which nearly spreads along the whole flagellum and is dynamically very stable.

(II) Transition to a dynamically stable curly-I state, which is accompanied by small transient regions of semicoiled form, which appear and disappear locally.

(III) Transition to a dynamically stable curly-I state with large transient regions of semicoiled form. The semicoiled domains appear repeatedly over time near the cell body ( $s = 0$ ) and move towards the free end of the flagellum, where they shed off.

(IV) An initial nearly full-length transition to the curly-I state, followed by a reverse transition to a full-length, relatively stable semicoiled state.

(V) Transition to a dynamically stable semicoiled form, the curly-I state is not reached.

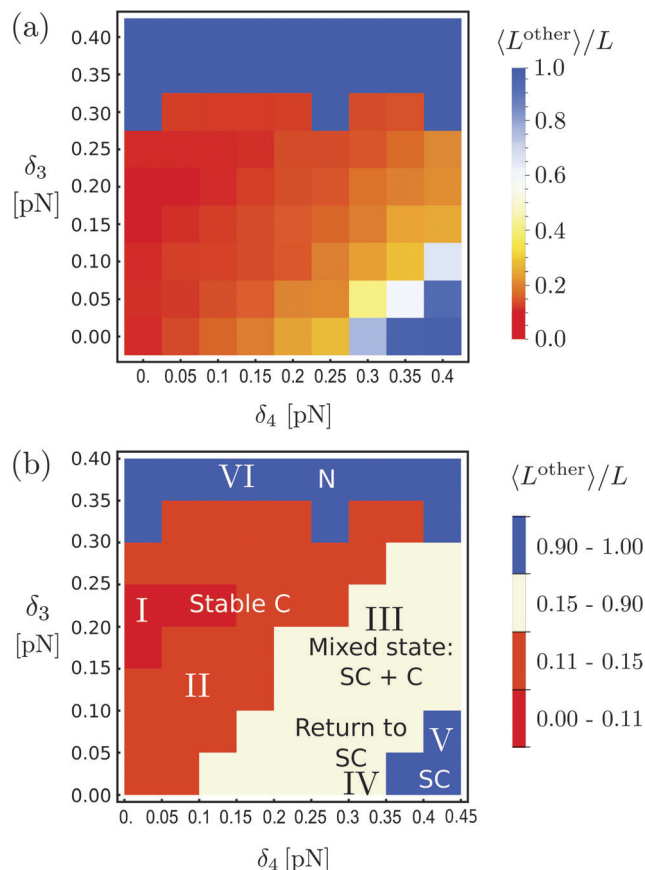
(VI) Dynamically stable normal form. No significant flagellar portion transforms to other polymorphic forms.

**4.2.3 Energy landscape and state diagram.** Combining information obtained from a range of values for  $\delta_3$  and  $\delta_4$ , we finally arrive at a comprehensive picture of the flagellar dynamics under reversal. Fig. 5(a) shows how the average length  $\langle L^{\text{other}} \rangle$  of the flagellar portion, which is not in the curly-I state, varies in the  $\delta_3$ – $\delta_4$  plane. Here  $\langle \dots \rangle$  represents an average over time excluding the initial period of transition from the normal form. So, a low value of  $\langle L^{\text{other}} \rangle$  implies a dynamically stable curly-I state, whereas a high value implies either large fluctuations in the curly-I state or no transition to this state. We already recognize that the curly-I state is unstable for high values of both  $\delta_3$  and  $\delta_4$ . However, more interestingly, we also notice that a very stable curly-I state occurs even for higher values of  $\delta_4$  provided we increase  $\delta_3$  accordingly.

Going into depth, we now monitor the detailed time evolution of the polymorphic states along the whole flagellum (like in Fig. 4) for the complete  $\delta_3$ – $\delta_4$  plane. We identify all the dynamic states listed in the previous section and find that states I–VI are characterized by values of  $\langle L^{\text{other}} \rangle$  in the ranges 011%, 1115%, 1590% and  $>90\%$  of the flagellar length. The respective regions in the  $\delta_3$ – $\delta_4$  plane are extracted from Fig. 5(a) and represented by colors in Fig. 5(b). The corresponding dynamic states are indicated by their roman numbers. Note that states III and IV are not distinguishable from each other by the range defined for  $\langle L^{\text{other}} \rangle$ . The same holds for states V and VI. However, the full time evolution of the polymorphic forms along the flagellum clearly identifies them as separate states and we mark their occurrence in the  $\delta_3$ – $\delta_4$  plane.

## 5 Discussion

A comparison of our findings with experimental results should, in principle, give the unknown parameters of our extended



**Fig. 5** Dynamic stability of curly-I state and state diagram for  $\delta_1 = \delta_2 = 0$  pN. (a) Mean length of the flagellar portion, which is not in the curly-I form,  $\langle L^{\text{other}} \rangle / L$ , represented in the  $\delta_3$ - $\delta_4$  plane by a color code. (b) Colored regions in the  $\delta_3$ - $\delta_4$  plane represent different length ranges of  $\langle L^{\text{other}} \rangle / L$  in units of the total flagellar length; dark red: 0–11%, light red: 11–15%, beige: 15–90%, and blue: >90%. Roman numbers indicate the distinct dynamic phases shown in Fig. 4.

Kirchhoff free energy. It should also reveal how accurately the elastic properties of the flagellum are described by this energy.

The relevance of our findings becomes clear, when we consider the full time evolution of a flagellum under reversal of the driving torque as observed in experiments.<sup>2,18</sup> For a real *E. coli*, a reversely rotated flagellum first transforms in full length to the semicoiled form, followed by the curly-I form, which persists until the rotation switches back to the CCW sense. Even though the appearance of the semicoiled domain is brief, it extends over the full flagellum and does not fluctuate into other forms until the curly-I domain grows from the cell body and takes over the whole flagellum. So, to model the flagellar dynamics under reversal correctly, the intermediate full-length semicoiled state should occur.

However, we do not observe such a behavior in the dynamic states reported in Fig. 4. The flagellum either transforms directly into the curly-I form [regions I and II in Fig. 5(b)] or it remains in the semicoiled state [region V in Fig. 5(b)]. Thus, for any combination of  $\delta_3$  and  $\delta_4$  there is never an intermediate transition to the full-length semicoiled state followed by an automatic transition to the curly-I form. This result of our

model clearly is in contrast to what is often observed for real bacteria. Non-zero but small values of  $\delta_2$  should not change this behavior because, as we have shown earlier,  $\delta_2$  does not affect the dynamics in any significant way.

One possible reason for this discrepancy with experiments is the harmonic approximation of the Kirchhoff free energy in the deformation  $d\Omega$ . For large deformations anharmonic terms become important. Inclusion of higher powers of  $d\Omega$  in the Kirchhoff free energy would result in a highly non-trivial energy landscape, which is expected to modify the flagellar dynamics observed in our present study. Possible reasons for inclusion of extra terms might also be related to the finer details of the molecular structure of the flagellum and the hook, not investigated fully so far. Effects of such extra terms in the Kirchhoff free energy, allowed by symmetries, will be examined in the future.

An alternative way to capture the experimental pathway of the observed polymorphic transitions in *E. coli* within the present model is the following. There could be an internal switch, realized by some biological mechanism, that causes an effective jump in the  $\delta_3$ - $\delta_4$  plane during the dynamics. Thus, the free energy in the first period of the flagellar reversal corresponds to the region V of Fig. 5(b), until the transformation to the semicoiled form is complete. Then, the values of  $\delta_3$ ,  $\delta_4$  switch to the region I + II for the remaining part of the dynamics as long as the reversal continues.

## 6 Conclusions

In conclusion, we examined the dynamics of a reversely rotated *E. coli* flagellum attached to a moving cell body by thoroughly exploring the elastic free energy landscape of the flagellum. We considered a general form of the extended Kirchhoff free energy that was shown to be most appropriate for both stretching and rotation-induced polymorphism. Minima of this free energy correspond to the known polymorphic forms of the flagellum. However, the relative values of the ground state energies of those minima are not known.

We systematically studied how changes in the ground state energies influence the transition of a flagellum in two cases: from a semicoiled to the curly-I state, and from a normal to the curly-I state, respectively. These transitions are relevant during flagellar reversal.

We find that under reverse rotation, a normal flagellum can transform to a curly-I state, whose stability depends sensitively on the relative ground state energies of the involved polymorphic forms. The transition to the curly-I form can even be forbidden, making the flagellum either to continue in the normal form or to transform to a stable semicoiled state. We have classified these distinct dynamical states and obtained a state diagram for varying ground state energies. From this, one infers that for any combination of the ground state energies in our model, an intermediate transition to a full-length semicoiled state followed by a transition to the final curly-I form cannot be realized. However, we suggest an alternative way to reproduce within

our model such a sequence of transitions observed for a real bacterium.

Our study provides a complete picture of how the elastic free energy landscape determines the dynamics of a reversely rotated flagellum attached to a movable cell body. Therefore, our findings are important for the proper modeling of the locomotion of a bacterium including its tumbling. We show that the full phenomenology of an *E. coli* flagellum cannot be realized by simply adjusting the parameters of the extended Kirchhoff free energy. This calls for alternative approaches. Investigating the importance of anharmonic terms in the free energy or how finer details of the hook<sup>53</sup> influence the flagellar dynamics could be two possibilities in this direction.

On the other hand, based on the established state diagram we suggested an *ad hoc* method to realize the correct polymorphic sequence of an *E. coli* flagellum. Implementing this method allows a thorough theoretical investigation of the complex and still not fully understood tumbling event of an *E. coli*. Moreover, since the elastic properties of bacterial flagella are similar, our method can also be applied to explore the sequence of polymorphic forms seen in other peritrichous bacteria during tumbling.

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