Powering a burnt bridges Brownian ratchet: A model for an extracellular motor driven by proteolysis of collagen

Saveez Saffarian,[1] Hong Qian,[2] Ivan Collier,[3] Elliot Elson,[4] and Gregory Goldberg[3,4]
[1]*Cell Biology Department, Harvard Medical School/CBR, 200 Longwood Avenue, Boston, Massachusetts 02115, USA*
[2]*Applied Mathematics Department, University of Washington, Box 352420, Seattle, Washington 98195-2420, USA*
[3]*Dermatology Department, Washington University in St Louis, 7741 Barnard Hospital, St Louis, Missouri 63110, USA*
[4]*Biochemistry and Molecular Biophysics Department, Washington University in St Louis, Box 8231, 660 South Euclid Avenue, St. Louis, Missouri 63110-1093, USA*
(Received 27 June 2005; published 10 April 2006)

Biased diffusion of collagenase on collagen fibrils may represent the first observed adenosine triphosphate-independent extracellular molecular motor. The magnitude of force generated by the enzyme remains unclear. We propose a propulsion mechanism based on a burnt bridges Brownian ratchet model with a varying degree of coupling of the free energy from collagen proteolysis to the enzyme motion. When constrained by experimental observations, our model predicts 0.1 pN stall force for individual collagenase molecules. A dimer, surprisingly, can generate a force in the range of 5 pN, suggesting that the motor can be of biological significance.

DOI: 10.1103/PhysRevE.73.041909 PACS number(s): 87.16.Ac

Type I collagen, a major constituent of extracellular matrix, is a triple-helical protein that consists of two $\alpha 1$(I) and one $\alpha 2$(I) polypeptide chains [1]. At physiological ionic strength and temperature and at neutral pH, the triple-helical monomers spontaneously assemble into staggered arrays forming nativelike collagen fibrils up to 500 nm in diameter with 300 nm periodicity. The precise mechanism of fibril assembly is a matter of considerable interest [2,3]. Thermodynamic study of this process showed that fibril growth is accompanied by a temperature-dependent positive enthalpy change of 514.6 kJ/mol at 30 °C [4]. Collagen fibrils are stable structures and are resistant to proteolysis by all but specialized metalloproteases, collagenases. Human interstitial collagenase, MMP-1, cleaves all three polypeptide chains of the triple-helical collagen monomer at a single specific site [5]. Progressive cleavage of collagen monomers at this site leads to a disassembly of the entire fibril [6].

We have found that proteolysis of collagen by MMP-1 involves a biased diffusion of the enzyme on the fibril surface [7]. The directional component of the otherwise random diffusion depends on the proteolytic activity of the enzyme. Modeling the experimental observations shows that restricting the diffusion of the enzyme to only one side of the cleaved collagen monomer helix is sufficient to explain the experimentally observed bias. This effectively creates a molecular motor with a mechanism similar to that of the burnt bridges Brownian ratchet [8,9]. Brownian ratchets were proposed as a model for actin and microtubule polymerization for which Brownian forces are the main active forces pushing the leading edge of the cell forward during cell locomotion [10–12]. Rectified Brownian motion has also been proposed as the propulsion mechanism for other molecular motors, including kinesin and *p*-type adenosine triphosphate (ATP)ases [13,14]. "Power stroke" mechanisms that rely on an internal force due to conformational changes in parts of the motor molecule have also been proposed for kinesin and myosin motors [15]. In theory, a combination of both mechanisms can be easily modeled. As an example, one can consider a Brownian particle which moves along an asymmetric one-dimensional potential. This particle experiences non-Brownian active forces on parts of these tracks. Such thermal ratchets have been reviewed in [16].

According to the burnt bridges Brownian ratchet mechanism, only Brownian (thermal) forces drive the enzyme motion. The energy released by the proteolysis of the collagen is utilized to erect a reflective boundary at each site cleaved by the enzyme. The proteolysis and subsequent unfolding of a collagen fibril, however, releases far more energy than the minimal amount required to erect this reflective boundary. The 62.6 kJ/mol of free energy due to the proteolysis of three polypeptide chains of the collagen monomer, in addition to the energy released upon fibril disassembly, adds up to a potential release of free energy around 585.7 kJ/mol [3]. Here, we present a framework to describe the behavior of the MMP-1 motor under varying conditions of coupling to the free energy liberated by collagen cleavage and fibril unpacking, thus creating a burnt bridges model with varying degrees of internal force coupling.

MMP-1 binds irreversibly to collagen fibrils and does not dissociate until the whole fibril is digested [6]. During and shortly after cleavage, the MMP-1 molecule is at the cleavage site positioned three-quarters of the length from one end of the collagen monomer. Each cleavage is followed by a local unfolding of the fibril which most likely propagates along the length of the monomer [6]. We propose that coupling of the free energy released by collagen cleavage and fibril unpacking results in a force exerted on the enzyme molecule. We assume that the cleavage of one triple helix does not perturb the adjacent helices, and so the force applied to the molecule upon cleavage can extend only to the boundary of the same monomer. As shown in Fig. 1, the distance δ—to which this force is applied—is a free parameter in the model. We assume that the force is constant to illustrate the consequences of a simple model.

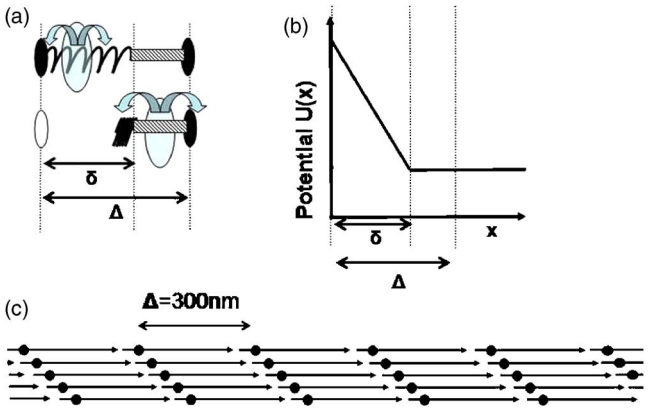

FIG. 1. (Color online) The powered burnt bridges model: The motor molecule performs a random walk on its substrate as shown in (a). The solid circle represents a potential cleavage site while the open circle represents a site already cleaved. Cleavage sites are positioned at a distance Δ along the track. Once cleavage occurs, the molecule is subjected to a constant force up to a distance δ from the digested site as shown in (b). A representation of collagen fibrils is shown in (c). A collagen fibril is composed of triple-helical monomers that line up to form asymmetric tracks. Each arrow on the fibril shows one triple helical monomer with the arrow pointing from the C to the N terminus of the monomer. Each fibril consists of many such parallel tracks that are offset by 60 nm from one another. Solid circles show cleavage sites which are positioned approximately three-quarter of the length from the N terminus of the monomers. In this context, the "spring" in the caption title and in Fig. 1(a) should not be regarded as a conventional Hookean spring, but rather as a device that produces constant force independent of compression.

With the above consideration, the following model emerges: An MMP-1 molecule diffuses along a one-dimensional track [Fig. 1(a)] with cleavage sites separated by a distance Δ positioned periodically along the track. When an enzyme molecule reaches a cleavage site, the probability of cleavage is P_c. After each cleavage, the molecule ends up on the right side of the cleavage and a constant force field F_{int} is generated, which extends from the cleaved site over a distance $\delta < \Delta$. When F_{int} or δ vanish, the current model becomes a pure Brownian ratchet [10,14], while at $\delta = \Delta$ and $P_c = 1$ it becomes completely powered by the internal force. The relative force field length (RFFL) is defined as: δ/Δ.

When the cleavage probability is 100%, cleavage occurs whenever the enzyme molecule passes a cleavage site thus prohibiting its return. In these situations, the time interval T between repeated cleavages is the exit time of a diffusion process. By exit time T_x, we mean the time a Brownian particle takes to reach a cleavage site the first time, starting at $x(0 < x < \Delta)$. Clearly T_x is a random variable; its expectation $T(x) = E[T_x]$, known as mean first passage time, is the solution to the differential equation [17]:

$$DT''_{xx} - [(U'_z + F_{\text{ext}})/\eta]T'_x = -1 \tag{1}$$

with the boundary conditions set to: $T'_z(0) = 0$, $T(\Delta) = 0$. The F_{ext} is positive when opposing the escape of the particle from the region. By defining U as follows:

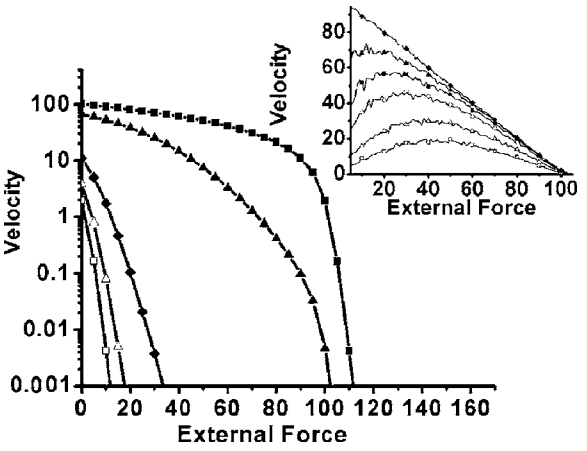

FIG. 2. The force-velocity response under varying RFFL: The force-velocity relationship is calculated from eq. (5). The force is normalized to the Brownian force which equals $1/\beta\Delta$ and the velocity is normalized to D/Δ. The $F_{\text{int}} = 100$ in all, $\delta/\Delta = 0$ (open squares), $\delta/\Delta = 0.3$ (open triangles), $\delta/\Delta = 0.6$ (solid diamonds), $\delta/\Delta = 0.9$ (solid triangle), and $\delta/\Delta = 1$ (solid squares). The inset shows the force activation of the motor when $P_c < 1$. The force-velocity relationship is calculated using the Monte-Carlo simulation outlined in the text $\delta/\Delta = 1$ in all and the curves show $P_c = 1$, 0.8, 0.6, 0.4, 0.2, and 0.1 in descending order.

$$U_{\text{eff}}(x) = U(x) + F_{\text{ext}}x, \quad \beta = \frac{1}{kT}. \tag{2}$$

With $U(x)$ as shown in Fig. 1, and subtracting a constant potential to provide continuity:

$$U_{\text{eff}}(x) = (F_{\text{int}} - F_{\text{ext}})(\delta - x) \quad 0 < x < \delta,$$

$$U_{\text{eff}}(x) = F_{\text{ext}}(x - \delta) \quad \delta < x < \Delta. \tag{3}$$

The solution to Eq. (1) is given as [18]:

$$T(0) = \frac{1}{D\beta^2}\left[[\exp(\beta F_c \delta) - 1]\left(\frac{1}{F_c^2} + \frac{(\exp[\beta F_{\text{ext}}(\Delta - \delta)] - 1)}{F_{\text{ext}}F_c}\right) + \frac{(\exp[\beta F_{\text{ext}}(\Delta - \delta)] - 1)}{F_{\text{ext}}^2} - \frac{(\Delta - \delta)\beta}{F_{\text{ext}}} - \frac{\delta\beta}{F_c}\right]. \tag{4}$$

In which we define the following: $F_c = F_{\text{ext}} - F_{\text{int}}$; the Brownian force $F_B = 1/\beta\Delta$ and the average velocity:

$$V = \frac{\Delta}{T(0)}. \tag{5}$$

The force-velocity curves for this model (for $P_c = 1$) computed with an internal force which is 100 times greater that of the Brownian force, $1/\beta\Delta$, are depicted in Fig. 2. As expected, the larger the RFFL, the higher the velocity at a given external force. Defining the stall force is complex. It is difficult to determine the parameters required to consider the cleavage process as a reversible reaction. Furthermore, for $\delta/\Delta < 1$, the mechanism of transport is mixed, with the enzyme molecule being driven by the internal force for $x < \delta$ and then moving by simple diffusion to encounter the next cleavage site. Therefore, to provide a simple illustration, we

define the stall force as the external force required to reduce the dimensionless velocity to 0.1. Although this definition is arbitrary, Fig. 2 demonstrates that a limiting force is fairly well defined as the velocity decreases toward 0.1.

The velocity for a thermal ratchet driven only by Brownian motion is readily derived by putting $\delta=0$.

$$V_{\mathrm{BR}}=\frac{D}{\Delta}\left(\frac{w_{\mathrm{BR}}^2}{e^{w_{\mathrm{BR}}}-1-w_{\mathrm{BR}}}\right), \tag{6}$$

where $w_{\mathrm{BR}}=\beta F_{\mathrm{ext}}\Delta$ is the dimensionless resistance force, in agreement with the results obtained from [10] for a Brownian ratchet.

The conditions of 100% cleavage probability might be hard to reach experimentally. Note that a P_c of 10% described the original experimental observations at 22 °C. When $0<P_c<100\%$, a direct analytical solution is more difficult and thus Monte Carlo simulations were performed. In each cycle of the simulation, which corresponds to a millisecond in real time, the step size of the molecule is determined from the Langevin equation and, under the conditions of no internal or external force, has a Gaussian distribution with a standard deviation of 50 nm. The cleavage sites are positioned 300 nm apart and a reflecting boundary is placed at each cleaved site. For example, if a molecule at position x is supposed to take a step dx and $x+dx$ is larger than l, the position of the reflective boundary, then the step dx is adjusted to $dx=2(1-x)-dx$ to enforce the reflective boundary condition. In the force velocity simulations, the molecule is positioned at a random location in the beginning first quarter of a 30 μm-long track. Once the molecule reaches the end of the track, the average velocity is calculated by dividing the length of the track by the time spent passing it. This procedure is repeated 100 times and the results are averaged. The resulting force velocity relationships are shown in the inset of Fig. 2 for varying P_c with RFFL $\delta/\Delta=1$ and an internal force $F_{\mathrm{int}}=100\frac{1}{\beta\Delta}$. A surprising effect of the model is the activation of the motor under external load. As shown in the inset of Fig. 2, the velocity of the motor initially increases under the application of external force to a maximum and then decreases again. This counterintuitive result can be explained easily. When $P_c<100\%$, the motor will likely overshoot the cleavage site. Then, the application of a small external force would push the molecule to revisit the missed cleavage site thus increasing the rate of cleavage and thereby the overall speed of the motor.

Now that we have described the behavior of the new model, we will apply it to experimental measurements of the motion of MMP-1 on collagen fibrils. In the original experiments, a laser beam was positioned on the collagen fibril, and the transport of fluorescently tagged MMP-1 molecules was studied through their contribution to fluorescence fluctuation autocorrelation functions, which demonstrated biased diffusion of the molecules on collagen fibrils [7]. It was also shown that the diffusion coefficient of the active enzyme was well approximated by that of an inactive mutant in which the proteolysis and diffusion mechanisms were uncoupled. The behavior of the inactive mutant demonstrated the requirement of enzymatic activity for directed motion of MMP-1.

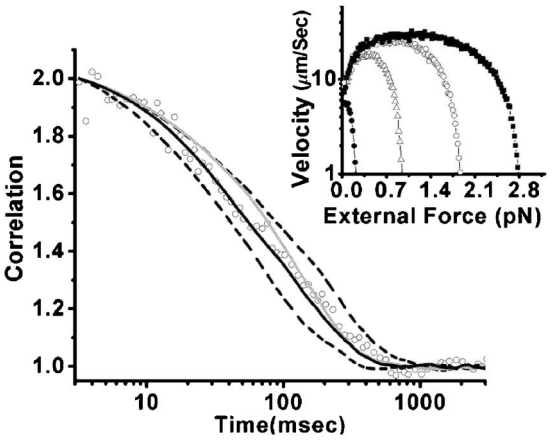

FIG. 3. The experimental and modeled correlation functions: Open circles represent an experimental correlation function measured previously [7] for the active MMP–1 molecule on a collagen fibril. The gray solid line is a fit to the correlation function using $P_c=10\%$ with $F_{\mathrm{int}}=0$. The top and bottom dashed lines represent the correlation function with $P_c=5\%$ with no force coupling for the upper curve and with $F_{\mathrm{int}}=\frac{100}{\beta\Delta}$ and $\delta/\Delta=1$ at the lower curve. The best fit is achieved when $\delta/\Delta=0.5$ and $F_{\mathrm{int}}>\frac{10}{\beta\Delta}$ as shown by the black line. The correlation functions for any internal force magnitude greater than 10 overlap (data not shown). The inset however shows the force velocity prediction with an RFFL=1, $P_c=0.05$, $F_{\mathrm{int}}=0.13$ pN (solid circles), 0.65 pN (open triangles), 1.3 pN (open circles) and 1.95 pN (closed squares) demonstrating that under the condition of external load the different internal forces would yield dramatically different behaviors.

To investigate whether our generalized model with an appreciable internal force could still explain the experimental correlation functions [7], we have produced a series of Monte Carlo simulations of which those with the closest behavior to the experimental correlation function are illustrated in Fig. 3. The experimental correlation functions have been obtained under the conditions of zero load and effectively measure the behavior of the molecules as they pass through the laser beam. Under zero-load conditions, the behavior of the correlation function is sensitive to the value of RFFL but not to the internal force as long as $F_{\mathrm{int}}>10F_B$. For internal forces higher than ten times the Brownian force and under no load, the motor spends most of its time in the Brownian ratchet driven area and thus its velocity becomes limited to the Brownian ratchet velocity. Thus, a very good agreement with the experimental data is reached with the cleavage probability P_c set to 5%, the RFFL $\delta/\Delta=0.5$ and $F_{\mathrm{int}}>10F_B$. Hence, to match the experimental measurements the RFFL of the motor can be around 0.5. We cannot, however, put a limit on the internal force of the motor by studying the experimental correlation function since under no load, all correlation functions with internal forces larger than $10F_B$ overlap. Notwithstanding the fact that other sets of parameters might fit the results equally well, this demonstrates the applicability of our model to the experimental measurements. The inset in Fig. 3 demonstrates the force-velocity relationship for different values of F_{int} at RFFL of 0.5. All of the different internal forces depicted in the inset result in identical zero-load correlation functions. It is predicted, however, that their re-

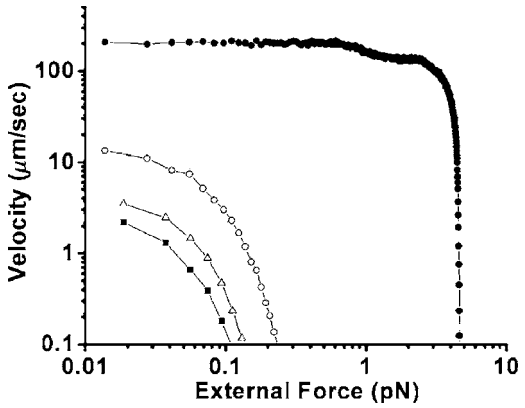

FIG. 4. The predicted force velocity behavior of MMP-1: Single MMP–1 molecule with $P_c=0.05$ and $F_{\text{int}}=0$ (solid squares), with $P_c=1$ and $F_{\text{int}}=0$ (open triangles), $P_c=1$, $F_{\text{int}}=100$, and $\delta/\Delta=0.5$ (open circles). A group of two MMP–1 molecules with $P_c=1$, $\delta/\Delta=0.5$ and $F_{\text{int}}=100$ (solid circles), the two molecules move on tracks that are offset by δ thus creating an effective $\delta/\Delta=1$.

sponses to load would be dramatically different.

The results presented in Fig. 4 summarize the calculated force-velocity behavior of the MMP-1 motor assuming that RFFL=0.5 and all of the energy released by cleavage and unpacking of the collagen fibril is used to push the molecule forward. Although the internal force applied to the molecule is rather large >4 pN, the stall force remains well below 1 pN, and the Brownian ratchet still dominates the motor activity.

The secreted MMP-1 enzyme has been shown to associate with the cell surface via interaction with integrins [19]. The locomotion of some cells, such as keratinocytes on collagen substrata, has been shown to depend on the cell surface-associated collagenolytic activity of the enzyme [20]. Under these circumstances, it is reasonable to assume that the biologically relevant cell surface-bound enzyme is clustered. MMP-1 is among very few enzymes that are capable of collagen proteolysis. Another enzyme is a membrane tethered metalloprotease MT1-MMP that plays a prominent role in many processes associated with cell invasion and motility [21,22]. MT1-MMP has been shown to dimerize on the cell surface [23,24]. Thus, we next considered the behavior of a collagenase dimer with each of two enzyme molecules traveling on adjacent tracks of a collagen fibril offset by 67 nm.

Linking of Brownian ratchets and consequent effects on their stall force have been previously studied in the context of microtubule polymerization in which up to 13 slightly shifted tracks of microtubules push against a common load [11]. These models, however, are powered solely through Brownian motion since there is no internal force generation mechanism in microtubule polymerization reactions, and thus the effects of linking the ratchets on the stall force are mostly linear. Coupled Brownian particles have also been studied in the context of flashing and rocking ratchets [25–28] and yield interesting characteristics which highly depend on the forms of proposed linkage.

For the purposes of our model, we have assumed that the two enzymes are connected with an inflexible bond and thus the movement of the enzymes with respect to one another is negligible. The dimer is tethered to the cell membrane via binding to integrins (MMP-1) or via a transmembrane domain (MT1-MMP). Then, the force exerted by the cluster could assist cell locomotion by driving cell protrusion along the collagen fibril. According to this model, this enzyme cluster would encounter cleavage sites on the offset adjacent tracks so that the Brownian powered section on one of the tracks overlaps with the internal force driven section on a parallel track. As a result, the grouped enzyme molecules would always be traveling powered by the internal force on one of its tracks, effectively making the RFFL of the group close to one. Then the cluster of MMP-1 molecules traveling on the collagen fibril could be viewed as a molecule traveling on a single track with increased RFFL and decreased cleavage site spacing. The frictional coefficient that determines the viscous resistance to the motion of a single enzyme molecule on a collagen fibril can be deduced from the measured diffusion coefficient, $D=8\pm1.5\times10^{-9}$ cm^2 s^{-1}. The "viscous" resistance to the motion of the clustered molecule would be determined by the sum of the individual molecule frictional coefficients. The force-velocity relationship for a collagenase dimer is shown in Fig. 4. The increase in the stall force for the two-molecule motor compared to a single enzyme molecule is almost an order of magnitude reaching 4.6 pN, a value on a par with ATP-driven intracellular motors. Thus, cell surface-anchored collagenases may function as molecular motors that assist with the movement of the cell protrusions along the underling collagen fibrils and play a prominent role in many processes associated with cell invasion and motility.

We have shown that matrix metalloproteases MMP-2 [29], MMP-1 [7], and MMP-9 (Collier *et al.* unpublished) utilize a remarkable surface diffusion mechanism for substrate interaction. The biased diffusion of collagenase on the surface of its substrate, collagen fibril, is the first example of an extracellular molecular motor that operates in an environment where conventional sources of energy, such as ATP, are not present. Thus, other mechanisms of spatial organization of the extracellular matrix can be employed. From the above calculations, it is clear that efficient coupling of the free energy in the collagen fibril can be sufficient to drive a Brownian ratchet molecular motor. The positioning and specificity of the motor molecules with respect to the substrate becomes vital to their functionality.

In view of these model predictions, it is important to develop an experimental system to measure the force velocity of a collagenase motor. Because the single MMP-1 molecule generates too small a force to be measured by optical trapping and preparing beads with adsorbed motor molecules in the proper arrangement to enable their cooperative operation, experimentally challenging other approaches will also have to be considered.

[1] K. Kadler, D. Holmes, J. Trotter *et al.*, Biochem. J. **15**, 1 (1996).
[2] C. Miles and A. Bailey, Micron **32**, 325 (2001).
[3] E. Leikina, M. Mertts, N. Kuznetsova *et al.*, Proc. Natl. Acad. Sci. U.S.A. **99**, 1314 (2002).
[4] G. Na, L. Phillips, and E. Freire, Biochemistry **28**, 7153 (1989).
[5] G. Fields, H. Van Wart, and H. Birkedal-Hansen, J. Biol. Chem. **262**, 6221 (1987).
[6] H. Welgus, J. Jeffrey, G. Stricklin *et al.*, J. Biol. Chem. **255**, 6806 (1980).
[7] S. Saffarian, I. E. Collier, B. L. Marmer *et al.*, Science **306**, 108 (2004).
[8] T. Antal, P. L. Krapivsky, and S. Redner, Phys. Rev. E **72**, 036121 (2005).
[9] J. Mai, I. M. Sokolov, and A. Blumen, Phys. Rev. E **64**, 011102 (2001).
[10] C. Peskin, G. Odell, and G. Oster, Biophys. J. **65**, 316 (1993).
[11] G. Sander van Doorn, C. Tanase, B. M. Mulder *et al.*, Eur. Biophys. J. **29**, 2 (2000).
[12] A. Mogilner and G. Oster, Eur. Biophys. J. **28**, 235 (1999).
[13] R. F. Fox, Phys. Rev. E **57**, 2177 (1998).
[14] R. F. Fox and M. H. Choi, Phys. Rev. E **63**, 051901 (2001).
[15] D. Keller and C. Bustamante, Biophys. J. **78**, 541 (2000).
[16] F. Jülicher, A. Ajdari, and J. Prost, Rev. Mod. Phys. **69**, 1269 (1997).
[17] H. M. Taylor and S. Karlin, *An Introduction to Stochastic Modeling* (Academic Press, San Diego, 1998).
[18] H. Qian, Mech. Chem. Biosyst. **1**, 267 (2004).
[19] J. Dumin, S. Dickeson, T. Stricker *et al.*, J. Biol. Chem. **276**, 29368 (2001).
[20] B. Pilcher, J. Dumin, B. Sudbeck *et al.*, J. Cell Biol. **137**, 1445 (1997).
[21] H. Sato, T. Takino, Y. Okada *et al.*, Nature (London) **370**, 61 (1994).
[22] A. Strongin, I. Collier, G. Bannikov *et al.*, J. Biol. Chem. **270**, 5331 (1995).
[23] Y. Itoh, A. Takamura, N. Ito *et al.*, EMBO J. **20**, 4782 (2001).
[24] D. Rozanov, E. Deryugina, B. Ratnikov *et al.*, J. Biol. Chem. **276**, 25705 (2001).
[25] Z. G. Zheng, Int. J. Mod. Phys. B **18**, 2498 (2004).
[26] Z. Csahok, F. Family, and T. Vicsek, Phys. Rev. E **55**, 5179 (1997).
[27] A. Igarashi, S. Tsukamoto, and H. Goko, Phys. Rev. E **64**, 051908 (2001).
[28] F. Julicher and J. Prost, Phys. Rev. Lett. **75**, 2618 (1995).
[29] I. E. Collier, S. Saffarian, B. L. Marmer *et al.*, Biophys. J. **81**, 2370 (2001).