Single-Molecule Rupture Dynamics on Multidimensional Landscapes

Yohichi Suzuki and Olga K. Dudko

Department of Physics and Center for Theoretical Biological Physics, University of California at San Diego, La Jolla, California 92093, USA

(Received 27 August 2009; published 25 January 2010)

We explore emergent effects of multidimensionality of the free energy landscape on single-molecule kinetics under constant force. The proposed minimal model reveals the existence of a spectrum of unusual scenarios for the force-dependent lifetime, all of which are shown to occur on a free energy landscape with a single transition state. We present an analytical solution that governs single-molecule responses to a constant force and relates them to microscopic parameters of the system.

DOI: 10.1103/PhysRevLett.104.048101 PACS numbers: 87.15.ad, 87.15.Cc

Single-molecule manipulation methods are making it possible to directly measure forces generated during key processes in the living cell. These methods have been applied to an expanding variety of fundamental biological problems, ranging from the mechanical properties of biopolymers and the strength of ligand-receptor interactions to the dynamics associated with enzyme catalysis [1,2]. In particular, force-clamp experiments allow monitoring of the response of a single biomolecule held under a constant stretching force. A chosen value of force is maintained through a continuous readjustment of the molecular extension [3] or via the passive force clamp [4]. From a time series of the extension, the time scale to rupture, or the lifetime $\tau(F)$, at each value of force is obtained. The $\tau(F)$ data can then be interpreted in microscopic terms by fitting to a theoretical model.

Force-induced biomolecular rupture involves a vast number of degrees of freedom both of the molecule being pulled and of the surroundings. To make it a tractable problem, one usually assumes that the instantaneous configuration of the molecule can be fully identified by a single variable—pulling coordinate x, e.g., the end-to-end distance of a biopolymer as it is being stretched. This assumption is justified when changes in x represent the slowest mode of the dissociation process while all other degrees of freedom rapidly attain Boltzman equlibrium. The slow motion is treated as a Brownian motion on the potential of mean force along the reaction coordinate x with the effect of other coordinates ''packed'' into the thermal bath. The lifetime $\tau(F)$ in the resulting 1D description is determined by diffusive crossing of an activation barrier tilted under the applied force, and can be calculated from Kramers theory [5–7].

Limitations of such a 1D description are apparent. If there is another degree of freedom (call it Q) which is as slow as or slower than x, the reduction of the multidimensional dynamics to a 1D process along x is no longer possible. Indeed, Hyeon and Thirumalai showed [8] that the extension (x) in the force-quench refolding of RNA hairpins is largely determined by local conformational changes in the dihedral angles (Q), indicating that x alone may not be an adequate reaction coordinate. Similarly, the extension of a protein held under a stretching force is likely to be correlated with the fraction Q of the native amino acid contacts [9], and the redistribution of Q not only can be as slow as changes in x but also can in fact represent the rate-limiting step of the protein unfolding. A multidimensional approach is then required to describe molecular rupture, with the free energy $G(x, Q)$ now being a function of at least two coordinates [10].

In this Letter we explore, in the framework of a minimal model, the range of responses of a single macromolecule or a molecular complex to constant pulling force when the dynamics of a degree of freedom (Q) other than the molecular extension (x) is essential for the rupture kinetics. We reveal the existence of a rich spectrum of qualitatively distinct scenarios for the force-dependent lifetime $\tau(F)$, among which is a ''rollover'': a seemingly counterintuitive effect of strengthening the system by low force followed by an accelerated rupture at higher forces. While nomonotonic force dependence of lifetime has been observed in experiments on catch-slip adhesion bonds in single cells [11] and in simulations of protein unfolding [12,13], it has been largely attributed to multiple coexisting pathways each selected in a different force range [12–17]. Here we demonstrate that a dynamics as simple as that on a single dissociation pathway [13] is sufficient to realize nonmonotonic lifetimes. Because of natural movement of the transition state with force, no additional deformation energy term [18] needs to be introduced artificially. Our model leads to an analytical solution that describes a variety of force dependencies of the lifetime in terms of microscopic properties of the system.

Free energy of the system is the sum of the intrinsic free energy, $G_0(x, Q)$, and the mechanical work $(-Fx)$ of the external constant force F acting in the direction of x. As is typical for this class of problems, $G_0(x, Q)$ has a well (the bound state) separated from the free state by a high barrier that has the minimum height at the saddle point (Fig. 1). A minimal model which incorporates the effect of the slow

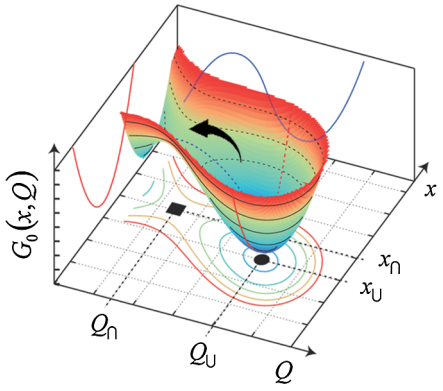

FIG. 1 (color online). Intrinsic free energy $G_0(x, Q)$ has a bound state at $(x_\cup, Q_\cup)$ separated from the free state by a high barrier at $(x_\cap, Q_\cap)$.

coordinate Q on the rupture kinetics under constant force is [19,20]

$$G(x, Q) = G_0(Q) + \tfrac{1}{2}k(Q)[x - x_0(Q)]^2 - Fx. \quad (1)$$

In the absence of force, the $x =$ const free energy curves are assumed to resolve the bound state and the transition state in an interval of values of x that includes the coordinate of the transition state, $x_\cap$ (Fig. 1). The $Q =$ const profiles of the free energy are harmonic with the curvature $k(Q)$ and the minimum at $x_0(Q)$. $k(Q)$ is a measure of the statistical dispersion in the values of the molecular extension at a given Q and can be thought of as a molecular stiffness, while $x_0(Q)$ is the most probable value of the extension at a given Q in the absence of force. To preserve the generality of the analysis, we do not restrict ourselves with any explicit functional forms of $G_0(Q)$, $k(Q)$, and $x_0(Q)$ until the end [Eq. (8)] of this Letter.

Under the assumption that the lifetime of the system is controlled by the diffusion of the probability density of the molecular configurations across a region of the saddle (Fig. 1), $\tau(F)$ can be found from the Langer's multidimensional generalization [21] of Kramers theory [5]:

$$\tau(F) = 2\pi\tau_+(F)\left(\frac{|\det \mathbf{H}_\cap(F)|}{\det \mathbf{H}_\cup(F)}\right)^{1/2} \exp[\beta\Delta G^\ddagger(F)], \quad (2)$$

where $\Delta G^\ddagger(F) = G(x_\cap(F), Q_\cap(F)) - G(x_\cup(F), Q_\cup(F))$ is the height of the activation barrier at force F, $\tau_+(F)$ is the unique positive root of $\det(\beta\mathbf{D}\mathbf{H}_\cap(F) + \tau_+^{-1}\mathbf{I}) = 0$, $\mathbf{D}$ is the diffusion matrix, $\mathbf{H}_{\cap/\cup}(F)$ is the Hessian matrix of $G(x, Q)$ at the transition state/bound state, and $\mathbf{I}$ is the unit matrix. Coordinates $(x_\cup(F), Q_\cup(F))$ of the bound state and $(x_\cap(F), Q_\cap(F))$ of the transition state are the solutions of $\{\partial G(x, Q)/\partial Q = 0, \partial G(x, Q)/\partial x = 0\}$. As a result of the reshaping of the free energy landscape in Eq. (1) by the force, the bound and transition states change their Q positions according to

$$\frac{\partial Q_{\cup/\cap}}{\partial F} = \frac{1}{\lambda_{\cup/\cap}(F)}\left[\frac{\partial x_0}{\partial Q} - \frac{F}{k^2(Q)}\frac{\partial k}{\partial Q}\right]\Bigg|_{Q_{\cup/\cap}(F)}, \quad (3)$$

where $\lambda_{\cup/\cap}(F)$ are the second Q derivatives of $G(x, Q)$ along the dissociation pathway, evaluated in the bound $(\cup)$/transition $(\cap)$ states (see [22] for details).

Because the major impact of force on the lifetime in Eq. (2) stems from the exponential factor, the qualitative behavior of $\tau(F)$ can be accessed based on the effect of the force on the barrier height $\Delta G^\ddagger(F)$. We find [22] that the rate at which the barrier height of the free energy surface in Eq. (1) changes with the force F is given by

$$\partial\Delta G^\ddagger/\partial F = -[x_\cap(F) - x_\cup(F)]. \quad (4)$$

If the extension in the transition state, $x_\cap(F)$, exceeds that in the bound state, $x_\cup(F)$, the rate of change in the barrier height in Eq. (4) is negative indicating that the force shortens the lifetime. If the extension is the same in the two states, Eq. (4) indicates no change to the barrier, the lifetime is essentially insensitive to force. If rupture mechanism requires that two residues approach each other in order to attain the transition state, stretching force applied at these residues will result in an increase of the barrier height [the right-hand side of Eq. (4) is positive] and thus in an extended lifetime. If two or more of the above scenarios are realized each in a different range of the applied force, the resulting molecular response will be a nonmonotonic lifetime $\tau(F)$, a phenomenon not expected in a conventional 1D description.

The difference in the extension in the transition state and in the bound state that appears in Eq. (4) can be expressed explicitly in terms of the two characteristics of the model in Eq. (1), $x_0(Q)$ and $k(Q)$:

$$x_\cap(F) - x_\cup(F) = x_0(Q_\cap(F)) - x_0(Q_\cup(F)) + F[k^{-1}(Q_\cap(F)) - k^{-1}(Q_\cup(F))]. \quad (5)$$

Based on Eqs. (4) and (5), we conclude that the barrier height, and thus the lifetime $\tau(F)$, is affected by the force when the bound state and the transition state are characterized by different values of x_0 and/or k. Below we dissect possible scenarios for $\tau(F)$ of the system in Eq. (1). Our approach is based on tracking changes, caused by the force, in the relative position of the bound and transition states as governed by Eq. (3). These changes determine the evolution of the barrier height with force, Eqs. (4) and (5), which, in turn, determines $\tau(F)$, Eq. (2). This approach eliminates the need for assumptions of explicit functional forms of $x_0(Q)$ and $k(Q)$, thereby enhancing the generality of conclusions. For details of analysis, see [22].

First, we note that when neither x_0 nor k vary with Q, the barrier in Eqs. (4) and (5) is insensitive to force and hence rupture is not affected by pulling. Next we explore possible scenarios for $\tau(F)$ when only one of the two characteristics of the model, x_0 or k, varies with Q. As summarized in Fig. 2, the range of scenarios even under such restricting conditions is remarkably diverse [see Figs. 2(b) and 2(d)] and includes monotonic as well as nonmonotonic profiles of $\tau(F)$, depending on the nature of $x_0(Q)$ [Fig. 2(a)] or of $k(Q)$ [Fig. 2(c)]. Unlike the conventional 1D description

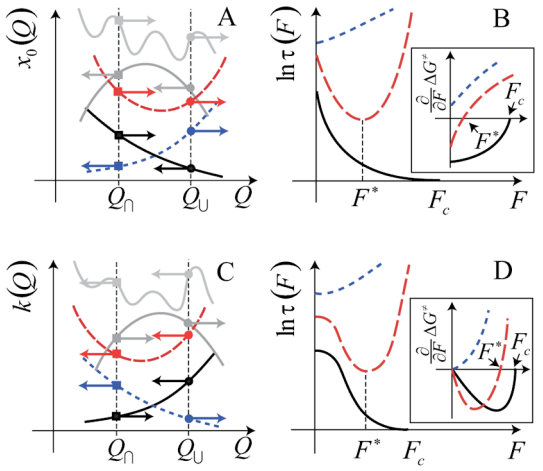

FIG. 2 (color online). Force-dependent lifetimes $\tau(F)$ of the system in Eq. (1). (a) Profiles of $x_0(Q)$ (solid black, dashed red, dotted blue) resulting in distinct scenarios for $\tau(F)$ at $k = \text{const}$. As force increases, the extrema $Q_\cup$ and $Q_\cap$ move (see arrows) according to Eq. (3). None of the other types of $x_0(Q)$ (e.g., solid gray lines) lead to a scenario for $\tau(F)$ qualitatively different from those in (b). (b) $\tau(F)$ scenarios at $k = \text{const}$ corresponding to each of the $x_0(Q)$ curves in (a). Inset: Rate of change of the barrier height with force at $k = \text{const}$, corresponding to each of the $x_0(Q)$ curves in (a). (c) and (d) The same as (a) and (b) but for $x_0 = \text{const}$.

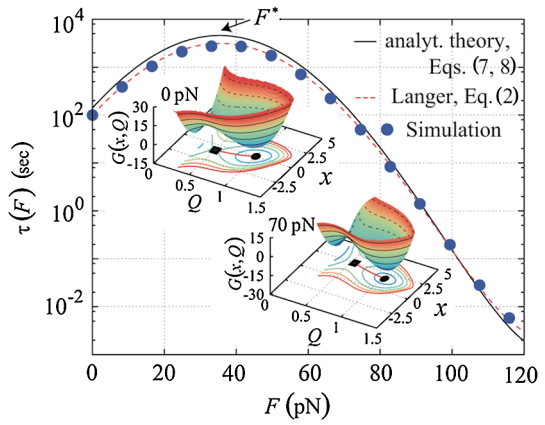

FIG. 3 (color online). Rollover in the lifetime from theory and simulations. Snapshots of the free energy landscape at zero force and at high force show distortion of the dissociation path in the (x, Q) plane. See [22] for parameter values.

with x being the reaction coordinate, the monotonic $\tau(F)$ is now governed not only by the dynamics along x but also by that along Q.

Let us turn to the general case when the states corresponding to different values of Q are characterized by different values of both the most probable extension $x_0(Q)$ and the molecular stiffness $k(Q)$. The evolution of the barrier height with force [Eqs. (4) and (5)] is now determined by the interplay between two factors, one being the difference in x_0 in the bound and transition states, and another being the difference in k in these two states. This interplay can potentially result in unlimited number of scenarios for $\tau(F)$, including those with alternating phases of increase and decrease. In particular, the difference $x_\cap(F) - x_\cup(F)$ in Eq. (4) is now allowed to change sign from negative to positive at a force F^*, resulting into the rollover in $\tau(F)$ (Fig. 3). The rollover can be realized when the transition state possesses two properties as opposed to the bound state: (i) short molecular extension and (ii) soft structure. Because of (i), stretching force will counteract the intrinsic mechanism of attaining the transition state; as a result, low force will slow down the rupture. On the other hand, a sufficiently high force will distort the pliable [due to (ii)] transition state such that rupture through a distorted barrier may eventually become as (or more) efficient as rupture through the unperturbed barrier. The two factors are balanced at $F = F^*$ where the rollover in the lifetime occurs. The described mechanism is principally different from a discrete switch between two coexisting pathways, as there is only one pathway in the model in Eq. (1). This study thus points out to an alternative mechanism of the rollover (Fig. 3) and other nonmonotonic scenarios (Fig. 4) in the lifetime. For a comparison of different models, see [22].

Rollover in $\tau(F)$ may be observed in the forced unfolding of a protein or a nucleic acid which is of a prolate shape in its folded state [23]: pulling on the ends of the polar diameter will tend to separate the residues that otherwise would not likely be that far apart in the transition state. Nonmonotonic $\tau(F)$ is expected in pulling experiments on knotted proteins and on ligand-receptor complexes with the ligand behaving as a "hook" [24,25] since pulling will

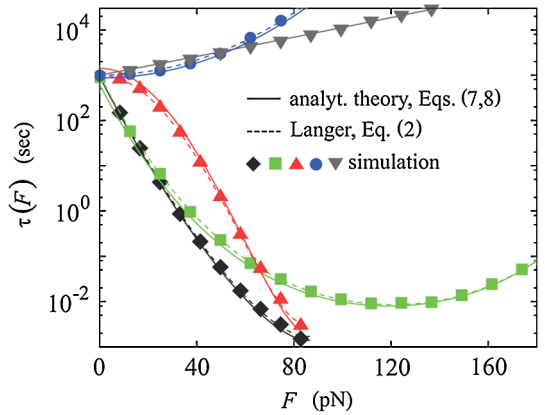

FIG. 4 (color online). Scenarios for the force-dependent lifetime that arise from the model in Eq. (1) when only one of the two characteristics of the model, the extension x_0 or the stiffness k, varies with Q. Lines: theory; symbols: simulations. Profiles in black diamonds, green squares, and gray triangles are due to the Q dependence of x_0 [$k = \text{const}$; compare with predictions in Fig. 2(b)]. Profiles in red triangles and blue circles are due to the Q dependence of k [$x_0 = \text{const}$; compare with Fig. 2(d)]. All the profiles are reproduced by analytical theory in Eqs. (7) and (8) (solid lines). See [22] for parameter values.

oppose unknotting or unhooking at least in some range of forces.

When Q is the slowest degree of freedom among all others, including the extension x, the 2D model in Eq. (1) can be reduced to a picture of diffusive barrier crossing on a potential of mean force along the reaction coordinate Q, which can be calculated by averaging Eq. (1) over x as $G(Q) = -\beta^{-1} \ln \int \exp[-\beta G(x, Q)] dx$ [20]:

$$G(Q) = G_0(Q) - F x_0(Q) - \frac{F^2}{2k(Q)} + \frac{1}{2\beta} \ln \frac{\beta k(Q)}{2\pi}. \quad (6)$$

If the stiffnesses of the bound and the transition states are comparable, the contribution of the logarithmic term to rupture kinetics can be neglected. We find that, for a broad class of models with $G_0(Q)$ being a linear-cubic function [6] and $x_0(Q)$ and $1/k(Q)$ each allowed to be any polynomial up to second degree in Q, the lifetime on the potential of mean force in Eq. (6) can be calculated from Kramers theory as

$$\tau(F) = \tau_0 / \Delta q(F) \exp[-\beta \Delta G^{\ddagger}(1 - \Delta q^3(F))]. \quad (7)$$

Here $\Delta q(F) \equiv \Delta Q(F)/\Delta Q^{\ddagger}$ is the distance along Q between bound and transition states normalized by its zero force value, and $\tau_0 = \pi \Delta Q^{\ddagger 2}/(3 D_Q \beta \Delta G^{\ddagger}) \exp(\beta \Delta G^{\ddagger})$ is the lifetime at zero force.

To illustrate the utility of Eq. (7), consider $G_0(Q) = \frac{2\Delta G^{\ddagger}}{\Delta Q^{\ddagger 3}}[Q - \Delta Q^{\ddagger}/2]^3 - \frac{3\Delta G^{\ddagger}}{2\Delta Q^{\ddagger}}[Q - \Delta Q^{\ddagger}/2]$, along with $x_0(Q) = \epsilon Q^2 - Q \frac{\Delta x^{\ddagger}}{\Delta Q^{\ddagger}}[1 + \epsilon \frac{\Delta Q^{\ddagger 2}}{\Delta x^{\ddagger}}] + \Delta x^{\ddagger}$ and $k(Q) = k_{\cup}^0 \frac{1 - \Delta k^{\ddagger}/k_{\cup}^0}{1 - (Q/\Delta Q^{\ddagger}) \Delta k^{\ddagger}/k_{\cup}^0}$. Here $\Delta x^{\ddagger}$ is the distance along x between the transition and bound states and $\Delta k^{\ddagger}$ is the difference in molecular stiffness in the bound ($k_{\cup}^0$) and transition states at $F = 0$. The nature of $x_0(Q)$ is specified by ϵ: $x_0(Q)$ is monotonic at $|\epsilon| < |\epsilon^*|$ [Fig. 2(a), solid black and dotted blue] and nonmonotonic at $|\epsilon| > |\epsilon^*|$ [Fig. 2(a), dashed red], where $\epsilon^* = \Delta x^{\ddagger}/\Delta Q^{\ddagger 2}$. With this choice of model functions, $\Delta q(F)$ in Eq. (7) is

$$\Delta q^2(F) = 1 - \frac{2\Delta x^{\ddagger}}{3\Delta G^{\ddagger}} F - \left(\frac{\Delta k^{\ddagger}/(3\Delta G^{\ddagger} k_{\cup}^{02})}{1 - \Delta k^{\ddagger}/k_{\cup}^0} - \frac{\epsilon^2 \Delta Q^{\ddagger 4}}{9 \Delta G^{\ddagger 2}} \right) F^2. \quad (8)$$

We establish the quality of our results through comparison with Brownian dynamics simulations of molecular rupture on the 2D free energy landscape in Eq. (1). Figures 3 and 4 demonstrate the agreement between the analytical theory in Eqs. (7) and (8) (solid lines) and simulations (symbols). The analytical solution also agrees with the lifetime obtained by numerical evaluation of the 2D Langer formula, Eq. (2); the two results become practically identical at high diffusion anisotropy, $D_x \gg D_Q$.

In summary, this study revealed the existence of unusual scenarios for the force-dependent lifetime in single-molecule dynamics at constant force. The scenarios occur for dynamics as simple as that on a 2D free energy landscape with a single transition state. Further advances in single-molecule techniques should enable discrimination between the mechanism of rupture involving a switch between two competing barriers and the mechanism where a single barrier is continuously altered with force.

We are grateful to Robert Best and Attila Szabo for valuable comments. This work was supported by the NSF CAREER Grant No. MCB-0845099, the NSF Center for Theoretical Biological Physics Grant No. PHY-0822283, and the Hellman Faculty Fund.

[1] W. Greenleaf, M. Woodside, and S. Block, Annu. Rev. Biophys. Biomol. Struct. **36**, 171 (2007).
[2] C. Bustamante, Annu. Rev. Biochem. **77**, 45 (2008).
[3] A. Oberhauser, P. Hansma, M. Carrion-Vazquez, and J. Fernandez, Proc. Natl. Acad. Sci. U.S.A. **98**, 468 (2001).
[4] W. Greenleaf, M. Woodside, E. Abbondanzieri, and S. Block, Phys. Rev. Lett. **95**, 208102 (2005).
[5] H. Kramers, Physica (Amsterdam) **7**, 284 (1940).
[6] O. Dudko, G. Hummer, and A. Szabo, Phys. Rev. Lett. **96**, 108101 (2006).
[7] L. Freund, Proc. Natl. Acad. Sci. U.S.A. **106**, 8818 (2009).
[8] C. Hyeon and D. Thirumalai, J. Phys. Condens. Matter **19**, 113101 (2007).
[9] N. Socci, J. Onuchic, and P. Wolynes, J. Chem. Phys. **104**, 5860 (1996).
[10] O. Dudko, A. Filippov, J. Klafter, and M. Urbakh, Proc. Natl. Acad. Sci. U.S.A. **100**, 11378 (2003).
[11] B. Marshall, M. Long, J. Piper, T. Yago, R. McEver, and C. Zhu, Nature (London) **423**, 190 (2003).
[12] D. West, P. Olmsted, and E. Paci, J. Chem. Phys. **124**, 154909 (2006).
[13] R. Best, E. Paci, G. Hummer, and O. Dudko, J. Phys. Chem. B **112**, 5968 (2008).
[14] D. Bartolo, I. Derenyi, and A. Ajdari, Phys. Rev. E **65**, 051910 (2002).
[15] E. Evans, A. Leung, V. Heinrich, and C. Zhu, Proc. Natl. Acad. Sci. U.S.A. **101**, 11281 (2004).
[16] V. Barsegov and D. Thirumalai, Proc. Natl. Acad. Sci. U.S.A. **102**, 1835 (2005).
[17] Y. Pereverzev, O. Prezhdo, M. Forero, E. Sokurenko, and W. Thomas, Biophys. J. **89**, 1446 (2005).
[18] O. Prezhdo and Y. Pereverzev, Acc. Chem. Res. **42**, 693 (2009).
[19] A. Berezhkovskii and V. Zitserman, J. Phys. A **25**, 2077 (1992).
[20] O. Dudko, G. Hummer, and A. Szabo, Proc. Natl. Acad. Sci. U.S.A. **105**, 15755 (2008).
[21] J. Langer, Ann. Phys. (N.Y.) **54**, 258 (1969).
[22] See supplementary material at http://link.aps.org/supplemental/10.1103/PhysRevLett.104.048101 for detailed derivation of the main results.
[23] K. Scott, L. Randles, and J. Clarke, J. Mol. Biol. **344**, 207 (2004).
[24] E. Lee, M. Gao, N. Pinotsis, M. Wilmanns, and K. Schulten, Structure **14**, 497 (2006).
[25] M. Bertz, M. Wilmanns, and M. Rief, Proc. Natl. Acad. Sci. U.S.A. **106**, 13307 (2009).