Strength limit of entropic elasticity in beta-sheet protein domains

Sinan Keten and Markus J. Buehler*

Laboratory for Atomistic and Molecular Mechanics, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave., Room 1-235A&B, Cambridge, Massachusetts 02139 USA

(Received 30 July 2008; published 16 December 2008)

Elasticity and strength of individual beta-sheet protein domains govern key biological functions and the mechanical properties of biopolymers including spider silk, amyloids, and muscle fibers. The worm-like-chain (WLC) model is commonly used to describe the entropic elasticity of polypeptides and other biomolecules. However, force spectroscopy experiments have shown pronounced deviations from the ideal WLC behavior, leading to controversial views about the appropriate elastic description of proteins at nanoscale. Here we report a simple model that explains the physical mechanism that leads to the breakdown of the WLC idealization in experiments by using only two generic parameters of the protein domain, the H-bond energy and the protein backbone's persistence length. We show that a rupture initiation condition characterized by the free energy release rate of H-bonds characterizes the limit of WLC entropic elasticity of beta-sheet protein domains and the onset of rupture. Our findings reveal that strength and elasticity are coupled and cannot be treated separately. The predictions of the model are compared with atomic force microscopy experiments of protein rupture.

DOI: 10.1103/PhysRevE.78.061913 PACS number(s): 87.14.E−, 82.37.Rs, 62.20.M−, 81.40.Lm

I. INTRODUCTION

Elasticity and strength of individual protein domains govern vital physiological processes and the mechanical properties of biopolymers including silks, amyloids, and muscle fibers [1–4]. Under small deformation, many proteins display entropic elasticity with a characteristic stiffening elastic behavior, often described using the worm-like-chain (WLC) model [5–7]. Isolated single chains of polypeptides and nucleic acids with weaker self-interactions agree particularly well with the WLC idealization [8,9]. Under large deformation, however, beta structures such as the I27 and I32 domains in titin often exhibit deviations from the WLC model, as they show distinct force peaks under perturbation due to the cooperative rupture of H-bonds under shear loading [10,11]. As illustrated in Fig. 1(a), a single set of WLC parameters falls short of explaining the entire deformation behavior of these nanostructures, and the condition that characterizes the transition from one WLC curve to another remains unclear. Similar phenomena observed from mechanical unfolding experiments of other biological molecules have thus rendered the applicability of WLC model to describe large deformation of folded protein structures questionable. Statistical mechanics approaches based on Kramer's diffusion model or the Bell formulation [12–16] have been successful in predicting the rupture strength of protein domains. However, such models cannot explicitly consider the physical mechanisms of the process such as H-bond rupture, and therefore provide only a phenomenological description of their mechanical properties without explicitly considering the structure and geometry (for example, size or length) of the protein domain. A mechanistic model that is capable of explaining the generic physical relationship between the elasticity and strength of protein domains has remained elusive.

We recently proposed a thermodynamics based fracture mechanics model (referred to here as the beta-strand strength model, BSSM) to describe H-bond rupture mechanisms in beta-proteins like titin I27 domain shown in Fig. 1(b), applied in earlier preliminary studies to predict the strength properties and size effects in beta-sheets[17,18]. Here we extend the BSSM framework to provide for the first time a physical description of the link between elasticity and rupture strength of a protein domain based on a simple, coherent formulation that can predict the key aspects of a protein domain's mechanical experimental signature as shown in Fig. 1.

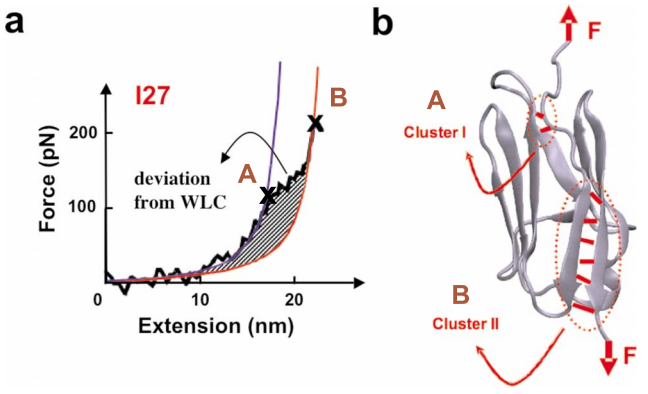

FIG. 1. (Color online) Example force extension profile of a beta-sandwich protein structure, as obtained from experimental analyses (rupture marked with an "×") [11]. Force-extension profiles of I27 domain shown in subplot (a) reveal a two-step process of unfolding, corresponding to rupture of two separate clusters of H-bonds (plot redrawn based on data from Ref. [11]). The curves reveal that the WLC model alone is not capable of describing the entire deformation range, and show that there exist a transition from one WLC curve to a second one at a force level of approximately 110 pN (that is, two sets of WLC parameters are required to describe the observed behavior, where the point of transition from one to the other cannot be predicted).

*Corresponding author; mbuehler@mit.edu; FAX: +1-617-258-6775.

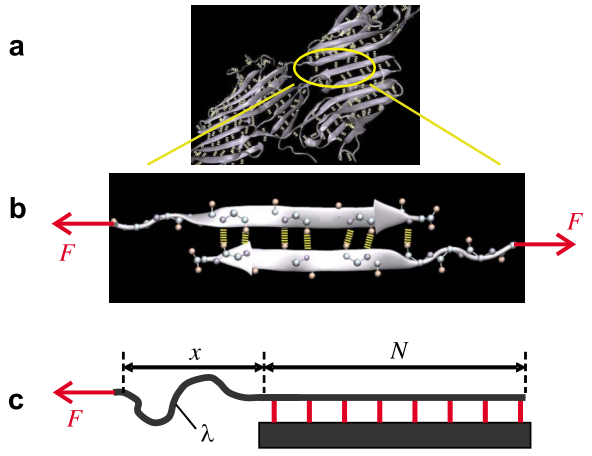

FIG. 2. (Color online) Schematic representation of model setup used in the beta-strand strength model (BSSM). Subplot (a) illustrates the characteristic structure of a beta-sheet protein domain. Subplot (b) shows a simple beta-sheet structure, the fundamental building block of larger beta-sheet rich protein structures. Subplot (c) illustrates the schematic of the BSSM consisting of a single polypeptide stabilized by a linear array of H-bonds with a free end at the left-hand side (corresponding to the unattached random coil protein part). The part of the polypeptide chain that is attached to the substrate (part drawn as straight line) is assumed to be relaxed before rupture occurs.

II. MODEL DEVELOPMENT

We provide a brief overview of the underlying principles of the BSSM framework. The BSSM framework describes the physical unit mechanisms that lead to the rupture of the protein domain during force spectroscopy experiments. Force peaks observed in these experiments have been linked to individual rupture events in beta proteins [see Fig. 2(a)], where key H-bonded domains unravel in a protein structure. This suggests that a single beta sheet such as the one shown in Fig. 2(c) can be considered as the unit building block acting as a mechanical clamp in a larger protein structure. Such an assembly of H-bonds is the smallest and simplest subsystem that can be considered in the unfolding of a protein domain. We model this beta-sheet protein building block as a polypeptide chain stabilized by H-bonds, as schematically shown in Fig. 2(b).

The BSSM model couples the external force applied to the protein structure to H-bond rupture mechanisms by using the Griffith-Irwin energy balance concept [19], modified here for the free energy of the system, to include entropic effects due to stretching of polypeptide chains, as opposed to internal energy as done generally for crystalline materials. Figure 2(b) displays a schematic corresponding to this simple model system, showing a system of N H-bonds. Stretching due to external force reduces the entropy of the free end of the polypeptide chain, causing a net change in the free energy of the system. Rupture of individual H-bond clusters dissipates the free energy imparted on the system. The core prediction of the BSSM is a strong size effect of the strength of H-bond clusters, revealing a transition between two physical mechanisms of rupture at a critical number of H-bonds, N_{cr}, which denotes the maximum number of bonds that can break simultaneously under uniform quasistatic shear deformation. We explain these effects in more detail. The rupture strength of a small cluster of H-bonds ($N<N_{cr}$) can be predicted by the Bell formulation [12,13], assuming bonds break simultaneously under this uniform loading condition. For systems larger than a critical number of bonds ($N\geqslant N_{cr}$), the Bell assumption ceases to hold, and only N_{cr} bonds break simultaneously at a constant force. This leads to a constant strength of the bond assembly regardless of the number of H-bonds present. The only input parameters that feed into the BSSM model are the H-bond energy and the persistence length of the protein polypeptide backbone, ξ_P. The persistence length of free polypeptide chains is reliably found to be $\approx$0.4 nm [3,20], (this particular value has been suggested for titin, spider silk proteins, and tenascin), whereas the H-bond energy depends on the particular protein structure considered (and the solvent conditions), and typically ranges from 2 to 8 kcal/mol.

III. THEORETICAL FRAMEWORK

Our analysis suggests that there exist three key deformation regimes for polypeptide chains stabilized by H-bonds. There is an initial low force entropic regime, where H-bond rupture does not take place and the polypeptide chain is reorganized due to the applied load. The elastic behavior in this regime is entropic and can be described by the worm-like-chain (WLC) model [6],

$$F_{\mathrm{WLC}}(\alpha) = \frac{k_B T}{4\xi_P}[(1-\alpha)^{-2} + 4\alpha - 1]. \tag{1}$$

In Eq. (1) k_B is Boltzmann's constant, T is the temperature, ξ_P is the persistence length of the molecule, and $\alpha=x/\lambda$ is the deformation variable defined as the ratio of end-to-end distance to the contour length of the chain, equivalent to the continuum theory concept of stretch.

The two other deformation regimes pertain to how H-bond rupture mechanisms change as a function of the size of the assembly. H-bonds show cooperative rupture behavior below a critical number of H-bonds [17,18]. In this regime of uniform rupture, the energy barrier to unfolding scales linearly with the number of H-bonds present in the system. The rupture force of a small cluster of H-bonds (at vanishing deformation rates) can be estimated by the Bell formulation as

$$F_{\mathrm{BELL}}(N) = \frac{1}{x_B}\left[k_B T \ln\left(\frac{1}{\omega\tau}\right) + E_{\mathrm{HB}} N\right], \tag{2}$$

where x_B=4 Å is the distance over which the force acts until bond rupture (=the transition state), $\omega=1\times10^{13}$ s^{-1} is the natural frequency of bond vibration [12], $\tau\approx$20 ps is the characteristic time scale of H-bond rupture as determined from both experiment and atomistic simulation studies [17,18], N is the number of H-bonds in the cluster considered [see, e.g., Fig. 2(c) for the geometry], and E_{HB} is the dissociation energy of a single H-bond. All of the parameters that appear in Eq. (2) can be estimated from geometric argu-

ments, direct experimental measurements, or molecular dynamics (MD) simulations.

For larger H-bond assemblies, the simultaneous H-bond rupture assumption ceases to hold because rupture propagates like a "crack" with a localized rupture front, and is then controlled by a free energy release rate condition. In this case rupture initiates locally and the rupture front propagates through the interface, in clusters of H-bonds that rupture simultaneously, at constant force (this process can be envisioned similar to a crack in a brittle crystal, which spreads and eventually leads to failure of the entire crystal). The maximum rupture force can be calculated using the energy balance concept from Griffith's fracture theory, as described in [18]. The onset of failure is characterized by the condition that the change in free energy W_P due to the extension of the fracture must balance the energy necessary to create new protein "surfaces," γ_{HB} (that is, polypeptide that is no longer attached to another polypeptide sequence since H-bonds are broken). We note that the parameter γ_{HB} is the one-dimensional (1D) equivalent of surface energy, and is defined as $\gamma_{\mathrm{HB}}=E_{\mathrm{HB}}/L_{x,0}$, where E_{HB} is the energy required to rupture a single H-bond, and $L_{x,0}$ is the lateral distance between two H-bonds. The units of γ_{HB} are energy per unit length, which is equivalent to force. The negative of the expression for the free energy change with respect to a rupture advance of one unit distance, δa, is called the energy release rate $G=-\delta W_P/\delta a$. This leads to the rupture initiation condition, similar to the Griffith fracture condition,

$$G = -\frac{\delta W_P}{\delta a} \overset{!}{=} \gamma_{\mathrm{HB}}. \tag{3}$$

The applied force that satisfies this condition, F_{MAX}, is the ultimate fracture strength of any large assembly of uniformly loaded H-bonds in a beta-sheet, and is independent of the cluster size N. A closed form expression for G can be developed by equating the energy dissipated by H-bond rupture to energy released by the loading and relaxation cycle of a WLC model description, as described in [17,18] (it is also noted that the results are very similar if modified WLC models are used, such as the discrete WLC model as discussed in [17]). Note that this fracture strength F_{MAX} depends only on the parameters E_{HB} and the persistence length, ξ_P, thus $F_{\mathrm{MAX}}(\xi_P, E_{\mathrm{HB}})$.

Based on this framework, the complete elastic-rupture force-extension behavior of a H-bond assembly with N H-bonds can be predicted, considering the three deformation regimes introduced above,

$$F(\alpha, N, \gamma_{\mathrm{HB}}, \xi_P) = \begin{cases} F_{\mathrm{WLC}}(\alpha), & \alpha < \alpha_{fr}, \\ F_{\mathrm{BELL}}(N), & \alpha \geqslant \alpha_{fr}, \quad N < N_{\mathrm{cr}}, \\ F_{\mathrm{MAX}}(\gamma_{\mathrm{HB}}, \xi_P), & \alpha \geqslant \alpha_{fr}, \quad N \geqslant N_{\mathrm{cr}}, \end{cases} \tag{4}$$

where α_{fr} is the stretch level that satisfies the rupture condition

$$F_{\mathrm{WLC}}(\alpha_{fr}) = \begin{cases} F_{\mathrm{BELL}} & \text{for } N < N_{\mathrm{cr}}, \\ F_{\mathrm{MAX}} & \text{for } N \geqslant N_{\mathrm{cr}}. \end{cases} \tag{5}$$

The most important aspect of this model is the realization that the WLC model for elasticity [see Eq. (1)] breaks down when the governing size-dependent fracture condition given in Eqs. (2) and (3) for the maximum rupture force is reached. The model given in Eqs. (4) and (5) provides a constitutive relationship for beta-strand protein domains loaded in shear, describing the elastic behavior as a function of the geometry of the protein domain (given by N) and the basic constituent physical properties (H-bond energy and persistence length). The model may also be applicable to other protein structures, in particular those that employ large clusters of H-bonds loaded in parallel.

In summary, our model predicts that there exist three key deformation regimes for a beta strand stabilized by H-bonds (for the geometry of beta-sheet protein domains in our model, see Fig. 2). At low forces, there is always an initial entropic regime during which H-bond rupture does not take place and the polypeptide chain is stretched. The WLC model provides a suitable description of the behavior in this low force regime. The two other two possible deformation regimes pertain to the particular size of the H-bond assembly, since the H-bond rupture mechanisms change as a function of the size of the assembly. These regimes must be distinguished as follows: The rupture force of a small cluster of H-bonds (below a critical number of H-bonds N_{cr}, thus $N < N_{\mathrm{cr}}$) can be described by the Bell formulation [12,17]. For larger H-bond assemblies ($N \geqslant N_{\mathrm{cr}}$), the Bell description (which intrinsically assumes homogeneous rupture of all H-bonds in the system) ceases to hold. This is because in this case the rupture of H-bonds physically behaves like the propagation of a crack and is controlled by the free energy release rate condition [19] rather than the Bell's statistical strength model, given by Eq. (1).

According to Eq. (3), free energy change per unit polypeptide length due to the relaxation of the strained polypeptide chain after detachment of the confining H-bonds, G, must equal the energy released by the rupture of H-bonds per unit length, $\gamma_{\mathrm{HB}} \sim E_{\mathrm{HB}}$. The applied force that satisfies this condition, F_{MAX}, is the ultimate maximum fracture strength of any large H-bond assembly loaded uniformly and is independent of the H-bond assembly size. The fracture force F_{MAX} depends only on the parameters E_{HB} and persistence length ξ_P. Based on this model, the complete elastic-rupture force-extension behavior of a H-bond assembly with N H-bonds can be predicted, considering the interplay of the three deformation regimes. The key aspect of this model is the realization that the WLC model for elasticity breaks down when the governing size-dependent fracture condition for force [given in Eq. (3)] is reached. Predictions of the model are illustrated in Fig. 3. Figure 3(a) depicts the three force regimes described by Eq. (4) as well as extension-relaxation curve predictions for various sizes of H-bond clusters. Figure 3(b) shows the rupture strength as a function of the cluster size.

IV. RESULTS

The model discussed in Sec. III can be used to explain the physics of bond rupture events observed in atomic force mi-

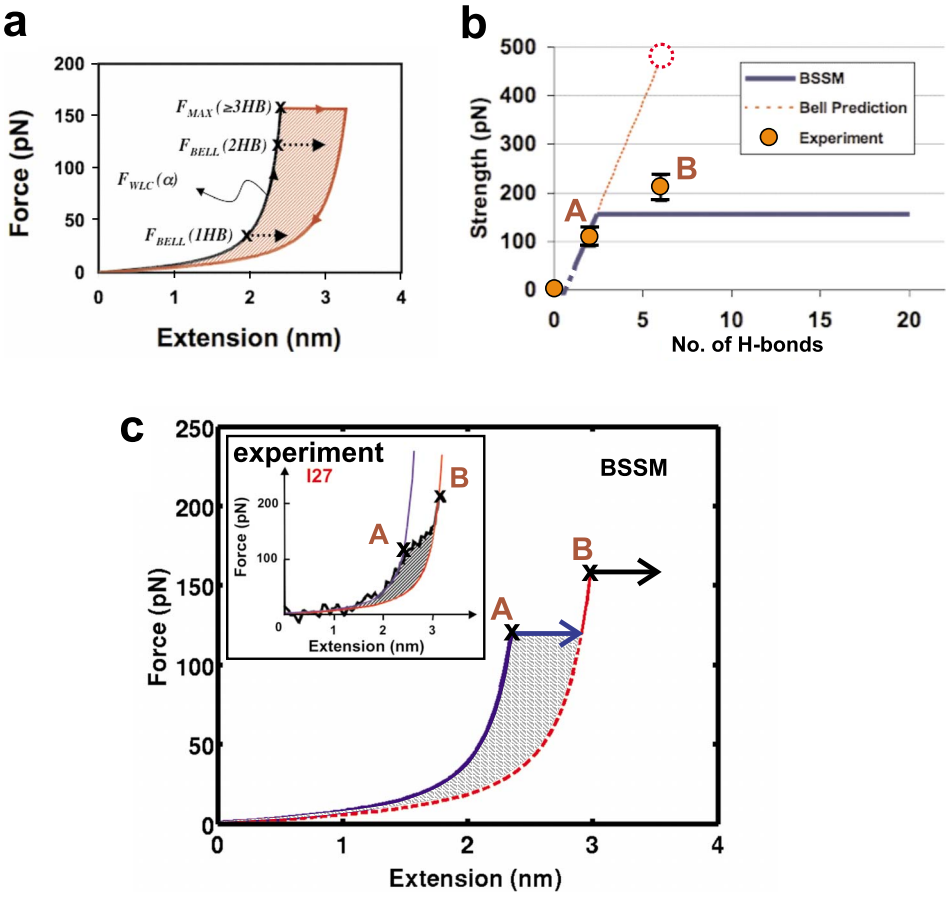

FIG. 3. (Color online) BSSM model predictions and direct comparison with experimental results of rupture mechanics of the titin I27 domain. Subplot (a) shows the elastic curve and peak force for different sizes of H-bond ("HB") clusters, with rupture marked by an "×." Extension and relaxation processes are described by the WLC equation [Eq. (1)], and the fracture force is predicted by Bell theory [Eq. 2] or for larger clusters by the Griffith energy balance condition [Eq. (3)]. At rupture, the force remains constant and extension is due to the increased contour length released by the rupture of H-bonds. The three force regimes predicted by Eqs. (4) and (5) are illustrated. Subplot (b) shows the rupture strength of a beta sheet (point of deviation from the WLC model), as a function of the number of interstrand H-bonds, N. While the Bell model (homogeneous shear assumption) predicts a continuous increase in force with increasing cluster size, BSSM predicts saturation after three hydrogen bonds in agreement with experimental data from I27 [geometry shown in Fig. 1(b)]. With a minimal number of constant parameters (persistence length of the polypeptide ξ_P and H-bond dissociation energy E_{HB}) and structural information (H-bond cluster size N), BSSM is able to predict the strength of different domains of a beta protein during an unfolding experiment. The dotted circle predicts the strength according to the Bell model; apparently it is much too large compared with the experimental observation. Subplot (c) illustrates the force-extension behavior of I27 under external loading, experimental data [11] (inset) and direct comparison with elastic behavior predicted by BSSM theory (blue and red curves). We note that the length scales in the experimental results [shown in the inset of (c)] have been normalized by the number of tandem repeats stretched experimentally, to obtain the extension length scales corresponding to rupture of a single domain. The shaded area corresponds to the energy dissipated by the rupture of two H-bonds. Note the indication of points A and B in this plot and in subplot (b), for the geometry shown in Fig. 1(b).

croscopy (AFM) or optical tweezers experiments. Here we focus on interpreting and explaining the characteristic rupture behavior observed in the classical AFM study by Fernandez *et al.* on the titin I27 domain [11], with a single set of parameters. This study considered a protein domain with two differently sized clusters of H-bonds (cluster I with two H-bonds and cluster II with six H-bonds, lying below and above the critical number of H-bonds N_{cr}).

Through a careful investigation of experimental data, Fernandez and his colleagues identified multiple force peaks and deviations from the WLC fit [11] to the force extension profile of the studies. The rupture of a single domain of the titin molecule occurs in a two-step process, involving first a hump barrier at which the first cluster of H-bonds break, and second a maximum rupture force at which the entire protein domain ruptures, followed by a rapid decay of the force. When the first cluster of H-bonds is removed by a proline mutation that breaks the cluster (that is, cluster I has zero H-bonds), the characteristic force peak and deviation of this cluster is not observed. The geometry of I27 domain and the behavior found in experiments on I27 and I32 domains are reviewed in Fig. 1. Since the structure of the I27 domain is well known and its mechanical response has been characterized in detail by several AFM studies and molecular dynamics simulation (MD) [11,21,22], it is a suitable benchmarking problem for validation of the BSSM.

We apply the BSSM to the structure shown in Fig. 1(b). We use the H-bond dissociation energy $E_{HB}=5.05$ kcal/mol and persistence length $\xi_p=0.4$ nm, according to the experimental values reported for I27 in Ref. [11]. Once these two parameters are fixed, we can predict the complete force-extension curve for this structure without fitting any additional parameters. A direct comparison of the predicted elasticity curve with the AFM experiment is shown in Fig. 3(c). We note that the length scales in the experimental results [shown in the inset of Fig. 3(c)] have been normalized by the number of tandem repeats stretched experimentally, to obtain the extension length scales corresponding to rupture of a single domain. This normalization is similar to that done in Fig. 2 of the original experimental paper [11], and explains the difference in extension length scales shown here versus the results shown above in Fig. 1(a). The normalization is necessary because in experimental studies tandem domains of I27 are covalently linked to build a concatamer, and the extensions considered in our analysis correspond to partial unfolding of each domain. The initial loading of the entire protein domain follows a WLC behavior until cluster I ($N=2$) breaks. Rupture occurs at 120 pN according to BSSM. This strength prediction is based on the Bell model, since for this protein domain $N_{cr}=2.41>N=2$. This rupture force is in close agreement with the experimental value of 108 pN. After rupture of cluster I, the contour length of the protein increases by the amount of 6.6 Å (corresponding to the free chain length exposed due to the rupture of two H-bonds). More load can be sustained by the strongest cluster in the protein consisting of six H-bonds, hence the force continues to increase on the shifted WLC curve (due to the increased contour length) until the rupture of cluster II, which leads to complete unraveling.

The key question we address now is, how much force can cluster II with six H-bonds resist at the point of rupture and how does this compare with cluster I with two H-bonds? The discussion of this issue illustrates the controversy associated with the current understanding. If one takes the same Bell formulation as we did for two H-bonds (for which a good agreement was observed between theory and experiment), rupture should occur at 471 pN [see Fig. 3(b)]. However, the experimentally observed value is between 190–220 pN [see Figs. 3(b) and 3(c)], significantly lower than this prediction, leading to a controversy in the interpretation of this phenomenon. According to the current understanding, this inconsistency can only be addressed by empirically selecting a second set of model parameters that describe the breaking of cluster II.

We find that this issue can be resolved by the realization that the rupture process for cluster II is governed by a different mechanism and must be described by the free energy release criterion [Eq. (3)], and not the Bell model [Eq. (2)]. The BSSM predicts that the maximum number of bonds that can break simultaneously for this protein domain is $N_{cr}=2.41$, suggesting that the rupture force should saturate beyond three H-bonds to a value of approximately 156 pN. This finding immediately explains the lower than expected rupture strength of cluster II and why the Bell model is not capable of predicting the strength of cluster II.

The theoretical framework developed here applies for near-equilibrium pulling rates where the constant force assumption of the energy balance criterion is valid. Although AFM experiments are carried out at relatively slow deformation rates, nonequilibrium processes may still be significant. We observe that the experimental force peak of 190–220 pN for the second cluster turns out to be higher than our prediction of 156 pN. It has been established that peak forces in experiments are highly rate dependent [13–15,23]. The instantaneous loading rates in the experiments after the rupture of the first cluster may be significantly higher than equilibrium conditions, and may provide an explanation for this discrepancy. Furthermore, the flat curve predicted by BSSM as a result of the assumption that the force remains constant during H-bond rupture has also not been observed by this experiment. Rather, the force seems to ramp up slightly at this point, thereby yielding a higher value for the second peak and also indicating that indeed the equilibrium condition has not been reached. Future AFM experiments at slower pulling rates and higher resolution may provide better validation for our theoretical predictions. Despite this slight disagreement, our simple model describes the overall force-extension behavior well.

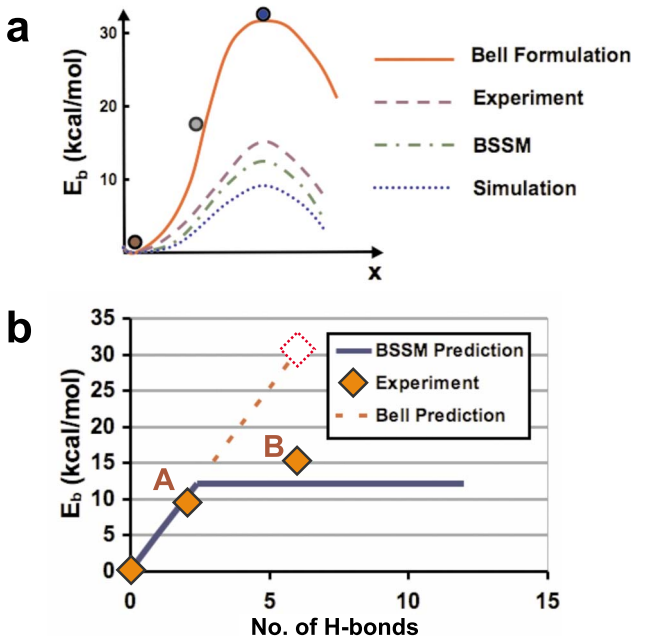

FIG. 4. (Color online) Comparison of the height of the overall energy barrier as a function of the number of H-bonds in a beta strand, N. Calculation of the height of the effective energy barrier for six H-bonds loaded in shear, based on experimental data, Bell theory, BSSM, and MD simulation results are shown in subplot (a). The results show close agreement between our model, experimental and computational predictions. As predicted by BSSM, the maximum height of the energy barrier that can be achieved by a cluster of H-bonds is limited, and therefore the Bell prediction fails for large clusters. Subplot (b) illustrates that Bell model overshoots experimentally observed energy barriers significantly (point shown as dotted square), for cases beyond the critical cluster size N_{cr}. The experimental value observed suggests an asymptotical behavior with constant energy barrier for $N>N_{cr}$. The BSSM prediction agrees well with this observation (points A and B are indicated to relate to the results shown in Fig. 3).

Figure 4 summarizes the energy barrier predictions for the

rupture of cluster II based on BSSM predictions, experimental values, as well as simulation results for a model three strand beta-sheet system [18]. We note that according to our model, the energy dissipated by cluster rupture (that is, through overcoming the energy barrier) depends on the force level and the change in contour length of the system rather than individually on initial and final states, in agreement with experimental studies [3,20].

The comparison described here confirms that BSSM is capable of explaining key events in rupture of proteins. The only input parameters are the H-bond dissociation energy and persistence length, which show limited variability for proteins [3,24–26].

V. DISCUSSION AND CONCLUSION

We summarize the major findings reported in this paper. The closed form expressions of the model [Eqs. (4) and (5)] explain the particular force peaks in protein unfolding experiments, based on either a Bell model prediction or a thermodynamics based free energy release condition [Eq. (3)], depending on the size of the H-bond cluster. This theoretical framework has been quantified and validated here for the AFM experiments on I27 [11] (Fig. 3), but should be generally applicable to other protein domains, in particular other beta structures that exhibit exceptional strength and elasticity in extracellular matrix, silks, and amyloids [3,23,27–31]. A specific comparison for other protein structures will be addressed in future work.

The Bell model or similar formulations can be used to predict strength of subcritical H-bond cluster sizes, as it can also be used to link energy barriers to peak force values (Fig. 4). However, using the Bell model to calculate force peaks of large cluster of H-bonds requires a homogeneous rupture assumption, which is physically impossible as stated by our model in Eq. (3), and would indeed lead to excessively high force peaks that approach the strength of covalent bonds, a phenomenon clearly not observed in experiment. We resolve this controversy by using the energy balance concept from fracture mechanics and applying it to the free energy competition between H-bonds and entropic elasticity of the protein backbone.

The WLC model alone cannot be used to describe the entire deformation range of protein domains. A thermodynamics viewpoint shows that strength and elasticity are coupled and cannot be considered independently as previously believed. The key contribution is that for most protein structures that employ critical and greater than critical size H-bond clusters, rupture is ultimately governed by the energy release rate G. A similar fracture mechanics concept has been used for nearly a century to explain materials failure of crystals, but has not yet been included in strength models for protein domains, explaining the disagreement of existing models with experimental observations.

ACKNOWLEDGMENTS

S. K. acknowledges support from Massachusetts Institute of Technology. This research was supported by the Army Research Office, by a National Science Foundation grant, by the Air Force Office of Scientific Research, as well as by the Office of Naval Research and DARPA. The authors acknowledge a supercomputing grant at the San Diego Supercomputing Center.

[1] B. L. Smith *et al.*, Nature (London) **399**, 761 (1999).
[2] T. P. Knowles, A. W. Fitzpatrick, S. Meehan, H. R. Mott, M. Vendruscolo, C. M. Dobson, and M. E. Welland, Science **318**, 1900 (2007).
[3] M. Rief, M. Gautel, A. Schemmel, and H. E. Gaub, Biophys. J. **75**, 3008 (1998).
[4] B. Isralewitz, M. Gao, and K. Schulten, Curr. Opin. Struct. Biol. **11**, 224 (2001).
[5] P. Wiggins, T. Van der Heijden, F. Moreno-Herrero, A. Spakowitz, R. Phillips, J. Widom, C. Dekker, and P. Nelson, Nat. Nanotechnol. **1**, 137 (2006).
[6] J. F. Marko and E. D. Siggia, Macromolecules **28**, 8759 (1995).
[7] A. Deniz, S. Mukhopadhyay, and E. Lemke, J. R. Soc., Interface **5**, 15 (2008).
[8] M. Buehler and S. Wong, Biophys. J. **93**, 37 (2007).
[9] C. Bustamante, S. B. Smith, J. Liphardt, and D. Smith, Curr. Opin. Struct. Biol. **10**, 279 (2000).
[10] D. J. Brockwell, E. Paci, R. C. Zinober, G. S. Beddard, P. D. Olmsted, D. A. Smith, R. N. Perham, and S. E. Radford, Nat. Struct. Biol. **10**, 731 (2003).
[11] P. E. Marszalek, H. Lu, H. B. Li, M. Carrion-Vazquez, A. F. Oberhauser, K. Schulten, and J. M. Fernandez, Nature (London) **402**, 100 (1999).
[12] G. I. Bell, Science **200**, 618 (1978).
[13] T. Ackbarow, X. Chen, S. Keten, and M. J. Buehler, Proc. Natl. Acad. Sci. U.S.A. **104**, 16410 (2007).
[14] E. Evans and K. Ritchie, Biophys. J. **72**, 1541 (1997).
[15] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, and E. Evans, Nature (London) **397**, 50 (1999).
[16] M. Rief, J. M. Fernandez, and H. E. Gaub, Phys. Rev. Lett. **81**, 4764 (1998).
[17] S. Keten and M. J. Buehler, Nano Lett. **8**, 743 (2008).
[18] S. Keten and M. J. Buehler, Phys. Rev. Lett. **100**, 198301 (2008).
[19] A. A. Griffith, Philos. Trans. R. Soc. London, Ser. A **221**, 163 (1921).
[20] A. F. Oberhauser, P. E. Marszalek, H. P. Erickson, and J. M. Fernandez, Nature (London) **393**, 181 (1998).
[21] M. Rief, M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub, Science **276**, 1109 (1997).
[22] H. Lu and K. Schulten, Biophys. J. **79**, 51 (2000).
[23] M. Sotomayor and K. Schulten, Science **316**, 1144 (2007).
[24] E. Oroudjev, J. Soares, S. Arcdiacono, J. B. Thompson, S. A. Fossey, and H. G. Hansma, Proc. Natl. Acad. Sci. U.S.A. **99**, 6460 (2002).

[25] T. E. Fisher, A. F. Oberhauser, M. Carrion-Vazquez, P. E. Marszalek, and J. M. Fernandez, Trends Biochem. Sci. **24**, 379 (1999).
[26] H. Lu, B. Isralewitz, A. Krammer, V. Vogel, and K. Schulten, Biophys. J. **75**, 662 (1998).
[27] A. S. Mostaert, M. J. Higgins, T. Fukuma, F. Rindi, and S. P. Jarvis, J. Biol. Phys. **32**, 393 (2006).
[28] J. F. Smith, T. P. J. Knowles, C. M. Dobson, C. E. MacPhee, and M. E. Welland, Proc. Natl. Acad. Sci. U.S.A. **103**, 15806 (2006).
[29] C. Y. Hayashi, N. H. Shipley, and R. V. Lewis, J. Biol. Chem. **24**, 271 (1999).
[30] E. H. Lee, M. Gao, N. Pinotsis, M. Wilmanns, and K. Schulten, Structure (London), **14**, 497 (2006).
[31] M. S. Z. Kellermayer, L. Grama, A. Karsai, A. Nagy, A. Kahn, Z. L. Datki, and B. Penke, J. Biol. Chem. **280**, 8464 (2005).