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A multi-timescale strength model of alpha-helical protein domains

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Abstract

Here we report a constitutive model that characterizes the strength of an alpha-helical protein domain subjected to tensile deformation, covering more than ten orders of magnitude in timescales. The model elucidates multiple physical mechanisms of failure in dependence on the deformation rate, quantitatively linking atomistic simulation results with experimental strength measurements of alpha-helical protein domains. The model provides a description of the strength of alpha-helices based on fundamental physical parameters such as the H-bond energy and the polypeptide's persistence length, showing that strength is controlled by energetic, nonequilibrium processes at high rates and by thermodynamical, equilibrium processes at low rates. Our model provides a novel perspective on the strength of protein domains at ultra-slow pulling speeds relevant under physiologic and experimental conditions.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Alpha-helical protein domains, together with beta-sheets and tropocollagen molecules, represent one of the most abundant protein structures found in biology. In addition to being part of larger protein structures (such as in enzymes and other globular proteins), alpha-helical protein domains also play an important mechanical and structural role in biology. For example, alpha-helix networks in intermediate filaments have been shown to provide mechanical integrity to cells, and also to support biological processes that involve mechanical signaling such as mechanotransduction or mechanosensation to regulate gene activation [1–4]. Therefore, in order to advance our understanding of such biological processes, a quantitative understanding of the mechanical behavior of alpha-helices is crucial. In addition to medical and biological applications, a detailed understanding of alpha-helices and alpha-helix based protein networks and their resulting mechanical properties could possibly lead to the creation of *de novo* synthetic alpha-helix based materials [5, 6].

The mechanical properties of alpha-helices must be understood through disparate timescales, reaching from picoseconds (e.g. during injuries, trauma, mechanical shock) to seconds and more (e.g. during regular physiological cellular processes) [3, 4, 7, 8]. However, currently there exists no model that describes the mechanical strength behavior of alpha-helical protein domains that considers associated physical mechanisms through this exhaustive range of timescales. Experiments have been carried out at relatively slow pulling rates (of the order of nanometers per second), and computer simulations (e.g. molecular dynamics simulations) have been carried out at much faster deformation rates (of the order of meters per second). The results of such experimental and computational studies have not yet been integrated. Understanding the behavior of proteins over multiple timescales and deriving the behavior at one timescale from the known behavior at another timescale is crucial to enable future biological research and to develop engineering design methods to create *de novo* biological protein materials. Currently no model has been reported that can predict experimentally accessible pulling speeds based on the analysis of molecular dynamics (MD) simulation results.

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Moreover, MD simulation studies typically cannot be directly extrapolated towards lower pulling speeds, since they predict unphysical phenomena such as negative strength values.

Here we resolve this issue by providing a self-consistent approach that allows us to predict the strength of alpha-helices over more than ten orders of magnitude in timescales, quantitatively linking atomistic simulation results with experimental results, based on fundamental physical parameters that include the energy and geometry of H-bonds (HBs) and the persistence length of the protein's backbone. The model captures the behavior of alpha-helices from 'slow' natural biological processes up to mechanical shock as it appears in accidents and injuries.

2. Computational approach

To demonstrate the link between computer simulation and experiment, we utilize a set of MD simulations that were reported in earlier simulation studies [9]. For convenience, here we review details of the computational atomistic modeling approach. For all atomistic simulations, we use a classical MD approach, implemented in the MD program NAMD [10] using the CHARMM22 force field [11]. All simulations are performed at a temperature of 300 K (NVT ensemble, temperature control using a Berendsen thermostat), with a time step of 1 fs. Careful energy minimization and finite temperature equilibration of all structures are simulated before the protein domain is mechanically loaded. The protein structure obtained from the Protein Data Bank (PDB identifier 1gk6, part of human vimentin intermediate filament) is solved completely in a TIP3 water skin. In all cases studied here, the entire protein is embedded in water, before and during deformation of the protein. This is essential to capture the correct HB rupture dynamics.

To apply forces to the molecule in order to induce deformation, we use steered molecular dynamics (SMD) [12], with SMD spring constant $k_{\mathrm{SMD}} = 10$ kcal mol^{-1} Å^{-2}. We obtain force versus displacement data by monitoring the time-averaged applied force (F) and the position of the atom that is pulled (x) over the simulation time.

To apply load, C_α atoms at one end are fixed and the force is applied on the C_α atom at the other end in the AH structure, with a pulling speed v. The tensile boundary conditions chosen for the AH domain are closest to the physiological conditions. Several other boundary conditions have been used (changing fixed and pulled atoms, pulling at different convolutions). No changes in the rupture forces have been observed, suggesting that the results reported here are robust with respect to changes in the boundary conditions.

3. Theoretical modeling and results

A cartoon of the AH protein and a schematic diagram of the tensile load boundary conditions used to study the rupture mechanism are shown in figure 1(A). As reported in previous work, MD simulations of AHs in explicit solvent were performed over four orders of magnitude of pulling speeds (from 0.05 to 100 m s^{-1} [9]). The rupture force of the AH

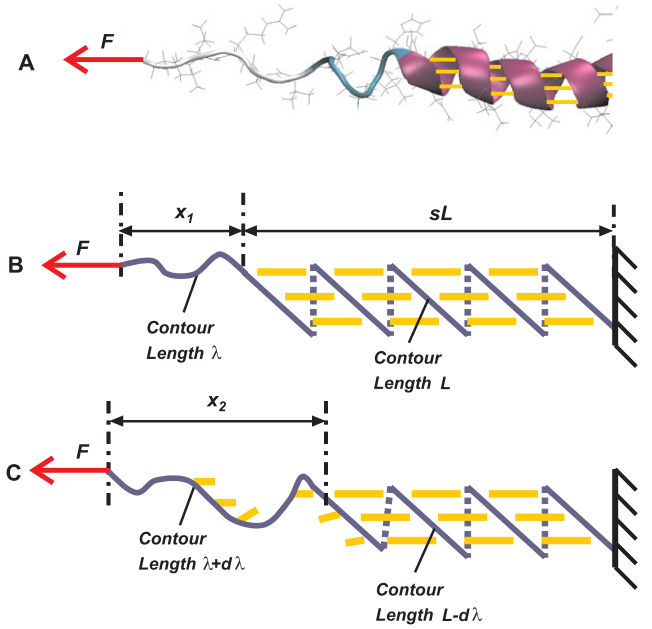

Figure 1. (A) The atomistic-scale protein structure of a single alpha-helix (AH) from a vimentin coiled-coil dimer. The helical backbone is stabilized by parallel arrangements of hydrogen bonds (HBs, yellow dashed lines). (B), (C) A schematic model system of an AH strained by an external force before and after onset of rupture, showing the process of releasing a segment of backbone polypeptide due to the rupture of HBs, thereby increasing the contour length of the free end entropic chain by dλ.

structure, identified at the point of breaking of the first HBs, is plotted as a function of the protein domain's lifetime τ in figure 2(A). When the system is not in equilibrium, as is the case for high deformation rates, the relation between τ and the applied force f can be described by a simple Bell model [13]:

$$\tau = \omega_0^{-1} \exp\left(\frac{E_\mathrm{b} - f x_\mathrm{b} \cos(\theta)}{k_\mathrm{B} T}\right), \qquad (1)$$

where E_b is the energy barrier of HB breaking, and x_b is the distance between the equilibrium state and the transition (=rupture) state of the protein domain (note that $v = \Delta x/\Delta t = x_\mathrm{b}/\tau$, where v is the externally applied pulling speed). Further, the parameter $\theta \approx 16°$ describes the angle between the applied force f and the orientation of the HBs, k_B is the Boltzmann constant, T is the absolute temperature, and $\omega_0 = 10^{13}$ s^{-1} is the natural frequency of bond vibration. It is noted that, in addition to the phenomenological model used here, other stochastic models exist that link timescales and pulling speeds to bond breaking forces; for a description of other models we refer to the literature [14–22]. The force as a function of timescale τ and the energy landscape parameters (ELPs, E_b and x_b) is given by

$$f(\tau, E_\mathrm{b}, x_\mathrm{b}, \theta) = (x_\mathrm{b} \cos(\theta))^{-1} [E_\mathrm{b} - k_\mathrm{B} T \ln(\omega_0 \tau)]. \qquad (2)$$

(For detailed explanations of these equations see [9].) For a given pair of ELPs, equation (2) leads to a straight line in the f–ln(τ) space. Direct MD simulation studies in explicit water confirm this predicted behavior, however, we observe

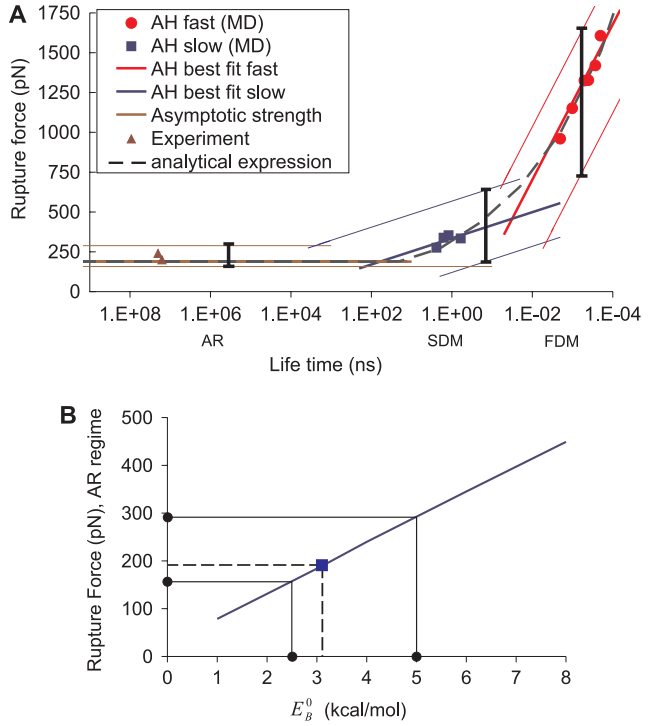

Figure 2. (A) Rupture force versus lifetime of the AH system at the onset of failure (=strength properties), including all three regimes over more than ten orders of magnitude of timescales. MD simulation results (as reported in [9]) suggest a change in mechanism from the fast deformation mode (FDM) to the slow deformation mode (SDM) on increasing the timescales. At approximately 350 pN the effective energy barriers under the applied force in the Bell model are comparable, and therefore mark the transition between FDM and SDM mechanisms. At longer timescales there is another change in deformation mechanism from the SDM to the asymptotic regime (AR), predicted here at a timescale of approximately 100 ns when $f_{\mathrm{AR}} > f_{\mathrm{SDM}}$. Experimental results confirm this prediction. Thin lines show the strength behavior for a broad range of HB energy values from 2.5 to 5 kcal mol^{-1} (marking error bars for uncertainties in the H-bond energy). The dashed line represents a closed analytical expression from the power law fit over all three regimes, provided in equation (11). (B) Dependence of the critical rupture force on E_{B}^0, in the AR. The strength of the system near equilibrium conditions (AR) depends linearly on E_{B}^0 (this parameter determines the energy release rate γ_s). The specific value of E_{B}^0, usually found in a range between 1 and 8 kcal mol^{-1}, varies between different solvent conditions and the specific sequence of the protein domain.

two distinct regimes, each of which follows the predicted linear logarithmic dependence of the unfolding force with respect to the life time of the structure (please see section 2 for details about the MD simulation setup). The analysis of the atomistic mechanisms of rupture together with the analysis based on Bell's model shows that the two slopes shown in figure 2(A) correspond to two distinct unfolding mechanisms with two different energy barriers (see table 1) [9]. In the fast deformation mode (FDM), the observed deformation mechanism and the calculated $E_{\mathrm{B}}^{\mathrm{FDM}}$ indicate that single HBs break sequentially, whereas in the slow deformation mode (SDM) three to four HBs break simultaneously (3.6 HBs form one alpha-helical convolution, which unfolds as a whole in this mode). The sequential breaking of HBs at high pulling speeds (short timescales, FDM) is due to the fact that HB breaking in the protein remains localized. This is because pulling occurs faster than the ability of the protein to mediate HB breaking induced 'plastic' deformation. In the SDM regime, however, pulling is slow enough that entire convolutions rupture under the applied force, leading to effectively higher energy barriers for unfolding [9].

At increasing timescales in the SDM the Bell model prediction leads to negative forces, an unphysical prediction. Furthermore, experimental values [23, 24] clearly do not lie on an extension of the slope predicted from the SDM regime, and rather suggest that the f–$\ln(v)$ curve approaches an asymptotic zero slope (see figure 2(A)). Could the Bell model be used to explain this behavior at vanishing pulling rates? Adopting the Bell model to describe this behavior would lead to an increase of x_{B} (since x_{B} controls the slope of the f–$\ln(\tau)$ curve), approaching infinity for slopes approaching zero. It is noted that in other models (e.g. the microscopic theory [19, 25]) a similar approach has been taken, where the value of x_{b} is defined as a function of pulling speed (equivalently, the timescale), leading to a continuous change of the slope of the f–$\ln(v)$ curve.

The approach of x_{b} to extremely large values is, however, unphysical, since the transition point x_{b} can not be larger than the finite contour length of the protein domain. This suggests that another mechanism must determine the protein rupture force. The key to understand this change in mechanism is the realization that at sufficiently long timescales the deformation of the system goes through equilibrium and is no longer controlled by a statistically activated process as described in the Bell model or equations (1)–(2). Thus the strength does not depend on the timescale of loading beyond a critical τ_{crit}, and is independent of pulling rate for very long timescales.

At long timescales $\tau > \tau_{\mathrm{crit}}$ entropic effects that stem from conformational changes of the polypeptide chains are activated and the strength is characterized by a free energy release rate condition. A similar approach has recently been reported for beta-sheets in [26]. Here we develop a model specific to AH protein domains. The aim here is to find the critical force that will initiate rupture of HBs in an alpha-helix at quasi-equilibrium deformation rates. Similar to the Griffith condition used to predict the onset of fracture in crystals [27], the free energy released by freeing polypeptide chains from their geometric confinement in helical convolutions must equal the energy required to break these HBs. The free energy balance condition at the onset of fracture requires that $G = -(A_2 - A_1 - F\delta)/\mathrm{d}\lambda = \gamma_s$, where γ_s denotes energy released by rupture of HBs per unit crack advance, $F\delta$ is the work done by the external force on the system, and A_1 and A_2 are the initial and final free energies of the protein backbone as determined from the worm-like chain elasticity theory. The free energy of the system before and after rupture is given as

$$A_1 = \lambda A_{\mathrm{WLC}} - \gamma_s L + L A_{\mathrm{FOLD}} \tag{3}$$

and

$$A_2 = (\lambda + \mathrm{d}\lambda) A_{\mathrm{WLC}} - \gamma_s (L - \mathrm{d}\lambda) + (L - \mathrm{d}\lambda) A_{\mathrm{FOLD}}, \tag{4}$$

Table 1. Comparison and summary of the three deformation regimes (FDM = fast deformation mode, SDM = slow deformation mode, AR = asymptotic regime) with their characteristic physical parameters and numerical values.

Mechanism, associated pulling speeds (in m s^{-1})	Timescale (in ns)	Force levels (in pN)	Physical parameters	Controlling physical mechanism and explanation
AR $v < 0.001$	$\tau > 100$	$F < 200$	$\gamma_s =$ 0.91 kcal mol^{-1} Å^{-1} $\xi_P = 4$ Å	Thermodynamical free energy release rate through equilibrium (asymptotic strength model)
SDM $0.001 < v < 0.4$	$0.05 < \tau < 100$	$200 < F < 350$	$E_b^{SDM} = 11.1$ kcal mol^{-1} $x_b^{SDM} = 1.2$ Å	Simultaneous rupture of HBs in one convolution, activated statistical process (Bell)
FDM $v > 0.4$	$\tau < 0.05$	$F > 350$	$E_b^{FDM} = 4.87$ kcal mol^{-1} $x_b^{FDM} = 0.2$ Å	Sequential rupture of HBs, activated statistical process (Bell)

where $A_{WLC} = \int_0^{\alpha} F_{WLC}(\alpha)\,d\alpha$ is the free energy state (energy per length) of the already unfolded free segments of the protein and $A_{FOLD} = \int_0^{s} F_{WLC}(\alpha)\,d\alpha$ is the free energy state of the folded segment of the chain. Hereby α is equal to the ratio of the end-to-end length of the free chain to its contour length $\alpha = x/\lambda$, equivalent to mechanical stretch, and the parameter s denotes the ratio of the end-to-end length of the alpha-helix to its contour length, L (the physical meaning of this parameter is that it describes how much contour length is stored per unit length of alpha-helix). We refer the reader to figure 1 for an illustration of the definition of variables. Equation (4) illustrates the interplay between entropic energy release in the stretched and relaxed segments of the chain with energetics of HB rupture, thereby coupling two key physical aspects of the protein unfolding problem. The energy contribution from the external force is given as

$$\delta W_F = -F(\alpha - s)\mathrm{d}\lambda. \tag{5}$$

Hence the critical condition for HB rupture can be given as

$$A_{WLC}(\alpha_{cr}) + F(s - \alpha_{cr}) + \gamma_s - A_{FOLD}(s) = 0, \tag{6}$$

where α_{cr} is the critical stretch level that initiates rupture. The strength regime described by equation (6) is referred to as the asymptotic regime (AR), and the force prediction is then found by the WLC model, through $f_{AR} = F_{WLC}(\alpha_{cr})$, leading to

$$f_{AR} = \frac{k_B T}{4\xi_P}\left[(1-\alpha_{cr})^{-2} + 4\alpha_{cr} - 1\right]. \tag{7}$$

With the core theory established, we can now substitute quantitative values for the parameters. The parameter γ_s describes the HB energy stored per unit length of AH and can be obtained from

$$\gamma_s = \frac{E_b^0}{L_0}, \tag{8}$$

where E_b^0 is the dissociation energy of a single bond and $L_0 = 0.33$ nm is the distance between adjacent HBs along the length of the helix. The parameter $s = 0.45$ can be estimated from atomistic simulations of the deformation mechanics of alpha-helices, where the unfolded length of the molecule can easily be calculated to find the ratio with initial end-to-end distance. These values are also in excellent agreement with the well-established alpha-helix pitch of 5.4 Å per convolution [28, 29]. We further note that the fracture model is independent of the size of the macromolecule and the helical domain. This is because the initial unfolded contour length does not influence the strength prediction.

Combining all three mechanisms (FDM, SDM, AR), the strength of an AH domain is

$$F(\tau; x_b^{FDM}, E_b^{FDM}, x_b^{SDM}, E_b^{SDM}, \theta, \xi_P, \gamma_s) = \max\left\{\begin{array}{c} f_{FDM}(\tau; x_b^{FDM}, E_b^{FDM}, \theta) \\ f_{SDM}(\tau; x_b^{SDM}, E_b^{SDM}, \theta) \\ f_{AR}(\xi_P, \gamma_s(E_b^0)) \end{array}\right\}. \tag{9}$$

The functions f_{FDM} and f_{SDM} can be calculated from equation (2), and f_{AR} can be calculated from equations (6)–(8). We estimate E_B^0 from the MD simulation results in the SDM, where the 3.6 HBs in one convolution break simultaneously, thus $E_b^0 = E_b^{SDM}/3.6 = 3.1$ kcal mol^{-1}, and therefore $\gamma_s = 0.91$ kcal mol^{-1} Å^{-1}. This relation between E_b^0 and E_b^{SDM} shows the ability of our model to link directly between the AR and results in the SDM. A similar link can be established between the AR and FDM, where the energy barrier found in FDM typically directly corresponds to the HB energy, since rupture occurs sequentially.

In summary, once the structural parameters (e.g. the ratio s, the persistence length ξ_P, the distance of HBs L_0) and energetic parameters (e.g. the energy of individual HBs, E_b^0) are determined from MD simulations, the force level in the AR can be calculated based on the theoretical link developed here, by solving equations (6)–(8). In analogy, the inverse calculation is possible as well. Rupture forces as they would occur at very high pulling speeds (e.g. as they appear during injuries) can be calculated from data generated at slow pulling speeds, for example through experimental analysis, by applying equation (2). Being able to calculate and predict the behavior at one timescale from observations in another timescale, as achieved in this model, underlines the coupled, multi-timescale character of our theory.

The value of E_b^0 determined from the MD simulation studies is in good agreement with earlier experimental and simulation results [30], where E_b^0 was reported to be in the

range of 3–6 kcal mol^{-1}. We choose the persistence length of a polypeptide chain as suggested from both experiment and theory to be $\xi_P = 4$ Å [31]. Based solely on these two parameters, E_b^0 and ξ_P, the force in the AR is calculated to be ≈189 pN. The AR regime is reached at a critical timescale of 100 ns (or equivalently at pulling speeds $v < 0.001$ m s^{-1}), when $f_{AR} > f_{SDM}$. The strength value of f_{AR} is plotted in figure 2(B) as a function of the HB energy E_b^0.

In order to facilitate the direct application of our model as a constitutive equation in a multi-scale simulation approach (e.g. as a strength model), we have fitted the results to an empirical relation that provides a single (empirical) mathematical expression that interpolates through all timescales (and thus all modes of deformation mechanisms). The force as a function of timescale is expressed as

$$F(\tau) = \xi_1(\tau) + h(\tau)(\xi_0 - \xi_1(\tau)), \tag{10}$$

where ξ_0 describes the force level in the AR regime, given by f_{AR}. The use of a smooth Heaviside function $h(\tau) = 1/(1 + (\tau_c/\tau)^k)$ enables us to describe the transition from AR to the rate dependent regimes (FDM and SDM). In this Heaviside function τ_c characterizes the timescale of the transition, and k determines the sharpness of the transition. The Heaviside function approaches $h(\tau) = 1$ for $\tau \ll \tau_c$, and is zero for $\tau \gg \tau_c$. The strength dependence on timescale in the FDM and SDM regimes is approximated using a power law of the form $\xi_1(\tau) = b_1\tau^{b_2}$. The parameters in equation (10) are fitted to reproduce the overall behavior shown in figure 2, leading to $\tau_c = 7.4$ ns, $k = 1$, $\xi_0 = 189$ pN, $b_1 = -330$ pN and $b_2 = -0.18$. The complete expression is

$$F(\tau) = b_1\tau^{b_2} + \frac{1}{1 + (\tau_c/\tau)^k}(\xi_0 - b_1\tau^{b_2}). \tag{11}$$

The fit to the simulation and experimental results is shown in figure 2(A) as the dashed line.

The model developed above (and the numerical interpolation given in equations (10) and (11)) is validated through quantitative comparison with experimental results. Experimental results of stretching and breaking single AH domains [23, 24] (with a length of less than 100 Å) report forces between 140 and 240 pN during unfolding. Figure 2(A) summarizes the described regimes and shows a quantitative comparison between the model prediction and MD simulation results as well as experimental results. In addition to the values used in this study that were based on earlier MD results, an envelope curve for E_b^0 ranging from 2.5 to 5 kcal mol^{-1} is included to illustrate how the predictions change under variations of the energy of HBs. We note that other experimental results [31–35] (not shown in figure 2) that consider AH spectrin repeats lie slightly below the predicted force range, of the order of 50 pN, which would require extremely low values of $E_b^0 \approx 1$ kcal mol^{-1}. A possible explanation for this behavior could be the difference in the observed unfolding mechanism, which is the unfolding of the anti-parallel coiled-coil repeat instead of rupture of individual HBs of an AH domain. For instance, in one of the studies x_b was estimated to be 15 Å [31–35], which is ten times higher than the x_b for a single HB, thus suggesting an alternative rupture mechanism.

4. Summary and discussion

The most important contribution of this paper is the development of a constitutive model (equations (9) and (11)) that describes the strength properties of AH protein domains over more than 10 orders of magnitude of timescales. Up until now such a model has not been reported, and to the best of our knowledge this model is the first to quantitatively link MD simulation results [9] and experimental AH strength values [23, 24] in a simple physical model as shown in figure 2(A). An important feature of the model reported in equation (9) is that it only includes basic parameters of the protein structure, that is, the HB energy and geometry, as well as persistence length. The strength properties of the AH protein domain, a universally found biological protein structure, are controlled by different mechanisms at distinct timescales, with strong strengthening under faster rates (shorter timescales).

According to our model, the strength at very slow pulling rates is controlled by energetics of HB rupture and entropic effects of the unfolding polypeptide backbone, and not by a continuously changing energy barrier that moves along the reaction coordinate x_b as suggested in the microscopic theory. Changes in the reaction coordinate x_b are only observed at relatively fast pulling rates, where it can be directly linked to changes in the physical mechanism of rupture (that is, the change from FDM to SDM as reported from MD simulation studies). Further, we have shown here that the deformation mechanisms that appear in MD simulations are likely different from those that appear in experiments. However, even though carried out at much faster pulling speeds, MD simulations allow us to determine basic parameters such as E_b^0. These parameters can then be used to predict force levels that appear *in vivo* or in experimental studies. This has been achieved here through the introduction of the model that characterizes the strength of alpha-helical protein domains in the AR regime. Our study could motivate new experiments, in particular those that would provide a systematic variation of deformation rates to probe the transitions between the regimes described here.

Since our model is derived from fundamental principles, such as the rupture energetics of HBs and entropic effects (which appear universally in almost any protein structure), it should be applicable to other protein structures (e.g. amyloid beta-helices or tropocollagen molecules). Our model becomes specific to a particular protein structure solely through parameters that define the geometry, such as L_0, s, θ and x_b, as well as related energetic parameters such as E_b^0 and γ. Environmental conditions such as salt concentration, pH and the exposure to water (e.g. due to geometric confinement into larger protein structures that shield from the direct exposure to water) are captured by the value of E_b^0, which describes the energy necessary for breaking a single HB. Different protein structures with different geometries in different environments feature different levels of E_b^0, and as a consequence the strength values as well as the timescale at which changes in deformation mechanism appear are expected to vary. The variation of

the strength, however, remains within relatively small error margins as shown in figure 2(A)/(B) (see error bars that show the change of strength across all regimes due to changes of the HB energy from 2.5 to 5 kcal mol^{-1}, and the analysis in figure 2(B) that shows the variation of strength for a broad range of values of the HB energy).

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