The stretching of single poly-ubiquitin molecules: Static versus dynamic disorder in the non-exponential kinetics of chain unfolding

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Static disorder has recently been implicated in the non-exponential kinetics of the unfolding of single molecules of poly-ubiquitin under a constant force [Kuo, Garcia-Manyes, Li, Barel, Lu, Berne, Urbakh, Klafter, and Fernández, Proc. Natl. Acad. Sci. U.S.A. **107**, 11336 (2010)]. In the present paper, it is suggested that *dynamic* disorder may provide a plausible, alternative description of the experimental observations. This suggestion is made on the basis of a model in which the barrier to chain unfolding is assumed to be modulated by a control parameter r that evolves in a parabolic potential under the action of fractional Gaussian noise according to a generalized Langevin equation. The treatment of dynamic disorder within this model is pursued using Zwanzig's indirect approach to noise averaging [Acc. Chem. Res. **23**, 148 (1990)]. In conjunction with a self-consistent closure scheme developed by Wilemski and Fixman [J. Chem. Phys. **58**, 4009 (1973); *ibid.* **60**, 866 (1974)], this approach eventually leads to an expression for the chain unfolding probability that can be made to fit the corresponding experimental data very closely. © *2011 American Institute of Physics*. [doi:10.1063/1.3582899]

I. INTRODUCTION

Chemical reactions that are influenced by spatial or temporal randomness in their surroundings represent a class of interesting time-dependent phenomena that Zwanzig, in an early and influential paper, has called "rate processes with [static or] dynamical disorder."[1] Such processes tend to deviate from the kinetics defined by Arrhenius's theory of reaction rates, typically exhibiting non-exponentialities in the decay of survival probabilities and other statistical measures of reactivity. Important dynamical information on reaction pathways is often concealed in these deviations from Arrhenius behavior, and can sometimes be inferred from careful single-molecule studies of particle dynamics.[2]

This was nicely illustrated in recent work by Kuo *et al.*[3] who used force-clamp spectroscopy to measure the increase in length of single poly-ubiquitin molecules that had been stretched by a constant force of between 90 to 190 pN applied to one end. They found that each of the nine compact ubiquitin domains that made up the polymer unfolded in sequence and increased its length in fixed steps of 20 nm. They also found that the duration of the individual unfolding steps was not a constant, but varied between fractions of a second to several seconds, and that a frequency histogram of the unfolding times of an ensemble of many thousand such poly-ubiquitin molecules (which is a direct measure of the probability that an unfolding event occurs in a time interval t) showed clear departures from exponential decay. As an explanation of these observations, Kuo *et al.* have suggested that because of heterogeneity in the populations of their conformational sub-states, different poly-ubiquitin molecules in different experimental measurements do not follow exactly the same trajectories or surmount exactly the same activation barriers to reach their final configurations. In other words, during the course of a given single-molecule measurement, a selected poly-ubiquitin molecule is locked into a conformation for which the barrier to unfolding is in general different from the barrier for any other molecule in a different experiment. Individual chains in the ensemble of molecules therefore evolve in an environment that is in effect, *statically* disordered. When the results of different experiments are combined, the disorder is averaged out according to the distribution of static barrier heights, and the effects are then manifested as deviations from Arrhenius behavior in the final, observed dynamics, specifically in the decay of the survival probability, $S(t)$, of the folded state.

Based on Zwanzig's model of static disorder,[1] Kuo *et al.* have derived an expression for the disorder-averaged $S(t)$ [see Eq. (5) in Sec. II] by generalizing Arrhenius's reaction rate theory to include Gaussian fluctuations around a mean barrier free energy. This expression, which does not have a simple closed form, can be very well fit (numerically) to data from six sets of experiments carried out at different applied forces after the mean k_F and the variance σ^2 of the barrier height fluctuations are adjusted for best fit. An expression for $S(t)$ that does have a closed form can be derived from Eq. (5) if one considers the limit of small barrier height fluctuations (small in relation to the thermal energy k_BT); this expression is

$$S(t) = \frac{\exp(-k_F t)}{\sqrt{1 + k_F \sigma^2 t/(k_B T)^2}}. \tag{1}$$

Although this expression is non-exponential, at early times it *is* exponential to a very good approximation, so the fact that under certain conditions the static disorder model predicts an

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exponential survival probability motivates a consideration of other possible routes to non-exponential kinetics.

One such possibility is, of course, *dynamic* disorder,[1] which refers to time-dependent fluctuations in a reaction rate that originate in time-dependent fluctuations of a "control" parameter r, such as a barrier height. But if these fluctuations are fast, they would lead effectively to a single average value of the rate constant, and the resulting kinetics would then be exponential, and so no longer satisfactorily describe the poly-ubiquitin data. The opposite limit—of slow fluctuations of the control parameter—corresponds to the static disorder limit considered by Kuo *et al.*, which *can* account for these data, but as just discussed, does not seem to produce the expected analytical forms for various probability distributions in certain limits. There still remains the possibility—as yet unconsidered—that the fluctuations of r actually occur on timescales intermediate between these extremes. This limit is, in fact, closer to the conventional understanding of the term dynamic disorder, and it involves "the entire history"[1] of r along a single-molecule trajectory for some interval of time t.

Incorporating the full time dependence of r into a theory of unfolding kinetics may actually be essential to its correct description for the following reason: protein conformational fluctuations are known to span a wide range of timescales.[4] This is evidenced by the widespread occurrence of sub-diffusive dynamics in the fluctuations of inter-residue distances, which can be traced to power law correlations in the time-dependent decay of protein memory functions.[5] There is therefore unlikely to be a clear separation of timescales between chain dynamics and barrier crossing, in general, so a treatment of reaction kinetics based on a model of either very fast or very slow r fluctuations may not be entirely realistic. It seems worthwhile therefore to explore the effects that the incorporation of a time-varying r into a model of poly-ubiquitin unfolding have on quantities like the survival probability. This is what we set out to do in this paper, which is essentially an adaptation of Zwanzig's general treatment of dynamic disorder to the model introduced by Kuo *et al*.

Section II provides some mathematical background on the role of static disorder in the first order kinetics of chain unfolding. Section III discusses these kinetics from the point of view of dynamic disorder, which is manifested in the fluctuations of a control parameter that in this paper is assumed to obey a generalized Langevin equation. The analysis of dynamic disorder in our model follows Zwanzig's "indirect" approach to noise averaging.[1] This approach leads to an equation for the survival probability that closely resembles a reaction-diffusion equation. The solution to this equation is provided in Sec. IV, in approximate form, using a self-consistent closure scheme developed by Wilemski and Fixman.[6] The time-dependent decay of the solution is examined in Sec. V, in the short and long-time limits. The paper concludes with a comparison of these results with data from Kuo *et al.*'s experiments, and a discussion of the implications of these results for understanding the origins of non-exponentiality in single-molecule stretching.

II. BACKGROUND AND REVIEW: THE STATIC DISORDER MODEL

One of the quantities that Kuo *et al.* extract from their data on poly-ubiquitin unfolding times is the ensemble-averaged probability density, $S(t)$, that a given chain survives in the folded state up to a time t under the action of a constant applied force. To determine this quantity theoretically, they consider a model in which the probability density for survival in the folded state in a given experiment is assumed to depend on a random variable r that modulates the height of the barrier that is crossed during unfolding. This probability—denoted $S(r, t)$—is in turn assumed to obey the following first order rate equation,

$$\frac{dS(r,t)}{dt} = -k(r)S(r,t), \tag{2}$$

where $k(r)$ is a rate constant whose dependence on r is taken to be

$$k(r) = k_F \exp(-\beta r). \tag{3}$$

Here, k_F is the rate constant for crossing a barrier of *fixed* height, with k_F given by $k_F = A\exp(-\beta\{\Delta G_{avg} - F\Delta x_{avg}\})$, where A is the usual pre-exponential factor, $\beta = 1/k_BT$, with k_B Boltzmann's constant and T the temperature, ΔG_{avg} is the average height of the free energy barrier in the absence of the applied force F, and Δx_{avg} is the average distance between the folded and transition states along the reaction coordinate. Kuo *et al.* make the further assumption that the variable r is governed by the probability distribution,

$$f(r) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[-r^2/2\sigma^2\right], \tag{4}$$

σ^2 being the variance of r. They then calculate (by numerical integration) a disorder-averaged survival probability, $\bar{S}(t)$, from

$$\bar{S}(t) \equiv \int dr f(r) S(r,t) = \int dr f(r) \exp\left[-k_F t \exp\left(-\beta r\right)\right] \tag{5}$$

and compare this quantity with the survival probability measured experimentally.

The analytical expression shown in Eq. (1) is obtained from Eq. (5) by considering the limit $\beta^2\sigma^2 \ll 1$.

III. THE DYNAMIC DISORDER MODEL

The starting point of our own calculations is the following rate equation for the evolution of the survival probability along a single-molecule trajectory:

$$\frac{dS(r(t))}{dt} = -k(r(t))S(r(t)), \tag{6}$$

where $r(t)$ is the same random variable introduced in Sec. II that modulates the barrier free energy, except that here it is explicitly time-dependent. Its stochastic variation is assumed—and this is the defining feature of the model—to be described

by a *generalized* Langevin equation (GLE),[7]

$$\lambda r(t) = -\alpha \int_0^t dt' K(t-t')\dot{r}(t') + \xi(t), \tag{7}$$

where α and λ are essentially timescale parameters (whose precise microscopic definition is not needed as they will be combined later on with other phenomenological parameters and then adjusted for best fit), and $K(t-t')$is a memory function that is related to the noise $\xi(t)$ by $K(|t-t'|) = (1/\alpha k_B T)\langle \xi(t)\xi(t')\rangle$. When $\xi(t)$ is white noise, Eq. (7) reduces to the ordinary Langevin equation [$K(t-t')$ becoming proportional to $\delta(t-t')$], and $r(t)$ evolves according to simple Brownian motion. In our model, we assume that $\xi(t)$ is described by the process known as fractional Gaussian noise (fGn),[8] which is defined completely by the following properties: $\langle \xi(t)\rangle = 0$ and $(1/\alpha k_B T)\langle \xi(t)\xi(t')\rangle = 2H(2H-1)|t-t'|^{2H-2}$, where H, the so-called Hurst index, is a number satisfying $1/2 \le H < 1$ and is a measure of the temporal range over which thermal forces in the medium are correlated. (The limit $H = 1/2$ represents the complete lack of correlation of these forces, while higher values of H represent force correlations that are "persistent.") The motivation behind the choice of fGn to describe the trajectories of r is the fact that fGn has been shown in several previous studies to provide a highly satisfactory model of the environmental fluctuations that underlie distance fluctuations in large proteins.[9] In the present context, its use is also dictated in some sense by the physics of the problem, which is that the control parameter r must exhibit bounded (i.e., not too large on average) fluctuations in time, while simultaneously exhibiting decaying correlations (so as to mimic the correlations that one expects will be present in large molecules with strongly coupled dynamical motions). The *simplest* equation that meets these requirements and yet can be derived rigorously from first principles[7] is, we believe, Eq. (7), a GLE that describes the motions of a fictitious particle confined to a harmonic potential and acted on by random forces with power law correlations.

Equation (6) can be solved formally to give

$$S[r] = \exp[-\int_0^t dt' k(r(t'))], \tag{8}$$

with $k(r)$ defined in Eq. (3). This relation defines S as a functional of r [which is itself a functional of the noise $\xi(t)$], and before it can be compared to its experimental counterpart, it must first be averaged over the distribution of $\xi(t)$. The direct averaging of $S[r]$ over the statistics of $\xi(t)$ is, in general, highly non-trivial, but Zwanzig has shown how the averaging may be performed indirectly.[1] The idea is to construct an equation for the joint probability density of $S(t)$ and $r(t)$ in which the noise has been averaged out starting from the "equations of motion" for these two variables. Equation (6) defines such an equation for $S(t)$. A related equation for the evolution of $r(t)$ has been derived elsewhere[10] using the methods of functional calculus;[11] without going into the details of the derivation (which are given in detail in Ref. 10), we merely note that the equation is given by

$$\dot{r}(t) = -\eta(t)r(t) + \theta(t), \tag{9}$$

where $\eta(t) = -d\ln\chi(t)/dt$, $\chi(t) = E_{2-2H}[-(t/\tau)^{2-2H}]$, $\tau = [\alpha\Gamma(2H+1)/\lambda]^{1/(2-2H)}$, and

$$\theta(t) = \chi(t)\frac{d}{dt}\chi(t)^{-1}\int_0^t dt'\phi(t-t')\xi(t'). \tag{10}$$

Here, $E_a(z)$ is the Mittag-Leffler function[12] [defined as $E_a(z) = \sum_{n=0}^{\infty} z^n/\Gamma(an+1)$], $\Gamma(b)$ is the gamma function, and $\phi(t)$ is the inverse Laplace transform of the function $\hat{\phi}(s) = 1/[\lambda + s\alpha\hat{K}(s)]$, the caret denoting the Laplace transform with respect to the variable s.

The probability density that at time t, $S(t)$ has the value S and $r(t)$ the value r is given, in general, by

$$P(S,r,t) = \langle \delta(S - S(t))\delta(r - r(t))\rangle, \tag{11}$$

where the angular brackets refer to an average over all realizations of the noise. From here, a lengthy but straightforward calculation (discussed in Ref. 10) shows that

$$\frac{\partial P}{\partial t} = k(r)\frac{\partial}{\partial S}SP + \eta(t)\frac{\partial}{\partial r}rP + \frac{1}{\lambda}\eta(t)k_B T\frac{\partial^2 P}{\partial r^2}. \tag{12}$$

By multiplying this equation by S and integrating over all S from 0 to 1 (and setting the surface term $P(1,r,t>0)$ to 0), one obtains an equation for the noise averaged survival probability $\bar{S}(r,t) \equiv \int_0^1 dS S P(S,r,t)$. This equation is

$$\frac{\partial \bar{S}}{\partial t} = -k(r)\bar{S} + D\bar{S}, \tag{13a}$$

where the operator D is defined as

$$D \equiv \eta(t)\left[\frac{\partial}{\partial r}r + \frac{1}{\lambda}k_B T\frac{\partial^2}{\partial r^2}\right]. \tag{13b}$$

IV. THE SURVIVAL PROBABILITY IN THE WILEMSKI-FIXMAN APPROXIMATION

Once Eq. (13a) is solved, and then integrated over r, the expression so obtained, viz., $\langle S(t)\rangle \equiv \int dr\bar{S}(r,t)$, can be compared with the experimentally measured survival probability. Unfortunately, an exact solution of this equation probably cannot be found analytically for the given rate constant expression [Eq. (3)]. However, the mathematical structure of Eq. (13a) is identical to the equation we had used earlier to study the non-exponential escape kinetics of DNA from an α-haemolysin nanopore.[13] That equation was solved approximately using a method developed by Wilemski and Fixman,[6] the basic idea of which is to write the exact $\bar{S}(r,t)$ as the product of two terms: one, an equilibrium survival probability $\bar{S}_{eq}(r)$ that is taken to depend only on r and that is assumed to describe the state of the system at the initial time $t = 0$, and the other, a purely time-dependent function that is determined self-consistently.

To implement this program, one first notes that the formal solution to Eq. (13a) under the assumed initial condition is

$$\bar{S}(r,t) = \bar{S}_{eq}(r) - \int_{-\infty}^{\infty} dr' \int_0^t dt' G(r,t-t'|r')k(r')\bar{S}(r',t'), \tag{14}$$

where the function G is the solution of $(\partial/\partial t - D)\,G(r, t - t'|r') = \delta(r - r')\delta(t - t')$. The solution of this equation is known; it is given by

$$G(r, t|r', 0) = \sqrt{\frac{\lambda}{2\pi k_B T(1-\chi^2(t))}} \times \exp\left[-\frac{\lambda(r - r'\chi(t))^2}{2k_B T(1-\chi^2(t))}\right]. \quad (15)$$

The limit $t \to \infty$ of Eq. (15) defines the function $\bar{S}_{eq}(r)$, which can shown to be $\bar{S}_{eq}(r) = \sqrt{\lambda/2\pi k_B T}\exp[-\lambda r^2/k_B T]$. Introducing the functions $w(t) \equiv \int_{-\infty}^{\infty} dr k(r)\bar{S}(r, t)$ and $\bar{w} \equiv \int_{-\infty}^{\infty} dr k(r)\bar{S}_{eq}(r)$, one now writes the Wilemski-Fixman approximation in the form $\bar{S}(r, t) \approx \bar{S}_{eq}(r)w(t)/\bar{w}$. After substituting this approximation into the right-hand side of Eq. (14), multiplying both sides of the equation by $k(r)$ and finally integrating over r, the following equation for the unknown function $w(t)$ can be derived:

$$w(t) = \bar{w} - \int_0^t dt' C(t - t')w(t')/\bar{w}, \quad (16a)$$

where

$$C(t - t') = \int_{-\infty}^{\infty} dr \int_{-\infty}^{\infty} dr' k(r)G(r, t - t'|r')k(r')\bar{S}_{eq}(r'). \quad (16b)$$

From the given expressions for $k(r)$, $G(r, t - t'|r')$, and $\bar{S}_{eq}(r)$, one easily evaluates $C(t)$ as

$$C(t) = k_F^2 \exp\left[\frac{\beta}{\lambda}(1 + \chi(t))\right]. \quad (17)$$

Knowing $C(t)$, it is possible, in principle, to exploit the convolution structure of Eq. (16a) to solve for $w(t)$ using Laplace transforms. But when $\chi(t)$ is a Mittag-Leffler function (as is the case in our fGn-GLE model), it seems unlikely that we can determine the Laplace transform of $C(t)$ in closed form. So as before we must resort to approximations.

V. THE SURVIVAL PROBABILITY IN THE SHORT AND LONG-TIME LIMITS

A. The short time limit

In the limit $t \to 0$, and from the definition of the Mittag-Leffler function, it is possible to approximate $\chi(t)$ by the expansion $\chi(t) = 1 + a_1 t^b + O(t^{2b})$, where $b = 2 - 2H$, $a_1 = 1/\tau^b\Gamma(3 - 2H)$, and $\tau = (\alpha\Gamma(2H + 1)/\lambda)^{1/b}$. $C(t)$, in turn, can then be approximated to this order by $C(t) \approx k_F^2 \exp(2\beta/\lambda)\exp(-a_2 t^b)$, where $a_2 = a_1\beta/\lambda$. Thus, at early times, $C(t)$ behaves essentially as a stretched exponential. To the same order, this behavior can be reproduced by $C(t) \approx k_F^2 \exp(2\beta/\lambda)E_b(-a_2\Gamma(b + 1)t^b)$. The reason for writing $C(t)$ in this form is that it can now be Laplace transformed using tabulated results[12] to yield,

$$\hat{C}(s) = \frac{a_4 s^{b-1}}{s^b + a_3}, \quad (18)$$

where $a_3 = a_2\Gamma(b + 1)$ and $a_4 = k_F^2 \exp(2\beta/\lambda)$. Hence, from Eq. (16a), we find that

$$\hat{w}(s) = \frac{\bar{w}(a_3 + s^b)}{a_3 s + a_4 s^b/\bar{w} + s^{b+1}} = \frac{\bar{w}s^{b-1}}{a_3 + s^b + a_4 s^{b-1}/\bar{w}} + \frac{a_3\bar{w}s^{-1}}{a_3 + s^b + a_4 s^{b-1}/\bar{w}}. \quad (19)$$

The formal expansion[14] of the two fractions on the right-hand side of Eq. (19) yields

$$\hat{w}(s) = \bar{w}\sum_{k=0}^{\infty}\left(-\frac{a_4}{\bar{w}}\right)^k \frac{s^{b-(b-(b-1)(k+1))}}{(a_3 + s^b)^{k+1}} + a_3\bar{w}\sum_{k=0}^{\infty}\left(-\frac{a_4}{\bar{w}}\right)^k \frac{s^{b-(b+1-(b-1)k)}}{(a_3 + s^b)^{k+1}}. \quad (20)$$

In this form, the inverse Laplace transform of $\hat{w}(s)$ can be obtained analytically, again using tabulated results;[12] we thus find that

$$w(t) = \bar{w}\sum_{k=0}^{\infty}\frac{(-1)^k}{k!}\left(\frac{a_4}{\bar{w}}\right)^k t^k E^{(k)}_{b,1+k(1-b)}(-a_3 t^b) + a_3\bar{w}\sum_{k=0}^{\infty}\frac{(-1)^k}{k!}\left(\frac{a_4}{\bar{w}}\right)^k t^{b+k} E^{(k)}_{b,1+b+k(1-b)}(-a_3 t^b), \quad (21)$$

where $E_{p,q}(x)$ is the so-called generalized Mittag-Leffler function,[12] which is defined by $E_{p,q}(x) = \sum_{n=0}^{\infty} x^n/\Gamma(pn + q)$, and $E^{(k)}_{p,q}(x)$ is the kth derivative of the function with respect to its argument. From these definitions, the early-time limit of $w(t)$ is found to be

$$w(t) = \bar{w}[E_{b,1}(-a_3 t^b) + O(t)] + a_3\bar{w}[t^b E_{b,1+b}(-a_3 t^b) + O(t^{b+1})] \approx \bar{w}E_b(-a_3 t^b), \quad (22)$$

where, in deriving the second equality in Eq. (22), we have made use of the identity $E_{p,1}(x) = E_p(x)$.

Substituting Eq. (22) into the Wilemski-Fixman approximation, we find that

$$\bar{S}(r, t) \approx \bar{S}_{eq}(r)E_b(-a_3 t^b).$$

Hence,

$$\langle S(t)\rangle \equiv \int dr\bar{S}(r, t) = E_b(-a_3 t^b). \quad (23)$$

The corresponding unfolding probability in this regime, $p(t)$, (defined by the relation $p(t) = -d\langle S(t)\rangle/dt$) is therefore given by[15]

$$p(t) = a_3 t^{b-1} E_{b,b}(-a_3 t^b). \quad (24)$$

B. The long-time limit

In the limit $t \to \infty$, the asymptotic behavior of the Mittag-Leffler function can used to approximate the function

$\chi(t)$ by the leading order expansion $\chi(t) \approx 1/(t/\tau)^b \Gamma(2H-1)$, where τ and b are the same parameters defined in Sec. V A above. The correlation function $C(t)$ [Eq. (17)] is therefore given approximately by $C(t) \approx a_5 + a_6 t^{-b}$, where $a_5 \equiv k_F^2 \exp(\beta/\lambda)$ and $a_6 \equiv a_5 \beta \tau^b / \lambda \Gamma(2H-1)$. Hence, the Laplace transform of $C(t)$ becomes $\hat{C}(s) \approx a_5 s^{-1} + a_6 \Gamma(1-b) s^{-(1-b)}$. From this expression we find that

$$\hat{w}(s) = \frac{\bar{w}}{a_5/\bar{w} + s + a_6 \Gamma(1-b) s^b / \bar{w}}. \tag{25}$$

Using the same expansion procedure outlined in Sec. V A, one can Laplace invert the above relation formally to produce

$$w(t) = \bar{w} \sum_{k=0}^{\infty} \frac{(-1)^k}{k!} \left(\frac{a_5}{\bar{w}}\right)^k t^k \times E^{(k)}_{1-b,1+bk}(-a_6 \Gamma(1-b) t^{1-b}/\bar{w}). \tag{26}$$

The Mittag-Leffler function in this equation is again expanded asymptotically to leading order, after which it is differentiated k times with respect to its argument. The resulting infinite series can be resumed in closed form to give

$$w(t) = \frac{\bar{w}^2}{a_6 \Gamma(1-b) t^{1-b}} E_{b,b}(-a_5 t^b / a_6 \Gamma(1-b)). \tag{27}$$

Hence, after invoking the Wilemski-Fixman approximation, the disordered averaged survival probability in this long-time regime becomes

$$\langle S(t) \rangle = \frac{\bar{w}}{a_6 \Gamma(1-b) t^{1-b}} E_{b,b}(-a_5 t^b / a_6 \Gamma(1-b)), \tag{28}$$

so the corresponding unfolding probability, $p(t)$, is[15]

$$p(t) = \frac{\bar{w}}{a_6 \Gamma(1-b)} \left[\frac{(1-b)}{t^{2-b}} E_{b,b}\left(-\frac{a_5 t^b}{a_6 \Gamma(1-b)}\right) + \frac{a_5}{a_6 \Gamma(1-b) t^{2-2b}} \left\{ E_{b,2b-1}\left(-\frac{a_5 t^b}{a_6 \Gamma(1-b)}\right) + (1-b) E_{b,2b}\left(-\frac{a_5 t^b}{a_6 \Gamma(1-b)}\right) \right\} \right]. \tag{29}$$

VI. RESULTS AND DISCUSSION

In their experiments on poly-ubiquitin stretching under a constant force, Kuo *et al.* have shown that the non-exponentiality in the probability of chain unfolding, $p(t)$, which cannot be reconciled with a model based on simple Arrhenius kinetics, can be plausibly explained by a model based on a static distribution of reaction barriers. Interestingly, recent simulation data by Li *et al.*[16] on the role of water in the forced unfolding of poly-ubiquitin suggest how a static distribution of transition states might occur during the process as a result of the intrusion of *variable* numbers of water molecules into the space between H-bonded beta strands, with the consequent weakening of the forces responsible for holding the chain in a compact configuration. As the authors themselves note, however, the timescales separating unfolding from water intrusion are quite large, so the evidence in favor of this particular mechanism of generating static disorder—though suggestive—is somewhat equivocal.

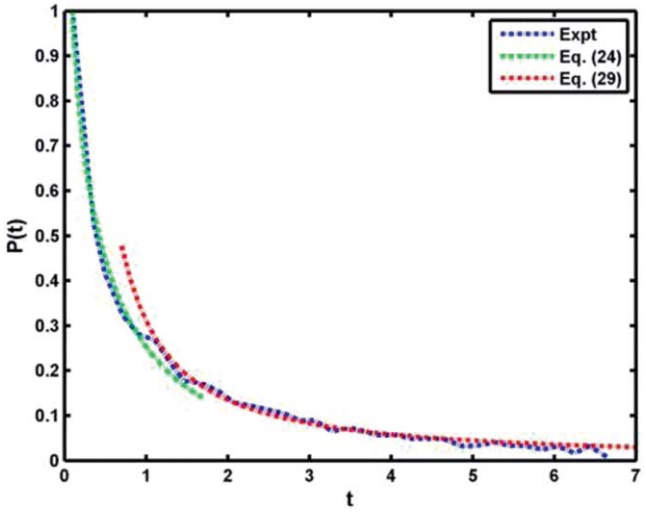

FIG. 1. The unfolding probability $p(t)$ as a function of the time t. The blue curve corresponds to the probability reconstructed from the experimental histogram of Fig. 1(c) in Ref. 3, as described in the text. The green curve corresponds to Eq. (24), with the parameters H and a_3 adjusted for best fit to the values 0.605 and 0.848, respectively. The red curve corresponds to Eq. (29), with H set to the same value of 0.605 used in the short time regime, and the parameters $a_5/a_6\Gamma(1-b)$, and $\bar{w}/a_6\Gamma(1-b)$ adjusted for best fit to the values 0.00094 and 1.732, respectively.

Dynamic disorder as an alternative explanation of the data in Ref. 3 cannot, therefore, be ruled out. A comparison of its predictions for $p(t)$ [Eqs. (24) and (29)] with the experimental $p(t)$ is shown in Fig. 1. The blue curve in this figure corresponds to the experimental data and has been reconstructed[17] from the actual data points in the histogram of Fig. 1(c) in Ref. 3 (which corresponds to the 110 pN force experiment). The green curve corresponds to the early-time limit given by Eq. (24), the parameters H and a_3 in that equation having been set to the best fit values of 0.605 and 0.848, respectively. The red curve corresponds to the long-time limit given by Eq. (29), the parameter H having been fixed at the value 0.605 obtained earlier for the short time behavior, and the parameters $a_5/a_6\Gamma(1-b)$ and $\bar{w}/a_6\Gamma(1-b)$ having been set, respectively, to the best fit values of 0.00094 and 1.732. (The parameters a_3 and $a_5/a_6\Gamma(1-b)$ can obviously be identified with decay constants, and $\bar{w}/a_6\Gamma(1-b)$ with a weighting factor, but because we have adopted a phenomenological approach in which a complex many-body problem has been reduced to a one-dimensional problem in the dynamical variable r, we cannot hope to specify the microscopic decay process that these parameters correspond to.) As is evident from Fig. 1, the degree of agreement between the experimental and theoretical curves is very close. The same degree of agreement cannot be achieved with $H = 0.5$ (the white noise limit), where the survival probability would be found to decay exponentially, suggesting that temporal correlations between the fluctuations in r are important in producing non-exponential unfolding kinetics.

Furthermore, the fact that the survival probability $S(t)$ is given by a generalized Mittag-Leffler function means that at early times it behaves essentially as a stretched exponential,

which is the kind of behavior that seems to characterize the decay of at least some regions of the experimental curves in Ref. 3.

The possibility that dynamic disorder underlies the non-exponential kinetics of poly-ubiquitin unfolding suggests that the chain might exist in a number of different conformational sub-states that interconvert amongst themselves at rates that span a range of timescales, including those comparable to the timescales of unfolding. Other large globular proteins are known to exhibit this timescale overlap between conformational fluctuations and barrier crossing dynamics, so it would not be surprising if poly-ubiquitin behaved in essentially the same way. In this scenario, the water intrusion that has been suggested to play a role in generating static disorder would probably have little direct bearing on the unfolding kinetics, unless it were to contribute to the thermal fluctuations and to produce the kind of temporal correlations among the random forces acting on the chain that lead to colored noise effects.

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