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Breaking bonds in the atomic force microscope: Theory and analysis

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A theoretical framework is developed to analyze molecular bond breaking in dynamic force spectroscopy using atomic force microscopy. An analytic expression of the observed bond breaking probability as a function of force is obtained in terms of the relevant physical parameters. The force-ramp mode is discussed in detail, which gives the best framework to extract the relevant physical parameters such as the potential depth and its width, if a set of widely different force-loading rates are used. We also show that the commonly used Ritchie-Evans model is incomplete and that it is only applicable for forces well below the maximum permitted by the potential. Statistical complications arising from the use of constant velocity experiments are discussed in detail.

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I. INTRODUCTION

The interactions between small molecular aggregates can be manipulated in nanoarchitectures to construct a molecular switch with bond forming and bond breaking providing the on/off states of a switch [1]. Likewise, bond breaking occurs when forces are applied in the unfolding of biomolecules [2,3]. This process implies the separation of the two molecular fragments along their reaction coordinate. For a diatomic molecule *AB* the reaction coordinate is the *A*-*B* distance and the energy surface is the intramolecular potential as a function of that distance. In the case of complex molecules, the separation of their dissociated fragments involves rotations relative to each other and changes in their internal structures. Here the reaction coordinate is to be understood as the minimum energy pathway in a multidimensional coordinate space in which the center of mass separation with local adjustments in relative orientation is the dominant one. Excitations in the rotational and internal vibrational degrees of freedom extend the reaction pathway into a multidimensional valley in the free energy surface. The latter can and has been calculated for many systems by first principles methods of quantum mechanics [4].

Evans and Ritchie [5] presented the first model for dynamic force spectroscopy using a simplified version of the bond potential and calculated the loading-rate-dependent breaking probability. Various authors have since extended their model, taking into account rebinding [6–8], or attempted to reconstruct the free energy profile [9–11], and to describe experiments with a constant force loading rate [12,13].

A lot of experimental and theoretical work is done in a setup where the atomic force microscope (AFM) cantilever is pulled with a constant velocity. This experiment has to be analyzed using statistical mechanics where the position control over the cantilever leads to statistical fluctuations in the applied force [14]. From Kramer's rate theory we know that the applied force is the natural variable of the problem, hence a proper transformation from the Helmholtz to the Gibbs ensemble in statistical mechanics should be performed. This complication is commonly circumvented by simply assuming that the force is linear in the cantilever position [6,7,15,16], which is a questionable practice as dynamic force spectroscopy relies on polymer linkers with nonlinear force extension relations. Moreover, there are fundamental statistical difficulties when transforming from the controlled time-dependent position $l(t)$ to the force $f(t)$. While the controlled force ensemble requires a well-defined force but allows for position fluctuations, the constant velocity experiment provides a controlled position and causes non-negligible force fluctuations for small systems such as an AFM.

The uniqueness of modeling any data for the purpose of extracting underlying microscopic parameters has not received sufficient attention. Here we set up an analytical theory based on previous work [12] that explains breaking force distributions and is used to extract relevant physical parameters. For a unique description, one needs at least three independent parameters: the activation energy, the attempt frequency, and the potential width. We will extract these from experimental examples and delineate a set of criteria to ensure uniqueness of such a procedure.

II. THEORY

We treat bond breaking by an external force as a thermally activated process for which we write down an Arrhenius rate equation for the probability $P(t)$ that the molecule is still intact at time t, $\dot{P}=-A\exp[-\beta\Delta V(f)]P$. $\Delta V(f)$ is the activation energy or energy difference between the free energy minimum of the bond and the barrier to be overcome in bond breaking under the influence of an applied force f, see Fig. 1. The prefactor A contains information about the changes in entropy due to the breakup of the molecule and also about the internal energy redistribution into the bond that eventually breaks. According to transition state theory it is given by $A=\kappa\nu q^{*}/q$ where ν is the attempt frequency, κ the accommodation coefficient, and q^{*}/q the ratio of the internal partition functions of the activation complex to that of the molecule in the initial state. In the simplest scenario ν can be interpreted as the attempt frequency to break the bond, i.e., roughly that of the oscillations around the mini-

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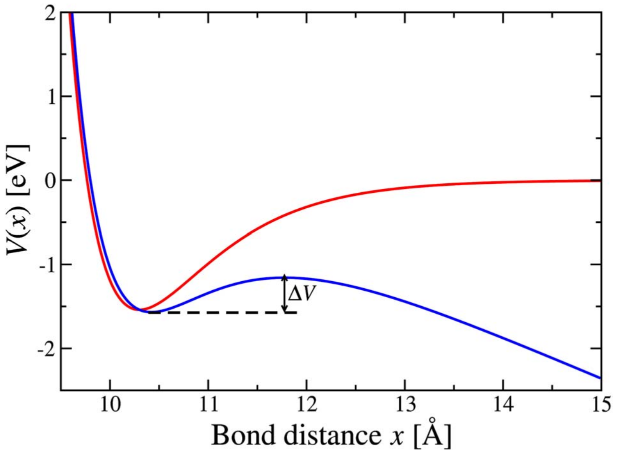

FIG. 1. (Color online) Morse potential as a function of the separation of the TP-Ru^{2+} complex from TP, without and with an external force applied. ΔV is the activation barrier height (Ref. [12]).

mum of the bond potential. This frequency is typically around 10^{12} s^{-1} but is drastically reduced in a liquid, mostly due to solvation effects. In addition, the accommodation coefficient is typically much smaller than unity for a reaction in a liquid, as is the ratio of the partition functions is also smaller than one so that one expects $A \ll \nu$.

In our model, we assume that the molecule-linker complex is in thermodynamic equilibrium with respect to its mechanical extension. The pulling processes are generally slow enough that one does not have to use nonequilibrium models of polymer stretching such as described in Ref. [17]. This also means that one can assume uniform tension throughout the molecule as it is done in virtually all of the literature on dynamic force spectroscopy and neglect the effects of dissipative forces. For extremely high force-loading rates, additional complications with a time-dependent propagating tension along the backbone would naturally arise [18,19] but are not considered here.

In the force ramp mode, the force is increased linearly in time with a force loading rate α, $f = f_0 + \alpha t$. Eliminating t in favor of f we get

$$\frac{dP}{df} = -\frac{A}{\alpha} \exp\{-\beta \Delta V(f)\} P, \tag{1}$$

with the analytic solution

$$P(f) = \exp\left[-\frac{A}{\alpha} \int_{f_0}^{f} \exp[-\beta \Delta V(f')] df' \right]. \tag{2}$$

We obtain the distribution of the bond breaking force by taking the derivative of the probability $P_b(f) = 1 - P(f)$ with respect to the force. The most probable bond breaking force f_{mp} as well as the distribution width Δf are given by setting the second and third derivative of Eq. (2) to zero, respectively.

To go further analytically we need to specify the bond potential $V(x)$ as a function of the reaction coordinate to obtain $\Delta V(f)$. For a simple molecular bond the Morse potential is known to capture all essential features including the all important dissociative state at large separation. In the presence of an external force f it reads

$$V(x) = V_0\{\exp[-2\gamma(x - x_0)] - 2\exp[-\gamma(x - x_0)]\} - f(x - x_0). \tag{3}$$

γ^{-1} is the range of the potential, $-V_0$ its depth, and x_0 is the position of its minimum at $f = 0$. In order to obtain the dissociation barrier $\Delta V(f) = V_{\max} - V_{\min}$ we take the potential difference between the two local extrema in Eq. (3). This barrier can be written in terms of the maximally permissible force $f_{\max} = \gamma V_0 / 2$ as

$$\frac{\Delta V(f)}{V_0} = \sqrt{1 - \tilde{f}} - \tilde{f} \tanh^{-1} \sqrt{1 - \tilde{f}}, \tag{4}$$

where $\tilde{f} = f / f_{\max}$. Moreover, it can be approximated to within a few percent over the complete force range $0 < \tilde{f} < 1$, by $\Delta V \approx V_0(1 - \tilde{f})^2$. This approximation is valid not only for the Morse potential, but also works remarkably well for other models such as the Lennard-Jones potential. It suggests that to a first approximation, the exact shape of the bond potential is of minor importance in comparison to its depth and width. The parabolic form of ΔV allows us to do the integral in Eq. (2) explicitly to find

$$P(\tilde{f}) \simeq \exp\left[-\frac{1}{2\alpha} \sqrt{\frac{\pi}{\beta V_0}} f_{\max} \times \{\mathrm{erf}(\sqrt{\beta V_0}) - \mathrm{erf}(\sqrt{\beta V_0}(1 - \tilde{f}))\} \right]. \tag{5}$$

We obtain the most probable breaking force f_{mp} from its second derivative, giving

$$1 - 2\frac{f_{\mathrm{mp}}}{\gamma V_0} = \frac{\gamma A}{4\beta\alpha} \exp\left[-\beta V_0 \left(1 - 2\frac{f_{\mathrm{mp}}}{\gamma V_0}\right)^2 \right]. \tag{6}$$

The rates $dP_b(f)/df = -dP(f)/df$, or the breaking force distributions, for a force ramp experiment are shown in Fig. 2(a) for three potentials with $v_0 = V_0 / k_B T = 20$, 40, 60, and three values of the force-loading rate $\tilde{\alpha} = \alpha / (\gamma A V_0) = 1$, 10^{-5}, and 10^{-10}. The shapes of these curves are certainly in agreement with experimental results [1,2], including their slight asymmetry.

To get a quantitative understanding we plot in Fig. 2(b) the most probable breaking force $\tilde{f}_{\mathrm{mp}} = f_{\mathrm{mp}} / f_{\max}$ and the width of the force distribution $\Delta \tilde{f} = \Delta f / f_{\max}$ as a function of $\tilde{\alpha}$. From Eq. (6) we see that $\lim_{\alpha \to \infty} f_{\mathrm{mp}} = f_{\max}$, which can be reached if either the loading rate α is very large or if the attempt frequency A is very low.

III. RESULTS

A fit of our theory to the experimental results on the breaking of the Terpyridine-Ru-Terpyridine (Tp-Ru-Tp) complex [1] yielded the parameters $\tilde{\alpha} = 2 \times 10^{-5}$ and $v_0 = 14$. This implies that $V_0 = 0.35$ eV and $\gamma V_0 \simeq 0.5$ eV/Å $= 0.7$ nN, in agreement with estimates from quantum chemical calcu-

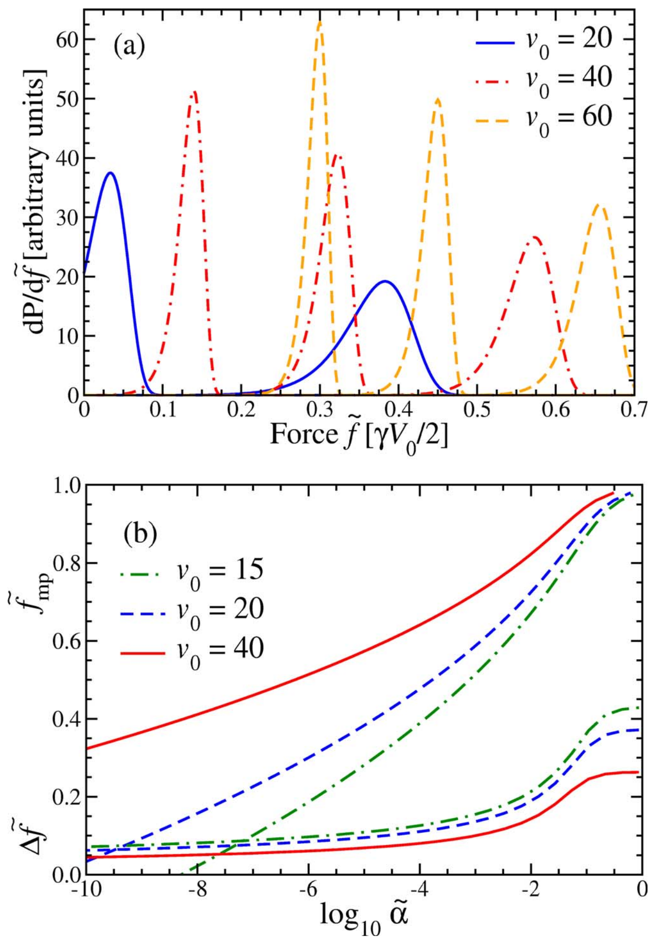

FIG. 2. (Color online) (a) Breaking force distribution $dP(\tilde{f})/d\tilde{f}$ for three choices of potentials and for three force load rates $\tilde{\alpha} = \alpha/(\gamma A V_0) = 10^{-10},\ 10^{-5},\ 10^{-1}$ (left to right within each group). (b) Most likely breaking force f_{mp} (top) and width of the force distribution (bottom) as a function of force load rate for three potential depths.

lations provided that asymmetry in the bond breaking and solvent effects are accounted for [12]. From $\tilde{\alpha}$ we obtain $A = 6\times10^5\ \mathrm{s}^{-1}$. Note that this fit is not unique and serves merely as an example; this nonuniqueness is addressed below.

Next, we would like to make the connection with the Ritchie-Evans version of the Bell model [5,20] employed in numerous experimental papers for the data analysis, for which the rate equation reads

$$\frac{dP}{dt} = -k^*_{\mathrm{off}} \exp[\beta f \Delta x_e]P, \tag{7}$$

where Δx_e is interpreted as the maximum elongation of the molecule in quasiequilibrium before breaking. Δx_e is not a constant as frequently assumed, but a function of f, as in our theory. One can obtain this simplified model from the present theory by assuming that the breaking force is much smaller that the maximum ($f \ll \gamma V_0/2$), which is generally applicable for small loading rates. Equation (1) is expanded to get a rate expression like Eq. (7) with $\Delta x_e = 4/\gamma$ and $k^*_{\mathrm{off}} = A\exp[-\beta V_0]$. This enables direct comparison with the TP-Ru-TP data by Kudera *et al.* [1], where we find $\Delta x_e = 2$ Å, and $k^*_{\mathrm{off}} = 0.5\ \mathrm{s}^{-1}$, agreeing with their values of 3.3 Å and $0.05\ \mathrm{s}^{-1}$ to within an order of magnitude. The reason for these discrepancies in the two parameters k^*_{off} and Δx_e is, of course, the approximate nature of the Ritchie-Evans model. The data extend over an interval $f_{\mathrm{mp}} \pm \Delta f/2$, and do not satisfy the condition $f \ll \gamma V_0/2$ for the top 20% of this range. Hence a least-squares fit with the Ritchie-Evans model can only yield approximate parameters. In concluding this discussion we note that the Ritchie-Evans model lacks an important physical parameter, namely the strength of the bond. It is thus limited to forces with $f \ll \gamma V_0/2$, a restriction not imposed in the general model.

TABLE I. The parameters V_0, γ, and A for the ubiquitin experiment, the corresponding k^*_{off} from the Ritchie-Evans model, and the maximum force $f_{\max} = \gamma V_0/2$ for each Morse potential.

V_0 [eV]	γ (Å^{-1})	A (s^{-1})	k^*_{off} (s^{-1})	Δx_e (Å)	$f_{\max}$ (pN)
0.236	1.76	1.06×10^2	9.78×10^{-3}	2.27	334
0.273	2.09	8.02×10^2	1.75×10^{-2}	1.91	457
0.319	2.29	6.58×10^3	2.28×10^{-2}	1.75	585
0.371	2.41	5.78×10^4	2.65×10^{-2}	1.66	717
0.403	2.47	2.21×10^5	2.82×10^{-2}	1.62	799
0.427	2.51	5.81×10^5	2.93×10^{-2}	1.60	857

A second example is the unfolding of ubiquitin, which was recently measured by Schlierf, Li and Fernandez [2]. Again, a fit to this data is nowhere near unique. Table I gives our parameters for six indistinguishable fits in Fig. 3 and the corresponding values of k^*_{off}= and Δx_e for the Ritchie-Evans model. It suggests a bond strength of the order of 0.3 to 0.4 eV, which is typical for strong or multiple van der Waals interactions. Notice that for all but the very first set of parameters, the Ritchie-Evans parameters are very close to those obtained in the original reference [2], $k^*_{\mathrm{off}} = 0.0375\ \mathrm{s}^{-1}$ and $\Delta x_e = 1.7$ Å. Note also that this whole experiment is done

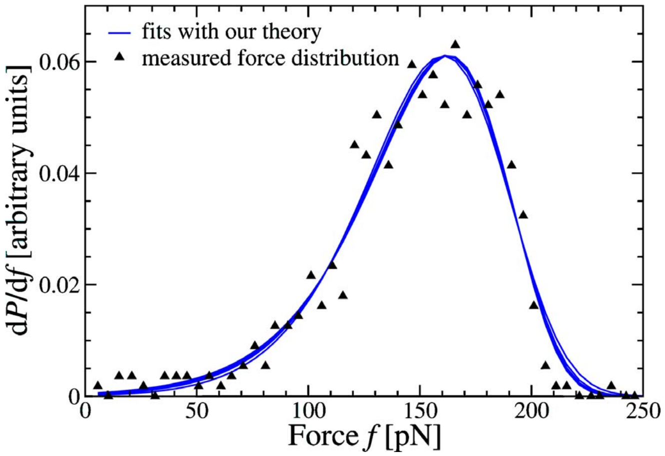

FIG. 3. (Color online) The unfolding forces for ubiquitin, measured in Ref. [2] and fitted with our theory. All parameters are given in Table I.

in the limit $f \ll f_{\max}$, which explains why the Ritchie-Evans model gives such good results. We would like to stress that a unique fit of all three physical parameters V_0, γ, and A is possible if additional data with different force loading rates is taken into account simultaneously.

Given the fitting ambiguities shown above, we will now try to establish criteria for experiments such that the extraction of the three independent parameters A, V_0, and γ is unique. Starting with the force ramp mode, there are several options: (i) measure the force distribution for several force loading rates α and fit them with the theoretical curve; (ii) measure the breaking force distributions when several strands are attached; and (iii) determine the maximum and width of the breaking force distribution for several force loading rates α; this is possible provided data with very good statistics are available.

We have first checked option (i) and found that a unique fit is obtained if we take three values of α that span two orders of magnitude. Our data were force distributions calculated from Eq. (5). The extracted fit parameters agree with the original input data with very high precision, but the uniqueness of the fit is quickly lost if the force loading rates differ by less than two orders of magnitude. Similarly, option (ii), i.e., using the force distributions for several attached strands (for 1, 2, and 3 attached strands), also leads to a unique fit if two force loading rates are used that differ by one order of magnitude.

So far our analysis of data to extract the underlying physical parameters controlling bond breakage is based on perfect theoretical breaking force distributions. Noise in experimental data and limited statistics complicate the fitting procedure significantly. One can only expect that the data analysis yields a unique set of parameters if good data are available, in particular spanning a wide range of force loading rates.

Some numerical difficulties in fitting experiments with the theoretical breaking force distribution arise from the fact that three parameters must be obtained simultaneously. This could be circumvented with an alternative fitting procedure, valid for fast pulling when forces in the vicinity of $f_{\max}$ can be reached. First, one integrates the experimental breaking force distribution $dP_b(f)/df$ to obtain $P_b(f)$ and then plots

$$\ln\left[\frac{1}{1-P_b(f)}\frac{dP_b(f)}{df}\right] = \ln A - \beta \Delta V(f) \qquad (8)$$

as a function of f. For small forces this function approaches $[\ln A - \beta V_0]$ and, as the force approaches its maximum, it yields $\ln A$. Next, one fits the function $[\ln A - \beta V_0(1 - f/f_{\max})^2]$ (which should be possible numerically in the small force regime) to get $f_{\max} = \gamma V_0/2$ and thus γ. This way, good first estimates for the three parameters are obtained, which can then be used for the curve fitting described in Eq. (5).

We now would like to discuss some of the complications arising from constant velocity experiments. Regardless of the experimental setup, the natural variable of the bond breaking problem is the applied force f. In the v=const experiment, however, the experimental control variable is the force's thermodynamic conjugate, namely the cantilever length l. As indicated previously, it is improper to assume that force and distance are simply related by $f = kx$ for some effective spring constant k. This is not only due to the nonlinearity of the polymer spacer force extension relation, but is a thermodynamic principle. In the constant velocity regime there are non-negligible force fluctuations in small systems (such as a dynamic force spectroscopy experiment), hence one cannot calculate a unique $f(t)$ relation for any given experiment. This particular difficulty has been previously noticed but was attributed to the inapplicability of Kramer's rate theory instead [21]. Finally, we would like to point out that non-negligible spacer dispersion such as modeled by Friedsam *et al.* [22] leads to more complications in fitting observed force spectra, as one would have to integrate a rate equation for each separate experimental run before achieving fitting accuracy similar to what we describe in this paper. All these theoretical difficulties can be quite comfortably circumvented in a constant force-loading rate experiment [2,3], which is somewhat more difficult to implement in practice but which provides the correct statistical ensemble in return.

IV. CONCLUSION

We have shown in this paper that a simple analytical theory can be adapted for the analysis of bond breaking in the atomic force microscope. Since the probability distribution of bond breaking forces have rather simple shapes, and not much structure apart from some asymmetry, a large set of data must be available to obtain the underlying physical parameters such as bond strength, bond width, and Arrhenius prefactor uniquely. Extracting the actual shape of the energy surface requires much additional work. *Large* means data obtained under different experimental conditions, such as force loading rates varying over two orders of magnitude. We emphasize that three independent parameters are necessary for a complete microscopic explanation of this situation, compared to two in models derived from Ritchie and Evans' work. Moreover, it is necessary to work in a force-controlled environment which provides the correct statistical framework for the proper analysis of experiments.

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