The load dependence of rate constants

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As experimental techniques in biophysics have progressed at the single molecule level, there has been considerable interest in understanding how external mechanical influences (such as load) affect chemical reactions. The majority of biophysical studies investigating load-dependent kinetics use an equation where the rate constant exponentially depends on force, which is sometimes called Bell’s equation. This equation requires the determination of two parameters that describe the potential energy-strain function: $k_0$, which is the reaction rate in the absence of load, and $x_c$, which is the difference in strain between the reactant and transition states. However, there have been theoretical studies based on Kramers’ theory suggesting that the rate constant should have load-dependent pre-exponential terms and nonlinear load-dependent terms in the exponential. Kramers’ theory requires an exact knowledge of the potential energy-strain function, which is in general not known for an experimental system. Here, we derive a general approximation of Kramers’ theory where the potential energy-strain function is described by five parameters, which can, for small loads, be reduced to four-, three-, and finally to two parameters (Bell’s equation). We then use an idealized physical system to validate our approximations to Kramers’ theory and show how they can predict parameters of interest (such as $k_0$ and $x_c$) better than Bell’s equation. Finally, we show previously published experimental data that are not well fitted by Bell’s equation but are adequately fitted by these more exact equations. © 2008 American Institute of Physics.

I. INTRODUCTION

In many biologically relevant chemical reactions, the reacting molecules experience a load. This external mechanical influence affects the rate of the chemical reaction as well as its equilibrium. The recent development and widespread use of single molecule assays, such as the laser trap, has allowed direct observation of the load dependence of chemical reactions. For example, optical trapping has led to the study of these “strain-dependent” chemical reactions in the field of processive motors and in the related field of muscle contraction (see, e.g., Smith and Sleep for a recent theoretical work and Veigel et al. and Kad et al. for direct experimental observations of this load dependence). In the field of protein folding, load-dependent unfolding observed by using atomic force microscopy provides information about a protein’s free energy landscape, especially around the transition state.

Most of these recent studies use an equation with the rate constant exponentially varying with load:

$$k(F) = k_0 \exp(Fx_c/k_BT),$$

where $F$ is the applied force, $x_c$ is some “characteristic distance” (usually interpreted as the distance along the reaction coordinate to the transition state), $k_BT$ is Boltzmann’s constant, $T$ is the absolute temperature, and $k_0$ is the rate of the reaction in the absence of load. Equation (1) is sometimes attributed to Bell or is sometimes justified by a simple analysis of the Arrhenius equation. For simplicity, we will refer to it as Bell’s equation.

Despite the ubiquity of Bell’s equation several theoretical studies have shown that higher powers of $F$ should appear in the exponential term. Recent experimental and theoretical work provide evidence that Bell’s equation can give poor estimates for the parameters $k_0$ and $x_c$. Furthermore, several careful theoretical analyses have shown that the expression for the rate constant as a function of force is of the form

$$k(F) = g(F)\exp(-\Delta V_F/k_BT),$$

where $g(F)$ is some function of force, and $\Delta V_F$ is the force-dependent potential energy barrier to reaction. If Bell’s equation, which is so commonly used in the analysis of load-dependent chemical reactions, is not always correct, we must understand when its predictions will be inaccurate and find alternative equations for these situations.

By using a simple, idealized mechanicochemical model, we show that the load dependence of rate constants is governed by the relation shown in Eq. (2), while under certain conditions, Bell’s equation provides an adequate approximation. However, in some biological applications, the simple assumptions of Bell’s equation break down, and a more complicated model must be used. To address these situations, we derive a new, more exact expression for the force dependence of rate constants. Our derivation may be generalized to find higher-order (though not analytic) approximations, or may be simplified to three lower-order approximations, the lowest being Bell’s equation. Thus, we present a new method
of understanding the force dependence of rate constants that can be easily and simply applied to experiment.

II. THE MODEL

We consider a fictitious molecular system with a completely specified potential energy as a function of strain. This idealized system is preferable to a real system because knowledge of the exact potential energy–strain relationship is only possible in the ideal case. Since the goal of modeling load-dependent kinetics is often to determine the properties of this potential, knowing the exact values of these properties provides a good way to evaluate any candidate model.

A. Physical model

The simple physical system is shown in Fig. 1. It consists of a small particle (assumed to be a point mass) moving in one dimension and attached to a zero-length linear spring with stiffness $k$. At some distance $x^*$, there is a potential well. Solvent molecules collide with the small particle, causing it to undergo Brownian motion.

The attractive potential (centered at $x^*$) has the characteristics of a Lennard–Jones potential (a phenomenological description of the interaction potential of two molecules), and we assume that the potential is centered a distance $L$ away from the axis along which the point mass moves (in order to avoid repulsive forces),

$$V = \frac{1}{2} k \sigma^2 + 4E \left( \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6 \right),$$

where $r = \sqrt{(e-x^*)^2 + L^2}$, $E$ is a measure of the depth of the binding potential, $\sigma$ is a measure of the width of the binding potential, and $e$ is the strain on the spring, which we assume to have zero rest length. The physical system and its potential energy are shown in Fig. 1.

B. The large ensemble limit

If we look at the strain as a function of time for a single system (see Fig. 2), we see two different behaviors. First, there is a low-variance oscillation about $e=x^*$. Second, there is a higher-variance oscillation about $e=0$. This observation is not surprising; the system has two attractors, and as solvent molecules collide with the point mass, it moves between the first (near $e=0$, which is less stiff) and the second (near $e=x^*$, which is more stiff). When the system is in the basin of attraction of the attractor near $e=x^*$, we say that it is “bound.” Otherwise, we say that the system is “unbound” (see Fig. 1).

For a large enough ensemble of these systems, the stochastic nature of the ensemble is negligible. We may then speak of a probability distribution, $W(e, \dot{e}, t)$, governed by the generalized Fokker–Planck equation. In the high-viscosity limit relevant to most biological reactions, we may simplify the generalized Fokker–Planck equation to the Smoluchowski equation in $w(e, t)$, where $W=P(\dot{e})w(e, t)$, with $P(\dot{e})$ being the equilibrium velocity distribution. If the system is in quasi-steady-state, then we may reduce an entire probability distribution to a single number: the probability of a system being bound. Quasi-steady-state implies that near $e=0$ and $e=x^*$, the probability distribution is approximately in equilibrium and the flux between these two distributions.

FIG. 1. Left: a schematic representation of a simple physical system with strain-dependent chemistry; right: the potential energy–strain curve of such a system indicating the bound state. The physical model is a point mass moving in one dimensional on a zero-length linear spring. The mass interacts with a binding site situated at $x^*$. The schematic is meant to appear very similar to drawings from Huxley (Ref. 25) since the essential reactions in skeletal muscle contraction are thought to have many of the properties of this system. The analysis presented in this paper also applies to processive motors, protein folding under load, and forcible detachment of cells in addition to muscle.

FIG. 2. Position of a 1D particle on a spring undergoing Brownian motion in the vicinity of an attractive potential. The particle goes between two attractors, one at $e=0$ and one at $e=x^*$ (dashed line). When the particle is in the basin of attraction of the attractor at $e=x^*$, the curve is drawn in black (and we call it bound). If not, the curve is drawn in gray and we say that the particle is unbound.
slowly varies with respect to the time necessary to obtain them (see Appendix A for details). Thus, the distribution is completely defined by a single parameter, which is the proportion of bound or unbound systems.

Then, defining the following variables, we may write a first order ordinary differential equation (ODE) for the binding rate (see Appendix A for an explicit derivation):

\[
\frac{dn_b}{dt} = k_a(1 - n_b) - k_d n_b,
\]

where \( n_b \) is the probability of being bound, \( k_a \) is the attachment rate constant, and \( k_d \) is the detachment rate constant.

In this form, the rate constants are functions of the potential energy surface and physical constants. For example,

\[
k_a = k_b \int_{x_c}^{\infty} \text{erf}\left( \frac{V_c - V(e)}{k_b T} \right) \exp(-V(e)/k_b T) d(e) \exp(-V(\infty)/k_b T) d(e) = k_b \int_{0}^{\infty} \exp(V(e)/k_b T) d(e) \int_{0}^{\infty} \exp(-V(\infty)/k_b T) d(e) = k_b \int_{x_c}^{\infty} \text{erf}\left( \frac{V_c - V(e)}{k_b T} \right) \exp(-V(e)/k_b T) d(e)
\]

where \( \gamma \) is the viscous drag coefficient, \( x_c \) is the position of the transition state, and \( V_c \) is the potential energy at the transition state (an expression for \( k_d \) is given in Appendix A).

This analysis was first performed by Kramers in 1940. In most biological applications, we will have relatively deep wells and so the numerator should usually be very close to 1, which is Kramers’ result. However, owing to our definition of a bound state, we get a slightly different result when we perform this analysis (see Appendix A). In all of our simulations, we used Eq. (5) since the numerator has a potentially important load dependence and will lead to more accurate predictions of \( k_a \) for shallow wells.

### III. THE FORCE DEPENDENCE OF RATE CONSTANTS

Equation (5) gives an exact expression for the force dependence of rate constants. However, to use this equation, we must have an exact knowledge of the potential energy as a function of strain. Furthermore, this equation very strongly depends on the shape of the potential energy–strain curve near the transition state, which is the most difficult portion of the curve to experimentally investigate. To find an experimentally useful equation for the force dependence of rate constants, we must make some approximations.

Upon load application, the distance between the potential energy minimum at the unbound state (which we arbitrarily define as \( \epsilon = 0 \)) and the local potential energy maximum at the transition state (which we define as \( \epsilon = x_c(F) \)) changes. We define this load-dependent distance as \( x_c(F) - x_c(0) = \Delta x_c \), and we assume that it is small with respect to \( x_c(0) \) (which we refer to simply as \( x_c \)). Then, we may write the following relation (explicitly derived in Appendix B) by neglecting terms of order \( (\Delta x_c)^2 \):

\[
k_a = k_0 \sqrt{1 - \frac{\kappa' F}{\kappa_1^2}} \exp\left( \frac{F x_c - F^2(1/2 \kappa + 1/2 \kappa_0)}{k_b T} \right),
\]

where \( k_0 \) is the rate of attachment in the absence of load, \( \kappa_0 \) and \( \kappa'_0 \) are the curvature and its derivative with respect to \( \epsilon \) at \( \epsilon = 0 \) in the absence of load, and \( \kappa_1 \) and \( \kappa'_1 \) are the negative curvature and its derivative with respect to \( \epsilon \) at the transition state in the absence of load.

Equation (6) is of the form of Eq. (2). It has five parameters that may be varied to fit the data: \( k_0, x_c, 1/\kappa_0 + 1/\kappa_0' + 1/\kappa_1^2 \), and \( \kappa_1'/\kappa_1^2 \). In some cases, it is possible to experimentally measure \( k_0, \kappa_0 \), and \( \kappa_0' \). Then there are only three free parameters. This equation is sufficiently general for most biological applications, although if necessary, additional parameters may be added by using the methods outlined in Appendix B. These higher-order approximations generally cannot be analytically expressed. We refer to Eq. (6) as the five parameter force-dependent rate model.

For some cases, we might require a less accurate approximation. If the elasticity of the unbound state, \( k \), is approximately constant, as we expect in the model shown in Fig. 1, or more generally neglecting the smallest terms (see Appendix B), we get an equation of the form

\[
k_a = k_0 \sqrt{1 - \frac{\kappa'_1 F}{\kappa_1^2}} \exp\left( \frac{F x_c - F^2(1/2 \kappa + 1/2 \kappa_0)}{k_b T} \right).
\]

Equation (7) has four parameters that may be varied to fit the data: \( k_0, x_c, 1/\kappa_0 + 1/\kappa_0' \), and \( \kappa_1'/\kappa_1^2 \). We refer to Eq. (7) as the four-parameter force-dependent rate model.

By neglecting the smallest terms in Eq. (7) we may write (see Appendix B)

\[
k_a = k_0 \exp\left( \frac{F x_c - F^2(1/2 \kappa_0 + 1/2 \kappa_0')}{k_b T} \right).
\]

We refer to this equation as the three-parameter force-dependent rate model.

Finally, neglecting terms of order \( \Delta x_c \), Eq. (8) reduces to a two-parameter force-dependent rate model, which is exactly Bell’s equation [Eq. (1)]:

\[
k_a = k_0 \exp\left( \frac{F x_c}{k_b T} \right).
\]
model predictions. Comparing these equations to more exact values of $k_a$ from our simple physical system reveals the relative contribution of the terms in Eq. (6) (see Fig. 3).

A. Comparing the models

Generally, a biochemist or biophysicist who uses a force-dependent rate model is interested in estimating properties of the potential from data. So, candidate models should be evaluated by how accurately they estimate these parameters. An idealized system affords an opportunity to test these models because we know all of the properties of the potential.

By using the molecular model shown in Fig. 1, we simulated a binding reaction with 13 different constant force values. Parameters were picked to simulate a reaction with a relatively weak spring and a large displacement on binding [$k = 0.5 k_B T$, $x^* = 9$ nm, $E = 25k_B T$, $\sigma = 0.75$ nm, and $L = 1.25 \sigma$ (parameter set A)]. The process was repeated with a stiffer spring and a smaller displacement [$k = 3.5 k_B T$, $x^* = 4$ nm, $E = 35.4 k_B T$, $\sigma = 0.75$ nm, and $L = 1.25 \sigma$ (parameter set B)]. The rate constant for attachment was estimated from numerical simulations of the Smoluchowski equation as described in Appendix C.

We fit the load-dependent rate constants from these simulations with three candidate models: the four-, three-, and two-parameter force-dependent rate models [Eqs. (7)–(9), respectively]. We did not examine the five-parameter model [Eq. (6)] because for our physical model, the curvature of the unbound state is close to constant and so the fifth parameter should have a very small effect. The fitting parameters, which started from random initial seeds, converged for each fit. As expected, the four-parameter model was indistinguishable from the data, the three-parameter model slowly deviated, while the two-parameter model rapidly deviated. The parameters predicted by the fits are summarized in Table I.

### IV. DISCUSSION

A careful analysis of Kramer’s theory leads to a series of simple equations that approximate the exact solution, the five-, four-, three-, and two-parameter force-dependent rate equations [Eqs. (6)–(9), respectively]. The simplest of these is the oft-used Bell’s equation [Eq. (1)]; the most general is of the form of previous equations based on Kramer’s theory [Eq. (2)]. Comparison of these equations with a simulation of a simplified system reveals increasingly good agreement with increasing complexity, as expected, providing independent confirmation of the approximations made in the derivation in Appendix B.

The parameters from Bell’s equation were much poorer estimates of the exact values than either the three- or four-parameter equations. In our simple model, by adding a single parameter to Bell’s equation, we gain considerable accuracy in our parameter predictions. However, although this simple

<table>
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<th>Parameter set B</th>
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<td>0.3379</td>
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<tr>
<td>$\kappa'/\kappa^2$ (nm/k$_B T$)</td>
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<tr>
<td>0.278</td>
<td>0.260</td>
<td>\cdots</td>
</tr>
<tr>
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</tr>
<tr>
<td>0.107</td>
<td>0.211</td>
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simulation suggests that the three-, four-, and five-parameter models have biological application, direct evidence is necessary to show biological relevance.

Deviations from Bell’s equation can be seen in some biologically relevant cases, even in published data fitted with Bell’s equation. For example, Marshall et al. reported an adequate fit to their data with Bell’s equation, yet the three- and four-parameter rate equations fit their data much better (see Fig. 4). We would therefore conclude that the data are best fitted by a three-parameter model and that \( \langle \tau \rangle_0 = 1.9 \) s and \( x_c = 0.32 \) nm (differences of 15% and 30%, respectively, from their reported values). Bell’s equation fit is especially poor when the data are plotted as a dissociation rate as a function of force (Fig. 4, inset), indicating that even an apparently adequate fit can lead to very poor estimates of physical parameters. The more complicated equations for strain-dependent rate constants are probably overlooked because of the qualitative agreement of the simpler Bell’s equation.

A. Comparison with previous studies

This is not the first study to show the limitations of Bell’s equation. For example, if one assumes a potential energy–strain function, one can approximate the solution for the attachment rate \( k_a \) from Kramers’ theory [Eq. (5)]

\[
V(x) = V_T(x/x_c)^2 \quad \text{for} \quad x < x_c, \quad \text{and} \quad -\infty \quad \text{for} \quad x \gg x_c \]

and “linear-cubic” \( V(x) = (3/2)V_T(x/x_c) - 2V_T(x/x_c)^3 \) potentials have the same general form. Consequently, this form has been proposed as a general relationship for the load dependence of a rate constant, which we refer to as the Dudko–Hummer–Szabo (DHS) equation:

\[
k_a \approx k_0 \exp \left( -\frac{2V_T}{k_BT} \left( 1 - \frac{V_T}{V_T'} \right)^{1/\nu} \right).
\]

This model has four free parameters \( k_0, x_c, V_T, \) and \( \nu \). For the physical model presented here, the fits from this equation were very similar to the fits from the four-parameter force-dependent rate equation (see Fig. 5). Thus, we conclude that for fitting experimental results, there should be little difference between parameters estimated from the fits of the DHS equation [Eq. (10)] and those from the four-parameter equation [Eq. (7)].

When one estimates physical parameters from noisy data, one must be careful not to use an equation with too many free parameters. For example, if a data set is not significantly different from the three-parameter force-dependent rate equation, then the physical parameters estimated from the four-parameter equation should be treated with suspicion as they are likely contaminated by the noise. Here, we provide a method to estimate particular parameters of interest
from the data, and also to estimate the maximum number of parameters in any fit. This latter estimate can be important when using an equation like the DHS equation to predict, say, the potential barrier height (V_T).

To demonstrate how our theory can be used in conjunction with previous theories, we added different amounts (5% and 0.05%) of random noise to our Smoluchowski solution with parameter set A. The noisier data (5%) could be well fitted by the three-parameter model while being significantly different from Bell’s equation. The less noisy data (0.05%) was well fitted by the four-parameter model while being significantly different from the three-parameter model. For the noisier data, the DHS equation predicts V_T=(12.1±4.7)k_B T, while with less noisy data, the DHS equation predicts V_T=(14.87±0.07)k_B T (the exact value is V_T=13.81k_B T). For the noisy data, the parameter estimate varies by more than ±35%, while for the less noisy data, the estimate varies by less than ±0.5%. Since this model has four parameters and is well fitted by a three-parameter model, we may understand this variation: the DHS equation is overspecified (see Fig. 5). Thus, our model gives us a means to evaluate the accuracy of parameters predicted by other models.

**B. Load dependence of entropy and Bell’s equation**

The five- and four-parameter force-dependent rate equations fundamentally differ from Bell’s equation in that they provide an estimate for the load dependence of the unbound and transition state entropies. To understand this difference and why Bell’s equation neglects these terms, it is useful to briefly examine the history of Bell’s equation.

Based on empirical observations, Zhurkov\(^{23}\) proposed the following equation for metal fracture:

\[
  k_a = \sqrt{k/m} \exp(-\Delta V_T(0) - Fx_c/k_B T),
\]

Where \(\Delta V_T(0)\) is the difference between the potential energy of the transition state and that of the unbound state at zero load. Equation (11) becomes identical (save for a factor of 1/2π) to the prediction of the transition state theory, with the approximation \(\Delta V_T(F) = \Delta V_T(0) - Fx_c\) (this potential energy approximation is correct to first order in \(\Delta x_c\), as shown in Appendix B). The difficulty in measuring \(k/m\) for different metals makes it quite likely that a factor of 1/2π could be missed. Later, Bell\(^{15}\) postulated this equation to hold for the rupture of protein-protein bonds.

Alternatively, the Arrhenius equation (a phenomenological relation proposed to fit the temperature dependence of rate constants) can be written as

\[
  k_a = A \exp(-\Delta V_T(F)/k_B T).
\]

Note that the prefactor \(A\) is assumed independent of applied load. By approximating \(\Delta V_T(F) = \Delta V_T(0) - Fx_c\), we again recognize the linear force-dependent model. However, neither of these equations is appropriate (in general) for a system governed by Kramers’ theory.

Even after some simplifying assumptions, the force dependence of Kramers’ theory differs fundamentally from Eqs. (11) and (12). We may write the equivalent expression of Eq. (11) for the system presented here as (see Appendix B):

\[
  k_a = \frac{1}{2\pi\gamma} \sqrt{\left(\kappa_0 - \kappa_0'\frac{F}{k_B T}\right)\left(\kappa_1 - \kappa_1'\frac{F}{k_B T}\right)} \times \exp(-\Delta V_T(F)/k_B T).
\]

In Eq. (13), it is apparent that there is an additional force dependence that is missing from equations derived from the transition state theory or from the Arrhenius equation assuming a force-independent preexponential factor. To give this load dependence an intuitive explanation, it is useful to use a thermodynamic form of the rate equation:

\[
  k_a \approx \exp(\Delta S_T/k_B) \exp(-\Delta V_T/k_B T),
\]

where we have assumed that volume and pressure changes are negligible in the course of the reaction. We can immediately see that the force dependence in Eq. (13) arises from the load dependence of entropy.

In broad terms, entropy is related to the volume that a particular state takes up in phase space. For a simple quadratic potential (in the high-viscosity limit of Kramers’ theory), the entropy is directly proportional to the logarithm of the curvature of the potential energy–strain curve. Thus, the force dependence seen in Eq. (13) arises from the fact that the curvature of the transition state, and therefore its entropy, is load dependent. The more load sensitive the curvature of a particular state is, the more load dependent the rate constant will be. A quadratic potential is very insensitive to strain, while a highly nonlinear potential is more sensitive. Therefore, the choice of a linear spring made this effect especially small in our idealized system.

Kramer’s theory, as opposed to transition state theory, is applicable to most reactions that occur in solution.\(^{24}\) In general, Kramer’s theory predicts that the change in entropy between the unbound and transition states is load dependent. Bell’s equation, whether derived from transition state theory or from the Arrhenius equation, neglects this load-dependent entropy. Here, we have shown that this approximation holds for small forces (i.e., small \(\Delta x_c\)) but leads to errors at larger forces. These larger forces occur in some biologically relevant situations.

**C. Conclusions**

Current experiments are reaching the limit of Bell’s equation, as shown in Fig. 4 and by Schlierf and Rief\(^{15}\) and Dudko et al.\(^{17}\) For most applications, it would be worthwhile to use one of the more exact approximations (either the five-, four-, or three-parameter equation) to fit data. Then, if the data still systematically deviate from the model, more complicated forms must be derived (by the methods outlined in Appendix B). If systematic deviations are not found, then one may estimate the parameters of the potential (1/\(\kappa_0 + 1/\kappa_1\), \(\kappa_0'/\kappa_0\) and \(\kappa_1'/\kappa_1\)) and can have more confidence in conclusions extrapolated from the fit (such as the value of the characteristic distance or the detachment rate in the absence of load). As more detailed measurements are possible and different systems are analyzed, it becomes critical to...
understand the underlying assumptions of these force-dependent rate equations so that we understand when they will fail and can apply the appropriate correction.

**ACKNOWLEDGMENTS**

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**APPENDIX A: DERIVING KRAMERS’ THEORY FROM THE SMOLUCHOWSKI EQUATION**

Starting from the Smoluchowski equation

\[
\frac{\partial w}{\partial t} = \frac{k_b T}{\gamma} \frac{\partial^2 w}{\partial \varepsilon^2} + \frac{1}{\gamma} \frac{\partial}{\partial \varepsilon} \left( \frac{dV}{d \varepsilon} w \right),
\]

(A1)

we make the following definition:

\[
j = \frac{k_b T}{\gamma} \frac{\partial w}{\partial \varepsilon} + \frac{1}{\gamma} \frac{dV}{d \varepsilon} w.
\]

(A2)

Then, we may rewrite Eq. (A1) as

\[
\frac{\partial w}{\partial t} = \frac{\partial j}{\partial \varepsilon}.
\]

(A3)

Equation (A3) is written in terms of the probability distribution \(w(x,t)\), but we would like to write our differential equation in terms of the bound and unbound states. We define the probability of being in the region of space where binding may occur \((x_c \leq \varepsilon \leq x)\), \(\eta_b\), as

\[
\eta_b(t) = \int_{x_c}^{x} w d\varepsilon,
\]

(A4)

where \(x_c\) is the local maximum in Fig. 1. Then, we can rewrite Eq. (A3), by assuming certain smoothness criteria are satisfied, as

\[
\frac{d\eta_b}{dt} = -j(x_c,t).
\]

(A5)

By rewriting Eq. (A2) and rearranging, we have

\[
j \frac{\gamma}{k_b T} \exp(V(x)/k_b T) = \frac{\partial}{\partial \varepsilon} (w \exp(V(x)/k_b T)).
\]

(A6)

If the potential energy barrier for binding is large compared to \(k_b T\), then the system is in “quasi-steady-state” in that we may assume that \(j\) is approximately a constant, and in the neighborhood of \(\varepsilon = 0\) and \(\varepsilon = x^+\),

\[
w_0 \approx N_0 (1 - \eta_b) \exp(-V(\varepsilon)/k_b T),
\]

\[
w_+ \approx N_0 \eta_b \exp(-V(\varepsilon)/k_b T),
\]

(A7)

where \(N_0\) and \(N_+\) are normalization constants:

\[
\frac{1}{N_0} = \int_{-\infty}^{x_c} \exp(-V(\varepsilon)/k_b T)d\varepsilon,
\]

(A8)

\[
\frac{1}{N_+} = \int_{x_c}^{\infty} \exp(-V(\varepsilon)/k_b T)d\varepsilon.
\]

By using the quasi-steady-state assumption, we can rewrite Eq. (A6) by assuming that \(j\) is a constant:

\[
j \approx k_b T (-N_0 (1 - \eta_b) + N_+ \eta_b) \gamma f_{x^+} \exp(V(\varepsilon)/k_b T)).
\]

(A9)

Now, if we rewrite Eq. (A5) with our new expression for \(j\) and rearrange a few terms, we have

\[
\frac{d\eta_b}{dt} = \frac{k_b T}{\gamma f_{x^+} \exp(V(\varepsilon)/k_b T)} (-N_0 \eta_b + N_+ (1 - \eta_b)).
\]

(A10)

Equation (A10) tells us about \(\eta_b\), but we want to know about \(\eta_b\), which is the number of bound molecules. We define \(n_b\) as

\[
n_b = \int_{x_c}^{\infty} \int_{-\varepsilon}^{\varepsilon} \sqrt{\frac{m}{2\pi k_b T}} \exp(-m\dot{\varepsilon}^2/2k_b T)wd\varepsilon d\varepsilon
\]

\[
= \int_{x_c}^{\infty} \text{erf} \left( \frac{V_c - V(\varepsilon)}{k_b T} \right) w d\varepsilon,
\]

(A11)

where

\[
\dot{\varepsilon}_{\text{max}} = \sqrt{\frac{2(V_c - V(\varepsilon))}{m}}.
\]

(A12)

With Eq. (A7), this becomes

\[
n_b = \int_{x_c}^{\infty} \text{erf} \left( \frac{V_c - V(\varepsilon)}{k_b T} \right) \eta_b N_+ \exp(-V(\varepsilon)/k_b T) d\varepsilon.
\]

(A13)

And if we define the following variables for simplicity:

\[
A = \int_{x_c}^{\infty} \text{erf} \left( \frac{V_c - V(\varepsilon)}{k_b T} \right) N_+ \exp(-V(\varepsilon)/k_b T) d\varepsilon,
\]

(A14)

\[
B = \frac{k_b T}{\gamma f_{x^+} \exp(V(\varepsilon)/k_b T)}
\]

(A15)

we may differentiate Eq. (A13) with respect to time and substitute in Eq. (A10):

\[
\frac{dn_b}{dt} \approx -(BN_c + BN_0) n_b + ABN_0.
\]

(A16)

where, if we define the following two variables:

\[
k_a = ABN_0
\]

(A17)

and

\[
k_d = BN_c + (1 - A)BN_0.
\]

(A18)

we reduce Eq. (A16) to the familiar form
\[ \frac{dh_b}{dt} = k_a (1 - n_b) - k_d n_b. \]  

(A19)

### APPENDIX B: SIMPLE FORCE-DEPENDENT RATE EQUATIONS FROM KRAMERS’ THEORY

The expression for \( k_a \) from our derivation of Kramers’ theory is

\[ k_a = ABN_0. \]  

(B1)

In general, \( A, B, \) and \( N_0 \) depend on force. For the following derivation, we assume that \( A \) is close to 1 and load independent (which is true for deep wells). We examine the force dependence of \( B \) and \( N_0 \) for an arbitrary potential:

\[ V(e, F) = V(e, 0) - F e. \]  

(B2)

We assume that three critical points of \( V \) exist, corresponding to the unbound, transition, and bound states. A Taylor expansion of \( V(e, F) \) about any one of the three critical points, \( x_m(F) \), gives

\[ V(e, F) = V(x_m(F), 0) + \frac{1}{2} \frac{d^2 V(e, 0)}{de^2} \Delta x_m^2 + O((\Delta x_m)^3). \]  

(B4)

where we have defined \( \Delta x_m = x_m(F) - x_m(0) \). We may find the value of \( x_m(F) \) by noting that the derivative of \( V(e, F) \) is zero there:

\[ \frac{dV(e, F)}{de} \bigg|_{x_m(F)} = \frac{dV(e, 0)}{de} \bigg|_{x_m(F)} - F = 0. \]  

(B5)

By doing a Taylor expansion of \( dV/de \) about \( x_m(0) \), we find

\[ -F + \frac{d^2 V(e, 0)}{de^2} \bigg|_{x_m(0)} \Delta x_m + O(\Delta x_m^2) = 0. \]  

(B6)

For convenience, we denote the curvatures of \( V \) at \( x_m(0) \) as \( k_m \). Then we may write

\[ x_m(F) = x_m(0) + \frac{F}{k_m} + O(\Delta x_m^2). \]  

(B7)

Then, plugging back into Eq. (B4), we have

\[ V(x_m(F), F) = V(x_m(0), 0) - F x_m(0) - \frac{F^2}{2k_m} + O(\Delta x_m^2). \]  

(B8)

### a. Potential energy at the critical point

A Taylor expansion about \( x_m(0) \) yields

\[ V(x_m(F), F) = V(x_m(0), 0) - F x_m(0) - F \Delta x_m 
+ \frac{1}{2} \frac{d^2 V(e, 0)}{de^2} \Delta x_m^2 + O(\Delta x_m^3). \]  

(B4)

b. Curvatures at the critical point

We do a Taylor expansion of the curvature of \( V(\epsilon, F) \) about \( x_m(0) \):

\[ \frac{d^2 V(\epsilon, F)}{de^2} \bigg|_{x_m(F)} = \kappa_m + \frac{d\kappa_m}{de} \frac{F}{\kappa_m} + O(\Delta x_m^2). \]  

(B9)

Plugging Eqs. (B8) and (B9) into Eq. (B3), we end up with

\[ V(\epsilon, F) = V(x_m(0), 0) - F x_m(0) - \frac{F^2}{2\kappa_m} 
+ \frac{1}{2} \left( \kappa_m + \frac{d\kappa_m}{de} \frac{F}{\kappa_m} \right) (\epsilon - x_m(F))^2, \]  

(B10)

where we neglect terms of order \( \Delta x_m^2 \) and \( (\epsilon - x_m(F))^3 \).

c. Finding the terms in Kramers’ equation

The expression for \( N_0 \) is then

\[ N_0 = \sqrt{\frac{\kappa_0 + (d\kappa_0/de)(F/k_0)}{2\pi k_b T}} \exp \left( \frac{V_0 - F x_0 - (F^2/2k_0)}{k_b T} \right), \]  

(B11)

where \( \kappa_0 \) is the curvature at the unbound state in the absence of load.

The expression for \( B \) is

\[ B = \frac{k_b T}{\gamma} \sqrt{\frac{\kappa_t - (d\kappa_t/de)(F/k_t)}{2\pi k_b T}} \times \exp \left( \frac{-V_t + F x_t - (F^2/2k_t)}{k_b T} \right), \]  

(B12)

where \( \kappa_t \) is the negative curvature at the transition state in the absence of load. We define it as the negative curvature to ensure that \( \kappa_t > 0 \).

Therefore, the general expression for \( k_a \), correct to order \( \Delta x_m^2 \) and \( (\epsilon - x_m(F))^3 \), is

\[ k_a = \sqrt{\left( \frac{\kappa_0 + \kappa_0'(F/k_0)}{\kappa_t - \kappa_t'(F/k_t)} \right)} \]  

\[ \times \exp \left( \frac{-V_f + F x_f - F^2(1/2\kappa_0 + 1/2\kappa_t)}{k_b T} \right), \]  

(B13)

where \( V_f \) is the difference in potential energy between the transition and unbound states.

This equation has five free parameters that may be varied to fit data \((k_0, \kappa_0'/\kappa_t', \kappa_0'/\kappa_t, x_t, \text{ and } 1/\kappa_t + 1/\kappa_0)\). Equation (B13) should be an excellent approximation for most biological applications. To put it in a more useful form, we may write

\[ k_a = k_0 \sqrt{\left( \frac{1 - \frac{1}{2\kappa_t'}}{\frac{1}{2\kappa_0'}} \right)} \left( \frac{1 + \frac{1}{2\kappa_0'}}{\frac{1}{2\kappa_t'}} \right) \times \exp \left( \frac{F x_f - F^2(1/2\kappa_0 + 1/2\kappa_t)}{k_b T} \right), \]  

(B14)
1. Simplifications

Neglecting the smallest term in Eq. (B14),
\[
k_a = k_0 \sqrt{1 + \left( \frac{\kappa_0}{\kappa_r} - \frac{\kappa_r}{\kappa_0} \right) F} \times \exp\left( \frac{\int x_c - F^2 (1/2 \kappa_r + 1/2 \kappa_0)}{k_b T} \right).
\] (B15)

This equation has four free parameters that may be varied to fit data \((k_0, \kappa_0/\kappa_r - \kappa_r/\kappa_0, x_c, 1/\kappa_r + 1/\kappa_0)\). In the special case that \(\kappa_0\) arises from an approximately linear spring (with spring constant \(k\)), as in our simple example shown in Fig. 1, then Eq. (B15) may be written as

\[
k_a = k_0 \sqrt{1 - \frac{\kappa_r}{\kappa_0} x_c} \exp\left( \frac{\int x_c - F^2 (1/2 \kappa_r + 1/2 \kappa_0)}{k_b T} \right).
\] (B16)

Note that if we neglect terms of order \(\Delta x_m (e - x_m)^2\) [as well as \(\Delta x_m^2\) and \((e - x_m)(F)^3\)] in the potential energy approximation [Eq. (B10)], we get

\[
k_a = k_0 \exp\left( \frac{\int x_c - F^2 (1/2 \kappa_r + 1/2 \kappa_0)}{k_b T} \right),
\] (B17)

which is a lower-order approximation of Eq. (B13). This equation has three free parameters that may be varied to fit data \((k_0, x_c, 1/\kappa_r + 1/\kappa_0)\).

If we further neglect terms of order \(\Delta x_m (e - x_m)\), we get

\[
k_a = k_0 \exp\left( \frac{\int x_c}{k_b T} \right).
\] (B18)

A still lower-order approximation of Eq. (B13), which is Bell’s equation.

Note that in each of the above equations, we may replace the stiffness of the unbound state, \(\kappa_0\), with the spring constant \(k\) in our simple mechanical model shown in Fig. 1. We do this throughout the paper.

APPENDIX C: RATE CONSTANT ESTIMATION

We integrated the Smoluchowski equation by using a Crank–Nicholson implicit/explicit method. We used two general methods to estimate rate constants from these numerical solutions. The first is appropriate for reactions that reach half the equilibrium bound value relatively quickly (\(\tau_{1/2} \approx 1 \mu s\)). The second is appropriate for reactions with \(\tau_{1/2} > 1 \mu s\).

1. Rate constant estimation: Rapid reactions

A unimolecular binding or unbinding reaction of the sort we consider here is not governed by a first order ODE. Instead, it is governed to acceptable accuracy by the Smoluchowski equation, which is a second order partial differential equation. The significant deviations between the two are typically over within tenths or hundredths of nanoseconds, so for most practical applications, a first order ODE is an excellent approximation to a unimolecular binding or unbinding reaction. The “gold standard” to which we would like to hold any model is the best fit in a least squares sense. Thus,

for relatively rapid reactions, we fit the solution to the Smoluchowski equation with a first order ODE and compared the parameters of that fit to those predicted by Kramers’ theory.

2. Rate constant estimation: Slow reactions

For slow reactions, it becomes impractical to integrate the Smoluchowski equation until it approaches equilibrium. Instead, we start with the assumption that the proportion of bound systems from the solution to the Smoluchowski equation, \(n_b(t)\), was generated by a first order ODE. In that case, we may estimate the overall reaction rate \((k_a + k_d)\) with the following equation:

\[
k_a + k_d = - \frac{d}{dt} \left( \ln \frac{d n_b}{dt} \right). 
\] (C1)

Additionally, since we may calculate the equilibrium constant exactly and we know that any solution to Smoluchowski’s equation tends to the equilibrium distribution, we may substitute \(K_{eq} k_a\) for \(k_d\) and solve for \(k_a\):

\[
k_a = \frac{1}{1 + K_{eq}} \frac{d}{dt} \left( \ln \frac{d n_b}{dt} \right). 
\] (C2)

For very slow reactions that start from approximately zero systems in the bound state \((n_b(0) \approx 0)\), we can simplify Eq. (C2) to

\[
k_a = \frac{d n_b}{dt}. 
\] (C3)

In Fig. 6, we use this technique to show that Smoluchowski’s equation converges to Kramers’ prediction of \(k_a\) within a few tens of nanoseconds.