Dynamic Response of Adhesion Complexes: Beyond the Single-Path Picture

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We analyze the response of molecular adhesion complexes to increasing pulling forces (dynamic force spectroscopy) when dissociation can occur along either one of two alternative trajectories in the underlying multidimensional energy landscape. A great diversity of behaviors (e.g. non-monotonicity) is found for the unbinding force and time as a function of the rate at which the pulling force is increased. We highlight an intrinsic difficulty in unambiguously determining the features of the energy landscape from single-molecule pulling experiments. We also suggest a class of “harpoon” stickers that bind easily but resist strong pulling efficiently.

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The last decades have witnessed a remarkable development of physical investigation methods to probe single molecules or complexes by various micromanipulation means. New techniques have been put forward to probe the unfolding of proteins and to quantify the strength of adhesion structures\textsuperscript{1.2}. An important step in this direction is the proposal of the group of Evans to use soft structures to pull on adhesion complexes or molecules at various loading rates (dynamic force spectroscopy)\textsuperscript{3}. Moving the other end of the soft structure by force that increases linearly in time at constant velocity induces on the complex a pulling force which increases linearly in time \( f = r t \). Measuring the typical rupture time \( t_{\text{typ}} \) yields a typical rupture force \( f_{\text{typ}} = r t_{\text{typ}} \) that depends on the pulling rate \( r \). This provides information as to the energy landscape of the bound complex. Indeed, in many situations one observes a linear increase of \( f_{\text{typ}} \) with \( \log(r) \), which can be understood within a simple adiabatic Kramers picture for the escape from a well (bound/attached state) over a barrier of height \( E \) located at a projected distance \( x \) from the well along the pulling direction. The progressive increase of the force results in a corresponding increase of the escape rate, so that, in agreement with some experiments\textsuperscript{1}, the typical rupture force increases logarithmically with \( r \): 

\[
    f_{\text{typ}} \approx k_B T / x \ln[r/(k_B T \omega)] ,
\]

where \( \omega \) is the escape rate in the absence of force. The rupture time on the other hand decreases with \( r \). The occurrence in some cases of two successive straight lines in a \( [f_{\text{typ}}, \log(r)] \) plot has been argued to be the consequence of having two successive barriers along the 1D escape path, the intermediate one showing up in the response at fast pulling rates\textsuperscript{1} (Figs. 3 and 4). Other theories have tried to back up more complete information as to the overall effective 1D potential landscape by an analysis of the probability distribution for rupture time and of the statistics of trajectories before rupture\textsuperscript{7,8}. Assemblies in series and in parallel of such 1D bonds have also been considered\textsuperscript{2,3,9,10}.

In this Letter we point out limitations arising from the a priori assumption of a single-path topology of the energy landscape for the interpretation of such experiments. From the analysis of simple examples with a two-path topology, we draw three conclusions: (i) first, the dependence of the rupture force and rupture time on the pulling rate can take various forms, including non-monotonic behavior (see e.g. Figs. 3 to 6). (ii) Second, the main features of the energy landscape can not be unambiguously deduced from a \( [f_{\text{typ}}, \log(r)] \) plot, as very different landscapes can yield similar curves (Fig. 3). (iii) Third, we propose simple “harpoon” designs (Fig. 1c and d) for functionally efficient stickers that can bind easily but resist strongly in a range of pulling forces (Fig. 1).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Sketch of the topology of the main valley of the energy landscape for a few examples. \( 0 \) denotes the fundamental bound state, \( A \) and \( B \) are local minima, and \( a, a', b \) and \( b' \) are passes to overcome. To the right (increasing values of \( x \)) of the last passes is the continuum that describes unbound states. (a) classical single-path scheme. (b,c,d) unbinding can occur through two alternative routes \( \alpha \) and \( \beta \).}
\end{figure}
FIG. 2. Classical picture for a single-path energy landscape (Fig. 1a): the probability density $P(f)$ for unbinding at force $f$ is plotted in grey-scale as a function of the pulling rate $r$. The typical force $f_{\text{typ}}$ (locus of the maximum of $P$) is highlighted with a dashed-line. Plotted curves correspond to $E_{\alpha} = 12, x_{\alpha} = 0.5, E_A = 9, x_A = 1$, and $E_a = 20, x_a = 2$. At very low pulling rates unbinding is not affected by the pulling and proceeds over barrier $a$ with a “spontaneous” rate $\omega_0 \exp(-E_a)$. For larger pulling rates the typical unbinding force $f_{\text{typ}}$ increases linearly with $\log(r)$, with a slope proportional to $1/x_a$. Increasing further the pulling rate can lead to a steeper slope $\propto 1/x_a$, corresponding to escape over the inner barrier $a'$. These asymptotes are depicted with solid lines. The dashed arrows along the drawings indicate which pairs of energy well and barrier are probed in these asymptotic limits. Inset: mean rupture time against pulling rate.

FIG. 3. Switch geometry (Fig. 1b): plot of the same quantities as in Figure 2 for $E_a = 20, x_a = 0.5$, and $E_b = 30, x_b = 2$. At low pulling rates unbinding is controlled by escape over $a$ whereas for large values of $r$ it occurs mostly over $b$: the slope of the unbinding force (average or typical) decreases from $1/x_a$ to $1/x_b$.

Obviously for real binding/adhesion complexes, there are numerous (conformational) degrees of freedom, and the configurational space is clearly multidimensional. This allows for complex energy landscapes and various topologies for the structure of their valleys and passes 12. Only the probing (pulling) is unidirectional. We note in passing that even for more macroscopic sticky systems, usual adhesion tests for soft adhesives often show up hysteresis loops associated with the existence of more than one degree of freedom 14. We do not attempt here an exhaustive exploration of effects allowed by the multidimensionality of the phase space, but rather focus on a few simple two-path topologies (Fig. 1a), to argue for the three points mentioned above.

The three examples we consider, sketched in Figure 1b, c, and d, correspond to simple hairpin schemes whereby detachment can proceed through two alternative routes $\alpha$ and $\beta$. These simple quasi 1D schematic situations can be conveniently dealt with using an adiabatic Kramers theory, which has been shown to be an efficient way of obtaining semi-quantitatively correct answers 14.

A common set of notations can be ascribed for all cases (Fig. 1a). From the fundamental bound state “0”, the route $\alpha$ for escape (detachment) is over barriers $a$, of height $E_a$ located at a projected distance $x_a$ from “0”. Alternatively, escape can occur through branch $\beta$, over barrier $b$, of height $E_b$, and projected distance $x_b$. All energies and projected distances are measured relative to the state “0” (i.e. $E_0 = 0$ and $x_0 = 0$). Intermediate barriers $a'$, $b'$ and local minima A and B may exist, with energies $E_{aa'}, E_{bb'}, E_A, E_B$ (all positive), and projected distances $x_{aa'}, x_{bb'}, x_A, x_B$. In line with typical values from experiments, we choose to write energies in units of $k_B T \approx 4$ pN nm and distances in nm.

Practically, we describe the time evolution of the probabilities of being in the potential minima (bound states) using “chemical” transition rates over the barriers as given by the Kramers formula. We furthermore assume the attempt frequencies to be constant and all equal to $\omega_0$ which provides the only intrinsic time-scale in the problem, so that the transition rate from minimum $I$ over the neighboring barrier $i$ is $\omega_0 \exp(-E_i-E_I)+f(t)/(x_i-x_I))$. For the plots of Figures 2 to 4 we take arbitrarily $\omega_0 = 10^8$ s$^{-1}$. Jump over the rightmost barrier ($a$ or $b$) of either path corresponds to rupture leading to escape to $x \to \infty$.

We focus on the case where either $E_{bb'}$ or $E_b$ is larger than $E_a$, so that $\alpha$ is the “natural” route by which attachment and detachment proceeds in the absence of pulling. We also limit ourselves to simple scenarios in which the force is linearly increased in time $f = rt$.

For further reference we recall the classical single-path scenario (Fig. 1a) in Figure 2 for a typical set of parameters, and then we turn to a brief analysis of the three
First case: switch – Topology as in Figure 1b. Escape occurs through either barrier $a$ or barrier $b$ both located downwards in the pulling direction ($x_a, x_b > 0$). The escape proceeds through path $\alpha$ at weak pulling rates as $E_a < E_b$, but if $x_b > x_a$ it can switch to path $\beta$ for pulling forces $f$ large enough such that $E_a - f x_a > E_b - f x_b$. The result (see Fig. 3) is then a succession of two straight lines of decreasing slopes in the $\log(r) \times \log(p)$ plot, the first one (slope $\propto 1/x_a$) characteristic of the spontaneous route $\alpha$ while the second (slope $\propto 1/x_b$) provides information on the alternative route $\beta$. In the trivial case $x_a > x_b$, route $\beta$ is never explored so that the classical single-path picture applies.

To clarify the calculation leading to the plot in Figure 3, we describe the evolution of the probability of attachment $p(t)$ at time $t$ of the system initially attached at time $t = 0$ [$p(0) = 1$] by

$$\partial_t p(t) = -\omega_0 (e^{-E_a f} + e^{-E_b f} - 1) - \omega_0 (e^{-E_a f} + e^{-E_b f} - 1) p(t)$$

Solving (1) numerically with $f = rt$ yields $p(t)$ and therefore the probability density $P(f) = -\frac{1}{f} \partial_f p(f)$ for the unbinding force. The typical values of $f$ are highlighted in the plots, with the whole distribution $P(f)$ suggested through a gray-scale. Similar procedures will be used in the following examples, with thermal equilibrium between the bound states assumed as initial conditions.

Second case: harpoon – Topology similar to the previous one but with $x_a < 0$ (Fig. 3c). The main feature here is that as the pulling force increases, the probability to escape over $a$ decreases. Therefore the system gets “stuck” in route $\beta$. If the barrier $E_b$ is infinite (left side of Fig. 3d), there is a finite probability $p_{\infty} = \exp(-\frac{\omega_0}{E_a})$ that unbinding never occurs. For a finite but high barrier $E_b$, pulling eventually results in unbinding but at high rupture forces (see Fig. 3d). The topology thus allows here to form “easily” (i.e. over barrier $a$) a “harpoon” sticker that can resist strong pulling. Correspondingly the mean unbinding time increases first with pulling rate (a phenomenology connected to the negative resistance analyzed in Ref. [13]), before decreasing for larger values when activated escape over $b$ dominates. Note that the probability distribution $P(f)$, now consists of two separate ensembles, which coexist over a narrow region of pulling rates. This is in contrast with Figure 3 where there is a continuous evolution of a single cloud.

Third case: combo – The alternative route consists of two barriers and a local minimum $B$ (Fig. 3d), and we focus on the case where $E_B$ is smaller than the two others. Thanks to the increased complexity and number of parameters in this case many scenarios can occur, covering features already unveiled in Figures 3 to 5 (e.g. switch and harpoon). More intricate pictures can also show up, as depicted in Figure 5. An explanation of this example is given in the caption, illuminating how for low or high

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FIG. 4. “Harpoon” geometry (Fig. 1c): plot of the same quantities as in Figure 2 for $E_a = 20$, $x_a = -2$, and $E_b = 40$, $x_b = 2$. Pulling here impedes unbinding through the “spontaneous” route $\alpha$, so that as soon as the rate is strong enough for pulling to affect unbinding, the escape is controlled by the larger barrier $b$, resulting in an upward jump of the typical unbinding force and time. Inset: the average unbinding time is here non-monotonic.

FIG. 5. “Selective harpoon” from the combo topology (Fig. 1d): same quantities as in Figure 2 for $E_a = 20$, $x_a = 2$, $E_B = 10$, $x_B = 0.5$, $E_B = 5$, $x_B = 1.5$, and $E_b = 27$, $x_b = 2.5$. At low pulling rates the spontaneous path $\alpha$ is used. Upon increase of $r$, larger forces are employed and the minimum $B$ becomes favorable as compared to 0. As $E_B$ is not too large, equilibration of population then empties 0 in $B$, so that escape eventually occurs from $B$ over $b$, resulting in a higher straight line of slope $\propto 1/(x_b - x_B)$. At even higher pulling rates, because $x_a > x_B$, the escape over $a$ becomes faster than this equilibration, and therefore, path $\alpha$ is used again. Barrier $a$ controls the behavior at low and high rates, but in an intermediate window, a stronger bonding is provided by barrier $b$. The typical (dashed line) or average unbinding force is non-monotonic.
pulling rates barrier $a$ controls the behavior, whereas for intermediate values, the secondary and stronger barrier $b$ limits unbinding. Two features are striking. First, the unbinding force (typical or average) is no more monotonic. Second, branch $\beta$ results in a strengthening of the barrier from $a$ to $b$ from $B$ in case (c). Neither the topology, nor the location of the probed segment of the energy landscape can be asserted from such data sets.

**Discussion** — With the three simple examples above, we have clearly enlarged the numbers of behaviors that one may obtain from a classical dynamic force spectroscopy method (see Figs. 3 to 5). Conversely, we also want to stress the second point (ii) mentioned in the introduction: simple patterns (e.g. the succession of two lines of increasing slopes) can be the outcome of many diverse landscapes. For example, Figure 6 displays force-rate curves similar to that of Figure 3, but that correspond to sensibly different landscapes. Not only are the typical and average unbinding forces very similar in the three cases, but so are the probability distributions for most values of $r$. Only close to the cross-over between the two straight lines can slight differences be detected. To distinguish more selectively possible landscapes, it may be necessary to use other temporal sequences than the simple $f = rt$, e.g. to reveal equilibration processes between local minima.

Eventually we would like to emphasize that the harpoon geometries proposed here constitute a very obvious paradigm for efficient stickers. Attachment of the sticker can proceed through route $\alpha$ with a possibly not too high barrier $E_a$. The harpoon configuration then allows to benefit from the much stronger $b$ barrier for a given window of pulling forces, making the sticker more efficient in these conditions. This “hook” design is obviously a favorable strategy for adhesion complexes, the function of which is to maintain adhesion under the action of well-defined tearing stresses. It would be surprising if advantage was not taken of this by some biological systems.

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