Effects of intermediate bound states in dynamic force spectroscopy

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We revisit here some aspects of the interpretation of dynamic force spectroscopy experiments. The standard theory predicts a typical unbinding force \( f^* \) linearly proportional to the logarithm of the loading rate \( r \) when a single energetic barrier controls the unbinding process; for a more complex situation of \( N \) barriers, it predicts at most \( N \) linear segments for the \( f^* \) vs. \( \log(r) \) curve, each segment characterizing a different barrier. We here extend this existing picture using a refined approximation, we provide a more general analytical formula, and show that in principle up to \( N(N+1)/2 \) segments can show up experimentally. As a consequence the interpretation of data can be ambiguous, for the characteristics and even the number of barriers. A further possible outcome of a multiple-barrier outcome is a bimodal or multimodal distribution of the unbinding force at a given loading rate, a feature recently observed experimentally.

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I. INTRODUCTION

The last decades have witnessed a revolution in the methods to observe and manipulate single bio-macromolecules or bio-molecules complexes. New micromanipulation techniques have especially been put forward to probe the folded structure of proteins and to quantify the strength of adhesion complexes (1). 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 or DFS) (7). Moving the other end of the soft structure at constant velocity induces on the end of the soft structure at constant velocity induces on the complex a pulling force that increases linearly in time \( f = rt \). Measuring the typical unbinding time \( t^* \) yields an unbinding force \( f^* = rt^* \) that depends on the pulling rate \( r \). The typical outcome of such experiments is a plot of \( f^* \) vs. \( \log(r) \) composed by a succession of straight lines with increasing slopes (force spectrum). It has been argued and that it is possible to deduce the value of some relevant structural parameters of the complex by analysing force spectra thanks to an adiabatic Kramers picture. This picture consist in considering the unbinding process as the thermally activated escape from bound states over a succession of barriers along a one-dimensional path crossing a mountainous energy landscape (7). Within this scheme, each straight line of the force spectrum witnesses the overcome of an energy barrier and its slope maps the barrier to a distance \( x \) along the pulling direction. This procedure has been shown to yield reasonable values for a few systems, and has been confirmed by numerical simulations (8).

Subsequently, theoretical studies have refined the above original model, e.g., by inclusion of rebinding events (11), study of time dependent loading rates (6), or consideration of more complex topographies (13) and topologies of the energetical landscape (12).

In the present paper we explore the potential influence of the existence of intermediate bound states on the experimental dynamic response of adhesion complexes as probed in DFS. To achieve this goal, we first revisit the analysis of the escape from a bound state consisting of an arbitrary number of barriers along a 1D path under the application of an external load (in line with earlier studies of Strunz et al. (13)), and then discuss the implications of this analysis for the interpretation of experimental data. In (III) the standard picture is recalled, together with its two underlying assumptions. In (IV), we first relax the \( a priori \) assumption of a deep fundamental bound state and provide a general expression that relates the typical rupture force to the loading rate (within a single escape rate approximation). The practical implications of this new formula [Eq. (15)] are discussed, and in particular we comment upon intrinsic ambiguities in inferring information from a \( [\log(r), f^*] \) plot. Then, we show in (V) that in the presence of multiple bound states it may be necessary to relax the other assumption (a single typical rupture force for each loading rate) as multimodal rupture force distributions naturally show up, a feature recently observed in lipid extraction experiments (14).

II. MODEL AND NOTATIONS

Figure 1 illustrates the energy landscape of a one-dimensional escape path with \( N \) energy barriers and wells. The position and energy of the \( i \)th energy well \( (i = 0 \) marking the fundamental bound state, and \( 1 \leq i \leq N-1 \) the intermediate ones) are denoted by \( x_i \) and \( E_i \), respectively. Similarly, the position and energy of the \( j \)th energy barrier are denoted by \( \tilde{x}_j \) and \( \tilde{E}_j \), respectively (where \( 1 \leq j \leq N \)). For convenience, without loosing generality, we set \( x_0 = 0 \) and \( E_0 = 0 \) for the fundamental bound state. The unbound “state” is on the right hand side of the \( N \)th barrier. If the energy differences \( \tilde{E}_i - E_i \) and \( \tilde{E}_{i+1} - E_i \) exceed \( k_B T \) the transition rates \( k_i^- \) (and \( k_i^+ \)) from the \( i \)th energy well over the left \( i \)th barrier (and right \( (i+1) \)th barrier, respectively) can be writ-
A. Single escape rate approximation

If for any experimentally relevant load $f$ the equilibration within the bound states is much faster than the escape to the unbound state, the unbinding can be described by a single load dependent escape rate $k(f)$. Following the calculation of Evans [10], if re-binding is negligible (which is the case in most experimental situations), the probability $P(t)$ of remaining in the bound state at time $t$ (the survival probability of the bond) then decreases as

$$\frac{dP(t)}{dt} = -k(rt)P(t). \quad (4)$$

The solution of this differential equation is $P(t) = \exp[-\int_{0}^{t} k(rt') \, dt']$. The probability density for unbinding between times $t$ and $t + \Delta t$ is $p_{f}(t) = -dP(t)/dt = k(rt)P(t)$, from which, after changing the variable from $t$ to $f$, one gets the probability density for the distribution of the unbinding force: $p_{f}(f) = (1/r)k(f)P(f/r)$. The typical unbinding force $f^{*}$ is defined as the peak of this probability density: $dp_{f}(f)/df|_{f=f^{*}} = 0$, which yields the simple formula

$$\frac{d\tau(f)}{df} \bigg|_{f=f^{*}} = -\frac{1}{r}, \quad (5)$$

where $\tau(f) \equiv 1/k(f)$ denotes the load dependent mean escape time. This formula gives the loading rate $r$ at which the typical unbinding force is $f^{*}$. For practical purposes it is often necessary to invert this relation to, e.g., predict the typical unbinding force for an experimentally imposed loading rate.

To set a reference for further comparison, we explicitly invert the above relation in case of a single barrier, i.e. when $\tau(f) = \tau(0) \exp(-f\bar{x}_{1})$, and obtain

$$f^{*} = \frac{k_{B}T}{\bar{x}_{1}} \ln \left[ \frac{r\tau(0)\bar{x}_{1}}{k_{B}T} \right]. \quad (6)$$

As mentioned in the introduction, the escape over a single barrier results in a single straight line in the force spectrum. The experimental observation of a linear segment consequently gives hints as to the structure of the energy landscape, in particular the slope of the segment permits to deduce a distance $\bar{x}_{1}$ between the energy well and the barrier.

B. Deeply bound fundamental state approximation

Assuming further that the fundamental bound state is much deeper than the intermediate ones: $E_{i}(f) - E_{0}(f) \gg k_{B}T$ for any experimentally relevant load $f$ (i.e. before unbinding has statistically almost certainly occurred, see Fig. 2a.), Evans has shown that the mean escape time from the fundamental bound state to the unbound state is well approximated by [10,14]

$$\tau(f) = \sum_{j=1}^{N} \frac{1}{k_{0,j}(f)} = \sum_{j=1}^{N} \frac{e^{-f\Delta x_{0,j}/k_{B}T}}{k_{0,j}(0)}. \quad (7)$$
FIG. 2 Sketch of two energy landscapes with one intermediate well. Dotted drawings: no external force. When a constant force is applied energies are lowered by \( f_x \) (dashed lines), the resulting landscapes appear in solid lines. The dotted arrows indicate which pair of wells and barriers control the kinetics at zero load. The solid arrows indicate the new limiting effective escape process at higher forces. (a) The escape from the fundamental bound state remains the limiting process whatever the pulling force. (b) The escape form the intermediate bound state energy becomes the limiting process at high forces.

This allows one to obtain an explicit \( r \) vs. \( f^* \) relationship by plugging Eq. (7) into Eq. (5), which yields the compact formula

\[
r = \left( \sum_{j=1}^{N} \frac{\Delta E_{0,j} e^{-f^* \Delta x_{0,j}/k_B T}}{k_B T (0)} \right)^{-1}. \tag{8}
\]

This equation predicts a spectrum \( f^* \) vs. \( \log(r) \) consisting of a succession of at most \( N \) segments of increasing slopes, each of which yielding an information \( \Delta E_{0,j} \) about an intermediate barrier.

**IV. BEYOND THE DEEPLY BOUND FUNDAMENTAL STATE APPROXIMATION**

In this section we relax the approximation made in the last subsection (IIIB), generalize accordingly equations (7) and (9), and discuss the experimental implications of this generalization.

**A. Refined theory**

In general, it is possible that for large enough forces one or more of the intermediate bound states become deeper than the fundamental bound state before unbinding has occurred (see Fig. 2 b). In such cases the above DBFS approximation breaks down. However we show below that it is still possible to compute rather simply the escape time \( \tau \) from the "bound state" to the "unbound state", provided we maintain the assumption of a single escape rate \( 1/\tau(f) \).

Let us put the system into its fundamental bound state, and let it evolve according to the transition rates given in Eqs. (1) and (2). Whenever the system gets into the unbound state (by making a transition over the outermost barrier) let us place it back into the fundamental bound state. The stationary state of an ensemble of such systems is characterized by a probability current, which is constant everywhere and equal to \( 1/\tau \) by definition. To calculate \( \tau \) we have to solve the following system of equations:

\[
P_i k_i^+ - P_{i+1} k_{i+1}^- = 1/\tau \quad 0 \leq i \leq N - 2, \tag{9}
\]
\[
P_N k_N^+ = 1/\tau, \tag{10}
\]
\[
\sum_{i=0}^{N-1} P_i = 1 \tag{11}
\]

where \( P_i \) denotes the probability of being in the \( i \)th bound state \( (0 \leq i \leq N - 1) \). The first \( N \) equations describe the probability current over each of the \( N \) barriers, and the last equation is just the normalization condition. These \( N + 1 \) linear equations uniquely determine the \( N + 1 \) variables \( (P_i \) and \( \tau \)), and can be solved easily in a recursive way. First, \( P_{N-1} \) can be expressed from Eq. (10), and then \( P_{N-2} \tau, \ldots, \) \( P_0 \tau \) recursively from Eq. (9) yielding

\[
P_i \tau = \frac{1}{k_i^+} + \frac{k_{i+1}^-}{k_i^+ k_{i+1}^+} + \cdots + \frac{k_{N-1}^-}{k_i^+ k_{i+1}^+ \cdots k_{N-1}^+} = \sum_{j=i+1}^{N} \frac{1}{k_{i,j}} \tag{12}
\]

where Eqs. (8), (11), and the definition (11) have been used. Note that because the \( k_{i,j} \) are only formal definitions, constructed as products and ratios of the single-barrier rates (1) and (2), they are meaningful even if \( \Delta E_{i,j} < 0 \). From the normalization (11) one can easily express \( \tau \) as

\[
\tau = \sum_{i=0}^{N-1} P_i \tau = \sum_{i=0}^{N-1} \sum_{j=i+1}^{N} \frac{1}{k_{i,j}}. \tag{13}
\]

The sum is dominated by the smallest effective rates, which are the bottlenecks of the unbinding process. Consequently, this formula remains a good approximation for \( \tau \) even if some of the barriers disappear at big loads, because the corresponding formal transition rates make negligible contributions. By indicating the load force \( f \) explicitly, we arrive at

\[
\tau(f) = \sum_{i=0}^{N-1} \sum_{j=i+1}^{N} \frac{1}{k_{i,j}(f)} = \sum_{i=0}^{N-1} \sum_{j=i+1}^{N} e^{-f \Delta x_{i,j}/k_B T} k_{i,j}(0), \tag{14}
\]

which generalizes (7). An analytic formula can be given for the \( f^* \) vs. \( r \) relationship by plugging Eq. (12) into Eq. (5) :

\[
r = \left[ \sum_{i=0}^{N-1} \sum_{j=i+1}^{N} \frac{\Delta x_{i,j} e^{-f^* \Delta x_{i,j}/k_B T}}{k_B T (0)} \right]^{-1}, \tag{15}
\]

This generalization of equation (8) is one of the main results of this paper. Let us briefly comment on immediate features of this new formula.
First, Eq. \(8\) is easily recovered from (15) assuming a DBFS. Indeed, the assumption \(E_i(f) \gg E_0(f)\) implies \(k_{0,i} \gg k_{i,j}\), if \(i > 0\) [see Eq. \(3\)] and therefore, the relation \(P_i/P_0 \ll 1\), if \(i > 0\) is deduced from Eq. \(12\). The probability to find the system in the fundamental bound state is close to 1. So, the sum over \(i\) in Eq. \(14\) is dominated by the contributions of the effective escape rates from the \(0\)th well only. Finally, the sum over \(i\) (labeling the intermediate states) is reduced to its sole first term too, and Eq. \(15\) becomes identical to Eq. \(8\).

Second, each of the \(N(N + 1)/2\) terms of Eq. \(15\) alone would yield a straight line in the \(f^*\) vs. \(\log(r)\) plot. However, at any loading rate the highest force value (the uppermost segment, corresponding to the most difficult transition) limits the unbinding process, therefore, the \(f^*(r)\) curve is expected to closely follow the upper envelope of these segments [see Fig. 2(a)]. Depending on the position of the lines, this upper envelope can consist of up to \(N(N + 1)/2\) linear segments.

Third, this last point is clearly at odds with the prediction within the DBFS approximation. Indeed assuming a DBFS corresponds to forbidding the display in the force spectrum of the \(N(N − 1)/2\) segments corresponding to the probing of the escape from an intermediate bound state (see Fig. 2 b.).

**B. Practical implications: Ambiguity in the determination of “structural” parameters**

We now insist on some practical implications of the above general description. We do not attempt a full inspection of all the possible dynamic responses of arbitrarily complex systems, but rather focus on two simple examples in order to stress that the main features of the energy landscape can in general not be unambiguously inferred from \(\log(r), f^*\) plots.

To emphasize the experimental relevance of this discussion, we use for the parameters values comparable to those observed in experimental systems. Specifically, we take the geometrical factors \(\alpha_i, s\) and \(\hat{\alpha}_i, s\) to be all equal to 1, \(\omega_0 = 10^8\) s\(^{-1}\) and \(k_B T = 4 \times 10^{-21}\) J.

1. Ambiguity in determining the barriers positions

Fig 3(a) and Fig 3(b) display two force spectra as obtained from Eq. \(15\). Both correspond to energy landscapes with two barriers. Though the two \(\log(r), f^*\) plots are almost identical they are related to very different sets of values for the energy levels and positions (along the pulling direction) of the wells and the barriers.

Fig 3 (a) corresponds to the situation where the standard picture to account for the two segments is suited [7]. At low force, the escape from the fundamental 0th state over the outermost barrier is the limiting process. The slope of the first segment is proportional to \(k_B T/\tilde{E}_1\). For the highest forces (above \(\sim 30\) pN) the energy of the external barrier is reduced below \(\tilde{E}_1\) and the deepest bound state remains located at \(x_0 = 0\). The process that mostly impedes the unbinding is the overcome of the innermost barrier \(\tilde{E}_1\) with a rate \(k_{0,1}\). The slope of the curve is now larger and proportional to \(k_B T/\tilde{E}_1\).

Fig 3 (b) corresponds to an energy landscape for which the above explanation is inappropriate. At low force the unbinding kinetic is controlled by the escape from the fundamental state over the outermost barrier again. But, for pulling forces larger than \(\sim 30\) pN this outer barrier remains the highest [see inset in Fig 3(b)]. However the slope of the spectrum increases as in the (a) case. The reason is that the deepest (and most occupied) bound state is now located at \(x = x_1\) and the presence of the second segment actually witnesses the escape from this intermediate state to the unbound state with a rate \(k_{1,2}\). The value of the second slope scales therefore with \(k_B T/(\tilde{E}_2 - x_1)\). Since the escape rate \(k_{1,2}\) in the (b) case is equal to the escape rate \(k_{0,1}\) in the (a) case, the two spectra in Fig. 3 turn out to be indistinguishable and cannot be *a priori*...
FIG. 4 Two very similar force spectra corresponding to energy landscapes with different numbers of intermediate wells. The rule of the line styles is the same as in Fig.3: (a) dotted lines: \( k_{0.3} \), dash-dotted lines: \( k_{0.2} \), dashed lines: \( k_{0.1} \). (b) dotted lines: \( k_{0.2} \), dash-dotted lines: \( k_{1.2} \), dashed lines: \( k_{0.1} \).

Parameter values: (a) \( (\bar{x}_1, \bar{E}_1) = (0.6 \text{ nm}, 1.4 \text{ k_B T}), (x_1, E_1) = (0.7 \text{ nm}, 12 \text{ k_B T}), (\bar{x}_2, \bar{E}_2) = (1.1 \text{ nm}, 19 \text{ k_B T}), (x_2, E_2) = (2 \text{ nm}, 16 \text{ k_B T}) \) and \( (\bar{x}_3, \bar{E}_3) = (2.5 \text{ nm}, 24 \text{ k_B T}) \). (b) \( (\bar{x}_1, \bar{E}_1) = (1.1 \text{ nm}, 19 \text{ k_B T}), (x_1, E_1) = (1.9 \text{ nm}, 10 \text{ k_B T}), (\bar{x}_2, \bar{E}_2) = (2.5 \text{ nm}, 24 \text{ k_B T}) \).

2. Ambiguity in determining the number of barriers

After having shown with the simple example above that ambiguity can exist in determining distances from dynamic force spectra, we show here that even more strikingly it is impossible in general to assess the number of wells and barriers. Again we use a simple example to do so.

Fig. 4 displays two force spectra obtained using Eq. (15). They are both well approximated by a succession of three segments with increasing slopes. Again, the two \([\log(f^*), f^*]\) curves are very similar although they are constructed from landscapes that do not even comprise the same number of peaks and wells.

In Fig 4(a) the three segments describe the escape from the same fundamental state over the three distinct energy barriers.

The larger the pulling force the closer the limiting barrier to the fundamental state [see inset in Fig. 4(a)].

In Fig. 4(b), the landscape consists of only two barriers. However, the force spectrum reveals that three different escape processes can limit the unbinding kinetic. At low forces \((f \lesssim 50 \text{ pN})\) the two observed linear segments results from the escape form the fundamental state over the two peaks at \( \bar{x}_1 \) and \( \bar{x}_2 \) respectively. Conversely, at high forces it is the escape from the deeply lowered intermediate state over the outer barrier that determines the escape rate (see inset in Fig. 4(b), drawing with dash-dot line). With the chosen parameters the effective rate \( k_{0.3}, k_{0.2} \) and \( k_{0.1} \) in Fig. 4(a) case correspond respectively to \( k_{0.2}, k_{0.1} \) and \( k_{1.2} \) in Fig. 4(b) case. Thus the two plots are indistinguishable and cannot be used to predict the number of barriers along the 1D escape path.

In conclusion of this subsection, we suggest great care in inferring features of the underlying energy landscape from dynamic force spectroscopy experiment. Our generalized equation may be helpful in dealing with the corresponding ambiguity as it allows (with some work) to generate various landscapes that can account for the observed data, whereas Eq. (15) can only yield a single set of parameters (e.g. those used for the plots in Fig. 4(a) and Fig. 4(a)).

V. BEYOND THE SINGLE ESCAPE RATE APPROXIMATION: MULTIMODAL UNBINDING FORCE DISTRIBUTIONS

Up to this point we have been considering a generalized theory in which the deeply bound fundamental state (DBFS) approximation is dropped, but the unbinding is still approximated as a simple first-order escape process. Indeed, the validity of Eq. (15) relies on the assumption that at any moment the distribution of the populations of the bound states can be well approximated by the distribution corresponding to a homogeneous stationary current.

This is, however, not always the case. As we stated earlier, the sum of the \( 1/k_{i,j}(f) \) terms in Eq. (14) is dominated by the smallest effective rate constant \( k_{i',j'}(f) \) corresponding to the slowest effective transition. A consequence of this is that all the bound states located to the left of barrier \( j' \) are close to equilibrium (because of the slow outflow over barrier \( j' \)), and the population of any state located to the right is negligible (because they practically belong to the unbound state). Now, if the slowest transition rate changes from \( k_{i',j'}(f) \) to \( k_{i'',j''}(f) \) as the loading force \( f \) is increased, and if \( j'' < j' \), then a considerable population might remain in the intermediate bound states between the new and the old limiting barriers, \( j'' \) and \( j' \) respectively. This residual population is incompatible with the new stationary current dominated by \( k_{i'',j''}(f) \), and must escape in a different way, yielding a secondary maximum of the unbinding force distribution (see Fig. 5(b)).

The escape of the majority of the population (located to the left of the new limiting barrier \( j'' \)) can still be characterized by Eq. (14) of our generalized theory. On the other hand, we have to slightly modify this formula to describe the escape of the residual population (trapped between the new and old limi-
Consequently, the absolute maximum of the unbinding force distribution always follows the upper envelope of the \(N(N+1)/2\) lines, however, some secondary maxima might also appear at lower forces, which follow the upper envelope of only a subset of the lines [comprising \((N-j''')(N-j''' + 1)/2\) elements]. Such secondary maxima of a multimodal force distribution give important information on the internal structure of the energy landscape of the unbinding path, and makes the determination of the number and positions of the energy wells and barriers less ambiguous. It is actually a nice achievement of our generalized theory to be able to make sense of the segments of secondary maxima in a unique frame for fitting parameters (see e.g. Fig. 5, where the segment corresponding to the secondary maximum corresponds to the transition from the well 1 over the barrier 2, a step neglected in the DBFS approximation). The possibility of a bimodal distribution for the case of a two-state system has already been reported by Strunz et al. [13], and our description systematizes and generalizes their findings.

To provide a simple illustration for the somewhat formal discussion above, we also focus on a system consisting of two bound states, as depicted in Fig. 5(a). Increasing the force, the limiting transition rate changes from \(k_{0,2}\) to \(k_{1,2}\) and then to \(k_{0,1}\). In the range of the loading rate \(r\) between about 10^4 and 10^5 pN/s the intermediate bound state (1) has enough time to accumulate a large population, which is then flushed by the \(k_{1,2}\) transition before the transition \(k_{0,1}\) flushes the rest from the fundamental bound state (0). In the range above 10^6 pN/s the intermediate bound state (1) cannot accumulate much of the population, but it still possesses a small fraction of the initial equilibrium distribution, which is again flushed by the \(k_{1,2}\) transition first.

Very recently the group of Evans actually reported the experimental occurrence of a bimodal force distribution [14]. The corresponding experiment consisted in pulling on “diC14 PE” lipids from a bilayer made of “C18:0/1 PC” lipids. With the help of our generalized theory, Evans and Williams were able to fit their data and interpret the results in terms of an energy landscape with two barriers [personal communication, see Ref. [3] in Ref. [14]].

VI. CONCLUSION

In this paper, we have revisited the standard theory used to account for the dynamic response of molecular stickers. Our refined description, valid for an arbitrarily complex one-dimensional energy landscape, has allowed us to highlight several practical consequences of the diversity of the possible unbinding scenarios. For example several markedly different energy landscapes can yield the same rupture force distribution. To resolve this ambiguity other experimental technics, e.g. flow chamber experiments [15], are then required. We have also identified the physical origin of multimodal unbinding force distributions and shown how their analysis provides informations on the unbinding pathways.
References

[1] T. R. Strick, M.-N. Dessinges, G. Charvin, N. H. Dekker, J.-F. Allemand, D. Bensimon and V. Croquette Rep. Prog. Phys. 66, 1 (2003).

[2] M. Rief, M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub, Science 276, 1109 (1997); M. S. Kellermayer, S. B. Smith, H. L. Granzier, and C. Bustamante, ibid. 276, 1112 (1997).

[3] M. G. Poirier, A. Nemani, P. Gupta, S. Eroglu, and J. F. Marko, Phys. Rev. Lett. 86, 360 (2001).

[4] D. A. Simons, M. Strigl, M. Hohenadl, and R. Merkel, Phys. Rev. Lett. 83, 652 (1999).

[5] T. Nishizaka, R. Seo, H. Tadakuma, K. Kinoshita, and S. Ishiwata, Biophys. J. 79, 962 (2000).

[6] A. Pierre, A. M. Benoliel, P. Bongrand, and P. A. van der Merwe, Proc. Natl. Acad. Sci. USA 93, 15114 (1996).

[7] E. Evans and K. Ritchie, Biophys. J. 72, 1541 (1997); R. Merkel, P. Nassoy, A. Leung, K. Ritchie, and E. Evans, Nature 397, 50 (1999).

[8] G. Hummer and A. Szabo, Proc. Natl. Acad. Sci. USA 98, 3658 (2001).

[9] H. Grubmüller, B. Heymann and P. Tavan, Science, 271, 997 (1996).

[10] E. Evans, Faraday Discuss. 111, 1 (1998); E. Evans, Annu. Rev. Biophys. Biomol. Struct. 30, 105 (2001).

[11] U. Seifert, Europhys. Lett. 58, 792 (2002).

[12] D. Bartolo, I. Derényi and A. Ajdari, Phys. Rev. E. 65, 051910-1 (2002).

[13] T. Strunz, K. Oroszlan, I. Schumakovitch, H.-J. Güntherodt, and M. Hegner, Biophys. J. 79, 1206 (2000).

[14] E. Evans and P. Williams, in Physics of bio-molecules and cells, edited by H. Flyvbjerg et al. (Springer, Berlin, 2002), pp. 145–204.

[15] A. Pierres, D. Touchard, A.-M. Benoliel, and P. Bongrand, Biophys. J. 82, 3214 (2002).