

# Optimal evaluation of single-molecule force spectroscopy experiments

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The forced rupture of single chemical bonds under external load is addressed. A general framework is put forward to optimally utilize the experimentally observed rupture force data for estimating the parameters of a theoretical model. As an application, we explore to what extent a distinction between several recently proposed models is feasible on the basis of realistic experimental data sets.

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## I. INTRODUCTION

Single-molecule force spectroscopy [1] refers to the experimental observation of chemical dissociation by pulling apart the molecular complex of interest at a constant velocity  $v$  until the bond breaks. The evaluation of the resulting rupture force data is a nontrivial task [2–8]: for one and the same  $v$ , the rupture forces are found to be randomly distributed over a wide range, and for different  $v$ , different such probability distributions are obtained. While both on the experimental and modeling sides a great amount of work has led to substantial progress and sophistication, much less effort has been spent to improve the still rather basic methods of connecting and comparing theory and experiment. This is the subject of our present work.

Our starting point is the probability density  $p_1(f|\boldsymbol{\mu}, v)$  that a dissociation event occurs at a pulling force  $f$ , given the pulling velocity  $v$  and *any theoretical model* with certain model parameters  $\boldsymbol{\mu}$ . Now, our main question is as follows: What is the optimal estimate of those model parameters  $\boldsymbol{\mu}$  that can be extracted from any given set of  $N$  rupture forces  $\mathbf{f}=\{f_i\}_{i=1}^N$  and pulling velocities  $\mathbf{v}=\{v_i\}_{i=1}^N$ ? Since the  $f_i$  are statistically independent, the probability of observing the given set of rupture forces  $\mathbf{f}$  reads

$$p(\mathbf{f}|\boldsymbol{\mu}, \mathbf{v}) = \prod_{i=1}^N p_1(f_i|\boldsymbol{\mu}, v_i). \quad (1)$$

The main result of our paper is that the optimal parameter estimate is obtained by simply maximizing Eq. (1) with respect to  $\boldsymbol{\mu}$ : no other “recipe” is able to yield estimates closer to the true parameter values systematically, i.e., on the average over many data sets  $\mathbf{f}$ .

## II. EXAMPLE

The explicit form of  $p_1$  in Eq. (1) depends on the specific model one is considering, and similarly for the meaning and even the number of the model parameters  $\boldsymbol{\mu}$ . While our general theory applies to any model, an illustrative and particularly simple example is provided by the most widely used model [1,2,9], viewing the dissociation as a rate process of the form  $\dot{n}(t)=-k(f(t))n(t)$ , where  $n(t)$  denotes the bond survival probability and  $k(f(t))$  the dissociation rate at the instantaneous pulling force  $f(t)$ , and adopting the approximations [1,2,9]

$$f(t) = \kappa vt, \quad k(f) = \exp\{\lambda + \alpha f\}. \quad (2)$$

Here,  $\kappa$  is the net elasticity of the setup and  $\kappa v$  is the so-called loading rate. While they are considered as known [1,2,9],  $\boldsymbol{\mu}=(\lambda, \alpha)$  are the two unknown model parameters in the case of our specific example at hand. Their physical meaning is discussed in detail, e.g., in Refs. [1,2]:  $k(0)=\exp\{\lambda\}$  represents the force-free dissociation rate and  $\alpha k_B T$  the dissociation length (distance between potential well and barrier along the reaction pathway), where  $k_B T$  is the thermal energy. Having thus completely specified the model [1,2,9], a straightforward calculation yields for this particular example the explicit result

$$p_1(f|\boldsymbol{\mu}, v) = \frac{e^{\lambda+\alpha f}}{\kappa v} \exp\left\{-\frac{e^{\lambda+\alpha f}-1}{\kappa v \alpha}\right\}. \quad (3)$$

## III. FORMAL ANALYSIS

The quantity in Eq. (1) is called the *likelihood* and plays a central role in Bayes’ theorem [10]

$$p(\boldsymbol{\mu}|\mathbf{f}, \mathbf{v}) = p(\mathbf{f}|\boldsymbol{\mu}, \mathbf{v})p(\boldsymbol{\mu}, \mathbf{v})/p(\mathbf{f}, \mathbf{v}). \quad (4)$$

The left-hand side represents the “likeliness” of  $\boldsymbol{\mu}$ , given the data  $\mathbf{f}, \mathbf{v}$ , and hence is clearly of central interest for our purposes. Considering also the right-hand side as a function of  $\boldsymbol{\mu}$ , it is equal to the likelihood from Eq. (1) times  $p(\boldsymbol{\mu}, \mathbf{v})$ , encapsulating all our knowledge about  $\boldsymbol{\mu}$  before the measurement, times a  $\boldsymbol{\mu}$ -independent factor  $1/p(\mathbf{f}, \mathbf{v})$ . We emphasize that we will not use the Bayesian formalism in our actual calculations below, only in their intuitive interpretation.

Next we exploit the fact that typically a quite large set of rupture data  $\mathbf{f}$  is available. Thus, focusing on large  $N$ , it is convenient to rewrite Eq. (1) as

$$p(\mathbf{f}|\boldsymbol{\mu}, \mathbf{v}) = \exp\{-Ns_N(\mathbf{f}, \boldsymbol{\mu}, \mathbf{v})\}, \quad (5)$$

$$s_N(\mathbf{f}, \boldsymbol{\mu}, \mathbf{v}) := -N^{-1} \sum_{i=1}^N \ln p_1(f_i|\boldsymbol{\mu}, v_i). \quad (6)$$

Furthermore, we assume that the relative frequency with which the different pulling velocities  $v$  are sampled converges toward a well-defined limit  $\rho(v)$  for  $N \rightarrow \infty$ . Finally, we assume that the rupture forces  $f_i$  have been sampled according to the “true” distribution  $p_1(f_i|\boldsymbol{\mu}_0, v_i)$  with unknown,

“true” model parameters  $\mu_0$ . Then it follows from the law of large numbers [11] that

$$s_N(\mathbf{f}, \mu, \mathbf{v}) \rightarrow s(\mu) := -\langle \ln p_1(f|\mu, v) \rangle \quad (7)$$

for  $N \rightarrow \infty$ , where  $\langle \cdot \rangle$  indicates an average over  $f$  and  $v$  with weight  $p_1(f|\mu_0, v)\rho(v)$ . Hence,  $s_N$  is an intensive, entropy-like quantity. Observing that  $s(\mu) - s(\mu_0)$  is a relative entropy of the form  $\langle \ln[p_1(f|\mu_0, v)/p_1(f|\mu, v)] \rangle$ , one can infer [11] that  $s(\mu)$  has a unique absolute minimum at  $\mu = \mu_0$ . For any given  $\mathbf{f}$  and  $\mathbf{v}$ , we denote by  $\mu^* = \mu^*(\mathbf{f}, \mathbf{v})$  the maximum of the likelihood  $p(\mathbf{f}|\mu, \mathbf{v})$  with respect to  $\mu$ , or, equivalently, the minimum of  $s_N(\mathbf{f}, \mu, \mathbf{v})$  in Eq. (5). Since  $s_N$  converges for large  $N$  toward  $s$  according to Eq. (7), also the minimum  $\mu^*$  of the former converges to the minimum  $\mu_0$  of the latter. Consequently, for  $\mu$  close to  $\mu^*$  and large  $N$ , we can expand  $s_N(\mathbf{f}, \mu, \mathbf{v})$  up to second order about its minimum at  $\mu^*$  and the Hessian matrix of  $s_N(\mathbf{f}, \mu, \mathbf{v})$  can be replaced by the Hessian  $H = H(\mu_0)$  of  $s(\mu_0)$ , i.e.,

$$s_N(\mathbf{f}, \mu^* + \Delta, \mathbf{v}) = s_N(\mathbf{f}, \mu^*, \mathbf{v}) + \Delta^\dagger H \Delta / 2. \quad (8)$$

For large  $N$  this is a very good approximation for all  $\mu$  values with an appreciable weight in Eq. (5), i.e.,

$$p(\mathbf{f}|\mu, \mathbf{v}) \propto \exp\{-N(\mu - \mu^*)^\dagger H(\mu - \mu^*)/2\}. \quad (9)$$

Within this narrow peak region, the factor  $p(\mu, \mathbf{v})$  in Eq. (4), though usually unknown in detail, can be considered as approximately constant, i.e.,  $p(\mu|\mathbf{f}, \mathbf{v}) \propto p(\mathbf{f}|\mu, \mathbf{v})$ . Given  $\mathbf{f}$  and  $\mathbf{v}$ , the likelihood from Eq. (1) thus quantifies the likeliness that the true model parameters are  $\mu$ .

Upon repeating the entire set of  $N$  pulling experiments with the same set of pulling velocities  $\mathbf{v}$ , a different set of rupture data  $\mathbf{f}$  will be sampled, yielding a different maximum likelihood estimate  $\mu^*$ . While the probability distribution of  $\mathbf{f}$  is given by Eq. (1) with  $\mu = \mu_0$ , what can we say about the distribution of the maximum likelihood estimates  $\mu^*$ ? To determine its first moments, we differentiate Eq. (8) and choose  $\Delta = \mu_0 - \mu^*$ , resulting in

$$\mu^* - \mu_0 = -H^{-1} \partial s_N(\mathbf{f}, \mu_0, \mathbf{v}) / \partial \mu. \quad (10)$$

Averaging over  $\mathbf{f}$  yields zero on the right hand side, as can be inferred from Eqs. (6) and (7) and the fact that  $\mu_0$  is the minimum of  $s$ . Hence,

$$\langle \mu^* \rangle = \mu_0, \quad (11)$$

i.e., the maximum likelihood estimate is “unbiased.” An analogous but somewhat more involved calculation [12] yields for the second moments the result

$$\langle (\mu^* - \mu_0)(\mu^* - \mu_0)^\dagger \rangle = (NH)^{-1}. \quad (12)$$

Observing that  $(NH)^{-1}$  is the covariance matrix of the distribution from Eq. (9), we arrive at our first main conclusion: For any given, sufficiently large data set  $\mathbf{f}$ , the expected deviation of the concomitant maximum likelihood estimate  $\mu^*$  from the true parameters  $\mu_0$  immediately follows from the “peak width” of the likelihood from Eq. (1), considered as a function of  $\mu$ .

Similarly, from the higher moments one can infer [12] that  $\mu^*$  is Gaussian distributed, yielding with Eq. (9) our

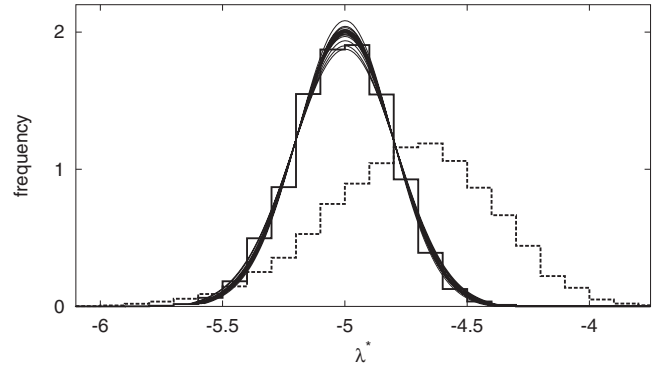


FIG. 1. Solid histogram: Numerically determined distribution of the first components of the maxima  $\mu^* = (\lambda^*, \alpha^*)$  of the likelihood from Eq. (1) for 10 000 computer experiments. For each of them,  $N=400$  rupture forces  $f$  were sampled according to Eq. (3), 100 for each of the four loading rates  $\kappa v = 50, 200, 1000, 5000$  pN/s and with true parameters  $\lambda_0 = -5$  and  $\alpha_0 = 0.1$  pN $^{-1}$ . These are typical numbers in real experiments [1]. Thin lines: The likelihood from Eq. (1) for the first 15 of the 10 000 experiments after integrating over  $\alpha$ , shifting the maximum to  $\lambda_0$ , and normalizing (some are almost indistinguishable). Dotted histogram: Distribution of the estimates for  $\lambda$  according to the standard method, as described in the main text.

second main conclusion: Apart from the peak position and a normalization factor, the likelihood from Eq. (1) for one given data set  $\mathbf{f}$  looks practically the same as the distribution of the maximum likelihood estimates  $\mu^*$  from many repetitions of the  $N$  pulling experiments.

Figure 1 illustrates these findings by means of the example from Eqs. (2) and (3). Since two-dimensional distributions are difficult to compare graphically, we focus on the marginal distributions for the first component  $\lambda$  of  $\mu = (\lambda, \alpha)$  (the findings for  $\alpha$  are similar). The close agreement of the 15 thin lines with the histogram in Fig. 1 very convincingly illustrates our two conclusions above.

In view of the argument below Eq. (9), it seems intuitively quite plausible that the maximum of the likelihood  $\mu^*$  should be the best possible guess for the unknown true parameters  $\mu_0$ . A more rigorous line of reasoning starts with an arbitrary “recipe” of estimating the true parameters  $\mu_0$  from a given data set  $\mathbf{f}$ , formally represented by some function  $\tilde{\mu}(\mathbf{f})$ . The only assumption is that this recipe is unbiased, i.e., upon repeating the same experiment many times, on the average, the true parameters are recovered,  $\langle \tilde{\mu}(\mathbf{f}) \rangle = \mu_0$ . By generalizing the well-known Cramér-Rao inequality [11], which in turn is basically a descendant of the Cauchy-Schwarz inequality, one can show [12] for any such recipe  $\tilde{\mu}(\mathbf{f})$  that

$$\langle (\tilde{\mu} - \mu_0)(\tilde{\mu} - \mu_0)^\dagger \rangle - (NH)^{-1} \geq 0, \quad (13)$$

i.e., the matrix on the left-hand side is non-negative definite. Comparison with Eq. (12) yields our third main conclusion: There is no unbiased estimator  $\tilde{\mu}$  of the true parameters  $\mu_0$  which on the average outperforms the maximum likelihood estimate  $\mu^*$ .

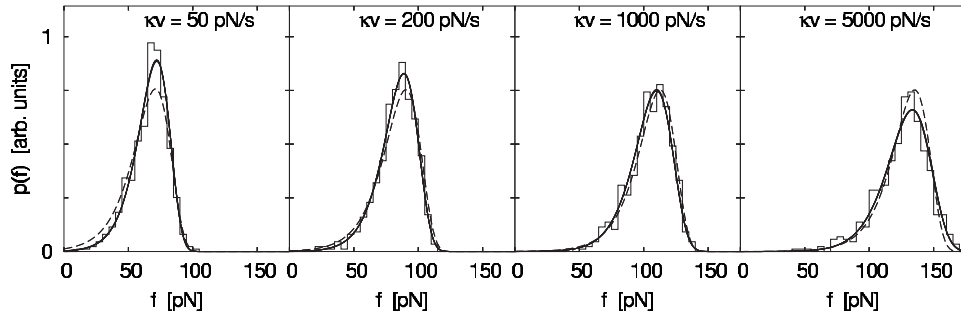


FIG. 2. Rupture force distribution for different loading rates  $\kappa v$ . Histograms: Numerically generated rupture forces according to Eq. (15) with  $\gamma=2/3$ ,  $\lambda_0=-5$ ,  $\alpha_0=0.1 \text{ pN}^{-1}$ ,  $\epsilon_0=15$ . For each  $\kappa v$ , we sampled 500 forces, i.e.,  $N=2000$ . Solid: maximum likelihood fit  $p_1(f|\mu^*, v)$  according to Eq. (15) for  $\gamma=1/2$  and  $2/3$  (not distinguishable in this plot). Dashed: same for  $\gamma=1$ . Upon repeating the entire numerical experiment, the resulting plots always look practically the same.

The remaining possibility that a biased estimator may be even better is rather subtle to treat rigorously, but intuitively this seems quite unlikely. Furthermore, in the above conclusion we exploited the relation in Eq. (12) which is strictly correct only for asymptotically large  $N$ . Finally, the criterion of minimizing the left hand side in Eq. (13) itself is in principle also debatable, but hardly in practice. Being unable to make any further progress along these lines, we directly compared the maximum likelihood estimate with other known recipes of evaluating single-molecule rupture data [1,2,4,5,7,8,13]. In all cases we found that the maximum likelihood was superior.

#### IV. THREE CASE STUDIES

We now describe three case studies. (1) In single-molecule force spectroscopy, the most widely used recipe for estimating parameters consist of the following steps. (i) Fit a Gaussian to the observed rupture force distribution for a fixed pulling velocity  $v$  and approximate the most probable rupture force  $f^*$  by the maximum of that Gaussian. (ii) Plot  $f^*$  for different  $v$  versus  $\ln(v)$  and fit the resulting points by a straight line. (iii) Assume that the model (2) and (3) is

applicable and deduce its model parameters  $\mu=(\lambda, \alpha)$  from the slope and the axis intercept of the straight line as detailed, e.g., in Refs. [1,2,4,5,13]. We have applied this procedure to each of the 10000 experiments in Fig. 1 and plotted the distribution of the resulting estimates for  $\lambda$  in Fig. 1. The conclusion is that the maximum likelihood estimate represents a substantial improvement compared to the so far “standard method” of data evaluation in this field.

(2). Generalizations of the rate from Eq. (2) of the form

$$k(f) = (1 - \gamma\alpha f/\epsilon)^{1/\gamma-1} e^{\lambda+\epsilon(1-(\gamma\alpha f/\epsilon)^{1/\gamma})} \quad (14)$$

with three model parameters  $\mu=(\lambda, \alpha, \epsilon)$  have recently attracted considerable interest [14]. Here,  $\lambda$  and  $\alpha$  have the same physical meaning as in Eq. (2),  $\epsilon:=E_b(0)/k_B T$  stands for the force-free activation energy barrier in units of the thermal energy  $k_B T$ , while  $\gamma \in \{1/2, 2/3, 1\}$  labels three different models: For  $\gamma=1$  the parameter  $\epsilon$  drops out and one recovers Eq. (2),  $\gamma=2/3$  reproduces the Kramers rate for a cubic reaction potential, and  $\gamma=1/2$  corresponds to a parabolic potential well with a cusp barrier [14]. The resulting rupture force distribution

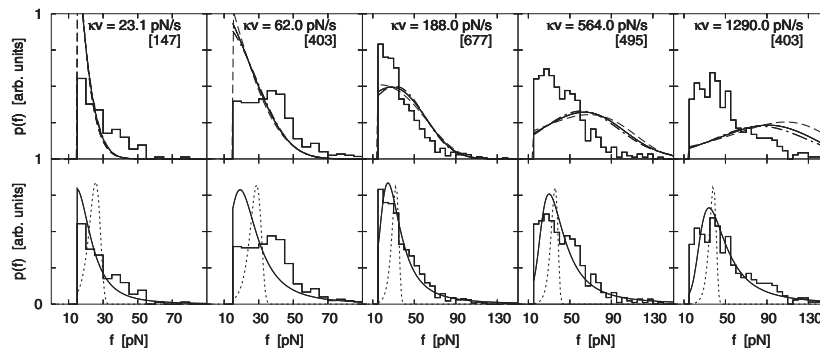


FIG. 3. Same as Fig. 2 but for experimental rupture forces from Ref. [8]. The number of rupture events for each loading rate is indicated in parentheses. Upper row: Experimental data (histograms) and maximum likelihood fit  $p_1(f|\mu^*, v)$  according to model (14) and (15) for  $\gamma=1$  (dashed),  $2/3$  (solid), and  $1/2$  (dash-dotted). Lower row: Experimental data (histograms), maximum likelihood fit  $p_1(f|\mu^*, v)$  according to model (16) (solid), and best fit according to the standard method, as described in the main text (dotted).

$$p_1(f|\boldsymbol{\mu}, v) = \frac{k(f)}{\kappa v} \exp\left(-\frac{e^\lambda e^{\epsilon[1-(1-\gamma\alpha f/\epsilon)^{1/\gamma}]}-1}{\alpha}\right) \quad (15)$$

with  $k(f)$  from Eq. (14) can be determined analogously to Eq. (3). There is an ongoing debate in the literature about which of the three models is most appropriate to evaluate experimental rupture data [14]. Taking for granted that one of the three models approximates the “truth” satisfactorily, choosing  $\boldsymbol{\mu}=\boldsymbol{\mu}^*$  is—according to our above conclusions—the closest one can get to the “full truth” on the basis of one given data set  $\mathbf{f}$ . In case of disagreement about the true  $\gamma$  value, a fully objective selection criterion seems impossible to define in principle. In practice, the usual criterion is the comparison with the basic true quantity observed experimentally, namely, the distribution of rupture forces. In view of Fig. 2, we conclude that under typical experimental conditions it is absolutely impossible to decide whether  $\gamma=1/2$  or  $\gamma=2/3$  is better, and even  $\gamma=1$  performs almost as well [15].

(3). In Fig. 3 the same comparison as in Fig. 2 is repeated, but now for real experimental data from Ref. [8]. Again, the models (14) and (15) with  $\gamma=1/2$  and  $2/3$  are hardly distinguishable;  $\gamma=1$  differs slightly more, while the standard method yields a completely different best fit. However, none of them satisfactorily describes the experimental reality. The same incompatibility is recovered for all other experimental data sets we analyzed so far (see also Ref. [7]). An almost perfect agreement (within the statistical uncertainty of the experimental data) is obtained by means of yet another recent extension [7] of the model (2) and (3), considering the parameter  $\alpha$  itself as randomly sampled from

$$\rho(\alpha) = (2\pi\sigma_\alpha^2)^{-1/2} \exp\{-(\alpha - \bar{\alpha})^2/2\sigma_\alpha^2\}, \quad (16)$$

resulting in a model with three parameters  $\boldsymbol{\mu}=(\lambda, \bar{\alpha}, \sigma_\alpha)$ . Possible reasons for such a heterogeneity of the dissociation rate are uncontrollable variations of the experimental conditions or of the complicated biomolecular complex itself [7].

## V. CONCLUSIONS

The maximum  $\boldsymbol{\mu}^*$  of the likelihood from Eq. (1) is the best possible estimate for the unknown model parameters  $\boldsymbol{\mu}$ , given an appropriate model and a (sufficiently large) set of rupture forces  $\mathbf{f}$ . The accuracy of this estimate follows from the dispersion of the (approximately Gaussian) likelihood peak about  $\boldsymbol{\mu}^*$ . The procedure is extremely simple and general. For example, the pulling velocities  $v_i$  may be all the same, all different, or distributed in any other way, and the pulling force  $f(t)$  may or may not increase linearly with time [only in the first case is there a well-defined loading rate  $\kappa$ ; cf. Eq. (2)].

By means of a least-squares fit one gets—by definition—the best possible agreement between theory and experiment with respect to any given deviation measure. A typical example is to optimize the agreement between experimental and theoretical rupture force distributions. In particular, the resulting agreement with the data in Figs. 2 and 3 would be (at least slightly) better than for any of the depicted theoretical lines. However, our present goal is not to optimally fit rupture force distributions but rather to fit the unknown model parameters as closely as possible: they are the quantities of prime interest, and any other kind of fitting procedure is mainly an intermediate step in order to estimate them. Using the rupture force distributions to fit parameters seems natural, but our paper shows that one can do better. Our present comparison of the rupture forces in Figs. 2 and 3 serves a different purpose: once the parameters are estimated as well as possible according to our method, the resulting rupture force distributions can be used as an independent consistency test for a given hypothetical model.

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- [1] R. Merkel, Phys. Rep. **346**, 343 (2001); E. Evans, Annu. Rev. Biophys. Biomol. Struct. **30**, 105 (2001).  
 [2] E. Evans and K. Ritchie, Biophys. J. **72**, 1541 (1997).  
 [3] M. Rief, J. M. Fernandez, and H. E. Gaub, Phys. Rev. Lett. **81**, 4764 (1998).  
 [4] R. Merkel *et al.*, Nature (London) **397**, 50 (1999).  
 [5] T. Strunz *et al.*, Biophys. J. **79**, 1206 (2000).  
 [6] B. Heymann and H. Grubmüller, Phys. Rev. Lett. **84**, 6126 (2000); U. Seifert, *ibid.* **84**, 2750 (2000); D. Bartolo, I. Derényi, and A. Ajdari, Phys. Rev. E **65**, 051910 (2002); B. Isralewitz, M. Gao, and K. Schulten, Curr. Opin. Struct. Biol. **11**, 224 (2001).  
 [7] M. Raible *et al.*, Biophys. J. **90**, 3851 (2006); Europhys. Lett. **73**, 628 (2006).  
 [8] N. Nguyen-Duong, K. W. Koch, and R. Merkel, Europhys. Lett. **61**, 845 (2003).  
 [9] G. I. Bell, Science **200**, 618 (1978).  
 [10] G. D’Agostini, Rep. Prog. Phys. **66**, 1383 (2003); V. Dose, *ibid.* **66**, 1421 (2003).  
 [11] T. M. Cover and J. A. Thomas, *Elements of Information Theory* (Wiley, New York, 1991).  
 [12] Details will be given elsewhere; see also H. Cramér, *Mathematical Methods of Statistics* (Princeton University Press, Princeton, NJ, 1946).  
 [13] M. Evstigneev and P. Reimann, Phys. Rev. E **68**, 045103(R) (2003).  
 [14] O. K. Dudko, A. E. Filippov, J. Klafter, and M. Urbakh, Proc. Natl. Acad. Sci. U.S.A. **100**, 11378 (2003); G. Hummer and A. Szabo, Biophys. J. **85**, 5 (2003); C. E. Maloney and D. J. Lacks, Phys. Rev. E **73**, 061106 (2006); O. K. Dudko, G. Hummer, and A. Szabo, Phys. Rev. Lett. **96**, 108101 (2006); F. Hanke and H. J. Kreuzer, Phys. Rev. E **74**, 031909 (2006); and further references in those works.  
 [15] S. Sills and R. M. Overney, Phys. Rev. Lett. **91**, 095501 (2003).