

Long Lifetime of Hydrogen-Bonded DNA Basepairs by Force Spectroscopy

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Supporting Information

SI Theory: Multiple parallel bonds

In this section we describe a slight extension of a model which has recently been proposed by Guo *et al.* (1) to describe the effect of multiple parallel bonds on the rupture force distribution and which has been employed in Figs. 6B and S1.

Let us assume that one pulls on N parallel bonds $i = 1, \dots, N$. The PEG linkers connecting the ligands and the receptors to the tip and to the surface, respectively, shall have the same total contour length L for all bonds. However, the offset d_i ($i = 1, \dots, N$) between the apex of the cantilever and the attachment point of the linker is in general different for each of the N bonds (see inset of Fig. 6A). For long linkers, i.e. large L , we can neglect deviations of the pulling direction from the normal direction to the surface. For each single bond i , the distance $s(t)=vt$ between tip and surface can then be written as a function of the force $f_i(t)$ which acts on this bond and of the total force $f(t)$ experienced by the AFM cantilever [cp. Eq. (2)]:

$$s(t) = vt = \frac{f(t)}{\kappa} + L \left[\coth \left(\frac{f_i(t)LK}{k_B T} \right) - \frac{k_B T}{f_i(t)LK} \right] - d_i \quad , \quad i = 1, \dots, N \quad , \quad (\text{S1})$$

where

$$f(t) = F(s(t)) = \sum_{i=1}^N f_i(t) \quad . \quad (\text{S2})$$

For a given retraction velocity v and given offsets d_i , Eqs. (S1) and (S2) represent a system of $N + 1$ coupled equations which uniquely fix the time-dependence of the single forces $f_i(t)$. Because the coupled equations cannot be solved analytically for $f_i(t)$, we have employed numerical methods, see e.g. Ref. (2), whenever these functions needed to be evaluated. Noting that the total force $f(t)$ in Eq. (S2) is a monotonically increasing function of time, this function can be (numerically) inverted and all quantities – in particular the single forces $f_i(t)$ – can alternatively be expressed as functions of the total force. Similarly, all functions of the total force can be expressed as functions of time. In the following, we will repeatedly switch between these alternatives. It is worth noting that, as a consequence of the different attachment points d_i , the forces $f_i(t)$ which act on the single bonds will be different, reflecting that the total force is in general unequally distributed among the parallel bonds which is not covered by the usual theories for multiple bonds, cp. discussion in Ref. (1).

According to a one-step rate process the survival probability of a single bond is given by

$$n_i(t) = \exp \left(- \int_0^t dt' k(f_i(t')) \right) \quad , \quad (\text{S3})$$

where $f_i(t)$ is the time-dependent force acting on that bond. The probability $n^N(t)$ that none of the bonds has ruptured before time t is given by the product of the single survival probabilities:

$$n^N(t) = \prod_{i=1}^N n_i(t) \quad . \quad (\text{S4})$$

Following Guo *et al.*(1), we assume that all bonds rupture nearly simultaneously if the offsets do not deviate too much from each other (see also Sect. *Simulation of force extension curves*). The distribution of rupture forces is then given by

$$p^N(f) = -\frac{d}{df} n^N(t(f)) = -\frac{1}{\dot{f}(t(f))} \dot{n}(t(f)) \quad , \quad (\text{S5})$$

where the dot denotes the derivative with respect to the time and where $\dot{f}(t(f))$ can be determined numerically as described below Eq. (S2).

While Eqs. (S1)-(S5) were derived for a given number of bonds N and given offsets d_1, \dots, d_N , we next have to account for the fact that the number N of parallel bonds will be different for each repetition of the pulling experiment. Moreover, it will be other molecules, attached to other linkers which form these bonds. Therefore, the offsets are themselves random variables drawn from a joint probability distribution $\rho^N(d_1, \dots, d_N)$ and the distribution of measured rupture forces has to be compared to:

$$p(f) = \sum_{N=1}^{\infty} \nu_N \int_0^{\infty} d_1 \dots \int_0^{\infty} d_N \rho^N(d_1, \dots, d_N) p^N(f) \quad , \quad (\text{S6})$$

where the relative frequency of a complex with N bonds is denoted by ν_N . The multiple integrals in Eq. (S6) can in general only be evaluated numerically, e.g. by employing a Monte Carlo scheme.

Finally, we need to specify, the distribution of the offsets d_i . In this work we assume that the d_i are independent and identically distributed, hence $\rho^N(d_1, \dots, d_N) = \prod_{i=1}^N \rho(d_i)$ with some distribution $\rho(d)$ which, in general, depends in a complicated way on the experimental details like the geometry of the AFM tip and on the association kinetics. This function cannot be uniquely fitted from our experimental data. We have therefore chosen a uniform distribution on the interval $[0; d_{max}]$ which corresponds for example to the situation that the linkers are uniformly distributed on a half sphere with radius d_{max} and that the attached molecules have equal probability of forming a bond.

When applying this model to the experimental data from Fig. 6 we have assumed that the PEG linker length and the Kuhn length are the same as those obtained from fitting Eq. (2) to the force extension curves shown in Fig. 1B. Furthermore, in the framework of the multiple bond model we have restricted our discussion to force dependent dissociation rates of the form (1) with $\gamma = 1$, i.e. to the usual Bell model. We found that for any realistic value of d_{max} , the quality of the fit of Eq. (S6) to the experimental data did not significantly depend on the exact choice. For the comparable low rupture forces of the A-T complex, it is in particular not significantly different from the fit obtained under the assumption that the force is equally distributed among the bonds.

Throughout this work the maximum offset was set to $d_{max} = 10\text{nm}$. This value is smaller than the estimated offset obtained from fitting Eq. (2) to the force extension curves shown in Fig. 1B. However, as discussed in the main text, this estimate is not very reliable. Furthermore, a closer inspection of Eqs. (S1)-(S6) shows that simultaneously adding a “small” value δd to all offsets shifts the lower limit of the measurable forces $f > F(s = 0)$ towards higher values and results for these forces in a renormalization of the distribution (S5). Such a shift has therefore no influence on the rupture force distribution (S6) if we normalize it according to the assumption that only rupture events above a certain sufficiently large threshold force can be detected. Hence, d_{max} has to be understood as the maximum possible difference of the offsets such that the corresponding rupture events are not filtered out when using the less strict selection criterion discussed in Sect. ‘Multiple rupture events’.

It is worth noting that in practice also the distribution of the PEG linker lengths is not infinitely sharp, as we have assumed above, but has some finite, albeit small, width. In the above described model this variation can be taken into account by viewing $\rho(d)$ as an effective distribution of the offset which can deviate from the true one. The opposite situation that the linkers length is randomly distributed while the offset is always the same is discussed in Ref. (1), leading to similar results.

SI Methods: Simulation of force extension curves

In Fig. 5B we additionally took into account that the N bonds do *not* rupture simultaneously, but within some finite time interval Δt : Immediately after the rupture of each bond a new force equilibrium arises for the remaining bonds which is again given by equations of the form (S1),(S2). In this new equilibrium, the total force experienced by the cantilever is lower while the forces f_i acting on the remaining bonds are higher than before the rupture of the single bond. Hence, one expects that the rupture of multiple bonds manifests itself as “force dips” in the measured force distance curves. However, under many experimentally realistic conditions the time interval Δt is often very small and/or the force dips are not very pronounced. Since the experimental force distance curves are perturbed by thermal and instrumental noise and have in general passed some low-pass filter to reduce this noise, it can in practice be very difficult to detect multiple rupture events. The fraction of such events which will falsely be classified as single bond rupture depends amongst others on the cantilever stiffness, linker length, retraction speed, bandwidth of the AFM, noise level, rate parameters, and on the method which has been used for the data pre-processing. While a detailed analysis of this point is clearly out of the scope of the present work, we have simulated force extension curves of the rupture of multiple parallel bonds on the computer. In this way one can get an impression under which circumstances

multiple rupture events might be misinterpreted as single rupture events. We now describe details of the simulation procedure which is similar to the algorithms described in Refs. (3,4).

We assume that N bonds have formed at time $t = 0$ with given offsets d_i ($i = 1, \dots, N$). As described in the previous section, the probability that a single bond has not ruptured up to time t is given by Eq. (S3) with time-dependent force which follows from Eqs. (S1) and (S2). For each of the bonds, we then draw a random number t_i from the probability distribution $-\frac{d}{dt}n_i(t)$ and determine the bond i_1 for which the smallest number has been drawn. This bond ruptures first with rupture time $t_{r,1} = t_{i_1}$ and the force-distance curve for times $t \leq t_{r,1}$ is given Eqs. (S1) and (S2). For $t > t_{r,1}$, the forces acting on the remaining $N - 1$ bonds follow from the same equations with $f_{i_1}(t) = 0$ and the new survival probabilities are given by Eq. (S3) where the lower limit of the integral has to be replaced by $t_{r,1}$. This procedure is then iteratively repeated until all bonds have ruptured. For a better comparison to measured force-distance curves we finally add a Gaussian noise with experimentally realistic amplitude to the simulated curves.

SI Figure:

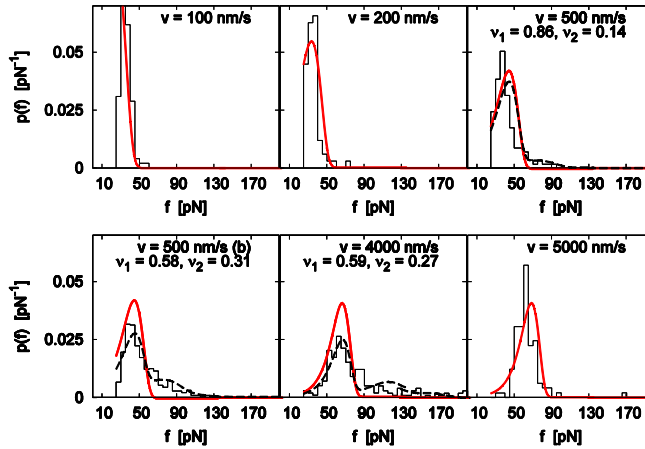


Fig S1: Same data set (T-2AA) as Fig. 3, but the filter settings for multiple rupture events have been loosened. In this data set loosening (only) the f_{\min} criteria as in Fig. 6 did not have significant influence to the distribution of the rupture forces, which already indicates that the linker density and/or the linker clustering appears to be lower compared to the T-A data set. We therefore loosened the second step in multiple rupture filtering, i.e. now also curves are accepted where the cantilever does not jump back immediately to the baseline. The solid red curves show the maximum likelihood fit for Bell's model from Fig. 3, showing that for $v=100, 200$ and $5,000\text{nm/s}$

the shapes of the force distributions did not change significantly. For $v = 500$ nm/s pulling velocity rupture forces were measured with two different cantilevers denoted by (a) and (b). Only for $v = 500$ nm/s ((a) and (b)) and $v = 4000$ nm/s the distributions clearly contain multiple rupture events. As in Fig. 6 we fitted those distributions with the multiple rupture model (dashed lines). The probabilities of observing single bonds (ν_1) and double bonds (ν_2) are indicated in the figures. As in Fig. 6 we assumed $\nu_N = 0$ for $N > 3$ implying $\nu_3 = 1 - \nu_1 - \nu_2$.

SI References

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