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Energy landscape roughness of the streptavidin–biotin interaction

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Molecular interactions between receptors and ligands can be characterized by their free energy landscape. In its simplest representation, the energy landscape is described by a barrier of certain height and width that determines the dissociation rate of the complex, as well as its dynamic strength. Some interactions, however, require a more complex landscape with additional barriers and roughness along the reaction coordinate. This roughness slows down the dissociation kinetics of the interaction and contributes to its dynamic strength. The streptavidin–biotin complex has been extensively studied due to its remarkably low dissociation kinetics. However, single molecule measurements from independent experiments showed scattered and disparate results. In this work, the energy landscape roughness of the streptavidin–biotin interaction was estimated to be in the range of 5–8k_BT using dynamic force spectroscopy (DFS) measurements at three different temperatures. These results can be used to explain both its slow dissociation kinetics and the discrepancies in the reported force measurements. **Copyright © 2007 John Wiley & Sons, Ltd.**

Keywords: **energy landscape roughness; dynamic force spectroscopy; atomic force microscopy; streptavidin–biotin; free energy; energy surface; energy landscape; temperature**

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INTRODUCTION

Molecular interactions between ligands and receptors, as well as the folding and unfolding of proteins, can be characterized by an energy landscape consisting of potentially multiple energetic barriers along a reaction coordinate. In thermally activated processes, the energy landscape determines the dissociation or unfolding rates of the process (Kramers, 1940; Hanggi *et al*., 1990; Pollak and Talkner, 2005). Different biophysical approaches have been employed to determine the dissociation rate of molecular interactions including titration calorimetric analysis and surface plasmon resonance technique. Typically, these techniques reveal the bulk, averaged kinetic, and equilibrium properties of the process, but do not provide information about the position of the transition states or the presence of intermediate substates.

A great number of molecular interactions between receptors and ligands support external forces under physiological conditions (Bell, 1978). The energy landscape of these interactions determines their dynamic strength, i.e., the maximum force the complex can resist before unbinding (Evans and Ritchie, 1997). Single molecule dynamic force spectroscopy (DFS) has been employed to characterize the energy landscape of receptor–ligand interactions in both purified systems and in their native, cellular environment (Merkel *et al*., 1999; Yuan *et al*., 2000; Li *et al*., 2003; Zhang *et al*., 2004; Wojcikiewicz *et al*., 2006). DFS consists of measurements of the force required to unbind individual receptor–ligand complexes, i.e., unbinding force (F), at different loading rates ($dF/dt = r_t$). The resulting force spectra (F vs. r_t) are usually interpreted in terms of the Bell model, which enables us to determine the unbinding rate at zero force (k^0) and the position of the energy barrier along the reaction coordinate (x_β) (Bell, 1978; Evans and Ritchie, 1997).

However, some systems require a more complex energy landscape involving multiple barriers and, even more, a continuous distribution of energy barriers, which, in addition, may require more refined theories (Derenyi *et al*., 2004; Marshall *et al*., 2005; Neuert *et al*., 2006). For example, Frauenfelder and coworkers showed that the relaxation of myoglobin after CO dissociation can be explained in terms of a rough landscape stemming from a hierarchical structure of substates within states (Ansari *et al*., 1985; Frauenfelder *et al*., 1991). Inspired by these work, Zwanzig (1988) derived a general expression for the effective diffusion of a particle in a rough potential and concluded that a rough energy surface can result in a dramatic slowing down of the effective diffusion. More recently, in the context of protein unfolding, Hyeon and Thirumalai (2003) investigated the effect of an applied force to a rough energy landscape. In this study, the authors showed that the amplitude of the energy roughness can be derived from DFS data obtained at different temperatures. Nevo *et al*. (2005) extended the theory to take into account possible variations in the potential width and applied the model to estimate the energy landscape roughness of the GTPase Ran–importin-β interaction.

One of the best characterized receptor–ligand systems is the streptavidin–biotin complex (Stayton *et al*., 1999). Its unusually slow dissociation kinetics makes this complex

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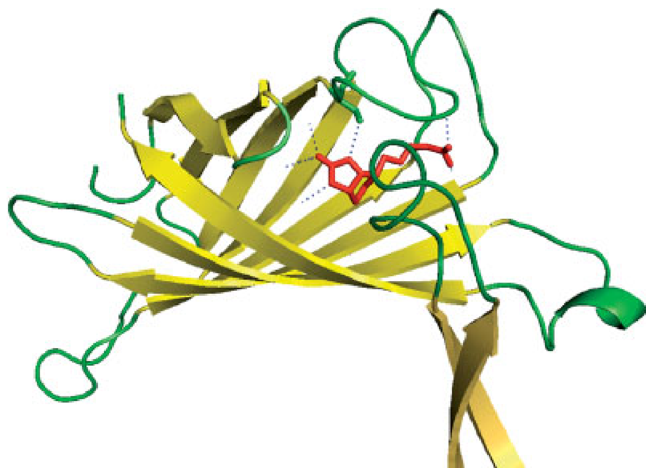

Figure 1. Ribbon diagram of the streptavidin (yellow and green) complex with biotin (ball-and-stick red) from the crystal structure reported by Weber *et al.* (1989). The flexible loop from the adjacent monomer is also shown. The H-bonds formed between the two molecules are represented as blue dashed lines.

very interesting from both applied and fundamental standpoints. The dissociation properties of the complex have been extensively studied using a variety of experimental techniques and computer simulations, and detailed structural information has been revealed from crystallographic analysis (Figure 1) (Weber *et al*., 1992; Chilkoti and Stayton, 1995; Grubmuller *et al*., 1996; Young *et al*., 2007). Moreover, the streptavidin–biotin system was one of the first to be studied at the single molecule level using force spectroscopy (Florin *et al*., 1994; Lee *et al*., 1994; Moy *et al*., 1994). Its free energy landscape has been well characterized by DFS measurements, which identified at least two energy barriers (Merkel *et al*., 1999; Yuan *et al*., 2000; Zhou *et al*., 2006). However, the single molecule unbinding forces reported in the literature appear to be dispersed, scattered, and very sensitive to the experimental conditions (Pincet and Husson, 2005). We hypothesize that the free energy surface of the streptavidin–biotin interaction has a large roughness amplitude that may explain the observed scattering in the experimental data and may be the origin of the low dissociation kinetics. To test this hypothesis we measured the unbinding forces required to dissociate single streptavidin–biotin complexes at various loading rates and at different temperatures using the atomic force microscope (AFM). The acquired dynamic force spectra were analyzed in terms of the Bell model and the roughness was estimated using the formalism developed by Nevo *et al*. (2005).

MATERIALS AND METHODS

Force apparatus

A custom built AFM was used to carry out force measurements of the streptavidin–biotin interaction. Briefly, a piezoelectric translator with strain gauge position sensor and feedback electronics (Physik Instrumente, Auburn, MA) was used to displace the cantilever in the vertical direction. The interaction between the AFM tip and the substrate was determined from the deflection of the AFM cantilever. A focused laser spot from a pigtail diode laser (Oz Optics, Ontario, Canada) was reflected off the back of the cantilever onto a two-segment photodiode to monitor the cantilever's deflection. The photodiode signal was then preamplified, digitized by a 16-bit analog-to-digital converter (Instrutech Corp., Port Washington, NY), and processed by a Pentium 4 PC. All force scan measurements were recorded at the desired temperature using unsharpened Si_3N_4 cantilevers (Thermomicroscopes, MLCT-3 4AUHW, Sunnyvale, CA). Cantilevers with nominal spring constant of approximately 0.010 N/m were calibrated by thermal fluctuation analysis according to Hutter and Bechhoefer (1993). The spring constant of the cantilever did not vary significantly with variations in temperature. Temperature of the sample was controlled using a Peltier element (Melcor, Trenton, NJ) coupled to the base of the sample holder.

Functionalization of AFM tips

Cantilevers were coated with biotinylated bovine serum albumin (biotin-BSA) and then coupled with streptavidin (Sigma, St. Louis, MO) (Yuan *et al*., 2000). Briefly, cantilevers were washed in acetone for 5 min, UV irradiated for 15 min, and then immersed overnight in 50–100 μl of biotin-BSA (Sigma; 0.5 mg/ml in 100 mM $NaHCO_3$, pH 8.6) in a 4°C humidified chamber. After several rinses in PBS, biotin-BSA coated tips were then coupled with streptavidin (Sigma; 0.5 mg/ml in 100 mM $NaHCO_3$) during a 10 min incubation. Unbound streptavidin molecules were washed away by several PBS rinses.

Biotinylated bead preparation

Cross-linked 4% agarose beads labeled with biotin were obtained from Sigma. Agarose beads were washed three times with PBS and resuspended in the same solution for measurements. To minimize bead movement during the force measurements, the biotin-labeled beads were plated on a culture dish coated with streptavidin.

AFM force measurements

Force curves were acquired bringing a streptavidin-coated AFM tip into contact with the biotinylated agar surface. The retraction speed was varied from 93 to 37 600 nm/s, covering nearly three orders of magnitude of the loading rate. The maximum indentation force was controlled to minimize the contact area between the tip and the bead, i.e., to minimize the number of bonds formed. To further reduce the adhesion frequency, either soluble streptavidin or biotin was titrated to the buffer solution until the desired adhesion frequency was observed. The reduction in the adhesion frequency in the presence of soluble streptavidin also confirmed the specificity of the interaction. Measurements were carried out at 17, 24, and 37 ± 0.5°C.

Retraction curves with unbinding events (<35%) were analyzed by measuring the unbinding force. The actual loading rate was determined from the slope just before unbinding in force–time plots. Individual unbinding forces (f)

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were determined from the peak in the retraction trace and corrected from the hydrodynamic drag force due to the surrounding liquid (Alcaraz *et al*., 2002). The hydrodynamic drag factor was determined at each temperature. All the detected unbinding events were grouped according to loading rates and histograms were generated. On average 210 unbinding events were used to obtain each histogram. A Gaussian curve was fitted to the probability distribution to determine the most probable unbinding force. The most probable loading rate for each group was obtained by fitting a log-normal curve to the loading rate histograms. Data are reported as mean $\pm$ SE. Changes in the rupture forces as a function of loading rate and temperature were analyzed by two-way analysis of variance (ANOVA). Statistical significance was assumed at $p < 0.05$.

RESULTS

Temperature dependent dynamic force spectra of the streptavidin–biotin interaction were derived from force–distance curves acquired at different retraction speeds to access a wide range of loading rates. The adhesion frequency was kept low to ensure that the acquired measurements correspond to the unbinding of a single streptavidin–biotin complex. Figure 2 shows six representative force curves in consecutive measurements from which only two revealed adhesion events. Representative histograms from comparable loading rates at 17 and 37°C are shown in Figure 3, with Gaussian curves fitted to estimate the most probable unbinding force. The most probable unbinding forces were

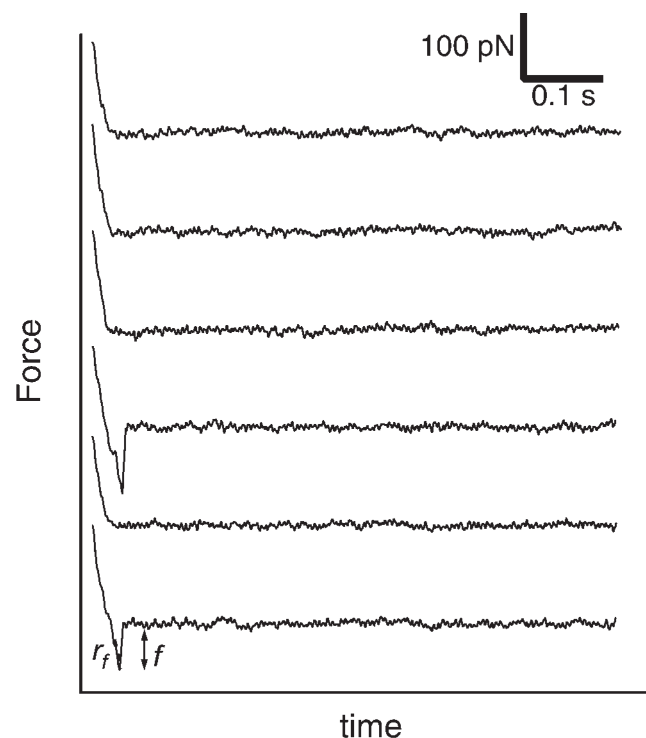

Figure 2. Representative examples of consecutive force–time curves acquired at 1.25 μm/s retraction speed. The unbinding force (f) and loading rate (r_f) were calculated for each unbinding event. Curves were filtered using a moving average filter for clarity.

plotted as a function of loading rates to obtain the dynamic force spectra of the interaction (Figure 4). A clear shift toward lower forces was observed when temperature increased. At each temperature, the dynamic force spectrum showed two loading regimes, governed by the inner (fast loading rates) and the outer (slow loading rates) barriers of the complex. The Bell model was used to determine the off-rate and potential width of each energy barrier

$$F = \frac{k_B T}{x_\beta} \ln\left(\frac{x_\beta r_f}{N k^o k_B T}\right) \tag{1}$$

where k_B is the Boltzmann constant, and T the absolute temperature. The term N was fixed to 2 to take into account the experimental configuration of two bonds in series (Evans, 2001). The transition between loading regimes was determined by maximizing the correlation coefficient of the fits of Equation (1). The Bell model parameters of the streptavidin–biotin interaction at 17, 24, and 37°C are tabulated in Table 1.

The surface roughness of the streptavidin–biotin energy landscape was computed using Nevo's modification of the Hyeon and Thirumalai approximation, which takes into account possible changes in the transition state position (Hyeon and Thirumalai, 2003; Nevo *et al*., 2005)

$$\varepsilon^2 \approx \frac{x_\beta(T_1) k_B T_1 x_\beta(T_2) k_B T_2}{x_\beta(T_2) k_B T_2 - x_\beta(T_1) k_B T_1}\left[\Delta F_0^\ddagger \left(\frac{1}{x_\beta(T_1)} - \frac{1}{x_\beta(T_2)}\right) + \frac{k_B T_1}{x_\beta(T_1)} \ln \frac{r_f(T_1) x_\beta(T_1)}{k^o(T_1) k_B T_1} - \frac{k_B T_2}{x_\beta(T_2)} \ln \frac{r_f(T_2) x_\beta(T_2)}{k^o(T_2) k_B T_2}\right] \tag{2}$$

where $\Delta F_0^\ddagger$ is the height of the potential, and $r_f(T_1)$ and $r_f(T_2)$ are loading rates at two different temperatures that give rise to the same unbinding force. The model assumes a Gaussian distributed amplitude of roughness, independent of the position along the reaction coordinate. The $\Delta F_0^\ddagger$ value of the outer transition state was taken from the latest reported value of $\sim 42 k_B T$ based on titration calorimetric analysis (Hyre *et al*., 2006). The activation energy obtained using the Arrhenius law (i.e., $k^0 \sim \exp(-\Delta F_0^\ddagger / k_B T)$) on our data ($60 k_B T$) was in reasonable agreement considering only three data points were available. The height of the inner transition state was estimated relative to the outer one knowing that the difference between barriers is given by $\Delta\Delta F_0^\ddagger = \ln(k_1^0/k_2^0)$, leading to a difference of $\sim 5.4 k_B T$ at room temperature. The Arrhenius law predicted an unreasonably low value of $\sim 2 k_B T$ for the height of the inner barrier based on the derived dissociation rates. Again the low number of data points and the displacement of the inner barrier position may contribute to the inapplicability of the Arrhenius equation. In fact, the Arrhenius equation is only a first approximation when the energy landscape presents small roughness (Hyeon and Thirumalai, 2003). Table 2 summarizes the energy landscape roughness at four force levels (i.e., 75, 90, 135, and 156 pN), two from each loading regime. Our estimations of the energy landscape roughness were consistent within each loading linear regime, i.e., within their localization along the reaction coordinate, being on average 5.6 ± 0.5 and $7.5 \pm 1.4 k_B T$ along the inner and outer barriers, respectively.

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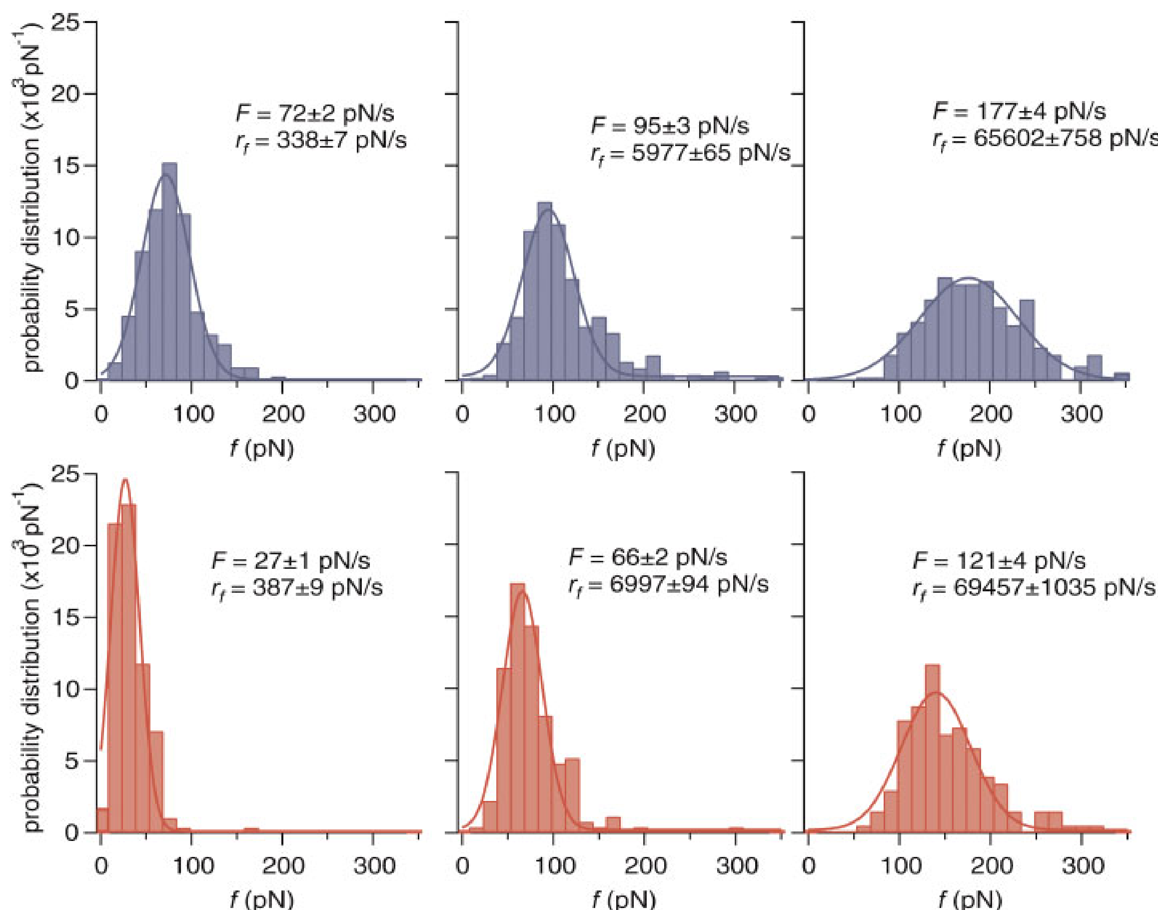

Figure 3. Unbinding forces (*f*) were grouped by loading rates and histograms were generated. Normalized histograms obtained from measurements at 17°C (up) and 37°C (down) at three similar loading rates (r_F) are shown for comparison. The solid lines are the corresponding Gaussian fits used to extract the most probable unbinding force (*F*). This figure is available in colour online at www.interscience.wiley.com/journal/jmr

DISCUSSION AND CONCLUSION

The dynamic force spectrum of the streptavidin–biotin complex at different temperatures was determined using the AFM in force spectroscopy mode. Temperature increase induced a marked drop in the unbinding forces of the complex over the entire range of loading rates. Fitting the acquired measurements from each of the two linear loading regimes to the Bell model [i.e., Equation (1)] enabled us to characterize the two main barriers in the streptavidin–biotin energy landscape. Moreover, knowledge of the position of the two barriers enabled us to determine how roughness was distributed along the reaction coordinate. As summarized in Table 2, there is a slight increase in the roughness amplitude from the inner barrier to the outer barrier.

The force spectroscopy methodology used in the present work has been previously used to determine the energy landscape of streptavidin–biotin and other receptor–ligand complexes (Merkel *et al*., 1999; Zhang *et al*., 2004; Sulchek *et al*., 2005; Wojcikiewicz *et al*., 2006). The finding of two energy barriers localized at 0.09 and 0.38 nm at room temperature is in good agreement with previous experimental results and molecular simulations of the same system (Grubmuller *et al*., 1996; Merkel *et al*., 1999; Yuan *et al*., 2000; Pincet and Husson, 2005; Zhou *et al*., 2006). The effect of temperature on the Bell parameters is summarized in Table 1. The Bell parameters revealed that the position of the outer barrier did not change considerably (<22%) within the range of temperatures tested, whereas the inner barrier widened by as much as 67% at the higher temperature (from 0.09 to 0.15 nm). This considerable widening of the inner barrier, together with a lowering of the outer barrier, led to a dynamic force spectrum with less differentiated loading regimes as shown in Figure 4. Changes on the barrier height and width in temperature have been already observed in other systems (Nevo *et al*., 2005; Schlierf and Rief, 2005; Janovjak *et al*., 2007). Our observations reveal softening of the inner transition state at the highest temperature, i.e., temperature increase shifted outwards the inner barrier, facilitating dissociation. This behavior may reflect destabilization of streptavidin at high temperatures due, perhaps, to the change in the distance and strength of hydrogen bonds at 37°C. As expected for a thermally activated process, the kinetic off rates obtained for the outer barrier followed a clear increase with temperature. However, this was not the case for the inner barrier, probably because of the shift in the barrier position at the highest temperature. Our barrier localizations are in agreement with the suggestion that the intermediate state of the complex is very similar to the ground state except for a hydrogen bond formed by the D128 residue is replaced by a water molecule (Hyre *et al*., 2002). Based on crystallographic and computational analyses, the

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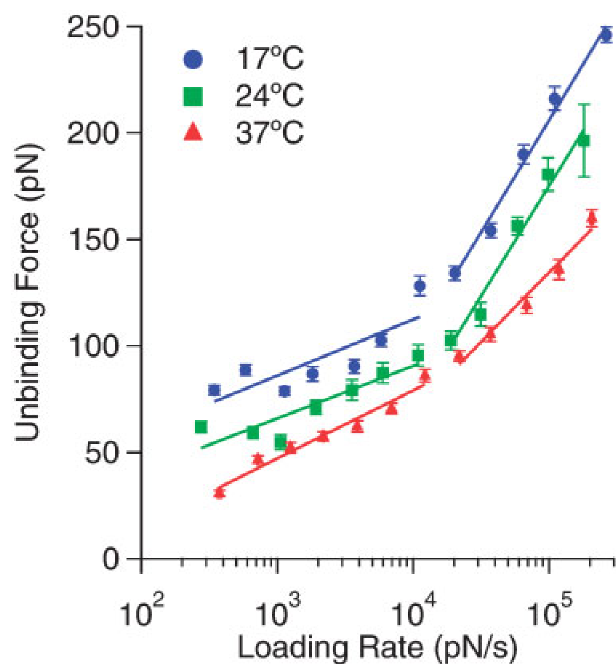

Figure 4. Dynamic force spectra (most probable unbinding force vs. loading rate) of the streptavidin–biotin interaction at 17°C (blue circles), 24°C (green squares), and 37°C (red triangles). The solid lines (same color convention) represent the best fits to Equation (1), from which information of the inner (high loading rates) and outer (low loading rates) barriers were extracted.

initial unbinding of the complex was predicted to result in a ~0.2 nm lengthening of the D128-biotin distance. This distance falls within our positions of the two energy barriers and may support their hypothesis.

Our estimates of the energy landscape roughness were based on the model of Nevo and collaborators (Nevo *et al*., 2005). As summarized in Table 2, the roughnesses of the inner and outer barrier of the streptavidin–biotin complex are ~5.6 and 7.5k_BT, respectively. Similar values have been obtained for other systems, such as the unbinding of GTPase Ran from its receptor importin-β (5.7k_BT), the unbinding of complementary DNA strands (10k_BT), and the unfolding of the transmembrane helices of bacteriorhodopsin (4–6k_BT) (Schumakovitch *et al*., 2002; Nevo *et al*., 2005; Janovjak *et al*., 2007). Even more, when the force clamp technique was used to measure the unfolding rate of ubiquitin, Brujic *et al*. (2006) found that their observed power-law distribution of unfolding rates can be explained by assuming an exponential distribution of energy barriers with a mean value of 6.6k_BT. The similarity in the results in such

Table 1. Interaction parameters (±95% confidence intervals) obtained from fitting Equation (1)

	Inner barrier		Outer barrier	
T (°C)	k_1^0 (1/s)	x_1 (nm)	k_2^0 (1/s)	x_2 (nm)
17	0.02 ± 0.13	0.35 ± 0.27	12 ± 9	0.09 ± 0.02
24	0.10 ± 0.33	0.38 ± 0.21	23 ± 16	0.09 ± 0.03
37	1.22 ± 0.93	0.31 ± 0.07	15 ± 17	0.15 ± 0.05

k_i^0, dissociation rate at zero force; x_i, potential width.

Table 2. Free energy landscape roughness (ε) estimated from Equation (2)

		ε (k_BT) for T_1, T_2 (°C)		
Barrier	F^* (pN)	17, 24	17, 37	24, 37
Outer	75	5.8	9.8	7.6
	90	6.7	8.1	7.3
Inner	135	4.9	5.8	5.9
	156	5.1	5.8	6.0

dissimilar systems suggests a common origin for the roughness.

The activation barrier of the streptavidin–biotin interaction is one of the highest for a noncovalent interaction and its origin is mainly enthalpic. The bound complex is formed by a network of at least seven hydrogen bonds, from which five reside deep in the binding pocket (Figure 1). In addition, important hydrophobic and van der Waals contributions from at least three tryptophan contacts stabilize the complex (Weber *et al*., 1989; Chilkoti *et al*., 1995; Weber *et al*., 1995; Freitag *et al*., 1998). Our estimated values of the energy landscape roughness correspond to 13–18% of the barrier height. This roughness of energy surfaces can be interpreted as the existence of different substates within the main states of the system (Frauenfelder *et al*., 1999). The streptavidin–biotin system has at least two energy barriers and, thus, an intermediate state. As mentioned above, this intermediate substate has been suggested to emerge from the stochastic diffusion of a water molecule in and out of the binding site replacing the hydrogen bond between biotin and the D128 streptavidin residue (Hyre *et al*., 2002). Thus, this intermittent substate may contribute to the roughness when the outer barrier governs the interaction (slow loading rates), giving rise to the 2k_BT difference between the roughness estimates along the reaction coordinate. In contrast, when the outer barrier is suppressed by an applied force and the interaction is governed by the inner barrier (high loading rates), the intermediate state is not observable and does not contribute to the averaged roughness. Thus, the higher roughness observed from the outer barrier is in agreement with the interpretation of a roughness caused by the existence of multiple substates. However, this does not explain the molecular origin of the overall roughness. As was already suggested by Kramers (1940), roughness can be an effect of the oversimplification implied when reducing the actual three-dimensional unbinding pathway to a one-dimensional pathway. However, due to the structurally restricted corridor by which biotin dissociates, the one-dimensional assumption appears to be reasonable for the present complex. The detailed structural information of the streptavidin–biotin complex enables us to speculate about the particular origin of the observed roughness. It is known that at least two of the formed H-bonds compete with water molecules from the solvent. This may lead to a population of slightly different energetic substates that contributed to the observed roughness. In addition, it has been shown for the streptavidin tetramers that a flexible loop in the adjacent streptavidin monomer closes the binding pocket, stabilizing the bound state (Stayton *et al*., 1999). Mutations of the W120 residue in this loop showed a dramatic drop in the

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outer barrier of $\sim 6k_BT$ (Wong, 1999; Yuan *et al*., 2000). The flexibility of this loop may also induce different structural configurations (substates) in the bound complex, with considerable energetic variations, inducing roughness. Since the contribution of W120 is known to be of van der Waals and hydrophobic nature (Chilkoti *et al*., 1995), we thus conclude that the energy landscape roughness stems from a complex distribution of substates with variations in structural conformations involving H-binding, van der Waals, and hydrophobic interactions.

Diffusion in a rough potential has been shown to decrease exponentially with the amplitude of roughness (Zwanzig, 1988). The energy landscape roughness of a receptor–ligand system is predicted to result in a dramatic drop of the dissociation rate. Thus, the large roughness detected in the streptavidin–biotin energy landscape may contribute to the slow dissociation kinetics of the complex. This slowing of the dissociation kinetics might be more pronounced at low temperatures. We indeed observed such an effect at the lowest temperatures tested, especially at the low loading rates regime, where we found higher roughness amplitude. The observed roughness may also explain the high scattering in the unbinding forces reported in the literature from force spectroscopy experiments (Pincet and Husson, 2005). Indeed, if the energy landscape is rough, it is reasonable to think that when biotin binds to streptavidin there are many substates in which the complex may fall. The system will hop from substate to substate until the ground, bound state is reached. Thus, slight variations in the contact time of the different experimental conditions may lead to detectable changes in the unbinding force. This is in agreement with the experiments reported by Pincet and Husson (2005), in which force changed considerably with the time the complex remained bound. The authors suggested a third energy barrier with very slow association kinetics that may be again explained if roughness is considered.

From the parameters obtained from our measurements (Tables 1 and 2), we can roughly sketch the smooth and rough energy landscape of our complex (Figure 5). It is important to remark that the roughness sketched in the figure has been randomly generated from a Gaussian distribution of barrier heights with ε standard deviation. In addition, the characteristic length of the roughness is unknown and was assumed to be much smaller than the dimensions of the barrier widths. However, the picture gives an idea of how the energy landscape can be modified if roughness is taken into account.

In summary, we have determined the dynamic force spectra at different temperatures of the streptavidin–biotin interaction using the AFM. Two loading regimes were observed and interpreted as being governed by an outer and an inner barrier. Fitting the Bell model to these regimes enabled us to determine the dissociation rate and the barrier positions at the tested temperatures. We also estimated the free energy landscape roughness of the streptavidin–biotin interaction. The linear regimes enabled us to localize the roughness amplitude along the reaction coordinate. Roughness increased considerably from the inner to the outer barrier, an observation consistent with the structure of the streptavidin–biotin complex.

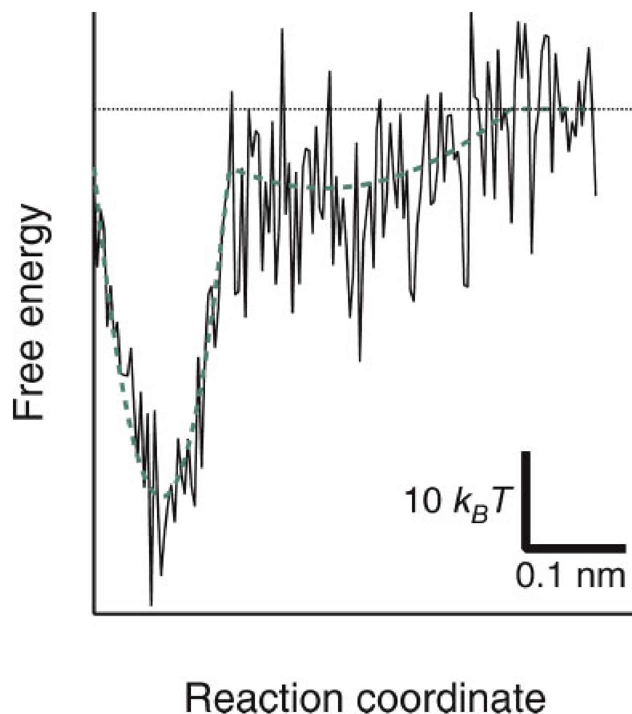

Figure 5. Sketch of rough (black thin line) and smooth (green thick dotted line) free energy landscapes for the streptavidin–biotin interaction assuming a Gaussian distributed roughness amplitude independent of the position along the reaction coordinate (zero mean, ε standard deviation). This figure is available in colour online at www.interscience.wiley.com/journal/jmr

Acknowledgments

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REFERENCES

Alcaraz J, Buscemi L, Puig-de-Morales M, Colchero J, Baro A, Navajas D. 2002. Correction of microrheological measurements of soft samples with atomic force microscopy for the hydrodynamic drag on the cantilever. *Langmuir* **18**(3): 716–721.

Ansari A, Berendzen J, Bowne SF, Frauenfelder H, Iben IET, Sauke TB, Shyamsunder E, Young RD. 1985. Protein states and protein quakes. *Proc. Nat. Acad. Sci. USA* **82**(15): 5000– 5004.

Bell GI. 1978. Models for specific adhesion of cells to cells. *Science* **200**(4342): 618–627.

Brujic J, Hermans RI, Walther KA, Fernandez JM. 2006. Single-molecule force spectroscopy reveals signatures of glassy dynamics in the energy landscape of ubiquitin. *Nat. Phy.* **2**(4): 282–286.

Chilkoti A, Stayton PS. 1995. Molecular-origins of the slow streptavidin-biotin dissociation kinetics. *J. Am. Chem. Soc.* **117**(43): 10622–10628.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002/jmr

Chilkoti A, Tan PH, Stayton PS. 1995. Site-directed mutagenesis studies of the high-affinity streptavidin-biotin complex—contributions of tryptophan residue-79, residue-108, and residue-120. *Proc. Nat. Acad. Sci. USA* **92**(5): 1754–1758.

Derenyi I, Bartolo D, Ajdari A. 2004. Effects of intermediate bound states in dynamic force spectroscopy. *Biophys. J.* **86**(3): 1263–1269.

Evans E. 2001. Probing the relation between force—Lifetime—and chemistry in single molecular bonds. *Annu. Rev. Biophys. Biomol. Struct.* **30**: 105–128.

Evans E, Ritchie K. 1997. Dynamic strength of molecular adhesion bonds. *Biophys. J.* **72**(4): 1541–1555.

Florin EL, Moy VT, Gaub HE. 1994. Adhesion forces between individual ligand-receptor pairs. *Science* **264**(5157): 415–417.

Frauenfelder H, Sligar SG, Wolynes PG. 1991. The energy landscapes and motions of proteins. *Science* **254**(5038): 1598–1603.

Frauenfelder H, Wolynes PG, Austin RH. 1999. Biological physics. *Rev. Mod. Phys.* **71**(2): S419–S430.

Freitag S, Trong IL, Chilkoti A, Klumb LA, Stayton PS, Stenkamp RE. 1998. Structural studies of binding site tryptophan mutants in the high-affinity streptavidin-biotin complex. *J. Mol. Biol.* **279**(1): 211–221.

Grubmuller H, Heymann B, Tavan P. 1996. Ligand binding: molecular mechanics calculation of the streptavidin biotin rupture force. *Science* **271**(5251): 997–999.

Hanggi P, Talkner P, Borkovec M. 1990. Reaction-rate theory—50 years after Kramers. *Rev. Mod. Phys.* **62**(2): 251–341.

Hutter JL, Bechhoefer J. 1993. Calibration of atomic-force microscope tips. *Rev. Sci. Instrum.* **64**(7): 1868–1873.

Hyeon CB, Thirumalai D. 2003. Can energy landscape roughness of proteins and RNA be measured by using mechanical unfolding experiments? *Proc. Nat. Acad. Sci. USA* **100**(18): 10249–10253.

Hyre DE, Amon LM, Penzotti JE, Le Trong I, Stenkamp RE, Lybrand TP, Stayton PS. 2002. Early mechanistic events in biotin dissociation from streptavidin. *Nat. Struct. Biol.* **9**(8): 582–585.

Hyre DE, Trong IL, Merritt EA, Eccleston JF, Green NM, Stenkamp RE, Stayton PS. 2006. Cooperative hydrogen bond interactions in the streptavidin-biotin system 10.1110/ps.051970306. *Protein Sci.* **15**(3): 459–467.

Janovjak H, Knaus H, Muller DJ. 2007. Transmembrane helices have rough energy surfaces. *J. Am. Chem. Soc.* **129**(2): 246–247.

Kramers HA. 1940. Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica* **7**(4): 304.

Lee GU, Kidwell DA, Colton RJ. 1994. Sensing discrete streptavidin biotin interactions with atomic-force microscopy. *Langmuir* **10**(2): 354–357.

Li FY, Redick SD, Erickson HP, Moy VT. 2003. Force measurements of the alpha(5)beta(1) integrin-fibronectin interaction. *Biophys. J.* **84**(2): 1252–1262.

Marshall BT, Sarangapani KK, Lou JH, McEver RP, Zhu C. 2005. Force history dependence of receptor-ligand dissociation. *Biophys. J.* **88**(2): 1458–1466.

Merkel R, Nassoy P, Leung A, Ritchie K, Evans E. 1999. Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. *Nature* **397**(6714): 50–53.

Moy VT, Florin EL, Gaub. HE. 1994. Intermolecular forces and energies between ligands and receptors. *Science* **266**(5183): 257–259.

Neuert G, Albrecht C, Pamir E, Gaub. HE. 2006. Dynamic force spectroscopy of the digoxigenin-antibody complex. *FEBS Lett.* **580**(2): 505–509.

Nevo R, Brumfeld V, Kapon R, Hinterdorfer P, Reich Z. 2005. Direct measurement of protein energy landscape roughness. *EMBO Rep.* **6**(5): 482–486.

Pincet F, Husson J. 2005. The solution to the streptavidin-biotin paradox: the influence of history on the strength of single molecular bonds. *Biophys. J.* **89**(6): 4374–4381.

Pollak E, Talkner P. 2005. Reaction rate theory: what it was, where is it today, and where is it going? *Chaos* **15**(2): 26116.

Schlierf M, Rief M. 2005. Temperature softening of a protein in single-molecule experiments. *J. Mol. Biol.* **354**(2): 497–503.

Schumakovitch I, Grange W, Strunz T, Bertoncini P, Guntherodt HJ, Hegner M. 2002. Temperature dependence of unbinding forces between complementary DNA strands. *Biophys. J.* **82**(1): 517–521.

Stayton PS, Freitag S, Klumb LA, Chilkoti A, Chu V, Penzotti JE, To R, Hyre D, Le Trong I, Lybrand TP, Stenkamp RE. 1999. Streptavidin-biotin binding energetics. *Biomol. Eng.* **16**(1–4): 39–44.

Sulchek TA, Friddle RW, Langry K, Lau EY, Albrecht H, Ratto TV, DeNardo SJ, Colvin ME, Noy A. 2005. Dynamic force spectroscopy of parallel individual mucin1-antibody bonds. *Proc. Nat. Acad. Sci. USA* **102**(46): 16638–16643.

Weber PC, Ohlendorf DH, Wendoloski JJ, Salemme FR. 1989. Structural origins of high-affinity biotin binding to streptavidin. *Science* **243**(4887): 85–88.

Weber PC, Wendoloski JJ, Pantoliano MW, Salemme FR. 1992. Crystallographic and thermodynamic comparison of natural and synthetic ligands bound to streptavidin. *J. Am. Chem. Soc.* **114**(9): 3197–3200.

Weber PC, Pantoliano MW, Salemme FR. 1995. Crystallographic and thermodynamic comparison of structurally diverse molecules binding to streptavidin. *Acta Crystallogr. D Biol. Crystallogr.* **51**: 590–596.

Wojcikiewicz EP, Abdulreda MH, Zhang X, Moy VT. 2006. Force spectroscopy of LFA-1 and its ligands, ICAM-1 and ICAM-2. *Biomacromolecules* **7**(11): 3188–3195.

Wang J, Chilkoti A, Moy VT. (1999). Direct force measurements of the streptavidin-biotin interaction. *Biomolecular Engneering* **16**(1–4): 45–55.

Young T, Abel R, Kim B, Berne BJ, Friesner RA. 2007. Motifs for molecular recognition exploiting hydrophobic enclosure in protein-ligand binding. *Proc. Natl. Acad. Sci. USA* **104**(3): 808–813.

Yuan C, Chen A, Kolb P, Moy VT. 2000. Energy landscape of streptavidin-biotin complexes measured by atomic force microscopy. *Biochemistry* **39**(33): 10219–10223.

Zhang XH, Bogorin DF, Moy VT. 2004. Molecular basis of the dynamic strength of the sialyl Lewis X-selectin interaction. *Chemphyschem* **5**(2): 175–182.

Zhang XH, Craig SE, Kirby H, Humphries MJ, Moy VT. 2004. Molecular basis for the dynamic strength of the integrin alpha(4)beta(1)/VCAM-1 interaction. *Biophys. J.* **87**(5): 3470–3478.

Zhou J, Zhang LZ, Leng YS, Tsao HK, Sheng YJ, Jiang SY. 2006. Unbinding of the streptavidin-biotin complex by atomic force microscopy: a hybrid simulation study. *J. Chem. Phys.* **125**(10): 104905.

Zwanzig R. 1988. Diffusion in a rough potential. *Proc. Natl. Acad. Sci. USA* **85**(7): 2029–2030.

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