Measuring Single-Bond Rupture Forces Using High Electric Fields in Microfluidic Channels and DNA Oligomers as Force Tags

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ABSTRACT The disruption force of specific biotin-streptavidin bonds was determined using DNA oligomers as force tags. Forces were generated by an electric field acting on a biotinylated fluorescently labeled DNA oligomer. DNA oligomers were immobilized via biotin-streptavidin bonds on the walls of microfluidic channels. Channel layout and fluid-based deposition process were designed to enable well-defined localized deposition of the oligomers in a narrow gap of the microchannel. Electric fields of up to 400 V/cm were applied and electric field induced desorption of DNA oligomers was observed. At $T \approx 30^{\circ}C$, field-induced desorption of both a 12 mer as well as a 48 mer yielded a streptavidin-biotin disruption force of 75 fN. Streptavidin-functionalized surfaces remained intact and could be reloaded with biotinylated oligomers. At $\approx 20^{\circ}C$, however, no field-induced unbinding of the oligomers was observed at electric field strength of up to 400 V/cm, indicating a significant temperature dependence of the bond strength.

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The question of the strength of a single chemical bond or of the interaction between biological receptors and their ligands has already stirred a host of research in the past. Most of this work made use of mechanical forces exerted on molecular bonds by the cantilever of a force microscope (1–7). Merkel et al. employed lipid vesicles to present biological receptors in their native environment and utilized deviations of the vesicle form from spherical shape to calculate rupture forces. In particular, the rate dependency of rupture forces is now well established (8–11). Forces on the order of 100 pN have been measured in case of the biotin-streptavidin system, depending on the speed of rupture. In this work, we propose a novel method and report preliminary results concerning electric field-induced rupture of biotin-streptavidin bonds (Fig. 1). Biotinylated and fluorescently labeled 12 mer and 48 mer DNA oligomers were used as force tags. The oligomers carry one negative elementary charge per phosphate group. Streptavidin was immobilized in microchannels via aminosilane and a bifunctional spacer (Fig. 1). The force acting on the bond may then be obtained according to

$$F = qE = -enE, \tag{1}$$

with q being the overall charge of the molecule given by the elementary charge, e, times the number of nucleotides, n, the oligomer consists of. E is the applied electric field. Microchannel structures with integrated electrodes for potential sensing were prepared by photolithography and wet chemical etching in glass (Fig. 2 *a*). Fluidic ports were drilled and a cover plate was glued on top to seal the channel structure. A narrow ($\approx 50\ \mu m$) gap provided for high internal channel resistance in this area (Fig. 2 *a*). Thus, most of the voltage applied drops across this narrow stretch of the channel resulting in maximum electric field strength. Low conductivity histidin buffer (50 mM, pH 7.05) was used to minimize Joule heating.

Two side channels connect at both ends of the gap. These are employed during the fluid-based immobilization process used to immobilize the oligomers exclusively in the gap. First, aminopropylsilane (APS) is immobilized by pumping an APS solution from port 1 to port 2 while blocking the cross channel (3,4), followed by *N*-hydroxysuccinimid-biotin (NHS-biotin) flow through ports 3 and 4, which is covalently bound to amino groups only in the gap region. Then, streptavidin is immobilized by flushing a solution of the protein through ports 1 and 2, which binds only in the gap region. Finally, biotin-DNA oligomers are selectively immobilized in the gap region as is confirmed by fluorescence microscopy. Voltage was applied in a ramp, at terminals 1 and 2 (Fig. 2 *a*), increasing over time at 2 V/s. Voltage drop over the gap region was measured using platinum thin film electrodes embedded in the channel. Integrated fluorescence intensity was determined in three regions of interest (ROI) indicated in Fig. 2 *b*: (*1*) within the gap, (*2*) outside the channel as a reference signal, and (*3*) in one of the side channels. Control experiments with fluorescein isothiocyanate-labeled streptavidin showed specific binding of the oligomers exclusively to the streptavidin. Also, when streptavidin functionalized surfaces were pretreated by biotin, no immobilization of biotinylated oligomers was observed, indicating that biotin oligomers indeed bind specifically via the biotin-streptavidin bond. Fig. 3 *a* shows data obtained in a typical experiment with a 12 mer biotin oligomer immobilized. Each

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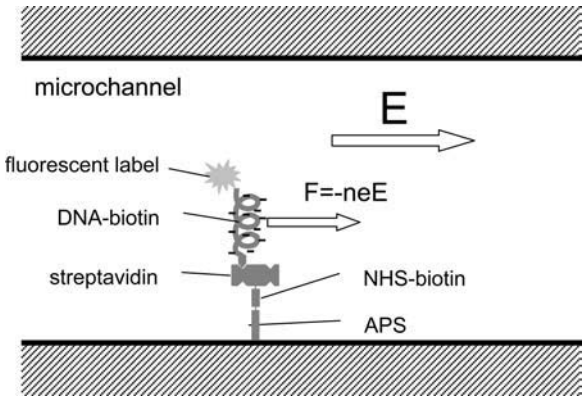

FIGURE 1 Scheme of experimental setup.

data point corresponds to a fluorescence image. Intensity remains virtually stable, indicating constant concentration of bound oligomers up until the electric field strength reaches a value sufficient to induce rupture of the biotin-streptavidin bond. Within a few seconds, the fluorescence intensity drops to background value. In case of the 12 mer oligomer, the rupture field strength was 440 ± 20 V/cm ($N = 3$). For the 48 mer, we obtained 90 ± 32 V/cm ($N = 4$), Fig. 3 *b*. This corresponds to a rupture force of 85 fN (12 mer) and 69 fN (48 mer) (Eq. 1). The following concerns arise and require discussion:

1. Since the bond force (≈77 fN on average) obtained is so much smaller than previously measured by atomic force microscopy measurements, could the biotin oligomers be merely physisorbed rather than attached by specific oligomer biotin-streptavidin bonds? Experiments with FITC-labeled streptavidin and CY3-labeled oligomers showed that both green and red fluorescence signals coincide both with respect to intensity and structure, indicating specific biotin-streptavidin bonds. In addition, if the streptavidin-functionalized surface was preincubated with biotin, no biotinylated oligomers will bind to the surface.
2. How do we know that actually the oligomer is desorbed while the streptavidin remains intact? After field induced desorption of the biotinylated oligomers, the fluorescence of the streptavidin was still present and could be loaded with another sample of biotinylated fluorescently labeled DNA oligomer, indicating intact streptavidin functionalization.
3. Could excessive temperatures due to Joule heating have damaged the streptavidin and caused biotinylated oligomers to be released? Current flow typically was on the order of a few microamperes. The power dissipation was ≤1 mW. Even with the relatively low heat conductivity of glass, the temperature increase will be <1 K even at the highest field strengths applied in this study.
4. The force measured for the rupture of the biotin-streptavidin bond is much lower than the value measured earlier by mechanical force spectroscopy (12). In our experiments, we used DNA oligomers without additional spacer between the oligomer and the biotin moiety, which

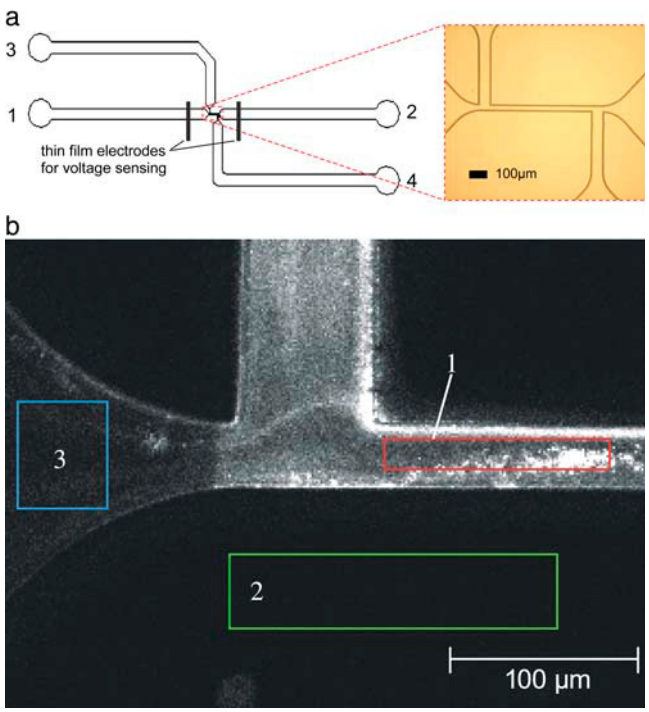

FIGURE 2 Channel layout (*a*) and fluorescence micrograph of channel (depth, 15–20 m) with immobilized oligomer (*b*).

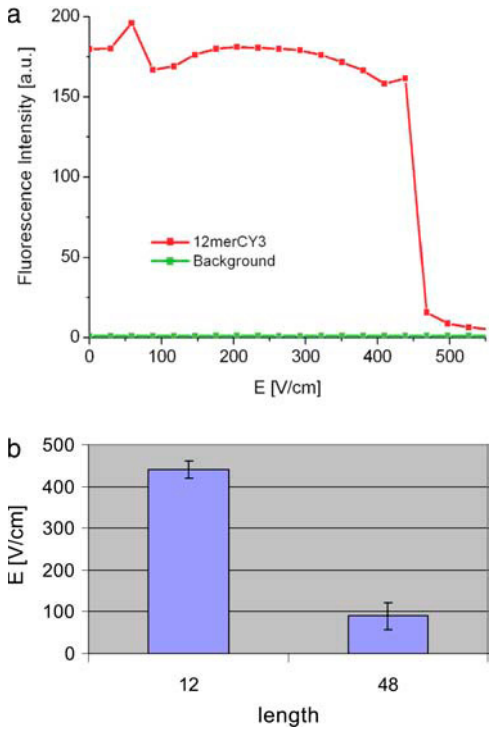

FIGURE 3 Field-induced desorption of a 12 mer (*a*) and electric field required for 12 mer and 48 mer, respectively (*b*).

might affect binding strength. Also, rupture force lessens with decreasing velocity (9). With our system, extremely low velocities are conceivable to occur during rupture. We have demonstrated electric field-induced dissociation of streptavidin-biotin bonds at forces much lower than previously reported from experiments utilizing force microscopy. Particularly, the temperature seems to have a pronounced effect on the magnitude of disruption forces, since although release of oligomers was observed repeatedly at 30°C, no bond rupture could be induced at $T = 20$°C, even at the highest field strength available to us ($\approx$500 V/cm) and using a 48 mer oligomer. The origin and the size of this effect have to be investigated further.

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