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Light-responsive Polymer Particles as Force Clamps for Mechanical Unfolding of Target Molecules

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Abstract

Single molecule force spectroscopy techniques are powerful tools to investigate the mechanical unfolding of biomolecules. However, they are limited in throughput and require dedicated instrumentation. Here, we report a force-generating particle that can unfold target molecules on-demand. The particle consists of a plasmonic nanorod core encapsulated with a thermoresponsive polymer shell. Optical heating of the nanorod leads to rapid collapse of the polymer, thus transducing light into mechanical work to unfold target molecules. The illumination tunes the duration and degree of particle collapse thus controlling the lifetime and magnitude of applied forces. Single molecule fluorescence imaging showed reproducible mechanical unfolding of DNA hairpins. We also demonstrate triggering of 50 different particles in <1 min, exceeding the speed of conventional atomic force microscopy. The polymer force clamp represents a facile and bottom-up approach to force manipulation.

Graphical Abstract

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Author Contributions

All authors have given approval to the final version of the manuscript. H.S., Z.L., B.D. and K.S. conceived and designed the experiments. H.S. and Z.L. designed and assembled the PFC laser illumination system. H.S. and Z.L. performed the DNA unfolding experiments. H.S., Y.L. and K.G. performed the surface modification. H.S. and V.M. performed the DNA modification. A.B. carried out the MATLAB analysis. J.Z. carried out the COMSOL simulation. The manuscript was prepared by H.S. and K.S. with input from all authors.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Supporting information (docx)

Supporting movie S1–S8 (avi)

Keywords

core-shell particle; responsive materials; force spectroscopy; gold nanoparticles; DNA unfolding

Tools such as atomic force microscopy (AFM)^{1–4} along with optical^{5–7} and magnetic⁸ tweezers have opened the door to studying the mechanical unfolding of biomolecules. However, these techniques are low throughput, require dedicated and complex instrumentation⁹ and also require linking the target molecule of interest to the force probe.¹⁰ One approach to increase the throughput is centrifuge force microscopy (CFM) where Wong and coworkers retrofitted a standard centrifuge to spin a sample containing microbeads anchored through a molecule of interest.^{11, 12} More recently, Liedl and colleagues showed that bracket-shaped DNA origami structures can clamp target DNA molecules at a defined extension.¹³ This was a noteworthy development and in contrast to CFM, avoids the need for instrumentation since the force generating unit is synthesized bottom-up. Nonetheless, each origami clamp is fixed and does not allow for dynamic modulation of the applied force. This is an important capability as recent evidence shows that the rate of folding (and unfolding) and the force response are highly dependent on the dynamic history of a bond.^{14, 15} Therefore, it is important to develop methods of dynamically applying forces using force-generating nanomaterials.

Herein, we report a polymer force clamp (PFC) which can deliver pN forces to target biomolecules of interest leading to controlled unfolding. The general design of the PFC is illustrated in Scheme 1 and is based on our recently published work, where these polymer particles were used to trigger receptor activation in living cells.¹⁶ This past work suggested that multiple layers of these particles can be crosslinked to DNA with biotin-streptavidin to apply pN scale forces, which led us to the current investigations. The PFC is comprised of a gold nanorod core ($\sim 25 \times 100$ nm) as a photothermal transducer¹⁷ coated with a 250 nm thermoresponsive poly-N-isopropylmethacrylamide (pNIPMAm) shell (Figure 1a and S1). We synthesized PFCs with a 250 nm polymer shell because modeling suggested that thermal energy is dissipated at a radius of 250 nm from the nanorod core (Figure S2). Therefore, near-infrared (NIR) illumination of the PFC leads to its collapse (from 550 nm to 310 nm based on DLS shown in Figure S3) generating an inward force applied to target biomolecules which are immobilized on a surface. To provide a convenient and modular handle for the attachment of target molecules, the PFC surface was decorated with alkyne groups amenable for Cu (I) catalyzed cycloaddition to any azide-modified target. The PFC particles were also labeled with fluorescein to validate the collapse of the particle following

NIR triggering. Although any NIR source can be used to control the collapse of the PFC, we employed a commercially-available galvo illuminator (Rapp Optoelectric, Germany) (Figure 1b) because this generated a 5 μm NIR spot controllable with μs time resolution (Figure S4).

Upon triggering by NIR, we observed an increase in the peak fluorescence intensity at the center of the fluorescein labeled PFC while the integrated intensity remained constant, thus showing optically-triggered particle collapse (Figure 1c and 1d). Timelapse imaging and line-scan analysis further confirm NIR-driven particle collapse (Figure S5 and Movie S1). These results show that we can perform simultaneous particle triggering and fluorescence imaging with high resolution.

We next investigated whether the PFC can mechanically clamp biomolecules. We focused our efforts on DNA hairpins because of their well-characterized digital force-response profiles and tunable mechanical properties.¹⁸ The $F_{1/2}$ is defined as the equilibrium force that leads to a 50% probability of hairpin unfolding and can be easily tuned by changing the GC content of a hairpin stem.¹⁹ In this study, we used two types of DNA hairpins with calibrated $F_{1/2}$ values of 4.7 pN and 13.1 pN (22% and 100% GC content stem, respectively).^{20, 21} To detect unfolding, we hybridized the hairpin with ligand strand and anchor strand that were functionalized with a fluorophore (Cy3B) and quencher (BHQ2), respectively, that undergo contact quenching (Figure 2a and Table S1). DNA hairpins were immobilized onto a glass coverslip that was decorated with 13 nm gold particles providing an additional quenching mechanism.^{22, 23} The surface anchored 4.7 pN DNA hairpin was coupled to the PFC using the click cycloaddition reaction (Figure S6). PFC surface density was reduced to facilitate control and characterization of DNA unfolding by individual particles (Figure S6). When the particle was illuminated at a 1 Hz frequency and 50 % duty cycle (500 msec illumination followed by 500 msec rest), we observed a rapid and concurrent increase in the fluorescein and Cy3B signal intensities (Figure 2b). Both channels reversibly responded to the NIR input and were spatially co-localized (Movie S2 and S3). We attributed the change in fluorescein signal to the physical collapse of the PFC while the enhancement of Cy3B signal is due to the mechanical unfolding of the 4.7 pN DNA hairpin. 50 different PFCs were randomly selected across a single substrate whose size changes were first confirmed by imaging in the fluorescein channel. Then, the change in Cy3B intensity (ΔI) of each particle was measured (Supplementary Method) and plotted in a histogram (Figure 2b). The mean ΔI was 1145 ± 490 a.u. which corresponds to unfolding of 7.6 ± 3.8 % of DNA hairpins under each particle (Figure S7 and S8). Given the DNA density as measured by AFM analysis (Figure S9) and bulk fluorescence measurements (Figure S10), this indicates that ~ 21 DNA hairpins ($F_{1/2} = 4.7$ pN) were mechanically unfolded within the brightest pixel area ($160 \text{ nm} \times 160 \text{ nm}$). Triggering different particles can be automated for rapid clamping measurements. Indeed, Figure S11 and Movie S4 show triggering of 49 PFCs and measuring associated DNA unfolding in less than one minute, which exceeds the throughput of a typical AFM clamping measurements by orders of magnitude.

Controls using non-extendable duplex DNA structure (Figure 2c) or covalently linking the particles to the glass instead of the DNA hairpin (Figure 2d and S12) confirmed that the Cy3B signal is due to mechanical unfolding of the DNA hairpin. In these controls, the fluorescein signal responded similarly to the experiments shown in Figure 2b, (Movie S5

and S6) but the I was 209 ± 36 a.u. (Figure 2c) and 92 ± 68 a.u. (Figure 2d, Movie S7 and S8). The weak Cy3B signals observed here are likely due to a combination of structural changes in the nicked DNA duplex following application of mechanical strain^{6, 24–26} as well as weak bleedthrough from the 785 nm NIR illumination (Figure S13). Furthermore, finite element analysis (Figure S14 and S15) predicts that the local temperature increase is insufficient to thermally denature the hairpin based on the melting curves for the DNA hairpins ($T_m=54$ °C and 70 °C for the 22% and 100% GC content stems, Figure S16). Taken together, the experiments and theoretical stimulations confirm that the PFC mechanically clamps and unfolds target molecule. Therefore, to the best of our knowledge, this represents the first example of a force-generating polymer particle that can be triggered to unfold target molecules on demand.

Next, we compared the unfolding of the $F_{1/2} = 4.7$ pN and 13.1 pN DNA hairpins (Figure 3a). Linescan analysis confirmed that PFC particles on both surfaces showed collapse upon NIR stimulation. However, the tension signal (I) for the 13.1 pN probes was significantly lower than that of the 4.7 pN probes (Figure 3b). A histogram plot of the I for $n = 50$ particles on two different samples with similar DNA density (for each type of DNA probe) is shown in Figure 3c. The I for the population of PFC particles shifted from a mean of 1145 ± 487 a.u. to 702 ± 498 a.u. when the probe $F_{1/2}$ was increased from 4.7 pN to 13.1 pN. Given that we used identical DNA surface densities in these samples and the unfolding was performed using the same batch of PFC particles, the difference in I is due to the innate difference in the free energy of unfolding the 4.7 and 13.1 pN hairpins. This suggests that more stable DNA hairpins require greater mechanical work to drive unfolding, and thus there are fewer unfolding events in the 13.1 pN sample. In other words, the experiment indicates that the PFC generates a limited amount of mechanical work that sets the total number of unfolded molecules during a collapse cycle with a given illumination profile (time and intensity).

To better define the force generated by each PFC as a function of laser power, we used the PFC to unfold DNA duplexes that are described as tension gauge tethers (TGTs), which denature at a threshold value of force, tension tolerance (T_{tol})^{27–30}. The T_{tol} is defined as the amount of force that leads to unfolding of a duplex within 2 sec. If the PFC applies a force above T_{tol} , then the tether will rupture and dequench the fluorophore (Figure 4a). The fraction of ruptured TGTs was calculated based on the fluorescence signal (Figure S17 and S18). We synthesized different TGT surfaces with $T_{tol} = 12$ pN, 33 pN and 58 pN by positioning the anchor of the upper strand. A 2 sec illumination at different laser powers was applied. Increasing the laser power leads to a general increase in the TGT signal. However, we found that the fraction of ruptured TGTs reached a plateau with the $2.75 \text{ mW} / \mu\text{m}^2$ laser power on the 12 pN TGT surface (Figure 4b) whereas a linear increase was observed on 33 pN TGT surface (Figure 4c). There was minimal TGT rupture of the $T_{tol} = 58$ pN probes (Figure 4d). These results define the range of applied forces using the calibrated TGT probes, and thus demonstrate that the average force experienced per molecule can be tuned and enhanced up to ~58 pN when employing the maximum illumination intensity of $3.39 \text{ mW} / \mu\text{m}^2$.

To demonstrate single molecule unfolding with our force-generating particles, we reduced the labeling ratio of DNA to 1:10000. This dye-labeling ratio resulted in a small fraction (~5%) of PFC particles that displayed single molecule fluorescence. Single molecule intensity was validated using control samples that were dequenched by hybridization and also by measuring single step photobleaching (Figure S19). To mechanically unfold single labeled DNA hairpins, we used a power density of 0.75 mW/ μm (matching the conditions used in Figure 2 and 3) with an on-time of 450 msec followed by a 450 msec rest time. Figure 5a shows representative time-lapse imaging of a single hairpin reversibly unfolding due to PFC-generated forces. The EMCCD camera acquisition (16.67 frame/sec) was synchronized with the NIR illumination pulse sequence in order to average the force-induced unfolding traces from different PFC particles. Figure 5b shows the average fluorescence intensity from a 5×5 pixel region of interest during a 17-sec NIR illumination sequence. Unfolding was characterized as a ~450 a.u. jump in fluorescence above the signal due to NIR background and could be measured for tens of cycles until the dye photobleached. A histogram of $n = 900$ single hairpin unfolding events from four different PFC particles exhibited a bimodal distribution of fluorescence intensity (Figure 5c). This distribution corresponds to the intensity of the folded and unfolded states of single DNA hairpins. Taken together, these experiments demonstrate that the PFC provides the ability to simultaneously manipulate and characterize the mechanical unfolding of individual target molecules.

Note that the PFC approach to unfolding carries several caveats. First, the applied force likely varies across the particle-surface junction. Molecules at the center of the junction likely experience weaker forces compared to the edges. Secondly, we observed particle-particle variability in DNA unfolding (Figure S20), which is due to the alignment of the linearly polarized source with the orientation of the nanorods (Figure S21).^{31, 32} Another source of variability is due to the monodispersity of the polymer shell and the gold nanorod, which is akin to tip-to-tip variability in AFM or bead-to-bead variability in optical/magnetic tweezer experiments. Nonetheless, when we probed 49 PFCs attached to the 13.1 pN DNA hairpin probe for two cycles, the individual particle response was generally reproducible (19% difference between first and second cycles) (Figure S11 and Movie S4).

In summary, our work demonstrates a novel approach for mechanically manipulating and unfolding target molecules. Forces are generated due to the volume phase transition of the polymer particle, and although in this report we focused on using light to trigger this process, it is possible to drive the volume phase transition using other stimuli such as pH, ionic strength, and temperature. Fundamentally, PFCs represent a bottom-up approach to force manipulation, where each particle is a force generating unit akin to an AFM force probe. Therefore, massive parallelization of mechanical unfolding is, in principle, possible using an ensemble of PFCs. This contrasts with conventional AFM, optical and magnetic tweezers where parallelization still represents a challenge.⁹ Finally, Time-resolved measurements of the volume phase transition of NIPAM nanogel particles indicate that the particle collapse occurs at the nanosecond time scale^{33, 34} and thus, PFCs can potentially offer force manipulation at the ns time scale, which is currently inaccessible to conventional methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

General design of the polymer force clamp (PFC). (a) Representative TEM image of PFC particles. Scale bar: 100 nm. (b) Optical configuration for simultaneous NIR illumination and fluorescence imaging. (c) Representative fluorescein images of labeled PFC particle irradiated with a 785 nm NIR laser beam at a power density of $0.75 \text{ mW}/\mu\text{m}^2$. Fluorescence within the blue circles was measured. Scale bar: 500 nm. (d) Plot displaying the mean and maximum intensity of PFC before and after NIR illumination. Error bars represent the standard deviation, $n = 5$.

Figure 2. Mechanical clamping of DNA hairpins. (a) Schematic diagram. (b–d) Representative fluorescein/Cy3B images and histogram of change in Cy3B intensity from 50 individual PFCs tethered to (b) DNA hairpins, (c) non-extendable DNA duplexes, and (d) PEG linker. Scale bar: 1 μm . NIR power density: 0.75 $\text{mW}/\mu\text{m}^2$.

Figure 3.

GC content influences unfolding. (a) Representative time-lapse fluorescein/Cy3B images of clamping the DNA hairpin with 22% and 100% GC content in a single illumination cycle (red circle, power density of $0.75 \text{ mW}/\mu\text{m}^2$, 1 Hz, 50% duty cycle). Scale bar: $5 \mu\text{m}$. (b) Line-scan analysis plot of force-clamp and DNA hairpin fluorescence in one unfolding cycle. (c) Histogram of Cy3B intensity values (unfolding) of 22% GC and 100% GC hairpin targets; $n = 50$.

Figure 4. Mechanical unfolding of TGT. (a) The first two fluorescence images show fluorescein-labeled PFC particles immobilized onto a 12 pN TGT surface at two magnifications. The third and fourth images show the Cy3B 12 pN TGT signal before and after NIR illumination, respectively. NIR power density = $2.75 \text{ mW}/\mu\text{m}^2$. Scale bar = $1 \mu\text{m}$, except for first image, which is $5 \mu\text{m}$. (b–d) Schematic and plots showing the fraction of ruptured TGTs as a function of laser power. TGT with $T_{\text{tol}} = 12, 33$, and 58 pN were tested.

Figure 5.

Monitoring the mechanical unfolding of a single DNA hairpin. (a) Representative time-lapse images of PFC-driven single hairpin unfolding. Scale bar: 2 μm . (b) Plot of the fluorescence intensity from data shown in panel a as a function of time (NIR stimulation). The signal during NIR illumination abruptly decreases at $t = 15$ s, which likely corresponds to photobleaching. The remaining signal during NIR illumination is due to background. The fluorescence intensity was calculated from a region of interest of 5×5 pixels. (c) Frequency histogram plotting the fluorescence intensity for 900 NIR illumination cycles collected from four different PFC particles. The signal was analyzed prior to photobleaching events.

Scheme 1.

Schematic comparing force spectroscopy using a conventional atomic force microscope and our polymer force clamp (PFC).