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Collective Conformations of DNA Polymers Assembled on Surface Density Gradients

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Supporting Information

ABSTRACT: To study dense double-stranded DNA (dsDNA) polymer phases, we fabricated continuous density gradients of binding sites for assembly on a photochemical interface and measured both dsDNA occupancy and extension using evanescent fluorescence. Despite the abundance of available binding sites, the dsDNA density saturates after occupation of only a fraction of the available sites along the gradient. The spatial position at which the density saturates marks the onset of collective stretching of dsDNA, a direct manifestation of balancing entropic and excluded-volume interactions. The methodology presented here offers a new means to investigate dense dsDNA compartments.

Dilute double-stranded DNA (dsDNA) molecules exhibit relaxed polymeric conformations at lengths beyond the persistence scale, $l_p \approx 50$ nm, which corresponds to 150 base pairs (bp).[1,2] At high density, overlap between polymers induces conformational changes that balance the restoring force of polymer elasticity and the swelling pressure due to excluded-volume repulsion.[3,4] Collective conformations in dsDNA are fundamentally important for understanding crowded cellular environments[5,6] and for the functionality of surface-bound dsDNA systems, such as hybridization arrays,[7−11] coated particle assemblies,[12,13] and synthetic biochemical compartments.[14−17] Most work on surface-bound dense DNA has focused on short molecules (≤100 bp),[18,19] where the polymer degrees of freedom are negligible, or on short single-stranded DNA.[20,21] In fact, longer dsDNA molecules (1 kbp) assembled on beads have been shown to exert compression forces as a result of collective polymer stretching.[22,23] Here we present direct measurements, free of external manipulation, of collective packing and extension of dsDNA polymers (0.3−2.5 kbp) on flat surfaces along continuous density gradients to values of interchain distances well below l_p.

To measure the collective extension of dsDNA brushes, we assembled dsDNA polymers along continuous density gradients (100 μm long) patterned on a glass prism coated with a photochemical monolayer (Figure 1a; see the Supporting Information for detailed methodology). The dsDNA polymers were chemically attached to the patterned surfaces through biotin−strepavidin (SA) conjugation; each dsDNA had one strand labeled at the 5′-end with Alexa488 and the other strand labeled at the 5′-end with biotin. The final assembly attained maximal density and was stable for days at room temperature. A total internal reflection fluorescence (TIRF) image was obtained by reflecting a 488 nm laser beam from the prism surface, exciting the fluorophores at the dsDNA ends along the exponential decay of the evanescent field perpendicular to the surface. To extract the dsDNA brush extension as a function of density, the epifluorescence (epiFL) image was taken concomitantly with the TIRF image (Figure 1b,c).[24−26] The

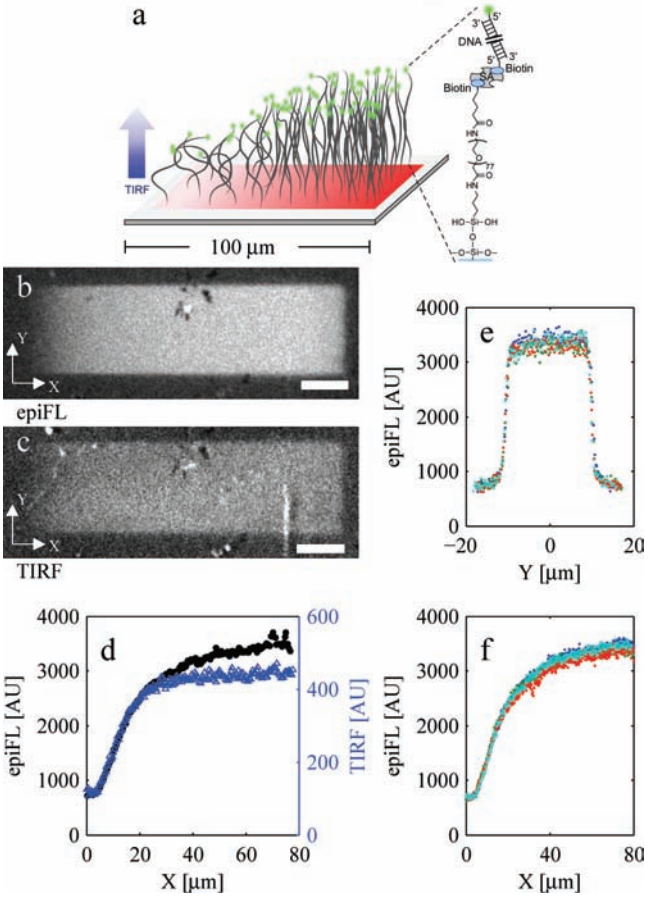

Figure 1. Measuring the collective assembly of dsDNA polymers: (a) Density gradient of dsDNA polymers assembled on a photochemical interface. (b, c) Epifluorescence (epiFL) and total internal reflection fluorescence (TIRF) images of 1300 bp dsDNA polymers. The scale bars correspond to 10 mm. (d) EpiFL and TIRF profiles of 1300 bp dsDNA averaged along *Y*. (e, f) Density profiles along the gradient (*X*) and symmetry axes (*Y*) of four gradient replicas (colored dots).

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images are related to $\sigma(X, Y)$, the local density of dsDNA molecules, and $h(X, Y)$, the local mean dsDNA height:

$$\mathrm{epiFL}(X, Y) \propto \sigma(X, Y)$$

$$\mathrm{TIRF}(X, Y) \propto \sigma(X, Y) \exp[-h(X, Y)/z_0]$$

where $z_0 \approx 100$ nm is the evanescent decay length. Both signals were taken from a region within the gradient and averaged along the symmetry axis (Y), resulting in one-dimensional profiles (Figure 1d). The incipient saturation of the TIRF profile with respect to the density profile, directly reports on increased mean dsDNA height (Figure 1d). The complete assembly methodology was highly reproducible, as reflected in the similarity among four replicas of each dsDNA gradient (Figure 1e,f).

Using identical binding site gradient patterns, we studied the assembly of SA and 300, 1300, and 2500 bp dsDNA (Figure 2a). We averaged the epiFL and TIRF profiles for several

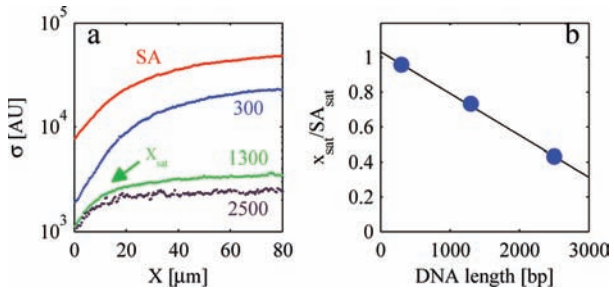

Figure 2. Saturation of dsDNA assembly: (a) Density profiles of SA and 300, 1300, and 2500 bp dsDNA. The saturation point X_{sat} is marked. (b) X_{sat} as a function of dsDNA length.

gradient replicas to obtain a single plot for each dsDNA length N. Each density profile saturated at a length-dependent maximal density $\sigma_{max}(N)$ that decreased with N, indicating that the dsDNA polymer assembly takes up only a small fraction of the available sites relative to SA. The interpolymer distances at maximal density for 1300 bp dsDNA amounted to 20−30 nm, as previously measured with radioactive-labeled dsDNA.[15] Defining the saturation point as $\sigma(X_{sat}) = 0.85\sigma_{max}$, we found that the density profiles of the longer DNAs saturated earlier along the axial position than that of the short dsDNA (Figure 2b). We deduce that longer polymers are maximally packed at a lower binding site density. Despite the abundance of binding sites and the excess of free dsDNA in the bulk solution during incubation, further assembly of the brush is hindered beyond σ_{max}, marking a regime where excluded-volume interactions of bound polymers dominate. In this regime, interpenetration of additional polymers is likely confined to a narrow outer region of the brush,[27] preventing their binding to the surface sites.

We next studied whether the saturation of assembly is correlated with extension of the dsDNA. Figure 3a,b presents TIRF profiles for SA and 300, 1300, and 2500 bp dsDNA plotted versus the density (epiFL). Expectedly, the SA epiFL and TIRF profiles are identical up to a constant factor (i.e., TIRF = C_0·epiFL), implying a constant height [i.e., $h/z_0 \approx -\log(\mathrm{TIRF/epiFL}) = -\log(C_0)$]. In contrast, the TIRF profiles for dsDNA polymers deviate from the epiFL signal at high densities, with the reduced TIRF signal per molecule most pronounced for 1300 bp dsDNA, which is direct evidence of a

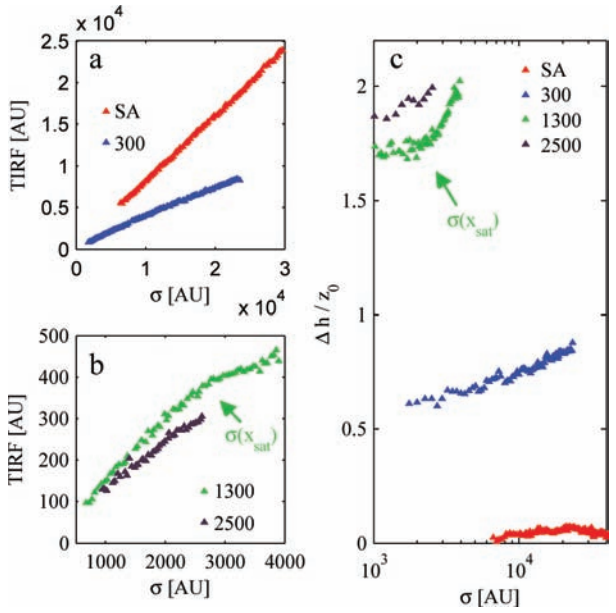

Figure 3. Collective extension: (a, b) TIRF gradient plotted against density σ (epiFL). (c) Extension of dsDNA relative to SA in units of the evanescent decay length $z_0 \approx 100$ nm.

height extension. Notably, the onset of the extension coincides with the density saturation point, $\sigma(X_{sat})$.

Figure 3c shows the height increase relative to SA, which is given by

$$\frac{\Delta h}{z_0} = -\ln\left(\frac{\mathrm{TIRF}}{\mathrm{epiFL}} \cdot \frac{\mathrm{epiFL_{SA}}}{\mathrm{TIRF_{SA}}}\right)$$

The extension of 300 bp dsDNA increases with density from $0.6z_0$ to $0.9z_0$. With $z_0 \approx 100$ nm, we find $h_{300}^{max} \approx 90$ nm, which agrees with previous measurements.[2] This short dsDNA is nearly rigid, comprising only 2 persistence lengths; hence, the height increase at high density is likely due to the alignment of randomly tilted molecules. For 1300 and 2500 bp dsDNA polymers, the height extends from $1.7z_0$ to $2z_0$ (corresponding to $h_{1300}^{min} \approx 170$ nm and $h_{1300}^{max} \approx 170$ nm) and from $1.8z_0$ to $2z_0$, respectively. Generally, the reduction of maximal density with dsDNA polymer length limits the measurable extension.

In summary, we offer a new methodology for studying the collective conformations of surface-bound dsDNA polymers using continuous surface gradients on a photochemical biochip. We directly observed height extension of the dsDNA polymers at high density. We further demonstrated that brush extension is correlated with saturation of the brush density, suggesting exclusion of free dsDNA from the brush during buildup. Experiments of collective assembly and extension as a function of ionic strength are underway but are beyond the scope of this communication. The method presented here could be used to study the kinetics of reactions in a dense dsDNA phase in a noninvasive manner.

■ ASSOCIATED CONTENT

Supporting Information

DNA sample preparation, biochip methodology, and imaging protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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