PROBING PHYSICAL PROPERTIES OF DNA-PROTEIN COMPLEXES USING NANOFLUIDIC CHANNELS

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ABSTRACT

We present the use of nanofluidic channels as a tool for determining physical properties of single DNA-protein complexes. By coating the nanochannels with a lipid bilayer we avoid sticking of proteins to the channel walls. RecA is a prokaryotic protein involved in recombination and DNA repair. We study filaments of RecA, bound to both double stranded (ds) and single stranded (ss) DNA. We determine the persistence length of RecA filaments on both dsDNA and ssDNA and obtain values in agreement with the literature. Neither the DNA nor the protein has to be attached to handles or surfaces, and the technique is directly transferable to Lab-on-a-Chip technologies for high throughput measurements in solution.

KEYWORDS: Nanofluidics, Single DNA Molecules, DNA-Protein Interactions, Lab-on-a-Chip

INTRODUCTION

Nanofluidic channels have become an important tool to investigate single DNA molecules both from a fundamental polymer physics perspective as well as in *e.g.* optical mapping techniques. Compared to traditional single molecule techniques, such as optical and magnetic tweezers, the DNA does not need to be attached to any handles to be analyzed. When a DNA molecule is confined in a channel with two dimensions smaller than the radius of gyration it will spontaneously stretch out along the channel.[1] The extension strongly depends on parameters such as degree of confinement and ionic strength of the surrounding solution. While equally relevant, less effort has been made to study the properties of DNA-protein complexes. A main reason for this is that the extreme surface-to-volume ratio in the nanochannels causes most proteins to stick to the channel walls. We have eliminated the problem with sticking by coating the channels with a lipid bilayer.[2]

RecA is an evolutionary conserved protein involved in homologous recombination and DNA repair, for which it forms helical filaments on single-stranded (ss) DNA in presence of ATP. *In vitro,* RecA-DNA filaments can be formed on both double- and single-stranded (ds or ss) DNA in presence of ATP or, preferably, a non-hydrolysable analog such as ATPγS. Using other techniques, persistence lengths ranging from 600-1000 nm have been reported for both ssDNA and dsDNA. RecA filaments bound to DNA is thus a suitable model system for initial experiments on how DNA-protein complexes behave under confinement.

Persson *et al*. introduced nanochannels with gradually changing dimensions, nanofunnels, to expose DNA molecules to a varying degree of confinement in a single chip.[3] These funnels can thus be used to study the same DNA molecule or DNA-protein complex at different degrees of confinement in a straightforward fashion. Nanofunnels have previously been used to study bare DNA, and are here, for the first time, used to study the physical properties of a DNA-protein complex; RecA-filaments formed on ss- and dsDNA.

EXPERIMENTAL

DNA sample preparation. λ-DNA was stained with YOYO-1 at a staining ration of 1:10 (dye:basepairs). The DNA concentration was 0.8 μM (basepairs) in 0.5xTBE buffer (pH 8.4), supplemented with 3% (v/v) β-mercaptoethanol to suppress photodamage of the DNA. The sample was incubated at room temperature for 2-3 hours before loading into the nanofluidic device.

DNA-protein sample preparation. RecA filaments were formed by mixing RecA and λ-DNA (ds) or ssDNA at 1.5 μM and 0.8 μM (basepairs), respectively, in 0.5xTBE buffer (pH 7.0) containing 5 mM $MgCl_2$ and 50 mM ATPγS. 50 mM DTT was added to the buffer to minimize oxygen radical induced photodamages. Long filaments (8-20 μm) were formed when the sample was incubated over night at 4°C. The ssDNA was prepared using rolling circle amplification using a protocol described elsewhere.[4]

Lipid passivation. The nanofluidic channel system was passivated using a lipid bilayer, as previously described.[2] The bilayer contained 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and Lissamine rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine lipids at 99:1 weight ratio. The fluorescent lipid made it possible to observe the bilayer formation by fluorescence microscopy, and to examine the status of the bilayer after storage (at 4°C).

The nanofluidic chip. The nanofluidic chip is schematically depicted in Figure 1. The devices were fabricated by standard micro- and nanofabrication techniques as described in detail elsewhere.[1] The channels are 140 nm deep and the width decreases gradually from 800 nm to 100 nm. The channel dimensions were measured using electron microscopy and profilometers before sealing. The devices were mounted in a chuck and the channels were wetted with buffer.

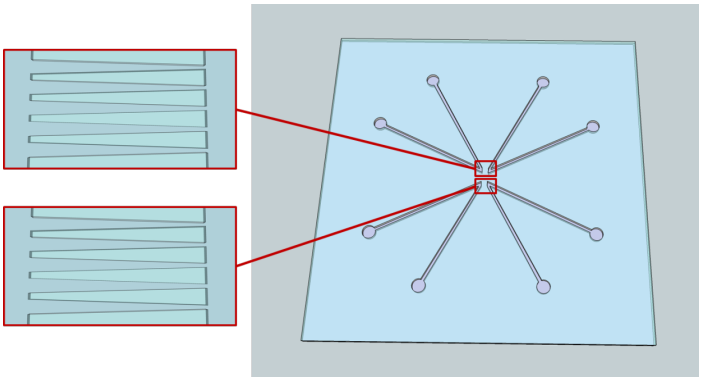

Figure 1: A schematic illustration of the nanofluidic chip design, which consists of two U-shaped microchannels connected at the center by the nanofunnels. One chip contains two separate channel systems.

RESULTS AND DISCUSSION

From snap-shot images (Figure 2, top left) we are able to discern that the RecA filaments formed on dsDNA (RecA-dsDNA) behave fundamentally different from YOYO-stained dsDNA (YOYO-dsDNA) when changing the degree of confinement. In the wide end, the YOYO-dsDNA covers the whole channel while the RecA-dsDNA undulates between the channel walls. Furthermore, when the channels dimensions are decreased, the YOYO-dsDNA is significantly extended while the RecA-dsDNA extension barely changes. Also, we observe considerable differences in dynamics of the two DNA complexes: While the YOYO-dsDNA extension changes threefold between the most and least extended state in a 750 nm wide channel, the RecA extension only varies ~5% (Figure 2, lower left).

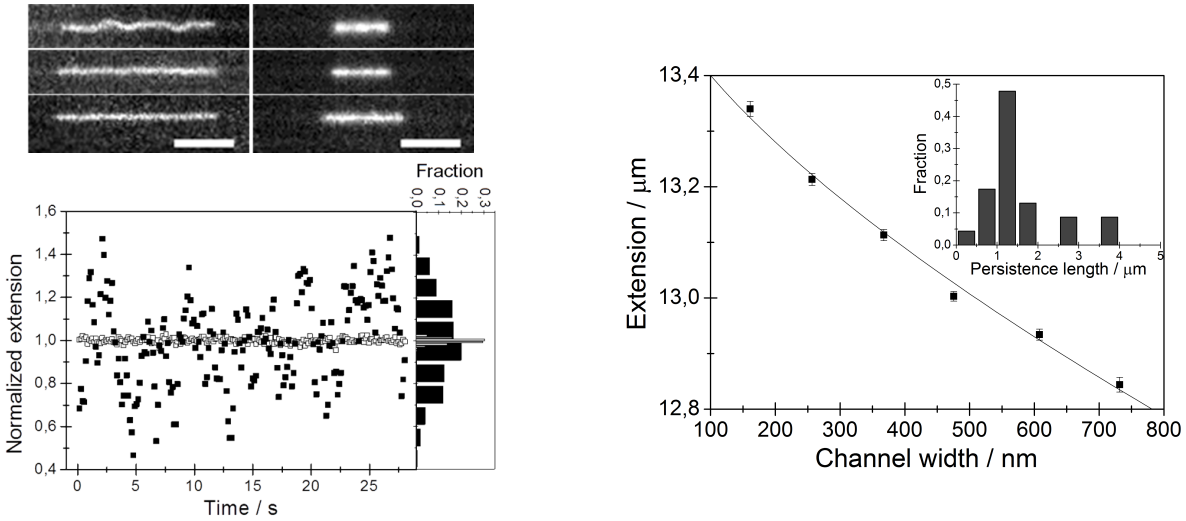

Figure 2: Top Left: Snap-shots of a fluorescent RecA filament (left) and a YOYO-stained dsDNA (right) at three different confinements, 730 nm (top), 370 nm (center) and 160 nm (bottom), respectively. Bottom Left: The relative extension of the RecA filament (open circles) and YOYO-stained dsDNA (solid squares) in the wide end of the funnel. The histograms demonstrate the length distribution for the RecA filament (white) and the YOYO-stained dsDNA (black), respectively. Right: The change in extension with channel width for a RecA filament in a 140 nm deep nanofunnel (solid symbols). The solid line is the fit to Eq. 1. Inset: Persistence lengths determined for 23 RecA filaments.

Measurements of the extension of a single filament at different channel dimensions enables determination of the persistence length. We use Odijk theory (Equation 1) to analyze our data, since the persistence length is expected to be larger than the width of the channel[5]:

$$r = L * \left(1 - B * \left[\left(\frac{D_1}{P}\right)^{2/3} + \left(\frac{D_2}{P}\right)^{2/3}\right]\right) \qquad (1)$$

In Equation 1, D_1 and D_2 are the dimensions of the funnel, r is the extension of the molecule and B has been numerically estimated to 0.085.[6] The unknown variables are thus the contour length of the molecule, L, and the persistence length, P. Fitting the extension at different confinements to this equation yields, for the specific filament in Figure 2 (right), a persistence length of 1.39 µm. By repeating the procedure for ~20 filaments we obtain an average persistence length for RecA on dsDNA, 1.15 ± 0.30 µm, that agrees well with the literature.[7]

We were also interested in studying RecA filaments formed on ssDNA (RecA-ssDNA) since this is the natural template for RecA in cells. RecA filaments formed on ssDNA appear very similar to the RecA-dsDNA filaments (fig. 3 left), as expected from earlier studies where it has been shown that the properties of the filament are mainly governed by the protein and not the DNA template. We obtain an average persistence length for the RecA-ssDNA filament of 1.61 ± 0.83 μm. The persistence length is longer and the standard deviation significantly larger than for dsDNA. While we do not yet have the full explanation for this observation, we note that the RecA-ssDNA formed by the RCA protocol (see experimental) is shorter than the RecA-dsDNA which means that we have a lower resolution in our measurements. For the Odijk theory to be valid, the polymer must be stretch to more than 90% of its contour length. We are currently working on alternative ways to analyze the data to discern if the large standard deviation is due to intrinsic differences between the complexes or experimental considerations.

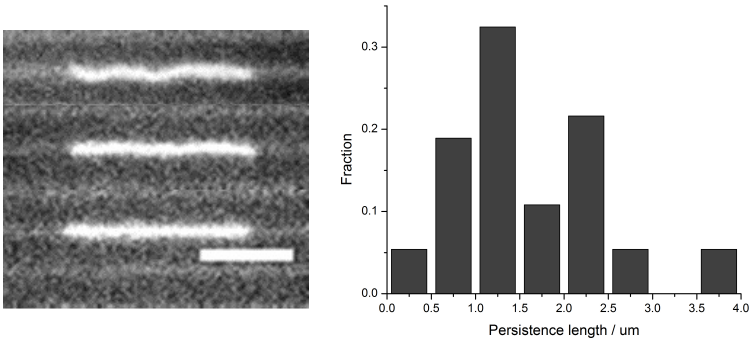

Figure 3: Left: Snap-shots of a fluorescent RecA filament formed on ssDNA at three different confinements, 730 nm (top), 370 nm (center) and 160 nm (bottom), respectively. Middle: Persistence lengths determined for 32 RecA filaments on ssDNA.

CONCLUSION

We demonstrate how physical properties of DNA-protein complexes can be investigated using nanofluidic channels. The experiments are performed in solution without the need to attach the DNA or protein to any surfaces or handles. This in turn means that we, in a future lab-on-a-chip device, will be able to do the same kind of analysis on DNA-protein complexes, such as chromatin, extracted from cells.

From a fundamental polymer physics perspective, the RecA filaments are very interesting since they have a persistence length that is more than one order of magnitude larger than for DNA. The RecA filaments will thus allow experimental studies of the Odijk regime with fluorescence microscopy in a way that is out of reach for dsDNA due to light diffraction.

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