A LIPID-BASED PASSIVATION SCHEME FOR NANOFLUIDICS

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ABSTRACT

Stretching DNA in nanochannels allows for direct, visual studies of genomic DNA at the single molecule level. In order to facilitate the study of the interaction of linear DNA with proteins in nanochannels, we have implemented a highly effective passivation scheme based on lipid bilayers. We show long-term passivation of nanochannel surfaces to several relevant reagents and demonstrate that the performance of the lipid bilayer is significantly better compared to standard bovine serum albumin-based passivation. Moreover, we demonstrate how the passivated devices allow us to monitor single DNA cleavage events during enzymatic degradation.

KEYWORDS

single molecules, nanofluidics, protein−DNA interactions, passivation, lipid bilayer

INTRODUCTION

A promising tool for single-molecule studies of biomolecules and biomolecular interactions is the nanofluidic chip, where macromolecules, such as DNA, can be stretched, directly visualized, manipulated, and probed on their own length scales without being constrained by tethering to beads or surfaces [1, 2]. Nanofluidic channels have been used both to understand the polymer physics of confined DNA [3] and for DNA mapping [4]. While some proof-of-principle experiments of DNA−protein interactions in nanochannels have been performed [2, 5], widespread use remains elusive due to the problem of nonspecific adhesion of the proteins to channel walls, which is worsened by the extreme surface-to-volume ratio in nanofluidics. The standard means of passivating surfaces for protein studies include saturating the surface with either bovine serum albumin (BSA) or caseins (from dry milk powder) or coating the surface with PLL-g-PEG. Although such methods have proven very useful for open surfaces and in microfluidics, they have limited applicability to nanofluidics since, relying on stochastic binding to the surface and/or competition with the sample of interest, they are prone to defects. Furthermore, the passivation agent is often charged so that it can stick to the surfaces, but this limits its usefulness for studies of interactions between oppositely charged molecules in nanofluidic systems.

In this study we demonstrate the use of LBLs as a passivation layer in nanofluidic networks [6], consisting of nanochannels and nanoslits, fabricated in fused silica. As opposed to immobilization-based passivation schemes, the use of LBLs provides a fluid, self-healing layer that is extremely smooth and inert to a wide variety of biomolecules. Using fluorescence microscopy we demonstrate the ability of LBLs to prevent sticking of protein-coated quantum dots and DNA−protein complexes.

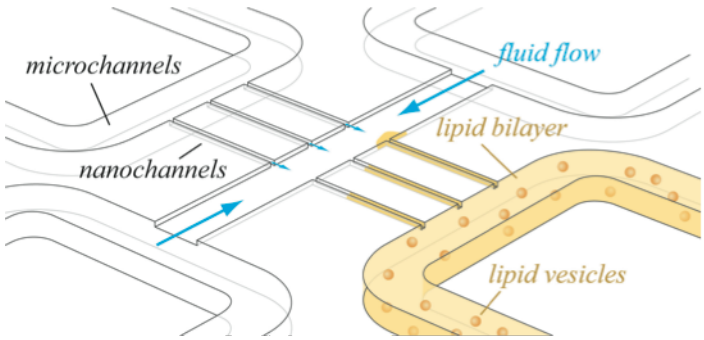

Figure 1: A schematic overview of the device. Four microchannels are used to bring in reagents to the nanofluidic structures in the center. In the illustrated scenario the right microchannel contains lipid vesicles and is coated with a LBL that spreads against a fluid flow into the nanochannels and the slit.

EXPERIMENTS

To form a LBL in nanochannels of dimensions on the order of 100 nm, we first deposited lipid vesicles in the microchannels, allowed them to rupture, and then let the formed LBL spontaneously spread into the nanostructures (Figure 1). We thus formed a uniform LBL in the nanochannels without introducing any vesicles into the channels. By imposing a counter flow of buffer opposite to the direction of the LBL spreading, we ensured that no lipid vesicles or debris entered into the nanochannels during the formation of the LBL. In order to evaluate the usefulness

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of LBLs as a passivation coating, we introduce three types of samples into our devices: streptavidin-coated quantum dots (streptavidin-QDs), fluorescently labeled RecA proteins and RecA−DNA complexes.

Bright streptavidin-QDs allowed us to evaluate any deficiencies in the ability of LBLs to prevent nonspecific protein binding, for example, due to small voids in the bilayer. The streptavidin-QDs were introduced into a nanofluidic chip consisting of a nanoslit (horizontal) and several nanochannels (vertical), both partially coated with a LBL (Figure 2a). The channels were flushed with streptavidin-QDs and subsequently with buffer. While the streptavidin-QDs to a large extent stick to the non-coated part, there is almost no sticking to the LBL- coated part of the nanostructure, demonstrating the effectiveness of the LBL coating. Sporadic streptavidin-QD binding can be seen, but binding to the few available defect sites saturates quickly and at low concentrations, which indicates that the streptavidin-QDs are bound to static defects in the LBL.

To compare the performance of the LBL passivation to that of standard passivation schemes, we characterized the sticking properties of streptavidin-QDs in nanochannels prepared according to standard protocols with BSA [6], see Figure 2b,c. For the BSA-coated nanochannels streptavidin-QDs can be readily flushed into the chip, but a significant number of them remain stuck to the channel walls even after thorough washing with buffer. In contrast, coating the nanochannels with a LBL leads to a significantly lower density of stuck streptavidin-QDs after washing.

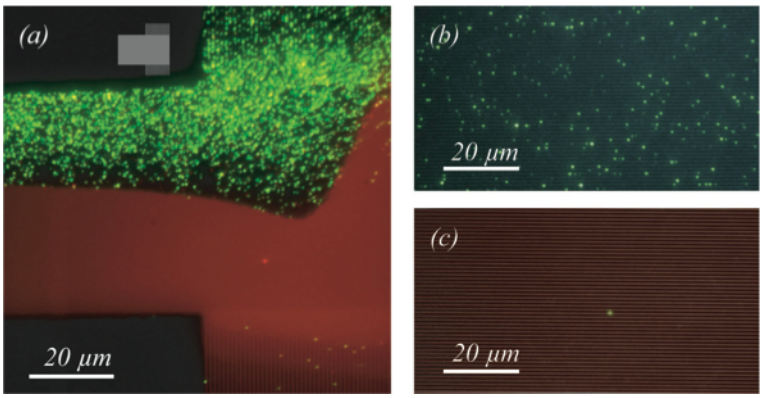

Figure 2. (a) Fluorescence micrograph of a nanoslit in the center and arrays of nanochannels in the upper and lower right-hand side corners (see the schematic in the inset) partially coated with a LBL (red). Bright green spots, corresponding to bound streptavidin-QDs, clearly indicate the propensity of nonspecific binding to the uncoated areas and, by contrast, show that the number of defects in the LBL is very low. (b) Fluorescence micrograph of streptavidin-QDs (green) in an array of BSA-coated nanochannels. (c) Fluorescence micrograph of streptavidin-QDs (green) in an array of LBL-coated nanochannels (red).

Lipid-coated nanochannels are potentially a powerful tool to directly visualize the organization and the dynamics of protein− DNA complexes. To demonstrate the antifouling properties of the LBL, we introduce a solution containing fluorescently labeled RecA proteins and nonstained λ-phage DNA into nanochannels partly coated with LBLs (Figure 3). RecA forms filaments on DNA that can be several micrometers long. RecA proteins that are not DNA bound are small, and diffuse fast in the microchannels, reaching the nanochannels first. Flushing the proteins through the nanochannels, starting in the LBL-coated end, reveals that while the proteins do not stick to the LBL-coated part, the untreated nanochannels light up quickly due to adsorption of the fluorescently tagged protein (lower parts of Figure 3). Subsequently, large RecA−DNA complexes can be seen to readily move in the lipid-coated nanochannels while they stick immediately upon contact with the untreated nanochannel.

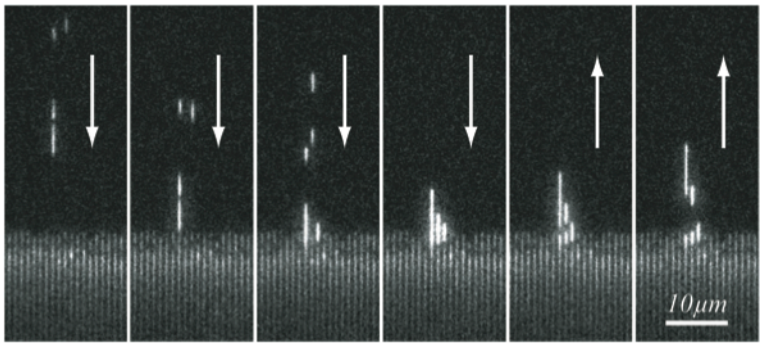

Figure 3. RecA bound to DNA approaching from the top in lipid-bilayer treated channels toward the untreated channels. The untreated channels are clearly visible to the bottom with nonspecifically bound fluorescently stained RecA proteins. Arrows indicate the direction of the fluid flow driving the motion of the DNA. The RecA−DNA complex is immediately bound once it makes contact to the untreated channels.

As an example of a dynamic process that we can observe in our devices, we demonstrate the activity of a working enzyme in the nanochannels by introducing λ-phage DNA in LBL- passivated nanochannels together with DNase I [7], an enzyme that cuts DNA at random locations. To be able to observe the actual cutting of the DNA, we introduced the DNA and the enzyme separately at different ends of the nanochannels. The DNA encounters the enzyme within the nanochannel, and because of the low concentration of the enzyme, individual cutting events are observed (Figure 4a). The DNA is typically entirely degraded within a time span of ~10 s. This corresponds to the expected diffusion time of the enzyme along the length of the λ-phage DNA in the channel, consistent with the known fast reaction kinetics of DNase I. As a negative control we rule out any significant contribution of photonicking to the degradation of the DNA by a comparison with DNA in nanochannels without enzyme (Figure 4b). Here the DNA remains intact over a ~5 min time scale.

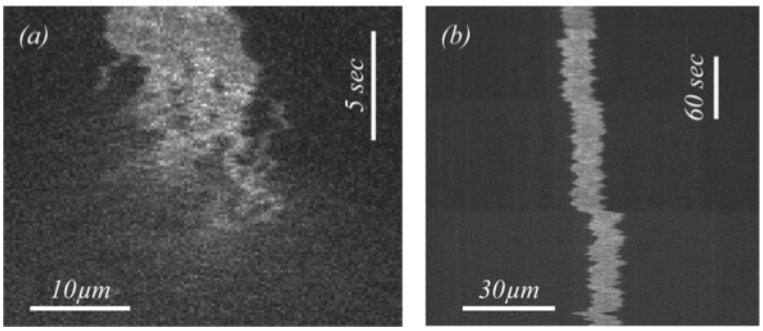

Figure 4. Kymographs of DNA in nanochannels. (a) An examples of λ-phage DNA in a nanochannel encountering DNase I enzymes. Single cuts are clearly visible. (b) λ-phage DNA in a lipid-coated nanochannel without DNase I.

In conclusion, we have demonstrated the performance of a LBL coating as an excellent passivation approach for nanofluidics in a range of applications. We have shown that a LBL prevents sticking of streptavidin-QDs and RecA proteins to the walls of a nanofluidic device. We have further demonstrated that the LBL passivation allows us to visualize RecA−DNA complexes as well as enzymatic digestion by DNase I along stretched DNA molecules in the nanochannels. We believe that the LBL passivation approach will be useful for systematic elucidation of kinetics and site specificity of protein−DNA interactions as well as for implementing DNA sequencing.

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