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A Nanoelectromechanical Biosensor Based on Precise Quantification and Control of DNA Orientation

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We utilize spectral self-interference fluorescent microscopy (SSFM) to measure fluorophore height with sub-nm precision to precisely quantify DNA orientation. A novel polymeric 3D scaffold is used to functionalize the sensor surface and to control orientation of the surface anchored DNA.

I. Introduction

Despite the completion of the human genome sequencing in 2003, progress towards understanding the regulatory mechanism of diseases through the use of genomic data has been slow. Part of the reason is that DNA sequence alone reveals little about DNA, RNA and protein function and deep understanding of the disease processes on a molecular level are still lacking. Protein-DNA interactions play a crucial role in these processes, such as in DNA replication, chromosome packaging, and transcription of DNA to RNA.

While the direct observation of protein-DNA interactions is difficult because critical size dimensions are on sub-nanometer scales, tools are available to study these interactions precisely. For example, NMR and X-Ray Crystallography provide 3D protein and DNA complex structures with atomic resolution [1,2] and FRET permits the investigation of protein-DNA dynamics in vivo with nanosecond temporal resolution [3]. However, they do not offer high-throughput capability to study the DNA sequence dependence, one of the most critical parameters influencing protein-DNA interactions. Traditionally, micro-arrayed methods are used to study sequence dependence [4]. Protein Binding Microarrays (PBM) are used to study over 23 million unique DNA sequence interactions with 104 different DNA binding proteins to determine the sequence dependent binding specificities[5]. However, they provide no information in regards to the specific DNA conformation changes that are induced by DNA-binding proteins.

Our aim in this work is to develop a high throughput microarrayed approach to precisely quantify DNA conformation. The platform permits precise in situ and real-time quantification of DNA orientation and conformation on a silicon dioxide surface through the application of a technique called spectral self-interference fluorescent microscopy (SSFM). SSFM is an interferometric technique that allows quantification of the vertical distance of a fluorophore to a reflecting surface with sub-nanometer resolution [6,7]. Through fluorescent labeling of DNA strands at different locations on the DNA, we use the fluorophore heights to precisely quantify the orientation and conformation of the surface immobilized DNA.

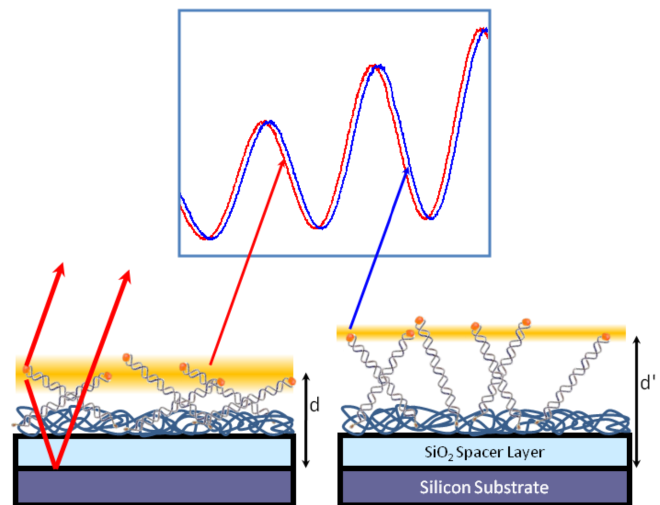

Figure 1: (Left) The sensor surface is functionalized with an amphoteric 3D polymeric binding scaffold. Amine functionalized dsDNA probes are end-grafted to the polymer. The ensemble height of fluorescent tags on short (10-100 base pair long) surface anchored double stranded oligonucleotides (dsDNA) is measured with sub nanometer resolution using spectral self-interference fluorescence microscopy (SSFM) (Right) The surface becomes negatively charged for buffer pH> 6.5 and dsDNA are repelled to assume a standing orientation.

II. Methods

Previously, we showed that on-chip detection of conformation changes in DNA through observed fluorophore-surface height changes requires induced orientation of the surface immobilized DNA in a standing position [8]. This was done by applying negative charge to the Au surface, on which the thiol functionalized dsDNA probes were end-grafted [9,10]. Here we adopt an entirely novel electromechanical approach to orient dsDNA on the SiO2 sensor surface; we designed a highly amphoteric polymer that adopts a net negative or positive charge depending on the buffer pH. The isoelectric point of the polymer was tested with electrosmotic flow. We observe an isoelectric point (pI) of about 6.5 for this polymer, indicating a net negative charge at pH > 6.5 and net positive charge at pH < 6.5. This charging of the polymer results in electrostatic forces between the polymer and immobilized DNA and we demonstrate the ability to manipulate the DNA orientation in a controlled fashion by adjustment of the buffer pH.

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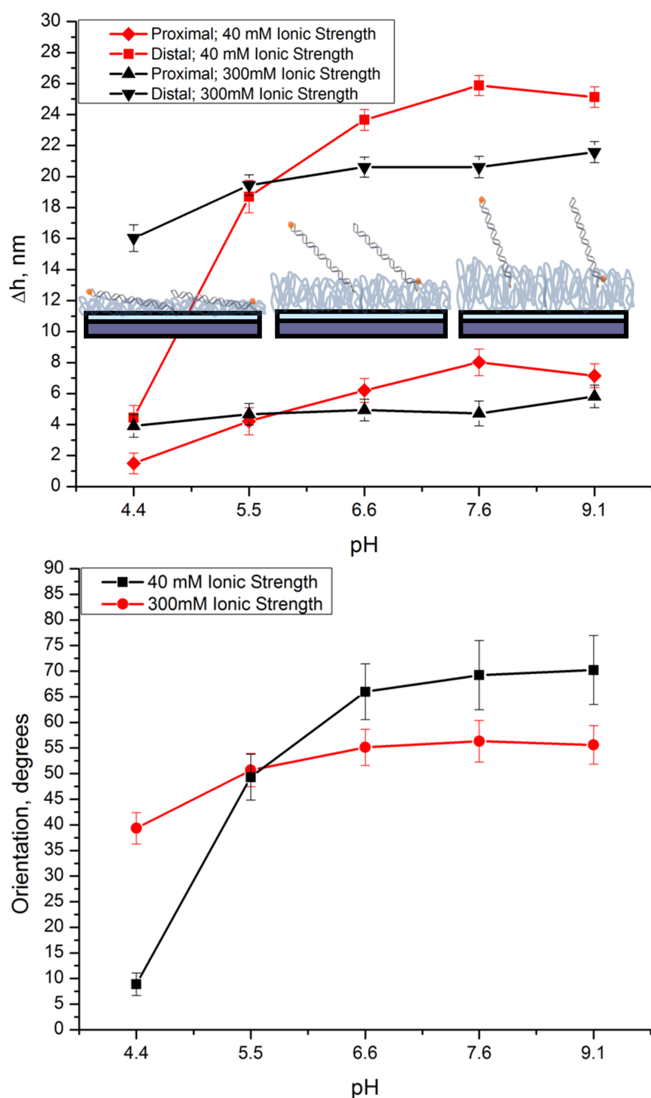

Figure 2: (*Top*) The mean height change of surface proximal and surface distal fluorphore tags on 60 base pair long dsDNA probes upon surface hyrdration as a function of the buffer pH. The ionic strength of the buffers is 40 mM and 260 mM NaCl is added to investigate the effect of ionic strength on the buffer swelling and probe orientation. The cartoon shows the polymer swelling and change in probe orientation at an ionic strength of 40 mM. The surface proximal fluorphore tags are used to measure the polymer swelling: the positively charged polymer collapses onto the negatively charged oxide surface (swelling is 3 nm) and negatively charged polymer is repelled from the oxide surface (swelling is 16 nm). Fixed charges within the polymer also repel each other and contribute to polymer swelling. The surface distal and surface proximal fluorophore heights allow precise quantification of dsDNA orientation: the dsDNA is oriented in a lying position by the positively charged polymer and in a standing position by the negatively charged polymer. *Bottom* The calculated probe orientation as a function of buffer pH at ionic strength of 40 mM and 300 mM. Low ionic strength allows the electric field to penetrate far from the charged polymer to effectively orient immobilized dsDNA

The positively charged polymer attracts the negatively charged dsDNA to orient it in a lying position while the negatively charged polymer repels the dsDNA to orient it in a standing position. An intense electric field (~10^6 V/cm) results near the interface of an ionic buffer and a charged surface due to the high concentration gradient of charges that accumulates. The characteristic length scale of this electric field is inversely proportional to the square root of the salt concentration: l_d = 0.3/sqrt(concentration(M)), nm. As a result, low ionic buffer concentrations more effectively orient the immobilized DNA because the electrostatic force is applied to a larger proportion of the dsDNA.

III. Conclusions

We designed a novel polymeric surface for the controlled orientation of surface anchored dsDNA probes. The precise sub-nm observation of fluorophore heights permits the precise quantification of probe orientation. This nanoelectomechanical biosensor is scalable and will facilitate future studies of high-throughput, microarrayed detection of the sequence dependence of protein induced DNA conformation changes.

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