Precise Quantification and Control of Surface Immobilized DNA Orientation

Philipp Spuhler[1], Laura Sola[2], Xirui Zhang[1], Margo Monroe[1], Joseph Greenspun[1], Marcella Chiari[2] and M. Selim Unlu[1]

[1]Biomedical Engineering, Boston University, 8 St Mary's St, Boston, MA 02215, pspuhler@bu.edu

[2]Istituto di Chimica del Riconoscimento Molecolare, Via Mario Bianco 9, Milano, 20131, Italy

Abstract

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

1. Introduction

When the Human Genome Project was started to map and identify the 20,000 – 25,000 genes, many scientists predicted that this mapping, through the application of bioinformatics, would lead to distinct correlations between genetic sequences and hereditary diseases. Sequencing of the human genome was completed in 2003, however, progress towards finding the roots of hereditary disease through the use of bioinformatics and 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 many of these processes, such as in DNA replication, site-specific recombination, 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 Förster Resonant Energy Transfer (FRET) permits the investigation of protein-DNA dynamics *in vivo* with nanosecond temporal resolution [3]. However, the DNA sequence dependence, one of the most critical parameters influencing protein-DNA interactions, is difficult to study with these techniques because they do not offer high-throughput capability. For example, a study to investigate protein interactions with all combinations of a (relatively short) ten nucleotide long protein binding sequence becomes impossible because there are over 1 million unique sequences. Yet, these types of studies are currently the only viable method to gain an understanding of genomic scale function of transcription factors and, consequently, a high-throughput approach is a necessity.

Traditionally, micro-arrayed methods are used to study sequence dependence [4]. For example, the Protein Binding Microarrays (PBM) developed by Bulyk and coworkers 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]. PBMs have greatly accelerated the understanding of transcription factor function. However, such they provide no information in regards to the 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 surface with sub-nanometer resolution [6,7]. Through fluorescent labeling of DNA strands at multiple locations on the DNA we use the fluorophore locations to precisely quantify the orientation and conformation of the surface anchored DNA.

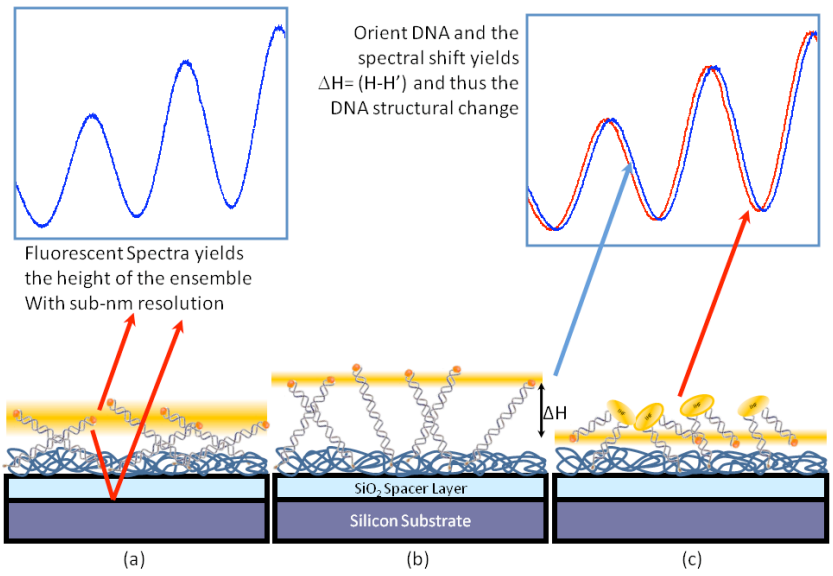

Figure 1: (a) The sensor surface is functionalized with a highly amphoteric 3D polymeric binding scaffold. Amine functionalized dsDNA probes are end-grafted to the polymer. The ensemble height of fluorescent tags on short (10-100 base pair long) surface anchored double stranded oligonucleotides (dsDNA) is measured with sub nanometer resolution using spectral self-interference fluorescence microscopy (SSFM) **(b)** The surface becomes negatively charged for buffer pH> 6.5. The negatively charged dsDNA are repelled and assume a standing orientation. The dsDNA probes are oriented with the negatively charged polymer. **(c)** Integration Host Factor to the dsDNA induces a bend of 160 at the binding location, resulting in a measurable fluorophore height change. The measured fluorophore height change allows precise quantification of protein induced conformation change in dsDNA.

2. Results and Discussion

2.1 An Amphoteric 3D Binding Scaffold

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 approach to orient dsDNA on the SiO_2 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.

Figure 2 shows a typical experiment for measurement and control of dsDNA orientation. 60mer oligonucleotides are labeled on surface proximal and surface distal ends and an array of 500, 100 m diameter, sized spots are robotically spotted on the SiO_2 chip. The height difference between surface distal and surface proximal fluorophores provides the oligonucleotide orientation. In a dry environment the probes and polymer collapse on the sensor surface, resulting in a horizontal probe orientation (Figure 2b). This indicates that surface density is too low to result in crowding effects between adjacent probes that could result in steric hindrance. The fluorophore –silicon heights are then measured for all spots in buffer (10 mM Tris/TES/NaOH, 50 mM NaCl, pH 7.5) and in dry conditions. Figure 2c shows the height difference of fluorophore heights in solution versus those in a dry environment. The surface proximal fluorophore is a measure of the polymer swelling. Positively charged polymer (pH <6.5) is attracted to negatively charged oxide surface and the negatively charged polymer (pH > 6.5) is repelled by the negatively charged oxide surface. Charging of the polymer also results in swelling because fixed charges within the polymeric scaffold repel each other. The height difference between surface distal and surface proximal fluorophores indicates the dsDNA orientation (Figure 2d).

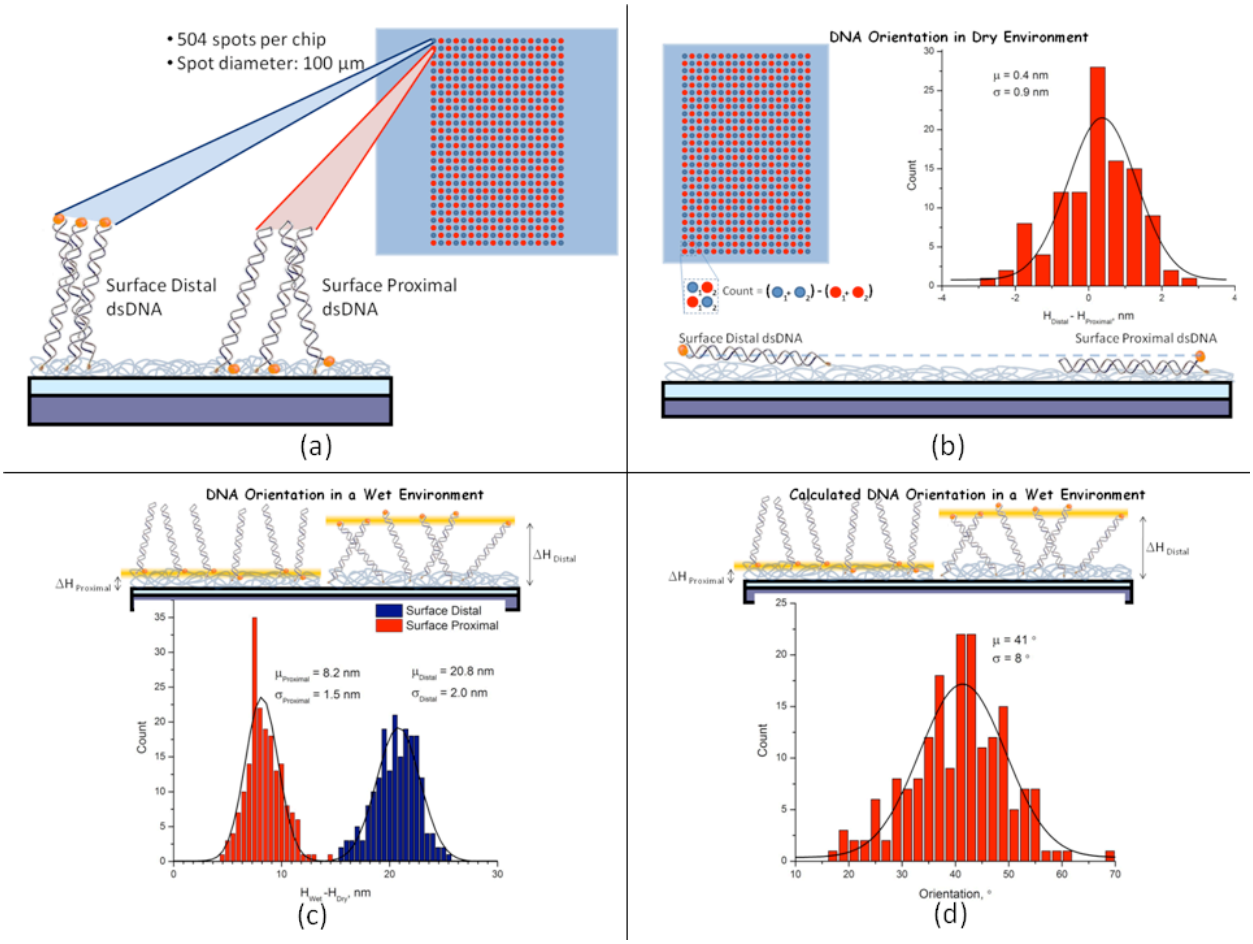

Figure 2: (a) An array of 18x28 60 base pair long dsDNA is spotted on a functionalized silicon dioxide chip. Spots in blue are fluorescently tagged at the surface distal end and spots in red are tagged at the surface proximal end. The spotting is made in a checkered pattern to provide symmetry in two dimensions to eliminate the influence of variations in the silicon dioxide thickness. **(b)** The mean height difference between surface distal and surface proximal fluorophores is 0.4 nm, indicating that the oligonucleotides collapse onto the surface in a dry environment. **(c)** The change in fluorophore –silicon height is measured for all spots in pH 7.5 buffer (10 mM Tris/TES/NaOH, 50 mM NaCl) versus height in dry conditions. A histogram of the fluorophore height difference in a wet versus dry environment, $\Delta H = H_{Wet} - H_{Dry}$, is plotted for surface distal and surface proximal fluorophores. **(d)** The mean angle assumed for dsDNA probes in wet conditions is higher than randomly oriented DNA (33) and indicates electrostatic repulsion between the polymer and dsDNA.

Figure 3a shows the controlled orientation of dsDNA probes through adjustment of buffer pH. 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 mobile 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 (Figure 3b).

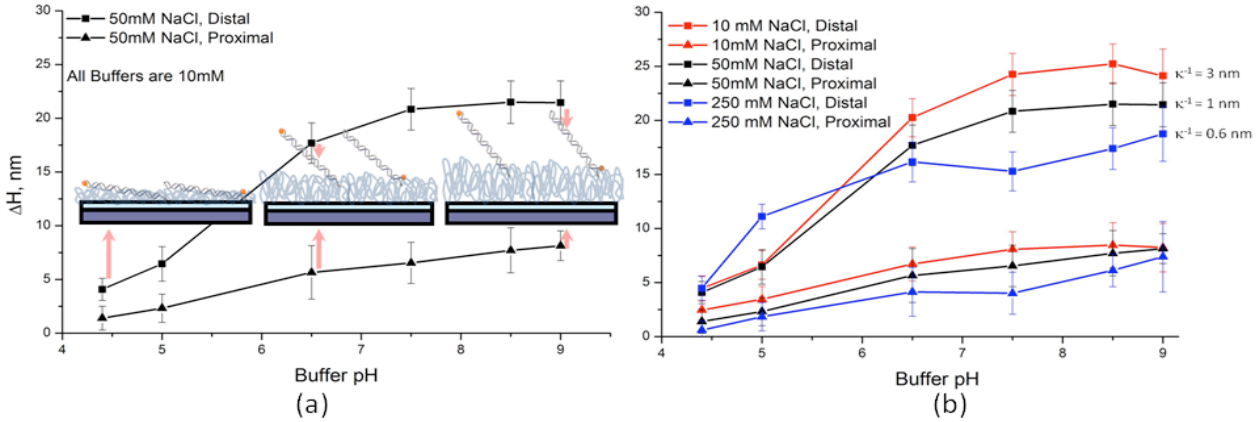

Figure 3: (a) Surface proximal dsDNA indicate polymer swelling: positively charged polymer collapses onto the negatively charged oxide surface and negatively charged polymer is repelled from the oxide surface. Fixed charges within the polymer also repel each other and contribute to polymer swelling. 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. **(b)** A low salt concentration allows the electric field to penetrate far from the charged polymer to more effectively orient immobilized dsDNA (b) Calculated orientations at pH 9 for NaCl concentrations 250 mM, 50 mM and 10 mM

3. 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. The platform is scalable and will facilitate future studies of high-throughput, microarrayed measurement of the sequence dependence of protein induced DNA conformation.

4. References

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