A Platform for *in situ* Real-Time Measurement of Protein Induced Conformational Changes of DNA

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Abstract- A platform for *in situ* and real-time measurement of protein induced conformational changes in dsDNA is presented. We demonstrate protein induced bending upon sequence specific binding of Integration Host Factor (IHF) to dsDNA probes.

I. INTRODUCTION

Several platforms were recently developed to study protein-DNA interactions. The most popular assays, ChIP-chips and DamID, have proven useful for measuring protein-DNA complexes *in vivo* but do not have high throughput capability. For *in vitro* measurements, Protein Binding Microarrays (PBMs) provide a high throughput approach but require cloning and expression of the proteins with an epitope tag. None of the aforementioned approaches provide information indicating the conformational changes of DNA that can result upon protein binding. Methods such as FRET and AFM are able to measure the conformational change of DNA upon the formation of a protein-DNA complex but do not allow high throughput measurements. For platforms wherein probes are surface-bound, such as SSFM, the random orientation of probes causes problems for the measurement of conformation [1]. Here we introduce a platform for *in situ* real-time measurement of protein-induced conformational changes on immobilized DNA, which allows control of probe orientation via an applied electric field. As the measurement is optical, the technique can be modified to make micro-arrayed measurements and is amenable for use as a high throughput assay.

II. EXPERIMENT

Since its interaction with DNA is well characterized, we utilize the sequence- specific Escherichia coli Integration Host Factor (IHF) to demonstrate the ability to measure protein induced dsDNA bending. The measurement principle is depicted in Figure 1. 2mm diameter Au electrodes adjacent to Pt counterelectrodes were patterned on a sapphire wafer. 80 base pair long double stranded DNA oligonucleotides (dsDNA) were functionalized with a thiol on the proximal end and with a Cy3 fluorophore on the distal end and end-grafted on the gold electrode inside a fluidic channel. Two different probe sequences were designed and immobilized on separate electrodes: Probe 1 contains the IHF H’ binding site, derived from the phage lambda attP sequence, near the proximal end, and Probe 2 contains the H’ binding site near the distal end. A microscope in reflected dark field mode (Olympus BX-RLA2) was used for the probe illumination (CW DPSS laser, 532nm, Newport Spectra Physics, Pro Millennia) and the fluorescence light detection. We filter the reflected light from the laser illumination using a long wave pass filter (550±5 nm, Newport, 10LWF-550) and a band pass filter (Semrock, FF01-582/75-25). The probe fluorescent emission intensity (Cy3, peak emission ~570 nm) is detected by a cooled photomultiplier (Hamamatsu, Photosensor Modules H7422) operating in the single-photon-counting mode.

The efficiency of non-radiative energy transfer (ET) between the fluorophores and surface plasmons in gold follows the distance dependency, $ET \propto d^{-3}$, where $d$ is the distance between the fluorophore and the gold surface [2]. This allows for real time observation of emitter – gold distance through the fluorescent

Figure 1a: Application of a negative potential repels the DNA probe from the surface and results in high fluorescence intensity due to inefficient energy transfer between Cy3 and the gold electrode while positive potentials pull the DNA probes to the surface, resulting in reduced fluorescent intensity.

Figure 1b: Application of a negative potential orients dsDNA probes in upright position and a large fluorescence intensity drop is observed when probe conformation changes due to IHF binding. (Not drawn to scale)
control orientation through the application of an electric potential between the gold surface and a platinum counter electrode in solution. Figure 2 shows fluorescent measurements of four dsDNA layers immobilized on separate electrodes as the applied potential is swept from -0.55 to +0.1 V. Brown and blue traces show measurements from electrodes having Probe #1 immobilized (H' binding sequence near the proximal end) and red and green traces show measurements from electrodes having Probe #2 immobilized (H' binding sequence near the distal end). The application of an electric potential creates a uniform ensemble of oligonucleotides and facilitates detection of conformational changes: for dsDNA oriented in an upright, i.e. “standing” position, a large drop in fluorescence is expected to occur due to IHF-induced bending upon binding. The sequence specificity of binding is then easily discernible, since bending near the proximal end of the dsDNA (Probe #1) pushes the fluorophore substantially closer to the gold surface than bending near the distal end (Probe #2). In contrast, with zero or positive applied potential (dsDNA in lying position), bending becomes less apparent.

Titration experiments showed significant onset of IHF induced oligonucleotide bending at 10 nM IHF, which is somewhat higher than would be expected since $k_D$ is reported to be in the range of $0.3 \times 10^{-9}$ to $2 \times 10^{-9}$ nM for specific IHF-oligonucleotide binding [7]. This can be explained by our use of low salt concentration, (50 mM NaCl and 10 mM Tris) which was shown to decrease the specificity of binding [8].

IV. Conclusions

We demonstrate a significant increase in sensitivity for detection of IHF-induced conformation changes in immobilized dsDNA by uniformly ordering immobilized dsDNA probes through application of an electric field. We also demonstrate the ability to detect differences in the bending location that results due to the sequence specific binding of IHF. The measurements are observable in real time, allowing for kinetics measurements to be made.

REFERENCES