# OPTICAL MAPPING OF TRANSCRIPTIONAL FACTOR BINDING SITES ON SINGLE DNA MOLECULES USING NANOFLUIDIC DEVICES

**K.K.Sriram[1, 2, 3], Jia-Wei Yeh[1], Yii-Lih Lin[1], Yi-Ren Chang[1], Chia-Fu Chou[1, 4, 5]**

[1]*Institute of Physics, Academia Sinica, Taiwan.*

[2]*Nanoscience and Technology/Taiwan International Graduate Program, Academia Sinica, Taiwan.*

[3]*Department of Engineering and System Sciences, National Tsing Hua University, Taiwan.*

[4]*Genomic Research Center, Academia Sinica, Taiwan.*

[5]*Research Center for Applied Sciences, Academia Sinica, Taiwan.*

## ABSTRACT

Transcriptional factors (TF) are proteins that bind to specific bases of DNA using DNA binding domains to carry out the process of transcription. Identification of TF binding sites is essential for understanding the regulatory circuits that control cellular processes such as cell division and differentiation as well as metabolic and physiological balance [1]. In this work, we deploy a proof-of-concept method adopting bio-conjugation, nanofluidic devices and single molecule techniques for direct mapping of TF binding sites on field stretched single DNA molecules. Here we demonstrated a simple and robust system for TF binding site identification, complementary to the existing techniques practiced in this field.

## KEYWORDS

Microfluidics, nanofluidics, Transcriptional Factors, Single Molecule Studies, Fluorescence Imaging.

## INTRODUCTION

In the recent years, single molecule techniques have a major contribution in the process of getting some insights on details at the molecular level, which in general are not feasible with ensemble experiments. Due to the advancement of micro/nano fabrication methods [2] [3], together with advances in fluorescence single molecule studies, we are now able to address previously unanswerable questions in relevant areas of research [4]. Various groups have tried and reported TF binding site mapping using single molecule techniques like optical tweezers [5], atomic force microscopy [6], molecular combing [7] etc. Here, we implemented a method that exploits the advantages of micro- and nano-fluidics, fluorescence single molecule imaging and analysis to map TF binding sites on genomic DNA molecules. With this, we could overcome some of the limitations and drawbacks involved in the existing techniques.

## EXPERIMENT

In this work, we use fluidic devices composed of micro- and nano-regions fabricated in fused silica substrates and are conformably sealed by a coverslip. The nano-region here is a nanoslit with tens of nanometers in depth. TF (E.coli RNA polymerase) bound genomic DNA molecules (λ-phage), coupled with fluorospheres of sizes similar to or larger than the depth of the nanoslits, at one of their ends (by biotin-streptavidin linking [8]), were trapped at the micro-nano interface, thus stretching them in the nanoslits in the presence of a small electric field. The fluorospheres also serve the purpose of identifying the orientation of DNA molecules and facilitate mapping of TF binding sites with improved resolution. Both DNA and proteins were fluorescently labeled to achieve high-resolution mapping of TF binding sites using an epi-fluorescence microscope (Figure 1).

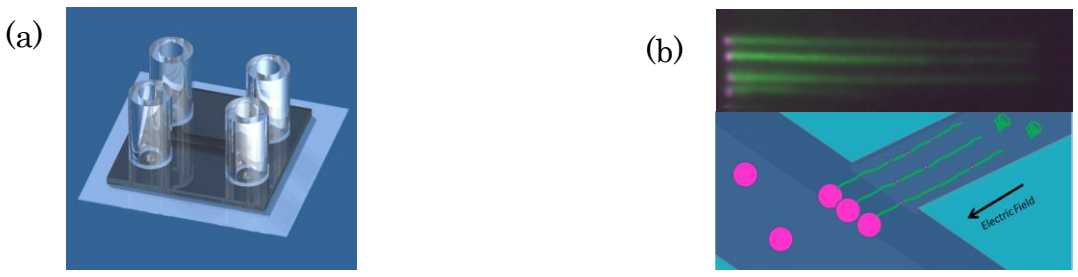


***Figure 1.*** *(a) Image of a completely assembled device. (b) Fluorescence image of fluosphere coupled λ-phage genomic DNA trapped at the micro-nano interface and stretched inside the nanoslit by an applied electric field. (Figure below shows a schematic representation of the scenario).*

Lambda phage DNA has two promoters and three pseudo-promoters for *E.coli* RNA polymerase holoenzyme (RNAP) [5]. We use this well-studied system to demonstrate the ability of our device in observing single DNA molecules with RNAP bound to them and measuring their binding locations. DNA-RNAP complexes are formed using formaldehyde crosslinking mechanism and RNAP is tagged with quantum dots (QDs) using primary-secondary antibody coupling. DNA molecules were labeled with YOYO-I nucleic acid stain (1:5 dye: base-pair ratio) (Figure 2).

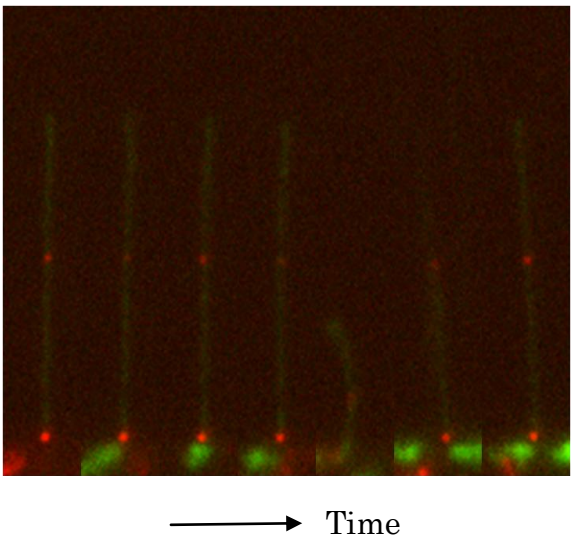


***Figure 2.*** *Time-lapse imaging shows the stretching of λ-genomic DNA (Green) with bound QD- labeled E.coli RNAP complex (Red) by applied electric field in the nanoslit.*

Experimental results show QDs localized along the stretched DNA molecules. A histogram was plotted with TF binding sites mapped from ~50 DNA molecules and our analysis shows that these positions are in good agreement with the promoter and pseudo-promoter regions for RNAP (Figure 3).

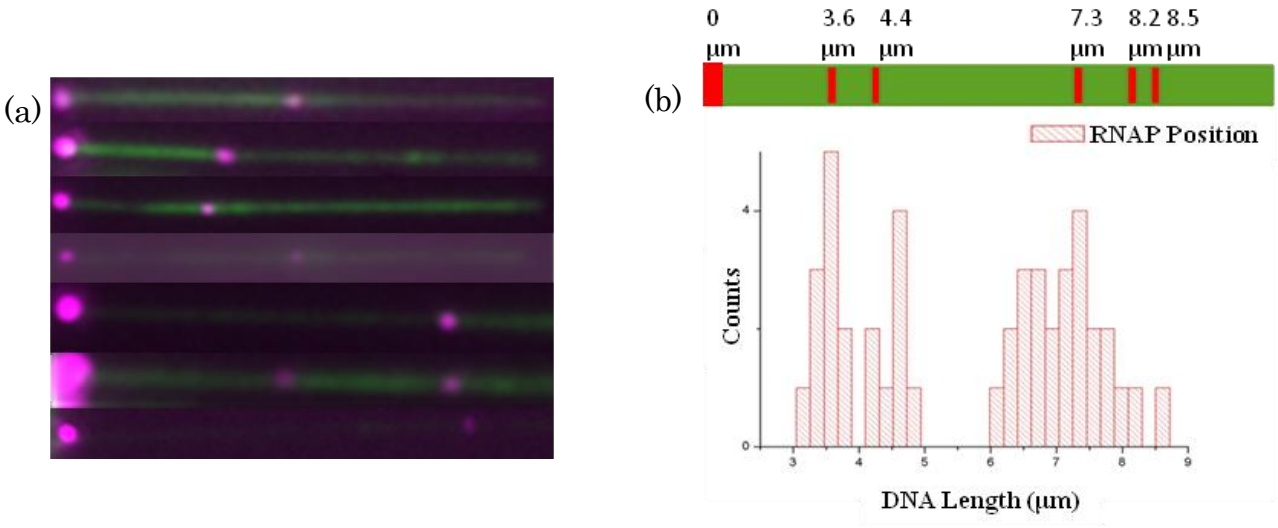


***Figure 3.*** *(a) Fluorescence images show λ-genomic DNA (green) with QD labeled E.Coli RNAP (Magenta) complex stretched inside the nanoslit. The large dots aligned on the left are the fluorospheres trapped at the micro-nano interfaces. (b) Localization of RNAP molecules bound to λ-genomic DNA stretched in the nanoslit. Histogram shows the values obtained from our experiments are in good agreement with known promoter (3.6 and 4.4µm) and pseudo-promoter (7.3, 8.2 and 8.5 µm) regions of E.coli RNAP [5].*

Advantages of our device include multiplexing, quick mapping to identify TF binding sites and ability to distinguish real complexes from non-specific ones. Our device also opens up the possibility for a lab-on-chip device in which in-vivo complexed DNA-protein samples can be extracted and protein- binding sites mapped [9], which may serve as a simple and robust system for complementary analysis to the existing techniques practiced in this field.

## ACKNOWLEDGEMENT

This work was supported by AS Nanoscience and Technology Program, AS Foresight Project (AS-97-FP-M02), and National Science Council (Taiwan) (99-2112-M-001-027-MY3). We thank Dr. Kuo-Tang Liao, Dr. Chia-Fen Hsieh and Mr. Leonardo Lesser-Rojas for helpful discussions, and technical support from the AS Nano Core Facilities.

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## CONTACT

***Dr. Chia-Fu Chou, Nanobioscience Lab, cfchou@phys.sinica.edu.tw**