

Nanotechnology E-Newsletter

March 2005

Direct imaging of transcription repressors bound to nano-channel elongated DNA

This note reports the direct imaging of the *E. coli-lac* repressor protein (LacI) bound to DNA (deoxyribonucleic acid): in this case, DNA with a single copy of the *Lac* operator (*lacO*) inserted in it (40.82kbp). One of the key challenges in transcription profiling is the observation of interactions of transcription-factor proteins with single DNA molecules. Current technology, such as DNA foot-printing analysis, looks at an ensemble of millions of DNA strands. In order to analyze the occupancy of transcription factor sites by individually-bound proteins, it is necessary that the DNA molecule be extended in a linear manner and that single proteins be imaged with high spatial resolution. We have developed a platform using single-molecule fluorescence imaging and nano-fabrication techniques to directly visualize transcription factor proteins on DNA to a resolution of 100 base pairs (bp).

In order to visualize the LacI, a protein was constructed that fused green fluorescent protein (GFP) and LacI. The monomeric GFP-LacI fusion proteins were bound *in vitro* to *lacO*-DNA constructs. The DNA molecules were then stained with the red fluorescent intercalator dye called BOBO-3, and the LacI-DNA complex molecules elongated in nano-channels with dimensions comparable to the persistence length of double-stranded DNA (60nm). Next, the DNA and protein were imaged using total-internal-reflection fluorescence (TIRF) microscopy.

Figure 1(a) shows the micro-/nano-fluidic device. The idea was to drive the DNA molecules into the micro-channel, and then into the nano-channels, using electrophoresis. Figure 1(b) shows GFP-

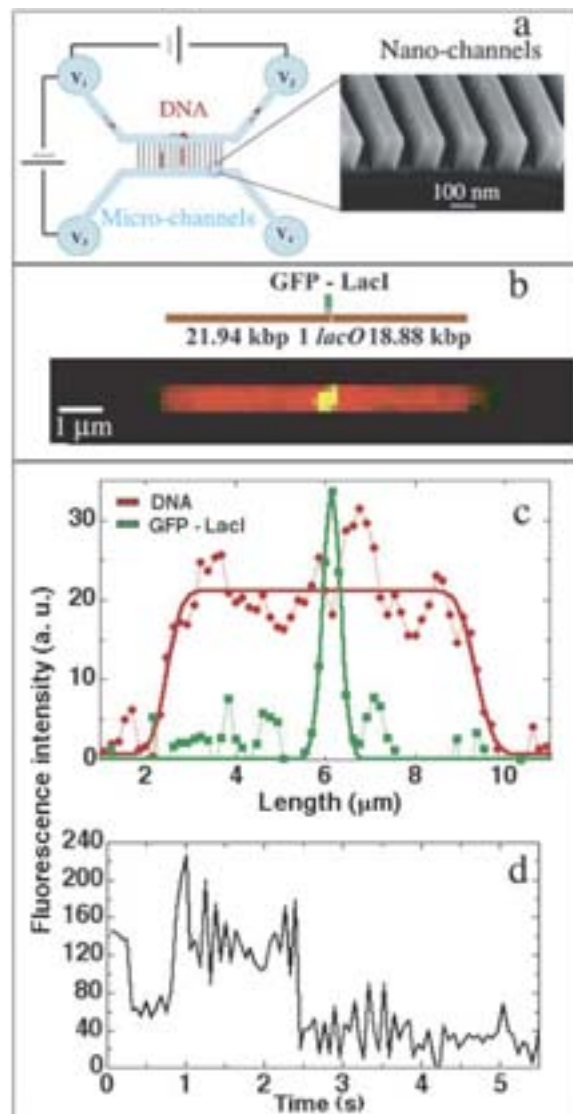


Figure 1. Micro- and nano-fluidic device (unsealed), and image and analysis of GFP-LacI bound to *lacO*-DNA. (a) Schematics of the micro-/nano-fluidic device. Blue regions are micro-channels and the bridging black lines are nano-channels along which the DNA molecules (red) are elongated. DNA molecules are guided consecutively into micro- and nano-channels using electrophoresis. Scanning electron microscopy images of an array of 80nm×100nm channels made using nano-imprint lithography.⁴ (b) Schematics of GFP-LacI bound to *lacO*-DNA, and (superposed) a frame-averaged image of LacI-DNA elongated in a 100nm×100nm channel. (c) Fluorescence intensity profiles and fits for the DNA and the GFP-LacI. (d) Fluorescence time trace of the bound GFP-LacI, which emitted 6.3×10^4 photons in its lifetime.

LacI bound to a *lacO*-DNA construct elongated in a nano-channel. The location of the bound protein was determined by curve-fitting the DNA fluorescence-intensity profile to a modified error function,^{1,2} and the protein profile to a Gaussian function.² The precision of the protein location is set by the measurement error associated with the positions of the two DNA ends (i.e. DNA length) and the position of the protein in the image. The error in DNA-length measurements decreases with N (the number of independent measurements¹) as $1/N^{1/2}$. The error in protein location measurements, on the other hand, decreases with M (the number of collected photons from a GFP-LacI molecule³) as $1/M^{1/2}$.

The DNA molecule shown in Fig. 1(b)

is an averaged image of 150 frames and the bound GFP-LacI is an averaged image of 40 frames. This gives an average M of 34 photons per frame. The protein is located 170bp off the target *lacO* site, and it is within the measurement error of approximately 190bp. By taking hundreds more images of the DNA, the bound location can be determined to the precision of 100bp, or limited by the number of photons had the GFP-LacI molecules a higher photon yield.² This GFP-LacI molecule was bound specifically to *lacO*: it is a monomer according to the photon yield of 6.3×10^4 photons, and the unitary bleaching event observed in the fluorescence time trace.²

Y. M. Wang, Keith Morton*, Xiao-Juan Guan*, Ling Guo*, Edward C. Cox*, James Sturm†, Steve Chou‡, Shirley Tilghman*, and Robert H. Austin

Department of Physics

*Department of Molecular Biology

†Department of Electrical Engineering

Princeton University

Princeton, NJ

E-mail: ymwang@Princeton.EDU

References

1. J. Tegenfeldt, C. Prinz, H. Cao, S. Chou, W. Reisner, R. Riehn, Y. M. Wang, E. C. Cox, J.C. Sturm, P. Silberzan, and R. H. Austin, *The dynamics of genomic-length DNA molecules in 100-nm channels*, **Proc. Nat. Acad. Sci.** **101**, pp. 10979-10983, 2004.
2. Y. M. Wang, J. Tegenfeldt, W. Reisner, R. Riehn, X. J. Guan, L. Guo, E. Cox, J. Sturm, and R. H. Austin, *Direct imaging of transcription repressor bound to nano-channel linearized DNA*, forthcoming publication.
3. R. E. Thompson, D. R. Larson, and W. W. Webb, *Precise nanometer localization analysis for individual fluorescent probes*, **Biophysical J.** **82**, pp. 2775-2783, 2002.
4. L. J. Guo, X. Cheng, and C. F. Chou, *Fabrication of Size-Controllable Nanofluidic Channels by Nanoimprinting and Its Application for DNA Stretching*, **Nano Lett.** **4**, pp. 69-73, 2004.