NIRT: Watching proteins bend DNA with Subnanometer Resolution

NSF NIRT Phys-0404286

Thomas T. Perkins (PI), Meredith D. Betterton (co-PI), James A. Goodrich (co-PI)

[1]JILA and Dept. of Molecular, Cellular and Developmental Biology, [2]Dept. of Applied Math, & [3]Dept. of Chemistry and Biochemistry, University of Colorado at Boulder

This NIRT combines theoretical predictions of DNA conformation with state-of-the-art instrumentation to elucidate the biochemically important problem of how protein-DNA interactions control gene expression. Specifically, our experiment is based on the physical kink (100°) induced in the DNA backbone by the binding of TATA-box binding protein (TBP). A conceptual picture of our experiment is shown in Fig. 1. From this and similar experiments, we hope to develop a better understanding of how proteins bind to and bend DNA, the first step in the process of transcribing DNA into RNA. Moreover, we expect that our subnanometer technology and polymer physics models of short DNA developed under this NIRT to be widely applicable to a variety of single molecule biophysics experiments.

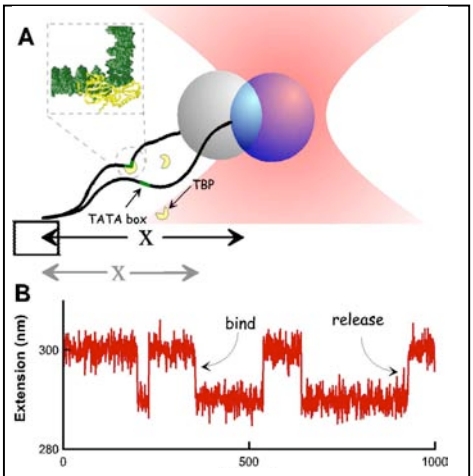

Figure 1: (**A**) A schematic of TBP binding to a single DNA molecule held under tension with optical tweezers. When TBP binds to the TATA-box, it introduces a kink, moving the trapped bead (*gray*). (**B**) An illustration showing the expected signal for TBP binding and release.

To achieve single molecule detection of TBP binding requires the ability to resolve subnanometer motion in an optical trapping microscope. Moreover, the on- and off-rates of TBP are quite slow 10s to 100s of seconds. Hence, this stability is required for long periods of times (100 seconds). In 2004, the Perkins lab published a new method, called differential back focal plane (BFP) detection for subnanometer resolution in an optical trapping microscope in 2D using stuck polystyrene beads.[2] In our first year of NIRT funding, we have generalized this technique to 3D. Two significant factors contributed to this experimental realization: (*i*) dramatically increasing the sensitivity and stability of BFP detection to vertical motion, and (*ii*) fabricating a regular array of nanometer-sized fiducial marks that were firmly coupled to the cover slip. This array of fiducial marks was fabricated from hydrogen silsesquioxane that cross-links to form glasslike features with tunable diameters down to 20 nm.[3] These posts yielded excellent optical signals. The short term vertical stability of 0.14 nm ($\overline{\sigma}_z$) was determined from the mean standard deviation in 1 s from a non-overlapping set of 1 s intervals over 100 s. Long term vertical stability was <1 nm over 100 s. Overall, as shown in Fig. 2, short-term 3D stability was excellent, achieving sub-Ångstrom in-plane stability ($\overline{\sigma}_x$ = 0.08 nm, σ_y = 0.07 nm, & σ_z = 0.14 nm).[4] In conclusion, we have developed a new

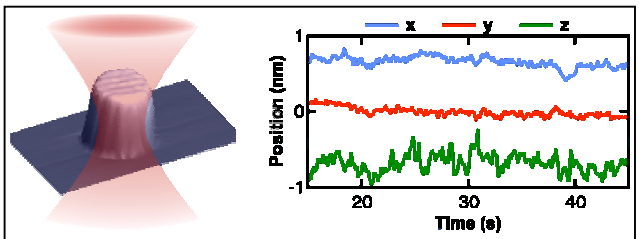

Figure 2: Demonstration of subnanometer 3D stabilization of an optical microscope.

method that provides for real-time stabilization of an optical microscope to better than 1 nm. This technological result will provide the foundation for our single molecule TBP studies as shown in Fig. 3.

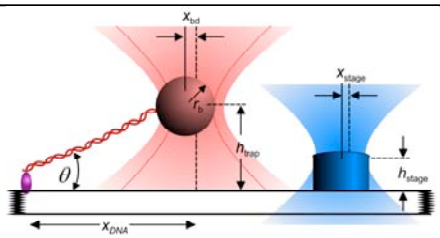

Figure 3. Optical trapping instrument with two detector lasers to subtract out stage noise. One laser (*blue*) tracks the stage noise in three dimensions. The second laser (*red*) tracks the motion of the bead in the trap (*dotted red*).

Theory. Interpretation of the experimental data will be aided by a model which predicts the change in DNA extension due to bends or loops formed in the DNA by proteins. We have developed a theoretical model applicable to DNA molecules with relatively short contour lengths (50-500 nm) with or without a bend in the DNA backbone. Our work modifies the successful WLC model of entropic elasticity to include chain-end boundary conditions, rotational fluctuations of the bead(s) attached to the ends of the polymer, and the presence of one (or more) bends in the polymer. This work will allow determination of the induced bend angle from single-molecule experiments. A paper describing this work was recently submitted[5] and another manuscript is in preparation.

Ensemble studies. Ensemble fluorescence resonance energy transfer (FRET) experiments are being performed to establish the average bend angle of TATA DNA bound by human TBP and TFIID and to determine the effect of other transcription factors on this bend angle. To date, solution FRET studies showed that our recombinant human TBP causes a 104° bend in the TATA DNA, which is in good agreement with published measurements. Interestingly, the addition of the TFIIA to the TBP/TATA complex decreases the FRET efficiency, giving a calculated bend angle of 80°. To confirm that the decrease in FRET observed upon addition of TFIIA is due to TFIIA entering the complex, the TBP/TFIIA/TATA complex has been separated from partial complexes using native gel electrophoresis. The in-gel FRET measurements on the TBP/TFIIA/TATA complex show a bend angle of 80°. A manuscript describing this work is currently in preparation.

References

[1] For further information about this project email tperkins@jila.colorado.edu

[2] Nugent-Glandorf, L.; Perkins, T. T. *Opt. Lett.* ***29***, 2611 (2004).

[3] Namatsu, H.; Takahashi, Y.; Yamazaki, K.; Yamaguchi, T.; Nagase, M.; Kurihara, K. *J. Vac. Sci. Technol. B* ***16***, 69 (1998).

[4] Carter, A. R., King, G. M., Ulrich, T. A., Halsey, W. Alchenberger, D., Perkins, T. T., *Submitted.*

[5] Li, J., Nelson, P. C., Betterton, M. D., *Submitted.*