Manipulation of DNA-protein Interactions at the Nanoscale

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PIs: **R.G. Larson[*], M.A. Burns[*], S. Pang[+], D.T. Burke[#],**
University of Michigan, Depts of ChE[*], EECS[+], and Human Genomics[#]

The goal of this project is to develop in vitro methods of studying protein-DNA interactions. Such interactions are crucial to DNA packaging, repair, and transcription, whose understanding is part of the next wave of biological research following the completion of the human genome project. A key milestone in our efforts is the creation of a micro-nano device similar to that depicted in Fig. 1, in which sub-micron nano-channels direct proteins to specific areas along a stretched DNA molecule, permitting direct imaging of DNA-protein interactions.

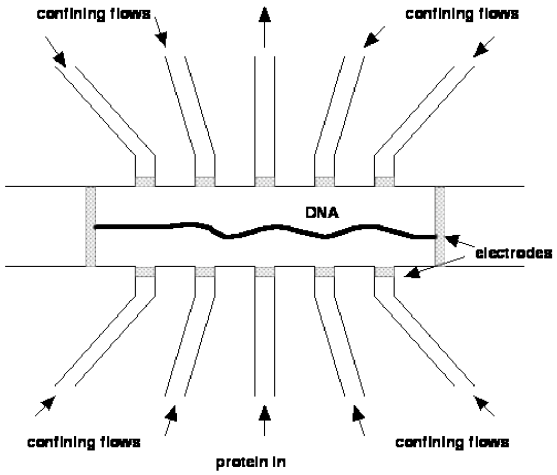
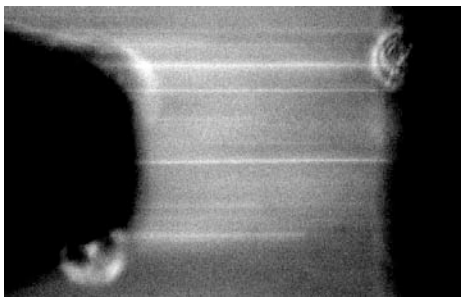


Fig. 1 Schematic platform for manipulating DNA/protein interactions at the nano-scale. Shaded patches are electrodes for anchoring DNA or for concentrating proteins.

Key steps to accomplishing this goal are 1) The creation of micro- and nano-channels in glass with electrode deposition, 2) stretching and aligning DNA molecules by "electrostretching" [1] or other methods within these channels, 3) fluorescently labeling proteins and tracking optically their interactions with DNA molecules, and 4) combining all of the above elements into a single integrated method. Accomplishing this ambitious program requires integration of microfabrication in glass and silicon, DNA manipulation using flow and electric fields, and labeling and microscopy of DNA and proteins. We have made progress on the first three of the above steps, and have begun to integrate steps 1 and 2.



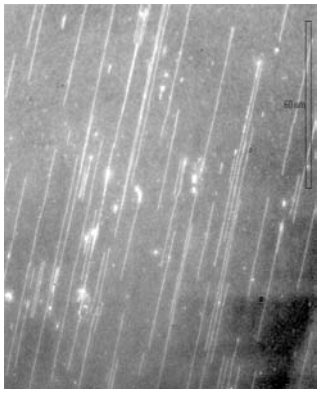


Fig. 2. DNA stretched using high-frequency electric field between electrodes (left) and using "molecular combing" (right). The electrode spacing is 20 μm.

Fig. 2 shows DNA molecules that we have stretched using an electric field in a microfabricated device between electrodes (left) and a flow field (right). The dynamic molecular combing method [2] uses a receding meniscus to stretch out the DNA. In Fig. 3, type II restriction endonuclease EcoRI was fluorescently labeled using TRITC so that its interaction with the DNA could be observed along with YOYO-1 labeled DNA and injected into the customized flow cell where cover glass with DNA stretched on its surface forms the bottom wall. Notice that the protein slides up the DNA molecule, whereas the flow is directed from left to right, and in other images one can see proteins convected from left to right by the flow (not shown). This is the first time such motion has been observed along DNA molecules combed onto a surface. Such motion on electrostretched DNA was observed earlier by Kabata et al. [3].

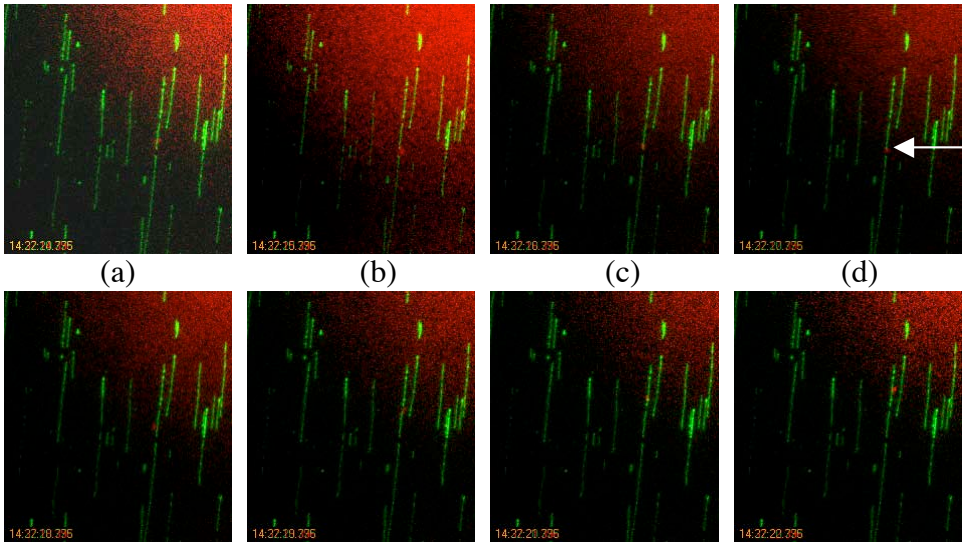
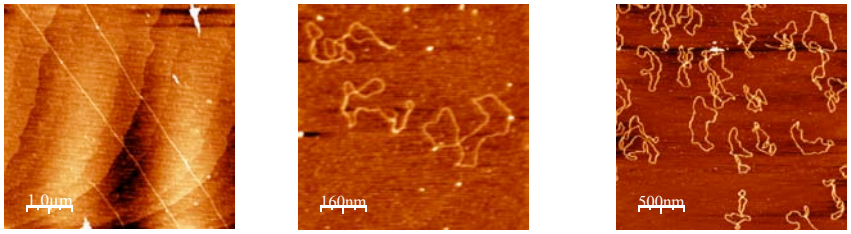


Fig. 3. (a) Photolabeled Eco RI (red TRITC stain) in contact with λ-DNA (green YOYO-1 stain) at times separated by one-second intervals. The red protein, indicated by an arrow in (d), slides along the DNA molecule.

Using diffusion measurements as well as light scattering, we have found that readily observable proteins are not single proteins, but rather clumps of proteins, with size a few hundred nanometers. True single proteins are not readily visible in water because they diffuse rapidly on the time scale of videomicroscopy. To overcome this difficulty, we are now tethering proteins to fluorescent 20 nm carboxylic-functionalize polystyrene beads, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) in conjunction with N-hydroxylsulfosuccinimide (sulfo NHS) to couple the exposed carboxylic groups of polymers to the primary amine groups of proteins. We observe similar migration of these protein-decorated beads along DNA molecules.

To check for the influence of fluorescently labeling, we use atomic force microcopy (AFM) since AFM can be carried out with or without labeling. YOYO-1 labeled DNA molecules were therefore deposited and stretched onto APTES treated Mica surface via suctioning method. Due to the size of long lambda DNA (Fig. 4(a), 48502 bp), an image obtained over a large scanning area yielded lower resolution than that of much shorter plasmid pBR322 DNA (Fig. 4(b), 4361 bp). For application with proteins, an image of pBR322 in solution was obtained in water (Fig. 4.(c)). Imaging with proteins is under way.

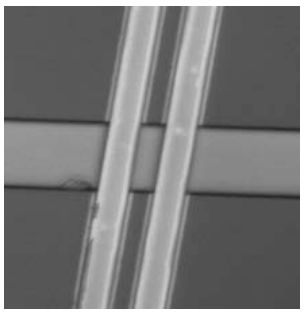


(a) (b) (c)

Fig. 4. (a) Bright straight lines are YOYO-1 labeled stretched λ-DNA, (b) pBR322 DNA dried onto mica imaged by AFM. (c) pBR322 DNA in solution imaged by AFM.

Future Plans The next steps are to complete the methods of micro and nano-channel construction developed in the Pang lab to produce a sandwich of glass and silicon, with glass on one side and silicon on the other. An example of the construction of the main channel with two electrodes for electrostretching DNA is shown in Fig. 5. The nano-channel sideports illustrated schematically in Fig. 1 now need to be constructed, and this will be a challenging problem.

Fig. 5. 25 μm deep, 150 μm wide Si channel (horizontal) bonded to glass with two electrodes (vertical) using PMMA as the sealant at 150 °C and 0.2 MPa. The electrodes on glass bonded together.



Additionally, we need to continue our study of protein labeling, including the use of nano-particle labeling, and check with AFM whether or not labeling influences the interaction of proteins with a stretched DNA molecule. We are also using gel shift assays with bulk samples in which we can measure the efficacy of both labeled and unlabeled restriction enzymes in cutting un-anchored DNA molecules in bulk solution. These studies, coupled with AFM and fluorescence microscopy experiments, are essential to determine the effects of surface anchoring of DNA, and of labeling of DNA and/or proteins, on the DNA/protein interactions. We are exploring several different proteins, including Eco RI (a restriction enzyme) and RecA, a recombinase involved in DNA repair. We also need to perfect the placement of electrodes and of control of electric fields and surface treatments to ensure reliable electrostretching of DNA molecules in a micro-channel. To date, the electro-stretching shown in Fig. 2 is not reliably obtained, and systematic studies are now improving reproducibility. All of this is in preparation for carrying out optical microscopy experiments on protein-DNA in confined micro- and nano-channels.

Potential Areas of Collaboration There are potential areas of collaboration with other NIRT grants in the creation of nanoarrays, manipulation of DNA molecules, and in microfabrication of nano-sized channels.

References

[1] Washizu, M., Kurosawa O, Arai I, Suzuki S Shimamoto N (1995). IEEE Transactions: Industrial Applications 31:447-456.
[2] A. Bensimon et al., *Science* **265**, 2096 (1994)
[3] Kabata, Okada, Washizu, Jpn J Appl Phys, 200
[4] For further information, contact rlarson@umich.edu