Observing Protein-DNA Interactions Using Double-Tethered DNA Curtains

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In this work, we combine nanopatterning with single-molecule biology to probe how repair proteins scan DNA strands for errors. Mismatch repair proteins such as the heterodimer Msh2-Msh6 repair DNA base-pair mismatches that lead to colorectal cancer and may influence the onset of other tumors.[1] These proteins move up and down DNA strands by 1-dimensional diffusion searching for mutations (i.e., base-pair mismatches) to repair. To directly visualize this process, DNA molecules are stretched out in buffer flow in a flowcell, an arrangement called "DNA curtains."[2,3] This is a very effective way of isolating individual DNA molecules for observation via total internal reflectance (TIRF) microscopy, however, it can be difficult to accurately observe protein diffusion under these conditions because hydrodynamic forces can affect protein motion. It is therefore desirable to tether both ends of the DNA strands to the surface of a microscope slide, so that protein diffusion can be observed without buffer flow.

Double-tethered "DNA curtains" are formed by flowing the DNA in a lipid bilayer across nanoscale barriers. The flow chamber is constructed from a fused silica slide patterned with sub-100 nm-wide Cr or Cr/Au barriers and 1 μm x 2 μm pentagonal pads oriented perpendicular to the flow (Fig. 1). The barriers are 15-30 nm tall. Anti-digoxigenin antibodies are introduced into the chamber, nonspecifically binding to the pentagons. DNA strands are linked by one end to a floating lipid bilayer, while the other end is tagged with digoxigenin. When buffer flow is applied, the DNA strands are elongated by the flow, and are mechanically tethered to the linear barriers on one end. The opposite end is chemically bound to the pentagons by the digoxigenin-anti-digoxigenin linkage (Fig. 2). Buffer flow can then be halted, and quantum dot-tagged proteins can be observed as they move along the DNA strands via 1-D diffusion. Furthermore, it is possible to double-tether two strands of DNA at a 90-degree angle, crossing them (Fig 3). In this way, we can examine whether proteins dissociate from DNA strands in order to find base-pair mismatches quickly.

The barriers are patterned on fused silica substrates by electron beam lithography and nanoimprint lithography. A ~ 4 μL flowcell is assembled on the slide using a borosilicate glass cover slip and ~ 25 μm-thick double-sided tape.[4] To illuminate the fluorescent quantum dot-tagged proteins, TIRF microscopy utilizes the evanescent field that is generated beyond a reflective surface present at the interface between two transparent materials with different refractive indices (e.g., a silica slide and the aqueous buffer solution). Figure 2 shows fluorescent images of DNA tethered to the barriers with the flow off, both before (a) and after (b) double-tethering. Figure 3 shows crossed DNA strands. Double-tethered DNA arrays eliminate the interference of the buffer flow's hydrodynamic forces with protein motion, enabling careful observation of repair protein motion.

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[2] T.A. Fazio, M.L. Visnapuu, S.J. Wind, and E.C. Greene, Langmuir **24** (2008)
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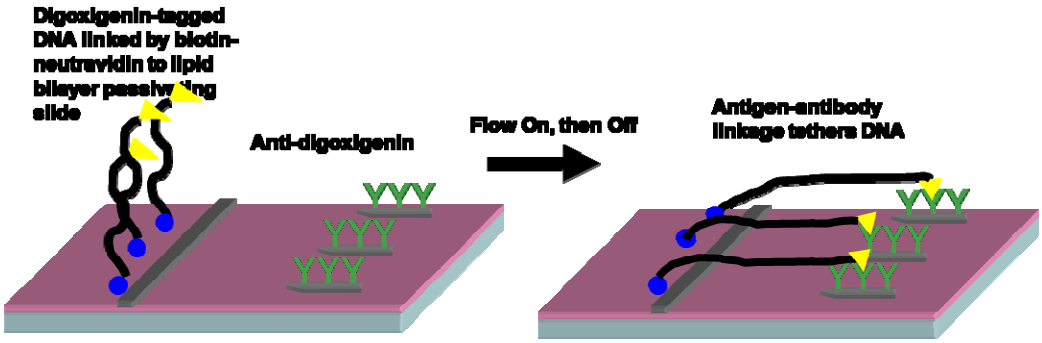

Figure 1. Schematic of double-tethered DNA. Biotin-tagged DNA is linked to a lipid bilayer by neutravidin. The opposite end of the DNA is tagged with digoxigenin. When buffer flow is turned on, DNA is mechanically tethered by a nanopatterned line and stretched out in flow. The digoxigenin binds to anti-digoxigenin nonspecifically bound to 1x2μm pentagons, completing the double-tether.

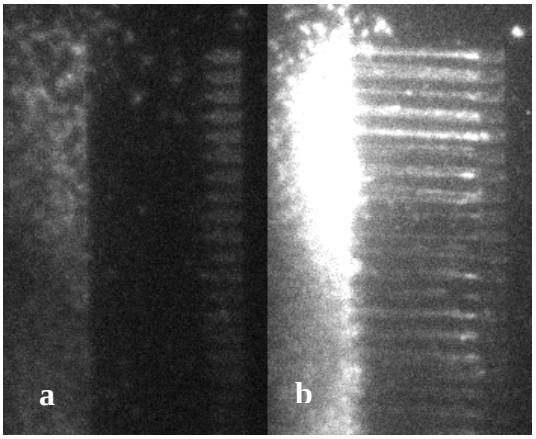

Figure 2. Double-tethered DNA curtains (a) with flow off, before double-tethering (b) with flow off, after double-tethering.

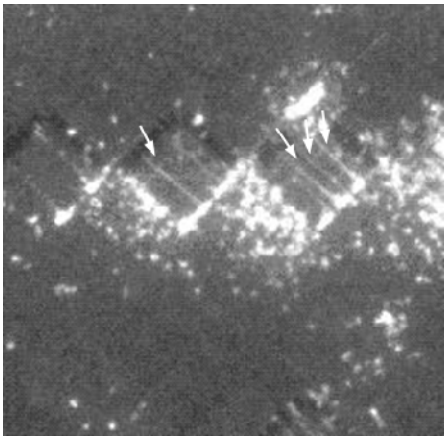

Figure 3. Arrows highlight crossed DNA molecules double-tethered using a box-shaped pattern.