Communication

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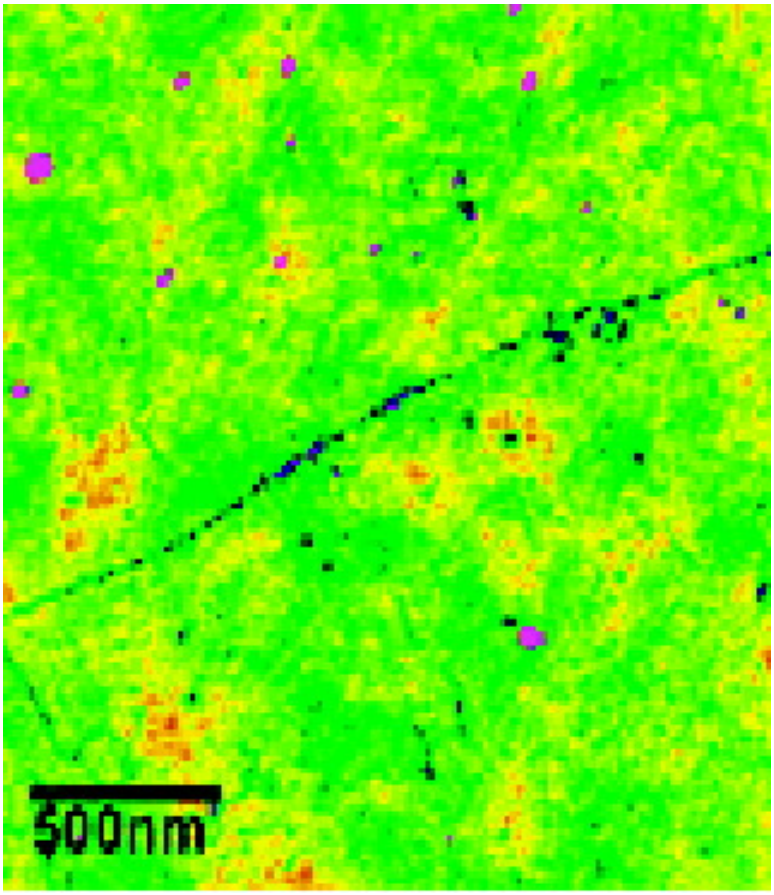

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Enzymatic Clipping of DNA Wires Coated with Magnetic Nanoparticles

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Since the discovery of the double-helical structure of DNA, many exciting advances have been made in the biological sciences to manipulate, modify, control, and amplify DNA in solution. One of the ways that DNA can be manipulated in solution is by fragmentation using restriction endonucleases. The BamH1 enzyme is one of the over 200 varieties of restriction endonucleases; it specifically recognizes the DNA sequence GGATCC and cleaves the strand at that point.[1] The pioneering work with DNA in molecular biology was restricted to that done in solution until recently when molecular combing techniques demonstrated that DNA can be stretched onto solid surfaces via the surface tension of a receding meniscus of a DNA solution and the air/solution interface.[2] Schwartz et al. reported how stretched DNA molecules maintained their biological activity and accessibility by showing the ability of the stretched DNA to be enzymatically digested using a mix of restriction endonucleases.[3] In recent years, molecular combing and other receding meniscus stretching techniques have been used to stretch DNA that has been templated with several materials, including nanoparticles, nanotubes, and polyelectrolytes. Such studies showed that DNA can serve as a metallization scaffold for metals such as Au, Cu, and Pt.[4−10] In this communication, we describe the ability of DNA to electrostatically assemble magnetic nanoparticles while retaining its biochemical recognition properties. We show that the templated DNA, once stretched, can be digested by the BamH1 restriction enzyme.

To prepare the samples, a 5 ng/mL solution of double-stranded nonmethylated λ-phage DNA (*E. coli* host strain GM 119 from Sigma-Aldrich) in 10 mM tris(hydroxymethyl)amino-methane (Tris) buffer was incubated with 5 ng of Fe_2O_3 magnetic nanoparticles (Figure 1a). The mixture was agitated on a vortex mixer for 1 h at room temperature. Prior to preparing the mixture, positively charged water-soluble Fe_2O_3 magnetic nanoparticles were synthesized using a method described by Li et al.[11] The average size of the particles as determined by tapping mode AFM (TMAFM) was 4.1 ± 0.9 nm, determined from the height of a line scan across a chosen particle. The particles were also characterized using XPS, FTIR, TEM, and XRD (Supporting Information). The weak electrostatic interactions between the positively charged Fe_2O_3 nanoparticles and the negatively charged DNA caused the particles to align along the DNA. The resulting templated DNA was then stretched onto freshly cleaned silicon oxide (WaferNet, CA) using molecular combing and subsequently rinsed with ultrapure water to remove any residual salts from the buffer before analysis by TMAFM. Typical experiments would yield SiO_x surfaces with varying concentrations of stretched DNA that was either completely or partially coated, Figure 1b.[12] A line profile across a bare DNA yields a height of 0.76 ± 0.10 nm, while a line scan across a DNA coated with magnetic nanoparticles yields a height of 1.6 ± 0.2 nm. We postulate that the difference in height between the particle coated

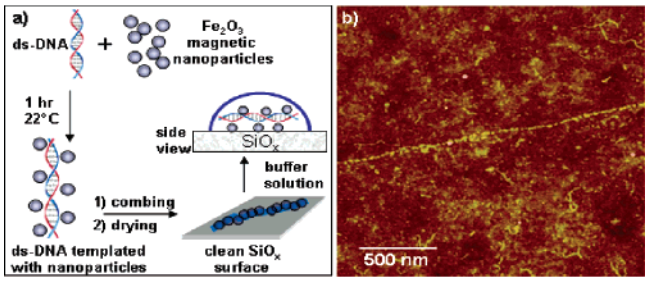

Figure 1. (a) Procedure for templating Fe_2O_3 nanoparticles onto DNA and subsequent stretching using the molecular combing technique. (b) TMAFM height image of a DNA strand coated with Fe_2O_3 nanoparticles.

DNA and the average size of nanoparticles is caused by the higher mobility and electrostatic interaction of the smaller particles to the DNA. Furthermore, our experiments showed that if one uses larger particles, the templated DNA cannot be easily stretched on surfaces. Magnetic force microscopy measurements were used to confirm the presence of nanoparticles in the assemblies with various particle sizes.[12]

The proof-of-concept experiment that we report in this communication demonstrates that stretched, surface-immobilized DNA that has been templated with nanoparticles retains its recognition properties. Prior to performing experiments on the surface, we verified that the coated molecules could be clipped by BamH1 in solution, Figure 2a. In gel 1, wells 1−4 contain the same amount of DNA. However, from the comparison between wells 1 and 3, it is evident that the stain is brighter in well 1, giving the impression of a higher quantity of DNA in that well. Since the ethidium bromide (EtBr) stain intercalates between the bases of the DNA molecule, the fact that wells 3 and 4 are not as bright suggests that the nanoparticles along the DNA are inhibiting the staining process. This is not surprising since the steric hindrance caused by the nanoparticles needs to be overcome. The EtBr can still intercalate since the particles are aligning along the phosphate backbone and are not thought to interact with the DNA bases. The endonuclease BamH1 is also hindered by the presence of the particles, but the structural transformation in the assembly remains accessible enough for the BamH1 to be able to act and recognize a specific sequence, as evident by the results in well 4 of gel 1. It is plausible to assume that the higher loading with particles along the DNA can eventually prevent the endonuclease from accessing the specific sequences. To explore this idea, we made assemblies by varying the ratios of DNA:nanoparticles and tested to see which ones would be clipped by BamH1 (gel 2 of Figure 2a). The endonuclease cuts the templated DNA into five fragments in all cases. These results strengthen the hypothesis that the particles are not disturbing the hydrogen bonding between the bases and are merely lining up along the contours defined by the phosphate backbone. We tested only a few different concentrations of DNA:nanoparticles and plan to perform comprehensive experiments to confirm whether or not the

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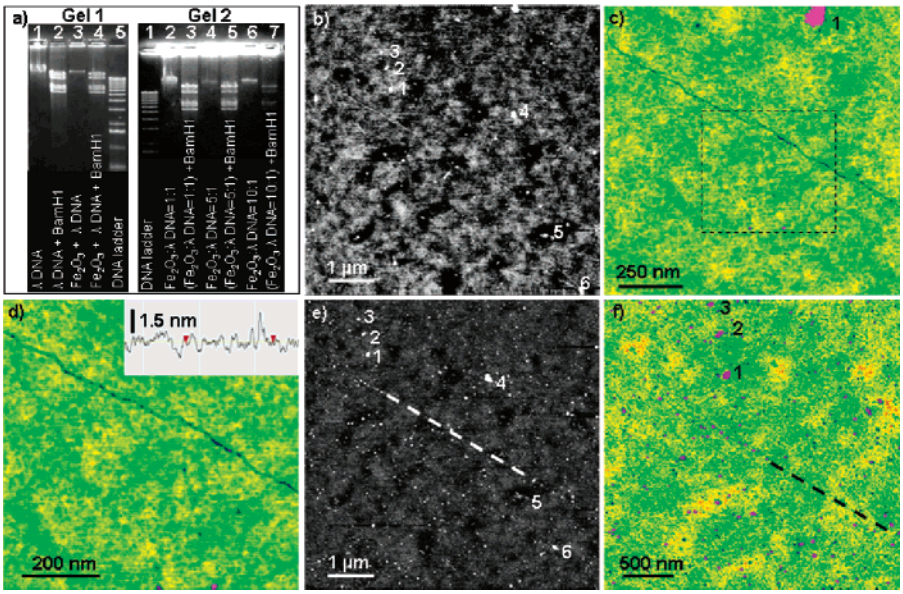

Figure 2. (a) Gel electrophoresis (0.8% agarose gel) of the assemblies described in the text. Part b shows a TMAFM height image of a stretched templated DNA on a SiO_x surface. The numbers on the image indicate surface imperfections used as markers. Part c is a higher resolution height image of the same surface as in b. The dashed square indicates the region shown in part d. The line scan inset shows the height of the templated DNA to be 1.8 nm. The surface was exposed to BamH1 as described in the text prior to acquiring the height image in e. The dashed line is used to indicate the portion of DNA that is missing on the basis of comparison with image b. Part f shows a higher resolution image of the surface after exposure to BamH1.

amount of particles on the DNA has an effect on the electrophoretic mobility of the templated structure. Such studies will also contribute to a better understanding of the factors that control the amount of nanoparticles on the DNA strand. Similar studies have been done with Au nanoparticles modified with DNA.[13] The gel results provide insight into the possible mechanism of the DNA fragmentation by BamH1 in solution. The mechanism is likely to be similar on the surface. The assembly is anchored to the SiO_x using the positively charged nanoparticles. Once the structure is hydrated with buffer solution (Figure 1a), the DNA becomes more accessible to BamH1 and the process depends on the steric hindrance induced by the presence of the nanoparticles along the phosphate backbone. We verified that bare DNA was cut by the BamH1 after it was stretched on surfaces modified with poly(allylamine hydrochloride) (see Supporting Information). Surfaces containing templated and stretched DNA were treated with 35 μL of BamH1 enzyme (Sigma-Aldrich) at a concentration of 10 units for periods of 10 min or less. Figure 2b−f shows the results of this experiment. An important finding from these experiments is that portions of the templated DNA can be detached from the surface after the clipping. This suggests that the electrostatic interactions between the positively charged nanoparticles used to template the DNA and the negatively charged oxide surface are not strong enough to hold smaller fragments of the templated DNA. One expects to observe five nicks on the basis of the gel results. We were unable to confirm this on a surface due to the suspected detachment of some templated fragments from the surface after treatment with BamH1. One reason for this result might be the fact that the strand is not coated uniformly and, as it is stretched, there are not an equal number of binding points to the surface along the length of the entire templated DNA molecule. Studies to test this hypothesis are underway in our laboratory. Our results so far cannot rule out the possibility that BamH1 clips some of the DNA on the surface in a nonspecific fashion after a certain period of time. Future experiments will introduce strategies to label the DNA on specific locations with larger beads in order to evaluate and control this possible nonspecificity.

In this communication, we introduce the use of restriction enzymes as an additional tool for DNA templated nanotechnology. We show that the BamH1 restriction enzyme can be used to fragment DNA after it has been templated with magnetic nanoparticles. The enzyme acts on the assemblies after they are immobilized and stretched on a surface. This technique may be successfully incorporated in higher order device structure nanofabrication.

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Supporting Information Available: Particle characterization: TEM, XPS, FTIR and XRD; AFM images of the bare, cut DNA; and enlarged versions of the figures presented in the text (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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