Dual restriction enzyme digest of cationic-gold-coated DNA scaffolds

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Abstract: DNA strands coated with AuNPs were cleaved by restriction enzymes while in solution or on a surface. Enzymatic activity was verified by gel electrophoresis prior to surface analysis. Cleavage results suggest that enzymes can recognize the AuNP-coated strands while on the surfaces, though specificity in digestion has not yet been verified. Development allows for advances in site specific localization of components using biological media.
Keywords: DNA, gold nanoparticles, restriction enzymes, surface, digestion

Introduction

Nanoscale devices are a current interest in many scientific disciplines. Some researchers have concentrated on DNA as a target for device design development, due to the specific interactions that are readily controlled (Whitesides et al 1991; Zhang et al 1993; Richter 2001; Chen et al 2004). Watson-Crick base-pairing, chain elongation, particle attachment, and temperature or pH dependent orientation have all been used to show that DNA has the ability to be specifically oriented and manipulated (Wang et al 1997; Cai et al 2001; Sönnichsen et al 2005; Viasnoff et al 2006; Mao et al 2007). The bottom-up strategies furnished by these properties allow for the development of inexpensively made functional structures, only requiring instrumentation for characterization. DNA is able to be hybridized and melted based upon the solution pH. This enabled researchers to uncurl structures to produce fluorescent outputs, which are readily detected, or curl strands to quench the output. Enzymes have also been attached to DNA tiles, and then used to manipulate solutions containing target analytes (Park et al 2005). These examples demonstrate that targets are able to be found by various moieties on surfaces using highly specific interactions. As a further addition, we are interested in displaying the capacity of particle-coated DNA to be cleaved in solution and on surfaces in a specific, consistent, inexpensive and high-throughput manner. We demonstrate here that gold-coated strand fragments can be prepared using well studied and commercially available enzymes.

The use of gold in these strategies provides various advantages when coupled with DNA manipulation. Theoretically, DNA nanostructures can be implemented in electronics technology, developing structures for devices (Richter 2001; Yonezawa et al 2002). Gold is an ideal candidate for electrostatic attachment since its conductivity and noble metal properties, or filled d-orbital configuration, prevent oxidation and degradation of electronic contacts. The ability to manipulate these architectures specifically when attached to semiconducting surfaces provides further advancement for the later site specific localization of specific functionalities such as memory storage (Zheng et al 2004; Gu et al 2005). In this case, restriction enzymes provide an amicable solution to what might be an otherwise complex task since manipulation can occur both in solution as well as on various surfaces. Recently this group provided evidence that strands can be severed with the restriction enzymes and then reattached using

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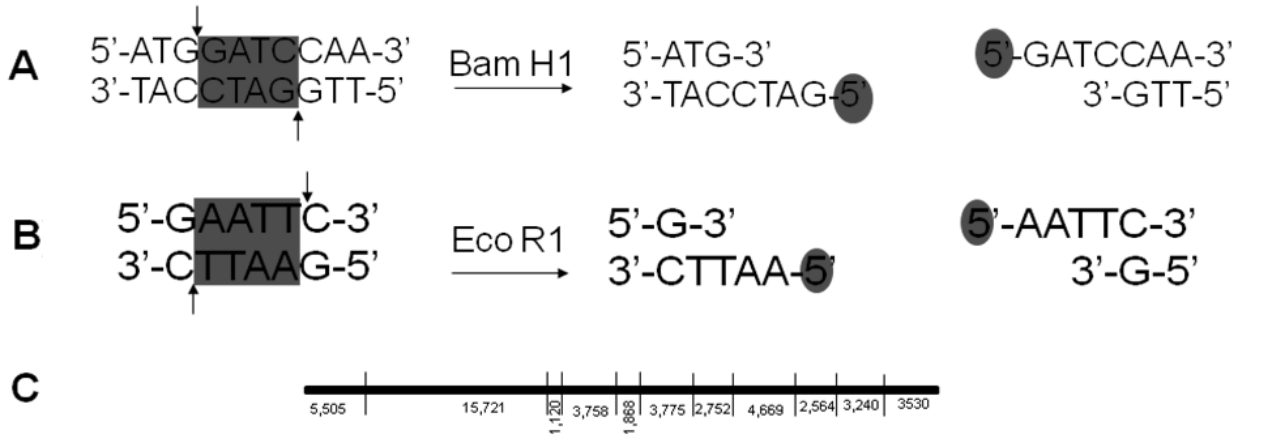

Figure 1 Schematic representation of restriction enzyme cleavages. The cleavage sites of BamH1 and EcoR1 are shown, in addition to the restriction map of the bacteriophage-λ 48 kBP sequence.

T4 DNA-ligase, even while coated with particles of varying composition (Kinsella et al 2007). The use of Au-NP's in these implementations serves as both a demonstration in ability as well as a functional step towards the creation of testable architectures.

Materials and methods

Gold-coated strands were prepared at a ratio of 1:5 unmethylated λ-phage DNA (Promega, WI) to 5 nm poly(L)-lysine coated gold particles (Ted Pella, Ca). Mixtures were vortexed for 30–60 minutes to allow attachment of the cationic particles to the DNA templates. Bare λ-phage DNA was used in control experiments with slight variations in methods between control and coated-strand parameters. Each gel analysis used solutions as provided by the supplier, stock dilutions were only performed when applying the solutions to the surfaces and performing UV/VIS verification. Cleavage site specificity of BamH1 and EcoR1 enzymes (Promega, CA) was verified by gel electrophoresis, using both bare and coated DNA samples. 15 × 25cm, 0.8% agarose gels were run at 120 V for 4 hours. Samples were loaded according to the type of digest as well as concentration. Three template concentrations were used in coated strand gels, though all at the same 1:5 ratio of DNA-to-particles.

Coated or uncoated DNA was applied to the clean silicon oxide surfaces in 2 μL droplets and then combed using a stream of nitrogen (Bensimon et al 1995). Cleavages were performed in a stepwise manner for bare DNA; strands were first cleaved by pipetting 2 μL of BamH1 and the surfaces were then imaged. Those surfaces were successively treated with EcoR1, followed by a second imaging step. During each treatment the enzymes were left to interact on the surface for 10–15 minutes before being twice rinsed with D.I. water. Coated strands were cleaved using a cocktail of both enzymes concurrently, with digestion times ranging between 1.5–10 minutes. Images were taken by tapping mode Atomic Force Microscopy (AFM) using silicon nitride tips (Veeco, NY) at scan rates of 1–2Hz.

Results and discussion

DNA strands were used as templates for gold nanoparticles (AuNP's) via electrostatic attractions between the negatively-charged phosphate backbone of the DNA and the positively-charged poly-(L)-lysine functionalized particles. A ratio of 1:5 (v/v) λ-DNA: particles stock solutions produced the most amicable coating as determined by UV/VIS spectrometry. Cleavage efficiency was determined in solution by gel electrophoresis. BamH1 and EcoR1 enzyme experiments were performed in solution, either in two single enzyme digestion steps or in a single step digestion with both enzymes, to determine digestion efficiency. Comparison of digests on bare strands provided evidence that both digest methods produced similar results, thus in the interest of time a single digestion step was used for coated strands. Figure 1 presents results from the gel separations, where enzymatic activity on coated strands was examined by leaving the DNA:particles ratio constant while increasing solution concentrations.

Overall the enzymes effectively cleaved the strands in a specific manner, when compared with bare strand cleavages. As the concentration of the particles on the templates increased, strands were cleaved with less specificity, suggesting a limit of enzyme recognition as a function of concentration. Additionally, the average standard deviation of the increase in peak intensities, as a function of increased

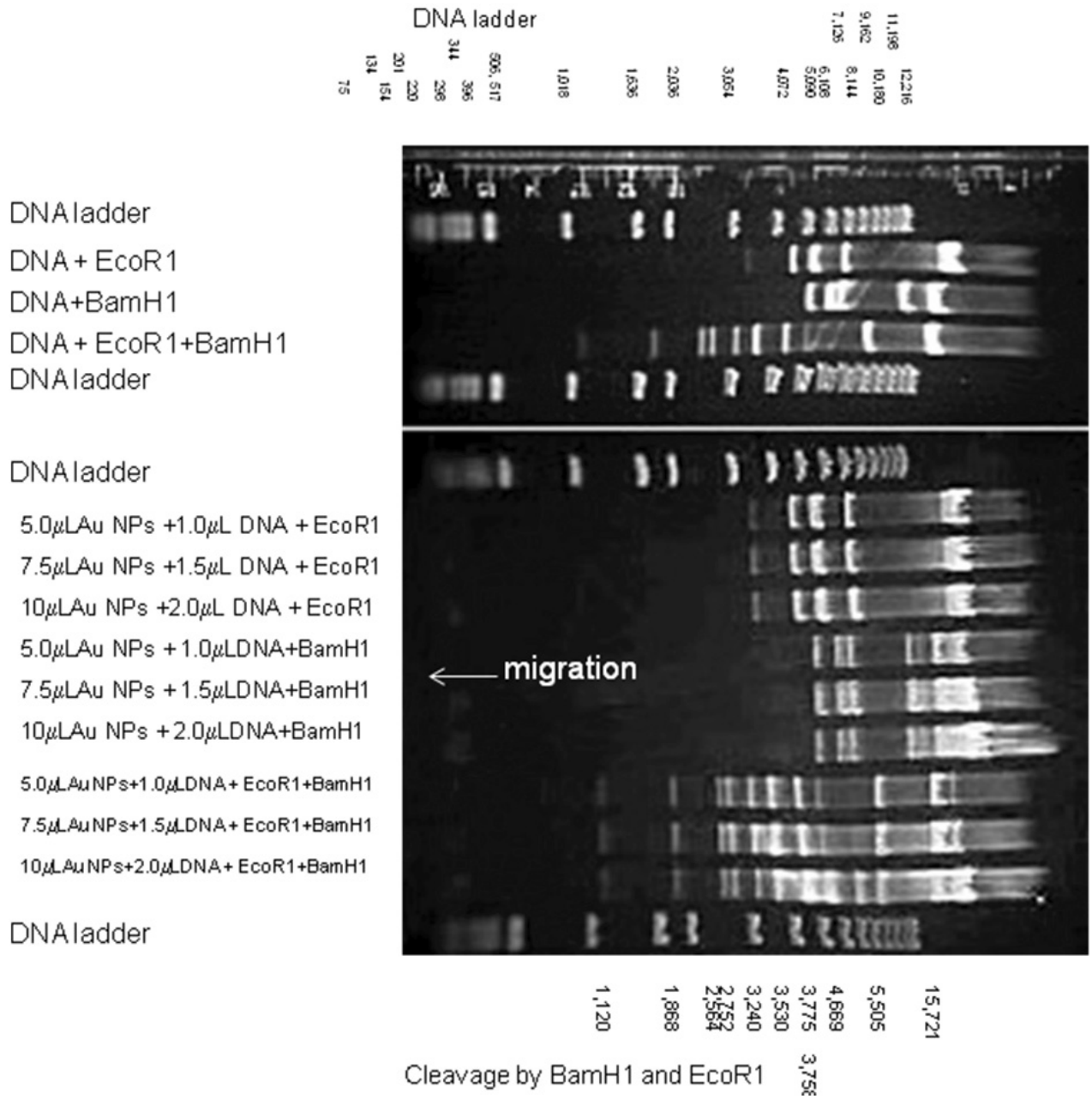

Figure 2 Gel electrophoresis separation of gold coated DNA strands cleaved with BamH1, EcoR1, and both enzymes. The first gel is bare DNA cleaved in solution then run on 0.8% agarose. The second gel is coated DNA run under the same conditions. Varied concentrations of coated strands show similar features, though BamH1 and dual digest lanes show evidence of nonspecific cleavages at increased concentrations. There is also some evidence of enzyme inactivity, especially for the Bam H1 enzyme, marked by the presence of a band above the 15,721 fragment.

concentration, was found to be 6.58 counts. In some cases there were decreased band intensities at higher concentration, to the point where outputs for the same band location were almost identical in the high and low concentration lanes. Though the above stated may be, there was a general increased in band intensity as concentration increased, with the exception of the highest concentration lanes in the BamH1 and dual digest experiments. This seems to imply that the enzymes were able to recognize the strands, but there is some evidence that both nonspecific cleavage and decreased cleavage efficiency occurred at higher concentrations. In the nonspecific cleavage examples, the strands do not seem to be cleaved at locations much distant from their recognition sites, but the variation in the bands is readily seen and additional bands are apparent where they should not occur. Taking the above stated into account, it seems that there is a peak activity when the concentration reaches 7.5 μL of Au-NPs to 1.5 μL λ-DNA when using BamH1 in the reaction mixture.

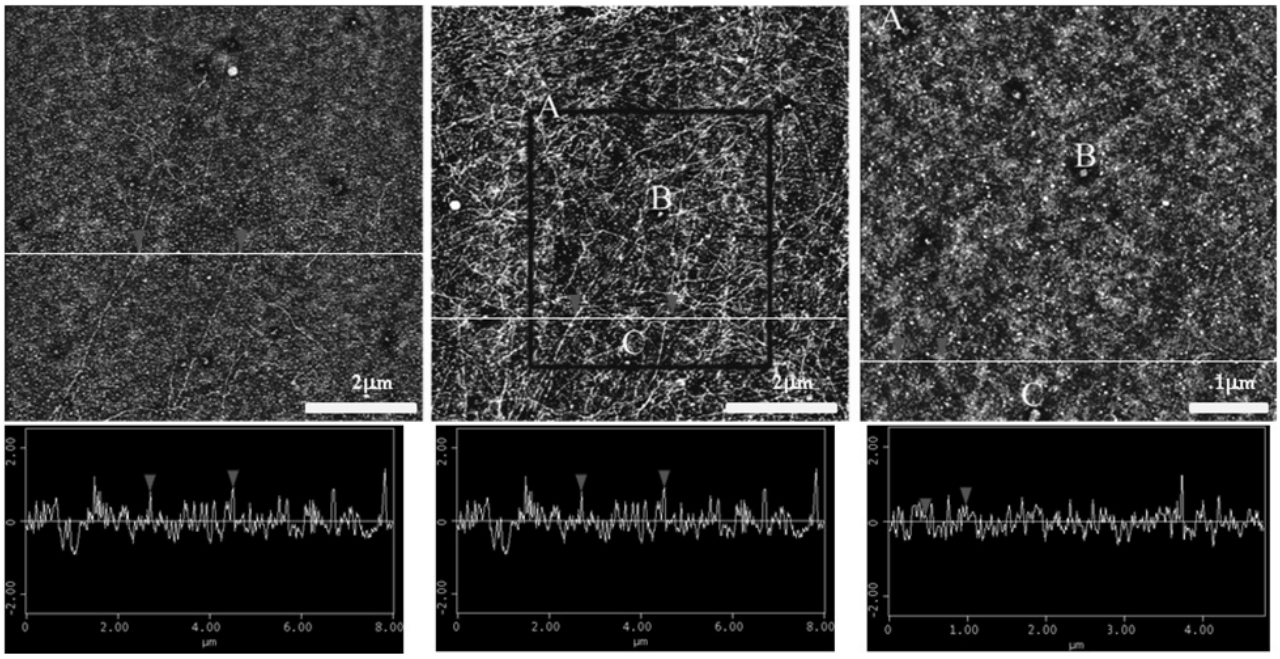

Figure 3 Template strands on silicon oxide surfaces. The initial image presents coating factors of the AuNP-DNA templates. The following images are the surface before and after cleavage experiments. The height profiles below each image are for that respective image, and arrows designate the location on the profile of the corresponding arrow on the AFM image. The box in the second image is the area in the third image, prior to cleavage by a combination of both enzymes. The letters **A**, **B**, and **C** designate surface discontinuities used to locate the area of interest, following digestion. DNA strands are shown in the last image to be heavily digested by the enzymes with features appearing less coated and separated from their complementary structures in many locations.

When placed on silicon oxide surfaces, variations in the concentration of strands occurred as expected, and a majority of the observed templates followed the previously documented trend of variable coating (Nakao et al 2003; Kinsella and Ivanisevic 2005; Nyamjav et al 2005). The strands were either elongated across the surface or intertwined with one another and stretched, mostly a result of variations in solution concentration. As can be seen in Figure 2, each configuration of the DNA templates displayed similar degrees of particle attachment to the strands, and it may be presumed, as particles were observed to also be loose on the surface, that neither implies a variation in the recognition of one entity for the other. Cleavage results suggested that enzymatic activity was retained when the templates were placed on the surface. The differences between the two variations in stretching did not seem to have a significant effect on the ability of the enzymes to locate the strands for cleavage. Variations in time for digestion showed similar results where, for example, digesting for a period of 1.5 minutes produced significant cleavages, comparable with the longer digest times. This is consistent with experiments in solution where UV/VIS measurements of enzymatic digestion of DNA show significant changes in absorption even at lower time scales. It can be assumed that the enzymes function in a similar fashion to those in cellular environments (Huang et al 1982; Castellano et al 2006). Likewise, the ability of the enzymes to locate the strands does not necessarily suggest that the enzymes are specifically cleaving at their proper palindrome sites (Thielking et al 1990; Janscak et al 1999).

Summary

We have demonstrated the ability to doubly cleave AuNP-coated DNA templates in solution and on a surface. In solution both EcoR1 and BamH1 restriction enzymes are able to cleave coated strands at or near the correct restriction sites. Coated strands are able to be stretched across silicon oxide; then selectively cleaved by the stated enzymes. Though the enzymes are able to recognize the templates on the silicon oxide, there is no clear indication that they are being specifically cleaved as in solution. This further development extends the possibility of site specific localization of components through the use of a biological media.

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