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ADP011016

TITLE: Site-Specific Attachment of gold Nanoparticles to DNA Templates

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TITLE: Materials Research Society Symposium Proceedings Volume 635.
Anisotropic Nanoparticles - Synthesis, Characterization and Applications

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Site-Specific Attachment of Gold Nanoparticles to DNA Templates

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ABSTRACT

DNA was used as a scaffold for the binding of gold nanoparticles using a standard chemical technique. A DNA template was designed with amino-modified thymines located every 3.7 nm, which would allow the attachment of the carboxylic acid functionalized gold nanoparticles. The gold particles were covalently bound to the amino groups on the DNA using standard 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) chemistry in the presence of a competitor to block excess gold binding sites. The products were analyzed by transmission electron microscopy (TEM) and atomic force microscopy (AFM).

INTRODUCTION

The need to produce regular arrangements of nanoparticles led to the idea of using DNA as a scaffold or template for assembly of nanoscale arrays. Beginning in the 1980s Seeman et al. experimented with combining DNA fragments to produce geometrical shapes, including cubes [1], triangles [2], two-dimensional arrays [3-5] and various forms of DNA knots [6,7].

Using DNA as a structural molecule has many advantages. It can be easily synthesized in lengths from 5 to over 100 nucleotides. It can be joined end to end to produce longer linear molecules or more complex shapes, and it can be modified at predetermined sites to allow for the attachment of other molecules in a specific manner.

The precise arrangement of nanoparticles to form an array is a difficult task. DNA has been used by others as a template for the attachment of particles. Mirkin et al. [8-10] and Alivisatos et al. [11,12] have successfully attached oligonucleotide derivatized nanoparticles to DNA using hybridization techniques. Niemeyer and coworkers have assembled biotinylated gold clusters on streptavidin-DNA oligonucleotides and subsequently hybridized the clusters to a complementary RNA template [13]. Cassell et al. assembled fullerene derivatives along the DNA backbone using cation exchange [14].

The present study was an attempt to use DNA as a scaffold for placement of gold nanoparticles at specific sites using a chemical reaction. Gold nanoparticles with an average diameter of 1.5 nm were synthesized with a mercaptosuccinic acid coating. Oligonucleotides were designed with amino-modified bases for attachment to carboxylic acid functionalized gold particles. The modified bases were separated by 10 base pairs (approximately 3.7 nm). The reaction between the amino group on the DNA and the carboxyl group on the gold particle was facilitated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). Analysis of the products by transmission electron microscopy

(TEM), and atomic force microscopy (AFM) showed that the gold particles are bound to the DNA.

EXPERIMENTAL DETAILS

Oligonucleotides were purchased from Oligos Etc. They were designed with C6-amino-modified thymines (X) separated by 10 base pairs and with compatible overhangs to permit ligation reactions to easily occur:

GATCTAXCAACGGCTCAXCCAA

X=T-(CH₂)₆-NH₂

TAGTTGCCGAGTAGGTTCTAGA

The 5' ends of the double-stranded oligonucleotide were phosphorylated and then the DNA was ligated. Enzymes were purchased from Promega.

Gold nanoparticles coated with mercaptosuccinic acid were synthesized as described elsewhere [15]. The gold was dissolved in water and the pH adjusted to ~3.8. Final concentration of the gold solution was 4.7 mg/ml. Approximately 50 µg gold nanoparticles were incubated at room temperature with ~1.5mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce) and ~2.0mg N-hydroxysuccinimide (Pierce) for 30-60 minutes.

Methylamine (Sigma) concentrations were calculated as multiples of gold binding sites on the DNA. Typical amounts used were 1X (equal to the concentration of modified thymines), 2X, 5X, 10X, and no methylamine. The methylamine was added to 2-10 µg DNA and then this mixture was added to the gold particles. Incubation was typically 2-4 hours at room temperature.

For AFM imaging, reaction mixtures were diluted 1:10,000 to 1:20,000 in 20mM Tris, 5mM KCl, 5mM MgCl₂, and 3mM ZnCl₂. Five µl of this solution was deposited on freshly cleaved mica. The samples were allowed to dry for 10-30 minutes, then washed first with water, then 50% ethanol, and finally ethanol. After drying overnight in a vacuum desiccator, imaging was carried out on a Nanoscope III AFM system (Digital Instruments).

For transmission electron microscope (TEM) imaging, reaction samples were typically diluted 1:60. The TEM grid was dipped into the solution and allowed to dry overnight before imaging on a Hitachi H-600 system.

RESULTS

Figure 1 shows an AFM image of the mercaptosuccinic acid coated gold particles used in the binding reaction with the DNA. The average diameter of the particles was 1.5 nm as measured by X-ray diffraction [15]. Each particle has multiple reactive carboxyl groups on its surface. In order to decrease the chances of one particle binding to many amino groups on the DNA, an agent was used to block some of the carboxyl groups on the gold. Methylamine was chosen because of its small size and similarity to the methylene side chain containing the amino group on the DNA.

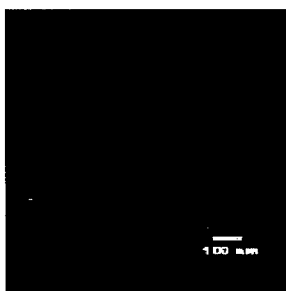


Figure 1. AFM image of mercaptosuccinic acid coated gold nanoparticles.

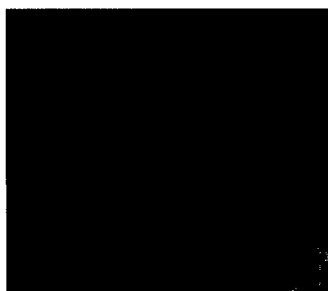
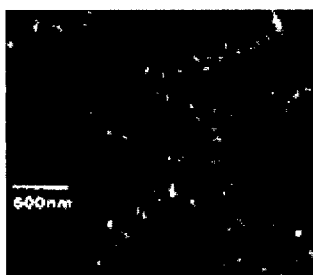


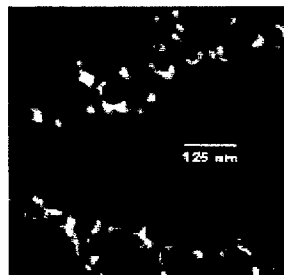
Figure 2. TEM image of DNA bound to gold nanoparticles at magnification of 250,000.

Figure 2 shows a transmission electron micrograph of DNA bound to the gold nanoparticles without the methylamine blocker. The gold particles are bound to the DNA but multiple strands of DNA are held together because of the many reactive sites on each gold particle. The particles cause cross-linking between different DNA strands and possibly between different sites on the same DNA strand, leading to an aggregate of DNA and gold.

AFM images of DNA bound to gold in the presence of methylamine are shown in Figure 3. The methylamine concentration in the reaction was equal to the concentration of amino-modified thymines on the DNA. Figure 3A shows linear double-stranded DNA bound to the gold nanoparticles. The looping seen in the image may be the result of sample deposition on the mica substrate, or there may still be some cross-linking between DNA strands due to the multiple binding sites on the gold particles. However, if cross-linking is occurring, it is greatly reduced by the addition of the methylamine (compare with Figure 2). Figure 3B shows a close-up view of the upper right quadrant of 3A. Analysis of the interparticle distance reveals that the closest resolvable particles are about 12.3 nm apart, a separation of 3-4 binding sites on the DNA. It is not clear at this time why all the binding sites are not occupied. Further studies are underway to address this issue.



A



B

Figure 3. AFM images of gold nanoparticles bound to DNA. **A.** Carboxylic acid functionalized gold particles bound to amino-modified thymines on the DNA using EDC and methylamine. **B.** Close-up view of upper right quadrant of **A**. An analysis of the spacing between particles indicates that the closest resolvable particles are separated by 3-4 binding sites on the DNA.

CONCLUSIONS

Gold nanoparticles functionalized with carboxylic acid groups have been chemically bound to amino-modified thymine bases on double-stranded DNA. The blocking agent methylamine was used to inactivate excess reactive groups on the gold particles, minimizing cross-linking between strands of DNA.

The advantages to this method are that particles can potentially be placed wherever a modified base is inserted during synthesis of the DNA. Therefore, arrangement of the particles would be dependent only on design of the DNA template and the size of the particle. A variety of functional groups can be used to modify DNA during synthesis and any particle that can be functionalized with a complementary reactive group can be bound to the DNA. The DNA product is double-stranded thus retaining the regularity of structure that makes DNA an attractive building block.

ACKNOWLEDGMENTS

This research was supported by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U. S. Department of Energy under contract DE-AC05-00OR22725.

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