DNA Nanotechnology

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Site-Specific Control of Distances between Gold Nanoparticles Using Phosphorothioate Anchors on DNA and a Short Bifunctional Molecular Fastener**

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Precise control of the locations of and distances between nanomaterials is a great challenge in nanoscale science and technology. Meeting this challenge is critical, not only to a fundamental understanding of quantum effects of these materials at nanometer scales,[1] but also to practical applications in nanoelectronics, photonics, and medicine. A number of methods have been reported.[2] Among them, "bottom-up" assembly, particularly using DNA molecules as templates to position nanomaterials, is promising,[3] as DNA has been shown to be a highly programmable molecule resulting in 2D[3c,e,4] and 3D[5] nanostructures. Despite the progress, methods to functionalize these DNA nanostructures with nanomaterials are limited. Alkane thiol modification on either end of DNA is usually used to attach DNA on gold nanoparticles (AuNPs), which in most cases introduces nicks or complications in designing DNA nanostructures.[6,7]

Herein we report a novel method to assemble nanoparticles along DNA strands with precise control of the position of and distance between nanoparticles using phosphorothioate-modified DNA (PS-DNA) coupled with a short bifunctional fastener (BF; Scheme 1). Similar to a molecular anchor, the phosphorothioate allows programmable placement of modifications during oligonucleotide synthesis that can control both the position and the number of modifications. The BF has an alkane thiol group at one end that can bind to a AuNP and an iodoacetamide group at the other end that can bind to a phosphorothioate group on a modified DNA backbone. This method can place nanomaterials at any selected backbone site of the DNA structure, making it possible to precisely control the position of the nanoparticles along DNA and the distances between them, without the need to functionalize AuNPs with a large number of DNA molecules[6] or purify monofunctionalized nanomaterials.[7] As the functionalization is made on the DNA backbone, at which the phosphorothioate is almost always available for binding, this method can be readily applied to 2D and 3D DNA nanostructures, without the need to introduce nicks on those structures, minimizing the risk of affecting the structural stability, as well as increasing the possibility of fastening nanomaterials at any desirable location on the DNA nanostructure. Instead of using DNA strands to connect AuNPs and DNA structures, the much shorter BF also holds the AuNPs in such a way that there is less freedom of movement on the DNA, allowing even more precise control of the positions of and distances between AuNPs.

To take advantage of different reactivities of alkane thiolate and phosphorothioate, we chose *N*,*N*′-bis(α-iodoacetyl)-2,2′-dithiobis(ethylamine) (BIDBE)[8] as a precursor to the bifunctional fastener (BF, *N*-iodoacetyl-2-mercaptoethylamine; see Scheme 1, right). BIDBE contains an iodoacetamide group at both ends and a disulfide bond in the middle. The iodoacetamide functional group has been shown to react with the phosphorothioate group specifically,[9] while the disulfide bond, upon reduction to an alkane thiol, can bind to AuNPs selectively.[10]

To demonstrate specific covalent attachment of the BF to PS-DNA, BIDBE was synthesized as reported previously,[8] and then treated with PS-DNA at 50 °C for 6 h, followed by addition of tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) to reduce the disulfide bond to a thiolate. Analysis of the reaction products by MALDI-TOF mass spectrometry indicated formation of BF-PS-DNA adducts (calculated molecular weight(M_w): 8275 Da; observed M_w: 8272 ± 8 Da (0.05–0.1 %); see Supporting Information, Figure S1). The reaction yield with optimal conditions (50 °C for 6 h) reached up to 90 %. In contrast, DNA without phosphorothioate modification did not show any coupling to BF, as evidenced

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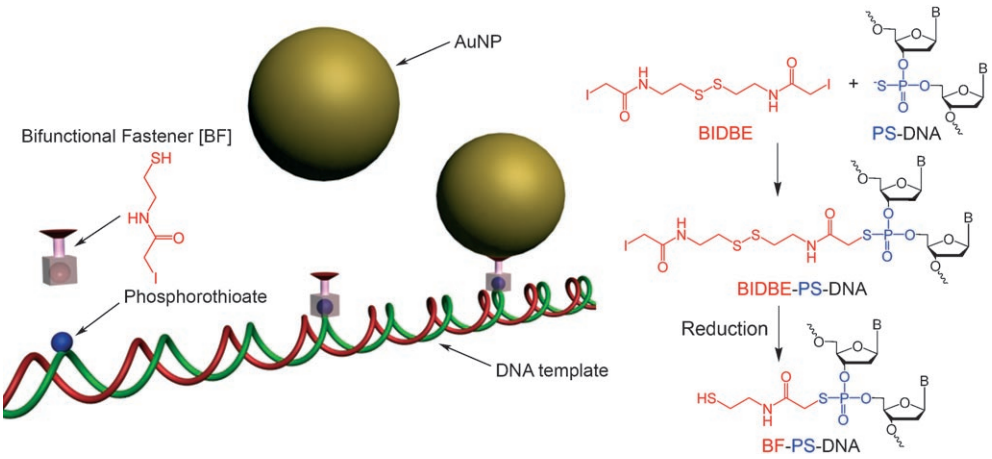
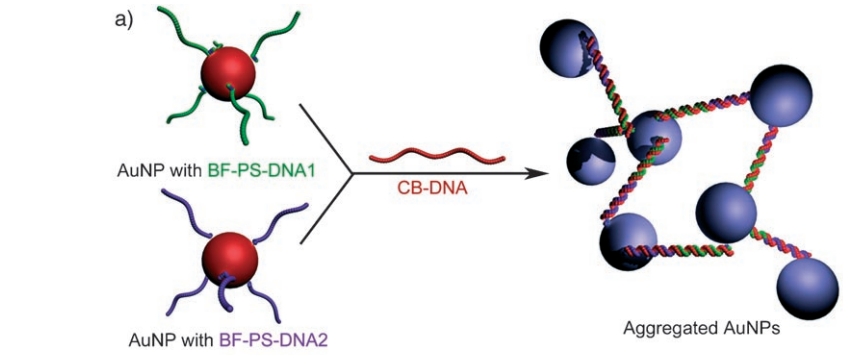
Supporting Information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Left: depiction of gold nanoparticle (AuNP) assembly on DNA by a phosphorothioate anchor and a short bifunctional fastener (BF). Right: the reaction between BIDBE and a phosphorothioate group on DNA and activation of the BF by disulfide bond reduction. BIDBE = *N*,*N*′-bis(α-iodoacetyl)-2,2′-dithiobis(ethylamine).

by the absence of a new peak in the mass spectrum under identical conditions.

To demonstrate conjugation of the BF-PS-DNA to AuNPs, a 33-mer DNA containing two adjacent phosphorothioate modifications at the 5′-end (PS-DNA1) was first coupled to BF using the above procedure, and then used to functionalize 13-nm AuNPs (Figure 1 a). In a different container, a parallel experiment was carried out using a second 33-mer DNA with noncomplementary sequence to the first oligonucleotide containing two adjacent phosphorothioate modifications at the 3′-end (PS-DNA2). Mixing the contents of the two containers did not result in a shift of the plasmon peak at 520 nm (data not shown), suggesting no AuNPs aggregation. This result is expected since no complementary DNA molecules are used. On the other hand, when a third 60-mer bridging DNA complementary to both the first and second DNA strands (CB-DNA) was added to the above solution, a red-shift of the plasmon peak to approximately 531 nm was observed (Figure 1 b), indicating hybridization-induced aggregation of AuNPs. After heating the aggregates to over 90 °C, which is above the 30-mer DNA melting temperature (ca. 70 °C), the plasmon peak blue-shifted back to the original position at 520 nm, thereby demonstrating redispersion. In contrast, when the bridging CB-DNA was replaced with a noncomplementary control DNA strand (Non-CB-DNA), no shift in the plasmon peak was observed, which shows that the AuNP aggregation is sequence-specific. To ensure that functionalization of the AuNPs was due to phosphorothioate modifications on the DNA, the PS-DNAs were replaced with non-phosphorothioate-modified DNA (Non-PS-DNA1 and Non-PS-DNA2, see Supporting Information, Table S1) that have gone through the BF treatment procedures. No plasmon shift



PS-DNA1: 5′-TT*T* TTA GCA TAT GAC TAT GTT ACT CGC TAT AGC-3′
PS-DNA2: 5′-GTA CTT GCA ATA TGT GCA ATG GCG AGG ATT T*T*T-3′
CB-DNA: 5′-AAT CCT CGC CAT TGC ACA TAT TGC AAG TAC GCT ATA GCG AGT AAC ATA GTC ATA TGC TAA-3′
Non-CB-DNA: 5′-AAT CGT ATA CTG ATA CAA TGA GCG ATA TCG CAT GAA CGT TAT ACA CGT TAC CGC TCC TAA-3′
Blue nucleotides with * have phosphorothioate modification on the backbone

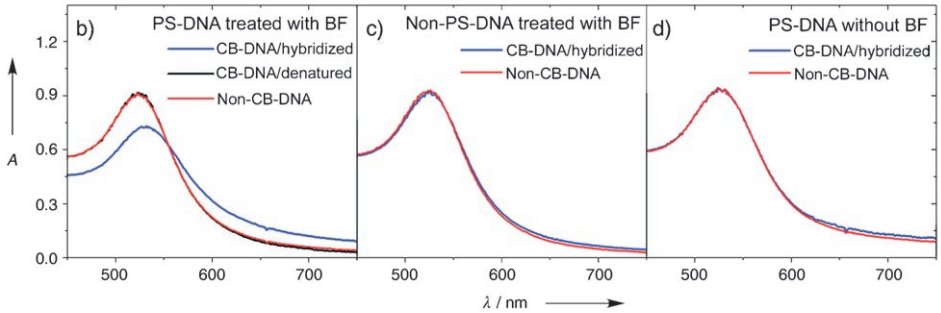


Figure 1. a) Functionalization of AuNPs with BF-PS-DNA1 and BF-PS-DNA2, addition of bridging CB-DNA, and aggregation. The DNA sequences are also given. b) UV/Vis spectra of the aggregation-dependent plasmon resonance of AuNPs functionalized with BF-PS-DNA. As a control, the UV/Vis spectra of AuNPs with BF-treated non-phosphorothioate-modified DNA (c), and PS-DNA without the BF treatment (d) are shown.

was observed, indicating lack of AuNP assembly (Figure 1c). Furthermore, to demonstrate the necessity of the bifunctional fastener for AuNP assembly, PS-DNAs (PS-DNA1 and PS-DNA2) without BF were employed in another control experiment, in which no change in the plasmon peak was observed either (Figure 1d). Therefore, both phosphorothioate modification and bifunctional fastener are necessary for the system to successfully attach AuNPs to the DNA template. This result differs from a previous study that showed phosphorothioate DNA alone can help form AuNP aggregates.[11]

To determine whether the method provides precise position and distance control of AuNPs along the PS-DNA, 5-nm AuNPs were assembled onto PS-DNA which was immobilized on a 30-nm-thick gold thin film on a silicon wafer. To increase the yield of AuNPs binding to phosphorothioate-modified sites and to make the linkage rigid, three adjacent phosphate moieties were modified to phosphorothioate to bind to a single AuNP. Therefore, to form a AuNP dimer with an 80-base-pair gap between NPs, a 100-mer DNA was used with positions 9, 10, 11, 89, 90, and 91 modified to phosphorothioate (2PS-Anchor DNA(80), see Supporting Information, Table S2). This modified DNA was treated with BIDBE by using the procedure shown in Scheme 1. After removing residual BIDBE molecules from the PS-DNA on a gel-filtration column, the BIDBE-coupled PS-DNA strand was treated with TCEP to reduce the disulfide bond in BIDBE to *N*-iodoacetyl-2-mercaptoethylamine (BF). This single-stranded 2PS-Anchor DNA(80) was then hybridized with 100-mer complementary DNA containing an alkane thiol modification with an 18-atom hexa-ethylene glycol (PEG) spacer on the 5′ end (CP-DNA) to form double-stranded 2PS-dsDNA(80). For further details, see Supporting Information, Table S2. The purpose of the alkane thiol end group and PEG spacer on CP-DNA are to form a tether to gold thin films and provide some spatial freedom on the double-stranded DNA (dsDNA) to increase reactivity between AuNPs and the BF-treated phosphorothioate-modified positions. Although single-stranded DNA can be used, dsDNA is preferred as it forms a more rigid structure, making it easier to control the AuNP positioning on the DNA.

Phosphorothioate modifications were positioned at least 9 base pairs from both ends of the dsDNA to increase the thiol–gold surface immobilization through the alkane thiol end group as opposed to the BFs on the phosphorothioate modifications. After 12 h of incubation of the above dsDNA immobilized on gold surface in a 5-nm AuNP solution, the AuNP assemblies were characterized using a field-emission scanning electron microscope (FE-SEM, Hitachi S-4700).

The SEM images show that a significant portion of AuNPs can be identified as dimers (Figure 2a). To prevent bias in counting dimers and measuring distances between the dimers, a population histogram of AuNP dimers on the gold surfaces was generated for 2PS-dsDNA(80) (Figure 3a). Using three SEM images for each sample, the pixel coordinates of the center of each nanoparticle were collected using an in-house computer program. From these coordinates, the distances between these nanoparticles were then calculated. The aggregates of three or more AuNPs or clustering of two or more pairs of NP dimers in proximity were excluded, as they might be due to non-oligonucleotide AuNP aggregation.

As the length of the dsDNA (100 bps, ca. 34 nm) used for assembly was below the persistence length (ca. 50 nm), the distances between AuNPs could be directly compared to the distances between phosphorothioate modifications of the PS-DNA. For 2PS-dsDNA(80), the predicted distance between two AuNP dimers is about 27.2 nm. As expected, it can be seen in the histogram in Figure 3a that the dominant distance for this DNA matches the predicted distance. To demonstrate that such a distance can be varied according to specific locations of the phosphorothioate modification, we repeated the above experiment using two different 100-mer dsDNAs with 70- and 60-base-pair spacing between the phosphorothioate modifications (called 2PS-dsDNA(70), with positions 9, 10, 11, 79, 80, and 81 phosphorothioate-modified, and 2PS-dsDNA(60), with positions 19, 20, 21, 79, 80, and 81

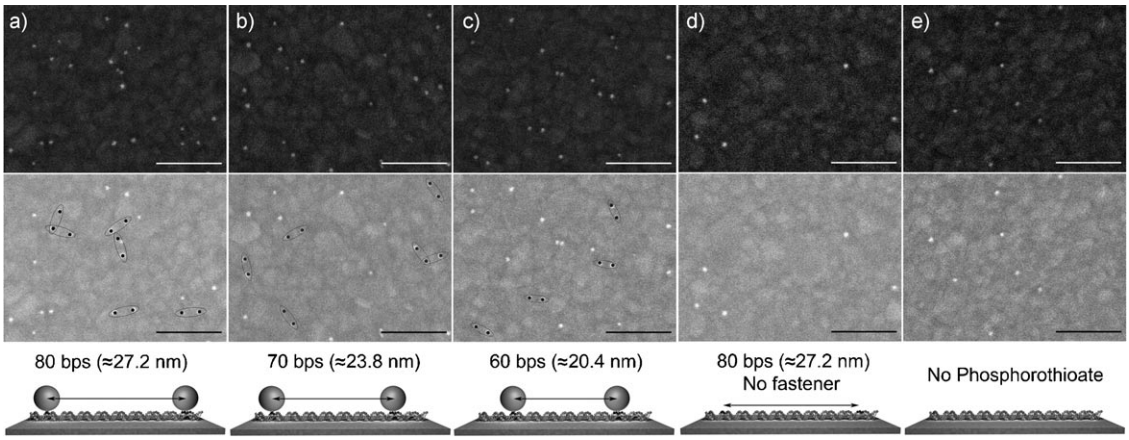


Figure 2. SEM images of AuNPs assembled on BF-treated 2PS-dsDNA with two triplet phosphorothioate modifications at spacings of a) 80 bps (ca. 27.2 nm), b) 70 bps (ca. 23.8 nm), and c) 60 bps (ca. 20.4 nm). AuNPs assembled on d) dsDNA with two triplet phosphorothioate modifications of 80-bps spacing but without bifunctional-fastener treatment and e) dsDNA without phosphorothioate modifications are shown as a control. The overlap of the SEM images with identified AuNP dimers and the AuNP-DNA assemblies (black dots and ellipses) are also shown for each SEM images. The scale bars are 100 nm; bp = base pair.

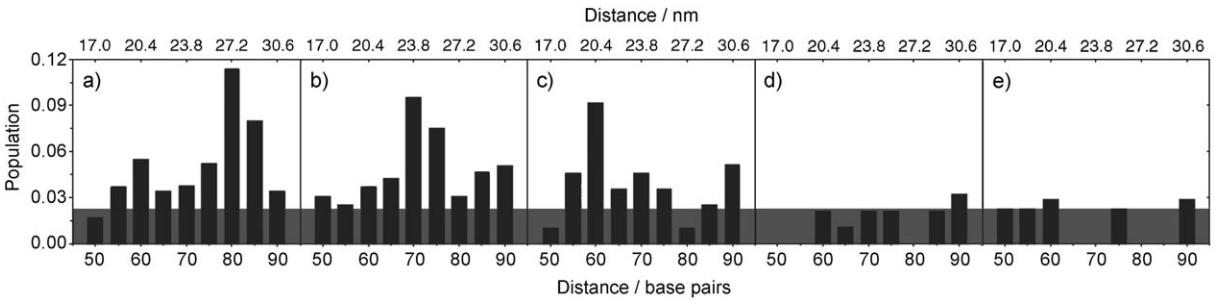


Figure 3. Histogram of the population of AuNP dimers assembled on DNA with different spacings between PS modifications. The distances between all NPs on the surfaces were measured and twice the number of dimers at a specific distance per total number of NPs on the surface was counted. The predicted spacings between the bifunctional fastener treated phosphorothioate modifications on DNA are a) 80 bps (ca. 27.2 nm), b) 70 bps (ca. 23.8 nm), and c) 60 bps (ca. 20.4 nm). In addition, statistics of control samples with d) phosphorothioate modified DNA (80 bps spacing) without bifunctional fastener treatment and e) non-phosphorothioate modified DNA immobilized on the surface are shown as controls. The average of the bars in both control samples was calculated and anything below this value was treated as background (dark region).

phosphorothioate-modified, see Supporting Information, Table S2). From the SEM images (Figure 2b and c) and population histogram analyses (Figure 3b and c) it appears that the dominant distances between the AuNP dimers match those predicted by the phosphorothioate modifications. In contrast, control surfaces immobilized using dsDNA with phosphorothioate modifications but without bifunctional fastener (2PS-dsDNA(80)-nofastener; Figure 2d) or without phosphorothioate modification (0PS-dsDNA; Figure 2e) had sparse AuNP distributions which are mainly due to non-specific binding of AuNPs to the gold surface.[12] Population histogram analyses of both control surfaces indicated no preference in the distance between AuNPs (Figure 3d and e).

In conclusion, a novel method has been developed using phosphorothioate DNA coupled with a short bifunctional fastener (BF) to precisely control both the specific location of and distance between AuNPs. This method has been demonstrated, both in solution through AuNP aggregation and disassembly based on PS-DNA hybridization and denaturation, and on surfaces by SEM imaging and aided by statistical analyses. The phosphorothioate DNA anchor and short bifunctional fastener make it possible to specifically functionalize DNA nanostructures (1D, 2D, and 3D) with various nanoscale materials while maintaining structural integrity and stability.

Experimental Section

BIDBE was synthesized by following the literature[8] procedure and was dissolved in DMF to prepare a 100-mM BIDBE solution. To couple DNA containing one phosphorothioate modification with BIDBE, DNA (1 mM, 10 μL), BIDBE solution (100 mM, 20 μL), and phosphate buffer (10 mM, 36 μL, pH 7.0) were mixed and heated at 50 °C for 5–6 h. The reaction yield depends heavily on the ratio between BIDBE and phosphorothioate. If the number of phosphorothioate modifications on DNA increases from 1 to *n*, the concentration of DNA should be lowered *n*-fold accordingly. The optimum ratio between BIDBE and phosphorothioate is 200:1. After reaction, excess BIDBE molecules and DMF were removed by a gel-filtration column.

Further experimental details of materials, AuNP aggregation and disassembly experiments, AuNP dimer formation on gold surfaces, SEM imaging, and image processing for histogram data collection are provided in the Supporting Information.

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