

FOCUS REVIEW

DNA nanostructures as scaffolds for metal nanoparticles

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The rapid development of DNA nanotechnology has enabled the precise bottom-up integration of metal nanoparticles (NPs) on a nanometer scale. The recent introduction of the DNA origami technique has facilitated even more complicated control of the positioning of not only 2 dimensional (D) but also 3D structures. The basic idea and recent distinctive examples of such metal NP arrays on DNA nanostructure are briefly reviewed.

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INTRODUCTION

Although top-down fabrications, such as photolithography and etching, have long driven advances in nanotechnology, they are now approaching their physical limits. Bottom-up fabrication techniques are expected to break through these limitations. However, these approaches cannot yet replace top-down techniques. Colloidal nanocrystals, including metals and semiconductors, exhibit distinctive properties not observed in the bulk state. Therefore, they are of particular interest for nanotechnology and nanoscience. For example, gold nanoparticles (AuNPs) exhibit unique optical and electrical properties, such as surface plasmon resonance.^{1,2} On the basis of their distinctive properties, metal NPs have been applied for biological sensing, diagnosis and other applications.^{3,4} Moreover, as new properties not observed in the distributed state are expected to emerge in the organized states,⁵ the precise arrangement of metal NPs in 1 dimensional (D) to 3D is one of the important technical objectives of bottom-up nanotechnology.^{6,7}

On the other hand, DNA is the ideal building block for the construction of self-organized nanostructures. A strand of DNA can specifically bind with its complementary counter strand by Watson–Crick base pairing to form a B-type double helix, which is most common for a pair of complementary DNA in nature and almost independent of their sequences. This fact is highly convenient for fabricating universal nanostructures, as each double helix consisting of various sequences of DNA has the same diameter and pitch. On the basis of this fact and other advantages, such as sequence programmability, reversible double helix formation and strand exchange, availability by automatic synthesis, the relative rigidity of the double helix, enzymatic scission and ligation ability, and easy amplification by a PCR, many DNA-based approaches to construct highly ordered or well-designed nanostructures have been reported.^{8–10}

Whereas DNA nanostructures prepared on the basis of the conventional ‘programmed self-assembly’ strategy have provided rather large and periodical patterns, smaller but more discrete and precisely controlled nanostructures can be prepared using the ‘DNA origami’ assembly technique (Figure 1). DNA origami is the latest technology in DNA nanotechnology, and was first reported by Rothemund¹¹ using atomic force microscopy (AFM) images of nanoscale geometric shapes as well as more complicated smiley faces and world map hemispheres. The key to this technique is the use of the commercially available M13mp18 phage genome, which is a 7249 nucleotide-long, circular and single-stranded DNA, as the scaffold for the assembly. With the aid of many appropriately designed short helper DNA strands (called ‘staple strands’), the phage genome is artistically, but entirely spontaneously, folded into a predetermined nanostructure, running back and forth to shape a raft-like plane with DNA helices. Since its introduction, this technique has been extensively studied, and the use of DNA origami has widened markedly. At present, DNA origami can create not only arbitrary 2D nanostructures but also 3D nanostructures, such as hollow polyhedrons or even more complicated nano-objects. More details on the design philosophy can be found in previous reviews.^{12–15}

Using DNA self-assemblies, including DNA origami as scaffolds, precisely controlled and well-organized metal NP arrays in 1D to 3D can be constructed. In this short review, recent progress in metal NP arrays based on DNA nanostructures, especially DNA origami, are introduced as new bottom-up type nanomaterials. These biomolecule–metal hybrid materials possess great potential for application in nanomachines, biological sensors and optical devices, as well as for electron-conductive nano-wire networks, in the next-generation of nanoscale functional materials.

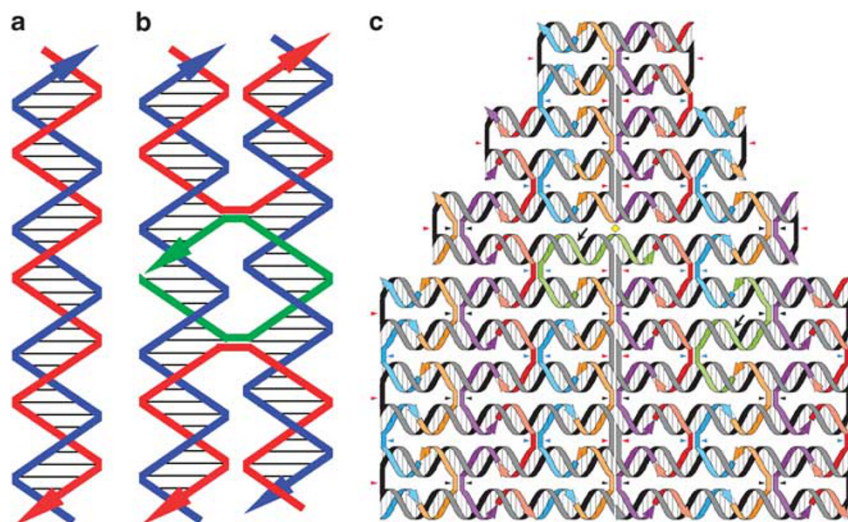


Figure 1 Schematic illustrations of the basic motifs used in structural DNA nanotechnology. (a) DNA duplex. (b) Double-crossover. (c) DNA origami. The triangles in (c) indicate the crossover points of the DNA strands. Figure (c) was reproduced with permission from the publisher.

METAL NP ARRAYS USING DNA SELF-ASSEMBLIES

As mentioned above, many examples of the construction of metal NP arrays using DNA assemblies as scaffolds are known (Figure 2).^{16–26} Such DNA-based metal NP assemblies were also introduced in recent reviews.^{10,16} 2D periodic AuNP arrays were prepared using DNA nano-tiles as scaffolds. In 2005, Pinto *et al.*¹⁷ succeeded in preparing linear arrays of AuNPs that had different sizes with periodic intervals on 2D DNA tiles based on double-crossover structures (Figure 2a). In this approach, two types of DNA strands with different sequences were introduced onto AuNPs of different sizes, and the complementary hybridization with the DNA introduced onto the tiles enabled the periodic arrangement of the AuNPs. However, the number of DNA strands introduced onto the AuNPs was not controlled. In 2006, Sharma *et al.*¹⁸ reported on 2D periodic AuNP arrays on a lattice-like DNA nanostructures based on a 4-way (Holliday) junction (Figure 2b). In this work, an AuNP attaching only to one DNA strand was purified and used. However, Lo *et al.*¹⁹ reported the size-specific loading of AuNPs into triangular tube-like DNA assemblies (Figure 2c). They also reported that the on-demand release of AuNPs entrapped in the DNA nanotube was possible using an addition of complementary DNA, which can partially unfasten the nanotubes.

Recently, the creation of small 3D polyhedron-like units consisting of DNA and metal NPs was reported. Mastroianni *et al.*²⁰ reported on the construction of a tetrahedron-like nanostructure with AuNPs connected to DNA, in which four AuNPs were located at the vertices of the tetrahedron (Figure 2d). These researchers also reported that chiral pyramidal structures were produced using four types of AuNPs of different sizes. Similar chiral AuNP assemblies have been independently prepared by Chen *et al.*²¹ Kim *et al.*²² also reported on pyramidal or octahedral structures created with AuNPs and DNA, in which the AuNPs were located at the vertices of the polyhedrons. These researchers called the method ‘aqueous-phase anisotropic sequential ligand replacement strategy’, and it could attach DNA strands up to six times at right angles to each other on an AuNP (Figure 2e).

In these approaches, to precisely prepare the designed structures with high specificity and efficiency, it is important to prepare the AuNPs to attach to a defined number of DNA strands using the

geometrically controlled relative positions on the AuNPs. The refinement of methods to introduce DNA via thiol–Au bonds have also been investigated to control the numbers and geometries of DNA strands on AuNPs.^{23,24} The purification of AuNPs that are attached to a defined number of DNA strands is usually performed using gel electrophoresis. However, to achieve effective separation by the number of DNA strands attached, a relatively longer DNA strand is required. We recently reported on a convenient method to purify AuNPs attached to a defined number of relatively short oligo-DNA strands using a non-covalent chain extension method and demonstrated the formation of simple AuNP assemblies (Figure 2f).²⁵ Moreover, we constructed linear AuNP arrays using AuNPs attached to two DNA strands at diametrically opposed positions on the AuNPs using polar singularities produced by self-assembled monolayer formation on AuNPs (Figure 2g).²⁶

ATTACHMENT OF METAL NPs TO PLANAR DNA ORIGAMI ASSEMBLIES

From the early stage of its studies, DNA origami has been considered a promising platform for the precise arraying of molecules and NPs. Theoretically, DNA origami can attain 3.5 Å resolution using the nucleotides in the scaffold, which are distributed all over the origami structure (the practical resolution for differentiating the surface of the DNA origami is ~6 nm). In addition, extensive studies on DNA chemistry have resulted in the development of various techniques to chemically modify DNA oligomers, and almost no limitation exists in attaching functional molecules to DNA today. Such modified DNA can be readily attached to DNA origami structures via hybridization to a receptor portion connected to a staple strand, or more directly, modified DNA can be used as a staple strand for the folding of the scaffold.

Rinker *et al.*²⁷ and Ke *et al.*²⁸ have reported on various nanoarrays formed on DNA origami scaffolds such as those of protein and mRNA. Selective positioning of AuNPs on a DNA origami scaffold was also accomplished within a short period (Figure 3a).²⁹ First, 1:1 conjugates of AuNP and DNA with a divalent thiolate–Au linkage were prepared in a 1:1 mixture of lipoic acid-modified DNA and bis-(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium

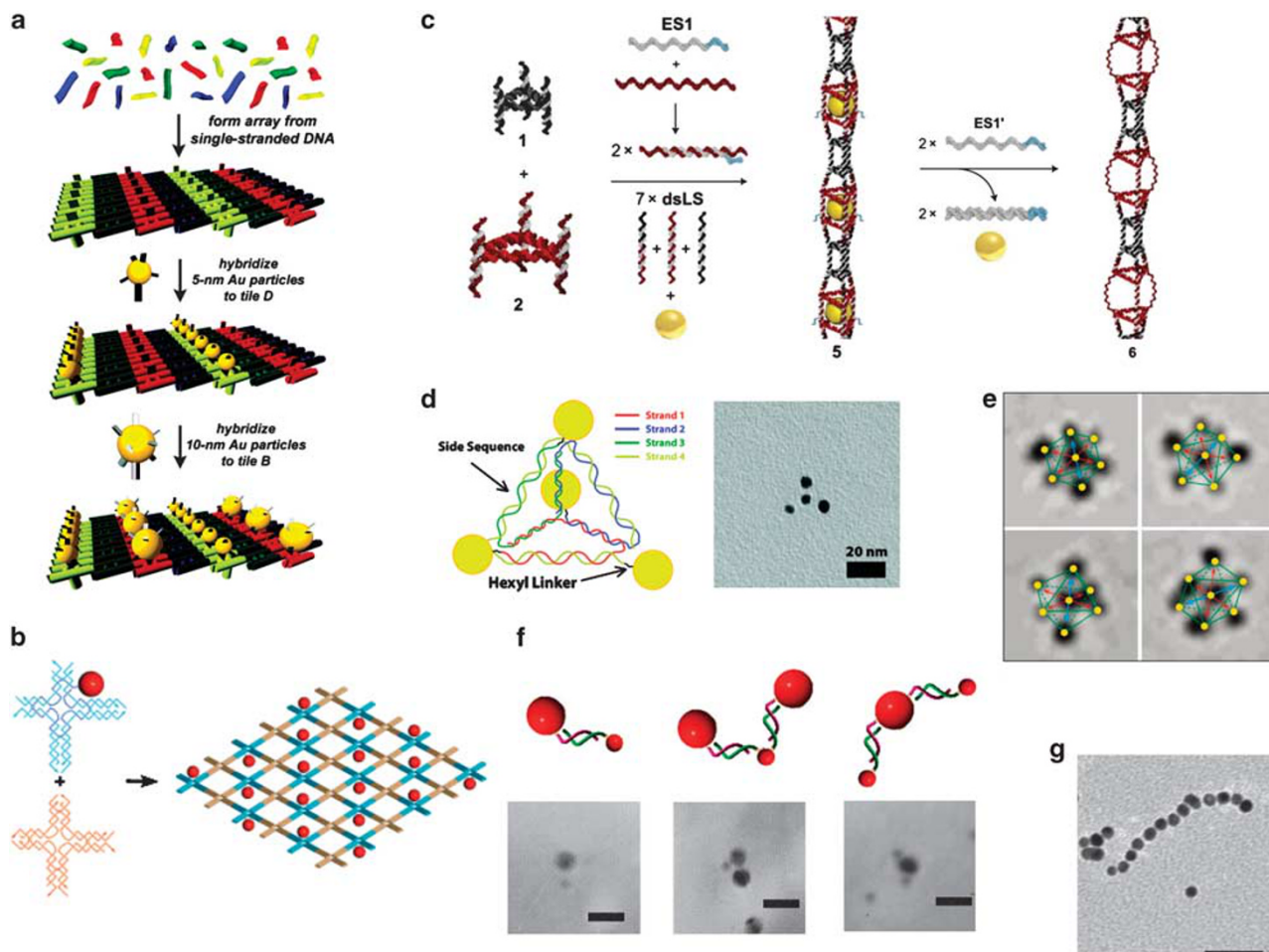


Figure 2 AuNP arrangements using DNA assemblies. (a) 2D AuNP assembly using DNA tiles based on double-crossover components.¹⁷ (b) Schematic representations of the lattice-like DNA-templated assembly of periodic AuNP nanoarrays.¹⁸ (c) Load and release of AuNP into and from triangular DNA nanotubes.¹⁹ (d) Schematic and transmission electron microscopy (TEM) image of DNA-nanocrystal pyramids.²⁰ (e) TEM images and the geometric interpretation of a 3D octahedral DNA-AuNP structure.²² (f) Schematic representation for large and small AuNP aggregates and TEM images using a purified AuNP attaching a defined number of DNAs. The scale bars are 20 nm.²⁵ (g) TEM images of linear AuNP assemblies using DNA/AuNP conjugates attaching DNAs at diametrically opposed positions. (a–f) Reproduced with permission from the publishers.

salt (BSPP)-coated 10 nm AuNPs. They were purified using agarose gel electrophoresis and were passivated using a layer of short oligonucleotides composed of five thymine residues modified with a monothiol group. This passivation enhanced the dispersibility of the AuNPs in salt-containing buffer solutions. The AuNP–DNA conjugate was used as one of the staple strands for a rectangular DNA origami structure. Using AFM imaging, the attached AuNP was clearly imaged on the resulting rectangular DNA origami. The yield of the AuNP attachments reached 91%, which was close to twice as high as the yield of the control origami structure using a monovalent AuNP–DNA conjugate. These researchers further examined the attachment of two AuNPs on an origami structure using another divalent AuNP–DNA conjugate in which the second AuNP was attached 47 nm from the first one. The yield of the dual attachment was also twice as high as that using monovalent conjugates.

In addition to AuNPs, Pal *et al.*³⁰ succeeded in assembling silver (Ag) NPs on a DNA origami structure (Figure 3b). The most significant advance of the study was the usage of multiple DNA strands conjugated to a particle, in contrast to the aforementioned

example using AuNPs with a predetermined number of DNA strands. AgNPs are more susceptible than AuNPs to oxidation and aggregation under high-salt conditions, which are essential for the preparation of DNA nanostructures. These researchers solved this problem by coating the AgNPs with multiple chimeric phosphorothioated DNA strands. The 9-mer phosphorothioate portion in the chimera binds to the surface of the 20-nm AgNPs, and the remaining 15-mer regular DNA segment provides binding affinity to the captured strands on a DNA origami structure. Three binding positions were prepared on a triangular DNA origami structure, and more than three captured strands were observed in a binding position with ~ 6 nm separation. The successful formation of a trimer AgNP array was clearly demonstrated using transmission electron microscopy imaging of the composites. The yield of the trimer was $\sim 62\%$. By combining the two strategies described thus far, a AuNP–AgNP heterodimer structure was also prepared. A 20-nm AgNP was attached to a triangular DNA origami structure bearing a 5-nm AuNP that was prepared as described in the previous paragraph. The center-to-center distance of the resulting heterodimer was estimated to be 13 nm using

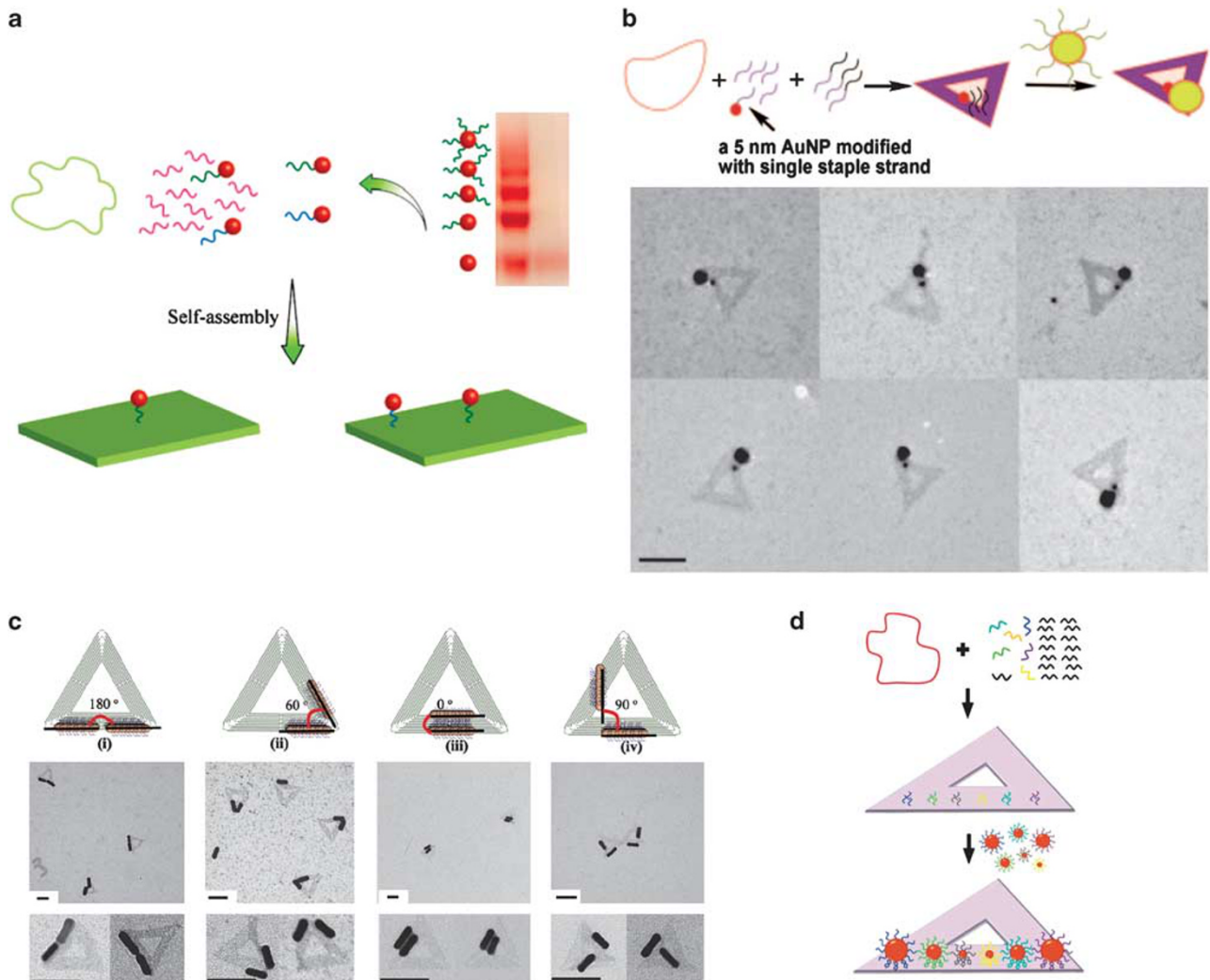


Figure 3 Planar DNA origami scaffolds modified with metal NPs (or NRs). (a) AuNP attachment to rectangular DNA origami.²⁹ (b) AuNP–AgNP heteroconjugation.³⁰ (c) AuNRs with predetermined orientation.³² (d) Bowtie-like alignment of AuNPs with different sizes.³³ The scale bars are 100 nm. These figures were reproduced with permission from the publishers.

the transmission electron microscopy images. Energy-dispersive X-ray spectroscopy confirmed the existence of both Ag and Au elements in the field.

These researchers also examined another approach to assemble fluorescent Ag nanoclusters on a triangular DNA origami structure.³¹ To nucleate Ag nanoclusters *in situ*, a maximum of 65 sugar-modified DNA strands were displayed on an arm of the triangular DNA origami structure using extended staple strands. AFM images of the triangles after the Tollens reaction demonstrated significant growth of the height of the arm bearing the sugar-modified DNA strands. Transmission electron microscopy imaging and energy-dispersive X-ray spectroscopy further confirmed the presence of Ag on the DNA scaffold.

Pal *et al.*³² also succeeded in assembling Au nanorods (AuNRs) together with AuNPs onto a DNA origami scaffold in a predetermined orientation (Figure 3c). AuNRs with multiple short (12 or 15 nucleotides) DNA strands were first prepared and then treated with a triangular DNA origami structure bearing five complementary strands per AuNR attached to the ends of select staple strands that were ~ 10.4 nm apart. The addition of two

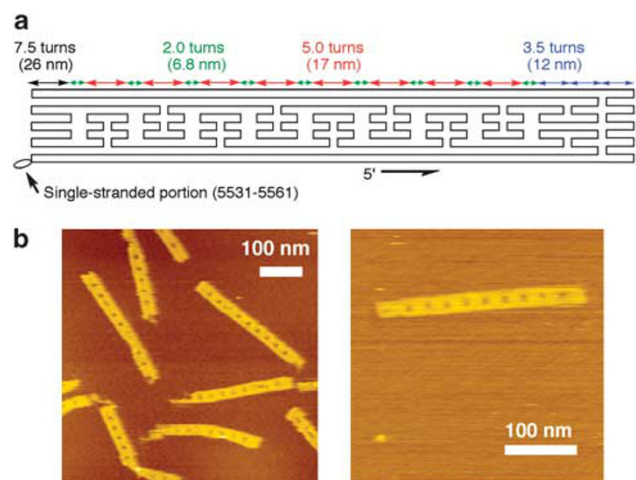


Figure 4 The folding pattern of the M13 scaffold in the punched DNA origami (a) and typical AFM images (b).³⁵ These figures were reproduced with permission from the publisher.

equivalents of AuNR, four cycles of slow annealing from 45 to 30 °C, and subsequent agarose gel purification produced fairly uniform AuNR-DNA origami composites with the anticipated orientation of the AuNR on the triangle. The researchers further proceeded to construct many discrete dimeric structures. The first AuNR was attached to one of the arms of the triangular DNA origami structure in a parallel orientation, and the second AuNR was immobilized on the arm to form either a relative angle of 180° (end-to-end AuNR organization), 0° (parallel organization), 90° or 60°. Each AuNR dimer exhibited individual resonance peak shifts in the extinction spectra that reasonably agreed with the predicted values.

Ding *et al.*³³ used a similar strategy to construct a linear chain of AuNPs with progressively decreasing sizes (Figure 3d). AuNPs of 15,

10 and 5 nm diameters were individually modified with DNA strands. Then, in total, 6 AuNPs (pairs of the 15, 10 and 5 nm AuNPs) were aligned into a bowtie-like arrangement on a triangular DNA origami sheet, creating three captured strands per attachment position. Scanning electron microscopy images clearly confirmed the desired alignment, and a 5-nm-plasmon band shift was observed between the DNA origami/AuNPs mixture before and after annealing.

DNA WELLS FOR DISCRETE METAL NP ARRAYS

In the aforementioned studies, all of the attachments of NPs to DNA origami structures were performed on the surface of the origami structure. We recently proposed a new strategy to assemble nanoarrays of individual NPs on DNA origami scaffolds.^{34–39} This strategy

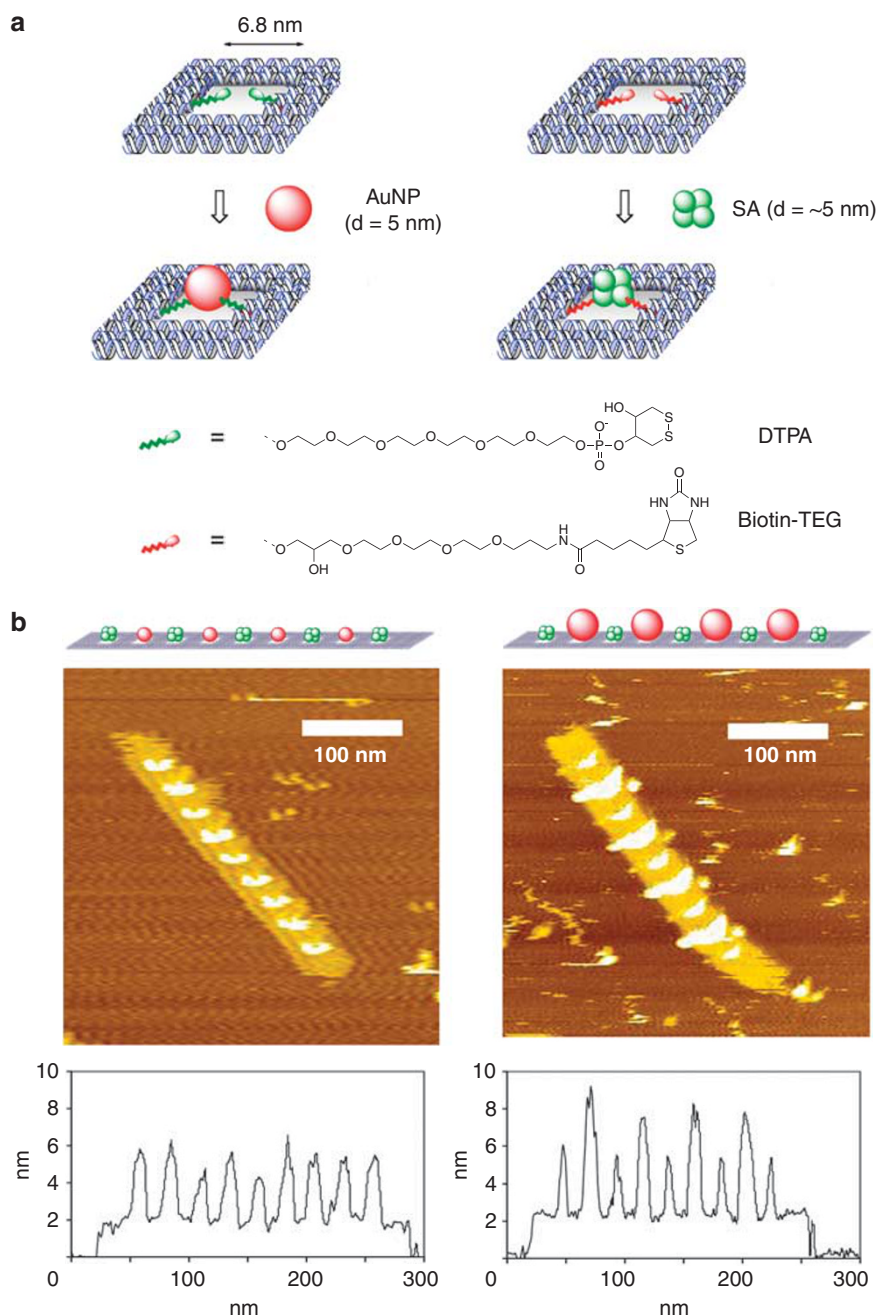


Figure 5 Arraying of NPs utilizing single-particle capture in DNA wells.³⁶ (a) Schematic illustration of the system. (b) Typical AFM image of alternating AuNP-SA heteroarray before (left) and after (right) Au enhancement. These figures were reproduced with permission from the publisher.

is based on our previous finding that a nm-sized cavity (a DNA well) embedded in 2-nm-thick DNA sheets, including DNA origami, can serve as a well to precisely capture one protein molecule inside and stably accommodate it under repetitive AFM scanning.^{34,35} This approach is applicable to other NPs of similar diameters, such as AuNPs.³⁶ We designed a stick-like punched DNA origami structure with nine wells with dimensions of $7 \times 14 \times 2$ nm (Figure 4). Two of the edges of each well were modified with a dithiol residue (dithiol phosphoramidite (DTPA)), which is known to form significantly stronger bonds with AuNPs than simple monothiol, similar to the lipoic acids used by Yan and Liu, via a 2.3-nm long triethylene glycol linker at the end of appropriate staple strands (the anchor strands; Figure 5a). When excess AuNPs are added to the solution of this punched origami structure, precisely one AuNP is captured in a well to produce an AuNP nanoarray with a 26-nm period. The size of the

well is crucial for single-particle capture. A well of twice the size often captures two particles inside. The AuNPs accommodated in the wells display tremendous stability under repetitive AFM scanning compared with those tethered on the origami surface (not in the wells) or those captured in the wells but attached by only one linker portion. This stability most likely occurs because of the bidentate binding of the AuNP in a well by both of the anchors, as is the case for protein nanoarrays.^{35,37} The site to capture AuNPs in the punched origami (*n*th well) can be freely selected simply by selecting the staple strand to be modified with DTPA. To the best of our knowledge, the present system is the first system that requires no pre-modification of the AuNPs with DNA but only the addition of unmodified AuNPs to the solution of punched DNA origami to pattern precisely one particle at a desired position. A simple system for nano-patterning of AuNPs has thus been established.

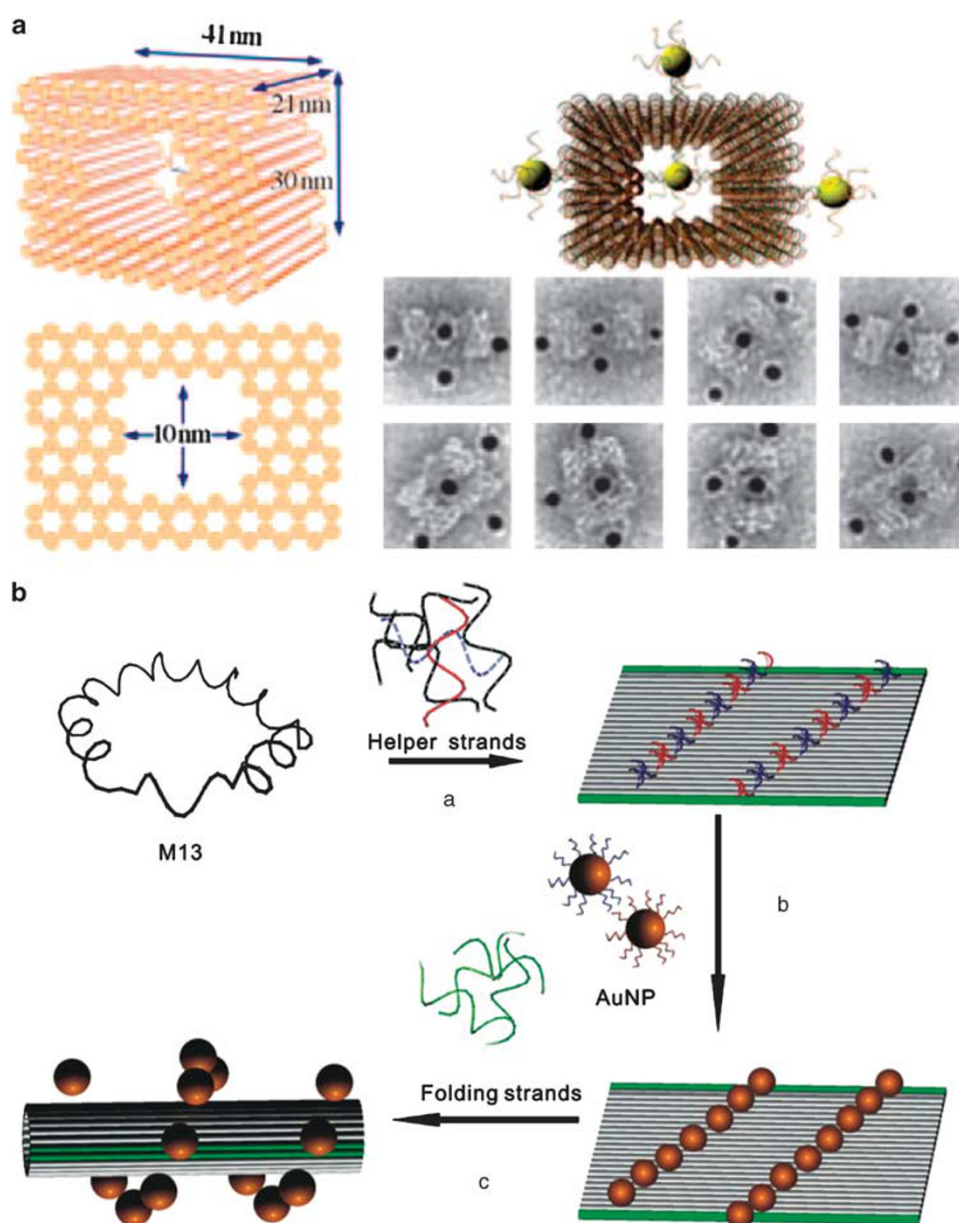


Figure 6 AuNPs attached to 3D DNA origami structures. (a) AuNPs on a DNA origami cage.⁴² (b) Helical AuNP array on a DNA origami tube.⁴⁴ These figures were reproduced with permission from the publishers.

To demonstrate the high addressability and chemoselectivity of the system, an alternating streptavidin (SA)/AuNP heteroarray with a 26-nm separation was created (Figure 5b). SA is a tetrameric protein with a 5-nm diameter that strongly binds to four biotins for each monomer and was our original target to capture in a well. Using four combinations of DTPA-modified anchor strands, the four even-numbered wells in the punched origami were selectively functionalized with dithiols. Similarly, the five odd-numbered wells in the origami were selectively biotinylated with the use of 5'-biotinylated anchor strands. The resulting punched origami with mixed functionalization was first incubated at 4 °C in the presence of excess AuNPs for 4 days. After the free AuNPs were removed from the solution using a gel-filtration microspin column, SA was added to the solution, and the resulting mixture was immediately imaged using AFM. All of the wells in the origami were filled with a particle of 5 nm diameter, although it was impossible to discriminate AuNPs from SA simply by shape analyses. We thus performed Au enhancement, which works similarly to Ag enhancement but deposits Au around the initial AuNPs. After the reaction was performed on a mica surface at room temperature for 3 h, the particles in the even-numbered wells were significantly and selectively enlarged up to 8 nm. The particles in the odd-numbered wells were intact even after the reaction, indicating that these particles are SA and that the AuNPs were only captured in the thiolated wells as expected.

As we have previously demonstrated using protein nanoarrays, the single-particle capture into a DNA well can be completely controlled in a reversible manner,³⁸ and any AuNP in nanoarrays can be independently placed and removed with the aid of a programmed strand displacement technique.⁴⁰ Owing to its strong binding ability, SA has been widely used to mediate the localization of enzymes in blot assays or enzyme immunoassays. A selection of SA-enzyme conjugates are thus commercially available today and have already been arrayed on DNA origami scaffolds with DNA wells.³⁹ SA/AuNPs heteroarrays have the potential to become useful tools for enzymatic studies with single-molecule resolution in the near future.

The similar attachment of AuNPs into cavities formed in a DNA origami sheet have been recently reported by Endo *et al.*⁴¹ These researchers prepared a square-shaped DNA origami structure with six 5 × 60 nm 'slits' separated by two DNA helices to align the AuNPs. One slit had five positions for AuNP attachment, and each of the positions was modified with two DTPA groups tethered to the DNA

helices on both sides of the slit. The addition of AuNPs to the DNA origami solution resulted in the formation of AuNP arrays of up to five particles either along a slit or across the slits. The yield of AuNP arrays along a slit was significantly lower than that across the slits, most likely because undesired multiple binding of the DTPA groups to a particle occurred between incompletely isolated attachment positions in a slit.

ATTACHMENT OF METAL NPs TO 3D DNA ORIGAMI ASSEMBLIES

Nanostructures created using the DNA origami technique are not limited to 2D sheets. Various fine 3D structures have also been produced. Zhao *et al.*⁴² recently constructed an interesting 3D DNA origami-AuNP composite (Figure 6a). A DNA origami cage with an outer dimension of 40 × 31 × 21 nm and a 10 × 10 × 21 nm inner cavity was prepared by applying the 'honeycomb lattice' design principle established by Douglas *et al.*⁴³ The inner cavity was used to accommodate a 5-, 10- or 15-nm AuNP covered with short DNA strands. The introduction of multiple capture strands, complementary to the strands on the AuNP, markedly enhanced the capture yield (that is, the inner cavity bearing three or four capture strands produced nearly a 100% yield). Because DNA nanostructures are intrinsically flexible, even a 15-nm AuNP, which has a much larger diameter than the theoretical size of the inner cavity, was successfully encapsulated in the DNA cage with a reasonably high yield (~68%). The attachment of additional AuNPs to the outer surfaces of the DNA cages was also successful. Up to four 5 nm AuNPs were integrated in a DNA cage. Three of these AuNPs were aligned using the inner cavity and the two opposite outer surfaces, and the other AuNP was attached to the outer surface between the former two.

In contrast to the previous example in which AuNPs were attached to pre-formed 3D nanostructures, Shen *et al.*⁴⁴ proposed a unique procedure to obtain AuNP arrays in a helical geometry (Figure 6b). A flat, rectangular DNA origami sheet (90 × 60 × 2 nm) was first decorated with 10 or 13 nm AuNPs. Up to 15 AuNPs were aligned in two oblique lines on the DNA origami sheet with a spacing of 16 nm. Next, the sheet was rolled up into a tube with the aid of additional DNA strands designed to staple the two long sides of the sheet, arranging the AuNPs into a helical geometry. The AuNP helices purified using agarose gel electrophoresis were clearly imaged using the transmission electron microscopy. In addition, the AuNP helices

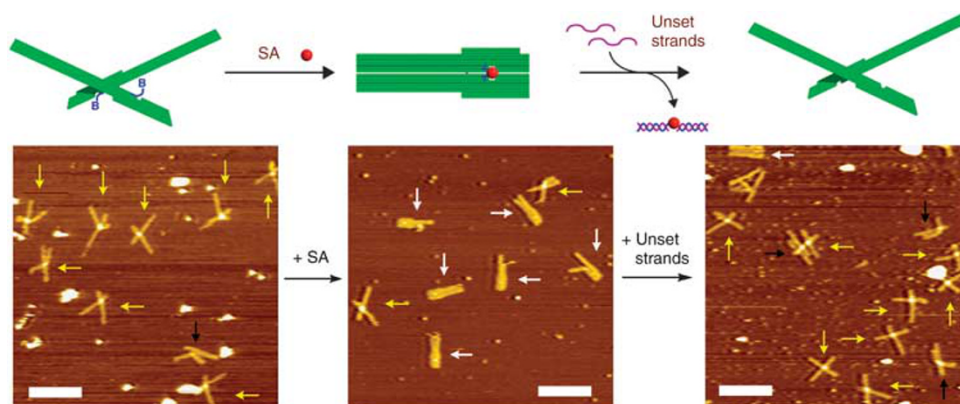


Figure 7 Nanomechanical DNA origami (DNA origami pliers) that pinch precisely one target molecule.⁴⁸ The two levers were modified with a biotin group. Such biotinylated DNA origami pliers markedly change their structure from an X-shaped open form (left) to a parallel closed form (center) by pinching an SA. Removal of the SA from the complex again opens the pliers (right). The scale bars are 300 nm. These figures were reproduced with permission from the publisher.

exhibited significant circular dichroism (CD) signals around the plasmonic resonance of the AuNPs (~525 nm), demonstrating the plasmonic chiral response of the AuNP helices.

CONCLUDING REMARKS AND OUTLOOK.

DNA self-assembly systems such as DNA origami are one of the most useful and versatile techniques to prepare precise arrangements of nano-objects such as molecules and NPs. In particular, to obtain non-symmetrical, non-periodical and well-designed nano-objects that have complex structures, DNA systems are the best building blocks. However, following an examination of the literature on metal NP-DNA composites,¹⁶ simple strategies for the construction of metal NP nanoarrays or the conjugation of NPs to DNA nanostructures appear to be almost exhausted, and studies on their optical, photonic or electrical properties are becoming increasingly important. One unsolved but important problem is the requirement of divalent ions (that is, the 10 mM Mg²⁺ or even higher concentration of monovalent ions) to preserve these DNA nanostructures, which is indeed an unfavorable condition for metal NP dispersion. A breakthrough in the method for the preparation of DNA nanostructures is desired.

The research field of DNA origami is in a similar situation. The recent trend in the field is to combine DNA origami with the dynamic motion of DNA machines, such as the use of a programmable nanoscale assembly line on a DNA origami stage that was demonstrated by Gu *et al.*,⁴⁵ a molecular spider (SA-enzymatic DNA conjugate) or DNA walker that walks along a track prepared on a DNA origami sheet.^{46,47} Recently, we have constructed nanomechanical DNA origami devices, DNA origami pliers and DNA origami forceps, which consisted of two levers connected at a fulcrum (Figure 7).⁴⁸ These nanomechanical origami devices experience a marked shape transition from an X-shaped open form to a parallel closed form (or the reverse) by interacting with various specific chemical/biochemical targets. Attaching multiple AuNPs to such nanomachines may permit the dynamic control of plasmonic resonance between metal NPs/NRs. Metal-biomolecule hybrid systems using a combination of DNA nanostructures with inorganic nano-objects are expected to have great potential to create new science and technology for not only static (stable) but also dynamic materials.

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