Nanoparticle Arrays

DNA-Encoded Self-Assembly of Gold Nanoparticles into One-Dimensional Arrays**

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DNA-templated material assemblies usually fall into two categories: nonspecific electrostatic (or intercalative)[1] and sequence-specific assemblies.[2–5] The latter take advantage of the Watson–Crick base pairing and can assemble materials into well-defined structures by DNA-based molecular recognition. This approach is promising in controlling both the complexity and regularity of nanostructures. In previous work this approach was used to build small assemblies of gold nanoparticles (AuNPs).[3] The basic scheme is to hybridize mono-DNA-functionalized AuNPs with template-DNA strands to form the desired structures. It is conceivable to integrate this strategy with more extended and complicated DNA nanostructures[2,6] for nanoparticle assembly. One immediate challenge is to build large arrays by DNA-encoded self-assembly. Herein, we report the DNA-encoded self-assembly of AuNPs into well-extended micrometers-long one-dimensional (1D) arrays. The resulting linear structures could potentially link the nanometric properties of materials with the convenience of micrometric maneuverability.

Rolling-circle DNA polymerization is well-suited for the preparation of long, repetitive DNA single strands as templates for nanoparticle assembly. During the polymerization, a DNA polymerase uses a short (less than 100 bases long), circularized, single DNA strand as a template to synthesize long (more than 10000 bases), linear, tandemly repetitive single DNA strands under isothermal conditions.[7] This technique has found important applications in gene detection.[7c] Here, we use the products of rolling-circle polymerization to assemble AuNPs into extended 1D arrays up to 4 μm long.

Figure 1 illustrates our strategy to assemble micrometers-long AuNP arrays: 1) functionalization of 5-nm AuNPs with DNA 1 (with a 5′ thiol group) to form a 1:1 AuNP/DNA 1 conjugate (AuDNA 1); 2) rolling-circle polymerization[7] to synthesize template DNA ($\mathbf{T}_{long}$) with a large number of repeats complementary to DNA 1; and 3) hybridization of AuDNA 1 with $\mathbf{T}_{long}$ to form micrometers-long AuNP assemblies.

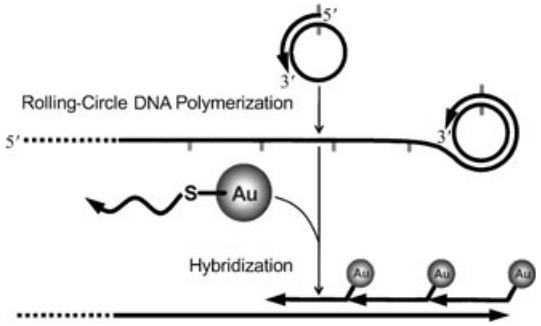

Figure 1. Synthesis of an extended gold nanoparticle array by combining DNA-encoded self-assembly and rolling-circle polymerization of DNA.

The reported methods[3] for synthesizing AuDNA 1 were adopted. The 53-base-long thiolated DNA 1 was first incubated with phosphine-capped 5-nm AuNPs in buffer 1 (see the Experimental Section) for over 3 h. Then, the AuDNA 1 conjugates were isolated by 3 % agarose gel electrophoresis in 10–20 % yields. Dark-red bands in the agarose gel corresponding to AuDNA 1 were easily determined (see the Supporting Information) by comparing the gel patterns to a pure AuNP band or by observing the evolution of the gel patterns while gradually increasing the amount of thiolated DNA 1 in the AuNP solution.

Before the assembly of micrometers-long AuNP arrays, we tested this scheme in the formation of smaller structures containing three to six AuNPs. Template-DNA strands ($\mathbf{T}_n$, $n = 3$–6, where n denotes the number of repeats in the template strands and each repeat is complementary to DNA 1) were prepared by ligation and purified by polyacrylamide gel electrophoresis (PAGE; 6 %, denaturing conditions). AuDNA 1 and the corresponding template strand were

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then mixed together in buffer 1 at different ratios, and an AuDNA 1/$\mathbf{T}_n$ molar ratio of 1:0.5 (in terms of the repeats) was finally chosen to produce the desired AuNP assemblies, since the excess of AuDNA 1 could compensate its slightly decreased hybridization ability. The AuNP assemblies were isolated by gel electrophoresis (3% agarose), eluted into buffer 2, and analyzed by transmission electron microscopy (TEM). The TEM images and statistical analyses in Figure 2 and Table 1 clearly show the formation of the desired structures in high yields.

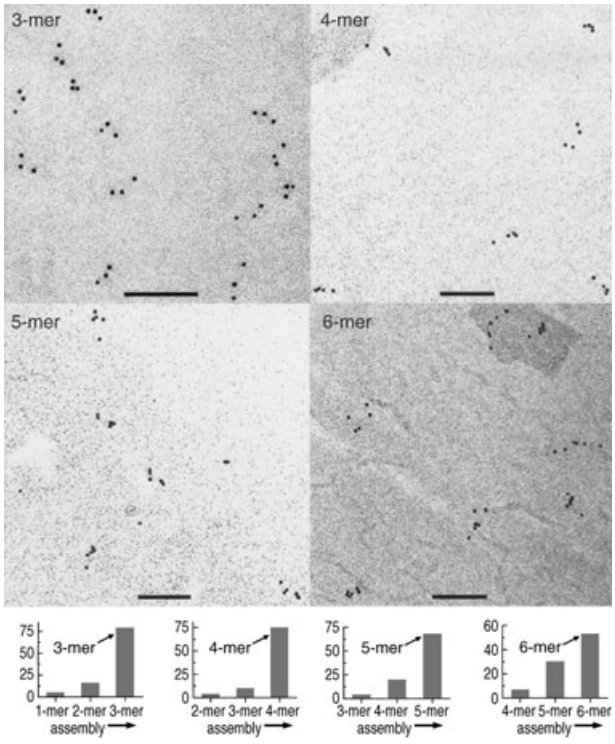

Figure 2. TEM images and statistical column charts showing the highly efficient assembly of gold nanoparticles into 3-, 4-, 5-, and 6-mers; all scale bars correspond to 100 nm.

Table 1: Typical yields for the AuNP assemblies from 3- to 6-mer obtained by TEM image analysis.[a]

	3-mer	4-mer	5-mer	6-mer
Counts of AuNPs [N_T]	619	345	466	660
Yield [%]	79	75	67.5	53

[a] N_T is the total number of observed AuNPs in the TEM images. The yield is defined as N_A/N_T, where N_A is the total number of AuNPs within the desired assembly. Particles located close to the edges of the TEM images were excluded.

The interparticle spacings of the AuNP assemblies were analyzed from the TEM images. To avoid the confusion in finding the correct particle pairs, only assemblies with easily distinguishable linear structures were chosen for the measurements. The results showed a center-to-center interparticle distance of (18.5 ± 6.9) nm (see the Supporting Information), consistent with the theoretical value of 18.0 nm for a 53-base-long DNA duplex. The observed interparticle distances had quite a wide distribution, which ranged from 6.1 to 33.9 nm. We attributed the spacing variation to the strong particle–particle and particle–substrate affinities, the bending, stretching, or partial unzipping of the DNA duplex during sample preparation, the flexibility of the linkage part between DNA and the AuNPs, and the 5-nm diameter of the AuNPs.[3b]

The synthesis of micrometers-long DNA template ($\mathbf{T}_{long}$) is key to the formation of extended AuNP arrays but is usually difficult. We solved this issue by rolling-circle DNA synthesis,[7] which used a circularized DNA 1 as the template, a 12-base-long DNA 2 as the primer, and a Phi29 DNA polymerase. The polymerization product ($\mathbf{T}_{long}$) contains many copies of the DNA 1 complement. $\mathbf{T}_{long}$ migrated very slowly in 1% agarose gel (Figure 3a) and is at least thousands

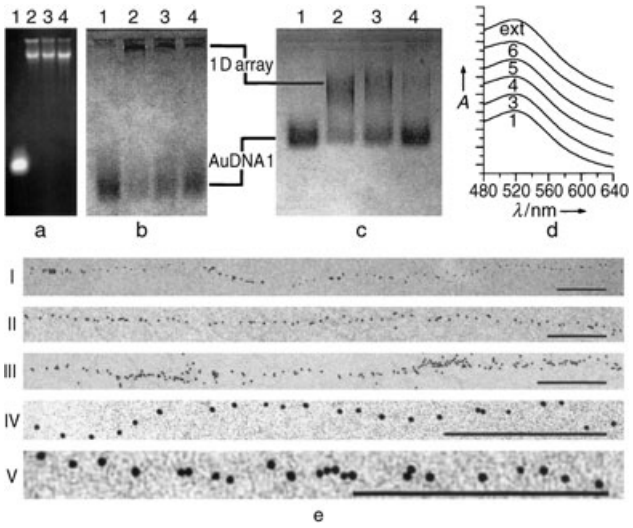

Figure 3. a) Agarose gel (1%) electrophoresis for the rolling-circle-synthesized products; lane 1: 53-base cDNA1, lanes 2–4: polymerized products obtained in different synthesis batches, which indicate good consistency. b, c) Agarose gel (b: 3%, c: 0.5%) electrophoresis of the extended 1D AuNP assembly; lane 1: AuDNA1, lanes 2–4: products obtained in 1:1, 1:0.5, and 1:0.25 ratios. d) Optical absorbance of the 1- (AuDNA1), 3-, 4-, 5-, and 6-mers and the extended (ext) 1D structure. e) TEM images of the extended 1D structures; (IV) and (V) are close-up views of part of the wires in (I) and (II); all scale bars are equivalent to 200 nm.

of bases long, with reference to a 100-base-pair DNA ladder (see the Supporting Information). The high-molecular-weight fraction was sliced from the gel, electrically eluted, precipitated from ethanol, and redispersed in doubly distilled H_2O (ddH_2O). The extended 1D arrays were prepared by combining AuDNA 1 and $\mathbf{T}_{long}$ at different ratios and annealing from 95 to 4 °C in buffer 1. The resulting aggregates were too large to migrate into a 3% agarose gel (Figure 3b), but they ran well in a 0.5% gel (Figure 3c). The aggregates at a ratio of 1:0.5 (AuDNA 1:repeat of $\mathbf{T}_{long}$) migrated slightly slower than those at a ratio of 1:1, which indicates that there were some unpaired segments on the template and thus an excess of

AuDNA 1 could improve the DNA hybridization. The aggregates at a ratio of 1:0.25 migrated the same as those at a ratio of 1:0.5, which implies that the template was saturated and thus incorporation of more particles was impossible. A ratio of 1:0.5 was chosen for the synthesis of the micrometers-long AuNP arrays.

Large-area atomic force microscopy (AFM), TEM, and field-emission scanning electron microscopy (FESEM) images showed 1D arrays of AuNPs up to 4 μm long aligned in a parallel fashion on TEM grids and silicon-wafer substrates (see the Supporting Information). The AuNP arrays could reach several micrometers in length, so it is reasonable to assume that the fluidic flow created during removal of the solution droplet could cause the long assemblies to be stretched and aligned. As shown in Figure 3e, the AuNPs were either well-separated within the appropriately stretched parts of the wires or entangled and coalesced on other parts. The visible absorbance spectra of the extended arrays and the 3- to 6-mers (Figure 3d) show no significant differences with that of AuDNA 1, in agreement with the large interparticle distances expected for a 53-base-pair DNA spacer.

In conclusion, we have successfully prepared extended 1D arrays of gold nanoparticles by combining rolling-circle polymerization and DNA-encoded self-assembly. The arrays obtained can dissolve in water and form a stable homogeneous solution, which enables subsequent modifications and easy manipulation. These results are an important step forward in obtaining extended nanoparticle arrays in a precise and controllable manner, which may help build structures with versatile functionalities. One of our further research goals is to design and fabricate more rigid and hierarchically complicated structures extending to one, two, or even three dimensions and to explore any potentially emerging physical properties.

Experimental Section

Buffer solutions: TBE buffer: tris(hydroxymethyl)aminomethane (Tris, 89 mM), ethylenediaminetetraacetate (EDTA, 2 mM), and boric acid (89 mM), pH 8.0. Buffer 1: 1:1 TBE + sodium chloride (0.2 M). Buffer 2: 1:1 TBE + sodium acetate (NaAc, 0.1 M). NEB Phi29 polymerase reaction buffer: Tris-HCl (50 mM), magnesium chloride (10 mM), ammonium sulfate (10 mM), and dithiothreitol (DTT, 4 mM).

Synthesis of $\mathbf{T}_n$ ($n = 3$–6): For the preparation of DNA templates $\mathbf{T}_n$, cDNA 1, a DNA strand complementary to DNA 1, was designed to leave four-base sticky ends at both sides of the duplex with DNA 1 that allow the formation of a ladder of duplexes. The corresponding templates were obtained by ligating cDNA 1 in the above product mixtures with a T4 DNA ligase (New England Biolabs, Inc., NEB). The 3- to 6-mers of cDNA 1 were then purified from a 6% denaturing polyacrylamide gel. DNA 1: 5′GAGTAGACCGTGCATCATGGACTAACCAGTGACCGCATCGGACAGCAGCCTGA3′; cDNA 1: 5′ACTCTCAGGCTGCTGTCCGATGCGGTCACTGGTTAGTCCATGATGCACGGTCT3′.

Rolling-circle synthesis of $\mathbf{T}_{long}$: Rolling-circle polymerization was conducted in solution (20 μL) at 37 °C for 90 min. The reaction was stopped by heating at 65 °C for 10 min. The reaction mixture contained 53-base-long circularized DNA 1 (0.1 μg), 12-base primer DNA 2 (5′CCATGATGCACG3′; 0.023 μg), Phi29 DNA polymerase (New England Biolabs, Inc., NEB; 5–10 units), NEB Phi29 polymerase reaction buffer, dNTP mix (1 mM), and BSA (0.1 mg mL^{-1}). The polymerization product was then dialyzed against ddH_2O for 16 h to remove salts and the dNTP monomers. The dialyzed sample was loaded onto 1% agarose gel containing ethidium bromide (0.5 μg mL^{-1}). For electrophoresis, the running buffer was half as concentrated as the TBE buffer and is designated 0.5 × TBE. The major band of the product (see the Supporting Information) was sliced and sealed into a dialysis membrane tube filled with 0.5 × TBE buffer. The product was electrically eluted from the gel block, extracted with butanol, precipitated with ethanol, and finally dispersed in ddH_2O (100 μL). The purified product was quantitated by measuring its UV absorbance at 260 nm. Typically, 3–5 μg of the $\mathbf{T}_{long}$ template was obtained.

Phosphine-capped gold nanoparticles (AuNPs): AuNPs (Ted Pella, Redding, CA; 5 nm) were stabilized by complexation with bis(*para*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (salt 1, Strem Chemicals, Newburyport, MA). Typically, salt 1 (2 mg) was added to the AuNP solution (10 mL), and the mixture was shaken at 22 °C overnight. The AuNPs were then precipitated by adding solid sodium chloride until the color turned blue. After centrifugation, the supernatant was carefully removed with a pipette, and the AuNP precipitate was redispersed in ddH_2O to a concentration of 1–4 μmol, as estimated from the optical absorbance at 520 nm (1 OD equals 0.083 μmol for 5-nm AuNPs).

AuDNA 1: Thiolated DNA 1 and phosphine-protected AuNPs (1–4 μM) were mixed in buffer 1 at a molar ratio of 1:1 (estimated by optical absorbance). The mixture was incubated at 22 °C for over 3 h. The product containing AuNPs conjugated with different numbers of DNA 1 appeared as a series of dark-red bands in a 3% agarose gel with 0.5 × TBE as running buffer. The sample band corresponding to AuDNA 1 was sliced from the gel and sealed in a dialysis membrane tube prefilled with 0.5 × TBE buffer. AuDNA 1 was then electrically eluted into the dialysis tube, and the inner tubing buffer was carefully collected.

AuNP assemblies: Purified AuDNA 1 (0.3–0.8 μM based on the absorbance of AuNPs at 520 nm) was mixed with DNA templates at molar ratios (AuDNA 1:repeat of the template) of 1:1, 1:0.5, and 1:0.25. The mixtures were incubated in buffer 1: 95 °C (1 min), 65 °C (5 min), 50 °C (10 min), 37 °C (20 min), 22 °C (20 min), and 4 °C (20 min). The products were separated by electrophoresis on 3 or 0.5% agarose gel with buffer 2 as the running buffer. The ratios corresponding to the highest yields of the desired products were chosen, and the expected product bands were electrically eluted into buffer 2 and kept at 4 °C.

Microscopic analyses: 1) The TEM sample was prepared by dropping sample solution (5 μL) onto a carbon-coated TEM grid (400 mesh, EMS Inc.). After 1 min, the solution was wicked from the edge of the grid with a piece of paper. TEM images were taken with a Philips CM-10 transmission electron microscope operated at 80 kV in the bright-field mode. 2) The sample on a silicon wafer for AFM and FESEM imaging was prepared by spotting sample solution (5 μL) onto the silicon substrate. The drop was blown off after 1 min by compressed air and the surface of the wafer was washed once with aqueous magnesium acetate solution (2 mM, 30 μL). The sample was first checked with a Nanoscope IIIa multimode atomic force microscope operated in the tapping mode in air, and then subjected to imaging with a Hitachi S-4800 FESEM operated at 1 kV.

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