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# DNA Bimodified Gold Nanoparticles

Tao Zhang,[†,‡] Yuanchen Dong,[‡] Yawei Sun,[‡] Ping Chen,[†] Yang Yang,[§] Chao Zhou,[‡,§] Lijin Xu,[*,†] Zhongqiang Yang,[*,‡] and Dongsheng Liu[*,‡]

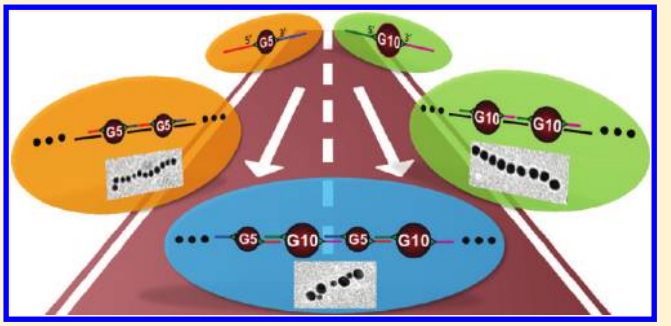
[†]Department of Chemistry, Renmin University of China, Beijing 100872, P. R. China

[‡]Key Laboratory of Organic Optoelectronics & Molecular Engineering of the Ministry of Education, Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China

[§]National Center for Nanoscience and Technology, Beijing 100190, P. R. China

Supporting Information

**ABSTRACT:** We report a general approach to bimodify gold nanoparticles (GNPs) with two different DNA strands via DNA template reaction. Two thioctic acid modified DNA strands, one at 5′ end and one at 3′ end, were attached to GNPs through bivalent thiol-gold bond. By sequence design, assemblies of 5 nm GNPs chains, 10 nm GNPs chains and alternative arrangement of 5 and 10 nm GNPs could be achieved. Gel electrophoresis, transmission electron microscope (TEM), UV−vis spectra were used to characterize the assemblies. It is believed that this new kind of bimodified GNPs with two different DNA strands at different ends would enrich the toolbox of DNA−GNP conjugates and provide diverse selectivity for further assembly.




## INTRODUCTION

Assemblies of nanoparticles have become increasingly important building blocks, offering well-defined geometric arrangement and collective properties for various applications.[1−3] Neighboring nanoparticles can affect the resonance frequency in a distance-dependent manner, and cause concomitant color changes, which have been widely employed to build up biosensors for detection and diagnosis.[4−6] Assembly of "nanoscale matter" features a bottom-up approach to miniaturize devices; meanwhile, the creation of assemblies of nanoparticles bridges nanoscopic objects and the macroscopic world to construct complex functional materials for nanoscience and technology.[7] Therefore, many efforts are underway to develop facile and efficient methods to organize nanoparticles into desirable structures.

Among the methods of assembling nanoparticles, DNA-directed assembly[8−11] is a promising strategy owing to its unique properties of DNA, such as programmability, molecular recognition, ease of synthesis and modification.[12,13] In 1996, Mirkin[14] and Alivisatos[15] independently reported a first example that DNA−gold nanoparticle (GNP) conjugates could serve as scaffolds for the construction of nanostructures through sequence-specific DNA hybridization. Following these pioneering works, a number of studies focused on using DNA as templates to form linear or ringlike GNP aggregates.[16−24] With the maturation of two and three-dimensional DNA nanostructure fabrication,[25,26] GNPs could be organized to specific positions of DNA architectures.[27,28] Most of the assemblies employed DNA monomodified GNP conjugates, i.e., only one single-stranded DNA (ssDNA) was attached to one GNP.

Along with the formation of monomodified GNP conjugates, a series of discrete modified GNPs containing various numbers of DNA strands were also generated, and the desired DNA−GNP conjugates needed further separation through agarose gel electrophoresis or high-performance liquid chromatography (HPLC). There have been a few reports on the preparation of bimodified GNPs,[29−31] however, their extended assemblies were rarely studied. It was reported that two DNA strands were generally attached at the same ends,[29−31] i.e., either both 5′ ends or both 3′ ends. Since DNA duplex runs in antiparallel direction, one DNA strand orients from the 5′ to 3′ end, and its complementary strand orients from the 3′ to 5′ end. Herein we described a new method to prepare bimodified GNPs with two different DNA strands at different ends, not only expanding diversity of building blocks but also providing more versatile assemblies, and finally studied their assembly behavior.

## EXPERIMENT

**Materials.** All DNA (including 5′ or 3′ amine-linker modified) were obtained from Invitrogen Corp. Bis(p-sulfonatophenyl)-phenylphosphine dehydrate dipotassium salt (BSPP), potassium carbonate anhydrous (99%), sodium chloride (NaCl, 99.5%),

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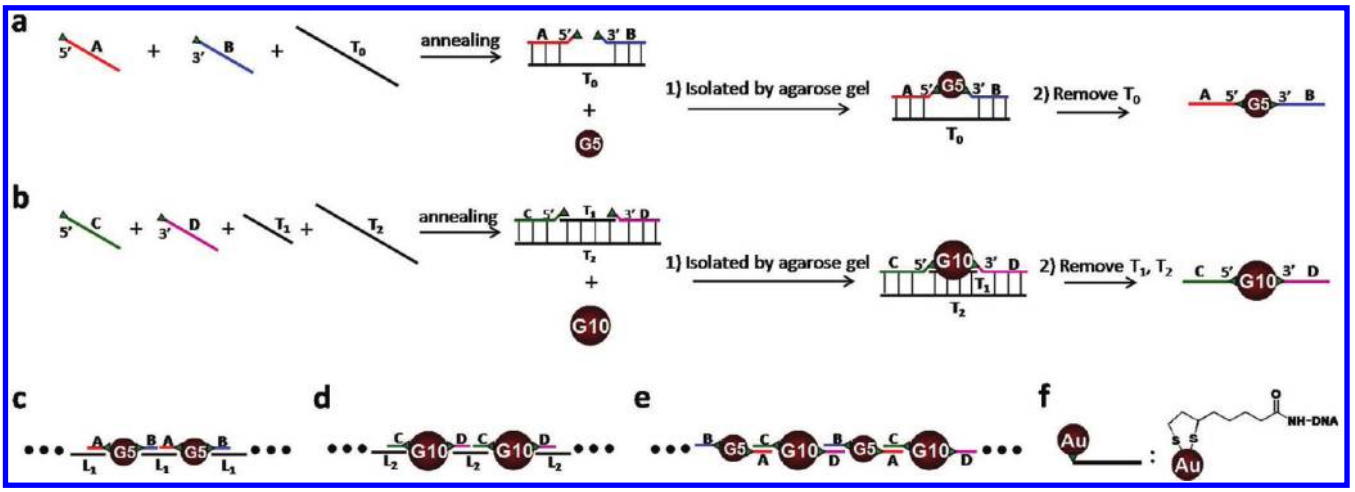
**Scheme 1. Scheme of the Preparation of DNA−GNP Conjugates via DNA Template Reaction**[a]




[a] (a) **A** and **B** bimodified **G5**. (b) **C** and **D** bimodified **G10**. (c−e) Schematic unit assembly of **G5**, **G10**, or both. (f) Scheme of thioctic linkage between DNA and GNPs. $\mathbf{ABT_0}$ and $\mathbf{T_1CDT_2}$ were constructed respectively by one pot reaction, annealing DNA strands in buffer (pH 8, 100 mM NaCl and 0.5×TBE at 95 °C, 5 min).

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 98%), 4-(dimethylamino)pyridine (DMAP, 99%) and *N*-hydroxysuccinimide (NHS, 98%) were obtained from Sigma. Thioctic acid was obtained from Alfa. Acetonitrile (ACN, HPLC grade, meets ACS specification, SK Chemical), acetic acid (99.8%, for biochemistry, Acros Organics), and triethylamine (99%, pure, Acros Organics) were used in HPLC. Water used in all experiments was ultrapure Milli-Q water (resistance >18 MΩ·cm).

**Template Design.** Our strategy to prepare DNA−GNP conjugates and their assembly principle is illustrated in Scheme 1. GNPs with a diameter of 5 nm[32] and 10 nm[33] were employed in this study, denoted as **G5** and **G10**, respectively. First, we sought to conjugate DNA with GNPs via DNA template reaction.[34] In past studies, DNA−GNP conjugates were generally synthesized from GNPs and ssDNA connected by a monovalent thiol−gold bond, which often leads to the collapse of DNA−GNP conjugates. Recent reports[27,32,35−37] have shown that thioctic acid-modified DNA could improve the conjugation efficiency due to the higher stability of the chelating bivalent thiol−gold bond. Herein we synthesized thioctic acid-modified DNA as described previously,[32] it contains three components: (i) a 3′- or 5′- thioctic functional group, depicted as a green triangle in the scheme; (ii) a 3T spacer to separate the main DNA strand and GNPs; (iii) the main DNA strand for hybridization.

According to the size of GNPs, different DNA templates of $\mathbf{ABT_0}$ and $\mathbf{T_1CDT_2}$, as shown in Scheme 1a,b, were designed for the modification of **G5** and **G10**, respectively. Note that two thioctic acid-modified DNA strands (one is modified at the 3′ end and another at the 5′ end) hybridize with a ssDNA to form a template. As DNA strands hybridize in the antiparallel direction, therefore, the two thioctic acid groups are placed head-to-head in the middle of the template. GNPs are attached to the template via covalent thioctic-gold linkage. Considering the subsequent linear assembly of DNA−GNP conjugates, a spacer equivalent to the size of the particle is designed locating between two thioctic acid groups, leading to the formation of DNA bimodified GNPs where the two DNA strands are attached to diametrically opposed positions on the GNP. In the case of **G5**, thioctic acid-modified DNA strands **A** and **B**, both contained 3T spacer closer to the thioctic groups and 15 bases, which hybridized with a ssDNA $\mathbf{T_0}$ containing 45 bases, thus a spacer containing 15 bases single strand (1 nm ≈ 3 bases)[38,39] on $\mathbf{T_0}$ was left between two thioctic groups. A double-stranded spacer was not used because half-integral helix twists of DNA (15 base pair ≈ 1.5 turns)[11,39] might separate two thioctic groups in the opposite direction. By contrast, in the case of **G10**, a single strand might be too long to resist its flexibility,[38] therefore, a length of 30 base pair duplex spacers (≈ 3 turns) was deliberately designed.

**DNA−GNP Conjugation.** In a typical experiment, two thioctic acid-modified DNA strands were mixed with corresponding template strands to form DNA duplexes; for example, modified DNA strands, **A** and **B**, were incubated with nonthiolated DNA strand, $\mathbf{T_0}$, in pH 8 buffer containing 100 mM NaCl and 0.5 × TBE, annealed (95 °C for 5 min) resulting in template, $\mathbf{ABT_0}$. Similarly, **C** and **D** were incubated with $\mathbf{T_1}$ and $\mathbf{T_2}$. It is noted that the nonthiolated DNA strand, $\mathbf{T_0}$, $\mathbf{T_1}$, and $\mathbf{T_2}$ not only bring thioctic acid-modified DNA strands in suitable distance for further conjugation with GNPs, but also lengthen the conjugating DNA strands to promote the separation of DNA−GNPs by agarose gel electrophoresis.[16,21] The obtained DNA templates $\mathbf{ABT_0}$ and $\mathbf{T_1CDT_2}$ directly conjugated with **G5** and **G10** in the same buffer (pH 8, 100 mM NaCl and 0.5 × TBE), respectively, followed by incubation at room temperature overnight. The obtained DNA duplexes then conjugated with GNPs via the formation of a bivalent thiol−gold bond (Scheme 1f).

**Purification Methods.** In order to achieve pure components, we explored 3% agarose gel electrophoresis to separate and characterize DNA bimodified conjugates. The agarose gels containing the desired bands were cut out and immersed in water. Most of DNA−GNP conjugates diffused out of the gel over 10 h. The subsequent removal of the nonthiolated DNA strands was achieved by incubating DNA−GNP conjugates in denatured buffer containing 100 mM NaCl, 0.5 × TBE, 3 M urea for 1 h. Then the mixture was centrifuged at 1.2 × $10^4$ rpm for >30 min, and the supernatant was carefully removed. The obtained DNA−GNPs conjugates without template strand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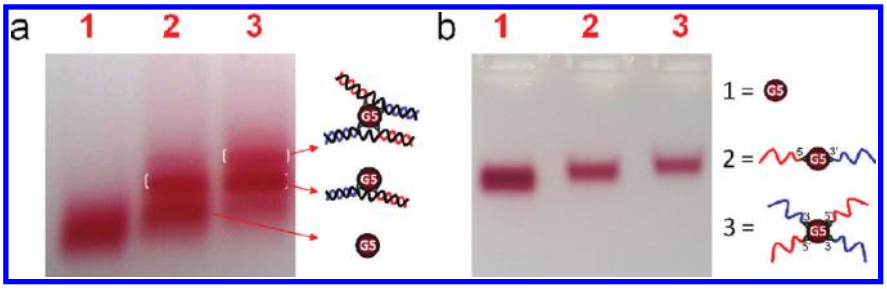


**Figure 1.** Agarose gel electrophoresis of **ABT$_0$-G5**. (a) Agarose gel electrophoresis of **ABT$_0$-G5** conjugates. The band with the red icon is the band with desired products. 3% agarose gel, 0.5 × TBE, 3.5 V/cm, 60 min, 4 °C. (b) Agarose gel isolation image of free **G5** and purified bimodified and tetra-modified **G5** after removal of its template by 3 M urea. 3% agarose gel, 0.5 × TBE, 3.5 V/cm, 18 min, 4 °C.

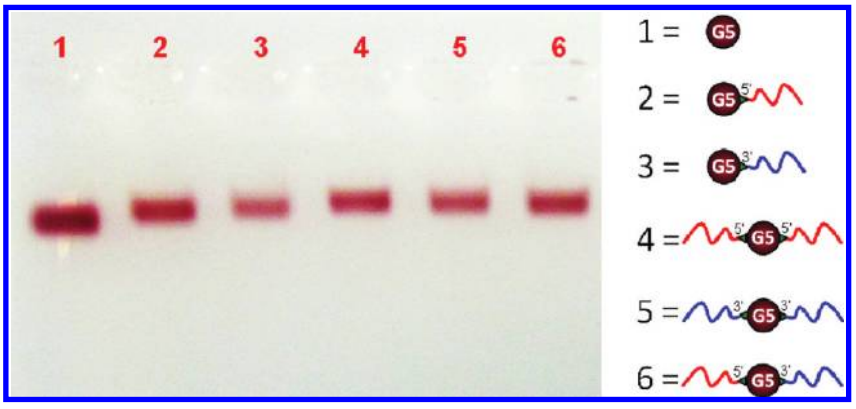


**Figure 2.** Agarose gel electrophoresis of different DNA numbers and strands of conjugated 5 nm GNPs. Lane 1, free **G5**; lane 2, **A-G5**; lane 3, **B-G5**; lane 4, **A$_2$-G5**; lane 5, **B$_2$-G5**; lane 6, **AB-G5**. 3% agarose gel, 0.5 × TBE, 3.5 V/cm, 20 min, 4 °C.

were rinsed in 100 mM NaCl and 0.5 × TBE and centrifuged, and repeated twice.

## RESULTS AND DISCUSSION

Figure 1a showed the agarose gel electrophoresis of conjugation of **G5** and **ABT$_0$**. The gel was visualized under white light as the red color of GNPs. Lane 1 corresponds to the free **G5**, lane 2 corresponds to the products when a molar ratio of **G5** to DNA was 1:0.5, and lane 3 corresponds to the products after increasing the ratio to 1:1. Each lane was loaded with a same amount of **G5**. In Figure 1a, comparing to lane 1, there were two additional discrete bands with reduced mobilities in lanes 2 and 3. The extra two bands in lane 3 are assigned to conjugates of **ABT$_0$-G5** and **(ABT$_0$)$_2$-G5**, respectively, due to their steadily decreased mobility caused by the increase of DNA. After removal of the DNA strands **T$_0$** by urea, a similar pattern of electrophoretic mobility of bare **G5** and the obtained two conjugates (**AB-G5** and **(AB)$_2$-G5**) could also be observed (Figure 1b).

In order to validate the ratio of DNA to GNPs in **AB-G5**, we constructed the DNA monoconjugated **G5** (**A-G5** and **B-G5**) and DNA bimodified **G5** (**A$_2$-G5** and **B$_2$-G5**) and compared their electrophoretic mobility with **AB-G5** on the same gel (Figure S1, Supporting Information). As shown in Figure 2, the mobility decreased in the order of free **G5** (lane 1), monoconjugated **G5** including **A-G5** (lane 2), **B-G5** (lane 3), and bimodified **G5** including **A$_2$-G5** (lane 4) **B$_2$-G5** (lane 5), and **AB-G5** (lane 6). It is notable that **AB-G5** displayed similar mobility to **A$_2$-G5** and **B$_2$-G5**, indicative of the presence of two DNA strands on **G5** in **AB-G5**.

Next we sought to determine the assembly behavior of such bimodified GNPs. We designed that the assembly of **AB-G5** and **CD-G10** was conducted by the aid of linker strand **L$_1$** and **L$_2$** (Scheme 1c−e). **CD-G10** were prepared by using the same strategy as that for **AB-G5** (see details in Figure S2). Linear chains of either **G5** or **G10** were observed in Figure 3a,b, respectively. Alternative arrangements between **AB-G5** and **CD-G10** through hybridization of **A** and **C**, **B** and **D** (Figure 3c) further supported our design of bimodified GNPs at different ends of DNA bonded to a gold surface. However, zoom-out transmission electron microscopy (TEM) images (Supporting Information Figures S3−S5) showed that self-assembled chains have a length of up to 15 nanoparticles. The limited number of DNA strands raises difficulties; especially, the chances of two DNA bimodified AuNPs colliding and reacting with each other are low, thus leading to a low yield. The nonspecific interaction between DNA and AuNPs is another factor preventing efficient assembly, and further efforts are underway. UV−vis spectra of three assemblies were also characterized (shown below the TEM images correspondingly).

To prove alternative arrangement between **AB-G5** and **CD-G10** was not caused by nonspecific interaction, mixture of **AB-G5** and free **G10** (lane 5), **CD-G10** and free **G5** (lane 6) were loaded in the same agarose gel (Figure 4). These two mixtures showed no smear: only one band in lane 5, which was in agreement with separate **AB-G5** (lane 2) and **G10** (lane 3), which had similar mobility, and two bands in lane 6: the faster band had same mobility as the sample in lane 1, and the slower band had same mobility as the sample in lane 4. Trailing bands

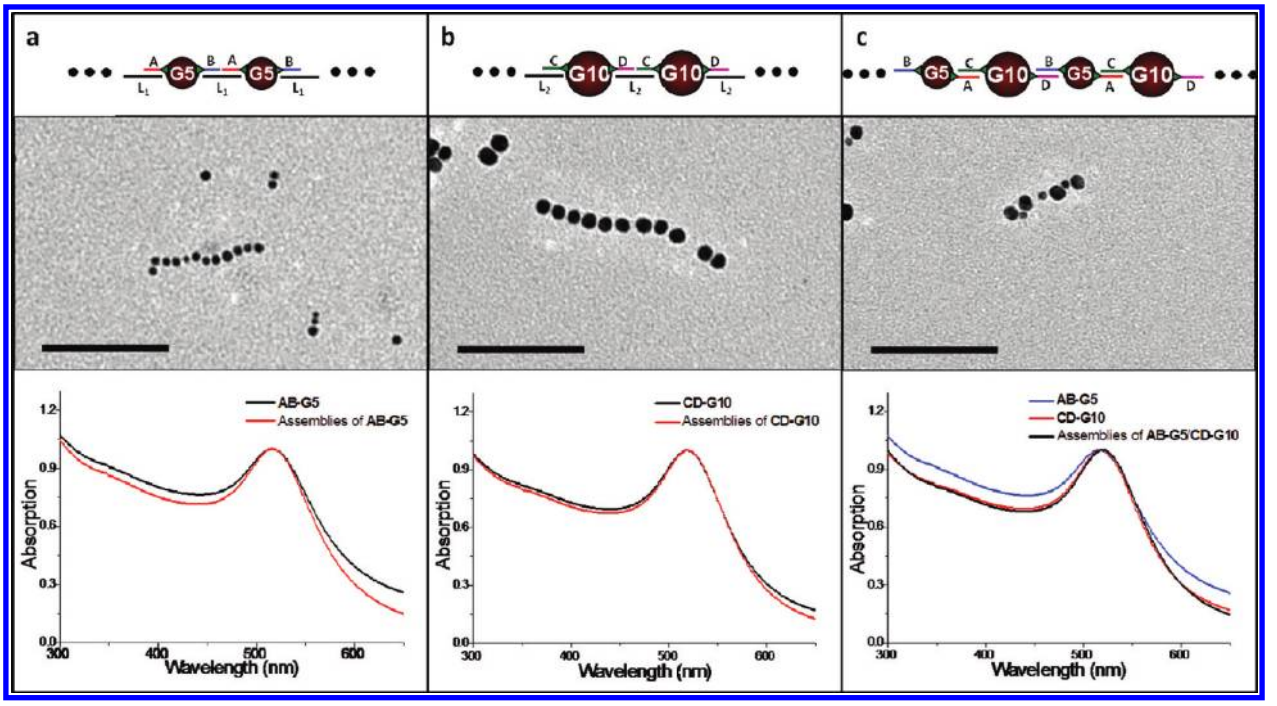


**Figure 3.** TEM images (the bar is 100 nm) and corresponding normalized UV−vis spectra of assemblies of (a) **AB-G5**, (b) **CD-G10**, and (c) **AB-G5**/**CD-G10**.

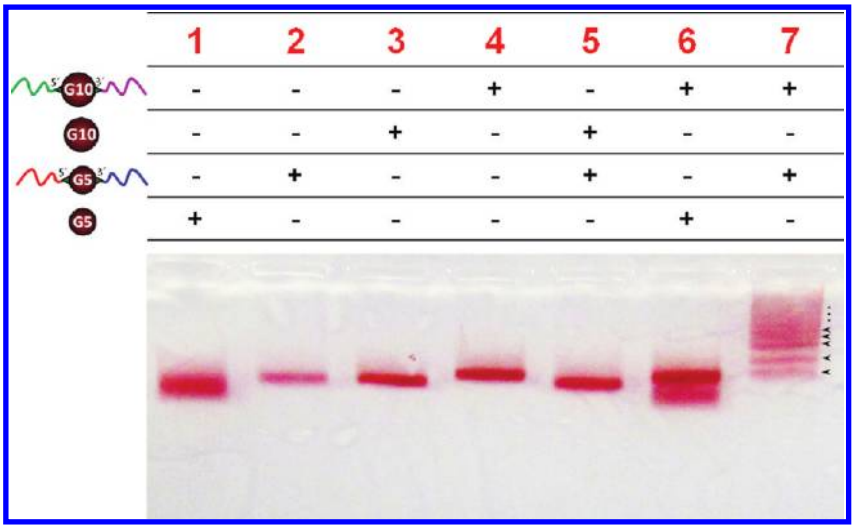


**Figure 4.** Electrophoresis of **AB-G5**/**CD-G10** assemblies and its controls. Lane 1, free **G5**; lane 2, **AB-G5**; lane 3, free **G10**; lane 4, **CD-G10**; lane 5, mixture of **AB-G5** and free **G10**; lane 6, mixture of **CD-G10** and free **G5**; lane 7, assemblies of **AB-G5**/**CD-G10**. 3% agarose gel, 3.5 V/cm, 25 min.

in lane 7 indicated there were a variety of assemblies formed from **AB-G5** and **CD-G10**, e.g., dimers, trimers, tetramers, and so forth.

## ■ CONCLUSIONS

In conclusion, we have successfully prepared DNA bimodified GNPs at different ends and explored their self-assembly behavior. The DNA−GNP conjugates were synthesized via DNA template reaction. The DNA−GNP conjugates were linked with a stable chelating bivalent thiol−gold bond, and different DNA templates were selected to modify GNPs according to the size of nanoparticles. Agarose gel electrophoresis was used to characterize and isolate the prepared conjugates. The TEM image indicated that such bimodified GNPs could be assembled into linear chains. Different forms of assemblies such as **G5** chains, **G10** chains, and spaced **G5** and **G10** chains could be achieved by careful design. The nanoparticle incorporated in this new kind of bimodified GNPs could be considered as a "big mismatch" in DNA strands, and would be available for being further knitted into complex DNA nanostructures via, for example, DNA origami technique.[40,41] It is also believed that our design to assemble DNA bimodified GNPs into one-dimensional nanostructures could not only provide a new method to modulate the assembly behavior of nanoparticles but also benefit the exploration of emerging physical properties of highly

ordered architectures. To further extend the template reaction to two dimensions (2D) and three dimensions (3D), by designing scaffolds such as punched DNA cages with functional DNA strand inside, more DNA strands with precise modification could be attached on GNP surfaces, which would bring more versatile and controllable assemblies in high dimensions.

## ■ ASSOCIATED CONTENT

**Supporting Information.** DNA sequences, agarose gel electrophoresis, TEM images, experimental details. This material is available free of charge via the Internet at http://pubs.acs.org/.

## ■ AUTHOR INFORMATION

### Corresponding Author

*E-mail: xulj@chem.ruc.edu.cn (L.X.); zyang@tsinghua.edu.cn (Z.Y.); liudongsheng@tsinghua.edu.cn (D.L.).

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