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DNA-directed self-assembly of gold nanoparticles into binary and ternary nanostructures

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Abstract
The assembly and characterization of gold nanoparticle-based binary and ternary structures are reported. Two strategies were used to assemble gold nanoparticles into ordered nanoscale architectures: in strategy **1**, gold nanoparticles were functionalized with single-strand DNA (ssDNA) first, and then hybridized with complementary ssDNA-labelled nanoparticles to assemble designed architectures. In strategy **2**, the designed architectures were constructed through hybridization between complementary ssDNA first, then by assembling gold nanoparticles to the scaffolding through gold–sulfur bonds. Both TEM measurements and agarose gel electrophoresis confirmed that the latter strategy is more efficient in generating the designed nanostructures.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The assembly of nanoparticles into ordered architectures is a potential route to achieve further construction and miniaturization of electronic and optical devices. Various physical and chemical methods, including electrostatic force [1, 2], hydrophobic force [3, 4], covalent bonding [5, 6] and synthesis [7, 8], and biomolecular interactions such as DNA hybridization [9, 10] have been used to successfully construct nanoscale architectures. However, preparing extended two- and three-dimensional architectures with synthetically programmable building blocks and assembly parameters remains a challenge faced by nanoparticle-based materials chemistry [11].

Much effort has been exerted in exploring the strategies to organize nanoparticles into ordered architectures [12–29]. Feldheim and co-workers [12, 13] demonstrated the use of phenylacetylene as building blocks to assemble silver and gold nanoparticles with pseudo-$D_{\infty h}$, D_{3h}, and T_d symmetries. Recently, biomolecule-directed strategies showed great promise in assembling nanoparticles into a wide diversity of architectures, because of their high efficiency, high specificity and genetic programmability [14–27]. Proteins cannot only be used as scaffolding to assemble ordered architectures through gold–sulfur bonds [14] and metallization [15], but also serve as linkers to assemble nanostructures via the highly specific biomolecular recognition systems of streptavidin–biotin [16], antibody–antigen [17], carbohydrate–selectin [18] and peptide based interactions [19]. Live viruses or microorganisms such as cowpea mosaic virus (CPMV) [20], tobacco mosaic virus (TMV) [21] and bacteria [22] can also serve as living templates to assemble ordered nanoscale architectures. DNA has been used as a synthetically programmable interconnection for the preparation of new materials with preconceived architectural parameters and properties [9, 10, 23–25]. Such nanoassembled materials have been shown to have potential applications in new detection systems such as biosensors [26] and

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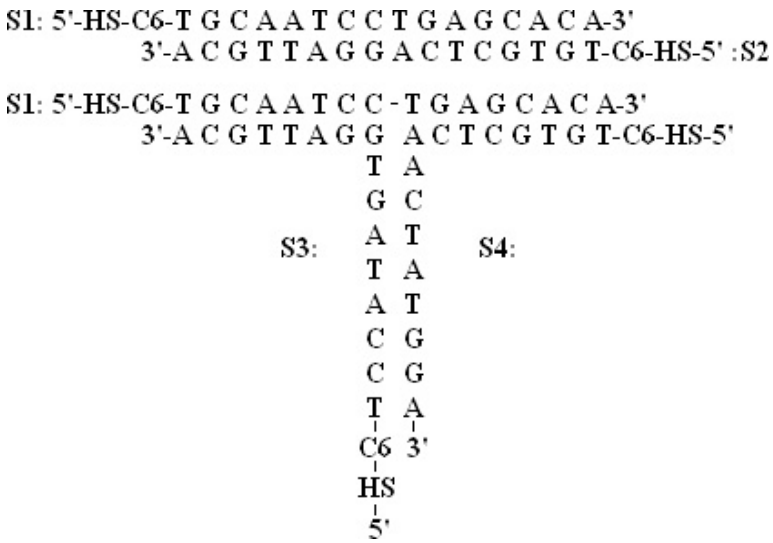

Scheme 1. Sequence of alkylthiol-capped oligonucleotides used in this study.

chemical sensors [27, 28], and in the construction of nanoelectronic devices [29].

While most reports have focused on the conjugation of ssDNA to gold nanoparticles, few studies have documented the fabrication of Au/double-strand-DNA (ds-DNA) conjugates [30], which has been extensively attempted but with limited success. In this study, two strategies have been proposed to assemble gold nanoparticles into binary and ternary nanoscale architectures based on sequence-specific DNA hybridization: in strategy **1**, gold nanoparticles are functionalized with single-strand DNA (ssDNA) first, and then hybridized with complementary ssDNA-labelled nanoparticles to assemble ordered architectures. In strategy **2**, the ordered architectures are constructed through hybridization between complementary ssDNA first, then by assembling gold nanoparticles to the scaffoldings through gold–sulfur bonds. Both TEM measurements and agarose gel electrophoresis confirmed that the latter strategy is more efficient in generating the designed nanostructures.

2. Experimental section

2.1. Preparation of oligonucleotide-modified gold nanoparticles

Aqueous dispersions of citrate-stabilized 10 and 30 nm diameter gold nanoparticles were prepared as described in detail elsewhere [31] using reagents supplied by Aldrich. The alkylthiol-capped oligonucleotides were purchased from Genemed Synthesis Inc (scheme 1). The immobilization of alkylthiol-capped oligonucleotides on gold nanoparticles has been described in detail elsewhere [31]. The oligonucleotide-modified gold nanoparticles were definitely stable to agglomeration in salt solution (in 0.3 M NaCl, 10 mM phosphate buffer (pH 7.0)) as evidenced by the lack of change in the UV–vis spectroscopy results of aqueous suspensions of particles. The increase in ionic strength is necessary in order to screen the electrostatic repulsion between the negatively charged particles and the negatively charged oligonucleotides.

2.2. Preparation of nanoparticle assemblies

To form nanoparticle assemblies, a stoichiometric amount of oligonucleotide-modified gold nanoparticles were combined in a microcentrifuge tube. Unless otherwise mentioned, nanoparticle assemblies were formed in 0.3 M NaCl, 10 mM phosphate buffer (pH 7.0). The mixture was incubated in a water bath at 70 °C for 5 min and was then allowed to cool slowly to room temperature (over ∼2 h) in the water bath.

2.3. Characterization

TEM studies were performed using a Phillips Tecnai 12 instrument. Carbon-coated copper TEM grids were prepared according to a modified literature procedure described briefly here: 5 μl of dilute aqueous sample was spotted onto a grid and left for 90 s before lightly touching one edge of the grid with filter paper to wipe off the moisture. The grids were then allowed to air dry prior to analysis. Gels were prepared with 1.5% agarose by weight in 0.5X tris-borate-EDTA (TBE) buffer. Electrophoresis was run at 5 V cm^{-1} and then visualized by a digital camera.

3. Results and discussion

Binary nanoparticle architecture was assembled with citrate-stabilized gold particles averaging 10 nm in diameter using two strategies (illustrated in figures 1(A) and 1(D), respectively). For the first set of experiments, alkylthiol-capped oligonucleotides S1 and S2 were immobilized on 10 nm gold particles, respectively. TEM and UV–vis results suggested that the size and optical properties of the gold nanoparticles did not significantly change upon modification with oligonucleotides. When a stoichiometric amount of S1-modified nanoparticles and S2-modified nanoparticles were combined, the colour of the mixture changed from red to purple gradually and some irreversible precipitation appeared on the bottom of the tube. The formation of assembled nanostructures should be responsible for this colour change [26–28]. TEM images (figure 1(B)) show binary assemblies of

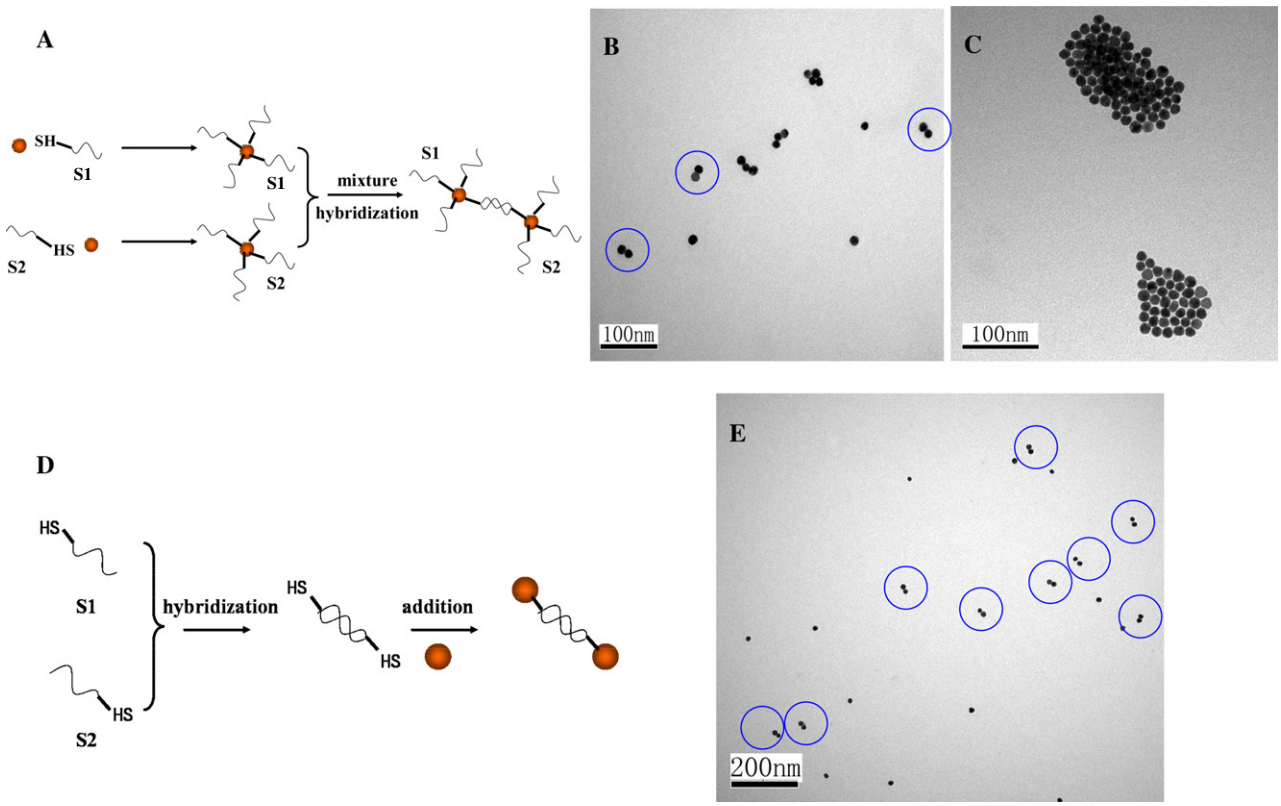

Figure 1. (A) Illustration of strategy **1** to prepare binary assemblies; ((B) and (C)) TEM images of Au nanoparticle dimers synthesized by strategy **1**; (D) Illustration of strategy **2** to prepare binary assemblies; (E) TEM images of Au nanoparticle dimers synthesized by strategy **2**.

Table 1. Statistical analysis of TEM images.

	Percentage of designed architectures[a]	
	Binary	Ternary
Bare Au nanoparticles	19.5 ± 8%	11.5 ± 8%
S1-labelled Au nanoparticles	16.8 ± 9%	9.8 ± 9%
S2-labelled Au nanoparticles	18.3 ± 9%	
S3-labelled Au nanoparticles		10.9 ± 10%
S4-labelled Au nanoparticles		11.5 ± 9%
Control experiments (non-complementary DNA)	17.2 ± 9%	10.7 ± 9%
Strategy **1**	38.7 ± 8%	30.9 ± 10%
Strategy **2**	73.6 ± 10%	56.1 ± 10%

[a] The percentage of designed architectures is calculated by dividing the number of gold nanoparticles used in assembling designed architectures by the total number of gold nanoparticles.

gold nanoparticles, as expected for linkages generated by complementary DNA hybridization. In order to verify that the assembly of binary nanoparticle architecture is DNA-directed, statistical analysis of TEM images was carried out and the data is listed in table 1. 'Bare' gold particles and ssDNA-functionalized gold particles could randomly form self-assembled dimers (∼19.5%, 16.8% and 17.3%, respectively). In a control experiment (with non-complementary DNA-modified Au nanoparticles), dimers could also be observed (∼20%). However, when complementary oligonucleotides were functionalized to gold particles, the percentage of the designed binary nanoparticle assembly increased to ∼40%. Though the exact number of oligonucleotides immobilized on each particle is beyond control at this stage, more than one oligonucleotide can be attached to each nanoparticle [32]. Thus, every nanoparticle can link many particles and a huge aggregation can be formed as a final result (figure 1(C)). These results indicated that strategy **1** was not perfect in generating desirable binary assemblies. In an alternative attempt, the two complementary alkylthiol-capped oligonucleotides S1 and S2 were hybridized into ds-DNA with thiol groups at each end (illustrated in figure 1(D)). Then, desirable binary nanoparticle assemblies can be obtained by conjugating gold nanoparticles to the preformed scaffolding through gold–sulfur bonds (figure 1(E)). In contrast to previous sets of experiments, no significant colour change or irreversible precipitation were observed, even if the mixture was allowed to stand for several hours. Statistical analysis of TEM images suggested

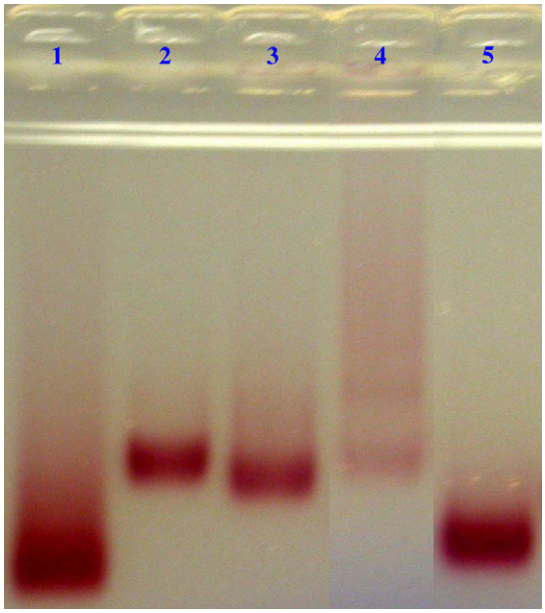

Figure 2. Agarose gel electrophoresis. Samples loaded are: lane 1, bare 10 nm gold particle; lane 2, 10 nm gold particle modified with S1; lane 3, 10 nm gold particle modified with S2; lane 4, Au nanoparticle dimers synthesized by strategy **1**; lane 5, Au nanoparticle dimers synthesized by strategy **2**.

that binary nanoparticle assemblies could be formed more efficiently by using strategy **2**, as evidenced by the increase to ∼70% of the designed binary nanoparticle assembly (table 1).

Gel electrophoresis is a powerful technique in biology and is widely applied in the separation of DNA of different sizes [33]. Recently, this technique was used to isolate small gold clusters [34, 35]. Gel electrophoresis was used herein to show the differences between the binary assemblies obtained by using these two methods. A typical image of a 1.5% agarose gel of Au nanoparticle–DNA conjugates is presented in figure 2. It is well known that particle mobility in a porous matrix (gel) under an electric field depends on both the size and charge of the particle. When modified with alkylthiol-capped oligonucleotides, the size of the gold nanoparticles increased due to the immobilization of ss-DNA on the gold surface, thus retarding the migration of gold nanoparticles, as shown in lane 1 to lane 3 in the gel image in figure 2. Lane 4 and lane 5 of figure 2 correspond to binary assemblies prepared by the first and the second method, respectively. Lane 4 shows a red smear band, which suggests that various assemblies such as dimers, trimers and multimers could be formed when hybridizing complementary ss-DNA-modified gold nanoparticles. The sample well still appeared red after electrophoresis, which indicated that some huge aggregation of gold nanoparticles could not migrate through the gel matrix

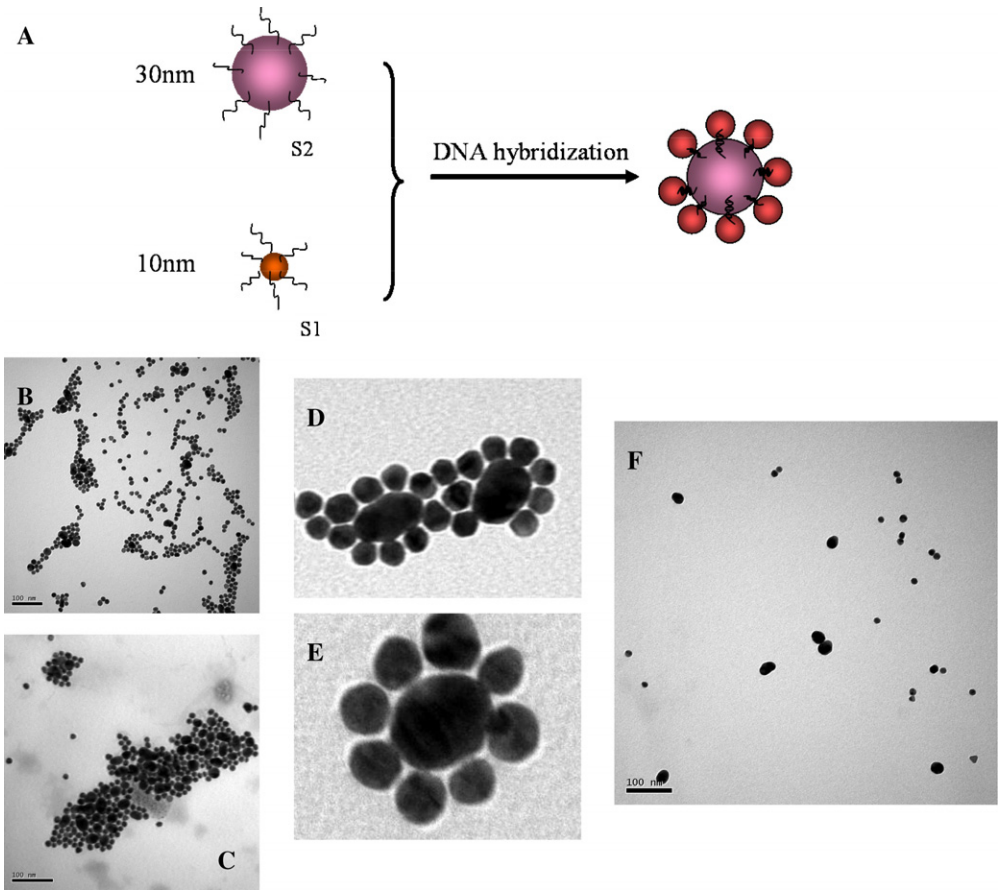

Figure 3. (A) Illustration of the strategy to prepare 'satellite'-shaped assemblies; ((B)–(E)) a nanoparticle satellite structure obtained from the reaction involving 9:1 S1-modified 10 nm particles/S2-modified 30 nm particle; (F) TEM image of control experiment.

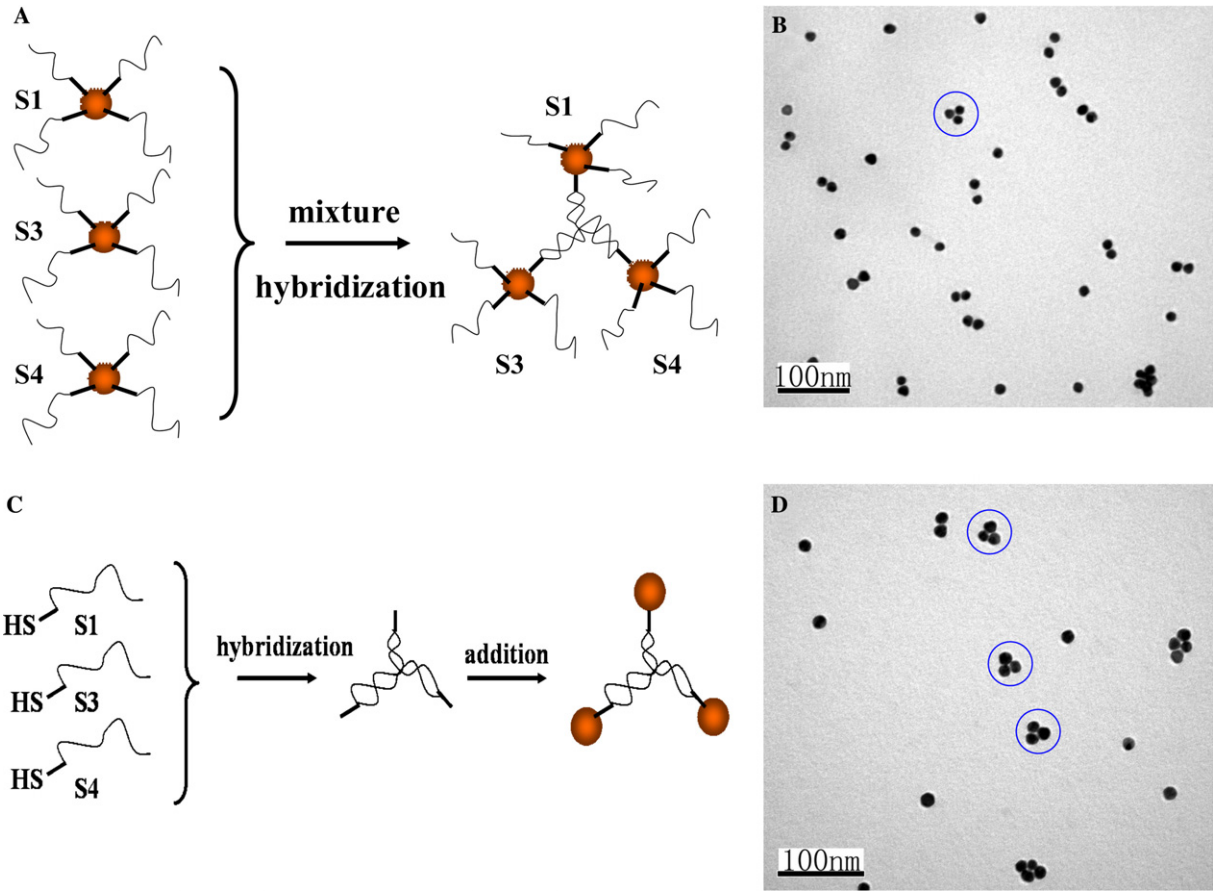

Figure 4. (A) Illustration of the strategy **1** to prepare ternary assemblies; (B) TEM images of Au nanoparticle trimers synthesized by strategy **1**; (C) illustration of the strategy **2** to prepare ternary assemblies; (D) TEM images of Au nanoparticle trimers synthesized by strategy **2**.

due to their large size. These results suggest that it is difficult to obtain controllable binary nanoparticle assemblies using the first method. However, a clear single band is shown in lane 5, which indicates that the assemblies formed using the second method are consistent in composition. From this gel image, we also found that binary nanoparticle assemblies prepared by the second method migrated more slowly than bare gold nanoparticles, but faster than ss-DNA modified gold particles. A possible reason is that few ds-DNA can attach to each gold particle due to steric and electrostatic repulsion [23, 35–37]. Based on all these results, we can conclude that the second method is more efficient in preparing binary assemblies.

Multiple binary nanoparticle architecture, 'satellite'-shaped architecture, was assembled with citrate-stabilized gold particles averaging 10 and 30 nm in diameter. In this study, the 10 nm particles were treated with alkylthiol-capped oligonucleotides S1 and the 30 nm particles were modified with alkylthiol-capped oligonucleotides S2. Then, the 10 nm modified particles were mixed with the 30 nm modified particles (figure 3(A)) at a series of ratios of 1:1, 2:1 and 100:1. When two nanoparticles were mixed at ratios of 1:1 and 2:1, barely any 'satellite'-shaped structures were observed by TEM, possibly due to the low probability of having 30 nm gold particles surrounded by enough 10 nm gold particles. However, at a ratio of 100:1, a few 'satellite'-shaped structures with many 'superfluous' 10 nm gold particles were observed. Based on a simple geometric calculation, one 30 nm gold particle can be surrounded by a maximum of ten 10 nm gold particles, which is consistent with the assembled 'satellite'-shaped structures. A ratio of 9:1 was selected for subsequent experiments, taking into consideration the particle size variation. TEM images of the DNA-directed assembly of gold nanoparticles revealed 'satellite'-shaped structures consisting of one 30 nm particle surrounded by seven to nine 10 nm particles (figures 3(B)–(E)). Similar structures have also been assembled by Mirkin's group using a different strategy [11]. Control experiments were performed by mixing 10 nm modified particles with 30 nm modified particles at a 9:1 ratio, with non-complementary oligonucleotides immobilized on gold particles. Under these conditions, randomly dispersed particles or aggregates, consisting of particles of the same size, were observed by TEM (figure 3(F)). No colour change and 'satellite'-shaped structures could be observed. These results further confirm that the sequence-specific DNA hybridization, not the non-specific interaction, should be responsible for the formation of 'satellite'-shaped structures.

Triangular ternary architecture was also assembled in this study using three oligonucleotides which are partially complementary and can help obtain three-arm conjugations (scheme 1) [38]. Gold nanoparticles were assembled through DNA scaffolding to generate trimers. Two sets of experiments using different strategies were carried out. For the first set

of experiments, alkylthiol-capped oligonucleotides S1, S3 and S4 were immobilized on 10 nm gold particles respectively. Then, the stoichiometric amount of S1-modified nanoparticles, S3-modified nanoparticles and S4-modified nanoparticles were combined to generate the designed structures (as illustrated in figure 4(A)). In the second attempt, the three alkylthiol-capped oligonucleotides S1, S3 and S4 hybridized to form triangular scaffolding with thiol groups at each end (illustrated in figure 4(C)). Then, desirable ternary nanoparticle assemblies could be obtained by conjugating gold nanoparticles to the preformed scaffolding through gold–sulfur bonds. TEM images (figures 4(B) and (D)) indeed show ternary assemblies of gold nanoparticles, as expected for linkages generated by complementary DNA hybridization. Similar to the formation of binary assemblies, huge aggregation of gold nanoparticles was also observed when using strategy **1**. Statistical analysis of TEM images suggested that ternary nanoparticle assemblies could be formed more efficiently by using strategy **2**; as evidence please note that the percentage of designed ternary nanoparticle assembly increased from ∼30% to ∼60% (table 1).

Based on our results, strategy **2** is more efficient in DNA-directed self-assembling ordered nanoscale architectures. Another advantage of strategy **2** over strategy **1** is that barely any huge aggregation is formed during the course of the self-assembly reaction. One disadvantage of strategy **2** is that preformed dsDNA can attach to any metal nanoparticles through sulfur–metal bonds, which limits its application in controlling the designed assembly of different types of components [25].

4. Conclusion

In this study, two strategies were proposed to assemble gold nanoparticles into ordered architectures and prepare discrete assemblies. In strategy **1**, gold nanoparticles were functionalized with single-strand DNA (ssDNA) first, and then hybridized with complementary ssDNA-labelled nanoparticles to assemble designed architectures. In strategy **2**, the designed architectures were constructed through hybridization between complementary ssDNA first, then by assembling gold nanoparticles to the scaffoldings through gold–sulfur bonds. Both TEM measurements and agarose gel electrophoresis confirmed that the latter strategy was more efficient in generating the designed nanostructures. The steric and electrostatic repulsion may have been responsible for the differences between these two strategies. In conclusion, our study shows how the molecular recognition properties of oligonucleotides can be used to direct the placement of nanoparticles into ordered architectures, which may have potential applications in the design, preparation, and manipulation of broad architectures for molecular imaging, nanoelectronics, or nanobiosensing technologies.

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References

[1] Boal A K, Galow T H, Ilhan F and Rotello V M 2001 *Adv. Funct. Mater.* **11** 461–5
[2] Guldi D M, Zilbermann I, Anderson G, Kotov N A, Tagmatarchis N and Prato M 2004 *J. Am. Chem. Soc.* **126** 14340–1
[3] Han L, Wu W, Kirk F L, Luo J, Maye M M, Kariuki N N, Lin Y H, Wang C M and Zhong C J 2004 *Langmuir* **20** 6019–25
[4] Ellis A V, Vijayamohanan K, Goswami R, Chakrapani N, Ramanathan L S, Ajayan P M and Ramanath G 2003 *Nano Lett.* **3** 279–82
[5] Haremza J M, Hahn M A, Krauss T D, Chen S and Calcines J 2002 *Nano Lett.* **2** 1253–8
[6] Banerjee S and Wong S S 2002 *Nano Lett.* **2** 195–200
[7] Banerjee S and Wong S S 2003 *J. Am. Chem. Soc.* **125** 10342–50
[8] Fu L, Liu Z M, Liu Y Q, Han B X, Hu P G, Cao L C and Zhu D B 2005 *Adv. Mater.* **17** 217–21
[9] Mirkin C A, Letsinger R L, Mucic R C and Storhoff J J 1996 *Nature* **382** 607–9
[10] Alivisatos A P, Johnsson K P, Peng X G, Wilson T E, Loweth C J, Bruchez M P and Schultz P G 1996 *Nature* **382** 609–11
[11] Mucic R C, Storhoff J J, Mirkin C A and Letsinger R L 1998 *J. Am. Chem. Soc.* **120** 12674–5
[12] Brousseau L C, Novak J P, Marinakos S M and Feldheim D L 1999 *Adv. Mater.* **11** 447–9
[13] Novak J P and Feldheim D L 2000 *J. Am. Chem. Soc.* **122** 3979–80
[14] Mcmillan R A, Paavola C D, Howard J, Chan S L, Zaluzec N J and Trent J D 2002 *Nat. Mater.* **1** 247–52
[15] Behrens S, Habicht W, Wagner K and Unger E 2006 *Adv. Mater.* **18** 284–9
[16] Niemeyer C M, Burger W and Peplies J 1998 *Angew. Chem. Int. Edn* **37** 2265–8
[17] Shenton W, Davis S A and Mann S 1999 *Adv. Mater.* **11** 449–52
[18] Hiddessen A L, Rodgers S D, Weitz D A and Hammer D A 2000 *Langmuir* **16** 9744–53
[19] Xu L, Guo Y, Xie R G, Zhuang J Q, Yang W S and Li T J 2002 *Nanotechnology* **13** 725–8
[20] Blum A S *et al* 2004 *Nano Lett.* **4** 867–70
[21] Fowler C E, Shenton W, Stubbs G and Mann S 2001 *Adv. Mater.* **13** 1266–9
[22] Rosi N L, Thaxton C S and Mirkin C A 2004 *Angew. Chem. Int. Edn* **43** 5500–3
[23] Storhoff J J and Mirkin C A 1999 *Chem. Rev.* **99** 1849–62
[24] Le J D, Pinto Y, Seeman N C, Musier-Forsyth K, Taton T A and Kiehl R A 2004 *Nano Lett.* **4** 2343–7
[25] Pinto Y Y, Le J D, Seeman N C, Musier-Forsyth K, Taton T A and Kiehl R A 2005 *Nano Lett.* **5** 2399–402
[26] Taton T A, Mirkin C A and Letsinger R L 2000 *Science* **289** 1757–60
[27] Liu J W and Lu Y 2003 *J. Am. Chem. Soc.* **125** 6642–3
[28] Liu J W and Lu Y 2006 *Angew. Chem. Int. Edn* **45** 90–4
[29] Keren K, Berman R S, Buchstab E, Sivan U and Braun E 2003 *Science* **302** 1380–2
[30] Loweth C J, Caldwell W B, Peng X G, Alivisatos A P and Schultz P G 1999 *Angew. Chem. Int. Edn* **38** 1808–12
[31] Storhoff J J, Elghanian R, Mucic R C, Mirkin C A and Letsinger R L 1998 *J. Am. Chem. Soc.* **120** 1959–64
[32] Demers L M, Mirkin C A, Mucic R C, Reynolds R A, Letsinger R L, Elghanian R and Viswanadham G 2000 *Anal. Chem.* **72** 5535–41
[33] Noolandi J 1998 *Advances in Electrophoresis* vol 5 (Deerfield Beach: VCH) pp 2–57
[34] Zanchet D, Micheel C M, Parak W J, Gerion D and Alivisatos A P 2001 *Nano Lett.* **1** 32–5
[35] Sandstro P and Kerman B A 2004 *Langmuir* **20** 4182–6

[36] Parak W J, Gerion D, Pellegrino T, Zanchet D, Micheel C, Williams S C, Boudreau R, Le Gros M A, Larabell C A and Alivisatos A P 2003 *Nanotechnology* **14** R15–27
[37] Seeman N C 2003 *Chem. Biol.* **10** 1151–9
[38] Yang M S and Millar D P 1996 *Biochemistry* **35** 7959–67