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End-to-end self-assembly and colorimetric characterization of gold nanorods and nanospheres via oligonucleotide hybridization

Bifeng Pan[1], **Limei Ao, Feng Gao**[1], **Hongye Tian, Rong He and Daxiang Cui**

Department of Bio-nano-Science and Engineering, National Key Laboratory of Nano/Micro Fabrication Technology, Institute of Micro-Nano Science and Technology, Shanghai JiaoTong University, 1954 Huashan Road, Shanghai 200030, People's Republic of China

E-mail: panbifeng@sjtu.edu.cn and gaofeng@sjtu.edu.cn

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Abstract
We report end-to-end assembly for tuning the optical properties of a gold nanorod/sphere based on oligonucleotide hybridization. The rationale behind the selection of the mercaptoalkyloligonucleotide molecule is based on the fact that the thiol group binds to the ends of the nanorods, which further assemble in an end-to-end fashion through hybridization with the target oligonucleotide. A highly selective, colorimetric polynucleotide detection method based on mercaptoalkyloligonucleotide-modified gold nanorod/sphere probes is also reported.

1. Introduction

The synthesis of inorganic materials with dimensions on the nanometre scale has greatly improved in the last ten years [1–7]. Future applications in nanoscale electronic devices [1, 2], magnetic data storage systems, optical devices [8], and biological sensing [9–11] require monodisperse semiconductor or metallic materials of controlled size and shape. Nanotechnology is fundamentally changing the way in which materials are synthesized and devices are fabricated [1]. For example, hierarchical integration of nanoscale building blocks (nanoparticles, nanorods, nanotubes, etc) into functional assemblies and further into multifunctional devices can be achieved through a 'bottom up approach' [3]. Self-assembly of Au nanorods driven by surfactants [4], DNA molecules [5], and their preferential end-to-end assembly using biotin–streptavidin connectors [6] have been demonstrated. Tailoring the optoelectronic properties of nanomaterials through the stepwise integration of nanoscale building blocks is one of the major challenges in the area of nanotechnology [7].

Thus far, only one gold nanoparticle system, which exclusively involves oligonucleotide-functionalized 13 nm diameter Au particles as the building blocks, has been extensively studied. Nevertheless, this initial one-component system (here, component refers to the type of particle) has already increased our understanding of particle–particle interactions on the nanometre length scale and has led to the development of a new and highly selective colorimetric detection technology for oligonucleotides [12, 13].

Here we report that gold nanorods, surface functionalized with mercaptoalkyloligonucleotide, can be assembled preferentially in an end-to-end fashion upon the addition of a target oligonucleotide. These results provide evidence that the chemical reactivity of nanomaterials is shape dependent and may be useful in construction of future nanoscale assemblies. Furthermore, we show how this oligonucleotide-directed assembly strategy can be used to prepare binary (two-component) assembly materials comprising two different-shaped oligonucleotide-functionalized nanoparticles. Importantly, the proof-of-concept demonstrations reported herein suggest that this strategy could be extended easily to a wide variety of multicomponent systems where nanoparticle building

[1] Authors to whom any correspondence should be addressed.

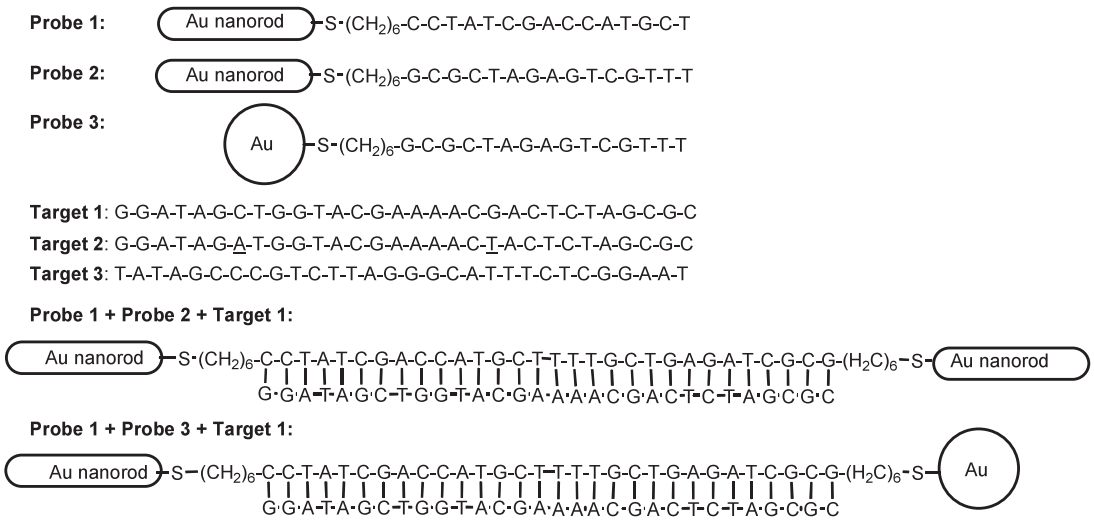

Figure 1. Scheme showing the end-to-end assembly of rod–rod and rod–sphere by surface functionalization with mercaptoalkyloligonucleotide, and subsequent addition of target oligonucleotide: target 1, complementary target; target 2, 2 bp mismatch; target 3, non-complementary target.

blocks that vary in chemical composition, size or shape are arranged in space on the basis of their interactions with complementary linking oligonucleotide.

2. Materials and methods

Mercaptoalkyloligonucleotide was purchased from Bioasia Biotech. Co. (Shanghai, China). To prepare the aqueous gold nanospheres, 4 ml of 0.5% sodium citrate was added to 100 ml of 0.01% boiling tetrachloroauric acid solution. The mixture was stirred until a deep wine red colour was obtained, indicating formation of gold nanoparticle suspension. Transmission electron microscopy showed that the prepared gold nanoparticles have a narrow distribution of particle sizes with a mean diameter of about 25 nm.

We synthesized gold nanorods of aspect ratio 3.0 according to the seed-mediated growth approach in water in the presence of a shape-directing surfactant, cetyltrimethylammonium bromide (CTAB) [14–16]. The nanorods thus prepared are covered with at least a bilayer of CTAB and are stable and soluble in water [15]. Binding of the mercaptoalkyloligonucleotide, in PBS buffer (0.1 M NaCl, 10 mM phosphate buffer, pH 7.0), to the gold nanorods (or spheres) was accomplished by adding 50 μl of 1 μM mercaptoalkyloligonucleotide to 1.0 ml of 0.10 μM rods (or spheres) in PBS, aged for 40 h at 25 °C, collected by centrifugation at 4000 rpm for 10 min, and resuspended. This procedure was used to prepare all of the nanoparticle probes as shown in figure 1. 2 μl of target oligonucleotide were titrated into the mercaptoalkyloligonucleotide-coated probes. This was mixed and incubated for 48 h at 25 °C. A simpler way to monitor hybridization is to spot 1–3 μl of the solution containing the probe/target aggregates on a C_{18} thin-layer chromatography (TLC) plate.

TEM pictures were taken with a JEM 100-CXII microscope (JEOL, Japan) at 100 kV. TEM samples were prepared by drop casting the solution used for spectroscopic investigation onto a carbon coated copper grid. UV–vis spectra were measured at 20 °C with a UNICAM UV 300 spectrometer (Thermo Spectronic, USA) equipped with a 10 mm quartz cell. The 200–1200 nm wavelength region was scanned as it includes the absorbance of the gold nanorod.

3. Results and discussion

To verify the rod–rod and rod–sphere assembly, TEM images were recorded in the absence and presence of target oligonucleotide under similar experimental conditions. The addition of complementary target oligonucleotide to the mercaptoalkyloligonucleotide-coated gold nanorod probes produced an unexpectedly high proportion of end-to-end-linked gold nanorods, which were separated by about 10–15 nm spacings—the size of a target oligonucleotide molecule (figure 2(b)). The thiol group binds strongly and preferentially to the nanorod end, and the hybridization thus allows for preferential linking of rods in an end-to-end arrangement (figures 2(b), (c)), whereas the usual preference for rod aggregation is side-to-side (figure 2(a)).

As shown in figure 2(d), the average diameter of gold nanospheres obtained was 25 nm. The sample deposited from a rod–sphere mixture that does not include target 1 shows a random distribution of rods and spheres (figure 2(e)). On the other hand, a linear rod–sphere assembly is generated by adding target 1 and the images are shown in figure 2(f).

An average of statistics revealed that ∼84% end-to-end linkages could be realized (figure 3(a)) while other binding events comprised ∼16% (figures 3(b) and (c)).

As indicated in this study, thiol derivatives preferentially bind to the {111} ends of the rods for further interactions through oligonucleotide hybridization. Our current results provide evidence that chemical linkages can organize nanorods in a nonrandom fashion, which may be exploited for the assembly of higher-order arrays of nanomaterials.

Gold in its nanometric dimension possesses shape dependent optical and electronic properties. For example,

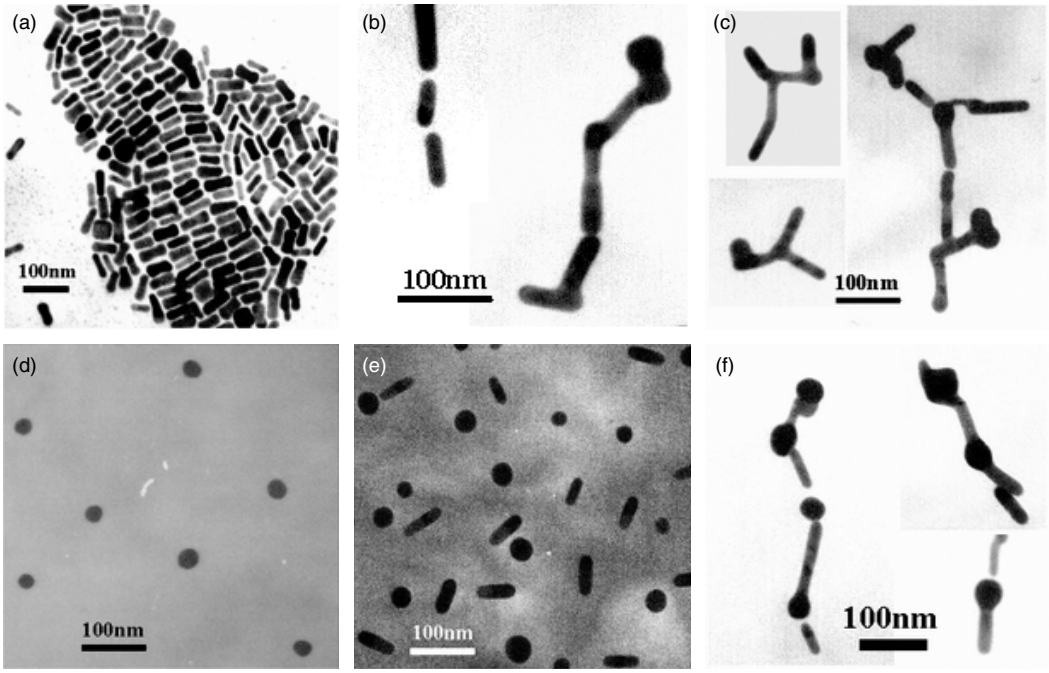

Figure 2. Comparison of the transmission electron micrographs for gold nanorod, nanosphere, and rod–rod/sphere assemblies in hybridization buffer (0.1 M NaCl, 10 mM phosphate buffer, pH 7.0) at 25 °C. (a) 10 nM gold nanorod solution, ((b), (c)) mixture of 49 μl of 10 nM probe 1 and 10 nM probe 2 in hybridization buffer, then 2 μl of the 50 nM target 1 was added and kept for 48 h at 25 °C (final target concentration 1 nM), (d) 10 nM gold nanospheres in PBS buffer, (e) mixture of 49 μl of 10 nM probe 1 and 10 nM probe 3 in the absence of target oligonucleotide, (f) mixture of 49 μl of 10 nM probe 1 and 10 nM probe 3 in hybridization buffer, then 2 μl of the 50 nM target 1 was added and kept for 48 h at 25 °C (final target concentration 1 nM).

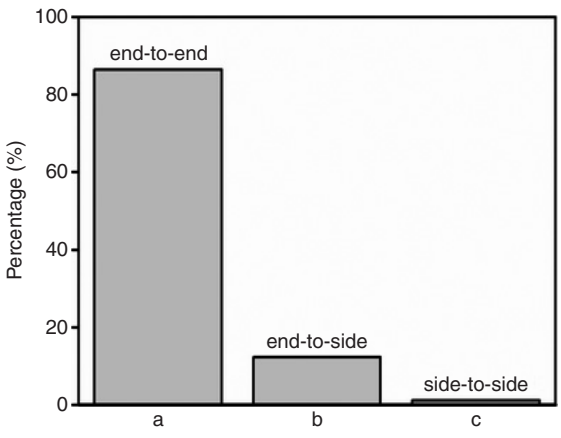

Figure 3. Proportion of numbers of (a) end-to-end, (b) end-to-side, and (c) side-to-side linkages observed in transmission electron micrographs. In this case 500 nanoparticles were counted.

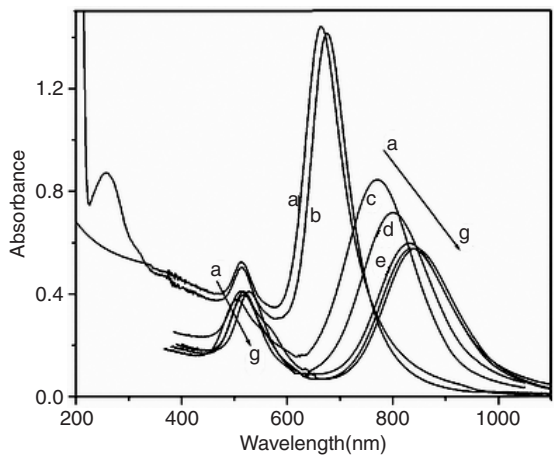

Figure 4. The UV–vis absorbance spectra of rod–rod assemblies were measured at different time intervals: (a) gold nanorod solution (aspect ratio 3.0) in the absence of the oligonucleotide; (b) mixture of probe 1 and probe 2 in the absence of target oligonucleotide; (c)–(g) mixture of 49 μl 10 nM probe 1 and 10 nM probe 2; 2 μl of 50 nM complementary target 1 was added and aged for (c) 10 min, (d) 40 min, (e) 120 min, (f) 24 h, and (g) 48 h at 25 °C.

Au nanorods possess two absorption bands, one at a shorter wavelength (∼520 nm) and the other at a longer wavelength which undergoes a bathochromic shift with increasing aspect ratio [14, 15]. The colour change induced by oligonucleotide hybridization can be monitored by UV–vis spectroscopy. Typical UV–vis spectra of the mercaptoalkyloligonucleotide-modified gold nanoparticles in the absence and in the presence of target 1 are shown in figures 4 and 5.

The short and long wavelength bands of Au nanorods decrease upon addition of target 1, accompanied by the formation of a red-shifted absorption band. The absorption bands at 530 and 680 nm slowly shifted toward longer wavelength with increasing time (traces c–g in figures 4 and 5). We attribute these changes to the further propagation of the rod–rod or rod–sphere assemblies. The initially formed rod–rod/sphere assemblies continue to grow through

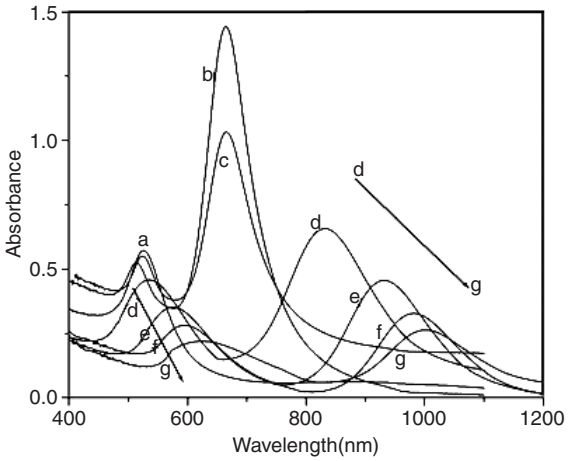

Figure 5. The UV–vis absorbance spectra of rod–sphere assemblies were measured at different time intervals: (a) gold nanosphere solution, (b) gold nanorod solution (aspect ratio 3.0), (c) mixture of probe 1 and probe 3 in the absence of the target oligonucleotide, (d)–(g) mixture of 49 μl 10 nM probe 1 and 10 nM probe 3; 2 μl of 50 nM complementary target 1 was added and aged for (d) 10 min, (e) 60 min, (f) 240 min, and (g) 24 h at 25 °C.

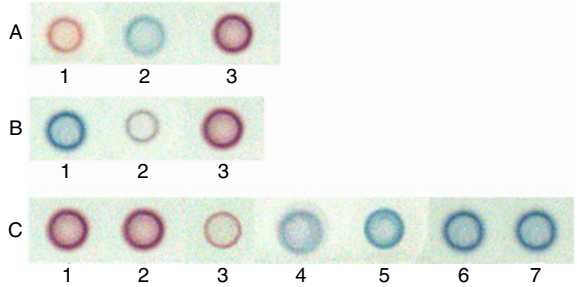

Figure 6. Selective colorimetric oligonucleotide detection based on rod–sphere assembly. (A1) 10 nM probe 3, (A2) 10 nM probe 1, (A3) mixture of probe 3 and probe 1 in the absence of target oligonucleotide. (B) Mixture of 49 μl of 10 nM probe 1 and 10 nM probe 3 in hybridization buffer, then 2 μl of the 50 pM (B1) complementary target 1, (B2) 2 bp mismatched target 2, and (B3) noncomplementary target 3 was added and kept for 48 h at 25 °C (final target concentration, 1 pM). (C) The colour changes of a mixture of probe 1 and probe 3 by adding target 1 with different final concentrations of (C1) 0.03 pM, (C2) 0.06 pM, (C3) 0.12 pM, (C4) 0.48 pM, (C5) 1.0 pM, (C6) 2.0 pM, and (C7) 10.0 pM in hybridization buffer.

oligonucleotide hybridization to yield larger assemblies of different sizes. The half width of the absorption bands increased with increasing incubation time. Various factors such as size and shape of nanoparticles and the charge distribution on particles influence the plasmon absorption [17, 18]. Since the rod–rod and rod–sphere assemblies of different sizes exhibit different spectral maxima, so broader absorption bands are observed.

The colour differentiation is enhanced by the C_{18}-silica support. The colour of the nanosphere solution is pink (figure 6(A1)), the nanorod colour is jade-green (figure 6(A2)), and the rod–sphere mixture is deep red (figure 6(A3)) in the absence of oligonucleotide. The spot was blue for a hybridized sample containing target 1 (figure 6(B1)), indicating that the nanoparticles were assembled at room temperature, as determined by the change in the UV–vis spectrum for the solution (figure 5). For target 2, the colour was intermediate (figure 6(B2)). In the presence of target 3 the spot remains deep red (figure 6(B3)). Colour change with different target 1 concentration is shown in figures 6(C1)–(C7), which shows that this two-component system can detect about 1 pM of a nucleotide.

4. Conclusions

In conclusion, we report that gold nanorods, surface-functionalized with mercaptoalkyloligonucleotide, can be assembled preferentially in an end-to-end fashion upon the addition of a target oligonucleotide. These results provide evidence that the chemical reactivity of nanomaterials is shape dependent and may be useful in construction of future nanoscale assemblies. This oligonucleotide hybridization-based assembly strategy can be used to prepare binary (two-component) assembly materials comprising two different-shaped oligonucleotide-functionalized nanoparticles. This two-component nanoparticle-based strategy should be more complicated than any one-component detection system based on a single-strand probe hybridizing with the target. Strategies such as ours that exploit rod–sphere assembly by oligonucleotide hybridization and use probes with signals sensitive to particle aggregation or distance should prove generally useful in designing highly discriminating nucleic acid detection systems. We do not yet know the ultimate sensitivity of the system, although with the unoptimized system ∼1 pM of an oligonucleotide can be detected. The method should be particularly useful in assays where expense and simplicity in instrumentation and operation are important.

Acknowledgments

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