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A flexible DNA modification approach towards construction of gold nanoparticle assemblies

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Materials and methods

1.1 Materials

Boric acid, acetic acid, ethylenediamine-tetraacetic acid, NaCl, MgCl2.6H2O, StainsAll®, formamide, tris(hydroxymethyl)-aminomethane (Tris), and ethidium bromide, bis(*p*-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt were purchased from Aldrich. 5-ethylthiotetrazole, controlled pore glass (CPG) and reagents for automated DNA syntheses were purchased from Glen research. Microcon® size-exclusion centrifugal filter devices were purchased from Millipore. Citrate coated AuNPs were made in-house according to the literatures [1].

1.2 Apparatus

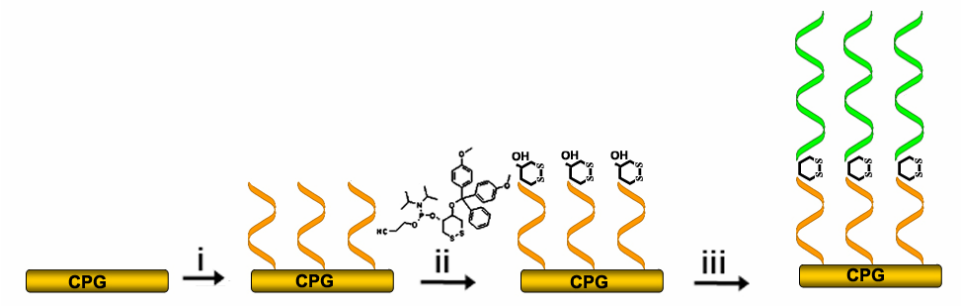
^{1}H NMR spectra were recorded on a Mercury plus 300 MHz using $CDCl_3$ as solvent unless otherwise noted. All chemical shifts were reported in parts per million (ppm), ^{1}H NMR chemical shifts were referenced to TMS (0 ppm) or residual $CHCl_3$ (7.26 ppm). ESI mass spectra were recorded on a Bruker Apex IV FTMS. Standard automated oligonucleotide solid-phase synthesis was performed on an Applied Biosystems 394 DNA synthesizer. UV-Vis spectra were measured on a Varian Cary 300 biospectrophotometer. High-performance liquid chromatography (HPLC) was performed using a Techcomp LC2000 series HPLC. Gel electrophoresis experiments were carried out on an acrylamide 20 × 20 cm vertical DYCZ24 electrophoresis unit, and an agarose 8.5 × 8 cm horizontal DYCZ31 separation minigel system. Transmission electron microscopy (TEM) images were obtained using a

JEM-2100 200 kV electron microscope.

1.3 Synthesis of dithiolphosphoramidite molecule, 1,2-dithiane-4-O-dimethoxytrityl-5-[(2-cyanoethyl) - N,N- diisopropyl)]-phosphoramidite (DTPA)

Target molecule was synthesized according to the method in literatures [2]. All air and water sensitive reactions were performed under nitrogen atmosphere. Toluene and tetrahydrofuran (THF) were distilled from sodium. Acetonitrile and pyridine were distilled from CaH_2. Compound DTPA: Yield 60.2 %, ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ 7.56-7.53 (dd, J = 8.1 Hz, J = 2.7 Hz, 2H), 7.43-7.39 (m, 4H), 7.33-7.15 (m, 3H), 6.86-6.81 (m, 4H), 3.80 (d, J = 1.5 Hz, 6H), 3.74-3.64 (m, 2H), 3.60-3.45 (m, 2H), 3.11-3.07 (m, 2H), 2.75-2.68 (m, 4H), 2.65-2.54 (t, J = 6.6 Hz, 1H), 2.35-2.29 (t, J = 6.6 Hz, 1H), 1.18-0.96 (m, 12H). ESI-MS: Calcd for $C_{34}H_{43}N_2O_5PS_2$: 654.2. Found: 655.2 ($M+H^+$), 677.2 ($M+Na^+$).

1.4 Synthesis of oligonucleotides



Scheme 1. DNA sequence of the appropriate length is (i) synthesized on CPG with a standard automated oligonucleotide synthetic protocols, which is then followed by (ii) the incorporation of a dithiolphosphoamidite molecule using a modified protocol (extended coupling/deprotection time of 6/2 min), and (iii) the second DNA arm of appropriate sequence is further synthesized on it with the standard automated oligonucleotide synthetic protocols. More dithiolphosphoamidite molecules can be incorporated into the same strand by following the ii) and iii) procedures.

The oligonucleotides were constructed on CPG supports using conventional phosphoramidite chemistry. For the sequences modified by one or more DTPA in the interior of the DNA sequence, the first arm, of appropriate sequence, was initially grown on the solid support using standard automated solid phase oligonucleotide synthetic protocols. DTPA was then incorporated. Subsequently, the second arm was synthesized using standard synthetic protocols (Scheme 1). Products were cleaved from the

support by treatment with concentrated NH_4OH for 16 h at 55°C. The NH_4OH solution was decanted and dried down to yield the crude DNA mixture. The crude mixture obtained was purified by preparative reverse-phase HPLC with 0.03 M triethylammonium acetate (TEAA), pH 7 and a 1%/min gradient of 95% CH_3CN/5% 0.03 M TEAA at a flow rate of 1 mL/min. Quantification was estimated based on UV-Vis absorbance at 260 nm.

Table 1 DNA sequences for the preparation of AuNP assemblies in Fig. 2. The sequences of all oligonucleotides are listed in the 5′ to 3′ direction, unless otherwise specified. X= cyclic disulfide group.

Name	Sequences
1 a	AAT TGA TAT GTC ACG AAT AAC ACA AAT CGG TCA GTA ATC T（X）CT TGA AGG TAG CAA ACG ACA GGT CCA AAT GAA GAT ACG AA
1 b	TCA TTG CTT CAG TAT CTA AGT AGT AGT GAC CAC TAG CTC T（X）TT CGT ATC TTC ATT TGG ACC TGT CGT TTG CTA CCT TCA AG
1 c	AGA TTA CTG ACC GAT TTG TGT TAT TCG TGA CAT ATC AAT T（X）AAG AAC GAT AGG TAT CAT CC
1 d	AGA GCT AGT GGT CAC TAC TAC TTA GAT ACT GAA GCA ATG A（X）GG ATG ATA CCT ATC GTT CTT
2 a	AAT TGA TAT GTC ACG AAT AAC ACA AAT CGG（X）G CAA ACG ACA GGT CCA AAT GAA GAT ACG AA
2 b	5'-CC GAT TTG TGT TAT TCG TGA CAT ATC AAT T-3'-(X)-3'-TC ATA GAT TCA TCA TCA CTG GTG ATC GAG A-5'
2 c	AG TAT CTA AGT AGT AGT GAC CAC TAG CTC T（X）TT CGT ATC TTC ATT TGG ACC TGT CGT TTG C
Control	(X) AGA TTA CTG ACC GAT TTG TGT TAT TCG TGA CAT ATC AAT TAAG AAC GAT AGG TAT CAT CC

Table 2 DNA sequences for the preparation of AuNP assemblies in Fig. 3 and Fig. 4. In Fig. 4, hairpin-loop neck regions are highlighted. The sequences of all oligonucleotides are listed in the 5′ to 3′ direction (5'→3'), unless otherwise specified. X= cyclic disulfide group.

Name	Sequence (5' → 3')
3 a	TTTTTTTTTTTTT(X)ATAGAAGGCATAACACGACAGGTCCAAGGTAAGATACGAT(X)TTTTTTTTTTTT
3 b	ATCGTATCTTAACCTTGGACCTGTCGTGTTATGCCTTCTAT
4 a	TTTTTTTTT (X) TAACACGACAGGTCCAAGGT (X) TTTTTTTTT
4 b	ACCTTGGACCTGTCGTGTTA
5 a	TAG AGA CGT(X)AAG ATA CGA TTA ACT AAC CTC ACC AAA CAG (X) ACC CGC ACT

	TTA TGA TAG TTC AAT GAG AAT (X) AAG A GA GCC
5 b	CTG TTT GGT GAG GTT AGT TAA TCG TAT CTT
5 c	ATT CTC ATT GAA CTA TCA TAA AGT GCG GGT
5 d	ACGTCTCTA**GCGG**AAGAACCCACAA**CCGC**GGCTCACTT
5 e	**GCGG**TTGTGGGTTCTT**CCGC**CTTCCTCTCG
5 f	CGAGAGGAAG**GCGG**AAGAACCCACAA**CCGC**

1.5. Native gel

The hybridization result was verified by native polyacrylamide gel electrophoresis (PAGE). In a typical experiment, samples of each building block in the correct molar ratios (final assembly 1.2 × 10^{-10} moles; 30 µL TAMg buffer) was added in 1 × TAEMg buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM $MgCl_2.6H_20$) and incubated at 95 °C for 10 minutes followed by slowly cooling to 5 °C over a period of 16 hours. All of the final assemblies were characterized using 8 % native polyacrylamide gel (run at constant current of 10 mA, 4°C and visualized using StainsAll®).

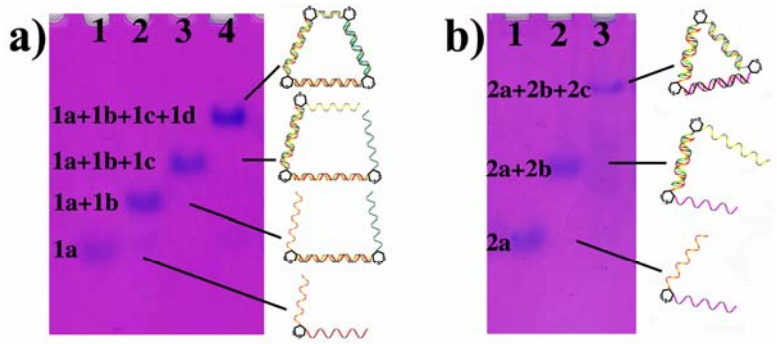


Fig. S1 Native polyacrylamide gel analysis of the assembly products. (a) Assembly of **1a-d**. The sequential addition of **1a-d** (lane 1-4) forms the trapezia structure in high yield. (b) Three building blocks **2a-c** (lane 1-3) hybridize for constructing the triangle structure.

The native polyacrylamide gel analysis was performed to prove the hybridization effects, where the single-stranded sequences **1a** and **2a** are sequentially titrated with the complementary strands **1b-d** (Fig. S1a, lanes 2-4) and **2b-c** (Fig. S1b, lanes 2-3). The assemblies of two, three, and four building blocks showed well-defined, single bands of steadily decreasing mobility. This behavior is consistent with the clean and quantitative

formation of discrete duplex assemblies containing the desired number of building blocks in each case.

1.6. Preparation of AuNP, DNA-AuNP conjugates, and AuNP constructions

Gold colloids with mean diameters of 5 nm were synthesized by the citrate/tannic acid method [1]. The citrate-stabilized gold colloids were subsequently exchanged with a negatively charged phosphine shell using bis(p-sulfonatophenyl)phenyl- phosphine dehydrate dipotassium salt. DNA-AuNP conjugates were prepared by mixing AuNPs with cyclic disulfide-modified ssDNA with a proper mole ratio and incubated for 12-48 h in PBS buffer (containing 100 mM NaCl). The method used for isolating DNA-AuNP monoconjugates, AuNP dimer and trimer from the reaction mixture is gel electrophoresis (1.5-2.5 % agarose gel at 5 V/cm, 0.5 × TBE buffer), followed by recovery of the appropriate band. Collected fractions were concentrated in Microcon 50000 MWCO centrifugal filters and hybridized with equimolar quantities of complementary conjugates to create AuNP constructions. AuNPs nanostructures were self-assembled in 1×TBE buffer with 0.3 M NaCl.

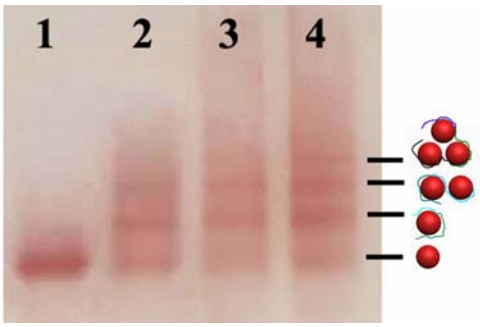


Fig. S2 Agarose gel (2.0 %, w/v) electrophoresis analysis of the conjugate products of poly-DTPA modified DNA and AuNPs. Lane 1, 5 nm AuNPs; lane 2, 3 and 4, conjugate mixture of 5 nm AuNPs and DNA modified with two, two and three DTPA, as indicated in Fig. 3a i), ii) and Fig. 4a. As shown in Fig. S2, the agarose gel electrophoresis demonstrated that the DNA functionalized nanoparticles display discrete lines with the formation of particle groupings.

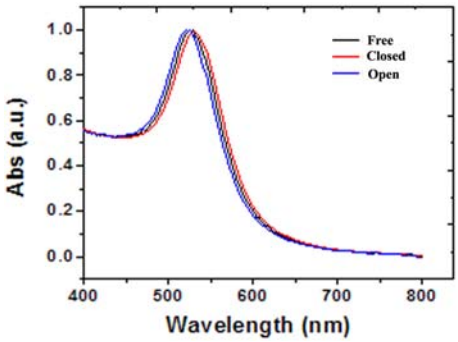


Fig. S3 UV-Vis spectra changes of the different assembly conditions. With the changes of the assembly conditions, there are little but differentiable changes in UV-Vis spectra.

[1] Handley DA. Methods for synthesis of colloidal gold. In: Hayat, M. A. Editor. Colloidal Gold: Principles, Methods and Applications. Academic press: San Diego, 1989. p. 13-22.

[2] Liepold P, Kratzmüller T, Persike N, Bandilla M, Hinz M, Wieder H, Anal. Bioanal. Chem. 2008, **391**, 1759-1772.