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Supporting Information

Efficient one-pot assembly of higher-order DNA nanostructures by chemically conjugated branched DNA

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SUPPLEMENTAL MATERIALS AND METHODS

1. General information

Materials and agents: All solvents and chemicals were purchased commercially and were used without additional purification. Every aqueous solution was made with Millipore water. All oligonucleotides were synthesized and characterized by Sangon Biotech (China). The concentration of each strand was adjusted to 100 μM. By using polyacrylamide gel electrophoresis (PAGE), the unmodified DNA strands were purified. The modified DNA strands were purified by high-performance liquid chromatography. We bought the following compunds from Sigma-Aldrich (China): Auric acid ($HAuCl_4$), Sodium citrate ($Na_3C_6H_5O_7$), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), Bis(p-sulfonatophenyl)phenyl phosphine dihydrate dipotassium salt (BSPP), and Silver nitrate (AgNO3). The DBCO-sulfo-NHS ester was provided by Sigma Aldrich (USA). We purchased the Amicon Ultra Centrifugal Filters (MWCO 3K and 100K) from Merck Millipore.

Characterizations: AFM images were obtained using a Multimode 8 atomic force microscope (Bruker) in ScanAsyst mode in air, at room temperature. TEM images were obtained using a TEM-HT7700 instrument. The UV spectrum was obtained using a UV-2600.

2. Experiment section

2.1 Synthesis of DBCO-modified DNA strand

150 μL of 100 μM (15 nmol, 1 eq.) single stranded DNA with NH_2 C6 modification in the 5' end was added into 22.5 μL of 20 mM (450 nmol, 30 eq.) DBCO-NHS solution dissolved in DMF (the DNA sequence is provided in Table S1). Then, 50 μL of Acetonitrile (ACN) and 1 μL of TEA were added subsequently. After stirring for 12 h at room temperature, the reaction mixture was precipitated in 0.3 M NaOAc and 75% ethanol at -80 ℃. Next, the excess DBCO-NHS was removed from the product (DNA-DBCO) by purification twice using 3 kDa MWCO centrifuge filters. Ultimately, for the last cross-linking step, the refined product was dissolved in 50 μL of ddH_2O.

2.2 Synthesis of branched DNA structures

A branched organic molecule with six azide groups (Di-PE-$6N_3$) was synthesized following our previous report.[1] The Di-PE-$6N_3$ was mixed with DNA-DBCO solution in three different molar ratios (1:1, 1:3, and 1:6) to obtain the branched DNA structures with different number of branches (B_n: B_2-B_6). After that, 5M NaCl was added, resulting in a final concentration of 1 M. PAGE was used to monitor the reaction while stirring it at 37 ℃ until no more products were produced, which could take up to three days. Ultimately, the chemically conjugated products B_n (where n = 2, 3, 4, 5, and 6, n represents the quantity of single-stranded DNA linked to every Di-PE-$6N_3$ molecule) were purified by PAGE and characterized by MALDI-TOF-MS.

2.3 Synthesis of tetrahedral DNA nanostructures

The DNA sequences used for the assembly of tetrahedral DNA nanostructures (TDN) are shown in Table S1.[2] The linear single-stranded DNAs (S1, S2, S3, and S4) were mixed at an equimolar ratio (1 μM) in 1×TAE/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate, pH = 8.3). The sample was maintained at 95 ℃ for 5 min and then cooled to room temperature using a PCR program for 12 h. The assembled TDN was characterized using 6% native PAGE at 4 ℃.

2.4 Preparation of higher-order DNA nanostructures

The linear single-stranded DNAs (S1*, S2, S3, and S4*) were mixed with the each B_n (B_2-B_6) in a molar ratio of 1:1 (each linear DNA : ssDNA of B_n) in 1×TAE/Mg^{2+} buffer, respectively. Then, the mixture was maintained at 95 ℃ for 5 min and cooled to room temperature using a PCR program for 12 h. The assembled higher-order DNA nanostructures were characterized by 0.8% agarose gel electrophoresis at 4 ℃.

2.5 AFM characterization

20 μL of the constructed DNA nanostructures was spotted on a freshly cleaved mica surface. After adsorbing for 5 min, the samples were washed three times with 200 μL deionized water. Then, the samples were dried at room temperature before imaging.

2.6 Synthesis of gold nanoparticles

An aqueous solution of $HAuCl_4 \cdot 3H_2O$ (1.0 mM, 250 mL) was added to a three-neck flask and heated to boiling temperature under stirring. Then, sodium citrate (38.8 mM, 25 mL) was added to the mixture. The reaction mixture was maintained in a boiling state for another 20 min before cooling to room temperature. The gold nanoparticles (AuNPs) were then washed twice with deionized water and centrifuged twice at 13000 rpm Finally, the AuNPs were redispersed in deionized water for subsequent modifications.

2.7 Modification of gold nanoparticles with thiolated ssDNAs

To increase the solubility of the AuNPs, they were first stabilized using bis(p-sulfonatophenyl)phenylphosphine (BSPP). A typical DNA modification method to increase concentration of sodium salt was adopted. AuNPs and thiolated ssDNA were mixed at a molar ratio of 1:675 in 1×TBE buffer containing 100 mM NaCl, and the solution was shaken for 12 h. The concentration of NaCl in the solution was adjusted to 200 mM and the solution was shaken for another 6 h. After that, the concentration of NaCl in the solution was adjusted to 300 mM, and the solution was shaken for another 12 h. DNA-modified AuNPs were washed three times with

deionized water to remove excess ssDNA by centrifugation at 13000 rpm for 15 min. Finally, DNA-modified AuNPs were redispersed in ddH_2O.

2.8 Organizing AuNPs on higher-order DNA nanostructures

DNA-modified AuNPs were mixed with the higher-order tetrahedral DNA nanostructures at a molar ratio of 5:1 (AuNP : TDN of B_nTDN) in 1.5×TAE/Mg^{2+} buffer by slow cooling (1 ℃/min) from 40 ℃ to 20 ℃ for six cycles. The co-assembled products (B_nTDN-AuNP) were purified by 0.6% agarose gel electrophoresis.

2.9 TEM characterization

10 μL of gold nanoparticles-modified higher-order DNA nanostructures (B_nTDN-AuNP) were spotted on a fresh copper grid. The sample was removed after adsorption for 10 minutes. Then, 10 μL of the uranyl acetate solution was adsorbed onto a grid for 2 minutes. Finally, the samples were dried at room temperature prior to imaging.

SUPPLEMENTAL FIGURES

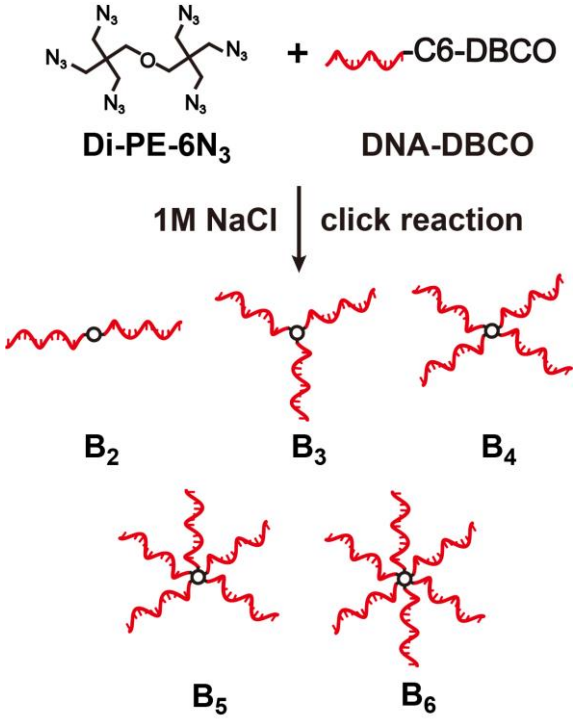

Fig. S1 Synthetic route for the construction of branched DNA structures (B_n: B_2-B_6).

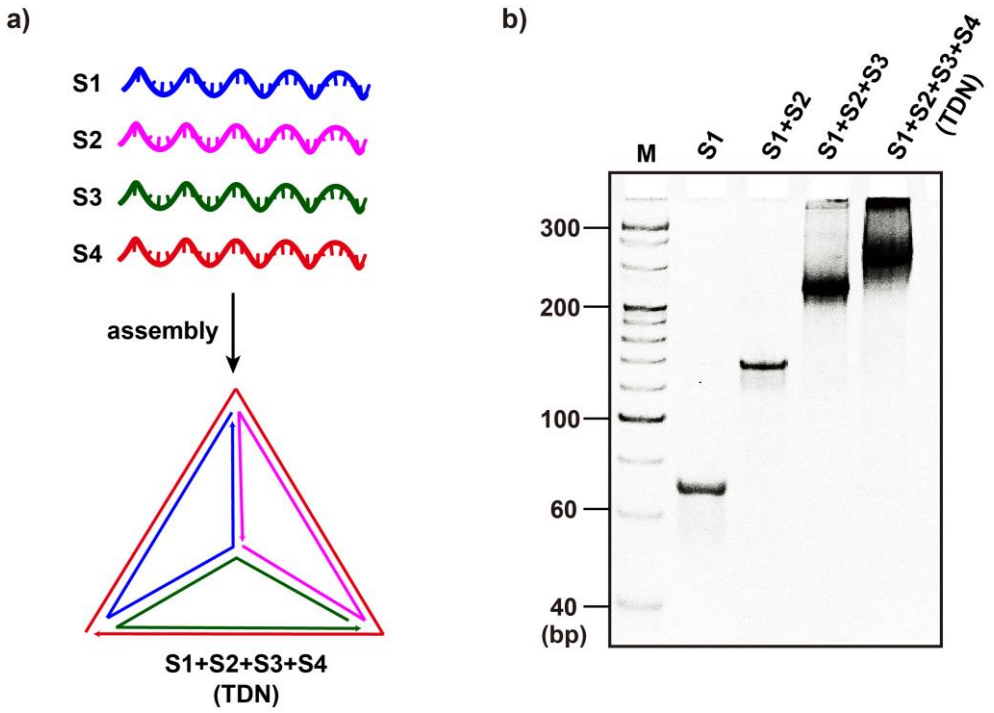

Fig. S2 (a) Illustration of the assembly of a tetrahedral DNA nanostructure (TDN). (b) 6% native PAGE analysis of step-by-step construction of TDN, M: DNA marker.

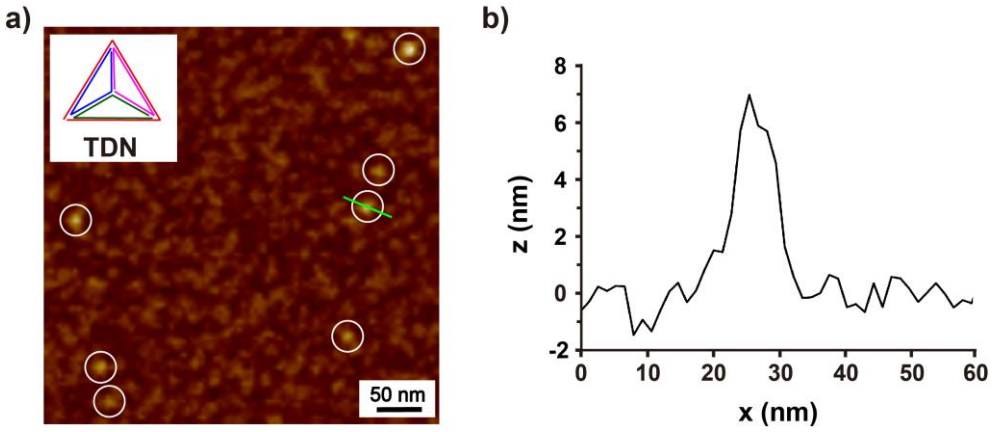

Fig. S3 (a) AFM image of tetrahedral DNA nanostructure (TDN), scale bar: 50.0 nm. (b) Height analysis of TDN.

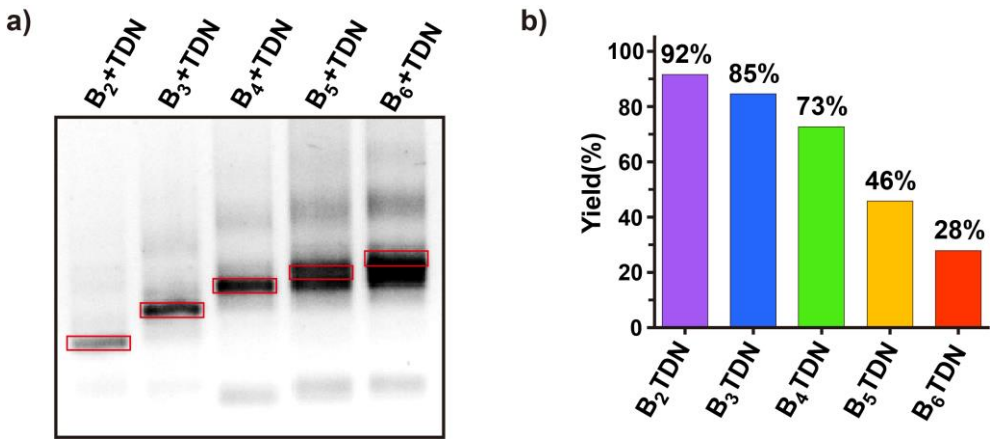

Fig. S4 (a) 0.8% agarose gel electrophoresis analysis of the unpurified higher-order tetrahedral DNA nanostructures (B_n+TDN). (b) Assembly yield of higher-order tetrahedral DNA nanostructures (B_nTDN).

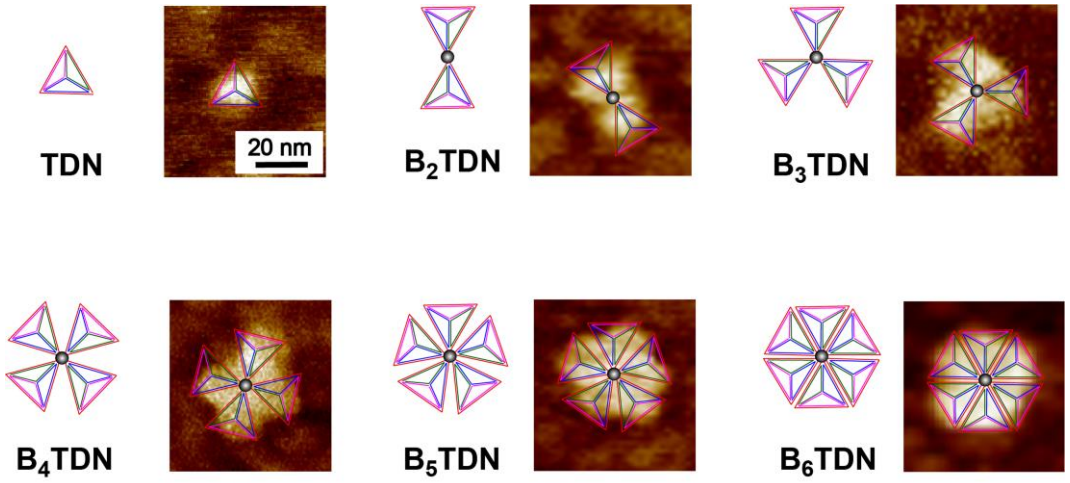

Fig. S5 Magnified AFM images of the higher-order DNA nanostructures (B_nTDNs), scale bar: 20 nm.

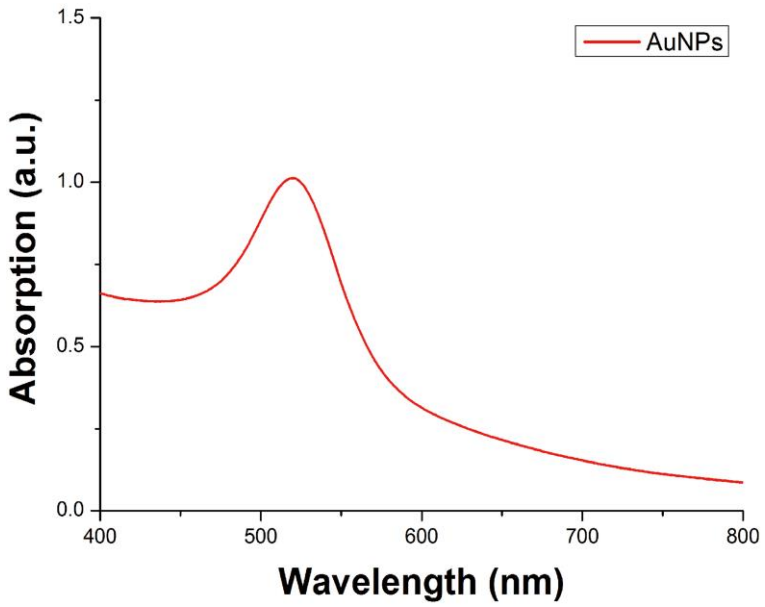

Fig. S6 UV-Vis spectra of gold nanoparticles (AuNPs).

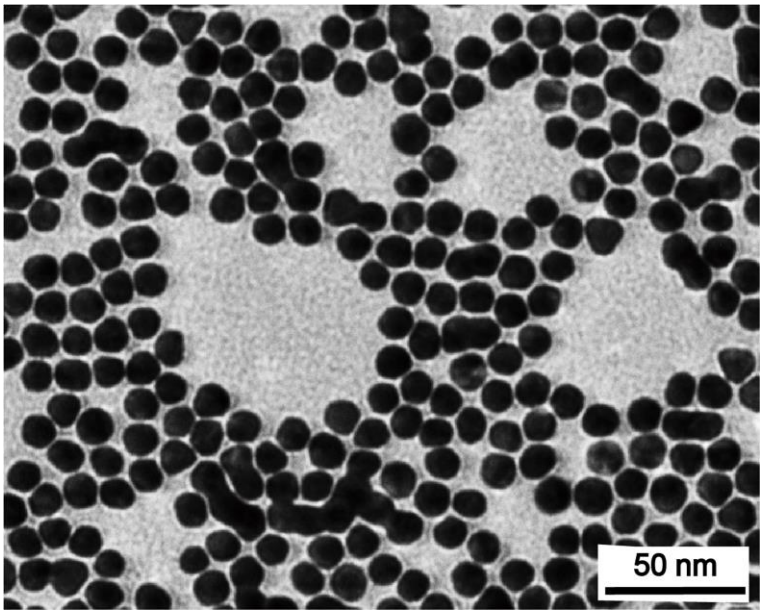

Fig. S7 TEM image of gold nanoparticles (AuNPs), scale bar: 50 nm.

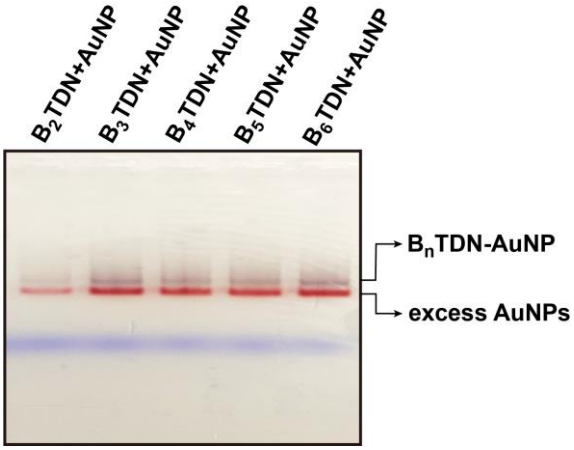

Fig. S8 0.6% agarose gel electrophoresis analysis of the unpurified gold nanoparticles loaded higher-order tetrahedral DNA nanostructures (B_nTDN-AuNP).

Full raw data of gels

Fig. 1a

Fig. 2a

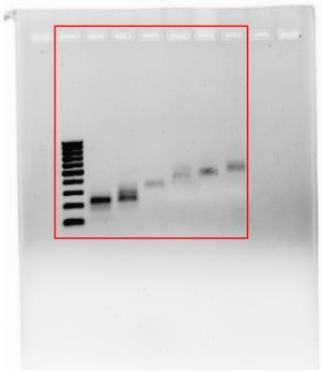

Fig. S2b

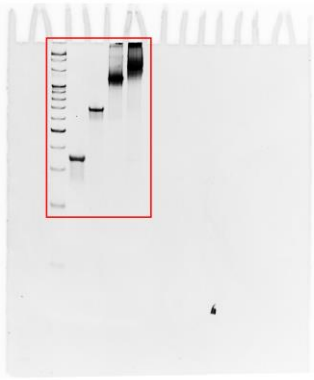

Fig. S4a

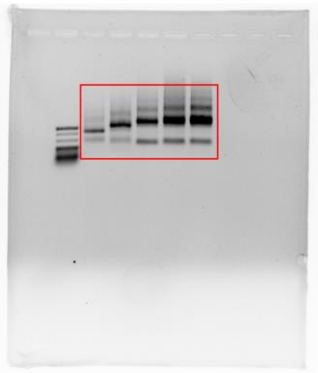

Fig. S8

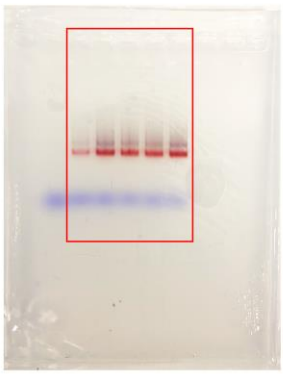

Table S1 DNA sequences for construction of higher-order DNA nanostructures.

Name	Sequence (5'-3')
DNA-NH_2	NH_2-TTTTTTTTTTAACATCTAGTACATGTCTAGTCAGTATCAT
S1	AGATACTGACTAGACATGTACTAGATGTACGATTACAGCTTGCTACACG ATCTACTAGTGGGAATTCAGACTTAGGAATGTTCG
S2	ATTTATCACCCGCCATAGTAGAGTTGACAACTAGATGTATGTCTAGATAA TCACTAACGAACATTCCTAAGTCTGAATTCCCAC
S3	AGTCAACTCTACTATGGCGGGTGATAAAAAGTAGATCGTGTAGCAAGC TGTAATCGATAATCACTAGATACTGACTAGACATGT
S4	AACATCTAGTACATGTCTAGTCAGTATCATAGTGATTATCTAGACATACA TCTAGTAACATGTCTAGTCAGTATCTAGTGATTA
S1*	AAAAAAAAAAAAAAACCCAAGATACTGACTAGACATGTACTAGATGTA CGATTACAGCTTGCTACACGATCTACTAGTGGGAATTCAGACTTAGGAA TGTTCG
S4*	AGTGATTATCTAGACATACATCTAGTAACATGTCTAGTCAGTATCTAGTG ATTA
DNA-SH	TTTTTTTTTTTTTTTAGCG-SH

SUPPLEMENTAL REFERENCES

1 Y. Li, J. Pei, X. Lu, Y. Jiao, F. Liu, X. Wu, J. Liu and B. Ding, *J. Am. Chem. Soc.*, 2021, **143**, 19893.

2 L. Zhang, Y. Wang, J. Karges, D. Tang, H. Zhang, K. Zou, J. Song and H. Xiao, *Adv. Mater.*, 2023, **35**, e2210267.