Electronic Supplementary Material (ESI) for Chemical Communications

A New Strategy Improves Assembly Efficiency of DNA Mono-Modified Gold Nanoparticles

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S1, 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%), 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 ultra-pure MilliQ water (resistance > 18 MΩ.cm).

S2, Synthesis[1] and Stabilization[2] of 5 nm Gold Nanoparticles

1 Prepare 1 mL of 4% $HAuCl_4$ solution in water.

2 Add 375 µL of 4% $HAuCl_4$ solution plus 500 µL of 0.2 M K_2CO_3 to 100 mL water, cooled to 4 °C in fridge. Mix well.

3 Dissolve sodium borohydride ($NaBH_4$) in water to get a concentration of 0.5 mg/mL. Prepare fresh.

4 Add five 1-mL aliquots of the $NaBH_4$ solution to the $HAuCl_4/K_2CO_3$ suspension with rapid stirring. A color change from bluish-purple to reddish orange is observed as the additions take place.

5 Stir for 5 min at 4 °C after the completion of $NaBH_4$ addition.

6 In order to get gold nanoparticles (AuNPs) with narrow size distribution, centrifuge the solution at 1.2×10^4 rpm for 10 min and collect supernatant.

7 BSPP (20 mg) was added to the AuNPs solution (50 mL, particle density 3×10^{15}/mL) and the mixture was shaken overnight at room temperature.

8 Sodium chloride (NaCl, solid) was added slowly to this mixture while stirring until the color changed from deep burgundy to light purple. The resulting mixture was centrifuged at 3000 rpm for 30 min and the supernatant was carefully removed with a pipette. AuNPs were then resuspended in 1 mL solution of BSPP (250 mg/L). Upon mixing with 1 mL methanol, the mixture was centrifuged, the supernatant was removed and the AuNPs were resuspended in 1 mL solution of BSPP (250 mg/L).[2,3]

9 The concentration of the AuNPs was estimated from the optical absorbance at ~ 520 nm by UV spectroscopy.[4]

S3, Synthesis of Thioctic Acid-DNA

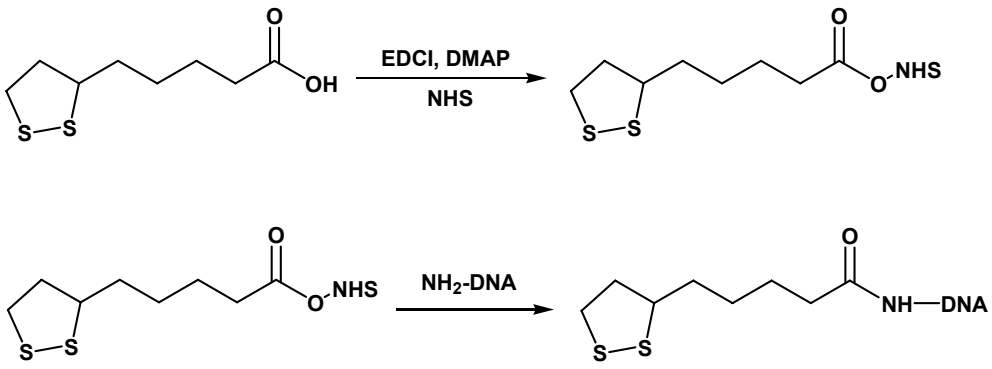


Figure S1. Scheme of synthesis of thioctic acid-DNA.

First, thioctic acid was modified with NHS. 2.06 g (10 mmol) thioctic acid, 1.26 g (11 mmol) NHS and 50 mg (0.41 mmol) DMAP were dissolved in 50 mL CH_2Cl_2. 2.3 g (12 mmol) EDCI was added and the solution was stirred at room temperature for 6 hrs. The solution was washed with H_2O (50 mL) and brine (50 mL), and the organic phase was dried with Na_2SO_4. Crude product was purified with column chromatography (silica, 200-300 mesh, ethyl acetate was used as eluant). 1.84 g yellow solid was obtained (yield 61%).

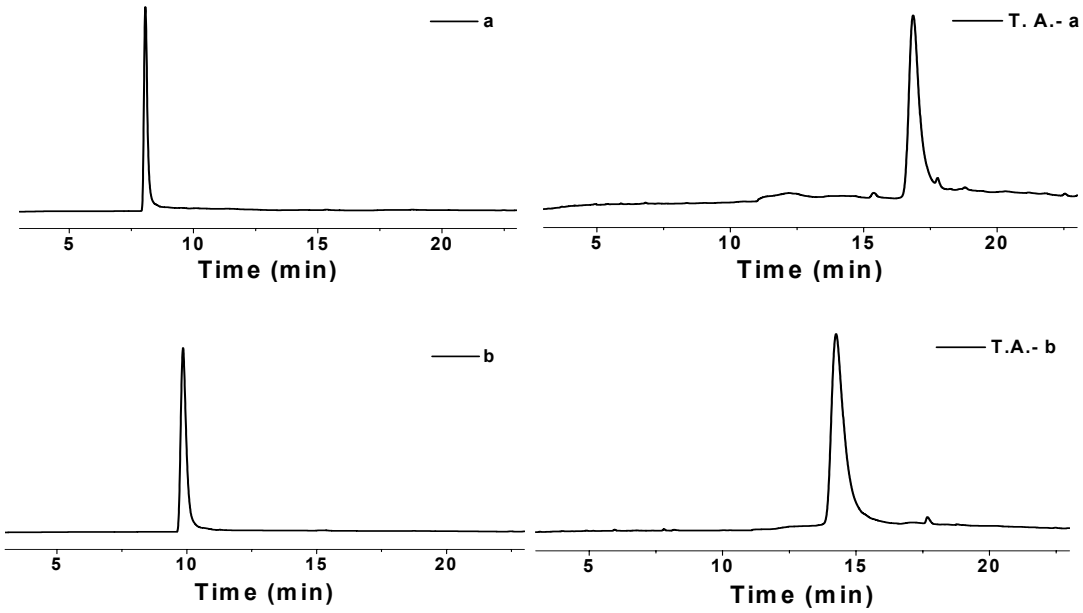
Then thioctic acid ester was added in excess (molar ratio of thioctic acid ester and amine linker modified DNA at more than 200:1) to amine linker modified DNA at a molar ratio of more than 200, in 50 mM pH 7 acetic acid-triethylamine (TEAA) buffer containing 70% acetonitrile (ACN) and 30% water. The mixture was incubated at room temperature over 10 hrs.[2]

The preparation of pH 7 TEAA could be found in Oligonucleotide Purification Application Notebook: www.waters.com/webassets/cms/library/docs/000396en.pdf

S4, Purification of Thioctic Acid-DNA by HPLC

Reverse-phase HPLC (Aglient 1200, Column type C_{18}, Gradient 40 min, 5%-25% ACN, buffer A: TEAA, 0.1 M, pH 7; buffer B: ACN) was used to purify the product. Fractions containing thioctic acid-DNA was collected, dried to powder and resuspended in water.[2]

The left and right spectra of each row represent the original NH_2-DNA and thioctic acid-DNA, respectively. Icon on the upper right corner is the abbreviation of each sample. For example, **a** represents DNA with sequence **a**, and **T. A.-a** represents thioctic acid-DNA with sequence **a**. We can see that amine modified DNA was eluted out of the column at the retention time less than 10 min, and thioctic acid-DNA was eluted out of the column at about 5 min later. The peak is clean, suggesting the yield is nearly 100%. Therefore, we just added water to precipitate the unreacted thioctic acid-NHS which hardly dissolves in water, and then removed it by filtration. Then the solution containing thioctic acid-DNA was dried to powder and resuspended in water.



S5, MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometer) of Thioctic Acid-DNA

Table 1. Summary of molecular weights of thioctic acid-DNA.

Name of the Sample	Calculated *m/z*	MALDI-TOF
Thioctic acid-**a**	4962	4967
Thioctic acid-**b**	11896	11916

S6, Preparation of ss-AuNPs and ds-AuNPs

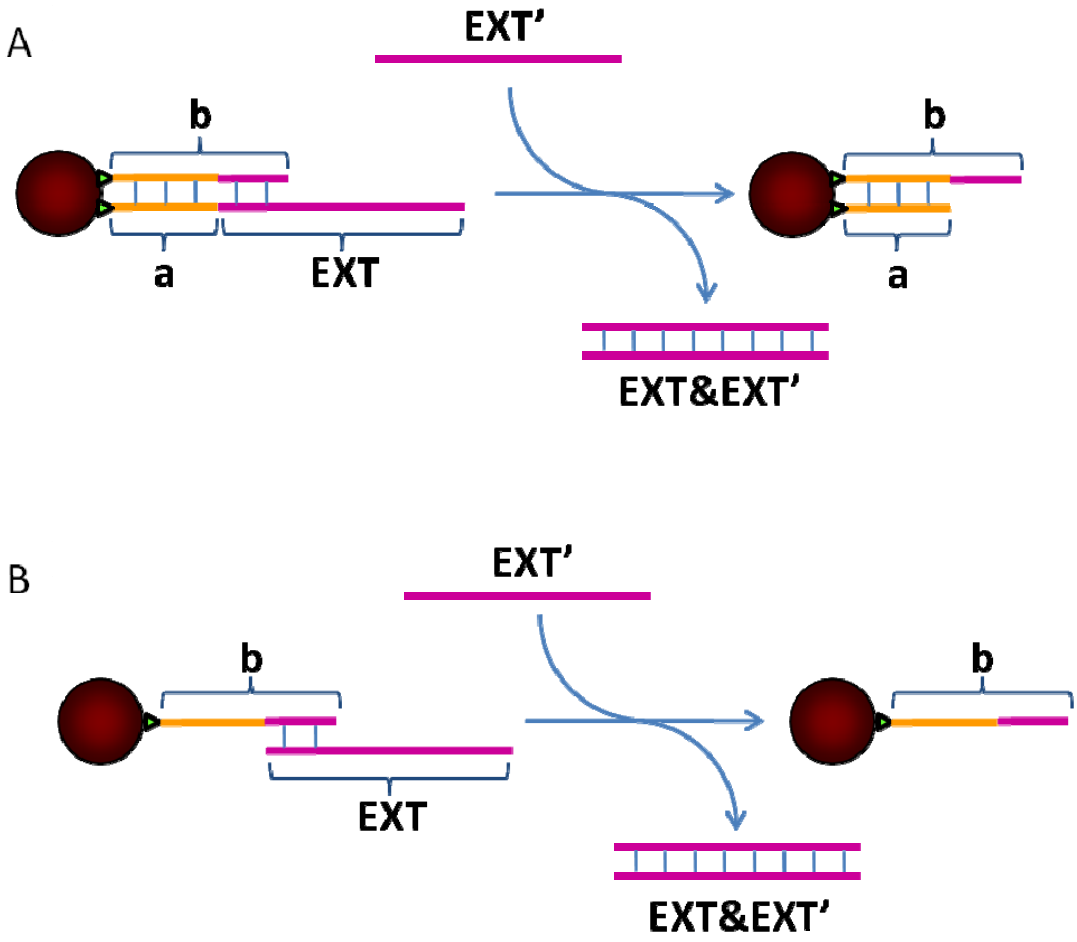


Figure S2. Schematic **EXT** isolation methods to get desired duplex **ab** modified gold nanoparticles (**ds-AuNPs**) and single strand **b** modified gold nanoparticles (**ss-AuNPs**).

According to the previous report, short strands (here, 38 bases **b** or 38 bases **b** and 15 bases **a**) were not long enough to isolate DNA modified 5 nm gold nanoparticles in agarose gel. Here we used the method that Sleiman[5,6] introduced (Figure S2) using one long strand (here, **EXT**) which was partially complementary to **b** to help to increase the separation between conjugated and unconjugated AuNPs by electrophoresis, followed by strand displacement of **EXT** by its complementary DNA strand, **EXT'**.

EXT was removed by adding excessive complementary strands **EXT'** and incubated at ambient temperature for 2 hrs in the solution of 0.5×TBE and 100 mM NaCl. The complementary duplexes (**EXT & EXT'**) were washed off by centrifuging at 1.2×10^4 rpm for 35 min. **EXT & EXT'** duplexes remained in the supernatant, and the desired AuNPs modified by **b** strand (**ss-AuNPs**) or **ab** double strand (**ds-AuNPs**) precipitated to the bottom. Remove the supernatant, and add buffer (0.5×TBE and 100 mM NaCl) to

rinse the precipitates. Repeat three times.

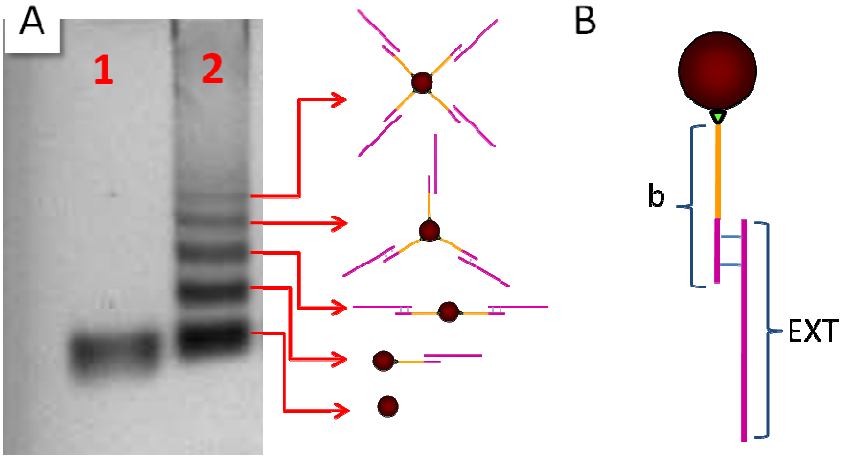


Figure S3, A, Agarose gel electrophoresis (3% agarose gel, 0.5×TBE, 6.7 V/cm, 60 min, 4 °C) of isolating DNA templates modified AuNPs. Left, the gel image under white light. Right, scheme of the desired products. The second to the lowest band was the preliminary products of **ss-AuNP** hybrized with **EXT**. **EXT** was removed following the same procedure in the section of **S6**. B, Schematic preliminary products of **ss-AuNP** hybrized with **EXT**.

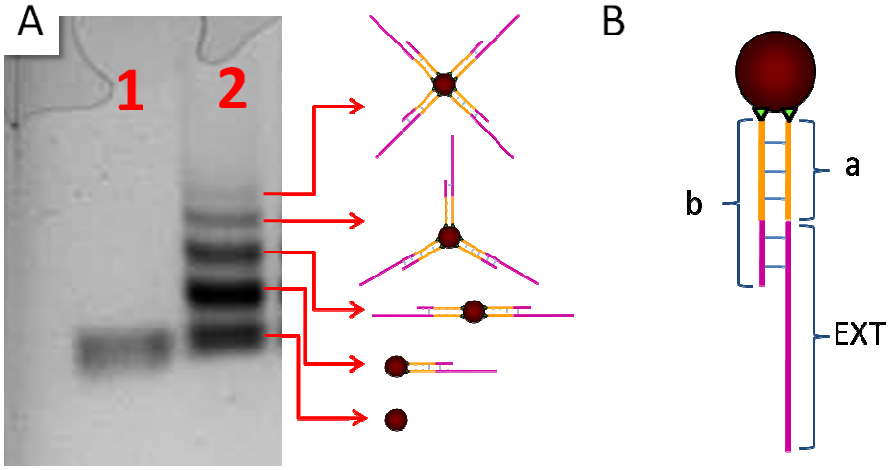


Figure S4. A, Agarose gel electrophoresis (3% agarose gel, 0.5×TBE, 6.7 V/cm, 60 min, 4 °C) of isolating DNA templates modified gold nanoparticles. Left, the gel image under white light. Right, scheme of the desired products. The second to the lowest band was the preliminary products of **ds-AuNP** with **EXT**. **EXT** was removed following the same procedure in the section of **S6**. B. Schematic preliminary products of **ds-AuNP** hybrized with **EXT**.

S7, Construction of Y-Scaffold DNA

The Y-scaffold was constructed by three ssDNA (Figure S5), Y_A, Y_B, Y_C is partially complementary in pairs and all of them have sticky ends shown in black which could complementary to **b** shown in magenta bounded to **ss-AuNP** and **ds-AuNP**. Agarose gel image (Figure S6) show details of components, Y_A, Y_B, Y_C, Y_A+Y_B, Y_B+Y_C, Y_A+Y_C, Y_A+Y_B+Y_C (Y-scaffold).

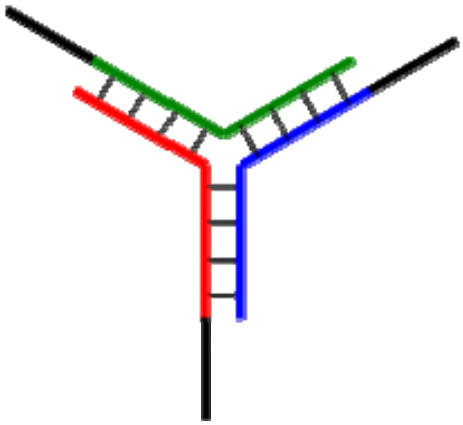


Figure S5. Schematic structure of Y-scaffold.

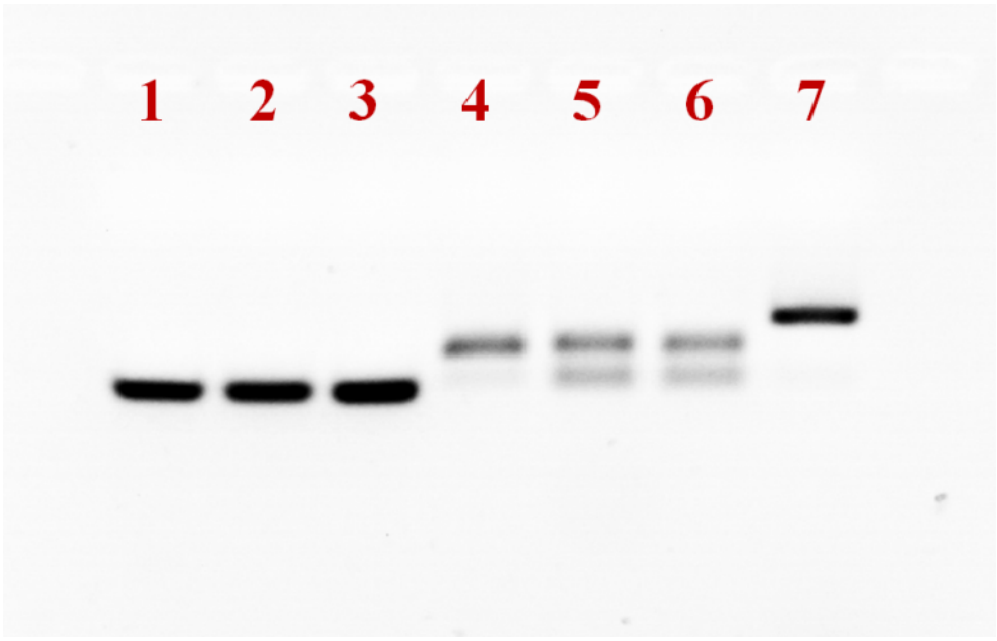


Figure S6. Agarose gel electrophoresis (3% agarose gel, 0.5×TBE, 8.3 V/cm, 80 min, 4 °C) of Y-scaffold. Lane 1, Y_A; Lane 2, Y_B; Lane 3, Y_C; Lane 4, Y_A+Y_B; Lane 5, Y_B+Y_C; Lane 6, Y_A+Y_C; Lane 7, Y_A+Y_B+Y_C (Y-scaffold).

S8, Agarose Gel Electrophoresis of Assemblies of ss-AuNPs or ds-AuNPs with Y-Scaffold and Optical Density Analysis

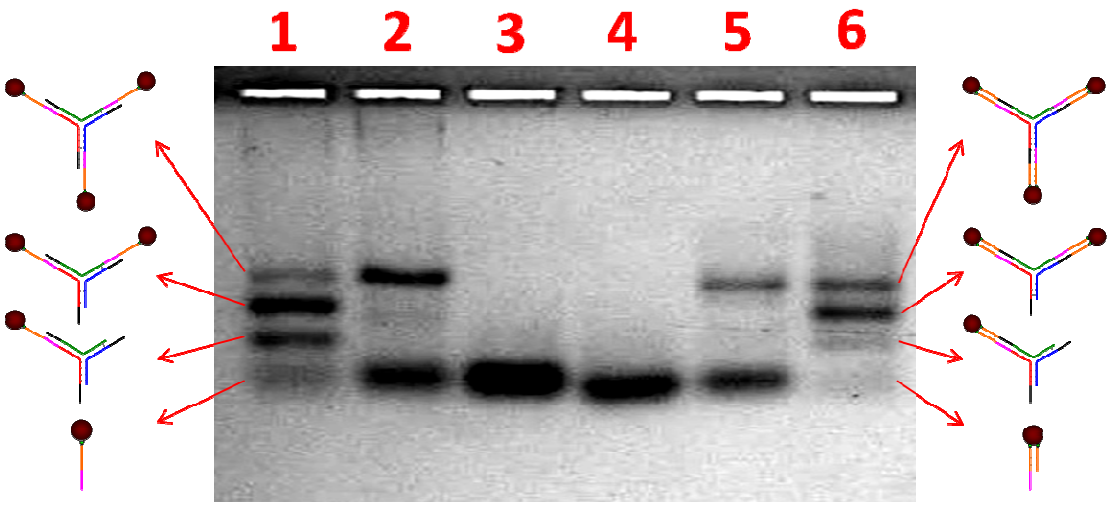


Figure S7. Agarose gel electrophoresis (3% agarose gel, 0.5×TBE, 6.6 V/cm, 48 min, 4 °C) of assemblies of **ss-AuNPs** or **ds-AuNPs** with Y-scaffold. Lane 1, ratio (sticky ends ratio of Y-scaffold and mono-AuNPs, similarly hereinafter) of Y-scaffold and **ss-AuNP** at 1:1 ; lane 2, ratio of Y-scaffold and **ss-AuNP** at 0.2:1; lane 3, free **ss-AuNPs**; lane 4, free **ds-AuNPs**; lane 5, ratio of Y-scaffold and **ds-AuNP** at 0.2:1; lane 6, ratio of Y-scaffold and **ds-AuNP** at 1:1. All lanes were loaded with the same amount of mono-AuNPs but different amounts of Y-scaffold.

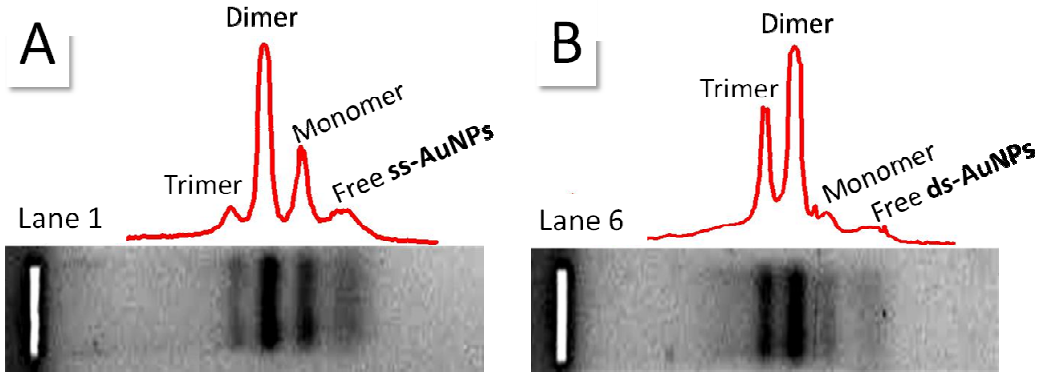


Figure S8. Optical density analysis of Lane 1 and 6 in Figure S7 by LANE 1D Analyzer V4.0 software (Beijing Sage Creation). From left to right, four discrete bands in A and B correspond to trimer, dimer, monomer and free mono-AuNPs, respectively. The trend map was shown above the gel image.

S9, TEM Images of Assemblies of ss-AuNPs or ds-AuNPs with Y-Scaffold

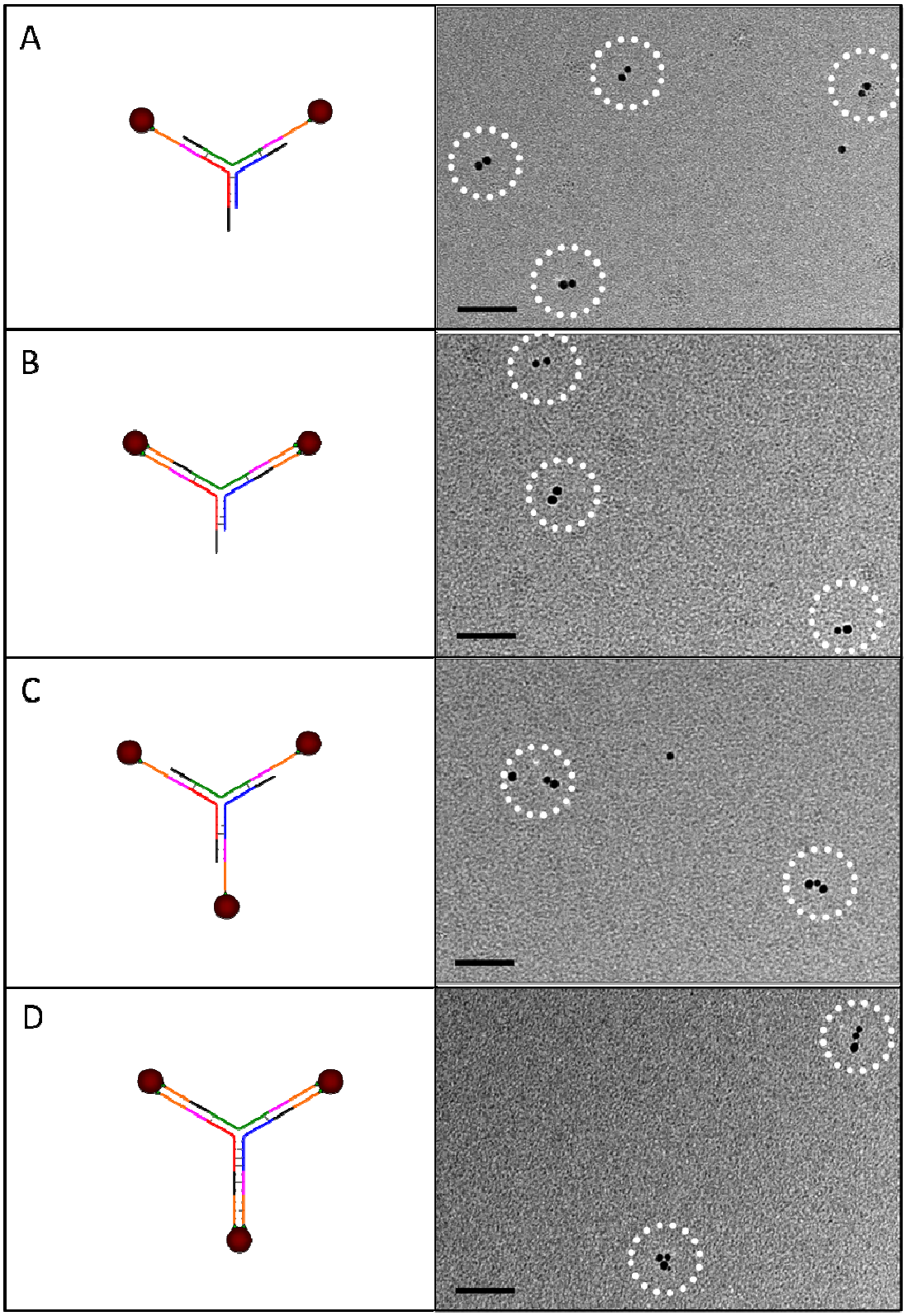


Figure S10. TEM images of assemblies of **ss-AuNPs** or **ds-AuNPs** with Y-Scaffold. A) Dimer, 2**ss-AuNPs**/Y-scaffold; B) Dimer, 2**ds-AuNPs**/Y-scaffold; C) Trimer, 3**ss-AuNPs**/Y-scaffold; D) Trimer, 3**ds-AuNPs**/Y-scaffold. The scale bar is 50 nm.

S10, DNA Sequences (5'-3')

Y_A: 5'-AGT GTT AGT GGA CCG ATG GAT GA C CTG TCT GCC TAA TGT TCG TAA G-3'

Y_B: 5'-AGT GTT AGT GGA CCG ATG GAT GA C TTA CGA CGC ACA AGG AGA TCA TGA G-3'

Y_C: 5'-AGT GTT AGT GGA CCG ATG GAT GA C TCA TGA TCT CCT TTA GGC AGA CAG G-3'

EXT: 5'-AGT GTT AGT GGA CCG ATG GAT GAT AGG ACG ACT TCT TGT TGT AGC ACG ACT TGG ACG CA-3'

EXT': 5'-TGC GTC CAA GTC GTG CTA CAA CAA GAA GTC GTC CTA TCA TCC ATC GGT CCA CTA ACA CT-3'

S11, References

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