Facile and efficient preparation of anisotropic DNA-functionalized gold nanoparticles and their regioselective assembly

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Supporting Information

Experimental Section

General chemical reagents were purchased from Sigma Aldrich and used without further purification. Amphiphilic diblock copolymers polystyrene-block-poly(acrylic acid) PS_{144}-*b*-PAA_{50}, M_n = 15000 for the PS block and M_n = 3600 for the PAA block, M_w / M_n = 1.20 was purchased from Polymer Source Inc.; 2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol (sodium salt) (PSH) was purchased from Avanti Polar Lipids; 200 mesh copper specimen grids with formvar/carbon support film (TEM grids) was purchased from Ted Pella Inc. Twenty nanometer AuNP was purchased from British BioCell International (BBI). Thirteen nanometer AuNP were prepared following literature procedures by sodium citrate reduction of $HAuCl_4$.[17] DNA oligomers were purchased from Integrated DNA Technologies and purified by standard desalting.

Sequence of DNA used for the synthesis and functionalization.

SH-A10: 5′ SH-AAA AAA AAA A 3′

SH-A30: 5′ SH-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA 3′

SH-R20: 5′ SH-TTT TTT TTT TCA ATG CTC TGG ATC TGT GGG 3′

D1: 5′ AAC AAT TAT ACT CAG CAA TTT TTT TTT T –SH 3′

D2: 5′ TTG CTG AGT ATA ATT GTT TTT TTT TTT T –SH 3′

D3: 5′ TTG CTG AGT ATA ATT GTTA GCGC 3′

D4: 5′ NH-TTT TTT TTT T AAG AAT TTA TAA GCA GAA 3′

D5: 5′ SH-TTT TTT TTT T TTC TGC TTA TAA ATT CTT 3′

Ultrapure water with resistivity > 18 $M\Omega \cdot cm^{-1}$ was used for all experiments.

Anisotropic particle synthesis

The encapsulation was carried out according to the procedure from our previously reported paper with minor modifications.[18] 2.5 µL of 1mM thiolated DNA was treated with 0.5 µL of 100 mM sodium acetate pH 5.5 and 1.5 µL of 10 mM tris(2-carboxyethyl)phosphine (TCEP). The mixture was left at room temperature for 1 hour before purifying once with Centricon-3k to remove excess TCEP. Citrate stabilized AuNPs (~20nm, 1.5 mL) solution was centrifuged to a volume of ~15 µL using 13100 *g* for 15 min. The TCEP treated DNA was added to the concentrated AuNP and diluted with mili-Q water to a volume of 100 µL. A reaction vial (6 mL) was added 340 µL of DMF solution, PS_{144}-*b*-PAA_{50} (60 µL, 4 mg/mL in DMF), AuNP-DNA mixture and hydrophobic ligand PSH (5 µL, 0.5 mg/mL in EtOH) in order. The reaction mixture would have a total final volume of 505 µL, where V_{DMF} / V_{H2O} = 4, [AuNP]= 3.45 nM, [DNA] = 4.95 µM, [PSH]= 6.77 µM. The mixture was heated at 95 °C for 2 hr, and then allowed to cool down gradually till room temperature. Similar procedures were used for other DNA strands. The nanoparticles were purified by centrifugation where 100ul of as-synthesized nanoparticles were diluted into 1400 uL mili-Q water to trap the nanoparticle in a kinetically stable state.

Functionalization/ligand exchange with selected DNA strands

To 5 µL of 1 mM thiol-DNA in Millipore water, 1 µL of 0.1 M sodium acetate buffer at pH 5.5 and 3.0 µL of 10 mM TCEP in Millipore water were added and mixed. This mixture was kept at room temperature for 1 hour and then purified by Centricon-3k to remove excess TCEP. 400 µL of as-synthesized nanoparticle were centrifuged one in water and another two times in 50% buffer A. The TCEP treated DNA was added to the purified particles and 100 µL of 50 mM Na-Citrate pH 3 was added to the mixture. The mixture was left overnight. Due to the low pH, the particles would sediment at the bottom of the tube but with sonication and addition of buffer A the particles would resuspended in solution. The particles were purified with buffer A via centrifuge 6 times to ensure no free DNA remained in the supernatant.

Buffer A: 0.1 M sodium phosphate buffer, pH 7.3, 0.05% Tween-20

Buffer B: 0.1 M sodium chloride and 0.1 M sodium phosphate buffer, pH 7.3

Assembly

For demonstrating the functionality of the asymmetric nanoparticle, excess of 5 nm AuNP/ 10 nm AuNP functionalized with D2 was added in large excess to the a-(D1)AuNP. The mixture was kept in buffer B and incubated at room temperature overnight. The excess 5 nm And 10 nm AuNP can be purified away via centrifugation with 1:1 buffer A and buffer B.

Ratiometric, Hetero and Homo Assembly.

The concentration of 13 nm a-(D2)AuNP, and 20 nm a-(D1)AuNP and 30 nm (D2)-AuNP were determined using the extinction coefficient 2.7×10^{8} $M^{-1}cm^{-1}$, 8.8×10^{8} $M^{-1}cm^{-1}$ and 4.7×10^{9} $M^{-1}cm^{-1}$ respectively. For the ratiometric system, snowman like assemblies were achieved when the ratio between the particles were controlled to be 1:1 while a ratio of 10:1 was used to give satellite structures. For hetero particle assembly, ratio of 1:1, 13nm a-(D2)AuNP and 20nm a-(D1)AuNP were used. For the homo particle assembly, D3 was added at 120 times of the particle concentration to give approximately 1:1 ratio of D1 to the linker. All the assemblies were performed in buffer B and imaged without further purification. Except for homo particle assembly, where the 0.5 M NaCl and 50 mM NaPi pH 7 were used.

DNA quantification

FAM-labeled DNA was functionalized on a-(DNA)AuNP. The AuNP concentration was first determined by UV-vis. Fluorescence of sample upon etching of the AuNP (50 µL) by KCN (10 µL, 100mM) was determined using a fluorometer. The fluorescence intensity was correlated to the DNA concentration present in the solution and divided by the number of particles to determine the number of DNA on each particle. To determine the amount of DNA that non-specifically attach to the particle, non-thiolated FAM DNA was used as a control.

Equipment and Characterizations

The particles synthesized as well as the nano-assemblies formed were analyzed using JEOL 2010LaB6/ 2100cryo transmission electron microscope (TEM) operated at 200 kV. Samples were prepared by mixing 4 µL of nanoparticle solution and 4 µL of $(NH_4)_6Mo_7O_{24}$ (40 mM) and then added onto a fomvar/carbon-coated copper TEM grid (Ted pella). $(NH_4)_6Mo_7O_{24}$ was used as a negative stain to increase the contrast

of the polymer. Nanoparticle concentrations were characterized by the absorbance at 535 nm using UV-Vis spectrophotometry (Hewlett–Packard 8453). Fluorescence measurements were carried out on a Fluoromax-2 fluorimeter (HORIBA Jobin Yvon inc., Edison, NJ) to determine the concentration of fluorophore labeled DNA.

Calculation of anisotropic particle yields.

We cannot rule out the possibility that particles that appear to be fully encapsulated may be a result of the particles viewed when the particle is aligned parallel to the beam. Particles are deemed anisotropic when the AuNP appears to be off-center from the polymer shell while AuNP which are positioned at the center of the polymer shell will be deemed fully encapsulated.

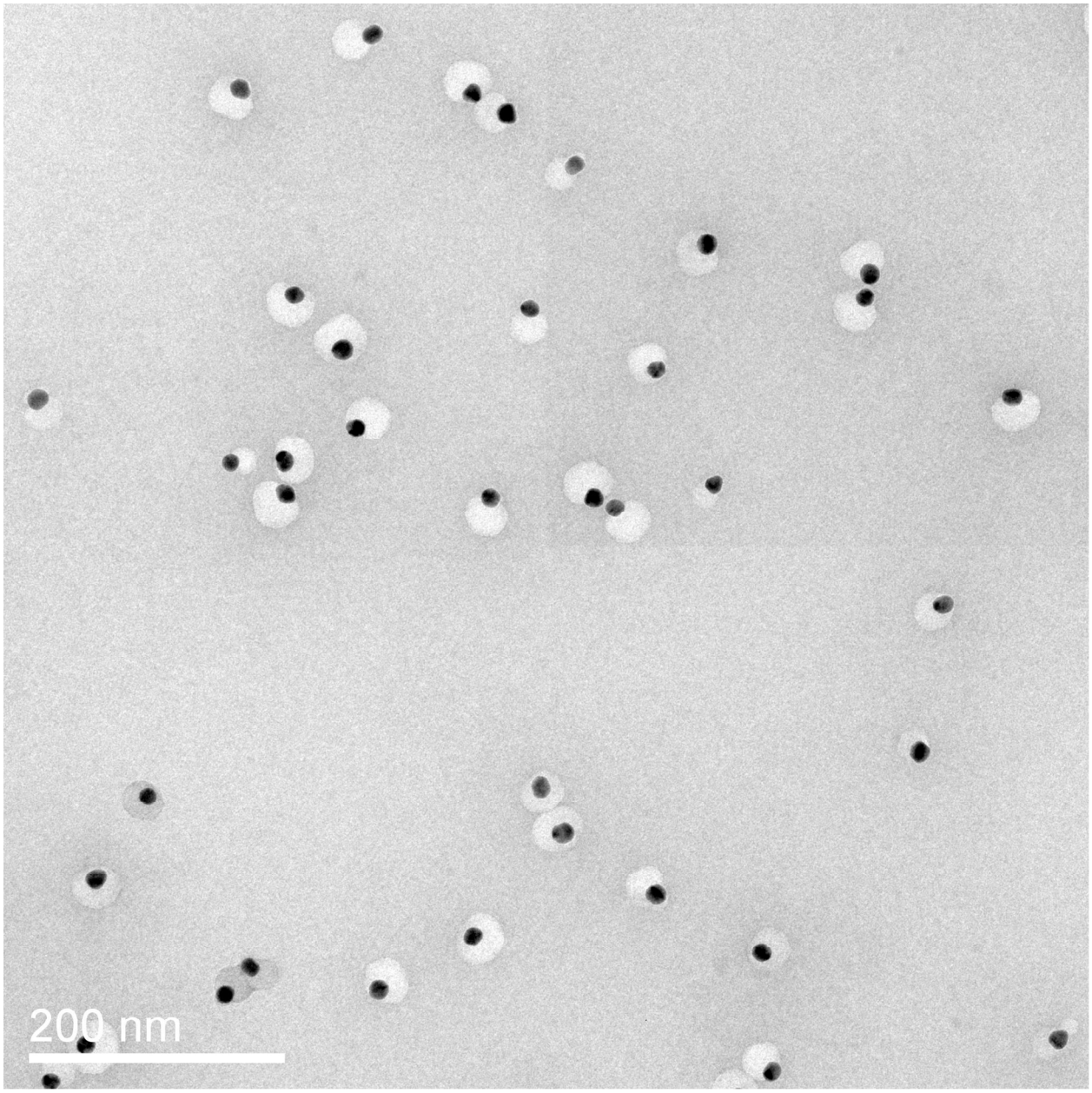


Figure S1. Large area view of a-AuNP synthesized with 15 nm AuNP and SH-A10 as the hydrophilic ligand.

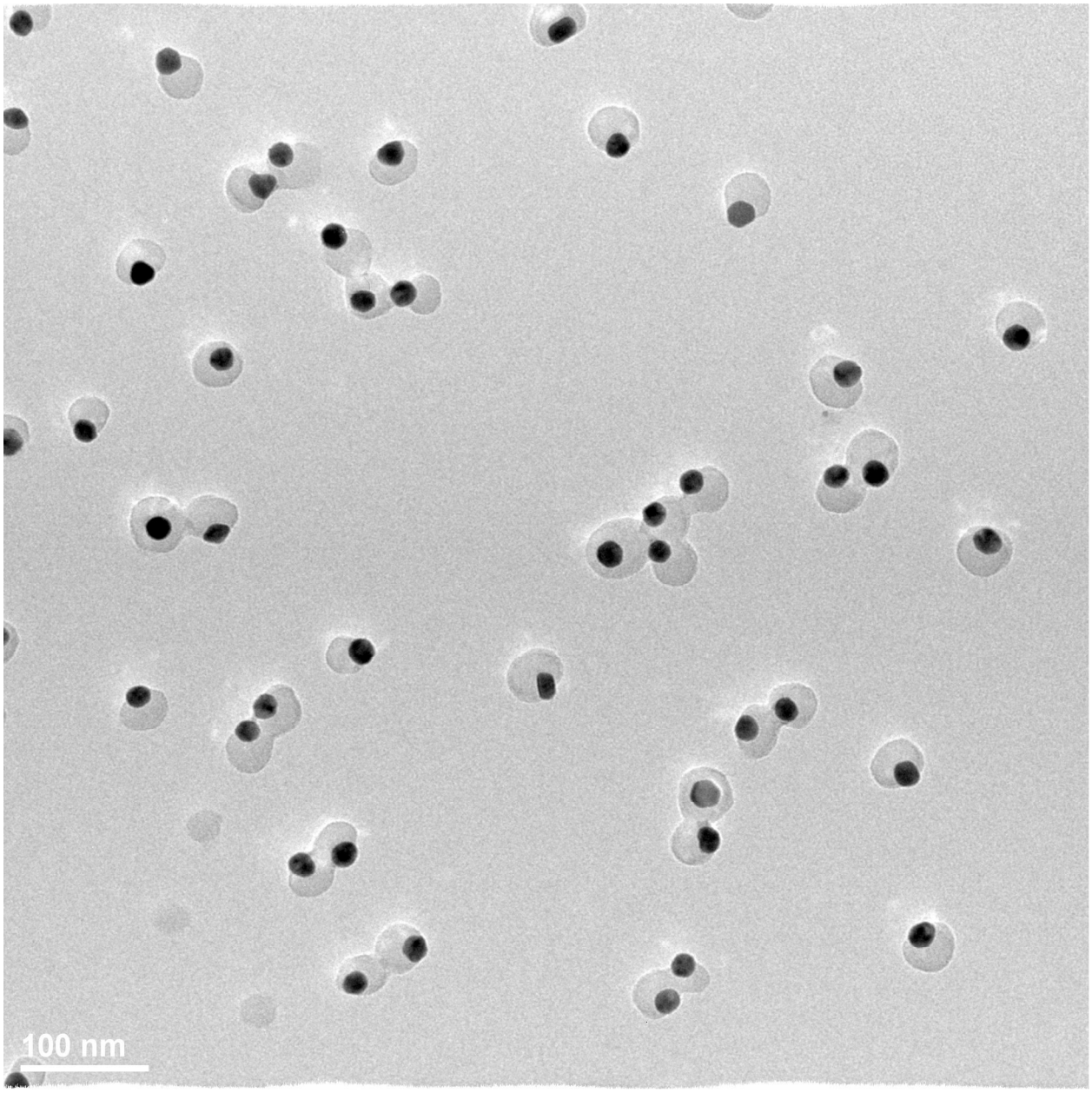


Figure S2. Large area view of a-AuNP synthesized with 20 nm AuNP and SH-A10 as the hydrophilic ligand.

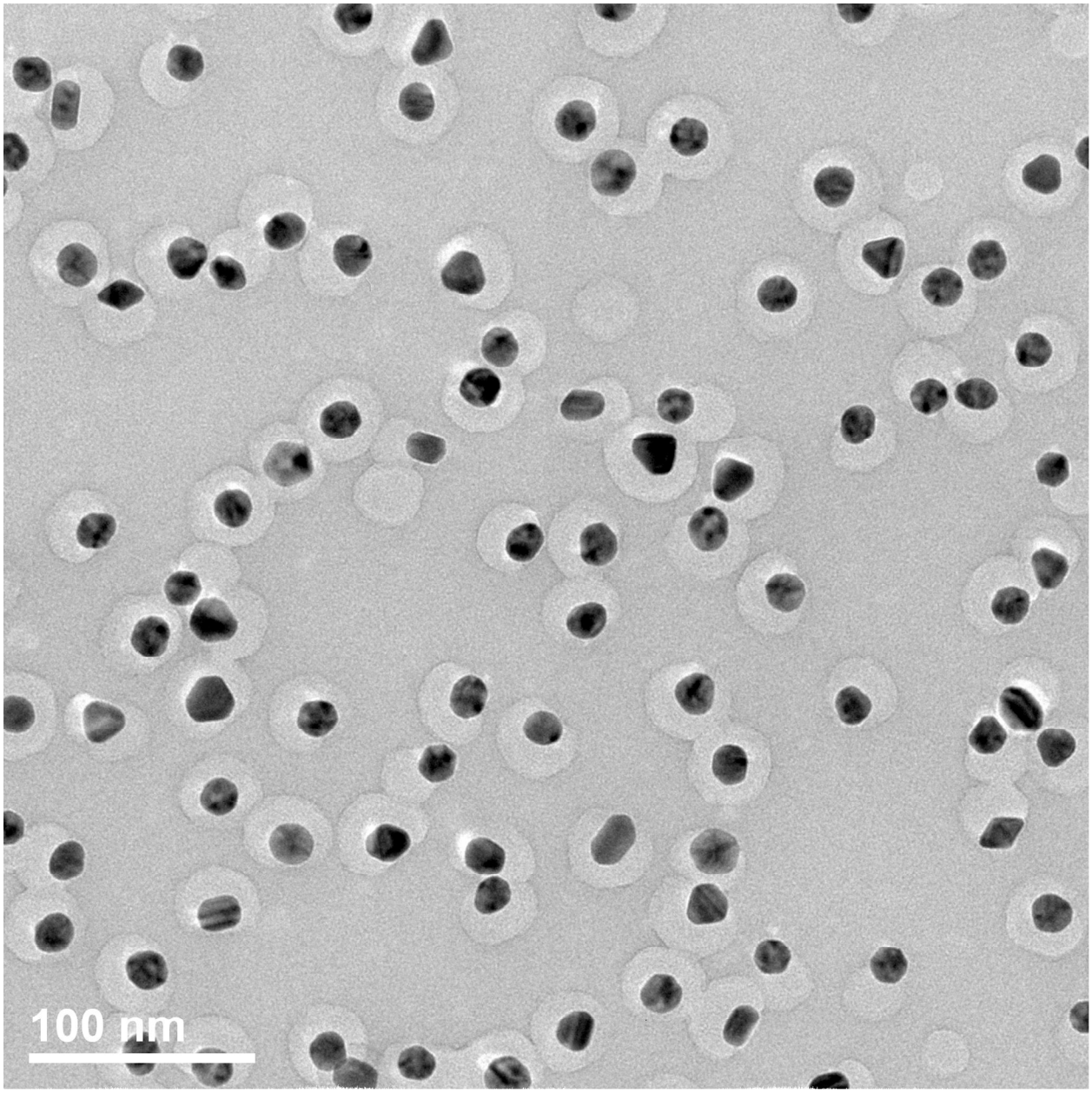


Figure S3. Large area view of a-AuNP synthesized with 20 nm AuNP and SH-A10 as the hydrophilic ligand. The ratio of PSH:DNA used is 2.2.

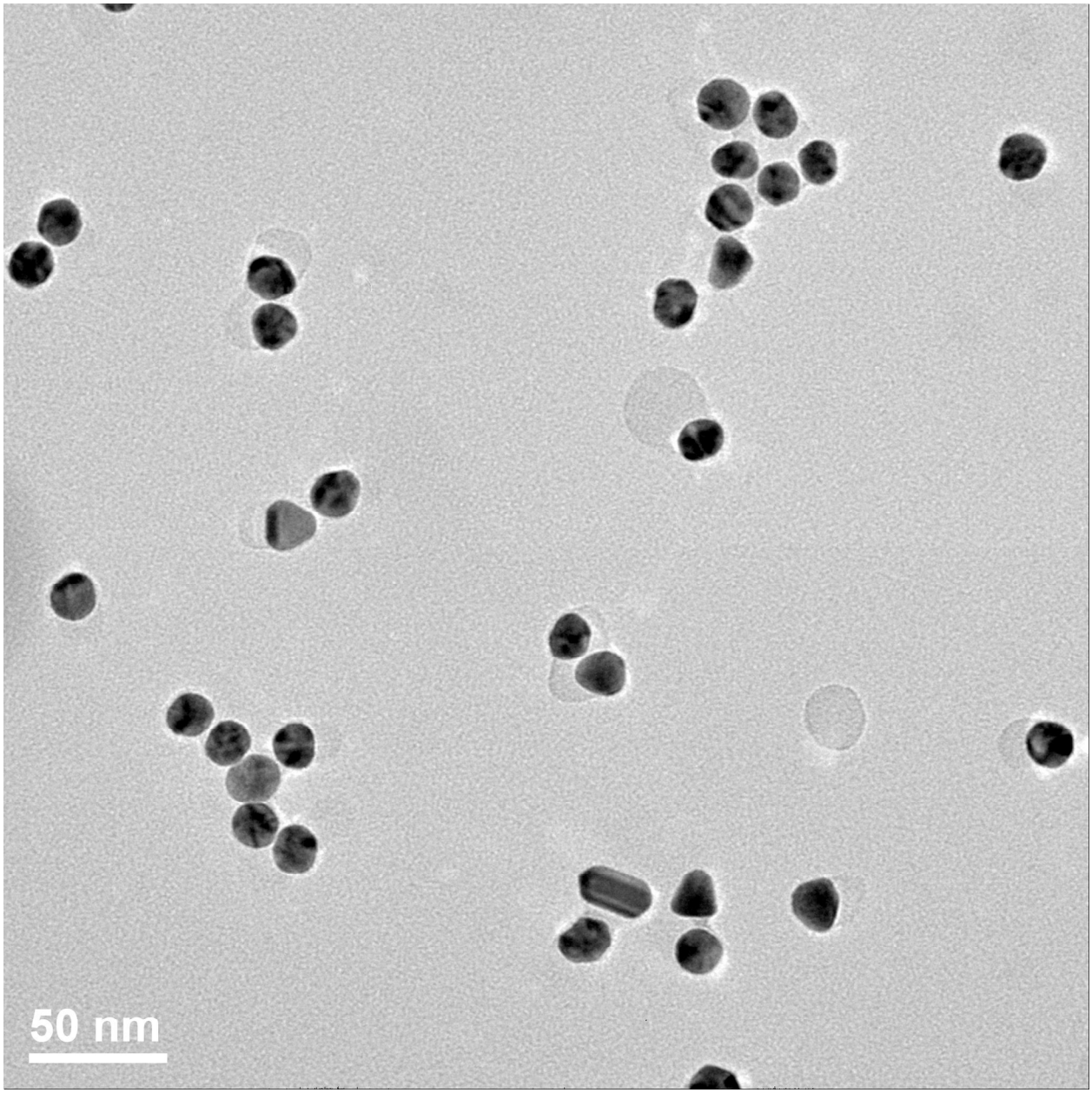


Figure S4. Large area view of a-AuNP synthesized with 20 nm AuNP and SH-A10 as the hydrophilic ligand. The ratio of PSH:DNA used is 1.

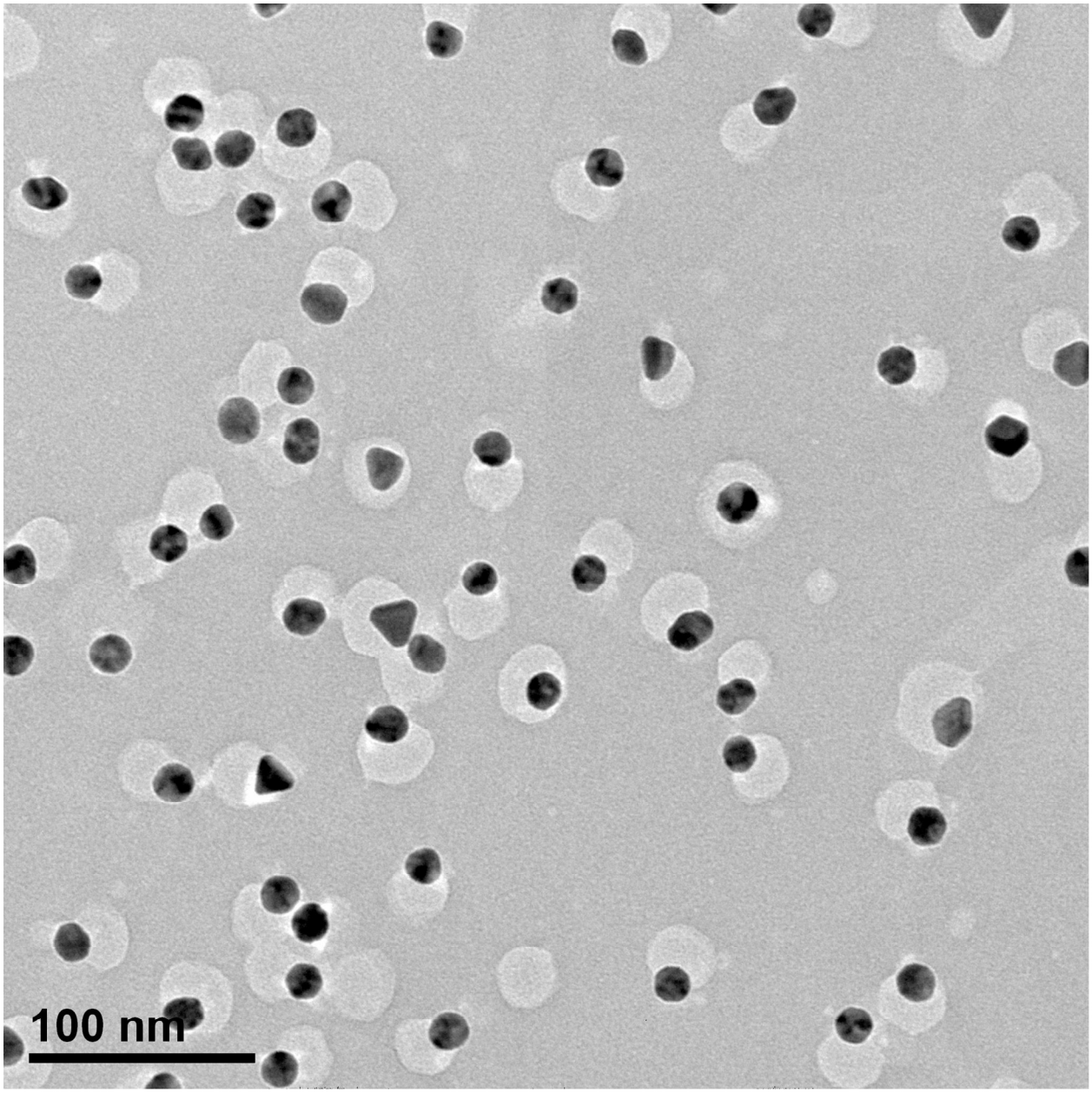


Figure S5. Large area view of a-AuNP synthesized with 20 nm AuNP and SH-A30 as the hydrophilic ligand. The ratio of PSH:DNA used is 2.2.

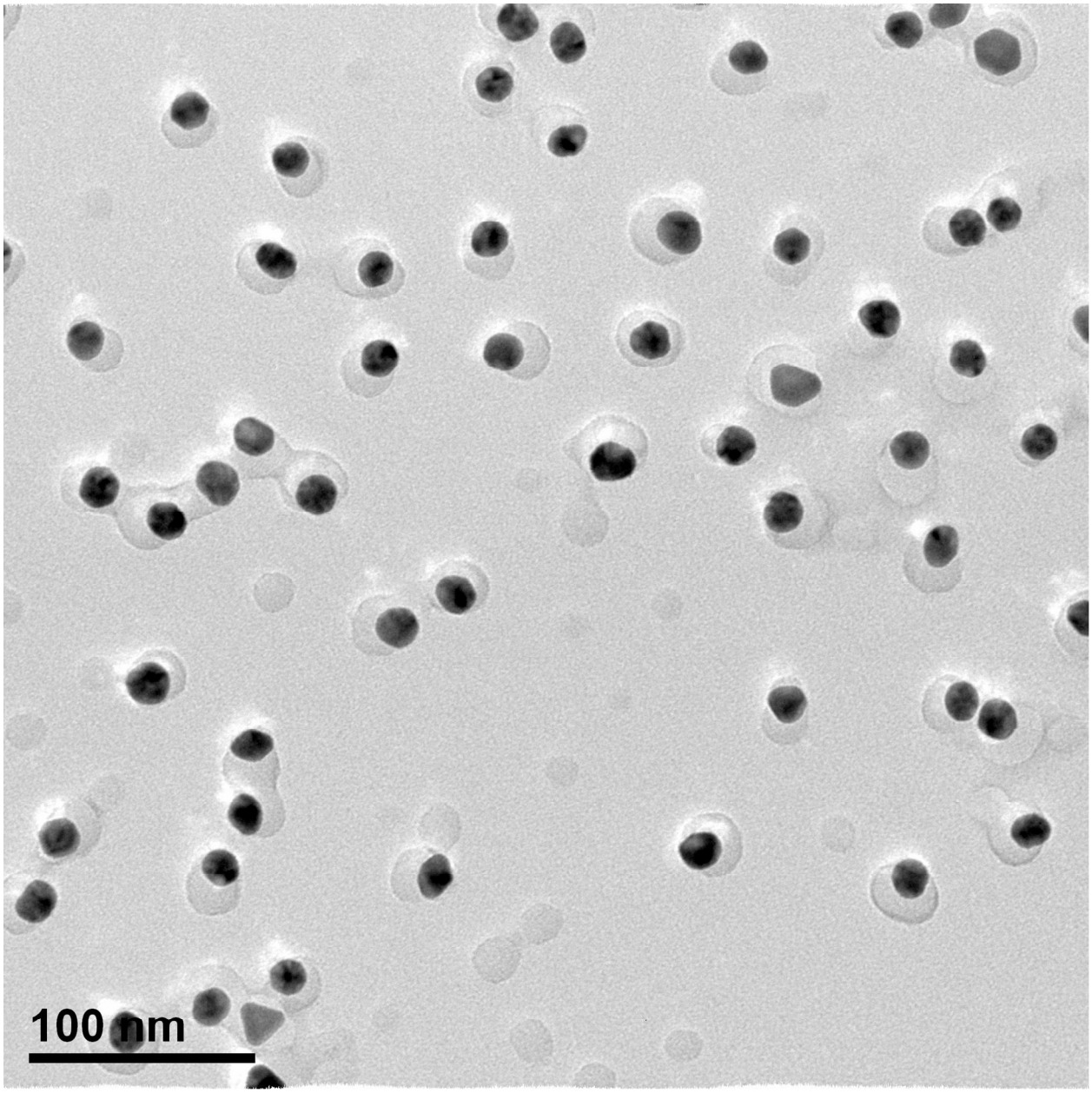


Figure S6. Large area view of a-AuNP synthesized with 20 nm AuNP and SH-R20 as the hydrophilic ligand. The ratio of PSH:DNA used is 5.6.

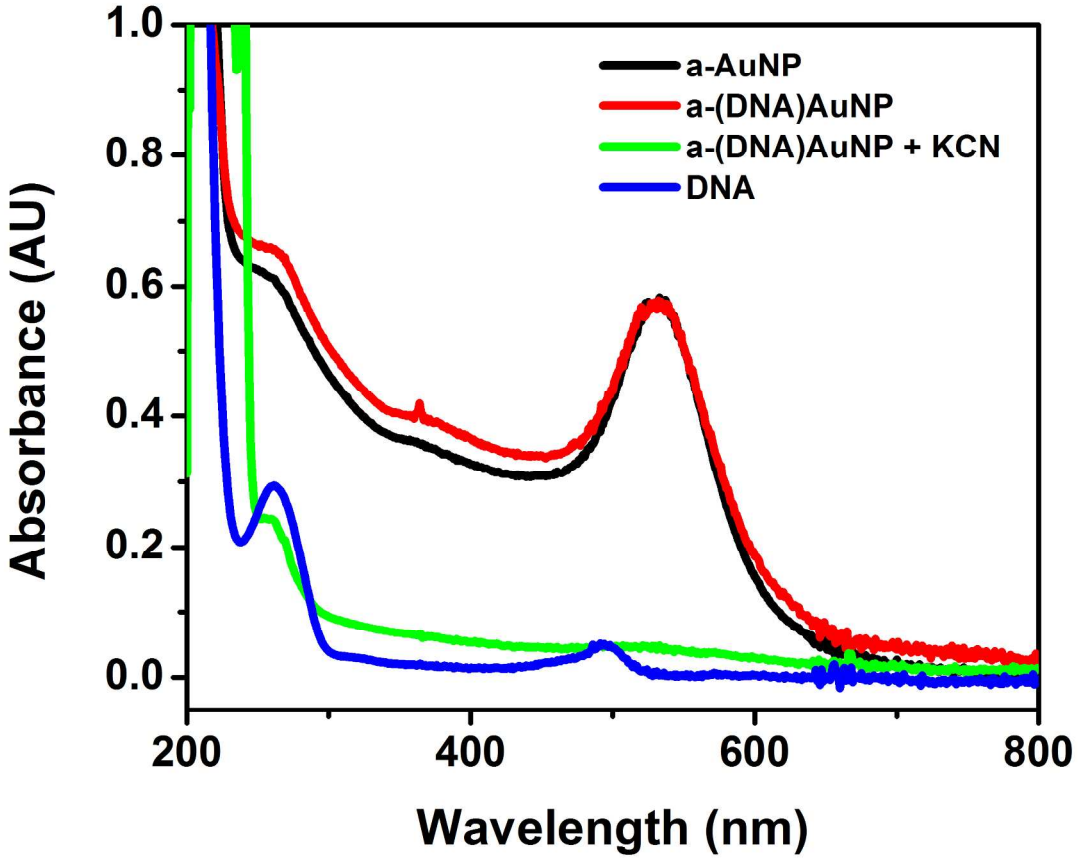


Figure S7. UV-vis absorbance for a-AuNP before and after ligand exchange with desired DNA. Also shown is the absorbance of a-DNA-AuNP after incubation with KCN to ensure all AuNP has been etched away and quantification of DNA can be carried out.

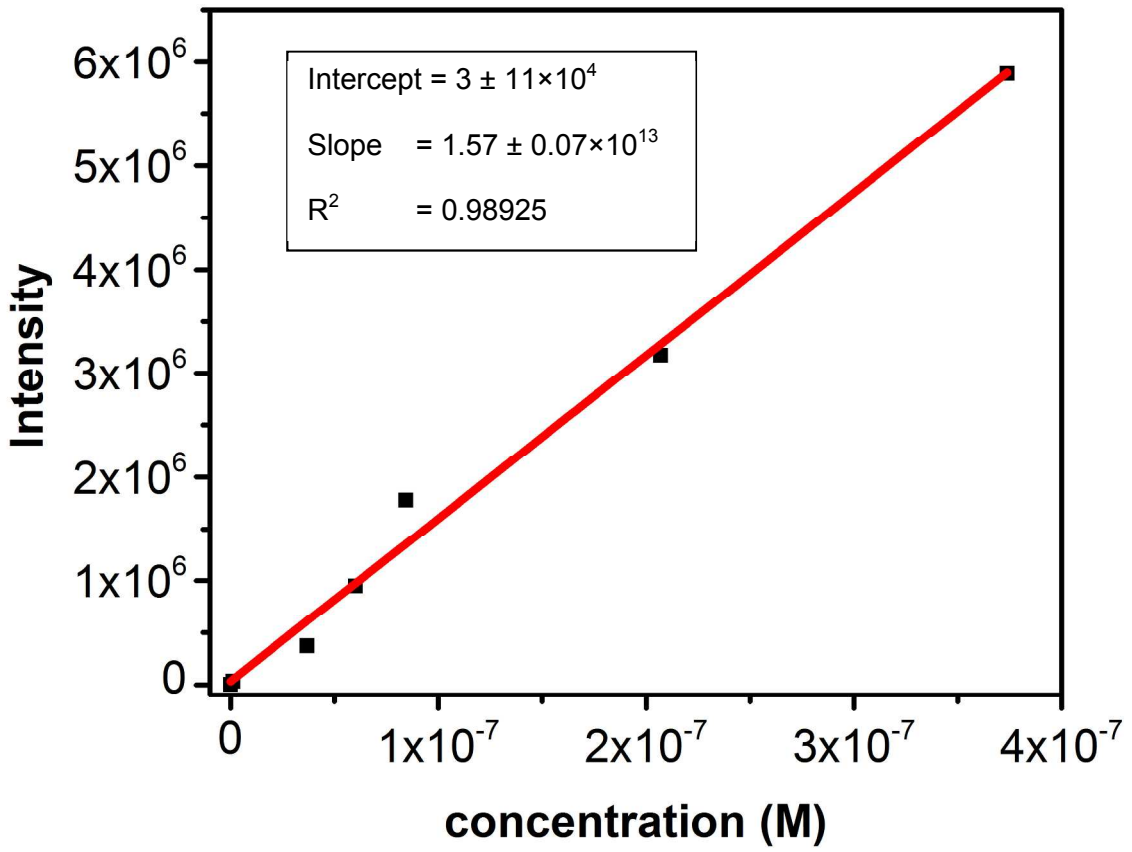


Figure S8. Fluorescence calibration curve for measuring DNA concentration on AuNP after synthesis.

Table S1. Table shows measurements and calculation to obtain amount of DNA per AuNP. Three measurements was taken for the sample and the concentration was calculated from the slope and intercept values obtained from this graph. The number of DNA on each particle was obtained by dividing the concentration of DNA with the concentration of particles.

	Thiolated DNA on AuNP	Non-thiolated DNA on AuNP
Concentration calculated from fluorescence (M)	2.41E-07	6.10E-10
	2.09E-07	6.68E-10
	2.08E-07	4.97E-10
Average concentration (M)	2.20E-04	2.20E-04
Standard deviation (M)	4.59E-11	1.47E-13
Volume of fluorophore (L)	2.20E-04	2.20E-04
No. of moles of DNA	4.82E-11	1.30E-13
No. of moles of AuNP	3.70E-13	3.86E-13
DNA/AuNP	**1.30E+02**	**3.37E-01**
Calculated error for DNA/AuNP	2.40E+01	

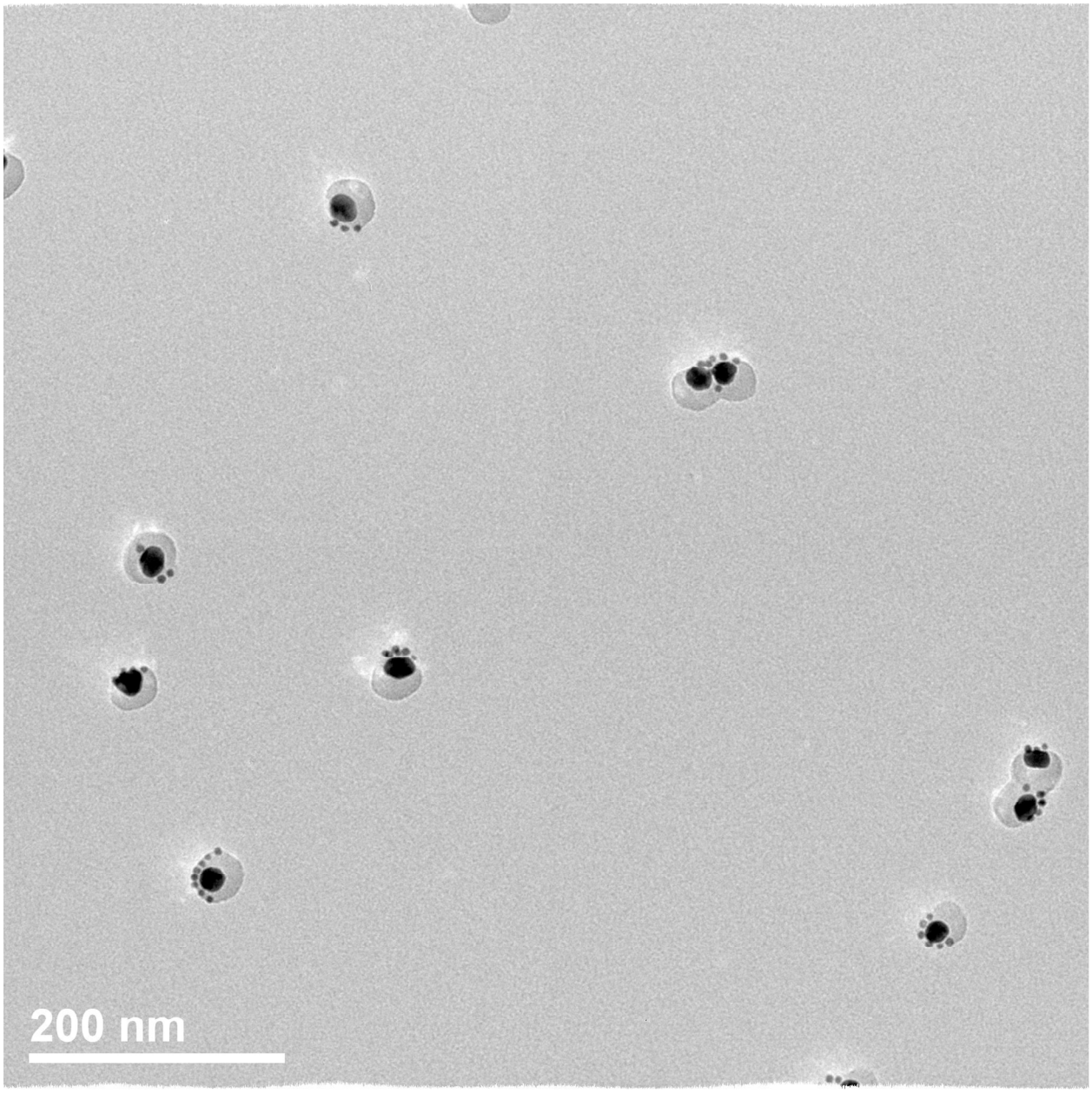


Figure S9. Large area view of selective hybridization of a-(D1)AuNP (20 nm) and c-(D2)AuNP (5 nm).

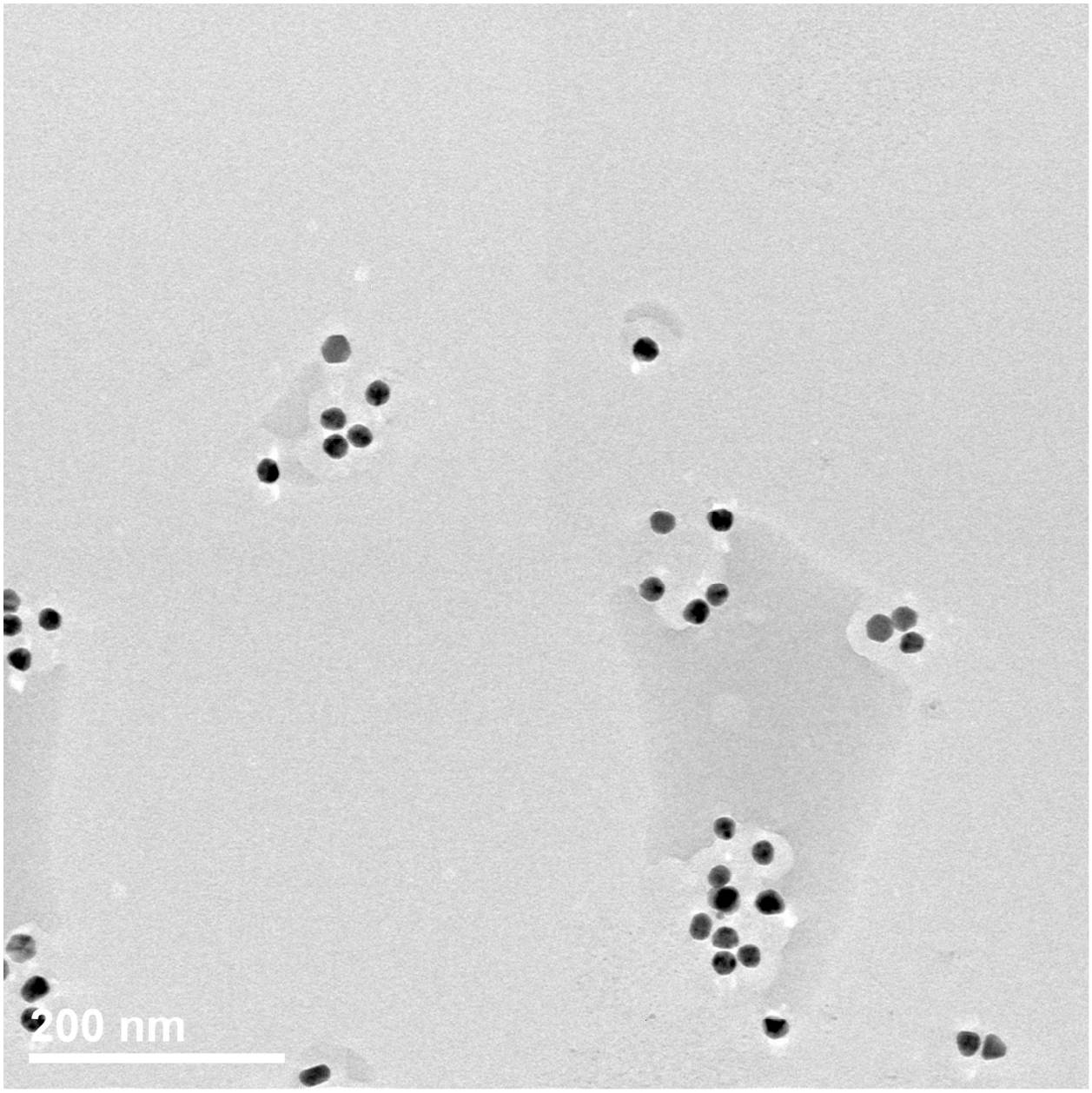


Figure S10. Large area view of selective hybridization of a-(D1)AuNP (20 nm) and 5 nm AuNP functionalized with non-complementary strand. No 5 nm AuNP was attached indicating the selectivity offered by the DNA strand.

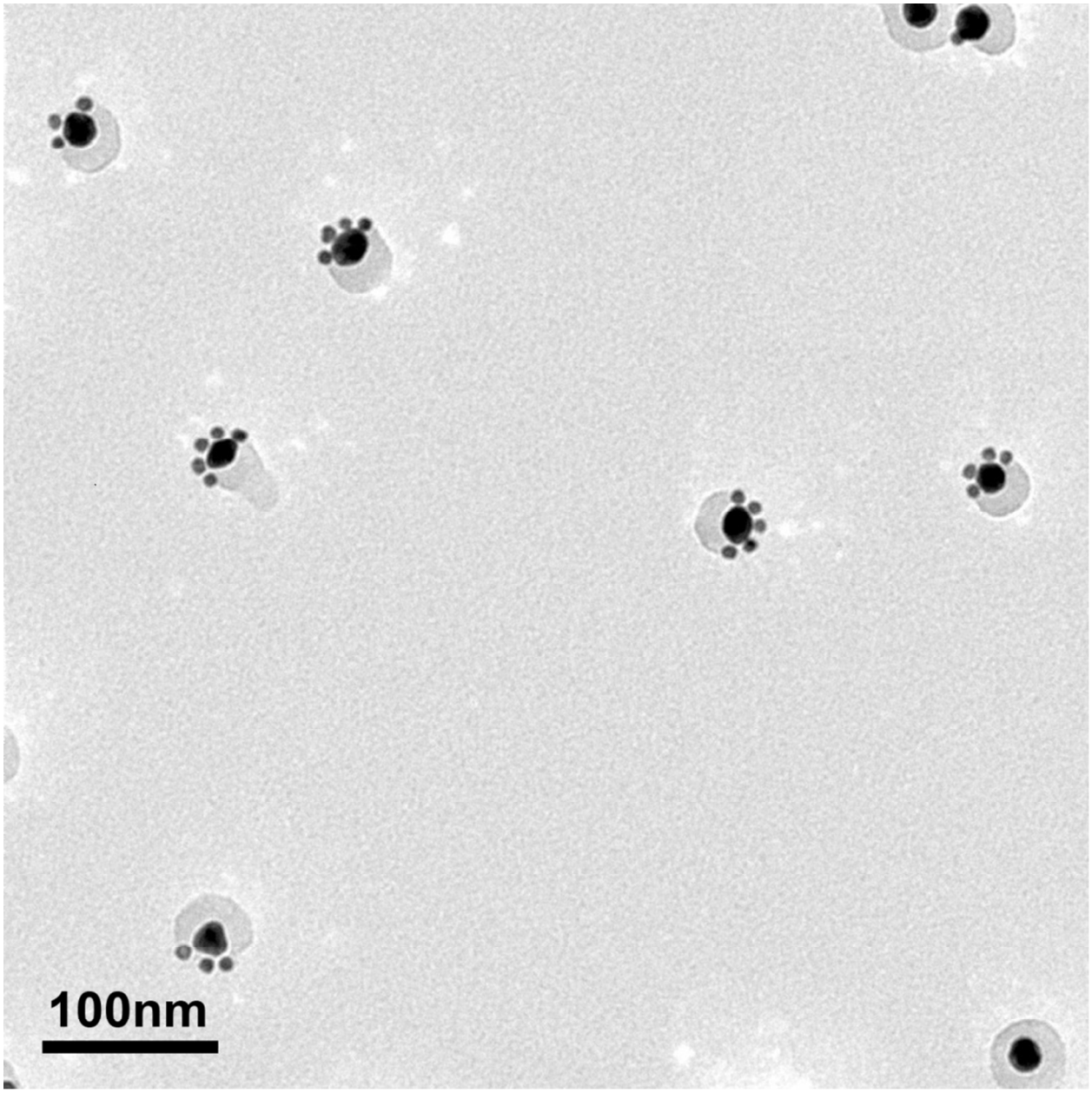


Figure S11. Large area view of selective hybridization of a-(D1)AuNP (20 nm) and c-(D2)AuNP (10 nm).

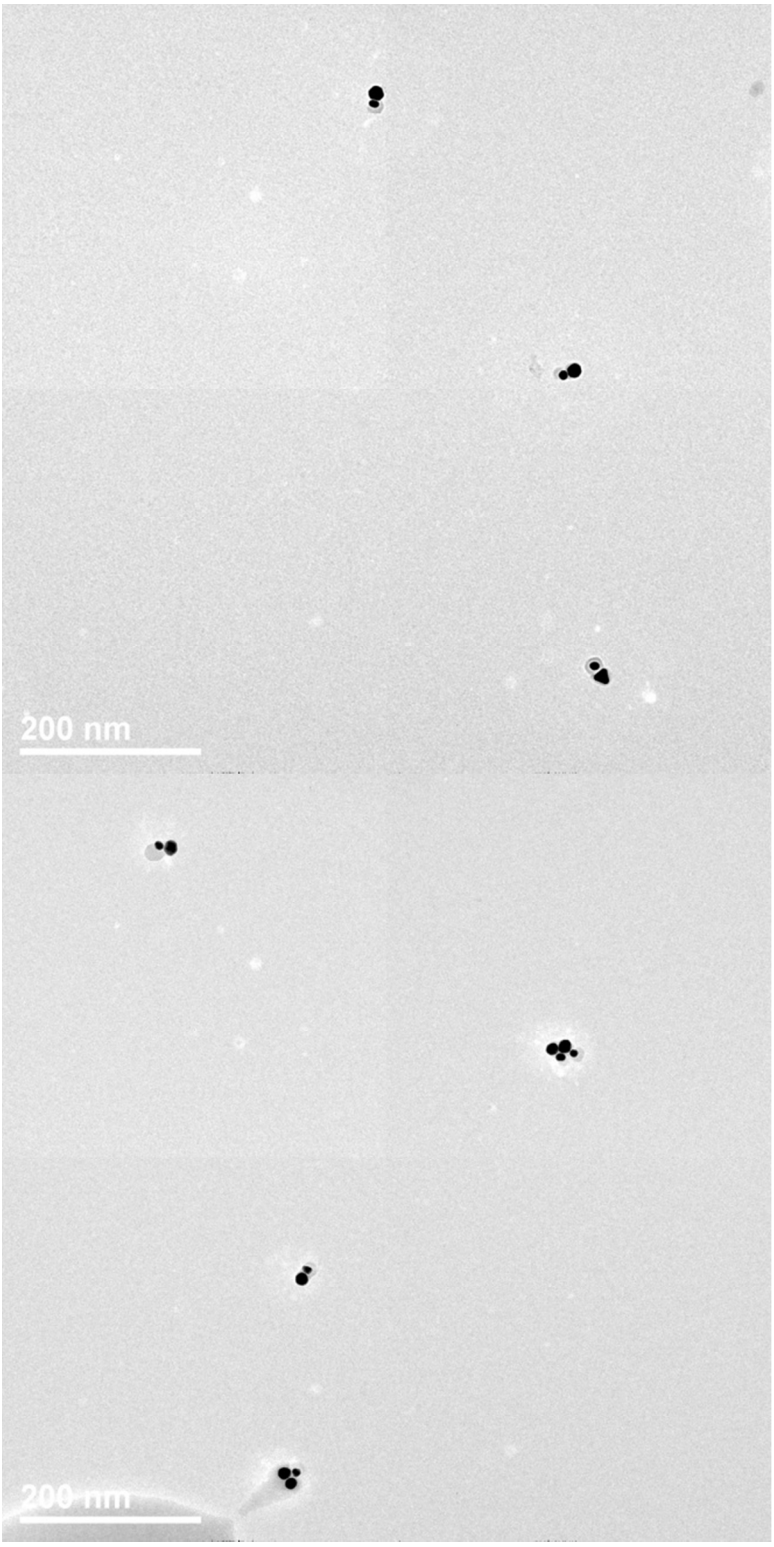


Figure S12. Additional TEM micrographs selective hybridization of a-(D1)AuNP (20 nm) and c-(D2)AuNP (30 nm) at ratio of 1:1.

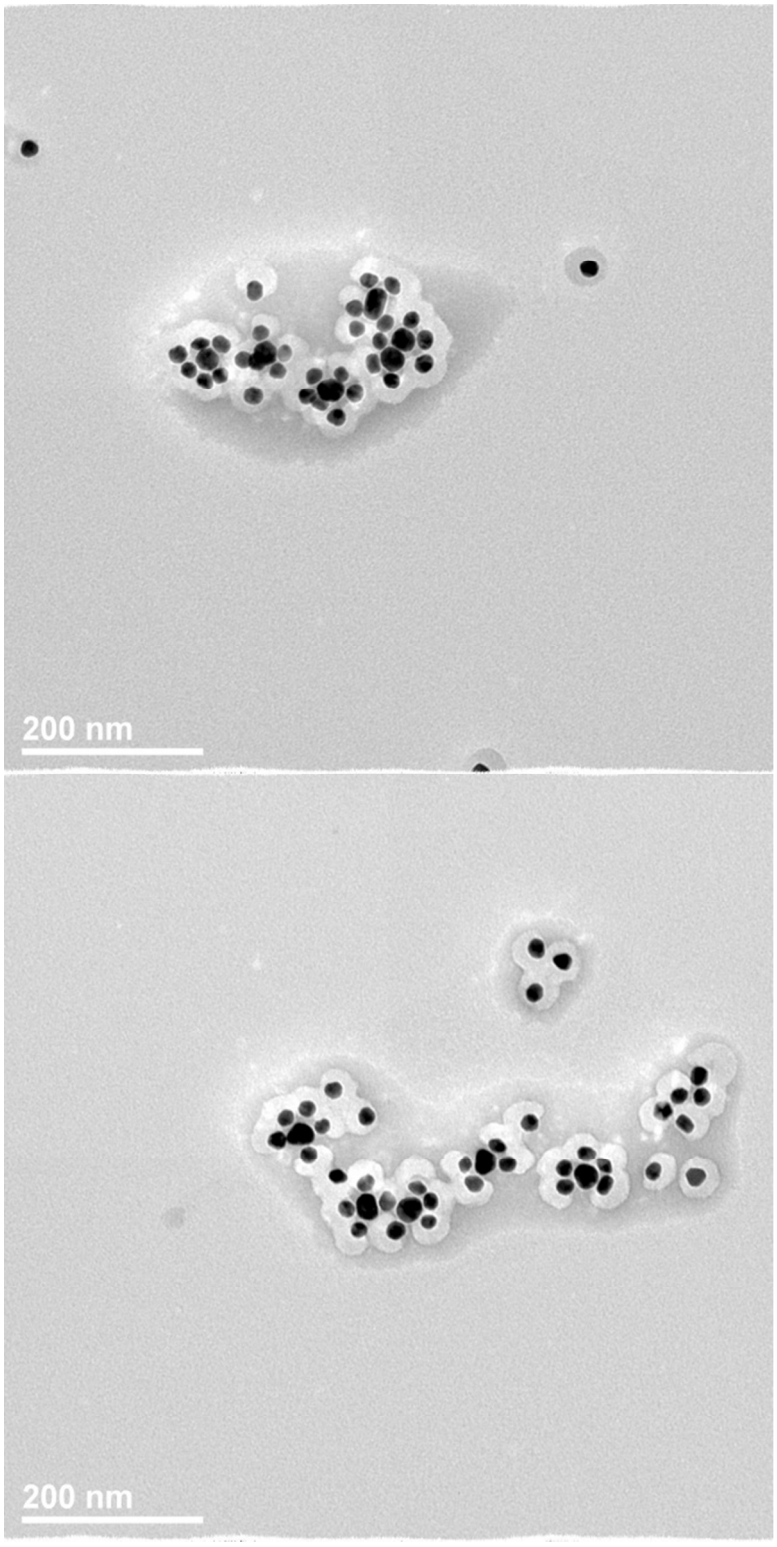


Figure S13. Additional TEM micrographs selective hybridization of hybridization of a-(D1)-AuNP (20 nm) and c-(D2)AuNP (30 nm) at ratio of 10:1.

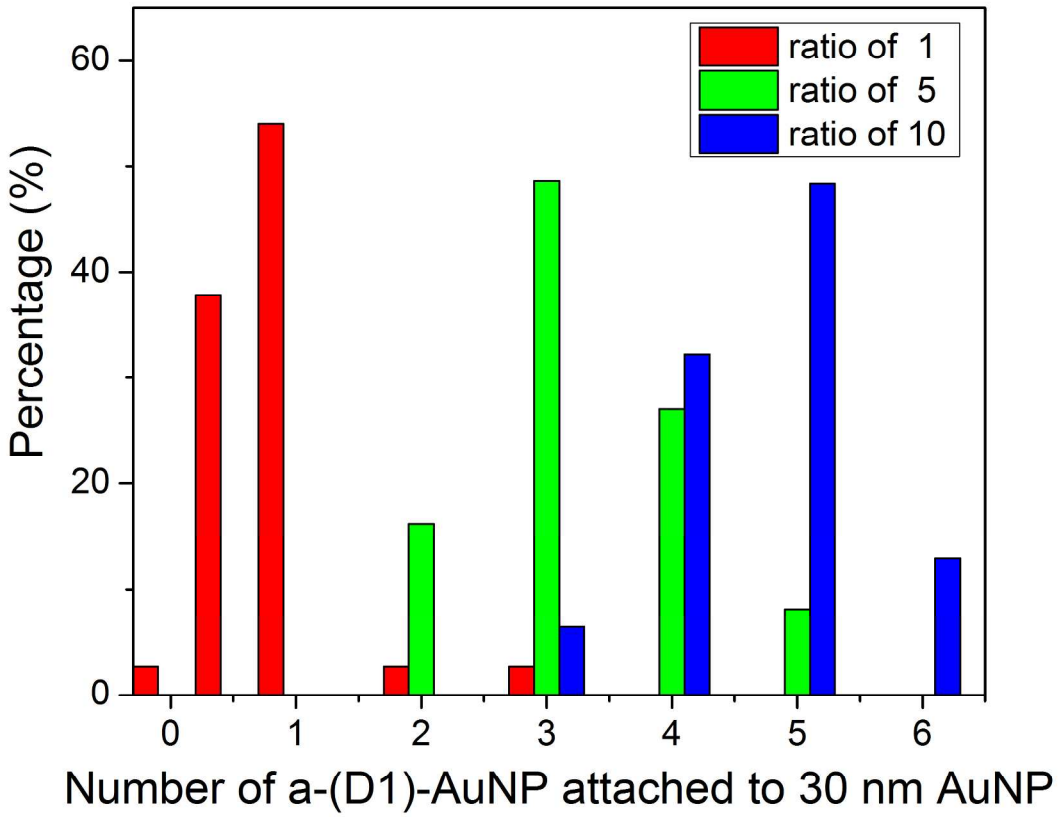
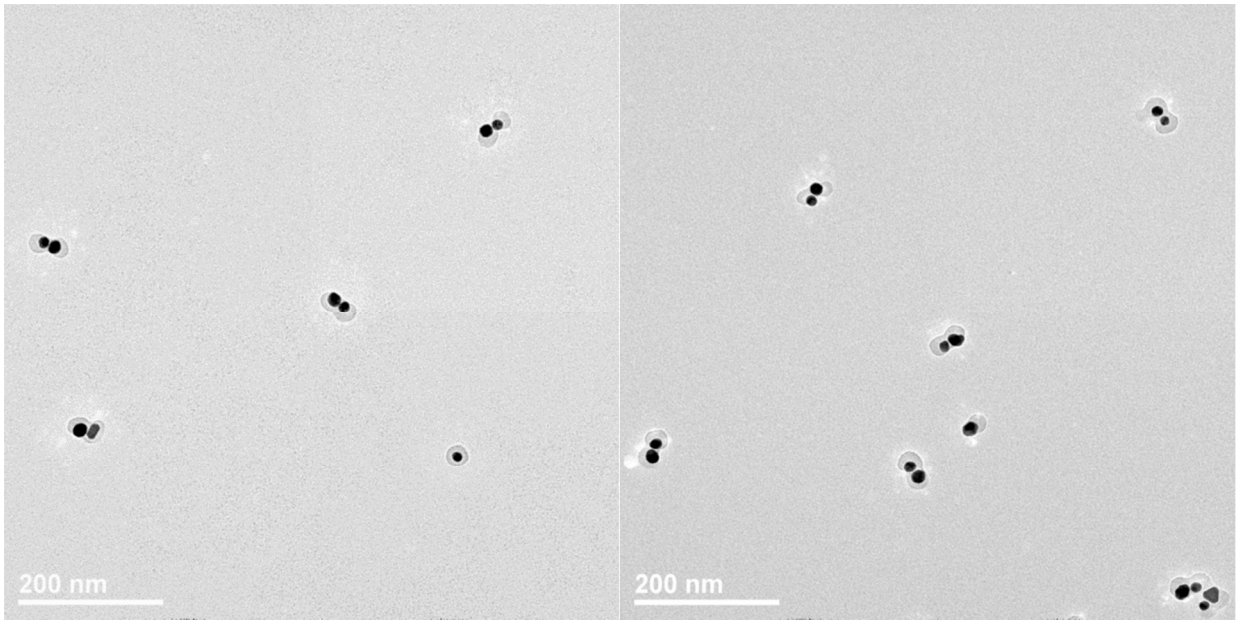


Figure S14. Bar graph showing distribution of hybridization of a-(D1)-AuNP (20 nm) and c-(D2)AuNP (30 nm) at ratios of 1:1, 5:1 and 10:1.



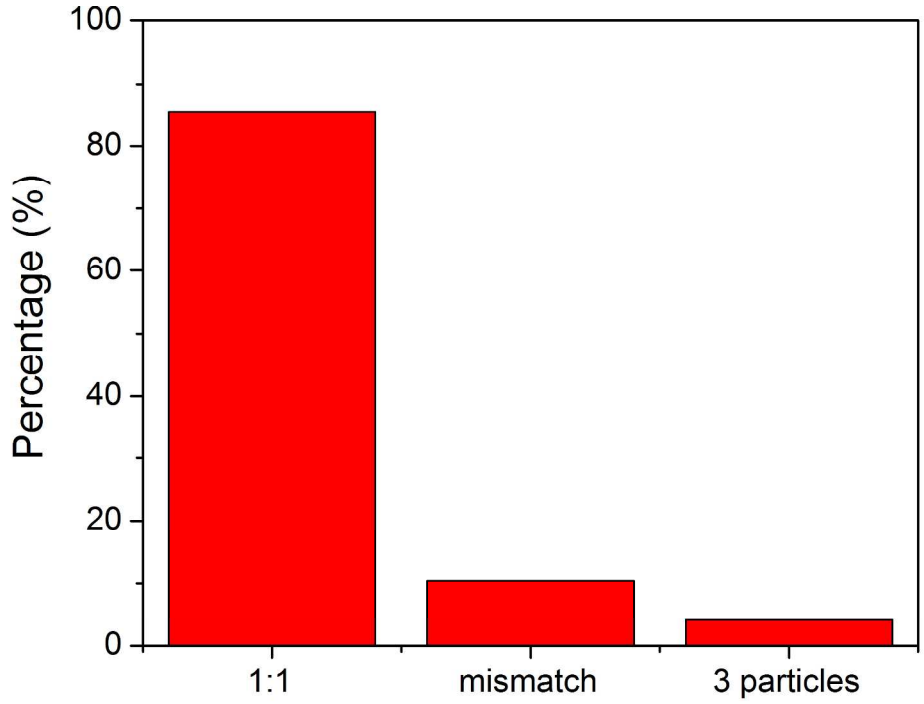
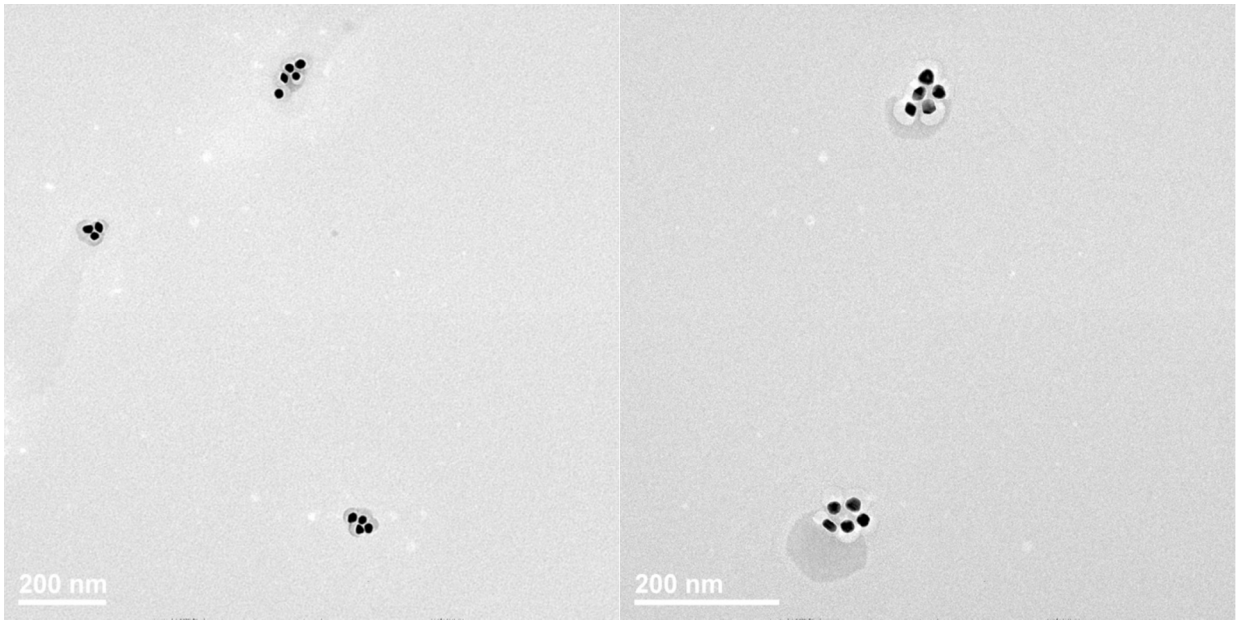


Figure S15. Additional TEM micrographs selective hybridization of a-(D1)AuNP (20 nm) and c-(D2)AuNP (13 nm).Graph at the bottom shows percentage of particles with correct dimer formation, mismatch and with 3 or more aggregates.



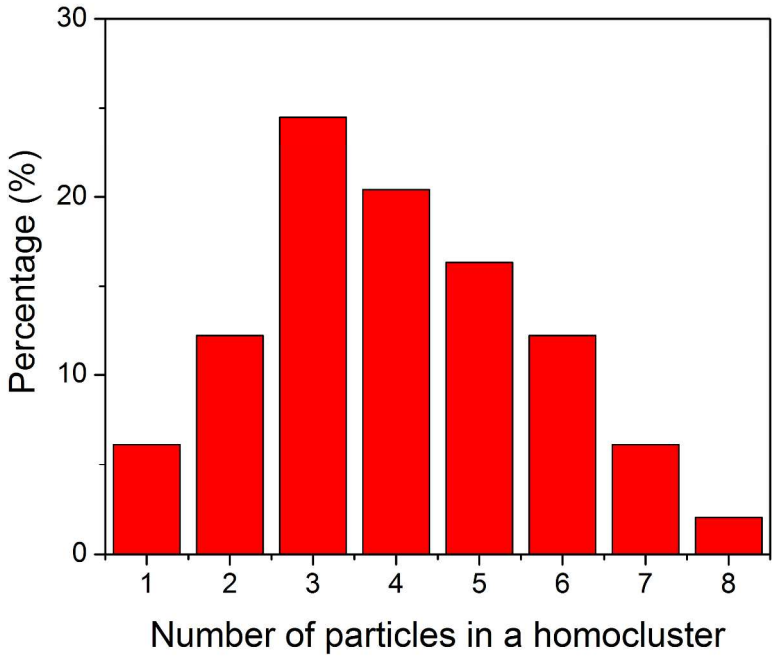


Figure S16. Additional TEM micrographs of homo-assembly of a-(D1)AuNP (20 nm) with D3 DNA and graph showing the distribution of particles in a homo-assembled cluster.