Supplementary Material (ESI) for Chemical Communications

Supplemental information for

Fabrication of Satellite Silver-Gold Nanoclusters Linked by DNA Hybridization

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DNA Sequences:

All DNA sequences were ordered from Integrated DNA Technology Inc (www.idtdna.com) and purified by denaturing PAGE gel electrophoresis.

1) The ps-9, ps-6 and ps-3 strands each contains 9, 6, or 3 phosphorothioate (ps) linkage groups marked as a * between the bases close to the 5' end, and they share a 56 base long recognition sequence.

ps-9 5' -C*A*T* G*C*G* G*G*C* TAA AAA TTT TTG TTT AGC TAT ATT TAA TAT GAT ATT CAA GAG GAA GGT TAT CTC CT-3'

ps-6 5'-C*A*T* G*C*G* TAA AAA TTT TTG TTT AGC TAT ATT TAA TAT GAT ATT CAA GAG GAA GGT TAT CTC CT-3'

ps-3 5'-C*A*T* TAA AAA TTT TTG TTT AGC TAT ATT TAA TAT GAT ATT CAA GAG GAA GGT TAT CTC CT - 3'

2) The complimentary strand to the recognition domain is modified with an amino-group at the 5' end, which then is further reacted with lipoic acid to gain two thiol groups at the 5'-end. Bidentate interaction between the lipoic acid modified DNA with AuNP surface is a stable linkage that allows a 1:1 conjugate of DNA-AuNP to be isolated and hybridized with the ps-9 modified AgNPs to form the bimetallic satellite structure.

Comp: 5' - /5AmMC6/TTT TTT TAG GAG ATA ACC TTC CTC TTG AAT ATC ATA TTA AAT ATA GCT AAA CAA AAA TTT TTA TTT AAA TTT TTT TTT TTT TTT TTT TTT TTT TTT T - 3'

The length of this strand is extended to 100mer by adding 31T and 7T at the 3' and 5' ends, in order to make the 1:1 AuNP:DNA conjugate easier to be separated with the bare AuNP and the 1:2 conjugate by agarose gel electrophoresis.

3) A pair of ps-po-DNAs that each contains 9-ps groups on the 5' end, a 6 base linker (A6) and 9-base recognition sequence that are complementary to each other. These are used to prepare the two sets of AgNPs that carry DNA sequences complimentary to each other to form the large aggregation through DNA hybridization, and thermal melting will recover the uniform dispersed AgNPs.

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Ps-9-A 5'-A*T*A* A*G*C* C*A*T* AAA AAA ATC GCG CGC-3'
Ps-9-A' 5'-A*T*A* A*G*C* C*A*T* AAA AAA GCG CGC GAT-3'

Experimental methods

Materials:
Silver nanoparticles (diameter 20 ± 5 nm given by the manufacturer) were purchased from Ted Pella Inc (catalog number 15705-5sc). The size and distribution were analyzed by TEM imaging and was found to be 32 ± 5 nm.
All oligonucleotides used for the experiments were obtained from Integrated DNA Technologies. (±)-α-Lipoic acid, N-hydroxysuccinimide (NHS), N,N´-dicyclohexylcarbodiimide (DCC) and Tris(carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich and used without further purification. Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) was purchased from Strem Chemicals Inc.

Conjugation of ps-DNA with 32 nanometer silver nanoparticle:
The silver colloid obtained from the company was concentrated 10 times by centrifugation (10,000 rpm, 20 min) and redispersion in nanopure water. To the 100 μL of silver nanoparticle solution, appropriate aliquots of ps-DNA, SDS solution and 0.1 M pH 7.4 phosphate buffer (PB) solution were added such that their final concentrations were 10 μM, 0.01% and 10 mM. The nanoparticle solution was kept for gentle shaking overnight. Then 4 M NaCl was added by small aliquotes over 24 hours to raise the final NaCl concentration to 500 mM. The solution was incubated overnight. Then the excess of Oligonucleotides were removed by centrifugation (10,000 rpm, 20 min) and redispered to a buffer solution that contains 10 mM PB, 500 mM NaCl and 0.01% SDS. This centrifugation and redispersion procedure was repeated three times.

Estimation of number of ps-DNA on each AgNP:
The approximated surface coverage of ps-DNA on AgNP was calculated by measuring the concentration of AgNPs and the DNA concentrations before and after the surface attachment. The concentration of the AgNps was calculated from the particle density given by the manufacturer ($4x10^{10}$/ml, OD ~ 0.706 at 407 nm, BB-international via Ted Pella) and OD measurement, assuming no change to the extinction coefficient of the particle when it is conjugated with DNA. The ps-DNA concentration before and after the attachment was calculated from the OD at 260 nm of the DNA solution before mixed with the AgNps and the supernatant after the incubation. From these, the number of ps-DNA per AgNP was estimated to be ~ 3900 and the surface area per ps-DNA is ~ 0.8 nm^2 or ~ 200 $pmol/cm^2$. As compared to the reported surface density of thiol-modifed DNA on AgNPs, this surface area per DNA molecule is in the reported range[s1]. From the surface area per DNA molecule, the phosphorothiolated portion of the ps9-DNA seems lying tangential on the AgNP surface with the phosphothioate backbone attached on the surface and the bases more likely perpendicular to the surface.

Preparation of 1:1 conjugation of DNA with 5 nm AuNP:

a. Activation of Lipoic acid to synthesize NHS ester of lipoic acid. DCC (2.10 g) was mixed with lipoic acid (2.06 g) in THF (10 mL) followed by the addition of NHS (1.15 g, 10

mM). The reaction mixture was filtered after stirring continuously for 72 hrs. The filtrate was evaporated to get a crystalline solid. NHS ester of lipoic acid was further purified by recrystallization from Toluene. S1 NMR analysis of the resulting product (Varian 400) confirmed the formation of NHS ester of lipoic acid.

b. Conjugation of lipoic acid with amine modified oligonucleotides. An ester of lipoic acid prepared as described above was added in excess to amine modified oligonucleotides in a solution of 70% acetonitrile and 30% water (pH ~ 8). The reaction mixture was kept overnight at room temperature. Lipoic acid conjugated oligonucleotides were purified by microspin G25 columns and used for the next step.

c. Phosphination and concentration of AuNPs. AuNPs (5 nm, Ted Pella Inc.) were stabilized with adsorption of BSPP. Phosphine coating increases the negative charge on the particle surface therefore, stabilizes the AuNPs in high electrolyte concentrations at a higher particle density. BSPP (15 mg) was added to the colloidal nanoparticles solution (50 mL, particle density $5.7x10^{12}$/mL) and the mixture was shaken overnight at room temperature. Sodium Chloride (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 resuspensed in 1 mL solution of BSPP (2.5 mM). Upon mixing with 1 mL methanol, the mixture was centrifuged, the supernatant was removed and the AuNPs were resuspended in 1 mL BSPP solution (2.5 mM). The concentration of the AuNPs was estimated from the optical absorbance at ~ 520 nm.

d. Preparation of AuNP-DNA conjugates with discrete number of DNA. The lipoic acid modified DNAs is incubated with equimolar ratio of phosphinated AuNPs in 0.5xTBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) containing 50 mM NaCl overnight at room temperature. AuNP-DNA conjugates with discrete numbers of oligonucleotides were separated by 3% agarose gel (running buffer 0.5% TBE, loading buffer 50% glycerol, 15 V/cm,25 μL load volume). The band with 1:1 ratio of AuNP/DNA was electroeluted into the glass fiber filter membrane, backed by dialysis membrane (MWCO 10000). AuNP-DNA conjugates were recovered using a 0.45 μm centrifugal filter device. Concentration of these AuNP-DNA conjugates was estimated from the optical absorbance at ~ 520 nm.

Preparation of Ag-core-Au-Satellite nanoclusters:

To the 100 μL 5 nM ps-DNA functionalized silver nanoparticle solution 1:1 Au nanoparticle-DNA(10 μL,500 nM) was added and gradually cooled from 65^{o}C to room temperature. The unbound gold nanoparticles were removed from the solution by low speed centrifugation (4000 rpm, 20 min). The unbound 5 nm AuNPs stay suspended and was removed in the supernatant.

TEM Imaging:
The TEM sample was prepared by dropping 2 μL of the sample solution on carbon coated grid (400 mesh, Ted pella). Before depositing the sample, the grids were negatively glow discharged using Emitech K100X machine. After 30 seconds, the sample was wicked from the grid by touching its edge with a piece of filter paper. To remove the excess salt, the grid was

washed by touching with a drop of water and excess water was wicked away by touching with a filter paper. TEM studies were conducted by using a Philips CM12 transmission electron microscope, operated at 80 kV in the bright field mode.

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TEM Images:

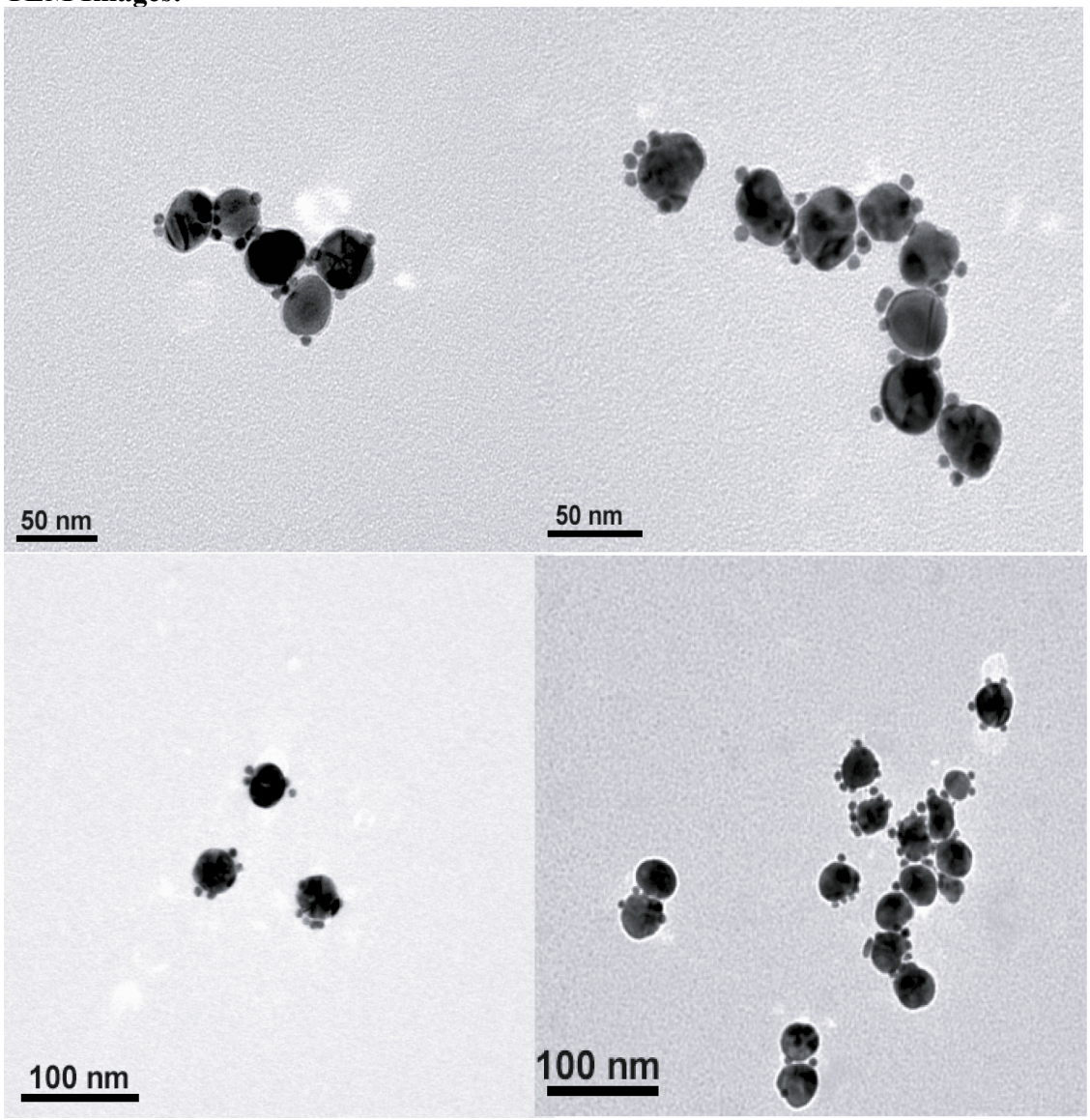

Figure S1. Zoom-out images of Ag core-Au satellite nanostructures

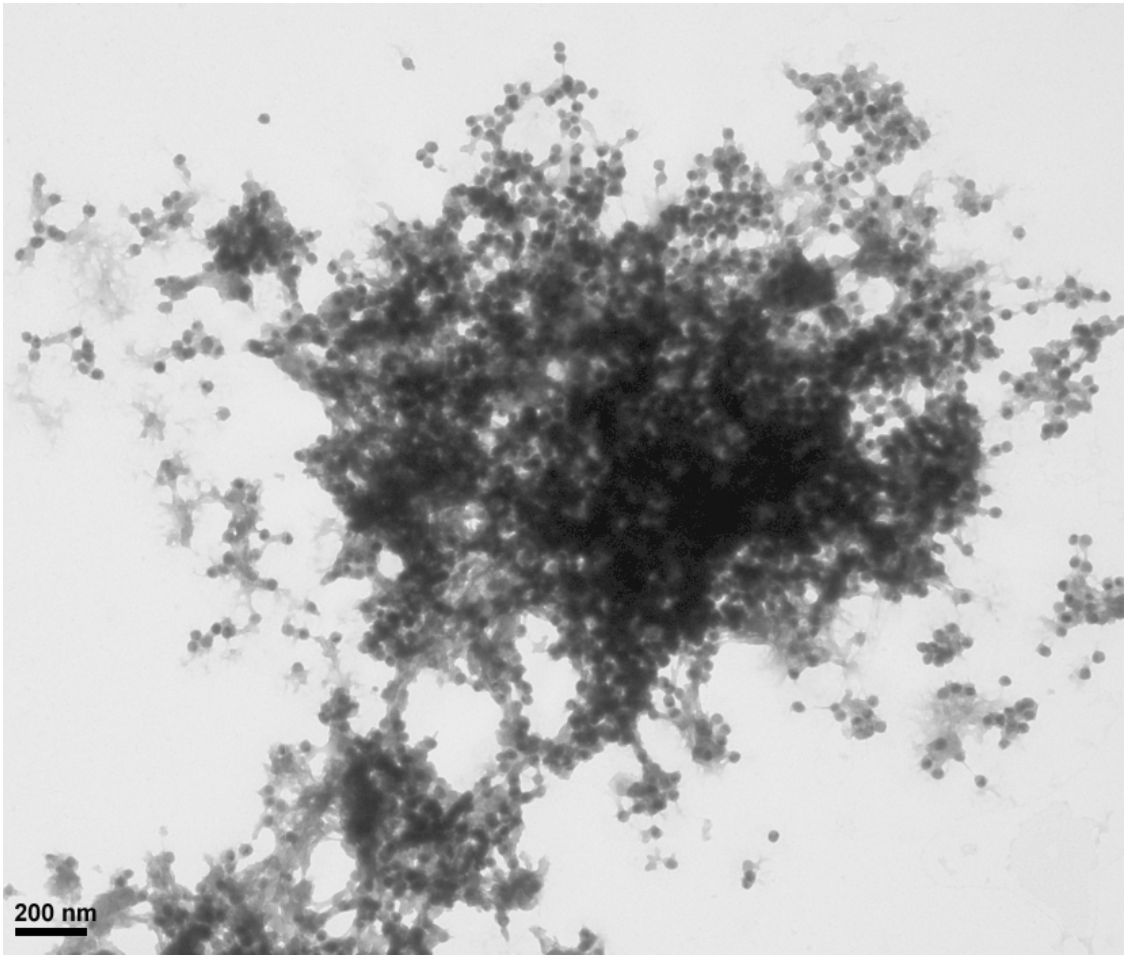

Figure S2. TEM image of the aggregation of silver nanoparticles through DNA hybridization. The DNA were observed by negatively staining with Uranyl acetate.

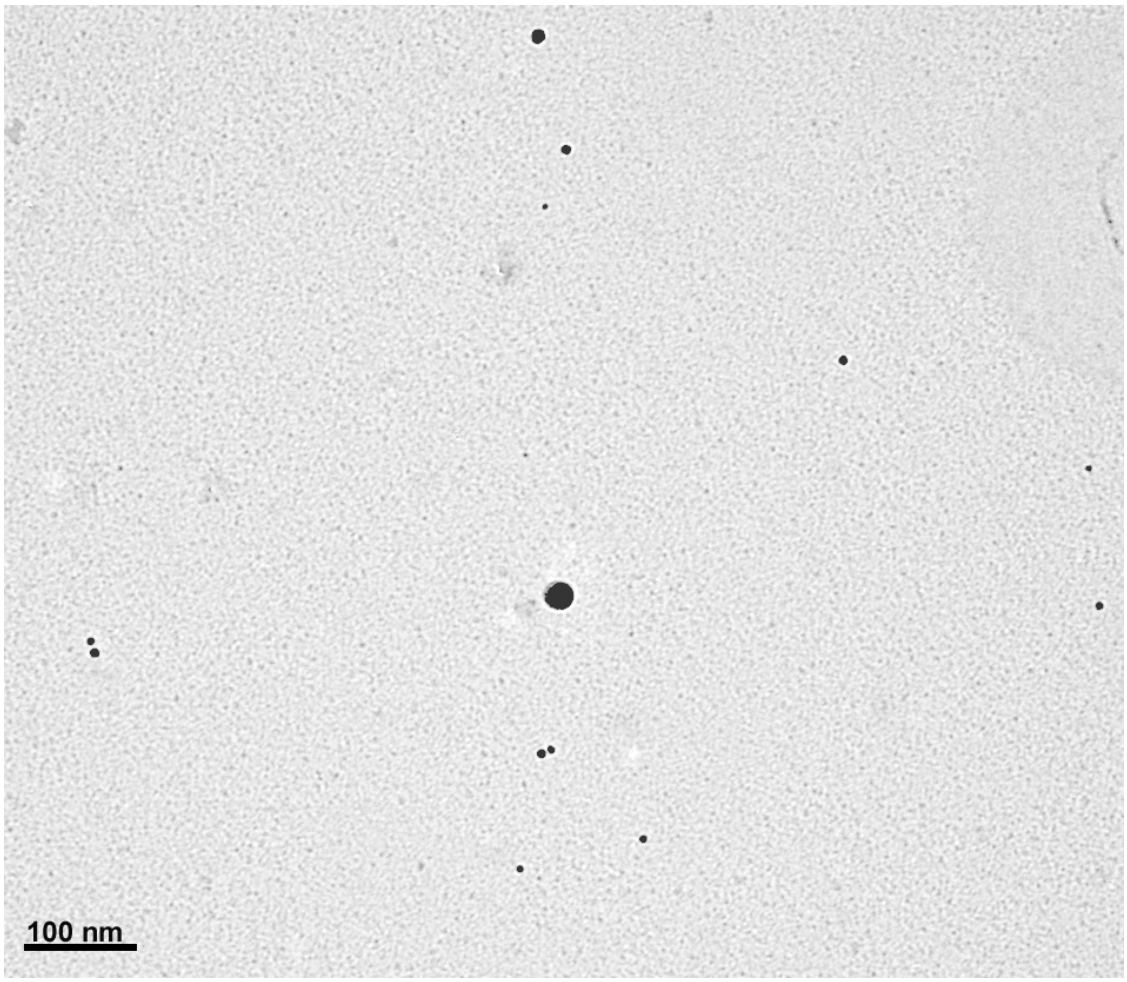

Figure S3. Negative control where the AgNP is modified with ps-DNA not complimentary to DNA attached to the AuNP. Therefore no formation of Ag core-Au satellite structure is observed.

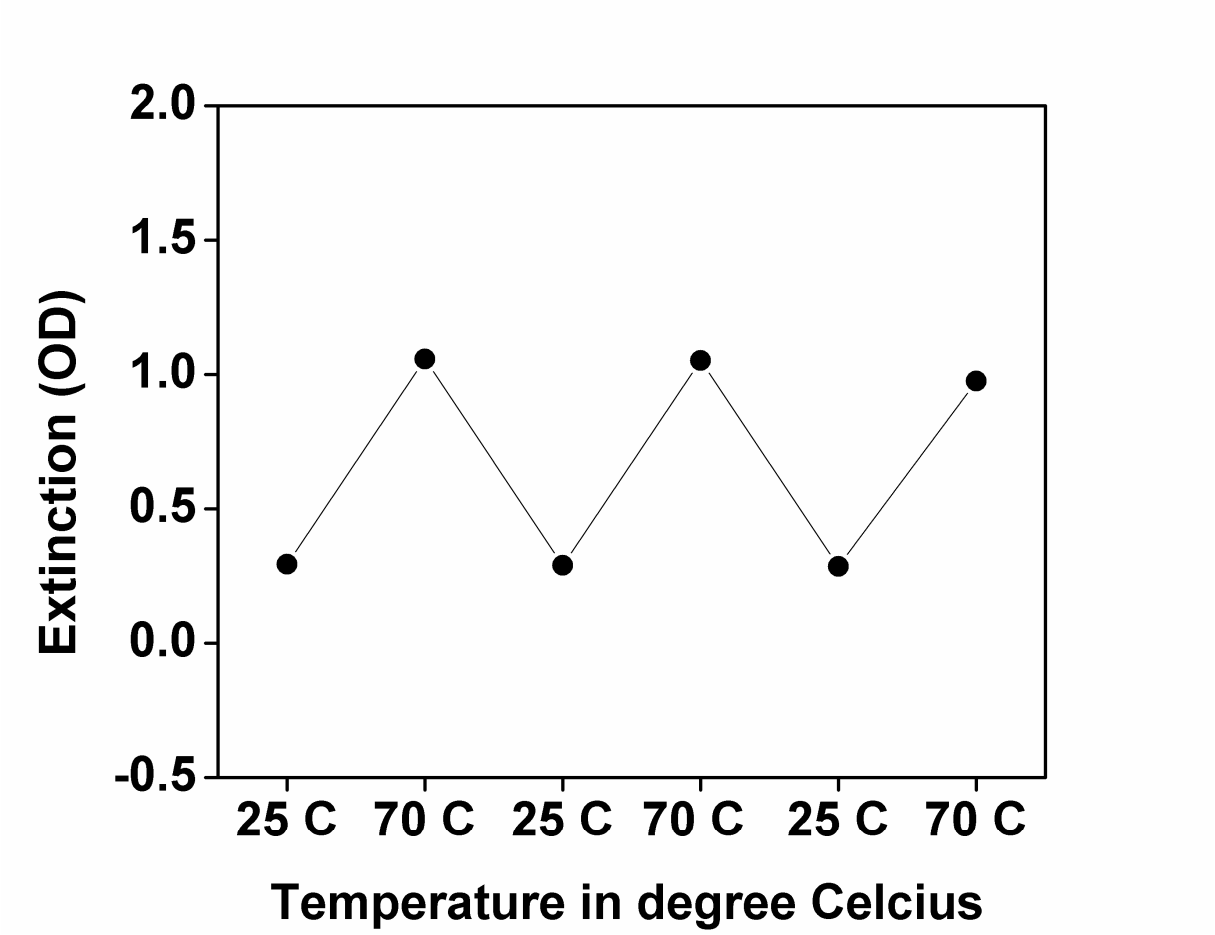

Figure S4: The absorption profile of AgNPs at 412 nm when the temperature is cycled between 25 ^{o}C (aggregated state) and 70 ^{o}C (separated state). AgNP aggregation was induced by hybridization of two complimentary strands of DNA attached to the surface of two sets of AgNps. The plot shows excellent reversibility, strongly support the mechanism of the aggregation is due to DNA hybridization.