Stable silver nanoparticle–DNA conjugates for directed self-assembly of core-satellite silver–gold nanoclusters

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The past two decades have seen increased use of nano-scale materials for bio-sensing, diagnostics and therapeutics.

Noble metal nanoparticles have been used extensively for these applications due to their biocompatibility and rich optoelectronic properties.

Metal nanoparticles conjugated with oligonucleotide have potential in bio-detection for targets as oligonucleotides, proteins and peptides, colorimetric detection of pH changes and in studying real-time molecular interactions.

The sequence specificity of Watson–Crick base pairing between two single stranded oligonucleotides allows the use of metal nanoparticle DNA conjugates as building blocks for bottom up nanotechnolology

The use of gold nanoparticles (AuNPs) has been more prevalent over silver nanoparticles (AgNPs) because AuNPs are easier functionalize by DNA modified with one or more sulfur moeties and are chemically more stable than AgNPs.

For example it is known that AgNPs are susceptible to oxidation and subsequent degradation or subject to irreversible aggregation in solutions containing metal cations.

These limitations have resulted in very few reports of AgNP–DNA conjugation.

Recently, AgNPs have gained much interest due to their high extinction coefficients compared to AuNPs, inherent catalytic properties and propensity to enhance Raman scattering.

It has been demonstrated that increasing the number of thiol moieties in the capping ligands resulted in enhanced stability of the AgNPs in high salt concentrations.

Despite this advancement, the synthesis of oligonucleotides labeled with multiple thiol groups remains cumbersome and involves special purification steps.

A novel strategy to functionalize AgNPs withchimeric phosphorothioate modified DNA (ps-po-DNA), which were then used in the fabrication of bimetallic coresatellite nanoclusters that each contains a silver core (diameter32 nm) surrounded by a number of 5 nm gold NPs.

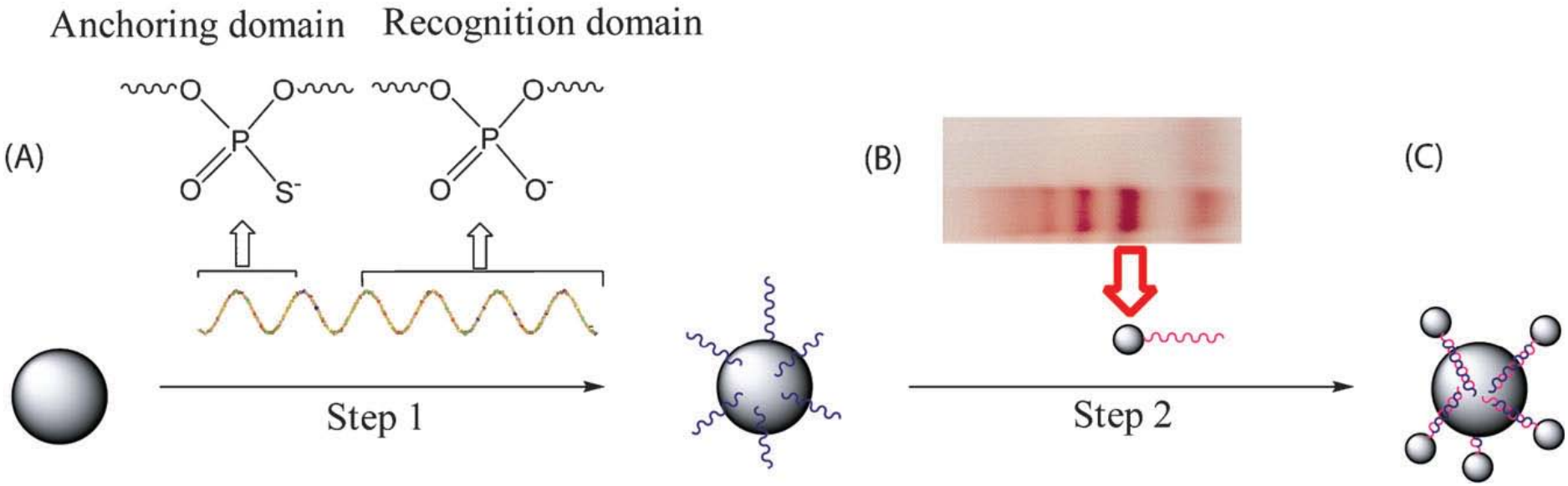


Fig. 1 Schematic representation of the functionalization of AgNPs with ps-DNA, and subsequent fabrication of Ag-core–Au-satellite nanoclusters.

A) The oligonucleotide contains tandem sequence of an anchoring domain that consists of nucleotides with phosphorothioate groups in the backbone and a recognition domain with the normal phosphate backbone.

(B) 1 : 1 conjugation of 5 nm AuNP with oligo of the complementary sequence of the recognition domain is prepared and purified by agarose gel electrophoresis.

(C) The first step is conjugation of ps-DNA to the surface of 32 nm AgNP. The second step is hybridization of the complimentary DNA conjugated to 5 nm AuNP to the DNA anchored on the surface of the 32 nm AgNP.

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.

The effect of the number of ps-linkages in the oligonucleotides on the stability of AgNPs was examined by a DTT-displacement experiment, monitored by UV-vis spectroscopy.

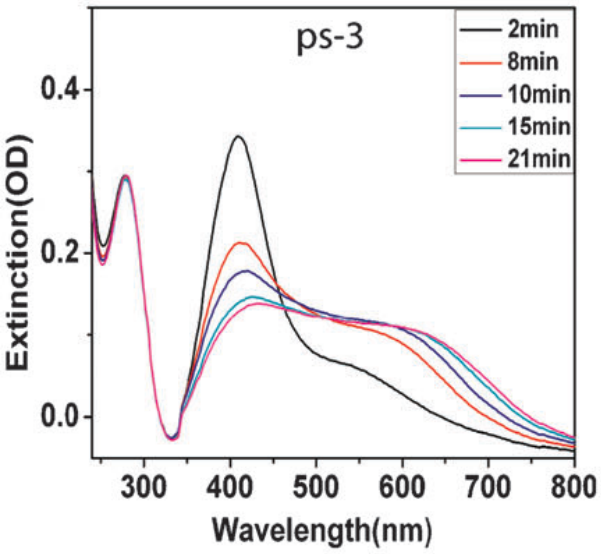
DTT is a reducing agent that is known to cleave the Ag–S bond and displace the thiol modified ligands from the AgNP surface, thus resulting in irreversible aggregation of the nanoparticles in a high salt condition.

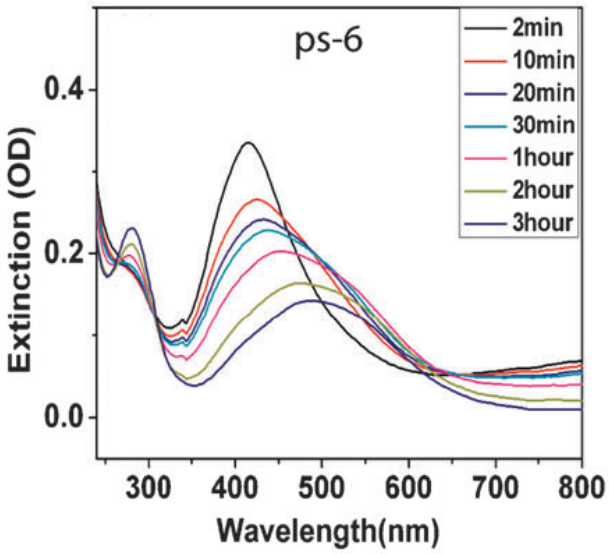
More stable the capping ligand is on the surface of the AgNPs, the slower the aggregation is in
the presence of DTT.

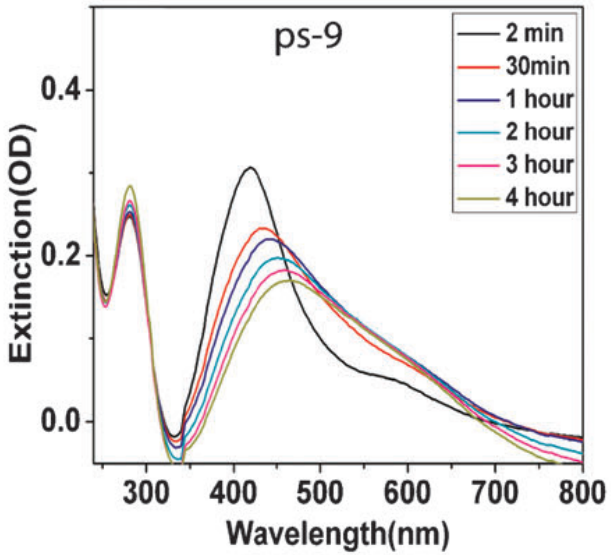
Three DNA strands, ps-3, ps-6 and ps-9, were used to functionalize the AgNPs.Upon addition of DTT, all samples showed a decrease of the absorbance at 400 nm and a red shift of the absorbance peak, indicating growing size of the aggregates with time.

The AgNPs functionalized with ps-3showed the lowest stability and functionalized with ps-9 showed the highest stability.

From this DTT displacement experiment we can conclude that the ps-DNA is specifically bound to the surface of the AgNPs and function as a protection layer against particle aggregation.







UV-vis absorbance assay after 15 mM DTT was added to the same concentration of ps-3, ps-6 and ps-9 functionalized AgNPs.

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) wasmixed with lipoic acid (2.06 g) in THF (10 mL) followed by the addition of NHS (1.15 g, 10mM).

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.

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 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 removed with a pipette. AuNPs were then resuspensed in 1 mL solution of BSPP (2.5 mM).

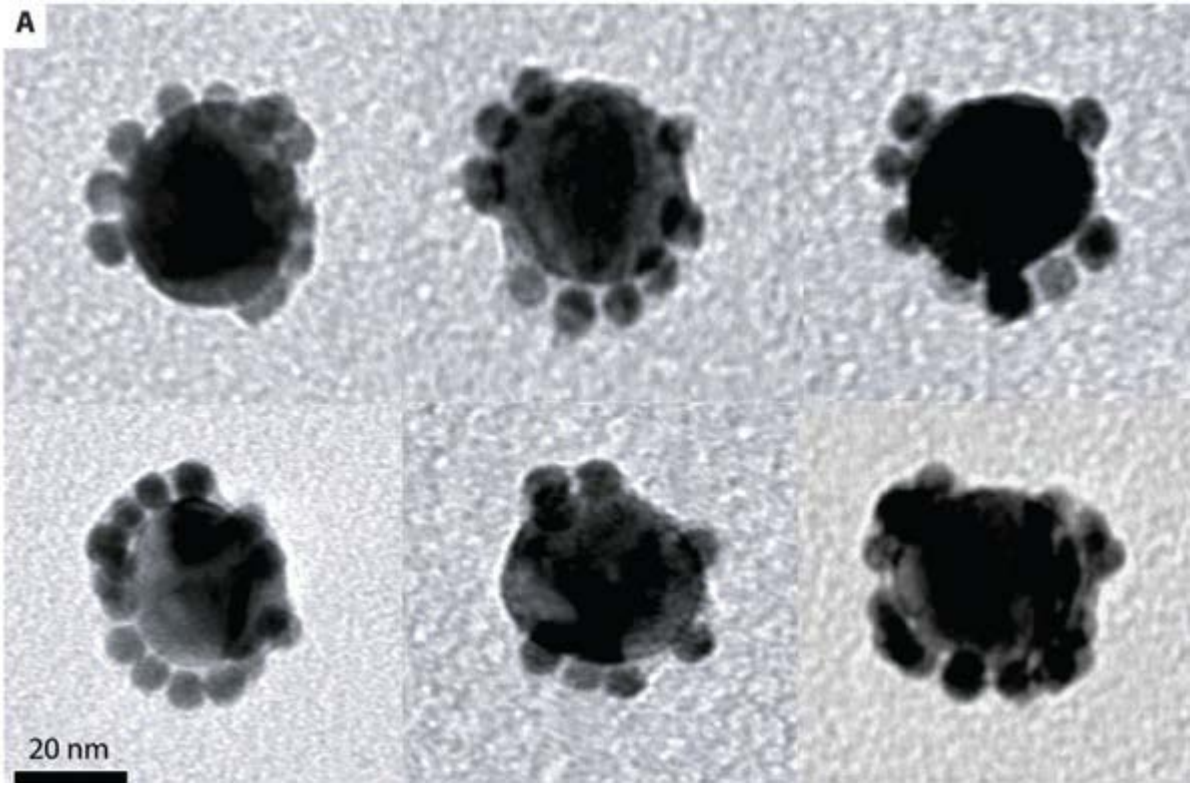
Upon mixing with 1 mL methanol, the mixture was centrifuged, the supernatant was removed and the AuNPs were resuspended in 1 mL BSPP solution (2.5 mM).The concentration of the AuNPs was estimated from the optical absorbance at ~ 520 nm.

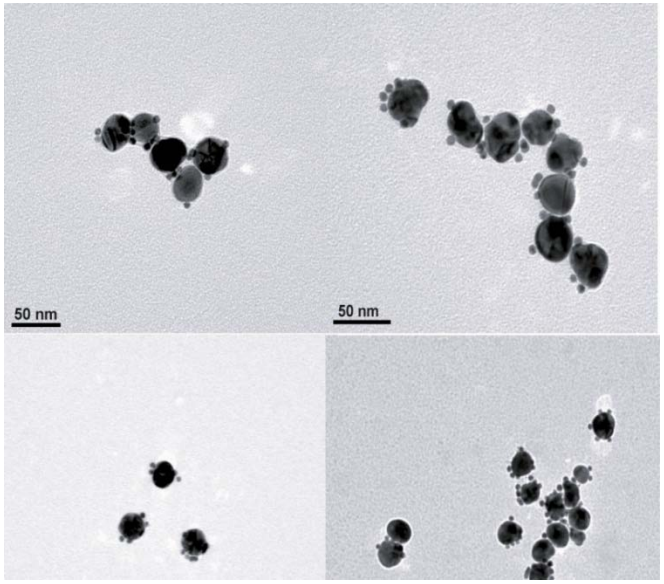
d. Preparation of AuNP-DNA conjugates with discrete number of DNA. The lipoic acid modified DNAs is incubated with equimolar ratio of phosphinated AuNPs in 0.5xTBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) containing 50 mM NaCl overnight at room temperature.
AuNP-DNA conjugates with discrete 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).

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.





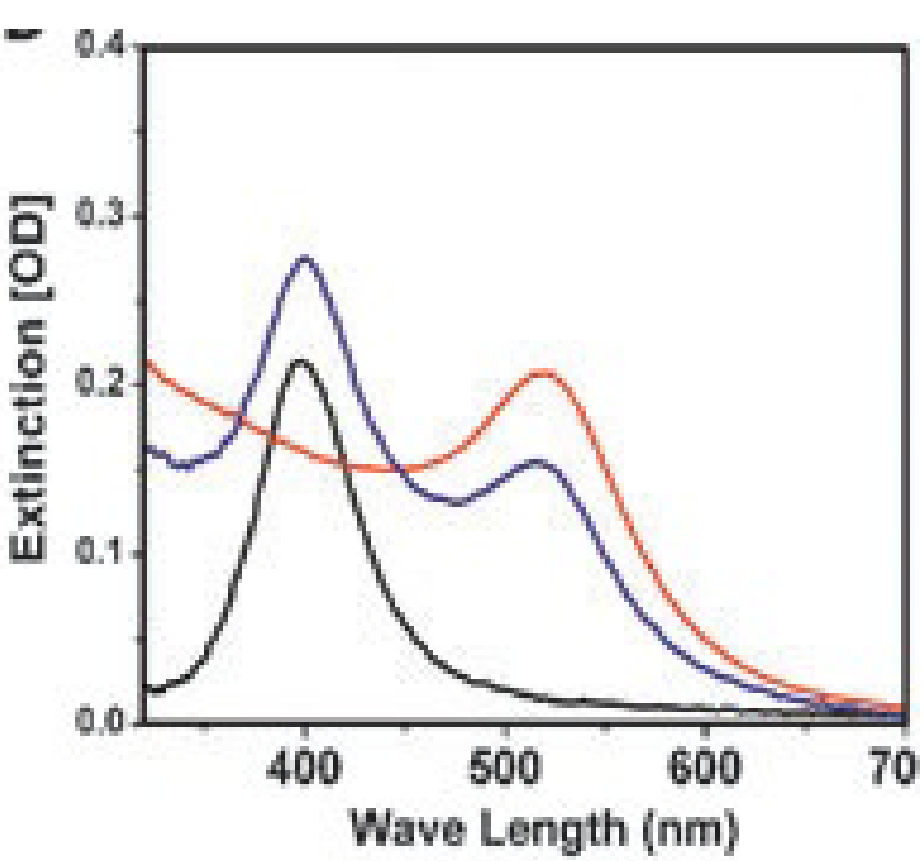
(A) Representative TEM images of 32 nm Ag-core-5 nm Au satellite cluster. The scale bar for each image is 20 nm.

The UV-vis spectra of the nanoclusters indicated the presence of the plasmonic absorption peaks for both AuNPs and AgNPs.
DLS studies showed a significant size difference between the ps-DNA functionalized AgNPs and the Ag–Au core-satellite structure.

The hydrodynamic radius for the ps-9 DNA modified AgNP is 24 nm (green), which is larger than the radius measured from TEM, 16.3 nm.

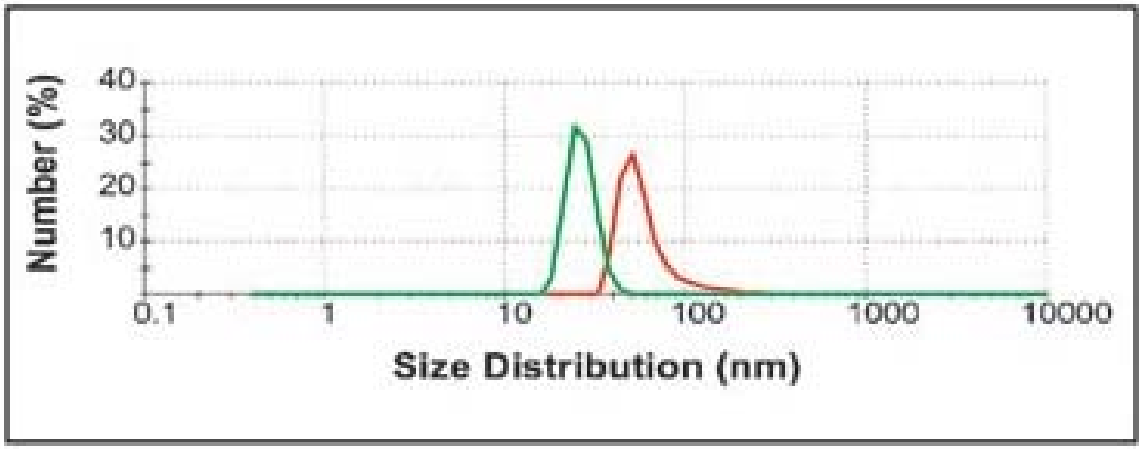
In TEM imaging, the DNA surface modification can not be observed due to significant lower electron density of DNA compared to that of the AgNPs.

But DLS measures the size by means of diffusion correlation in aqueous solution. The loose layer of ssDNA strands (56mers extending from the surface) on the AgNP surface if assume a random coiled conformation in aqueous solution would increase the hydrodynamic radius ofthe particle by 8–10 nm.



UV-vis spectra of 32 nm AgNP with ps-9 (black), 5 nm AuNP with a single DNA (red), AgNP core-AuNP satellite (blue).

C) DLS shows the hydrodynamic Radius for the AgNP and AgNP core-AuNP satellite24 nm (green) and 50 nm (red), respectively.



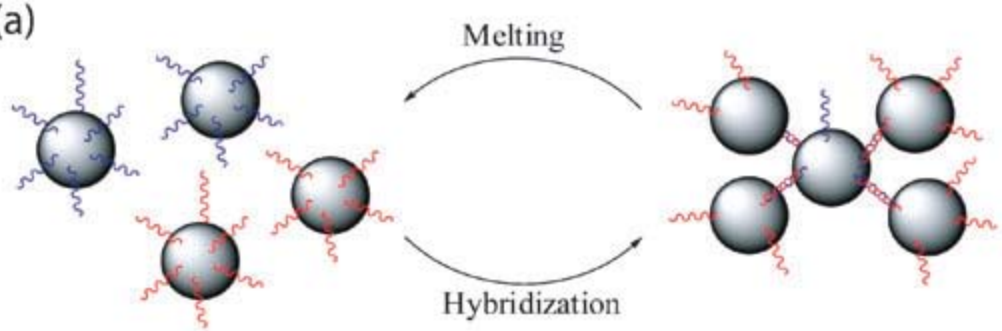
Colorimetric assay using two sets of AgNPs, each functionalized with an oligonucleotide that containsa ps-9 domain and a recognition domain complementary to each other .

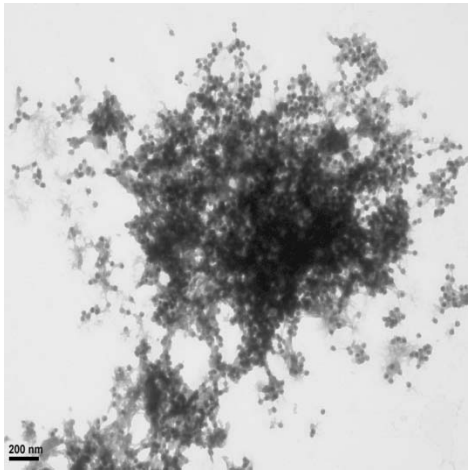
When the two sets of functionalized AgNPs were mixed together, they formed aggregates due to hybridization of the complimentary recognition domains of the DNAs.

This aggregation resulted in a characteristic broadening of the peaks and dampening of the silver plasmonic resonance absorbance peak.

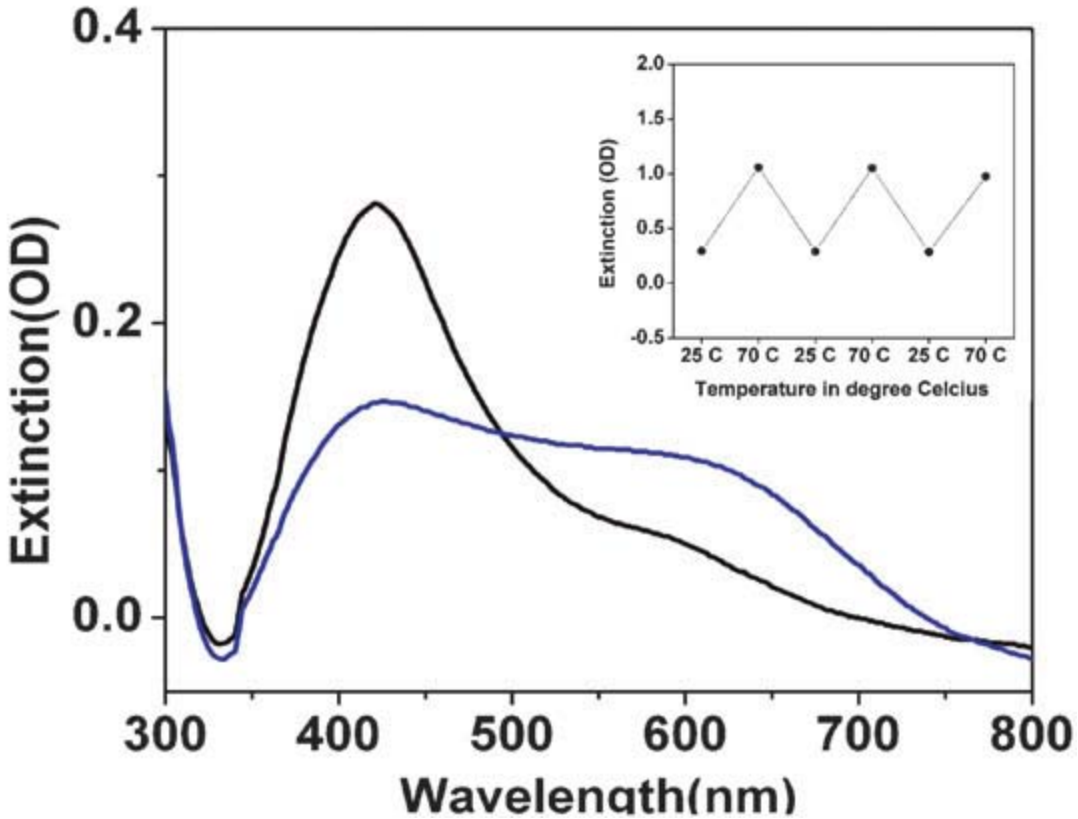
When the aggregates were heated to 70 ^{0}C, the absorption profile of dispersed AgNPs was recovered, suggesting the reversibility of DNA mediated aggregation.

(a)


Melting
Hybridization


200 nm

(b)


Extinction(OD)
0.4
0.2
0.0
300
400
500
600
700
800
Wavelength(nm)
Extinction (OD)
2.0
1.5
1.0
0.5
0.0
-0.5
25 C
70 C
25 C
70 C
25 C
70 C
Temperature in degree Celcius

summary

Development of an easy to use and robust strategy to achieve AgNP and DNA conjugates that are stable in buffer conditions that are amenable to DNA hybridization.

Such AgNP–DNA conjugates open up opportunities to assemble hierarchical nanostructures that may find use in nanophotonics and biosensing application.

THANK YOU