Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B


**Supporting Information**

# Chiral Nanoparticle Pyramids for Ultrasensitive Endonuclease Detection

# Experimental

## Materials.

The bis-(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BPS), trisodium citrate ($C_6H_5Na_3O_7$•$2H_2O$, ≥99.0%) and tetrachloroauric acid trihydrate ($HAuCl_4$•$3H_2O$, 99.9%) were obtained from Sigma Aldrich Chemical Company (Atlanta, GA, USA). All other chemicals were of analytical grade purity or better and were used as received without further purification. All DNA oligonucleotides (sequences shown in Table S1, supporting information) were obtained from Sangon (Shanghai, China). The stock solutions of DNA were prepared with 100 mM Tris-HCl solution (pH=7.5) and kept frozen at -18$^{o}$C during storage. DNase I was purchased from Aladdin Industrial Corporation (Shanghai, China). The cleavage buffer for DNase I was 10 mM Tris-HCl solution (pH=7.5) containing 10 mM $MgCl_2$ and 0.5 mM $CaCl_2$. Ultrapure water (18 MΩ·cm) was purified with a Millipore filtration system and used in all experiments. All glassware was cleaned with aqua regia (HCl : $HNO_3$ in a 3 : 1 ratio by volume) for 12 h before use.

## Instruments

Transmission Electron Microscopy (TEM) images were obtained using a JEOL JEM-2100 transmission electron microscope operating at an acceleration voltage of 200 kV. The chirality of the pyramid nanostructures was characterized by MOS-450/AF-Circular dichroism. UV-Visible (UV-Vis) spectra were collected on a UNICO 2100PC UV/Vis spectrophotometer. The size distribution was obtained using a Malvern Zetasizer Nano ZS instrument. A 633 nm laser source and backscattering detector at 173$^{o}$ were used for the dynamic light scattering (DLS) experiment.

## Synthesis and Functionalization of AuNPs

Two different-sized AuNPs ($AuNP_1$ = 15 ± 2 nm, and $AuNP_2$ = 25 ± 3 nm) were synthesized by reduction of $HAuCl_4$ using trisodium citrate. Briefly, 2.5 mL of freshly prepared trisodium citrate (1% by weight) was quickly added to 100 mL of boiling aqueous $HAuCl_4$ (0.25 mM) under vigorous stirring and reflux. After boiling for 30 min,

the reaction was complete and $AuNP_1$ were obtained. The 1.6 mL trisodium citrate (1% by weight) was used to prepare $AuNP_2$. The synthesized AuNPs were cooled to room temparaure and stored at 4°C. AuNP-DNA conjugates were then prepared. Briefly, the AuNPs were first modified with BPS. Then, the BPS-protected AuNPs were mixed with DNA oligonucleotides in a molar ratio of 1 : 5. After incubation for 24 h, the samples were centrifuged to remove free oligonucleotides. The concentration of AuNP-DNA conjugates was calculated using Lambert-Beer's law: $c = A_{450} / (b\varepsilon_{450})$, $A_{450}$ is the absorbance value at 450 nm, $\varepsilon_{450}$ is the corresponding extinction coefficients for each AuNP size, $\varepsilon_{450}$ (15 nm) = $2.18\times10^8$ $M^{-1}cm^{-1}$, $\varepsilon_{450}$ (25 nm) = $1.1\times10^9$ $M^{-1}cm^{-1}$, b = 1 cm.

**Self-assembly of AuNP Pyramids.**

The AuNP pyramids were constructed by mixing four equal amounts of AuNP-DNA conjugates using 1×TBE buffer (pH = 7.5, containing 50 mM NaCl) as the hybridization buffer. After overnight incubation at room temperature, the pyramids with high yield were obtained. Five different types of AuNP pyramids were constructed: all four $AuNP_1$s (denoted as $4AuNP_1$); three $AuNP_1$s + one $AuNP_2$ ($3AuNP_1$+$1AuNP_2$); two $AuNP_1$s + two $AuNP_2$s ($2AuNP_1$+$2AuNP_2$); one $AuNP_1$ + three $AuNP_2$s ($1AuNP_1$+$3AuNP_2$); four $AuNP_2$s ($4AuNP_2$).

**Assay of DNase I Activity.**

The stability of AuNP pyramids was improved by adding thiolated poly(ethylene glycol) molecules (mPEG-SH, Mw = 1000) to the pyramid solution at a molar ratio of 500 : 1. After 30 min incubation, the solution was centrifuged at 5,000 g for 10 min and then suspended in 0.5 mL of DNase I buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH =7.5) at a final concentration of 2 nM. Different amounts of DNase I were then added to the solution to give final volumes of 200 μL. To ensure the full reaction, the mixture was incubated for 30 min at 37°C. The CD spectra were then recorded to determine DNase I activity.

**Table S1.** ssDNA sequences used to form AuNP pyramids

| Name | Sequence |
|---|---|
| ***Y1*** | HS-5'-TTT GCC TGG AGA TAC ATG CAC ATT ACG GCT TTC CCT ATT AGA AGG TCT CAG GTG CGC GTT TCG GTA AGT AGA CGG GAC CAG TTC GCC-3' |
| ***Y2*** | HS-5'-TTT CGC GCA CCT GAG ACC TTC TAA TAG GGT TTG CGA CAG TCG TTC AAC TAG AAT GCC CTT TGG GCT GTT CCG GGT GTG GCT CGT CGG-3' |
| ***Y3*** | HS-5'-TTT GGC CGA GGA CTC CTG CTC CGC TGC GGT TTG GCG AAC TGG TCC CGT CTA CTT ACC GTT TCC GAC GAG CCA CAC CCG GAA CAG CCC-3' |
| ***Y4*** | HS-5'-TTT GCC GTA ATG TGC ATG TAT CTC CAG GCT TTC CGC AGC GGA GCA GGA GTC CTC GGC CTT TGG GCA TTC TAG TTG AAC GAC TGT CGC-3' |

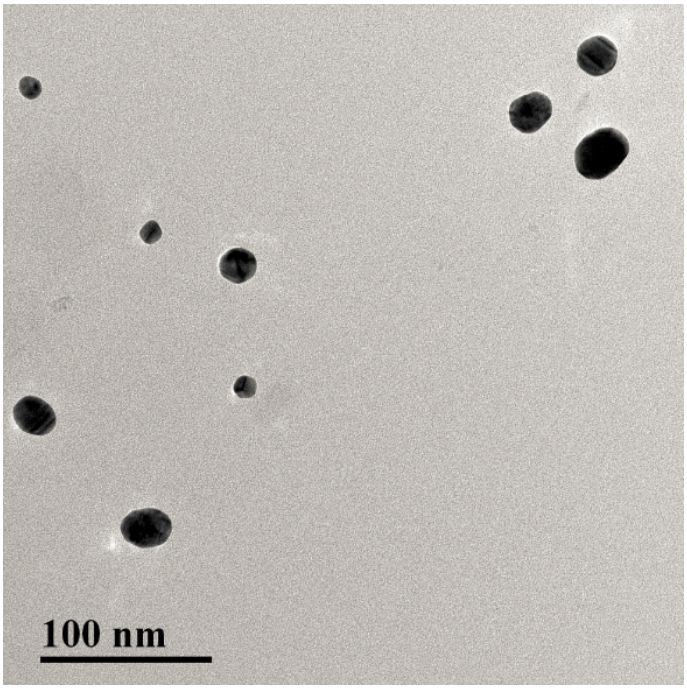


**Figure S1.** TEM picture of the mixture of $AuNP_1$ and $AuNP_2$.

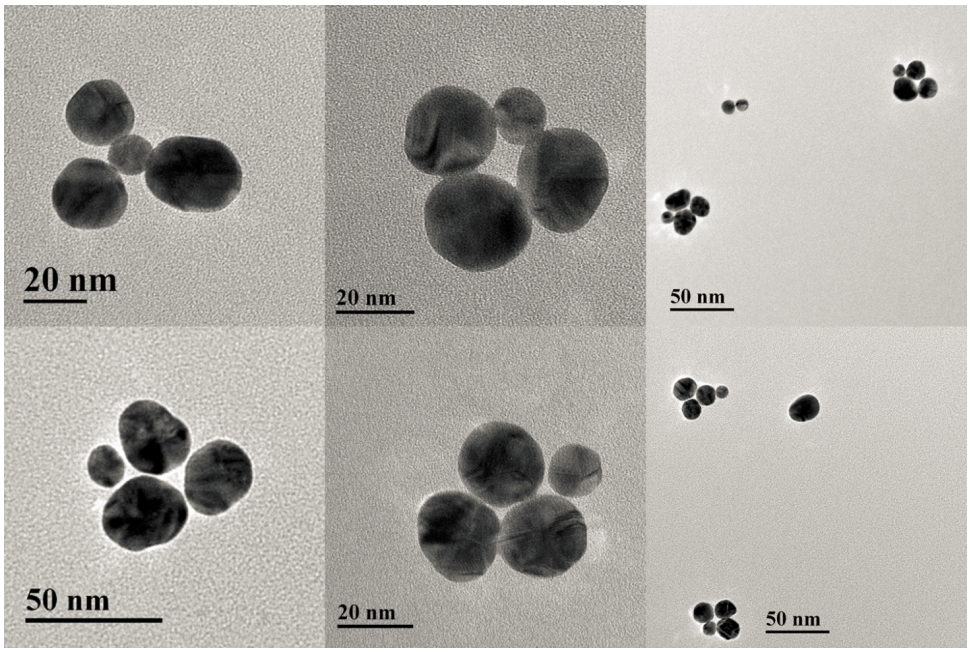


**Figure S2.** Some other TEM images of $3AuNP_2$+$1AuNP_1$ pyramid.

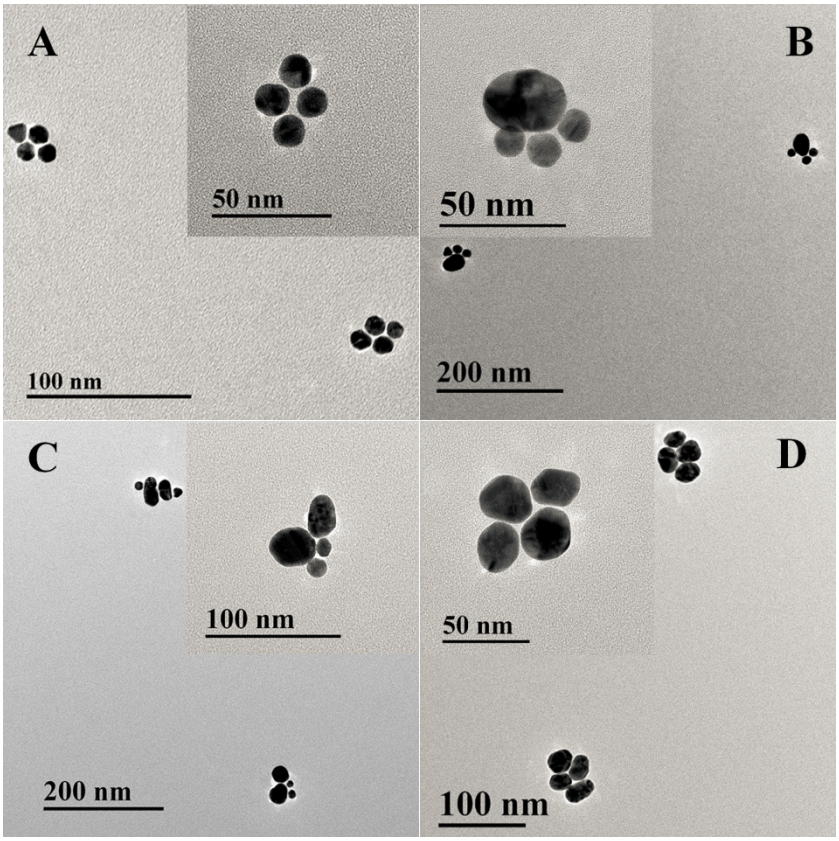


**Figure S3.** TEM images of $4AuNP_1$ pyramid (A), $1AuNP_2$+$3AuNP_1$ pyramid (B), $2AuNP_2$+$2AuNP_1$ pyramid (C), $4AuNP_2$ pyramid (D).

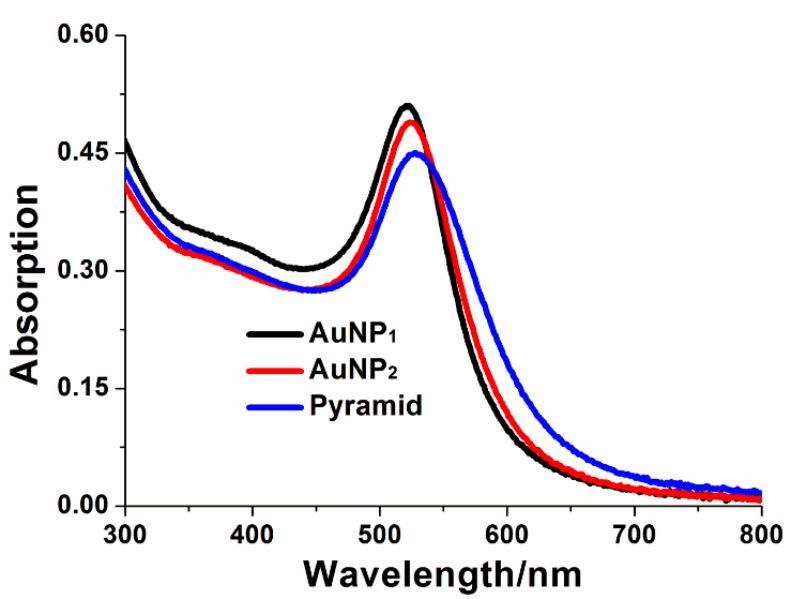


**Figure S4.** UV-Vis spectra of $AuNP_1$, $AuNP_2$ and $1AuNP_1+3AuNP_2$ pyramid.

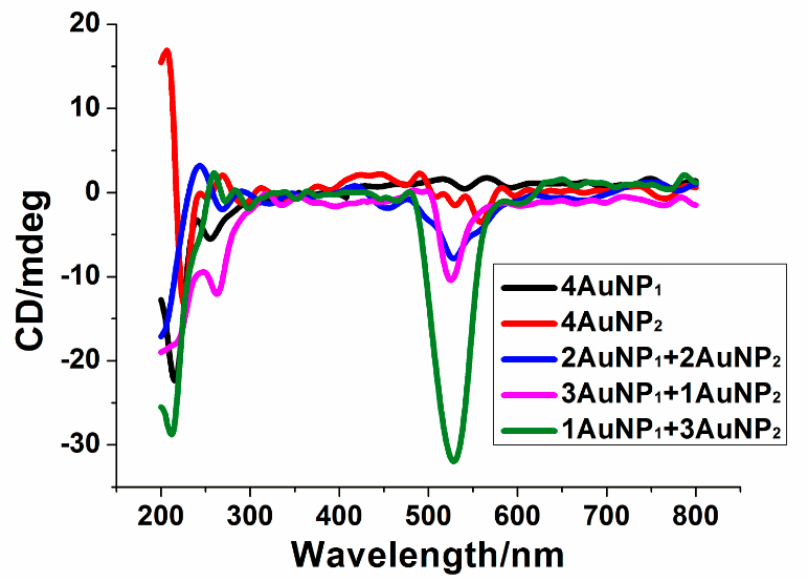


**Figure S5.** CD spectra of the five kinds of AuNP pyramids.

**Table S2.** Detection of DNase I in FBS samples

| sample | Original concentration (U/mL) | Spiked concentration (U/mL) | Determined mean ± SD[a] (U/mL) | Recovery (%) |
|---|---|---|---|---|
| FBS 1 | 0.034 | 0.05 | 0.079±0.005 | 93.3±5.0 |
| | | 0.1 | 0.132±0.004 | 97.7±5.2 |
| FBS 2 | 0.025 | 0.05 | 0.081±0.003 | 109.1±7.1 |
| | | 0.1 | 0.131±0.005 | 106.3±4.5 |

a: SD was calculated based on three parallel experiments for each sample.