Supporting Information

Materials

All single-stranded DNA used in this paper were synthesized and purified using polyacrylamide gel electrophoresis by Shanghai Sangon Biotechnology. The DNA sequences are shown in Table S1. Dipotassium bis(p-sulfonatophenyl)phenylphosphane dehydrate (DBD), tetrachloroauric acid ($HAuCl_4$) and sodium citrate were purchased from Sigma-Aldrich (St. Louis, MO, SA) and were used without further purification. Unless specifically indicated, all other chemicals were purchased from Shanghai Chemical Reagents Company (Shanghai, China). Ultrapure water from a Milli-Q device (18.2 MΩ, Millipore, Molsheim, France) was used in this research. All glassware was cleaned with freshly prepared *aqua regia* and then washed with ultrapure water prior to use.

Measurements

Particle size distribution and zeta potential were measured by a Zetasizer Nano ZS system (Malvern). The 633 nm laser was used for the DLS measurement. UV/vis spectra were recorded on a UNICO 2100 PC UV/vis spectrophotometer and analyzed with the Origin Lab software. The CD spectra were recorded on a J-710 spectropolarimeter (Jasco, Japan). JEOL JEM-2100 operating at an acceleration voltage of 200 kV was utilized for Transmission electron microscopy (TEM) images and energy dispersive X-ray spectroscopy (XEDS). Prior to TEM examination, 7.2 μL of the samples was dried onto a carbon film of TEM copper grids.

Synthesis of Au NPs

25 nm Au NPs were prepared by sodium citrate reduction. $HAuCl_4$ (49.2 mL, 0.01%) was heated to boiling and an aqueous solution of trisodium citrate (0.8 mL, 1wt%, freshly prepared) was quickly added under vigorous stirring and reflux. After several minutes, the color of the solution changed from blue to bright red. After boiling for 15 min, the solution was cooled down to room temperature and stored at 4 ℃. Au atom concentration of Au NPs was determined by UV/vis spectroscopy (25 nm Au NP equivalent to 2 nM).

10 nm Au NPs were synthesized by reduction of $HAuCl_4$ using sodium citrate-tannin acid. Firstly, 2.5 mL $HAuCl_4$ (0.4% by weight) was added to 77.5 mL water. After stirring, the mixture named A solution was prepared. Second, B solution was prepared as follows: 4 mL sodium citrate (1wt%),

0.1 mL tannic acid (1wt%) and 0.1 mL K_2CO_3 (25mM) were added to 15.8 mL water under stirring. Third, A and B solutions were heated to 60 ℃ for several minutes, and then B solution was quickly added to A solution under high-speed stirring. The solution was kept at 60 ℃ for 30 min under reflux until the color turned to reddish orange and did not change further, and then cooled to room temperature and stored at 4 ℃. The Au concentration of the Au NPs was determined by UV/vis spectroscopy (10 nm Au NP equivalent to 20 nM).

Preparation of Single-Stranded DNA-Modified Au NPs.

An aqueous 1 mL of 25nm Au NPs solution was stirred with 100 μL DBD (100 μg/mL) at room temperature for more than 10 h, and then the Au NPs modified with DBD were purified by centrifugation at 7200 rpm for 10 min, and re-suspended in 500 μL 0.5×Tris-borate buffer. 0.4 μL of 1 μM DNA2/ DNA4 /DNA6 solution was added to 100 μL of this solution and the resulting mixture (Au NPs/ssDNA=1/2, molar ratio) incubated for 12 h in 0.5×Tris-borate buffer, 50 mM NaCl with shaking, and then the Au NPs modified with single-stranded DNA were purified by centrifugation at 7200 rpm for 10 min, and re-suspended in the same volume of 0.5×Tris-borate buffer, and stored at 4 ℃.

An aqueous 10nm Au NPs solution (1 mL) was stirred with 200 μL DBD (100 μg/mL) at room temperature for more than 10 h, and then the Au NPs modified with DBD were purified by centrifugation at 13000 rpm for 10 min, and re-suspended in 500 μL 0.5×Tris-borate buffer. 2 μL of 1 μM DNA1/ DNA3/ DNA5 solution was added to 100 μL of this solution and the resulting mixture (Au NPs/ssDNA=1/1, molar ratio) incubated for 12h in 0.5×Tris-borate, 50 mM NaCl with shaking, and then the Au NPs modified with single-strand DNA were purified by centrifugation at 13000 rpm for 10 min, and re-suspended in the same volume of 0.5×Tris-borate, and stored at 4 ℃.

Detection methods

82 μL 25nm AuNPs-DNA4 solution and 8 μL 10nm AuNPs-DNA3 solution was injected into a 200 μL centrifuge tube. Then 10 μL Ag^+ at different concentrations was added to the centrifuge tube, respectively. The mixture was incubated on a shaker at room temperature for 1 h. Finally, the tube was equilibrated for 2 min. The CD spectra were recorded from 200 to 800 nm. The sample was detected using the same procedures as those used for the standards. The tap water sample was

filtered through a 0.22 μm membrane filter.

Detection system affected by DNA length

DLS, as a widely used characterization method that can provide information about the change in overall size of the dimers in solution. From the DLS data in Figure 4D, the hydrodynamic diameters of the dimers assembled by 13, 26, and 39 bp DNA were determined to be 46.33±3.5, 54.79 ±2.2, and 59.49±4.1 nm, respectively, in agreement with a progressive increase in the size of the heterodimer with the length of the DNA linker. The CD saturated intensities at 525 nm for heterodimers linked by 13, 26, and 39 bps were -68.88, -113.08, and -33.61 mdeg, respectively (Fig. 3A).

Calculating the CD and absorbance spectra

Simulations of CD and absorbance spectra were performed using CST Microwave Studio®. In the simulation, the elliptic sphere was used as Au NP. Simulation was carried out in the following parameters: elongation factors for 10 nm Au NPs and 25 nm Au NPs are 1.21 and 1.29. The gap between heterodimer is 8 nm. Heterodimer takes relative orientation specified by 10°.

Calculations of CD spectra: Calculation of optical properties was carried out based on different elements of a standard scattering matrix, also known as *S-matrix*, corresponding to amplitudes of different scattering modes. Their value is dependent on the probabilities of different interactions of NPs superstructures with light. The CD spectra were calculated from the matrix elements describing the transmission of RCP and LCP, T_{RCP} and T_{LCP} having the same polarization in the incoming beam. A similar matrix coefficient, T_{RLP}, describes the transmissions of LCP out of the model structure with RCP input. In turn, another coefficient, $T_{LRP,}$ corresponds to the transmissions of RCP out of the model structure with LCP as incoming light.

Calculating the anisotropy factor (g-factor)

The optical activity of chiral systems is often quantified using the g-factor:

$g = \Delta\varepsilon / \varepsilon$ Eq. 6

where $\Delta\varepsilon$ and ε are the molar circular dichroism and molar extinction coefficient, respectively.

Molar ellipticity $[\theta]$=mdeg/ light path(cm)/concentration(M)

Molar ellipticity $[\theta] = 3000 * \Delta\varepsilon$

$\Delta\varepsilon$= mdeg/ light path (cm)/concentration (M)/3000

ε is calculated by the absorbance (A), $\varepsilon = A/CL$. C is the concentration of the solution, L is the thickness of the solution=optical path length. Using the CD and the absorbance spectrum of the samples, the value of the g-factor can be calculated.

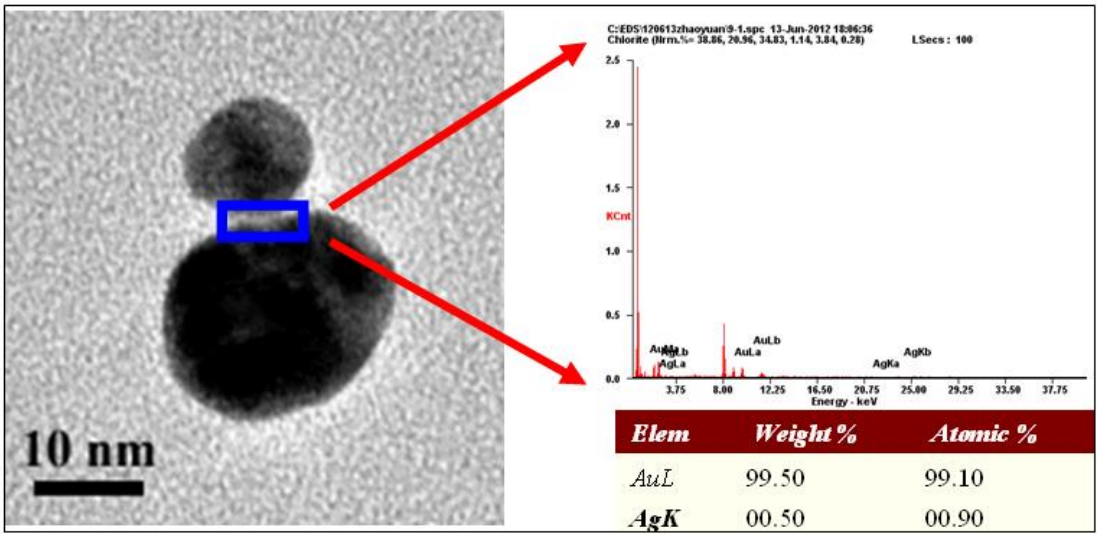

Figure S1. TEM image and XEDS spectrum of a gap in a single heterodimer.

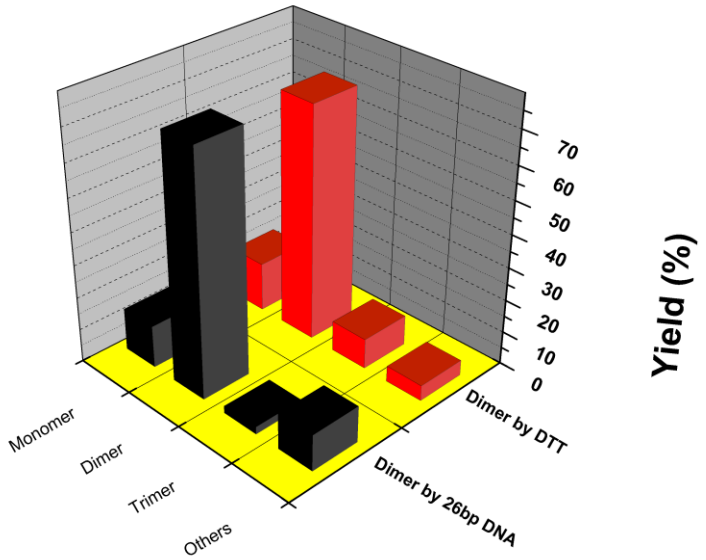

Figure S2. Yields for heterodimers assembled by 26bp DNA (10 nM Ag^{+}) and D,L-DTT (100 nM D, L-DTT), respectively. Note that, with the corresponding concentrations of inducers, all chiroptical activities of heterodimers were saturated.

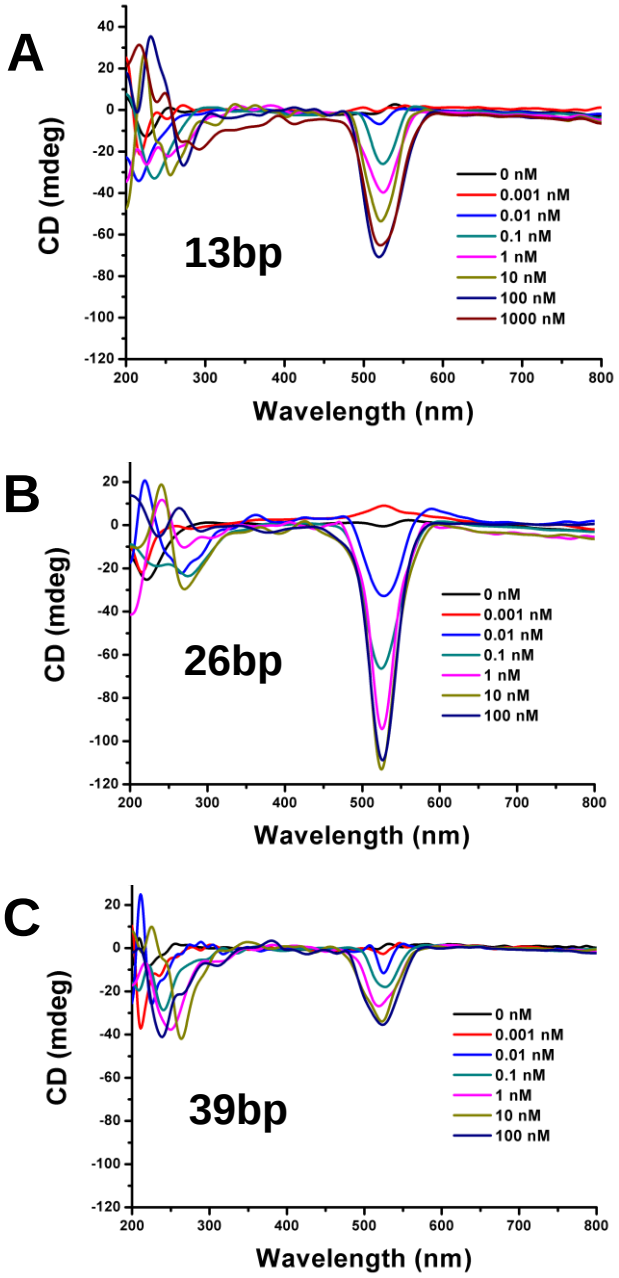

Figure S3. CD spectra of heterodimers mediated by different DNA lengths with varying Ag^{+} concentration.

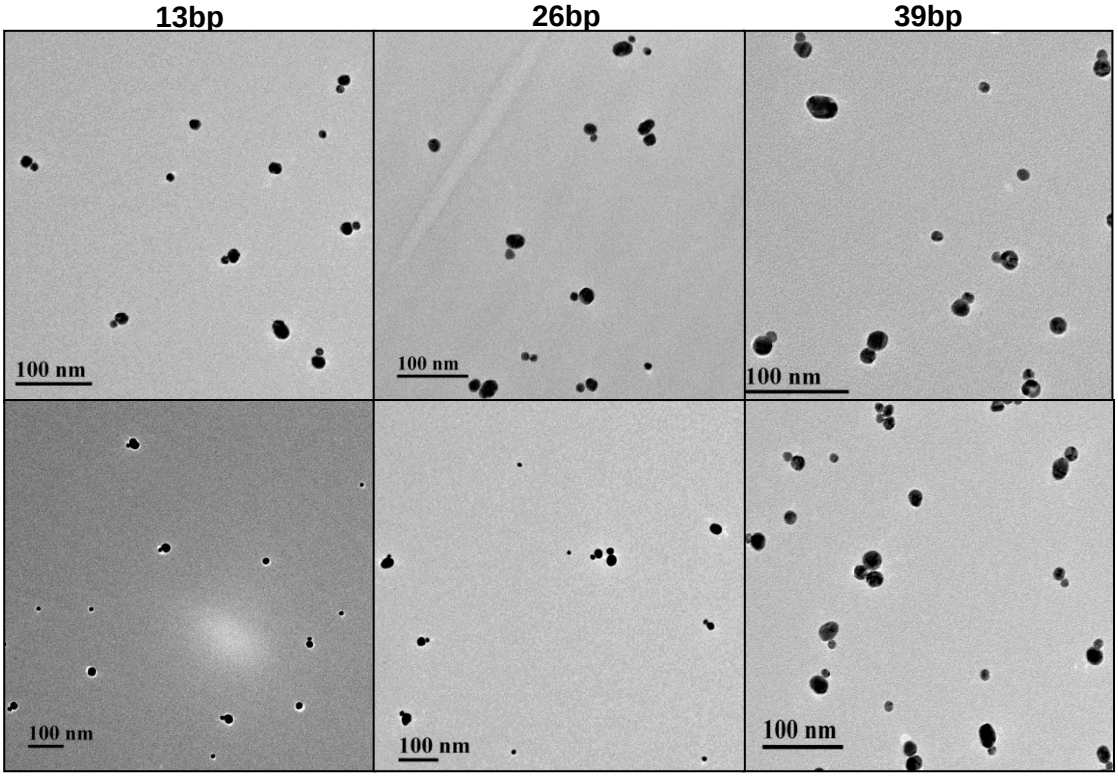

Figure S4. Representative TEM images for heterodimers assembled by different DNA lengths.

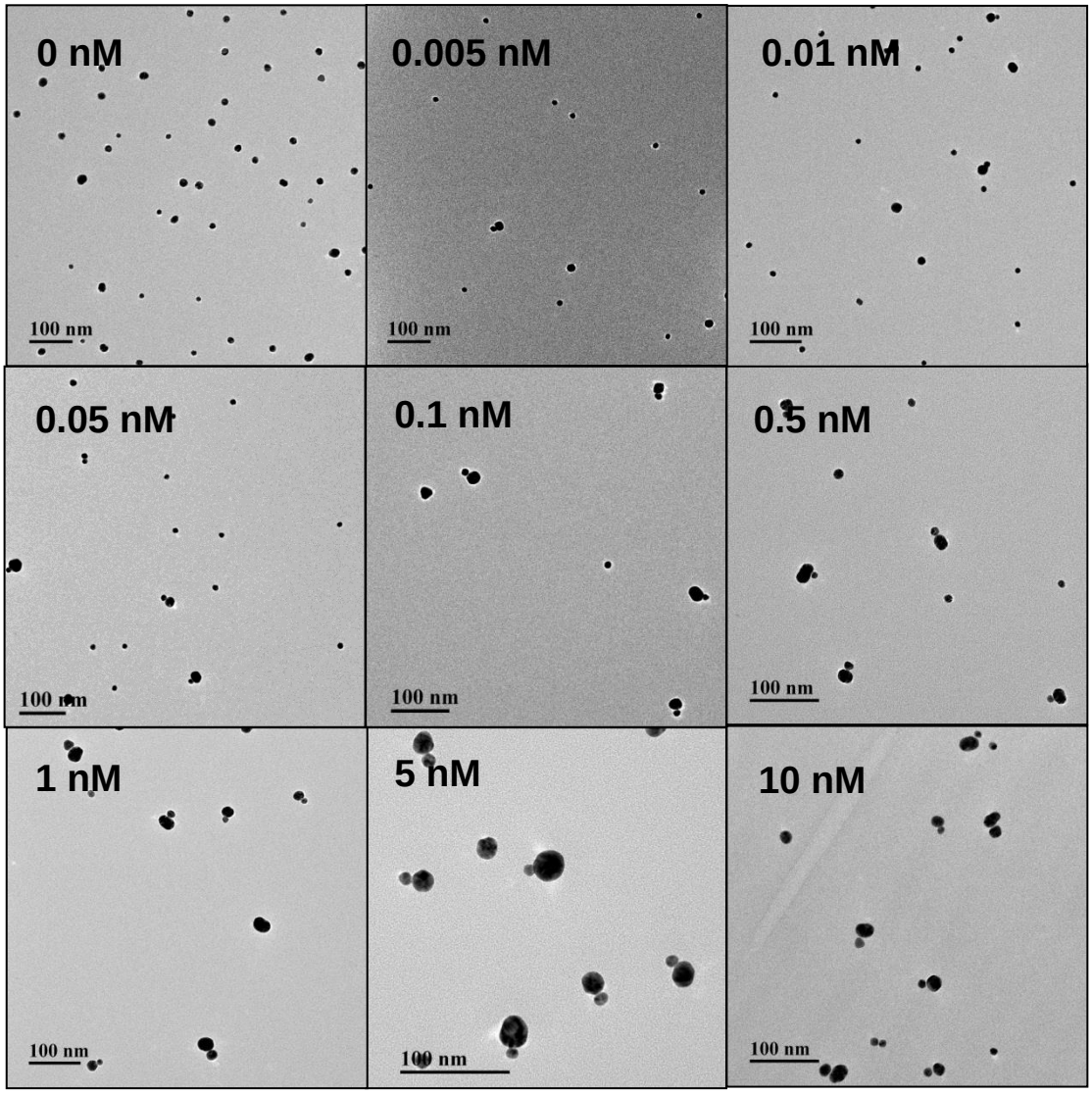

Figure S5. Representative TEM images for plasmonic chiroptical sensors in the range of 0-10 nM silver ions.

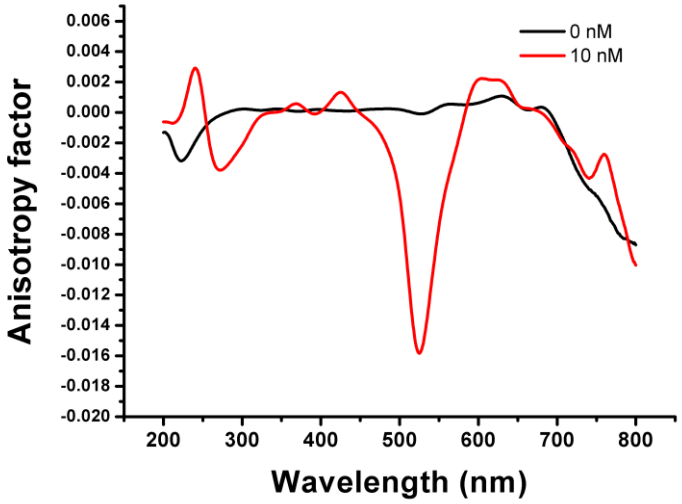

Figure S6. The g-factor of plasmonic NPs heterodimers.

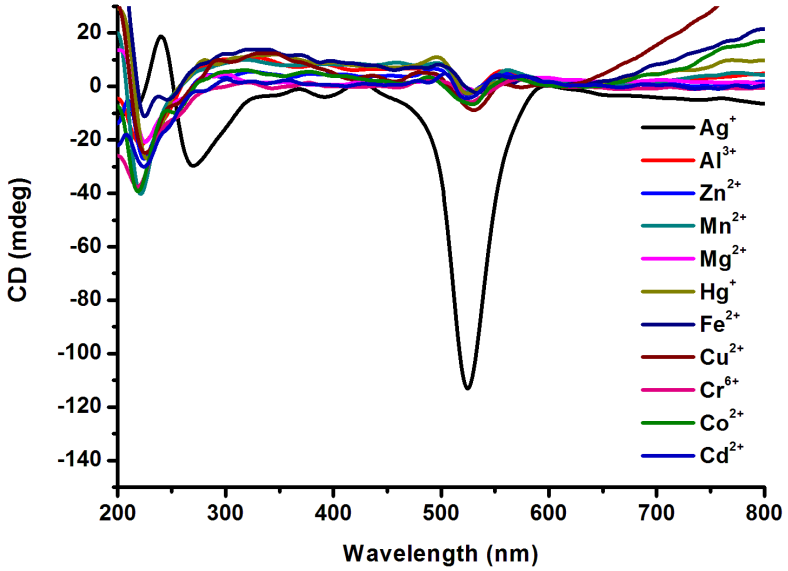

Figure S7. Specificity of the plasmonic chiroptical sensor for various metal ions. The concentration of silver ions is 10 nM and that of others metal ions is 100 nM in all cases.

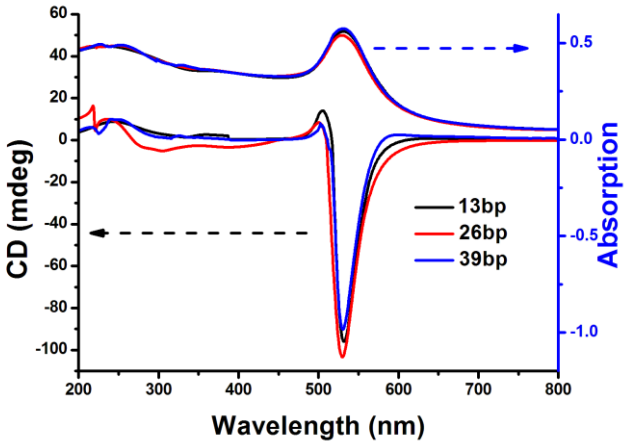

Figure S8. Calculated CD spectra and UV/Vis absorbance spectra of heterodimer formed by different DNA lengths.

Table S1. DNA sequence used in experiments (Each strand bears a hexyl-thiol linker at its 3' end).

Types	Sequence (5'-3')
DNA1	TCTCTTCTCTTCA
DNA2	TCAACACAACACA
DNA3	CTCTCTTCTCTTCTCTCTTCTCTTCA
DNA4	TCAACACAACACACAACACAACACAC
DNA5	CTCTCTTCTCTTCTTCTCTTCTCTTCTCTCTTCTCTTCA
DNA6	TCAACACAACACACAACACAACACAACAACACAACACAC