Supporting Information

Insight on chirality encoding from small thiolated molecule to plasmonic Au@Ag and Au@Au nanoparticles

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S.1 - Synthesis of AuBP84 and AuBP140

Gold bipyramids were prepared by seed-mediated growth method starting from overgrown pentatwinned gold seeds according to the fully described protocol previously reported by our group.[1]

Pentatwinned gold seeds preparation and overgrowth

Pentatwinned gold seeds were prepared by rapid reduction of $HAuCl_4$ salt in presence of CTAC. A quantitative of 1.7 g of commercial powder CTAC was added to a 250 mL round-bottom flask and dissolved in 73 g of Milli-Q water under stirring (40 x 20 mm^2 oval magnetic stirring bar was used). Once CTAC was completely dissolved, 800 µL of 25 mM $HAuCl_4$ was introduced followed by 1 mL of 0.1 M of citric acid solution. The mixture was then cooled to 19-21 °C with the help of an ice-water bath. To start the reduction of the gold salt 1 mL of 50 mM $NaBH_4$/NaOH (containing 50 mM of $NaBH_4$ and 50 mM of NaOH) solution was pipetted in 1-2 s, under vigorous stirring (800-1000 rpm). The color changed from pale yellow to brownish orange as indication of the nanoparticles formation. To remove all the H_2 produced, the reaction was stirred for 1 minute and the rest of unreacted $NaBH_4$ decomposed by adding 0.6 mL of 0.1 M of a citric acid solution. Then, the stirring was slowed to 200-300 rpm and the flask was heated at 85 °C with the help of an oil bath for 1 h. To proceed with the overgrowth, the temperature of the oil bath was then set at 45-50 °C and the stirring increased to 600-700 rpm. Separately, a growth solution was prepared by mixing 38 mL of 140 mM CTAB, 4.4 mL of 25 mM $HAuCl_4$, 800 µL of 40 mM $AgNO_3$, 1.1 mL of 0.1 M NaOH, and 2.4 mL of 0.4 M ethanolic HQL in a 50 mL beaker. The so obtained growth solution was then preheated to 40 °C and then poured into the seed solution. The mixture was then kept at 45-50 °C for 40 min. The overgrown seeds were then centrifuged at 11k rpm for 1 h, the red/orange supernatant was discarded and the particles re-dispersed in 6.5 mL of 1 mM CTAB. The process was repeated 3 times until the supernatant was colorless.

Synthesis of AuBP84

To prepare gold bipyramids a growth solution was slowly dropped into a suspension of overgrown seeds. **Seed solution**: 320 µL of previously obtained overgrown seeds and 12 µL of $AgNO_3$ (40 mM) were added to 0.75 mL CTAB (140 mM) in a closed vial. The vial was then heated in a water bath 2 min at 60°C before adding the growth solution. **Growth solution:** In another vial, 0.5 mL of $HAuCl_4$ (100 mM) was added under vigorous stirring to 1.5 mL CTAB (140 mM), followed by 90 µL of 1,1,3,3-tetramethylguanidine (0.8 M), 30 µL of $AgNO_3$ (40 mM) and finally by 325 µL catechol (0.8 M in 25:75 EtOH/H_2O). The growth solution was stirred for 2 min (the color changed from orange to deep brown). Finally, the growth solution was incorporated into the hot seed solution under stirring, in 3-4 minutes using a pipette. Then, the heating was maintained at 60°C for 20 min. The AuBPs suspension was centrifuged at 8000 rpm for 5 min adding 150 µL of EtOH to reduce the viscosity. Once washed with 5 mM CTAB the particles can be finally dispersed in 2 mL of 5 mM CTAB.

To prepare gold bipyramids a growth solution was slowly dropped in to a suspension of overgrown seeds. **Seed solution**: 160 µL of previously obtained overgrown seeds and 12 µL of $AgNO_3$ (40 mM) were added to 0.75 mL CTAB (140 mM) in a closed vial. The vial was then heated in a water bath 2 min at 60°C before adding the growth solution. **Growth solution:** In another vial, 0.5 mL of $HAuCl_4$ (100 mM) was added under vigorous stirring to 1.5 mL CTAB (140 mM), followed by 90 µL of 1,1,3,3-tetramethylguanidine (0.8 M), 30 µL of $AgNO_3$ (40 mM) and finally by 325 µL catechol (0.8 M in 25:75 EtOH/H_2O). The growth solution was stirred for 2 min (the color changed from orange to deep brown). Finally, the growth solution was incorporated into the hot seed solution under stirring, in 4-5 minutes using a pipette. Then, the heating was maintained at 60°C for 20 min. The AuBPs suspension was centrifuged at 8000 rpm for 5 min adding 150 µL of EtOH to reduce the viscosity. Once washed with 5 mM CTAB the particles can be finally dispersed in 2 mL of 5 mM CTAB.
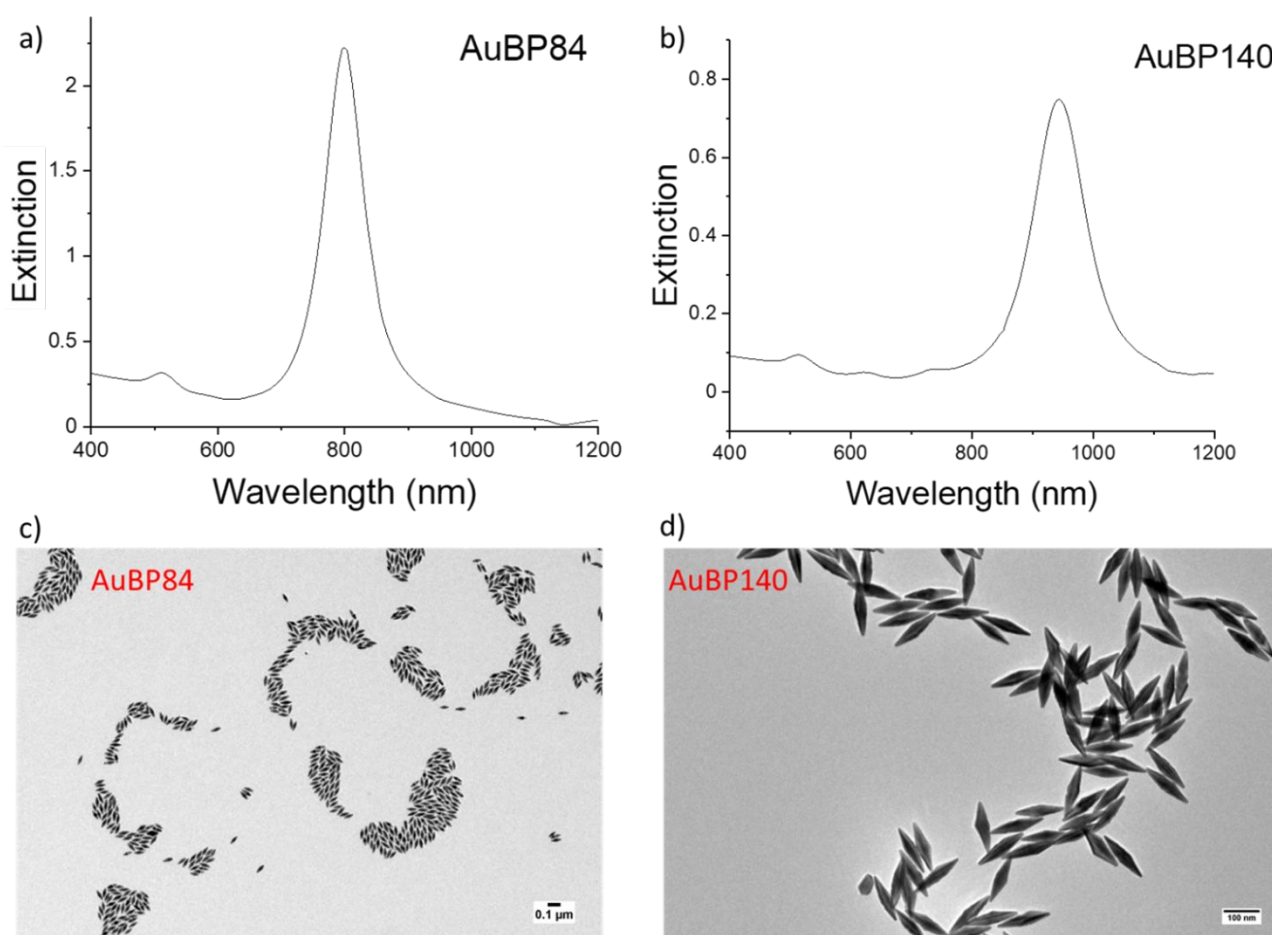

Figure S1. AuBPs characterization. Extinction spectra of (a) AuBP84 and (b) AuBP140. Transmission electron microscopy images of (c) AuBP84 and (d) AuBP140.

Table T1. TEM statistics over 200 NPs. The volume and the surface of the single particle are calculated using the formulas reported in Figure S2.

AuBP	D (nm)	L (nm)	d (nm)	$V_{1\ particle}$ (nm^3)	$S_{1\ particle}$ (nm^2)
AuBP84	26 ± 2	84 ± 6	9 ± 1	20329 ± 2781	4537 ± 412
AuBP140	36 ± 3	140 ± 8	9.5 ± 0.8	59494 ± 4462	9800 ± 735
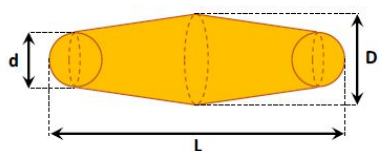
$$V_{1\,particle} = \frac{\pi}{6}\left(\frac{L-d}{2}(D^2 + Dd + d^2) + d^3\right)$$

$$S_{1\,particle} = \pi(D + d)\sqrt{\left(\frac{L-d}{2}\right)^2 + \left(\frac{D-d}{2}\right)^2} + \pi d^2$$

Figure S2. Truncated cones with spherical tips model used to calculate the volume and the surface of a single particle.

Table T2. Molar extinction coefficients at 400 nm for different AuBPs used. Au^0 concentration was obtained by ICP and correlated to extinction at 400 nm to obtain ε_{400} using Lambert-Beer law.

AuBP	ε_{400} ($L\cdot mg^{-1}\cdot cm^{-1}$)	Extinction at 400 nm	$[Au^0]$ ($mg\cdot L^{-1}$)
AuBP84	0.0141	0.3643	25.8
AuBP140	0.0149	0.5148	34.4

Au^0 concentration was obtained by ICP and correlated to extinction at 400 nm to obtain ε_{400} using Lambert-Beer law. NPs concentration can be obtained dividing the total Au^0 volume by the volume of one single particle ($V1_{particle}$) (calculated with the relation in table T1 and Figure S2). The $[Au^0]$ in the suspension of AuBP84 and AuBP140 used as core was set to 0.5 g/L. 50 µL contains 25 µg of Au^0 corresponding to V_{Au} = $1.29\cdot10^{-6}$ cm^3 (using ρ_{Au} = 19.32 g/cm^3). The AuBP84 concentration for 50 µL used in Figure 4 in the main text is then $2.11\cdot10^{-9}$ mol/L while for AuBP140 is $7.22\cdot10^{-10}$ mol/L.
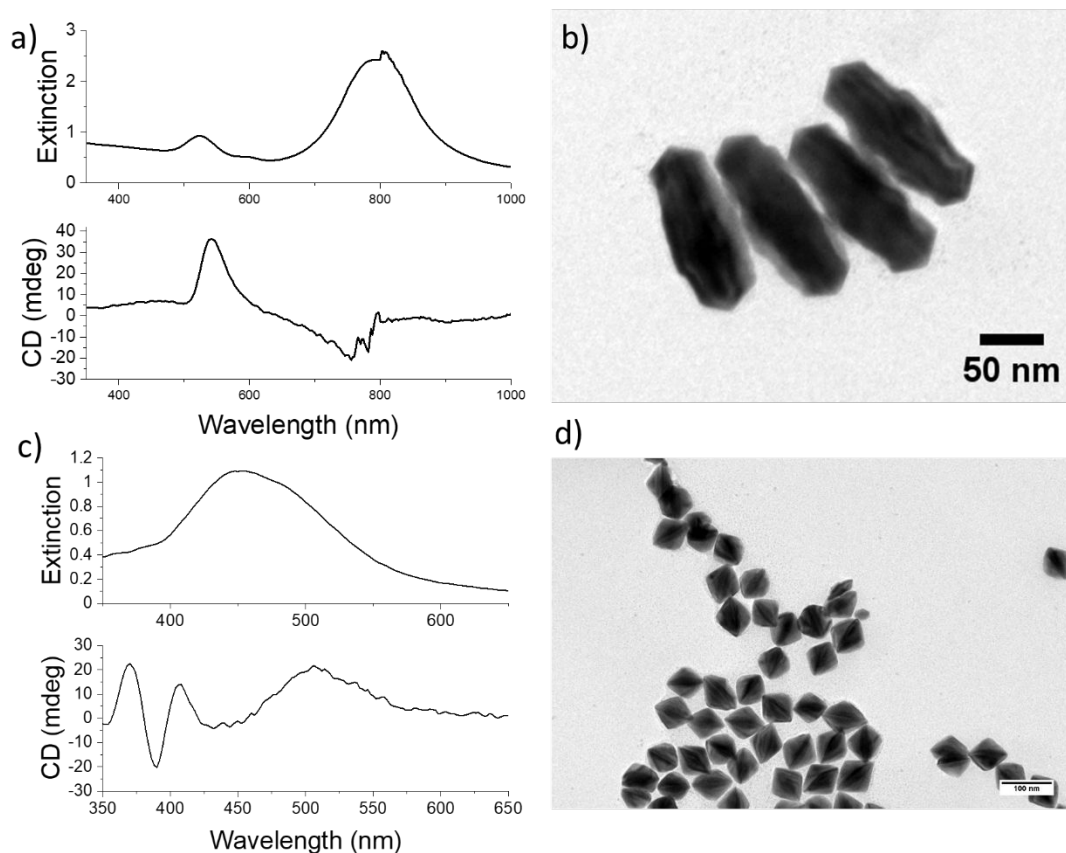

Figure S3. (a) Extinction and CD spectra of AuBP140@Au and (b) related TEM image. (c) Extinction and CD spectra of AuBP84@Ag and (d) related TEM image.
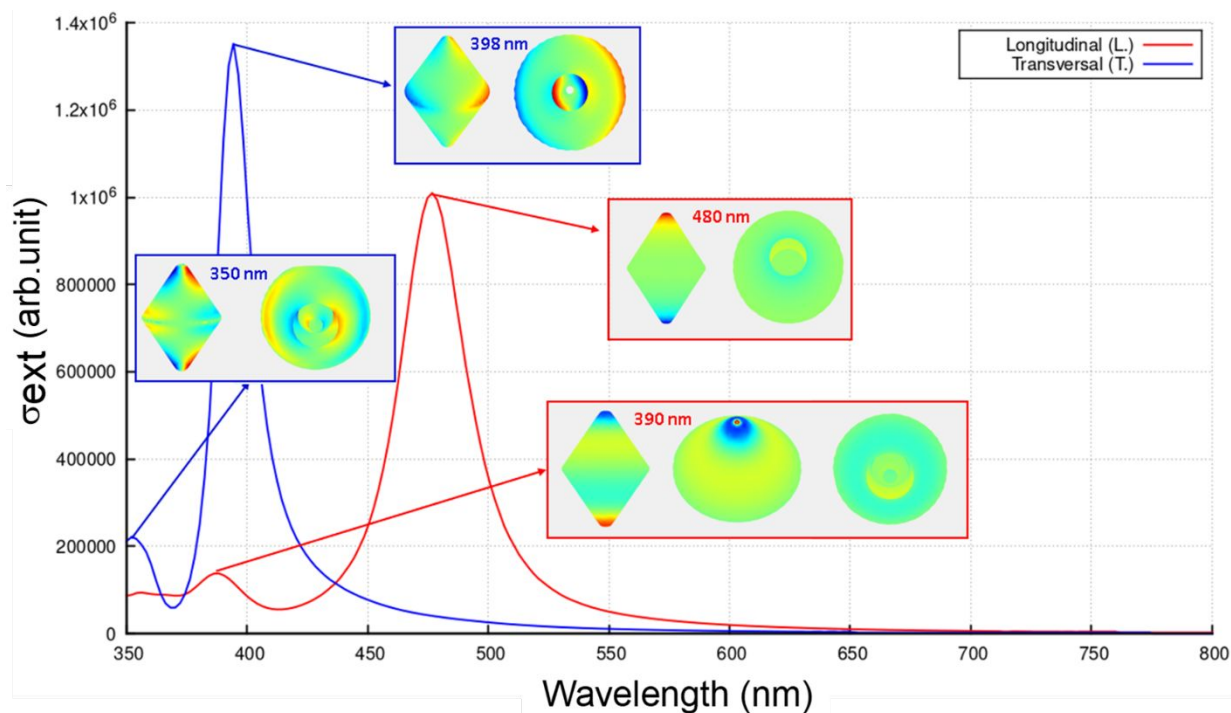
Figure S4. Quasi-static simulations of the extinction spectrum of the AuBP140@Ag model structure (see main text, computational details) upon an incoming electric field oriented either along the transversal (blue curve) or longitudinal direction (red curve).
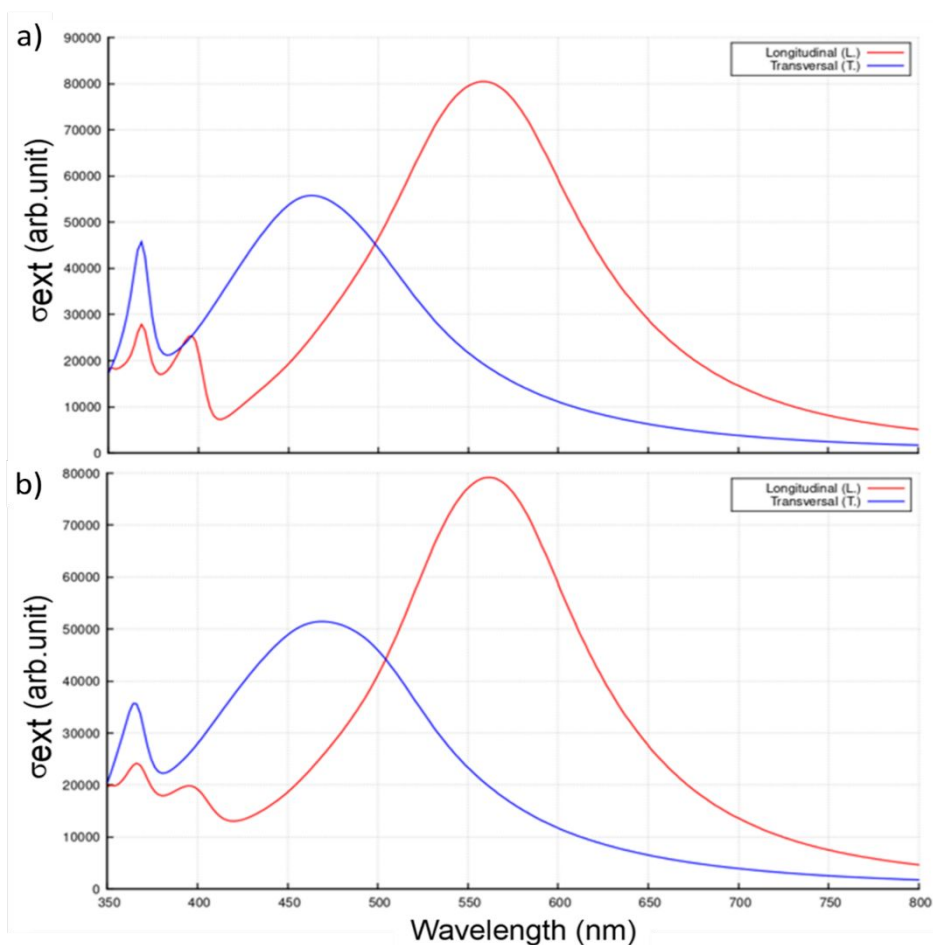

Figure S5. Simulated extinction spectra of the AuBP140@Ag model structure (b) with or (a) without the Au core obtained by solving the full-Maxwell equations.
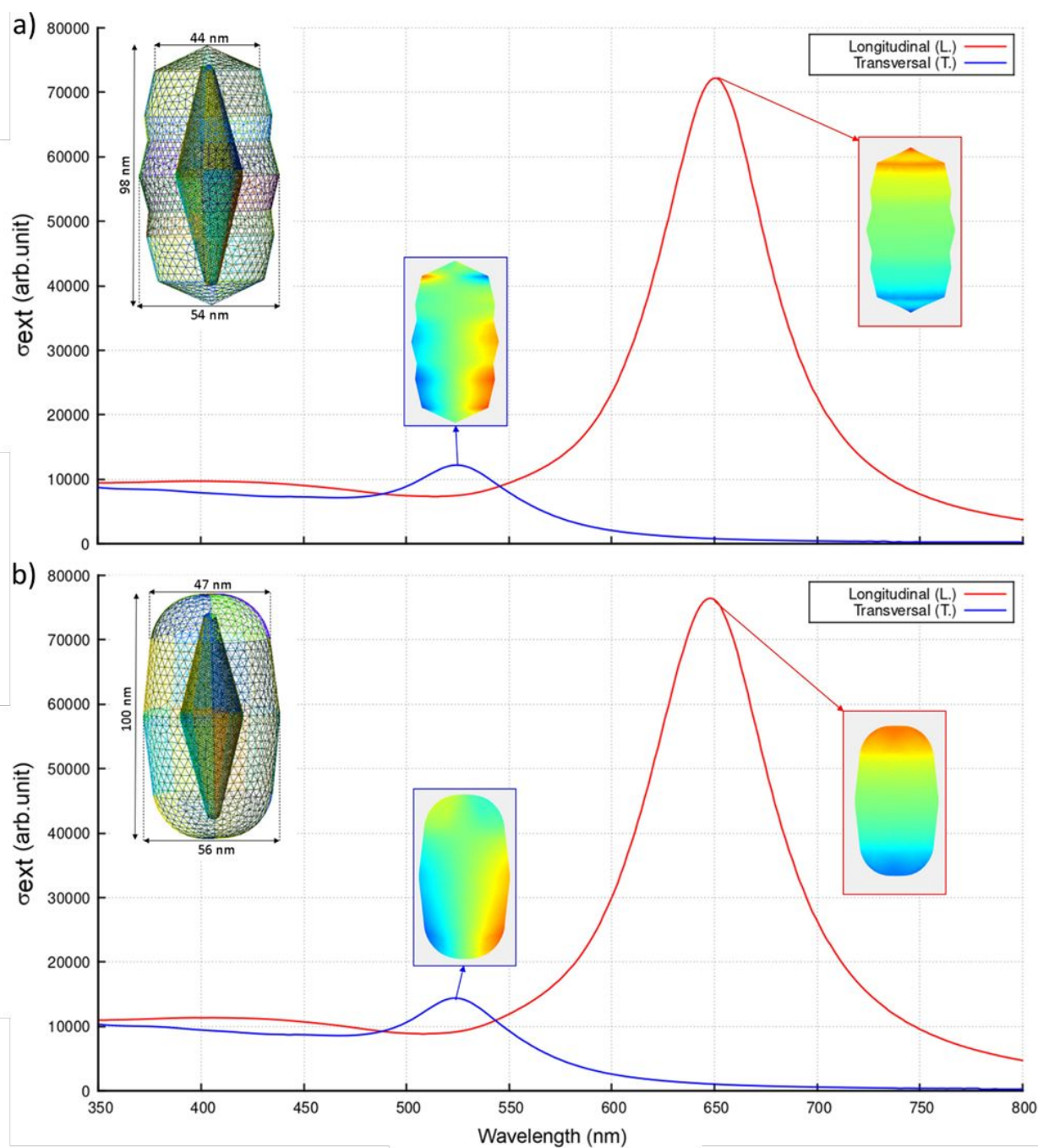
Figure S6. Simulated extinction spectra of two different simplified gnocchi-like AuBP84@Au model NPs (panels a,b) based on the geometrical scheme reported in Figure 6b, main text. The outer Au layer is made transparent for clarity. The inner Au BP is 85(height) x 26(width) nm. Remarkably, despite the simplicity of the outer Au layer morphology employed in the simulations, the computed extinction spectra are in good agreement with the experimental counterpart shown in Figure 3b, solid black curve. The peak energies of the two main resonances are well reproduced and they mainly exhibit dipolar character, as expected. Notably, the absence of the wavy lateral surface in b) does not lead to prominent changes in the extinction spectra.
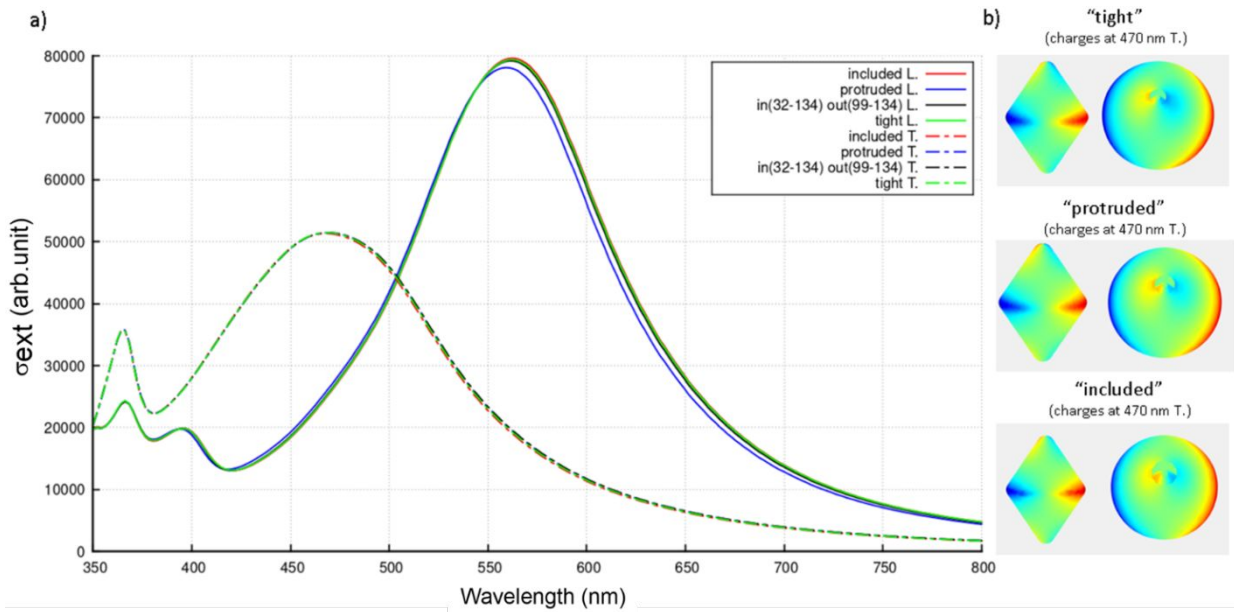
Figure S7. (a) Comparison among the simulated extinction spectra of three distinct AuBP140@Ag bipyramids featuring different tip structures. In the "tight" model the outer silver holes at the apexes, which leave the Au core tips exposed (see computational details and main text), are narrower than those of the reference AuBP140@Ag structure, whose spectra are reported in black. In the "protruded" case the inner gold core is protruding out of the silver shell, whereas in the "included" model the silver outer layer is completely covering the inner gold core. Solid curves correspond to longitudinal polarization of the incoming electric field, whereas dashed ones correspond to transversal polarization. (b) Surface charges at 470 nm (transversal polarization).
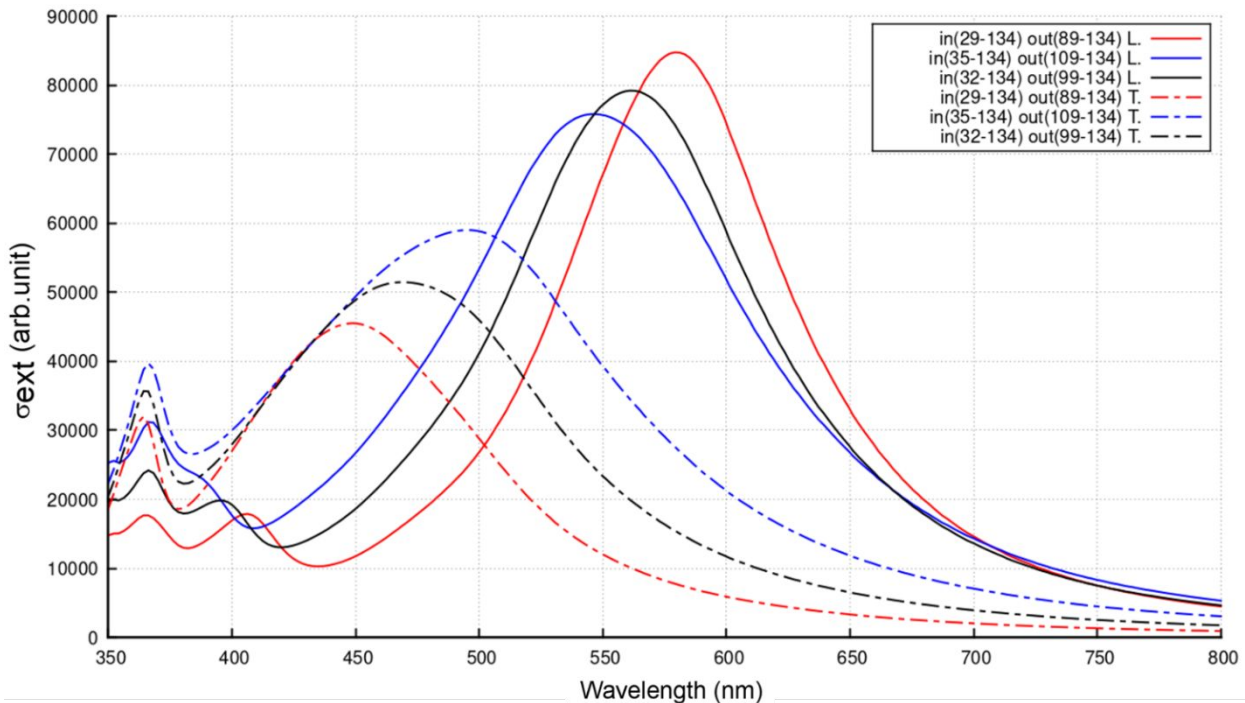

Figure S8. Comparison among the simulated extinction spectra of three distinct AuBP140@Ag featuring different aspect ratios. The shorthand notation used in the legend stands for the width-length dimensions of the inner gold AuBP core and outer silver shell expressed in nm (*i.e.*, inner(width-length) outer(width-length)). The results in black are related to the reference AuBP140@Ag structure. Solid curves correspond to longitudinal polarization of the incoming electric field, whereas dashed ones correspond to transversal polarization.
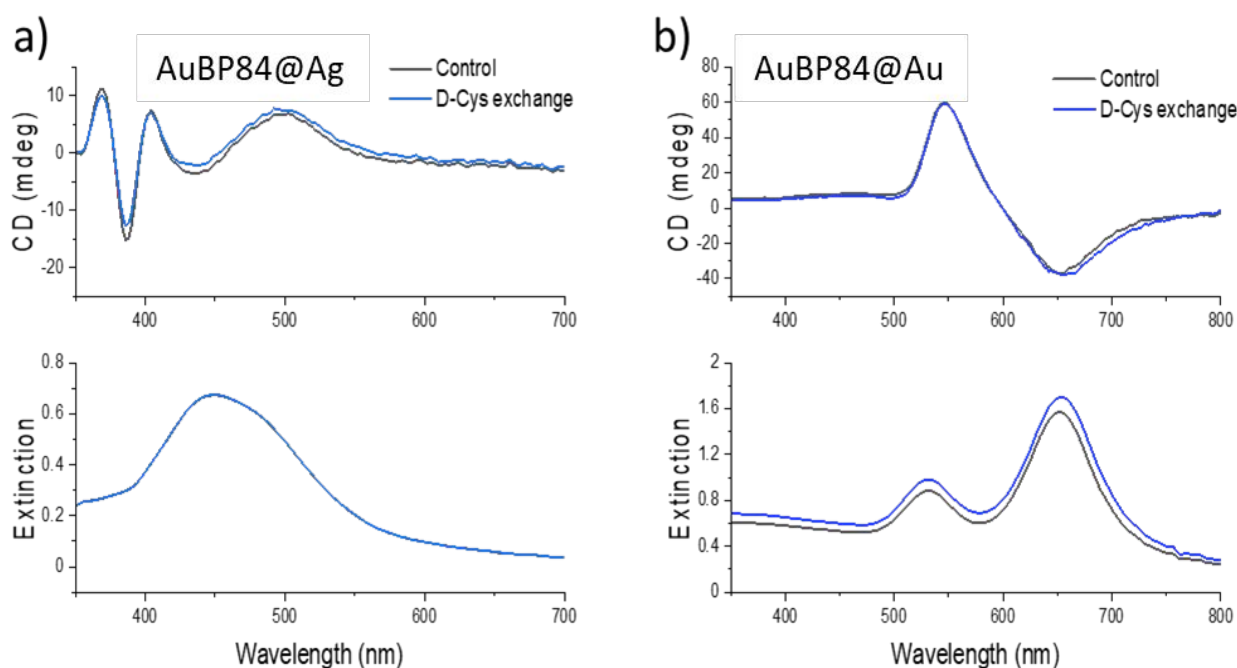
Figure S9. Effect of adding D-Cys. Extinction spectra and CD spectra of (a) AuBP84@Ag and (b) AuBP84@Au. To exchange the L-Cys by D-Cys, the particles were washed by centrifugation, then the D-Cys concentration was set at 10 µM and the mixture incubated for 1 h at 60-70°.

S.2 - NaBH$_4$ treatment and cysteine titration

NaBH$_4$ treatment

A volume of 1 mL of AuBP84@Au or AuBP140@Ag synthesized in standard conditions (see Methods in the main text) was centrifuged at 10k rpm for 3 minutes and re-dispersed in 1 mM CTAB. The operation was repeated three times to remove all the cysteine in solution except the one adsorbed on the surface. The particles were then centrifuged again and the supernatant removed. Then, NaBH$_4$ was added to the pellet. The final concentration of NaBH$_4$ was 25mM and the final volume was 100 µL. The suspension was incubated 10 minutes at RT and then centrifuged again to collect the clear supernatant containing the freed cysteine. Before measurement, the remaining NaBH$_4$ was decomposed incubating the supernatant for 24h at 25 °C.

A reference sample was produced incubating AuBP84 seeds with Cys (20 µM) to produce AuBP84@Cys. Once produced this reference sample was treated like the core-shell NPs samples.

Cysteine titration

The cysteine titration was performed using a thiol detection assay kit (Cayman, 700340). A volume of 50 µL of the sample was added to 50 µL of a solution containing the fluorescent dye and a buffer (pH 7.4, phosphate/EDTA). The fluorescence was recorded after 5 minutes of incubation using an excitation wavelength of 390 nm and an emission of 515 nm. A calibration curve was made for Cys concentration ranging from 0 µM to 1 µM in similar conditions as the sample (1mM CTAB and 1 day after incubation of 25mM NaBH$_4$).

Surface coverage

To evaluate the surface coverage, the number of the molecules measured experimentally was divided by the total surface area which corresponds to the area of a single particle times the number of particles present in 1 mL of synthesized chiral NPs. To calculate the area of a single particle the model in Figure S2 was used. The number of particles was equivalent to the number of AuBPs added, calculated in Table T2. The results are shown in Table T3.
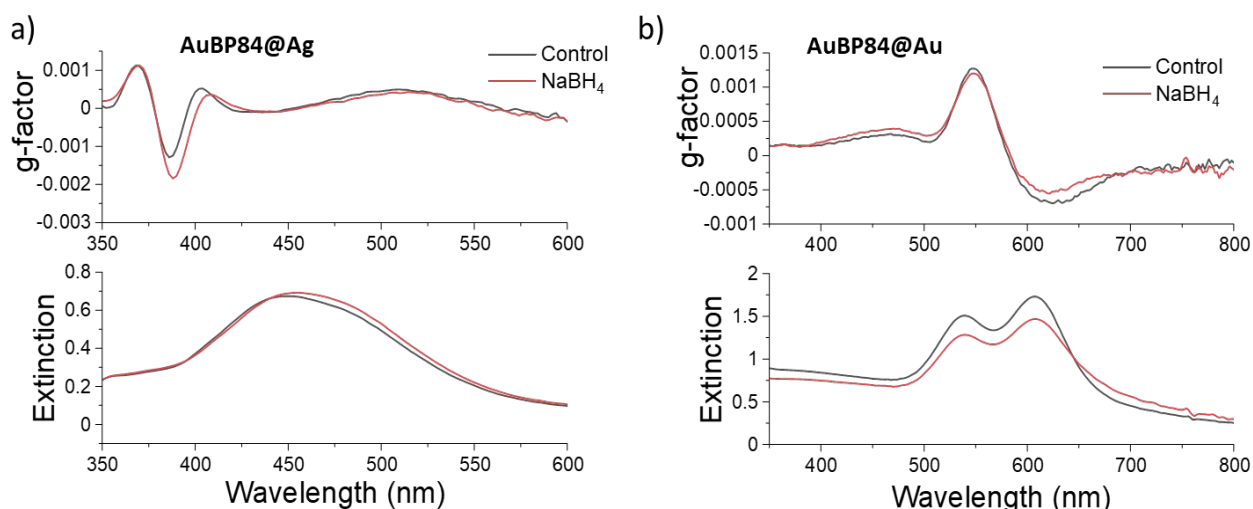
Figure S10. Effect of reductive desorption of cysteine with NaBH₄ on the surface of the NPs. Extinction and g-factor spectra of (a) AuBP84@Ag and (b) AuBP84@Au. The changes in the extinction spectra are attributed to a partial aggregation of the sample as a result of the NaBH₄ treatment.
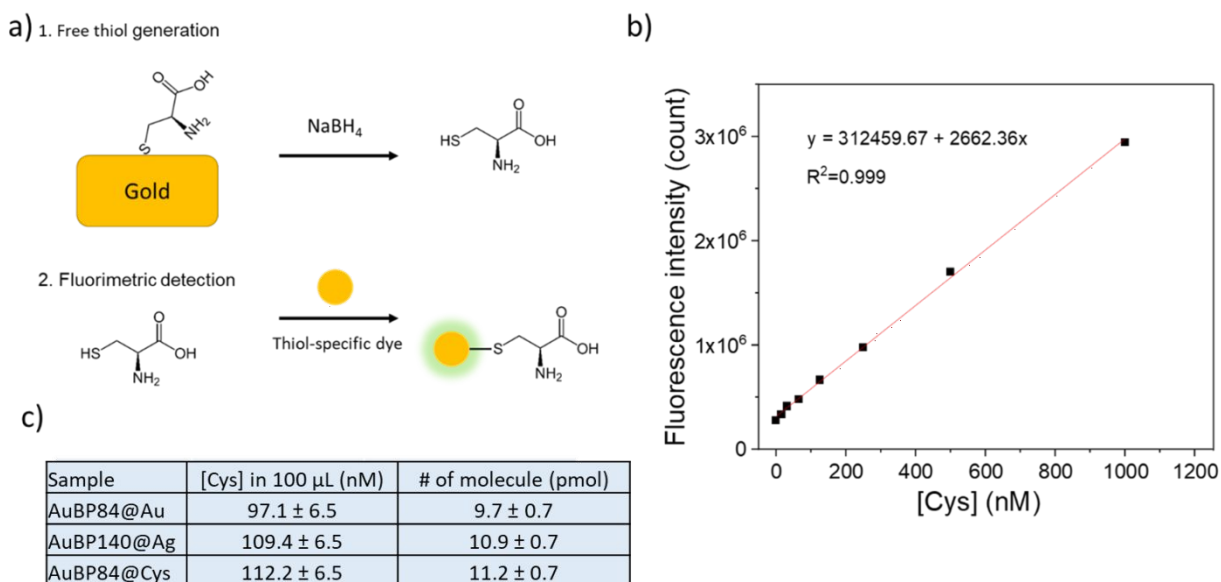

Sample	[Cys] in 100 µL (nM)	# of molecule (pmol)
AuBP84@Au	97.1 ± 6.5	9.7 ± 0.7
AuBP140@Ag	109.4 ± 6.5	10.9 ± 0.7
AuBP84@Cys	112.2 ± 6.5	11.2 ± 0.7

Figure S11. Cysteine titration. (a) Schematic representation of the experimental procedure: The cysteine was freed in solution after reduction with NaBH₄. A fluorescent derivate was generated when the thiol reacted spontaneously with the dye. (b) Calibration curve from 0 µM to 1 µM of L-Cys with fluorometric assay. (c) Cysteine quantification for AuBP84@Au and AuBP140@Ag and AuBP84@Cys. The uncertainty refers to the standard deviation calculated by measuring 3 different samples of 100 nM cys.

Table T3. Calculation of the surface coverage for AuBP84@Au and AuBP140@Ag and AuBP84@Cys.

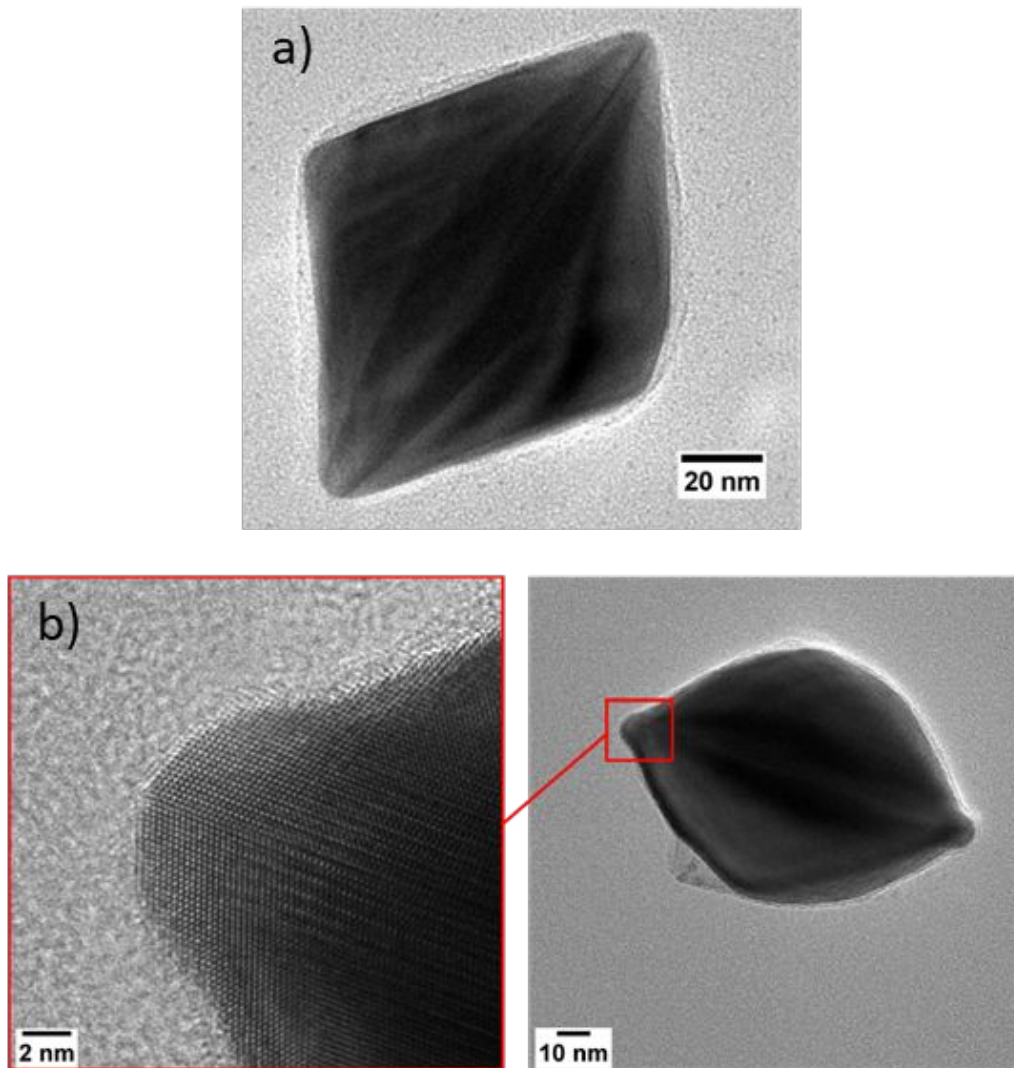| NPs	$S_{1\ particle}$ (nm²)	$N_{particles}$	S_{total} (cm²)	S coverage (nmol·cm⁻²)
AuBP84@Au	14384	$3.18 \cdot 10^{10}$	4.57	0.0021
AuBP140@Ag	25559	$8.70 \cdot 10^{9}$	2.22	0.0049
AuBP84	4537	$3.18 \cdot 10^{10}$	1.44	0.0054

Figure S12. Electron microscopy characterizations of AuBP140@Ag. (a) TEM images showing the AuBP core inside the Ag shell. (b) HRTEM of AuBP140@Ag.
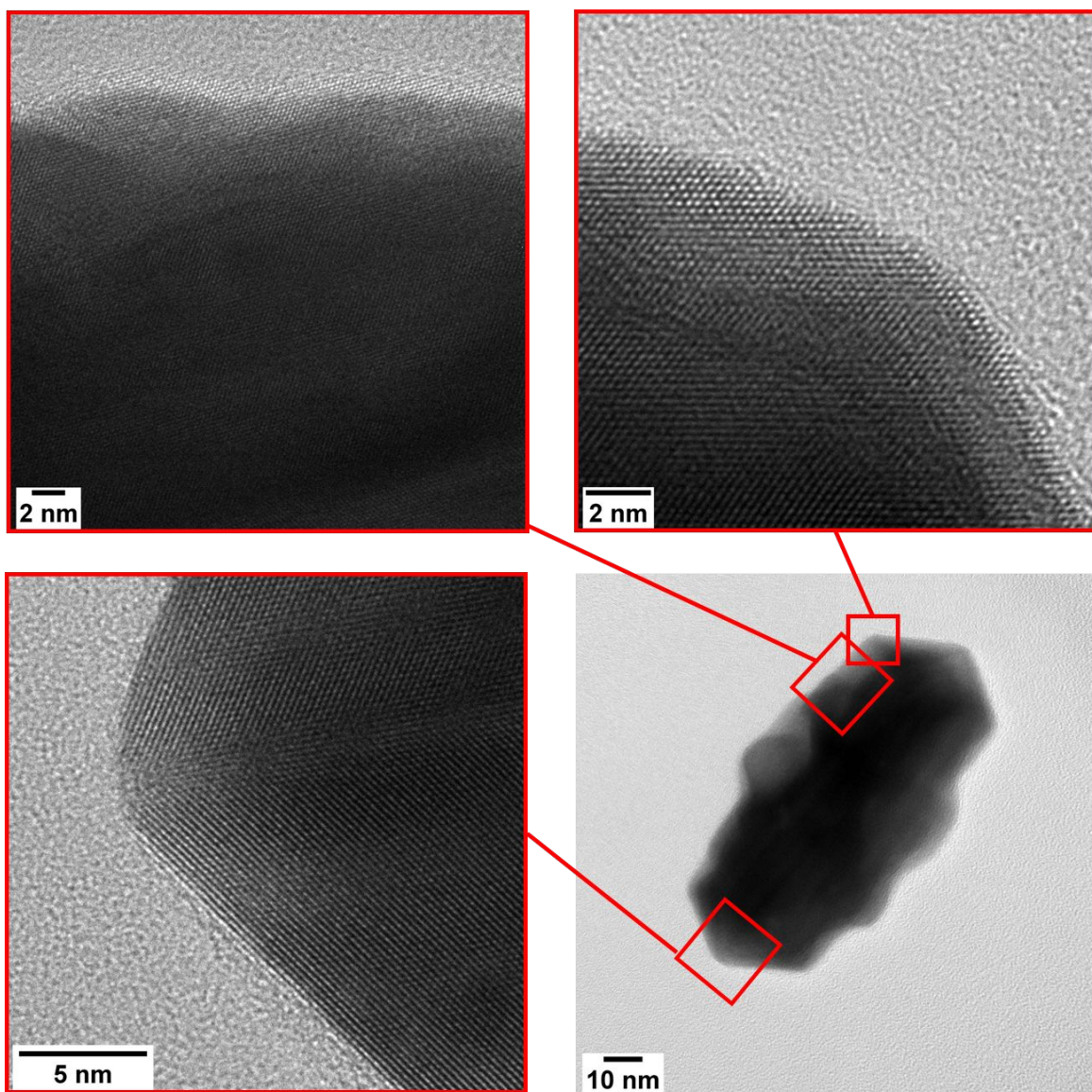
Figure S13. Electron microscopy characterizations of AuBP84@Au. HRTEM of AuBP84@Au confirms the crystalline nature of the wavy lateral side.
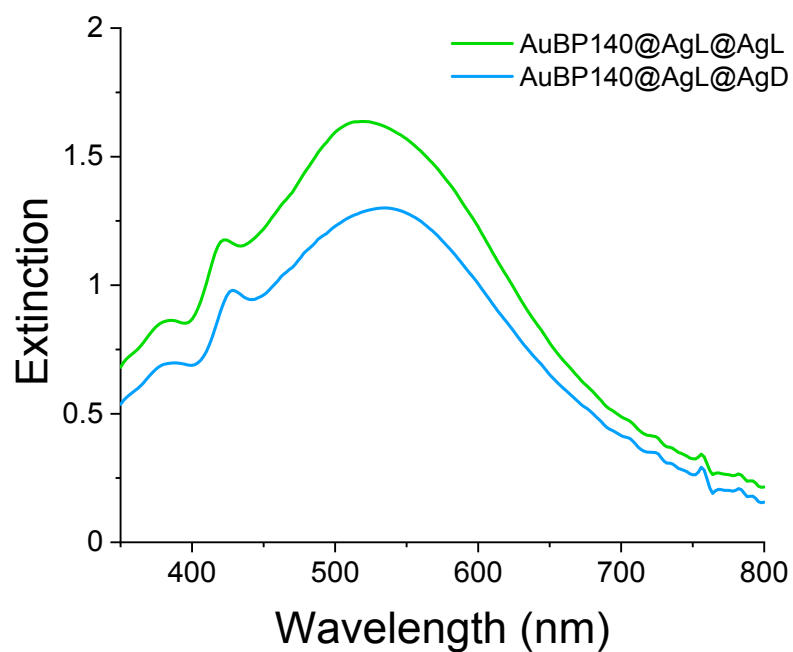
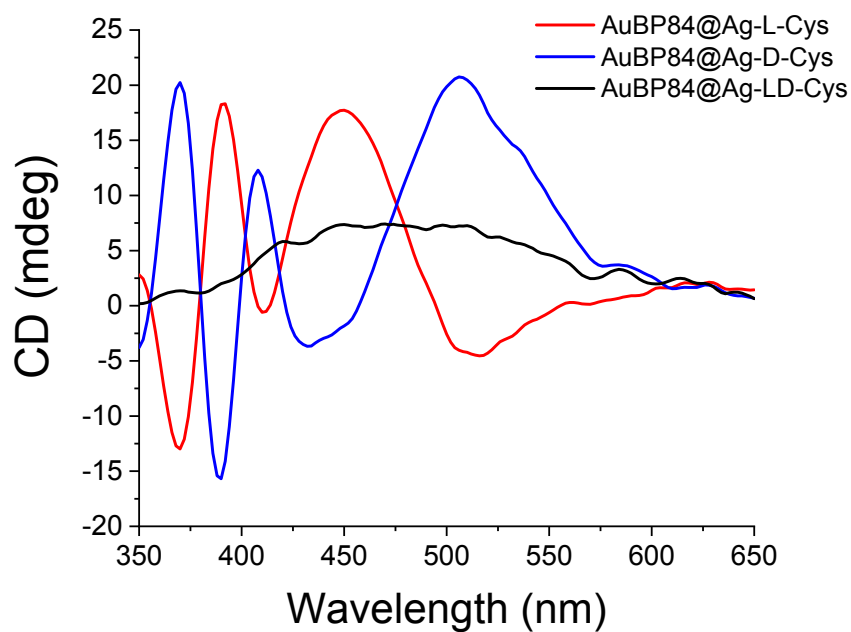**Figure S14.** Extinction spectra of AuBP140@AgL@AgD and AuBP140@AgL@AgL.

Figure S15. Impact on the CD spectrum for AuBP84@Ag obtained using L-Cys, D-Cys and racemic mixture of Cys.
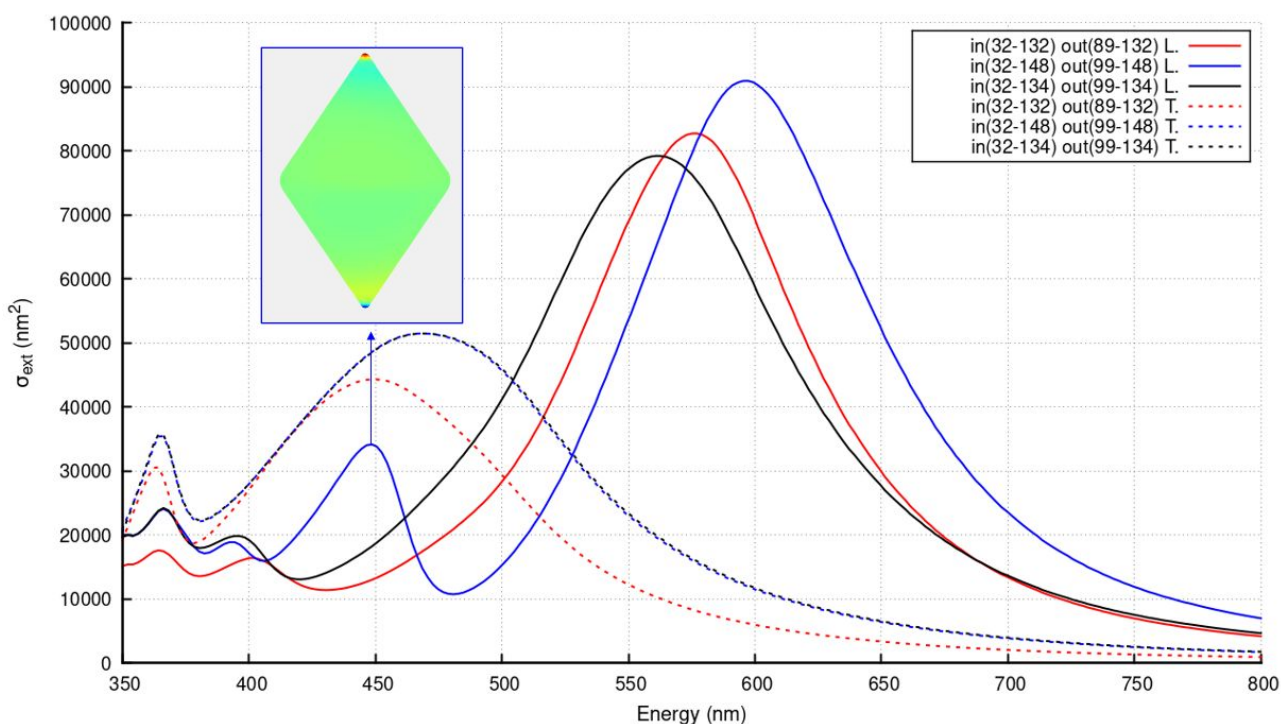
Figure S16. Simulated extinction spectra of three distinct AuBP140@Ag structures whose dimensions were chosen based on the TEM statistical analysis of the 20µL-sample (Figure 4d, main text). The shorthand notation used in the legend stands for width-length dimensions of the inner gold AuBP core and outer silver shell expressed in nm (*i.e.*, inner(width-length) outer(width-length)). The black curve is related to the reference AuBP140@Ag structure described in the "Computational details" (main text), whereas blue and red curves correspond to the two structures whose dimensions are on the sides of the statistical distribution above-mentioned. Solid curves correspond to longitudinal polarization of the incoming electric field, whereas dashed ones correspond to transversal polarization. The extinction spectrum of the structure related to the blue curve features a small peak around ≈ 450 nm (longitudinal polarization) that is not present in the other cases, and it is associated with a weak concentration of surface charge density close to the tips (the color bar has been scaled by 1/5 compared to figure 3d, main text, to emphasize the charge pattern). All other peaks and corresponding surface charge densities are in good agreement with each other, thus providing solid evidence of the robustness of the computational results.
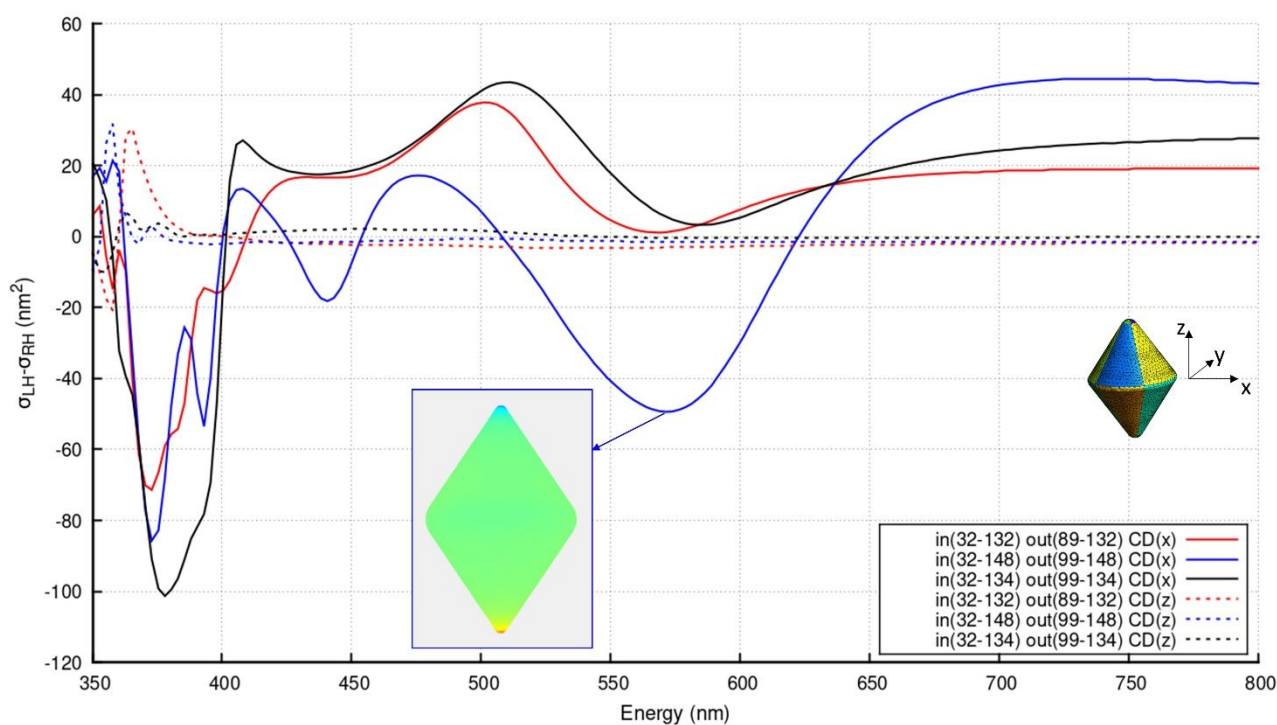
Figure S17. Simulated CD response of the three different AuBP140@Ag structures obtained by inducing a spiral motif on the Ag outer layer of the corresponding structures of Figure S16 (more details about this chiral asymmetry can be found in "Computational details", main text). Solid curves correspond to an incoming wave propagating along the x-direction and electric field polarization rotating in the zy plane. Dashed curves correspond to an incoming wave propagating along the z-direction and electric field polarization rotating in the xy plane. Notably, the chiral response of the structure associated with the blue curve features a negative peak around ≈ 575 nm (blue arrow) that is not so pronounced in the other two cases but is in line with the experimental mild negative signal around 600 nm (see solid red curve Figure 3a, main text). Despite this minor difference, the three spectra look similar to each other and the main negative peak, which is also clearly detected experimentally, is always appearing around 375 nm.

Supplementary Reference

(1)   Chateau, D.; Desert, A.; Lerouge, F.; Landaburu, G.; Santucci, S.; Parola, S. Beyond the Concentration Limitation in the Synthesis of Nanobipyramids and Other Pentatwinned Gold Nanostructures. *ACS Appl. Mater. Interfaces* **2019**, *11*, 39068–39076