Supporting Information

Serum Albumins Guided Plasmonic Nanoassemblies with Opposite Chiralities

Zhao-Yi Wang[a,‡], Ning-Ning Zhang[b,‡], Jin-Cheng Li[a], Jun Lu[b,c], Li Zhao[d], Xue-Dong Fang[a,*], Kun Liu[b,*].

[a.] *China-Japan Union Hospital of Jilin University, Changchun, 130033, P.R. China*

[b.] *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun, 130012, P.R. China*

[c.] *Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States*

[d.] *College of Life Science, Jilin University, Changchun, 130012, P.R. China*

[*] Xue-Dong Fang and Kun Liu are co-corresponding authors.

[‡] These authors contributed equally.

Materials and Methods

1. Materials

Human serum albumin (HSA, ≥96%), bovine serum albumin (BSA, ≥98%) hexadecyltrimethylammonium bromide (CTAB), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), Sodium polystyrenesulfonate (Na-PSS, M_w = 70 kDa), $_L$-ascorbic acid (AA) and $_L$-cystine ($_L$-CYS, >99%) were purchased from Sigma-Aldrich. Porcine serum albumin (PSA), sheep serum albumin (SSA), and equine serum albumin (ESA) were purchased from

Equitech-Bio. Inc. The sodium citrate (98%) and 5,5′-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent, TDAB) were purchased from Aladdin. Braford reagent was purchased from Beyotime. All chemicals were used without any treatment. Deionized water (18.2 MΩ) was used in all the experiments.

2. Synthesis and of GNRs

GNRs were synthesized by the method reported previously[1]. The gold seeds were prepared by adding a freshly prepared, ice-cold $NaBH_4$ solution (0.50 mL, 10 mM) into CTAB (3.5 mL, 0.10 M) and $HAuCl_4$ (0.12 mL, 15 mM) mixture at 25.5 °C. The seed solution was stirred for 2.0 min and stored in 25 °C bath for 30 min. The growth solution was the mixture of 8.86 mL H_2O, CTAB (0.36g, 1.0 mM), $AgNO_3$ (0.40 mL, 4.0 mM), $HAuCl_4$ (0.50 mL, 15 mM), and AA (0.12 mL, 0.078 M). Finally, 0.10 mL seed solution was added to the growth solution. The mixture was then incubated at 27.0 °C for 12 h.

3. Regrowth of GNRs

The as-synthesized GNRs needed further regrowth to get more uniform GNRs. Aqueous solutions of AA (0.12 mL, 0.078 M), $AgNO_3$ (0.40 mL, 4.0 mM), and $HAuCl_4$ (0.50 mL, 15 mM) were added to 10 mL above GNR solution, and then was incubated at 27 °C for 4.0 h. The regrowth process was repeated for three times to obtain the GNRs with LSPR peak at about 700 nm.

4. Preparation of citrate-stabilized GNRs

The ligand exchange was performed according to the previous protocol with minor adjustment[2].10 mL of freshly prepared CTAB-coated GNRs were centrifuged at 9000 rpm for 15 min, and nearly 95% of the supernatant was decanted. The remainder was redispersed in 0.15 wt% Na-PSS to a final volume of 10 mL, and allowed to stabilize for at least 1 h. Next, the above solution was centrifuged again and redispersed in 0.15 wt% Na-PSS for at least 1 h. Finally, PSS-stabilized GNRs (10 mL) were centrifuged and redispersed into 10 mL of 5 mM sodium citrate and allowed to sit for 12 h. The zeta-potential of GNRs changed from +52.6 mV to -41.5 mV after the positively charged CTAB ligands were replaced by the negatively charged sodium citrate, indicating the ligand exchange is successful.

5. SAs Induced the Chiral Assembly of GNRs

To obtain the GNRs for assembly, 1.0 mL of the regrowth GNRs was centrifuged at 9000 rpm for 15 min. The supernatant was removed, and then 500 μL 4.0 °C stored PBS solution was added. Next, 500 μL PBS (10 mM, pH 7.4) solution containing 6.0 μM SAs was added into the fresh prepared GNRs PBS solution quickly, and mixed evenly with pipette. The solution was stored at 4.0 °C for assembly process. The chiral assembly pH and ionic strength modulation were realized by adjusting the PBS buffer with HCl, NaOH, and NaCl, namely, the PBS (pH 5.4 or 6.4 or 7.4 or 8.4, ionic strength 5 mM or 10 mM or 20 mM) were used as solvent for reaction.

6. Quantitation of Free Thiol of SAs

Ellman's reagent (TDNB) was used to quantify the free thiol groups of different SAs. The SAs (5.0 μM) or CYS standards (0-200 μM) were dissolved in PBS (5.0 mM, pH 7.4). The 100 μL of SAs or $_{L}$-CYS standards were added to each reaction well of a 96-well plate. A volume of 100 μL TDNB (0.52 mg/ mL) and EDTA (0.30 mg/ mL) in PBS (5.0 mM, pH 8.0) were added to the reaction well. The mixtures were incubated for 3.0 min. Then, the absorbance was recorded at 412 nm by a Microplate reader. The free thiol concentrations of SAs were obtained from the calibration curve got from the CYS standards.

7. Characterization

CD spectra were measured with a Chirascan V100 circular dichroism spectrometer (Applied Photophysics Ltd.). Extinction spectra were recorded by a PerkinElmer Lambda 950 UV−vis−NIR spectrometer. Zeta potential were measured by a Zetasizer Nano Series (Nano-S90, Malvern Instruments). Surface enhanced Raman spectrum (SERS) was recorded using a HR800 Raman spectrometer (Horiba-Jobin Yvon), and the 785 nm laser was used as light source. The 10 μL assembled solution was dropped onto a quartz plate for SERS measurements. Cryo-TEM experiments were measured on FEI Talos F200C TEM instrument (200 kV) equipped with a SC 1000 CCD camera (USA Gatan, Inc.). Image post-processing used the 3D reconstruction software Inspect 3D and Avizo. Transmission electron microscopy (TEM) images obtained from a Tecnai G2-F30ST with an accelerating voltage of 200 kV on carbon-coated Cu grids. About 1.0 μL assembled solution was dropped on Cu grids and then evaporated quickly at room temperature. A sequence alignment of the SAs produced using the ClustalW2 server[3]. Tecan Infinite 200 Pro Microplate Reader was used to quantify the free thiol groups and the adsorbed SAs on GNRs.

Supporting Figures

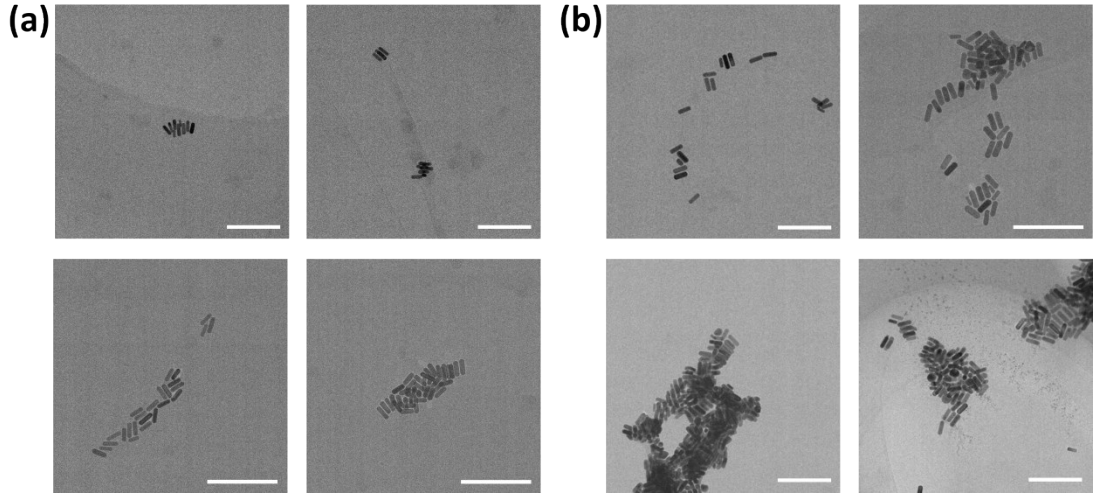

Figure S1. The cryo-TEM images of nanoassemblies guided by (a) HSA and (b) BSA at pH value of 7.4, ionic strength of 10 mM. The scale bars are 200 nm.

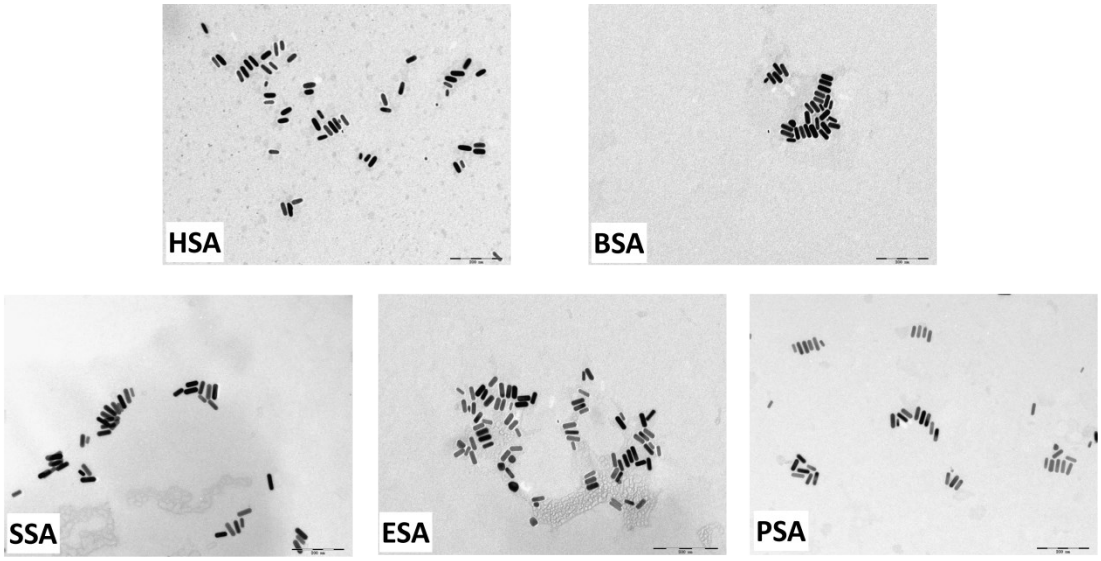

Figure S2. The TEM images of nanoassemblies guided by HSA, BSA, ESA, PSA, and SSA at pH value of 7.4, ionic strength of 10 mM. The scale bars are 200 nm.

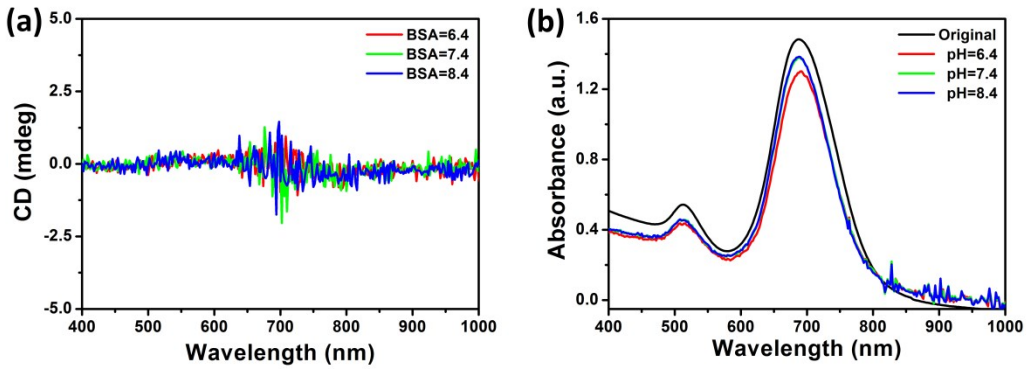

Figure S3. Vis-NIR region (a) CD and (b) UV-Vis absorbance spectra of BSA guided citrate-coated GNR assemblies under different pH conditions with the ionic strength of 10 mM.

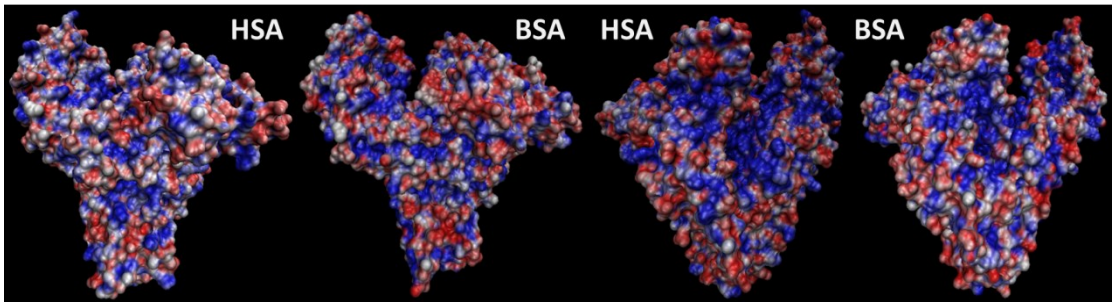

Figure S4. Charged distribution on the surface of HSA and BSA visualized by VMD at pH 6.4. The left two pictures show molecules in the orientation with domain I on the right and further domains are arranged clockwise, whereas in the right two pictures domain I is on the left and the domains are arranged anticlockwise.

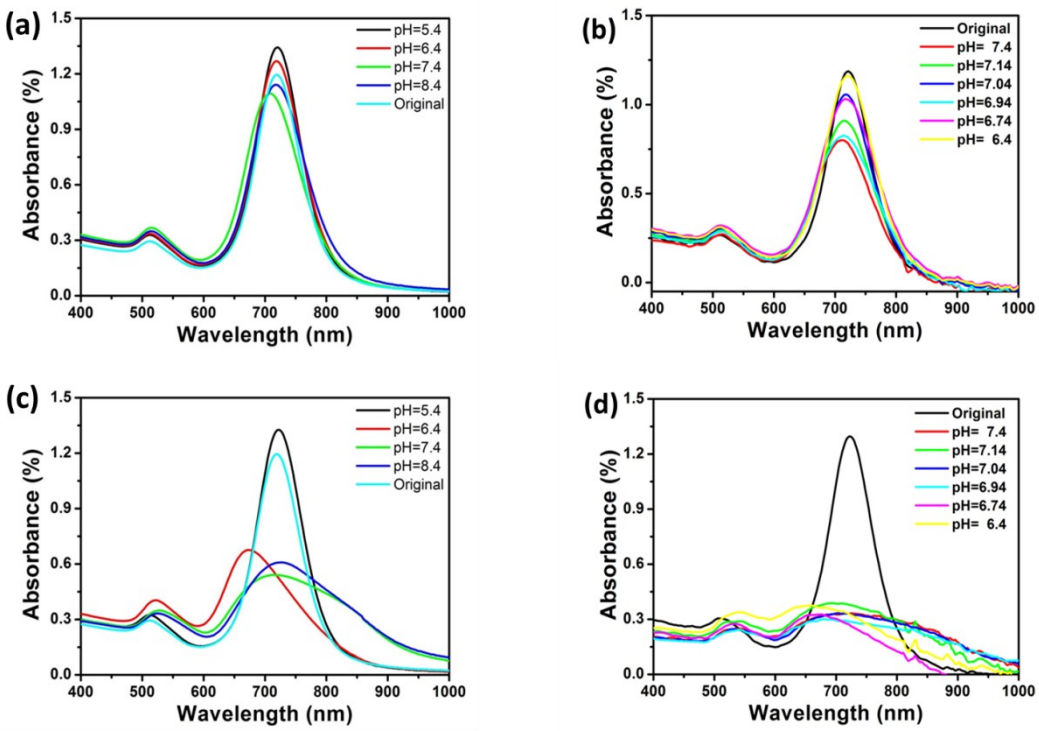

Figure S5. The effect of pH to the chiral plasmonic assemblies: the UV-Vis absorbance spectra of HSA (a) and BSA (c) at a difference interval of ~1.0 pH unit, the UV-Vis absorbance spectra of HSA (b) and BSA (d) at a difference interval of ~0.20 pH unit.

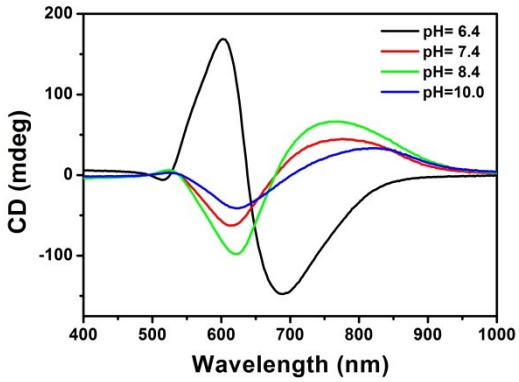

Figure S6. The CD spectra of BSA induced chiral assembly by varying the pH from 6.4 to 10.0 at the interval of ~1.0 pH unit. The concentration of BSA was 3.0 μM and the ionic strength was 10 mM.

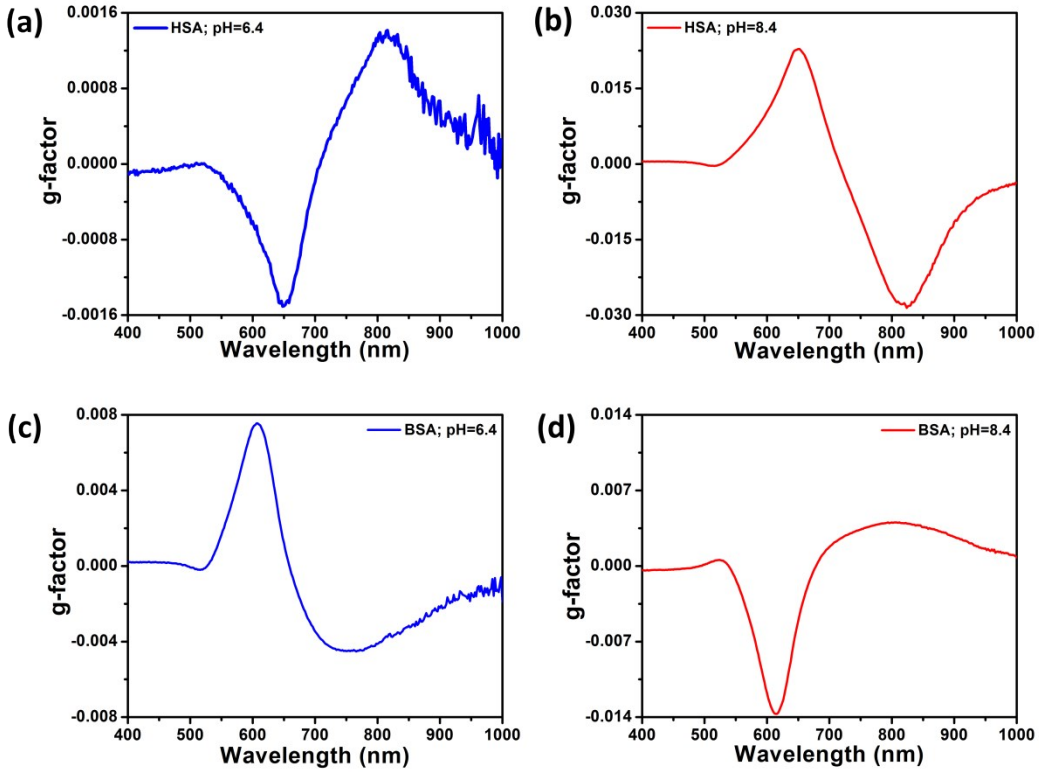

Figure S7. The anisotropy factors (*g*-factor), defined as *g*-factor = Δε/ε, of SA/GNRs assemblies at different pH values. (a) and (b) are *g*-factors calculated according to the CD and UV results for HSA guided chiral assembly at pH 6.4 and 8.4, respectively. (c) and (d) are *g*-factors calculated according to the CD and UV results for BSA guided chiral assembly at pH 6.4 and 8.4, respectively.

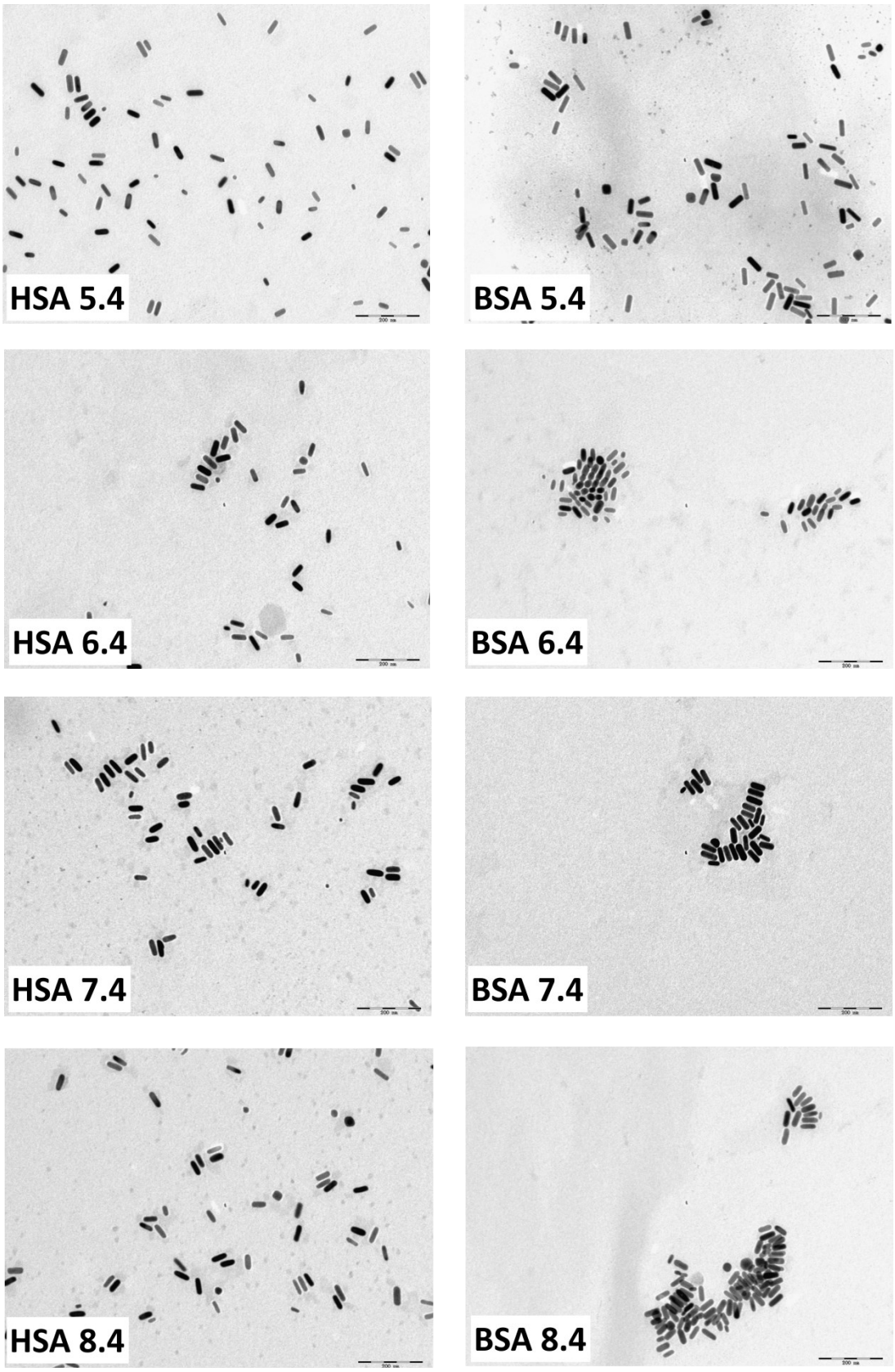

Figure S8. TEM images of HSA (left panel) and BSA (right panel) guided nanoassemblies at the pH range from 5.4 to 8.4. The ionic strength was set to 10 mM. The scale bars are 200 nm.

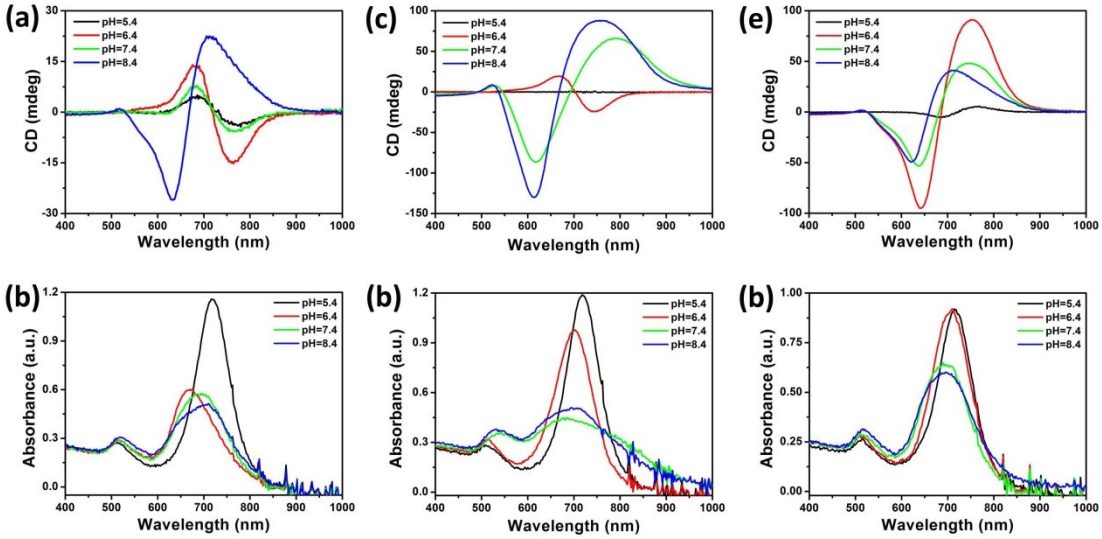

Figure S9. The effect of pH value to the chiral plasmonic assemblies: the CD spectra of PSA (a) and ESA (b), and SSA (c) at a difference interval of ~1.0 pH unit. The concentrations of HSA and BSA are PSA, ESA, and SSA were 3.0 μM. The ionic strength was set to 10 mM.

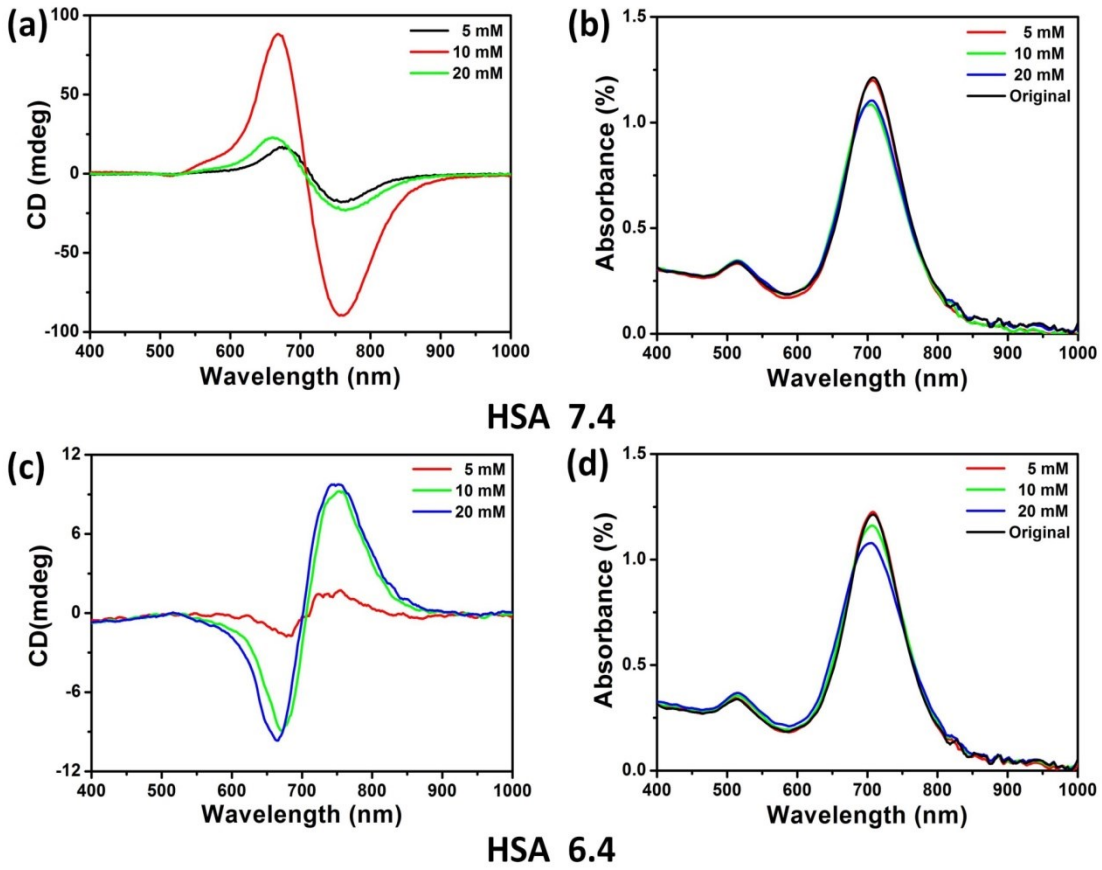

Figure S10. The effect of ionic strength to the chiral plasmonic assemblies: Vis-NIR region (a, c) CD and (b, d) UV absorbance spectra of HSA guided Au NRs assemblies under various pH conditions (a, b: pH = 7.4; c, d: pH = 6.4) and by the addition of NaCl to increase the ionic strength.

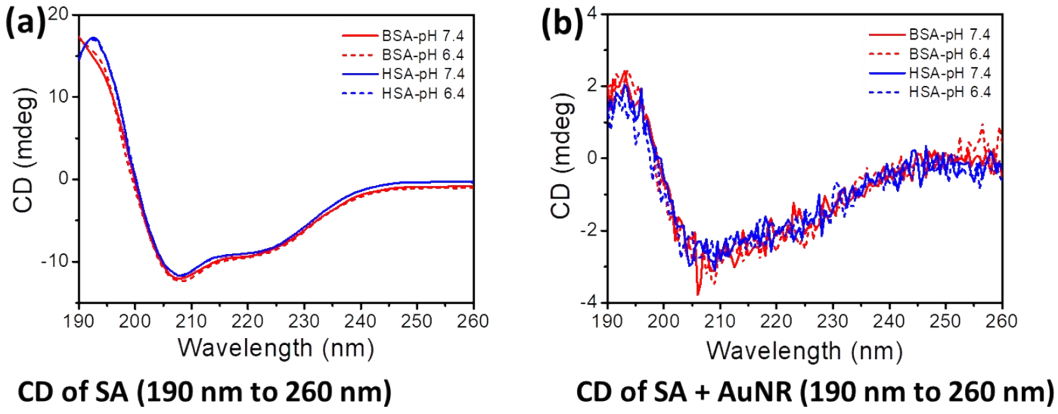

Figure S11. The DUV CD spectra of (a) SAs in CTAB solutions and (b) chiral plasmonic Au NRs/SA assemblies.

Table S1. ζ-potential characterization of the chiral assemblies.

	HSA pH 7.4	HSA pH 6.4	BSA pH 7.4	BSA pH 6.4
ζ of the assemblies (mV)	**15.5**	**35.3**	**14.4**	**21.3**

References

[1] Nikoobakht, B.; El-Sayed, M. A. Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method. *Chem. Mater.,* **2003**, *15*, 1957−1962.

[2] Mehtala, J. G.; Zemlyanov, D. Y.; Max, J. P.; Kadasala, N.; Zhao, S.; Wei, A. Citrate-Stabilized Gold Nanorods. *Langmuir,* **2014**, *30*, 13727−13730.

[3] Thompson, J. D.; Higgins, D. G.; Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* **1994**, *22*, 4673-4680.