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## Supporting Information

### **Heat-Enhanced Symmetry Breaking in Dynamic Gold Nanorod Oligomers: the Importance of the Interface Control**

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## 1. Experimental details

**Materials:** Chlorauric acid ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) and silver nitrate ( $\text{AgNO}_3$ ) were purchased from Beijing Chemical Reagent Company. Sodium borohydride ( $\text{NaBH}_4$ ), L-ascorbic acid (AA) and trisodium citrate were purchased from Alfa Aesar. L-cysteine (L-CYS), D-cysteine (D-CYS), L-cystine, cysteamine, L-Homo-cysteine, N-acetyl-L-cysteine (NAC) and disodium chromoglycate (DSCG) were purchased from Sigma. Cetyltrimethylammonium bromide (CTAB), L-glutathione (L-GSH) and L-oxidized glutathione (L-GSSG) were purchased from Amresco.  $\text{HS-C}_{11}\text{-(EG)}_6\text{-NH}_2 \cdot \text{HCl}$  (PEG-NH<sub>2</sub>) and  $\text{HS-C}_{11}\text{-(EG)}_6\text{-OCH}_3$  (PEG-OMe) were purchased from ProChimia. Milli-Q Water (18 M $\Omega$ ·cm) was used for the preparation of all solutions.

**Instruments:** CD measurements were conducted on a JASCO J-810 CD spectrometer and the bandwidth was set at 5 nm. Extinction spectra were obtained using a Cary 50 UV-vis-NIR spectrophotometer. SEM images were taken using a Hitachi S-4800 SEM with a voltage of 10 kV. TEM images were taken using a Tecnai G2 20 S-TWIN TEM with an accelerating voltage of 200 kV. Zeta potentials were obtained using a zeta potential and size analyzer (Malvern, Zeta-sizer nano ZS). Raman spectra were obtained in the solution phase using a Renishaw InVia Raman microscope. Spectra measurement: All extinction and CD spectra were obtained directly with solution samples, which were put into a 1cm  $\times$  1cm quartz curvete. For JASCO-810 CD spectrometer, the extinction spectra were the sum of the extinction of the left circular polarized light (LCP) and that of the right circular polarized light (RCP).

### 1. Synthesis of Gold nanorods and fabrication of their chiral assemblies

(1) Gold nanorods were synthesized using a seed-mediated growth procedure.

**Preparation of Au seed solution:** CTAB-capped seeds were prepared by chemical reduction of  $\text{HAuCl}_4$  with  $\text{NaBH}_4$ : freshly prepared, ice-cold  $\text{NaBH}_4$  (0.01 M, 10mL) was injected into a mixture of CTAB (0.1 M, 7.5 mL),  $\text{HAuCl}_4$  (23 mM, 0.1mL) and deionized water (1.8 mL) under magnetic stirring for 3 min. The color of the solution turned from yellow to brown, indicating the formation of Au seeds. The seed solution was kept undisturbed at 30°C for 2-5 h before use.

GNRs with LSPR maximum at  $\sim$ 730 nm (Au730),  $\sim$ 560 nm (Au560),  $\sim$ 800 nm (Au800),  $\sim$ 880 nm (Au880), were prepared as follows:

**Au730:** The Au730 growth solution was made by mixing CTAB (0.1 M, 100 mL),  $\text{HAuCl}_4$  (25 mM, 2 mL),  $\text{AgNO}_3$  (0.1 M, 120  $\mu\text{L}$ ), and AA (0.1 M, 552  $\mu\text{L}$ ). The seed solution (120  $\mu\text{L}$ ) was added to the growth solution to initiate the growth of GNRs. The well-mixed solution was kept undisturbed

for 12 h, then AA (0.1 M, 552  $\mu$ L) was added twice at 40 min intervals. The total reaction time was for 24 h.

*Au560*: The Au560 growth solution was made by mixing CTAB (0.1 M, 100 mL), HAuCl<sub>4</sub> (25 mM, 2 mL), AgNO<sub>3</sub> (0.1 M, 45  $\mu$ L), H<sub>2</sub>SO<sub>4</sub> (1 M, 1 mL), CuCl<sub>2</sub> (0.1 M, 100  $\mu$ L), and AA (0.1 M, 800  $\mu$ L). The seed solution (240  $\mu$ L) was added to the growth solution to initiate the growth of GNRs. The mixture was kept undisturbed for 12 h, then HAuCl<sub>4</sub> (0.025 M, 285  $\mu$ L) was added and the reaction continued for 2 h.

*Au800*: The Au800 growth solution was made by mixing CTAB (0.1M, 100 mL), HAuCl<sub>4</sub> (25 mM, 2 mL), AgNO<sub>3</sub> (0.1 M, 100  $\mu$ L), H<sub>2</sub>SO<sub>4</sub> (1 M, 1 mL), CuCl<sub>2</sub> (0.1 M, 200  $\mu$ L), and AA (0.1 M, 800  $\mu$ L). The seed solution (240  $\mu$ L) was added into the growth solution to initiate the growth. The mixture was kept undisturbed for 12 h.

*Au 880*: The Au880 growth solution contains CTAB (0.1 M, 100 mL), HAuCl<sub>4</sub> (25 mM, 2 mL), AgNO<sub>3</sub> (0.1 M, 110  $\mu$ L), H<sub>2</sub>SO<sub>4</sub> (1 M, 1 mL) and AA (0.1 M, 800  $\mu$ L). The seed solution (1 mL) was added to the growth solution to initiate the growth of GNRs. The mixture was kept undisturbed for 50 min, followed by the addition of HAuCl<sub>4</sub> (25 mM, 1 mL) and AA (0.1 M, 400  $\mu$ L) at 30 min interval. The reaction mixture was incubated for 2-3 h.

After synthesis, all GNR preparations were centrifuged twice (9200 rpm, 10 min) and redispersed in 2 mM CTAB solution.

(2) *Fabrication of GNR side-by-side assemblies*. Trisodium citrate solution (20 mM, 25  $\mu$ L) was added to gold nanorod suspension (0.1 nM, 1mL) containing 2 mM CTAB to trigger the formation of assemblies. The mixture was first kept at 70°C for 3 min to form side-by-side assemblies, and then quenched rapidly in ice-cold water for 1 min. The degree of assembly can be controlled by changing the concentration of trisodium citrate (0.48-0.52 mM) and/or the assembly time (3-10 min).

(3) *Formation of chiral assemblies by adsorption of chiral thiols*. The thiol was added into the suspension of assembled GNRs. For L-CYS, 10  $\mu$ L of different concentrations of L-CYS were added to 1mL GNR suspension (0.1 nM, 1mL). The mixture was incubated at 30°C for 30 min to obtain chiral assemblies. Amplification of the PCD signal was achieved by thermal annealing at temperatures higher than 30°C for 30 min. For studying the temperature effect, assemblies were heated at different temperatures (30, 40, 50, 55, or 60°C) in the presence of 0.7  $\mu$ M L-CYS. For derivatives of L-CYS and other chiral molecules, the concentration of thiol molecules was fixed at

0.7  $\mu\text{M}$ . Briefly, these molecules were added at 0.7  $\mu\text{M}$  to GNR assemblies and the mixture was equally divided into two parts and incubated at 30°C and 60°C, respectively. For GNRs with different aspect ratios, GNRs with LSPR maxima at 560nm, 800nm, or 880nm were employed. L-CYS (0.7  $\mu\text{M}$ ) was added to the assemblies and they were equally divided into two parts to incubated at 30°C and 60 °C for 30min, respectively.

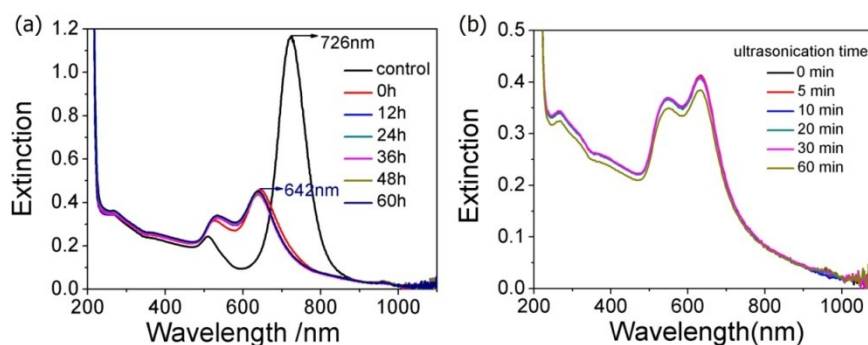
## 2. Heat-assisted PCD enhancement in GNR oligomers.

*Influence of temperature on L-CYS adsorption:* L-CYS (0.07 mM, 10  $\mu\text{L}$ ) was added to GNR suspension (0.1 nM, 1mL). The mixtures were incubated for 30 min at 30 or 60°C. After cooling to ambient temperature, trisodium titate (25  $\mu\text{L}$ , 20 mM) was added to initiate assembly and the samples incubated at 30 °C for 30 min. Extinction and CD spectra were then measured.

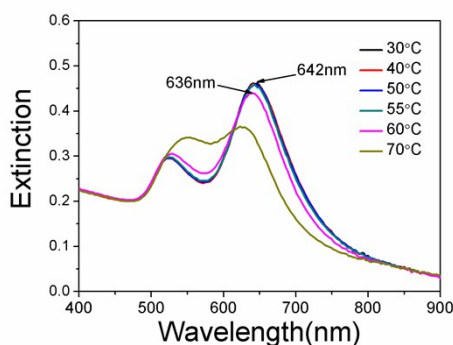
*Effect of heat treatment before L-CYS adsorption on PCD amplification:* Assemblies (1mL) that were pre-fabricated at 70°C were first heated at 60°C for 30 min. After cooling to ambient temperature, L-CYS (0.07 mM, 10  $\mu\text{L}$ ) was added and the mixture was incubated at 30°C for 30 min. Extinction and CD spectra were then measured.

3. *Ligand titration with other thiols.* The prepared assemblies were first incubated with 0.7  $\mu\text{M}$  L-CYS at 30°C or 60°C for 30 min were employed to study the effect of ligand exchange on the PCD. For chirality transfer, different concentrations of D-CYS (10  $\mu\text{L}$ ), the enantiomer of L-CYS, were added to the solution (1 mL) and incubation was carried out for 30min at 30°C to study how the PCD changed with variations in D-CYS concentration. To study the function of interface layers, different concentrations of PEG-NH<sub>2</sub> and PEG-OMe (10  $\mu\text{L}$ ) were added to the assembly solutions (1 mL) and incubated for 30min at 30°C.

## 2. The stability of non-chiral GNR oligomers



**Figure S1.** (a) The extinction spectra of GNR monomers (control) and the SS oligomers during 60h aging. (b) The extinction spectra of GNR oligomers under ultrasonic treatment for different periods.



**Figure S2.** The extinction spectra of GNR oligomers modified with 0.7  $\mu\text{M}$  L-CYS after heat treatment at different temperatures for 30 min, respectively.

### 3. Calculation of g factor

The *g factor* was calculated by the formulas below:

$$\frac{\Delta A}{A} = \frac{\Delta \epsilon * c * l}{\epsilon * c * l} = \frac{CD(mdeg)}{32980 * A}$$

$c$  and  $l$  are the GNR concentration (0.1 nM) and optical path (1 cm), respectively.

**Table S1** Anisotropic factor (*g factor*) obtained from different gold and silver nanostructures

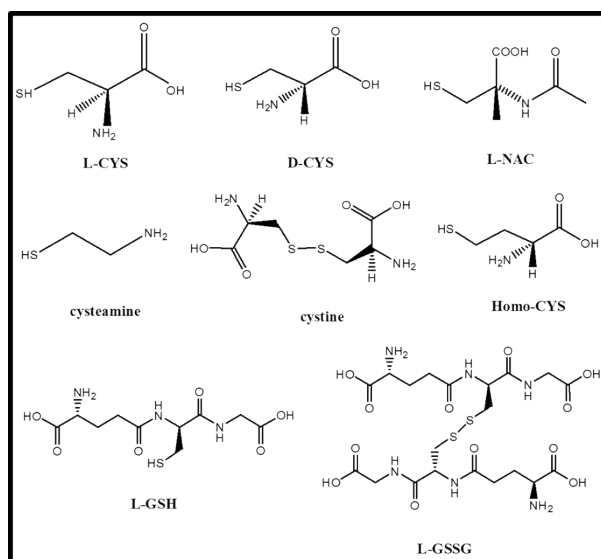
Table S1 Anisotropic factor ( <i>g factor</i> ) obtained by various methods		
Plasmonic nanostructures	<i>g factor</i>	Reference
gold nanoparticles grown in situ on peptide nanotubes	5.00E-05	1
end-to-end assemblies of Au bipyramids linked by DNA	4.36E-04	2
side-by-side assembly of Au nanorods linked by cysteine	9.61E-04	3
Ag nanoparticle chains linked by cholate	1.00E-03	4
end-to-end assemblies of Au nanorods linked by cysteine	1.17E-03	5
Au nanorod dimers linked by chiral quantum dots	1.45E-03	6
Ag nanocubes modified with DNA ( <b>multipolar mode</b> )	4.40E-03	7
side-by-side assembly of L-cysteine modified Au nanrods	9.30E-03	8
Additional metallic shell deposition on DNA linked heterodimers of Au NPs	1.21E-02	9
DNA-linked pyramids comprising metal nanoparticles and quantum dots	1.90E-02	10
Au nanorod helix assembled via DNA origami	2.00E-02	11
Au-Ag nanoparticle heterodimers linked by protein	2.05E-02	12
Au nanorods adsorbed on chiral fibers	2.20E-02	13
Au@Ag nanoparticles arranged into a helix on a DNA origami	2.50E-02	14
helical arrangement of Au nanorods on lipid templates	2.70E-02	15
<b>Cysteine-modified gold nanorods side-by-side assemblies after thermal annealing</b>	<b>6.5E-02</b>	<b>here</b>

### Reference

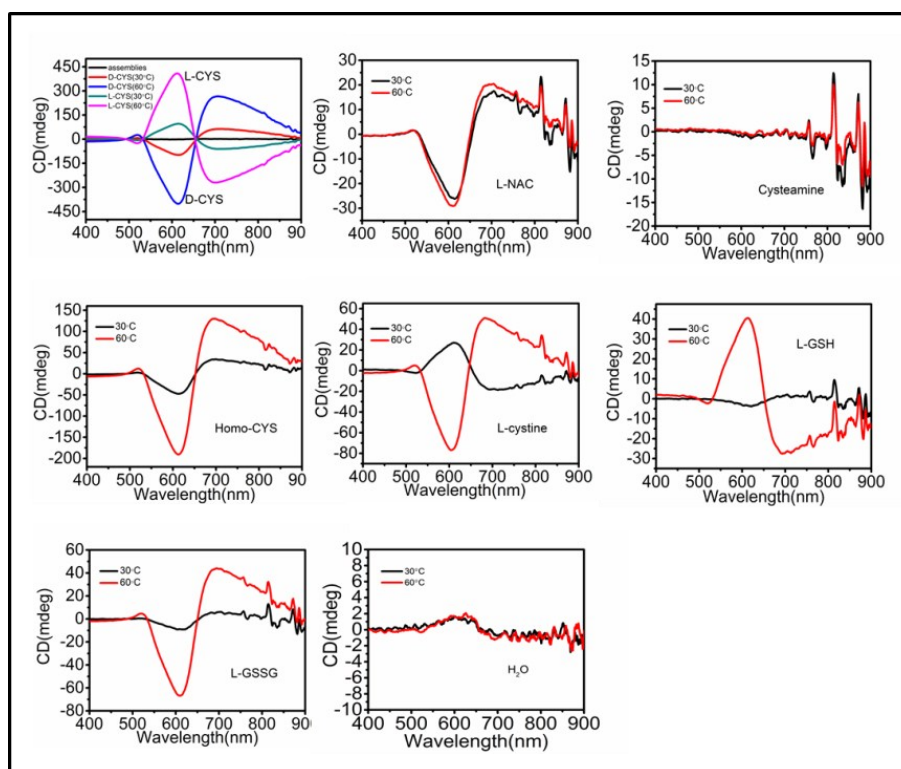
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#### 4. Heat-assisted PCD Amplification for different thiolated chiral molecules

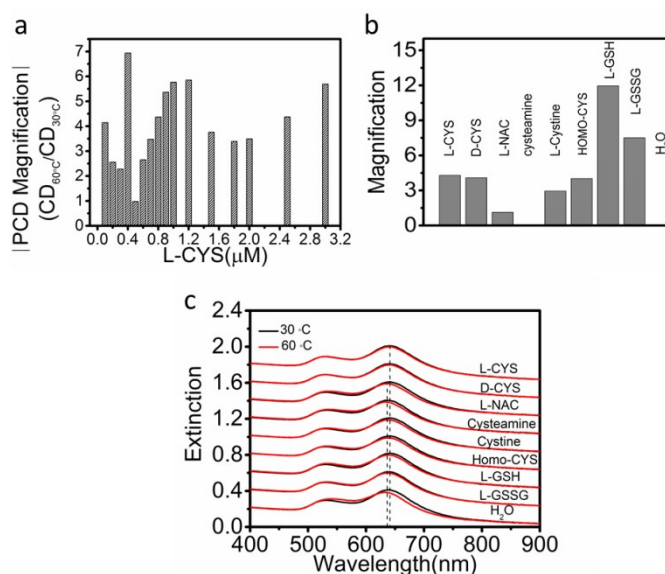


**Figure S3-1.** Molecular structures of employed thiolated molecules.



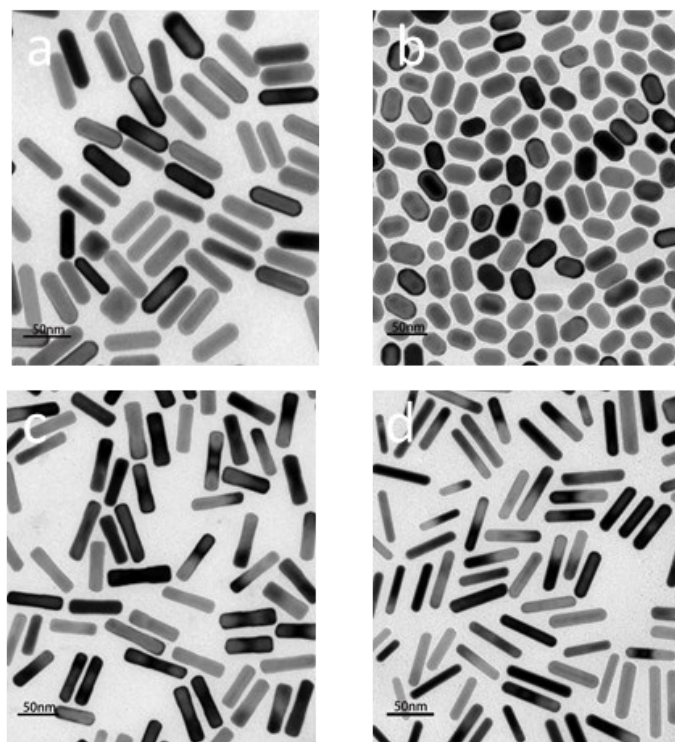
**Figure S3-2.** CD spectra of oligomers modified with different thiolated molecules before and after heating treatment at 60°C for 30 min. The concentration of the molecules is 0.7  $\mu\text{M}$ .

Note: Except cysteine, the concentration for other chiral thiols was not optimized to achieve maximum PCD signals.



**Figure S3-3.** Ratio of  $\text{PCD}_{60^\circ\text{C}}/\text{PCD}_{30^\circ\text{C}}$  at 600 nm for [L-CYS] (a) or for different molecules at 0.7  $\mu\text{M}$  concentration (b). The extinction spectra of the oligomers modified with different molecules before and after heat treatment at 60°C for 30 min (c).

## 5. Extension of heat enhancement effect to GNRs with different aspect ratios and sizes



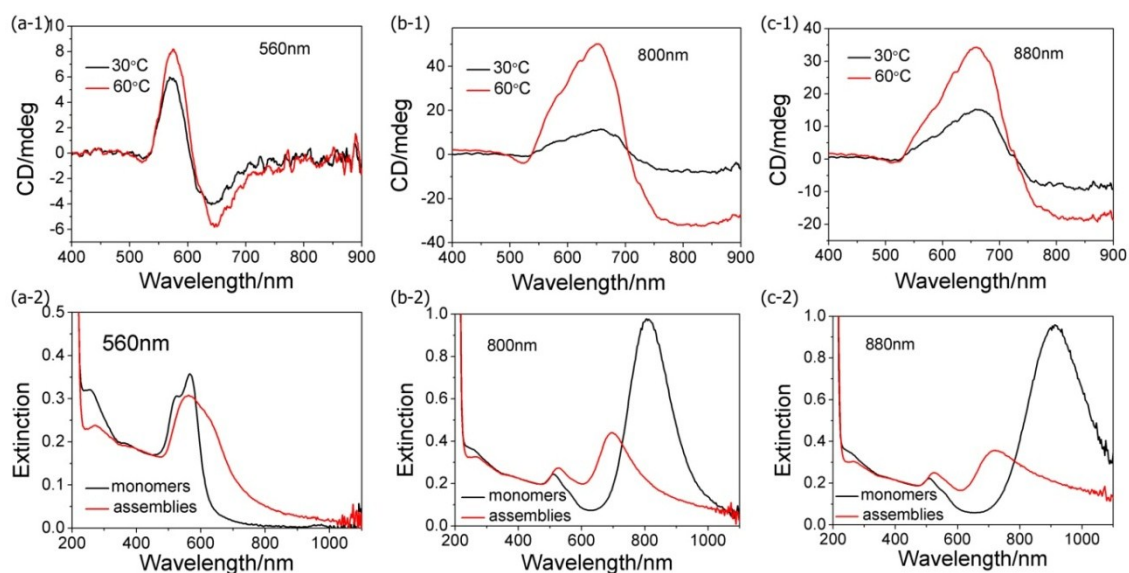
**Figure S4.** TEM images of discrete GNRs of Au730 (a), Au560 (b), Au800 (c), and Au880 (d).

**Table S2** Statistic size information of GNRs

	<b>Diameter/nm</b>	<b>Length/nm</b>	<b>Anisotropic Ratio (AR)</b>
<b>Au730</b>	$19.0 \pm 2.1$	$58.8 \pm 4.1$	$3.1 \pm 0.3$ (9.7% sd)
<b>Au560</b>	$23.3 \pm 1.9$	$36.8 \pm 4.6$	$1.6 \pm 0.2$ (12.5% sd)
<b>Au800</b>	$16.7 \pm 2.4$	$60.5 \pm 6.5$	$3.7 \pm 0.5$ (13.5% sd)
<b>Au880</b>	$12.6 \pm 2.1$	$57.6 \pm 8.7$	$4.7 \pm 0.7$ (14.9% sd)

**Table S2.** The mean sizes of discrete GNRs with different aspect ratios. The data were obtained by a statistic average of 500 individual GNRs, respectively.



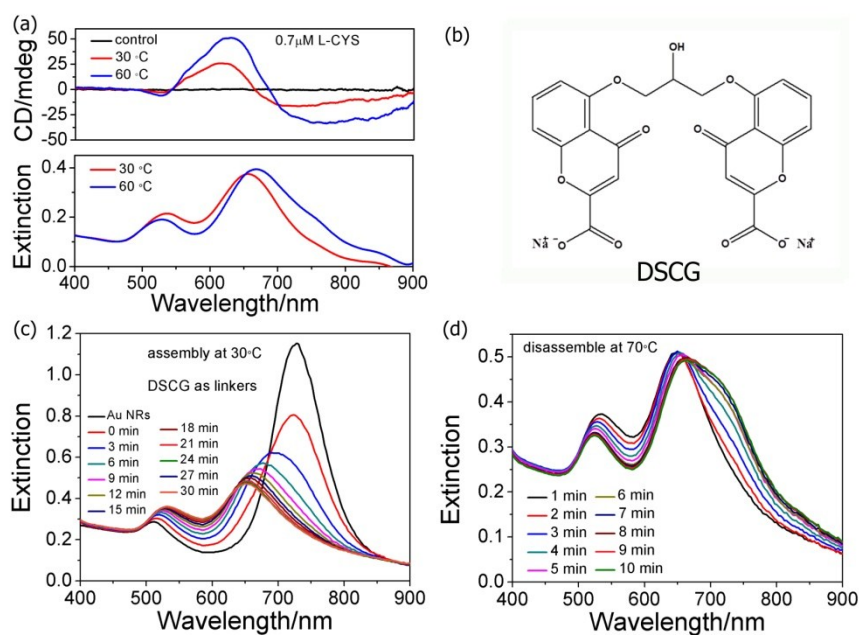


**Figure S5.** PCD enhancement effect of oligomers for the GNRs with different LSPR maximum. The LSPR positions of the discrete GNRs are 560, 800 and 880 nm, respectively. The CD spectra (upper) and extinction spectra (below) of oligomers obtained by incubation with  $0.7\mu\text{M}$  L-CYS at  $30^\circ\text{C}$  or  $60^\circ\text{C}$  for 30min.

Note: The assembly conditions for these GNRs are not optimized to achieve the maximum PCD.

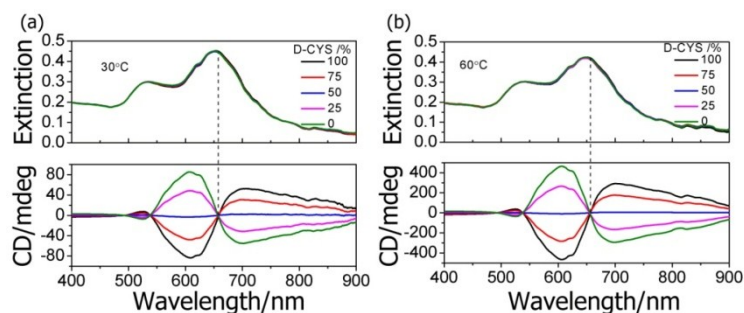
## 6. PCD amplification using DSCG as the linker to fabricate the GNR oligomers.

$33\mu\text{L}$  DSCG (disodium cromoglicate,  $40\text{mM}$ ) was added into  $0.1\text{nM}$  GNRs suspension at  $30^\circ\text{C}$ . After 30 min,  $10\mu\text{L}$  L-CYS ( $0.07\text{mM}$ ) was added and the mixture was kept in water bath of 30 or  $60^\circ\text{C}$  for 30min. As control, the oligomers without chiral molecules modification were heated at  $70^\circ\text{C}$  for 30min.

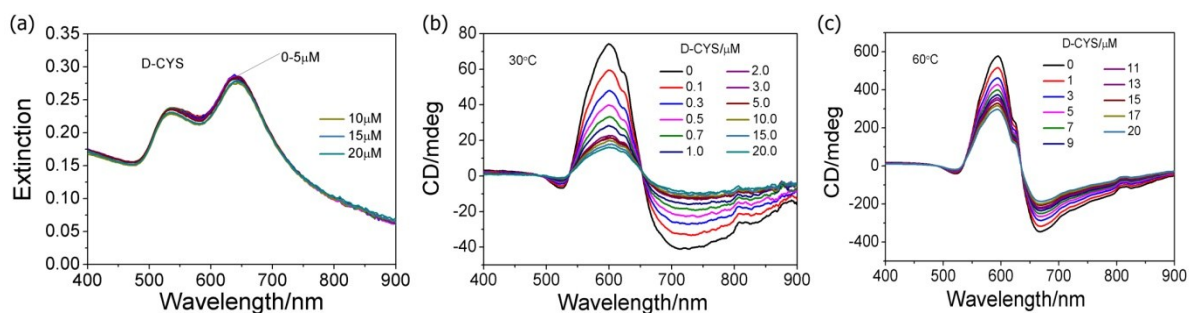


**Figure S6. Influence of assembly degree on PCD signal.** (a) The CD spectra and extinction spectra of oligomers fabricated with DSCG as linkers at 30°C and modified with 0.7 μM L-CYS before and after heating treatment at 60°C for 30min. (b) The chemical structure of DSCG. (c) Evolution of extinction spectra using DSCG as linker at 30°C. (d) Evolution of extinction spectra of the oligomers upon 70°C heat treatment. Control is the GNR oligomers linked by DSCG but without cysteine adsorption.

### 7. Effect of enantiomeric molecule (D-CYS) on PCD response

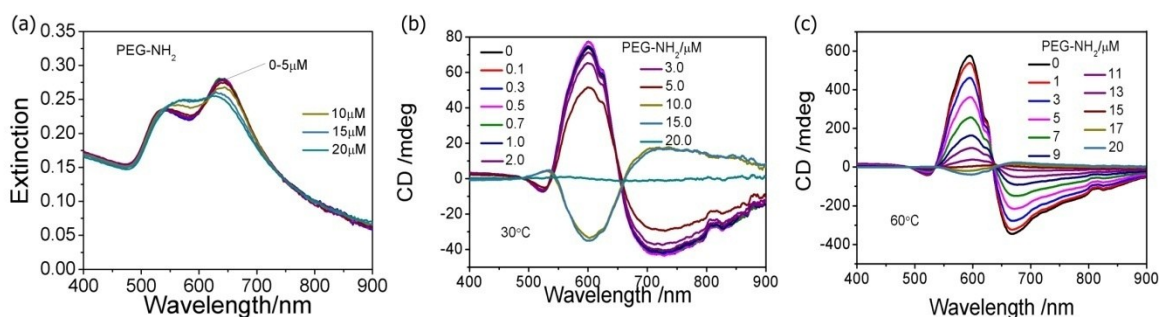


**Figure S7.** Effect of L- and D-CYS co-adsorption at different ratios on PCD response: The mixture of the enantiomers was added to the oligomers solution at 30°C (a) and 60°C (b) for 30min.  $[L-CYS] + [D-CYS] = 0.7 \mu M$ .



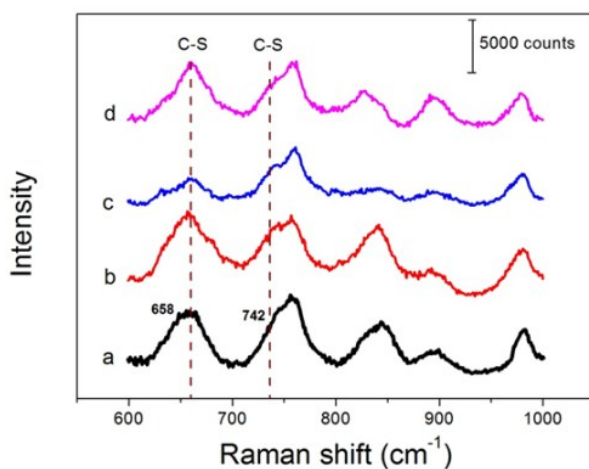
**Figure S8.** Effect of post-replacement with D-CYS on extinction spectra (a) and CD spectra (b) of 30°C-treated oligomers. (c) The change of CD spectra of 60°C-treated oligomers with the titration of D-CYS. Post-replacement experiments are conducted at 30°C.

### 8. Effect of replacement with long chain thiols on PCD amplification



**Figure S9.** Effect of the titration with PEG-NH<sub>2</sub> at 30°C: (a) the extinction spectra and CD spectra of 0.7 μM L-CYS modified GNR oligomers (30°C- treated) and (c) (60°C- treated) after addition of PEG-NH<sub>2</sub>.

Slight LSPR blue-shift may indicate the enhanced coupling of rods inside oligomers due to decreased rod gap after partial ligand exchange of CTAB bilayer by PEG-NH<sub>2</sub>.



**Figure S10.** Effect of PEG-NH<sub>2</sub> (10 μM) on SERS spectra of adsorbed L-CYS after forming GNR end-to-end assemblies: a) 10 μL L-CYS of 0.07 mM was added into 1 mL GNR monomer suspension (0.1 nM) and incubated at 30°C for 30 min; b) 10 μL L-CYS of 0.07 mM was added

into 1 mL gold nanorod monomer suspension (0.1 nM) and incubated at 60°C for 30 min; c) a +10  $\mu$ L PEG-NH<sub>2</sub> of 1 mM and the mixture was incubated at 30°C for 30 min; and d) a + 10 $\mu$ L PEG-NH<sub>2</sub> of 1mM and the mixture was incubated at 60°C for 30 min. After adjusting the pH values of sample suspensions to pH = 2, GNRs assembled through hydrogen bonds between L-CYS molecules and the SERS spectra were collected using 785 nm excitation.

No obvious change in C-S stretching modes of L-CYS at 657 and 742 cm<sup>-1</sup> were observed, indicating that mainly CTAB molecules on rod surface were replaced by PEG-NH<sub>2</sub>.

**Table S3 size and zeta potential**

	2mM CTAB	2mM CTAB +0.7 $\mu$ M L-CYS	2mM CTAB +0.7 $\mu$ M L-CYS +20 $\mu$ M L-CYS	2mM CTAB +0.7 $\mu$ M L-CYS +20 $\mu$ M D-CYS	2mM CTAB +0.7 $\mu$ M L-CYS +20 $\mu$ M PEG-NH <sub>2</sub>	2mM CTAB +0.7 $\mu$ M L-CYS +20 $\mu$ M PEG-OMe
Size(d.nm)	7.49 $\pm$ 0.245	7.68 $\pm$ 0.085	7.42 $\pm$ 0.057	8.22 $\pm$ 0.042	8.46 $\pm$ 0.086	9.08 $\pm$ 0.214
Zeta Potential ( $\xi$ .mV)	48.3 $\pm$ 1.20	48.5 $\pm$ 1.42	47.9 $\pm$ 2.99	46.7 $\pm$ 0.87	47.9 $\pm$ 2.06	33.4 $\pm$ 1.66

**Table S3.** The size and zeta potential of the discrete GNRs dispersed in 2mM CTAB aqueous solution before and after incubation with different thiolated molecules at 30°C for 30 min.