Specific Detection of Cysteine and Homocysteine: Recognizing One-Methylene Difference Using Fluorosurfactant-Capped Gold Nanoparticles

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Supporting Information

1. Experimental Details

1.1 Chemicals. Triton X-100 (4-(C_8H_{17})C_6H_4(OCH_2CH_2)$_n$OH), Zonyl FSN-100 (F(CF_2CF_2)$_{1-7}$$CH_2CH_2O$($CH_2CH_2O$)$_{0-15}$H), hydrogen tetrachloroaurate(Ш) trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, 20 standard amino acids, Hcy, homocystine, glutathione (Glu), cysteinylglycine, glucose, and tris(2-carboxy-ethyl)phosphine (TCEP) were purchased from Sigma-Aldrich. All solutions were prepared with deionized water (Mili Q, Millipore). The pH of the phosphate buffer solution (PBS) was adjusted with NaOH or HCl.

1.2 Nanoparticle synthesis. All glassware used for preparation of GNPs was thoroughly washed with freshly prepared aqua regia (HNO_3 : HCl =1:3), rinsed extensively with deionized and ultrahigh purity water sequentially, and then dried in an oven at 100 °C for 2-3 h. Colloidal GNPs with average diameters of 12 nm and 40 nm were prepared following the literature procedure.[1] Briefly, a 60 mL solution of 0.01 % $HAuCl_4$ was brought to a vigorous boil with stirring in a round-bottom flask fitted with a reflux condenser, and then different amounts of 1.0 % sodium citrate was added to the stirring and refluxing $HAuCl_4$ solution (for 12 nm and 40 nm GNPs, 4.5 mL and 0.6 mL sodium citrate were used, respectively). The solution was maintained at the boiling point with continuous stirring for about 15 min. After the solution was allowed to cool to room temperature with continued stirring, 240 μL 10 % FSN-100 was added. The suspension was stored at 4 °C until further use. Assuming spherical particles and density equivalent to that of bulk gold (19.30 g/cm^3), the concentration of the GNPs was calculated (12 nm GNPs, ~5.5 nM; 40 nm GNPs, ~0.14 nM). The TEM specimens were prepared by depositing an appropriate amount of the FSN-capped GNPs onto

the carbon-coated copper grids, and excess solution was wicked away by a filter paper. The grid was subsequently dried in air.

1.3 Apparatus. Transmission electron microscopy (TEM) images were taken using a Philips microscope (Tecnai 20) operated at an acceleration voltage of 200 kV. UV-vis data were recorded on a Hewlett-Packard 8453 diode-array UV-vis spectrophotometer using quartz cuvettes with an optical path length of 1 cm at room temperature.

1.4 Oxidation of free-reduced Cys and Hcy in human urine. Prior to the preparation of calibration standards, free-reduced Cys and Hcy in the human urine sample were oxidized. A 20 mL human urine sample was placed into a 50 mL volumetric flask, and then the pH of the sample was adjusted to 8.1 with 1.0 M NaOH. After 5.0×10^{-8} M Fe^{3+} was plunged into the urine sample solution, the sample solution was saturated with ultra-pure oxygen for 20 min. Hereafter, the pH of the urine sample was adjusted to 6.0 with 1.0 M HCl, and was degassed by bubbling purified nitrogen for 30 min.

1.5 Calibration standards. Stock solutions of 10 mM Cys and Hcy were daily prepared with water (deaerated with purified nitrogen). The working solutions with various Cys and Hcy concentrations were obtained by appropriate dilution with water and processed without delay. For preparation of calibration standards in the human urine, a 250 μL of the urine sample (after oxidation of free-reduced Cys and Hcy) was placed in a centrifuge tube, and gently vortex-mixed with 500 μL ice-cold methanol, kept them at room temperature for 15 min to assure complete protein precipitation, and then centrifuged at 13000 rpm for 10 min. The clear supernate was filtered through a 0.22 μm filter, followed by the addition of standard solutions with various concentrations.

1.6 Determination of tCys and tHcy in human urine samples. A 2.5 μL portion of 0.2 M TCEP in pH 6 PBS was added to 250 μL urine sample in a centrifuge tube. The mixture was kept at 60 ^{0}C for 30 min. After cooling to room temperature, the sample was gently vortex-mixed with 500 μL ice-cold methanol, and kept them at room temperature for 15 min to assure complete protein precipitation before centrifuging at 13000 rpm for 10 min. The clear supernate was filtered through a 0.22 μm filter. For the determination of total concentration of Cys and Hcy, a 60 μL portion of supernate was added to a 1.5 mL 12 nm FSN-capped GNP solution containing 5 mM PBS (pH ~ 6) and 100 mM NaCl; for the determination of the tHcy, a 114.6 μL portion of supernate was added to a 1.5 mL 40 nm FSN-capped GNP solution containing 10 mM PBS (pH ~6). Then, the colorimetric evolution of the solutions was measured.

2. Aggregation of the FSN-capped GNPs induced by Cys and Hcy.

Our previous study showed that capping of 12 nm GNPs with FSN ligands resulted in ~4 nm red-shift (from 521 nm to 525 nm) of the SPR peak.[2] For 40 nm GNPs, no red-shift of the SPR peak (at 533 nm) was observed. The colloidal solution of 40 nm FSN-capped GNPs is deep-red in color. The TEM image shows that the GNPs are roughly spherical.

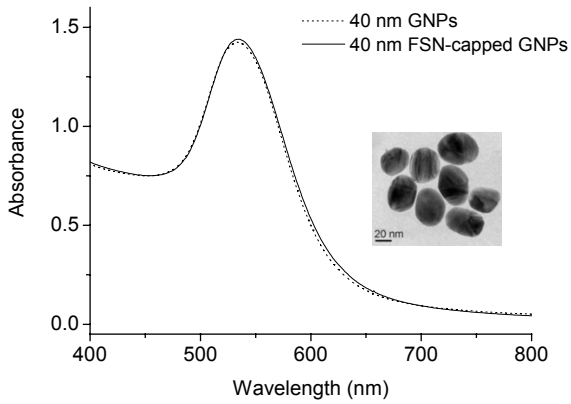


Figure S1. UV-vis spectra of 40 nm GNPs with or without FSN capping. Inset, TEM image of the FSN-capped GNPs.

Like the small sized FSN-capped GNPs,[2] obvious color change of the colloidal solution occurred upon the addition of Cys or Hcy, indicating the formation of the GNP aggregate. Figure S2 shows the spectral change of the colloids in responding to Hcy. With the decrease of the SPR peak at ~533 nm, a new peak appeared at ~790 nm. Similar results were also obtained in the case of Cys.

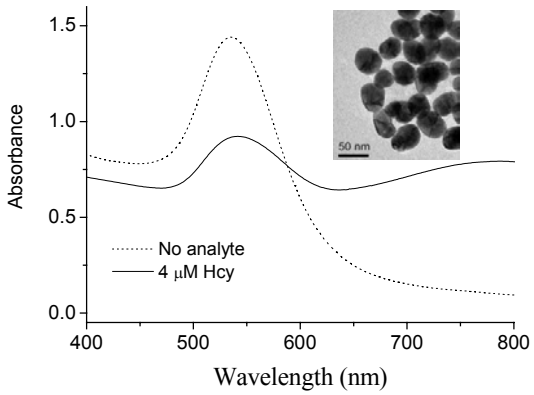
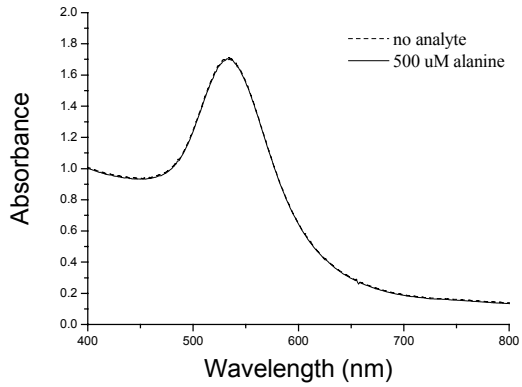
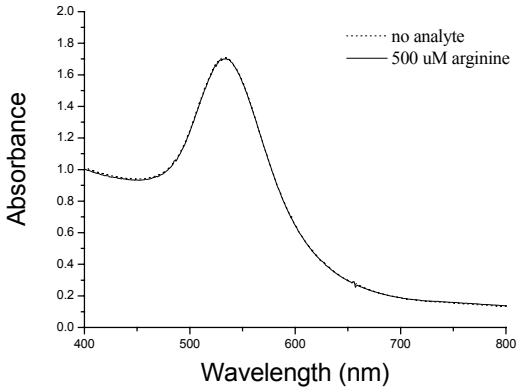


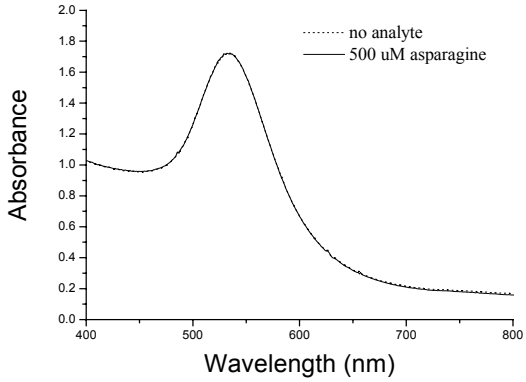
Figure S2. UV-vis spectra of 40 nm FSN-capped GNP colloids in the presence of Hcy. The solutions contained 5 mM PBS and 45 mM NaCl (pH ~6.0). The spectra were acquired at 1200 s after the addition of Hcy. Inset, TEM image of the aggregate of the FSN-capped GNPs.

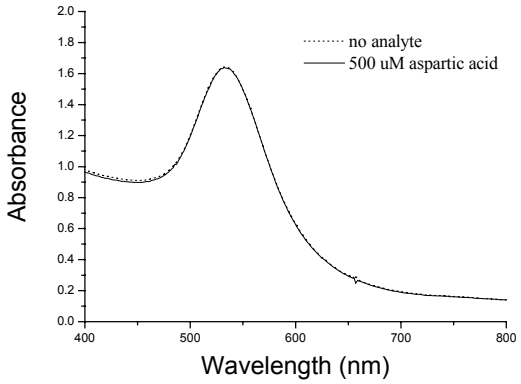
3. Interactions of 40 nm FSN-capped GNPs with a variety of molecules.

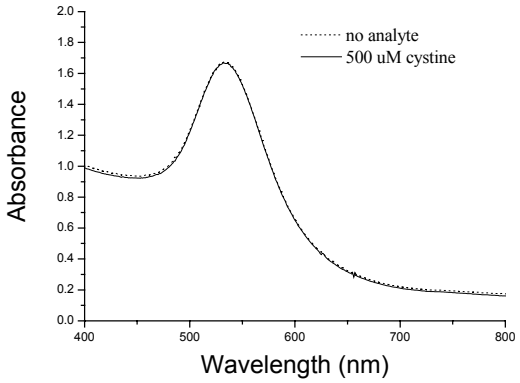
The interactions of 40 nm FSN-capped GNPs with a variety of biomolecules were examined. The colloids did not respond to standard amino acids other than Cys, the oxidized dimeric forms of Cys and Hcy, and biomolecules such as Glu, cysteinylglycine, and glucose.

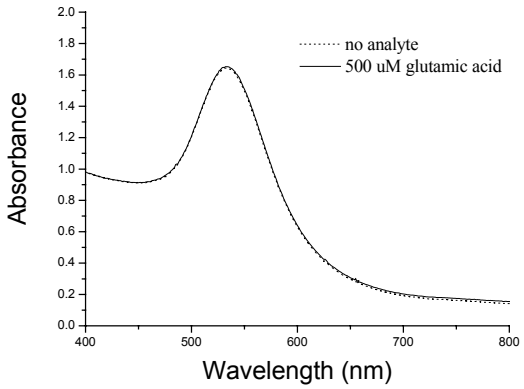


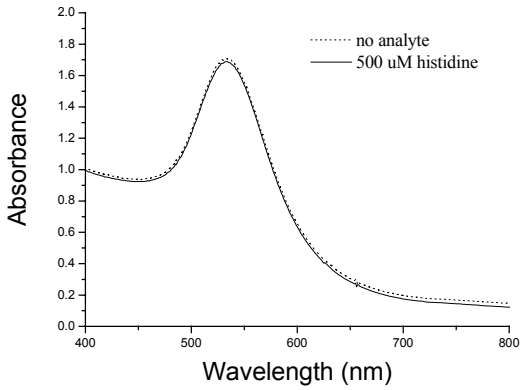








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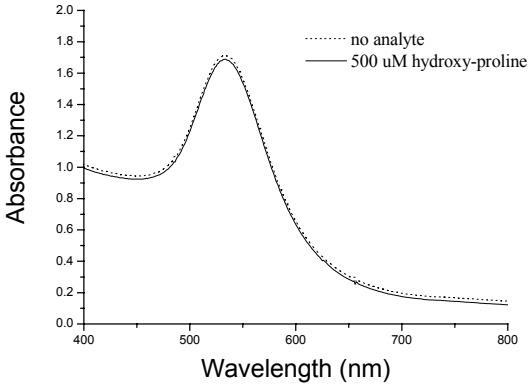
Absorbance
Wavelength (nm)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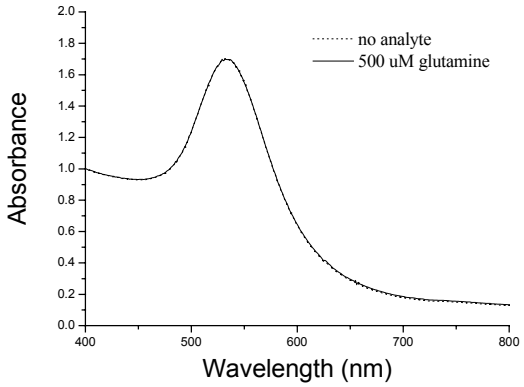
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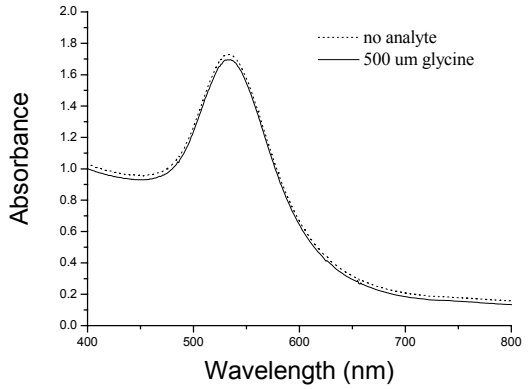
Absorbance
Wavelength (nm)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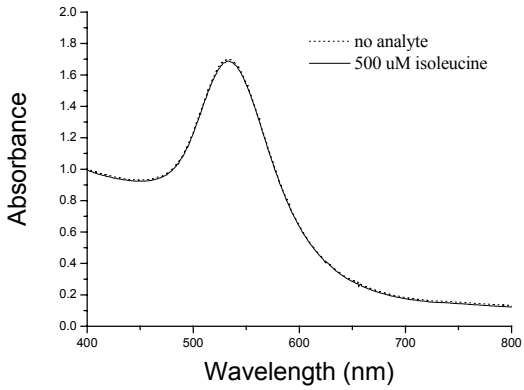
Absorbance
Wavelength (nm)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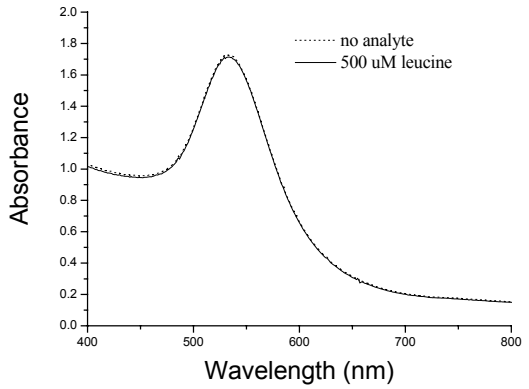
Absorbance
Wavelength (nm)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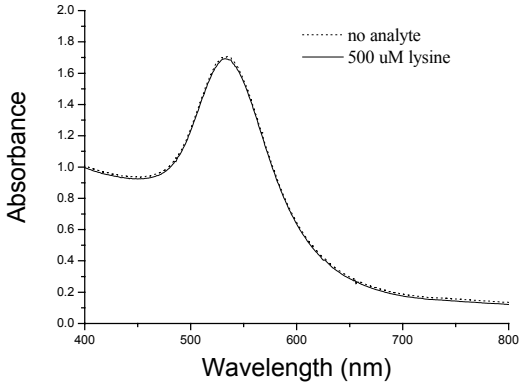
Absorbance
Wavelength (nm)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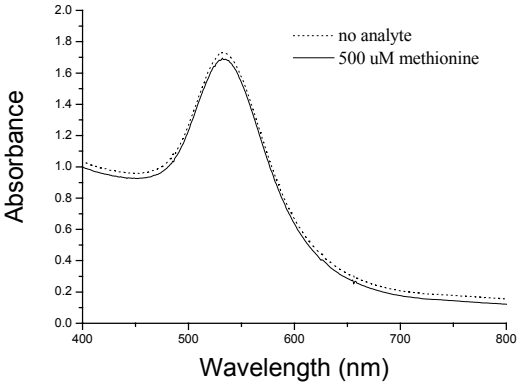
Wavelength (nm)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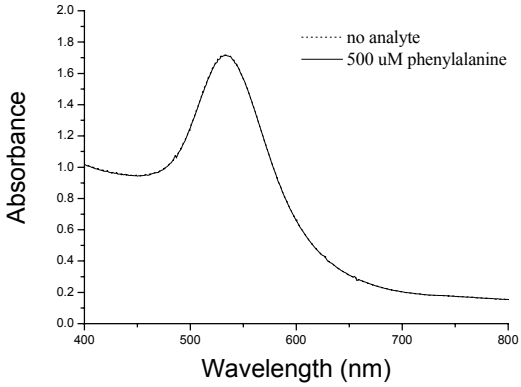
Wavelength (nm)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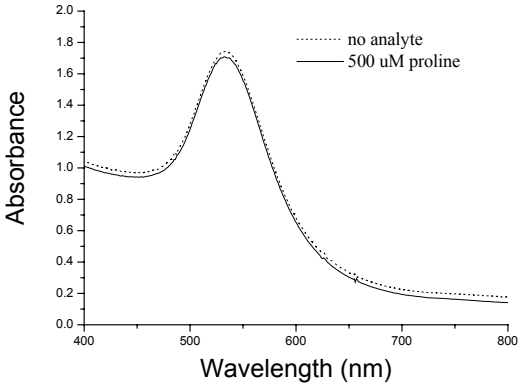
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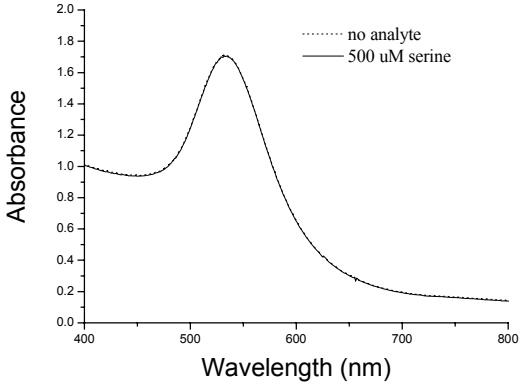
Absorbance
Wavelength (nm)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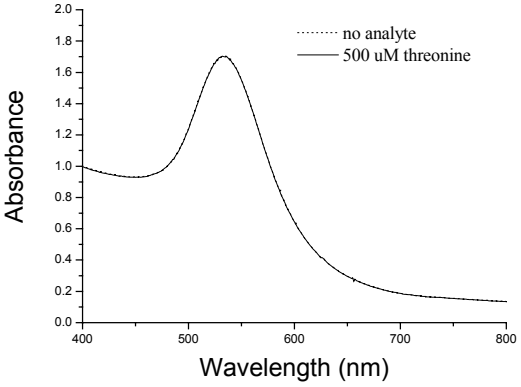
Wavelength (nm)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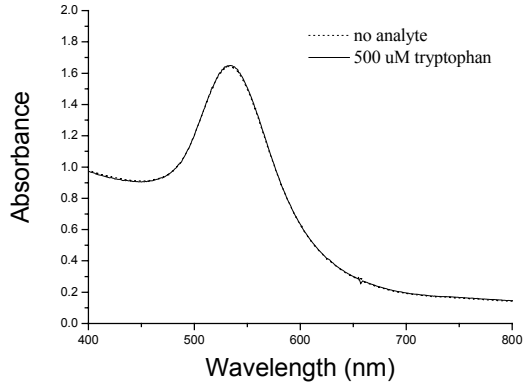
Absorbance
Wavelength (nm)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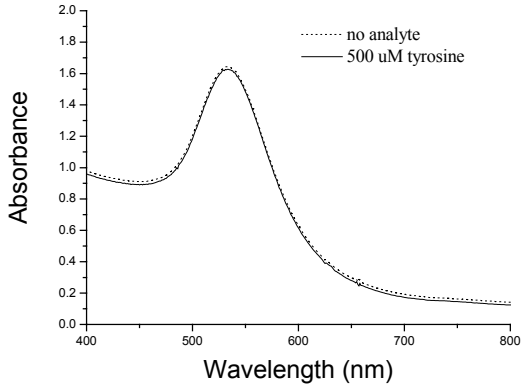
Absorbance
Wavelength (nm)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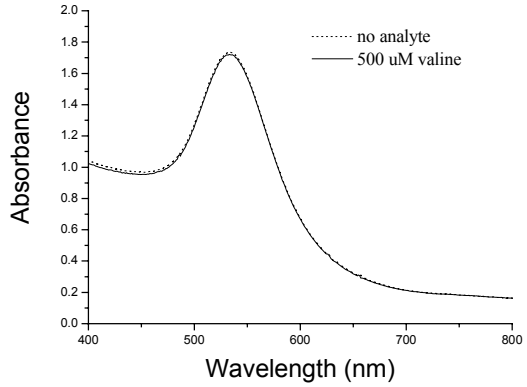
Absorbance
Wavelength (nm)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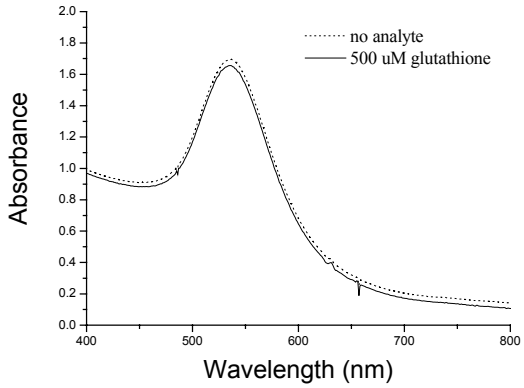
500 uM valine
Absorbance
Wavelength (nm)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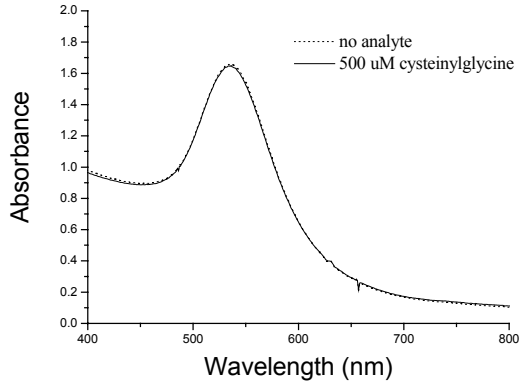
Absorbance
Wavelength (nm)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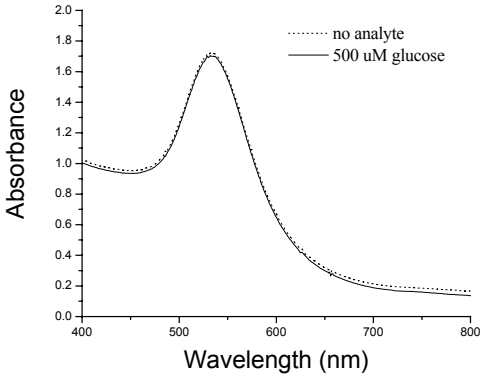
500 uM cysteinylglycine
Absorbance
Wavelength (nm)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500 uM glucose
Absorbance
Wavelength (nm)

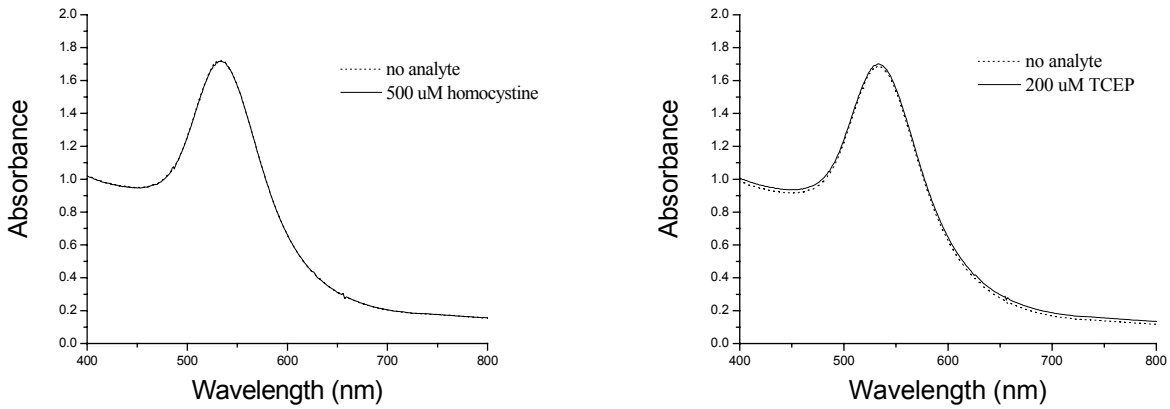

Figure S3. UV-vis spectra of 40 nm FSN-capped GNP colloids in the presence of a variety of molecules. The solutions contained 5 mM phosphate (pH ~6) and 45 mM NaCl. The spectra were measured at 30 min after the addition of the analytes.

5. Further evidences for the proposed aggregation mechanism.

5.1 Effect of pH. The effect of solution pH on the colorimetric evolution was also investigated. In the presence of low salt (< 10 mM), the maximum rate of solution color change induced by Hcy was observed at pH around 6, near the pI of the attached Hcy; lower or higher pH would result in slower aggregation, which could be due to the change of the charging state of Hcy molecules. This result supports the assumption of the GNP cross-linking via the electrostatic interaction between the bound amino acid species. As the solution ionic strength increased, however, the pH effect became less and less significant, suggesting the important role of the London-van der Waals attraction force in driving the process.

5.2 Reponses to small non-biological thiols. To further verify the different aggregation routes, the responses of 40 nm FSN-capped GNPs to some small thiol molecules, such as 3-mercapto-1-propanesulfonic acid and 2-mercaptoethylamine, were examined. These molecules (~10 μM) did not induce the color change of the colloidal solution with low ionic strength (2 mM phosphate, pH ~6); however, the addition of 100 mM NaCl led to color change from deep-red to blue immediately in each case. This suggests that these small thiols were able to replace some FSN ligands and attach to the GNPs, rendering the colloids less stable. When solution ionic strength was high enough to shield the interparticle electrostatic repulsion, the London-van der Waals attraction force could drive the GNP aggregation; however, under low-salt conditions, the aggregation could not proceed unless appropriate cross-linkages were established between the GNPs. Note that, although the small thiol

molecules may also lead to the GNP aggregation in high-salt solution, they will not interfere with the detection of Cys and Hcy because of their absence in most biological matrices.

6. Selectivity of the FSN-capped GNPs towards Cys and Hcy in urine sample.

Figures S5 and S6 show that, in presence of the dilute human urine, the colloidal solution of 12 nm FSN-capped GNPs responded to both of Cys and Hcy, while the colloidal solution of 40 nm FSN-capped GNPs responded to Hcy only. Other biomolecules could not induce the color change. Clearly, the high selectivity of the colorimetric response towards authentic Cys and Hcy could preserve in the assay of real samples.

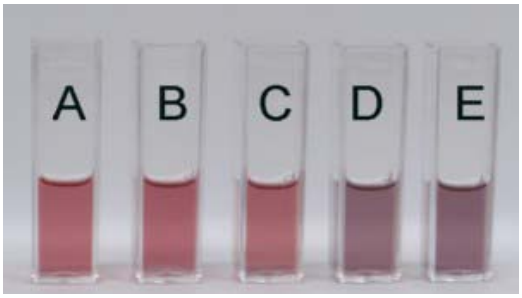

Figure S5. Colorimetric responses of 12 nm FSN-capped GNP colloids to various analytes. A, no analyte; B, cystine and 19 standard amino acids other than Cys (each 10 μM); C, 100 μM Glu; D, 4 μM Cys; E, 4 μM Hcy. The solutions contained 5 mM PBS (pH ~6), 100 mM NaCl and 50 times diluted human urine.

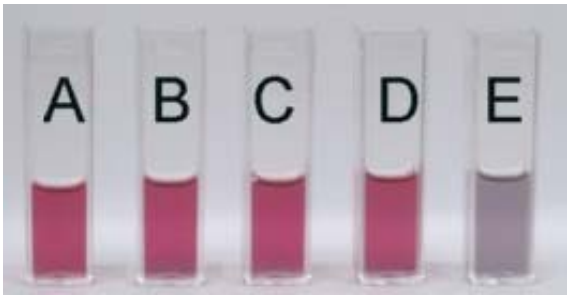

Figure S6. Colorimetric responses of 40 nm FSN-capped GNP colloids to various analytes. A, no analyte; B, cystine and 19 standard amino acids other than Cys (each 10 μM); C, 100 μM Glu; D, 50 μM Cys; E, 8 μM Hcy. The solutions contained 2 mM PBS (pH ~6) and 50 times diluted human urine.

References

1. Z.-Y. Zhong, S. Patskovskyy, P. Bouvrette, J. H. T. Luong and A. Gedanken, *J. Phys. Chem B.*, 2004, **108**, 4046.

2. C. Lu, Y. Zu, V. W. W. Yam, *Anal. Chem.*, 2007, **79**, 666.