Supporting Information

A Colorimetric Sensor Array Based on Gold Nanoparticles and Amino Acids for Identification of Toxic Metal Ions in Water

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S1. EXPERIMENTAL SECTION

Materials. Sodium citrate tribasic dehydrate, gold(III)chloride trihydrate ($HAuCl_4$), 11-mercaptoundecanoic acid (MUA), lysine, cysteine, histidine, tyrosine, arginine, $Hg(NO_3)_2.H_2O$, $Cd(NO_3)_2.4H_2O$, $Fe(NO_3)_3.9H_2O$, $Pb(NO_3)_2$, $Al(NO_3)_3.H_2O$, $Cu(NO_3)_2.6H_2O$, $Cr(NO_3)_3.9H_2O$, $AgNO_3$, $Ca(NO)_3.4H_2O$, $Zn(NO_3)_2.6H_2O$, $Co(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Sr(NO_3)_2$, KNO_3, NaCl, and $FeCl_2.4H_2O$ were obtained from Sigma-Aldrich (St Louis, MO). All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed bed system.

Synthesis of MUA capped AuNPs. First, citrate capped AuNPs were prepared according to Turkevich method.[1] Briefly, 12 mL preheated sodium citrate solution (1%, w/w) was added to the 100 mL of boiling water containing 8.5 mg of $HAuCl_4$ salt. The solution was vigorously stirred while heating for 20 min until color of the solution turned to deep red. After reaction completed, the volume of AuNP solution was adjusted to 100 mL.

To modify the AuNP surface with MUA, 10 mL of aqueous solution of MUA (3.25 mg) containing NaOH (0.5 M, 30 µL) was added to citrate capped AuNPs solution (50 mL) while heating. Then, the solution was stirred for 1 hour.[2] Solution was cooled to room temperature and volume of the MUA capped AuNP solution was adjusted to 50 mL. Finally, MUA capped AuNPs were washed with water twice to remove the unbound MUA molecules.

Determination of amino acid concentrations in the assay. To determine the suitable amino acid concentrations that will be used in the colorimetric sensor array UV-Vis spectra of AuNPs (0.1 nM) in the presence of different amino acid concentrations between 0 µM and 500 µM were recorded using a UV-Vis spectrophotometer at different time intervals up to 1 h. The colorimetric responses were calculated by dividing the extinction of AuNPs at 625 nm by the extinction of 525 nm (Ex_{625}/Ex_{525}).

Colorimetric detection of metal ions. The experiments were performed in a 96 well plate. 50 μL MUA capped AuNPs (0.1 nM) was mixed with 5 μL of cysteine (1 mM) or 20 μL of other amino acids (1 mM) (lysine, cysteine, histidine, tyrosine, and arginine) at determined concentrations. Then, metal ions (Hg^{2+}, Cd^{2+}, Fe^{3+}, Pb^{2+}, Al^{3+}, Cu^{2+}, and Cr^{3+}) in water at different concentrations were added to this solution to give final metal ions concentrations in the range of 2 μM to 50 μM. Final volume of assay was completed to 100 μL with water. Finally, color changes were detected by the naked eye and/or by using a plate reader. The colorimetric responses were recorded at 625 nm and 525 nm. The colorimetric responses were calculated by dividing the extinction of AuNPs at 625 nm by the extinction of 525 nm (Ex_{625}/Ex_{525}). The photographs were taken with a digital camera (12.1 megapixels).

Characterization. Morphology of the AuNPs was characterized using a TEM (Tecnai G2 F30, FEI). Raman spectra were collected using a DXR Raman Microscope (Thermo Scientific). Zeta potentials of the AuNPs were measured using a Zetasizer (Nanoseries, Malvern). Absoprtion spectra of AuNPs were obtained using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu). A plate reader (Spectra max M5, Molecular Devices) used to read the absorption values at 525 nm and 625 nm of the colorimetric assay.

Data analysis. The colorimetric responses of the array against metal ions at different concentrations were analysed using hierarchical cluster analysis (HCA) as the pattern recognition technique. The analyses were performed by built-in HCA algorithms of MATLAB software. Euclidean distance method was used for the obtaining the dendrograms (see next section for more information).

S2. HCA ANALYSIS

MATLAB code for HCA:

```
X = [enter data]
D = pdist (X)
Squareform (D)
Z = linkage (X,'complete','euclidean');
H = dendrogram (Z,'Orientation','right')
Set (H,'LineWidth',1)
```

-The distance between two data points were calculated using Euclidean distance, which is expressed as follow;

$$d(a,b) = \sqrt{\sum_{i=1}^{n}(a_i - b_i)^2}$$

S3. ADDITIONAL FIGURES

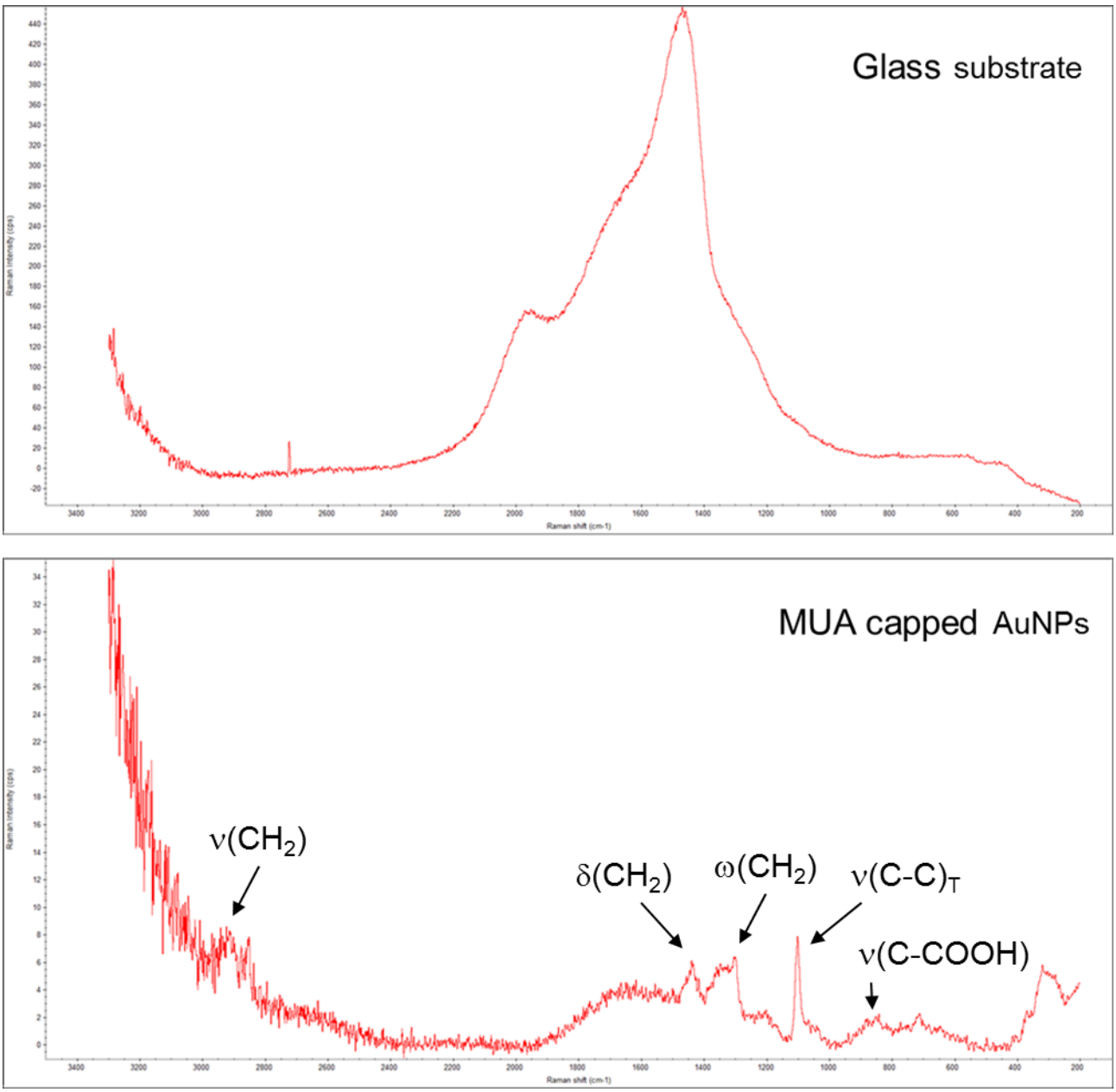


Figure S1. Raman Spectra of glass substrate and MUA capped AuNPs and amino acid treated AuNPs which were dried on glass substrates.

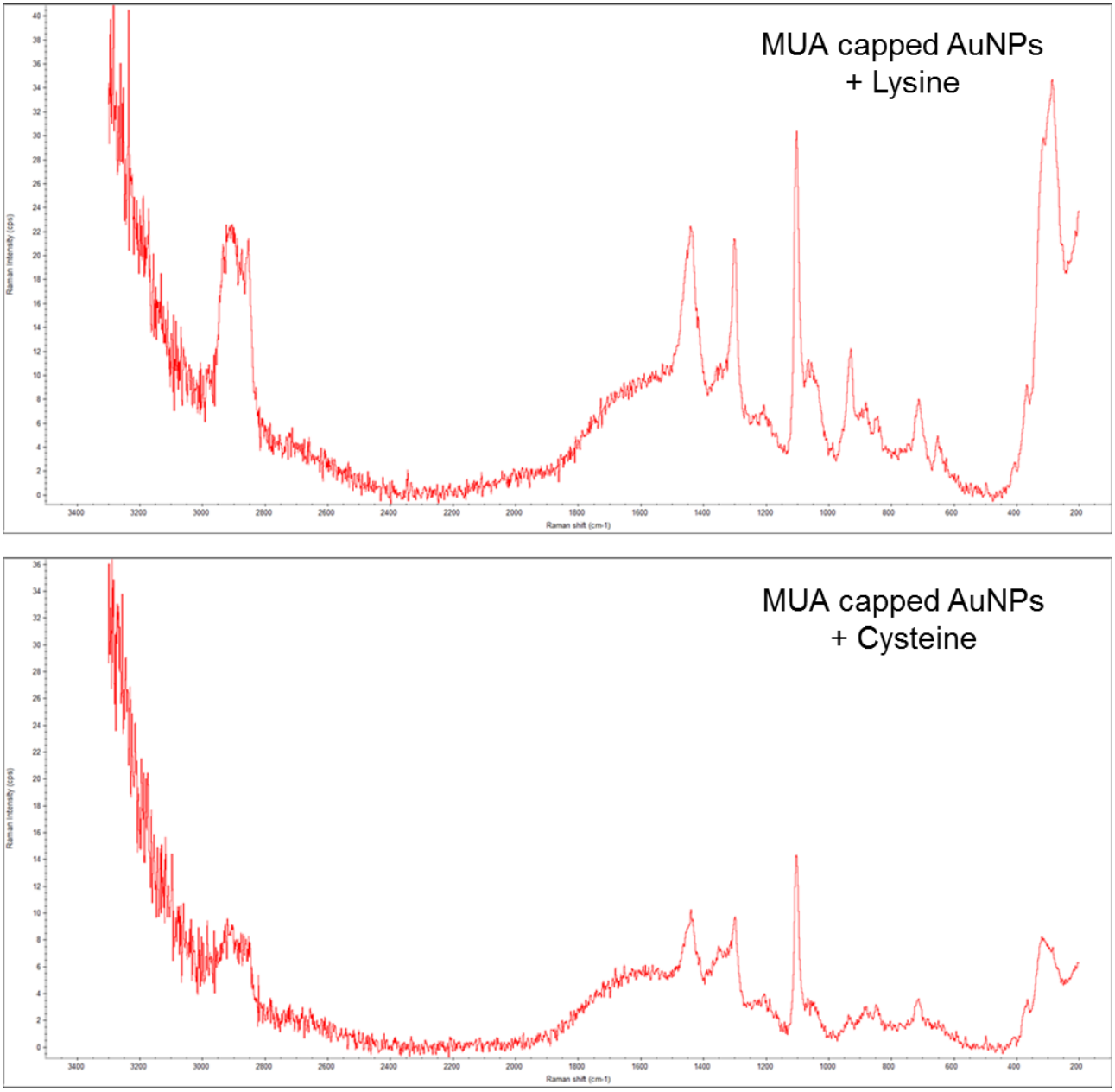
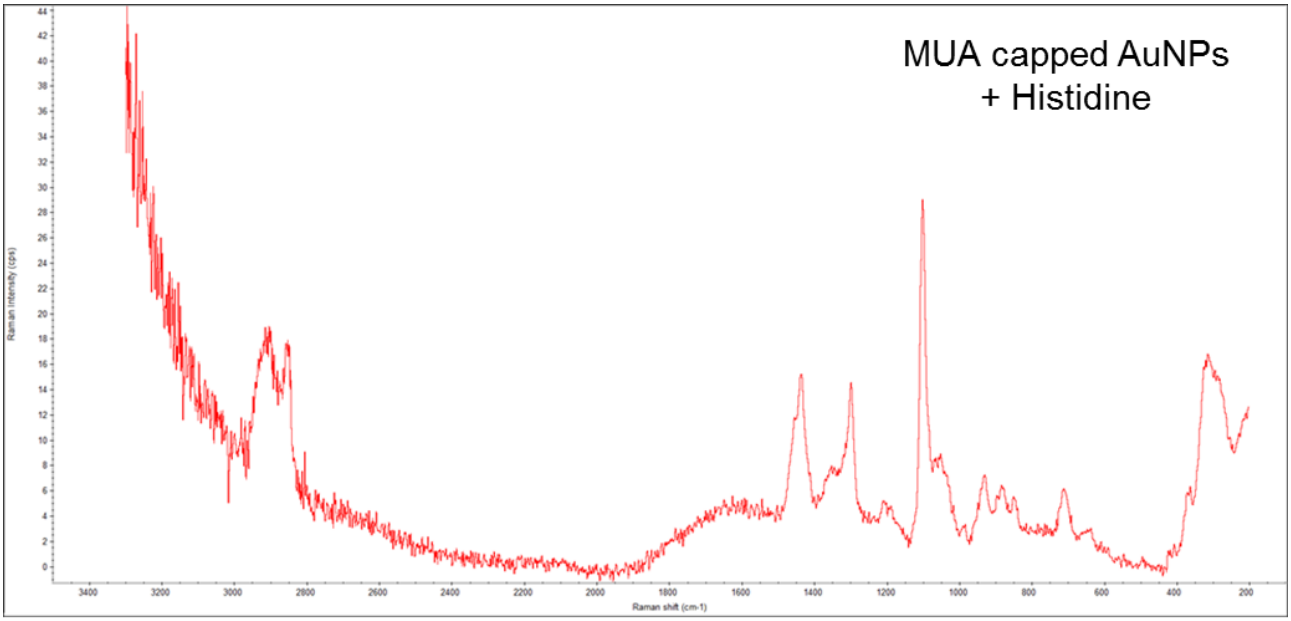


Figure S1. Cont'd.



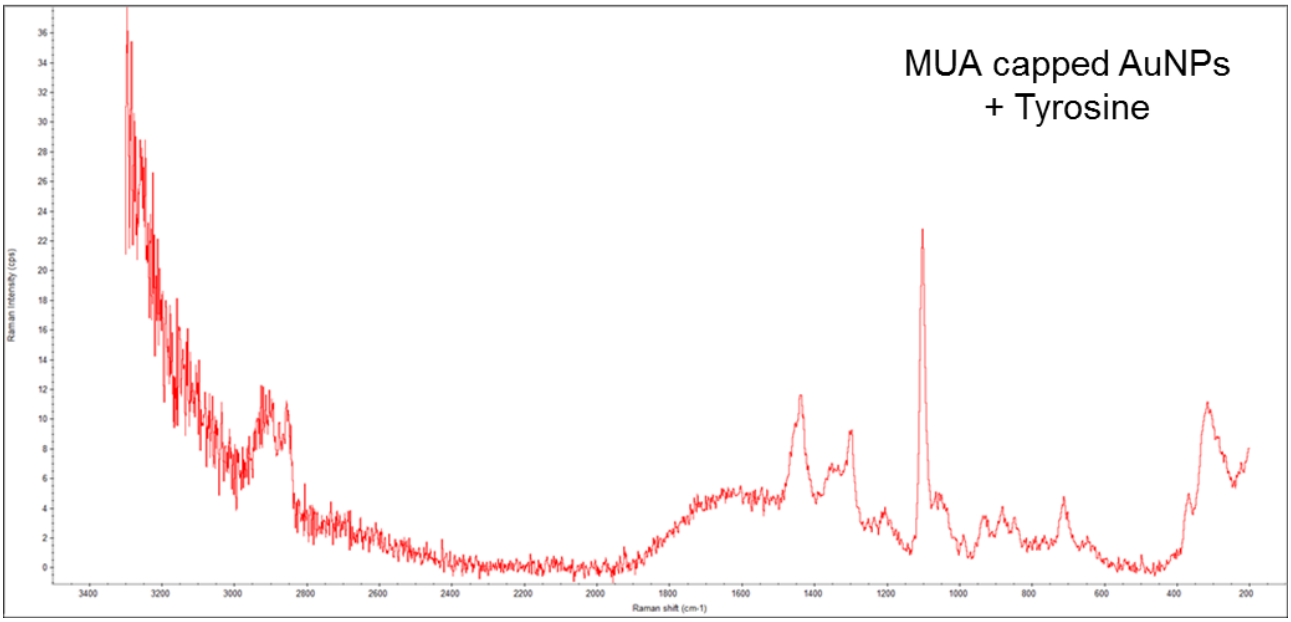


Figure S1. Cont'd.

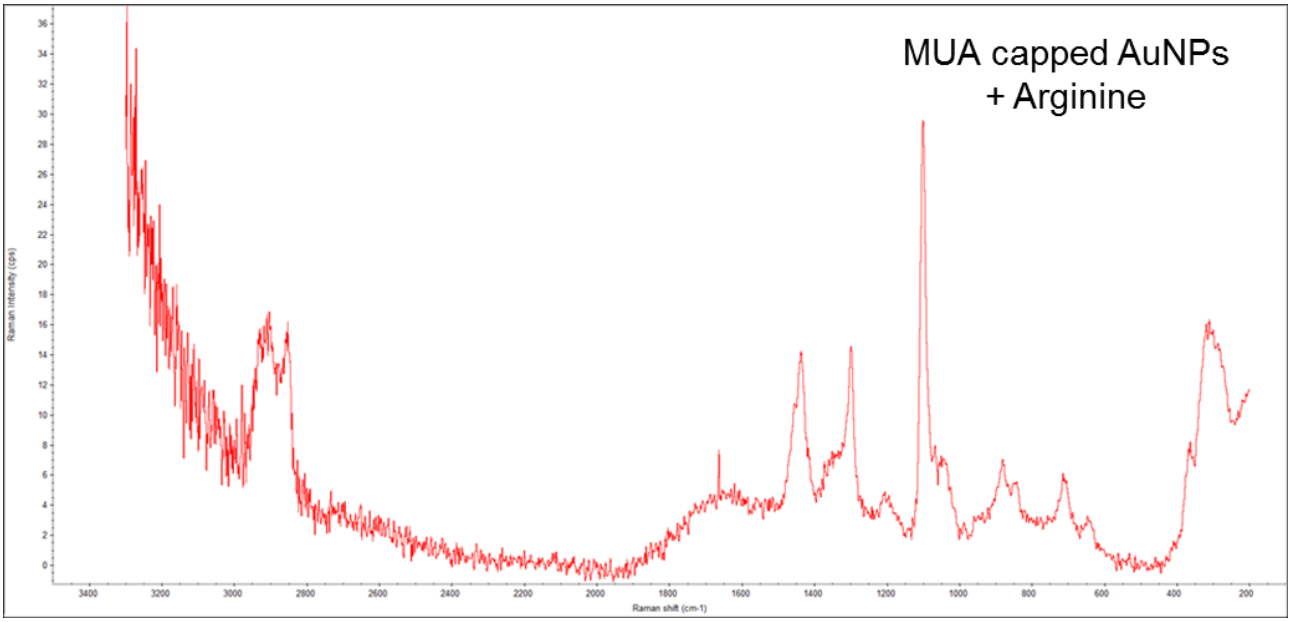


Figure S1. Cont'd.

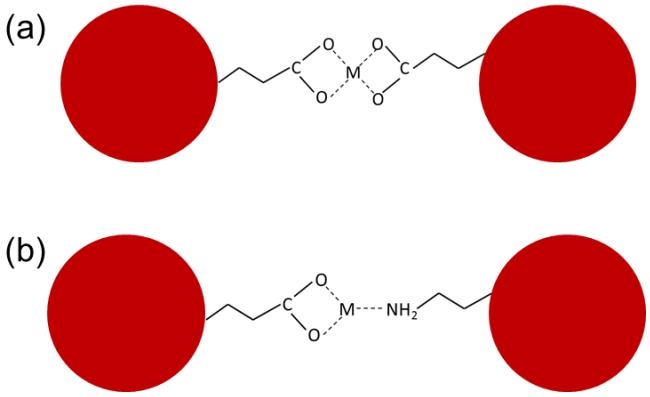


Figure S2. Structure of MUA capped AuNPs and some possible interaction between metal ions and amino acid or MUA molecules.

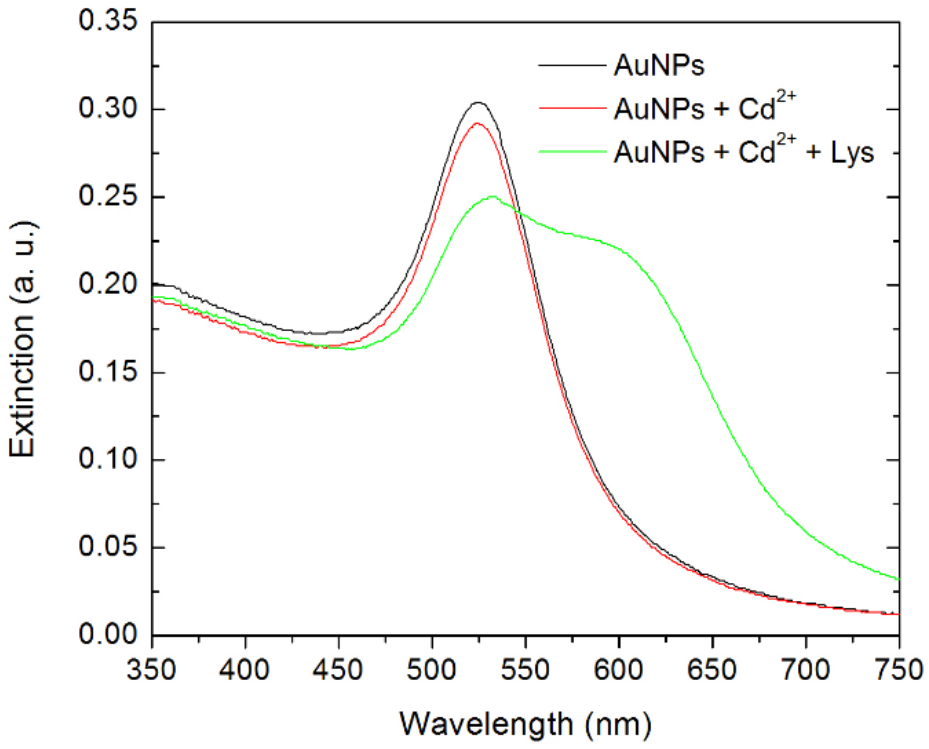


Figure S3. Representative UV-Vis spectra for AuNPs in the presence or absence of metal ions and amino acids.

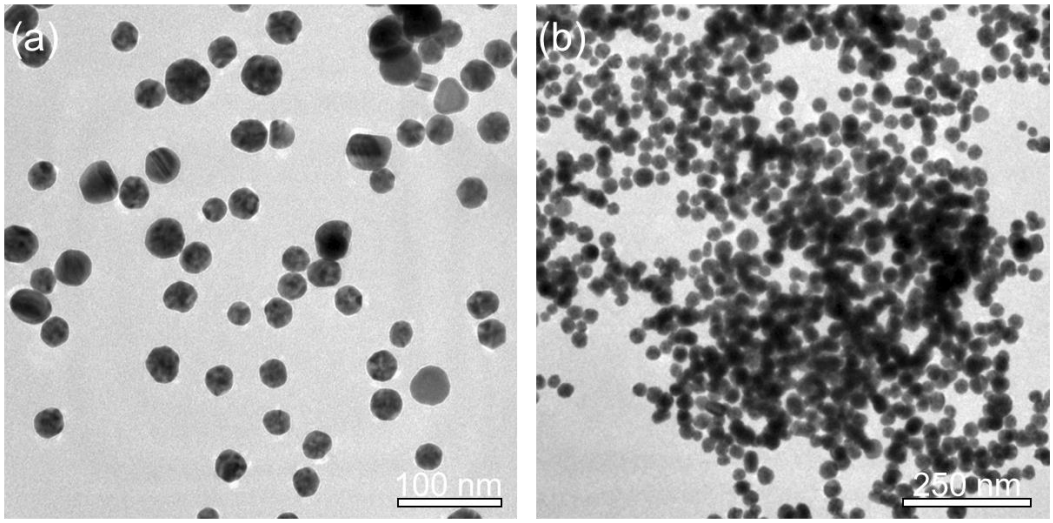


Figure S4. TEM images of well-dispersed as prepared MUA capped AuNPs (a) and aggregated AuNPs in the presence of 20 µM of Cd2+ and 200 µM of lysine.

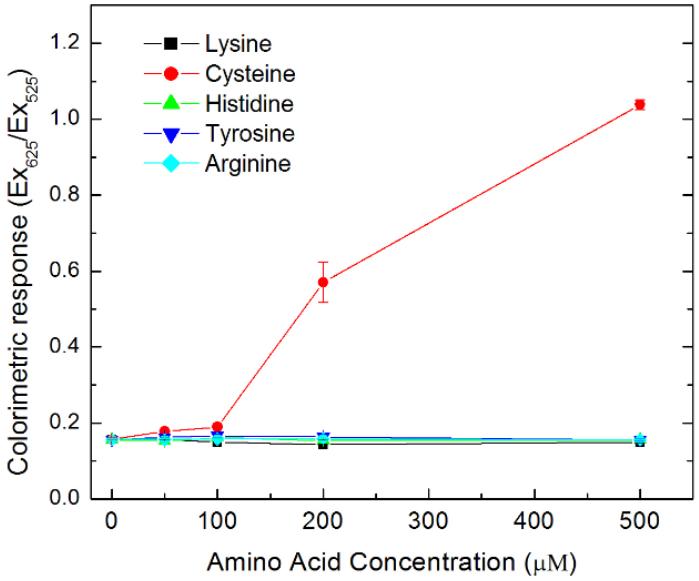


Figure S5. Colorimetric response (Ex_{625}/Ex_{525}) of AuNPs depending on amino acid concentrations in the absence of metal ions.

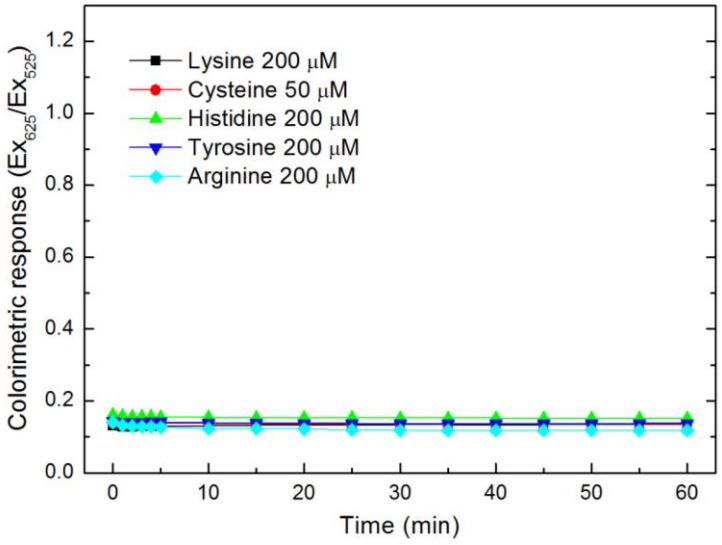


Figure S6. Time dependent colorimetric response (Ex_{625}/Ex_{525}) of AuNPs in the presence of amino acids at their concentrations used in the assay.

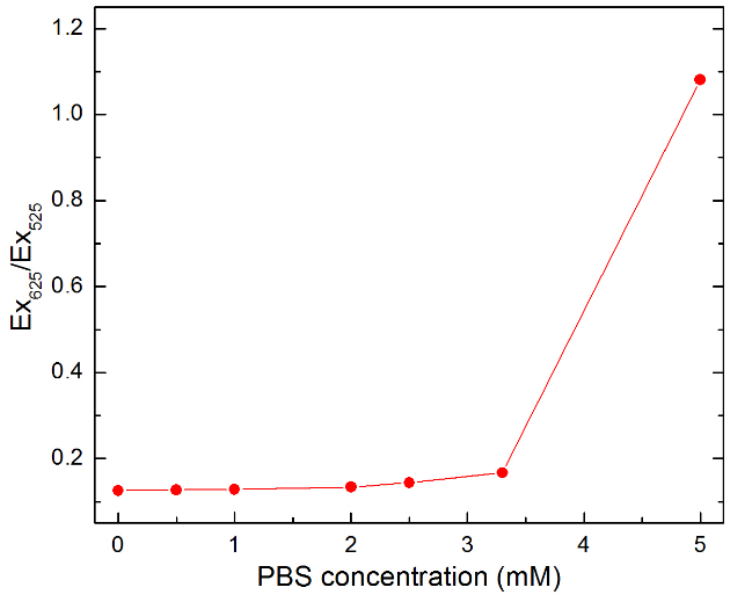


Figure S7. Effect of salinity on the stability of MUA capped AuNPs.

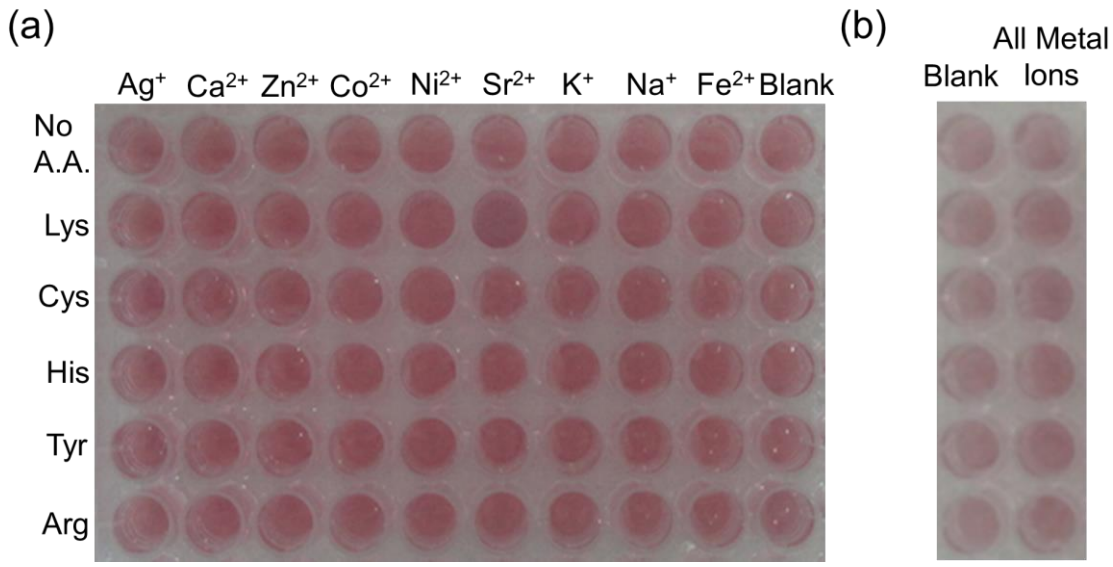
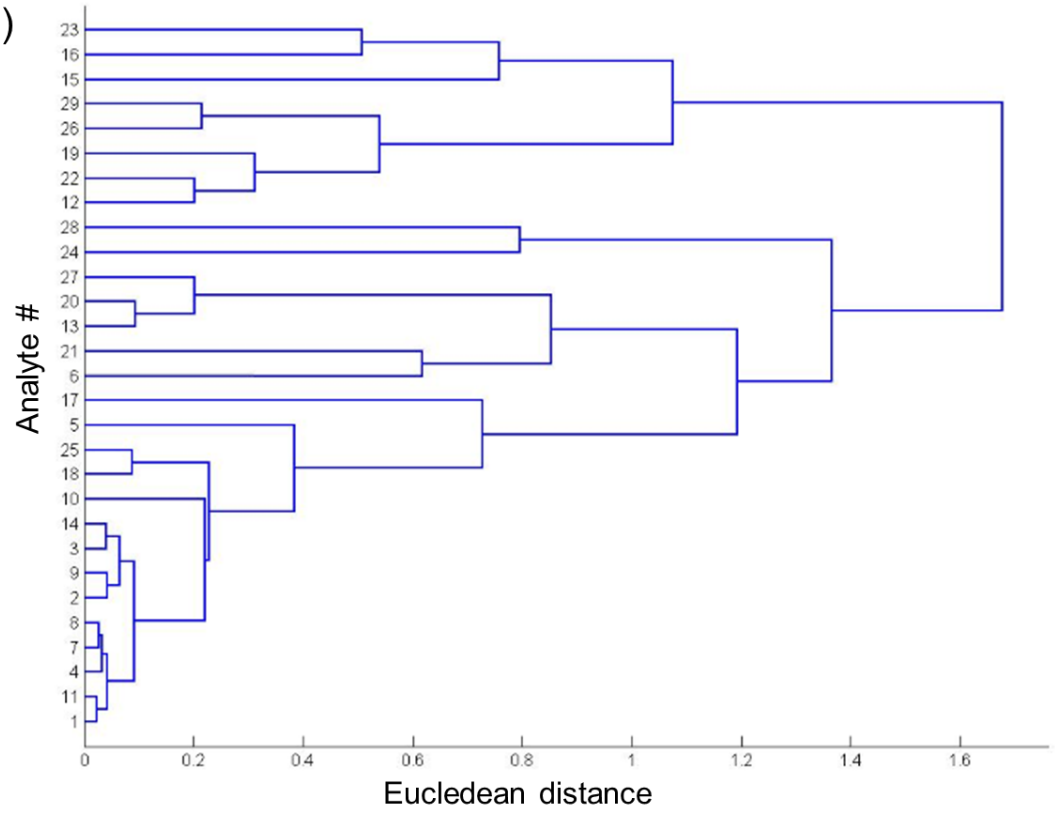


Figure S8. Representative photograph of the colorimetric sensor array response against (a) 20 μM of 9 nonresponsive metal ions with the assay (b) mixture of all metal ions.

(a)



(b)

Analyte#	Metal ion	Analyte#	Metal ion	Analyte#	Metal ion
1	Blank	11	Cd^{2+} (10 μM)	21	Cu^{2+} (20 μM)
2	Hg^{2+} (2 μM)	12	Fe^{3+} (10 μM)	22	Cr^{3+} (20 μM)
3	Cd^{2+} (2 μM)	13	Pb^{2+} (10 μM)	23	Hg^{2+} (50 μM)
4	Fe^{3+} (2 μM)	14	Al^{3+} (10 μM)	24	Cd^{2+} (50 μM)
5	Pb^{2+} (2 μM)	15	Cu^{2+} (10 μM)	25	Fe^{3+} (50 μM)
6	Al^{3+} (2 μM)	16	Cr^{3+} (10 μM)	26	Pb^{2+} (50 μM)
7	Cu^{2+} (2 μM)	17	Hg^{2+} (20 μM)	27	Al^{3+} (50 μM)
8	Cr^{3+} (2 μM)	18	Cd^{2+} (20 μM)	28	Cu^{2+} (50 μM)
9	Hg^{2+} (10 μM)	19	Fe^{3+} (20 μM)	29	Cr^{3+} (50 μM)
10	Cd^{2+} (10 μM)	20	Pb^{2+} (20 μM)		

Figure S9. (a) Dendrogram showing the discrimination between all the tested metal ions at different concentrations. (b) Table listing the names and concentrations of analytes in (a).

S4. SUPPORTING TABLE

Table S1. Zeta potentials of MUA capped AuNPs before and after interacting with different amino acids.

Sample	Zeta potential (mV)
MUA capped AuNPs	-12.3 ± 2.0
Lysine + MUA capped AuNPs	-14.1 ± 2.2
Cysteine + MUA capped AuNPs	-14.2 ± 0.9
Histidine + MUA capped AuNPs	-13.7 ± 0.6
Tyrosine + MUA capped AuNPs	-16.2 ± 1.2
Arginine + MUA capped AuNPs	-16.0 ± 1.4

REFERENCES

(1) Enüstün, B. V.; Turkevich, J. Coagulation of Colloidal Gold. *J. Am. Chem. Soc.* **1963**, *85*, 3317.

(2) Kim, Y.; Johnson, R. C.; Hupp, J. T. Gold Nanoparticle-Based Sensing of "Spectroscopically Silent" Heavy Metal Ions. *Nano Lett.* **2001**, *1*, 165-167.