Supporting Information

Lysozyme-Stabilized Gold Fluorescent Cluster: Synthesis and Its Application in Hg^{2+} Sensor

Hui Wei,[a] *Zidong Wang,*[b] *Limin Yang,*[ac] *Shiliang Tian,*[a] *Changjun Hou,*[c] *and Yi Lu**[ab]

[a]Department of Chemistry, University of Illinois, Urbana-Champaign, Urbana, Illinois 61801, USA;

[b]Department of Materials Science and Engineering, University of Illinois, Urbana-Champaign, Urbana, Illinois 61801, USA;

[c] College of Bioengineering, Chongqing University, Sha Ping Ba Street 174, Chongqing, 400044, China.

Fax: (+1) 217-244-3186; E-mail: yi-lu@illinois.edu

Table S1. The recipe for optimizing the ratio of lysozyme to $HAuCl_4$. Lysozyme and $HAuCl_4$ were added into water, mixed for 5 minutes, then NaOH was added and further incubated at 37ºC overnight.

Sample	1	2	3	4
5 mg/mL lysozyme	0.25 mL	0.25 mL	0.25 mL	0.25 mL
2mM $HAuCl_4$	0.5 mL	0.25 mL	0.1 mL	0.05 mL
water	0 mL	0.25 mL	0.4 mL	0.45 mL
1M NaOH	20 µL	20 µL	20 µL	20 µL

Table S2. The recipe for optimizing the concentrations of lysozyme and $HAuCl_4$. 0.25 mL lysozyme and 0.25 mL $HAuCl_4$ were added into 0.25 mL water, mixed for 5 minutes, then NaOH was added and further incubated at 37ºC overnight.

Sample	5	6	2	7	8	9
lysozyme	20 mg/mL	10 mg/mL	5 mg/mL	2 mg/mL	1 mg/mL	0.5 mg/mL
$HAuCl_4$	8 mM	4 mM	2 mM	0.8 mM	0.4 mM	0.2 mM
1M NaOH	80 µL	40 µL	20 µL	8 µL	4 µL	2 µL

Table S3. The recipe for optimizing the concentrations of NaOH. 0.1 mL 10 mg/mL lysozyme and 0.1 mL 4 mM $HAuCl_4$ were added into 0.1 mL water, mixed for 5 minutes, then NaOH was added and further incubated at 37ºC overnight.

Sample	10	11	12	13	14	15	16	17	18	19	20
1M NaOH/ µL	0	1	2	5	10	15	20	25	30	35	40

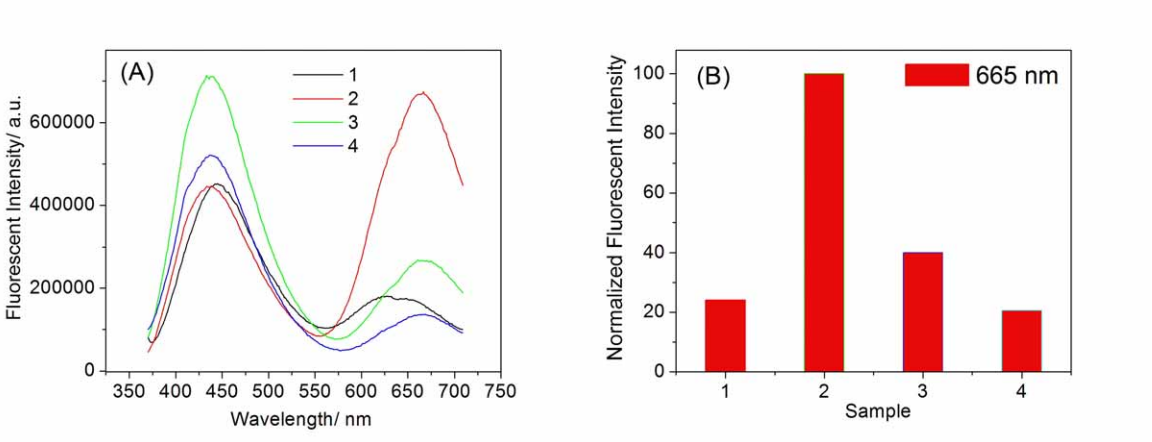

Figure S1. Fluorescent curves for optimizing the ratio of lysozyme to $HAuCl_4$ (A), and the corresponding histogram (B). The excitation wavelength was 360 nm.

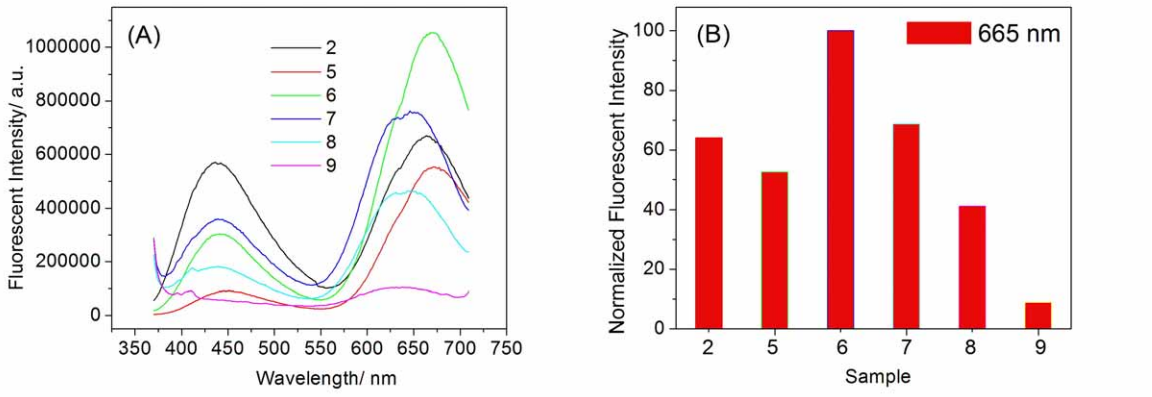

Figure S2. Fluorescent curves for optimizing the concentrations of lysozyme to $HAuCl_4$ (A), and the corresponding histogram (B). The excitation wavelength was 360 nm.

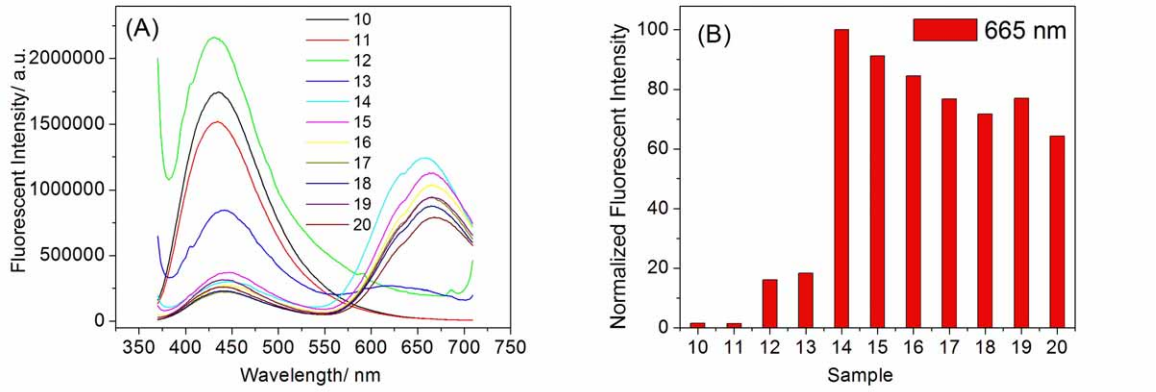

Figure S3. Fluorescent curves for optimizing the concentration of NaOH (A), and the corresponding histogram (B). The excitation wavelength was 360 nm.

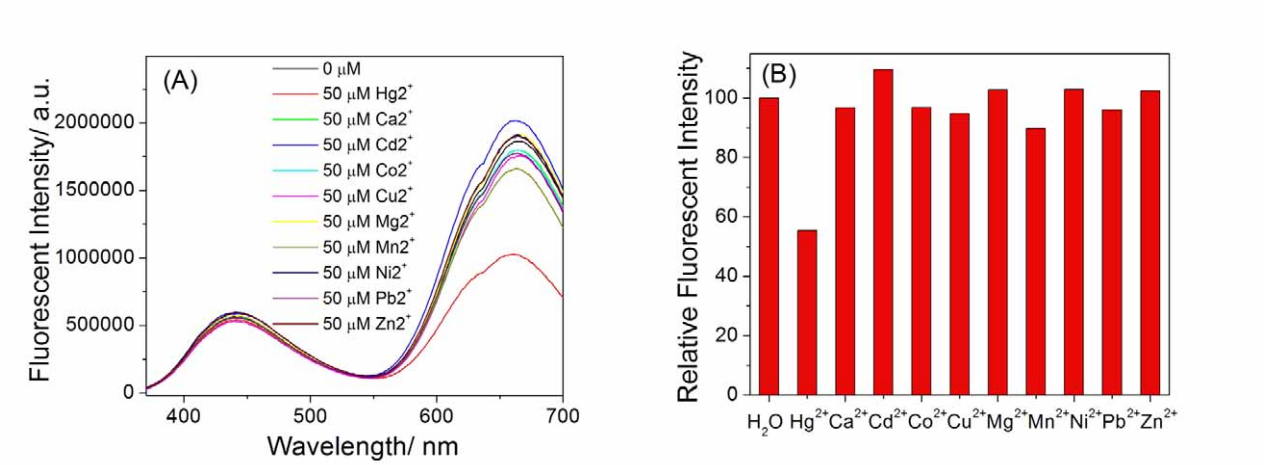

Figure S4. (A) The fluorescent spectra of as-prepared LsGFC probes (~ 34 μM) in the absence and presence of 50 μM different metal ions, (B) the relative fluorescent intensity at 660 nm vs. metal ions. The excitation wavelength was 360 nm.

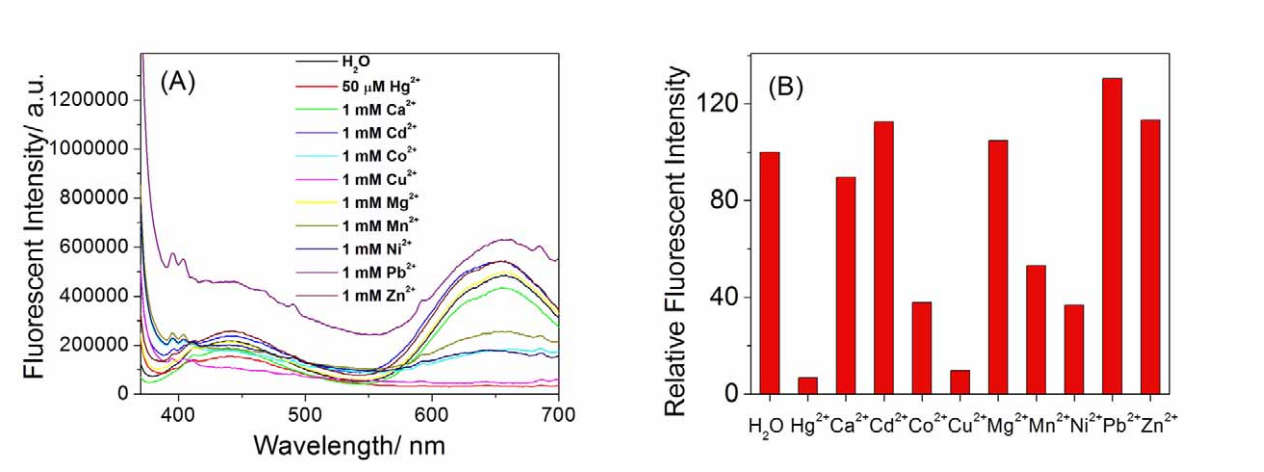

Figure S5. The fluorescent spectra of 10% diluted probes LsGFC (~ 3.4 μM) in the absence and presence of 50 μM Hg^{2+} (or 1 mM different metal ions), (B) the relative fluorescent intensity at 657 nm vs. metal ions. The excitation wavelength was 360 nm.

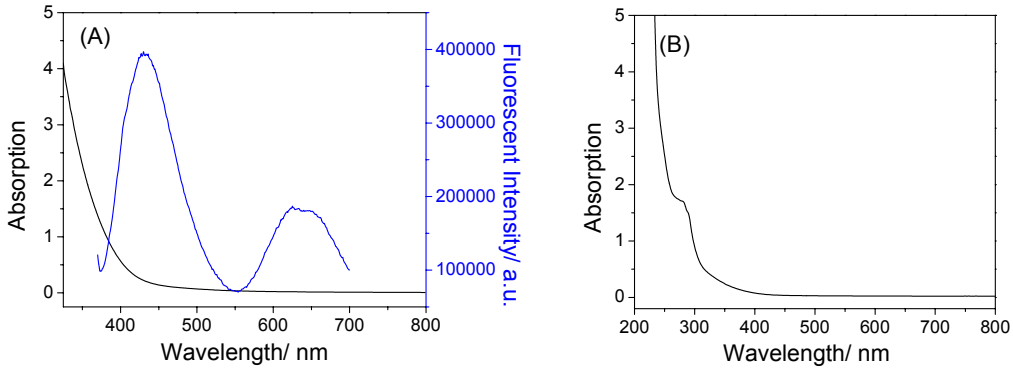

Figure S6. (A) Absorption (left) and fluorescent (right) spectra of the as-prepared gold cluster stabilized by CRABP. (B) Absorption of the 10% diluted gold cluster stabilized by CRABP. The excitation wavelength was 360 nm.

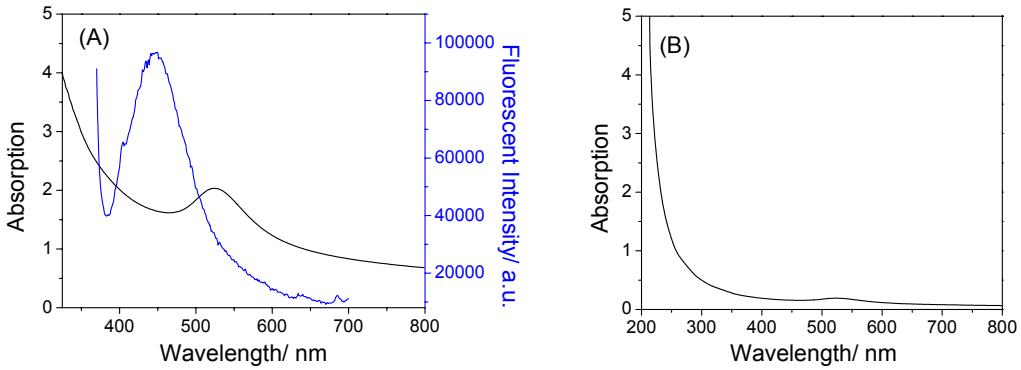

Figure S7. (A) Absorption (left) and fluorescent (right) spectra of the as-prepared gold particles stabilized by catalase. (B) Absorption of the 10% diluted gold particles stabilized by catalase. The excitation wavelength was 360 nm.

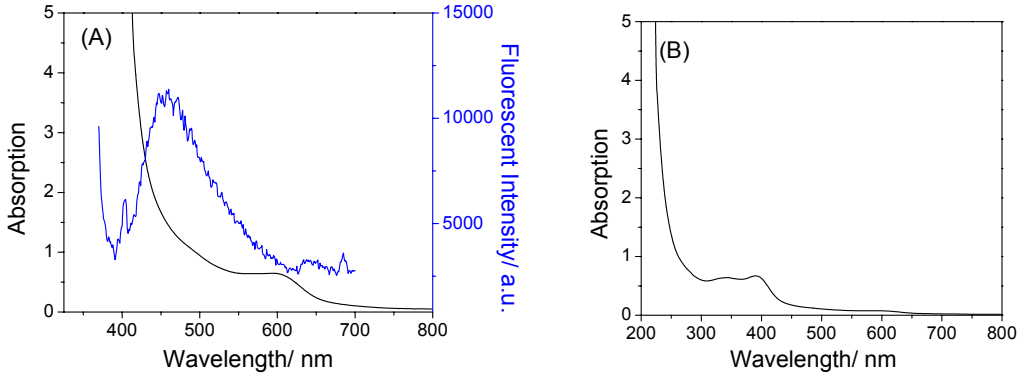

Figure S8. (A) Absorption (left) and fluorescent (right) spectra of the as-prepared gold particles stabilized by myoglobin. (B) Absorption of the 10% diluted gold particles stabilized by myoglobin. The excitation wavelength was 360 nm.

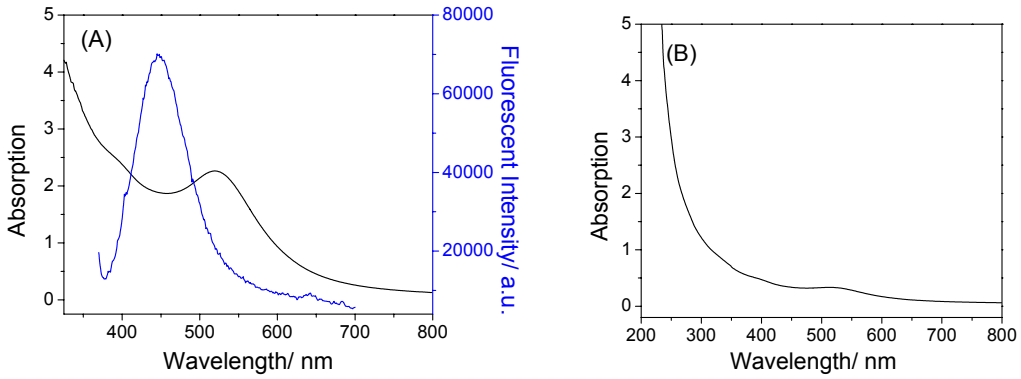

Figure S9. (A) Absorption (left) and fluorescent (right) spectra of the as-prepared gold particles stabilized by HRP. (B) Absorption of the 10% diluted gold particles stabilized by HRP. The excitation wavelength was 360 nm.

Optimal Conditions for Preparation of LsGFC. Based on the data in Figures S1, S2 and S3, the optimal conditions for preparation of LsGFC is: 0.1 mL 10 mg/mL lysozyme and 0.1 mL 4 mM $HAuCl_4$ were added into 0.1 mL water, mixed for 5 minutes, then 10 μL 1M NaOH was added and further incubated at 37ºC overnight.

Estimation the Concentrations of the LsGFC. In Ying's work, their gold cluster stabilized by BSA consisted of 25 gold atoms with a size of ~0.8 nm (J. P. Xie, Y. G. Zheng and J. Y. Ying, *J. Am. Chem. Soc.*, 2009, **131**, 888-889). Our gold cluster was larger and had a size of ~1 nm, so it should consist of 38 gold atoms, which was consistent with previous result (P. P. H. Cheng, D. Silvester, G. Wang, G. Kalyuzhny, A. Douglas, and R. W. Murray, *J. Phys. Chem. B*, 2006, **110**, 4637-4644). Thus, the LsGFC concentration could be estimated with the following equation: [LsGFC]=[Au atoms]/38. So the concentration of the as-prepared LsGFC probes, the 10% diluted LsGFC probes, and the 1% diluted LsGFC probes were 34 μM, 3.4 μM, and 0.34 μM, respectively.

Synthesis of GFCs with other Proteins. To test whether any protein can be used to synthesize GFC, we did the following experiments. Cellular retinoic acid-binding protein II (CRABP), catalase from bovine liver, myoglobin from equine skeletal muscle or peroxidase from horseradish (HRP) were used instead of lysozyme to prepare the GFC under basic conditions. Specifically, 0.1 mL 10 mg/mL protein and 0.1 mL 4 mM $HAuCl_4$ were added into 0.1 mL water, mixed for 5 minutes, then 10 μL 1M NaOH was added and further incubated at 37ºC overnight. The as-prepared samples were used for fluorescent and absorption spectroscopic characterization. The results were shown in Figures S6, S7, S8 and S9.

Only CRABP could produce fluorescent gold clusters while other three proteins produced plasmonic gold nanoparticles. The fluorescent emission of GFC stabilized by CRABP centered at ~ 635 nm. The plasmonic peaks of catalase stabilized gold particles; myoglobin stabilized gold particles, and HRP stabilized gold particles were centered at 528 nm, 600 nm, and 520 nm, respectively. These results indicated that not all the proteins could be used to prepare fluorescent gold particles. However, much more careful studies would be needed to further elucidate the reaction mechanism of fluorescent gold particles formation by using proteins.