Supporting Information

Regulation of the Enzymatic Activities of Lysozyme by the Surface Ligands of Ultrasmall Gold Nanoclusters: The Role of Hydrophobic Interactions

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Syntheses of AuNCs

The synthesis procedure of DHLA-AuNCs was according to the literature method with minor modifications[1]. 78 mg LA was added to 200 mL aqueous solution containing 700 μL NaOH (2 M). The mixture was stirred for 20 min to completely dissolve the LA, followed by the addition of $HAuCl_4$ solution (4.8 mL, 1% by mass). The solution turned from light yellow to colorless upon stirring for another 5 min. Subsequently, an aqueous solution of $NaBH_4$ (4.8 mL, 50 mM) was added dropwise to the mixture under rapid stirring. The mixture was stirred overnight. The DHLA-AuNCs were concentrated using a 10 kDa ultrafiltration tube and dialyzed with a 3500 Da dialysis bag for 24 h in phosphate buffered saline (PBS) (0.01 M, pH 7.4) buffer. The buffer solution was renewed every 4 h.

The synthesis procedure of GSH-AuNCs was according to the literature method with minor modifications[2]. The freshly prepared aqueous solutions of $HAuCl_4$ (20 mM, 0.50 mL) and GSH (100 mM, 0.15 mL) were mixed with 4.35 mL of ultrapure water at 25 °C. The reaction mixture was heated to 75 °C under gentle stirring (500 rpm) for 12 h. An aqueous solution of strongly orange-emitting Au NCs was formed. The orange-emitting Au NCs were precipitated with 3-fold amount of 2-propanol, and then resuspended in ultrapure water and precipitated with 2-propanol three times. The purified Au NCs were dried overnight at 37 °C in vacuum, and the final product in the powder form could be dispersed in phosphate buffered saline (PBS) and further purified by dialysis against 0.01 $mol \cdot L^{-1}$ PBS buffer for 24 h, and the buffer solution was renewed every 4 h.

Characterizations of AuNCs

The concentration of Au in DHLA-AuNCs or GSH-AuNCs was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Then, according to the literature[1-3], Au_{22} may be the predominant species in the DHLA–AuNCs we synthesized, and Au_{36} was used as the average particle composition of GSH-AuNCs. Combined with these conditions, the concentrations of gold nanoclusters were measured.

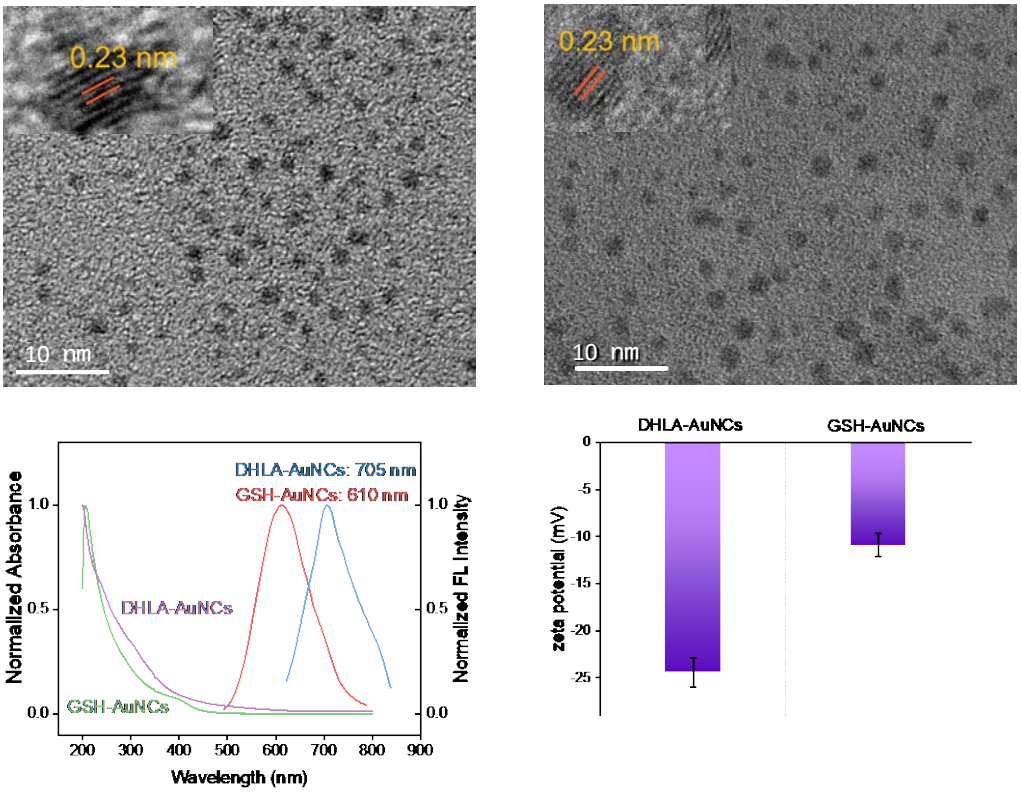

Figure S1. The TEM images of DHLA-AuNCs (a) and GSH-AuNCs (b). Scale bars in the insets of (a) and (b) were 5 nm and 10 nm, respectively. (c) The UV-vis absorption and emission spectra of DHLA-AuNCs and GSH-AuNCs, respectively. (d) The zeta potential of DHLA-AuNCs and GSH-AuNCs.

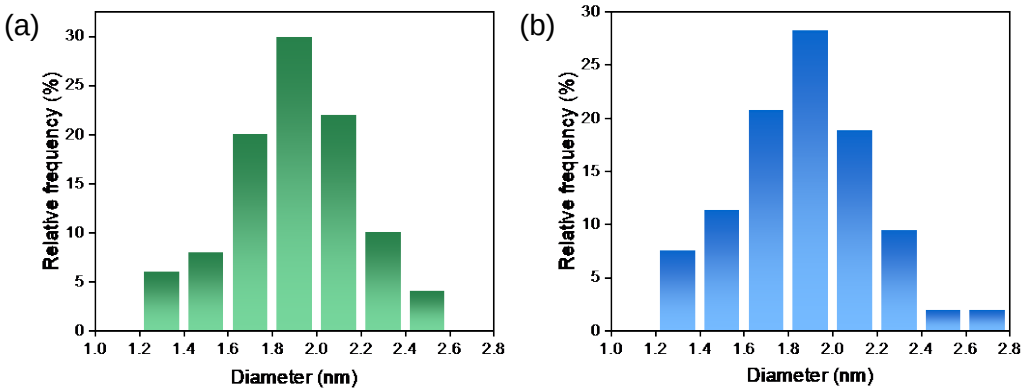

Figure S2. The corresponding size statistical results from TEM of DHLA-AuNCs (a) and GSH-AuNCs (b), respectively.

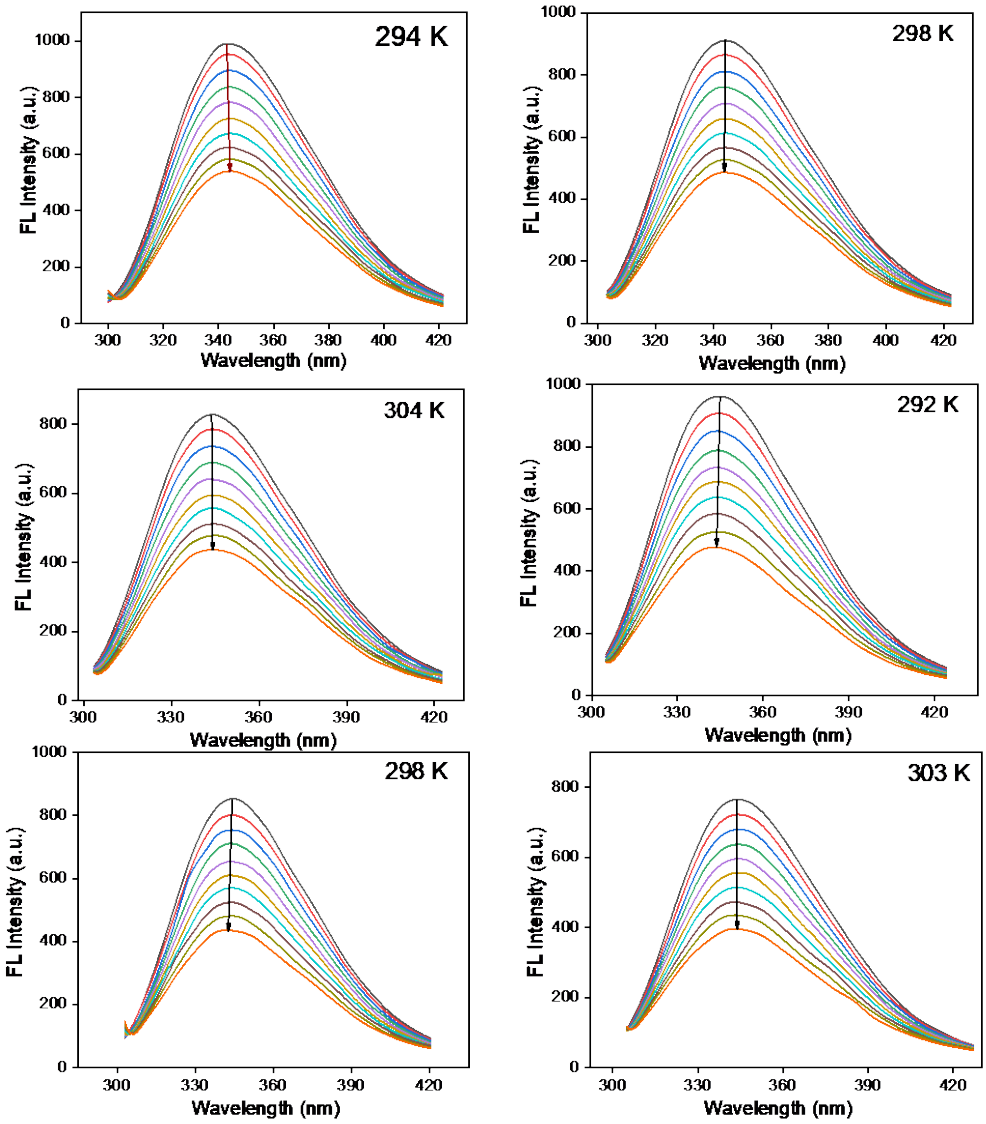

Figure S3. The fluorescence quenching spectra of Lys titrated by DHLA-AuNCs at 294K (a), 298 K (b), 304 K (c). Fluorescence quenching spectra of Lys titrated by GSH-AuNCs at 292 K (d), 298 K (e), 303 K (f).

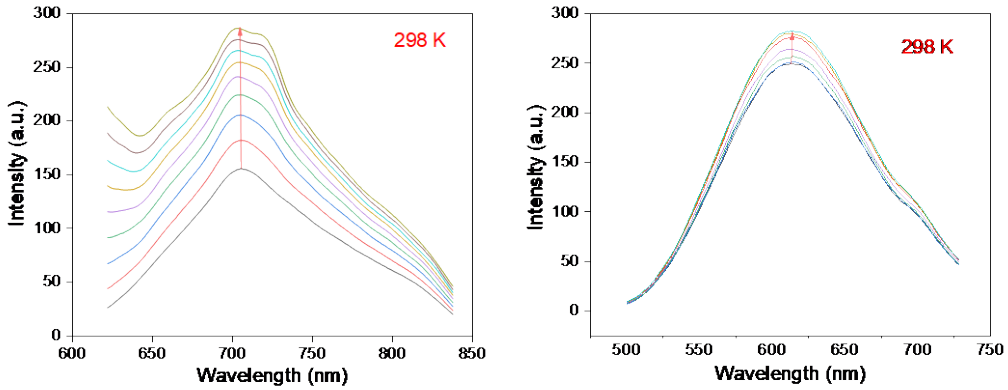

Figure S4. The fluorescence spectra of DHLA-AuNCs (a) and GSH-AuNCs (b) titrated by Lys at 298 K.

Table S1. The fluorescence lifetime of Lys incubated with DHLA-AuNCs at different molar ratios.

AuNCs: Lys	τ_1 (ns)	α_1	τ_2 (ns)	α_2	τ_{av}(ns)	χ^2
0:1	1.67	60%	3.33	40%	2.33	1.069
0.5:1	1.77	73%	3.97	27%	2.36	1.291
1:1	1.51	60%	3.55	40%	2.33	1.202
2:1	0.73	57%	3.62	43%	1.97	1.250

Table S2. The fluorescence lifetime of Lys incubated with GSH-AuNCs at different molar ratios.

AuNCs: Lys	τ_1 (ns)	α_1	τ_2 (ns)	α_2	τ_{av}(ns)	χ^2
0:1	2.04	65%	4.48	35%	2.89	1.055
0.5:1	2.06	69%	4.49	31%	2.81	0.963
1:1	2.14	75%	4.79	25%	2.80	1.031
2:1	2.12	73%	4.55	27%	2.78	1.040

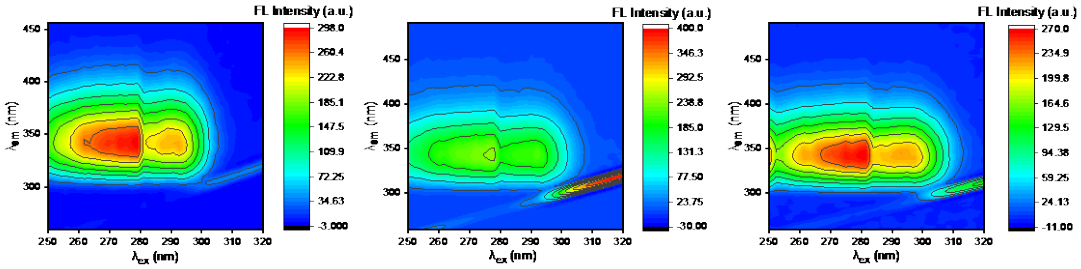

Figure S5. 3D fluorescence spectra of Lys (a) and Lys incubated with DHLA-AuNCs (b) and GSH-AuNCs (c), respectively.

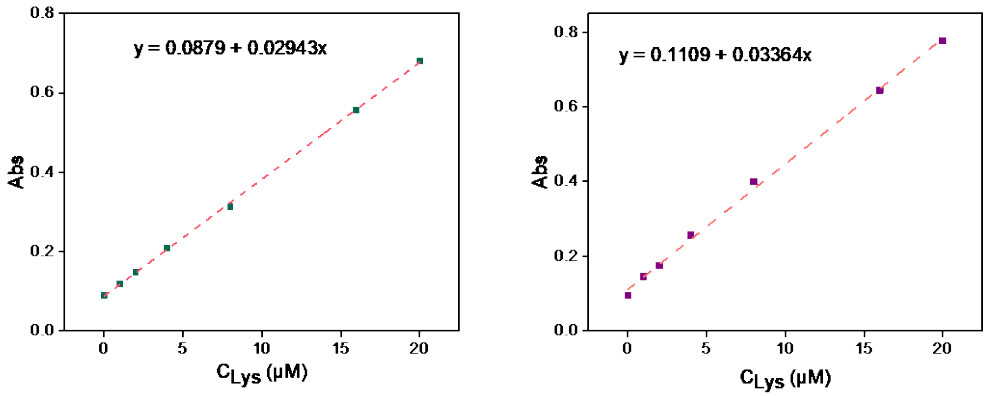

Figure S6. The BCA working curves of Lys for DHLA-AuNCs-Lys complex (a) and GSH-AuNCs-Lys complex (b), respectively.

Table S3. The concentration of DHLA-AuNCs solution when centrifugalizing at different speed.

Speed (rpm)	c_{AuNCs} (μM)
0	3.33
3000	3.29
6500	3.09
12000	3.22

Table S4. The concentration of Lys in the supernatant (c_{ex}) and the amount that bound with DHLA-AuNCs (c_{bound}) quantified by BCA. The c_{ex} at 0:1 was set as zero to better show the results.

Lys : DHLA-AuNCs	c_0 (μM)	Abs	c_{ex} (μM)	c_{bound} (μM)
0:1	0.000	0.088	0.000	0.000
0.25:1	0.625	0.090	0.047	0.578
0.5:1	1.250	0.089	0.014	1.236
0.75:1	1.875	0.089	0.022	1.853
1:1	2.500	0.092	0.141	2.359
2:1	5.000	0.166	2.649	2.351
4:1	10.000	0.310	7.533	2.467

Table S5. The concentration of Lys in the supernatant (c_{ex}) and the amount that bound with GSH-AuNCs (c_{bound}) quantified by BCA. The c_{ex} at 0:1 was set as zero to better show the results.

Lys : GSH-AuNCs	c_0 (μM)	Abs	c_{ex} (μM)	c_{bound} (μM)
0:1	0.000	0.110	0.000	0.000
0.25:1	0.625	0.119	0.263	0.362
0.75:1	1.875	0.126	0.4671	1.408
1:1	2.500	0.127	0.500	2.000
2:1	5.000	0.131	0.619	4.381
4:1	10.000	0.169	1.733	8.267
6:1	15.000	0.331	6.584	8.416

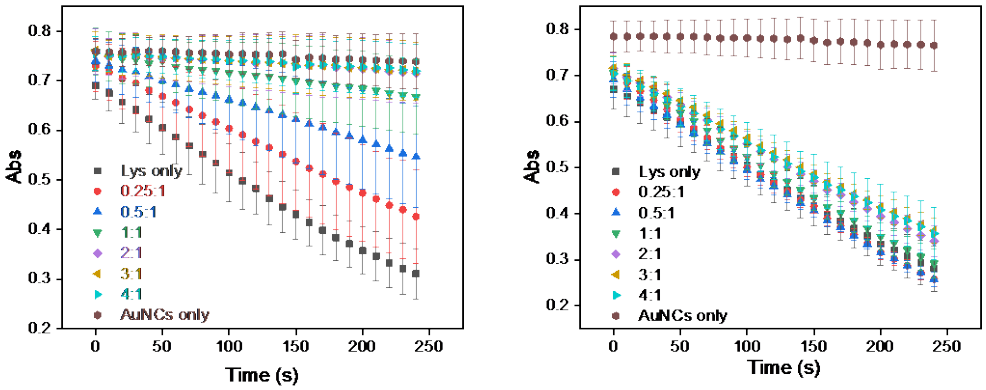

Figure S7. The absorbance changes of *Micrococcus luteus* induced by Lys incubated with different molar ratio of DHLA- AuNCs (a) and GSH-AuNCs (b), respectively.

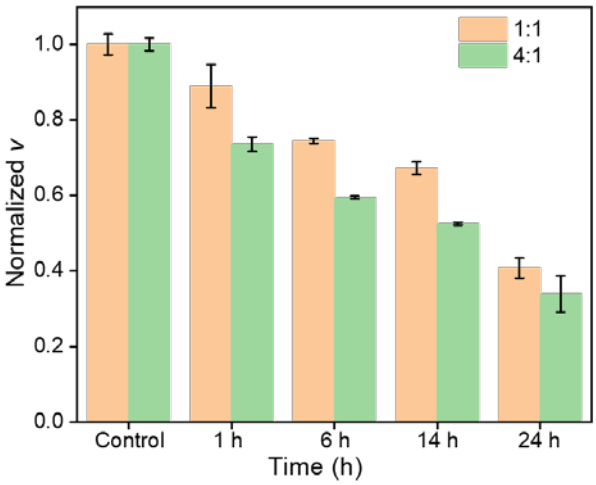

Figure S8. The normalized *v* of lysozyme when incubated with DHLA-AuNCs (molar ratio of DHLA-AuNCs : Lys = 1: 1 or 4:1) for different times.

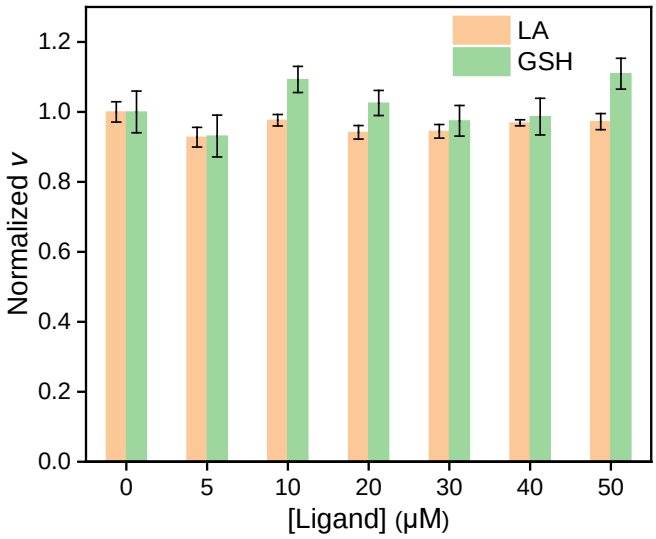

Figure S9. Normalized *v* of Lys-catalyzed reactions under different concentrations of LA and GSH.

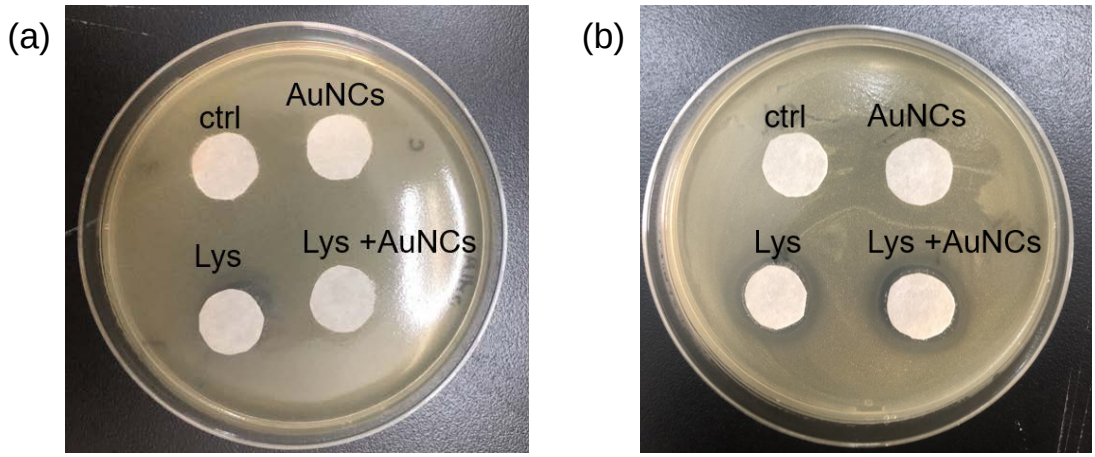

Figure S10. Anti-bacterial activity of Lys in the presence of DHLA-AuNCs (a) and Lys-GSH-AuNCs (b) against *Micrococcus luteus* determined by the filter paper disk agar diffusion method.

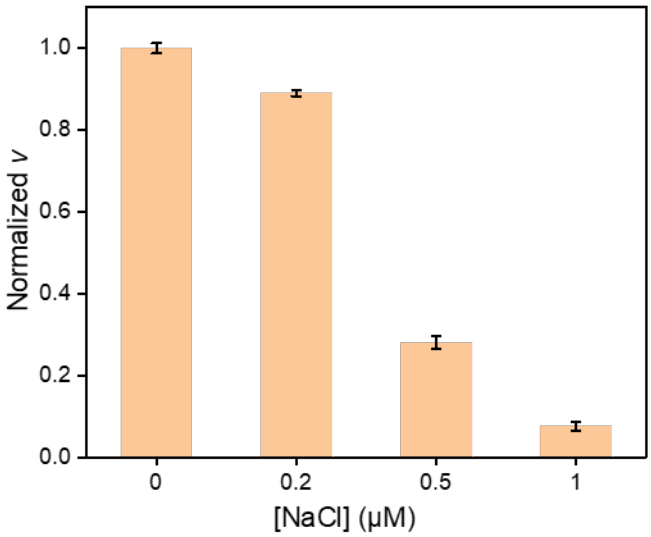

Figure S11. The normalized *v* of Lys when incubating with different concentration of NaCl.

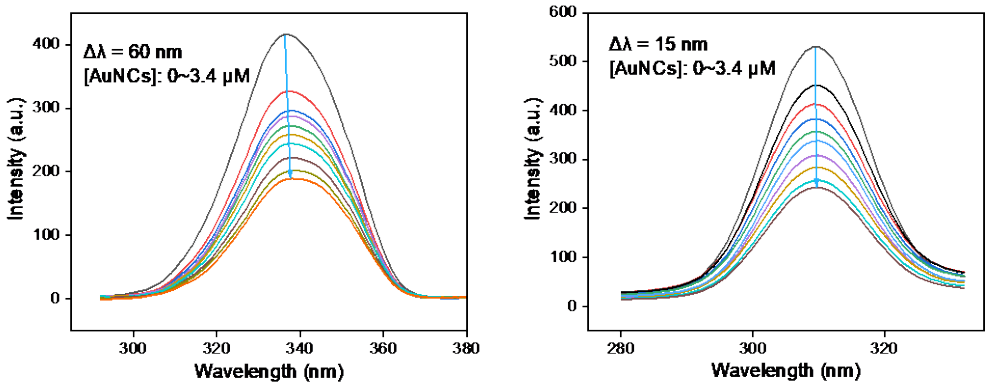

Figure S12. Synchronous fluorescence of Lys in the absence and presence of GSH-AuNCs. (a) Δλ = 60 nm. (b) Δλ = 15 nm.

Table S6. The contents of secondary structures of lysozyme when incubated with DHLA-AuNCs. *c*(Lys) = 10 μM.

c(DHLA-AuNCs) (μM)	α-helix (±3%)	β-sheet (±2%)	Turns (±1%)	Unordered (±1%)	Total
0	29.7%	19.3%	23.8%	27.2%	100.0%
1	27.4%	21.0%	22.6%	29.5%	100.5%
2	25.2%	21.6%	22.7%	28.6%	98.1%
5	18.2%	29.0%	23.5%	26.1%	96.8%
10	12.0%	53.2%	17.2%	18.2%	100.6%

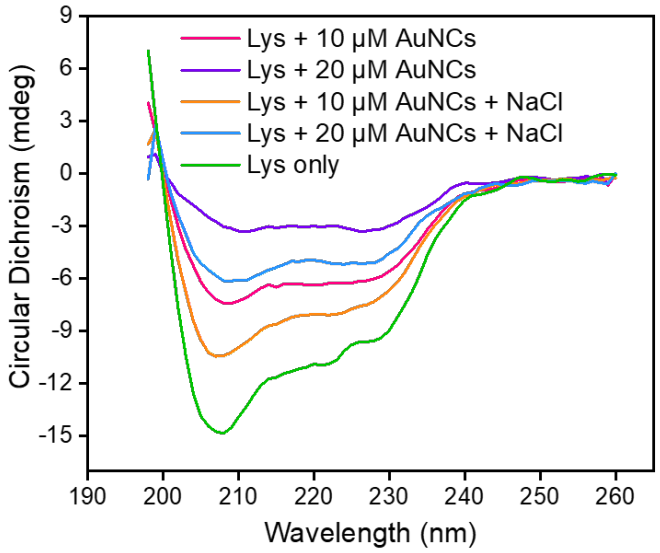

Figure S13. CD spectra of Lys incubated with DHLA-AuNCs at different molar ratios in the absence and presence of NaCl. [NaCl] = 0.2 M.

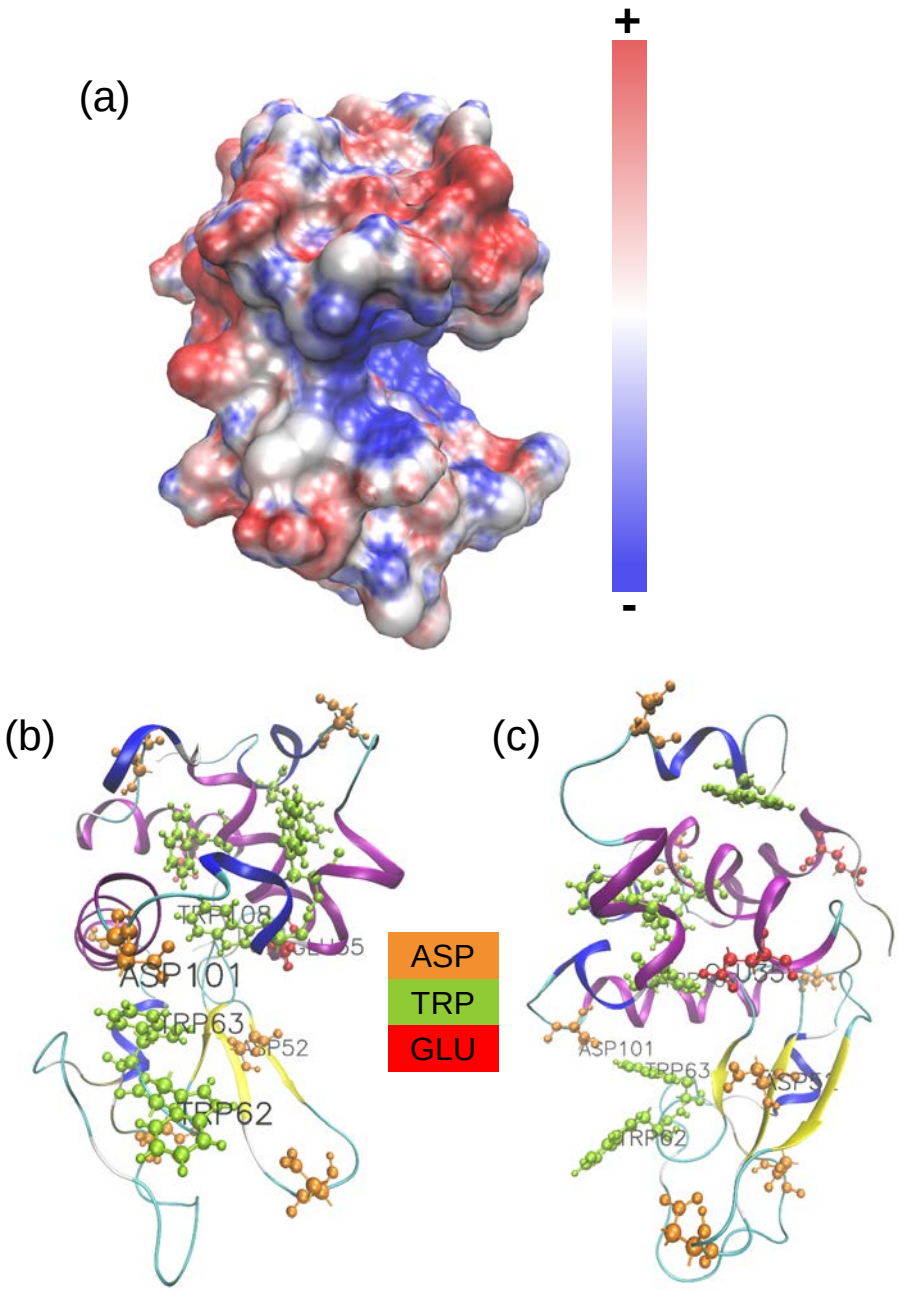

Figure S14. Surface potential diagram of Lys (a). Schematic representations of Lys at front side (b) and lateral side (c). These images were made with VMD (Visual Molecular Dynamics).

REFERENCES

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3. Yin, M. M.; Dong, P.; Chen, W. Q.; Xu, S. P.; Yang, L. Y.; Jiang, F. L.; Liu, Y. Thermodynamics and Mechanisms of the Interactions between Ultrasmall Fluorescent Gold Nanoclusters and Human Serum Albumin, gamma-Globulins, and Transferrin: A Spectroscopic Approach. *Langmuir* **2017**, *33*, 5108-5116.