Experimental Section

Materials and equipment

Hydrogen Tetrachloroaurate used for the synthesis of glutathione coated gold nanoparticles (GS-AuNPs) was purchased from Fisher Scientific (U.S.). All the other chemicals were obtained from Sigma-Aldrich and used as received without further purification. The luminescence spectra were collected by a PTI QuantaMaster^{TM} 30 Fluorescence Spectrophotometer (Birmingham, NJ). Absorption spectra were acquiredfrom a Varian 50 Bio UV-Vis spectrophotometer. Hydrodynamic diameter of the nanoparticles (NPs) in the aqueous solution was analyzed by a Brookhaven 90Plus Dynamic Light Scattering Particle Size Analyzer (DLS). Transmission electron microscopy (TEM) images were obtained with a 200 kV Jeol 2100 transmission electron microscope. Circular dichroism spectroscopy was performed using iTC200 microcalorimeter.

Synthesis of the different emissive glutathione coated gold nanoparticles (GS-AuNPs)

The 810 nm-emitting GS-AuNPs were synthesized by a thermal reduction method as described in our previous reports.[1] Briefly, 50 mL of 2.4 mM L-glutathione solution was mixed with 150 μL 1M $HAuCl_4$ under vigorous stirring (glutathione-to-$HAuCl_4$ ratio = 0.8:1). The mixture was then heated in a 95^{o}C oil bath to obtain highly luminescent gold nanoparticles. The dual-emissive GS-AuNPs were synthesized with the same thermal reduction method as described above using 50 mL of 3.6 mM glutathione solution (glutathione-to-$HAuCl_4$ ratio = 1.2:1). When the glutathione concentration further increased to 4.8 mM (50 mL, glutathione-to-$HAuCl_4$ ratio = 1.6:1), the 600 nm-emitting GS-AuNPs formed.

The synthesized GS-AuNPs were purified by centrifuging at 21, 000 g to remove the large aggregates after the reaction. The supernatant was further purified by adjusting the solution pH to 3 ~ 4, and then adding ethanol into the solution, followed by centrifuging the solution at 4000 g for 5 min to discard the supernatant. The precipitates were then resuspended in PBS buffer, and then purified again with size exclusive column.

X-ray AbsorptionSpectroscopy (XAS) Studies

XAS spectra were acquired using beamline CLS@APS Sector 20 of the Advanced Photon Source at Argonne National Laboratories, Argonne, IL. Wavelength selection was performed using a double-crystal Si(111) monochromator, with higher harmonic rejection achieved by detuning to 80% of the maximum incident X-ray intensity. Samples were analyzed in the liquid phase at room temperature using Teflon sample holders equipped with Kapton film windows. Absorption spectra were obtained using standard gas ionization detectors, and energy-calibrated using metal foil

references.

XAS data processing and fitting was performed using WinXAS.[2] Raw spectra were background-subtracted and normalized using simple polynomial functions. The EXAFS data from the resulting spectra were then isolated via spline-fitting and Fourier-transformed to obtain the FT-EXAFS spectra. Structural parameters, most notably coordination numbers (CNs) and bond lengths, were obtained by fitting the FT-EXAFS spectra using Au–N and Au–S scattering paths. Due to the fact that N and O have very similar scattering properties, it is not possible to distinguish between the two in these FT-EXAFS spectra; thus, a single Au–N path was used, encompassing contributions from both N and O atoms. For each sample, single Debye-Waller parameter (σ^2) and E0-shift (ΔE_0) values were used for both the Au–N/O and Au–S scattering path, in order to reduce the number of free-running variables in the fit. It should be noted that the EXAFS region of 3 to 12 $Å^{-1}$ was used for each sample, with FT fitting performed from 1.2 to 2.45 $Å^{-1}$.

Comparison of surface coverage of GSH on 600 nm-emitting, 810 nm-emitting and dual-emissive GS-AuNPs

Elemental analysis showed that the 600 nm-emitting GS-AuNPs were composed of 18.94% C, 2.77% H, and 5.72% N, the 810 nm-emitting GS-AuNPs were composed of 12.08% C, 1.72% H, and 3.72% N, and the dual-emissive GS-AuNPs were composed of 13.29% C, 1.29% H, and 4.57% N. These data were used to compare the ligand surface coverage of the three types of GS-AuNPs in a following way:

1. Chemical formulas of GS-AuNPs can be expressed as Au_xSG_y, wherein SG = glutathione containing a deprotonated thiol group ($C_{10}H_{16}N_3O_6S$, 306 g/mol), ***x*** = number of Au atom, ***y*** = number of glutathione. The (average glutathione-to-Au ratio of GS-AuNPs) = *y*/x.

Considering the three types of GS-AuNPs have the same size (2.5 nm) and the same surface area, the ratio between the surface coverage of glutathione on different types of GS-AuNPs can be calculated using the glutathione-to-Au ratios (*y*/x). For example, regarding the two types of single-emissive GS-AuNPs, the ratio between their surface coverage of glutathione = [glutathione-to-Au ratio of 600 nm-emitting NPs] / [glutathione-to-Au ratio of 810 nm-emitting NPs]. To compare the dual-emissive and single-emissive GS-AuNPs in surface coverage of glutathione, the ratio between their surface coverage of glutathione = [glutathione-to-Au ratio of dual-emissive NPs] / [glutathione-to-Au ratio of 810 nm-emitting NPs], or [glutathione-to-Au ratio of dual-emissive NPs] / [glutathione-to-Au ratio of 600 nm-emitting NPs].

Therefore, the key is to obtain the *x* and *y* values for each type of GS-AuNPs.

2. Calculation of *x*

For a spherical metal nanoparticle, the number of metal atom in this particle (N) can calculated using this equation:

$R = r_s \cdot N^{1/3}$, R = particle radius, r_s = Wigner-Seitz radius.

For a 2.5 nm AuNP (R = 1.25 nm; r_s = 0.145 nm for Au), x = N =640.

3. Calculation of *y* using the measured mass fraction of C

$\mathbf{Au_{640}SG_y}$, where SG = $C_{10}H_{16}N_3O_6S$ (306 g/mol), Au: 197 g/mol

For ***600 nm-emitting GS-AuNPs***, mass fraction of C was measured to be 18.94%

$[10 \times y \times 12] / [640 \times 197 + y \times 306] = 18.94\%$

$y = 385$

Elemental analysis calculated (%) for $\mathbf{Au_{640}SG_{385}}$: H 2.53, N 6.63; found: H 2.77, N 5.72.

For ***810 nm-emitting GS-AuNPs***, mass fraction of C was measured to be 12.08%

$[10 \times y \times 12] / [640 \times 197 + y \times 306] = 12.08\%$

$y = 183$

Elemental analysis calculated (%) for $\mathbf{Au_{640}SG_{183}}$: H 1.61, N 4.22; found: H 1.72, N 3.72.

For ***dual-emissive GS-AuNPs***, mass fraction of C was measured to be 13.29%

$[10 \times y \times 12] / [640 \times 197 + y \times 306] = 13.29\%$

$y = 211$

Elemental analysis calculated (%) for $\mathbf{Au_{640}SG_{211}}$: H 1.77, N 4.65; found: H 1.29, N 4.57.

4. Calculation of average glutathione-to-Au ratio

For 600 nm-emitting GS-AuNPs ($\mathbf{Au_{640}SG_{385}}$), glutathione-to-Au ratio = y/x = 385/640 = 0.60

For 810 nm-emitting GS-AuNPs ($\mathbf{Au_{640}SG_{183}}$), glutathione-to-Au ratio = y/x = 183/640 = 0.29

For dual-emissive GS-AuNPs ($\mathbf{Au_{640}SG_{211}}$), glutathione-to-Au ratio = y/x = 211/640 = 0.33

5. Comparison of ligand surface coverage of 600 nm-emitting and 800 nm-emitting GS-AuNPs

Considering the two types of single-emissive GS-AuNPs have the same size and same surface area, the ratio of their surface coverage of glutathione = [glutathione-to-Au ratio of 600 nm-emitting NPs]

/ [glutathione-to-Au ratio of 810 nm-emitting NPs] = 0.60 / 0.29 = 2.07

This number indicated the surface coverage of glutathione on the 600 nm-emitting NPs was 2-fold of that on the 810 nm-emitting GS-AuNPs.

6. Comparison of ligand surface coverage of dual-emissive and single-emissive GS-AuNPs

Regarding the dual-emissive and 600 nm-emitting GS-AuNPs, the ratio between their surface coverage of glutathione = [glutathione-to-Au ratio of dual-emissive NPs] / [glutathione-to-Au ratio of 600 nm-emitting NPs] = 0.33/0.60 = 0.55

Regarding the dual-emissive and 810 nm-emitting GS-AuNPs, the ratio between their surface coverage of glutathione = [glutathione-to-Au ratio of dual-emissive NPs] / [glutathione-to-Au ratio of 810 nm-emitting NPs] = 0.33 / 0.29 = 1.14

These results suggested the surface coverage of glutathione on the dual-emissive NPs was 55% of that on the 600 nm-emitting GS-AuNPs but 14% higher than that on the 810 nm-emitting GS-AuNPs.

Reference

[1] a) C. Zhou, G. Hao, P. Thomas, J. Liu, M. Yu, S. Sun, O. K. Oez, X. Sun, J. Zheng, *Angew. Chem. Int. Ed.***2012**, *51*, 10118-10122; b) J. Liu, M. Yu, C. Zhou, S. Yang, X. Ning, J. Zheng, *J. Am. Chem. Soc.***2013**, *135*, 4978-4981.

[2] T. Ressler, *J. Synchrotron Radiat.***1998**, *5*, 118-122.

Supplementary Data

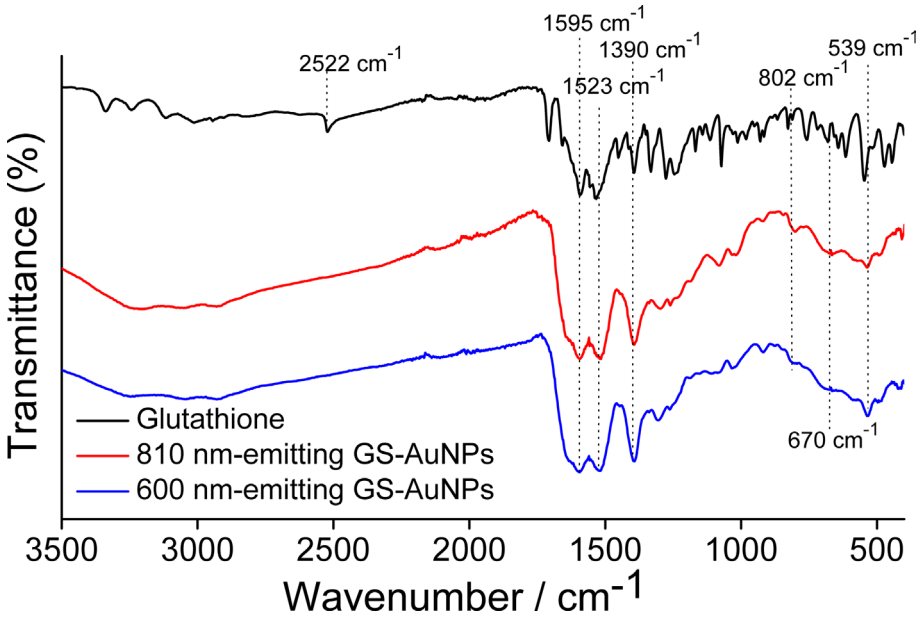

Figure S1. IR spectra of the single-emissive GS-AuNPs.

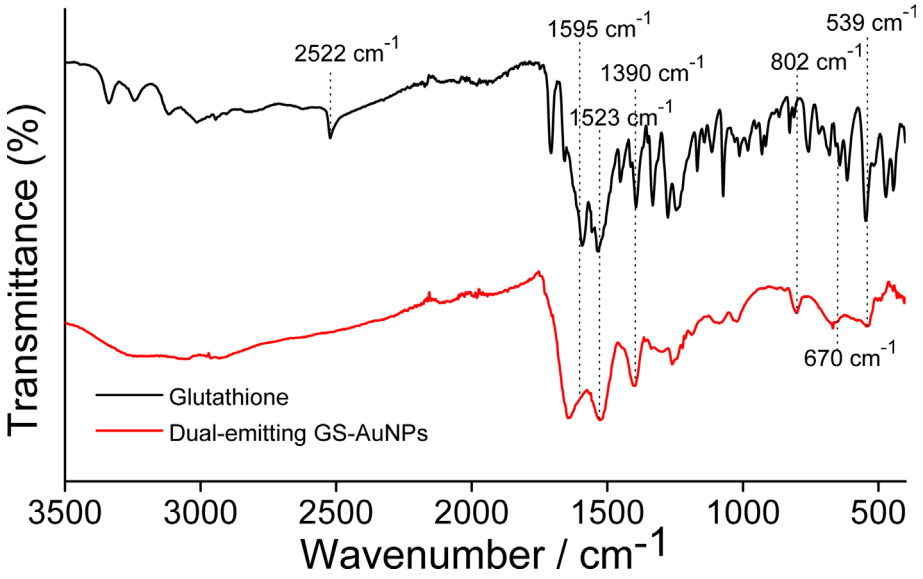

Figure S2. IR spectra of the dual-emissive GS-AuNPs.

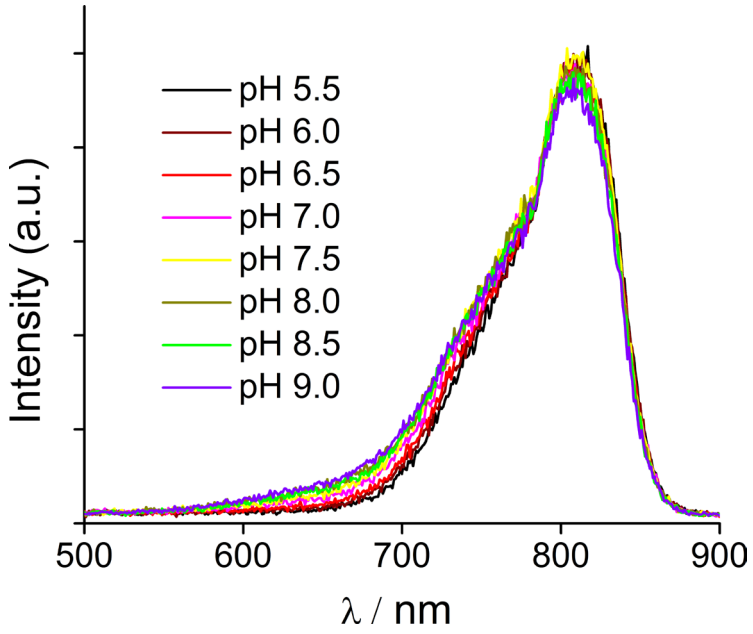

Figure S3. The luminescent spectra of 810nm-emitting GS-AuNPs at different pHs. The results showed that the luminescent intensity of 810nm-emitting GS-AuNPs would not change significantly from pH 5.5 to pH 9.0 in the solution.

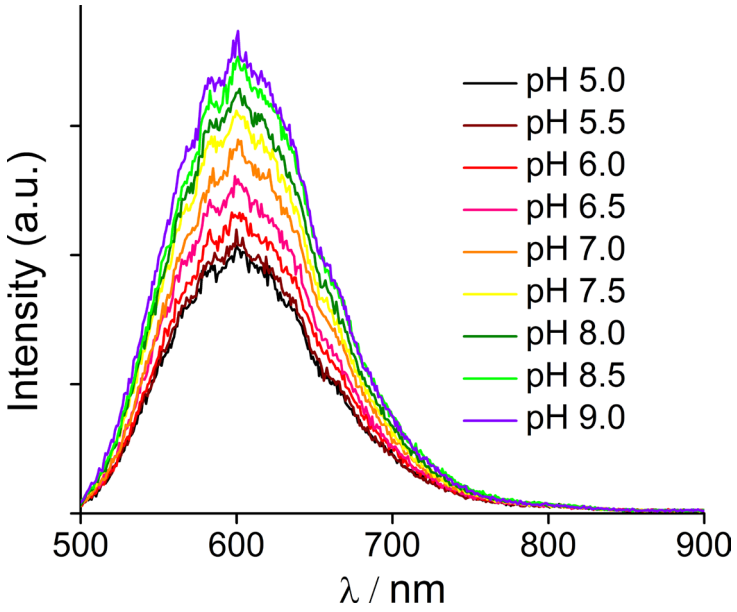

Figure S4. The pH-dependent luminescence spectra of 600 nm-emitting GS-AuNPs. The results showed that the luminescence intensity of 600nm-emitting GS-AuNPs increased with the increase of pH in the solution. The ratio of increase was 1.8 fold from pH 5.0 to pH 9.0.

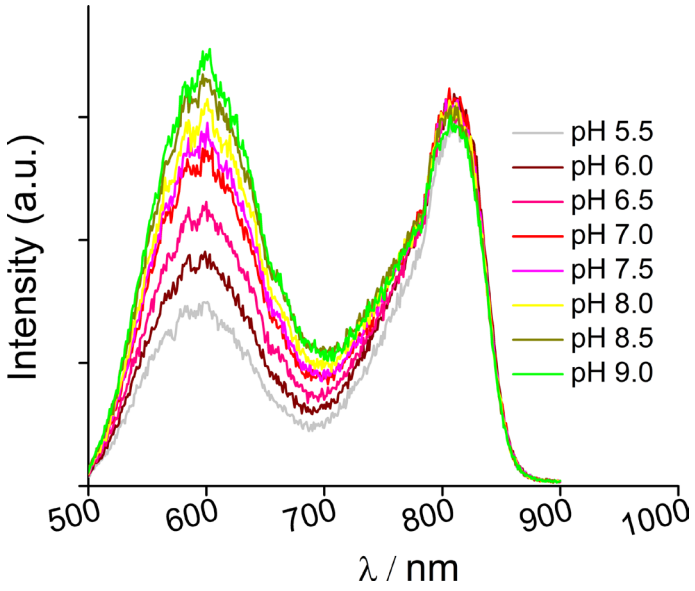

Figure S5. The pH-dependent luminescence spectra of the mixture of 600 nm- and 810-emitting GS-AuNPs.

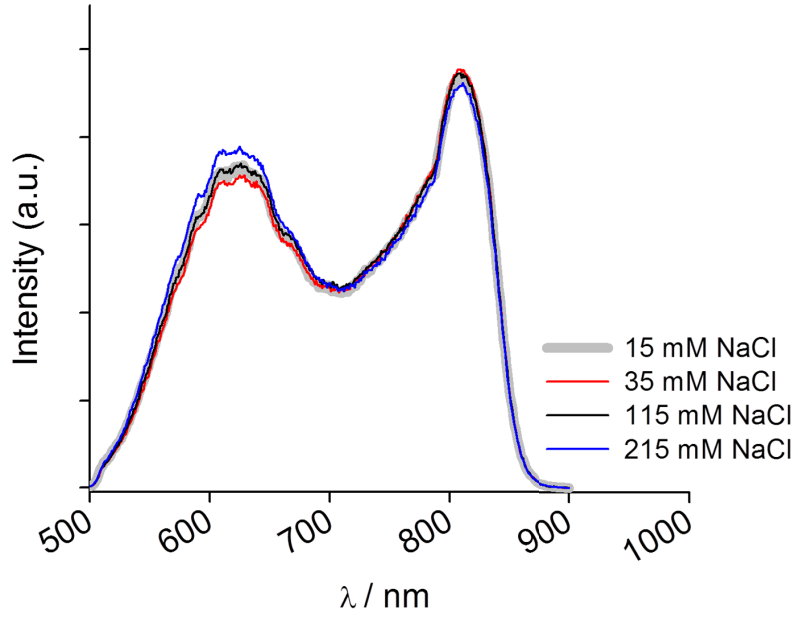

Figure S6. The luminescence spectra of dual-emissive GS-AuNPs at different concentrations of NaCl from 15, 35, 115 to 215 mM (pH 7). Aqueous solutions of 0.1, 0.5 and 1 M NaCl were prepared using deionized water. The dual-emissive GS-AuNPs were dissolved in PBS containing 15.4 mM NaCl. The same volume (50 μL) of deionized water, 0.1 M NaCl, 0.5 M NaCl, and 1 M NaCl were respectively added to 300 μL PBS solution of dual-emissive GS-AuNPs, resulting in the final NaCl concentrations of 15, 35, 115 and 215 mM. The pH value of all the samples was adjusted to pH 7 (measured by pH meter).

Table S1. Quantitative structural information of the 600 nm-and 810 nm-emitting GS-AuNPs at pH 7.0 in PBS solution from the EXAFS fitting result. CN = average coordination number.

Sample	Path	CN (atoms)	Bond Distance (Å)	Debye-Waller ($Å^2$)	E_0 Shift (eV)
600 nm-emitting GS-AuNPs	Au-S	1.8	2.32	0.002	6
	Au-N	0.5	2.09		
810 nm-emitting GS-AuNPs	Au-S	1.4	2.32	0.002	7
	Au-N	0.9	2.10		

Table S2. Average lifetimes of 600 nm and 800 nm emissions of dual-emissive GS-AuNPs at different pHs.

pH	Em = 600 nm	Em = 810 nm
9.0	166.8 ns	1.17 μs
7.5	138.7 ns	1.20 μs
5.0	64.3 ns	1.67 μs

Table S3. Quantitative structural information of the dual-emissive GS-AuNPs at different pHs from the EXAFS fitting result. CN = average coordination number.

pH	Path	CN (atoms)	Bond Distance (Å)	Debye-Waller ($Å^2$)	E_0 Shift (eV)
9.0	Au-S	**1.3**	2.32	0.001	8
	Au-N	**1.0**	2.11		
7.0	Au-S	**1.6**	2.32	0.003	7
	Au-N	**0.8**	2.10		
5.0	Au-S	**2.3**	2.31	0.003	5
	Au-N	**0.1**	2.04		

Table S4. Quantitative structural information of 600 nm-emitting GS-AuNPs at different pHs from the EXAFS fitting result. CN = average coordination number.

pH	Path	CN (atoms)	Bond Distance (Å)	Debye-Waller ($Å^2$)	E_0 Shift (eV)
9.0	Au-S	**2.0**	2.32	0.003	5
	Au-N	**0.3**	2.06		
7.0	Au-S	**1.8**	2.32	0.002	6
	Au-N	**0.5**	2.09		
5.0	Au-S	**2.1**	2.32	0.003	6
	Au-N	**0.3**	2.07		

Table S5. Quantitative structural information of 810 nm-emitting GS-AuNPs at different pHs from the EXAFS fitting result. CN = average coordination number.

pH	Path	CN (atoms)	Bond Distance (Å)	Debye-Waller ($Å^2$)	E_0 Shift (eV)
9.0	Au-S	**1.3**	2.32	0.001	7
	Au-N	**0.9**	2.09		
7.0	Au-S	**1.4**	2.32	0.002	7
	Au-N	**0.9**	2.10		
5.0	Au-S	**1.5**	2.32	0.001	8
	Au-N	**0.9**	2.11		