Molecular engineering of the $Au_{25}(SG)_{18}$ nanocluster at the single cluster level to brighten the NIR-II fluorescence

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Experimental Section

Synthesis of $Au_{25}(SG)_{18}$ nanoclusters

The synthesis of $Au_{25}(SG)_{18}$ nanoclusters followed a reported method[1]. Aqueous solutions of $HAuCl_4$ (3.75 mL, 40 mM) and glutathione (4.5 mL, 50 mM) were added to ultrapure water (141.75 mL) under vigorous stirring, followed by NaOH addition to adjust the pH of the reaction solution to 11.0. And then, the reaction vessel was saturated with CO for 2 min. The reaction mixture was allowed to proceed under gentle stirring (500 rpm) at room temperature for 24 h. The precipitate was collected using the rotary evaporator, washed with methanol several times, and vacuum-dried. 40 mg of $Au_{25}(SG)_{18}$ nanoclusters were obtained.

Synthesis of PDA-$Au_{25}(SG)_{18}$ nanoclusters

0.2 mL aqueous solution of PDA (1 mM) and 0.2 mL aqueous solution of $Au_{25}(SG)_{18}$ nanoclusters (100 μM) were added to 1.48 mL phosphate buffer (20 mM, pH = 11.0) . The mixture was allowed to react for 2 h at room temperature and then

stored in a 4 °C refrigerator for further use.

Characterizations of $Au_{25}(SG)_{18}$ nanoclusters

UV-visible (UV-vis) absorption spectrum of $Au_{25}(SG)_{18}$ clusters in aqueous solution was recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectrum of $Au_{25}(SG)_{18}$ clusters in aqueous solution was recorded on Edinburgh (FLS980, USA). The transmission electron microscopy (TEM) images were recorded in a Thermo Fisher Talos F200S G2 electron microscope (Thermo Fisher, USA).

Photostability study.

$Au_{25}(SG)_{18}$ nanocluster and PDA- $Au_{25}(SG)_{18}$ nanocluster in PBS (2 mg/ml) was exposed with a laser (808nm, 5 W/cm^2, 30 min). During these measurements, the absorptions of above solutions were monitored.

NIR-II fluorescence imaging

NIR-II fluorescence imaging system（NIROPTICS Series III 900/1700）was used for dynamic NIR-II fluorescenc imaging. 200 μL PDA-**$Au_{25}(SG)_{18}$ nanoclusters solution**（5.0 mg/mL）were injected through tail vein by using a 28-gauge syringe needle. The mouse was mounted on platform below the laser（808 nm）. The emitted light from the animal was filtered through 900 nm long-pass filters (Thorlabs) with an exposure time of 200 ms.

In vivo Toxicity

C57 mice were divided into $Au_{25}(SG)_{18}$ treated and PDA-$Au_{25}(SG)_{18}$ treated groups, with 3 mice in each group. Au25 clusters (10.0 mg/mL, 200 μL) were intravenously injected into mice. At 1, 3 and 14 days, the liver, kidney, spleen, heart

and lung were collected and fixed in 4% neutral buffered formalin for at least 48 hours. All tissues were embedded into paraffin and sectioned into slices. Pathology analysis was then performed by hematoxylin and eosin (H&E) staining. The slides were observed using a digital light microscope.

In vivo pharmacokinetics

C57 mice were divided into $Au_{25}(SG)_{18}$ treated and PDA-$Au_{25}(SG)_{18}$ treated groups, with 3 mice in each group. Au_{25} nanoclusters solution（5.0 mg/mL, 200 μL） were injected into C57 male mice through tail veins (n=3). After administration, blood of each mouse was drawn from inner canthus at different time points of 1minute, 10minutes, 0.5h, 1h, 2h, 6h, 12h, 24h and 48 h respectively for the pharmacokinetics analysis according to the amount of Au element by inductively coupled plasma mass spectrometry.

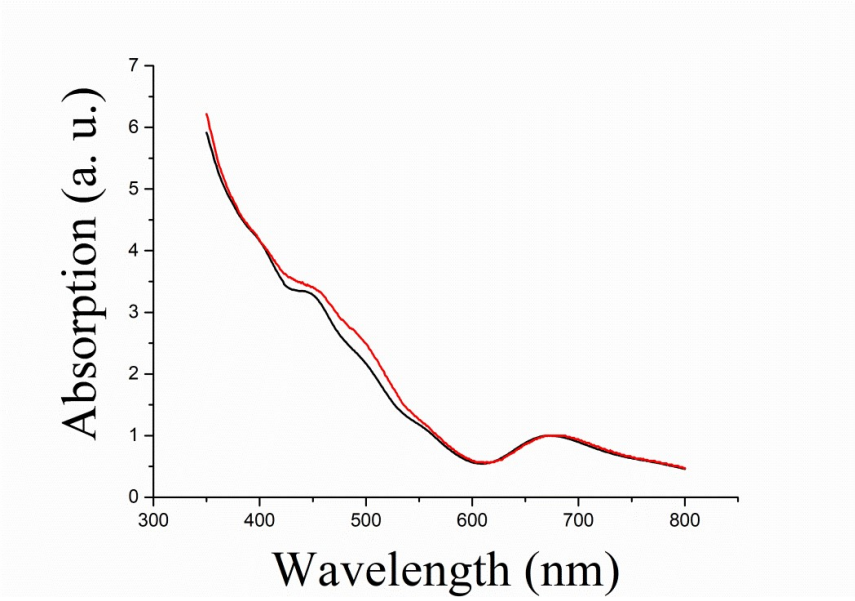

Fig. S1 UV–vis absorption spectra of $Au_{25}(SG)_{18}$ NCs (black) and PDA-$Au_{25}(SG)_{18}$ NCs (red) in H_2O.

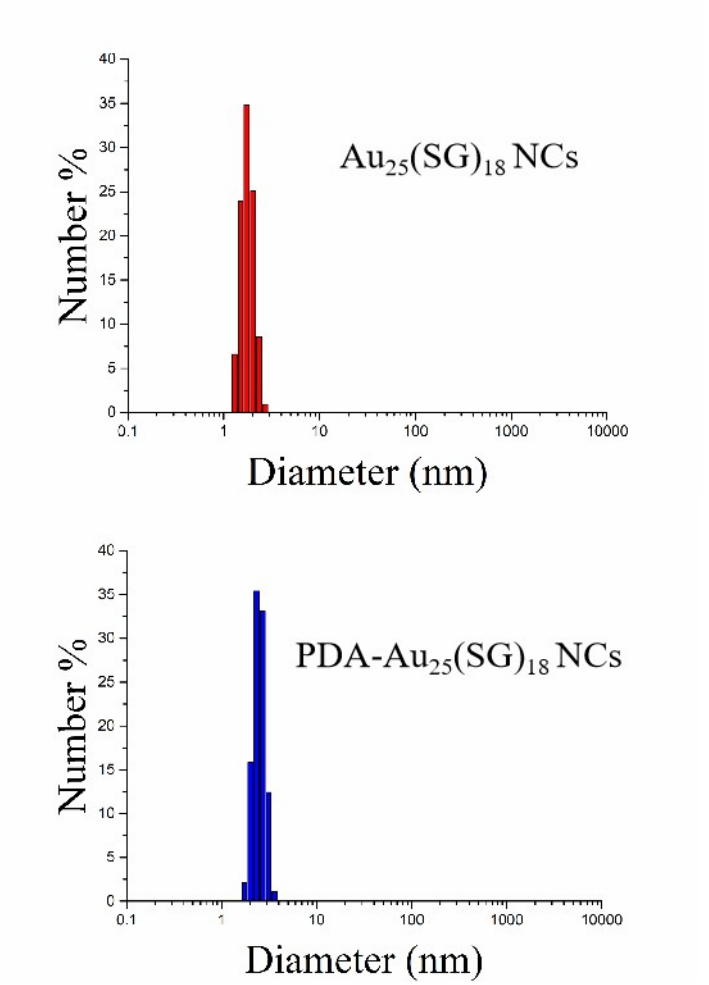

Fig. S2 DLS measurements of $Au_{25}(SG)_{18}$ NCs and PDA-$Au_{25}(SG)_{18}$ NCs.

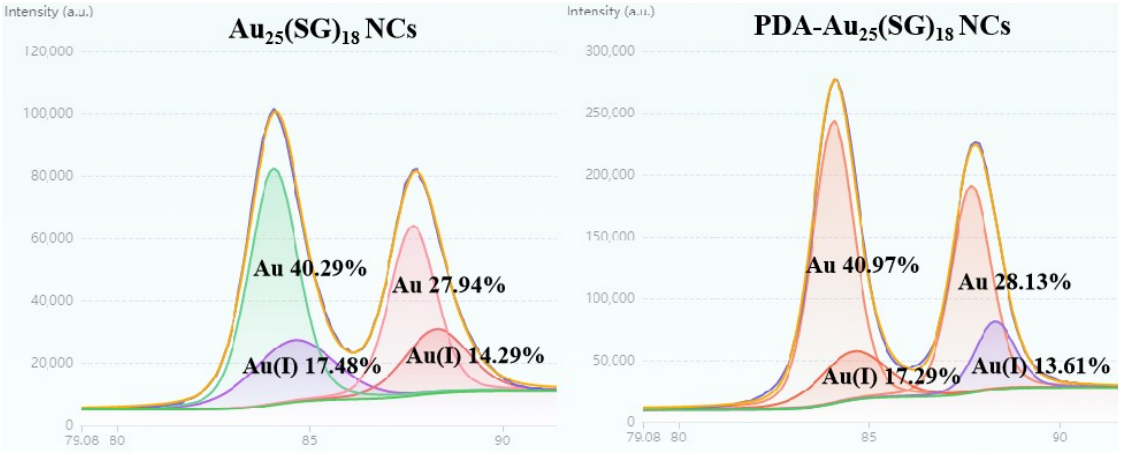

Fig. S3 The XPS fit analysis of $Au_{25}(SG)_{18}$ NCs and PDA-$Au_{25}(SG)_{18}$ NCs.

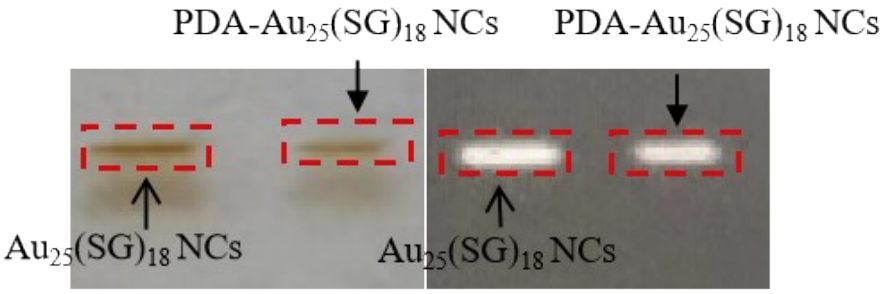

Fig. S4 Digital photos of the PAGE gels of $Au_{25}(SG)_{18}$ NCs and PDA-$Au_{25}(SG)_{18}$ NCs under visible (left) and Laser (right).

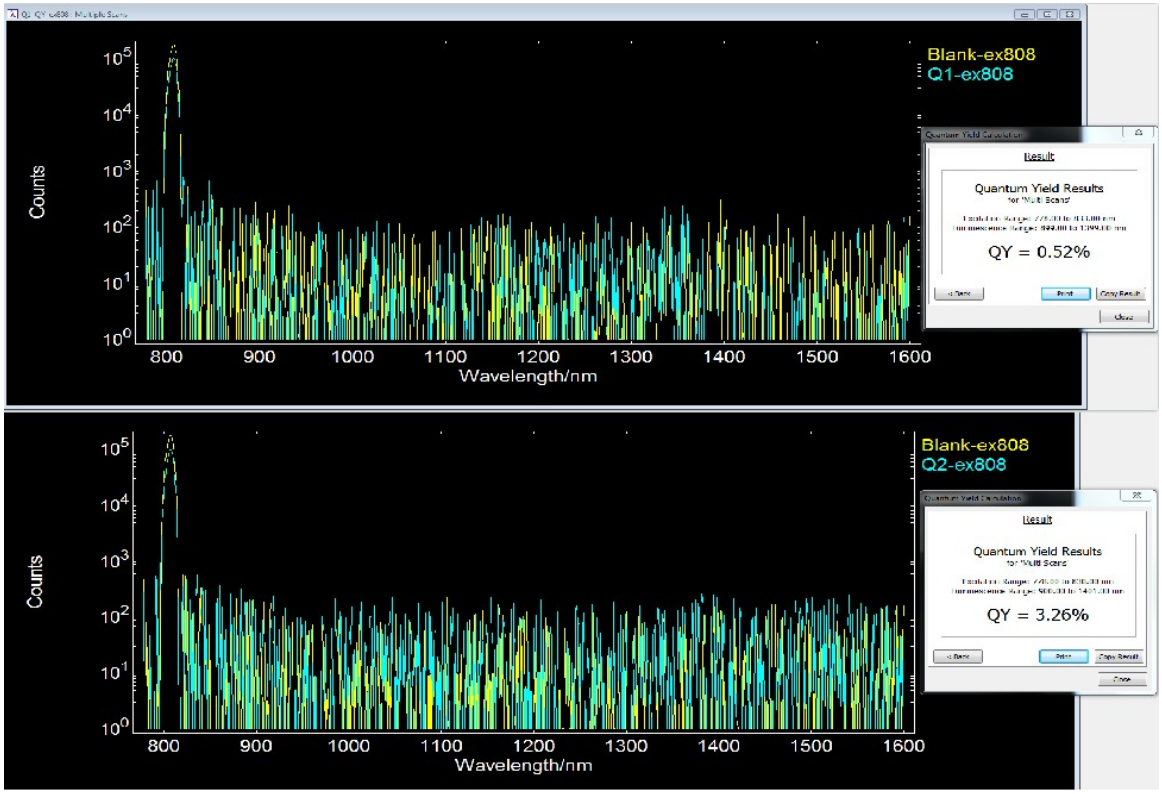

Fig. S5 Luminescence decay of $Au_{25}(SG)_{18}$ nanoclusters (a) and PDA[5]-$Au_{25}(SG)_{18}$ nanoclusters (b) in aqueous solutions at 1040 nm

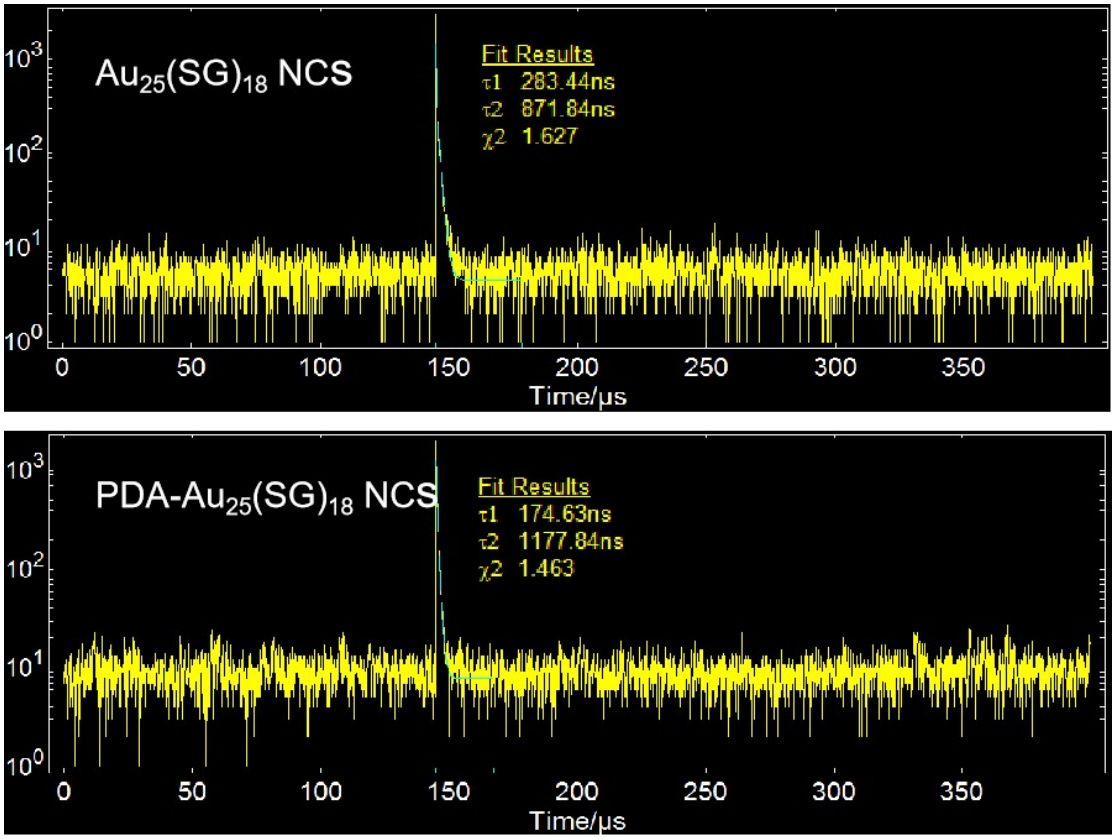

Fig. S6 Luminescence decay of $Au_{25}(SG)_{18}$ nanoclusters (a) and PDA[5]-$Au_{25}(SG)_{18}$ nanoclusters (b) in aqueous solutions at 1040 nm.

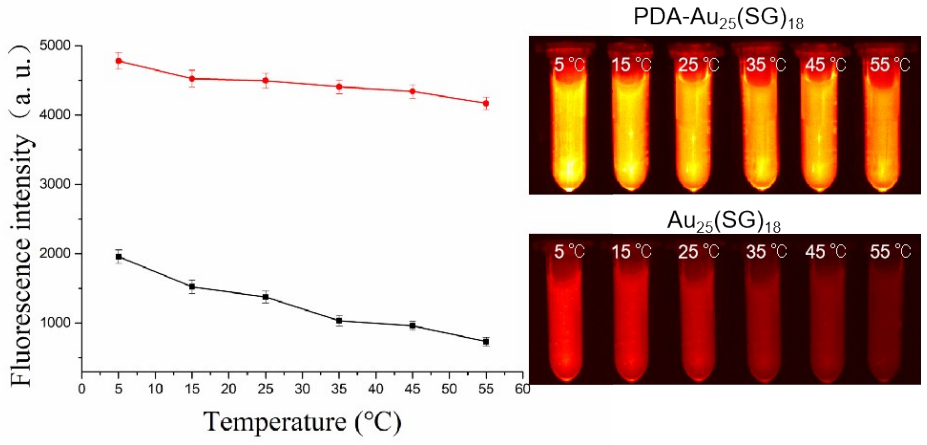

Fig. S7 Fluorescence intensity variations of $Au_{25}(SG)_{18}$ NCs (red) and PDA-$Au_{25}(SG)_{18}$ NCs (black) in aqueous solutions with temperature. Inset: the corresponding NIR-II fluorescence images in aqueous solutions at different temperature.

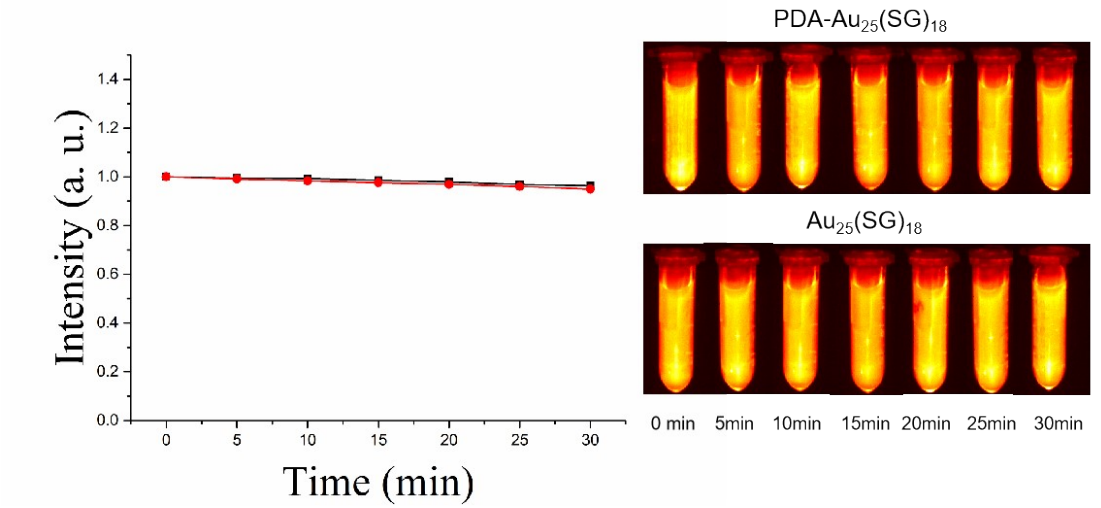

Fig. S8 Absorbance intensity change of $Au_{25}(SG)_{18}$ NCs (red) and PDA-$Au_{25}(SG)_{18}$ NCs (black) in H_2O. Inset: the corresponding NIR-II fluorescence images at different time.

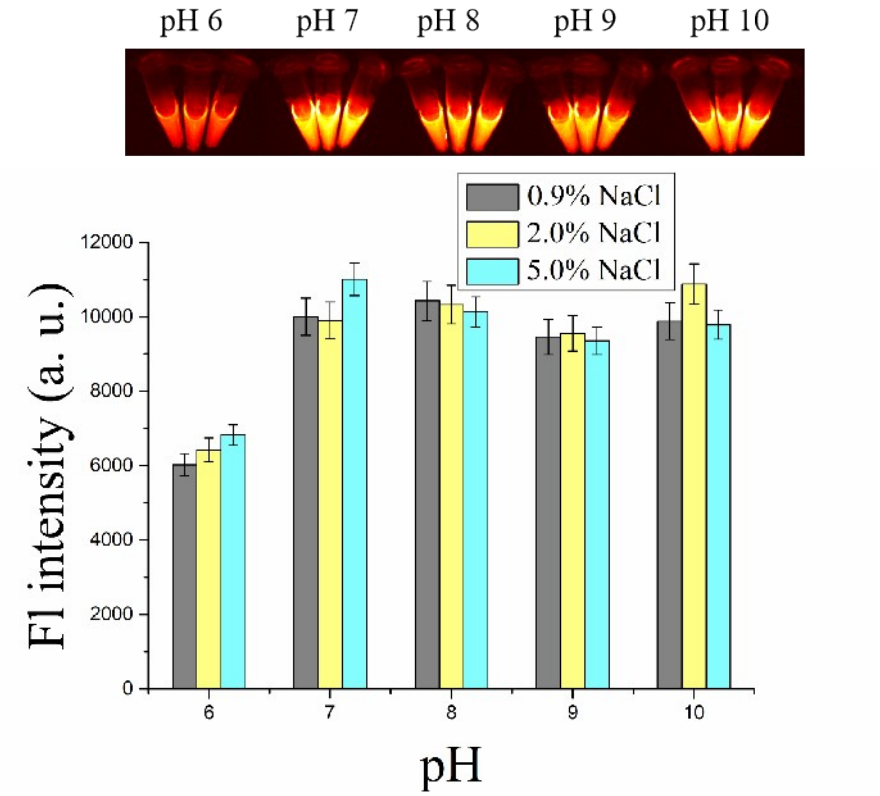

Fig. S9 The pH-dependent fluorescence intensity of PDA-$Au_{25}(SG)_{18}$ NCs in different ion strength. Inset: the corresponding NIR-II fluorescence images in different pH and ion strength, , the ion strength are 0.9%, 2.0% and 5.0% in every image from left to right.

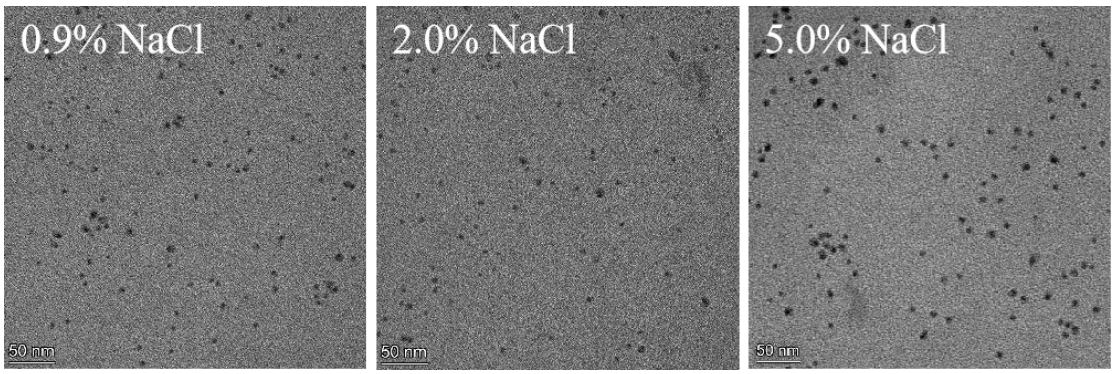

Fig. S10 TEM images of PDA-$Au_{25}(SG)_{18}$ NCs in different ion strength. . Scale bar=50 nm.

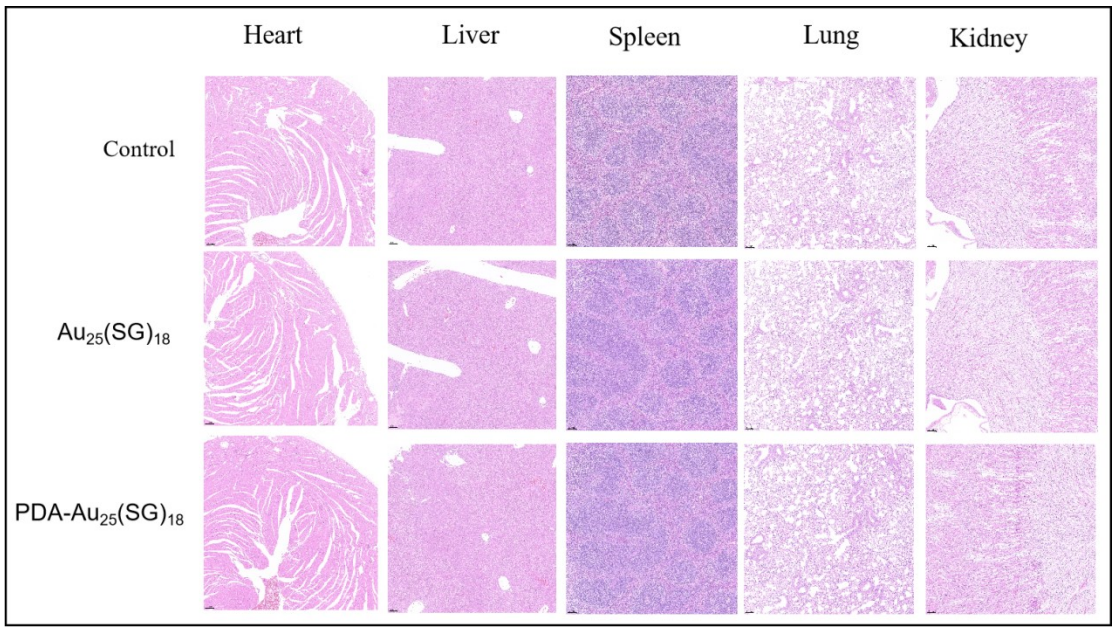

Fig. S11 Pathological data from the heart, liver, spleen, lung, and kidney of mice treated with $Au_{25}(SG)_{18}$ and PDA-$Au_{25}(SG)_{18}$ at the concentration of 1.0 mg/mL 14 days after injection. Scale bar is 0.1mm. No significant toxic responses were found.

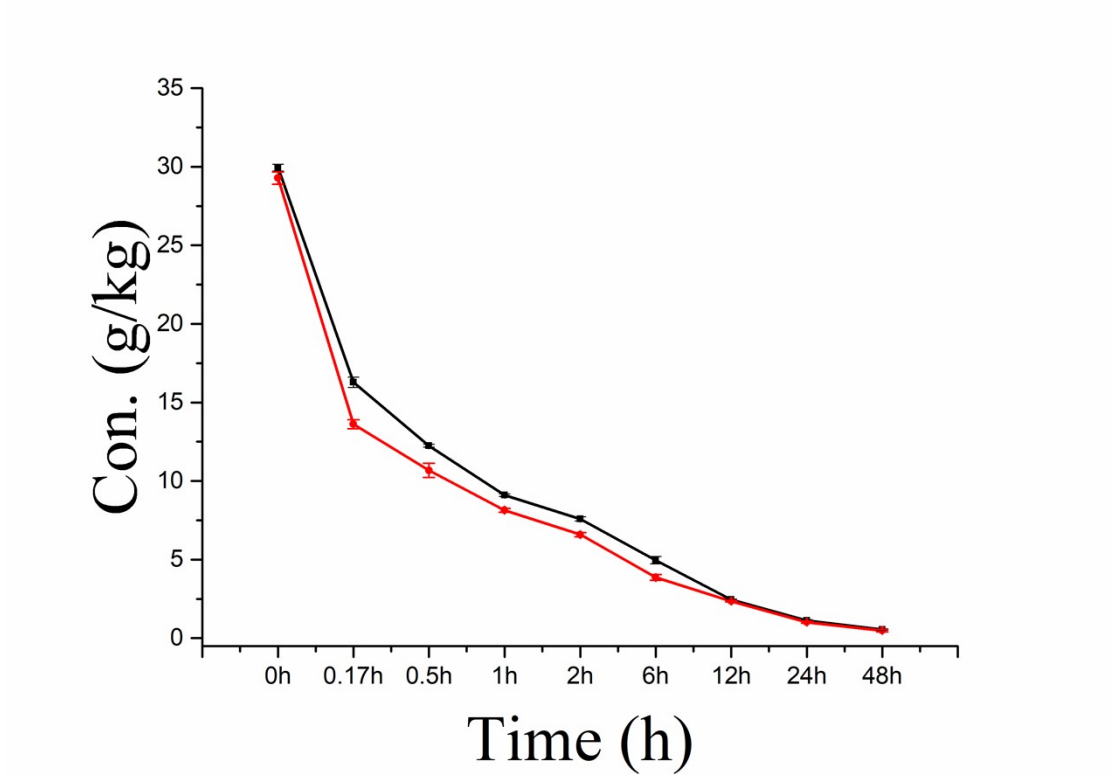

Fig. S12 Time-dependent biood concentration of gold element determined by ICP-MS.

Supplementary Table 1 EXAFS fitting parameters at the Au L3–edge of $Au_{25}(SG)_{18}$

NCs.

Path	CN^a	R(Å)^b	σ^2(Å^2)^c	ΔE_0(eV)$_d$
Au-S	1.4 ± 0.1	2.32±0.01	0.0026	9.1
Au-Au	3.1 ± 1.1	2.81±0.01	0.0147	-3.1

[a]CN, coordination number; [b]R, distance between absorber and backscatter atoms; [c]σ2, Debye-Waller factor to account for both thermal and structural disorders; [d]ΔE_0, inner potential correction.

Supplementary Table 2 Optical parameters of $Au_{25}(SG)_{18}$ NCs and PDA-$Au_{25}(SG)_{18}$ NCs obtained from FL measurements.

Sample	QY (%)	τ (μs)	K_r (s^{-1}, $\times 10^5$)	K_{nr} (s^{-1}, $\times 10^5$)
$Au_{25}(SG)_{18}$ NCs	0.52	0.87	0.06	11.4
PDA-$Au_{25}(SG)_{18}$ NCs	3.26	1.17	0.27	8.5

References

1. Liu H, Hong G, Luo Z, et al. Atomic-Precision Gold Clusters for NIR-II Imaging. *Adv Mater.* Nov 2019;31(46):e1901015.