# Surface-Regulated Injection Dose Response of

# **Ultrasmall Luminescent gold Nanoparticles**

Jiayi Zhu, Zhipeng Zhao, Huarui Chen, Xinglin Chen, and Jinbin Liu\*

Key Laboratory of Functional Molecular Engineering of Guangdong Province, School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640 (China).

#### **Experimental Procedures**

#### Materials

All reagents were commercially available and used without further purification unless specifically noted. Hydrogen Tetrachloroaurate (HAuCl<sub>4</sub>·3H<sub>2</sub>O) and Glutathione (GSH) used for synthesis of GSH coated gold nanoparticles (GS-AuNPs) were purchased from Sigma-Aldrich (St Louis, MO, USA). Other chemicals were purchased from Sigma-Aldrich and were used as received without further purification. Ultrapure water (18.2 M $\Omega$ ·cm<sup>-1</sup>) was achieved from a Pall water purification system (Cascada III. I. USA). All glassware was cleaned with fresh aqua regia (HCl/HNO<sub>3</sub> = 3:1, v/v) before use.

#### **General instruments**

The luminescence spectra were recorded on a LS 55 luminescence spectrophotometer (PerkinElmer, USA). The absorption spectra were obtained from a UV-Vis Spectrophotometer UV-2600 (Shimadzu, Japan). Transmission electron microscopy (TEM) images were obtained with a JEOL JEM 2100F TEM (Japan) with an accelerating voltage of 200 kV. Elemental analyzer was carried out on a Vario EL cube (Elementar, Germany). Gold concentrations were measured by inductively coupled plasma mass spectrometer (ICP-MS) from Thermo Fisher Scientific iCAP RQ (Germany). The *in-vivo* imaging was

performed using a UVP ChemStudio PLUS 815 imaging system (Analytikjena, USA). The *in-vivo* X-ray imaging was conducted with an *In-Vivo* Xtreme FX Pro (Bruker, Germany).

#### Surface Coverage of GSH on GS-AuNPs

The calculation of surface coverage was conducted according to our previously reported methods.<sup>1, 2</sup> Chemical formulas of GS-AuNPs can be expressed as  $Au_xGS_y$ , here GS = glutathione containing a deprotonated thiol group (C<sub>10</sub>H<sub>16</sub>N<sub>3</sub>O<sub>6</sub>S, 306 g/mol), x = number of Au atom, y = number of GS. Considering the three types of GS-AuNPs have the similar size of 2.2 nm, and the similar surface area, the surface coverage of GS on the different types of GS-AuNPs can be calculated with the GS-to-Au ratio of GS-AuNPs = y/x. All calculated parameters from element analyses were listed in Table S1.

#### Calculation of x

For a spherical metal nanoparticle, the number of metal atom in this particle *x* can be calculated by this equation:  $R = r_s \cdot N^{1/3}$ , R = particle radius,  $r_s =$  Wigner-Seitz radius.

For a 2.2 nm AuNP (R = 1.1 nm;  $r_s = 0.145$  nm for Au),  $x = N = 436.59 \approx 437$ .

Calculation of y using the measured mass fraction of C

 $Au_{437}GS_{y}$ , where  $GS = C_{10}H_{16}N_{3}O_{6}S$  (306 g/mol), Au: 197 g/mol

For H-AuNPs, the average mass fraction of C was measured to be 16.67%

 $[10 \times y \times 12] / [437 \times 197 + y \times 306] = 16.67\%$ 

 $y = 208.02 \approx 208$ 

The surface coverage of GS on AuNPs, GS-to-Au ratio = y/x = 208/437 = 0.476 = 47.6%

Elemental analysis calculated (%) for Au<sub>437</sub>GS<sub>208</sub>: H 2.22, N 5.83, S 4.45; found: H 3.74, N 5.77, S 6.25.

For L-AuNPs, the average mass fraction of C was measured to be 10.46%

 $[10 \times y \times 12] / [437 \times 197 + y \times 306] = 10.46\%$ 

 $y = 102.34 \approx 103$ 

The surface coverage of GS on AuNPs, GS-to-Au ratio = y/x = 103/437 = 0.236 = 23.6%

Elemental analysis calculated (%) for Au<sub>437</sub>GS<sub>103</sub>: H 1.39, N 3.65, S 2.78; found: H 2.95, N 3.51, S 5.79.

#### Animal and tumor xenograft model

The BALB/c mice (female, 6 weeks old, ~25 g) and BALB/c nude mice (female, 4-6 weeks old, ~25 g), were obtained from the Guangdong medical laboratory animal center. All experiments involving animals were conducted with approval from the Ethics Committee of Laboratory Animal of Institute of Animal Health, Guangdong Academy of Agricultural Sciences.

Tumor implantation: The human breast cancer cell line MDA-MB-231 (purchased from ATCC company) was cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin at 37 °C in humidified atmosphere containing 5% CO<sub>2</sub>. The cell suspension (in DMEM with 10% (v/v) FBS) was then mixed with matrigel (2:1 v/v) and injected subcutaneously upper near the mammary fat pad area of the nude mouse with a volume of 100 µL dense suspension (containing about 1×10<sup>6</sup> cells) for each mouse. The tumor was allowed to grow about 10 days and reach 6-8 mm size before study.

#### Statistical analysis

The data were presented as means  $\pm$  standard deviations (SD). Statistical analyses were performed using a Student's t-test. NS, no significance, \*p < 0.05, \*\*p <0.01 and \*\*\*p <0.001.

## **Supplementary Data**



**Fig. S1** Characterization of the synthesized AuNPs. The absorption and emission spectra (a-c), and TEM images (d-f) of AuNPs.



Fig. S2 Core-size (a-c) and hydrodynamic diameter (d-f) of the AuNPs.



**Fig. S3** Analysis of serum protein binding of AuNPs using agarose gel electrophoresis at different concentrations (mg/mL: 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0, gold atom determined by ICP-MS). The solution of AuNPs was incubated in DPBS solution supplemented with 10% ( $\nu/\nu$ ) FBS for 30 min under 37 °C. (a) The images were obtained under 365 nm excitation (upper panel) and stained with Coomassie brilliant blue 250 to locate FBS (lower panel). (b) Gold amount in each band was measured by ICP-MS.



**Fig. S4** Agarose gel electrophoresis analysis of mice plasma binding of ultrasmall AuNPs at different concentrations (gold atom, 2.0 to 0.4 mg/mL).



**Fig. S5** Time-dependent X-ray density curves derived from bladder (a) and liver (b) obtained from noninvasive X-ray images of mice after injection of H-AuNPs, L-AuNPs, and B-AuNPs within 60 min, respectively.



**Fig. S6** Excretion efficiency of H-AuNPs, L-AuNPs, and B-AuNPs at a single high dose (N = 3 for each group) within 7 days post-injection.



**Fig. S7** Biodistribution of H-AuNPs, L-AuNPs, and B-AuNPs at a single high dose (N = 3 for each group) in major organs at 1 h, 48 h and 7 d after intravenous injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



**Fig. S8** Noninvasive fluorescence imaging of the dynamic distribution of L-AuNPs with different injection doses. (a) Fluorescence images of the mice after intravenous injection with L-AuNPs at different doses (10.5 and 50.8 mg/kg, N = 3 for each group) within 2 h post injection. Time-dependent fluorescence intensities from the bladder and liver areas, and the fluorescence ratios of bladder/liver for L-AuNPs at injection dose of 10.5 mg/kg (b) and 50.8 mg/kg (c).



Fig. S9 Excretion efficiency of L-AuNPs at different injection doses (0.5, 2.1, 10.5, and 50.8 mg/kg, N = 3 for each group) within 7 days post-injection.



Fig. S10 Excretion efficiency of L-AuNPs at different injection doses (0.5, 2.1, 10.5, and 50.8 mg/kg, N = 3 for each group) within 7 days post-injection.



Fig. S11 Biodistribution of L-AuNPs at doses of 0.5, 2.1, 10.5, and 50.8 mg/kg (N = 3 for each group) in kidney and liver at 1, 48 h, and 7 d after intravenous injection.



**Fig. S12** Biodistribution of L-AuNPs at doses of 0.5, 2.1, 10.5, and 50.8 mg/kg (N = 3 for each group) in major organs at 1, 48 h, and 7 d after intravenous injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



Fig. S13 Excretion efficiency of H-AuNPs and B-AuNPs at single low doses (0.5 mg/kg, N = 3 for each group) within 7 days post-injection.



**Fig. S14** Biodistribution of H-AuNPs and B-AuNPs at single low doses (0.5 mg/kg, N = 3 for each group) in major organs at 1, 48 h and 7 d after intravenous injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



**Fig. S15** The total retention of different AuNPs at different injection doses in major organs (*e.g.*, liver, spleen, lung, and kidney) at 7 days post-injection.



**Fig. S16** a) Luminescent images the L-AuNPs in solution (Left: PBS; Right: L-AuNPs). b) Ex-vivo images of the liver and kidney taken from the mouse 30 min p.i. after i.v. injection of PBS and L-AuNPs (10.2 mg/kg) (Excitation: 455-495 nm, Emission: 800 nm longer).



**Fig. S17** Representative TEM images and the core size distributions of the L-AuNPs before injection (a) and excreted from the mice into urine (b). The L-AuNPs from the urine collected within 30 min post-injection were dropped on a TEM grid and dried before observation.



**Fig. S18** H&E staining images of kidney and liver after injection of H-AuNPs (50.8 mg/kg), L-AuNPs (50.8 mg/kg), and B-AuNPs (26.4 mg/kg) at 12 h p.i.. Scale bar is 100 mm.



Fig. S19 Blood pharmacokinetics of H-AuNPs at different doses (0.5 and 50.8 mg/kg, N = 3 for each group) within 72 h post-injection.



Fig. S20 Blood pharmacokinetics of L-AuNPs at different doses (0.5, 2.1, 10.5, and 50.8 mg/kg, N = 3 for each group) at within 72 h post-injection.



Fig. S21 Blood pharmacokinetics of B-AuNPs at different doses (0.5 and 26.4 mg/kg, N = 3 for each group) within 72 h post-injection.



**Fig. S22** Biodistribution analysis of H-AuNPs with a single-dose (2.1, 10.5, and 50.8 mg/kg, N = 3 for each group) in mice bearing MDA-MB-231 tumors at 24 h post-injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



**Fig. S23** Biodistribution analysis of L-AuNPs with a single-dose (2.1, 10.5, and 50.8 mg/kg, N = 3 for each group) injection in mice bearing MDA-MB-231 tumors at 24 h post-injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



**Fig. S24** Biodistribution analysis of B-AuNPs with a single-dose (0.5 and 26.4 mg/kg, N = 3 for each group) in mice bearing MDA-MB-231 tumors at 24 h post-injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



**Fig. S25** Biodistribution analysis of H-AuNPs with repeated doses (2.1 and 10.5 mg/kg, N = 3 for each group) every 3 h, for five doses in mice bearing MDA-MB-231 tumors at 24 h post-injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).



**Fig. S26** Biodistribution analysis of L-AuNPs with repeated doses (2.1 and 10.5 mg/kg, N = 3 for each group) every 3 h, for five doses in mice bearing MDA-MB-231 tumors at 24 h post-injection (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Sk: skin, Mu: muscle, Bl: blood, St: stomach, In: intestine, Br: brain).

Materials	Mass mg	С %	Н %	N %	S %
	3.846	16.46	3.706	5.69	6.145
H-AuNPs	4.154	16.7	3.725	5.8	6.247
	4.041	16.84	3.775	5.83	6.345
	3.839	10.55	3.007	3.52	5.822
L-AuNPs	4.146	10.4	2.9	3.5	5.801
	3.664	10.43	2.952	3.51	5.754

**Table S1.** The chemical formulas parameters of GS-AuNPs obtained from the element analyses.

Table S2. Summary of the excretion efficiency of AuNPs at different injection doses.

Materials	Injection does mg/kg	3 h	3 h	48 h	48 h	7 d	7 d
		Urine (%ID)	Feces (%ID)	Urine (%ID)	Feces (%ID)	Urine (%ID)	Feces (%ID)
	50.8	59.40	0.06	70.14	2.32	73.83	2.97
L-AuNPs	10.5	25.01	6.04	33.82	19.83	39.53	23.00
	2.1	20.71	2.15	28.94	22.54	32.76	25.41
	0.5	19.31	0.88	26.06	19.88	29.40	26.35
	50.8	68.32	1.58	73.77	5.28	78.42	6.62
n-Aumrs	0.5	28.93	0.36	41.22	19.68	46.87	22.09
B-AuNPs	26.4	0.54	0.36	5.18	5.24	9.03	15.87
	0.5	0.73	0.04	9.20	10.40	18.52	20.93

Materials	Injection does mg/kg	Heart (%ID/g)	Liver (%ID/g)	Spleen (%ID/g)	Lung (%ID/g)	Kidney (%ID/g)
L-AuNPs	50.8	3.76	9.99	6.60	5.95	17.31
	10.5	4.85	13.18	10.60	5.86	12.15
	2.1	5.96	15.96	11.49	7.17	11.53
	0.5	7.78	33.66	14.10	9.53	25.36
H-AuNPs	50.8	1.09	0.79	0.55	1.70	9.11
	0.5	2.47	1.77	1.61	3.32	13.34
B-AuNPs	26.4	7.41	9.71	7.77	7.97	16.47
	0.5	4.55	16.20	15.57	6.12	13.17

**Table S3.** Summary of the biodistribution of AuNPs at different injection doses at 1 h post-injection.

**Table S4.** Summary of the biodistribution of AuNPs at different injection doses at 48 h post-injection.

Materials	Injection does mg/kg	Heart (%ID/g)	Liver (%ID/g)	Spleen (%ID/g)	Lung (%ID/g)	Kidney (%ID/g)
L-AuNPs	50.8	1.07	8.05	7.54	1.55	9.40
	10.5	1.44	9.99	9.79	6.07	9.94
	2.1	2.34	20.11	16.84	3.44	12.77
	0.5	1.79	24.78	9.87	2.27	28.54
H-AuNPs	50.8	0.98	2.39	2.49	2.13	13.65
	0.5	2.23	7.10	6.83	3.92	25.06
B-AuNPs	26.4	5.93	30.91	52.22	5.41	35.68
	0.5	1.17	27.79	25.11	5.13	10.37

Materials	Injection does mg/kg	Heart (%ID/g)	Liver (%ID/g)	Spleen (%ID/g)	Lung (%ID/g)	Kidney (%ID/g)
	50.8	1.38	8.21	10.06	1.89	5.69
L-AuNPs	10.5	1.72	12.28	16.91	1.88	6.91
	2.1	1.94	20.47	13.76	2.73	7.23
	0.5	1.61	24.32	9.87	3.90	10.44
H-AuNPs	50.8	0.59	1.99	5.29	1.87	8.27
	0.5	1.16	3.73	11.67	3.28	13.99
B-AuNPs	26.4	4.41	39.60	35.26	4.41	21.71
	0.5	3.44	34.60	28.27	3.48	18.12

**Table S5.** Summary of the biodistribution of AuNPs at different injection doses at 7 d post-injection.

**Table S6.** Pharmacokinetics parameters of AuNPs at different injection doses.

Materials	Injection does mg/kg	Distribution half-life $(t_{1/2}\alpha, \min)$	Elimination half-life $(t_{1/2\beta}, h)$	Area under the curve ( <i>AUC</i> , %ID h/g)	Plasma clearance ( <i>CL</i> , mL/h)
	50.8	12.60	2.42	76.79	1.296
L-AuNPs	10.5	18.06	2.45	107.18	1.005
	2.1	7.56	4.10	193.60	0.487
	0.5	5.76	4.32	329.76	0.342
H-AuNPs	50.8	15.78	4.93	76.69	0.651
	0.5	2.82	3.24	105.08	0.380
B-AuNPs	26.4	358.80	5.58	183.96	0.511
	0.5	38.40	0.73	65.49	1.548

### References:

- 1 L. Gong, Y. Chen, K. He and J. Liu, ACS Nano, 2019, 13, 1893-1899.
- J. Zhu, K. He, Z. Dai, L. Gong, T. Zhou, H. Liang and J. Liu, *Anal. Chem.*, 2019, 91, 8237-8243.