Angewandte
Eine Zeitschrift der Gesellschaft Deutscher Chemiker
Chemie

Supporting Information

Tailoring Renal Clearance and Tumor Targeting of Ultrasmall Metal Nanoparticles with Particle Density

*Shaoheng Tang, Chuanqi Peng, Jing Xu, Bujie Du, Qingxiao Wang, Rodrigo D. Vinluan III, Mengxiao Yu, Moon J. Kim, and Jie Zheng**

anie_201609043_sm_miscellaneous_information.pdf

Materials and Equipment

All chemicals were purchased from Fisher Scientific and were used as received without further purification. Transmission electron microscopy (TEM) images were obtained using a JEOL 2100 transmission electron microscope with a 200 kV accelerating voltage. Hydrodynamic diameters (HDs) of the samples in the aqueous solution were analyzed using a Brookhaven 90Plus Dynamic Light Scattering (DLS) Particle Size Analyzer. Zeta potentials of the samples were analyzed by a Brookhaven ZetaPALS zeta potential analyzer. pH was measured by a Accumet AB15 pH meter and a Accuphast microprobe electrode. Absorption spectra were taken using a Varian 50 Bio UV-Vis spectrophotometer Luminescence spectra were collected by a PTI QuantaMasterTM 30 Fluorescence Spectrophotometer (Birmingham, NJ, USA). In vivo NIR fluorescence imaging was performed using a Carestream Molecular imaging system In-Vivo FX PRO (Billerica, MA, USA).

GS-AgNPs synthesis.

200 μL of 1 M $AgNO_3$ solution was added to 40 mL of 16 mM glutathione solution in a 40 ml three-necked flask. The mixture was then heated at 95 ^{o}C oil bath for 37 h. The NPs were precipitated out of the supernatant by adding 1 M NaOH and ethanol, followed by centrifuging at 4,000 g for 5 min. The precipitates were suspended in PBS buffer.

ICP-MS analysis

Each tissue sample was completely digested by 2 mL of aqua regia at room temperature overnight. Subsequently, the solution was diluted to 10 mL using 0.5% HCl and 2% HNO_3. Samples were passed through a 0.22-mm filter to remove any undigested debris prior, and then subjected to ICP-MS measurement. The analysis of Au content was performed on ICP-MS (Agilent 7900).

Experimental Animals

The animal studies were performed in compliance with guidelines set by the University of Texas System Institutional Animal Care and Use Committee. Male balb/c mice of 6-8 weeks old weighing 20~25 g were used for the biodistribution and SPECT imaging studies. Nude mice (Purchased from Charles River Inc., Strain code 01B74) of 8 weeks old weighing 20~25 g were used for the in vivo fluorescence imaging studies. The animals were housed in groups of three under standard environmental conditions (23±1 ºC, 50±5% humidity and a 12/12 h light/dark cycle) and maintained with free access to water and a standard laboratory diet.

Biodistribution and Pharmacokinetics Studies

Female BALB/C mice were injected with 1.4 mg of the four kinds of NPs to evaluate the tissue distributions. Mice were sacrificed at 7 min and 48 h post injection (p.i.). The organs of interest (brain, tail, intestine, membrane, stomach, skin, muscle, bone, tumor, lung, heart, kidney, spleen and liver) were harvested, weighed and quantified using ICP-MS. Standards were prepared and counted along with the tissue samples to calculate the percentage-injected dose per gram (%ID/g). To determine the pharmacokinetic parameters, mice injected with the four kinds of NPs and blood samples were drew at 2 min, 5 min, 10 min, 30min, 1 h, 3 h, 5h, 8h, 12h, 24 h, and 48 h p.i. and quantified using ICP-MS. The pharmacokinetic parameters were calculated based on a two-compartment open model.

In Vivo Fluorescence Imaging

Female BALB/C mice were first anesthetized with isofluorane (3%) and oxygen. The pre-injection fluorescence image (Excitation: 710/50 nm, emission: 830/50 nm, integration time: 30 seconds) was obtained right before the IV injection of GS-AuNPs and GS-AgNPs into the mice. The mice were then intravenously injected 200 μL GS-AuNPs and GS-AgNPs and placed on the imaging bed of the Carestream Molecular In Vivo Imaging FX PRO imaging system (Woodbridge, CT). During the imaging, the inhalational anesthesia (isoflurane) was received by the mouse through a nose cone attached to the imaging bed. The fluorescence images were then acquired every 30 seconds with the exactly same imaging setting. All the post injection images were acquired at the same parameter settings and are scaled to the same maximum values. Imaging bed temperature was kept at 37°C at all time.

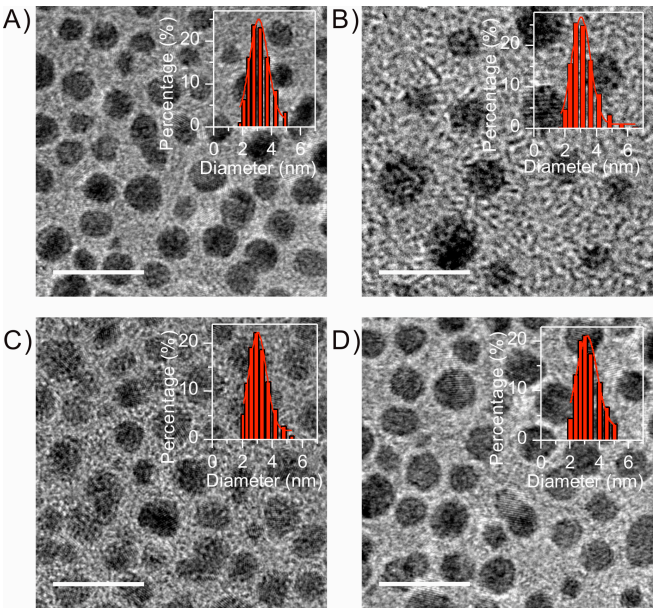

Figure S1. Typical transmission electron microscopy images and hydrodynamic diameter distributions of the four kinds of GS-NPs (a) GS-AuNPs (b) GS-AgNPs (c) GS-Au/Ag(1)NPs (d)GS-Au/Ag(2)NPs. scale bar=5 nm

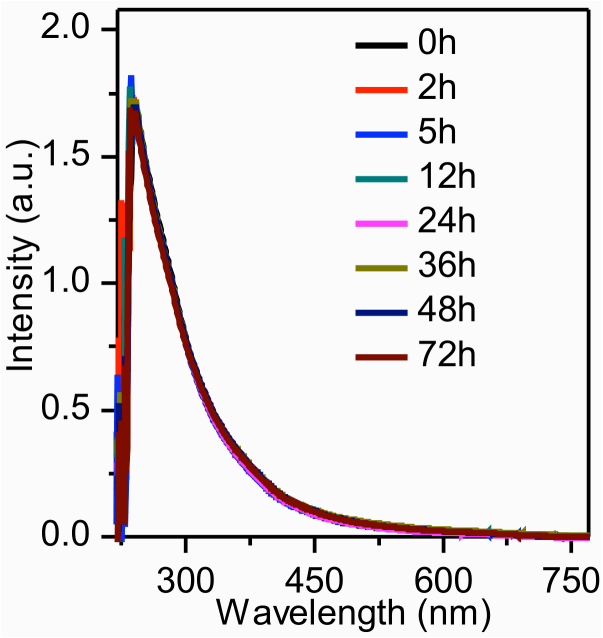

Figure S2. UV-vis absorption spectra of GS-AgNPs after incubation in the PBS (phosphate buffer saline) containing 10% fetal bovine serum (FBS) at 37 ℃ for 0, 2, 5, 12, 24, 36, 48 and 72 h.

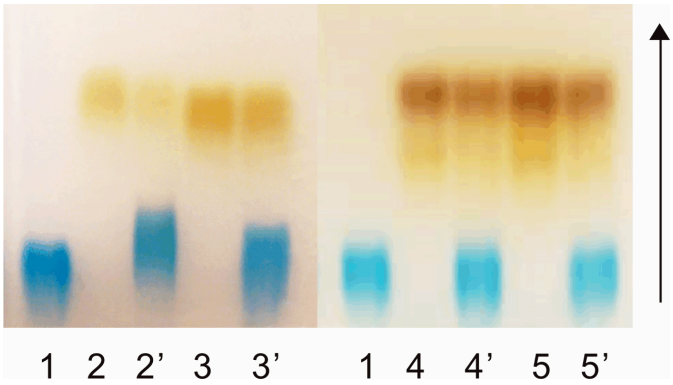

Figure S3. Agarose gel electrophoresis of the four kinds of NPs with or without incubation with fetal bovine serum (FBS). GS-AuNPs (well 2 and 2’), GS-AgNPs (well 3 and 3’), GS-Au/Ag(1)NPs (4 and 4’) and GS-Au/Ag(2)NPs (5 and 5’) were incubated in the absence (well 2, 3, 4, 5) or presence (well 2’, 3’, 4’, 5’) of 10% (v/v) CBB250-stained FBS at 37 ^{o}C for 30 min. Blue band in wells 1= CBB250-stained FBS proteins.

Table S1. Pharmacokinetic parameters of GS-AgNPs, GS-Au/Ag(1)NPs, GS-Au/Ag(2)NPs and GS-AuNPs

Parameter (unit)	GS-AgNPs	GS-Au/Ag(1)NPs	GS-Au/Ag(2)NPs	GS-AuNPs
$t_{1/2\alpha}$ (min)	1.55±0.44	2.41±0.21	3.49±0.88	5.12±1.08
$t_{1/2\beta}$ (h)	22.24±2.63	21.36±3.31	20.29±3.55	16.49±1.67
AUC (mg mL^{-1}×h)	0.621	1.82	2.46	2.59
CL (mL h^{-1})	2.25	0.77	0.57	0.54
V_d (mL)	8.97±0.07	4.18±0.04	3.68±0.1	2.98±0.08

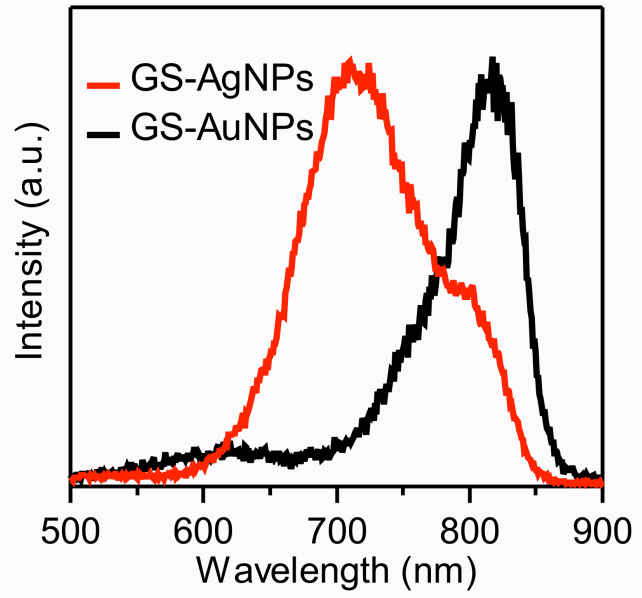

Figure S4. Fluorescence emission spectra of GS-AgNPs and GS-AuNPs

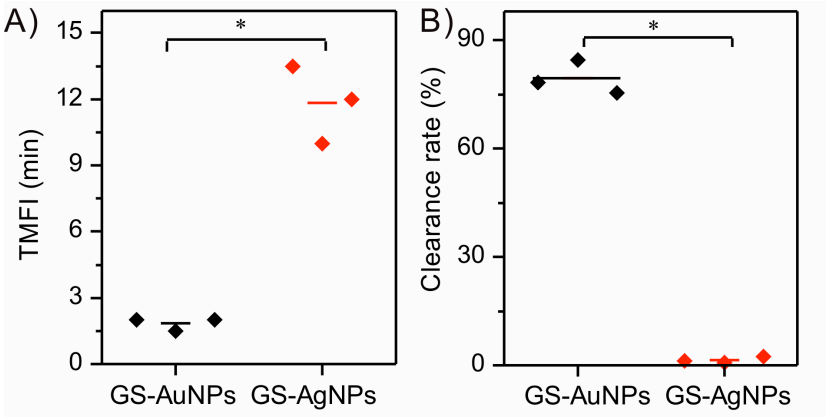

Figure S5. Statistical analysis of the two parameters extracted from the kidney TFICs of two group of mice (n=3) injected with GS-AuNPs and GS-AgNPs, repectively. The parameters include A) time to reach the maximum fluorescence intensity (TMFI) and B) clearance rate (defined as the clearance percentage at 60 min=[(peak value-intensity at 60 min p.i.) / peak value] x 100%).*p<0.005.

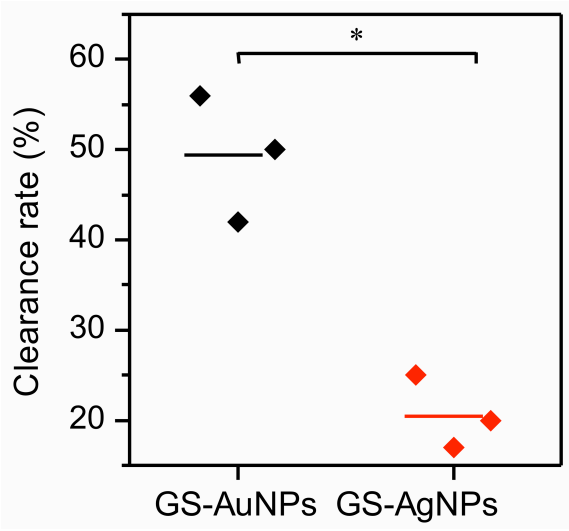

Figure S6. Statistical analysis of clearance rate of GS-AuNPs and GS-AgNPs from background of two group of mice (n=3). (defined as the clearance percentage at 60 min=[(peak value-intensity at 60 min p.i.) / peak value] x 100%).*p<0.005.

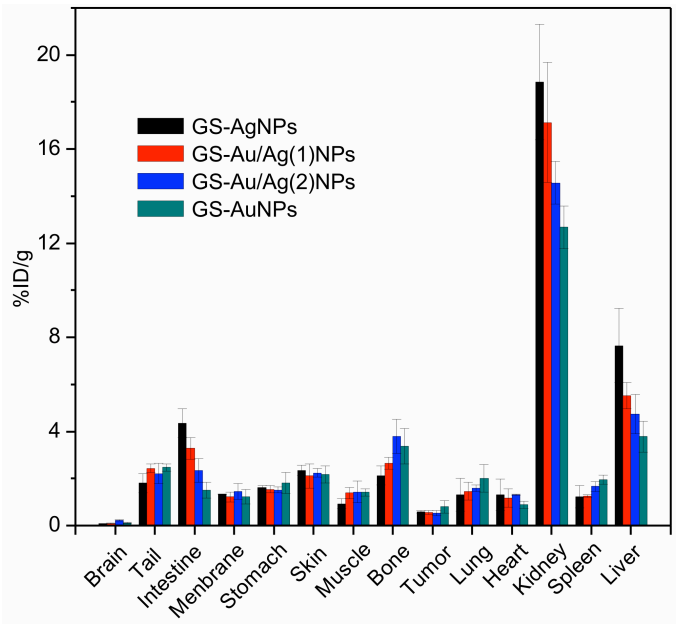

Figure S7. Biodistribution of four kinds of GS-NPs in various organs at 7 min p.i..

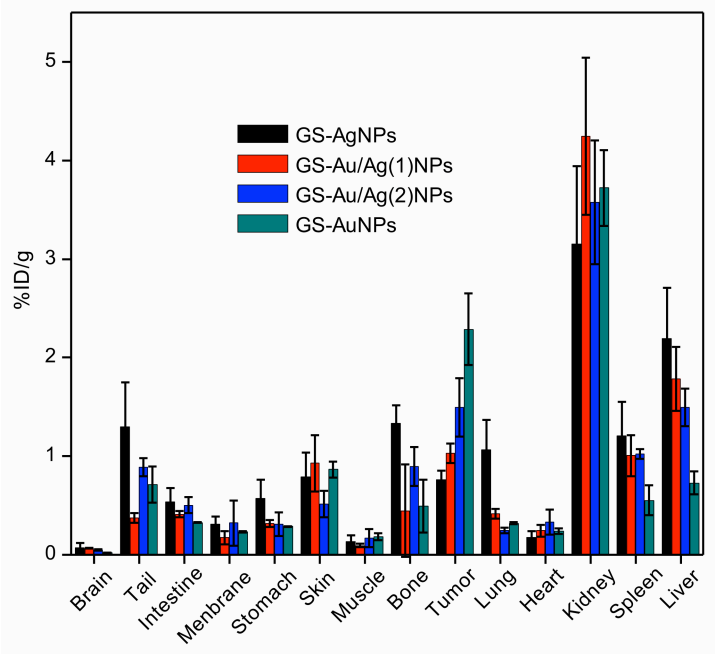

Figure S8. Biodistribution of four kinds of GS-NPs in various organs at 48 h p.i.