# Supporting Information

# Cancer Photothermal Therapy with ICG-conjugated Gold Nanoclusters

Xingya Jiang, Bujie Du, Yingyu Huang, Mengxiao Yu and Jie Zheng*

Department of Chemistry and Biochemistry, The University of Texas at Dallas, 800 West Campbell Road, Richardson, Texas 75080, USA

*Corresponding email: jiezheng@utdallas.edu

## Contents

Supporting figures

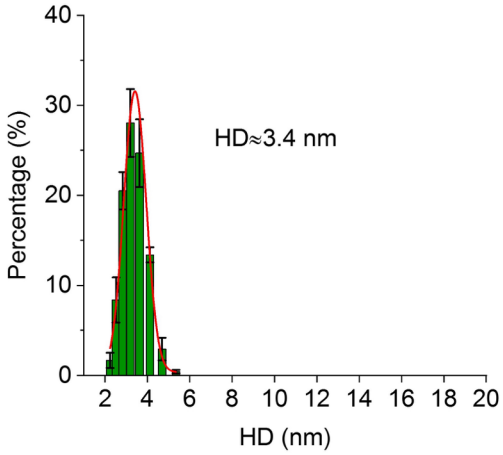


**Figure S1.** Hydrodynamic diameter (HD) of $ICG_4$-GS-Au25 in PBS buffer. The HD of $ICG_4$-GS-Au25 in PBS is 3.4±0.5nm without larger aggregation.

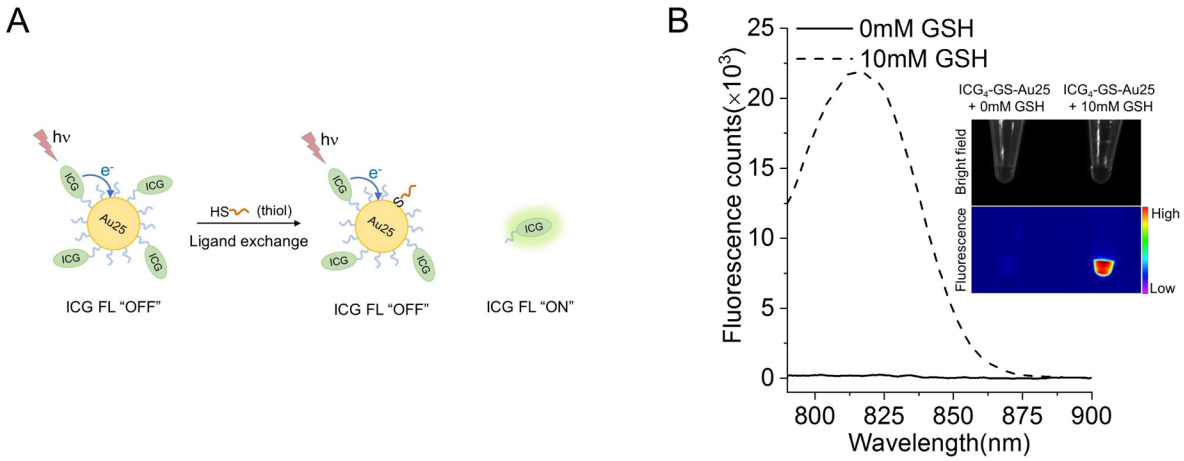


**Figure S2.** Fluorescence activation of $ICG_4$-GS-Au25 by thiol molecules. (A) Scheme of the ICG fluorescence activation through ligand exchange with other thiol molecules. (B) Fluorescence emission spectra of the same $ICG_4$-GS-Au25 in PBS containing 0 mM reduced glutathione (GSH) or 10 mM GSH (pH adjusted to ~7) with 760 nm exaction. Inserted is the fluorescence image acquired using *in vivo* animal imaging system.

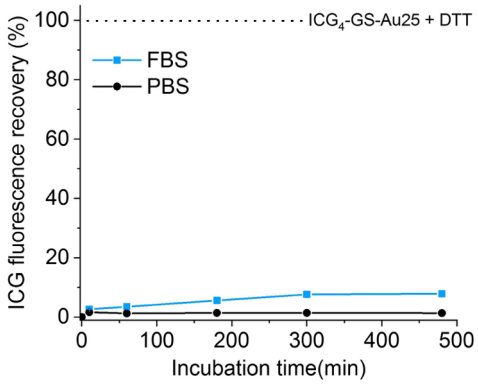


**Figure S3.** Stability test of $ICG_4$-GS-Au25 in PBS and FBS media. ICG fluorescence of $ICG_4$-GS-Au25 incubated in PBS or FBS was normalized to the ICG fluorescence of $ICG_4$-GS-Au25 incubated in 20mM DTT, which could release all ICG from the surface of Au25 nanocluster.

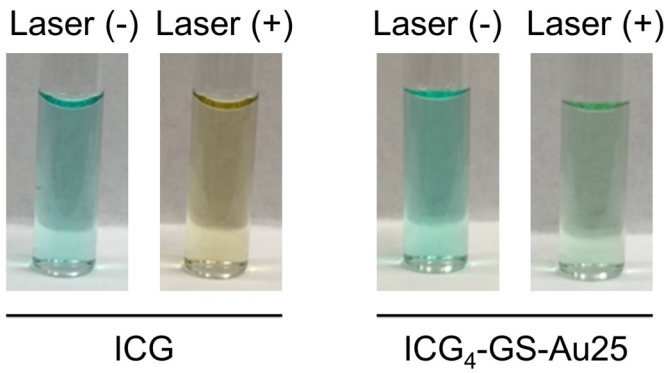


**Figure S4.** Photochemical stability test of $ICG_4$-GS-Au25 and free ICG. Color images of $ICG_4$-GS-Au25 and free ICG solutions before and after 808nm laser irradiation for 6 mins with a power density of $0.5 W/cm^2$.

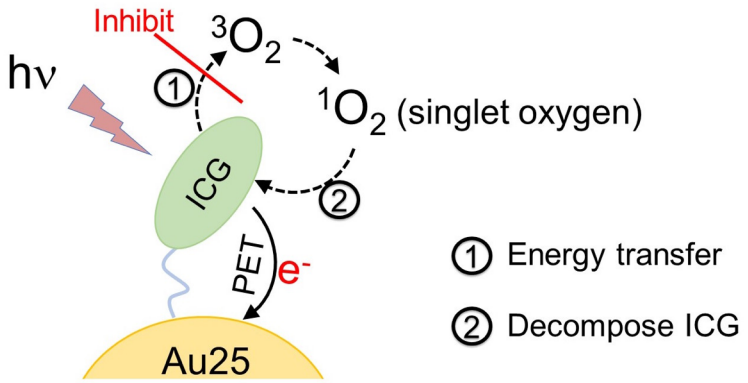


**Figure S5.** Proposed mechanism for the enhanced ICG photochemical stability of $ICG_4$-GS-Au25. After conjugation of ICG onto GS-Au25, the photo-induced electron transfer (PET) process (transfer of excited state electron from ICG to Au25) inhibits the transfer of energy from excited electrons in ICG to nearby oxygen molecules and thus inhibit the generation of reactive singlet oxygen locally, which in turn can slow down the singlet oxygen-induced decomposition of ICG.

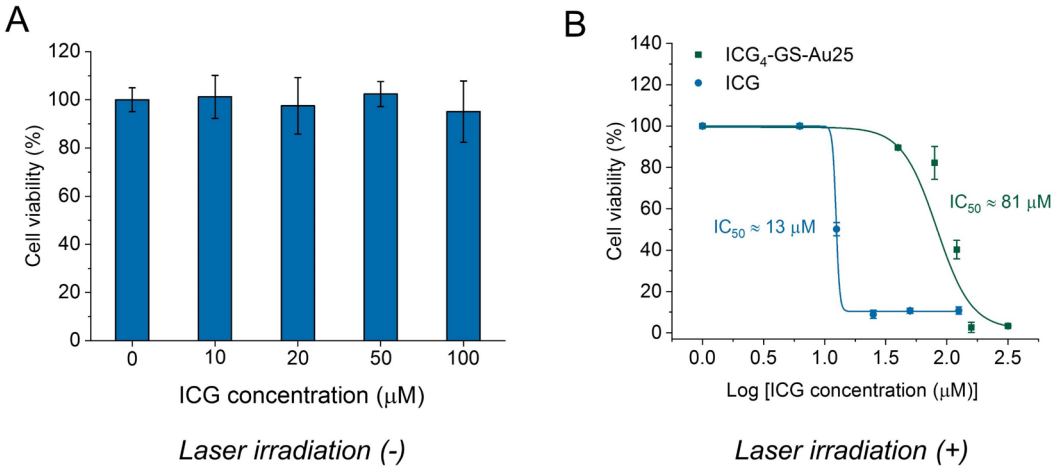


**Figure S6.** Cytotoxicity and phototoxicity of free ICG *in vitro*. (A) Cell viability of MCF-7 cells incubated with various concentration of free ICG in darkness, which indicates that free ICG itself does not exhibit significant cytotoxicity without light exposure. (B) The dose response curves of the cell viability of MCF-7 cells incubated with different concentrations of $ICG_4$-GS-Au25 or free ICG for 2 hours, followed by 808 nm laser irradiation (0.5 W/cm$^2$) for 5 mins. Cell viability was quantified by MTT assay.

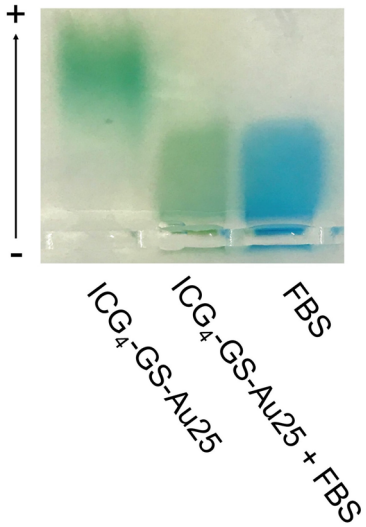


**Figure S7.** Serum protein binding analysis of $ICG_4$-GS-Au25. $ICG_4$-GS-Au25 was incubated with Fetal Bovine Serum (FBS) at 37°C for 10 min and analyzed by 2% agarose gel electrophoresis for protein binding profile.

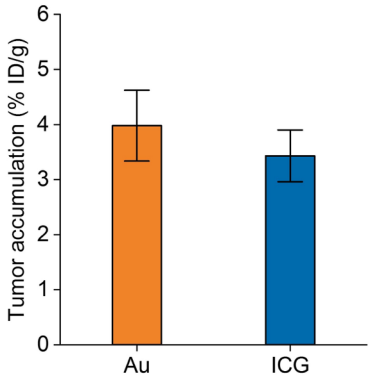


**Figure S8.** Tumor accumulation of ICG and Au at 24h after intravenous injection of $ICG_4$-GS-Au25.

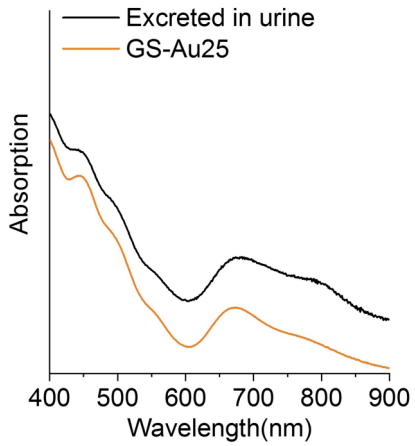


**Figure S9.** Absorption spectrum of the Au nanoclusters excreted in urine after i.v. injection of $ICG_4$-GS-Au25 in BALB/c mice. The absorption spectrum of excreted Au nanoclusters in urine matches the characteristic absorption spectrum of the Au25 cluster but without the ICG absorption, suggesting the ICG is dissociated from Au25 *in vivo* before renal elimination of the Au25.

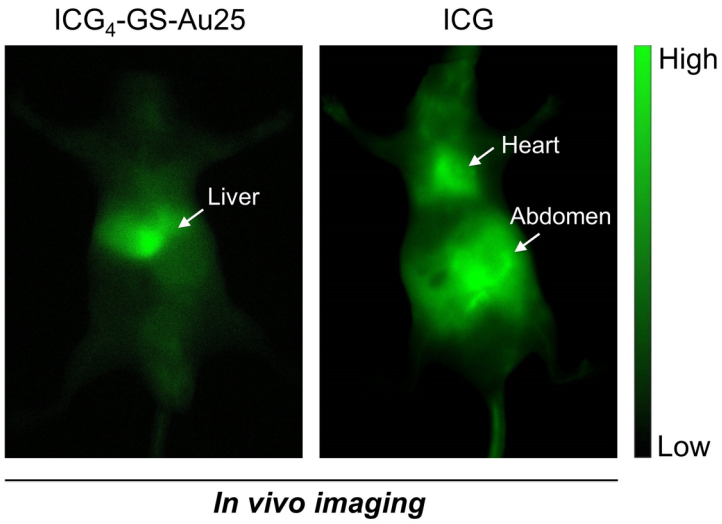


**Figure S10.** *In vivo* fluorescence imaging of mice injected with $ICG_4$-GS-Au25 or free ICG. Immediately after intravenous injection (~20s p.i.), free ICG lighted up the whole body, particularly the heart and abdomen because of the rich in blood. In contrast, $ICG_4$-GS-Au25 remained relatively non-fluorescent in the extrahepatic circulation but was fluorescently activated inside the liver, indicating that the *in vivo* dissociation of $ICG_4$-GS-Au25 happened in the liver.

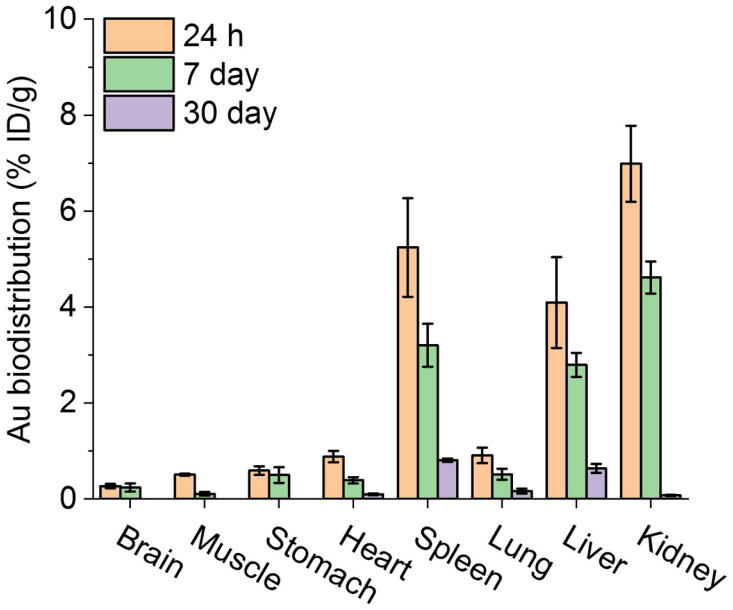


**Figure S11.** Biodistribution of Au in BALB/c mice i.v. injected with $ICG_4$-GS-Au25 at 24h, 7day and 30day post injection.

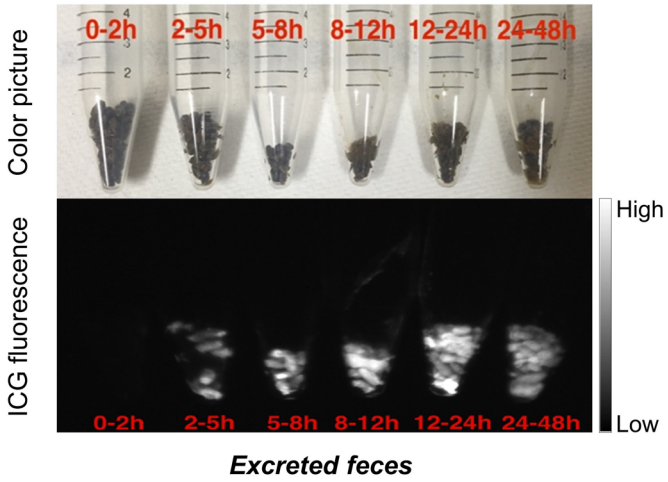


**Figure S12.** Fluorescence images of the feces excreted by BALB/c mice i.v. injected with $ICG_4$-GS-Au25. Strong ICG fluorescence could be observed from the feces of mice 2h after the injection of $ICG_4$-GS-Au25, indicating that the dissociated ICG was efficiently eliminated through the hepatobiliary pathway.