Supporting Information

Near-infrared fluorescence imaging of cancer cells and tumors through specific biosynthesis of silver nanoclusters

Shengping Gao[1], Donghua Chen[1], Qiwei Li[1], Jing Ye[1], Hui Jiang[1], Christian Amatore[2], and Xuemei Wang[1]*

[1]State Key Laboratory of Bioelectronics (Chien-Shiung Wu Lab), School of Biological Science and Medical Engineering, Southeast University, Nanjing, 210096, China

[2] Ecole Normale Supérieure, UMR CNRS-ENS-UPMC 8640 and LIA CNRS XiamENS NanoBioChem Departement de Chimie, 24 rue Lhomond 75005 Paris (France)

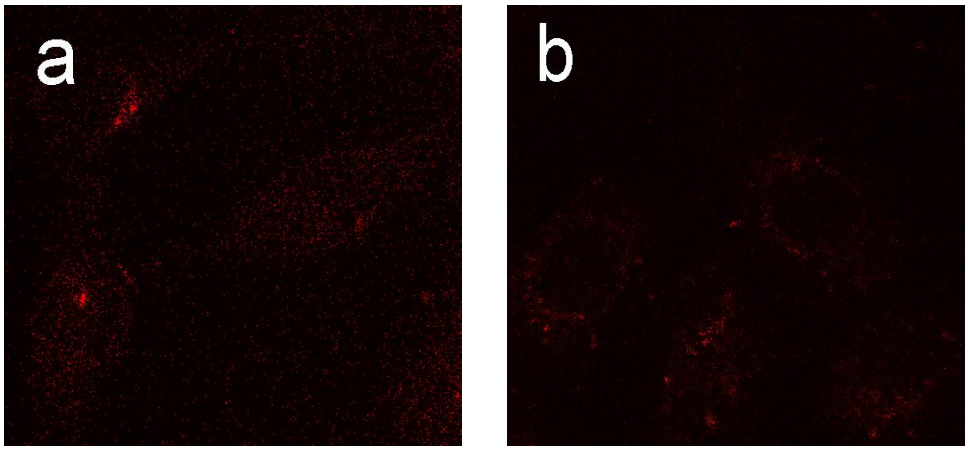

Figure S1| Effect of different concentrations of $[Ag(GSH)]^+$ on the intracellular accumulation in normal embryo liver cells (L02 cells): (a) L02 cells alone as control; (b) L02 cells incubated in the presence of $[Ag(GSH)]^+$ 100.0 μg/mL. The cells were observed under a confocal fluorescence microscope at the fluorescence excitation wavelength of 590 nm.

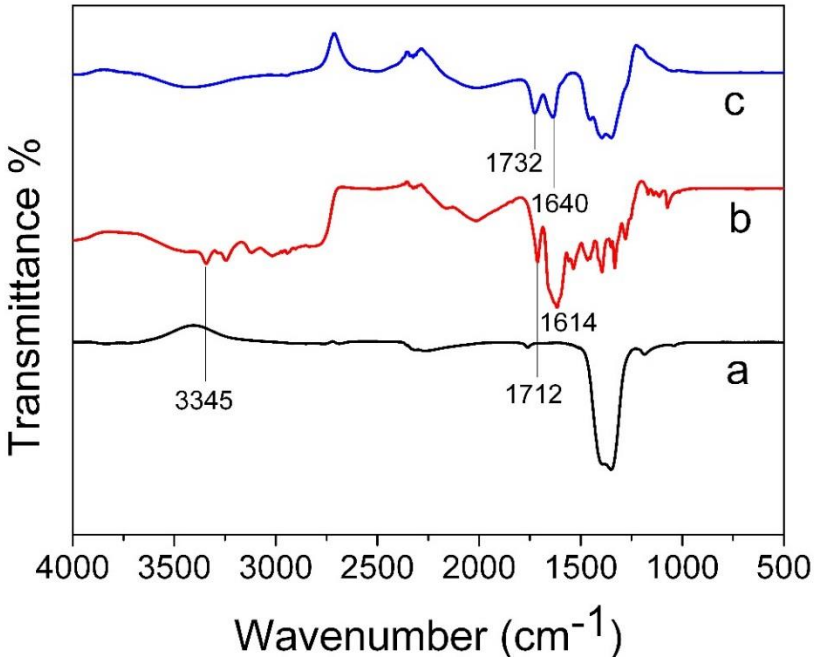

Figure S2 | FT-IR spectra of $AgNO_3$ (a) GSH (b) and $[Ag(GSH)]^+$ complexes (c).

FTIR spectra of GSH and its Ag^+ complexes were taken by using a thermo fisher scientific FTIR spectrophotometer (American, Nicolet Co.) in the interval 4000-400 cm^{-1}, and the samples were prepared in D_2O solution. The FTIR spectrum of the complex between GSH and Ag+ is presented in Figure S2. There were some significant changes observed after the formation of the complex. The absorption bands at 3345 cm^{-1} attributed to $–NH_2$ were missing and at 1712cm^{-1} characteristic of >C=O was shifted to 1732cm^{-1}. The band at 1614 cm^{-1} corresponding to the carboxylate anion $-COO^-$ was shifted to 1640 cm^{-1}. The analytical band characteristic of the asymmetric valent vibrations of $-COO^-$ at 1598 cm^{-1}, was shifted to 1637 cm^{-1} after the complex formation[1-3]. The spectra confirmed that Ag^+ ions bond predominantly with –COOH and $–NH_2$ functional groups of GSH.

References:

1 Dimitrova, M., Turmanova, S. & Vassilev, K. Complexes of glutathione with heavy metals as catalysts for oxidation. *Reaction Kinetics, Mechanisms and Catalysis* **99**, 69-78 (2010).

2 Fiorucci, A. R., Saran, L. M., Cavalheiro, E. T. G. & Neves, E. A. Thermal stability and bonding in the silver complexes of ethylenediaminetetraacetic acid. *Thermochim Acta* **356**, 71-78 (2000).

3 Wang, CX, Xu, L, Zhang, D, Shi, XD, Dong, FX, Yu, K, Lin, Q, Yang, B, Fluorescent silver nanoclusters as effective probes for highly selective detection of mercury(II) at parts-per-billion levels, *Chemistry – An Asian Journal* **7**, 1652–1656 (2012).

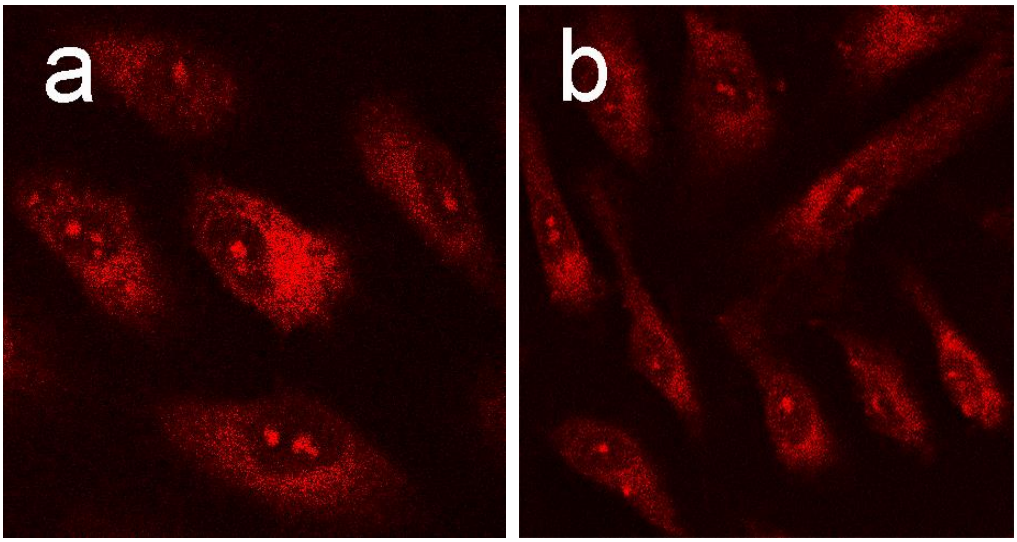

Figure S3| Effect of $[Ag(GSH)]^+$ on the intracellular accumulation in different cancer cells: (a) HepG2 cells; (b) A549 cells. All the cells were incubated in the presence of $[Ag(GSH)]^+$ 100.0 μg/mL. The cells were observed under a confocal fluorescence microscope at the fluorescence excitation wavelength of 532 nm.

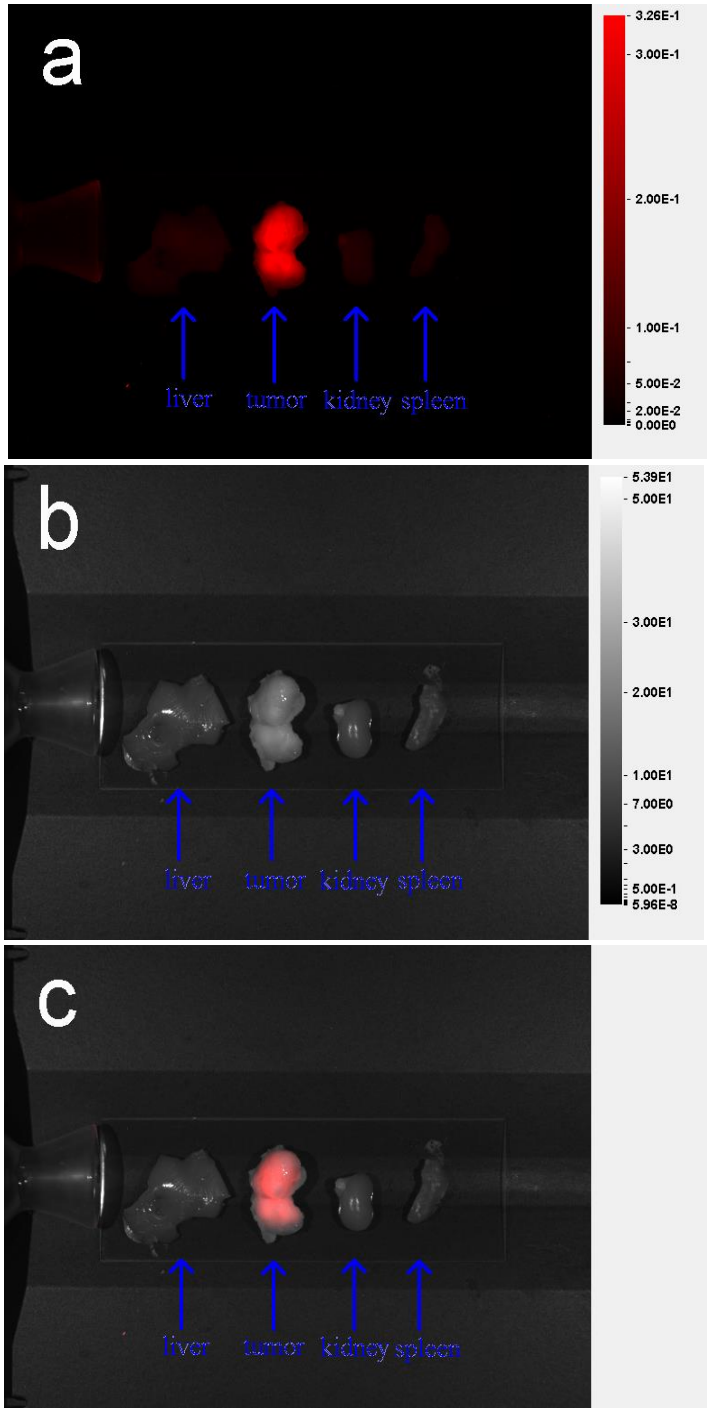

Figure S4 | Representative NIR fluorescence ex vivo images of excised HeLa tumors and other visceral organs in $[Ag(GSH)]^+$-injected xenograft tumor mouse. (A) Comparison of (a) NIR fluorescence and (b) morphological images. (B) Overlay of morphological and fluorescence images shown in A. The relevant fluorescence excitation wavelength was 590 nm

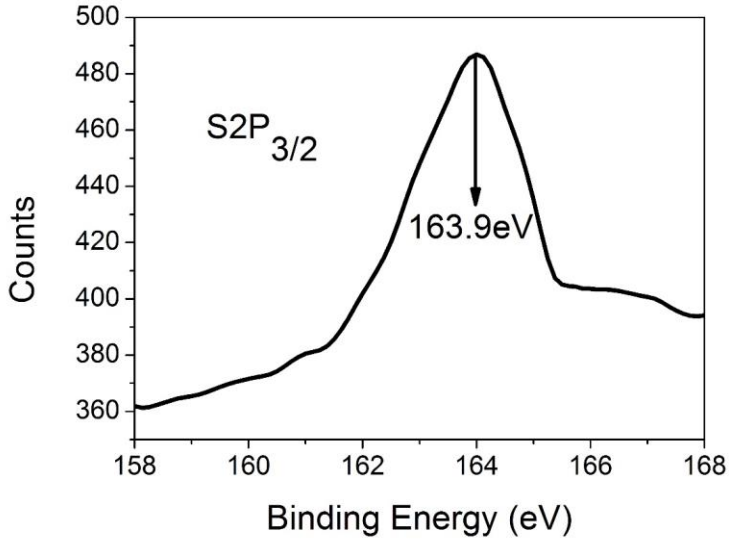

Figure S5 | XPS evidencing the $S2P_{3/2}$ photo-electron emission from silver nanoclusters (Ag NCs) biosynthesized in situ by HeLa cells after incubation with $[Ag(GSH)]^{+}$. When Ag+ is in cells in the presence of GSH for [Ag(GSH)]+ solutions, the redox phenomenon could be observed, where silver goes to the relevant oxidation state. X-ray photoelectron spectroscopy (XPS) spectra reveal that a ~163.9eV binding energy (i.e., for $S2P_{3/2}$, higher than that of the reduced GSH) is due to the formation of disulfide linkage[4,5]. As a corollary of the reduction of the noble metal, GSH was readily oxidized to GSSG (oxidized) species, which evidently involves the disulfide linkage and renders stability to the cluster[6].

References:

4 Bourg, M. C., Badia, A. & Lennox, R. B. Gold-sulfur bonding in 2D and 3D self-assembled monolayers: XPS characterization. *J Phys Chem B* **104**, 6562-6567 (2000).

5 Nesbitt, H. W., Bancroft, G. M., Pratt, A. R. & Scaini, M. J. Sulfur and iron surface states on fractured pyrite surfaces. *Am Mineral* **83**, 1067-1076 (1998).

6 Ganguly, M., Pal, A., Negishi, Y. & Pal, T. Synthesis of Highly Fluorescent Silver Clusters on Gold(I) Surface. *Langmuir* **29**, 2033-2043 (2013).