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Supporting Information for

Hybrid Polymeric Nano-capsules Loaded with Gold Nanoclusters and Indocyanine Green for Dual-modal Imaging and Photothermal Therapy

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Two-photon photostability of AuNCs

The photostability of AuNCs were evaluated by two-photon confocal microscopy equipped with a femtosecond Ti: sapphire oscillator. The AuNCs were irradiated by a 820 nm laser with a input power density of 12 W for 60 min. Fluorescent images were acquired every 5 min. An open source software package (Imaging J) was utilized to quantify the fluorescent intensity. [1]

Entrapment efficiency of ICG

To estimate the entrapment efficiency of ICG, nano-capsules were lyophilized, weighted and dissolved in trichloromethane. ICG in the solution was extracted by DI water. The samples of extraction were immediately transferred into quartz cuvettes for fluorescence detection, the excitation wavelength was 760 nm for ICG, and the emission spectra of ICG were acquired from 790 to 900 nm at 5 nm intervals. [2] All measurements and sample treatments were handled in the dark with identical setting-up. The fluorescence calibration curves of ICG were obtained to verify spectral characteristics and linearity range. The concentrations of ICG were estimated from the corresponding intensity at the peak emission wavelength, by using the ICG calibration curves. ICG or AuNCs entrapment efficiency (E.E%) and loading capacity (L.C%)were calculated as following:

$$E.E\% = \frac{\text{Mass of ICG/AuNCs in nano-capsules}}{\text{Mass of ICG/AuNCs initially added}} \times 100\% \quad \text{(S1)}$$

$$L.C\% = \frac{\text{Mass of ICG/AuNCs in nano-capsules}}{\text{Mass of nano-capsules}} \times 100\% \quad \text{(S2)}$$

Photoacoustic (PA) imaging in the phantom

Different concentrations of AuIP nano-capsules were injected into the tubular hydrogel phantom of agarose with the ICG as control.[3] The phantom samples contained a concentration gradient of ICG or equivalent ICG-loaded nano-capsules, including 0, 2.5, 5, 10, 20, 40 μg/mL. The phantom was scanned by a photoacoustic (PA) imaging system (MOST 128, iTheraMedical, Germany). The PA signal intensity was evaluated through mean pixel intensity of the same area of the images, the

images were acquired at the same intensity of laser pulse.

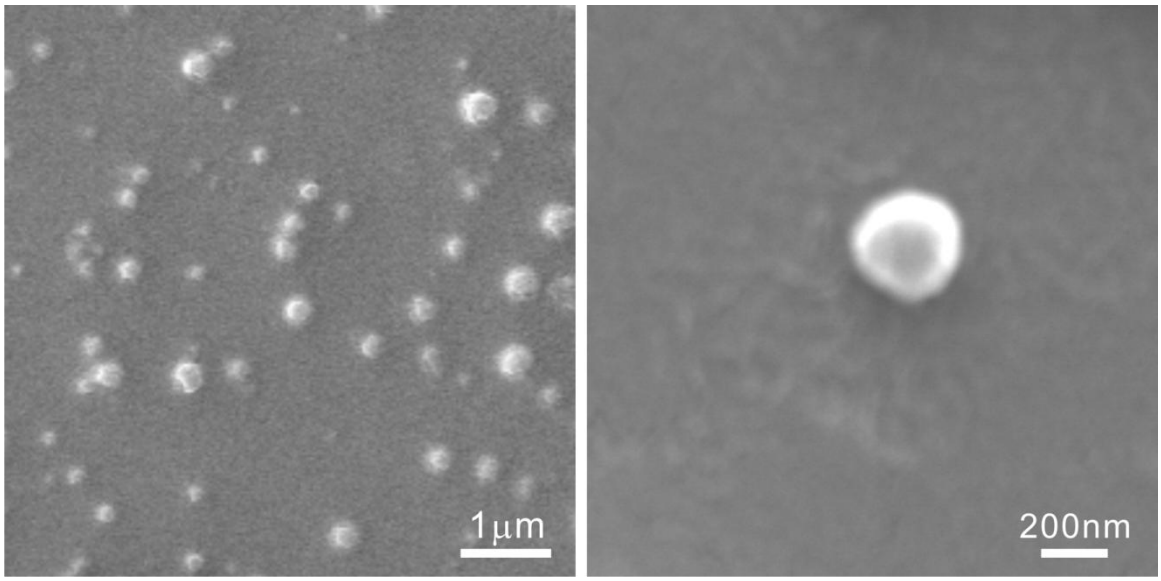

Figure S1. SEM images of AuIP nano-capsules.

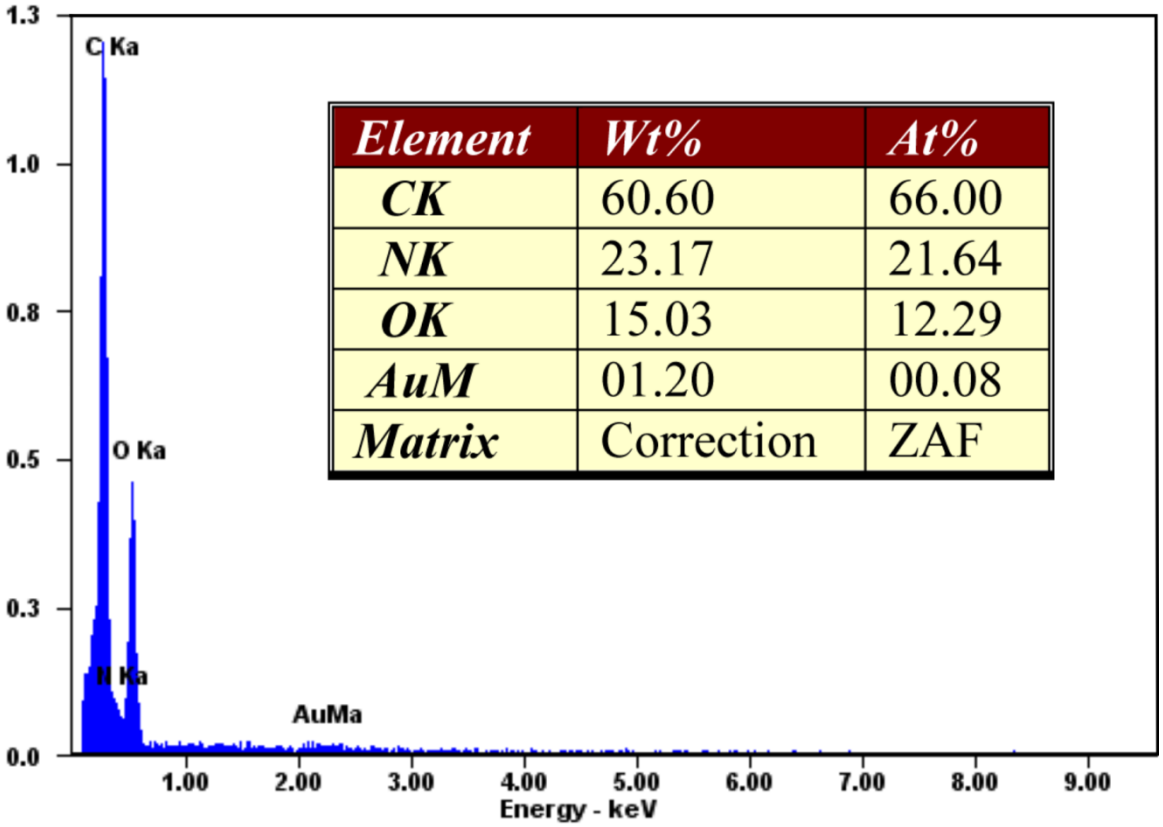

Figure S2. The EDS analysis of AuIP nano-capsules (The inset: the composition and ratios of the main elements).

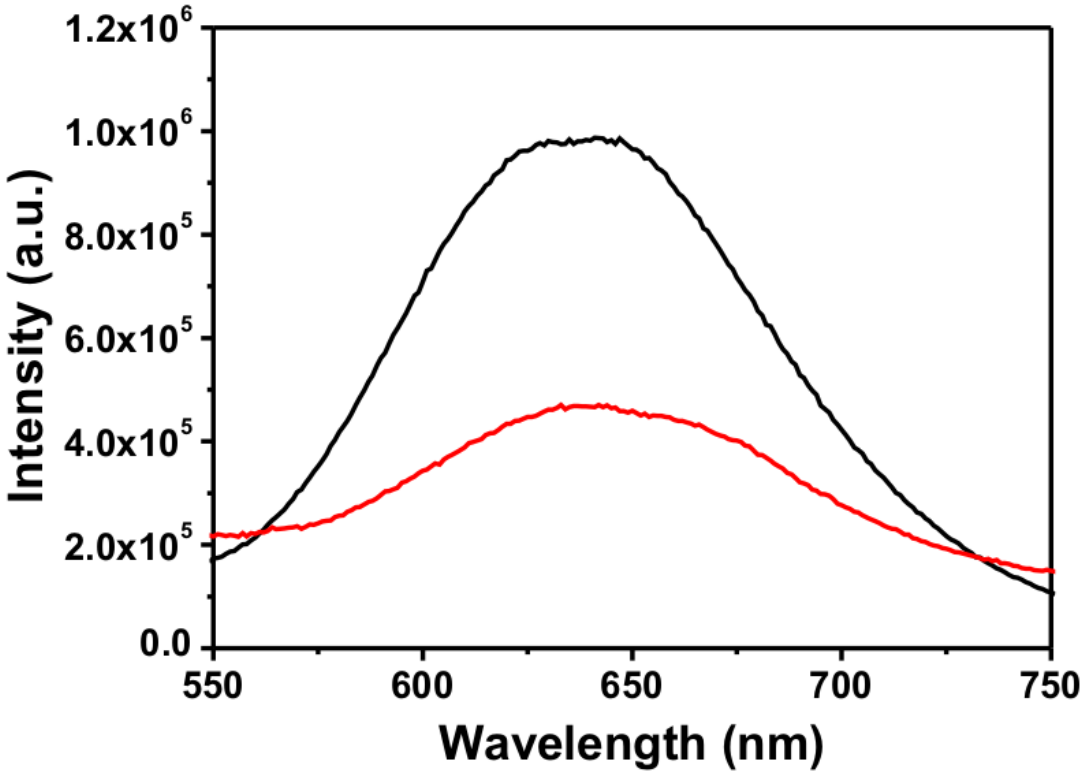

Figure S3. Comparison of the fluorescent intensities in the one-photon fluorescence mode (λ_{ex} = 405 nm), black curve: AuNCs solution, red curve: AuNCs loaded nano-capsules.

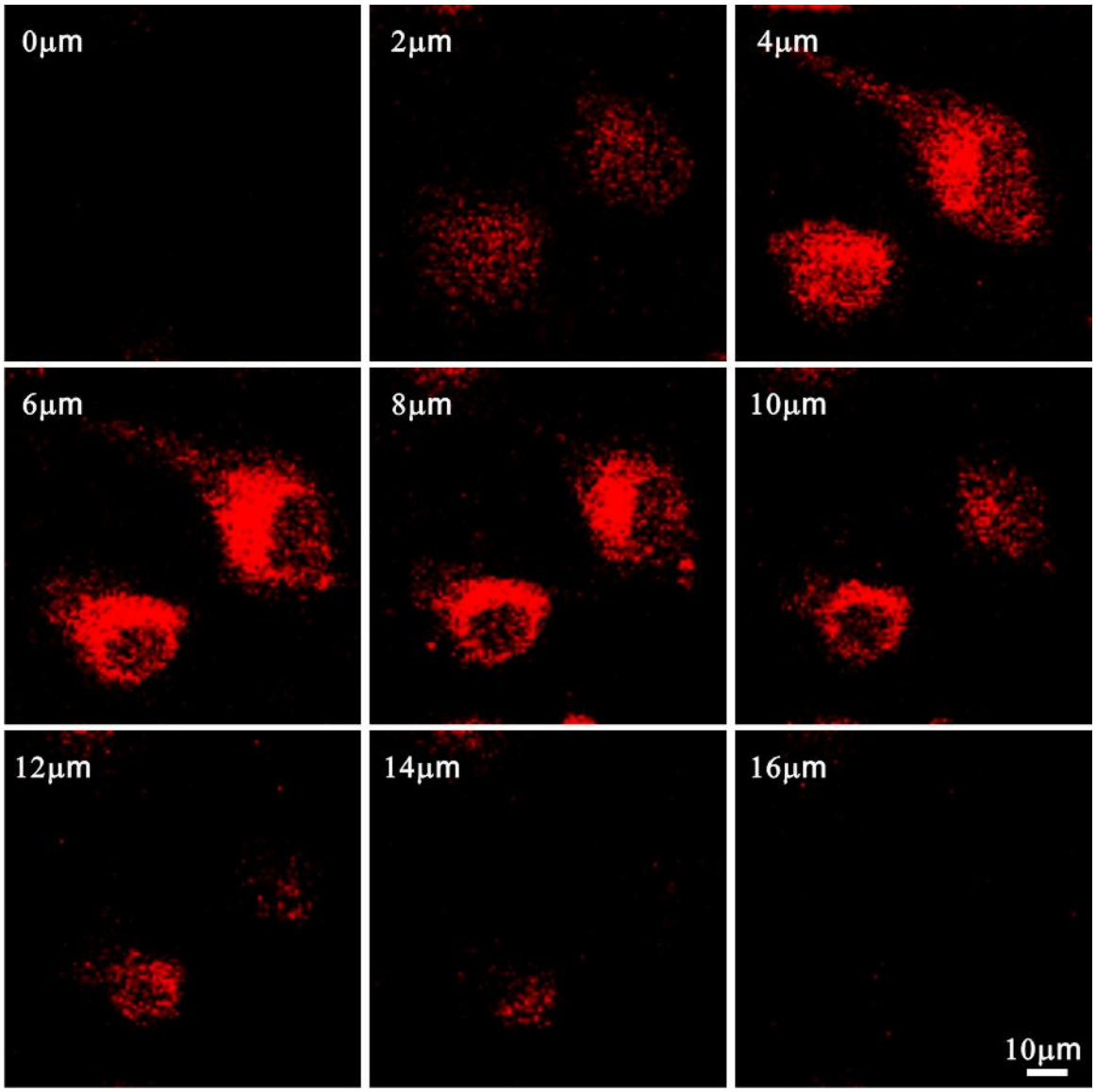

Figure S4. Two-photon confocal Z-stack sectioning of U87-MG cells treated with AuIP-RGD nano-capsules for 24h

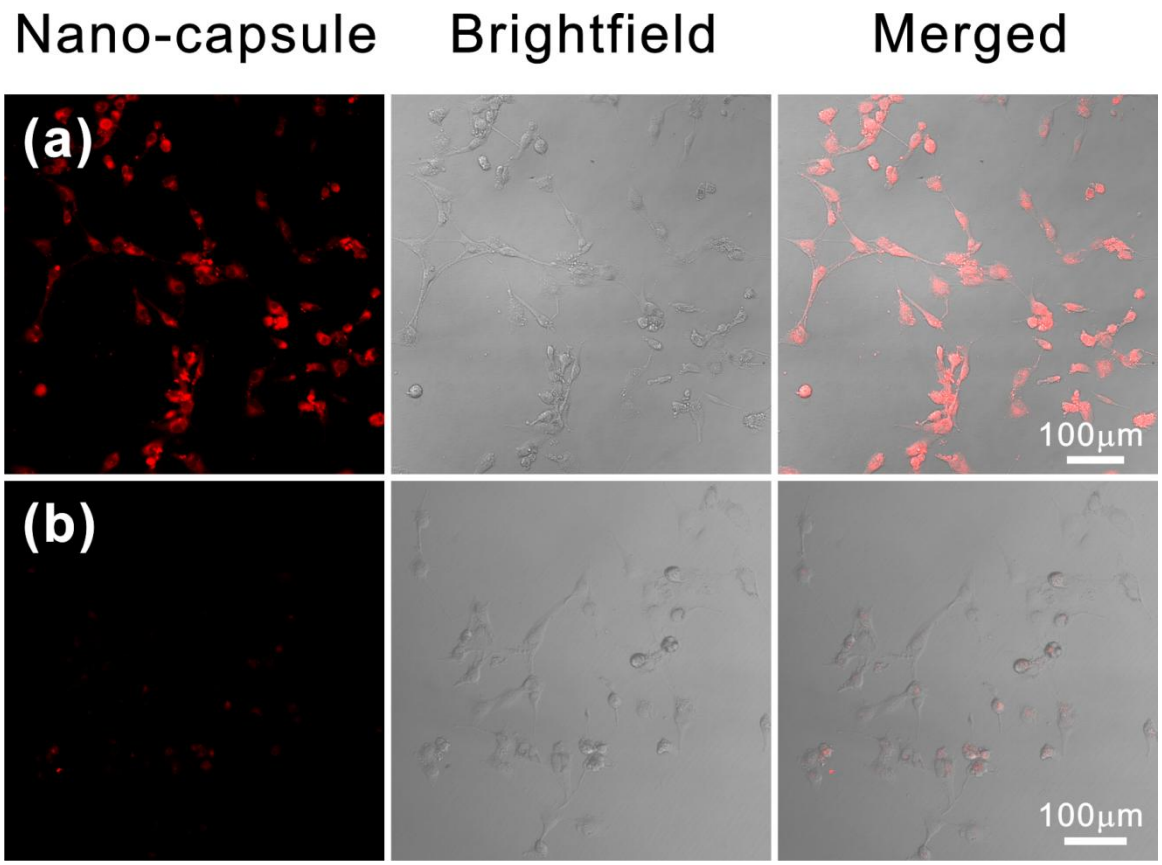

Figure S5. One-photon confocal images of U87-MG cells incubation with (a) AuIP-RGD nano-capsules and (b) AuIP nano-capsules for 24h.

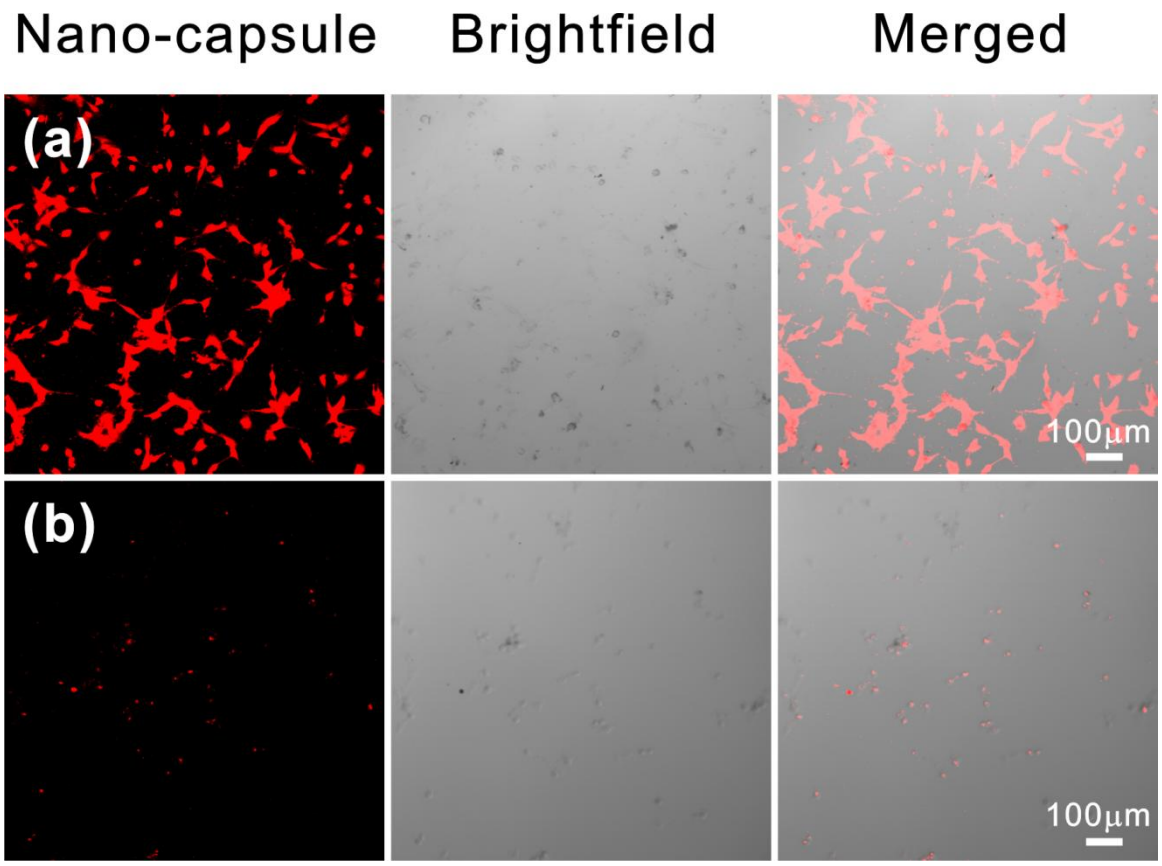

Figure S6. Two-photon confocal images of U87-MG cells incubation with (a) AuIP-RGD nano-capsules and (b) AuIP nano-capsules for 24h.

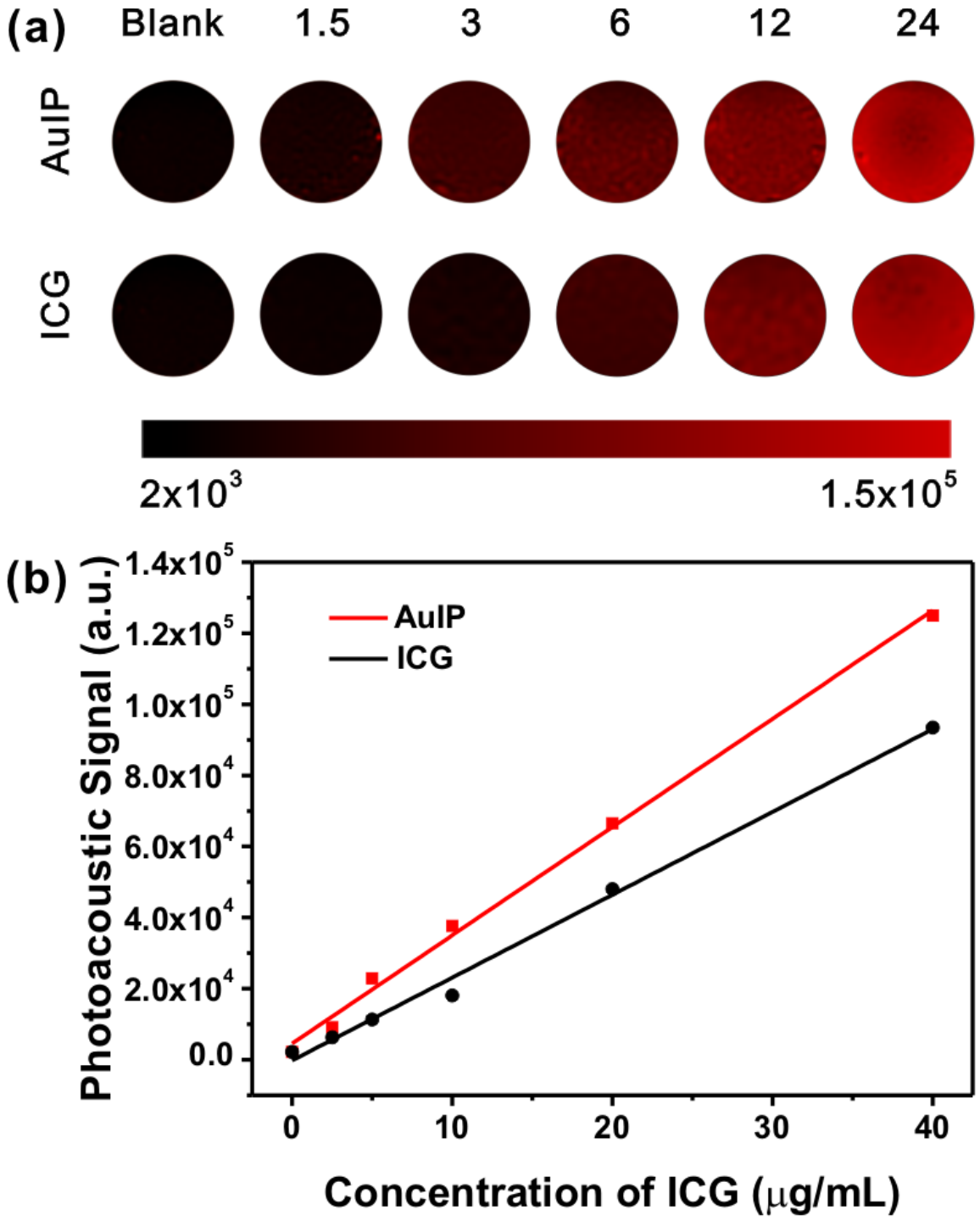

Figure S7. (a) Sectional photoacoustic images, and (b) photoacoustic signals analysis of tissue-mimicking phantom made of agarose gel, dependent on the concentration of ICG.

References

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2. A. Topete, D. Melgar, M. Alatorre-Meda, P. Iglesias, B. Argibay, S. Vidawati, S. Barbosa, J. A. Costoya, P. Taboada and V. Mosquera, *J. Mater. Chem. B*, 2014, **2**, 6967-6977.
3. M. Xu and L. V. Wang, *Rev. Sci. Instruments*, 2006, **77**, 041101.