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Electronic Supplementary Information

BSA-bioinspired gold nanorods loaded with immunoadjuvant for treatment of melanoma by combined photothermal therapy and immunotherapy†

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Part of the experimental details

Characterization techniques

The ultraviolet-visible-NIR (UV-Vis-NIR) spectra were performed using a Cary 5000 UV-Vis-NIR spectrophotometer (Santa Clara, CA). For transmission electron microscopy (TEM) measurements, we used a Hitachi H-7500 Electron microscope (Tokyo, Japan) operating at 80 kV. A diluted sample suspension was deposited onto carbon-coated copper grid and air dried before measurements. A NanoBrook Omni instrument (Brookhaven Instruments corporation, Long Island, NY) was used for dynamic light scattering (DLS) and zeta potential measurements.

Cell culture

B16-F10 melanoma cells, RAW264.7 macrophages, and DC2.4 cells were purchased from the American Type Culture Collection (ATCC; Bethesda, MD). Bone-marrow-derived dendritic cells (BMDCs) were generated from the bone marrow of C57BL/6 mice. All cancer cells were cultured with DMEM containing 10% FBS and 1% penicillin and streptomycin.

***In vitro* R837 release**

The *m*PEG-GNRs@BSA/R837 nanocomplexes were dispersed into 1 mL of PBS (pH 7.4, [R837] = 200 μg/mL) or acetate buffer (pH 5.0, [R837] = 200 μg/mL) and placed in a dialysis bag (MWCO = 10,000), hermetically tied, and suspended in 9 mL of corrosponding buffer solution. The entire system was kept in a vapor-bathing constant temperature vibrator at 37 °C. Buffer medium (1 mL) was taken out at each predetermined time interval and measured by UV-Vis spectrometer. The volume of the outer phase buffer was maintained constant by replenishing 1 mL of the corresponding buffer solution.

***In vitro* cytotoxicity assay**

For in vitro cytotoxicity assay of *m*PEG-GNRs@BSA, B16-F10 cells (5000 cells per well) were seeded into a 96-well plate. After overnight incubation, the cells were co-cultured with mPEG-GNRs@BSA at various Au concentrations for 24 h. A standard 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was then performed to obtain the relative cell viabilities according to the manufacturer's instruction.

***In vitro* photothermal therapy**

B16-F10 cells were seeded into a six-well plate at a density of 1×10^6/well, and under cultivation for 12 h. Then, the cells were co-cultured with *m*PEG-GNRs@BSA at the Au concentration of 11.5 μg/mL (58.4 μM) for 6 h, and the cells were collected in 1.5 mL-tubes without medium *via* digestion and centrifugation. Hereafter, the collected cells were irradiated by a 1064 nm NIR laser (1.0 W/cm^2) at different power density for 10 min. Then the cells were cultured for another 2 h and stained with trypan blue (Hyclone; Logan, UT). The cell viability was measured by TC20TM Automated Cell Counter (Bio-Rad; Hercules, CA).

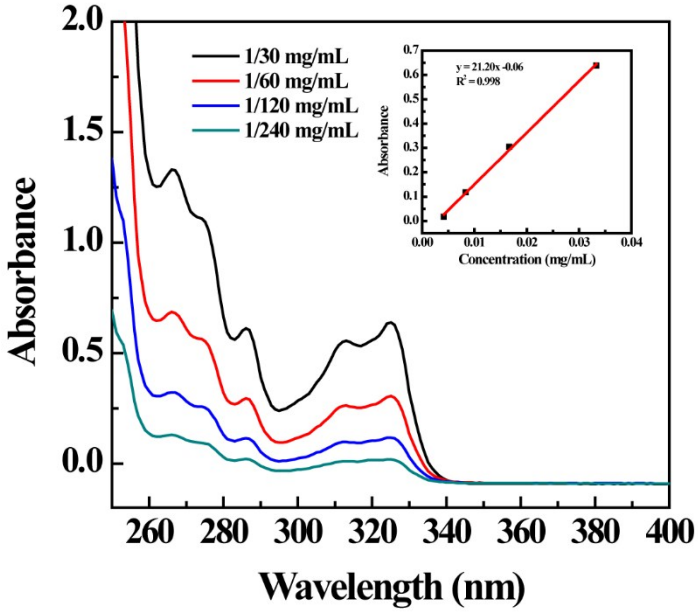

Fig. S1 UV-Vis spectra of R837 methanol solution at different concentrations, and inset is the standard curve of the R837.

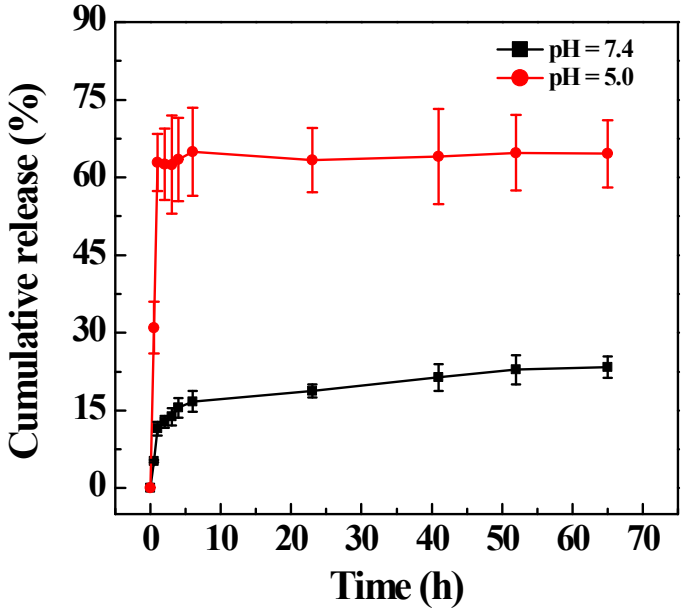

Fig. S2 Cumulative release of R837 from the *m*PEG-GNRs@BSA/R837 nanocomplexes in PBS (pH 7.4) and acetate buffer (pH 5.0) at 37 ^{o}C.

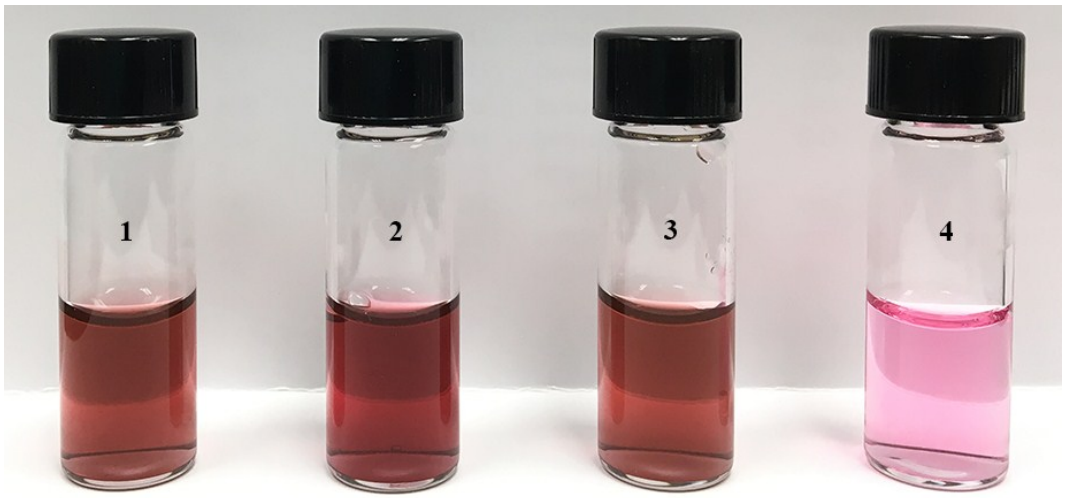

Fig. S3 Photos of *m*PEG-GNRs@BSA/R837 nanocomplexes dispersed in water (1), PBS (2), and cell culture medium (3). Blank cell culture medium is shown in (4).

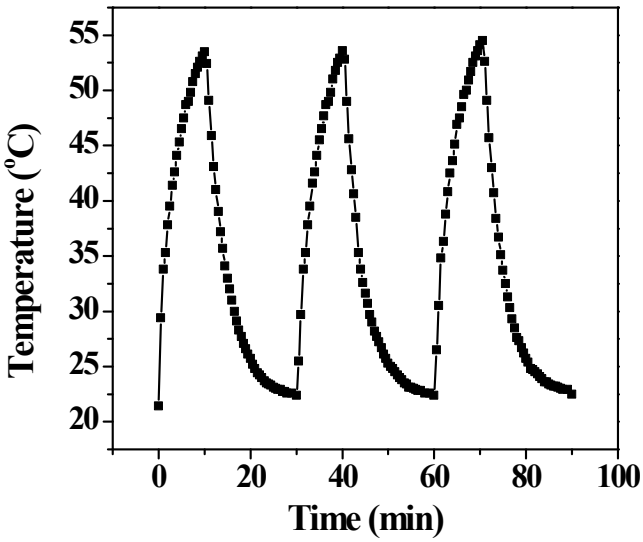

Fig. S4 Heating of a suspension of the *m*PEG-GNRs@BSA/R837 nanocomplexes in water at the Au concentration of 10 μg/mL for three laser on/off cycles (1.0 W/cm^2).

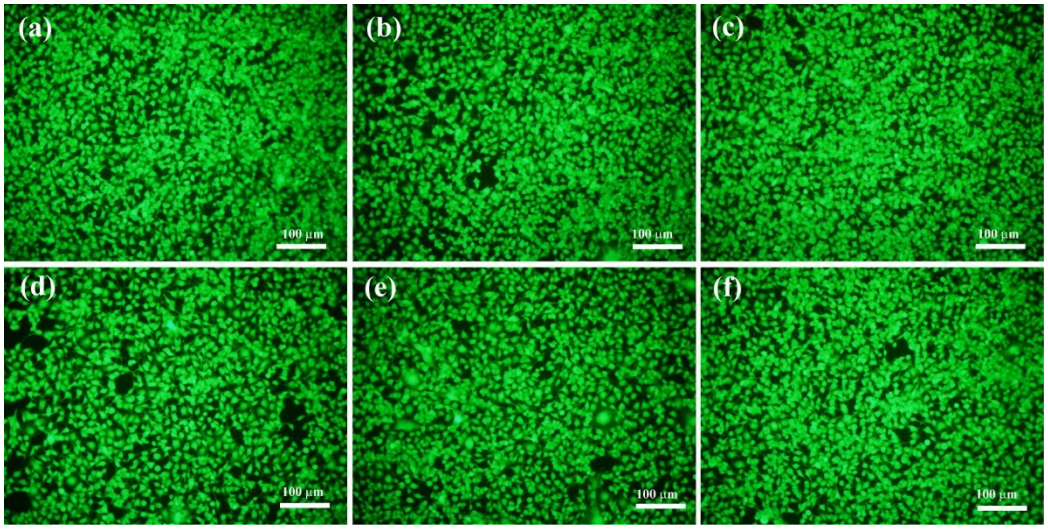

Fig. S5 Inverted fluorescence microscopic images of B16-F10 cells treated with the *m*PEG-GNRs@BSA at the Au concentrations of 0 μM (a), 10 μM (b), 20 μM (c), 40 μM (d), 80 μM (e), and 100 μM (f), respectively for 24 h. The cells were stained with calcein AM.

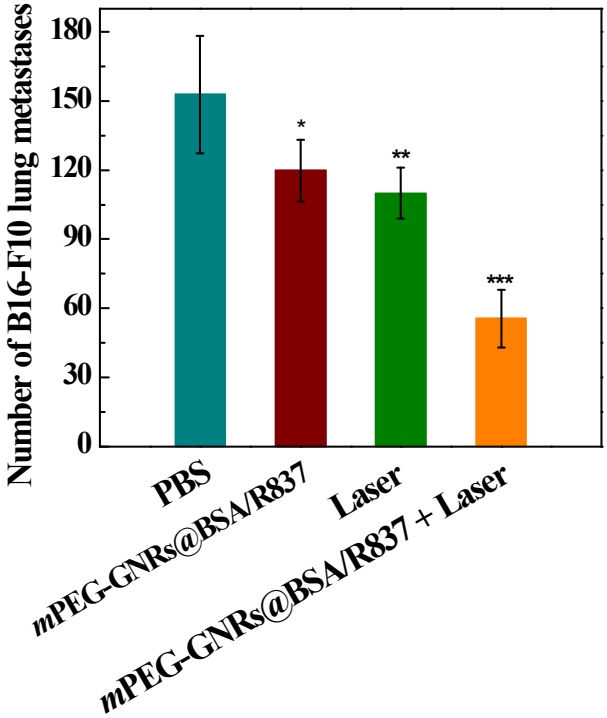

Fig. S6 Number of B16-F10 lung metastases 15 days after various treatments (statistical analysis was performed and all data were compared with the PBS control).