

Electronic Supplementary Information

Fabrication of Graphene and AuNP Core Polyaniline Shell Nanocomposites as Multifunctional Theranostic Platforms for SERS Real-time Monitoring and Chemo-photothermal Therapy

Supporting Figure Captions

Figure S1 The AFM image of GO-PVP A) and the height profile in selected location B).

Figure S2 Energy dispersive X-ray (EDX) analysis of GO-Au@PANI.

Figure S3 Dynamic light scattering (DLS) data of GO-Au@PANI in water.

Figure S4 The FT-IR spectrum of the nanostructures. As shown in FTIR spectra, characteristic peak at 1563 cm^{-1} corresponding to C=C band in graphene but also the PVP absorptions at 1422 , 1635 and 1285 cm^{-1} , which are ascribed to the vibrations of CH_2 , C=O and C-N in PVP, respectively. The bands at 1275 cm^{-1} (C-N stretching mode in a secondary aromatic amine), 1210 cm^{-1} (an aromatic C-H in-plane deformation), 1634 and 1529 cm^{-1} (quinonoid and benzenoid ring stretching vibrations) in the FTIR spectra Au@PANI proved the existence of PANI.

Figure S5 The GO-Au@PANI we prepared was stable in aqueous solution, phosphate buffered saline (PBS) and RPMI-1640 cell medium 10% fetal bovine serum (FBS) for 0 h A) and 48 h B).

Figure S6 The UV-Vis-NIR absorption spectra of GO-Au@PANI before and after NIR laser irradiation (808 nm , 2.5 W cm^{-2} , 30 min).

Figure S7 Representative photographs of tumor-bearing mice before treatment and at

day 0,8,16 after treatment.

Figure S8 Blood analysis datas of mice 16 days after GO-Au@PANI photothermal treatment and GO-Au@PANI/DOX chemo-photothermal treatment (808 nm, 2.5Wcm^{-2}). Age-matched healthy mice were used as the control group. These findings did not indicate a trend of toxicity. ALT = alanine aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase, BUN = kidney function marker urea nitrogen, A/G = albumin/globin ratio.

Figure S1

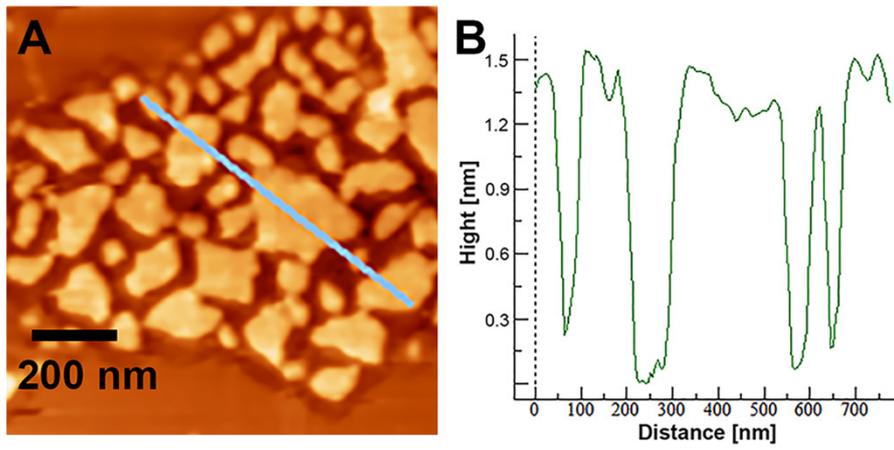


Figure S2

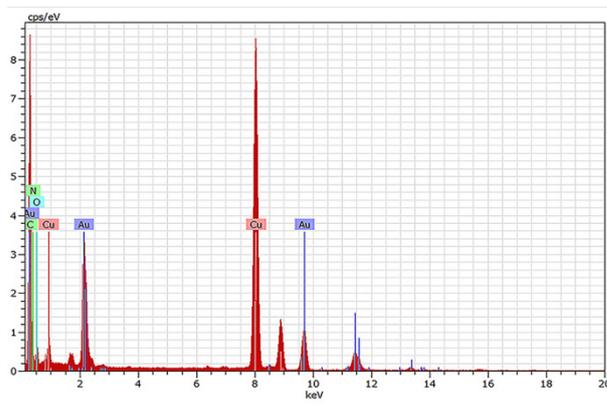


Figure S3

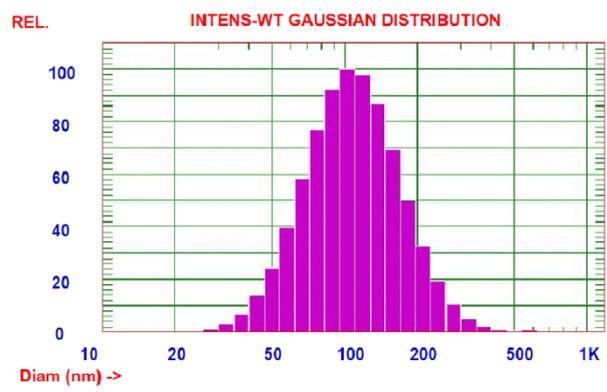


Figure S4

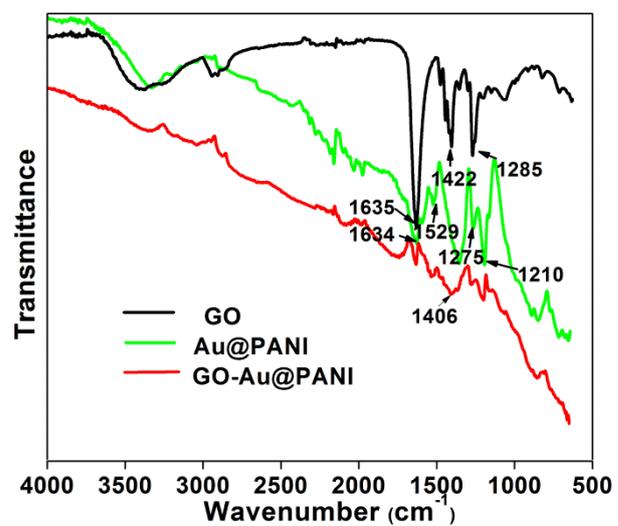


Figure S5

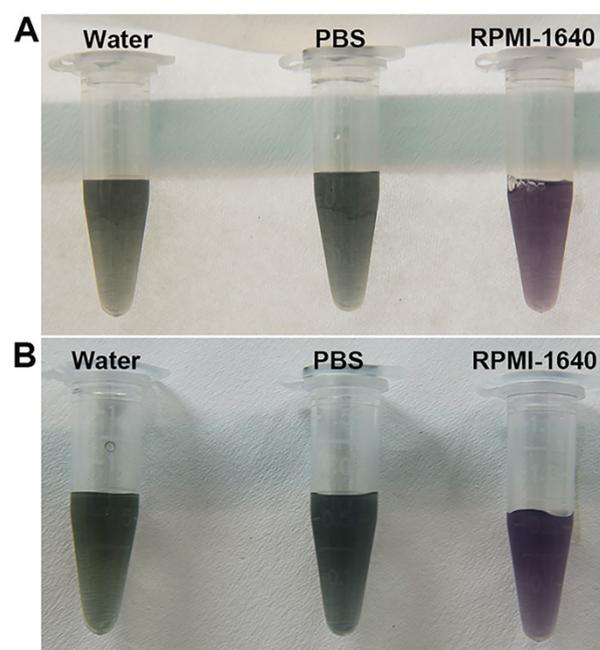


Figure S6

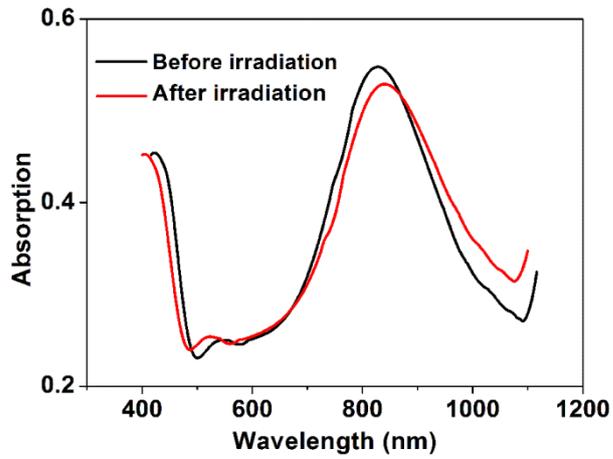


Figure S7

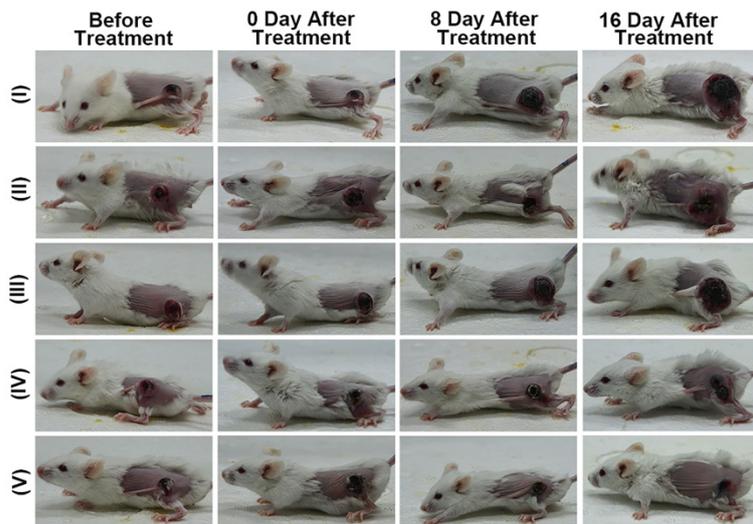
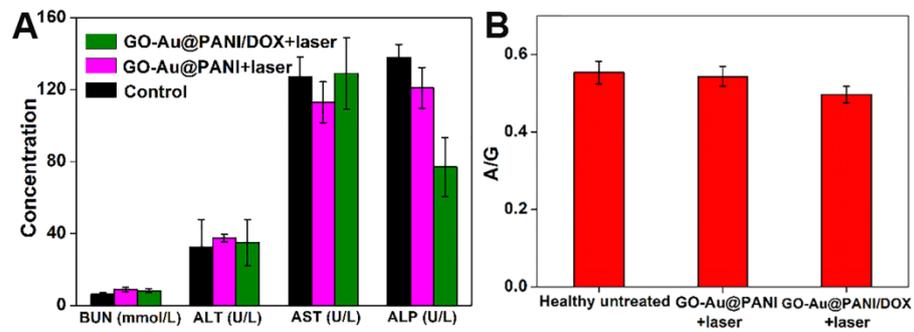


Figure S8



Experimental Section

Synthesis of GO-Au@PANI nanocomposites

Water soluble GO was prepared by oxidizing graphite according to a modified Hummer's method [1]. To obtain NGO, 10 mg of GO was cracked by ultrasonic probe at 620 W for 2 h and the concentration of the final solution was 2 mg mL^{-1} . PVP, was functionalized on NGO to serve as a biocompatible stabilizer of NGO in physiological environment [2]. GO-PVP were prepared according previous report [3]. Typically, 2 mg mL^{-1} aqueous solution of PVP ($M_w = 30000$) was added to the above as-purified NGO solution dropwise and the mixture was continuously stirred at $50 \text{ }^\circ\text{C}$ for 18 h. Afterwards, the resulting mixture was dialyzed against deionized water using a 100 kDa filter (Millipore) to remove the unbound PVP.

AuNP seeds were prepared by the conventional sodium borohydride reduction of chloroauric acid according to the literature [4]. In a typical synthesis, HAuCl_4 aqueous solute (0.6 mL, 30 mM) was diluted to 40 mL with ice-cold deionized water, to which a potassium carbonate (0.2 mL, 0.2 M) was added. No apparent colour change was seen within a few minutes after completely mixing. Thereafter, a total of 4 mL of freshly prepared ice-cold NaBH_4 (0.5 mg mL^{-1}) was quickly injected, which immediately turned from dark yellow to purple black. The reaction was allowed for 5 min under rapid stirring and the final color was salmon pink. Then, the AuNPs were successfully prepared.

Next, a mixture of aniline (100 mM, 30 μ L) and SDS (40 mM, 300 μ L) was added to the AuNPs (3 mL) under stirring. Then the reaction mixture was followed by addition of acidic $(\text{NH}_4)_2\text{S}_2\text{O}_8$ solution (15 mM in 1 M HCl, 15 μ L) and kept stirring for 6 h to ensure complete polymerization. The purified Au@PANI nanocomposites were collected by centrifugation and washing three times.

To obtain GO-Au@PANI, Au@PANI solution were added to large excess of GO-PVP solution. After overnight stirring, excess GO-PVP was removed by centrifugation. Then, the obtained GO-Au@PANI sample was redispersed in water after purified by repeated rinsing and centrifugation. The resulting GO-Au@PANI solution was stored at 4 $^{\circ}$ C for the future applications.

Characterization

TEM images were obtained using JEM-2010HR transmission electron microscope (JEOL, Japan) operated at 200 kV equipped with an energy-dispersive X-ray (EDX) spectrum. UV-vis absorption spectra were recorded using a UV-Vis spectrometer (UV-3200S, MAPADA, Shanghai, China). Raman spectra were recorded using a Renishaw inVia microspectrometer (Derbyshire, England) equipped with both 785 nm (semiconductor laser) and 514 nm excitation (Ar⁺ laser). FT-IR spectra were collected using a Nicolet 6700 FT-IR spectrometer. The photothermal effect of GO-Au@PANI was studied by monitoring the temperature changes of various concentrations of GO-Au@PANI (GO content: 0, 1, 2, 5 and 10 μ g

mL⁻¹) aqueous solution under irradiated by a continuous-wave diode NIR laser (808 nm, 2.5 W cm⁻²) by using a submerged thermocouple thermometer.

Drug loading and release study of GO-Au@PANI

To load DOX onto GO-Au@PANI, different concentrations of DOX in same volume were simply mixed with GO-Au@PANI (100 µg mL⁻¹) to achieve different mass ratio. After overnight stirring, the reaction reached the equilibrium state. Unbound and physically adsorbed DOX molecules were removed by repeated washing and filtration through a 100 kDa filter (Millipore). The resulting GO-Au@PANI/DOX was resuspended and stored at 4 °C. To evaluate the DOX-loading efficiency (DLE), the content of DOX in the supernatant was measured by fluorescence spectroscopy. The drug loading efficiency was calculated by the following equation:

$$\text{DLE}\% = (W_{\text{initial DOX}} - W_{\text{DOX in supernatant}}) / W_{\text{initial DOX}} \times 100\%$$

where $W_{\text{initial DOX}}$ and $W_{\text{DOX in supernatant}}$ were the weights of DOX in original and residual DOX solution, respectively. To study the release behavior of DOX, the GO-Au@PANI/DOX suspension was added into dialysis bag and dialyzed in PBS at pH 7.4 and 5.5, respectively. The laser-triggered drug release behavior was studied in a similar procedure. At predetermined time intervals, the sample was irradiated with a NIR laser (808 nm, 2.5 W cm⁻²) for 10 min. The amount of DOX released was determined by the fluorescence spectroscopy (excitation at 480 nm).

SERS imaging of tumor cells using GO-Au@PANI

The 4T1 murine breast cancer cell line was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO), penicillin (100 units mL⁻¹), and streptomycin (100 mg mL⁻¹). The cells were seeded in culture flasks and incubated at 37 °C in a fully humidified atmosphere containing 5% CO₂. For Raman imaging, the cells were seeded onto quartz substrates in cell culture petri dishes (35 mm). After 24 h incubation, the culture medium was replaced with fresh medium containing the GO-Au@PANI for 8 h. Then the cells were washed with PBS several times and then fixed with neutral buffered formalin (4% formaldehyde) for 10 min. All Raman imaging was performed using a Renishaw inVia confocal Raman system (controlled using WiRE 3.2 software) with a 785 nm laser coupled to a Leica DM-2500M microscope. Before measurement, the instrument was calibrated with a silicon standard whose Raman peak is centered at 520 cm⁻¹. The laser light source which passed through a 50× objective (~1 μm laser spot size) was illuminated on the sample, and the Raman signals were collected using a CCD Camera. Raman mapping was performed in a streamline mode at wavenumber center 1350 cm⁻¹ and the integration time was 2 s.

Cellular dynamics of nanostructures by SERS-fluorescence spectroscopy

To investigate cellular uptake process of the GO-Au@PANI, Raman imaging was carried out after the cells incubation with GO-Au@PANI for 0.5, 1, 2, 4 and 8 h, respectively. To track the intracellular drug release, the cells were incubated with GO-Au@PANI/DOX for 6 h at 37 °C. The DOX release

dynamics was then monitored during a period of 45 min using a SERS-fluorescence microscopy. SERS imaging was performed using the characteristic signals of PANI (1100-1200 cm^{-1}) under 785 nm laser excitation. Fluorescence mapping was carried out under the same microspectrometer with the laser excitation centred at 514 nm. Fluorescence spectral maps were collected in the streamline mode at 0.5 s exposure time at the wavelengths range from 590 nm to 640 nm.

***In vitro* experiments**

Five thousand 4T1 cells were plated in a 96-well plate for 24 h to allow the cells to attach. Different concentrations (0, 0.5, 1, 2, 5, 7.5, 10, 20, 50 and 100 $\mu\text{g mL}^{-1}$) of GO-Au@PANI were introduced into each well. The 4T1 cells were then allowed to incubate for 24 h. The standard MTT assay was carried out to evaluate the cell viabilities of the nanocarriers. For in vitro cancer therapy, the cells were exposed to various concentrations of free DOX, GO-Au@PANI, GO-Au@PANI/DOX, respectively. Then the cells were or were not irradiated by the NIR laser at an output power of 2.5 W cm^{-2} with illumination for 1 min after 6 h of incubation. The anti-tumor effects of these treatments were evaluated by the MTT assay. For fluorescence analysis, 4T1 cells were grown onto sterile glass coverslips in cell culture petri dishes (35 mm) overnight to 70-80% confluence. After that, the cells were exposed to DOX, GO-Au@PANI and GO-Au@PANI/DOX at the DOX concentration of 3 $\mu\text{g mL}^{-1}$. Then the cells were irradiated with an 808 nm laser for 10 min and incubated at 37 $^{\circ}\text{C}$ for

an additional 4 h. Before fluorescence imaging, the cells were fixed with 4% neutral buffered formalin for 10 min and then stained with Hoechst 33258 dye ($4 \mu\text{g mL}^{-1}$) for 10 min in the dark. After three times washings with PBS, the fluorescent pictures of the cells were taken with a fluorescence microscope (Leica DM-2500, Leica Microsystems, Wetzlar, Germany).

***In vivo* antitumor effect**

Female Balb/c mice (5 weeks old) were purchased from laboratory animal center of Sun Yat-Sen university. All animals were treated ethically and in compliance with protocols approved by South China Normal University Animal Care and Use Committee. 4T1 cells (1×10^6) suspended in 100 μL of PBS were implanted into a dorsal subcutis of female Balb/c mice. When the tumor volume reached to 200 mm^3 (around 2 weeks) on average, the mice were randomly divided into 5 groups ($n = 5$ per group), minimizing the differences of weights and tumor sizes among groups. The mice were then intratumorally injected with 100 μL PBS (control, Group I), PBS+laser (Group II), DOX (Group III), GO-Au@PANI+laser (Group IV) or GO-Au@PANI/DOX+laser (Group V) at the DOX concentration of 1 mg kg^{-1} . For NIR laser treated groups, the tumor regions of mice were exposed to the NIR laser (808 nm , 2.5 W cm^{-2}) for 2 min after 12 h injection. The surface temperature of the tumor was simultaneously imaged by an infrared thermal camera (Fluke Ti 200, Fluke Corp, Washington, USA). The dimension of tumors were monitored every two days. The length and width of the tumors were measured by a digital caliper.

The tumor volume was estimated according to the following formula: (tumor width)² × (tumor length)/2. Relative tumor volumes were calculated as V/V₀, where V₀ was the tumor volume at initiation of the treatment.

***In vivo* toxicity assessment**

Body weights of animals were monitored every two days. Body weight change was defined as the relative body weight normalized to their initial weight. For blood analysis and histological examination, the animals were sacrificed 16 days after treatment, and the blood samples and major organs (heart, liver, spleen, lungs and kidney) were collected. All the blood parameters (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase and blood urea nitrogen) were measured in the first affiliated hospital of Jinan University. For histological examination, the organs from various groups were fixed in 4% formalin and processed routinely into paraffin for H&E staining using standard techniques. The slices were examined under the Leica DM-2500M microscope.

References

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