Supplementary Information

Drug loaded multilayered AuNRs for combined photothermal and chemo-therapy

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**Part 1: Optical properties of the nanorods**

The images of rods solution obtained from different synthesis condition were collected by a digital camera at white light. The synthesized rods of different reaction time (3 min to 1020 min), different AgNO3 content (0 µL to 450 µL) and different seed content (12 µL to 60 µL) were evaluated by spectroscopic measurement (Lambda 35 UV-Vis spectrophotometer) at the range of 400 nm-1000 nm. AuNRs/DOX-PE-BSA were dispersed in PBS solution with different concentrations (6.0 - 80 µg / mL) and measured by UV-Vis spectrometer at a wavelength of 481.5 nm.

The impact of reaction time, the content of AgNO3 solution and seed solution on the optical properties of AuNRs were investigated. Figure S1 shows the UV-Vis-IR absorbance spectra of AuNRs at different reaction time (3 min to 1020 min). The absorbance spectra of AuNRs at different reaction time (within 65 min) are displayed in Figure S1A. The LSPR bands were blue-shifted as the reaction time extended. Figure S1B shows the absorbance spectra in a long time range (32 min to 1020 min). The LSPR peaks stopped blue-shifting after 5 h, indicating the completion of the growth process at such time point. The white light photos of AuNRs solution in Fig. 3B are corresponding to seed solution (1), AuNRs at reaction time of 60 min (2), 100 min (3) and 2 h (4) respectively.

The content of AgNO3 solution also correlated to the absorbance spectra of AuNRs. The single absorbance peak at 547 nm separated into two surface plasmon resonance bands after AgNO3 solution was added into the reaction solution (Figure S2). The
LSPR bands characteristic of AuNRs changed as the content of AgNO₃ solution increased within the range of 0 - 450 µL (Figure S2A-B). Additionally, as can be seen in Fig. S2C, the color of AuNRs solution changed from bluish green to deep purple red as the content of AgNO₃ solution increased (50 µL to 325 µL). The aspect ratios of AuNRs also changed within this range (Figure S2D). The influence of the seed solution content on the LSPR peak of AuNRs was also investigated (Figure S2E). As the seed solution content increased from 12 µL to 48 µL, the LSPR peak of AuNRs increased from 750 nm to 824 nm. However, the LSPR peak blue-shifted to 786 nm as the seed solution content changed to 60 µL. The standard absorbance spectrum and the appearance of AuNRs solution adopted for further application at cell and animal level are displayed in Figure S2F. After continuous optimization of the synthetic conditions and rigid purification process via centrifugation, AuNRs with purple red appearance in solution, LSPR peak at 765 nm and aspect ratio of 4.0 were screened out for NIR thermal therapy.
Figure S1: A) The Uv-Vis-IR absorbance spectra for AuNRs at different reaction time (3 min to 65 min); B) The Uv-Vis-IR absorbance spectra for AuNRs at different reaction time (32 min to 1020 min). 1: seed solution; 2: AuNRs of 60 min reaction time; 3: AuNRs of 100 min reaction time; 4: AuNRs of 2 h reaction time.
Figure S2: A) The characteristic LSPR bands of AuNRs changed as different content of AgNO₃ solution (0 - 175 µL); B) The characteristic LSPR bands of AuNRs changed as different volume of AgNO₃ solution (200 - 450 µL); C) The longitudinal plasmon resonance maximum of AuNRs as a function of the volume of AgNO₃ solution added; Insert: the appearance of AuNRs solution changed from bluish green to deep purple red as the content of AgNO₃ solution increased from 50 µL to 325 µL. D) The longitudinal plasmon resonance maximum of AuNRs as a function of their aspect ratio.
aspect ratios; E) The LSPR peak of AuNRs as a function of the seed solution (12 µL to 48 µL); F) The standard absorbance spectrum and the solution appearance of AuNRs.

**Part 2: Standard Curve**

As shown in Figure S3A, the absorbance peaks of PSS and BSA are at 244 nm and 279 nm, respectively. The lack of absorbance peak characteristic of PDDA could be ascribed to the absence of the aromatic heterocyclic and unsaturated double bonds in PDDA structure. The characteristic absorbance peak of DOX is at 485 nm (Figure S2B). As a result, the absorbance exerts no interference to the construction of calibrated curve. Given the standard curve of DOX in the concentration range of 6.0 - 80.0 µg/mL \((Y=0.0182X+0.0248, R^2 = 0.9989)\) (Figure S3B), the saturated drug loading capacity of DOX in the AuNRs-PE-BSA was determined to be approximately 5.4 %.

**Figure S3:** A) the absorbance spectra of PSS and BSA; B) The absorbance spectra
and standard curve of DOX at a concentration range of 6.0 - 80.0 μg/mL.