Supporting Information

Antimicrobial gold nanorods with dual-modality photodynamic inactivation and hyperthermia

Wen-Shuo Kuo, Chich-Neng Chang, Yi-Ting Chang, Chen-Sheng Yeh*

Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan.

*To whom correspondence should be addressed. E-mail: csyeh@mail.ncku.edu.tw
Experimental section

Materials

Hydrogen tetrachloroaurate (III) hydrate (HAuCl₄) was purchased from Alfa Aesar Co. (Ward Hill, MA, USA). Cetyltrimethylammonium bromide (CTAB), sodium citrate and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Polymers of polyacrylic acid (PAA) (M.W. ~50000 Da) were obtained from Polysciences Inc. (Warrington, PA, USA). Toluidine blue O (TBO) was purchased from Acros Organics Co. (Geel, Belgium). LIVE/DEAD BacLight Bacterial viability kits and Singlet Oxygen Sensor Green Reagent were acquired from Invitrogen Corp. (Carlsbad, CA, USA). All chemicals and reagents were of analytical grade.

Synthesis of Au-PAA-TBO nanorods

The Au nanorods were synthesized using the seedless growth method.¹ The mean length and width of the Au nanorods were approximately 35 nm and 9.3 nm, respectively, and their aspect ratios (length divided by width) were about 3.8. The positively charged surface of the Au nanorods was changed to a negatively charged surface by mixing the nanorods with polyacrylic acid (PAA) (M.W. ~50000). Excess PAA in the solution was removed by centrifuging the rod solution at 7500 rpm for 10 min. The resulting precipitates were redispersed in deionized water. The excess TBO
was dissolved in aqueous solution and as-prepared Au-PAA nanorods were mixed in a tube. EDC was added to the tube and incubated for 1 h. The mixture was centrifuged at 7500 rpm for 10 min and then the supernatant was collected. The precipitates were washed several times with deionized water. The Au-PAA-TBO nanorods were obtained and redispersed in deionized water. The concentration of Au nanorods was determined using an atomic absorption (AA) spectrometer (Solaar, UK).

**Characterization**

Electron micrographs using transmission electron microscopes (JOEL 1200, at 80KV; JEOL 3010, at 300KV; and PHILIPS CM-200, at 200KV, Japan) were obtained by placing a drop of the sample on a copper mesh coated with an amorphous carbon film; the solvent was then evaporated in a vacuum desiccator. The formation of amide bonds between TBO and PAA after addition of EDC was confirmed by an electron-impact (EI) mass measurements using a Magnetic Sector Mass Analyzer (JEOL, JMS-700). The UU-Vis absorption spectra were recorded on a spectrometer (Agilent 8453, USA). FTIR spectra were collected using a spectrometer (PerkinElmer RX1, USA). The data of zeta potential was measured using a spectrometer (Manern Nano-ZS90, UK).

**Estimating the average amount of TBO per Au nanorod**

The average amount of TBO per Au nanorod was estimated using UV-Vis spectra. The excess TBO was dissolved in deionized water, and then it was measured and the absorbance was recorded using UV-Vis spectra. The same quantity of TBO was then coated with Au-PAA nanorods for 1 h using covalent conjugation. The mixture was
centrifuged at 7500 rpm for 10 min and then the supernatant was collected. The absorbance difference between pure TBO and the collected supernatant was calculated, and then the molar concentration of TBO coated on Au-PAA nanorods was estimated using Beer’s Law. The amount of Au nanorods was quantified by atomic absorption (AA) spectrometer. Finally, the average amount of TBO per Au nanorod was derived.

Culturing methicillin-resistant *Staphylococcus aureus* (MRSA)

This study used methicillin-resistant *Staphylococcus aureus* (ATCC 27659). Bacteria were grown in a brain heart infusion (BHI) (DIFCO 0418; BD Biosciences, San Jose, CA, USA) at 37 °C. The growth bacteria were centrifuged and the pellet resuspended in PBS. The OD$_{600}$ was adjusted to 0.05.

Determining bacteria viability rates after laser exposure

Method 1: Bacteria (OD$_{600}$ ~0.05) were added to TBO, PAA-TBO, and Au-PAA-TBO nanorods, respectively, and incubated for 6 h in a 1.5-mL tube at 37 °C. After they had been incubated, the mixture was centrifuged and the pellet of bacteria were diluted (OD$_{670}$ ~0.15) and exposed to a 633-nm HeNe laser (fixed output power: 0.06 W cm$^{-2}$, beam spot: 40.8 mm$^2$) (Coherent Inc., Santa Clara, CA, USA) or an 808-nm continuous-wave (CW) diode laser (maximal output power: 3.1 W cm$^{-2}$, 16 mm$^2$ of laser beam spot area) (Enwave Operonics, Inc., Model FSL-808-450MS-FS), and then
SYTO 9/propidium iodide (PI) (Live/Dead BacLight Bacterial viability Kits, Invitrogen) was added and mixed. Measurements were obtained using a fluorescence spectrophotometer (F-2500, Hitachi) according to the manufacturer’s instructions.²

Method 2: Bacteria (OD₆₀₀ ~0.05) were added to TBO, PAA-TBO, and Au-PAA-TBO nanorods, respectively, and incubated for 6 h in a 1.5-mL tube at 37 °C. After incubation, the mixtures were centrifuged and the pellets of bacteria were diluted to OD₆₀₀ ~0.15 and exposed to a 633-nm HeNe laser (fixed output power: 0.06 W cm⁻², beam spot: 40.8 mm²) (Coherent Inc., Santa Clara, CA, USA) or an 808-nm continuous-wave (CW) diode laser (maximal output power: 3.1 W cm⁻², 16 mm² of laser beam spot area) (Enwave Operonics. Inc., Model FSL-808-450MS-FS). The bacteria (OD₆₀₀ ~0.15) were then diluted to a dilution factor of 10⁻⁴ and plated on the BD brain heart infusion (BHI) agar plates (DIFCO 0418; BD Biosciences, San Jose, CA, USA). The plated were incubated aerobically at 37 °C for 12-16 h. After incubation, the number of bacteria surviving (CFU mL⁻¹) was determined.

Singlet oxygen detection

Bacteria (OD₆₀₀ ~0.05) were added Au-PAA-TBO nanorods (1.8 × 10⁹) and to TBO alone, respectively, and then incubated for 6 h at 37 °C. After they had been incubated, the mixture was centrifuged and the bacteria were diluted (OD₆₇₀ ~0.15) and exposed to a 633-nm HeNe laser (0.06 W cm⁻²), and then 1 μM of Singlet Oxygen Sensor
Green Reagent (Invitrogen) (Ex/Em: 488/525 nm) was added. Measurements were obtained using a fluorescence spectrophotometer (F-2500, Hitachi) according to the manufacturer’s instructions.\textsuperscript{3-5}

Temperature dependence of irradiation time for Au-PAA-TBO nanorods with 808-nm laser irradiation

The temperature elevation of Au-PAA-TBO nanorods (1.8 \times 10^9 Au nanorods) was measured using an 808-nm CW diode laser (3.1 W cm\textsuperscript{-2}) in a range of irradiation times (5-40 min). A thermocouple (TES-1319A–K type; Tes Instrument, Shanghai, China) was immersed in the composite solutions to determine bulk temperature of solution.

References


**Fig. S1** Mass (electron impact) spectroscopy of PAA-TBO complexes after EDC conjugation. The m/z 299 and 329 fragments indicated the formation of amide bonds.
**Fig. S2** UV-Vis spectra of Au nanorods, Au-PAA nanorods, and Au-PAA-TBO nanorods at a particle concentration of $4.4 \times 10^9$ Au nanorods mL$^{-1}$. (Inset: UV-Vis spectra of pure PAA and TBO).
Fig. S3 FTIR spectra of (a) PAA, (b) TBO, and (c) Au-PAA-TBO nanorods.
Fig. S4 Bacteria were incubated with Au-PAA-TBO nanorods in the dark for 24 h and then their viability was evaluated using (a) a Live/Dead kit (Invitrogen) and (b) colony forming unit counting method. The nanorods were delivered in a range of dosages (range: from $2.2 \times 10^8$ to $3.5 \times 10^9$ Au nanorods). The presented data was an average of 4 runs in each experimental condition.
Fig. S5 PACT experiments were performed against methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Bacteria treated Au-PAA-TBO nanorods were exposed to a HeNe 633 nm laser (0.06 W cm$^{-2}$, beam spot: 40.8 mm$^2$) and Au nanorods were delivered in the dose of 1.8x10$^9$ Au nanorods (The quantities of free TBO and TBO conjugated on Au nanorods were fixed at 6.25 nM.). All the experiments exploiting Au-PAA-TBO nanorods and TBO were done in completely dark room. Bacterial viability was evaluated by the colony forming unit counting method. The presented data was an average of 10 runs in each experimental condition.
Fig. S6 The temperature profile of Au-PAA-TBO nanorods ($1.8 \times 10^9$ Au nanorods) irradiated using a 808 nm CW diode laser ($3.1 \text{ W cm}^{-2}$, beam spot: $16 \text{ mm}^2$) as a function of irradiation time. The presented data was an average of 5 runs in each experimental condition.
Fig. S7 Photothermal ablation experiments were performed on MRSA. Bacteria-treated Au-PAA-TBO nanorods (1.8 × 10^9 Au nanorods) were exposed to an 808-nm CW laser (3.1 W cm^{-2}, beam spot: 16 mm^2). The viabilities were evaluated using (a) a Live/Dead kit and (b) colony forming unit counting method. The presented data was an average of 5 runs in each experimental condition.
Fig. S8 Bacteria-treated Au-PAA-TBO nanorods and bacteria alone were exposed to a HeNe 633 nm (0.06 W cm$^{-2}$, beam spot: 40.8 mm$^{2}$) for 1 min and a continuous-wave (CW) diode 808 nm laser (3.1 W cm$^{-2}$, beam spot: 16 mm$^{2}$) for 25 min. Au nanorods were delivered at the doses of (a) $1.8 \times 10^9$ and (b) $0.9 \times 10^9$ Au nanorods. The viabilities were evaluated using colony forming unit counting method. The presented data was an average of 10 runs in each experimental condition.