SUPPORTING INFORMATION

Highly effective photodynamic inactivation of *Escherichia Coli* pathogens using gold nanorods/SiO_2 core-shell nanostructures with embedded verteporfin.

S1. Experimental part

S1.1. Materials

1.4Cetyl trimethylammonium bromide (CTAB), silver nitrate ($AgNO_3$), hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \times 3H_2O$), tetraethylorthosilicate (TEOS), ascorbic acid, sodium hydroxide (NaOH), verteporfin, 9,10-anthracenediylbis(methylene)dimalonic acid (ABDA) and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich and used as received.

S1.2. Synthesis of gold nanorods (Au NRs)

Au NRs were synthesized according to a seed-mediated, surfactant-assisted procedure adapted from the works of El-Sayed[1] and Murphy[2] and co-workers. Seed solution was prepared by mixing $HAuCl_4$ (10 mM, 125 μL) with an aqueous solution of CTAB (0.2 M, 5 mL). To this solution was added ice-cold $NaBH_4$ (0.01 M) and the solution was stirred for 5 min. The resulting seed solution became brownish yellow, and aged for 5 min at room temperature before use.

For the growth solution, 75 μL, 85 μL or 95 μL in order to synthesize Au NRs with longtitudal plasmons at 700, 800 and 900 nm, respectively, were added to of 10 mM $AgNO_3$ and 500 μL of $HAuCl_4$ (10 mM) and mixed with 5 mL of water and 5 mL of CTAB (0.2 mM) solution. The solution was acidified to pH 3-4 with 200 μL of 1% HCl, then 60 μL of 0.1 M ascorbic acid solution was added. Finally 48 μL of seeds was injected into the growth solution. Reaction was performed at 25 °C without stirring. The excess of CTAB surfactant was discarded from the final product by centrifugation at 10000 rpm for 30 min and then the precipitate was re-dispersed in 10 mL of DI water to yield Au NRs in a concentration of 10 nM. The concentration was derived spectrophotometrically from the extinction coefficient determined at 700, 800 and 900 nm longtitudal plasmons to be 3.5×10^9 , 4.8×10^9 and 6.2×10^9 M^{-1} cm^{-1}.[3]

S.1.3.Preparation of Au NRs@SiO_2 particles

To a solution of as-prepared 10 nM Au NRs (1 mL in DI water), 187 μL of 20 mM tetraethoxysilane (TEOS) solution in ethanol was added. The reaction was run in a 2 mL plastic eppendorf due to a strong interaction of glassware with TEOS. The pH of the solution was adjusted to 10 by means of 10 mM NaOH. Next, vigorous stirring of horizontally placed eppendorf (belly dancer, 300 rpm) for 24 h at 23°C was performed to properly grow the silica layer over CTAB coated AuNRs. Importantly, the concentration of CTAB prior to silica coating was carefully controlled to be precisely 1 mM. The excess of the reagents as well as simultaneously formed empty silica particles has been separated from AuNRs@SiO_2 by means of centrifugation (8000 rpm, 30 min). Supernatant was carefully removed and a pellet was re-dispersed in 1 mL of DI water to give 5 nM AuNRs@SiO_2 with a silica shell of 20±3 nm in thickness.

S.1.4. Loading of Au NRs@SiO_2 particles with verteporfin (VP)

To a solution of 5 nM AuNRs@SiO_2 (1 mL in DI water) were added 20 μL of verteporfin (1 mM in DMSO). The reaction mixture was stirred for 20 h at room temperature. The formed AuNRs@SiO_2-VP nanocomposite was purified by means of centrifugation (8000 rcf, 15 min) and re-dispersed in distilled water. The supernatant was used to determine the residual verteporfin concentration spectrophotometrically (λ = 692 nm, ε = 13500 M^{-1} cm^{-1}, in PBS).[4] 4 μM Verteporfin were adsorbed on 5 nM AuNRs@SiO_2, corresponding to ≈ 800 molecules per nanorod.

Estimation of Au NRs in 1 mL:

$$N_{Au\ NRs} = 5 * 10^{-9} \times 6.02 * 10^{23} \times 10^{-3} = 3 * 10^{12}\ particles\ per\ 1\ mL$$

Estimation of VP molecules per Au NRs in 1 mL:

$$N_{VP} = 4 * 10^{-6} \times 6.02 * 10^{23} \times 10^{-3} = 2.4 * 10^{15}\ molecules\ per\ 1\ mL$$

$$N = \frac{N_{VP}}{N_{Au\ NRs}} = \frac{2.4 * 10^{15}}{3 * 10^{12}} = 800\ molecules$$

S.1.5. Single oxygen detection

Singlet oxygen generation was monitored through the chemical oxidation of an aqueous solution of 9,10-anthracenediylbis (methylene)dimalonic acid (ABDA) (10 µM) in presence of VP (4 µM) and AuNRs@SiO_2 and Au NRs@SiO_2-VP each at 230 pM respectively. The decrease in the ABDA absorbance at 378 nm was first monitored under irradiation at 710 nm and a laser power of 0.5 W cm^{-2} using a continuous laser (CW, unmodelocked MIRA-900, France) or a femtosecond pulsed titanium:sapphire laser (Chameleon model, Coherent) that delivers 100 fs pulses at a repetition rate of 80 MHz. The laser beam was focused onto a cuvette (1 cm path length and 2 mm width) containing 1 mL of solution. Irradiation was stopped at 1, 2, 5, 10, 20, 30 min and absorption spectra were recorded.

S.1.6. $^1O_2$ quantum yield estimation

To estimate the $^1O_2$ quantum yield (Φ_x) of VP and AuNRs@SiO_2 and Au NRs@SiO_2-VP nanostructures, (ln A/A_0) of ABDA at 378 nm was plotted *vs.* the photo-irradiation time and fitted to a pseudo-first-order kinetic model. The $^1O_2$ quantum yield was estimated using methylene blue (MB) as reference (Φ_{MB} = 0.52 in H_2O) according to equation 1:

$$\Phi_x = \Phi_{MB} \cdot \frac{k_x}{I_x} \cdot \frac{I_{MB}}{k_{MB}}$$

where, Φ_{MB} us the $^1O_2$ quantum yield of MB in H_2O, k_x and k_{MB} the rate constants for the photoreaction of ABDA with AuNRs and MB, respectively, and I_x and I_{MB} the optical density of AuNRs and MB in H_2O at 710 nm wavelengths of irradiation.

S.1.7. Photodynamic ablation of bacteria solutions

All bacteria were used from fresh night pre-culture (LB medium, OD_{600} = 1.0). In a total volume of 110 µL in 96-wells plate, bacteria (10^4 cfu mL^{-1}) have been mixed with the corresponding testing compounds PBS (control), VP (5 µM, 0.5 % DMSO), Au NRs (100 pM), AuNRs@SiO_2 (100 pM), Au NRs@SiO_2-VP (100 pM) in a sterile PBS. Both irradiated and non-irradiated controls have been created (3 wells per condition). The laser beam diameter was adjusted exactly to the square of the well to be ~ 0.3 cm^2. Wells were irradiated for 10 and 30 min at 1 W cm^{-2}. The conditions of the experiment are constructed in a way to complete the treatment within 2 h to prevent long exposure of bacteria to PBS solution.

Bacteria titer assay was used to determine the residual concentration of bacteria in wells. To perform a titer assay, 10-fold dilutions of a bacteria stock 10^4 cfu mL^{-1} are prepared, and 10 µL aliquots are dropped on the LBagar + Kanamycin plates. After an incubation period of 20 h at 37°C in a conventional thermostat, colonies were counted.

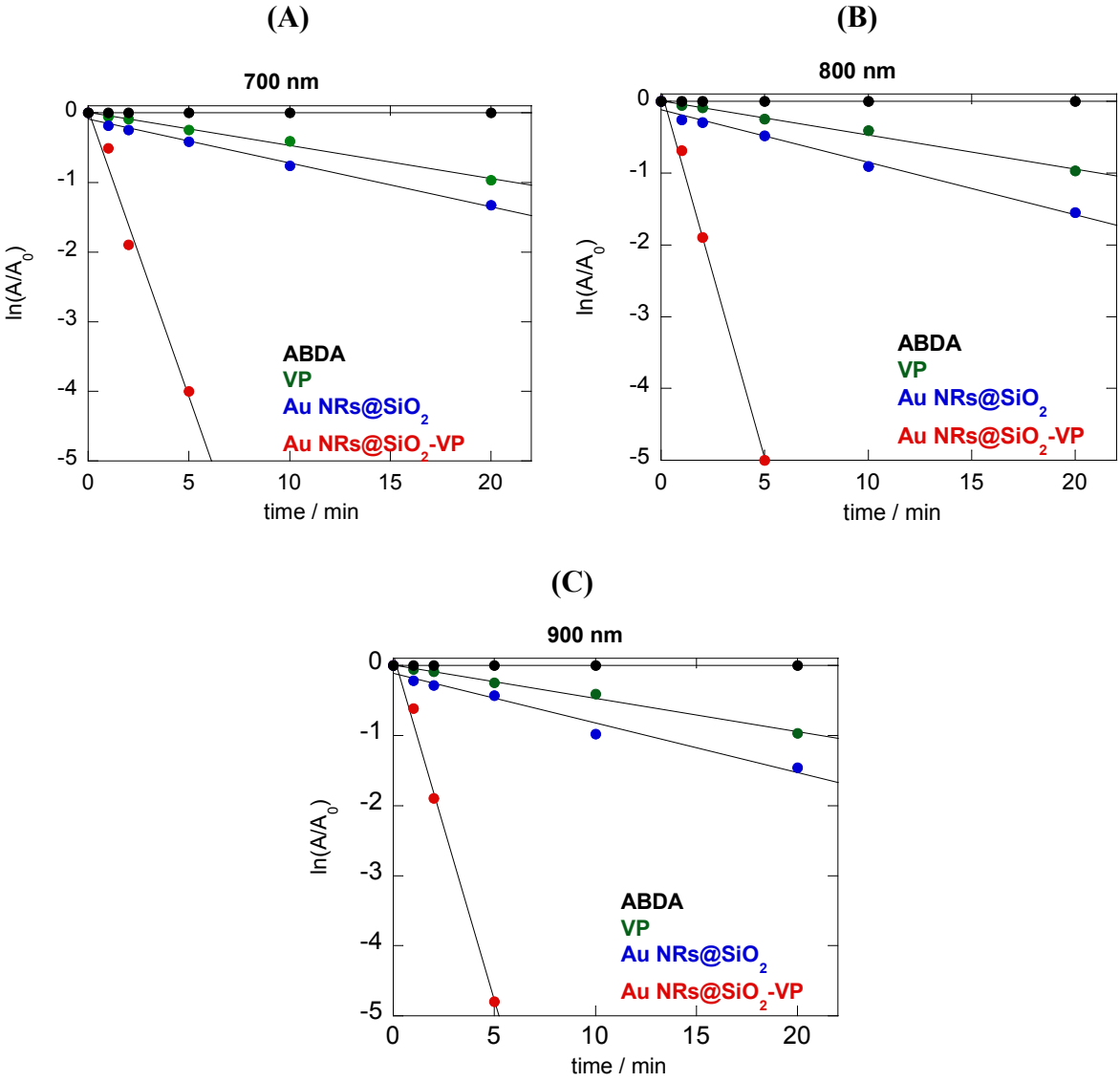
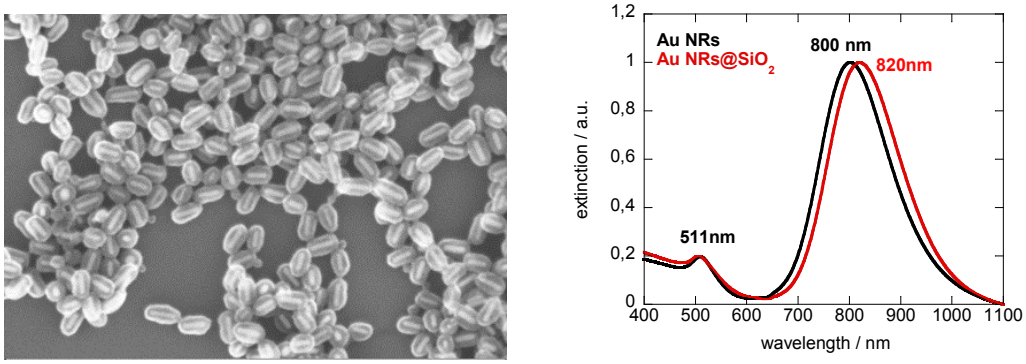
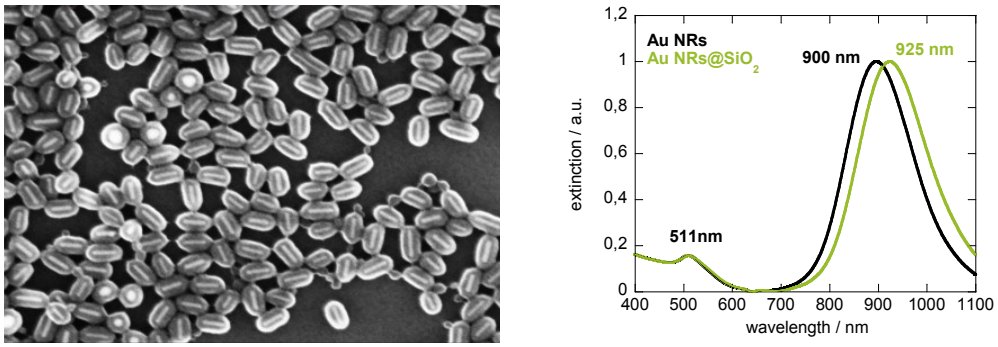


Figure S1. Pseudo-first order plots for VP (4 µM), AuNRs@SiO_2 (230 pM), and AuNRs@SiO_2-VP (230 pM+4µM) with longitudinal plasmon bands at ≈700 nm (A), ≈800 nm (B) and ≈900 nm (C).

(A)



(B)



(C)

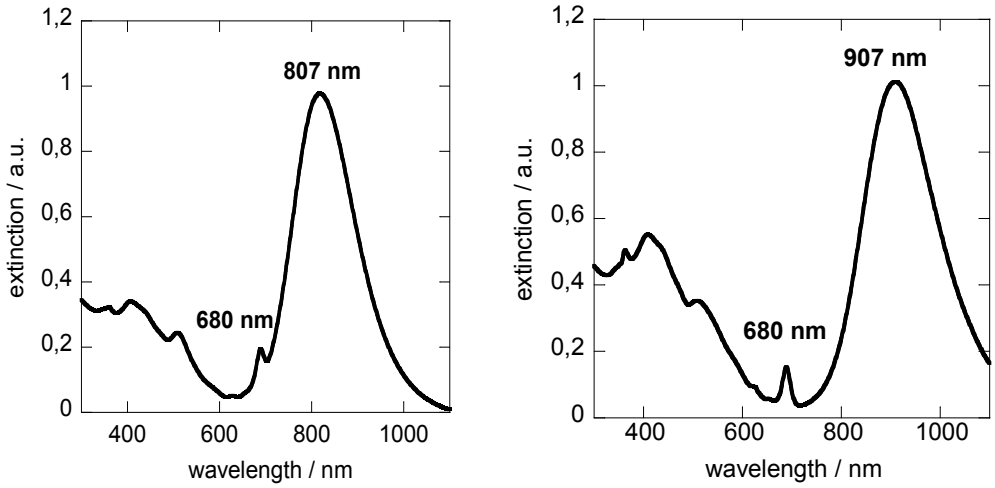


Figure S2. SEM and UV/Vis absorption spectra of Au NRs@SiO_2 nanostructuers with longitudinal plasmon bands at 800 (A) and 900 nm (B) scale 200 nm ; (C) and UV/Vis absorption spectra Au NRs@SiO_2-VP nanostructuers with longitudinal plasmon bands at 800 and 900 nm

References

(1) Nikoobakht, B.; El-Sayed, M. A. *Chem. Mater.* **2003**, *15*, 1957.
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(3) Orendorff, C. J.; Murphy, C. J. *J. Phys. Chem. B* **2006**, *110*, 3990.
(4) Aveline, B.; Hasan, T.; Redmond, R. W. *Photochem. Photobiol.* **1994**, *59*, 328.