

Supporting information for:

Photogenerated Charge Carriers and Reactive Oxygen Species in ZnO/Au Hybrid Nanostructures with Enhanced Photocatalytic and Antibacterial Activity

Weiwei He^{†‡}, Hyun-Kyung Kim^{‡§}, Wayne G. Wamer[‡], David Melka[‡], John H. Callahan[‡], Jun-Jie Yin^{‡}*

[†]Key Laboratory of Micro-Nano Materials for Energy Storage and Conversion of Henan Province, Institute of Surface Micro and Nano Materials, Xuchang University, Henan 461000, P. R. China

[‡]Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD 20740, USA

[§]Food Safety Bureau, Ministry of Food and Drug Safety, Osong Health Technology Administration Complex 363-700, Republic of Korea

*Corresponding Author: junjie.yin@fda.hhs.gov

MATERIALS AND METHODS

Chemical and Materials. An aqueous dispersion of zinc oxide nanoparticles (20 wt%, 30-40 nm) was purchased from US Research Nanomaterials, Inc (Houston, TX). The spin-trap 5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO) was purchased from Applied Bioanalytical Labs (Sarasota, FL). Chloroauric acid hydrated, Rhodamine B (RhB), methylene blue (MB), NaN_3 , DMSO and superoxide dismutase (SOD) were purchased from Sigma Chemical Co. (St. Louis, MO). 1-Hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) were purchased from Alexis, Enzo Life Sciences, Inc. (NY, USA). 4-Oxo-2,2,6,6-tetramethylpiperidine (4-Oxo-TEMP), diethylene triamine pentaacetic acid (DTPA), xanthine, xanthine oxidase, salicylic acid and standard buffer solutions were all purchased from Sigma-Aldrich (St. Louis, MO). Milli-Q water (18 M Ω cm) was used for preparation of all solutions.

Synthesis of ZnO/Au hybrid nanostructures. In a typical synthesis, 5 ml of 1.0 mg/ml ZnO suspension was mixed with 0.1 ml of 0.5 mg/ml HAuCl_4 aqueous solution in a quartz tube and sealed. The molar percentage of Au to ZnO in this representative reaction is 0.2%. The above solution was sonicated for 3 min, then irradiated for 15 min with a 450 W Xenon lamp. A clearly evident color change from white to dark grey was observed, indicating the formation of ZnO/Au hybrid nanostructures. We refer to the product of this reaction as ZnO/Au0.2%. Similarly, other ZnO/Au hybrid nanoparticles having different molar percentages of Au were synthesized by simply changing the amount of HAuCl_4 added. We observed as more HAuCl_4 was added, a darker product was produced. Finally, the precipitate was collected by centrifugation, washed three times with double distilled water, and diluted to 5.0 ml with water for further experiments.

Characterization. Transmission electron microscopy (TEM) and high resolution TEM (HRTEM) images were captured on a JEM 2100 FEG (JEOL) transmission electron microscope (accelerating voltage of 200 kV) located at the NanoCenter, University of Maryland, College Park, MD. Energy-dispersive X-ray analysis (EDX) was conducted using the same microscope. The samples for TEM analysis were prepared by adding drops of the redispersed colloidal solutions onto standard holey carbon-coated copper grids, which were then air dried at room temperature. The crystal structure of synthesized products was characterized by X-ray diffraction (XRD, Bruker D8 Advance diffractometer) monochromatized Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$). UV-Vis absorption spectra were obtained using a Varian Cary 300 spectrophotometer. The surface area of ZnO and ZnO/Au structures with different Au loading were analyzed using Autosorb® iQ™ surface area analyzer and AsiQwin™ software.

The photocatalytic activities of ZnO and various ZnO/Au hybrid nanostructures were evaluated by measuring the degradation of methylene blue (MB) and salicylic acid (SA) in aqueous solutions. 0.1 mg/ml of ZnO or as-prepared ZnO/Au photocatalyst was dispersed in a 20 mL aqueous solution containing 0.2 mg/l MB or 0.5 mM SA. The solution was continuously stirred in the dark for about 1 h to establish an adsorption-desorption equilibrium between the photocatalyst and substrates. Then, the suspension was irradiated using a 800 W Xenon lamp filtered to deliver simulated sunlight. During irradiation, the solution was stirred to maintain a suspension. At selected time intervals, aliquots of suspension were removed and centrifuged. The residual concentration of the MB or SA in the supernatant was monitored using a Varian Cary 300 spectrometer.

Growth of bacteria and test of antibacterial activity of ZnO/Au nanostructures. Pure bacterial cultures of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus subsp. aureus*

(ATCC 29213) were chosen as gram negative and positive model organisms, respectively, for antibacterial activity experiments. Both bacteria were grown overnight on BHIA (Brain Heart Infusion Agar, BD-Difco) plates in an incubator at 37°C. Bacterial growth was harvested using a sterilized swab and resuspended in the 0.01 × PBS buffer to achieve a viable cell concentration of about 1.5 to 2.0 × 10⁹ cells/ml. This suspension of bacteria was used as a stock suspension for antibacterial activity tests.

Antibacterial activities of nanomaterials were tested in 96-well plates. One hundred eighty µl of the bacterial stock suspension was transferred into each well and 20 µl of 0.05 mg/mL nanomaterials was added to each well. The 96-well plates containing bacteria and nanomaterials were irradiated under a solar simulator (Model 91190-1000, Oriel, Stratford, CT) for 10 minutes and then incubated at 37°C for 1 hour. The bacteria and nanomaterial mixture in each well was then serially diluted and 10 µl from each dilution was spread onto BHIA agar plates. The plates were incubated at 37°C overnight, and the resulting bacterial growth was enumerated in colony forming units (CFU). The bacteria survival rate was calculated as follows:

$$\text{Survival rate (\%)} = 100 \times \text{CFU (cell + nano + rad)} / \text{CFU}_o \text{ (cell)}$$

where CFU (cell + nano + rad) is the number of colony forming units measured after plating cells irradiated in the presence of nanomaterials, and CFU_o (cell) is the number of colony forming units measured after plating cells exposed to neither nanomaterials nor simulated sunlight. All tests were conducted in triplicate and repeated at least twice to confirm reproducibility.

Electron spin resonance spectroscopy. All the ESR measurements were carried out using a Bruker EMX ESR spectrometer (Billerica, MA) at ambient temperature. A solar simulator consisting of a 450 W Xenon lamp filtered to provide simulated sunlight was used in

ESR studies. Fifty microliter aliquots of control or sample solutions were put into quartz capillary tubes with internal diameters of 0.9 mm and sealed. The capillary tubes were inserted in the ESR cavity, and the spectra were recorded during irradiation at selected times. All ESR measurements were carried out using the following settings for detection of the spin adducts: 20 mW microwave power, 100 G scan range and 1 G field modulation.

The spin trap BMPO was used to verify the formation of superoxide ($\bullet\text{OOH}$) and hydroxyl radicals ($\bullet\text{OH}$) during exposure of ZnO and ZnO/Au hybrid nanostructures to simulated sunlight. 4-oxo-TEMP was used to demonstrate the generation of singlet oxygen during irradiation of samples. CPH and TEMPO were used as spin labels for studying the holes and electrons generated during photoexcitation of ZnO/Au hybrid nanostructures. In these experiments, the intensity of the ESR signal was measured as the peak-to-peak height of the second line of ESR spectrum. ESR spectra were recorded from the sample mixture, containing spin probes (BMPO, 4-Oxo-TEMP, CPH or TEMPO) and nanoparticles (ZnO or ZnO/Au), after exposure to simulated sunlight for selected times. For comparison, the controls either without catalysts or without irradiation were also recorded. The final concentration of each component is described in each figure caption.

To further distinguish each ROS, DMSO, SOD and NaN_3 were employed separately to test their scavenging effect on the ESR signal for hydroxyl radical, superoxide and singlet oxygen, respectively. To test the effect of superoxide on various spin probes, xanthine and xanthine oxidase (XOD) were mixed together to generate superoxide. The control sample contained 0.05 mM DTPA, 1mM xanthine and various spin probes in water, to which 0.2 U/mL XOD was introduced to initiate the production of superoxide. Hydroxyl radicals were produced by irradiating 1 M hydrogen peroxide.

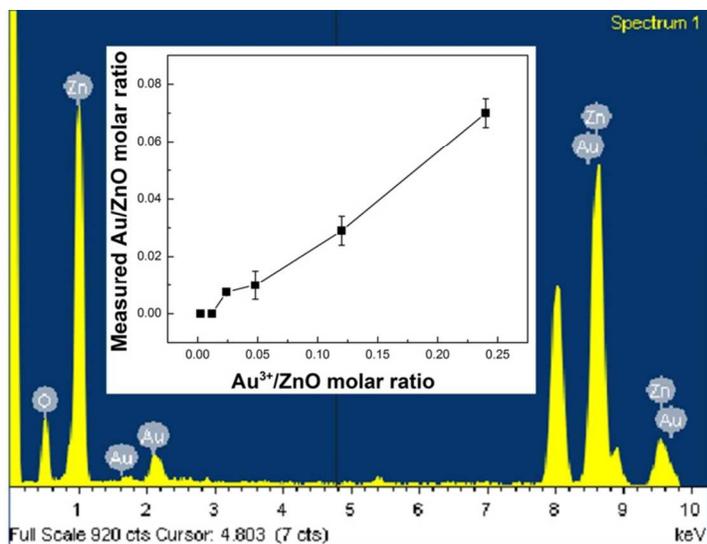


Figure S1. Energy-dispersive X-ray (EDX) analysis of ZnO/Au hybrid nanoparticles. Inset: Effect of the added $\text{HAuCl}_4^-/\text{ZnO}$ molar ratio on the obtained Au/ZnO molar ratio in the nanostructures measured by EDX analysis. Au/ZnO ratio from EDX vs $\text{HAuCl}_4^-/\text{ZnO}$ ratio.

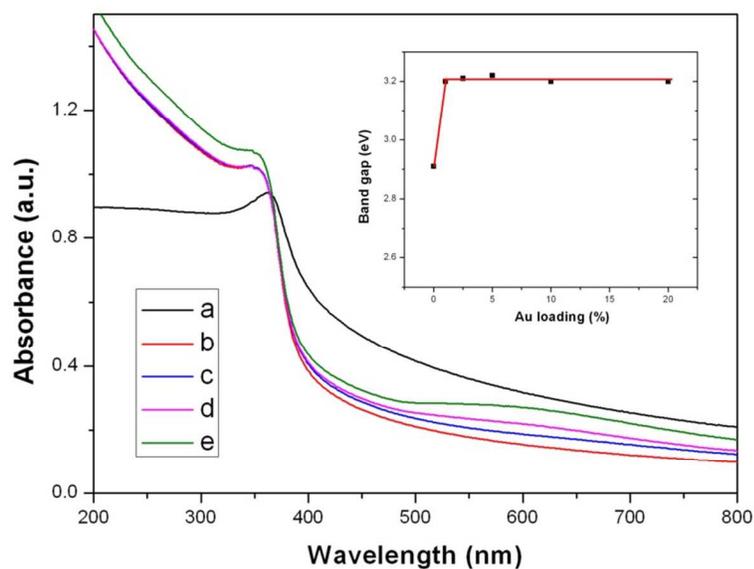


Figure S2. UV-Vis spectra of ZnO (a) and various ZnO/Au hybrid nanostructures formed at different $\text{HAuCl}_4^-/\text{ZnO}$ molar ratios of 0.2% (b), 1.0% (c), 2.0 (d) and 4.0% (e). Inset: band gap

changes calculated from the spectra corresponding to ZnO and different ZnO/Au hybrid nanostructures.

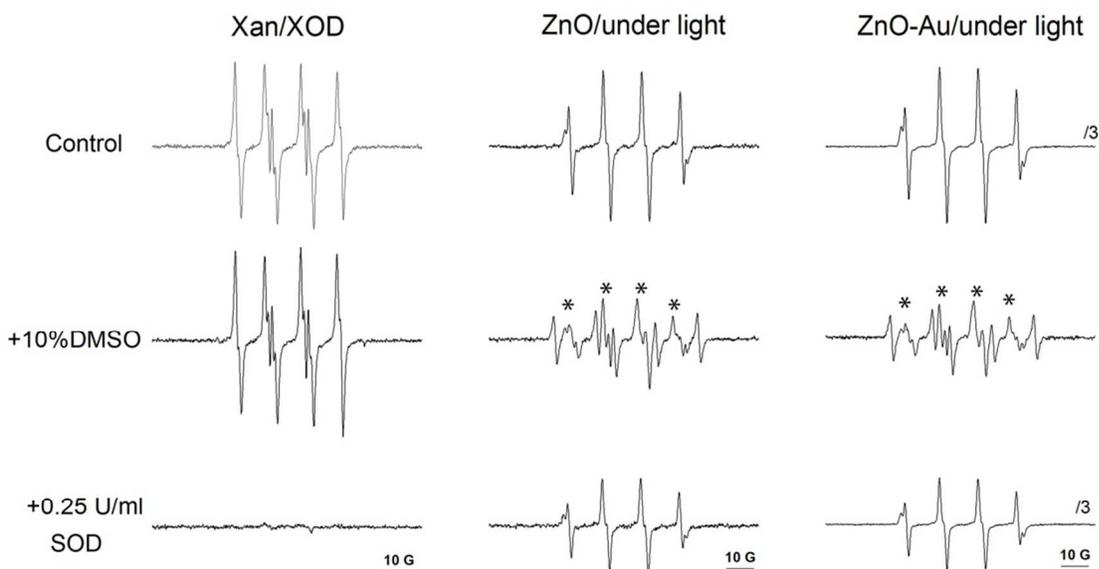


Figure S3. Effect of DMSO and SOD on superoxide generated by mixing xanthine (Xan) and xanthine oxidase (XOD) and ESR signal generated from ZnO and ZnO-Au hybrid nanostructures under simulated sunlight. In the Xan/XOD oxidase system, ESR spectra were obtained from samples containing 0.05 mM DTPA, 1 mM Xan, 0.2U/ml XOD, and 10% DMSO (or 0.25 U/ml SOD), spectra were recorded at 5 min after mixing. For ZnO and ZnO/Au, the spectra were obtained at 5 min under irradiation from samples containing 25 mM BMPO and 0.1 mg/ml ZnO or ZnO/Au2% nanostructures without and with 10% DMSO or 0.25 U/ml SOD.

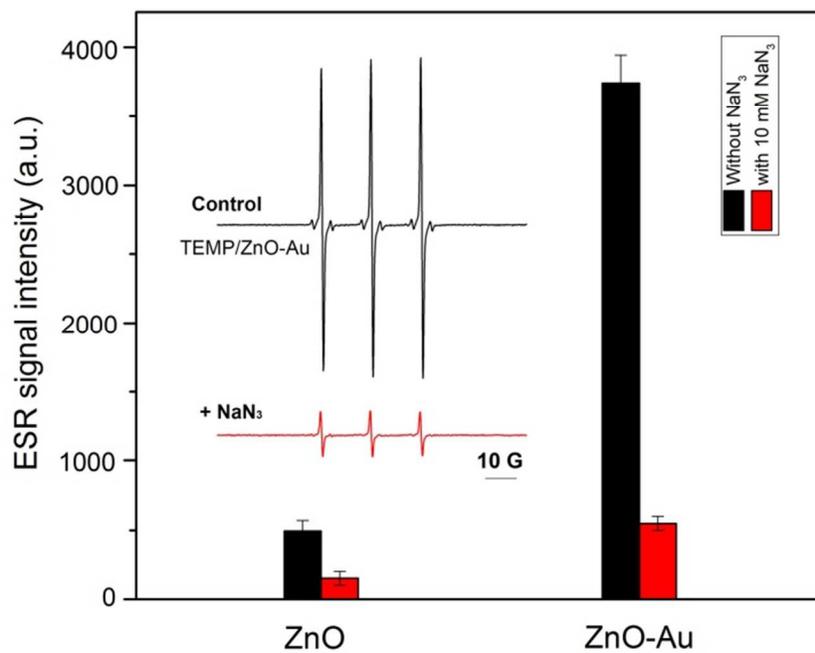


Figure S4. Effect of NaN₃ on ESR signal intensity from spin labels 4-Oxo-TEMP in the presence of ZnO or ZnO/Au hybrid nanostructures. (Conditions: 10 mM 4-Oxo-TEMP, 10 mM NaN₃, 0.1 mg/ml ZnO or ZnO/Au hybrid nanostructures with Au/ZnO loading ratio 4%, irradiation for 3 min with simulated sunlight.)

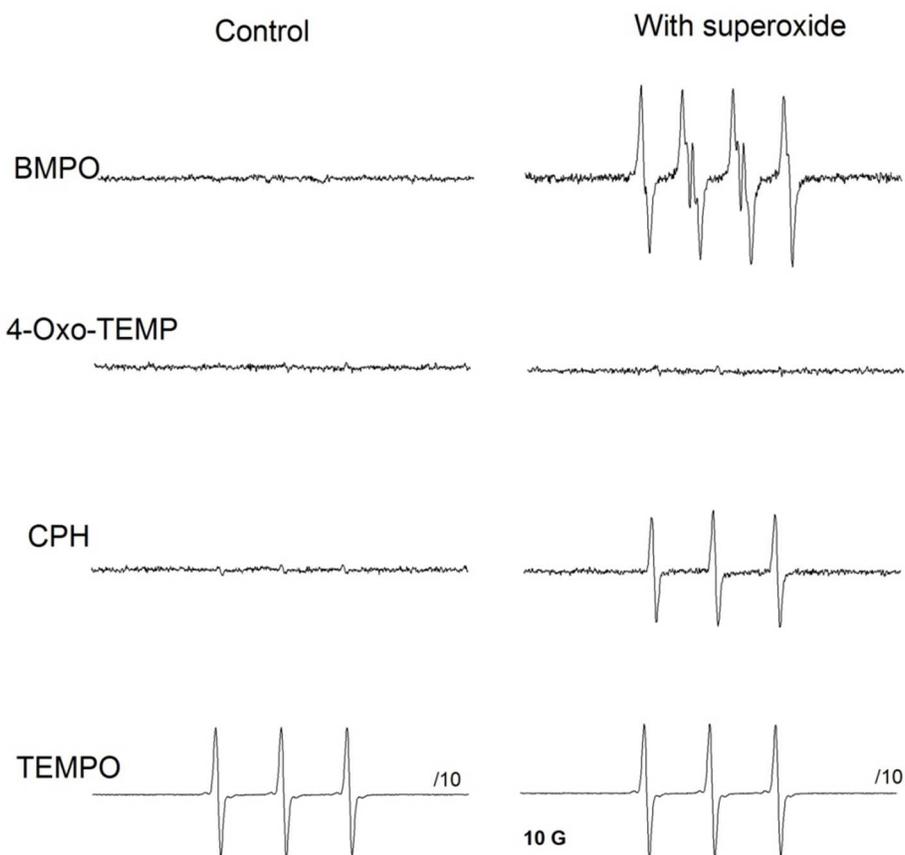


Figure S5. Effect of superoxide on different spin probes. The conditions used to form superoxide are same as in Figure S3. The control spectra were obtained from samples containing 0.05 mM DTPA, 1 mM xanthine and 25 mM BMPO (or 10 mM 4-Oxo-TEMP, or 0.02 mM CPH, or 0.02 mM TEMPO) before adding 0.2U/ml XOD to initiate the generation of superoxide.

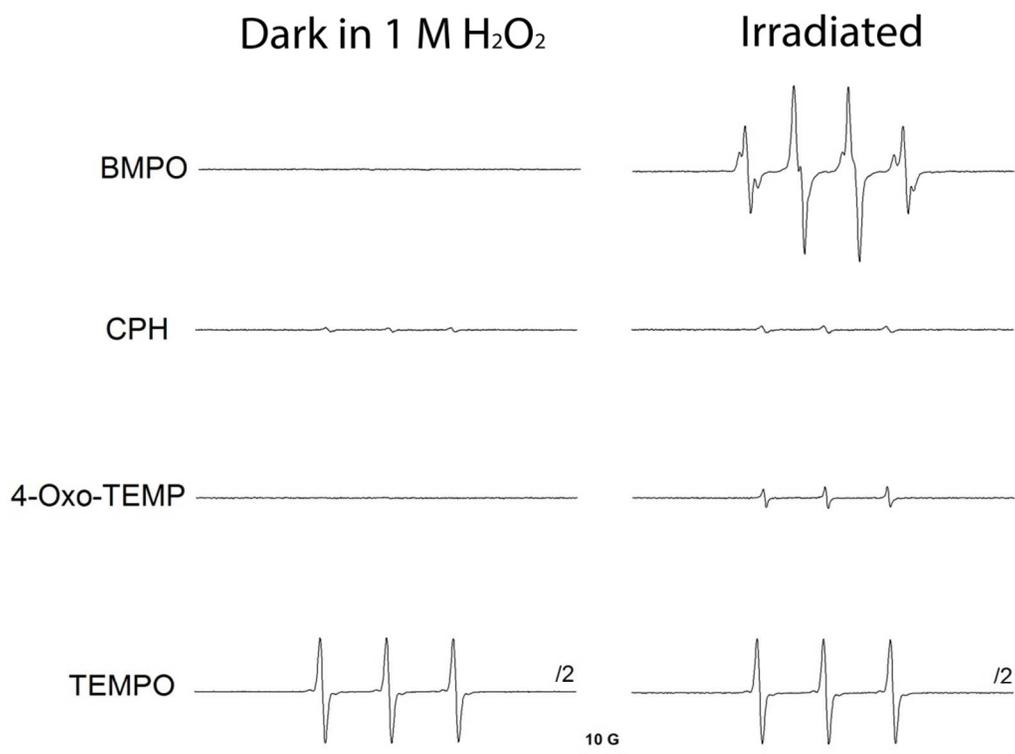


Figure S6. Effect of hydroxyl radicals on different spin probes. ESR spectra were obtained from samples containing 1 M H₂O₂, and different spin probes (25 mM BMPO, 10 mM 4-oxo-TEMP, 0.02 mM CPH and 0.02 mM TEMPO) in the dark or irradiated with simulated sunlight. Spectra were recorded at before and after irradiation for 3 min. The hydroxyl radical was generated by decomposition of hydrogen peroxide during exposure to UV radiation in simulated sunlight.

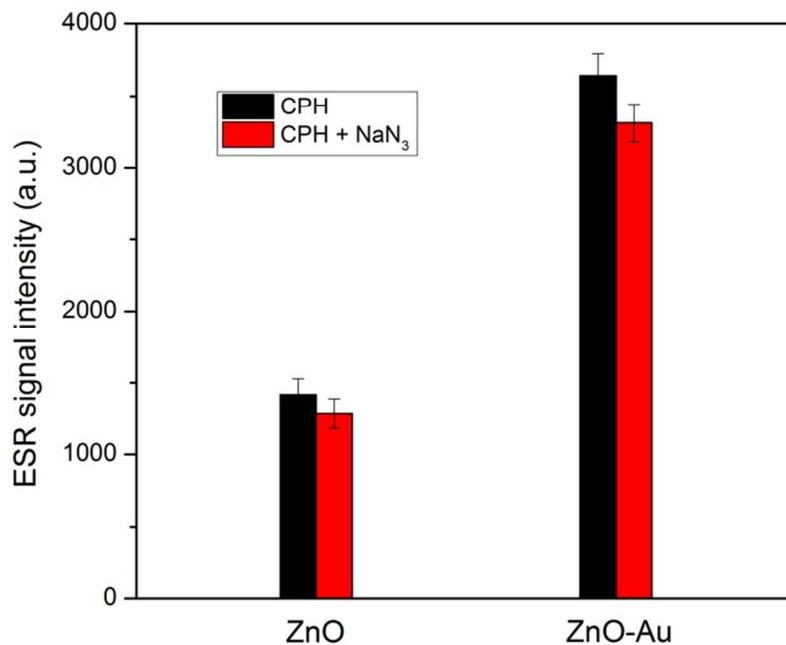


Figure S7. Effect of NaN₃ on ESR signal intensity from CPH in the presence of ZnO or ZnO/Au hybrid nanostructures. (Conditions: 0.02 mM CPH, 10 mM NaN₃, 0.1 mg/ml ZnO or ZnO/Au hybrid nanostructures with Au/ZnO loading ratio 4%, irradiation for 3 min with simulated sunlight.)

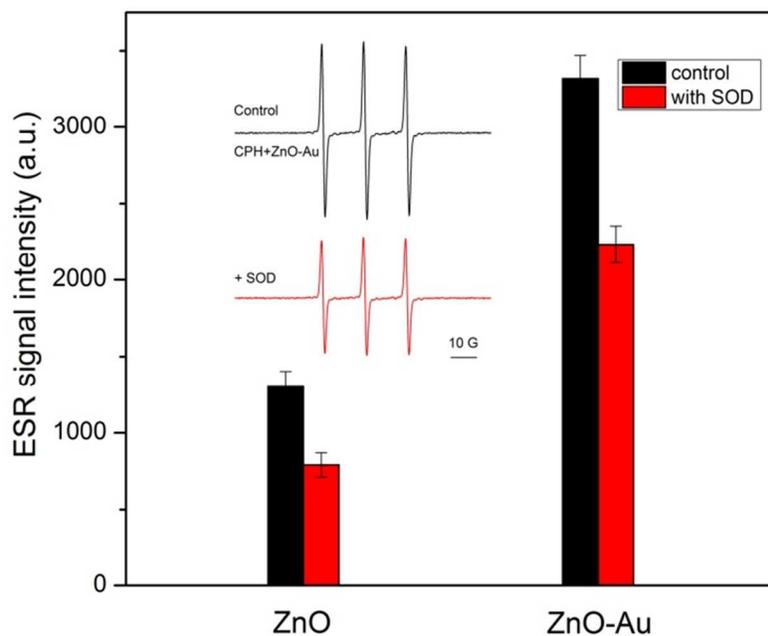


Figure S8. Effect of SOD on ESR signal intensity generated from CPH oxidation in the presence of ZnO or ZnO/Au hybrid nanostructures. (Conditions: 0.02 mM CPH, 0.25 U/ml SOD, 0.1 mg/ml ZnO or ZnO/Au hybrid nanostructures with a molar loading ratio of Au to ZnO of 4%, irradiation for 3 min with simulated sunlight.)

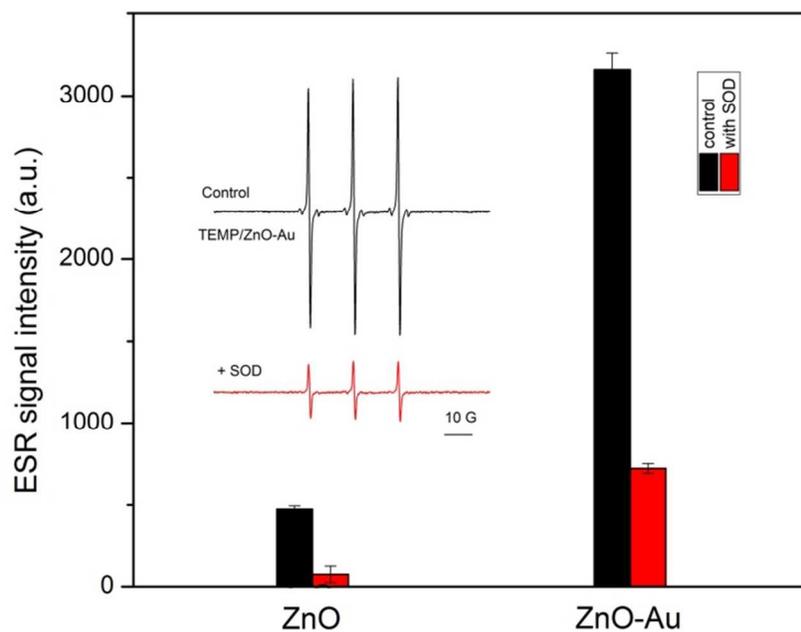


Figure S9. Effect of SOD on ESR signal intensity generated from spin labels 4-Oxo-TEMP in the presence of ZnO or ZnO/Au hybrid nanostructures. (Conditions: 10 mM 4-Oxo-TEMP, 0.25 U/ml SOD, 0.1 mg/ml ZnO or ZnO/Au hybrid nanostructures with a molar loading ratio of Au to ZnO of 4%, irradiation for 3 min with simulated sunlight.)

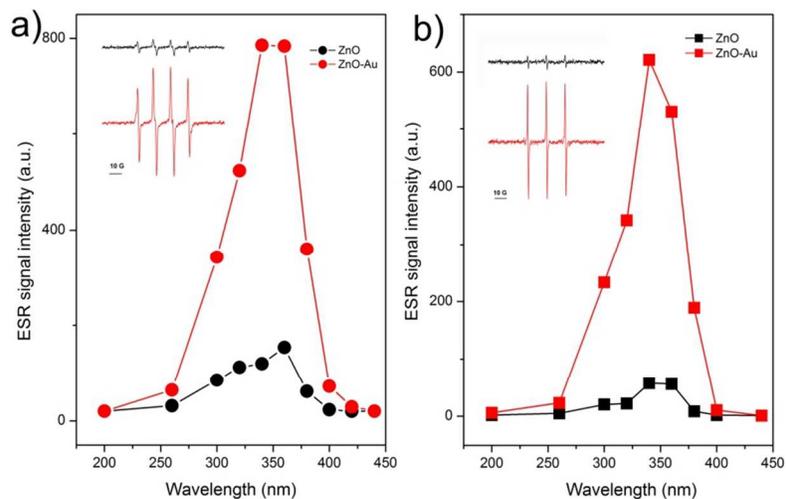


Figure S10. Wavelength dependent enhanced ROS generation of (a) hydroxyl radical and (b) singlet oxygen ESR intensity versus monochromatic light wavelength. Insets represent the corresponding ESR spectrum obtained in presence of ZnO (black) or ZnO/Au 4% (red) under irradiation with 360 nm wavelength. The ESR signal intensities were calculated from corresponding ESR spectra obtained from samples containing 25 mM BMPO (a) or 10 mM 4-Oxo-TEMP (b), and 0.1 mg/ml ZnO or 0.1 mg/ml ZnO/Au with Au to ZnO loading ratio of 4% suspensions, after irradiation for 3 min under tunable wavelength monochromatic light.