Supporting Information

Dynamic Detection of Endogenous Hydroxyl Radicals at Single-Cell Level with Individual Ag-Au Nanocages

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Experimental Details

Materials. All chemicals were analytical grade and used without further purification. Sodium chloride (NaCl), methyl thiazolyl tetrazolium (MTT), sodium hypochlorite (NaClO), dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), sodium hydroxide (NaOH), ferrous sulfate ($FeSO_4$), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), ethylenediaminetetraacetic acid (EDTA) and hydrogen peroxide (H_2O_2) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Ethylene glycol (EG), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), silver trifluoroacetate (CF_3COOAg), sodium hydrosulfide hydrate ($NaHS \cdot xH_2O$), 5-bromine salicylic acid (5-BrSA), hexadecyl trimethyl ammonium Bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), ascorbic acid (AA) and phorbol 12-myristate 13-acetate (PMA) were purchased from Aladdin Ltd. (Shanghai, China). Poly(vinylpyrrolidone) (PVP, Mw= 55000), diethylamine NONOate sodium salt hydrate (DEA NONOate), hypoxanthine (HX) and xanthine oxidase (XOD) were obtained from Sigma–Aldrich. The polyethylene glycol (mPEG-SH, Mw= 5000) was purchased from Shanghai Yare Biotech,Inc. Arginine-glycine-aspartic-cysteine acid peptide (RGDC) was purchased from GL Biochem (Shanghai) Ltd. All aqueous solutions were prepared using deionized (DI) water with a resistivity of 18.2 MΩ·cm.

Instruments. The UV-Vis absorption spectra were taken using a UV-Vis spectrometer UV-3600 (Shimadzu, Japan). Transmission electron microscopy (TEM) images were measured on a JEOLJEM 200CX transmission electron microscope using an accelerating voltage of 100 kV. Dynamic light scattering (DLS) experiments were performed at 25 °C using a Brookhaven BI-200SM instrument. Zeta-potentials of nanoparticles were measured at room temperature using ZETASIZER nanoseries (Nano-ZS, Malvern). MTT assay was recorded at 490 nm using a Bio-Rad 680 microplate reader. SPR resonance angle was measured by Nikon Ti-2 microscope. The dark-field images and spectra measurements were carried out on Nikon Ti-E microscope equipped with a broadband light source (EQ-99XFC LDLS, Energetiq Technology), a color-cooled digital camera (DS-RI1, Nikon), a monochromator (Acton SP2300i, PI) equipped with a spectrograph CCD (PIXIS 400BR_excelon, PI) and a grating (grating density: 300 Lmm^{-1}; blazed wavelength: 500 nm).

Synthesis of core-shell Au@Ag nanorods. Uniform Au@Ag nanorods were synthesized according to previous report.[1] The first step was to synthesize gold nanorods which were prepared via a typical seed-mediated growth process with some modification. The seed solution was prepared by mixing a 5 mL of 0.5 mM $HAuCl_4$ with 5 mL of 0.2 M CTAB solution, followed by immediately injecting 0.6 mL of cold fresh 0.01 M $NaBH_4$ with vigorous stirring. The color of solution changed from yellow to brown, and the stirring was stopped after 2 min reaction. The seed solution was aged at room temperature for 30 min before use. The growth solution was prepared by dissolving 9.0 g CTAB and 1.1g 5-BrSA in 250 mL DI water with stirring. After dissolution, 12 mL of 4 mM $AgNO_3$ solution was added, and the mixture was kept undisturbed at 30 °C for 15 min, and then 250 mL 1 mM $HAuCl_4$ solution was added. After slow stirring for 15 min, 2 mL of 0.064 M AA was added, and the solution was vigorously stirred until it faded. Subsequently, 0.8 mL of seed solution was injected into the growth solution. The resultant mixture was stirred for 30 s and left undisturbed at 30°C for 12 h for nanorods growth. The final products were isolated by centrifugation at 8500 rpm for 30 min and the precipitates were redispersed in 10 mL DI water.

The obtained Au nanorods were used for the preparation of Au@Ag core-shell nanorods. In brief, 6 mL of 2 mM $AgNO_3$, 3 mL of 0.1 M AA, and 500 μL of the isolated Au nanorods were added into 140 mL of 0.86 wt% PVP. After heated to 40 ºC, 6 mL of 0.1 M NaOH was added to improve the reducing ability of AA. The color of the solution changed from brown to blue swiftly. After cooled for 30 min at room temperature, the as prepared Au@Ag nanorods were collected by centrifugation at 7000 rpm for 15 min and redispersed in in DI water.

Cell viability assay. For the cell viability assay, HeLa cells were seeded and cultured for 24 h in 96-well plates. Then the NCs with different concentrations were added to each well and incubated for different times, followed by the addition of 10 μL MTT solution (5 mg/mL) and incubation at 37 ℃ for 4 h. After that, 100 μL dimethyl sulfoxide was added and shaken for 10 min, and the optical density (OD) at a wavelength of 490 nm was measured using a microplate reader to obtain the cell viability. Relative cell viability was expressed as: ([OD]test/[OD]control) × 100%. Each experiment was repeated at least three times.

Supporting Figures

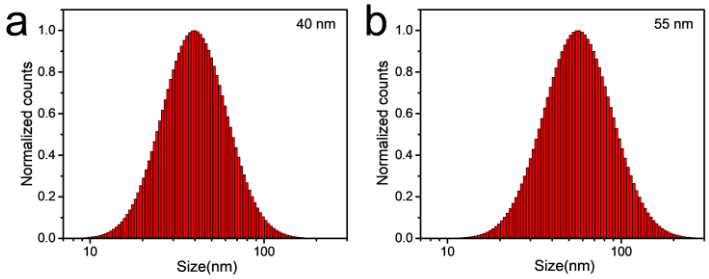

Figure S1. The hydrodynamic size distribution of 40 nm and 55 nm Ag-Au nanocages.

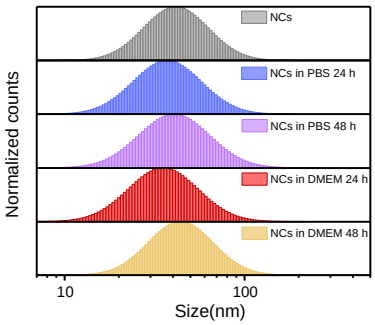

Figure S2. Stability of Ag-Au@PEG/RGD nanocages (NCs). DLS reslults of NCs, 1 nM NCs incubated with PBS (10 mM, pH 7.4) for 24 h and 48 h, and 1 nM NCs treated with DMEM cell culture medium (containing 10% fetal bovine) for 24 h and 48 h.

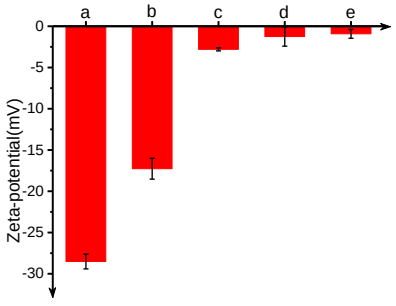

Figure S3. Zeta-potentials of Ag-Au nanocages (a), Ag-Au@PEG nanocages (b) and Ag-Au@PEG nanocages modified by RGD peptide of 10000 (c), 30000 (d), and 50000 (e) molar concentration excess.

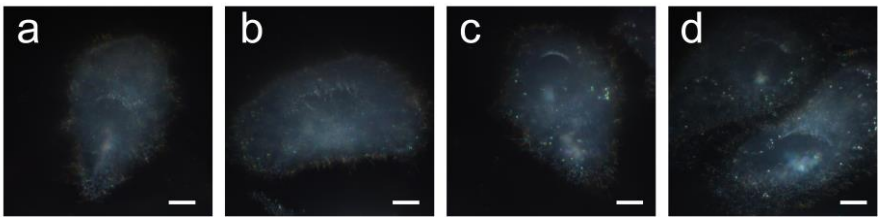

Figure S4. DFM images of HeLa cells treated with 0.1 nM nanoprobes modified by RGD peptide of 0 (a), 10000 (b), 30000 (c), and 50000 (d) molar concentration excess for 30 min. Scale bar is 5 μm.

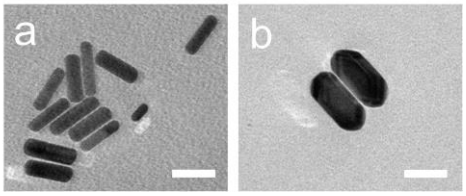

Figure S5. TEM micrograph of the Au nanorods as nanoprobe core (a) and Au@Ag nanorods (b). Scale bar is 50 nm.

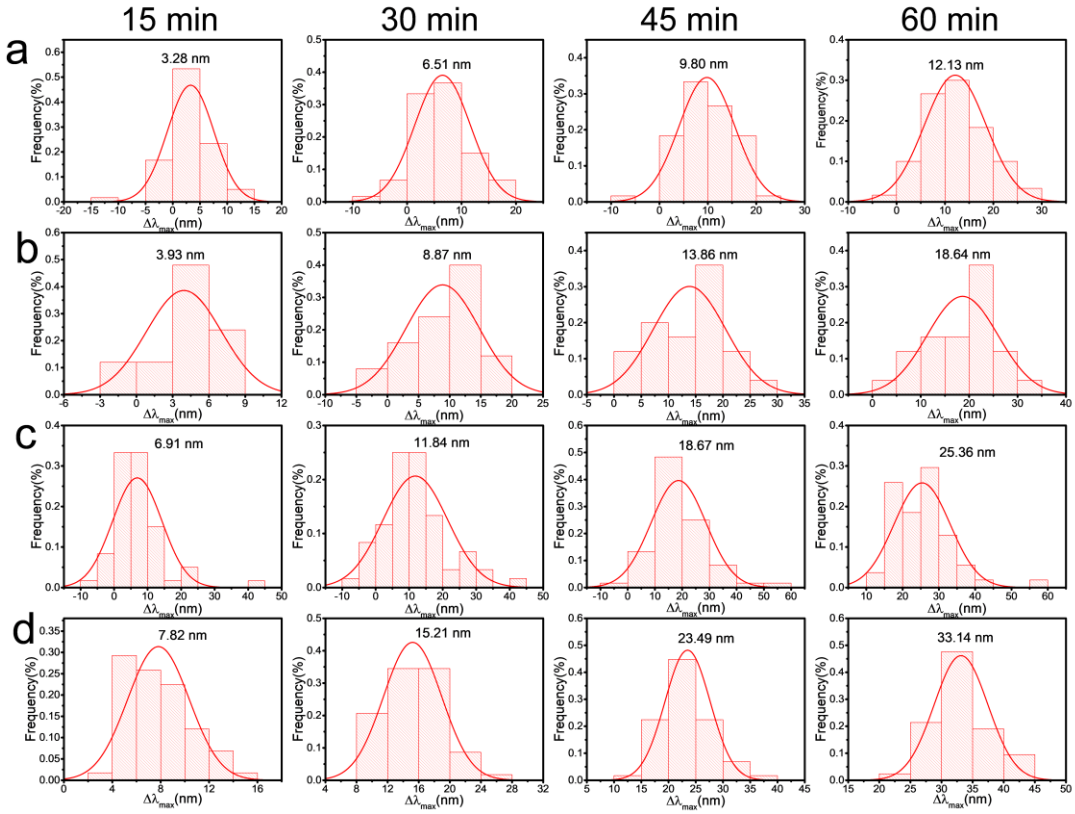

Figure S6. Time-dependent $\Delta\lambda_{max}$ distributions of Ag-Au@PEG/RGD nanocages when exposed to 100 pM (a), 1 nM (b), 10 nM (c), and 100 nM (d) •OH.

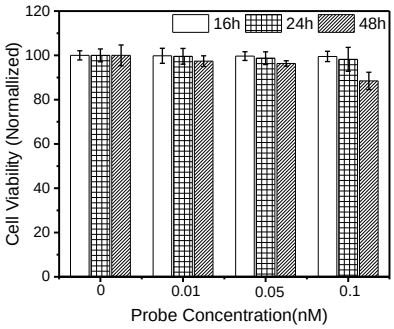

Figure S7. Viability of HeLa cells incubated with 0, 0.01, 0.05 and 0.1 nM nanoprobes for 16, 24, and 48 h.

References

1. Chen, Z.; Li, J.; Chen, X.; Cao, J.; Zhang, J.; Min, Q.; Zhu, J.-J., Single Gold@Silver Nanoprobes for Real-Time Tracing the Entire Autophagy Process at Single-Cell Level. *J. Am. Chem. Soc.* **2015,** *137,* 1903-1908.