Supporting Information

A Ratiometric Fluorescence Probe for Monitoring Hydroxyl Radical in Live Cells Based on Gold Nanoclusters

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1. Quantum yield calculations

In order to calculate the quantum yield (Φ) of AuNCs probe, a method was taken from "A Guide to Recording Fluorescence Quantum Yields" produced by Horiba Ltd. at http://www.horiba.com/uk/scientific/products/fluorescence-spectroscopy/application-notes/quantum-yields/.

Rhodamine B dissolved in ethanol was chosen as the reference, the data of integrated photoluminescence intensities and UV absorbance values were plotted (seven concentrations) and the slopes of the AuNCs and Rhodamine B were determined. The quantum yield was calculated using the following equation:

$$\Phi_x = \Phi_{ST}(m_x/m_{ST})\ (\eta^2_x/\eta^2_{ST})$$

where Φ is the quantum yield, m is slope, η is the refractive index of the solvent, ST is the standard and X is the sample.

And the quantum yield of AuNCs probe was calculated to be ~5 %.

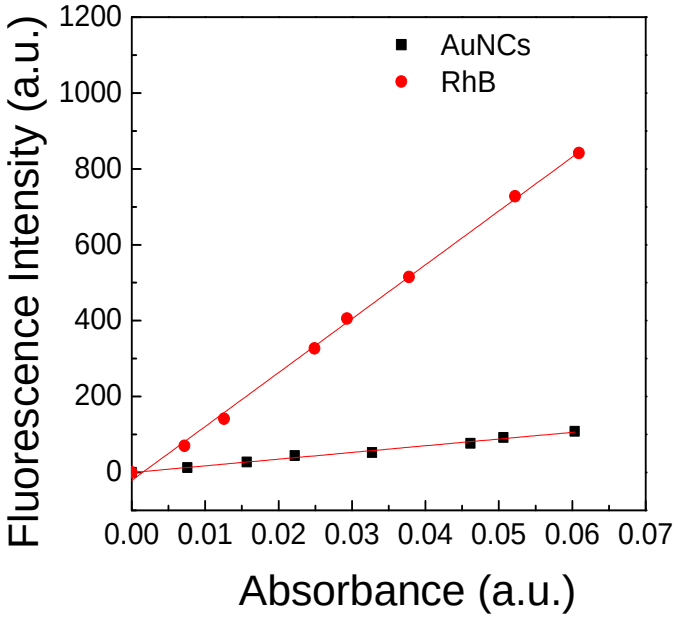

Figure S1. Photoluminescence (excited at 480 nm) and absorbance (at 480 nm) of AuNCs probe and rhodamine B.

2. The optimized mass concentration ratio between AuNCs and HPF

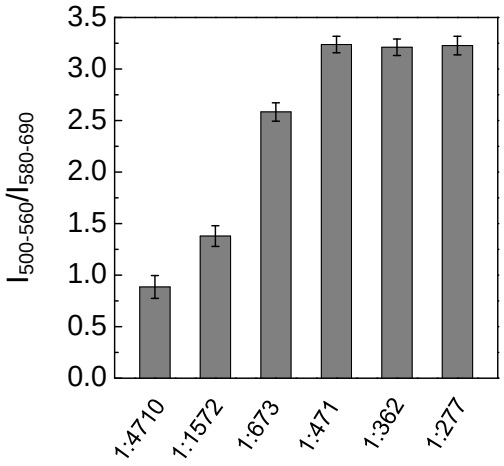

Figure S2. The optimized results of mass concentration ratio between HPF and BSA gold cluster. The horizontal axis shows AuNC@HPF probe with different ratios (C_{HPF}:$C_{Au\ cluster}$): 1:4710, 1:1572, 1:673, 1:471, 1:362, 1:277, and the vertical axis shows the fluorescence intensity ratio $I_{500\text{-}560}/I_{580\text{-}690}$ of AuNC@HPF after adding 150 μM ·OH to the fluorescent probe.

3. Reaction Time

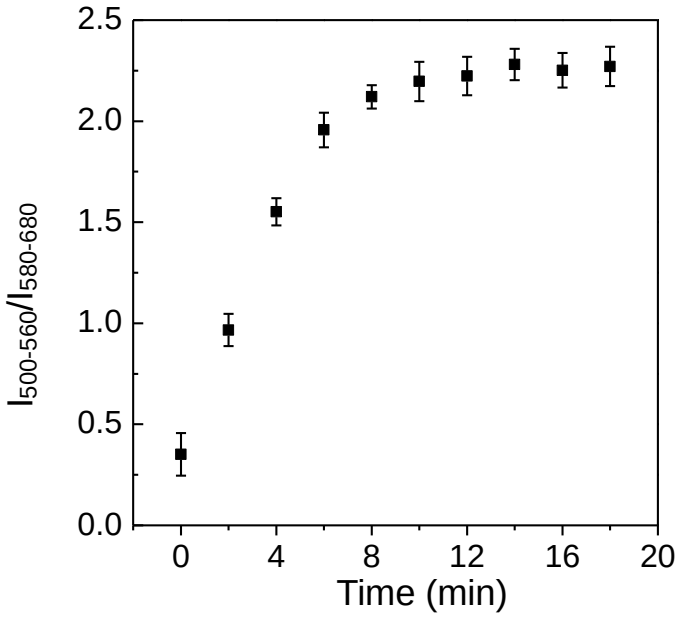

Figure S3. The relationship between the fluorescence ratio ($I_{500\text{-}560}/I_{580\text{-}680}$) and reaction time.

4. The reaction of probe with H_2O_2 or Fe^{2+}

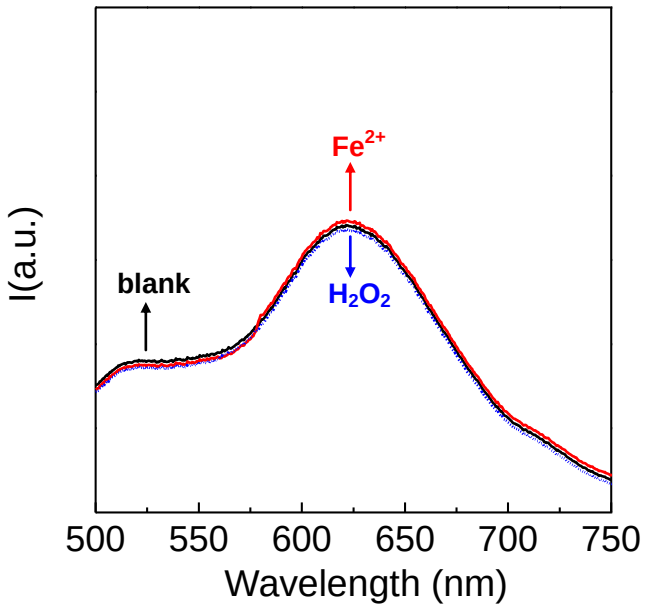

Figure S4. Fluorescent responses of AuNC@HPF with addition of Fe^{2+} (100 μM) and H_2O_2 (600 μM), respectively, upon 488 nm excitation.

5. The selectivity test against amino acids and glucose

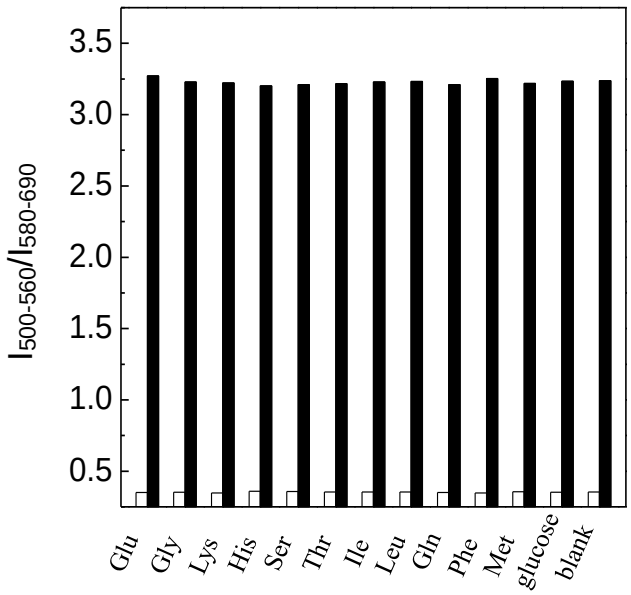

Figure S5. Fluorescence responses of AuNC@HPF in PBS (pH = 7.4) upon 488 nm excitation toward glucose (1 mM) and amino acids (10 μM).

6. pH stability

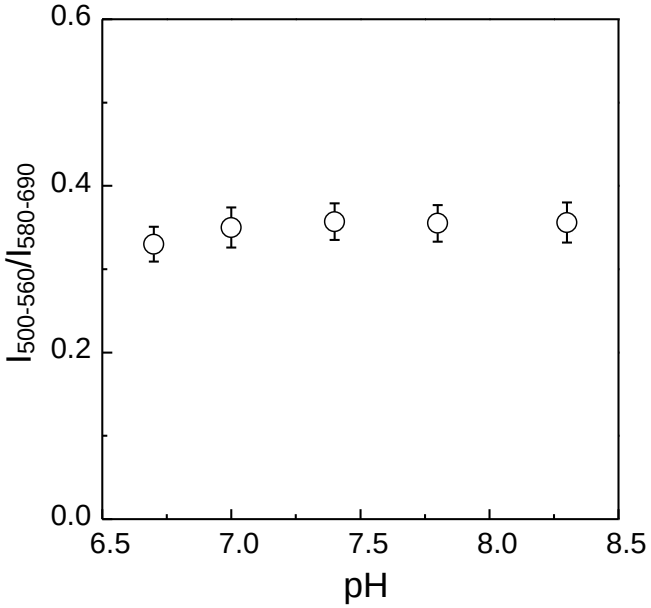

Figure S6. Fluorescent responses of AuNC@HPF (standard solution) upon 488 nm excitation in PBS with pH ranging from 6.7 to 8.3.

7. Nuclear staining experiments

To make sure the location of the AuNC@HPF probe in Hela cells, nuclear staining experiment was performed. AuNC@HPF probe was first incubated with Hela cells in cell culture media for 1 h, and Hoechst 33342 (a fluorescent nuclear stain) was introduced and incubated for 10 min. Then LPS was added to stimulate $^{\bullet}OH$ for 45 min, and the confocal fluorescence images of different channels were obtained, as shown in Figure S7. The overlay of confocal fluorescence image and bright-field image of Hela cells confirmed that the probe of AuNC@HPF localized in the region of the cytosol and near the nucleus.

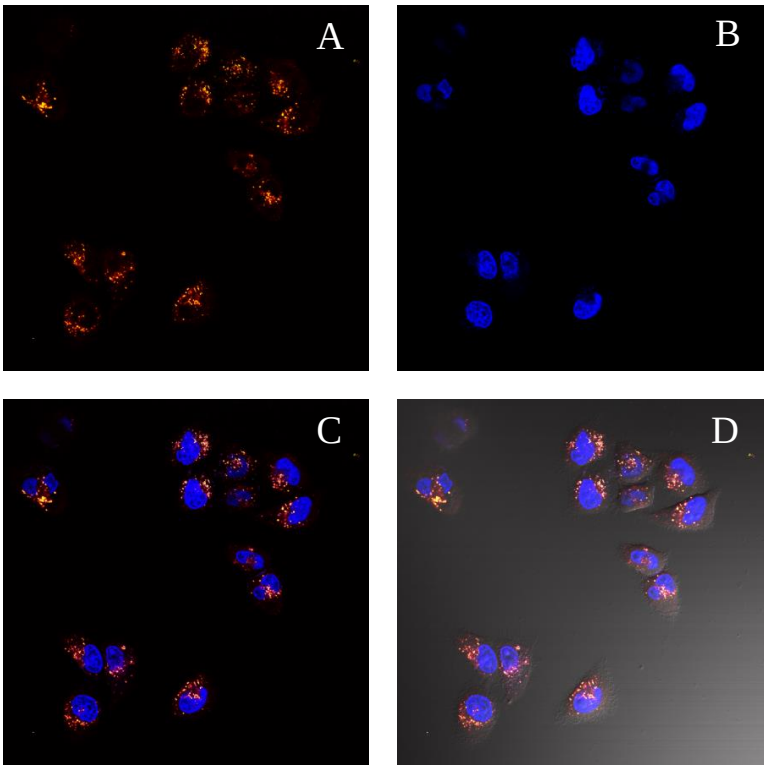

Figure S7. (A) The confocal fluorescence image of AuNC@HPF probe, (B) The confocal fluorescence image of cell nuclear stained by hoechst 33342, (C) The overlay image of (A) and (B), and (D) The overlay of confocal fluorescence image (C) and the bright-field image of Hela cells.

8. Confocal fluorescence images of AuNC@HPF with separated channels in live cells

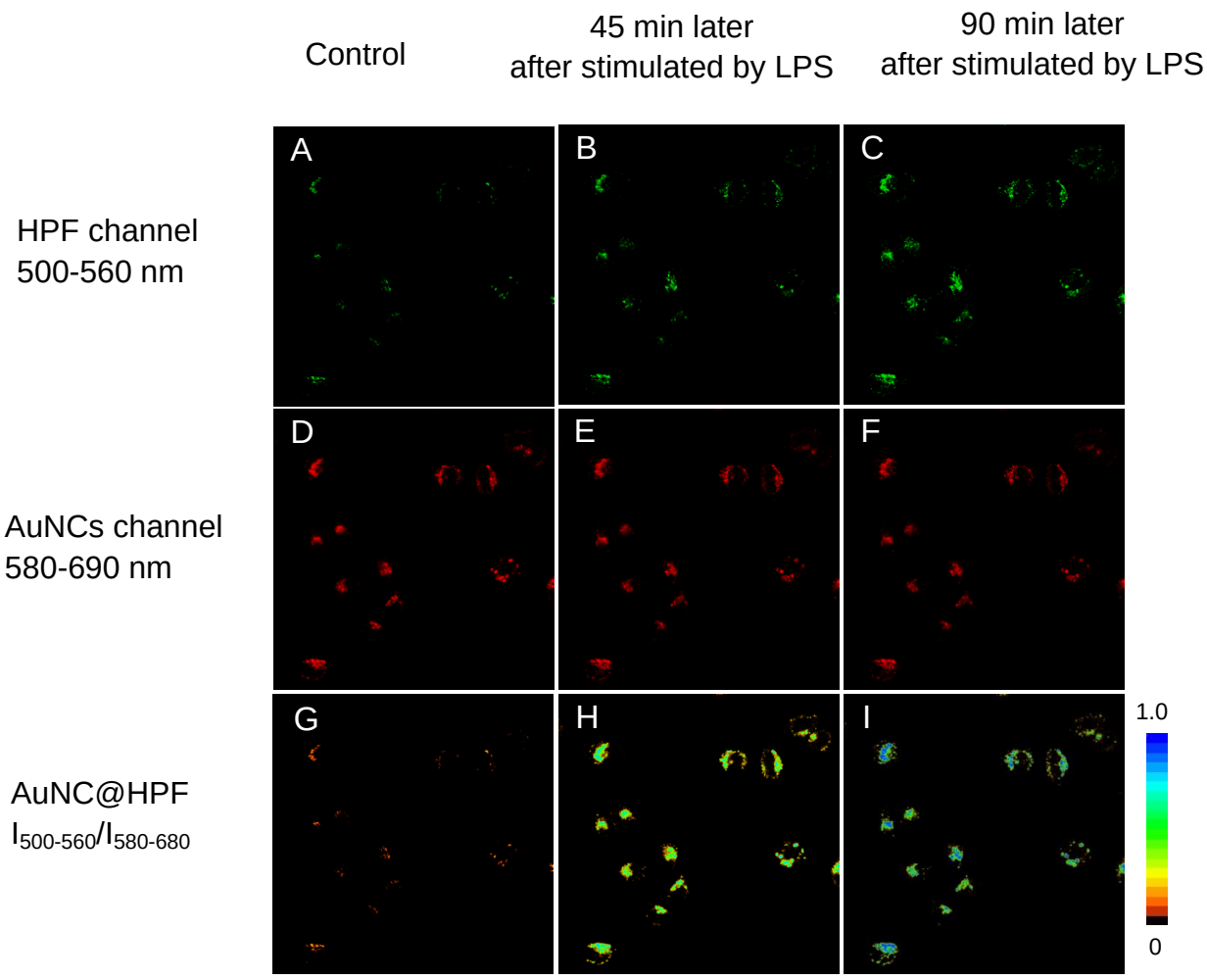

Figure S8. The confocal fluorescence images of HPF channel (A-C) and AuNCs channel (D-F). (G-I) Pseudocolored ratiometric images ($I_{500\text{-}560}/I_{580\text{-}680}$) of the two channels. (A, D, G) The confocal fluorescence images before addition of LPS, (B, E, H) after stimulated by LPS for 45 min, and (C, F, I) after stimulated by LPS for 90 min.