

## Supporting Information

### **Chemically Engineered Nanoparticle-Protein Interface for Real Time Cellular Oxidative Stress Monitoring**

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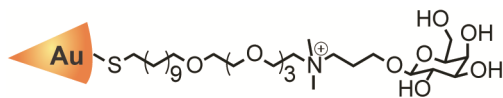
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## Experimental Section

**General** HAuCl<sub>4</sub> used for gold nanoparticle synthesis was purchased from Strem Chemicals Inc (Newburyport, MA), all other chemicals in this study were purchased from Sigma-Aldrich or Fisher Scientific, and used as received. Transmission electron microscopy (TEM) was performed on a JEOL-2010 microscope with an accelerating voltage of 200 kV, the diameter of gold nanoparticles was analyzed using ImageJ. Dynamic Light Scattering (DLS) profile and Zeta potential of nanoparticles were measured on Malvern Zetasizer (Nano series, Malvern Instruments Inc, USA) with a He-Ne laser (633nm) and a backscattering angle of 173° at the room temperature. The GFP fluorescence was recorded on a Molecular Devices SpectraMax M3 microplate reader, with excitation at 475 nm and emission at 510 nm.

## Synthesis of AuNP-Gal nanoparticle



1-pentanethiol protected 2 nm gold nanoparticle (AuNP-C5) and galactose ligand were synthesized according to previous reports.<sup>[1]</sup> AuNP-Gal was prepared through the place-exchange reaction between AuNP-C5 and galactose ligand. Briefly, 40 mg of galactose ligand was added to a 3 mL CH<sub>2</sub>Cl<sub>2</sub> solution of 10 mg AuNP-C5.<sup>[1b]</sup> The reaction mixture was stirred for another 48 h at room temperature, followed by solvent evaporation *in vacuo*. The resulted residue was then washed extensively with a mixture of hexanes and DCM to remove 1-pentanethiol and excessive galactose ligand. The crude nanoparticle was then dialyzed against water for 3 days using SnakeSkin™ Dialysis Tubing (Thermo Scientific, 10,000 MWCO). Finally, molecular cut off filtration (10,000 MWCO) was performed 5 times to ensure the purity and to concentrate the galactose-functionalized gold nanoparticles.

**Green fluorescent protein (GFP) modification** Recombinant GFP was expressed according to our previous report.<sup>[2]</sup> PB-GFP was prepared by reacting native GFP with 4-nitrophenyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonate (NBC) at different ratios.<sup>[3]</sup> Briefly, 0.7 mg GFP was dissolved in 0.1 M NaHCO<sub>3</sub> buffer solution (pH = 8.5) at a protein concentration of 1 mg/mL. To above solution, 150  $\mu$ L DMSO solution containing 0.25, 0.5, or 1 mg NBC was added to prepare PB5-GFP, PB9-GFP, and PB20-GFP, respectively. The reaction mixtures were then stirred at room temperature for additional 10 h, followed by ultrafiltration purification using Amicon® Ultra Centrifugal Filters (MWCO = 10,000, Millipore, MA). Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to characterize modified GFP. A saturated sinapinic acid stock solution was prepared in 50% acetonitrile, 50% H<sub>2</sub>O, and 0.1% TFA. An equal volume of 1 mM PB-GFP solution was added to the matrix stock solution. 2.5  $\mu$ L of this mixture was spotted on the sample carrier, and MALDI-MS analysis was performed on a Bruker Autoflex III mass spectrometer.

**PB-GFP fluorescence titration with AuNP-Gal** PB-GFP (100 nM) was titrated with increased concentration of AuNP-Gal, ranging from 0 to 90 nM. GFP fluorescence ( $\lambda_{\text{ex}} = 475$  nm,  $\lambda_{\text{em}} = 510$  nm) in the presence of AuNP-Gal was normalized to that without AuNP-Gal addition. Nonlinear least-squares curve fitting analysis was used to estimate the binding constant ( $K_a$ ) between PB-GFP and AuNP-Gal according to our previous reports.<sup>[4]</sup>

**H<sub>2</sub>O<sub>2</sub>-modulated AuNP/PB20-GFP fluorescence response** AuNP-Gal/PB20-GFP complexes was incubated with different concentrations of H<sub>2</sub>O<sub>2</sub>, ranging from 0 to 150  $\mu$ M at 37 °C. These AuNP-Gal/PB20-GFP conjugates were pre-prepared in a fixed PB20-GFP concentration (100 nM) and PB20-GFP to NP ratio (4 : 1) in 10% FBS containing PBS for 15 minutes. PB20-GFP fluorescence was monitored for 120 minutes, and the fluorescence values

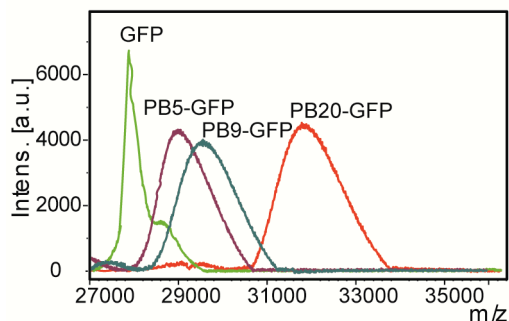
were presented relative to the PB20-GFP control. All experiments were performed independently at least three times.

**Selective H<sub>2</sub>O<sub>2</sub> assay using AuNP/PB20-GFP interface** AuNP-Gal/PB20-GFP complexes (pre-prepared in a fixed PB20-GFP concentration (100 nM) and PB20-GFP to NP molar ratio (4 : 1) in 10% FBS containing PBS for 15 minutes) was treated with 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> or other ROS (final concentration was 100  $\mu$ M) at 37 °C, the PB20-GFP fluorescence was monitored every 5 minutes for 120 minutes, and presented relative to the AuNP-Gal/PB20-GFP controls. ROS were generated as follows: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), *tert*-butyl hydroperoxide (TBHP) and hypochlorite (ClO<sup>-</sup>) were introduced from commercially available aqueous solution, nitric oxide (NO•) was generated from PROLI NONOate. The catalase concentration, when present, was 0.5 mg/mL. All experiments were performed in triplicate.

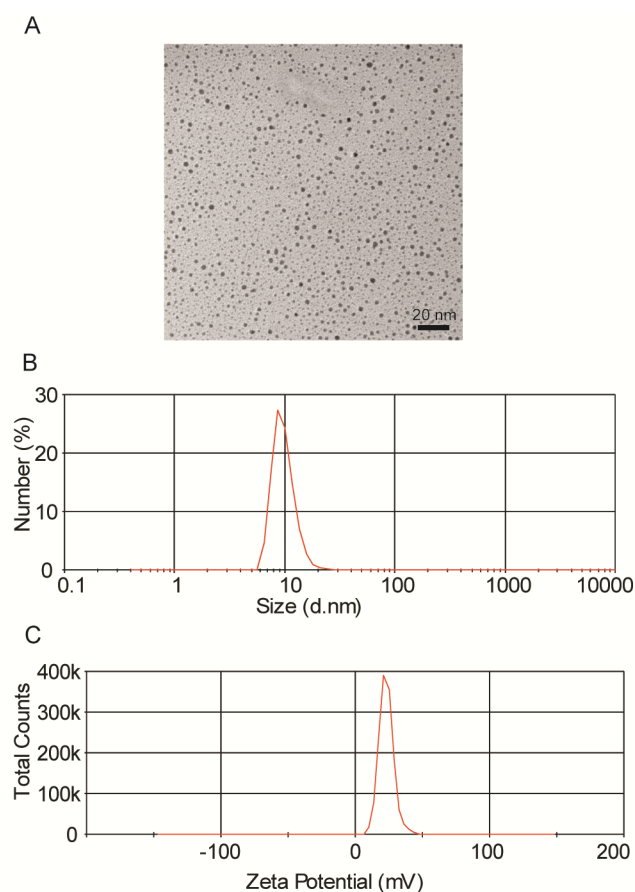
**Cell Culture** Jurkat cells were purchased from ATCC. Cells were maintained in RPMI-1640 medium (Life technologies), supplemented with 10% FBS, and 1% antibiotics (Cellgro). Cells were cultured in a humidified atmosphere containing 5% CO<sub>2</sub> at 37 °C and sub-cultured every four days.

**Cellular assays using AuNP/PB20-GFP** Jurkat cells ( $1 \times 10^4$  cells in 100  $\mu$ L culture medium) were split into black 96-well plates, to which AuNP-Gal/PB20-GFP complexes (pre-prepared in a fixed PB20-GFP concentration (100 nM) and PB20-GFP to NP molar ratio (4 : 1) in 10% FBS containing PBS for 15 minutes) were added. For the spiked H<sub>2</sub>O<sub>2</sub> assay, H<sub>2</sub>O<sub>2</sub> with concentration increased from 0 to 40  $\mu$ M was added to above cells, followed by GFP fluorescence monitoring every 10 minutes for 120 minutes at 37 °C. For real time cellular oxidative stress study, Jurkat cells were treated with either PMA (0.5  $\mu$ M) or a combination of PMA (0.5  $\mu$ M) and catalase (0.5 mg/mL), followed by the addition of AuNP-Gal/PB20-GFP complexes. The fluorescence restoration of GFP was similarly recorded within 120 minutes,

and presented relative to the AuNP-Gal/PB20-GFP controls. All experiments were performed independently at least three times.



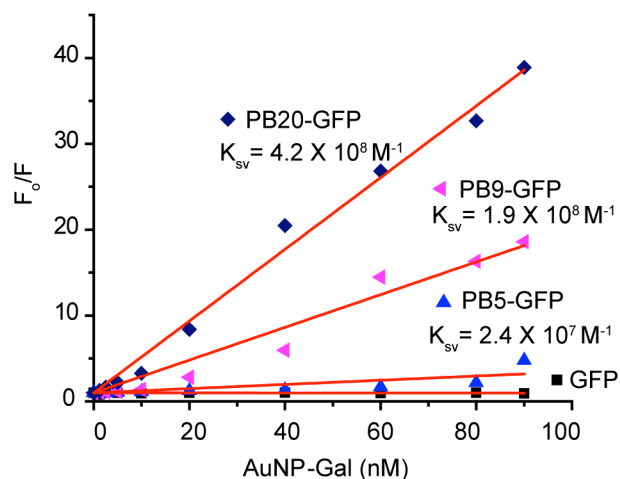
**Figure S1.** MALDI-TOF mass spectra of GFP and PB-modified GFP.



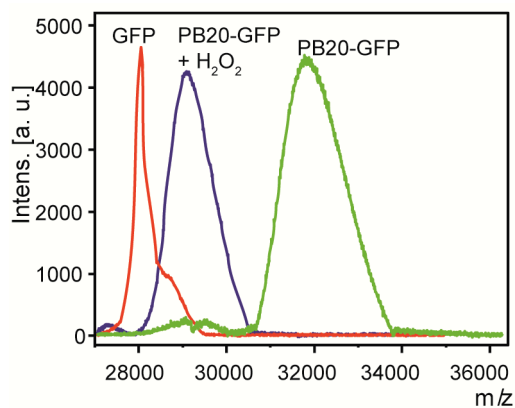
**Figure S2.** Characterizations of AuNP-Gal. (A) TEM image of AuNP-Gal. (B) Dynamic light scattering (DLS) diameter of AuNP-Gal ( $9.6 \pm 1.2$  nm, recorded in 5 mM phosphate buffer at pH 7.4 at a concentration of  $1 \mu\text{M}$ ). (C) Zeta-potential of AuNP-Gal ( $20.2 \pm 2.5$  mV, recorded in 5 mM phosphate buffer at pH 7.4 at a concentration of  $1 \mu\text{M}$ ).

**Table S1.** Apparent binding constants for the AuNP-Gal/PB-GFP complexes, as determined by fitting the GFP fluorescence titration curves.

Protein	Apparent binding constant ( $K_a$ )
PB5-GFP	$(6.6 \pm 1.9) \times 10^7 \text{ M}^{-1}$
PB9-GFP	$(8.6 \pm 1.2) \times 10^7 \text{ M}^{-1}$
PB20-GFP	$(2.0 \pm 0.5) \times 10^8 \text{ M}^{-1}$

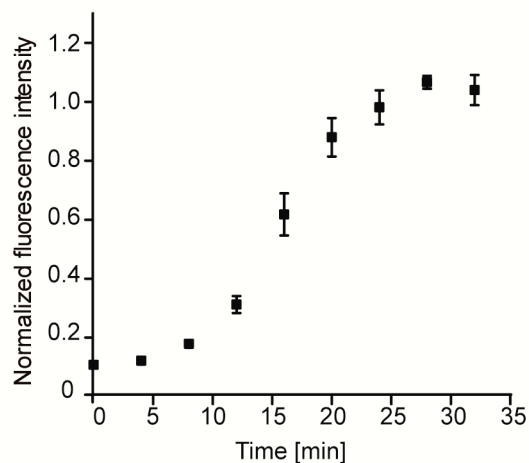


**Figure S3.** Stern-Volmer plots for quenching of the GFP, PB5-GFP, PB9-GFP, and PB20-GFP fluorescence by AuNP-Gal are shown.  $K_{sv}$ : Stern-Volmer constant.

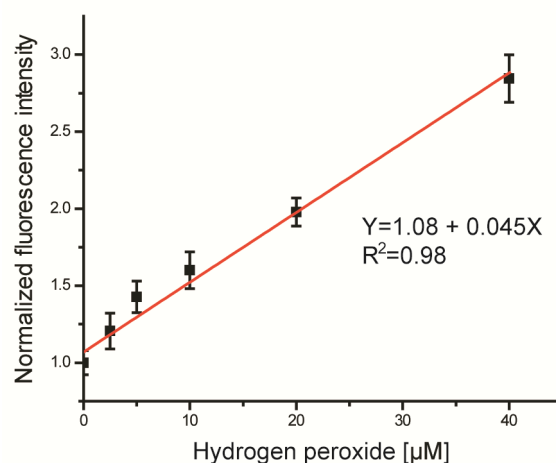


**Figure S4.** MALDI-TOF mass spectra of GFP, PB20-GFP before and after H<sub>2</sub>O<sub>2</sub> treatment.

30  $\mu$ M PB20-GFP was treated with 30 mM H<sub>2</sub>O<sub>2</sub> for MALDI-TOF analysis.



**Figure S5.** Time course of PB20-GFP fluorescence response of AuNP-Gal/PB20-GFP (100 nM of PB20-GFP) complex treated with 1 mM H<sub>2</sub>O<sub>2</sub>. The fluorescence was presented relative to the PB20-GFP controls. Error bar represents the standard deviation of three independent studies.



**Figure S6.** Fold increase of AuNP-Gal/PB20-GFP fluorescence after 120 min. of incubation with  $H_2O_2$  at indicated concentration in the presence of Jurkat cells. Error bar represents the standard deviation of three independent studies.

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[3] M. Wang, S. Sun, C. I. Neufeld, B. Perez-Ramirez, Q. Xu, *Angew. Chem. Int. Ed.* **2014**, *53*, 13444.

[4] C. C. You, M. De, G. Han and V. M. Rotello, *J. Am. Chem. Soc.* **2005**, *127*, 12873.