Supporting Information for

Horseradish Peroxidase-Mediated, Iodide-Catalyzed Cascade Reaction for Plasmonic Immunoassays

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MATERIALS AND METHODS

Materials. Horseradish peroxidase (HRP), sodium iodide and cysteine were purchased from Sigma-Aldrich (Shanghai, China). Primary antibodies and HRP-conjugated antibodies were purchased from Jackson ImmunoResearch (Pennsylvania, USA). Hepatitis C virus (HCV) ELISA kit was purchased from AutoBio (Zhengzhou, China). 4-(2-Hydroxyethyl)piperazine-1-ethane-sulfonic acid sodium salt (HEPES), chloroauric acid ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, hydrogen peroxide and the other reagents required for the experiments were of analytical grade and used as received.

Synthesis of AuNPs. AuNPs were synthesized by the citrate-mediated reduction of $HAuCl_4$. Briefly, $HAuCl_4$ solution (1 mM, 100 mL) was heated under reflux with stirring, followed by the addition of trisodium citrate solution (38.8 mM, 10 mL). The red-colored solution was allowed to cool to room temperature and a polyethersulfone membrane (0.22 μM) was used to remove large clusters to obtain the AuNPs.

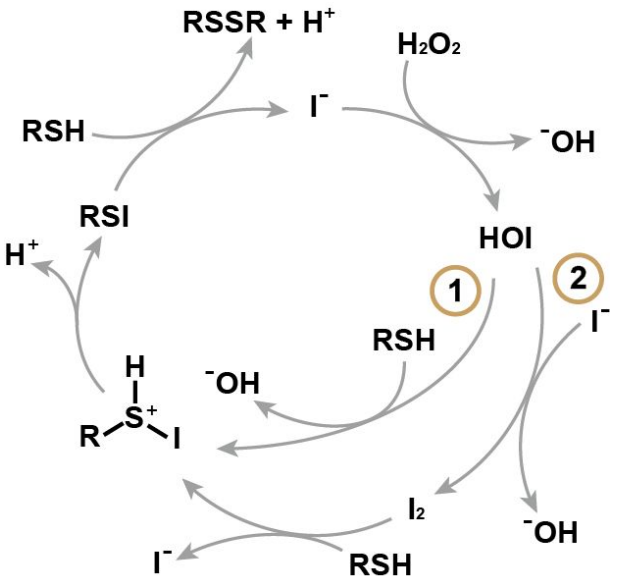
AuNPs for iodide sensing. For iodide sensing, 100 μL of the prepared AuNPs was incubated with hydrogen peroxide (20 μM), cysteine (20 μM) and different concentrations of sodium iodide, followed by the addition of HEPES buffer solution (20 mM, pH 7.0) to reach a final volume of 200 μL. The mixture of the solution was allowed to react for about one hour to induce the aggregation of AuNPs. The surface plasmon resornance (SPR) absorption of AuNPs was measured by a microplate spectrophotometer (TECAN, Infinite®200 PRO).

AuNPs for HRP sensing. For HRP sensing, hydrogen peroxide (200 μM), sodium iodide (200 μM) and different concentrations of HRP were added in H_2O-HCl solution (pH = 3) to reach a final volume of 100 μL. The mixture was kept to react at 37 °C for an hour. 20 μL of the mixture was used to incubate with hydrogen peroxide (20 μM), cysteine (20 μM) and 100 μL of the AuNPs, followed by the addition of HEPES buffer solution (20 mM, pH 7.0) to reach a final volume of 200 μL. The mixture of the solution was kept to react for about one hour and the SPR absorption of AuNPs was measured.

Plasmonic immunoassay. For the model protein (rabbit anti-human IgG) immunoassay, human IgG was coated and blocked with bovine serum albumin on the 96-well plate. Rabbit anti-human IgG of varying concentrations was added to incubate at 37 °C for 30 minutes, followed by the washing and addition of goat anti-rabbit IgG labeled with HRP (0.4 μg/mL). The wells were washed for five times after incubation at 37 °C for 30 minutes. Hydrogen peroxide (400 μM), sodium iodide (400 μM) and H_2O-HCl solution (pH = 3) were added to reach a final volume of 50 μL. The mixture was kept to react at 37 °C for an hour, and 20 μL of the mixture was used for iodide sensing. For hepatitis C virus (HCV) immunoassay, HCV ELISA kit that has been coated with HCV antigen was used. Clinical samples of HCV-infected patients were provided by Shandong Academy of Medical Sciences. 500-fold diluted human serum (including real samples, negative samples and blank control) was added to react at 37 °C for 30 minutes, followed by the introduction of goat anti-human IgG labeled with HRP (0.4 μg/mL). After incubation at 37 °C for 30 minutes, the wells were

washed for five times before hydrogen peroxide (400 μM), sodium iodide (400 μM) and H_2O-HCl solution (pH = 3) were added for HRP and subsequent iodide sensing.

SUPPLEMENTARY FIGURES



Scheme S1 A schematic illustration of the proposed mechanism for iodide-catalyzed oxidation of thiol compounds (such as cysteine and glutathione) to form disulfide compounds (such as cystine and glutathione disulfide) by H_2O_2. Adapted with permission from [1]. Copyright 2007 © Georg Thieme Verlag Stuttgart · New York.

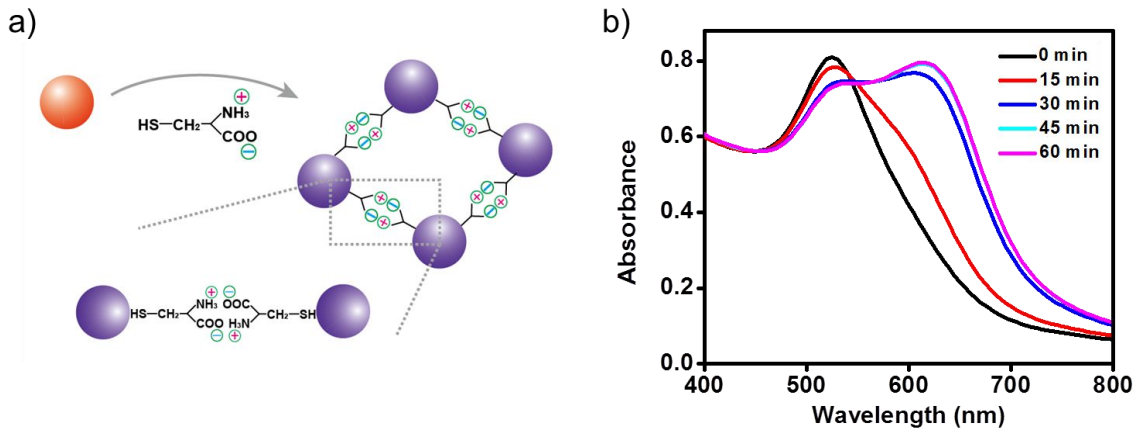


Figure S1 (a) Schematic cysteine-stimulated aggregation of AuNPs. (b)

Time-dependent UV/vis spectra of cysteine-stimulated aggregation of AuNPs.

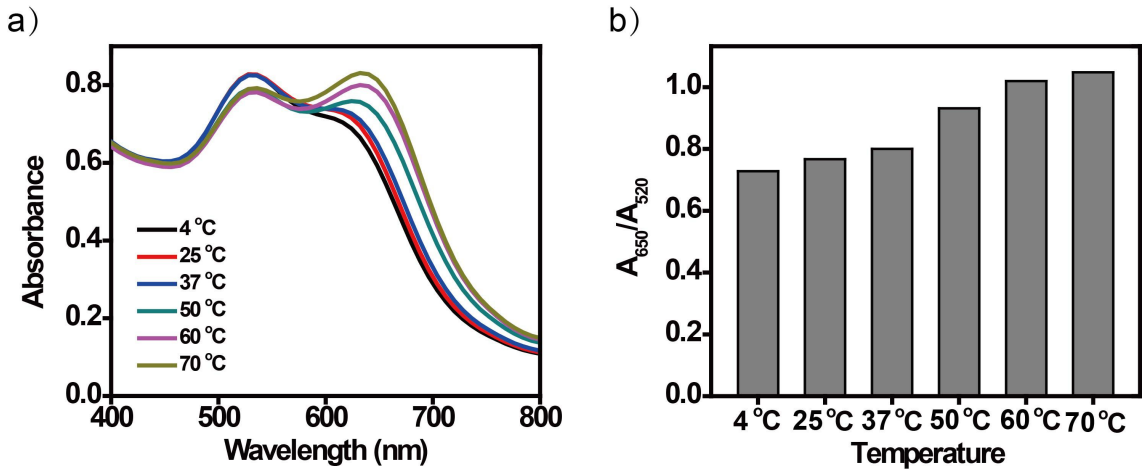


Figure S2 Cysteine-induced aggregation of AuNPs at different temperatures (4 °C, 25 °C, 37 °C, 50 °C, 60 °C, 70 °C). (a) UV/vis spectra. (b) A_{650}/A_{520} from the UV/vis spectra. The concentration of cysteine is 40 μM, and the incubation time is 20 minutes.

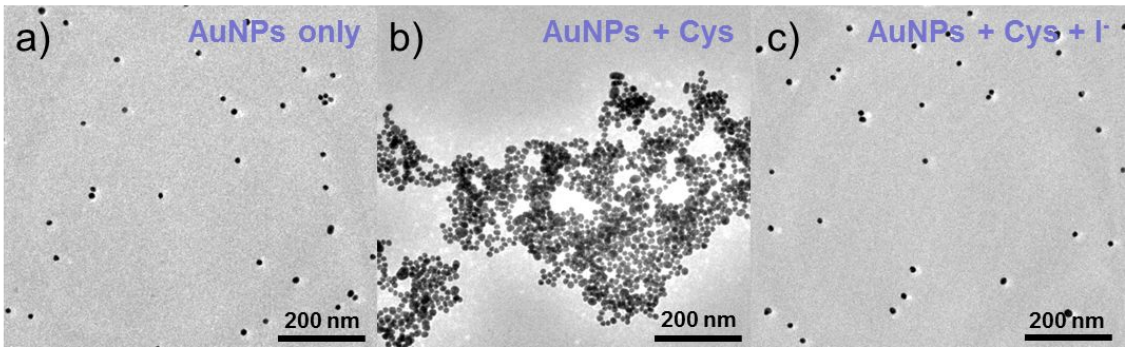


Figure S3 TEM characterizations of AuNPs. (a) AuNPs only (b) AuNPs and cysteine (c) AuNPs after iodide-catalyzed oxidation of cysteine.

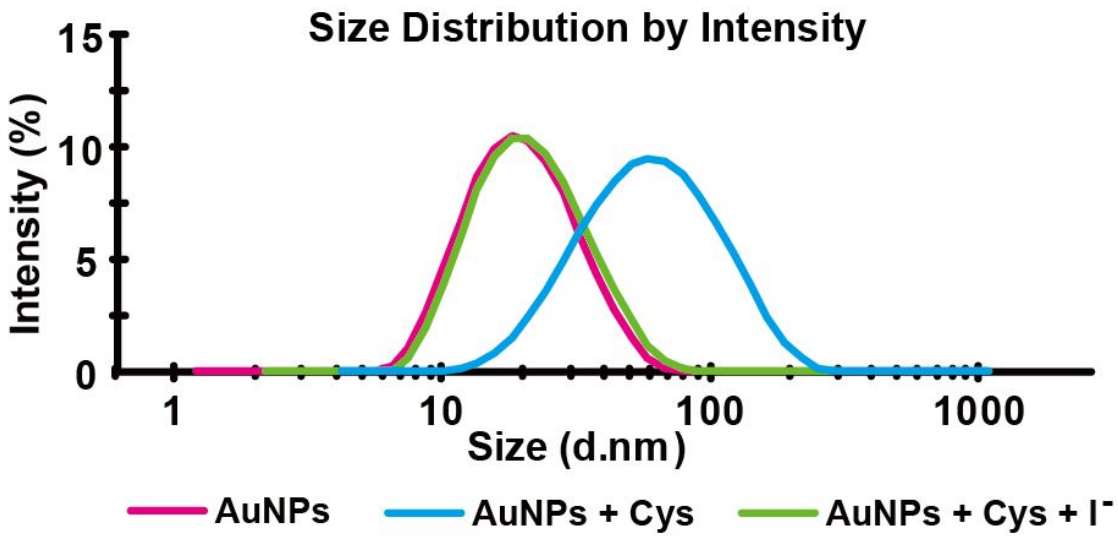


Figure S4 DLS characterization of AuNPs, AuNPs incubated with cysteine, and AuNPs after iodide-catalyzed oxidation of cysteine.

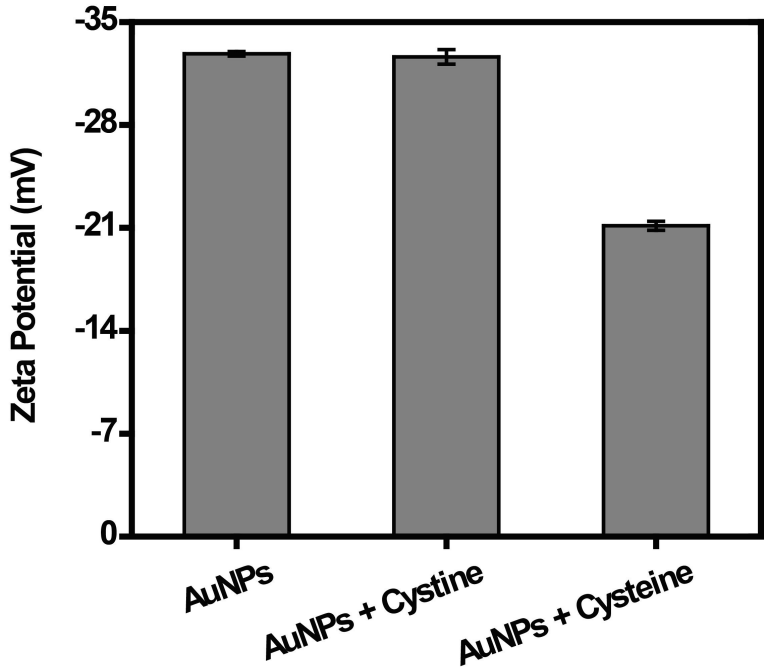


Figure S5 Zeta-potential characterization of AuNPs, AuNPs incubated with cystine that derives from iodide-catalyzed oxidation of cysteine, and AuNPs incubated with cysteine.

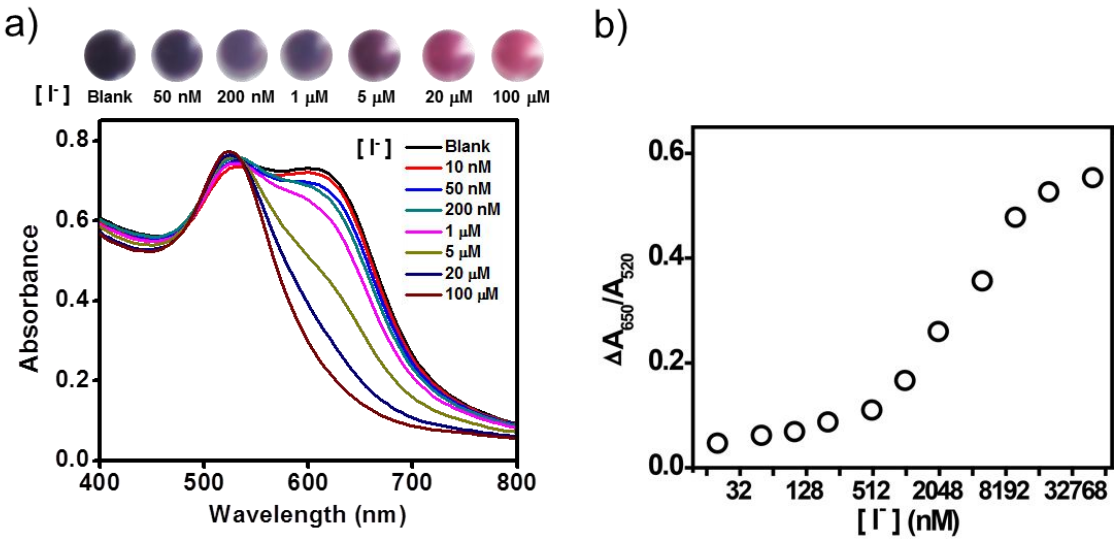


Figure S6 The sensitivity for iodide sensing. (a) UV/vis spectra and corresponding photographs of the AuNPs solution after iodide-catalyzed reaction with varying iodide concentrations (b) A_{650}/A_{520} value from the UV/vis spectra.

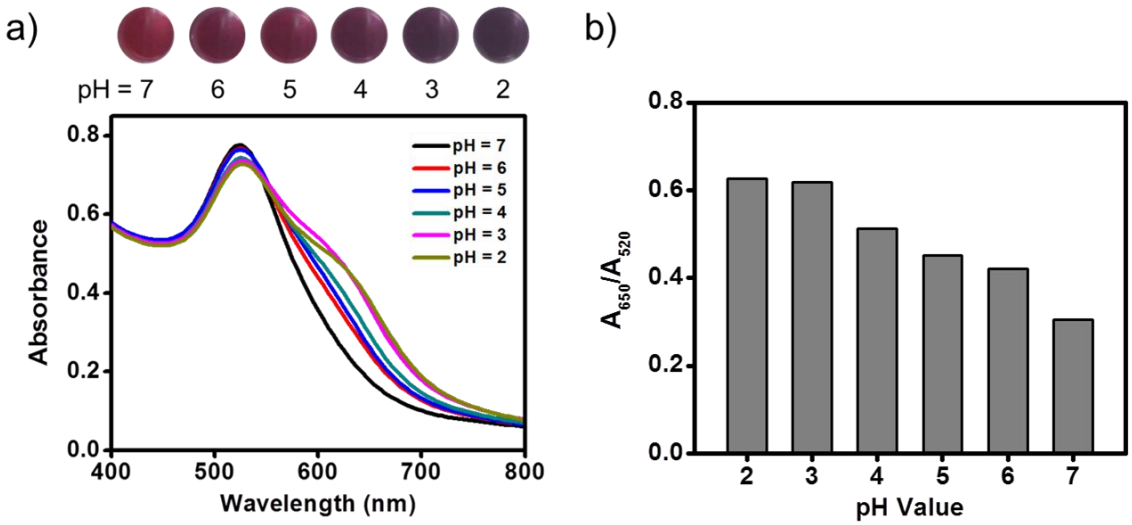


Figure S7 HRP-catalyzed oxidation of iodide under different pH values. (a) UV/vis spectra and corresponding photographs of the AuNPs solution (b) A_{650}/A_{520} value from the UV/vis spectra.

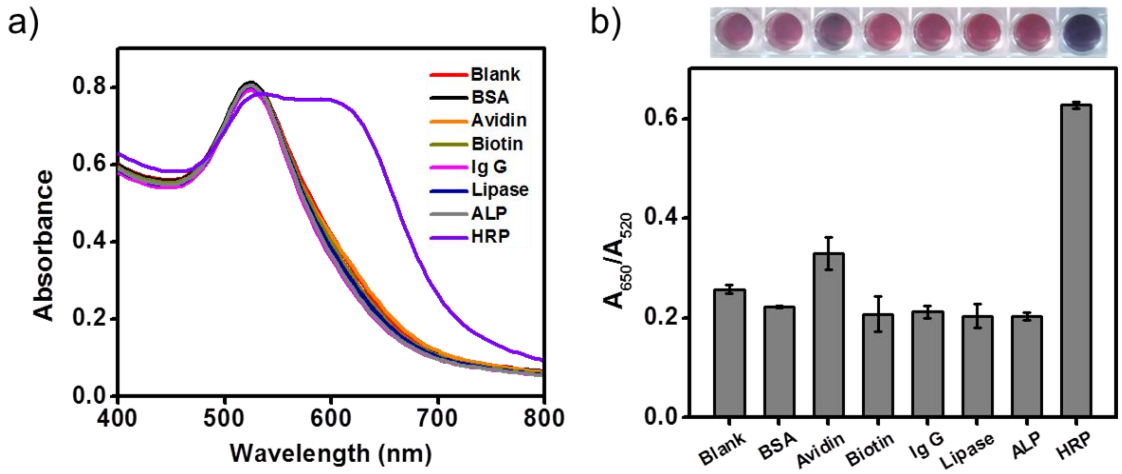


Figure S8 Response of potentially interfering proteins and enzymes towards HRP-catalyzed oxidation of iodide for HRP sensing. (a) UV/vis spectra. (b) A_{650}/A_{520} from the UV/vis spectra and corresponding photographs of the AuNPs solution.

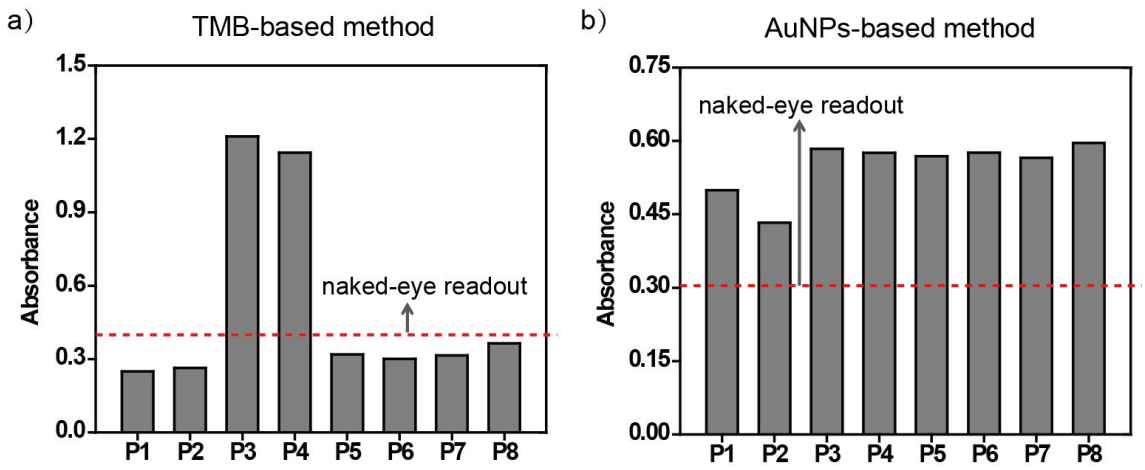
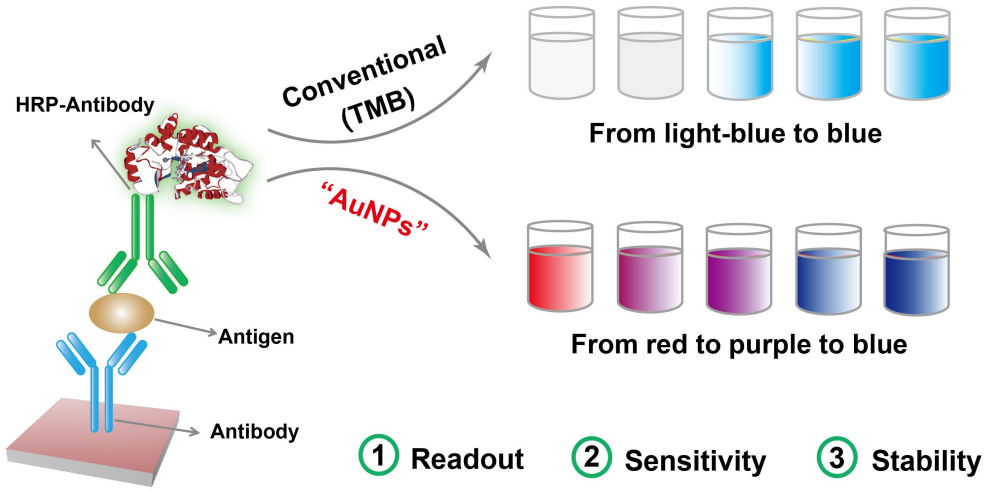
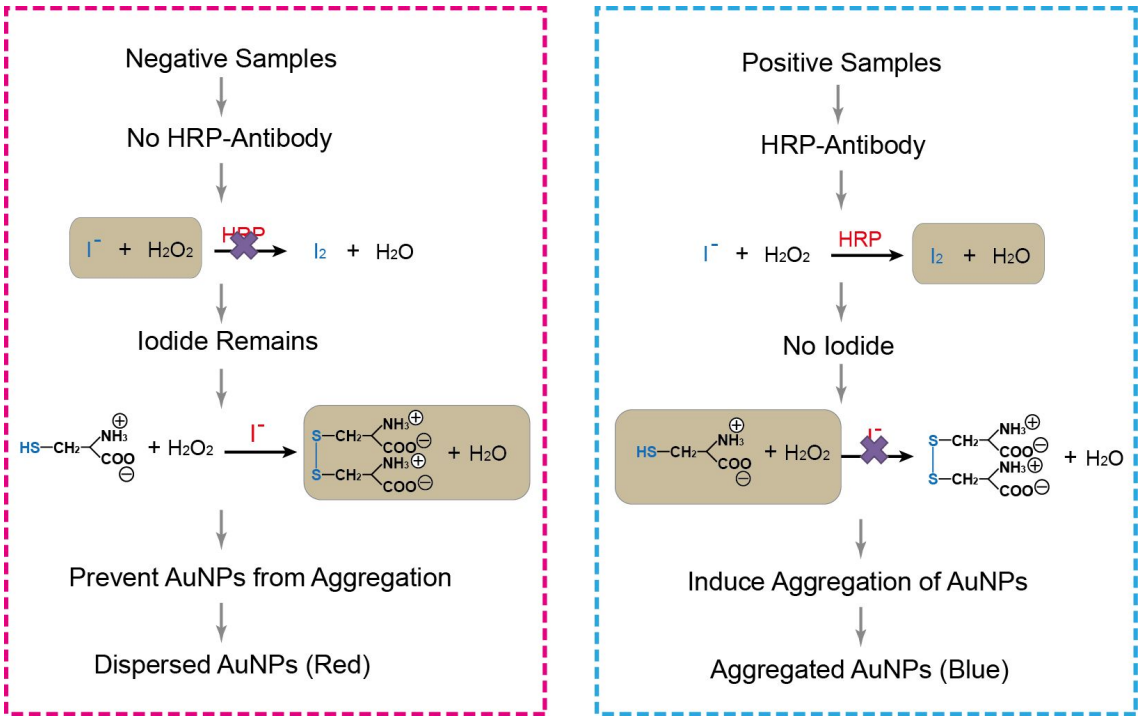


Figure S9 The assay results of anti-HCV antibodies in real clinical diagnosis. P1 to P8 represent the sera of different HCV-infected patients that are clinically diagnosed to be positive.



Scheme S2 A schematic comparison between conventional TMB-based ELISA and AuNPs-based plasmonic ELISA. Conventional TMB-based ELISA relies on distinguishing different shades of the blue-colored product. AuNPs-based plasmonic ELISA allows a more convenient readout with different colors from red to purple to blue with enhanced sensitivity.



Scheme S3 A schematic illustration of the molecular events occurred in the assaying process of negative samples and positive samples.

Reference

(1) Kirihara, M.; Asai, Y.; Ogawa, S.; Noguchi, T.; Hatano, A.; Hirai, Y. *Synthesis* **2007**, 3286-3289.