

Supporting information

Materials

Acrylic acid, acrylamide, bisacrylamide, ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (Shanghai, China). Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium borohydride (NaBH_4) and ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) were purchased from Alfa Aesar (Shanghai, China). Hydrogen peroxide (H_2O_2) was obtained from Beijing Chemicals (Beijing, China). Glucose, fructose, lactose, and maltose were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Glucose oxidase (GOx, from *Aspergillus Niger*) was purchased from BBI (Ontario, Canada). Human serum was obtained from a healthy adult male. Apple juice and orange juice (100% juice content) were purchased from Huiyuan Beverage and Food Group Co. (Beijing, China). Dialysis tubing was purchased from Pierce (2000 MWCO). Fructose, lactose, maltose, and glucose stock solutions were stored overnight at room temperature before use.¹ All reagents were of analytical reagent grade. All solutions were prepared with ultra-pure water (18.25 M Ω cm) from a Millipore system.

Apparatus and Characterization

Fluorescence measurements were carried out on Jasco-FP-6500 spectrofluorometer (Jasco International Co. LTD. Tokyo, Japan) using a quartz cell of 1 cm path length at room temperature. UV-vis absorption spectra measurements were carried out on a Cary 300 UV/vis spectrophotometer at room temperature. Glucose detection assay with the hexokinase method was carried out on an automatic Biochemistry Analyzer

(Olympus AU640, Olympus America Inc., Center Valley, PA).² Transmission electron microscope (TEM) measurements were carried out on a JEOL JEM-2010EX transmission electron microscope with a tungsten filament at an accelerating voltage of 200 kV. FTIR characterization was carried out on a BRUKE Vertex 70 FTIR spectrometer. All of the photographs were taken by a digital camera (Canon, Japan).

H₂O₂ triggered sol-gel transition

A stock solution of 30% acrylamide/bisacrylamide (29:1) was prepared for use. To verify the sol-gel transition could be triggered by H₂O₂ through a Fenton reaction, 800 μL 30% acrylamide/bisacrylamide aqueous solutions, 50 μL Fe²⁺ (1 M) and 200 μL pH 4.0 Na₂HPO₄ (25 mM) were mixed and give a homogeneous fluidic solution. Then, by addition of 10 μL H₂O₂ (10 mM) into the mixture, sol-gel transition took place immediately.

Fluorescence detection of H₂O₂

To design an optical fluorescent sensor, a stock solution of 30% acrylic acid/bisacrylamide (29:1) was prepared for use. To prepare the sensor solution, 220 μL 30% acrylic acid/bisacrylamide aqueous solutions, 30 μL Fe²⁺ (100 mM), 45 μL Na₂HPO₄ (25 mM, pH 4.0) buffer solution and 5 μL Ru(bpy)₃²⁺ (680 μM) aqueous solutions were mixed. Then, by addition 5 μL H₂O₂ of different concentrations into the mixture, sol-gel transition took place immediately and the volume of the gel shows a H₂O₂-concentration-dependent fashion. The mixture was diluted to 600 μL after incubation for 10 min at room temperature. Quantitative analysis was performed

by monitoring the fluorescence emission intensity at 586 nm of the aqueous medium with the excited wavelength at 458 nm.

Fluorescence detection of glucose

20 μL GOx (40 mg/mL) and 200 μL of glucose (40 mM) were dissolved in 0.5 mM Na_2HPO_4 (pH 7.0) buffer and the mixture was kept at 37 $^\circ\text{C}$ in a water bath for 1h. Then, the incubation solution was diluted to different concentration and taken out to add to the reaction mixture, which contain 220 μL 30% acrylic acid/bisacrylamide aqueous solutions, 30 μL of 100 mM Fe^{2+} , 45 μL of 25 mM Na_2HPO_4 (pH 4.0) buffer solution and 5 μL of 680 μM $\text{Ru}(\text{bpy})_3^{2+}$ aqueous solutions. Sol-gel transition took place immediately and the volume of the gel is glucose-concentration-dependent. The mixture was diluted to 600 μL after incubation for 10 min at room temperature. Quantitative analysis was performed by monitoring the fluorescence emission intensity at 586 nm of the aqueous medium with the excited wavelength at 458 nm and collected within a range of 520-700 nm. In control experiments, 5 mM maltose, 5 mM lactose, and 5 mM fructose were used instead of glucose for the experiment ¹.

Synthesis of acrylic acid modified AuNPs

Briefly, 10 μL of acrylic acid was added into 20 mL HAuCl_4 (1 mM) solution and the resulting solution was then kept continuously stirring for 10 min. Then, under vigorous stirring, 600 μL NaBH_4 (10 mM) was added slowly and the resulting solution was then kept continuously stirring for 15 min until an orange-red mixture was obtained. The mixture was stored in a refrigerator at 4 $^\circ\text{C}$ and then diluted to 2-fold for use after incubate for 3 days. The concentration of the prepared AuNPs

dispersion was determined with UV-vis spectrometry reported previously and found to be 5.6 nM in this study.

H₂O₂ induce AuNPs aggregation

To verify the aggregation of AuNPs was induced by H₂O₂ through a Fenton reaction, 5 μL Fe²⁺ (20 mM, in the form of [Fe(EDTA)]) was added to a 190 μL AuNP dispersion (5.6 nM), after that, 5 μL H₂O₂ (0.4 mM) was added and the resulting solution was allowed to incubate for 10 min. UV-vis absorption spectra were collected within a range of 300-800 nm at room temperature. As control experiments, sole addition of 5 μL Fe²⁺ (20 mM) or 5 μL H₂O₂ (0.4 mM) to the 190 μL AuNP dispersion (5.6 nM) was examined.

Colorimetric detection of H₂O₂

For colorimetric detection of H₂O₂, 5 μL Fe²⁺ (20 mM, in the form of [Fe(EDTA)]) was added to a 190 μL AuNPs dispersion (5.6 nM), after that, 5.0 μL H₂O₂ of different concentrations was added and the resulting solution was allowed to incubate for 10 min. UV-vis absorption spectra were collected within a range of 300-800 nm at room temperature.

Colorimetric detection of glucose

10 μL of 40 mg/mL GOx and 200 μL of glucose (10 mM) were dissolved in 0.5 mM Na₂HPO₄ (pH 7.0) buffer and the mixture was kept at 37 °C in a water bath for 1h. Then, the incubation solution was diluted to different concentration and taken out 5 μL to add to the AuNPs-Fe²⁺ solution, which containing 190 μL AuNPs dispersion (5.6 nM) and 5 μL Fe²⁺ (20 mM, in the form of [Fe(EDTA)]). UV-vis absorption

spectra were collected within a range of 300-800 nm at room temperature. In control experiments, 0.5 mM maltose, 0.5 mM lactose, and 0.5 mM fructose were used instead of glucose for the experiment¹.

Colorimetric detection of glucose

For glucose determination in human serum, the sample was firstly treated by spin dialysis at 10 000 rpm for 40 min and then diluted 2-fold using 0.5 mM Na₂HPO₄ buffer (pH 7.0) for the incubation with GOx in the next step. For glucose determination in fruit juice, apple juice or orange juice were firstly treated by centrifugation at 12 000 rpm for 40 min and the supernatants were diluted 7.5-fold using 0.5 mM Na₂HPO₄ buffer (pH 7.0) for the incubation with GOx³. For colorimetric detection, the incubation solution was diluted further before added to the detection solution.

Reference

- [1] Y. J. Song, K. G. Qu, C. Zhao, J. S. Ren and X. G. Qu, *Adv. Mater.*, 2010, **22**, 2206.
- [2] DIN EN ISO 15197: In Vitro Diagnostic Test Systems Requirements for Blood Glucose Monitoring Systems for Self-Testing in Managing Diabetes Mellitus (ISO 15179:2003). Geneva: World Health Organization, 2003.
- [3] V. Sanz, S. de Marcos, J. R. Castillo and J. Galban, *J. Am. Chem. Soc.*, 2005, **127**, 1038.

Table S1. Zeta potential of the nanoparticles

| Sample | Zeta potential (mV) |
|--------------------------------------------------------------------|---------------------|
| Acrylic acid-AuNPs | -8.97 ± 0.55 |
| Acrylic acid-AuNPs-H ₂ O ₂ -Fe ²⁺ | -15.25 ± 1.15 |

Table S2. Different method for determination of glucose in three real samples.

| Samples | Fluorescence method (mM) | Colorimetric method (mM) | Hexokinase method (mM) |
|--------------|--------------------------|--------------------------|------------------------|
| Human serum, | 5.24 | 5.29 | 5.27 |
| apple juice | 102.76 | 115.18 | 107.83 |
| orange juice | 111.62 | 136.73 | 117.53 |

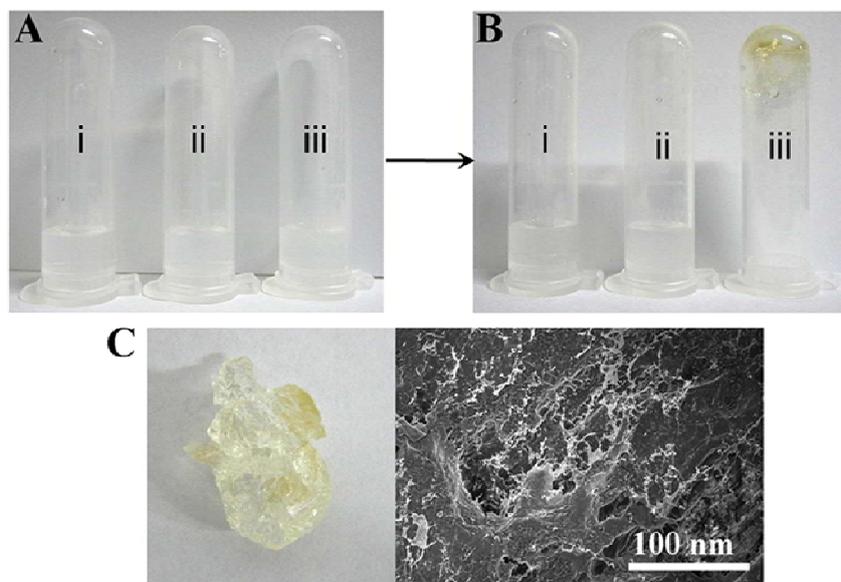


Fig. S1 Optical images of the sol-gel transition of acrylamide/bisacrylamide aqueous solutions. (A) i-iii, the aqueous solution of 800 μL 30% acrylamide/bisacrylamide (29:1) in 200 μL pH 4.0 Na_2HPO_4 (25mM) buffer. (B) i, addition 50 μL Fe^{2+} (1 M) to Ai. ii, addition 50 μL H_2O_2 (50 mM) to A ii. iii, addition 50 μL Fe^{2+} (1 M) and 50 μL H_2O_2 (50 mM) to Aiii. (C) Optical photograph (left) and SEM image (right) of the hydrogel.

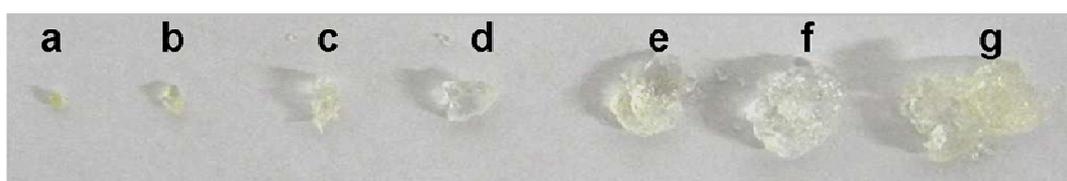


Fig. S2 Optical images of the hydrogel formation in response to different H_2O_2 concentration in acrylamide/bisacrylamide-pH 4.0 Na_2HPO_4 - Fe^{2+} system. a: 50 μM ; b: 100 μM ; c: 250 μM ; d: 500 μM ; e: 1 mM; f: 2 mM; g: 5 mM.

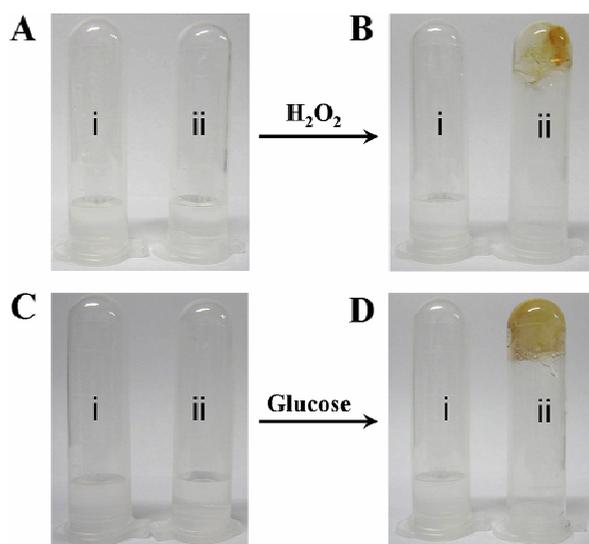


Fig. S3 Optical images of the sol-gel transition of acrylic acid/bisacrylamide aqueous solutions in response to H₂O₂. (A) i, ii, the aqueous solution of 800 μ L 30% acrylic acid/bisacrylamide (29:1) with 50 μ L Fe²⁺ (1 M) in 200 μ L pH 4.0 Na₂HPO₄ (25mM) buffer. (B) i, addition 50 μ L water to Ai. ii, addition 50 μ L H₂O₂ (50 mM) to Aii. (C) i, ii, the aqueous solution of 800 μ L 30% acrylic acid/bisacrylamide (29:1) with 50 μ L Fe²⁺ (1 M) in 200 μ L pH 4.0 Na₂HPO₄ (25mM) buffer. (D) i, addition 50 μ L water to Ci. ii, addition 50 μ L glucose (50 mM)-GOx solution, which has been incubated in pH 4.0 Na₂HPO₄ (25 mM) buffer solution at 37 °C for 1h, to Cii.

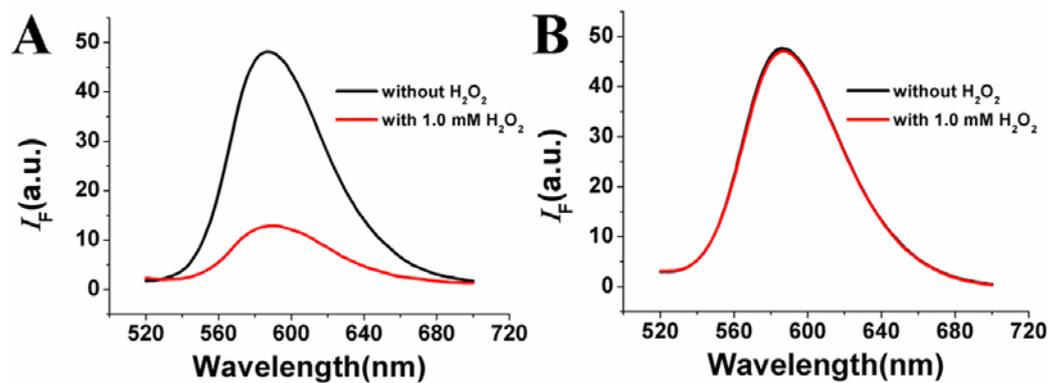


Fig. S4 Fluorescence spectra of $\text{Ru}(\text{bpy})_3^{2+}$ in the aqueous medium of pH 4.0 PBS-acrylic acid/bisacrylamide- Fe^{2+} - $\text{Ru}(\text{bpy})_3^{2+}$ system (A) and pH 4.0 PBS-acrylamide/bisacrylamide- Fe^{2+} - $\text{Ru}(\text{bpy})_3^{2+}$ system (B). Excitation wavelength was 458 nm.

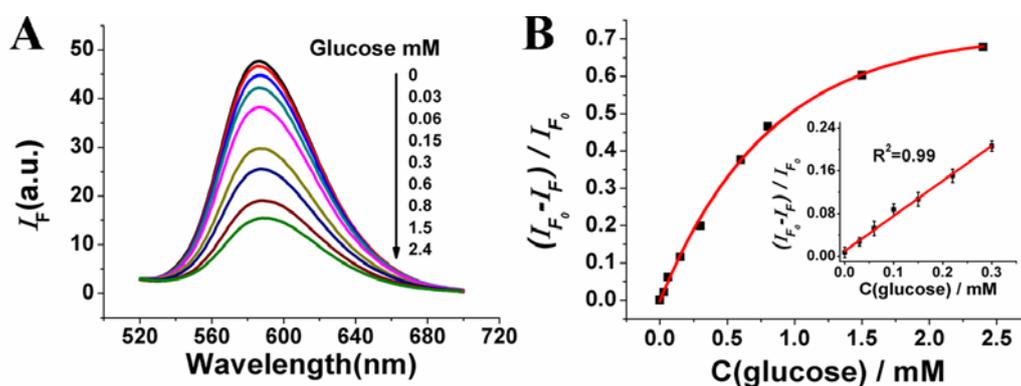


Fig. S5 Sensitivity tests of the sol-gel detection of glucose. (A) Fluorescence emission spectra of the aqueous medium of pH 4.0 PBS-acrylic acid/bisacrylamide- Fe^{2+} - $\text{Ru}(\text{bpy})_3^{2+}$ system after addition of different concentration of glucose (after incubated with GOx). (B) Quantification of glucose concentration by monitoring the relative fluorescence intensity $((I_{F_0} - I_F) / I_{F_0})$, where I_{F_0} and I_F are fluorescence emission intensity at 586 nm of the aqueous medium without or with glucose. Excitation wavelength was 458 nm.

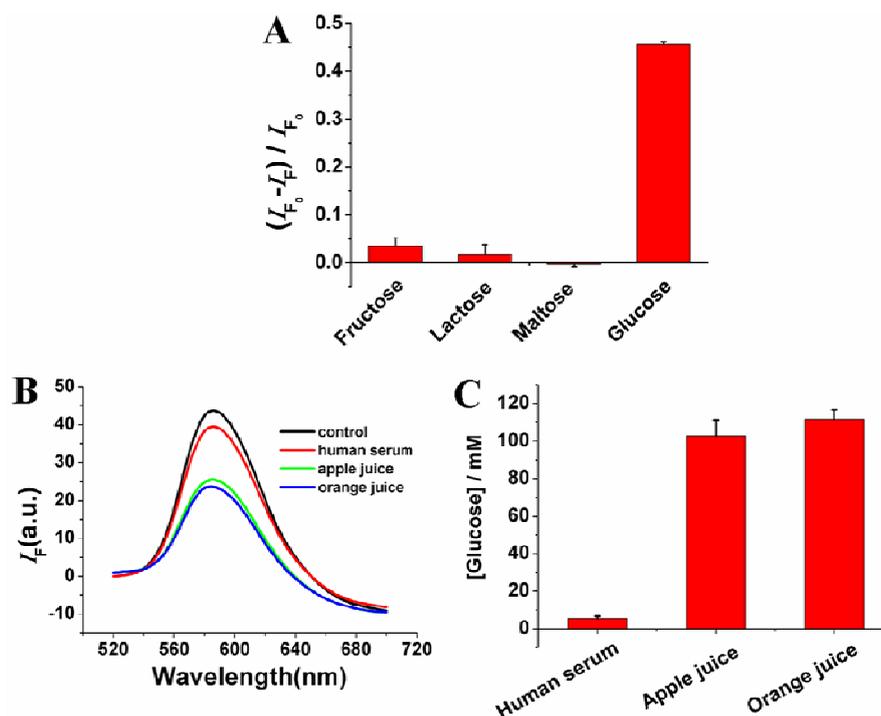


Fig. S6 (A) Selectivity analysis for glucose detection by monitoring the relative fluorescence. The analyte concentrations were as follows: 5 mM fructose, 5 mM lactose, 5 mM maltose, and 0.8 mM glucose. The error bars represent the standard deviation of three measurements. (B) The fluorescence spectra for different samples (buffer solution or diluted human serum and fruit juice samples) after incubation with GOx. Human serum, apple juice, and orange juice were diluted 40-, 150-, and 150-fold, respectively. (C) The glucose concentration in the sample according to the calibration curve of Fig. S5B.

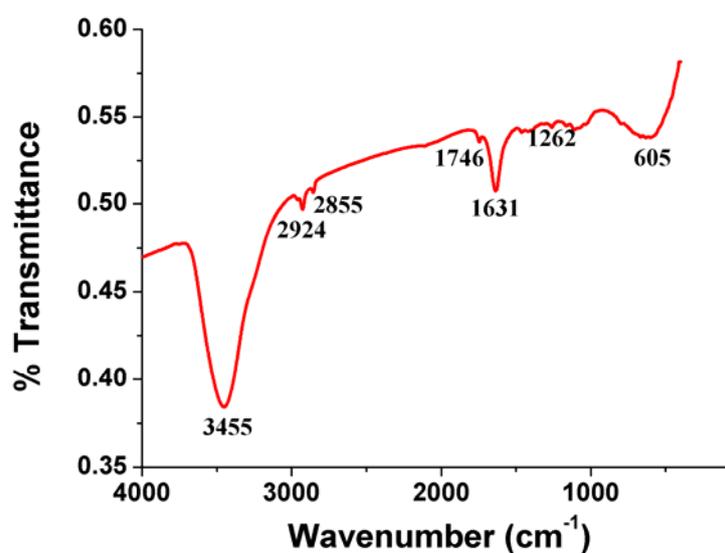


Fig. S7 FTIR Spectra of acrylic acid modified AuNPs.

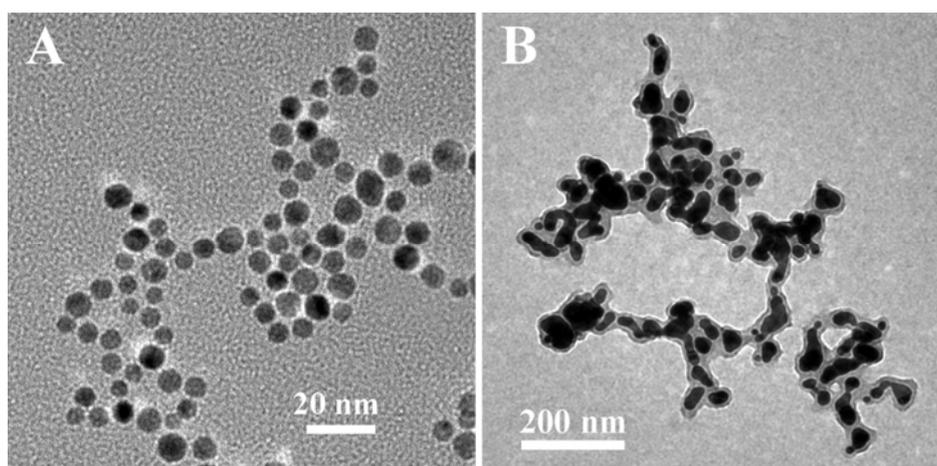


Fig. S8 TEM images of (A) acrylic acid modified AuNPs, (B) 0.5 mM Fe²⁺ (in the form of [Fe(edta)]) and 12.5 μM H₂O₂ added.

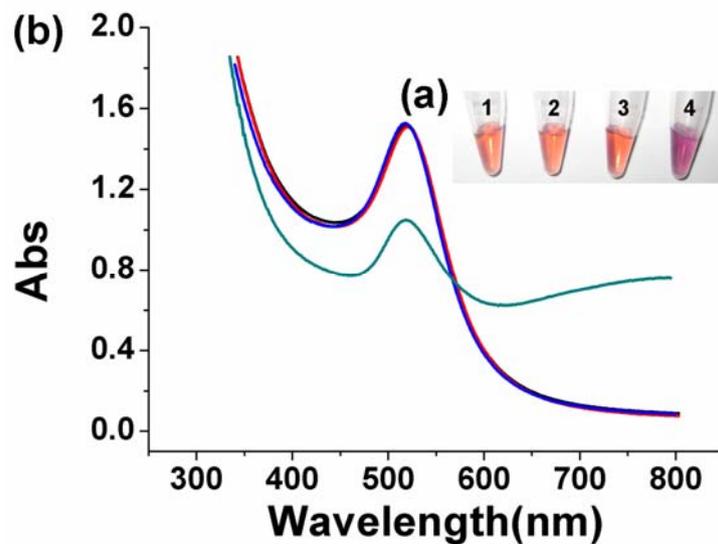


Fig. S9 H_2O_2 -induced aggregation of AuNPs. (a) Photographs and (b) UV/Vis spectra of AuNPs dispersions, prepared first by addition of 5 μL H_2O (black curve, vial 1), 5 μL H_2O_2 (0.5 mM; red curve, vial 2), 5 μL Fe^{2+} (20 mM, in the form of $[\text{Fe}(\text{edta})]$; blue curve, vial 3) or 5 μL H_2O_2 (0.5 mM) and 5 μL Fe^{2+} (20 mM, in the form of $[\text{Fe}(\text{edta})]$; green curve, vial 4) to 190.0 μL AuNPs (5.6 nm).

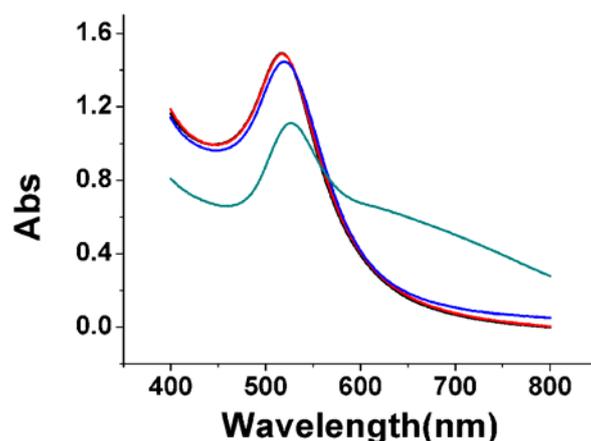


Fig. S10 Glucose-induced aggregation of AuNPs. UV/Vis spectra of AuNPs dispersions, prepared first by addition of 5 μL H_2O (black curve), 5 μL glucose (1.5 mM; after incubated with GOx, red curve), 5 μL Fe^{2+} (20 mM, in the form of $[\text{Fe}(\text{edta})]$; blue curve) or 5 μL H_2O_2 (1.5 mM, after incubated with GOx) and 5 μL Fe^{2+} (20 mM, in the form of $[\text{Fe}(\text{edta})]$; green curve) to 190.0 μL AuNPs (5.6 nm).

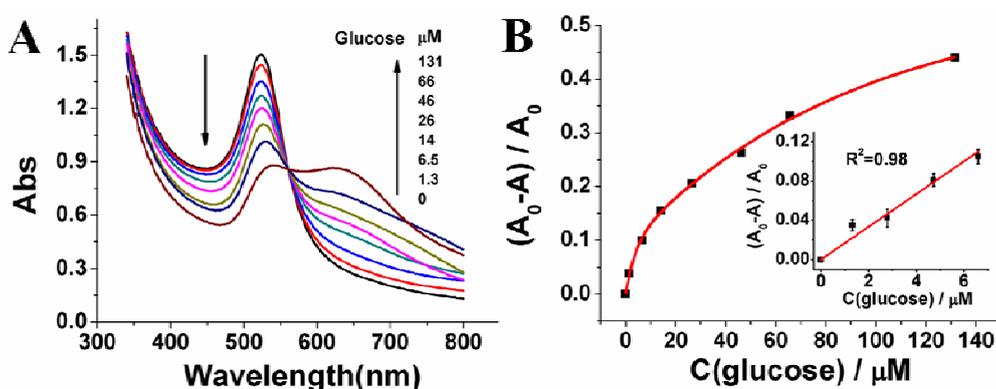


Fig. S11 (A) UV/visible absorption spectra of AuNPs- Fe^{2+} reaction mixture after addition of different concentration of glucose (after incubated with GOx). (B) Quantification of glucose concentration by monitoring the relative UV/visible absorption at 519 nm: $(A(0)_{519} - A_{519}) / A(0)_{519}$, where $A(0)_{519}$ and A_{519} are UV/visible absorption at 519 nm of the reaction mixture without or with glucose.

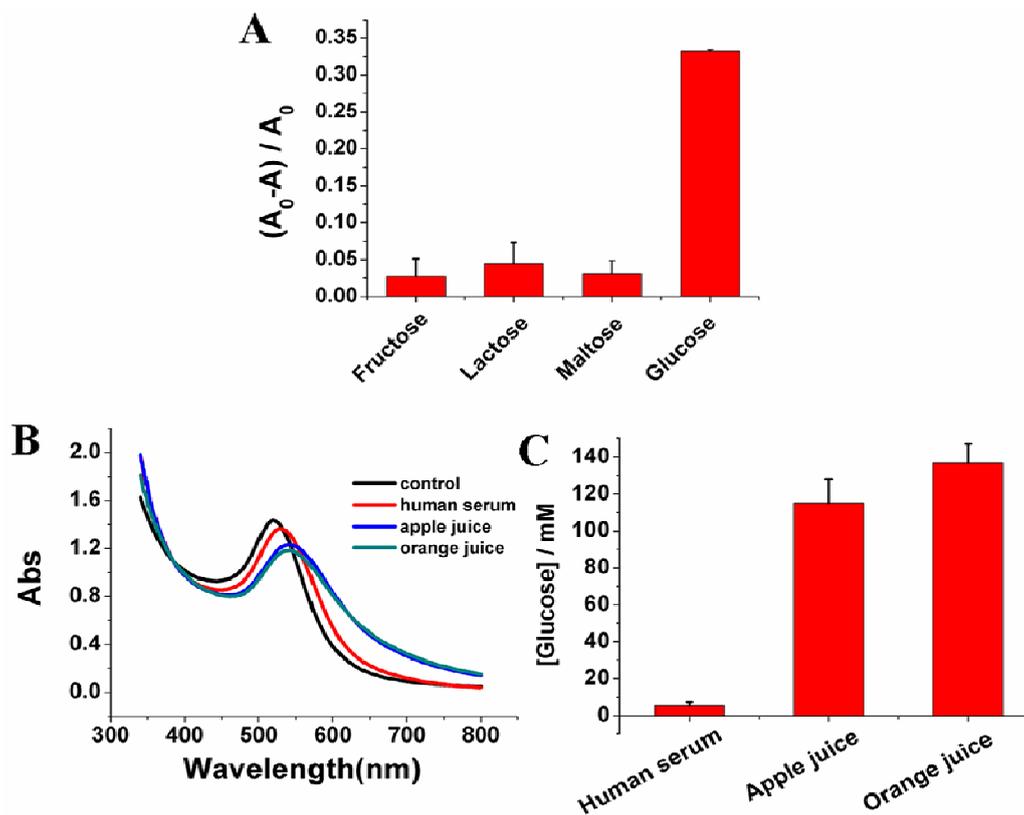


Fig. S12 (A) Selectivity analysis for glucose detection by monitoring the relative relative UV/visible absorption at 519nm of the AuNPs reaction solution. The analyte concentrations were as follows: 0.8 mM fructose, 0.8 mM lactose, 0.8 mM maltose, and 0.066 mM glucose. The error bars represent the standard deviation of three measurements. (B) The fluorescence spectra for different samples (buffer solution or diluted human serum and fruit juice samples) after incubation with GOx. Human serum, apple juice, and orange juice were diluted 1000-, 5000-, and 5000-fold, respectively. (C) The glucose concentration in the sample according to the calibration curve of Fig. S11B.