

Plasmonic blood glucose monitor based on enzymatic etching of gold nanorods

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Experimental Section

Materials and Measurements.

Ascorbic acid was purchased from Alfa Aesar (Ward Hill, MA), $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, AgNO_3 , NaBH_4 , FeSO_4 , hexadecyltrimethylammonium bromide (CTAB), H_2O_2 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). D-Glucose was purchased from Bio Basic Inc. (Ontario, Canada). EDTA and Glucose oxidase (GOx) were purchased from Sangon (Shanghai, China). Dopamine (DA) and uric acid (UA) were purchased from Sigma-Aldrich (St. Louis, Mo). Millipore Microcon centrifugal filter devices (NMWL 3KDa) were purchased from Fisher (Montreal, Canada). All chemical reagents were of analytical grade and used without further purification. All solutions were prepared using ultrapure water ($18.25 \text{ M}\Omega \text{ cm}$) from the Millipore Milli-Q system. Serum samples were obtained from the hospital of Hunan University (Changsha, China). The concentration of all reagents in this work was final concentration except the concentration of analytes (H_2O_2 or glucose) was initial concentration in the samples to be analyzed.

Absorbance experiments were performed on Synergy Mx multimode microplate reader (BioTek Instruments, Inc.). The UV-Vis absorbance spectra were scanned from 450 to 900 nm at 30°C . Each sample was repeated three times to obtain the mean values ($n = 3$).

Preparation of gold nanorods.

GNRs were prepared according to silver ion-assisted seed-mediated growth approach previously reported in literature¹ with moderate modification. Briefly, 10 mL of 0.1 M CTAB was mixed with 0.25 mL of 0.01 M HAuCl₄ in a 20-mL glass bottle with gentle mixing. 0.6 mL of freshly prepared, ice-cold 0.01 M NaBH₄ solution was then added quickly to the mixture solution, followed by rapid inversion for 2 min. The color of the solution changed from dark yellow to brownish yellow, indicating the seed solution formation. The seed solution was kept at room temperature for at least 2 h before use. To prepare the GNRs growth solution, 2.0 mL of 0.01 M HAuCl₄ and 0.4 mL of 0.01 M AgNO₃ aqueous solution were mixed with 40 mL of 0.1 M CTAB in a 50-mL glass bottle, then 0.8 mL of 1.0 M HCl was injected into the mixture and the pH of the solution was adjusted to 1-2. After gently mixing the solution, 0.32 mL of 0.1 M ascorbic acid was added and the growth solution was obtained. Finally, 0.096 mL of seed solution was injected into the growth solution with gently mixing for 10 s, and the color of the solution gradually changed to dark red in the first 30 min. Left it undisturbed at room temperature for 24 h to ensure full growth of GNRs.

The oxidation of GNRs was accomplished by adding an appropriate volume of H₂O₂ (35 wt %) into the obtained starting GNRs. The oxidation process was monitored by taking UV-Vis absorbance spectra as a function of time. Shorter GNRs were obtained at intermediate stages of oxidation by centrifugation and resuspension in 0.1 M CTAB for use.

Absorbance spectrum and TEM Characterizations.

UV-Vis absorbance spectra of GNRs solutions were taken using Synergy Mx multimode microplate reader. Low-magnification TEM images were acquired on a JEOL JEM-2100F. For TEM characterization, GNRs (1 mL each) were centrifuged at 10000 r/min for 20 min, and then redispersed into ultrapure water (1 mL each), centrifuged again at 5000 r/min for 20 min, and finally redispersed into ultrapure water (0.2 mL each). An amount of 0.01 mL of each resulting GNRs solution was drop-cast carefully onto a lacey-Formvar TEM grid stabilized with a thin layer of carbon and allowed to dry in air overnight before TEM imaging.

GNRs based Sensor for H₂O₂ detection.

It has been reported that the aspect ratio of GNRs in solution decreases with increasing temperature.² In order to avoid thermal reshaping of GNRs and get a better result, in the following studies, the reaction was carried out by incubating the system at 30 °C for 3 h.

For H₂O₂ sensing, 10 µL of 2.5 mM FeSO₄, 10 µL of 2.5 mM EDTA and 20 µL of H₂O were added in a 200 µL PCR tube, followed by adding 48 µL of GNRs and 2 µL of 1M HCl. For simplicity, the above-mentioned reaction system is designated as GNRs reaction solution in the next section. Then 10 µL H₂O₂ with different concentrations (0.1 mM - 100 mM) was added. After rapid inversion, the mixture solution was gently mixed at 30 °C for 3h, then the samples were transferred to a 96-well plates. H₂O (10 µL) was used instead of 10 µL of H₂O₂ for the control. The microwell plate loaded with the samples was placed into the Synergy Mx multimode microplate reader, and the protocol was set by the procedure of measuring the absorption spectra from 450 to 900 nm at 30 °C. The signal is defined as the shift of longitudinal surface plasmon resonance (LSPR), ΔW ($\Delta W = W_1 - W_2$, W_2 and W_1 are the longitudinal surface plasmon wavelength of sample and control).

For investigation of the effect of the Fenton reaction to speed up the oxidation of GNRs, a series of samples consisting of 48 µL of GNRs, 2 µL of 1M HCl, 20 µL of H₂O, 10 µL of H₂O₂ (100 mM and 10 mM, respectively) and 20 µL of Fe²⁺-EDTA or H₂O were prepared. To study the influence of the initial aspect ratios of GNRs on LSPR response, a series of samples containing 48 µL solution of different kinds of GNRs with varying aspect ratio, 2 µL of 1 M HCl, 40 µL of H₂O and 10 µL of 100 mM H₂O₂ were prepared.

GNRs based Sensor for Glucose detection.

The glucose reaction solutions were composed of 90 µL of glucose (initial concentrations, 0, 100 µM, 250 µM, 500 µM, 750 µM, 1 mM, 2.5 mM, 5 mM, 10 mM, 25 mM, and 50 mM, respectively) and 10 µL of 0.4 mg mL⁻¹ GOx. After 1 h of incubation in a water bath at 37 °C for GOx-catalyzed reaction, 10 µL of the resulting solution was added to the GNRs reaction solutions. The incubation and detection

procedure was the same as shown in the above-mentioned H_2O_2 sensing experiment.

To test the selectivity of the proposed glucose sensor, 20 μM DA, 50 μM AA and 20 μM UA were used instead of glucose, respectively. To investigate the effect of the analogues of glucose on the performance of this sensor, 25 mM sucrose, lactose and fructose were used instead of glucose, respectively. The remaining steps were the same as above-mentioned procedure. To confirm that the oxidation of GNRs was induced by the generation of H_2O_2 from the GOx-catalyzed oxidation of glucose, 10 μL of 0.2 mg mL^{-1} catalase, an enzyme that catalyzes H_2O_2 disproportionation, was added to the glucose reaction solution containing 4.5 mM glucose (final concentration).

Analysis of Glucose in Human Serum Samples

The human serum samples were separated by centrifugation on Amicon Ultra centrifugal filter device with a 3,000 molecular weight cutoff according to the manufacturer's instructions. It made it possible to obtain the glucose from the serum samples, as the macromolecular and proteins remained in the concentrated fractions. Followed procedure was the same as the previously mentioned process for glucose detection.

1. W. Ni, X. Kou, Z. Yang and J. Wang, *ACS Nano*, 2008, **2**, 677-686.
2. L. Gou and C. J. Murphy, *Chemistry of Materials*, 2005, **17**, 3668-3672.

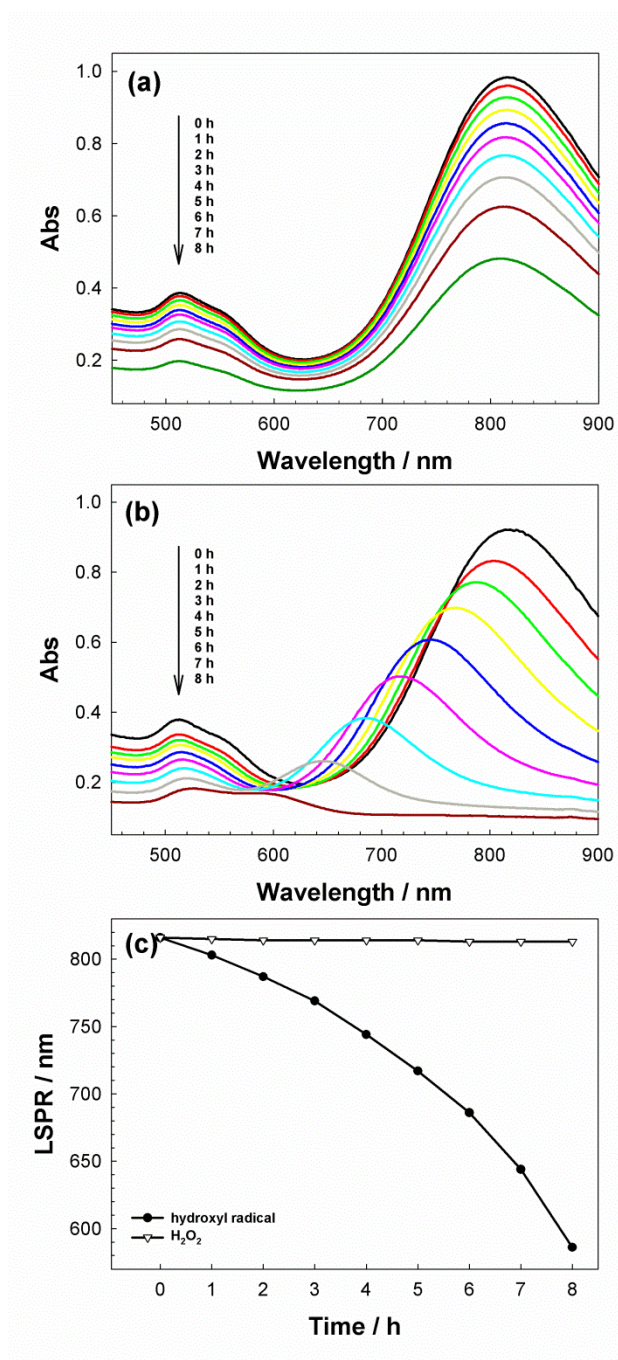


Fig. S1 The effect of the Fenton reaction in the oxidation of GNRs. The UV-Vis spectra change of GNRs in the oxidation etching process (a) with 1 mM H₂O₂; (b) with hydroxyl radical generated by Fenton reaction, C_{H₂O₂} = 1 mM, C_{Fe²⁺-EDTA} = 0.25 mM; (c) the LSPR shift of the GNRs as the function of oxidation time with H₂O₂ or hydroxyl radical; hydroxyl radical was generated by Fenton reaction, C_{H₂O₂} = 1 mM, C_{Fe²⁺-EDTA} = 0.25 mM.

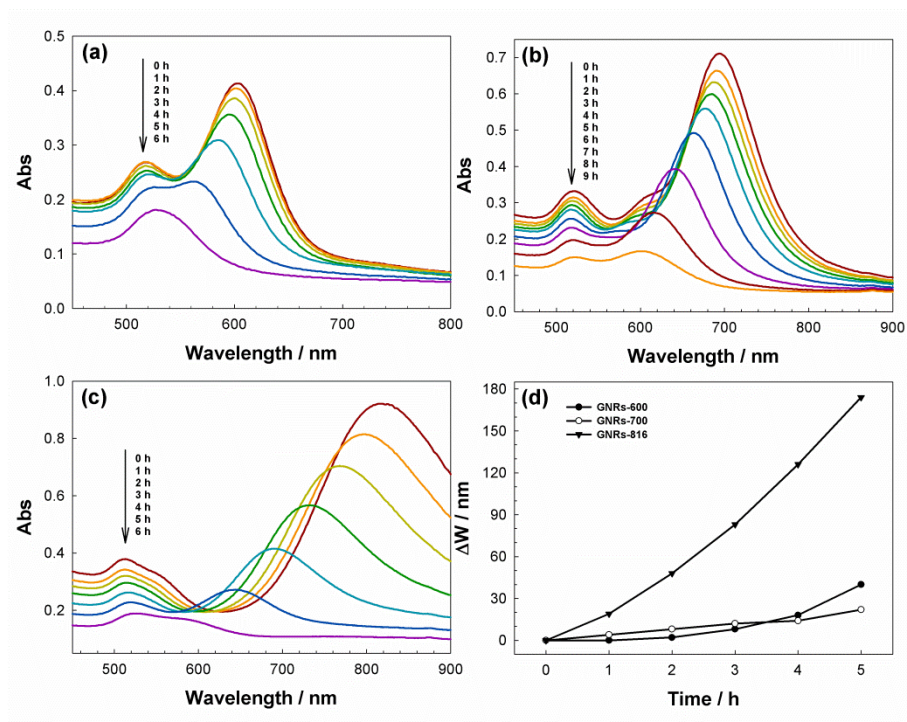


Fig. S2 The UV/Vis spectra change of GNRs in the oxidation etching process via Fenton reaction with different aspect ratios of GNRs. (a) GNRs-600; (b) GNRs-700; (c) GNRs-816; (d) The variation of the LSPR shift of three different GNRs with H_2O_2 via Fenton reaction. $C_{H_2O_2} = 10$ mM, $C_{Fe^{2+}-EDTA} = 0.25$ mM.

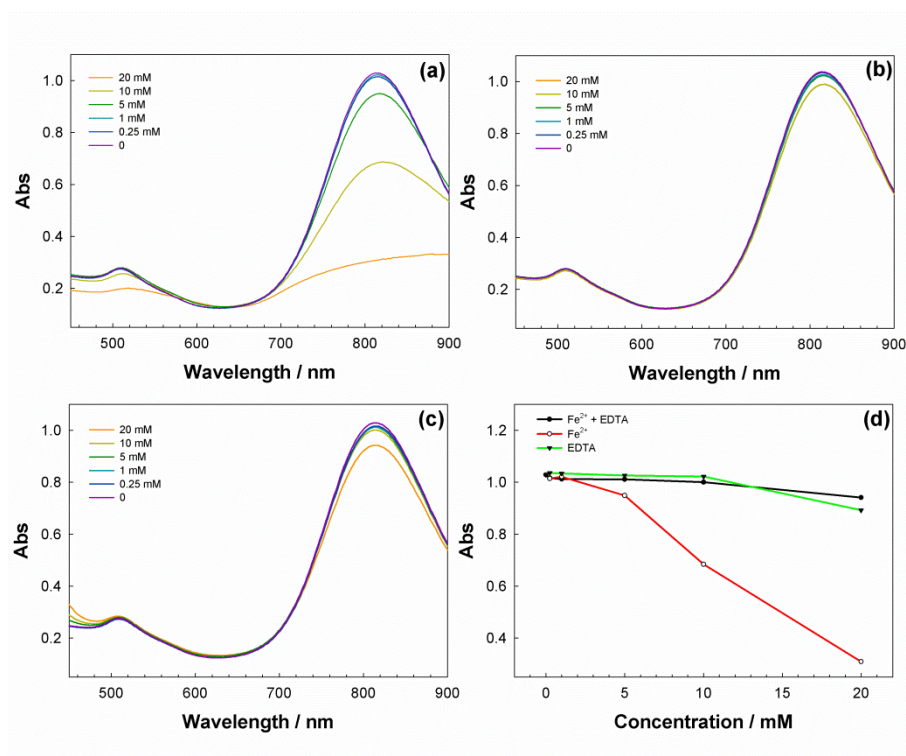


Fig. S3 The UV/Vis spectra of GNRs upon addition of different concentration of Fe^{2+} (a), EDTA (b) and both Fe^{2+} and EDTA of the same concentration (c) (final concentrations: 20, 10, 5, 1, 0.25, 0 mM, respectively), $t = 12$ h; (d) the relative absorption intensity of LSPR peak at 816 nm dependent on the different concentration of Fe^{2+} , EDTA, and Fenton reagent Fe^{2+} -EDTA in the absence of H_2O_2 .

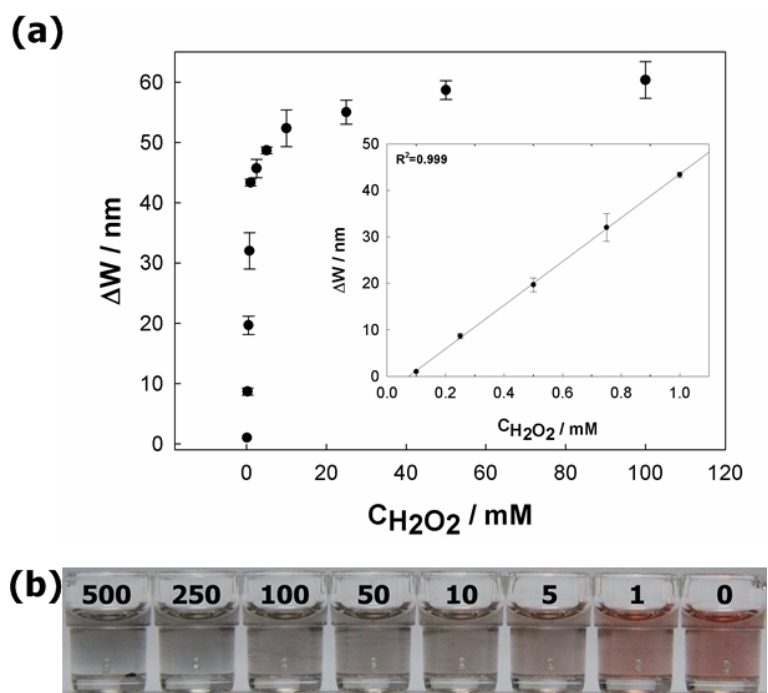


Fig. S4 **(a)** The relationship between the concentrations of H_2O_2 and the variation of absorption wavelength, the inserted figure is the calibration curve for the detection of H_2O_2 ($\text{CH}_2\text{O}_2 = 0.1 - 1$ mM). Each point reflects the average of three independent experiments. Error bars indicate standard deviations. (initial concentration: 100, 50, 25, 10, 5, 2.5, 1, 0.75, 0.5, 0.25, 0.1 mM, respectively). $t = 3$ h, $\text{C}_{\text{Fe}^{2+}\text{-EDTA}} = 0.25$ mM. **(b)** Color change of the plasmonic sensor with the decrease of H_2O_2 concentrations (initial concentration: 500, 250, 100, 50, 10, 5, 1, 0 mM, respectively). $\text{C}_{\text{Fe}^{2+}\text{-EDTA}} = 1$ mM, $t = 30$ min.

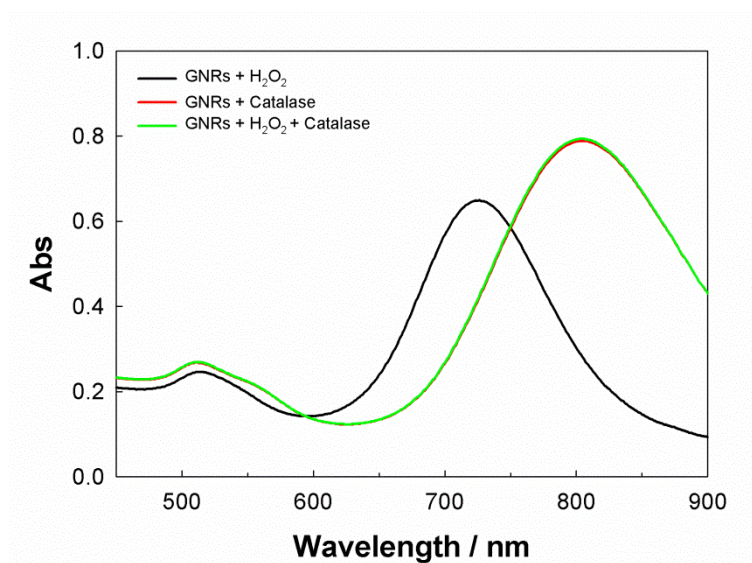


Fig. S5 UV/Vis spectra of GNRs prepared with the addition of 10 μ L catalase (0.2 mg mL⁻¹) in the presence or absence of 10 mM H₂O₂ (final concentration).

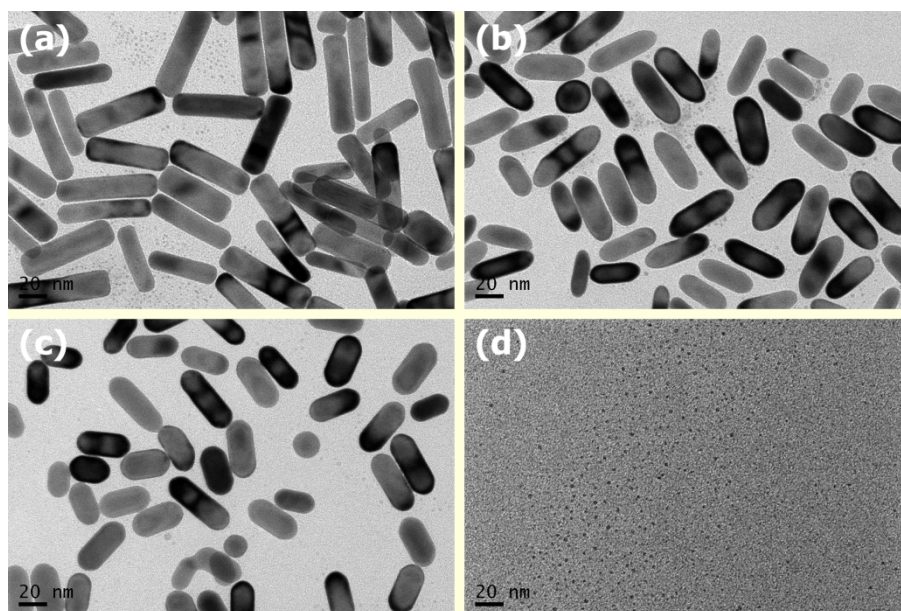


Fig. S6 TEM micrographs of GNRs before (a) and after oxidation for 30 min (b), 2 h (c), and 6 h (d) with 4.5 mM glucose in the presence of GOx via Fenton reaction. $C_{\text{Fe}^{2+}\text{-EDTA}} = 1$ mM. Scale bar: 20 nm.

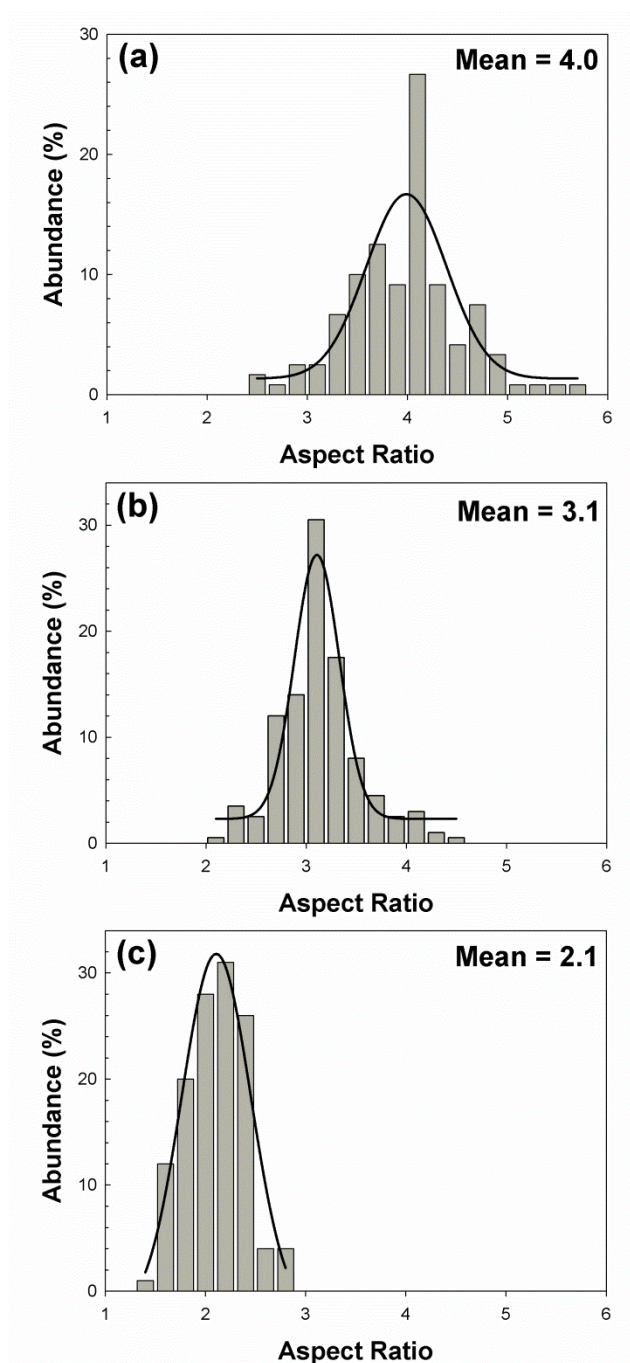


Fig. S7 Distributions of aspect ratios analyzed from the corresponding TEM micrographs of GNRs before (a) and after oxidation for 30 min (b), and 2h (c).

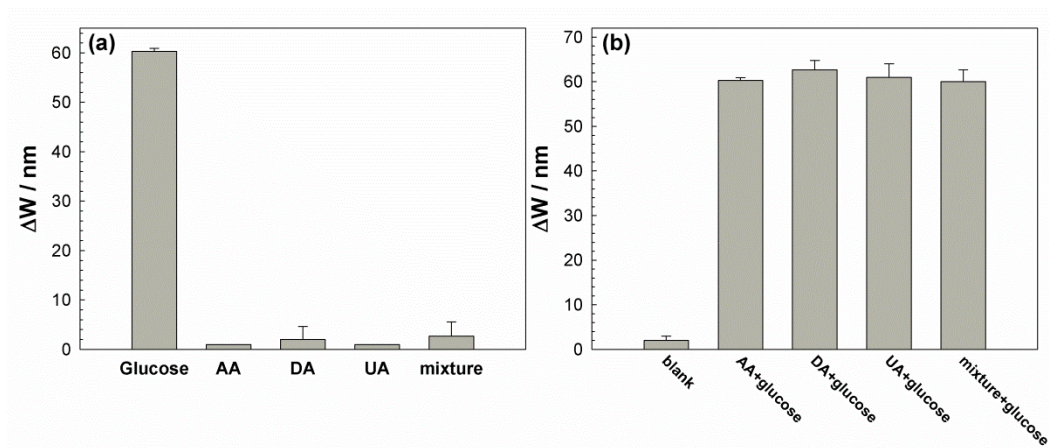


Fig. S8 Selectivity tests of the proposed glucose sensor to normal interferences in traditional glucose assay. ΔW : the LSPR shift of the sensing system in response to (a) 20 μM DA, 50 μM AA or 20 μM UA and (b) the mixture of 5 mM glucose and 20 μM DA, 50 μM AA or 20 μM UA.

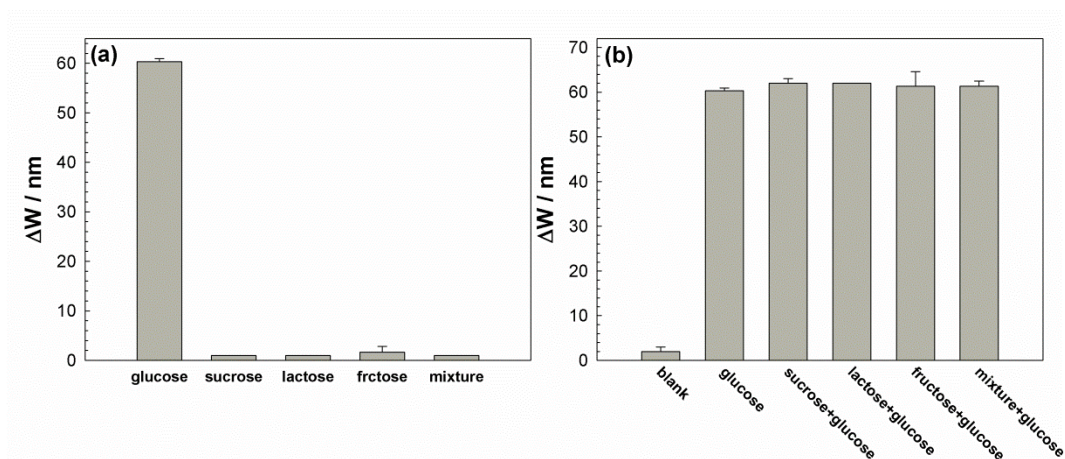


Fig. S9 (a) Selectivity tests of the proposed glucose sensor to normal analogues in traditional glucose assay. ΔW : the LSPR shift of the sensing system in response to (a) 25 mM sucrose, 25 mM lactose or 25 mM fructose and (b) the mixture of 5 mM glucose and 25 mM sucrose, 25 mM lactose or 25 mM fructose.

Table S1 Determination of blood glucose in human serum samples.

Samples	Glucose found by the proposed sensor (mM)	Glucose found by ASCA AG-II (mM)	RSD (%)
1	5.34 ± 0.17	5.26	1.5
2	5.44 ± 0.68	5.56	2.2
3	6.23 ± 0.42	6.22	0.2