Supporting Information for

A high-throughput colorimetric assay for glucose detection based on glucose oxidase-catalyzed enlargement of gold nanoparticles

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Experimental Section

Materials and Instruments. Gold nanoparticles (AuNPs, 83 nM) with a diameter of 5 nm were purchased from TED PELLA, Inc. Glucose oxidase (GOx) and HSA was purchased from Sigma Aldrich. Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), hydrogen peroxide (H_2O_2) were purchased from Aladdin (Shanghai, China). Amino acids and saccharides were purchased from Guangfu Chemical Reagent (Tianjin, China). Glucose Assay Kit was purchased from Biosino Bio-Technology and Science Inc. The 96-well polystyrene plate was purchased from R&D systems. The serum samples were obtained from the Third Xiangya Hospital, Central South University (China). Deionized water (Milli-Q grade, Millipore) with a resistivity of 18.2 MΩ·cm was used throughout this study. The UV−vis spectra of AuNP solutions were recorded with a Hitachi U-3900 UV−vis spectrophotometer. The absorbance of AuNP solutions in 96-well plates were collected at 530 nm by a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek Instruments, Inc.). TEM images

were obtained by using a JEOL 1400 model at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) was performed on a Zeta Sizer Nano ZS (Malvern Zetasizer 300HS and He/Ne laser at 632.8 nm at scattering angles of 90 at 25 ^{o}C).

Procedures for Glucose Sensing. All the experiments were carried out in the 96-well plate. Glucose in PBS (0.01 M, pH= 7.4), $HAuCl_4$ (0.16 mM) and 5 nm AuNPs (8.3 nM) were added in sequence. After adding all the reactants, kinetic of the absorbance at 530 nm in 30 minutes was measured and the UV-vis spectra were measured soon afterwards.

Detection of glucose in clinical samples. For blood glucose sensing, all the human serum samples were ultra-filtrated by a 3 kDa ultrafiltration tube to remove large molecules and unknown precipitates, followed by diluting for 10-folds with deionized water to locate in the linear range of our assay. Then, the obtained samples were assayed under the conditions above.

Glucose kit-based assay. We applied the commercial glucose kit to measure the levels of both healthy and patient samples, and the obtained results were compared with those from the AuNP-based assay. We performed the detection procedures strictly following the recommended

procedures as received.

Evaluation of the clinical test performance. Sensitivity and specificity are two basic parameters used for evaluating the detection performance of a diagnostic system in clinical test. Based on the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN), the clinical test performance can be defined as:

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Selectivity = \frac{TN}{TN + FP}$$

Receiver operating characteristic (ROC) curve is an overall indicator for sensitivity and specificity in a clinical test. A ROC space is defined by setting 100 % − specificity % and sensitivity as x and y axes respectively, based on which, the ROC curves were created using GraphPad Prism 6.0.

RGB analysis of paper-based method. According to the RGB color model, red (R), green (G) and blue (B) are three primary color components which can create any color. By analyzing the relative intensity of red color, we can evaluate the red chromaticity level (r) of the grown AuNPs on filter paper, which is corresponding to the concentration of glucose added. The red chromaticity level (r) was calculated as below, [S1]

$$r = \frac{R}{R + G + B},$$

where R, G, and B values were obtained from Photoshop.

Ethics statement

Our study was approved by the institutional review board of Nankai University. The use of the clinical samples was approved by both the patients and the Ethics Committee of the Third Xiangya Hospital, Central South University (China).

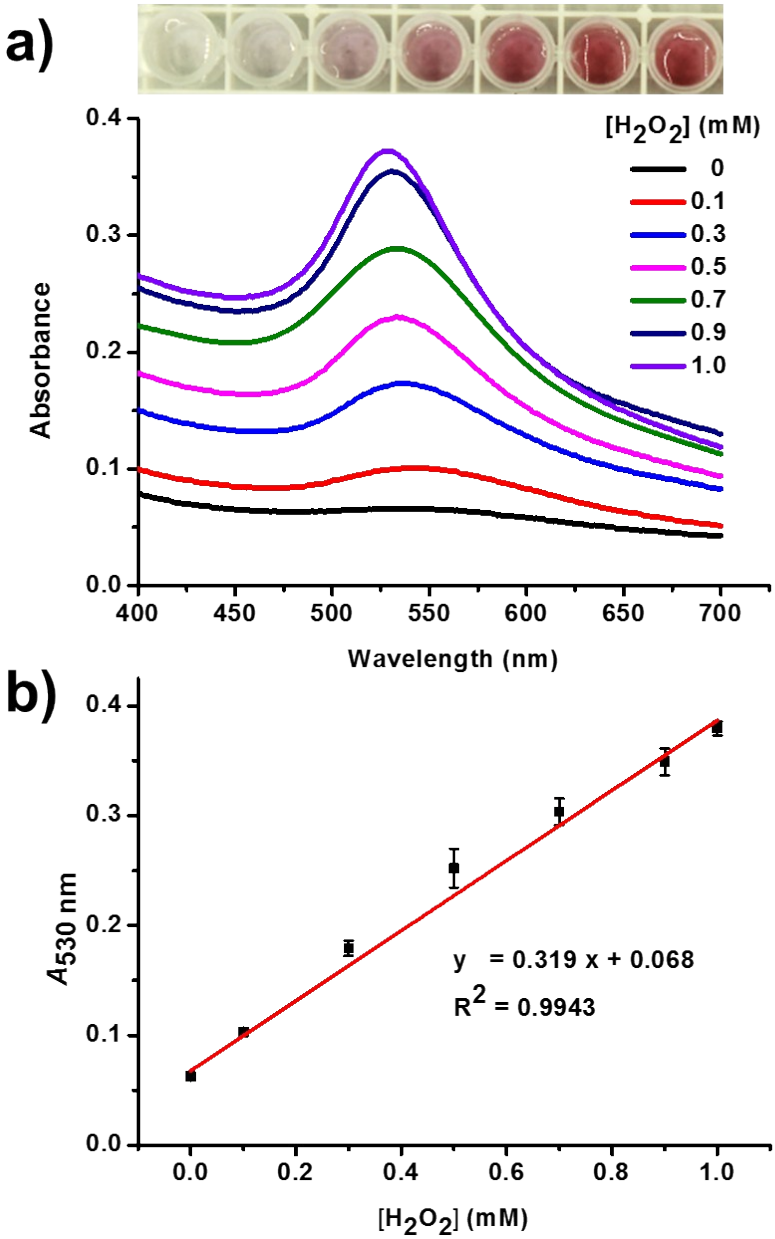


Fig. S1 H_2O_2-catalysed growth of 5 nm AuNPs in the presence of $HAuCl_4$. a) Images and corresponding UV-Vis spectra of the obtained AuNPs solutions after the addition of different concentrations of H_2O_2. b) A_{530nm} values versus various concentrations of H_2O_2. Error bars represent standard deviations of three independent measurements. ([5 nm AuNPs]=8.3 nM, [$HAuCl_4$]=0.16 mM)

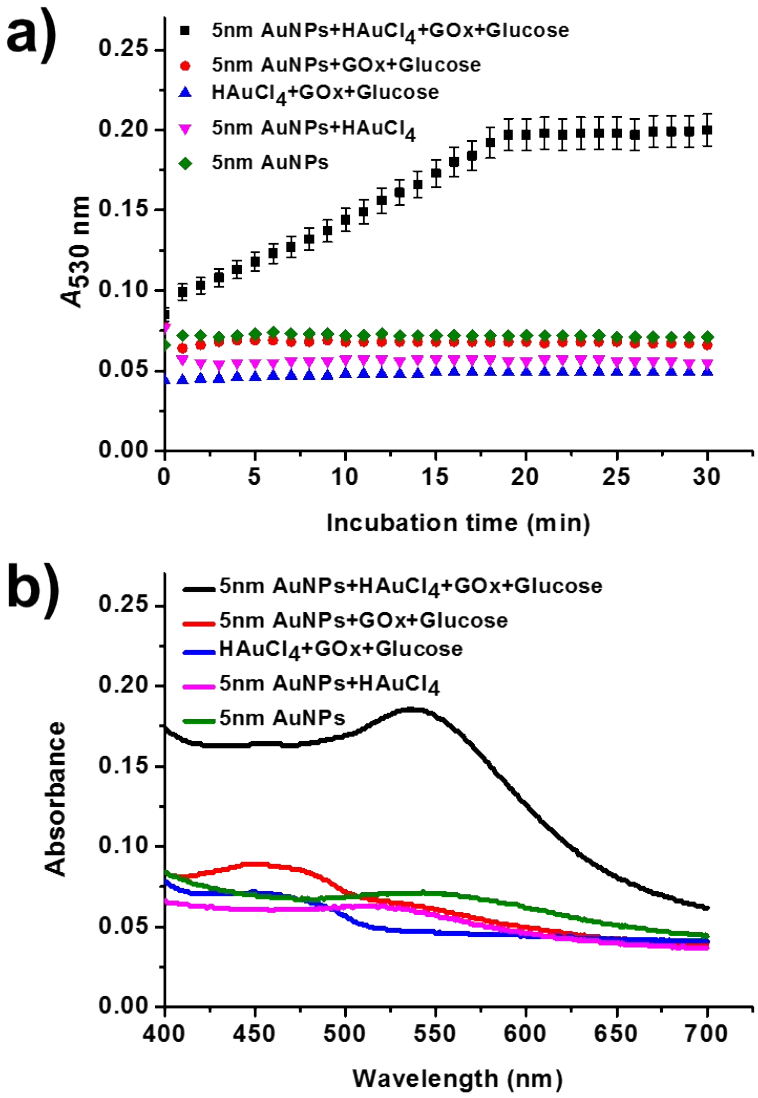


Fig. S2 a) Kinetic process and b) spectra (at 30 min) of 5 nm AuNPs solutions with and without the presence of $HAuCl_4$ and/or GOx and Glucose, respectively. ([5 nm AuNPs] = 8.3 nM, [$HAuCl_4$] = 0.16 mM, [GOx] = 1 mg/mL, [Glucose] = 1 mM)

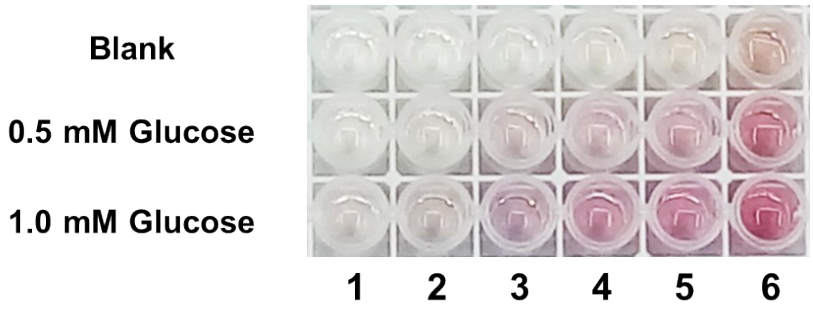


Fig. S3 Images of AuNPs solutions with different concentrations of 5 nm AuNPs. The concentration of AuNPs in wells 1-6 are 0.8, 1.7, 4.2, 8.3, 16.6, and 43 nM, respectively. ([$HAuCl_4$] = 0.16 mM, [GOx] = 1 mg/mL).

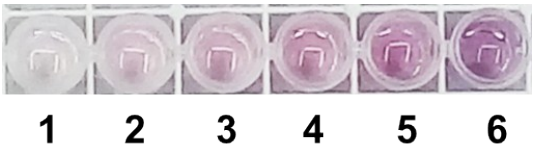


Fig. S4 Images of AuNPs solutions with different concentrations of $HAuCl_4$. The concentrations of $HAuCl_4$ in wells 1-6 are 0.04, 0.08, 0.12, 0.16, 0.20, and 0.24 mM, respectively. ([5 nm AuNPs] = 8.3 nM, [GOx] = 1 mg/mL, [Glucose] = 1 mM)

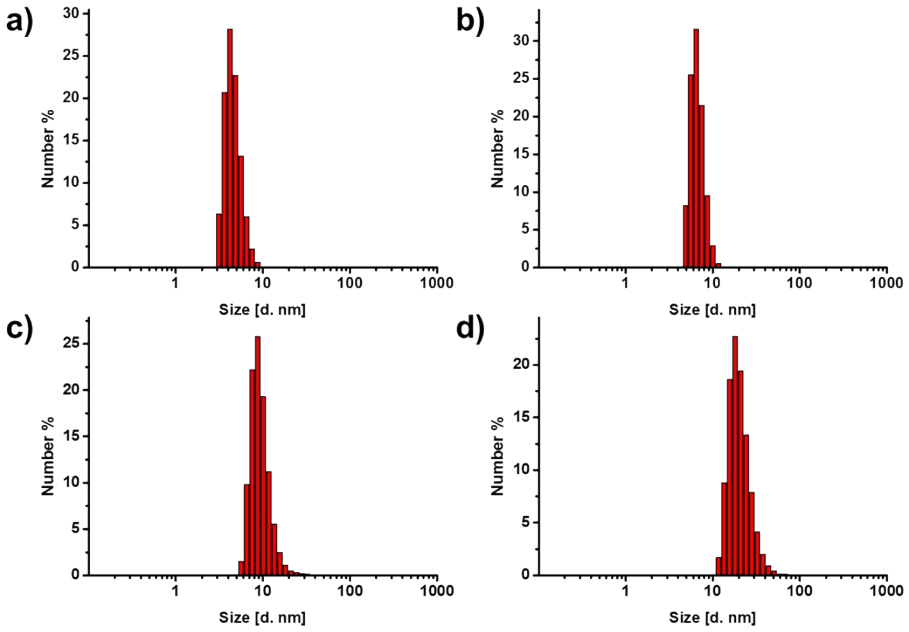


Fig. S5 Dynamic light scattering (DLS) analysis of 5 nm AuNP seeds a) and those after treatment with b) 0.5, c) 1.0 and d) 2.0 mM of glucose in the presence of 1.0 mg/mL GOx and 0.16 mM $HAuCl_4$, respectively.

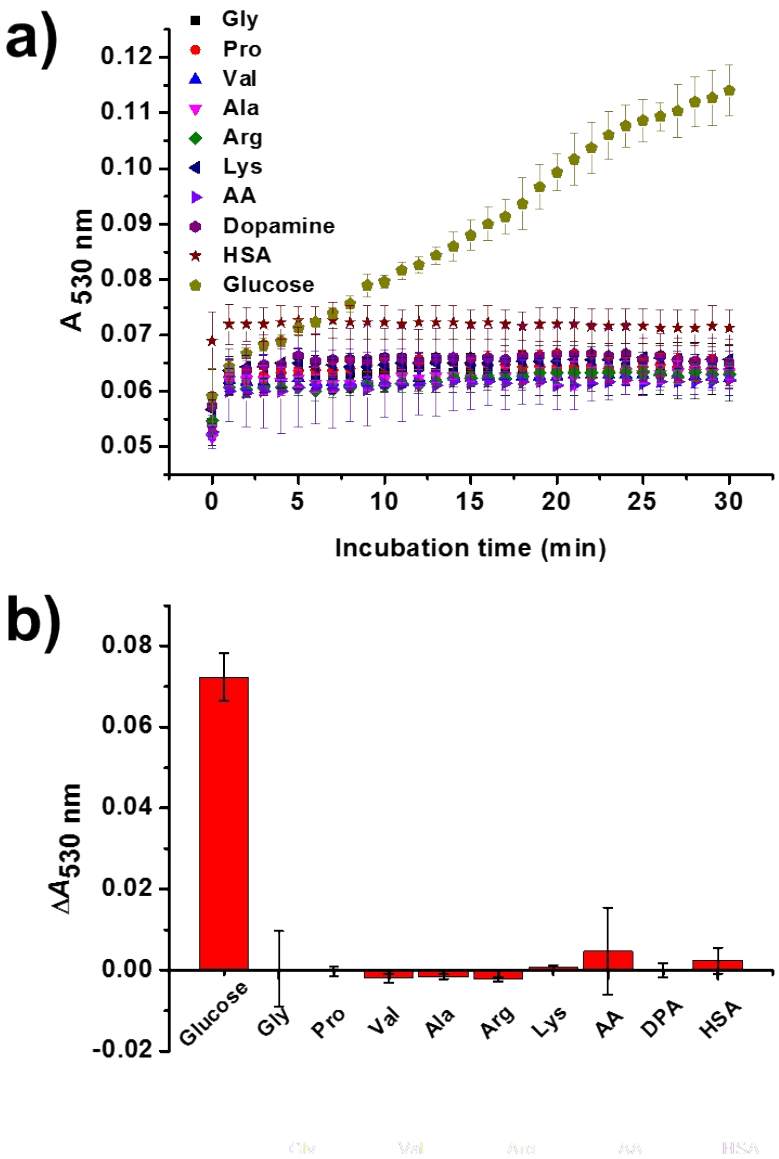


Fig. S6 AuNPs systems towards various potential interfering substances in serum. a) Kinetic process and b) $\Delta A_{530\ nm}$ of the AuNPs sensor incubating with glucose , various amino acid, ascorbic acid (AA), dopamine, and HSA, respectively. The concentrations of various amino are 28, 27, 16, 30, 5, 1.6, 5 μM, respectively; the concentration of AA, dopamine and HAS are 5 μM, 3 nM, and 4.2 mg/mL, respectively. The concentration of glucose is 500 μM. $\Delta A_{530\ nm} = A–A_0$, where A is the absorbance of AuNP solutions after incubating with various interfering substances and glucose, and A_0 is that after incubating with blank sample (deionized water).

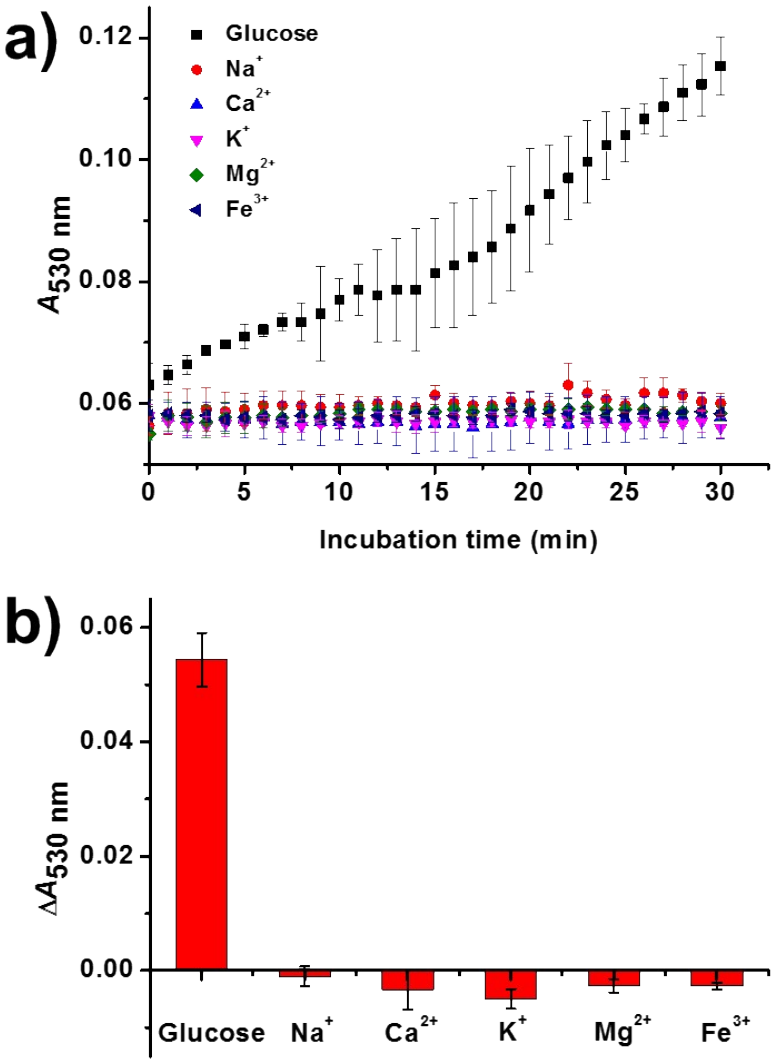


Fig. S7 AuNPs systems towards various potential metallic ions. a) Kinetic process and b) $\Delta A_{530\ nm}$ of the AuNPs sensor incubating with glucose and various metallic ions, respectively. The concentration of glucose is 500 μM. The concentrations of metallic ions are 0.2 mM. $\Delta A_{530\ nm} = A - A_0$, where A is the absorbance of AuNP solutions after incubating with various interfering substances and glucose, and A_0 is that after incubating with blank sample (deionized water).

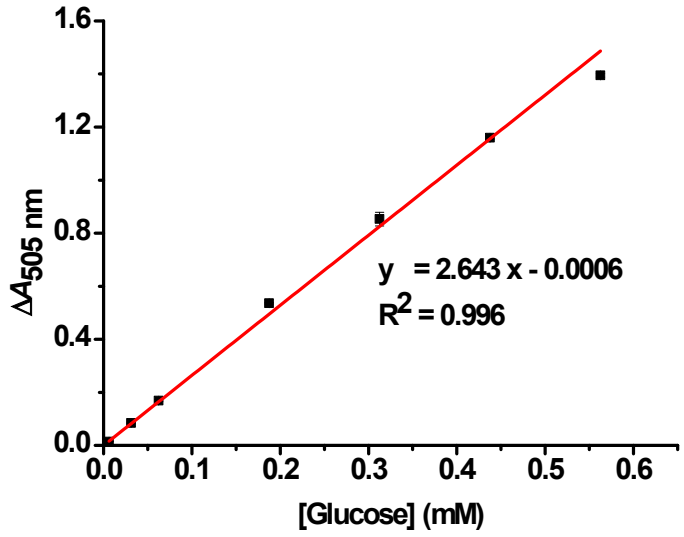


Fig. S8 Plot of $\Delta A_{505\ nm}$ values versus various concentrations of glucose in the glucose kit method. $\Delta A_{505\ nm} = A - A_{0,}$ where A is the absorbance of the various concentrations of glucose after incubating with the working solution of glucose kit, A_0 is that of the blank sample (deionized water) after incubating with the working solution of glucose kit.

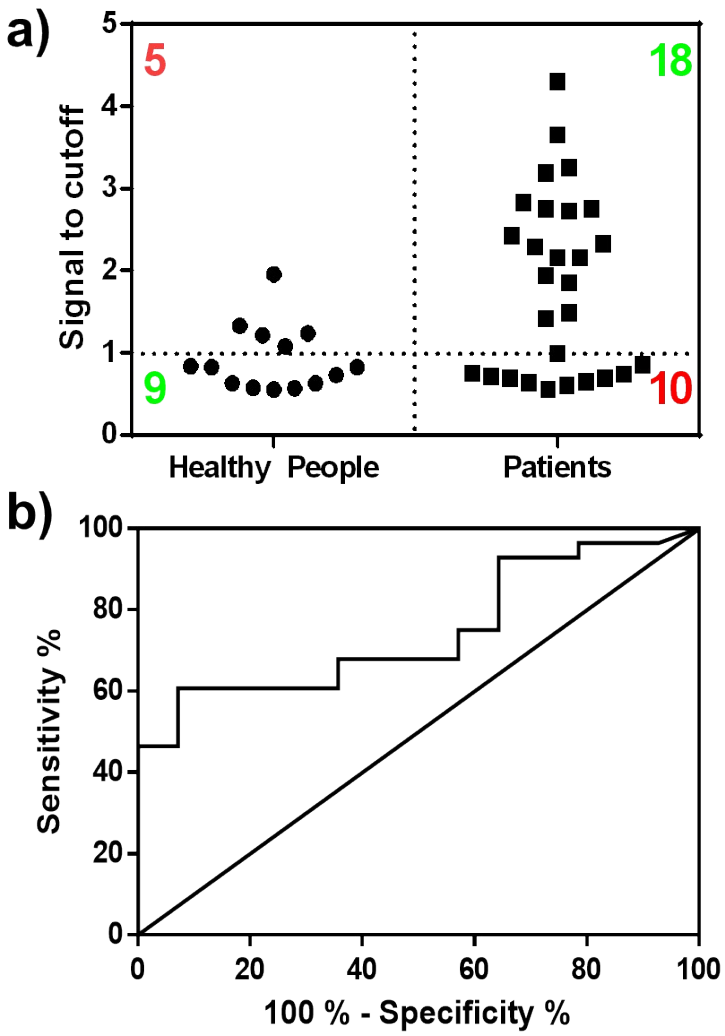


Fig. S9 Results of clinical screening for the glucose kit-based method. a) Vertical scatter plots of signal to cutoff values for negative (filled circles) and positive (filled squares) diabetes samples. b) Sensitivity and specificity measured by the received-operating characteristic (ROC) curves.

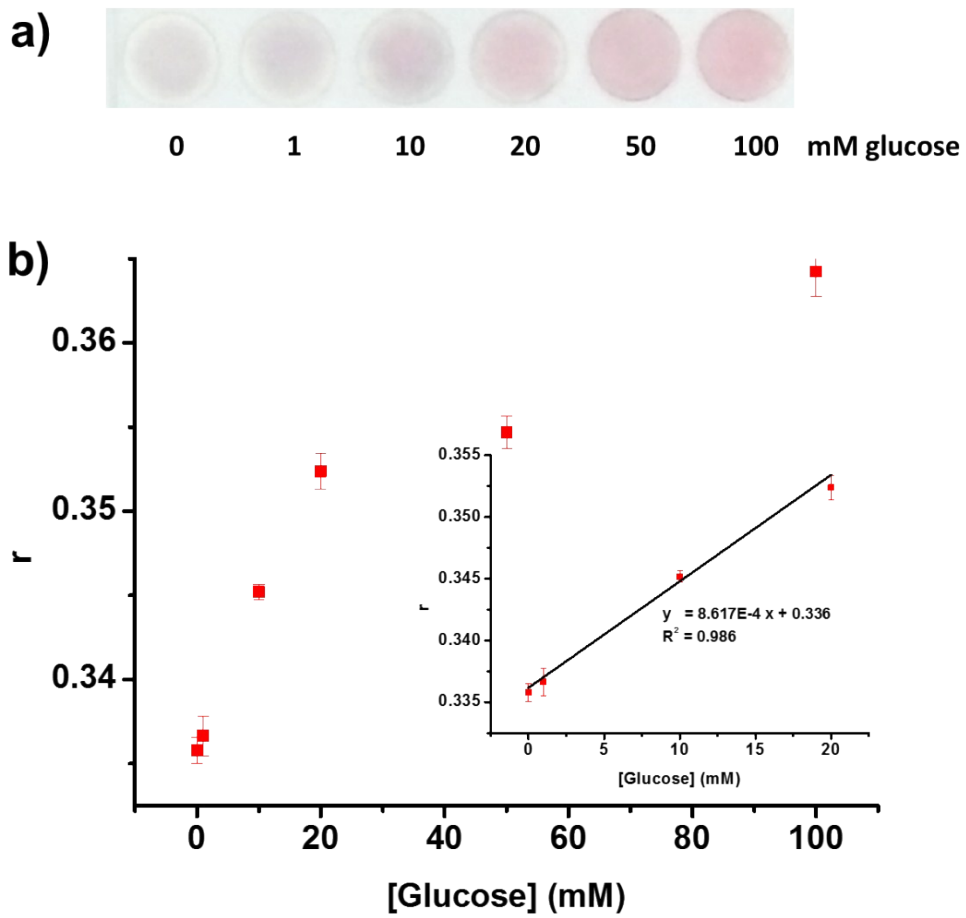


Fig. S10 Colorimetric analysis of the paper-based assay for glucose. a) Images of filter papers after incubating GOx (1 mg/mL), 5 nm AuNPs (8.3 nM) and HAuCl4 (0.16 mM) with various concentrations of glucose for 30 min. b) Plots of r (red chromaticity level) versus various concentrations of glucose. r = R/(R+G+B), where R (Red), G (Green) and B (Blue) are the three primary color components, whose values were obtained by Photoshop software.

Table S1 Results of clinical screening by AuNPs growth-based assay.

Healthy people

Blank	No.90	No.91	No.95	No.96	No.97	No.99	No.100
0.064	0.079	0.07	0.069	0.066	0.069	0.087	0.077
0.063	0.086	0.071	0.07	0.068	0.069	0.093	0.075
0.064	0.077	0.069	0.072	0.07	0.065	0.091	0.073
	No.101	No.102	No.103	No.104	No.105	No.106	No.107
	0.102	0.105	0.087	0.085	0.082	0.077	0.107
	0.103	0.108	0.095	0.086	0.083	0.076	0.112
	0.107	0.112	0.096	0.084	0.082	0.077	0.105

Patients

Blank	No.1	No.3	No.4	No.6	No.7	No.8	No.9
0.07	0.084	0.089	0.132	0.092	0.077	0.079	0.094
0.06	0.079	0.088	0.127	0.095	0.076	0.079	0.096
0.061	0.074	0.098	0.12	0.088	0.076	0.077	0.099
	No.10	No.12	No.13	No.14	No.15	No.16	No.17
	0.123	0.119	0.096	0.095	0.181	0.083	0.139
	0.141	0.112	0.088	0.107	0.182	0.085	0.14
	0.127	0.112	0.098	0.094	0.192	0.092	0.135
	No.18	No.19	No.21	No.22	No.23	No.25	No.30
	0.123	0.113	0.146	0.132	0.1	0.103	0.073
	0.123	0.108	0.151	0.134	0.103	0.109	0.071
	0.128	0.116	0.154	0.139	0.102	0.094	0.074
	No.47	No.48	No.49	No.58	No.59	No.68	No.69
	0.082	0.074	0.111	0.11	0.09	0.102	0.101
	0.085	0.074	0.111	0.11	0.085	0.098	0.103
	0.066	0.071	0.117	0.112	0.088	0.106	0.103

Table S2 Results of clinical screening by glucose kit method.

Healthy people

Blank	No.90	No.91	No.95	No.96	No.97	No.99	No.100
0.041	0.104	0.085	0.066	0.05	0.096	0.059	0.043
0.046	0.098	0.087	0.068	0.051	0.1	0.046	0.045
0.051	0.096	0.085	0.066	0.049	0.096	0.068	0.045
	No.101	No.102	No.103	No.104	No.105	No.106	No.107
	0.045	0.162	0.096	0.067	0.045	0.042	0.048
	0.048	0.151	0.118	0.066	0.046	0.046	0.085
	0.045	0.154	0.104	0.066	0.043	0.061	0.064

Patients

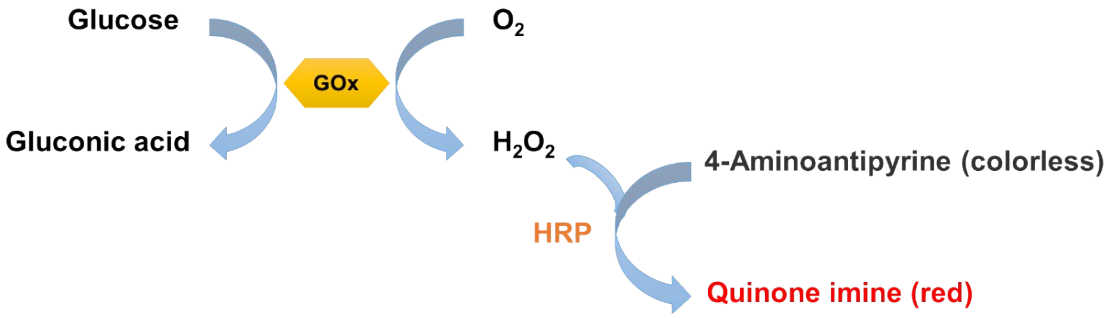
Blank	No.1	No.3	No.4	No.6	No.7	No.8	No.9
0.044	0.219	0.151	0.255	0.045	0.056	0.055	0.079
0.043	0.217	0.146	0.254	0.046	0.057	0.058	0.082
0.043	0.218	0.147	0.255	0.074	0.057	0.064	0.077
	No.10	No.12	No.13	No.14	No.15	No.16	No.17
	0.079	0.219	0.193	0.185	0.343	0.171	0.046
	0.063	0.22	0.197	0.188	0.338	0.171	0.046
	0.061	0.221	0.193	0.186	0.352	0.177	0.051
	No.18	No.19	No.21	No.22	No.23	No.25	No.30
	0.221	0.223	0.292	0.261	0.156	0.171	0.067
	0.219	0.224	0.292	0.268	0.156	0.171	0.045
	0.219	0.232	0.291	0.25	0.154	0.176	0.043
	No.47	No.48	No.49	No.58	No.59	No.68	No.69
	0.056	0.044	0.112	0.181	0.05	0.055	0.119
	0.056	0.044	0.112	0.183	0.048	0.055	0.119
	0.069	0.045	0.115	0.185	0.055	0.056	0.12

Table S3 Clinical information of the 14 healthy individuals and 28 patients suffered from diabetes mellitus.

Healthy				Patient							
No.	Patient ID	Age (year)	Gender	No.	Patient ID	Age (year)	Gender	No.	Patient ID	Age (year)	Gender
90	422982	20	Female	1	421835	60	Male	18	421339	62	Male
91	422984	47	Male	3	416253	60	Male	19	422559	51	Male
95	426266	47	Male	4	323098	70	Female	21	281395	46	Male
96	351958	70	Male	6	334737	55	Female	22	265910	52	Male
97	423899	66	Female	7	421304	68	Female	23	209839	50	Female
99	424059	39	Male	8	290661	70	Female	25	420944	66	Female
100	426238	21	Female	9	422579	54	Female	30	188875	60	Male
101	426547	52	Female	10	229572	80	Male	47	422349	62	Female
102	388403	66	Male	12	291479	72	Male	48	421113	46	Male
103	331328	67	Female	13	309249	77	Female	49	422349	47	Female
104	105097	88	Male	14	206358	54	Male	58	421250	50	Male
105	424044	58	Male	15	423396	65	Female	59	422049	43	Female
106	265924	46	Female	16	259799	51	Female	68	155987	82	Male
107	268177	29	Female	17	79940	81	Male	69	153450	85	Male

Table S4 Comparison of the performances of the various GOx-based nanoprobes for glucose sensing with the present AuNP-based assay.

nanoprobes	signal readout	LOD (μM)	probe modification	detection condition	ref
CdSe/ZnS QDs	fluorescence	100	yes	N/A	[S2]
Mn-doped ZnS QDs	phosphorescence	3	yes	clinical serum	[S3]
Si QDs	fluorescence	0.68	yes	clinical serum	[S4]
Ag NPs	colorimetric	0.17	yes	N/A	[S5]
Ag nanoprisms	colorimetric	0.2	no	Pre-add Fe^{2+} in clinical serum	[S6]
Au NPs	colorimetric	28	yes	spiked urine	[S7]
Au NCs	fluorescence	5	yes	clinical serum	[S8]
Au NPs	colorimetric	49	no	clinical serum	This work



Scheme S1 Mechanism of the glucose kit.

References:

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