Electronic Supplementary Information

Enhanced ELISA with handheld pH meters and enzyme-coated microparticles for the portable, sensitive detection of proteins

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Experimental

Materials and apparatus

Human oncogenic protein (HOP, BCR-ABL p190), biotinylated second antibody for HOP, microtitter plates that were immobilized with monoclonal primary antibody for HOP, conventional ELISA kit for optical detection of HOP, reagent kit for optical detection of H_2O_2, bovine serum albumin (BSA), human serum albumin (HSA), and streptavidin are the products of Sangon Biotechnology Co. Ltd. (Shanghai, China). Glucose oxidase (GOx, from Aspergillus niger, 100−250 U mg^{-1}), glutaraldehyde and normal human serum (NHS) were obtained from Sigma-Aldrich. Amine-coated superparamagnetic microparticles (diameter ca. 0.1 − 0.5 μm) were from Tianjin BaseLineChrom Tech Research Centre (Tianjin, China). All other chemicals were of analytical grade and were used as received without further purification. Ultrapure water (with a specific resistivity of 18.2 MΩ·cm) was obtained from an ultrapure water system (UPS-II-20L) provided by Chengdu Yuechun Technology Co., Ltd. (Chengdu, China). The involved buffered solution includes 10 mM phosphate-buffered saline (PBS, 10 mM phosphate buffer, pH 5.8 or 7.0) solution.

Handheld pH meter with a resolution of 0.01 pH (SIN-PH173) is the product of Hangzhou Sinomeasure Automation Technology Co., Ltd. (China). Benchtop microplate spectrophotometer (Multiskan FC) is from Thermo Scientific Inc. (USA). Optical characterization of enzymatic activity of the functionalized microparticle probes was performed on a Varian Cary 50 ultraviolet-visible spectrophotometer. Glucose meter (ONETOUCH UltraEasy) is the product of Johnson & Johnson Medical (USA).

Preparation and optical characterization of microparticle probes functionalized with GOx and streptavidin

In brief, 0.5 mL of amine-coated superparamagnetic microparticle suspension (1 mg mL^{-1}) was mixed with 7 mL of 2.5% (w/v) glutaraldehyde in water. The mixture was incubated for 3 h at room temperature. After these aldehyde-activated microparticles were isolated magnetically and washed twice with PBS buffer (pH 7.0) to remove unreacted glutaraldehyde molecules, they were dispersed in 5 mL of 5 mg mL^{-1} GOx and 1 mg mL^{-1} streptavidin in PBS buffer (pH 7.0), followed by 12 h incubation under stirring. 1 mL of 10 mg mL^{-1} BSA was further used to block the remaining aldehyde groups on these probes. After magnetic separation and washings, the resultant functionalized microparticles were re-suspended in 5 mL of PBS buffer (containing 1 mg mL^{-1} BSA, pH 7.0) and stored at 4 °C until used. The as-prepared functionalized microparticles could remain stable with no significant change in bioactivity for two months.

The enzymatic activity of the GOx coated on the microparticle probe (for specifically oxidizing glucose to generate gluconic acid and H_2O_2) was additionally investigated using a commercially-available reagent kit for optical detection of H_2O_2. According to the instructions of the assay kit, 100 μL of the as-prepared GOx-coated microparticle probe (0.1 mg mL^{-1}) was incubated with 100 μL of 10 mM glucose solution in ultrapure water for 1 h. After magnetic separation, the reaction solution was subsequently mixed with 50 μL of the working solution containing Fe^{2+} and a specific dye for additional 20 min incubation. No names of the two reagents were given due to the patent protection. The H_2O_2 produced via the GOx-catalyzed oxidation reactions oxidized the Fe^{2+} to Fe^{3+} that further bound the dye to form a Fe^{3+} -dye complex. This complex possessed a characteristic adsorption peak at 580 nm in the ultraviolet-visible spectrum; its absorbance was proportional to the level of H_2O_2 that positively depended on the enzymatic activity of the GOx. In the comparison experiment, all these procedures were carried out except that 100 μL of original GOx solution (5 mg mL^{-1}) was used instead of the GOx-immobilized microparticle probes.

Preparation of GOx-streptavidin conjugates

Briefly, 1 mL of 1 mg mL^{-1} streptavidin, 1 mL of 2 mg mL^{-1} GOx in PBS buffer (pH 7.0) and 5 mL of 2.5% (w/v) glutaraldehyde in water were incubated at 4°C overnight. The mixture solution was then dialyzed in a dialysis bag (retained molecular weight 3.5 kDa) to remove the excess cross-linker. Next, the dialyzed solution was diluted with PBS buffer (pH 7.0) to its original volume. The resulting GOx-streptavidin conjugates were stored at 4 °C until used.

Assay procedures of pH meter-based ELISA for HOP detection

In a typical assay, 100 μL of a HOP sample in 10 mM PBS buffer (pH 7.0) or undiluted NHS was added into one well of the microtitter plates that were immobilized with monoclonal primary antibody and incubated for 1 h. The well was then washed twice with PBS buffer (pH 7.0), and 100 μL of 1 mg mL^{-1} biotinylated second antibody in 10 mM PBS buffer (pH 7.0) was further added to recognize and bind the HOP analyte. After 1 h incubation, unbound second antibody was washed away with PBS buffer. Subsequently, 100 μL of functionalized microparticle suspension (0.1 mg mL^{-1}) was added and incubated for 20 min, followed by a washing treatment. A 30 mg mL^{-1} glucose solution in ultrapure water (100 μL) was then used to generate gluconic acid and H_2O_2 via the GOx-catalysed oxidation of glucose. After 1 h incubation, the final liquid-phase reaction solution consisting of the two products and possible residual glucose was mixed with 1 mL of 25 mM KCI in ultrapure water for pH measurement using the handheld pH meter.

In the comparison experiment, all these procedures were carried out except that GOx-streptavidin conjugates were used instead of the functionalized microparticle probes. In the specificity experiments, all these procedures were carried out but using BSA, HSA, NHS, or

HOP in NHS instead of the HOP in 10 mM PBS buffer (pH 7.0). All the experiments ware performed at room temperature (25 °C).

Assay procedures of conventional ELISA for optical detection of HOP

For each well of the monoclonal primary antibody-immobilized microtitter plates, 100 μL of a HOP sample in 10 mM PBS buffer (pH 7.0) was added and incubated for 1 h at 37 °C. After the well was washed with PBS buffer (pH 7.0), 100 μL of a biotinylated second antibody solution in 10 mM pH 7.0 PBS buffer (1 mg mL^{-1}) was further added, followed by another 1 h incubation at 37 °C. After unbound second antibody was washed away with PBS buffer, 100 μL of streptavidin-conjugated with horseradish peroxidase (HRP) was added and incubated for 1 h. After a washing treatment, the addition of 3,3',5,5'-tetramethylbenzidine (TMB) and H_2O_2 produced a soluble end product that was pale blue in color. The TMB reaction was stopped with 2 M H_2SO_4 (resulting in a yellow color), and read at 450 nm using a bench-top microplate spectrophotometer.

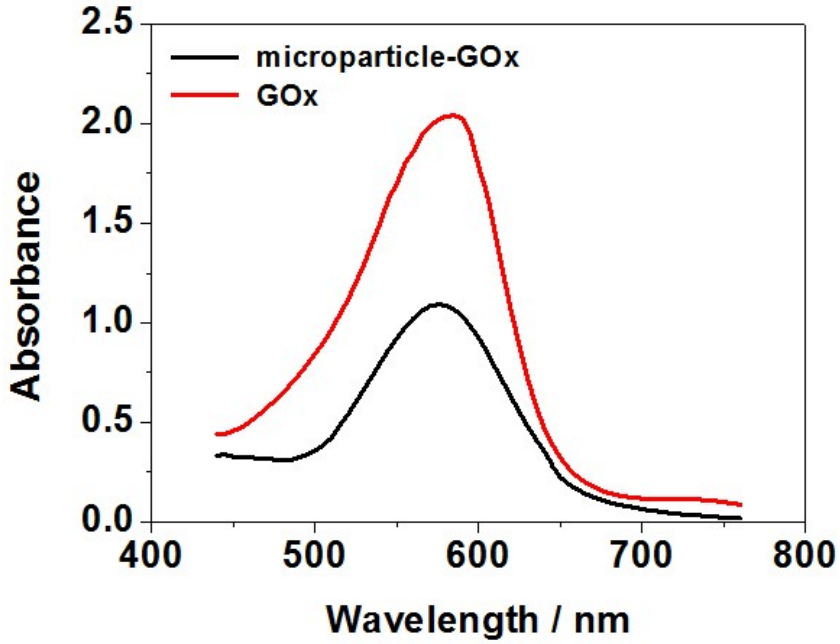

Fig. S1 Ultraviolet-visible spectra obtained from an original GOx solution (5 mg mL^{-1}) (red curve) and microparticle-GOx probes (prepared from 0.1 mg mL^{-1} microparticle suspension and 5 mg mL^{-1} original GOx solution) (black curve) after they were incubated with 10 mM glucose solution in ultrapure water for 1 h and in turn mixed with a working solution (in the commercially-available H_2O_2 assay kit) that contained Fe^{2+} and a specific dye for additional 20 min. The GOx on the microparticles or the free GOx in the solution specifically oxidized glucose to generate gluconic acid and H_2O_2. The H_2O_2 produced then oxidized the Fe^{2+} to Fe^{3+} that further bound the dye to form a Fe^{3+}-dye complex with a characteristic adsorption peak at 580 nm. The absorbance was proportional to the H_2O_2 level that depended on the enzymatic activity of the GOx. An obvious adsorption peak at 580 nm was observed from the microparticle-GOx probe (black curve), directly demonstrating that the GOx had been successfully covalently conjugated on the microparticles when using the glutaraldehyde as a linker reagent and still maintained good activity for the specific catalytic oxidation of glucose. Its absorbance was lower than that obtained from the free GOx solution (red curve), which was due to that only limited number of GOx molecules had been bound on the microparticles. The cross-linker glutaraldehyde also caused some losses in their enzymatic activity.

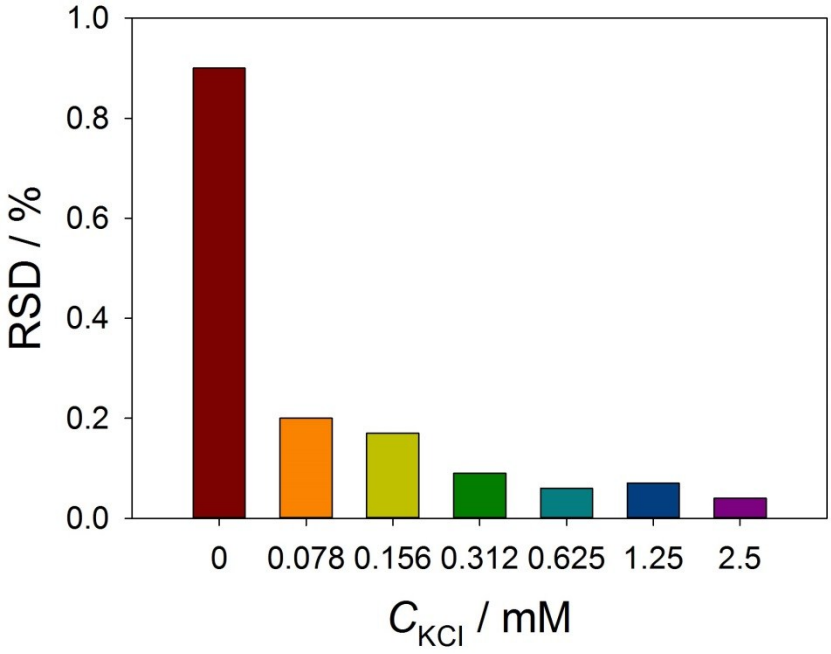

Fig. S2 Relative standard deviation (RSD) results obtained from the pH measurement of different concentrations of KCI solutions in ultrapure water (with a resistivity of 18.2 MΩ·cm) each of which was measured ten times.

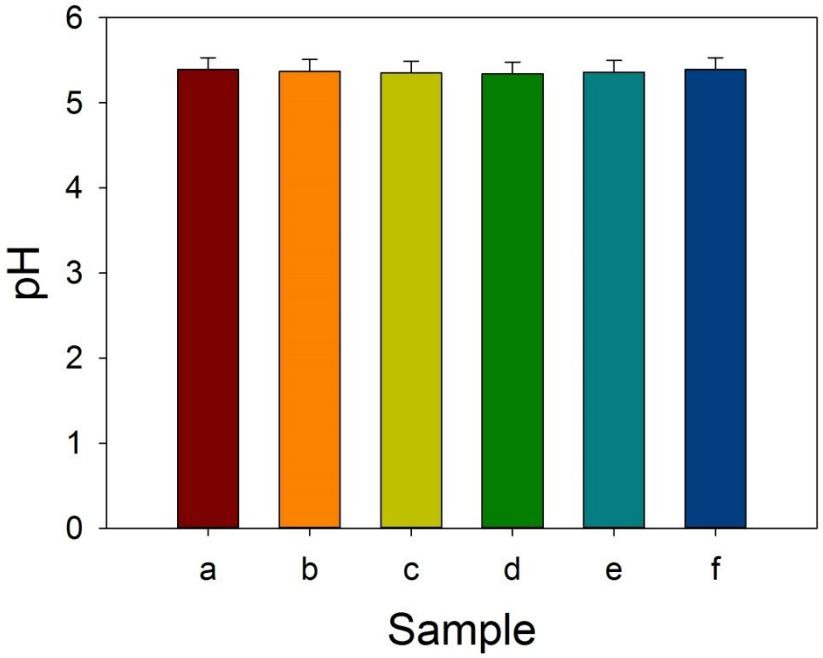

Fig. S3 The pH values obtained from 10 μg mL^{-1} gluconic acid solutions (in ultrapure water) that contain 2.5 mM KCI and different concentrations of glucose: a) 0.5, b) 1, c) 5, d) 10, e) 20, and f) 30 mg mL^{-1}. The error bars reflect the standard deviations from five repetitive experiments of each sample.

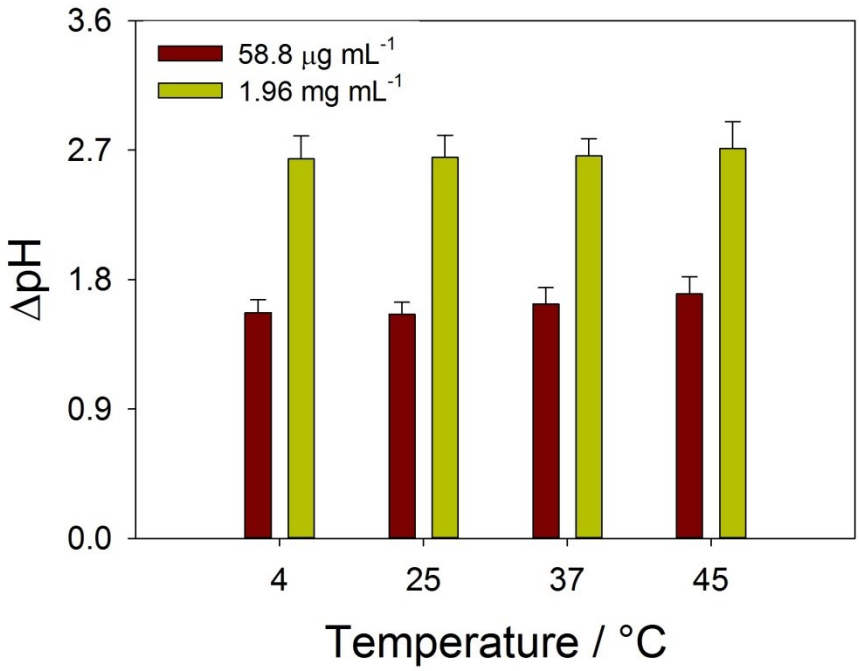

Fig. S4 The pH changes (ΔpH) between 28.8 μg mL^{-1} and 1.96 mg mL^{-1} gluconic acid in 2.5 mM KCI aqueous solution and the background solution (2.5 mM KCI aqueous solution without gluconic acid) obtained at different temperatures for pH measurement. The error bars reflect the standard deviations from five repetitive experiments of each sample.

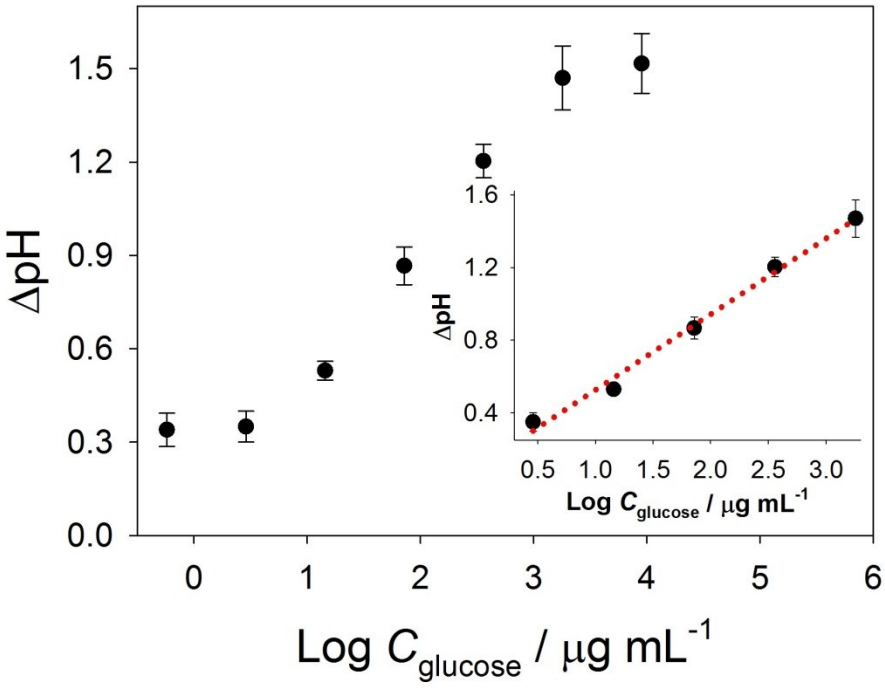

Fig. S5 The exponential relationship between the pH changes (ΔpH) obtained from different glucose samples and the logarithm values of their concentrations (Log$C_{glucose}$). Each glucose sample was incubated with GOx-coated microparticle probes to generate gluconic acid and H_2O_2. The pH of the resultant reaction mixture in 2.5 mM KCI aqueous solution was measured using a handheld pH meter. The pH change is defined as $\Delta pH = pH_s - pH_b$, where pH_s and pH_b are the pH values recorded by the pH meter for the glucose sample and the 2.5 mM KCI aqueous solution (background solution), respectively. The insert displays the corresponding calibration curve in a glucose concentration range of 2.88 μgmL^{-1} – 1.80 mg mL^{-1} with a regression equation of $y = 0.39545x + 0.14357$ ($R = 0.9870$). The limit of detection (LOD) for glucose was estimated to be 870 ngmL^{-1} (3σ). Each data point represents the average value of three repetitive experiments. The error bars reflect the standard deviations from the average values.

Table S1 Comparison of the new ELISA and conventional ELISA

	New ELISA	Conventional ELISA
Reader	Handheld pH meter [a]	Bench-top spectrophotometer [b]
Cost	~$ 30	~$ 157000
Portability	Yes	No
User	Minimally-trained	Well-trained
Limit of HOP detection	570 fg mL^{-1}	8 pg mL^{-1}

[a] The handheld pH meter (SIN-PH173) is the product of Hangzhou Sinomeasure Automation Technology Co., Ltd. (China); [b] The benchtop spectrophotometer (Multiskan FC) is from Thermo Scientific Inc. (USA).

Table S2 Recovery of HOP in PBS and human serum samples

Sample	Added (pg mL^{-1})	Found [c] (pg mL^{-1})	Recovery [c] (%)	RSD (%, *n*=6)
1[a]	0.6	0.58	96.6	2.3
2[a]	1.2	1.22	101.6	1.1
3[a]	2.5	2.56	102.4	1.9
4[a]	5.0	5.10	102.0	3.9
5[a]	10	9.78	97.8	4.0
6[a]	20	19.50	97.5	2.7
7[a]	40	39.90	99.7	2.6
8[b]	10	9.42	94.2	5.7
9[b]	20	18.85	94.3	8.5
10[b]	40	41.18	102.9	7.1

[a] The HOP samples in PBS solution; [b] The HOP samples in undiluted human serum; [c] Recovery for each sample refers to a ratio of the found value (calculated from the corresponding ΔpH value and the regression equation shown in the caption of Fig. 3B in the manuscript) and the added concentration of HOP.

Table S3 Comparison of the performance of the new portable ELISA and the portable ELISAs previously reported

Analyte	Analytical platform	Portable quantitative reader	LOD [b]	Ref.
Human oncogenic protein	Microtitter plate	Handheld pH meter	570 fg mL^{-1}	This work
Prostate specific antigen; HIV-1 capsid antigen p24	Microtitter plate	NA [a]	1 ag mL^{-1}; 1 ag mL^{-1}	1
HIV related protein gp120	Microtitter plate	NA [a]	10 ag mL^{-1}	2
Rabbit IgG; Neuropeptide Y	Paper	Digital camera	1.1 ng mL^{-1}; 9.8 ng mL^{-1}	3
Ferritin	Paper	Colour reflection densitometer	80 ng mL^{-1}	4
Human epididymis protein 4	Plastic microchip	Cell phone and charge-coupled device	19 ng mL^{-1}	5
Staphylococcal enterotoxin B	Plastic microchip	Charge-coupled device	100 pgmL^{-1}	6

[a] NA indicates 'not available', because the two assay methods only enable qualitative analysis; [b] LOD indicates limit of detection.

References

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Table S4 Comparison of the performance of handheld pH meter and glucose meter for the glucose detection

Linear range	Limit of detection	Reader
2.88 μg mL^{-1} – 1.80 mg mL^{-1}	870 ng mL^{-1}	Handheld pH meter [a]
198 μg mL^{-1} – 5.99 mg mL^{-1}	198 μg mL^{-1}	Glucose meter [b]

[a] The glucose detection performance of pH meter-based method was obtained from Fig. S5; [b] The glucose meter (ONETOUCH UltraEasy) is the product of Johnson & Johnson Medical (USA).