Electronic Supplementary Information

New two dimensional liquid-phase colorimetric assay based on old iodine-starch complexation for simple, low-cost, portable, naked-eye detection and quantification of analytes

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Experimental

Reagents and apparatus

Glucose oxidase (from *Aspergillus niger*, ⩾100 U/mg), glucose, human serum, cysteamine, hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), sodium citrate, formamide, dimethyl sulfoxide, dimethyl formamide, and ethanol were the products of Sigma-Aldrich (USA). Hydrogen peroxide (H_2O_2, 30 wt% in H_2O) and potassium iodide (KI) was obtained from Thermo Fisher Scientific (USA). Soluble starch was from May & Baker Ltd. (UK). All other chemicals were of analytical grade and were used as received without further purification. The involved buffered solution includes 10 mM phosphate buffer solution (PBS, pH 5.3 or 7) prepared from Na_2HPO_4 and KH_2PO_4 and 10 mM sodium acetate-acetic acid buffer solution (SABS, pH 3). All stock and buffer solutions were prepared with deionized water (with a specific resistivity of 18.2 MΩ·cm), unless specifically stated otherwise.

Optical characterization of iodine product, iodine-starch complex was performed on an ultraviolet/visible (UV/Vis) spectrometer (Varian Cary 50). Images of all solutions and real-time processes of the iodine-starch complexation were recorded using a smart phone (Apple iPhone 5s).

Assay procedures for detection of H_2O_2 and glucose

In a typical two dimensional liquid-phase colorimetric assay (2D LPCA) for H_2O_2, 50 μL of a sample in 10 mM PBS (pH 7) was incubated with 50 μL of 0.2 M KI in 10 mM SABS (pH 3) in a test tube with marked bars for 10 min at room temperature (ca. 25 °C) to produce iodine products. These iodine molecules those are insoluble in water further bind excessive iodine ions to form linear soluble triiodide ions. Then, 50 μL of 6 wt% starch in formamide was dropped into this reaction solution. The linear soluble triiodide ions could slip into the coil of the starch molecules causing an intense blue or black color in the upper of the final

mixture solution. Since the formamide had a higher density than H_2O, it rapidly sedimentated during the dropwise addition and made the reaction mixture's bottom show colorless. The intensity and length of the blue or black color was proportional to the iodine-starch complex level that positively depended on the H_2O_2 concentration in the sample. Counting the blue or black length-related marked bars of the test tube by the naked eye allowed for the quantitative detection of the analyte.

Moreover, for the assay of glucose in 10 mM PBS (pH 7) or 20 % human serum (diluted with 10 mM PBS, pH 7), 25 μL of a buffer sample or a serum sample was firstly incubated with 25 μL of 1 mg/mL glucose oxidase in 10 mM PBS (pH 5.3) for 30 min at room temperature (ca. 25 °C) to generate H_2O_2. Then, the following experiments were carried out according to the general procedures shown in the H_2O_2 assay above.

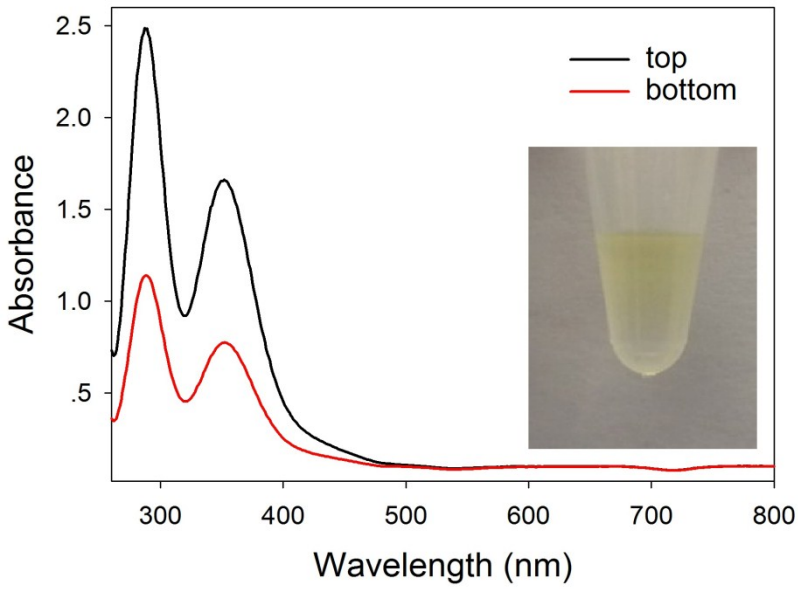

Fig. S1. UV/Vis spectra obtained from the top and bottom phases of a mixture containing iodine produced after 10 min-incubation of 0.2 M KI in 10 mM SABS (pH 3, 50 µL) and 1.5 mM H_2O_2 in 10 mM PBS (pH 7, 50 µL) followed by dropwise addition of formamide (50 µL). The image of the yellow mixture is shown as the insert.

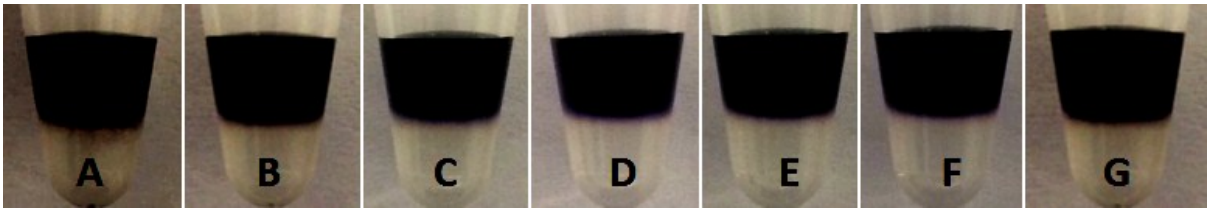

Fig. S2. Stability investigation for a black iodine-starch complex obtained by 10 min-incubation of 0.2 M KI in 10 mM SABS (pH 3, 50 µL) and 1.5 mM H_2O_2 in 10 mM PBS (pH 7, 50 µL) followed by dropwise addition of 6 wt% starch in formamide (50 µL). The images were recorded at different times: (A) 6 s, (B) 30 s, (C) 20 min, (D) 40 min, (E) 1 h, (F) 2 h, and (G) 3 h.

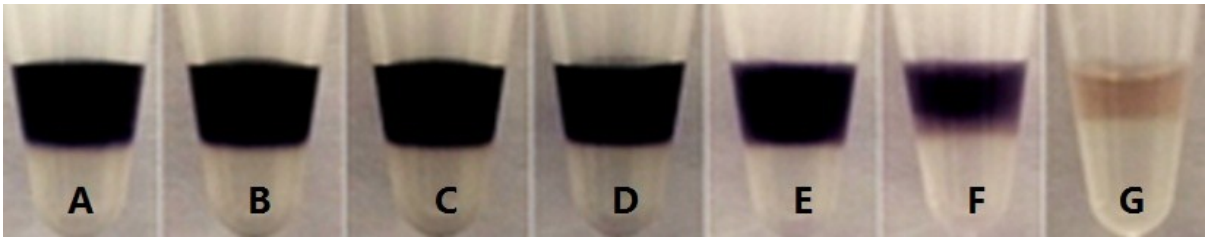

Fig. S3. Temperature investigation for a black iodine-starch complex obtained by 10 min-incubation of 0.2 M KI in 10 mM SABS (pH 3, 50 μL) and 1.5 mM H_2O_2 in 10 mM PBS (pH 7, 50 μL) followed by dropwise addition of 6 wt% starch in formamide (50 μL). The images were recorded at different temperatures: (A) 4, (B) 25, (C) 30, (D) 40, (E) 50, (F) 60, and (G) 70 °C. No significant changes are observed in the length of the black phase in the temperature range from 4 to 50 °C.

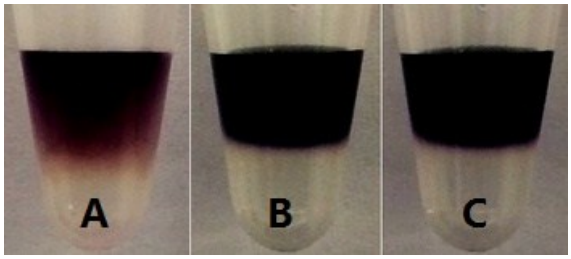

Fig. S4. Images of iodine-starch complex created from 10 min-incubation of 0.2 M KI in 10 mM SABS (pH 3, 50 μL) and 1.5 mM H_2O_2 in 10 mM PBS (pH 7, 50 μL) followed by dropwise addition of 6 wt% starch in different organic solvents (50 μL each): (A) dimethyl formamide, (B) formamide, and (C) dimethyl sulfoxide. The solubility of the starch in dimethyl formamide and dimethyl sulfoxide is low, so the corresponding 6 wt% starch solutions were prepared by diluting a 20 wt% starch solution in H_2O with the two solvents. Ethanol was also tested; however, it was found that the starch was insoluble in mixtures of H_2O and ethanol. Thus, the dropwise addition of insoluble starch into the yellow iodine mixture was not conducted.

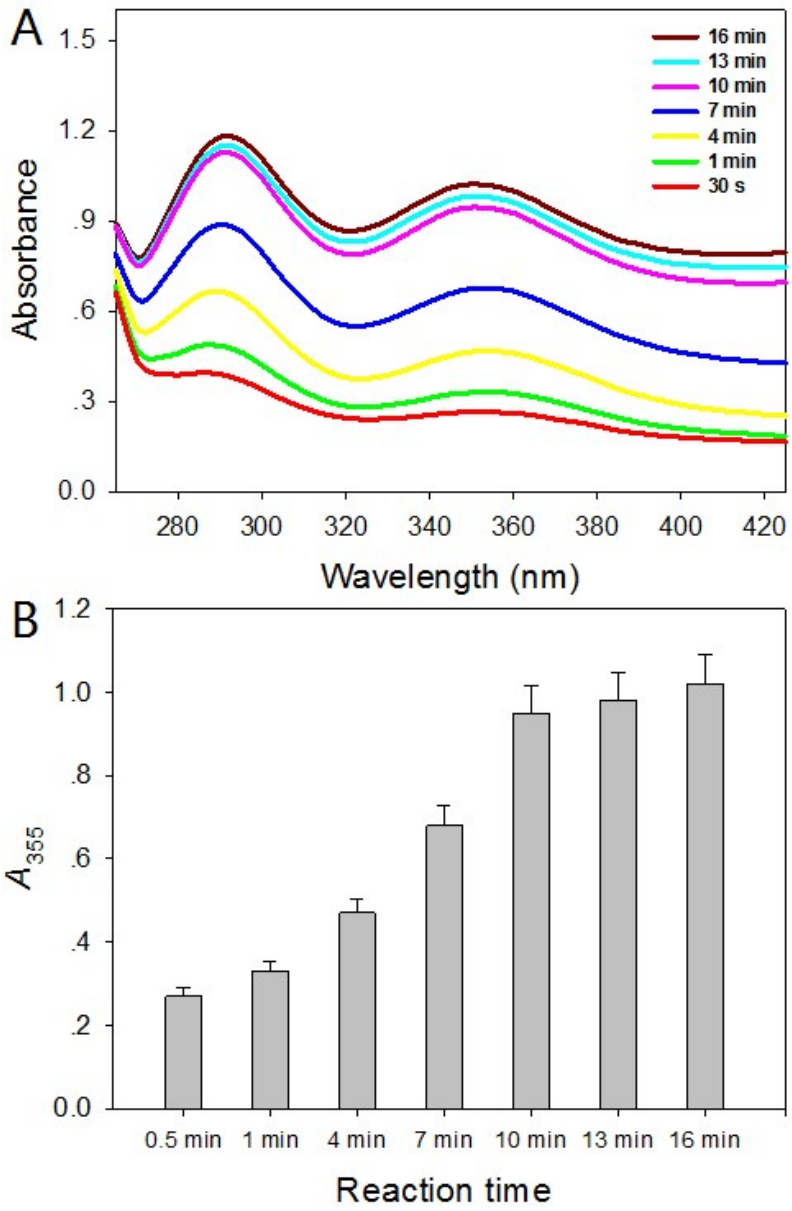

Fig. S5. Optimization of the time for redox reaction between KI and H_2O_2. (A) UV/Vis spectra obtained from mixtures of 0.2 M KI and 0.37 mM H_2O_2 (v/v = 1/1) at different reaction times. (B) Absorbance values at 355 nm (A_{355}) of the iodine products generated at different reaction times. Each error bar represents a standard deviation across three replicate experiments. 10 min was chosen as the optimal reaction time.

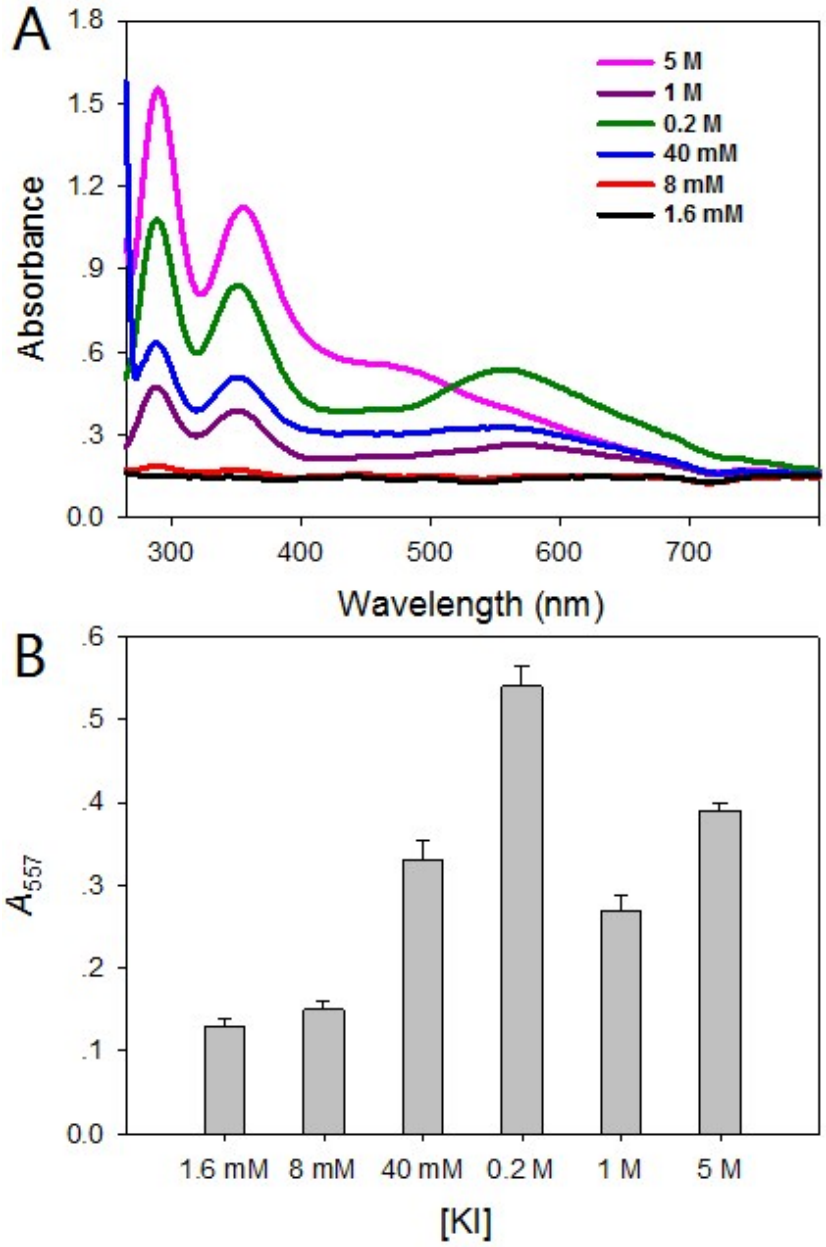

Fig. S6. Optimization of the KI concentration ([KI]). (A) UV/Vis spectra obtained from 10 min-incubation of 0.37 mM H_2O_2 and KI solutions at different concentrations (v/v = 1/1), followed by dropwise addition of 50 μL of 6 wt% starch in formamide into 100 μL of each resultant mixture. (B) Absorbance values at 557 nm (A_{557}) of the iodine-starch complex products generated using different KI concentrations. Each error bar represents a standard deviation across three replicate experiments. 0.2 M was chosen as the optimal KI level as it resulted in the largest absorbance.

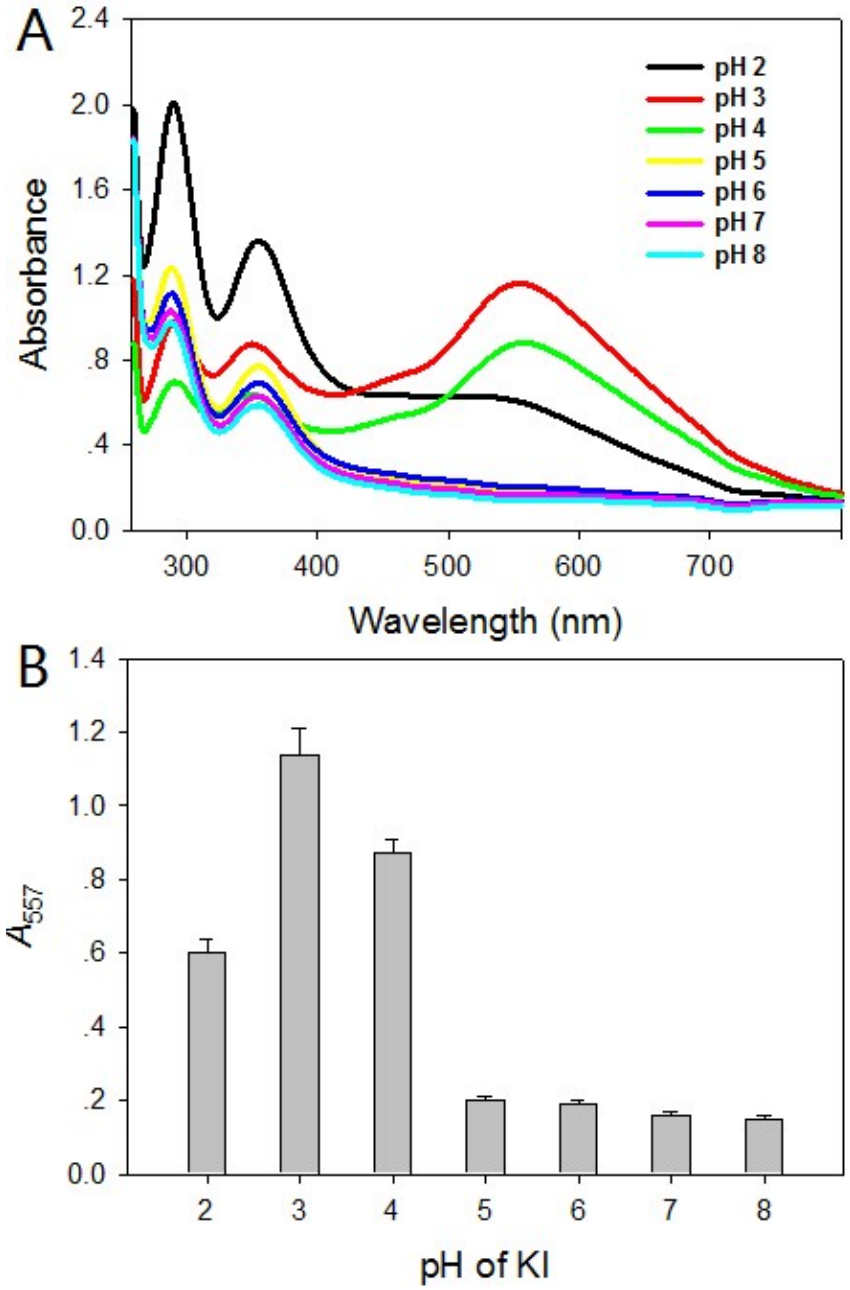

Fig. S7. Optimization of pH of the KI solution. (A) UV/Vis spectra obtained from 10 min-incubation of 0.5 mM H_2O_2 and 0.2 MKI solutions with different pHs, followed by dropwise addition of 50 µL of 6 wt% starch in formamide into 100 µL of each resultant mixture. (B) Absorbance values at 557 nm (A_{557}) of the iodine-starch complex products generated with different pHs of KI solutions. Each error bar represents a standard deviation across three replicate experiments. The pH of 3 was chosen as the optimal value as it resulted in the largest absorbance.

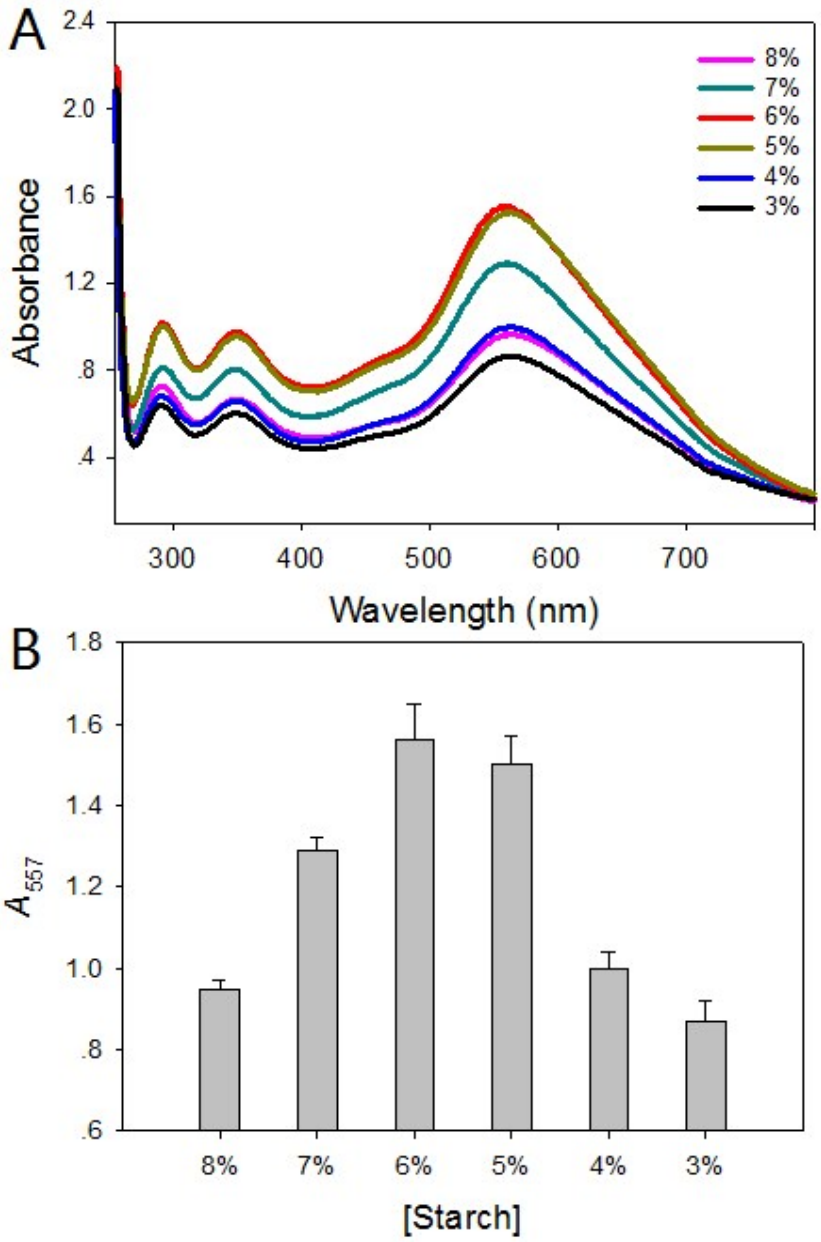

Fig. S8. Optimization of the starch level ([starch]). (A) UV/Vis spectra obtained from 10 min-incubation of 0.75 mM H_2O_2 and 0.2 M KI (pH 3) (v/v = 1/1), followed by dropwise addition of 50 μL of different levels of starch in formamide into 100 μL of each resultant mixture. (B) Absorbance values at 557 nm (A_{557}) of the iodine-starch complex products created using different starch levels. Each error bar represents a standard deviation across three replicate experiments. 6 wt% was chosen as an optimal starch level as it resulted in the largest absorbance.

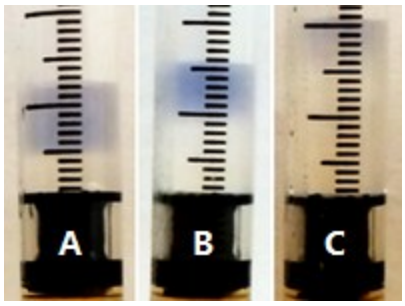

Fig. S9. Images obtained from dropwise additions of different volumes of 6 wt% starch in formamide into 100 μL of 0.18 mM H_2O_2 and 0.2 M KI (pH 3) (v/v = 1/1) after 10 min-incubation: (A) 25, (B) 50, and (C) 100 μL. 50 μL was chosen as an optimal starch volume as it gave the clearest colorimetric result.

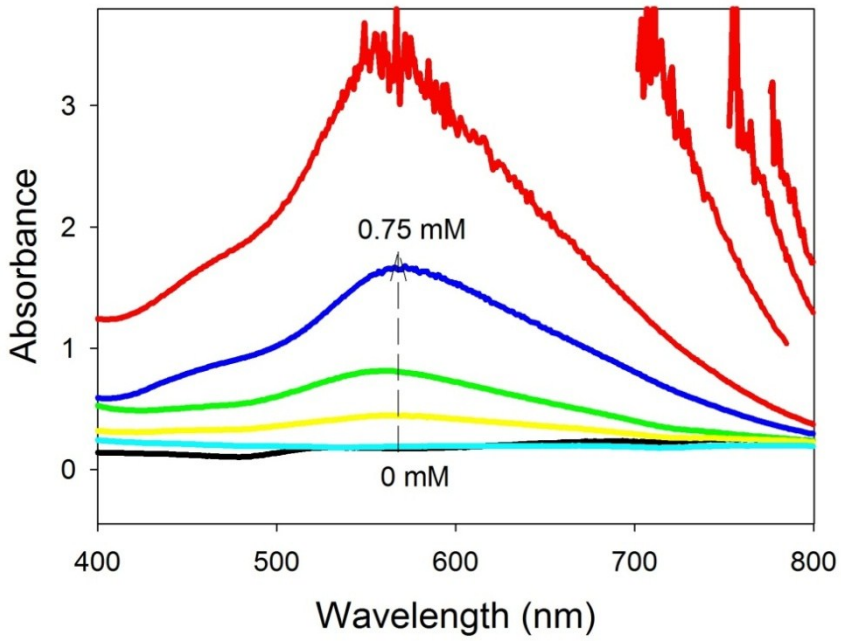

Fig. S10. UV/Vis spectra obtained from 10 min-incubation of different samples with varying concentrations of H_2O_2 and 0.2 M KI (v/v = 1/1), followed by dropwise addition of 50 μL of 6 wt% starch in H_2O into 100 μL of each resultant mixture. The red curves with overflow (off-scale) signals were obtained from the H_2O_2 samples with concentrations from 1.5 to 12 mM.

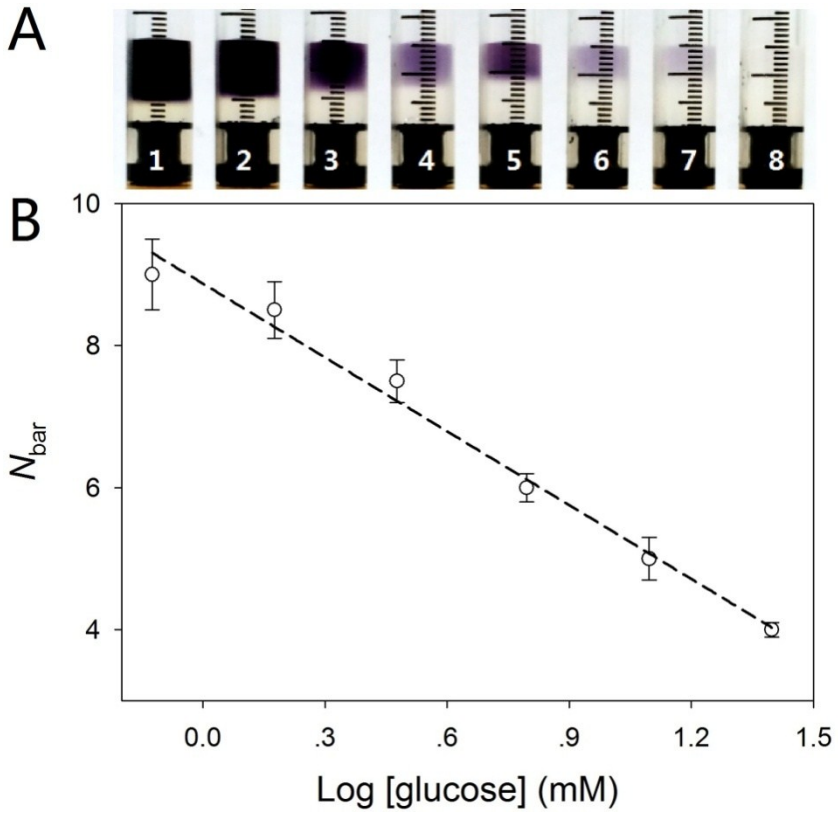

Fig. S11. (A) Colorimetric results obtained from different glucose samples in 10 mM PBS (pH 7) using the 2D LPCA: (1) 25, (2) 12.5, (3) 6.3, (4) 3.1, (5) 1.5, (6) 0.75, (7) 0.37, and (8) 0.18 mM. (B) The calibration curve describes the linear relationship between the number of bars (N_{bar}) on the marked test tubes and the logarithm of glucose concentrations (Log [glucose]). Each error bar represents a standard deviation across three replicate experiments.

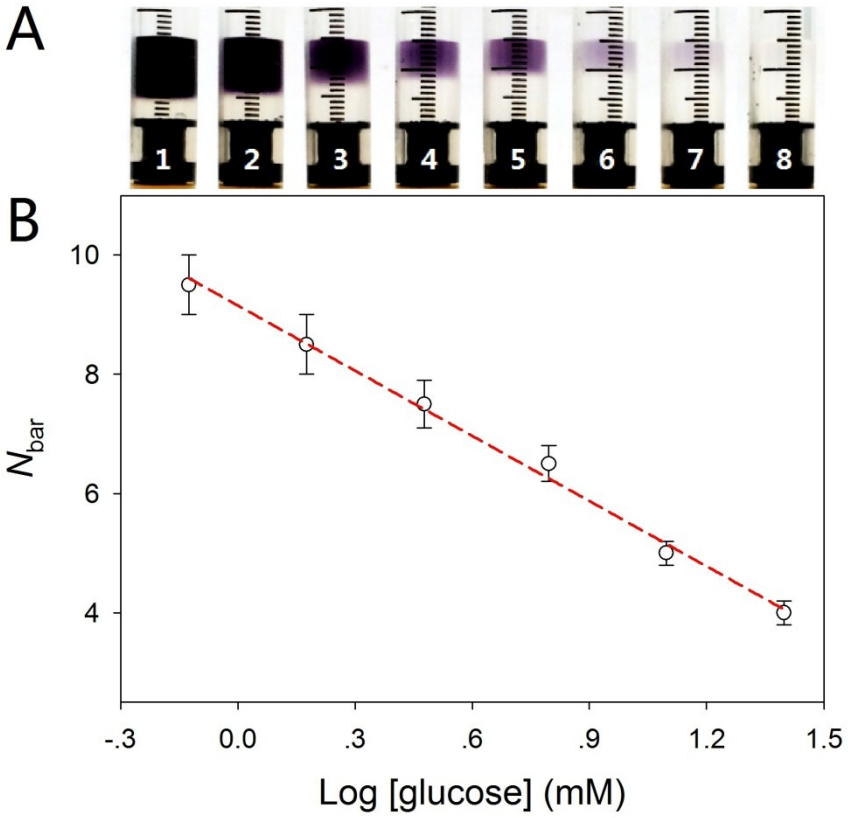

Fig. S12. (A) Colorimetric results obtained from different glucose samples in 20 % human serum using the 2D LPCA: (1) 25, (2) 12.5, (3) 6.3, (4) 3.1, (5) 1.5, (6) 0.75, (7) 0.37, and (8) 0.18 mM. (B) The calibration curve describes the linear relationships between the number of bars (N_{bar}) on the marked test tubes and the logarithm of glucose concentrations (Log [glucose]). Each error bar represents a standard deviation across three replicate experiments.

Table S1. Physicochemical properties of the solvents for starch

solvent	**solubility for soluble starch**	**dispersibility for soluble starch aqueous solution**	**density (g/mL)**
formamide	high	quit high	1.134
dimethyl sulfoxide	low	high	1.100
H_2O	quit high	quit high	1.000
dimethyl formamide	quit low	low	0.944
ethanol	no	no	0.789