Electronic Supplementary Information

Facile colorimetric detection of glucose based on an organic Fenton reaction

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Experimental Section

Materials. Chloranil and N, N-Dimethylformamide (DMF) were obtained from Aladdin Inc. (Shanghai, China). 30% H_2O_2, lactose and sucrose were obtained from Beijing Chemical Reagent Company (Beijing, China). D(+)-glucose was obtained from Sino-American Biotechnology Co., Ltd. (Luoyang, China). D-fructose was purchased from Bio Basic Inc. (Canada). Maltose was bought from Merck (Germany). Choline chloride was purchased from Alfa Aesar. Glucose oxidase (from *Aspergillus niger*, GOx) was purchased from Amresco Inc. (USA). Choline oxidase (from *Alcaligenes species*, ChOx) and uric acid were purchased from Sigma. Other chemicals were of analytical grade and were used as received. Ultra-pure water (18.2 MΩ) was used throughout the experiment.

Apparatus. Absorption spectra were recorded on a Cary 50 Scan UV-Visible spectrophotometer (Varian, USA) at room temperature. Scanning-kinetics measurements were recorded with a time interval of 10 min.

Investigation. In the case of using 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer as supporting medium, 219 μL buffer was mixed with 1 μL H_2O or 1 μL of 1.0 M H_2O_2, followed by addition of 4 μL of 100 mM chloranil (in DMF). To test possible interaction between H_2O_2 and DMF, 219 μL buffer was mixed with 1 μL of 1.0 M H_2O_2, followed by addition of 4 μL DMF. In the case of using water as supporting medium, 219 μL water was mixed with 1 μL H_2O or 1 μL of 1.0 M H_2O_2, followed by

addition of 4 μL of 100 mM chloranil (in DMF). In the case of using a low concentration of chloranil, 219 μL 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer was mixed with 1 μL H_2O or 1 μL of 1.0 M H_2O_2, followed by addition of 4 μL of 1.68 mM chloranil (in methanol). The final concentration of chloranil was 30 μM in this case.

Colorimetric Detection of Glucose. 20 μL of 20 mg/mL GOx (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0) was mixed with 200 μL of different concentration of glucose solution (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0), and the mixture was kept in 37 °C water bath for 30 min. Then the tube was taken out, followed by addition of 4 μL of 100 mM chloranil (in DMF). Chloranil was dispersed in solution via a gentle shake. The tube was then left undisturbed at room temperature, and 180 μL of solution was transferred to the quartz UV-visible cell to record absorption spectra after 300 min. To test the possible interaction between glucose and chloranil, 20 μL of buffer was used instead of GOx to sense 5 mM glucose. When testing other saccharides, procedures were done as mentioned above.

To accelerate the kinetics of the organic Fenton reaction in glucose detection, 20 μL of 20 mg/mL GOx (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0) was mixed with 200 μL of 1 mM glucose solution (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0) or buffer, and the mixture was kept in 37 °C water bath for 30 min. Then the tube was taken out and 1 μL of 500 mM NaOH was added, followed by a sonication for a few seconds to disperse the alkali. 4 μL of 100 mM chloranil (in DMF) was added afterwards and scanning-kinetics measurements were recorded immediately.

To test the possible influence of DMF on the bioactivity of GOx, 20 μL of 20 mg/mL GOx and 200 μL of 1 mM glucose was mixed in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0), 2% (v/v) DMF buffer, and the mixture was kept in 37 °C water bath for 30 min. 4 μL of 100 mM chloranil (in DMF) was added to the taken-out tube and absorption spectra were recorded after 300 min.

Glucose detection in a human blood serum sample was also performed. The human serum sample was obtained from a local hospital and the concentration of glucose was determined by the hospital beforehand. 10 μL of serum sample was mixed with 190

μL of 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer, and then 20 μL of 20 mg/mL GOx (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0) was added. The mixture was kept in 37 °C water bath for 30 min and then the tube was taken out to add 4 μL of 100 mM chloranil (in DMF). Absorption spectra were recorded after 300 min.

Colorimetric Detection of H_2O_2. 20 μL of 110 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer was mixed with 200 μL of different concentration of H_2O_2, and then 4 μL of 100 mM chloranil (in DMF) was added. Chloranil was dispersed in solution via a gentle shake. The tube was then left undisturbed at room temperature, and 180 μL of solution was transferred to the quartz UV-visible cell to record absorption spectra after 60 min.

Colorimetric Detection of Choline. Procedures were done as the glucose detection procedures except that 20 μL of 5 mg/mL ChOx (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0) was mixed with 200 μL of different concentration of choline solution (in 10 mM NaH_2PO_4-Na_2HPO_4, pH 7.0), and absorption spectra were recorded 180 min later after addition of chloranil.

Note: In the process of glucose, H_2O_2 or choline detection, precipitates of chloranil formed at the bottom of the tubes since saturated chloranil in the final solution was used. Avoid shaking or inverting the tube before the absorption characterization and just leave the tube undisturbed in the equilibrating time. Carefully transfer 180 μL of the supernatant to the quartz UV-visible cell. The inconsistency of the precipitation process between different tubes in the parallel tests may, however, decrease the stability of the experiments to some extent.

All assays were performed in 1.5 mL microcentrifuge tubes. Photographs were taken before the absorption characterization. All reported values represent the mean and standard error of the mean of three measurements conducted in three independent assay performances. All concentrations of analytes referred in the article are the original concentrations of the 200 μL test solution.

Discussion

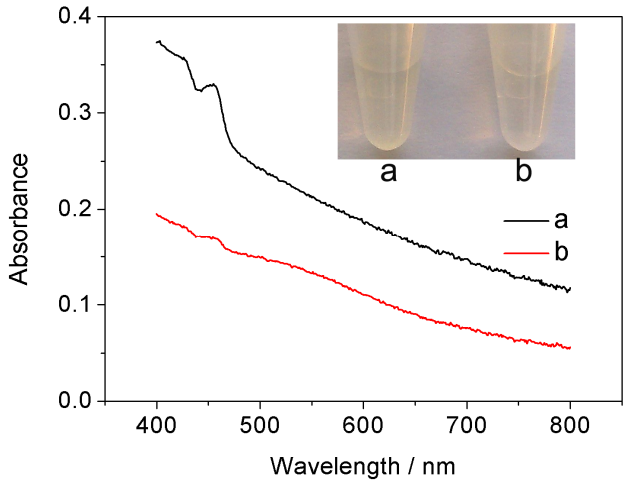

Fig. S1 Absorption spectra of chloranil (dissolved in DMF) treated with H_2O (a) and H_2O_2 (b) in water. Inset: Visual observation.

It was reported that chloranil could be hydrolyzed in strong alkaline solution and the hydrolysis reaction could also occur at near-neutral pH, albeit more slowly.[1] Hydrogen peroxide accelerated the rate of chloranil decomposition,[1] in which process •OH was generated.[2,3] Our result presented here is a bit different from the previous report,[1] where a low concentration of chloranil could decompose slowly without addition of H_2O_2. The difference originated from the different concentration of chloranil used. In the previous report,[1] stock solution of chloranil was prepared in methanol while the solubility of chloranil in methanol was poor (less than 5 mM determined by the titration experiment at 15 °C), and chloranil with a final concentration of 30 μM in buffer was used. In our experiments, stock solution of chloranil was prepared in DMF while the solubility of chloranil in DMF was relatively high (more than 160 mM determined by the titration experiment at 15 °C), and saturated chloranil in buffer was used. OH^- was consumed as chloranil was hydrolyzed, and the hydrolysis reaction was very slow in the absence of H_2O_2.[1] To hydrolyze a high concentration of chloranil in a fixed period of time completely was not easy, and few amounts of chloranil hydrolysis in solution could not cause an absorbance appearance at 530 nm. These were confirmed by the scanning-kinetics measurements.

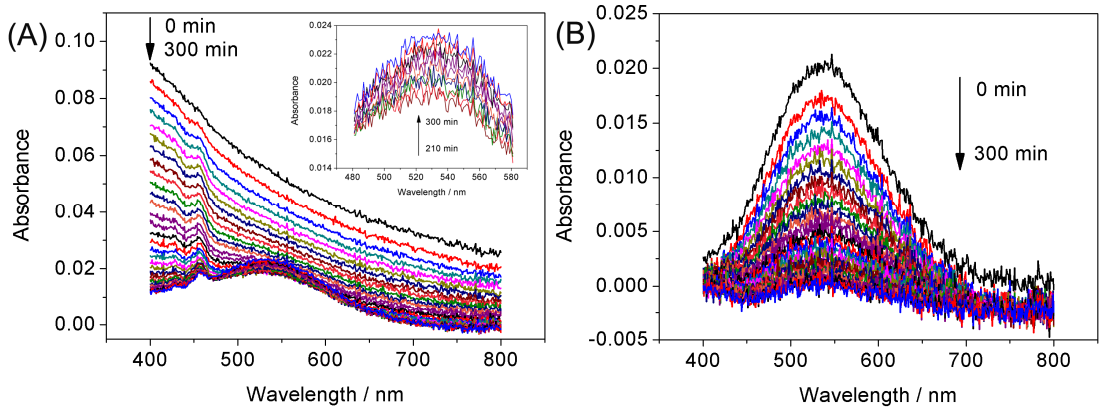

Fig. S2 Changes in absorption spectra with time for 30 μM chloranil treated with H_2O (A) or H_2O_2 (B) in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer. Inset (A): Detailed changes in the time range from 210 to 300 min.

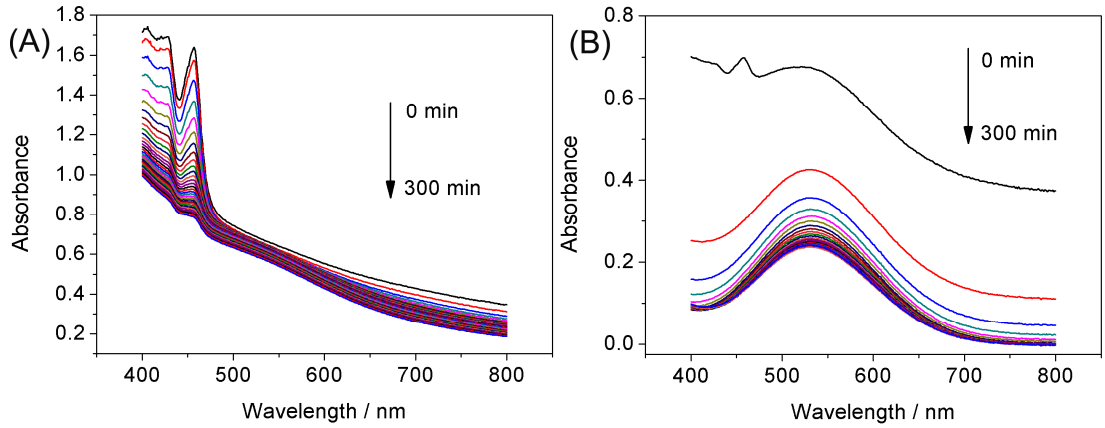

Fig. S3 Changes in absorption spectra with time for saturated chloranil treated with H_2O (A) or H_2O_2 (B) in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer.

Fig. S2 to Fig. S3 demonstrated that the different case of treating 30 μM chloranil or saturated concentration of chloranil with H_2O or H_2O_2 in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer. When treating 30 μM chloranil with H_2O, the absorption showed a gradual decrease in the beginning period of time, and then an absorbance at 530 nm appeared and gradually increased as time elapsed (Fig. S2A), indicating the hydrolysis of chloranil. The decreasing trend in the whole wavelength range was due to the decreasing molar extinction coefficient at 530 nm as chloranil decomposed.[1] Thus a low concentration of chloranil would undergo hydrolysis in the absence of H_2O_2 in the recorded period of time, which was consistent with the previous report.[1] However, no obvious absorbance at 530 nm could be observed in the absorption spectra when treating saturated chloranil with H_2O (Fig. S3A), indicating

that the saturated chloranil could not be easily hydrolyzed. When treating 30 μM chloranil with H_2O_2, the organic Fenton reaction rate was very fast and maximum absorbance at 530 nm appeared in the first scan (Fig. S2B). Then the absorbance at 530 nm gradually decreased, which would be unfavorable for quantifying. When treating saturated chloranil with H_2O_2, chloranil decomposition rate was also fast, however, stable absorption curves could be obtained as time elapsed (Fig. S3B). Using saturated chloranil not only magnified the value of absorbance, which was beneficial to monitoring and quantifying, but also led to an obvious visible color change. In contrast, the color change from colorlessness to faint purple in the case of using 30 μM chloranil was not favorable for visual determination (Fig. S4). Therefore, saturated chloranil was used in the experiment. Under the current condition, chloranil hydrolysis reaction proceeded slowly and the color of solution remained pale yellow in a fixed period of time; while the presence of H_2O_2 could decompose chloranil through an organic Fenton reaction, accompanied by a color change to purple.

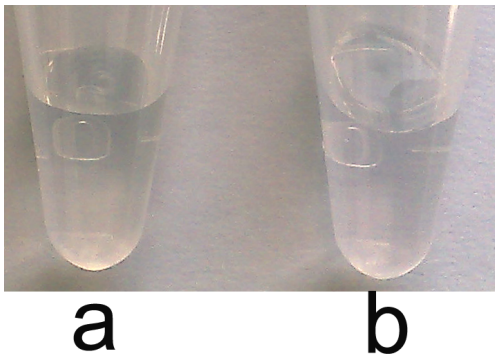

Fig. S4 Visual observation of 30 μM chloranil treated with H_2O (a) or H_2O_2 (b) in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) buffer after a reaction time of 5 min.

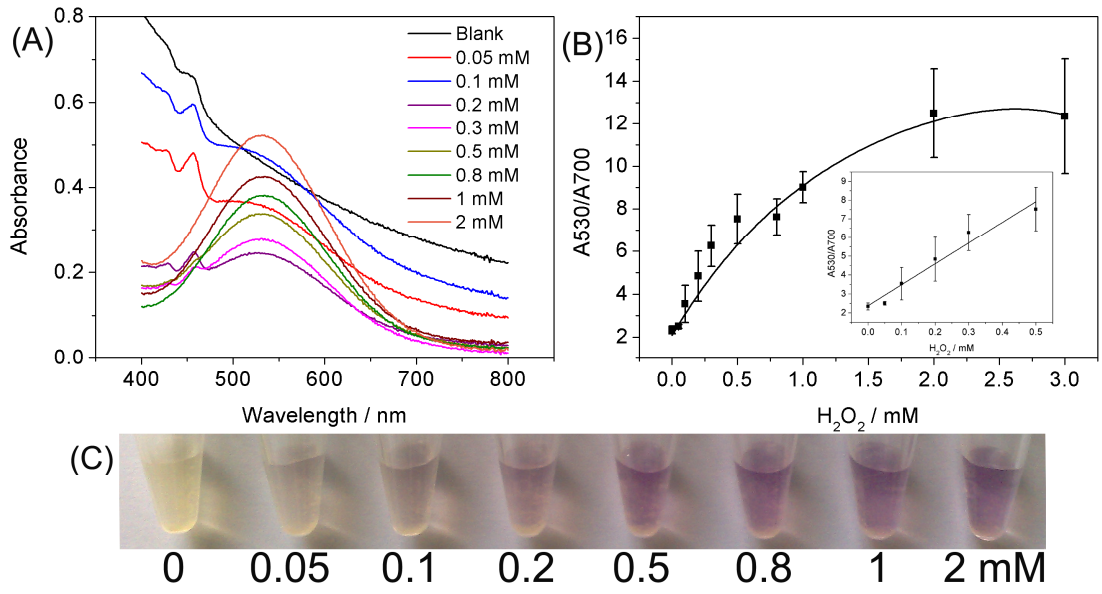

Fig. S5 Absorption spectra (A), plot of absorption ratio (A530/A700) versus H_2O_2 concentration (B) and visual observation (C) of the H_2O_2 sensing system treated with different concentration of H_2O_2 within the range from 0 to 3 mM. Inset (B): Derived calibration curve.

Chloranil was shown to be a sensitive colorimetric indicator towards the concentration of H_2O_2. Fig. S5A showed the absorption spectra when treating the H_2O_2 sensing system with water or different concentration of H_2O_2. In the absence of H_2O_2, a strong absorption band from 400 nm to 480 nm was observed and no absorption peak at 530 nm was revealed. This suggested that chloranil could be hardly decomposed without H_2O_2. In the presence of 0.05 mM H_2O_2, however, an absorption band around 530 nm appeared, indicating the presence of H_2O_2 reacted with chloranil and decomposed chloranil. The absorbance at 530 nm was intensified and increased along with the increasing concentration of H_2O_2, thus the system responded positively to the H_2O_2 concentration. This was attributed to that more H_2O_2 led to a greater degree of chloranil decomposition. The ratio value (A530/A700) was a sensitive function toward the H_2O_2 concentration, which increased in the concentration range from 0 to 2 mM and saturated at 3 mM (Fig. S5B). A good linear correlation was obtained within the concentration range from 0.002 mM to 0.5 mM (Fig. S5B, inset), and the detection limit was calculated to be 2×10^{-6} M. As the concentration of H_2O_2 increased, color of the solution gradually turned from pale yellow to purple (Fig. S5C). A darker purple color was obtained in the presence of a higher concentration of H_2O_2,

which was consistent with the absorption change and resulted from a greater degree of chloranil decomposition by H_2O_2.

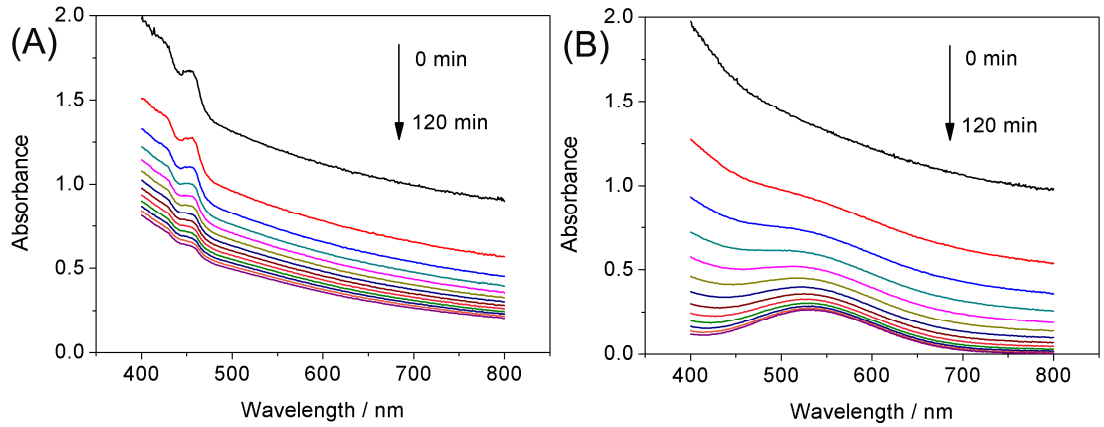

Fig. S6 Changes in absorption spectra with time for the glucose sensing system treated with buffer (A) or 1 mM glucose (B), in which case moderate alkali was introduced before chloranil addition.

The kinetics of the organic Fenton reaction in glucose detection could be effectively accelerated by introducing appropriate amount of alkali before chloranil addition. In such a case, absorption gradually decreased and no absorption peak around 530 nm could be observed in the recorded time range from 0 to 120 min in the absence of glucose (Fig. S6A). While in the presence of 1 mM glucose, the absorbance centered at 530 nm was gradually intensified and stable absorption curves could be obtained after 120 min (Fig. S6B). Therefore the equilibrating time was shortened from 300 min (Fig. 2B) to 120 min by a simple alkali addition. This was attributed to the fact that the rate of reaction between chloranil and H_2O_2 was higher under alkaline conditions.[1] It should be noted that although the introduction of more alkali may further accelerate the organic Fenton reaction theoretically, too much alkali introduction could, however, also lead to a high background or false positive signals in the absence of glucose since chloranil itself could be hydrolyzed under strongly alkaline conditions.[1]

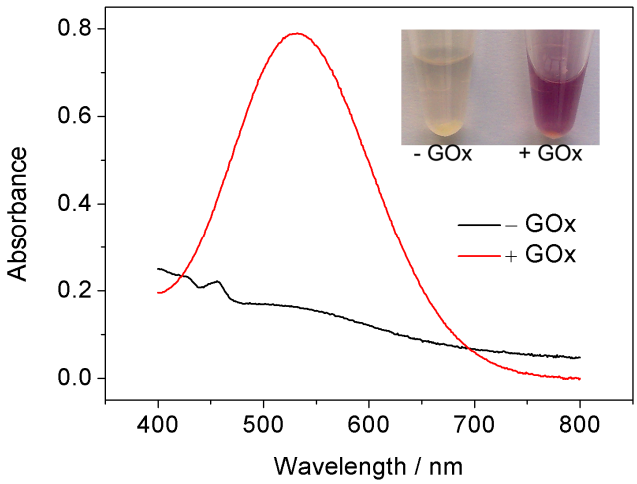

Fig. S7 Absorption spectra of the glucose sensing system treated with 5 mM glucose in the absence or presence of GOx. Inset: Visual observation.

In the absence of GOx, glucose could not be easily oxidized to produce gluconic acid and H_2O_2. Fig. S7 demonstrated that a high concentration of glucose would not react with chloranil, confirming the generated H_2O_2 from the enzymatic reaction decomposed chloranil and led to the absorption/color change.

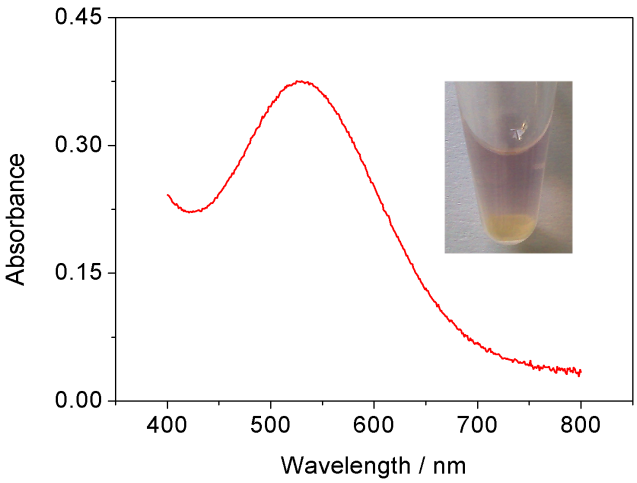

Fig. S8 Absorption spectra and visual observation of the glucose sensing system treated with 1 mM glucose, in which case GOx-catalyzed glucose oxidation was performed in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0), 2% (v/v) DMF buffer.

DMF was used as an organic solvent to dissolve chloranil. Note that the presence of a small volume fraction of DMF was shown to have little influence on the bioactivity of GOx. The GOx-catalyzed glucose oxidation was performed in 10 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0), 2% (v/v) DMF buffer. In such a case treated with 1 mM

glucose, a characteristic absorbance at 530 nm in the absorption spectra as well as a red-purple color was observed (Fig. S8). The obtained value of absorption ratio (8.24) did not deviate significantly from that of the GOx-catalyzed glucose oxidation in aqueous phosphate buffer (8.51) to sense 1 mM glucose, thus DMF hardly affected the bioactivity of GOx. This was in accordance with the previous reports in which DMF could be used to help to remain the bioactivity of enzymes[4-5] or to help GOx to remain the native structure and maintain its bioactivity to sense glucose.[6] Moreover, in our experiment DMF was added after GOx-catalyzed glucose oxidation with a final volume fraction of 1.8%, therefore DMF had little influence on the bioactivity of GOx in the sensing system.

The glucose sensing system was also applied to detect glucose concentration in the human serum sample. The concentration of the glucose in the serum was calculated to be 2.48 mM (n=3) by the method, which was lower than that determined by the hospital (4.89 mM). The influence of the serum on the system should result in the inaccuracy of the measured result. Meanwhile, glucose itself could decompose in the serum in vitro, which could be also responsible for the lower detected concentration.

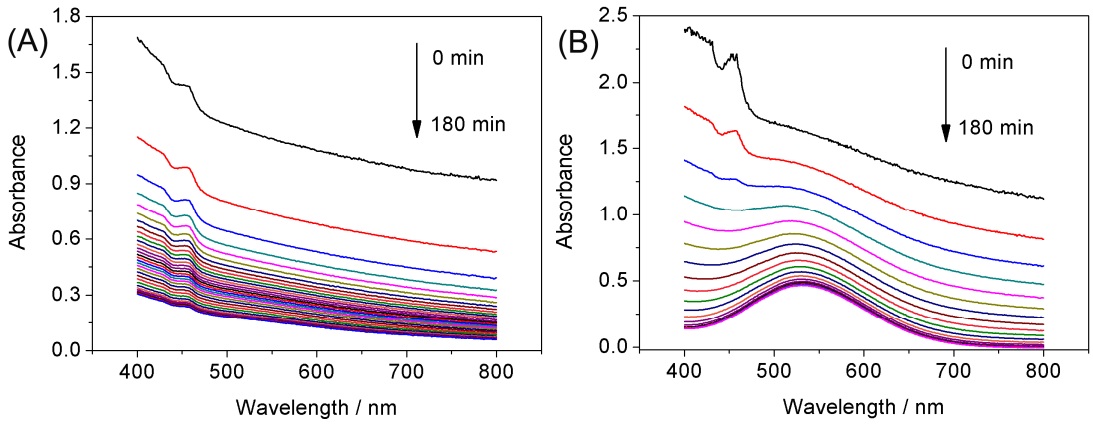

Fig. S9 Changes in absorption spectra with time for the choline sensing system treated with buffer (A) or 1 mM choline (B).

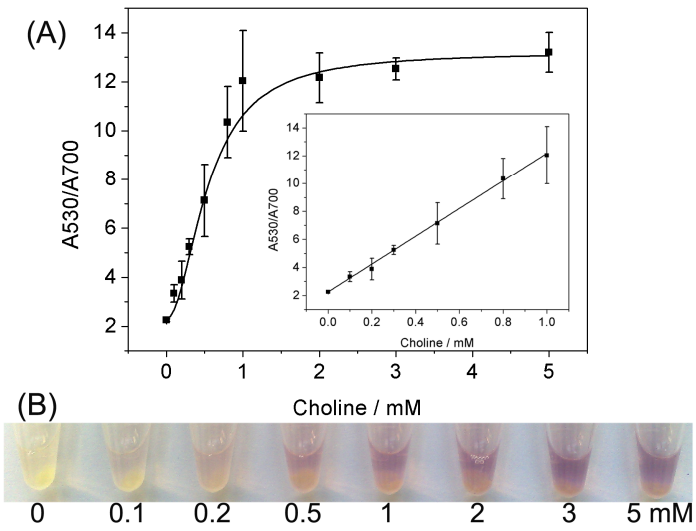

Fig. S10 Plot of absorption ratio (A530/A700) versus choline concentration (A) and visual observation (B) of the choline sensing system treated with different concentration of choline within the range from 0 to 5 mM. Inset (A): Derived calibration curve.

The same principle was extended to detect choline by virtue of ChOx-catalyzed reaction of choline and O_2 to betaine and H_2O_2. The amount of generated H_2O_2 should be proportional to the concentration of choline, according to which choline could be determined by chloranil decomposition by H_2O_2. Scanning-kinetics measurements showed that stable absorption curves could be obtained 180 min later after the addition of chloranil (Fig. S9), thus 180 min was chosen as the equilibrating time before absorption spectra were recorded. As expected, the value of absorption ratio (A530/A700) correlated sensitively and positively with the concentration of choline (Fig. S10A). The ratio value showed a good linear correlation toward the choline concentration within the range from 0.02 mM to 1 mM (Fig. S10A, inset) and saturated at 5 mM; the detection limit was calculated to be 0.02 mM. The color of the solution turned gradually from pale yellow to purple along with the increasing concentration of choline (Fig. S10B), indicating that more H_2O_2 was generated from the oxidation of a higher concentration of choline and induced a greater degree of chloranil decomposition. A concentration down to 0.1 mM choline caused a visible color change. The lower detection limit of choline sensing system than glucose sensing system was probably due to the fact that in the enzyme-catalyzed substrate oxidation one molar choline generated two molar H_2O_2 whereas glucose only

generated the equivalent mole. Control experiments confirmed that the color change resulted from the decomposition of chloranil by H_2O_2 rather than the possible reaction between chloranil and choline (Data not shown).

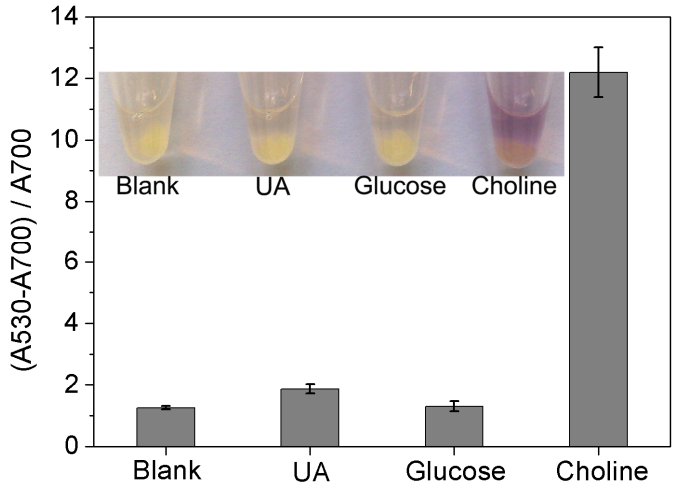

Fig. S11 Relative increase in absorption ratio of the choline sensing system treated with buffer, saturated UA, glucose (5 mM) and choline (5 mM).

Selectivity tests were performed using saturated uric acid (UA) and 5 mM glucose as controls. The values of relative increase in the absorption ratio when treating the choline sensing system with UA and glucose were similar to the blank case and much lower than that of the case treated with choline (Fig. S11), indicating that the system responded selectively to choline. Accordingly, only the presence of choline induced a solution color change to purple, while the presence of other samples retained the color pale yellow (Fig. S11, inset).

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