Electronic Supplementary Information

Aggregation-induced emission luminogen-based fluorescence detection of hypoxanthine: a probe for biomedical diagnosis of energy metabolism-related conditions

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Table of Contents

Materials and methods:

Chemicals and instrumentation

N-(2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzylidene)-4-(1,2,2-triphenylvinyl)aniline (TPE-HPro) was synthesized by our group. All other chemicals and reagents were commercially available and used as received without further purification. Potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), potassium hydroxide (KOH), acetonitrile (ACN), xanthine oxidase (XO), hypoxanthine, urea, glucose, lactic acid, citric acid, ascorbic acid, xanthine, bovine serum albumin (BSA), sodium bicarbonate ($NaHCO_3$), calcium chloride ($CaCl_2$), sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), sodium sulfate (Na_2SO_4), ammonium chloride (NH_4Cl) were all purchased from Sigma-Aldrich. Pure water was produced by Millipore Milli-Q Plus Water Purification System.

Fluorescence intensity and spectrum were recorded by Varioskan™ LUX multimode microplate reader.

Systematic study

The solution for hypoxanthine detection was prepared by mixing 90 μL of PBS buffer (50 mM, pH 7- 12) with 10 μL TPE-HPro stock solution (200- 1000 μM, in ACN) thoroughly. Then 1 μL XO stock solution (20- 400 unit/mL) was added into the solution, followed by addition of 1 μL hypoxanthine (0- 8 mM, in PBS buffer). The reaction was carried out at 37 °C for 0- 60 min. The fluorescence measurement was conducted with an excitation wavelength of 373 nm and an emission wavelength of 530 nm. The relative fluorescence intensity (ratio of fluorescence intensity in the presence of hypoxanthine to that without hypoxanthine, I/I_0) was calculated and plotted.

Determination of hypoxanthine in buffer

The working buffer employed 50 μM TPE-HPro, 1 unit/mL XO in ACN/PBS solution (1:9 v/v, pH 10.3). Standard solutions containing from 5×10^{-6} to 3.2×10^{-4} M of hypoxanthine were tested in the working buffer with three replicates. The relative fluorescence intensity was calculated and plotted versus hypoxanthine concentration.

Interference study

The fluorescence responses to common interfering substances in the working buffer were recorded. These included urea (8 mM), lactate (500 μM), glucose (100 μM), citrate (100 μM), xanthine (20 μM), ascorbate (8 μM) and BSA (0.2 mg/mL), which were compared with the fluorescence response to hypoxanthine (80 μM).

Determination of hypoxanthine in artificial urine

Artificial urine was prepared by mixing lactic acid (1.1 mM), citric acid (2.0 mM), $NaHCO_3$ (25 mM), urea (170 mM), $CaCl_2$ (2.5 mM), NaCl (90 mM), $MgSO_4$ (2.0 mM), Na_2SO_4 (10 mM), KH_2PO_4 (7.0 mM), K_2HPO_4 (7.0 mM) and NH_4Cl (25 mM) in pure water. The pH of artificial urine was adjusted to 10.3 with KOH. The supernatant was then collected for testing.

The XO activity (1- 5 unit/mL) was re-optimized for the hypoxanthine determination in artificial urine.

Standard solutions containing from 1×10^{-5} to 1.2×10^{-4} M of hypoxanthine were tested in the artificial urine with three replicates. The relative fluorescence intensity was calculated and plotted versus hypoxanthine concentration.

Additional figures:

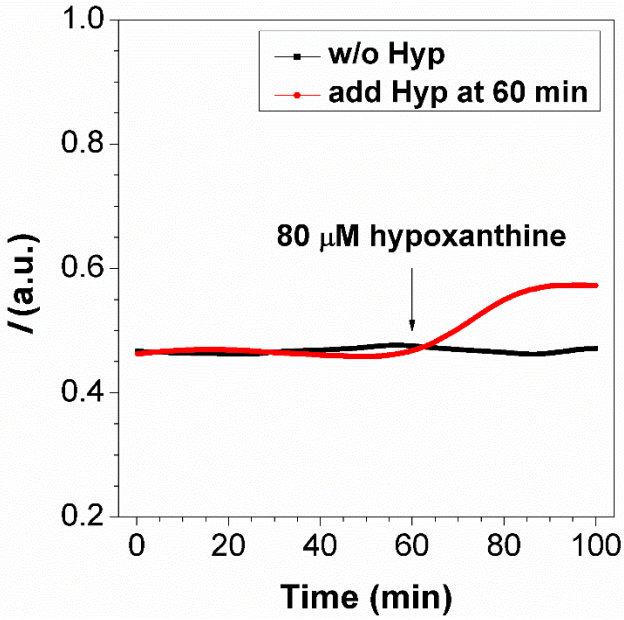

Fig. S1 Enzyme activity test after its incubation in working buffer for 60 min. Hypoxanthine was added at 60 min to check whether the enzyme was still alive. Excitation wavelength, 373 nm; emission wavelength, 530 nm; temperature, 37 °C; incubation time, 0- 100 min; water fraction, 90%; [TPE-HPro], 50 μM; pH 10.3; [XO], 1 unit/mL; [Hypoxanthine], 80 μM.

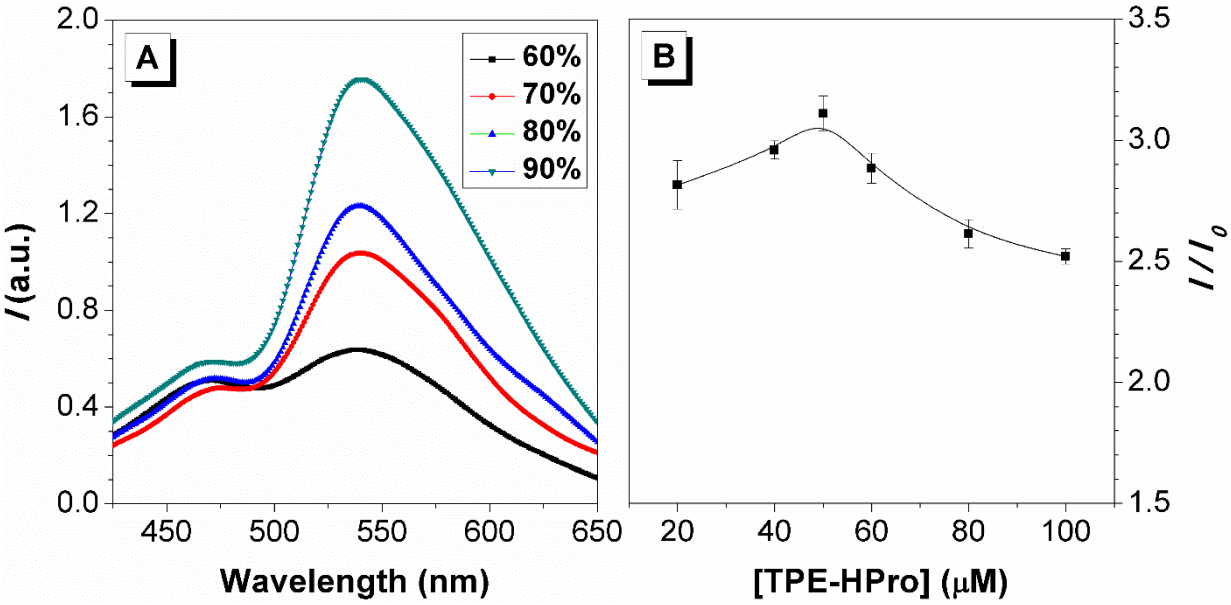

Fig. S2 Investigation of AIE feature and optimization of water fraction and TPE-HPro concentration. (A) Fluorescence spectra of TPE-HPro in ACN/buffer mixtures with different water fractions. (B) Plot of relative fluorescence intensity at 530 nm versus TPE-HPro concentration. Excitation wavelength, 373 nm; temperature, 37 °C; incubation time, 60 min; water fraction, 90%; [TPE-HPro], 50 μM; pH 10.3; [XO], 1 unit/mL; [Hypoxanthine], 80 μM.

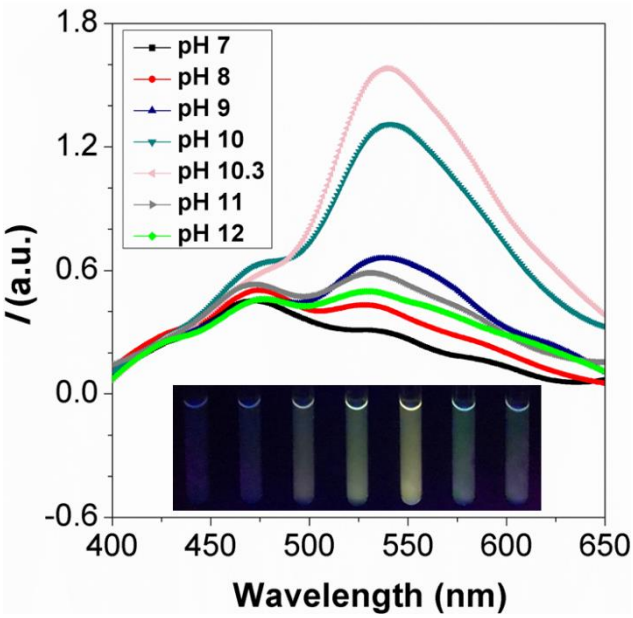

Fig. S3 The effect of pH on detection performance. Fluorescence spectra of TPE-HPro in solutions at different pH. Excitation wavelength, 373 nm; temperature, 37 °C; incubation time, 60 min; water fraction, 90%; [TPE-HPro], 50 μM; pH 7- 12; [XO], 1 unit/mL; [Hypoxanthine], 80 μM. Inset: photographs of solutions with increasing pH values from left to right taken under 365 nm UV illumination.

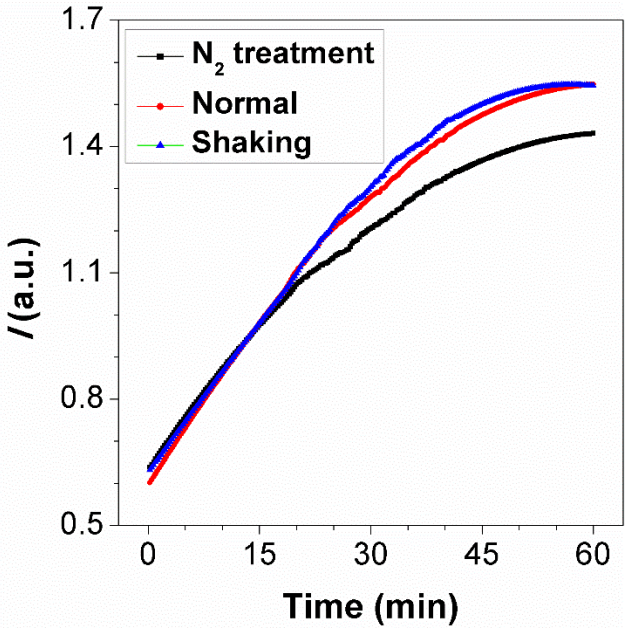

Fig. S4 The effect of oxygen supply on detection performance. Different conditions of oxygen supply: bubbling nitrogen through working buffer for 30 min before reaction; normal reaction; continuous shaking during reaction. Excitation wavelength, 373 nm; emission wavelength, 530 nm; temperature, 37 °C; incubation time, 0- 60 min; water fraction, 90%; [TPE-HPro], 50 μM; pH 10.3; [XO], 1 unit/mL; [Hypoxanthine], 80 μM.

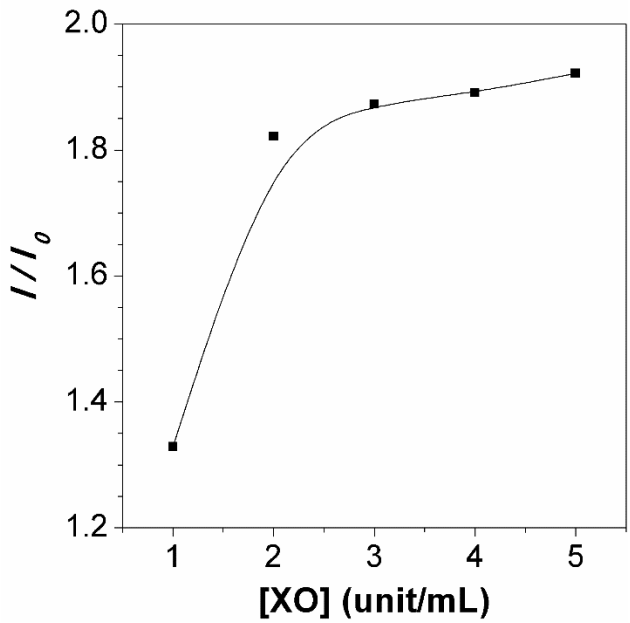

Fig. S5 Optimization of XO activity in artificial urine. Excitation wavelength, 373 nm; emission wavelength, 530 nm; temperature, 37 °C; incubation time, 60 min; water fraction, 90%; [TPE-HPro], 50 μM; pH 10.3; [Hypoxanthine], 80 μM.