Electronic Supporting Information (ESI)

One-pot synthesis of metal-organic framework-based drug carrier for intelligent glucose-responsive insulin delivery

Yan Duan,[ab] Fanggui Ye,*[a] Yuanlin Huang,[a] Yuemei Qin,[a] Caimei He,[a] Shulin Zhao*[a]

[a.] *State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, College of Chemistry and Pharmacy, Guangxi Normal University, Guilin 541004, China.*

[b.] *Guangxi Colleges and Universities Key Laboratory of Regional Ecological Environment Analysis and Pollution Control of West Guangxi, Baise University, Baise 533000, China*.

1. Instruments and chemicals

All chemicals were analytical reagent grade and used without further purification. Glucose (99%), 2-methylimidazole (Hmim, 99%), Zinc nitrate hexahydrate ($Zn(NO_3)_2$ $6H_2O$, 99%) and pynanine (99%) were purchased from Aladdin chemistry Co. Ltd. Polyvinylpyrrolidone (PVP, MW∼10 000) was purchased from TCI. Insulin (27.5 U/mg), insulin loaded fluorescein isothiocyanate (FITC-Ins) and glucose oxidases (GOx) were purchased from Sangon Biotech. Ultrapure water (Millipore 18.2 MΩ•cm) used throughout the whole experiments.

Scanning electron microscope (SEM) images were performed with a Quanta 200 FEG SEM (Philips, Netherland). Transmission electron microscopy (TEM) images were carried out on a Tecnai F-20 electron microscope operating at 200 kV (FEI, USA). X-ray diffraction (XRD) patterns were performed with an X'Pert PRO diffractometer (PANalytical, Netherland) using Cu_{Ka} radiation. Thermo gravimetric analysis (TGA) in air was taken on a LABSYS evo TGDSC equipment (Setaram Instrumentation, France). Fluorescence analysis was performed with a LS55 Fluorescence Spectroscopy (PE, USA). Confocal laser scanning microscope (CLSM) images were taken on a Fluoview FV1000 confocal microscope (Olympus, Japan). Inductively coupled plasma mass spectrometry (ICP-MS) was carried out on an X Series ICP-MS (Agilent, USA). Circular dichroism (CD) spectrums were recorded by J-810 spectrophotometer (JASCO, Japan). UV-Vis absorption spectra were recorded on a model Cary 60 spectrophotometer (Agilent, USA).

2. The synthesis of the Ins-GOx/ZIF-8 and ZIF-8

For the Ins-GOx/ZIF-8 composites, 40 mg insulin, 20 mg GOx and 2 mg PVP were added into an aqueous solution of Hmim (160 mM, 20 mL). For insulin, 40 mg insulin was dissolved in 200 μL diluted HCl solution (pH 3.0, 20 mM), and then adjusted to pH 10.3 with 100 mM NaOH. An aqueous solution of zinc nitrate hexahydrate (40 mM, 20 mL) was also prepared. The two solutions were mixed and

stired for 10 s, and then incubated for 30 min at room temperature. The unloaded insulin was collected by centrifugal separation with a centrifugal filter (25000 Da molecular masscutoff). The Ins-GOx/ZIF-8 was obtained by centrifugal, washed and sonicated two times with water and ethanol, and dried in vacuum at room temperature. The encapsulation efficiency (EE) and drug loading capacity (DLC) of insulin were tested by using a Coomassie Plus protein assay. The absorbance was measured at a wavelength of at 595 nm in UV-Vis absorption spectra. The EE and DLC were calculated using the following formula:

$$\text{Encapsulation efficiency (EE)} = \frac{\text{total amount of insulin added - free insulin}}{\text{total amount of insulin added}} \times 100\%$$

Formula S1

$$\text{Drug loading capacity (DLC)} = \frac{\text{total amount of insulin added - free insulin}}{\text{weight of nanoparticles}} \times 100\%$$

Formula S2

For the pure ZIF-8, methanol solutions of zinc nitrate hexahydrate (40 mM, 20 mL) mixed with methanol solutions of 2-methylimidazole (160 mM, 20 mL), and stired for 10 s. Then, the mixing solution was incubated for 24 h at room temperature. ZIF-8 was obtained by centrifugal separation, washed and sonicated two times with methanol, and dried in vacuum at room temperature.

3. Detection of pH change of GOx-Ins/ZIF-8 solution at different concentration of glucose

The pH in solution was monitored by introducing a fluorescent pH-sensitive probe (pyranine). The excitation spectrum of the pyranine was recorded under different pH using an LS55 fluorescence spectroscopy. The pynanine solution (100 μL, 10 μM) was added into PBS solution (900 μL, 20 μM) with different pH (pH=7.4, 7.0, 6.5, 6.0), and then shaked for 15s. The emission wavelength was fixed at 515 nm, the excitation wavelength was scanned from 350 nm to 500 nm.

GOx-Ins/ZIF-8 was dispersed in 3mL PBS solution (pH 7.2) with different concentrations of glucose (0, 1, 4 mg/mL) and incubated for 1 h at 37 °C. Then

centrifuging, 900 μL of liquid supernatant mixed with 100 μL pynanine (10 μM), shaking for 15s. Then the excitation spectra of the solution were measured using LS55 Fluorescence Spectroscopy with the above standard programme.

4. In vitro Insulin Release Studies.

The Ins-GOx/ZIF-8 of 4 mg was added into PBS solution (20 mM, pH 7.2, 2 mL) with three glucose concentrations (0, 1, 4 mg/mL) and evaluated the release of insulin. Incubation at 37 °C for predetermined time, 100 μL of the supernatant was taken out for analysis and 100 μL of fresh media was added into the well to keep a constant volume. The insulin concentration in the withdrawn sample was determined by using a Coomassie Plus protein assay.

The insulin-release studies in glucose solution simulating the daily blood glucose fluctuations were performed using a method similar to that described above. First, 4 mg GOx-insulin/ZIF-8 was dispersed in 2 mL of PBS solution containing 4 mg/mL glucose at 37 °C. After 15 min, the solution was centrifuged, and all of the supernatant was replaced with 1 mL PBS solution containing glucose. The 1 or 4 mg/mL glucose solutions were introduced alternatively at a designed time point.

5. Circular dichroism spectroscopy

The stability of the released insulin was determined by analysis of the conformation of released insulin using circular dichroism (CD), and the resulting spectrum was compared to standard insulin. The standard insulin was dissolved in PBS solution (0.02 M, pH 7.2) at the final concentration of 0.5 mg/mL. CD measurements were carried out on a Jasco J-810 CD spectropolarimeter at 25 °C with a cell length of 1.0 cm. For the far UV CD spectra, samples were scanned from 190 to 250 nm and accumulated 5 times, at a resolution of 0.2 nm and scanning speed of 700 nm/min.

6. Ins-GOx/ZIF-8 stability in the presence of trypsin and polar organic solvents

The Ins-GOx/ZIF-8 was incubated for 1h in phosphate buffer saline containing

polar organic solvents (50% methanol and 50% ethanol) and trypsin (1 mg/mL), simulated gastric fluid (SGF, with pepsin (1%, w/v)) and simulated intestinal fluid (SIF, with trypsin (1%, w/v)). Tiny amount of the enzyme solution was taken out and diluted by phosphate buffer saline to appropriate concentration and immediately subjected to the above enzymatic active assays. The Ins-GOx/ZIF-8 was collected by centrifugation and washed three times with deionized water, and immediately subjected to enzymatic activity assays and insulin release studies. The Ins-GOx /ZIF-8 was added to PBS (1 mL) with 4 mg/mL glucose concentrations and incubated at 37 °C for 4 h to evaluate the release of insulin.

To determine the enzymatic activity, free GOx (1 μg/mL) and GOx/ZIF-8 (30 μg/mL) was added into 1 mL of PBS solution (10 mM, pH 7.2) containing 0.1 mg/mL of HRP, 0.7 mM TMB and 4 mg/mL concentrations of glucose. The resultant mixture was incubated for 25 min at room temperature. The absorption spectrum of the solution was measured using UV-Vis absorption spectra, and the absorbance at wavelength 655 nm was recorded.

7. In vitro cytotoxicity of Ins-GOx/ZIF-8 and ZIF-8

The in vitro cytotoxicity of nanoparticles was tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Specifically, HeLa cells were seeded in 96-well plate and were incubated for 24 h. Series dilutions of Ins-GOx/ZIF-8 and ZIF-8 solution ranging from 10 μg/mL to 100 μg /mL were added into each well, and were cultured for another 24 h. After this, 100 μL MTT solution was added to each well, and incubating with cells for 4 h. MTT was removed, and 100 μL DMSO was added into each well using dissolving the purple formazan crystal. The cell viability was measured by reading the absorbance at 570 nm, and samples were normalized to non-treated cells.

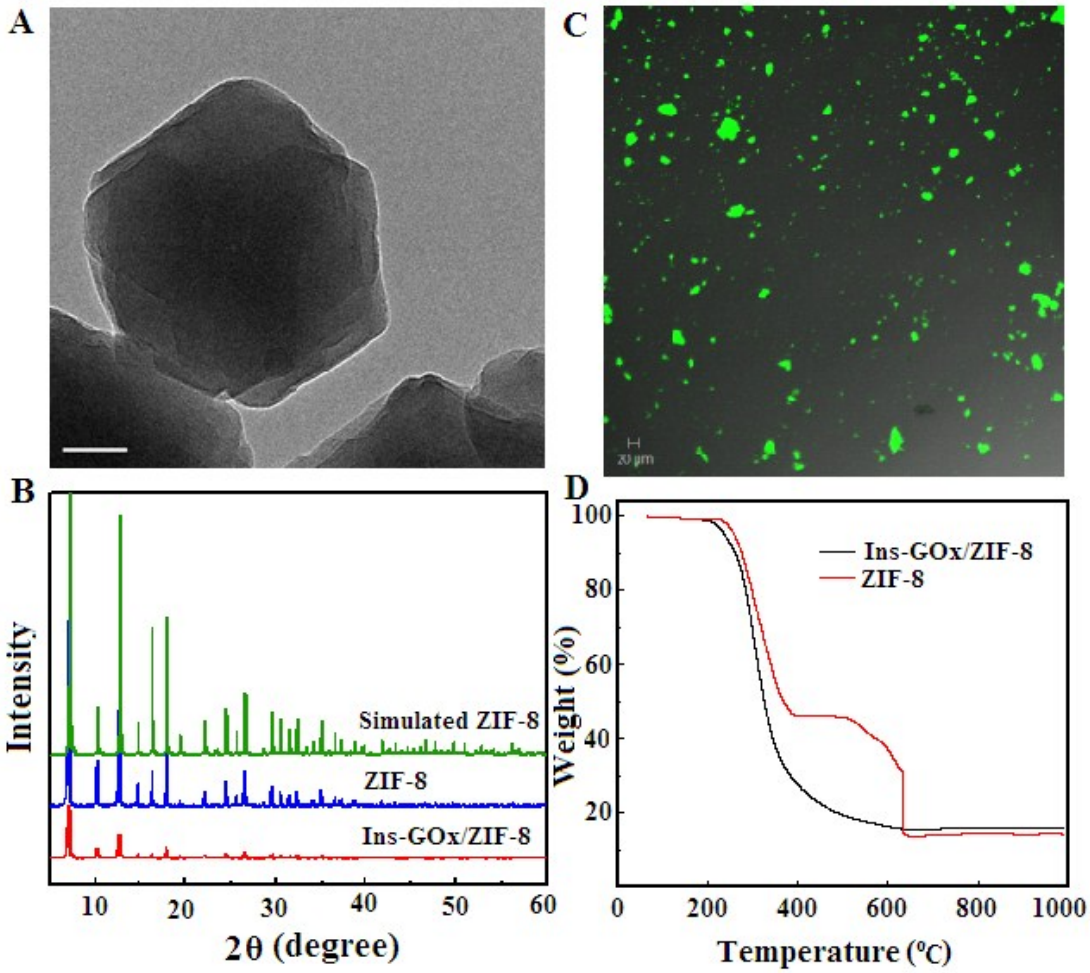

Fig. S1. (A) TEM images of ZIF-8. (B) XRD patterns of pure ZIF-8 (blue), Ins-GOx/ZIF-8 (red) and simulated ZIF-8 (green). (C) Confocal microscopy image of the Ins-GOx/ZIF-8 composite, in which insulin was labeled with FITC (green). (D) TGA curves of the Ins-GOx/ZIF-8 composite and ZIF-8 in air.

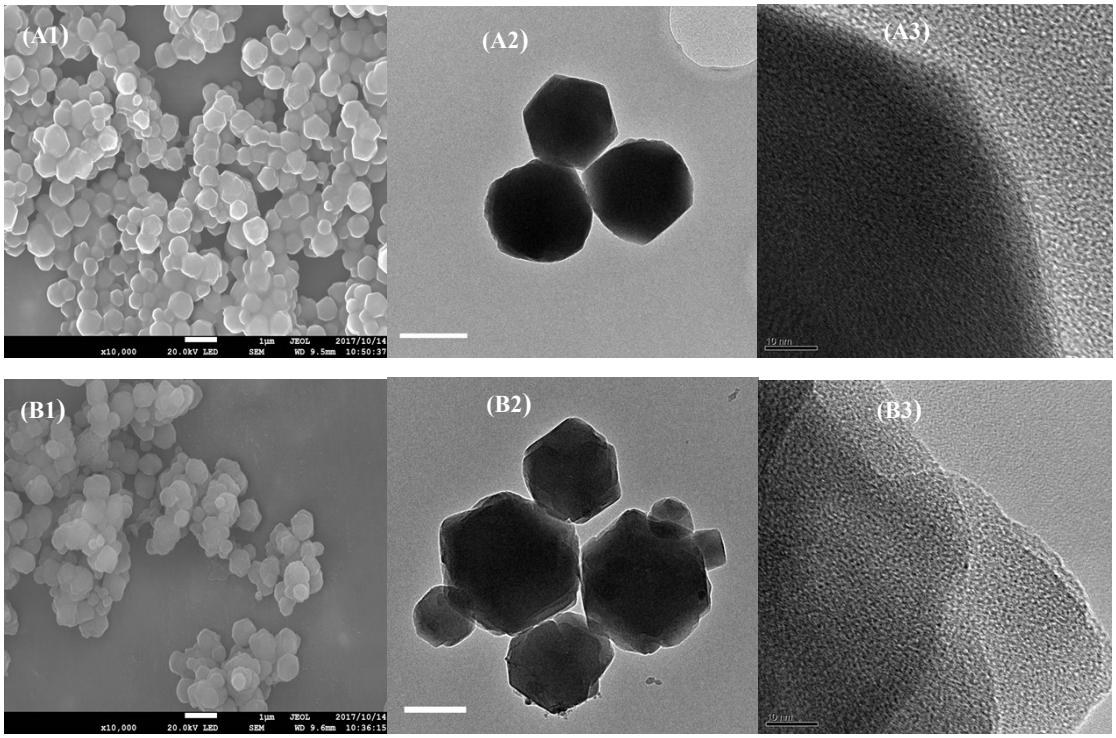

Fig. S2. (A1) SEM images of ZIF-8, (A2-3) TEM images of ZIF-8. (B1) SEM images of Ins-GOx/ZIF-8 composites, (B2-3) TEM images of Ins-GOx/ZIF-8 composites.

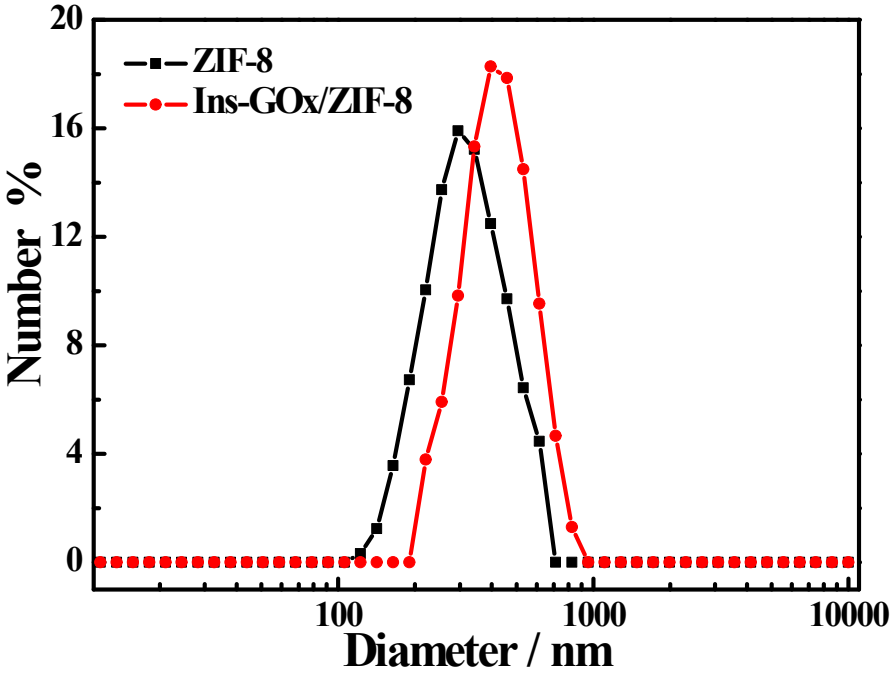

Fig. S3. The size distribution of ZIF-8 (back) and Ins-GOx/ZIF-8 (red) dispersed in water.

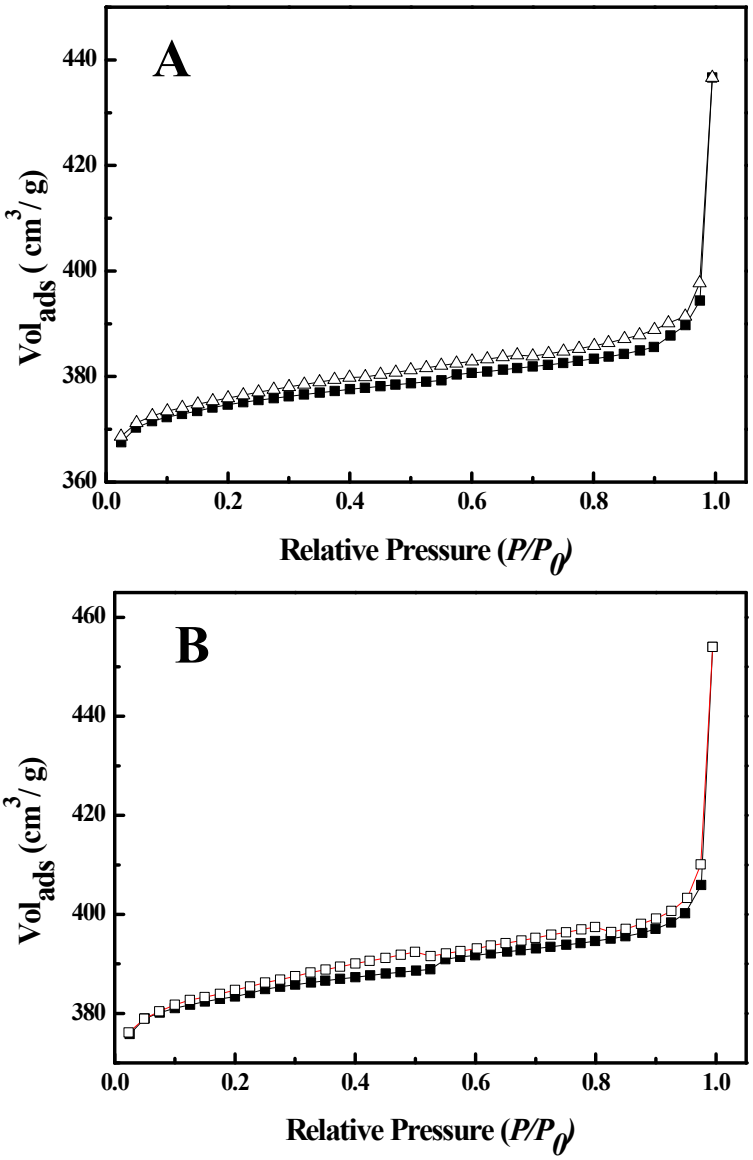

Fig. S4. The nitrogen adsorption–desorption isotherms for ZIF-8 nanoparticles (A) and Ins-GOx/ZIF-8 material (B).

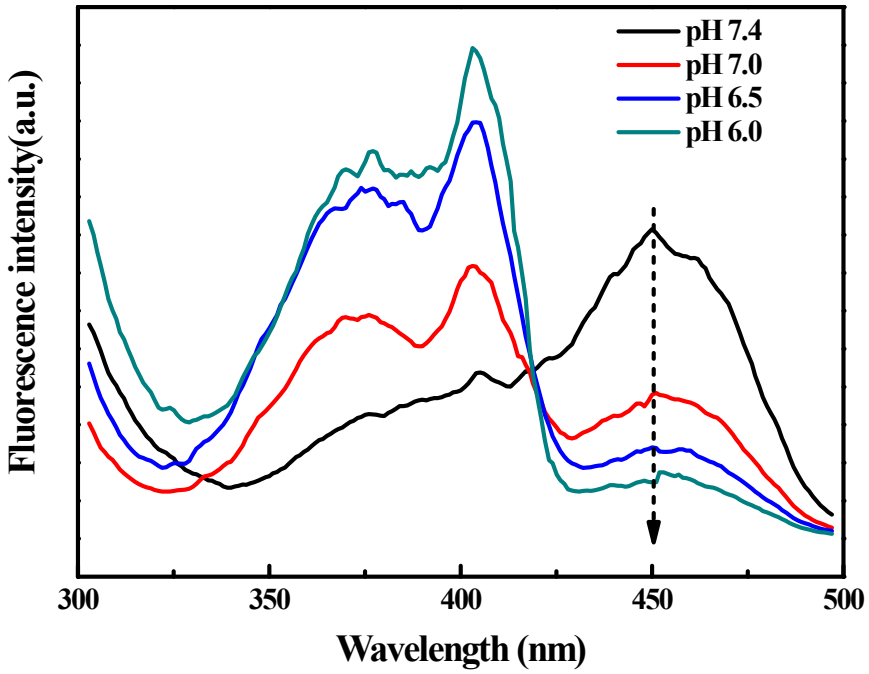

Fig. S5. Excitation fluorescence spectra of pyranine under different pH at emission wavelength of 515 nm, the fluorescent intensity of pyranine decreases at excitation wavelength of 450 nm and increases at 405 nm when pH reduced from 7.2 to 6.0.

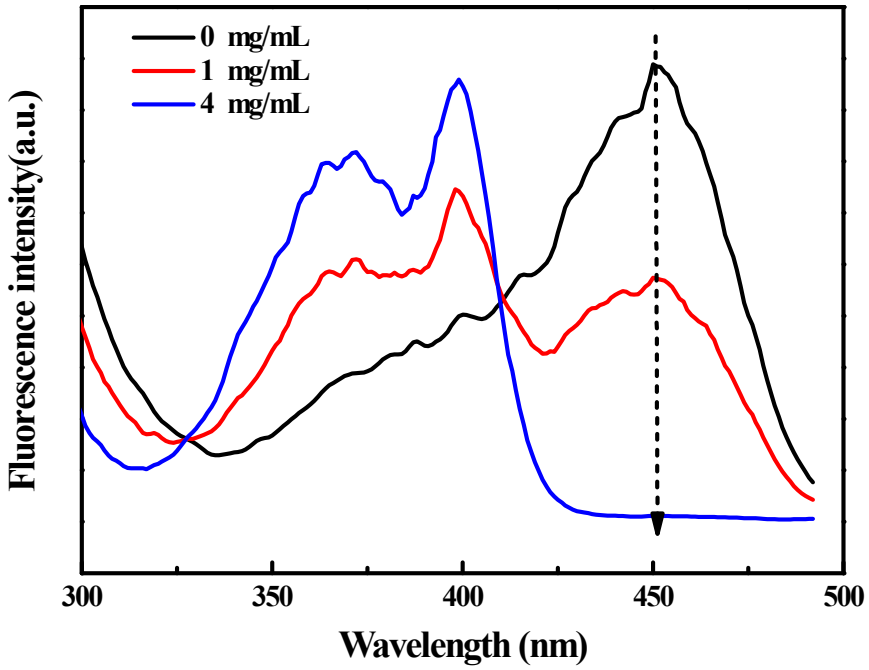

Fig. S6. Excitation fluorescence spectra of pyranine under different glucose concentration.

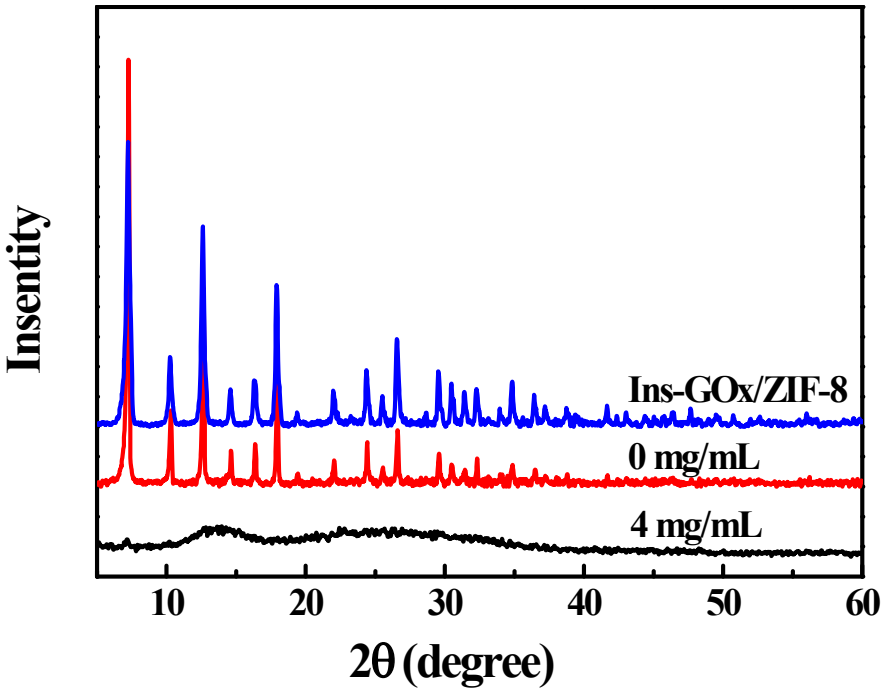

Fig. S7. XRD pattern of Ins-GOx/ZIF-8 which was incubated in 0 mg/mL (red) and 4 mg/mL (black) glucose solution for 24 h.

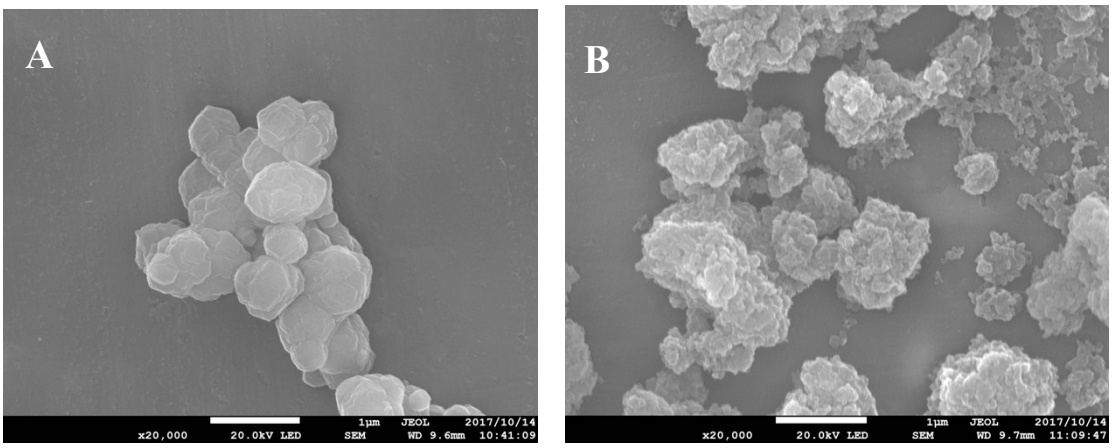

Fig. S8. SEM images of Ins-GOx/ZIF-8 incubated in 0 mg/mL (A) and 4 mg/mL (B) glucose solution for 24 h.

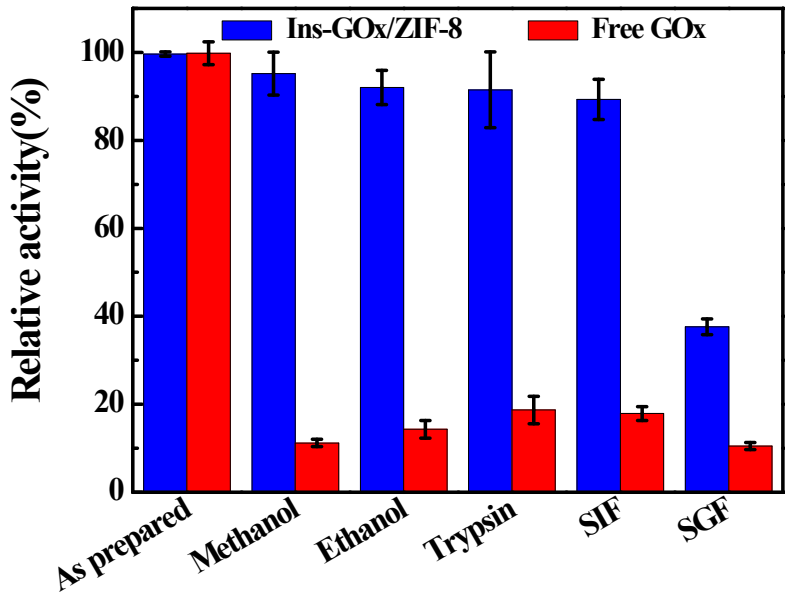

Fig. S9. Relative activities of free GOx and Ins-GOx/ZIF-8, after incubation in methanol, ethanol, trypsin, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) for 1h, respectively.

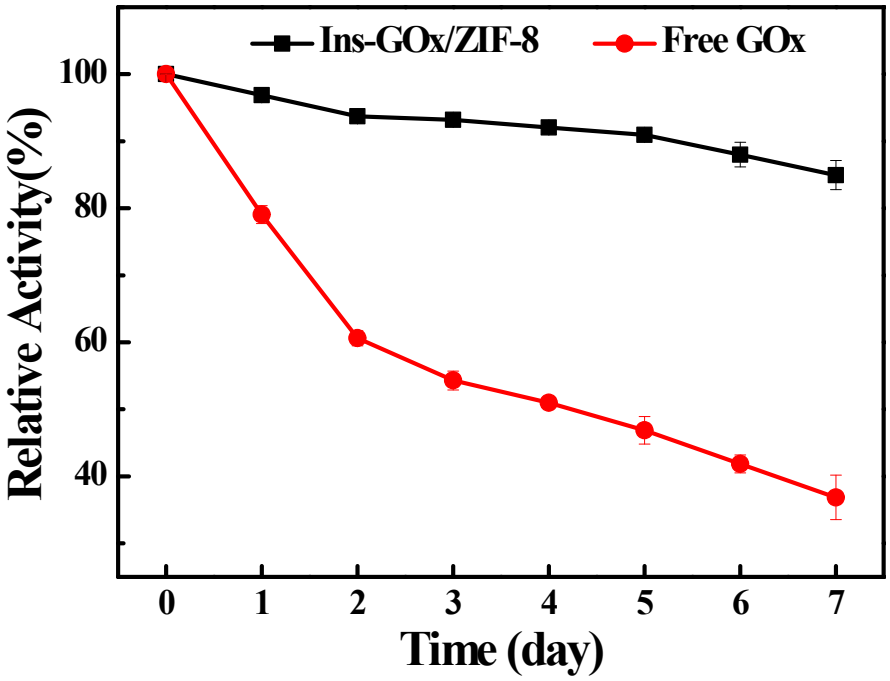

Fig. S10. Long-term stability of the Ins-GOx/ZIF-8 composite and free GOx incubated in PBS solution (pH 7.2) at room temperature.

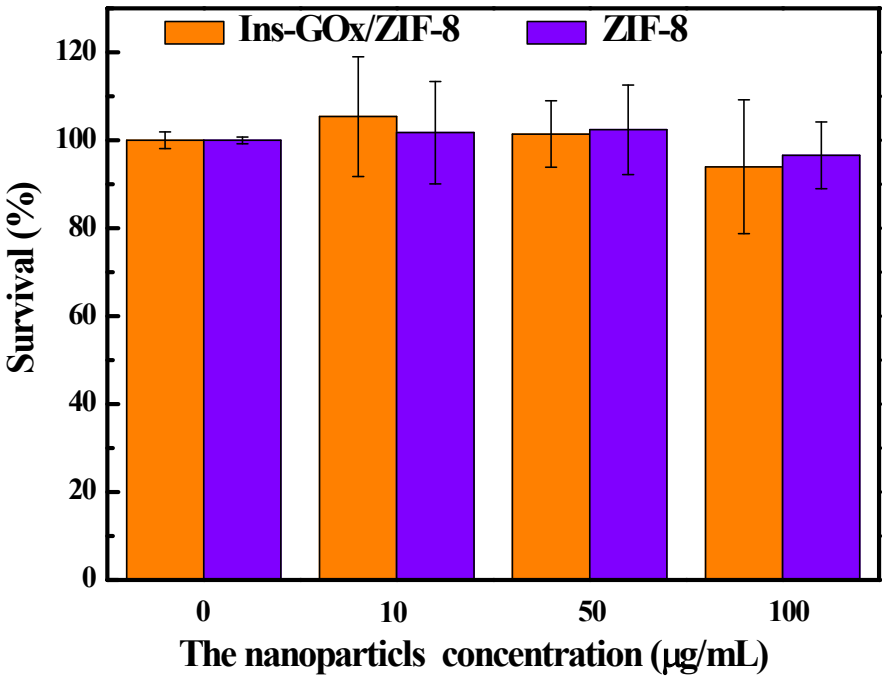

Fig. S11. Cell viability after incubated with ZIF-8 and Ins-GOx/ZIF-8.

Table S1. Diameters measured by DLS and surface zeta potential of materials

Sample	Size (nm)	Zeta potential (mV)
ZIF-8	347.2	-3.53
Ins-GOx/ZIF-8	427.5	45.1

Table S2. BET specific surface values and pore volumes calculated from the N_2 adsorption-desorption isotherms for materials

Sample	S_{BET} (m^2/g)	Pore volume (cm^3/g)
ZIF-8	1449	0.646
Ins-GOx/ZIF-8	1219	0.501