*Supplementary Information for:*

# Hemoglobin Coated Oxygen Storagable Metal-organic Framework as A Promising Artificial Oxygen Carrier

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## 1. General Synthetic Information

Bovine hemoglobin, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EDC) and N-hydroxysuccinimide (N-Hydroxysuccinimide, NHS) were purchased from Sigma (Shanghai, China). $HAuCl_4 \cdot 4H_2O$ (Au > 48%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Potassium ferrocyanide ($K_4Fe(CN)_6$) and hydrogen peroxide ($H_2O_2$, 30% wt) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). The diluted $H_2O_2$ aqueous solutions were freshly prepared before being used. Phosphate buffer solution (PBS) was prepared by mixing disodium hydrogen phosphate (0.2 M) with sodium dihydrogen phosphate (0.2 M). All chemical reagents were analytical grade. Deionized water was used for all the experiments. The deoxygenated water was obtained from double-distilled water by bubbling nitrogen for at least 30 min. Standard solutions were freshly prepared for each experiment and kept in a glass flask with a rubber septum.

All electrochemical measurements were performed with a CHI660 electrochemical workstation (CH Instruments Co., USA). The electrochemical cell consisted of a three electrode system with a modified glassy carbon electrode (GCE, f = 3 mm) as a working electrode, a platinum wire as a counter electrode and a saturated calomel electrode (SCE) as a reference electrode. A magnetic stirrer and a stirring bar provided the convective transport during the amperometric studies. Current-time data was recorded after a steady-state current had been achieved. Scanning electron microscopy (SEM) and Energy Dispersive Spectrometer (EDS) images were obtained with a Hitachi s4800 SEM (Hitachi, Ltd., Japan). Transmission electron microscopy (TEM) were obtained with FEI Talos F200X TEM. Indium tin oxide electrode (ITO) was used for SEM image. Fourier transform infrared (FTIR) was obtained with Nicolet iS10 FT-IR (Thermo Fisher Scientific, USA). Hyperbaric oxygen chamber (5L, 9 bar maximum, homemade by Institute of Special Environmental Medicine, Nantong University) was used for MOFs to adsorb oxygen. All measurements were done at room temperature of 25 ± 0.2 $^{o}$C in a Faraday cage.

## 2. Preparation of Hb-HKUST-1 particles.

2.1 HKUST-1 preparation

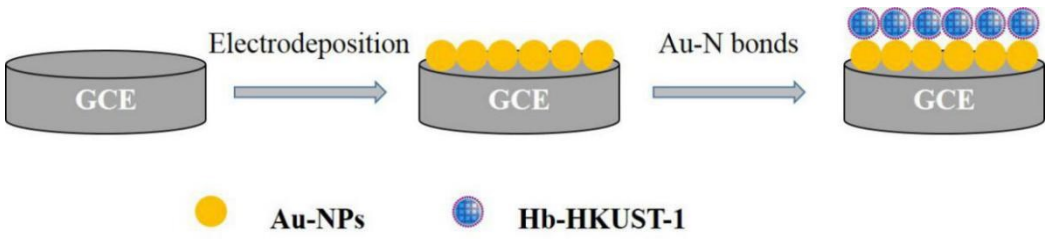
HKUST-1 was synthesized by dissolving 1,3,5-benzene tricarboxylic acid (5g, 23.8 mmol) and $Cu(NO_3)_3 \cdot 3H_2O$ (17.7g, 76.1 mmol) in a mixture of ethanol (220 mL), water (220 mL) and dimethyl formamide (16 mL). After incubating at 80 $^oC$ for 20h, the blue HKUST-1 particles were formed and isolated by filtration and washed with methanol (200 mL).

2.2 Synthesis of Hb-HKUST-1

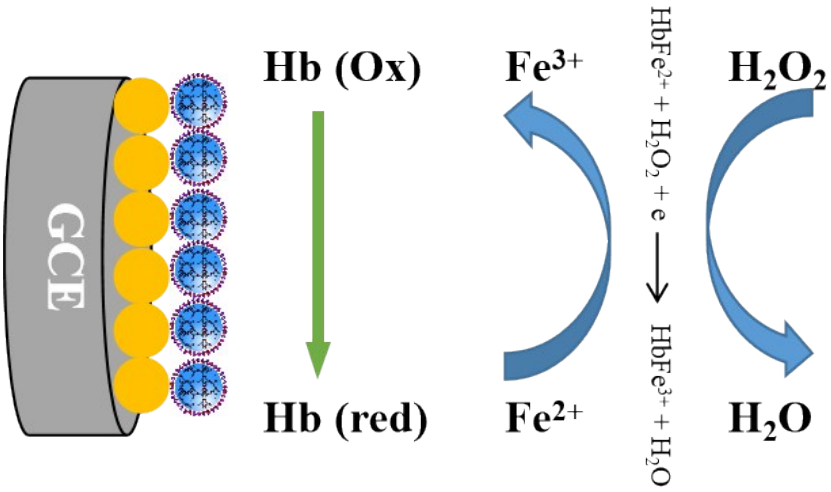
HKUST-1 (0.5 g) was stirred with EDC (2 g, 10.4 mmol) and NHS (2.4 g, 20.8 mmol) in PBS solution (pH 7.4) for 2h to activate carboxyl group. Hb was then added to the solution and stirred at room temperature for another 12 hours. Hb-HKUST-1 particles were then centrifuged at 5000 rpm for 20 min and washed 3 times with PBS. The brown particles were dried under vacuum for 1 h. Prior to sorption measurements, HKUST-1 was activated under high vacuum ($10^{-3}$ Torr) at room temperature.

## 3. Preparations of GCE/Au-NPs/Hb-HKUST-1

The bare GCE were firstly polished with abrasive paper and then with alumina (0.25 and 0.05 m) slurry on micro-cloth pads, then on ultrasonication in distilled water for 30 s and dried in air. Au-NPs were electro-deposited on the surface of GCE as reported in the literature.[1] In brief, the Au-NPs deposition was carried out by cyclic voltammetry (CV) from 0.00 to 0.70 V for 40 cycles at the scan rate of 0.10 V $s^{-1}$ in 1.0 M KCl solution containing 0.50 mM $HAuCl_4$. After that, the Au-NPs modified GCE (GCE/Au-NPs) was washed thoroughly with water to remove the un-reacted chemicals. Then, GCE/Au-NPs electrode was immersed in the Hb-HKUST-1 dispersion for 6 h. During this process, the Hb-HKUST-1 dispersion bath-sonicated for 5 min for every 1 h. Finally, the modified electrode (GCE/Au-NPs/Hb-HKUST-1) was stored in 4 $^oC$ before use. The fabrication procedure of GCE/Au-NPs/Hb-HKUST-1 electrode is shown in Scheme 2.




**Scheme S1.** Schematic process for the construction of GCE/Au-NPs/Hb-HKUST-1.




**Scheme S2.** The transmembrane electrocatalytic process for reduction of $H_2O_2$ by Hb.

**4. The structure of HKUST-1.**

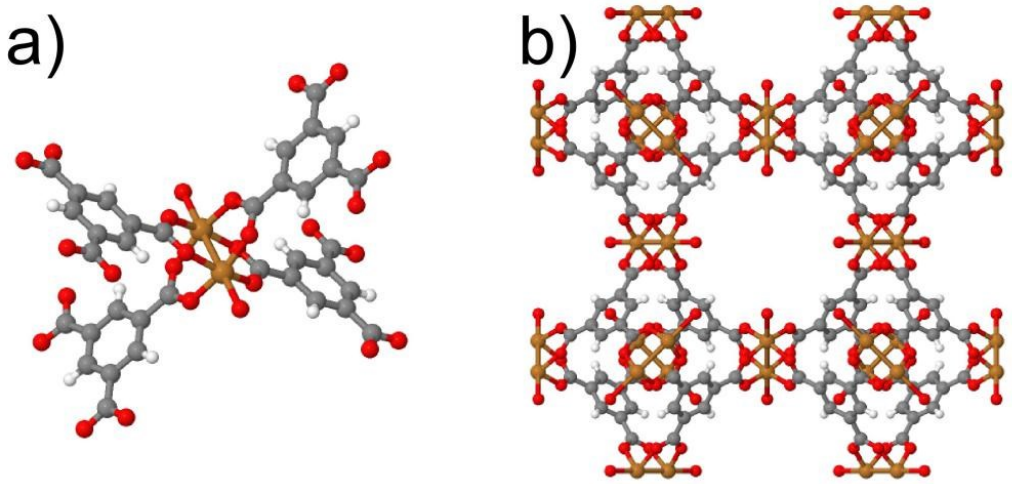



**Figure S1.** a) Core structure, and b) Cartoon of the porous HKUST-1 viewed from different axes (made with chemtube3d). Gray (Carbon), White (hydrogen), Red (oxygen), and brown (copper).

**5. EDS analysis of HKUST-1 and Hb-HKUST-1.**

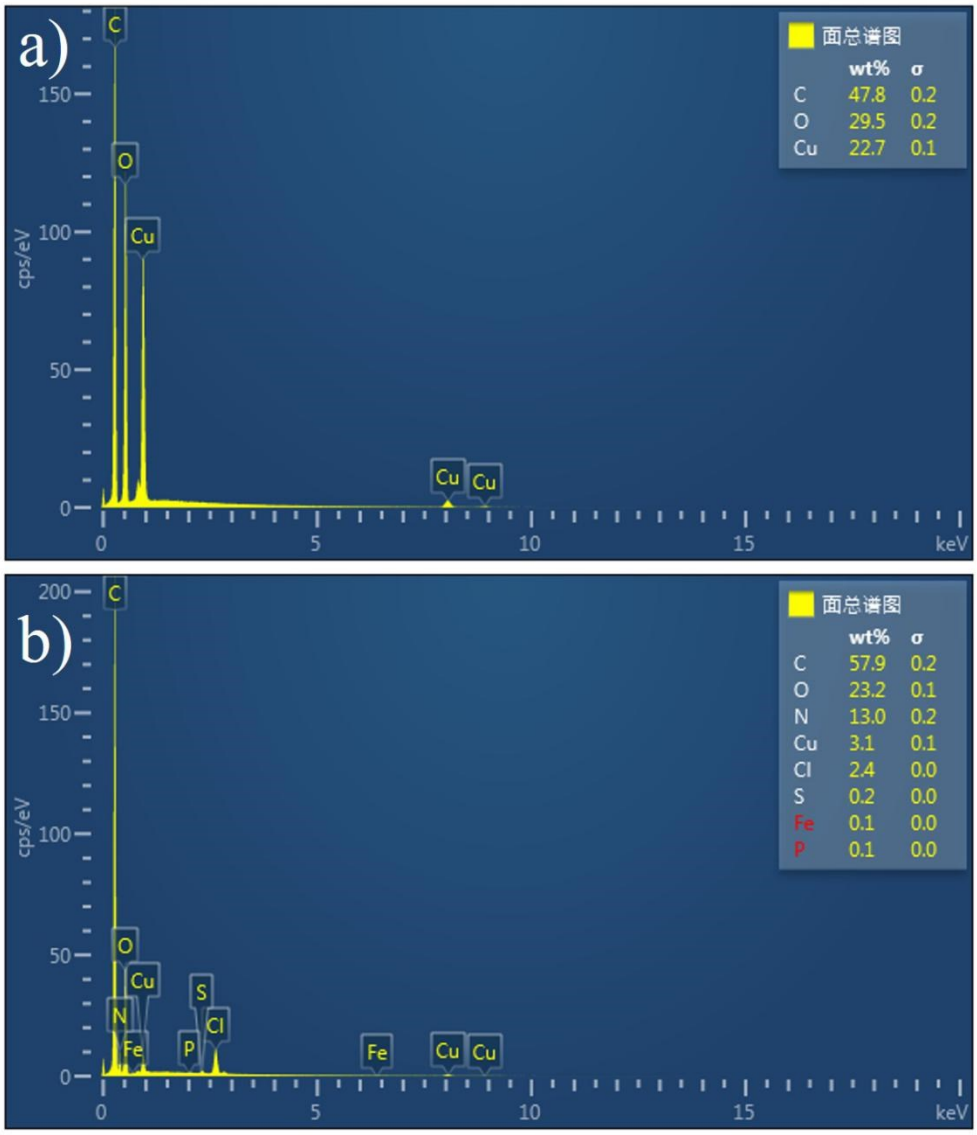



**Figure S2.** EDS analysis of a) HKUST-1 and b) Hb-HKUST-1. The appearance of N, Fe indicate that Hb was successfully grafted to HKUST-1 particles.

## 6. Stability analysis of Hb-HKUST-1.

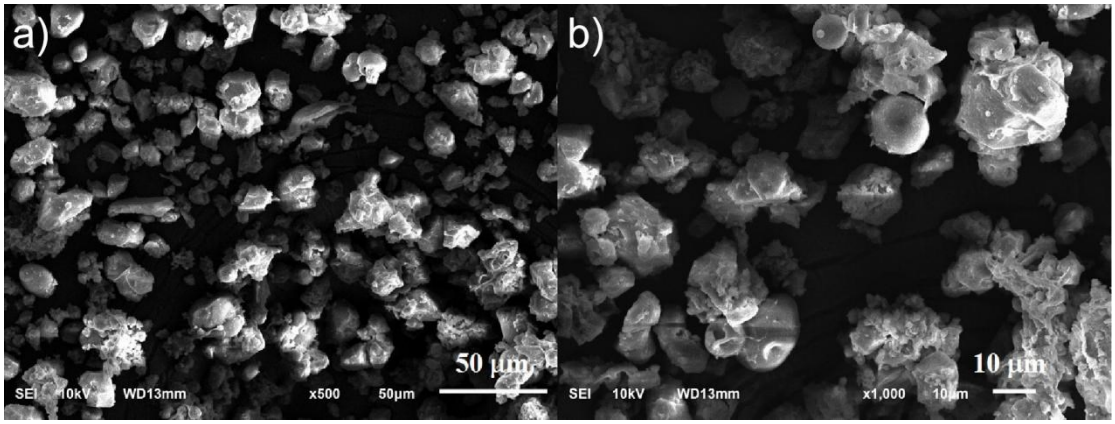



**Figure S3.** SEM image of Hb-HKUST-1 microparticles, and b) Enlarged image of Hb-HKUST-1 microparticles after incubated with PBS solution for 24h.

## 7. Absolute oxygen capacity of Hb-HKUST-1.

Absolute oxygen adsorption isotherms at 5 bar for Hb-HKUST-1 were measured three times at room temperature.

| Hb-HKUST-1 | 1 | 2 | 3 |
|---|---|---|---|
| **Before activated with $O_2$** | 2.3569 | 3.2121 | 3.3504 |
| **After activated with $O_2$** | 2.3932 | 3.2623 | 3.4039 |
| **Absolute $O_2$ adsorbed** | 0.0363 | 0.0502 | 0.0535 |
| **$O_2$ content** | 1.52% | 1.54% | 1.57% |

**Table S1.** Oxygen adsorption ability of Hb-HKUST-1 (5 bar) at room temperature.

**8. FTIR spectra of HKUST-1 and Hb-HKUST-1.**

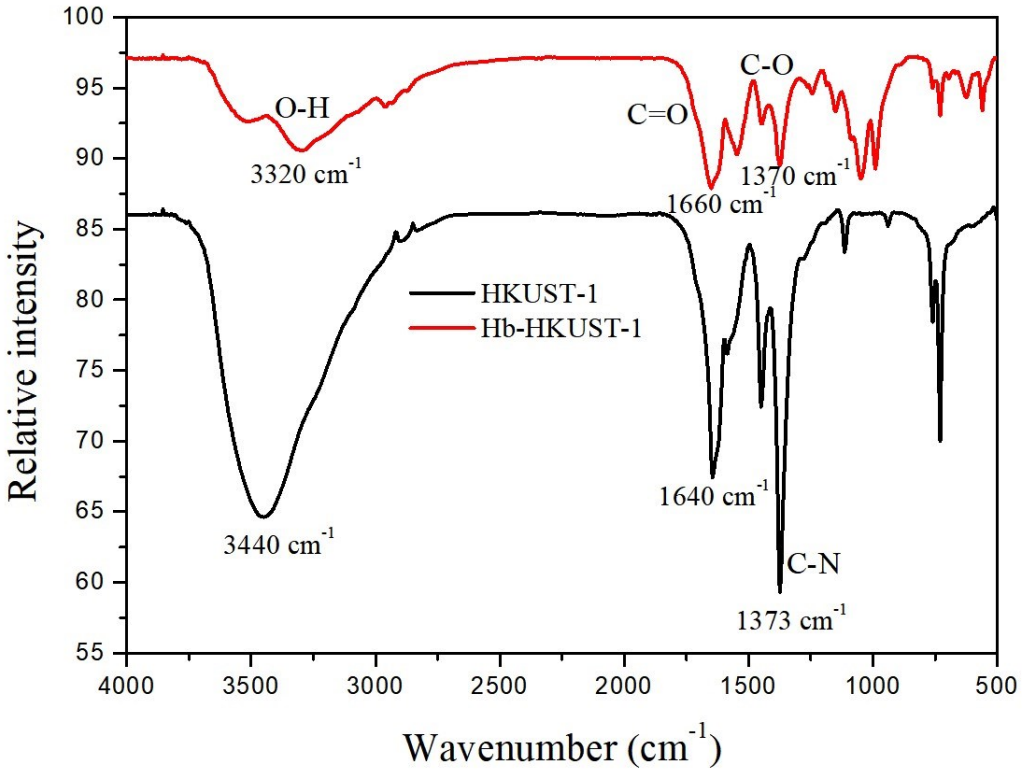



**Figure S4.** Fourier transform infrared (FTIR) spectra of microparticles HKUST-1 (red) and Hb-HKUST-1 (black).

**9. UV-Vis spectra of HKUST-1 and Hb-HKUST-1.**

The absorption spectra were recorded using a VARIAN Fluorescence Spectrophotometer at 20 °C. The samples were each tested at the wavelength appropriate for HKUST-1 and Hb-HKUST-1. The data points were collected at 1 nm increments with a 0.1 s integration period. All spectra were corrected for background absorption by subtracting a blank scan of the buffer system.

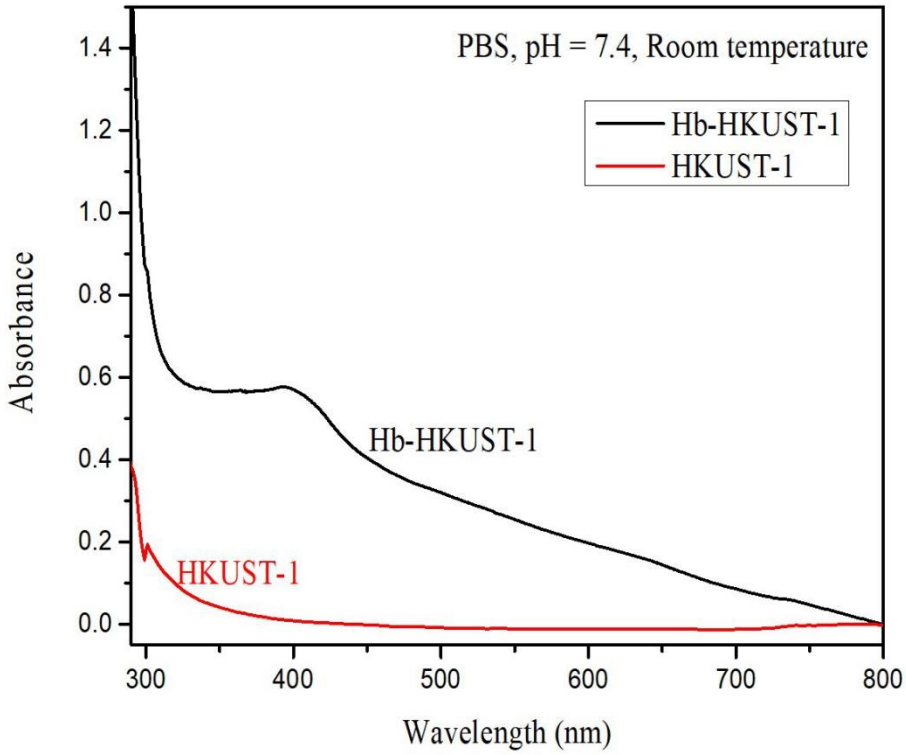



**Figure S5.** UV-vis absoption spectra of a) HKUST-1 solution at pH = 7.4 PBS, b) Hb-HKUST-1 solution at pH = 7.4 PBS.

**10. Cyclic voltammograms of different electrodes in PBS.**

CV curves of the bare GCE, GCE/Au-NPs, GCE/Au-NPs/ Hb-HKUST-1 and GCE/Au-NPs/Hb-HKUST-1 charged with oxygen (5 bar for 10 minutes) tested in PBS (pH 7.4).

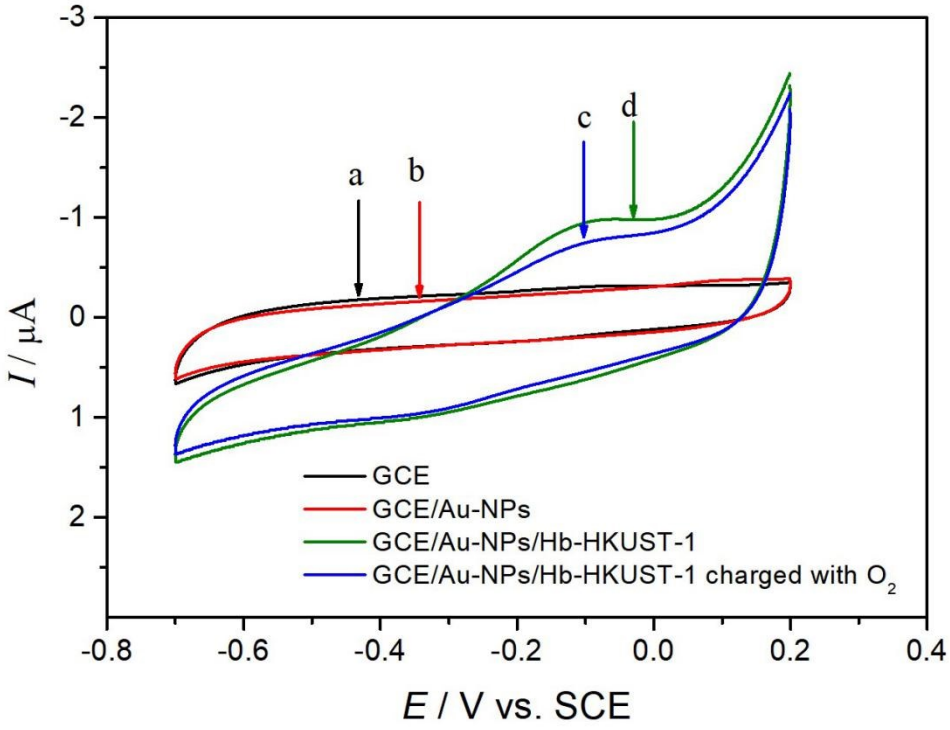



**Figure S6.** Cyclic voltammograms of (a) bare GCE, (b) GCE/Au-NPs, (c) GCE/Au-NPs/Hb-HKUST-1, (d) GCE/Au-NPs/Hb-HKUST-1 supplied with oxygen in 0.2 M PBS (pH=7.4) at the scan rate of 50 mV $s^{-1}$.

**11. Amperometric response to oxygen in PBS.**

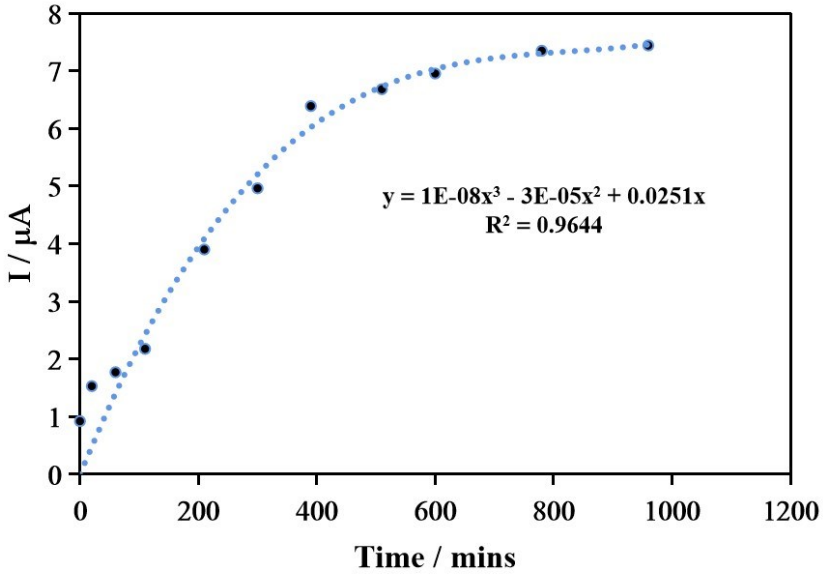



**Figure S7.** Amperometric responses of oxygen charged Hb-HKUST-1 at GCE/Au-NPs/ Hb-HKUST-1 at the working potential of -0.220 V in PBS (pH 7.0).

**12. Toxicity of Hb-HKUST-1 to Hela cells.**

The cytotoxicity of Hb-HKUST-1 microparticles (MPs) was evaluated by the standard MTT assay. Briefly, HeLa cells were seeded in 96-well U-bottom plates at a density of 2.0 $\times 10^4$ cells/well, and incubated with Hb-HKUST-1 at varied concentrations (0, 5.0, 10.0, 20.0, 40.0, 80.0 and 100.0 μg/ml) at 37℃ for 24 h. Then, the culture media were discarded, and 10μL of the MTT solution (5 mg/mL in dulbecco's modified eagle medium (DMEM)) was added to each well, followed by incubation at 37℃ for 4 h. The supernatant was abandoned, and 100 μL of Formazan solvent was added to each well to dissolve the formed formazan. After shaking the plates for 4 h, absorbance values of the wells were read with a microplate reader at 570 nm. The cell viability rate (VR) was calculated according to the equation. The cell viability was expressed as a percent of the control culture value, and it was calculated using the following equation: Cells viability (%) = ($OD_{MPs}$ - $OD_{blank}$)/ ($OD_{control}$ - $OD_{blank}$) × 100%.

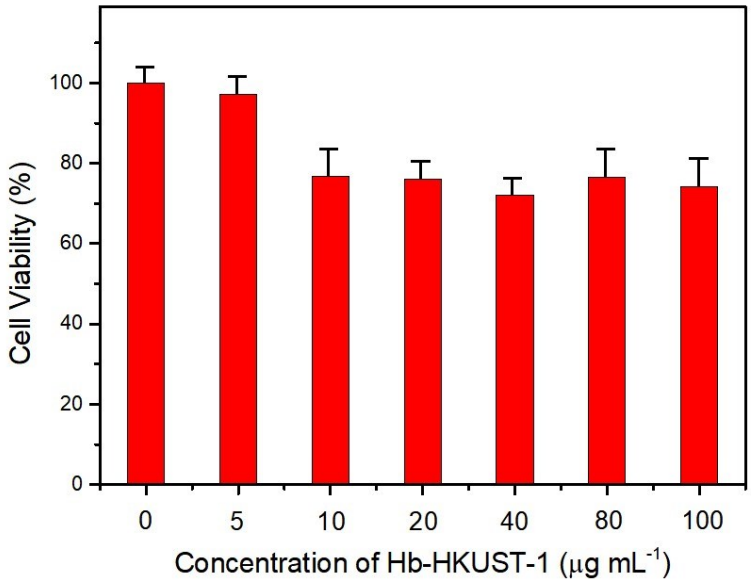



**Figure S8.** Effects of Hb-HKUST-1 at varied concentrations (0-100 μM) on the viability of Hela cells. The viability of Hela cells without Hb-HKUST-1 is defined as 100%. The results are the mean ± standard deviation of five separate measurements.

1. H. Y. Gu, S. Y. Lu, Q. Y. Jiang, C. M. Yu, G. Li and H. Y. Chen, *Analytical Letters*, 2006, **39**, 2849-2859.