SUPPORTING INFORMATION

In-situ hybridization of enzymes and their metal-organic framework analogues with enhanced activity and stability by biomimetic mineralisation

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Materials

Cytochrome c (CAS#9007-43-6, from equine heart), glucose oxidase (CAS#9001-37-0, type VII, from Aspergillus niger), horseradish peroxidase (CAS#9003-99-0, Type VI), Candida rugose lipase (CAS#9001-62-1), trypsin (CAS#9002-07-05), D-glucose, hydrogen peroxide (30 wt.% in water), fluorescein isothiocyanate (FITC) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were purchased from Sigma Aldrich.

Synthesis of CuBDC and enzyme-CuBDC composite

In a typical experiment, at room temperature, a solution of cupric sulfate (100 mM, 1 mL) was added into a solution containing both enzymes (0.5 mg/mL) and sodium salt of 1, 4-benzenedicarboxylate (BDC) (50 mM, 1 mL, pH 6.0). By simply mixing the two solution, enzyme-CuBDC composites were immediately (about 1 minute) obtained as precipitates, followed by washing with deionized water three times and centrifugation to recover the products. CuBDC was synthesized by mixing BDC sodium salts solution (without enzymes) with copper solution.

Enzymatic assays

All of the enzyme quantifications and activity assays were carried out with a UV–Visible spectrophotometer (SHIMADZU UV-2450 spectrophotometer).

Activity assays for Cyt c: H_2O_2 and ABTS were used as substrates for the activity assay. The final assay solution contains 50 μL 1 μM Cyt c, Cyt c-CuBDC or Cyt c&CuBDC containing solution (equivalent molar weight of Cyt c for Cyt c-CuBDC, Cyt c&CuBDC, equivalent mass weight of Cyt c for CuBDC), 900 μL 0.5 mM ABTS and 50 μL H_2O_2 at different concentrations (0-50 mM) in 50 mM Tris-HCl buffer (pH 7.4). The increase in absorbance at 405 nm was recorded for 2 min. Millimolar extinction coefficient of oxidized ABTS at A_{405} nm is 36.8. The fastest rate is usually in the first minute. The enzyme activity was calculated from the slope of absorbance versus time curve. The activity assays for HRP and its HRP-CuBDC composites are almost the same using different enzyme (0.05 μM) and H_2O_2 concentration (0-100 μM).

Activity assays for GOx: Glucose and ABTS were used as substrates for the activity assay of Cyt c and HRP. The final assay solution contains 50 μL 0.01 μM GOx (equivalent molar weight of GOx for GOx-CuBDC, GOx&CuBDC), 50 μL 0.2 μM HRP, 850 μL 0.5 mM ABTS and 50 μL glucose different concentrations (0-100 mM) in 50 mM Tris-HCl buffer (pH 7.4). The increase in absorbance at 405 nm was recorded for 2 min. Millimolar extinction coefficient of oxidized ABTS at A405 nm is 36.8. The fastest rate is usually in the first minute. The enzyme activity was calculated from the slope of absorbance versus time curve.

Encapsulation efficiency for Cyt c-CuBDC and GOx-CuBDC

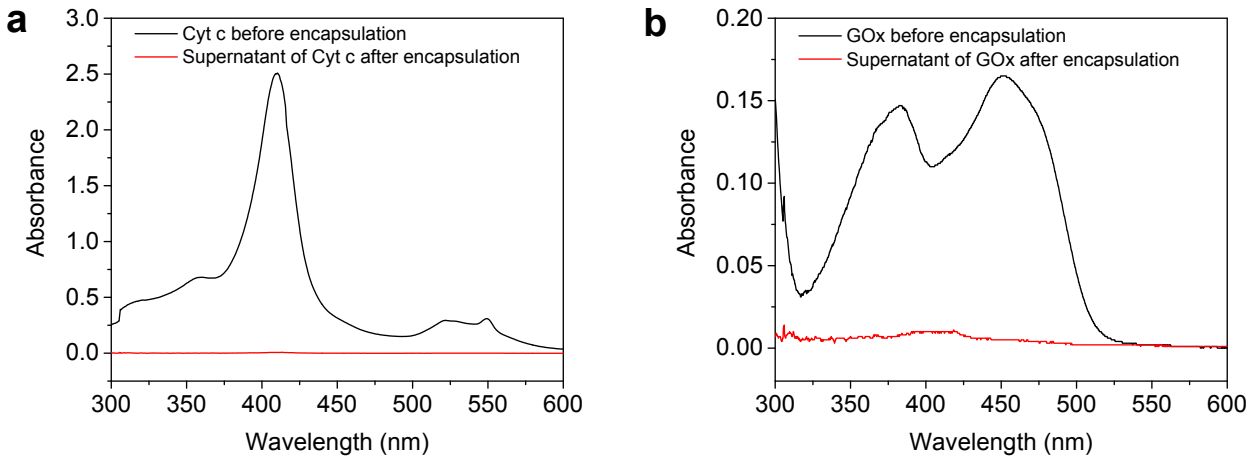


Figure S1 UV-Vis Spectra of (a)Cyt c or (b)GOx before and after encapsulate into CuBDC.

Table S1 BET surface area measurements of CuBDC and Cyt c-CuBDC

Sample	CuBDC	Cyt c-CuBDC-1	Cyt c-CuBDC-2	Cyt c-CuBDC-3
Enzyme loading amount (mg)	0.0	1.0	2.0	4.0
BET surface area (m^2/g)	63.0	61.4	53.9	45.8

Effect of Cu^{2+} concentration on the activity of free Cyt c.

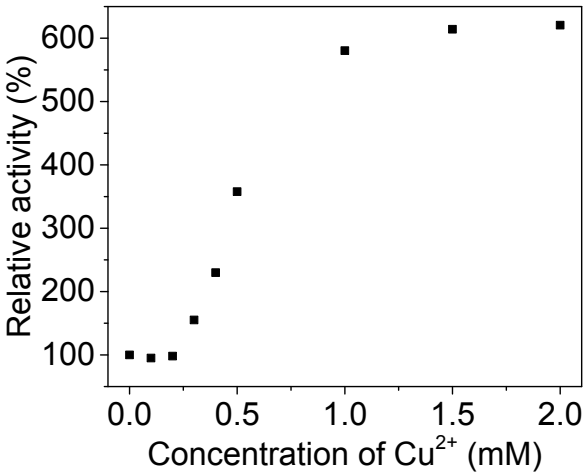


Figure S2 The effect of Cu^{2+} concentration on the activity of Cyt c.

Activity assays for HRP, CRL and TRY that incorporated with CuBDC

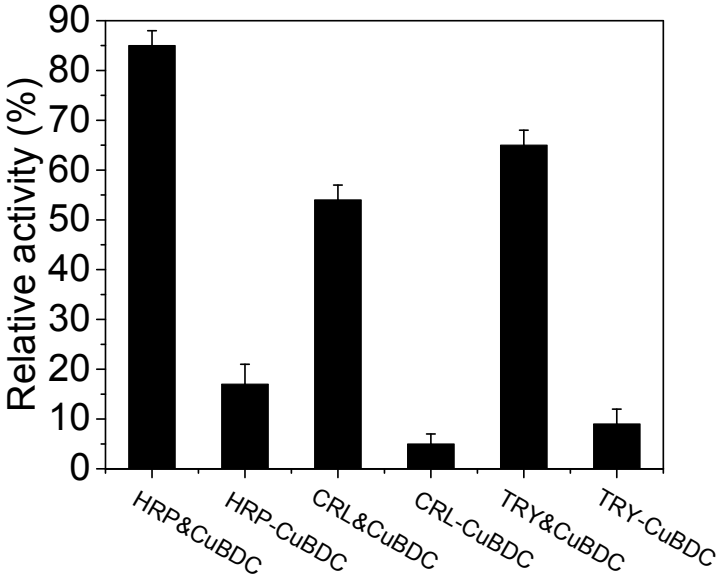
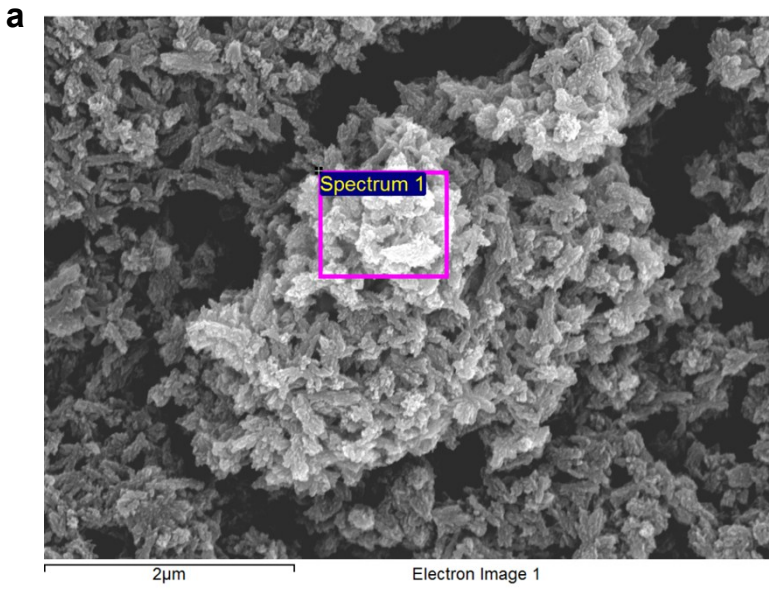
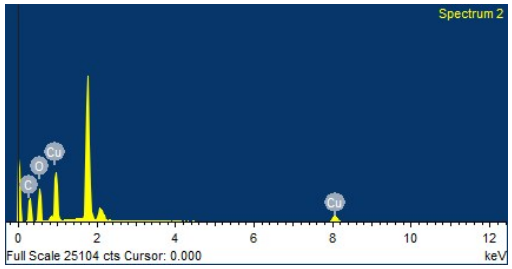


Figure S3 The relative activity of enzyme-CuBDC or enzyme&composite composites compared to its free form of enzyme.

Elemental analysis by SEM-EDS



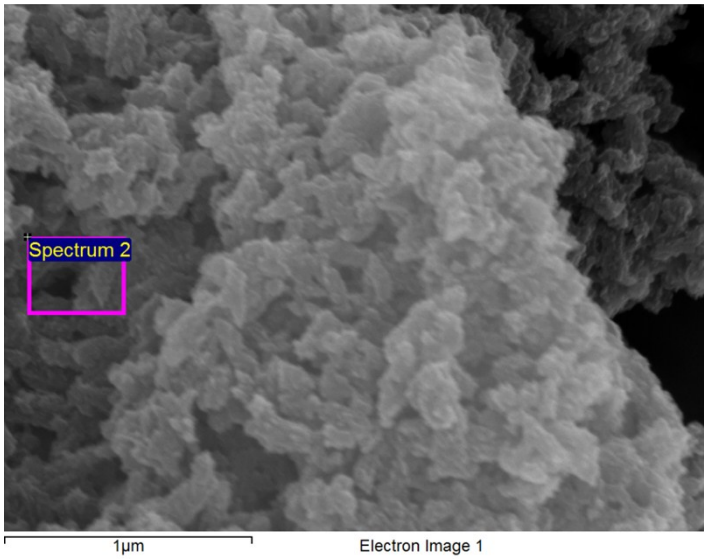


c

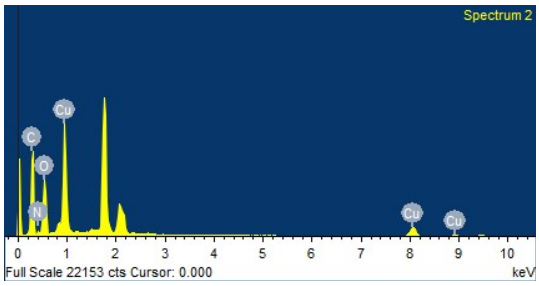
Element	Weight%	Atomic%
C	41.10	62.78
O	23.25	26.92
Cu	35.65	10.30
Totals	100.00	

Figure S3 elemental analysis by SEM-EDS for CuBDC

a



b



c

Element	Weight%	Atomic%
C	41.53	61.92
N	2.72	3.48
O	22.55	25.24
Cu	33.20	9.36
Totals	100.00	

Figure S4 elemental analysis by SEM-EDS for Cyt c-CuBDC