Electronic Supplementary Information (ESI)

Enhancing the Enzymatic Inhibition Performance of Cu-based Metal-Organic Frameworks through Shortening the Organic Ligands

Ming Xu[a],‡, Hong Liang[a],‡, Sha-Sha Meng[a] and Zhi-Yuan Gu[a,*]

a.Jiangsu Key Laboratory of Biofunctional Materials, Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, China.

‡ M.X. and H.L. contributed equally.

** Corresponding Author: guzhiyuan@njnu.edu.cn*

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Section S1. Chemicals and Instrumentations

All chemicals employed were of analytical grade and used as supplied without further purification. The copper trifluoromethanesulfonate ($Cu(OTf)_2$), 4,4-bipyridine (bpy), $Cu(NO_3)_2 \cdot 3H_2O$, iminazole, acetone, N-2-hydroxyethylpiperazine-N'-3-propanesulfonic acid (HEPPS), NaOH and dimethyl sulfoxide (DMSO) were purchased from Aladdin Industrial Inc (Shanghai, China). N-succinyl-L-phenylalanine-p-nitroanilide (SPNA) was purchased from Sigma (St. Louis, MO). The α-Chymotrypsin (ChT) was purchased from Sangon Biotech (Shanghai) Co., Ltd (Shanghai, China). Ethanol (EtOH) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Powder X-ray diffraction (PXRD) patterns were obtained from Rigaku D/MAX-2500 diffractometer with a CuKα radiation (1.54056 Å). Transmission electron microscopy (TEM) analyses were performed on JEOL JEM-2100F transmission electron microscopy operated at an accelerating voltage of 200 kV. The absorbance was recorded by a Hitachi UH5300 UV-Vis spectrophotometer. The fluorescence emission peaks were measured on a Hitachi F4600 fluorescence spectrometer.

Section S2. Synthesis and Characterization of Cu-MOF

The Cu-MOF were prepared according to the reported literature procedure with only a few changes[1]. Typically, an aqueous solution of $Cu(OTf)_2$ (30.0 mM, 10.0 mL) was added to a 22-mL glass vial upon which 1 mL of pure ethanol was layered meticulously. Then, an ethanol solution of bpy (60.0 mM, 10.0 mL) was layered onto the ethanol layer to obtain a threelayers system, which was left to stand for three weeks at room temperature. The dark blue crystals were collected by centrifugation, and washed with 20 mL acetone and ethanol five times, respectively.

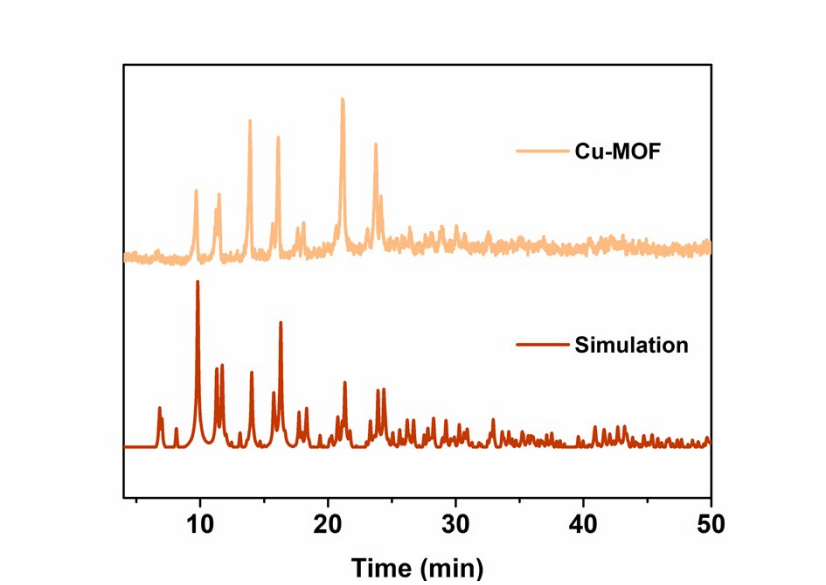

Figure S1. PXRD patterns of synthesized Cu-MOF and simulated Cu-MOF.

Section S3. Activity of ChT with Different Concentrations of $Cu(im)_2$

The $Cu(im)_2$ was dissolved with HEPPS buffer (100 mM, pH = 7.4). To obtain the activity of ChT, the substrate (SPNA, 160 μL) was added to the ChT solution (80 μg/mL, 1840 μL). The ChT (80 μg/mL) was incubated with $Cu(im)_2$ for 10 min. The concentrations of $Cu(im)_2$ varied from 0 to 60 μg/mL. The concentration of SPNA was 2 mM in the solvent mixture of ethanol/DMSO (90%/10%). Enzyme activity was collected by monitoring the absorbance of PNA every 2 min for 20 min in 30 °C at 410 nm with Hitachi UH5300 spectrophotometer. For comparison, the activity of ChT (80 μg/mL) was monitored as well.

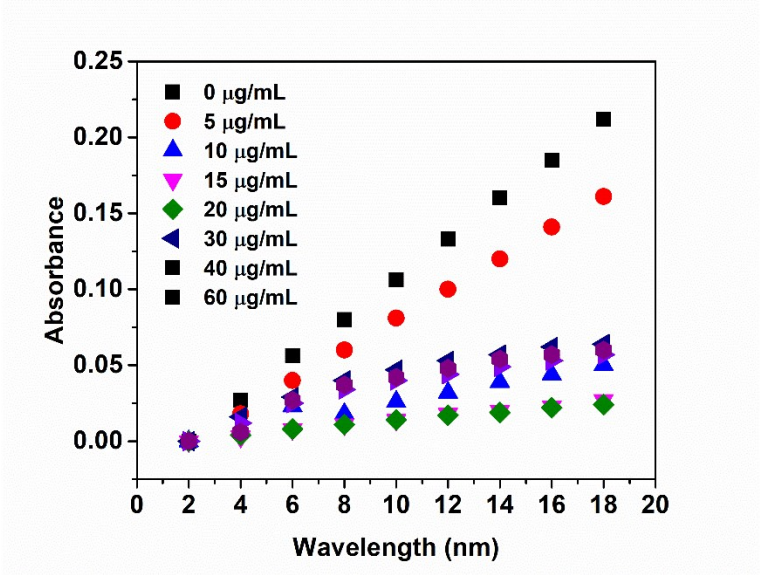

Figure S2. Activity of ChT (80 μg/mL) with different $Cu(im)_2$ concentrations (0, 20, 30, 40, 60, 80 and 100 μg/mL).

Section S4. Activity of ChT with the Incubation of $Cu(im)_2$ in the Presence of NaCl at Different Concentrations.

All of the substances were dissolved in HEPPS buffer (100 mM, pH = 7.4) at 30 ^{o}C. Different concentrations of NaCl were added to 80 μg/mL ChT either before the addition of 10 μg/mL $Cu(im)_2$ (before incubation) or after the 10-min incubation with 10 μg/mL $Cu(im)_2$ (after incubation). The concentration of NaCl varied from 0 to 600 mM in 100 mM HEPES buffer (pH = 7.4). For comparison, the same series of NaCl were added to 80/μg·mL ChT without $Cu(im)_2$ and the activity was recorded as well. To obtain ChT activity, the substrate SPNA (160 μL) was added to the ChT solution (80 μg/mL, 1840 μL). The concentration of SPNA was 2 mM in the solvent mixture of ethanol/DMSO (90%/10%). Enzyme activity was collected by monitoring the absorbance of PNA every 2 min for 20 min in 30 ^{o}C at 410 nm with Hitachi UH5300 spectrophotometer. For comparison, the activity of ChT (80 μg/mL) was monitored as well.

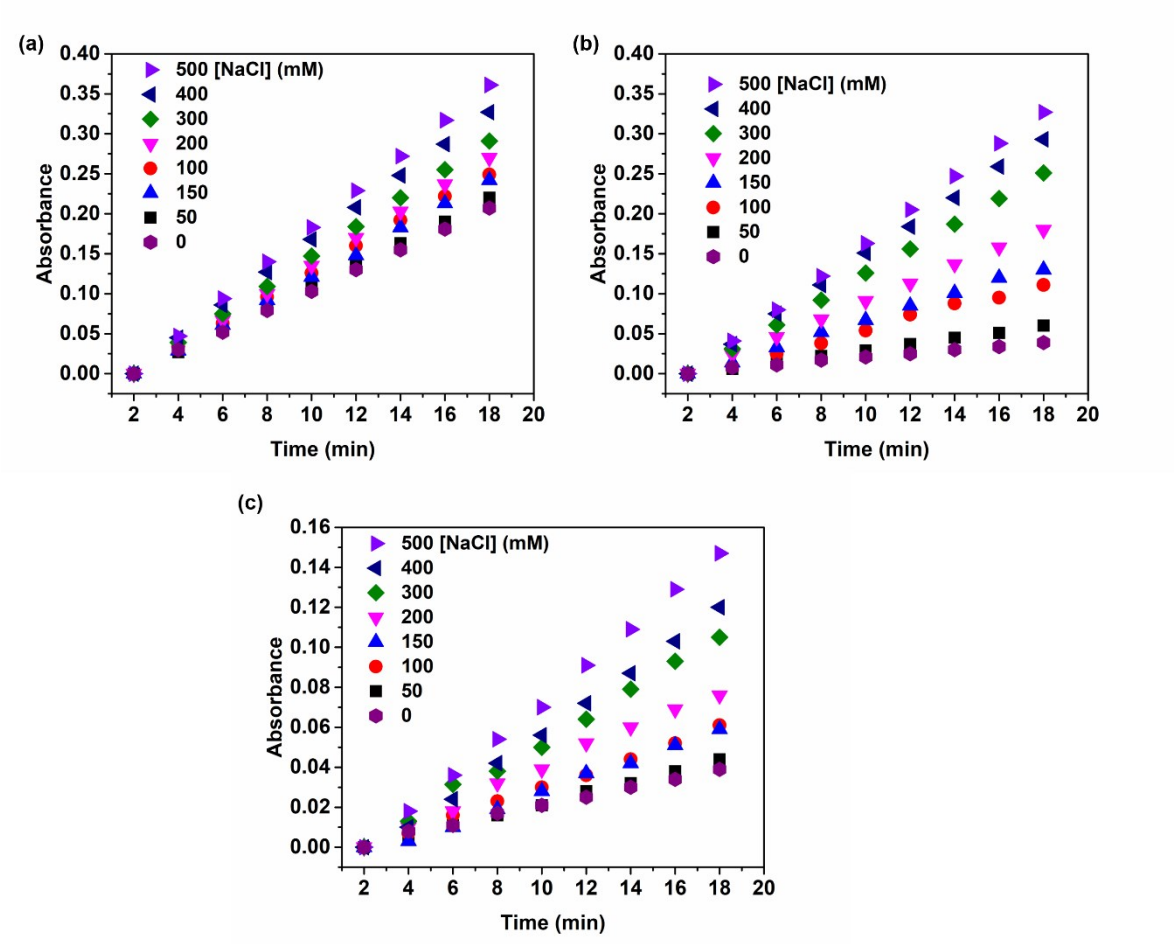

Figure S3. (a) Activity of ChT (80 μg/mL) with different concentrations of NaCl in the absence of inhibitors. Activity of ChT (80 μg/mL) with different concentrations of NaCl before (b) and after (c) incubation with $Cu(im)_2$ (10 μg/mL) as an inhibitor.

Section S5. Activity of ChT with the Incubation of $Cu(im)_2$ in the Presence of Histidine at Different Concentrations.

All of the substances were dissolved in HEPPS buffer (100 mM, pH = 7.4) at 30 ^{o}C. Different concentrations of histidine were added to 80 μg/mL ChT after the 10 min incubation with 10 μg/mL $Cu(im)_2$. The concentration of histidine varied from 0 to 600 μM in 100 mM HEPES buffer (pH = 7.4). For comparison, the catalysis activity of histidine with different concentrations from 0 to 60 mM were recorded as well. To obtain ChT activity, the substrate SPNA (160 μL) was added to the ChT solution (80 μg/mL, 1840 μL). The concentration of SPNA was 2 mM in the solvent mixture of ethanol/DMSO (90%/10%). Enzyme activity was collected by monitoring the absorbance of PNA every 2 min for 20 min in 30 ^{o}C at 410 nm with Hitachi UH5300 spectrophotometer. For comparison, the activity of ChT (80 μg/mL) was monitored as well.

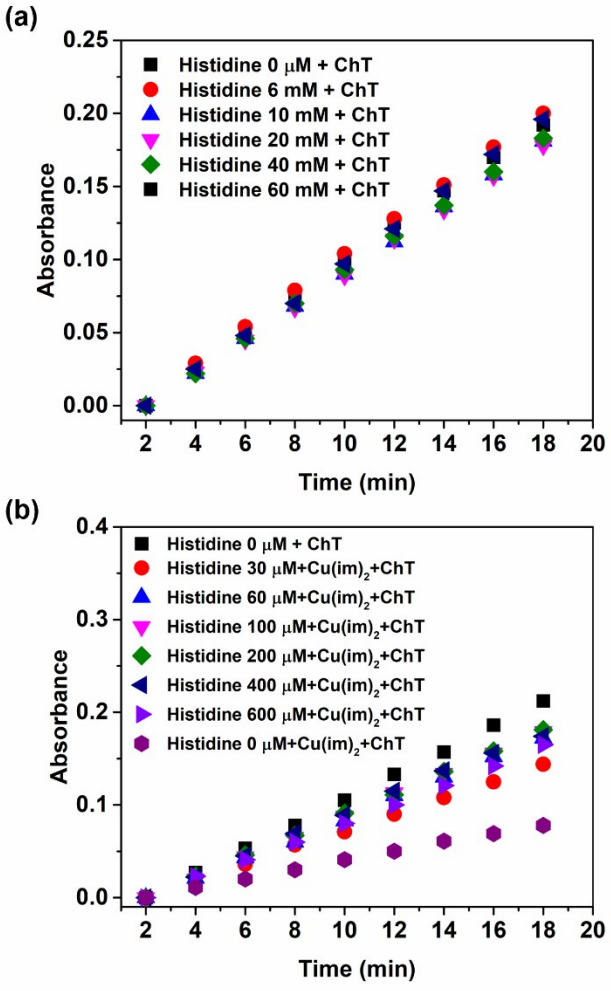

Figure S4. (a) Activity of ChT (80 μg/mL) with different concentrations of histidine in the absence of $Cu(im)_2$ (10 μg/mL). (b) Activity of ChT (80 μg/mL) with different concentrations of histidine with and without $Cu(im)_2$ (10 μg/mL).

Reference

1. M. Xu, S. Yuan, X.-Y. Chen, Y.-J. Chang, G. Day, Z.-Y. Gu and H.-C. Zhou, *J. Am. Chem. Soc.*, 2017, **139**, 8312-8319.