Two-Dimensional Metal–Organic Framework Nanosheets as an Enzyme Inhibitor: Modulation of the α-Chymotrypsin Activity

Ming Xu,†,‡ Shuai Yuan,§,⊥ Xin-Yu Chen,† Yu-Jie Chang,† Gregory Day,‡ Zhi-Yuan Gu,§,⊥⊥ and Hong-Cai Zhou‡,§,⊥

†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
‡Department of Chemistry, Texas A&M University, College Station, Texas 77843-3255, United States
§Department of Materials Science and Engineering, Texas A&M University, College Station, Texas 77842, United States

Supporting Information

ABSTRACT: Two-dimensional metal–organic framework (MOF) nanosheets are utilized as effective enzyme inhibitors, providing an inspiring means to enhance the control of cellular processes as well as improve our understanding of the surface chemistry between MOFs and enzymes. In this paper, we demonstrated that the activity of α-chymotrypsin (ChT) can be effectively inhibited with 96.9% inhibition by 2-D Cu(bpy)2(OTf)2 nanosheets, while Zn(bim)2 nanosheets show no significant inhibition effect toward ChT. Kinetic studies revealed that the material acts as a competitive inhibitor toward ChT. Furthermore, fluorescence and circular dichroism spectroscopy reveal that the 2-D MOF nanosheets do not change the secondary structure of the enzyme. The Cu(II) center of the 2-D nanosheets binds the 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) molecules in the buffer, leading to an electrostatic interaction between the nanosheets and the enzyme. In addition, the irreversible coordination interactions between Cu(II) center and His-57 played an important role during the inhibition process, as supported by ionic strength experiments and UV absorbance changes of Cu(II) d–d transitions. As a result, the substrate is prevented from reaching the active sites of the enzyme causing enzyme inhibition. The modulation of enzyme activity by 2-D MOF nanosheets opens up a new direction for the exploration of the MOF–bio interface in physiological and catalytic systems.

INTRODUCTION

Metal–organic frameworks (MOFs) with high specific surface areas, tunable pores, and diverse functionalities have been explored extensively for gas adsorption and separation, energy storage, catalyst, and carbon dioxide capture.1–7 Recently, MOFs were utilized in the stabilization of enzymes for catalysis in extreme conditions as well as in biomedical applications, such as drug delivery, bioimaging, and photodynamic therapy.8–17 Although the applications are in diverse fields, understanding the fundamentals of MOF–protein interactions will be key in developing these technologies.18 At the same time, few researchers are focused on the modulation of enzyme activity within the MOF–bio interface, although general metal-based coordination compounds have been extensively studied in biomedical research.19

Modulation of enzyme activity provides an influential means to enhance the control of cellular processes such as DNA replication, signal transduction, and metabolism.11 Gold nanoparticles, carbon nanotubes, graphene oxide, functionalized MoS2, dendrimers, small organic molecules, and metal–organic complexes have all been used to alter the activity of biomolecules.20–25 Compared with other solid state materials, MOFs contain precisely designed structure and surface properties that are ordered on the molecular level through the judicious selection of ligand and metal nodes. This provides MOF structures with a high degree of tunability, enabling the modulation of enzymatic activity to be rigorously controlled. However, MOFs have seldom been investigated for enzyme inhibition to the best of our knowledge. In order to be applicable to enzyme inhibition, MOFs are usually required to be stable and dispersible in water. However, bulk crystals of MOFs give inhomogeneous dispersion in water while nanoscale MOFs easily form aggregates in solution, which hinders the analysis.26–28 Besides, the pore apertures of most MOFs are not large enough for the enzymes. Recently, several examples of two-dimensional (2-D) MOF nanosheets have been synthesized.29–35 Although 2-D MOF nanosheets have highly accessible active sites and homogeneous features, their successful applications were limited to membrane separation, energy storage, iodine capture, MALDI-TOF matrices and fluorescent sensing.36–39 2-D MOF nanosheets have good dispersibility and large surface areas for adequate protein binding. In contrast to some small molecules and biological
molecules, MOF nanosheets can easily enter cells. Due to the advantages of 2-D MOF nanosheets, we employed such a material to form a MOF–bio interface, enabling us to study the modulation of enzyme activity. α-Chymotrypsin (ChT, EC 3.4.21.1) was selected as model enzyme because it is an important serine protease in the alimentary canal and its modulation of enzyme activity. 

The synthesis method for 2-D Zn2(bim)4 (bim = benzimidazole) nanosheets, while the synthesized two MOF nanosheets with different structure has been well characterized (Figure 1c,d).40 Important serine protease in the alimentary canal and its 3.4.21.1) was selected as model enzyme because it is an important serine protease in the alimentary canal and its modulation of enzyme activity.

Investigating the modulation of ChT is of great importance in the field of biology, as immoderate ChT activity may lead to cystic fibrosis, inflammatory arthritis, and other medical disorders.31,42 ChT can be inhibited by several materials, such as gold nanoparticles, due to the interactions between ChT and the inhibitive materials, such as hydrophobic interactions, electrostatic interactions, hydrogen bonding, and π–π stacking.34,43–45 The ChT has a hydrophobic “hot spot” surrounded by cations on the surface, which allows for both hydrophobic and electrostatic interactions. The surface charges and organic compositions of 2-D MOF nanosheets offer great opportunities to take advantage of these interactions, possibly allowing for the tuning of the enzyme activity.

To facilitate the interactions at the MOF–bio interface without interference from a polymer coating, a top-down synthesis strategy was chosen, although macromolecule-assisted bottom-up synthesis could also result in a well-controlled morphology for the 2-D MOF nanosheets.36,46 Herein, we synthesized two MOF nanosheets with different metals. One is 2-D Zn2(bim)4 (bim = benzimidazole) nanosheets, while the other is Cu-based 2-D MOF nanosheets (Figure 1a,b), namely, Cu(bpy)2(OTf)2 (Cu-MOF, bpy = 4,4′-bipyridine, OTf = trifluoromethanesulfonate).34,35,47,48 Both MOFs have similar layered structures and large surface area with divalent metal ions and nitrogen-containing organic ligands, showing good water stability. However, the isostructural bpy/OTf based nanosheets with other metal centers is highly moisture sensitive, which implements its applications as enzyme inhibitors.49 To investigate the possible interactions between ChT and the 2-D MOF nanosheets, we used N-succinyl-l-phenylalanine-p-nitroanilide (SPNA) as a substrate that can be hydrolyzed by ChT forming a reaction product with a characteristic UV–vis absorption at 410 nm (Scheme 1). By observing the rate of the reaction before and after incubating ChT with 2-D MOF nanosheets, the enzyme activity and modulation effect can be well evaluated.

Scheme 1. Enzymatic Hydrolysis Reaction

In this paper, we demonstrated that the activity of ChT (80 μg·mL⁻¹) can be effectively inhibited with 96.9% inhibition by the 2-D Cu-MOF nanosheets at a concentration of 100 μg·mL⁻¹, while Zn2(bim)4 shows no significant inhibition effects toward ChT. From the kinetic data, we found that the material acted in competitive inhibition mode. Furthermore, with the fluorescence spectroscopy and circular dichroism patterns, it can be concluded that the material does not change the secondary structure of the enzyme. Besides the electrostatic interaction, an irreversible coordination interaction between a Cu(II) center and the His-57 fragment were also observed during the incubation process, strongly inhibiting the enzyme.

EXPERIMENTAL METHODS

The 2-D MOF nanosheets were obtained by physical exfoliation from their bulk MOFs. Bulk Cu-MOF was prepared according to the reported literature procedure with only a few changes.34,47,48 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 three-layer system, which was left to stand for 3 weeks at room temperature. The dark blue crystals were collected by centrifugation, and washed with 20 mL of acetone and ethanol five times each. To obtain 2-D Cu-MOF nanosheets, single crystals of Cu-MOF were placed in distilled water and stirred for 9 h. After removal of the sediment, a dispersed solution of nanosheets was obtained. The wet 2-D Cu-MOF nanosheets were collected by centrifugation at 12000 rpm for 20 min and then dried at room temperature. The synthesis method for 2-D Zn2(bim)4 nanosheets is described in Supporting Information.

X-ray diffraction (XRD) patterns were obtained from a Rigaku D/ MAX-2500 diffractometer with a Cu Kα radiation (1.54056 Å), with data recorded from 2° to 50°. Transmission electron microscope (TEM) images were made on a JEOL JEM-2100F electron microscope operated at an accelerating voltage of 200 kV. To prepare samples for TEM analysis, 2-D Cu-MOF nanosheets were dispersed in water and ultrasonicated for 1 h. The dispersed solution (0.5 mg·mL⁻¹) was then dropped onto a copper-supported ultrathin carbon film (Beijing Zhongjingkeyi Technology Co., Ltd., China). Scanning electron microscope (SEM) images and energy dispersive spectroscopy (EDS) spectra were collected on a JSM-7600F (JEOL Ltd.) scanning electron microscope.

All enzyme experiments were performed in HEPES buffer (100 mM, pH 7.4) at 30 °C. The concentration of ChT was 80 μg·mL⁻¹, while the concentration of 2-D MOF nanosheets ranged from 0 to 100 μg·mL⁻¹. ChT was incubated with those 2-D MOF nanosheets, observing the rate of the reaction before and after incubating ChT with 2-D MOF nanosheets, the enzyme activity and modulation effect can be well evaluated.
hydrolysis substrate (SPNA, 160 μL) was added to the MOF–ChT solution (1840 μL). The concentration of SPNA was 2 mM in the solvent mixture of ethanol/DMSO (90%/10%). Enzyme activity was calculated by monitoring the absorbance of p-nitroaniline (PNA) every 2 min for a total of 20 min at 410 nm with a Hitachi UH5300 spectrophotometer. Different concentration of NaCl solution was added to 80 μg·mL⁻¹ ChT either before or after incubation to study the ionic strength effect. The concentration of NaCl solution was varied from 0 to 600 mM in 100 mM HEPES buffer (pH 7.4). For comparison, NaCl solution at the same concentrations was added to an 80 μg·mL⁻¹ ChT solution without 2-D Cu-MOF nanosheets. To demonstrate the advantage of 2-D Cu-MOF nanosheets, the inhibition effects of Cu(OTf)₂py₄ complex and Cu(OTf)₂ were evaluated under different ionic strengths. To investigate the function of the HEPES molecule, two more Good’s zwitterionic buffers, 4-morpholinoethanesulfonic acid (MES) and N-(2-hydroxyethyl) piperazine-N’-(2-hydroxypropanesulfonic acid) monohydrate (HEPSSO) were also used instead of HEPES at the same concentration (100 mM).³⁰

In order to calculate inhibition constant, the concentration of SPNA was varied from 0.5 to 4 mM with 80 μg·mL⁻¹ ChT and 40 μg·mL⁻¹ 2-D Cu-MOF. Kᵦₑ and Kₑ were calculated according to the Michaelis-Menten equation in the control experiment in which the rate of ChT with different SPNA concentrations was measured in the absence of inhibitor. The inhibition constants Kᵦₑ and α were calculated using the mixed-model inhibition equation. Furthermore, dissociation constant (Kᵦₑ) and molar binding radios (α, the number of ChT bonding to single Cu(II) center site) were evaluated by nonlinear least-squares curve-fitting analysis of activity assay.³¹³²

Different incubation times between ChT and 2-D Cu-MOF nanosheets were investigated with both fluorescence spectroscopy (Hitachi F-4600) and circular dichroism (CD, AppliedPhotophysics Chirascan) to evaluate the possible secondary structure changes of ChT. The mixture of ChT (80 μg·mL⁻¹ and 2-D Cu-MOF nanosheets (40 μg·mL⁻¹) was excited at 295 nm, and the emission spectra were recorded from 300 to 450 nm with ChT and denatured ChT as control experiments. The denatured ChT was obtained by heating ChT at 100 °C for 30 min. The CD spectra were measured on a CD spectrophotometer with quartz cuvettes of 1 mm path length at room temperature. The spectra were recorded from 190 to 250 nm. The ChT concentration was 80 μg·mL⁻¹, and the 2-D Cu-MOF nanosheet concentration was 40 μg·mL⁻¹. NaOH (100 mM) and HCl (100 mM) were added into water (pH = 7.4) to replace the HEPES buffer in order to get clear CD background. The CD spectra of 2-D Cu-MOF nanosheets was measured to eliminate any background effects.

The coordination interactions between Cu(II) center and His-57 were confirmed through the UV spectra of 2-D Cu-MOF nanosheets in water, in HEPES buffer, and incubated with and without ChT in HEPES buffer from 0 to 4 h. The UV spectra were recorded from 300 to 900 nm on a Hitachi UH5300 spectrophotometer.³⁰³¹ In order to get the significant d–d transition band in UV absorbance from 400 to 800 nm, the concentration of 2-D Cu-MOF nanosheets was 200 μg·mL⁻¹. To confirm the active site, different concentrations of histidine solution from 0 to 590 μM were added into 80 μg·mL⁻¹ ChT after incubation with 40 μg·mL⁻¹ Cu-MOF nanosheets. For comparison, the catalytic activity of histidine and the activity of ChT with 590 μM histidine without MOF were recorded as well.

## RESULTS AND DISCUSSION

### Materials Characterization.

The MOF nanosheets were fully characterized and their water stability was tested before the MOF nanosheets were utilized for enzyme inhibition. The XRD pattern of the prepared bulk Cu-MOF is consistent with the simulated pattern (Figure 2a).³⁷³⁸ The bulk Cu-MOF showed layered structures in SEM images (Figure S1a). After exfoliation, 2-D Cu-MOF nanosheets were successfully obtained and confirmed by SEM, EDS, and TEM (Figure S1−S3). Under TEM analysis conditions, the nanosheets preferentially roll up to form nanorolls (Figure 2b). Similar nanoroll structures were also observed in AFM (Figure S4).

This phenomenon is attributed to the different exfoliation method compared to the previous paper, which has also been supported by Kondo et al.³⁴ To confirm their water stability, the 2-D Cu-MOF nanosheets were dispersed in water at room temperature. The Tyndall scattering of this system was unchanged for 18 h indicating good water stability of this 2-D material (Figure S5). The UV absorbance of 2-D Cu-MOF nanosheets dispersed in HEPES buffer over 4 h showed no difference indicating good stability of the nanosheets in this buffer (Figure S6). The successful synthesis of Zn₂(bim)₄ and 2-D Zn₂(bim)₄ nanosheets were also confirmed by the XRD and TEM (Figure S7,8).

### Activity Assays.

In order to investigate the inhibition effect between two MOF nanosheets and ChT, SPNA was chosen as a substrate and catalytically hydrolyzed by ChT in a HEPES buffer (pH 7.4) at 30 °C. The reaction rate of the hydrolysis was used to determine the enzyme activity. To make a fair evaluation of the capacity of the inhibitor, the inhibited enzyme activity was normalized to the ChT activity in the same incubation time without any inhibitors (Figure S9−14). The ChT (80 μg·mL⁻¹) was incubated with 2-D MOF nanosheets at different concentrations ranging from 0 to 100 μg·mL⁻¹ for 1 h. The ChT activity shows no significant decrease with the incubation of 2-D Zn₂(bim)₄ nanosheets (Figure 3a, S13). In contrast, ChT activity performance remarkably decreased with increasing concentration of 2-D Cu-MOF nanosheets. In total, 96.9% of ChT activity was inhibited with 2-D Cu-MOF.
nanosheets at a concentration of 100 μg·mL⁻¹ (Figure 3a, S12). Compared to other materials, 2-D Cu-MOF nanosheets showed a superior inhibition effect, while the Zn₂(bim)₄ nanosheets did not exhibit noticeable levels of inhibition for ChT (Figure S15).

We propose that the different inhibition effects with the two kinds of 2-D MOF nanosheets might suggest different metal configurations in different coordination environment. According to previous publications, the main driving force for an electrostatic interaction between ChT and an inhibitor resulted from the positively charged residues surrounding the active sites of ChT and the negative charged functional groups on inhibitors. The stable fully coordinated Zn(II) in the 2-D Zn₂(bim)₄ nanosheets would be unable to bind HEPES molecules, leaving the overall Zn₂(bim)₄ nanosheets positively charged like their ZIF precursor and thus preventing Zn₂(bim)₄ from inhibiting ChT. On the other hand, the six coordinate Cu engaged in Jahn–Teller distortional effects in the 2-D Cu-MOF nanosheets could easily exchange OTf⁻ ligands with HEPES and form a negatively charged surface, leading to an electrostatic interaction toward ChT.

Because of the poor inhibition effect of 2-D Zn₂(bim)₄ nanosheets, we chose 2-D Cu-MOF nanosheets with moderate concentrations of 40 μg·mL⁻¹ for the following investigation. The incubation time was evaluated to show the bonding kinetics between ChT and 2-D Cu-MOF nanosheets (Figure 3b). A longer incubation time resulted in better inhibition effect in the first 1 h while the time of incubation exhibited no significant influence on inhibition effect after 1 h. The incubation time of 1 h was selected for all following experiments to make sure the electrostatic bonding between ChT and 2-D Cu-MOF nanosheets formed to its fullest extent. The successful bonding between ChT and 2-D Cu-MOF nanosheets was supported by the particle size distribution result, as after incubating with the 2-D Cu-MOF nanosheets the average size of ChT is much larger than the natural one (Figure S16).

2-D Cu-MOF Nanosheet–ChT Inhibition Kinetics. The inhibition mode was investigated to explore the potential bonding sites of ChT for 2-D Cu-MOF nanosheets. The Michaelis–Menten equation and the mixed-model inhibition equation were plotted with different concentrations of substrate (S) and inhibitor (I) to calculate the maximum reaction rate (v_max), the Michaelis–Menten constant (K_m), and the inhibition constants (K_i and α). The mixed-model inhibition equation is given as

\[
\frac{1}{v} = \frac{1}{v_{\text{max}}} + \frac{K_m}{v_{\text{max}}[S]} + \frac{K_i}{v_{\text{max}}[I]} \left(\frac{1}{[I]} + \frac{1}{[S]}\right)
\]

The value of α is used to describe the type of inhibition, whether the inhibition is competitive, noncompetitive, or uncompetitive. When α = 1, the inhibitor does not affect the substrate combining with the enzyme, which is noncompetitive inhibition. When α ≫ 1, the inhibitor binds with the enzyme at its active site preventing the binding of the substrate, in a process termed competitive inhibition. When α ≪ 1, the inhibitor binds with the enzyme causing an enhancement of substrate binding, preventing the catalytic process, in this case hydrolysis, from occurring, in a process termed uncompetitive inhibition.

From the slope and intercept of the Lineweaver–Burk plot, we can determine k_cat = 1.687 × 10⁻² s⁻¹ and K_m = 1681.4 μg·mL⁻¹ (Figure 4), typical values for ChT. We obtained K_i = 12.25 μg·mL⁻¹ as well as α = 12.05, implying that the 2-D Cu-MOF nanosheets inhibit ChT through competitive inhibition.

Figure 4. Lineweaver–Burk plot without inhibitors (black) and mixed-model inhibition equation plot with 40 μg·mL⁻¹ 2-D Cu-MOF nanosheets as an inhibitor (red).
In other words, 2-D Cu-MOF nanosheets bind with ChT at its active sites and prevent any further interaction of the substrate toward the enzyme.

Investigation of ChT Secondary Structure Changes Induced by 2-D Cu-MOF Nanosheets. Fluorescence and CD spectroscopy were used to investigate whether the competitive inhibition between 2-D Cu-MOF nanosheets and ChT would change the secondary structure of the enzyme. In our research, natural ChT in HEPES buffer (pH 7.4) has its characteristic fluorescence emission peak at 331 nm under excitation of 295 nm, while the weak fluorescence of 2-D Cu-MOF nanosheets would not affect the measurements (Figure S17). Denatured ChT shows a significant red shift with its fluorescence emission peak at 353 nm due to the exposure of Trp residues. After incubation with 2-D Cu-MOF nanosheets at a concentration of 40 μg·mL⁻¹ for 0 h, 4 and 24 h, the peak of ChT shows no shift, which indicates that ChT retained its natural secondary structure (Figure 5a). To authenticate these results, CD spectra were recorded as a secondary source for information regarding possible changes to the secondary structure of ChT during 2-D Cu-MOF nanosheet inhibition. Uninhibited ChT shows two characteristic minima at 230 and 202 nm, while denatured ChT has one distinctive minimum at 200 nm (Figure 5b). Almost no change in the CD data of ChT was observed with preincubated with 2-D Cu-MOF nanosheets from 0 to 24 h, which corroborated that the conformation of ChT exhibits no change in the presence of 2-D Cu-MOF nanosheets. The retention of the ChT structure during the incubation indicates that the competitive inhibition from 2-D Cu-MOF nanosheets through electrostatic interaction does not change the secondary structure of the enzyme.

Effect of Ionic Strength. To further prove that the driving force for the interaction between ChT and 2-D Cu-MOF nanosheets is electrostatic interaction, we changed the ionic strength of the solution before and after incubation with 2-D Cu-MOF nanosheets, in order to determine if the electrostatic interaction can be weakened by high ionic strength. Different concentrations of NaCl, from 0 to 600 mM, were used to change the ionic strength. For each NaCl concentration, the activity of ChT (80 μg·mL⁻¹) before and after incubation with 2-D Cu-MOF nanosheets (40 μg·mL⁻¹) for 1 h was normalized by the ChT activity without inhibitor at the same salt concentration. When the NaCl was added before incubation, the enzyme activity showed a significant recovery of activity dependent on the concentration of NaCl (Figure 6).

Specifically, when the concentration of NaCl was increased to 400 mM, 2-D Cu-MOF nanosheets were unable to inhibit ChT activity. However, when NaCl was added after incubation, the activity of ChT showed almost no recovery. These results indicate that ChT could not bind to 2-D Cu-MOF nanosheets under high ionic strength conditions, confirming the importance of electrostatic interactions between the ChT and inhibitor for binding. However, once ChT has bound to the material after incubation, the enzymatic activity cannot be restored even at high NaCl concentrations. A purely electrostatic interaction should see recovery of ChT activity as the NaCl concentration increases, as it should destabilize the 2-D Cu-MOF–ChT interaction as it does in the preincubation experiments. In other words, the electrostatic interactions provide a kinetic rational for the inhibition of ChT by 2-D Cu-MOF nanosheets, but other thermodynamic driving forces might be available to maintain inhibitor binding once the 2-D Cu-MOF nanosheets have begun interacting with the ChT active site.

The Coordination Interactions. Herein, we propose that this possible force is a coordination interaction between the

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Figure 5. (a) Tryptophan fluorescence and (b) CD spectra of ChT (80 μg·mL⁻¹) with and without 2-D Cu-MOF nanosheets (40 μg·mL⁻¹) as inhibitor for 0, 4, and 24 h. The fluorescence and CD spectra of denatured ChT (80 μg·mL⁻¹) were also recorded.

Figure 6. Activity of ChT (80 μg·mL⁻¹) before and after incubation with 2-D Cu-MOF nanosheets (40 μg·mL⁻¹) in the presence of NaCl at different concentrations.
Lewis acidic copper metal site and the ChT His-57 residue. The coordination interactions were not only evidenced by the ionic strength experiments but also by the strong electrostatic interactions between 2-D Cu(OTf)$_2$(bpy)$_2$ nanosheets and ChT in HEPES buffer. The strong Lewis acidity of the Cu(II) center in Cu-MOF nanosheets resulting from the dissociation of OTf$^-$ is likely to be attacked by the nucleophilic nitrogen of deprotonated HEPES (pK$_a = 7.5$).$^{56}$ Although in electroneutral status, the Cu-MOF nanosheets might also bond to positively charged residues of ChT through the electrostatic interaction with the negatively charged surface of the nanosheet—HEPES compounds. Two more Good’s zwitterionic buffers MES and HEPPSO were used instead of HEPES to support this conclusion. MES molecule could hardly bind with Cu$^{2+}$ while HEPPSO reveals significant interaction with Cu$^{2+}$, which is similar to HEPES.$^{58}$ As shown in Figure S18, HEPPSO supports almost the same inhibition effect as HEPES, but MES shows lower inhibition toward ChT with the incubation of 2-D Cu-MOF nanosheets. The buffer molecules play a significant role in the binding with the Cu-MOF nanosheets. The inhibition effect would be further strongly regulated by this binding.

The UV patterns in Figure 7 also give evidence to support the binding between HEPES and Cu(II) centers. As shown in the inset pattern in Figure 7a, the UV absorbance of MOF samples show an obvious change around 600 nm when dispersed in HEPES buffer as compared with spectra performed in unbuffered water. These changes correspond to the change of d-d transition around the Cu(II) centers. An obvious color change was also observed upon the addition of buffer, which, in principle, reflects the changes in the chemical environment around the Cu(II) centers. Although the absorbance suggested the aggregation of nanosheets in HEPES buffer, no precipitation was observed throughout the test, and the nanosheets in HEPES still performed as an effective inhibitor toward ChT.

The structure of the catalytic triad in uninhibited ChT (Asp-102, His-57, and Ser-195) has been well established. The carboxylate group of Asp-102 forms a compressed hydrogen bond with His-57, increasing the alkalinity of the imidazole nitrogen with the pK$_a$ going from 7.0 to 12.0 (Figure S19). The coordinative interaction between the copper metal site and ChT His-57 residue was elucidated by UV spectra. The pK$_a$ of His-57 is larger than that of HEPES; the nitrogen of His-57 might easily replace the nitrogen of HEPES, attacking the Cu(II) center of the 2-D Cu-MOF nanosheets (Figure S20). Upon the addition of ChT, the UV absorbance also changes, especially in the region from 400 to 800 nm, which is consistent with our arguments regarding the Cu(II) center’s interaction with the HEPES molecule through coordination bonds. In addition, the decrease of absorbance baseline reveals the disaggregation of 2-D Cu-MOF nanosheets. Other than baseline drift, the absorbance of d-d transition around 600 nm exhibited almost no change after 1-h incubation of ChT with 2-D Cu-MOF nanosheets, which is consistent with the enzyme activity data (Figure 3b).

Different concentrations of histidine solution were used to confirm the binding of His-57 toward 2-D Cu-MOF nanosheets. With the increase of histidine concentration, the activity of ChT recovered (Figure S21–23). The influence of histidine indicated that the Cu-MOF nanosheets bind with His-57 on the enzyme. The strong affinity between the enzyme and Cu-MOF nanosheets is demonstrated by the significantly low K$_d$ of 1.04 × 10$^{-7}$ M, which is smaller than the K$_d$ between ChT and other materials (Figure S24).$^{31,52,57}$ It is consistent with the data that the interaction between Cu-MOF nanosheets would be eliminated only when the molar ratio of histidine-to-ChT reaches as high as 184:1.

The coordination interaction could occur only when the Cu(II) center and His-57 were at a suitable distance, which was highly reliant on the electrostatic interaction between 2-D Cu-MOF nanosheets and ChT. It is evidenced by the ionic strength experiment that coordination interactions could not occur if high concentrations of NaCl were added before incubation; in those instances, the enzyme activity could be recovered. On the other hand, coordination interactions already occurred in the incubation samples and these coordination interactions changed little with increasing concentration of NaCl added after incubation, leading to no recovery of enzyme activity. Therefore, the electrostatics act as a kinetic factor that controls the accessibility of inhibitor. Meanwhile, the formation of a coordination bond between His-57 and the Cu(II) center represents a thermodynamic sink, which explains the irreversible inhibition of enzymatic activity. For comparison, the inhibition effect of Cu(OTf)$_2$ salt and the Cu(OTf)$_2$(bpy)$_2$ complex toward ChT could hardly be regulated by changing the ionic strength of the solution (Figure S25).
CONCLUSIONS

In summary, we have demonstrated 2-D Cu-MOF nanosheets can act as an effective enzyme inhibitor through competitive binding of the active site of ChT, instead of changing the enzyme's native conformation. The competitive inhibition resulted from not only the electrostatic interaction between the nanosheets and the enzyme but also irreversible coordination interactions. Ionic strength experiments suggest that the electrostatic interaction is primarily a kinetic effect and the binding of the Cu(II) center by His-57 provides the thermodynamic sink that fully inhibits the enzyme. The modulation of enzyme activity by 2-D MOF nanosheets will open a new direction for exploration of the MOF–bio interface in physiological and catalytic systems, providing influential means to enhance the control of cellular processes as well as our understanding of the surface chemistry between MOF and enzymes.

ASSOCIATED CONTENT

1 Supporting Information

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Experimental details, figures, tables, and inhibition data (PDF)

AUTHOR INFORMATION

Corresponding Authors
*guzhiyuan@njnu.edu.cn
*zhou@chem.tamu.edu

ORCID

Zhi-Yuan Gu: 0000-0002-6245-4759
Hong-Cai Zhou: 0000-0002-9029-3788

Author Contributions

M. Xu and S. Yuan contributed equally.

Notes

The authors declare no competing financial interest.

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