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A Water-Soluble Copper-Immobilized Covalent Organic Framework Functioning as an “OFF–ON” Fluorescent Sensor for Amino Acids

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In this paper, we describe the construction of a new fluorescent hydroxyl- and hydrazone-based covalent organic framework (TFPB-DHTH COF) through the one-pot polycondensation of 1,3,5-tris(4-formylphenyl)benzene (TFPB) and 2,5-dihydroxyterephthalohydrazide (DHTH) under solvothermal conditions. The synthesized TFPB-DHTH COF possessed high crystallinity, a regular pore structure, excellent chemical stability, and sites featuring functional groups suitable for interactions with metal ions. In addition, the TFPB-DHTH COF was highly soluble in water and buffer and displayed a strong fluorescence emission under UV irradiation. The fluorescence of the TFPB-DHTH COF was quenched selectively by Cu²⁺ ions, forming a non-fluorescent copper-immobilized TFPB-DHTH COF (Cu@TFPB-DHTH COF); the off fluorescent state of the Cu@TFPB-DHTH COF was efficiently and selectively switched on upon the addition of cysteine (Cys) and L-histidine (L-His). Therefore, the Cu@TFPB-DHTH COF functioned as an “OFF–ON” fluorescent sensor for the detection of Cys and L-His. When incorporating N-ethylmaleimide and Ni²⁺ ions, we could separately and selectively detect Cys and L-His, respectively. Our new sensing assay could be completed within 30 min; it provided linear relationships with respect to concentrations of Cys and L-His of 2–100 and 2–200 µM, respectively, with limits of detection of 340 and 520 nM, respectively. Our strategy highlights the possibility of using water-soluble COFs as novel fluorescent sensors for amino acids, with promise for applications industrially or in environmental remediation.

Introduction

Cysteine (Cys) is an essential small thiol-functionalized amino acid for living cells, where it plays crucial roles in various pathological and physiological processes (e.g., redox-balance regulation, anti-aging, metabolism, biocatalysis, protein synthesis, and detoxification), while also being associated with many diseases involving cancers. Cys is a significant structural and functional component of many enzymes and proteins. Nevertheless, abnormal levels of Cys in the human body can place human health at risk. For example, a high level of Cys can be a vital factor for amyotrophic lateral sclerosis and Parkinson’s, Alzheimer’s, and cardiovascular diseases. In addition, a lack of Cys can lead to symptoms such as liver damage, hair discoloration, developmental delay, and hematopoietic dysfunction. Therefore, it is important to develop a simple selective, sensitive and reliable methods method for the detection of Cys. L-Histidine (L-His) is a natural amino acid featuring an aromatic imidazole unit; it is mainly present in the active-catalytic sites of various enzymes. L-His plays important roles in decreasing the risk of internal microtrauma bleeding and in monitoring the metal transport processes of essential biological bases. Chronic L-His deficiency is associated significantly with Friedreich’s ataxia, epilepsy, Parkinson’s disease, and the inability to develop normal erythropoiesis; the overexpression of L-His in biological fluids (serum and urine) can cause hereditary metabolic disorder. Therefore, reliable and highly sensitive detection and quantification of L-His is thus of considerable significance for therapeutic systems including the clinical treatment and medicinal science. Several analytic techniques have been reported previously for the quantitative detection of Cys and L-His, including those based on UV–Vis absorption spectroscopy, electrochemical analysis, capillary electrophoresis, spectrofluorimetry, immunoassaying, and high-performance liquid chromatography (HPLC). These techniques, however, have various shortcomings: they can be time-consuming and/or operationally inconvenient, and they can require cumbersome and/or expensive instrument. Recently, photophysical analytical techniques, particularly fluorescence-based versions, have become widespread for their high sensitivity, low detection limits, low cost, convenience, and suitability for bioimaging. The design of novel fluorescent probes including fluorophoric molecules, metal nanoclusters, and carbon dots (CDs), has also gained recognition. The detection mechanisms of these fluorescent probes have depended mainly on interactions between the functional groups of the probes and the thiol/amino groups of Cys or the imidazole/amino groups of L-His. In addition, mechanisms involving the coordination displacement of ligand–metal probes by Cys and L-His have also been reported. For example, Park et al. described the label-free detection of Cys through the fluorescence turn-on of metallophilic Hg²⁺–Au nanoclusters upon coordination of its Hg²⁺ moieties with Cys through its thiol group. Moreover, Yan et al. demonstrated water-soluble CDs as a novel fluorescent probe for the detection of Cys through the turn-on fluorescence of a CD–Hg²⁺ complex after Hg²⁺–Cys coordination. Das et al. developed a ligand-displacement
Covalent organic frameworks (COFs) are drawing interest as fluorescent probes for the detection of Cys and l-His. Similar to conventional crystalline porous materials [e.g., zeolites and metal organic frameworks (MOFs)], COFs possess precise predesigned structures and tailorable functionality, thereby allowing structural and chemical control over their particular functions. In addition to overcoming the shortcomings of the weakly crystalline structures and irregular pore distributions of most inorganic porous materials, COFs can also operate without the potential for collapse that often occurs for MOFs in organic solvents and water. Moreover, COFs can display high thermal and chemical stabilities, low densities, extremely high surface areas, regular porosity, excellent crystalline forms, and tunable structures and pore sizes. Accordingly, COFs are promising candidate materials for various applications, including the removal of pollutants from wastewater, (photo)catalysis, energy storage, gas separation and uptake, sensing, solar cells, the detection and removal of toxic metal ions, and photodynamic therapy.

Various COFs displaying fluorescence characteristics have been constructed previously and applied as chemosensors for pH, electron-rich arenes, mercury ions, 2,4,6-trinitrophenol, and anions. For biosensing applications, COF materials can have several attractive features, including high conductivity, high loading capacity, and biocompatibility. First, the uniform and crystalline structures of COFs can accelerate the migration of charged particles and, thereby, enhance their biosensor response rates. Second, the regular porosity of COFs can cause them to have large specific active surface areas and high surface free energies, leading to very high loading capacities when combined with organic molecules. Third, the biocompatibility of COF materials can provide appropriate microenvironments for biomolecules, thereby maintaining their activity. Together, these features appear to make COFs more desirable than conventional porous materials based on organic polymers and inorganic materials.

The distinctive photophysical and chemical properties of COFs encouraged us to develop one for the detection of l-His and l-Cys. Biosensors based on COFs remain rare and, to the best of our knowledge, the use of water-soluble COFs for the detection of amino acids has not been reported previously. In this paper, we describe the preparation of a water-soluble copper-immobilized COF (Cu@TFPB-DHTH COF) through the polycondensation of 1,3,5-tris(4-formylphenyl)benzene (TFPB-3CHO) and 2,5-dihydroxyterephthalohydrazide (DHTH) under solvothermal conditions (forming the TFPB-DHTH COF) and subsequent incorporation of Cu^{2+} ions (Scheme 1). The TFPB-DHTH COF displayed high chemical stability and a strong fluorescence emission, while the Cu@TFPB-DHTH COF exhibited a non-fluorescent emission. Interestingly, the addition of Cys or l-His to the fluorescence-off state of the Cu@TFPB-DHTH COF stimulated the snatching and seizure of the Cu^{2+} ions and, thereby, switched on the fluorescence emission of the TFPB-DHTH COF (Scheme 2). Therefore, we also studied the use of the Cu@TFPB-DHTH COF as a water-soluble “OFF–ON” fluorescent sensor for the detection of Cys and l-His.
Scheme 2. Schematic representation of the fluorescence sensing of Cys and L-His using the Cu@TFPB-DHTH COF.

Experimental section

Materials

Tetrakis(triphenylphosphine)palladium(0), 2,5-dihydroxy-1,4-benzenedicarboxylic acid, and 1,3,5-tribromobenzene were purchased from Acros. 4-Formylphenylboronic acid, Cys, L-His, and 10X PBS buffer were obtained from Sigma–Aldrich. Potassium carbonate, mesitylene, and hydrazine hydrate (98%) were purchased from Alfa Aesar. Methanol (MeOH), ethanol (EtOH), tetrahydrofuran (THF), 1,4-dioxane, glacial acetic acid, acetone, and dichloromethane (DCM) were obtained in analytical grade and used without purification.

Measurements

Transmission electron microscopy (TEM) was performed using a JEOL-2100 scanning electron microscope, operated at an accelerating voltage of 200 kV. Field-emission scanning electron microscopy (FE-SEM) was conducted using a JEOL JSM-7610F scanning electron microscope; the COF sample was subjected to Pt sputtering for 100 s prior to observation. The TFPB-DHTH COF sample for UV–Vis adsorption spectroscopy was dissolved in water and transferred to a small quartz cell (0.2 × 1.0 × 4.5 cm³); the spectrum was recorded using a JASCO V-770 UV-visible-NIR spectrophotometer. Fluorescence emission spectra were recorded using a LabGuide X350 spectrometer; samples were placed in a quartz cuvette having a path length of 1 cm and a slit width of 10 × 10 nm. The photoluminescence quantum yield (PLQY) of the TFPB-DHTH COF was recorded using a HORIBA Fluorolog-3 photon counting spectrophotometer system equipped with a Quanta-φ 6-inch integrating sphere. All pH measurements were performed using a SUNTEX SP-2100 pH meter.

Synthesis of 2,5-Dihydroxyterephthalohydrazide (DHTH)

A 100-mL two-neck round-bottom flask was charged with 2,5-dihydroxy-1,4-benzenedicarboxylic acid diethyl ester48 (2.00 g, 7.86 mmol), EtOH (80 mL), and hydrazine hydrate (10 mL). The mixture was heated under reflux at 90 °C for 20 h, forming a large amount of a pale-yellow precipitate. The solid was filtered off under vacuum and washed several times with EtOH. DHTH (1.5 g, 85%) was obtained after two recrystallizations of the crude solid from EtOH (Scheme S1). FTIR: 3397, 3307, 3044, 1630, 1541, 1505, 1349, 1255, 1161, 955 cm⁻¹ (Fig. S1).

1H NMR (500 MHz, DMSO-d₆) δ (ppm): 11.57 (s, 2OH, 2H), 10.01 (s, 2NH, 2H), 7.33 (s, 2CH, 2H), 4.67 (s, 2NH₂, 4H) (Fig. S2).

13C NMR (100 MHz, DMSO-d₆) δ (ppm): 167.37, 151.50, 119.73, 115.59 (Fig. S3).

Synthesis of 1,3,5-Tris(4-formylphenyl)benzene (TFPB-3CHO)

A 100-mL two-neck round-bottom flask was charged with 1,3,5-tribromobenzene (1.00 g, 3.18 mmol), tetrakis(triphenylphosphine) palladium(0) (184 mg, 0.160 mmol), potassium carbonate (2.64 g, 19.1 mmol), and 4-formylphenylboronic acid (2.86 g, 19.1 mmol). The mixed solids were evacuated under pressure for 30 min and then a mixture of 1,4-dioxane (40 mL) and water (7.5 mL) was added slowly under
a stream of N$_2$. The resulting mixture was heated at 110 °C for 40 h, then cooled to room temperature over 1 h. The mixture was poured into ice-water (200 mL), producing a white precipitate. After vacuum filtration, the crude solid was washed several times with water and MeOH (Scheme S2). The crude solid was purified through a short chromatographic column (SiO$_2$; EtOAc/n-hexane, 1:9) to yield TFPB-3CHO as a white solid (990 mg, 80%). FTIR: 3040, 2811, 2717, 1685, 1604, 1563, 1420, 1380, 1214, 1170 cm$^{-1}$ (Fig. S4).

1H NMR (500 MHz, DMSO-d$_6$) $\delta$ (ppm): 10.08 (s, 3CHO, 3H), 8.18 (d, $J = 8.8$ Hz, 6H), 8.16 (s, 3H), 8.05 (d, $J = 8.8$ Hz, 6H) (Fig. S5).

13C NMR (100 MHz, DMSO-d$_6$) $\delta$ (ppm): 194.14, 146.07, 141.39, 136.08, 130.80, 128.53, 126.86 (Fig. S6).

**Synthesis of TFPB-DHTH COF**

In a 25-mL tube, TFPB-3CHO (100 mg, 0.256 mmol, 1 eq.) and DHTH (86.9 mg, 0.384 mmol, 1.5 eq.) were suspended in a mixture of 1,4-dioxane and mesitylene (1:3, 5 mL) and an aqueous solution of acetic acid (6 M, 0.4 mL). The tube was degassed through several freeze/pump/thaw cycles and then sealed off by flame and heated at 120 °C. After 72 h, the mixture was cooled to room temperature, producing a solid that was filtered off and washed several times sequentially with 1,4-dioxane, MeOH, acetone, and THF. The TFPB-DHTH COF (92% yield) was obtained as a yellow-brownish solid after drying under vacuum at 120 °C for 24 h (Scheme 2).

**Fluorescence quenching of TFPB-DHTH COF in the presence of Cu$^{2+}$ ions**

To examine the effect of Cu$^{2+}$ ions on the fluorescence of the COF, an aqueous solution (0.1 mL) of copper acetate [Cu(OAc)$_2$], copper nitrate [Cu(NO$_3$)$_2$], or copper chloride (CuCl$_2$), at a final concentration of 200 µM, was added to an aqueous solution of TFPB-DHTH COF in 1x PBS (pH 7.4, 0.9 mL) at a final concentration of 0.125 mg mL$^{-1}$. The mixture was shaken strongly using a shaker and equilibrated at room temperature for 15 min. The fluorescence emission spectrum of the mixture was recorded. For the sensitivity test, aqueous CuCl$_2$ solutions (0.1 mL) of various concentrations were added to an aqueous solution of TFPB-DHTH COF in 1x PBS (pH 7.4, 0.9 mL) at a final concentration of 0.125 mg mL$^{-1}$. After shaking and 15 min of equilibration, fluorescence emission spectra of the mixtures were recorded.
Fig. 2. (a) PXRD patterns of the TFPB-DHTH COF: experimental pattern (black); simulated Pawley refined pattern (red dots); their difference pattern (blue); and simulated patterns for the completely eclipsed AA-stacking (olive) and staggered AB-stacking (violet) succession models. (b, c) Top views of (b) the completely eclipsed AA-stacking and (c) staggered AB-stacking succession models. (d) Nitrogen adsorption–desorption isotherm of the TFPB-DHTH COF at 77 K. (e) Pore size distribution of the TFPB-DHTH COF.

were measured. For the selectivity test, aqueous solutions (0.1 mL) of various metal ions at a final concentration of 200 µM was added to an aqueous solution of TFPB-DHTH COF in 1x PBS (pH 7.4, 0.9 mL) at a final concentration of 0.125 mg mL⁻¹. After shaking of the mixture and 15 min of equilibration, fluorescence emission spectra were recorded.

**Fluorescence sensing of Cys and l-His**

A stock aqueous solution of Cu@TFPB-DHTH COF was prepared through the addition of 0.87 mM aqueous CuCl₂ (4.6 mL) to an aqueous solution of TFPB-DHTH COF in 1x PBS (pH 7.4, 15.4 mL) at a concentration of 0.162 mg mL⁻¹. The mixture was shaken strongly using a shaker and equilibrated at room temperature for 60 min. The final concentrations of CuCl₂ and TFPB-DHTH
COF were 200 µM and 0.125 mg mL⁻¹, respectively. For the detection of Cys, aqueous solutions of Cys in 1x PBS (pH 7.4, 0.25 mL) of various concentrations were added to the stock solution of Cu@TFPB-DHTH COF (0.9 mL) and 2 mM Ni²⁺ (0.12 mL). Each mixture was shaken vigorously and equilibrated at room temperature for 15 min before measuring its fluorescence emission spectrum. For the detection of l-His, aqueous l-His solutions (0.25 mL) of various concentrations were added to the stock solution of Cu@TFPB-DHTH COF (0.9 mL) and of 2 mM NEM (0.1 mL). Each mixture was shaken vigorously and equilibrated at room temperature for 15 min before recording its fluorescence emission spectrum. For selectivity tests, other essential amino acids—alanine (Ala), aspartic acid (Asp), glutamine (Gln), glycine (Gly), glutamic acid (Glu), lysine (Lys), phenylalanine (Phe), tryptophan (Trp), proline (Pro), valine (Val), and tyrosine (Tyr)—were tested individually.

**Results and discussion**

Scheme 1a displays the synthesis of our new TFPB-DHTH COF. The use of solvothermal conditions with TFPB-3CHO and DHTH in 1,4-dioxane and mesitylene (1:3, v/v), in the presence of a catalytic amount of acetic acid (6 M), produced the TFPB-DHTH COF. Fourier transform infrared (FTIR) and solid state ¹³C nuclear magnetic resonance (NMR) spectroscopy verified the molecular structure of the TFPB-DHTH COF. The FTIR spectrum of DHTH featured a very broad signal in the range 3670–3307 cm⁻¹ for the vibration of the OH groups, overlapped with signals at 3397 and 3307 cm⁻¹ for the vibration of hydrazide (NHNH₂) group, a signal at 1630 cm⁻¹ for the vibration of the amide carbonyl (NHCO) group, and signals at 1613 and 1537 cm⁻¹ for the vibrations of aromatic (C=C) bonds [Fig. 1A(a) and Fig. S1]. The FTIR spectrum of the triformyl monomer TFPB-3CHO featured signals at 3040, 2811, 2717, 1685, and 1605 cm⁻¹ for the
vibrations of the aromatic C–H bonds, the Fermi double-resonance of the aldehydic C–H bonds, the aldehydic C=O bonds, and the aromatic groups, respectively [Fig. 1A(b) and Fig. S4]. The FTIR spectrum of the TFPB-DHTH COF did not feature the signals of the aldehydic C–H bonds (at 2811 and 2717 cm$^{-1}$) or the aldehydic C=O groups (at 1685 cm$^{-1}$) of TFPB-3CHO, nor the signals of the NHH$_2$N$_2$ groups (at 3397 and 3307 cm$^{-1}$) of DHTH, suggesting complete consumption of these monomers. Moreover, new signals appeared at 1644 and 1602 cm$^{-1}$ for the vibrations of the C=O and C=N groups, respectively, consistent with the formation of acyldiazone Schiff base (CONH–N=C) linkages and then the successful formation of the TFPB-DHTH COF [Fig. 1A(c)].

The $^{13}$C NMR spectrum of DHTH [Fig. 1B(a) and Fig. S3] featured a signal at 167.37 ppm representing the carbon nuclei of its C=O groups, as well as high-intensity signals at 151.50 ppm for the aromatic carbon nuclei attached to the hydrazide group (C–CONHNH$_2$). 6:30 AMthe signals of other aromatic carbon nuclei appeared at 119.73, and 115.59 ppm. The $^{13}$C NMR spectrum of TFPB-3CHO exhibited a peak at 194.14 ppm representing its aldehydic carbon nuclei [C(=O)–H], as well as signals in the range from 146.07 to 128.86 ppm for the aromatic carbon nuclei [Fig. 1B(b) and Fig. S6]. Solid state $^{13}$C NMR spectroscopy confirmed the successful condensation between DHTH and TFPB-3CHO to form the TFPB-DHTH COF, with the disappearance of the signal for the aldehydic carbon nuclei (at 194.14 ppm) of TFPB-3CHO and the appearance of new signals at 168.26 and 152.38 ppm for the C=O and C=N groups, respectively, of the formed acyldiazone Schiff base (CONH–N=C) [Fig. 1B(c)], in addition to signals of the other aromatic carbon nuclei at 158.38, 149.68, and 130.22–110.93 ppm.

To study the crystal structure of the TFPB-DHTH COF, we recorded its powder X-ray diffraction (PXRD) pattern with
Fig. 5. (a) Fluorescence emission spectra of the TFPB-DHTH COF dissolved in 1x PBS buffer (pH 7.4) in the presence of copper.
salts having various counter ions (Cu\(^{2+}\) concentration: 200 µM). (b) Fluorescence emission intensities at a wavelength of 520 nm of the TFPB-DHTH COF dissolved in 1x PBS buffer (pH 7.4) in the presence of Cu\(^{2+}\) and other metal ions (metal ion concentration: 200 µM). (c) Fluorescence emission spectra of the TFPB-DHTH COF dissolved in 1x PBS buffer (pH 7.4) in the presence of various concentrations of Cu\(^{2+}\) ions. (d) Calibration curve of the fluorescence emission intensity of the TFPB-DHTH COF with respect to the concentration of Cu\(^{2+}\) ions. Measurements were made at a TFPB-DHTH COF concentration of 0.125 mg mL\(^{-1}\) and under excitation at 365 nm.

angles \(20\) ranging from 1 to 30°. The as-synthesized TFPB-DHTH COF featured an experimental pattern (Fig. 2a, black curve) with an intense peak at a value of \(20\) of 2.16°, corresponding to the [100] plane. In addition, the PXRD pattern of the COF featured a set of reflected peaks at low-angle range, at values of \(20\) of 3.79, 4.40, and 5.83°, that we attribute to its [110], [200], and [210] planes, respectively. In the high-angle region, a broad reflected peak appeared at a value of \(20\) of 25.74°, matching the [001] plane (Fig. 2a). This experimental PXRD pattern confirmed that our TFPB-DHTH COF possessed a microcrystalline framework and a long-range arrangement. We applied the Bragg equation to calculate the \(d\)-spacing between the [100] planes \((d_{100})\) and the interlayer distance between the packed layers of the TFPB-DHTH COF. The as-synthesized COF exhibited a value of \(d_{100}\) and an interlayer distance of 4.01 nm and 3.45 Å, respectively (Table S1). To gain insight into the two-dimensional (2D) packing of the TFPB-DHTH COF, we applied Material Studio software to simulate two possible crystal packing models (completely eclipsed AA-stacking and staggered AB-stacking succession models) of our COF (Figs. 2b and 2c and Fig. S7), and then estimated the theoretical PXRD patterns of these models. Notably, the locations of the experimental PXRD peaks of the TFPB-DHTH COF (Fig. 2a, black curve) matched well with those of the simulated PXRD pattern generated through Pawley refinement of the completely eclipsed AA-stacking model of our COF (Fig. 2a, red dots). This matching was confirmed by negligible differences (Fig. 2a, blue curve) between the experimental and simulated PXRD patterns. The optimized unit cell parameters of the TFPB-DHTH COF, estimated from this refinement of the eclipsed AA-stacking model, were \(a = b = 46.231\) Å, \(c = 4.078\) Å, \(\alpha = \beta = 90°\), and \(\gamma = 120°\) (Fig. 2b and Figs. S7a and S7b, Table S2). Furthermore, comparisons of the experimental PXRD pattern of our TFPB-DHTH COF (Fig. 2a, black curve) with the calculated PXRD patterns of the completely eclipsed AA-stacking (Fig. 2a, green curve) and staggered AB-stacking (Fig. 2a, purple curve) successions models revealed consistency with the completely eclipsed AA-stacking model and high variance with the staggered AB-stacking model, suggesting completely eclipsed AA-stacking of the 2D layers in our TFPB-DHTH COF.

We estimated the surface area (360 m\(^2\) g\(^{-1}\)) and total pore volume (0.58 cm\(^3\) g\(^{-1}\)) of the TFPB-DHTH COF from its adsorption/desorption curve, by applying the Brunauer–Emmett–Teller (BET) model. In addition, we used nonlocal density functional theory (NLDFT) to investigate the pore size of the TFPB-DHTH COF, validating a narrow pore size distribution with a pore width of 3.77 nm (Fig. 2e and Table S1). We visualized the morphology of the TFPB-DHTH COF by using FE-SEM and TEM. Fig. S8 reveals that the crystalline TFPB-DHTH COF underwent self-assembly during its formation to produce uniform micrometer-scale tubules. Almost all of these TFPB-DHTH COF tubules were assembled into a set of bundles, presumably arising because of strong hydrogen bonding among the surfaces of the COF. Nevertheless, after sonication in polar protic solvents (e.g., ETOH), separated COF tubules could be observed. TEM imaging of the TFPB-DHTH COF confirmed the micrometer-scale tubular morphology of our COF; it also revealed that the average length and diameter of the tubular TFPB-DHTH COF were 295 ± 32 and 750 ± 45 nm, respectively (Fig. S9).

We used thermogravimetric analysis (TGA) to investigate the thermal and chemical stability of our synthesized TFPB-DHTH COF. Fig. S10 reveals that the TFPB-DHTH COF exhibited sufficient thermal stability, with decomposition temperatures \(T_{\text{d}}\) of 322 and 321 °C under \(N_2\) and air atmospheres, respectively. In addition, we tested the chemical stability of our TFPB-DHTH COF by dispersing 20 mg of the as-synthesized COF in various solvents [1,4-dioxane, THF, acetone, aqueous KOH (1 M), aqueous HCl (1M), MeOH, DMF, water, and 1x PBS buffer] for 2 days, collecting the solid through filtration under vacuum, washing the sample several times with THF, and finally drying it at 100 °C for 20 h. The PXRD patterns and \(N_2\) gas adsorption and desorption isotherms of the collected COF samples revealed high chemical stability of the TFPB-DHTH COF in these organic and aqueous solvents, as revealed no changes in the intensities or positions of the signals (Fig. S11) in addition to approximate similar surface area values (Table S3). We attribute the high thermal stability of the TFPB-DHTH COF to the strong intramolecular hydrogen bonds between the C=O groups of the hydrazone Schiff base (CONH–N=C) units and the OH groups in the ortho positions of the aromatic rings; we suspected that these units would also act as bidentate sites for the selective bonding of metal ions.

We investigated the electronic excitation and luminescence of the TFPB-DHTH COF by measuring its fluorescence emission spectrum in various solvents. We prepared solutions of our COF crystallites in water at
concentrations of 0.5, 0.25, 0.125, and 0.0625 mg mL\(^{-1}\), and then excited these solutions at 365 nm. Fig. 3a reveals that the fluorescence spectrum of each aqueous solution of the TFPB-DHTH COF exhibited an intense emission maximum at 520 nm. Interestingly, the fluorescence intensity of our COF increased from 16,430 to 31,161 to 41,453 a.u. upon decreasing the concentration of the COF from 0.5 to 0.25 to 0.125 mg mL\(^{-1}\) (Fig. 3b), respectively, indicative of aggregation-caused quenching (ACQ) of the TFPB-DHTH COF. We attribute the decrease in the fluorescence intensity to 29,677 a.u. at a concentration of our COF of 0.125 mg mL\(^{-1}\) (Fig. S13) revealed that the band gap of our TFPB-DHTH COF was 3.16 eV. The photoelectron spectrum of the TFPB-DHTH COF revealed a HOMO energy of 5.53 eV (Fig. 4d).

Finally, we determined the energy of the lowest unoccupied molecular orbital (LUMO) of our TFPB-DHTH COF to be 2.37 eV, by subtracting the band gap from the HOMO energy.

Our new TFPB-DHTH COF displayed high fluorescence emission, a uniform distribution of acylhydrazone and OH groups, excellent crystallinity, and good solubility in water and buffer; therefore, we were encouraged to investigate its applications for fluorescent sensing. First, we evaluated the quenching effect of the TFPB-DHTH COF dissolved in 1x PBS buffer (pH 7.4) after the addition of copper (Cu\(^{2+}\)) ions. We added aqueous solutions of copper salts with different counter ions [Cu(OAc)\(_2\), Cu(NO\(_3\))\(_2\), CuCl\(_2\)] to a solution of TFPB-DHTH COF in water. Fig. 5a reveals that the fluorescence emission intensity of the TFPB-DHTH COF was quenched dramatically after the addition of these copper salts. The approximately equal quenching efficiencies upon the addition of these copper salts suggested that the counter anions (OAc\(^-\), NO\(_3\)^-, Cl\(^-\)) had only marginal effects on the recognition of the Cu\(^{2+}\) ions by the TFPB-DHTH COF.

We investigated the selective quenching of the fluorescence of the TFPB-DHTH COF by Cu\(^{2+}\) ions by observing the change in the fluorescence emission intensity of our COF in the presence of 12 different metal ions (MCl\(_2\): M\(^{2+}\) = Na\(^+\), Li\(^+\), Ag\(^+\), Mg\(^{2+}\), Ba\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Fe\(^{3+}\), Fe\(^{2+}\), Hg\(^{2+}\), Cu\(^{2+}\)) (Fig. 5b). Among these tested metal ions, only Cu\(^{2+}\) ions caused strong quenching of the fluorescence of the TFPB-DHTH COF; the other metal ions had only slight fluorescence quenching effects. Alkali metal ions (Na\(^+\), Li\(^+\), Ag\(^+\)), alkaline earth ions (Mg\(^{2+}\), Ba\(^{2+}\), Ca\(^{2+}\)), and the transition metal Zn\(^{2+}\) ion had negligible effects on the fluorescence emission intensity of our COF; Al\(^{3+}\) and Cr\(^{3+}\) ions quenched approximately 10% of the fluorescence intensity, while the Fe\(^{3+}\), Fe\(^{2+}\), and Hg\(^{2+}\) ions both quenched it by approximately 60%. These findings confirmed the outstanding selectivity and excellent quenching response of our TFPB-DHTH COF toward Cu\(^{2+}\) ions, with a quenching efficiency of approximately 90% (Fig. 5b). To optimize the fluorescence quenching with Cu\(^{2+}\) ions, we evaluated the fluorescence emission intensity of our COF in water in the presence of CuCl\(_2\) at various concentrations in 1x PBS buffer (pH 7.4).

Fig. 5c reveals that the fluorescence emission intensity of an aqueous solution of TFPB-DHTH COF at a concentration of 0.125 mg mL\(^{-1}\) decreased gradually upon increasing the concentration of Cu\(^{2+}\) ions from 0.5 to 200 μM, but remained nearly unaffected thereafter when the concentration of Cu\(^{2+}\) ions increased to 300 μM. Approximately 90% of the fluorescence emission intensity of our COF was quenched in the presence of 200 μM of Cu\(^{2+}\) ions, representing a mole ratio of 1:6.8 (TFPB-DHTH COF/Cu\(^{2+}\)). Thus, full quenching of the fluorescence of TFPB-
DHHT COF required at least 6 mole equivalents of Cu$^{2+}$ ions. The fluorescence emission of the COF and the concentration of Cu$^{2+}$ ions had a linear relationship in the range from 2 to 200 μM, characterized by the equation $Y = 34,392 - 125C$, where $Y$ is the fluorescence intensity and $C$ is the concentration of Cu$^{2+}$ ions in solution; the correlation coefficient ($R^2$) was 0.968 (Fig. 5d). We estimated the limit of detection (LOD) of Cu$^{2+}$ ions to be 12 nM, based on $3\sigma/k$ (where $\sigma$ is the standard deviation of six blanks and $k$ is the slope of the calibration curve). The Cu$^{2+}$ ion has significant coordination ability toward fluorescent materials and COFs having amino, C=O, and OH groups in their structures; this coordinative binding induces electron transfer from the excited state of the fluorescent materials and COFs to the Cu$^{2+}$ ion, leading to dramatic quenching of fluorescence.69-72

Similarly, we attribute the fluorescence quenching of our TFPB-DHTH COF to strong binding between Cu$^{2+}$ ions and the active sites (C=O and OH groups) in the pore walls of our COF. This binding would induce electron transfer from the excited state of the COF to the Cu$^{2+}$ ions. This electron transfer process and the coordination interaction between Cu$^{2+}$ ion and TFPB-DHTH COF were confirmed by X-Ray photoelectron spectroscopy (XPS) measurements. Fig. S14 reveals that the TFPB-DHTH COF featured a set of XPS peaks of C1s, N1s, and O1s, while Cu@TFPB-DHTH COF

![Fig. 6.](image-url)
(pH 7.4) in the presence of various concentrations of l-His. (d) Calibration curve of the fluorescence emission intensity of the Cu@TFPB-DHTH COF with respect to the concentration of l-His. Measurements were made at concentrations of the Cu@TFPB-DHTH COF and the Cu$^{2+}$ ions of 0.125 mg mL$^{-1}$ and 200 μM, respectively, under excitation at 365 nm.

featured and Cu2p peak in addition to other XPS peaks of C1s, N1s, and O1s. Figs. S15a,b also reveals that the binding energy of O1s orbital shifted from 532.37 eV in TFPB-DHTH COF to 533.26 eV in Cu@TFPB-DHTH COF. This positive energy of O1s orbital shifted from 532.37 eV in TFPB-DHTH COF. To determine the optimal conditions for the “OFF–ON” fluorescence emission of our new Cu@TFPB-DHTH COF at 520 nm increased in the presence of 200 μM of Cu$^{2+}$ ions, we prepared a corresponding stock solution of the Cu@TFPB-DHTH COF to test the turn-on fluorescence sensing of Cys and l-His.

The thiol functional groups of two Cys moieties can undergo coordination with a Cu$^{2+}$ ion to form a stable 2:1 Cys/Cu$^{2+}$ complex. The thiol–Cu$^{2+}$ interaction is relatively stronger than the coordination between a Cu$^{2+}$ ion and C=O, OH, and amino groups.71,74,75 In addition, the imidazole group of l-His can interact strongly with a Cu$^{2+}$ ion to produce a stable 2:1 l-His/Cu$^{2+}$ complex; again, the binding affinity is higher than that between a Cu$^{2+}$ ion and C=O, OH, and amino groups.71,74 Consequently, we suspected that addition of Cys or l-His to the Cu@TFPB-DHTH COF might induce snatching and seizure of its Cu$^{2+}$ ions, thereby switching on the fluorescence emission of our Cu@TFPB-DHTH COF.

Therefore, we tested the Cu@TFPB-DHTH COF assembly as an “OFF–ON” fluorescence probe for the detection of Cys and l-His, operating through competitive coordination among the TFPB-DHTH COF, Cu$^{2+}$ ions, and Cys/l-His influencing the fluorescence emission of the TFPB-DHTH COF. To determine the optimal conditions for the “OFF–ON” fluorescence detection of Cys and l-His, we varied the pH and the incubation time. Figs. S16a and S16c reveal that the fluorescence emission intensity of the aqueous solution of the Cu@TFPB-DHTH COF was recovered maximally, after the incubation time approached 15 min and then remained constant thereafter. Therefore, for subsequent detection of Cys and l-His, we employed an incubation time of 15 min.

Under these optimal conditions, we evaluated the “OFF–ON” fluorescence detection of Cys and l-His and examined the relationship between the fluorescence recovery intensity and the concentration of Cys and l-His. For Cys, the intensity of the fluorescence emission peak at 520 nm increased upon increasing of the concentration of Cys in the range from 2 to 400 μM in the presence of 300 μM of Ni$^{2+}$ ions; it did not change any more upon increasing the concentration of Cys thereafter to 500, 1000, or 1500 μM (Figs. 6a and 6b). The inset to Fig. 6b reveals a linear relationship between the fluorescence intensity at 520 nm and the Cys concentration over the range from 2 to 100 μM.

The regression equation for Cys detection was $I = 8126.21 + 221.76[Cys]$, where $I$ is the fluorescence intensity at 520 nm and [Cys] is the concentration of Cys (μM); the correlation coefficient ($R^2$) was 0.9997. The LOD of Cys when using our new “OFF–ON” Cu@TFPB-DHTH COF fluorescence probe was equal to 340 nM, based on a signal-to-noise value (S/N) of 3. Likewise, upon incremental addition of l-His at concentrations in the range from 2 to 400 μM, the fluorescence intensity of our new Cu@TFPB-DHTH COF at 520 nm increased in the presence of 200 μM of NEM. The fluorescence intensity remained approximately unchanged when the concentration of l-His was greater than 500 mM (Figs. 6c and 6d). The inset to Fig. 6d reveals that the fluorescence intensity at 520 nm exhibited a linear relationship with the concentration of l-His within the range from 2 to 200 μM. The linear curve provided a regression equation for l-His detection of $I = 7947.02 + 127.77[I-His]$, where $I$ is the fluorescence intensity at 520 nm and [l-His] is the concentration of l-His (μM); the correlation coefficient ($R^2$) was 0.9993. We calculated the LOD of l-His to be 520 nM. Compared with other reported methods for the detection of Cys and l-His (Table S4), our present method is among the most highly selective. In addition, our detection strategy has several advantages over those previous methods, including high thermal and chemical stability, no need for chemical modification, excellent crystallinity, high solubility in water, and low cost.
could detect Cys quantitatively when using our Cu@TFPB-DHTH COF probe; similarly, we could achieve the quantitative detection of l-His in the presence of NEM. Furthermore, Fig. S17 reveals that the presence of Ni²⁺ ions in the detection system did not affect the fluorescence recovery efficiency of Cys; neither did the presence of NEM affect the recovery efficiency of l-His. Therefore, our new sensing assay displayed good selectivity toward Cys and l-His, respectively.

Conclusions
We have prepared a new fluorescent OH- and hydrazone-functionalized COF (TFPB-DHTH COF) displaying high crystallinity, excellent chemical stability, and suitable functional groups for interaction with metal ions; its solutions in water and buffer exhibited very high fluorescence emission under UV irradiation. The fluorescence emission of the TFPB-DHTH COF was switched off selectively upon the addition of Cu²⁺ ions, due to electron transfer from the excited state of the COF to the Cu²⁺ ions. The formed Cu@TFPB-DHTH COF functioned as an “OFF–ON” fluorescent sensor for the specific detection and differentiation of Cys and l-His in the presence of Ni²⁺ ions and NEM, respectively. Our new sensing assay could be completed within 30 min, with LODs of 340 and 520 nM for Cys and l-His, respectively. Thus, this facile “OFF–ON” detection assay for Cys and l-His, separately. Our new sensing assay could be completed within 2+ ions and NEM, due to electron transfer from the excited state of the COF to the Cu

Conflicts of interest
“There are no conflicts to declare”.

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