Metal–Organic Framework-Based Selective Sensing of Biothiols via Chemidosimetric Approach in Water

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ABSTRACT: Selective detection of biothiols holds prime importance owing to the role of varied concentrations of biothiols in various diseases, thus demanding extensive research for developing materials toward selective sensing. In this study, we targeted postsynthetic modification (PSM) approach for imparting desired functionality to chemically stable UiO-66-NH₂ metal–organic framework, which exhibits highly selective sensing toward biothiols. The appended dinitrobenzenesulfonyl group reacts with biothiols via chemidosimetric approach. As a consequence, the probe exhibits a turn on response, which holds immense importance for facile biological studies.

INTRODUCTION

Cysteine and glutathione among other biothiols play a vital role in the human physiology and biological activities, and their deficiency has been linked to various neurodegenerative disorders.1,2 These biothiols are known to serve in various cellular activities, for example, cysteine serves as a precursor to glutathione that maintains the redox activity within the cellular environment. This feature makes it very important in the maintenance of cellular stress. Deficiency in the cysteine content within the cells leads to retarded growth, liver damage, impaired antioxidant defenses, depressed immune functions, etc. On the other hand, glutathione maintains the levels of cysteine (reduced form) by controlling the redox environment within the intracellular compartments. Oxidative stress management is performed by glutathione via radical ion-trapping mechanism. These biothiols are also involved in internal cellular transduction and pose interference in molecular recognition.3,4 Hence, it is crucial to develop biocompatible materials for measuring their concentration within intracellular environments. Cysteine concentrations are directly linked with various diseases, making it significant to develop probes for detection of such thiols via intracellular bioimaging. Until now various methods have been developed for sensing of biothiols like capillary electrophoresis, high-performance liquid chromatography, UV/vis spectroscopy, mass spectroscopy, electrochemical methods, etc.5–7 These methods pose limitations such as high experimental costs, complex sample processing, etc., making them difficult for use in real-time cellular analysis. However, fluorescence-based methods have gained significant attention owing to various advantages, including highly sensitive and selective detection, facile experimentation technique, high cost efficiency, and instant data procurement for rapid analysis essential for real-time biological sample analysis.8,9 Luminescence-based sensing involves two kinds of approach: (1) interaction-based, wherein changes within the highest occupied molecular orbital–lowest unoccupied molecular orbital band gap of the donor acceptor leads to generation of signal in form of different photoluminescence emission profiles and (2) reaction-based, wherein analyte selectively reacts with the probe, leading to signal generation. To impart high selectivity to the fluorescent probes, reaction-based approach, that is, “chemidosimetric approach” offers a great deal of advantage in terms of selectivity over other known methods due to the presence of functionalized receptors mimicking lock and key model in the enzymatic reactions in the biological systems.10,11 Thus, dual facet combining the facile measurable technique and the highly selective reaction-based approach may offer exciting results in the field of sensing of biothiols. Fluorescence-based probes offer another significant advantage as being a noninvasive technique; thus, development of such probes for real-time biothiols sensing within the intracellular fluids should be explored.12 In the literature, various small molecule/organic probes for biothiol sensing are available, but these probes offer disadvantage as they tend to aggregate and are less soluble in water medium, thus making organic-based probes difficult for its utilization in real-time applications.13 Hence, there is a need for development of novel materials for analyte recognition.

Metal–organic frameworks (MOFs), evolved as a class of potentially porous materials, have shown extensive applications...
in the fields of sorption, sensing, catalysis, etc.\textsuperscript{14–16} MOFs are extended frameworks constructed from organic linkers and metal ions, wherein the organic linkers can be functionalized to impart specificity.\textsuperscript{17} MOFs have exhibited tremendous potential in the field of sensing owing to the presence of nanocavities with highly aligned recognition sites combined with the preconcentration effect, thus leading to fast response mechanism.\textsuperscript{18–21} To tune the selectivity and sensitivity of a probe, linker design strategy plays a vital role due to the facile tunable nature.\textsuperscript{22} To assign specific functionality, linkers can be functionalized via two approaches: presynthetic approach and postsynthetic approach. The presynthetic approach involves functionalization of linker with appended functionalities prior to MOF synthesis.\textsuperscript{23} Linker functionalization via postsynthetic modification (PSM) was pioneered by Cohen and co-workers.\textsuperscript{24,25} Until now, numerous reports have shown the distinct advantage of postsynthetic modification in MOFs that leads to superior performance compared to the pristine MOF.\textsuperscript{26} The PSM approach plays a pivotal role in sensing applications by imparting selective recognition sites post MOF synthesis.\textsuperscript{27} Thus, MOFs can act as nanoluminescent cavity with appended functionalities for photoluminescence-based studies in real-time applications.\textsuperscript{28} MOFs can be well dispersed in water and thus pose an advantage for such analysis. For biothiol sensing, we sought to incorporate functional sites specific for biothiols. Although there are a few reports that have shown selective sensing of biothiols, extensive developments are demanded.\textsuperscript{29,30} Existing literature revealed various organic reaction-based probes, but we restricted our search for probes that behave in a “turn on” fashion. It is well known that probes that respond in a turn on fashion can be studied with ease within the biological systems due to high signal-to-noise ratio as measurement occurs against a dark background. Enthused from this, we sought targeted attachment of highly electron-withdrawing functional groups like 2,4-dinitrosulfonyl chloride (DNS), sought targeted attachment of highly electron-withdrawing functional groups like 2,4-dinitrosulfonyl chloride (DNS), imparting selective recognition sites post MOF synthesis.\textsuperscript{27} MOFs can act as nanoluminescent cavity with appended functionalities for photoluminescence-based studies in real-time applications.\textsuperscript{28} MOFs can be well dispersed in water and thus pose an advantage for such analysis. For biothiol sensing, we sought to incorporate functional sites specific for biothiols. Although there are a few reports that have shown selective sensing of biothiols, extensive developments are demanded.\textsuperscript{29,30} Existing literature revealed various organic reaction-based probes, but we restricted our search for probes that behave in a “turn on” fashion. It is well known that probes that respond in a turn on fashion can be studied with ease within the biological systems due to high signal-to-noise ratio as measurement occurs against a dark background. Enthused from this, we sought targeted attachment of highly electron-withdrawing functional groups like 2,4-dinitrosulfonyl chloride (DNS), which is known as a highly specific and selective probe for biothiols (Figure 1).\textsuperscript{31}

![Figure 1](image-url)  
Figure 1. Schematic representation showing mechanistic pathway followed by UiO-66-DNS for biothiols sensing.

**RESULTS AND DISCUSSION**

The proposed idea was to integrate the specificity of the reaction and immobilization of the functional group that may exhibit a faster turn on response (Scheme 1).

Among other requirements, the nontoxic nature of probe is essential in real-time biological system; hence, we chose UiO-66-NH\(_2\), a highly robust Zr-MOF, known for its wide range of chemical stability, as a template for postsynthetic modification.\textsuperscript{32} UiO-66-NH\(_2\) was synthesized via a solvothermal method by a reported procedure.\textsuperscript{33} Initial characterization by powder X-ray diffraction (PXRD) revealed its bulk phase purity (Figure 2A). The as-made compound was desolvated following reported procedure for performing the postsynthetic modification (Figure S3). As strategized, UiO-66-NH\(_2\) was postsynthetically modified by reaction with dinitrosulfonyl chloride at room temperature to yield UiO-66-DNS (Experimental Section, SI). To evaluate framework integrity post reaction, characterization of UiO-66-DNS was performed using PXRD, thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FT-IR), energy dispersive X-ray (EDX) spectrometry, elemental analysis, and field emission scanning electron microscopy (FESEM). PXRD analysis revealed integrity of framework after PSM with retention of the highly crystalline nature of the sample (Figure S1). FT-IR spectra showed peaks around \(\sim 1160 \text{ cm}^{-1}\) for the stretching frequency of S–O bond and around \(\sim 1350 \text{ cm}^{-1}\) for N–O bond for the incorporation of DNS (Figure 3A). Elemental analysis showed sulfur content, which can be correlated to the successful grafting of DNS group to amine sites. Thermogravimetric analysis exhibited an initial weight loss of 12% owing to the presence of occluded solvent molecules during the postsynthetic modification protocol. UiO-66-DNS was activated at 120 °C, thus obtaining an activated phase of the material (Figure S3). FESEM analysis revealed that the morphology of the sample was maintained, and EDX analysis revealed sulfur content as anticipated (Figure S4). Thus, successful fabrication of UiO-66-DNS was characterized thoroughly by the above-mentioned techniques. UiO-66-DNS was probed by UV–vis spectroscopy, which showed maximum absorbance wavelength around \(\lambda_{\text{max}} \sim 350 \text{ nm}\) (Figure 3B). For an initial check regarding the performance of the probe for biothiols sensing, UiO-66-DNS was dispersed in water and photoluminescence profile was recorded upon excitation at \(\sim 325 \text{ nm}\) after addition of 20 \(\mu\text{L}\) of cysteine solution with a buffer time of 2 min. Photoluminescence profile showed emission spectra with the peak maximum \(\lambda_{\text{max}}\) centered around \(\sim 432 \text{ nm}\) (Figure 2B). After consecutive addition of 200 \(\mu\text{L}\) of cysteine, an overall \(\sim 48\)-fold enhancement in intensity profile was observed (Figure 2B).

This change may be attributed to the release of highly electron-deficient DNS moiety that quenches the fluorescence intensity of the initial probe (Figure 1).

Stability of the framework was examined after treatment of UiO-66-DNS with cysteine and glutathione. PXRD analysis confirmed the retention of the framework with the pattern showing highly crystalline nature (Figure S2). TGA also showed a similar pattern to UiO-66-NH\(_2\), from which it can be inferred that the framework exhibits a similar thermal stability profile (Figure S3). Retention of morphology was also confirmed by FESEM analysis, wherein UiO-66-DNS and cysteine-treated UiO-66-DNS showed the similar hexagonal-shaped morphology (Figure S6). Inspired from the above
results for cysteine sensing, we sought to test the effect of other biothiols like glutathione, which is large in size compared to cysteine (Figure S18). Photoluminescence studies were carried out in water medium in dispersed phase. The photoluminescence profile exhibited smooth emission at ∼432 nm (Figure S9). Upon successive addition of 20 μL of glutathione and recording photoluminescence profile after an equilibration time of 2 min, we observed an overall ∼26-fold enhancement in photoluminescence due to the release of electron-deficient DNS group (Figure S9). The enhancement in luminescence was also visible to the naked eye under UV light. In terms of kinetics of the probe, UiO-66-DNS showed faster response toward cysteine compared to glutathione in terms of the extent of enhancement. This observation can be explained by comparing the analytes in terms of sterics (Figure S18). The less bulky cysteine molecule may exhibit facile diffusion and thus can interact easily with the reaction probe, leading to considerably higher enhancement in photoluminescence intensity. Glutathione being bulkier in size may have restricted diffusion and consequently less interactions with the appended reaction sites, leading to less enhancement in photoluminescence intensity. The sensitivity of probe is an important criterion for analyzing the probe performance. Hence, limit of detection (LOD) calculations were performed according to reported protocols and the value was found to be around 9.8 μM for cysteine sensing29 (Figure S17). This low detection limit can be attributed to prealigned reaction centers present within the nanosized porous cavities of the framework that may lead to a fast intramolecular charge-transfer (ICT) process occurring between the linker and the DNS moiety. Upon removal of strong electron-withdrawing DNS moiety (Figure 1), the ICT may get quenched, thus leading to a significant enhancement in intensity profile. Both sensitivity and selectivity play a vital role in the design of sensory probe. To check the selectivity of the probe, we analyzed the photoluminescence performance of the probe UiO-66-DNS in the presence of other amino acids, keeping the concentration constant. As anticipated, other amino acids like phenylalanine, leucine, valine, isoleucine, tryptophan, and glycine showed negligible enhancement in the fluorescence intensity of UiO-66-DNS (Figures S9–S14). The performance of other amino acids was recorded after adding 200 μL of various amino acid solution to 2 mg of UiO-66-DNS; thereafter, photoluminescence profile was recorded after an equilibration time of 2 min. No substantial enhancement in the luminescence intensity was observed (Figure 4). The observed selectivity can be attributed to the presence of prealigned highly reactive functional sites, i.e., dinitrobenzenesulfonyl chloride moiety, which selectively reacts with the thiol functionality of cysteine, thus exhibiting selectivity over other amino acids. From this, we conclude the importance of the reaction-based approach in MOFs for imparting high selectivity and sensitivity. Further, this probe...
may be utilized for in vivo cell imaging for measuring cysteine concentration as cysteine holds tremendous significance because its concentration has been linked to various diseases.

In summary, we have functionalized UiO-66-NH2 via postsynthetic modification for imparting selectivity for biothiol sensing. As anticipated, the probe exhibited selective and specific sensing toward biothiols over other amino acids. We believe that the organic functionalization approach to impart selective sites of recognition within the nano porous domains of MOF leads to an appreciable value of LOD ~ 1.18 ppm. We hope this report stimulates research within the field of MOF for selective recognition of various biologically relevant amino acids.

■ EXPERIMENTAL SECTION

Materials. 2-Aminoterephthalic acid and 2,4-dinitrobenzenesulfonyl chloride were purchased from Sigma-Aldrich. ZrCl4 was purchased from Merck. All dry solvents and other chemicals were procured locally. All of these chemicals were used without further purification.

Physical Measurements. All fluorescence measurements were done on a Jobin Yvon Fluoromax-4 spectrophotometer. Powder X-ray diffraction patterns were recorded on a Bruker D8 Advanced X-ray diffractometer using Cu Kα radiation (λ = 1.5406 Å) in the 5−40° 2θ range. The IR spectra were acquired using a NICOLET 6700 FT-IR spectrophotometer using KBr pellet in the 400−4000 cm−1 range. Solid-state UV−visible spectra were recorded on a PerkinElmer UV−visible spectrometer. Gas adsorption measurements were studied using a Belsorp-max instrument from Bel Japan. The SEM images were obtained using an FEI Quanta three-dimensional dual beam Fourier transform scanning electron microscope at 30 kV.

Synthesis of UiO-66-NH2 (1). UiO-66-NH2 was synthesized according to a slightly modified previously reported protocol.33 A mixture of ZrCl4 (83 mg, 0.35 mmol) and 2-aminoterephthalic acid (63 mg, 0.35 mmol) was dissolved in 4 mL of N,N-dimethylformamide (DMF), and the resulting solution was placed in a Teflon-lined Parr stainless steel vessel (17 mL) and sealed. The sealed vessel was then placed in an oven and allowed to heat at 120 °C for 24 h. After slow cooling to room temperature, a pale yellow crystalline powder (1) was isolated by filtration and further washed with DMF and MeOH three times. Thereafter, 1 was dipped in MeOH for exchange. The MeOH-exchanged MOF was filtered and heated at 100 °C under vacuum for 12 h to get guest-free UiO-66-NH2 (1). The thus obtained 1 was used for all measurements.

Synthesis of Postsynthetically Modified UiO-66-NH2-PSM (1-PSM). UiO-66-DNSCl was synthesized via a slightly modified already reported protocol.34 UiO-66-NH2 (100 mg, 0.37 mmol), 2,4-dinitrobenzenesulfonyl chloride (117.3 mg, 0.44 mmol), and dichloromethane (5 mL) were taken in a 50 mL round-bottom flask. The above mixture was maintained at 0 °C, and pyridine (44 μL, 0.55 mmol) was added subsequently under stirring conditions for 1 h. Further, the reaction mixture was filtered and washed with water and acetone and activated at 120 °C for 24 h.

Preparation of the Analytes. Cysteine, glutathione, phenylalanine, leucine, valine, isoleucine, tryptophan, and glycine purchased from Sigma-Aldrich were used as received.

For photoluminescence studies, 1 mM stock solution of various amino acids was prepared in distilled water, and 2 mg of MOF was used for all of the photoluminescence and absorption studies.

■ ASSOCIATED CONTENT

 Supporting Information
 The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01891.

PXRD data, TGA plots, FT-IR spectra, FESEM images, fluorescence spectra (PDF)

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Notes
The authors declare no competing financial interest.

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■ REFERENCES

(1) Heafield, M. T.; Fearn, S.; Steventon, G. B.; Waring, R. H.; Williams, A. C.; Sturman, S. G. Plasma cysteine and sulphate levels in patients with motor neurone, Parkinson’s and Alzheimer’s disease. Neurosci. Lett. 1990, 110, 216−220.
(2) Herzenberg, L. A.; Rosa, S. C. D.; Dubs, J. G.; Roederer, M.; Anderson, M. T.; Ela, S. W.; Deresinski, S. C.; Herzenberg, L. A. Glutathione deficiency is associated with impaired survival in HIV disease. Proc. Natl. Acad. Sci. U.S.A. 1997, 94, 1967−1972.
(3) Yin, C.; Huo, F.; Zhang, J.; Martínez-Máñez, R.; Yang, Y.; Lv, H.; Li, S. Thiol-addition reactions and their applications in thiol recognition. Chem. Soc. Rev. 2013, 42, 6032−6059.
(4) Dalton, T. P.; Shertzer, H. G.; Puga, A. Regulation of Gene Expression by Reactive Oxygen. Annu. Rev. Pharmacol. Toxicol. 1999, 39, 67−101.
(5) Luo, Y.; Zhang, L.; Liu, W.; Yu, Y.; Tian, Y. A Single Biosensor for Evaluating the Levels of Copper Ion and L-Cysteine in a Live Rat Brain with Alzheimer’s Disease. Angew. Chem. 2015, 127, 14259−14262.
(6) Liu, J.; Sun, Y.-Q.; Huo, Y.; Zhang, H.; Wang, L.; Zhang, P.; Song, D.; Shi, Y.; Guo, W. Simultaneous Fluorescence Sensing of Cys and GSH from Different Emission Channels. *J. Am. Chem. Soc.* 2014, 136, 574–577.

(7) Qian, Q.; Deng, J.; Wang, D.; Yang, L.; Yu, P.; Mao, L. Aspartic Acid-Promoted Highly Selective and Sensitive Colorimetric Sensing of Cysteine in Rat Brain. *Anal. Chem.* 2012, 84, 9570–9584.

(8) Chen, X.; Zhou, Y.; Peng, X.; Yoon, J. Fluorescent and colorimetric probes for detection of thiols. *Chem. Soc. Rev.* 2009, 39, 2120–2135.

(9) Lee, M. H.; Kim, J. S.; Sessler, J. L. Small molecule-based ratiometric fluorescence probes for cations, anions, and biomolecules. *Chem. Soc. Rev.* 2015, 44, 4185.

(10) Zhou, Y.; Yoon, J. Recent progress in fluorescent and colorimetric chemosensors for detection of amino acids. *Chem. Soc. Rev.* 2012, 41, 52–67.

(11) Guo, Z.; Nam, S. W.; Park, S.; Yoon, J. A highly selective ratiometric near-infrared fluorescence cyanine sensor for cysteine with remarkable shift and its application in bioimaging. *Chem. Sci.* 2012, 3, 2760–2765.

(12) Nagarkar, S. S.; Saha, T.; Desai, A. V.; Talukdar, P.; Ghosh, S. K. Metal-organic framework based highly selective fluorescence turn-on probe for hydrogen sulphide. *Sci. Rep.* 2014, 4, No. 7053.

(13) Mei, J.; Hong, Y.; Lam, J. W. Y.; Qin, A.; Tang, Y.; Tang, B. Z. Aggregation-Induced Emission: The Whole Is More Brilliant than the Parts. *Adv. Mater.* 2014, 26, 5429–5479.

(14) Li, J.-R.; Kuppler, R. J.; Zhou, H.-C. Selective gas sorption and separation in metal-organic frameworks. *Chem. Soc. Rev.* 2009, 38, 1477–1504.

(15) Lustig, W. P.; Mukherjee, S.; Rudd, N. D.; Desai, A. V.; Li, J.; Ghosh, S. K. Metal-organic frameworks: functional luminescent and photonic materials for sensing applications. *Chem. Soc. Rev.* 2017, 46, 3242.

(16) Lee, J.; Farha, O. K.; Roberts, J.; Scheidt, K. A.; Nguyen, S. T.; Hupp, J. T. Metal-organic framework materials as catalyst. *Chem. Soc. Rev.* 2009, 38, 1450–1459.

(17) Zhou, H.-C.7’; Kitagawa, S. Metal-Organic Frameworks (MOFs). *Chem. Soc. Rev.* 2014, 43, 5415–5418.

(18) Hu, Z.; Diebert, B. J.; Li, J. Luminescent metal-organic frameworks for chemical sensing and explosive detection. *Chem. Soc. Rev.* 2014, 43, 5815–5840.

(19) Allendorf, M. D.; Bauer, C. A.; Bhakta, R. K.; Houk, R. J. T. Luminescent metal-organic frameworks. *Chem. Soc. Rev.* 2009, 38, 1330–1352.

(20) Li, Y.-A.; Yang, S.; Li, Q.-Y.; Ma, J.-P.; Zhang, S.; Dong, Y.-B. UiO-68-ol NMOF-Based Fluorescent Sensor for Selective Detection of HClO and Its Application in Bioimaging. *Inorg. Chem.* 2017, 56, 13241–13248.

(21) Zhang, Y.; Yuan, S.; Day, G.; Wang, X.; Yang, X.; Zhou, H.-C. Luminescent sensors based on metal-organic frameworks. *Coord. Chem. Rev.* 2018, 354, 28–45.

(22) Lu, W.; Wei, Z.; Gu, Z.-Y.; Liu, T.-F.; Park, J.; Park, J.; Tian, J.; Zhang, M.; Zhang, Q.; Gentle, T.; III, Bosch, M.; Zhou, H.-C. Tuning the structure and function of metal-organic frameworks via linker design. *Chem. Soc. Rev.* 2014, 43, 5561–5593.

(23) Nguyen, J. G.; Cohen, S. M. Moisture-Resistant and Superhydrophobic Metal-Organic Frameworks Obtained via Post-synthetic Modification. *J. Am. Chem. Soc.* 2010, 132, 4560–4561.

(24) Cohen, S. M. Postsynthetic Methods for the Functionalization of Metal-Organic Frameworks. *Chem. Rev.* 2012, 112, 970–1000.

(25) Cohen, S. M. The Postsynthetic Renaissance in Porous Solids. *J. Am. Chem. Soc.* 2017, 139, 2855–2863.

(26) Karmakar, A.; Kumar, N.; Samanta, P.; Desai, A. V.; Ghosh, A. V. A Post-Synthetically Modified MOF for Selective and Sensitive Aqueous-Phase Detection of Highly Toxic Cyanide Ions. *Chem. - Eur. J.* 2016, 22, 864–868.

(27) Aguilera-Siglat, J.; Bradshaw, D. A colloidal water-stable MOF as a broad-range fluorescent pH sensor via post-synthetic modification. *Chem. Commun.* 2014, 50, 4711–4713.