CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

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To be cited as: *Chem. Eur. J.* 10.1002/chem.201703438

Link to VoR: http://dx.doi.org/10.1002/chem.201703438

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10.1002/chem.201703438

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Enzyme Assisted Metal-Organic Framework Sensing System for Diethylstilbestrol Detection

Wen-Juan Li, Lan Chang, Qiao Liu, Di Ning, Xi-Yuan Yao, Yue Li,* and Wen-Juan Ruan*[a]

Abstract: As novel fluorescent sensing materials, metal-organic frameworks (MOFs) have shown great potential in environmental monitoring. However, most of the researches are limited to traditional pollutants, while the application of MOFs to the detection of the pollutants with more complicated structures, such as endocrine disrupting chemicals (EDCs), has rarely been explored. The difficulties faced in the sensing of EDCs include their electronic stability and the structural similarity among homologues, which could be overcome by the incorporation of enzymatic reaction. In this work, the first example of enzyme assisted MOF fluorescent sensing was developed for the analysis of diethylstilbestrol (DES, a synthetic estrogen). In this system, DES is firstly oxidized by HRP/H_2O_2 quantitatively to its quinone form, and then the quinone product is selectively captured by a stilbene based luminescent MOF to induce fluorescence response. By the tandem sensitization and filtration of enzymatic reaction and MOF adsorption, this method shows high sensitivity (DL = 89 nM) and can distinguish DES from other similar-structured EDCs.

Introduction

Metal-organic frameworks (MOFs) have attracted great attention as a novel family of fluorescent sensing materials.[1] The wide choice of metal ions and organic linkers makes the optical properties of MOFs highly tunable, and their channel structures could serve as a powerful tool for analyte screening and enrichment in the sensing process. However, most of the researches in this area are focused on the detection of traditional pollutants, such as, nitro compounds,[2] heavy metal ions,[3] and VOCs,[4] while the applications of MOFs to the analysis of the pollutants with more complicated structures are still very rare yet.[5] Over the last few decades, the occurrence of endocrine disrupting chemicals (EDCs), which could be defined as the compounds that have potential adverse effects on body via interactions with the endocrine system, has become a worldwide issue of increasing environmental concern.[6] As far as we know, there is no report of using MOFs for the spectroscopic analysis of EDCs. The primary challenge faced in the fluorescent sensing of EDCs is the electronic stability of this kind of pollutants, which prevents them to induce fluorescent response. Additionally, the structures among the homologues of EDCs are usually very close, so the discrimination of them is quite difficult.[7] Enzymatic reactions have the characteristics of high substrate specificity and high conversion selectivity. The incorporation of enzymatic reaction to MOF sensing can solve both of these problems. In this system, enzymatic reaction specifically converts the target pollutant to the activated product to induce the fluorescent response of MOF, while other homologues remain unreacted and do not interference the analysis. The high conversion selectivity of enzymatic reaction ensures that the signal produced by the activated product could truly reflect the original quantity of target pollutant.

Diethylstilbestrol (DES) is a synthetic estrogen which had been used as feed additive in livestock industry to promote animal growth[7] and as drug clinically to prevent pregnancy complications.[8] Due to its stability, the metabolism of DES in human body needs much longer time than natural estrogens.[9] Researchers have proved that long-term exposure to DES would increase the risk of immunological dysfunctions,[10] birth defects[11] and some kinds of cancers.[12] Due to these detrimental effects, DES has been banned by many countries, including USA and several European countries. However, the worldwide reports about illegal use of DES in animal production remind that the residue of DES in food is still a real threat to human health.[13] To control its abuse, various methods, including chromatographic techniques,[14] enzyme-linked immunosorbent assays (ELISAs)[15] and electrochemical analyses,[16] have been developed for the detection of DES. Nevertheless, these methods intrinsically suffer from the drawbacks of equipment-requiring, time-consuming, requiring strict pretreatment and susceptibility to matrix effects. Therefore, a convenient, rapid and propagable DES analysis method is still in urgent need yet.

It has been reported that, in the presence of peroxidase, DES is oxidized by H_2O_2 to its quinone form, diethylstilbestrol-4',4''-quinone (DESQ).[17] Like other quinone compounds, DESQ is expected to be electron-deficient and much easier to accept electron than the substrate. On the other hand, the contact of luminescent MOF with electron-deficient compound usually induces emission quenching.[18] Based on these facts, herein, a MOF based sensing method is designed for the detection of DES with the assistance of horseradish peroxidase (HRP). In this system, a stilbene based luminescent MOF (Zr-sbdc) is used as the sensor. DES is firstly oxidized by HRP/H_2O_2 quantitatively to DESQ, and then the formed DESQ is captured by Zr-sbdc to cause fluorescence response. By the tandem filtration of enzymatic reaction and MOF adsorption, this method shows high selectivity and can distinguish DES from a series of similar-structured EDCs. To the best of our knowledge, this work is the first example of enzyme assisted MOF fluorescent sensing.

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Results and Discussion

Characterization of MOF

Zr-sbdc was obtained as white power from the solvothermal reaction between $ZrOCl_2$ and trans-4,4'-stilbenedicarboxylic acid (H_2sbdc) in the mixed solvent of DMF and acetic acid (3:1, v/v). PXRD analysis (Figure 1a) showed that this compound presents a UiO-type connection with all the diffraction peaks could be readily indexed to $[Zr_6O_4(OH)_4(sbdc)_6]$, a recently reported member of this MOF series.[19] This backbone structure was also supported by elemental and thermogravimetric analyses (Figure S1 in the Supporting Information), which determined the formula of $[Zr_6O_4(OH)_4(sbdc)_6](DMF)_3(H_2O)_{30}$. UiO-type MOFs are famous for the high stability and extensive channel structure. The N_2 adsorption experiment of our sample gave the pore volume of 1.05 $cm^3\ g^{-1}$ and BET surface area of 2080 $m^2\ g^{-1}$ (Figure S2 in the Supporting Information). Electron microscope observation showed that Zr-sbdc has an ordered morphology of monodisperse octahedrons with dimensions of ~150 nm (Figure 1b). The smooth crystal planes and sharp edges exhibit the good crystallinity of this sample. The small particle size and porous structure of Zr-sbdc are good for its dispersion in the media and contact with the analyte, and thus facilitate the following fluorescence tests.

The photoluminescence of Zr-sbdc was directly measured with its aqueous suspension. Upon the excitation at 329 nm, Zr-sbdc gave a prominent emission band with the maximum at 376 nm and extending to the visible range (Figure 2a). The nearly identical position and similar shape of this emission band to the fluorescent spectrum of free $sbdc^{2-}$ ligand (376 nm, Figure 2b) indicates that the photoluminescence of Zr-sbdc is essentially ligand-centered. Containing the stilbene structure, free $sbdc^{2-}$ ligand is unstable under irradiation due to the torsion about the central C=C bond to the nonfluorescent *cis*-isomer.[20] In our experiments, even the exciting light used in fluorescent measurement could cause a continuous weakening of its emission. In contrast, this photoisomerization was largely impeded in Zr-sbdc, because the ligand configuration was fixed upon the coordination to inorganic nodes. Negligible change of the fluorescent spectrum of Zr-sbdc was observed with the prolongation of testing time. The photostability of Zr-sbdc is necessary for its fluorescent sensing applications.

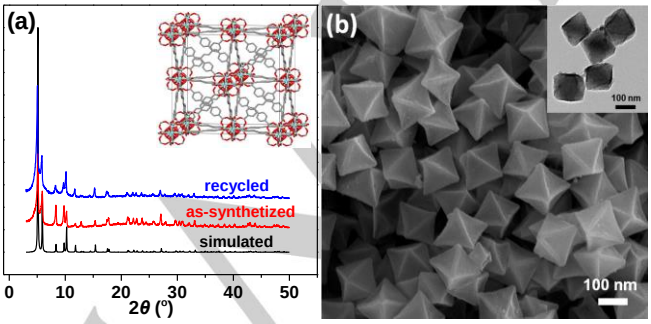


Figure 1. a) PXRD pattern and b) SEM image of Zr-sbdc. Inset of a): framework structure of $[Zr_6O_4(OH)_4(sbdc)_6]$.[19] Inset of b): TEM image of Zr-sbdc.

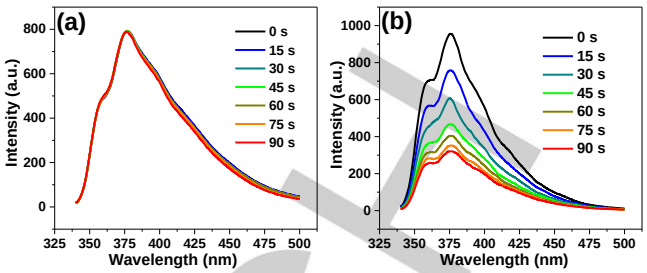


Figure 2. Fluorescent spectra of the aqueous a) suspension of Zr-sbdc (15 mg L^{-1}) and b) solution of $sbdc^{2-}$ ligand (6.5 μM, dissolving H_2sbdc by 2 equiv. of NaOH), λ_{ex} = 329 nm.

Detection of DES

Taking advantage of the photoluminescence of Zr-sbdc, it was used as the sensor for the enzyme assisted detection of DES. The sensing experiments were carried out mainly at pH = 6.0, because HRP exhibits the highest activity at near neutral conditions.[21] As shown in Figure 3, DES caused little change on the emission of Zr-sbdc. Then, HRP and overdosed H_2O_2 (Figure S3 in the Supporting Information) were added successively to induce the oxidation of DES, and a remarkable quenching of fluorescence was observed. The emission intensity decreased to 5% of the original value in 1 min and then maintained stable, showing the reaction had gone to completion. In contrast, the addition of HRP and H_2O_2 to the Zr-sbdc suspension without DES did not change the fluorescent spectrum much, which indicates that, even under the activation of HRP, H_2O_2 cannot work as an efficient quencher. The fluorescence quenching observed with the coexistence of DES, HRP and H_2O_2 is likely to be caused by the formed DESQ product.[17] In another experiment, DES was oxidized in the solution containing HRP and H_2O_2, extracted with $CDCl_3$ and subjected to ^{1}H NMR analysis. The recorded ^{1}H NMR spectrum

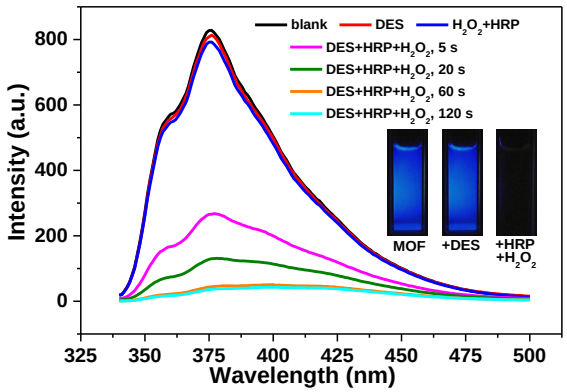


Figure 3. Fluorescent spectra of Zr-sbdc suspension before (black) and after the addition of DES (red), after the addition of H_2O_2 and HRP (blue), after the addition of DES, H_2O_2 and HRP and placed for 5 (magenta), 20 (green), 60 (orange) and 120 s (cyan). Inset: the photographs showing the original fluorescence of the suspension of Zr-sbdc and its decreased fluorescence after DES, enzyme and H_2O_2 addition. 15 mg L^{-1} Zr-sbdc, 60 μM DES, 0.5 mg L^{-1} HRP, 200 μM H_2O_2, in Bis-Tris buffer (20 mM, pH = 6.0)

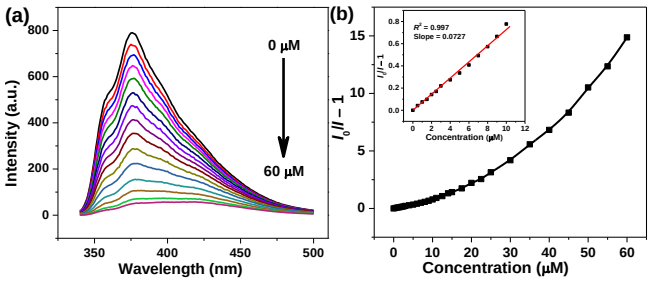


Figure 4. a) Fluorescent spectra of Zr-sbdc at different concentrations of DES. 15 mg L^{-1} Zr-sbdc, 0−60 μM DES, 0.5 mg L^{-1} HRP, 200 μM H_2O_2, in Bis-Tris buffer (20 mM, pH = 6.0). b) Fluorescence response of Zr-sbdc to different amounts of DES. Inset: linear response of Zr-sbdc to DES in the low concentration range of 0−10 μM.

clearly validated the formation of DESQ under enzymatic oxidation conditions (Figure S4 in the Supporting Information). To confirm its quenching effect, DESQ was prepared separately[22] and added to the suspension of Zr-sbdc, and a similar fluorescence intensity decrease was observed (Figure S5 in the Supporting Information). Because the enzymatic transformation of DES to its quinone form is quantitative,[17] the extent of fluorescence quenching after reaction completion could indirectly reflect the original amount of DES.

The sensitivity of this method was evaluated by fluorescence titration experiments. Different amounts of DES were added to the suspension of Zr-sbdc, followed by the addition of the same amount of HRP and H_2O_2 (Figure 4a). We use $I_0/I - 1$, where I_0 and I are respectively the fluorescence intensities before and after DES, HRP and H_2O_2 addition, to represent the fluorescent response induced by DES (Figure 4b). In the low concentration range of 0−10 μM, the fluorescence response is proportional to the content of DES (R^2 = 0.997), which is in accordance with the Stern-Volmer equation ($I_0/I = 1 + K_{SV} \cdot [Q]$, K_{SV} is the Stern−Volmer constant). From the slope of the fitting line and the deviation of fluorescent measurement, the detection limit of this method is calculated to be 89 nM ($3\sigma/k$, σ is the standard deviation of I_0/I measurements and k is the slope of the fitting line). This low detection limit indicates that the sensitivity of our enzyme assisted sensing is among the best of recently reported spectroscopy based DES analytic methods[23] and even comparable with some electrochemical technologies.[16b,16c] At higher concentrations, the response plot deviates from linearity and displays an upward curvature. This superlinear behavior could be explained by self-adsorption or energy-transfer processes because of the spectra overlap between Zr-sbdc emission and DESQ absorption (Figure S6 in the Supporting Information).[24]

To test the pH effect on this sensing method, we measured Zr-sbdc emission before and after the addition of DES, H_2O_2 and HRP at different pH values (Figure S7 in the Supporting Information). Generally, the emission intensity of Zr-sbdc maintained constant in the pH range from 4.0 to 8.0. Within this pH range, Zr-sbdc could give fluorescent response to the oxidation product of DES, although the sensitivity exhibited some degree of decrease when the pH was below 4.5 or above 7.5. Based on these results, the optimum pH range of our sensing method is determined to be 4.5−7.5.

The recognition of a specific hazard from other similar-structured compounds is of great significance to practical application. Besides DES, we also test the fluorescence responses of this method to other EDCs, including dienestrol (DE), hexoestrolum (HEX), diethylstilbestrol dipropionate (DESDP), dienoestroldiacetate (DEDA), β-estradiol (E2), estriol (E3), daidzein (DZE), bisphenol A (BPA), dichlorodiphenyl-trichloroethane (DDT), 3,3',4,4'-tetrachlorobiphenyl (PCB77), di-*n*-octyl-phthalate (DOP), hydroquinone (HQ) and catechol (CE). In contrast to DES, none of these compounds could notably affect the fluorescent spectrum of Zr-sbdc (Figure 5a). The high selectivity of this enzyme assisted sensing was further confirmed by competition experiments, in which DES was added to the suspensions of Zr-sbdc coexistent with other EDCs. As shown in Figure 5b, the fluorescent signal induced by DES was nearly unperturbed in these systems. It is noteworthy that, among the tested EDCs, the structures of DE, HEX, DESDP and DEDA are quite similar to that of DES, but our method can recognize DES over these compounds without difficulty. This fine structural discrimination is rare for the reported MOF sensing systems.[5a,5b]

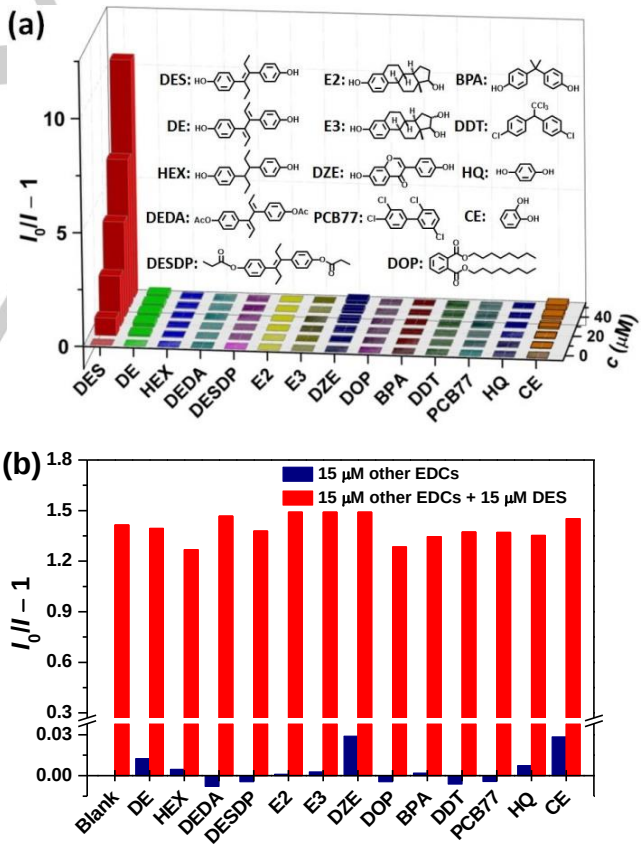


Figure 5. a) Fluorescent response of Zr-sbdc toward DES and other EDCs after the addition of HRP/H_2O_2, 15 mg L^{-1} Zr-sbdc, 0−50 μM EDCs, 0.5 mg L^{-1} HRP, 200 μM H_2O_2, in Bis-Tris buffer (20 mM, pH = 6.0). b) Competitive selectivity of Zr-sbdc/HRP/H_2O_2 toward DES in the presence of other EDCs.

Sensing mechanism

To better understand the high selectivity of this enzyme assisted MOF sensing, a series of investigations were carried out. Since the fluorescence quenching with DES addition is expected to be via a photo-induced electron transfer (PET) from MOF to DESQ, firstly, we calculated the frontier orbitals of the tested EDCs and their oxidation products, and compared the energy levels with the band structure of Zr-sbdc. As discussed above, the photoluminescence of Zr-sbdc is ligand centered, so the positions of its conduction and valance bands could be represented respectively by the LUMO and HOMO of H_2sbdc. As shown in Figure 6, the LUMO energy levels of the tested EDCs (including DES) are all above that of H_2sbdc, indicating that the electron injections from excited MOF to them are thermodynamically forbidden. This comparison is consistent with the experimental phenomenon that the solely addition of these compounds cannot quench the emission of Zr-sbdc. Under the oxidation of HRP/H_2O_2, except DES, HQ and CE, the other EDCs are not affected (Figure S8 in the Supporting Information) and thus cannot induce fluorescence response in the sensing system. The enzymatic oxidation of DES, HQ and CE transforms them to DESQ, *p*-benzoquinone (p-BQ) and *o*-benzoquinone (*o*-BQ), respectively,[17,25] which largely enhances the electron affinity. In contrast to their substrates, the LUMOs of quinone products lie at lower energy levels than the conduction band of Zr-sbdc. This result on one side confirms the PET mechanism occurring in DES sensing. However, in our experiments, the oxidation products of HQ and CE did not quench the emission of Zr-sbdc despite the fact that the excited-state electron transfer is also energetically feasible for them. This contradiction suggests that the PET mechanism and the substrate specificity of enzymatic reaction are not enough to explain the high selectivity of our sensing system.

Then, the quenching process was studied with transient fluorescence method (Figure S9a in the Supporting Information). The emission decay of Zr-sbdc was fit best by a biexponential function with a short subnanosecond term (τ_1 = 0.33 ns) and a longer term (τ_2 = 2.22 ns, Table S1 in the Supporting Information). This biexponential decay could be explained by the inhomogeneous rotation or flipping kinetics of phenyl ring, since the ligand configuration is not completely fixed in Zr-sbdc.[26] With the increase of the addition amount of DESQ, the lifetimes

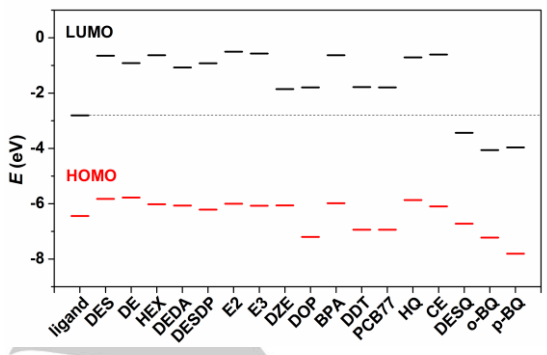


Figure 6. Frontier orbital energies of tested EDCs and quinone products.

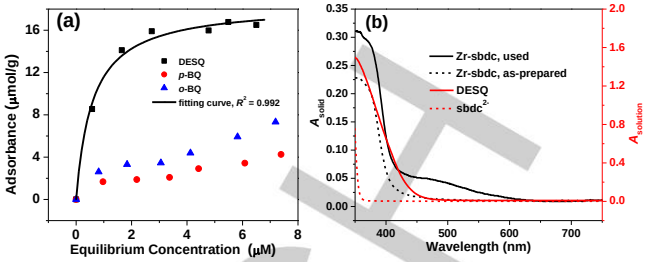
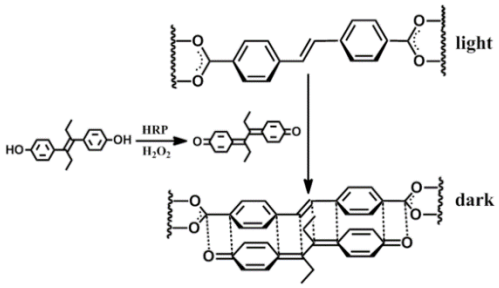


Figure 7. a) Adsorption of DESQ, *p*-BQ and *o*-BQ on Zr-sbdc. b) UV-vis diffuse reflectance spectra of as-prepared and used Zr-sbdc sensor. The absorption spectra of DESQ and $sbdc^{2-}$ in solution were also provided for comparison.

of these two terms, as well as their relative contributions, were hardly changed, which is in contrast to the remarkable decrease of steady-state fluorescent intensity (I_0/I increased from 1 to 5.2) (Figure S9b in the Supporting Information). The invariant fluorescence lifetime is typical for a static quenching pathway.

In the static quenching mechanism, the sensor would form a nonfluorescent complex with the analyte in the ground state, and this pre-association determines the quenching efficiency. To further illustrate the DES selectivity, the adsorption of DESQ on Zr-sbdc was measured and compared with those of *p*- and *o*-BQs (Figure 7a). In these experiments, the solutions of quinones were freshly prepared by the enzymatic oxidation of DES, HQ and CE. Zr-sbdc was added after reaction completion, and the suspensions were stirred for 5 min to achieve the adsorption−desorption equilibrium (Figure S10 in the Supporting Information). The quinone concentrations before and after the adsorption process were quantified by spectrophotometric method. It was observed that the adsorption amount of DESQ is much higher than those of BQs. Considering the structure of DESQ, we expect that its adsorption occurs through the formation of π-stacking complex with the $sbdc^{2-}$ ligand of Zr-sbdc (like the case of BQ-HQ complex, Scheme 1). The formation of this kind of complex is usually accompanied by the appearance of a red-shifted band of charge-transfer absorption in the UV-vis spectrum.[27] To confirm it, the Zr-sbdc sensor was separated after DES sensing and subjected to diffuse reflection spectroscopy analysis. As shown in Figure 7b, the absorption band of used Zr-sbdc sensor could extend to the wavelength above 600 nm. In contrast, none of Zr-sbdc, $sbdc^{2-}$ or DESQ gives absorption above 450 nm. Therefore, the absorption of



Scheme 1. Sensing mechanism of Zr-sbdc to DES.

used Zr-sbdc in the range of 450−600 nm can only be attributed to the complex formed between $sbdc^{2-}$ ligand and DESQ. To rule out the structural change of Zr-sbdc, the used sensor was characterized by different methods. The recorded PXRD pattern, IR spectrum and TEM image of the used sensor (Figure 1a, Figures S11 and S12 in the Supporting Information) were almost the same as those of the as-prepared one, showing that Zr-sbdc maintained stable in the sensing process and the observed fluorescence and absorption changes are not due to the structural change of the sensor. It is reasonable that the matched size between DESQ and $sbdc^{2-}$ ligand maximizes the orbital overlap between them, and thus makes the adsorption of DESQ much stronger than BQs.

Further information about DESQ adsorption comes from DFT calculation. Although DESQ has *cis* and *trans* two possible conformations (Figure S13 in the Supporting Information), the energies of *cis*-configured DESQ molecule and its complex with H_2sbdc are both lower than the corresponding energies of the *trans*- one (Figure S14 and Table S2 in the Supporting Information). Therefore, the following discussion is limited to the adsorption of *cis*-configured DESQ. The formation of the complex between H_2sbdc and *cis*-configured DESQ is energetically favorable with a binding energy of –12.44 kcal mol^{-1}. In accordance with the superior performance of DESQ in adsorption experiments, this value is more negative than the binding energies of H_2sbdc with *p*- and *o*-BQs (–7.07 and –9.28 kcal mol^{-1}, respectively). The scrutinization about the optimized structure of DESQ-H_2sbdc complex would illustrate the nature of the intermolecular interaction (Figure 8). Because of the steric hindrance caused by ethyl groups, DESQ presents a bent geometry, which makes only one quinomethane ring locate parallel to the benzo ring of H_2sbdc to form π-π stacking. The distance between ring centroids is 3.65 Å, similar to the cases of BQs (Figure S15 in the Supporting Information). Additionally, the quinone O atom on the other quinomethane ring gives short distances (2.83 and 2.93 Å) with two of the benzo H atoms of H_2sbdc to form H-bonds. This cooperation of π-π stacking and H-bonds leads to the strong affinity between DESQ and H_2sbdc. The HOMO of DESQ-H_2sbdc complex distributes mainly around H_2sbdc ligand, while the LUMO locates at DESQ (Figure S16 in the Supporting Information). This orbital arrangement indicates that, upon irradiation, the excited electron of H_2sbdc would shift to DESQ to form the charge transfer excited state.

The fitting of DESQ adsorption with Langmuir isotherm ($Q_e = Q_{max} \cdot K_{eq} \cdot c_e/(1 + K_{eq} \cdot c_e)$, where Q_{max} is the saturated adsorption capacity, Q_e and c_e are respectively the adsorption amount and liguid-phase concentration at equilibrium, K_{eq} is the equilibrium constant) gave K_{eq} of 1.68 μM^{-1} and Q_{max} of 18.5 μmol g^{-1} (also shown in Figure 7a). With the value of K_{eq} and the determined structure of Zr-sbdc (ρ_{MOF} = 760 g L^{-1}), we calculated that, in the low DESQ concentration range, the concentration of adsorbed DESQ in Zr-sbdc could reach 2.4×10^4 times higher than its concentration in solution (because $K_{eq} \cdot c_e << 1$, the Langmuir isotherm is simplified to $Q_e = Q_{max} \cdot K_{eq} \cdot c_e$, and the ratio of $c_e^{MOF} : c_e^{soltuion}$ could be obtained by $Q_{max} \cdot K_{eq} \cdot \rho_{MOF}$). This high partition coefficient is rare for MOF sensing systems,[5a] and indicates that the channel structure of Zr-sbdc

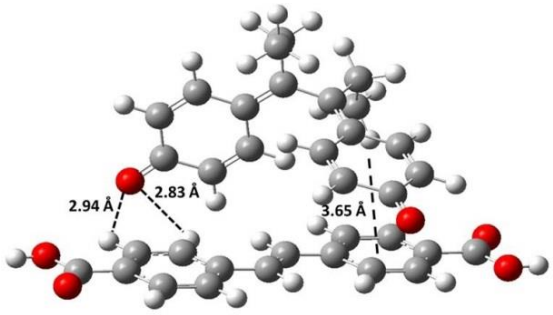


Figure 8. Optimized geometry of the complex formed between H_2sbdc and *cis*-configured DESQ.

serves well in the enrichment of DESQ and thus enhances the sensitivity of fluorescence response. To confirm this assumption, fluorescent titration experiments were carried out on $sbdc^{2-}$ ligand. Although the emission of free ligand could still be quenched by the oxidation product of DES, the sensitivity is much lower than that of Zr-sbdc (Figure S17 in the Supporting Information). Even at the highest DES concentration tested in Zr-sbdc system of 60 μM ($I_0^{Zr\text{-}sbdc}/I^{Zr\text{-}sbdc} - 1 = 14.9$), $sbdc^{2-}$ only gave a small fluorescence response of 0.3. This comparison clearly proves the sensitization effect of MOF adsorption on the fluorescent sensing of DES.

Real sample analysis and reusability

In order to evaluate the feasibility of the proposed method for the detection of DES in real samples, two kinds of food samples, milk and fish extract, were analyzed with it. The results obtained by the standard addition method are summarized in Table 1. It can be seen that the average recoveries were in the range of

Table 1. Analytical results of DES in the real samples.[a]

Sample	Spiked [μM]	Found [μM]	Recovery [%]
	0	–	–
	5	4.95±0.10	99.0±1.9
Milk	10	10.11±0.12	101.1±1.2
	15	15.18±0.17	101.2±1.2
	20	20.42±0.17	102.1±0.8
	0	–	–
	5	4.89±0.02	97.9±0.5
Fish extract	10	9.89±0.16	98.9±1.6
	15	14.69±0.11	98.0±0.7
	20	19.81±0.25	99.0±1.3

[a] 20 μL milk or fish extract and standard DES solution were added simultaneously to Zr-sbdc suspension.

97.9–102.1% and the relative standard deviations were lower than 1.9%. These results demonstrate the potential applicability of this enzyme assisted MOF sensing in food safety analysis.

The reusability of Zr-sbdc was tested by recycling experiments (Figure S18 in the Supporting Information). Between each run, the used Zr-sbdc sensor was separated by filtration, simply recovered by washing with ethanol, dried and used to the next run. The fluorescent intensity of Zr-sbdc after recycling six times could still retain ~80% of the initial value, and its response to DES was nearly unaffected, which demonstrates the high stability and reusability of Zr-sbdc in DES sensing.

Conclusions

In summary, a stilbene based nanoscale MOF was synthesized and used to construct the first enzyme assisted MOF fluorescent sensing system. Although DES cannot cause the fluorescence response of Zr-sbdc, this analyte can still be indirectly detected through the quenching induced by its enzymatic oxidation product. This method exhibited fine structural discrimination and can selectively sense DES over other similar-structured EDCs. Both enzymatic reaction and MOF adsorption contribute to this high selectivity. The substrate specificity of HRP oxidation screens out the homologues that cannot form quinone products, and the following adsorption process ensures that, among quinone products, only DESQ quenches the fluorescence of Zr-sbdc. MOF adsorption also acts a preconcentration function and thus enhances the analytic sensitivity. Our results illustrate that using enzymatic reaction to extend analyte scope and enhance response selectivity is a promising improvement direction of MOF sensing.

Experimental Section

Materials

All used chemicals were obtained commercially and used as received without further purification. The solution of DESQ was prepared according to the literation method.[22] The aqueous solutions used in fluorescent experiments were made up with ultrapure water.

Synthesis of Zr-sbdc

$ZrOCl_2$•$8H_2O$ (96.7 mg, 0.3 mmol) and H_2sbdc (80.5 mg, 0.3 mmol) were dissolved in a mixed solvent of DMF (21 mL) and acetic acid (7 mL) and heated at 120 °C for 8 h with continuous stirring. After cooling down to room temperature, the white precipitate of Zr-sbdc was separated by centrifugation, washed with DMF and methanol in sequence, and then dried in vacuum at 80 °C for 6 h. Anal. Calcd. (%) for $[Zr_6O_4(OH)_4(sbdc)_6](DMF)_3(H_2O)_{30}$ (Mw = 3036.6): C, 41.53; H, 4.81; N, 1.38. Found: C, 41.57; H, 4.82; N, 1.46.

Fluorescent measurement

The powder of Zr-sbdc (3 mg) was immersed in 200 mL Bis-Tris buffer (20 mM, adjusted by HCl to pH = 6.0) and dispersed by ultrasonic apparatus for 12 h. Then let it stand for a day to get a stable suspension (0.015 g L^{-1}). 3 mL of the suspension was used in each fluorescent measurement and placed in a 1×1 cm^2 optical quartz cuvette with continuous stirring at 25 °C. After the sequential addition of DES, HRP and H_2O_2, the fluorescent spectra were recorded at intervals until stable.

Analysis of real sample

Fish extract was freshly prepared before analysis by the following procedures: a piece of yellow croaker (2.5 g) was crushed to homogenate using a mortar and pestle, followed by adding 10 mL of acetonitrile, extracting with ultrasonic agitation for 20 min and centrifuging to get supernatant fluid. In standard addition experiments, milk or fish extract (20 μL) and a certain volume of standard DES solution were simultaneously added to the Zr-sbdc suspension (3 mL), then HRP and H_2O_2 were injected to start the reaction. The fluorescent intensity recorded after reaction completion was used to determine the concentration of DES.

Calculation details

The DFT calculation about the interaction between H_2sbdc and quinones was conducted using the Gaussian 09 program. ωB97X-D functional[28] was selected because this functional was justified to allow a good reproduction of the π-π interactions.[29] The geometry was optimized at ωB97X-D/6-31G(d,p) level to find the energy minimum, and a higher level of ωB97X-D/6-311++G(d,p) was employed for the single point energy calculation.[30] The molecules of quinone and H_2sbdc were optimized separately at first. Then, the obtained geometries of these species were put together with different relative positions as the initial configurations of the geometric optimization of DESQ-H_2sbdc complex. The structural optimization of DESQ molecule could obtain *cis*- and *trans*-conformations, which were both used to construct the initial complex configurations. In the optimization of quinone-H_2sbdc complex, only the quinone molecules were left relaxed, while the atoms of H_2sbdc were fixed to replicate bulk behaviour. Afterward, the energies of the optimized geometries from different initial configurations were compared to determine the geometry with the minimum energy. The binding energies (BEs) were calculated as:

$$BE = E_{complex} - E_{H_2sbdc} - E_{quinone}, \quad (1)$$

where $E_{complex}$, E_{H_2sbdc} and $E_{quinone}$ are the energies of the complex formed by H_2sbdc and quinone, isolated H_2sbdc and quinone, respectively. The counterpoise correction was further taken to eliminate the basis set superposition error (BSSE).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21673121 and 21307062).

Keywords: metal-organic frameworks • sensors • enzyme assist • diethylstilbestrol • tandem filtration

[1] a) L. E. Kreno, K. Leong, O. K. Farha, M. Allendorf, R. P. Van Duyne, J. T. Hupp, *Chem. Rev.* **2012**, *112*, 1105-1125; b) Z. Hu, B. J. Deibert, J. Li, *Chem. Soc. Rev.* **2014**, *43*, 5815-5840; c) B. Li, H.-M. Wen, Y. Cui, W. Zhou, G. Qian, B. Chen, *Adv. Mater.* **2016**, *28*, 8819-8860; d) M.-X. Wu, Y.-W. Yang, *Adv. Mater.* **2017**, *29*, 1606134.

[2] a) S. S. Nagarkar, B. Joarder, A. K. Chaudhari, S. Mukherjee, S. K. Ghosh, *Angew. Chem. Int. Ed.* **2013**, *52*, 2881-2885; b) B. Joarder, A. V. Desai, P. Samanta, S. Mukherjee, S. K. Ghosh, *Chem. Eur. J.* **2015**, *21*, 965-969.

[3] K. S. Lim, S. Y. Jeong, D. W. Kang, J. H. Song, H. Jo, W. R. Lee, W. J. Phang, D. Moon, C. S. Hong, *Chem. Eur. J.* **2017**, *23*, 4803-4809.

[4] W. Xie, W.-W. He, S.-L. Li, K.-Z. Shao, Z.-M. Su, Y.-Q. Lan, *Chem. Eur. J.* **2016**, *22*, 17298-17304.

[5] B. Wang, X.-L. Lv, D. Feng, L.-H. Xie, J. Zhang, M. Li, Y. Xie, J.-R. Li, H.-C. Zhou, *J. Am. Chem. Soc.* **2016**, *138*, 6204-6216; b) Z. Hu, W. P. Lustig, J. Zhang, C. Zheng, H. Wang, S. J. Teat, Q. Gong, N. D. Rudd, J. Li, *J. Am. Chem. Soc.* **2015**, *137*, 16209-16215; c) J.-N. Hao, B. Yan, *Adv. Funct. Mater.* **2017**, *27*, 1603856.

[6] a) E. Diamanti-Kandarakis, J.-P. Bourguignon, L. C. Giudice, R. Hauser, G. S. Prins, A. M. Soto, R. T. Zoeller, A. C. Gore, *Endocr. Rev.* **2009**, *30*, 293-342; b) W. V. Welshons, K. A. Thayer, B. M. Judy, J. A. Taylor, E. M. Curran, F. S. vom Saal, *Environ. Health Perspect.* **2003**, *111*, 994-1006.

[7] R. M. Harris, R. H. Waring, *Maturitas* **2012**, *72*, 108-112.

[8] O. W. Smith, *Obstet. Gynecol. Surv.* **1949**, *4*, 190-191.

[9] H. Tapiero, G. Nguyen Ba, K. D. Tew, *Biomed. Pharmacother.* **2002**, *56*, 36-44.

[10] M. I. Luster, R. E. Faith, J. A. McLachlan, G. C. Clark, *Toxicol. Appl. Pharmacol.* **1979**, *47,* 279-285.

[11] R. M. Giusti, K. Iwamoto, E. E. Hatch, *Ann. Intern. Med.* **1995**, *122*, 778-788.

[12] E. R. Greenberg , A. B. Barnes , L. Resseguie , J. A. Barrett , S. Burnside , L. L. Lanza , R. K. Neff , M. Stevens , R. H. Young , T. Colton, *New Engl. J. Med.* **1984**, *311*, 1393-1398.

[13] M. W. Pfaffl, B. Reck, R. Dreher, H. H. D. Meyer, *Anal. Chim. Acta* **2003**, *483*, 401-412.

[14] a) C. Basheer, A. Jayaraman, M. K. Kee, S. Valiyaveettil, H. K. Lee, *J. Chromatogr. A* **2005**, *1100*, 137-143; b) A. Peñalver, E. Pocurull, F. Borrull, R. M. Marcé, *J. Chromatogr. A* **2002**, *964*, 153-160.

[15] G. Degand, P. Schmitz, G. Maghuin-Rogister, *J. Chromatogr. B* **1989**, *489*, 235-243.

[16] a) J. Bai, X. Zhang, Y. Peng, X. Hong, Y. Liu, S. Jiang, B. Ning, Z. Gao, *Sens. Actuators B* **2017**, *238*, 420-426; b) J. S. Aragao, F. W. P. Ribeiro, R. R. Portela, V. N. Santos, C. P. Sousa, H. Becker, A. N. Correia, P. de Lima-Neto, *Sens. Actuators B* **2017**, *239*, 933-942; c) Q. Bin, W. Wei, Y. W. Chi, G. N. Chen, *Anal. Biochem.* **2005**, *336*, 196-201.

[17] S. Liao, H. G. Williams-Ashman, *Biochim. Biophys. Acta* **1962**, *59*, 705-707.

[18] S. Pramanik, C. Zheng, X. Zhang, T. J. Emge, J. Li, *J. Am. Chem. Soc.* **2011**, *133*, 4153-4155.

[19] J. Zhang, S. Yao, S. Liu, B. Liu, X. Sun, B. Zheng, G. Li, Y. Li, Q. Huo, Y. Liu, *Cryst. Growth Des.* **2017**, *17*, 2131-2139.

[20] a) R. Zhu, Y. Zhao, T. Sun, H. Liu, Y. Zhang, X. Li, *Colloid. Polym. Sci.* **2015**, *293*, 2469-2475; b) J. Saltiel, *J. Am. Chem. Soc.* **1967**, *89*, 1036-1037.

[21] a) C.-X. Lei, S.-Q. Hu, G.-L. Shen, R.-Q. Yu, *Talanta* **2003**, *59*, 981-988; b) G. Bayramoğlu, M. Y. Arıca, *J. Hazard. Mater.* **2008**, *156*, 148-155.

[22] J. G. Liehr, B. B. DaGue, A. M. Ballatore, J. Henkin, *Biochem. Pharmacol.* **1983**, *32*, 3711-3718.

[23] a) K. Yin, F. Yu, D. Liu, Z. Xie, L. Chen, *Sens. Actuators B* **2016**, *223*, 799-805; b) J. Wang, H. Z. Ye, Z. Jiang, N. S. Chen, J. L. Huang, *Anal. Chim. Acta* **2004**, *508*, 171-176.

[24] a) Z.-Q. Shi, Z.-J. Guo, H.-G. Zheng, *Chem. Commun.* **2015**, *51*, 8300-8303; b) Q. Zhang, A. Geng, H. Zhang, F. Hu, Z.-H. Lu, D. Sun, X. Wei, C. Ma, *Chem. Eur. J.* **2014**, *20*, 4885-4890.

[25] L. Gianfreda, G. Iamarino, R. Scelza, M. A. Rao, B*iocatal. Biotransfor.* **2006**, *24*, 177-187.

[26] a) N. B. Shustova, B. D. McCarthy, M. Dincă, *J. Am. Chem. Soc.* **2011**, *133*, 20126-20129; b) Y. Ren, J. W. Y. Lam, Y. Dong, B. Z. Tang, K. S. Wong, *J. Phys. Chem. B* **2005**, *109*, 1135-1140.

[27] B. D. McCarthy, E. R. Hontz, S. R. Yost, T. Van Voorhis, M. Dincă, *J. Phys. Chem. Lett.* **2013**, *4*, 453-458.

[28] J.-D. Chai, M. Head-Gordon, *Phys. Chem. Chem. Phys.* **2008**, *10*, 6615-6620.

[29] W. Wang, Y. Zhang, Y.-B. Wang, *J. Chem. Phys.* **2014**, *140*, 094302.

[30] a) S. Kasuriya, S. Namuangruk, P. Treesukol, M. Tirtowidjojo, J. Limtrakul, *J. Catal.* **2003**, *219*, 320-328; b) Y. Wu, H. Chen, D. Liu, J. Xiao, Y. Qian, H. Xi, *ACS Appl. Mater. Interfaces* **2015**, *7*, 5775-5787.

10.1002/chem.201703438

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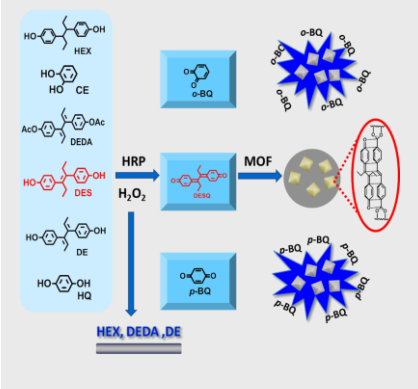
Layout 1:

FULL PAPER

Enzyme assistd MOF sensing system is designed for the first time for the fluorescent detection of diethylstilbestrol. Within it, diethylstilbestrol is specifically oxidized by HRP/H_2O_2 to its quinone form, which is selectively captured by a stilbene based luminescent MOF to induce fluorescence response. By the tandem filtration of enzymatic reaction and MOF adsorption, fine structural discrimination of analyte is achieved.



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