Energy-Transfer Metal–Organic Nanoprobe for Ratiometric Sensing with Dual Response to Peroxynitrite and Hypochlorite

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Supporting Information

ABSTRACT: An energy-transfer metal–organic nanoprobe is designed for ratiometric sensing with dual response to both peroxynitrite (ONOO⁻) and hypochlorite (ClO⁻). Here, a nanoscale metal–organic framework (NMOF) acts as the energy donor and molecular probe as the acceptor to construct a Förster resonance energy transfer (FRET) nanosystem. Biocompatible dextran conveniently binds to the NMOF surface through multiple weak coordination interactions to improve water dispersibility and cell uptake. Dextran can also coordinate with the molecular probe with arylboronic acid group, which enables the convenient grafting of molecular probes to the NMOF surface to construct energy-transfer nanoprobes. Because of efficient FRET, the bright blue fluorescence of NMOF is quenched, whereas red emission from the acceptor is enhanced. Upon reacting with ONOO⁻, the probe departs from NMOF and the fluorescence of NMOF is recovered because of the interruption of FRET. When reacting with ClO⁻, the phenothiazine moiety in the molecular probe is oxidized into phenothiazine-S-oxide, which leads to more efficient energy transfer and the fluorescence shifts from red to orange. The nanoprobes are also successfully applied to the detection of ONOO⁻ and ClO⁻ in living cells.

INTRODUCTION

Metal–organic frameworks (MOFs), self-assembled from metal ions and organic ligands, are considered as a novel family of materials with diverse and designable structures. They have gained great attraction owing to their potential applications in energy storage, gas adsorption, catalysis, separation, and so on. With the particle sizes of MOFs well controlled to the nanoscale as nanoscale MOFs (NMOFs), they exhibit great potential applications in bioimaging, drug delivery, photodynamic therapy, sensors, and so forth. NMOFs present a high synthetic flexibility because the directional interactions between metal ions and ligands can be systematically controlled and tuned. NMOFs also show intrinsic benefits of the combination of metal ions and organic ligands. NMOFs with good dimensions between 20 and 200 nm have been reported with regular or some irregular shapes.

Various kinds of surface functionalization of the NMOFs could improve their stability and cell internalization, as well as grafting biomolecules, which is a key step toward biomedical applications. Proteins, DNA, RNA, peptides, and polymers, are usually applied to functionalize NMOFs. Dextran derived from the natural materials, with excellent biocompatibility and water solubility, has been used as a matrix or functionalization material for NMOFs. As the surface functionalization material, dextran could control the interaction between nanoprobes and the analytes as biological media. Here, we report a simple yet useful surface functionalization method to form ratiometric fluorescent nanoprobes with dual response to both peroxynitrite (ONOO⁻) and hypochlorite (ClO⁻).

Reactive oxygen species (ROSs) are essential signaling molecules, which are implicated in various diseases, such as autoimmunity disease, asthma, kidney disease, cardiovascular diseases, and even cancers. Both ONOO⁻ and ClO⁻ are powerful natural oxidants that could react with proteins, DNA, RNA, and other biomolecules and play important roles in physiology and pathology in living system. The precise roles of them in biological activities remain elusive, although they are of great important. Thereby, the accurate and reliable analytical methods to detect ONOO⁻ and ClO⁻ are necessary to elucidate their functions. In the past decades, lots of fluorescence ROS sensors have been developed based on various fluorophores, including fluorescein, rhodamine, coumarin, boron-dipyrromethene, metal complexes, and so on. Ratiometric fluorescent nanoprobes have the advantages of long-term tracking and good stability, and they work through changes at two different wavelengths to increase the dynamic ranges of the measurement, which is significant during the detection in living cells.

In this work, the nanoprobe was consisted of NMOF, the surface functionalization material (dextran), and a small molecular probe (BPP). The arylboronate group from BPP could react with ONOO⁻ to form hydroxyl derivatives, and the...
phenothiazine group of BPP could be oxidized into phenothiazine-5-oxide\textsuperscript{36} after reacting with ClO$^-$. Dextran was selected as a desired agent for NMOF surface functionalization and combination with BPP. The proposed structures of dextran with BPP to form Dex-BPP are shown in Scheme 1, which could bind with bis(boronates) in its pyranose form through the 4-OH, 5-CH$_2$OH, and 1,2-diols.\textsuperscript{37} In the meantime, the unreacted 1,2-diols of dextran could coat onto the surface of NMOF through multiple weak coordination with metal ions. After surface functionalization of Dex-BPP onto the NMOF, the Förster resonance energy transfer (FRET) was built using NMOF as the donor and the BPP probe as the accepter. Upon reacting with ONOO$^-$, BPP detached from NMOF, resulting in the interruption of the FRET between them. When the NMOF coating with Dex-BPP named MD reacted with ClO$^-$, compared with the original BPP structure, the oxidized sulfoxide form showed higher efficiency of the energy transfer. On the basis of the fluorescent change of the FRET system, a ratiometric fluorescent nanoprobe has been demonstrated, which shows different responses to ONOO$^-$ and ClO$^-$. 

**METHODS**

**Preparation of the NMOF Probe.** Dextran (0.2 g) was dissolved in 10 mL of deionized water and mixed with the BPP solution [1.55 mg of BPP in 5 mL of dimethylformamide (DMF)]. Triethylamine was used to adjust the pH of the solution to 8–9. The coating reaction was carried out at room temperature overnight to obtain the Dex-BPP solution. Meanwhile, NMOF was dispersed in DMF (5 mL, 0.5 mg/mL) and mixed with 1 mL of the Dex-BPP solution and continued to stir overnight. The MD product was collected by centrifugation and then washed with DMF and water three times.

**General Experiment for ONOO$^-$ and ClO$^-$ Testing of the NMOF Probe.** The experiments of photophysical characterization were carried out at room temperature. Absorption spectra (Shimadzu UV-3600 spectrophotometer) and fluorescence spectra (Fluorolog-3 spectrophotometer) measurements were performed. The MD was dispersed in deionized water. ONOO$^-$ was prepared by the reported methods,\textsuperscript{38} and ClO$^-$ was prepared by directly diluting commercially available NaClO. To test the fluorescence responses of the MD toward ONOO$^-$, ClO$^-$, or other reactive agents, MD stock solutions were diluted with phosphate-buffered saline (PBS) and treated with desired concentration of analytes. The fluorescence spectra under the excitation of 340 nm were measured 20 min after the addition of ROS. The emission spectra were scanned from 360 to 650 nm.

**Cell Culture and Imaging.** The cytotoxicity assay was carried out by using the methyl thiazolyl tetrazolium (MTT) Cell Proliferation and Cytotoxicity Assay Kit (Beyotime, China) against HeLa cells. The cervical cancer line Hela cells were kindly provided by Faculty of Health Sciences, University of Macau. Hela cells were incubated in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin solution. All cells were maintained at 37 °C, 5% CO$_2$, a humidified environment. For imaging ONOO$^-$, Hela cells were seeded into glass-bottomed culture dishes and allowed to adhere for 24 h.\textsuperscript{39,40} MD (10 mg/L) was added and incubated for 30 min and then incubated with 1 mM ONOO$^-$ donor SIN-1 for 30 min at 37 °C in the same medium. After the incubation, cells were washed twice by using prewarm PBS buffer. Fixative solution (Histochoice Mb Tissue Fixative, Amresco) was added for 15 min at room temperature and then washed three times using PBS buffer. Cells without SIN-1 were also employed as the control in the same process. For imaging ClO$^-$, HeLa cells were seeded into glass-bottomed culture dishes and allowed to adhere for 24 h. MD (10 mg/L) was added and incubated for 30 min, and then, 5 μM NaClO was added and incubated for another 20 min. After the incubation, cells were treated the same as before. The samples were observed by Carl Zeiss Confocal LSM710.
RESULTS AND DISCUSSION

Synthesis and Characterization of the NMOF Probe. The NMOF probe was prepared according to our previous work with some modification as described in the Supporting Information. As shown in Figure 1a,b, the maximum absorption peak of NMOF was 340 nm and the emission wavelength of NMOF was 410 nm. The molecular probe with the arylboronate group and phenothiazine group was designed to sense ONOO− and ClO−, respectively, and the synthetic route is shown in Scheme S1. The arylboronate group could react with ONOO− to form hydroxy derivatives, and divalent sulfur in the phenothiazine group could be oxidized into sulfoxide by ClO−. BPP showed weak fluorescence in polar medium because of the strong intramolecular charge transfer (ICT). It was revealed that loading molecules with strong ICT into pores of MOF or linking onto MOF surface could enhance fluorescence because of the change of the polarity of the local environment. In this nanoprobe system, as expected, efficient energy transfer from the NMOF to the molecule BPP was observed after a small amount of BPP was coated onto the surface of the NMOF. Upon reacting with ONOO−, BPP detached from NMOF and the FRET between them was destroyed, which led to the recovery of NMOF fluorescence and the fading of the BPP fluorescence. When MD reacted with ClO−, compared with the original BPP structure, oxidized sulfoxide showed higher efficiency of the energy transfer.

In this system, dextran was applied as the role of bridge between NMOF and BPP to form the FRET system as described in the Supporting Information. After surface coating, the structure of the NMOF was almost as before coating.

Figure 1. (a) Absorption spectra of NMOF, Dex-BPP, and MD; (b) fluorescence spectra of NMOF and MD with an excitation wavelength at 340 nm; (c) absorption spectra of BPP, BPP with ONOO−, and BPP with ClO− together with fluorescence spectra of NMOF; (d) photographs of fluorescence changing of NMOF (10 mg/L), MD (10 mg/L), MD (10 mg/L) with 10.0 μM ONOO−, and MD (10 mg/L) with 10.0 μM ClO−. Excitation of the UV lamp: 365 nm; and (e) fluorescence lifetimes at 420 nm of NMOF (10 mg/L), MD (10 mg/L), MD (10 mg/L) with 10.0 μM ONOO−, and MD (10 mg/L) with 10.0 μM ClO− excited under 340 nm.
The fluorescence responses of the NMOF-based nanoprobe MD to ONOO⁻ and ClO⁻ were studied. MD emitted purple fluorescence with an emission peak at 595 nm, which was consistent with the fluorescence of the BPP molecule. Upon the addition of ONOO⁻, MD exhibited a hypsochromic shift of the fluorescence spectra with isosbestic points at 515 nm as shown in Figure 3a. With the increase of ONOO⁻ concentration, the emission peak of MD at 595 nm decreased in concert with an increase of the blue band peaked at 410 nm. The ratio of the fluorescence intensities of MD at 410 nm to 595 nm in Figure 3b linearly correlated with ONOO⁻ concentration from 0.00 to 1.00 μM, which indicated an accurate detection limit. Upon the addition of ClO⁻, the emission peak of MD at 570 nm increased with the increase of ClO⁻ concentration; in the meantime, the band with blue color at 410 nm was kept stable as shown in Figure 3c. The ratio of the fluorescence intensities of MD at 570 nm to 410 nm in Figure 3d also linearly correlates with ClO⁻ concentration from 0.00 to 10.00 μM. The results of the MD probe to ONOO⁻ and ClO⁻ corresponded to the sensing mechanism as presented in Scheme 1. The photostability experiment was performed, and the results are shown in Figure S6; the emission curve showed almost no change after the illumination.

To evaluate the selectivity of MD to ONOO⁻ and ClO⁻, the MD probe was reacted with common ROSs and metal ions, including ONOO⁻, ClO⁻, H₂O₂, NO⁻, HO⁺, ·OH, r-BuOOH, Cu²⁺, Zn²⁺, and Ca²⁺. The fluorescence spectra are shown in Figure 4a; ONOO⁻ and ClO⁻ caused obvious ratiometric fluorescent response of the spectra. Furthermore, other ROSs and metal ions could not promote an apparent change in the MD spectra when present in 10 equivalent excess. The fluorescence intensity ratios F⁴₁₀nm/F⁵₉₅nm of MD to ONOO⁻ and F⁵₇₀nm/F⁴₁₀nm of MD to ClO⁻ as shown in Figure 4b,c in contrast to other samples were enhanced about 5 times and 2 times, respectively. These results indicated that MD displayed selectivity toward ONOO⁻ and ClO⁻ to other common ROSs.

MTT cell viability assay was performed to evaluate the cytotoxicity of MD, and the results showed that MD has low toxicity. The desirable fluorescence properties of MD for ONOO⁻ and ClO⁻ prompted us to utilize it for detection of intracellular ONOO⁻ or ClO⁻. As shown in Figure 5a, when Hela cells were incubated with MD, they exhibited red fluorescence, which means that MD was cell-permeable and still had the FRET effect in cells. When the Hela cells were treated with SIN-1, the red fluorescence was quenched and the blue fluorescence of NMOF was recovered as presented in Figure 5b, indicating that MD could image the intracellular ONOO⁻ with dual color switching. After being treated with...
NaClO (5 μM) for 20 min, intense yellow fluorescence emerged in the cells in Figure 5c. The merged cell pictures in Figure 5 confirmed that the fluorescence signals were just located in the intracellular area. The good cell membrane permeability and significant dual color switching enabled MD as an excellent nanoprobe for ratiometric detection of ONOO− and ClO−.

### CONCLUSIONS

In conclusion, we have developed a novel metal–organic nanoprobe for ratiometric sensing with dual response to both ONOO− and ClO− through FRET. Dextran is introduced to conveniently bind to the surface of the energy donor NMOF and combine with the energy acceptor molecular probe. This nanoprobe exhibits excellent photostability, high brightness, good water solubility, and cell internalization, which is successfully applied to the detection of ONOO− and ClO− in living cells with different two-color switches. The novel probe is a promising tool for the investigations of ONOO−- and ClO−-related diseases. The design strategy of energy-transfer nanoprobes presented herein might be extended to construct other ratiometric fluorescent nanoprobes for a variety of applications.

### ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01489.

Synthetic methods of NMOF and Dex-BPP; 1H NMR, 13C NMR, and FT-IR spectroscopy spectra of the nanoprobe; photostability experiments of MD; and MTT assay result of MD (PDF)

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Notes

The authors declare no competing financial interest.

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