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Rapid and facile ratiometric detection of an *anthrax* biomarker by regulating energy transfer process in bio-metal-organic framework

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ABSTRACT

A ratiometric fluorescent sensor based on luminescent bio-metal-organic framework was prepared by exchanging both Tb^{3+} and Eu^{3+} cations into anionic bio-MOF-1. Due to a highly efficient energy transfer from Tb^{3+} to Eu^{3+} (> 89%), emission color of Tb/Eu@bio-MOF-1 was orange-red even though Tb^{3+} was the dominant content in this Tb/Eu co-doping material. More interestingly, this energy transfer process could be modulated by dipicolinic acid (DPA), an unique biomarker for bacillus spores. With DPA addition, corresponding DPA-to-Tb^{3+} energy transfer was gradually enhanced while the energy transfer from Tb^{3+} to Eu^{3+} was significantly weakened. By regulating the energy transfer process in Tb/Eu@bio-MOF-1, visual colorimetric sensing of DPA in porous MOF was realized for the first time. Detection limit of Tb/Eu@bio-MOF-1 for DPA was 34 nM, which was much lower than an infectious dosage of *Bacillus anthracis* spores (60 μM) for human being. Besides, Tb/Eu@bio-MOF-1 showed a remarkable selectivity over other aromatic ligands and amino acids. More importantly, this porous ratiometric sensor worked equally well in human serum. These particularly attractive features of Tb/Eu@bio-MOF-1 made the direct, rapid and naked-eye detection of DPA for practical application possible.

1. Introduction

Anthrax is one of the acute infectious disease which infects both animals and humans. *Bacillus anthracis* has been reported as a potential bioterrorism agent since the *anthrax* attack in the U. S. in 2001, and still attracts broad attention throughout the world (Webb, 2003; Yung et al., 2007). A number of attempts have been made to detect bacillus spores such as bacteriology, serology-immunoassays and polymerase chain reactions (PCR) (Das et al., 2015; Hurtle et al., 2004). However, traditional biological methods usually require lengthy cycles, complicated operation, expensive reagents and professional analysis, making them not suitable for on-line monitoring (Su et al., 2016). Rapid, sensitive and straightforward detection of bacillus spores is crucial for disease and bioterrorism prevention (Ai et al., 2009). Recently, optical techniques for dipicolinic acid (DPA, a unique biomarker and major constituent of bacterial spores) detection have caused wide concern due to their low cost, fast response and easy portability. Surface enhanced Raman spectroscopy (SERS) and surface plasmon resonance (SPR) biosensors have been explored to detect biological molecules, respectively (Gao et al., 2015; Lepage et al., 2013; Zhang et al., 2005). Another convenient optical sensing technique for biological molecules is based on lanthanide luminescence method, which is rapid, selective and highly sensitive (Oh et al., 2011; Yilmaz et al., 2010). In previous reports, terbium (Tb) has been proved effective for DPA detection (Rosen et al., 1997; Tsukube and Shinoda, 2002). However, its nonselective binding for anionic interferents limits its further application. Europium (Eu) based nanocomposite has shown improved selectivity (Ai et al., 2009). However, Tb-based and Eu-based sensors determine DPA concentration through emission intensity variation which is greatly influenced by excitation, environmental and instrumental factors (Li et al., 2013). Sensing reliability can be improved by using a ratiometric fluorescent sensor, which measures the ratio between analyte signal and reference signal (Guan et al., 2015; Zou et al., 2014).

Luminescence from lanthanide ions is usually weak due to their forbidden f-f transitions. Many proposals have been reported to enhance their luminescence such as coordinating to organic ligands or being doped into special matrices (Eliseeva and Bunzli, 2010; Shen and Yan, 2015a; Zhang et al., 2015). There are several advantages in introducing lanthanide centers into metal organic

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frameworks (Chen et al., 2010; White et al., 2009). Firstly, MOFs have a number of photons emitted in per unit volume, which enhances lanthanide luminescence (An et al., 2011). Secondly, their excellent stability and porous rigid scaffold protect lanthanide ions from complex environment. Thirdly, their high adsorption volume and large surface area can gather analyte and amplify reaction zone (Wang et al., 2012). Most importantly, MOFs structures can be precisely designed and manipulated to meet specific needs.

Herein, we present a direct and rapid strategy for colorimetric detection of *Bacillus anthracis* biomarker DPA using Tb^{3+}/Eu^{3+} co-doped Bio-MOF (here-after denoted as Tb/Eu@bio-MOF-1). Luminescent Tb/Eu@bio-MOF-1 was synthesized via exchanging Tb^{3+} and Eu^{3+} cations into anionic bio-MOF-1. The resulting MOF exhibited orange-red emission color due to a highly efficient energy transfer from Tb^{3+} to Eu^{3+}. After meeting DPA, DPA-to-Tb^{3+} energy transfer was gradually enhanced while the energy transfer from Tb^{3+} to Eu^{3+} was significantly weakened, leading to fluorescence color change from orange-red to green. For the first time, we realized visual colorimetric sensing of DPA by regulating the energy transfer process in Tb/Eu@bio-MOF-1.

2. Materials and methods

2.1. Chemicals and materials

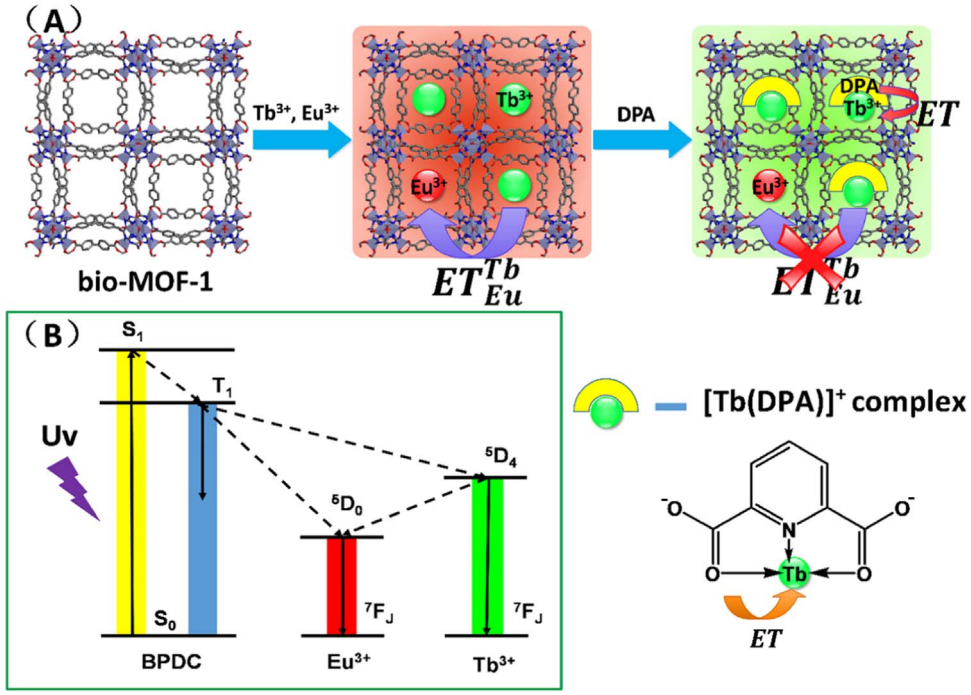
Adenine, 4,4′-biphenyl dicarboxylic acid (BPDC), zinc acetate dihydrate, 2,6-pyridinedicarboxylic acid (DPA) were purchased from commercial sources. $Eu(NO_3)_3$●6 h_2O was obtained by dissolving Eu_2O_3 in concentrated nitric acid with agitation, and then heating this solution to dryness. In addition to adding hydrogen peroxide, $Tb(NO_3)_3$●6 h_2O was obtained by the same way from Tb_4O_7. Unless otherwise mentioned, all purchased analytical grade solvents were used without further purifications. Double distilled water was used in this work.

2.2. Characterization

X-ray powder diffraction (XRD) patterns were obtained from a Bruker D4 X-ray diffractometer (Germany) with Cu $K\alpha_1$ radiation (λ=1.5405 Å, 40 kV, 30 mA). Scanning electron microscopy (SEM) images were measured on a Hitachi S-4800 microscope. Contents of Tb^{3+}, Eu^{3+} and Zn^{2+} in our work were determined by an iCAP6300 inductively coupled plasma-optical emission spectrometer (ICP-OES, US Thermo Scientific). Samples were prepared by decomposing the powder samples into concentrated nitric acid, followed by ultrasonic treatment and dilution to 1% nitric acid solution. N_2 adsorption and desorption isotherms were measured at liquid nitrogen temperature, using a Nova l000 analyzer (US Quantachrome Corporation Company). Samples were degassed in vacuum at 150 °C for at least 10 h before adsorption and their surface area values were calculated according to Brunauer-Emmett-Teller (BET) equation. Fluorescence excitation and emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer using a 450 W xenon lamp as excitation source. Emission lifetimes were measured with a Lecroy Wave Runner 6100 Digital Oscilloscope (1 GHz) using a tunable laser as excitation source (Continuum Sunlite OPO) and calculated by exponential fitting.

2.3. Preparation of bio-MOF-1

Construction route for Tb/Eu@bio-MOF-1 sensor and DPA sensing mechanism are depicted in Scheme 1. Bio-MOF-1 was synthesized through a solvothermal method as reported previously (An et al., 2009). Typically, adenine (0.25 mmol), 4,4′-biphenyl dicarboxylic acid (0.5 mmol) and zinc acetate dihydrate (0.75 mmol) were dissolved in DMF (27 mL) and water (2 mL) and stirred vigorously for 60 min, then nitric acid (2 mmol) was added. After these reagents were well mixed, this mixture was sealed in a 40 mL Teflon-lined stainless-steel autoclave, heated at 130 °C for 48 h. Rod-shaped crystals were collected and washed with DMF and dichloromethane (CH_2Cl_2) several times and dried in vacuum at 60 °C overnight.



Scheme 1. (A) Synthesis strategy of Tb/Eu@bio-MOF-1 and its principle for the detection of DPA; (B) Schematic diagram of the ligand-metal energy transfer from triplet state of the BPDC to Ln^{3+} ions and the metal-metal energy transfer from $^5D_4$ of the Tb^{3+} to $^5D_0$ of the Eu^{3+} ions.