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Imidazolium Ionic Liquid Functionalized Eu^{3+}-ZIF Rapidly and Sensitively Detects Lysine

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Abstract

An eco-friendly and green rare-earth hybrid material, Eu^{3+}-ZIF@IL, was synthesized using ionic liquids as a medium through a straightforward hydrothermal method. This material maintains the inherent stability and high porosity of ZIFs while enhancing fluorescence detection capabilities via the integration of ionic liquids. This innovation has yielded a probe with superior fluorescent properties, making it especially effective for the recognition and detection of biomolecules. In practical applications, Eu^{3+}-ZIF@IL exhibits high selectivity and sensitivity towards lysine, achieving effective analysis in complex biological matrices with a detection limit as low as 26.42 μM. This demonstrates its outstanding performance and significant potential in the market for fluorescent probes.

Keywords

Ionic Liquid; Rare Earth Hybrid Probe; Sensing Detection; Zeolitic Imidazolate Framework; Lysine.

1. Introduction

Amino acids constitute the basic structure of biological proteins[1] and play crucial roles in organisms. They participate in metabolism, maintain cellular redox environments, balance the human nervous system, and provide energy sources for organisms, truly serving as the source of life [2]. In particular, essential amino acids, as precursors and intermediates of normal physiological activities in the human body, are key parameters and important indicators for diagnosing diseases [3-5]. With the advancement of instrumentation technology, analytical methods such as high-performance liquid chromatography (HPLC) [6], capillary electrophoresis [7], and mass spectrometry [8] have been widely used for amino acid detection. Although these methods exhibit high accuracy and selectivity, their widespread application is limited due to their time-consuming nature, high cost, and complex operation. In contrast, fluorescence-based detection methods [9] have increasingly attracted attention due to their high sensitivity and simplicity. In this regard, while much research has focused on probe fluorescence quenching characteristics, there has been relatively less research on probe fluorescence turn-on mechanisms. Fluorescence turn-on probes typically exhibit higher signal-to-noise ratios and can effectively avoid false responses, making them more suitable for practical detection needs.

Over the past few decades, researchers have reported various fluorescence turn-on probes for amino acid sensing, including organic small molecules [10], carbon dots [11], polymer dots [12], and metal-organic frameworks (MOFs) [13]. The fluorescence turn-on detection mechanisms of these probes can be mainly divided into two categories: aggregation-induced emission (AIE) and the "on-off-on" model. The AIE mechanism, first discovered by the Tang research group [14], points out that certain thiophene molecules emit almost no light in solution but exhibit significantly enhanced luminescence

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in aggregated states or solid films. This luminescence enhancement is caused by aggregation. In the "on-off-on" model [15, 16], researchers first introduce a quenching group into a highly emissive fluorescence probe to turn off luminescence (on to off). When the analyte is added, it disrupts the interaction between the probe and the quenching group, thus restoring the high fluorescence emission of the original probe (off to on).

Zeolitic imidazolate frameworks (ZIFs) [17, 18], as a promising subclass of metal-organic frameworks (MOFs), are emerging materials in the field of porous materials. ZIFs are widely used in catalysis, electrochemistry, and gas adsorption. Their excellent thermal and chemical stability also makes them suitable for industrial applications such as flue gas separation and natural gas purification. Previous research has mainly focused on modifying the framework to enhance the selectivity of ZIFs in adsorption processes. Li and his research team [19] synthesized a symmetric zwitterionic liquid $[C_6BIm_2]$ $[NTf_2]_2$ as a sterically hindered solvent to construct a porous ionic liquid based on low-viscosity zeolitic imidazolate frameworks (ZIFs). The good chemical and thermal stability of ionic liquids allows ZIF-67 to maintain a stable state under a wide range of temperatures and chemical environments.

In this paper, we designed and studied an imidazolium-based ionic liquid as a carrier to encapsulate Eu^{3+}-ZIF through covalent linkage. First, Eu^{3+}-ZIF was synthesized through a hydrothermal method. Then, [BMIM][Cl] and the carboxylic acid ligand DPA were used to promote fluorescence generation and stability in water, resulting in Eu^{3+}-ZIF@IL. This probe can effectively detect lysine in water with high selectivity, with a detection limit of 23.42 μM. Notably, the fluorescence response of the probe to other amino acids, including arginine, glutamic acid, threonine, and aspartic acid, is completely different from that of lysine, which has practical significance in biochemical and environmental monitoring fields.

2. Experimental

2.1 Reagents and Chemicals

1-methylimidazole, 2,6-pyridinedicarboxylic acid, $EuCl_3 \cdot 6H_2O$, 3-butyl-1-methyl-1H-imidazol-3-ium chloride, arginine, glutamate, threonine and aspartic acid were purchased from Adamas-Beta Co., Ltd. (Shanghai, China). Anhydrous ethanol, 2,2'-azobis(2-methylpropionitrile), N, N-dimethylformamide and L-cysteine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycine, alanine, phenylalanine and lysine was purchased from Aladdin Chemical Regent Co., Ltd. (Shanghai, China).

2.2 Characterization

Fourier transform infrared spectroscopy (FT-IR) in KBr pellets was obtained on a Nexus 912 AO446 spectrophotometer in the range of 4000−400 cm^{-1}. The X-ray photoelectron spectroscopy (XPS) analysis was carried out on K-α+ supplied by Thermo Fisher Scientific. Among them, monochromatic Al Kα source was the X-ray source. Fluorescence spectra were measured with a RF-6000 spectrophotometer (wavelength resolution was 0.5 nm) using a xenon lamp as an excitation source.

2.3 Experimental Procedures

The synthesis process of rare earth hybrid probe Eu^{3+}-ZIF@IL is shown in Scheme 1 as follows.

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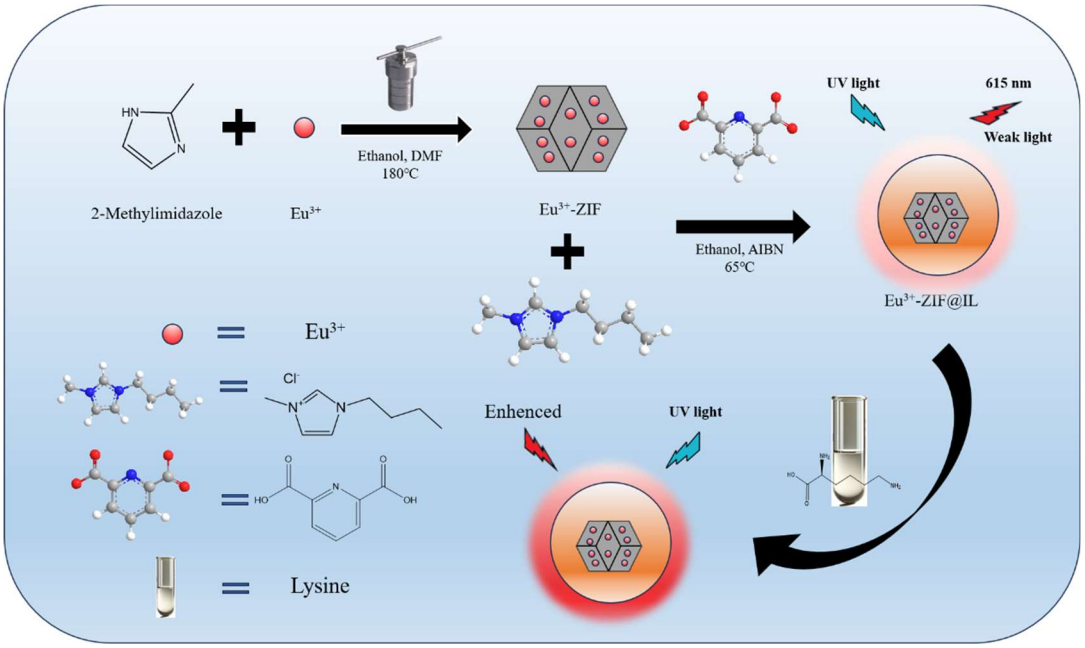

Scheme 1. Experimental Process of Eu^{3+}-ZIF@IL

2.3.1 Preparation of Ionic Liquid Eu^{3+}-ZIF

The synthesis method of Eu^{3+}-ZIF is based on the previous report [20]. 0.041 g of 2-methylimidazole (0.5 mM) and 0.037 mg of $EuCl_3 \cdot 6H_2O$ (0.1 mM) were added to a mixed solution of 2 mL ethanol and 10 mL DMF, respectively. Ultrasonic treatment was then performed for 10 minutes to obtain a uniform solution before mixing and ultrasonic treatment for 5 minutes. The mixture was then transferred to a 50 ml Teflon container and reacted at 180 °C for 24 hours. Finally, the obtained white crude product was thoroughly washed three times with water and absolute ethanol. After drying, the resulting white solid was Eu^{3+}-ZIF.

2.3.2 Preparation of Ionic Liquid Eu^{3+}-ZIF@IL

50 mg of Eu^{3+}-ZIF was ultrasonically dispersed in 10 ml of absolute ethanol. Then, 5 ml of a 10 mM DPA solution was gradually added dropwise to the Eu^{3+}-ZIF reaction system and reacted at room temperature for 2 hours. Subsequently, 0.5 g of 1-butyl-3-methylimidazolium chloride was added to the system. After purging with N_2 for 30 minutes, 10 ml of an ethanol solution containing 0.01 g of AIBN was slowly injected into the solution. The entire system was then heated to 65 °C and continuously stirred under a closed condition for 10 hours. After the reaction, the white product was washed three times with absolute ethanol and then placed in a vacuum oven for drying for 12 hours to obtain the final product, Eu^{3+}-ZIF@IL.

2.3.2 Sensing Detection of Lysine (Lys)

A uniform solution of Eu^{3+}-ZIF@IL was prepared and then added dropwise to a series of Lys solutions with different concentrations. The fluorescence intensity changes were measured using a fluorescence spectrometer. The concentration range of the added Lys solutions was from 100 μM to 500 μM. The fluorescence emission spectra were measured at an excitation wavelength of 278 nm, with the emission wavelength of Eu^{3+}-ZIF@IL being 615 nm. For all fluorescence tests, the slit width of the instrument was set to 2 nm.

3. Results and Discussion

3.1 Material Characterizations

The surface structures of [BMIM][Cl], Eu^{3+}-ZIF, and Eu^{3+}-ZIF@IL were analyzed in detail using Fourier Transform Infrared Spectroscopy (FTIR). Fig.1 exhibits an absorption peak at 3369 cm^{-1}, which is attributed to the stretching vibration of the N-H bond. The absorption peaks located at 1558 cm^{-1} and 1504 cm^{-1} in Eu^{3+}-ZIF correspond to the stretching vibrations of C-N and N-H bonds,

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respectively. Notably, the C-Cl stretching vibration at 1076 cm^{-1}, the C-C stretching vibration at 1250 cm^{-1}, and the C=C stretching vibration at 1595 cm^{-1} reveal the close interaction between the ionic liquid [BMIM][Cl] and Eu^{3+}-ZIF, which is fully consistent with the hybrid material ZIF@[BMIM][Cl] described in the literature [21]. Additionally, the increased sharp peak at 1438 cm^{-1} further validates the tight binding between the sample [BMIM][Cl] and Eu^{3+}-ZIF, indicating the intact surface structure of Eu^{3+}-ZIF@IL.

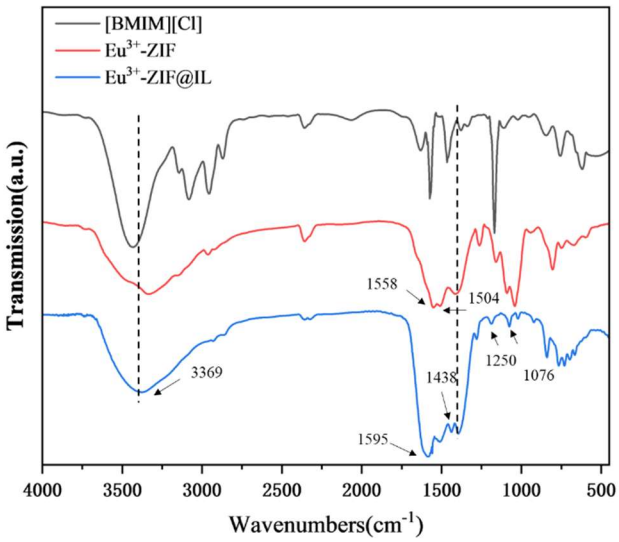

Fig.1 The infrared spectra of [BMIM][Cl], Eu^{3+}-ZIF andEu^{3+}-ZIF@IL

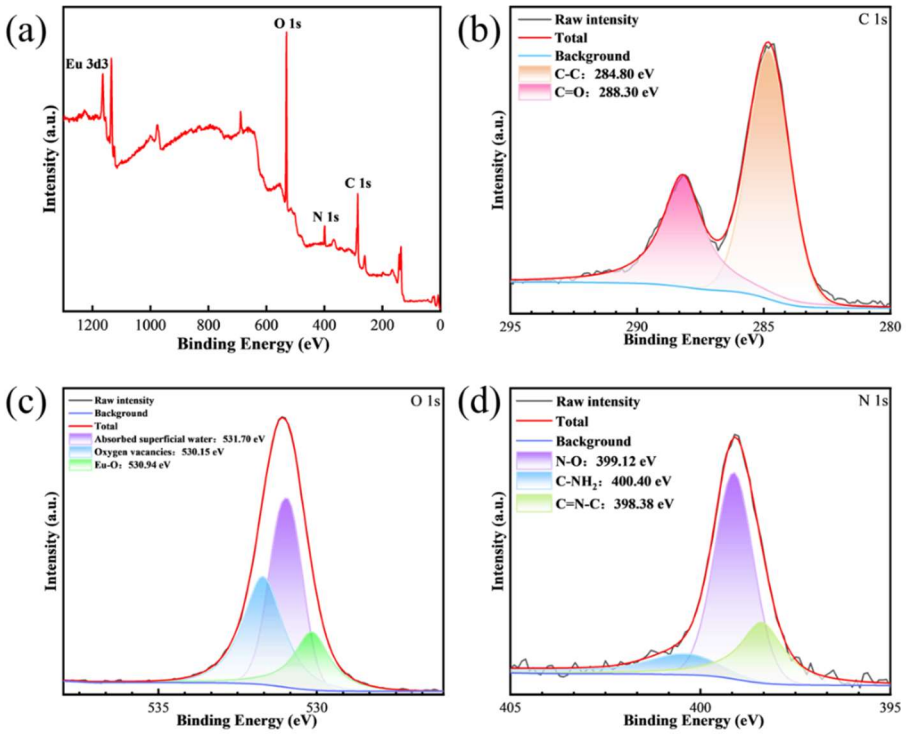

Fig. 2 (a) The XPS full spectrum of Eu^{3+}-ZIF@IL; (b) The spectrum of C1s for Eu^{3+}-ZIF@IL; (c) The spectrum of O1s for Eu^{3+}-ZIF@IL; (d) The spectrum of N1s for Eu^{3+}-ZIF@IL.

The elemental composition of the successfully prepared rare earth hybrid probe Eu^{3+}-ZIF@IL was analyzed through XPS. Fig. 3.2 displays characteristic peaks for C, O, N, and Eu at 284.8 eV, 531.2

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eV, 399.2 eV, and 1134.4 eV, respectively. Fig. 2 represent the fine spectra of C 1s, O 1s, N 1s, and Eu 3d, respectively. For the C 1s spectrum, two peaks appear at 284.8 eV and 288.30 eV, corresponding to the characteristic peaks of C-C/C=C and C-O, respectively. The O 1s spectrum exhibits peaks at 530.15 eV, 530.9 eV, and 531.7 eV, indicating the presence of Eu-O in the sample, which confirms the coordination bonding between DPA and Eu. For the N 1s spectrum, the three peaks at 398.38 eV, 399.12 eV, and 400.40 eV can be attributed to C=N-C, N-O, and C-NH-C, respectively. The XPS results are consistent with the FT-IR spectral data, further demonstrating the successful synthesis of Eu^{3+}-ZIF@IL.

3.2 Fluorescence Properties and Chemical Sensing Properties of Eu^{3+}-ZIF@IL

At room temperature, the excitation and emission spectra of the prepared Eu^{3+}-ZIF@IL were measured using a Shimadzu fluorescence spectrometer. As shown in Fig.3., the optimal excitation wavelength of Eu^{3+}-ZIF@IL is 278 nm, and the optimal emission wavelength is 615 nm. The emission peak is attributed to the characteristic transition of Eu^{3+} from $^5D_0$-$^7F_2$.

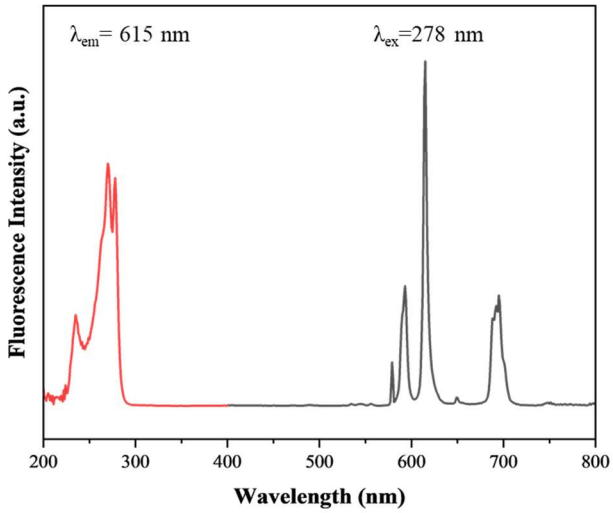

Fig. 3 The fluorescence spectrum of Eu^{3+}-ZIF@IL

3.3 The Fluorescent Sensing of Lysine by Eu^{3+}-ZIF@IL

3.3.1 Analysis of Detection Feasibility

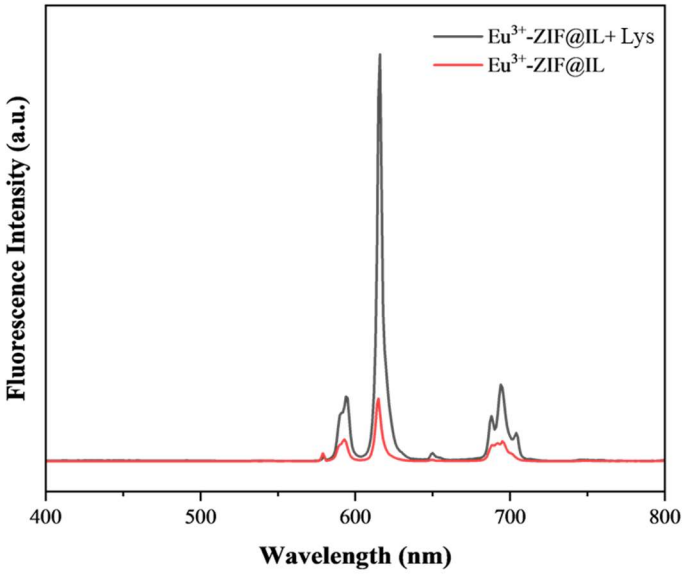

Fig. 4 Fluorescence enhancement phenomenon of Eu^{3+}-ZIF@IL upon addition of lysine

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By measuring the fluorescence emission spectra of the probe Eu^{3+}-ZIF@IL before and after the addition of lysine, its sensing response to lysine as a fluorescent probe can be studied. As shown in Fig.4, a significant enhancement in fluorescence intensity is observed at the characteristic emission peak of Eu^{3+}-ZIF@IL upon the addition of lysine. This indicates that lysine has a certain influence on the energy transfer of the rare earth ion Eu^{3+}, possibly due to charge transfer or aggregation-induced emission mechanisms. The comparison of fluorescence spectra before and after adding lysine further demonstrates the feasibility of using Eu^{3+}-ZIF@IL for the detection of lysine.

3.3.2 Fluorescence Stability of Eu^{3+}-ZIF@IL

To investigate the sensitivity of Eu^{3+}-ZIF@IL in detecting lysine (Lys), fluorescence response tests were conducted with this rare earth hybrid probe against varying concentrations of Lys. Initially, we examined the fluorescence response of Lys as a function of time and pH. After adding a certain amount of Lys aqueous solution to the probe, the fluorescence intensity was measured at intervals of 1 minute. It was observed that the fluorescence intensity began to stabilize after 10 minutes (as shown in Fig. 5a) Additionally, as the pH increased, the fluorescence response remained stable within the pH range of 3 to 7; however, a significant enhancement in fluorescence was observed when the pH exceeded 7 (as shown in Fig. 5b). This phenomenon may be attributed to changes in the coordination environment of the rare earth ions under alkaline conditions. Since most scenarios for amino acid detection occur in acidic environments, we conclude that this probe is feasible for the detection of Lys.

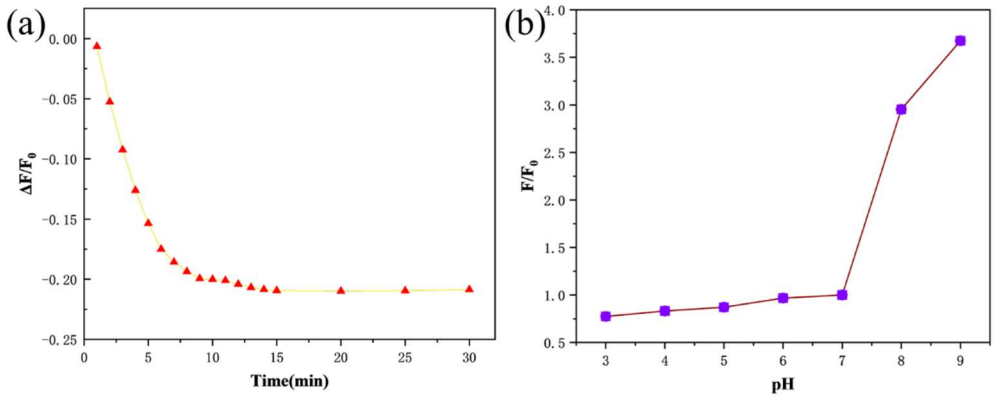

Fig. 5 (a) Fluorescence variation of Eu^{3+}-ZIF@IL upon addition of lysine;
(b) Fluorescence variation of Eu^{3+}-ZIF@IL at different pH values

3.3.3 Analysis of Eu^{3+}-ZIF@IL's Response to Lysine Concentration

Fig.6 demonstrates that the fluorescence intensity of Eu^{3+}-ZIF@IL increases as the concentration of lysine (Lys) rises. This analysis reveals that the probe exhibits a favorable fluorescence response to lysine within the concentration range of 100-500 μM. The enhancement trend exhibits a linear relationship, and the fluorescence response value (F/F_0) correlates well with the concentration of lysine. The linear regression equation obtained after fitting is $y = 0.00649x + 0.28$ ($R2 = 0.99103$). This can be described using the Stern-Volmer equation $F/F_0 = 1 + K_{SV}C$, where C represents the concentration of lysine solution, KSV is the Stern-Volmer constant, and F and Fi represent the luminescence intensities at 615 nm before and after adding lysine at different concentrations, respectively. Based on the LOD = 3σ/S formula, the detection limit of lysine for the probe is calculated to be 23.42 μM. Here, σ is the standard deviation obtained from 20 consecutive scans of the Eu^{3+}-ZIF@IL probe without Lysine, and S is the slope of the linear regression equation. This further corroborates that the Eu^{3+}-ZIF@IL probe possesses excellent fluorescence sensing characteristics for detecting lysine, making it a superb sensor for measuring lysine concentration levels.

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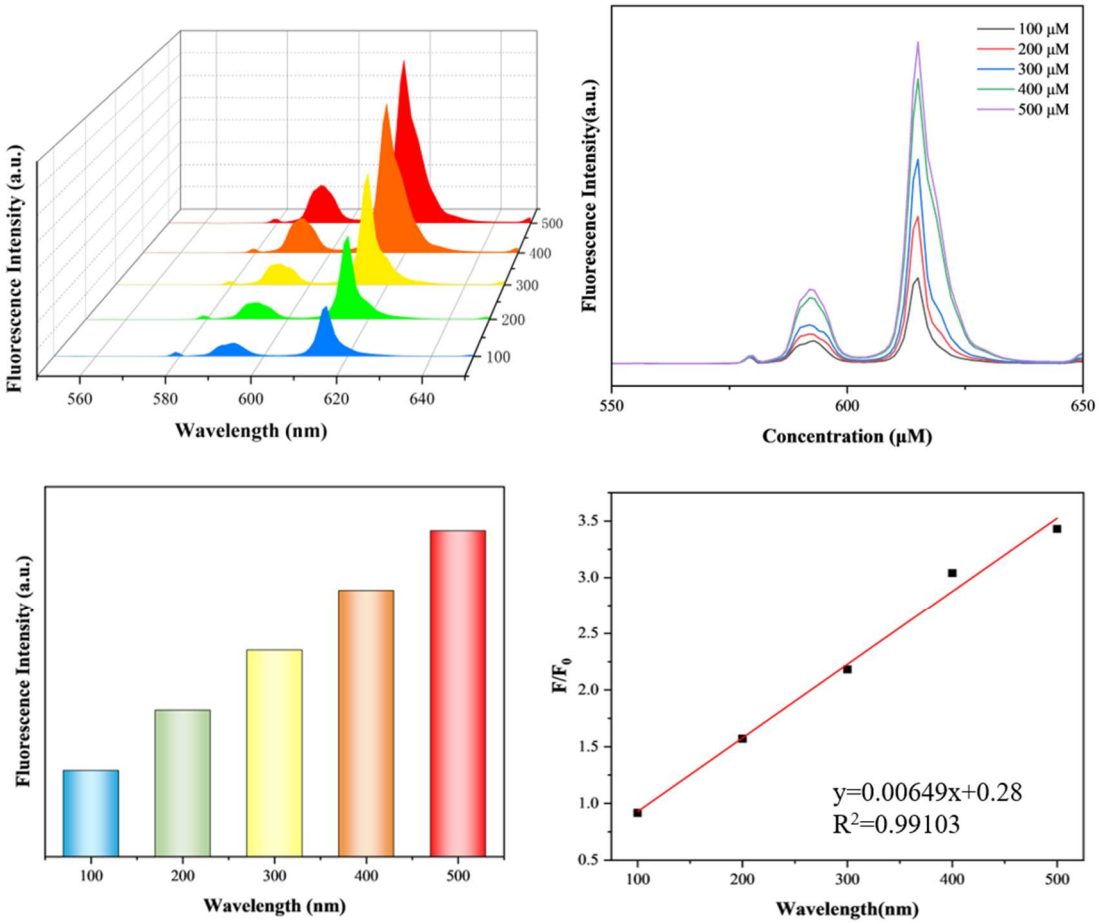

Fig. 6 Eu^{3+}-ZIF@IL Fluorescence spectra with increasing lysine concentration

3.3.4 Anti-Interference Performance of Eu^{3+}-ZIF@IL

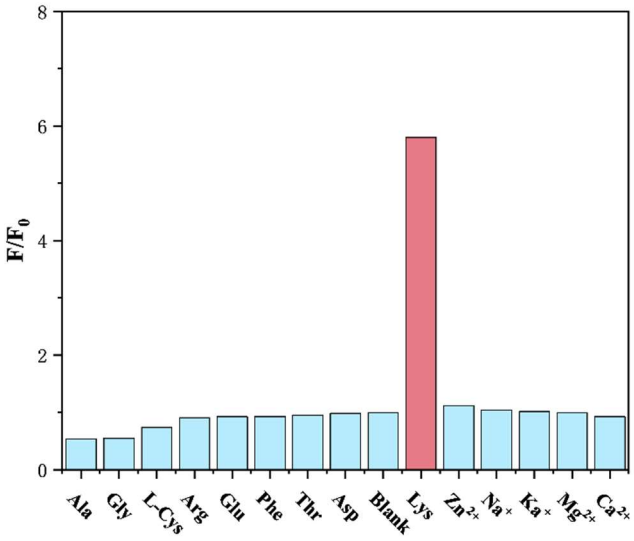

Fig. 7 Fluorescence intensity of Eu^{3+}-ZIF@IL in different solutions

To further investigate the specificity and selectivity of Eu^{3+}-ZIF@IL in the detection of lysine (Lys), its interference with other common amino acids was explored. These amino acids included glycine, arginine, phenylalanine, alanine, cysteine, aspartic acid, and threonine. Additionally, the effects of various metal ions such as Na^{+}, K^{+}, Zn^{2+}, Ca^{2+}, and Mg^{2+} were also examined. In this study, the concentration of Lys was maintained at 100 μM, while the concentration of all other interfering

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substances was set at 1 mM.The results demonstrated that Lys effectively enhanced the fluorescence of Eu^{3+}-ZIF@IL, as shown in Fig. 7. In contrast, the presence of other amino acids or metal ions did not have a significant impact on the fluorescence of Eu^{3+}-ZIF@IL. This finding clearly indicates that Eu^{3+}-ZIF@IL exhibits high specificity and selectivity towards Lys, with minimal interference from other amino acids or metal ions.

Therefore, Eu^{3+}-ZIF@IL can be utilized as a rare-earth hybrid probe for the detection of lysine, offering both high sensitivity and specificity. Its excellent anti-interference performance ensures accurate and reliable Lys detection even in complex biological or environmental samples.

4. Conclusion

This study successfully synthesized an eco-friendly, green, rare earth hybrid material, Eu^{3+}-ZIF@IL, using a hydrothermal method with ionic liquids as the solvent. This material not only retains the high stability characteristic of ZIFs but also significantly enhances its fluorescence detection capabilities through the incorporation of ionic liquids. As a fluorescence probe, Eu^{3+}-ZIF@IL demonstrates exceptional performance in detecting lysine, characterized by high sensitivity and specificity. It effectively identifies and amplifies the fluorescence signal of lysine, achieving a detection limit as low as 23.42 μM. Despite the presence of interference from various common amino acids and ions, it maintains a stable response, proving its broad applicability in biochemistry, the food industry, and pharmaceuticals. Its superior fluorescence properties suggest significant potential in the fluorescence probe market, potentially driving advancements and innovation in related industries.

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