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A naphthalimide-based fluorescent probe for highly selective detection of pyrophosphate in aqueous solution and living cells

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ABSTRACT

A naphthalimide-based fluorescent ensemble probe **NPM**-Cu for selectively detecting PPi in aqueous solution (10 mM HEPES, pH 7.4) has been developed. Moreover, the application of **NPM**-Cu in living cells fluorescence imaging was carried out as well.

1. Introduction

Over the past two decades, the development of novel fluorescent probes capable of detecting anions has attracted increasing attention since many anions play important roles in biological, industrial, and environmental processes.[1] Among the various anions, pyrophosphate (PPi) is considered to be an important target because it involves in several biochemical reactions, such as the hydrolysis of adenosinetriphosphate (ATP), DNA polymerization, and other metabolic processes.[2] Recent research has revealed that the level of PPi is related to various diseases, such as arthritis and Mönckeberg's arteriosclerosis.[3] Moreover, patients with calcium pyrophosphate dehydrate (CPPD) crystals and chondrocalcinosis have also been shown to have a high level of synovial fluid PPi.[4] Therefore, the selective detection of PPi in aqueous solution, especially in biological tissues is of great importance. In view of its simplicity, high sensitivity and real-time detection, fluorescent technique has been regarded as one of the most promising method of detecting PPi.[5,6]

To date, several fluorescent probes for PPi have been reported.[5,6] These reported fluorescent probes can be catalogued into two main types. One type[5] is based on the electrostatic interaction or H-bonding interaction between the receptors and PPi and the other type[6] is based on metal ion complexes. These fluorescent probes provided effective methods for the detection of PPi. However, few of these probes can be applied to detect PPi in aqueous solution. Recently, Zhu et al. developed a novel fluorescent sensor showing turn-on fluorescence to pyrophosphate anion with high selectivity in 100% aqueous solution.[6n] Moreover, some of them lack good specificity for PPi, especially against its analogues such as ATP and AMP.[6a,6m] In addition, some of the known PPi fluorescent probes undergo fluorescence quenching upon responding with PPi, which is not only disadvantageous for a high signal output during detection but also undesirable for analytical purposes.[6k] Therefore, there is great need for the development of structurally simple fluorescence '*turn-on*' probes that can detect PPi selectively in aqueous solution and living cells.

During the past few years, we have focused our research efforts on the development of the novel fluorescent probes and fluorescent materials.[7] For example, recently, we prepared a naphthalimide-based fluorescent ensemble probe **NPC** by an efficient synthetic approach for selectively detecting His in aqueous solution.[7a] Similarly, we synthesized a naphthalimide-based compound **NPM**, which exhibited highly selective fluorescence *turn-off* for Cu^{2+} in aqueous solution.[7c] The addition of Cu^{2+} into the solution of **NPM** induced an almost complete fluorescence quenching (Scheme 1).

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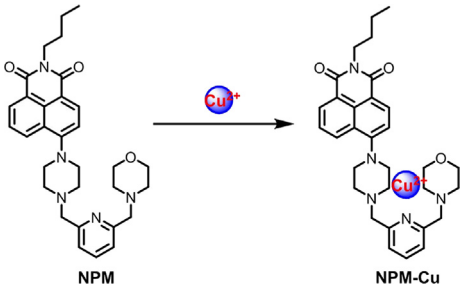

Scheme 1. The structures of probes **NPM** and **NPM-Cu**.

Interestingly, in continuation of our research on **NPM**, we found that the ensemble **NPM-Cu** exhibited a highly sensitive and selective fluorescence *turn-on* response toward PPi in aqueous solution (10 mM HEPES, pH 7.4) and living Hela cells.

2. Results and discussion

Firstly, the emissive property of **NPM-Cu** on changing the pH was determined. As shown in Fig. 1, there was nearly no change in the fluorescence intensity of **NPM-Cu** in the pH range of 10.0 to 7.2, suggesting that **NPM-Cu** was stable in this pH range. When pH<7.2, the emission intensity of **NPM-Cu** gradually increased; this may be due to the protonation of N atoms in **NPM**. Therefore, it demonstrated that **NPM-Cu** could work in the pH range of 7.2–10.0.

The fluorescence response of **NPM-Cu** toward a series of anions such as PPi, ADP, ATP, AMP, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, S^{2-}, SCN^-, OAc^-, and SO_3^{2-} in aqueous solution (10 mM HEPES, pH 7.4) was investigated. As shown in Fig. 2, the addition of PPi to a solution of **NPM-Cu** induced a significant enhancement of fluorescence with the emission maximum at 532 nm. However, under identical

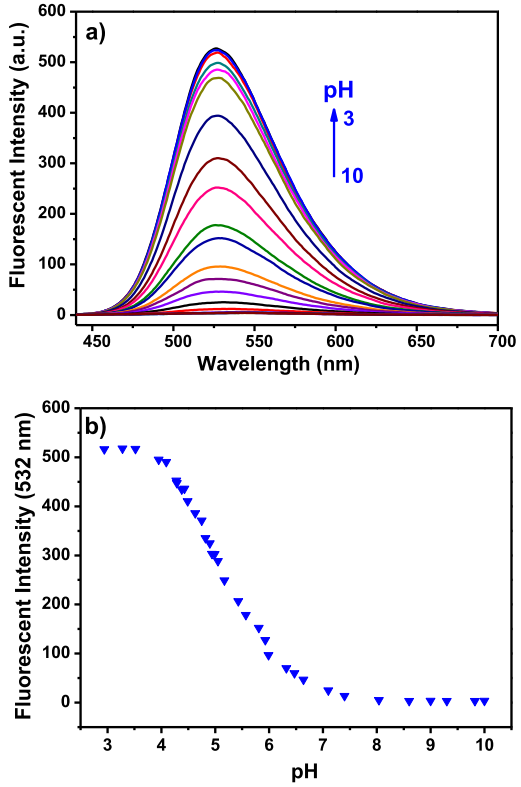

Fig. 1. The influence of pH on the fluorescence spectra (a) of **NPM-Cu** (10 μM **NPM** in presence of 20 μM Cu^{2+}) in water. (b) Curve of the fluorescence intensity of **NPM-Cu** at 532 nm versus pH. (slits: 2.5, 5 nm).

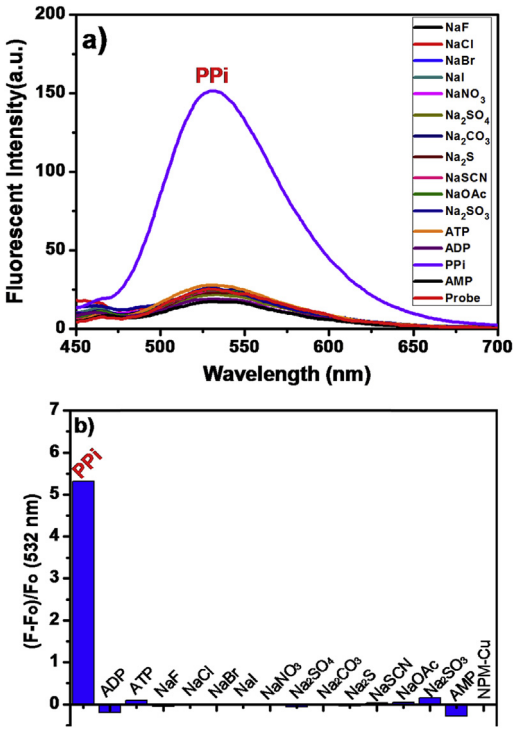

Fig. 2. (a) Fluorescence spectra of **NPM-Cu** (the complex of 10 μM **NPM** and 20 μM Cu^{2+}) in the presence of various anions (all anions were 200 μM); (b) Fluorescence emission response of **NPM-Cu** toward various anions in aqueous solution (10 mM HEPES, pH 7.4).

conditions, nearly no fluorescence intensity changes were observed in emission spectra with the other anions. The results indicated that **NPM-Cu** was a selective fluorescence *turn-on* probe for PPi in aqueous solution (10 mM HEPES, pH 7.4).

Then the detailed response of **NPM-Cu** to PPi was investigated as shown in Fig. 3. With the titration of PPi into the solution of **NPM-Cu**, the fluorescence increased gradually and nearly without a change in the wavelength of the maximum. It is worth noting that the fluorescence intensity of **NPM-Cu** at 532 nm was linear with regard to the concentration of PPi (Fig. 3b). The obtained results implied that **NPM-Cu** was sensitive to PPi and can be potentially used to quantitatively detect PPi in aqueous solution.

The sensing mechanisms of **NPM** to Cu^{2+} and PPi were proposed as follows. Firstly, upon titration with Cu^{2+} induced the fluorescence quenching of **NPM** was due to the well-known paramagnetic effect of Cu^{2+},[8] which quenched the excited state of the fluorophore **NPM** by energy and/or electron transfer. Subsequently, the addition of PPi into the solution of **NPM-Cu** induced the emission enhancement should be attributed to the dissociation of Cu^{2+} from the **NPM-Cu** complex. It has been suggested that PPi was capable of snatching Cu^{2+} from Cu^{2+}-complexes and then forming a new stoichiometric complex PPi-Cu,[6n,9] which was further confirmed by Job's plot analysis (Fig. 4). Thus, based on the absorption (Fig. S2) and fluorescence titration spectra, and the Job's plot data, the sensing mechanism of probe **NPM-Cu** with PPi was proposed as shown in Scheme 2. The addition of PPi induced Cu^{2+} removed from its **NPM-Cu** complex thereby the paramagnetic effect of Cu^{2+} to **NPM** was inhibited, which led to a recovery of the fluorescence emission of **NPM**.

To our knowledge, fluorescent probes for imaging PPi in living cells are very rare.[6n,6q] In order to further demonstrate the potential application of **NPM-Cu** in biological systems, fluorescence imaging experiments of **NPM-Cu** in living cells were performed. As shown in Fig. 5, a bright fluorescence was observed when Hela cells were treated with the **NPM** probe only. By contrast, no fluorescence was