A Naphthalimide Fluorescent Sensor for Zn^{2+} Based on Photo-induced Electron Transfer

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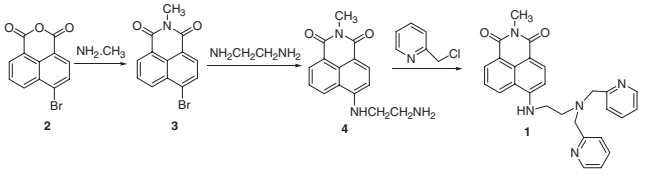
A new Zn^{2+} fluorescent sensor **NIDPA** (**1**) which takes 1,8-naphthalimide as a reporting group and di-2-picolylamine (**DPA**) as a recognizing group has been synthesized via simple steps. Based on photo-induced electron transfer (**PET**) mechanism, **NIDPA** has a nearly 5-fold fluorescent enhancement under simulated physiological conditions corresponding to the binding of Zn^{2+}. Apparent dissociation constant for Zn^{2+} (K_d) is in the sub-*n*M range, and Ca^{2+}, Mg^{2+}, Fe^{3+}, Ni^{2+}, and Cr^{3+} have little influence on fluorescence enhancement.

Zn^{2+} is one of the most important transition metal ions found in physiology, where it has multiple roles in both extra- and intra-cellular functions.[1,2] Some of the roles have been long known, such as in gene transcription and metallloenzyme.[2,3] Other functions are just being discovered and investigated, such as zinc's role in synaptic neurotransmission[4] and in mediation neuronal excitotoxicity.[5] Currently, there is great interest in the development of fluorescent sensors for exploring the role of Zn^{2+} in medicine and biology as well as in the environment.[6–10] However, there is still scope for improvement in the design of such sensors as they often suffer from disadvantages such as sensitivity to H^+, Ca^{2+}, and Mg^{2+}, short excitation and emission wavelengths, small Stokes shifts and cumbersome synthesis.

Herein, we report the design and properties of (**NIDPA**, **1**), a new Zn^{2+} selective and sensitive fluorescent **PET** sensor synthesized in a few steps with high yields. In **NIDPA**, 4-aminonaphthalimide, with large Stockes shift and desirable spectroscopic properties is used as fluorophore, and *N*,*N*-bis(2-pyridylmethyl) ethylenediamine (DPA), a selective chelator for Zn^{2+}, as receptor. The nitrogen atom of tertiary amine is both the chelating site of Zn^{2+} and the electron-source for **PET**. The synthetic procedure is shown in Scheme 1. **4** was prepared according to the similar method previously described with 70% yield.[11] **NIDPA** (**1**) was easily synthesized via the reaction of 2-chloromethylpyridine and **4** with 50% yield, and characterized by spectroscopic data.[12]



Scheme 1. Synthesis of **NIDPA**.

The absorption maximum wavelength of **NIDPA** was 453 nm, emission maximum wavelength was 549 nm, and fluorescence quantum yield was 0.04 under physiological conditions (pH 7.4, $I = 0.1$ (NaCl)) with EDTA to scavenge adventitious metal ions, which was determined by using fluorescein in 0.1 N NaOH ($\Phi = 0.85$) as a standard. The low quantum yield of the bound-free sensor results from **PET** quenching, which is due to the electron transfer from the nitrogen atoms in **DPA** moiety to the fluorophore. Upon addition of Zn^{2+}, the fluorescence intensity was increased by nearly 5-fold, and the quantum yield of the Zn^{2+} complex was increased up to 0.17. The absorption maximum wavelength and the emission maximum wavelength were blue shifted to 443 and 539 nm, which accounts for the coordination of the amino group on naphthalene with Zn^{2+}.

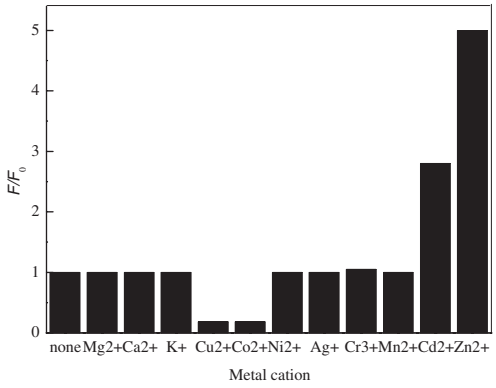


Figure 1. Fluorescence response of 1 μM **NIDPA** to various metal cations. Bars represent the final integrated fluorescence response (F) over the initial intergtated emission (F_0). Initial spectrum was acquired in 100 mM NaCl, 10 mM Tris, 1 μM EDTA pH 7.4 at 25 °C.

The fluorescence response of **NIDPA** to various cations is shown in Figure 1. As expected, other physiologically important cations which exist at high concentration in living cells such as Ca^{2+}, Mg^{2+}, Na^+, and K^+ did not give rise to any changes in the fluorescence emission of **NIDPA** even at high concentration (5 mM). These results are presumably due to the poor complexation of alkaline metals or alkaline earth metals with the chelator of **NIDPA**. Similar results were obtained for some first-row transition metal cations, their fluorescence spectra were slightly influenced by addition of Fe^{3+}, Ni^{2+}, and Cr^{3+} (5 μM). Cu^{2+} and Co^{2+} quenched the fluorescence to a little extent, probably because there is electron or energy transfer between metal cation and fluorophore, which is known as the fluorescence quenching mechanism.[13] But these cations would have little influence in vivo, since they are not present to a significant extent in ordinary biological system.

The sensors based on **PET** are usually disturbed by proton in the detection of metal cations, so the low sensitivity to the operative pH is very important.[14] In the presence of saturating Zn^{2+}, the fluorescence intensity of **NIDPA** decreases along with the increase of pH value, but when pH >7.0, the fluorescence intensity is almost a constant (Figure 2). From the change of fluorescence intensity integrated from 480 to 700 nm, a sigmoidal curve is ob-

served, which gives a pK_a of 5.8, which corresponds to protonation of the tertiary nitrogen atom of **DPA**. So, it is almost stable over the physiological pH range.

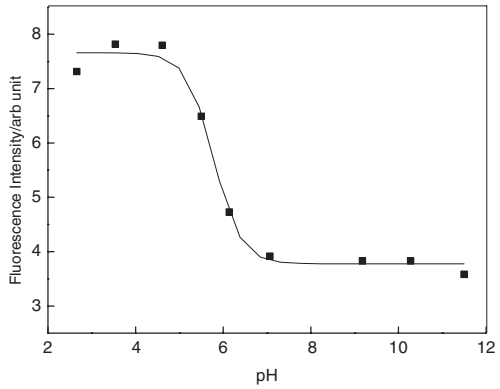


Figure 2. Effect of pH on the fluorescence intensity of 1 μM **NIDPA**–Zn^{2+} in phosphatic buffers (excitation at 453 nm) at 25 °C.

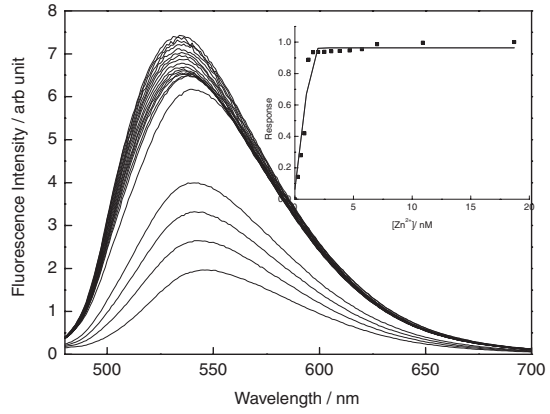


Figure 3. Fluorescence emission spectra of 1 μM **NIDPA** in buffered Zn^{2+} solutions with free Zn^{2+} concentrations from 0 to 20 nM, for the final several spectra, additional $ZnSO_4$ was added to provide the concentration of free Zn^{2+} to 25 μM. Inset: fluorescence response obtained by integrating the emission spectra between 480 and 700 nm, subtracting the baseline (0 Zn^{2+}) spectrum and normalizing to the full scale. These data were measured in 10 mM Tris-HCl solutions (pH 7.4) containing 100 mM NaCl, 10 mM NTA, and 0–10 mM $ZnSO_4$. The slit width was 4 nm for both excitation and emission.

In order to determine the ability of **NIDPA** to complex with Zn^{2+}, the apparent dissociation constant, $K_{d,}$, was determined using Zn^{2+} and pH-buffered solutions[8b] (Figure 3). From the sigmoidal curve, K_d is 0.83 nM. Hence, **NIDPA** can be used to determine free Zn^{2+} concentrations at low levels, which is sensitive for application in mammalian cells. Furthermore, a Hill plot analysis revealed maximum fluorescence obtained at 1:1 ratio, which suggested that **NIDPA** should form a 1:1 complex with Zn^{2+}.

In summary, we have developed a simple and sensitive fluorescent probe **NIDPA** (**1**) for Zn^{2+} with *N*,*N*-bis(2-pyridylmethyl)ethylenediamine as acceptor for Zn^{2+} and 1,8-naphthalimide as a fluorophore. It is excited at 453 and emits at 539 nm with 5-fold fluorescence enhancement after complexation with Zn^{2+}. The quantitative response range is in the sub-*n*M range, and its fluorescence is not induced by other biologically important cations such as K^+, Na^+, Ca^{2+}, Fe^{3+}, Ni^{2+}, and Mg^{2+} under physiological conditions.

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References and Notes

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12 For **1**: mp 163–164 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (d, 1H, $J = 8.4$), 8.64 (d, 1H, $J = 7.2$), 8.57 (d, 2H, $J = 4.8$), 8.43 (d, 1H, $J = 8.4$), 7.82 (s, 1H), 7.72 (t, 1H, $J = 8.0$), 7.58 (t, 2H, $J = 8.0$), 7.41 (d, 2H, $J = 7.6$), 7.16 (t, 2H, $J = 5.6$), 6.54 (d, 1H, $J = 8.4$), 4.03 (s, 4H), 3.54 (s, 3H), 3.42 (s, 2H), 3.09 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 164.6, 156.7, 148.8, 137.4, 134.7, 131.2, 130.9, 129.6, 128.9, 128.1, 124.6, 124.3, 123.0, 122.7, 120.9, 112.0, 59.5, 51.2, 40.7, 27.1. API–ES–MS (positive) m/z: 452 ($[M + H]^+$).
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