A New Red Fluorescent Probe for Hg^{2+} Based on Naphthalene Diimide and Its Application in Living Cells, Reversibility on Strip Papers

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Abstract: A new fluorescent probe (**NDI-5**) towards Hg^{2+} ions has been designed and synthesized based on naphthalene diimide (NDI), which showed extremely weak fluorescence emission in solution owing to the twisted intermolecular charge transfer (TICT) between the NDI core and the receptor. However, upon the addition of Hg^{2+} ions, a “turn-on” fluorescence response with red emission was obvious with 32-fold enhancement of the quantum yield, and the detection limit was as low as 1.3 μM . Furthermore, **NDI-5** can be applied to MCF-7 cells to realize the detection of Hg^{2+} ions in the living systems, and the reversibility with the coordination of KI can be conducted in solution and test stripes for several times.

1. Introduction

As one of the most hazardous pollution and dangerous elements to biological and environmental systems, mercury ions are widely distributed in water, soil and air [1-4]. It can easily pass through skin, respiratory and gastrointestinal tissues, and cause damage to brain, the liver, kidney, immune and center nervous system, due to its high affinity to thiol group in proteins and enzymes [5-9]. Thus, it is extremely important to develop the analytical approach to detect Hg^{2+} with high sensitivity and selectivity. Thanks to the great effort of scientists, many fluorescent probes towards Hg^{2+} have been successfully reported by using proteins, nucleic acids, DNAs, nanoparticles, and several types of small molecules and some other biomacromolecules as the receptors [10-19]. Compared with those with blue and green emission, red fluorescent probes exhibited some advantages for the applications in the living systems, such as favorable tissue penetration, low light scattering and minimal auto-fluorescence and background-fluorescence [20-23]. However, in order to achieve the strong red fluorescence, the selection of the electron donors and acceptors is an extremely essential and hard work. As the strong electron acceptors and big π -conjugated systems, naphthalene diimide (NDI) derivatives have attracted considerable attention with the wide applications in organic field-effect transistors (OFET) [24-25], polymer photovoltaic cells, electrical energy storage, supramolecular assembly, and so on [26-31]. Moreover, based on our previous work, the substituted NDI derivatives bearing tertiary amine moieties usually induced twisted intramolecular charge transfer (TICT), resulting in the quenching of fluorescence. But the strong fluorescence can be regained by the coordination of a specific metal ion for the suppression of TICT affect [32]. Thus, in consideration with its desired electronic and structural properties, NDI can act as one of the ideal components for the creation of red fluorescent probes.

In this text, we reported a novel red fluorescent probe based on NDI unit (**NDI-5**), in which bis[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine acted as electron donor and receptor to capture Hg^{2+}

(Chart 1). This probe displayed a fast and dramatic fluorescence “turn-on” response to Hg^{2+} ions with the maximum emission wavelength (λ_{max}) of 651 nm. The high sensitivity was obtained with the limited detection of 1.3×10^{-6} mol/L. Furthermore, it could be applied into the living cells and realize the repeatable detection of Hg^{2+} as the test strips. The possible TICT mechanism ensured that **NDI-5** was sensitive to the macro-/micro-environment, such as viscosity, polarity and pH, which played key roles in biological processes. Herein, we would like to present the synthesis, structural characterization, sensing property, and possible applications of **NDI-5** as test strips and in cell imaging in detail.

Chart 1. The detection of Hg^{2+} ions by the fluorescent probe **NDI-5**

2. Experimental section

2.1 Material and Instrumentations

Compound **1** [32] and **2** [33] were obtained according to the literatures. NaNO_3 , KNO_3 , LiCl , $\text{Ba}(\text{NO}_3)_2$, AgNO_3 , $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, MgSO_4 , CaCl_2 , $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{Hg}(\text{ClO}_4)_2 \cdot 3\text{H}_2\text{O}$ were purchased from Sinopharm Chemical Reagent Co., Ltd. All other chemicals were used as received without further purification. Doubly distilled water was used to prepare solutions of different metal ions.

^1H and ^{13}C NMR spectroscopy study was conducted with a Varian Mercury 300 spectrometer using tetramethylsilane (TMS; $\delta = 0$ ppm) as internal standard. UV-visible spectra were obtained using a Shimadzu UV-2550 spectrometer. Fluorescence spectra were recorded with Hitachi F-4600

fluorescence spectrophotometer. The ESI mass spectra were measured on a Finnigan LCQ advantage mass spectrometer. GC-MS spectra were performed by Thermo DSQ II.

Scheme 1. The synthetic route of **NDI-5**.

2.2 Synthesis of compound 3: Under an atmosphere of nitrogen, compound **1** (324 mg, 0.50 mmol) and compound **2** (104 mg, 0.40 mmol) were dissolved into 2-methoxyethanol (16 mL) in a dry Schlenk (100 mL) and then the resultant mixture was stirred at 125 °C for 4 hours. After being cooled to room temperature, the mixture was poured into water (100 mL), extracted with dichloromethane (100 mL) for three times, the organic layers were dried by anhydrous Na_2SO_4 . The crude product was further purified by silica gel column chromatography using chloroform and ethyl acetate (1:2) as an eluent to give compound **3** as a red-purple solid (142 mg, 42.8%). ^1H NMR (CDCl_3) δ (ppm): 8.84 (s, 1H, ArH), 8.29 (s, 1H, ArH), 5.49 (s, 2H, ArH), 4.12 (br, 8H, -N-CH₂-), 3.99 (br, 4H, -N-CH₂-), 2.04 (s, 6H, -CH₃), 1.93 (s, 6H, -CH₃), 1.62 (br, 4H, -CH₂-), 1.38-1.31 (m, 12H, -CH₂-), 0.97-0.89 (m, 12H, -CH₃). m/z $[\text{M}+1]^+$: calcd., 828.37, found, 828.68.

2.3 Synthesis of compound NDI-5: Under an atmosphere of nitrogen, compound **3** (190 mg, 0.23 mmol) and 2-ethylhexylamine (316 mg, 2.29 mmol) were dissolved in DMF (10 mL) and refluxed for 1 hour. After cooled to room temperature, the mixture was poured into 0.5 mol/L HCl (100 mL), extracted with dichloromethane (100 mL) for three times, and the organic layers were dried by anhydrous Na_2SO_4 . Then the crude product was further purified by silica gel column chromatography using chloroform and ethyl acetate (1:1) as an eluent to give compound **NDI-5** as a dark blue solid (115 mg, 57.2%). ^1H NMR (CDCl_3) δ (ppm): 9.76 (s, 1H, -NH), 8.23 (s, 1H, ArH), 8.18 (s, 1H, ArH), 5.58 (s, 2H, ArH), 4.11 (m, 8H, -N-CH₂-), 3.82 (br, 4H, -N-CH₂-), 3.45 (br, 2H,

-N-CH₂-), 2.08 (s, 6H, -CH₃), 2.01 (s, 6H, -CH₃), 1.92 (br, 2H, -CH-), 1.78 (t, $J = 6.6$ Hz, 1H, -CH-), 1.56-1.31 (m, 24H, -CH₂-), 1.00-0.87 (m, 18H, -CH₃). ¹³C NMR (CDCl₃) δ (ppm): 166.50, 163.32, 161.73, 138.92, 127.44, 127.04, 123.56, 121.37, 118.83, 104.75, 100.50, 53.19, 46.62, 46.20, 44.27, 43.75, 39.12, 37.77, 31.06, 30.59, 28.54, 24.42, 23.82, 23.01, 14.05, 13.20, 10.85, 10.59. m/z [M+1]⁺: calcd., 877.60, found, 877.88, MS-TOF m/z Anal.calcd. for: C₅₂H₇₆N₈O₄, 877.6023; found, 877.6050.

2.4 Preparation of NDI-5 solutions. NDI-5 (3.5 mg, 4.0 μ mol) was dissolved in acetone (10 mL) to afford the stock solution with the concentration of 4.0×10^{-4} mol/L. This stock solution was diluted to desired concentrations with the appropriate solvent medium when needed.

2.5 Preparation of solutions of metal ions. 0.1 mmol of each inorganic salt (AgNO₃, Al(NO₃)₃ 9H₂O, Ba(NO₃)₂, CaCl₂, 3CdSO₄ 8H₂O, CoCl₂ 6H₂O, Cr(NO₃)₃ 9H₂O, Cu(NO₃)₂ 3H₂O, FeSO₄ 7H₂O, Fe(NO₃)₃ 9H₂O, Hg(ClO₄)₂ 3H₂O, KNO₃, LiCl, MgCl₂ 6H₂O, MnSO₄ H₂O, NaNO₃, NiCl₂ 6H₂O, Pb(NO₃)₂, ZnCl₂) was dissolved in distilled water (10 mL) to afford 1.0×10^{-2} mol/L aqueous solution. The stock solutions were diluted to desired concentrations with distilled water when needed.

2.6 The changes of UV-vis absorption spectra of NDI-5 with the addition of Hg²⁺ ions. The solution of NDI-5 (2.0×10^{-5} mol/L) was prepared in acetone. A solution of NDI-5 (2.0 mL) was placed in a quartz cell (10.0 mm width) and the absorption spectrum was recorded. The Hg²⁺ ions were added and the corresponding absorption spectrum was recorded at room temperature.

2.7 The changes of fluorescence spectra of NDI-5 with different metal ions. The solution of NDI-5 (2.0×10^{-5} mol/L) was prepared in acetone. The solutions of metal ions (1.0×10^{-2} mol/L) were prepared in distilled water. A solution of NDI-5 (2.0 mL) was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded. Different metal ions were introduced and the corresponding fluorescence spectra were recorded at room temperature each time.

2.8 Competition experiments of Hg^{2+} with other metal ions. The solution of **NDI-5** (2.0×10^{-5} mol/L) was prepared in acetone. **NDI-5** (2.0 mL) was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded. 1.0 equiv of Hg^{2+} was added and the corresponding fluorescence spectra were recorded at room temperature, subsequently, a kind of metal ions (except Hg^{2+}) was introduced and the corresponding fluorescence spectra were also recorded.

2.9 Fluorescence titration of NDI-5 with Hg^{2+} ions. The solution of **NDI-5** (1.0×10^{-5} mol/L) was prepared in acetone. The solution of Hg^{2+} ions (1.0×10^{-2} mol/L) was prepared in distilled water. A solution of **NDI-5** (3.0 mL) was placed in a quartz cell (10.0 mm width) and fluorescence spectrum was recorded. The Hg^{2+} ions solution was added in portions and the corresponding fluorescence spectra were recorded at room temperature each time.

2.10 Cell culture. The breast cancer cell lines (MCF-7) was obtained from China Center for Type Culture Collection. The cell culture media and supplements were obtained from Gibco Corp. MCF-7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM). All media were supplemented with 10 % fetal bovine serum (FBS) and 1% Penicillin-Streptomycin. Cells were grown at 37 °C and 5% CO_2 (HERACELL 150i, Thermo Scientific). Cells were harvested using accutase to ensure that surface antigens are not degraded by prolonged exposure to more traditional reagents, such as Trypsin-EDTA.

2.11 Microscopy and imaging methods. Fluorescence cell imaging was performed with scanning microscopy. Excitation was carried out at 510-550 nm, and emissions were collected at the wavelength longer than 600 nm. The MCF-7 cells were washed with HEPES (20 mM, pH= 7.40) for three times to remove the excess of original medium. Then MCF-7 cells were incubated with **NDI-5** (1.0×10^{-4} mol/L) (note: ca. 50% DMSO was added for solubility reasons) for 0.5 h. Then the excess of **NDI-5** was removed and washed with HEPES for three times before imaging. Then MCF-7 cells were further incubated with Hg^{2+} ions for another 0.5 h. Fluorescence images were obtained on a

laser scanning microscope with an objective lens ($\times 20$).

3. Results and discussion

3.1 The optical response of NDI-5 towards Hg^{2+} ions

The absorption and fluorescence spectra of **NDI-5** towards Hg^{2+} ions were shown in Figure 1. The probe **NDI-5** exhibited extremely weak fluorescence with the quantum yield (Φ) of 0.28% for the possible TICT process. Excitingly, upon addition of Hg^{2+} ions, the fluorescence intensity of **NDI-5** increased rapidly with 38-fold enhancement ($\Phi = 8.83\%$). It exhibited bright red fluorescence with the λ_{max} of emission at 651 nm. And the time course study showed that the response of **NDI-5** to Hg^{2+} only required 1 minute to reach the equilibrium (Figure S1), indicating the quick detection process. Also, after the addition of Hg^{2+} ions, the blue-shift of the corresponding absorption spectra (23 nm) was obvious, along with the color of solution changing from slight blue to bluish (the photos in Figure 1A). It indicated that the nitrogen atom linked to the NDI core has taken part in the coordination with Hg^{2+} ions, thus weaken the electron donating ability of bis[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine.

Figure 1. The UV-vis spectra (A) and fluorescence spectra (B) of **NDI-5** (2.0×10^{-5} mol/L) with/without Hg^{2+} ions (1.0 equiv.). Inset: the photos of **NDI-5** and **NDI-5**- Hg^{2+} under sunlight (A) and UV-lamp (B).

Furthermore, with the aim to investigate the possible TICT mechanism, the influence of viscosity, polarity and pH on the fluorescence of **NDI-5** have been conducted and analyzed in detail. As shown

in Figure 2, the fluorescence intensity of **NDI-5** in ethanol was enhanced dramatically upon the addition of glycerin, and when the fraction of glycerin was 70%, the maximum intensity of fluorescence achieved 3.5-fold of that in ethanol, may be due to the rotation of the bis[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine moiety around carbon-nitrogen was partly frozen by the high viscosity of glycerin. Also, the maximum emission wavelength of fluorescence became red-shift (about 16 nm) for the high polarity of glycerin. Furthermore, various solvents with different polarities have been incorporated to investigate the fluorescent recognition of polarity, which were shown in Figure S2. In the solvents with low polarities such as cyclohexane and toluene, the fluorescence emission of **NDI-5** was relatively strong. However, when the solvent changed to THF, acetone or acetone/water (v/v, 1:1) with high polarity, the fluorescence intensity decreased in a large degree, along with the red-shifts of fluorescence spectra. The possible reason was that the fluorescence intensity of **NDI-5** was quenched by the charge separation at the excited state, in which the high polarity of the solvent stabilized the charge separation of the TICT relaxation process.

Furthermore, the theoretical calculation of **NDI-5** has been conducted by using density functional theory (DFT) calculations with Gaussian 09 program as shown in Table 1 [34]. In the ground state, the dihedral angle between the NDI core and the receptor (bis[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine) was about 52.71° in the optimized structure of **NDI-5**. The HOMO was delocalized through the whole π system, while the LUMO located in the NDI core. However, in the excited state, the receptor unit was nearly perpendicular to the NDI core with a dihedral angle about 89.96° , and the HOMO was restricted on the receptor unit as the electron donor, while the LUMO was located in the NDI core, leading to the charge separation state. Thus, **NDI-5** exhibited nearly no fluorescence in the solution since the TICT process in the excited state.

Figure 2. (A) The fluorescent spectra of **NDI-5** in ethanol (2.0×10^{-5} mol/L) upon the addition of glycerin. (B) Plot of fluorescence intensity and $\lambda_{\max}$ of **NDI-5** in ethanol (2.0×10^{-5} mol/L) with the different fractions of glycerol.

	Optimized structure	HOMO	LUMO
Ground state	52.71°
Excited state	89.96°

Table 1. The optimized structure and HOMO, LUMO of **NDI-5** in ground state and excited state, which were calculated using TDDFT calculations with Gaussian 09 program.

With the aim to suppress the possible TICT process, the acidic environment was necessary to the protonation of bis[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine, and the result was obvious as shown in Figure 3. When the pH value was gradually decreased from 4.05 to 0.80, the fluorescence intensity was enhanced about 11-fold with the “turn-on” response (the photos in Figure 3). This sensitive response may be useful to image intracellular pH in macrophage cells targeting acidic microenvironments. Similarly, the coordination between bis[2-(3,5-dimethylpyrazol-1-yl)ethyl]amine and Hg^{2+} could suppress the possible TICT process in a large degree, leading to the enhanced fluorescence intensity.

Figure 3. The fluorescent responses of **NDI-5** in the acetone/water mixture (v/v, 1:1) under different pH values. Inset: The fluorescence intensity at 651 nm of **NDI-5** with various pH values. The photos of **NDI-5** with some pH values under UV-lamp, pH values from left to right: 0.80, 1.10, 1.27, 1.54, 4.05, 5.05, 6.97, 12.02.

3.2 The selectivity of **NDI-5** towards Hg^{2+} ions

To assess the specificity of **NDI-5** towards Hg^{2+} , the fluorescence response of **NDI-5** (20 μM) to various metal ions were investigated, which were shown in Figure 4. Hg^{2+} ions induced the enhancement of fluorescence intensity of **NDI-5** about 38-fold, while environment relevant alkali and alkaline earth cations, including K^+ , Ca^{2+} , Na^+ , Mg^{2+} , Li^+ , Ba^{2+} and other transition-metal ions Zn^{2+} , Cd^{2+} , Pb^{2+} , Cu^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} , nearly had no influence on the fluorescence intensity. However, Fe^{3+} , Cr^{3+} , and Al^{3+} have a little interference with small increases of fluorescence intensity, which were still much lower than that of **NDI-5**- Hg^{2+} . The addition of Ag^+ could cause the blue-shift of the fluorescence spectrum and a little enhancement of fluorescence intensity, which did not have much influence on the detection of Hg^{2+} ions. To further investigate the selectivity of **NDI-5** towards Hg^{2+} ions in the presence of other metal ions, competitive experiments were performed (Figure S3). The enhancement of fluorescence intensity was still obvious in the presence of Hg^{2+} ions and other competing metal ions, indicating the relatively good selectivity of **NDI-5** towards Hg^{2+} ions. Besides, the counter anions did not exhibit obvious influence on the fluorescence spectra of **NDI-5** (Figure

S4).

Figure 4. Fluorescence spectra (A) and the changes of fluorescence intensity (B) of **NDI-5** (2.0×10^{-5} mol/L) in acetone in the presence of various metal ions (1.0 equiv.). Inset: the photos of **NDI-5** with different metal ions under UV-lamp: 1) blank, 2) Hg^{2+} , 3) Li^+ , 4) Ba^{2+} , 5) Mn^{2+} , 6) Co^{2+} , 7) Ni^{2+} , 8) Fe^{2+} , 9) Fe^{3+} , 10) Ag^+ , 11) Zn^{2+} , 12) Mg^{2+} , 13) Na^+ , 14) Ca^{2+} , 15) K^+ , 16) Pb^{2+} , 17) Cd^{2+} , 18) Cr^{3+} , 19) Al^{3+} , 20) Cu^{2+} .

3.3 The sensitivity of **NDI-5** towards Hg^{2+} ions and binding mode of the complex **NDI-5-Hg²⁺**

Besides the selectivity of the fluorescence probe, another important parameter was the sensitivity, that was, the detection limit of **NDI-5** towards Hg^{2+} ions. Thus, the titration experiment of **NDI-5** towards different concentrations of Hg^{2+} ions was conducted (Figure 5). For the single **NDI-5**, the weak fluorescence can be nearly ignored. However, upon addition of Hg^{2+} ions, the fluorescence intensity at 651 nm gradually enhanced with the increasing concentrations of Hg^{2+} ions. When the concentration of Hg^{2+} ions was up to 10 μM , the same with that of **NDI-5**, the fluorescence intensity increased to its maximum, indicating a 1:1 stoichiometry for the **NDI-5-Hg²⁺** complex. It was further confirmed by the job's plot and ESI-MS spectrum (Figure S5 and Figure S6). Calculated from the titration curve, the binding constant was $4.24 \times 10^6 \text{ M}^{-1}$ and the detection limit (S/N=3) [35] towards Hg^{2+} ions was $1.3 \times 10^{-6} \text{ mol/L}$, exhibiting the high sensitivity with strong coordination ability.

To gain a clear understanding of the binding mode of **NDI-5** and Hg^{2+} ion, ^1H NMR spectra of **NDI-5** with/without Hg^{2+} ions were conducted (Figure 6). Hg^{2+} can affect the signals of the

hydrogen atoms closed to the binding site [36]. Treatment with 1.0 equiv. of Hg^{2+} ions resulted in the downfield shift of H_a ($\Delta\text{ppm} = 0.31$) and H_b ($\Delta\text{ppm} = 0.32$) of the NDI core, indicating the nitrogen atom of the receptor, which was linked to the NDI core, has participated in the binding process with Hg^{2+} ions. And the peak of H_e in the 3,5-dimethylpyrazol unit was shifted about 1.13 ppm. Also, significant downfield shifts of H_d ($\Delta\text{ppm} = 0.76$) and H_c ($\Delta\text{ppm} = 0.11$) in the flexible chains were observed respectively, suggesting that the nitrogen atoms in the pyrazol ring have been coordinated with Hg^{2+} ions.

Figure 5. Fluorescence spectra of **NDI-5** (1.0×10^{-5} mol/L) in acetone upon the addition of various concentrations of Hg^{2+} ions.

Figure 6. ^1H NMR spectra (300 MHz, acetone- d_6) of **NDI-5** without/with the addition of Hg^{2+} ions (1.0 equiv.)

3.4 The reversible response of NDI-5 towards Hg^{2+} ions

The reversibility is one of the most important factors for fluorescence chemosensors in practical applications. The reversibility of **NDI-5** was investigated by cycling the reaction of the probe with Hg^{2+} and KI, due to the high affinity of KI towards Hg^{2+} [37]. As shown in Figure 7, in acetone solution, the extremely weak fluorescence of **NDI-5** became the bright red emitting after the addition of Hg^{2+} , and the existence of KI (2.0 equiv.) can quench the fluorescence of the **NDI-5**- Hg^{2+} complex thoroughly. And the presence of KI alone had no influence on the emission of **NDI-5**. Since the changes of the fluorescence intensity were derived from the reaction between KI and Hg^{2+} , the fluorescence could be recovered again after the addition of another batch of Hg^{2+} (1.0 equiv.). These reversible cycles, fluorescence off and on, could be repeated for five or more times under the same condition, demonstrating the good reversible property of **NDI-5** towards Hg^{2+} and I^- .

Figure 7. (A) The fluorescence spectra of **NDI-5** (2.0×10^{-5} mol/L) in acetone with Hg^{2+} and KI. Inset: the photos of **NDI-5** with Hg^{2+} ions and KI under UV-lamp. (B) The changes of fluorescence intensity at 651 nm of **NDI-5** in acetone (2.0×10^{-5} mol/L) upon alternate additions of Hg^{2+} and KI. Inset: the photos of **NDI-5** in acetone by the addition of corresponding Hg^{2+} or KI for several times under UV-lamp.

3.5 The application of NDI-5 on test strip papers and in living cells

Motivated by the favorable features of **NDI-5** in solution, and with the aim to make the practical application conveniently, test strips were prepared by immersing filter papers into the solution of **NDI-5** (4×10^{-4} mol/L) and then drying in air, which exhibited nearly no fluorescence

under UV lamp. Afterwards, the test strips were immersed into the solution of Hg^{2+} ions with different concentrations. As shown in Figure 8, accompanying with the increase of the concentrations of Hg^{2+} , an obvious “turn-on” process of the fluorescence could be detected. Importantly, even though the concentration of Hg^{2+} was as low as 2 μM , the red fluorescence of the test strip was obvious. Subsequently, the reversibility in solution was further evaluated through strips experiment, which was closed to the detection of real samples. The probe **NDI-5** still served as an effective fluorescence switch modulated by Hg^{2+}/KI for several times, suggesting that **NDI-5** could act as a reversible sensor towards Hg^{2+} ions in real application.

Figure 8. Test strips with **NDI-5** immersed in the solution of Hg^{2+} and KI for several times under UV-lamp. The photos in the right side: The photos of fluorescence response of **NDI-5** test strips exposed to different concentrations of Hg^{2+} under UV lamp. From bottom to up, the concentrations of Hg^{2+} were 0, 2.0×10^{-6} , 2.0×10^{-5} , 1.0×10^{-4} , 1.0×10^{-3} and 1.0×10^{-2} mol/L, respectively.

To further demonstrate the practical application of the probe in the biochemical and physiological field, **NDI-5** was utilized in the image of the MCF-7 cells. MCF-7 cells were incubated with **NDI-5** (1.0×10^{-4} mol/L) for 30 mins, and followed by washing with HEPES (20 mM, pH = 7.40) for three times, as shown in Figure 9B, nearly no fluorescence was observed. However, once the cells were further incubated with Hg^{2+} ions for another 30 mins, a significant red fluorescence was detected (Figure 9E). Bright-field measurements confirmed that the cells after treatment with Hg^{2+} and **NDI-5** were viable throughout the imaging experiments (Figure 9A and 9D). As depicted in Figure 9C and 9F, the overlay of fluorescence and bright-field images revealed that the fluorescence signals were localized in the whole cell, indicating the good cell-membrane

permeability of **NDI-5**. These results suggested that probe **NDI-5** can be used for detecting Hg^{2+} in living cells and in vivo potentially.

Figure 9. Fluorescent images of living MCF-7 cells: (A), (B) and (C) were bright field image, dark field image and overlay image of dark and bright field of the cells incubated with **NDI-5** (1.0×10^{-4} mol/L) for 30 mins, respectively; (D), (E) and (F) were bright field image, dark field image and overlay image of dark and bright field of cell incubated with **NDI-5** (1×10^{-4} mol/L) for 30 mins, then further incubated with Hg^{2+} (1.0×10^{-4} mol/L) for another 30 mins.

4. Conclusion

In conclusion, we have synthesized a reversible red fluorescent probe (**NDI-5**) towards Hg^{2+} ions, which mainly consisted of naphthalene diimide as the strong electron withdrawing unit and bis[2-(3,5-dimethylpyrazol-1 yl)ethyl]amine as the receptor. As the coordination of Hg^{2+} ions can suppress the possible twisted intermolecular charge transfer (TICT) in the whole molecule, the probe **NDI-5** exhibited a “turn-on” fluorescence response to Hg^{2+} ions rapidly and selectively. Due to the high affinity of KI to Hg^{2+} , the probe can be utilized as the test strips repeatedly for several times. Also, the good cell-membrane permeability of **NDI-5** made the detection of Hg^{2+} ions in living cells effectively.

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