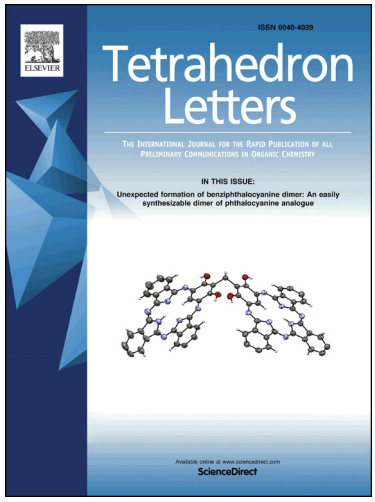

Turn-on fluorescent detection of strong acids based on a naphthalimide-indoline hybrid

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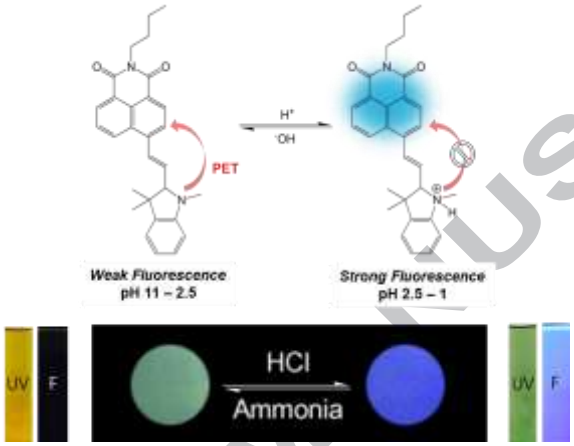

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ABSTRACT

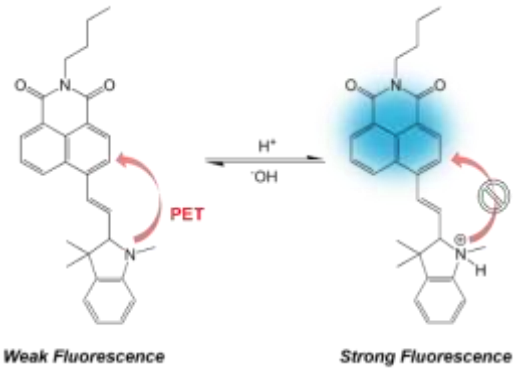
A naphthalimide-indoline hybrid (**1**) was developed as a pH-sensitive turn-on fluorescent probe. Probe **1** displays a weak fluorescence intensity in pH span of 2.5-11.0 owing to a photo-induced electron transfer (PET) from the indoline moiety to the naphthalimide. However, the PET process is suppressed under the pH of 2.5, showing a strong fluorescence signal at 430 nm. The turn-on fluorescent change of **1** is selective for the acidity (H^+) over other anions, metal ions, redox species and it displays a good reversibility. Moreover, glass TLC plates coated with probe **1** can readily detect acid vapors at an ambient atmosphere.

Introduction

Measuring pH is very important in in biology, industry, and environments.[1,2] In biology, pH regulates the activity of enzymes involved in a variety of biological processes. For example, mammalian cells have some organelles such as mitochondria, lysosome, endoplasmic reticulum, etc.,[3] and their pH ranges from 4 to 8 depending on the biological functions in the cell. In addition, the pH of gastric acid ranges from 1.5 to 3.5 in the human stomach and it plays a crucial role in digestion by activating digestive enzymes.[4] The inappropriate pH has been associated with various human diseases.[5] However, in the industry, strong acids such as HCl, HF, and etc., are widely used for glass etching, metal cleaning, and electronic manufacturing. Exposure to the acids can cause severe chemical burns and fatal systemic toxicity.[6,7] Thus, acid liquids and vapors must be strictly regulated in manufacturing environments and their leak should be monitored rapidly. Therefore, an effective and rapid detection of pH is extremely important to human being.

So far, glass electrodes are commonly used in pH measurement. But, this method has limitations in analysis environments because it is subject to chemical attack by strong acids, concentrated alkaline solutions, diluted HF, and etc.[8,9] In addition, the glass electrode can be easily broken and thus it should be handled with care. Currently, a number of fluorescent probes are exploited for pH measurement.[10-12] The probes provide changes in the fluorescence signal to pH with a high sensitivity, and a rapid response time. But their working range is usually pH 4-11 and only a few fluorescent sensors are available for the detection of the extremely acidic pH region.[13,14]

In this study, we presented a naphthalimide-indoline hybrid (**1**) as an acidic pH-sensitive turn-on fluorescent probe. As illustrated in Scheme 1, probe **1** shows a weak fluorescence in neutral and basic solutions, presumably due to a photo-induced electron transfer (PET) that might occur from the nitrogen atom of indoline moiety to the naphthalimide part. However, in case of the acidic solution, a protonation of the indoline induces a suppression of the PET process, which gives rise to a fluorescence increase. Thus, probe **1** provides a fluorescence change in a turn-on manner towards pH variations. Photophysical changes of **1** to pH variations and its sensing mechanism was investigated using absorption and fluorescence spectroscopy, as well as ^{1}H NMR spectroscopic studies.



Scheme 1. Proposed pH-dependent fluorescent turn-on mechanism of probe **1**.

Results and discussion

Reagents and Materials

All reagents, including perchlorate (ClO_4^-) salts of metal ions, tetrabutylammonium (TBA) salts of anions, thiols such as glutathione (GSH), cysteine (Cys), homocysteine (Hcy), sodium

hydrosulfide used for SH_2 generation) and other chemicals for synthesis and analysis were purchased from Aldrich, TCI, Alfa and used as received. All solvents were HPLC reagent grade, and distilled water was used in the analytical experiments. NMR was recorded at Bruker 400 MHz instrument and all chemical shifts are reported in ppm value using TMS as an internal reference. ESI-MS data were obtained using liquid chromatography mass spectrometer (LC/MS) at the Korea Basic Science Institute.

UV/Vis absorption and Fluorescence Spectroscopy

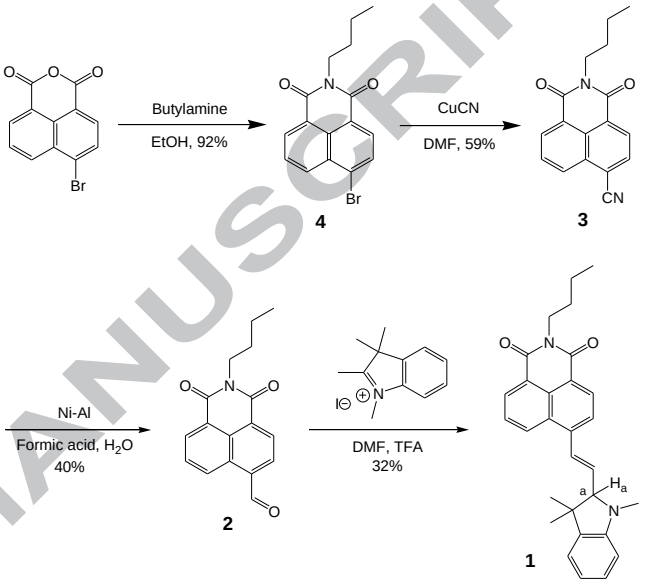
Stock solutions of probes, perchlorate salts of metal ions, and TBA salts of anions were prepared in CH_3CN. The pH buffer solutions were prepared by using 50 mM of potassium chloride (for pH 1-2 buffer), potassium hydrogen phthalate (for pH 3-5 buffer), potassium dihydrogen phosphate (for pH 6-8 buffer), sodium tetraborate (for pH 9-10 buffer), and sodium bicarbonate (for pH 11 buffer). The pH was adjusted by adding 0.1 M of NaOH or 0.1 M of HCl solution. Stock solutions of reactive oxygen species were prepared by using literature procedures.[15] Briefly, H_2O_2, *tert*-butylhydroperoxide (HOO^tBu), and hypochlorite (NaOCl) were delivered from 35%, 70%, and 11-14% aqueous solutions respectively. Hydroxyl radical (HO•) and *tert*-butoxy radical (^tBuO•) were generated by the reaction of 10 mM $(NH_4)_2Fe(SO_4)_2$, with 10 mM H_2O_2 or HOO^tBu, respectively. Superoxide (O_2^-) was delivered from 10 mM of potassium oxide (KO_2) in 10 mM pH 7.4 PBS solution. The Cu^+ was delivered from $[Cu(MeCN)_4][PF_6]$ in CH_3CN solution.[16] UV/Vis absorption and fluorescence spectra were recorded using UV-2600 (Shimadzu), and RF-6000 (Shimadzu) spectrophotometers, respectively. Excitation was provided at 390 nm with excitation and emission slit widths at 5 nm, respectively.

Synthetic procedures

Synthesis of **2-4**: Compounds **2**[17], **3**[18], and **4**[19] were prepared according to the literature procedures.

Synthesis of **1**: A mixture of **2** (120 mg, 0.43 mmol), 1,2,3,3-tetramethyl-*3H*-indolium iodide (260 mg, 0.86 mmol) and trifluoroacetic acid (TFA) (3.0 mL, 26.4 mmol) in dimethylformamide (DMF) (12 mL) was stirred under nitrogen. The resulting solution was heated at reflux for 3h at 120 °C. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (EA), and washed with water. The organic layer was then collected, and dried with anhydrous Na_2SO_4. After removal of the solvents, the crude product was purified by silica gel column chromatography using EA/hexanes (v/v, 1:7) as the eluent to yield **1** as a yellow solid (60 mg, 32%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.99 (t, *J* = 7.0 Hz, 3H); 1.17 (s, 3H); 1.44-1.50 (m, 5H); 1.71-1.76 (m, 2H); 2.79 (s, 3H); 3.62 (d, *J* = 8.5 Hz, 1H); 4.19 (t, *J* = 7.2 Hz, 2H); 6.54-6.58 (dd, J_1 = 15.8 Hz, J_2 = 8.3 Hz, 1H); 6.61(d, *J* = 8.0 Hz, 1H); 6.81 (t, *J* = 7.2 Hz, 1H); 7.08 (d, *J* = 7.0 Hz, 1H); 7.17 (t, *J* = 7.5 Hz, 1H); 7.48 (d, *J* = 15.5 Hz, 1H); 7.79 (t, *J* = 7.5 Hz, 1H); 7.92 (d, *J* = 7.5 Hz, 1H); 8.51 (d, *J* = 8.5 Hz, 1H); 8.58 (d, *J* = 7.5 Hz, 1H); 8.64 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 14.0, 20.5, 24.7, 26.1, 30.3, 35.0, 40.4, 45.0, 80.8, 108.3, 119.0, 121.9, 122.0, 123.3, 124.7, 127.0, 127.9, 128.7, 129.5, 129.6, 130.1, 131.2, 131.3, 135.4, 138.8, 141.0, 151.4, 164.1, 164.4 ppm. ESI-MS *m/z* (M^+) calcd for $C_{29}H_{30}N_2O_2$ 438.2307, found 439.23 06 ($M+H^+$).

A naphthalimide-indoline hybrid (**1**) was synthesized as depicted in Scheme 2. Compounds **2**[17], **3**[18], and **4**[19] were synthesized according to the literature procedures. Condensation of **2** with 1,2,3,3-tetramethyl-*3H*-indolium iodide in presence of trifluoroacetic acid (TFA) as a catalyst in DMF gave probe **1**. The structures of **1-4** were confirmed by ^{1}H NMR, ^{13}C NMR, and ESI-MS analyses (Figs. S4-S6). Interestingly, unlike other fluorophore-indolium hybrid materials,[20] probe **1** showed a doublet peak at 3.62 ppm assigned to the proton (H_a) of indoline moiety in ^{1}H NMR spectrum (Fig. S4). In addition, the tertiary carbon (C_a) of probe **1** was clearly observed at 80.8 ppm in ^{13}C NMR spectrum (Fig. S5).



Scheme 2. Synthetic routes to a naphthalimide-indoline hybrid (**1**).

*Optical properties of probe **1***

First of all, UV/Vis absorption and fluorescence spectral changes of **1** were monitored in various pH solutions. In Fig. 1a, as the pH changed from 11 to 1.0, fluorescence intensity (FI) at 430 nm was increased. The FI at 430 nm was very weak in pH span of 11 to 3.0, while 29-fold increase was seen as the pH changed from 3.0 to 1.0 (see inset of Fig. 1a). In addition, a blue fluorescence was clearly observed in the pH 1.0 buffer solution. However, negligible changes were seen in the absorption spectra of **1** in Fig. S1. These findings support the notion that the pH-dependent fluorescent turn-on signal is due to a PET process as suggested in scheme 1. Moreover, probe **1** exhibited a gradual fluorescence increase at 430 nm when the pH acidifies from 3.0 to 1.0 in Fig. 1b, and it was plotted in Fig. 1c. A plot of pH *vs* $\log[(I_{max}-I)/(I-I_{min})]$ was also produced based on the Henderson-Hasselbach type equation ($\log[(I_{max} - I)/(I - I_{min})] = pK_a - pH$) (Fig. 1d).[21] The pK_a of probe **1** turned out to be 1.60 ± 0.018, which implied that probe **1** could be used for measuring the pH range of 2.5 ~ 1.0. We thus demonstrated that probe **1** can give a fluorescence turn-on at the below pH 2.5, and could be useful for monitoring of the highly toxic acid solutions such as HCl and HF.

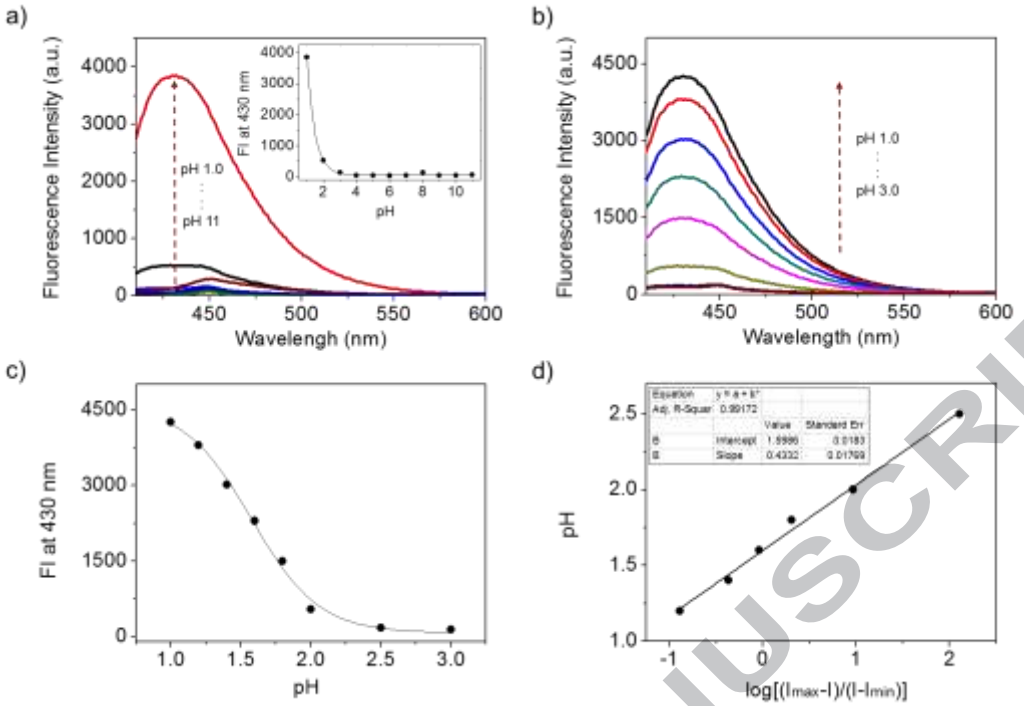


Fig. 1. Fluorescence response of **1** to pH variations. (a) Fluorescence spectra of **1** (10.0 μM) at different pH values (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11). Inset: plot of fluorescence intensity (FI) at 430 nm *vs* pH. (b) Fluorescence spectra of **1** (10.0 μM) recorded at pH span of 1-3 (1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0). (c) Plot of FI at 430 nm *vs* pH obtained from (b). (d) Plot of pH *vs* $\log[(I_{max}-I)/(I-I_{min})]$, where I is the observed fluorescence intensity of **1** at 430 nm. The y-intercept is the pK_a value (1.60 ± 0.018) of **1**. All data were obtained using an excitation at 390 nm in 50.0 mM buffer solution containing 10% (v/v) CH_3CN at room temperature.

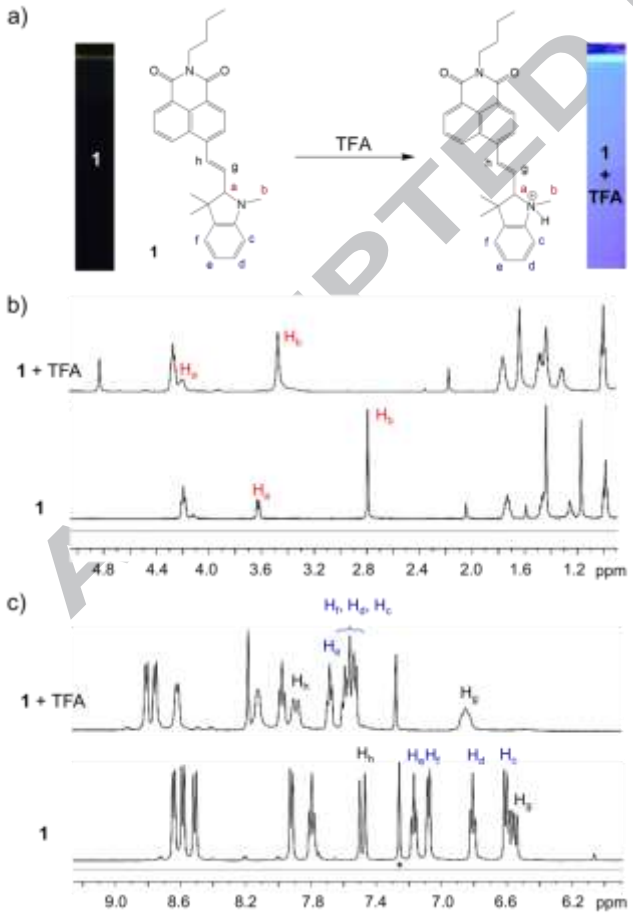


Fig. 2. (a) Fluorescence change observed upon addition of TFA (trifluoroacetic acid) to the solution of **1** in $CDCl_3$. (b and c) Partial ^1H NMR spectra of **1** with excess TFA in $CDCl_3$.

To get insight into the pH-dependent fluorescence turn-on mechanism of probe **1**, ^1H NMR analyses were carried out using trifluoroacetic acid (TFA) in $CDCl_3$. Upon titration of **1** with TFA, the proton peaks corresponding to naphthalimide and indoline parts were seen to shift to lower field (Figs. S2 and S3). Particularly, as shown in Fig. 2, the shifts for protons H_a, H_b, and aromatic protons (H_c, H_d, H_e, and H_f) of indoline part are dramatic in the presence of TFA. The protons H_g and H_h were also seen to shift to lower field during the titration. This observation is ascribed to the deshielding of protons for naphthalimide and indoline that results when the nitrogen group of indoline part is protonated by TFA. Moreover, we clearly observed a blue fluorescence upon addition of TFA to the solution of probe **1** in $CDCl_3$ (see Fig. 2a) These findings are consistent with a protonation of indoline part of **1** and the pH-dependent PET process as depicted in Scheme 1.

In order to confirm the selective fluorescence response of probe **1** to acidity (H^+), the possible interference of other analytes was tested. The fluorescence changes of **1** were tested in the presence of various anions (F^-, Cl^-, I^-, HPO_4^-, HSO_4^-, OAc^-, OH^-, ClO_4^-, and CN^-), and metal ions (Na^+, K^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Mg^{2+}, Ba^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Cu^+, Cu^{2+}, Fe^{2+}, and Fe^{3+}), as well as redox species (H_2O_2, •OH, HOO^tBu, $•O^tBu$, $•O_2^-$, ClO^-, SH_2, GSH, Cys, and Hcy). As shown in Fig. 3, under pH 1.0 solution, probe **1** showed a significant fluorescence increase at 430 nm. However, in case of other anion, metal ions, and redox species under pH 7.4 solution, an insignificant change was seen. These results tell us that probe **1** can be used as a selective turn-on fluorescent probe for monitoring acidic pH in biology and industry without any interferences from other interferants.

The reversibility of the pH-dependent fluorescence change was also investigated. As displayed in Fig. 4, a solution of probe **1** showed a reversible fluorescent off-on signal when the solution was changed to the acidic or basic for three cycles. This is fully consistent with the proposed mechanism of **1** as suggested in scheme

1. As a result, this revealed that probe **1** is useful for measuring an extremely acidic pH with its good reversibility.

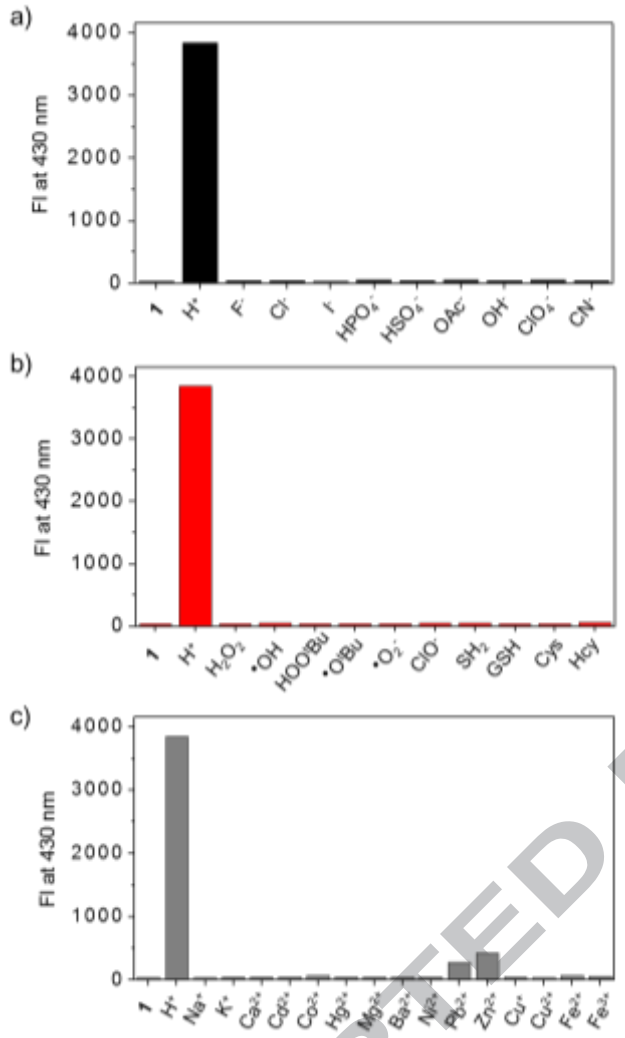


Fig. 3. Fluorescence changes of **1** (10.0 μM) upon addition of (a) anions, (b) redox species and (c) metal ions (10 equiv, respectively) in pH 7.4 buffer solutions (PBS solution was used for anions and redox species; HEPES solution was used for metal ions; 10.0 mM, respectively) containing 10% (v/v) CH_3CN. A pH 1 buffer solution (50 mM) was used as a source of proton (H^+). All data were obtained using an excitation at 390 nm.

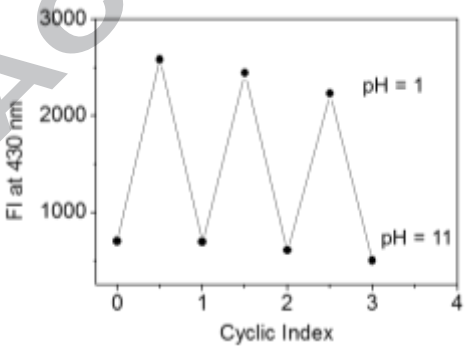


Fig. 4. Reversibility of **1** at acidic (pH 1) and basic (pH 11) conditions. The pH value was adjusted by using HCl or NaOH solutions. All data were recorded using an excitation at 390 nm in aqueous solution containing 10% (v/v) CH_3CN at room temperature.

Furthermore, we tested whether probe **1** can detect acid vapors or other environmentally abundant vapors (i.e., ammonia, methylamine, hydrazine, acetaldehyde, and H_2O_2). To this end, silica gel-coated glass TLC plates were soaked in a CH_3CN solution of probe **1** and dried. The plates stained with probe **1** were then exposed to vapors for 1 minute, respectively, under ambient conditions. As seen in Fig. 5a, upon exposure of acid vapors such as HCl and TFA, a strong blue fluorescence was seen in the TLC plates. In case of other acids such as HNO_3, H_2SO_4, AcOH, and formic acid, a little or no fluorescence changes were seen. We could explain that insufficient amounts of acid vapors were generated at room temperature owing to their relatively low vapor pressures. In addition, no detectable fluorescence increase was observed upon exposure of other vapors, including ammonia, methylamine, hydrazine, acetaldehyde, and H_2O_2. Moreover, reversible fluorescence change of probe **1** was seen in the TLC plate (Fig. 5b). These results provide support for the notion that probe **1** can selectively detect acids based on a pH-dependent PET effect that gives rise to fluorescent off-on changes both in solution and glass TLC plate.

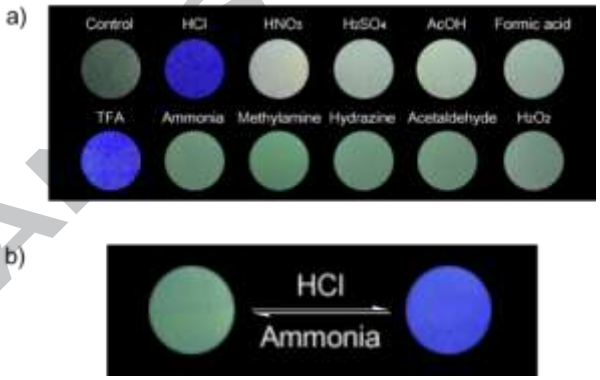


Fig. 5. (a) Fluorescence responses of probe **1** (5.0 mM)-stained glass TLC plates after exposure to various vapors for 1.0 min, respectively. (b) Reversible fluorescence change of probe **1** in the TLC plate.

Conclusions

Acidic pH-sensitive turn-on fluorescent probe (**1**) was developed using a naphthalimide-indoline hybrid. Under the neutral and basic solutions, probe is almost non-fluorescent by a PET occurring from an indoline part to the naphthalimide. However, under the acidic solution, probe **1** undergoes a suppression of the PET process, which gives rise to a fluorescence increase at 430 nm. This was also investigated by ^1H NMR analyses using $CDCl_3$ solution of **1** with TFA. In addition, the fluorescence increase is clearly observed in pH span of 1.0-2.5, which is related to the acidic pH range needed in biology and industry. Moreover, the fluorescence increase of **1** is selective for acidic solutions over other anions, metal ions, and redox species, and probe **1** also has a good reversibility. Furthermore, glass TLC plates coated with probe **1** can selectively detect acid vapors (HCl and TFA) over other environmentally abundant vapors (i.e., ammonia, methylamine, hydrazine, acetaldehyde, and H_2O_2). Therefore, probe **1** would be useful for detecting strong acids in solution and when **1** is present on solid supports such as glass TLC plate, and etc.

Acknowledgments

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Supplementary Material

Supplementary data related to this article can be found http://////////////

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Highlights:

- A pH-sensitive naphthalimide-indoline hybrid was synthesized.
- The naphthalimide-indoline hybrid showed a fluorescent turn-on signal in the pH range of 1.0-2.5.
- The fluorescent turn-on of probe was selective for acidic solutions over other ions and reactive species.
- The fluorescence change of probe to acidity showed a good reversibility.
- Glass TLC plates coated with the probe can selectively detect acid vapors.