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FRET based selective and ratiometric 'naked-eye' detection of CN⁻ in aqueous solution on fluorescein–Zn–naphthalene ensemble platform

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ABSTRACT

We have developed a FRET-based ratiometric fluorescent probe for the detection of CN^- using a fluorescein–Zn–naphthalene ensemble (NFH·Zn²⁺). The sensing mechanism was ascribed by displacement approach. The chemosensor exhibits high selectivity and sensibility for CN^- . The speculation was supported by fluorescence emission spectra, UV–vis spectrum, ¹H NMR titration experiments, and mass spectra. The interconversion of probe NFH and NFH·Zn²⁺ via the complexation/decomplexation by the modulation of Zn²⁺/CN⁻ mimics INHIBIT gate. In addition, it also shows an excellent performance in 'dip stick' method.

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The recognition of anions represent an interesting and emerging field of research due to the important roles of anions in industrial and biological processes.¹ Among different anions of interest, the detection of cyanide is of great importance due to its high toxicity² in physiological systems. It can affect many functions in the human body, including the vascular, visual, central nervous, cardiac, endocrine, and metabolic systems.³ Cyanide containing salts are widespread chemicals found in surface water originating not only from industrial waste but also from biological sources.⁴ Serious problems⁵ due to accidental release of cyanide into the environment are often caused by various industrial processes such as gold mining, electroplating etc. Therefore, various methods for the detection of cyanide have been developed. However, all these methods have limitations that they require time consuming procedure and more sophisticated instrumentation with high detection limits.⁶ Therefore, the development of optical chemosensors for cyanide has attracted considerable attention in recent decades because of their simplicity, low cost, and rapid measurement.⁷ For the optical detection of cyanide ions generally three approaches like hydrogen-bonding interaction,⁸ nucleophilic addition reaction,⁹ and displacement approach¹⁰ have been followed. The sensing phenomena through hydrogen-bonding interaction mostly suffer by the interference of F⁻ and AcO⁻ ions. The nucleophilic addition reactions mainly happen in organic to

http://dx.doi.org/10.1016/j.tetlet.2014.05.018 0040-4039/© 2014 Elsevier Ltd. All rights reserved. semi-aqueous media. In displacement approach, almost all reactions belong to copper–cyanide affinity, that is, at first, fluorescence of the receptor is quenched by copper with the help of its paramagnetic character and then on addition of cyanide the quenched fluorescence is recovered. Thus the displacement approach is limited to a fluorescence 'off–on' type sensor.

To overcome this limitation our choice is a ratiometric sensor. Ratiometric sensor is exceptionally useful compared to a conventional 'off-on' sensor, as the former allows the measurement of emission intensities at two different wavelengths, and thereby, the correction for environmental effects.¹¹ This can be achieved by the use of FRET based receptor moiety. A FRET based process is independent of the concentration of a single fluorescent dye and one can quantify the analyte concentration by using the ratio of intensities of the well resolved fluorescence peaks with reasonable intensities at two different wavelengths for analyte-free and analyte-bound probe.¹² Normally, dyes based on FRET processes are usually linked by a non-conjugated spacer, and the energy transfer occurs through space. In FRET-based energy cassettes large pseudo-Stokes shifts have occurred and overlap between acceptor and donor moiety is a necessary condition. To design small-molecule FRET ratiometric fluorescent probe for ratiometric detection of anions, it is required to formulate a FRET platform, which consists of an energy donor and an energy acceptor. In general, the emission spectrum of the donor should have reasonable overlap with the absorption spectrum of the acceptor.¹³ In most of the cases FRET process is turned on due to binding but here we report the reverse process, that is, FRET process is turned off (Scheme 1).

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Toward this end, herein we wish to disclose the first FRET based ratiometric fluorescent probe (fluoroscein–Zn–naphthalene ensemble, or Zn complex of naphthalene fluorescein hybrid, i.e., NFH) following displacement approach for the detection of CN⁻ in aqueous media.

The synthesis of chemosensor (NFH) is outlined in Scheme 2. 2-Hydroxy-1-naphthaldehyde, fluorescein hydrazine and chemosensor (NFH) are synthesized according to reported procedure.¹⁴ The structure of the receptor is confirmed by ¹H NMR and HRMS spectra.

In NFH chemosensor naphthalene and fluorescein chromophores act as an energy donor and an acceptor, respectively. First, the emission of the naphthalene chromophore and the absorption of the ring-opened fluorescein dye were measured (Fig. 1). From this figure, it is clear that there is an overlap between these two spectra, indicating that the FRET from the naphthalene chromophore to the fluorescein moiety took place.

Figure 2a shows the absorption spectra of NFH as a function of Zn^{2+} concentration in aqueous solution at room temperature. Addition of Zn^{2+} ion the absorption band at 370 nm is decreased rapidly and the absorption intensity at 436 nm is increased with an isosbestic point at 397 nm, which corresponds to the absorption of fluorescein. The absorption stabilized after the amount of added Zn^{2+} reached 1 equiv and a significant color change from colorless to yellow could be observed easily by the naked eye (Fig. 2a, inset). Job's plot shows a 1:1 stoichiometry between NFH and Zn^{2+} (Fig. S1).

The probe NFH, when excited at 371 nm, exhibits a weak emission at 415 and 524 nm. In the absence of Zn^{2+} , the excited energy of the naphthalene donor could not be transferred to the fluorescein acceptor, as the fluorescein acceptor is in the closed form. Thus, the FRET should be off in the free NFH. Upon addition of an increasing amount of Zn^{2+} , a dramatic enhancement of the



Scheme 1. Approach for the detection of cyanide ions on the basis of Zn-complex.



Figure 1. The overlap (shown with green shade) between the emission and absorption spectra of the donor (naphthalene) and acceptor (fluorescein) moieties.

emission at 524 nm at the expense of the emission at 431 nm, exhibited a clear green fluorescence (Fig. 2b). Almost complete enhancement happened when 1 equiv of Zn^{2+} was added to the solution of NFH (Supporting information).

The sensor NFH–Zn²⁺ for cyanide detection was prepared in situ by addition of 1 equiv of ZnCl₂ to CH₃OH/H₂O (3:7, v/v) solution of NFH. Now, the UV–vis and fluorescence studies were carried out at pH 7.1. The yellow color of the NFH·Zn²⁺ disappeared upon addition of an increasing concentration of CN⁻ (Fig. 3, inset). The absorbance at 436 nm decreased and it increased at 370 nm with an



Figure 2. (a) UV-vis absorption titration spectra and (b) Fluorescence spectra of NFH ($c = 2.0 \times 10^{-5}$ M) in presence of Zn²⁺ ($c = 2.0 \times 10^{-4}$ M, 0–200 µl) at pH 7.1 in CH₃OH/H₂O (3:7, v/v).



Scheme 2. Reaction scheme for preparation of NFH.

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Figure 3. (a) Absorbance spectra of Zn^{2+} ensemble upon addition of CN^- at pH 7.1. The naked eye color change of NFH + Zn^{2+} with addition of CN^- (inset) (b) Ratiometric response of NFH + Zn^{2+} ensemble (2.0 × 10⁻⁵ M) toward CN^- .



Figure 4. Fluorescence emission spectra of Zn^{2+} ensemble upon addition of CN^- at pH 7.1 (λ_{ex} = 371). The fluorescence color change of NFH + Zn^{2+} with addition of CN^- (inset).

isosbestic point at 397 nm upon the addition of an increased concentration of CN^- (Fig. 4). The addition of 1.2 equiv of CN^- to NFH·Zn²⁺ turned the original yellow colored solution into a colorless solution. The decrease of yellow color of the solution containing the NFH·Zn²⁺ complex strongly suggested that the ring opened amide form of NFH·Zn²⁺ was converted to the spirolactam form of NFH in the presence of CN^- . The selectivity of the UV response of NFH·Zn²⁺ was verified in the presence of different anions such as Br⁻, Cl⁻, I⁻, F⁻, ADP, ATP, PPi, OAc⁻, Pi, SH⁻, SCN⁻, N³⁻ (Fig. 5a).

Addition of CN⁻ to the solution of the complex of NFH·Zn²⁺ brought the reverse change in the fluorescence spectra (Fig. 4) via reverse FRET phenomenon. The fluorescence of NFH·Zn²⁺ at 524 nm dramatically decreased along with small enhancement at 415 nm with a clear isoemission point at 452 nm upon the addition of CN⁻. The changes of the emission intensities became constant and enhanced fluorescence of NFH·Zn²⁺ was recovered by 98% eventually when the amount of CN⁻ (2.0×10^{-4} M) added reached 1.2 equiv).

As there were more errors when the volume of the mixture was gradually increasing, it could not achieve better fluorescence recovery later. So, it is clear that all of the anions, except CN^- , are practically insensitive to the fluorescence of the NFH·Zn²⁺ complex and binding of CN^- ions to Zn^{2+} led to regeneration of the cyclic lactam form and confirms the reversible binding of fluorescein dyad to Zn^{2+} . One of the most important features of NFH– Zn^{2+} is its high selectivity toward CN^- over other competitive anions. Only ADP and ATP interfere a little more in this case (Fig. S3). The sensitivity for CN^- was calculated on the basis of the linear relationship between the emission intensity at 552 nm



Figure 5. (a) Ratiometric response of NFH–Zn²⁺ (2.0×10^{-5} M) toward anions in UV–vis titration. (b) Fluorescence response of NFH–Zn²⁺ (2.0×10^{-5} M) toward anions (1 equiv) with emission spectroscopy.



Figure 6. Color changes on test paper (a) NFH; (b) NFH in presence of Zn^{2+} ; (c) NFH Zn^{2+} in presence of CN^- under ambient and UV light.

and the CN⁻ concentration. Moreover, when the same equiv of Zn^{2+} was added for its interaction with CN⁻, which showed fluorescence recover for 6 switching cycles (Fig. S4). The equilibrium competition constant¹⁵ was 170 M⁻¹ on the basis of the fluorescence titration data in HEPES buffer solution at pH 7.1. The data fit to a displacement model that provides an equilibrium competition constant (Fig. S5). Finally, the detection limit was measured to be 0.509 μ M. According to the World Health Organization (WHO), CN⁻ concentrations lower than 1.9 μ M are acceptable in drinking water.¹⁶ This means that this system is sensitive enough to monitor cyanide concentrations in drinking water (Fig. S6).

Now, we became interested in studying the binding affinity of NFH–Zn²⁺-ensemble for CN⁻ using ¹H NMR and ¹³C NMR spectroscopy. Interestingly, the peak at 11.493 ppm (for the naphthalene OH proton) shifted to 11.508 ppm due to binding with CN⁻. Moreover, the peak at 8.569 ppm shifted to 8.304 ppm and most of the aromatic protons shifted to upfield due to formation of NFH. In the case of ¹³C NMR, the peaks at 206.65 ppm (for spirolactum carbon atom), 201.37 ppm (for emine carbon atom) and 171.51 ppm (9 carbon in fluorescein), respectively, shifted to 163.33 ppm, 152.24 ppm, and 79.18 ppm, respectively, when CN⁻ was added to NFH-Zn²⁺ ensemble. Thus, from ¹H NMR and ¹³C NMR titration we conclude that NFH–Zn²⁺ ensemble behaves as a very smart probe for CN⁻.

To further look into the nature of the interaction between NFH– Zn^{2+} and CN^- , HRMS mass spectrometric experiments were carried out. When 1 equiv of CN^- was added to this complex, the peak at m/z 562.0517 corresponding to $(NFH-H^++Zn^{2+})^+$ disappeared and only the peak at m/z 501.1495 corresponding to $[NFH + H^+]^+$ was observed. This suggests that the binding affinity of CN^- toward Zn^{2+} is much more than that of NFH toward Zn^{2+} and thus the removal of Zn^{2+} from the complex by the CN^- induced ring-closure reaction.

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Scheme 3. (a) General representation of the symbol of an INHIBIT Gate (Formal Combination of a NOT,YES and AND Gate); (b) the corresponding truth table of this logic gate; (c) the output signals of this logic gate in the presence of different inputs.

Many sensors for ${\rm Zn}^{2+}$ and ${\rm CN}^-$ detection could only be performed in solution, which would limit their applications under special circumstances such as on-site detection in situ. To investigate the practical application of chemosensor NFH, test strips were prepared by immersing filter papers into a CH₃OH/H₂O binary solution of NFH (2×10^{-4} M) and then drying in air. As shown in Figure 6, the obvious color and fluorescence changes were observed with Zn²⁺ and CN⁻ solution. Therefore, the test strips could directly detect Zn^{2+} and CN^- in CH_3OH/H_2O binary solutions. Development of such dipsticks is useful as instant qualitative information is obtained without resorting to the instrumental analysis.

This type of reversible behavior of Zn^{2+} and CN^- toward NFH mimics the INHIBIT logic gate (integrated¹⁷ by combining a NOT, a YES, and an AND gate). In this logic gate, Zn^{2+} and CN^- are used as input. For input, the presence of Zn^{2+} and CN^{-} is defined as '1' state and the absence of the molecules defined as '0' state. One of them, that is, Zn²⁺ in this case should lead to absorption and fluorescence in its occupied state, equivalent to a YES operation (input 1, Scheme 3). The interaction of the other input, that is, CN⁻ in this case (input 2, Scheme 3) with its corresponding receptor should lead to absorption and fluorescence quenching, thereby implementing the necessary NOT gate. The receptor, that is, NFH (occupied or free) acts in parallel on the absorption and fluorescence output signal, which implements the required AND function. In the presence of both inputs, the quenching (by input 2) should override the absorption and fluorescence enhancement by input 1, in accordance with the truth table shown in Scheme 2.

In conclusion, we have unveiled a new zinc complex-based ratiometric, colorimetric, and fluorogenic sensor for CN⁻ anion. To the best of our knowledge, it may be the first FRET based ratiometric fluorescent probe with displacement approach for the detection of CN⁻ in aqueous media using Zn ensemble. The detection limit of CN^- is quite low (0.509 μ M) using this ensemble. The color changes would be useful for visual detection which is costeffective and convenient without resorting to any instrument. This system also mimics INHIBIT gate.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.05. 018.

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