



Cyanine dye-based chromofluorescent probe for highly sensitive and selective detection of cyanide in water

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ABSTRACT

Cyanine dye Cy5 was used to be a probe for highly selective detection of trace cyanide in water by using a convenient two-phase strategy. The detection limit of both the fluorescent and colorimetric assay for cyanide is below 1.9 μM , the maximal allowance level for drinking water set by the World Health Organization.

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Anion recognition has been a challenging area in supramolecular chemistry and has aroused extensive research activities in recent decades with special focus on the design and synthesis of artificial chemosensors capable of effective and selective sensing of different anions.^{1,2} In this regard, substantial progress has been achieved for recognizing general anions such as halide, carboxylate, phosphate, and so on.^{3–5} However, anions such as cyanide that may cause much mischief to human beings and to the environment, have not yet been sensed or investigated thoroughly enough. It is known that cyanide that is 0.5–3.5 mg per kg of body weight is lethal for human beings.⁶ Recent studies have shown that the lethal cyanide concentration in the blood of victims of fire accidents is 23–26 μM .⁷ Despite the wide awareness of the fact that binding of cyanide to cytochrome oxidase reduces the activity of this enzyme and inhibits oxygen utilization by cells,⁸ the use of cyanide is still the current practice in industries such as gold mining, electroplating, and metallurgy.⁹

Most of the present cyanide sensors are based on the strong coordination ability or on the nucleophilic character of the cyanide anion. Chemosensors that rely on the formation of complexes between cyanide and heavy transition metal systems¹⁰ or boron derivatives¹¹ have been reported. Nucleophilic addition reactions of cyanide to the electron-deficient carbon containing systems (e.g., oxazine, pyrylium, squaraine, trifluoroacetophenone, acridinium salts, benzil, β -vinyl substituted calix[4]pyrrole, etc.)¹² were also developed. However, there are several limitations associated with many of these currently available cyanide sensors: such as (i) These sensors work only in organic solvents; (ii) They have poor

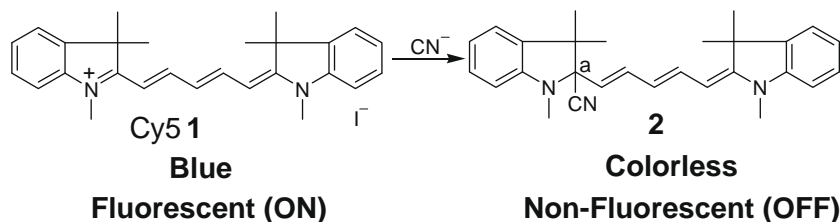
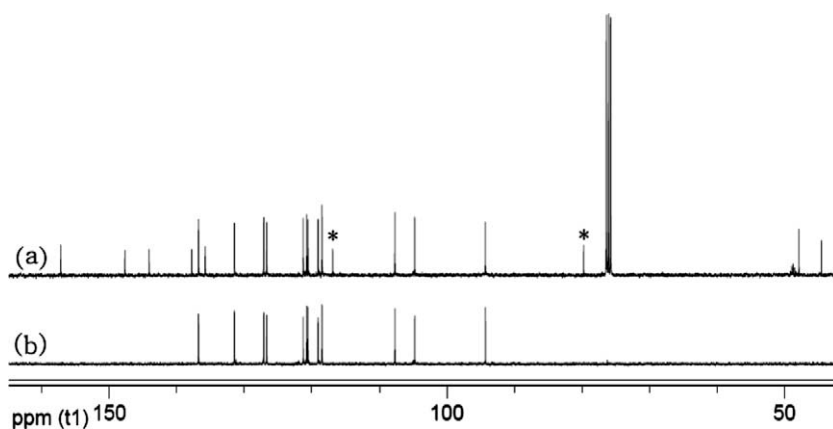
selectivity over fluoride or acetate; (iii) They show low sensitivity; and (iv) They possess short-wavelength absorption or emission.

As we know, the colorimetric probes have been attracted for their easily observable color change, offering qualitative and quantitative information without using expensive equipment. On the other hand, fluorescent probes may present advantages such as high sensitivity, simplicity, and feasibility as a diagnostic tool for biological concerns.^{12f} Consequently, chromofluorescent probes are in great demand. Especially, the probes with absorption and emission maxima in the near-infrared (NIR) region around 650–900 nm are believed to be superior to those short-wavelength probes for their reduced scattering and low background emissions.⁵ However, cyanide probes with both NIR absorption and emission were rarely found before.¹³

Cyanine dyes contain two nitrogen centers (one of which is positively charged) which are connected by a polymethine bridge with odd number of carbons. They are well-known for their large extinction coefficients ($>10^5$ in many cases, so high sensitivity may be achieved), strong fluorescence, and easily tunable absorption and emission maxima by varying the heterocyclic structure or the length of the polymethine bridge. Cyanine dyes have been widely used in various fields such as fluorescent labeling of biomolecules,¹⁴ photodynamic therapy,¹⁵ optical data storage,¹⁶ and molecular recognition including sensing of metal ions, nitric oxide (NO), sugars, and nucleic acids, etc.¹⁷ However, detection of common inorganic anions by utilizing cyanine dyes has not been reported. Murthy and co-workers reported that the hydrocyanines can be synthesized by reducing the iminium cations of the cyanine dyes with NaBH_4 .¹⁸ Inspired by their work, we envisioned that the nucleophilic addition of cyanide to the iminium cation moiety of

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Scheme 1. Proposed cyanide-sensing mechanism of **1**.Figure 1. Partial ^{13}C NMR (a) and DEPT 135 (b) spectra of compound **2** in CDCl_3 .

the cyanine dye may achieve a specific sensing of this anion. Here, we report the first observation of highly selective and sensitive detection of cyanide in water by using the commercially available NIR cyanine dye, 1,1',3,3',3',3'-Hexamethylindodicarbocyanine iodide **1** (i.e., Cy5, Scheme 1).

The pattern of interaction between **1** and cyanide was first examined by ^1H NMR titration experiment (Fig. S1). Upon addition of cyanide in aliquots, the signals attributed to compound **1** disappeared with the appearance of a set of new signals. The ^1H NMR signals remained essentially unchanged as more than 1.0 equiv cyanide was introduced to the solution. This suggests a 1:1 reaction stoichiometry between compound **1** and cyanide. Reaction of excess KCN with compound **1** in DMSO yielded product **2** (for synthesis, see Supplementary data) whose ^1H NMR spectrum is consistent with the one shown in Figure S1. This, together with the ^{13}C NMR and DEPT 135 spectra of compound **2** (also see Supplementary data), indicate that compound **2** should be the product of the nucleophilic addition reaction between cyanide

and Cy5 (Scheme 1). The two quaternary carbon signals at 79.7 and 116.9 in ^{13}C NMR spectrum of **2** clearly suggest the existence of C_a and a cyano-group, respectively (Fig. 1). High resolution mass

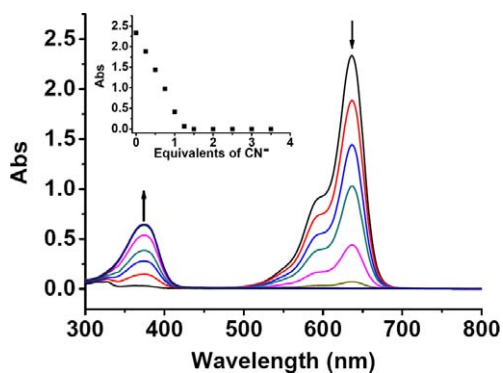


Figure 2. Changes in the absorption spectra of Cy5 (10 μM) upon addition of increasing amounts of CN^- as Bu_4N^+ salt (0–3.5 equiv) in CH_3CN at 22 $^\circ\text{C}$. The inset shows the absorbance changes at 637 nm as a function of equivalents of CN^- .

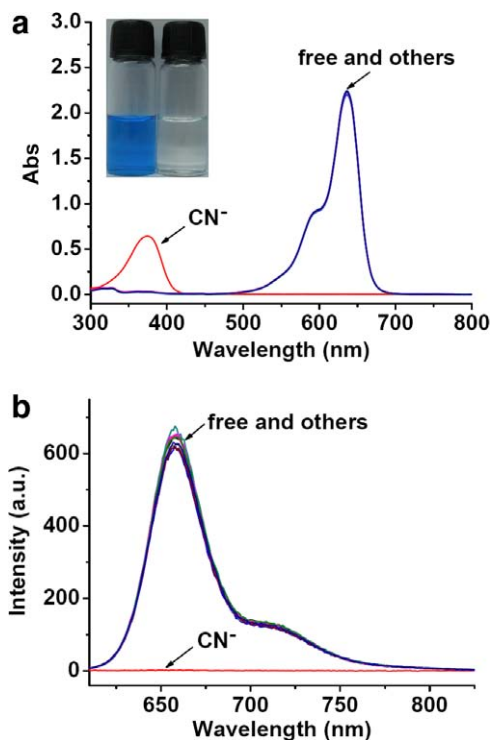


Figure 3. (a) The absorption spectra changes when Cy5 (10 μM) was treated with various anions as Bu_4N^+ salts (CN^- , 2 equiv; F^- , 30 equiv; Cl^- , Br^- , AcO^- , H_2PO_4^- , HSO_4^- , NO_3^- , ClO_4^- , SCN^- , 100 equiv) in CH_3CN at 22 $^\circ\text{C}$ in 1 min. The inset shows the color changes of Cy5 before and after addition of CN^- (2 equiv). (b) The emission spectra changes when Cy5 (1 μM) was treated with various anions as Bu_4N^+ salts (CN^- , 8 equiv; others, 100 equiv) in CH_3CN at 22 $^\circ\text{C}$ in 1 min (excitation at 600 nm).

spectrometry (HRMS) analysis of **2** was also conducted (Supplementary data). The mass peaks at m/z 383.2485 (calculated value, 383.2482) corresponding to $[2-\text{CN}]^+$ was clearly demonstrated, which provided further evidence for the nucleophilic addition mechanism.

The absorption spectrum of Cy5 (10 μM) in CH_3CN exhibited a band centered at 637 nm (Fig. 2). Upon addition of increasing amounts of Bu_4CN , this band faded gradually with the concomitant appearance of a new absorption band at 375 nm, implying a destruction of the large π conjugation. The UV-vis behavior of Cy5 after addition of CN^- further confirmed the aforementioned reaction mechanism. The association constant of Cy5 with CN^- in CH_3CN was too large to be determined accurately ($>10^5 \text{ M}^{-1}$) by using nonlinear curve fitting analysis.¹⁹ The colorimetric sensitivity of Cy5 toward CN^- in CH_3CN was examined. Addition of 2 equiv of CN^- to 1 μM solution of Cy5 induced an obvious color change from pale blue to almost colorless (Fig. S2), corresponding to a detection limit of about 2 μM . Figure 3a shows the absorption spectra changes of Cy5 in the presence of common inorganic anions of current interest (CN^- , F^- , Cl^- , Br^- , AcO^- , H_2PO_4^- , HSO_4^- , NO_3^- , ClO_4^- and SCN^-). It can be seen that the reaction was completed in 1 min upon addition of 2 equiv of CN^- with a color change from deep blue to colorless, whereas all other anions induced no obvious changes in their respective absorption spectra even when the anions were added in large excess.

Excitation of Cy5 (1 μM) in CH_3CN at 600 nm exhibited a strong emission band at 658 nm (Fig. 3b). Respective addition of

100 equiv of F^- , Cl^- , Br^- , AcO^- , H_2PO_4^- , HSO_4^- , NO_3^- , ClO_4^- and SCN^- caused no significant changes in its fluorescence spectrum. In sharp contrast, addition of 8 equiv of CN^- resulted in a complete quenching of the fluorescence of Cy5 within 1 min (fluorescent off).

It is worthy of note that the present probe demonstrates high selectivity and sensitivity toward CN^- over other competitive anionic species by using both colorimetric and fluorescent detecting methods. This is understandable because CN^- is the strongest nucleophile among all the anions tested. To investigate the possibility of utilizing the superior binding of Cy5 with CN^- for detecting trace of cyanide in water, the acetonitrile–water mixtures were first tested but failed to exhibit meaningful color change due to the strong solvation effect of water. Detection of anions in water is a great challenge up to now for the poor performance of receptors in this media due to the solvation effect. However, under biphasic conditions, this limitation can be avoided effectively. Gabbai et al. has reported a cationic borane receptor which can capture fluoride from water with high efficiency by using biphasic strategy.²⁰ In our case, the two-phase system was also used successfully for the detection of cyanide in water. Cy5 can be co-dissolved with Bu_4NBr (0.1 M) in CH_2Cl_2 . Mixing the resulted solution of Cy5 (10 μM) with KCN (70 μM) in CHES buffer (pH 9.50, 50 mM, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:1$) resulted in an obvious color change of the organic phase from blue to colorless. In contrast, no detectable color change was observed when Cy5 (10 μM in CH_2Cl_2) was treated with the Na^+ or K^+ salts of other potentially competing anions (KF, KCl, KBr, NaOAc, NaH_2PO_4 , NaHSO_4 , NaNO_3 , NaClO_4 , 1000 μM) in CHES buffer (Fig. 4a).

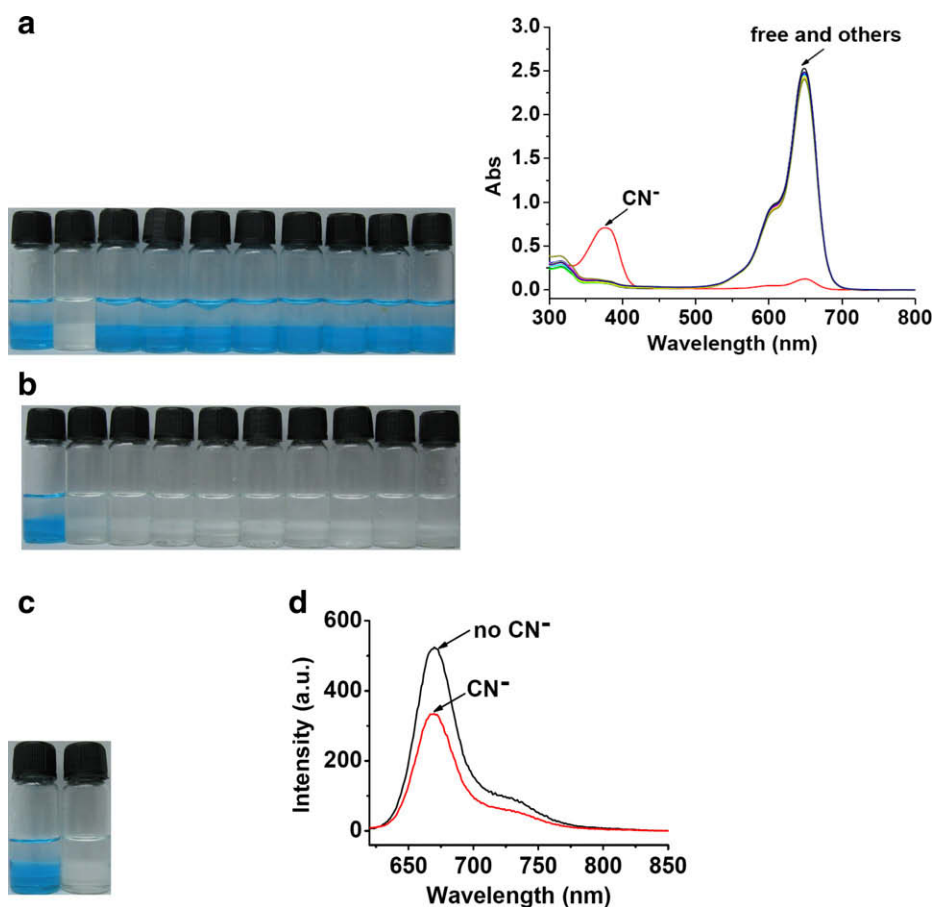


Figure 4. Two-phase detection experiments with Cy5 in the presence of a phase-transfer catalyst (Bu_4NBr , 0.1 M) in CH_2Cl_2 and anions as Na^+ salts or K^+ salts (KCN, KF, KCl, KBr, NaOAc, NaH_2PO_4 , NaHSO_4 , NaNO_3 , and NaClO_4) in CHES buffer (pH 9.50, 50 mM). (a) $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:1$, $[\text{Cy5}] = 10 \mu\text{M}$, left: from left to right: no anion, 70 μM CN^- , 1000 μM of F^- , Cl^- , Br^- , AcO^- , H_2PO_4^- , HSO_4^- , NO_3^- , ClO_4^- , respectively; right: the corresponding absorption spectra. (b) $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:1$, $[\text{Cy5}] = 10 \mu\text{M}$, from left to right: no anion, 70 μM CN^- , 1000 μM other anions + 70 μM CN^- . (c) $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:1$, $[\text{Cy5}] = 10 \mu\text{M}$, left: 1000 μM every kind of anions except CN^- , right: 70 μM CN^- + 1000 μM every kind of other anions. (d) $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1.5:1$, $[\text{Cy5}] = 0.5 \mu\text{M}$, fluorescence spectra changes of organic phase after treatment with and without 1 μM of KCN in CHES buffer, excitation at 600 nm.

Even when the CN^- (70 μM) was mixed with other anions in CHES buffer, Cy5 still can recognize it by similar color change from blue to colorless (Fig. 4b). More importantly, under the detecting conditions (i.e., 10 μM of Cy5 in CH_2Cl_2), the presence of all other anions (F^- , Cl^- , Br^- , AcO^- , H_2PO_4^- , HSO_4^- , NO_3^- , ClO_4^- , 1000 μM each) in CHES buffer ($\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:1$) also did not show any interference to the specific sensing of CN^- by Cy5. A distinct color change was only seen for CN^- (Fig. 4c). The colorimetric limit of detection was then estimated. When 1 μM of Cy5 in CH_2Cl_2 was treated with 1.5 μM of KCN in CHES buffer ($\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:7$), a color change of the organic phase was observed from light blue to almost colorless (Fig. S3), meaning that our probe can be used to detect the presence of trace cyanide in water via a naked-eye discernible color change at a detection limit below the maximum permissible level (1.9 μM) for drinking water by the WHO.²¹ To our knowledge, detection of cyanide in water less than 1.9 μM is a challenging task by colorimetric method. There is only one example reported that can meet this criterion.^{12d} However, heating at 50 °C was needed in this case. Considering the advantages of low cost and easy to operate, our method is attractive for practical application.

The interference experiments and detection limit of Cy5 for cyanide detection by fluorescent method were also evaluated by using the two-phase system (Fig. S4 and Fig. 4d). Under the testing conditions (10 μM of Cy5 in CH_2Cl_2), the presence of all other anions (1000 μM each) in CHES (pH 9.50, 50 mM, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:3$) except for cyanide did not induce significant changes of the fluorescence of the organic phase after shaking. In contrast, addition of the 100 μM KCN in CHES buffer caused the fluorescence of Cy5 almost completely quenched (Fig. S4). Figure 4d shows the detection limit evaluation for Cy5 by fluorescent method. Treatment of 0.5 μM of Cy5 in CH_2Cl_2 with 1 μM of KCN in CHES buffer (pH 9.50, 50 mM, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 1:5$) resulted in about 36% fluorescence decrease. Therefore, Cy5 can also be used to detect the WHO-suggested maximum-allowed cyanide concentration in drinking water by fluorescent method.

In summary, we have established a colorimetric and fluorescent probe Cy5 for the detection of cyanide by making use of the strong nucleophilic character of cyanide to the positively charged imine moiety of the cyanine dye. A two-phase system was successfully used to overcome the drawback of poor performance of Cy5 in water. The present sensing system has the following advantages: (1) it is a NIR sensor; (2) the sensing of our probe is not affected by the common interferents such as F^- and AcO^- ; (3) both the colorimetric and fluorescent detection limits are lower than WHO guidelines of 1.9 μM ; (4) our probe is commercially available. To improve the performance of cyanine dye in water, systematic research on the effect of the polymethine chain's length and the heterocyclic structure to the detection limit is in progress in our laboratory. Positive results have been obtained and will be reported in due course.

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Supplementary data

Supplementary data associated with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2009.09.079.

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