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A new triphenylamine fluorescent dye for sensing of cyanide anion in living cell

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ABSTRACT

A triphenylamine-based fluorescent probe **1** with larger Stokes shift (~141 nm) has been synthesized, which can make an efficient nucleophilic addition by reacting with CN^- in water. Upon addition of CN^-, the red fluorescence of **1** was distinctly quenched. Importantly, **1** exhibits a highly selective response toward CN^- over other anions in water and a high sensitivity (detection limit $\leqslant$ 11 nM). Moreover, it has potential application to track CN^- levels in living cells by confocal laser scanning microscopy (CLSM).

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Cyanide ion, as a highly toxic anion, has drawn considerable attention for its detection and determination.[1] The high toxicity of cyanide lies in its ability to bind with the ferric iron in cytochrome oxidase and inhibit oxygen utilization by cells.[2] According to the guidelines of the World Health Organization (WHO), the acceptable level of CN^- is lower than 1.9 μM in drinking water and 20 μM is considered as its lethal level.[3] By now, the detection limit of previous studies can only reach the micromolar concentration, which is higher than the physiologically lethal level (~20 μM).[4] Moreover, in the presence of other anions, such as fluoride and acetate, the selectivity of CN^- in water is still a great challenge.[5]

More recently, there has been increasing interest in designing the chemodosimeter for the sensing of cyanide with the purpose of minimizing the interference of other anions, and this usually has been developed in different solvent systems by the nucleophilic addition of CN^- to pyrylium,[6] pyrazolone,[7,8] tri-fluoroacetophenone,[9] benzil derivatives,[10] Meldrum's acid[11], etc.[12] However, many achievements have been mainly focused on the design of recognized groups, which always attach on the commercial fluorophores with short excited/emission wavelength. For example, coumarin was an excellent fluorophore with brightness emission around 490 nm, but small Stokes shift make obvious overlap between absorption and emission spectra which is a big puzzle for selecting an optimized excitation wavelength. Among several strategies, Föter resonance energy-transfer (FRET) mechanism was used for larger Stokes shift[13] in which the excitation energy of the energy-donor is transferred to the energy-acceptor. On the other hand, two signal moieties in one sensory unit make the bigger molecular weight and larger scale.

Therefore, we are interested in developing a specific fluorescent sensor with a larger Stokes shift for sensitive CN^- detection in water and living cells. Our idea is to use the nucleophilic addition reaction of CN^- with a dicyanovinyl group as the strategy for the design of a probe to track CN^- levels in living cells with high sensitivity. Triphenylamine was used as a fluorophore whose strong donating property can induce an effective redshift of the maximum emission peak with the connection of a smaller electron-withdrawing group. For the purpose, the ICT-based sensor **1** was designed and synthesized (Scheme 1 and Fig. S1). Compound **1** contains two pyridine groups and a dicyanovinyl group which is connected by a triphenylamine.[14] Pyridine-attached groups can not only improve the water-solubility of **1** but also serve as an electron-withdrawing group. More importantly, a dicyanovinyl-introduced group plays a vital role as a cyanide reactive site.[15] As shown in Figure 1A, **1** indicated two strong absorption bands at ca. 360 and 465 nm in water, which were ascribed to the ICT process. The obvious red emission at 612 nm was found as shown in Figure 1B with a Stokes shift larger than 140 nm indicating that a full emission spectrum can be obtained by choosing maximum absorbance as excitation wavelength and a small overlap between absorption and emission spectra. Upon the addition of CN^-, a solution of **1** can result in a color change from orange to colorless accompanying a gradual decrease in absorption intensity. The result indicated the generation of stabilized anionic species **1**-**CN**

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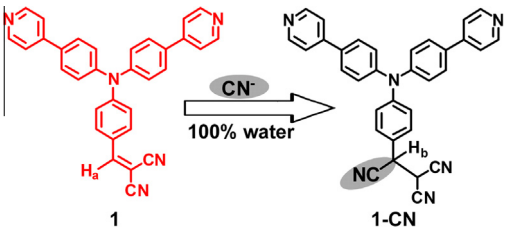

Scheme 1. Structures of **1** and **1-CN**.

which can be confirmed by ^{1}H NMR, ^{13}C NMR, HRMS, and IR spectra (Figs. S2–S5).

Fluorescent properties of sensor **1** were also studied by adding CN^- to a pure water solution of **1** (2.5 μM) at room temperature. As shown in Figure 1B, the emission **1** at 612 nm (λ_{ex} = 465 nm) decreased rapidly upon the addition of CN^-. When 2.4 equiv CN^- was added to the solution of **1** (Φ_f = 0.16, pH 7, Fig. S1), a fivefold (F_0/F) decrease in fluorescence intensity at 612 nm was observed. These facts indicate that **1** can serve as a 'naked-eye' sensor for CN^-.

This is further supported through TD-DFT calculations on **1** and **1-CN** compounds. The molecular orbitals of these compounds are presented in Figure 2. The HOMO orbital of **1** is localized on the entire molecule including the donor and acceptor. The LUMO orbital is concentrated on the acceptor side of dicyano-vinyl and LUMO+1 is on the pyridine groups, thus resulting in a charge transfer from triphenylamine to dicyano-vinyl and pyridine. Interestingly, the electron density of **1-CN** compound is significantly contributed to the cyanation of the anionic species at the HOMO level, but the LUMO state stretches out to the pyridine moiety. However, the electron density of the phenyl group connected with the cyanine anion is low. ICT process in sensor **1** is also weak. Furthermore, we also calculated the lowest excitation energies of **1** and **1-CN**

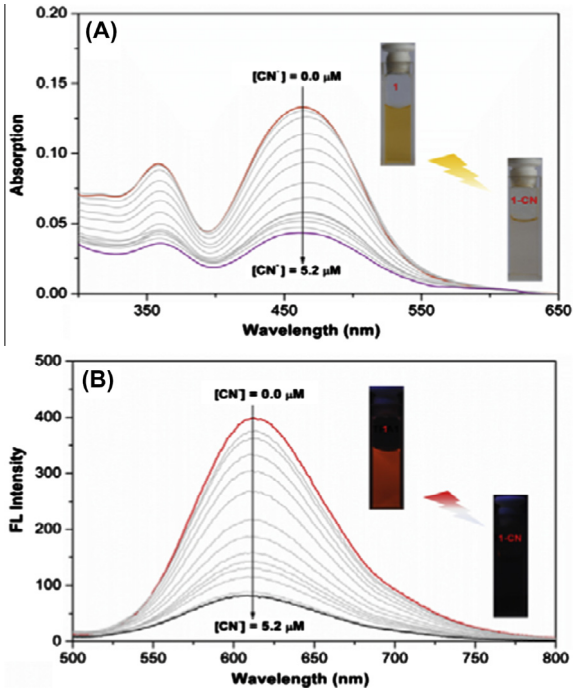

Figure 1. (A) Absorption titration of **1** (2.5 μM) with the addition of CN^- (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, 4.8, and 5.2 μM) in water at room temperature (inset: the color change images of **1**). (B) Emission titration of **1** (λ_{ex} = 465 nm) with addition of CN^- (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, 4.8, and 5.2 μM) in water at room temperature (inset: the fluorescent change images of **1**).

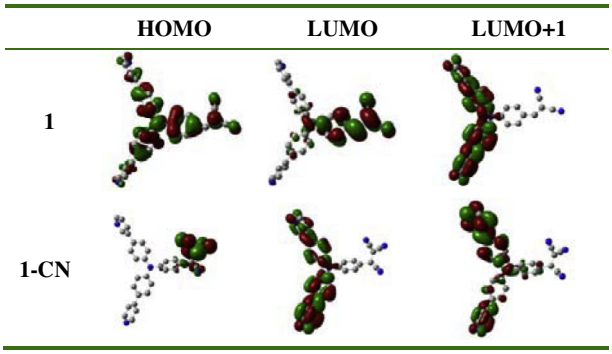

Figure 2. Electron density maps of the frontier molecular orbitals of **1** and **1-CN**.

(Table 1). Calculation results indicated the absorbance at short wavelength became stronger than that at long wavelength in **1-CN**, which is close to the experimental results (Table 1). Although the computational absorption spectra underestimated the experimental absorption maxima in general as shown in Table 2, analogous spectroscopic behaviors were reproduced qualitatively. Moreover, absorption band at 471 nm in **1-CN** is close to the experimental results (465 nm).

To better evaluate the cyanide-selective nature of **1**, fluorescence changes caused by the addition of other anions, including F^-, Cl^-, Br^-, I^-, CO_3^{2-}, HCO_3^-, SO_3^{2-}, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, SCN^-, NO_2^-, $S_2O_8^-$, AcO^-, S^{2-} and N_3^- were investigated. A pure water solution of **1** (2.5 μM), containing 40 equiv of each of these anions, were maintained for 20 min at room temperature and then subjected to fluorescence analysis. As shown in Figure 3, anions other

Table 1
Calculated TD-DFT (MPW1K) excitation energies for the lowest transition (eV, nm), oscillator strengths (*f*), and composition in terms of molecular orbital contributions, and experimental absorption maxima

	State	Composition[a]	*E* (nm)	*f*	Exp. (nm)[c]
1	S1	94% H → L	414.15	1.2040	465
	S2	88% H → L+1	309.95	0.7433	360
1-CN	S1	98% H → L	471.62[b]	0.0083	465
	S2	83% H → L+1	419.19[b]	0.0152	360

[a] H = HOMO, L = LUMO, L+1 = LUMO+1.
[b] TD-DFT calculation in vacuo.
[c] In H_2O.

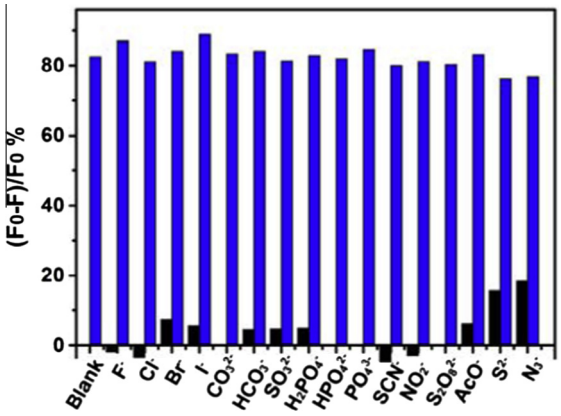

Figure 3. Various anions selectivity profiles of **1** (2.5 μM) in the presence of various anions in water (pH 7): (black bars) emission ratios of $(F_0 - F)/F_0$ at 612 nm in the presence of 40 equiv of F^-, Cl^-, Br^-, I^-, CO_3^{2-}, HCO_3^-, SO_3^{2-}, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, SCN^-, NO_2^-, $S_2O_8^-$, AcO^-, S^{2-} and N_3^-; (blue bars) fluorescence intensity in the presence of various anions, followed by 4.0 equiv of CN^-.

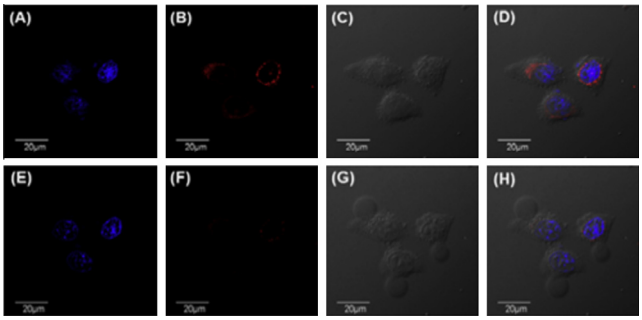

Figure 4. (A–H) CLSM images (A) HeLa cells incubated with Hoechst 33258 for 15 min at 37 °C. (B) is then stained with 10 μM **1** in PBS for 15 min at 37 °C. (C) and (D) are bright-field and overlay images of (A), (B) and (C), respectively. (E) HeLa cells incubated with Hoechst 33258 for 15 min at 37 °C. (F) is stained with 10 μM **1** in PBS for 15 min at 37 °C, then further treated with 40 μM CN^- at 37 °C for 2 h. (G) and (H) are bright-field and overlay images of (E), (F), and (G), respectively (Hoechst 33258: λ_{ex} = 405 nm, λ_{em} = 460 ± 30 nm; **1**: λ_{ex} = 488 nm, λ_{em} = 590 ± 50 nm).

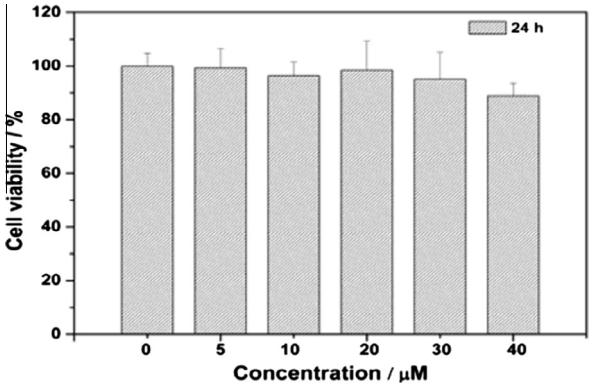

Figure 5. Cell viability values (%) estimated by MTT proliferation test at different concentrations of **1**. HeLa cells were cultured in the presence of **1** at 37 °C for 24 h.

than CN^- do not lead to significant changes in the fluorescence intensity. As a result, the selectivity profile for CN^- over other anions is remarkably high. In addition, titration spectrum shows the detection limit of **1** for CN^- was estimated to be 11 nM (Fig. S6). It should be noted that **1** can also be applied for detecting the level of CN^- in drinking water by the requirement of WHO (1.9 μM).

Furthermore, the application of **1** in the monitoring of intracellular CN^- concentrations was investigated by confocal laser scanning microscopy (CLSM). As shown in Figure 4B, a red luminescence was observed inside the HeLa cells. Overlay of luminescence and brightfield images demonstrated that the red luminescence was evident in the cytoplasm over the nucleus and membrane, which was also confirmed by xz cross-sectional image (Fig. 4D and Fig. S7). When the HeLa cells were again incubated with 40 μM CN^- at 37 °C for 2 h, the red luminescence was distinctly decreased (Fig. 4F) while the blue luminescence was slightly enhanced (Fig. 4H). Finally, these results indicate **1** can be taken advantage of tracking CN^- levels in living cells.

The toxicity of **1** for Hela cells was measured by using a standard MTT assay (Fig. 5). After Hela cells were cultured with **1** (40 μM) in PBS at 37 °C for 24 h, cell viabilities were estimated to be more than 90%. Even at a much higher concentration of **1** (100 μM), cell viabilities were still over 60% (Fig. S8). Hence, probe **1** has very low cytotoxicity.

In conclusion, we have demonstrated that **1** can be used as a highly selective and sensitive probe for detecting the nanomolar level of CN^- in water. Furthermore, **1** is taken up by cells and can thus be used for the cell image by CLSM. As **1** does not possess any general cytotoxicity it might be of interest to use for a specific bioimaging in vitro and in vivo. We are currently working on developing a turn-on fluorescent probe for detecting picomolar concentration of CN in water.

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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.2013.07.011.

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