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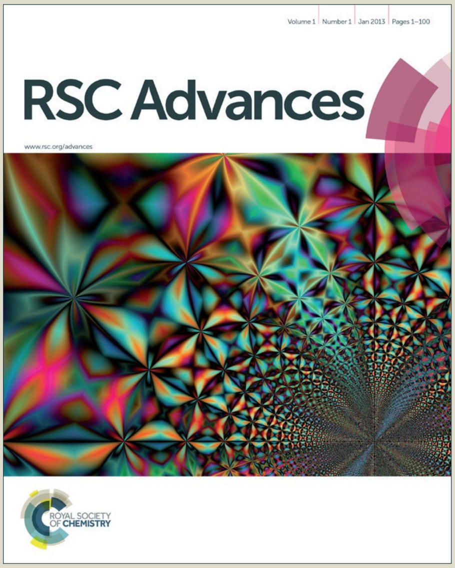

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PAPER

A colorimetric and "turn-on" fluorimetric chemosensor for selective detection of cyanide and its application in food sample

Jing-Han Hu,*[a] You Sun,[a] Jing Qi,[a] Peng-Xiang Pei,[a] Qi Lin,[b] You-Ming Zhang[b]

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A novel highly selective isonicotiamide-based chemosensor HY for cyanide was successfully designed and synthesized, which showed both colorimetric and fluorescent turn-on responses for cyanide ions in aqueous. This structurally simple chemosensor could detect CN^- anion over other anions in aqueous solution $DMSO/H_2O$ (v/v = 7:3) undergo deprotonation reaction. Results showed that the chemosensor HY exhibited 14-fold enhancement in fluorescence at 552 nm and showed yellow fluorescence under the UV-lamp after the addition of CN^- in aqueous solution. An obvious change in color from pale yellow to yellow that could be detected by naked eye. We have successfully utilized the above CN^- and H^+ for the fabrication of INHIBIT molecular logic gates, using CN^- and H^+ as chemical inputs and the fluorescence intensity signal as the output. Moreover, the detection limit on fluorescence response of the sensor to CN^- is down to 5.12×10^{-8} M by titration method. Test strips based on HY were obtained, which could be used as a convenient and efficient CN^- test kit to detect CN^- in aqueous solution. Furthermore, the sensor could detect the CN^- in food sample as a sample and effective method.

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Introduction

Anions have very crucial roles in biological systems and chemical processes.[1-2] Among various anions, cyanide ion has attained significance because of a highly toxicity.[3-4] Cyanide can form a stable complex with cytochrome *c* oxidase in the blood of humans, which generates inhibition of the function of the enzyme, resulting in cytotoxic hypoxia and cellular asphyxiation.[5-8] Even so, cyanide is an important compound widely used in many chemical processes, such as synthetic fibers, plastics manufacturing, herbicides, and the gold-extraction process.[9-11] The Environmental Protection Agency (EPA) has set the MCL (MCL: maximum contaminant level) of 2.7×10^{-6} M for cyanide in order to regulate safe levels for drinking water systems. [12] Hence, the development of a rapid and accurate detection method for CN^- can be of rather interest. Many solutions have been proposed to detect CN^-, for example, electrochemical, and chromatography.[13-14] Among those methods, optical sensors which utilize chemical reactions that produce fluorometric and colorimetric responses have appealed to scientists, as their higher selectivity, sensitivity and especially low cost.[15-18] However, only a few of them are "turn-on" fluorescent sensors.[19-21]

Fluorescence and colorimetric detections of anions have been successfully developed based on various mechanisms, such as intramolecular charge transfer (ICT),[22] photo-induced electron transfer (PET),[23] excited state intramolecular proton transfer (ESIPT),[24] and metal-ligand charge transfer (MLCT).[25] Among the different intelligent strategies in designing cyanide sensors, the deprotonation approach is often used due to easy design and comprehension. Anion sensors usually containing groups such as amides, thiourea, pyrrole and hydroxyl having acidic proton recognize anions via hydrogen-bonding interactions or deprotonation.[26-27]

Our research group has a longstanding interest in molecular recognition.[28-29] In this work, we have synthesized a new chemosensor **HY** synthesized with 4-(Diethylamino)salicylaldehyde and isonicotinyl hydrazide by only one step. In aqueous solution, fluorescence enhancements as well as colorimetric changes were observed with CN^-. Many selective receptors have been researched in organic media. Actually, in biological and environmental systems, ion-receptor interactions usually occur in aqueous media. The active hydrogen atom could easily combine with strong alkaline ion CN^- and allow the receptors to tolerate a substantial amount of water from the solvent.[30] In addition, we utilized the characteristics of an "OFF–ON–OFF" fluorescent switch fabricating of INHIBIT molecular logic gates, which CN^- and H^+ as chemical inputs and

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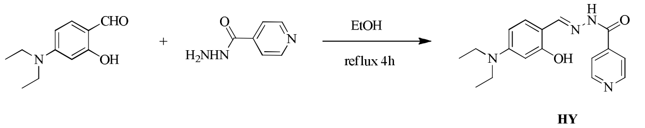
the fluorescence intensity signal as the output. As practical applications, the sensor **HY** could successfully detect CN^- in bitter seeds, which encourage the use of sensor in our lives. The detection limit in fluorescence response of the sensor to CN^- was down to 5.21 $\times 10^{-8}$ M. The mechanism of this process was verified by spectroscopic methods including ^{1}H NMR, UV-vis, and mass spectrometry.

Results and Discussion

The host **HY** was readily synthesized by only one step condensation of 4-(Diethylamino)salicylaldehyde and isonicotinyl hydrazide. (Scheme 1) The chemosensor **HY** has been characterized by ^{1}HNMR, ^{13}C NMR, and ESI-MS. (Fig. S1–S3†) The structure of **HY** was further confirmed by single-crystal X-ray diffraction. A single crystal of **HY** was obtained by solvent diffusion method using ethanol. The O (2)···H (3) bond length was 0.82 nm, and the distance of H (3) ···N (3) was 1.89 nm. (Fig. 1) A serious of colorimetric and fluorimetric recognition abilities of **HY** were investigated by adding anionic species such as F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, CN^- and SCN^- to the DMSO/H_2O (v/v = 7:3).



Scheme 1 Synthesis of receptor **HY**.

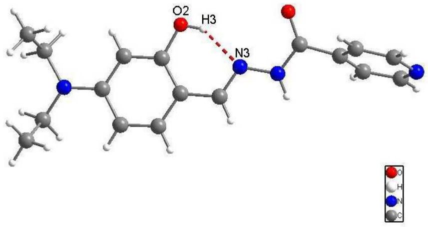


Fig. 1 Single-crystal X-ray structure of sensor **HY**.

In the UV-vis spectrum of a solution of **HY** (2.0 $\times 10^{-5}$ M) showed the strong and broad absorption at 380 nm. However when 50 equiv. CN^- were added, a new absorption appeared at 423 nm (Fig. 2). These changes in the absorption of **HY** were also nearly nonresponsive to the other anions such as F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, CN^- and SCN^-. Meanwhile only the addition of cyanide anion displayed expected color change from pale yellow to yellow, which could be distinguished by naked eyes in contrast to other anions. These results suggested that sensor **HY** shows excellent selectivity for CN^- over all other.

The compound **HY** alone displayed a weak, single fluorescence emission band at 535 nm when excited at 375 nm in aqueous media DMSO/H_2O (v/v = 7:3) solution. Meanwhile, only CN^- rendered a remarkable "Turn-On" fluorescence response with 14-fold increase in fluorescence intensity at 552 nm, as shown in Fig. 3, which responded with a fluorescence color change from colorless to yellow under UV lamp. Whereas, other anions such as F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, and SCN^-revealed a negligible change in the fluorescence color and emission intensity. The fluorescence profiles at 552 nm showed a high selectivity for CN^- in contrast to other anions. Indeed, such fluorescence enhancement could be distinguished with naked eyes.

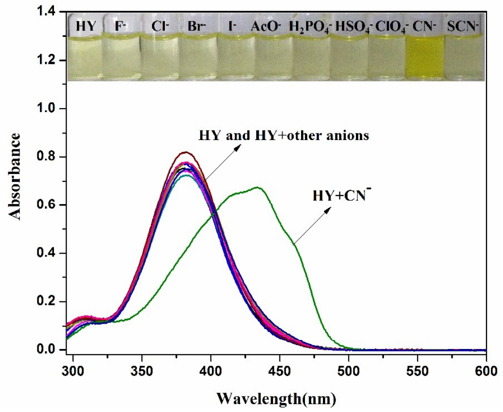


Fig. 2 Absorbance spectra data for a mixture of **HY** and anions: F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, CN^- and SCN^- (50 equiv.) in the DMSO/H_2O (v/v = 7:3) solution. Inset: photograph of **HY** upon adding of F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, CN^- and SCN^-.

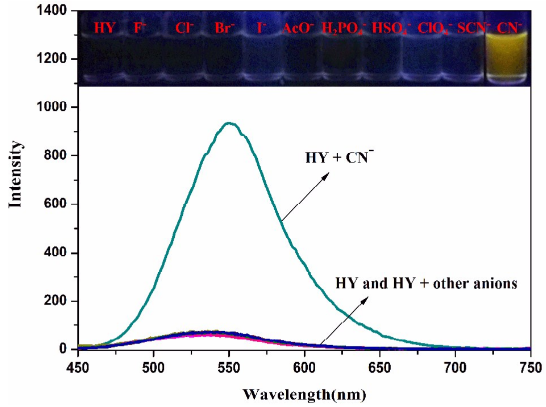


Fig. 3 Fluorescence emission data for a mixture of **HY** and anions: F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, ClO_4^-, CN^- and SCN^- (50 equiv.) in the DMSO/H_2O (v/v = 7:3) solution (λ_{ex}= 375 nm). Inset: photograph of **HY** upon adding of F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^- ClO_4^-, CN^- and SCN^-.

The interaction of sensor **HY** with CN^- (0.01 M) was further studied by UV-vis titration experiments. As shown in Fig. 4, the increasing concentrations of CN^- (0.01 M) from 0.0–7.28 equiv. in DMSO/H_2O (v/v = 7:3) lead to a decrease in the 380 nm

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absorption bands, associated with the 423 nm band increase. That showed one bathochromic shift in the absorption band with Δλ = 43 nm. In addition, a well defined isosbestic point was also noted at 399 nm, which indicated an interconversion into single discrete chemical species during the titration process.

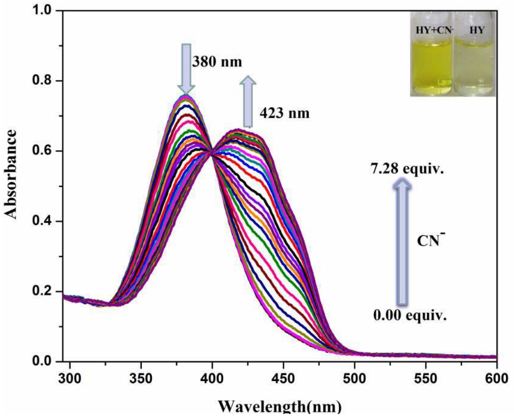

Fig. 4 Absorbance spectra of **HY** in the presence of different concentration of CN^- (0.0-7.28 equiv.) in DMSO/H_2O (v/v = 7:3). Inset: photograph of **HY** and upon adding of CN^-.

The colorimetric and fluorescent sensing abilities were also investigated by respectively adding pure water solution of various anions (50 equiv.) to the DMSO/H_2O (v/v = 7:3) solutions of sensor **HY** (2.0×10^{-5} M). The results showed that only addition of cyanide anion displayed same changes. (Fig. S5†)

Fluorescent titration was carried out to gain more insight into the recognition properties of receptor **HY** as a CN^- probe (Fig. 5). The hydrogen of hydroxyl group and one nitrogen atom could form intramolecular hydrogen bond, when the molecules were stimulated, which proton migration between oxygen and nitrogen atoms consumed some energy leading **HY** fluorescence weak. With increasing addition of CN^- (0.1 M), the emission band at 552 nm of chemosensor **HY** steadily increased. About 14.31 equiv. of cyanide ions was required for the complete change of the fluorescent response.

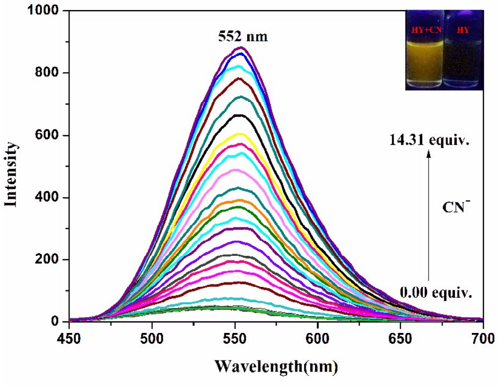

Fig. 5 Fluorescence spectra of **HY** in the presence of different concentration of CN^- (0.0-14.31 equiv.) in DMSO/H_2O (v/v = 7:3) solution. Inset: photograph of **HY** and upon adding of CN^-.

In Fig. 6, it was easily seen that the fluorescence intensity change was almost linear with the on concentrations of CN^- (0.1 M). The detection limit could be determined to be 5.12 $\times10^{-8}$ M, which is calculated on the basis of 3δ/S (where δ is the standard deviation of the blank solution and S is the slope of the calibration curve), (Fig. S4†) which was much lower than the maximum contaminant level (MCL) of 2.7 $\times10^{-6}$ M for cyanide in drinking water by the World Health Organization (WHO).

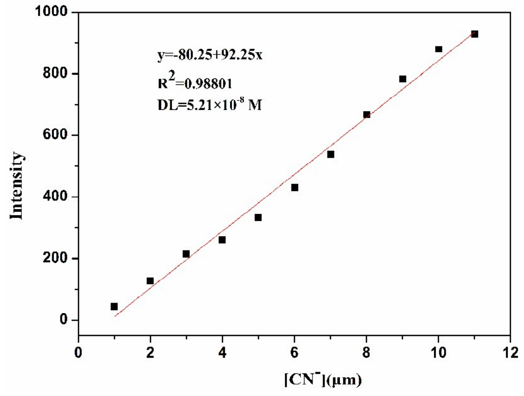

Fig. 6 Fluorescence detection limit spectra of **HY** (2.0 $\times10^{-5}$ M) in DMSO/H_2O (v/v = 7:3) solution upon addition of an increasing concentration of CN^- (0.1 M).

A serious of experiments were carried out to investigate the CN^- recognition mechanism of **HY**. In order to gain an insight into the stoichiometry between **HY** and CN^-, the method of continuous variations was used as shown in Fig. 7. As expected, a job plot was implemented, demonstrating a 2:1 stoichiometry for CN^--**HY**.

To explore the sensing mechanism of senor **HY** to CN^-, the ^{1}H NMR titration was investigated, which illustrated the characteristic structural changes occurring upon interaction with CN^-. As shown in the Fig. 8, sensor **HY** showed three single peaks at 11.98, 11.25 and 8.43 ppm in DMSO–d_6, which corresponded to the protons of –OH, –NH and –CH=N. After adding 0.1 equiv. of CN^- in DMSO–d_6, the –OH and –NH peaks at 11.98 and 11.25 ppm both disappeared. It was due to when basic ions existed, strong acid was readily deprotonated. Meanwhile with the increase of cyanide, the proton peak of aromatic and pyridine ring both gradually showed upfield shift that indicated the increase of electron density through charge delocalization in the conjugated system. Moreover, from the ESI-MS data, we could also see an obvious peak at m/z 357.1754 assignable to [**HY** — $2H^+ + 2Na^+ + H^+$] (m/z = 357.34). (Fig. S6†)

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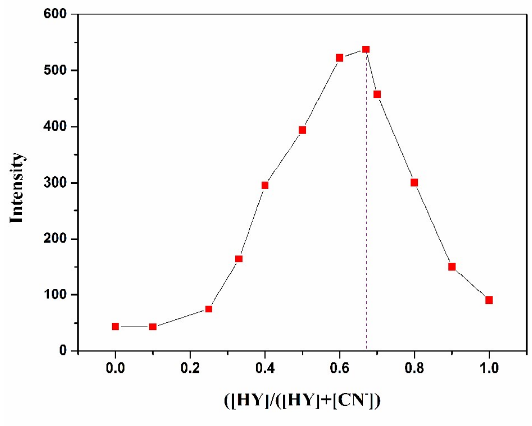


Fig. 7 Job plot of **HY** and CN⁻, which indicated that the stoichiometry of CN⁻-**HY** was 2:1.

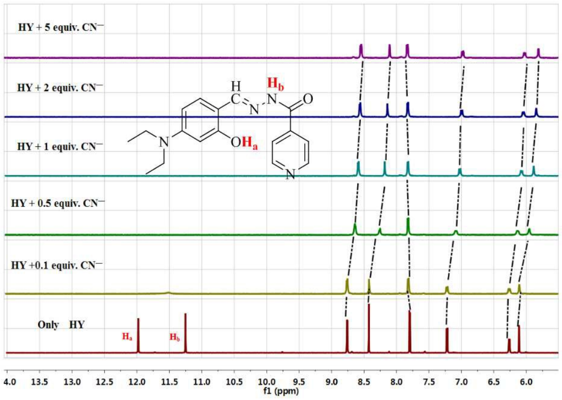
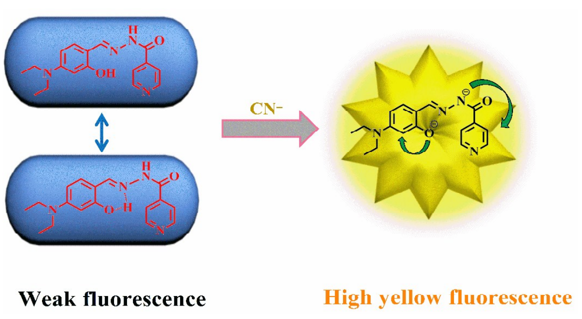


Fig. 8 ^{1}H NMR spectra of free **HY** (400 MHz, DMSO-d_6) and in the presence of varying amounts of CN⁻.

Those common results further suggested that the mechanism of sensing CN⁻ was the combination of intramolecular hydrogen bonds and deprotonation, which the reaction ratio of **HY** and CN⁻ is 1:2. On account of the above findings, we propose that the reaction mechanism in this system may proceed through the route depicted in Scheme 2.



Scheme 2 The proposed reaction mechanism.

The pH dependence of sensor **HY** in DMSO/H_2O (v/v = 7:3) solution was also checked by UV-vis and fluorescence spectroscopy. Cyanide ions were added to the buffer solution of **HY** at different pH values. The detection can work well in the pH range 9.0-13.0. (Fig. S7†) The reversibility of a chemosensor was one of the essential aspects for its applications. Therefore, the reversibility of **HY** was observed by the alternate addition of CN⁻ and H⁺, as shown in Fig. 9. The addition of CN⁻ to a solution of **HY** remarkably showed "ON" behavior through complex formation, which the fluorescence intensity of **HY** clearly enhanced at 552 nm. After addition of H⁺ into a solution containing **HY** and CN⁻, the emission intensity of **HY** diminishes hence acted as "OFF" switch. On further addition of CN⁻ solution, the fluorescence intensity was revived again, indicating the reversible behavior of **HY** for CN⁻ ions. This "OFF–ON–OFF" switching process could be repeated several times with little fluorescence efficiency loss.

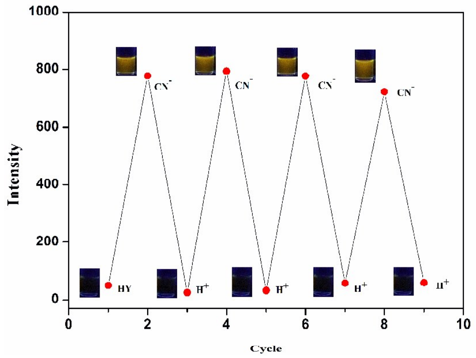
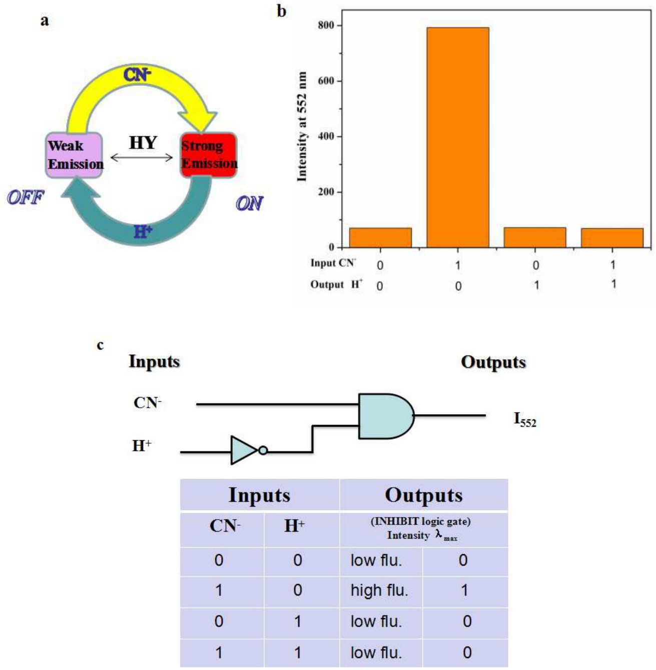


Fig. 9 Fluorescence study showing reversibility of CN⁻ coordination to **HY** by H⁺.



Inputs		Outputs	
CN⁻	H⁺	(INHIBIT logic gate) Intensity λ_{max}	
0	0	low flu.	0
1	0	high flu.	1
0	1	low flu.	0
1	1	low flu.	0

Fig. 10 a) Schematic presentation of "OFF-ON" system for **HY** in the presence of CN⁻ and H⁺, b) Performance of **HY** in the INHIBIT gate

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mode. The bars show the fluorescence output of **HY** at 552 nm in the presence of CN^- and H^+ as inputs, c) Molecular logic gate table and the respective symbolic representation of the INHIBIT logic gate function.

Based on the reversible fluorescent switch of our system, we use it as a two-input molecular logic gate, while the emission intensity at 552 nm acted as the output. When the output was zero, there are three conditions (I) both the CN^- and H^+ are absent, (II) only H^+ was present, (III) both CN^- and H^+ were present, which corresponded to the gate being closed, so termed as “OFF” (quenched fluorescence emission). When CN^- alone was present, the output is one and this corresponds to the gate being open and hence termed as “ON” (strong fluorescence emission). Therefore, **HY** could act as a logic gate as shown in Fig. 10. The repeated behavior of the system by fluorescence change could clearly suggest that **HY** was reversible and a reusable sensor for CN^-.

To facilitate the use of **HY** for the detection of cyanide, test strips were prepared by immersing filter papers into a DMSO/H_2O (v/v = 7:3) binary solution of **HY** (0.01 M) followed by its exposure to air for drying. These test strips containing **HY** was utilized to detect CN^-. As shown in the Fig. 11, when cyanide anions were added on the test kits, obviously, the change of color was observed from gray to light green in visible light. At the same time, fluorescent tests were also done by same method. Meanwhile, under the 365 nm UV lamp the addition of cyanide made the fluorescence color changed immediately from blue to yellow on the test papers. Hence, the test strips could conveniently detect CN^- in solutions.

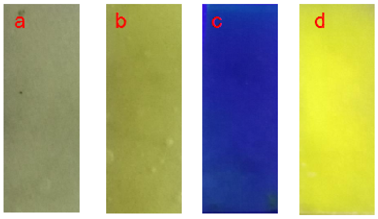

Fig. 11 Photographs of **HY** on test strips (a) only **HY** by naked eyes, (b) after immersion into solutions with CN^- by naked eyes, (c) only **HY** under UV lamp, (d) after immersion into solutions with CN^- under UV lamp.

In order to investigate its applicability of **HY** in our lives, one hundred grams of crushed bitter seeds were put into a flask by addition of 300 mL of water and 0.5 g of NaOH. And the mixture solution containing cyanide need be filter after stirring 20 min. Then we made the pH=9 which diluted the solution with fresh double water. As shown in Fig. 12, upon the addition of the cyanide solution into **HY**, the fluorescence intensity increased. And the color change from colorless to yellow observed by the naked eyes under UV lamp (365 nm).

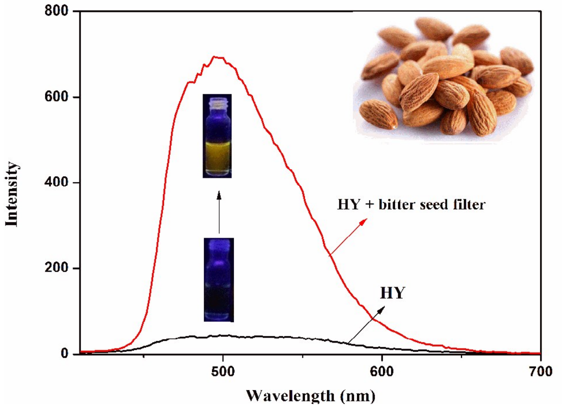

Fig. 12 Fluorescence spectral response of **HY** (20 μM) in diluted bitter seeds.

Comparison with other reported cyanide selective sensors, the sensor **HY** was synthesized in simple, low cost procedure. As shown in bellow table, sensor **HY** had preferable properties and applications, so the compound **HY** can be used as a potential fluorescent sensor for CN^- recognition. (Table S1†)

Conclusions

In conclusion, we have synthesized a dual channel, efficient, and simple chemosensor **HY**, which showed special selectivity and high sensitivity UV−vis absorption and fluorescence recognition for CN^- in DMSO/H_2O (v/v = 7:3) solutions. Moreover, the detection limit on fluorescence response of the sensor to CN^- is down to 5.12×10^{-8} M. The CN^- induced fluorescence enhance process could be totally reversed by adding H^+ and the switching process could be repeated several times with little fluorescence loss, which can be utilized as the output to build an INHIBIT logic gate by using CN^- and H^+ as inputs. Hence, this sensing system shows many advantages. Notably, this sensor was successfully applied to the detection of cyanide in bitter seeds. We believe that these characteristics of **HY** make it attractive for further molecular modifications and underlying applications as a colorimetric and fluorimetric sensor for CN^-.

Acknowledgment

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Notes and references

[a] College of Chemical and Biological Engineering, Lanzhou Jiaotong University, Lanzhou, Gansu, 730070, P. R. China
E-mail: hujinghan62@163.com
[b] College of Chemical and Chemical Engineering, Northwest Normal University, Lanzhou, Gansu, 730070, P. R. China
E-mail address: zhangnwnu@126.com.

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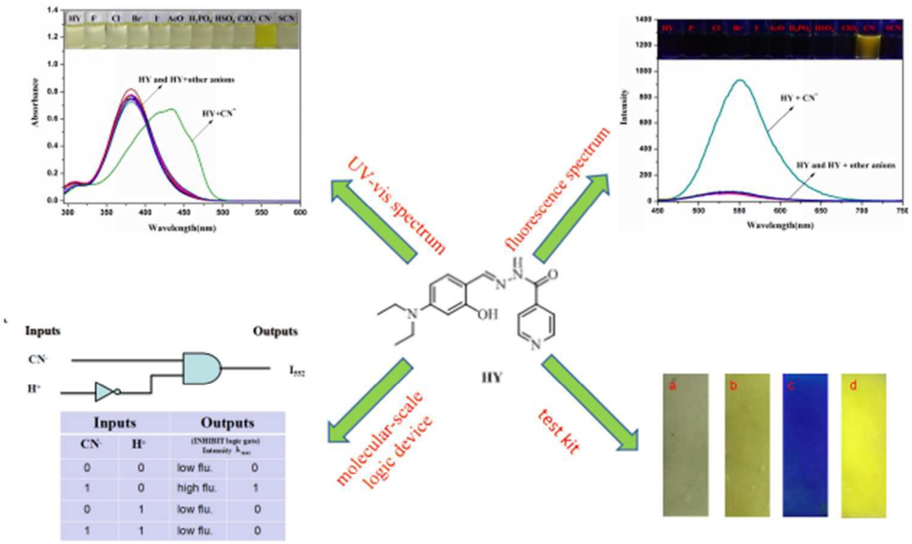
Graphical Abstract

A colorimetric and “turn-on” fluorimetric chemosensor for selective detection of cyanide and its application in food sample

Jing-Han Hu,*[a] You Sun,[a] Jing Qi,[a] Peng-Xiang Pei,[a] Qi Lin,[b] You-Ming Zhang[b]



Inputs		Outputs (INHIBIT logic gate) Intensity λmax	
CN⁻	H⁺		
0	0	low flu.	0
1	0	high flu.	1
0	1	low flu.	0
1	1	low flu.	0

Graphical abstract: A simple cyanide highly selective and sensitive fluorescent enhanced chemosensor **HY** had been designed and synthesized.