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Conjugated Polyfluorene-based Reversible Fluorescent Sensor for Cu(II) and Cyanide Ions in Aqueous Medium

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A blue emissive polyfluorene bearing sulfate functionality in the side chain (PFS), as a sensory polymer to selectively report the presence of Cu^{2+} (detection limit down to 2.5 μM) based on the fluorescence "turn-off." Surprisingly, the quenched luminescence of PFS by Cu^{2+} could be turned on after the addition of CN^- (detection limit down to 6 μM), making PFS a sensitive, selective, and reversible cyanide probe.

The development of highly selective and low-limit fluorescent chemosensors capable of sensing target guests via changes in fluorescent signals is a field of great interest, due to their high sensitivity and potential applications in chemistry and biology.[1] Among the reported fluorescent chemosensors, conjugated polymer-based fluorescent (CPF) chemosensors attracted much attention in these years because of the high sensitivity of these sensors due to the enhanced electronic communication.[2] The transfer of excitation energy along the whole backbone of the CPF to the chromophore reporter results in the amplification of fluorescent signals[2,3] which makes them apt to use as the optical platforms in highly sensitive chemical and biological sensors.[4] So far, good CPF chemosensors for the detection of toxic anions are still scarce, although many anions are involved in chemical, biological, and environmental processes of particular relevance.[5] For an example, extremely toxic cyanide, which can directly lead to the death of human beings in a few minutes, though is still widely used as industrial material in gold mining, electroplating, plastics production, and other fields.[6] Though it is difficult to measure the exact lethal dosage or exposure limit, recent studies have shown that the lethal cyanide concentration in the blood of fire victims is ca. 20–30 μM.[7] There have been a number of small molecular fluorescent chemosensors reported for selective sensing of cyanide.[8] However, a problem lies in the reversibility of these chemosensors. There are few systems which utilize the strong affinity of cyanide ions to transition metals[9] via complexation and result a change in the optical properties of a fluorescent probe to provide a method for detection. Polymer-based cyanide sensors are scarcely seen and mostly work in organic media.[10] In aqueous media, it is yet unexplored. Hence, it is very challenging to synthesize a polymer-based fluorescent probe to selectively, efficiently, and repeatedly detect cyanide ions in water.

Polyfluorene and its designed copolymers are among the most potential blue-light-emitting materials for electronic and optoelectronic application such as light-emitting devices (LEDs),[11] photovoltaics,[12] and field effect transistors[13] and chemosensors[10] due to high quantum yield and thermal stability.

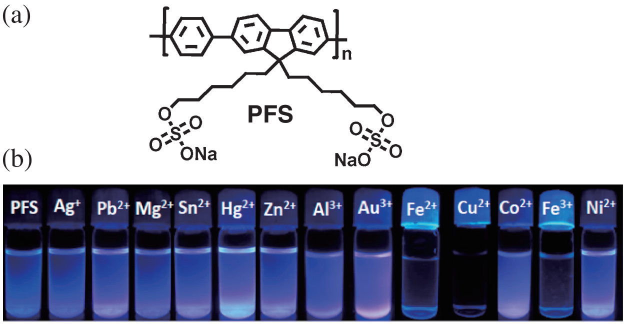

Figure 1. (a) Molecular structure of PFS polymer and (b) fluorescence response of aqueous solution of PFS upon addition of 50 μM of various metal ions excited at 365 nm using a UV lamp.

From this point, we have designed a water-soluble polyfluorene (PFS) with sulfate ions at the terminal of the alkyl chain of a fluorene segment (Figure 1a), which may sense metal ions selectively and sensitively based on the fluorescence "turn-off" and after the addition of selective anions, the quenched fluorescence of polyfluorene by metal ions may return (turn on).[14] Poly[9,9-bis(6-sulfooxyhexyl)fluorene-*alt*-1,4-phenylene] sodium salt (PFS) has been successfully synthesized by the Suzuki cross-coupling reaction of monomer **2** with 1,4-benzene diboronic acid and followed by the post polymer functionalization with phenol. Subsequent acidification by concd H_2SO_4 has been performed to introduce the sulfate group in the alkyl groups of each fluorene monomer (Scheme S1)[15] and 64% sulfate ions are incorporated into the polymer. Average molecular weight of polymer is ca. 8000 (PDI = 1.3) as determined by GPC study using polystyrene as standard.

The presence of sulfate groups imparts water solubility to PFS, and it shows absorption maxima at 334 nm and a strong blue emission at wavelength of 423 nm. The optical property of the conjugated polymer is affected in presence of various metal ions that can effectively anchor with the binding sites of the polymer. Salts of common metal ions including Ag^+, Al^{3+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Au^{3+}, Zn^{2+}, Cu^{2+} are added (50 μM) to 2 mL of PFS (1 mg/100 mL) and the corresponding UV–vis and fluorescence spectroscopy are monitored. Optical photograph of PFS and PFS mixed with each metal ion (PFS–M^{n+}, $n = 1$, 2, or 3) are taken under UV light irradiation (Figure 1b) which show the quenching of blue fluorescence of polyfluorene in addition of Cu^{2+}, Fe^{3+}, and Fe^{2+}. UV–vis spectra of the PFS and PFS–M^{n+} (SI, Figure S1)[15] reveal a noteworthy blue shift of 334 nm absorption peak to 315 nm with a great loss of absorbance in the case of Cu^{2+} (chloride salt). However, loss of absorbance or the blue shifting is not so prominent for other metal ions.

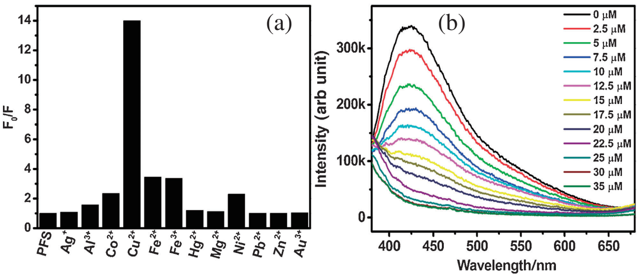

Figure 2. (a) Responses of sensor PFS and PFS–M^{n+} (M stands for bi- or trivalent metal) in water (50 μM). The excitation was at 360 nm, and the emission was at 423 nm. (b) Fluorescence emission spectra of the PFS in 2 mL of aqueous solution with successive addition of Cu^{2+}.

PL spectra of the PFS and PFS–M^{n+} (SI, Figure S2)[15] reveal the decrease of the emission peak at 423 nm only in the presence of Cu^{2+} and Fe^{3+} among all the metal ions used. Co^{2+} and Ni^{2+} have also shown a small decrease but in the case of Cu^{2+} the emission of 423 nm peak is completely diminished. The F_0/F (where F_0 is intensity of 423 nm peak of PFS only and F is the intensity at 423 nm of PFS–M^{n+}) plot for all metal ions suggests that PFS fluorescence is greatly quenched by Cu^{2+} (Figure 2a).

We have further investigated the concentration dependence of Cu^{2+} on the fluorescence intensity of PFS. As shown in Figure 2b, quenching of photoluminescence is observed at very low concentration of Cu^{2+} (2.5 μM), and the fluorescent intensity is decreased drastically upon further increase in Cu^{2+} concentration. At the concentration of ca. 25 μM of Cu^{2+}, no detectable luminescence is seen. The luminescence intensity vs. Cu^{2+} concentration (SI, Figure S3a)[15] has showed that at 25 μM of Cu^{2+}, the highest quenching has occurred. The quenching efficiency of Cu^{2+} is further derived using the Stern–Volmer equation (Figure S3b) and the calculated Stern–Volmer constant (K_{sv}) is $0.115 \times 10^6\,M^{-1}$. Under the experimental conditions, F_0/F of PFS solution are nearly proportional to the amount of Cu^{2+} added (2.5 μM, $R^2 = 0.9968$) indicating the binding capability of PFS between the safe and toxic levels of inorganic copper in drinking water.[16]

Again the morphology study from FESEM clearly supports the complexation of polymer PFS with the Cu^{2+}, since the micelle-like structure of the polymer (SI, Figure S4)[15] in water is completely destroyed to produce a network-like structure in the presence of Cu^{2+}. The above data suggest that PFS can report the presence of trace amounts of Cu^{2+} ions in water based on the fluorescence turn-off. It may be due to the efficient energy transfer from the conjugated backbone to the copper ions, leading to the quenching of the strong luminescence of the polymer chains which are coming closer via complexation with Cu^{2+} ions to allow interchain excitation energy transfer.[2c,17]

So, if the interaction between the Cu^{2+} ions and the sulfate is interrupted in PFS–Cu,[2] or the Cu^{2+} ions does not coordinate with sulfate group, the strong luminescence of PFS might not get quenched. The species, which either disrupt the interaction between the Cu^{2+} ions and the sulfate group of PFS or replace the Cu^{2+} ions from the PFS–Cu^{2+} complex to form more stable new complexes, may recover the quenched luminescence of PFS.[2c,17] Our quest for a fluorescent probe for cyanide (CN^-) may be realized, since anions which snatch Cu^{2+} ions from the PFS–Cu^{2+} complex via complexation to regenerate characteristic luminescence can be quantified using the above phenomenon. PFS–Cu^{2+} is an ideal luminescent probe for above quantification due to its remarkable fluorescence quenching behavior. To evaluate the anion selectivity of PFS–Cu^{2+} complex, the fluorescence intensity of the complex has been investigated in the presence of various anions including F^-, Cl^-, Br^-, I^-, CN^-, NO_2^-, NO_3^-, and ClO_4^-. Recovery of luminescence is only seen upon addition of 100 μM of CN^- (SI, Figure S5).[15] Further F/F_0 plot (where F is fluorescence intensity after addition of anion in PFS–Cu^{2+} complex and F_0 is fluorescence intensity of PFS–Cu^{2+} complex of 423 nm peak) vs. various anion (Figure 3a) shows the complete recovery of photoluminescence at 423 nm of PFS selectively by cyanide ions only. The picture of the vial presents the fluorescent "turn on" of the quenched blue emission PFS–Cu^{2+} with CN^- (inset of Figure 3a).

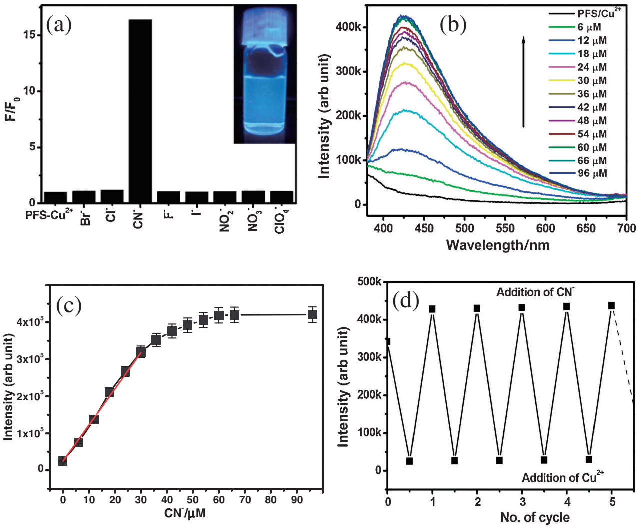

Figure 3. (a) Bar diagram depicting the effect of anions (100 μM) upon PFS–Cu^{2+} complex. The excitation was at 360 nm, emission 423 nm and slit 2/2, Inset: Vial containing solution of PFS–Cu^{2+}/CN excited at 365 nm. (b) Fluorescence emission spectra of the PFS–Cu^{2+} complex in aqueous solution with successive addition of CN^-. (c) The fluorescence titration profile at 423 nm upon the addition of CN^- (excitation at 360 nm) and first part of the plot is linear (marked with red line) with the standard error of 3.4%. (d) Reversibility study of PFS chemosensor upon successive addition of Cu^{2+} and CN^- in aqueous solution.

A qualitative scheme of the overall turn off with addition of Cu^{2+} on PFS and again turn on after addition of cyanide to that PFS–Cu^{2+} complex is presented in Figure S6. Successive turn off and turn on by alternating addition of Cu^{2+} and CN^- to PFS–Cu^{2+} is rechecked by UV–vis spectroscopy (SI, Figure S7).[15] The addition of CN^- to PFS–Cu^{2+} complex reproduces the characteristic PFS band at 334 nm. This data clearly indicates that the introduction of cyanide ion into PFS–Cu^{2+} complex produces the CuCN complex and thus producing free PFS (Figure S6).[15] Thus, PFS can be overwhelmingly used to probe Cu^{2+} and CN^- selectively and efficiently in aqueous medium beyond the lethal limit. The reversibility of recovering behavior of the fluorescence of PFS–Cu^{2+} has been studied in detail in

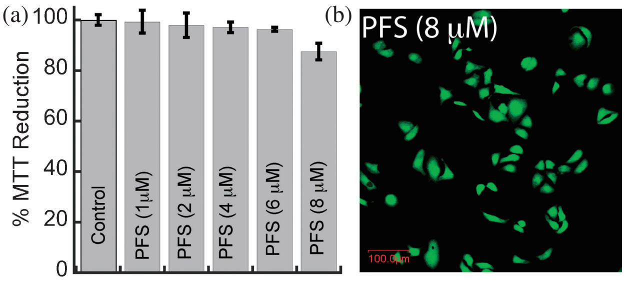

Figure 4. (a) MTT assay of PFS solution at indicated concentration and (b) cell viability study at 8 μM of PFS solution.

Figure 3b. The completely quenched fluorescence of PFS–Cu^{2+} is turned on after the addition of CN$^-$, at very low concentration (6 μM). Further increasing the concentration of CN$^-$ has led to stronger fluorescence and after addition of 60 μM of CN$^-$ to the PFS–Cu^{2+} complex the fluorescence intensity of the 423 nm peak has reached a plateau (Figure 3c). As the first part of that plot is linear we can propose that our aqueous soluble polyfluorene-based PFS–Cu^{2+} complex chemosensor readily detects the cyanide ion in water from 6 μM which is much lower than the lethal amount of the cyanide ion (20–30 μM).

The FESEM image (Figure S4)[15] of PFS–Cu^{2+} complex after addition of CN$^-$ evidently reveal the retention of spherical aggregation structure of PFS which confirms the PFS is free after addition of CN$^-$. The repeatability, reliability as well as reversibility study of that PFS-based chemosensor have been also done by alternate addition of Cu^{2+} and CN$^-$ in the same aqueous solution of PFS (Figure 3d). We have performed up to the 5th cycle and up to that cycle this chemosensor system is very promising, useable and beyond that this system should be effective also. Again from inductively coupled plasma optical emission spectrometry (ICP-OES), it is confirmed that we can remove 83% Cu^{2+} used for complexation with PFS by formation of white precipitate with cyanide ions.

The toxicity data using MTT assay suggest that PFS up to 8 μM is not cytotoxic as HeLa cells showed 90% MTT reduction in presence of PFS. The cytotoxicity was further evaluated by calcein AM which showed that cells are healthy in presence of PFS (Figure 4). This data suggest that PFS can be used to detect Cu^{2+} and CN$^-$ in any physiological sample without any cytotoxicity.

In conclusion, a water-soluble polyfluorene derivative has been designed, synthesized, and applied to detect sensitively, selectively, and reversibly Cu^{2+} and CN$^-$ ions via a "turn-off"–"turn-on" mechanism. The detection limit of CN$^-$ goes down to 6 μM, well below of lethal limit as well as the removal of the Cu–cyanide complex has given chance to use this polymer as a filtering substance.

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