ACS APPLIED MATERIALS & INTERFACES

Research Article

Cite This: ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

www.acsami.org

Copper Complex-Embedded Vesicular Receptor for Selective Detection of Cyanide Ion and Colorimetric Monitoring of Enzymatic Reaction

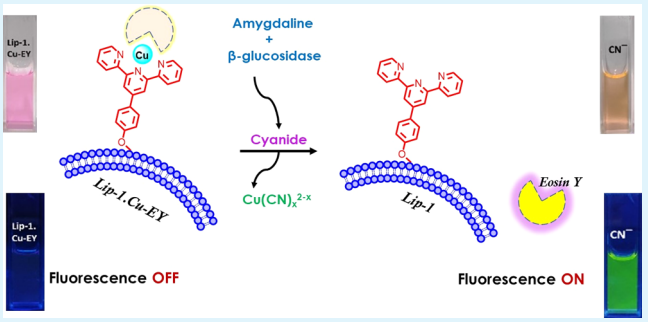
Rahul Kaushik,[†] Rahul Sakla,[†] Amrita Ghosh,[†] Sapna Dama,[‡] Ashwani Mittal,[‡] and D. Amilan Jose*[,†]

[†]Department of Chemistry, National Institute of Technology (NIT)-Kurukshetra, Kurukshetra 136119, Haryana, India

[‡]Skeletal Muscle Lab, Institute of Integrated and Honors Studies, Kurukshetra University, Kurukshetra 136119, Haryana, India

Supporting Information

ABSTRACT: Detection of environmentally important ion cyanide (CN^-) has been done by a new method involving displacement of both metal and indicator, metal indicator displacement approach (MIDA) on the vesicular interface. Terpyridine unit was selected as the binding site for metal (Cu^{2+}), whereas Eosin-Y (**EY**) was preferred as an indicator. About 150 nm sized nanoscale vesicular ensemble (**Lip-1.Cu**) has shown good selectivity and sensitivity for CN^- without any interference from other biologically and environmentally important anions. Otherwise, copper complexes are known for the interferences of binding with phosphates and amino acids. The **Lip-1.Cu** nanoreceptor also has the possibility to be used for real-time colorimetric scanning for the released HCN via enzymatic reactions. **Lip-1.Cu** has several superiorities over the other reported sensor systems. It has worked in 100% aqueous environment, fast response time with colorimetric monitoring of enzymatic reaction, and low detection limit.



KEYWORDS: *chemical biosensor, copper complex, cyanide, vesicles, β-glucosidase assay*

INTRODUCTION

Cyanide (CN^-) is considered to be one of the most fatal anions present in the environment and could enter the human body by many routes.[1−3] The central nervous system could be damaged even by a very low concentration of cyanide, which is fatal to human as well as marine lives within few minutes.[3] Nevertheless, CN^- is still used in many industrial and biochemical processes. The release of the CN^- anion from cyanogenic plants through enzymatic reactions increases the interest in developing new methods that could be used to recognize CN^- in aqueous media and various food products.[4] Although several methods and probes have been discovered, only a limited number of probes can be utilized in pure water at neutral condition.[5−11] Maximum probes reported in the literature work in semiaqueous medium or with a high percentage of organic solvent.[12−21] Thus, there is an urgent need to invent a simple chemical sensor system to detect cyanide in aqueous medium, especially in biological and environmental samples. It is essential to develop new probes for bioimaging applications that allow the direct detection of bacteriogenic cyanide in the infected lungs.[22] Moreover, for the detection of cyanide in the bio-sample, the probe requires the properties of decent water solubility, high sensitivity, and good selectivity. Furthermore, simple synthetic procedure is also vital for real applications.

A variety of fluorescence and colorimetric probes for the recognition of cyanide ion is established on coordination with metal ions or complex,[23] displacement of copper ion[3,8,24−32] from copper complex, hydrogen-binding interaction,[33−37] proton transfer,[38−41] and reaction-based.[42−44] However, for the detection of CN^- in biological environment, it is very significant to develop a new strategy or method to detect CN^- at very low concentrations in 100% aqueous medium. This challenge could be overcome by introducing the cyanide receptors on the surface of the vesicle. Vesicles are known for their biocompatibility, monodispersed nature, and facile preparation.[45] The functionalized vesicular surfaces are ideal to work at physiological conditions and are perfect for the detection of endogenously produced anions in living beings.[46−49]

Numerous systems are designed and developed to identify cyanide ions. Among them, metal complexes utilizing the affinity of cyanide for metals have attracted much attention.[28,29,50,51] Sensors based on metal-ion displacement have found to be applicable for detecting CN^- in aqueous media, but those working under pure water medium are not well

Received: September 24, 2019
Accepted: November 19, 2019
Published: November 19, 2019

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

known. The metal displacement approach with copper as the metal center is well known,[52] because the generation of steady $[Cu(CN)_x]^{n-}$ complex in solution gives rise to change in optical signal.[29,30]

Considering the potential application of copper complex for cyanide sensor, here we have reported an innovative vesicle-based fluorescent and colorimetric sensor for cyanide using the metal indicator displacement assay (MIDA), where both metal and indictor displaced upon interaction with specific analytes.[52,53] Previously, DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine)-based vesicles functionalized with Cu-DPA complex (DPA = di-(2-picolyl)amine) have been used for hydrogen sulfide detection using the MIDA approach.[53] Vesicles play significant roles to solubilize the system in water and also to improve the sensitivity of the sensors. The copper complex on the surface of the vesicles is accountable for the high selectivity of cyanide in aqueous medium. The indicator Eosin-Y (EY) displaced along with copper is responsible for the change in fluorescence, UV−vis and color property.

Herein, we have described a sensing ensemble composed of indicator dye and copper complex functionalized on vesicles that could serve as an innovative colorimetric and fluorescent sensor for the detection of CN^- in water with high sensitivity. This approach is new for CN^- sensor, and it may help to eliminate the tedious synthesis, improve biocompatibility, and provide an ideal condition to work with biological samples.

EXPERIMENTAL SECTION

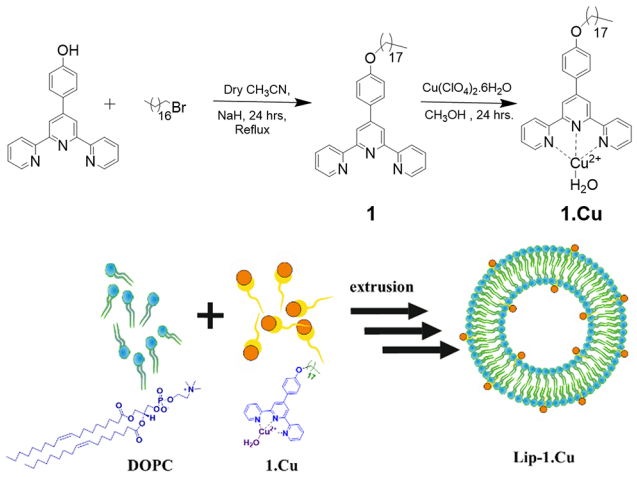
Synthesis of 4′-(4-(Octadecyloxy)phenyl)-2,2′:6′,2″-terpyridine (1). A mixture of 1-bromooctadecane (614 mg, 1.84 mmol), 4′-(4-hydroxyphenyl)-2,2′:6′,2″-terpyridine (500 mg, 1.53 mmol), and NaH (70 mg, 1.84 mmol) in a 100 mL round-bottom flask was refluxed in distilled CH_3CN for 24 h under N_2 atmosphere. The reaction was monitored using TLC, and the solvent was evaporated under vacuum. Then, extraction was done with chloroform and water to remove the NaH. The organic layer was dried using anhydrous Na_2SO_4 and evaporated under vacuum. The crude obtained was purified by using silica gel column chromatography in hexane:ethyl acetate solvent mixture. Yield: 640 mg (72%) (see the Supporting Information for detailed characterization of **1**).

Synthesis of Copper Complex of 4′-(4-(Octadecyloxy)-phenyl)-2,2′:6′,2″-terpyridine (1.Cu). 4′-(4-(Octadecyloxy)-phenyl)-2,2′:6′,2″-terpyridine (**1**) (100 mg, 0.17) and $Cu(ClO_4)_2 \cdot 6H_2O$ (65 mg, 0.17 mmol) were stirred for 24 h at room temperature in a $CHCl_3$−MeOH mixture. The solvent was evaporated and the crude obtained was redissolved in dichloromethane. The product containing dichloromethane was washed with water, and the organic layer was extracted and evaporated under vacuum. The resulting solid was washed with diethyl ether to obtain pure product **1.Cu**. Yield: 65 mg. ESI-MS *m*/*z* (%): $[1.Cu + 2H_2O]^+$ calculated: 676.35, experimental: 675.22 (100%).

RESULTS AND DISCUSSION

A new amphiphilic copper complex of 4′-(4-(octadecyloxy)-phenyl)-2,2′:6′,2″-terpyridine (**1.Cu**) was prepared by a two-step procedure and characterized by routine analytical methods (see Experimental Section in the Supporting Information (SI) and Figure S1). Copper complexes are well-known receptors to detect CN^- due to strong coordination with Cu^{2+}, which form a very stable complex $[Cu(CN)_x]^{n-}$.[24,25,54−56] However, most of the reported copper complexes working in semiaqueous system could not be appropriate for the CN^- detection in biological system.[25−27] Consequently, to tackle the problem of biocompatibility, we have prepared an amphiphilic copper complex (**1.Cu**) that could be easily incorporated into vesicles surface and work in biologically appropriate pure aqueous environment. **1.Cu** was incorporated into the phospholipid 1,2-dioelyl-*sn*-glycero-3-phosphocholine (DOPC) to form nanoscale vesicular receptor **Lip-1.Cu** (Scheme 1).

Scheme 1. Synthetic Scheme of 1.Cu and Schematic Representation of Formation of Lip-1.Cu Using 1.Cu and DOPC Lipid via Extrusion Method



The hydrophobic nature of **1.Cu** could be overcome by incorporating it into the lipid layer using a simple protocol, i.e., thin-film hydration method.[57] The preparation of bilayers vesicles-embedded metal complex (**Lip-1.Cu**) was done by using **1.Cu** (5 mol %) and synthetic lipid DOPC at physiological pH.

The nanoscale vesicles (**Lip-1.Cu**) were prepared by the extrusion method through 100 nm pore size polycarbonate membranes. The homogeneous solution of **Lip-1.Cu** was clear and expected to be pure; hence, the solution containing vesicle was not additionally purified. Dynamic light scattering (DLS) studies calculated the average size of the vesicles **Lip-1.Cu** as 150 ± 10 nm (Figure S2). From the transmission electron microscopy (TEM) images (Figure 1) of the vesicles, it is seen that the vesicles are in the size range of 100−150 nm.

Receptor-modified vesicles (**Lip-1.Cu**) were kept in the dark at 4 °C. Then, all of the studies were done within 5 days. It was also observed that the dilution of the stock vesicle solution could not influence the average size of the vesicles **Lip-1.Cu**. The effect of size after dilution was confirmed by DLS studies.

The indicator displacement approach is considered to be convenient, reliable, and efficient owing to: (a) liberty to choose the indicator, (b) work in complete aqueous environment, and (c) quick and real-time detection.[52] Taking these into account, we have used Eosin-Y (**EY**) indicator due to its high quantum yield, very high solubility in water, and undergoes complex formation with Cu^{2+}. The paramagnetic Cu^{2+} binds with **EY** through its free carboxylic group and consequently quenches the fluorescence of EY in pure aqueous medium.

For sensing studies, the probe **Lip-1.Cu-EY** was synthesized in situ by combining the modified vesicles (**Lip-1.Cu**) and indicator (**EY**) in the buffer solution. The formation of **Lip-1.Cu -EY** sensing ensemble was verified by systematic titration using UV−vis and emission spectroscopy (Figure 2). All of the UV−vis and emission studies were carried out in aqueous

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

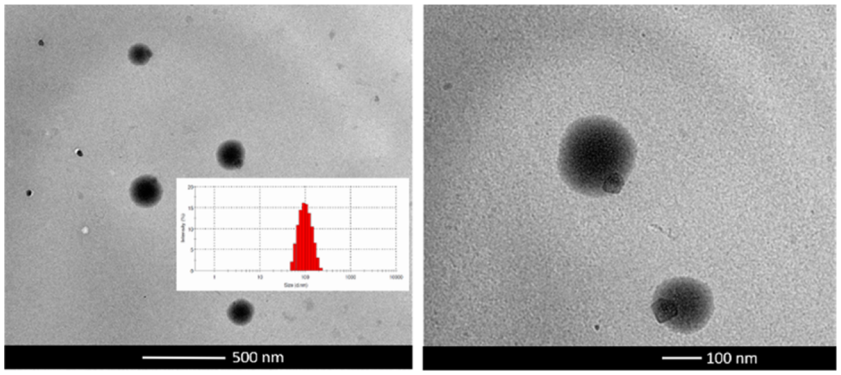


Figure 1. HR-TEM images of vesicles **Lip-1.Cu** prepared in aqueous solution. (Inset) DLS analysis.

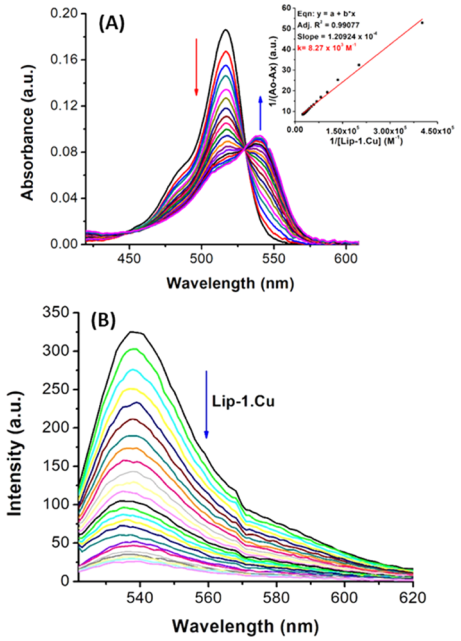


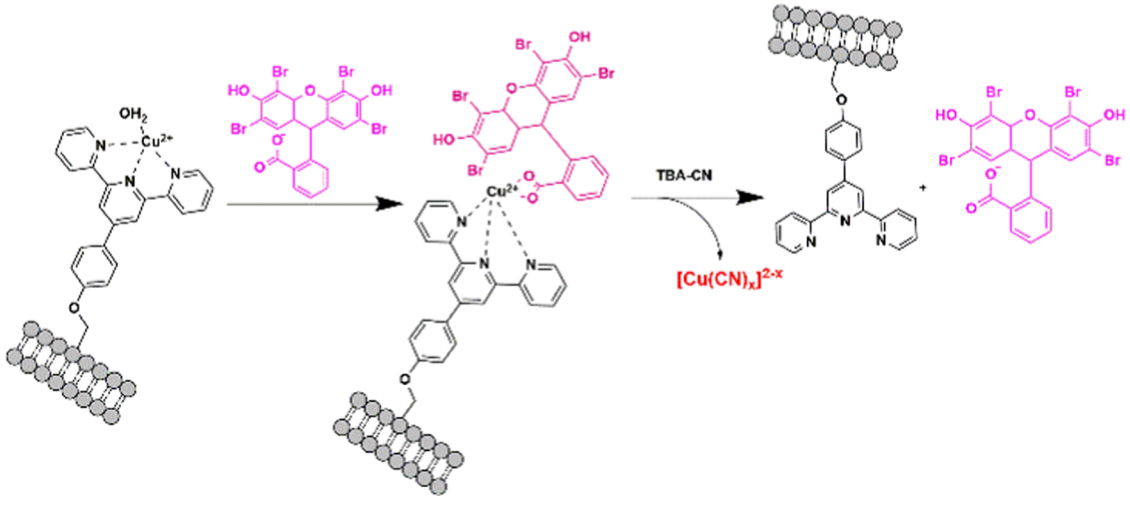
Figure 2. (A) UV−vis absorption titration of **EY** (2 μM) with **Lip-1.Cu** (0−4.5 μM). (B) Emission titration of **EY** (2 μM) with **Lip-1.Cu** (0−4.5 μM) in aqueous buffer medium at RT.

medium (HEPES buffer, 20 mM, pH 7.4). In a systematic absorbance titration, a characteristic absorbance peak of **EY** (2 μM, $\epsilon = 9.3 \times 10^4$ $mol^{-1}cm^{-1}$) was observed at 515 nm, with consecutive addition of **Lip-1.Cu** (0−4.5 × 10 μM), absorbance spectra showed a red shift of 25 nm along with a ratiometric change with decrease at 515 nm and enhancement at 540 nm (Figure 2A), and a color change from light pink color to dark pink color could be clearly visible by the naked eye.

In the emission spectrum, a strong emission band of **EY** was observed at 540 nm. The intensity band at 540 nm was quenched with the successive addition of **Lip-1.Cu** (λ_{exc} = 515 nm) (Figure 2B). The decrease in emission intensity could be attributed to the paramagnetic characteristics of Cu^{2+} center. The spectroscopic variation in the UV−visible and emission spectra is owing to the association of carboxylic acid group of the **EY** to the copper complex embedded on the surface of the vesicles (Scheme 2). The formation of the **Lip-1.Cu-EY** ensemble was established by calculating the binding constant (K_{SV}) to be 8.27×10^3 M^{-1} (Figure 2A, inset).

The indicator displacement approach (IDA) is widely known for the recognition of biologically important analytes such as ATP, pyrophosphate, and CN^-,[58] but is rarely known using the receptors embedded on vesicles or the probes, which can work in pure aqueous medium.[59] In the IDA approach, it is important that the indicator (EY) itself should not show any change with the analyte (i.e., CN^-). To confirm this, the effect

Scheme 2. Sensing Mechanism for Cyanide Detection via MIDA Approach



DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

of CN^- on EY was studied with the help of absorption and emission spectroscopy. It was found that even after the addition of 600 equivalents of CN^-, there is no change in the UV−vis and emission spectra of EY (Figure S3).

To the best of our knowledge, IDA for CN^- detection in receptor-embedded vesicles is not known in the literature. Therefore, the capability of **Lip-1.Cu-EY** for the selective sensing of CN^- was examined over other important anions such as (F^-, Cl^-, Br^-, I^-, and SO_4^{2-}), phosphates (ATP, pyrophosphate, ADP, AMP, $H_2PO_4^-$, and HPO_4^{2-}), reactive sulfur species (methionine, $S_2O_3^{2-}$), and reactive nitrogens (NO, NO_2^-, and N_3^-) in aqueous HEPES buffer solution.

The UV−vis spectrum (Figure 3A) showed that except CN^-, ATP, and PPi, all other analytes exhibit an insignificant

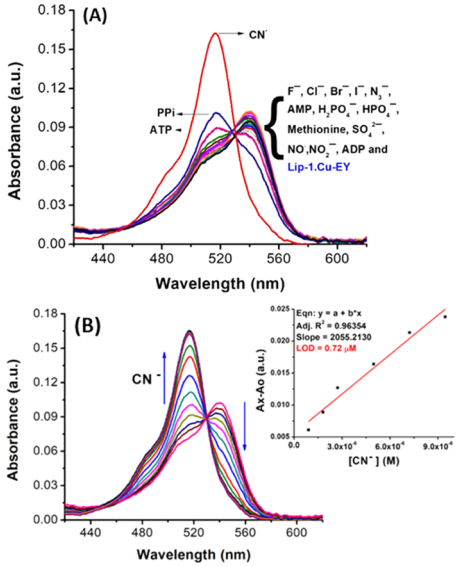


Figure 3. (A) Selectivity studies of **Lip-1.Cu-EY** with cyanide (4.0×10^{-5} M) and other analytes such as methionine (4.5×10^{-4} M), $S_2O_3^{2-}$ (4.5×10^{-4} M), NO (4.5×10^{-4} M), NO_2^- (4.5×10^{-4} M), N_3^- (4.5×10^{-4} M), ATP (4.5×10^{-4} M), ADP (4.5×10^{-4} M), AMP (4.5×10^{-4} M), PPi (4.5×10^{-4} M), $H_2PO_4^-$ (4.5×10^{-4} M), HPO_4^{2-} (5×10^{-4} M), F^- (5×10^{-4} M), Cl^- (5×10^{-4} M), Br^- (5×10^{-4} M), I^- (5×10^{-4} M), and SO_4^{2-} (5×10^{-4} M) using UV−visible absorption spectroscopy at pH 7.4. (B) UV−vis absorption titration of **Lip-1.Cu-EY** with CN^- (0−4.0×10^{-5} M). (Inset) LOD plot for CN^- by UV−vis absorption spectrum.

spectral change. However, in the presence of CN^-, the intensity band at 515 nm reduced with an enhancement at 540 nm. The changes observed for ATP and PPi are significantly lesser compared to CN^-, and this little change could be either due to the chelate formation of copper metal center with the free oxygen atoms of adenosine triphosphate (ATP) and pyrophosphate (PPi) or due to the dissociation of copper metal from the **Lip-1.Cu-EY** ensemble (Figure S4).[60−63] The absorption spectrum of the ensemble **Lip-1.Cu-EY** in the presence of CN^- resembles the absorption spectrum of **EY**, which confirms the disassembly of the **EY** and copper metal from the vesicles. Copper has been used as the most common metal center for cyanide sensing as the formation of $[Cu(CN)_x]^{2-x}$ (x = 2 or 4) species is stable.[24] Therefore, the introduction of CN^- to the **Lip-1.Cu-EY** ensemble displaces the copper metal ion to form $[Cu(CN)_x]^{2-x}$ and release the indicator dye **EY**, and the absorption spectrum of EY was restored.

Further, systematic UV−vis titration has been carried out with CN^- to determine the LOD and binding constant of **Lip-1.Cu-EY** with cyanide. With successive addition of CN^- (0−4×10^{-5} M) to the **Lip-1.Cu-EY** ensemble (Figure 3B), the UV−vis spectrum showed a reversible change with the retrieval of the absorption peak at 515 nm along with the formation of an isosbestic point at 530 nm. The solution color was slowly transformed from dark pink to pale pink. The limit of detection (LOD) and binding constant for CN^- were calculated as 0.72 μM (Figure 3B, inset) and 2.087×10^4 M^{-1} (Figure S5), respectively. These calculations were done based on absorption titration. This confirmed that the **Lip-1.Cu-EY** working ensemble is efficient to detect cyanide and its chemical activity is advantageous over the other reported sensors.

Detection of cyanide with the competitive species was also tested. As shown in Figure S6, the competitive studies revealed that coexistence of various species including biological phosphates ATP and PPi barely interfere the detection of CN^-. These studies confirmed that **Lip-1.Cu-EY** could be appropriate even when the CN^- coexists with competitive anions in water.

EY is well known for its characteristic fluorescent behavior.[64] EY has a strong emission band at wavelength of 540 nm, when excited at 515 nm. We have found that the strong emission of EY at 540 nm is quenched with the successive addition of **Lip-1.Cu** for the emergence of **Lip-1.Cu-EY** ensemble.

Therefore, **Lip-1.Cu-EY** is non-fluorescent in pure aqueous medium. For selectivity, we have recorded the emission spectrum of **Lip-1.Cu-EY** with different competitive analytes (Figure 4A). The emission of **Lip-1.Cu-EY** (at 540 nm) was enhanced only with CN^-, PPi, and ATP in the following order: CN^- (4-fold) ≫ PPi (0.8-fold) > ATP (0.5-fold) (Figures S7 and S8). However, other analytes barely show any significant change in the emission spectrum at 540 nm. Competitive studies were also carried out with coexistence of various species including biological phosphates ATP and PPi. The presence of other species barely interfere the detection of CN^- and confirm the recognition of CN^- even in the presence of other analytes (Figures S9 and S10). It should be noted that the interference from hydrogen sulfide (H_2S) may be expected because H_2S is known to form stable CuS by displacing copper.[52] The interference of H_2S could be easily avoided by adding an equivalent amount of hydrogen peroxide (H_2O_2) (Figure S11). Peroxide is known to remove H_2S by oxidation process, and it is not active toward the probe.[65]

Further, the sensitivity studies with CN^- were carried out via systematic emission titration (Figure 4B). With successive addition of CN^- (0−4×10^{-5} M) to the non-fluorescent **Lip-1.Cu-EY** ensemble, the spectrum showed a reversible change with the recovery of the emission band at 540 nm and became fluorescent due to the displacement of **EY**. The limit of detection (LOD) for CN^- was determined as 0.33 μM (Figure 4B, inset), which closely matches with the LOD calculated from UV−vis titrations and much lesser than the threshold limit of cyanide ion (1.9 μM), recommended by WHO in safe drinking water[66] or the concentration of HCN (about 26 μM) found in fire victims.[67]

Fluorescence lifetime experiments were also executed to confirm the detection mechanism (Figure S12). The lifetime of **EY** was recorded as 1.13 ns, and after the formation of the **Lip-1.Cu-EY** ensemble, the lifetime increases to 1.32 ns, and consequently decreases to 1.20 ns after the addition of CN^-. The lifetime results confirmed the displacement of metal and

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

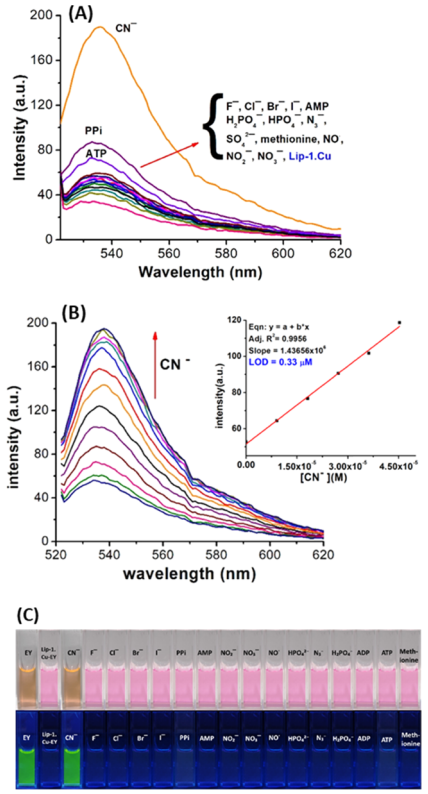


Figure 4. (A) Selectivity studies of **Lip-1.Cu-EY** with cyanide (4.0×10^{-5} M) and other analytes such as methionine (4.5×10^{-4} M), $S_2O_3^{2-}$ (4.5×10^{-4} M), NO (4.5×10^{-4} M), NO_2^- (4.5×10^{-4} M), N_3^- (4.5×10^{-4} M), ATP (4.5×10^{-4} M), ADP (4.5×10^{-4} M), AMP (4.5×10^{-4} M), PPi (4.5×10^{-4} M), $H_2PO_4^-$ (4.5×10^{-4} M), HPO_4^{2-} (5×10^{-4} M), F^- (5×10^{-4} M), Cl^- (5×10^{-4} M), Br^- (5×10^{-4} M), I^- (5×10^{-4} M), and SO_4^{2-} (5×10^{-4} M) emission spectroscopy in aqueous medium. (B) Emission titration of **Lip-1.Cu-EY** ensemble with CN^- (0–4.0×10^{-5} M) in buffer. LOD plot for CN^- using emission spectroscopy (inset B). (C) Naked eye color change with different analytes in normal light and under UV lamp.

indicator (**EY**) from **Lip-1.Cu-EY**. The change in size of vesicles upon dilution was checked by DLS experiments, and the results showed that after dilution, the size of the vesicles remains almost same. Also, the size of **Lip.-1.Cu**, **Lip-1.Cu-EY**, and **Lip-1.Cu-EY+CN⁻** does not change significantly; these results confirmed that the size of the vesicles remains almost same throughout the sensing mechanism for the detection of CN^- (see details in SI Experimental Section and Figures S13–S17).

The displacement of indicator from **Lip-1.Cu-EY** ensemble must be quick for the time-dependent measurement of the CN^-. To confirm that, real-time studies using absorption and fluorescence spectroscopy were performed where we observed that the MIDA sensing mechanism shows fast response (Figure S18). This validated that the **EY** could act as an ideal indicator in **MIDA** for CN^- sensing in vesicular solution.

Further, to understand the role of vesicles on CN^- binding and displacement mechanism, the binding constant and LOD of CN^- with amphiphilic probe **1.Cu-EY** were compared to those of **Lip-1.Cu-EY**. Probe **1.Cu-EY** is not soluble in water; therefore, the binding studies of **1.Cu-EY** with all anions were performed in aqueous THF solution (HEPES:THF (95:5, v/v) mixture) as shown in Figure S19. Solubility problem of **1.Cu** in water itself confirms the important role of vesicles in **Lip-1.Cu-EY**. Moreover, as shown in Table S1, vesicles-embedded **1.Cu** (**Lip-1.Cu-EY**) showed an enhanced binding constant and LOD (20 times higher) with CN^- ion compared to simple **1.Cu**. This result confirms the role of vesicles for the improved sensitivity of **1.Cu** for CN^- detection in pure aqueous environment. **Lip-1.Cu-EY** has been compared to the previously reported metal complex-based cyanide sensors (Table S2). The comparison table suggests that the present probe has advantages with respect to biocompatibility and sensitivity.

The present **Lip-1.Cu** system for the CN^- detection has shown many advantages such as (a) it can work in pure aqueous medium, which eliminates the use of organic solvents, (b) detection is very fast, (c) the pure aqueous medium also rules out the weak interaction between copper and other competitive anions and results in improving the selectivity, (d) the liberty to use indicator with absorbance and emission at longer wavelength, and (e) the LOD is analogous or superior to other systems due to increase in the number of active sites for CN^-.[8,10,11,24,27,66,68]

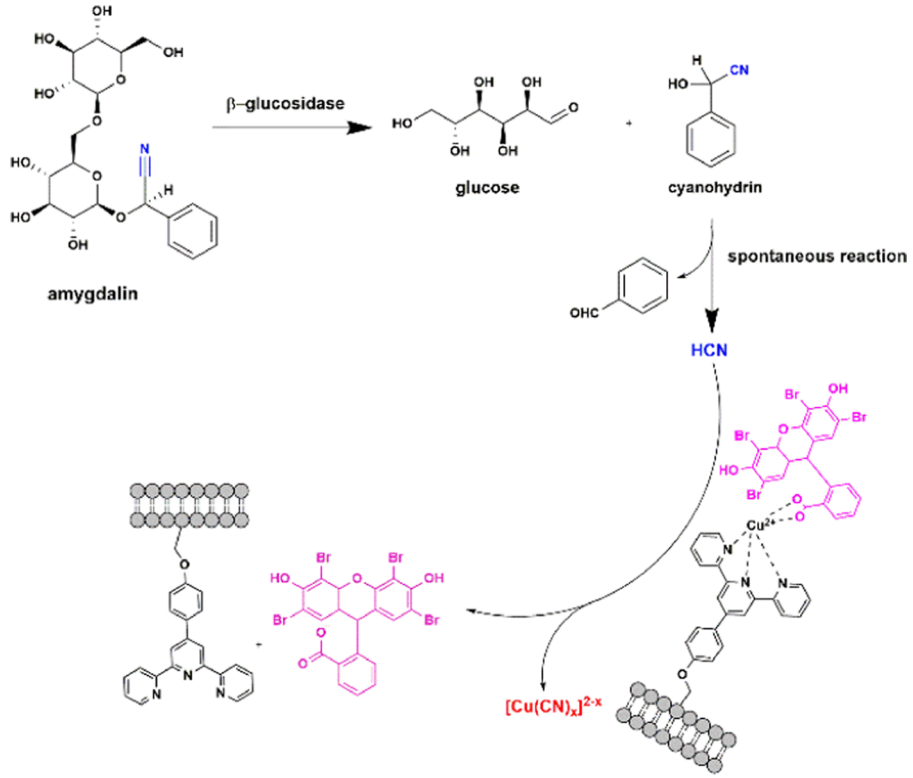
The low LOD and quick response suggest that by means of MIDA, the **Lip-1.Cu-EY** would possibly be suitable for monitoring enzymatic reaction where cyanide ion involved. A cyanogenic diglucoside amygdalin (D-mandelonitrile-β-D-glucosido-6-β-D-glucoside) generally originated in seed of apricots, plums, apples, cassava roots, and bitter almond.[38] Amygdalin itself is non-toxic, but various investigations showed that enzymatic hydrolysis of amygdalin leads to the conversion of glucose and mandelonitrile. Next, the spontaneous transformation of the unstable mandelonitrile to HCN and benzaldehyde takes place (Scheme 3). The produced HCN is able to cause toxicity through inhibition of cytochrome oxidase.

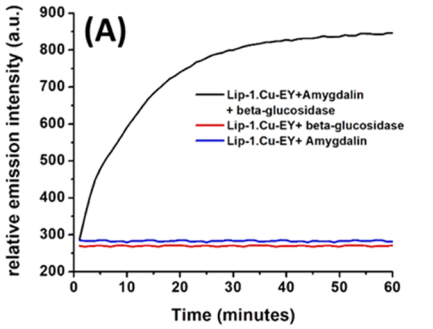
The cyanide ion generated through enzyme cleavage of the amygdalin could be detected by probe and can be useful for measuring enzyme activity in vitro. Accordingly, the enzymatic hydrolysis reaction of amygdaline and β-glucosidase in water (pH 7.4) was accomplished by color change, UV–vis and emission spectral changes. As shown in Figure 5A, fluorescence intensity of **Lip-1.Cu-EY** (2 μM) at 530 nm was recorded in the presence of enzyme β-glucosidase (0.01 UN/mL) and amygdalin (3.5 mM) as a function of time. In the absence of β-glucosidase or only in the presence of amygdaline, fluorescence intensity at 540 nm was in *off* state, whereas a consequent enhancement in the fluorescence intensity was found with amygdalin in the presence of β-glucosidase. Visible color change was also observed due to the enzymatic reaction, which could be used for the real-time colorimetric detection of CN^- released via enzymatic reaction (Figure 5B). At pH 7.4, cyanide (pK_a = 9.21) is anticipated to mostly exist as HCN (~99%). The kinetics of β-glucosidase assay was investigated by varying amygdalin concentrations from 1.2×10^{-4} to 6.0×10^{-4} M (Figure S20) and keeping the enzyme concentration constant (0.01 UN/mL). From the kinetic study, the Michaelis–Menten rate constant (K_m) value of β-glucosidase activity was measured as 2.75 mM at RT.

Thus, the HCN generated from amygdalin by hydrolysis reacted with the **Lip-1.Cu-EY** to displace EY from the nanoassembly and regenerate the emission intensity on state at 535 nm. Control experiments in the absence of amygdalin under similar experimental conditions were performed where we observed no increment in the fluorescence intensity or change in the absorbance (Figure S21). This observation

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587–47595

Scheme 3. Cyanogenic Glucosidase Assay Mechanism Using Amygdalin





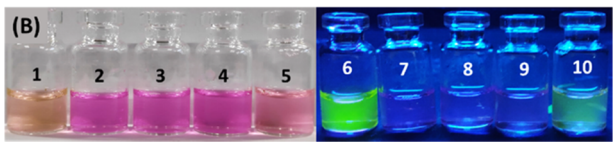


Figure 5. (A) Enzymatic assay of **Lip-1.Cu-EY** ensemble and amygdalin (6.0×10^{-4} M) with or without β-glucosidase enzyme (0.01 UN/mL) in buffer at RT; λ_{exc} = 515 nm. (B) Photographic images of (1) **EY** (2.5×10^{-5} **M**), (2) **Lip-1.Cu-EY** only, (3) **Lip-1.Cu-EY** with β-glucosidase (0.01 UN/mL), (4) **Lip-1.Cu-EY** with amygdalin (6.0×10^{-4} M), and (5) **Lip-1.Cu-EY** with amygdalin (6.0×10^{-4} M) and β-glucosidase (0.01 UN/mL) in normal light. Photographic images of (6) **EY** (2.5×10^{-5} M), (7) **Lip-1.Cu-EY** only, (8) **Lip-1.Cu-EY** with β-glucosidase (0.01 UN/mL), (9) **Lip-1.Cu-EY** with amygdalin (6.0×10^{-4} M), and (10) **Lip-1.Cu-EY** with amygdalin (6.0×10^{-4} M) and β-glucosidase (0.01 UN/mL) under UV lamp (the image was taken after 5 min of incubation time).

proves that the emission enhancement was exclusive because of the reaction of HCN/CN^- formed via hydrolysis of amygdalin by β-glucosidase. These results confirmed that the sensor **Lip-1.Cu-EY** could be applied for real-time monitoring of HCN released via enzymatic reaction.

To check the effect of lipids on cyanide binding, we have also prepared the **1.Cu** assembled vesicles by using DPPC and DSPC lipids (Figures S22 and S23). These lipids were selected based on their increase in transition temperature (see Table S3). As like DOPC, the vesicles formed by using DPPC and DSPC are stable for a week. The cyanide-ion binding of these vesicles was carried out by following the same method as DOPC vesicles. DPPC and DSPC vesicles-incorporated **1.Cu** shows sensitive binding to CN^- ion, and the binding constant and LOD calculated for these vesicles are compared in Table S3. We have noticed that the CN^- sensing ability of these vesicles is slightly different from each other. The change in sensitivity may be due to different transition temperatures of lipids. Also, we found that fluid-phase DOPC vesicles are better for anion binding studies than rigid DSPC vesicles (Table S3).

Further, the sensitivity of **Lip-1.Cu-EY** for CN^- in C2C12 (mouse skeletal muscle) cell lines were measured by fluorescence microscopy. The cells incubated with **EY** (20 μM), **Lip-1.Cu-EY**, and **Lip-1.Cu-EY** with CN^- (400 μM), in buffer at 37 °C were incubated overnight and imaged through fluorescence microscopy. Live cell images display a strong fluorescence with **EY** (Figure S27A) and a weak fluorescence with **Lip-1.Cu-EY** (Figure S27B). The strong fluorescence is recovered with cyanide ion due to displacement of copper (Figure S27C). This observation is in agreement with the solution studies.

CONCLUSIONS

In concluding remarks, a new approach for detection of CN^- through displacement of both metal center and indicator (MIDA process) on vesicular interface has been reported. We

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

have chosen terpyridine unit as binding site for Cu^{2+} and **EY** as an indicator for sensing mechanism in 100% aqueous medium. The vesicular ensemble **Lip-1.Cu-EY** showed good selectivity and sensitivity for CN^- without any interference from other anions. Otherwise copper complexes are known for the interferences of binding with phosphates and amino acids. The **Lip-1.Cu-EY** ensemble is probably utilized for real-time monitoring of HCN release via enzymatic reactions, whereas most of the reported CN^- probes work in semiaqueous environment, give late response, and lack applications in biological samples. Therefore, this probe (**Lip-1.Cu-EY**) has numerous merits compared to the other reported sensor systems. Irrespective of the small molecular probes, we can modulate the lipid membrane of the vesicular surface according to the cellular environment in the biological sample and also could change the indicator accordingly with different excitation and emission wavelengths. These applications predict and open a new area of research based on vesicles-based MIDA approach for anion sensing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.9b17316.

General procedures of UV−visible absorption and fluorescence experiments; interference studies; detection limit; and binding study plots (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: amilanjosenit@nitkkr.ac.in.

ORCID

Rahul Sakla: 0000-0001-5538-1830

Amrita Ghosh: 0000-0002-8735-8747

D. Amilan Jose: 0000-0003-2978-3400

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the Department of Chemistry, NIT Kurukshetra, for the research facilities. D.A.J. acknowledges the SERB-DST, Core Research grant no. EMR/2017/004085, CSIR grant no. 01 (2855)/16/EMR-II and DBT Project No. BT/PR23533/NNT/28/1301/2017 for the financial support. R.K. thanks the financial support of CSIR, India, for the Senior Research Fellowship (09/1050/(0009)/2018-EMR-I). R.S. thanks HSCST, Haryana, for the financial support. A.G. acknowledges CSIR for SRA fellowship.

■ REFERENCES

(1) Long, L.; Huang, M.; Wang, N.; Wu, Y.; Wang, K.; Gong, A.; Zhang, Z.; Sessler, J. L. A Mitochondria-Specific Fluorescent Probe for Visualizing Endogenous Hydrogen Cyanide Fluctuations in Neurons. *J. Am. Chem. Soc.* **2018**, *140*, 1870−1875.

(2) Xu, Z.; Chen, X.; Kim, H. N.; Yoon, J. Sensors for the Optical Detection of Cyanide Ion. *Chem. Soc. Rev.* **2010**, *39*, 127−137.

(3) Wang, F.; Wang, L.; Chen, X.; Yoon, J. Recent Progress in the Development of Fluorometric and Colorimetric Chemosensors for Detection of Cyanide Ions. *Chem. Soc. Rev.* **2014**, *43*, 4312−4324.

(4) Niu, Q.; Sun, T.; Li, T.; Guo, Z.; Pang, H. Highly Sensitive and Selective Colorimetric/Fluorescent Probe with Aggregation Induced Emission Characteristics for Multiple Targets of Copper, Zinc and Cyanide Ions Sensing and Its Practical Application in Water and Food Samples. *Sens. Actuators, B* **2018**, *266*, 730−743.

(5) You, G. R.; Park, G. J.; Lee, J. J.; Kim, C. A Colorimetric Sensor for the Sequential Detection of Cu^{2+} and CN^- in Fully Aqueous Media: Practical Performance of Cu^{2+}. *Dalton Trans.* **2015**, *44*, 9120−9129.

(6) Tigreros, A.; Rosero, H.-A.; Castillo, J.-C.; Portilla, J. Integrated Pyrazolo[1,5-a]Pyrimidine−Hemicyanine System as a Colorimetric and Fluorometric Chemosensor for Cyanide Recognition in Water. *Talanta* **2019**, 395−401.

(7) Liu, S.; Yang, M.; Liu, Y.; Chen, H.; Li, H. A Novel "Turn-on" Fluorescent Probe Based on Triphenylimidazole-Hemicyanine Dyad for Colorimetric Detection of CN− in 100% Aqueous Solution. *J. Hazard. Mater.* **2018**, *344*, 875−882.

(8) Jung, K. H.; Lee, K.-H. Efficient Ensemble System Based on the Copper Binding Motif for Highly Sensitive and Selective Detection of Cyanide Ions in 100% Aqueous Solutions by Fluorescent and Colorimetric Changes. *Anal. Chem.* **2015**, *87*, 9308−9314.

(9) Das, S.; Biswas, S.; Mukherjee, S.; Bandyopadhyay, J.; Samanta, S.; Bhowmick, I.; Hazra, D. K.; Ray, A.; Parui, P. P. A Cyanide Selective Off−on Fluorescent Chemosensor with in Vivo Imaging in 100% Water: Solid Probe Preferred over in Situ Generation. *RSC Adv.* **2014**, *4*, 9656−9659.

(10) Bhowmick, I.; Boston, D. J.; Higgins, R. F.; Klug, C. M.; Shores, M. P.; Gupta, T. Naked Eye Detection of Cyanide in Water with Coii Bis(Terpyridine) Complexes. *Sens. Actuators, B* **2016**, *235*, 325−329.

(11) Guo, Y.-Y.; Tang, X.-L.; Hou, F.-P.; Wu, J.; Dou, W.; Qin, W.-W.; Ru, J.-X.; Zhang, G.-L.; Liu, W.-S.; Yao, X.-J. A Reversible Fluorescent Chemosensor for Cyanide in 100% Aqueous Solution. *Sens. Actuators, B* **2013**, *181*, 202−208.

(12) Gwon, S.-Y.; Lee, E.-M.; Kim, S.-H. Hemicyanine-Based Colorimetric Chemosensors: Different Recognition Mechanisms for CN- Sensing. *Spectrochim. Acta, Part A* **2012**, *96*, 77−81.

(13) Hao, Y.; Nguyen, K. H.; Zhang, Y.; Zhang, G.; Fan, S.; Li, F.; Guo, C.; Lu, Y.; Song, X.; Qu, P.; Liu, Y.-N.; Xu, M. A Highly Selective and Ratiometric Fluorescent Probe for Cyanide by Rationally Altering the Susceptible H-Atom. *Talanta* **2018**, *176*, 234−241.

(14) Maurya, N.; Bhardwaj, S.; Singh, A. K. Selective Colorimetric and Fluorescence 'Turn-on' Sensor for Ag^+ and in-Situ Sensing of CN^- (Off-on-Off) Via Displacement Approach. *Mater. Sci. Eng., C* **2017**, *74*, 55−61.

(15) Ramachandran, E.; Vandarkuzhali, S. A. A.; Sivaraman, G.; Dhamodharan, R. Phenothiazine Based Donor-Acceptor Compounds with Solid-State Emission in the Yellow to NIR Region and Their Highly Selective and Sensitive Detection of Cyanide Ion in Ppb Level. *Chem. - Eur. J.* **2018**, *24*, 11042−11050.

(16) Salomon-Flores, M. K.; Bazany-Rodriguez, I. J.; Martinez-Otero, D.; Garcia-Eleno, M. A.; Guerra-Garcia, J. J.; Morales-Morales, D.; Dorazco-Gonzalez, A. Bifunctional Colorimetric Chemosensing of Fluoride and Cyanide Ions by Nickel-Pocop Pincer Receptors. *Dalton Trans.* **2017**, *46*, 4950−4959.

(17) Shaily; Kumar, A.; Ahmed, N. Indirect Approach for Cn-Detection: Development of "Naked-Eye" Hg^{2+} Induced Turn-Off Fluorescence and Turn-on Cyanide Sensing by the Hg^{2+} Displacement Approach. *Ind. Eng. Chem. Res.* **2017**, *56*, 6358−6368.

(18) Sun, T.; Niu, Q.; Li, Y.; Li, T.; Hu, T.; Wang, E.; Liu, H. A Novel Oligothiophene-Based Colorimetric and Fluorescent "Turn on" Sensor for Highly Selective and Sensitive Detection of Cyanide in Aqueous Media and Its Practical Applications in Water and Food Samples. *Sens. Actuators, B* **2018**, *258*, 64−71.

(19) Tirfoin, R.; Aldridge, S. A Molecular Traffic Light': Highly Selective Cyanide Sensing in Aqueous Media by a CpFe(Indenyl)-Functionalized Borane. *Dalton Trans.* **2013**, *42*, 12836−12839.

(20) Yang, X.; Li, Y.; Ding, Y.; Zhao, Z.; Zhang, Y.; Liu, X.; Fu, Z.; Cui, Y.; Sun, G.; Zhang, G.; Yan, M. Ratiometric & Reversible Fluorescent Sensing of NaCN by an Effective Probe Based on Phenanthro[9,10-D]Imidazole Platform. *Sens. Actuators, B* **2017**, *253*, 478−487.

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

(21) Zhao, H.-W.; Wu, G.; Sun, X.-Y.; Chao, J.-B.; Li, Y.; Jiang, L.; Han, H. A Highly Selective and Ratiometric Molecular Probe for Cyanide Sensing Based on a Phenothiazine-Hemicyanine Dye. *J. Lumin.* **2018**, *201*, 474−478.
(22) Nam, S.-W.; Chen, X.; Lim, J.; Kim, S. H.; Kim, S.-T.; Cho, Y.-H.; Yoon, J.; Park, S. In Vivo Fluorescence Imaging of Bacteriogenic Cyanide in the Lungs of Live Mice Infected with Cystic Fibrosis Pathogens. *PLoS One* **2011**, *6*, No. e21387.
(23) Huang, S.-Y.; Pierre, V. C. A Turn-on Luminescent Europium Probe for Cyanide Detection in Water. *Chem. Commun.* **2018**, *54*, 9210−9213.
(24) Kaushik, R.; Ghosh, A.; Singh, A.; Gupta, P.; Mittal, A.; Jose, D. A. Selective Detection of Cyanide in Water and Biological Samples by an Off-the-Shelf Compound. *ACS Sens.* **2016**, *1*, 1265−1271.
(25) Bhalla, V.; Singh, H.; Kumar, M. Triphenylene Based Copper Ensemble for the Detection of Cyanide Ions. *Dalton Trans.* **2012**, *41*, 11413−11418.
(26) Rathi, P.; Chahal, M. K.; Sankar, M. Highly Electron Deficient Tetrabenzoquinone-Appended Ni(II) and Cu(II) Porphyrins: Spectral, Solvatochromatic, Electrochemical Redox and Tuneable F− and CN− Sensing Properties. *New J. Chem.* **2017**, *41*, 11962−11968.
(27) Yadav, N.; Singh, A. K. Dicarbohydrazide Based Chemosensors for Copper and Cyanide Ions Via a Displacement Approach. *New J. Chem.* **2018**, *42*, 6023−6033.
(28) Zheng, Z.-B.; Huang, Q.-Y.; Han, Y.-F.; Zuo, J.; Ma, Y.-N. Ruthenium(II) Complex-Based Chemosensors for Highly Sensitive and Selective Sequential Recognition of Copper Ion and Cyanide. *Sens. Actuators, B* **2017**, *253*, 203−212.
(29) Mohammadi, A.; Zabihi, F. S.; Chaibakhsh, N. Chemical Sensor Using Metal-Organic Complex: Preparation, Characterization and Application for Highly Selective Detection of Cyanide Ions in Mixed Aqueous-Organic Media. *J. Photochem. Photobiol., A* **2018**, *367*, 384−389.
(30) Mohammadi, A.; Yaghoubi, S. Development of a Highly Selective and Colorimetric Probe for Simultaneous Detection of Cu^{2+} and CN− Based on an Azo Chromophore. *Sens. Actuators, B* **2017**, *251*, 264−271.
(31) Park, G. J.; Hwang, I. H.; Song, E. J.; Kim, H.; Kim, C. A Colorimetric and Fluorescent Sensor for Sequential Detection of Copper Ion and Cyanide. *Tetrahedron* **2014**, *70*, 2822−2828.
(32) Nair, R. R.; Raju, M.; Jana, K.; Mondal, D.; Suresh, E.; Ganguly, B.; Chatterjee, P. B. Instant Detection of Hydrogen Cyanide Gas and Cyanide Salts in Solid Matrices and Water by Using Cu(II) and N(III) Complexes of Intramolecularly Hydrogen Bonded Zwitterions. *Chem. - Eur. J.* **2018**, *24*, 10721−10731.
(33) Feng, E.; Fan, C.; Wang, N.; Liu, G.; Pu, S. A Highly Selective Diarylethene Chemosensor for Colorimetric Detection of CN^- and Fluorescent Relay-Detection of Al^{3+}/Cr^{3+}. *Dyes Pigm.* **2018**, *151*, 22−27.
(34) Mondal, J.; Manna, A. K.; Patra, G. K. Highly Selective Hydrazone Based Reversible Colorimetric Chemosensors for Expeditious Detection of CN- in Aqueous Media. *Inorg. Chim. Acta* **2018**, *474*, 22−29.
(35) Wang, K.; Yu, X.; Zhu, Y.; Xing, M.; Liang, M.; Sun, Y.; Cao, D.; Guan, R.; Liu, Z. Indole Derivatives for Cyanide Detection Based on Nucleophilic Addition and Hydrogen-Bond Interaction. *J. Mol. Struct.* **2018**, *1173*, 647−652.
(36) Chow, C.-F.; Ho, P.-Y.; Wong, W.-L.; Gong, C.-B. A Multifunctional Bimetallic Molecular Device for Ultrasensitive Detection, Naked-Eye Recognition, and Elimination of Cyanide Ions. *Chem. - Eur. J.* **2015**, *21*, 12984−12990.
(37) Chow, C.-F.; Ho, P.-Y.; Wong, W.-L.; Lu, Y.-J.; Tang, Q.; Gong, C.-B. Catalyst Displacement Assay: A Supramolecular Approach for the Design of Smart Latent Catalysts for Pollutant Monitoring and Removal. *Chem. Sci.* **2017**, *8*, 3812−3820.
(38) Jose, D. A.; Elstner, M.; Schiller, A. Allosteric Indicator Displacement Enzyme Assay for a Cyanogenic Glycoside. *Chem. - Eur. J.* **2013**, *19*, 14451−14457.
(39) Kim, M.; Mergu, N.; Son, Y.-A. Imidazole-Containing Ratiometric Receptor for the Selective and Sensitive Detection of Cyanide and Fluoride Via Deprotonation and a Receptor-Anion Ensemble for Cu^{2+} Sensing. *J. Lumin.* **2018**, *204*, 244−252.
(40) Kumar, P. S.; Lakshmi, P. R.; Elango, K. P. An Easy to Make Chemoreceptor for the Selective Ratiometric Fluorescent Detection of Cyanide in Aqueous Solution and in Food Materials. *New J. Chem.* **2019**, *43*, 675−680.
(41) Yalçın, E.; Alkis, M.; Seferoglu, N.; Seferoglu, Z. A Novel Coumarin-Pyrazole-Triazine Based Fluorescence Chemosensor for Fluoride Detection Via Deprotonation Process: Experimental and Theoretical Studies. *J. Mol. Struct.* **2018**, *1155*, 573−581.
(42) Wang, S.; Fei, X.; Guo, J.; Yang, Q.; Li, Y.; Song, Y. A Novel Reaction-Based Colorimetric and Ratiometric Fluorescent Sensor for Cyanide Anion with a Large Emission Shift and High Selectivity. *Talanta* **2016**, *148*, 229−236.
(43) Yu, X.; Wang, K.; Cao, D.; Wu, Q.; Guan, R.; Xu, Y.; Sun, Y.; Liu, Z. Simple Benzothiazole Chemosensor for Detection of Cyanide Anions Via Nucleophilic Addition. *Chem. Heterocycl. Compd.* **2017**, *53*, 42−45.
(44) Zheng, Z.-H.; Li, Z.-K.; Song, L.-J.; Wang, Q.-W.; Huang, Q.-F.; Yang, L. A Biocompatible Colorimetric Triphenylamine-Dicyanovinyl Conjugated Fluorescent Probe for Selective and Sensitive Detection of Cyanide Ion in Aqueous Media and Living Cells. *Sensors* **2017**, *17*, 405.
(45) Grimaldi, N.; Andrade, F.; Segovia, N.; Ferrer-Tasies, L.; Sala, S.; Veciana, J.; Ventosa, N. Lipid-Based Nanovesicles for Nanomedicine. *Chem. Soc. Rev.* **2016**, *45*, 6520−6545.
(46) Banerjee, S.; König, B. Molecular Imprinting of Luminescent Vesicles. *J. Am. Chem. Soc.* **2013**, *135*, 2967−2970.
(47) Cho, E.; Kim, H.; Choi, Y.; Paik, S. R.; Jung, S. Polydiacetylenyl B-Cyclodextrin Based Smart Vesicles for Colorimetric Assay of Arginine and Lysine. *Sci. Rep.* **2016**, *6*, No. 31115.
(48) Li, S.; Zhang, L.; Jiang, J.; Meng, Y.; Liu, M. Self-Assembled Polydiacetylene Vesicle and Helix with Chiral Interface for Visualized Enantioselective Recognition of Sulfinamide. *ACS Appl. Mater. Interfaces* **2017**, *9*, 37386−37394.
(49) Li, X.; Liu, W.; Yue, X.; Song, P.; Yin, Y.; Meng, M.; Xi, R. A Competitive Immunoassay Using Hapten-Modified Polydiacetylene Vesicles for Homogeneous and Sensitive Detection of Sodium Benzoate. *Sens. Actuators, B* **2018**, *258*, 1060−1065.
(50) Lou, X.; Qiang, L.; Qin, J.; Li, Z. A New Rhodamine-Based Colorimetric Cyanide Chemosensor: Convenient Detecting Procedure and High Sensitivity and Selectivity. *ACS Appl. Mater. Interfaces* **2009**, *1*, 2529−2535.
(51) Kang, J. H.; Lee, S. Y.; Ahn, H. M.; Kim, C. A Novel Colorimetric Chemosensor for the Sequential Detection of Ni^{2+} and CN^- in Aqueous Solution. *Sens. Actuators, B* **2017**, *242*, 25−34.
(52) Kaushik, R.; Ghosh, A.; Amilan Jose, D. Recent Progress in Hydrogen Sulphide (H_2S) Sensors by Metal Displacement Approach. *Coord. Chem. Rev.* **2017**, *347*, 141−157.
(53) Kaushik, R.; Sakla, R.; Ghosh, A.; Selvan, G. T.; Selvakumar, P. M.; Jose, D. A. Selective Detection of H_2S by Copper Complex Embedded in Vesicles through Metal Indicator Displacement Approach. *ACS Sens.* **2018**, *3*, 1142−1148.
(54) Jung, H. S.; Han, J. H.; Kim, Z. H.; Kang, C.; Kim, J. S. Coumarin-Cu(II) Ensemble-Based Cyanide Sensing Chemodosimeter. *Org. Lett.* **2011**, *13*, 5056−5059.
(55) Liu, Y.; Lv, X.; Zhao, Y.; Liu, J.; Sun, Y.-Q.; Wang, P.; Guo, W. A Cu (II)-Based Chemosensing Ensemble Bearing Rhodamine B Fluorophore for Fluorescence Turn-on Detection of Cyanide. *J. Mater. Chem.* **2012**, *22*, 1747−1750.
(56) Das, S.; Biswas, S.; Mukherjee, S.; Bandyopadhyay, J.; Samanta, S.; Bhowmick, I.; Hazra, D. K.; Ray, A.; Parui, P. P. A Cyanide Selective Off-On Fluorescent Chemosensor with in Vivo Imaging in 100% Water: Solid Probe Preferred over in Situ Generation. *RSC Adv.* **2014**, *4*, 9656−9659.

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595

(57) Zhang, H. Thin-Film Hydration Followed by Extrusion Method for Liposome Preparation. In *Liposomes: Methods and Protocols*; D'Souza, G. G. M., Ed.; Springer: New York, NY, 2017; pp 17−22.
(58) Nguyen, B. T.; Anslyn, E. V. Indicator−Displacement Assays. *Coord. Chem. Rev.* **2006**, *250*, 3118−3127.
(59) Xia, H.; Li, J.; Zou, G.; Zhang, Q.; Jia, C. A Highly Sensitive and Reusable Cyanide Anion Sensor Based on Spiropyran Functionalized Polydiacetylene Vesicular Receptors. *J. Mater. Chem. A* **2013**, *1*, 10713−10719.
(60) Malik, A. H.; Hussain, S.; Tanwar, A. S.; Layek, S.; Trivedi, V.; Iyer, P. K. An Anionic Conjugated Polymer as a Multi-Action Sensor for the Sensitive Detection of Cu^{2+} and PPi, Real-Time Alp Assaying and Cell Imaging. *Analyst* **2015**, *140*, 4388−4392.
(61) Yu, W.; Qiang, J.; Yin, J.; Kambam, S.; Wang, F.; Wang, Y.; Chen, X. Ammonium-Bearing Dinuclear Copper(II) Complex: A Highly Selective and Sensitive Colorimetric Probe for Pyrophosphate. *Org. Lett.* **2014**, *16*, 2220−2223.
(62) Xu, Z.; Ren, Y.-Y.; Fan, X.; Cheng, S.; Xu, Q.; Xu, L. A Naphthalimide-Based Fluorescent Probe for Highly Selective Detection of Pyrophosphate in Aqueous Solution and Living Cells. *Tetrahedron* **2015**, *71*, 5055−5058.
(63) Guo, Z.; Zhu, W.; Tian, H. Hydrophilic Copolymer Bearing Dicyanomethylene-4H-Pyran Moiety as Fluorescent Film Sensor for Cu^{2+} and Pyrophosphate Anion. *Macromolecules* **2010**, *43*, 739−744.
(64) Hu, M.; Feng, G. Highly Selective and Sensitive Fluorescent Sensing of Oxalate in Water. *Chem. Commun.* **2012**, *48*, 6951−6953.
(65) Patra, S. K.; Sheet, S. K.; Sen, B.; Aguan, K.; Roy, D. R.; Khatua, S. Highly Sensitive Bifunctional Probe for Colorimetric Cyanide and Fluorometric H_2S Detection and Bioimaging: Spontaneous Resolution, Aggregation, and Multicolor Fluorescence of Bisulfide Adduct. *J. Org. Chem.* **2017**, *82*, 10234−10246.
(66) Agarwalla, H.; Gangopadhyay, M.; Sharma, D. K.; Basu, S. K.; Jadhav, S.; Chowdhury, A.; Das, A. Fluorescent Probes for the Detection of Cyanide Ions in Aqueous Medium: Cellular Uptake and Assay for B-Glucosidase and Hydroxynitrile Lyase. *J. Mater. Chem. B* **2015**, *3*, 9148−9156.
(67) Kumari, N.; Jha, S.; Bhattacharya, S. A Chemodosimetric Probe Based on a Conjugated Oxidized Bis-Indolyl System for Selective Naked-Eye Sensing of Cyanide Ions in Water. *Chem. - Asian J.* **2012**, *7*, 2805−2812.
(68) La, M.; Hao, Y.; Wang, Z.; Han, G.-C.; Qu, L. Selective and Sensitive Detection of Cyanide Based on the Displacement Strategy Using a Water-Soluble Fluorescent Probe. *J. Anal. Methods Chem.* **2016**, No. 1462013.

DOI: 10.1021/acsami.9b17316
ACS Appl. Mater. Interfaces 2019, 11, 47587−47595