

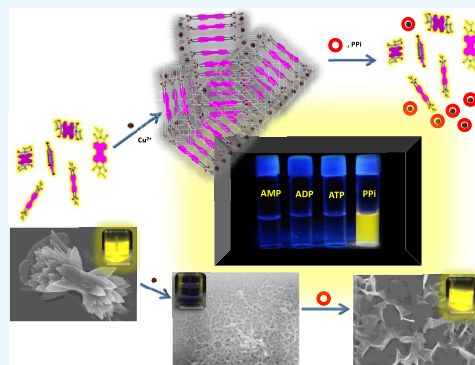
Selective Detection of Pyrophosphate Anions in Aqueous Medium Using Aggregation of Perylene Diimide as a Fluorescent Probe

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Supporting Information

ABSTRACT: A water-soluble perylene diimide, aspartic acid-functionalized perylene diimide (APDI), has shown significant sequential “turn-off” and “turn-on” responses toward Cu^{2+} and inorganic pyrophosphate (PPi), respectively. APDI was found to show selectivity toward Cu^{2+} and inorganic PPi over adenosine monophosphate, adenosine diphosphate, and adenosine triphosphate. The detection has been studied by absorption and emission spectroscopy techniques. Incorporation of Cu^{2+} into the solution of APDI results in a distinct quenching of the fluorescence intensity, while there was no spectral change in the presence of other metal ions. The formed APDI– Cu^{2+} ensemble can turn on its fluorescence signal when PPi is present. The detection of PPi could be traced by looking at the change in color of the solution under the naked eye. No interference was observed from other anions, making the APDI– Cu^{2+} aggregate a highly selective biosensor for PPi.



1. INTRODUCTION

The most ingenious creation in the whole universe is unarguably the creation of life, and when there is life, there are deterrents to it. One of the most devastating and long-lasting deterrents to life is disease. Diseases are ubiquitous as oxygen in our life. Hence, it automatically produces a huge demand for the correct diagnosis along with proper treatment of a disease. In the research field, inorganic pyrophosphate has tipped the scales of our attention toward itself immensely for being responsible for prior biological roles in our body. People after the age of 30s or 40s suffer from common bone malfunctions. Common bone diseases such as calcium pyrophosphate deposition disease (CPDD) arise from the accumulation of pyrophosphate dehydrate crystals in joints^{1–5} and tissues surrounding them. It promotes the joint cartilage to break down, causing various forms of painful arthritis often mistaken as gout⁶ and other conditions.⁷ Generally, the occurrence of this disease increases with age but can happen in young ages as well. Hence, proper diagnosis of pyrophosphate is very much required. Pyrophosphate is produced when adenosine triphosphate (ATP) is hydrolyzed under cellular conditions and present in DNA replication, regarded as an important parameter in case of real-time DNA sequencing which can result in early cancer detection. Early detection of abnormal levels of inorganic pyrophosphate (PPi) in the synovial or intercellular fluids can lead to saving lives by preventing CPDD or cancer in its inchoate stages.^{8–10} Apart from the sophisticated, costly, and time-consuming conventional ways of detection like atomic absorption spectroscopy, inductively coupled plasma mass spectroscopy, voltammetry, computed tomography, and radiography, we need to develop techniques that are cost-effective and easy in handling.^{11–14}

Although various types of fluorescent chemosensors have been reported to selectively detect PPi, there are only few reports on aqueous medium existing in the literature.^{15–23} Czarnik et al. reported the sensing of PPi in pure aqueous solution using anthracene derivatives.²¹ Hong et al. developed a naphthalene-based fluorescent sensor that was selective for PPi over ATP, which can work over a wide range of pH.²² Yoon et al. first reported a naphthalene diimide-based fluorescent chemosensor for PPi at physiological pH taking advantage of excimer formation.¹

Perylene diimide (PDI) derivatives have attracted much attention in sensing applications due to their high fluorescent quantum yield, high photostability, and large extinction coefficient but have the disadvantage of being insoluble in water due to severe aggregation resulting from π – π stacking.^{24–42} Many approaches have been reported to synthesize water-soluble perylene diimide.^{43–47} Recently, perylene diimide-based sensors have been developed to detect cations, anions, and biomolecules.^{28,29,34,38,45–47,49} However, simple and high-synthetic-yield PDI-based chemosensors for PPi in 100% aqueous medium are rare in the literature. Muthuraj and his co-workers made a three-component-based PDI–HIS + GO + Cu^{2+} system to detect PPi in aqueous medium, which is quite sophisticated, and the preparation process requires sensitive handling of the GO sheet for making it completely water-dispersible.⁴⁶ Shi et al. have developed a PDI-based PDI–GlyAsp/ Cu^{2+} system for detecting pyrophosphate anions in

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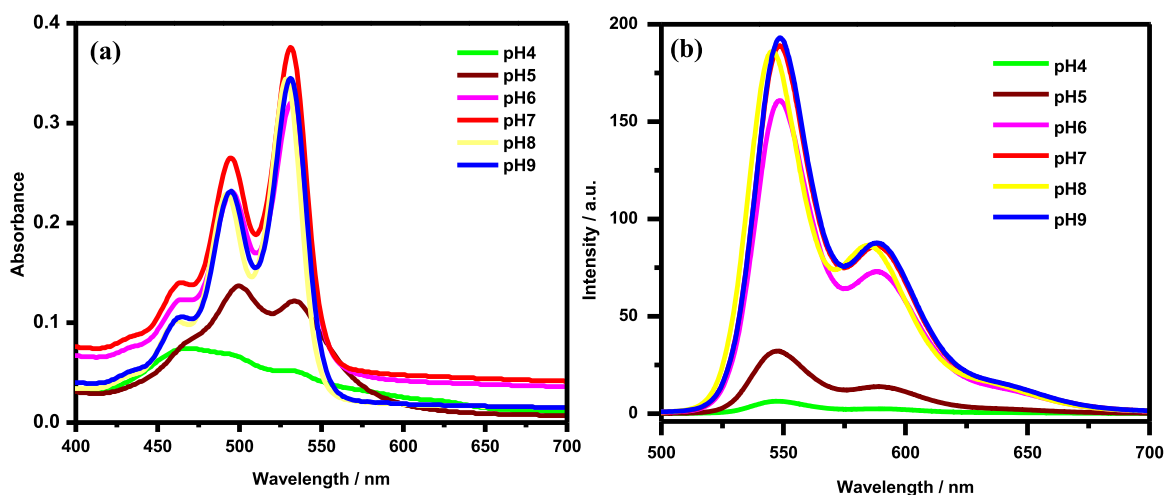


Figure 1. (a) UV–vis spectra of APDI ($10\ \mu\text{M}$) at different pHs in aqueous medium at $25\ ^\circ\text{C}$ and at a scan speed of $100\ \text{nm}$ and path length of $10\ \text{mm}$. (b) Fluorescence graph of APDI ($10\ \mu\text{M}$) at different pHs in aqueous medium. The spectra were recorded at $25\ ^\circ\text{C}$ with slit of $5/5\ \text{nm}$ and $\lambda_{\text{ex}} = 440\ \text{nm}$.

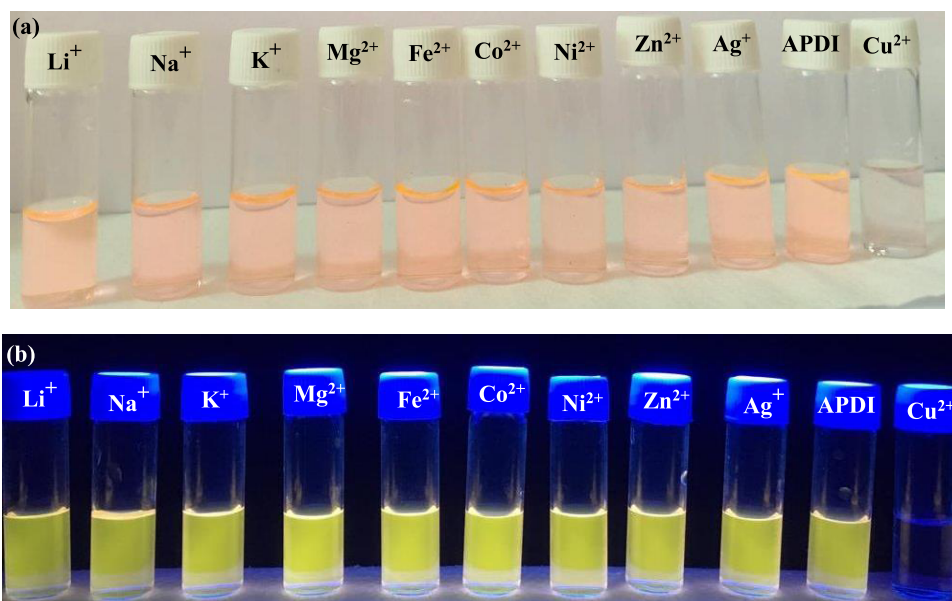


Figure 2. Photographs of APDI solutions ($10\ \mu\text{M}$) in the presence of various metal ions ($100\ \mu\text{M}$): (a) under visible light and (b) under UV light excited at $365\ \text{nm}$.

aqueous medium, but this method required alkaline medium, which might not be appropriate for protein or biomolecules sensitive toward alkali.⁴⁷ Herein, we report a PDI-based chemosensor in 100% water medium, which can selectively detect PPI over other coexisting anions adenosine monophosphate (AMP), adenosine diphosphate (ADP), and ATP. Our developed chemosensing ensemble probe requires a simple preparation method involving no sophisticated handling and simple aggregation–disaggregation mechanism of PDI; it is soluble perfectly in neutral aqueous medium, showing distinct color change visibly in the presence of PPI. The synthesis of aspartic acid-functionalized perylene diimide (APDI) has been described in our previous report. The aspartic acid-appended PDI derivative, APDI, shows bright orange in its monomeric form in aqueous medium. APDI in the presence of copper has been shown to quench its fluorescence intensity selectively, bright orange color of APDI turning to pale pink due to π – π stacking interaction between perylene cores as Cu^{2+} helps in

bringing the moieties closer to each other. The idea behind singling out Cu^{2+} among all of the other metal ions is that Cu^{2+} has very strong affinity toward PPI.^{47,48} Thus, in the presence of PPI, the APDI– Cu^{2+} complex disaggregates to form a Cu^{2+} –PPI complex and sets the dye free in the solution, which is responsible for the reappearance of the original fluorescent color.

2. RESULTS AND DISCUSSION

2.1. 1 UV–Vis and Fluorescence Titration of APDI at Different pHs. A set of six different APDI solutions ($10\ \mu\text{M}$) was prepared, and the pH was adjusted at 4, 5, 6, 7, 8, and 9 by adding aq HCl/NaOH solution. The APDI solutions of different pHs at the same concentration of $10\ \mu\text{M}$ were kept for 20 min. It has been found that the acidic one changed its color from orange to pale pink (Figure S1). The optical measurements were performed to see the changes in their ground state and excited state. The solution mixtures were made homogeneous by

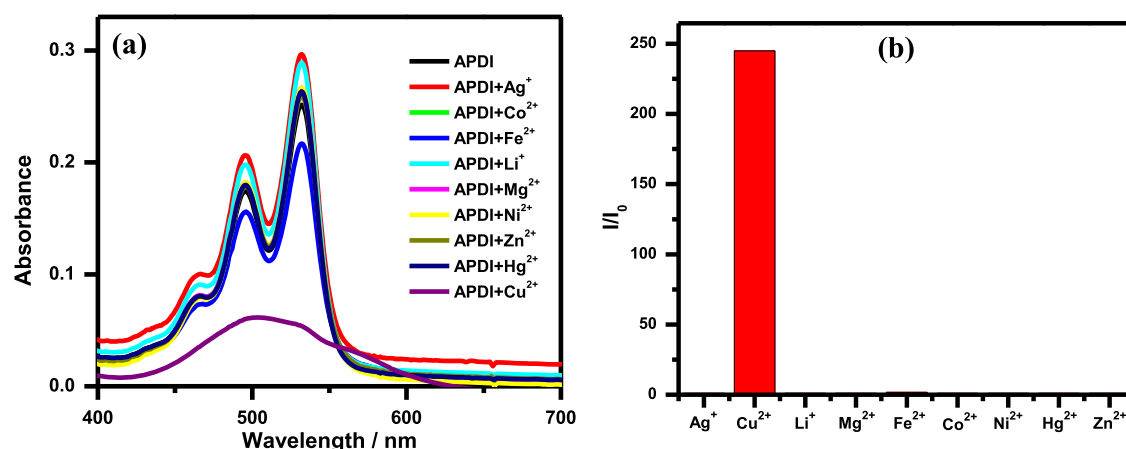


Figure 3. (a) UV–vis absorption spectra of APDI (10 μM) upon addition of respective metal ions (100 μM) were measured at 25 $^{\circ}\text{C}$ in a cell having path length of 10 mm in HEPES buffer (pH 7.4). (b) Dependence of the emission intensity factor (I/I_0) was obtained by excitation of the 10 μM APDI fluorophore at the said excitation wavelength of 440 nm, slit of 5/5 nm, and path length of 10 mm in HEPES buffer (pH 7.4).

shaking well before all experiments. UV–vis and photoluminescence (PL) spectra were taken at 25 $^{\circ}\text{C}$. It has been observed that at lower pH, i.e., at pH 4, aggregation occurs at its maximum and that in neutral pH conditions, it gives intense monomeric peak. At pH 7, APDI mainly shows three absorption peaks at 531, 494 and 463 nm and a weak broad band at 430 nm, indicating the transition of $S_0 \rightarrow S_1$, with respective vibronic structure $\nu = 0 \rightarrow \nu' = 0, 1, 2$ and 3 transitions respectively (Figure 1a).⁴⁷ Due to the presence of a large hydrophobic π -conjugated core of APDI derivatives, they aggregate^{30,31,41,56} at lower pH, resulting in decrease of absorption band at 531 and 494 nm. The fluorescence intensity of APDI is sensitive to the pH of the medium. The maximum fluorescence intensity was observed in neutral solution having peaks at 547 and 589 nm (Figure 1b), in which deprotonation of the hydrophilic carboxylic groups on the APDI side chains likely hinders the formation of strongly coupled aggregates. On the contrary, the fluorescence intensity was significantly decreased in acidic medium due to protonation of the carboxylate groups, which induces the formation of stable aggregates. The π -stacked aggregates of APDI derivatives are stable in the protonated state, but their supramolecular structure changes in neutral and basic media. In the aggregate, monomeric units slide over their adjacent ones and increase the intermolecular distance upon deprotonation. Intermolecular hydrogen bonds may help maintaining the stability of the protonated aggregate. Fluorescence spectra showed a pH dependence of APDI derivatives with a nonlinearly increasing response ranging from pH 4 to 9, which can be identified by naked eye when the color changes from pale pink to orange.

2.2. Study of Specific Complexation of APDI with Cu^{2+} Ions. It is well known that the carboxyl group ($-\text{COOH}$) has a very high binding^{45,47,50} tendency toward Cu^{2+} , Ni^{2+} , Cd^{2+} , and Zn^{2+} ions. Since the APDI derivative comprises four chelating carboxylic groups ($-\text{COOH}$), we have pursued to find the most apposite coordination metal ion pair. Hence, we have carried out complexation of APDI with different metal ions, viz, Li^+ , Ag^+ , Mg^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , and Cu^{2+} in *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) buffer at room temperature. As shown in Figure 2, sensor APDI in the presence of the Cu^{2+} ion shows a change of color from orange to pale pink and fluorescence turns off under UV light. To check the selectivity of the sensor APDI for Cu^{2+} , the same

experiments were carried out using Li^+ , Ag^+ , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , and Hg^{2+} metal ions. Interestingly, no change of color was found either under the naked eye or under UV light in the presence of other tested metal ions (Figure 2). A distinct UV–vis spectrum was observed having a peak at 504 nm with a shoulder at 566 nm, and the three absorption peaks at 463, 494, and 531 nm were completely diminished upon addition of only Cu^{2+} ions, while other metal ions such as Li^+ , Ag^+ , Mg^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , and Hg^{2+} showed negligible spectral changes (Figure 3a). This result indicates that our designed chemosensor, APDI, can act as a potential candidate for sensing Cu^{2+} ions under the naked eye in pure aqueous medium.

Emission intensity enhancement (I/I_0) (Figure 3b) was obtained by excitation of the APDI fluorophore at the said excitation wavelength of 440 nm during cation-selective chromogenic chemosensing, through a competitive experiment (Li^+ , Ag^+ , Mg^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , and Hg^{2+}). The fluorescence enhancement factors (EFs) of APDI in the presence of various metal ions can be seen in Figure 3b. In the presence of Cu^{2+} ions, the fluorescence intensity of APDI decreased by over 250-fold, whereas the EFs of Li^+ , Ag^+ , Mg^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , and Hg^{2+} were nearly equal to zero. APDI thus exhibits an obvious “turn-off” fluorescence response and shows an extremely high selectivity toward the Cu^{2+} in aqueous media. APDI showed significant fluorescence quenching with Cu^{2+} only, identifying it as an excellent turn-off sensor for Cu^{2+} .

2.3. Complexation Titration of APDI with Cu^{2+} Ions.

After 12 h of keeping the APDI solutions (10 μM) in HEPES buffer (pH 7.4) with Cu (II) ions, it has been found that the color changes to pale pink, and these solutions have shown fluorescence turn-off under UV light (excitation at 365 nm) as the concentration of Cu^{2+} ions varies from lower to higher (Figure S2). Afterward, we have performed spectroscopic measurements such as UV–vis and PL to observe the electronic changes in the ground state as well as in the excited state of the newly formed APDI– Cu^{2+} complex with respect to free APDI. Each solution was shaken well and made homogeneous before taking any absorption or emission spectrum at 25 $^{\circ}\text{C}$. Results showed a gradual decrease in absorption and emission spectra as the concentration of copper ion increased. The absorption bands of APDI at 463, 494, and 531 nm were diminished gradually, and a new broad spectrum appeared at 504 nm with a shoulder at 566 nm, which indicates that addition of Cu^{2+}

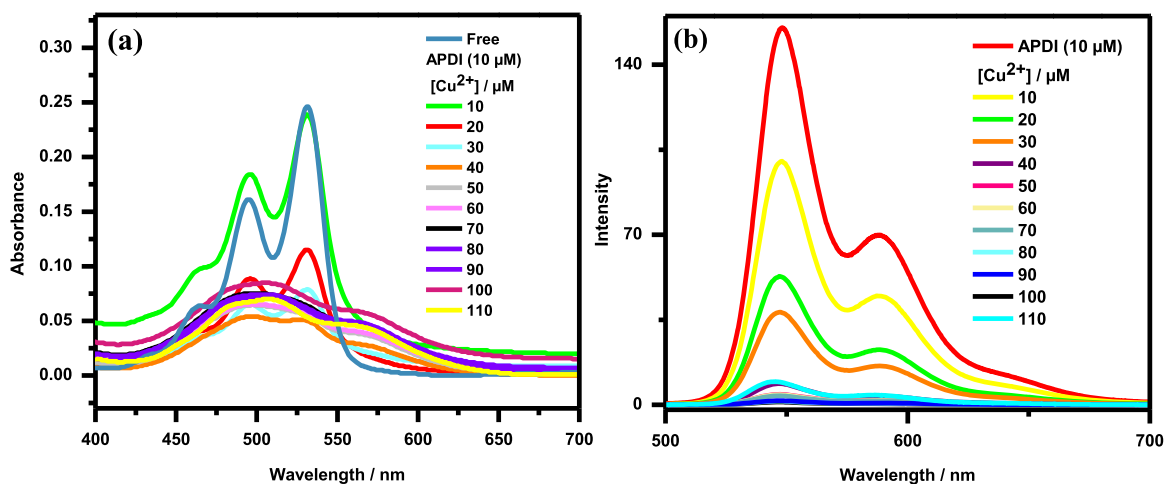


Figure 4. (a) Absorption spectra of 10 μM APDI with different concentrations of Cu^{2+} at 25 $^{\circ}\text{C}$, at a scan speed of 100 nm and path length of 10 mm in HEPES buffer (pH 7.4). (b) Emission spectra of 10 μM APDI with different concentrations of Cu^{2+} at 25 $^{\circ}\text{C}$, $\lambda_{\text{ex}} = 440$ nm, path length = 10 mm, and slit = 5/5 nm in HEPES buffer (pH 7.4).

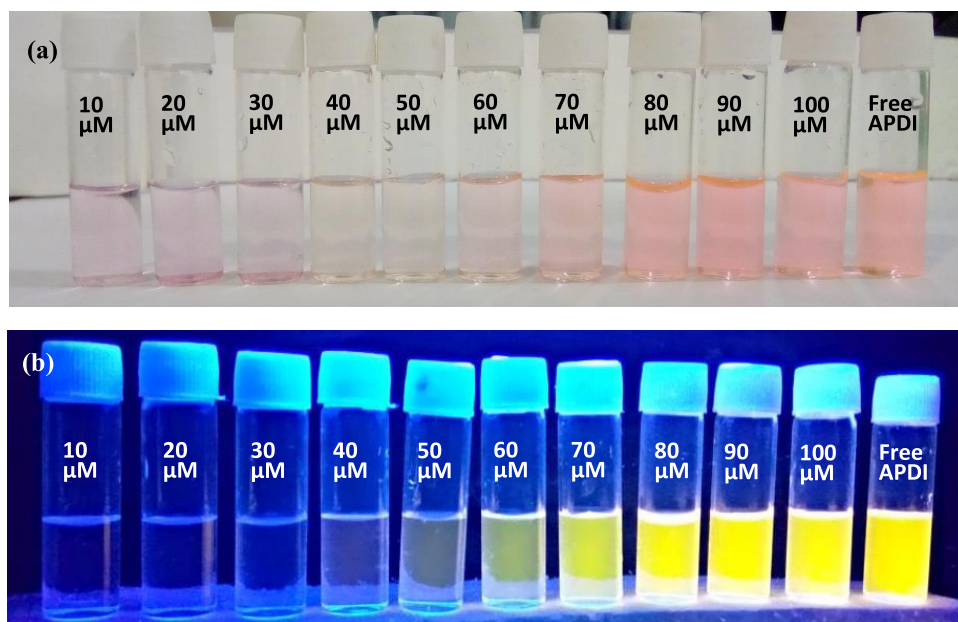


Figure 5. Photos of response of APDI– Cu^{2+} aggregates [APDI (10 μM) + Cu^{2+} (100 μM)] in aqueous HEPES buffer solution (pH 7.4) with increasing concentration of PPI: (a) under normal light and (b) under UV light having excitation at 365 nm.

produced a new ensemble (Figure 4a). It has further been seen that for free APDI, A_{0-0}/A_{0-1} is 1.52, whereas in the presence of 100 μM Cu^{2+} ions, the A_{0-0}/A_{0-1} ratio is 0.92, which supports the formation of aggregates.^{47,51} The binding of Cu^{2+} could also be traced by fluorescence titration as strong quenching of the APDI emission at 547 nm (Figures 4b and S3) was observed upon gradual addition of the metal ion. This change indicates that Cu^{2+} induced aggregation of APDI by bringing two perylene moieties closer to favor π – π stacking between them. Thus, APDI exhibits an obvious turn-off fluorescence response and shows an extremely high selectivity toward Cu^{2+} in aqueous media.

2.4. Application of the APDI– Cu^{2+} Ensemble for Selective Sensing of PPI. As Cu^{2+} has binding affinity toward PPI,⁵² we have also tested our ensemble for the detection of PPI. A set of competitive complexations with different concentrations of PPI was performed. Surprisingly, the orange color of

the solutions having concentrations above 40 μM PPI reappeared under visible and yellow under UV light, respectively (Figure 5). It is evidently clear that the absorption peak of APDI redevelops at 531 and 494 nm for 0–0 and 0–1 electronic transitions; on the other hand, the aggregation band at 566 nm decreased (Figure 6a). These results prove the disassembly of APDI– Cu^{2+} aggregates. Subsequently, the emission intensity also became stronger upon gradual increase of concentration of PPI (Figure 6b). Further addition of PPI above 100 μM did not make any changes in absorption and emission spectra. The result is due to the competitive binding of PPI with Cu^{2+} , which resulted in the disassembly of the APDI– Cu^{2+} complex, which is responsible for reappearance of the original color of monomeric APDI, resulting in the reappearance of the free APDI emission peak at around 547 nm (Figure 6b). Benesi–Hilderbrand analysis (Figure S4) gives the apparent binding constant for the interaction between APDI– Cu^{2+} and PPI, which was calculated

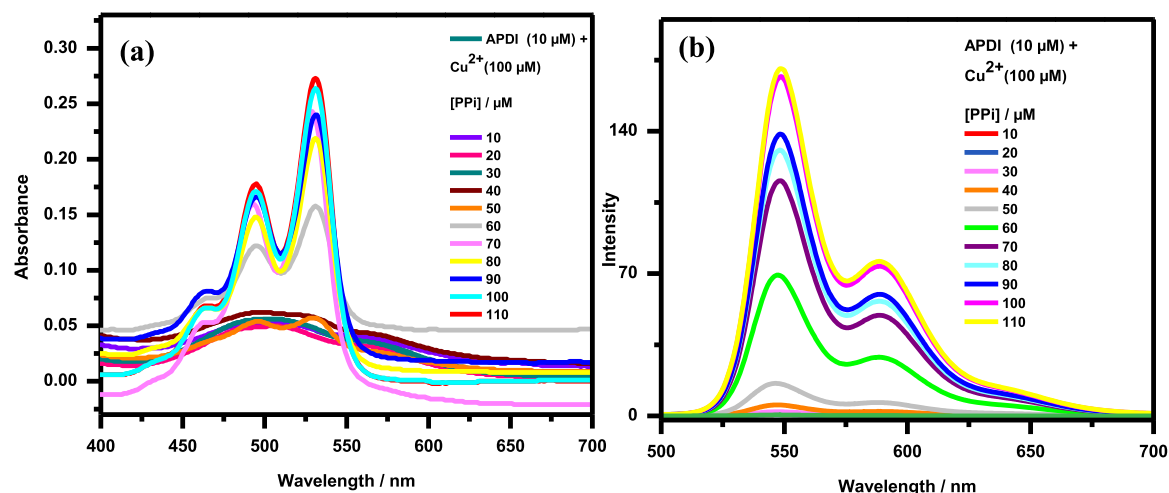


Figure 6. (a) Absorption spectra of APDI–Cu²⁺ aggregates [APDI (10 μM) + Cu²⁺ (100 μM)] titrated with PPI (0–110 μM) in HEPES buffer (pH 7.4) solution at a scan speed of 100 nm and path length of 10 mm. (b) Emission spectra of the APDI–Cu²⁺ aggregate [APDI (10 μM) + Cu²⁺ (100 μM)] titrated with PPI (0–110 μM) in HEPES buffer (pH 7.4) solution at 25 °C, λ_{ex} = 440 nm, path length = 10 mm, and slit = 5/5.

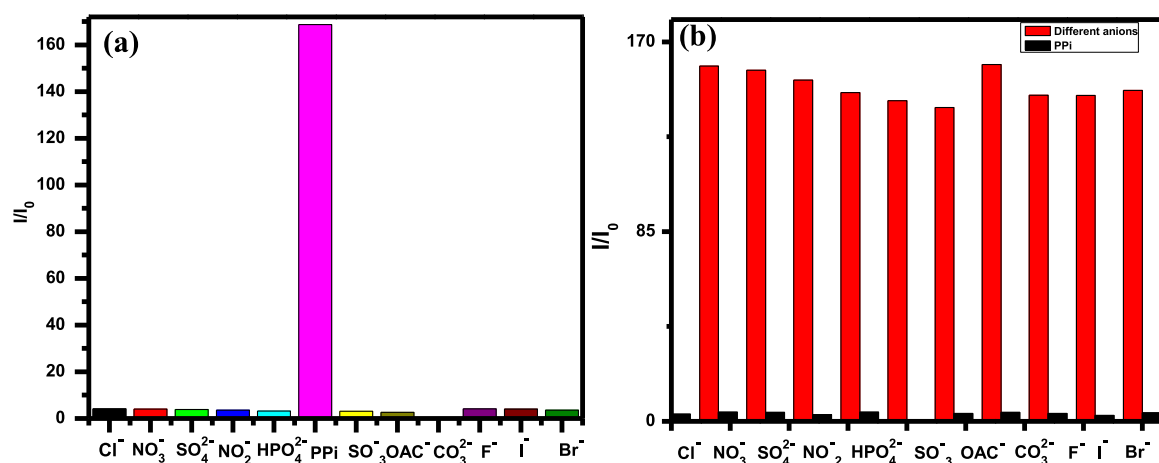


Figure 7. (a) Fluorescence response of APDI–Cu²⁺ aggregates in HEPES buffer upon addition of various anions. (b) Fluorescence response bar plot of the APDI–Cu²⁺ aggregate upon addition of various anions in aqueous HEPES buffer. Red bar: the integrated fluorescence response after addition of 100 μM PPI; black bar: fluorescence response with different anions at 100 μM; excitation wavelength: 440 nm; and emission wavelength: 547 nm.

to be 8.9×10^5 . The limit of detection for PPI was calculated to be 1.08×10^{-7} M using the $3\sigma/S$ equation (Figure S5).

2.5. Specific and Selective Binding of APDI–Cu²⁺ Ensemble Exclusively with PPI over Other Common Anions. It is required to check the interferences of other common anions to apply the developed APDI–Cu²⁺ probe for selective detection of PPI. It has been found that the APDI–Cu²⁺ aggregate only binds with PPI and releases monomeric APDI in solution, whereas it remains inactive toward other common anions. Figure S6 shows the selective recovery of the color of monomeric APDI and fluorescence under UV light. The selectivity toward the PPI anion only can be examined by performing fluorescence measurements of APDI upon addition of common anions, viz., Cl[−], NO₃[−], SO₄^{2−}, NO₂[−], HPO₄^{2−}, SO₃[−], OAC[−], CO₃^{2−}, F[−], I[−], and Br[−] under identical conditions. The emission intensity increment (I/I_0) of the APDI–Cu²⁺ ensemble vs different anions at 547 nm (Figure 7a) clearly shows that the APDI–Cu²⁺ ensemble selectively responds to PPI and does not exhibit any remarkable change with other anions. Furthermore, the fluorescence response of the APDI–Cu²⁺ ensemble toward PPI was investigated in the presence of excess of other anions. Figure 7b reveals the dramatic recovery of

fluorescence when PPI was added into the solution containing common anions. These results indicate the selectivity of the present chemosensing ensemble probe APDI–Cu²⁺ for the detection of biologically important inorganic pyrophosphate.

2.6. Response of APDI–Cu²⁺ Probe toward AMP, ADP, and ATP. As the in situ prepared APDI–Cu²⁺ ensemble exhibits good selectivity for PPI over general anions, the studies were further extended to organic biorelevant molecules bearing a phosphate moiety, viz., ATP, ADP, and AMP. Although many reports exist in the literature that report detection of PPI, but only few reports are there on selective recognition of PPI over AMP, ADP, and ATP.^{15,23,53,54} To the best of our knowledge, this is the first report on a perylene diimide-based biosensor for the recognition of PPI where AMP, ADP, and ATP do not interfere. The in situ prepared APDI–Cu²⁺ ensemble was treated with 100 μM ATP, ADP, AMP, and PPI, respectively. Interestingly, PPI showed instant color change to orange, whereas other did not (Figure 8). The addition of PPI resulted in quick recovery of fluorescence at conditions identical to those used for free APDI, and absorption bands at 531 and 494 nm for 0–0 and 0–1 electronic transitions reappeared, whereas the aggregation peak of APDI–Cu²⁺ at 566 nm was diminished

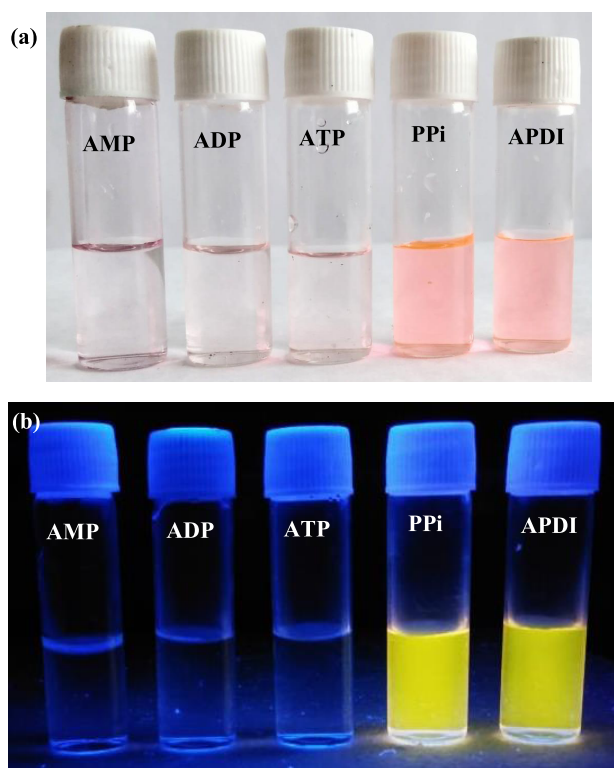


Figure 8. Photos of response of different phosphate anions (100 μM) added to APDI– Cu^{2+} aggregates [APDI (10 μM) + Cu^{2+} (100 μM)] in aqueous HEPES buffer solution (pH 7.4): (a) under normal light (from left to right: AMP, ADP, ATP, PPi, and pure APDI solution) and (b) under UV light having excitation at 365 nm (from left to right: AMP, ADP, ATP, PPi, and pure APDI solution).

(Figure 9a). Figure 5 shows the gradual increase of luminescent properties of APDI on incremental addition of PPi to the in situ formed APDI– Cu^{2+} aggregate. In contrast, ADP, ATP, and AMP showed weaker response as compared to PPi (Figure 8). As observed in Figure 9b, when 100 μM ATP and ADP were added to APDI– Cu^{2+} aggregates, slight fluorescence enhancement was observed. The enhancement in the presence of ADP is larger than that in the presence of ATP, while AMP did not

induce any detectable change of the fluorescence intensity of the APDI– Cu^{2+} complex. It is also an interesting result that the fluorescence enhancement caused by PPi is 4 times larger than that caused by ATP or ADP (Figure S7b). Thus, the present developed novel biosensor based on a water-soluble perylene diimide– Cu^{2+} aggregate for PPi, which is a very important finding in the context of selectivity. We have also tested the reversibility of APDI in the detection of Cu^{2+} and PPi. As depicted by Figure 10, the fluorescence intensity of APDI alternatively quenched and enhanced when alternatively Cu^{2+} and PPi were added to the solution of APDI. This result proves the formation of aggregate after addition of Cu^{2+} and disassembly of the aggregate in the presence of PPi.

2.7. Morphological Investigation of Aggregation and Disaggregation Phenomena. Further evidence for aggregation and disaggregation was obtained from the morphological changes of APDI, APDI + Cu^{2+} aggregates, and APDI + Cu^{2+} in the presence of PPi by field emission scanning electron microscopy (FESEM). The FESEM images (Figure 11) of pure APDI showed scattered flakelike structures (Figure 11a). The Cu^{2+} ion induced the formation of aggregation as shown in Figure 11b. It is clear from the images that APDI forms long fibers when Cu^{2+} is present in the solution, which finally forms an entangled network structure in the dried state. The aggregated APDI + Cu^{2+} fibers are disaggregated rapidly in the presence of PPi, regaining the flakelike structure of the APDI monomer (Figure 11c). The above results further confirm that pure APDI aggregates in the presence of Cu^{2+} and disaggregates on addition of PPi.

3. CONCLUSIONS

We have successfully developed a new biosensor based on a water-soluble perylene diimide derivative, which can selectively detect PPi without interferences from common anions, that is, AMP, ADP, and ATP. Our finding suggests that water-soluble perylene diimide can act as a multifunctional chemosensor for Cu^{2+} ions and PPi in pure aqueous medium. By tracing the change of color, it distinguishes Cu^{2+} ion from other metal ions and PPi from common anions (even from AMP, ADP, and ATP). Spectroscopic measurements show the formation of aggregates of APDI with Cu (II) ions and disaggregation in the

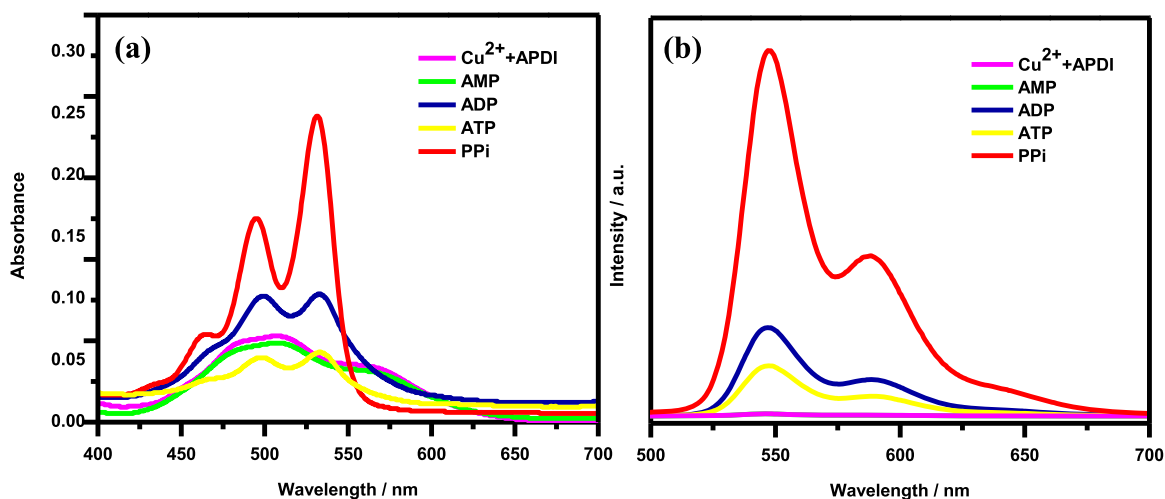


Figure 9. (a) Absorbance change and (b) emission change of the sensor APDI– Cu^{2+} aggregate [APDI (10 μM) + Cu^{2+} (100 μM)] in aqueous HEPES buffer solution (pH 7.4) upon addition of 100 μM AMP, ADP, ATP, and PPi (potassium salt). In both the experiments, cell path length = 10 mm and measurement temperature = 25 $^{\circ}\text{C}$. In the case of fluorescence measurements, the excitation wavelength was 440 nm.

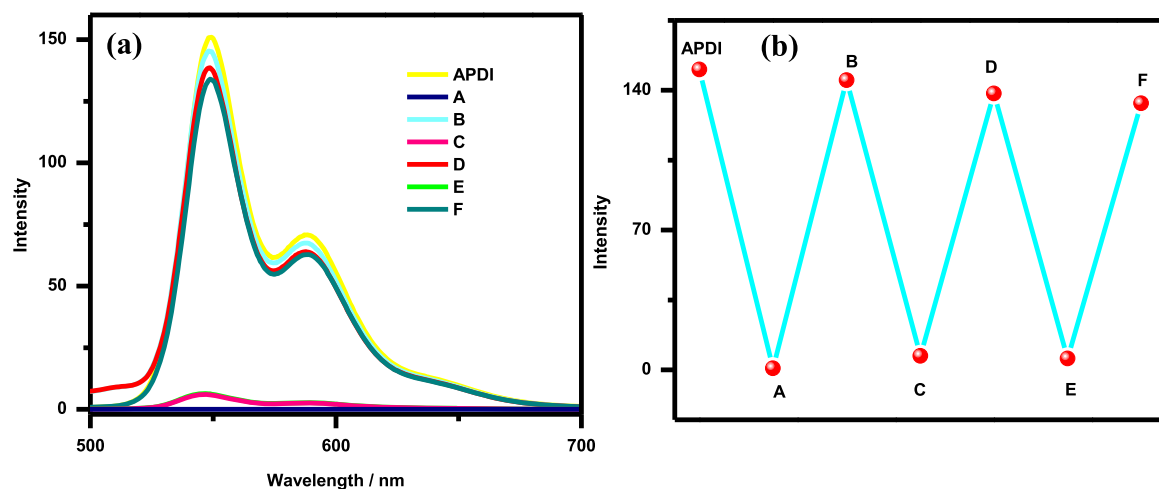


Figure 10. (a) Plot of fluorescence spectra upon addition of Cu^{2+} and PPI repeatedly to the solution of APDI at an excitation wavelength of 440 nm, slit of 5/5, temperature of 25 °C; A = APDI (10 μM) + Cu^{2+} (100 μM), B = APDI (10 μM) + Cu^{2+} (100 μM) + PPI (100 μM), C = APDI (10 μM) + Cu^{2+} (100 μM) + PPI (100 μM) + Cu^{2+} (100 μM), D = APDI (10 μM) + Cu^{2+} (100 μM) + PPI (100 μM) + Cu^{2+} (100 μM) + PPI (100 μM), E = APDI (10 μM) + Cu^{2+} (100 μM) + PPI (100 μM) + Cu^{2+} (100 μM) + PPI (100 μM) + Cu^{2+} (100 μM) + PPI (100 μM), and F = APDI (10 μM) + Cu^{2+} (100 μM) + PPI (100 μM) + Cu^{2+} (100 μM) + PPI (100 μM) + Cu^{2+} (100 μM) + PPI (100 μM). (b) Reversibility graph for the detection of PPI in aqueous HEPES buffer having pH 7.4 upon alternate addition of Cu^{2+} (100 μM) and PPI (100 μM) ions, tracing the fluorescence intensity at 547 nm.

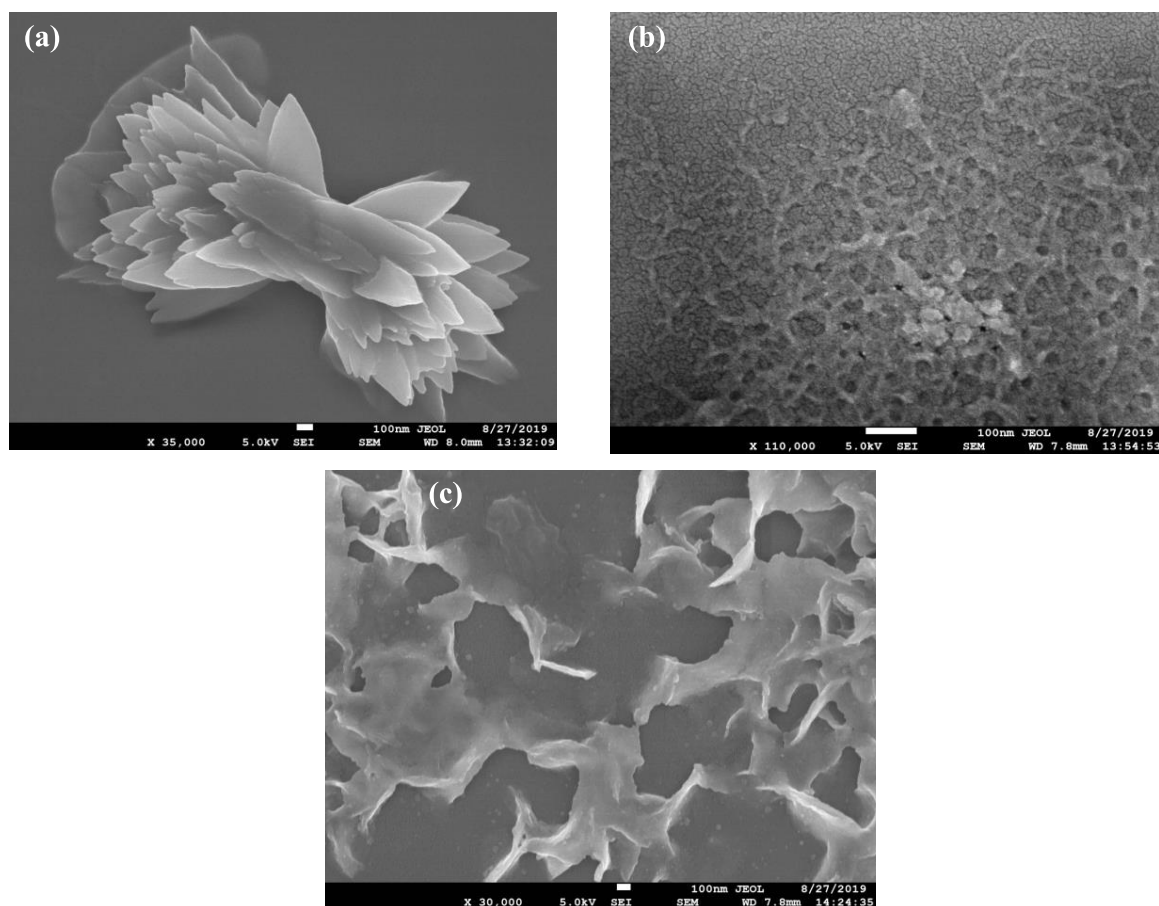
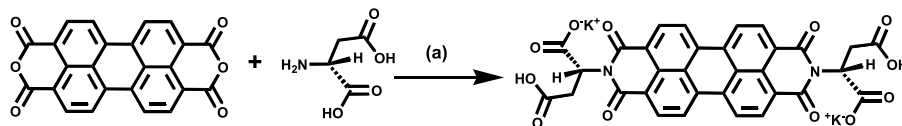


Figure 11. FESEM images of (a) APDI monomer (10 μM), (b) APDI– Cu^{2+} aggregates [APDI (10 μM) + Cu^{2+} (100 μM)], and (c) APDI– Cu^{2+} aggregates in the presence of PPI [APDI (10 μM) + Cu^{2+} (100 μM) + PPI (100 μM)].

presence of PPI anions, which has further been proved by morphological investigations (FESEM). Our achievement is curated with the excellent photochemical stability, chemical inertness toward oxidation, and high quantum yield of the APDI probe. The one-step synthesis of the probe with biocompatible

amino acid functionalities is cost-effective for large-scale production. Our low-cost analytical probe plays an instrumental role in selective detection of PPI in the presence of AMP, ADP, and ATP in 100% aqueous medium. The absence of any acidic/basic condition in our method constitutes a platform for the

Scheme 1. Synthetic Procedure of APDI: (a) DMSO, H₂O, 100 °C/L-Aspartic Acid, KOH, H₂O, 3 h, 74% Yield

exploitation and applications in a biological environment including blood serum, urine, etc. The APDI–Cu²⁺ ensemble probe showed selective recognition of PPI with very low detection limit of 1.08×10^{-7} M in purely aqueous medium. Hence, development of the colorimetric biosensor in an aqueous medium for pyrophosphate has great potential in the fields of cell imaging and diagnosis.

4. EXPERIMENTAL SECTION

4.1. Materials and Instrumentation. Perylene-3,4,9,10-tetracarboxylic dianhydride and aspartic acid were purchased from Sigma-Aldrich and used as received. Aspartic acid-functionalized perylene diimide (APDI) was synthesized following previous reports.^{55,56} For all aqueous mixtures and for spectroscopic studies, water of spectroscopy or high-performance liquid chromatography (HPLC) grade was used. HEPES buffer (pH 7.4) was used to prepare solutions for spectroscopic measurements. ¹H NMR spectra were recorded at room temperature on 500 MHz spectrometers (Bruker). ¹H NMR chemical shifts (δ) were reported in parts per million (ppm). ¹H NMR shifts were referenced to the residual hydrogen peak of D₂O (4.60 ppm). Splitting patterns were denoted as s (singlet) and br (broad). Fourier transform infrared (FTIR) spectra were recorded using KBr pellets of samples in an FTIR 8400S instrument (Shimadzu). UV–vis spectra were measured using a Hitachi U2910 model-2J1-0013 UV–vis spectrophotometer at 25 °C in a cell having a path length of 10 mm and scan speed of 100 nm. The fluorescence measurement was carried out using a cell having a path length of 10 mm in Hitachi F-4600 FLSPECTROPHOTOMET model 5J2-0004 at an excitation wavelength of 440 nm, excitation and emission slit of 5/5 nm, and scan speed of 240 nm. The morphologies after aggregation and disaggregation were observed by FESEM. A drop of the corresponding solution was casted on a glass cover slip and then dried at room temperature. A JEOL, JSM 6700F, instrument operating at 5 kV was used for imaging. Samples were coated with platinum for 90 s to reduce surface potential.

4.2. Synthesis of APDI. Perylene-3,4,9,10-tetracarboxylic dianhydride (750 mg, 8.15 mmol) was suspended in 50 mL of dimethyl sulfoxide (DMSO) and heated to 100 °C in a reflux apparatus. Aspartic acid (8.67 g; 65.2 mmol) was dissolved in 8 mL of 8 M KOH solution and added dropwise to the suspension. The mixture was stirred at 100 °C for 3 h and then cooled to room temperature. The residue was collected by filtration and washed with a mixture of DMSO and water (2:1). Furthermore, the residue was dissolved in a minimum amount of hot water and precipitated by the addition of acetone. Then, the procedure was repeated four times. The solid material was then collected by filtration, washed twice with acetone, and dried under vacuum to get the desired product, yield: 74% (Scheme 1).

¹H NMR, (500 MHz, D₂O, 25 °C) δ : 8.18 (br, 4H), 7.89 (br, 4H), 5.97 (br, 2H), 3.24 (d, 2H), 3.05 (br, 2H). ¹³C NMR, δ : 180.08, 176.70, 164.46, 133.64, 131.47, 128.11, 124.68, 123.17, 121.97, 54.44, 52.73, 38.86. FTIR (KBr), ν_{max} = 750, 810, 866, 1132, 1256, 1364, 1569, 1640, 1687, 3348 cm⁻¹. UV/vis (H₂O), λ_{max} /nm (ϵ /M⁻¹ cm⁻¹) 531 (27268), 494 (18146), 463 (9453).

Fluorescence (H₂O), λ_{max} /nm: 547, 586, fluorescence quantum yield (Φ_f) = 0.68.

4.3. Preparation of Solutions for Spectroscopic Measurements. **4.3.1. Preparation of Solutions for pH-Dependent UV–Vis and Fluorescence Studies.** The APDI stock solution (20 mL, 1 mM) was prepared by dissolving 12.44 mg of APDI in 20 mL of HPLC water. Then, 10 μ M APDI solution was prepared by diluting the stock solution with proper dilution.

4.3.2. Preparation of Solutions for Study of Specific Complexation of APDI with Cu²⁺. The stock solution of Cu²⁺ ions (40 mM) was prepared by dissolving 152 mg of Cu(II) nitrate salt in 100 mL of HEPES buffer. Then, different concentration solutions of copper(II) ions such as 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μ M along with 10 μ M APDI were made respectively with proper dilution. The solutions were shaken well and kept for 12 h for the complete complexation to be occurred before any measurements.

For different cations (Li⁺, Ag⁺, Mg²⁺, Fe²⁺, Co²⁺, Ni²⁺, Zn²⁺, Hg²⁺) other than the copper(II) ion, stock solutions were made at 1 mM concentration in HEPES buffer (pH 7.4) and diluted as desired. As APDI moieties contain K⁺ ions in the synthesized product, spectroscopic data of Na⁺ and K⁺ ions have not been included although measurements have been done for the control test.

4.3.3. Preparation of Solutions for APDI–Cu²⁺ Ensemble for Selective Sensing of PPI. Main stock solution was prepared as follows: 12 mM PPI was prepared by dissolving 41 mg of PPI in 10 mL of HEPES buffer aqueous solution (pH 7.4) and used for PPI titration experiments. The APDI–Cu²⁺ ensemble was prepared by mixing 10 μ M APDI with 100 μ M Cu²⁺ in HEPES buffer, and sufficient time (12 h) was given to form a stable complex to produce 100% conversion of APDI to APDI–Cu²⁺ aggregates. The in situ formed ensemble was used for the PPI detection experiment. Different concentrations of PPI solutions (3 mL) such as 10, 20, 30, 40, 50, 60, 80, 90, and 100 μ M with the in situ formed APDI–Cu²⁺ aggregate ([APDI] = 10 μ M and [Cu²⁺] = 100 μ M) in HEPES buffer were prepared and kept for 12 h.

For all anions (Cl⁻, NO₃⁻, SO₄²⁻, NO₂⁻, HPO₄²⁻, SO₃²⁻, OAC⁻, CO₃²⁻, F⁻, I⁻, Br⁻, AMP, ADP, and ATP) other than PPI, stock solutions were made at 1 mM concentration in HEPES buffer (pH 7.4), which was diluted as desired for spectroscopic measurements.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02405.

Photos of APDI solution at different pH and concentration of Cu²⁺ ion; Fluorescence titration linear plot; Benesi–Hildebrand analysis; calibration curve; photographs of various anions added to the APDI–Cu²⁺ aggregate in HEPES buffer; chemical structure of phosphate-containing compounds; and UV–vis and fluorescence plots of APDI in presence of PPI (PDF).

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Notes

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