ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

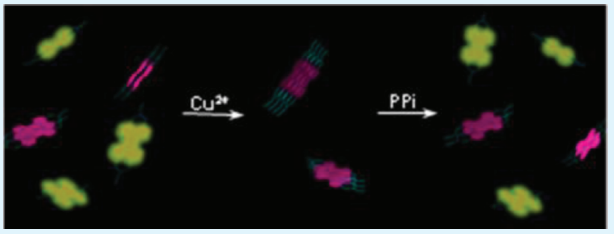
A Turn-on Fluorescent Sensor for Pyrophosphate Based on the Disassembly of Cu^{2+}-Mediated Perylene Diimide Aggregates

Xueling Feng, Yuxiu An, Zhiyi Yao, Chun Li,* and Gaoquan Shi

Department of Chemistry, Tsinghua University, Beijing 100084, China

Supporting Information

ABSTRACT: A complex between an anionic perylene diimide derivative (PDI-GlyAsp) and cupric ion has been prepared and applied to be turn-on fluorescent probe for the detection of pyrophosphate (PPi) in 100% aqueous solution. The complex formation process and PPi detection have been studied by absorption and emission spectroscopy. It was confirmed that the introduction of cupric ion into PDI-GlyAsp solution resulted in the assembly of PDI-GlyAsp into PDI-GlyAsp/Cu^{2+} aggregates, leading to the fluorescence quenching of PDI-GlyAsp. Upon addition of PPi into the above solution led to the disassembly of the aggregates due to the competitive binding of PPi with Cu^{2+} in the PDI-GlyAsp/Cu^{2+} complex, and a recovery of PDI-GlyAsp emission was observed. Therefore, the PDI-GlyAsp/Cu^{2+} complex can be applied as a turn-on fluorescent probe for detecting PPi with high selectivity and sensitivity.



KEYWORDS: *pyrophosphate, perylene diimide, fluorescence, biosensor, aggregation*

INTRODUCTION

Colorimetric and fluorescent sensing biologically important anions has attracted considerable interest in recent years.[1−3] Among them, pyrophosphate (PPi) is of particular interest because it plays pivotal roles in many biological processes such as cellular metabolism,[4] DNA polymerization, and some enzymatic reactions.[5,6] It is also known that abnormal PPi levels are related to some severe medical conditions.[7−10] Thus, it is important to easily monitor the concentrations of PPi in aqueous solutions.[11−13] Although several optical strategies have been developed to detect PPi, such as synthesized receptors based on multiple hydrogen bonding,[14−17] metal−ligand interactions,[18−21] indicator displacement approach,[22,23] and competitive binding with metal ion,[24] it remains a challenge to find new approaches that could improve the simplicity, selectivity, and sensitivity of PPi detection in 100% aqueous media.

Recently, fluorescent chemosensors based on water-soluble perylene diimide (PDI) derivatives have received increasing attention because of their outstanding photochemical stabilities and high fluorescence quantum yields.[25−29] However, simple and easily synthesized PDI probes for sensing anions remain rare. Herein, we report a novel "turn-on" fluorescent assay for PPi, which is based on the competitive binding of PPi and a water-soluble PDI-GlyAsp conjugate with cupric ion. To the best of our knowledge, this is the first example of a PDI derivative for sensing and discriminating PPi with other inorganic anions in 100% aqueous solution. The design rationale for turn-on fluorescent PPi detection is illustrated in Figure 1. PDI-GlyAsp can form a complex with Cu^{2+} ion through metal−ligand interaction, and was quenched efficiently because of the formation of nonemitting aggregates with the forbidden low-energy excitonic transition. Upon the introduction of PPi into the system, the competitive binding with the Cu^{2+} ion promotes the disassembly of PDI-GlyAsp/Cu^{2+} complex. Accordingly, the complex of PDI-GlyAsp and Cu^{2+} formed in aqueous solution would be collapsed, and the disassembly of the complex can be monitored easily by recording the fluorescence recovery of PDI chromophores. Therefore, a turn-on fluorescent method for the detection of PPi can be established.

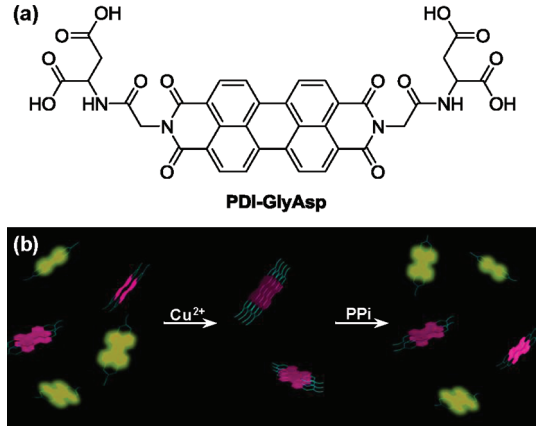


Figure 1. (a) Chemical structures of PDI-GlyAsp. (b) Assembly disassembly process of PDI-GlyAsp in the presence of Cu^{2+} ion and PPi.

Received: April 27, 2011
Accepted: January 5, 2012
Published: January 5, 2012

EXPERIMENTAL SECTION

Materials. 3,4,9,10-Perylenetetracarboxylic acid bisanhydride was a product of Anshan HIFI Chemicals Co., Ltd., China. All other chemicals were purchased from Aldrich and Beijing Chem. Reagents Co. (Beijing, China) and were used as received. Ultrapure water (18.2 MΩ cm at 25 °C) was obtained with a Millipore filtration system.

Measurements. Absorption and emission spectra were collected by using a Hitachi 3010 UV−visible spectrometer and a LS55 fluorescence spectrometer (PerkinElmer), respectively. ^{1}H NMR spectra were carried out on a JNM-ECA300 spectrometer (JEOL). The limit of detection (LOD) of the probe toward PPi was calculated by using the equation LOD = 3σ/m, where σ is the standard deviation of the blank, and m is the slop of the calibration plot. The quantum yield (Φ) measurement for PDI-GlyAsp was performed in DMF solution, and the excitation wavelength was 495 nm and fluorescein was employed as a standard (Φ = 0.925 in 0.1 M NaOH aqueous solution).

Synthesis of PDI-GlyAsp Conjugate. 3,4,9,10-Perylenetetracarboxylic acid bisanhydride (196 mg, 0.5 mmol), glycyl-L-aspartic acid (200 mg, 1.05 mmol), and 2.0 g of imidazole were added into a Schlenk tube and heated at 140 °C for 8 h with stirring under N_2 atmosphere. The reaction mixture was allowed to cool to 90 °C, and then poured into water and filtered. Then, the filtrate was acidified with 2.0 M HCl, and the precipitate was filtered and washed with water and dried under vacuum at 80 °C to give the product of PDI-GlyAsp (350 mg, 95%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 8.65 (s, 2H), 8.53 (d, 2H), 8.25 (d, 2H), 4.68 (s, 4H), 4.55 (t, 2H), 2.70, (m, 2H), 2.58 (m, 2H); MOLDI-TOF MS (m/z): 736.0917 [M + H$^+$]; the quantum yield in DMF was measured to be 0.68 refer to fluorescein in 0.1 M NaOH aqueous solution (Φ = 0.925).

RESULTS AND DISCUSSION

Synthesis and Solution Properties of PDI-GlyAsp. PDI-GlyAsp was synthesized in high yield and purity by one-step reaction between perylenetetracarboxylic bisanhydride and glycyl-L-aspartic acid in molten imidazole following a simple purification procedure. As expected, PDI-GlyAsp exhibits good solubility in alkali aqueous solution due to the electrostatic repulsion between its multiple negative charges. To thoroughly examine its solution properties, the absorption spectra of PDI-GlyAsp in HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid) buffer (10 mM, pH 7.4) over a wide concentration range of 0.1 to 20 μM were recorded (Figure 2a). It can be seen that at a low concentration (0.1 μM), the spectrum shows three absorption bands (532, 495, and 466 nm) and a weak broad shoulder around 415 nm, being characteristic of the $S_0 \rightarrow S_1$ transition with well-resolved vibronic structures corresponding to $\nu = 0 \rightarrow \nu' = 0$, 1, 2, and 3 transitions, respectively.[30] Moreover, the ratio of 0−0 to 0−1 transition in absorption intensity was calculated to be around 1.57, being close to the normal Frank-Condom progressions ($A_{0-0}/A_{0-1} \approx 1.6$) for the free PDI molecules.[31] These results indicated that PDI-GlyAsp is in the monomeric form in HPES buffer at a low concentration. With increasing the concentration, the absorption peaks are red-shifted and the transitions from the ground state to the higher levels of electronic states (0−1, 0−2, and 0−3) are enhanced gradually with respect to the 0−0 transition. Finally, the vibronic peaks exhibit an inversion in intensity with movement to high concentrations, in which the most intense absorption maximum was the 0−1 transition at 499 nm, and the lowest energy vibronic band (0−0) was red-shifted to 535 nm. Moreover, there exist isosbestic points at 548 and 505 nm. These observations indicate that there are two species in solution, and most likely PDI-GlyAsp exists in equilibrium between free monomer and dimer in the concentrations examined here.[32−34]

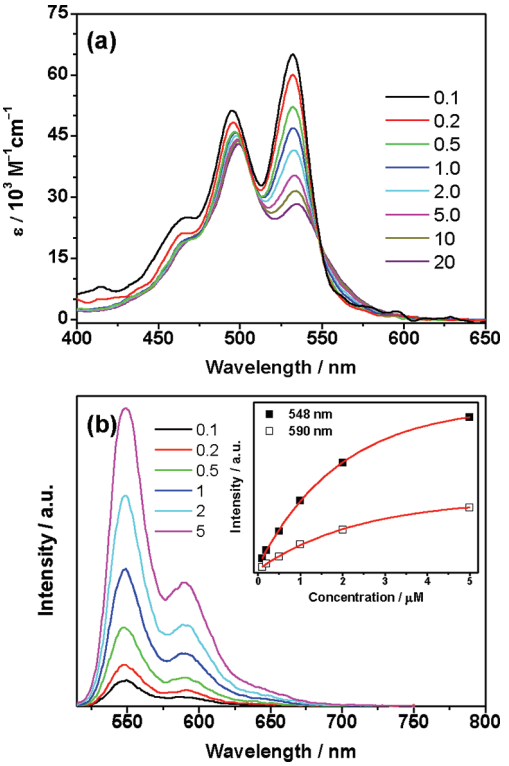


Figure 2. (a) Absorption and (b) emission spectra of PDI-GlyAsp in HEPES buffer (10 mM, pH 7.4) with the concentrations as indicated. Excitation wavelength: 495 nm. Inset: plots of emission intensity versus the concentration of PDI-GlyAsp.

Intermolecular $\pi-\pi$ stacking plays a decisive role in controlling the emissive properties of fluorophore, thus, concentration-dependent emission spectra were also recorded to monitor the solution behavior of PDI-GlyAsp in HEPES buffer (Figure 2b). It can be seen that all emission spectra depict two emission maxima around 548 and 590 nm, indicating the presence of emitting monomeric form of PDI-GlyAsp in solution. Moreover, a nonlinear increase of the emission intensities at 548 and 590 nm with increasing concentration was observed (inset of Figure 2b). Based on these spectral results and considering the fact that the emission at 590 nm has no distinct overlap with the absorptions, one can conclude that no emitted photons were reabsorbed by ground-state PDI-GlyAsp molecules. The self-quenching at higher concentrations can be attributed to the intermolecular interaction of dimers.[32]

Cu^{2+}-Induced Aggregation of PDI-GlyAsp. It is known that multiple L-aspartic acid pendants in one fluorophore facilitate the binding with metal cations.[35] Therefore, PDI-GlyAsp with a modest concentration of 5.0 μM was chosen for examining its sensing ability, in which PDI-GlyAsp exists in the dimeric form mainly. The dimers are thought to be a kind of supramolecular oligomer,[33] doubling the apparent molecular weight and the L-aspartic acid pendants, and to be beneficial for the binding with metal cations. Figure 3 compares absorption and emission spectra of PDI-GlyAsp (5.0 μM) and its mixtures with different amounts of Cu^{2+} ions in HEPES buffer. It can be seen from Figure 3a that upon addition of increasing amounts of Cu^{2+} ions, the absorption bands of PDI-GlyAsp are

dx.doi.org/10.1021/am201616r | ACS Appl. Mater. Interfaces 2012, 4, 614−618

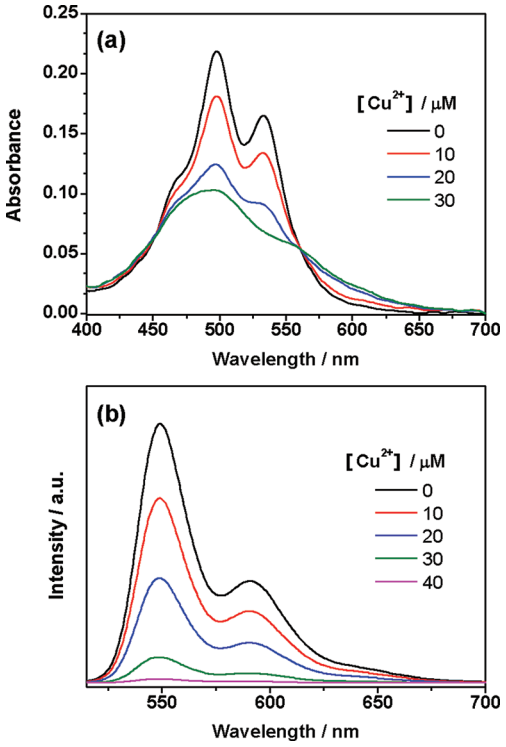


Figure 3. (a) Absorption and (b) emission spectra of PDI-GlyAsp (5.0 μM) upon the stepwise introduction of different amounts of Cu^{2+} ions as indicated in HEPES buffer (10 mM, pH 7.4). Excitation wavelength: 495 nm.

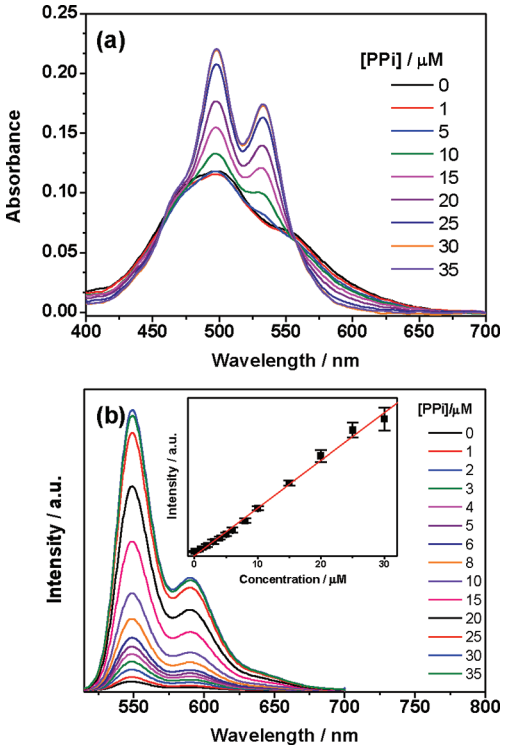


Figure 4. (a) Absorption and (b) emission spectra of PDI-GlyAsp/Cu^{2+} upon titration with PPi (0–35 μM) in HEPES buffer (10 mM, pH 7.40). Inset: emission intensity at 548 nm upon titration with PPi. Excitation wavelength: 495 nm.

weakened gradually and a new broad shoulder attributed to the extended oligomers are developed at longer wavelength (around 590 nm). These spectral results reflect that the addition of Cu^{2+} ions intensifies the π–π stacking among perylene moieties and facilitates the formation of PDI-GlyAsp aggregates.[29,34] Simultaneously, the emission from the perylene chromophores decreases gradually (Figure 3b) due to the formation of larger nonemitting aggregates with the forbidden low-energy excitonic transition, and the observed emission is characteristic of PDI chromophores in the monomeric from.[32,33] These observations indicate that Cu^{2+} ion can induce the aggregation of PDI-GlyAsp effectively through the synergistic effect of Cu^{2+} coordination with aspartic acid moieties and π–π stacking between PDI aromatic planes.

Turn-On Fluorescent Detection of PPi. Given the fact that PPi has strong affinity toward Cu^{2+} with respect to carboxylate,[24] one can expect that the introduction of PPi into PDI-GlyAsp/Cu^{2+} solution would competitively bind with Cu^{2+}, disrupting the PDI-GlyAsp aggregates. As a result, PDI-GlyAsp monomer molecules are released, leading to the recovery of PDI fluorescence, and a turn-on fluorescent signal is detected. To ascertain the sensing ability of PDI-GlyAsp/Cu^{2+} toward PPi, we chose HEPES buffer containing 5.0 μM PDI-GlyAsp and 30 μM Cu^{2+} as the probe for a variety of mono- and divalent anions.[36] In this case, the intensity of PDI-GlyAsp fluorescence was quenched by more than 97% at 548 nm with respect to that of a solution containing PDI-GlyAsp only. Figure 4 illustrates the absorption and emission spectra of PDI-GlyAsp/Cu^{2+} upon titration with PPi in HEPES buffer. It is clearly seen that upon addition of increasing amounts of PPi, the absorption bands associated with the PDI-GlyAsp aggregates (590 nm) decrease gradually in intensity, and the ones related to 0–0 and 0–1 transitions are developed. Simultaneously, the emission intensity becomes stronger. As equimolar amount of PPi (relative to Cu^{2+}) was added to the solution, 30-times enhancement of the fluorescence intensity (approximately 100% of the initial intensity of PDI-GlyAsp) was observed, and both absorption and emission spectra recovered the features of PDI-GlyAsp in HEPES buffer. Addition of extra PPi scarcely induced any further changes in absorption and emission spectra. These results indicate that the competitive binding of PPi with Cu^{2+} resulted in the dissociation of PDI-GlyAsp/Cu^{2+} complex, which is responsible for the reappearance of the fluorescence due to the equilibrium moving to the free PDI-GlyAsp monomeric form. Notably, there exists a good linear relationship between fluorescence intensity and the concentration of PPi (R = 0.997 from 0.1 to 30 μM), indicating that this approach is applicable for quantitative detection of PPi, and the limit of detection was calculated to be 2.0×10^{-7} M.

Selectivity of the Probe for PPi. To evaluate the specificity of PDI-GlyAsp/Cu^{2+} toward PPi, we carried out fluorescence titration with other anions including F^-, Cl^-, Br^-, I^-, $CH_3CO_2^-$, HPO_4^{2-}, NO_3^-, NO_2^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-}, and HSO_3^- under identical conditions. It is clearly seen that the most striking effects are observed for PPi, confirming the probe is selectively response to PPi (Figure 5a). Furthermore, the fluorescence response of PDI-GlyAsp/Cu^{2+} toward PPi was examined in the presence of excess amounts of these anions. As shown in Figure 5b, slight fluorescence recovery can be detected even in the presence of 20 equiv. of these anions, whereas as 10 μM PPi was introduced into these solutions, a dramatic fluorescence recovery was observed. These results indicate that no remarkable interference to the PPi detection

dx.doi.org/10.1021/am201616r | ACS Appl. Mater. Interfaces 2012, 4, 614–618

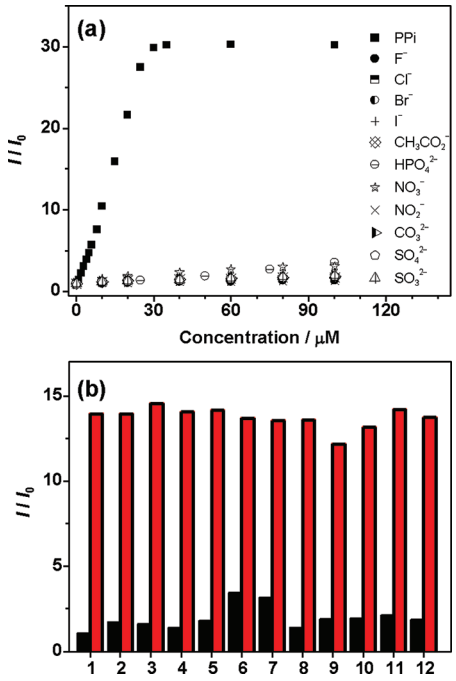


Figure 5. (a) Dependence of the emission intensity enhancement (I/I_0) of PDI-GlyAsp/Cu^{2+} at 548 nm on the different anions at various concentrations in HEPES buffer (10 mM, pH 7.4). (b) Fluorescence response of PDI-GlyAsp/Cu^{2+} upon addition of various anions in HEPES buffer (10 mM, pH 7.4). Black bars: upon addition of 20 equiv. of anions; Red bars: the integrated fluorescence response upon subsequent addition of 2 equiv. of PPi to the solution containing the probe and 20 equiv. of anions (relative to PDI-GlyAsp). Excitation wavelength: 495 nm. 1, F^-; 2, Cl^-; 3, Br^-; 4, I^-; 5, $CH_3CO_2^-$; 6, HPO_4^{2-}; 7, NO_3^-; 8, NO_2^-; 9, CO_3^{2-}; 10, SO_4^{2-}; 11, SO_3^{2-}; 12, HSO_3^-.

was found in the presence of these chemical species, and PDI-GlyAsp/Cu^{2+} is really a highly selective chemosensor material for PPi in aqueous solution. It is noted here that this approach exhibits satisfactory selectivity for PPi with respect to phosphate, a main interferent and usually coexisting under many circumstances.[13] However, the probe also has a positive response toward ATP and ADP (see Figure S1 in the Supporting Information). Therefore, interference by these species must be considered in developing assays based on the present sensor.

Fluorescent Assay for Alkaline Phosphatase (ALP) Activity. To demonstrate the potential of the PDI-based PPi sensor in a practical application, a fluorescent assay based on the PDI-GlyAsp/Cu^{2+} complex was constructed to monitor the activity of ALP. In a typical experimental condition, a solution containing 5 μM PDI-GlyAsp, 30 μM Cu^{2+}, and 10 μM PPi in 10 mM HEPES buffer (pH 7.40) was prepared and incubated for 10 min at 25 °C. ALP with the given concentration was then added into the mixture, and the time course of the emission intensity of PDI-GlyAsp at 548 nm was monitored immediately (λ_{ex} = 495 nm). It can be seen from Figure 6 that the emission intensity at 548 nm originated from the monomeric PDI-GlyAsp was gradually decreased with the incubating time from 0 to 15 min. The control experiment demonstrates that ALP has little effect on the emission property. These observations indicate that the emission intensity changes were due to the collapse of PPi/Cu^{2+} metal–ligand complex by the ALP-catalyzed hydrolysis of PPi, and the formation of nonemitting

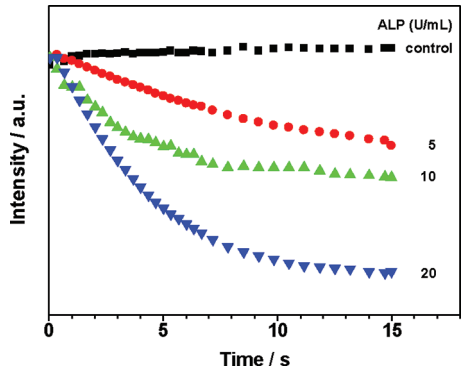


Figure 6. Emission intensity of PDI-GlyAsp (5 μM) at 548 nm vs incubating time in the hydrolysis of PPi (10 μM) with various concentrations of ALP in 10 mM HEPES buffer (pH 7.40). Excitation wavelength: 495 nm.

Cu^{2+}-mediated aggregates of PDI-GlyAsp with the forbidden low-energy excitonic transition. Moreover, the emission intensity decrease accelerates as the ALP concentration increased. This preliminary study indicates that the PDI-GlyAsp/Cu^{2+} probe provides an effective, real-time fluorescent assay for ALP activity.

CONCLUSIONS

We have designed and synthesized a water-soluble PDI derivative, PDI-GlyAsp, through a simple one-step reaction in high yield, and developed a simple turn-on fluorescent sensor for PPi in 100% aqueous solution based on Cu^{2+}-mediated PDI-GlyAsp aggregates. In aqueous solution, PDI-GlyAsp exhibits strong monomer fluorescence, which was effectively quenched by the addition of Cu^{2+} ions due to the formation of PDI-GlyAsp/Cu^{2+} aggregates. Upon addition of PPi, fluorescence recovery was observed due to the competitive binding of PPi with Cu^{2+} and the dissociation of the PDI-GlyAsp/Cu^{2+} aggregates. We expect that the present findings will not only provide an important clue to design fluorescent sensors for PPi, but also open a door to develop PDI-based sensors for anions.

ASSOCIATED CONTENT

Supporting Information

Emission intensity response of PDI-GlyAsp/Cu^{2+} and BPTA-PDI at 548 nm upon titration with ATP, ADP, AMP, and PPi in HEPES buffer (10 mM, pH 7.40). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: chunli@mail.tsinghua.edu.cn. Tel: +86-10-6279-8909. Fax: +86-10-6277-1149.

ACKNOWLEDGMENTS

The authors gratefully thank the National Natural Science Foundation of China (50973054, 91027028, 50873902) for financial support of this work.

REFERENCES

(1) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486.
(2) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419.
(3) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094.

(4) Farre, E. M.; Geigenberger, P.; Willmitzer, L.; Trethewey, R. N. *Plant Physiol.* **2000**, *123*, 681.
(5) Ronaghi, M.; Haramohamed, S.; Pettersson, B.; Uhlen, M.; Nyren, P. *Anal. Biochem.* **1996**, *242*, 84.
(6) Steinberg, K. M.; Okbu, D. T.; Zwick, M. E. *Anal. Chem.* **2008**, *80*, 520.
(7) Doherty, M.; Belcher, C.; Regan, M.; Jones, A.; Ledingham, J. *Ann. Rheum. Dis.* **1996**, *55*, 432.
(8) Rutsch, F.; Schauerte, P.; Kalhoff, H.; Petrarulo, M.; August, C.; Diekmann, L. *Acta Paediatr.* **2000**, *89*, 1265.
(9) Rutherford, R. B.; Foster, B. L.; Bammler, T.; Beyer, R. P.; Sato, S.; Somerman, M. J. *J. Dent. Res.* **2006**, *85*, 505.
(10) Richette, P.; Bardin, T.; Doherty, M. *Rheumatology* **2009**, *48*, 711.
(11) O'Neil, E. J.; Smith, B. D. *Coord. Chem. Rev.* **2006**, *250*, 3068.
(12) Zapata, F.; Caballero, A.; Espinosa, A.; Tárraga, A.; Molina, P. *J. Org. Chem.* **2008**, *73*, 4034.
(13) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23.
(14) Vance, D. V.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397.
(15) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463.
(16) Aldakov, D.; Anzenbacher, P. *J. Am. Chem. Soc.* **2004**, *126*, 4752.
(17) Zhao, X.; Schanze, K. S. *Chem. Commun.* **2010**, *46*, 6075.
(18) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, T. *J. Am. Chem. Soc.* **2002**, *124*, 3920.
(19) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777.
(20) Huang, X.; Guo, Z.; Zhu, W.; Xie, Y.; Tian, H. *Chem. Commun.* **2008**, 5143.
(21) Kim, M. J.; Swamy, K. M. K. K.; Lee, M.; Jagdale, A. R.; Kim, Y.; Kim, S.-J.; Yoo, K. H.; Yoon, J. *Chem. Commun.* **2009**, 7215.
(22) Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 3811.
(23) McDonough, M. J.; Reynolds, A. J.; Lee, W. Y. G.; Jolliffe, K. A. *Chem. Commun.* **2006**, 2971.
(24) Zhao, X.; Liu, Y.; Schanze, K. S. *Chem. Commun.* **2007**, 2914.
(25) Che, Y.; Yang, X.; Zang, L. *Chem. Commun.* **2008**, 1413.
(26) Peneva, K.; Mihov, G.; Herrmann, A.; Zarrabi, N.; Börsch, M.; Duncan, T. M.; Müllen, K. *J. Am. Chem. Soc.* **2008**, *130*, 5398.
(27) Chen, X.; Jou, M. J.; Yoon, J. *Org. Lett.* **2009**, *11*, 2181.
(28) Szekle, H.; Schübel, S.; Harenberg, J.; Krämer, R. *Chem. Commun.* **2010**, *46*, 1667.
(29) Wang, B.; Yu, C. *Angew. Chem., Int. Ed.* **2010**, *49*, 1485.
(30) Würthner, F.; Chen, Z.; Dehm, V.; Stepanenko, V. *Chem. Commun.* **2006**, 1188.
(31) Li, A. D. Q.; Wang, W.; Wang, L.-Q. *Chem. —Eur. J* **2003**, *9*, 4594.
(32) Ma, T.; Li, C.; Shi, G. *Langmuir* **2008**, *24*, 43.
(33) Zhao, L.; Ma, T.; Bai, H.; Lu, G.; Li, C.; Shi, G. *Langmuir* **2008**, *24*, 4380.
(34) Rehm, S.; Stepanenko, V.; Zhang, X.; Rehm, T. H.; Würthner, F. *Chem. —Eur. J.* **2010**, *16*, 3372.
(35) Qin, C.; Wu, X.; Gao, B.; Tong, H.; Wang, L. *Macromolecules* **2009**, *42*, 5427.
(36) The PDI-GlyAsp/Cu^{2+} complexes in HEPES buffer were prepared and aged for 4 h before the anion titration experiments, and no precipitate was observed throughout the experiment.