Nanomolar Pyrophosphate Detection in Water and in a Self-Assembled Hydrogel of a Simple Terpyridine-Zn2+ Complex

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · FEBRUARY 2014

Impact Factor: 12.11 · DOI: 10.1021/ja4128949 · Source: PubMed

4 AUTHORS:

Sandip Bhowmik
University of Jyväskylä
13 PUBLICATIONS **153** CITATIONS
SEE PROFILE

Biswa Nath Ghosh
University of Jyväskylä
14 PUBLICATIONS **95** CITATIONS
SEE PROFILE

Varpu Marjomäki
University of Jyväskylä
88 PUBLICATIONS **2,030** CITATIONS
SEE PROFILE

Kari Rissanen
University of Jyväskylä
668 PUBLICATIONS **10,793** CITATIONS
SEE PROFILE

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Nanomolar Pyrophosphate Detection in Water and in a Self-Assembled Hydrogel of a Simple Terpyridine-Zn^{2+} Complex

Sandip Bhowmik,[†,‡] Biswa Nath Ghosh,[†,‡] Varpu Marjomäki,[§] and Kari Rissanen*[,†]

[†]Department of Chemistry, Nanoscience Center, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

[§]Department of Biological and Environmental Science, Nanoscience Center, University of Jyväskylä, Survontie 9, 40500 Jyväskylä, Finland

Supporting Information

ABSTRACT: A simple terpyridine-Zn(II) complex is shown to act as an efficient and highly selective fluorescent sensor for pyrophosphate in water at physiological pH. The sensor complex showed an unprecedented fluorescence response (~500 fold increase) and a record nanomolar sensitivity (detectable fluorescent response at 20 nM and LOD ~ 0.8 nM). It has successfully been used to stain and record confocal fluorescence microscopy images of HeLa cells. Moreover, the complex was found to self-assemble into a hydrogel which was subsequently used to coat disposable paper strips for easy, low-cost detection of pyrophosphate.

In the past couple of decades, considerable effort had been invested in developing receptor probes to sense biologically significant ions at physiologically relevant concentrations.[1−4] Anions such as pyrophosphate (PPi) and ATP play a key role in energy transduction and several major metabolic processes.[5−7] The PPi concentrations can provide critical information on important processes such as DNA replication and hence can be used in cancer diagnosis by monitoring telomerase elongation process.[8] Detection and quantification of PPi can also help identifying diseases such as chondrocalcinosis or calcium pyrophosphate dihydrate (CPPD) crystal deposition disease.[9,10] Considering its importance, several techniques have been developed for the detection of PPi and other related NPPs (nucleotide polyphosphates).[11−14] Among these the fluorescence-based detection systems being the most popular mainly due to its high sensitivity, fast response, and the possibility of *in vivo* and *in vitro* imaging.[15−18] Although several fluorescent chemosensors have been developed for this purpose, certain key issues still remain to be addressed. Due to strong hydration of anions and low solubility of organic fluorescent probes, a majority of the studies are limited to organic or mixed aqueous media.[19−24] Although a few recent studies have addressed this issue, the sensitivity in water (or in organic or mixed media) was invariably limited to micromolar levels.[25−29] This meant that the rapidly growing fields, such as cancer diagnosis based on PPi detection, had to be solely dependent on fluorescent protein-based techniques, as the existing chemosensors lack the required sensitivity.[30−33] Hence, there is a growing demand for highly selective and sensitive probes for PPi detection for diagnostics and medical applications.

Herein, we report nanomolar level detection of PPi in water at physiological pH utilizing a simple fluorescent terpyridine-Zn^{2+} receptor. The receptor exhibited excellent PPi binding selectivity over other anions and structurally analogues NPPs.

The ligand 4′-(4-*N*,*N*′-dimethylaminophenyl)-2,2′:6′,2″-terpyridine **L** was synthesized according to the literature method.[34] Stirring of the dichloromethane solution of **L** with $ZnCl_2$ in methanol in 1:1 mixture resulted in the formation of metal complex **$ZnCl_2$L**. The crystal structure confirmed the formation of the 1:1 metal complex in which Zn^{2+} adopts a distorted trigonal bipyramidal N_3Cl_2 coordination (Figure 1).

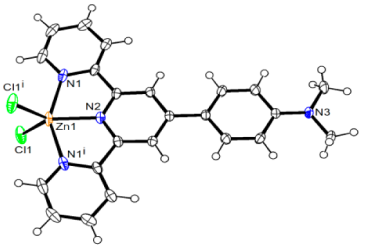

Figure 1. ORTEP plot of the molecular structure of **$ZnCl_2$L**. Thermal ellipsoids are shown at 50% probability level. Selected bond distances (Å) and angles (°): Zn1−N1 2.200(2), Zn1−N2 2.089(3), Zn1−$N1^i$ 2.200(2), Zn1−Cl1 2.2604(6), Zn1−$Cl1^i$ 2.2604(6), Cl1−Zn1−$Cl1^i$ 110.15(4), N1−Zn1−N2 74.62(5), N2−Zn1−$N1^i$ 74.62(5). Symmetry element: $i = -x + 2, y, -z + 1/2$.

The changes in the UV−Vis spectra of **$ZnCl_2$L** were monitored in the presence of different anions (as sodium salts) in water buffered with 0.01 M HEPES, pH 7.4, at 298 K. The buffered complex solution was yellow in color and its UV−Vis spectrum exhibited three major bands at 285, 320, and 410 nm. No significant changes in the spectra were observed for anions such as F^-, Br^-, I^-, SO_4^{2-}, AcO^-, NO_3^-, CO_3^{2-}, HPO_4^{2-}, and PO_4^{3-}.

However, the addition of PPi and other nucleotide triphosphates resulted in a sharp bathochromic shift (~ 30 nm) and a decrease in the peak intensity of 410 nm band (Figure 2a). The red shift was visibly evident in the slight change in color of the resulting solution and clearly indicated PPi-to-receptor binding. More drastic optical changes were observed in the fluorescence spectra of **$ZnCl_2$L**. Although **$ZnCl_2$L** was found to

Received: December 26, 2013

   dx.doi.org/10.1021/ja4128949 | *J. Am. Chem. Soc.* XXXX, XXX, XXX−XXX

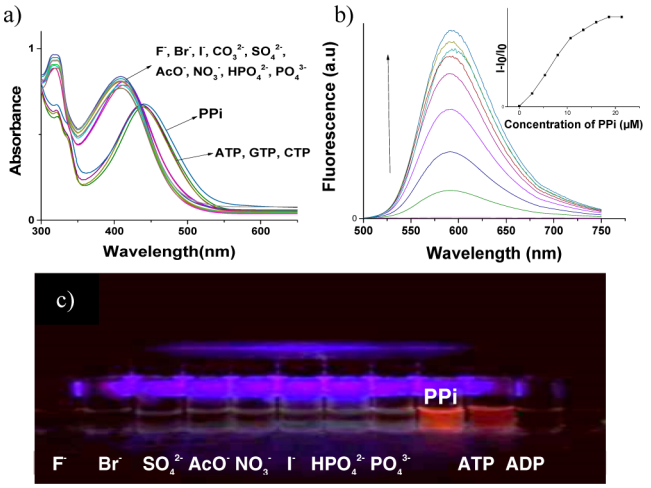

Figure 2. (a) UV−Vis spectra of $\mathbf{ZnCl_2L}$ (50 μM) in 10 mM HEPES buffer (pH 7.4) with different anions (50 μM). (b) Emission spectra of $\mathbf{ZnCl_2L}$ (50 μM) in 10 mM HEPES buffer (pH 7.4) upon addition of PPi (2.6−22 μM) (inset: change in emission @ 591 nm; λ ext @ 440 nm). (c) Emission of $\mathbf{ZnCl_2L}$ (50 μM) under 365 nm UV lamp with different anions (15 μM).

be fluorescent in the solid state, its fluorescence was effectively quenched in water.

An unprecedented ~500-fold enhancement in fluorescence intensity of $\mathbf{ZnCl_2L}$ was observed at 591 nm upon addition of 50 μM of PPi (Figure 2b,c). The titration plot depicting the change in fluorescence intensities ($I/I_0 - 1$) as a function of PPi concentrations showed the typical saturation characteristics. However, it revealed an unusual binding stoichiometry of 1:3 (PPi:$\mathbf{ZnCl_2L}$) which was confirmed unambiguously by a Job plot analysis conducted between PPi and $\mathbf{ZnCl_2L}$ (see SI). The excellent fluorescence response and the binding stoichiometry prompted us to investigate the lower detection limit of PPi in water. We were pleasantly surprised to find that probe was able to detect PPi at nanomolar levels (~20 nM; lowest limit of detection (LOD) was calculated to be ~0.8 nM) (Figure 3a).

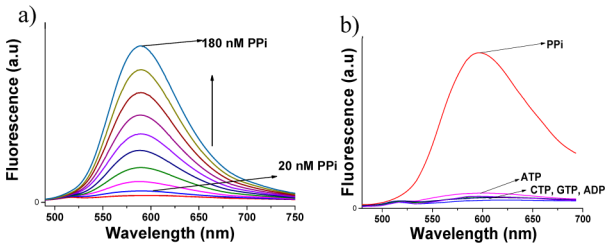

Figure 3. (a) Emission spectra of $\mathbf{ZnCl_2L}$ (50 μM) in 10 mM HEPES buffer (pH 7.4) upon addition of PPi (20−180 nM) (λ_{ex} = 440 nm). (b) Emission spectra of $\mathbf{ZnCl_2L}$ (50 μM) in 10 mM HEPES buffer (pH 7.4) upon addition of 200 nM PPi, ATP, ADP, CTP, and GTP) (λ_{ex} = 440 nm).

Only negligible changes in the fluorescence intensities of $\mathbf{ZnCl_2L}$ in presence of other nucleotide phosphates were observed compared to PPi (Figure 3b). The stronger binding affinity toward PPi can probably be attributed to higher negative charge density. On the other hand, almost no change in the fluorescence was induced by other anions such as F^-, Br^-, I^-, SO_4^{2-}, AcO^-, NO_3^-, CO_3^{2-}, HPO_4^{2-}, and PO_4^{3-} even when present at much higher concentrations (10 μM) compared to PPi (60 nM) (Figure 4a). Competitive binding studies were also carried out in the presence of these anions. As shown in Figure

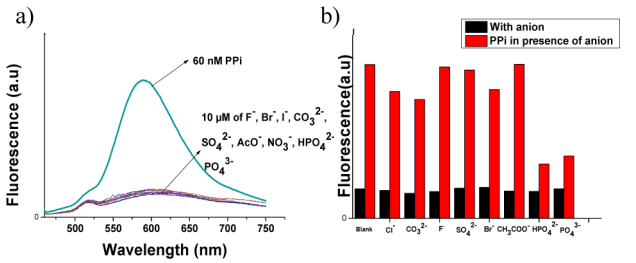

Figure 4. (a) Emission of $\mathbf{ZnCl_2L}$ (50 μM) in 10 mM HEPES buffer (pH 7.4) in presence of different anions. (b) Competitive binding affinity of $\mathbf{ZnCl_2L}$ (50 μM) toward PPi (50 nM, 0.001 equiv) in the presence of 1 equiv of different anions in water (10 mM HEPES buffer, pH 7.4).

4b, the fluorescence intensities of $\mathbf{ZnCl_2L}$ did not change upon addition of 1 equiv (50 μM) of any of these anions. However addition of only 0.001 equiv (50 nM) of PPi resulted in a remarkable enhancement in the fluorescence intensity further asserting the excellent selectivity and sensitivity of the PPi binding receptor. However it should be noted that a substantial decrease in the fluorescence response from PPi was observed in the presence of HPO_4^{2-} and PO_4^{3-} anions. Yet, considering the competitive inhibition from a large excess of other phosphate anions, the quenching effect is quite expected.

We further investigated the applicability of $\mathbf{ZnCl_2L}$ in cell imaging. HeLa cells were grown with DMEM (containing 10% fetal bovine serum and antibiotics) for 16 h and used at subconfluency. The cells were first washed with 10 mM HEPES buffer, pH 7.4, containing 137 mM NaCl, then treated with 10 μM $\mathbf{ZnCl_2L}$ for 30 min in the same buffer at 37 °C, and washed extensively with buffer before imaging. Images were recorded after laser excitation at 488 nm and using emission wavelengths between 555 and 655 nm for orange-red fluorescence. As depicted in Figure 5, $\mathbf{ZnCl_2L}$ was successfully employed in the

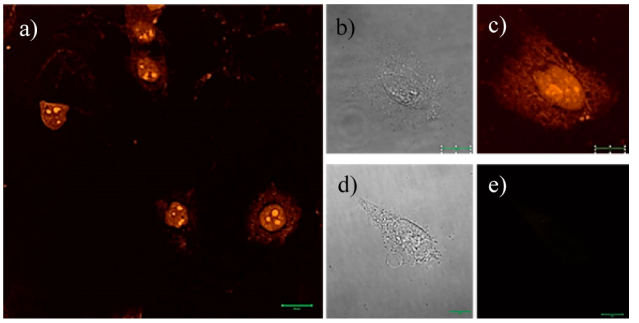

Figure 5. Confocal fluorescence microscopy images of (a) HeLa cells incubated with probe $\mathbf{ZnCl_2L}$ (50 μM), (c) single HeLa cell, and (e) single HeLa cell without staining (control). Differential interference contrast images of (b) single HeLa cell incubated with probe $\mathbf{ZnCl_2L}$ (50 μM) and (d) single HeLa cell without staining (control).

PPi staining of HeLa cells which exhibited a bright orange-yellow emission. Due to the excellent sensitivity of the receptor almost the entire cell could be mapped, even regions with minimal PPi concentrations. The maximum emission intensities were recorded at the nuclei as well as emissions were also observed from the membranous cytoplasmic structures.

dx.doi.org/10.1021/ja4128949 | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

As a promising device development, ligand **L** was found to form a self-assembled hydrogel (Figure 6c) in the presence of

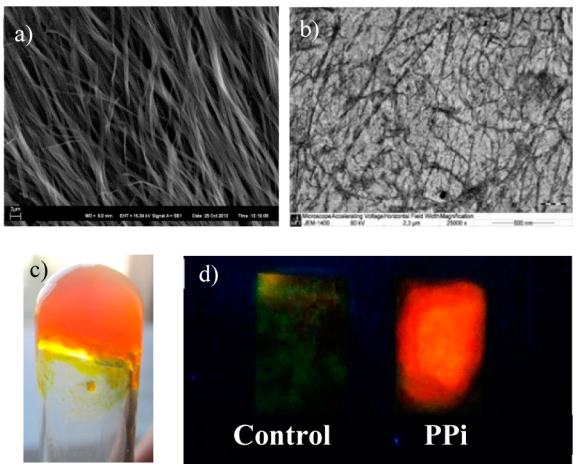

Figure 6. (a) SEM and (b) TEM images of hydrogels of **L** (6 mM) in presence of $\mathbf{ZnCl_2}$ (6 mM) in 0.1N HCl. (c) Photograph of the hydrogel. (d) Gel-coated paper strips under 365 nm UV lamp.

Zn(II) in slightly acidic media (SI for details on gelation experiment). The observations were in good agreement with our recent findings with analogues terpyridines.[35] A detailed investigation of the morphology through scanning electron and transmission electron microscopy (SEM and TEM, respectively) revealed the gel to have a typical fibrous structure with fiber dimensions varying from 50 to 120 nm (Figure 6a,b).

It occurred to us that these hydrogels could be utilized for rapid detection of PPi by the $\mathbf{ZnCl_2L}$ gel coating on a suitable surface. Gel coating is a very well-known technique for the fabrication of sensor devices. Several recent examples of gel-based fluorescent sensors have demonstrated the advantages of using the ordered, nanostructured gel surface for sensing rather than solution.[36−38] As a conclusion of this study, we investigated the applicability of our gel system for PPi sensing, keeping in mind the advantage that our PPi receptor itself is the hydrogelator. This simplified the sensing protocol greatly as the PPi receptor hydrogel could be used directly for coating, thus making the typical procedure of probe-doping into conventional gel matrices (most commonly silica) redundant.[39−41] We prepared disposable paper strips coated with $\mathbf{ZnCl_2L}$ hydrogel (SI) for this purpose. As expected, a bright orange emission from the strip was observed upon drop-casting PPi (1.6 $\mu g/cm^2$) solution onto it (Figure 6d), while the other anions failed to produce any significant emission. We are currently quantifying the results and will optimize the technique for commercial use.

In conclusion, we report a simple terpyridine-Zn(II) complex for efficient and selective sensing of PPi in water. The $\mathbf{ZnCl_2L}$ receptor complex shows remarkable fluorescent response (~500-fold) and an excellent sensitivity toward PPi that allows a subnanomolar level detection (LOD ~ 0.8 nM). The $\mathbf{ZnCl_2L}$ complex is successfully employed to stain HeLa cells for fluorescent imaging. Moreover, $\mathbf{ZnCl_2L}$ was found to form a hydrogel which was subsequently used to make gel coated paper strips for easy, low-cost detection of PPi. We believe these novel findings would be beneficial for the development of commercially viable chemosensor alternatives to enzyme and protein based assays in diagnostics and other clinical applications.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, UV−Vis and fluorescence study, gelation procedure, X-ray single crystal structure analysis (CCDC 977466). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

kari.t.rissanen@jyu.fi

Author Contributions

‡These authors contributed equally

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Mr. Petri Papponen and Mr. Hannu Salo are kindly acknowledged for their help in TEM and SEM measurements, respectively. The Academy of Finland (KR, grant no. 256259, 265328, 263377 and 263256; VM, 257125) is kindly acknowledged for financial support.

■ REFERENCES

(1) Guha, S.; Saha, S. *J. Am. Chem. Soc.* **2010**, *132*, 17674.
(2) Rostami, A.; Wei, C. J.; Guerin, G.; Taylor, M. S. *Angew. Chem., Int. Ed.* **2011**, *50*, 2059.
(3) Zapata, F.; Caballero, A.; White, N. G.; Claridge, T. D.; Costa, P. J.; Felix, V.; Beer, P. D. *J. Am. Chem. Soc.* **2012**, *134*, 11533.
(4) Egawa, T.; Hirabayashi, K.; Koide, Y.; Kobayashi, C.; Takahashi, N.; Mineno, T.; Terai, T.; Ueno, T.; Komatsu, T.; Ikegaya, Y.; Matsuki, N.; Nagano, T.; Hanaoka, K. *Angew. Chem., Int. Ed.* **2013**, *52*, 3874.
(5) Lipscomb, W. N.; Strater, N. *Chem. Rev.* **1996**, *96*, 2375.
(6) Horton, H. R.; Moran, L. A.; Scrimgeour, K. G.; Perry, M. D.; Rawn, J. D. *Principles of Biochemistry*, 4th ed.; Pearson Prentice Hall: Upper Saddle River, NY, 2006.
(7) Ryle, M. J.; Seefeldt, L. C. *J. Biol. Chem.* **2000**, *275*, 6214.
(8) Hirose, M.; Abe-Hashimoto, J.; Ogura, K.; Tahara, H.; Ide, T.; Yoshimura, T. *J. Cancer Res. Clin. Oncol.* **1997**, *123*, 337.
(9) Wright, G. D.; Doherty, M. *Ann. Rheum. Dis.* **1997**, *56*, 586.
(10) Tsui, F. W. L. *Curr. Rheumatol. Rep.* **2012**, *14*, 155.
(11) Anzenbacher, J. P.; Palacios, M. A.; Jursíkova, K.; Marquez, M. *Org. Lett.* **2005**, *7*, 5027.
(12) Quinlan, E.; Matthews, S. E.; Gunnlaugsson, T. *J. Org. Chem.* **2007**, *72*, 7497.
(13) Shin, I.-S.; Bae, S. W.; Kim, H.; Hong, J.-I. *Anal. Chem.* **2010**, *82*, 8259.
(14) Su, X.; Zhang, C.; Xiao, X.; Xu, A.; Xu, Z.; Zhao, M. *Chem. Commun.* **2013**, *49*, 798.
(15) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777.
(16) Anbu, S.; Kamalraj, S.; Jayabaskaran, C.; Mukherjee, P. S. *Inorg. Chem.* **2013**, *52*, 8294.
(17) Li, C.; Numata, M.; Takeuchi, M.; Shinkai, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 6371.
(18) Schäferling, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 3532.
(19) Mikata, Y.; Ugai, A.; Ohnishi, R.; Konno, H. *Inorg. Chem.* **2013**, *52*, 10223.
(20) Zhang, J. F.; Kim, S.; Han, J. H.; Lee, S.-J.; Pradhan, T.; Cao, Q. Y.; Lee, S. J.; Kang, C.; Kim, J. S. *Org. Lett.* **2011**, *13*, 5294.
(21) Sokkalingam, P.; Kim, D. S.; Hwang, H.; Sessler, J. L.; Lee, C.-H. *Chem. Sci.* **2012**, *3*, 1819.
(22) Rao, A. S.; Singha, S.; Choi, W.; Ahn, K. H. *Org. Biomol. Chem.* **2012**, *10*, 8410.
(23) Das, P.; Ghosh, A.; Kesharwani, M. K.; Ramu, V.; Ganguly, B.; Das, A. *Eur. J. Inorg. Chem.* **2011**, 3050.

(24) Caltagirone, C.; Bazzicalupi, C.; Isaia, F.; Light, M. E.; Lippolis, V.; Montis, R.; Murgia, S.; Olivari, M.; Picci, G. *Org. Biomol. Chem.* **2013**, *11*, 2445.
(25) Zhu, W.; Huang, X.; Guo, Z.; Wu, X.; Yu, H.; Tian, H. *Chem. Commun.* **2012**, *48*, 1784.
(26) Xu, Z.; Singh, N. J.; Lim, J.; Pan, J.; Kim, H. N.; Park, S.; Kim, K. S.; Yoon, J. *J. Am. Chem. Soc.* **2009**, *131*, 15528.
(27) Ojida, A.; Takashima, I.; Kohira, T.; Nonaka, H.; Hamachi, I. *J. Am. Chem. Soc.* **2008**, *130*, 12095.
(28) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23.
(29) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M.; Kim, Y.; Kim, S.-J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828.
(30) Ronaghi, M.; Uhlén, M.; Nyrén, P. *Science* **1998**, *281*, 363.
(31) Kim, J.-H.; Ahn, J.-H.; Barone, P. W.; Jin, H.; Zhang, J.; Heller, D. A.; Strano, M. S. *Angew. Chem., Int. Ed.* **2010**, *49*, 1456.
(32) Xu, S.; He, M.; Yu, H.; Cai, X.; Tan, X.; Lu, B.; Shu, B. *Anal. Biochem.* **2001**, *299*, 188.
(33) Karasawa, K.; Sano, Y.; Arakawa, H. *Luminescence* **2014**, *29*, 52.
(34) Wang, J.; Hanan, G. S. *Synlett* **2005**, 1251.
(35) Ghosh, B. N.; Bhowmik, S.; Mal, P.; Rissanen, K. *Chem. Commun.* **2013**, *50*, 734.
(36) Ren, Y.; Kan, W. H.; Thangadurai, V.; Baumgartner, T. *Angew. Chem., Int. Ed.* **2012**, *51*, 3964.
(37) Yildirim, A.; Budunoglu, H.; Deniz, H.; Guler, M. O.; Bayindir, M. *ACS Appl. Mater. Interfaces* **2010**, *2*, 2892.
(38) Kartha, K. K.; Babu, S. S.; Srinivasan, S.; Ajayaghosh, A. *J. Am. Chem. Soc.* **2012**, *134*, 4834.
(39) Dansby-Sparks, R. N.; Jin, J.; Mechery, S. J.; Sampathkumaran, U.; Owen, T. W.; Yu, B. D.; Goswami, K.; Hong, K.; Grant, J.; Xue, Z.-L. *Anal. Chem.* **2010**, *82*, 593.
(40) Wada, A.; Tamaru, S.-I.; Ikeda, M.; Hamachi, I. *J. Am. Chem. Soc.* **2009**, *131*, 5321.
(41) Hiruta, Y.; Yoshizawa, N.; Citterio, D.; Suzuki, K. *Anal. Chem.* **2012**, *84*, 10650.