JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Chemically-Modulated Photoluminescence of Graphene Oxide for Selective Detection of Neurotransmitter by "Turn-On" Response

Su-Ji Jeon, Seon-Yeong Kwak, DaBin Yim, Jong-Min Ju, and Jong-Ho Kim*

Department of Chemical Engineering, Hanyang University, Ansan 426-791, Republic of Korea

Supporting Information

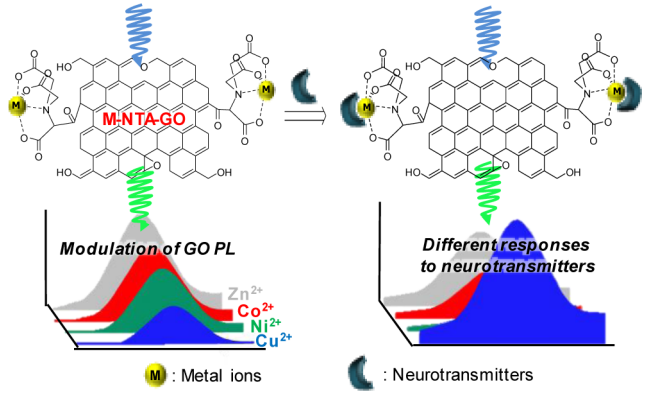
ABSTRACT: Designing artificial nanomaterials capable of selectively detecting targets without the use of expensive and fragile antibodies is of great interest in the applications of nanomedicine. Here, we show that the photoluminescence (PL) of graphene oxide (GO) was chemically modulated for the selective detection of a neurotransmitter without the use of antibodies. GO was functionalized with nitrotriacetic acid (NTA) on which four different metal ions were chelated (M-NTA-GO), which led to its different PL responses to neurotransmitters. In particular, the Cu-NTA-GO hybrid was able to selectively detect norepinephrine at nanomolar concentrations in a simple manner via its "turn-on" PL. Moreover, it was successfully applied to the selective detection of norepinephrine secreted from living PC-12 cells.

Graphene oxide (GO), a two-dimensional nanosheet of oxidized graphene,[1] is a new type of luminescent nanomaterial, which emits intense photoluminescence (PL) in the visible and near-infrared (NIR) ranges of the electromagnetic spectrum.[2] Its PL is tunable from the visible to the NIR window by varying the oxidation density on the basal plane and the edge.[2b,3] In addition, it has been reported that GO PL is much more stable against photodegradation as compared to conventional organic fluorophores.[4] These unique photophysical properties of GO may be helpful to overcome some deficiencies that organic fluorophores have displayed in biosensing and bioimaging. However, GO PL has been rarely used as a signal transduction tool for sensing biomolecules. Only a few bioimaging and biosensing applications based on the PL of GO have been reported.[2d,e] To date, GO has been extensively explored as a universal quencher for organic fluorescent dyes in bioassays.[5] Therefore, developing GO fluorescence-based optical biosensors for selective detection of target biomolecules is of great interest.

In order to impart recognition selectivity to nanomaterial-based biosensors for detection of target molecules, antibodies have been essential components to be conjugated to the nanomaterial surface. However, they are very expensive and susceptible to degradation by physical perturbations.[6] Moreover, immunoassays like enzyme-linked immunosorbent assay (ELISA) for the detection of neurotransmitters involve complicated multistep procedures.[7] Motivated by these deficiencies, artificial recognition phases such as imprinted polymers[8] and polymer corona phase[9] have been developed for the selective detection of target molecules without the use of antibodies. Creating artificial materials capable of selectively recognizing target molecules may be helpful to address some deficiencies of antibody-based assays, but the creation of these artificial materials remains a significant challenge.

Here, we report a novel chemical approach for modulating the PL response of GO to neurotransmitters for selective detection without the use of antibodies. To impart selectivity to GO for detection of a neurotransmitter, GO was functionalized with nitrotriacetic acid (NTA) in which four different metal ions including Zn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+} were coordinated (denoted M-NTA-GO). Chelation of metal ions onto NTA-GO led to different PL responses to several neurotransmitters such as glutamic acid (Glu), adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), dopamine (Dopa), and norepinephrine (NorEpi) that are involved in nervous system chemical processes and biological reactions[10] (Scheme 1). We also explored the fundamental mechanism responsible for the PL responses of M-NTA-GO hybrids to the neurotransmitters. Finally, Cu-NTA-GO was applied to the selective detection of NorEpi secreted from living cells.

Scheme 1. Schematic Diagram for Chemical Modulation of GO PL for the Selective Detection of a Neurotransmitter



First, GO was prepared by a modified Hummers method,[1] and functionalized by 2-chloroacetic acid to introduce more carboxyl groups (CM-GO). Then, *N*,*N*-bis(carboxymethyl)-L-lysine was coupled to the carboxyl groups of CM-GO to introduce NTA groups (NTA-GO) that can chelate the metal ions[11] (Figure S1). The obtained NTA-GO was characterized

Received: April 29, 2014
Published: July 18, 2014

dx.doi.org/10.1021/ja504276z | J. Am. Chem. Soc. 2014, 136, 10842−10845

by atomic force microscopy (AFM), Fourier transform infrared (FT-IR) spectroscopy, and elemental analysis (EA). NTA-GO had a lateral size of 70−200 nm with a height of 0.8 nm (Figure S2). The FT-IR spectra clearly showed the characteristic vibrational modes for an amide (H−N−C═O 1664 cm^{-1}) and a NTA group (C−N 1348 cm^{-1}, carboxyl C═O 1608 cm^{-1}) in NTA-GO. EA also revealed that the number of nitrogen in NTA-GO substantially increased as compared to that of CM-GO (Table S1). These results clearly suggest that NTA groups were covalently attached to GO.

Next, we examined the PL response of NTA-GO to four metal ions (Zn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}). The NTA-GO in PBS (pH 7.4) was treated with each metal ion at a concentration of 10−100 μM, and then its PL was measured under 350 nm excitation. For comparison, the PL response of pristine GO to the metal ions was also measured. As shown in Figure 1a, NTA-

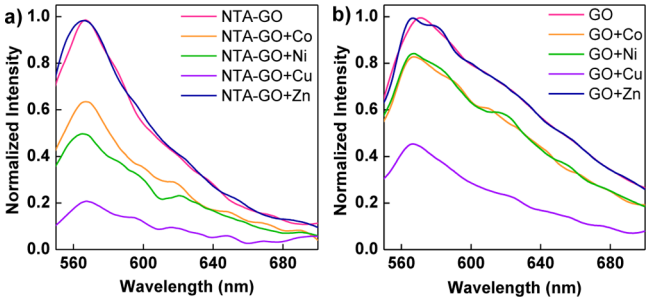


Figure 1. PL response of NTA-GO and pristine GO to four metal ions. (a) PL spectra of NTA-GO and (b) pristine GO in the presence of metal ions.

GO showed intense PL in the visible range of 550−700 nm. However, it was quenched upon addition of the metal ions except Zn^{2+}, while the absorption of NTA-GO remained constant (Figure S3). This result indicates that the PL quenching of NTA-GO occurred via electron transfer from its lowest unoccupied molecular orbital (LUMO) to the metal ions. Cu^{2+} ion was found to quench the PL of NTA-GO most effectively (80%), and Ni^{2+} and Co^{2+} ions caused the quenching of 50 and 35%, respectively, from its original intensity. This quenching effect of the metal ions was illustrated by comparing their reduction potentials.[12] Cu^{2+} has a positive reduction potential (+0.34 eV), indicating that it should be the most effective quencher. Both Ni^{2+} and Co^{2+} ions have similar values of reduction potential (−0.25 and −0.28 eV), and Zn^{2+} ion has the most negative value (−0.76 eV). Therefore, Zn^{2+} should be a poorer quencher than Ni^{2+} and Co^{2+} ions.

As compared to the quenching response of pristine GO to the metal ions (Figure 1b), NTA-GO exhibited much greater quenching responses at the same concentration of metal ions. This enhanced quenching response was ascribed to the higher affinity of an NTA group for the ions, increasing their local concentration at the interface. It was also observed that the PL quenching of NTA-GO and pristine GO linearly increased with an increase in the concentration of the metal ions (Figure S4). This quenching response was consistent with the Stern−Volmer equation[13] in this range of concentrations, which allowed calculation of the quenching rate constants (k_q) of four metal ions for the PL of NTA-GO and pristine GO. As shown in Table 1, the quenching rate constants of the metal ions for the PL of NTA-GO ($k_{NTA\text{-}GO}$) were much larger than those of the PL of pristine GO (k_{GO}). Besides, the PL quenching

Table 1. Quenching Rate Constants of Four Metal Ions for GO and NTA-GO PL

	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
k_{GO} ($M^{-1}\cdot s^{-1}$)	2.69×10^8	1.71×10^8	2.21×10^9	–
k_{NTAGO} ($M^{-1}\cdot s^{-1}$)	1.71×10^9	3.02×10^9	6.06×10^9	–

response of NTA-GO gradually increased with increasing the content of NTA groups on the material (Figure S5). This result indicates that the PL response of GO to the metals can be modulated by chemical functionalization. The complex formation of NTA-GO with the metal ions (M-NTA-GO) was confirmed by energy dispersive X-ray (EDX) and FT-IR spectroscopy. As shown in the EDX spectra (Figure S6), the characteristic peaks for each metal ion were clearly observed in M-NTA-GO hybrids. In addition, FT-IR spectra showed that the vibrational modes for C═O (1718−1731 cm^{-1}), C−N (1357−1360 cm^{-1}), and C−O (1127−1142 cm^{-1}) in the NTA group were shifted toward higher frequencies after chelation of the metal ions (Figure S6e).

Next, we examined the PL response of four M-NTA-GO hybrids (M = Co, Ni, Cu, and Zn) to the neurotransmitters including Dopa, Glu, NorEpi, ADP, and ATP (Figure 2). After addition of Dopa to the M-NTA-GO hybrids in PBS (Figure 2a), the PL of Cu-NTA-GO only increased as a function of Dopa concentration ,while Co-NTA-GO and Ni-NTA-GO

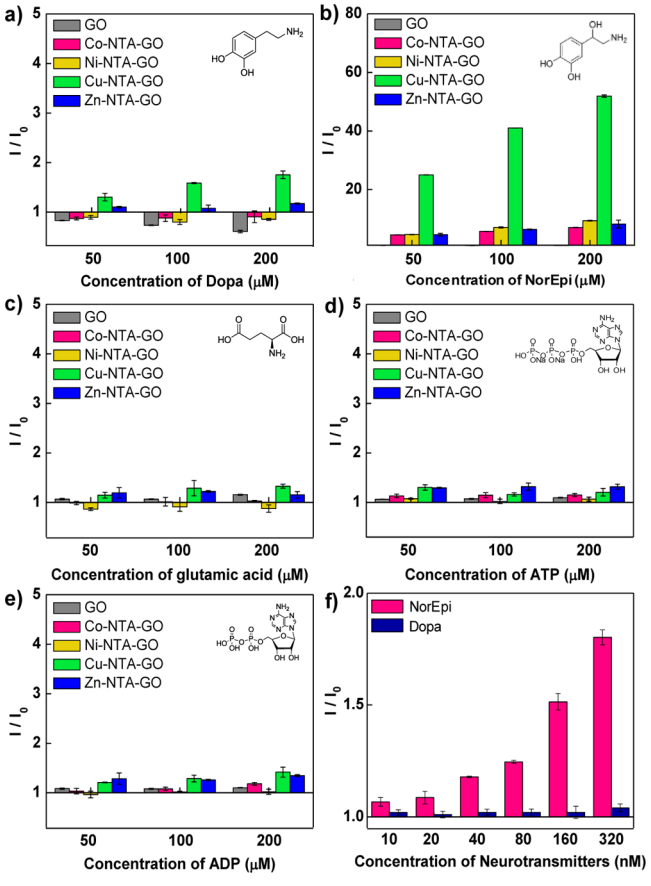


Figure 2. PL response of M-NTA-GO hybrids and pristine GO to (a) Dopa, (b) NorEpi, (c) Glu, (d) ATP, and (e) ADP. (f) PL response of Cu-NTA-GO to NorEpi and Dopa at nanomolar concentrations. I_o and I are the PL intensities before and after addition of neurotransmitters.

dx.doi.org/10.1021/ja504276z | J. Am. Chem. Soc. 2014, 136, 10842−10845

showed a slight quenching response. The PL of pristine GO was also quenched by Dopa.[13a] This result suggests that the PL response of GO to Dopa can be modulated by functionalizing it with the NTA-metal complexes. Next, a different neurotransmitter, NorEpi was added to the M-NTA-GO hybrids. Upon addition of NorEpi to Cu-NTA-GO, its PL dramatically increased to 50 times higher than the original intensity (Figure 2b). This "turn-on" response to NorEpi was 1 order of magnitude greater than that of Dopa, indicating that the PL of Cu-NTA-GO was much more sensitive to NorEpi. In addition, it was found that the PL response of Cu-NTA-GO to NorEpi increased with increasing the content of NTA groups on GO (Figure S7). However, other hybrids including Co-NTA-GO, Ni-NTA-GO, and Zn-NTA-GO showed much smaller PL increases in the presence of NorEpi. The different PL responses to the catecholamine derivatives suggest that the recognition selectivity of the GO can be modulated by metal incorporation. For Glu, ATP and ADP, no noticeable PL response was observed (Figure 2c–e).

We further investigated the sensitivity of Cu-NTA-GO for detection of NorEpi and Dopa at concentrations from 10 to 320 nM. As shown in Figure 2f, Cu-NTA-GO still showed a "turn-on" PL response to NorEpi even at 10 nM. However, it did not respond to Dopa at all nanomolar concentrations. Other interferences such as ascorbic acid (AA), uric acid (UA), and epinephrine (EP) were examined as well. AA and UA did not cause any PL increase for Cu-NTA-GO (Figure S8a). EP slightly increased its PL (Figure S8b), but it was still smaller than NorEpi did. The limit of detection (LOD) of Cu-NTA-GO for NorEpi detection was found to be 10.1 nM (Figure S8c). These results suggest that Cu-NTA-GO was able to selectively detect NorEpi at nanomolar concentrations through a "turn-on" PL response. The chemical functionalization of GO with an NTA-metal complex gave rise to the selective PL response to the specific neurotransmitter.

Then, we explored the mechanism for the selective response of Cu-NTA-GO to NorEpi. First, the effect of the neurotransmitter affinity to a copper ion on the PL response was examined. According to the previous literature,[14] NorEpi has much higher binding affinity to a copper ion than Dopa, Glu, ADP, and ATP. We also calculated association constants between the metal cations on GO and neurotransmitters based on the PL response of M-NTA-GO hybrids to neurotransmitters (Table S2). The PL response of Cu-NTA-GO to the neurotransmitters correlated well with their affinity strength to a copper ion (Figure 3a). The higher the affinity was, the larger was the "turn-on" response of Cu-NTA-GO PL. Next, we investigated the effect of the functional moieties of NorEpi on the PL response of Cu-NTA-GO. The addition of ethanolamine to Cu-NTA-GO did not cause any PL increase (Figure 3b). Pyrocatechol, not bearing an ethanolamine moiety, also showed no noticeable PL increase. In addition, 2-amino-1-phenylethanol not bearing a 1,2-diol moiety in a phenyl ring did not cause any increase in Cu-NTA-GO PL. These experimental results revealed that the functional moieties could not induce a PL increase in Cu-NTA-GO.

We then measured the reduction potential of the neurotransmitters as well as M-NTA-GO hybrids using cyclic voltammetry (CV) (Figure S9) and compared all values (Figure 3c). The values of reduction potential for four metal ions on NTA-GO slightly shifted, but almost similar to the known ones. The potential energy diagram shows that the potentials of both NorEpi and Dopa were higher than one of a

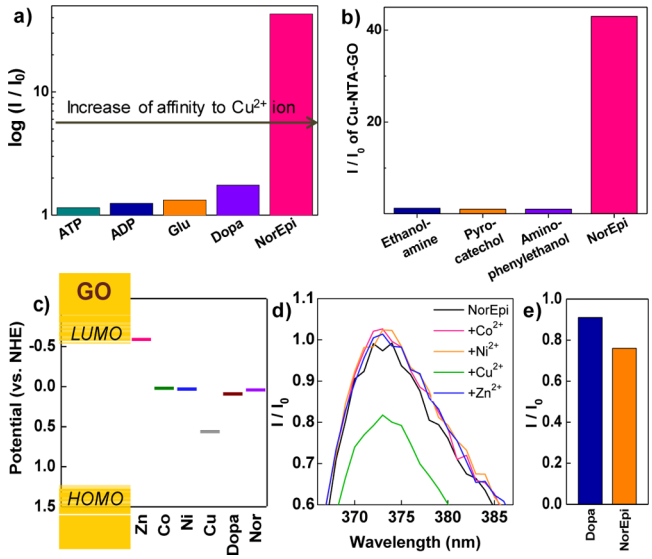


Figure 3. Mechanism study for the PL response of Cu-NTA-GO to NorEpi. (a) Correlation between the PL response of Cu-NTA-GO to the neurotransmitters (100 μM) and their affinity to Cu^{2+}. (b) Effect of the functional moieties in NorEpi on the PL of Cu-NTA-GO. (c) Diagram for the relative potential energies of GO, four metal ions, Dopa and NorEpi. (d) Emission spectra of NorEpi (100 μg/mL) in the presence of metal ions (300 μM). (e) Relative emission quenching of Dopa and NorEpi by Cu^{2+}.

copper ion, indicating that their electrons can be transferred to the Cu^{2+} ion. However, the other metal ions including Co^{2+}, Ni^{2+} and Zn^{2+} had similar or higher potentials than NorEpi and Dopa, which made electron transfer from the neurotransmitters to the metal ions unfavorable. This electron transfer from NorEpi and Dopa to the Cu^{2+} ion was clearly observed in the emission spectra (Figures 3d and S10). The emission intensity of NorEpi and Dopa at 374 nm was diminished only in the presence of the Cu^{2+} ion, while their absorption did not change (Figure S11a,b). In addition, the emission of NorEpi was more quenched by Cu^{2+} ion than that of Dopa due to the higher affinity of NorEpi to Cu^{2+} (Table S2). The absorption of Cu-NTA-GO in the presence of NorEpi or Dopa remained constant as well (Figure S11). These results indicate that this favorable electron transfer from NorEpi to Cu^{2+} ion resulted in preventing the transfer of the excited electrons in the LUMO of NTA-GO to the Cu^{2+} ion, which led to recombination of the electron–hole pair in NTA-GO. This recovered recombination in the presence of NorEpi caused the restoration of the quenched PL of NTA-GO.

Finally, we applied the Cu-NTA-GO hybrid to the selective detection of NorEpi secreted from living pheochromocytoma-12 (PC-12) (Figure 4a). It has been reported that chemical hypoxia using KCN induces an increased release of NorEpi in PC-12 cells.[15] KCN was added into the cell medium 4 h after incubation of the PC-12 cells. As shown in Figure 4b, the PL intensity of Cu-NTA-GO slightly increased upon addition of the cell media before stimulation of the cells with KCN (red line). However, after addition of KCN for stimulation, a dramatic PL increase was observed as a function of incubation time, indicating that the concentration of NorEpi in the cell media increased. Notice that the PL of pristine GO did not change upon addition of the cell media even after stimulation with KCN (Figure 4b, black line). This control experiment proves that the PL increase of the Cu-NTA-GO sensor was

dx.doi.org/10.1021/ja504276z | J. Am. Chem. Soc. 2014, 136, 10842–10845

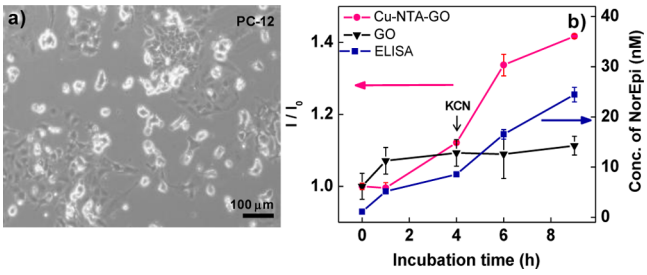


Figure 4. Cellular detection of NorEpi secreted from PC-12 cells with Cu-NTA-GO. (a) Optical image of PC-12 cells. (b) PL response of Cu-NTA-GO and GO to NorEpi released from PC-12 and the quantification of NorEpi using ELISA.

caused by NorEpi released from the stimulated cells. We also measured the concentration of NorEpi secreted from the PC12 cells using ELISA.[7] As shown in Figure 4b (blue line), after stimulation of the cells with KCN, the concentration of NorEpi in the media dramatically increased as a function of incubation time. This concentration increase was well correlated with the PL response of the Cu-NTA-GO sensor. This result verified that the Cu-NTA-GO sensor was able to selectively detect NorEpi released from the living cells. Compared to ELISA, for which procedures involve complicated and time-consuming multisteps using antibodies, the Cu-NTA-GO sensor is able to detect NorEpi much more rapidly and readily in a single-step procedure without using natural antibodies. The LOD of this system for NorEpi detection was comparable to the values of terbium ion-based sensors (0.64–80 nM) and ELISA (7.4 nM) but lower than that of electrochemical methods (60–67 nM) as shown in Table S3.

In summary, we demonstrated that the chemical modulation of GO PL allowed design of an optical biosensor for the selective detection of a specific neurotransmitter without the use of antibodies. Cu-NTA-GO was successfully applied to the selective detection of NorEpi secreted from the living cells. We expect that this novel chemical approach for designing a selective biosensor without using antibodies will be used in diverse sensing platforms.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and supporting data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

kjh75@hanyang.ac.kr

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Basic Science Research Program (2012-R1A1A1012516 and 2008-0061891) through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning, and the New & Renewable Energy of the Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government Ministry of Trade, Industry, and Energy (20123021010010).

■ REFERENCES

(1) Hummers, W. S.; Offeman, R. E. *J. Am. Chem. Soc.* **1958**, *80*, 1339–1339.

(2) (a) Sun, X. M.; Liu, Z.; Welsher, K.; Robinson, J. T.; Goodwin, A.; Zaric, S.; Dai, H. J. *Nano Res.* **2008**, *1*, 203–212. (b) Luo, Z. T.; Vora, P. M.; Mele, E. J.; Johnson, A. T. C.; Kikkawa, J. M. *Appl. Phys. Lett.* **2009**, *94*, 111909. (c) Loh, K. P.; Bao, Q. L.; Eda, G.; Chhowalla, M. *Nat. Chem.* **2010**, *2*, 1015–1024. (d) Jung, J. H.; Cheon, D. S.; Liu, F.; Lee, K. B.; Seo, T. S. *Angew. Chem., Int. Ed.* **2010**, *49*, 5708–5711. (e) Zhao, X. H.; Kong, R. M.; Zhang, X. B.; Meng, H. M.; Liu, W. N.; Tan, W. H.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2011**, *83*, 5062–5066.

(3) (a) Chien, C. T.; Li, S. S.; Lai, W. J.; Yeh, Y. C.; Chen, H. A.; Chen, I. S.; Chen, L. C.; Chen, K. H.; Nemoto, T.; Isoda, S.; Chen, M. W.; Fujita, T.; Eda, G.; Yamaguchi, H.; Chhowalla, M.; Chen, C. W. *Angew. Chem., Int. Ed.* **2012**, *51*, 6662–6666. (b) Xin, G.; Meng, Y.; Ma, Y.; Ho, D.; Kim, N.; Cho, S. M.; Chae, H. *Mater. Lett.* **2012**, *74*, 71–73.

(4) Roy, M.; Kusurkar, T. S.; Maurya, S. K.; Meena, S. K.; Singh, S. K.; Sethy, N.; Bhargava, K.; Sharma, R. K.; Goswami, D.; Sarkar, S.; Das, M. *3 Biotech* **2013**, *3*, 1–9.

(5) (a) Lu, C. H.; Yang, H. H.; Zhu, C. L.; Chen, X.; Chen, G. N. *Angew. Chem., Int. Ed.* **2009**, *48*, 4785–4787. (b) Balapanuru, J.; Yang, J. X.; Xiao, S.; Bao, Q. L.; Jahan, M.; Polavarapu, L.; Wei, J.; Xu, Q. H.; Loh, K. P. *Angew. Chem., Int. Ed.* **2010**, *49*, 6549–6553. (c) Jang, H.; Kim, Y. K.; Kwon, H. M.; Yeo, W. S.; Kim, D. E.; Min, D. H. *Angew. Chem., Int. Ed.* **2010**, *49*, 5703–5707. (d) Piao, Y.; Liu, F.; Seo, T. S. *Chem. Commun.* **2011**, *47*, 12149–12151.

(6) (a) Skottrup, P. D.; Nicolaisen, M.; Justesen, A. F. *Biosens. Bioelectron.* **2008**, *24*, 339–348. (b) Saerens, D.; Huang, L.; Bonroy, K.; Muyldermans, S. *Sensors* **2008**, *8*, 4669–4686.

(7) Wu, R.; Zhou, M.; Das, P.; Dong, W.; Ji, Y.; Yang, D.; Miksa, M.; Zhang, F.; Ravikumar, T. S.; Wang, P. *Am. J. Physiol.: Endocrinol. Metab.* **2007**, *293*, E1697–E1702.

(8) (a) Hoshino, Y.; Kodama, T.; Okahata, Y.; Shea, K. J. *J. Am. Chem. Soc.* **2008**, *130*, 15242–15243. (b) Haupt, K. *Nat. Mater.* **2010**, *9*, 612–614. (c) Shi, H. Q.; Tsai, W. B.; Garrison, M. D.; Ferrari, S.; Ratner, B. D. *Nature* **1999**, *398*, 593–597.

(9) Zhang, J. Q.; Landry, M. P.; Barone, P. W.; Kim, J. H.; Lin, S. C.; Ulissi, Z. W.; Lin, D. H.; Mu, B.; Boghossian, A. A.; Hilmer, A. J.; Rwei, A.; Hinckley, A. C.; Kruss, S.; Shandell, M. A.; Nair, N.; Blake, S.; Sen, F.; Sen, S.; Croy, R. G.; Li, D. Y.; Yum, K.; Ahn, J. H.; Jin, H.; Heller, D. A.; Essigmann, J. M.; Blankschtein, D.; Strano, M. S. *Nat. Nanotechnol.* **2013**, *8*, 959–968.

(10) (a) Solanto, M. V. *Behav. Brain Res.* **2002**, *130*, 65–71. (b) Elenkov, I. J.; Wilder, R. L.; Chrousos, G. P.; Vizi, E. S. *Pharmacol. Rev.* **2000**, *52*, 595–638. (c) Braunwald, E. *New Engl. J. Med.* **2008**, *358*, 2148–2159.

(11) Hochuli, E.; Dobeli, H.; Schacher, A. *J. Chromatogr.* **1987**, *411*, 177–184.

(12) Zumdahl, S. S.; Zumdahl, S. A. *Chemistry*; Houghton Mifflin: Boston, MA, 2007; p 843.

(13) (a) Chen, J. L.; Yan, X. P.; Meng, K.; Wang, S. F. *Anal. Chem.* **2011**, *83*, 8787–8793. (b) Permyakov, E. A. *Luminescent spectroscopy of proteins*; CRC Press: Boca Raton, FL, 1993; p 21.

(14) Boersma, A. J.; Brain, K. L.; Bayley, H. *ACS Nano* **2012**, *6*, 5304–5308.

(15) Kuo, J. S.; Cheng, F. C.; Shen, C. C.; Ou, H. C.; Wu, T. F.; Huang, H. M. *J. Cell Biochem.* **2000**, *79*, 191–201.