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Phosphorescent Sensor for Phosphorylated Peptides Based on an Iridium Complex

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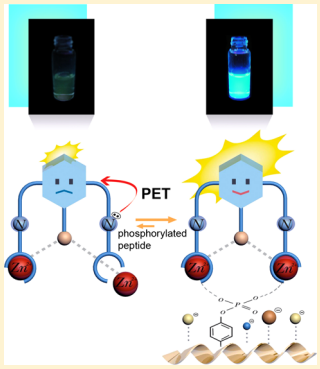
Supporting Information

ABSTRACT: A bis[(4,6-difluorophenyl)pyridinato-*N*,$C^{2'}$]iridium(III) picolinate (FIrpic) derivative coupled with bis(Zn^{2+}–dipicolylamine) (ZnDPA) was developed as a sensor (**1**) for phosphorylated peptides, which are related to many cellular mechanisms. As a control, a fluorescent sensor (**2**) based on anthracene coupled to ZnDPA was also prepared. When the total negative charge on the phosphorylated peptides was changed to −2, −4, and −6, the emission intensity of sensor **1** gradually increased by factors of up to 7, 11, and 16, respectively. In contrast, there was little change in the emission intensity of sensor **1** upon the addition of a neutral phosphorylated peptide, non-phosphorylated peptides, or various anions such as CO_3^{2-}, NO_3^-, SO_4^{2-}, phosphate, azide, and pyrophosphate. Furthermore, sensor **1** could be used to visually discriminate between phosphorylated peptides and adenosine triphosphate in aqueous solution under a UV–vis lamp, unlike fluorescent sensor **2**. This enhanced luminance of phosphorescent sensor **1** upon binding to a phosphorylated peptide is attributed to a reduction in the repulsion between the Zn^{2+} ions due to the phenoxy anion, its strong metal-to-ligand charge transfer character, and a reduction in self-quenching.



INTRODUCTION

The degree of phosphorylation of proteins is of great interest because it is related to cellular control mechanisms in many different processes, including metabolic pathways, cell growth and differentiation, membrane transport, and apoptosis.[1,2] Phosphorylation of serine, threonine, and tyrosine residues on the protein surfaces is regulated by a balance between the activities of kinases and phosphatases. Abnormal regulation of protein phosphorylation is either a cause or a consequence of major diseases such as cancer, diabetes, and rheumatoid arthritis, while defects in genes that produce protein kinases and phosphatases underlie a number of inherited and acquired disorders including leukemia, lymphomas, and immune diseases.[3,4] Thus, methods for detecting phosphorylated proteins would be of great use in the study of biological events.

To understand the complicated signal transduction mechanisms involved in these events, it is desirable to develop versatile methods and molecular probes that can selectively recognize proteins and enzymes. Several groups have devoted considerable efforts toward the development of receptors for phosphate or phosphate-containing biomolecules.[5−17] The Hamachi group reported the first fluorescent chemosensors for phosphorylated peptides in aqueous solution, which were based on anthracene coupled to a bis(Zn^{2+}–dipicolylamine) (ZnDPA) unit (**2**, Figure 1).[9] The sensitivity of sensor **2** greatly increased as the total net charge of the peptide being examined became more negative, because a greater negative charge more favorably suppressed the electrostatic repulsion between the first Zn^{2+} ion and a second incoming Zn^{2+} ion. This reduced electrostatic repulsion subsequently reduced the degree of photoinduced electron transfer (PET) quenching, resulting in an increased emission intensity.[9,11] Unfortunately, sensor **2** was not able to discriminate between phosphorylated peptides and adenosine triphosphate (ATP), which plays a crucial role in phosphorylation events.[1,2] This is problematic if one wants to use **2** as a practical tool for sensing phosphorylated proteins. Furthermore, the sensitivity of sensor **2** for phosphorylated peptides still needs to be improved.

RESULTS AND DISCUSSION

Phosphorescent Sensor for Phosphorylated Peptides.

Herein we report the first phosphorescent sensor for phosphorylated peptides. Our sensor (**1**, Figure 1) is based on an iridium complex attached to a ZnDPA unit. Several features distinguish our sensor from fluorescent analogues such as **2**. Bis[(4,6-difluorophenyl)pyridinato-*N*,$C^{2'}$]iridium(III) picolinate (FIrpic), a well-known sky-blue dopant in organic light-emitting diodes, was selected as the emitting unit on account of its high quantum efficiency (Φ_{PL} = 0.42).[18−20] Because of their strong metal-to-ligand charge transfer (MLCT) character, phosphorescent complexes are very sensitive to their surrounding environments, and therefore, an enhancement in the luminescence of the bound phosphorescent sensor may result from the increased hydrophobicity of the surrounding environment of the bound sensor in

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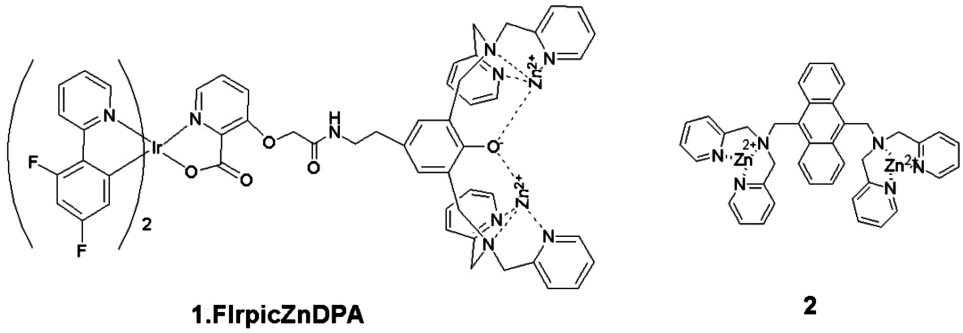

Figure 1. Structures of phosphorescent sensor **1** (FIrpicZnDPA) and fluorescent sensor **2**.

comparison with the environment of the free sensor. Furthermore, phosphorescent sensors have several advantages compared with fluorescent sensors, including decreased self-quenching (due to a large Stokes shift), high photostability, and a long lifetime, which is required for discrimination of the sensor signal from the autofluorescence of proteins.[21−25] It is well-known that ZnDPA moieties generate an anion-binding site through the formation of a phenoxo-bridged dinuclear metal complex.[26,27] Oxygen anions from a phosphate moiety bind to the dinuclear zinc complex by coordinating to the metal ions.[23,26,27] Furthermore, as depicted in Figure 2, the phenoxy

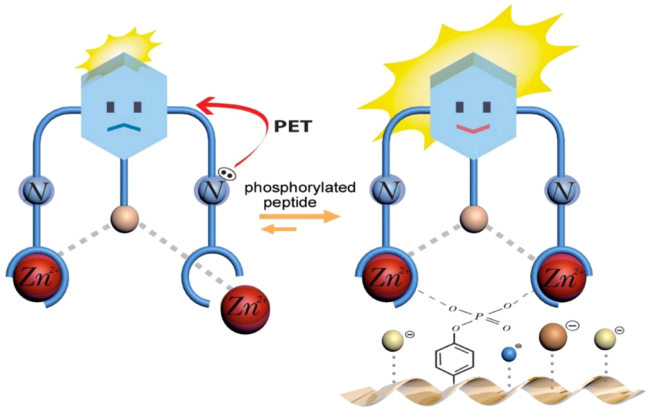

Figure 2. Schematic diagram of the complex between the phosphorescent sensor **1** and a phosphorylated peptide.

anion of sensor **1** can reduce the electronic repulsion between Zn^{2+} ions. The reduction in electronic repulsion increases the emission intensity by suppressing PET quenching more efficiently. Thus, sensor **1** exhibits a higher emission intensity than, for example, fluorescent sensor **2**, which does not have the coordinating oxygen anion. For this reason, our system is expected to provide a much greater luminescence intensity as a result of both the overall increased hydrophobicity of the surrounding environment of phosphorescent sensor **1** upon binding to phosphorylated peptides and the reduced electrostatic interaction between the Zn^{2+} ions in the ZnDPA unit.

Phosphorylated Peptide Sensing Ability. Phosphorescent sensor **1**[23] and fluorescent sensor **2**[9] were prepared according to methods described in the literature. It is known that the luminescence of phosphorescent sensor **1** results from both singlet MLCT (^{1}MLCT) [dπ(Ir) → π^*(N−O)] and triplet ligand-centered (^{3}LC) transitions in the FIrpic moiety.[23] As a result, the photophysical properties of phosphorescent sensor **1** are very sensitive to the surrounding environment because of its MLCT character.[22,23] The maximum emission of sensor **1** is observed at 475 nm when it is excited in the MLCT region (λ_{ex} = 380 nm) and is almost identical to that of FIrpic. Because of its large Stokes shift (Figure S3 in the Supporting Information), this sensor also exhibits reduced self-quenching. In contrast, the maximum emission of fluorescent sensor **2** appears at 420 nm when it is excited at the same wavelength (λ_{ex} = 380 nm), and it shows a small Stokes shift (Figure S4 in the Supporting Information). This means that there is an overlap between the absorption and emission spectra, which can be a cause of self-quenching.

To verify the sensitivity of these sensors to phosphorylated compounds, we compared their photoluminescence spectra. When phosphorylated tyrosine (p-Tyr) was added to aqueous solutions of **1** and **2** (HEPES buffer, 10 mM, pH 7.2), the emission intensity from both sensors showed a 2-fold increase, indicating that these sensors bound strongly to the phosphorylated species (Figure 3). Surprisingly, the luminescence intensity of phosphorescent sensor **1** increased sharply upon the addition of less than 1 equiv of p-Tyr, while that of sensor **2** did not appear to change until a molar excess of p-Tyr was added. Furthermore, we investigated the sensing abilities of sensors **1** and **2** for phosphorylated peptides II (total net charge −4) and III (total net charge −2) (Figure 4). Table 1 lists the sequences of peptides I through IV along with their total net charges (from −6 to 0). The emission intensity of sensor **1** increased 11-fold in the presence of peptide II and 7-fold with peptide III, while that of sensor **2** changed little with either peptide II or III. These results are in good agreement with those of the p-Tyr titration. Thus, phosphorescent sensor **1** is more sensitive to phosphorylation than **2** in both simple and complicated systems. As a control, we tested sensor **1** with non-phosphorylated peptides V and VI. The sequences of peptides V and VI are exactly the same as those of II and III, respectively (Table 1). There was little change in the emission intensity when the non-phosphorylated peptides V and VI were added, indicating that phosphorescent sensor **1** can distinguish between phosphorylated and non-phosphorylated peptides.[28] In addition, the enhancement of the emission intensity was proportional to the total net charge.

The sensing ability of sensor **1** was also tested for peptides with total charges of −6 (peptide I) and 0 (peptide IV) in order to prove the effect of the total net charge of the peptide (Figure 5). It turned out that the emission intensity of sensor **1** remarkably increased in proportion to the total negative charge of the peptide substrate (see Figure 6a for details). This is due to the reduced quenching effect. Thus, the luminescence intensity of **1** increased over 16-fold upon the addition of less than 1 equiv of peptide I (net charge −6), whereas there was

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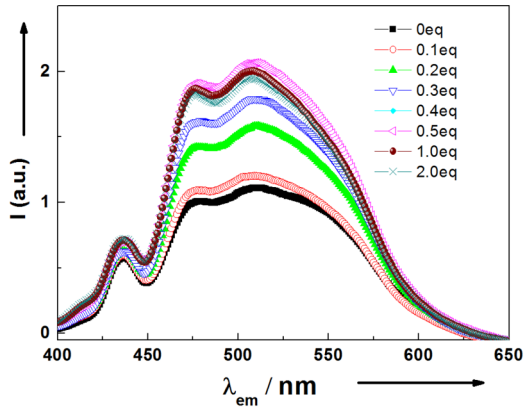
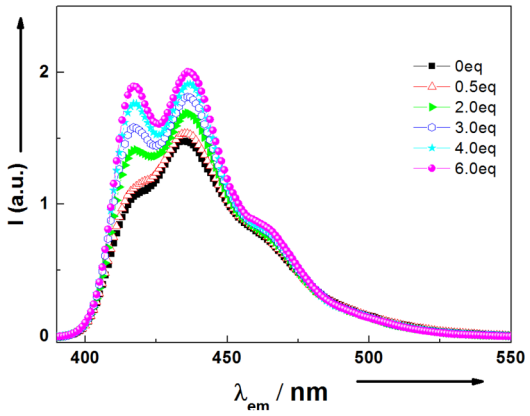


(a) Sensor **1** with p-Tyr



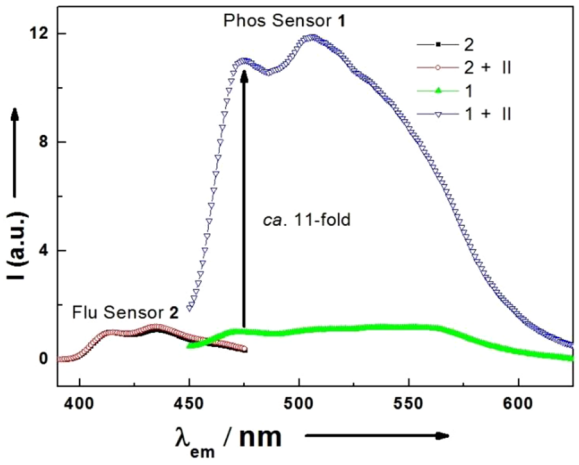
(b) Sensor **2** with p-Tyr

Figure 3. Changes in luminescence emission of (a) sensor **1** (10 μM) and (b) sensor **2** (10 μM) with increasing concentration of phosphorylated tyrosine (p-Tyr). The emission spectra (λ_{em} = 475 nm for **1** and λ_{em} = 415 nm for **2**) were measured in a 10 mM HEPES buffer (pH 7.2) at room temperature.

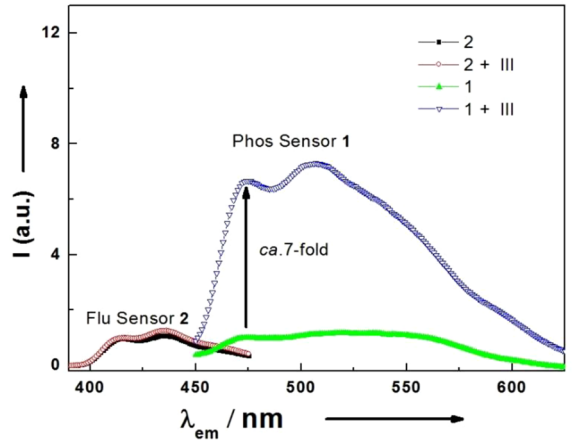
scarcely any emission change even when more than 2 equiv of peptide IV (net charge 0) was added (Figure 5). In summary, the emission intensity of sensor **1** was enhanced with increasing total negative charge of the peptide in the order of IV (0) < III (−2) < II (−4) < I (−6).

Furthermore, sensor **1** showed little change in luminescence upon the addition of various anions such as CO_3^{2-}, NO_3^-, SO_4^{2-}, phosphate, azide, and pyrophosphate over a concentration range of 10^{-6}–10^{-5} M (Figure 6b). This means that sensor **1** clearly has a high sensitivity only for phosphorylated peptides and not for other anions, simple phosphates, or pyrophosphates.

The binding constants (K) for peptides and nucleotides obtained by analyzing the saturation binding data under the assumption of 1:1 binding are summarized in Table 1. It is clear that the binding affinity depends strongly on the net negative charge of the phosphorylated peptide. The binding constant of sensor **1** becomes greater with increasing negative charge of the phosphorylated peptide: 5.0×10^5 for net charge −2 (peptide III), 9.6×10^5 for net charge −4 (peptide II), and 16×10^5 for net charge −6 (peptide I). This is consistent with previously reported results.[9,11] Thus, phosphorescent sensor **1** shows the highest binding constant toward peptide I (16×10^5) among the peptides examined. This indicates that phosphorescent



(a) **1** (10 μM) and **2** (10 μM) with and without peptide II (1.4 equiv)



(b) **1** (10 μM) and **2** (10 μM) with and without peptide III (2.0 equiv)

Figure 4. Changes in the luminescence of sensors **1** (10 μM) and **2** (10 μM) with (a) peptide II (1.4 equiv) and (b) peptide III (2.0 equiv) in 10 mM HEPES buffer (pH 7.2) at room temperature (λ_{ex} = 380 nm).

Table 1. Peptide Sequences, Net Charges, and Binding Constants (K) with Sensors 1 and 2

			$10^{-5} \cdot K$	
peptide	sequence	net charge	**1**	**2**
I	M-E-I-pY-A-E-E-L-D-M-A	−6	16	−[a]
II	T-S-T-E-P-Q-pY-Q-P-G-E-N-L	−4	9.6	−[a]
III	R-R-L-I-E-D-A-E-pY-A-A-R-G	−2	5.0	−[a]
IV	E-A-I-pY-A-A-P-F-A-K-K-K	0	−[a]	−[a]
V	T-S-T-E-P-Q-Y-Q-P-G-E-N-L	−4	−[a]	−[a]
VI	R-R-L-I-E-D-A-E-Y-A-A-R-G	−2	−[a]	−[a]
ATP			25	2.6
ADP			13	1.2
p-Tyr			7.1	0.8

[a]The binding constant could not be obtained because of low emission changes.

sensor **1** can discriminate with a high degree of sensitivity between phosphorylated peptide sequences according to the total amount of negative charge. However, fluorescent sensor **2** exhibits little emission change as the net negative charge of the peptide sequence increases (Figures 4 and 6a).

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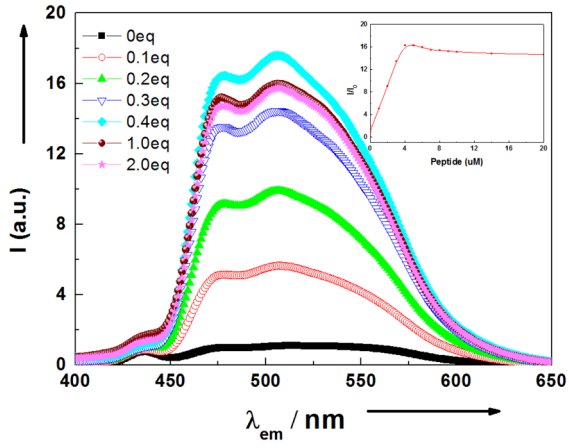
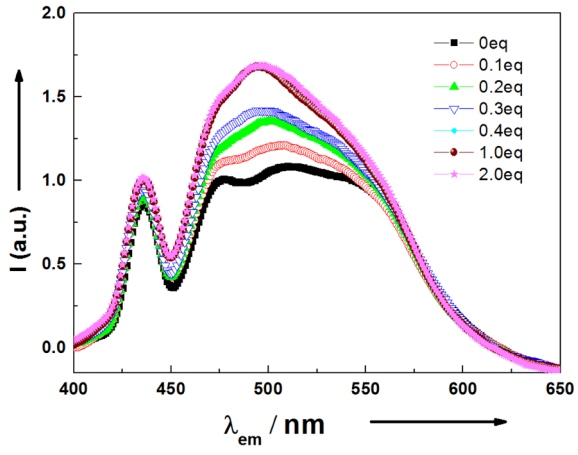
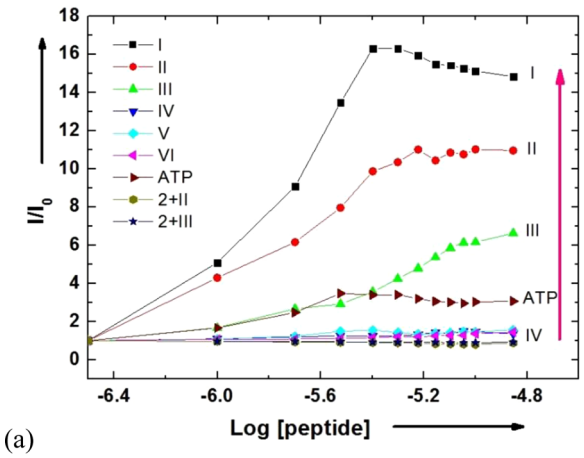


(a) **1** (10 μM) + peptide I

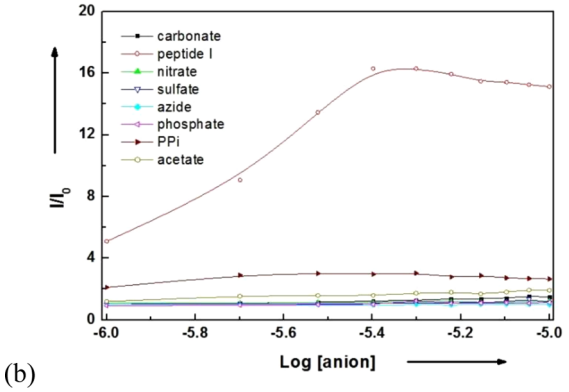


(b) **1** (10 μM) + peptide IV

Figure 5. Changes in the luminescence spectrum of **1** (10 μM) upon the addition of various amounts of (a) peptide I and (b) peptide IV. All of the spectra were measured in an aqueous HEPES buffer (pH 7.4) at room temperature (λ_{ex} = 380 nm). The inset in (a) shows the luminescence titration curve of **1** with peptide I.



(a)



(b)

Figure 6. Luminescence emission responses to the concentrations of (a) peptides I–VI and ATP for **1** (10 μM) and peptides II and III for **2** (10 μM) and (b) various anions for **1** (10 μM). The emission spectra (λ_{em} = 475 nm for **1** and λ_{em} = 415 nm for **2**) were measured in 10 mM HEPES buffer (pH 7.2) at room temperature.

There may be several reasons for the much higher signal amplification of phosphorescent sensor **1** upon binding to phosphorylated peptides compared with that of fluorescent sensor **2**. First, the phenoxy anion of **1** may reduce the electrostatic repulsion between zinc ions. The greater luminescence enhancement also results from the higher hydrophobicity of the surrounding environment of the bound phosphorescent sensor **1** upon binding to a phosphorylated peptide compared with the environment of free sensor **1** or phosphorescent sensor **1** bound to relatively hydrophilic phosphate derivatives (ATP, ADP, PPi). This is the case because phosphorescent sensor **1** is more sensitive to hydrophobic environments provided by the substrate than fluorescent sensor **2** as a result of its strong MLCT character. Furthermore, because of its large Stokes shift, sensor **1** has little chance of self-quenching. Finally, since it has a nonplanar and sterically bulky structure, sensor **1** shows a lower tendency to self-aggregate in solution compared with sensor **2**, which has a relatively flat structure.

Comparison between ATP and Phosphorylated Peptides. Next, we wondered whether sensor **1** can distinguish between phosphorylated peptides and phosphate derivatives such as ATP and ADP. The ZnDPA unit is known to have a strong binding affinity toward ATP and ADP,[26,27] which has made discrimination between phosphorylated peptides and ATP or ADP using this type of fluorescent sensor very difficult. However, when ATP or ADP was added to an aqueous solution of **1**, the emission intensity increased only slightly (less than 4-fold) compared with when peptide I (16-fold) or II (11-fold) was added, even though sensor **1** also binds strongly to ATP and ADP (Table 1 and Figure 6a). As shown in Figure 7, we can visually discriminate phosphorylated peptide I from ATP and non-phosphorylated peptide V by adding sensor **1** in a buffer solution under UV irradiation.

In order to see whether our system can indeed detect phosphorylated peptides in the presence of other phosphate-containing biomolecules, we examined the competitive binding of sensor **1** with phosphorylated peptides in the presence of excess ATP, ADP, and pyrophosphate (PPi). When a mixture of sensor **1** (10 μM), ATP (10 equiv), ADP (10 equiv), and PPi (10 equiv) in buffer solution was added to peptide I (1 equiv), the emission intensity of sensor **1** still showed a ca. 2-fold increase (Figure 8a). We observed a similar increase in the presence of 100 equiv of ATP, ADP, and PPi.

Sensing of the Phosphorylated Model Peptide of Protein p53. On the basis of the above results, we examined the ability of sensor **1** to sense the phosphorylated model peptide of protein p53, which plays a central role in protecting

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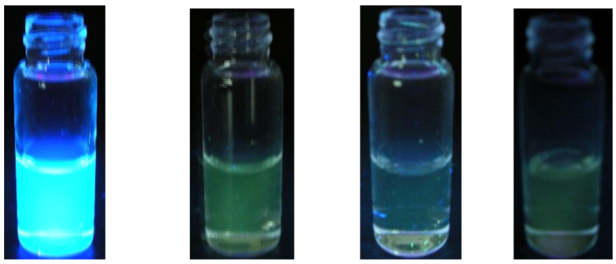

Figure 7. Luminescence intensity of **1** (10 μM) under UV irradiation in 10 mM HEPES buffer (pH 7.2) upon addition of (a) peptide I (20 μM), (b) ATP (20 μM), or (c) peptide V (20 μM) or with no additive (d).

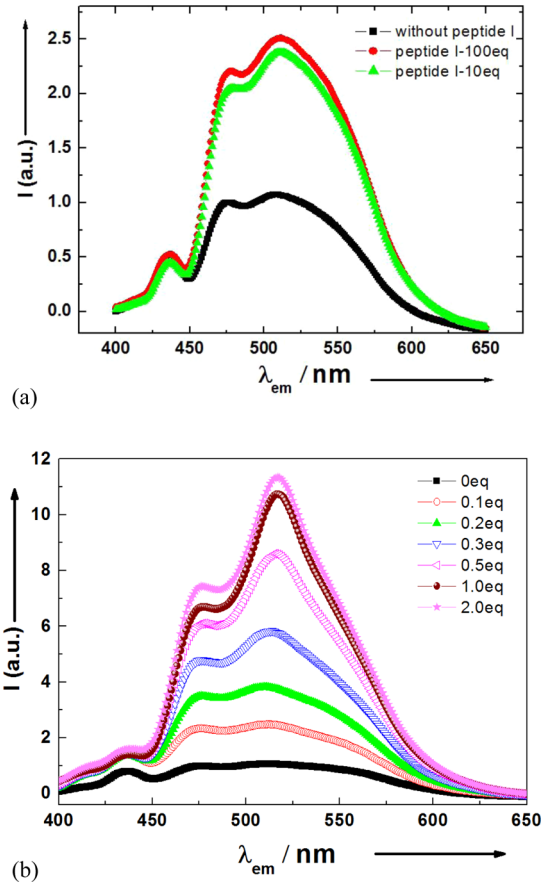

Figure 8. (a) Competitive binding experiments. Emission intensities of **1** (10 μM) in the presence of 10 equiv of ATP, ADP, and PPi in buffer solution without peptide I (black ■) and after addition of 1 equiv of peptide I (green ▲) and of **1** (10 μM) in the presence of 100 equiv of ATP, ADP, and PPi in buffer solution after addition of 1 equiv of peptide I (red ●) are shown. (b) Changes in the luminescence spectrum of **1** (10 μM) upon the addition of synthetic p53 peptide. All of the spectra shown were measured in an aqueous HEPES buffer (10 mM, pH 7.4) at 25 °C (λ_{ex} = 380 nm).

the body from cancer.[29,30] Normally, the p53 tumor suppressor is found at low levels in the human body. However, if cells are damaged, p53 levels rise and the protein binds to multiple regulatory sites, leading to the production of proteins that either halt cell division or initiate the process of programmed cell death (apoptosis). In response to DNA damage, p53 is phosphorylated at multiple sites by protein kinases. Thus, detection of phosphorylated p53 should be a useful cancer diagnosis technique. To date there have been few reports on p53 protein sensors. Therefore, we wondered whether our sensor **1** could be used to detect a synthetic p53 peptide phosphorylated at serine 392 ([pSer392]-p53). Surprisingly, it turned out that the emission intensity of sensor **1** sharply increased (ca. 8-fold) when the synthetic p53 peptide was added in an aqueous environment (Figure 8b). This result demonstrates that sensor **1** can successfully detect the presence of phosphorylated p53.

CONCLUSION

In conclusion, we have developed the new phosphorescent sensor **1**, composed of FIrpic coupled with a ZnDPA unit, that can discriminate phosphorylated peptides not only from non-phosphorylated peptides but also from various anions and nucleotides including ATP. As the total negative charge of a phosphorylated peptide increases, the emission intensity of sensor **1** increases greatly by up to 16-fold. This is largely due to the increased hydrophobicity of the environment surrounding the bound phosphorescent sensor, the phenoxy anion in sensor **1**, and the negative charge of the peptides, which reduces the PET quenching effect. In contrast, the control fluorescent sensor **2** can barely discriminate phosphorylated peptides from non-phosphorylated peptides and ATP in comparison with phosphorescent sensor **1**. This enhanced luminescence upon binding to phosphorylated peptides can be used for kinase assays. Finally, the fact that sensor **1** is able to detect synthetic phosphorylated p53 peptide indicates that our system might be developed into a potential step toward a new approach for the detection of cancer.

EXPERIMENTAL SECTION

Instrumentation and Methods. UV−vis spectra were recorded on a commercial spectrophotometer. Fluorescence spectra were recorded at 25 °C. Peptides I−VI, ATP, and various anions were purchased from a commercial supplier and used as received.

Synthesis. The sensors **1**[23] and **2**[9] were synthesized according to previously described methods.[9,23] All of the reagents were purchased from commercial suppliers and used as received.

Fluorescent Titrations. First, 10 μM solutions of sensors **1** and **2** were prepared from 1 mM stock solutions of sensors **1** and **2**, respectively, in dimethyl sulfoxide, by dilution with 10 mM HEPES buffer solution. The binding constants were determined by fluorescent titration of sensors **1** and **2** (10 μM) in 10 mM HEPES buffer with peptides I−VI, ATP, ADP, p-Tyr, and various anions. The prepared sensor solution was used as a solvent in the preparation of 1 mM stock solutions of peptides I−VI, ATP, ADP, p-Tyr, and various anions. Therefore, in spite of the volume change due to successive addition of the guest stock solution, a constant concentration was maintained throughout the fluorescent titrations. Details of the procedure for determining the binding constants are as follows. The above-mentioned stock solutions of peptides I−VI, ATP, ADP, p-Tyr, and various anions were successively added to the host solution in a 1 cm quartz fluorescence cell. The changes in the emission at λ_{max} (475 nm) were monitored for different concentrations of peptides, ATP, ADP, p-Tyr, and various anions. With the assumption of 1:1 complexation, the binding constants were evaluated by least-squares parameter estimation. The calculated curves fitted the changes in emission intensity well.

Competitive Binding Experiments. First, 10 μM solutions of sensor **1** with 100 or 1000 μM ATP, ADP, and PPi in 10 mM HEPES buffer were prepared, and then the emission intensities excited in the MLCT region (λ_{ex} = 380 nm) were measured. Next, 1 equiv of peptide I was added to these solutions, and the emission intensities excited in the MLCT region (λ_{ex} =380 nm) were measured again.

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Titrations of Synthetic Phosphorylated Peptide p53. The phosphorylated peptide p53 was purchased from Aldrich. The exact peptide sequence was not disclosed, except that it was phosphorylated at serine 392. However, it is generally known that aspartic acid, which is negatively charged at physiological pH and would reduce PET quenching, exists on both sides of serine 392. The titration was followed by the above-mentioned fluorescent titration methods.

■ ASSOCIATED CONTENT

Supporting Information

Job's plots between sensors (**1** and **2**) and p-Tyr, UV and PL spectra of sensors **1** and **2**, and titration PL spectrum of sensor **1** with various peptides (II–VI) and ATP. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lawrence, D. S. *Acc. Chem. Res.* **2003**, *36*, 353.

(2) Ahn, N. *Chem. Rev.* **2001**, *101*, 2207.

(3) Chauhan, A.; Chauhan, V. P. S.; Murakami, N.; Brockerhoff, H.; Wisniewski, H. M. *Brain Res.* **1993**, *629*, 47.

(4) Grundkeiqbal, I.; Iqbal, K.; Tung, Y.-C.; Quinlan, M.; Wisniewski, H. M.; Binder, L. I. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4913.

(5) Huston, M. E.; Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1989**, *111*, 8735.

(6) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397.

(7) Katayev, E. A.; Sessler, J. L.; Khrustalev, V. N.; Ustynyuk, Y. A. *J. Org. Chem.* **2007**, *72*, 7244.

(8) Hirsch, A. K. H.; Fischer, F. R.; Diederich, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 338.

(9) Ojida, A.; Mito-oka, Y.; Inoue, M.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256.

(10) Ojida, A.; Inoue, M.; Mito-oka, Y.; Hamachi, I. *J. Am. Chem. Soc.* **2003**, *125*, 10184.

(11) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454.

(12) Yoshimura, I.; Miyahara, Y.; Kasagi, N.; Yamane, H.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 12204.

(13) Ojida, A.; Honda, K.; Shinmi, D.; Kiyonaka, S.; Mori, Y.; Hamachi, I. *J. Am. Chem. Soc.* **2006**, *128*, 10452.

(14) Anai, T.; Nakata, E.; Koshi, Y.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2007**, *129*, 6232.

(15) Ishida, Y.; Inoue, M.; Inoue, T.; Ojida, A.; Hamachi, I. *Chem. Commun.* **2009**, 2848.

(16) Zhang, T. Z.; Edwards, N. Y.; Bonizzoni, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2009**, *131*, 11976.

(17) Jeong, H.-J.; Ohmuro-Matsuyama, Y.; Ohashi, H.; Ohsawa, F.; Tatsu, Y.; Inagaki, M.; Ueda, H. *Biosens. Bioelectron.* **2013**, *40*, 17.

(18) Adachi, C.; Kwong, R. C.; Djurovich, P.; Adamovich, V.; Baldo, M. A.; Thompson, M. E.; Forrest, S. R. *Appl. Phys. Lett.* **2001**, *79*, 2082.

(19) Tokito, S.; Iijima, T.; Suzuri, Y.; Kita, H.; Tsuzuki, T.; Sato, F. *Appl. Phys. Lett.* **2003**, *83*, 569.

(20) Holmes, R. J.; Forrest, S. R.; Tung, Y. J.; Kwong, R. C.; Brown, J. J.; Garon, S.; Thompson, M. E. *Appl. Phys. Lett.* **2003**, *82*, 2422.

(21) Lo, K. K.-W.; Hui, W.-K.; Chung, C.-K.; Tsang, K. H.-K.; Lee, T. K.-M.; Li, C.-K.; Lau, J. S.-Y.; Ng, D. C.-M. *Coord. Chem. Rev.* **2006**, *250*, 1724.

(22) Kwon, T.-H.; Kwon, J.; Hong, J.-I. *J. Am. Chem. Soc.* **2008**, *130*, 3726.

(23) Kwon, T.-H.; Kim, H. J.; Hong, J.-I. *Chem.—Eur. J.* **2008**, *14*, 9613.

(24) You, Y.; Lee, S.; Kim, T.; Ohkubo, K.; Chae, W. S.; Fukuzumi, S.; Jhon, G.-J.; Nam, W.; Lippard, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 18328.

(25) Woo, H.; Cho, S.; Han, Y.; Chae, W. S.; Ahn, D.-R.; You, Y.; Nam, W. *J. Am. Chem. Soc.* **2013**, *135*, 4771.

(26) Lee, D. H.; Im, J. H.; Son, S. U.; Chung, Y. K.; Hong, J.-I. *J. Am. Chem. Soc.* **2003**, *125*, 7752.

(27) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777.

(28) Unfortunately, we could not purchase the non-phosphorylated peptide I for use as a control. However, even without experiments on non-phosphorylated peptide I, control experiments with non-phosphorylated peptides V and VI obviously showed that sensor **1** has less binding affinity for non-phosphorylated peptides.

(29) Xue, W.; Zender, L.; Miething, C.; Dickins, R. A.; Hernando, E.; Krizhanovsky, V.; Cordon-Cardo, C.; Lowe, S. W. *Nature* **2007**, *445*, 656.

(30) Bernal, F.; Tyler, A. F.; Korsmeyer, S. J.; Walensky, L. D.; Verdine, G. L. *J. Am. Chem. Soc.* **2007**, *129*, 2456.