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Unique Sandwich Stacking of Pyrene-Adenine-Pyrene for Selective and Ratiometric Fluorescent Sensing of ATP at Physiological pH

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Abstract: A pincer-like benzene-bridged sensor **1** with a pyrene excimer as a signal source and imidazolium as a phosphate anion receptor was synthesized and investigated for ATP sensing. A unique switch of excimer vs monomer pyrene fluorescence of **1** is observed in the presence of ATP due to the charcteristic sandwich $\pi-\pi$ stacking of pyrene-adenine-pyrene. On the other hand, four other bases of nucleoside triphosphates such as GTP, CTP, UTP, and TTP can interact only from the outside with the already stabilized stacked pyrene-pyrene dimer of **1**, resulting in excimer fluorescence quenching. The fluorescent intensity ratio of monomer-to-excimer for **1** upon binding with ATP (I_{375}/I_{487}) is much larger than that upon binding with ADP and AMP. This difference is large enough to discriminate ATP from ADP and AMP. As one of the biological applications, sensor **1** is successfully applied to the ATP staining experiments. Sensor **1** is also applied to monitor the hydrolysis of ATP and ADP by apyrase. The results indicate that **1** is a useful fluorescent sensor for investigations of ATP-relevant biological processes.

Introduction

The development of recognition and sensing systems for anions has received considerable attention in recent years,[1] because anions are ubiquitous in biological systems and play significant roles in wide areas of biology, pharmacy, and environmental sciences.[2] Sensors based on anion-induced changes in fluorescence are particularly attractive because of the simplicity and high spatial and temporal resolution of fluorescence.[3] Adenosine-5′-triphosphate (ATP) is a multifunctional nucleotide containing three negatively charged phosphate groups. It plays several important roles in cell biology, mainly as the universal energy currency in living cells[4] and a signaling molecule to coordinate responses to energy status, in part by modulating ion channels[5] and activating signaling cascades.[6] Apart from its roles in energy metabolism and signaling, ATP is also incorporated into nucleic acids by polymerases in the processes of DNA replication and transcription.[7] Visible imaging of ATP can offer useful information about the production and consumption of ATP in real time, which were suggested in some cases to be spatially restricted in cells.[8] Accordingly, there have

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been considerable efforts to develop fluorescent[9] or colorimetric[9e,10] sensors for ATP.

Recently, Anslyn and co-workers[9g] reported the intelligent combinatorial library-based sensors to differentiate between structurally similar compounds of ATP and guanosine 5′-triphosphate (GTP), with the help of principal component analysis (PCA). Hamachi and co-workers utilized a zinc dipicolyl-appended xanthone,[9c] acridine,[9d] or anthracene[9i] as new fluorescent chemosensors for ATP. We also reported a new water-soluble imidazolium anthracene derivative, which not only differentiates the structurally similar compounds GTP and ATP but also acts as a potential fluorescent chemosensor for GTP in 100% aqueous solution.[11a] Wang and Chang[11b] and Ramaiah et al.[11c] showed excellent results regarding GTP-selective fluorescent chemosensors. On the other hand, UTP and UDP selective fluorescent chemosensor was reported recently by our group.[11d] The latest case was reported by Yellen and co-workers using the cpFP approach to create an improved cellular ATP biosensor based on GlnK1.[8]

However, it is still a challenging task to discriminate a certain nucleoside triphosphate among various nucleoside triphosphates such as ATP, GTP, CTP, UTP, and TTP. Indeed, in most reports,[8−11] it is not easy to find an example in which all of these five nucleoside triphosphates were examined to evaluate the selectivity, because all of these sensors only contain the recognition site for triphosphate groups. The distinguishability of ATP from adenosine diphosphate (ADP) and adenosine monophosphate (AMP) is also urgently desired for an improved sensor, since ATP is made from ADP or AMP, and its use in metabolism converts it back into these precursors. The ratio between ATP and AMP is used as a way for a cell to sense how much energy is available and to control the metabolic pathways that produce and consume ATP. Moreover, there are strong interactions between ATP and ADP on some proteins such as PII proteins, and the competition between ATP and ADP leads these proteins to sense the "energy charge" of the bacteria.[12] Then a fluorescent sensor, which can signal the exact concentration of ATP free from hindrance of other nucleoside triphosphates as well as ADP and AMP, is needed.

Herein, we report a new ATP selective fluorescent sensor, which can display a unique ratiometric fluorescent change only with ATP among similar nucleoside triphosphates. The ratiometric fluorescence signals can effectively discriminate ATP from ADP and AMP. Ratiometric fluorescent measurements observe changes in the ratio of the intensities of the emission at two wavelengths.[13] Thus, ratiometric fluorescent sensors have an important feature that they can use to evaluate the analyte concentration and provide built-in correction for environmental effects. Up to now, many investigations were conducted to make ratiometric fluorescent probes for metal ions such as Zn^{2+},[14] Ag^{+},[15] Pb^{2+},[16] and Cu^{2+}.[17] In contrast, ratiometric fluorescent sensors for anions mainly focused on small-sized anions such as F^{-} [18] and CN^{-},[18d,19] but hardly were developed for nucleoside triphosphates. Hamachi et al. reported sensors for ratiometric detection of ATP derivatives,[9d] but the selectivity lacks for various nucleosides. To the best of our knowledge, compound **1** is the first ratiometric fluorescent sensor for ATP with high selectivity. It is worth mentioning that a relatively simple imidazolium receptor can discriminate ATP efficiently from other nucleoside triphosphates by ionic hydrogen bonding[20] between imidazolium $(C-H)^{+}$ and triphosphate group and by $\pi-\pi$ stacking interaction[21] between pyrene and adenine base. The unique binding mechanism is proposed on the basis of fluorescence measurements, NMR experiments, and theoretical calculations.

Results and Discussion

Synthesis. For the synthesis of sensor **1**, 1-pyrenemethanol (**2**) was treated with phosphorus tribromide in toluene to give 1-bromomethylpyrene **3** in 91% yield. **3** was then reacted with 3.5 equiv of **4** in THF to give monoimidazolium ion **5** in 93% yield. This intermediate was heated at 90 °C in DMF with compound **6** for 48 h to give the desired sensor **1** in 27% yield (Scheme 1).

Fluorescence Behavior of 1 with Phosphate-Containing Anions. Figure 1 explains the fluorescent changes of **1** upon addition of $H_2PO_4^{-}$ (Pi), pyrophosphate (PPi), CTP, UTP, TTP, GTP, and ATP. Compound **1** displays two distinct and well-known fluorescent spectra of pyrene moieties in which a peak at 375 nm can be attributed to the monomeric emission and another peak at 487 nm comes from the excimer formation. As in Figure 1, **1** displays fluorescent quenching of excimer peaks upon addition of nucleoside triphosphates. On the other hand, Pi or PPi shows no change or induces very small quenching effects. The quenching effects of these excimer peaks are ATP $\cong$ GTP > TTP $\cong$ UTP > CTP. However, most importantly, only ATP induces a large enhancement in the monomeric fluorescent peak of **1**. This unique change allows an easy discrimination of ATP from the structurally similar nucleoside triphosphates. From

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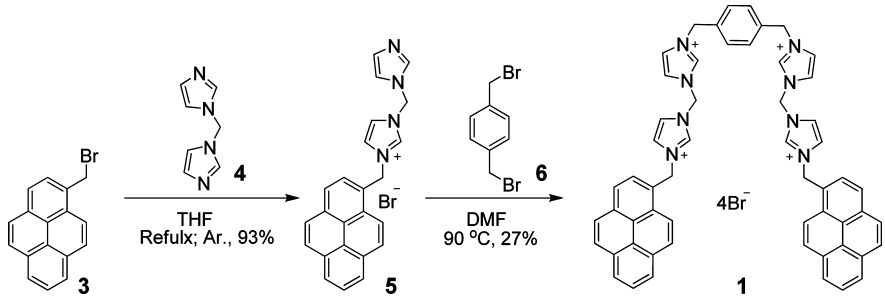
Scheme 1. Synthesis of Compound **1**



the fluorescent titration experiments (Figure 2), the association constant for ATP is calculated to be 1.03×10^4 M^{-1} (error < 15%).[22]

Investigation of Binding Pattern. It has been reported that different nucleoside bases interact with anthracene with different synergistic effects of π-stacking and electrostatic interactions.[11c] Also, they induce different fluorescence responses because of quenching by guanine and enhancing by adenine, where the guanine and adenine are perpendicular to the anthracene with the dipole moments of 6.37 and 1.45 D, respectively.[11a] As explained in our previous study, given that the dipole moment of solvent water is ~3 D, molecules solvating the anthracene with larger/smaller dipole moments than the solvent water tend to enhance/reduce the vertical emission transition and thereby reduce/enhance the fluorescent transition.[11a] In the present case, depending on the mono and excimer fluorescence responses, adenine displays different binding modes for the two pyrenes, in contrast to the other four bases. As in Scheme 2, we postulate that guanine, cytosine, thymine, or uracil interact from the

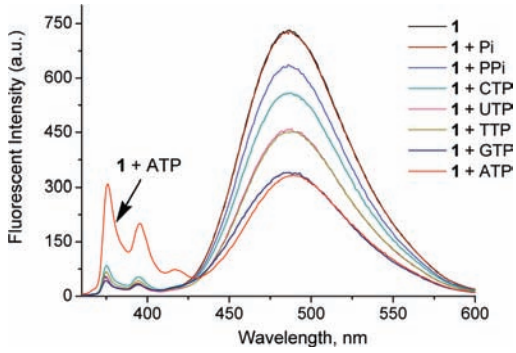


Figure 1. Fluorescent emission changes of **1** (10 μM) upon addition of sodium salt of Pi, PPi, CTP, UTP, TTP, GTP, and ATP (20 equiv) at pH 7.4 (20 mM HEPES) (excitation at 345 nm).

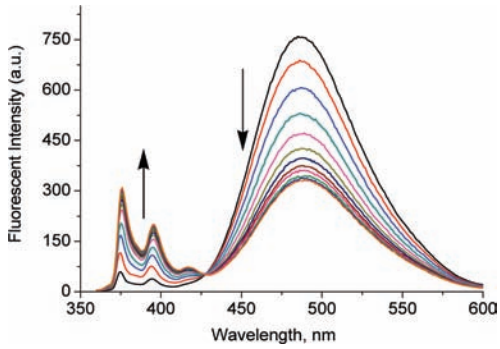
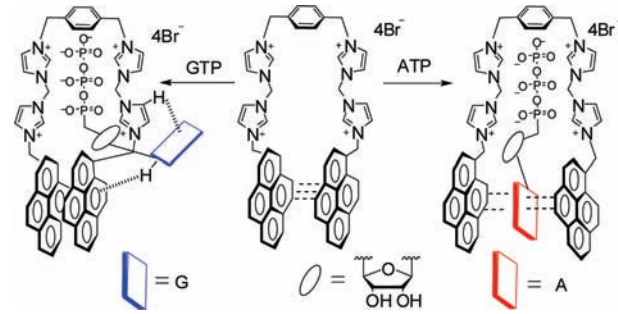


Figure 2. Fluorescent titrations of **1** (10 μM) upon addition of sodium salt of ATP at pH 7.4 (20 mM HEPES) (excitation at 345 nm).

Scheme 2. Proposed Binding Mode of **1** with ATP and GTP



outside with the already stabilized stacked pyrene−pyrene dimer, which quenches the excimer fluorescence because of the large dipole moments. On the other hand, adenine can be in between the two pyrene moieties and hence separated pyrene moieties give rise to the pyrene monomer fluorescence.

To gain support for the proposed binding pattern, ^{1}H NMR titration experiments of **1** with ATP and GTP and 2D NOESY of **1** with 1 equiv of ATP and 1 equiv of GTP are investigated (Figures S8−S11 in the Supporting Information). Figure 3 shows quite different ^{1}H NMR signals of **1** with 1 equiv of ATP and 1 equiv of GTP. Addition of 1 equiv of ATP to the solution of **1** in DMSO causes downfield shifts of the protons of imidazolium moieties, such as H_3 (0.14 ppm), H_4 (0.21 ppm), H_5 (0.06 ppm), H_9 (0.58 ppm), and H_{10} (0.62 ppm). This indicates the strong ionic hydrogen bond interaction between imidazolium and phosphate groups. More importantly, there are changes only in chemical shifts of the signals, which indicate that ATP interacts with two pyrene-bisimidazolium ligands equally. On the other hand, upon addition of 1 equiv of GTP, on the basis of NOESY analyses, the H_1−H_8 peaks of **1** split into two peaks with different upfield chemical shifts except H_4 (with downfield shifts). Particularly, the group of H_2, H_3, H_5 and H_6 experiences large upfield shifts of 0.81, 2.97, 1.17, and 0.53 ppm, respectively. It is known in the literature that when the imidazolium hydrogen forms the CH−π interaction, there are large upfield chemical shifts for the imidazolium hydrogen atoms.[23] These data mean that GTP interacts with two pyrene-bisimidazolium ligands unequally. The imidazolium ring closer to the pyrene would have the CH−π interaction with the guanine ring, which is possible when the guanine moiety of GTP interacts outside the pyrene−pyrene dimer (Scheme 2). This hypothesis is supported by theoretical calculations, which will be discussed

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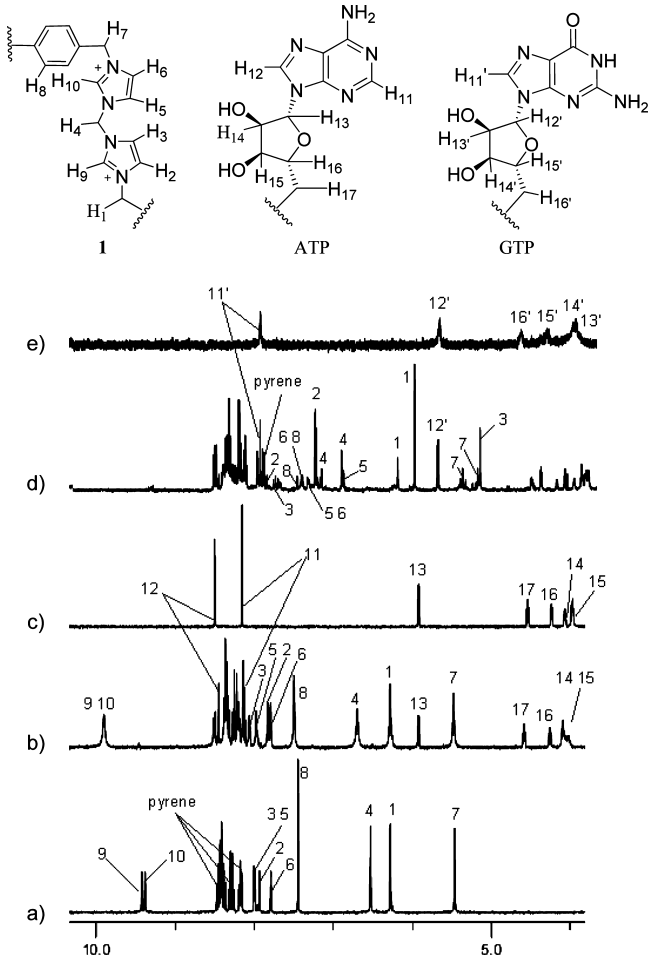

Figure 3. Partial 400 MHz ^{1}H NMR spectra for (a) **1** (2.4 mM), (b) **1** + ATP (1 equiv), (c) ATP, (d) **1** + GTP (1 equiv), and (e) GTP in DMSO-d_6. ATP and GTP were dissolved in D_2O as stock solution at 0.55 and 0.095 mol/L, respectively.

later. For **1**-GTP complex, imidazolium hydrogens H_9 and H_{10} disappear with solvation in D_2O because of the lower solubility of GTP in D_2O than ATP. The NOE from H_{11} (with slight upfield shift) of ATP to pyrene hydrogens (H_{py}) (Figure S10 in the Supporting Information) and the weak NOE from H_{11} of GTP to H_{py} (Figure S11 in the Supporting Information) reveal that, in **1**-ATP and **1**-GTP complexes, adenine and guanine are located closely to at least one pyrene moiety. Notably, there is no cross-peak between H_{12} of ATP and H_{py}, indicating that only the pyrimidine moiety of adenine is close to the pyrene dimer. Since there are no cross-peaks between the protons of ribose and those of pyrenes as well as imidazolium moieties, the ribose in ATP or GTP is outside the pincer-like receptor. All the above NMR results support the symmetrical binding mode of **1** with ATP and asymmetrical binding mode of **1** with GTP.

In addition, insights into the binding pattern of nucleotides were investigated theoretically by comparing the binding patterns and energetics of two structurally most similar nucleotides, ATP and GTP complexed with **1**. We used the density functional theory (B97-D/TZV2P level)[24] that can describe the π-interactions. Treatment of aqueous solvation effects are considered by doing conductor-like screening model (COSMO)[25] single-point energy calculations on the gas-phase-optimized geometries. The most stable form of **1**−4Br$^-$ has a perfect $\pi-\pi$ interaction between the two pyrene moieties. In fact, the presence of Br$^-$ anions helps stabilize the $\pi-\pi$ interaction between the two pyrene moieties, and hence it gives a strong excimer peak at 487 nm. For the **1**−ATP complexation, the **1**−ATP-i complex where the adenine moiety is stacked in between the two pyrene rings is found to be more stable (~4.6 kcal/mol in water) than the structure where the adenine moiety interacts outside the top of one of the already stacked pyrene rings (**1**−ATP-o) (Figure 4). In the **1**−ATP-i complex binding pattern, the unique fluorescent property of the pyrene moiety was restored, whereas in the **1**−ATP-o complex binding pattern, the fluorescent excimer property was still retained. Therefore, with the increasing concentration of ATP in the solution of **1**, more **1**−ATP-i complexes will be formed. In addition, in the **1**−ATP-i complex all imidazolium moieties form (C−H)$^+$---O$^-$ ionic H-bonds and the H_{12} of the imidazole moiety of adenine has no interaction with the pyrene ring as it is displaced outside the stacked π-systems. On the other hand, the **1**−GTP-o complex is found to be 12.8 kcal/mol more stable in water than the **1**−GTP-i complex (Figures 4). Quite interestingly, the pyrimidine moiety of guanine forms the CH$-\pi$ interaction with the H_5 of imidazolium moiety. As the imidazolium ring is the positively charged moiety, this kind of interaction was recently denoted as $\pi^+-\pi$ interaction,[26] which has much larger binding energies than the conventional CH$-\pi$ and $\pi-\pi$ interactions.[27] Meanwhile, the $H_{11'}$ of the imidazole moiety in guanine forms the CH$-\pi$ interaction with the pyrene ring. GTP cannot be

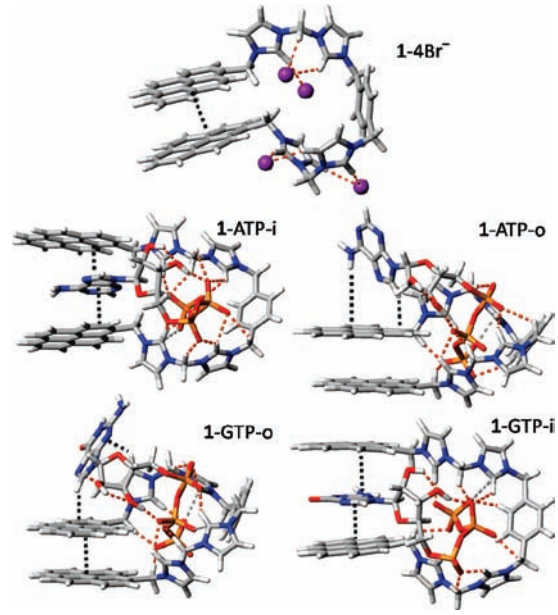

Figure 4. Calculated structures of **1** binding with 4Br$^-$, ATP, and GTP (i: stacked in between two pyrene rings; o: interacting outside two pyrene rings). Important CH---O$^-$/OH---O hydrogen bonds are denoted by orange dotted lines. Ion pair interactions (between positively charged imidazolium moiety and negatively charged oxygen atoms of phosphate group) are denoted by gray dotted lines. CH$-\pi/\pi-\pi$ interactions are denoted by black dotted lines.

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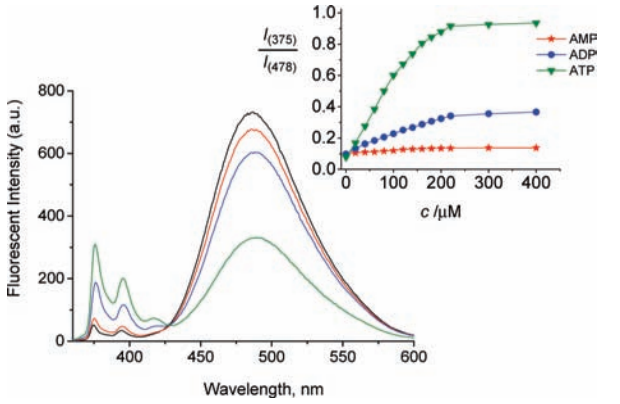

Figure 5. Fluorescent emission changes of **1** (10 μM) upon addition of sodium salt of AMP, ADP, and ATP (20 equiv) at pH 7.4 (20 mM HEPES) (excitation at 345 nm). Inset: Ratiometric calibration curve I_{375}/I_{487} as a function of the concentration of AMP, ADP, or ATP.

stacked between the two pyrene rings because of the presence of oxygen atom in the guanine moiety. We also expect the similar binding pattern in the cases of CTP, TTP, and UTP because of the presence of oxygen atoms in cytosine, thymine, and uracil moieties, respectively. In fact, in the calculated structure of the **1**−GTP-i complex, the oxygen atom and imidazole ring of guanine are quite displaced in comparison with that of the **1**−ATP-i complex. As the **1**−GTP-i complex binding mode would not form in the solution, the unique fluorescent pattern due to the pyrene ring would not be observed when GTP forms a complex with **1**.

ATP Selectivity. To further test adenine's unique role in this sensing system, we examine the fluorescence of **1** with the addition of ADP and AMP. In Figure 5, ADP and AMP display similar trends in the fluorescent changes, but much fewer changes. AMP induces almost negligible changes in both monomeric and excimer emissions. ADP displays a very small fluorescent quenching effect in excimer emission and a relatively larger enhancement in monomeric emission. However, the ratio for ATP (I_{375}/I_{487}) is much larger than that for ADP, which is large enough to discriminate ATP and ADP. More importantly, the curve can serve as the calibration curve for detection of ATP. The observed selectivity for ATP over ADP and the emission change caused by ATP, ADP, and AMP may be ascribed to the difference in electrostatic interaction due to their anionic charges and the difference in the adenine ring stacking between the two pyrene moieties. In ADP and AMP, the way that the phosphate groups interact with the imidazolium moieties of **1** would not be in a favorable structural condition for the adenine moiety to be stacked in between the two pyrene moieties.

Then, the competitive binding experiments are conducted in the presence of Pi, PPi, CTP, UTP, TTP, and GTP at 40 equiv, respectively, with the subsequent addition of 20 equiv of ATP. As shown in Figure 6, no significant variation in the intensity ratio (I_{375}/I_{478}) is found except for ATP. Ratiometric calibration curve I_{375}/I_{487} as a function of ATP's concentration works well even in the presence of 40 equiv of GTP (Figure 7). **1** has the ability to sense ATP with high selectivity even in the mixtures with other nucleoside triphosphates. At the same time, these results indicate again that the binding mode of adenine with the pyrene dimer is different from those of the other four nucleobases.

Biological Application of 1. We further investigate the biological application of **1** in cultured cells (HeLa cells). HeLa

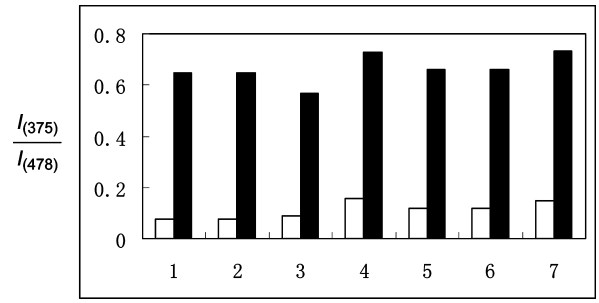

Figure 6. Fluorescence responses of **1** to various anions at pH 7.4 (20 mM HEPES) (excitation at 345 nm). White bars represent the addition of 40 equiv of the appropriate anion to a 10 μM solution of **1**. Black bars represent the subsequent addition of 20 equiv of ATP to the solution. (1) No anion, (2) Pi, (3) PPi, (4) CTP, (5) UTP, (6) TTP, (7) GTP.

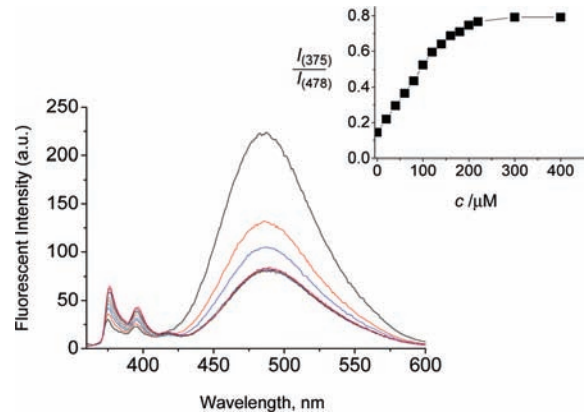

Figure 7. Fluorescent emission changes of mixture solution of **1** (10 μM) with 40 equiv of GTP upon addition of sodium salt of ATP (20 equiv) at pH 7.4 (20 mM HEPES) (excitation at 345 nm). Inset: Ratiometric calibration curve I_{375}/I_{487} as a function ATP's concentration.

cells are grown with DMEM for 16 h and used at 80% confluence. One group of cells is treated with 10 μM oligomycin for 30 min and the other group is not. Then cells are exposed to the 100 μM ATP sensor for 5 min and are washed with PBS. Microscopic observation is assessed in the fluorescent microscope (TE2000-U, Nikon). Images are recorded at the excitation wavelength of 340−380 nm and the emission wavelength of 435−385 nm for blue fluorescence. As shown in Figure 8, probe **1** is successfully applied to the ATP staining experiments. Oligomycin is known to decrease cellular ATP levels.[28] Probe **1** clearly displays blue fluorescence in the HeLa cells; on the other hand, the addition of oligomycin induces the fluorescence quenching.

Finally, with the ability of **1** to recognize ATP from PPi-containing anions, particularly ADP and AMP, chemosensor **1** can be applied to set up a ratiometric fluorescence assay for ATP-relevant enzyme activity. For example, apyrase is a hydrolytic enzyme that converts ATP or ADP into AMP and inorganic phosphate (Pi).[29] During the apyrase-catalyzed ATP and ADP hydrolysis, the monomer emission at 375 nm decreases and the excimer emission at 478 nm increases concurrently. In Figure 9, the plots of the emission ratio I_{478}/I_{375} show that the reaction rate is accelerated with an increase in apyrase concentration. Thus, this convenient real-time monitoring system for

(28) Gong, Y.; Sohn, H.; Xue, L.; Firestone, G. L.; Bjeldanes, L. F. *Cancer Res.* **2006**, *66*, 4880–4887.
(29) Zimmermann, H. *Trends Pharmacol. Sci.* **1999**, *20*, 231–236.

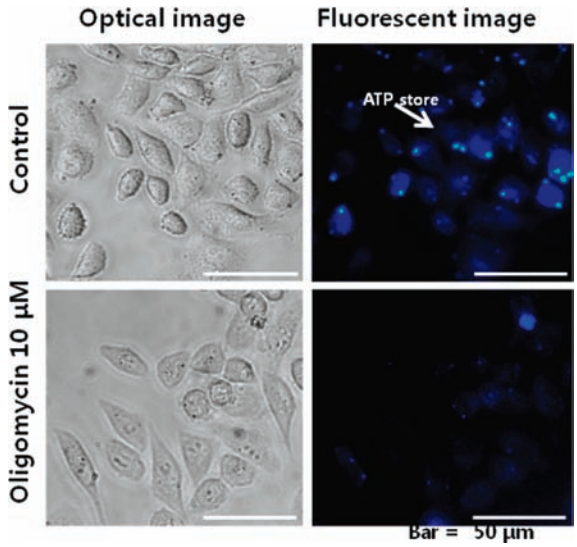

Figure 8. Images of HeLa cells incubated with probe **1** (100 μM) and their images after addition of olygomicyn (10 μM).

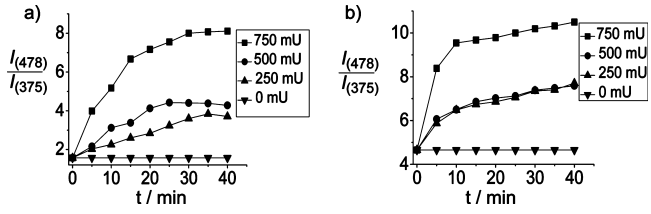

Figure 9. Time-trace plots of (a) ATP and (b) ADP hydrolysis of apyrase monitored by the emission ratio I_{478}/I_{375}. [**1**] = 10 M, [ATP] = [ADP] = 200 M, HEPES buffer (20 mM, pH 7.4), excitation at 345 nm.

apyrase activity would be helpful to elucidate their actions in biological systems.

Conclusions

We have shown that a new water-soluble and fluorescent imidazolium receptor **1** effectively and selectively recognizes the biologically important ATP over other structurally similar nucleoside triphosphates in aqueous solution of physiological pH 7.4. Compound **1** displays a large fluorescent quenching effect in its excimer emission, but a selective fluorescent enhancement in its monomeric emission. The fluorescent intensity ratio for ATP (I_{375}/I_{487}) is much larger than that for ADP and AMP, which is large enough to discriminate ATP from ADP and AMP. Different binding modes between ATP and other nucleoside triphosphates are proposed on the basis of fluorescence measurements, NMR experiments, and theoretical calculations. We believe that these novel findings will be important information to design sensors for nucleoside triphosphates.

Experimental Section

Materials and Methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230−400 mesh ASTM; Merck). ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 MHz or Varian 500 MHz. Chemical shifts were given in parts per million and coupling constants (J) in hertz. Fluorescence emission spectra were obtained using RF-5301/PC spectrofluorophotometer (Shimadzu).

Synthesis of Compound 3. The suspension of 1-pyrenemethanol **2** (2 g, 8.6 mmol) in toluene (100 mL) was cooled to 0 °C followed by addition of phosphorus tribromide (1 mL, 10.5 mmol) via syringe. The mixture was stirred at 0 °C for 1 h and then warmed to room temperature, during which the reaction became homogeneous. Saturated Na_2CO_3 solution (50 mL) was added slowly, and the reaction was stirred until it cooled to room temperature. The phases were separated, and the organic phase was washed with H_2O (50 mL × 2) and brine (50 mL × 2) and dried over Mg_2SO_4. The yellow filtrate was concentrated to minium volume. The yellow needlelike solid was collected and dried. The mother liquid was concentrated again, and the crystallization process was repeated. The total product was 2.3 g in 91% yield. Mp = 136 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 5.23 (s, 2H), 8.02 (m, 5H), 8.21 (m, 3H), 8.35 (d, J = 9.3 Hz, 1H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 32.3, 122.8, 124.6, 124.8, 125.1, 125.6, 126.3, 127.3, 127.7, 128.0, 128.2, 129.0, 130.5, 130.7, 131.2, 131.9.

Synthesis of Compound 5. A solution of 1-bromomethylpyrene **3** (0.2 g, 0.68 mmol) and bisimidazole **4** (0.35 g, 2.4 mmol) in 60 mL of THF was refluxed for 24 h under argon. After cooling to room temperature, the precipitate was filtered and washed with ether. Desired product compound **5** was obtained as a white solid (283 mg, 93%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 6.27 (s, 2H), 6.43 (s, 2H), 7.0 (s, 1H), 7.49 (s, 1H), 7.95−8.49 (m, 12H), 9.48 (s, 1H). ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 50.3, 56.9, 119.4, 122.1, 122.3, 123.4, 123.4, 123.6, 124.1, 125.2, 125.8, 126.0, 126.7, 126.8, 127.2, 128.24, 128.3, 128.7, 128.9, 129.2, 130.1, 130.6, 131.6, 136.7, 137.7. HRMS (FAB) calcd for $C_{24}H_{19}N_4$, $[M - Br]^+$ 363.1610; found, 363.1614.

Synthesis of Compound 1. To the solution of compound **5** (150 mg, 0.34 mmol) in 30 mL of DMF was added compound **6** (46 mg, 0.17 mmol) in 2 mL of DMF. The solution was then heated at 90 °C for 48 h under the protection of argon. The precipitate was filtered and washed with CH_2Cl_2 to give mg product **1** (52 mg, 27%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 5. 51 (s, 4H), 6.32 (s, 4H), 6.63 (s, 4H), 7.52 (s, 4H), 7.88 (s, 2H), 8.03 (m, 4H), 8.10−8.42 (m, 20H), 9.56 (s, 2H), 9.64 (s, 2H). ^{13}C NMR($CDCl_3$, 62.5 MHz): δ 50.9, 52.2, 58.7, 122.9, 123.0, 123.6, 123.9, 124.1, 124.6, 125.7, 126.3, 126.5, 127.2, 127.4, 127.7, 128.7, 128.8, 129.2, 129.3, 129.7, 130.6, 131.1, 132.1, 135.3, 138.3. HRMS (FAB) calcd for $C_{56}H_{46}Br_3N_8$, $[M - Br]^+$ 1069.1375; found, 1069.1378.

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Supporting Information Available: Fluorescence titration of **1** with PPi, CTP, UTP, TTP, GTP, AMP and ADP. ^{1}H NMR titration experiments of **1** with ATP and GTP in DMSO-d_6. 500 MHz NOESY spectra of **1** with 1 equiv of ATP and GTP in DMSO-d_6. NMR spectra of **1**, **3**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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