DOI: 10.1002/asia.201100120

Fluorescent Sensing and Discrimination of ATP and ADP Based on a Unique Sandwich Assembly of Pyrene-Adenine-Pyrene

Zhaochao Xu,[a, b] David R. Spring,*[b] and Juyoung Yoon*[a]

Dedicated to Professor Eun Lee on the occasion of his retirement and 65th birthday

Abstract: It is still a challenging task to discriminate adenosine-5′-triphosphate (ATP) from various nucleoside triphosphates, such as GTP, CTP, UTP, and TTP. The ability to distinguish ATP from adenosine diphosphate (ADP) by fluorescent signals is also urgently desired. Herein, we report two pyrene-based zinc complexes as nucleoside polyphosphate receptors with high selectivity for ATP and ADP based on fluorescence and NMR studies. A unique pyrene–adenine–pyrene sandwich assembly was observed in the case of compound **1** with ATP or ADP, resulting in the increase of monomer fluorescence intensity; whereas the other bases of nucleoside triphosphates, such as GTP, CTP, UTP, and TTP were not sandwiched, resulting in a switch in the monomer–excimer fluorescence of pyrene. The different binding patterns of various nucleobases with a pyrene–pyrene assembly make **1** a highly selective fluorescent sensor for ANP (N = di, tri). In the case of compound **2**, the first 0.5 equivalents of ATP induced a strong excimer emission, whilst ADP induced a large enhancement in the monomeric fluorescent peak. This fluorescence change makes **2** an efficient sensor to discriminate ATP from ADP.

Keywords: adp · assembly · atp · fluorescent sensing · pyrene

Introduction

The development of recognition and sensing systems for nucleoside polyphosphates has been extensively investigated[1] because nucleoside polyphosphates play central roles in many biological processes.[2] For example, adenosine-5′-triphosphate (ATP) mainly functions as the universal energy currency in living cells[3] and as a signaling molecule to coordinate responses to energy status, in part by modulating ion channels[4] and activating signaling cascades.[5] 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.[6] 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.[7] Sensors based on analyte-induced changes in fluorescence are particularly attractive owing to the simplicity and high spatial and temporal resolution of fluorescence.[8] A number of fluorescent sensors have been designed for ADP,[9] GMP,[10] GTP,[11] TTP,[12] UTP,[13] etc. In particular, the recognition[14] and fluorescent sensing[15] of ATP has drawn much more attention. However, it is still a challenging task to discriminate a particular nucleoside triphosphate among various nucleoside triphosphates such as ATP, GTP, CTP, UTP, and TTP. Indeed, in most reports,[9–15] it is not easy to find an example in which all of these five nucleoside triphosphates were examined to evaluate the selectivity, because most of these sensors only contain the recognition site for triphosphate groups. The ability to distinguish ATP from adenosine diphosphate (ADP) by fluorescence is also highly desirable for improved sensors, as ATP is made from ADP, and its use in metabolism converts it back into ADP and phosphate (Pi). The discrimination of ATP and ADP is important for sensing applications because it enables the detection of all

[a] Dr. Z. Xu, Prof. J. Yoon
Department of Chemistry and Nano Science and
Department of Bioinspired Science (WCU)
Ewha Womans University
Seoul, 120-750 (Korea)
E-mail: jyoon@ewha.ac.kr

[b] Dr. Z. Xu, Dr. D. R. Spring
Department of Chemistry
University of Cambridge
Cambridge, CB2 1EW (UK)
E-mail: spring@ch.cam.ac.uk

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/asia.201100120.

ATP-dependent processes. The detection of ADP could therefore be used to monitor the progress of numerous reactions, including ATPases that produce ADP and Pi, and kinases that produce ADP and a phosphorylated product. Moreover, there are strong interactions between ATP and ADP on some proteins like PII proteins, and the competition between ATP and ADP leads these proteins to sense the 'energy charge' of the bacteria.[16] Therefore, a fluorescent sensor that can sense and discriminate ATP and ADP free from the hindrance of other nucleoside polyphosphates is still needed.

A nucleotide is a multifunctional molecule which is composed of a nucleobase (nitrogenous base), a five-carbon sugar (either ribose or 2′-deoxyribose), and between one and three phosphate groups. Most of the fluorescent sensors bind nucleotides via electrostatic interactions between the cationic binding sites (ammonium groups or metal complex) of the receptor and the negatively charged polyphosphate groups. It is then predictable that such a sensor has a larger affinity with nucleoside triphosphates than with nucleoside diphosphates, nucleoside monophosphates, and other inorganic phosphate anions like pyrophosphate. In order to achieve better recognition of a nucleotide, a multifunctional receptor has to be built. In addition to the anion-binding sites, receptors need to contain other binding sites that are capable of interacting with the nucleobase and/or the sugar moiety. Interactions with the nucleobase may be achieved either by π-stacking, in which the distinction between different nucleobases rests on differences in stacking energies, or by sites capable of forming complementary hydrogen-bonding patterns, which should lead to molecular recognition between nucleobases. Lehn and co-workers reported one of the first synthetic multifunctional receptors for nucleotides, which contained a macrocyclic polyammonium moiety as an anion binding site and an acridine side-chain for a stacking interaction with adenine.[17] Since that report, π-stacking interactions have been widely considered in the design of artificial receptors for nucleoside polyphosphates.[11a–c,18] In our recent report, we found that adenine can form a unique pyrene–adenine–pyrene sandwich stack with a fixed pyrene dimer that affords a unique switch of excimer–monomer pyrene fluorescence to sense ATP selectively at physiological pH.[19] However, the other four bases of nucleoside triphosphates (GTP, CTP, UTP, and TTP) can only interact from the outside with the already stabilized stacked pyrene–pyrene dimer, thereby resulting in quenching of the excimer fluorescence.[19] It seems reasonable to comprehend that adenine can be inserted between two pyrene molecules whilst the other four bases (i.e. guanine, cytosine, thymine, and uracil) do not, because different nucleoside bases interact with aromatic rings with different synergistic effects of π-stacking and electrostatic interactions.[20] However, the question is whether this difference is due to inherent properties of adenine to form the sandwich complex with pyrene, rather than a fixed pyrene dimer. Herein, we report the synthesis of mono-pyrene containing compound **1** which is connected to a zinc complex that acts as the receptor for phosphate anions, and we report the first sandwich pyrene–adenine–pyrene assembly with ATP or ADP based on experimental evidence including fluorescence measurements and NMR experiments, which can recognize ANP (N = di, tri) from other nucleoside polyphosphates. More importantly, compound **2**, with a longer distance between pyrene and the zinc complex, can only form a sandwich assembly with ADP. Therefore, compound **2** also shows the ability to discriminate between ATP and ADP fluorescently.

Abstract in Korean:

본 논문은 파이렌-아데닌-파이렌 상호작용을 이용하여 ATP와 ADP를 구별할 수 있는 새로운 형광센싱 시스템에 관한 연구 결과를 설명하고 있다.

Results and Discussion

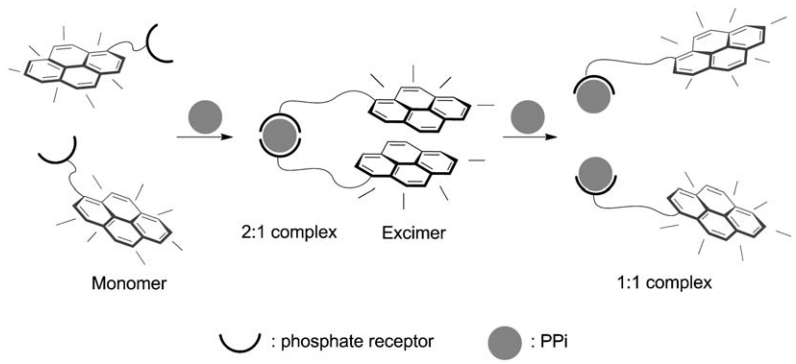
Design and Synthesis

The fluorescence and monomer/excimer fluorescence switch of pyrene have been widely investigated as probes of DNA,[21] RNA,[22] and nucleotides.[12a,19,23] However, in most cases, its use as a probe suffers from the efficient quenching of its fluorescence by nucleobases.[24] We discovered that adenine can insert into a tweezer-like pyrene dimer to form a sandwich complex whilst the other four bases (i.e. guanine, cytosine, thymine, and uracil) do not.[19] One reasonable way of further investigating the interacting modes of pyrene with different nucleobases from a general point of view, is to break the two-armed 'tweezer-ligands' into two free ligands, each composed of only one pyrene molecule linked by a phosphate receptor, as it is well-known that a pyrene/phosphate-receptor conjugate-based compound can form a pyrene excimer through self-assembly induced by pyrophosphate (Scheme 1).[25] Therefore, we can study whether the nucleoside polyphosphates can also induce the formation of the pyrene excimer; if so, then we can also study the interaction between the pyrene–pyrene assembly and different nucleobases.

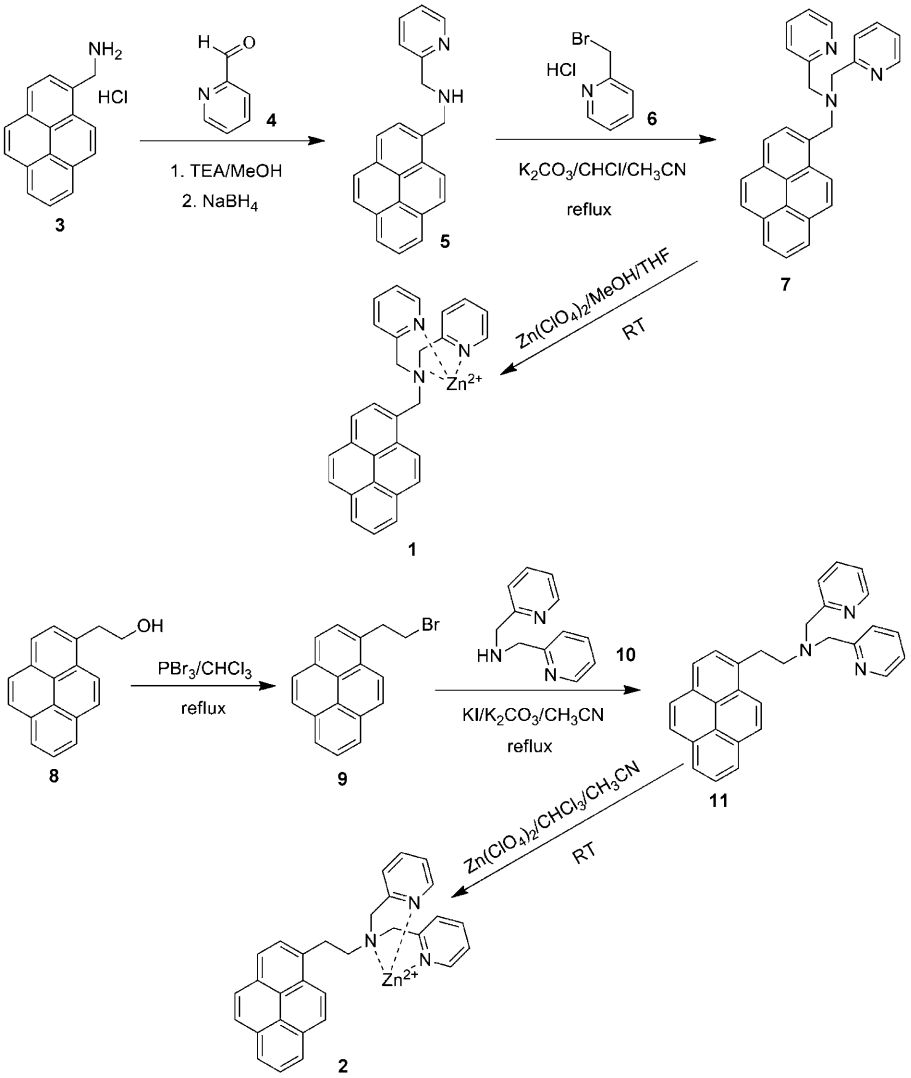
Owing to the difficulty in the low-yielding synthesis of the bi-imidazolium species,[19] we selected a zinc(II) complex with a bis(2-pyridylmethyl)amine (DPA) unit as phosphate receptor, which has been widely studied as a receptor for phosphate anions,[26] and thus synthesized compounds **1** and **2** as shown in Scheme 2.

Fluorescence Detection with Phosphate-Containing Anions

The fluorescence responses of **1** and **2** to the presence of $H_2PO_4^-$ (Pi), pyrophosphate (PPi), AMP, ADP, ATP, CTP, GTP, TTP, and UTP were investigated in buffered solution (HEPES 20 mM, pH 7.4, 25 °C). In 1999, Teramae and co-workers reported a pyrene-functionalized monoguanidinium receptor which was found to self-assemble to form a 2:1 (receptor/PPi) complex with PPi, with a correspondingly re-



Scheme 1. Step binding mode of pyrene/phosphate-receptor conjugates with PPi.



Scheme 2. Synthesis of compounds **1** and **2**.

markable change in the ratio of emission intensities of excimer to monomer owing to the pyrene fluorophore (Scheme 1).[25a] It should be noted that only when the self-assembly had formed did the pyrene-based sensor show a change in fluorescence. Increasing the PPi concentration above 0.5 equivalents relative to the receptor concentration formed a 1:1 (receptor/PPi) complex (Scheme 1) and resulted in an increase in the intensity of short-wavelength emission (monomer), whilst the excimer fluorescence intensity decreased.[25a] Thus, the pyrene monomer/excimer emission switch can be used to determine the stoichiometry and structure of the host–guest complex. Our compounds **1** and **2** displayed similar fluorescence responses in the presence of PPi. Figure 1a and 1c show structured emission bands at 370–450 nm which were assigned to a pyrene monomer emission. The addition of up to 0.5 equivalents of PPi relative to the concentration of **1** or **2** caused a significant decrease in the monomer emission and more-intense red-shifted emission bands centered at 475 nm were observed, which were attributed to the formation of a pyrene excimer, (Figure 1a and 1c); these bands indicated the formation of a **1**/PPi or **2**/PPi adduct with a 2:1 stoichiometry. Furthermore, on addition of another 0.5 equivalents of PPi, the intensity of short-wavelength emission (monomer) of **1** increased, while the excimer fluorescence intensity decreased, which indicated the formation of a **1**/PPi adduct of 1:1 stoichiometry (Figure 1b). However, further addition of another 1.5 equivalents of PPi did not change the fluorescence of **2**/PPi (2:1 complex) significantly. This result indicates that **2**/PPi (2:1 complex) is the main species in aqueous solution, and is much more stable than **1**/PPi (2:1 complex; Figure 1d). ^{1}H NMR studies of **1** with PPi support the above conclusion that a 2:1 complex is formed. As shown in Figure 2, upfield shifts for the methylene, pyridyl, and pyrenyl protons were observed when 0.5 equivalents of PPi was added. The only difference between **1** and **2** is the chain length between pyrene and the receptor (zinc

CHEMISTRY
AN ASIAN JOURNAL

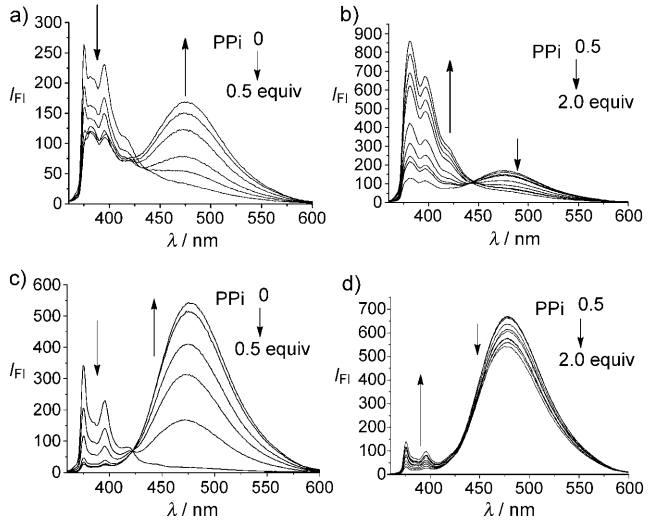


Figure 1. a) Fluorescent emission changes of **1** (10 μM) upon addition of 0–0.5 equiv PPi, b) 0.5–2 equiv PPi at pH 7.4 (20 mM HEPES, excitation at 345 nm), c) Fluorescent emission changes of **2** (10 μM) upon addition of 0–0.5 equiv PPi, and d) 0.5–2 equiv PPi. MeOH = methanol, THF = tetrahydrofuran.

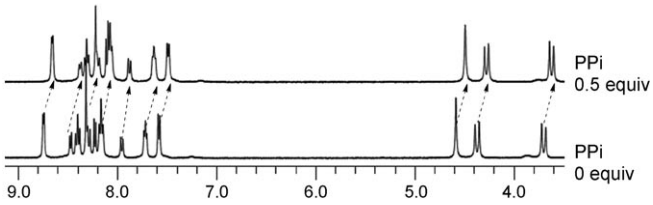


Figure 2. Partial 400 MHz ^{1}H NMR spectra for 1 and 1+PPi (0.5 equiv).

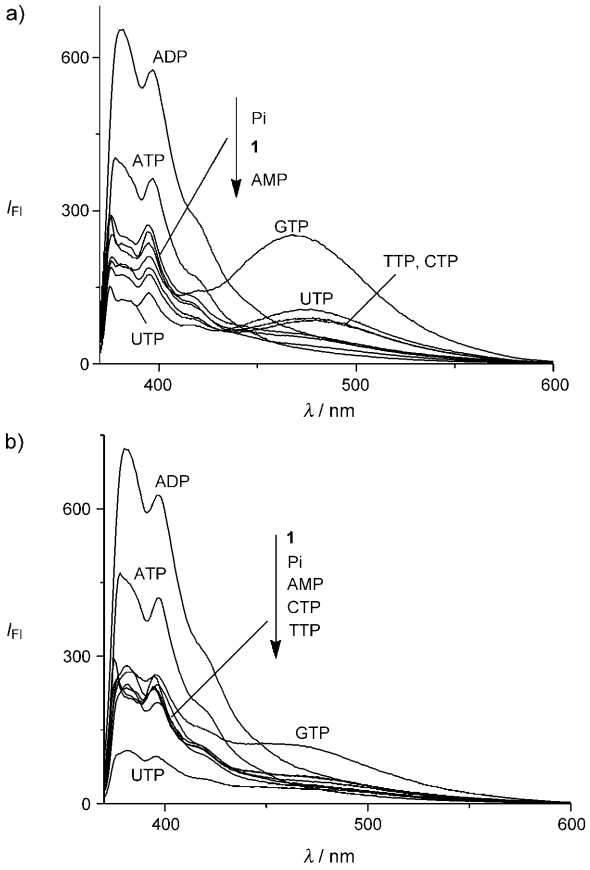


Figure 3. Fluorescent emission changes of **1** (10 μM) upon addition of a) 0.5 equiv, and b) 2 equiv Pi, AMP, ADP, ATP, CTP, UTP, TTP, and GTP at pH 7.4 (20 mM HEPES, excitation at 345 nm).

complex), which plays an important role in the formation of the assembly.

As in Figure 3 and 4, compounds **1** and **2** display quenched excimer fluorescence upon addition of CTP, GTP, TTP, and UTP. Taking **2**/CTP as an example (Figure 5a), with the first 0.5 equivalents UTP (the fluorescence titrations of **1** and **2** with GTP, TTP, and UTP can be found in the Supporting Information, Figure S1–S3, S6–S8), the pyrene–XTP–pyrene assembly was formed and the intensity of monomer emission decreased, while the excimer fluorescence intensity increased. With the addition of more CTP, **2** displayed fluorescent quenching of the excimer peak (Figure 5b). Pi or AMP shows no change or induces only very small quenching effects (Figure 3, 4; also see the Supporting Information, Figure S4, S5, S9, S10). However, ATP or ADP induce a large enhancement in the monomeric fluorescent peak of **1** without the appearance of excimer emission (Figure 3, Figure 6a, b). This unique change allows an easy discrimination of ATP from the structurally similar nucleoside triphosphates. Notably, in the case of compound **2**, the first 0.5 equivalents of ATP induce a strong excimer emission (Figure 6c, d). ADP induces a large enhancement in the monomeric fluorescent peak (Figure 6e). This fluorescence change makes **2** an efficient sensor to discriminate ATP from ADP.

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 owing to quenching by guanine and enhancement by adenine, where the guanine and adenine are perpendicular to the anthracene with dipole moments of 6.37 and 1.45 D, respectively.[11a] As explained in our previous study, given the dipole moment of solvent water is approximately 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 monomer and excimer fluorescence responses, adenine displays different binding modes for the two pyrene molecules, in contrast to other four bases.

In the presence of CTP, GTP, TTP, and UTP, the excimer emissions of compounds **1** and **2** confirm that nucleoside polyphosphates can also induce pyrene–pyrene assembly.

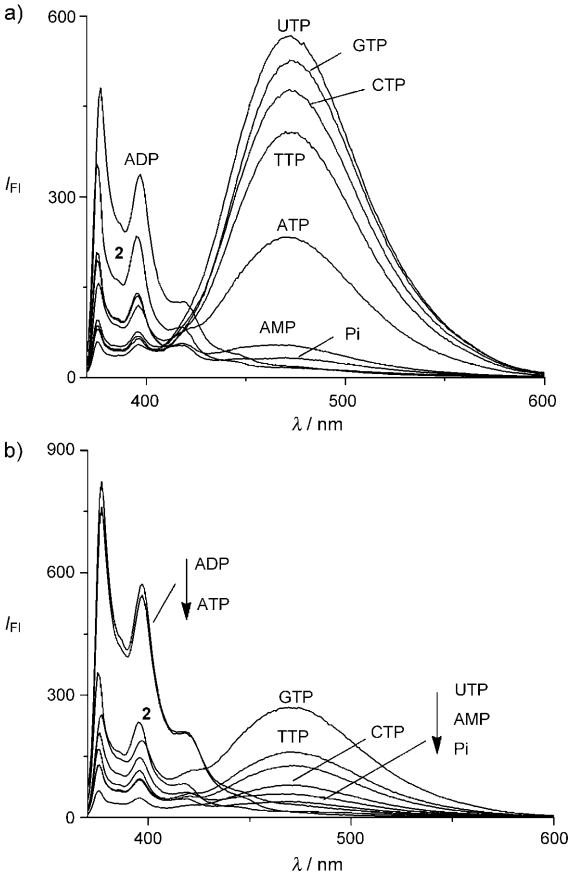


Figure 4. Fluorescent emission changes of **2** (10 μM) upon addition of a) 0.5 equiv, and b) 2 equiv Pi, AMP, ADP, ATP, CTP, UTP, TTP, and GTP at pH 7.4 (20 mM HEPES, excitation at 345 nm).

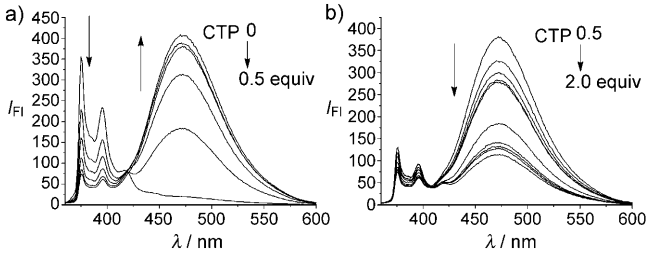


Figure 5. Fluorescent emission changes of **2** (10 μM) upon addition of a) 0–0.5 equiv CTP, b) 0.5–2 equiv CTP at pH 7.4 (20 mM HEPES, excitation at 345 nm).

Owing to the different synergistic effects of π-stacking and electrostatic interactions between different nucleoside bases and aromatic rings, these four bases, guanine, cytosine, thymine, and uracil, quenched the pyrene fluorescence to different extents. Also, these fluorescence responses indicate that guanine, cytosine, thymine, and uracil were outside the pyrene–pyrene assembly.

More importantly, the binding pattern of **1** with ATP and ADP was proposed to form pyrene–adenine–pyrene assemblies based on the monomer fluorescence enhancement and

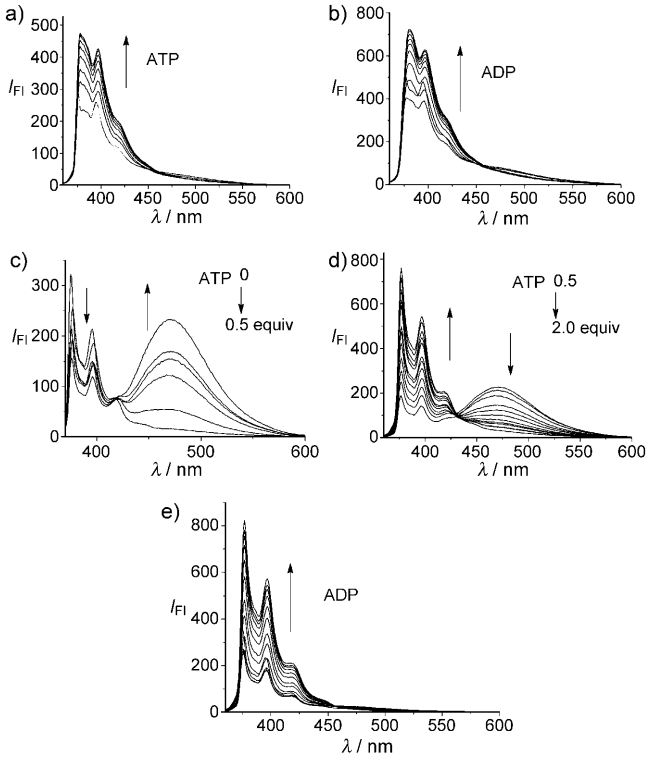


Figure 6. Fluorescent emission changes of a) **1** (10 μM) upon addition of 0–2 equiv ATP, b) 0–2 equiv ADP, c) **2** (10 μM) upon addition of 0–0.5 equiv ATP, d) 0–0.5 equiv ATP, e) 0–2 equiv ADP at pH 7.4 (20 mM HEPES, excitation at 345 nm).

H NMR spectroscopy titration experiments. The triphosphate or diphosphate induced the pyrene–pyrene assembly, but adenine was in-between the two pyrene moieties, and hence separated pyrene moieties give rise to the monomer fluorescence without the appearance of excimer emission. Figure 7 shows different ^{1}H NMR signals of **1** with 0.5 equivalents ADP, 0.5 equivalents ATP, and 0.5 equivalents GTP, respectively. Addition of 0.5 equivalents of ADP to the solution of **1** in dimethyl sulfoxide causes upfield shifts in the methylene (H^5, 0.09 ppm), pyridyl (H^2, 0.17 ppm; H^3, 0.43 ppm; H^4, 0.17 ppm), and pyrenyl protons (H^7, 0.17 ppm; H^8–H^{15}). Similarly, the addition of 0.5 equivalents of ATP to the solution of **1** in dimethyl sulfoxide causes upfield shifts of the methylene (H^5, 0.16 ppm), pyridyl (H^2, 0.35 ppm; H^3, 0.58 ppm; H^4, 0.1 ppm), and pyrenyl protons (H^7-H^{15}). This observation means that **1**/ADP or ATP (2:1 complex) is the main species in aqueous solution. The NOE correlations between H^{16} (with slight upfield shift) of ADP (or ATP) and the pyrene hydrogen atoms (H_{py}; Figure 8a) revealed that in the **1**/ADP and **1**/ATP complexes, adenine is located closely to at least one pyrene moiety. More importantly, there were only changes in chemical shifts of the signals, which indicated that ATP or ADP interacts with two pyrene ligands equally. Therefore, these NMR experiments indicated that adenine was inserted between two pyrene moelcules. Notably, there was no cross-peak between the H^{17} atom of ADP and H_{py}, thus indicating that only the pyri-

CHEMISTRY
AN ASIAN JOURNAL

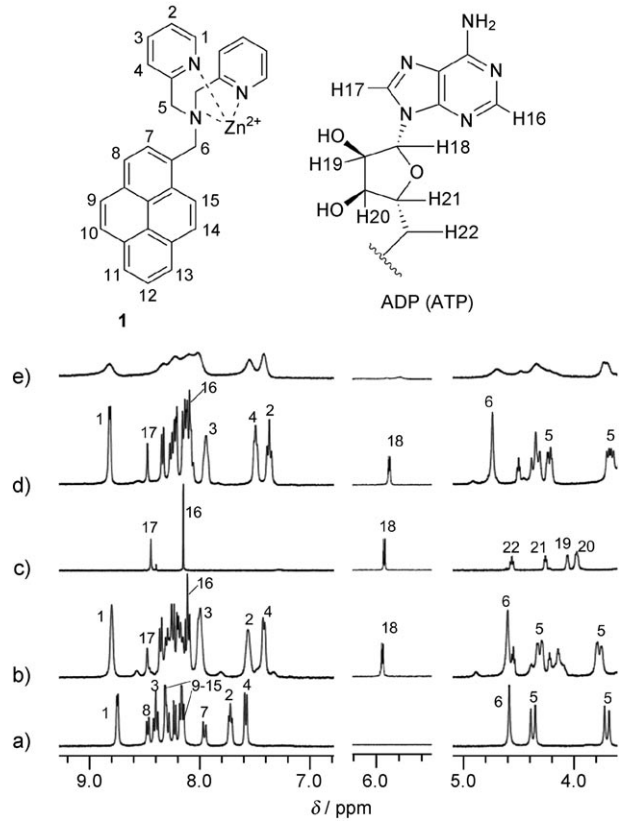


Figure 7. Regions from the 400 MHz ^{1}H NMR spectra for a) **1** (14.8 mM), b) **1**+ ADP (0.5 equiv), c) ADP (or ATP), d) **1**+ ATP (0.5 equiv), and e) **1**+GTP (0.5 equiv) in [D$_6$]DMSO.

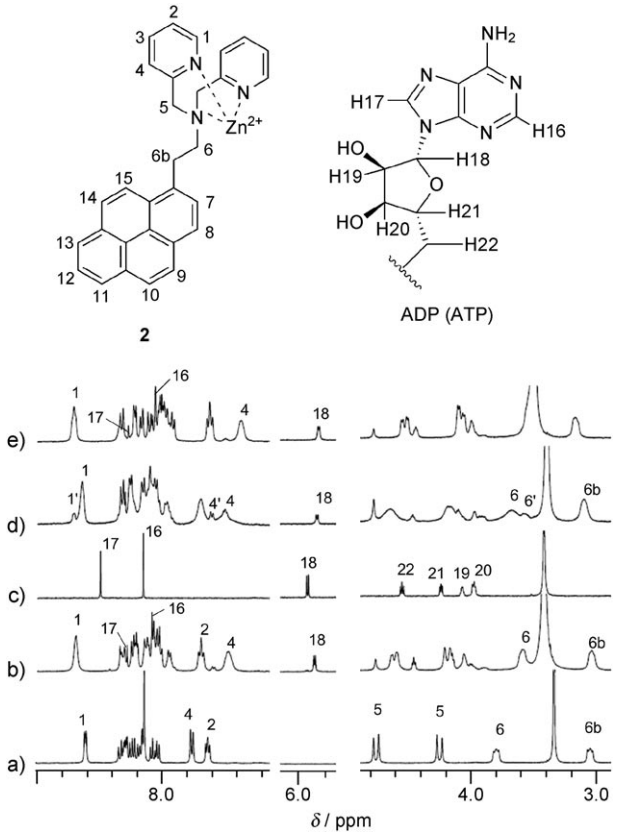


Figure 9. Regions from the 400 MHz ^{1}H NMR spectra of a) **2** (13 mM), b) **2**+ ADP (0.5 equiv), c) ADP (or ATP), d) **2**+ ATP (0.5 equiv), and e) **2**+ ATP (1 equiv) in [D$_6$]DMSO. DMSO = dimethyl sulfoxide.

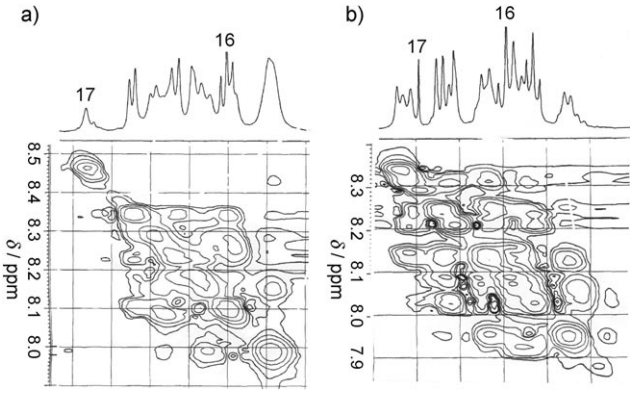


Figure 8. Regions from the 500 MHz NOESY spectrum of a) **1**, and b) **2** with 0.5 equiv of ADP in [D$_6$]DMSO.

midine moiety of adenine is close to the pyrene dimer (Figure 8a).

In accordance with fluorescence examination, the ^{1}H NMR of **2** with 0.5 equivalents ADP indicates the formation of the **2**–ADP–**2** assembly (Figure 9). The NOE from H^{16} (with slight upfield shift) of ADP to pyrene hydrogens (H$_{py}$) (Figure 8b) reveals that in **2**/ADP complex, adenine is located closely to at least one pyrene moiety. Notably, there is also a cross-peak between H^{17} of ADP and H$_{py}$, thereby indicating the existence of pyrene–adenine–pyrene π-stacking interactions. Howerver, the appearance of an excimer emission of **2** with less than 0.5 equivalents ATP indicates that adenine is outside the pyrene dimer in the case of **2**/ATP. Upon addition of 0.5 equivalents of ATP, the methylene (H^6) and pyridyl protons (H^4) of **2** are split into two peaks with different upfield chemical shifts, and H^1 has a downfield shift. Following the continuous addition of a further 0.5 equivalent of ATP, the excimer emission of **2** disappeared with a corresponding increase of monomer emission (Figure 6d). The ^{1}H NMR spectra of **2**/ATP (1:1) is almost the same as **2**/ADP (2:1) (Figure 9e). Therefore, we conclude that in the solution of **2**/ATP (1:1), 2:1 (**2**/ATP) is still the main species. The different binding modes of **1** and **2** with ADP and ATP may be attributed to the different linker lengths of **1** and **2**.

Conclusions

We have reported two pyrene-based zinc complexes as nucleoside polyphosphate receptors with high selectivity for ATP and ADP based on fluorescence and NMR studies. A unique pyrene–adenine–pyrene sandwich assembly was observed for compound **1** in the presence of ATP or ADP,

which resulted in the increase of monomer fluorescence intensity. The other four bases of nucleoside triphosphates (i.e. GTP, CTP, UTP, and TTP) can only be outside with the pyrene–pyrene assembly (induced by polyphosphates), thereby resulting in a switch in the monomer–excimer fluorescence of pyrene. The different binding patterns of various nucleobases with pyrene–pyrene assemblies make **1** a highly selective fluorescent sensor for ANP (N = di, tri). In the case of compound **2**, the first 0.5 equivalents of ATP induced a strong excimer emission, whilst ADP induced a large enhancement in the monomeric fluorescent peak. This fluorescence change makes **2** an efficient sensor to discriminate ATP from ADP.

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). Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with the thickness of 1 mm. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded using Bruker 250 MHz or 400 MHz instruments. Chemical shifts were given in ppm and coupling constants (J) in Hz. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

*Synthesis of Compound **5***

A mixture of 1-pyrenemethylamine hydrochloride **3** (1.07 g, 4.0 mmol), 2-pyridinecarboxaldehyde **4** (0.43 g, 2.0 mmol), and triethylamine (1.4 mL, 10 mmol) in methanol (80 mL) was stirred under argon at room temperature for 3 h. $NaBH_4$ (0.6 g, 15.9 mmol) was added in portions. Then, the solution was stirred for another half hour and the solvent was evaporated to 1/3 of its original volume under vacuum. Water (20 mL) and $NaHCO_3$ (0.7 g) were added to the concentrated solution, which was then stirred for 10 min, and extracted with chloroform (3×50 mL). The combined organic phase was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 98:2) to give the product as pale yellow oil (1.15 g) in 89% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 2.45 (s, 1H), 4.07 (s, 2H), 4.46 (s, 2H), 7.14 (s, J = 6.2 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 8.4 Hz, 1H), 8.01–8.40 (m, 8H), 8.36 (d, J = 9.0 Hz, 1H), 8.64 ppm (d, J = 4.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 51.43, 55.15, 121.90, 122.23, 123.66, 124.73, 124.86, 124.95, 125.04, 125.06, 125.95, 127.01, 127.09, 127.33, 127.54, 129.22, 130.64, 130.95, 131.39, 134.25, 136.28, 149.30, 160.24 ppm.

*Synthesis of Compound **7***

2-(bromomethyl)pyridine hydrochloride (**6**; 453 mg, 2.2 mmol) and K_2CO_3 (600 mg) were added to a solution of *N*-(pyren-1-ylmethyl)(pyridin-2-yl)methanamine (**5**; 700 mg, 2.2 mmol) in 60 mL dry acetonitrile. The mixture was then heated at reflux for 6 h under nitrogen and monitored by TLC. After the reaction was completed, the solvent was removed under reduced pressure. The crude product was then purified by column chromatography on alumina ($CHCl_3$/EtOAc = 1:1, v/v) to give product as a pale yellow oil in 85% yield (760 mg). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 3.72 (s, 4H), 4.14 (s, 2H), 6.81 (t, J = 6.8 Hz, 2H), 7.27 (m, 4H), 7.65–7.89 (m, 8H), 8.31 (d, J = 9.2 Hz, 1H), 8.31 ppm (d, J = 5.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ = 57.15, 60.57,121.99, 123.30, 124.05, 124.47, 124.70, 124.94, 125.82, 126.99, 127.07, 127.40, 128.23, 129.82, 130.71, 130.80, 131.23, 132.52, 136.29, 148.92, 159.63 ppm. HRMS (ESI) calcd for $C_{29}H_{24}N_3$ $[M+H]^+$ 414.1970, found 414.1972.

*Synthesis of Compound **1***

$ZnClO_4 \cdot 6H_2O$ (504 mg, 1.35 mmol) in 1 mL methanol was added dropwise to a solution of **7** (560 mg, 1.35 mmol) in 20 mL of $CHCl_3/CH_3CN$ (10:1), and the mixture was stirred for 30 min at room temperature. Then 50 mL ether was added slowly. After stirring for 10 h, the precipitate was collected to give **1** (66%) without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 3.70 (d, J = 10.2 Hz, 2H), 4.37 (d, J = 10.2 Hz, 2H), 4.59 (s, 2H), 7.58 (d, J = 5.0 Hz, 2H), 7.72 (t, J = 3.9 Hz, 2H), 7.96 (d, J = 5.8 Hz, 1H), 8.17 (t, J = 4.8 Hz, 3H), 8.22 (d, J = 5.8 Hz, 1H), 8.30 (m, 3H), 8.40 (t, J = 4.8 Hz, 2H), 8.47 (d, J = 5.0 Hz, 1H), 8.75 ppm (d, J = 5.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ = 50.95, 54.67, 116.95, 121.64, 122.69, 123.02, 123.66, 123.77, 123.83, 124.41, 124.81, 125.46, 126.23, 126.95, 127.03, 127.10, 128.92, 129.61, 129.68, 130.06, 139.60, 146.69, 153.17 ppm. HRMS (FAB) calcd for $C_{29}H_{23}ClN_3O_4Zn$ $[M+ClO_4]^+$ 576.0663, found 576.0665.

*Synthesis of Compound **9***

A suspension of 1-pyreneethanol **8** (1 g, 4 mmol) in toluene (100 mL) was cooled to 0 °C followed by addition of phosphorus tribromide (0.5 mL, 5.3 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 had cooled to room temperature. The phases were separated, and the organic phase was washed with H_2O (50 mL×2), brine (50 mL×2), and dried over Mg_2SO_4. The yellow filtrate was concentrated to minimum volume. The yellow needle-like solid was collected and dried. The mother liquid was concentrated again and the crystallization process was repeated (combined yield = 1.1 g, 88%). m.p. = 124 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 5.23 (s, 2H), 8.02 (m, 5H), 8.21 (m, 3H), 8.35 ppm (d, J = 9.3 Hz, 1H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ = 32.28, 122.80, 124.58, 124.84, 125.07, 125.61, 126.26, 127.32, 127.67, 128.01, 128.22, 129.03, 130.51, 130.73, 131.17, 131.92 ppm.

*Synthesis of Compound **11***

2-bromoethylpyrene **9** (500 mg, 1.62 mmol), di-(2-picolyl)amine (DPA, **10**; 320 mg, 1.62 mmol), K_2CO_3 (500 mg), and potassium iodide (30 mg) were added to acetonitrile (50 mL). After stirred and refluxed for 10 h under a nitrogen atmosphere, the mixture was cooled to room temperature and the solvent was removed under reduced pressure, which was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 100:1) to afford **11**. Yield: 525 mg (76%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 2.89 (t, J = 7.6 Hz, 2H), 3.41 (t, J = 7.6 Hz, 2H), 3.88 (s, 4H), 6.88 (t, J = 6.8 Hz, 2H), 7.11–7.28 (m, 4H), 7.62 (d, J = 8.0 Hz, 1H), 7.76–7.99 (m, 8H), 8.39 ppm (d, J = 5.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ = 30.99, 55.67, 60.47, 121.93, 122.82, 123.40, 124.65, 124.82, 124.93, 124.97, 125.78, 126.64, 127.14, 127.47, 127.95, 128.87, 129.88, 130.85, 131.35, 134.69, 136.28, 148.97, 159.62 ppm. HRMS (ESI) calcd for $C_{30}H_{26}N_3$ $[M+H]^+$ 428.2127, found 428.2127.

*Synthesis of Compound **2***

$ZnClO_4 \cdot 6H_2O$ (504 mg, 1.35 mmol) in 1 mL methanol was added dropwise to a solution of **11** (300 mg, 0.7 mmol) in 20 mL $CHCl_3/CH_3CN$ (10:1), and the mixture was stirred for 30 min at room temperature. Then 50 mL ether was added slowly. After stirring for 10 h, the precipitate was collected to give **2** (60%) without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 3.06 (t, J = 5.0 Hz, 2H), 3.80 (t, J = 5.0 Hz, 2H), 4.25 (d, J = 10.2 Hz, 2H), 4.76 (d, J = 10.2 Hz, 2H), 7.63 (t, J = 3.9 Hz, 2H), 7.77 (d, J = 5.0 Hz, 2H), 8.03–8.35 (m, 11H), 8.62 ppm (d, J = 3.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ = 24.78, 54.92, 55.29, 121.90, 122.88, 123.06, 123.44, 123.62, 123.89, 124.06, 125.16, 125.71, 126.28, 126.42, 126.59, 127.08, 128.48, 129.18, 129.72, 132.05, 139.48, 146.48, 153.58 ppm. HRMS (FAB) calcd for $C_{30}H_{25}ClN_3O_4Zn$ $[M+ClO_4]^+$ 590.0820, found 590.0827.

Acknowledgements

This work was supported by grants from the National Research Foundation (NRF; 20100018895, 20100001481), the WCU (R31-2008-000-10010-0), a Herchel Smith Postdoctoral Fellowship Fund, and the Frances and Augustus Newman Foundation.

[1] a) Y. Zhou, Z. Xu, J. Yoon, *Chem. Soc. Rev.* **2011**, DOI:10.1039/C0CS00169D; b) X. J. Zhao, C. Z. Huang, *TrAC Trends Anal. Chem.* **2010**, *29*, 354–367; c) C. Spangler, M. Schaeferling, O. S. Wolfbeis, *Microchim. Acta* **2008**, *161*, 1–39; d) P. D. Beer, P. A. Gale, *Angew. Chem.* **2001**, *113*, 502–532; *Angew. Chem. Int. Ed.* **2001**, *40*, 486–516; e) S. K. Kim, D. H. Lee, J.-I. Hong, J. Yoon, *Acc. Chem. Res.* **2009**, *42*, 23–31; f) E. J. O'Neil, B. D. Smith, *Coord. Chem. Rev.* **2006**, *250*, 3068–3080; g) S. V. Khlyntseva, Y. R. Bazel, A. B. Vishnikin, V. Andruch, *J. Anal. Chem.* **2009**, *64*, 657–673; h) T. Sakamoto, A. Ojida, I. Hamachi, *Chem. Commun.* **2009**, 141–152; i) A. V. Eliseev, H.-J. Schneider, *J. Am. Chem. Soc.* **1994**, *116*, 6081–6088; j) S. Mizukami, T. Nagano, Y. Urano, A. Odani, K. Kikuchi, *J. Am. Chem. Soc.* **2002**, *124*, 3920–3925.

[2] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Wlater, in *Molecular Biology of the Cell*, *4th ed.*, Garland Science, New York, **2002**, p. 120.

[3] J. R. Knowles, *Annu. Rev. Biochem.* **1980**, *49*, 877–919.

[4] F. M. Ashcroft, F. M. Gribble, *Diabetologia* **1999**, *42*, 903–919.

[5] P. B. Dennis, A. Jaeschke, M. Saitoh, B. Fowler, S. C. Kozma, G. Thomas, *Science* **2001**, *294*, 1102–1105.

[6] a) A. Kornberg, *J. Biol. Chem.* **1988**, *263*, 1–4; b) X. Shen, G. Mizuguchi, A. Hamiche, C. Wu, *Nature* **2000**, *406*, 541–544.

[7] J. Berg, Y. P. Hung, G. Yellen, *Nat. Methods* **2009**, *6*, 161–166.

[8] a) Z. Xu, J. Yoon, D. R. Spring, *Chem. Soc. Rev.* **2010**, *39*, 1996–2006; b) Z. Xu, S. K. Kim, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 1457–1466; c) Z. Xu, X. Chen, H. N. Kim, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 127–1137; d) C.-H. Lee, H. Miyaji, D.-W. Yoon, J. L. Sessler, *Chem. Commun.* **2008**, 24–34; e) T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Kruger, F. M. Pfeffer, *Coord. Chem. Rev.* **2006**, *250*, 3094–3117; f) R. Martínez-Máñez, F. Sancanón, *Chem. Rev.* **2003**, *103*, 4419–4476; g) J. F. Callan, A. P. de Silva, D. C. Magri, *Tetrahedron* **2005**, *61*, 8551–8588; h) X. Chen, Y. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 2120–2135; i) P. A. Gale, *Acc. Chem. Res.* **2006**, *39*, 465–475; j) D. T. Quang, J. S. Kim, *Chem. Rev.* **2010**, *110*, 6280–6301; k) Y. Zhou, J. Won, J. Y. Lee, J. Yoon, *Chem. Commun.* **2011**, *47*, 1997–1999.

[9] a) D. D. Hackney, *ACS Chem. Biol.* **2010**, *5*, 353–354; b) S. Kunzelmann, M. R. Webb, *ACS Chem. Biol.* **2010**, *5*, 415–425; c) L. Vial, P. Dumy, *J. Am. Chem. Soc.* **2007**, *129*, 4884–4885.

[10] a) V. Amendola, G. Bergamaschi, A. Buttafava, L. Fabbrizzi, E. Monzani, *J. Am. Chem. Soc.* **2010**, *132*, 147–156; b) A. K. Nair, P. P. Neelakandan, D. Ramaiah, *Chem. Commun.* **2009**, 6352–6354.

[11] a) J. Y. Kwon, N. J. Singh, H. Kim, S. K. Kim, K. S. Kim, J. Yoon, *J. Am. Chem. Soc.* **2004**, *126*, 8892–8893; b) S. Wang, Y.-T. Chang, *J. Am. Chem. Soc.* **2006**, *128*, 10380–10381; c) P. P. Neelakandan, M. Hariharan, D. Ramaiah, *J. Am. Chem. Soc.* **2006**, *128*, 11334–11335; d) S. K. Kim, B.-S. Moon, J. H. Park, Y. I. Seo, H. S. Koh, Y. J. Yoon, K.-D. Lee, J. Yoon, *Tetrahedron Lett.* **2005**, *46*, 6617–6620; e) S. C. McCleskey, M. J. Griffin, S. E. Schneider, J. T. McDevitt, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 1114–1115; f) S. Amemiya, P. Buhlmann, Y. Umezawa, *Chem. Commun.* **1997**, 1027–1028.

[12] a) Z. Zeng, L. Spiccia, *Chem. Eur. J.* **2009**, *15*, 12941–12944; b) T.-H. Kwon, H. J. Kim, J.-I. Hong, *Chem. Eur. J.* **2008**, *14*, 9613–9619; c) M. Shionoya, T. Ikeda, E. Kimura, M. Shiro, *J. Am. Chem. Soc.* **1994**, *116*, 3848–3859.

[13] X. Chen, M. J. Jou, J. Yoon, *Org. Lett.* **2009**, *11*, 2181–2184.

[14] a) F. Sancenón, A. B. Descalzo, R. Martínez-Máñez, M. A. Miranda, J. Soto, *Angew. Chem.* **2001**, *113*, 2710–2713; *Angew. Chem. Int. Ed.* **2001**, *40*, 2640–2643; b) A. Ishida, Y. Yamada, T. Kamidate, *Anal. Bioanal. Chem.* **2008**, *392*, 987–994; c) A. Ghosh, A. Shrivastav, D. A. Jose, S. K. Mishra, C. K. Chandrakanth, S. Mishra, A. Das, *Anal. Chem.* **2008**, *80*, 5312–5319; d) Z. Kejík, K. Záruba, D. Michalík, J. Šebek, J. Dian, S. Pataridis, K. Volka, V. Král, *Chem. Commun.* **2006**, *42*, 1533–1535; e) C. Li, M. Numata, M. Takeuchi, S. Shinkai, *Chem. Asian J.* **2006**, *1–2*, 95–101; f) S. M. Butterfield, M. L. Waters, *J. Am. Chem. Soc.* **2003**, *125*, 9580–9581; g) P. L. Sazani, R. Larralde, J. W. Szostak, *J. Am. Chem. Soc.* **2004**, *126*, 8370–8371.

[15] a) C. Li, M. Numata, M. Takeuchi, S. Shinkai, *Angew. Chem.* **2005**, *117*, 6529–6532; *Angew. Chem. Int. Ed.* **2005**, *44*, 6371–6374; b) Y. Kanekiyo, R. Naganawa, H. Tao, *Chem. Commun.* **2004**, 1006–1007; c) C. Bazzicalupi, S. Biagini, A. Bencini, E. Faggi, C. Giorgi, I. Matera, B. Valtancoli, *Chem. Commun.* **2006**, 4087–4089; d) Z. Yao, X. Feng, W. Hong, C. Li, G. Shi, *Chem. Commun.* **2009**, 4696–4698; e) A. J. Moro, P. J. Cywinski, S. Korsten, G. J. Mohr, *Chem. Commun.* **2010**, *46*, 1085–1087; f) H. Wu, C. He, Z. Lin, Y. Liu, C. Duan, *Inorg. Chem.* **2009**, *48*, 408–410; g) S. E. Schneider, S. N. O'Neil, E. V. Anslyn, *J. Am. Chem. Soc.* **2000**, *122*, 542–543; h) A. Ojida, I. Takashima, T. Kohira, H. Nonaka, I. Hamachi, *J. Am. Chem. Soc.* **2008**, *130*, 12095–12101; i) V. Pivovarenko, O. B. Vadzyuk, S. O. Kosterin, *J. Fluoresc.* **2006**, *16*, 9–15; j) Y. Miao, J. Liu, F. Hou, C. Jiang, *J. Lumin.* **2006**, *116*, 67–72; k) A. B. Descalzo, M. D. Marcos, R. Martinez-Máñez, J. Soto, D. Beltrán, P. Amorós, *J. Mater. Chem.* **2005**, *15*, 2721–2731; l) K. M. K. Swamy, S. K. Kwon, H. N. Lee, S. M. Shanthakumar, J. S. Kim, J. Yoon, *Tetrahedron Lett.* **2007**, *48*, 8683–8686.

[16] a) D. M. Wolfe, Y. Zhang, G. P. Roberts, *J. Bacteriol.* **2007**, *189*, 6861–6869; b) P. Jiang, A. J. Ninfa, *Biochemistry* **2007**, *46*, 12979–12996.

[17] M. W. Hosseini, A. J. Blacker, J.-M. Lehn, *J. Am. Chem. Soc.* **1990**, *112*, 3896–3904.

[18] a) J. A. Aguilar, E. Garcia-España, J. A. Guerrero, S. V. Luis, J. M. Llinares, J. F. Miravet, J. A. Ramirez, C. Soriano, *J. Chem. Soc. Chem. Commun.* **1995**, 2237–2238; b) M. Morikawa, M. Yoshihara, T. Endo, N. Kimizuka, *J. Am. Chem. Soc.* **2005**, *127*, 1358–1359; c) M. I. Burguete, E. García-España, L. López-Diago, S. V. Luis, J. Miravet, D. Sroczynski, *Org. Biomol. Chem.* **2007**, *5*, 1935–1944; d) C. Bazzicalupi, A. Bencini, S. Biagini, E. Faggi, S. Meini, C. Giorgi, A. Spepi, B. Valtancoli, *J. Org. Chem.* **2009**, *74*, 7349–7363; e) A. K. Nair, P. P. Neelakandan, D. Ramaiah, *Chem. Commun.* **2009**, 6352–6354.

[19] Z. Xu, N. J. Singh, J. Lim, J. Pan, H. N. Kim, S. Park, K. S. Kim, J. Yoon, *J. Am. Chem. Soc.* **2009**, *131*, 15528–15533.

[20] a) W. Saenger in *Principles of Nuclei Acid Structure*, Springer-Verlag, New York, **1988**; b) J. Rebek, Jr., B. Askew, P. Ballester, C. Buhr, S. Jones, D. Nemeth, K. Williams, *J. Am. Chem. Soc.* **1987**, *109*, 5033–5035; c) A. D. Hamilton, D. Van Engen, *J. Am. Chem. Soc.* **1987**, *109*, 5035–5036.

[21] a) M. E. Østergaard, P. Cheguru, M. R. Papasani, R. A. Hill, P. J. Hrdlicka, *J. Am. Chem. Soc.* **2010**, *132*, 14221–14228; b) D. M. Kolpashchikov, *Chem. Rev.* **2010**, *110*, 4709–4723; c) P. Conlon, C. J. Yang, Y. Wu, Y. Chen, K. Martinez, Y. Kim, N. Stevens, A. A. Marti, S. Jockusch, N. J. Turro, W. Tan, *J. Am. Chem. Soc.* **2008**, *130*, 336–342; d) A. Okamoto, Y. Ochi, I. Saito, *Chem. Commun.* **2005**, 1128–1130.

[22] a) U. Förster, K. Lommel. D. Sauter, C. Grunewald, J. W. Engels, J. Wachtveitl, *ChemBioChem* **2010**, *11*, 664–672; b) G. Wang, G. V. Bobkov, S. N. Mikhailov, G. Schepers, A. Van Aerschot, J. Rozenski, M. Van der Auweraer, P. Herdewijn, S. De Feyter, *ChemBioChem* **2009**, *10*, 1175–1185; c) A. A. Marti, X. Li, S. Jockusch, Z. Li, B. Raveendra, S. Kalachikov, J. J. Russo, I. Morozova, S. V. Puthanveettil, J. Ju, N. J. Turro, *Nucleic Acids Res.* **2006**, *34*, 3161–3168; d) M. K. Smalley, S. K. Silverman, *Nucleic Acids Res.* **2006**, *34*, 152–166.

[23] a) F. Schmidt, S. Stadlbauer, B. König, *Dalton Trans.* **2010**, *39*, 7250–7261; b) H.-W. Rhee, C.-R. Lee, S.-H. Cho, M.-R. Song, M. Cashel, H. E. Choy, Y.-J. Seok, J.-I. Hong, *J. Am. Chem. Soc.* **2008**, *130*, 784–785; c) Y. Kanekiyo, R. Naganawa, H. Tao, *Chem. Commun.* **2004**, 1006–1007; d) F. D. Lewis, Y. Zhang, R. L. Letsinger, *J. Am. Chem. Soc.* **1997**, *119*, 5451–5452.

[24] a) M. Manoharan, K. L. Tivel, M. Zhao, K. Nafisi, T. L. Netzel, *J. Phys. Chem.* **1995**, *99*, 17461–17472; b) T. L. Netzel, M. Zhao, K. Nafisi, J. Headrick, M. S. Sigman, B. E. Eaton, *J. Am. Chem. Soc.* **1995**, *117*, 9119–9128.
[25] a) S. Nishizawa, Y. Kato, N. Teramae, *J. Am. Chem. Soc.* **1999**, *121*, 9463–9464; b) H. K. Cho, D. H. Lee. J.-I. Hong, *Chem. Commun.* **2005**, 1690–1692.
[26] a) M. J. Kim, K. M. K. Swamy, K. M. Lee, A. R. Jagdale, Y. Kim, S.-J. Kim, K. H. Yoo, J. Yoon, *Chem. Commun.* **2009**, 7215–7217; b) H. N. Lee, Z. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S.-J. Kim, J. Yoon, *J. Am. Chem. Soc.* **2007**, *129*, 3828–3829; c) H. N. Lee, K. M. K. Swamy, S. K. Kim, J.-Y. Kwon, Y. Kim, S.-J. Kim, Y. J. Yoon, J. Yoon, *Org. Lett.* **2007**, *9*, 243–246.

Received: February 8, 2011
Published online: April 19, 2011

