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**PAPER**

# pH-Responsive self-duplex of $^{Py}$A-substituted oligodeoxyadenylate in graphene oxide solution as a molecular switch†

**Jeong Wu Yi,[a] Jaesung Park,[b] Kwang S. Kim[b] and Byeang Hyean Kim*[a]**

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In this paper, we demonstrated a highly discriminated and reliable molecular switch based on the interaction between the self-duplex of $^{Py}$A-substituted oligodeoxyadenylate and graphene oxide in aqueous solution. This system showed a clear on/off state through the association and dissociation of $^{Py}$A-modified oligodeoxynucleotide with graphene oxide in manipulated pH conditions, high amplitude efficiency for at least 50 cycles, and rapid response within seconds. Our molecular switch system has high reproducibility and simple operation by using pH stimulus.

## Introduction

Fluorescent nucleic acid systems are widely applied in various fields, from fundamental biological probes to optical devices, because hydrogen bonding between complementary bases yields a well-ordered structure and an ability to predict the secondary structure.[1] Nucleic acids are also used as a scaffold for arranging aromatic fluorophore assemblies, either by insertion into the DNA base pairs or by stacking *via* the duplex.[2] Among the numerous aromatic fluorophores, pyrene is popular because it has high quantum efficiency and the fluorescence is susceptible to environmental conditions. Moreover, proper stacking of pyrenes gives the excimer fluorescence, which is widely used with fluorescent devices for nucleic acid systems.[3]

Previously we reported a unique fluorescent phenomenon of pyrene-modified oligodeoxyadenylates; specifically, the insertion of pyrene-substituted deoxyadenosine ($^{Py}$A) in oligodeoxyadenylates, a 1,4-relationship, gives a reddish fluorescence emission at 570–580 nm.[4] We have already applied this novel property to detect single nucleotide polymorphisms in DNA and to probe a specific protein.[5]

Optical devices based on fluorescent nucleic acids, especially molecular switch and logic gate devices, were mainly developed to embody a molecular computer system. These devices based on fluorescent nucleic acids operate well under manipulated conditions, but the high background signal and waste accumulation remain problems to be solved.[6]

Recently, researchers have focused on the hydrophilicity and fluorescence-quenching phenomena of graphene oxide (GO). The oxidized functional groups of GO, a highly oxidized form of graphene, allow it to be suspended in aqueous and organic solvents at high concentrations, and the residual aromatic regions offer sites for interaction with nucleobases and aromatic fluorophores. By using these properties, we found an improved signal to background ratio of quencher-free molecular beacon with GO, and other research groups have widely applied this finding to develop biosensors, electronic materials, and delivery system for therapeutic oligonucleotides.[7] In spite of its highly efficient quenching ability, use of GO is limited in molecular devices based on fluorescent oligonucleotide systems. Because a complementary strand is needed to reproduce fluorescence emission in a GO–oligodeoxyadenylate system, the double strand accumulates as waste and a continuous supply of each strand is needed to perform the reversible system. Consequently, we took a new approach to design a molecular device based on fluorescent oligonucleotide systems with GO.

We focused on the charge interaction between the oxidized surface of GO and the phosphate backbone of oligonucleotides. Even though these two components have a negative charge–charge repulsion, the π–π interaction between the residual aromatic surface of GO and the aromatic parts in fluorescent oligonucleotides, nucleobases and fluorophores, compensate for this repulsion. We thought accurate pH changes could control the association and dissociation of the fluorescent oligonucleotides from GO without the need for complementary strands. The pH modulation is a popular external stimulus in a DNA nano-device with a pH-responding cytosine-rich strand, known as an i-motif or triplex structure, because pH change is rapid and easily controllable; furthermore, it can be designed as an electrical device by using water electrolysis.[8]

[a]*Laboratory for Modified Nucleic Acid Systems, Department of Chemistry, Division of IBB, Pohang University of Science and Technology, Pohang, South Korea. E-mail: bhkim@postech.ac.kr; Fax: 82 54 279 3399; Tel: 82 54 279 2115*

[b]*Center for Superfunctional Materials, Department of Chemistry, BK School of Molecular Science, Pohang University of Science and Technology, Pohang, 790-784, South Korea. E-mail: kim@postech.ac.kr; Fax: 82 54 279 3399; Tel: 82 54 279 2110*

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Herein, we report a new type of molecular switch based on the pH-responsive interaction between a self-duplex of $^{Py}$A-substituted oligodeoxyadenylate and GO in an aqueous medium. Fig. 1 depicts the pH-driven molecular switch consisting of oligodeoxyadenylate-containing pyrene-modified deoxyadenosine with GO.

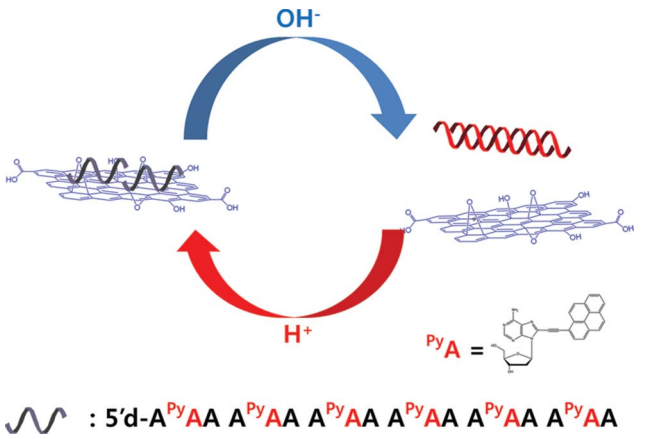


**Fig. 1** Schematic representation of the molecular switch based on the interaction between self-duplex of $^{Py}$A-substituted oligodeoxyadenylate and GO.

## Results and discussion

Table 1 shows designed fluorescent oligonucleotides containing $^{Py}$A. **ODN A** and **C** are modified oligodeoxyadenylates, and **ODN B** and **D** are control sequences.

We checked the fluorescence emission and circular dichroism (CD) spectra of oligonucleotides under different pH conditions in the absence of GO. $^{Py}$A-substituted oligodeoxyadenylates, **ODN A** and **C**, showed negligible shifts in fluorescence intensity with pH changes; however, their control sequences, **ODN B** and **D**, showed significantly reduced fluorescence intensity in acidic conditions (Fig. S2 in ESI†). We speculated that the protonated cytosines and guanines in the control sequences caused an undefined structure and reduced their fluorescence intensity in acidic conditions.

The CD spectra of the pyrene-substituted oligonucleotides (**ODN A**–**D**) showed discriminated results regarding their structural differences with their fluorescent spectra with varying pH. **ODN A** at pH 7.0 displayed a CD spectrum with a strong positive maximum at 217 nm and a shoulder at 232 nm, a weak positive band at 275 nm, and negative bands located at 250 and 206 nm. This spectrum is characteristic of single-stranded polyadenylates, which has previously been reported.[9] This characteristic CD spectrum was conserved at high and low pH conditions. Unexpectedly, **ODN B** at pH 7.0 and 9.0 displayed CD spectra similar to that of **ODN A**, but the exact structure could not be determined (Fig. S3 in ESI†). CD spectra of multi-pyrene-substituted oligonucleotides could be clearly discriminated. **ODN C** at pH 7.0 and 9.0 displayed characteristic CD spectra of self-duplex structure with a diminished positive band at 217 nm, intensive positive band at 278 nm with a shoulder at 260 nm, a 320-nm positive band caused by pyrene, and a negative band located at 250 nm.[4,10] **ODN D** did not show any characteristic CD spectra from varying pH (Fig. 2).

**Table 1** Fluorescent oligonucleotides used in this study

| Code | Sequence |
|---|---|
| **ODN A** | 5′d-A$^{Py}$AA AAA AAA AAA AAA AAA |
| **ODN B** | 5′d-T$^{Py}$AC GAC TAT GAC CAG TAG |
| **ODN C** | 5′d-A$^{Py}$AA A$^{Py}$AA A$^{Py}$AA A$^{Py}$AA A$^{Py}$AA A$^{Py}$AA |
| **ODN D** | 5′d-T$^{Py}$AC G$^{Py}$AC T$^{Py}$AT G$^{Py}$AC C$^{Py}$AG T$^{Py}$AG |

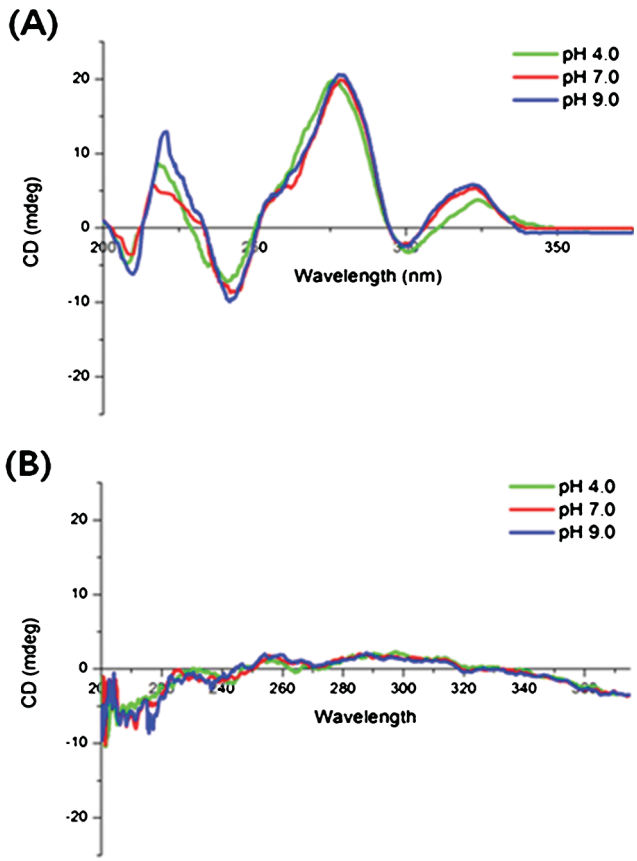


**Fig. 2** Comparison between the CD spectra of multi-$^{Py}$A-substituted oligodeoxyadenylate and multi-$^{Py}$A-substituted hetero oligonucleotide at variable pH conditions. (A) **ODN C** and (B) **ODN D**.

To determine the optimal concentration of GO, we checked the fluorescence emission of $^{Py}$A-substituted fluorescent oligonucleotides at various concentrations of GO. In the absence of GO, the fluorescence emission spectra of mono-$^{Py}$A-substituted strands, **ODN A** and **B**, were recorded at 460 nm caused by $^{Py}$A, and those of the multi-$^{Py}$A-substituted strands, **ODN C** and **D**, were recorded at the more red-shift wavelength, 575 and 520 nm, respectively.

The mono $^{Py}$A-substituted oligonucleotides, **ODN A** and **B**, were almost quenched at 0.121 mg ml$^{-1}$ of GO (Fig. S4 in ESI†). However, the multi $^{Py}$A-substituted oligonucleotides, **ODN C** and **D**, gave an interesting result. Despite the same number of substituted $^{Py}$As, the oligodeoxyadenylate, **ODN C**, was almost quenched at 0.123 mg ml$^{-1}$ GO, but the control strand, **ODN D**, was almost quenched at 0.048 mg ml$^{-1}$ GO (Fig. 3). This was interpreted to mean that multi-$^{Py}$A-substituted oligodeoxyadenylate (**ODN C**) did not interact well with the surface of GO, despite the presence of multi-pyrenyl moieties.

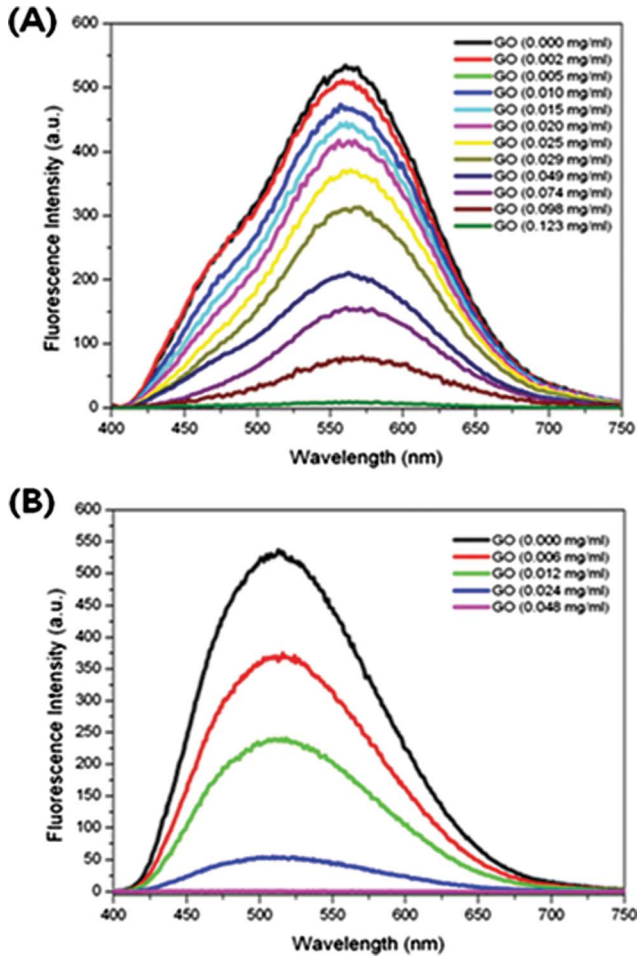


**Fig. 3** Fluorescence emission spectra of $^{Py}$A-substituted oligonucleotides (**ODN C** and **D**) in the presence of GO at various concentrations in 10 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$ at 20 °C. (A) **ODN C** and (B) **ODN D** fluorescence emission spectra in GO solution.

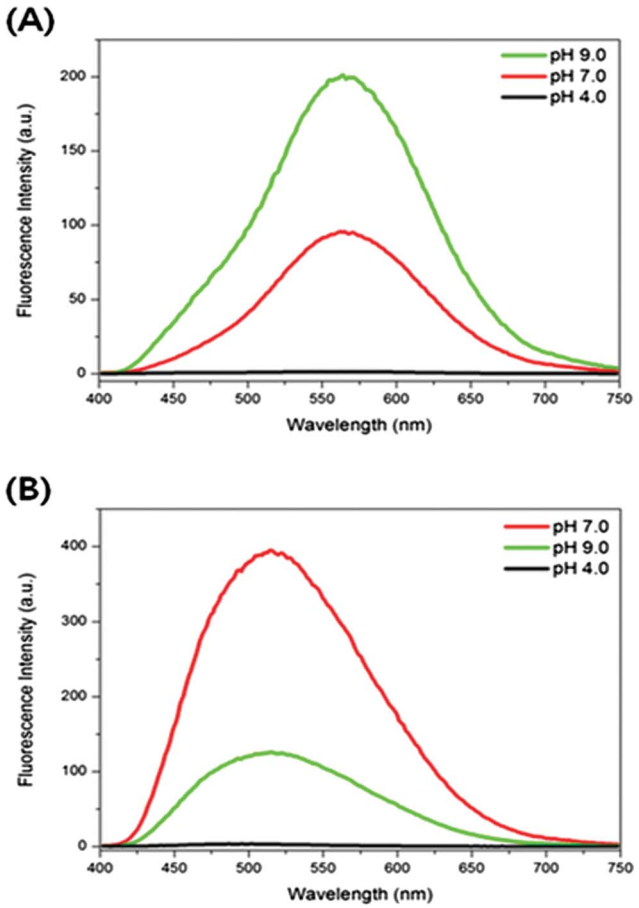


**Fig. 4** Fluorescence emission spectra of $^{Py}$A-substituted oligonucleotides (**ODN C** and **D**) at a fixed concentration of GO related to pH shifts. Spectra were recorded by continuously changed pH, from pH 7.0, 4.0 to 9.0 at 20 °C in 10 mM Tris–HCl (pH 7.0), 10 mM $MgCl_2$, 100 mM NaCl. Each concentration of ODN was 3.0 μM. (A) **ODN C** and (B) **ODN D** fluorescence emission spectra in GO solution.

Before performing the cycle for a molecular switch, we checked the fluorescence change of each strand at a fixed concentration of GO related to pH shifts. We chose the concentration of GO that had half the fluorescence intensity compared to that without GO. Each concentration of GO in the **ODN**/GO system was as follows: 0.035 mg ml$^{-1}$ (**ODN A** and **ODN B**), 0.040 mg ml$^{-1}$ (**ODN C**), and 0.012 mg ml$^{-1}$ (**ODN D**).

Generally, all $^{Py}$A-substituted oligonucleotides showed the almost quenched state in acidic conditions, pH 4.0, and regeneration of fluorescence in basic conditions, pH 9.0. However, **ODN D**, a multi-$^{Py}$A–substituted hetero strand, showed a low efficiency of fluorescence regeneration. The regenerative fluorescence intensity of **ODN D** did not reach the original intensity at neutral conditions, although other fluorescent oligonucleotides showed increased fluorescent intensity relative to that at neutral conditions. Because the multiple pyrenes in the hetero-strand provided strong binding with the surface of GO without any stable structure at basic conditions, **ODN D** could not be released from the surface of GO efficiently. Compared with **ODN D**, **ODN C** showed a conserved amplitude, because its self-duplex structure disturbed the binding between the pyrenyl moiety and GO surface (Fig. 4, Fig. S5 in ESI†).

Next, we investigated whether $^{Py}$A-substituted oligonucleotides were capable of performing a reversible pH-induced switch with GO from pH 4.0 to pH 9.0. To each buffered solution of 3.0 μM fluorescent oligonucleotide at pH 7 with GO, we added acid (2 N HCl) and base (2 N NaOH) alternately to switch the pH of the solution from 4.0 to 9.0, reversibly. Molecular switching of $^{Py}$A-modified fluorescent oligonucleotides with GO was visualized by monitoring the fluorescence signal at each respective wavelength at which signals were highly discriminated for the association and dissociation forms with GO at the initial cycle. However, multiple cycling of each molecular switching system showed clearly different decreasing rates of amplitude efficiency. The decrease in the intensity of mono-$^{Py}$A-substituted oligonucleotides, **ODN A** and **ODN B**, was clearly observed within 10 cycles. The **ODN A**, mono-$^{Py}$A-substituted oligodeoxyadenylate, was shown the decreased amplitude below 80% of fluorescence on-signal over 11 cycles. The **ODN B**, control strand of **ODN A**, was shown to have an even weaker conservation of amplitude relative to **ODN A** (Fig. S6 in ESI†).

 

In the multi-$^{Py}$A-substituted oligonucleotides, significantly different results were found. **ODN C**, the multi-$^{Py}$A-substituted oligodeoxyadenylate, sustained an amplitude over 92% yields. Over 50 cycles the diminution in the amplitude of the system, **ODN C**/GO, was negligible. In contrast to **ODN C**, **ODN D** showed a completely degenerated amplitude below 50% yields after the third cycle, indicating that the self-duplex structure of the modified oligodeoxyadenylate is an essential factor for a long duration of our molecular switch system (Fig. 5).

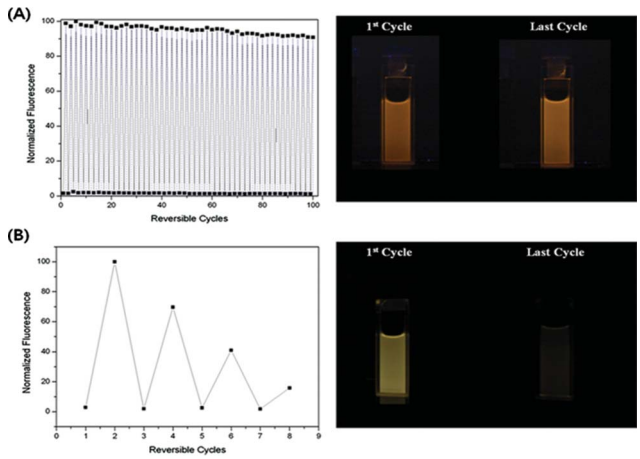


**Fig. 5** Cycling of the molecular switch as observed by fluorescence spectroscopy. Excitation at 380 nm and emission monitored at 570 nm (**ODN C**) and 520 nm (**ODN D**) in 10 mM Tris-HCl buffer containing 100 mM NaCl and 10 mM $MgCl_2$ at 20 °C. (A) Cycling of the **ODN C**/GO system and corresponding photographs of the first and last (50th) cycling solutions in a basic environment (pH 9.0) under UV irradiation. (B) Cycling of the **ODN D**/GO system and corresponding photographs of the first and last (4th) cycling solutions in a basic environment (pH 9.0) under UV irradiation.

Since self-assembly of $^{Py}$A-substituted oligodeoxyadenylate (**ODN C**) was involved in the unique fluorescence emission at 570 nm, the operation of designed molecular switch could be easily detected by fluorescence spectra. Moreover, the **ODN C**/GO system clearly showed a switchable signal *via* pH changes because GO gave sufficiently quenched state at pH 4.0.

## Conclusion

In summary, we have designed and performed a molecular switch based on self-assembly of multi-$^{Py}$A-substituted oligodeoxyadenylate (**ODN C**) that can operate by pH regulation in a GO solution. This molecular switch signal was visualized in a high pH environment (pH 9.0) with the dissociation and self-assembly of **ODN C** in GO solution, which results in an increase in fluorescence signal at 570 nm. The binding and response of the **ODN C** to the GO surface was rapid and capable of discriminating the off-state at a low pH (pH 4.0). Moreover, the **ODN C**/GO system showed a high reproducibility of fluorescence signal through multiple cycles and displayed the real on/off state *via* the different interactions between GO and pyrenyl-modified oligodeoxyadenylate through different pH conditions. This is the first attempt to establish a reversible device based on the interaction between DNA and GO. This system will provide possibilities for developing new pH-driven nano-devices.

## Experimental section

### Preparation of GO solution

We prepared GO according to the modified Hummers method and investigated its structural properties by using an atomic force microscope (MMAFM-2/393EX, Digital Instruments) and an infrared and Raman spectrometer (RAMALOG-10I, SPEX).[11] Manufactured graphite oxide, 2.24 mg, was dissolved in 10 ml of deionized water and ultrasonicated by a sonicator (B1510, BRANSON) for 2 h. Then the GO solution was centrifuged at 2800*g* for 10 min. The small amounts of graphites and aggregates of GO at the bottom of the centrifuge tube were discarded, and the supernatant was collected. AFM image showed that the thickness of the GO sheet was about 1 nm, which indicates a good suspension consisting of monolayer GO in water (Fig. S1 in ESI†).

### Preparation of $^{Py}$A-substituted fluorescent oligonucleotides

The modified nucleotide pyrene-substituted deoxyadenosine ($^{Py}$A) was synthesized through Sonogashira coupling of an ethynyl pyrene at the C8-position of a 2′-deoxyadenosine.[12] We used standard phosphoramidite methods and a DNA synthesizer (POLYGEN, Germany) to incorporate $^{Py}$A into the designed fluorescent oligonucleotides [18-mer oligodeoxynucleotide (ODNs) **A–D**] given in Table 1.[13]

Synthesized oligonucleotides were purified by using high performance liquid chromatography (Agilent 1100 series, USA) and characterized by using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer (PE Biosystems Voyager System 4095 instrument) in the positive-ion mode and a 1 : 1 mixture of 3-hydroxypicolinic acid (0.35 M) and ammonium citrate (0.1 M) as the matrix; the accelerating voltage was 25 kV (Table S1 in ESI†). Purified oligonucleotides were dissolved in 1.0 ml of nuclease-free water as the stock solution and quantified by using a UV-vis spectrophotometer. All oligonucleotides used in this study were prepared at a concentration of 3.0 μM or 1.5 μM with pH 7.0 Tris-buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, and 100 mM NaCl).

UV-vis absorption spectra and fluorescence emission spectra of designed fluorescent oligonucleotides were recorded by using a UV-vis spectrophotometer (CARY 100 Conc, VARIAN) and a spectrofluorophotometer (CARY Eclipse, VARIAN), respectively.

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