Helical pyrene-array along the outside of duplex RNA†

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A helical pyrene-array was formed by incorporation of multiple pyrenes into the sugar residues of duplex RNA leading to strong excimer fluorescence.

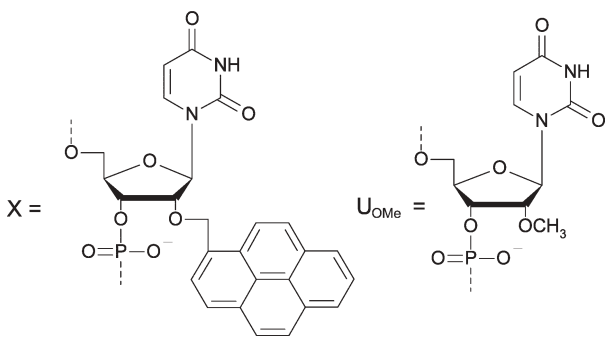
Aromatic π-arrays have attracted considerable interest because of their applicability to molecular devices conducting excitonic energy and electron migration. DNA and RNA can be suitable building blocks for the construction of nano-scale molecular architectures, since they have unique helical structures, molecular recognition properties, and well-developed strategies to incorporate a wide variety of π-aromatics.[1] From this point of view, several attempts have been made to construct aromatic chromophore-arrays using DNA.[2] For instance, the DNA involving multiple Methyl Red moieties in sequence form H* aggregate in the single stranded state which is converted into another aggregated structure by hybridization.[2a] Multiple incorporation of pyrenes at the terminal residues of the single stranded DNA results in pyrene excimer fluorescence.[2b] In these systems, however, the chromophore arrays of interest were formed within the single stranded region of DNA. In addition, extra nucleotides at the termini of the arrays are necessary to form duplex structures, because the non-nucleosidic residue carrying the chromophore has no base pairing ability. We have previously developed a method for incorporation of polycyclic aromatic hydrocarbons into the 2′-sugar position of DNA or RNA, and found that the attached π-aromatic is projected outward from the modified RNA duplexes.[3] When two pyrenes were introduced consecutively in duplex RNA, pyrene excimer emission was observed with a high quantum yield.[4] We therefore expected that our method might be easily applicable to the incorporation of multiple pyrenes into double helical RNA to generate π-aromatic arrays. Herein we describe the first example of helical pyrene-arrays along double stranded RNA with a remarkably strong excimer fluorescence.

The RNAs possessing multiple pyrenes employed in the present work were synthesized according to the method described in an earlier publication[3a] and are listed in Scheme 1. Table 1 indicates the T_m values for duplexes of pyrene-modified RNA (**P1**–**P4**) and its corresponding unmodified RNA (**P0**) formed with rA_{20} in a pH 7.0 buffer solution containing 0.1 M or 1 M NaCl. Under these conditions, the T_ms of the **P*n*** (***n*** $\geqslant$ 1) duplexes were slightly different by 0 to 3 °C from those of the **P0** duplex. The incorporation of multiple pyrenyl groups at the 2′-position of the RNA duplexes, therefore, does not show any significant effects on duplex stability.

Absorption spectra of the single stranded pyrene-RNAs exhibited overlapping pyrene and nucleobase absorptions below 300 nm and the 0–0 absorption bands for the ^1L_a transition of pyrene in the region of 300–370 nm.[5] No shift of the ^1L_a transition absorption bands were observed with the number of incorporated pyrenes. In contrast, the pyrene absorption band shifted to higher energy with an increasing number of incorporated pyrenes for double stranded **P*n*** (***n*** $\geqslant$ 2) (Fig. 1). Furthermore, the peak-to-valley intensity ratio (P_A) decreased from 1.20 for the **P1** duplex to 1.13 for the **P4** duplex.[6]

Fig. 2 shows the fluorescence spectra of the pyrene modified RNA in the double stranded state with excitation in the red-edge of the absorption band (350 nm). In the case of **P1**, the pyrene

P0; 5′- $(U_{OMe})_{19}$ U -3′
P1; 5′- $(U_{OMe})_9$ X $(U_{OMe})_9$ U -3′
P2; 5′- $(U_{OMe})_9$ XX $(U_{OMe})_8$ U -3′
P3; 5′- $(U_{OMe})_9$ XXX $(U_{OMe})_7$ U -3′
P4; 5′- $(U_{OMe})_8$ XXXX $(U_{OMe})_7$ U -3′



Scheme 1 Sequences of pyrene modified RNAs.

Table 1 T_m values of **P*n*** duplexes

	T_m[a]/°C	
	Buffer A[b]	Buffer B[c]
ds**P0**	45.0	61.9
ds**P1**	46.5	60.7
ds**P2**	47.5	59.8
ds**P3**	46.6	58.7
ds**P4**	42.7	58.8

[a] Measurements were carried out at 260 nm for a 1 : 1 mixture of oligonucleotides with an increase in temperature from 20 to 80 °C with a rate of 0.5 °C min^{-1}. rA_{20} was use for the complementary strand. [b] In a buffer containing 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0. [c] In a buffer containing 0.01 M sodium phosphate and 1 M NaCl, adjusted to pH7.0 .

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† Electronic supplementary information (ESI) available: Melting curves for the pyrene modified RNA duplex. See http://dx.doi.org/10.1039/b507808c

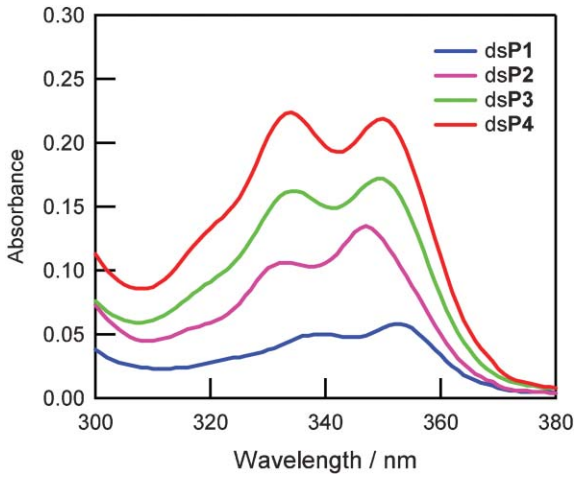

Fig. 1 Absorption spectra of **P*n*** (***n*** $\geqslant$ 1) duplexes (5×10^{-5} M). Measurements were carried out at room temperature in a buffer containing 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0.

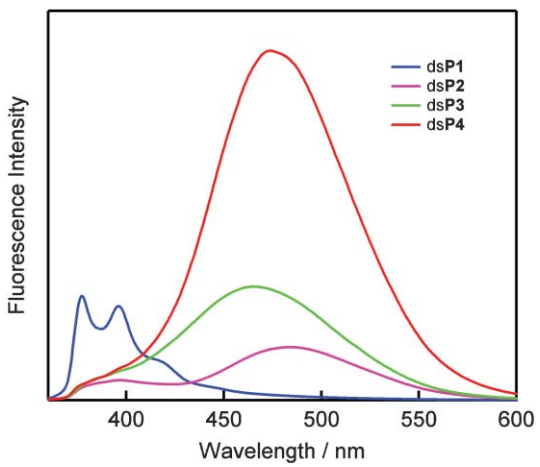

Fig. 2 Fluorescence spectra (λ_{ex} = 350 nm) of **P*n*** (***n*** $\geqslant$ 1) duplexes (5×10^{-5} M). Measurements were carried out at room temperature in a buffer containing 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0.

monomer emission was observed with peaks of 378 and 396 nm. When multiple pyrenes were incorporated, pyrene excimer emission was observed at around 480 nm as well as monomer emission. In particular, the 350 nm excitation for **P3** and **P4** yielded extremely strong excimer emission while the monomer emission was barely observed. Interestingly, with an increase in the number of incorporated pyrenes, the excimer intensity becomes much stronger than the estimated intensity by the simple sum of the pyrene excimer emission compared between ds **P2** and **P4**. The emission maximum of the excimer shifted to shorter wavelengths with an increase in the number of pyrenes. Biexponential fluorescence decays with short- and long-lived components were observed for **P3** and **P4**. The content of the short-lived component for **P4** is larger than that for **P3**.[7] Similar biexponential fluorescence decays have been reported for 1,*n*-bis(1-pyrenyl)-alkanes; the long- and short-lived components are ascribed to fully and partially overlapped excimer geometry, respectively.[8]

Fig. 3 shows the CD spectra of the **P*n*** duplexes. A typical CD signal of the A-form RNA helix in the range of 200–300 nm and a

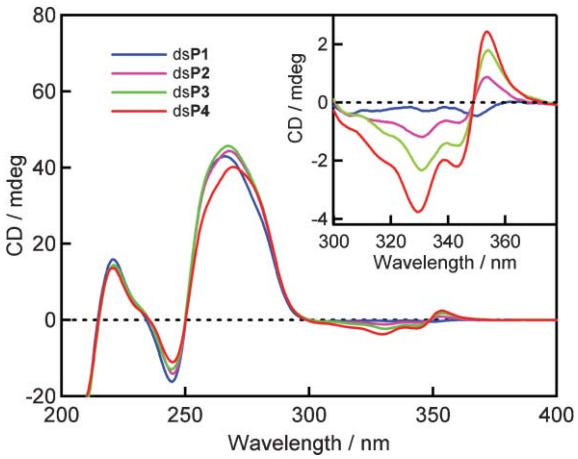

Fig. 3 CD spectra of **P*n*** (***n*** $\geqslant$ 1) duplexes (5×10^{-5} M). Measurements were carried out at room temperature in a buffer containing 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0.

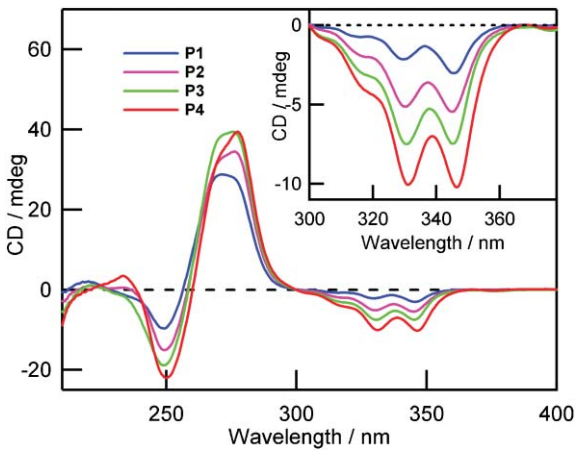

Fig. 4 CD spectra of single stranded **P*n*** (***n*** $\geqslant$ 1) (5×10^{-5} M). Measurements were carried out at room temperature in a buffer containing 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0.

negative induced CD signal in the range of 300–380 nm appeared for the **P1** duplex. For the **P*n*** (***n*** $\geqslant$ 2) duplexes, positive Cotton effects occurred in the range of the ^1L_a transition of pyrene and the positive Cotton effect was intensified with an increase in the number of pyrenes. On the other hand, no exciton coupled CD signal was observed for the single stranded **P*n***; all the vibrational bands within the ^1L_a transition of pyrene appeared at the same polarization (negative) as shown in Fig. 4. The intensity of the induced CD signal linearly increased with an increase in the number of the incorporated pyrenes.

All the observations made in the absorption, fluorescence and CD spectral studies are consistent with the proposed structures of the helical pyrene-arrays shown in Fig. 5. In the **P*n*** (***n*** $\geqslant$ 2) constructs, a helical pyrene-array is formed along the outside of the right hand RNA duplex. The pyrenes in the arrays take partially overlapped conformations with π-stacking in the ground state. The pyrene excimer fluorescence becomes exceptionally strong with an increase in the number of incorporated pyrenes. We anticipate that the present approach would be applicable to construct a variety of π-aromatic arrays of interest using a RNA double helix.

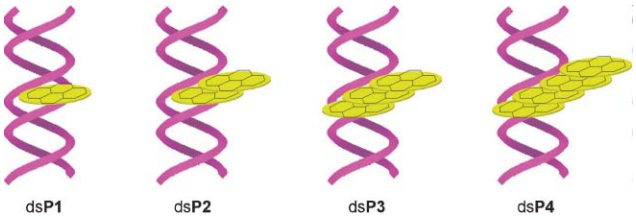

Fig. 5 Proposed structures for helical pyrene-array formed outside double stranded RNA.

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6 The peak-to-valley intensity ratio (P_A) can be used for evaluating the association of pyrenes. In this work, P_A was calculated from the absorption intensity of the red-edge band and that of the adjacent minimum at shorter wavelength. See: F. M. Winnik, *Chem. Rev.*, 1993, **93**, 587–614.

7 The calculated fluorescence lifetimes were 20.6 ns (22.6%), 71.0 ns (77.4%) for **P3**, and 21.5 ns (41.3%), 68.0 ns (58.7%) for **P4**. Fluorescence lifetime measurements were carried out at room temperature in the same buffer used for the steady state fluorescence measurements.

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