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# Fluorescent Sequence-Specific dsDNA Binding Oligomers

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Sequence-specific detection methods for double-stranded DNA (dsDNA) that obviate the need for denaturation would provide useful tools for bioorganic chemistry and genetics.[1] Previous efforts, such as molecular beacons[2] or peptide nucleic acid−thiazole orange (PNA-TO) conjugates,[3] require harsh denaturation conditions for hybridization to single-stranded DNA.[2−4] Previous efforts from our laboratory for the sequence-specific detection of dsDNA have focused on pyrrole−imidazole (Py-Im) polyamide−fluorophore conjugates, such as tetramethylrhodamine (TMR) or thiazole orange (TO), that bind in the minor groove of DNA.[5−7] TMR fluorescence was shown to be quenched when the fluorophore was covalently linked to the ring nitrogen of a pyrrole recognition element within a polyamide.[5] Remarkably, fluorescence was restored in a sequence-dependent manner upon binding to dsDNA.[5] Similarly, polyamide−TO intercalator conjugates also demonstrate fluorescence enhancement in the presence of match dsDNA.[6]

Having established Py-Im polyamide−dye conjugates as a suitable platform for sequence-specific fluorescent dsDNA detection,[5,6] we sought to develop a new class of fluorescent DNA binders wherein the fluorescent moiety is an integrated part of the recognition modules. We report here the design of sequence-specific fluorescent dsDNA-binding oligomers (Figure 1) which incorporate multiple 6-5 fused dimer recognition modules[8] and show a marked

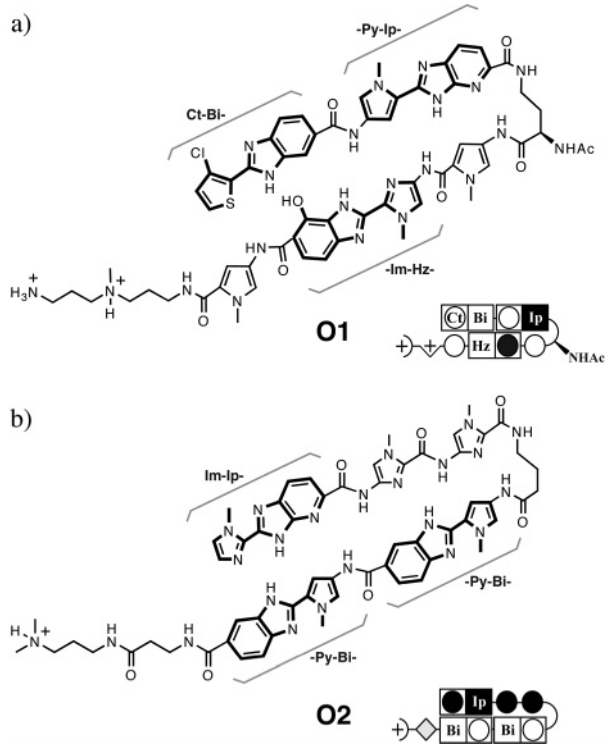


***Figure 1.*** Structure of oligomers. (a) Oligomer **O1** containing Ct-Bi-, -Py-Ip-, and -Im-Hz- recognition modules. (b) Oligomer **O2** containing Im-Ip- and two -Py-Bi- recognition modules.

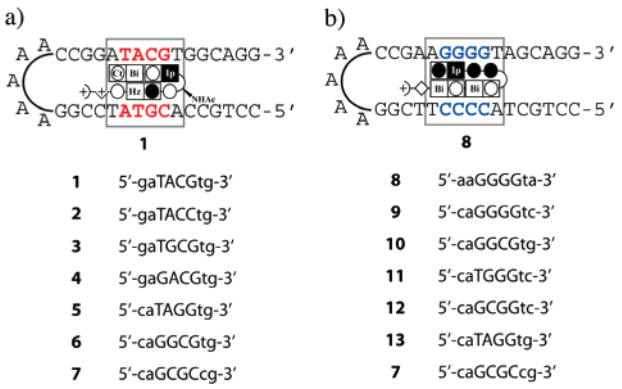


***Figure 2.*** Design of dsDNA library. (a) dsDNA sequences used for **O1**. (b) dsDNA sequences used for **O2**.

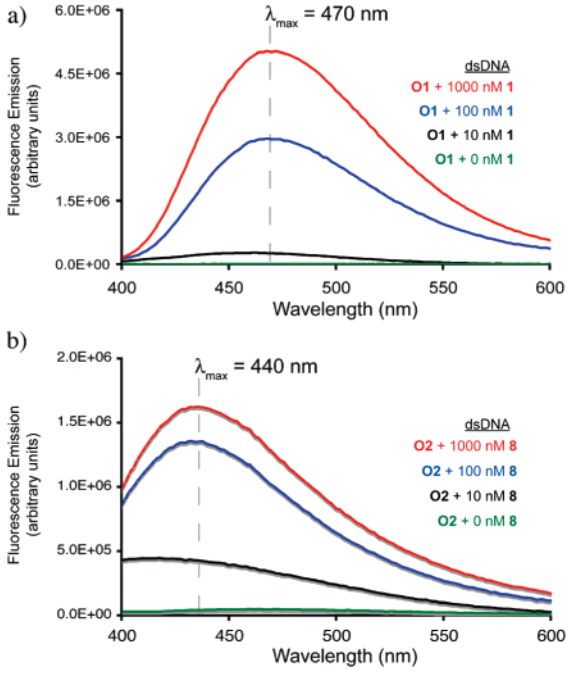


***Figure 3.*** Fluorescence emission spectra of **O1** and **O2** (1 $\mu$M) after 12 h incubation with their match binding site dsDNA ($\lambda_{Ex}$ = 340 nm). (a) Data for compound **O1**. (b) Data for compound **O2**. The emission was shown to plateau beyond 1 equiv DNA. (See Supporting Information for plots.)

fluorescent enhancement upon excitation at 340 nm in the presence of dsDNA. Oligomer **O1** contains the chlorothiophene−benzimidazole (Ct-Bi-), pyrrole−imidazopyridine (-Py-Ip-), and imidazole−hydroxybenzimidazole (-Im-Hz-) recognition modules, whereas oligomer **O2** contains imidazole−imidazopyridine (Im-Ip-) and two pyrrole−benzimidazole (-Py-Bi-) modules. The binding affinities of **O1** and **O2** targeted to two biologically important sequences, 5′-ATACGT-3′ (**O1**) and 5′-WGGGGW-3′ (**O2**), were determined



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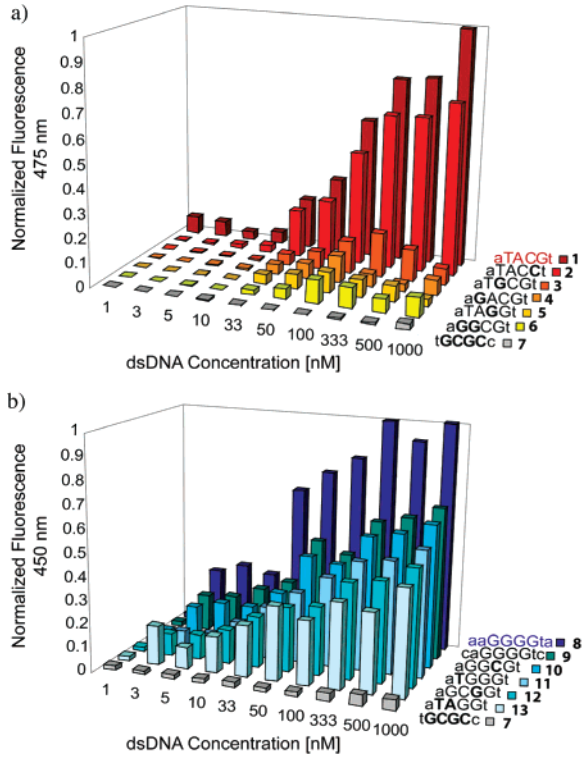


***Figure 4.*** Plot of dsDNA concentration versus normalized fluorescence for each dsDNA. (a) Data for compound **O1**. (b) Data for compound **O2**.

to be $K_a = 1.6 \times 10^9$ M$^{-1}$ and $2.6 \times 10^9$ M$^{-1}$, respectively, by quantitative DNase I footprinting.[9−11]

A library of dsDNA hairpins containing six base-pair match and mismatch binding sites for **O1** and **O2** was used to investigate their emission properties (Figure 2). The dsDNA library for **O1** and **O2** contained match sites (**1** and **8**, respectively), single base-pair (bp) mismatch sites (**2**−**5** and **10**−**12**, respectively), double bp mismatch sites (**6** and **13**, respectively), and full mismatch sites (**7** for both oligomers). The dsDNA **9** contains the 4-G match site of oligomer **O2**; however, the flanking sequence has been changed to emphasize the effect on binding. The presence of G·C bp under the tail is expected to lower the binding affinity of **O2** as compared to that of dsDNA **8**.[12]

Oligomers **O1** and **O2,** (1 $\mu$M concentration) were each incubated with an increasing concentration (1 nM to 1 $\mu$M) of dsDNA, and their emission spectra were recorded after excitation at 340 nm. The oligomers exhibited a marked increase in fluorescence upon addition of dsDNA containing their match site **1** and **8**, respectively (Figures 3 and 4).[13] Oligomer **O1** showed a moderate decrease in fluorescence intensity in the presence of dsDNA **2**, but proved to be much more sensitive to the incorporation of single base-pair mismatches at the alternate positions in dsDNAs **3**−**5** (Figure 4a). The incorporation of multiple base-pair mismatches in dsDNAs **6** and **7** showed a significant diminution in fluorescence intensity for **O1**. Oligomer **O2** exhibited a similar trend in sequence specificity, with a moderate decrease in fluorescence intensity observed upon incorporation of single base-pair mismatches (**9**−**12**) and a more significant decrease with multiple mismatches (**13** and **7**, Figure 4b).

Sequence-specific DNA binding molecules containing a fluorescent switch integrated as part of the recognition modules provides a method to detect DNA sequences without denaturation and in the absence of conjugation to a dye molecule. Fluorescent oligomers may be useful as site-specific chromosome paints for telomeric and centromeric repeats[14a,b] and could provide insight into cellular trafficking of DNA binding compounds.

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**Supporting Information Available:** Experimental procedures, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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