Supplementary Material for Chemical Communications

Targeting of Mixed Sequence Double-Stranded DNA Using Pyrene-Functionalized 2'-Amino-α-L-LNA

Patrick J. Hrdlicka, T. Santhosh Kumar and Jesper Wengel

Supporting Information

Synthesis of nucleosides 1-3 and ON1-ON7

Sodium triacetoxyborohydride mediated reductive amination[1] of nucleoside **1**[2] afforded alcohol **2** in 67% yield. The following standard O3'-phosphitylation gave phosphoramidite **3** in 88% yield,[3] which was used as a building block for incorporation of 2'-*N*-(pyren-1-yl)methyl-2'-amino-α-L-LNA monomer **X** into mixed sequence ONs (Scheme 1, Table 1) on a 0.2 μmol scale using an automated DNA synthesizer. Standard procedures were used except for extended coupling times for phosphoramidite **3** (30 min, using 1*H*-tetrazole as catalyst), effecting step-wise coupling yields of ~95% for phosphoramidite **3** and >99 % for unmodified deoxyribonucleotide phosphoramidites. After deprotection and cleavage from solid support (32% aq. ammonia, 55 °C, 12 h), the modified ONs were precipitated and repeatedly washed with ethanol, and their composition and purity (> 80%) verified by MALDI-MS analysis (see caption to Table 1) and ion-exchange HPLC, respectively.

Thermal denaturation studies

Concentrations of oligonucleotides were calculated using the following extinction coefficients (OD/μmol): G, 10.5; A, 13.9; T/U, 7.9; C, 6.6; pyrene, 20.2. Each strand (~1.0 μM) was thoroughly

mixed, and the complex denatured by heating to 80 °C followed by cooling to the starting temperature of the experiment. Quartz optical cells with a pathlength of 1.0 cm were used. Melting temperatures (T_m values/°C) were measured on a Perkin Elmer Lambda 35 UV/VIS spectrometer equipped with a PTP-6 Peltier temperature programmer and determined as the maximum of the first derivative of the thermal denaturation curve (A_{260} vs. temperature) recorded in medium salt buffer (100 mM NaCl, 0.1 mM EDTA and pH 7.0 adjusted with 10 mM NaH_2PO_4/5 mM Na_2HPO_4). A temperature range from 5-10 °C to 60-80 °C and a ramp of 1.0 °C/min were used.

Steady-state fluorescence studies

Fluorescence measurements were performed on a PerkinElmer LS 55 luminescence spectrometer equipped with a Peltier temperature controller. Quartz optical cells with a pathlength of 1.0 cm were used. Measurements were conducted using 1.0 μM of strands in T_m buffer. Corrections were made for solvent background but no attempts were made to eliminate dissolved oxygen in the buffer solution. Solutions were heated to 80 °C followed by cooling to 19 °C (± 0.1 °C) prior to measurements. Steady-state fluorescence emission spectra (360-600 nm) were obtained as an average of 5 scans at an excitation wavelength of 340 nm using an excitation slit of 4.0 nm, emission slit of 2.5 nm and scan speed of 120 nm/min.

Strand invasion experiments – representative procedure

1.0 equivalent of each **ON4** and **ON7** were mixed and diluted with T_m-buffer to give 500 μL of a 2.0 μM solution, which was fully denatured by heating and slowly cooled. Similarly, 2.0 equivalents of each **ON1** and **ON2** (total volume 500 μL, 4.0 μM in T_m buffer) were mixed, denatured and cooled. The two solutions were combined at 19 °C, thoroughly mixed and the steady-

state fluorescence emission spectra recorded at 19 °C (without any further denaturing or annealing steps), using the parameters described above.

Details of molecular modeling procedure

A standard B-type DNA-DNA duplex was built using the SPARTAN '02 program and subsequently modified within the MacroModel V7.2 suite of programs [R. D. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, C. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.* 1990, **11**, 440]. The charge from the phosphordiester backbone was neutralized with Na^+-ions, which were placed approximately 3 Å from the negatively charged oxygen atoms. All atomic positions, except those in the sugar and (pyren-1-yl)methyl moiety of monomer **X**, were frozen, and the duplex minimized using the Polak-Ribiere conjugate gradient method and the all-atom AMBER force field [S. J. Weiner, P. A. Kollmann, D. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta and P. K. Weiner, *J. Am. Chem. Soc.* 1984, **106**, 765] as implemented in MacroModel V7.2. A dielectric constant of 80 relative to vacuum was applied. Non-bonded interactions were treated without cut-offs. The minimized structure was then (using the same constraints as described above) submitted to 3 ns of stochastic dynamics (300 K, timestep of 2.0 fs, SHAKE all H), during which 300 structures were sampled and subsequently minimized.

Table S1. Absorption maxima of oligonucleotides in the range 300-400 nm.[a]

ON	λ_{max}
ON3	348.0/332.0
ON4	346.5/331.0
ON5	348.0/331.5
ON6	348.0/331.5
ON7	347.5/331.0
ON3:**ON2**	350.0/334.5
ON4:**ON2**	351.0/333.5
ON5:**ON2**	350.5/335.0
ON6:**ON1**	350.0/333.5
ON7:**ON1**	350.0/334.0
ON3:**RNA**	349.0/333.0
ON4:**RNA**	349.0/333.0
ON5:**RNA**	350.0/332.0
ON6:**RNA**	348.0/332.0
ON7:**RNA**	349.0/331.5

[a] Measurements were performed on solutions containing 1.0 μM of each strand at room temperature on a Shimadzu UV-160A spectrophotometer in the range 300-400 nm, using a quartz optical cell with a 1.0 cm pathlength.

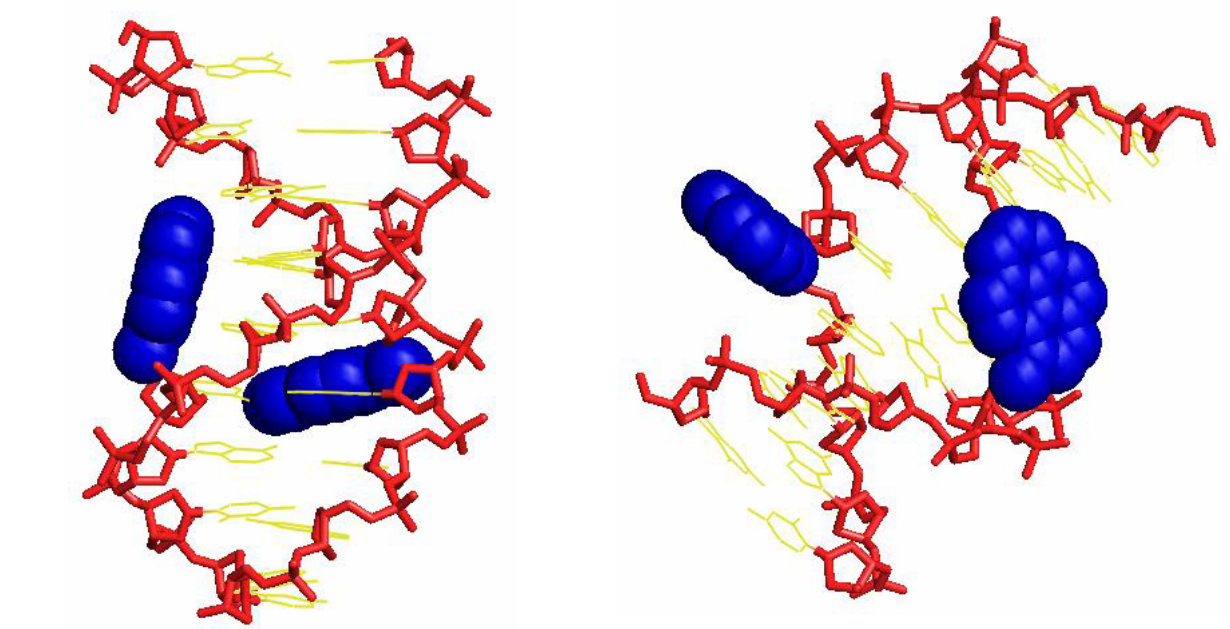

Figure S1. Two representations of the lowest energy structure of **ON4**:**ON7**. For clarity hydrogens, sodium ions and bond orders have been omitted. Colouring scheme: nucleobases, yellow; sugar-phosphate backbone, red; pyren-1-yl-methyl moiety, blue.

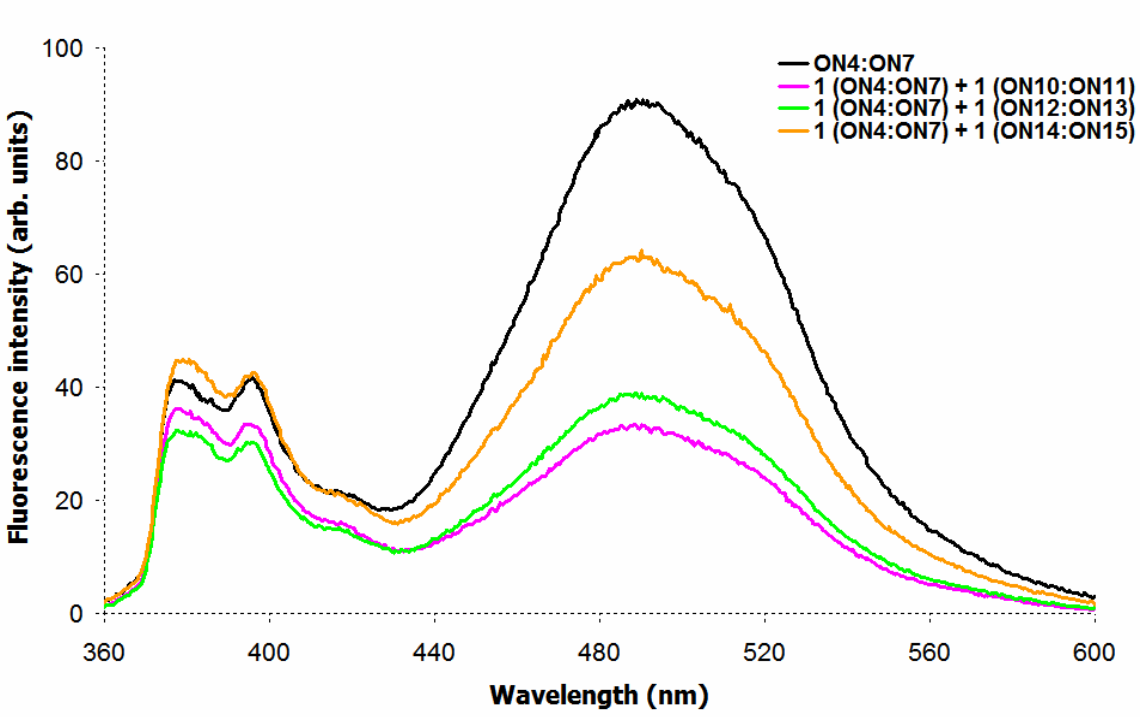

Figure S2. Steady-state fluorescence emission spectra of molecular recognition experiments (at 19 °C) using 1 equiv. of the +1 zipper probe (**ON4**:**ON7**) and 1 equiv. of fully base paired 9-mer dsDNAs (**ON10**:**ON11**, **ON12**:**ON13** and **ON14**:**ON15**) containing a single mismatch (central position) with respect to the probe. **ON10**, **ON12** and **ON14**: 5'-d(GTG A**B**A TGC)-3' where **B** = A, C and G, respectively; **ON11**, **ON13** and **ON15**: 3'-d(CAC T**B**T ACG)-5' where **B** = T, G and C, respectively.

References

1 A. F. Abdel-Magid, K. G. Carson, B. D. Harris, C. A. Maryanoff and R. D. Shah, *J. Org. Chem*. 1996, **61**, 3849.

2 T. S. Kumar, A. S. Madsen, J. Wengel and P. J. Hrdlicka. Manuscript in preparation.

3 Selected experimental data for phosphoramidite **3**: MALDI-HRMS *m/z* 1008.4072 ($[M + Na]^+$, $C_{58}H_{60}N_5O_8P{\cdot}Na^+$ Calc. 1008.4110); ^{31}P NMR (CH_3CN + DMSO-d_6) δ 150.7, 148.0.