Supplementary Material (ESI) for Chemical Communications

Insertion of Two Pyrene Moieties into Oligodeoxyribonucleotides for the Efficient Detection of Deletion Polymorphisms

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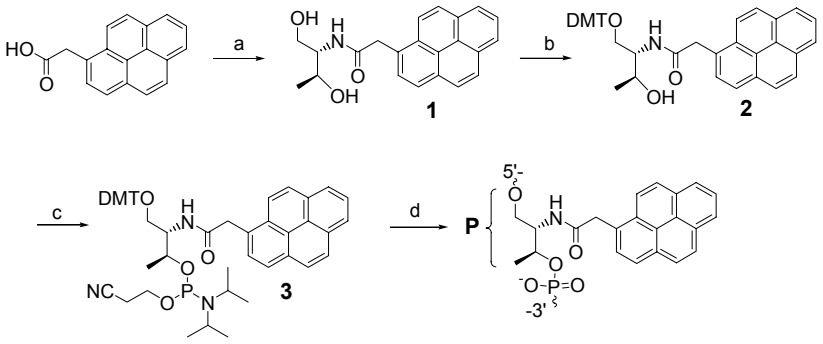
Supplemental Scheme 1. Synthesis of modified DNA tethering Pyrene moiety.



(a) D-threoninol, DCC, HOBt, DMF, r.t., 82 %; (b) DMT-Cl, CH_2Cl_2, Pyridine, 0→r.t., 91 %; (c) 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite, CH_3CN, 0→r.t., quant.; (d) DNA synthesizer.

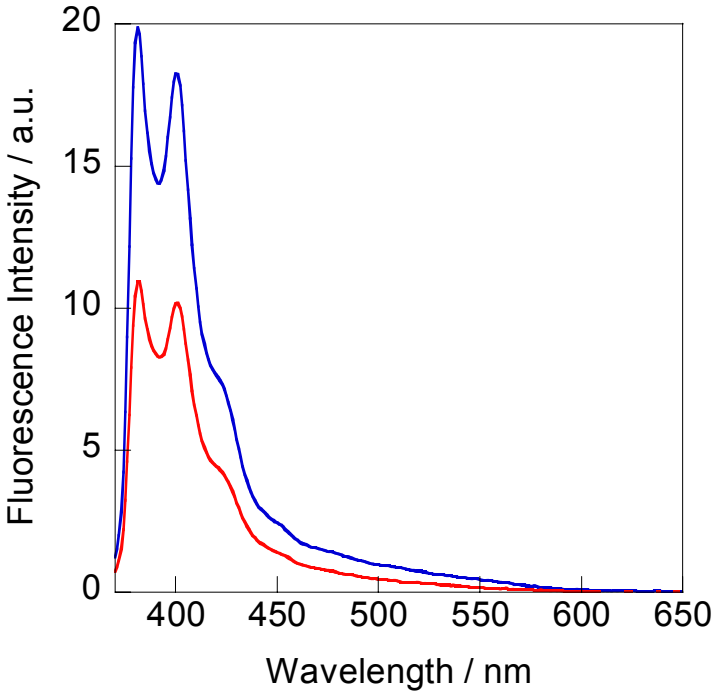
The phosphoramidite monomer tethering pyrene was synthesized as follows:

1-Pyreneacetic acid was coupled with D-threoninol in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in DMF. After the reaction mixture was stirred at room temperature for 24 h, the solvent was removed and the remained oil was subjected to silica gel column chromatography (CH_3OH : $CHCl_3$ = 20:1, R_f = 0.26) to afford compound **1** (yield 82%). ^{1}H NMR for **1** [500 MHz, DMSO]: δ = 8.45-8.04 (m, 9H, aromatic protons of pyrene), 7.79 (d, 1H, -N*H*CO-), 4.64 (s, 2H, -CH_2O*H*, -CH(CH_3)O*H*), 4.29 (dd, 2H, -C*H*$_2$C$_{16}$H$_9$), 3.91 (m, 1H, -C*H*(OH)CH_3), 3.60 (m, 1H, HOCH_2C*H*(NHCO-)-), 3.49 and 3.40 (dd, 2H, -C*H*$_2$-OH), 0.99 (d, 3H, -CH(OH)C*H*$_3$)

Dry pyridine solution containing **1** and N,N-diisopropylethylamine was cooled on ice under nitrogen. Then, 4,4'-dimethoxytrityl chloride (DMT-Cl) and small amount of 4-(dimethylamino)pyridine in CH_2Cl_2 was added to the above mixture. After 3 h of vigorous stirring, the solvent was removed by evaporation, followed by silica gel column chromatography (AcOEt : hexane : Et_3N = 75:25:3, R_f = 0.47) to afford **2** (yield 91 %): ^{1}H NMR for **2** [500 MHz, $CDCl_3$(TMS)]: δ = 8.30-6.53 (m, 22H, aromatic protons of DMT, pyrene), 6.17 (d, 1H, -N*H*CO-), 4.41 (s, 2H, -C*H*$_2$C$_{16}$H$_9$), 3.90 (m, 1H, -OCH_2C*H*(NHCO-)-), 3.83 (m, 1H, -C*H*(OH)CH_3), 3.70 and 3.69 (s, 6H, -C$_6$H$_4$-OC*H*$_3$), 3.22 and 3.04 (dd, 2H, -C*H*$_2$-ODMT), 0.94 (d, 3H, -CH(OH)C*H*$_3$)

In dry acetonitrile (10 mL) under nitrogen, **2** and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite were reacted with 1*H*-tetrazole. Prior to the reaction, **2** and 1*H*-tetrazole were dried by coevaporation with dry acetonitrile (three times). After 2 h, the product was taken into ethyl acetate. The organic solution was washed with saturated aqueous solution of $NaHCO_3$ and of NaCl, and dried over Na_2SO_4. Finally the solvent was removed *in vacuo,* and the oily product **3** was directly used for the DNA synthesis.

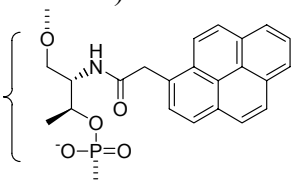
All the modified oligonucleotides in **Scheme 1b** in the main text were synthesized on an automated DNA synthesizer by using the phosphoramidite monomer **3** and other conventional ones from GLEN RESEARCH Co. Coupling efficiency of the monomer **3** was as high as the conventional ones as judged from the coloration of released trityl cation. After the recommended work-up, they were purified by the reversed-phase HPLC; a Merck LiChrospher 100 RP-18(e) column, 0.5 mL/min, a linear gradient 7.5-15 % (30 min) acetonitrile/water containing 50 mmol dm^{-3} ammonium formate, detection at 260 nm.

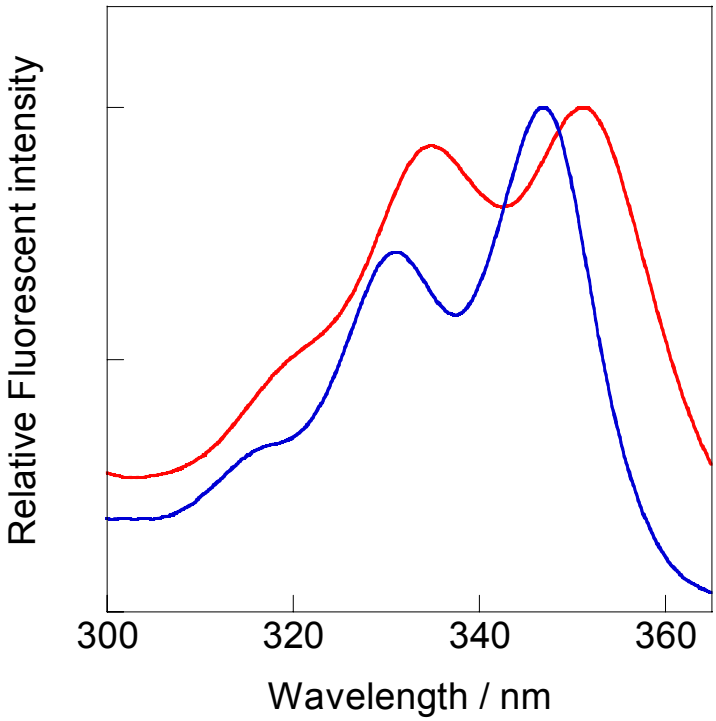


Supplemental Figure 1. Fluorescent emission spectra of single-stranded **P1** (blue line) and **P1**/**N** (red line) at 0 ˚C. Excitation wavelength was 345 nm. T_m of **P1**/**N** was 48.8 ˚C. [DNA] = 5 μM, [NaCl] = 100 mM, pH 7.0 (10mM phosphate buffer)

P1 : 5'-GGT-ATC-**P**-GCA-ATC-3'
N : 3'-CCA-TAG-CGT-TAG-5'

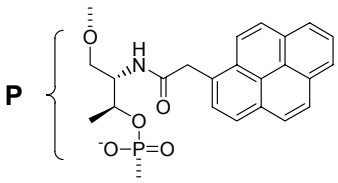
P

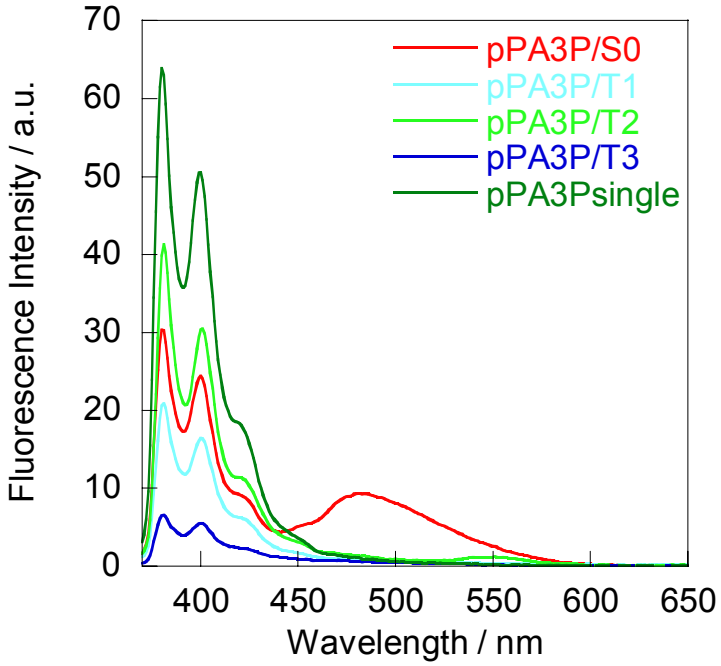




Supplemental Figure 2. Fluorescent excitation spectra of **PAP/N** at 0 ˚C monitored at 483 nm (blue line) and 377 nm (red line). The spectra were normalized at peak maxima. [DNA] = 5 μM, [NaCl] = 100 mM, pH 7.0 (10mM phosphate buffer)

PAP : 5'-GGT-ATC-**P**A**P**-GCA-ATC-3'
N : 3'-CCA-TAG-CGT-TAG-5'





Supplemental Figure 3. Fluorescent emission spectra of single-stranded **$_p$PA3P, $_p$PA3P/T3** (wild type), **$_p$PA3P/T2** (one-base deletion mutant), **$_p$PA3P/T1** (two-base deletion mutant), **$_p$PA3P/N** (three-base deletion mutant) at 0 ˚C. Excitation wavelength was 345 nm.
[DNA] = 5 μM, [NaCl] = 100 mM, pH 7.0 (10mM phosphate buffer)

$_p$PA3P : 5’-GGT-ATC-**$_p$P**AAA**$_p$P**-GCA-ATC-3’
T3 : 3’-CCA-TAG-TTT-CGT-TAG-5’
T2 : 3’-CCA-TAG-TT-CGT-TAG-5’
T1 : 3’-CCA-TAG-T-CGT-TAG-5’
N : 3’-CCA-TAG-CGT-TAG-5’