Supporting Information

Electrochemistry of DNA Monolayers Modified With a Perylenediimide Base Surrogate

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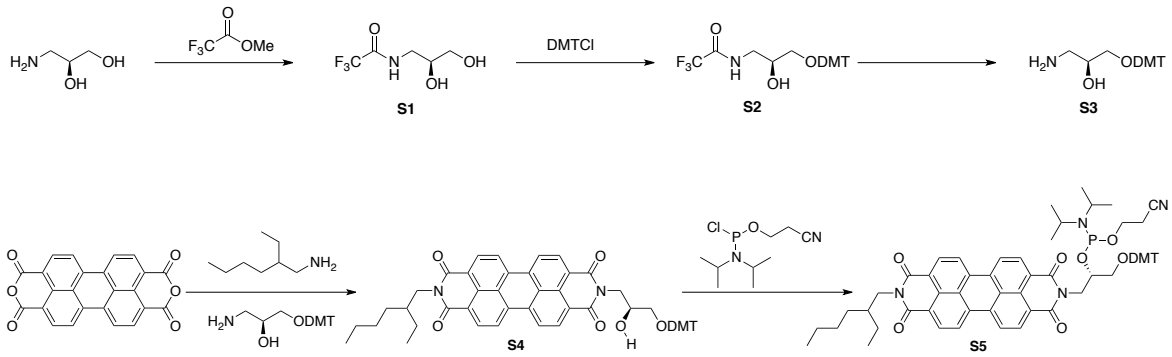
(I) Details of the perylene-3,4,9,10-tetracarboxylic diimide synthesis.

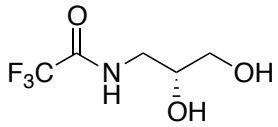


Supporting Figure 1. Schematic of the synthesis of the perylenediimide phosphoramidite **S5**.

(A) General Description of the Characterization of the Reaction Products. All intermediates and products for the phosphoramidite synthesis were characterized with nuclear magnetic resonance spectroscopy and mass spectrometry. The electrospray mass spectrometry (ESI MS) data was obtained at the University of California, Irvine Mass Spectrometry Facility on a Waters LCT Premier electrospray time-of-flight instrument. The ^{1}H NMR spectra were obtained on either a Bruker DRX500 or an AVANCE600 instrument. The ^{13}C NMR spectra were obtained on a Bruker DRX500 outfitted with a CryoProbe (Bruker TCI 500 MHz, 5 mm diameter tubes). The ^{31}P NMR spectra were acquired on a Bruker AVANCE instrument. Chemical shifts were reported in ppm for ^{1}H, ^{13}C, ^{19}F, and ^{13}C NMR. The chemical shifts for the NMR data were referenced as follows: for samples in $CDCl_3$, the ^{1}H NMR was referenced to tetramethylsilane (TMS) at 0.00, and the ^{13}C NMR was referenced to $CDCl_3$ at 77.23; for samples in CD_3OD, the ^{13}C NMR was referenced to the solvent peak at 49.00; for samples in CD_2Cl_2, the ^{1}H NMR was referenced to the solvent peak at 5.32, and the ^{13}C NMR was referenced to the solvent peak at 54.00. The chemical shifts for the ^{31}P NMR spectra were referenced *via* correction based on ^{1}H NMR calibration according to the 2008 IUPAC recommendations.[1] The data are labeled as

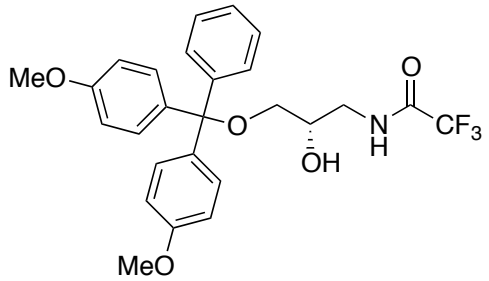
follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br s = broad singlet), the coupling constants (in Hertz), and the integration value.

(B) Synthesis and Characterization of the (S)-3-trifluoroacetamido-1,2-propanediol (S1).



The product was synthesized according to established literature protocols.[2,3] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 – 6.81 (br s, 1H), 3.96 – 3.88 (p, *J* = 5.8 Hz, 1H), 3.78 – 3.71 (dd, *J* = 11.2, 4.0 Hz, 1H), 3.69 – 3.56 (m, 2H), 3.46 – 3.37 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 159.72 – 158.84 (q, *J* = 36.7 Hz), 120.95 – 114.11 (q, *J* = 285), 71.23, 65.12, 43.82. ^{19}F NMR (376 MHz, CD_3OD) δ –77.58; ESI MS found 210.01 and 397.06 (M + Na and 2M + Na).

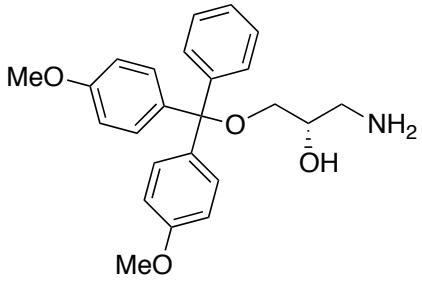
(C) Synthesis and Characterization of the (S)-3-trifluoroacetamido-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol (S2).



The product was synthesized according to established literature protocols.[2,3] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.38 (m, 2H), 7.32 – 7.27 (m), 7.25 – 7.21 (m, 1H), 6.86 – 6.81 (m, 4H), 6.70 – 6.62 (br s), 3.94 – 3.88 (m, 1H), 3.81 – 3.77 (s, 6H), 3.63 – 3.57 (ddd, *J* = 13.9, 6.7, 3.8 Hz, 1H), 3.33 – 3.295 (dd, *J* = 7.2, 4.8 Hz), 3.295 – 3.26 (dd, *J* = 9.9, 4.5 Hz, 1H), 3.17 – 3.12 (dd, *J* = 9.7, 6.1 Hz, 1H), 2.44 – 2.41 (d, *J* = 4.6 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.87, 150.00,

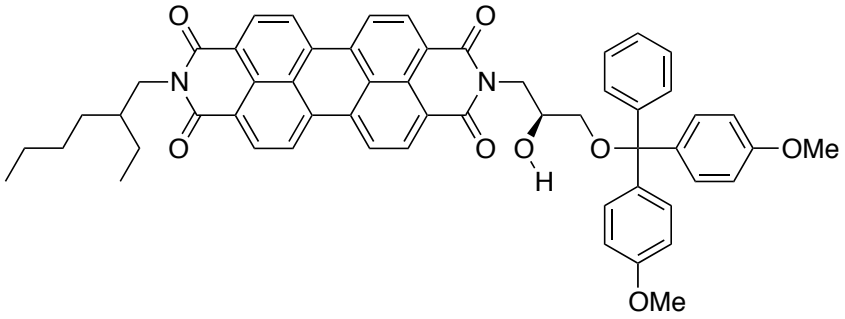
144.52, 136.22, 135.61, 135.58, 130.14, 128.21, 128.14, 127.26, 123.97, 113.49, 86.84, 69.12, 64.93, 55.45, 42.78; ESI MS found 512.14 (M + Na).

(D) Synthesis and Characterization of the (S)-3-amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol (S3).



The product was synthesized according to established literature protocols.[2,3] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 – 7.42 (m, 2H), 7.36 – 7.26 (m, 6H), 7.24 – 7.19 (tt, *J* = 7.25, 1.5 Hz, 1H), 6.86 – 6.79 (apar dt, *J* = 9, 2.75 Hz, 4H), 3.79 (s, 6H), 3.78 – 3.72 (m, 1H), 3.17 – 3.12 (d, *J* = 5.4 Hz, 2H), 2.85 – 2.80 (dd, *J* = 13.0, 4.0 Hz, 1H), 2.76 – 2.71 (dd, *J* = 13, 7.0 Hz, 1H), 1.97 (br s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.66, 144.97, 136.13, 130.20, 128.30, 128.01, 126.98, 113.31, 86.28, 71.14, 65.67, 55.37, 44.56; ESI MS found 416.14, 787.29, and 809.29 (M + Na, 2M + 1, 2M + Na).

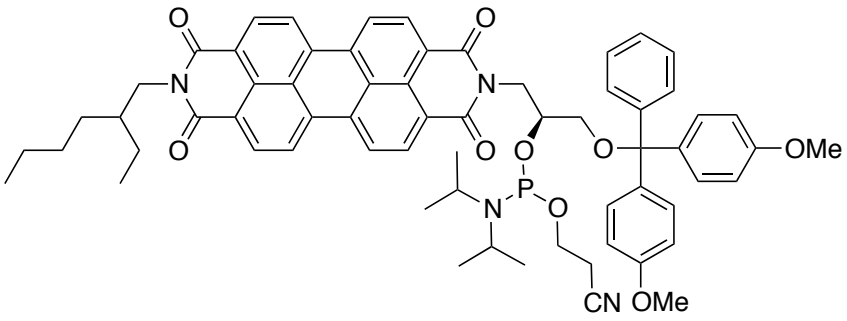
(E) Synthesis and characterization of the perylenediimide (S4).



The product was synthesized according to a procedure adopted from the literature.[3] First, 0.681 g (1.73 mmol, 1.01 equiv) 3,4,9,10-perylenetetracarboxylic dianhydride and 0.638 g of anhydrous $Zn(OAc)_2$ (3.48 mmol, 2.02 equiv) were combined in an oven-dried round bottom flask after cooling the flask under Ar. Anhydrous pyridine (46 mL) was then added via syringe, and the flask was fitted with a water-cooled condenser. The mixture was heated to reflux, and after 1.5 h, solutions of 2-ethyl-1-hexylamine (0.57 mL, 3.46 mmol, 2.0 equiv) and (S)-3-amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol (**S3**, 0.678 g, 1.72 mmol, 1.0 equiv) each dissolved in 12 mL anhydrous pyridine were successively added to the refluxing reaction mixture. The vials containing each amine were then rinsed with 3 mL anhydrous pyridine, and the rinse solution was added to the refluxing reaction mixture *via* syringe. This mixture was stirred rapidly and maintained at reflux for ~23 hours under argon. The reaction was subsequently allowed to cool to room temperature, concentrated to ~1/10 of the original volume by rotary evaporation, diluted in CH_2Cl_2 (100 mL), and filtered through a pad of celite, which was rinsed with CH_2Cl_2 (3 x 100 mL). The filtered solution was poured into a separatory funnel, which was rinsed with CH_2Cl_2 (3 x 10 mL). The crude mixture was extracted with aqueous KOH (1 M, 3 x 100 mL), dried for 10 min over anhydrous Na_2SO_4, and filtered. Next, silica gel (8 mL) was added to the crude product solution, and the mixture concentrated by rotary evaporation to produce a dry load for chromatography. The dry load was added to a silica gel column (90 mL silica gel, 3.2 cm O.D. column), and the products were eluted with mixtures of CH_2Cl_2:acetone (from 99:1 to 80:20 in 1200 mL total eluent). A 200 mL pre-fraction was collected in a flask, and the remaining volume was collected in 27 mL fraction tubes. Fractions 25 – 33 were concentrated to afford 0.314 g (21 %) of a dark red solid. ^{1}H NMR (500 MHz, CD_2Cl_2, mixture of diastereomers) δ 8.18 – 8.05 (m, 4H), 7.86 – 7.78 (m, 4H), 7.58 – 7.50 (dt, J = 8.4, 1.0 Hz, 2H), 7.44 – 7.36 (d, J = 7.8 Hz, 4H),

7.34 – 7.28 (t, *J* = 7.5 Hz, 2H), 7.24 – 7.19 (t, *J* = 7.25 Hz, 1H), 6.88 – 6.79 (d, *J* = 8.0 Hz, 4H), 4.57 – 4.48 (ddd, *J* = 13.6, 9.4, 2.0 Hz, 1H), 4.35 – 4.25 (m, 1H), 4.19 – 4.11 (dt, *J* = 13.7, 3.4 Hz, 1H), 4.09 – 3.95 (m, 2H), 3.77 (s, 6H), 3.40 – 3.31 (dd, *J* = 10.5, 6.5, Hz, 1H), 3.32 – 3.23 (d, *J* = 4.5 Hz, 2H), 1.90 – 1.85 (m, 1H), 1.45 – 1.24 (m, 8H), 0.99 – 0.92 (q, *J* = 7.0 Hz, 3H), 0.91 – 0.85 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_2Cl_2) δ 163.88, 163.86, 163.56, 159.13, 150.37, 145.68, 136.60, 134.00, 133.98, 133.63, 133.61, 131.10, 130.91, 130.88, 130.65, 130.63 129.03, 128.88, 128.69, 128.37, 127.26, 125.57, 125.54, 123.29, 123.27, 123.12, 123.02, 122.90, 113.60, 86.54, 69.73, 69.69, 66.45, 55.72, 44.71, 44.46, 38.48, 38.43, 31.25, 31.21, 29.23, 29.14, 24.56, 24.50, 23.69, 23.65, 14.49, 10.95, 10.87; ESI MS found 901.31 (M + Na).

(F) Synthesis and characterization of the perylenediimide phosphoramidite (S5).

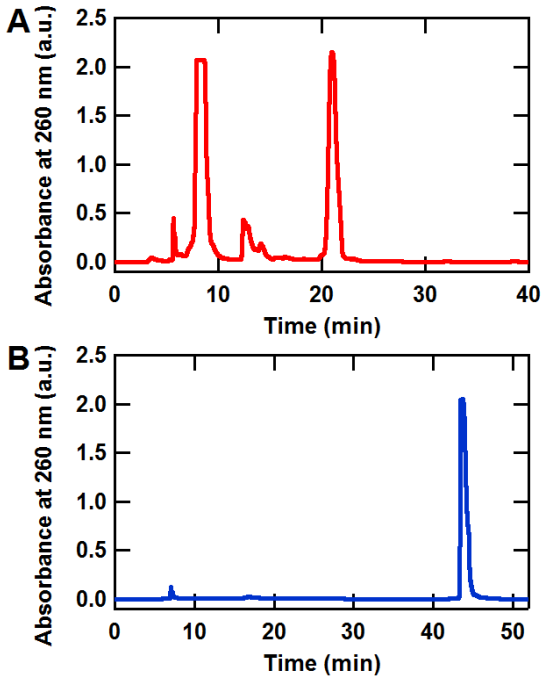


The phosphoramidite **S5** was synthesized according to a procedure adopted from the literature.[3] First, 1.519 g (1.728 mmol, 1.0 equiv) of the perylenediimide (**S4),** dichloromethane (17 mL), and distilled triethylamine (1.2 mL, 8.57 mmol, 4.96 equiv) were added to an oven-dried round bottom flask cooled under argon. Once the **S4** substrate dissolved to produce an opaque red solution, 0.395 mL *N,N*-diisopropylamino-β-cyanoethyl chlorophosphoramidite (1.72 mmol, 1.0 equiv) was added *via* syringe, with the bottle kept under argon pressure. After stirring for 4 hours, the solution was poured into a separatory funnel, rinsed and diluted with CH_2Cl_2 (3 x 10 mL), and extracted with aqueous $NaHCO_3$ (sat., 3 x 10 mL). The organic phase was dried for 5

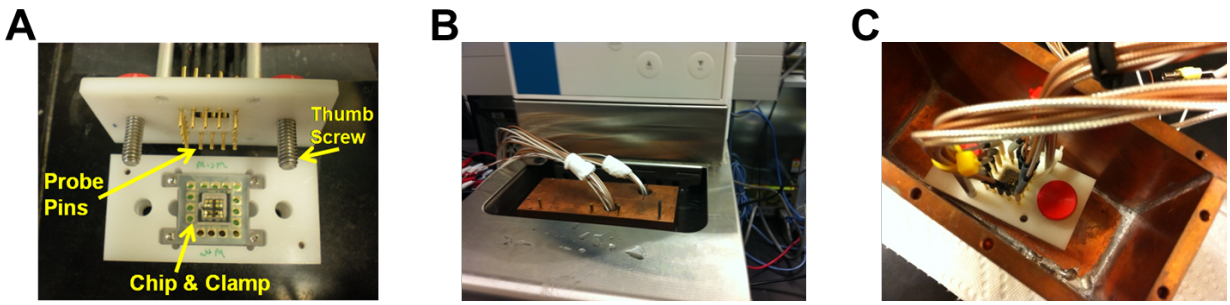
min over Na_2SO_4, filtered, and concentrated by rotary evaporation/vacuum drying to yield 1.817 g (97%) of a dark red solid. ^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.59 – 8.45 (m, 8H), 7.49 – 7.38 (m, 2H), 7.36 – 7.09 (m, 7H), 6.81 – 6.65 (m, 4H), 4.66 – 4.59 (dd, *J* = 13.3, 8.7 Hz, 0.5H), 4.57 – 4.43 (m, 1.5H), 4.35 – 4.28 (m, 0.5H), 4.25 – 4.04 (m, 2.5H), 3.75 – 3.57 (m, 8H), 3.56 – 3.34 (m, 3.5H), 3.23 – 3.18 (dd, *J* = 9.6, 5.2 Hz, 0.5H), 3.16 – 3.10 (dd, *J* = 9.7, 5.3 Hz, 0.5H), 2.77 – 2.73 (td, *J* = 5, 1 Hz, 0.5H), 2.45 – 2.39 (td, *J* = 6.5, 3.7 Hz, 1H), 2.34 – 2.28 (t, *J* = 6.7 Hz, 1H), 1.97 – 1.90 (m, 1H), 1.45 – 1.13 (m, 8H), 1.08 – 1.01 (dd, *J* = 14.1, 6.8 Hz, 6H), 1.01 – 0.85 (m, 12H); ^{13}C NMR (126 MHz, CD_2Cl_2) δ 164.09, 163.87, 163.71, 159.03, 145.45, 136.69, 134.99, 134.89, 131.66, 131.49, 130.64, 130.51, 130.46, 129.74, 128.65, 128.26, 128.19, 127.14, 126.77, 123.84, 123.81, 123.71, 123.60, 123.54, 118.08, 113.50, 113.46, 113.41, 113.34, 86.65, 66.25, 58.84, 55.68, 55.65, 55.59, 44.66, 43.69, 43.59, 43.44, 43.07, 38.50, 31.26, 29.25, 24.87, 24.81, 24.67, 24.57, 23.64, 23.20, 20.64, 14.44, 10.97; ^{31}P NMR (243 MHz, CD_2Cl_2) δ 149.16 – 148.88 (sextet, *J* = 9.2 Hz, 1P); ESI MS found 1101.49 (M + Na).

References

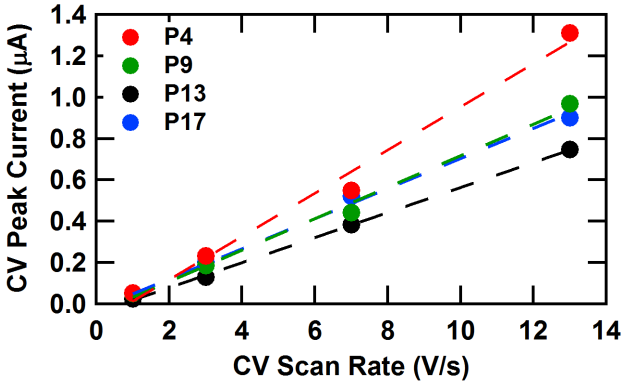
1. Harris, R. K.; Becker, E. D.; Cabral de Menezes, S. M.; Granger, P.; Hoffman, R. E.; Zilm, K. W. Further Conventions for NMR Shielding and Chemical Shifts. *Pure Appl. Chem.* **2008**, *80*, 59–84.
2. Azhayev, A. V.; Antopolsky, M. L. Amide Group Assisted 3'-dephosphorylation of Oligonucleotides Synthesized on Universal A-supports. *Tetrahedron*, **2001**, *57*, 4977–4986.
3. Wagner, C.; Wagenknecht, H.-A. Perylene-3,4:9,10-tetracarboxylic Acid Bisimide Dye as an Artificial DNA Base Surrogate. *Org. Lett.* **2006,** *8,* 4191–4194.



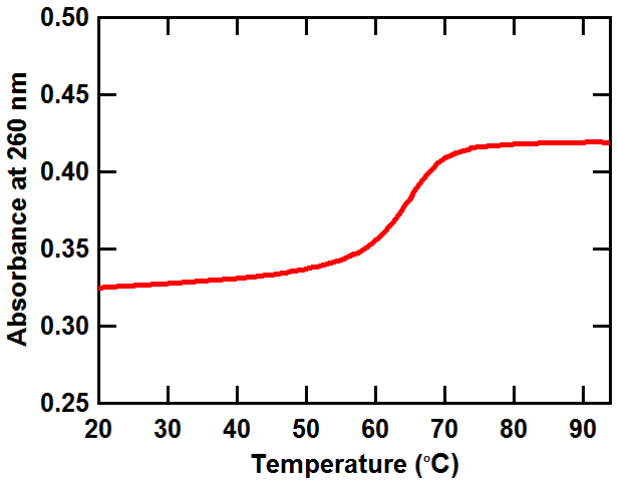
Supporting Figure 2. (A) Typical HPLC chromatogram corresponding to the purification of the **P9** oligonucleotide with the 4,4'-dimethoxytrityl moiety on. The gradient was evolved from 5% acetonitrile and 95 % 50 mM ammonium acetate, pH = 8 buffer to 75% acetonitrile and 25 % 50 mM ammonium acetate, pH = 8 buffer over 30 minutes. (B) Typical HPLC chromatogram corresponding to the purification of the **P9** oligonucleotide with the 4,4'-dimethoxytrityl moiety off. The gradient was evolved from 5% acetonitrile and 95 % 50 mM ammonium acetate, pH = 8 buffer to 15% acetonitrile and 85 % 50 mM ammonium acetate, pH = 8 buffer over the first 35 minutes. Then, the gradient was evolved from 15% acetonitrile and 85 % 50 mM ammonium acetate, pH = 8 buffer to 50% acetonitrile and 50 % 50 mM ammonium acetate, pH = 8 buffer over the next 5 minutes and held at 50 % acetonitrile and 50 % 50 mM ammonium acetate, pH = 8 buffer for another 5 minutes. The **P9** DNA sequence was 3'-TTCTATAT*P*TCGTGCGT-5', where the *P* indicates the location of the perylene.



Supporting Figure 3. Photographs of the custom built setup employed for temperature-controlled electrochemical measurements. (A) Photograph of the housing used for mounting chips featuring multiplexed gold electrodes. The clamped chip shown in the figure is connected to external electrochemical hardware through the mount. (B) Photograph of the test mount in a custom copper box (lid not shown). (C) Photograph of the copper box assembly when submerged in a temperature-controlled water bath, which maintained temperature uniformity over the entire electrochemical test assembly.



Supporting Figure 4. Cyclic voltammetry peak current as a function of scan rate for the **P4** (red), **P9** (green), **P13** (black), and **P17** (blue) DNA monolayers. The perylenediimide-modified monolayers all exhibited a linear dependence of the peak current on the scan rate, as expected for surface bound species.



Supporting Figure 5. The absorbance of a **P13** DNA duplex as a function of temperature. The plot displays a single melting transition.