Supplementary Information

Denaturation of dsDNA Immobilised at a Negatively Charged Gold Electrode is not caused by Electrostatic Repulsion

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Oligonucleotide Synthesis

Standard DNA phosphoramidites, solid supports and additional reagents were purchased from Link Technologies, Sigma and Applied Biosystems. Synthesis columns were obtained from Link Technologies for 3'- C7-aminoalkyl oligonucleotides and the dithiol phosphoramidite was purchased from Glen Research. All oligonucleotides were synthesized on an Applied Biosystems 394 automated DNA/RNA synthesizer using standard 0.2 or 1.0 µmole phosphoramidite cycles of acid-catalyzed detritylation, coupling, capping and iodine oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and in all cases were >98.0%. All β-cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use. The coupling time for normal (A, G, C, T) monomers was 35 s and the coupling time for the dithiol monomers was extended to 600 s. Cleavage of oligonucleotides from the solid support and de-protection was achieved by exposure to concentrated aqueous ammonia for 60 min at room temperature followed by heating in a sealed tube for 5 h at 55 °C. The 3'-Cy3-labelled oligonucleotides were synthesized by post-synthetic labeling of 3'-aminoalkyl oligonucleotides which were assembled using C7-aminolink solid support described above.

To incorporate the Cy3 chromophore at the 3'-end of C7-aminoalkyl oligonucleotide, 50-150 nmol of the oligonucleotide in 80 µL of 0.5 M Na_2CO_3/$NaHCO_3$ buffer (pH 8.75) was incubated overnight at room temperature with 625 µg of the succinimidyl ester of Cy3 (Invitrogen) in 30 µL of DMSO. The excess dye from the reaction was removed by NAP-10 Sephadex columns (GE Healthcare) before being purified by reversed-phase. Reversed-phase HPLC purification was carried out on a Gilson system using an Phenomenex column (C8), 10 mm x 250 mm, pore size 100 Å.

The following HPLC conditions were used: run time 20 min, flow rate 4 mL per min, binary system, gradient: time in min (% buffer B);0 (0); 3 (0); 3.5 (15); 15 (60); 16 (100); 17 (100); 17.5 (0); 20 (0). Elution buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate with 50% acetonitrile pH 7.0. Elution of oligonucleotides was monitored by ultraviolet absorption at 295 nm. Texas Red oligonucleotides gave two product peaks corresponding to the 5- and 6- regioisomers of Texas Red. The first peak (5-isomer) was collected and used in the subsequent SERS experiments and the second peak was discarded. After HPLC purification oligonucleotides were desalted using NAP-10 Sephadex columns (GE Healthcare), aliquoted into eppendorf tubes and stored at –20 ºC. All oligonucleotides were characterized by electrospray mass spectrometry and capillary electrophoresis.

Peptide Nucleic Acid Synthesis

Standard PNA monomers, Fmoc Xal resin were purchased from Link Technologies Ltd; *N*, *N*-dimethylformamide (DMF), piperidine were peptide synthesis grade and purchased from Rathburn Chemicals; 2-(1H-7-Azabenzotriazole-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU) and amino acids were bought from Novabiochem and CEM co-operation ltd; di-isopropylethylamine (DIPEA), 2,6-Lutidine, acetic anhydride, Trifluoroacetic acid (TFA), *m*-cresol, 1,2 Ethanedithiol (EDT) and Triisopropylsilane (TIS) were bought from Sigma Aldrich. All the PNA were synthesized on PerSeptive Biosystems Expedite Nucleic Acid/PNA synthesiser modified for PNA chemistry. The synthesis was performed on a 2 µmole scale with standard procedure for PNA synthesis by the Fmoc strategy otherwise indicated. Acetylation at *N*-terminal of some PNAs was performed manually on solid support with the following procedure. The capping reagent that used for the PNA synthesis on automate synthesiser (200 µL, Ac_2O) was used and left at room temperature for 10 min, washed with DMF (5 x 1 mL), a mixture of DMF and dichloromethane (1:1 v/v, 5 x 1 mL), DCM (5 x 1 mL) and diethylether (Et_2O, 5 x 1 mL). The resin was dried under stream of argon for 10 minutes before cleavage and de-protection. The resulting resin was transferred to a Millipore Ultrafree-MC PTFE 0.2 µm filtered microcentrifuge tube and the cleavage and de-protection procedure similar as standard but using a mixture of TFA:H_2O:EDT:TIS in a ratio of 92.5:2.5:2.5:2.5. PNA was labelled post-synthetically using Cy3 NHS ester (GE Healthcare, Cat No. PA13101). The PNA (29 ODs) was dissolved in buffer (0.5 M $NaHCO_3$/Na_2CO_3 pH 8.75, 100 µL). The PNA did not fully dissolve in this buffer alone but it fully dissolved when the NHS ester of Cy3 (0.5 mg in 50 µL DMSO) was added. The mixture was left at room temperature for 5 hours and then purification of the PNA was carried out by reversed-phase HPLC on a Gilson system using a Ascentis C18 (Supelco), 10 mm x 250 mm, pore size 10 micron. The following protocols were used: Run time 35 mins, flow rate 3 mL per min, binary system, gradient: Time in mins (% buffer B); 0 (5); 5 (10); 24 (30); 25 (95); 29 (95); 30 (10); 35 (10). Elution buffer A: 0.1 % TFA water and buffer B: 0.08 %TFA acetonitrile. Elution of PNAs was monitored by ultraviolet absorption. PNAs were then freezed dried and redissolved in water at working concentration.

Structures of the modifications to DNA and PNA

Structural details of the surface anchor and Cy3 label modifications for DNA and PNA are shown below.

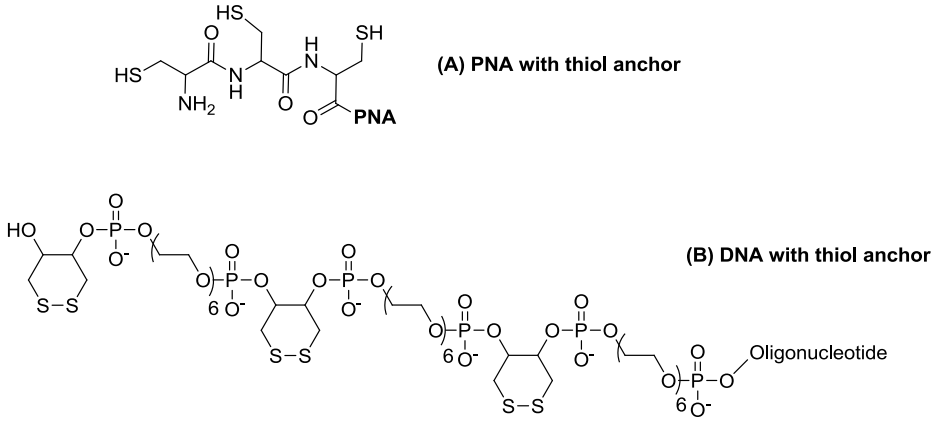

Figure S1: Structure of the thiol anchor for (A) PNA and (B) DNA

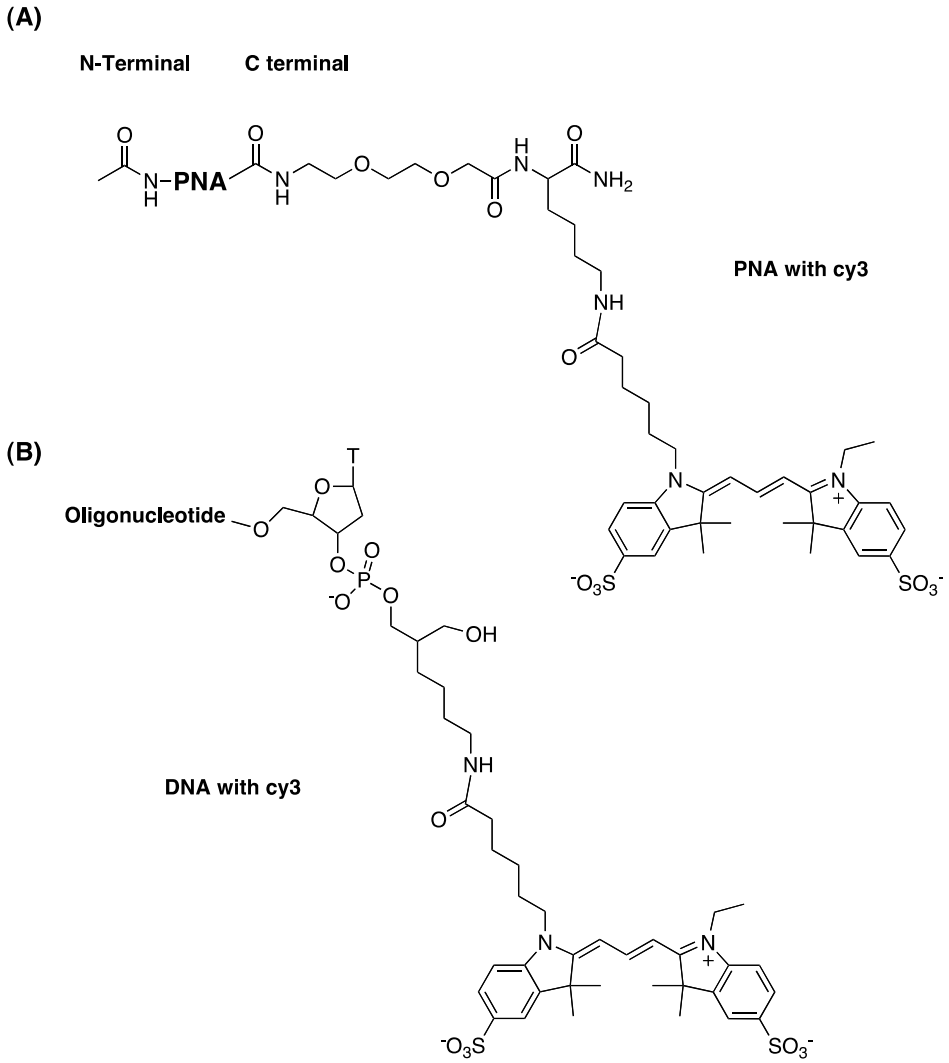

Figure S2: Structure of the Cy3 modification for (A) PNA and (B) DNA

Additional Electrochemical Melting Data

Melting curves are shown for denaturation of DNA and PNA at ionic strengths of 0.01 M and 1 M. Melting Potentials (determined from the mid-point of the sigmoidal fit) are given in Table 1 in the manuscript.

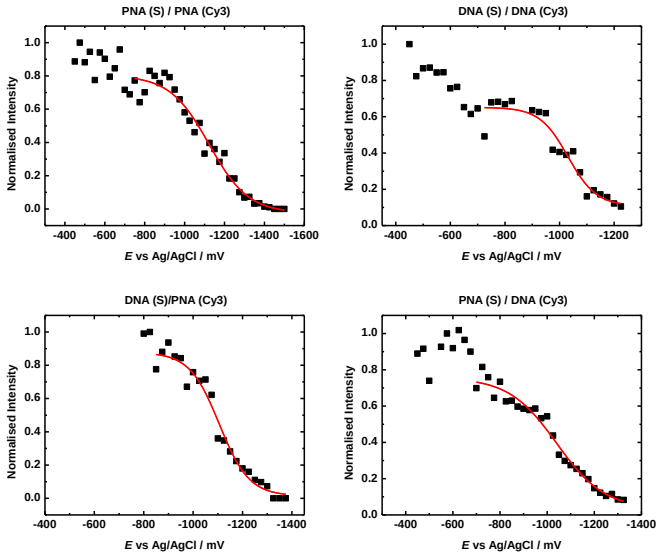

Figure S3: Electrochemical Melting of duplexes constructed of DNA and/or PNA at a sphere segment void surface. The peak intensity at 1590 cm^{-1} for (A) PNA/PNA(Cy3), (B) DNA/DNA(Cy3) (C) DNA/PNA(Cy3) and (D) PNA/DNA(Cy3) is plotted as a function of the applied potential. Sigmoidal curves have been fitted to the region corresponding to denaturation of duplex. The potential was swept a scan rate of 0.05 mV s^{-1} in a 10 mM Tris buffer (pH 7.2) with added NaCl (I = 0.01 M). Spectra were acquired with a 2.7 mW 633 nm excitation laser and have been background subtracted and normalized to maximum.

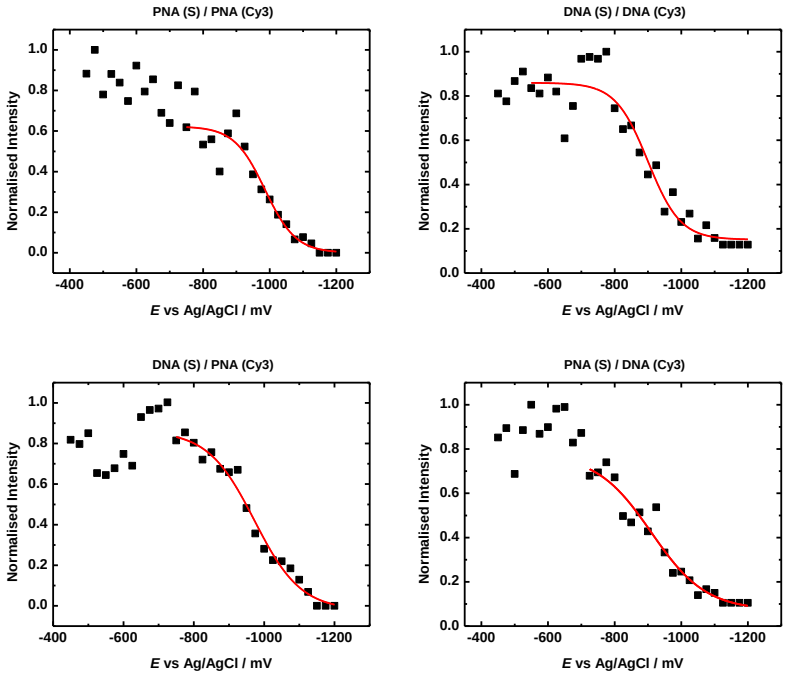

Figure S4: Electrochemical Melting of duplexes constructed of DNA and/or PNA at a sphere segment void surface. The peak intensity at 1590 cm^{-1} for (A) PNA/PNA(Cy3), (B) DNA/DNA(Cy3) (C) DNA/PNA(Cy3) and (D) PNA/DNA(Cy3) is plotted as a function of the applied potential. Sigmoidal curves have been fitted to the region corresponding to denaturation of duplex. The potential was swept a scan rate of 0.05 mV s^{-1} in a 10 mM Tris buffer (pH 7.2) with added NaCl (I = 1 M). Spectra were acquired with a 2.7 mW 633 nm excitation laser and have been background subtracted and normalized to maximum.

Comparison to Previous Data

In our previous work, we developed a model for the relationship between melting temperature and melting potential based on a series of electrochemical melting experiments with 5 fully-complementary DNA duplexes of differing sequence but of the same length (*Bioelectrochemistry*, **2012**, ***85***, 7-13). We found that the data obtained for the DNA/DNA duplex here was a good match to our existing model whilst the melting potentials of the DNA/PNA duplexes were not well predicted by our existing model. The PNA/PNA duplex had a melting temperature greater than 80 ^{o}C, beyond the range of our instrumentation, whilst the melting potential was only -1024 mV vs. Ag/AgCl.

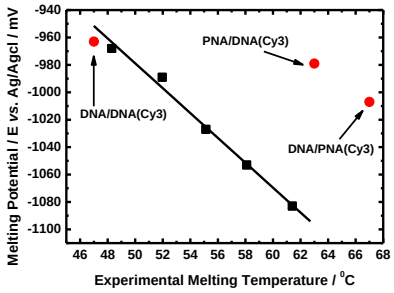

Figure S5: Melting Potentials for the DNA (S) / DNA (Cy3), PNA (S)/DNA (Cy3) and DNA (S)/PNA (Cy3) plotted vs. their corresponding melting temperature as determined by UV-Vis spectroscopy in solution (red circles) overlain with previously-published results obtained for a series of 5 duplexes of varying composition (black squares). The buffer conditions used to determine the melting potential and melting temperatures were identical; 10 mM Tris (pH 7.2), 0.1 M NaCl).

Calculations & Assumptions for the Double Layer Potential Profile Shown in Figure 5

To estimate the electric potential profile as a function of distance *x* from a charged surface of potential ϕ_0, as shown in Figure 5, we used the Gouy-Chapman-Stern model (A. J. Bard & L. R. Falkner, *Electrochemical Methods: Fundamentals & Applications,* **2nd Edition**, John Wiley & Sons, New Jersey, 551-552), assuming a potential of -0.8 V at the surface, and a value of 0.79 nm for the outer Helmholtz plane (the approximate radius of a hydrated sodium ion).

The potential is assumed to drop linearly between the electrode surface and the outer Helmholtz plane (that is, the distance of the closest approach of hydrated Na^+ to the surface), and an exponential dependence of potential as a function of distance is assumed in the diffuse layer.

$$\phi_x = \phi_{x=x_H} \exp\left(-k\left(x - x_H\right)\right)$$

where φ is the potential at distance *x*, and k^{-1} is the Debye length, given by:

$$k^{-1} = \frac{1}{\sqrt{8\pi b_j N_A I}}$$

where N_A is the Avogadro constant, b_j is the Bjerrum length (~ 0.7 nm at 25 °C) and *I* is the ionic strength of the electrolyte.

For the purposes of this model the presence of mercaptohexanol under-layer was ignored.

Stability of the Thiol Anchor Groups

We have demonstrated the stability of the DNA thiol anchor (Figure S1a) in our earlier work (*J. Am. Chem. Soc*, **2008**, ***130***, (46) 15589-15601) through re-hybridising a target after application of cathodic potentials and showing that the functionalised substrates with probe strands can be re-used. We have used a similar method to demonstrate stability of the PNA anchor at cathodic potentials. After completing an electrochemical melting experiment, a PNA functionalised substrate was rinsed with buffer before immersing into a fresh solution of PNA target for 12 h. The SER Spectrum was then recorded again to demonstrate that the PNA probe remained undamaged.

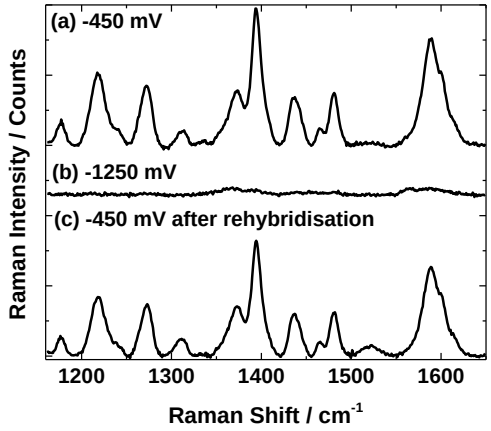

Figure S6. SER spectra of a Cy-3 labelled PNA target bound to an immobilised PNA probe at a sphere segment void surface (a) at -450 mV; (b) at -1250 mV and (C) at -450 mV after rinsing with buffer and hybridising to a new PNA target. Spectra were collected in static mode with a single 30s acquisition at a laser power of 2.7 mW.

Thermal Melting Curves for *I* = 0.1 M

Solution-based thermal denaturation was carried out on a Varian Cary 4000 UV-Visible spectrophotometer. 1 µM solutions (1 ml) of dsDNA duplexes were prepared in a 10 mM Tris (pH 7.2) with added NaCl and a total ionic strength of 0.1 M. The temperature was ramped at 1 °C per min after an initial annealing step. Denaturation of duplex was measured from changes in UV adsorption spectrum at 260 nm.

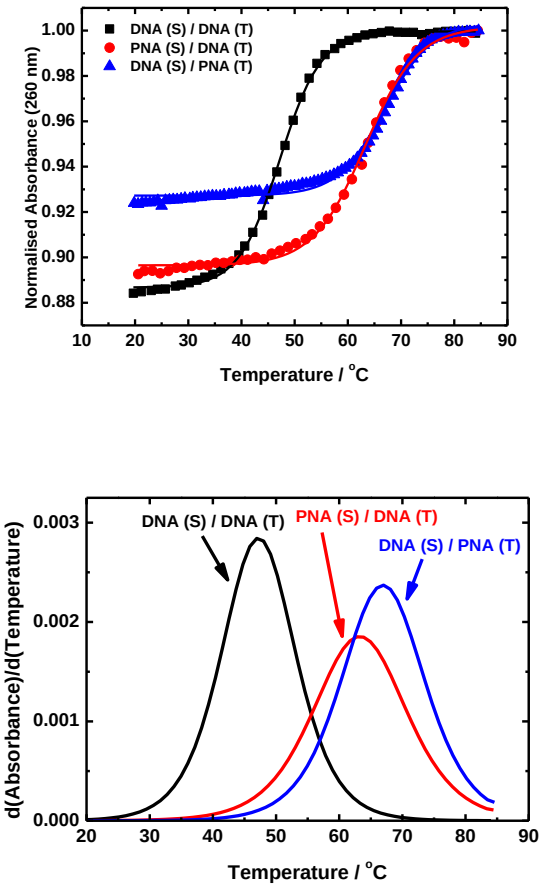

Figure S7. The change in absorbance at 260 nm and first derivative plots indicative of the thermal stability of DNA and PNA containing duplexes. The temperature of 10 mM Phosphate buffer (pH 8.1) with added NaCl (*I* = 0.1 M) containing 1 µM oligomer concentration was ramped at 1 °C min^{-1} after an initial annealing step.