Supporting Information

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Analysis of Short Tandem Repeats by using SERS Monitoring and Electrochemical Melting**

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Experimental Section

SSV preparation

Glass microscope slides were coated with a 10 nm chromium adhesion layer followed by a 200 nm layer of gold using a standard thermal evaporation. After deposition the slides were cut into pieces (2 x 1.5 cm) and cleaned for 90 min by sonication in isopropyl alcohol. After cleaning, the pieces were washed in deionised water and then submerged in 10 mM cystamine in ethanol for 72 h. Upon removal from cystamine solution, the gold pieces were again washed in deionised water before assembly of 600 nm polystyrene spheres (Duke Scientific). Spheres were arranged by cutting out and discarding a trapezium shaped area from a 2 x 1.5 cm area of parafilm, placing the film onto the prepared gold surface, covering with a glass cover slip and heating at 60 °C on a hot plate to seal. The 600 nm sphere solution was then introduced into the arrangement so it was in contact with the gold surface; the arrangement was then placed at an angle of 15° for 24 h at 15 °C^[10]. The closely packed monolayer of 600 nm polystyrene spheres obtained after removal of the glass and parafilm served as a template for the subsequent electrodeposition of gold. Electrodeposition of gold was carried out using 19.9 ml of commercial gold electroplating solution (ECF 60, Technic Inc) to which 0.1 ml of an additive (Brightener E3, Technic Inc) had been added to produce a suitable finish. Masking of the gold surface was carried out using varnish, the area intended for gold electrodeposition was left unmasked and in an electroplating bath was submerged beneath the surface of the electroplating solution. Electrodeposition was carried out under potentiostatic control at -0.72 V vs a homemade saturated calomel electrode (SCE) and using a platinum mesh counter electrode. Deposition was terminated when the amount of charge necessary to deposit the gold to a height of 0.8 *D* (480 nm) had been passed. The SSV substrate was then sonicated in *N,N*-dimethylformamide for 1 h in order to dissolve the polystyrene spheres.

PCR Amplification

PCR was carried out using 20 ng of target sequence (table 1) present in a reaction volume of 20 µl. The reaction volume contained 0.5 µM Texas Red labelled forward primer, and 0.05 µM reverse primer, 0.5 mM dNTPs (Qiagen, Crawley, UK), 1 x Qiagen PCR Buffer (Qiagen, Crawley, UK), 3 mM MgCl₂ and 1 unit of hot start DNA polymerase (Qiagen, Crawley, UK). PCR reactions were performed using a thermocycler and by employing an activation step of 95 °C for 15 min, followed by 35 cycles of 95 °C (20 s), 55 °C (30 s), and 72 °C (30 s).

Probe immobilization and DNA Hybridisation

The thiol modified probe sequence was diluted to a concentration of 1 µM in pH 7.0 10 mM Tris 1M NaCl buffer. The SSV substrate was exposed to probe solution for 48 hrs at 4 °C followed by exposure to 10 µM 6-Mercaptohexanol in pH 7.0, 10 mM Tris 1M NaCl buffer for 30 min at 4 °C. The Texas Red labelled PCR product was then added to 250 µl of pH 7.0, 10 mM Tris 100 mM NaCl buffer and allowed to hybridise with capture probe at 4 °C for 30 min.

Electrochemical Melting.

Electrochemical melting experiments were performed in a custom built cell (Ventacon, Winchester, UK). The SSV chip was connected into the cell before introducing pH 7.0, 10 mM Tris 10 mM NaCl measurement buffer into the cell. A 25 mm diameter circular glass cover slip was placed on top of the SSV and the cell lid screwed down prior to secure attachment to the microscope stage. The cell was connected to an EcoChemie micro Autolab III potentiostat/galvanostat. A series of potentials from -0.3 V to -1.5 V was applied. Potential steps of -100 mV were applied every 300 s with SERS spectra being recorded approximately 290 s after application of the potential step. Experiments were performed where a linear sweep of potential was applied using a scan rate of 0.5 mV s⁻¹ starting from -0.4 V and reaching -1.7 V or less. SERS spectra were recorded every 25 mV. Experiments were carried out at room temperature.

Raman Instrumentation and Data Analysis

Spectra were acquired using a UL-WD 50x objective on a Renishaw 2000 Raman microscope. A 632.8 nm He-Ne laser was employed with a laser spot diameter of 1 µm. Spectra were recorded for 5 – 10 s in a static mode centered at 1450 cm⁻¹ with a laser power of 2.4 mW.

For calculation of peak heights, a background subtraction was carried out using a manual baseline function within Origin 7.0 (OriginLab, Northampton, MA). A Boltzmann function was fitted to melting curves using Origin 7.0.

Reagents

Oligonucleotide and Texas Red labelled oligonucleotide sequences (see table 1) were purchased from ATDBio (Southampton, UK). Millipore water 18.3 M Ω cm was employed throughout the study. Ethanol, *N,N*-dimethylmethanamide and isopropyl alcohol were purchased from Fisher Scientific (Loughborough, UK). Cystamine was purchased from Sigma (Poole, Dorset, UK).

SERS spectra recorded from D16S539 polymorphs 10 – 14 during a sweep of potential (0.5 mV s^{-1})

Figure S1. D16S539 - 10 Repeats. Potential scanned at -0.5 mV s^{-1} and spectra recorded at 25 mV intervals between -0.550 and -1.150 V.

Figure S2. D16S539 - 11 Repeats. Potential scanned at -0.5 mV s^{-1} and spectra recorded at 25 mV intervals between -0.600 and -1.300 V.

Figure S3. D16S539 - 12 Repeats. Potential scanned at -0.5 mV s^{-1} and spectra recorded at 25 mV intervals between -0.600 and -1.525 V.

Figure S4. D16S539 - 13 Repeats. Potential scanned at -0.5 mV s^{-1} and spectra recorded at 25 mV intervals between -0.600 and -1.700V.

Figure S5. D16S539 - 14 Repeats. Potential scanned at -0.5 mV s^{-1} and spectra recorded at 25 mV intervals between -0.800 and -1.800 V .

Figure S6. Replicate melting curves for polymorphs 10 and 11 of D16S530. Points represent mean values and bars standard deviations ($n = 3$).

Multiplex Measurements

Electrochemical Melting Experiments with 2 STRs

Electrochemical melting experiments were conducted as described above, using 10 mM Tris Buffer + 10 mM NaCl and -100 mV potential steps over the range -0.3 V to -1.5 V vs Ag/AgCl. Raman instrumentation, cell setup, laser power and data acquisition were as described above.

Capture probe, template and primer sequences for DYS19

[illegible]

X = dithiol monomer; H = hexaethylene glycol spacer

Dual immobilisation and preparation of SSVs for experimentation

Gold SSV substrates were prepared as described above using 600 nm diameter polystyrene spheres. Capture probes for D16S539 and DYS19 were diluted to a concentration of 0.05 μM each in pH 7.0 10 mM Tris Buffer + 1 M NaCl. SSVs were submerged in the immobilisation solution for 48 h. After immobilisation SSVs were rinsed in deionised water. Blocking was then carried out by submerging the SSVs in 10 μM 6-mercaptohexanol for 1 h. A hybridisation solution was prepared by diluting 20 μL of D16S539 and 20 μL of DYS19 PCR in 460 μL of 10 mM Tris Buffer + 100 mM NaCl. After blocking, the SSVs were rinsed in deionised water and subsequently incubated at 4 $^{\circ}\text{C}$ for 1 h in the hybridisation solution containing DYS19 and D16S539 PCR product. SERS measurements and electrochemical melting experiments were then conducted.

Results

Capture probes for D16S539 and DYS19 were co-immobilised on SSV substrates. The PCR product for D16S539 (labelled with Texas Red) and the PCR product for DYS19 (labelled with Cy3) were then co-hybridised on the substrate for 1 h. SERS spectra were recorded and electrochemical melting experiments performed under the normal conditions. The signals for the two different Raman labels are clearly distinguishable (Figures S7 and S8) and can be separately analysed to derive the electrochemical melting curves (Figure S9).

Figure S7. Multiplex SERS spectra for DYS19 – 13R (Cy3) and D16S539 11R (Texas Red) recorded at different potentials.

Figure S8. Multiplex SERS spectra for DYS19 – 13R (Cy3) and D16S539 12R (Texas Red) recorded at different potentials.

Figure S9. Melting Curves for DYS19 – 13R (Cy3 peak at 1585 cm^{-1}) and D16S539 – 11 and 12R (Texas Red peak at 1501 cm^{-1}) using data taken from Figures S7 and S8.