Supplementary Material (ESI) for Chemical Communications

Highly sensitive detection of dye-labelled DNA using nanostructured gold surfaces

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Supplementary Material

i) Materials

Gold surfaces (Klarite™) were obtained from Mesophotonics (Southampton, UK) and used as supplied. Dyes labelled oligonucleotides were purchased from Eurogentec (Hampshire, UK), Invitrogen (Paisley, UK) and ADT Bio (Southampton, UK) and were purified by HPLC before use. The labelled oligonucleotides (shown in table 1 below) were diluted to various concentrations using sterile water (18.2 MΩ.cm).

Dye label	λ_{max} (nm)	**Oligonucleotide sequence**
ROX	585	5'-GCGTCATCGTATACACAGGAGCAG-3'
Cy7	748	5'-CATTATGTGCTGCCAATC-3'
BODIPY 650	650	5'- GCC TTG ACT GTG GTT TTA GC-3'
Capture strand	-	5'-GCT AAA ACC ACA GTC AAG GC-3'

Table 1: Dye labelled oligonucleotides used in this study,

ii) 1-{[5-(1,2-dithiolan-3-yl)pentanoyl]oxy}-2,5-pyrrolidinedione

The linker was synthesised by the following method. *N*-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC.HCl) (1.840 g, 9.6 mmol, 1.2 eq) was dissolved in DCM (anhydrous, 20 mL), to which, 1.7 mL diisopropylethylamine (DIPEA) (anhydrous, 9.6 mmol, 1.2 eq) was added and stirred for 10 min. To the solution *N*-hydroxysuccinimide (1.290 g, 11.2 mmol, 1.4 eq) was added and the reaction mixture stirred in an ice bath. Thioctic acid (1.643 g, 8.0 mmol, 1.0 eq) was dissolved in anhydrous DCM (10 mL) and added to the reaction over 5 min and stirred overnight. The reaction mixture was washed with HCl (5% (v/v), 50 mL × 2) and water (distilled, 50 mL). The organic layer was dried over Na_2SO_4. Solvents were concentrated *in vacuo* to yield 2.236 g of the product, as a yellow, solid. The residue was dry-loaded on Na_2SO_4 and the product purified by flash column chromatography giving to the product in 83 % yield. δ_H(400 MHz; DMSO) 1.4-1.7 (6H, m, CH_2 × 3), 1.84-1.93 (1H, m, CH), 2.38-2.46 (1H, m, CH), 2.68 (2H, t, J 7.2, CH_2), 2.81 (4H, s, succinimidyl CH_2 × 2), 3.12-3.21 (2H, m, CH_2), 3.56-3.65 (1H, m, CH). δ_C(400 MHz; DMSO) 24.415, 25.830, 28.026, 30.425, 34.217, 38.485, 56.011, 169.287, 170.612. $C_{12}H_{17}NO_4S_2$ requires, C, 47.51; H, 5.65; N, 4.62; S, 21.14 %, found: C, 47.71; H, 5.87; N, 4.50; S, 21.22 %; $[M+NH_4^+]$ requires, 321.0937; found, 321.0937.

iii) Raman spectra acquisition

Spectra were recorded at 632.8 nm λ_{ex} (HeNe) and 785 nm (diode) using a Renishaw (Gloucestershire, UK) System 1000 and a Leica DM/LM microscope. Backscattered light was collected at 180º to the surface using both 20x/0.4 (Olympus) long working distance and 50x/0.75 (Leica) objectives. Dielectric edge (632.8 nm) and Holographic notch (785 nm) filters were used to reject Rayleigh scattering and a 1200 lines/mm grating was used to resolve the spectra onto a red-enhanced *RenCam* deep-depletion charge-coupled device. The beam was attenuated to provide ~2.5 mW at the sample. The spot size could be varied by the use of a beam expander assembly and. The smallest possible laser illumination area is diffraction limited to ~0.5 μm (Spot diameter = 0.61 λ / NA). Typically, defocusing to 76% yielded a spot size of 50 μm using this setup. Spectra were also recorded using 785 nm λ_{ex} using a Mesophotonics SE1000

instrument. Other laser lines used to examine the enhancement of the surfaces were 532 nm (frequency doubled diode), 514.5 nm (Ar^+) and 406 nm (Kr^+).

iv) Drop coat tests

Samples of dye labelled oligonucleotide (500 nL) were deposited directly onto the SERS surface and allowed to dry in air. Spectra were taken across the sample area and each concentration was repeated five times. Signal relative standard deviations were also recorded using surfaces modified with a mask that allowed the samples to dry within a well. The masks had been designed to match the beam profile of the SE1000 instrument.

v) Self-assembled monolayers of oligonucleotide capture strands

A self-assembled film of the thioctic acid N-succinimidyl ester was formed on the Klarite™ surface by immersion in a 0.1 $mg.ml^{-1}$ dimethylformamide (DMF) solution for 30 minutes. After this time the surface was further washed with DMF (5 x 10 ml) and 99.8 % ethanol (5 x 10 ml). Attachment of the oligonucleotide capture strand was performed in aqueous conditions (1 μM) over 1.5 hours. Surfaces were rinsed using a PBS buffer. Despite the competing hydrolysis process, surface attachment was confirmed due to the large increase in the surface wettability of the oligonucleotide modified surface when compared to a surface functionalised by the linker alone. Excess oligonucleotide capture strands were removed from the surface by rinsing in PBS (5 x 20 ml). Slides were prepared incorporating distinct regions functionalised with a mismatch and a complementary capture sequence. The overall modification is shown below in figure 1.

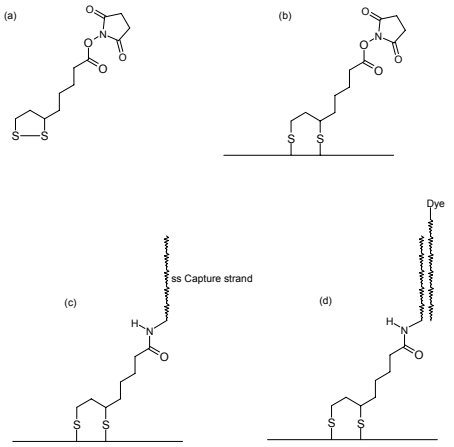

Figure 1: Modification of Klarite™ surface with a oligonucleotide capture strand via a thioctic acid derived NHS-ester linker. (a) 1-{[5-(1,2-dithiolan-3-yl)pentanoyl]oxy}-2,5-pyrrolidinedione, (b) linker attached to the Au surface via thiol bonds, (c) Attachment of ss Capture DNA strand, (d) Hybridisation of dye labelled complementary strand (BODIPY 650).

vi) Hybridisation of dye labelled complementary strand

The modified gold surfaces were fully immersed in a hybridisation solution containing the dye labelled complementary probe, 1M NaCl, 10mM Tris-HCl, 1mM EDTA and 0.01% SDS. Hybridisation was allowed to proceed at room temperature within an enclosure to prevent evaporation. After 1 hour the surfaces were rinsed with PBS buffer to remove non-hybridised oligonucleotides. It is important to ensure the slides are sufficiently washed as some dye labeled oligonucleotides bind non-specifically to unmodified surfaces.

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† Electronic Supplementary Information (ESI) available: Synthesis, SAM formation and hybridisation. See http://dx.doi.org/10.1039/b000000x/