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Supporting Information

Sensitive SERS Nanotags for Use with 1550 nm (Retina-Safe) Laser Excitation

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Experimental

Synthesis of Dye 1 [4-((1E,3E)-5-(2,6-di(selenophen-2-yl)-4H-thiopyran-4-ylidene)penta-1,3-dien-1-yl)-2,6-di(selenophen-2-yl)thiopyrylium hexafluorophosphate]

4-methyl-2,6-di(selenophen-2-yl)thiopyrylium hexafluorophosphate (0.100 g, 0.194 mmol), *N*-3-(phenylamino)allylidene)benzenaminium hexafluorophosphate (35.7 mg, 96.9 μmol), NaOAc (15.9 mg, 0.194 mmol), AcOH (1.0 mL) and Ac_2O (1.0 mL) were combined and heated to 95 °C for 10 min prior to cooling to ambient temperature and diluting with CH_3CN (3.0 mL). Ether (5 mL) was added to precipitate the product. Product yield was 61.0 mg (68%) of **Dye 1** as a copper-bronze solid, mp 210-212 °C: ^1H NMR [500 MHz, CD_2Cl_2] δ 8.34 (d, 4 H, *J* = 5.5 Hz), 7.95 (t, 1 H, *J* = 13.0 Hz), 7.82 (br s, 4 H), 7.60-7.46 (br s, 4 H), 7.44 (t, 4 H, *J* = 4.5 Hz), 6.75 (t, 1 H, *J* = 11.0 Hz), 6.52 (d, 2 H, *J* = 13.0 Hz); λ_{max} (CH_2Cl_2) 959 nm ($\varepsilon = 2.7 \times 10^5$ $M^{-1}cm^{-1}$); HRMS (ESI) *m/z* 776.7717 (calcd for $C_{31}H_{21}S_2{}^{80}Se_4{}^+$: 776.7740). **Anal.** Calcd for $C_{31}H_{21}S_2Se_4 \cdot PF_6$: C, 40.54; H, 2.30. Found: C, 40.84; H, 2.51.

Synthesis of Dye 2 [4-((1E,3E)-5-(2,6-di(thiophen-2-yl)-4H-selenopyran-4-ylidene)penta-1,3-dien-1-yl)-2,6-di(thiophen-2-yl)selenopyrylium hexafluorophosphate]

4-Methyl-2,6-di(thiophen-2-yl)selenopyrylium hexafluorophosphate (80.0 mg, 0.171 mmol), *N*-3-(phenylamino)allylidene)benzenaminium hexafluorophosphate (31.5 mg, 85.6 μmol), NaOAc (14.0 mg, 0.171 mmol), AcOH (2.0 mL) and Ac_2O (2.0 mL) were combined and heated to 90 °C for 3 min prior to cooling to ambient temperature and diluting with CH_3CN (3.0 mL). Ether (5 mL) was added to precipitate the product. Product yield was 33.1 mg (47%) of **Dye 2** as a copper-bronze solid, mp 206-208 °C: ^1H NMR [500 MHz, CD2Cl2] δ 8.00 (t, 2 H, *J* = 11.0 Hz), 7.64-7.63 (m, 8 H), 7.56 (br s, 4 H), 7.20 (t, 4 H, *J* = 4.5 Hz), 6.82

(t, 1 H, *J* = 12.0 Hz), 6.65 (d, 2 H, *J* = 12.5 Hz); λ_{max} (CH_2Cl_2) 880 nm ($\varepsilon = 6.6 \times 10^4$ M^{-1} cm^{-1}), 986 nm ($\varepsilon = 2.6 \times 10^5$ M^{-1} cm^{-1}); HRMS (ESI) *m/z* 680.8821 (calcd for $C_{31}H_{21}S_4{}^{80}Se_2{}^+$: 680.8851). **Anal.** Calcd for $C_{31}H_{21}S_4Se_2 \cdot PF_6$: C, 45.15; H, 2.57. Found: C, 45.24; H, 2.65.

4-Methyl-2,6-di(selenophen-2-yl)thiopyrylium hexafluorophosphate and 4-methyl-2,6-di(thiophen-2-yl)selenopyrylium hexafluorophosphate were synthesised and characterised as reported in a previous publication by Bedics *et al.*[1] *N*-3-(phenylamino)allylidene)benzene-aminium hexafluorophosphate was synthesised and characterised as reported in a previous publication by Panda *et al.*[2]

Synthesis and Characterisation of Nanotags

The HGNs were synthesised and characterised as reported in a previous publication by Bedics *et al.*[1] They have a localised surface plasmon resonance (LSPR) at 715 nm and a final concentration of 6 nM.

The 100 nm AuNPs (supplied by BBI Solutions) and commercial nanotags (purchased from Cabot Security Material Inc.) had concentrations of 3.73 nM and 3.37 nM respectively. A Nanosight LM10 was used initially to obtain concentrations in particles per L and following this the molar concentrations were calculated.

Before SERS measurements were performed, the concentrations of the nanoparticle solutions were adjusted to the same level via the procedure described in the instrument technical notes for the Nanosight LM10. Briefly, the original concentrated solutions were diluted with deionised water until 5 to 30 nanoparticles per CCD frame were obtained. The imaging videos were recorded for 90 seconds and analysed using the provided software (NTA 2.1). The required dilution factor was then applied to the 100 nm Au and HGN solutions so that each nanoparticle solution had a concentration of 3.4 nM (the same as the commercial nanotag).

Investigation into the SERS properties of the 1550 nm nanotags were carried out by mixing nanoparticle solution (100 nm AuNPs or HGNs - 450 μL) with Raman reporter solution (dye 1 or 2 - 50 μL, 10 μM); while the commercial nanotags were used as supplied (500 μL). The SERS measurements were performed using a Real Time Analyzer FT-Raman spectrometer (Connecticut, USA) coupled to an external EDFA (erbium-doped fibre amplifier) laser source. The external laser obtained from Innovative Photonic Solutions (model number RBP1546-C; New Jersey, USA) has an excitation at 1546 nm. All the measurements had a 10 second acquisition time and a laser power operating at 100 mW. Each sample was prepared in triplicate and 5 scans of each replicate were recorded.

For the SERS particle dilution studies, the chalcogen nanotags were prepared by mixing nanoparticle solution (100 nm AuNPs or HGNs - 250 μL) with Raman reporter solution (dye 1 or 2 - 50 μL, 10 μM) and deionised water (250 μL). The commercial nanotags were prepared by adding colloidal solution (250 μL) to deionised water (250 μL) and analysed over the concentration range of 1.7 nM to 80 pM, with the subsequent dilutions being made in deionised water. All other experimental conditions were kept the same as those previously stated. The limit of detection (LOD) was calculated to be 3 times the standard deviation of the blank, divided by the gradient of the straight line. Error bars represent one standard deviation resulting from 3 replicate samples and 5 scans of each.

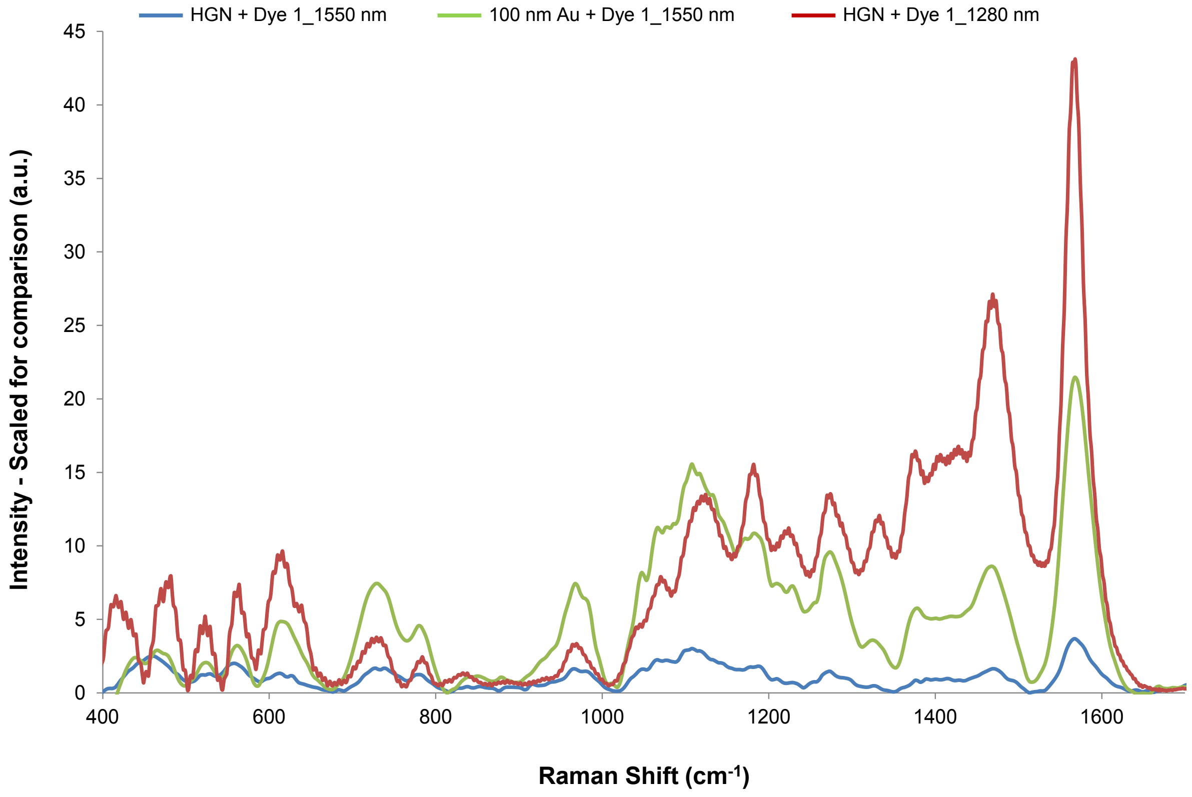

Figure S1 - SERS spectra for chalcogenopyrylium nanotags using a laser excitation at 1550 nm. The blue line represents dye 1 plus HGNs, the green line represents dye 1 plus 100 nm AuNPs and the red line is the SERS spectrum for dye 1 plus HGNs with a 1280 nm laser excitation as a comparison. A laser excitation of 1550 nm and an exposure time of 10 seconds were employed in this analysis. All spectra have been background corrected.

References

1. M. Bedics, H. Kearns, J. Cox, S. Mabbott, F. Ali, N. Shand, K. Faulds, J. Benedict, D. Graham and M. Detty, *Chemical Science*, 2015, **6**, 2302 - 2306.

2. J. Panda, P. R. Virkler, and M. R. Detty, *Journal of Organic Chemistry*, 2003, **68**, 1804 - 1809.