Electronic Supporting Information For:

“Multidentate macromolecules for functionalisation, passivation and labelling of metal nanoparticles”

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Chemicals and reagents

Copolymer of styrene-maleic anhydride with different maleic anhydride (MA) contents (25, 33, 50 wt\%) denoted by PSMA1900 (Mn \textasciitilde 1900), PSMA1700 (Mn \textasciitilde 1700) and PSMA1600 (Mn\textasciitilde 1600) were supplied by Aldrich and used without further purification. 5-aminobenzotriazole, sodium nitrite and N-(1-naphthyl)ethylene diamine were also supplied by Aldrich and used without further purification. Solvents such as diethylether, acetone, ethyl acetate, methanol, and ammonia of analytical grade were obtained from commercial sources (Sigma-Aldrich Chemical Company, UK) and used without purification. Tetrahydrofuran (THF) was dried and deoxygenated with a Pure-Solv 400 solvent purification system by Innovative Technology Inc., USA. Triethylamine was refluxed with NaOH followed by distillation and stored in 4Å° molecular sieve for use. Streptavidin-coated superparamagnetic beads (4 mg / ml) were obtained from New England Bioloabs, U.K. Synthetic oligonucleotides were purchased from ATDbio, UK. Neodymium Iron Boron permanent magnets (1 mm x 1mm) were purchased from E-Magnets UK., Sheffield, U.K.

Buffer systems:
Conjugation buffer: 25 mM MES (2-morpholinoethanesulfonic acid) buffer pH 6.
Hybridisation buffer: 10 mM MgCl\textsubscript{2}, 10 mM Tris-HCl, 40 mM KCl buffer pH 7.
Binding and washing buffer: 0.5 M NaCl, 20 mM Tris-HCl, 1mM EDTA) adjusted to pH 7.5
Buffers were filtered through a 0.2 μm pore size syringe filter (Whatman) prior to use.
Instrumentation

UV-Visible spectroscopy was performed on a Varian Cary Bio 300 spectrometer. The path length of the cuvette was 1 cm. Fourier-transform infra-red spectra were recorded in a Nicolet Impact 400 D spectrometer using KBr pellet method. $^1$H NMR spectra were obtained in DMSO-d$_6$ solutions, on a Bruker DPX400 (400 MHz) NMR spectrometer. SERRS analysis was performed using a Renishaw inVIA Raman spectrometer. The excitation source was an Argon ion laser (514 nm) producing ~30 mW at sample without attenuation. Back-scattered light ($180^\circ$) was collected using a Leica DMLM microscope equipped with a long-working distance objective (x20 / NA 0.4). The inelastically scattered light was analysed using a grating (1800 lines/mm) centred at 1200 cm$^{-1}$ and a RenCam CCD. Spectra were baseline corrected using GRAMS software and plotted in Microsoft Excel. Each spectrum was the result of three 10-sec accumulations.

Synthesis of SERRS-active macromolecules (polymer dyes):

The generic reaction scheme for the synthesis of dye-labelled linear polymers from poly(styrene-co-maleic anhydride) is shown above (Scheme 1).

Synthesis of PD-ABTNEDAs [Modification of PSMA with N-[4-(-5'-azobenzotriazolyl)naphthalen-1-yl)ethylene diamine]

$N$-[4-(-5'-Azobenzotriazolyl)naphthalen-1-yl)ethylene diamine (ABTNEDA), was synthesized as reported in our earlier study.$^1$ For the synthesis of PD1900-ABTNED
Scheme 1, ABTNEDA was taken (242 mg, 0.65 mmol) with dry THF (20 ml) in a 100 ml two necked round-bottom flask equipped with a magnetic stirrer, a reflux condenser, and an inlet used for nitrogen purging as well as addition of reagents using syringe. To the above mixture, a solution of 200 mg of PSMA1900 (0.11 mmol) dissolved in dry THF (10 ml) was added with constant stirring in an oil bath at 60° C with nitrogen purging. Then a solution of triethylamine (mole equivalent to ABTNEDA dye) in 5 ml dry THF was added slowly to the flask through a syringe over a period of 30 min. The reaction mixture was stirred at the same temperature for 24 hrs. The precipitate obtained was filtered and washed repeatedly with THF to remove the excess unreacted ABTNEDA and dried in vacuum at 70° C to yield 73% PD1900-ABTNEDA. PD1700-ABTNEDA and PD1600-ABTNEDA were prepared by adopting the above procedure by reacting 200 mg of PSMA1700 (0.12 mmol) or 200 mg of PSMA1600 (0.12 mmol) with ABTNEDA, 325 mg 0.88 mmol or 460 mg 1.25 mmol to yield 58% PD1700-ABTNED and 67% PD1600-ABTNEDA respectively. Hereafter we describe the PD1900-ABTNEDA, PD1700-ABTNEDA and PD1600-ABTNEDA as polymer dyes.

[PD1700-ABTNEDA and PD1600-ABTNEDA were prepared by adopting the above procedure by taking the mole ratio of ABTNEDA:PSMA1700 or PSMA1600 as 7.3 or 10, to yield 58% and 67% of PSMA1700-ABTNED and PSMA1600-ABTNED respectively.]

Characterisation

FT-IR spectroscopy confirmed the modification of various PSMA with ABTNEDA according to the reaction scheme 1. Figure 1 shows the FT-IR spectra of PSMA1900 and the polymer dyes, where the absorption bands at 1857 and 1779 cm⁻¹ are the characteristic bands assigned to asymmetrical and symmetrical C=O stretching of maleic anhydride moieties, respectively in the unmodified PSMA1900. Whereas the asymmetrical (≈1857 cm⁻¹) and symmetrical (≈1779 cm⁻¹) C=O stretching absorption band of the anhydride are disappeared in the polymer dyes, indicating that the anhydride has been converted and new signals corresponding to the carboxylate C=O stretching vibrations (≈1575 cm⁻¹) and amide group C=O stretching vibrations
(\sim 1667-1700 \text{ cm}^{-1}) \text{ are clearly noted from the spectra. New broad bands are being observed at } 3350 \text{ cm}^{-1} \text{ region, attributed to the N-H stretching in the polymer dyes.}

\textbf{Figure 1.} FT-IR spectra of PD1900 (a) and PD1900-ABTNEDA (b), PD1700-ABTNEDA (c) and PD1600-ABTNEDA (d).

In the $^1$H NMR spectrum of PSMA1900, the broad resonance peak between 0.9 and 2.5 $\delta$ ppm and the resonance peak between 6.3 and 7.5 $\delta$ ppm are due to the methylene/methine protons on the backbone chains and aromatic ring protons of styrene respectively (Figure 2). The methine protons of maleic anhydride appear between 2.8 and 3.4 $\delta$ ppm. The $^1$H NMR spectrum of PD1900-ABTNEDA, showed a resonance peaks between 6.1 and 7.7 $\delta$ ppm for their aromatic protons due to styrene units in the backbone of the copolymer and naphthalene/NH-benzotriazolyl units of ABTNEDA grafted in the opening reaction between the anhydride unit of PSMA1990 with amine containing ABTNEDA. The peaks for methylene protons attached to -NH observed between 3.1 and 3.6 $\delta$ ppm. In addition, it is worth mention that a small peaks around 4.4.1 $\delta$ ppm is attributed to –NH protons. Small broad peaks at 8, 8.3 and 9 $\delta$ ppm are due to the benzotriazole-protons.
Silver Colloid Preparation

All glassware was cleaned using *aqua regia* (HNO₃:H₂SO₄), followed by thorough rinsing with distilled water, prior to use. EDTA (0.947 g, 3.24 mmol) was added to a large beaker of distilled water (2 L). The beaker was heated on a hotplate, and the EDTA solution was mixed with an overhead stirrer. Sodium hydroxide (0.350 g, 8.75 mmol) dissolved in distilled water (20 ml) was then added to the beaker. Once the mixture reached boiling, silver nitrate (0.088 g, 0.52 mmol) in distilled water (20 ml) was added. The suspension was allowed to cool to room temperature and was poured into a plastic bottle for storage.

UV-Vis (H₂O): $\lambda_{\text{max}} = 413$ nm

Figure 2. ¹H NMR spectra of (a) PSMA1900, and (b) PD1900-ABTNEDA.
Preparation of SERRS-active macromolecule/silver colloid mixtures

An aqueous solution of the SERRS-active macromolecule (1 x 10^-4 M 0.5 ml) was added to fixed amount of silver colloid (3 ml) and water (1.5 ml) to give final concentrations of 1 x 10^-5 M, 1 x 10^-6 M, 1 x 10^-7 M and 1 x 10^-8 M polymer dye in solution. Samples were left to stand at room temperature for a period of 24 hrs prior to the measurements being taken.

UV-Vis measurements:
An aliquot of the polymer dye and silver colloid dispersion (200 µl) was diluted with distilled water (1800 µl)

SERRS measurements:
An aliquot of the polymer dye and silver colloid dispersion (200 µl) was pipetted into a disposable 96 - well plate and the microscope focused on the solution surface.
Stability of SERRS-active macromolecule/silver colloid mixtures:

![SERRS spectrum of silver nanoparticles labelled with PD1600 after 12 hours (solid line) and 1 month (dotted line).](image)

**Figure 3.** SERRS spectrum of silver nanoparticles labelled with PD1600 after 12 hours (solid line) and 1 month (dotted line).

**Preparation of Streptavidin-coated magnetic beads:**

Streptavidin-coated magnetic particles (50 μl, 4 mg/ml) were mixed in an eppendorf with an equal volume of binding and washing buffer. A permanent magnet was used to pull the magnetic beads to one side of the tube and the supernatant was removed and discarded. This washing process was repeated three times by re-suspending the beads in binding and washing buffer (50 μl). The beads were then re-suspended in binding and washing buffer (250 μl). An aliquot of the stock solution (20 μl) was diluted further with binding and washing buffer (250 μl, final concentration ~1.6 x 10^{-2} mg/ml) and stored at 4 °C. This dilute stock solution was the one used in further experiments.

**Derivatisation of SERRS-active macromolecules with oligonucleotides (PD1900-oligo)**

Purified PD 1900 polymer dye (100 μl, 10^{-4} M in conjugation buffer) was mixed with amino-oligonucleotide probe (50 μl, 1.5 x 10^{-4} M in H₂O, 5’Biotin- GTA TCT ATA TTC ATC ATA GGA AAC ACC ATT – NH₂), conjugation buffer (200 μl) and left for 45 minutes at room temperature under gentle stirring. Freshly prepared 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC-HCl, Aldrich) solution
(50 μl, 10 mg / ml in conjugation buffer) was then added to the reaction mixture, mixed well and placed in a fridge at 4 °C for 16 hrs.

Un-reacted oligonucleotide and reaction by-products were removed by centrifuging the samples at 6000 rpm for 10 mins, removing the supernatant and re-suspending the coloured residue in fresh conjugation buffer (500 μl). This washing step was repeated twice with conjugation buffer and once with double-distilled H₂O (500 μl).

The washed polymer-dye bioconjugate was incubated for 16 hrs at room temperature with EDTA silver colloid (300 μl) and double-distilled H₂O (200 μl).

Samples to be used in the DNA binding experiment were further treated by centrifuging the samples at 6000 rpm for 10 minutes, removed the supernatant, washing in binding and washing buffer (200 μl), centrifuged again, re-suspended in binding and washing buffer (80 μl) and mixed with a streptavidin-coated bead solution (20 μl, ~1.6 x 10⁻² mg / ml). The mixture was incubated for 30 mins at room temperature and washed five times with binding and washing buffer (100 μl).

**SERRS spectra of magnetic beads immobilised on microtitre well-plate:**

Bead samples in binding and washing buffer (100 μl) were placed in different wells of a 96-well microtitre plate. Permanent magnets were then positioned directly underneath the corresponding wells to force the magnetic beads to the bottom of the well. The plate was mounted on an x-y-stage and a x20 (LRO) was then used to focus on the beads immobilised on the surface of the microtitre well-plate. Mapping software (Wire 2.0) was then used to take multiple spectra over a defined region in 10 μm steps (514 nm excitation, grating centred at 1400 cm⁻¹, 10% power of 30 mW, 1 second, 1 accumulation). This data was then used to generate a “Raman map” based on the intensity of the most intense band at 1419 cm⁻¹ (corresponding to the SERRS dye).

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