# Multidentate macromolecules for functionalisation, passivation and labelling of metal nanoparticles†

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**A new class of SERRS-active macromolecule designed to protect silver nanoparticle surfaces against salt corrosion whilst retaining colloidal stability of the particles is reported.**

The optical properties of silver and gold nanoparticles make them suitable for the construction of small scale sensors which can be detected by plasmon resonance, surface-enhanced resonance Raman scattering (SERRS), colorimetry, or fluorescence.[1] To achieve maximum speciation, it is preferred to functionalise the particles through covalent attachment of sensing species. However under assay conditions, many common surface attachment groups can be displaced from the surface by competing species. With silver nanoparticles, one common problem is the action of sodium chloride on the surface to displace labels such as dyes, and earlier studies by others suggest that a suitable protective shell may help stabilise the particles in solution.[2] To overcome this problem, a new class of polymeric ligand which contains multiple copies of surface complexing dye labels per polymer molecule has been synthesised. The polymers protect the silver surface from corrosion by sodium chloride and retain the functionalised particles in suspension. Here we demonstrate dramatically-improved performance for sensitive detection using SERRS and give an initial example showing that the tags are effective in a high salt buffer analytical procedure.

The dye used to synthesize these ligands was *N*-[4-(5-azobenzotriazolyl)naphthalen-1-yl]ethylene diamine [**1**] which has been shown previously to give intense SERRS.[3] This dye has three key structural features: (1) an azo chromophore, which is SERRS-active; (2) a benzotriazole motif, which chemisorbs to metal surfaces; (3) an ethylamine fragment, through which the dye can be tethered to a macromolecule of choice. Multiple copies of the dye were introduced into the side-chains of a family of commercially available reactive pre-polymers *via* a polymer-analogous reaction (Fig. 1). The reactive macromolecules selected for this purpose were synthetic copolymers of styrene and maleic anhydride (PSMA), which are commercially available in a variety of molar masses and styrene to maleic anhydride ratios. The anhydride units within these macromolecules allow for facile introduction of a range of chemical species,[4] such as the alkylamine-ended SERRS-active dye [**1**].

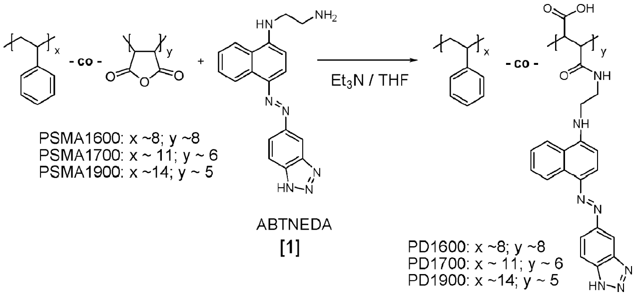

**Fig. 1** Ring opening of poly(styrene-co-maleic anhydride) copolymers by the SERRS-active dye [**1**] to give SERRS-active macromolecules.

Fig. 2 shows a model of the type of dye-labelled macromolecule synthesised. As modelled, we propose that it contains clear hydrophobic and hydrophilic regions. Hydrophobicity is provided by the benzotriazole azo dye and styrene groups. The hydrophilic region arises from the carboxylate groups on the polymer backbone which provide solubility in polar media at neutral pH. The benzotriazole group was chosen due to its property as a passivating agent for silver and also because the use of monomeric benzotriazole dyes has been very effective for studying surface-enhanced resonance Raman scattering (SERRS) from silver surfaces.[5] In this respect, the key feature of these dyes is that they contain both a chromophore, which absorbs in the visible region of the electromagnetic spectrum

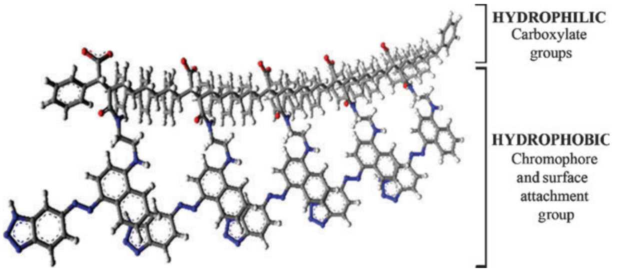

**Fig. 2** Schematic representation of the chemical structure of the polymer dyes. Oxygen atoms of the carboxylates are highlighted in red and N–N bonds in the attachment groups in blue.

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and is hence in resonance with the exciting radiation, and a benzotriazole moiety which chemisorbs to metal surfaces.

Another feature of these polymers is that there is a degree of backbone flexibility, which allows organisation of the polyvalent species on the metal surface. Furthermore, one side of the molecule is more hydrophilic than the other, consequently the molecule will tend to organise with the more hydrophobic benzotriazole groups pointing inward. This conformation provides very effective multiple attachment points to the metal nanoparticles while providing a hydrophilic outer layer, as well as providing an added driver for surface adhesion. The multiple strong surface attachment groups and polymer backbone protecting the surface is intended to provide passivation as well as strong adhesion to the surface.

Three polymers were synthesized and investigated (PD1600, PD1700, and PD1900). The parent PSMA polymers had number average molecular weights of 1600, 1700 and 1900, with maleic anhydride contents of 50%, 33% and 25% (w/w), respectively. On average, PD1600 contained 8 molecules of the SERRS active dye complexing agent [**1**] per 16 monomer residues, PD1700 contained 6 per 17 monomer residues, and PD1900 contained 5 per 19 monomer residues. The carboxylic acid function is not affected by the attachment of these dyes but, as we will show, it is available for the tethering of other molecules such as biomolecules for sensing.

SERRS was detected using a standard Raman spectrometer. The technique requires that a dye molecule is adsorbed on a roughened metal surface, and silver is particularly effective in providing strongly enhanced scattering with visible excitation.[6] It is known that intense signals are obtained mainly from the first layer of analyte in contact with the metal surface.[7] Thus, the appearance of a SERRS signal is a clear indication of strong attachment of at least some of the benzotriazole side chains to the surface. Silver colloid was prepared in alkaline conditions in the presence of EDTA, as reported by Heard and co-workers, with an average diameter of 39 nm.[8] An aqueous solution of the polymer dye was then added to the colloidal silver particles. Fig. 3 shows sharp intense spectra from all three of these colloidal suspensions. The final concentration of polymer was $2 \times 10^{-7}$ M. This is approximately the amount of polymer dye required to achieve monolayer coverage across the surface. As expected, the ability of the unattached (monomeric) dye to pack with more chromophores per unit surface area has resulted in somewhat more intense spectra. The relative intensities and frequencies of the bands in the spectra are very similar to those in the SERRS spectrum of the monomeric dye used to form the side chain. This confirms that attachment through the side-chains must be present at approximately the same orientation to the surface as the monomeric dye. The band just above 1400 $\text{cm}^{-1}$ results from displacements of the atoms in the aromatic rings and the C–N bridge groups, and the major band just below 1400 $\text{cm}^{-1}$ is due to the azo stretch. Other intense bands are mainly due to different in-plane ring displacements.[9]

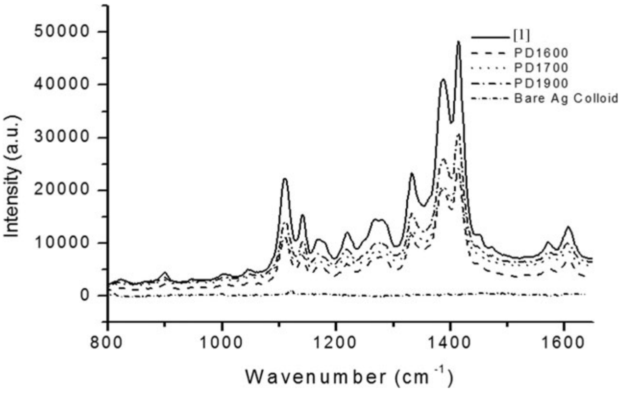

**Fig. 3** SERRS spectra obtained from silver colloid loaded with the azo dye [**1**] and the three SERRS-active macromolecules, PD1600, PD1700 and PD1900. The SERRS spectrum of bare colloid (no dye present) is included for reference purposes. Dye concentrations: $2 \times 10^{-7}$ M.

Sodium chloride is often present in biological buffers, but it corrodes silver surfaces through the formation of an insoluble layer of silver chloride.[2] Addition of saline solution to functionalised silver nanoparticles was used to make an initial assessment of the ability of the polymer dyes to passivate the surface. The frequency and shape of the main band in the UV-visible spectrum of silver colloid is related indirectly to particle size and shape through the interaction of the surface plasmon with light. The frequency of the main band can give an indication of the average particle size, the full width at half-height (FWHH) can give an indication of the particle size distribution, and aggregation is indicated by a red shift and a decrease in the extinction coefficient of the main band and the appearance of a second band at a longer wavelength. Unaggregated silver colloid absorbs visible light just above 400 nm. When the monomeric dye is added to the colloid, the spectrum broadens and the peak shifts to slightly longer wavelength (Fig. 4a) mainly due to the surface modification and possibly some particle–particle interactions. However, addition of sodium chloride to the colloid causes immediate loss of the peak due to precipitation of the unstable colloid. This is a result of corrosion of the surface by the sodium chloride to form a silver chloride surface layer thus reducing the effective surface charge and hence colloid stability. When the polymer dye was added to colloid, a very similar shift to that found with the monomeric dye was obtained. However, addition of sodium chloride did not cause precipitation and the peak in the UV-visible spectrum, although altered, was still present (Fig. 4b). Furthermore, these suspensions remain stable for months. After one month the SERRS spectrum was similar to that of the starting suspension but with a small drop in intensity (see ESI†). Silver colloidal particles are negatively charged and this charge keeps the particles in suspension. The presence of the carboxylate anion ensures that at neutral pH this stability will be maintained after addition of the polymer.

The free carboxylate groups on the polymer dye provide potential anchoring groups for covalent coupling of different molecules such as oligonucleotides, antibodies and enzymes. In this initial example of the use of the new polymers, a biotin-labelled oligonucleotide (30 mer, 5′-biotin, 3′-amine) was conjugated to a polymer dye (PD1900) *via* amide formation using carbodiimide chemistry. The new conjugate (PD1900–oligo) was then incubated overnight with silver colloid to promote adsorption of the conjugate onto the nanoparticle surface. The particles remained colloidally stable, as the

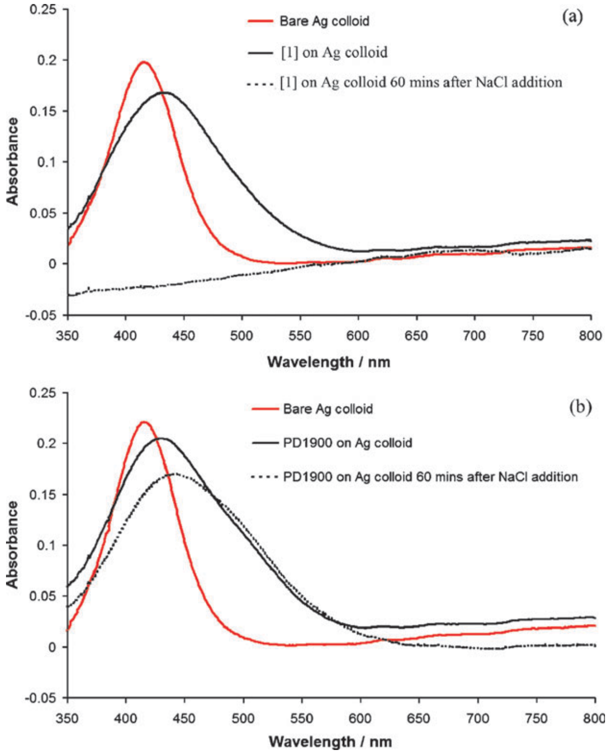

**Fig. 4** Electronic spectra of dye [**1**] loaded (a) and PD1900 loaded (b) silver colloid measured before, and 60 minutes after, exposure to 1.5 M NaCl. Dye/polymer dye concentrations: $1 \times 10^{-5}$ M.

polyanionic nature of the oligonucleotide provides electrostatic repulsion between the particles. To demonstrate their potential as tags for biorecognition, the labelled nanoparticles were mixed with streptavidin superparamagnetic magnetic beads (1 μm, New England Biolabs, UK) in the presence of a high salt buffer (0.5 M NaCl, 20 mM Tris-HCl, 1 mM EDTA, pH 7.5). The nanoparticles labelled with PD1900–oligo were trapped onto the bead surface due to the known strong interaction between biotin and the streptavidin protein.[10] A small permanent magnet was used to separate the beads onto the side of a microtitre well during the washing steps. A Raman map of the beads confirmed the capture of the silver nanoparticles coated with PD1900–oligo as shown in Fig. 5. This was not observed when nanoparticles labelled with PD1900 but lacking the biotin oligonucleotide linker were used.

To conclude, a new class of polymeric ligand particularly effective for passivation and labelling of silver surfaces has been developed. A surface charge is retained aiding suspension of nanoparticles. The polymer dye shows strong attachment to the surface and confers protection against sodium chloride corrosion. In addition, carboxylic acid groups are available for further conjugation, either for the addition of biomolecules for sensors or inclusion of particles in a polymer matrix. The example described shows one possible use of these materials.

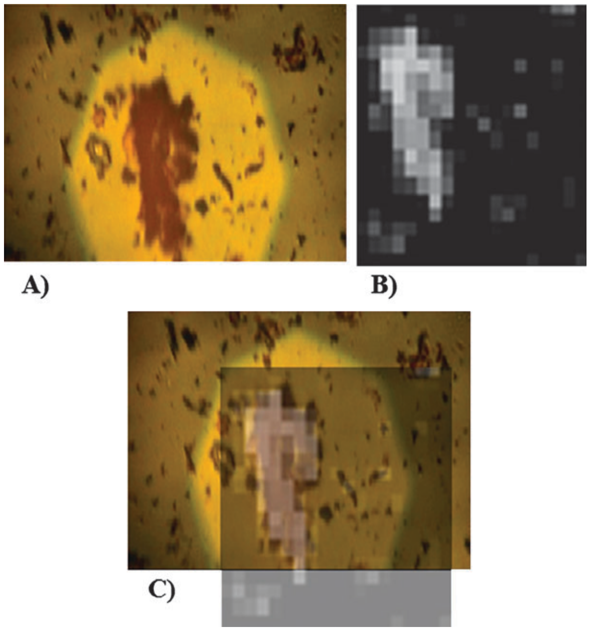

**Fig. 5** (A) Optical image of a glass slide covered with streptavidin superparamagnetic beads coated with PD1900–oligo adsorbed onto silver nanoparticles, (B) Raman map generated based on the PD1900 band at 1419 cm$^{-1}$ and (C) overlaid images showing signal overlap.

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