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

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Abstract

Chalcogenopyrylium nanotags demonstrate an unprecedented SERS performance with a retina safe, 1550 nm laser excitation. These unique nanotags consisting of chalcogenopyrylium dyes and 100 nm gold nanoparticles produce exceptional SERS signals with picomolar detection limits obtained at this extremely red-shifted and eye-safe laser excitation.

Raman scattering provides good molecular specificity however, it is dependent on the 4th power of the excitation frequency, thus scattering is weak when infrared (IR) excitation is used.[1] The scattering can be enhanced by attaching or trapping molecules close to the surface of metallic nanostructures, thus giving rise to a phenomenon known as surface enhanced Raman scattering (SERS).[2] SERS can give rise to enhancements several orders of magnitude greater than conventional Raman scattering. Hence it can be intense well into the infrared.[1-3]

SERS nanotags are *in situ* probes which consist of metallic nanoparticles and Raman reporters. They provide extremely sensitive and selective analytical tools for studying chemical and biological systems.[4] To date, noble metal nanoparticles, commonly silver and gold, are utilised as suspension based SERS substrates, as they are stable materials and resonant in the visible region.[1, 4] However, by changing the size, shape, surface chemistry and/or aggregation of the nanoparticles it is possible to shift the localised surface plasmon resonance (LSPR) several hundred nanometres into the IR region.[5]

Currently there is a lot of interest in designing SERS nanotags for use in the IR region due to the greater depth of light penetration into biological samples, such as blood and tissue samples, compared to the visible region.[6] Furthermore, there is reduced autofluorescence, limited photobleaching and the IR region provides an uncongested spectral window for optical analysis due to the absorption and scattering of many molecules, in particular bio-molecules being at a minimum.[6-9] To exploit this, there is a great need to design SERS nanotags, which give reproducible response, can provide strong enhancements and, in particular, are optically active in the IR region.[10, 11]

Medical research has mainly focussed on designing nanotags for use with 785 nm laser excitations and recently with 1064 nm laser excitations for photo-thermal ablation treatment.[12-15] SERS nanotags operating at 785 nm,[5, 13, 16, 17] 1064 nm[18-21] and very recently at 1280 nm have been reported.[11] This work however, is focussed on designing SERS nanotags which give strong SERS responses with 1550 nm laser excitation which is retina-safe. To date, we are only aware of 4 publications which have explored this laser wavelength in which only 2 of these employ SERS.[3, 22-24] One report was by Farquharson *et al.*, who detected the food contaminant 'melamine' at a concentration of 1 mg/mL by using silver doped sol-gels as the SERS substrate.[23] The other publication was by Smith *et al.* in 2010. This work determined that 100 nm gold nanoparticles (AuNPs) encapsulated with the non-resonant Raman reporter, BPE (1,2-bis(4-pyridyl)ethylene) at a concentration of 10 μM, produced the optimum conditions for SERS analysis at this wavelength. It was hoped that these results would provide the basis for future advancements in homeland security applications.[3]

Herein, we report that 100 nm AuNPs modified with chalcogenopyrylium dyes give strong SERS responses with picomolar (pM) detection limits using this retina-safe excitation. We have previously demonstrated the SERS capabilities of these dyes, with pM detection limits being observed with a 1280 nm laser excitation.[11] In that work, a small library of chalcogenopyrylium dyes were tested with hollow gold nanoshells (HGNs) as the SERS substrate and it was found that 2-thienyl and 2-selenophenyl substituents make excellent attachment groups for absorbing strongly onto gold surfaces. Furthermore, it was demonstrated that by increasing the number of sp^2 carbons in the chalcogenopyrylium backbone from 1 to 3 caused a significant increase in the SERS response. Based on these findings, 2 new dyes were synthesised with 5 sp^2 carbons in the backbone to generate a pentamethine bridge. The two dyes, namely

dye 1 and 2 have absorbance maxima at 959 and 986 nm, respectively. The fine tuning of these wavelengths into the NIR region arises by interchanging the chalcogen atoms in the backbone and in the ring systems. Increasing the number of sp^2 carbons in the core also causes the absorption maximum to red-shift.[11]

Following initial studies,[3, 11] HGNs and 100 nm AuNPs were modified with dye 1 and their ability as 1550 nm SERS nanotags investigated. Figure S1, ESI, shows that the 100 nm AuNPs make for better SERS substrates with a 4-fold increase in SERS intensity over the HGNs being observed. Therefore in the following experiments 100 nm AuNPs were solely used in the design of the 1550 nm chalcogenopyrylium nanotags.

To fully understand the SERS capabilities of these nanotags, they were compared with commercially available tags. These nanotags consist of large AuNPs (~100 nm in size) encapsulated with the non-resonant reporter BPE and silica. Figure 1, shows the results of this SERS comparison using the 1550 nm laser excitation. It should be noted that in these studies no inorganic salt was added to the nanotags to enhance the SERS response. Several of the chalcogenopyrylium dyes have previously been shown to produce intense SERS signals in the absence of KCl, and this is possibly due to a strong interaction occurring between the Raman reporter and nanoparticle surface, inducing self-aggregation.[11] This partial aggregation is still sufficient to produce intense SERS at 1550 nm (figure 1).

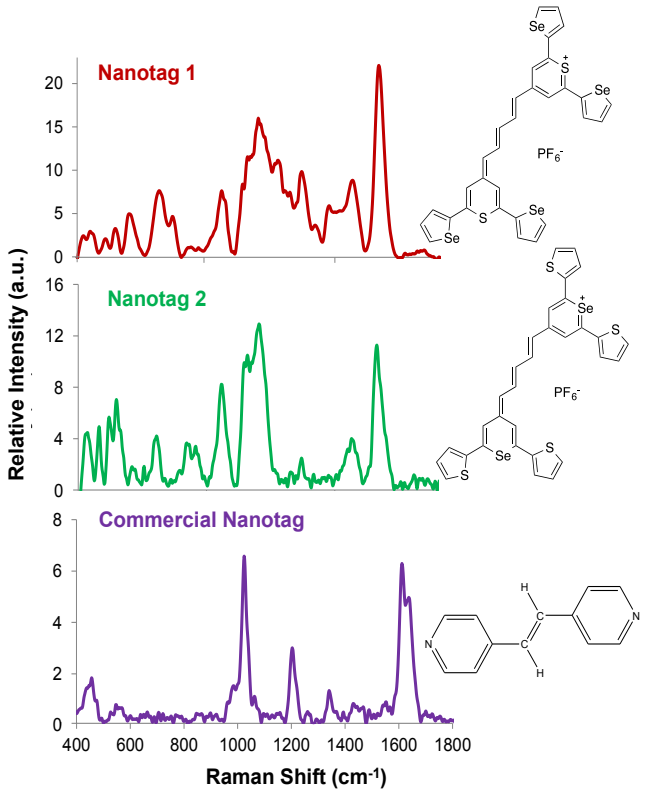

Figure 1 – SERS comparison study between the commercial nanotag and the chalcogenopyrylium nanotags 1 and 2 (100 nm AuNPs plus dyes 1 and 2 (10 μM)). All the nanotags had a concentration of 3.4 nM. A laser excitation of 1550 nm and an exposure time of 10 seconds were employed in this analysis. All spectra have been background corrected. Dye 1 has an absorbance maxima at 959 nm, dye 2 at 986 nm and the commercial tag does not absorb in the visible/IR region.

It can be observed in figure 1 that all three nanotags, which are highly aromatic, produce vibrationally rich and intense SERS spectra with the 1550 nm laser excitation. Nanotag 1 however, produced the most intense SERS response followed by nanotag 2 and then the commercial nanotag. When comparing the most intense peak at ~1600 cm^{-1} which arises due to heterocyclic aromatic ring stretching within the molecule;[10, 25] nanotag 1 has an increase of ~3 times that of the commercial nanotag while nanotag 2 is twice as intense. Furthermore, dye 2 with four 2-thienyl substituents gave a weaker SERS signal compared to dye 1 with four 2-selenophenyl substituents. This suggests that the selenophene group adheres more effectively to the gold surface than thiophene and supports previous reports where selenolates have shown a greater affinity for gold surfaces than thiolates and phenyls.[11, 26, 27]

Due to the exceptional SERS response obtained with these nanotags, particle dilution studies were conducted in order to calculate limits of detection (LOD) and further determine the level of sensitivity that can be achieved at this extremely red-shifted laser excitation. The LOD

study was conducted over the concentration range 1.7 nM to 80 pM. The initial particle concentration was 1.7 nM and subsequent dilutions were made in water until no signals from the nanotags were observed. The peak at ~1600 cm^{-1} was used to calculate the LOD since it was the most intense peak in the spectrum. Figure 2 shows that a linear response was followed for all three nanotags. The LOD was calculated to be 3 times the standard deviation of the blank, divided by the gradient of the straight line which can be observed in each of the plots in figure 2.

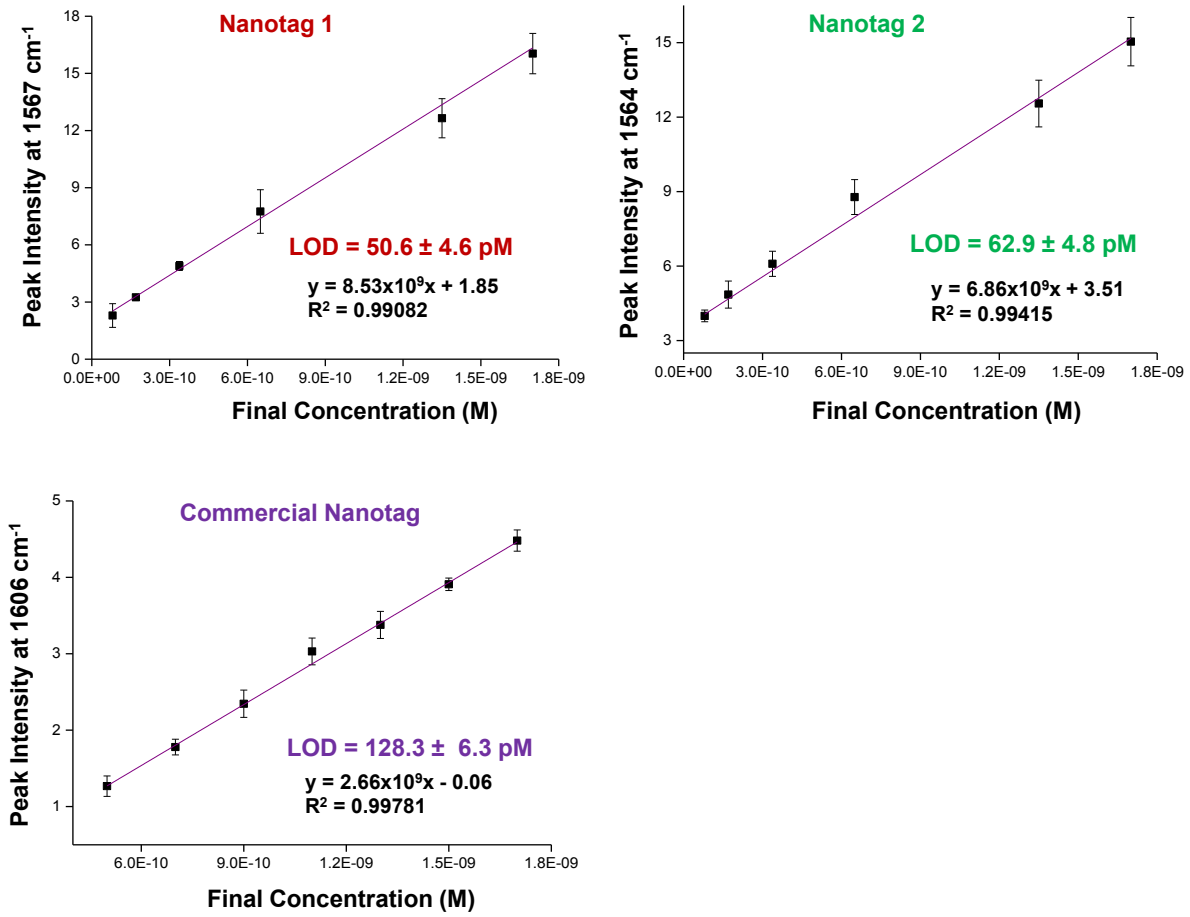

Figure 2 - SERS particle dilution studies for chalcogenopyrylium nanotags 1 and 2 and the commercial nanotag over the concentration range 1.7 nM to 80 pM. A laser excitation of 1550 nm and an exposure time of 10 seconds were employed in this analysis. Error bars represent one standard deviation resulting from 3 replicate samples and 5 scans of each.

Chalcogen nanotags 1 and 2 produced the lowest LODs with values of 50.6 ± 4.6 pM and 62.9 ± 4.8 pM, respectively. The commercially available nanotag gave the highest LOD value at 128.3 ± 6.3 pM. This value is approximately 2-2.5 times greater than the pentamethine chalcogen nanotags, demonstrating the superiority of these newly developed SERS nanotags

for use at this retina-safe excitation wavelength. Obtaining SERS signals in the IR region is extremely difficult, [10] thus it was an unexpected and yet extraordinary result to obtain SERS signals with pM detection limits at 1550 nm. Furthermore, these nanotags have demonstrated the ultra-low sensitivity that can be achieved by SERS in the IR region, thus providing the basis for future advancements in bio-chemical, medical and optical applications.

Conclusion

In conclusion, the combination of 100 nm AuNPs encapsulated with chalcogenopyrylium dyes have shown limits of detection in the picomolar range, which is extremely low as Raman scattering is intrinsically weak at this excitation wavelength. In addition, these unique nanotags have proven to be better than commercially available ones and the fact that they are compatible with a range of NIR laser excitations, demonstrates their flexibility which could be essential for future advancements in biomedical and optical applications specific to the IR range.

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Acknowledgements

D.G. acknowledges the Royal Society for support in the form of a Wolfson Research Merit Award. This work was also supported by Dstl and the Engineering and Physical Sciences Research Council [grant number EP/J500550/1]. M.A.B. and M.R.D. acknowledge the National Science Foundation (CHE-1151379) for partial support of this work. The research data associated with this paper will become available at the following link:
http://dx.doi.org/10.15129/2be0cb21-e1c0-4817-b382-cfe85dae0c71