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Extreme Red Shifted SERS Nanotags

Matthew A. Bedics, Hayleigh Kearns, Jordan M. Cox, Sam Mabbott, Fatima Ali, Neil C. Shand, Karen Faulds, Jason B. Benedict, Duncan Graham and Michael R. Detty

Surfaced enhanced Raman scattering (SERS) nanotags operating with 1280-nm excitation were constructed from reporter molecules selected from a library of 14 chalcogenopyrylium dyes containing phenyl, 2-thienyl, and 2-selenophenyl substituents and a surface of hollow gold nanoshells (HGNs). These 1280 SERS nanotags are unique as they have multiple chalcogen atoms available which allow them to adsorb strongly onto the gold surface of the HGN thus producing exceptional SERS signals at this long excitation wavelength. Picomolar limits of detection (LOD) were observed and individual reporters of the library were identified by principal component analysis and classified according to their unique structure and SERS spectra.

Introduction

Surface-enhanced Raman scattering (SERS) has been utilized as an extremely sensitive analytical tool in the study of biological systems. The combination of a metallic nanoparticle and an organic dye reporter molecule provide SERS nanotags that can be used to detect target molecules using laser Raman spectroscopy or SERS microscopy. SERS nanotags and SERS reporters have been designed and utilized with the 785-nm laser vs. 800-nm light is documented, superior penetration of 1280-nm light in turbid media such as human tissue is minimal in the 600-800-nm range and greater penetration depth into biological tissues.

SERS nanotags operating with 1280-nm excitation were constructed from reporter molecules selected from a library of 14 chalcogenopyrylium dyes containing phenyl, 2-thienyl, and 2-selenophenyl substituents and a surface of hollow gold nanoshells (HGNs). These 1280 SERS nanotags are unique as they have multiple chalcogen atoms available which allow them to adsorb strongly onto the gold surface of the HGN thus producing exceptional SERS signals at this long excitation wavelength. Picomolar limits of detection (LOD) were observed and individual reporters of the library were identified by principal component analysis and classified according to their unique structure and SERS spectra.

Results and Discussion

Reporter Library Synthesis and Properties

The library of dyes 1-14 was constructed by condensation of 4-methylthiopyrylium and 4-methylselenopyrylium salts 15 either with chalcogenopyranones or with (4-chalcogenopyranone)acetalddehyde derivatives in acetic anhydride to give monomethine dyes 1-8 or trimethine dyes 9-14, respectively (Figure 1). For trimethine dyes 9-14, values of λmax in CHCl3 were in the range of 1.1 × 105 to 1.5 × 105 M-1 cm-1 (Table 1). For trimethine dyes 9-14, values of λmax in CHCl3 varied from 826 nm for dye 10 to 826 nm for dye 14 while values of ε were in the range of 2.0 × 105 to 2.8 × 105 M-1 cm-1 (Table 1). The interchange of S and Se atoms in the chalcogenopyranylideneacetalddehyde derivatives 17 in acetic anhydride to give monomethine dyes 1-8 or trimethine dyes 9-14, respectively (Figure 1).
Fig. 1. Chalcogenopyrylium monomethine (1-8) and trimethine dyes (9-14) from building blocks 15-17.

Table 1. Values of the absorption maximum ($\lambda_{\text{max}}$), molar extinction coefficient ($\varepsilon$) and calculated LOD values from the SERS experiment with associated standard deviation (s.d.) error for chalcogenopyrylium dyes 1-14.

<table>
<thead>
<tr>
<th>Dye</th>
<th>$\lambda_{\text{max}}$, nm (CH$_2$Cl$_2$)</th>
<th>$\varepsilon$, M$^{-1}$ cm$^{-1}$ (CH$_2$Cl$_2$)</th>
<th>LOD, pM$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>653</td>
<td>$1.3 \times 10^5$</td>
<td>21.8 ± 1.9</td>
</tr>
<tr>
<td>2</td>
<td>676</td>
<td>$1.3 \times 10^5$</td>
<td>29.4 ± 2.7</td>
</tr>
<tr>
<td>3</td>
<td>699</td>
<td>$1.5 \times 10^5$</td>
<td>32.8 ± 2.4</td>
</tr>
<tr>
<td>4</td>
<td>676</td>
<td>$1.2 \times 10^5$</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>698</td>
<td>$1.1 \times 10^5$</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>6</td>
<td>724</td>
<td>$1.3 \times 10^5$</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>659</td>
<td>$1.4 \times 10^5$</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>687</td>
<td>$1.1 \times 10^5$</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>806</td>
<td>$2.5 \times 10^5$</td>
<td>9.1 ± 0.7</td>
</tr>
<tr>
<td>10</td>
<td>784</td>
<td>$2.0 \times 10^5$</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>11</td>
<td>810</td>
<td>$2.5 \times 10^5$</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>789</td>
<td>$2.2 \times 10^5$</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>13</td>
<td>813</td>
<td>$2.8 \times 10^5$</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>14</td>
<td>826</td>
<td>$2.3 \times 10^5$</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

$^a$ SERS - LOD ± s.d.

SERS Spectra and SERS Nanotags with Dyes 1-14

Raman scattering tends to be weak in the near infrared (NIR) region due to its dependence on the 4th power of the excitation frequency, but can be significantly enhanced by trapping molecules close to the gold surface of HGNs. The enhancement obtained from SERS is related to the frequency of the surface plasmon excited on the metal rather than the 4th power law.$^{15}$ Therefore, to make SERS a viable method at 1280 nm, the surface plasmon resonance (SPR) should have some resonance with the NIR excitation source. The SERS spectrum of dye 14 is shown in Figure 2a with the SERS spectra of dyes 1-13 in Figure S1 of the ESI. The HGNs have a SPR at 720 nm as shown in the extinction spectrum in figure S2 of the supplementary information. Moreover, the extinction spectra showing that the SPR does not shift upon functionalizing HGNs with these dyes or with the addition of KCl, can also be seen in figure S2. It is important to note that the plasmon resonance frequencies obtained do not match the excitation frequency at 1280 nm, however, it has been reported previously that for effective SERS to be achieved, the excitation wavelength does not need to match the wavelength of the plasmon maximum.$^{17}$$^{18}$

Fig. 2. a) SERS spectrum of dye 14 (10 µM) analyzed with HGNs (SPR recorded at 720 nm) and KCl (30 mM). The spectrum has been background corrected. b) SERS particle dilution study for dye 14 with HGNs and KCl over the concentration range 1.93 nM to 6 pM. The limit of detection was calculated to be 1.5 ± 0.1 pM. Peak height at 1590 cm$^{-1}$ was analysed by subtracting background ‘HGN only’ signal from each data point. Error bars represent one standard deviation resulting from 3 replicate samples and 5 scans of each. 1280-nm laser excitation was used for both a) and b) and an exposure time of 3 s for a) and 7 s for b) were employed in this analysis.

The 1280-nm SERS nanotags, consist of three important components, the first being the SERS substrate. In addition to strong SERS properties,$^{18}$ HGNs have small size (usually 50-80 nm), spherical shape and a strong tunable plasmon band from the visible to the NIR region.$^{19}$ Commonly, Ag and Au
spherical nanoparticles with plasmon bands in the visible region are used as SERS substrates. However, dyes 1-14 on these nanoparticles produced significantly weaker SERS signals than those observed with the HGNs (see Figure S3, ESI) due to their lack of red-shifted SPR.

The second necessary component of SERS nanotags is the Raman reporter. Since the SERS effect decreases exponentially as a function of distance from the nanoparticle, the Raman reporter must be near the gold surface. Dyes 1-14 incorporate S and Se atoms in the chalcogenopyrylium core and in the 2-thienyl and 2-selenophenyl groups on select members of this library to provide novel attachment points to gold for Raman reporters. Earlier studies have shown that thiophenes and selenophenes are both capable of forming self-assembled monolayers on gold. Selenolates have also been shown to have greater affinity for gold than thiolates.

As shown in Figure 1 and in Figure S1 (ESI), dyes 1-14, which are highly aromatic, produce vibrationally rich and intense SERS spectra with a laser excitation of 1280 nm. The trimethine dyes 9-14 produce more intense signals than their monomethine counterparts (dyes 1-8), with the selenophene-substituted reporters producing stronger SERS spectra than the thiophene-substituted dyes. The SERS spectra for dyes 1-13 were acquired with a 7-s acquisition time with the 1280-nm laser. The SERS spectrum of dye 14 with four 2-selenophenyl substituents was collected with only 3-s acquisition time due to the signal intensity saturating the spectrometer. Both 13 and 14 are significantly red-shifted with light absorption maxima >800 nm, making them NIR active. Dye 13 with four 2-thienyl substituents gave a weaker SERS signal compared to dye 14 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.

The third component in the SERS nanotag is the aggregating agent, usually a simple inorganic salt such as KCl that screens the Coloumbic repulsion energy between the nanoparticles, allowing the reporter molecules to adhere more closely to the nanoparticle surface. Although the aggregating agent with an optimum concentration at 30 mM was necessary for most of the dyes, it is important to note that with dyes 13 and 14, KCl was not required for intense signals to be observed (Figure S4, ESI). This is possibly due to a strong interaction occurring between the reporter and HGN surface inducing self-aggregation. Partial aggregation observed from these nanotags perhaps widens the scope for future SERS applications where aggregating agents are not required and the aggregation of the nanoparticles comes solely from a biological recognition event such as DNA-DNA interactions, DNA-protein interactions, peptide-protein interactions or sugar-protein interactions. These nanotags could be used as alternative reporters in biological applications such as photothermal ablation therapy or optical coherence tomography where there is a great need for NIR active materials.

X-ray Crystallographic Studies of Trimethine Dye 14

X-ray structural studies have shown that the chalcogenopyrylium/chalcogenopyranyl rings and the methine carbon of chalcogenopyrylium monomethine dyes related to 1-8 are coplanar and computational studies predict similar coplanarity in trimethine dyes 9-14. In the chalcogenopyrylium dyes bearing phenyl substituents at the 2-, 2′-, 6-, and 6′-positions, the phenyl rings for steric reasons cannot be coplanar with the chalcogenopyrylium/chalcogenopyranyl rings and the methine carbon. Other studies have shown that a 2-thienyl group can be coplanar with an attached thiopyryl ring. By having reduced steric interactions with an attached pyranyl ring, the 2-thienyl substituent has more conformational freedom to optimize interactions with a gold surface to which it is attached in the monomethine and trimethine dyes of this study.

X-ray crystallographic analysis of single crystals of dye 14 indicate that the thiopyrylium/thiopyranyl trimethine core and the four 2-selenophenyl substituents are highly coplanar as shown in Figure 3 (angles between the mean-planes of the aromatic groups are given in the ESI). In essence, all six chalcogen atoms can be involved in binding the reporter to the gold surface. Furthermore, the 2-selenophenyl substituents can rotate from coplanarity with the thiopyrylium/thiopyranyl trimethine core to a conformation to optimize binding to the HGN surface.

![Fig. 3. Thermal ellipsoid plots of the primary disorder species of dye 14 at the 50% probability level viewed a) from above and b) approximately parallel to the molecular plane. Atom colors are as follows: Se (dark grey), S (yellow), C (light grey), and H (white). Solvent molecules and counter-ion have been omitted for clarity.](image-url)
0.1 pM was calculated. The LOD was calculated to be 3 times the standard deviation of the blank, divided by the gradient of the straight line in Figure 1b. LODs for dyes 1-13 were also measured and are compiled in Table 1. Trimethine dyes 9-13 all gave LODs of <10 pM with LODs of 1.8 ± 0.2 and 1.5 ± 0.1 pM, respectively, for 11 and 13, which were comparable to dye 14. Monomethine dyes 5, 7, and 8 gave LODs of 3.4 to 5.4 pM, respectively, while monomethine dyes 1-3 gave LODs of 22 to 33 pM. The 1280-nm SERS signals from reporters 4 and 6 were too weak to allow determination of LODs.

The non-resonant commercial dyes BPE (1,2-bis(4-pyridyl)ethene) and AZPY (4,4-azopyridine), which are commonly used with gold nanosubstrates for SERS analysis,7,29 were also tested with the HGNs at this laser wavelength but failed to produce a SERS signal (Figure S5, ESI).

Principal Component Analysis (PCA) with Reporter Dyes 1-14

Dyes 1-14 can be separated out and individually identified in a reproducible manner based on their unique structures and SERS spectra through multivariate analysis in the form of principal component analysis (PCA). PCA is employed to reduce the dimensionality of the spectroscopic data thus facilitating identification of variations in the SERS spectra.30 PCA was carried out on 14 data sets consisting of three replicate spectra obtained from each individual dye. The resulting principal component (PC) scores plot (Figure 4) clearly illustrates three unique groupings. The red cluster contains the trimethine dyes 9-14; these reporter molecules produced the most intense SERS signals and all contain 3 sp² carbons in their trimethine bridge. The blue clustering highlights the monomethine dyes (1-3,5,7,8), which are good Raman reporters and produce intense SERS spectra with HGNs and KCl while the green cluster contains the two dyes which produce only weak signals with HGNs.

Conclusions

In conclusion, a set of new extreme red shifted SERS nanotags have been designed and synthesized to demonstrate unprecedented performance using 1280-nm excitation, a set of chalcogenopyrylium dye reporter molecules, and HGNs. These nanotags show a LOD in the picomolar range. The dye molecules are unique NIR reporters as they have multiple chalcogen attachment groups which allow them to bind strongly to the gold surface of the HGN and thus produce intense SERS signals. Dyes 1-14 with the more widely used gold nanoparticles or HGNs with conventional Raman reporters such as BPE were unable to match the combined performance of the chalcogenopyrylium dyes and HGNs indicating the unexpected and superior performance of SERS nanotags based on the combination of these dyes and the tunable HGNs. This significant result now makes SERS nanotags available for future use in a wide range of optical applications with 1280-nm excitation including deep tissue analysis and will provide a basis for future studies into harnessing their unique spectral properties.

Acknowledgements

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Notes and references

† Electronic Supplementary Information (ESI) available: General experimental details for the synthesis and characterization of dyes 1-14, intermediates 15-17, and HGNs; general description of the chemometrics used for principal component analysis; figures of SERS spectra of
dye/HGN nanotags with 1280-nm excitation; extinction spectrum for HGNs; SERS spectra of Au and Ag nanoparticles and dye 13 aggregated with KCl; SERS spectra of dyes 13 and 14 on HGN not aggregated with KCl; details of mean plane angle calculations, tables of crystallographic data, atomic coordinates and equivalent isotropic displacement parameters, anisotropic placement parameters, bond lengths, bond angles, and hydrogen atom coordinates and isotropic displacement parameters for dye 14. CCDC reference numbers.