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Mixed-monolayer glyconanoparticles for the detection of cholera toxin by surface enhanced Raman spectroscopy†

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We have produced silver glyconanoparticles for the sensitive (56 ng mL⁻¹), low volume and rapid detection of cholera toxin B-subunit (CTB) in synthetic freshwater (simulating the ion compositions of natural waters in which CTB could be found). This is achieved by monitoring the changes in surface enhanced Raman scattering (SERS) intensity of a Raman reporter bound to the glyconanoparticle surface. The particles selectively aggregate upon interaction with CTB, causing an increase in the measured SERS signal. The particles are designed to mimic the interactions involving the cell surface GM1 ganglioside and CTB. This is achieved by using a combination of polyethylene glycol linkers terminated with either galactose or sialic acid.

Nanoparticles have many potential applications in healthcare, including improved drug delivery and biodetection.1–3 Selective and sensitive bio-detectors can be produced by functionalising metallic nanoparticles with biomolecules such as DNA or antibodies.4 Gold and silver nanoparticles functionalised in this way, bind complementary target biomolecules and assemble to form aggregates which is indicated by the appearance of plasmonic-coupling bands in the extinction spectrum.5–8 In addition to biomolecules, Raman active molecules can be coated onto the particles giving rise to surface enhanced Raman scattering.9,10 Target-mediated aggregation of the nanoparticles creates inter-particle hot spots of electromagnetic energy which further enhances the Raman signals of the surface bound Raman reporter.11 SERS sensitivity rivals that of UV-visible extinction and fluorescence spectroscopy.12,13 Additionally, the molecularly specific Raman signals can allow for multiplexed detection by using multiple Raman reporters which is important for the analysis of multi-analyte samples.14–16 SERS nanoparticle biosensing has been applied to the detection of biomolecules (including DNA and antigens) along with the imaging and mapping of cells and their associated substructures.13,15,17 In addition to oligonucleotides and proteins, nanoparticles can be functionalised with carbohydrates and associated species for use in glyconanotechnology. This includes enzyme inhibition, oncology and the detection of lectins (carbohydrate binding proteins) or toxins related to disease.18–21 Carbohydrates, present extensively on cell surfaces, provide multifunctional binding sites involved in crucial endogenous processes as well as in pathogen establishment.22–24

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generation of glyconanoparticles which detect the CTB bacterial target both selectively and sensitively and in samples which simulate the environment in which the toxin could be found, namely synthetic freshwater samples which contain the ionic components of natural bodies of water. The approach for generating CTB-reactive particles involved deconstruction of the GM1 ganglioside to identify the major components in CTB binding. Galactose is a major component of the binding between the toxin and GM1 (see Fig. 1A), however, alone this interaction is weak (Gal–CTB \( K_d = 52 \text{ mM} \)).\(^{26,27}\) By simultaneously binding sialic acid in a symbiotic interaction, as is the case for GM1, the binding pocket is significantly stabilised (GM1–CTB \( K_d = 0.3 \text{ nM} \)).\(^{24}\)

Previous research on CTB-reactive glyconanoparticles includes the development of lactose-coated gold nanoparticles for the detection of CTB at 54 nM (3 \( \mu \text{g ml}^{-1} \))\(^{18}\) Our novel, mixed-monolayer galacto-sialonanoparticles (GSNPs) allow for detection of CTB with greater sensitivity than previously achieved because of the GM1-mimicking surface and by using SERS for detection.

50 nm colloidal silver nanoparticles, prepared using ethylendiaminetetraacetic acid (EDTA) reduction, were used to produce the biosensor. The Raman molecule used in analysis was the benzotriazole dye molecule 4-((1H-benzo[d][1,2,3]triazol-6-yl)-diazepen)-3,5-dimethoxyphenol (RB1) previously prepared by our group.\(^{28}\) The carbohydrates were subsequently tethered to the nanoparticle surface via thiolated polyethylene glycol (PEG) linkers. PEG provides steric protection that helps discourage aggregation by increasing the minimum interparticle distance in the colloid. Additionally, non-specific binding of biological or chemical species present in a sample is reduced. PEGylated galactose and sialic acid were produced by amide coupling (see ESI,\(^\dagger\) Section 6 and Fig. S2 for details) and used to functionalise the RB1-coated silver nanoparticles with GM1 mimics.

The SERS-active GSNPs were characterised by UV-visible extinction spectroscopy, dynamic light scattering and gel electrophoresis (see ESI,\(^\dagger\) Table S4 and Fig. S3). The surface coverage-activity relationship was evaluated by functionalising the particles with galactose and sialic acid linkers of different lengths and altering the ratio between the linkers. While the PEG coating, together with size of CTB, leaves space between the particles upon CTB binding, plasmonic coupling has been shown to occur up to a distance of 70 nm.\(^{29,30}\) It was therefore proposed that upon aggregation of GSNPs with CTB, an increased SERS response would be measured. The GSNPs were incubated with 80 nM CTB for 5 minutes and the resulting aggregation measured by monitoring changes in SERS intensity of the RB1 peak at 1364 cm\(^{-1}\) (see Fig. 1B).

The importance of both sialic acid and galactose in binding GM1 is demonstrated in Fig. 2. The greatest SERS intensity change for the glyconanoparticles is noted with the mixed galactose/sialic acid (15 : 1) monolayer. There is little interaction and hence low SERS increase when using sialic acid or galactose as the sole surface coating.

Our investigations reveal a maximum normalised SERS enhancement of 3.2 at a PEGylated carbohydrate ratio (galactose to sialic acid) of 15 : 1 (see Table 1), where the two carbohydrate linker types are of different lengths (PEG\(_{12}\)Gal and PEG\(_{8}\)Sia). The extension of the sialic acid from the nanoparticle surface with respect to the galactose encourages maximal binding and hence aggregation. This arises from the minimal steric interference from surrounding galactose molecules with sialic acid. The reduced amount of sialic acid on the surface with respect to the galactose indicates the importance of isolated galactose/sialic acid–CTB interactions. The interaction, aggregation and hence SERS enhancement is reduced when the sialic acid is...
Table 1  CTB-mediated SERS enhancement with different galacto–sialo surface ratios and PEG chain lengths. The normalised SERS enhancement values are listed below the corresponding galactose to sialic acid ratio.

<table>
<thead>
<tr>
<th>Coverage type</th>
<th>Normalised SERS enhancement at 1364 cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal/PEG 1:1</td>
<td>1.2 (+0.16)</td>
</tr>
<tr>
<td>Gal/PEG 3:1</td>
<td>2.0 (+0.20)</td>
</tr>
<tr>
<td>Gal/PEG 15:1</td>
<td>3.5 (+0.19)</td>
</tr>
<tr>
<td>Gal/PEG 30:1</td>
<td>3.0 (+0.21)</td>
</tr>
</tbody>
</table>

* The enhancement is a ratio of the RB1 signal at 1364 cm⁻¹ prior to aggregation with 80 nM CTB to the same signal measured 5 minutes after CTB addition in HEPES buffer, pH 7.4. The laser excitation wavelength used is 514.5 nm with a 1 second acquisition, 3 accumulations and 3 replicates measured.

Fig. 3  RB1 SERS spectra of GSNPs before and after addition of (top left) CTB or (top right) ConA in synthetic freshwater. (Lower middle) Normalised SERS intensity of 1364 cm⁻¹ peak measured 5 minutes after addition of the indicated concentration of CTB with 5 replicates per concentration. The limit of detection determined is 1 nM. The laser excitation wavelength used is 514.5 nm with a 1 second acquisition and 3 accumulations, 5 replicates measured.

Notes and references