Dye Coded Nanoparticles as SERRS Labels for Ultrasensitive Detection of Proteins and Antibodies

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ABSTRACT

One of the fundamental challenges of proteomics is to simultaneously increase sensitivity and throughput in detection. We have developed a novel surface enhanced resonance Raman scattering (SERRS) detection method that is amenable for almost any biological target. Linking materials were designed and synthesized to incorporate as one molecule i) a SERRS reporter ii) thiol groups for nanoparticle functionalisation, and iii) a bioconjugation group. These novel materials enable the facile fabrication of nanoparticle-biomolecule conjugates that exhibit a strong SERRS response. We have applied these materials to a number of proteomic targets in several assay formats. Rapid screening is facilitated by the use of ultra fast Raman line mapping.

Keywords: SERRS, nanoparticles, bioconjugation

1 INTRODUCTION

Recent developments in nanobiotechnology have led to new classes of multifunctional devices for biological and chemical analysis that exhibit significantly improved sensitivity and specificity when compared to systems currently in use.[1] The term nanobiotechnology has been coined to describe the interface of molecular biology and nanoengineering, focusing on the synthesis of nanoscale devices for use in molecular recognition. Of the newly developed technologies, one of the most promising involves utilization of the unique optical properties of metal, often gold, nanoparticles. Functionalisation of nanoparticles and nanostructures has led to a promising range of sensors with a wide range of applications including plasmonic biosensors [2] and SERS reporters [3,4].

SE[R]RS (surface enhanced [resonance] Raman scattering) is an extremely sensitive spectroscopic technique that offers several advantages over the more conventional fluorescence/colorimetric based antibody assays. Raman spectroscopy is a technique in which inelastic scattering of light from molecules is recorded to generate a quantized spectra with peaks corresponding to changes between vibrational energy levels. Surface-enhanced Raman Scattering (SERS) is an attractive method where the molecule of interest is immobilized to a roughened solid surface, massively increasing the intensity spectral response. The technique gives an information rich spectrum of narrow spectral lines related to specific vibrational frequencies, unique to individual molecules and is ideally suited for screening of multiple analytes with high sensitivity in close spatial proximity. The combination of surface enhanced Raman spectroscopy with resonance Raman scattering leads to the composite technique of SERRS. By utilizing a specific reporter molecule, generally a dye molecule that has electrical absorbance peaks close to the wavelength of the laser used to excite, the Raman signal can be improved by 10^{10} – 10^{15}, to the point where single molecules can be detected [2]. Moreover, SERRS studies using dye-labelled oligonucleotides have shown that a quantitative response can be achieved, significant for detecting small changes in the level of dye-labelled DNA, perhaps emphasising the diagnostic relevance of this technique [5]

The development of a robust, easy to use technique for the sensitive detection of specific proteins would have tremendous advantage over current diagnostic methods. There are many common disorders that would benefit from an early detection system, giving advantages to both patient and financially to the health service. ELISA's (enzyme-linked immunosorbent assays), Western blots and more recently microarrays are methods that are regularly used to determine the presence/levels of protein in a sample, each with its own limitations. Time costs, poor limits of detection and an inability to screen multiple analytes are areas of potential improvement. Fluorescence based detection techniques are of great use in the *in vivo* analysis of protein interactions, however the wide spectral bandwidth observed means fluorescence based reporters have limited use in multiplex analysis.

We have developed dye-coded nanoparticles for use as SERRS labels and applied them to a range of biologically relevant targets. The nanoparticle is carefully functionalized with a linker material; the linker is key to providing nanoparticles stability, a Raman dye and a functional group for use in bioconjugation. The linker materials are selectively enhanced using appropriate wavelengths of excitation to yield efficient discrimination between targets. Even using a single wavelength source, discrimination is achieved by the effective combination of the strongest spectral lines from the dye labels within the multiplex.

2 DYE CODED NANOPARTICLES

2.1 Functionalized nanoparticles

Gold and silver nanoparticles were prepared according to published protocols [6]. Nanoparticles were functionalized by the addition of a tuned linker molecule. Each linker contained; terminal thiol (-SH) and acid (-COOH) groups for attachment to the metal surface and biomolecule conjugation respectively, along with a central polyethylene glycol (PEG) unit that had been synthetically optimized to have a Raman tag covalently attached, as shown in figure 1. Several tags were available for use, each with a unique spectral fingerprint allowing multi-variant and multi-wavelength analysis.

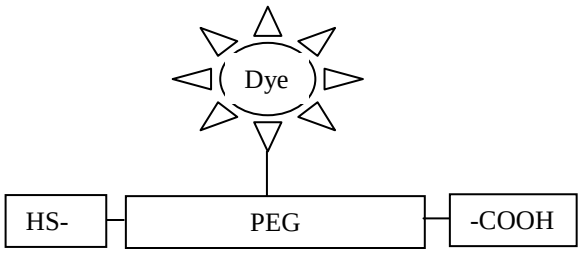

Figure 1. A generic representation of a SERRS linker. The complete unit contains four parts. Thiol (HS-) for gold attachment, PEG for water solubility, a dye for enhanced Raman signal and an acid group (-COOH) for biomolecule addition.

2.2 Bioconjugation to nanoparticles

The terminal acid group was used to covalently attach biomolecules via the formation of an amide bond, the coupling occurs preferentially with lysine residues available on the protein surface. Preliminary results suggest that 10-100 protein molecules surround a single 15nm gold nanoparticle (data not shown), depending on the materials or conditions used.

3 RESULTS AND DISCUSSION

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3.1 SERRS from a membrane

Previous studies have shown that for many dyes effective SERRS spectra can be obtained using aggregated nanoparticles and a fibre-probe system where the laser is focused into a plastic cuvette or a microtiter plate.[7] To demonstrate the versatility of the SERRS technique, a sandwich assay was carried out on a nitrocellulose membrane. Varying concentrations of a secondary antibody conjugated to a gold nanoparticle were used to detect a primary antibody immobilized on a membrane surface. Gold nanoparticles exhibit a red color when viewed in transmission and it is this property that is often used for detection. Progressive dilutions decrease the intensity of the coloration observed. When using a concentrated gold solution on the membrane, a red band appears where the primary antibody was immobilized. As the gold solution is diluted, the visibility of the band decreases to the point where it is no longer visible to the naked eye. By using an ultra-fast Raman line mapping system and a laser excitation corresponding to the SERRS dye used, a map can be obtained across the area the primary antibody was streaked. The map shows areas of distinct peaks where the dye is present contrasting to areas of low background noise, as shown in figure 2.

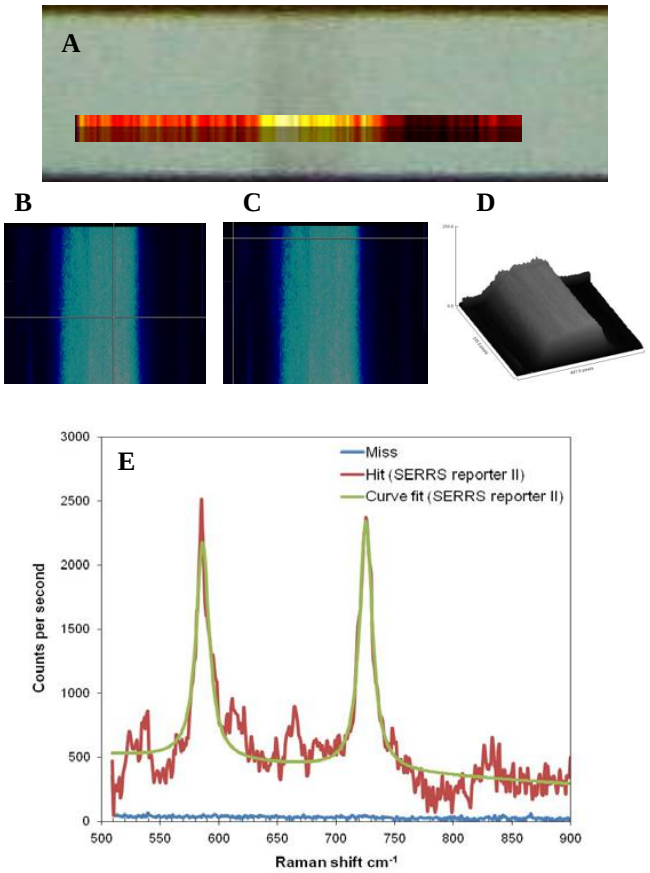

Figure 2. Ultra fast Raman line mapping. An antibody band on a nitrocellulose surface was used to capture a functionalised gold nanoparticle. Multiple SERRS spectra are analysed across the (slightly visible) capture band, shown in image **A**. The inset image has yellow/white colours representing areas of high SERRS response, darker areas have minimal SERRS response. The crosshairs in image **B** (hit) and **C** (miss) show the coordinates of the exact spectra compared. Image **D** has a 2-D image across the band. Example hit and miss spectra are shown in image **E.**

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Diluting the concentration of the secondary antibody gold solution effected gold concentration immobilized on the capture band. This resulted in decreased band width across the capture strip, as shown in figure 3.

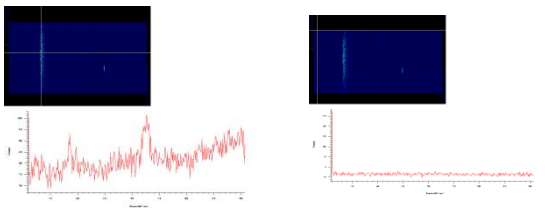

Figure 3. SERRS mapping of dilute gold conjugate solution. The nitrocellulose no longer had a visible red band indicating where the nanoparticle had been captured. The mapping showed a minor capture band from which SERRS responses could be obtained.

4 CONCLUSION

We have demonstrated that an increase in sensitivity can be obtained by using encoded SERRS nanoparticles. The use of SERRS as the primary detection method in such assays will also lead to several other significant advantages including the ability to simultaneously identify multiple targets from the distinct spectra observed. We have shown that SERRS from a nitrocellulose membrane is possible and that excellent discrimination can be achieved.

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