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Surface-enhanced Raman encoded polymer stabilized gold nanoparticles: Demonstration of potential for use in bioassays

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ABSTRACT

The preparation of biotinylated, self-assembled polymer stabilized gold nanoparticle hybrids encoded with a SERS active compound is described. The polymers used for nanoparticle stabilization are carefully designed for this purpose and are synthesized by the RAFT polymerization process, as the thiocarbonylthio end group provides a functional handle for anchoring the polymers to the gold surface. Functionalised biotin moieties are attached to the hybrid nanoparticles via Cu-catalyzed azide-alkyne cycloaddition. Binding of the biotinylated hybrid nanoparticles to streptavidin was confirmed by nanoparticle detection and identification by the SERS spectrum of the surface-bound SERS active compound, quinolinethiol. This investigation includes the requisites that constitute a bioassay, demonstrating the potential of polymer-coated hybrid nanoparticles for this purpose.

1. Introduction

Fluorescence labelling has found considerable use for detection of binding events in bioassays due to its inherent sensitivity and ease of implementation [1]. However, due to broad intrinsic band widths, the number of analytes in an assay is limited. Fluorescence labelling also has other inherent problems, including autofluorescence of the substrate, photobleaching of the dyes, and the need to use multiple excitation sources [2–4]. An alternative strategy for bioassays is based on gold nanoparticles (AuNPs), with surface enhanced Raman spectroscopy (SERS) as the detection technique. SERS has the potential for greater specificity than fluorescence and does not suffer from the obstacles noted above [2–5]. The narrow bandwidths associated with the SERS technique ($\sim$20 cm^{-1}) provides the potential to achieve much greater levels of multiplexing than is possible with fluorescence, significantly improving the prospects for increased assay throughput. Recently, a number of papers have reported the use of AuNPs labelled with a SERS active compound (SAC) for use in protein bioassays [2–7]. The function of the SAC is to detect and also differentiate between different AuNPs and has been likened to a "molecular barcode". The use of NPs brings its own problems, the most important of which is the poor long-term stability of the NP suspensions. Our strategy to overcome this has been to prepare SAC-encoded AuNPs stabilized by RAFT polymers [8], through surface binding of the polymer to Au via the thiocarbonylthio group [9–19].

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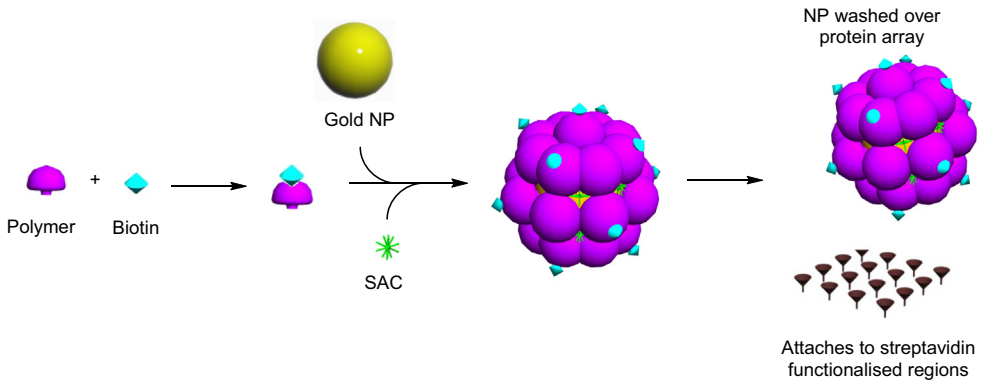
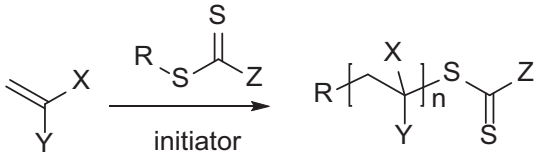


Fig. 1. Schematic showing the assembly of hybrid NPs for use in bioassays with SERS detection.



Scheme 1. Generic scheme for RAFT polymerization.

Here we demonstrate a model bioassay in which binding to a streptavidin substrate is detected by SERS. This was achieved through the synthesis of polymer stabilized AuNPs functionalized with a biotin moiety, introduced through the polymer end-group. A schematic of this strategy is given in Fig. 1. Whilst biotinylated polymer-stabilized AuNPs have been previously in the literature [17], with turbidity of the solution used to detect a binding event to avidin, detection of the event by SERS as described herein provides more definitive proof of bioconjugation.

There are a number of approaches for the synthesis of polymers with defined architecture; one of the most successful in recent years has been the reversible addition-fragmentation chain transfer (RAFT) process [18,19]. RAFT polymerization is applicable to a wide range of monomers and, under appropriate conditions, produces polymers with controlled molecular weight and low dispersity. These properties are brought about by use of a thiocarbonylthio chain transfer (or RAFT) agent [20]. A feature of RAFT polymerization is the conservation of the thiocarbonylthio functionality as the ω-end-group in the final polymer (see Scheme 1). This provides a functional handle in which to incorporate additional functionality [21,22]. The polymers were designed to have one end-group ('ZCS2') to specifically bind to the surface of the AuNPs, pendant polyethyleneglycol (PEG) chains, which exhibit low non-specific protein binding and the α-end-group ('R' group) carries an alkyne functionality, amenable to the Cu-catalyzed click reaction, which can be used to attach biologically active moieties required for bioassays.

2. Results and discussion

For the assembly of the hybrid nanoparticles, we targeted the synthesis of a polymer containing poly(ethylene glycol) (PEG) pendant groups through the RAFT polymerization of PEG methacrylate (PEGMA), because PEG is both water soluble and inhibits non-specific binding to proteins [23].

2.1. RAFT agent synthesis

To introduce alkyne functionality in the polymer end-group, we prepared prop-2-ynyl 2-phenyl-2-(phenylcarbonothioylthio)acetate (**5**), bearing the secondary benzylic propargyl ester 'R' group in moderate yield over two steps. Benzylic esters [24,25] allow for simple introduction of end-group functionality whilst delivering control over the polymerization of methacrylic monomers [22,23]. Esterification of α-bromophenylacetic acid **1** with propargyl alcohol using DCC coupling gave the bromo ester **2** in 37% yield (see Scheme 2). Subsequent reaction with benzodithioate salt **4** (prepared from PhMgBr **3** and CS_2) gave the alkyne functional dithiobenzoate RAFT agent **5** in 38% yield.

2.2. RAFT polymerization

Polymerization of PEGMA **6**, in the presence of the acetylene RAFT agent **5**, gave low dispersity alkyne-functional poly (PEGMA) **7** after purification by precipitation (M_n = 7800, Đ = 1.10) (see Scheme 3). The presence terminal acetylene unit

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