Biosensors and Bioelectronics 79 (2016) 661–668

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Single functional magnetic-bead as universal biosensing platform for trace analyte detection using SERS-nanobioprobe

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ARTICLE INFO

*Article history:*
Received 5 November 2015
Received in revised form
30 December 2015
Accepted 31 December 2015
Available online 31 December 2015

*Keywords:*
Surface-enhanced Raman scattering
Nanobiosensor
Magnetic-bead
Small molecule
Protein

ABSTRACT

SERS biosensor has demonstrated remarkable potential to analyze various bio/chemical targets with ultrahigh sensitivity. However, the development of universal SERS biosensing platforms with a uniform and reproducible structure that can quantitatively detect a broad range of trace analytes remains a significant challenge. The production of SERS nanotags with abundant Raman reporters and rational structure to conjugate with detection biomolecules is another key to design SERS-nanobioprobes. Here, we introduce a facile single magnetic-bead biosensing platform, formed by combining the captured antibodies/antigens conjugated magnetic-beads and the Au@Raman-Reporters@Ag sandwich-based nanorod tags labeled nanobioprobes. The advantage of the robust sandwich-structure-based nanotags is attributed not only to the high density Raman reporters contained inside, with high EF value because of enhanced electromagnetic field density, but also to the flexibility for bioconjugation of the detection biomolecules. The 3-D structure of the functional magnetic-bead provides a perfect platform to rapidly capture and enrich biomolecules. Ultrasensitive detection of two small molecules and a protein was achieved in samples, respectively.



## 1. Introduction

Ultrasensitive detection of trace analytes is essential in many fields, from clinical diagnostics and drug discovery, to public safety and environmental protection (Overington et al., 2006; Swierczewska et al., 2012; Longo et al., 2013; Ndieyira et al., 2014). Surface-enhanced Raman scattering (SERS) has demonstrated tremendous potential for the analysis of various biological and chemical targets, including small molecules, DNA, proteins, and cells, at the trace, and even at single-molecule levels (Nie and Emery, 1997; Qian and Nie, 2008; Grubisha et al., 2003; Zhang et al., 2013). SERS-based nanobiosensors originate from the combination of SERS, nanomaterial and biomaterial forms. Generally, the SERS-based biosensor may be grouped into three fundamental classes: planar SERS-active surfaces acting as biosensing platforms, SERS nanotags serving as labels on biomolecules, and the combination of both. Biosensors based on planar SERS-active surfaces have been widely developed (Grubisha et al., 2003; Anker et al., 2008; Stewart et al., 2008; Li et al., 2013). Because nanostructural geometry can markedly affect Raman signals, a lack of control over factors such as size and shape variation of nanostructures, non-identical internanostructural distances, and inhomogeneous distributions of analyte molecules on surfaces can adversely influence optimal performance of these structures (Anker et al., 2008; Stewart et al., 2008; Li et al., 2013). These biosensors with both high sensitivity and high reproducibility typically require sophisticated and expensive nanofabrication techniques. Furthermore, given the 2-D configurations, the available density of SERS-active sites within the detection volume is limited (Li et al., 2013). Additionally, the 2-D SERS substrates rely on the analytes' ability to diffuse slowly to in bulk solution to reach the SERS-active sites. Therefore, the corresponding SERS detection method is time-consuming and unfavorable for fast, in-line, real-time analysis. Construction of a universal SERS biosensing platform with a uniform and reproducible structure that can quantitatively detect a broad range of trace analytes is still considered a costly and significant challenge.

SERS nanotag, integration of silver or gold nanostructures and Raman active molecules, has been recently explored for detection, sensing, and imaging (Stewart et al., 2008; Li et al., 2013; Marc et al., 2013; Wang et al., 2013). The SERS nanotage system possesses several intrinsic benefits in quantitative assay of trace analytes. First, "hot spots" generated in the nanoscale junctions and interstices of the SERS nanostructures afford enormous Raman enhancement factors on the order of $10^{14}$–$10^{15}$, leading to superb

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http://dx.doi.org/10.1016/j.bios.2015.12.108
0956-5663/

detection sensitivity (Nie and Emery, 1997; Qian and Nie, 2008; Zhang et al., 2013). Second, the robust structures of SERS nanotags and their high densities of the SERS active molecules provide unique and uniform signals, which are significant valuable in quantitative analysis (Li et al., 2013). Third, when conjugated with biomolecular targeting ligands such as monoclonal antibodies, peptides, DNA sequences or small molecules, these SERS nanotag labeled nanobioprobes can be used to target their receptors with high specificity and affinity (Anker et al., 2008). Additionally, given the highly resolved Raman spectral signature and the wide excitation wavelength of SERS active molecules, SERS nanobioprobes are ideally suited for simultaneous detection of multiple species in complex samples (Marc et al., 2013; Li et al., 2012). Au@Ag core-shell nanotags have elicited considerable attention for their higher SERS activity than that of monometallic gold nanoparticles (Marc et al., 2013). In terms of plasmonic nanoparticle geometries, the Au@Ag NRs is considered to be most suitable because of its controllable monodispersity and aspectratio, broad plasmon resonance tenability from near-UV to the IR range, and increased sharpness and strength of longitudinal SPR bands (Wu et al., 2012). Some immunoassay methods have been reported using Au@Ag NRs nanobioprobes (Grubisha et al., 2003; Wu et al., 2012; Li et al., 2012). However, most of those assays labeled the SERS report molecules on the surface of Au@Ag NRs and did not match their LSPR with the Raman excitation wavelength (Qian and Nie, 2008). Moreover, the aggregation of nanobioprobes will form nanojunctions between two plasmonic surfaces to generate additional SERS "hotspots" (Li et al., 2010), which thus lead to an abnormally high level of SERS signals and inevitably affects the accuracy of detection results.

An ideal SERS-based biosensing platform would consist of a reproducible and standardized substrate with controllable and highly active SERS-based nanobioprobes. Herein, a facile and reproducible single magnetic-bead biosensing platform (SMBP) has been developed for ultrasensitive and highly selective bioassay of trace analytes. Single uniformly sized magnetic-beads with captured antibodies/antigens served as nanobiosensing substrate. The sandwich structure of Au@Raman reporters@Ag core-shell nanorods (Au@RR@Ag NRs) was assembled layer-by-layer relative to the Raman tags. The combination of functional magnetic-beads and Au@RR@Ag NRs based bionanoprobes has allowed the highly sensitive and selective detection of trace analytes, including small molecules and proteins. The 3-D structure of the functional magnetic-beads provides a perfect platform for the capture and enrichment of biomolecules and allows rapid binding between antibody and antigen in homogeneous solution by reducing the space steric effect. The sandwich structure of Au@RR@Ag NRs facilitates modification of various biomolecules through well-known self-assembling chemistry and preparation of the needed SERS-based nanobioprobes. This sandwich structure can embed a number of active Raman reporters with high EF value related to enhanced electromagnetic field density, thereby improving the sensitivity of the SERS nanobiosensor, while greatly inhibiting the unwanted Raman signals induced by "hot spots" formed from the aggregation of nanobioprobes. We demonstrated the ability of the SMBP in ultrasensitive detection of small molecules and proteins with established mechanisms.

## 2. Experimental sections

### 2.1. Materials and chemicals

N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), N-(3-dimethylamino propyl)-N'-ethylcarbodiimide hydrochloride (EDC), Tween® 20, Bovine serum albumin (BSA), 1-ethyl-3 (-3dimethylamino-propyl) carbodiimide hydrochloride (EDC), ovalbumin (OVA), p-Aminothiophenol (PATP) or 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), 3-Mercaptopropionic acid (MPA), CTAB, $HAuCl_4$, $NaBH_4$, and $AgNO_3$ was purchased from Sigma (Shanghai, China). MC-LR, MC-RR, MC-YR, MC-LW, MC-LF, and nodularin were obtained from Alexis (ALX-350-012). Dialysis Kit with 5 kDa molecular weight cut-off was purchased from GE Healthcare (USA). All solutions were prepared with ultrapure water from a Millipore Milli-Q sytem. All the other reagents, unless specified, were supplied by the Beijing Chemical Agents (China). All chemicals were of analytical reagent (AR) grade.

Monoclonal anti-MC-LR antibody and monoclonal anti-BPA antibody were produced by our research group. Mouse immunoglobulin G and goat anti-mouse immunoglobulin G was purchased from Sigma (Shanghai, China). All protein samples were used as received without further purification and diluted in the assay buffer (1 μM phosphate buffer solution with pH 7.4) prior to sensing measurement. Human serum (from clotted human male whole blood, 40–90 mg/ml total protein) was also purchased from Sigma (Shanghai, China).

### 2.2. Synthesis of sandwich-structure based Au@RR@Ag NRs nanotags

Gold nanorods (Fig. S1) were synthesized by a previously reported seeding growth method (Babak and Mostafa, 2003). To prepare Au@RR@Ag sandwich structure, 20 mL of the as-prepared Au NRs were centrifuged twice at 8000 rpm for 10 min to remove the excess reagents. The precipitate was re-dispersed in 10 mL of deionized water. Subsequently, 10 μL of 10 mM p-Aminothiophenol (PATP) or 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) ethanol solution was added and the mixture was reacted for 2 h under continuous stirring. The PATP (or DNTB) modified Au NRs were rinsed by centrifugation and re-suspension with the same volume of deionized water. Au@RR@Ag NRs were then prepared as previously described (Xiang et al., 2008) with some modifications. Briefly, 2 mL of the PATP (or DNTB) modified Au nanorods solution was added to 4 mL of 0.04 M CTAB aqueous solution with vigorous stirring at 28 °C. Up to 130 μL of 0.1 M ascorbic acid, varying amounts of 1 mM $AgNO_3$, and 240 μL of 0.1 M NaOH were added sequentially. The color of the solution gradually changed in 2 min, indicating the formation of Au@RR@Ag NRs. Followed by purification, the core-shell nanorods solution was concentrated to 2 mL with deionized water. Au@RR@Ag NR nanotags was carboxyled by MPA for next modification.

### 2.3. Conjugation of SERS nanotags with detection antibodies (denoted as SERS nanobioprobes)

The SERS nanobioprobes were prepared by conjugating an amine of antibodies to carboxyl-coated Au@RR@Ag NR nanotags through the EDC/sulfo-NHS chemistry. The EDC and Sulfo-NHS reagents were used together, to enhance the conjugation efficiency. 50 μL of 8 μM stock solution of Au@RR@Ag NR nanotags were diluted to 0.5 mL using a 10 mM borate buffer (pH 7.4), and up to 0.5 mL of 3 mg/mL antibodies was added. After being well-mixed, 10 μL of a 10 mg/mL as-prepared EDC/NHS solution was added to the mixture, which was gently stirred for 1.5 h at room temperature for conjugation. To block the unreacted carboxyl sites on the Au@RR@Ag NR nanotags surface, 0.5 mL of 10 mg/mL BSA was added to the mixture and allowed to react for an additional 1 h. The mixture then was filtered through a 0.2 μm PES syringe filter to remove any large aggregates before being transfer to a clean centrifugal ultrafiltration unit (100 kDa cutoff; GE Healthcare, USA). To remove any excess unbound antibody, the solution was centrifuged at 3000 rpm for five buffer exchanges (50 mM borate buffer, pH 8.3). After completing ultracentrifugation, the