Biosensors and Bioelectronics 77 (2016) 306–314

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A virus-based nanoplasmonic structure as a surface-enhanced Raman biosensor

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ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form
10 September 2015
Accepted 13 September 2015
Available online 21 September 2015

Keywords:
Virus-like particles
Cowpea mosaic virus
Raman
SERS
Gold nanoparticles
Self-assembly

ABSTRACT

Fabrication of nanoscale structures with localized surface plasmons allows for substantial increase in sensitivity of chem/bio sensors. The main challenge for realizing complex nanoplasmonic structures in solution is the high level of precision required at the nanoscale to position metal nanoparticles in 3D. In this study, we report a virus-like particle (VLP) for building a 3D plasmonic nanostructure in solution in which gold nanoparticles are precisely positioned on the VLP by directed self-assembly techniques. These structures allow for concentration of electromagnetic fields in the desired locations between the gold nanoparticles or "hot spots". We measure the efficiency of the optical field spatial concentration for the first time, which results in a *ten-fold* enhancement of the capsid Raman peaks. Our experimental results agree with our 3D finite element simulations. Furthermore, we demonstrate as a proof-of-principle that the plasmonic nanostructures can be utilized in DNA detection down to 0.25 ng/μl (lowest concentration tested), while the protein peaks from the interior of the nanoplasmonic structures, potentially, can serve as an internal tracer for the biosensors.

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1. Introduction

Surface-enhanced Raman spectroscopy (SERS) is a surface-sensitive analytical technique in which the Raman signal intensities of molecules adsorbed onto rough nanostructured noble metal surfaces are significantly enhanced relative to free molecule signals (Schlücker, 2014). Since its discovery in the 1970s, (Fleischmann et al. 1974) the technique has drawn increased attention due, at least in part, to its utility as both a qualitative and quantitative analytical tool, the former by virtue of a species' unique Raman spectrum and the latter by its potential to achieve spectral intensity enhancement factors (EFs) appropriate for single molecule detection. The main challenge in realizing such efficient SERS-active structures is the preparation of composite material architectures exhibiting precisely localized plasmonic interactions. The requisite plasmonic nanostructures are ideally fabricated using noble metal nanoparticles (NPs), which are positioned on or in a supporting inert dielectric structure with controlled nanoscale spacing in well-defined 2D or 3D geometries. Such nanostructures promulgate SERS activity *via* localization and concentration of electromagnetic fields as "hot spots" between adjacent NPs, which facilitate Raman spectral intensity enhancement.

The fabrication of SERS-active architectures has been realized by both "top-down" and "bottom-up" approaches. Top-down approaches often utilize sophisticated techniques such as e-beam lithography, (Aćimović et al. 2009; Bahns et al. 2009) oblique angle deposition, (Driskell et al. 2008; Malvadkar et al. 2010) metallization, (Mu et al. 2010; Shao et al. 2012) and/or etching, (Becker et al. 2009) among others, (Fan et al. 2010; Sarkar et al. 2014; Yang et al. 2015) in combination with wet chemistry to tune plasmon resonance frequencies *via* NP shape (Ortiz and Skrabalak 2014) and composition (e.g., alloy or core–shell species) modifications, (Ferrando et al. 2008; Zhao et al. 2013a) to fabricate 2D SERS architectures on planar surfaces. Such 2D architectures can be fabricated over macroscopic length scales, with SERS EFs $> 10^{13}$ and attomolar analyte detection levels (De Angelis et al. 2011; Tan et al. 2014) achieved for properly designed structures. However, 2D architectures may present limitations for certain applications where the excitation of the plasmons is required to be orientation-independent.

In contrast, 3D architectures are predicted to exhibit orientation-independent plasmonic signatures (Alù and Engheta 2009;

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http://dx.doi.org/10.1016/j.bios.2015.09.032
0956-5663/Published by Elsevier B.V.

Barrow et al. 2012; Engheta 2007; Urban et al. 2013) due to their high symmetry. Furthermore, solution-based methods using bottom-up approaches provide a cost-effective alternative for large scale production of 3D plasmonic nanostructures. Bottom-up approaches are dominated by template or scaffolding self-assembly methods more amenable to preparation of freestanding 3D SERS architectures in solution capable of functioning as mobile taggants, tracers, or probes. The simplest such architecture comprises two gold (Au) NPs connected by a molecular linker, (Busson et al. 2011; Wang et al. 2013) though the presence of the linker in the hot spots between the NPs can hinder analyte access. Fortunately, certain high symmetry biomolecules, such as DNA (Barrow et al. 2012; Zhao et al. 2013b) and viral protein capsids (or genetically engineered derivatives thereof), (Zahr and Blum 2012); (Spano et al. 2007) possess sufficiently stable 3D structures bearing surface functional groups capable of binding NPs at precise locations with fixed NP–NP separation distances on their surfaces. NP binding at these sites leads to nanoscale noble metal NP-biomolecule composites in which hindrance of analyte access to the hot spots is minimized. Furthermore, these 3D architectures are ideally sized for interaction with biological materials *in vitro* or *in vivo* and macroscopic surfaces for SERS biosensing.

We have been studying viral protein capsids (Chen et al. 2009; Smith et al. 2013; Soto and Ratna 2010) as scaffolds for fabrication of 3D bio-organic/inorganic nanohybrids for advanced optoelectronics and metamaterials applications (Blum et al. 2004, 2005, 2006, 2007, 2011; Zhou et al. 2012). We recently reported self-assembly of a "BC-nanocluster" (BC-NC) (Fontana et al. 2014) comprising Au NPs (ranging from 17 nm to 34 nm diameter) covalently bound to the surface of 30 nm diameter cowpea mosaic virus (CPMV) protein capsid. The nanostructure exhibits multiple plasmonic interactions among its bound Au NPs consistent with its icosahedral symmetry, with major features of its bulk absorbance spectrum in good agreement with 3D finite-element simulation predictions. Based on our previous findings, in the current work, we utilize 24–30 nm diameter Au NPs to fabricate nanoclusters (NCs) on virus-like-particles (VLPs) and compare them to their BC-NC analogues (Scheme 1). The VLP capsid resembles the wild type (WT) CPMV (Lin et al. 1999) but it lacks the genetic material (RNA) inside the capsid. We selected the VLP as an alternative scaffold based on its similarity to WT-CPMV (available reactive residues on the exterior capsid), its successful utilization in materials applications, (Jaafar et al. 2014); (Aljabali et al. 2010) and lack of nucleic acids that makes it an ideal platform for sensing DNA. We report herein, for the first time, measurements of the SERS response of both of our NCs. Furthermore, when we compare both NCs their SERS responses are similar, paving the way for future use of hollow VLP-NCs as nanoreactors for complex materials preparations and nanostructured 3D assemblies for SERS sensing platforms.

2. Materials and methods

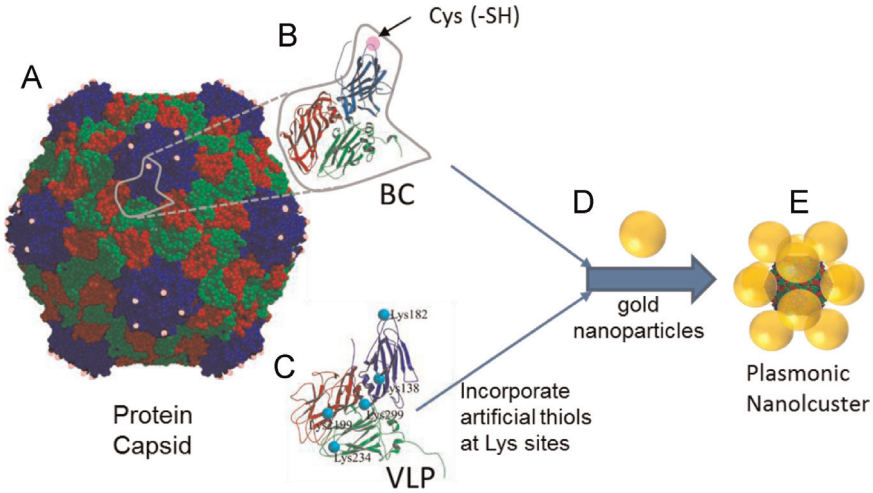
All chemicals were purchased from USA sources and used as received unless otherwise noted. All buffers and aqueous solutions were prepared with deionized water (Milli-Q water; 18 MΩ cm^{-1}). Buffers were filtered sterilized using 0.2 μm Nalgene filters (Fisher Scientific, Pittsburg, PA). *From this point on we will refer to the genetically modified BC-CPMV (60 cys per capsid) as simply BC and the WT-CPMV empty capsid as VLP.* BC was produced by J. Johnson's group and the VLP at G. Lomonosoff's laboratory by methods already published (Sainsbury et al. 2014).

2.1. Hi-trap desalting column, UV-Visible characterization, and quantification

A 5 ml pre-packed Hi-Trap desalting column (GE Healthcare Biosciences, Piscataway, NJ) was equilibrated with 25 ml of appropriate buffer for each application. The column was used for buffer exchange or to remove small molecules from reactions mixtures. Elutions from the column (1.5 ml each; via manual injection of buffer) were evaluated by UV–visible (UV–vis) spectroscopy to identify the capsid-containing fraction. A Cary 5000 UV–vis–NIR spectrometer (Agilent Technologies, Santa Clara, CA) was used for all UV–vis spectroscopy measurements using 1 ml disposable cuvettes (Fisher Scientific). Quantification of the capsids was based on the absorbance peak of the capsids (λ max) and corresponding extinction coefficient (ε). For the VLP: λ max is 280 nm, ε=1.28 ml mg^{-1} cm^{-1} and for BC: λ max is 260 nm, ε=8 ml mg^{-1} cm^{-1}.

2.2. Dynamic light scattering (DLS)

Particle size was determined using a Brookhaven Instruments ZetaPALS dynamic light scattering (DLS) system equipped with a 658 nm diode laser, using 1 cm path-length cuvettes. Three milliliters of sample were used. Ten measurements were taken per data



Scheme 1. Plasmonic Nanoclusters synthesis. (A) The protein capsid (30 nm in diameter) generated from the PDB file (1NY7). (B) Protein subunit where pink circles represent Cys incorporated *via* genetic engineering for a total of 60 thiols per capsid, BC-mutant. (C) Protein subunit of the VLP showing the locations of the natural-occurring Lys, artificial thiols are incorporated at the Lys sites using known chemistries. (D) Gold nanoparticles (24–30 nm in diameter) are bound to the VLP or BC by directed self-assembly resulting in (E) plasmonic nanoclusters. Structures are not drawn to scale.