SERS aptasensor from nanorod-nanoparticle junction for protein detection

Yuling Wang  
*Purdue University - Main Campus*, wang399@purdue.edu

Kyuwan Lee  
*Purdue University - Main Campus*, lee70@purdue.edu

Joseph Irudayaraj  
*Purdue University - Main Campus*, josephi@purdue.edu

Follow this and additional works at: [http://docs.lib.purdue.edu/nanopub](http://docs.lib.purdue.edu/nanopub)

Part of the Nanoscience and Nanotechnology Commons
SERS aptasensor from nanorod–nanoparticle junction for protein detection†

Yuling Wang, Kyuwan Lee and Joseph Irudayaraj*

Received (in Cambridge, UK) 21st September 2009, Accepted 4th November 2009
First published as an Advance Article on the web 18th November 2009
DOI: 10.1039/b919607b

A multicompontent nanostructure comprising of gold nanorod–nanoparticle (AuNR–AuNP) composites was fabricated to detect thrombin at subnanomolar concentrations in diluted human blood serum. Simulation and experiments revealed that the strong electromagnetic coupling resonance at the nanorod–nanoparticle junction of these probes can be used to construct highly sensitive SERS aptasensors.

Due to their high affinity, specificity and stability, aptamers, which are specific DNA or RNA strands,1 have been popular as ideal diagnostic reagents and as potential antibody replacements. They have been integrated with biomolecular devices to create electrochemical,2 optical3 or other apta-sensors. Since the first discovery of surface enhanced Raman scattering (SERS),4 past work has shown that the Raman scattering cross-section of a molecule can be increased by factors of up to 107–108,5 making it a viable alternative to fluorescence sensors. Theories have postulated that this enhancement could presumably emanate from the large electromagnetic (EM) field produced by the surface plasmon resonance (SPR) on metal nanostructures, particularly in “hot spots” of an ensemble of structures. Recent experiments and calculations have shown that the EM field can be greatly enhanced at the gaps or junctions of nanostructures, to create “hot spots”.6 Thus, with a decrease in interparticle distance between two nanostructures, the degree of their surface plasmon coupling increases and the enhanced fields from each particle begin to interfere coherently at the particle-nanorod junctions to give rise to high enhancements.7 Based on these considerations, fabrication of junctions based on EM hot spots by SERS using smaller size particles could result in devices with high sensitivity and specificity, especially when molecules such as DNA and proteins reside at these junctions.8

Gold nanorods (AuNRs) have attracted much attention because of their anisotropy in dimensions giving rise to a transverse and a longitudinal localized plasmon resonance in the visible-near-infrared region. This unique property has enabled AuNRs to be used as two-photon and Raman scattering probes for biomedical applications such as molecular and cell imaging, biosensing, bioassay, and photothermal therapy.9 Moreover, recent reports have shown that the strong light scattering property of AuNRs can be successfully exploited in disease diagnostics through the assembly of gold nanoparticles (AuNPs) and AuNRs.

Taking advantage of SERS hot spots and the optical property of AuNRs, we propose a novel SERS aptasensor based on AuNR–AuNP junctions to detect human z-thrombin in human blood serum.

Fabrication details of these composite structures are presented in Scheme 1. AuNRs were first functionalized by anti-thrombin antibody based on our previous method,11 to target the target z-thrombin protein. TEM imaging shows the as-prepared AuNRs to be uniform in shape and size with an average length and aspect ratio of about 50 nm and 3.9, respectively (Fig. 1A). The two distinct plasmon absorption bands at 513 and 727 nm (curve c in Fig. 1D), respectively denote the transverse and longitudinal nanorod plasmon resonance.12 After modification by anti-thrombin antibodies, the longitudinal plasmon peak slightly red-shifted to 730 nm (curve d in Fig. 1D), confirming the antibody functionalization protocol standardized in our previous works.13 AuNPs stabilized by citrate were labelled by thrombin-binding aptamer (TBA) and Raman reporters (mercaptobenzoic acid, MBA) according to previous reports,14 to create a protein-sandwich between nanorods and nanoparticles for SERS detection. TEM image in Fig. 1B shows a good monodispersity of 13 nm AuNPs with the respective plasmon peak at 518 nm. After decorating with the Raman reporter and TBA, the plasmon peak red-shifted to 523 nm confirming the binding (curve a, b in Fig. 1D). This shift is most likely due to the surface chemistry changes of AuNPs through the replacement of citrate-ligand layer by aptamers and Raman reporters, due to the formation of TBA and Raman reporter modified AuNPs complex.10 Human z-thrombin was then allowed to bind with AuNRs through the antibody–antigen interaction and AuNPs through the aptamer–protein binding to form AuNR–AuNP junctions, that give rise to a strong localized surface plasmon resonance (LSPR) under the laser excitation to produce an enhanced Raman signal as a measure of the bound protein. Since 13 nm diameter particles were used, the signal from particles not bound to nanorods will be negligible and will not contribute to any enhancement because of the size effect (shown in curve a of Fig. 3A).15

The AuNR–AuNP constructs were confirmed by TEM images as shown in Fig. 1C where the physical makeup of AuNPs bound to AuNRs is illustrated. Another interesting phenomenon observed is the binding of some AuNPs to two AuNRs due to the presence of two active sites in thrombin to tether the AuNRs through aptamer and protein interactions (additional TEM micrographs are shown in Fig. S1, ESI†).
enhancement factor of structures by finite difference time domain (FDTD) shows an AuNRs–AuNPs junction with different orientation) of these the electromagnetic field at the junction (Fig. S2, ESI w coupling between AuNRs and AuNPs. Simulation results of magnitude when AuNPs are functionalized at the ends of this enhancement could be increased by 2–3 orders of magnitude.

With an increase in concentration of thrombin, additional AuNPs are expected to bind to the AuNRs surface). The formation of AuNR–AuNP junctions and the surface plasmon coupling between nanorods and nanoparticles can be demonstrated by the UV-Vis absorption spectra (curve e of Fig. 1D), which show a slight shift of the SPR bands in both intensity and wavelength. The SPR band of AuNPs shifted from 518 nm to 529 nm upon attachment to AuNRs while that of AuNRs showed a red-shift from 730 nm to 738 nm in the longitudinal and 513 nm to 529 nm in the transverse localized plasmon resonance upon binding. The relative intensity of the ratio of the longitudinal versus the transverse band decreased from 4.6 to 1.9. Our observations agree well with the previous findings by Pierrat et al.,16 and validates the electromagnetic coupling between AuNRs and AuNPs. Simulation results of the electromagnetic field at the junction (Fig. S2, ESI†, AuNRs–AuNPs junction with different orientation) of these structures by finite difference time domain (FDTD) shows an enhancement factor of $\sim 10^7$ when excited by the 633 nm laser. This enhancement could be increased by 2–3 orders of magnitude when AuNPs are functionalized at the ends of AuNRs, contributing to further improvement in the signal for target-thrombin detection (Fig. 2).

A concentration profile experiment illustrating the sensitivity of the SERS aptasensor strategy to detect human $\alpha$-thrombin in Tris-HCl buffer through the AuNR–AuNP junction is demonstrated in Fig. 3. An obvious increase in the intensity of characteristic peaks (1078 and 1587 cm$^{-1}$, assigned to the $\nu_{(\text{bend})}$ and $\nu_{(\text{ring})}$ aromatic ring vibrations, respectively$^{17}$) of MBA is noted with respect to an increase in the concentration of $\alpha$-thrombin. As the number of AuNR–AuNP junctions increase due to an increase in the formation of the antibody–protein–aptamer complex, a corresponding increase in the SERS signal due to EM enhancement could be observed. Because of the strong electromagnetic field coupling that resides at these junctions (enhancement factor of $\sim 10^7$ from simulation and calculation), it was possible to detect 220 pM of $\alpha$-thrombin (curve b in Fig. 3B), comparable to the reported SERS aptasensor $\alpha$-thrombin assay.$^{18}$

Control experiments were conducted to assess the selectivity and specificity of the aptasensor SERS approach to detect bovine serum albumin (BSA) and IgG (even at a concentration as high as 10 $\mu$M, Fig. 3). Interaction of AuNRs with control proteins resulted in very weak reporter Raman signals, confirming the absence of nanorod–nanoparticle junctions in the control, verifying the specificity and selectivity of the aptasensor for $\alpha$-thrombin recognition. Another control experiment was performed using unmodified AuNRs and AuNPs in the presence of thrombin, yielding no detectable signal. The proposed aptasensor can also be used with multiplexed Raman reporter labels to detect a variety of targets. In Fig. S3 (ESI†) we show the fingerprint of three different Raman reporters with distinct Raman bands to detect thrombin providing the possibility of detecting multiple proteins when appropriate capture molecules are used.

The practical use of this novel SERS aptasensor was demonstrated in a complex biological matrix such as human blood serum (HBS). The SERS signal of MBA obtained from 5% HBS incubated at different concentrations of $\alpha$-thrombin is shown in Fig. 4. The intensity of the Raman reporter signal for $\alpha$-thrombin detection decreased by $\sim 20\%$ in HBS compared with that from Tris-HCl buffer for the same concentration due to the background of HBS as expected. Results indicate the lowest concentration of detectable $\alpha$-thrombin in the presence of complex biofluids (curve b in Fig. 4) as 887 pM, demonstrating excellent promise in clinical diagnostics.

In summary, we present a unique and simple SERS aptasensor concept using smaller diameter nanoparticles based on enhanced signal due to strong EM coupling that resides at
We would like to acknowledge partial support from the Showalter Trust grant and the NSF-IBDP grant (PI is Ben-Amotz).

Notes and references

11 C. Wang and J. Irudayaraj, Small, 2008, 4, 2204;