SERS opens a new way in aptasensor for protein recognition with high sensitivity and selectivity†

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SERS aptasensors for protein recognition based on Au nanoparticles labeled with aptamers and Raman reporters have been developed, which opens a new way for protein recognition of high sensitivity and selectivity.

Aptamers are specific DNA or RNA strands selected from a huge combinatorial library using the "systematic evolution of ligands by exponential enrichment" (SELEX) procedure, which have the ability to recognize specific molecular targets ranging from small inorganic or organic substances to proteins or cells.[1–3] Since their first discovery in the 1990s aptamers, because of their binding ability, are starting to appear in a series of applications in biosensors,[4] imaging probes,[5] MALDI targets,[6] and drugs.[7] Recently, optical or electrochemical aptamer biosensors based on enzyme,[8] fluorophore,[9] nanoparticle labels or on a binding-induced label-free detection[10] have been developed. However, until now, few papers have been published using Raman to detect the targets based on aptamers, which may be due to the low detection sensitivity of Raman scattering. But since the development of surface enhanced Raman Scattering (SERS),[11] the Raman scattering cross-section of a molecule can be increased by factors up to 10^{14}–10^{15}, comparable to fluorescence. This great enhancement is presumably from the large electromagnetic (EM) field produced by hot spots, which reside in the nanoscale junctions or interstices in metal nanostructures such as dimers or aggregates.[12] Nie's group and Kneipp's group have reported single molecule detection by SERS, indicating the high sensitivity of SERS for ultrasensitive chemical or biological detection.[13] Moskovits's group have recently reported the detection of DNA by SERS based on EM hot spots, in which the probe molecules reside, further indicating the ability of SERS for the ultra-sensitive detection for biological molecules.[14] Moreover, SERS has the inherent advantages over fluorescence[15] that (1) Raman scattering has the very narrow bandwidth of a typical Raman peak (<1 nm), while fluorescent bands can be as high as 50 nm in width; (2) the dye-labelled SERS active substrates can provide richer spectral information than fluorescence based signatures, which are often limited by spectral overlap of the chromophores and by background signals due to other components in the sample.

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Herein, we describe a unique method in SERS aptasensor for protein detection, taking α-thrombin as a model protein. Based on the fact that one α-thrombin molecule could bind two 15-mer aptamers (TBA) at the same time,[4,16] a sensing interface with a sandwich type system of TBA/α-thrombin/TBA–Au nanoparticles (AuNPs) was fabricated, in which AuNPs were labeled by Raman reporters (R6G). EM hot spots can be fabricated by deposition of Ag nanoparticles (AgNPs) on AuNPs and the large EM coupling effect is presumably produced at the hot spots between AuNPs and AgNPs where the Raman reporters reside.[12,14] Taking advantage of the high sensitivity of SERS and the specificity of aptamer to protein recognition, the aptasensor is anticipated to have high sensitivity and selectivity for protein detection.

The fabrication of the SERS aptasensor process is shown in Fig. 1. Au substrate was chosen to bind the 5′ thiolated TBA and was then blocked by 2-mercaptoethanol (ME) to prevent the nonspecific binding of the DNA, NPs or impurities on the Au substrate.[14] α-Thrombin was then bound to the TBA through one of the active sites. The assembly processes of α-thrombin on Au substrate have been investigated by electrochemical cyclic voltammetry (CV) as shown in Fig. S1 (ESI†). A decrease in the peak current upon the immobilization of α-thrombin on the Au electrode indicated that α-thrombin with a concentration of 15 nM was bound to the Au substrate successfully, which is in agreement with the literature.[17]

AuNPs stabilized by citrate and modified by TBA and Raman reporters were prepared according to the procedures in the literature.[18] TEM images show that the diameter of the AuNPs is ~13 nm with the surface plasmon absorption at 519 nm (Fig. S2a and Fig. S3a, ESI†), and after the modification, AuNPs still retain

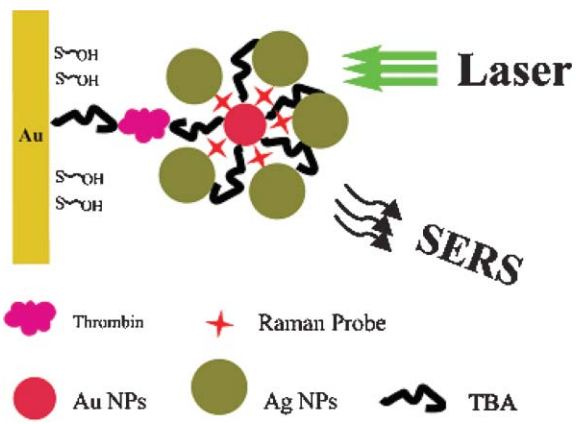

Fig. 1 Schematic illustration of the fabrication process of SERS aptasensor for protein recognition.

nearly monodispersion with the surface-plasmon band redshifting to 520 nm after centrifugation (Fig. S2b and S3c, Fig. S4, ESI†). In addition, UV-Vis absorption spectra of AuNPs modified by TBA and Raman reporters with different times indicate the stability of AuNPs can be retained for at least one week (Fig. S5, ESI†).

Through the other binding site of α-thrombin, the AuNPs labelled by TBA and Raman reporters can then be bound to α-thrombin immobilized on the Au substrate. The SEM image shown in Fig. 2A shows AuNPs modified by TBA and Raman reporters are uniformly distributed on the Au substrate through the interaction of α-thrombin to TBA, demonstrating that the sensing interface can be successfully fabricated. Before Ag enhancing, no Raman signal (curve b in Fig. 2B) could be detected due to a lack of EM enhancement for AuNPs (~13 nm) in visible light at 514.5 nm excitation, which was determined by the interband transition of gold[19] (requiring an excitation wavelength above 560 nm to produce EM) and the size effect (13 nm) in uniform distribution of AuNPs on Au substrate. (It is also difficult to produce Raman enhancement even using 633 nm as the excitation wavelength as reported by Mirkin's group.[20]) Therefore, we adopted a simple method to enhance the Raman signal of the reporters by depositing 18 μL of AgNPs (AgNPs colloid were diluted to a concentration of 4.5×10^{-11} M to prevent the formation of heavy aggregation) on the modified AuNPs surface and drying in the air.[21] Then, the hot spots were formed on the AuNPs surface and the Raman signal of the reporters could be greatly enhanced due to the large EM coupling effect produced by the hot spots between AuNPs and AgNPs. Due to the adsorption of R6G on AuNPs through electrostatic interaction, R6G was not displaced and the SERS signal was produced due to the aggregation effect of AgNPs rather than R6G transfer to the AgNPs in solution. From curve a in Fig. 2B, it can be seen that the intensity of the peaks are obviously enhanced upon Ag deposition and the peaks are characteristic of the Raman signal of R6G. The peaks at *ca.*1123 and 1178 cm^{-1} are assigned to the C–H in-plane bend mode and those at *ca.* 1360, 1504, and 1648 cm^{-1} are assigned to the C–C stretching modes.[22] A point to note is that the detection sensitivity was greatly improved mainly from the EM coupling effect produced by the hot spots on AuNPs and slightly from the resonance effect of R6G in the visible light.

SERS spectra of Raman reporters (R6G) upon Ag enhancement with different concentrations to investigate the sensitivity of the aptasensor for α-thrombin detection are shown in Fig. 3A . One can see that with the increase of the concentration of α-thrombin, the intensity of the probe greatly increases because more AuNPs will be bound to the substrate to produce more hot spots inducing large EM. The detection limit can reach as low as 0.5 nM (curve f in Fig. 3A, more clearly shown in Fig. S6, ESI†), which is comparable with the reported aptasensors for α-thrombin detection in the literature,[4e,8a] and indicates the high sensitivity of the SERS aptasensor for protein recognition. It should be noted that no linear relationship is obtained between the Raman

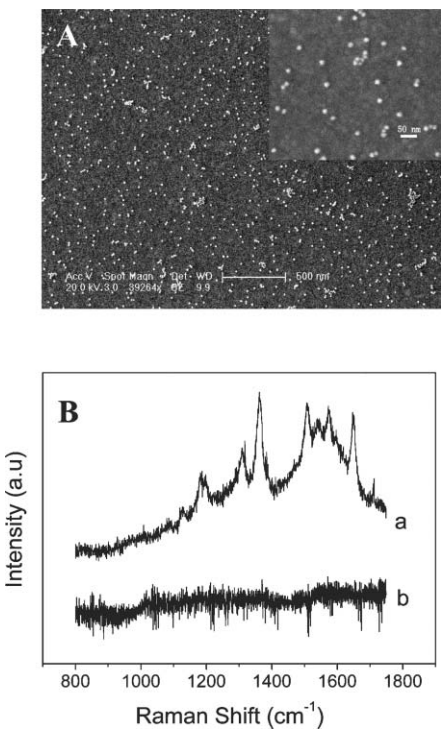

Fig. 2 (A) SEM image of AuNPs binding on the Au substrate (the scale bar is 500 nm) and the inset gives the high magnification of SEM image of AuNPs on Au substrate (the scale bar is 50 nm). (B) SERS spectra of Raman reporters (R6G) on AuNPs with (a) and without (b) Ag enhancement, the concentration of α-thrombin is 15 nM.

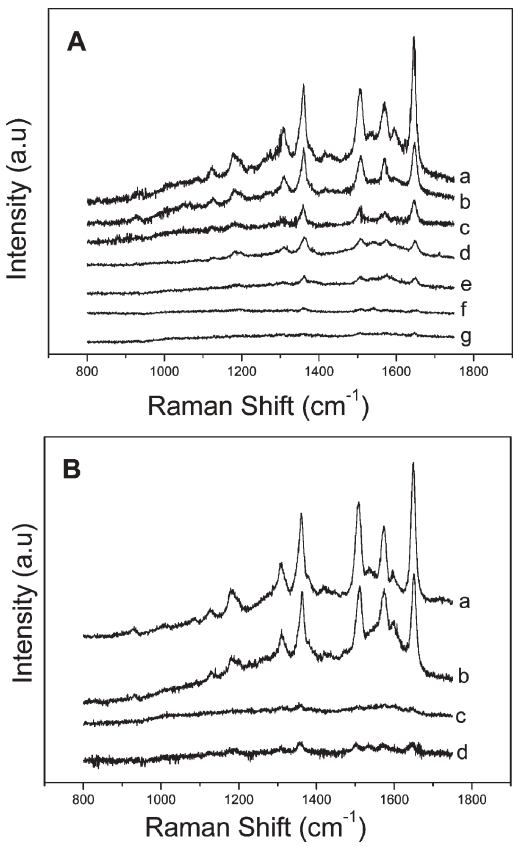

Fig. 3 SERS spectra of Raman reporters (A) with different concentrations of α-thrombin: a. 120 nM, b. 60 nM, c. 30 nM, d. 15 nM, e. 5 nM, f. 0.5 nM and g. 0 nM and (B) control measurements of SERS spectra of Raman reporters with different proteins: a. 100 nM α-thrombin, b. mixture of 200 nM β-thrombin, 200 nM γ-thrombin and 100 nM α-thrombin, c. mixture of 200 nM β-thrombin and 200 nM γ-thrombin, d. 1 μM BSA.

intensity and the concentration of the protein, similar to that of DNA detection as described previously,[23] which is mainly due to the aggregation of the AgNPs on the surface inducing a non-uniformity of the sample, so that the distribution of the hot spots are non-uniform. However, this method opens a new highly sensitive way to recognize and qualitatively detect protein. Further research will improve the uniformity of the SERS surface to quantify the protein detection. This work is in progress.

Control experiments were conducted to reveal the selectivity and specificity of the recognition reaction for detection of bovine serum albumin (BSA), β and γ- thrombin as shown in Fig. 3B. Upon the interaction of the Au–TBA substrate with foreign proteins, very weak Raman signals of the reporters were detected, indicating that no binding of the TBA modified Au substrate to the other proteins occurred, and almost no adsorption of AuNPs modified with TBA and Raman reporters on the Au substrate took place. Detection of α-thrombin from the mixture of β and γ- thrombin has also been investigated as shown in the curve b of Fig. 3B, from which α-thrombin can be detected in the mixture proteins, indicating the high selectivity and specificity of the SERS aptasensor for α-thrombin detection.

In summary, we have developed a unique and simple SERS aptasensor for protein recognition based on AuNPs labeled with aptamer and Raman reporters. Due to the strong EM coupling effect produced by the hot spots residing in the AuNPs and AgNPs, the aptasensor has high sensitivity with the detection limit of 0.5 nM. Based on the high binding activity of aptamer with the protein, the aptasensor also shows very high selectivity. More importantly, the overall process is amenable for extension to protein arrays, which provide a SERS platform for multiple protein detection using different Raman reporters based on aptamer–protein recognition and show potential applications in proteomics of great promise.

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