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REVIEW

Surface-enhanced Raman scattering for protein detection

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Abstract Proteins are essential components of organisms and they participate in every process within cells. The key characteristic of proteins that allows their diverse functions is their ability to bind other molecules specifically and tightly. With the development of proteomics, exploring high-efficiency detection methods for large-scale proteins is increasingly important. In recent years, rapid development of surface-enhanced Raman scattering (SERS)-based biosensors leads to the SERS realm of applications from chemical analysis to nanostructure characterization and biomedical applications. For proteins, early studies focused on investigating SERS spectra of individual proteins, and the successful design of nanoparticle probes has promoted great progress of SERS-based immunoassays. In this review we outline the development of SERS-based methods for proteins with particular focus on our proposed protein-mediated SERS-active substrates and their applications in label-free and Raman dye-labeled protein detection.

Keywords SERS · Protein detection · SERS-active substrate · Label-free detection · Raman dye-labeled detection

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Introduction

Background

Raman scattering, inelastic scattering of a photon, can be dramatically enhanced (10^2–10^{14}) by adsorption of molecules on a surface of noble metal, transition metal, or semiconductor substrates [1–5]; this is called surface-enhanced Raman scattering (SERS). With more than 30 years of development, SERS has been attracting increasing attention in the fields of physics, chemistry, surface science, nanoscience, and biomedical science [6–12]. It is very useful for detecting conformational changes and structural differences regarding preferred orientations of molecules on a metal surface [13, 14]. The SERS technique has proved to be a very effective analytical tool because of its high sensitivity, high selectivity, and fluorescence-quenching properties.

Recently, SERS and surface-enhanced resonance Raman scattering (SERRS) have been widely used as powerful tools for ultrasensitive chemical analysis down to the single-molecule level under favorable circumstances. Rapid development of SERS-based biosensors leads to its realm of applications from chemical-biochemical analysis to nanostructure characterization and biomedical applications [11, 12, 15, 16].

Proteins are essential for living organisms, because they are main components of the physiological metabolic pathways of cells [17, 18]. To really understand biological processes, we need to explore how proteins function in and around cells. Thus, a large-scale study of proteins, called the "Human Proteome Project" (HPP), was initiated in a quest for protein structures and functions soon after the accomplishment of the "Human Genome Project" (HGP) [19–21].

The proteome has been defined as the entire complement of proteins expressed by a cell, organism, or tissue type [22], and

proteomics is often divided into two branches (structural and functional). Conventional identification methods for proteins usually consist in protein separation and purification (e.g., 2D PAGE, HPLC), enzyme digestion, and mass spectral (MS) analysis. MS fingerprints and bioinformatics are usually used together to predict protein sequences and three-dimensional structure. X-ray crystallography and NMR spectroscopy are used to determine protein structures. Another important aspect of proteomics is the study of protein-ligand interactions, because the interactions between proteins and their ligands are crucial for many biological functions. Yeast two-hybrid assay, chemiluminescence, and fluorescence-based methods are commonly used to investigate interactions between proteins [23].

Why detect proteins by SERS?

With the development of proteomics, it is indispensable to develop new detection methods for high-throughput protein analysis. Most biological methods have the disadvantages being very time-consuming, consuming large amounts of materials, and resulting in low product yield, which would be stumbling blocks for high-efficiency proteomics [23]. For fluorescence-based methods, broad emission spectra from molecular fluorophores make multiplexing impossible, and the drawback of susceptibility to photobleaching may greatly weaken their detection limits. In contrast, SERS-based methods for biomolecules have great advantages over fluorescence-based methods in terms of photostability and spectral multiplexing [12, 16]; they are also much more sensitive than chemiluminescence-based methods. More and more studies have proved the great potential of SERS in protein identification and detection of protein-ligand interactions [16].

Based on target objectives, we divide current SERS-based methods for proteins into two types—label-free and Raman dye-labeled. The label-free strategy is to detect proteins directly by adsorption of proteins on SERS-active substrates, and analyze them by acquiring vibrational information about the proteins themselves. For example, hemoproteins (e.g., cytochrome *c*, myoglobin, and hemoglobin) [24–27] were well characterized by SERRS. Raman dye-labeled methods detect proteins indirectly by SERS of Raman dyes that are linked to probes. In the latter methods, metal (e.g., gold, Ag/SiO_2) nanoprobes with various dyes (Cy3, Cy5, MBA, DSNB, Rh B) [28–31] are usually used to detect target proteins.

In our studies we have developed several SERS-based methods for direct [32] and indirect [33, 34] detection of proteins. The greatest difference between our methods and other SERS-based methods is that our strategy is to prepare SERS-active substrates for target proteins on the basis of the strong interactions between proteins and metal nanoparticles. Moreover, we use SERS-active substrates in biological protein detection systems without labeled metal nanoprobes, and thus combine SERS with biological techniques, taking their advantages together. Our objective is to improve conventional detection methods for proteins in terms of sensitivity, simplicity, and photostability by SERS.

SERS-active substrates for proteins

Early SERS-based protein studies usually used common SERS-active substrates (e.g., metal electrode and colloid) that were prepared for small molecules. Because of selection rules of SERS, there are significant differences between the SERS spectra of macromolecules and those of small molecules. According to previous studies [35], SERS spectra of adsorbed macromolecules may not closely resemble their normal Raman spectra, because only the residues near the metal surface will be enhanced. Recently, adaptive SERS substrates for macromolecules such as DNA and proteins have attracted increasing attention.

Electrode, metal colloid, and island films

Roughened metal electrodes are usually prepared by performing several oxidation-reduction cycles (ORC), and the effect of potential on the SERS spectra of target proteins is sometime investigated [35, 36]. Silver electrode surfaces can be coated by self-assembled monolayers (SAM) that carry charged head groups, for example carboxylate (CO_2-SAM), and which enable variation of the charge density of the monolayer surface and provide biocompatible surfaces [37–39].

Silver colloid produced by the Lee and Meisel [40] citrate reduction method is often used in SERS studies, and colloidal gold is widely used as Raman dye labeled probes in immunoassays [28–30]. Keating et al. found that protein: Ag/Au colloid conjugates could induce stable SERS and control protein orientation [41]. In addition, silver colloid mixed with protein samples was sometime dried to improve SERS signals [42].

Metal island films are generally fabricated by vacuum evaporation with an electron beam. Drachev et al. prepared a type of adaptive silver substrate for soft adsorption of proteins by modified silver island films [43, 44]. The layer-by-layer (LBL) technique was also used to assemble multi-layer silver nanoparticles, which could induce strong SERS [45].

Protein-mediated substrates

In biochemistry, silver staining is one of the most popular non-radioactive methods for protein detection. It is available for detection of proteins separated by gel electrophoresis with 100-fold greater sensitivity than Coomassie

brilliant blue staining [46]. The basic mechanism underlying silver staining of proteins is that silver ions bonded to amino acid side-chains (primary COO^-, −SH) are reduced to free metallic silver by a reductant (citric acid, formaldehyde, or photoreduction) [47, 48].

In most SERS-based studies, SERS-active substrates are first prepared and then analytes are assembled on these substrates for further SERS detection. In our studies [32, 34], we use a different method to produce a SERS-active substrate which is mediated by target proteins and used for detection of these protein. We developed a new silver staining method for proteins with high sensitivity by using silver colloid, an excellent SERS-active substrate, as a staining reagent. Silver nanoparticles can be adsorbed by immobilized target proteins, because strong interactions (i.e., hydrophobic, electrostatic, and covalent interactions) between metal nanoparticles and the proteins result in aggregation of silver nanoparticles and a consequent SERS effect. In this way, we combine protein staining and SERS with silver colloid and take advantages of both to detect proteins.

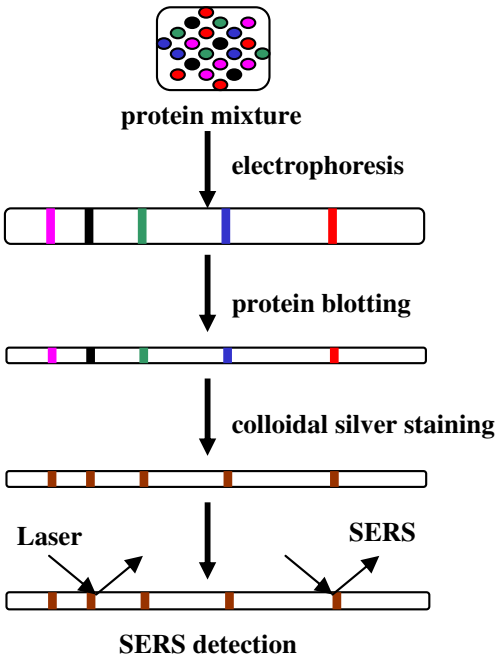

Fig. 1 "Western-SERS" procedure for label-free multi-protein detection (from Ref. [32], reproduced by permission of the American Chemical Society)

Label-free detection methods

Simple and conjugated proteins

Proteins that contain only amino acids are called simple proteins; conjugated proteins are those made up of a protein and a prosthetic group attached by covalent bonds or by weak interactions to the apoprotein [17]. For simple proteins, early SERS studies mainly focused on investigating modes of adsorption of amino acid residues, or preferred adsorption groups, for example, carboxyl and amino groups, on a metal surface [35, 49]. SERS spectra of the same simple protein from different research groups are often significantly different because of selective enhancement of amino acids near a metal surface or denaturation of proteins resulting from interactions between proteins and metal surfaces [35]. Some studies of proteins furnished SERS of amino acid side residues only whereas some groups did indeed obtain SERS signals from both amide groups and side residues [49].

Hemoproteins are the most conjugated proteins characterized by SERRS, and, unlike those of simple proteins, SERRS spectra of these proteins are much easier to analyze, because almost all SERRS bands arise from a heme group. Molecule orientation on a metal surface [41, 50], electron-transfer mechanism [37, 51] and single-molecule [52, 53] detection are all hot topics for SERRS studies of cytochrome *c*. Xu et al. [54] first reported single-molecule (SM) SERRS of hemoglobin, and dynamic oxygen release in hemoglobin was also observed by use of SERS by Etchegoin et al. [26]. Moreover, a recent study by Feng et al. achieved characterization of the native structure of myoglobin by SERRS using a home-designed flow system [55].

Multi-protein detection

A protein mixture from cells or tissues is usually separated by electrophoresis and then transferred by electroblotting on to immobilizing membranes, which is called protein

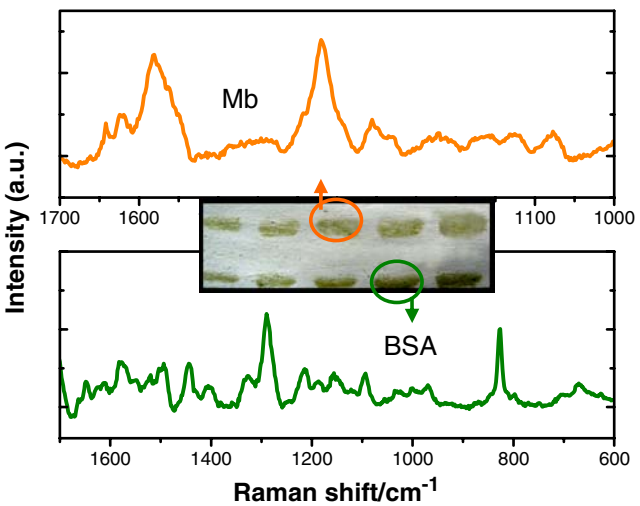

Fig. 2 SERRS of myoglobin and SERS of BSA on an NC membrane by the proposed "Western-SERS" procedure

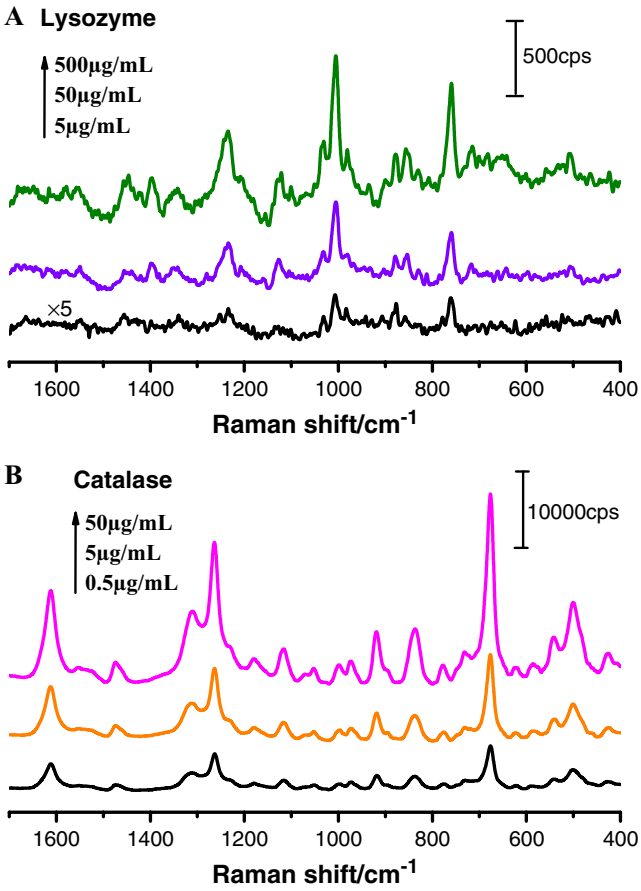

Fig. 3 Concentration-dependent SERS of (A) lysozyme and (B) catalase in aqueous solutions

blotting, electrophoretic transfer, or Western blot, for identification of individual or specific classes of protein by immunochemical detection methods [56, 57]. Based on Western blot and SERS, we developed a new analytical procedure for label-free protein detection designated "Western SERS", consisting of protein electrophoresis, Western blot, colloidal silver staining, and SERS detection (Fig. 1) [32]. We combined SERS and Western blot by using colloidal silver staining, combining their advantages. The most important feature of this method is that it enables multi-protein detection on one nitrocellulose (NC) membrane, which is inaccessible for other SERS-based detection methods.

A novel method of silver staining for Western blot that uses a silver colloid, an excellent SERS-active substrate, was first proposed in this study [32]. After protein blotting, the colloidal silver is used to stain all proteins on an NC membrane, which enables multi-protein detections. Silver nanoparticles are adsorbed along the net of the membrane to which proteins have already adhered, resulting in the formation of silver aggregates along the net of the NC membrane with SERRS or SERS emerging [16]. SERRS of myoglobin (Mb) and SERS of BSA on a NC membrane were observed by the proposed method (Fig. 2).

This "Western-SERS" method offers the dual advantages of simplicity and high sensitivity. Compared with Western blot and mass spectrometry, we can detect label-free proteins directly on an NC membrane without time and reagent-consuming procedures. Moreover, the detection limit of Western-SERS is almost consistent with the detection limit of colloidal silver staining (2 ng/band), and SERS signals do not self-quench, unlike fluorescence. Thus, the new method has great potential for identifying proteomic components or proteins of differential expression in some proteomes.

Detection of proteins in aqueous solutions

As mentioned above, it is difficult to obtain reproducible SERS spectra from roughened metal surfaces and dried colloids, especially for proteins with no chromophores,

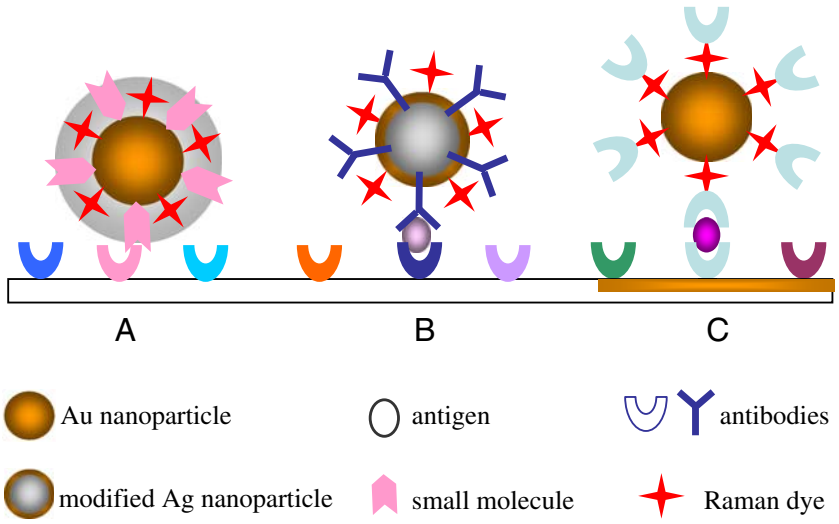

Fig. 4 Versatile metal nanoparticle probes for SERS-based immunoassays

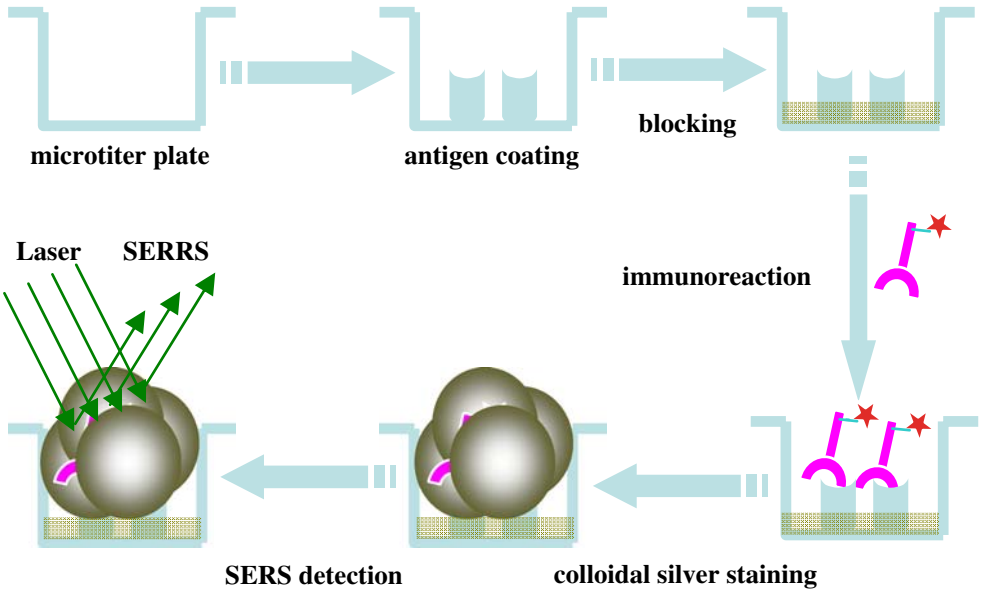

Fig. 5 Schematic diagram of FITC-linked immunoassay (from Ref. [33], reproduced by permission of the American Chemical Society)

because of irreproducible SERS substrates and different orientation of analytes on a metal surface. Also, no previous SERS-based study of proteins has enabled routine detection of label-free proteins with high sensitivity in an aqueous solution because halide ions, which are commonly used aggregation reagents, can form a strongly bonded surface layer which repels adsorption of proteins [58, 59].

In our new study, we designed an H^+ and protein-mediated strategy to induce strong SERS in aqueous solutions. We use an acidified sulfate as an aggregation agent, and target proteins are diluted by this aggregation agent before SERS measurements. Weak binding of SO_4^{2-} makes it possible to induce much stronger SERS, probably as a result of the high solubility product of silver sulfate [60]. On the other hand, excess H^+ cations are also indispensable, because under low pH conditions (lower than the p*I* of most proteins), all the target proteins carry net positive charges, enabling easy adsorption on a COO^- coated silver surface by electrostatic interactions.

In this way, we have greatly improved detection methods for label-free proteins in aqueous solutions. After being mixed with protein containing aggregation agent, silver colloid aggregated instantaneously and could then be used immediately for SERS measurement. We obtained concentration-dependent SERS spectra of simple and conjugated proteins with high sensitivity in aqueous solutions (Fig. 3). Moreover, when silver nanoparticles are aggregated by the proposed protein-associated aggregation agent, target proteins with net positive charges are sandwiched among silver nanoparticles in aqueous solutions, so vibrational information about the whole proteins would probably be displayed in their SERS spectra with remarkably enhanced reproducibility. Therefore, the detection procedure for label-free proteins has combined simplicity, rapidness, sensitivity and reproducibility, and has great potential in practical label-free protein detection.

Raman dye-labeled detection methods

Nanoparticle probe-based detection methods

Owing to the much higher Raman cross-section of Raman-active small molecules, Raman dye-labeled strategy is very useful for high-sensitivity detection of biorecognition [28–31]. In recent years, gold nanoparticle probes with versatile Raman dyes have become popular for SERS-based immunoassays [28–30]. Raman-active dyes and analytes are sometime both directly linked to metal nanoparticles [28,

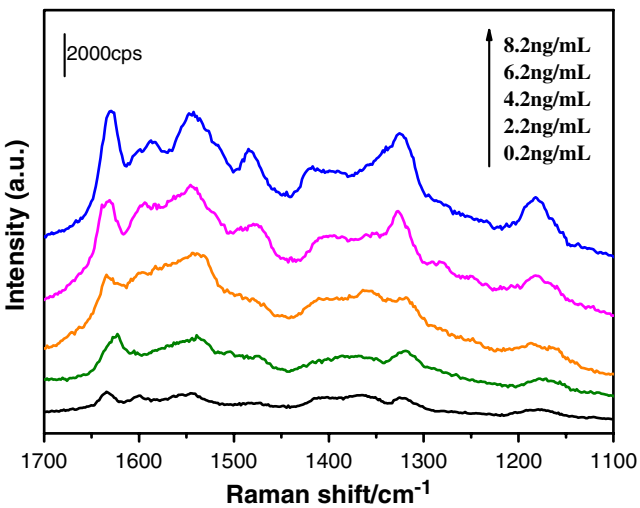

Fig. 6 Concentration-dependent SERRS of FITC in FITC-labeled immunoassay

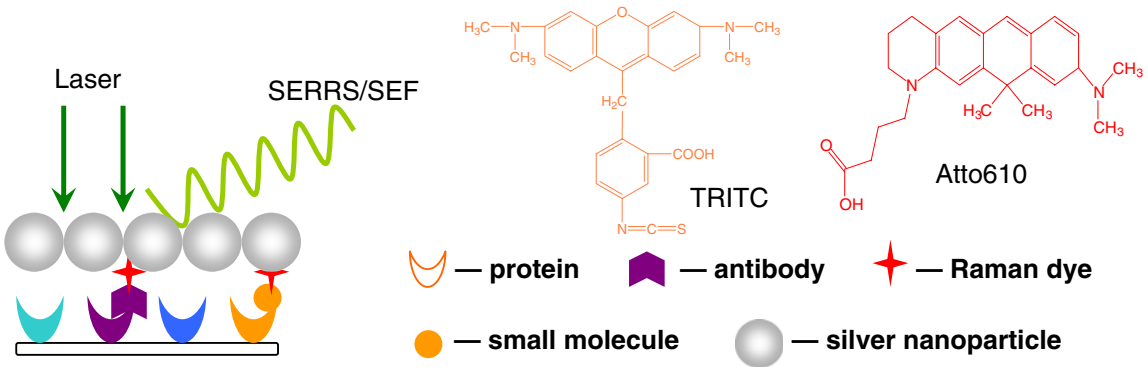

Fig. 7 SERS-based procedure for detection of protein-ligand interactions and two Raman dyes

29] (Fig. 4 A, B). Another method is first to link the Raman dyes to the analytes and then to attach metal nanoparticles [30] (Fig. 4C). In order to improve low SERS enhancement by small gold nanoparticles, Ag^+ cations are sometimes reduced on gold probes after immunoreactions (Fig. 4A), and several modified procedures (Fig. 4B) are used, for example Ag/Au, Ag/SiO_2, polyelectrolyte-coated Ag nanoparticles, [31, 61, 62], or modification of gold island film on solid chips [30] (Fig. 4C).

Although these metal probes have great advantages of high sensitivity and high selectivity, the complex procedure for synthesis of nanoparticle probes may restrict their applications to high-throughput protein detections. Moreover, binding sites of target analytes to their ligands (especially those containing sulfhydryl groups) may be destroyed by metal nanoparticles [17]. In contrast, fluorescent probes are widely used in practical biochemistry and biomedicine with advantages of safety and sensitivity [63]. In this section, we present our SERS-based methods in a fluorescent probe-based system without labeled metal nanparticle probes [33, 34].

Colloidal silver staining-based detection methods

FITC-linked immunoassay

As is well known, in enzyme-linked immunoabsorbent assay (ELISA), an enzyme acts as an amplifier, and target proteins are indirectly detected as chemiluminescence from products of enzyme-catalyzed reaction [64]. Thus, besides two immunoreactions (antigens are commonly sandwiched between two antibodies), the ELISA procedure also consists of enzyme-catalyzed reaction, which is very cumbersome and reagent-consuming. By using fluorescein isothiocyanate (FITC) as a Raman probe, we have developed a simple and sensitive method for immunoassay based on SERRS [33]. For the first time, a SERRS-based immunoassay on the bottom of a microtiter plate has been reported. We have applied the main pretreatment method of ELISA in the proposed method, and combined the advantages of good solid supports of ELISA and the high sensitivity of SERRS.

Figure 5 shows the procedure of the proposed FITC-linked immunoassay. The method has two major advan-

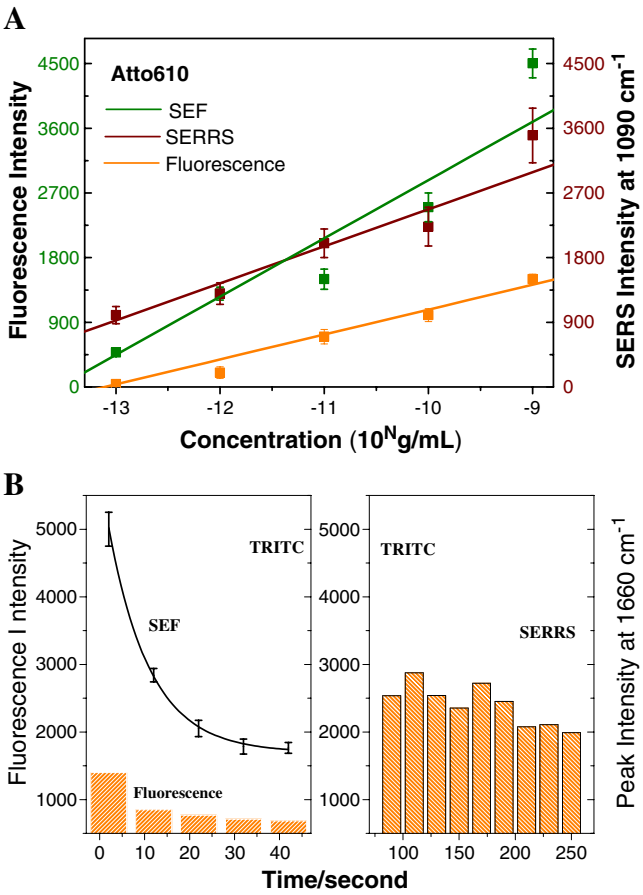

Fig. 8 Concentration-dependent SERRS, SEF, and fluorescence intensities of Atto610 (A) and time-dependent SERRS, SEF, and fluorescence intensities of TRITC (B)

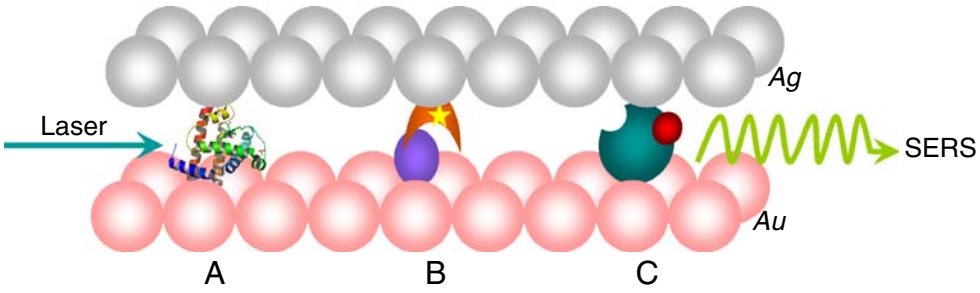

Fig. 9 Proposed sandwich metal substrate for versatile protein detection: (A) protein identification, (B) immunoassay, and (C) drug-screening

tages for immunoassay. First, we can determine target antigens via SERRS fingerprints of FITC molecules that are directly attached to antibodies, and thus the process is simple and reagent-saving. Second, by using SERRS of FITC, this method is sensitive enough to detect antigens at a concentration of 0.2 ng mL^{-1} (concentration-dependent SERRS spectra of FITC are shown in Fig. 6), which is comparable with the detection limit of Sandwich ELISA [65] and much lower than the FITC fluorescence-based method. Therefore, the proposed SERRS-based immunoassay may have great potential in high-sensitivity and high-throughput immunoassays.

Detection of protein-ligand interactions

Protein chips with versatile applications play an important role in high-throughput proteomic studies. The purpose of functional protein arrays is to quickly probe the activity of a given protein against many targets simultaneously, and the currently preferred detection method for protein arrays is fluorescence [66–68]. Based on advantages of SERS over fluorescence described above, we developed a SERS-based procedure for detection of protein-ligand interactions [34].

As shown in Fig. 7, after interactions between proteins and their corresponding ligands on aldehyde-functionalized glass slides, we employed colloidal silver staining to produce active substrates for SERS. TRITC and Atto610 were used as Raman and fluorescence reporters for determinations of the interactions between human IgG and TRITC-anti-human IgG and that between avidin and Atto 610-biotin; 514.5 and 568 nm laser lines were used for SERRS of TRITC and Atto610, respectively. As a result, we observed both SERRS and SEF by use of the proposed system. Probably, some fluorescent labels, which are very close to silver nanoparticles, are fluorescence quenched, whereas others, relatively farther from the silver surfaces because of the three-dimensional structure of the protein, are enhanced to different degrees [69, 70].

This procedure exploits several advantages of simplicity over other SERS and SEF-based related methods because of the protein staining-based strategy for silver nanoparticle assembly, high sensitivity from SERRS and SEF (Fig. 8A), and high photostability (Fig. 8B) compared with fluorescence-based protein detections. Therefore, the proposed method for detection of protein-ligand interactions has great potential in high-sensitivity and high-throughput chip-based protein function determination.

Sandwich substrate for versatile protein detection

SERS spectra of an analyte from Ag substrates are susceptible to changes because of different orientation on the metal surfaces or conformational changes because of intermolecular interactions, as described in the sections "SERS-active substrates for proteins" and "Label-free detection methods". For reproducible SERS-based proteins, metal sandwich substrates bridged by proteins have been

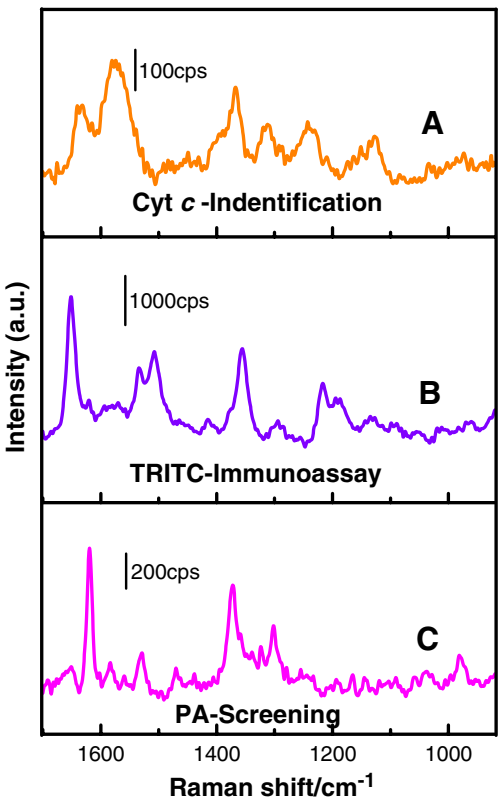

Fig. 10 SERS of cytochrome *c*, TRITC and pipemidic acid (PA) from the Au/Ag sandwich substrate

created in our recent study (Fig. 9). The sandwich architectures are fabricated on the basis of a layer-by-layer (LbL) technique [71, 72]. The first gold monolayer is prepared by self-assembly of gold nanoparticles on a poly (diallyldimethylammonium chloride) (PDDA)-coated glass slide. The sandwich structure is fabricated by the interactions between proteins in the middle layer and the metal nanoparticles in two metal layers [73].

Many SERS studies have revealed that SERS spectra from gold substrates are much more stable than those from silver substrates, while the average SERS enhancement ability of gold nanoparticles is much weaker than that of silver ones [74]. The most significant feature of this method compared with other SERS-based methods for protein detection is that a self-assembling gold nanoparticle monolayer is used for both capturing proteins and producing the SERS-active substrate with the second silver layer, making the proposed sandwich substrate more accessible and sensitive than other gold based SERS methods, and more reproducible than other silver-based SERS measurements.

Enormous SERS "hot spots" may emerge among metal aggregates formed on the edges between the two metal layers and between the junctions of metal nanoparticles [75] in the second metal layer. SERRS/SERS spectra with both high sensitivity and reproducibility, which are crucial for an analytical method, were obtained. By using these metal sandwich substrates, SERS-based versatile protein detection (i.e., identification, immunoassay, and drug screening) (Fig. 10) have been effectively carried out. The reproducibility of target molecules from Au/Ag sandwiches is comparable with those from Au/Au sandwiches. Moreover, SERRS intensities about seven times stronger can be obtained from the Au/Ag sandwiches compared with those from the Au/Au sandwiches. All the results presented in this study indicate that the proposed sandwich strategy holds great promise in SERS-based structural and functional proteomic studies.

Conclusion and perspectives

SERS-based detection methods outperform conventional assays in terms of sensitivity, selectivity, and stability. Unique features of proteins allow proteins to interact readily with metal nanoparticles, on the basis of which nanoparticle probes can be prepared for biorecognition, and protein-mediated SERS-active substrate is also accessible. Our protein-mediated strategy can be used for both protein identification and detection of protein-ligand interaction, and for both label-free and Raman dye-labeled detection with the advantages of simplicity, high sensitivity and low consumption. However, there are still some challenges for SERS detection of proteins; for example, the accuracy of quantitative detection because of inhomogeneous SERS-active substrates. In addition, few SERS studies have been conducted on detection of label-free protein–protein interactions because of large differences between SERS spectra from changing orientation of proteins without conjugated chromophores.

The promise of all these SERS-based assays is in practical biomedicine and biodiagnostics. A good attempt is that, as an excellent substrate of SERS, colloidal gold has recently been used for in-vivo targeting of a tumor [76]. Disease-related agents, for example viral pathogens [77] and *Mycobacterium avium* subsp [78] can be detected by SERS at a much lower detection limit than conventional methods, thus potentially enabling earlier detection of these diseases. However, most SERS-based assays have not been studied in real-world applications, and future advances will require more biocompatible SERS-active substrates and close collaboration between chemists and experts in biomedical fields

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References

1. Fleischmann M, Hendra PJ, McQuillan AJ (1974) Chem Phys Lett 26:163–166
2. Jeanmaire DL, Van Duyne RP (1977) J Electroanal Chem 84:1–17
3. Albrecht MG, Creighton JA (1977) J Am Chem Soc 99:5215–5217
4. Tian ZQ, Ren B, Wu DY (2002) J Phys Chem B 106:9463–9483
5. Wang YF, Zhang JH, Jia HY, Li MJ, Zeng JB, Yang B, Zhao B, Xu WQ, Lombardi JR (2008) J Phys Chem C 112:996–1000
6. Chang RK, Furtak TE (eds) (1982) In: Surface enhanced Raman scattering. Plenum, New York
7. Kerker M, Thompson BJ (eds) (1990) Selected papers on surface-enhanced Raman scattering. Society of Photo Optics
8. Tian ZQ, Ren B (2004) Annu Rev Phys Chem 55:197–229
9. Dieringer JA, McFarland AD, Shah NC et al (2006) Faraday Discuss 132:9–26
10. Weatherby S (ed) (2006) Surface enhanced Raman spectroscopy. Royal Society of Chemistry
11. Stiles PL, Dieringer FA, Shah NC, Van Duyne RP (2008) Annu Rev Anal Chem 1:601–626
12. Graham D, Goodacre R (ed) (2008) Special issue on surface enhanced Raman scattering, Chem Soc Rev 37(5)
13. Shanmukh S, Jones L, Driskell J et al (2006) Nano Lett 6:2630–2636
14. Zou S, Weaver MJ (1998) Anal Chem 70:2387–2395
15. Aroca R (2006) Surface-enhanced vibrational spectroscopy. Wiley

16. Kneipp K, Moskovits M, Kneipp H (eds) (2006) In: Surface-enhanced Raman scattering: physics and applications. Springer, Berlin Heidelberg New York
17. Wang JY, Zhu SG, Xun CF (2002) Biochemistry. High Education PressChina
18. Voet D, Voet JG (2004) Biochemistry, Vol 1, 3rd edn. Wiley, Hoboken, NJ
19. Twyman RM (2004) Principles of proteomics. BIOS Scientific Publishers, New York
20. Wilkins MR, Williams KL, Appel RD, Hochstrasser DF (1997) Proteome research: new frontiers in functional genomics. Springer, Berlin Heidelberg New York
21. Liebler DC (2002) Introduction to proteomics: tools for the new biology. Humana Press, Totowa, NJ
22. Wasinger VC, Cordwell SJ, Anne CD (1995) Electrophoresis 16:1090–1094
23. Westermeier R, Naven T (2002) Proteomics in practice: a laboratory manual of proteome analysis. Wiley-VCH, Weinheim
24. Eng LH, Schlegel V, Wang D, Neujahr HY, Stankovich MT, Cotton T (1996) Langmuir 12:3055–3059
25. Lecomte S, Wackerbarth H, Soulimane T, Buse G, Hildebrandt P (1998) J Am Chem Soc 120:7381–7382
26. Etchegoin P, Liem H, Maher RC, Cohen LF, Brown RJC, Milton MJT, Gallop JC (2003) Chem Phys Lett 367:223–229
27. Bizzarri AR, Cannistraro S (2002) Appl Spectrosc 56:1531–1537
28. Cao YC, Jin R, Nam JM, Thaxton CS, Mirkin CA (2003) J Am Chem Soc 125:14676–14677
29. Xu SP, Ji XH, Xu WQ, Li XL, Wang LY, Bai YB, Zhao B, Ozaki Y (2004) Analyst 129:63–68
30. Grubisha DS, Lipert RJ, Park HY, Driskell J, Porter MD (2003) Anal Chem 75:5936–5943
31. Gong JL, Liang Y, Huang Y, Chen JW, Jiang JH, Shen GL, Yu RQ (2007) Biosens Bioelectron 22:1501–1507
32. Han XX, Jia HY, Wang YF, Lu ZC, Wang CX, Xu WQ, Zhao B, Ozaki Y (2008) Anal Chem 80:2799–2804
33. Han XX, Cai LJ, Guo J, Wang CX, Ruan WD, Han WY, Xu WQ, Zhao B, Ozaki Y (2008) Anal Chem 80:3020–3024
34. Han XX, Kitahama Y, Tanaka Y, Guo J, Xu WQ, Zhao B, Ozaki Y (2008) Anal Chem 80:6567–6572
35. Grabbe ES, Buck RP (1989) J Am Chem Soc 111:8362–6366
36. Niaura G, Gaigalas AK, Vilker VL (1996) J Electroanal Chem 416:167–178
37. Murgida DH, Hildebrandt P (2004) Acc Chem Res 37:854–861
38. Murgida DH, Hildebrandt P (2001) Angew Chem Int Ed 40:728–731
39. Hildebrandt P, Murgida DH (2002) Bioelectrochem Bioenerg 55:139–143
40. Lee PV, Meisel D (1982) J Phys Chem 86:3391–3395
41. Keating CD, Kovaleski KM, Natan MJ (1998) J Phys Chem B 102:9404–9413
42. Pavan Kumar GV, Ashok Reddy BA, Arif M, Kundu TK, Narayana C (2006) J Phys Chem B 110:16787–16792
43. Drachev VP, Thoreson MD, Khaliullin EN, Davisson VJ, Shalaev VM (2004) J Phys Chem B 108:18046–18052
44. Drachev VP, Nashine VC, Thoreson MD, Ben-Amotz D, Davisson VJ, Shalaev VM (2005) Langmuir 21:8368–8373
45. Pieczonka NPW, Goulet PJG, Aroca RF (2006) J Am Chem Soc 128:12626–12627
46. Switzer RC, Merril CR, Shifrin S (1979) Anal Biochem 98:231–237
47. Oakley BR, Kirsch DR, Morris NR (1980) Anal Biochem 105:361–363
48. Merril CR, Pratt ME (1986) Anal Biochem 156:96–110
49. Stewart S, Fredericks PM (1999) Spectrochim Acta Part A 55:1615–164
50. Macdonald IDG, Smith WE (1996) Langmuir 12:706–713
51. Yue H, Khoshtariya D, Waldeck DH, Grochol J, Hildebrandt P, Murgida DH (2006) J Phys Chem B 110:19906–19913
52. Delfino I, Bizzarri AR, Cannistraro S (2005) Biophys Chem 113:41–51
53. Delfino I, Bizzarri AR, Cannistraro S (2006) Chem Phys 326:356–362
54. Xu HX, Bjerneld EJ, Kall M, Borjesson L (1999) Phys Rev Lett 83:4357–4360
55. Feng M, Tachikawa H (2008) J Am Chem Soc 130:7443–7448
56. Towbin H, Staehelin T, Gordon J (1979) Proc Natl Acad Sci 76:4350–4354
57. Burnette WN (1981) Anal Biochem 112:195–203
58. Bell SEJ, Sirimuthu NMS (2006) J Am Chem Soc 128:15580–15581
59. Bell SEJ, Sirimuthu NMS (2005) J Phys Chem A 109:7405–7410
60. Bell SEJ, Mackle JN, Sirimuthu NMS (2005) Analyst 130:545–549
61. Kim K, Park HK, Kim NH (2006) Langmuir 22:3421–3427
62. Cui Y, Ren B, Yao JL, Gu RA, Tian ZQ (2006) J Phys Chem B 110:4002–4006
63. Guilbault GG (1990) Practical fluorescence, 2nd edn. Marcel Dekker, New York
64. Kemeny DMJ (1992) Immunol Meth 150:57–76
65. Rosi NL, Mirkin CA (2005) Chem Rev 105:1547–1562
66. Blackstock WP, Weir MP (1999) Trends Biotechnol 17:121–127
67. MacBeath G, Schreiber SL (2000) Science 289:1760–1763
68. Zhu H, Snyder M (2003) Curr Opin Chem Biol 1:55–63
69. Lakowicz JR, Geddes CD et al (2004) J Fluoresc 14:425–441
70. Geddes CD, Lakowicz JR (2002) J Fluoresc 12:121–129
71. Li XL, Xu WQ, Zhang JH, Jia HY, Yang B, Zhao B, Li BF, Ozaki Y (2004) Langmuir 20:1298–1304
72. Ruan WD, Wang CX, Ji N, Lu ZC, Zhou TL, Zhao B, Lombardi JR (2008) Langmuir 24:8417–8420
73. Carney J, Braven H, Seal J, Whitworth E (2006) IVD Technol 11:41–51
74. Zeman EJ, Schatz GC (1987) J Phys Chem 91:634–643
75. Jiang J, Bosnick K, Maillard M, Brus L (2003) J Phys Chem B 107:9964–9972
76. Qian XM, Peng XH, Ansari DO, Yin-Goen Q, Chen GZ, Shin DM, Yang L (2008) Nat Biotechnol 26:83–90
77. Driskell JD, Kwarta KM, Lipert RJ, Porter MD, Neill JD, Ridpath JF (2005) Anal Chem 77:6147–6154
78. Yakes BJ, Lipert RJ, Bannantine JP, Porter MD (2008) Clin Vaccine Immunol 15:227–234