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Acid Cleavable Surface enhanced Raman Tagging for Protein Detection

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Abstract

Dye conjugation is a common strategy improving the surface enhanced Raman detection sensitivity of biomolecules. Reported is a proof-of-concept study of a novel surface enhanced Raman spectroscopic tagging strategy termed as acid-cleavable SERS tag (ACST) method. Using Rhodamine B as the starting material, we prepared the first ACST prototype that consisted of, from the distal end, a SERS tag moiety (STM), an acid-cleavable linker, and a protein reactive moiety. Complete acid cleavage of the ACST tags was achieved at a very mild condition that is 1.5% trifluoroacetic acid (TFA) aqueous solution at room temperature. SERS detection of this ACST tagged protein was demonstrated using bovine serum albumin (BSA) as the model protein. While the SERS spectrum of intact ACST-BSA was entirely dominated by the fluorescent signal of STM, quality SERS spectra can be readily obtained with the acid cleaved ACST-BSA conjugates. Separation of the acid cleaved STM from protein further enhances the SERS sensitivity. Current SERS detection sensitivity, achieved with the acid cleaved ACST-BSA conjugate is ~5 nM in terms of the BSA concentration and ~1.5 nM in ACST content. The linear dynamic range of the cleaved ACST-BSA conjugate spans four orders of magnitudes from ~10 nM to ~100 µM in protein concentrations. Further improvement in the SERS sensitivity can be achieved with resonance Raman acquisition. This cleavable tagging strategy may also be used for elimination of protein interference in fluorescence based biomolecule detection.

INTRODUCTION

With its single molecule sensitivity demonstrated with selected molecules such as Rhodamine 6G,¹ and adenine,² bis(benzimidazo) perylene³ surface enhanced Raman spectroscopy (SERS) has drawn significant interest for its potential for ultrasensitive bioanalytical application.^{4–5} Recent progress in nanoscience and technology has greatly enhanced our ability in SERS substrate design and fabrication.^{6–9} Development of a robust and effective SERS method for biomolecule detection, however, remains technically challenging.

Due to the low intrinsic SERS activity of biomolecules,¹⁰ SERS based biomolecule detection typically employs tagging methods, hoping that the SERS detection sensitivity of the labeling dye can be translated to the biomolecule-dye conjugate. Current SERS tagging methods can be broadly divided into two categories. The first class of the tagging methods, developed by Mirkin's and Porter's group utilizes preassembled SERS-tag/nanoparticle complexes as the reporters.^{11–14} Since SERS tags are directly immobilized on the

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nanoparticle, the SERS activity of the reporter complexes can be easily controlled and is less likely affected by the conjugated biomolecule. As a result this SERS tagging method can be extremely sensitive and robust in bioanalytical applications. However, the large size of the nanoparticle complex limits the compatibility of this tagging technique with common biomolecule separation methods including HPLC and the capillary electrophoresis. Currently the application of this tagging strategy largely limits to array based analysis where the target analyte is immobilized on a solid support.^{15, 16} The second class of methods, pioneered by Graham's and Vo-dinh's group,^{17–19} involves direct conjugation of the SERS tag with a biomolecule, followed by incubation of the tagged biomolecule complex with a SERS substrate for the spectral acquisition. While this tagging strategy offers a clear advantage for its compatibility with biomolecule separation and characterization techniques such as HPLC, gel electrophoresis and mass spectrometric analysis, which is important for the general applicability of a bioanalytical technique, the SERS sensitivity of this approach can be severely compromised, particularly in protein analysis. This may explain why there are very few reports of successful SERS detection of protein-dye conjugates despite their wide availability.

Using silver nanoparticle (AgNP) as the SERS substrate we recently demonstrated that protein drastically reduces the SERS of the dye molecule that is either mixed or conjugated with protein.²⁰ Several effects are responsible for the reduced dye SERS signal. Firstly, with its large hydrodynamic radius, the protein adsorbed or co-adsorbed onto the nanoparticle surface invariably enlarges the interparticle distance between aggregated AgNPs, which can drastically reduce electromagnetic enhancement (EM) for the dye molecules sitting in the interstitial area. It was estimated that compared to EM enhancement for the molecules in the interstitial area of the aggregated nanoparticles coated with Rhodamine 6G alone, the EM enhancement is reduced by 4 orders of magnitude for the molecules in the interstitial area of the aggregated nanoparticle that is coated with bovine serum albumin (BSA).²⁰ Secondly, it is commonly known that protein has high affinity to noble metal nanoparticle. As a result, protein competes with dye molecules for direct adsorption onto the SERS active surfaces, which can lead to insufficient dye fluorescence quenching as well as low chemical and electromagnetic enhancement of the dye molecules. Another factor, which can also keep the dye from direct contact with the SERS active surface, is the protein shielding. Since most of the SERS active dyes are hydrophobic, they are more likely to locate in the interior area of the globular protein. If protein maintains or largely maintains its native conformation on the nanoparticle surfaces, the chance for the dye molecules to direct interact with nanoparticle can be very small. Collectively, these effects may explain why fluorescence enhancement, instead of SERS enhancement, is a more commonly reported phenomena for protein-dye conjugate adsorbed on nanoparticles.^{21–23}

To effectively take advantage of the ultrahigh SERS activity of dye molecule for biomolecule detection, we developed a novel SERS tagging strategy using a newly designed SERS tag termed as acid cleavable SERS tag (ACST). As shown in scheme 1 for protein detection, our ACST consisted of the Rhodamine B (Rh-B) based SERS tag moiety (STM, shown in red), an acid cleavable linkage (blue) and a protein reactive group (green). The design of the acid labile linkage was based mainly on the work by Mende and Seitz who used this acid labile linkage for solid state peptide synthesis.²⁴ Our protein reactive group is a derivative of iodoacetamide that reacts readily with reduced cysteines and has been used extensively for protein/peptide modifications.^{25, 26}

To our knowledge, there have been only few works using cleavable linkage for fluorescence and SERS detection of biomolecule,^{27–30} and the only prior SERS work that used a cleavable SERS tagging strategy is the SERS detection of prostate-specific antigen (PSA).²⁷ By conjugation of a SERS active dye with PSA peptide substrate immobilized on a

nanoplasmonic resonator, Sun et al demonstrated that the PSA enzyme can be detected and quantified by the SERS signal reduction of the dye molecule resulted from enzymatic cleavage of the peptide linker between dye and nanoplasmonic resonator.²⁷ One important distinction of our cleavable tagging technique from the previous cleavable SERS and fluorescence tagging methods is in the design of the cleavable linkage. Essentially all the previous works use enzyme-cleavable linkage. While these methods provide excellent specificity, the reaction conditions for the enzymatic cleavage has to, presumably, be carefully controlled to maintain the enzyme activities.^{27–29} In contrast, in this work the linkage between the SERS active dye and biomolecule is designed to be cleavable with simple chemicals such as trifluoroacetic acid (TFA). As a result, our cleavage reaction can be much more robust and much easier to implement.

Because of the cleavability of the linkage between the STM moiety and the protein reactive moiety, STM can be detached and separated from the ACST tagged protein. As a result protein interference with STM SERS acquisition can be entirely eliminated, which theoretically ensures that the ultrahigh SERS sensitivity of the labelling dye can be fully taken advantage of the SERS detection of protein-dye conjugates.

In addition to its application in SERS detection of the dye-conjugated proteins, this acid-cleavable dye tagging strategy may also be used for improving the quantification accuracy and sensitivity of the fluorescence and UV-visible quantification of dye conjugated proteins. Indeed, cleavable fluorescence tagging has been demonstrated by Graham et al for detection of enzymatic activity of Trypsin.²⁸ It is well established that due to the electrostatic, hydrophobic interaction between protein amino acid residues and the dyes, protein conjugation changes the fluorescence quantum yields and/or UV-visible peak position and molar extinction coefficient of the dye molecules.³¹ The degree of the spectral interference depends on not only the protein and dye identities, but also the conjugation ratio, location of the conjugation, solvent composition (pH, ionic strength, temperature, etc).³² As a result, accurate fluorescence or UV-vis quantification of protein-dye conjugation is challenging. An acid cleavable dye tagging method such as the one described here would provide a clear path for elimination of protein interference in fluorescence measurement of the labelling dyes.

EXPERIMENTAL

Material and Reagents

Unless stated otherwise, all the chemicals and reagents including BSA used were purchased from Sigma-aldrich. 12% Tris-HCl precasted 10 wells ready gels and all the electrophoresis reagents was purchased from BioRad. High purity water ($>18 \text{ M}\Omega \text{ cm}^{-1}$) from Nanopure water purification system was used in all the experiments.

Synthesis of ACST and STM

ACST was prepared according to Scheme 2 shown above. Compound **1** was prepared based on the published procedure.²⁴ Detailed synthetic procedure for compound **2–5** and their NMR characterization is described in the supporting material (Scheme S-1). STM was synthesized according to the published procedure.³³

BSA conjugation with ACST

45 mg of BSA was dissolved in 1 ml of pH 9.5 carbonate buffer, to which equal volume of 120 μM ACST in ethanol was added. The reaction mixture was made to sit in the dark at room temperature for 1 hr with occasional stir. Free ACST was first removed with PD 10 column (AnaSpec. Inc.) using 50 mM carbonate buffer as eluant. The collected ACST-BSA fraction was further purified using an ultrafiltration tube with filtration membrane of 10000

MWCO (Millipore Inc). The ACST-BSA retained by the filtration membrane was reconstituted with water and further purified with membrane dialysis (MWCO 10000). Conjugation ratio of the ACST-BSA was determined with an Evolution 300 UV-visible spectrophotometer (Fisher Scientific).

Acid Cleavage of ACST

The acid cleavage reactions were conducted with trifluoroacetic acid (TFA) as the cleavage reagent at room temperature. To determine the optimal TFA concentration for the cleavage reaction, series of ACST and TFA mixtures were prepared where the concentration of ACST (~20 μ M) was kept constant and the concentrations of TFA were 25%, 12.5%, 6.25%, 3.1%, 1.5% respectively. Progression of the acid cleavage reaction was monitored with electrospray ionization (ESI)-MS (Agilent technologies). MS spectra were taken periodically for each cleavage solution and the relative abundance of the ACST and STM was monitored until complete disappearance of the ACST molecular ion.

SDS PAGE analysis

The electrophoresis was conducted with 12% Tris-HCl ready gel using a PowerPac™ Basic SDS PAGE system (BioRad). Briefly, into three lanes of the ready gel were loaded ~4 nmol of BSA control (without ACST conjugation), ACST-BSA in 1.5% TFA, ACST-BSA alone respectively. To investigate the possible protein degradation induced by ACST conjugation or TFA acid cleavage, protein ladder was also loaded to the gel on a separated lane. After separation, the gel was removed from the electrophoresis cell and fluorescence image of the gel was acquired with a UV transilluminator (Spectroline) before the BSA bands were identified using colloidal Coomassie Blue staining.

Ultrafiltration purification of ACST-BSA and separation of STM in TFA treated ACST-STM

Ultrafiltration was used for purification of PD-10 column purified ACST-BSA and for separation of acid cleaved STM from the TFA treated ACST-BSA. All the ultrafiltration was conducted with an Eppendorf bench top centrifugation machine with a motor speed of 13,000 rpm. For ACST-BSA purification, 450 μ L of PD-10 column purified ACST-BSA was loaded onto a 500 μ L ultrafiltration tube (membrane MWCO: 10,000). After 30 minutes' centrifugal filtration, the solution retained by the membrane was dispersed in water for further purification with membrane dialysis. The main difference in the experimental procedure between ultrafiltration separations of STM in the TFA treated ACST-BSA and ultrafiltration purification of the ACSTBSA conjugate is an ethanol washing step used in the STM separation. Briefly, after ultrafiltration of 400 μ L ACST-BSA of a proper concentration in 1.5% TFA solution, 400 μ L ethanol was added to the ultrafiltration tube to wash the STM trapped in the filtration membrane. The filtrates from both the sample and washing solution were combined for the SERS spectral acquisition. It should be emphasized that the washing step is very important. Without washing, the quality of the SERS spectrum obtained with the filtrate is much poorer due to the STM trapping.

SERS measurements

All the SERS spectra were obtained using LabRam HR (Jobin Yvon Horiba) instrument with Ag colloids as SERS substrates. Ag colloids were prepared according to Lee-Meisel.³⁴ Briefly, 27 mg of silver nitrate was dissolved in 150 ml water and heated to boiling, then 3 ml of 1% sodium citrate was added and the resulting solution was continued to boil for 1 hr. when not used, the AgNP was stored in a 4°C refrigerator. For a typical SERS spectral measurement, 10 μ L sample solution was first mixed with 10 μ L of AgNP solution, and the mixture was vortex for ~1 min before the addition of 10 μ L of 1% KCl as the aggregation agent. The resulting solution was again vortex for ~1 min before depositing onto a Ramchip

slide (Z&S Tech) for SERS acquisition.³⁵ It should be noted that the Ramchip slide is a normal Raman substrate (no surface enhancement) that has essentially no fluorescence and Raman background interference.

The SERS spectra were obtained with 633 nm HeNe laser using laser power on sample of 1.3 mW. All the spectra were collected with a 10× objective (NA=0.25, Olympus) corresponding to a laser spot on the sample of ~20 μm in diameter. The spectral acquisition times varied from 0.1s to 50 s.

SERS quantification of ACST-BSA

Series of ACST-BSA solutions with concentrations ranging from 10 nM to 100 μM were prepared using 1.5% TFA as solvent. After the solution was sit at room temperature for 6 hours to allow complete acid cleavage of the cleavable linker, 400 μL of the ACST-BSA solutions were transferred to an ultrafiltration tube for separation of the STM from the protein moiety as described above. For each sample the filtrate and ethanol washing solution was combined for SERS spectral acquisition. Five spectra were acquired for each ACST-BSA solution.

RESULT AND DISCUSSION

Acid cleavage of ACST

ACST acid cleavability was demonstrated with the ESI-MS spectra in Fig 1 that were acquired with intact ACST, and ACST treated with 1.5% TFA at different incubation times. Without TFA, the MS spectrum of the sample was entirely dominated by the m/z peak at 872.3, which is in excellent agreement with the singly charged molecular ion of ACST (m/z: cal. 872.3), confirming the successful preparation of the ACST. Complete ACST acid cleavage has been achieved with TFA at all the tested concentration as judged by the appearance of the STM peak (m/z: cal. 511.3, exp. 511.4) and the disappearance of the ACST peak in the MS spectra of the TFA treated samples. Depending on the concentration of TFA, the reaction times required for complete ACST cleavage are vastly different (Fig S-1). For example, with 25% TFA complete ACST cleavage was largely completed within 10 minutes, it took more than 3 hours for the 1.5% TFA sample to complete the cleavage reaction. It should be noted that even though no attempt has been made to separate and recover the product of the ACST acid cleavage reaction for further characterization, the excellent agreement between the calculated MS peak for STM and MS peak in the MS spectrum of the TFA treated samples indicates that STM is indeed released by acid cleavage of ACST as we proposed in Scheme 1.

It is worth noting that compared to 87.5% TFA used by Mende and Seitz for the acid cleavage reaction, the reaction conditions we have explored are much more milder.²⁴ With TFA of concentration as low as 1.5%, complete ACST cleavage was observed at room temperature in our experiment. Clearly, using TFA of low concentration is important to avoid possible protein damage such as acid catalyzed protein hydrolysis. As a result, the acid cleavage reaction for all the subsequent experiments in this study was carried out with 1.5% TFA at room temperature.

Acid cleavage of ACST-BSA

The photographs of SDS gel electrophoresis in Fig. 2 demonstrate the successful ACST-BSA conjugation and its successful ACST cleavage. The pinkish color at the gel-front in lane 3 in the fluorescence image (Fig 2, left) was resulted from the cleaved STM. The absence of the STM fluorescence in the BSA band of lane 3 indicates the effectiveness of acid cleavage. The identical separation profiles for the BSA control (lane 2, right), and the

intact and TFA treated ACST-BSA conjugates (lane 1 and 3, respectively, right panel) indicate that neither ACST/BSA conjugation nor the acid cleavage have a significant adverse effect on the protein integrity.

Quantification determination of the ACST conjugation ratio ($[ACST]/[BSA]$) was carried out based on the UV-vis spectrum of the ACST-BSA conjugates shown in Fig 3 where the 563 nm peak absorbance is from the STM moiety and the 280 nm peak is from the protein moiety. Using the molar absorptivity of 96,100 reported for the STM moiety,³⁶ and 43,890 for the BSA,³⁷ the ACST conjugation ratio is estimated to be 0.3, or 30% of BSA was labeled. It is important to note that even though BSA has 35 cysteine groups, there is only one free sulfhydryl group moiety in native BSA.³⁸ The rest of the cysteines are in oxidized form forming disulfide bridges between the cysteine residues. The lone “free” sulfhydryl moiety may also react with endogenous sulfhydryl compounds such as homocysteine, glutathione etc to form disulfide bonds that can further reduce the free sulfhydryl group for ACST conjugation.³⁸ Previous literature reported that the amount of free sulfhydryl groups in native BSA ranging from 0.36 to 0.69 mol/mol BSA.^{38, 39} Taking all the factors into consideration, the 30% ACST labeling ratio achieved under our current experimental condition is not unreasonable.

SERS spectra of ACST and STM

With this ACST tagging method, the SERS sensitivity of the ACST-protein conjugates is ultimately determined by SERS sensitivity of the STM moiety. As a result, exploring the STM SERS characteristics and the experimental conditions for optimal SERS detection of STM is importance for the optimization of the SERS detection scheme of the ACST-protein conjugate. It should be noted that UV-visible and fluorescence characteristics of STM have been studied,³⁶ to our knowledge, however, Raman including surface enhanced Raman of this new Rhodamine dye has not been explored.

Fig 4 shows the SERS spectra of (a) Rhodamine B, (b) STM and ACST (c) without and (d) with TFA treatment. Evidently, the SERS spectra of ACST, with and without TFA, were entirely dominated by the SERS feature of STM, indicating that the SERS activity of STM is drastically higher than that of the protein reactive moiety in the ACST construct. This result is not surprising given the ultrahigh SERS activities (single molecules in some cases) observed with various cationic Rhodamine dyes.¹

Another noteworthy observation in Fig. 4 is the remarkable similarity in the STM and Rh-B SERS spectral features. As a matter of fact the only difference in the spectra is the small differences in the relative peak intensities in the doublet around 1530 cm^{-1} region. Detailed reason for this spectral variation is out of the scope of current work. This result clearly demonstrates that amidation of the carboxyl group in Rh-B has no significant impact on its spectral profiles (shape). Similar effect has been observed with another Rh-B derivative in which the carboxyl group in Rh-B is esterified.⁴⁰

In stark contrast to the high similarity in their spectral shape, STM and Rh-B differs tremendously in their pH dependence of the SERS sensitivity. Unlike Rh-B whose SERS signal intensity is strongly pH dependent and its SERS signal is over 100 times higher in a 0.1M HCl solution than that in a water or 0.1M NaOH solution, the pH dependence of STM SERS signal is much less dramatic. High quality SERS spectra were observed in acidic, neutral and basic solutions, and the spectral intensity at the acidic solution is only about 3 times higher than that at the neutral and basic pH (Fig S-2). A $\sim 100\text{ pM}$ SERS sensitivity has been achieved with STM dissolved in water (Fig S-3). The detailed reason for the differential pH dependence of the STM and Rh-B SERS activity is beyond the scope of the current work. Similar pH dependence was reported by McCabe et al for the SERS spectrum

of Rb-B and esterified Rb-B.⁴⁰ It most likely relates to the fact that unlike Rh-B that is mainly neutral at neutral and basic solution, the STM is a cationic ion so it likely has stronger affinity to the negatively charged AgNP used for the SERS spectral acquisition. In addition to modifying the analyte structures, HCl and NaOH may also change the surface properties of the nanoparticle, affecting how nanoparticle interacts with analyte molecules. Clearly, the high SERS sensitivity of STM and its relative insensitivity to the pH changes is important for its practical applications in a broad range of biological conditions.

SERS spectra of ACST-BSA conjugate

Effectiveness of the ACST tagging method for SERS detection of protein-dye conjugate is demonstrated with Fig 5 where the SERS spectra of intact ACST-BSA, ACST-BSA/TFA mixture and filtrate of ACST-BSA/TFA mixture were shown. While quality SERS spectra were obtained with the TFA treated ACST-BSA in which the BSA concentration was as low as 50 nM, no SERS feature could be detected with intact ACST-BSA conjugates even when the BSA concentration was as high as 1 μ M (data not shown). Instead, the spectrum of the intact ACST-BSA was dominated by the fluorescence signal of STM moiety that itself exhibits appreciable fluorescence signal with 633 nm excitation laser (Fig S-4). The dominance of the STM fluorescence and the absence of the STM SERS signal in the intact ACST-BSA conjugate indicate that without acid cleavage, the STM was mostly shielded by protein from direct contact with nanoparticles, leading to ineffective STM fluorescence quenching and poor SERS enhancement. This result is consistent with our recent study that no SERS signal were observed with protein conjugated fluorescein isothiocyanate.²⁰ Indeed, previous research indicate that when the dye separates from metal nanoparticle surfaces beyond certain distance, instead of quenching, nanoparticle enhances the fluorescence signal of dye molecule.^{21, 23} Conversely the appearance of the STM SERS signal and the drastically attenuated STM fluorescence in the SERS spectrum of the TFA treated ACST-BSA sample indicate that once cleaved from protein moiety, most STM molecules were absorbed onto the AgNP under our experimental conditions.

Even though acid cleavage of the ACST-BSA drastically enhances the SERS detectability of labeling dye, its detection sensitivity, which is ~50 nM in protein and ~15 nM in ACST is significantly lower than the 100 pM sensitivity determined with free STM due most likely to the protein co-adsorption onto the AgNP surfaces. This result is consistent with our previous observation that compared to that of Rhodamine 6G (R6G) alone sample, the SERS signal of R6G in an equal molar R6G/BSA sample is reduced by 2 to 3 orders of magnitudes.²⁰ The STM signal attenuation can be resulted from competitive protein/dye adsorption onto the AgNP surface or non specific protein/STM interactions.

As expected, separation of acid cleaved STM from protein further improves the SERS sensitivity of the ACST-BSA. Even though the nominal STM concentration for spectrum (c) in Fig 5 is two times lower (because of the ethanol washing) than that for spectrum (b), the spectral signal to noise ratio of spectrum (c) is markedly better. With our current acid cleavage, ultrafiltration and ethanol washing scheme, quality SERS spectrum was obtained with filtrate of the TFA treated ACST-BSA that is 5 nM in BSA and 1.5 nM in ACST (Fig S-5). It is important to note that such a detection sensitivity is still more than 10 times worse than the ~100 pM detection limit determined with purified STM, indicating future improvement in the detection sensitivity of ACST-BSA is possible by optimization of the ACST cleavage and separation process.

Quantification of the protein-dye conjugates

Utility of the SERS detection of this SERS tagging strategy for quantification of ACST labeled BSA was shown in Fig 6 where ACST-BSA of different concentrations was

prepared using 1.5% TFA as solvent, followed by ultrafiltration of the cleaved dye from the protein moiety. The spectra shown in the left is the representative SERS spectra obtained with cleaved ACST-BSA of different concentrations and the calibration curve in the right showed the correlation between 619 cm^{-1} peak of STM with the protein concentration.

The results in Fig 6 (B) showed a strong correlation between STM SERS spectral intensity and protein concentration, indicating the possibility of estimation of the protein concentration with a dynamic range from 100 μM to 10 nM in protein concentrations. Clearly further improvement of the quantification sensitivity, accuracy and the dynamic range is possible by improving the STM separation schemes and by using a resonance Raman excitation conditions.

CONCLUSION

In conclusion, we prepared the first acid cleavable SERS tag and demonstrated that SERS detection sensitivity of dye tagged protein can be drastically improved with the ability of detaching and separating the SERS tag from the protein moiety. Current SERS detection sensitivity, achieved with the acid cleaved ACST-BSA conjugate is 5 nM in terms of the BSA concentration and 1.5 nM in ACST content. The linear dynamic range of the cleaved ACST-BSA conjugate spans four orders of magnitudes from ~10 nM to ~100 μM in protein concentrations. Further improvement in the SERS sensitivity is possible by (i) increasing the labeling ratio of the ACST tags, (ii) using resonance Raman excitation condition, and (iii) optimizing the ACST cleavage and protein removal. By combining the ACST tagging strategy with the isotope encoded SERS internal references method with which an isotope encoded STM is to used as internal references,⁴¹ the quantification accuracy of ACST is expected to be drastically improved. In addition to its potential applications for quantitative protein or proteomics analysis, this combined technique may also be used for SERS quantification of protein post-translational modifications by imparting target specificity on the protein reactive group in the ACST construct.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Dieringer JA, Lettan RB, Scheidt KA, Van Duyne RP. J. Am. Chem. Soc 2007;129:16249–16256. [PubMed: 18052068]
2. Kneipp K, Kneipp H, Kartha VB, Manoharan R, Deinum G, Itzkan I, Dasari RR, Feld MS. Phy. Rev. E 1998;57:R6281–6284.
3. Constantino CJL, Lemma T, Antunes PA, Aroca R. Anal. Chem 2001;73:3674–3678. [PubMed: 11510833]
4. Deb SK, Davis B, Knudsen GM, Gudihall R, Ben-Amotz D, Davisson VJ. J. Am. Chem. Soc 2008;130:9624–9625. [PubMed: 18593116]
5. Graham D, Mallinder BJ, Whitcombe D, Watson ND, Smith WE. Anal. Chem 2002;74:1069–1074. [PubMed: 11924965]
6. Lim D-K, Jeon K-S, Kim HM, Nam J-M, Suh YD. Nat Mater 2010;9:60–67. [PubMed: 20010829]
7. Liang H, Li Z, Wang W, Wu Y, Xu H. Adv. Mater 2009;21:4614–4618.
8. Levin CS, Kundu J, Barhoumi A, Halas NJ. Analyst 2009;134:1745–1750. [PubMed: 19684894]

9. Moskovits M. *Nature* 2010;464:357–357. [PubMed: 20237551]
10. Doering WE, Nie S. *Anal. Chem* 2003;75:6171–6176. [PubMed: 14615997]
11. Nam J-M, Thaxton CS, Mirkin CA. *Science* 2003;301:1884–1886. [PubMed: 14512622]
12. Grubisha DS, Lipert RJ, Park H-Y, Driskell J, Porter MD. *Anal. Chem* 2003;75:5936–5943. [PubMed: 14588035]
13. Porter MD, Lipert RJ, Siperko LM, Wang G, Narayanan R. *Chem. Soc. Rev* 2008;37:1001–1011. [PubMed: 18443685]
14. Driskell JD, Kwart KM, Lipert RJ, Porter MD, Neill JD, Ridpath JF. *Anal. Chem* 2005;77:6147–6154. [PubMed: 16194072]
15. Orth R, Sieber SA. *The Journal of Organic Chemistry* 2009;74:8476–8479. [PubMed: 19821566]
16. Carlson EE, Cravatt BF. *Nat Meth* 2007;4:429–435.
17. Graham D, Mallinder BJ, Whitcombe D, Smith WE. *ChemPhysChem* 2001;2:746–748.
18. Wang H-N, Vo-dinh T. *Nanotechnology* 2009;20:065101/065101–065101/065106. [PubMed: 19417369]
19. Wabuyele MB, Vo-Dinh T. *Anal. Chem* 2005;77:7810–7815. [PubMed: 16316192]
20. Zhang D, Ansar SM, Vangala K, Jiang D. *J. Raman Spectrosc.* In press. DOI: 10.1002/jrs.2548.
21. Bardhan R, Grady NK, Cole JR, Joshi A, Halas NJ. *ACS Nano* 2009;3:744–752. [PubMed: 19231823]
22. Xie F, Baker MS, Goldys EM. *Chem. Mater* 2008;20:1788–1797.
23. Tam F, Goodrich GP, Johnson BR, Halas NJ. *Nano Lett* 2007;7:496–501. [PubMed: 17256995]
24. Mende F, Seitz O. *Angew. Chem. Int. Ed* 2007;46:4577–4580.
25. Williams DK, Meadows CW, Bori ID, Hawkridge AM, Comins DL, Muddiman DC. *J. Am. Chem. Soc* 2008;130:2122–2123. [PubMed: 18220392]
26. Sechi S, Chait BT. *Anal. Chem* 1998;70:5150–5158. [PubMed: 9868912]
27. Sun C, Su K-H, Valentine J, Rosa-Bauza YT, Ellman JA, Elboudwarej O, Mukherjee B, Craik CS, Shuman MA, Chen FF, Zhang X. *ACS Nano* 2010;4:978–984. [PubMed: 20121209]
28. McKenzie F, Steven V, Ingram A, Graham D. *Chem. Commun* 2009:2872–2874.
29. Tsai C-S, Liu P-Y, Yen H-Y, Hsu T-L, Wong C-H. *Chem. Commun* 46:5575–5577.
30. Ingram A, Byers L, Faulds K, Moore BD, Graham D. *J. Am. Chem. Soc* 2008;130:11846–11847. [PubMed: 18700767]
31. Venkatraman P, Nguyen TT, Sainlos M, Bilsel O, Chitta S, Imperiali B, Stern LJ. *Nat Chem Biol* 2007;3:222–228. [PubMed: 17351628]
32. Grunwaldt G, Haebel S, Spitz C, Steup M, Menzel R. *J. Photoch. Photobio. B* 2002;67:177–186.
33. Nguyen T, Francis MB. *Org. Lett* 2003;5:3245–3248. [PubMed: 12943398]
34. Lee PC, Meisel D. *J. Phys. Chem* 1982;86:3391–3395.
35. Zhang D, Ansar SM. *Anal. Chem* 2010;82:5910–5914. [PubMed: 20536157]
36. Mende F, Seitz O. *Angew. Chem. Int. Ed* 2007;46:4577–4580.
37. Pace C, Vajdos F, Fee L, Grimsley G, Gray T. *Protein Sci* 1995;4:2411–2423. [PubMed: 8563639]
38. Yasuhara T, Nokihara K. *Anal. Chem* 1998;70:3505–3509. [PubMed: 9726170]
39. Riener C, Kada G, Gruber H. *Analytical and Bioanalytical Chemistry* 2002;373:266–276. [PubMed: 12110978]
40. McCabe AF, Graham D, McKeown D, Smith WE. *Journal of Raman Spectroscopy* 2005;36:45–49.
41. Zhang D, Xie Y, Deb Shirshendu K, Davison VJ, Ben-Amotz D. *Anal. Chem* 2005;77:3563–3569. [PubMed: 15924390]

Fig 1. ESI-MS spectra of (a) intact ACST, and (b–e) ACST in 1.5% TFA solution. Spectra (b–e) was taken after ACST mixed with TFA for 10 mins, 1 hour, 2hours, 3 hours and 6 hours respectively. Spectra were offset for clarity.

Fig 2. Photographs of SDS gel image that is (*left*) obtained with a UV lamp and (*right*) after BBC staining. Samples on lane 1 to 4 are intact ACST-BSA, BSA, 1.5% TFA treated ACST-BSA and protein ladder.

Fig 3.
UV-visible spectrum of ACST-BSA.

Fig 4. SERS spectra of (a) Rhodamine B, (b) STM, (c) ACST and (d) TFA treated ACST. Spectra are scaled and offset for clarity.

Fig 5. SERS spectrum of (a) intact ACST-BSA conjugate (b) mixture of ACST-BSA and 1.5% TFA and (c) ultrafiltrate of ACST-BSA and TFA mixture combined with equal volume of ethanol washing solution. The BSA concentration is 50 nM for (a), (b) and (c). Spectra were scaled and offset for clarity

Fig 6. (A) Representative SERS spectra obtained with STM cleaved from ACST-BSA of protein concentrations of (a) 100 μM , (b) 10 μM , (c) 1 μM , (d) 100 nM and (e) 10 nM. (B) Correlation between the STM SERS intensity at 619 cm^{-1} with protein concentration. The error bar represented one standard deviation.

Scheme 1.

(A). Structure of the acid-cleavable SERS tag (ACST) and the proposed acid cleavage reaction. ACST consisted of the SERS tag moiety (STM, in red), acid cleavable linker (in blue) and protein reactive group (in green). (B) Cartoon representation of SERS detection of protein using acid-cleavable SERS tags. When the dye-conjugated protein directly applied for SERS measurements, only fluorescence signal is observed. By acid cleavage of the dye-protein conjugation followed by ultrafiltration of the protein/dye mixture, SERS signal of the dye molecules is readily detected.

Scheme 2.

Synthesis of ACST tag. Reagents and conditions: (a) Piperazine, Et₃N (b) Rhodamine B, HBTU, DIPEA (c) Pd(PPh₃)₄, Ph₃SiH (d) 2-iodoacetic acid, HBTU, DIPEA. HBTU = 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, DIPEA: ethyldiisopropylamine