Supporting Information

Quantitative Evaluation of Proteins with Bicinchoninic Acid (BCA): Resonance Raman and Surface-enhanced Resonance Raman Scattering-based Methods

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Experimental section

Materials. Bovine serum albumin (BSA), human serum albumine (HSA), cytochrome *c* (cyt *c*), peroxidase from horseradish (HRP), human IgG and fetal bovine serum (FBS), QuantiPro BCA assay kit (QA, QB and QC), sodium citrate tribasic dihydrate and silver nitrate were purchased from Sigma-Aldrich Co., Ltd. at highest purity available and used as received without further purification. Ultrapure water (18.0 MΩ cm^{-1}) was used throughout the present study.

The phosphate-buffered saline (PBS; 0.01 M, pH 7.4) used in this study contains 0.8% NaCl, 0.02% KH_2PO_4, 0.02% KCl and 0.12% $Na_2HPO_4 \cdot 12H_2O$.

Colloidal silver was prepared by the aqueous reduction of silver nitrate (10^{-3} M, 200 mL) with trisodium citrate (1%, 4 mL). A TEM image of silver colloid and the plasmon absorption maximum of the silver colloid at 456 nm are shown in the Supporting Information (SI_Figure 4 and SI_Figure 5).[1]

Preparation of RR and SERRS-based protein detection reagents.

RR-based method. QuantiPro BCA assay kit:

QA reagent is prepared with sodium carbonate, sodium tartrate and sodium bicarbonate in 0.2 M NaOH at pH 11.25.

QB reagent is 4 % (w/v, 0.1 mol/L) BCA solution at pH 8.5.

QC reagent is 4 % (w/v, 0.16 mol/L) copper (II) sulfate pentahydrate solution.

The QuantiPro working reagent was prepared by mixing 25 parts of reagent QA and 25 parts of reagent QB. After reagent QA and QB have been combined, 1 part of reagent QC (copper II sulfate) was added and mixed until uniform in color.

SERRS-based method. QuantiPro BCA assay kit:

Reagent A was prepared with sodium carbonate, sodium tartrate and sodium bicarbonate in 0.2 M NaOH at pH 11.25.

Reagent B is 0.4 % (w/v, 0.01 mol/L) BCA solution at pH 8.5.

Reagent C is 0.4 % (w/v, 0.016 mol/L) copper (II) sulfate pentahydrate solution.

The working reagent was prepared by mixing 25 parts of reagent A and 25 parts of reagent B. After reagent A and B have been combined, 1 part of reagent C was added and mixed.

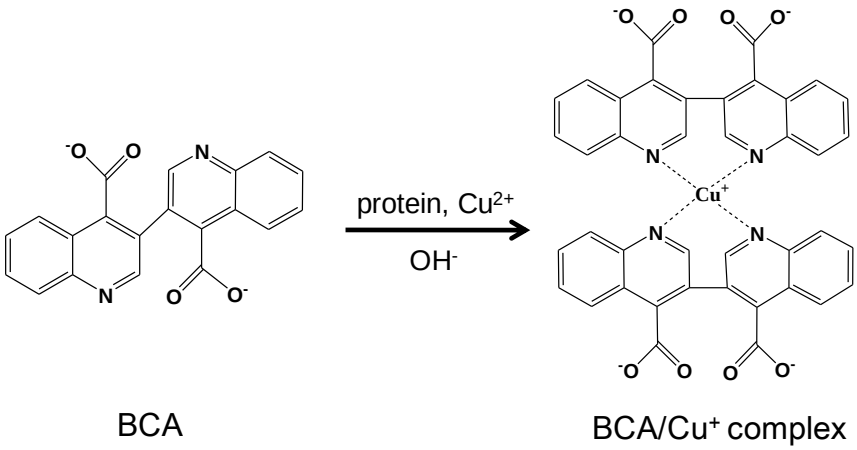
Pretreatment of protein samples (RR and SERRS-based methods). BSA, HSA, human IgG, cyt *c*, HRP and FBS were diluted with PBS buffer. To determine the protein concentrations, BSA concentrations of 50 μg/mL, 5 μg/mL, 500 ng/mL, 50 ng/mL, 5 ng/mL and 500 *p*g/mL were prepared for RR-based assay. HSA, human IgG, cyt *c* and HRP solutions at different concentrations (5 μg/mL

and 500 ng/mL) were used to study protein-to-protein variability, and the FBS dilution ratio was 10^4 for the evaluation of protein concentrations. The QuantiPro assay consists of 1 part protein sample and 1 part prepared QuantiPro working reagent and is placed at 37 ºC for two hours. The blank sample contains 1 part PBS buffer with 1 part prepared QuantiPro working reagent and is kept at 37 ºC for two hours.

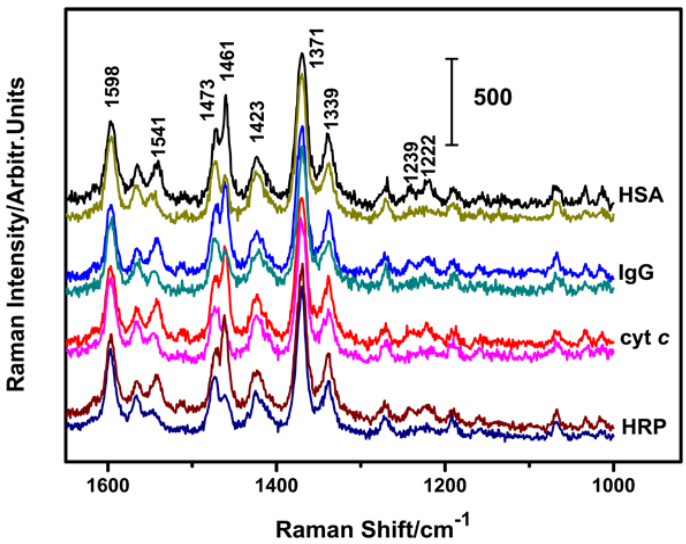
BSA, HSA, human IgG, cyt *c*, HRP and FBS were diluted in PBS buffer. To determine the protein concentrations, BSA concentrations of 100 μg/mL, 10 μg/mL, 1 μg/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 *p*g/mL, 10 *p*g/mL and 1 *p*g/mL were used for SERRS-based assay. HSA, human IgG, cyt *c* and HRP solutions at the same concentration (100 ng/mL) were used to study protein-to-protein variability, and the FBS dilution ratio was 10^6 for the evaluation of protein concentrations. According to the coupling reaction,[2] 1 part protein sample was mixed with 1 part prepared working reagent and placed at 37 ºC for two hours. The blank sample contained 1 part PBS with 1 part prepared working reagent and was placed at 37 ºC for two hours.

After the coupling reaction, RR-based assay of each sample was performed directly. SERRS-based assay was performed with the mixed solution consisting of 1 part of each sample and 9 parts silver colloid.

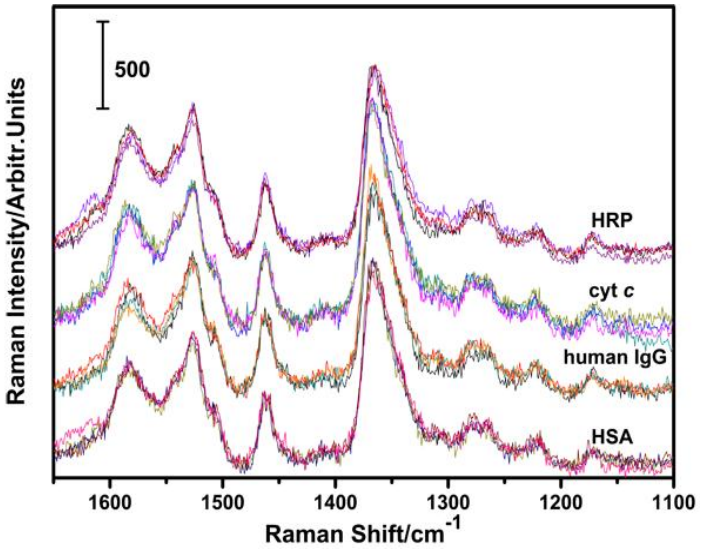
Instruments. After the couple reaction, the reaction solution was drawn with capillary and the laser was irradiated in the center of capillary. RR and SERRS spectra were recorded using a Jobin Yvon/HORIBA LabRam ARAMIS Raman spectrometer. The radiation from an air cooled frequency doubled Nd:Yag laser (532 nm) was used as an excitation source. The typical exposure time for each RR and SERRS measurement in this study were 30 s and 10 s with one accumulation (exposure time dependent RR and SERRS spectra were shown in SI_Figure 7 and SI_Figure 8). Absorbance spectra were recorded on a Scinco UV-spectrometer. Transmission electron microscopy (TEM) image of silver colloid was obtained using a Hitachi H-8100 microscope with an acceleration voltage of 200 kV.



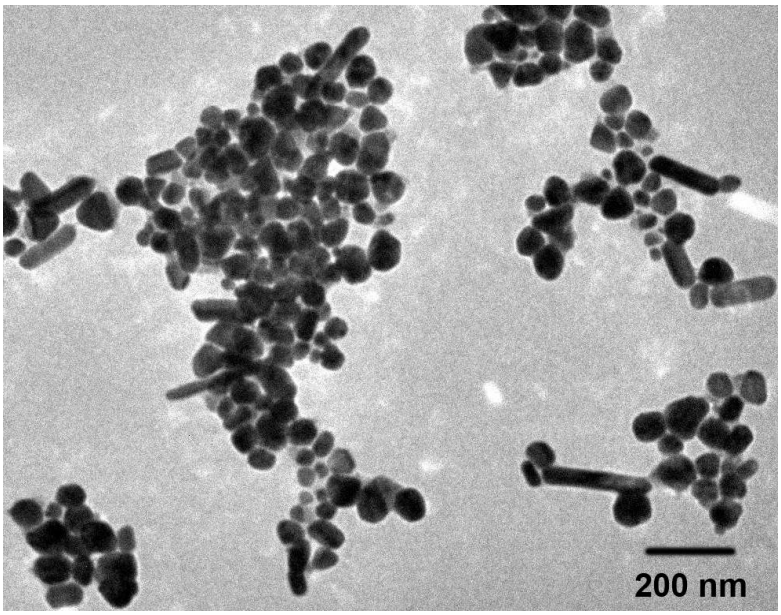
SI_Figure 1. Formation of the BCA/Cu^{+} complex.



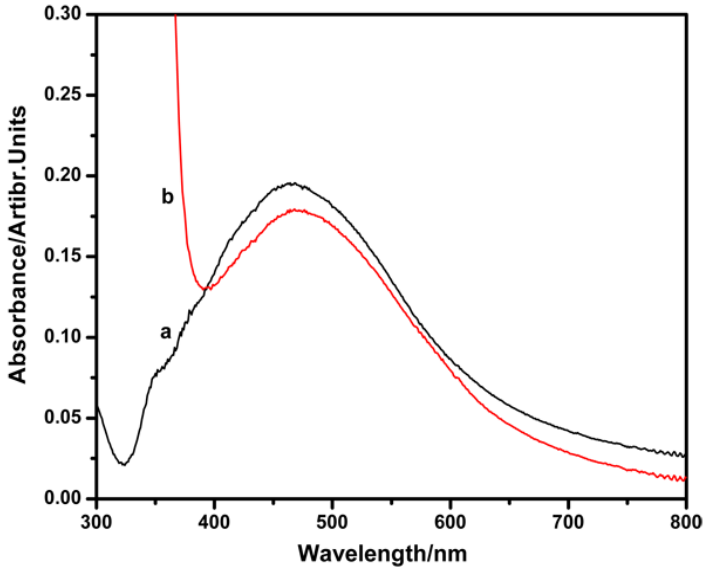
SI_Figure 2. RR spectra of HSA, human IgG, cyt *c* and HRP at concentrations of 5 μg/mL and 500 ng/mL. The BCA concentration was 2.5×10^{-2} mol/L in the present system.



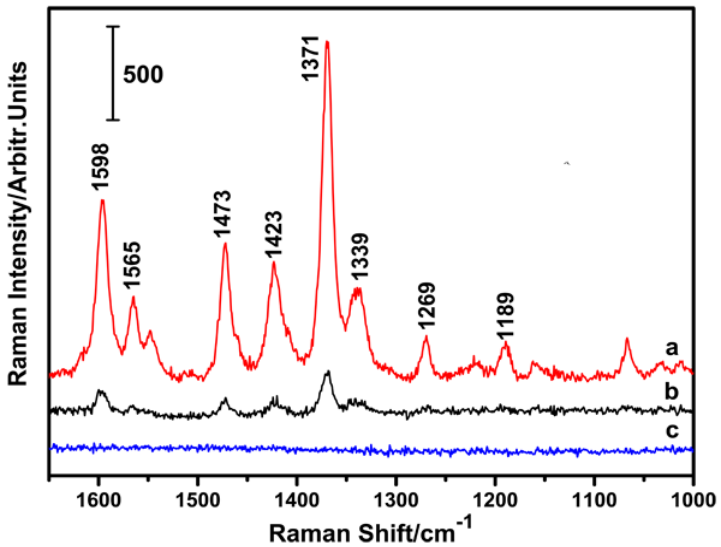
SI_Figure 3. SERRS spectra of four different proteins (HSA, human IgG, cyt *c* and HRP) at the same concentration (100 ng/mL). The BCA concentration was 2.5×10^{-4} mol/L in the present system.



SI_Figure 4. A TEM image of silver colloid.



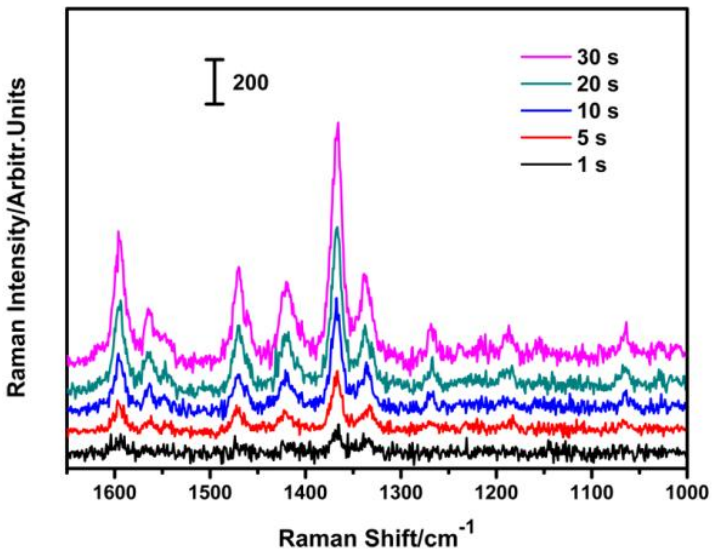
SI_Figure 5. UV-vis spectra of silver colloid (a) and the mixture of silver colloid and working reagent (b).



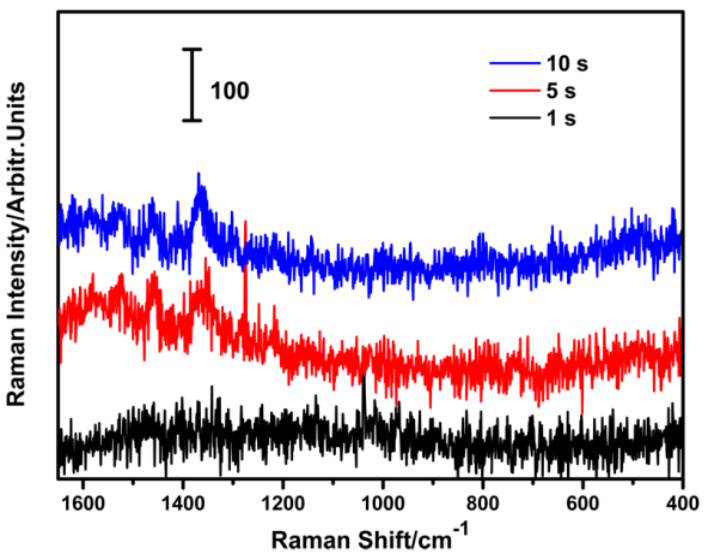
SI_Figure 6. Raman spectrum of the BCA working reagent at (a) 5.0×10^{-2} mol/L, the concentration-dependent SERS spectra of the BCA working reagent at (b) 5.0×10^{-3} and (c) 5.0×10^{-4} mol/L in silver colloid. The volume ratio of working reagent to Ag colloid was 1:9.

SI_Figure 6 shows the Raman spectra of the working reagent and SERS spectra of the working reagent mixed with silver colloid, and no new or highly enhanced Raman bands can be observed in the SERS spectra of the mixture compared to the reagent's normal Raman spectra. Therefore, BCA may weakly

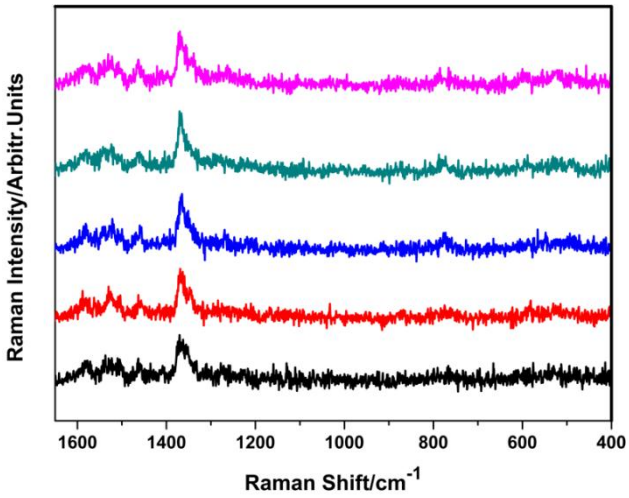
bind to the silver colloid and have no SERS. We can also conclude from the UV-vis spectra that there is no significant shift after the addition of the working reagent to the silver colloid (SI_Figure 5), indicating that the BCA molecule may weakly bind to the silver colloid.



SI_Figure 7. Exposure time dependent RR spectra. BSA concentration is 50 ng/mL. The BCA concentration was 2.5×10^{-2} mol/L in the present system. This RR-based method undergoes less signal-to-noise variation with exposure time increasing.



SI_Figure 8. Exposure time dependent SERRS spectra. BSA concentration is 10 pg/mL. The BCA concentration was 2.5×10^{-4} mol/L in the present system. This SERRS-based method undergoes less signal-to-noise variation with exposure time increasing.



SI_Figure 9. Reproducible SERRS spectra with five independent measurements. BSA concentration is 100 pg/mL. The BCA concentration was 2.5×10^{-4} mol/L in the present system. The proposed method possesses higher SERRS reproducible for protein quantitation analysis.

Reference

1. P. C. Lee and D. Meisel, *J. Phys. Chem.* 1982, **86**, 3391–3395.
2. P. K. Smith, R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson and D. C. Klenk, *Anal. Biochem.* 1985, **150**, 76-85.