Supplementary Material (ESI) for Analyst

Supporting Information

Acid Cleavable Surface enhanced Raman Tagging for Protein Detection

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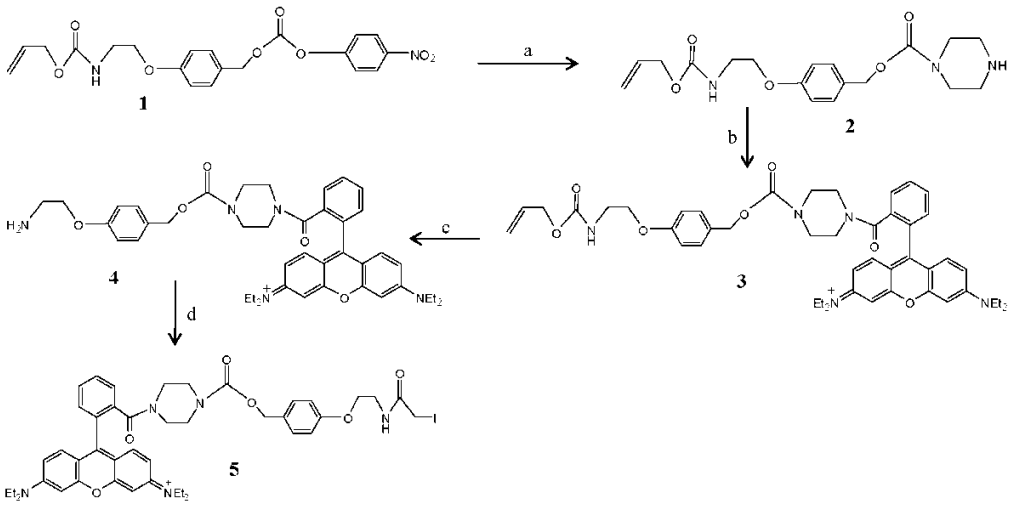
1. Synthesis of acid cleavable SERS tag (ACST):

The synthetic scheme of ACST is shown below:



Scheme 1: Preparation of the ACST.

Compound 1: Compound **1** was prepared based on the published procedure.[1]

Compound 2: Compound **1** (120 mg, 0.29 mmol), piperazine (100 mg, 1.2 mmol) and Et_3N (0.162 mL, 1.2 mmol) were dissolved in dichloromethane (10 mL) and the mixture was stirred for 18 hr at RT. The mixture was concentrated in vacuo and redissolved in EtOAc (20 mL), washed with H_2O (3 x 20 mL), sat. aq. $NaHCO_3$ (3 x 20 mL), and brine (25 mL). The resulting compound was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/MeOH 8:1) to afford compound **2** (45 mg, 43%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.28 (d, 2H, J = 8.7 Hz), 6.90 (d, 2H, *J* = 9.0 Hz), 5.85-5.98 (m, 1H), 5.14-5.31 (m, 2H), 5.03 (s, 2H), 4.52 (d, 2H, *J* = 5.4 Hz), 4.00 (t, 2H, *J* = 5.7 Hz), 3.48 (t, 2H, *J* = 5.7 Hz), 3.42 (t, 4H, J = 5.4 Hz), 2.73 (bs, 4H).

Compound 3: To a stirring solution of compound **2** (100mg, 0.28 mmol) in dichloromethane (20 mL) was added HBTU (157 mg, 0.41 mmol), DIPEA (0.14 mL, 0.83 mmol) and rhodamine B (172 mg, 0.36 mmol). The reaction mixture was stirred at room temperature for 3 hr and then quenched with dilute HCl. The organic layer was thoroughly washed with dilute HCl and brine, dried over Na_2SO_4, and concentrated

under vacuum. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 30:1) to afford compound **2** (178 mg, 82%) as a dark purple solid. ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.67-7.73 (m, 2H), 7.54-7.57 (m, 1H), 7.33-7.56 (m, 1H), 7.25-7.29 (m, 2H), 7.22 (d, 2H, *J* = 9.6 Hz), 6.87-6.92 (m, 2H), 6.85 (d, 2H, J = 8.7 Hz), 6.76-777 (m, 2H), 5.83-8.96 (m, 1H), 5.53 (bs, 1H), 5.16-5.31 (m, 2H), 5.01 (s, 2H), 4.54 (d, 2H, *J* = 5.4 Hz), 4.01 (t, 2H, *J* = 5.4 Hz), 3.52-3.67 (m, 10H), 3.36 (bs, 8H), 1.30 (t, 12H, J= 7.2 Hz).

Compound 4: Compound **3** (110 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (10 mL). Ph_3SiH (0.05 mL, 0.40 mmol) and $Pd(PPh_3)_4$ (10 mg) was added and the mixture was stirred for 18 h at RT under nitrogen. The mixture was concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/MeOH 20:1) to furnish compound **4** (66 mg, 67%) as a dark purple solid. ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.66-7.74 (m, 2H), 7.51-7.56 (m, 1H), 7.23-7.34 (m, 3H), 7.22 (d, 2H, *J* = 9.3 Hz), 6.90-6.92 (m, 2 H), 6.86 (d, 2H, *J* = 8.4), 6.75 (bs, 2H), 4.99 (s, 2H), 4.00 (t, 2H, J = 4.2 Hz), 3.55-3.63 (m 8H), 3.47 (s, 2H), 3.33 (bs, 10H), 3.10-3.11 (m, 2H), 1.32 (t, 12H, J= 7.5 Hz).

Compound 5: To a solution of compound **4** (100mg, 0.14 mmol) in dichloromethane (10 ml) was added succinic anhydride (70 mg, 0.7 mmol) and Et_3N (0.10 mL, 0.72 mmol) at room temperature. The resulting solution was stirred at room temperature for 12 hr. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH 20:1) to afford compound **5** (99 mg, 87%) as a dark purple solid. ^{1}H NMR (CD_2Cl_2, 300 MHz), δ (ppm): 7.69 (m, 2H), 7.54 (m, 1H), 7.36 (m, 1H), 7.24 (m, 4H), 6.87 (m, 4H), 6.75 (bs, 2H), 6.51 (bs, 1H), 4.99 (bs, 2H), 4.02 (m, 2H), 3.67 (bs, 2H), 3.60 (m, 10H), 3.29 (bs, 8H), 1.29 (m, 12H).

2. ACST cleavage with TFA of different concentrations: The acid cleavage of ACST was carried out with TFA solutions at concentrations of 1.5 %, 3 %, 6.25 %, 12.5 %, and 25 % respectively. The progress of the reaction was monitored with ESI-MS until the disappearance of ACST peak at 872.3. Fig.2 shows the experimental data obtained with 25%, 12.5%, 6.25%, 3 % TFA solutions. The ESI-MS spectra of ACST mixed with 1.5% TFA was shown in the primary article.

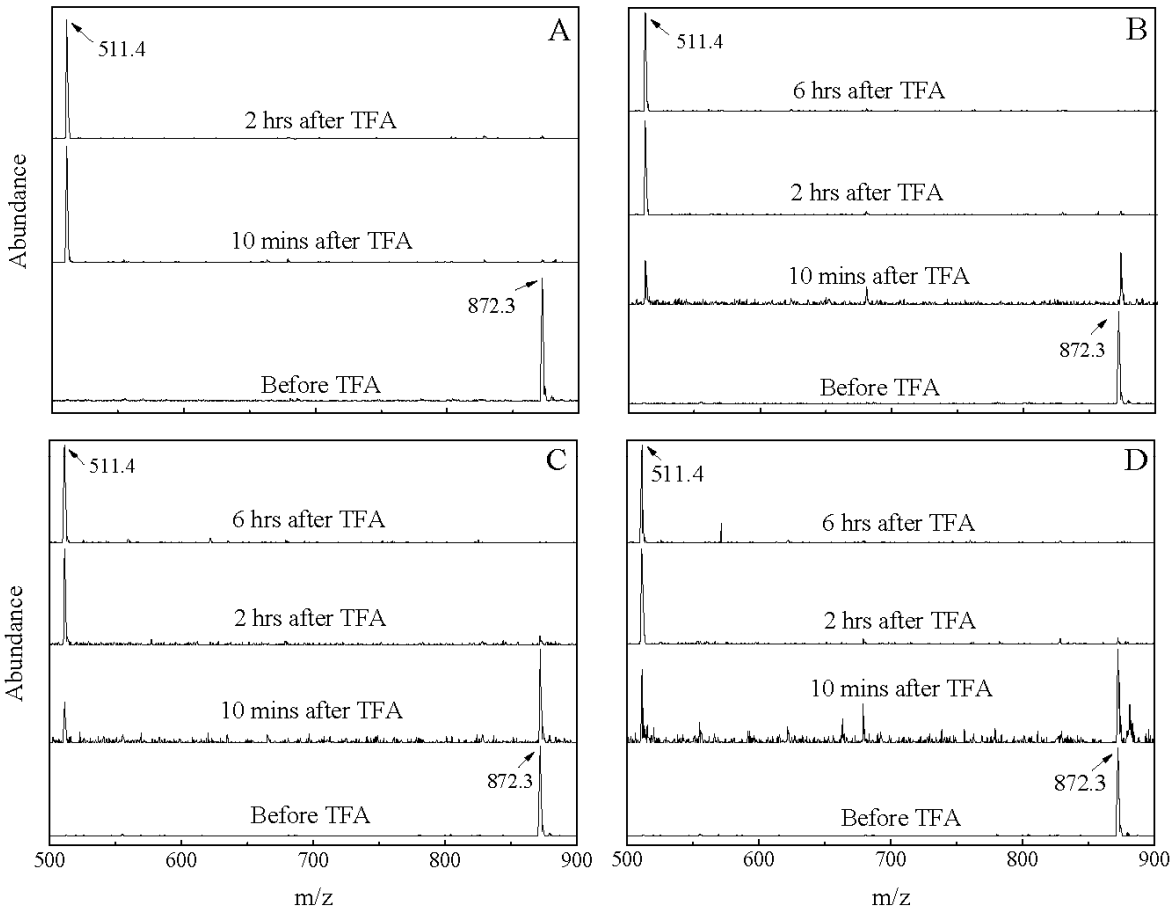


Fig S-1: ESI-MS spectra of ACST cleavage at TFA concentrations of (A) 25%, (B) 12.5%, (C) 6.25%, and (D) 3%. The (a) spectrum in each plot is intact ACST and (b-e) spectra are the ones taken after TFA mixed with ACST for 1 hour, 2 hours, 3 hours and 6 hours respectively.

3. pH dependence on SERS signal of STM and Rhodamine B: A 0.1 μM STM and Rhodamine B solutions were prepared with 0.1 M NaOH, water and 0.1 M HCl to investigate the effect of pH on SERS intensity of Rhodamine B and STM. As shown in Fig S-2, the SERS signal of Rhodamine B, that is monitored based on the 619 cm^{-1} peak, is over 100 times higher in the acidic solution than that in neutral and basic solutions, while the STM SERS signal at 619 cm^{-1} remains relatively constant.

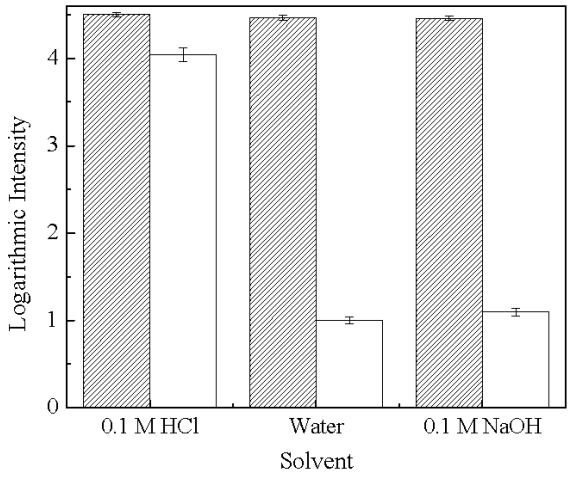


Fig S-2: Comparison of the SERS intensity of the 619 cm^{-1} peak of 0.1 μM STM (filled) and 0.1 μM Rhodamine B (not filled) in 0.1 M HCl, water and 0.1 M NaOH.

4. SERS detection sensitivity of STM: The STM SERS sensitivity was determined with a series of STM aqueous solutions with final concentrations varied from 1 μM to 100 pM.

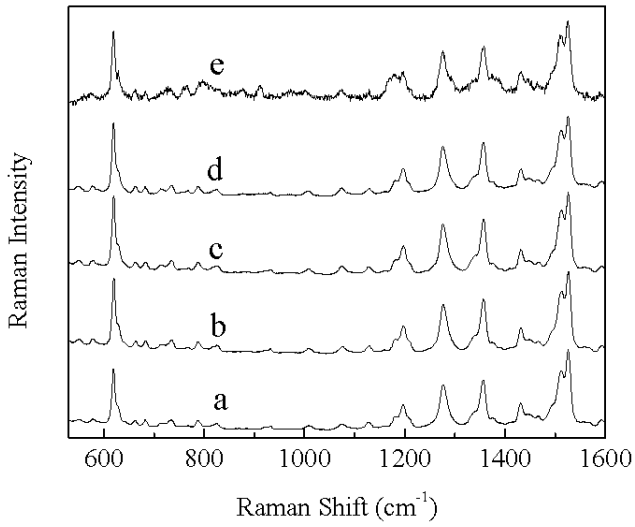


Fig S-3: SERS spectra of STM with concentration of (a) 1 μM (b) 100 nM (c) 10 nM (d) 1 nM (e) 100 pM. The spectra were scaled and offset for clarity.

5. Fluorescence signal of STM under 633 nm excitation laser: Fig S-4 shows the solution Raman spectrum of ~10 µM STM obtained with 633 nm excitation laser. Evidently, without AgNP, the normal Raman of STM are totally dominated by its fluorescence feature.[2]

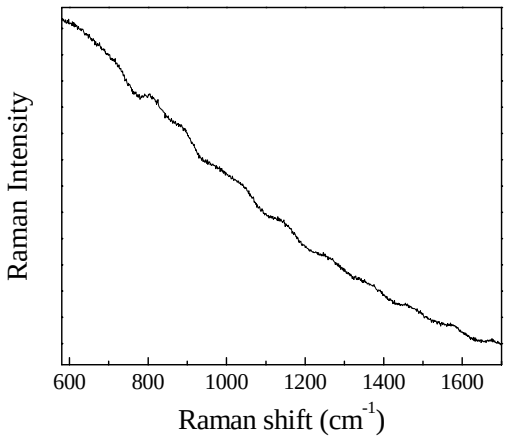


Fig S-4: Solution Raman spectrum of ~10 µM STM.

6. SERS spectrum of the filtrate of TFA treated ACST-BSA:

After mixing 5 nM ACST-BSA (concentration of BSA) with equal volume of 1.5% TFA solution, the mixture was left sit in the dark for 6 hours to allow the completion of the acid cleavage reaction. 400 µL ACST-BSA/TFA mixture was transferred to the ultrafiltration tube for ultrafiltration followed by 400 µL ethanol washing. The filtrate from the TFA cleavage solution and ethanol washing was combined for SERS spectral measurement.

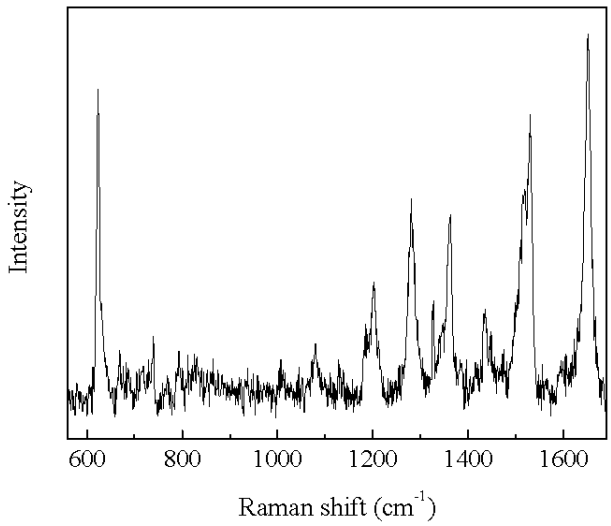


Fig S-5: The SERS spectrum of TFA treated ACST-BSA filtrate. The BSA and the ACST concentration in the acid cleavage solution was 5 nM, 1.5 nM respectively.

References:

(1) Mende, F.; Seitz, O. *Angew. Chem. Int. Ed.* **2007**, *46*, 4577-4580.
(2) Nguyen, T.; Francis, M. B. *Org. Lett.* **2003**, *5*, 3245-3248.