Electronic Supplemental Information for

A universal strategy for one-pot synthesis of SERS tags

Jin Li,[a] Huiqiao Liu,[a] Pengfei Rong,[b] Wen Zhou,[a,c] Xiaohu Gao,[c] and Dingbin Liu*[a,d]

[a] College of Chemistry, Research Center for Analytical Sciences, State Key Laboratory of Medicinal Chemical Biology, and Tianjin Key Laboratory of Molecular Recognition and Biosensing, Nankai University, Tianjin 300071 (China)

[b] Department of Radiology, the Third Xiangya Hospital, Central South University, Changsha, Hunan 410013 (China)

[c] Department of Bioengineering, University of Washington, Seattle, Washington 98195 (USA)

[d] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300071, China

E-mail: liudb@nankai.edu.cn

Table of contents

A. Synthetic chemistry

General information. Tetrahydrofuran (THF) was distilled under nitrogen over sodium benzophenone ketyl. Diisopropylethylamine (i-Pr_2NEt) and 1,2-dichloroethane (CH_2Cl_2) were distilled over CaH_2 under nitrogen. I-Pr_2NEt was degassed with argon stream for 1 h before use. All commercial chemicals were used without further purification. ^1H NMR spectra were recorded on a Varian 400 MHz spectrometer. Electron spray ionization mass spectra (ESI-MS) were obtained on a JMS-LC mate mass spectrometer. Electron ionisation mass spectra (EI-MS) were recorded on a Thermoquest Trace or a Thermo-Finnigan DSQ.

Thioacetic acid S-(4-iodo-phenyl) ester (A1). Compounds A1, 18 and 19 were synthesized according to the literature.[1] All of the reagents were thoroughly dried and flushed with argon before use. Zn powder (2.275 g, 35.0 mmol) and dichlorodimethylsilane (4.245 mL, 35.0 mmol) in dry 1,2-dichloroethane (80 mL) were added to an oven-dried flask equipped with a magnetic stir bar. 4-Iodo-benzenesulfonyl chloride (3.025 g, 10.0 mmol) and N,N-Dimethyl-acetamide (2.80 mL, 30.0 mmol) in dry 1,2-dichloroethane (80 mL) was added dropwise. The mixture was stirred at 75 ºC for 4.5 h and potassium carbonate (0.76 g, 5.5 mmol) was added. The mixture was cooled to room temperature and acetyl chloride (2.84 mL, 40.0 mmol) was added. The mixture was stirred overnight and filtered. The resulting solution was diluted with 40 mL dichloromethane, washed with brine and dried over magnesium sulfate. The resulting solid was purified by column chromatography on silica gel (petroleum ester as eluent) to provide 2.28 g of white solid product in 82% yield. ^1H NMR ($CDCl_3$, 400

MHz): δ 7.73 (d, 2 H, J = 12.0 Hz), 7.14 (d, 2 H, J = 8.0 Hz), 2.43 (s, 3 H). ESI-MS calcd for C_8H_7OSI: 278.1100, found: 279.1612 $[M+H]^+$.

Thioacetic acid S-(4-trimethylsilanylethynyl-phenyl) ester (18). Trimethylsilylacetylene (1.4 mL, 10.0 mmol), A1 (2.085 g, 7.5 mmol), $Pd(PPh_3)Cl_2$ (0.263 g, 0.375 mmol), CuI (0.072 g, 0.375 mmol), degassed i-Pr_2NEt (2.65 mL) and THF (12 mL) were added to a flame-dried vessel and allowed to stir at 50 ºC for 1 day. The reaction mixture was then poured into water, and the aqueous layer was extracted 3 times with dichloromethane. After drying the combined organic layers over magnesium sulfate, the solvent was removed in vacuo. The resulting crude product was purified by column chromatography on silica gel (petroleum ester and petroleum ester/dichloromethane 4:1 as eluent) to provide 1.75 g of light yellow solid product in 94% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.47 (d, 2 H, J = 8.0 Hz), 7.33 (d, 2 H, J = 8.0 Hz), 2.41 (s, 3 H), 0.25 (s, 9 H). ESI-MS calcd for $C_{13}H_{16}OSSi$: 248.4200, found: 249.0764 $[M+H]^+$.

Thioacetic acid S-(4-ethynyl-phenyl) ester (19). The 18 (0.935 g, 3.765 mmol) was dissolved in THF in a plastic vessel. A mixed solution of acetic anhydride/acetic acid (2.4 mL: 2.4 mL) and 1.0 M tetrabutylammonium fluoride (1.295 g, 4.105 mmol) in THF (5 mL) was added dropwise at -15 ºC and the solution was stirred for 15 min. After quenched with silica gel, the mixture was then poured into water and extracted with diethyl ether for 4 times. The extract was washed with brine and dried over magnesium sulfate. After filtration, the solvent was evaporated in vacuo to afford a crude product, which was further purified by a flash chromatography on silica gel (petroleum

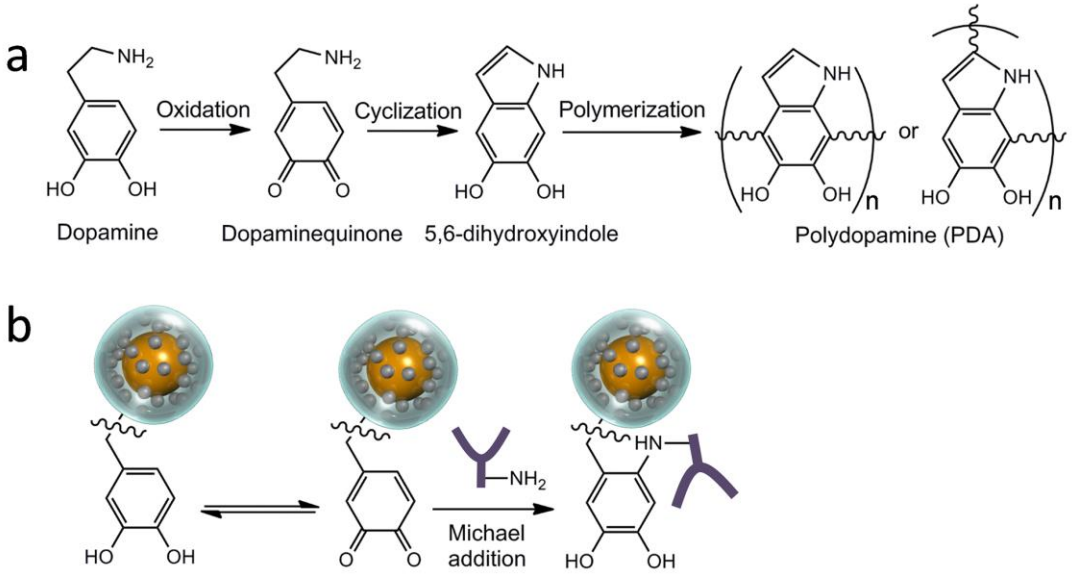
ester/dichloromethane from 5:1 to1:1 as eluent) to provide 0.63 g of light yellow liquid product in 92% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52(d, 2 H, J = 8.0 Hz), 7.37(d, 2 H, J = 8.0 Hz), 3.16 (s, 1 H), 2.43 (s, 3 H). ESI-MS calcd for $C_{10}H_8OS$: 176.2300, found: 177.0372 $[M+H]^+$.

S-(4-(phenylethynyl)phenyl) ethanethioate (20). Compound 20 was synthesized according to the literature.[2] Phenylacetylene (0.612 g, 6 mM), A1 (1.39 g, 5 mM), $Pd(PPh_3)_2Cl_2$ (0.335 g, 0.478 mmol) and CuI (0.091g, 0.478 mmol) were added to a Schlenk flask containing THF/Hunig's base solvent mixture (12 mL: 12 mL). The reaction mixture was deoxygenated by performing three freeze pump-thaw cycles, after which the flask was backfilled with nitrogen and sealed. The mixture was then stirred for 3 days at 55 °C until thin-layer chromatography (TLC) showed the reaction to be completed. The volatiles were then removed in vacuo and the remaining solids were further purified by column chromatography on silica gel to provide 0.91 g of light yellow soild product in 72% yield. ^{1}H NMR: ($CDCl_3$, 400 MHz) δ 7.57-7.47 (m, 4 H), 7.41-7.33 (m, 5 H), 2.44 (s, 3 H). ESI-MS calcd for $C_{16}H_{12}OS$: 252.3300, found: 253.0682 $[M+H]^+$.

1-(Phenylethynyl)-4-(mercapto)benzene (21). Compound 21 was synthesized according to the literature.[3] 20 (200 mg) was dissolved in chloroform-methanol mixture (2.5 mL: 7.5 mL) and 2.5 M HCl solution in methanol (10 mL) was added. The mixture was refluxed in nitrogen atmosphere for 5 h. After that, the reaction mixture was concentrated in vacuo and the crude product was purified by column chromatography on silica gel. Yield: 133 mg (80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.51-7.46 (m, 2 H),

7.39 (d, 2 H, J = 8.0 Hz), 7.34-7.30 (m, 3 H), 7.24 (d, 2 H, J = 12.0 Hz), 3.53 (s, 1 H). EI-MS calcd for $C_{14}H_{10}S$: 210.2900, found: 210.1000 $[M]^+$.

B. Supplementary Data



Scheme S1. Reaction mechanism of dopamine polymerization and conjugation of antibodies. (a) The dopamine catechol is first oxidized to quinone under a weak alkaline condition, then cyclizes, and ultimately polymerizes to form PDA homopolymers. (b) The exposed dopaminequinone on the PDA shell reacts with the amine group on antibody *via* Michael addition reaction.

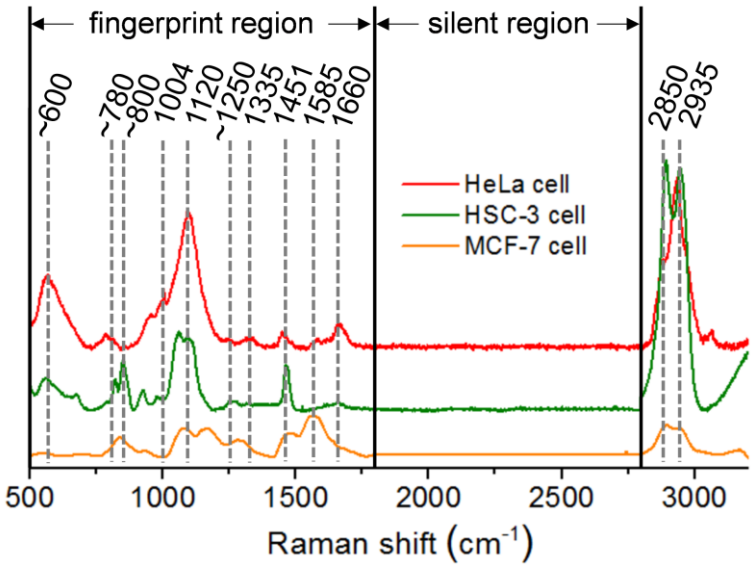


Figure S1. Raman spectra of three single human cancer cells. Multiple Raman bands are shown in the fingerprint region (<1800 cm^{-1}) while no signals can be observed in the cellular Raman-silent region (1800-2800 cm^{-1}). The spectra were obtained with an integration time of 2 s and 30 mW of 532 nm laser power. ~ 600 cm^{-1}: Cytochrome; ~780 cm^{-1}: Lipid and protein (lipid symmetric –O-C-C-N- stretch Tyr) ; ~800 cm^{-1}: Phosphoric acid; 1004 cm^{-1}: Phenylalanine; 1120 cm^{-1}: Lipid and protein (C-N backbone and lipid trans-conformations) ; ~1250 cm^{-1}: Amide-III; 1335 cm^{-1}: Adenine; 1451 cm^{-1}: Protein and lipid (-CH_2 bending and methylene deformation); 1585 cm^{-1}: Cytochrome; 1660 cm^{-1}: C=C, Amide-I; 2850 cm^{-1}: CH_2; and 2935 cm^{-1}: CH_3.

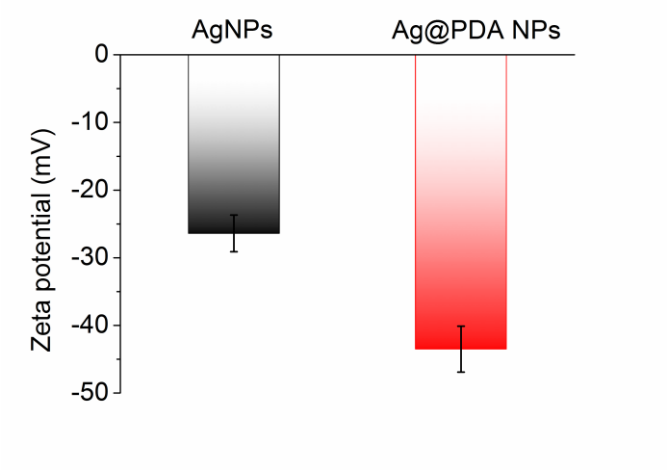


Figure S2. Zeta potential measurements of AgNPs and Ag@PDA NPs.

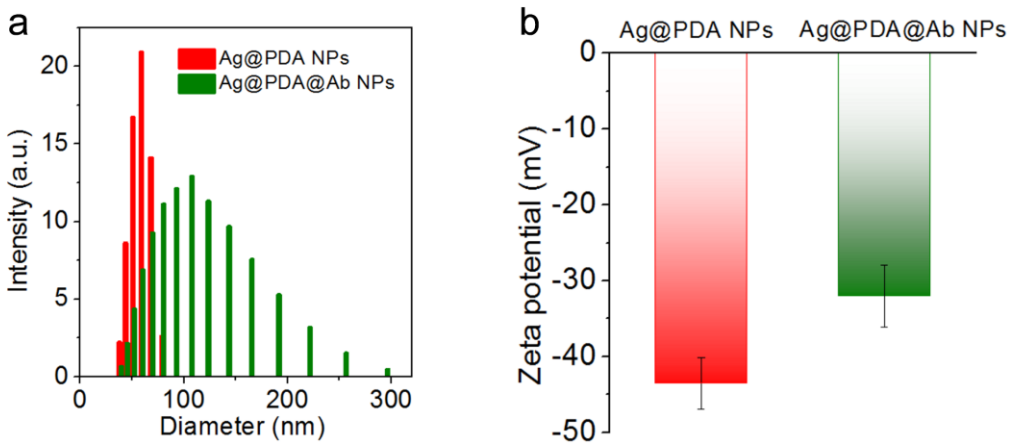


Figure S3. (a) DLS data and (b) zeta potentials of Ag@PDA NPs and those functionalized with HER2 antibodies (Ag@PDA@Ab NPs). With the antibody conjugation, the hydrodynamic diameter of the NPs increases from roughly 60 to 106 nm while the zeta potential drops from roughly -44 to -32 mV. The antibody conjugation procedure is as following. In brief, 2.2 μL of HER2 antibodies (1 mg/mL) were added to the Ag@PDA NPs (0.3 nM) suspended in 200 μL of deionized water and incubated at 4 °C for 12 h. Then, 5 μL of 10 mg/mL bovine serum albumin was added and incubated at 37 °C for 1 h to block unreacted catechol groups.

Table S1. Physiochemical properties of AgNPs, Ag@PDA NPs, and Ag@PDA@Ab NPs.

Samples	Core size [nm][a]	Shell thickness [nm][a]	Physical size [nm][a]	Hydrodynamic size [nm] (PDI)[b]	Zeta potential [mV][b]
AgNPs	N.A.[c]	N.A.[c]	30.1±3.2	41.9±0.9（0.16）	-26.4±2.7
Ag@PDA NPs	30.5±3.1	5.0±0.6	41.8±1.9	60.1±1.2（0.19）	-43.5±3.4
Ag@PDA@Ab NPs	29.8±2.7	5.1±0.9	44.2±2.8	106.2±3.5（0.24）	-32.0±4.1

[a]The values are calculated from TEM images of over 80 nanoparticles. [b]The values are obtained from DLS measurement of nanoparticles in Milli-Q water. Results are given in mean ± SD (PDI = polydispersity index) of six measurements. [c]Measurement is not applicable to the sample.

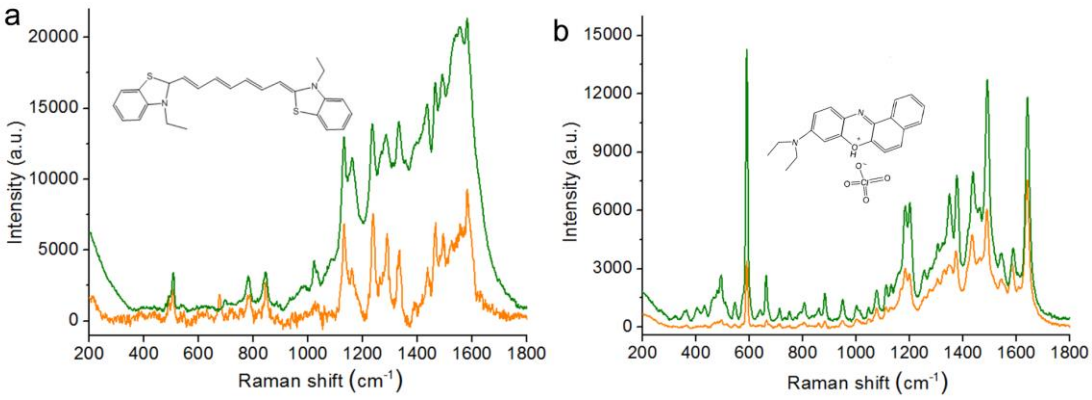


Figure S4. (a) Raman spectra of DTTC- and (b) Nile blue (NB)-coded SERS tags prepared by the one-pot incorporation (green) and conventional covalent conjugation (orange) approaches. All spectra were obtained with an integration time of 2 s and 30 mW of 532 nm laser power.

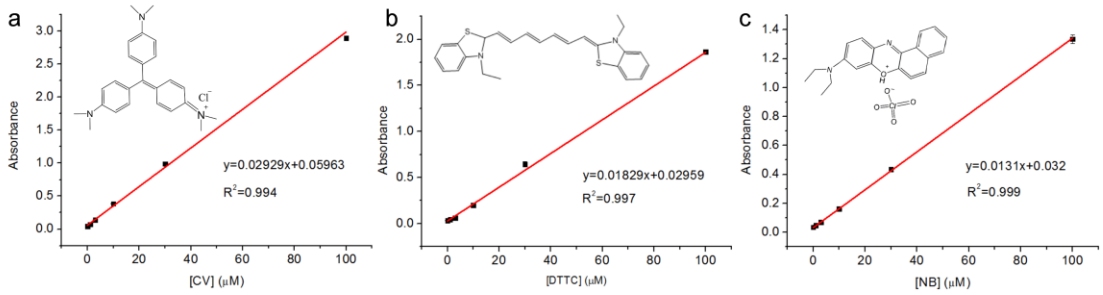


Figure S5. Calibration curves created by plotting the absorbance at 588 nm, 648 nm, and 594 nm with various concentrations of (a) CV, (b) DTTC, and (c) NB respectively.

To evaluate the average amounts of dye molecules in the two tags prepared *via* the conventional covalent conjugation method and one-pot incorporation approaches, we prepared two RR-coded tags solutions under the same conditions at room temperature. The dye amounts in the two solutions are the same. Then, the two solutions were centrifuged at 12000 rpm for 20 min to remove the pure tags, respectively. Next, the concentrations of free dye molecules in the two supernatants were calculated based on the calibration curves collected from UV-vis absorbance. Finally, we calculated average amounts of dye molecules in the two tags *via* sigma-minus method.

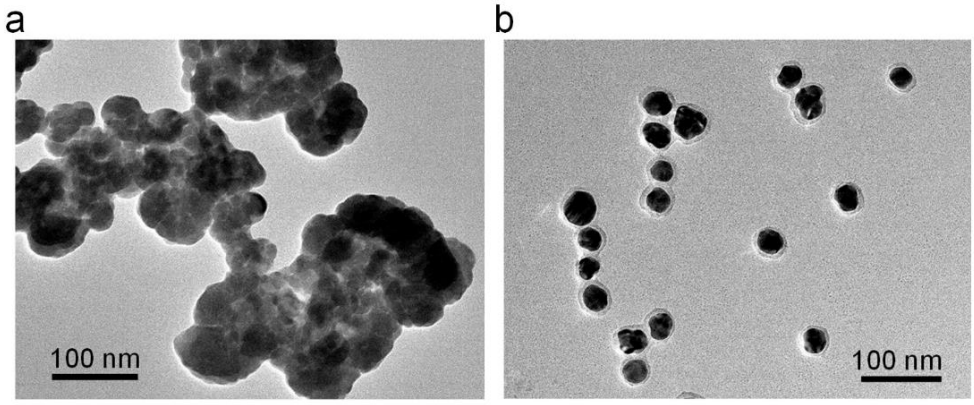


Figure S6. TEM images of (a) aggregated AgNPs caused by hydrophobic PMB molecules in conventional covalent conjugation strategy and (b) PMB-coded monodispersed SERS tags prepared by *in situ* incorporation strategy.

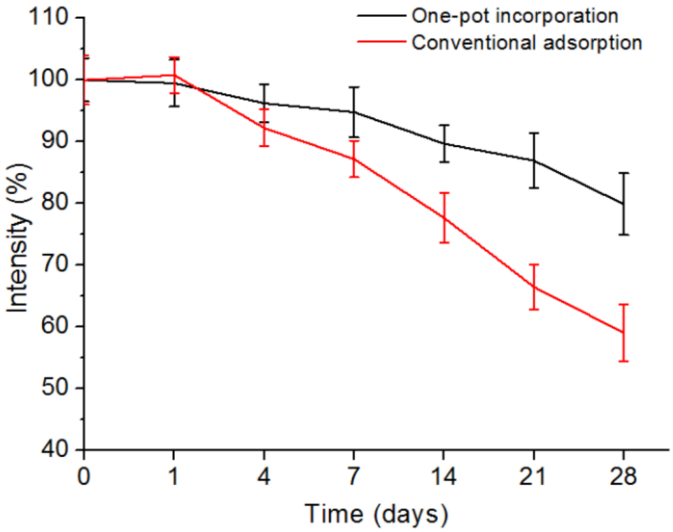


Figure S7. Comparison of long-term colloidal stability between 4-MB-coded SERS tags (0.2 nM) prepared by the conventional covalent conjugation (red) and one-pot incorporation (black) approaches in PBS. The time-dependent signal decrease is likely due to two facts: colloidal precipitation and desorption of reporters from AgNP surfaces.

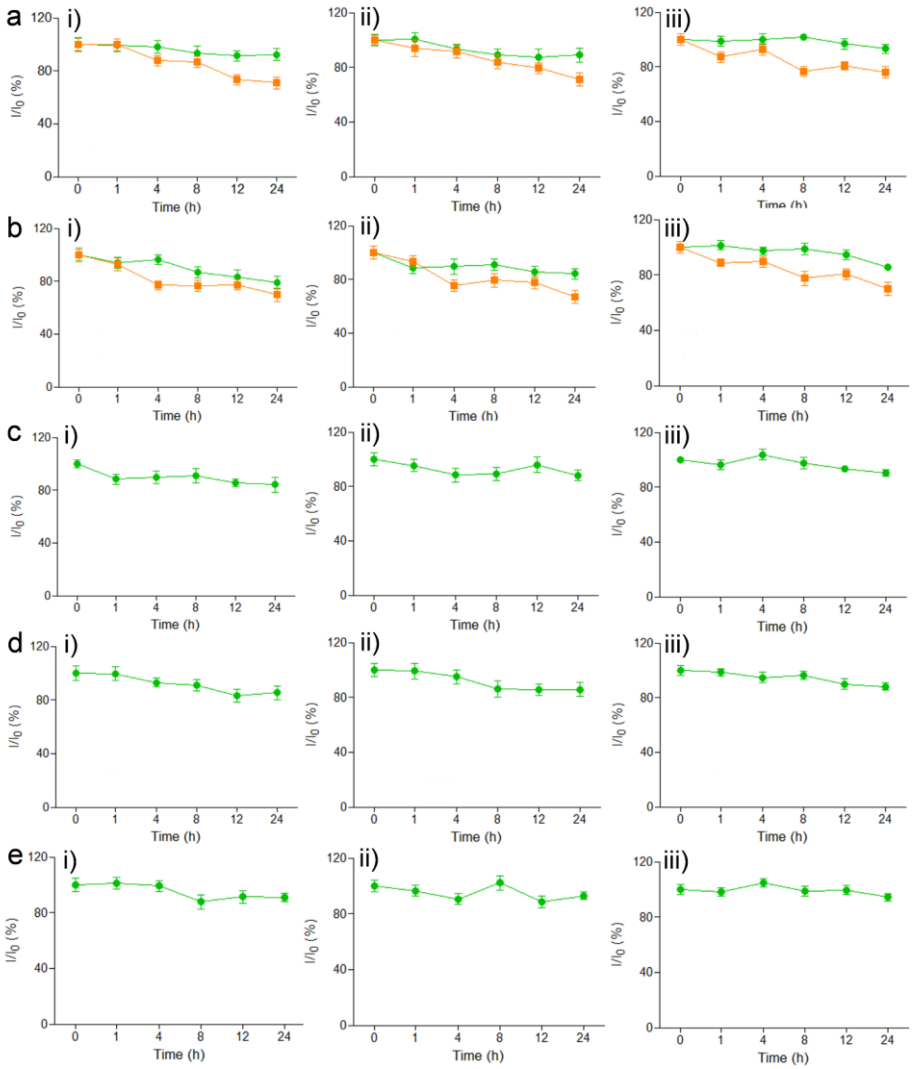


Figure S8. Signal stability of SERS tags prepared by the conventional covalent conjugation (orange) and one-pot incorporation (green) methods in various biologically related fluids: (i) PBS, (ii) cell culture medium (RPMI medium 1640 plus 10% FBS solutions), and (iii) cell lysis buffer. The SERS tags are prepared with the reporters: (a) CV, (b) 4-MB, (c) TPS, (d) 2-propynylamine, and (e) PMB. Note that TPS, 2-propynylamine, and PMB are unable to be employed as reporters in the conventional SERS tags, so their Raman intensities are inaccessible. I_0 represents the Raman intensity of the SERS tags immediately after yielding the tags (time=0 h) while I represents the Raman intensity of the SERS tags at desired time.

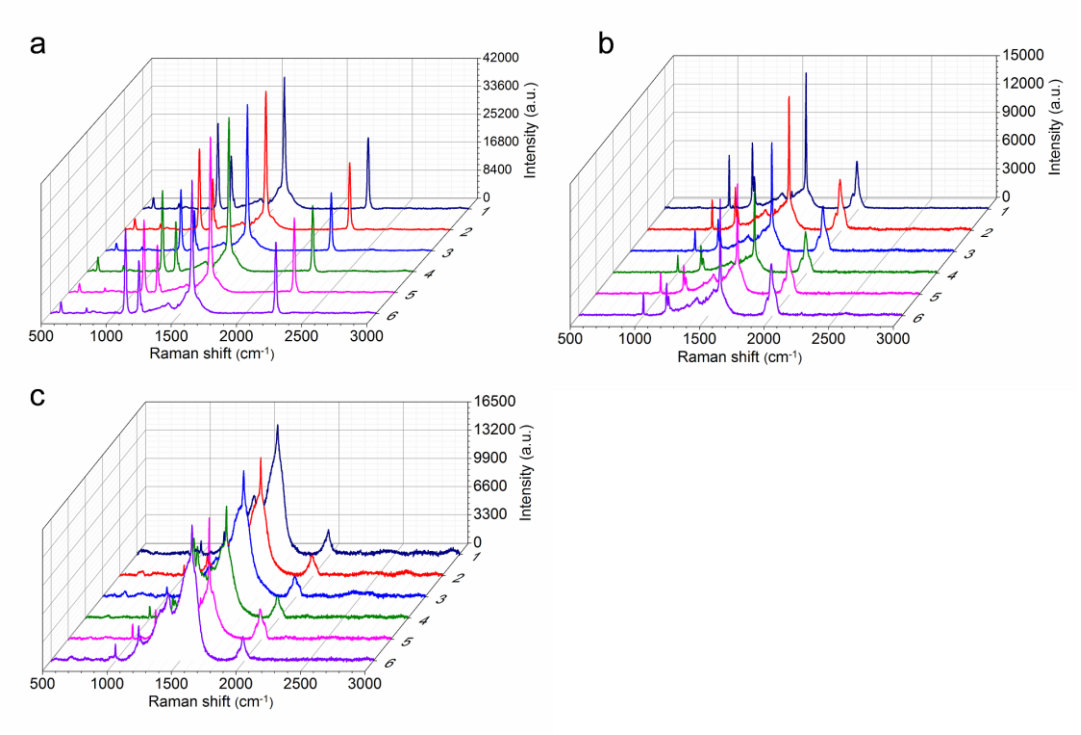
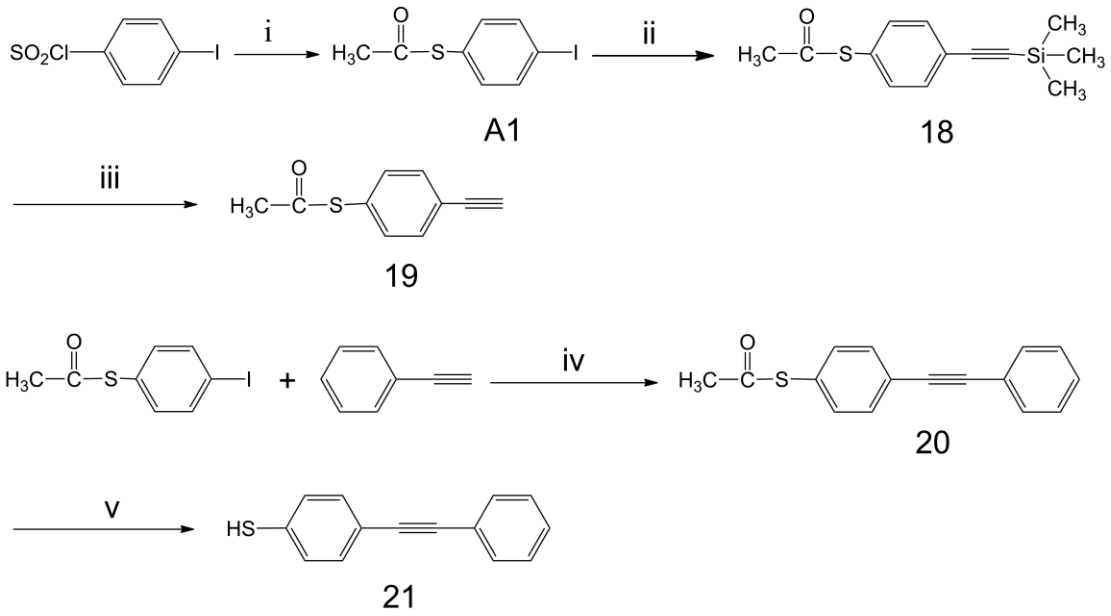


Figure S9. Signal reproducibility studies of (a) 4-MB-coded, (b) EB-coded, and (c) TPS-coded SERS tags prepared by the one-pot incorporation strategy. The experiments were carried out in six parallel samples. The concentration of 4-MB, EB, and TPS was 100 μM, 1 mM, and 1 mM respectively.



Scheme S2. Synthesis of compound A1, 18, 19, 20 and 21. Reagents and conditions: (i) Zn, *i*-Pr_2NEt, AcCl, K_2CO_3, overnight, rt; (ii) TMSA, $Pd[(PPh_3)_4]$, Et_3N, CuI, 3 h, rt; (iii) TBAF, Ac_2O, AcOH, rt; (iv) $Pd(PPh_3)_2Cl_2$, CuI, 2-3 days, 55 °C; (v) HCl-MeOH, $CHCl_3$, 5 h, rt.

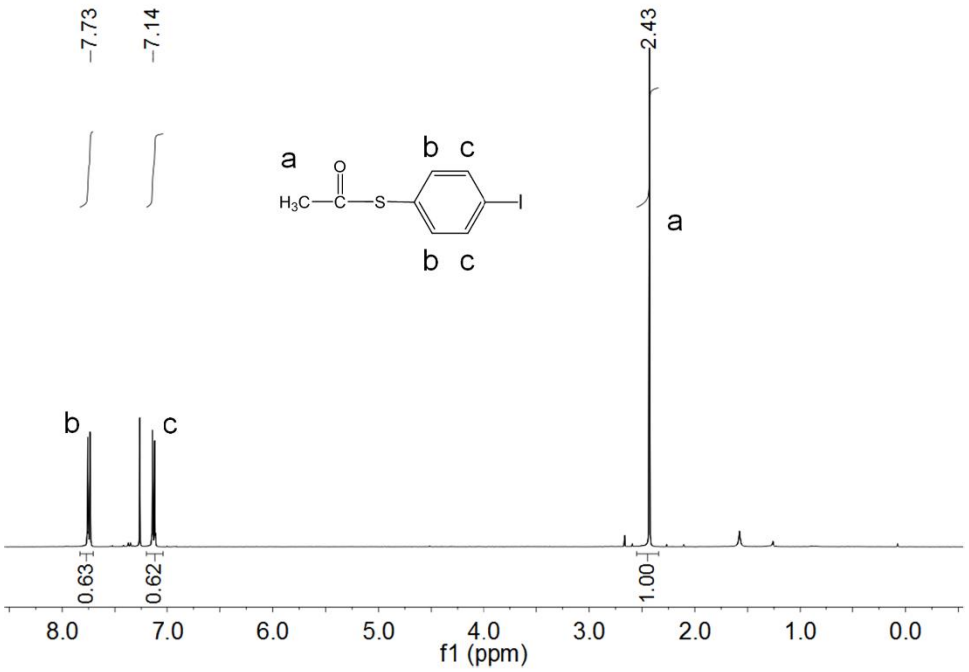


Figure S10. ^{1}H NMR (400 MHz in $CDCl_3$) of compound A1.

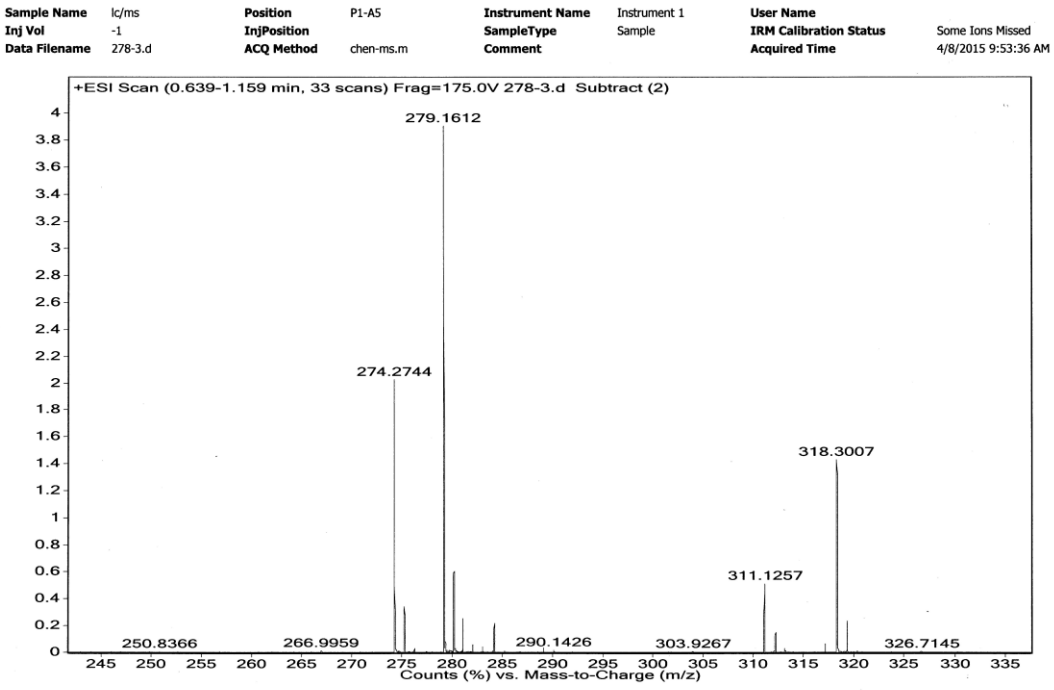


Figure S11. ESI-MS spectra of compound A1.

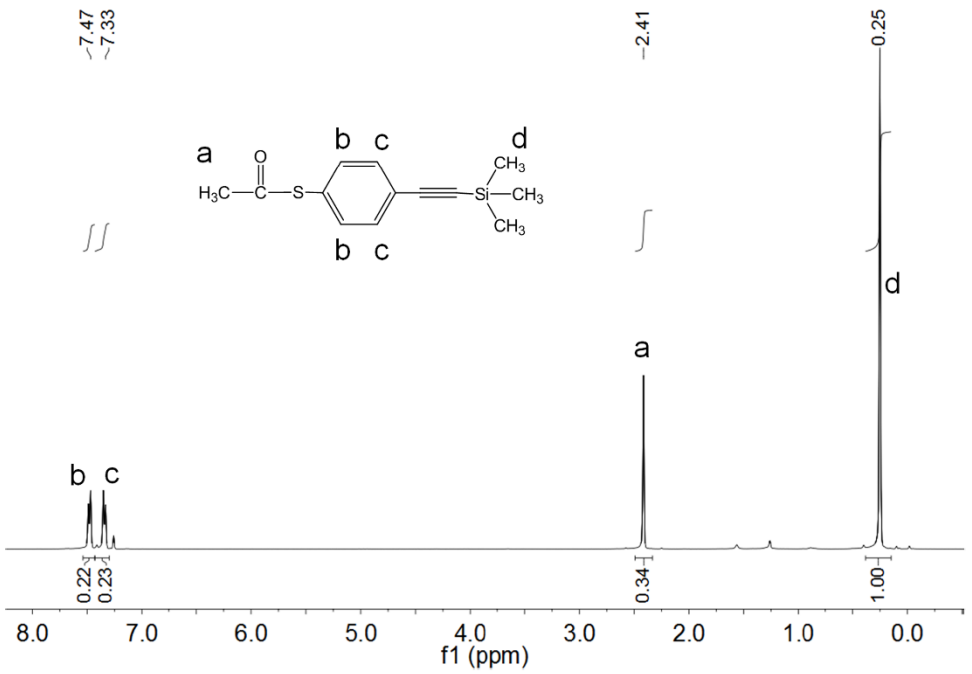


Figure S12. ^{1}H NMR (400 MHz in $CDCl_3$) of compound 18.

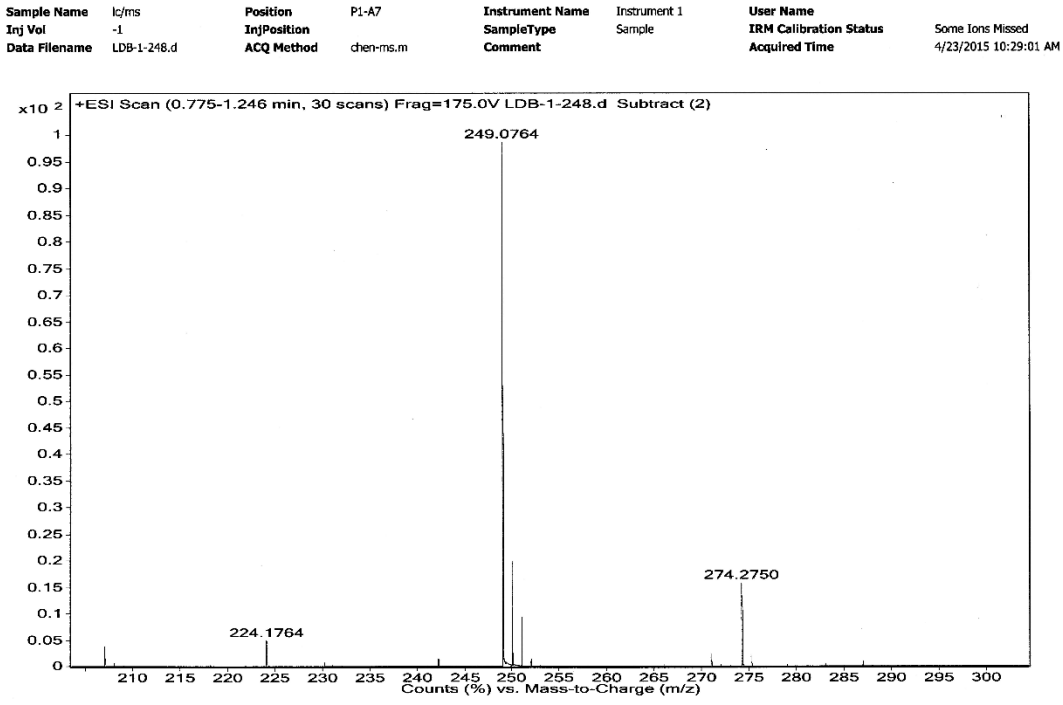


Figure S13. ESI-MS spectra of compound 18.

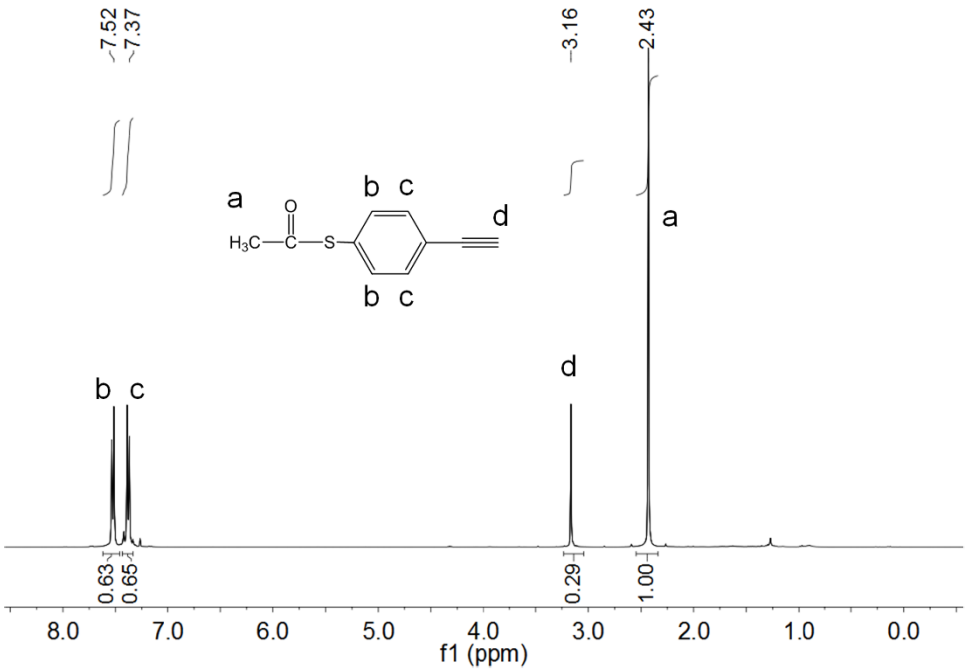


Figure S14. ^{1}H NMR (400 MHz in $CDCl_3$) of compound 19.

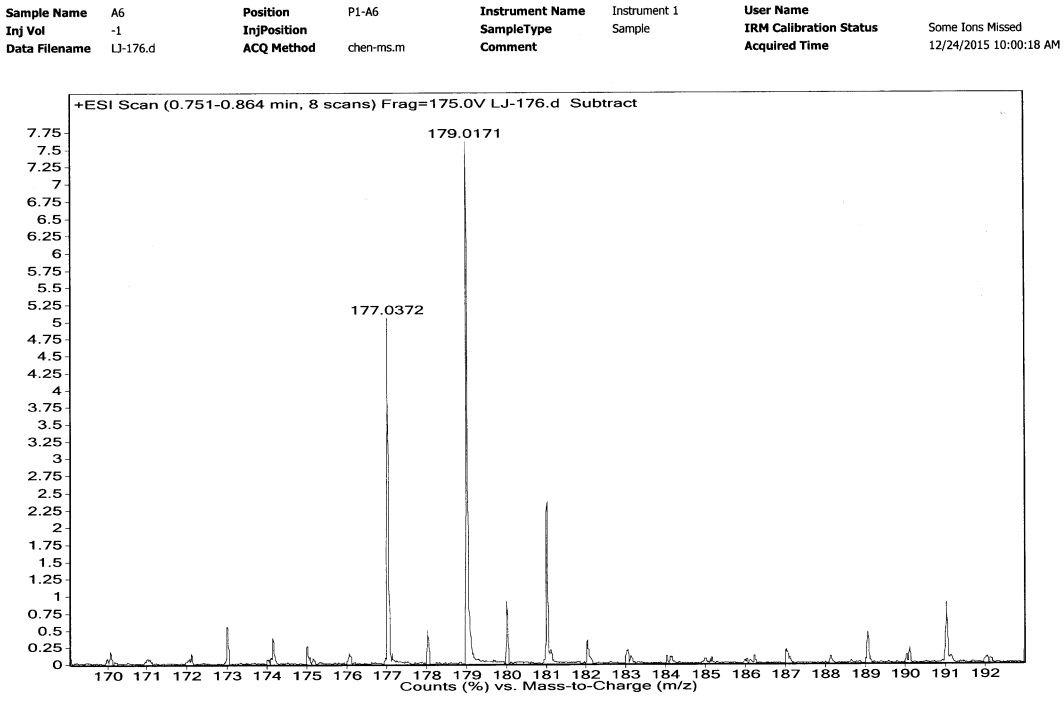


Figure S15. ESI-MS spectra of compound 19.

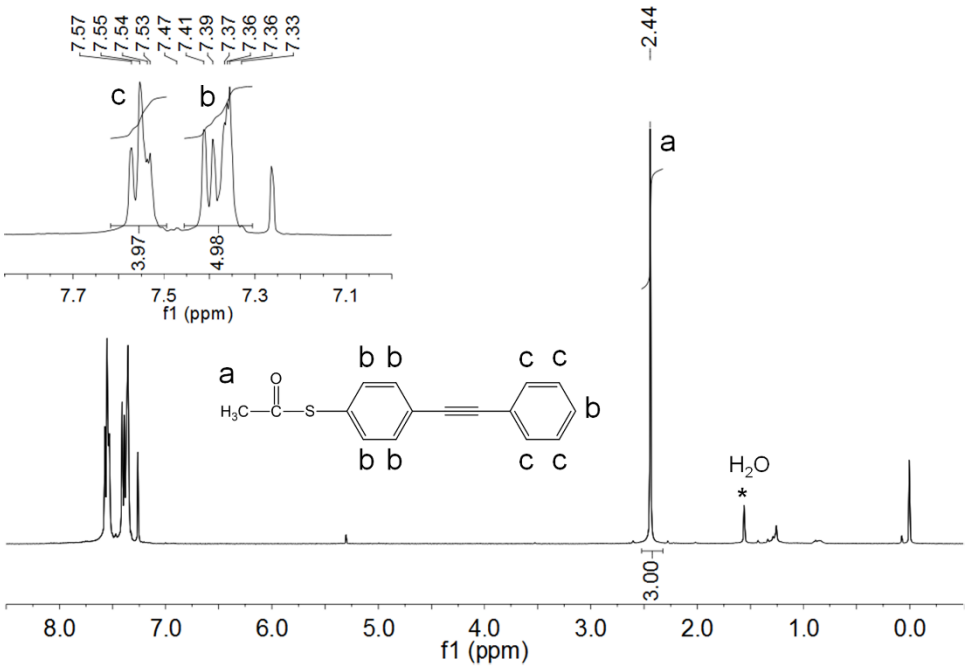


Figure S16. ^{1}H NMR (400 MHz in $CDCl_3$) of compound 20.

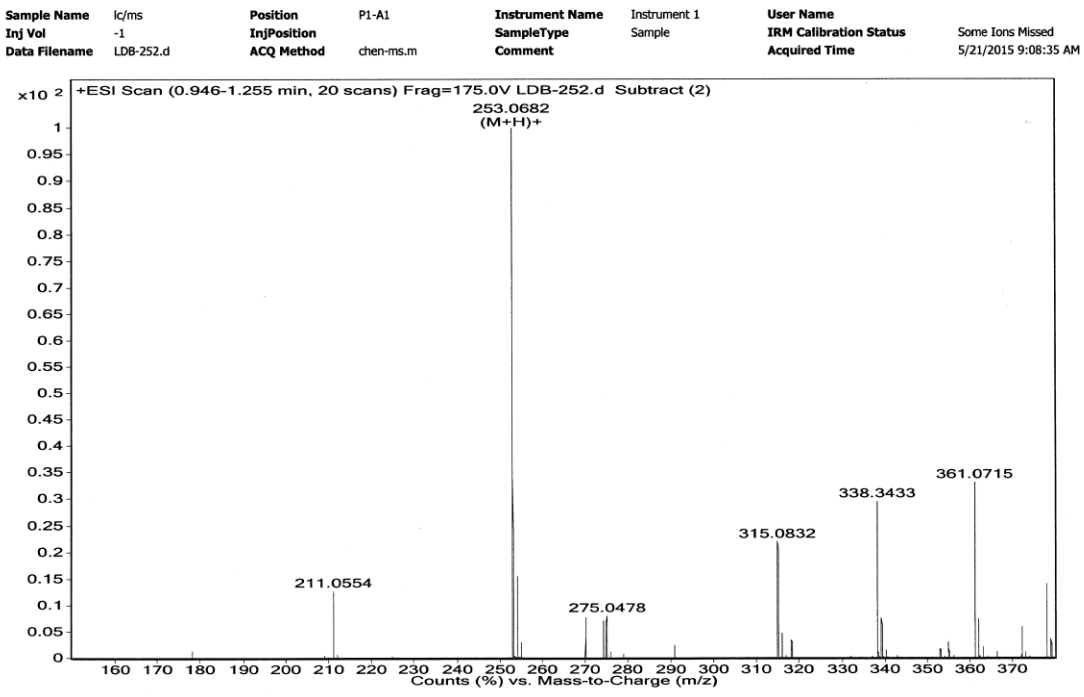


Figure S17. ESI-MS spectra of compound 20.

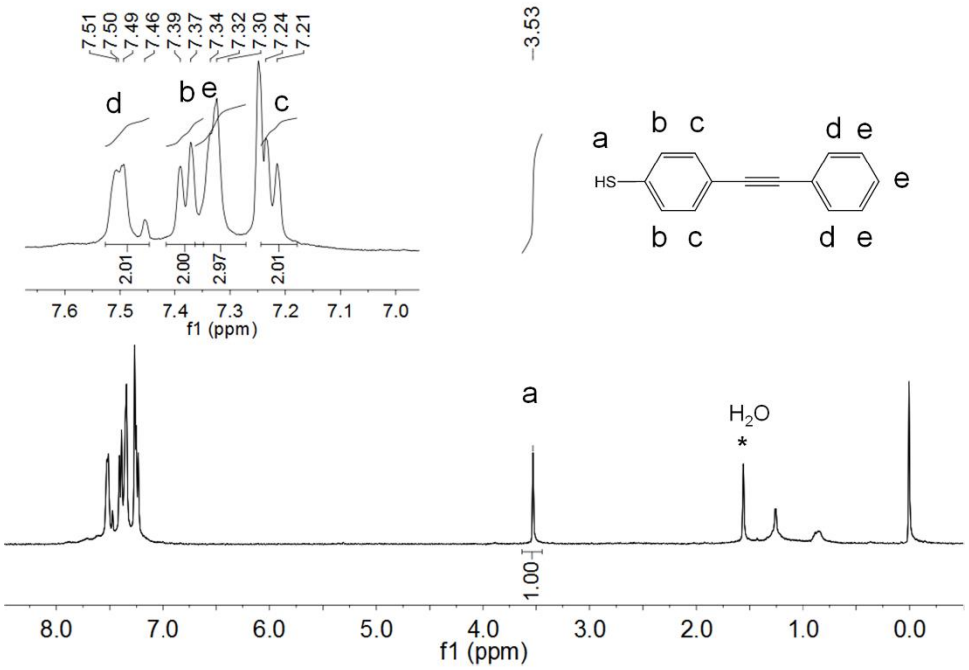


Figure S18. ^{1}H NMR (400 MHz in $CDCl_3$) of compound 21.

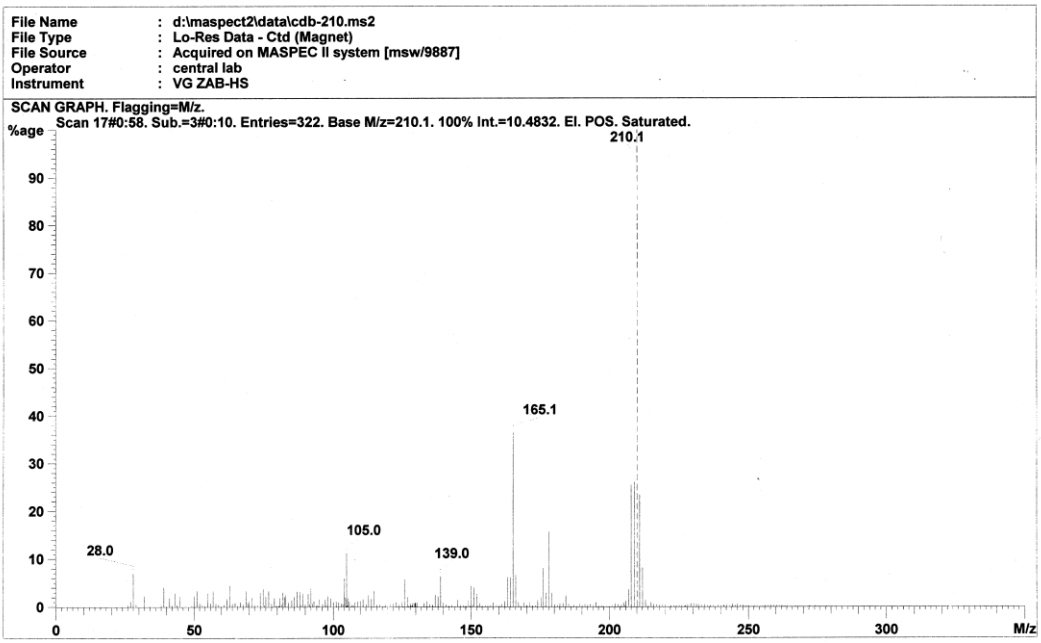
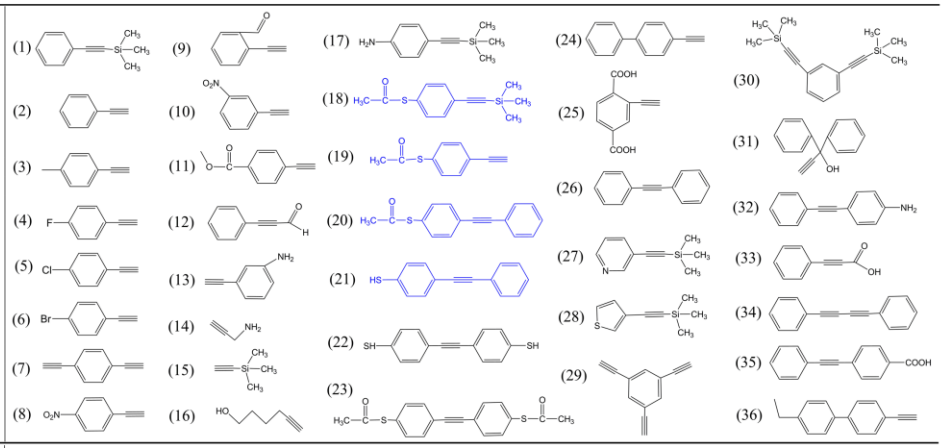
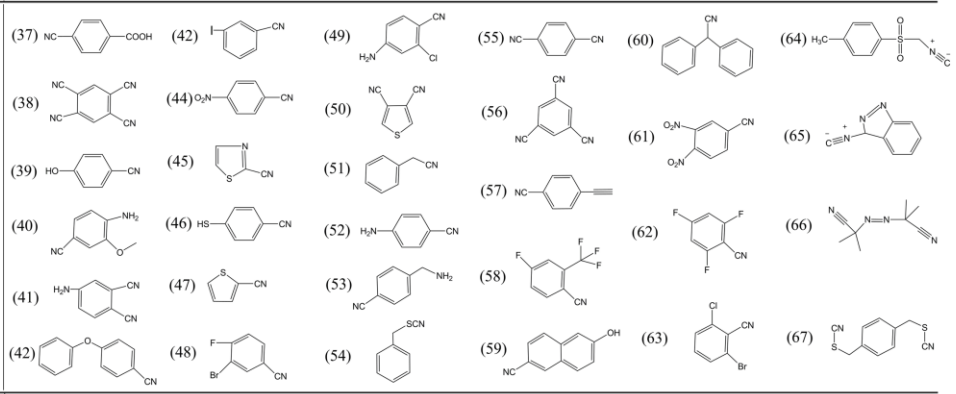
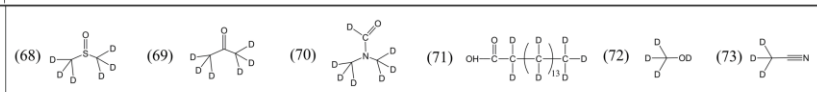
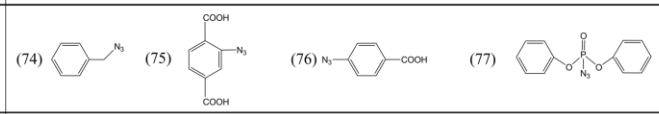
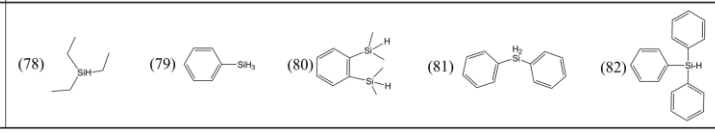


Figure S19. EI-MS spectra of compound 21.

Table S2. Molecular structures of 82 molecules and their corresponding codes.

C≡C	
C≡N	
C–D	
N_3	
Si–H	

* Among these molecules, 4 molecules were synthesized and the other 78 molecules are commercially available. The synthesized molecules are marked in blue.

Table S3. Raman data of the SERS tag library.

Plate code	Raman shift (cm^{-1})	Intensity (a.u.)	Plate code	Raman shift (cm^{-1})	Intensity (a.u.)
1	1985	2792	40	2223	4642
2	1985	4950	41	2232	7999
3	1974	18126	42	2223	6843
4	1982	5208	43	2229	740
5	1983	1682	44	2223	643
6	1988.5	2114	45	1975	1882
7	2209	24997	46	2231	19737
8	1987	1900	47	1972	849
9	1988	690	48	2228	2385
10	1977	4422	49	2222	10116
11	2000	1885	50	2223.7	9007
12	2203	4604	51	2222	1508
13	1974	1799	52	2206	383
14	1987	7461	53	2231	5378
15	1971	3175	54	2118.9	8834
16	1987	1785	55	1971	1901
17	1963	13086	56	1989	1792
18	2156	6082	57	2232	1329
19	2109	6957	58	2214	4170
20	2214	4179	59	2228	2284
21*[a]	2216	96206	60	1979	12770
22	2211	5712	61	2220	1749
23	2208	804	62	1973	2075
24	1979	45710	63	2225	3561
25	1982	5243	64	2205	6247
26	2219	1369	65	2166	8035
27	1982.9	3138	66	1966	1022
28	1981	23938	67	2114	1062
29	1985	5389	68*[b]	2121	3573
30	1977	2316	69*[b]	2116	614
31	1988	3096	70*[b]	2077	1466
32	2210	6015	71	2096	1309
33	2224	1335	72*[b]	2226	1061
34	2217	5529	73*[b]	1966	4421
35	2216	8501	74	2218	2499
36	1979	7370	75	2220	5310
37	2218	1505	76	1979	2468
38	2240	5830	77	1977	1452
39	2222	2039	78	2045	360

79	2214	2999	81	2211	2280
80	2222	1656	82	2217	1564

*a The reporter concentration for preparing 21-coded tags was 200 μM.

*b For 68, 69, 70, 72, and 73, the RRs were directly mixed with the mixture of AgNPs (0.2 nM) dopamine (0.1 mg/mL) solutions respectively and the reporter concentration for preparing each kind of tags was kept in 10 mM; The concentration for other reporters were identified to be 1 mM.

Table S4. The clinical information of the patients.

Patient	Age	Symptom	Over-expressed biomarkers	Metastasis degree
Patient 1	43	Infiltrating ductal carcinoma of the right breast	HER2(3+), EGFR+	Ⅱ-Ⅲ
Patient 2	65	Infiltrating ductal carcinoma of the right breast	ER+, PR(2+)	Ⅱ-Ⅲ
Patient 3	56	Infiltrating ductal carcinoma of the left breast	HER2(3+), ER+	Ⅲ
Patient 4	53	Benign fibroadenoma of breast	——	——

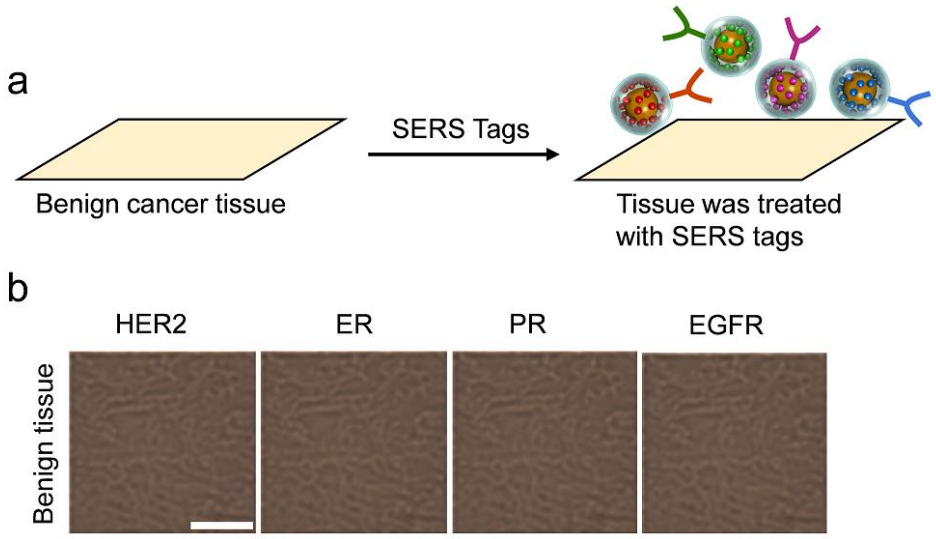


Figure S20. Control experiments by treating the benign cancer tissue with the four SERS tags coded with No.2, 68, 35, and 38 bf-RRs. (a) Schematic of the probing system, where the cancer biomarkers are down-expressed in the benign tissue, and no SERS signals from the tags were obtained (b). Scale bar = 50 μm. The SERS images were obtained by 532 laser power (30 mW), with an integration time of 2 s and a step size of 2 μm in StreamLine high-speed acquisition mode.

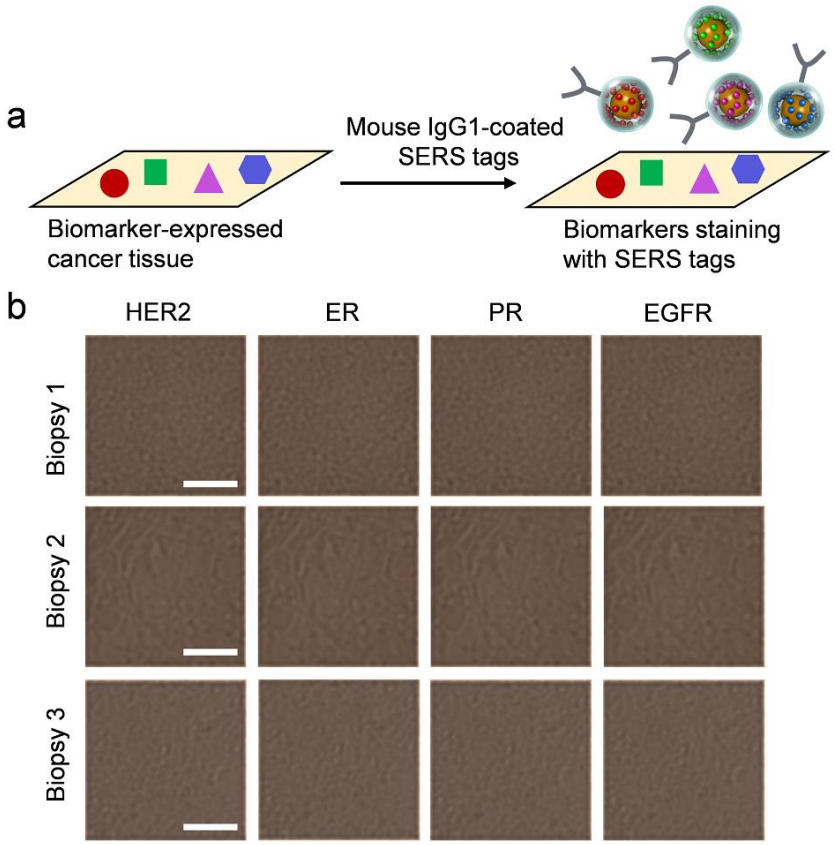


Figure S21. Control experiments by probing the biomarkers in breast cancer biopsies from the same three patients using mouse IgG1-coated SERS tags coded with No.2, 68, 35, and 38 bf-RRs. (a) Schematic of the probing system, where the mouse IgG1 is unable to recognize the cancer biomarkers, and no SERS signals from the tags were obtained (b). Scale bar = 50 μm. The SERS images were obtained by 532 laser power (30 mW), with an integration time of 2 s and a step size of 2 μm in StreamLine high-speed acquisition mode.

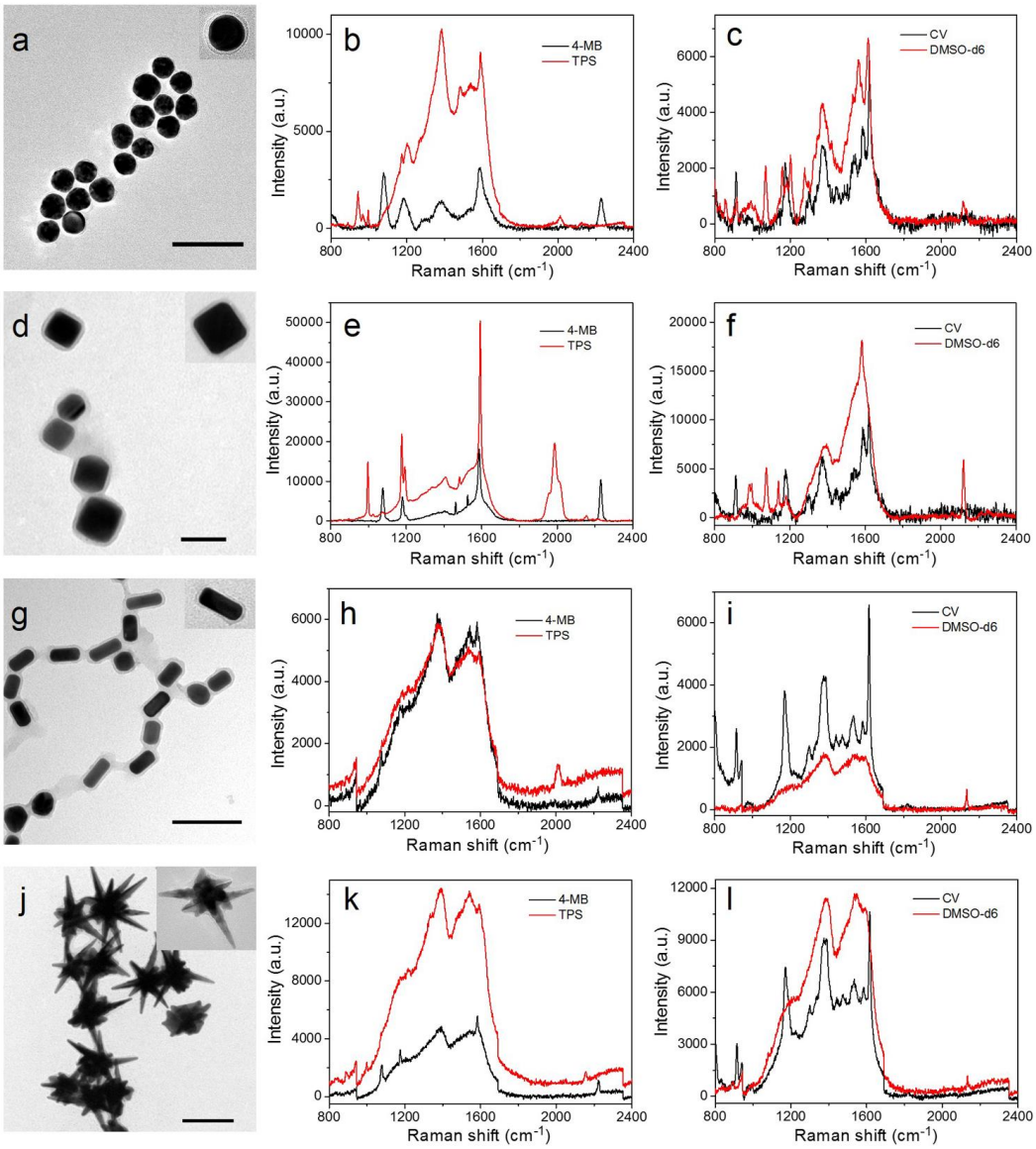


Figure S22. The substrate versatility of the one-pot incorporation strategy for SERS tags. TEM images of PDA-coated a) AuNPs (Au@PDA NPs), d) Ag nanocubes (Ag@PDA NCs), g) Au nanorods (Au@PDA NRs), and j) Au nanostars (Au@PDA NSs). The NP concentration was fixed to be 0.2 nM; while dopamine concentration was fixed to be 0.05 mg/mL. The scale bars in a), d), g), and j) are 100, 50, 100, and 100 nm respectively. SERS spectra of 4-MB- and TPS-coded b) Au@PDA NPs, e) Ag@PDA NCs, h) Au@PDA NRs, and k) Au@PDA NSs. 4-MB and TPS are the

molecules bearing anchoring groups and without anchoring groups respectively. The RR concentrations of 4-MB and TPS are 100 μM and 1 mM respectively. SERS spectra of CV- and DMSO-d6-coded c) Au@PDA NPs, f) Ag@PDA NCs, i) Au@PDA NRs, and l) Au@PDA NSs. CV and DMSO-d6 are molecules showing a relatively large and small Raman scattering cross-section respectively. The RR concentrations of CV and DMSO-d6 are 10 μM and 1 M respectively. All SERS spectra for Au@PDA NPs, Au@PDA NRs, and Au@PDA NSs were obtained with an integration time of 2 s and 20 mW of 633 nm laser power. All SERS spectra for Ag@PDA NCs were acquired with an integration time of 2 s and 30 mW of 532 nm laser power. The Au nanorods and Ag nanocubes were purchased from xianfeng nanomaterial technology Co., Ltd. (Nanjing, China). Au nanoparticles (AuNPs) were prepared through reduction of chloroauric acid with hydroxylamine hydrochloride.[4] Briefly, hydroxylamine hydrochloride (0.4 mM, 1 mL) was quickly added to the 13 nm sized AuNPs (0.196 nM, 125 mL) solution, and then an aqueous solution of $HAuCl_4$ (100 mM, 10 mL) was added dropwise with vigorous stirring for another 30 min. Finally, 1.6 mL of the 10% w/w trisodiumcitrate solution was added into the above solution and the mixture was stirred for another 30 min to obtain the AuNPs. Au nanostars (AuNSs) were prepared *via* a seed-growth method.[5] Briefly, 0.2 mL of $HAuCl_4$ (100 mM) was added into 60 mL of deionized water, followed by adding 80 μL of 1 M HCl and 0.6 mL of 13 nm sized AuNP seeds (1 nM). The solution was stirred for 2 min, and then 160 μL of 10 mM $AgNO_3$ was injected into the mixture; subsequently, 400 μL of L-ascorbic acid (100 mM) was added

rapidly and the solution turned green immediately. Finally, 0.6 mL of CTAB (100 mM) was added into the mixture to obtain the AuNSs.

C. Supplementary References

(1) K. Liu, X. Wang and F. Wang, *ACS Nano*, 2008, **2**, 2315−2323.

(2) R. W. Zehner, B. F. Parsons, R. P. Hsung and L. R. Sita, *Langmuir*, 1999, **15**, 1121−1127.

(3) R. Klajn, K. J. M. Bishop, M. Fialkowski, M. Paszewski, C. J. Campbell, T. P. Gray and B. A. Grzybowski, *Science*, 2007, **316**, 261−264.

(4) H. Di, H. Liu, M. Li, J. Li and D. Liu. *Anal. Chem.,* 2017, **89**, 5874−5881.

(5) Y. Zhang, W. Zhou, Y. Xue, J. Yang and D. Liu. *Anal. Chem.*, 2016, **88**, 12502-12507.