Two-Dimensional SERS Encoding Method for On-Bead Peptide Sequencing in High-Throughput Bioanalysis

(Supplementary information)

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General Information

Tetraethylorthosilicate (TEOS), 3-mercaptopropyl trimethoxysilane (MPTS), silver nitrate (AgNO3, 99.999+ %), octylamine (OA), sodium silicate aqueous solution (~26.5%), N, N-diisopropylethylamine (DIPEA), ethylene glycol (EG), 2-amino-4-chlorobenzenethiol (2-ACBT), 4-azidophenacyl bromide (4-APB), 2-amino-4-(trifluoromethyl)benzenethiol (2-ATFT), 4-aminothiophenol (4-ATP), 3-amino-1,2,4-triazole-5-thiol (3-ATT), 2-bromobenzenethiol (2-BBT), 4-bromobenzenethiol (4-BBT), benzylmercaptane (BMT), benzenethiol (BT), benzyl disulfide (BZDSF), 3-cyanobenzoic acid (3-CBA), 4-cyanobenzylaldehyde (4-CBAL), 2-chlorobenzenethiol (2-CBT), 4-chlorobenzenthiol (4-CBT), 3,4-dichlorobenzenethiol (3,4-DCT), 3,5-dichlorobenzenethiol (3,5-DCT), 3,4-dimethoxythiophenol (3, 4-DMOBT), 2,5-dimethoxythiophenol (2,5-DMOBT), 3,4-dimethylbenzenthiol (3,4-DMT), 3,5-dimethylbenzenthiol (3,5-DMT), 2-fluorobenzenethiol (2-FBT), 4-fluorothiophenol (4-FBT), 4-isopropylbenzenethiol (4-IBT), 3-mercaptobenzoic acid (3-MBA), 4-mercaptobenzoic acid (4-MBA), 2-mercaptobenzimidazole (2-MBI), 2-mercapto-5-methylbenzimidazole (2-MMBI), 2-mercapto-1-methylimidazole (2-MMI), 2-mercapto-6-methylpyridine (2-MMP), 4-methoxybenzenethiol (4-MOBT), 2-mercaptopyrimidine (2-MPY), 4-mercaptotoluene (4-MT), 4-nitrophenyl disulphide (4-NPDSF), 2-naphthalenethiol (2-NT), pentachlorothiophenol (PCTP), 5-phenyl-1H-1,2,4-triazole-3-thiol (5-PHTT), phenyl iso-thiocyanate (PITC), 5-(4-pyridyl)-1,3,4-oxadiazole-2-thiol (5-PODAT), 4-(pyridin-4-yl) pyridine (4-PPD), 1-phenyltetrazole-5-thiol (1-PTET), 2-quinolinethiol (2-QT), 2-thiazoline-2-thiol (2-TAT), 1H-1,2,4,-triazole-3-thiol (1H-TAT), 2-thiouracil (2-TU), (+)-biotin N-hydroxysuccinimide ester, tri-isopropylsilane (TIPS), bovine serum albumin (BSA), and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (NH4OH, 27%), absolute ethanol (99.9%), ethanol (95%), methanol, dichloromethane (DCM), N-methylpyrrolidone (NMP), piperidine, and trifluoroacetic acid (TFA) were purchased from Daejung (Busan, South Korea). TentaGel (TG) microbeads (0.22 mmol NH_2/g, 35 μm) were purchased from Rapp Polymere (Tübingen, Germany). Fluorenylmethyloxycarbonyl (Fmoc)-amino acids, 1-hydroxybenzotriazole (HOBt), and (1H-benzotriazol-1-yloxy) [tris(dimethylamino)] phosphonium hexafluorophosphate (BOP) were purchased from Bead Tech Inc. (Seoul, South Korea). Fmoc-photolabile linker (Fmoc-PLL) was purchased by Advanced ChemTech (Louisville, KY, USA). All reagents were used without further purification. Deionized (DI) water was used for all experiments.

Fabrication of two-dimensional Raman labeled SERS nano-identifiers (2D-SERS IDs)

The 500-nm sized silica nanoparticles (NPs) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and adjusted concentration with ethanol for further fabrication step. These silica NPs were then functionalized with a thiol group. Silica NPs (300 mg) was dispersed in 6 mL ethanol containing 300 μL of MPTS and 60 μL of aqueous ammonium hydroxide (27%). After the mixture was stirred for 6 h at 25 ºC, the MPTS-treated silica NPs were centrifuged and washed with ethanol several times. A 50 mg of MPTS-treated silica NPs was thoroughly dispersed in 50 mL of $AgNO_3$ solution (3 mM in ethylene glycol). An 41.3 μL portion of octylamine (5 mM) was then rapidly added into the dispersed MPTS-treated silica NPs. The resulting dispersion was stirred for 1 h at 25 ºC. Afterwards, the resulting Ag-embedded silica NPs was centrifuged and washed with ethanol several times for purification. And then, two kinds of Raman label compounds included in 1 mL of ethanol solutions and each concentrations were adjusted to 10 mM were simultaneously added into 10 mg of Ag-embedded silica NPs. The resulting dispersion was shaken for 30 min at 25 ºC. The Ag-coated silica NPs coded with Raman label compounds were centrifuged and washed with ethanol 2 times. To encapsulate Ag-embedded silica NPs with silica shell, the Ag-embedded silica NPs were dispersed in 15 mL of sodium silicate aqueous solution (14.4 μL, 0.036 *wt*% SiO_2). The dispersion was stirred with a magnetic bar for 15 h at 25 ºC. Ethanol (60 mL) was added to the reaction mixture while mixing vigorously with magnetic bar and then the dispersion was stirred for additional 3 h for the formation of a thin silica shell. Then, 250 μL of aqueous ammonium hydroxide (27%) and TEOS(25 μL) which injected three times each at 1 h time intervals were added to the mixed dispersion. The mixture was stirred for 24 h at 25 ºC. Finally, resulting 2D-SERS IDs were centrifuged and washed with ethanol several times.

Synthesis of solid-phase HPQ-containing peptides

The peptides were synthesized on TentaGel (50 mg) using conventional Fmoc chemistry. The TG beads were treated with pre-activated Fmoc-amino acid solution that was prepared with Fmoc-amino acid (3 equiv.), BOP (3 equiv., 14.6 mg), HOBt (3 equiv., 4.4 mg), and DIPEA (5 equiv., 9.58 μ L) in NMP (2 mL), for 1 h at 25 °C. The amino acid-loaded resin was washed alternately with NMP (×3), DCM (×3), and methanol (×3). A 20% piperidine/NMP solution was used to remove the Fmoc groups. After complete peptide synthesis, the terminal amine group was capped with an acetyl group, and side chain protection groups were cleaved with TFA/TIPS/H_2O (95:2.5:2.5) for 1 h, followed by five washes with NMP.

Encoding bio-ligands with 2D-SERS IDs on TG beads

After the peptide syntheses were completed, the TG microbeads were encoded with a combination of 2D-SERS IDs corresponding to peptide seqence code including each amino acid at each coupling step. All introduced 2D-SERS IDs were physically adsorbed and embeded on the microbead surface via solution driven swalling and shrinking process. In order to encode for mutiple coupling steps, 0.1 wt% of 2D-SERS IDs for each coupling step was added to TG beads and the mixture was then stirred for 5 min. The excess 2D-SERS IDs were removed by washing with ethanol and vacuum filtration.

Streptavidin binding reaction and analysis of fluorescence signal

An equal amount of HPQ-penta peptide TG beads and a biotin-TG beads mixture (10 mg) was incubated with 10 μ L of streptavidin-coated fluorescent NPs (SA-F-NPs, 1.0% w/v, SPHERO streptavidin-coated blue particles, ~400 nm in diameter) for 30 min. Then, the resulting TG beads were washed with PBS solution (×3), DI water (×3), and vacuum filtration. Fluorescence images of TG beads were obtained by a confocal laser-scanning microscope (SP8 X STED, Leica; Germany) with an ultraviolet emission line (405 nm) and detection in the 523 ± 75 nm channel. The fluorescence intensities of the TG beads were analyzed with the Leica Application Suite Advanced Fluorescence software (Leica; Germany)

SERS measurement from 2D-SERS IDs-coded TG beads

To characterize the 2D-SERS IDs, SERS measurements were performed using a confocal micro-Raman system (JY-Horiba, LabRam 300) equipped with an optical microscope (Olympus, BX41). The SERS signals were collected using a 50× (Olympus, 0.50 NA) and 100× objective lens (Olympus, 0.90 NA) in a back-scattering geometry and detected using a spectrometer equipped with a thermoelectrically cooled CCD detector. The 532 nm line of a diode-pumped solid-state laser (CrystaLaser, CL532-100-S) was used as an excitation source for Raman measurements. The laser power at the sample was 2.7 mW with the 50× objective lens, and 1.0 mW with the 100× objective lens. For identification of encoded 2D-SERS IDs, 15 μm × 15 μm Raman scanning on the surface of polymer microbead was performed by point-by-point mapping using a 100× objective lens with a 1s acquisition and 1 μm step-size.

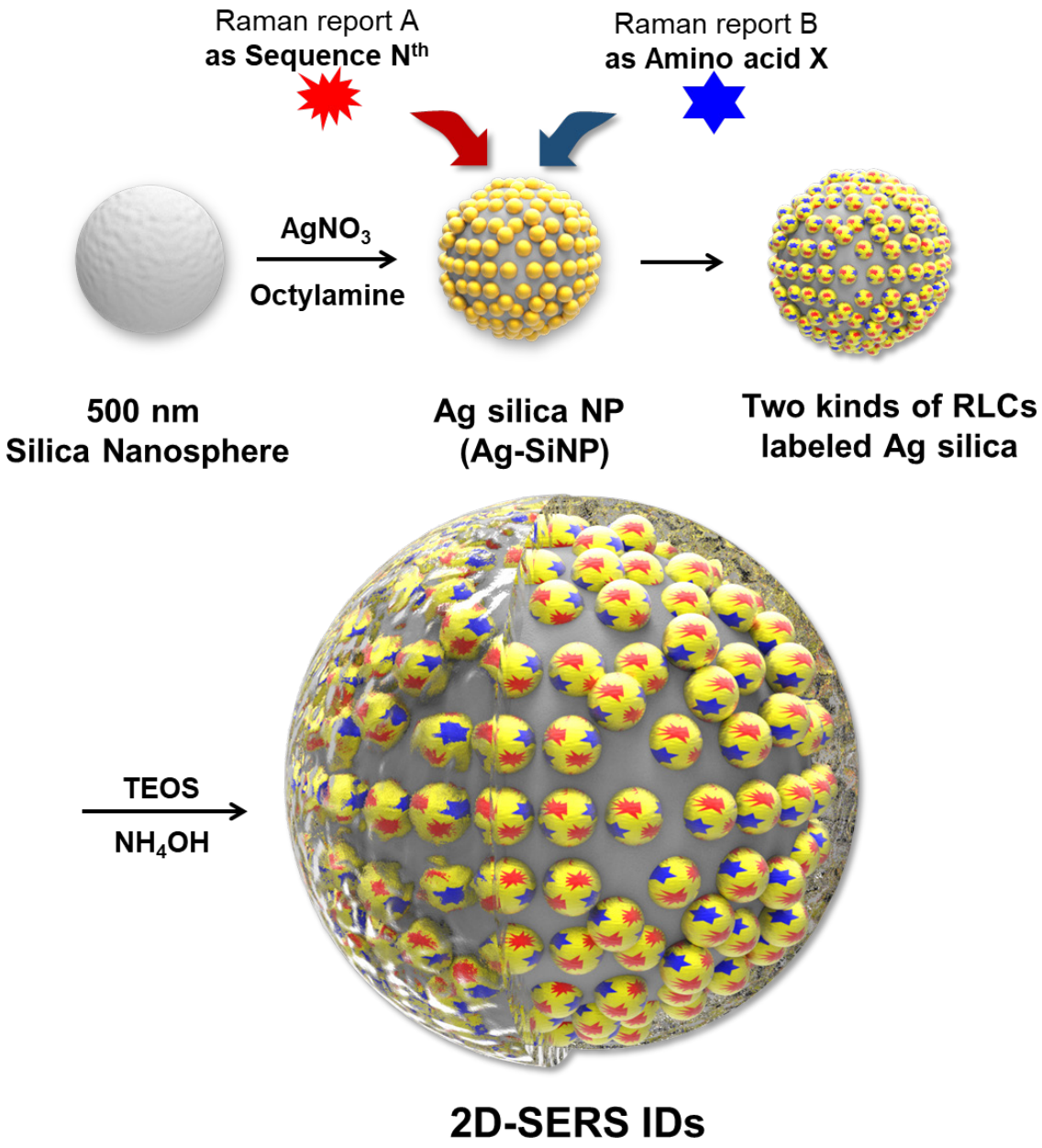

Figure S1. Schematic procdure of fabrication of two-dimensional Raman labeled SERS nanoidentifier (2D-SERS IDs).

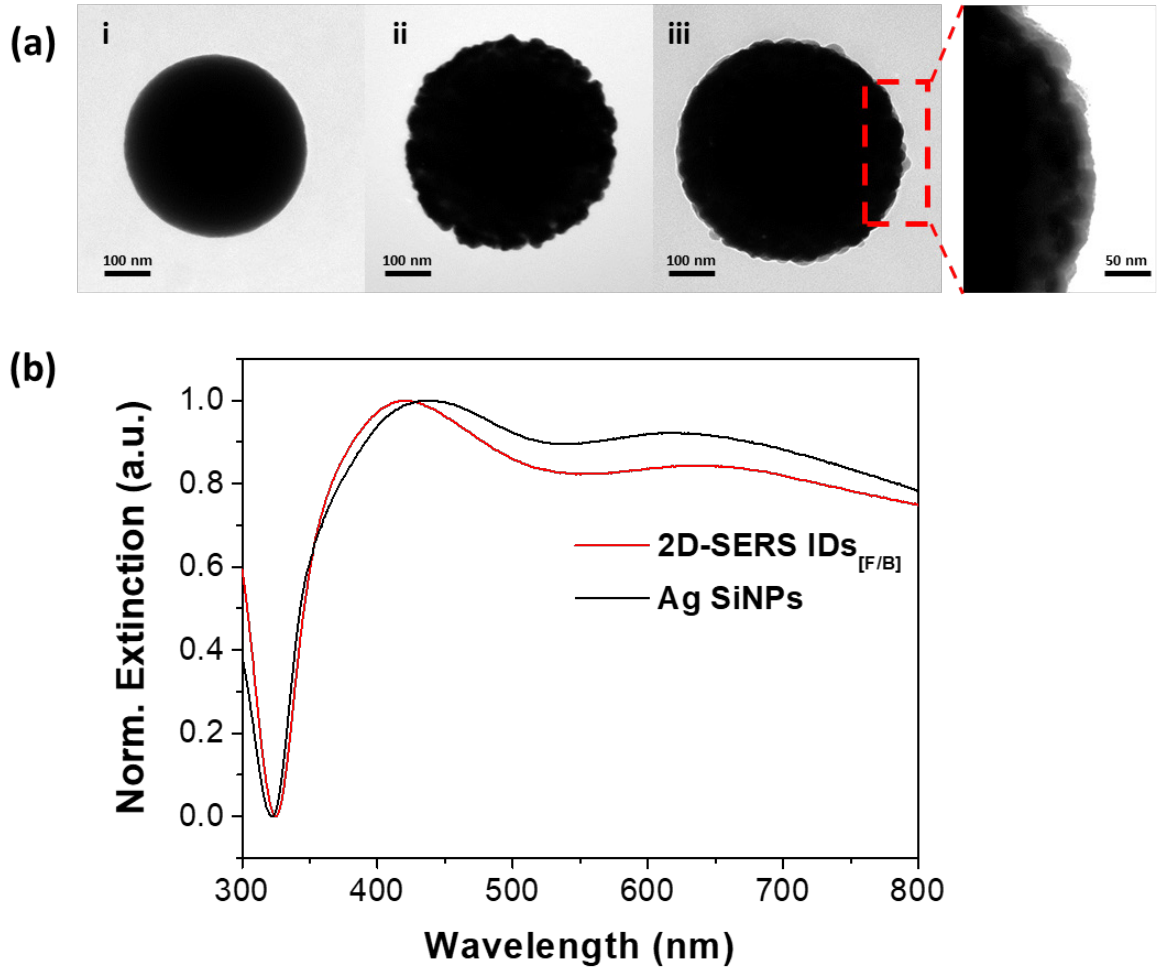

Figure S2. (a) Transmission electron microscopic images of (i) silica nanoparticle, (ii) silver nanoparticles embedded silica nanoparticle (Ag-SiNP), and (iii) silica shell coated two-dimensional Raman labeled nanoidentifier (2D-SERS ID) (inset of (iii) : high-magnification image of surface of 2D-SERS ID). (b) UV-visible absorption spectra of Ag-SiNPs and 2D-SERS IDs$_{[4FBT/BT]}$.

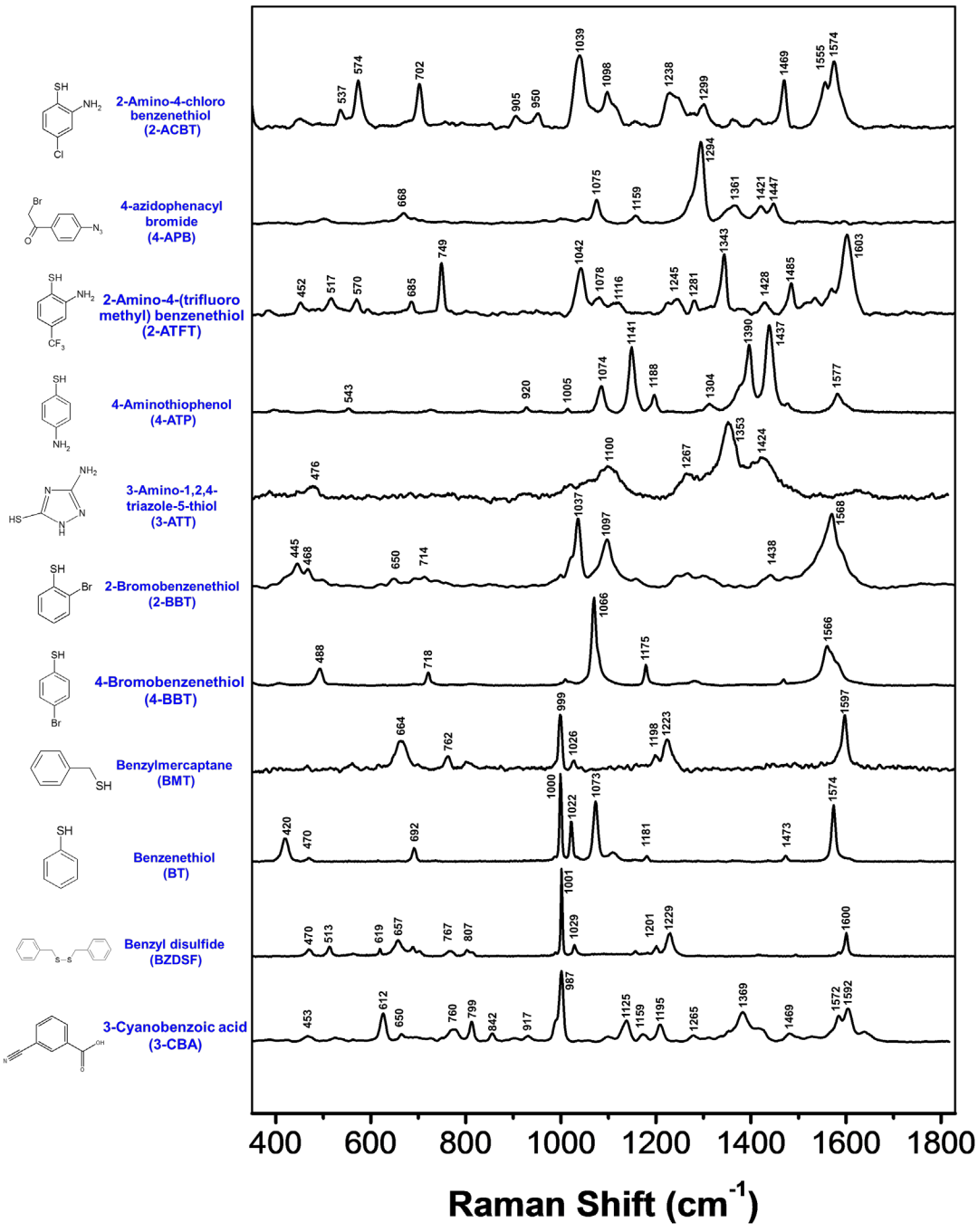

Figure S3. Forty-four kinds of Raman label compounds (chemical structures and names) and their corresponding SERS spectra (1/4)

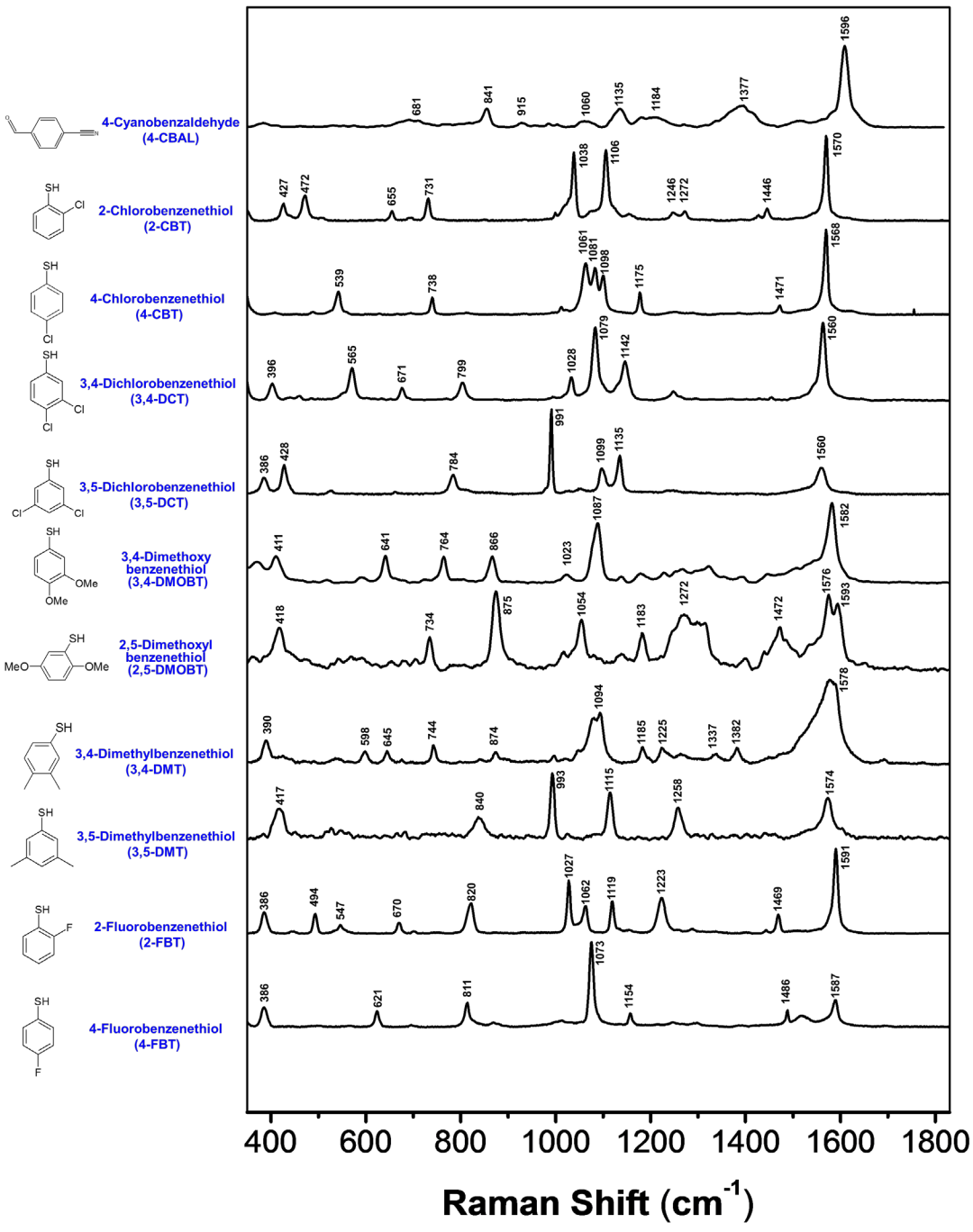

Figure S3. Forty-four kinds of Raman label compounds (chemical structures and names) and their corresponding SERS spectra (2/4)

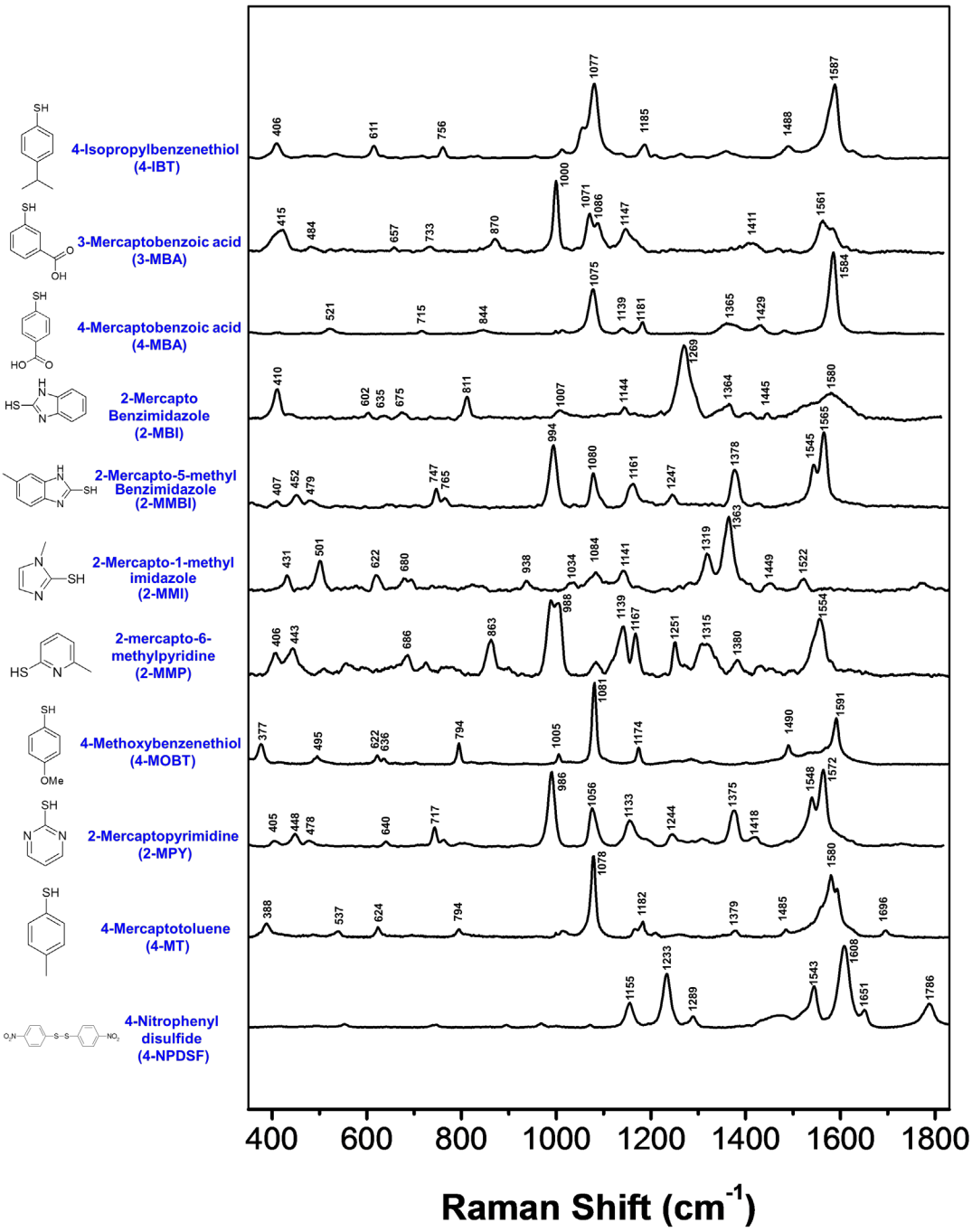

Figure S3. Forty-four kinds of Raman label compounds (chemical structures and names) and their corresponding SERS spectra (3/4)

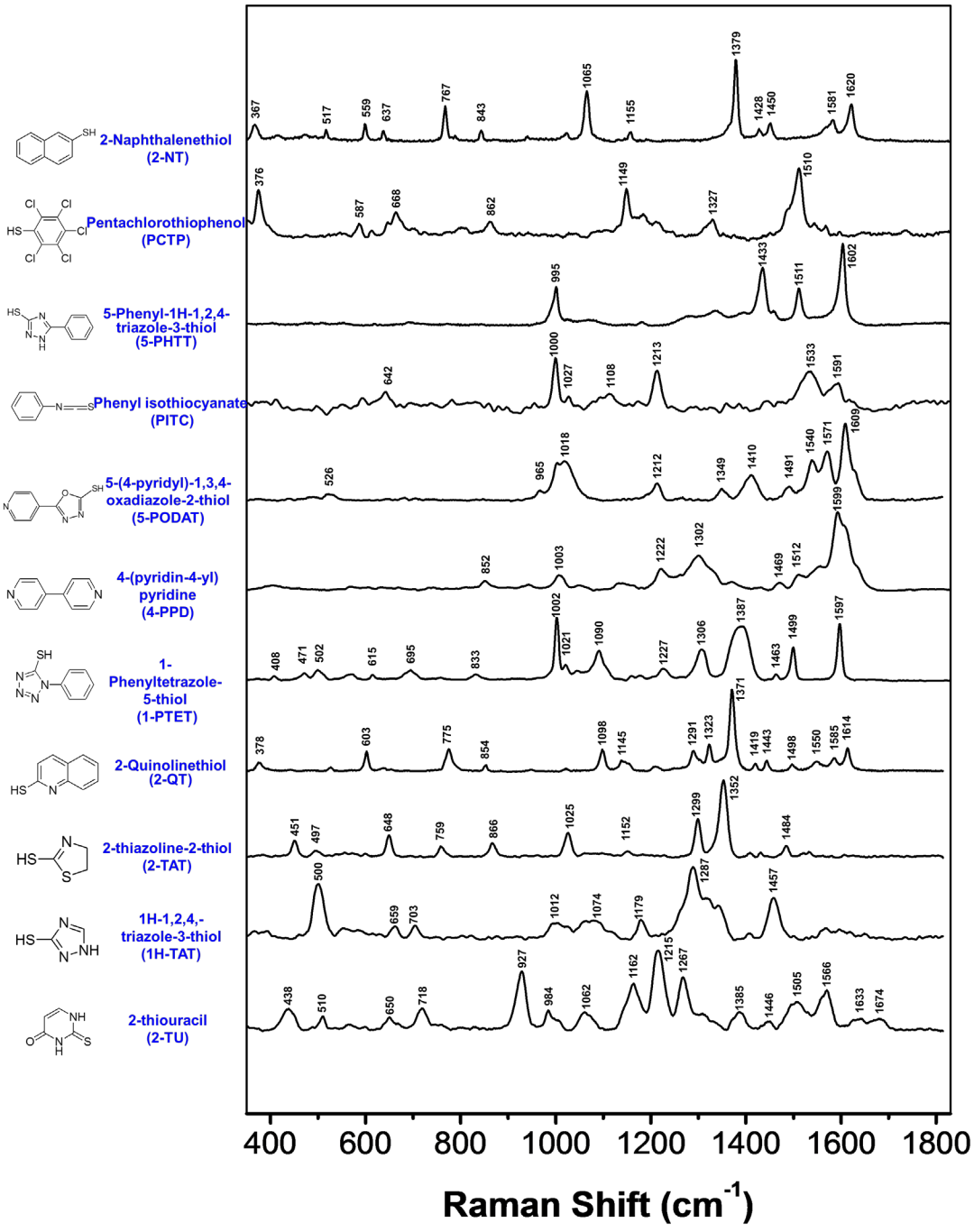

Figure S3. Forty-four kinds of Raman label compounds (chemical structures and names) and their corresponding SERS spectra (4/4)

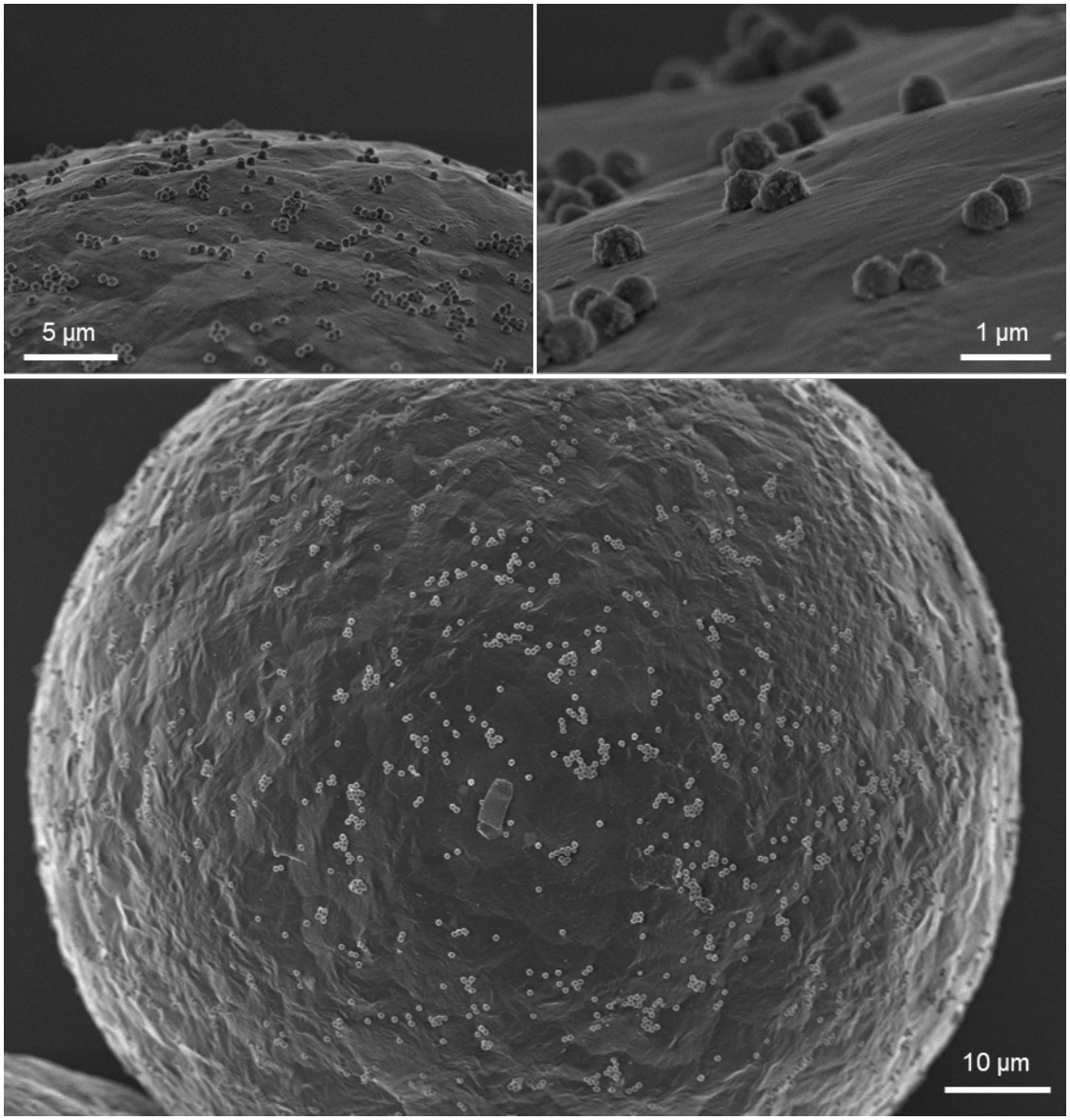

Figure S4. Field emission scanning electron microscope (FE-SEM) images of 2D-SERS IDs$_{[4\text{-FBT/BT}]}$ encoded TentaGel microbead.

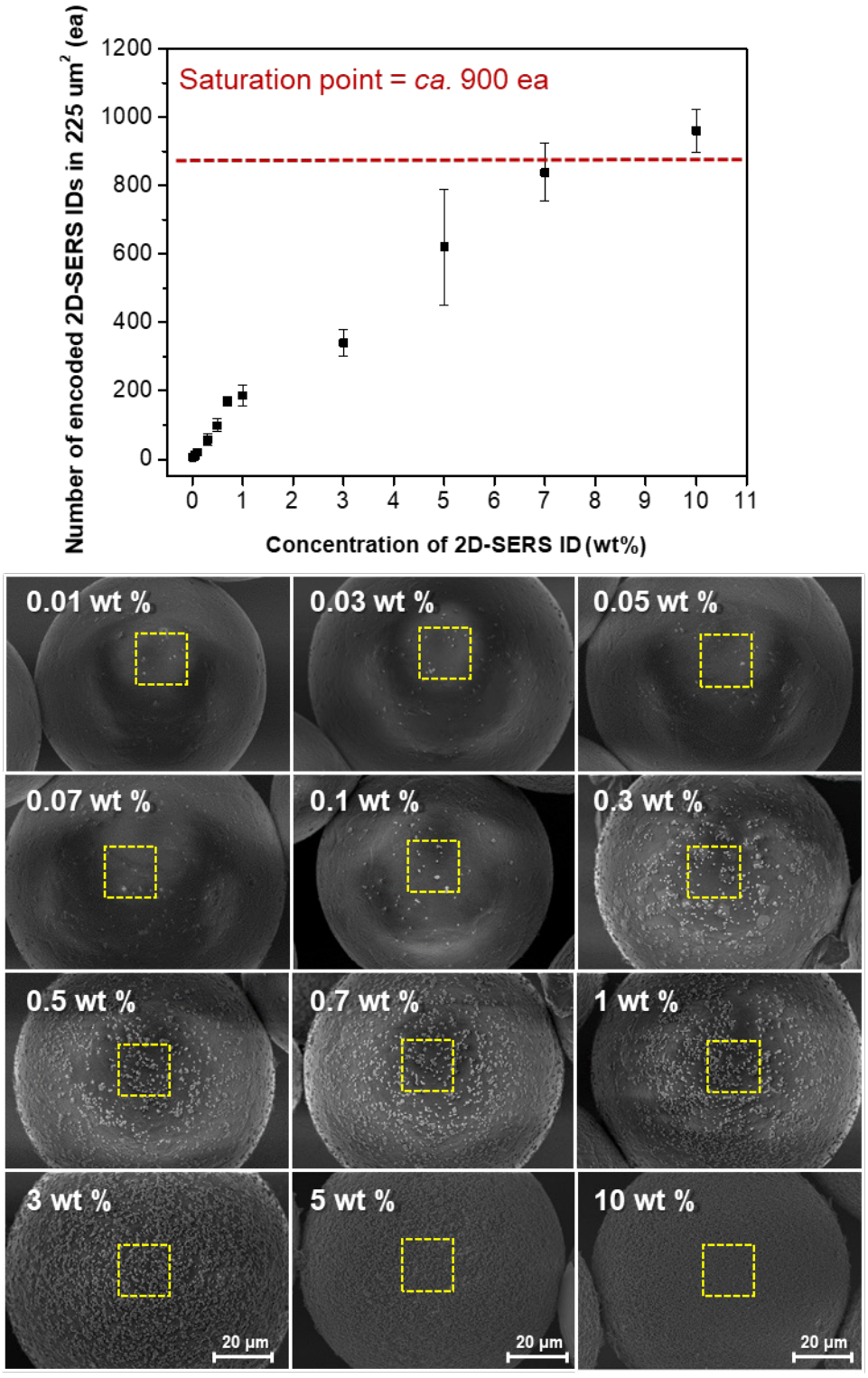

Figure S5. (a) The number of encoded 2D-SERS IDs in 15 μm × 15 μm (225 μm^2) surface area of TentaGel bead and (b) FE-SEM images of the 2D-SERS IDs encoded TentaGel microbeads after being treated with various concentrations of 2D-SERS IDs (0, 0.03, 0.5, 0.07, 0.1, 0.3, 0.5, 0.7, 1, 3, 5, and 10 wt%)

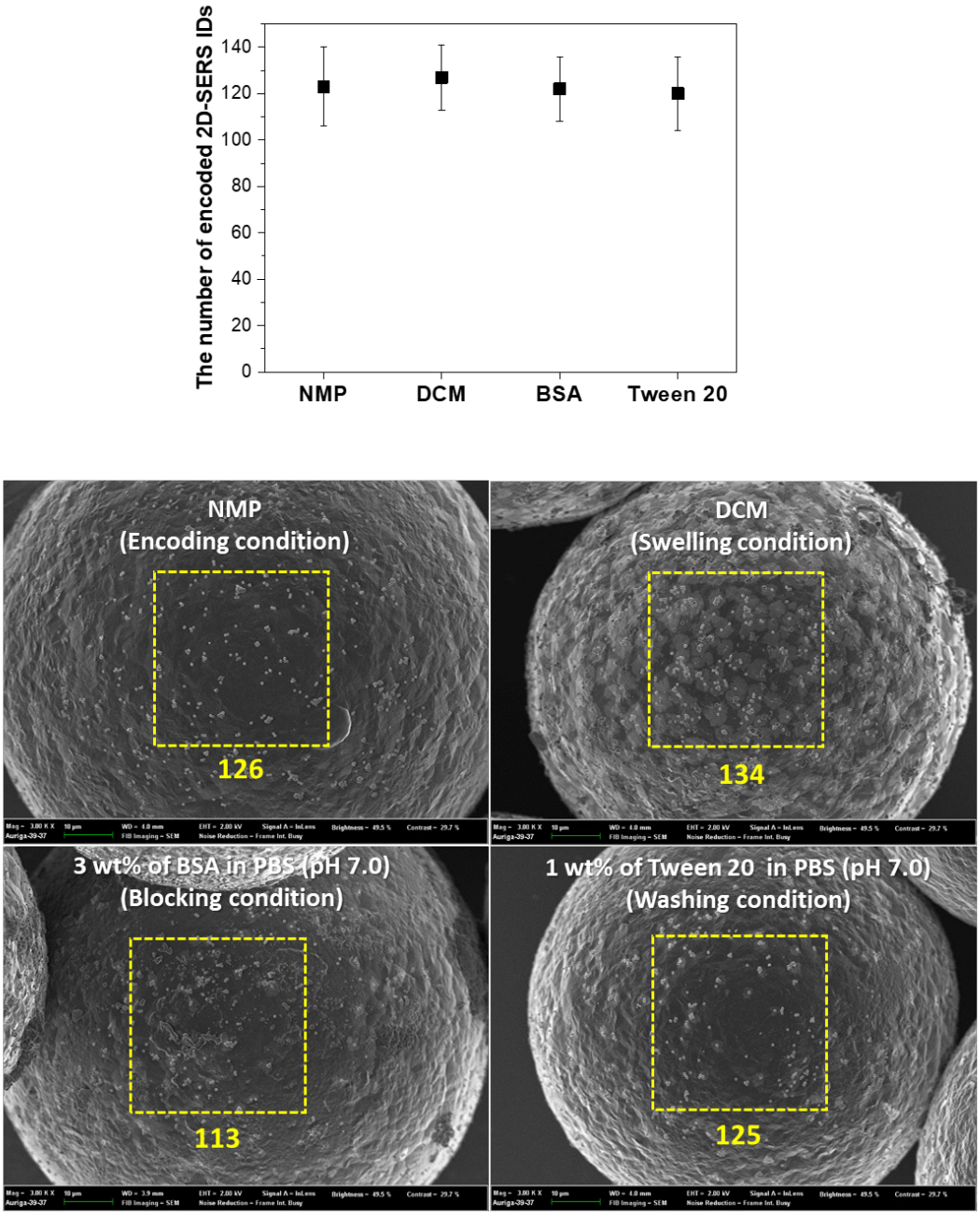

Figure S6. The average number of 2D-SERS IDs encoded on the TG and represenative FE-SEM images of 2D-SERS IDs$_{[4\text{-FBT/BT}]}$ encoded TG bead's surface after the encoded TG beads were treated with various solvents such as encoding (NMP), swelling (DMF), blocking (3 wt% of BSA in PBS), and washing conditions (1 wt% of Tween 20 in PBS).

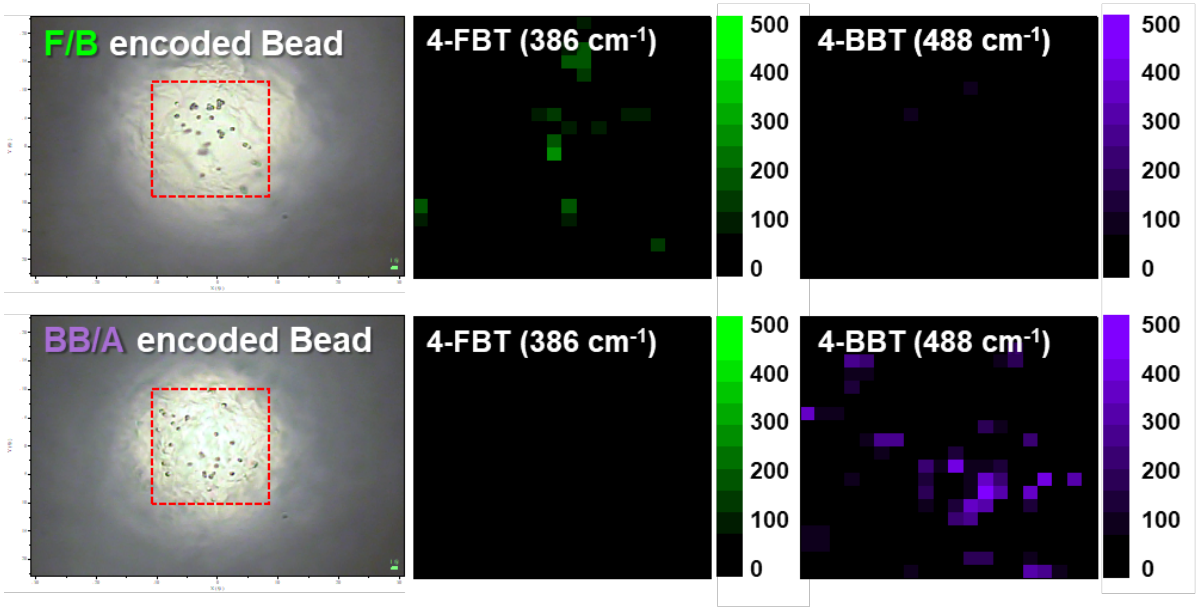

Figure S7. The evalution of cross contamination between 2D-SERS IDs$_{[4FBT/BT]}$ encoded TG bead and 2D-SERS IDs$_{[4BBT/4ATP]}$ during bioassay. Representative SERS intensity maps at 386 cm^{-1} for 4-FBT, and 488 cm^{-1} for 4-BBT from randomly selected 2D-SERS IDs encoded TG-beads in the mixture after mixing two differently encoded TG beads in PBS for 1 h.

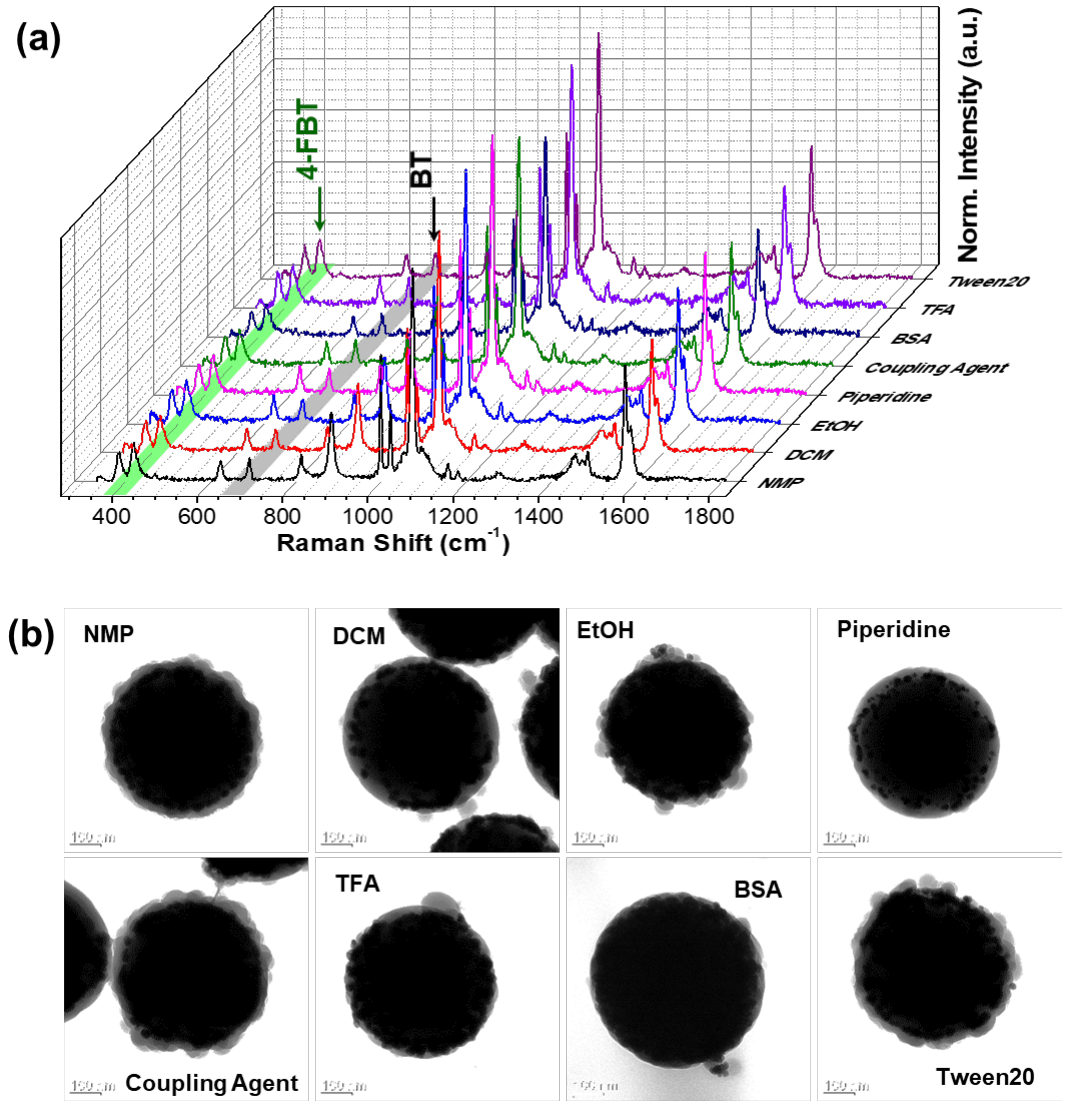

Figure S8. (a) SERS spectra and (b) TEM images of 2D-SERS $ID_{[4\text{-FBT/BT}]}$ after being treated with various solvents such as encoding (NMP), swelling (DCM), shrinking (EtOH), coupling (BOP, HOBt, and DIPEA in NMP), side chain removing (95% TFA), blocking (3% BSA in PBS), and washing (0.1% Tween-20 in PBS) conditions..

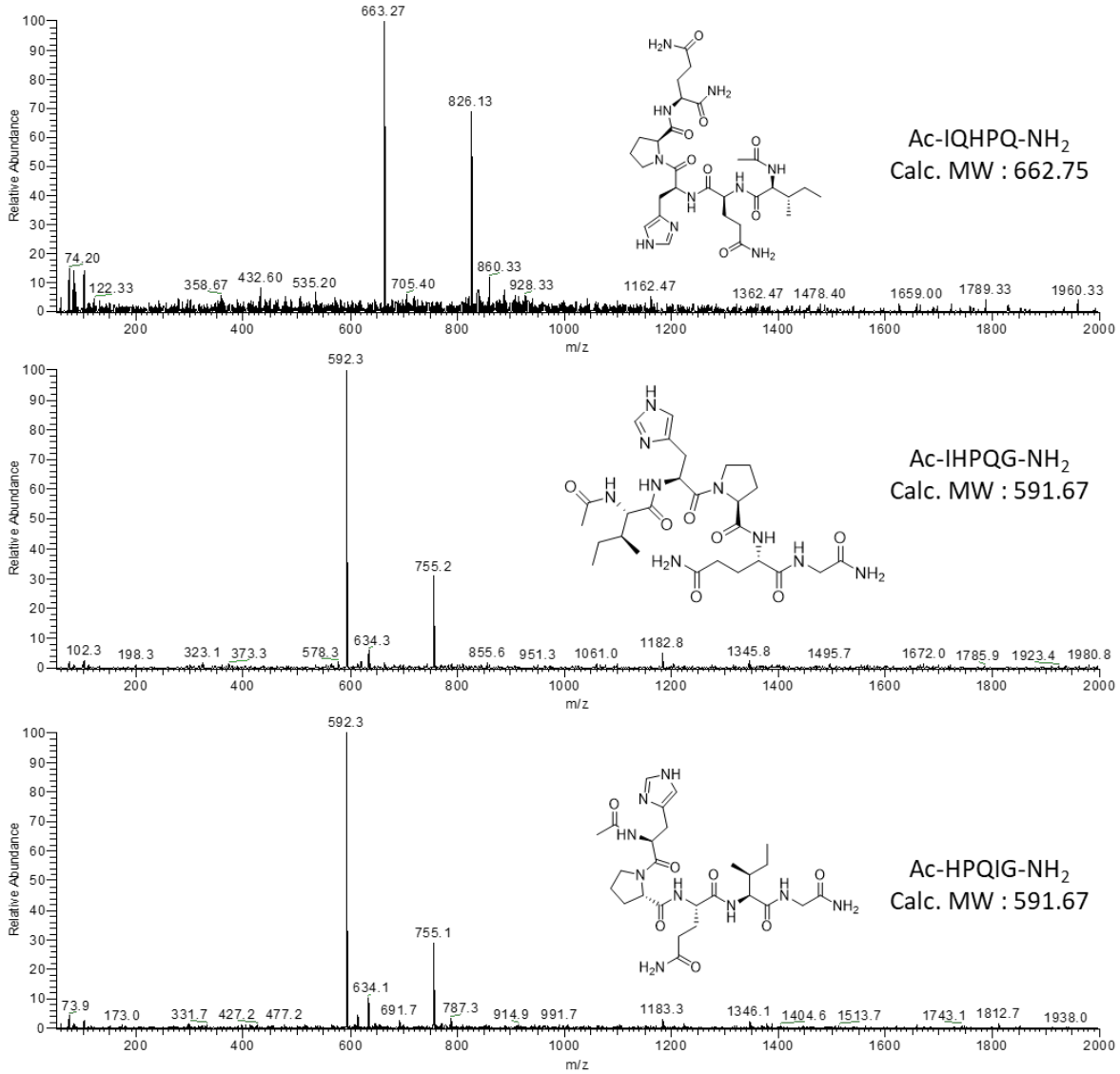

Figure S9. MALDI Analysis of three kinds of synthesized peptide (IQHPQ, IHPQG, and HPQIG)

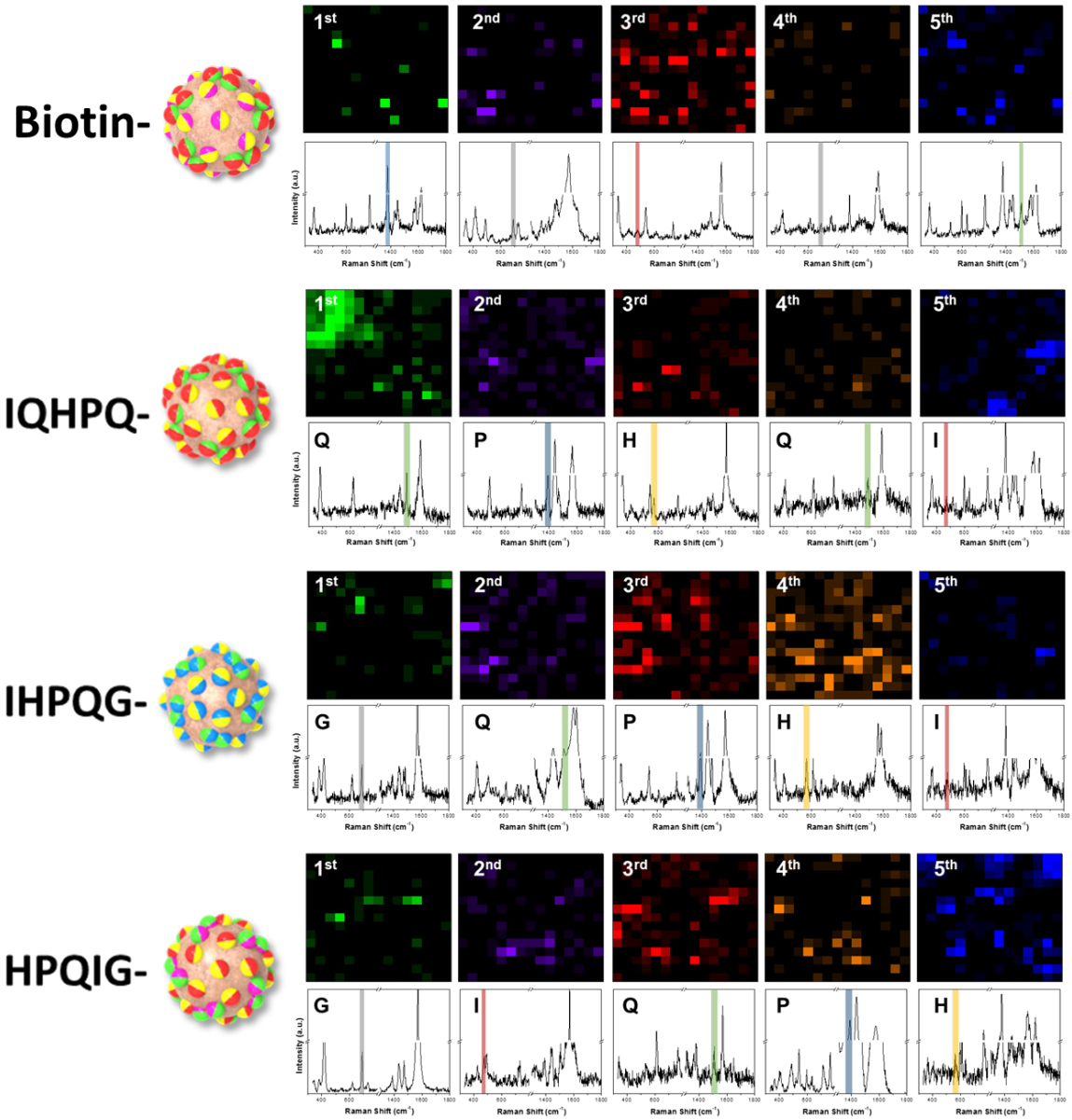

Figure S10. The representive 2D-SERS IDs codes for HPQ-cotained penta-peptides and biotind ligands on the TG beads.