

Electronic Supplementary Information

Encoding peptide sequences with surface-enhanced Raman spectroscopic nanoparticles

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Materials and General Procedure

Materials

Tetraethylorthosilicate (TEOS), 3-mercaptopropyl trimethoxysilane (MPTS), ethylene glycol (EG), poly(vinyl pyrrolidone) (PVP), 1-hydroxybenzotriazole (HOBt), *N,N*-diisopropylethylamine (DIEA), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), *N*-methylpyrrolidone (NMP), benzenethiol (BT), 4-mercaptotoluene (4-MT), 2-naphthalenethiol (2-NT), 4-aminothiophenol (4-ATP), 4-isopropylbenzenethiol (4-IBT), 3, 5-dimethylbenzenethiol (3, 5-DMT), 3, 4-dimethylbenzenethiol (3, 4-DMT), 4-methoxybenzenethiol (4-MOBT), 3, 4-dimethoxythiophenol (3, 4-DMOBT), 3, 5-dimethoxythiophenol (3, 5-DMOBT), 4-chlorobenzenethiol (4-CBT), 2-chlorobenzenethiol (2-CBT), 3,4-dichlorobenzenethiol (3, 4-DCT), 3, 5-dichlorobenzenethiol (3, 5-DCT), 2-fluorobenzenethiol (2-FBT), 4-fluorothiophenol (4-FBT), 4-bromobenzenethiol (4-BBT), 2-bromobenzenethiol (2-BBT), 2-mercapto-6-methylpyridine (2-MMP), 2-mercapto-1-methylimidazole (2-MMI), 2-mercapto-5-methylbenzimidazole (2-MBI), 5-phenyl-1H-1,2,4-triazole-3-thiol (5-PHTT), 2-amino-4-(trifluoromethyl)benzenethiol (2-ATFT), 2-amino-4-chlorobenzenethiol (2-ACBT), 3-mercaptobenzoic acid (3-MBA), 4-nitrophenyl disulfide (4-NPDSF), phenyl isothiocyanate (PITC), 3-cyanobenzoic acid (3-CBA), 4-cyanobenzaldehyde (4-CBAL) and 4-(pyridine-4-yl)pyridine (4-

PPD) were purchased from Sigma-Aldrich Inc. and used without further purification. TentaGelTM microbead (0.25 mmol NH₂/g, 35 μm) was purchased from Rapp Polymere (Tübingen, Germany). Fmoc-amino acids were purchased from BeadTech Inc. (Seoul, Korea). 1-Hydroxybenzotriazole (HOBt), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) were purchased from GL Biochem Ltd. (Shanghai, China).

Preparation of Ag nanoparticle-embedded silica nanospheres

Tetraethylorthosilicate (TEOS, 1.6 mL) was dissolved in 40 mL of absolute ethanol into which a 3 mL portion of aqueous ammonium hydroxide (27 %) was added. The resulting mixture was vigorously stirred using magnetic bar for 20 h at 25 °C. The silica nanoparticles (NPs) were centrifuged and then washed with ethanol several times to remove the excess reagents. These silica NPs were then functionalized with thiol group. Silica NPs (300mg) was dispersed in 6 mL ethanol containing 60 μL of MPTS and 150 μL of aqueous ammonium hydroxide (27 %). The mixture was stirred for 12 h at 25 °C. The resulting MPTS-treated silica NPs were centrifuged and washed with ethanol several times. A 100 mg portion of MPTS-treated silica NPs was thoroughly dispersed in 100 mL of AgNO₃ solution (3 mM in ethylene glycol). An 82.7 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 °C. Afterwards, the Ag NP-embedded silica NPs was centrifuged and washed with ethanol several times for purification.

Fabrication of SERS dots

A 500 μL portion of MPTS (0.2 M in ethanol) and 250 μL of Raman label compound (0.2 M in ethanol) were simultaneously added into 10 mg of Ag NP-embedded silica NPs. The resulting dispersion was shaken for 1 h at 25 °C. The Ag NP-embedded silica NPs, bearing both MPTS and Raman label compound, were centrifuged and washed with ethanol several times. To encapsulate Ag NP-embedded silica NPs with silica shell, the Ag NP-embedded silica NPs were dispersed in 15 mL of poly(vinyl pyrrolidone) solution (0.02 wt% PVP, M_w 40,000) and sodium silicate aqueous solution (7.2 mL, 0.03 wt% SiO₂). The dispersion was stirred with a magnetic bar for 20 h at 25 °C. Ethanol (60 mL) was added to the reaction mixture while mixing vigorously with magnetic bar and then the dispersion was stirred for additional 6 h for the formation of a thin silica shell. Finally, 250 μL of aqueous ammonium hydroxide (27 %) and 40 μL of TEOS were added to the reaction mixture and were stirred for 24 h at 25 °C. Finally, SERS dots were centrifuged and washed with ethanol several times.

Encoding tripeptides with SERS dots on microbeads

The peptide was synthesized on Fmoc-Rink amide-TentaGel™ (100 mg, Fmoc-Rink-TG) resins using conventional Fmoc chemistry accompanied by SERS dot encoding. After removing the Fmoc group from Fmoc-Rink-TG resin with 20% piperidine/NMP for 10 min, Fmoc-amino acid (3 equiv) was coupled to the free amino group using BOP (3 equiv., 25.2 mg), HOBt (3 equiv., 7.7 mg) and DIEA (3 equiv., 13.6 μ L) in NMP (0.5 mL) for 1 h at 25 °C. The first amino acid-loaded resin was washed alternately with NMP, DCM and MeOH. After removing Fmoc group from the first amino acid with 20% piperidine/NMP, one type of SERS dot (final concentration: 0.3 wt% to resin) was added to the resin suspension to encode the first amino acid. The resulting mixture was shaken for 5 min at 25 °C. As for the SERS dots that was not absorbed, they were removed by filtration. This process which involves coupling of amino acids and subsequent encodings with SERS dots was repeated.

Raman instrument

A micro-Raman system (JY-Horiba, LabRam 300) was utilized for the Raman measurement. The signal was collected by a $\times 10$ objective lens (Olympus, 0.40 NA), and a $\times 100$ objective lens (Olympus, 0.90 NA) with the back-scattering geometry equipped with a thermoelectrically cooled CCD detector. The 514.5 nm laser line from a continuous wave Ar ion laser (Melles Griot, 35-MAP-321) was used for the micro Raman measurements. The laser power at the sample was 7.0 mW with a $\times 10$ objective lens. In the case of using a $\times 100$ objective lens, the laser power at the sample was 1.0 mW, the acquisition time was 10 s and the beam diameter size was 1 μ m.

Barcode presentation method

We presented Barcode using the SERS spectrum of 4-BBT as an example. (see Fig. S1) First, relevant marker bands (492, 722, 1071, 1179, and 1585 cm^{-1}) were selected. And then, selected marker bands were normalized to the most intense Raman band (1071 cm^{-1}). The barcode x_i was finally presented from integration values of the Raman intensities $I(x)$ around the Raman bands with full width at half maximum (FWHM).

Supplementary Figures

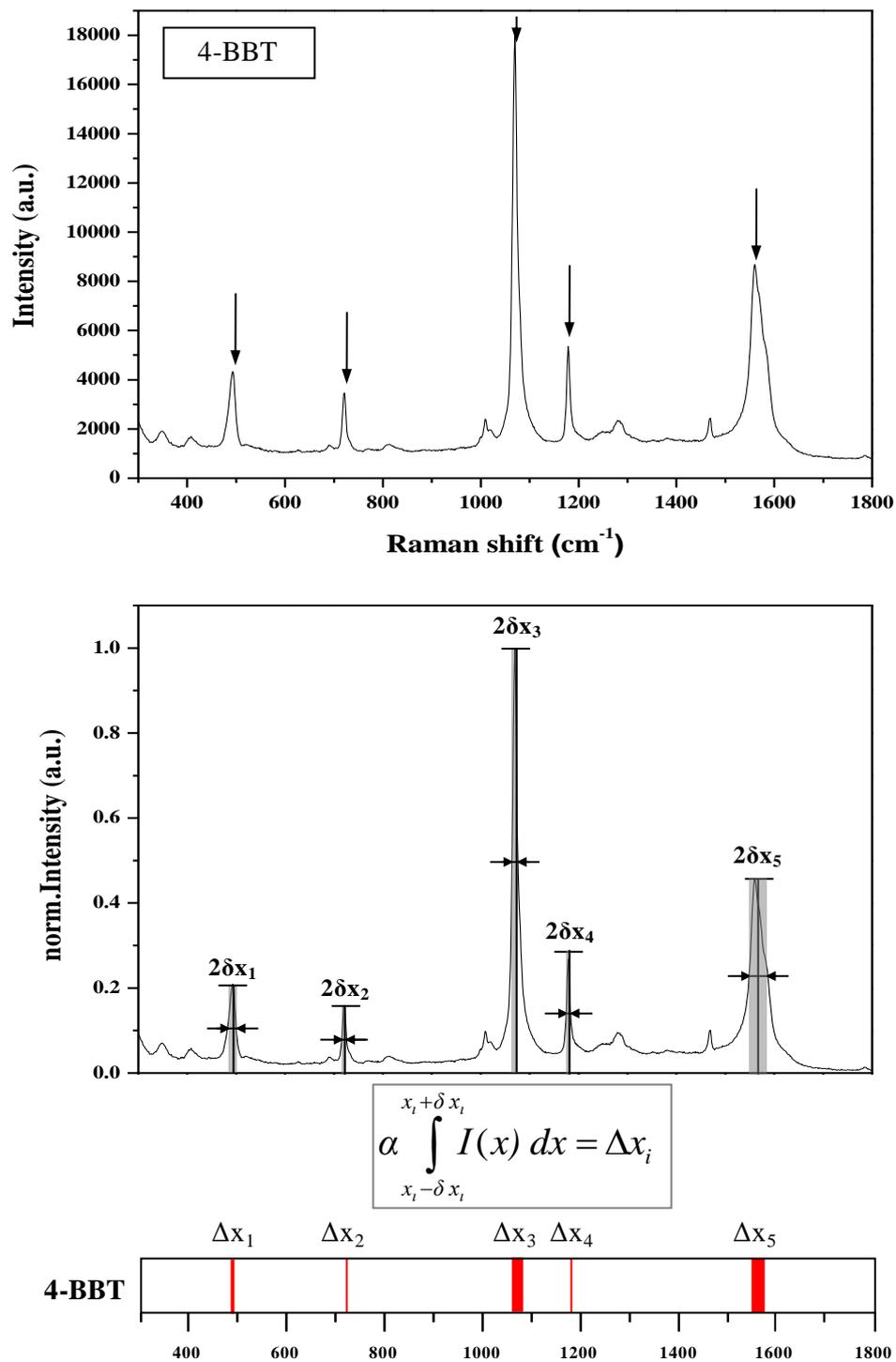
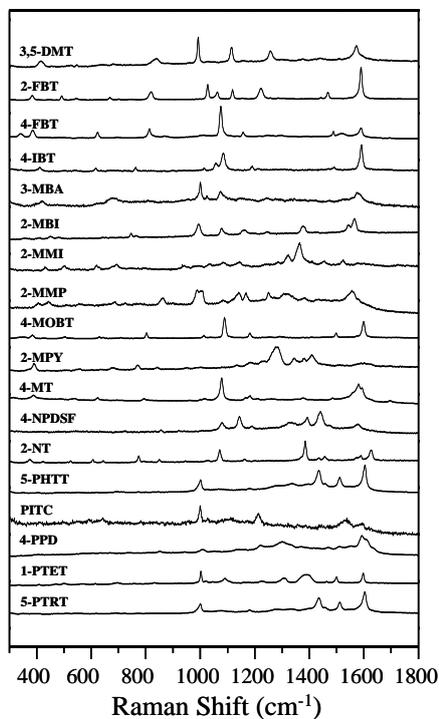
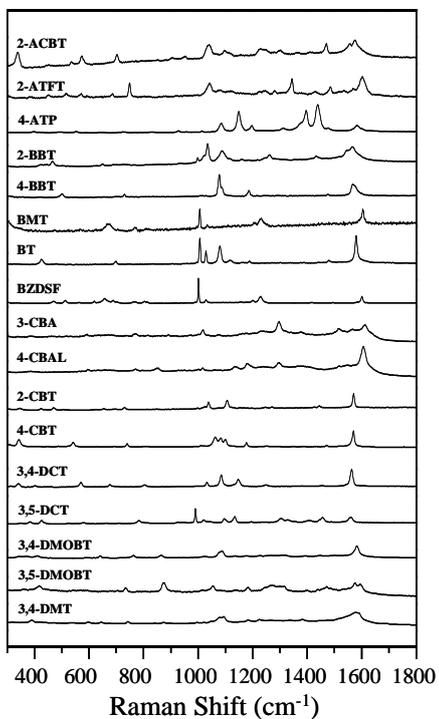
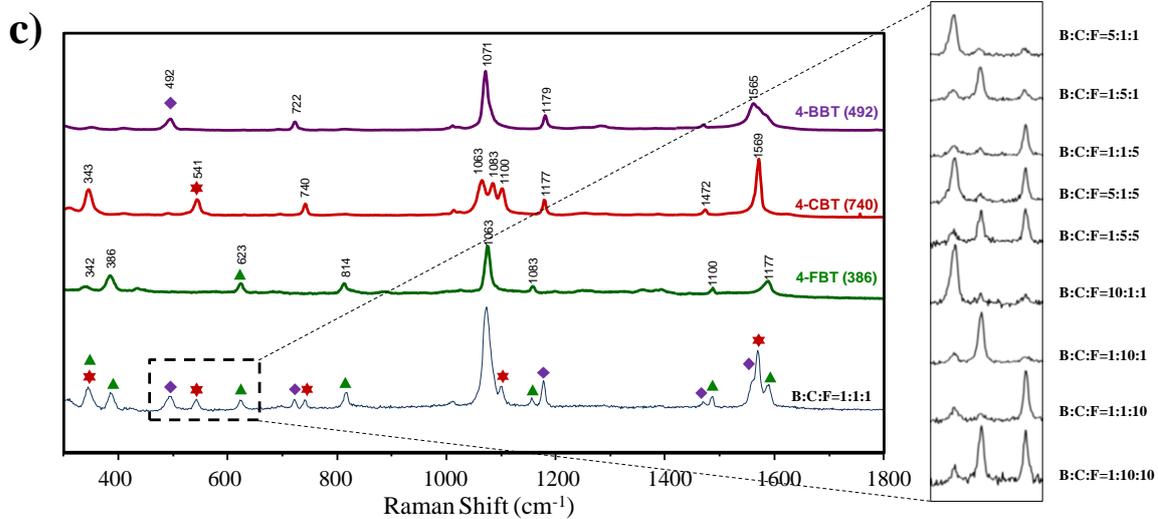
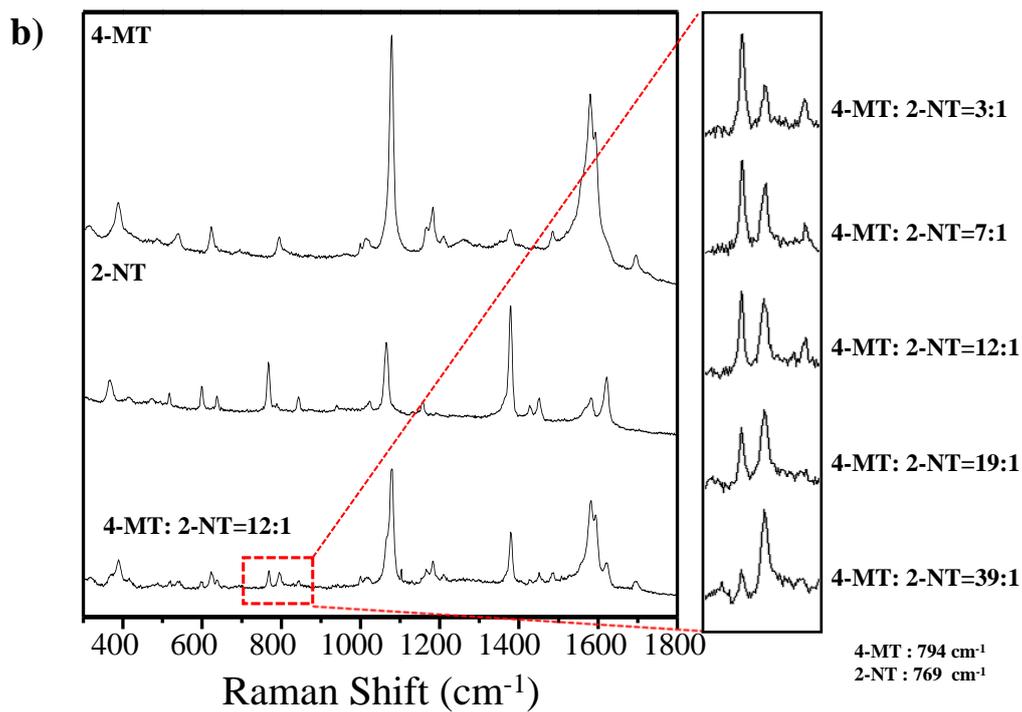


Fig. S1 An example of barcode presentation from integration values of the Raman intensities $I(x)$ around the Raman bands with FWHM.

a)

 2-Amino-4-chloro benzenethiol (2-ACBT)	 2-Amino-4-(trifluoro methyl) benzenethiol (2-ATFT)	 4-Aminothiophenol (4-ATP)	 2-Bromobenzenethiol (2-BBT)	 4-Bromobenzenethiol (4-BBT)	 Benzylmercaptane (BMT)
 Benzenethiol (BT)	 Benzyl disulfide (BZDSF)	 3-Cyanobenzoic acid (3-CBA)	 4-Cyanobenzaldehyde (4-CBAL)	 2-Chlorobenzenethiol (2-CBT)	 4-Chlorobenzenethiol (4-CBT)
 3,4-Dichlorobenzenethiol (3,4-DCT)	 3,5-Dichlorobenzenethiol (3,5-DCT)	 3,4-Dimethoxy benzenethiol (3,4-DMOBT)	 3,5-Dimethoxy benzenethiol (3,5-DMOBT)	 3,4-Dimethylbenzenethiol (3,4-DMT)	 3,5-Dimethylbenzenethiol (3,5-DMT)
 2-Fluorobenzenethiol (2-FBT)	 4-Fluorobenzenethiol (4-FBT)	 4-Isopropylbenzenethiol (4-IBT)	 3-Mercaptobenzoic acid (3-MBA)	 2-Mercapto-5-methyl Benzimidazole (2-MBI)	 2-Mercapto-1-methyl imidazole (2-MMI)
 2-mercapto-6-methylpyridine (2-MMP)	 4-Methoxybenzenethiol (4-MOBT)	 2-Mercaptopyrimidine (2-MPY)	 4-Mercaptotoluene (4-MT)	 4-Nitrophenyl disulfide (4-NPDSF)	 2-Naphthalenethiol (2-NT)
 5-phenyl-1H-1,2,4-triazole-3-thiol (5-PHTT)	 Phenyl isothiocyanate (PITC)	 4-(pyridin-4-yl)pyridine (4-PPD)	 1-Phenyltetrazole-5-thiol (1-PTET)	 5-Phenyl-1,2,3-triazole-3-thiol (5-PTRT)	





d)

$$T = \sum_{k=1}^M \frac{L!}{(L-k)!k!} P(i, k)$$
$$\sum_{k=1}^2 \frac{35!}{(35-k)!k!} (5^k - 5 + 1) = 12,530$$

T : The total number of possible encoding signature

L : The number of available Raman labels

M : The number of possible incorporated Raman labels

i : The number of possible intensity levels

$$P(i, k) = i^k - i + 1$$

$K = 1$ to M

Fig. S2 Raman labels for peptide encodings; a) Thirty five different kinds of Raman label compounds as Raman labels and their SERS spectra. b) Raman spectra obtained from a combination of 4-MT and 2-NT with different molar ratios (4-MT:2-NT, 3:1, 7:1, 12:1, 19:1, 39:1). c) Raman spectra obtained from a combination of 4-BBT (B), 4-CBT (C) and 4-FBT (F) with different molar ratios (B:C:F=1:1:1, 5:1:1, 1:5:1, 1:1:5, 5:1:5, 1:5:5, 10:1:1, 1:10:1, 1:1:10 and 1:10:10). d) Theoretical calculation of available Raman labels based on a combination of 4-MT and 2-NT.¹⁾

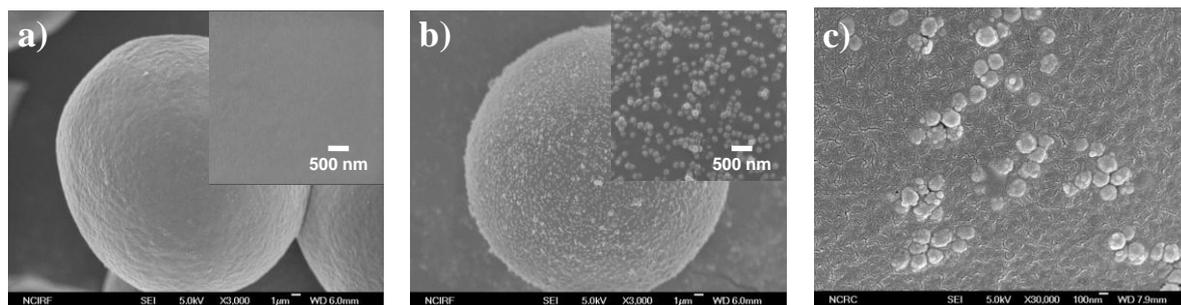


Fig. S3 FE-SEM images of SERS dot-encoded microbeads; a) Fmoc-Gly-TentaGel beads treated with SERS dot_{4MT}. b) H-Gly-TentaGel beads treated with SERS dot_{4MT}. c) Magnified image of SERS dot adsorbed on the surface of TentaGel beads.

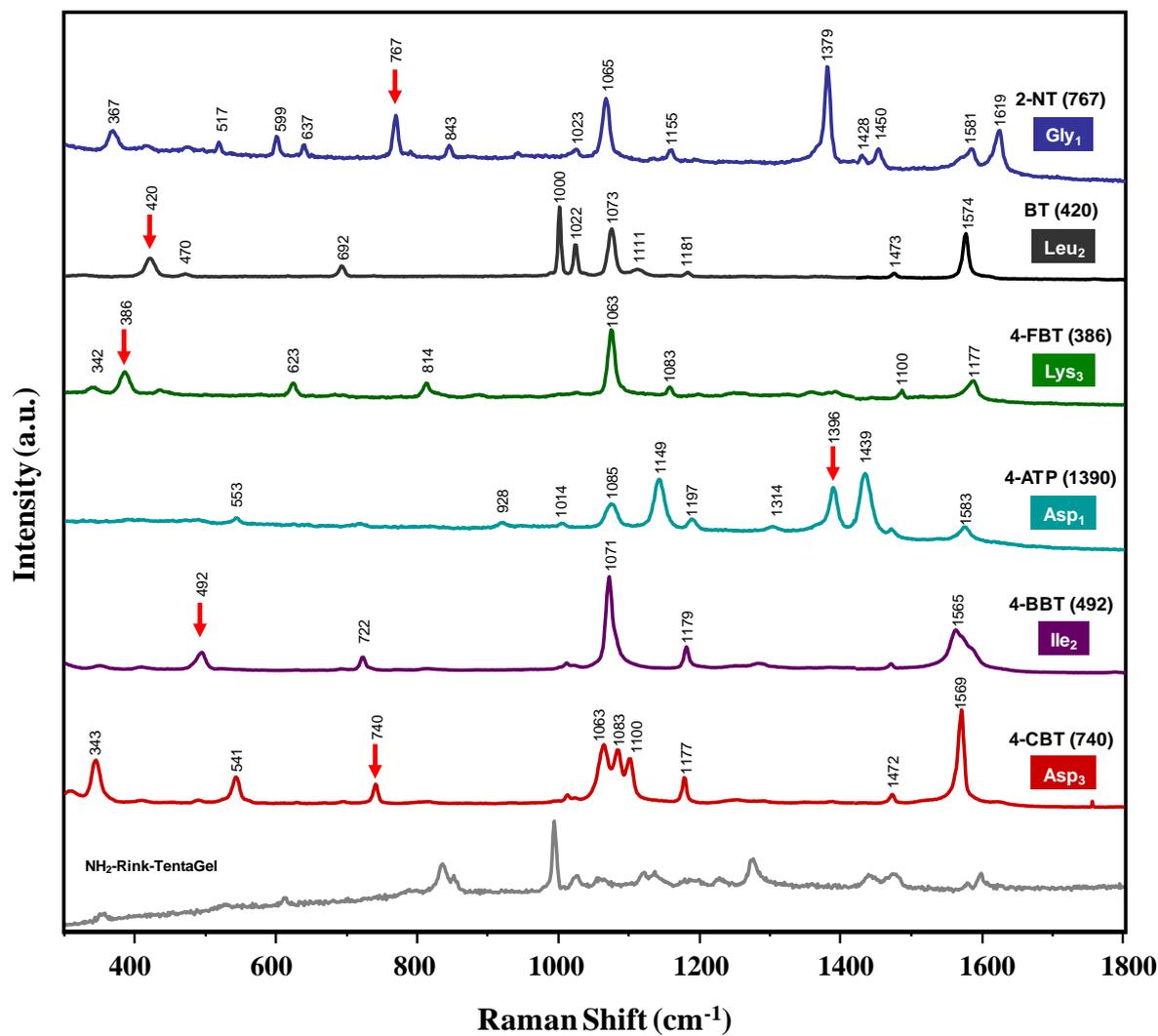


Fig. S4 Raman spectra of six types of SERS dots and Rink-TentaGel bead (background). The representative Raman peaks (red arrows) of the SERS dots could be selected without overlapping from others.

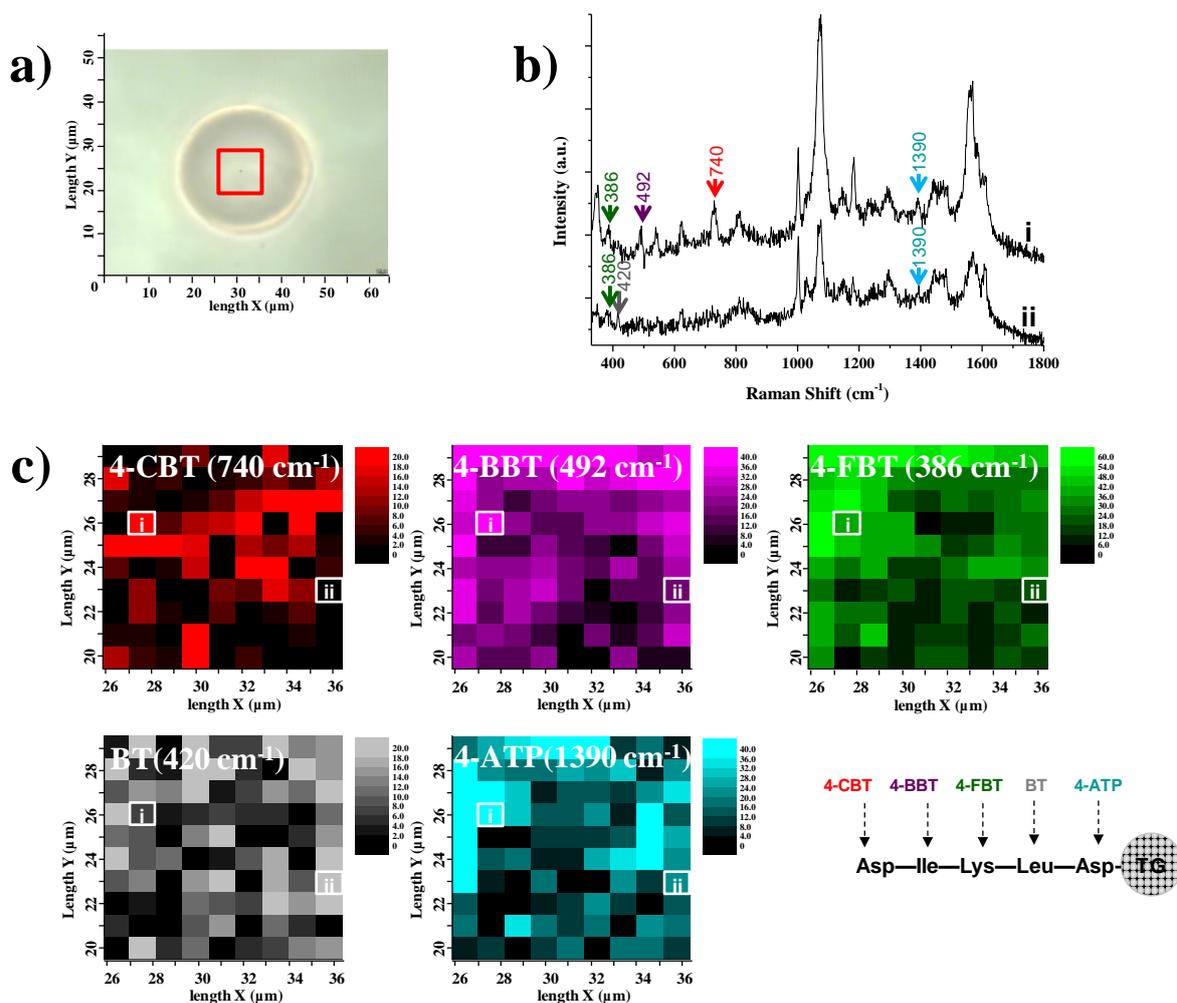
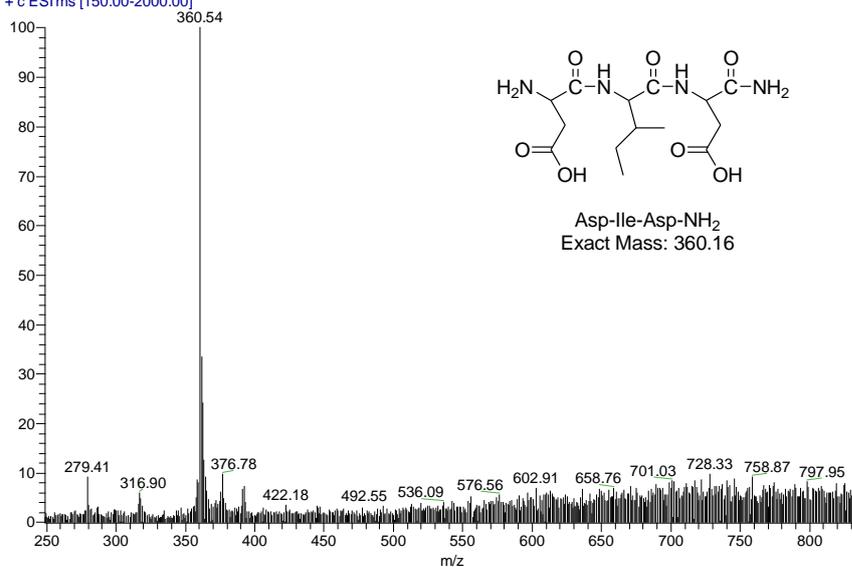


Fig. S5 Penta-peptide encoding with SERS dots; a) Optical picture of SERS dot-encoded bead having penta-peptide sequence. b) Raman spectra of Asp-Ile-Lys-Leu-Asp-TentaGel bead encoded with different types of SERS dots from SERS mappings (point i and ii), showing the specific peaks for each Raman label (4-FBT, 386 cm⁻¹; BT, 420 cm⁻¹; 4-BBT, 492 cm⁻¹; 4-CBT, 740 cm⁻¹; 4-ATP, 1390 cm⁻¹). c) SERS mapping of penta-peptide (Asp-Ile-Lys-Lue-Asp) generated from SERS dot_{4-CBT} for first Asp, SERS dot_{4-BBT} for Ile, SERS dot_{4-FBT} for Lys, SERS dot_{BT} for Leu and SERS dot_{4-ATP} for second Asp.

DID_090922174739 #1-107 RT: 0.02-2.74 AV: 107 NL: 3.31E5

T: + c ESI ms [150.00-2000.00]



oval_090922171714 #3-144 RT: 0.07-3.77 AV: 142 NL: 3.09E5

T: + c ESI ms [150.00-2000.00]

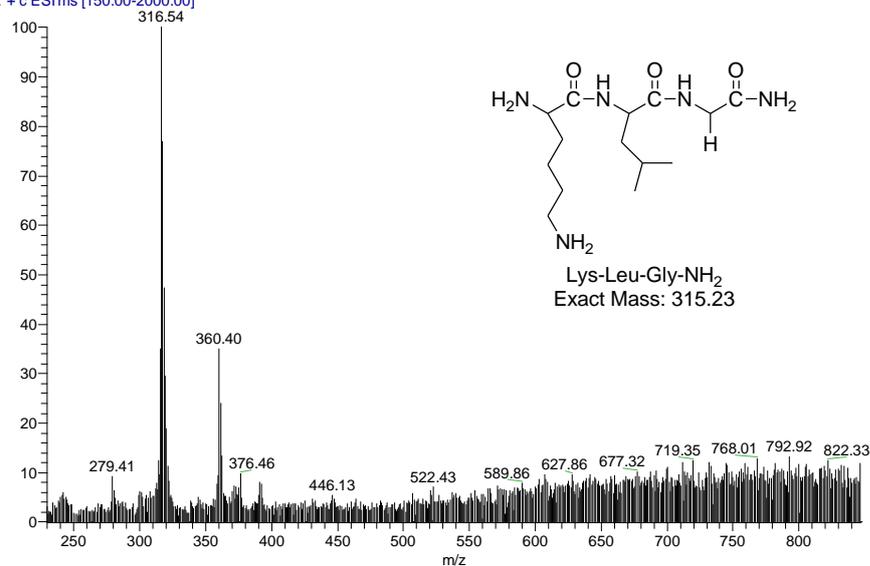


Fig. S6 ESI-MS spectra of Asp-Ile-Asp-NH₂ and Lys-Leu-Gly-HN₂.

Reference

1. X. Su, J. Zhang, L. Sun, T. W. Koo, S. Chan, N. Sundararajan, M. Yamakawa, and A. A. Berlin, *Nano Lett.*, 2005, **5**, 49-54