Electronic Supporting Information

Multi-dimensional On-particle Detection Technology for Multi-category Disease Classification

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1. Method

Preparation of porous silicon (pSi) and porous silicon microparticles (PSMPs). Porous Si was obtained by anodization of a boron-doped silicon wafer (resistivity 0.5 ~ 1.2 mΩ cm) of [100] crystal orientation in an electrolyte mixture of aqueous hydrofluoric acid (48 % by mass, Alatin Corp.), dimethylsulfoxide (DMSO) and distilled water (volume ratio = 2: 4: 1). Porosity of samples was controlled by the current density used in the electrochemical etch. The electrochemical cell was rinsed with deionized water after the etching step, and then refilled with a solution containing HF (3.3 % by mass), prepared by diluting aqueous HF with ethanol (CAUTION: HF is highly toxic and contact with skin should be avoided). The porous Si film was detached from the silicon substrate by application of a constant current (current density of 18 mA cm^{-2} for 3 min). The film was placed in a vial containing ethanol, which was capped and then subjected to ultrasound in a JY88-IIN (Ningbo Xingyi Ultrasonic Instrument Co. China) ultrasonic bath (for 15 min).

Chemical modification of porous Si.

Three types of surface modification were used in this work: Quaternization (Q-pSi), Carboxylation (C-pSi), and Au-plating (Au-pSi).

Q-pSi: The quaternizing surface was prepared by oxidizing the particles in ozone for 20 min using an ozone generator (Nanjing Xinmeng Eco-Technologies Co. Ltd.). The oxidized particles was then treated with a solution of (N,N-Diethyl-3-aminopropyl)trimethoxysilane (25 μL, Sigma-Aldrich Co.) in DMSO (1 mL) for 15 min. The particles were rinsed with DMSO and then the particles were placed in a flask containing a solution of 1-Chloropropane (Aladdin Industrial Inc.) (3 mL) in acetone (1 mL), which was then heated to reflux for 3 h. The particles were then rinsed with ethanol and dried in a stream of nitrogen.

C-pSi: The carboxyl species were grafted to the porous Si particle surface by thermal hydrosilylation: the particles were placed in a flask containing a deoxygenated (by nitrogen purge) solution of undecylenic acid (1 mL) in toluene (10 mL), which was

then heated to reflux for 3 h. Unreacted undecylenic acid was removed by rinsing with toluene and ethanol, and the sample was dried in a stream of nitrogen.

Au-pSi: Au-plated PSMPs was prepared by the electrochemical etching of single-crystal Si wafers (P++, 0.1 mΩ cm, Siltronix Co., France) mounted on a Teflon etching cell. Electrochemical etching was performed at a constant current of 30 mA for 120 s in electrolyte solution of 48 % HF and ethanol (4: 1, v/ v). Au films were electrochemically deposited on the obtained porous Si particle in the etching Teflon cell, but filled with $HAuCl_4$ (0.03 %, w/ v) solution. The pSi chip and a platinum counter electrode acted as the cathode and anode, respectively, and the electro-deposition was performed at a constant current of 5 mA for 1 min. After the samples were rinsed in deionized water, the pSi film was detached from silicon substrate by electrical polishing in 3.3 % HF (49 %, sigma) ethanol solution with constant current at 22 mA for 3 min. The PSMPs was obtained by sonicating the detached pSi film in ethanol for 5 min. The sample was rinsed with deionized water and dried with nitrogen gas.

Characterization of porous Si microparticles.

SEM: The surface morphologies of pSi and modified pSi were characterized by a field emission scanning electron microscope (SEM, Model Utral 55 field emission scanning electron microscope, CorlzeisD, Germany) using an accelerating voltage of 3 kV.

XPS: The elements on the surface of pSi and modified pSi were analyzed by a X-ray photoelectron spectrophotometer (XPS, VG ESCALAB MKII). The spectrometer was calibrated to the position of the 2p line of Si at a binding energy of 99.15 eV. Curve fitting of the core XPS lines was carried out using Origin software with a Gaussian-Lorentzian product function and a nonlinear Shirley background subtraction. Si 2p peak was used to calibrate the peak position in high resolution XPS analysis. The binding energies in references are: 103.4 eV (SiO_2), 402.1 eV (Me_4NCl), 284.6 eV (CH_2-CH_2), 286.8 eV ($MeCH_2OOCMe$), 83.8 eV and 87.5 eV (Au).[1, 2]

DRIFTS: The surface group of pSi and modified pSi were characterized by diffuse reflectance infrared Fourier transform spectra (DRIFTS, Nicolet iS 10, Thermo Fisher Scientific, U.S.A). Samples were filled into the DRIFT sample cup and detected at room temperature. The measurements were converted to the Kubelka–Munk mode at 0.4 cm^{-1} resolution.

Size-Exclusion and cross-reactive affinity of PSMP. The size-exclusion effects of pSi were performed using serum sample. The serum sample was incubated with pSi that etched at different conditions for 10 min. Psi with large pore was fabricated at a constant current of 80 mA for 60 s (porosity = 52.2 %, pore diameter ≈ 14 nm, measured by SEM). The electrochemical etching of pSi with small pore was performed at a current of 10 mA for 60 s (porosity = 20.3 %, pore diameter ≈ 9 nm, measured by SEM). Then 1.0 μL of α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in a mixture of water, acetonitrile and TFA (50: 50: 0.1, v/ v) was added to each spot, and the samples were air-dried before insertion into the instrument. Analyses were performed in linear positive-ion mode, using delayed extraction. The detected mass range is 1 ~ 50 kDa, and spectra were acquired in linear positive-ion mode using a 25 kV acceleration voltage and 220 ns pulsed ion extraction time.

The cross-reactive affinity of PSMPs were performed using RGDC (pI = 6.2, MW = 450 Da, ChinaPeptides Co., Ltd.), TP2 (PLIYLRLLRGQF-$CONH_2$, pI = 10.8, MW = 1.49 kDa, ChinaPeptides Co., Ltd.), and insulin (pI = 5.3, MW = 5.8 kDa, Wanbang Bio-pharm. Co.) as model peptides. Briefly, the testing samples were incubated with Q-pSi, C-pSi, or Au-pSi in PBS buffer (pH=7.4) for 10 min, while the control experiment was incubated in the absence of PSMPs. The particles were then spotted onto the MALDI plate. 1.0 μL of α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in a mixture of water, acetonitrile and TFA (50: 50: 0.1, v/ v) was added to each spot, and the samples were air-dried before insertion into the instrument. Analyses were performed in linear positive-ion mode, using delayed extraction. The detected mass range is 0.4 - 10 kDa, and spectra were acquired in linear positive-ion mode using a 25 kV acceleration voltage and 220 ns pulsed ion extraction time.

Collection of human serum samples. Blood samples were collected in 5 mL glass tubes, and allowed to clot at room temperature for up to 30 min. The clotted samples were then centrifuged at 4 °C for 5 min at 1000 rpm (1000 G), and aliquots of sera (upper phase) were made up and stored frozen at - 80 °C until use. The samples would be pre-fractionated using PSMPs with three different surface functionalities.

PSMPs based serum sample preparation. PSMPs formulations with the three surface modifications were tested. Thawed serum samples were diluted 1: 10 in distilled water. PSMPs (0.3 mg) were added to a vial containing the test solution (100 μL), and then shaken at room temperature for 30 min. The suspension was centrifuged at 8000 rpm for 2 min, and the supernatant was removed from the PSMPs. The PSMPs (incubated with serum) were resuspended in distilled water and thoroughly washed to remove any physisorbed species. The particles were isolated by centrifugation (2000 rpm for 1 min). Peptides captured by the particles were then detected by MALDI-TOF MS as described below.

On-particle detection of peptides/ proteins by MALDI-TOF MS. MALDI-TOF mass spectra were obtained using an Ultraflextreme MALDI-TOF/TOF (Bruker Daltonics Corp.) equipped with a smart beam laser. The particles were suspended in 100 μL distilled water and spotted onto the MALDI plate. 1.0 μL of α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in a mixture of water, acetonitrile and TFA (50: 50: 0.1, v/ v) was added to each spot, and the samples were air-dried before insertion into the instrument. Analyses were performed in linear positive-ion mode, using delayed extraction. The detected mass range is 0.4 - 10 kDa, and spectra were acquired in linear positive-ion mode using a 25 kV acceleration voltage and 220 ns pulsed ion extraction time. Spectra have been collected automatically using the AutoXecute™ software (Bruker Daltonics Corp.) for fuzzy-controlled adjustment of critical instrument settings to generate raw data of optimized quality. For each mass spectrum, 1600 laser shots were averaged. The raw spectra were processed with FlexAnalysis software (Bruker Daltonics Corp.).

Data analysis. The MATLAB software has been used for all data interpretation steps, which start with a data pretreatment, including normalization and internal signal alignment.

Student's t test: Student's t test was then used to find existence that statistically different from each two groups. For each pair of classes (CRC versus health, Liver cancer versus health and CRC versus Liver cancer), the number of characteristic peaks (p value < 10^{-7}) was listed in **Table 1**.

ClinProTools analysis: The whole data pretreatment was completed using default settings and performed automatically. ClinProTools provides a list of peaks sorted according to the statistical significance to differentiate between each two classes. A p value was calculated for each peak, which indicates the probability of differences in observed peak intensity.

Cluster analysis: The MALDI data (*m/z* peak intensities) were normalized and median centered. Pearson correlation was used to calculate the distance between the samples, and average linkage clustering was performed.

Artificial Neural Network. The hidden layer of the network was composed of five hidden nodes. The model was trained with 70 % training data, 15 % for validation, and 15 % for test. There were 20 CRC samples, 22 healthy samples and 19 liver cancer samples in the training and validation set, and 4 CRC samples, 2 healthy samples and 5 liver cancer samples in the test set. The training process stopped when reached a best validation performance.[3]

2. Supplementary Figure

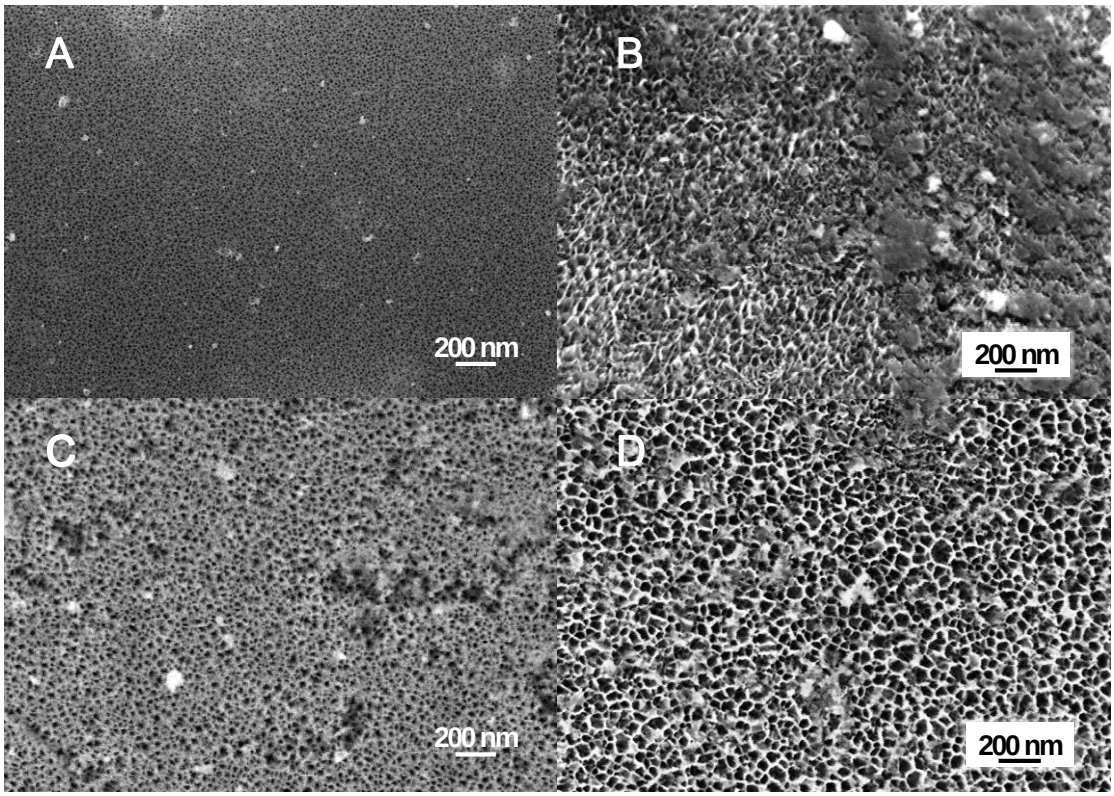

Fig. S1 (A) Plan-view scanning electron microscope (SEM) image of fresh-etched pSi. (B) SEM image of PSMPs with quaternizing surface. (C) SEM image of PSMPs modified by hydrosilylation with undecylenic acid. (D) SEM image of PSMPs with Au modified surface. The pore diameter of the modified PSMPs is about 10 nm measured by SEM image.

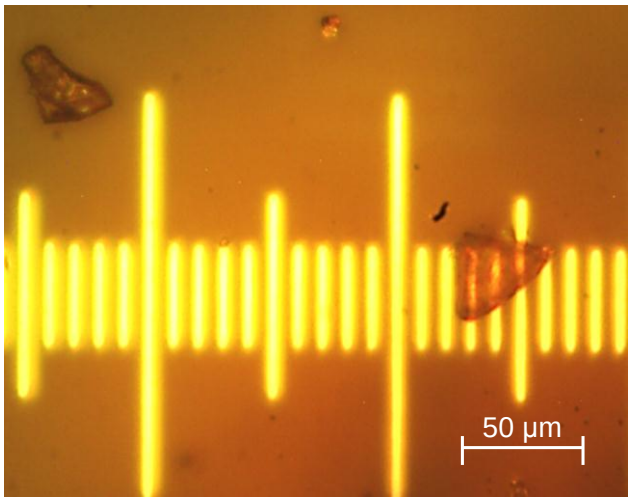

Fig. S2 Bright-field optical microscopy image of as-formed porous Si microparticles. The particle size was 30-50 µm.

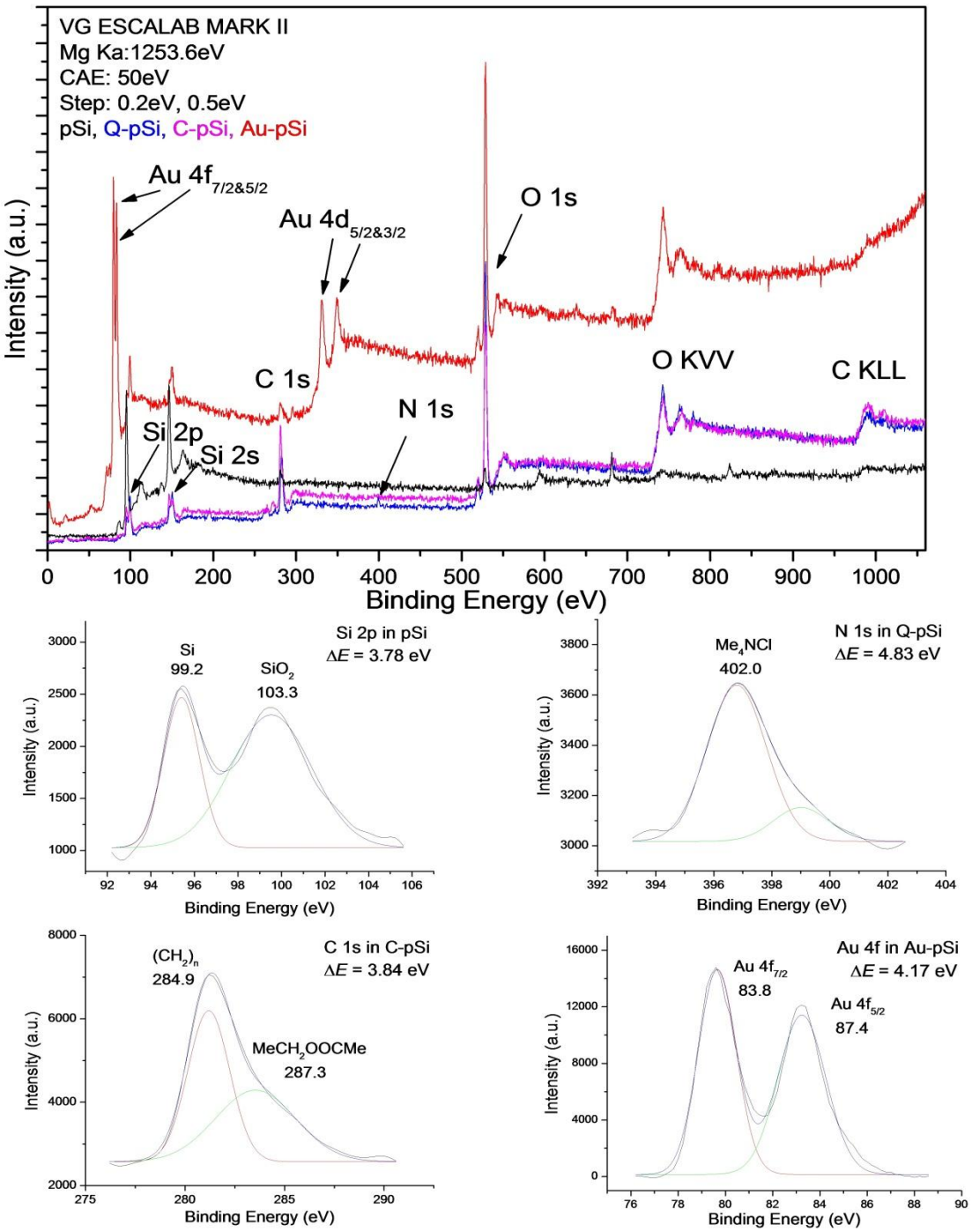

Fig. S3 (A) X-ray photoelectron spectroscopy of pSi sample (black line) and porous silicon samples after different modification: Q-pSi (blue line), C-pSi (pink line), and Au-pSi (red line). Typical survey scan was in the 0 - 1100 eV range. (B) Si 2p peak of pSi particles. (C) N 1s peak of Q-pSi. (D) C 1s peak of C-pSi. (E) Au 4f peak of Au-pSi. Si 2p peak was used to calibrate the peak position in high resolution XPS analysis. The binding energies in references are: 103.4 eV (SiO_2), 402.1 eV (Me_4NCl), 284.6 eV (CH_2-CH_2), 286.8 eV ($MeCH_2OOCMe$), 83.8 eV and 87.5 eV (Au). The fresh etched PSMPs sample shows Si 2p, Si 2s, and a low-intensity C 1s peak,

which is mainly due to adventitious carbon contamination. The Si 2p peak was observed at about 103 eV due to the partial oxidation of PSMPs. For the carboxyl surface, the relative intensities of the C 1s and O 1s lines increase concomitantly with a decrease in the intensity of Si 2p and Si 2s lines. As for the Q-pSi sample, a low-intensity of N 1s peak at 402.0 eV was observed. For the Au-pSi, a high-intensity of Au 4f double-humped peak is shown at 83.8 and 87.5 eV.

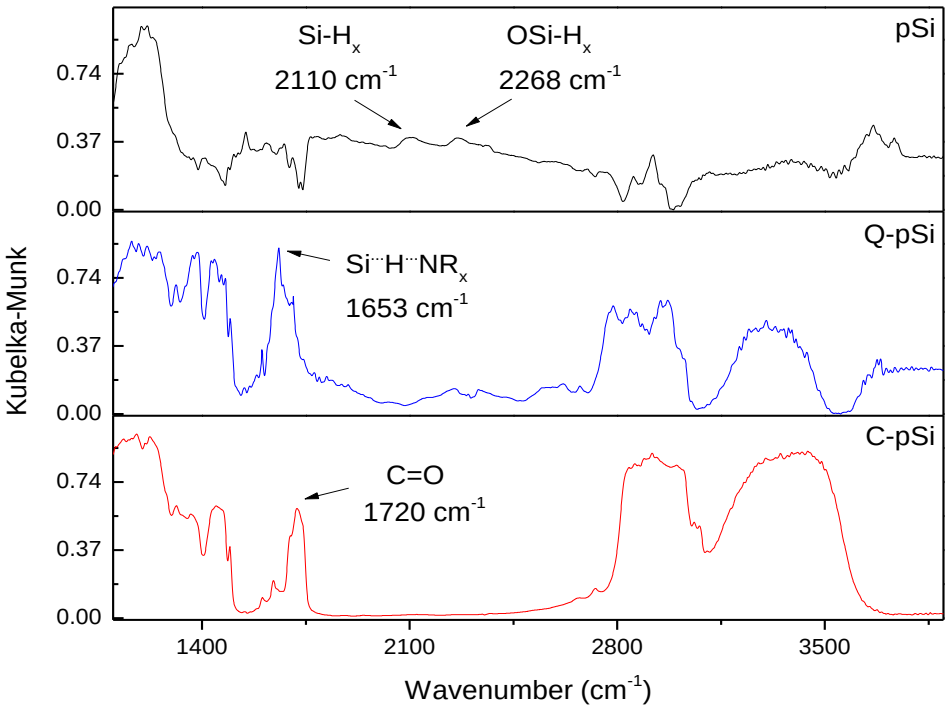

Fig. S4 Diffuse reflectance FTIR spectra of porous Si particle surfaces: (A) porous Si (Black line); (B) Q-pSi (Blue line) and (C) C-pSi (Red line). The intensity of the IR peaks at 2110 cm^{-1} and 2268 cm^{-1} corresponding to the Si-H_x bonds and OSi-H_x bonds decreased compare to the modified particles, whereas other IR peaks at 1653 cm^{-1} in Q-pSi and 1720 cm^{-1} in C-pSi grew significantly, referring to the Si···H···NR_x bonds and C=O bonds.[4-6]

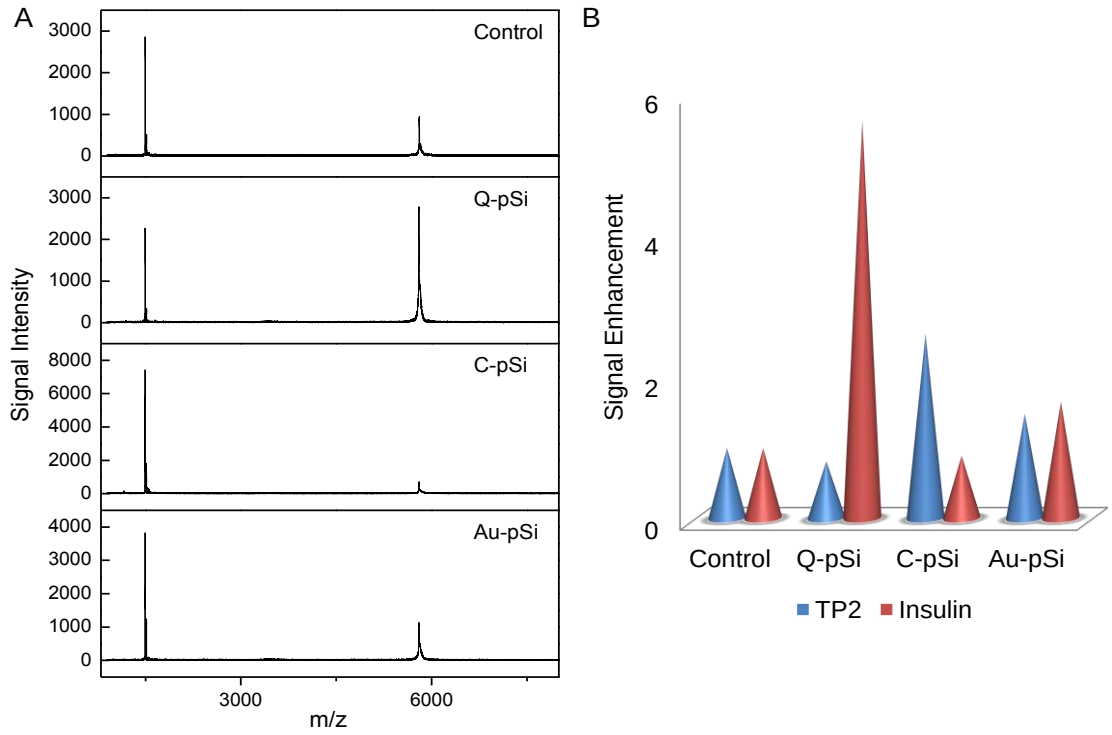

Fig. S5 MALDI mass spectra of insulin (MW = 5.8 kDa, pI = 5.3) and TP2 (MW = 1.5 kDa, pI = 10.8) on PSMPs with different modification. (A) mass spectrum of mixed sample (insulin + TP2) captured by Q-pSi, C-pSi, and Au-pSi, respectively. Control experiment was tested on a standard MALDI plate. (B) Relative peak intensity enhancement of TP2 (blue) and insulin (red) on Q-pSi, C-pSi, and Au-pSi.

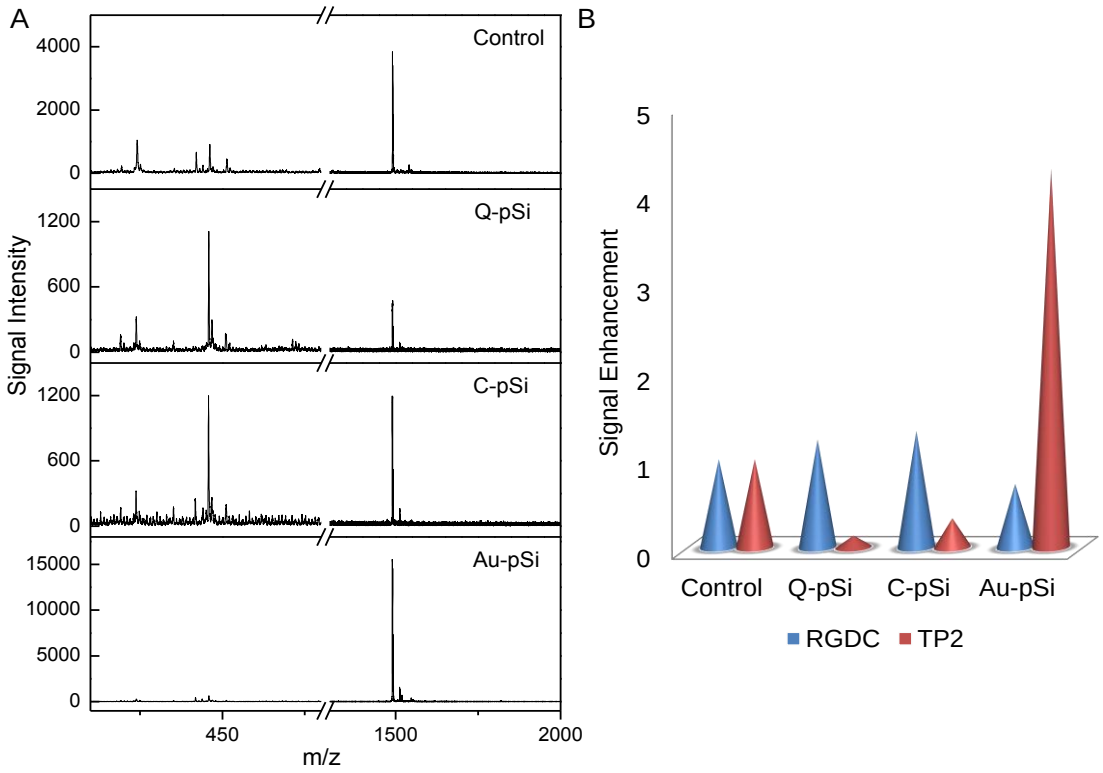

Fig. S6 MALDI mass spectra of RGDC (MW = 450 Da, pI = 6.2) and TP2 (MW = 1.5 kDa, pI = 10.8) on PSMPs with different modification. (A) mass spectrum of mixed sample (RGDC + TP2) captured by Q-pSi, C-pSi, and Au-pSi. Control experiment was tested on a standard MALDI plate. (B) Relative peak intensity enhancement of RGDC (blue) and TP2 (red) on Q-pSi, C-pSi, and Au-pSi.

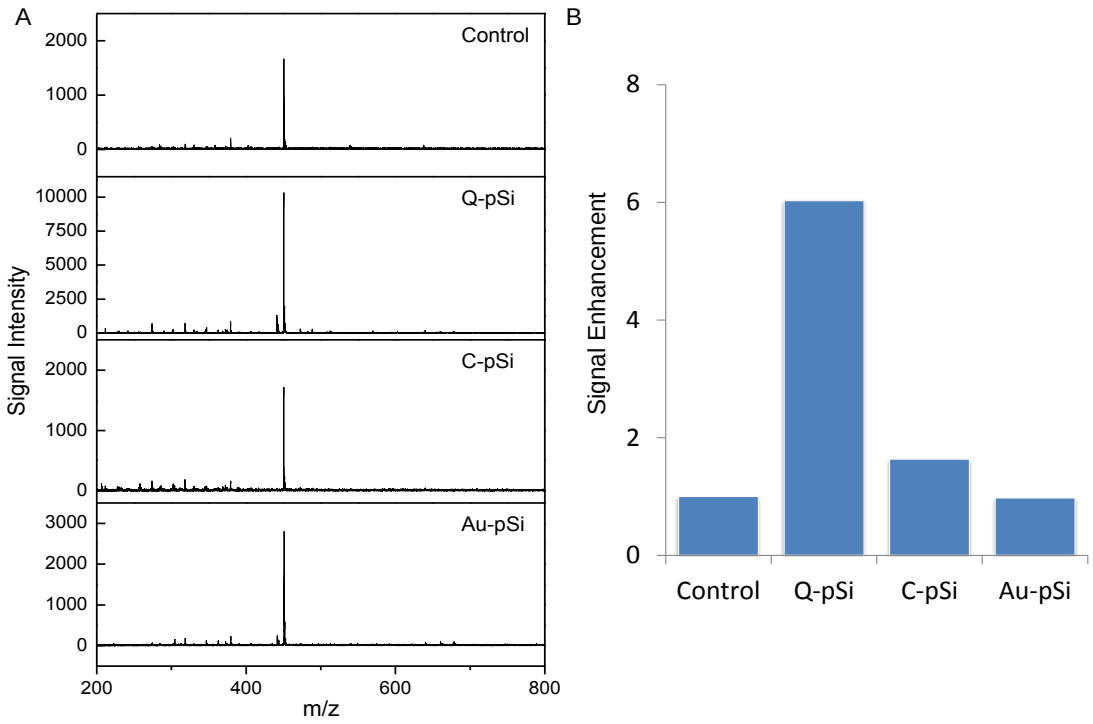

Fig. S7 MALDI mass spectra of RGDC (MW = 450 Da, pI = 6.2) on PSMPs with different surface modification. (A) Mass spectrum of RGDC captured by Q-pSi, C-pSi, and Au-pSi. Control experiment was tested on a standard MALDI plate. (B) Relative peak intensity enhancement of RGDC on Q-pSi, C-pSi, and Au-pSi compare with it on MALDI plate (control).

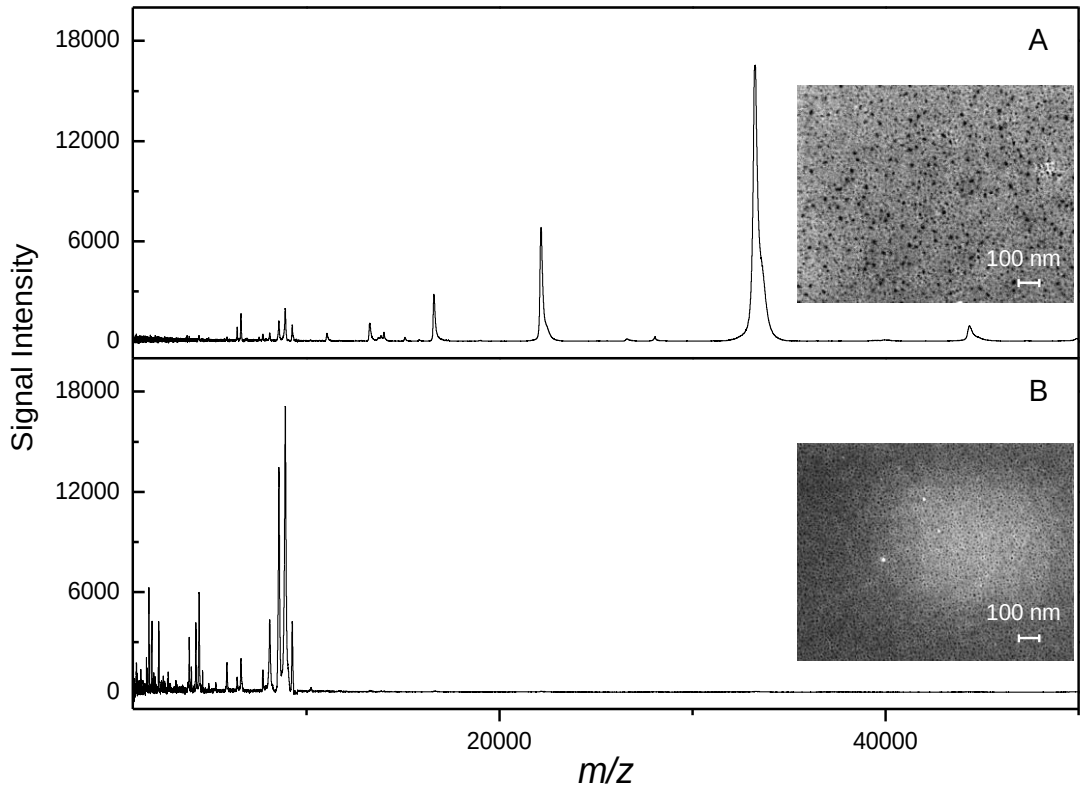

Fig. S8 MALDI-TOF mass spectra of serum sample tested on pSi with different pore sizes, which can be well controlled by changing the etching condition. (A) Mass spectra of serum sample obtained on pSi, which was electrochemically etched at a constant current of 80 mA for 60 s, the porosity of pSi is ~52.2 % and pore diameter is ~14 nm as indicated in SEM image of pSi (inset of A). (B) Mass spectra of serum sample obtained on pSi, which was electrochemically etched at a constant current of 10 mA for 60 s, the porosity of pSi is around 20.3 %, pore diameter is ~9 nm as indicated in the SEM image of pSi (inset of B).

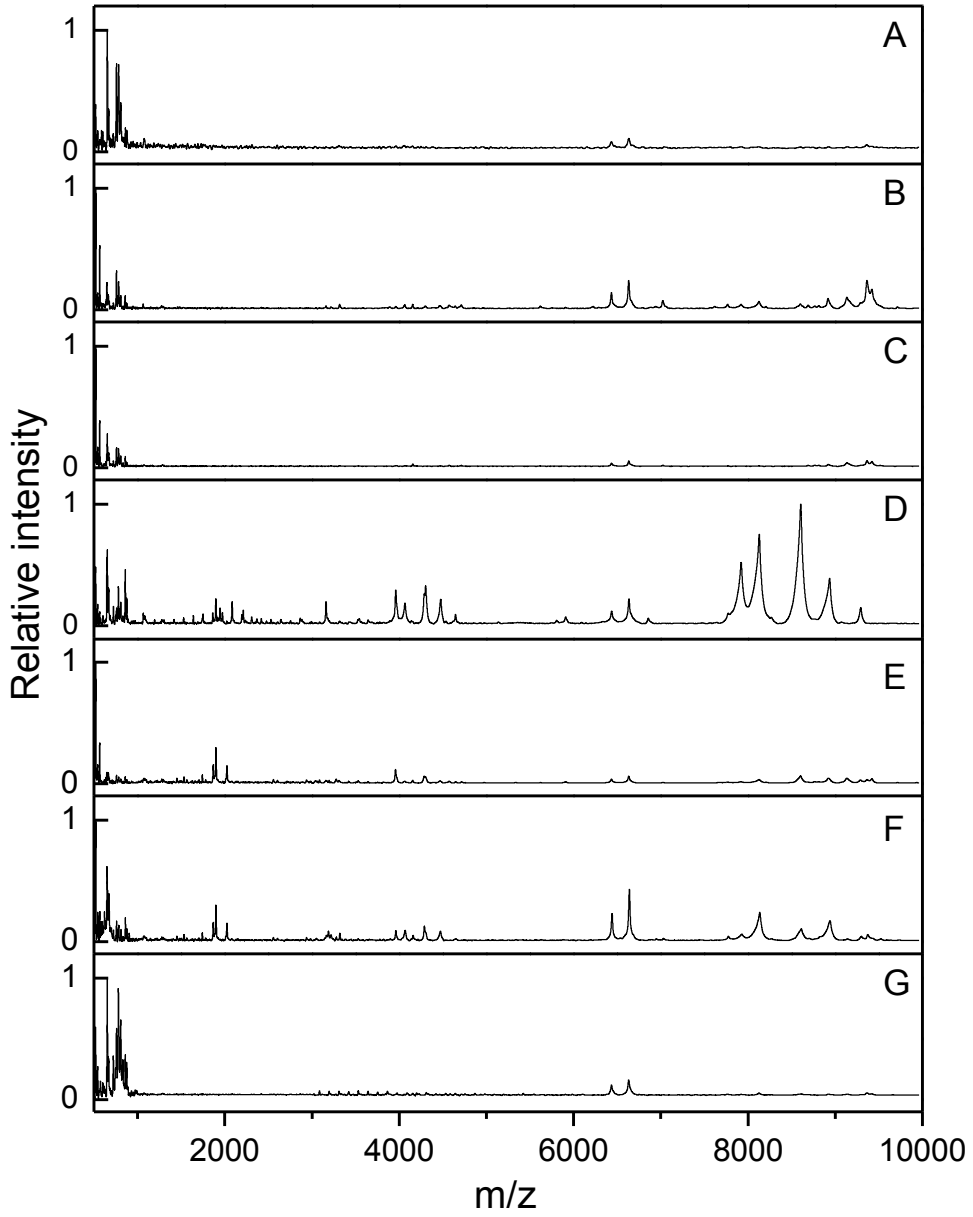

Fig. S9 MALDI-TOF mass spectra of serum sample with/ without elution step after treated by PSMPs with different surface chemistry. The normalized MS spectrum from the same serum sample detected on MALDI plate (A), MS spectrum of on-particle detection with three different surface functionalities: Q-pSi (B), C-pSi (D), and Au-pSi (F). And the MS spectrum of solution eluted from Q-pSi (C), C-pSi (E), and Au-pSi (G). The peptides captured in PSMPs were eluted with 10 μL of the elution solution (50 % ACN aqueous solution + 0.1 % TFA). As analysed by flexAnalysis software (Bruker Daltonics), the total peak number (S/ N > 3) in each spectral was calculated. There are 7 peaks in A, 46 peaks in B, 20 peaks in C, 115 peaks in D, 47 peaks in E, 42 peaks in F, and 20 peaks in G. The shown mass range is from 500 Da to 10 kDa.

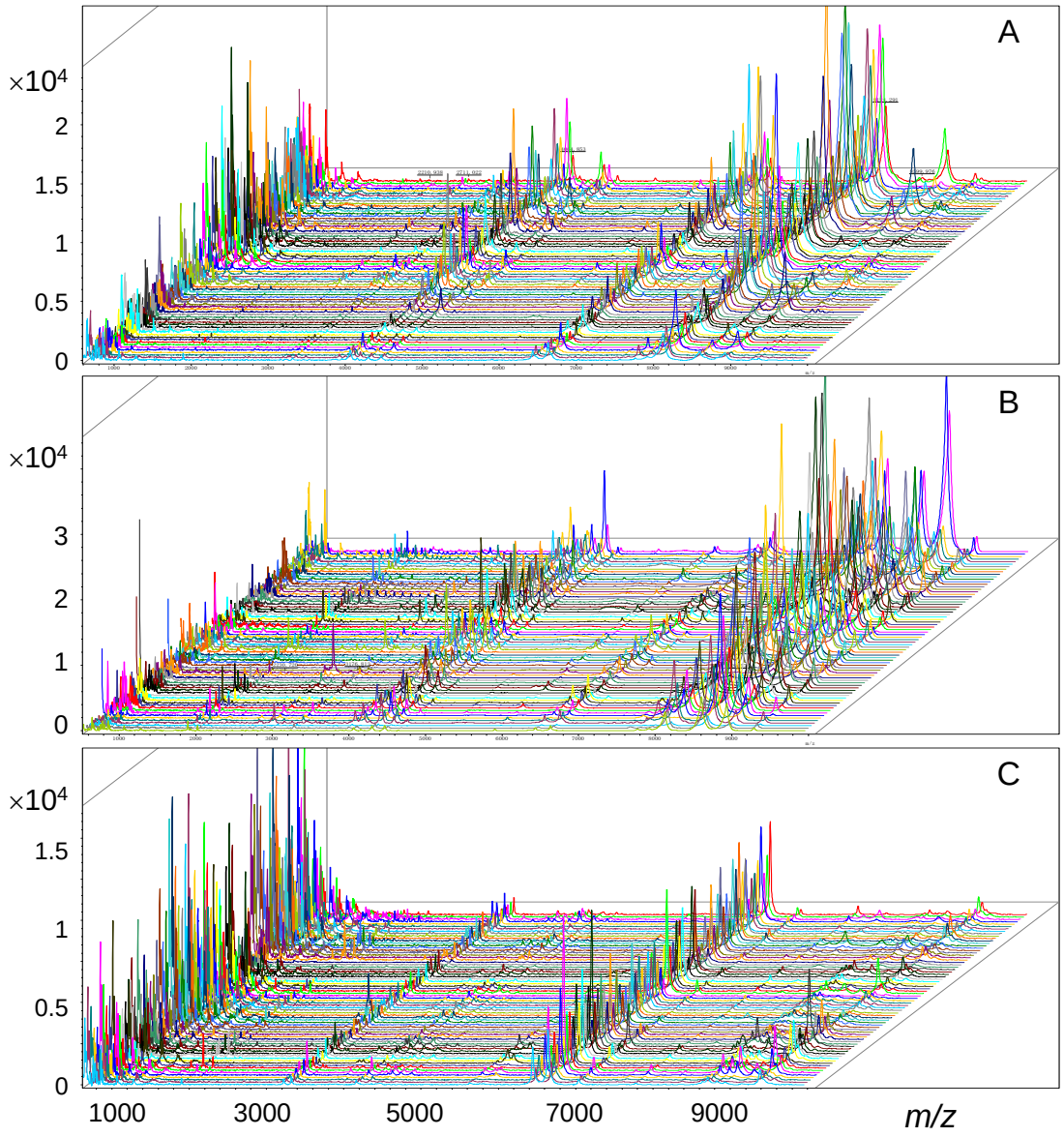

Fig. S10 MALDI-TOF mass spectral stacks of CRC, health and liver cancer samples. Each of the stacks contains 72 spectra without intensity normalization. Among them, 24 CRC patients, 24 liver cancer patients, and 24 healthy volunteers. A, B, and C indicates three types of PSMPs: Q-pSi, C-pSi, and Au-pSi, respectively.

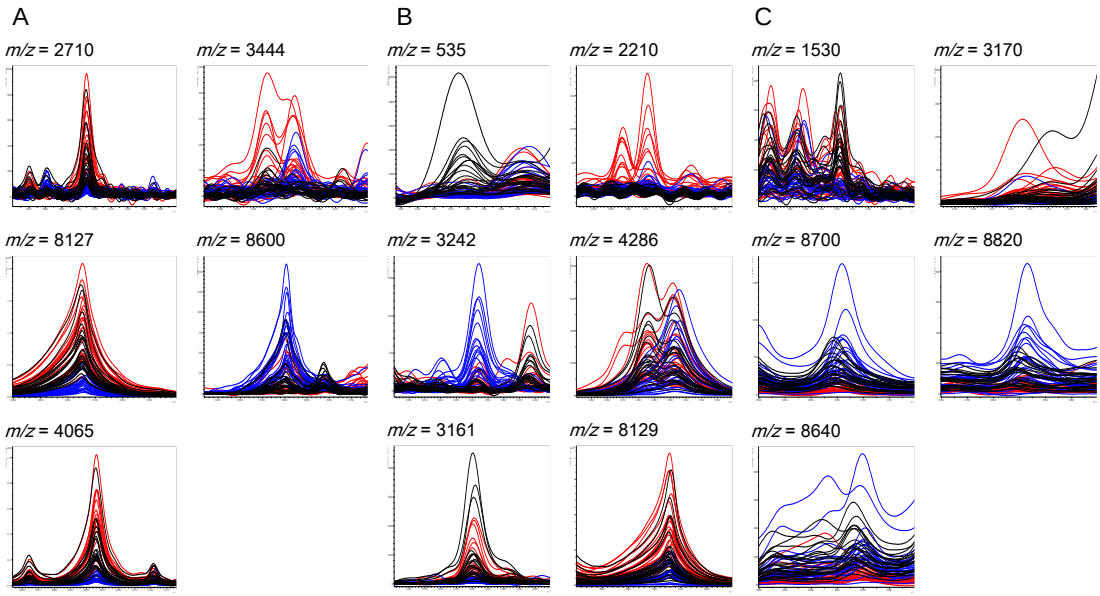

Fig. S11 Raw MALDI-TOF mass spectral overlays of the 16 most distinct peaks from serum peptide profiles obtained on three types of PSMPs. Each of the overlays contains 72 spectra without normalization; 24 CRC patients (red traces), 24 liver cancer patients (blue traces), and 24 healthy volunteers (black traces). The mono-isotopic mass (*m/z*) is shown for each peptide-ion peak. A, B, and C indicates Q-pSi, C-pSi, and Au-pSi, respectively.

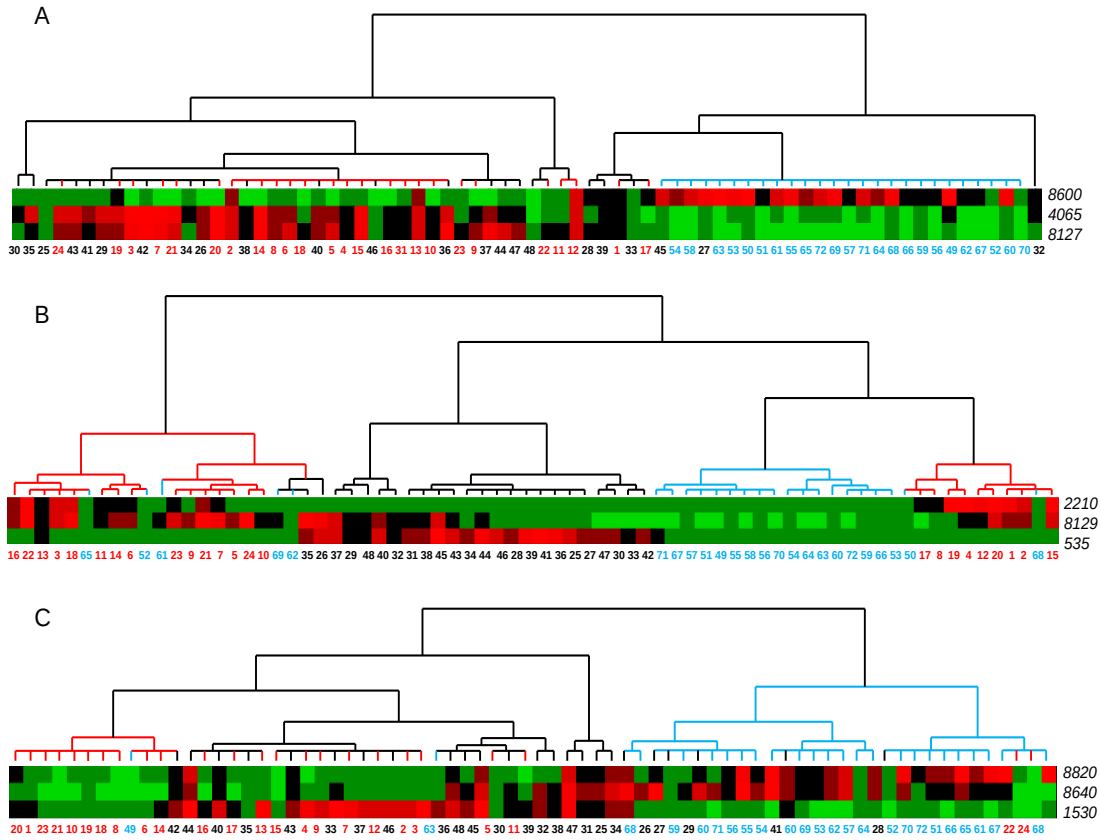

Fig. S12 Cluster analysis and heat map view of mass spectra data selected from Q-pSi (A), C-pSi (B), and Au-pSi (C). Serum samples are from CRC patients (No. 1~24), healthy volunteers (No. 25~48), and liver cancer patients (No. 49~72). The red, black and green color in the heat map represents high, medium, and low intensity/ absent peak intensity. Branches and clusters in the cluster tree are color-coded: CRC patients in red; healthy person controls in black and liver cancer patients in blue.

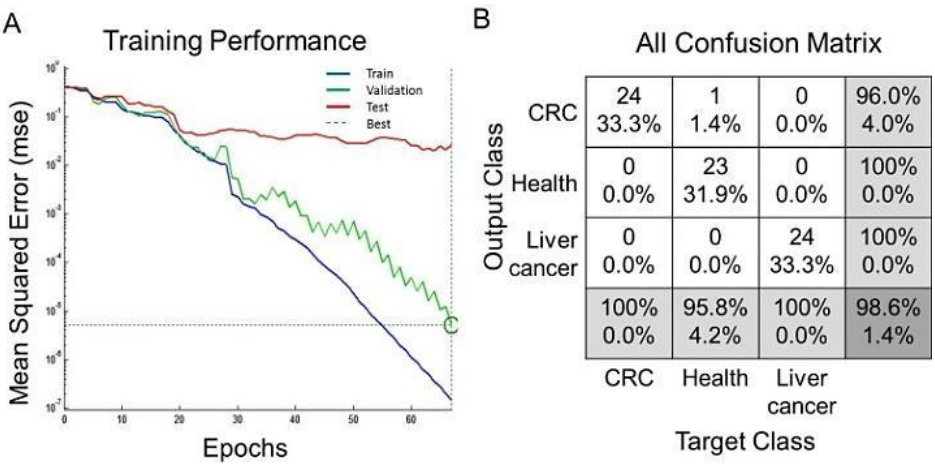

Fig. S13 (A) The training performance of the Artificial Neural Networks, with 70 % training data (blue), 15 % for validation (green), and 15 % for test (red). The best validation performance is 5.146e-006 at epoch 67. (B) The confusion matrix of the integrated Artificial Neural Networks. The hidden layer of the network was composed of five hidden nodes.

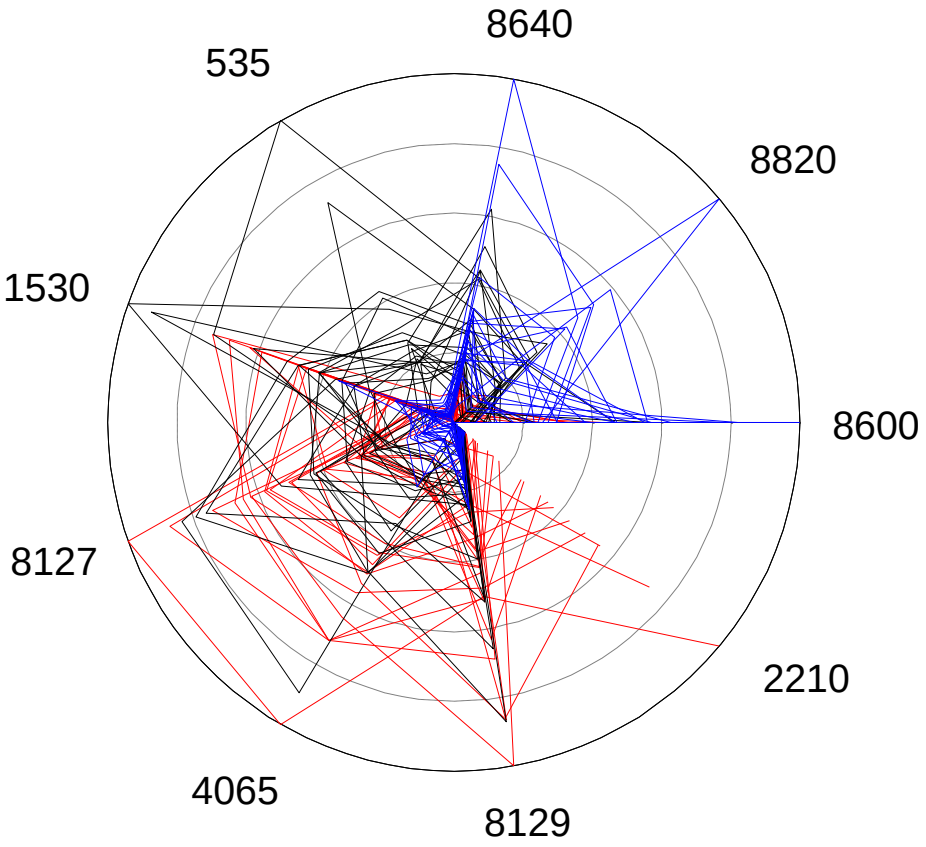

Fig. S14 Polar radiation pattern overlays of CRC (red traces, n = 24), health (black traces, n = 24) and liver cancer (blue traces, n = 24), polar-axis indicates m/z values of the 9 selected featured peaks.

3. Supplementary Table

Table S1. Demographic features of all the patients and healthy volunteers.

Sample type	Number of samples	Male/female ratio	Age, mean, years	Age, range, years
CRC	24	15/9	56	20-72
Liver cancer	24	13/11	60	38-81
Healthy Volunteers	24	12/12	58	51-79

Table S2. Top 40 features selected by ClinProTools software in profiles tested with Q-pSi.*

*The circled peaks were that matched with the result calculated using student's t test. The peaks in red circle were those selected for classification.

ClinProTools Version: 3.0 build 22
Number of peaks: 95
Sort Mode: p value tta

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	Ave3	StdDev1	StdDev2	StdDev3	CV1	CV2	CV3
X	84	8127.36	16.27	< 0.000001	< 0.000001	0.0356	29.56	27.69	13.29	6.71	11.68	4.47	22.72	42.2	33.68
X	55	4064.7	7.66	< 0.000001	< 0.000001	0.00573	12.67	11.47	5.01	4.76	4.66	1.95	37.57	40.57	38.85
X	38	2210.28	0.4	< 0.000001	< 0.000001	0.035	0.79	0.71	1.12	0.14	0.14	0.19	18.32	19.75	17.19
X	85	8603.03	3.34	< 0.000001	< 0.000001	< 0.000001	0.71	1.07	4.04	0.58	1.05	1.93	82.25	98.74	47.82
X	60	4301.87	1.33	< 0.000001	< 0.000001	< 0.000001	0.79	0.94	2.13	0.29	0.45	0.77	36.16	47.48	36.08
X	35	1897.01	1.49	< 0.000001	< 0.000001	0.0000166	1.05	1.18	2.53	0.48	0.56	0.88	45.63	47.78	34.73
X	37	2082.01	0.49	0.00000366	0.0000105	0.0128	0.81	0.71	1.21	0.28	0.17	0.32	35.17	24.21	26.24
X	40	2660.97	0.69	0.00000723	< 0.000001	< 0.000001	0.61	0.73	1.3	0.17	0.16	0.51	28.58	22.31	38.99
X	48	3444.12	0.52	0.0000073	0.00000148	< 0.000001	0.72	0.51	1.03	0.25	0.1	0.42	35.25	19.64	40.99
X	16	779.99	5.48	0.00000942	0.0000107	0.00000814	8.36	4.39	9.87	4.76	1.95	4.3	56.99	44.34	43.55
X	83	7920.37	4.01	0.0000183	0.0000393	0.000744	4.96	6.78	2.78	2.11	3.31	1.57	42.59	48.78	56.68
X	82	7808.75	0.51	0.0000183	0.000051	0.000564	1.14	1.29	0.78	0.41	0.38	0.23	36.07	29.85	29.75
X	45	3241.78	0.78	0.0000275	< 0.000001	< 0.000001	0.66	0.76	1.44	0.14	0.17	0.66	20.92	22.81	45.98
X	71	6228.03	0.14	0.0000284	0.000111	0.0565	0.32	0.44	0.46	0.09	0.16	0.08	27.67	36.17	17.12
X	18	807.02	2.97	0.0000307	0.000111	0.00000864	5.06	3.34	6.31	2.56	1.06	2.45	50.62	31.76	38.88
X	69	5864.16	0.17	0.000035	0.0000461	0.575	0.36	0.43	0.52	0.08	0.09	0.12	23.49	21.26	22.29
X	34	1740.54	0.67	0.0000418	0.00000101	< 0.000001	0.82	0.93	1.49	0.35	0.63	0.47	42.97	68.11	31.77
X	62	4616.39	1.42	0.0000488	0.00142	< 0.000001	2.07	1.94	0.65	1.55	1.6	0.15	74.84	82.59	23.05
X	75	6854.39	0.78	0.0000488	0.000051	< 0.000001	1.27	1.06	0.49	0.88	0.68	0.09	69.6	64.27	18.42
X	2	493.38	21.18	0.000137	0.00042	0.0289	59.38	38.2	44.64	17.6	9.29	15.31	29.63	24.32	34.29
X	41	2709.51	0.54	0.000153	0.000782	0.0000936	1.61	1.56	1.07	0.65	0.54	0.24	40.63	34.23	22.84
X	15	758.1	6.08	0.000267	0.000617	0.0000032	11.83	6.03	12.12	8.17	3.41	5.86	69.04	56.55	48.4
X	78	7184.25	0.37	0.000267	0.00133	< 0.000001	0.73	0.82	0.45	0.37	0.47	0.07	50.52	57.08	14.57
X	53	3960.61	1.52	0.000282	0.000215	0.0000803	2.23	3.05	1.53	0.9	1.53	0.65	40.31	50.03	42.31
X	36	1944.55	0.34	0.000287	0.000215	0.0325	0.83	0.82	1.16	0.24	0.2	0.3	28.46	24.66	26.05
X	63	4644.94	0.5	0.000331	0.00183	0.0567	1.39	1.49	1	0.4	0.56	0.29	28.93	37.59	29.5
X	17	785.73	3.69	0.000378	0.000883	0.00000806	7.24	4.14	7.83	4.72	2.12	3.56	65.25	51.3	45.44
X	54	3992.61	0.28	0.000415	0.00195	0.0111	1.01	1.08	0.8	0.25	0.3	0.16	24.42	28.01	20.56
X	42	3159.58	0.54	0.000548	0.00133	0.0000158	1.54	1.64	1.1	0.68	0.54	0.32	44.25	33.09	28.72
X	79	7478.43	1.31	0.00085	0.000376	< 0.000001	1.27	1.79	0.48	1.24	1.81	0.11	97.91	101.06	23.62
X	65	5338.27	0.13	0.00111	0.000782	0.11	0.46	0.58	0.59	0.11	0.15	0.11	22.82	24.94	19.13
X	23	1064.48	3.41	0.00122	0.0096	< 0.000001	4.29	6.18	2.78	2.08	4.49	1.27	48.41	72.59	45.7
X	10	647.3	11.15	0.00198	0.00905	0.000723	19.06	23.72	12.57	7.03	14.56	6.59	36.89	61.39	52.43
X	19	852.9	3.59	0.00211	0.0222	< 0.000001	5.05	6.98	3.39	2.48	4.89	1.41	49.03	70.11	41.71
X	29	1287.4	0.3	0.00223	0.0107	0.323	1.09	1.39	1.37	0.25	0.55	0.25	23.25	39.4	18.19
X	68	5802.23	0.36	0.00237	0.0768	< 0.000001	0.98	0.94	0.62	0.54	0.53	0.14	55.04	56.32	21.88
X	86	8749.78	0.19	0.00237	0.000596	0.000531	0.37	0.35	0.54	0.2	0.13	0.19	55.02	36.71	36.11
X	5	518.88	4.91	0.00363	0.00214	0.95	18.1	13.19	15.96	4.04	4.62	4.05	22.33	35.03	25.37
X	7	541.2	1.54	0.00365	0.00788	< 0.000001	5.25	4.16	5.71	1.18	0.91	3.37	22.4	21.81	59.07
X	31	1297.03	0.48	0.00494	0.0298	0.000144	1.12	1.6	1.36	0.25	0.75	0.34	22.01	46.81	24.68

Table S3. Top 40 features selected by ClinProTools software in profiles tested with C-pSi.*

*The circled peaks were that matched with the result calculated using student's t test. The peaks in red circle were those selected for classification.

ClinProTools Version: 3.0 build 22
Number of peaks: 95
Sort Mode: p value tta

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	Ave3	StdDev1	StdDev2	StdDev3	CV1	CV2	CV3
X	90	8268.3	1.68	< 0.000001	< 0.000001	0.000831	2.37	1.78	0.69	0.86	0.85	0.36	36.34	47.91	52.32
X	89	8128.99	25.64	< 0.000001	0.00000152	0.00000875	38.6	25.71	12.96	15.88	14.74	6.84	41.15	57.33	52.77
X	45	3243	1.99	< 0.000001	< 0.000001	< 0.000001	0.72	0.68	2.67	0.52	0.3	1.25	72.02	43.57	46.76
X	44	3160.64	2.75	0.0000119	< 0.000001	< 0.000001	2.67	3.68	0.93	1.63	2.82	0.27	61.01	76.65	29.37
X	88	7921.62	8.55	0.0000141	0.0000184	< 0.000001	11.91	11.58	3.37	7.67	8.17	2.82	64.4	70.55	83.69
X	80	6855.46	1.1	0.0000201	0.000166	< 0.000001	1.6	1.51	0.5	1.04	1.01	0.37	65.12	66.56	74.86
X	56	4065.34	5.83	0.0000366	0.0000163	0.0000019	9.23	5.63	3.39	4.56	3.19	1.98	49.42	56.66	58.44
X	71	5803.61	0.52	0.0000431	0.00168	< 0.000001	1.06	1.04	0.54	0.59	0.48	0.16	55.92	46.45	29.62
X	36	2022.81	3.62	0.000105	0.00000151	< 0.000001	1.2	2.39	4.82	1	1.44	3.41	83.54	60.03	70.73
X	60	4285.53	4.21	0.000122	0.000206	0.00000663	6.46	7.47	3.26	3.83	4.19	1.61	59.24	56.01	49.46
X	66	5250.98	0.32	0.000256	0.0000027	0.00000239	0.41	0.43	0.72	0.1	0.09	0.28	25.31	21.09	38.55
X	87	7812.48	0.97	0.000328	0.000562	< 0.000001	1.83	1.65	0.86	1.1	0.91	0.43	60.12	55.01	50.08
X	51	3814.89	0.63	0.000328	0.000142	< 0.000001	0.74	1.36	0.88	0.29	0.52	0.52	39.58	38.4	58.83
X	34	1936.4	0.65	0.000814	0.000364	0.0000567	0.87	1.31	1.52	0.37	0.43	0.74	43.09	32.57	48.47
X	8	535.33	5.92	0.00105	< 0.000001	< 0.000001	1.37	7.29	3.37	1.13	7.09	3.4	82.6	97.24	100.72
X	18	780.23	4.84	0.00105	0.000169	< 0.000001	4.3	3.11	7.95	4.12	1.7	4.81	95.67	54.75	60.55
X	58	4136.12	0.29	0.00105	0.00198	0.0843	1.01	0.94	0.72	0.3	0.27	0.18	29.34	28.98	25.4
X	84	7479.91	0.84	0.00157	0.0548	< 0.000001	1.25	0.93	0.41	1.07	0.91	0.12	85.88	97.54	30.12
X	54	3959.2	6.22	0.00172	0.000231	< 0.000001	6.91	9.71	3.5	4.67	7.16	3.16	67.56	73.72	90.4
X	82	7185.67	0.28	0.00198	0.0889	< 0.000001	0.61	0.45	0.33	0.33	0.26	0.08	54.33	56.12	23.98
X	43	2992.64	0.68	0.00231	0.000206	< 0.000001	0.68	0.64	1.32	0.37	0.3	0.72	55.22	46.96	54.9
X	48	3527.51	0.41	0.00255	0.00125	< 0.000001	0.78	1.08	0.67	0.22	0.46	0.17	27.99	42.55	25.58
X	86	7769.45	0.64	0.00256	0.00244	0.182	1.89	1.95	1.32	0.61	0.64	0.55	32.42	32.64	41.69
X	46	3276.33	0.61	0.00256	0.000238	< 0.000001	0.86	1.29	0.68	0.84	0.67	0.18	97.52	52.41	27.11
X	55	3996.04	0.35	0.0034	0.00902	0.00801	1.19	1.15	0.85	0.43	0.45	0.24	36.06	38.68	28.4
X	27	1075.99	0.51	0.00375	0.0033	0.0198	0.93	1.44	1.32	0.39	0.59	0.47	41.93	41.09	35.42
X	20	807.04	3.08	0.00393	0.000425	< 0.000001	3.09	2.32	5.4	3.05	1.18	3.58	98.51	50.91	66.26
X	40	2861.82	2.52	0.00393	< 0.000001	< 0.000001	1.14	0.81	3.33	0.4	0.17	7.56	35.05	20.98	226.92
X	30	1741	0.78	0.00393	0.00477	0.000543	1.06	1.46	1.84	0.45	0.65	0.93	42.72	44.44	50.48
X	63	4617.45	0.56	0.00419	0.00532	< 0.000001	1.22	1.03	0.66	0.7	0.58	0.34	57.84	56.81	51.97
X	28	1088.89	0.52	0.00457	0.00729	0.000134	1.11	1.64	1.52	0.41	0.57	0.68	37.12	34.75	44.78
X	24	1048.43	0.57	0.00461	0.00902	0.0000133	0.97	1.54	1.32	0.32	0.71	0.67	32.93	46.29	51.1
X	91	8604.77	11.91	0.00499	0.0027	0.025	17.73	19.64	29.63	7.24	10.3	13.27	40.84	52.45	44.78
X	19	785.79	2.38	0.0092	0.00244	< 0.000001	2.81	2.34	4.72	3.15	1.23	2.92	111.86	52.74	61.96
X	75	6289.3	0.13	0.0092	0.0104	0.505	0.36	0.37	0.49	0.14	0.13	0.13	37.86	34.99	27.24
X	1	493.64	12.09	0.00933	0.00579	0.0000105	19.59	31.68	23.57	10.6	12.67	15.04	54.1	39.99	63.82
X	38	2211.26	0.66	0.00933	0.0581	< 0.000001	1.54	0.89	1.15	1.31	0.19	0.4	84.61	21.76	35.21
X	4	517.43	3.14	0.0131	0.0176	0.0000146	5.68	8.82	7.96	2.82	3.62	5.49	49.67	40.99	69.05

Table S4. Top 40 features selected by ClinProTools software in profiles tested with Au-pSi.*

*The circled peaks were that matched with the result calculated using student's t test. The peaks in red circle were those selected for classification.

ClinProTools Version: 3.0 build 22
Number of peaks: 191
Sort Mode: p value tta

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	Ave3	StdDev1	StdDev2	StdDev3	CV1	CV2	CV3
X	181	8699.7	1.98	0.00000314	0.00000512	< 0.000001	0.76	1.58	2.74	0.36	0.68	1.68	48	42.95	61.23
X	60	1532.18	1.27	0.00000314	0.00000512	0.00000356	1.77	2.27	1	0.88	0.9	0.29	49.61	39.55	29.21
X	183	8819.89	1.04	0.00000314	0.00000724	< 0.000001	0.41	0.76	1.44	0.17	0.27	0.87	41.45	34.95	60.02
X	180	8635.69	0.93	0.00000314	0.00000512	0.0000199	0.63	1.27	1.56	0.27	0.44	0.85	42.08	34.99	54.26
X	125	4410.22	0.29	0.000114	0.000754	0.0000282	0.45	0.57	0.74	0.09	0.12	0.26	19.83	20.59	35.09
X	124	4348.49	0.23	0.000191	0.000241	0.0113	0.5	0.65	0.73	0.11	0.13	0.2	22.52	20.37	27.39
X	146	5799.82	0.35	0.000281	0.000499	< 0.000001	0.44	0.59	0.79	0.12	0.12	0.38	26.16	19.76	48.16
X	190	9426.97	1.1	0.000321	0.0000106	< 0.000001	1.06	0.82	1.91	0.32	0.37	0.93	29.94	45.7	48.54
X	95	3170.35	0.61	0.000321	0.000099	< 0.000001	1.19	0.93	0.58	0.56	0.65	0.14	46.77	70.3	23.95
X	50	1298.76	0.78	0.000385	0.00083	0.0626	1.98	2.17	1.39	0.8	0.52	0.53	40.42	23.72	38.33
X	52	1315.96	0.48	0.000385	0.000903	0.456	1.26	1.43	0.95	0.42	0.31	0.32	32.9	21.9	33.76
X	19	670.45	5.47	0.000607	0.00165	0.761	14.31	13.2	8.85	5.78	3.03	3.54	40.41	22.99	40.05
X	189	9371.15	4.8	0.000699	0.0000152	< 0.000001	2.92	2.18	6.98	1.52	1.43	4.45	51.99	65.72	63.67
X	43	1087.77	1.34	0.00086	0.00146	0.1	3.1	3.33	2	1.38	0.99	0.97	44.38	29.56	48.61
X	165	7064.35	0.31	0.0012	0.000281	0.000257	0.45	0.44	0.75	0.16	0.15	0.29	35.79	33.91	38.3
X	145	5756.25	0.41	0.00133	0.0000171	< 0.000001	0.46	0.63	0.87	0.13	0.14	0.69	28.54	22.89	79.15
X	31	860.03	10.76	0.00204	0.00992	0.0211	25.42	23.11	14.65	14.68	7.71	6.7	57.75	33.37	45.71
X	41	1071.38	1.59	0.00227	0.00358	0.00195	4.08	3.94	2.49	2.02	1.2	1.21	49.57	30.54	48.71
X	164	7028.21	1.81	0.00297	0.000241	< 0.000001	1.46	1.21	3.02	0.73	0.61	1.9	50.04	50.16	62.79
X	45	1104.11	0.78	0.00297	0.00161	0.0494	1.72	2.01	1.23	0.63	0.68	0.58	36.53	33.85	47.13
X	56	1488.38	0.51	0.00297	0.00145	0.00371	1.67	1.61	1.16	0.77	0.32	0.4	45.91	19.85	34.66
X	33	876.16	4.69	0.00328	0.00585	0.258	12.36	12.77	8.08	5.7	4.02	3.93	46.17	31.51	48.63
X	96	3187.77	1.11	0.00328	0.00562	< 0.000001	1.64	1.95	0.84	1.04	1.89	0.32	63.06	97.12	38.2
X	177	8573.05	0.47	0.00328	0.00022	< 0.000001	0.43	0.76	0.91	0.3	0.3	0.68	68.99	39.08	74.54
X	182	8773.34	0.21	0.00416	0.00585	0.000588	0.4	0.52	0.61	0.13	0.13	0.25	32.73	25.09	41.59
X	24	759.29	3.66	0.00444	0.00198	< 0.000001	5.35	3.96	7.63	3.46	2.16	3.68	64.73	54.59	48.31
X	16	648.31	11.78	0.00483	0.0137	0.573	35.76	37.79	26.01	15.77	8.41	11.11	44.11	22.26	42.7
X	51	1309.08	0.48	0.00514	0.00537	0.143	1.66	1.57	1.17	0.56	0.41	0.39	33.95	25.94	33.05
X	1	439.78	2.34	0.00517	0.0043	0.00000172	3.28	3.95	5.62	1.13	1.92	2.55	34.37	48.63	45.32
X	64	1710.54	0.29	0.00526	0.00145	0.0000178	1.08	1	0.79	0.4	0.2	0.2	36.88	19.9	25.49
X	144	5714.48	0.16	0.00526	0.000654	< 0.000001	0.34	0.43	0.5	0.1	0.09	0.23	28.2	19.89	45.56
X	48	1277.4	1.48	0.00633	0.00828	0.0515	4.01	4.18	2.71	2.11	1.26	1.37	52.48	30.19	50.5
X	17	654.17	11.04	0.00647	0.0227	0.000376	23.84	18.21	12.81	14.44	7.45	5.83	60.56	40.91	45.56
X	188	9330.53	0.78	0.00855	0.000099	< 0.000001	0.73	0.61	1.39	0.36	0.22	0.97	49.4	35.34	69.69
X	49	1293.49	1.07	0.00868	0.00558	0.0709	2.71	3.05	1.98	1.17	0.98	0.96	43.13	32.17	48.62
X	18	664.52	5.17	0.00892	0.0142	0.535	18.6	18.85	13.68	7.19	4.84	5.04	38.67	25.67	36.85
X	143	5622.64	0.5	0.0111	0.000859	< 0.000001	0.72	0.71	1.21	0.3	0.2	0.61	42.09	28.58	50.65
X	21	679.83	1.31	0.0113	0.0102	0.292	4.38	4.19	3.07	1.61	1.13	1.24	36.67	26.93	40.38
X	185	8925.29	1.03	0.0113	0.00558	< 0.000001	1.19	0.87	1.9	0.85	0.45	1.31	71.72	51.56	69.25
X	29	843.92	0.8	0.0119	0.00207	0.00118	2	1.93	1.2	1.08	0.68	0.8	53.95	34.97	66.45
X	118	4158.27	0.71	0.012	0.00537	0.017	1.22	1.45	1.94	0.51	0.54	0.79	41.87	37.5	40.75

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