Supplementary Information

Copper adhesive tape attached to reverse side of non-conductive glass slide to achieve protein MALDI-imaging in FFPE-tissue sections

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Supplementary Information--Experimental Section

Chemicals and Materials. Sinapinic acid (SA), α-cyano-4-hydroxycinnamic acid (CHCA), bradykinin (1-7) peptide, cytochrome c (Cyt c), bovine serum albumin (BSA), transferrin (TFR), hydrogen trichloride, xylene, ethanol, methanol, LC/MS-grade acetonitrile (ACN), trifluoroacetic acid (TFA), and hematoxylin & eosin (H&E) staining solutions were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water used throughout the experiments was obtained from a Milli-Q system (Millipore USA). Copper adhesive tapes (part no. 1181) were purchased from 3M (St. Paul, MN). The rat brains were derived from 8-week-old adult male Sprague-Dawley rats (Shanghai Super-B&K Laboratory Animal Corp. Ltd, Shanghai, China). The use of animal organs for this study was approved by the Ethics Committee of College of Life and Environmental Sciences, Minzu University of China.

Preparation of Protein and Peptide Standards. Standard solutions of proteins (Cyt c, BSA, and TFR) were prepared at a concentration of 1 mM in ultrapure water. And 10 μg/mL bradykinin (1-7) peptide were prepared in ultrapure water containing 0.1% TFA.

Tissue Sample Preparation. The rats were sacrificed by decapitation and immediately dissected to obtain the brains for formalin fixation and/or fresh frozen (FF) tissue sectioning. For formalin fixation, the rat brain tissues were fixed with 4% formalin in a hydrogen trichloride buffer (pH 7.4) for 24 h and dehydrated by the ascending concentration gradient elution of ethanol and xylene. The brain is then embedded in paraffin wax and stored at room temperature after cooling,[1] and then the rat brain FFPE tissue was sectioned into 10 μm thick slices in a Leica CM1860 cryostat (Leica Microsystems Inc., Wetzlar, Germany) at room temperature (25 ^{o}C) without starting the cooling program and these serial tissue slices were then mounted

on the AnchorChip target plate (Bruker Daltonics, Bremen, Germany) and different kinds of glass slides, including two non-conductive glass slides and one ITO coated glass slide (Bruker Daltonics, Bremen, Germany) for the following MALDI-TOF MS detection. For FF tissue sectioning, the fresh rat brain was flash-frozen by slowly immersing them in liquid nitrogen to avoid shattering after harvest, followed by the cryo-sectioning at -20 ºC into 10 μm thick slices in the Leica CM1860 cryostat. The tissue sections were then immediately thaw-mounted on the non-conductive glass slides.

Paraffin Removal. Paraffin was removed by twice washing in xylene for 3 min. Next, the slides were immersed in a series of ethanol aqueous solutions with decreasing concentrations (*i.e.*, 100%, 95%, and 70% ethanol). Among, FFPE tissue slides were immersed in 100% ethanol for twice 3-min wishing, once 1-min washing was performed in both of 95% and 70% ethanol aqueous solutions. Finally, FFPE tissue slides were immersed in water for twice 3-min rehydration before drying at room temperature.[2]

Copper adhesive tape attached to the reverse side of glass slide. Serial copper adhesive tapes with the cut length of 87 mm × 25 mm were symmetrically attached on the reverse side of the normal non-conductive glass slides (75 mm × 25 mm × 1 mm), the excess parts of copper adhesive tapes were then folded up and sticked onto the front of glass slides (**Fig. 1**). A UNI-T UT33D$^+$ Multimeter (UNI-Trend Technology Corp. Ltd, Dongguan, China) was used to evaluate the copper adhesive tape attached glass slides electrical conductivity.

Matrix Coating. SA was prepared at 20 mg/mL in 80:20 ACN: water, containing 0.2% TFA. CHCA was prepared at 1.7 mg/mL in 50:50 ACN/water containing 0.1% TFA. Using a GET-Sprayer (I) (HIT Co., Ltd, Beijing, China) sprayed the matrix

solution onto the tissue sections. The matrix solution was sprayed on the tissue sections for five cycles (5 s spray, 60 s drying time), and a thin layer of matrix was pre-planted. After air-drying in the ventilated smoke hood, the matrix solution was uniformly sprayed on the same tissue section and recirculated for 40 times. The optical images of the tissue sections were taken using an Epson Perception V550 Photo Scanner (Suwa, Japan).

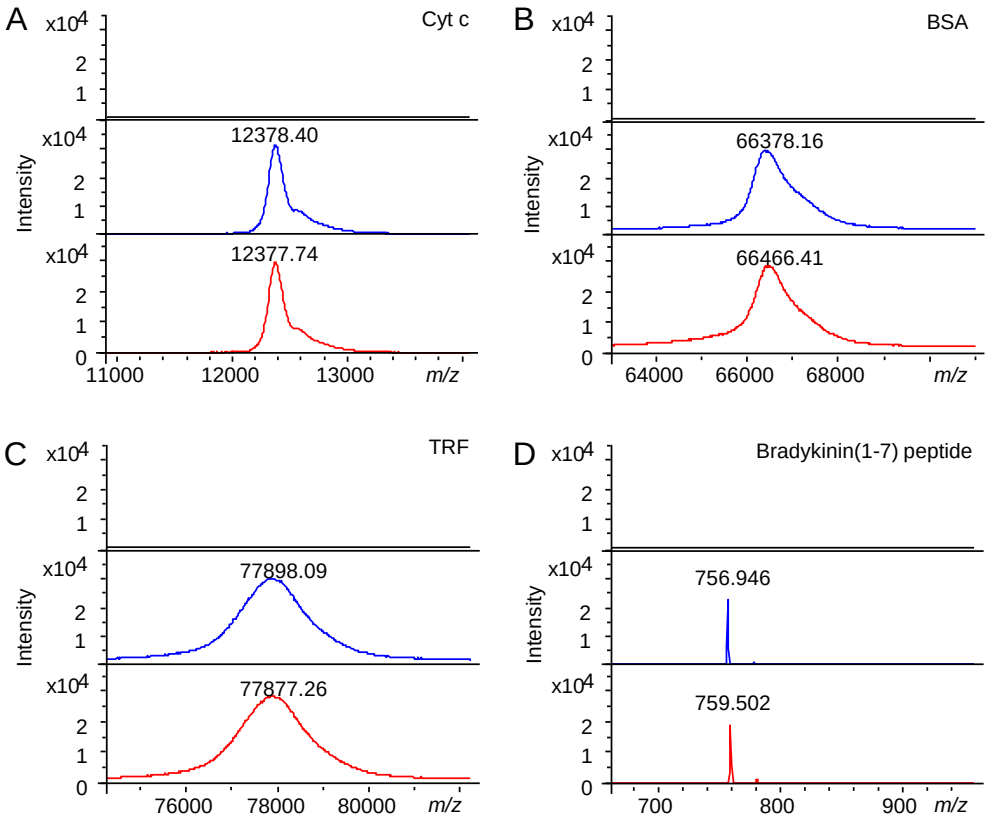
MALDI-MS. MALDI-TOF-MSI was performed in the positive-ion detection mode with the mass range of *m/z* 2500 to 35000, by using the Bruker Autoflex Speed MALDI-TOF/TOF Mass Spectrometer (Bruker Daltonics, Billerica, MA) equipped with a 2000-Hz solid-state Smartbeam Nd: YAG UV Laser (355 nm) (Azura Laser AG, Berlin, Germany). To obtain MALDI-MS profiling data, mass spectra were recorded from an accumulation of 20 laser scans, each scan was accumulated from 500 laser shots. For the acquisition of MALDI-MS imaging data, 100-μm laser grating step size was used to detect endogenous proteins in rat brain tissue sections *in-situ* and each scan (pixel) was accumulated from 500 laser shots. MS profiling data analysis was performed by Bruker *FlexAnalysis* v.3.4 software (Bruker Daltonics, Bremen, Germany). Image reconstruction was performed by using the Bruker *FlexImaging* v.4.1 software (Bruker Daltonics, Bremen, Germany).

Hematoxylin and Eosin Staining. After MALDI-MSI analysis, the tissue sections were washed by methanol to remove the matrix layer, and followed by hematoxylin and eosin (H&E) staining according to a previous study.[3] The H&E stained sections were also scanned using an Epson Perception V550 Photo Scanner to obtain standard histological optical images.

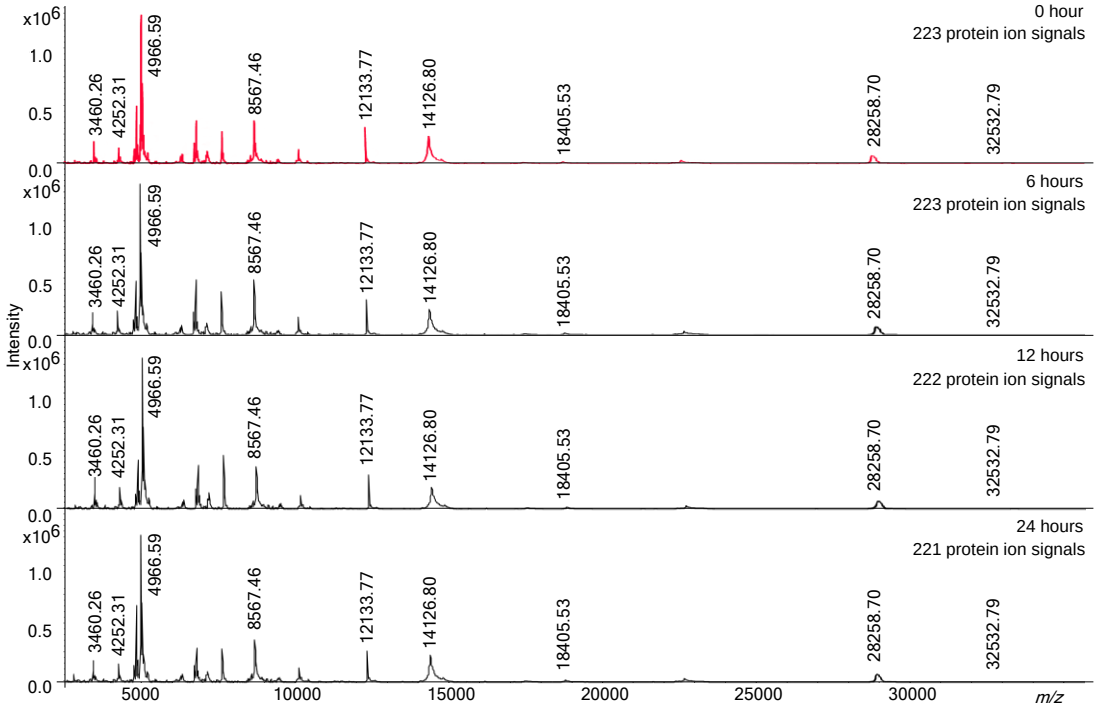
Supplementary Information--Results and Discussion

The three proteins (*i.e.,* cytochrome c (Cyt c), bovine serum albumin (BSA) and transferrin (TFR)) were selected as protein standards to evaluate the effect of the copper adhesive tape to the reverse side of glass slide method for MALDI-TOF MS detection. One μL of each standard solution was mixed with another 1 μL of SA matrix solution and spotted onto non-conductive glass slide, ITO coated glass slide, and copper adhesive tape attached glass slide, respectively, to form analyte-matrix co-crystallization for the following MALDI-MS analysis. Fig. S1A, S1B and S1C shows the mass spectra of the three selected protein standards (*i.e.*, Cyt c, BSA, and TFR) detected on three kinds of glass slides by MALDI-TOF MS in the positive-ion mode using SA as a matrix. The comparison shows that the protein standards on copper adhesive tape attached glass slides and ITO coated glass slides can be successfully detected by MALDI-TOF MS, while no obvious protein ion signal can be detected on non-conductive glass slides. The results clearly demonstrated the potential feasibility of this new method for *in-situ* detection and imaging of protein from FFPE tissue sections on non-conductive glass slides by MALDI-MSI. In addition, to further evaluate our new method for the detection of smaller proteins or peptides, 10.0 μg/mL bradykinin (1-7) peptide was used as a low mass weight standard for MALDI-MS on glass-slide detection using CHCA as the matrix. As shown in Fig. S1D, it can be obviously found that our new method also performed very well in the detection of peptide, indicating the potential application of this new method in the *in-situ* detection and imaging of proteins or peptides in FFPE tissue sections attached on non-conductive glass slides.

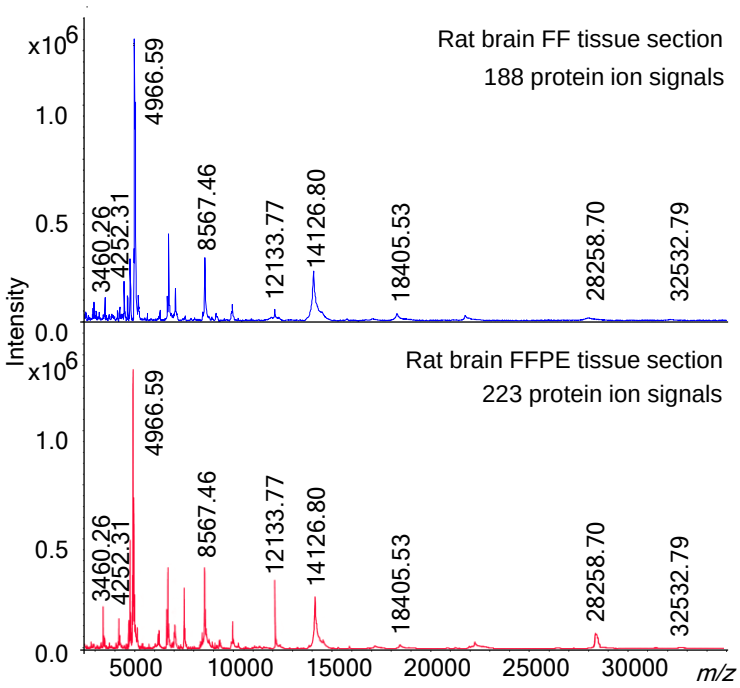
Supporting Information Figures



Supplementary Information Fig. S1 Comparison of MALDI-TOF mass spectra of selected protein and peptide standards detected in the positive-ion mode using SA and CHCA as the matrices, respectively. The black spectra were detected on a non-conductive glass slide, the blue spectra were detected on an ITO coated glass slide, and the red spectra were detected on a copper adhesive tape attached glass slide.



Supplementary Information Fig. S2 Comparison of mass spectra of protein detected in a rat brain FFPE tissue section with different high-vacuum storages (0, 6, 12, and 24 h) by MALDI-TOF MS in the positive-ion mode using SA as the matrix.



Supplementary Information Fig. S3 Comparison of mass spectra of protein *in-situ* detection from rat brain FFPE tissue and rat brain FF tissue on copper adhesive tape attached glass slides by MALDI-TOF MS in the positive-ion mode using SA as the matrix.

Supplementary Information Table

Supplementary Information Table S1. Comparison of protein ion signal detection in rat brain FFPE or FF tissue sections attached onto non-conductive glass slides (FFPE), ITO glass slides (FFPE), AnchorChip target plate (FFPE), and copper adhesive tape attached glass slide (FFPE and FF) by MALDI-TOF/TOF MS in the positive-ion mode using SA as a matrix.

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
2504.87	✗	✓	✓	✓	✓
2517.36	✗	✓	✓	✓	✓
2534.64	✗	✓	✓	✓	✓
2637.31	✗	✓	✗	✓	✓
2698.67	✗	✓	✓	✗	✓
2730.15	✗	✓	✓	✗	✓
2831.85	✗	✓	✓	✓	✓
2851.74	✗	✓	✗	✓	✓
2872.34	✗	✓	✗	✗	✓
2945.69	✗	✓	✓	✗	✗
2969.96	✗	✓	✓	✓	✓
2984.12	✗	✓	✓	✓	✓
3007.57	✗	✓	✓	✓	✓
3152.62	✗	✓	✓	✓	✓
3189.06	✗	✓	✓	✓	✓
3328.45	✗	✓	✓	✓	✓
3349.10	✗	✓	✗	✓	✓
3362.74	✗	✗	✓	✓	✓
3381.85	✗	✗	✓	✓	✓
3421.48	✗	✓	✓	✗	✓
3460.26	✗	✓	✓	✓	✓

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
3481.96	✗	✓	✓	✓	✗
3498.50	✗	✓	✓	✓	✓
3520.24	✗	✗	✓	✓	✓
3534.76	✗	✓	✓	✓	✓
3547.45	✗	✓	✗	✓	✓
3558.82	✗	✗	✗	✓	✓
3571.68	✗	✗	✗	✓	✓
3585.45	✗	✗	✓	✓	✓
3637.21	✗	✗	✓	✓	✓
3722.74	✗	✓	✗	✗	✓
3771.23	✗	✗	✗	✓	✓
3791.56	✗	✗	✗	✓	✓
3856.48	✗	✓	✓	✓	✓
3867.98	✗	✓	✗	✗	✓
4099.40	✗	✗	✗	✓	✓
4113.44	✗	✗	✗	✓	✓
4136.47	✗	✗	✗	✓	✓
4181.14	✗	✗	✓	✓	✓
4213.69	✗	✗	✗	✓	✓
4237.19	✗	✓	✓	✓	✗
4252.31	✗	✓	✓	✓	✓
4275.03	✗	✓	✓	✓	✓
4288.24	✗	✓	✓	✓	✓
4304.89	✗	✗	✓	✓	✓
4330.34	✗	✓	✗	✓	✓
4352.26	✗	✗	✗	✓	✓
4367.46	✗	✗	✓	✓	✓
4389.36	✗	✗	✗	✓	✓
4419.94	✗	✗	✗	✓	✓

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
4495.86	✗	✓	✓	✗	✓
4532.54	✗	✓	✓	✓	✓
4574.14	✗	✓	✓	✗	✗
4621.94	✗	✓	✓	✓	✗
4663.32	✗	✓	✓	✓	✓
4696.45	✗	✓	✗	✗	✓
4739.97	✗	✗	✓	✓	✓
4750.43	✗	✓	✓	✓	✓
4776.19	✗	✗	✗	✓	✓
4789.13	✗	✓	✓	✓	✓
4812.57	✗	✓	✓	✓	✓
4834.48	✗	✗	✓	✓	✓
4850.65	✗	✓	✓	✓	✓
4872.78	✗	✓	✓	✓	✓
4889.84	✗	✓	✓	✓	✓
4910.88	✗	✓	✓	✓	✓
4939.86	✗	✓	✓	✓	✓
4966.59	✗	✓	✓	✓	✓
4988.46	✗	✓	✓	✓	✓
5004.96	✗	✓	✓	✓	✓
5026.67	✗	✓	✓	✓	✓
5043.03	✗	✓	✓	✓	✓
5064.85	✗	✓	✓	✓	✓
5080.58	✗	✓	✓	✓	✓
5103.06	✗	✓	✓	✓	✓
5118.55	✗	✓	✓	✓	✓
5143.40	✗	✓	✓	✓	✓
5172.90	✗	✓	✓	✓	✓
5199.35	✗	✓	✓	✓	✓

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
5211.63	✗	✓	✗	✓	✗
5231.61	✗	✗	✗	✓	✓
5254.38	✗	✗	✓	✓	✗
5354.26	✗	✓	✓	✓	✗
5375.35	✗	✗	✗	✓	✓
5414.62	✗	✓	✓	✓	✗
5452.56	✗	✓	✓	✓	✓
5767.54	✗	✗	✓	✓	✓
5820.60	✗	✓	✓	✗	✗
5976.67	✗	✓	✓	✗	✗
5999.26	✗	✓	✓	✗	✓
6025.89	✗	✓	✗	✗	✗
6050.40	✗	✗	✗	✓	✓
6069.48	✗	✓	✓	✓	✓
6086.32	✗	✓	✗	✓	✓
6105.48	✗	✗	✗	✓	✗
6172.11	✗	✓	✗	✓	✗
6206.87	✗	✓	✓	✗	✗
6225.51	✗	✓	✓	✓	✓
6252.78	✗	✓	✓	✓	✓
6264.18	✗	✗	✗	✓	✓
6278.18	✗	✓	✓	✓	✓
6316.77	✗	✓	✓	✓	✓
6327.08	✗	✗	✓	✓	✓
6351.20	✗	✗	✗	✓	✓
6369.58	✗	✓	✗	✓	✗
6635.79	✗	✗	✗	✓	✓
6650.74	✗	✓	✓	✓	✓
6672.25	✗	✗	✓	✓	✓

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
6689.28	✗	✗	✗	✓	✓
6707.12	✗	✗	✗	✓	✓
6721.52	✗	✓	✓	✓	✓
6744.26	✗	✗	✗	✓	✓
6759.62	✗	✓	✓	✓	✓
6782.24	✗	✗	✓	✓	✗
6798.02	✗	✓	✓	✓	✓
6820.30	✗	✗	✗	✓	✓
6836.97	✗	✓	✓	✓	✓
6857.24	✗	✓	✓	✓	✗
6880.32	✗	✓	✓	✗	✗
6907.15	✗	✓	✓	✓	✓
6929.07	✗	✓	✓	✓	✓
6942.17	✗	✗	✗	✓	✓
6980.52	✗	✗	✗	✓	✓
7006.12	✗	✗	✗	✓	✓
7023.01	✗	✓	✓	✓	✓
7041.24	✗	✗	✓	✓	✗
7068.56	✗	✓	✓	✓	✓
7101.74	✗	✓	✓	✓	✓
7139.53	✗	✓	✓	✓	✓
7150.49	✗	✗	✗	✓	✓
7174.04	✗	✓	✓	✗	✓
7265.56	✗	✗	✓	✓	✗
7369.05	✗	✗	✗	✓	✗
7415.05	✗	✓	✓	✓	✓
7539.49	✗	✓	✓	✓	✓
7563.59	✗	✓	✓	✓	✓
7577.39	✗	✗	✗	✓	✗

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
7601.27	✗	✗	✓	✓	✓
7615.49	✗	✗	✓	✓	✓
7640.30	✗	✗	✗	✓	✗
7655.41	✗	✗	✗	✓	✗
7843.34	✗	✗	✗	✓	✗
7938.15	✗	✗	✗	✓	✓
8030.93	✗	✗	✗	✓	✓
8326.85	✗	✓	✓	✓	✓
8363.19	✗	✓	✓	✓	✓
8386.41	✗	✗	✗	✓	✓
8401.44	✗	✓	✓	✓	✓
8423.44	✗	✗	✓	✓	✗
8453.15	✗	✓	✓	✓	✓
8477.81	✗	✓	✓	✓	✓
8492.22	✗	✓	✓	✓	✓
8512.37	✗	✗	✗	✓	✗
8532.34	✗	✗	✗	✓	✓
8550.60	✗	✗	✓	✓	✓
8567.46	✗	✓	✓	✓	✓
8589.45	✗	✗	✗	✓	✓
8605.71	✗	✓	✓	✓	✓
8627.55	✗	✗	✗	✓	✗
8644.26	✗	✓	✗	✓	✗
8664.74	✗	✗	✓	✓	✓
8682.22	✗	✗	✓	✓	✓
8703.76	✗	✗	✗	✓	✓
8721.27	✗	✗	✗	✓	✗
8742.85	✗	✓	✗	✓	✓
8755.29	✗	✓	✓	✗	✗

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
8773.87	✗	✗	✗	✓	✓
8792.65	✗	✗	✗	✓	✓
8813.13	✗	✗	✓	✓	✓
8832.08	✗	✓	✓	✓	✓
8855.17	✗	✗	✗	✓	✓
8878.26	✗	✗	✗	✓	✗
8947.50	✗	✗	✗	✓	✗
8984.58	✗	✗	✓	✓	✗
9080.49	✗	✗	✗	✓	✗
9167.38	✗	✓	✓	✓	✓
9206.11	✗	✓	✓	✓	✓
9294.07	✗	✓	✓	✓	✓
9331.69	✗	✓	✓	✓	✓
9346.46	✗	✗	✓	✓	✓
9370.04	✗	✗	✗	✓	✓
9409.35	✗	✗	✗	✓	✗
9913.20	✗	✗	✗	✓	✓
9930.01	✗	✓	✗	✓	✓
9953.07	✗	✗	✓	✓	✓
9979.83	✗	✓	✓	✓	✓
10003.52	✗	✗	✗	✓	✓
10018.46	✗	✓	✓	✓	✗
10043.94	✗	✗	✓	✓	✗
10056.98	✗	✗	✗	✓	✓
10080.16	✗	✗	✗	✓	✓
10119.32	✗	✗	✗	✓	✗
10158.55	✗	✗	✗	✓	✓
10186.20	✗	✗	✗	✓	✓
10203.69	✗	✗	✓	✓	✓

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
10263.27	✗	✗	✗	✓	✗
10301.58	✗	✗	✓	✓	✗
10609.98	✗	✗	✗	✓	✗
10648.67	✗	✗	✗	✓	✓
11084.94	✗	✗	✓	✓	✓
11104.73	✗	✗	✗	✓	✓
11187.01	✗	✗	✓	✓	✓
11309.47	✗	✗	✗	✓	✓
11347.87	✗	✗	✗	✓	✓
11545.54	✗	✗	✗	✓	✓
12090.04	✗	✗	✓	✓	✓
12133.77	✗	✓	✓	✓	✓
12158.39	✗	✗	✗	✓	✓
12172.41	✗	✗	✓	✓	✓
12191.85	✗	✗	✗	✓	✓
12209.37	✗	✗	✗	✓	✓
12231.73	✗	✗	✗	✓	✗
12340.54	✗	✗	✓	✓	✓
12374.23	✗	✗	✗	✓	✓
12395.13	✗	✗	✗	✓	✗
12412.81	✗	✗	✗	✓	✗
13246.73	✗	✗	✗	✓	✓
13824.61	✗	✗	✓	✓	✓
13863.70	✗	✗	✓	✓	✓
13901.13	✗	✗	✗	✓	✗
14010.30	✗	✗	✗	✓	✓
14126.80	✗	✓	✓	✓	✓
14199.47	✗	✗	✗	✓	✗
14330.90	✗	✓	✓	✗	✗

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
14465.72	✗	✓	✗	✗	✓
14527.81	✗	✓	✓	✓	✓
14566.16	✗	✗	✗	✓	✗
14576.59	✗	✓	✗	✗	✓
14602.79	✗	✗	✗	✓	✗
14815.34	✗	✓	✓	✗	✗
15284.31	✗	✗	✓	✓	✓
15852.04	✗	✗	✗	✓	✗
17113.42	✗	✓	✓	✗	✗
17268.66	✗	✗	✗	✓	✓
18405.53	✗	✓	✓	✓	✓
18601.89	✗	✓	✓	✗	✗
18633.28	✗	✓	✗	✗	✗
18801.87	✗	✗	✓	✓	✗
20782.90	✗	✗	✗	✓	✓
21178.94	✗	✗	✗	✓	✗
21892.34	✗	✗	✓	✓	✓
22014.57	✗	✓	✓	✓	✗
22171.52	✗	✓	✗	✓	✓
22195.04	✗	✓	✓	✗	✗
22210.03	✗	✓	✓	✗	✗
22304.37	✗	✓	✓	✗	✗
22330.13	✗	✓	✓	✗	✗
22345.17	✗	✓	✓	✗	✗
22571.30	✗	✓	✓	✗	✗
22636.12	✗	✓	✗	✗	✗
27904.62	✗	✓	✓	✗	✗
27986.24	✗	✓	✓	✗	✗
28034.32	✗	✓	✗	✗	✗

Protein ion signals (*m/z*)	Rat brain FFPE tissue* sections				Rat brain FF tissue** sections
	Non-conductive glass slide	ITO coated glass slide	AnchorChip target plate	Copper adhesive tape attached glass slide	Copper adhesive tape attached glass slide
28212.54	✗	✓	✓	✗	✗
28258.70	✗	✓	✓	✓	✓
28287.37	✗	✗	✓	✗	✗
28324.56	✗	✗	✓	✓	✓
28437.32	✗	✓	✓	✗	✗
28476.08	✗	✓	✗	✗	✗
28500.32	✗	✓	✓	✗	✗
28531.84	✗	✓	✓	✗	✗
28602.23	✗	✓	✗	✗	✗
28850.49	✗	✓	✓	✗	✗
28872.44	✗	✓	✓	✗	✗
28893.74	✗	✗	✗	✓	✓
29109.61	✗	✓	✓	✗	✗
29170.89	✗	✓	✓	✗	✗
31295.18	✗	✗	✓	✓	✗
32532.79	✗	✓	✓	✓	✓
Total number of protein ion signals	**0**	**145**	**163**	**223**	**188**

Note: *FFPE tissue, formalin fixed paraffin-embedded tissue; **FF tissue, fresh frozen tissue.

References

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