Supporting Information for

Enhancing Surface-Assisted Laser Desorption Ionization Mass Spectrometry Performance by Integrating Plasmonic Hot-Electron Transfer Effect through Surface Modification

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Experimental

Chemical and Materials. Deionized water was provided by a Mili-Q Integral water purification system. Pyrogallol (PG) and bicine were purchased from J&K (Beijing). Amino acids, HPLC peptide standard mixture, and acetic acid were provided by Sigma and Alfa Aesar. $AgNO_3$ was purchased from Sinopharm Chemical Reagent Co., Ltd and Beijing Chemical Production Quality Supervision and Inspection Station Beijing Huateng Standard Research Center for testing and calibration technology.

Nanodiamond (average size 100 nm) was provided by Shenzhen diamond abrasive and grinding tools co. LTD (Shenzhen, China); gold nanorod (AuNR, 30×10 nm) was synthesized by other labs in our institute; hexagonal boron nitride (h-BN, diameter 0.5-5 μm, thickness <50 nm) as well as SiO_2 nanoparticles (average diameter 150 nm) were provided by XF nano, INC (Nanjing, China); polystyrene nanoparticles (average size 260 nm) was provided by Suzhou Nanomicro Technology Company, Ltd (Suzhou, China); porous silicon microparticles (average size of 6 μm, aperture diameter 100 Å) was provided by Agela Technologies (Tianjin, China).

Surface modification process. The surface modification process was done according to Phillip B. Messersmith's work in 2013 when he and his coworkers first published this bio-inspired phenolic surface coating method[1]. Kinds of nano/micro materials were first suspended in deionized water with concentrations of 1 mg/ml. For deposition of adherent PG layer, 0.5 mL of the material suspension was first centrifuged and superantant water was removed. Then 500 μL 0.1 mg/mL PG, pH 7.8 (100 mM bicine) was added, and the suspension was sonicated for 20 min. The addition of 500 μL1 mM $AgNO_3$ was then followed, and the suspension was sonicated for another 10 min. 10 μL of 12 mM acetic acid was added to stop the reaction. The addition of acetic acid would change the pH of the solution, limiting the degree of PG oxidation, thus stop the Ag^+ reduction reaction. The final suspension was then centrifugated and washed with 1 mL water, and finally suspended in 1 mL deionized water for use. So the final concentration of the PG-Ag modified material was 0.5 mg/mL, and the concentration of silver was 0.5 mM.

For the modification of macrosurfaces, like ITO glass surface, the ITO glass was first washed with deionized water and ethanol. Then immerse it into 0.2 mg/mL PG solution for 0.5-24 h at R. T. with mild agitation on a magnetic stirring apparatus. Subsequently, the ITO glass was removed from the modification solution, rinsed with deionized water and dried with nitrogen gas. Then the ITO substrate was immersed into 100 mM $AgNO_3$ solution for 0.5-24 h. After rinsed again with deionized water and dried with nitrogen, the ITO glass was ready to be used as SALDI substrate.

Although the mechanism and influence factors of this bio-inspired surface coating method are still under investigation, some progress has been made[2].

Mass spectrometry analysis. SALDI analysis was performed on a Bruker Ultraflextreme LDI-

TOF/TOF mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a smartbeam Nd: YAG 355 nm laser with reflection in negative ion mode. The maximum laser energy (outlet) was 100 µL, and can be adjusted between 0% and 100%. During all the testing, 15% - 30% energy, 1000 Hz, 200 shots were used unless otherwise stated. The accelerating voltage was 20.00 kV, extraction voltage was 17.70 kV, lens voltage was 6.4 kV and reflector voltage of 21.1 kV.

Ultraviolet-visible (UV-Vis) spectrometer, Transmission electron microscope (TEM) and Scan electron microscope (SEM)： The UV-Vis spectrometer used here was TU-1900 Spectrometer (Beijing, China). The TEM used for AuNP characterizing was Hitachi HT7700, and JEM-2011 for other material characterization. The SEM used for characterizing AgNI-on-ITO was a field emission SEM (JEOL 6701F).

Animals

Healthy Kunming mouse (20−25 g) and Sprague−Dawley rats (250−260 g) were from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal experiments were performed according to the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication, No. 3040-2, revised 1999, Bethesda, MD) and were approved by the Animal Care and Use Committee of the Chinese Academy of Sciences.

Establishment of MCAO model

Establishment of the MCAO model was carried out as described previously. [3]

Tissue Dissecting

Sprague−Dawley rat 24 h post MCAO was euthanized with chloral hydrate (350 mg/kg, i.p.), and then brain tissue was removed and snap-frozen in liquid nitrogen. Brain tissue from normal Kunming mouse was dissected and flash-frozen with the same method. All tissues were stored at −80 °C until further preparation.

MS Imaging

All tissues were sectioned at 10 µm thickness by a Leica CM1950 cryostat and transferred onto a piece of indium tin oxide (ITO) coated glass slides. Then the ITO glass slide with tissues was placed in a vacuum desiccator for about 30 min followed by matrix application and placed in the vacuum desiccator again for another 30 min before subjected to mass spectrometer for imaging analysis.

AgNI-on-hBN and hBN were dispersed in 80% methanol water solution with 2 mg/mL concentration respectively. An electrospray deposition device[4] was used to apply the material suspension on the tissue slice.

MS imaging was carried out in negative ion mode with a spatial resolution of 150 µm, a laser

frequency of 1000 Hz, and a sum-up of 200 laser shots per spot. The laser energy used for imaging was 60% of the full laser energy.

Structure Confirmation

Bruker 9.4T solariX FT-ICR mass spectrometer was used to get accurate m/z for the metabolites detected in brain tissues. Then these m/z values were searched against databases (Human Metabolome Database, http://www.hmdb.ca/; and LIPID MAPS, http://www.lipidmaps.org/) to get putative IDs. Further MS/MS was done by Ultraflextreme MALDI-TOF/TOF MS in the LIFT mode to further confirm the ID of the metabolites detected.

For MS/MS detection, the laser operated at 200 Hz and the MS/MS spectra were the sum of 500 shots for product ions. MS/MS spectra in the reflector mode were acquired with the pulsed ion extraction time of 80 ns, the ion source 1 is 7.5 kV, the ion source 2 is 6.75 kV, the lens voltage of 3.5 kV, the reflector voltage of 29.50 kV, the lift 1 voltage of 19.00 kV and the lift 2 voltage of 4.45 kV.

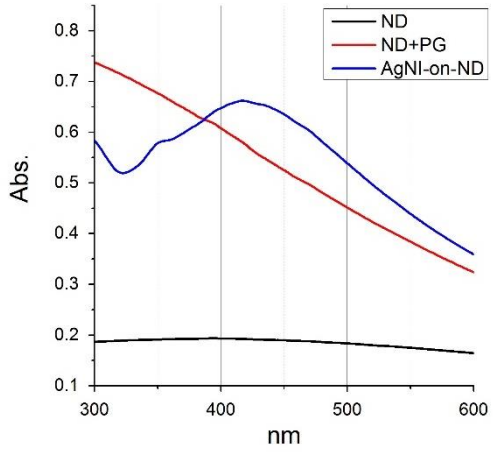

Figure S1. UV-Vis distinction spectra of bare ND, ND after first step modification (denoted as ND+PG), and final AgNI-on-ND.

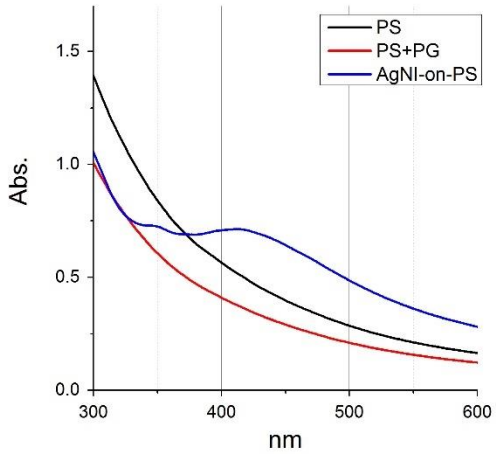

Figure S2. UV-Vis distinction spectra of bare PS nanoparticles, PS after first step modification (denoted as PS+PG), and final AgNI-on-PS.

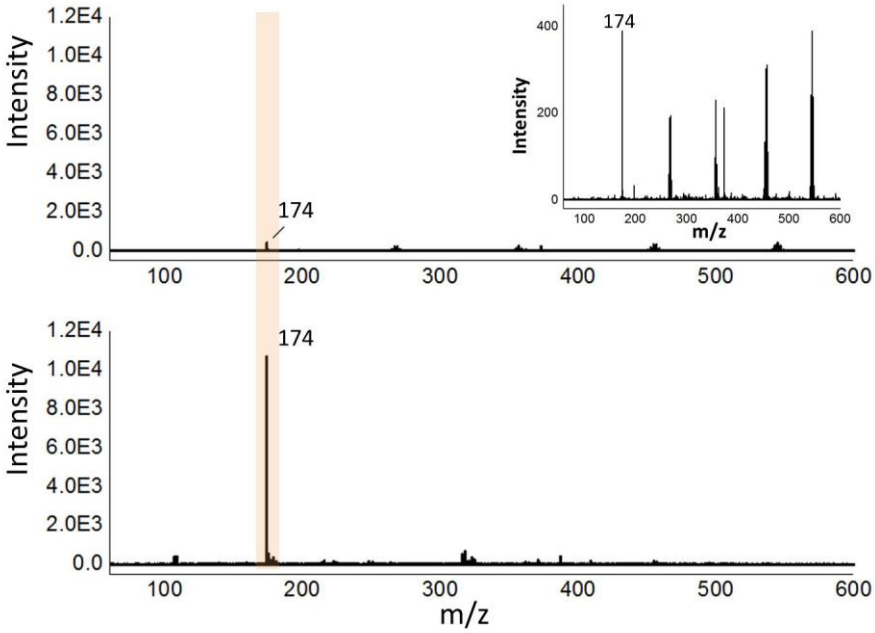

Figure S3. SALDI performance of AuNR (top) and AgNI-on-AuNR (bottom) with 0.5 nmol N-Ace-Asp as analyte. The inset is the enlarged view of the corresponding AuNR SALDI mass spectrum. The cluster peaks beside m/z 174 are Au clusters.

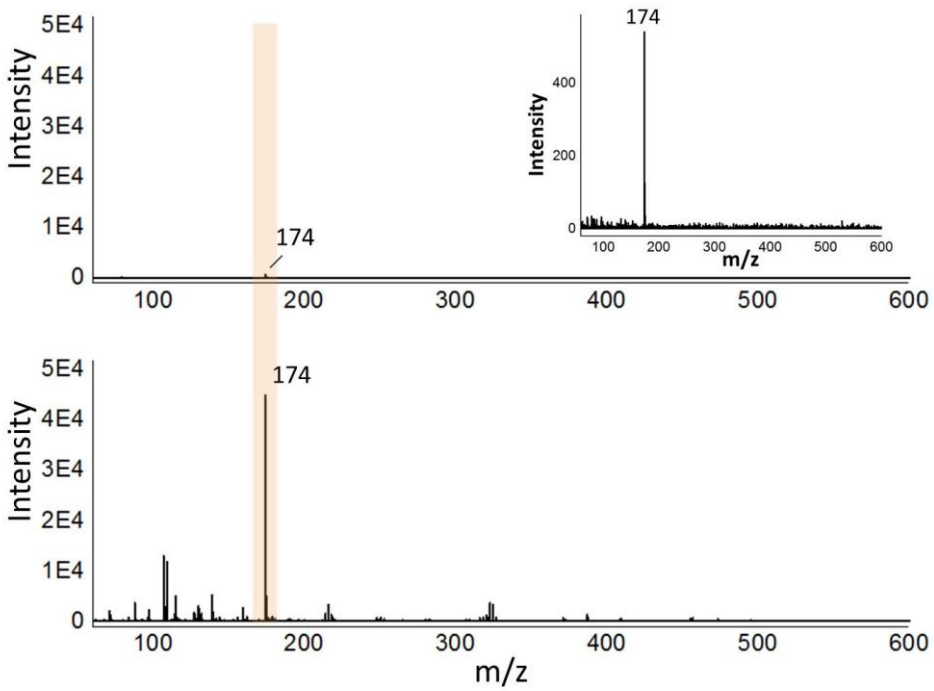

Figure S4. SALDI performance of hBN before modification (top) and AgNI-on-hBN (bottom) with 0.5 nmol N-Ace-Asp as analyte. The inset is the enlarged view of the corresponding h-BN SALDI mass spectrum.

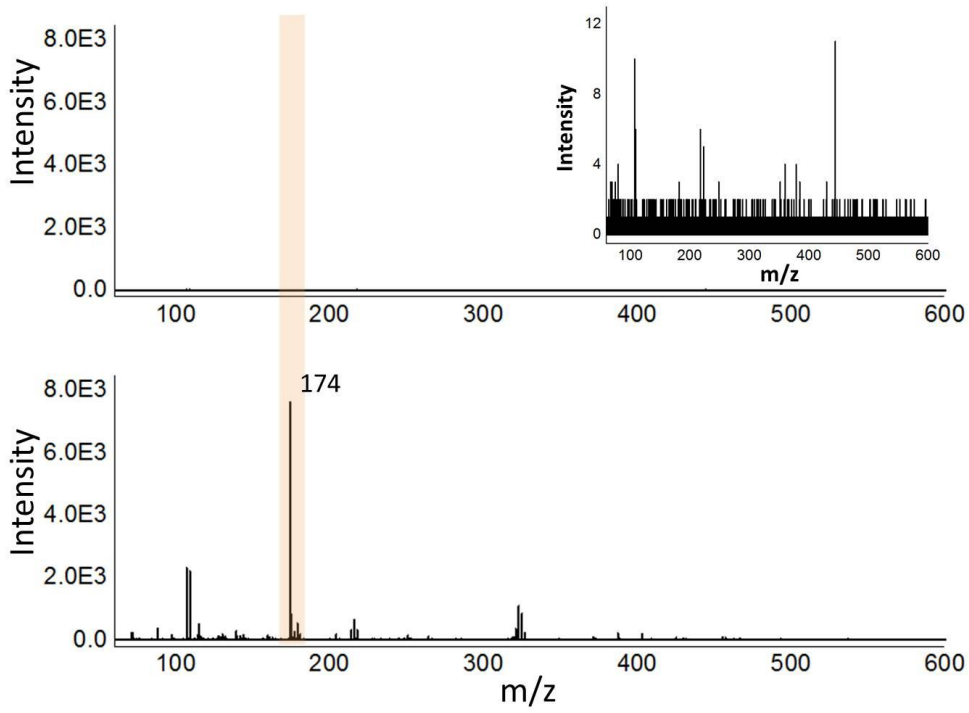

Figure S5. SALDI performance of SiO_2 nanoparticles (top) and AgNI-on-SiO_2 (bottom) with 0.5 nmol N-Ace-Asp as analyte. The inset is the enlarged view of the corresponding SiO_2 SALDI mass spectrum.

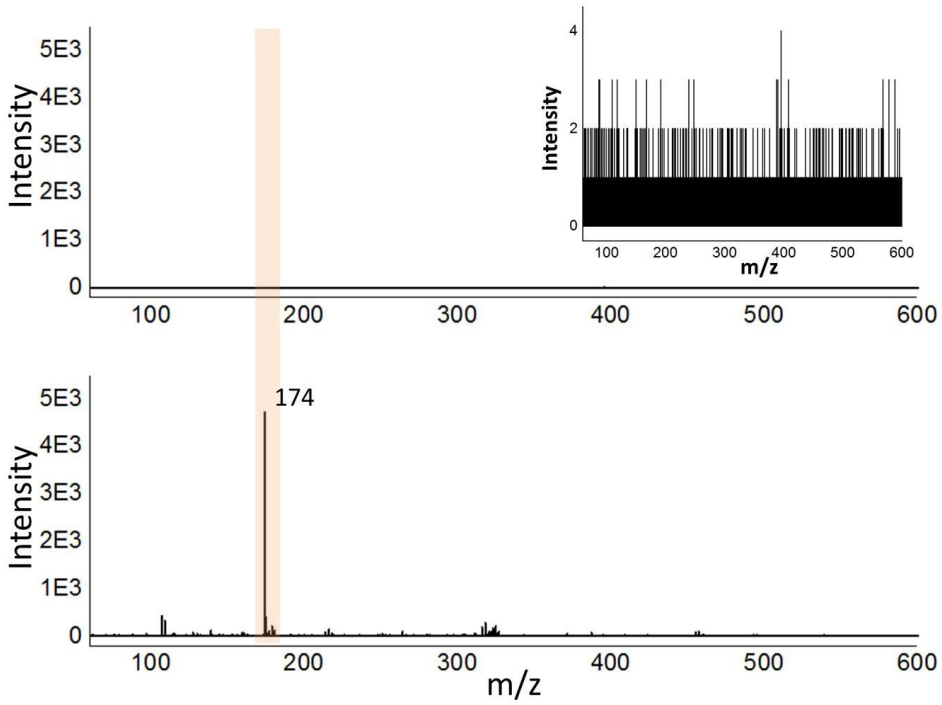

Figure S6. SALDI performance of bare porous Si micro particles (top) and AgNI-on-Si (bottom) with 0.5 nmol N-Ace-Asp as analyte. The inset is the enlarged view of the corresponding porous Si micro particles SALDI mass spectrum.

Table S1. Sulfatide assigned in diluted mouse brain extraction detected with bare h-BN as SALDI materials.

m/z	S/N	intensity	Molecular species	ion
806.5	11	46.2	d18:1-N18:0 ST	$[M-H]^-$
888.6	85	481	d18:1-N24:1ST	$[M-H]^-$
890.6	16	87.1	d18:1-N24:0ST	$[M-H]^-$

Table S2. Sulfatide assigned in diluted mouse brain extracts with AgNI-on-hBN. Data in red are the signals also detected with bare h-BN but here with much enhanced signal intensities and S/N.

m/z	S/N	intensity	Molecular species	ion
778.5	7	98.5	d18:1-N16:0 ST	$[M-H]^-$
806.5	52	709	d18:1-N18:0 ST	$[M-H]^-$
822.5	16	219	d18:1-OHN18:0 ST	$[M-H]^-$
860.6	10	125	d18:1-N22:1 ST	$[M-H]^-$
862.6	28	358	d18:1-N22:0 ST	$[M-H]^-$
876.6	7	88	d18:1-N23:0ST d18:1-OHN22:1ST	$[M-H]^-$
878.6	41	494	d18:1-OHN22:0ST	$[M-H]^-$
886.6	10	121	d18:1-N24:2ST	$[M-H]^-$
888.6	241	2894	d18:1-N24:1ST	$[M-H]^-$
890.6	86	1040	d18:1-N24:0ST	$[M-H]^-$
904.6	20	224	d18:1-N25:1ST d18:1-OHN24:1ST	$[M-H]^-$
906.6	37	417	d18:1-OHN24:0ST	$[M-H]^-$

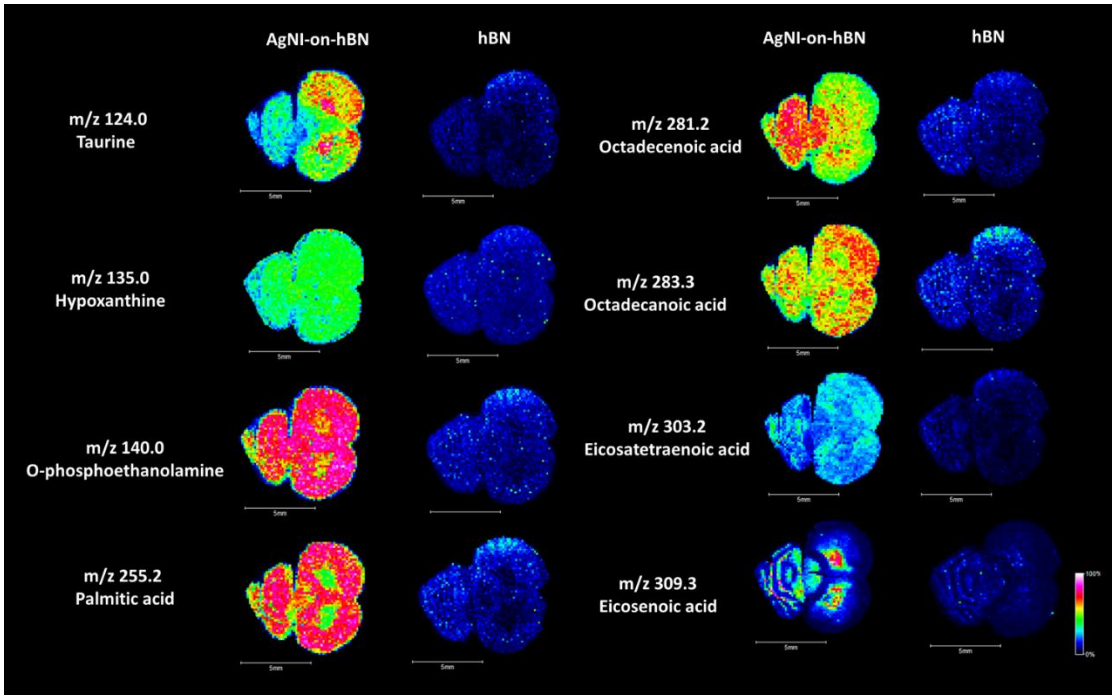

Figure S7. Representative ion images of small molecule metabolites in a health mouse brain tissue with AgNI-on-hBN and hBN as assisting material respectively.

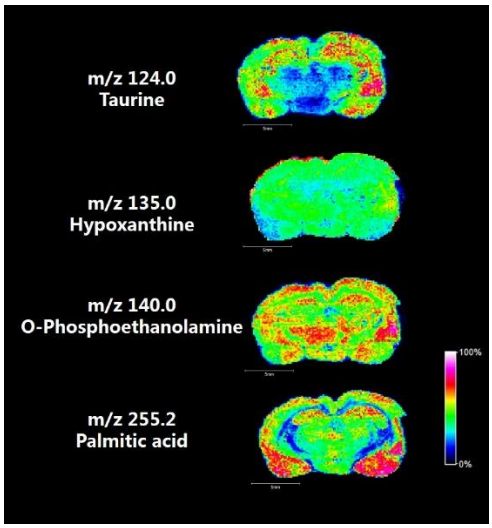

Figure S8. MS imaging of normal rat brain tissue with AgNI-on-hBN as assisting material, distribution of four small molecule metabolites. This figure shows that for normal brain tissue, the distributions of metabolites on left and right brain are symmetrical.

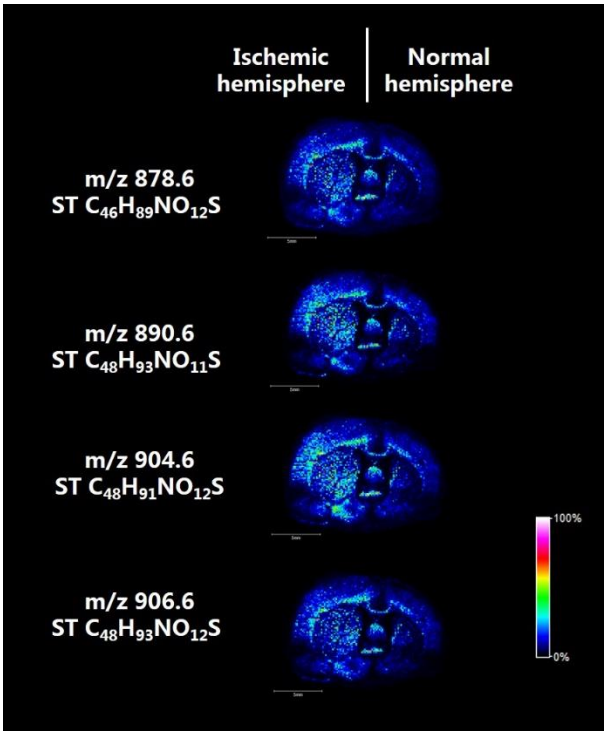

Figure S9. MS imaging of ischemic rat brain tissue with AgNI-on-hBN as assisting material, distribution of 4 out of 8 STs.

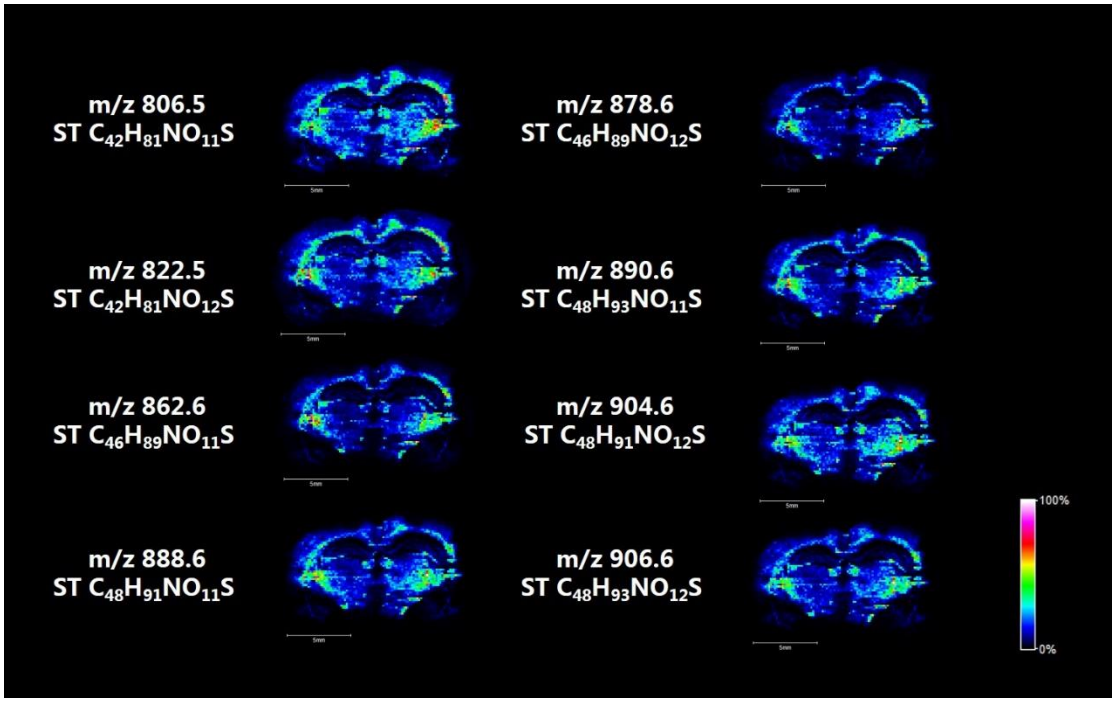

Figure S10. MS imaging of normal rat brain tissue with AgNI-on-hBN as assisting material, distribution of eight STs. This figure shows that for normal brain tissue, the distributions of these STs on left and right brain are symmetrical.

Table S3. Identification of metabolites in rat brain tissue by FTICR MS in negative ion mode using AgNI-on-hBN as assisting material.

Identification	**Experimental m/z**	**Theoretical m/z**	**Parent ion**	**molecular formula**	**ppm**
Taurine	124.00741	124.00739	$[M-H]^-$	$C_2H_7NO_3S$	0.17742
Hypoxanthine	135.03162	135.03124	$[M-H]^-$	$C_5H_4N_4O$	2.85185
Palmitic acid	255.23304	255.23295	$[M-H]^-$	$C_{16}H_{32}O_2$	0.33725
O-Phosphoethanolamine	140.01189	140.01182	$[M-H]^-$	$C_2H_8NO_4P$	0.51429
ST	806.54585	806.54576	$[M-H]^-$	$C_{42}H_{81}NO_{11}S$	0.11538
ST	822.54133	822.54067	$[M-H]^-$	$C_{42}H_{81}NO_{12}S$	0.80049
ST	862.60952	862.60836	$[M-H]^-$	$C_{46}H_{89}NO_{11}S$	1.34919
ST	878.60336	878.60327	$[M-H]^-$	$C_{46}H_{89}NO_{12}S$	0.10023
ST	888.62400	888.62401	$[M-H]^-$	$C_{48}H_{91}NO_{11}S$	-0.00788
ST	890.64020	890.63966	$[M-H]^-$	$C_{48}H_{93}NO_{11}S$	0.61011
ST	904.61901	904.61892	$[M-H]^-$	$C_{48}H_{91}NO_{12}S$	0.09735
ST	906.63503	906.63457	$[M-H]^-$	$C_{48}H_{93}NO_{12}S$	0.50552

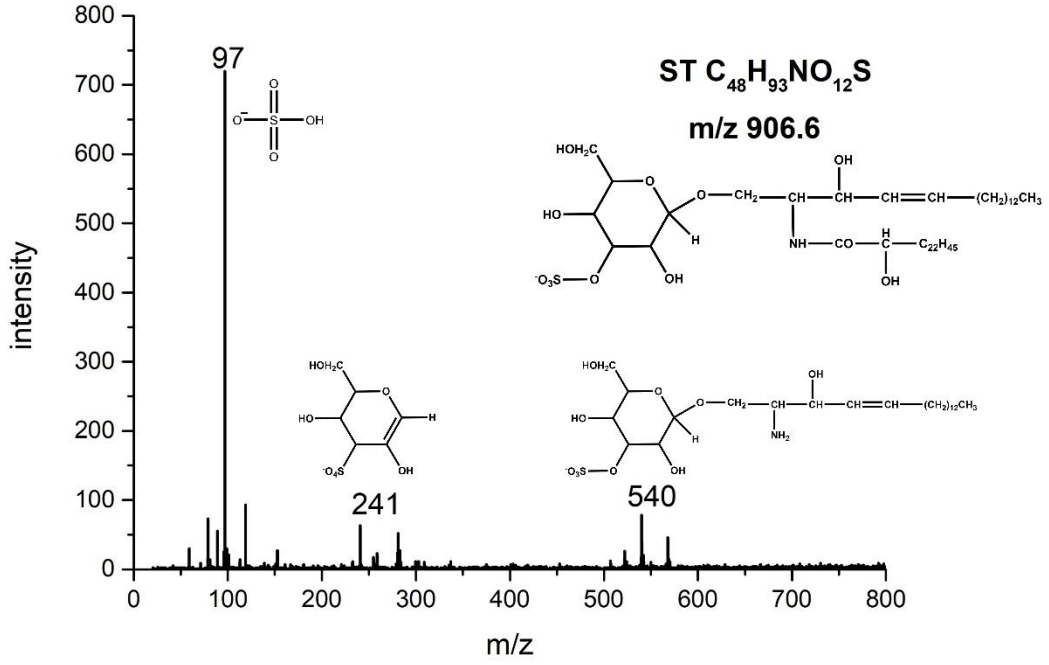

Figure S11. Representative in situ MS/MS spectrum of sulfatide (m/z 906) in negative ion mode.

Reference

1. T. S. Sileika; D. G. Barrett; R. Zhang; K. H. A. Lau; P. B. Messersmith, *Angewandte Chemie International Edition.* 2013, **52**, 10766-10770.
2. (a) Y. Chen; Q. Liu, *Journal of Membrane Science.* 2019, **570-571**, 176-183; (b) S. Geißler; A. Barrantes; P. Tengvall; P. B. Messersmith; H. Tiainen, *Langmuir.* 2016, **32**, 8050-8060.
3. E. Z. Longa, P. R. Weinstein, S. Carlson and R. Cummins, *Stroke*, 1989, **20**, 84-91
4. S. L. Li, Y. Y. Zhang, J. A. Liu, J. J. Han, M. Guan, H. Yang, Y. Lin, S. X. Xiong and Z. W. Zhao, *Sci Rep-Uk*, 2016, **6**.