Electronic Supplementary Material (ESI) for Analyst.

A general purpose MALDI matrix for the analyses of organic, peptide and protein molecules

Supporting Information

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Laser Energy for Small Molecules Analyses

Sample Preparation and Instruments: The 4-hydroxy-3-nitrobenzonitrile solution (25 mg/mL) was prepared in acetonitrile and stored at 4 ℃. The MRFA solution (10 μg/mL) was premixed with the 4-hydroxy-3-nitrobenzonitrile solution in the ratio of 1:1, then 1 μL of the mixture was pipetted on a polished stainless steel (MTP 384) plate. An autoflex mass spectrometer (Bruker Daltonics, Germany) were used in the positive ionization mode. Parameters of the laser and the mass spectrometer were set to analyze small molecules. Laser beam battenuation was set as: 75%, 70%, 65%, 60% and 55%, respectively.

MALDI-TOF Analysis: The MALDI-TOF mass spectra analyzing MRFA (10 μg/mL) with the matrix 4-hydroxy-3-nitrobenzonitrile by different laser beam battenuation are shown in Figure S1. The signal intensity, signal-to-noise (S/N) and peak resolution were considered to select the best laser energy. The MRFA's $[M+H]^+$ ions have very low peak intensity when the laser beam attenuation is 75% and 70% (Figure S1a and Figure S1b). And the S/N ratio of MRFA's $[M+H]^+$ peaks are 99 and 161, respectively. When the laser beam attenuation is tuned to 60% and 55%, although the MRFA's peak intensity are high, the matrix's fragment ion peaks are observed around 500 Th (Figure S1d and Figure S1e). The S/N ratio of MRFA's $[M+H]^+$ peaks are 212 and 235, respectively. In Figure S1c, MRFA's $[M+H]^+$ peak has a good intensity and the background is clean, and the S/N ratio of MRFA's $[M+H]^+$ peak reachs the maximum of 385.

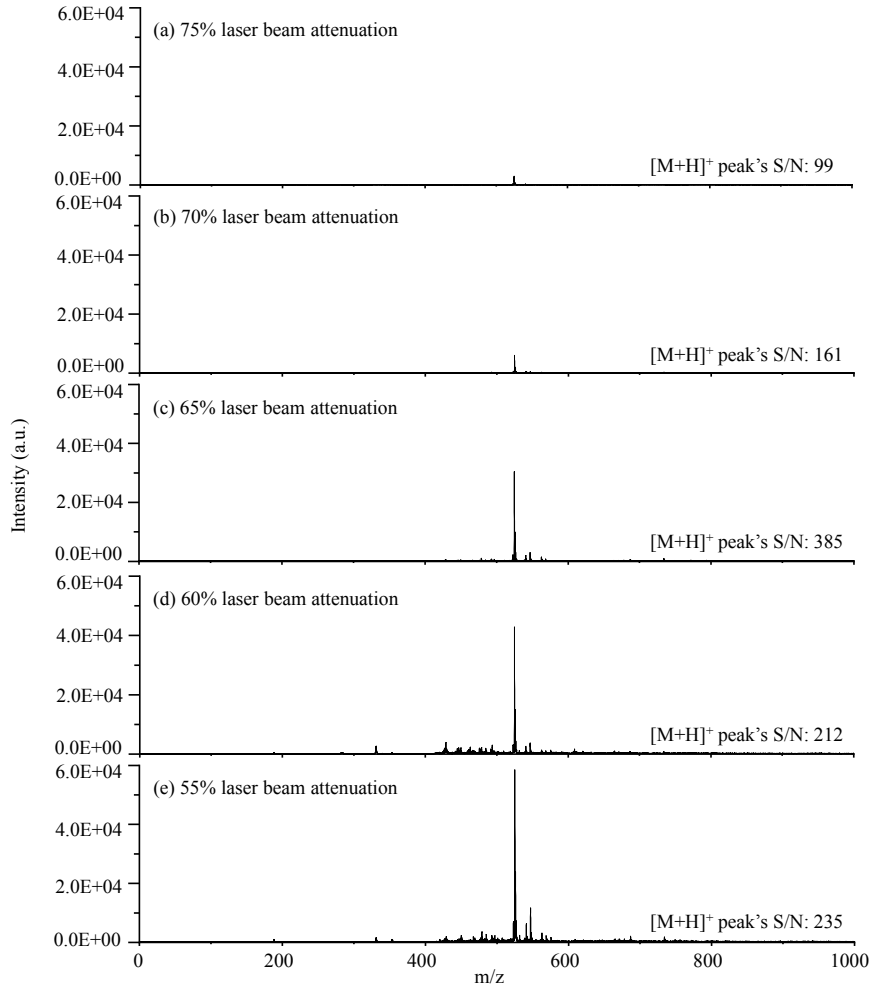

Figure S1. MALDI-TOF mass spectra of MRFA solution at 10 μg/mL with matrix of 4-hydroxy-3-nitrobenzonitrile at different laser energy.

Analysis of Peptides

Sample Preparation and Instruments: The SA solution I and the SA solution II were prepared in TA30, and the DHB solution (20 mg/mL) was prepared in TA30. Peptide MRFA was dissolved in water to prepare different concentrations of 10 μg/mL, 1 μg/mL and 0.1 μg/mL. The sample preparation method of the matrix DHB was the same as the general method. For the matrix SA, we used the double layer method to prepare the samples. Parameters of the laser and the mass spectrometer were set to analyze peptides, and the laser beam battenuation was set as 35%.

MALDI-TOF Analysis: Figure S2 plots the mass spectra of MRFA at different concentrations of 10 μg/mL, 1 μg/mL and 0.1 μg/mL with the matrices of DHB and SA, respectively. When using DHB as the matrix, the molecular ions of MRFA ($[M+H]^+$ at m/z 524.707) are observed in the first two samples (Figure S2a and Figure S2b), and a small peak of MRFA is shown in the third sample (Figure S2c). The signal-to-noise (S/N) ratio of MRFA ions are 329, 112 and 6, respectively. The background interference coming from the matrix DHB is mainly in the mass region of 500 Th. When using SA as the matrix, MRFA's molecular ions are observed in Figure S2d and Figure S2e. And the S/N ration of MRFA ions 18 and 3. Because the laser energy density of DHB and SA are higher than that of CHCA, the laser beam battenuation was set as 35%. As shown in Figure S2, the baselines of the mass spectra are significantly elevated.

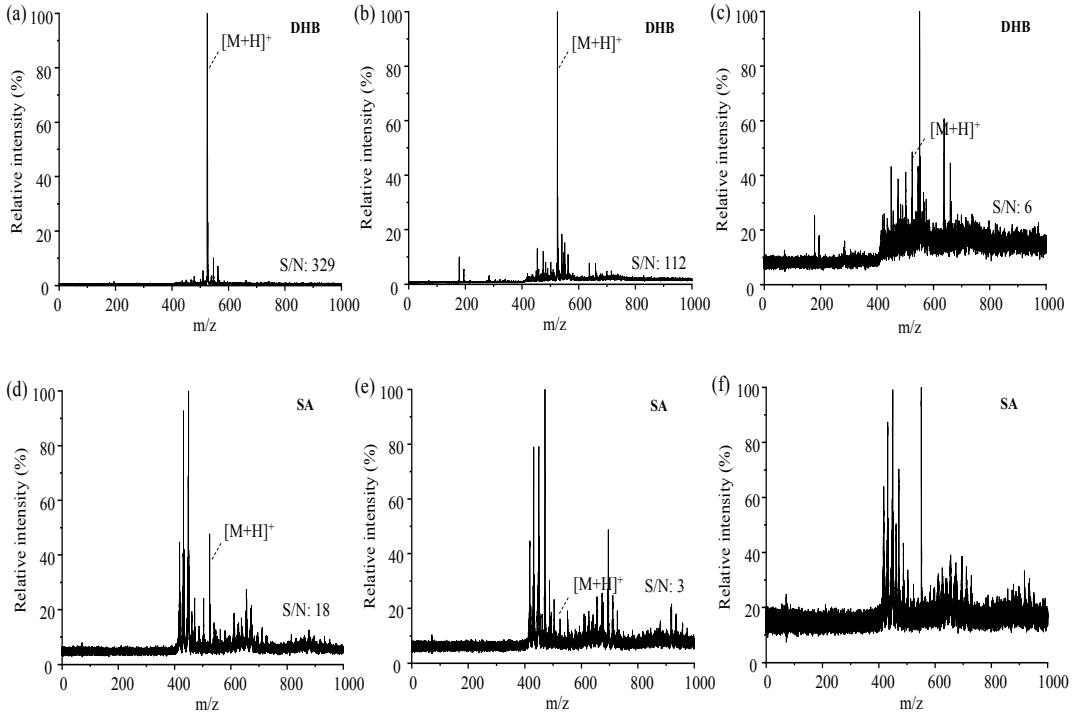

Figure S2. MALDI-TOF mass spectra of different concentrations of MRFA solutions at 10 μg/mL; 1 μg/mL and 0.1 μg/mL using matrices of DHB and SA, respectivly.

Analysis of Proteins

Sample Preparation and Instruments: Protein BSA was dissolved in water to prepare different concentrations of 0.1 mg/mL, 0.01 mg/mL and 0.001 mg/mL, and protein IgG was dissolved in water (0.1% trifluoroacetic acid) to prepare different concentrations of 1 mg/mL, 0.1 mg/mL and 0.01 mg/mL. For 4-hydroxy-3-nitrobenzonitrile, we used the general method to prepare the samples. We chose the matrix SA for comparison and used the double layer method to prepare the samples. Parameters of the laser and the mass spectrometer were tuned to analyze proteins.

MALDI-TOF Analysis: The MALDI-TOF mass spectra of the BSA solutions at different concentrations of 0.1 mg/mL, 0.01 mg/mL and 0.001 mg/mL are shown in Figure S3. When the concentrations of the BSA solutions are 0.1 mg/mL and 0.01 mg/mL, single and doubly charged BSA ions are observed with the matrices of 4-hydroxy-3-nitrobenzonitrile and SA, respectively. And the BSA's peak intensity of the first sample is higher than that of the second sample. When the BSA's concentration reduces to 0.001 mg/mL, the ions couldn't be detected in both case. Figure S4 plots the mass spectra of the IgG solutions (0.1% trifluoroacetic acid) at different concentrations of 1 mg/mL, 0.1 mg/mL and 0.01 mg/mL. Single and multiple charged ions of IgG are observed with two types of matrices in the first two samples. And when the concentration of the IgG solution reduces to 0.01 mg/mL, the IgG ions couldn't be detected.

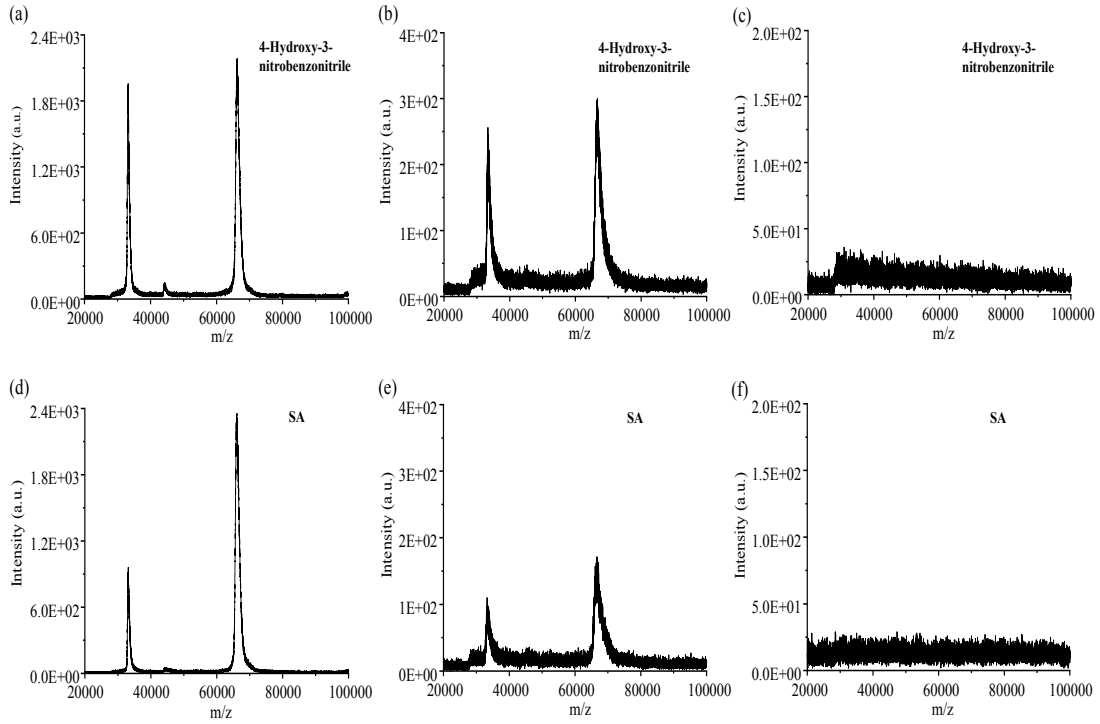

Figure S3. MALDI-TOF mass spectra of BSA solutions at 0.1 mg/mL; 0.01 mg/mL and 0.001 mg/mL with matrices of 4-hydroxy-3-nitrobenzonitrile and SA, respectively.

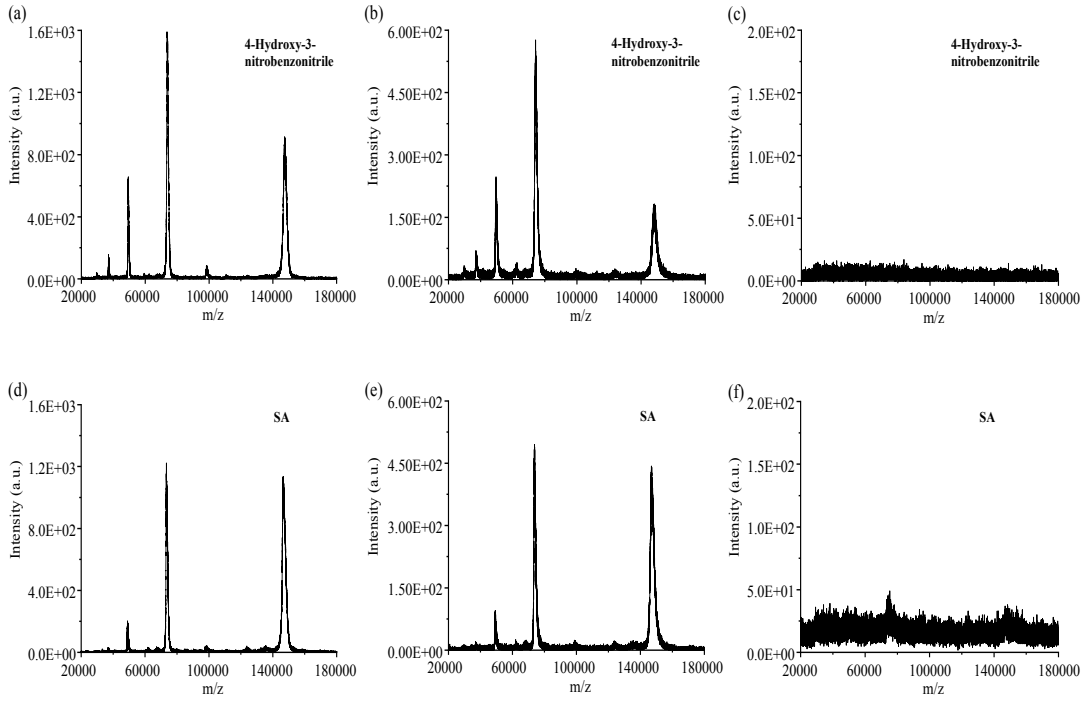

Figure S4. MALDI-TOF mass spectra of IgG solutions containing 0.1% trifluoroacetic acid at 1 mg/mL, 0.1 mg/mL and 0.01 mg/mL with matrices of 4-hydroxy-3-nitrobenzonitrile and SA, respectively.

Analysis of Mouse Brain tissue

Sample Preparation and Instruments: Mouse brain tissue was taken by the standard method and stored at - 80 ℃. Frozen mouse brain tissue (4.5 mg) was added into 500 μL cold saline solution, and it was ground by a grinder in an ice water bath. The tissue homogenate was centrifuged by 3000 r/min for 10 min, and the supernatant was taken. The supernatant was diluted in the ratio of 1:10 and 1:100. The sample solution was premixed with the matrix in the ratio of 1:1, then 1 μL of the mixture was pipetted on the polished stainless steel plate. The instrument parameters were tuned to analyze phospholipids.

MALDI-TOF Analysis: The RSD of shots and samples of the mouse brain tissue's sample diluents with the matrix 4-hydroxy-3-nitrobenzonitrile are shown in Figure S5. Five samples of the mouse brain tissue's homogenate were used as parallel experiments and the peak areas of $[(PC32:0)+H]^+$ at m/z 735.313 were used for analyses. The RSD of shots and samples are 29.03% and 31.61%, respectively. Figure S6 polts the mass spectra of the mouse brain tissue's homogenate using the matrices of 4-hydroxy-3-nitrobenzonitrile and DHB, respectively. In these sample diluents, there are more phospholipid peaks and higher peak's intensity when using 4-hydroxy-3-nitrobenzonitrile as the matrix (Figure S6a).

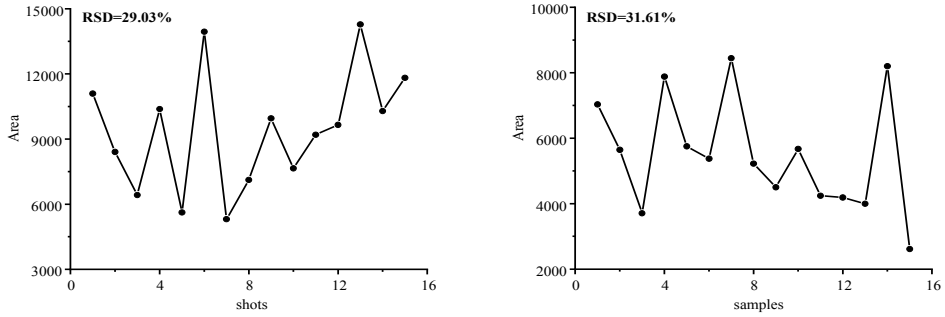

Figure S5. RSD of shots (left) and samples (right) of mouse brain tissue's sample diluents in the ratio of 1:100.

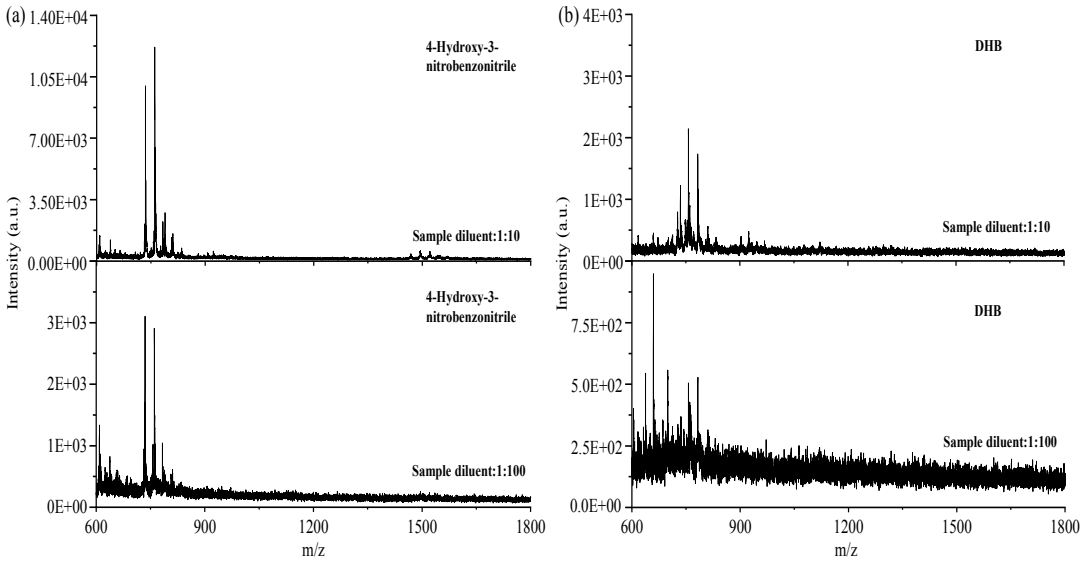

Figure S6. MALDI-TOF mass spectra of mouse brain tissue's homogenate using (a) 4-hydroxy-3-nitrobenzonitrile and (b) DHB as matrices, respectively.

Analysis of Bacteria

Sample Preparation and Instruments: *Escherichia coil* ATCC 25922 was obtained from Institute of Microbiology (Beijing, China) and LB agar was purchased from Solarbio (Beijing, China). All microbial experiments were perfomed in a biosafety level II laboratory. *E. coil* was grown on the LB agar plates at 37 ℃ for 12-15 h, and a single bacterial colony of *E. coil* was picked by a wire loop to spread on the polished stainless steel plate. 1 μL of the 4-hydroxy-3-nitrobenzonitrile solution was mixed with 1 μL of water (2.5% trifluoroacetic acid), then 1 μL of the mixture was pipetted on the thin bacteria *E. coil* layer. A software package MALDI Biotyper (Bruker Daltonics, Germany) were used. The MBT method was used to analyze microbial samples. Parameters of the laser were set as: laser beam focus: 85%, repetition rate: 1000 Hz and number of shots: 200, and parameters of the mass spectrometer were set as: PIE delay: 170 ns, ion source voltage 1: 19.5 kV, ion source voltage 2: 18.17 kV, lens voltage: 7 kV and linear detector voltage: 2.691 kV.

MALDI-TOF Analysis and Pattern Recognition:

Figure S7 plots and compares the mass spectra of *E. coil* with matrices of (a) 4-hydroxy-

3-nitrobenzonitrile and (b) CHCA, respectively. Results show that the protein peaks of *E. coil* are basically similar in Figure S7a and Figure S7b. When using 4-hydroxy-3-nitrobenzonitrile as matrix, the lagre protein peaks of *E. coil* are slightly more than that of CHCA. The pattern recognition results of *E. coil* by MALDI Biotyper are shown in the illustrations, and the comparison results of *E. coil* are similar. The score of bacteria species (*Escherichia coil* ATCC 25922 CHB) is 2.076 when 4-hydroxy-3-nitrobenzonitrile was used as the matrix; while it is 1.901 when commercial matrix CHCA was used.

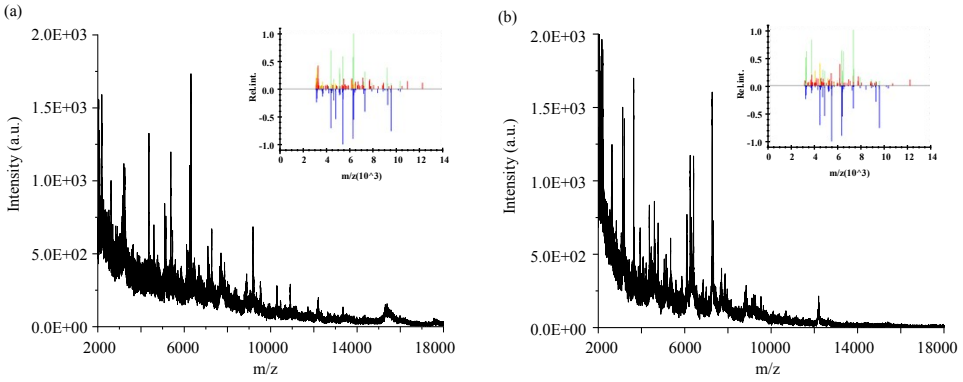

Figure S7. MALDI-TOF mass spectra of E. coil with matrices of (a) 4-hydroxy-3-nitrobenzonitrile and (b) CHCA, respectively. The pattern recognition results are shown in the inset.