Electronic Supplementary Information

Two-Dimensional Metal-Organic Framework Nanosheets as Matrix for Laser Desorption/Ionization of Small Molecules and Monitoring Enzymatic Reactions under High Salt Concentrations

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EXPERIMENTAL SECTION

Materials and Chemicals.

All chemicals were at least of analytical grade. The conventional MALDI matrix, α-cyano-4-hydroxy cinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) were obtained from Bruker Daltonics (Bruker, Germany). Dopamine and amino acids of glutamic acid (Glu), histidine (His), and tryptophan (Trp) were all purchased from Sigma (St. Louis, MO). Nucleo-bases (uracil (U), thymine (T), adenine (A), guanine (G)), serotonin, testosterone and bisphenol A (BPA) were obtained from Aladdin Co. Ltd (Shanghai, China). The enzyme of acetylcholine esterase and its substrate acetylcholine were purchased from Sigma (St. Louis, MO). HPLC grade methanol (MeOH) and acetonitrile were from Merck (USA). Deionized water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), iron nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), trimesic acid, benzimidazole and 2-methylimidazole were purchased from Aladdin Co. Ltd (Shanghai, China). Methanol (MeOH), n-propanol, nitric acid (HNO_3), hydrofluoric acid (HF) and N,N-dimethylformamide (DMF) were purchased Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Please see Table S1 for details.

Characterization.

X-ray diffraction (XRD) patterns were obtained from a Rigaku (Tokyo, Japan) D/MAX-2500 diffractometer with a CuKα radiation (1.54056Å). Transmission electron microscopy (TEM) images were made on a JEOL JEM-2100F transmission electron microscopy operated at an accelerating voltage of 200 kV. Atomic force microscopy (AFM) measurements were performed with a PicoPlus in tapping mode (Agilent, US). The scanning electron microscopy (SEM) images were recorded on a JSM-7600F (JEOL Ltd).

Synthesis of Matrices

1.Synthesis of 2-D Metal-Organic Framework Nanosheets.

The 2-D $Zn_2(bim)_4$ nanosheets was synthesized by liquid exfoliation after hydrothermal transformation from MOF ZIF-7 (ZIF=zeolite imidazolate framework) according to a previous report with minor modification. Typically, 500 mL of N,N-dimethylformamide (DMF) was added to a solid mixture of $Zn(NO_3) \cdot 6H_2O$ (1.5125 g) and benzimidazole (3.8475 g). After stirring for 1 h, the solution was kept statically at room temperature for 72 h. ZIF-7 nanoparticles were obtained, which were collected by centrifugation and thoroughly washed with methanol. The obtained product was dried at 50 °C overnight, then subsequently dried at 120 ºC for 48 h in a vacuum oven. For hydrothermal phase transformation, the obtained ZIF-7 was redispersed in distilled water at a concentration of 0.5 wt % and then refluxed at 100 ºC for 24 h. The turbid mixture was filtered and washed with distilled water and methanol, and then dried at 50 ºC overnight. White powder of pristine $Zn_2(bim)_4$ was obtained and further confirmed by the XRD characterization. In a typical process of liquid exfoliation, 80 mg of pristine $Zn_2(bim)_4$ was ultrasonicated in 120 mL of a mixed solvent of methanol and n-propanol (1:1, v/v) for 2 h. After

centrifuged at 3000 rpm for 5 min, the collected supernatant was further centrifuged at 12000 rpm for 20 min. The precipitant of the 2-D $Zn_2(bim)_4$ nanosheets (~1.5 mg) was obtained and dried at 50 ^oC overnight.

2.Synthesis of ZIF-8

ZIF-8 was synthesized at room temperature according to Cravillon et al.[1] 2-methylimidazole (648.9 mg, 7.904 mmol) dissolved in 20 mL ethanol were dissolved in a vial. Then $Zn(NO_3)_2 \cdot 6H_2O$ (293.3 mg, 0.987 mmol) in 20 mL ethanol were added. After stirring for 1 h, The product Was washed three times with ethanol and collected by centrifuging at 10000 r.p.m. for 10 min. The product was dried at 100℃ in a drying oven.

3.Synthesis of MIL-100

MIL-100(Fe) was synthesized under hydrothermal conditions according to Férey et al.[2] A mixture of iron nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$, 404 mg, 1.0 mmol), trimesic acid ($C_9H_6O_6$, 141 mg, 0.67 mmol), HNO_3 (0.025 ml), HF (0.035 ml) and H_2O (5 ml) was placed in Teflon autoclave placed in a drying oven. The mixture was heated at 150°C for 12 h. The resulting light orange powder sample was collected by filtration, washed with purified water and dried at room temperature. An activation conditions was applied by further heating 8 h with water and EtOH. The MIL-100(Fe) was vacuumed and heated at 80 °C overnight.

Matrix and Sample Preparation for MALDI-TOF MS.

The matrices of 2-D $Zn_2(bim)_4$ nanosheets, pristine $Zn_2(bim)_4$, ZIF-8 and MIL-100(Fe) were all dispersed in methanol/H_2O (1:1, v/v) and sonicated for 10 min to form matrix suspension (0.5 mg mL^{-1}). The CHCA matrix (0.7 mg mL^{-1}) was dissolved in 0.1% TFA in water/acetonitrile (15/85, v/v). The concentrations of stock solutions for all the following analytes were 10 mM. Solvents were water/MeOH (1:1, v/v) for amino acids and serotonin. Four nucleobases were dissolved in hot water. The solvents for BPA and testosterone were acetonitrile and MeOH, respectively. All solutions were stored at 4 °C for further use.

Enzymatic reaction was monitored using the 2-D $Zn_2(bim)_4$ nanosheets as a matrix. The substrate of acetyl-choline (ACh, 400 nM) was catalyzed under enzyme acetylcholine esterase (AChe, 4 nM) in the buffer of NH_4HCO_3 (10 mM) under 37°C. The reaction was monitored by spotting 1 uL of the mixture at reaction time of 0 min, 3 min, 6 min, 9 min, 12 min, 15 min, and 18 min, respectively.

MALDI-TOF MS Analysis.

MS spectra were obtained with MALDI TOF/TOF MS (Bruker Daltonics, Germany) in reflected positive-ion mode and negative-ion mode. The instrument has been equipped with a "Smartbeam^{TM}-II technology" laser. Mass calibrations were performed externally using the peaks from CHCA and DHB. The laser intensity and the matrix/analyte molar ratio are optimized using dopamine as a model sample with the CHCA matrix in negative-ion mode (Fig. S5a). Laser intensity of 10%, 50%, 80% was tested. The high S/N is obtained for dopamine in

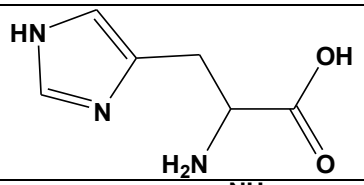
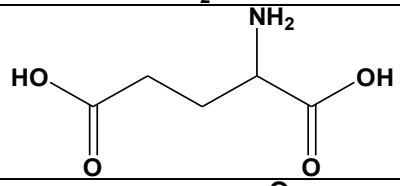
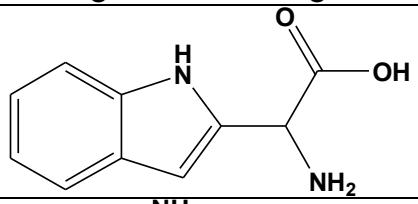
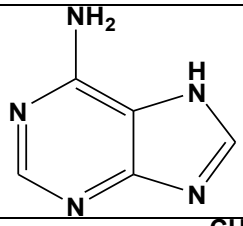
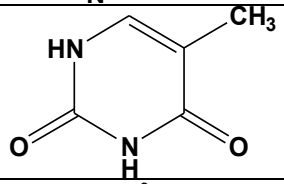
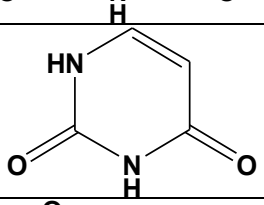
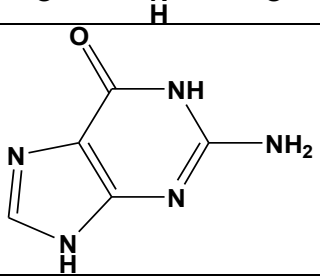
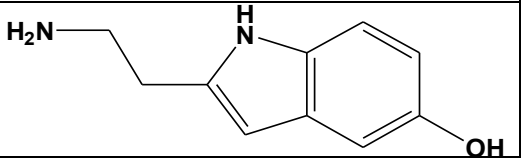
the laser intensity of 50% and 80%. The laser intensity of 50% was finally selected due to severe background interference ions with the laser intensity of 80%. Meanwhile, the matrix/analyte molar ratio is also optimized (Fig. S5b). The optimization of matrix concentrations for CHCA matrix is from 0.35 to 1.40 mg mL^{-1} (from 2 mM to 7 mM) with the fixed concentration of analyte of 10 mM. The matrix/analyte molar ratio is from 1:5 to 7:10. The CHCA matrix concentrations of 0.7 mg mL^{-1} (molar ratio of 2:5) was finally selected. These conditions are favorable to CHCA matrix. Furthermore, all the experimental data for the comparison of different matrices were finished under the same laser intensity (50%) and similar matrix concentration (0.7 mg mL^{-1} for CHCA and 0.5 mg mL^{-1}).

The laser intensity and the sampling frequency are adjusted to 50% and 1000 Hz, respectively. Each recorded mass spectrum was generated by accumulating data from 500 individual laser shots. The stainless steel MALDI plate was spotted using the following protocol for all experiments. Firstly, 1 μL of the analyte solution was deposited on a stainless steel plate with 384-well target and air-dried. Then, 1 μL of matrix suspension was deposited on the top of analytes.

(1) Cravillon, J.; Münzer, S.; Lohmeier, S.-J.; Feldhoff, A.; Huber, K.; Wiebcke, M. *Chem. Mater.* 2009, *21*, 1410-1412.

(2) Yoon, J. W.; Seo, Y.-K.; Hwang, Y. K.; Chang, J.-S.; Leclerc, H.; Wuttke, S.; Bazin, P.; Vimont, A.; Daturi, M.; Bloch, E.; Llewellyn, P. L.; Serre, C.; Horcajada, P.; Grenèche, J.-M.; Rodrigues, A. E.; Férey, G. *Angew. Chem. Int. Ed.* 2010, *49*, 5949-5952.

Table S1. List of small molecules detected in this work.

Compounds		Chemical formula	Structure
Histidine	His	$C_6H_9N_3O_2$	
Glutamic acid	Glu	$C_5H_9NO_4$	
Tryptophan	Trp	$C_{11}H_{12}N_2O_2$	
Adenine	A	$C_5H_5N_5$	
Thymine	T	$C_5H_6N_2O_2$	
Uracil	U	$C_4H_4N_2O_2$	
Guanine	G	$C_5H_5N_5O$	
Serotonin	-	$C_{10}H_{12}N_2O$	

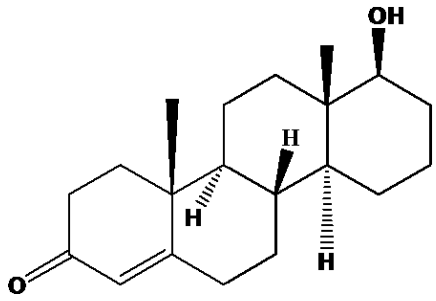
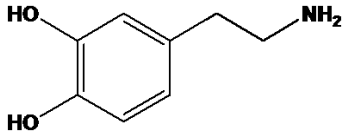
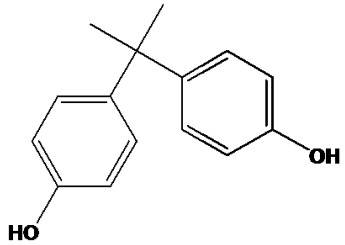
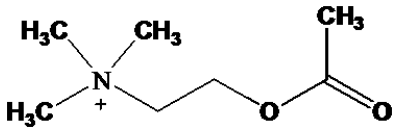
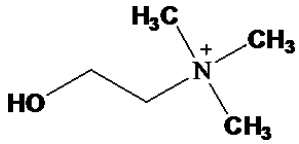
Testosterone	-	$C_{19}H_{28}O_2$	
Dopamine	-	$C_8H_{11}O_2N$	
Bisphenol A	BPA	$C_{15}H_{16}O_2$	
Acetylcholine	Ach	$C_7NH_{16}O_2$	
Choline	-	$C_5H_{14}NO$	

Table S2. MALDI-TOF Analysis of Four Nucleobases in Dual Ion Modes with the Matrices of 2-D MOF Nanosheets, CHCA and three MOFs.[a]

Ion mode	m/z	Detected ions	2-D $Zn_2(bim)_4$ nanosheets	CHCA	MOFs		
					Pristine $Zn_2(bim)_4$	MIL-100(Fe)	ZIF8
＋	136	[A+H]+	*	*	*	*	*
	159	[A+H+Na]+	*	*			
	188	[T+K+Na]+	*				
	113	[U+H]+	*		*		*
	152	[G+H]+	*	*	*	*	*
	174	[G+Na]+	*	*	*	*	*
	190	[G+K]+	*	*	*	*	*
－	134	[A-H]-	*	*	*	*	*
	125	[T-H]-	*		*	*	*
	111	[U-H]-	*	*	*	*	*
	150	[G-H]-	*	*	*	*	*
＋/－	Identified ions[b]		11	8	9	8	9
	Missing rate[c]		0%	27%	18%	27%	18%
＋	Background interference ions[d]		3	23	19	16	8
－			0	20	2	5	3

[a] The black stars in the table mark identified ions with specific matrices. [b] Identified ions mean the number of identified ions. [c] Missing rate was calculated as the ratio of missing ions to total ions. [d] Background interference ions were foreign ions with with S/N >6.

Table S3. Limit of detection (LOD) for dopamine analysis with different matrices.

Ion mode	m/z	Detected ions	LOD (S/N=3, μM)				
			2-D $Zn_2(bim)_4$ nanosheets	CHCA	MOFs		
					Pristine $Zn_2(bim)_4$	MIL-100(Fe)	ZIF8
＋	192	$[M+K]^+$	10	5	62	185	416
－	152	$[M-H]^-$	7	1	24	30	340

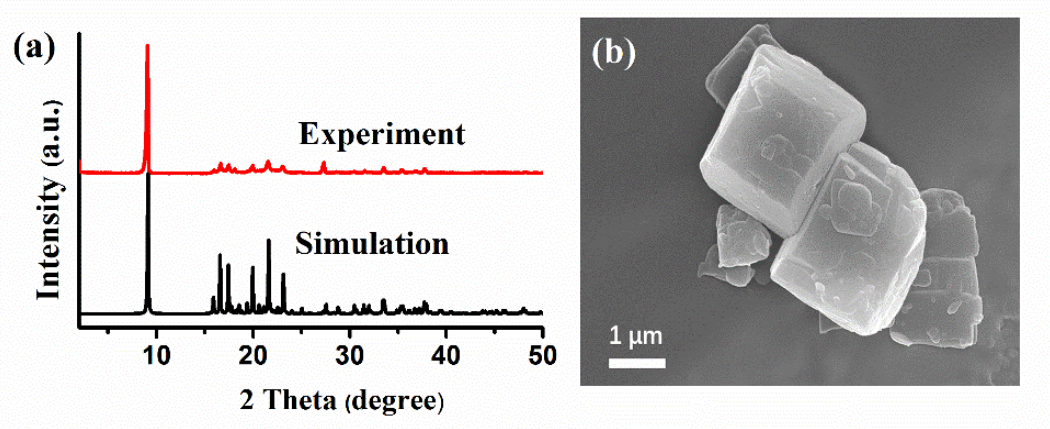


Fig. S1 (a) powder XRD patterns of pristine $Zn_2(bim)_4$. (b) SEM image of pristine $Zn_2(bim)_4$.

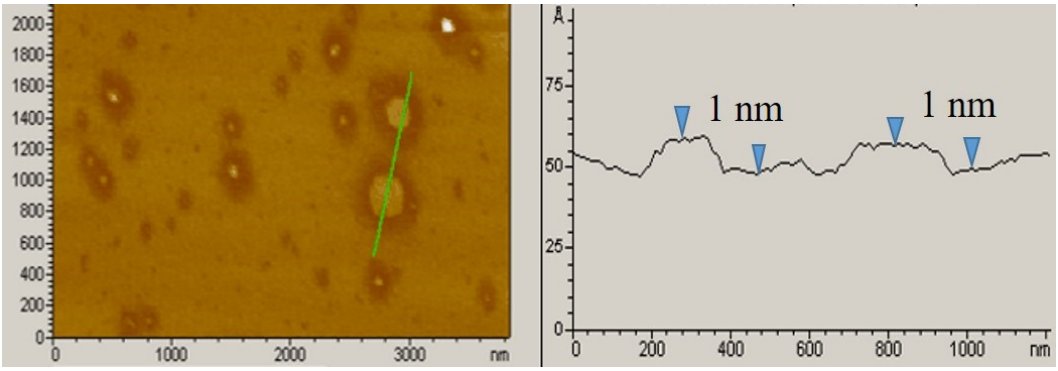


Fig. S2 AFM image of 2-D MOF nanosheets. The samples for AFM measurements were prepared by placing a drop of 2-D MOF nanosheets dispersion on a mica plate.

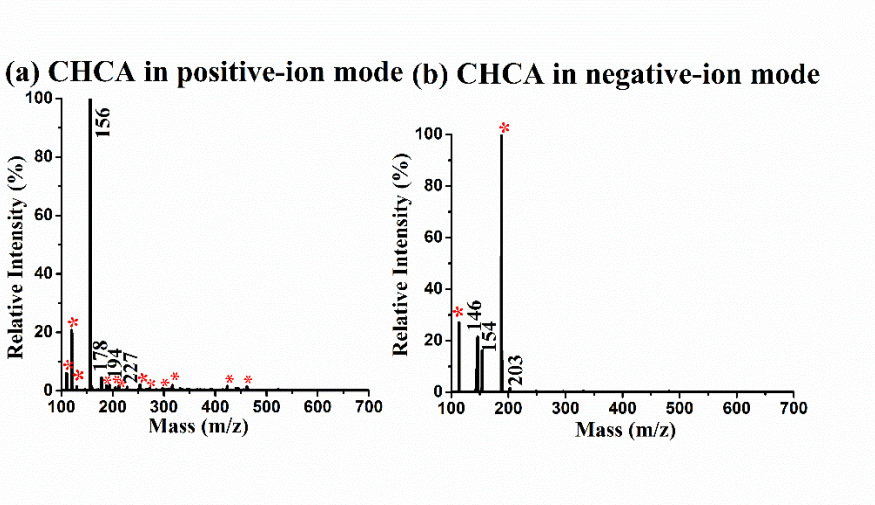


Fig. S3 CHCA assisted laser desorption ionization mass spectra of three amino acids (Glu, His and Trp) in positive-ion (a) and negative-ion modes (b). Background interference ions were shown with red marks.

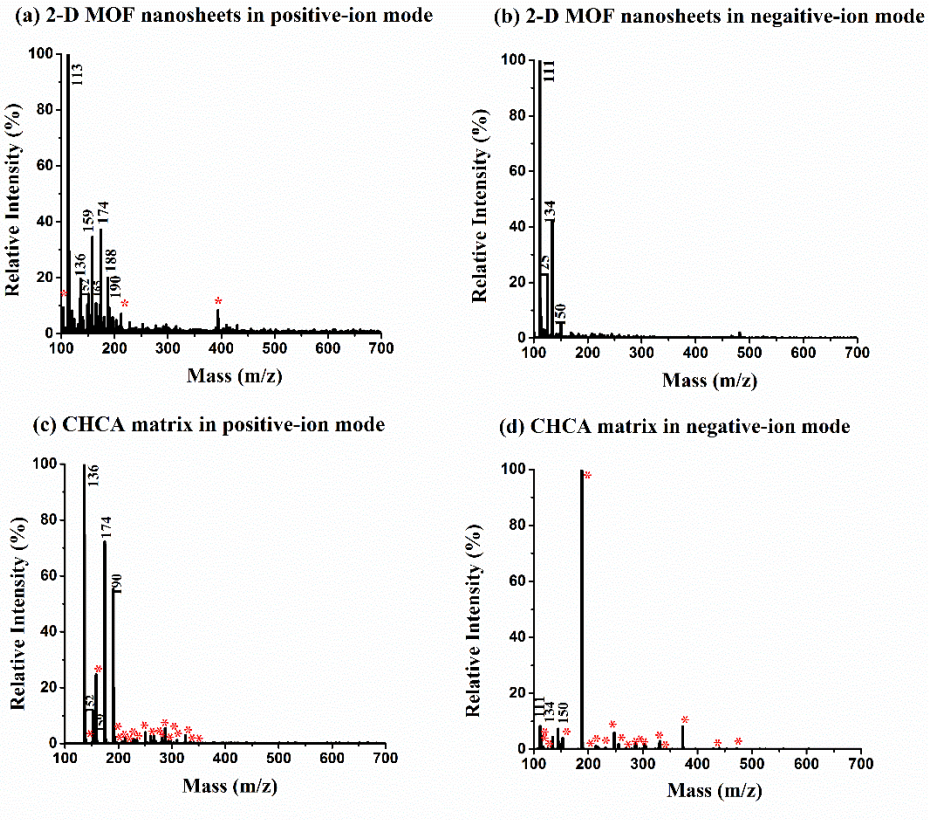


Fig. S4 Two-dimensional $Zn_2(bim)_4$ nanosheets (a, b) and CHCA (c, d) assisted laser desorption ionization mass spectra of four nucleobases (A, T, U and G) in positive-ion (a, c) and negative-ion modes (b, d). Background interference ions were shown with red marks.

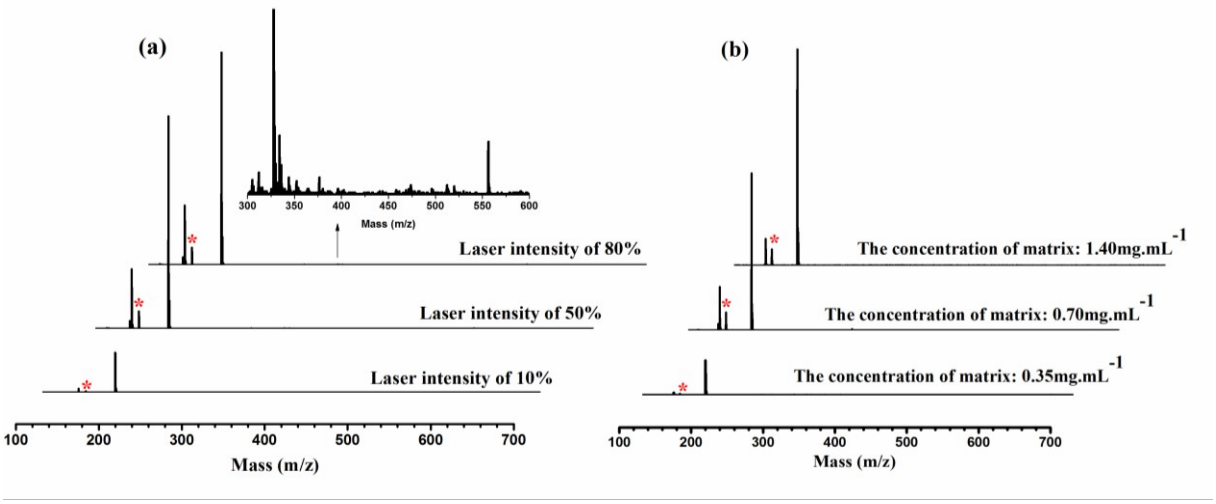


Fig. S5 The MS spectra of dopamine (10 mM) under laser intensity from 10% to 80% (a) and under different matrix/analyte molar ratio (b) with the matrix of CHCA in negative mode. The mark of * represents m/z 152, $[M-H]^-$. The optimization of matrix concentrations for CHCA matrix from 0.35 to 1.40 mg mL^{-1} (from 2 mM to 7 mM) are now added with the fixed concentration of analyte concentration of 10 mM. The matrix/analyte molar ratio is from 1:5 to 7:10. The laser intensity of 50% and the CHCA matrix concentrations of 0.7 mg mL^{-1} (molar ratio of 2:5) were finally selected, respectively.

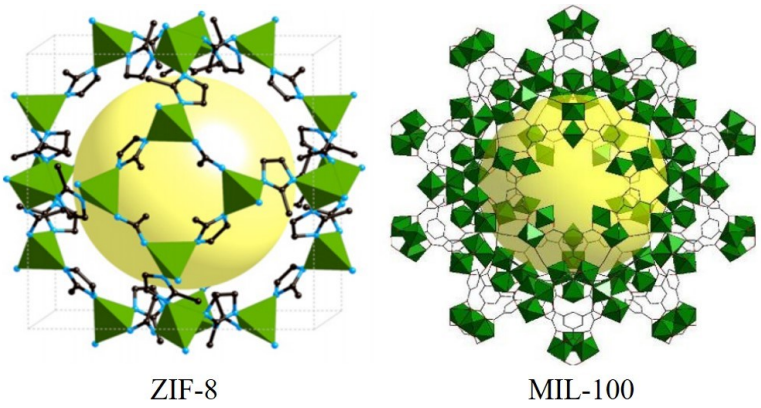


Fig. S6 Crystal Structures of ZIF-8 and MIL-100(Fe).

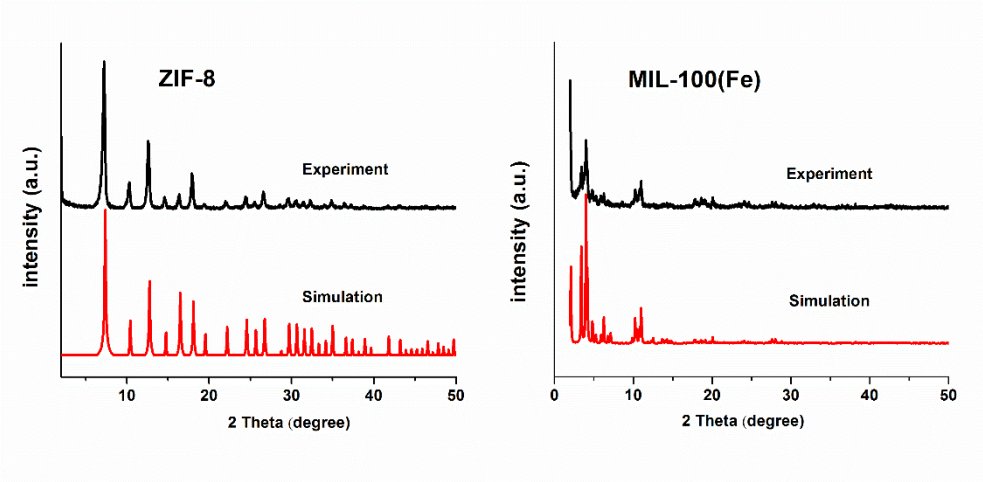


Fig. S7 Powder X-Ray diffraction patterns for ZIF-8 and MIL-100(Fe).

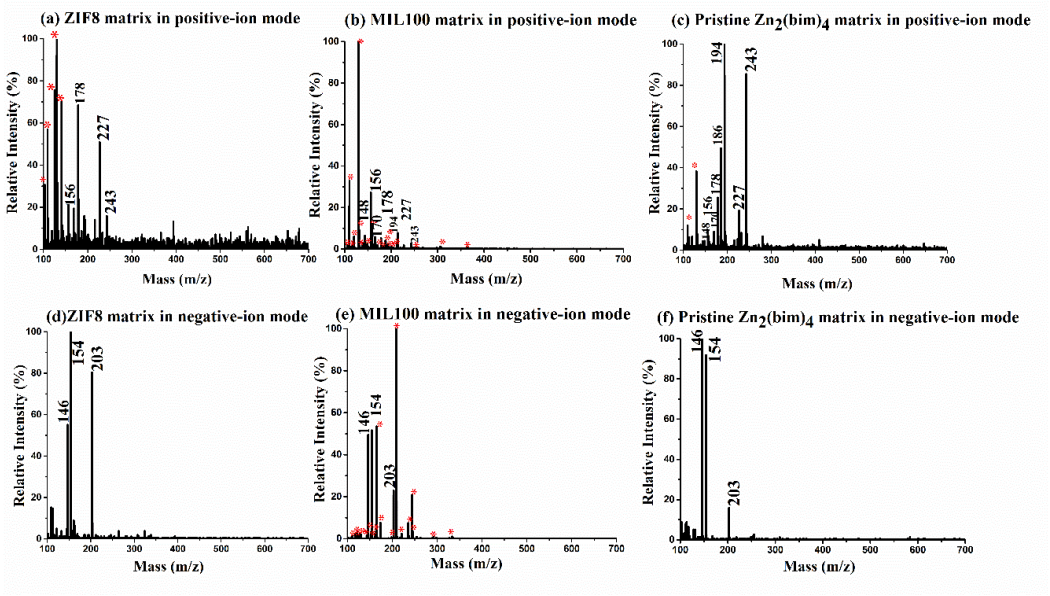


Fig. S8 MALDI-TOF mass spectra of amino acids mixture containing Glu, His and Trp with three MOF matrices (ZIF-8, MIL-100 and pristine $Zn_2(bim)_4$) in positive-ion and negative-ion mode. Background interference ions were shown with red marks. Representative eighteen peaks were selected marked in (b).

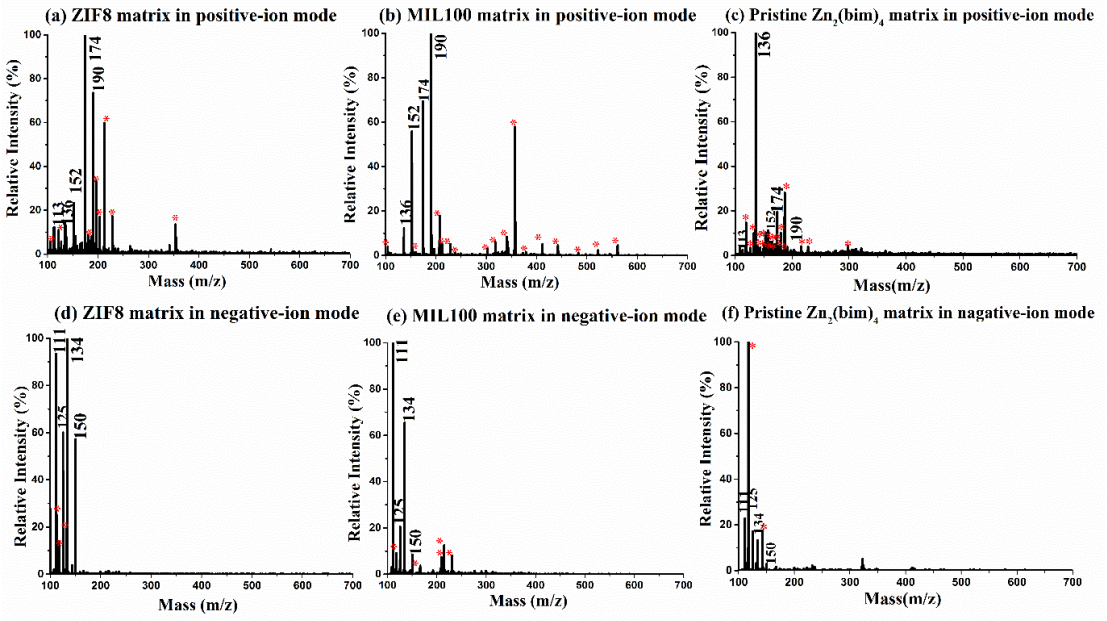


Fig. S9 MALDI-TOF mass spectra of four nucleobases containing A, T, U and G with three MOF matrices (ZIF-8, MIL-100 and pristine $Zn_2(bim)_4$) in positive-ion and negative-ion mode. Background interference ions were shown with red marks.

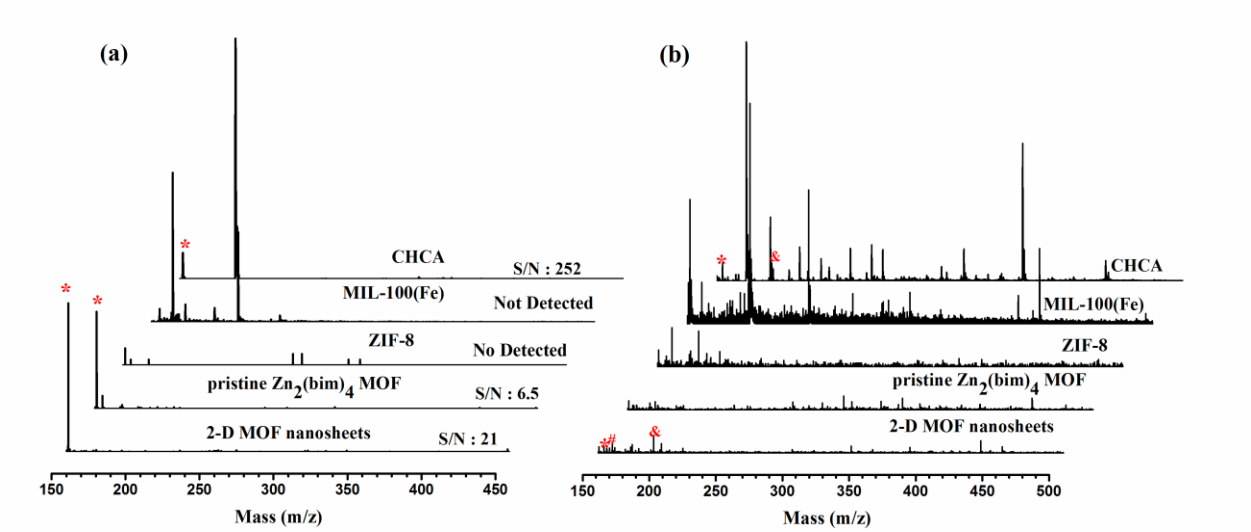


Fig. S10 MS spectra obtained for dopamine (50 μM) in negative ion mode (a) and positive mode (b) with different matrices to show 2-D $Zn_2(bim)_4$ nanosheets have a good sensitivity. In the negative mode, the mark of * represents m/z 152, $[M-H]^-$. In the positive mode, the mark of * represents m/z 154, $[M+H]^+$ (S/N=5.4 for 2-D nanosheets). The mark of # represents m/z 176, $[M+Na]^+$ (S/N=6.9 for 2-D nanosheets), while the mark of & represents m/z 192, $[M+K]^+$ (S/N=15.0 for 2-D nanosheets).

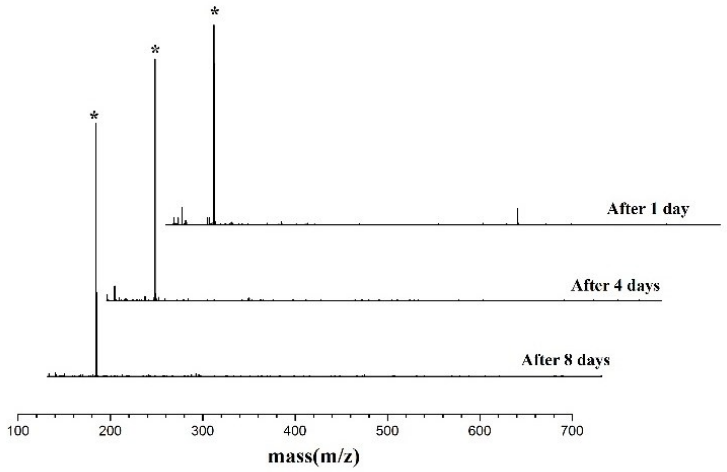


Fig. S11 MS spectra obtained for dopamine (2.5 mM, m/z 152, $[M-H]^-$) after 1 day, 4 days, and 8 days in negative ion mode with the matrix of 2-D $Zn_2(bim)_4$ nanosheets to show the good stability of nanosheets suspension.

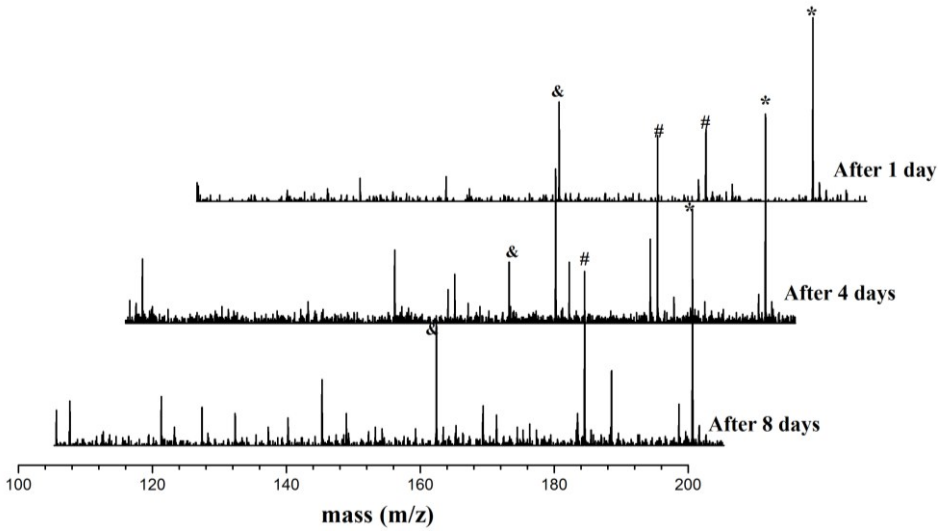


Fig. S12 MS spectra obtained for dopamine (2.5 mM) after 1 day, 4 days, and 8 days in positive ion mode with the matrix of 2-D $Zn_2(bim)_4$ nanosheets to show the good stability of the nanosheets suspension. The mark of & represents m/z 154, $[M+H]^+$. The mark of # represents m/z 176, $[M+Na]^+$. The mark of * represents m/z 192, $[M+K]^+$.

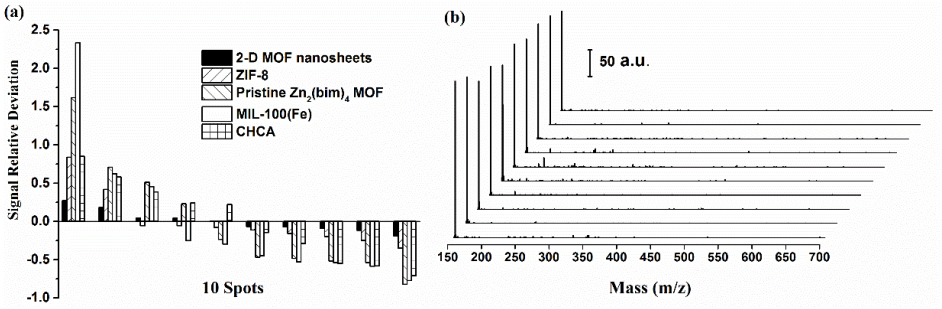


Fig. S13 (a) Signal relative deviation of MS intensity obtained in ten spots for dopamine (2.5 mM) analysis in negative ion mode with different matrices to show the good reproducibility of 2-D $Zn_2(bim)_4$ nanosheets. Relative deviation was calculated by $\text{relative deviation} = \frac{I_i - I_{Mean}}{I_{Mean}}$. (b) MS spectra obtained for ten parallel spots with the matrix of 2-D $Zn_2(bim)_4$ nanosheets for dopamine (m/z 152, $[M\text{-}H]^-$).

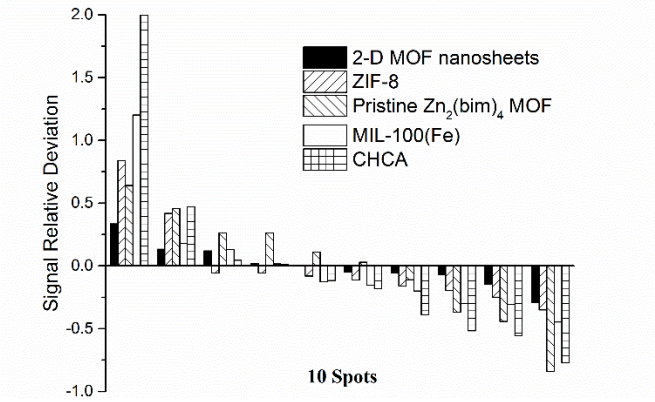


Fig. S14 Signal relative deviation for MS intensity obtained in ten spots for dopamine (2.5 mM) analysis in positive ion mode with different matrices to show the good reproducibility of 2-D $Zn_2(bim)_4$ nanosheets. Relative deviation was calculated by $\text{relative deviation} = \frac{I_i - I_{Mean}}{I_{Mean}}$.

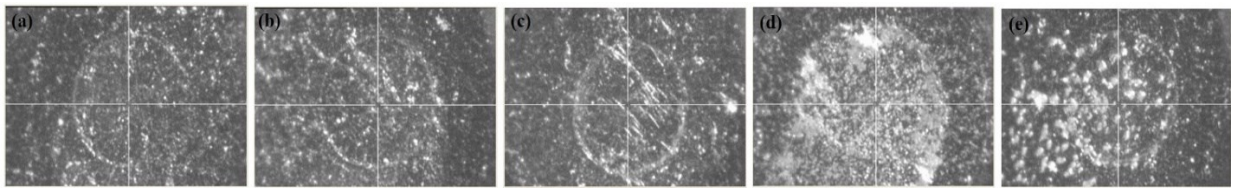


Fig. S15 Optical images of dried samples spotted with different matrices of (a) 2-D MOF nanosheets, (b) pristine $Zn_2(bim)_4$ MOF, (c) ZIF-8, (d) MIL-100(Fe), and (e) CHCA on the stainless steel target. Normally, 1 μL of the analyte solution was deposited on a stainless steel plate and air-dried. Then, 1 μL of matrix solution was deposited on the top of analytes.

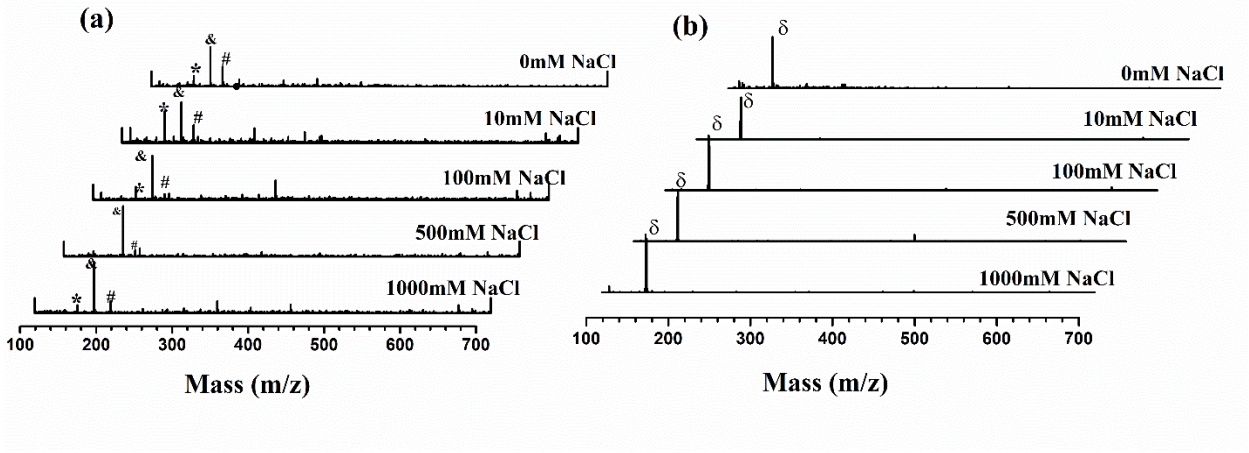


Fig. S16 MS spectra obtained for His (10 mM) with NaCl concentrations from 0 mM to 1000 mM in positive ion mode (a) and negative mode (b) with the matrix of 2-D $Zn_2(bim)_4$ nanosheets to show the good salt tolerance. The marks in (a): *, m/z 156, $[M+H]^+$; &, m/z 178, $[M+Na]^+$; #, m/z 194, $[M+K]^+$. The marks in (b): δ, m/z 154, $[M-H]^-$.

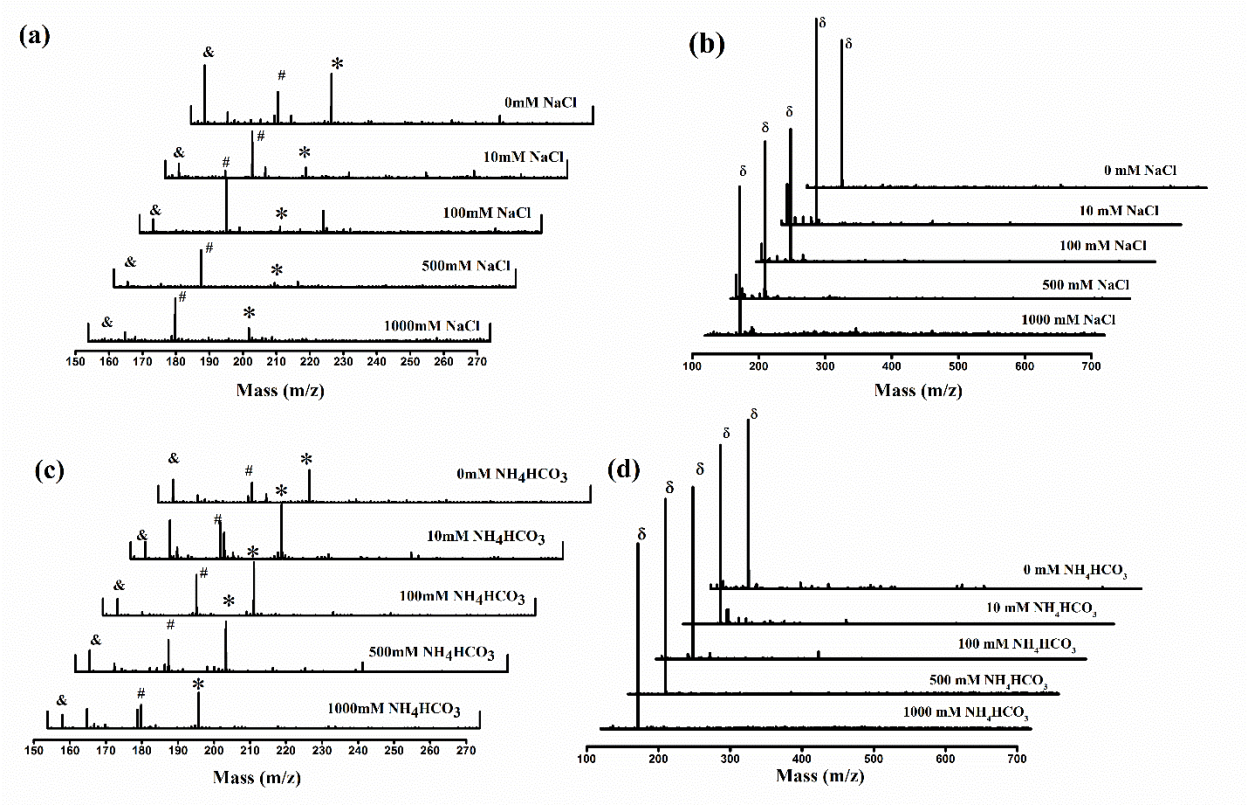


Fig. S17 MS spectra obtained for dopamine (2.5 mM) with NaCl (a and b) and NH_4HCO_3 (c and d) concentrations from 0 mM to 1000 mM in positive ion mode (a and c) and negative mode (b and d) with the matrix of 2-D $Zn_2(bim)_4$ nanosheets to show the good salt tolerance. In the positive mode, the mark of & represents m/z 154, $[M+H]^+$, the mark of # represents m/z 176, $[M+Na]^+$, the mark of * represents m/z 192, $[M+K]^+$. In the negative mode, the mark of δ represents m/z 152, $[M-H]^-$.