

Supporting information for
Fluorographene nanosheets: a new carbon-based matrix for
the detection of small molecules by MALDI-TOF MS

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EXPERIMENTAL SECTION

Chemicals and reagents

Graphite fluoride powders (fluorographite, (CF_{0.25})_n, Alfa Aesar), graphene oxide and carboxylated carbon nanotube (Nanjing XFNANO Materials Tech Co, Ltd) were used as received without additional treatment. Amino acids containing Gln, Pro, Leu, Asp, Arg, Tyr, Trp, Asn, His, Phe, Ser, Thr and Val were purchased from Beijing chemical reagents. Peptides containing Gly-Ala, Gly-His, Gly-Asp, Gly-Tyr, Val-Tyr-Val, Tyr-Gly-Gly-Phe-Leu and Asp-Arg-Val-Tyr-Ile-His-Pro-Phe were purchased from Shanghai Jili biochemical. Glutathione, hypoxanthine, creatinine, creatine, hypoxanthine riboside, taurine, N-acetyl aspartic acid, vitamin C, citric acid, Estradiol, 17 α -ethynylestradiol, huperzine-A and Doxorubicin hydrochloride were purchased from Sigma-Aldrich. Nitro-polyaromatic hydrocarbon, melatonin (MT) and uric acid (UA) were purchased from J&K Scientific. The 2, 4, 6-trinitrotoluene (TNT) and pentaerythritol tetranitrate (PETN) were provided by the Police of Haidian District, Beijing. Uric acid-1, 3-¹⁵N₂ was bought from Cambridge Isotope Laboratories, Int. (Andover, MA) and used as received. Acetonitrile and chloroform were of HPLC grade. Water used was deionized using a Milli-Q ultrapure water purification system (Merck, Ltd, USA).

MALDI-TOF MS analysis and MSI

Amino acids, peptides, glutathione, hypoxanthine, creatinine, creatine, hypoxanthine riboside, taurine, N-acetyl aspartic acid, vitamin C, citric acid, estradiol, huperzine-A and doxorubicin hydrochloride were dissolved in water as a concentration of 0.5 m M. 17 α -ethynylestradiol and nitro-polyaromatic hydrocarbon were dissolved in methanol as a concentration of 0.5 m M. MT was dissolved in ethanol at 1 m M as a stored solution. TNT and PETN were dissolved in acetone

at 1 m M. UA and UA-1, $3\text{-}^{15}\text{N}_2$ was dissolved in 10 m M NaOH solution in 50°C . FG was dissolved as a concentration of 0.1 mg/mL. The human serum was 2-fold diluted with acetonitrile and centrifuged to remove the high-abundance proteins, remaining the supernatant collected for negative ion mode MALDI-TOF MS analysis with the matrix of FG. For MALDI-TOF analysis, FG solution and analyte solution were mixed with the volume ratio of 1:1, then $1\ \mu\text{L}$ mixture was pipetted on the MALDI plate and air dried. MALDI-TOF MS analysis was performed on an Ultraflextreme MALDI-TOF/TOF MS (Bruker Daltonics, Billerica, MA) in negative reflection mode. The mass spectrometer was equipped with a smartbeam Nd: YAG pulsed laser operated at 355 nm. The acceleration and reflection voltage were 19 and 20 KV. The laser pulse energy was adjusted between 0 and $100\ \mu\text{J}$ per pulse by regulating the laser power energy from 0% to 100%. The mass spectra were acquired at an average of 500 laser shots and 1000 Hz frequency. A mixture of sugars was used for negative-ion mode mass calibration. The mass spectra were processed by FlexAnalysis v3.4. For MALDI-TOF MSI, The imaging data was acquired in negative ion mode with laser spot size setting at $200\ \mu\text{m}$ and summed up by 200 shots at a laser repetition rate of 2000 Hz. Imaging data were processed by Fleximaging v3.0 or Biomap v3.8.

Sample preparation for MALDI-TOF MS detection of MT in human serum

The human serum was first diluted with 2-fold acetonitrile to precipitate the high-abundance proteins, remaining the supernatant to be collected. Then melatonin (MT) was spiked into the supernatant to a final concentration of $100\ \mu\text{M}$. Then $1\ \mu\text{L}$ of the solution was mixed with $1\ \mu\text{L}$ of FG (diluted in ACN) and $1\ \mu\text{L}$ of the mixture was spotted on the target plate for MALDI-TOF MS detection.

Sample preparation for MALDI-TOF MS detection of SA in human saliva

The female volunteer was instructed to not eat food or drink for 2h before the saliva collection. During the collection, no physical or chemical stimulation was used, although the imagination in the volunteer' mind might have slightly stimulated the salivary flow. Saliva was collected for 10 min. After collection, 300 μ L of saliva was mixed with 900 μ L of acetonitrile and centrifuged at 1000 rpm for 10 min to precipitate the high-abundance proteins. The supernatant was filtered by 0.45 μ m membrane filter. Then 1 μ L of the saliva was mixed with 1 μ L of FG matrix (diluted in ACN) and 1 μ L mixture was spotted on the target plate for MALDI-TOF MS detection.

Sample preparation for in-situ MALDI-TOF MS detection of MCF-7 cells

The MCF-7 cells were cultured on the ITO glass and washed by 150 m M ammonium acetate solution. After washing, the cells were frozen rapidly by liquid nitrogen and freeze-dried for 12 hours. Then 10 μ L FG matrix was spotted on the surface of cells and after air-dried, the cells were detected by MALDI-TOF MS directly.

Sample preparation for MALDI-TOF MS detection of DOX in human serum

The human serum was first diluted with 2-fold acetonitrile to precipitate the high-abundance proteins, remaining the supernatant to be collected. Then doxorubicin with different concentrations was added into the human serum for MALDI-TOF MS analysis.

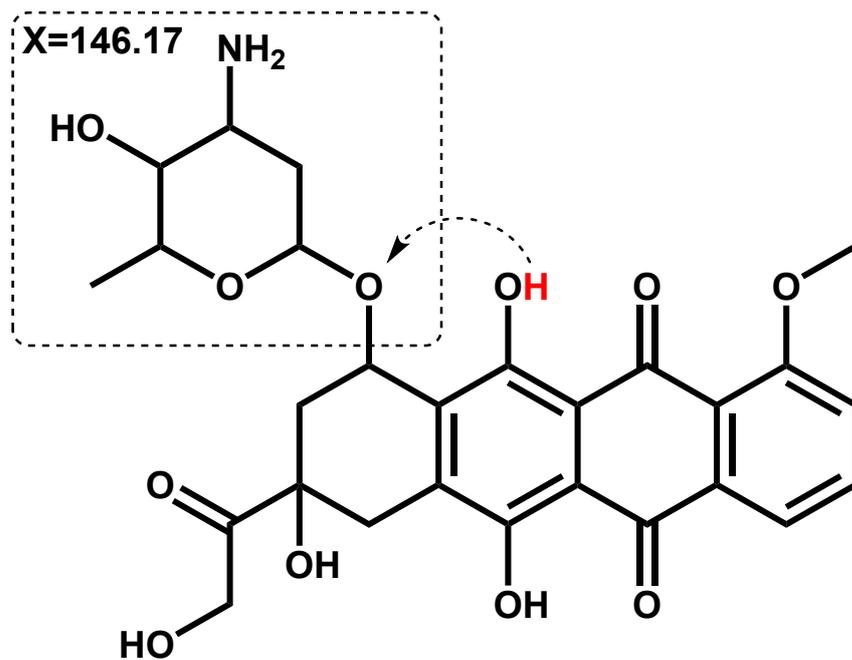
Sample preparation for uric acid analysis

For the analysis of uric acid in urine, the calibration curve was obtained in artificial urine. Uric-free artificial urine was prepared according to the literature with slight modification by dissolving 606.8 mg of urea, 22.5 mg of creatinine, 74.2 mg of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, 158.5 mg of NaCl, 112.5 mg of KCl, 40.2 mg of NH_4Cl , 22.2 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 12.2 mg of MgSO_4 , 8.5mg of NaHCO_3 , 1.0 mg of $\text{Na}_2\text{C}_2\text{O}_4$, 64.5 mg of Na_2SO_4 , 28.3 mg of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, and 6.9 mg of

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 50 mL of deionized water. The 0.2, 0.5, 1.0, 3.0, 5.0 mM uric acid solutions with 2 mM internal standard in artificial urine (diluted 20 times with water) were used in the calibration curve. For the detection of uric acid in real samples, fresh urine sample were collected from a health male volunteer, and warmed to 50°C to dissolve the precipitated uric acid and then diluted with 3-fold water immediately. Subsequently, 100 μL of the uric acid-1, 3- $^{15}\text{N}_2$ solution (10 mM) was mixed with 100 μL of the diluted urine then diluted to 500 μL with water. 1 μL of the mixture was mixed with 1 μL of FG matrix and then 1 μL of the sample was dropped onto the target plate to MALDI-TOF MS analysis.

Table S1. 24 kinds of solvents dispersing FG.

PE	hexane	cyclohexane	isooctane	pentanol	CH ₂ Cl ₂
butanol	IPA	EtOH	THF	EA	EF
dioxane	acetone	acetonitrile	DMF	MeOH	CCl ₄
ethor	toluene	EG	DMSO	NMP	isooctanol



Scheme S1. Fragment process of DOX by MALDI-TOF MS using FG as matrix in negative ion mode. Laser intensity: 70%.

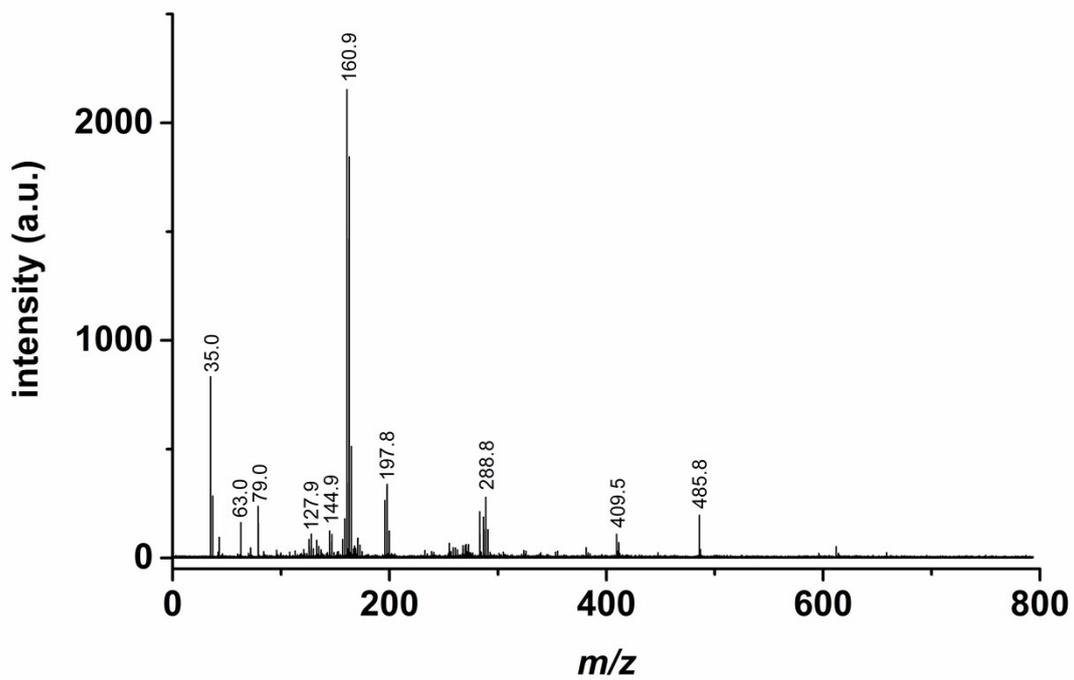
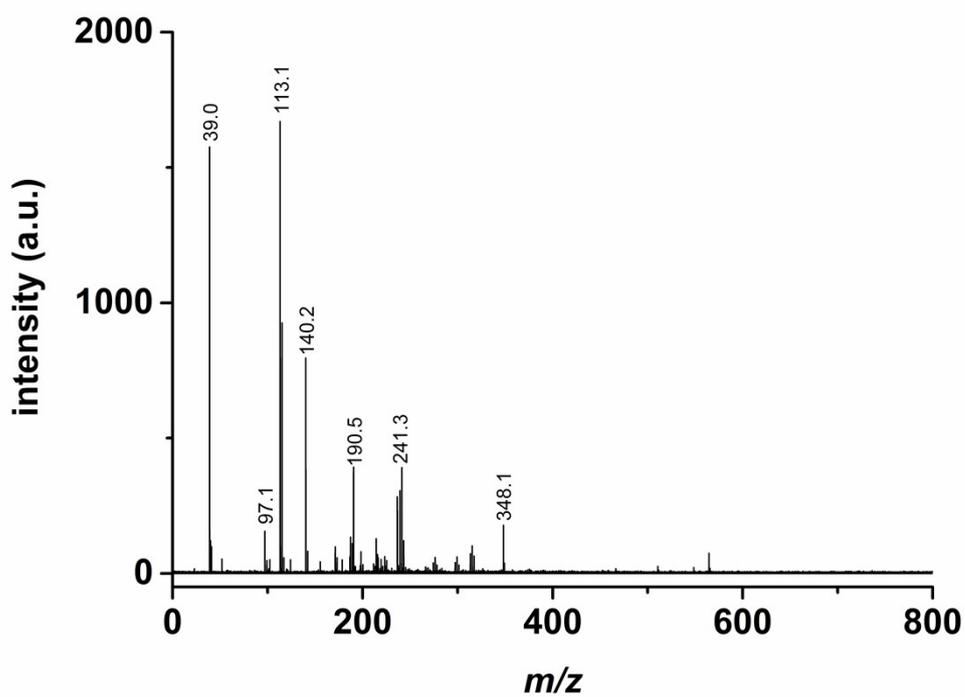


Fig. S1 MS spectra of FG in positive ion mode and negative ion mode.

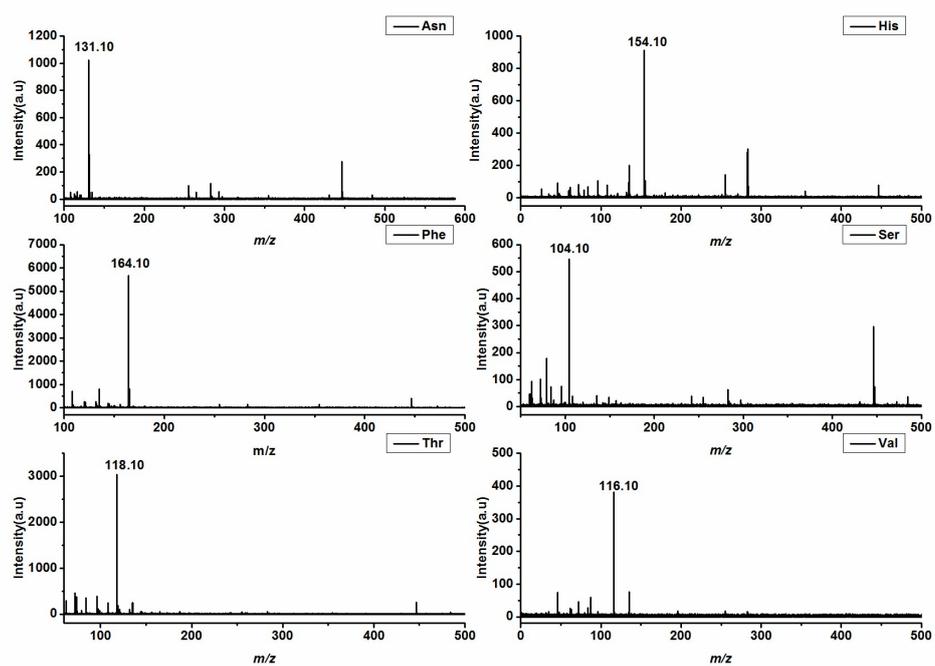


Fig. S2 MALDI-TOF mass spectra of amino acids: Asn (m/z 131.1), His (m/z 154.1), Phe (m/z 164.1), Ser (m/z 104.1), Thr (m/z 118.1), Val (m/z 116.1) using FG as matrix in negative ion mode. The amount of each analyte was 500 pmol.

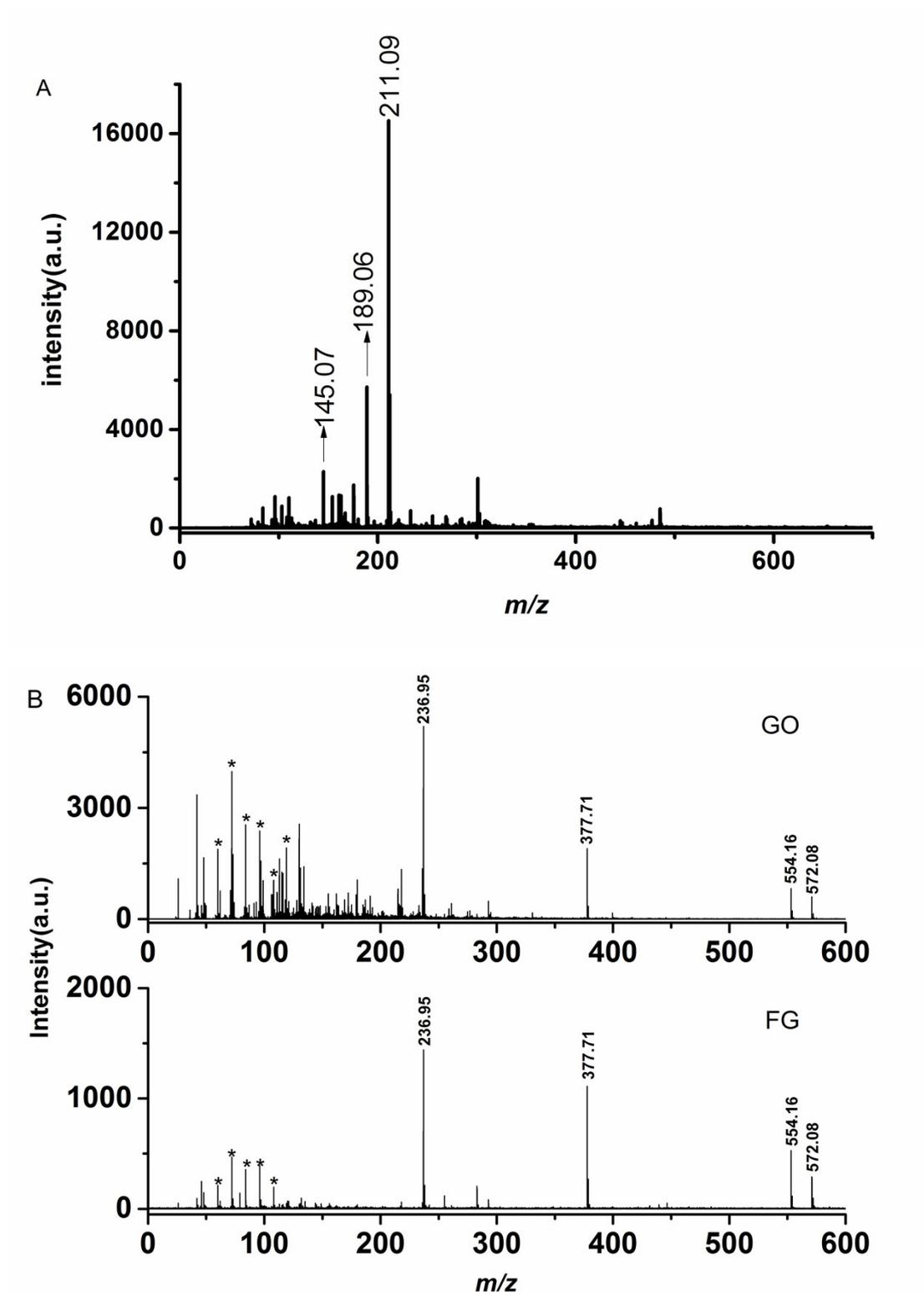


Fig. S3 A: MALDI-TOF mass spectra of three oligopeptides Gly-Ala (m/z 145.07), Gly-His (189.06) and Gly-Asp (m/z 211.09) mixture by using FG as matrix in negative ion mode. B: MALDI-TOF mass spectra of four peptides Gly-Tyr (m/z 236.95), Val-Tyr-Val (m/z 377.71), Tyr-Gly-Gly-Phe-Leu (m/z 554.16) and Asp-Arg-Val-Tyr-Ile-His-Pro-Phe (m/z 572.08) by using GO and FG as matrix in negative ion mode. * Represent carbon cluster anions at 60, 72, 84, 96, 108, 120 for GO and 60, 72, 84, 96, 108 for FG. The amount of each analyte was 500 pmol.

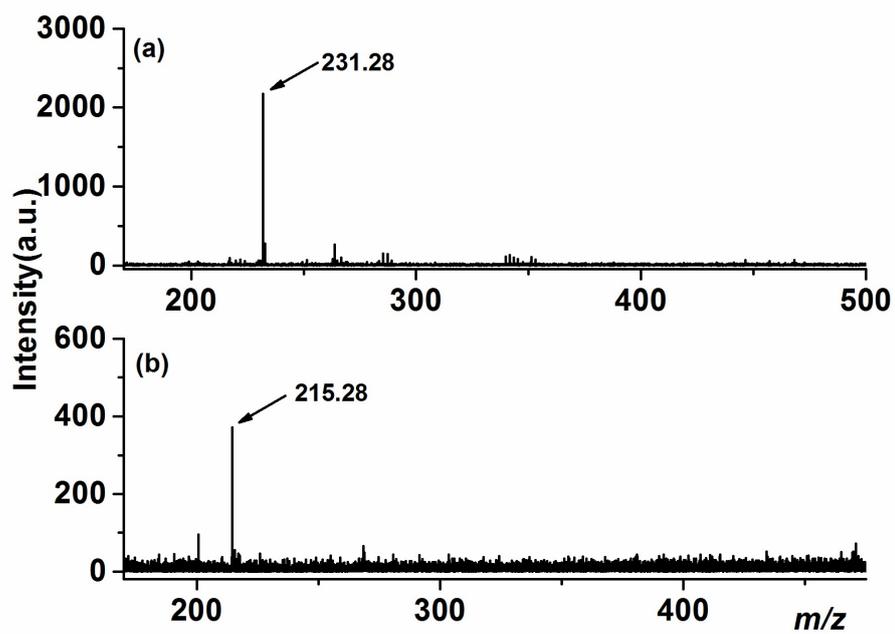


Fig. S4 Mass spectra of: (a) 1 m M MT in EtOH, m/z 231.28 refers to the $[M-H]^-$ ions of MT; (b) 100 μ M MT spiked in human urine, m/z refers to the $[M-CH_3-H]^-$ ions; by using FG in negative ion mode. Laser intensity: 70%.

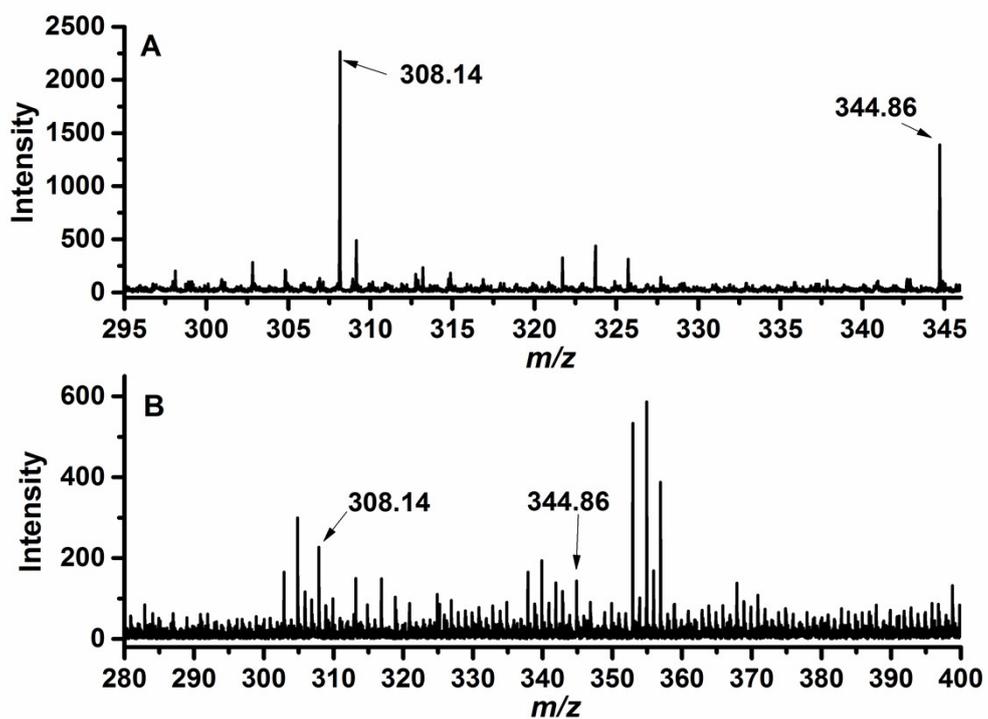


Fig. S5 Mass spectra of (A): 1m M SA in negative ion mode using FG as matrix, m/z 308.14 and 344.86 refer to $[M-H]^-$ and $[M+Cl]^-$ of SA; (B) mass spectra of saliva from a female volunteer, m/z at 308.14 and 344.86 refer to the $[M-H]^-$ and $[M+Cl]^-$ of endogenous SA in saliva.

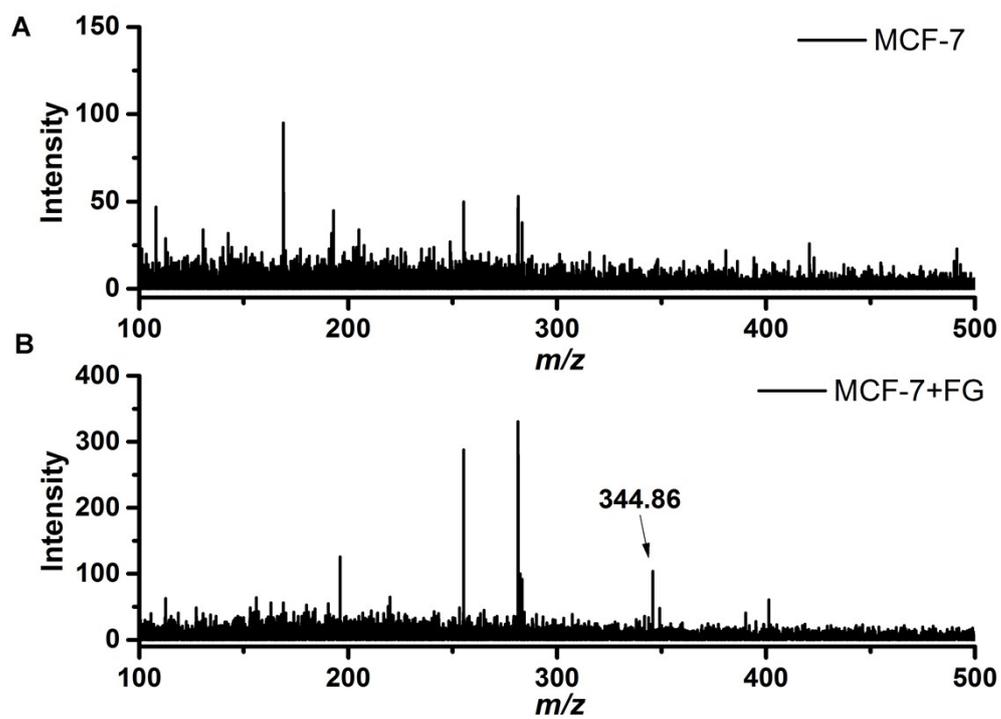
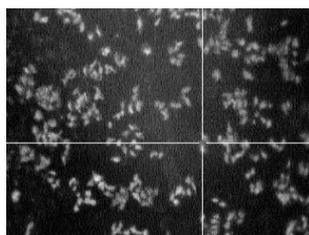


Fig. S6 Photograph of MCF-7 cells under vacuum and the mass spectrum of (A) MCF-7 cells without FG matrix; (B) MCF-7 cells with FG matrix, m/z 344.86 refers to the $[M+Cl]^-$ of SA.

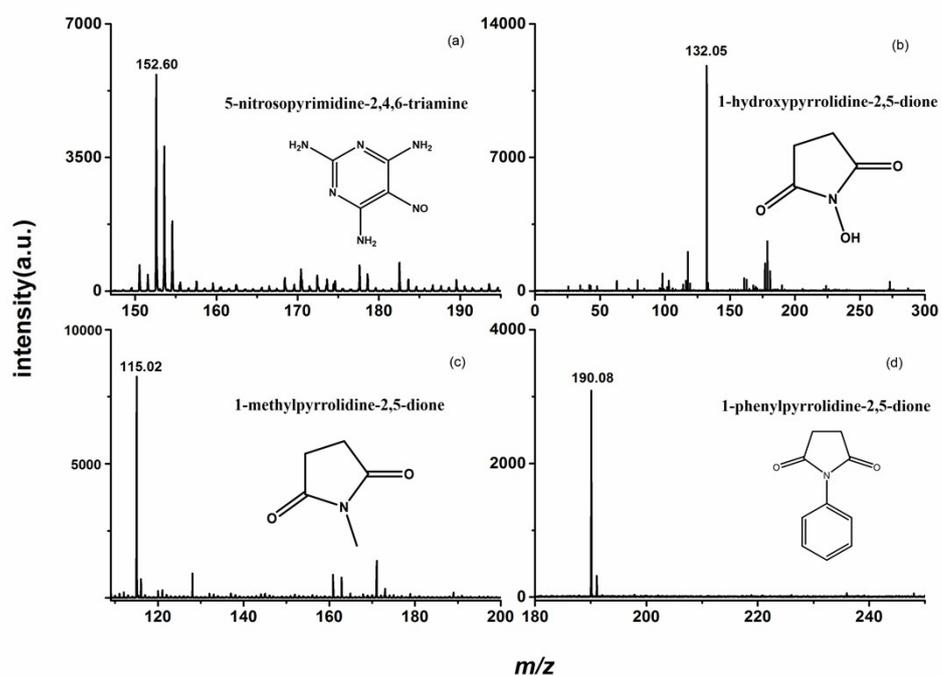


Fig. S7 MALDI-TOF mass spectra of nitroso-compounds: 5-nitrosopyrimidine-2, 4, 6-triamine (m/z 152.60) and imide-compounds: 1-hydroxypyrrolidine-2, 5-dione (m/z 132.05), 1-methylpyrrolidine-2, 5-dione (m/z 115.02) and 1-phenylpyrrolidine-2, 5-dione (m/z 190.08). The amount of each analyte was 500pmol.

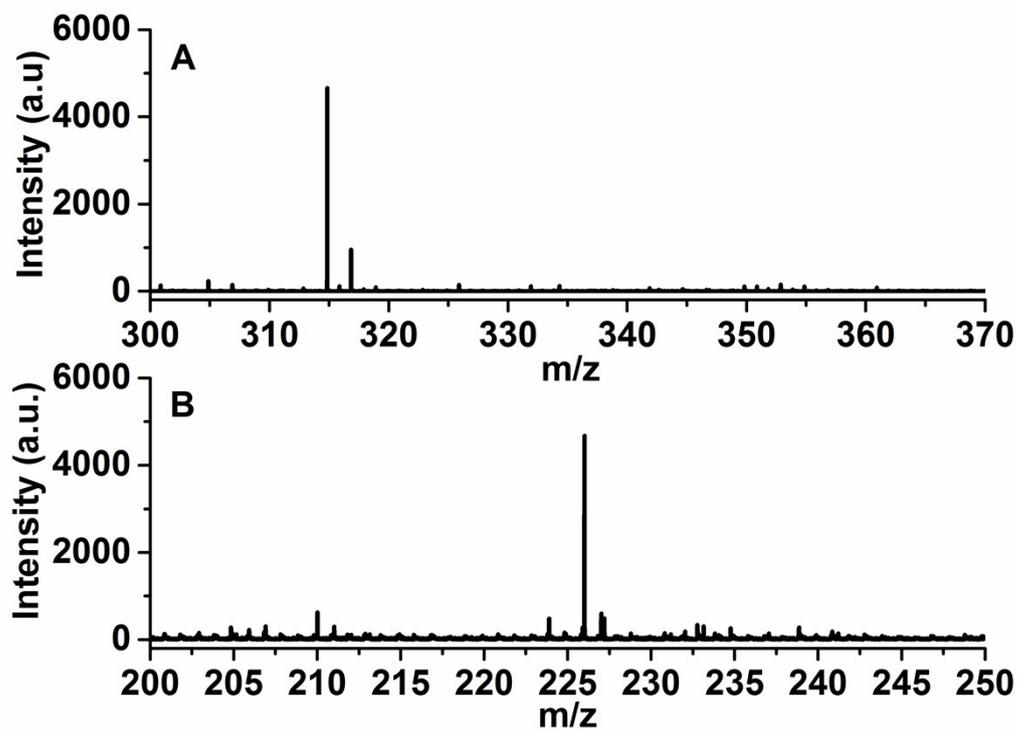


Fig. S8 MALDI-TOF MS spectra of explosives with FG as matrix in negative ion mode. A: pentaerythritol tetranitrate (PETN), m/z 315.01, $[M-H]^-$; B: 2, 4, 6-trinitrotoluene (TNT), m/z 226.01, $[M-H]^-$. The amount of each analyte is 500 pmol.

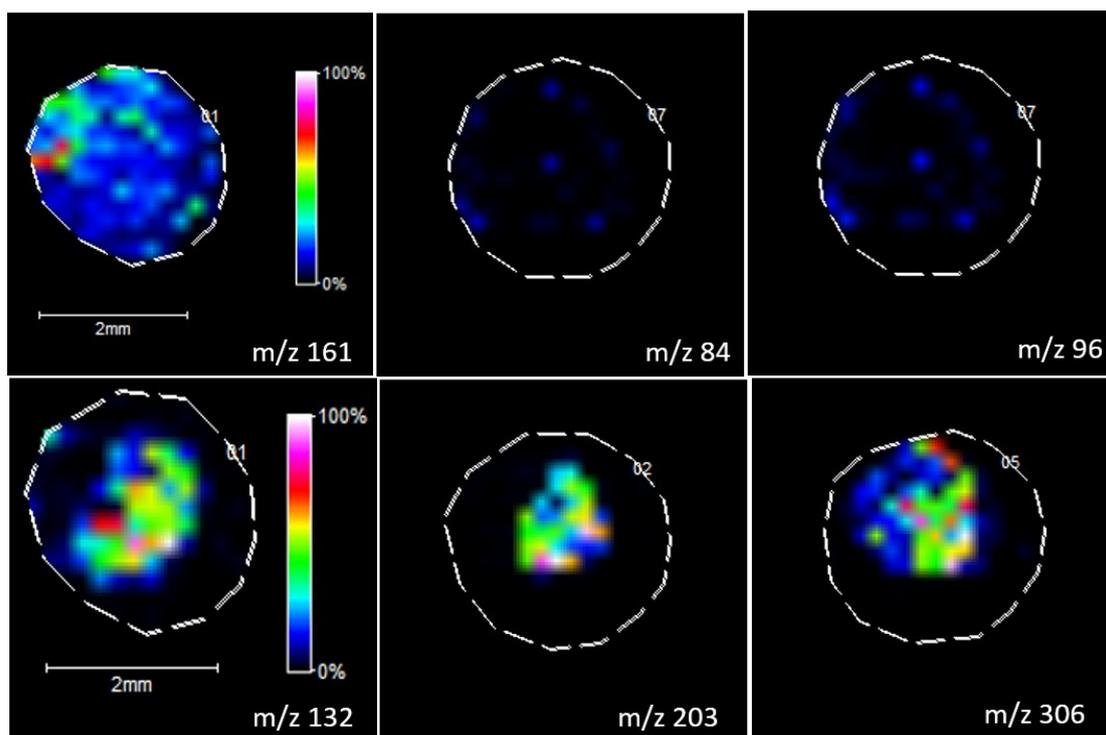


Fig. S9 MALDI-TOF mass spectrometry imaging of sample points: m/z 161, m/z 84, and m/z 96 for FG; m/z 132 for Asp, m/z 203 for Trp and m/z 306 for GSH using FG as matrix in negative ion mode. The upper line indicated the background signal of FG at m/z 161 and the lower line showed the chosen representative analytes Asp at m/z 132, Trp at m/z 203 and GSH at m/z 306. The amount of each analyte was 500 pmol.

