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Supporting Information

Development of N, S-doped carbon dots as a novel matrix for the analysis of small molecules by negative ion MALDI-TOF MS

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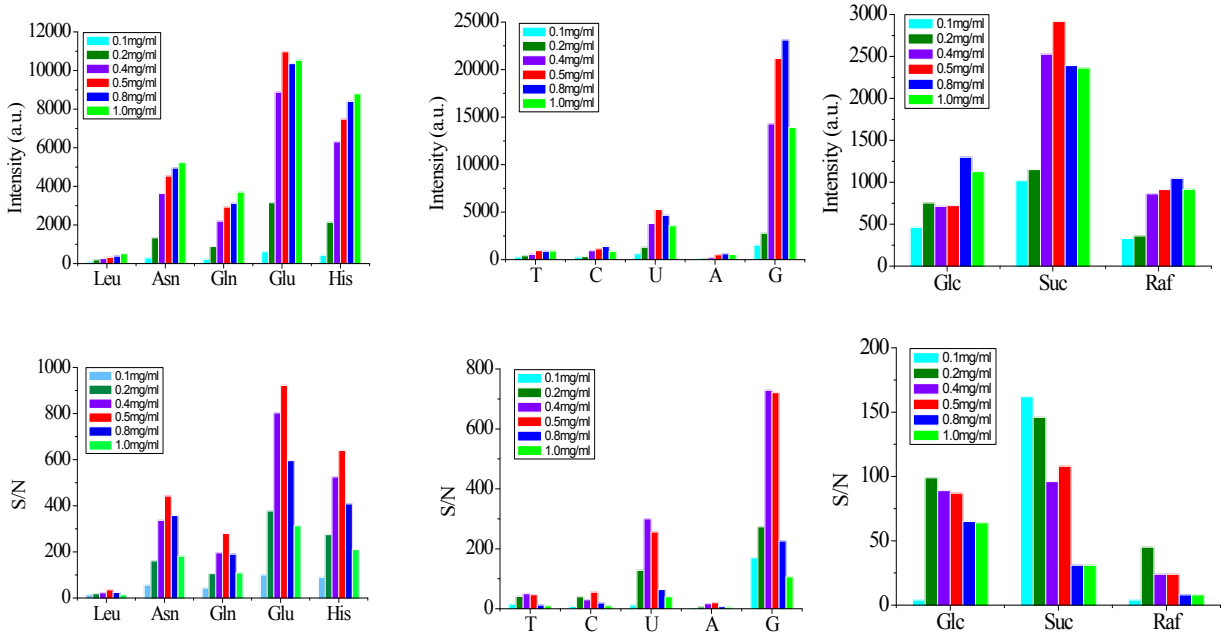

Fig. S1 Comparison of small molecules ion signals and S/N for different concentration of N, S-CDs as matrix. The concentration of N, S-CDs matrix was set as 0.1, 0.2, 0.4, 0.5,0.8,1.0mg/ml. As shown in the figure, the N, S-CDs at concentration of 0.5mg/ml had good performance.

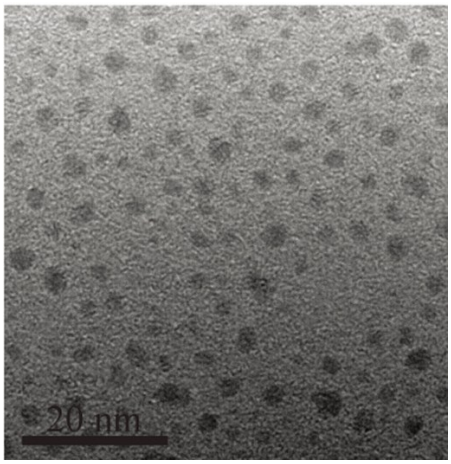

Fig. S2.TEM images of the obtained N, S-CDs.

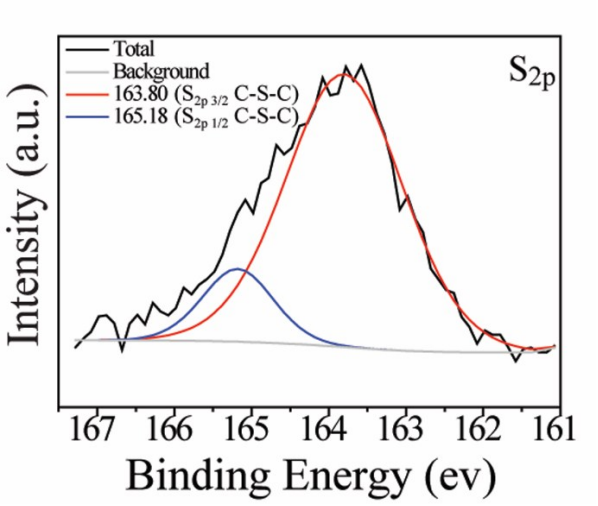

Fig. S3 High-resolution XPS spectra of S2p peaks of the N, S-CDs

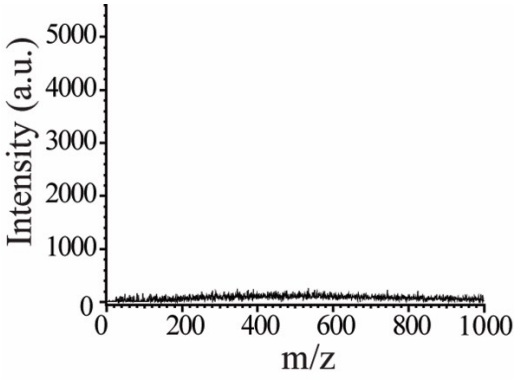

Fig. S4 LDI-TOF mass spectra of N, S-CDs in negative ion modes in the mass range of m/z 0 to 1000.

MALDI-TOF MS Analysis of Nucleoside.

To further research the performance of N, S-CDs as a matrix for analysis of small molecules, nucleoside solution containing T (MW 242.2), C (MW 243.2), U (MW 244.2), A (MW 267.2), and G (MW 283.2) were tested. With CHCA in positive ion mode (Fig.S5), nucleosides were only detectableat fairly low intensity corresponding to $[M + H]^+$ ion C (244.1),$[M + Na]^+$ ion T(265.1) and $[M + H]^+$ ions T (281.1), U (283.1), A (306.1), G (322.1). Similarly, the intrinsic matrix-related ions of CHCA (marked with asterisk)dominated the spectrum. When using N, S-CDs in positive ion mode (Fig.S5B), the sodiumadduct $[M + Na]^+$and potassium adduct $[M + K]^+$ ions of T (265.1, 281.1), C (266.1, 282.2), U (267.2, 283.2) and potassium adduct $[M + K]^+$ ion of G (322.1) can be identified in the spectra. However, we cannot distinguish the m/z 306.1 was $[A + K]^+$ or $[G + Na]^+$ and this indicated the defect of positive ion mode.

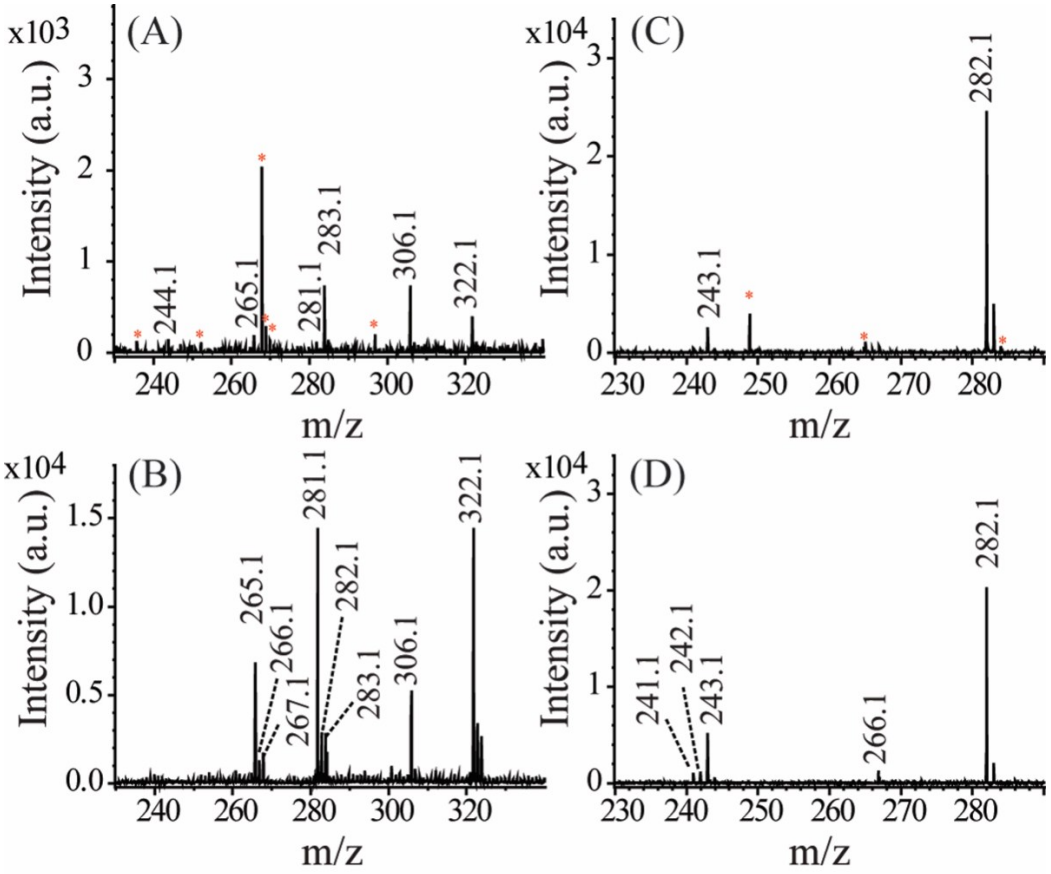

Fig. S5 MALDI-TOF MS spectra of nucleoside mixture solution by using (A) CHCA matrix in positive ion mode; (B) N,S-CDs matrix in positive ion mode; (C) 9AA matrix in negative ion mode; and (D) N,S-CDs matrix in negative ion mode. The nucleoside solution contains T (MW 242.2), C (MW 243.2), U (MW 244.2), A (MW 267.2), and G (MW 283.2) and the concentration of each analyte was set as 1 mM. Laser intensity: 60%.

When we used 9AA as matrix in negative ion mode (Fig.S5C), only two of the nucleosides can be detected including $[M - H]^-$ ions of U (243.1) and G (282.1). Meanwhile signals related to the 9AA matrix were obvious. However, with the matrix of N, S-CDs in negative ion mode (Fig.S5D), there were apparent $[M - H]^-$ ions of all the five nucleosides corresponding to T (241.1), C (242.1), U (243.1), A (266.1) and G (282.1) without any matrix-related ions.

MALDI-TOF MS Analysis of Peptides.

Although CHCA is the most commonly usedmatrix for peptide analysis by MALDI-MS, their signalswould be located at the m/z range of the matrix and compromisedby the matrix background. In this experiment, five peptidesof Tyr-Gly-Gly (MW 295.12), Tyr-Phe (MW 328.14), Phe-Gly-Phe-Gly (MW 426.48),Tyr-Gly-Gly-Phe-Leu (MW 555.62) and Arg-Ser-Gly-Phe-Tyr(MW 628.69) were analyzed by MALDI-TOFMS with CHCA, 9AA and N, S-CDs. With the matrix of CHCA in positive ion mode (Fig. S6A), only three peptides can be easily identified with $[M + H]^+$ ions of Phe-Gly-Phe-Gly (427.4), Arg-Ser-Gly-Phe-Tyr (629.6), $[M + Na]^+$ ions of Tyr-Gly-Gly (318.1),Arg-Ser-Gly-Phe-Tyr (651.6) and $[M + K]^+$ ions of Phe-Gly-Phe-Gly (334.1), Arg-Ser-Gly-Phe-Tyr (667.6). Other ions including matrix-related ions and those hardly identified (marked with asterisk) dominated the spectrum. In the case of N, S-CDs matrix in positive ion mode (Fig. S6B),the sodiumadduct $[M + Na]^+$ ions of all the peptides were reflected in the spectra, corresponding to Tyr-Gly-Gly (318.1), Tyr-Phe (351.1), Phe-Gly-Phe-Gly (449.4), Tyr-Gly-Gly-Phe-Leu (578.6) and Arg-Ser-Gly-Phe-Tyr (651.6). The potassium adduct $[M + K]^+$ ions of Phe-Gly-Phe-Gly (465.4), Tyr-Gly-Gly-Phe-Leu (594.6), Arg-Ser-Gly-Phe-Tyr (667.6) and $[M + H]^+$ ion of Arg-Ser-Gly-Phe-Tyr (629.6) can be detected. There were many analyte-related ions cannot be verified (marked with asterisk) made the spectra more complicated.

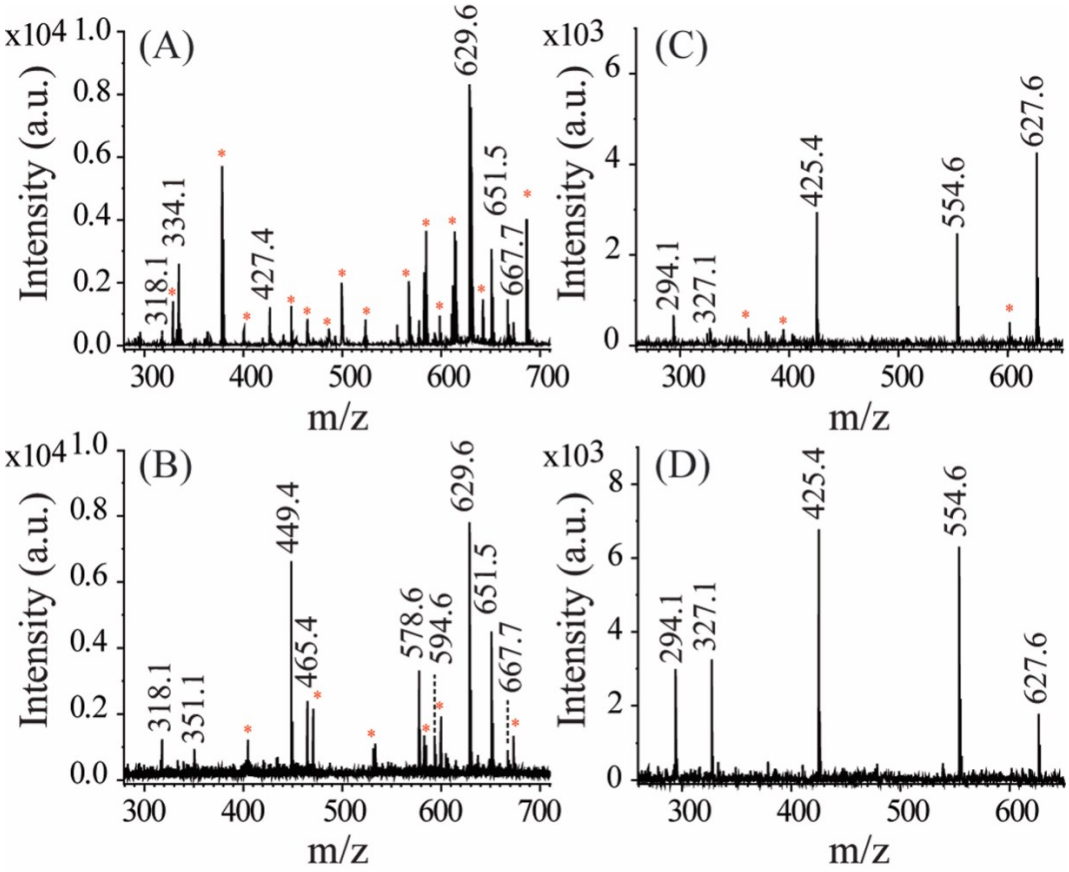

Fig. S6 MALDI-TOF MS spectra of peptide mixture solution by using (A) CHCA matrix in positive ion mode; (B) N,S-CDs matrix in positive ion mode; (C) 9AA matrix in negative ion mode; and (D) N,S-CDs matrix in negative ion mode. The peptide solution contains Tyr-Gly-Gly (MW 295.12), Tyr-Phe (MW 328.14), Phe-Gly-Phe-Gly (MW 426.48), Tyr-Gly-Gly-Phe-Leu (MW 555.62) and Arg-Ser-Gly-Phe-Tyr (MW 628.69) and the concentration of each analyte was set as 1 mM. Laser intensity: 60%.

The five peptides can be detected in negative ion mode with matrix of both 9AA (Fig. S6C) and N, S-CDs (Fig. S6D). They all displayed as $[M - H]^-$ ions corresponding to Tyr-Gly-Gly (294.1), Tyr-Phe (MW 327.1), Phe-Gly-Phe-Gly (425.4), Tyr-Gly-Gly-Phe-Leu (554.6) and Arg-Ser-Gly-Phe-Tyr (627.6). There were two main superiorities of N, S-CD matrix than 9AA observing the spectra, the signals were higher and the background was interference-free. In this sense, negative ion

mode could be considered as a better option to detect the small molecules in the low mass region when N, S-CDs are applied.

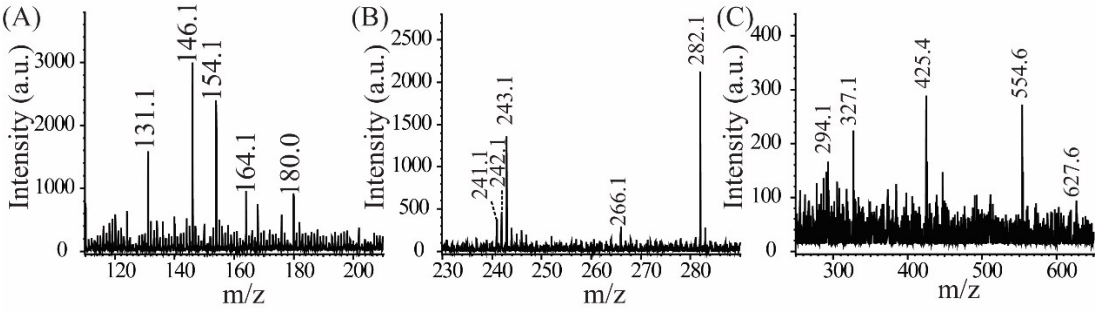

Fig. S7 MALDI-TOF MS spectra of (A) amine acids (B) nucleosides and (C) peptides by using N-CDs matrix in negative ion mode. The amino acids solution contains Asn (MW 132.12), Glu (MW 147.13), His (MW 155.16), Phe (MW 165.19),Tyr (MW 181.19),the nucleoside solution contains T (MW 242.2), C (MW 243.2), U (MW 244.2), A (MW 267.2), G (MW 283.2) and the peptide solution contains Tyr-Gly-Gly (MW 295.12), Tyr-Phe (MW 328.14), Phe-Gly-Phe-Gly (MW 426.48), Tyr-Gly-Gly-Phe-Leu (MW 555.62) Arg-Ser-Gly-Phe-Tyr (MW 628.69) and the concentration of each analyte was set as 1 mM. Laser intensity: 80%.

MALDI-TOF MS Analysis of Anticancer Drugs.

In our study, four common anticancer drugs of vorinostat (MW 264.15), vatalanib (MW 346.10) sunitinib (MW 398.21), and etoposide (MW 588.18) were, respectively, analyzed in negative ion mode with the aid of matrix N, S-CDs. For comparation, 9AA was used as a matrix to detect these small molecule anticancer drugs with the same method of N, S -CDs. As presented in Figure 5a, four drug molecules at concentration of 1 mM were all detectable as the $[M - H]^-$ ions at 263.1, 346.1, 398.2 and 588.2 at fairly low intensity with the 9AA matrix. The matrix-related ions of 9AA

(marked with asterisk) dominated the spectra which seriously suppressed analyte signals. On the contrary, with the matrix of N, S-CDs, the signals were much higher without any interference from matrix besides the impurities in drugs. It demonstrated N, S-CDs is more suitable for analyzing small molecule drugs than 9AA in negative ion mode.

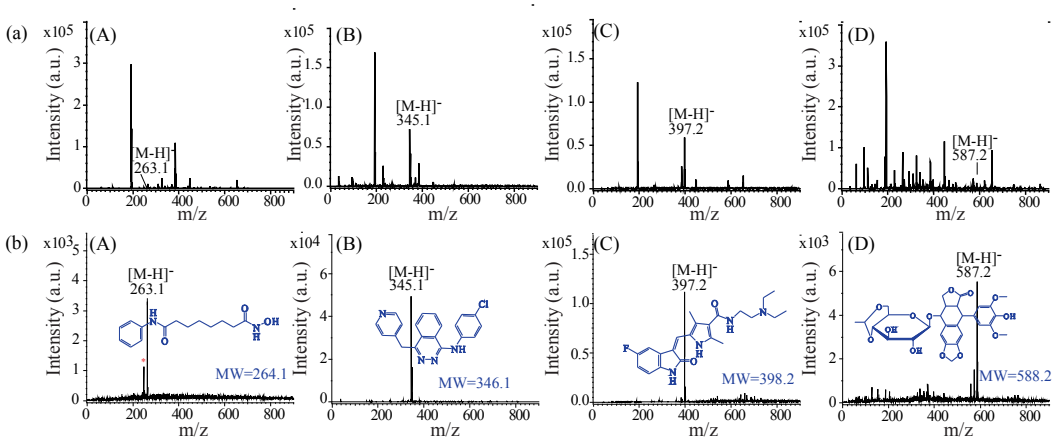

Fig.S8 MALDI-TOF MS spectra of (A) vorinostat, (B) vatalanib, (C) sunitinib and (D) etoposide using (a) 9AA matrix and (b) N,S-CDs matrix in negative ion mode. The concentration of each analyte was set as 1 mM. Laser intensity: 60%.

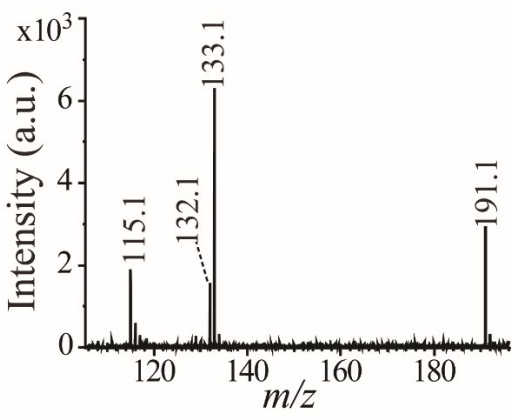

Fig. S9 MALDI-TOF MS spectra of carboxylic acid mixture solution by using N,S-CDs matrix in negative ion mode. The carboxylic acid solution contains Fumaric acid (MW 116.07), L-Aspartic acid (MW 133.1), DL-Malic (MW 134.09) and Citrate (MW 628.69) and the concentration of each analyte was set as 1 mM.

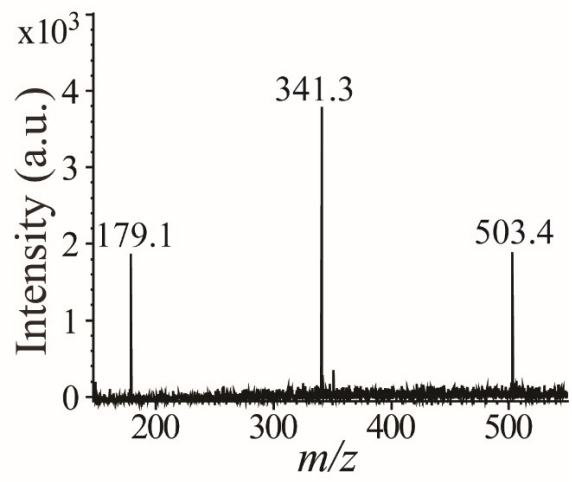

Fig. S10 MALDI-TOF MS spectra of saccharides mixture solution by using N,S-CDs matrix in negative ion mode. The saccharides solution contains glucose (MW 180.16), maltose (MW 342.30) and raffinose (MW 504.40) and the concentration of each analyte was set as 1 mM.

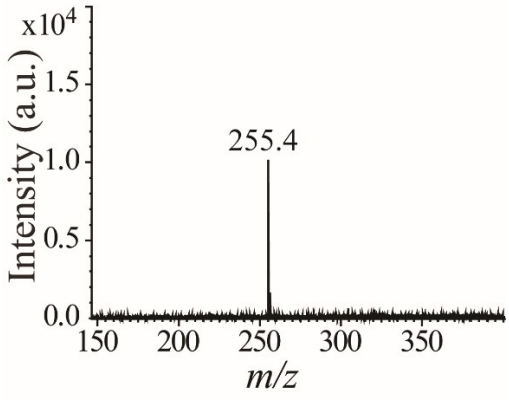

Fig. S11 MALDI-TOF MS spectra of palmitic acid (MW 256.42) by using N,S-CDs matrix in negative ion mode. The concentration of analyte was set as 1 mM

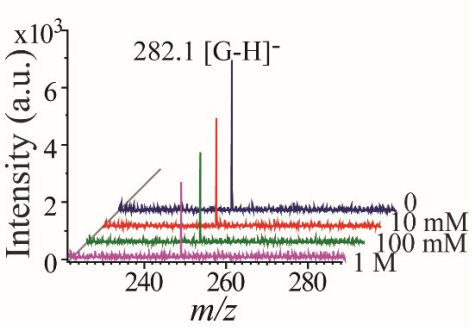

Fig. S12 MALDI-TOF MS spectra of guanine analyzed by using matrix N, S-CDs in negative ion mode with no additional salt, 10 mM , 100 mM and 1 M NaCl.

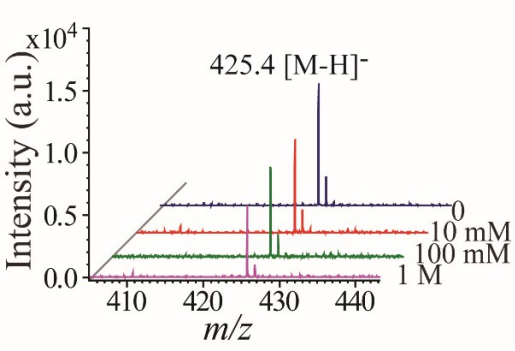

Fig. S13 MALDI-TOF MS spectra of Phe-Gly-Phe-Gly analyzed by using matrix N, S-CDs in negative ion mode with no additional salt, 10 mM , 100 mM and 1 M NaCl.

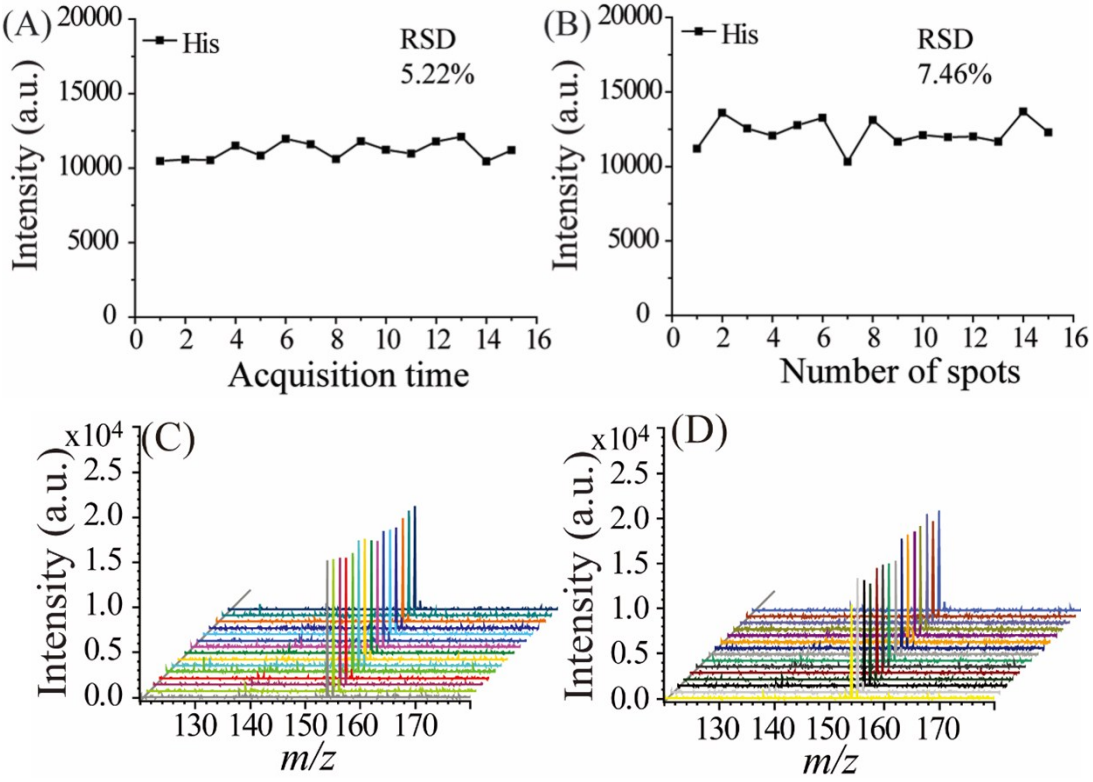

Fig. S14 (A)MS signal intensity of His repeatedly acquired from one sample spot by using N, S-CDs matrix in negative ion mode. Continuous 15 spectra were obtained by applying laser shots on random positions uniformly located on the spot. (B)MS signal intensity of His repeatedly acquired from 15 different samples pots by using N, S-CDs

matrix in negative ion mode.(C) and (D) are the original MS spectra of (A) and (B), respectively. The concentration of His was 1mM. Laser intensity: 60%.

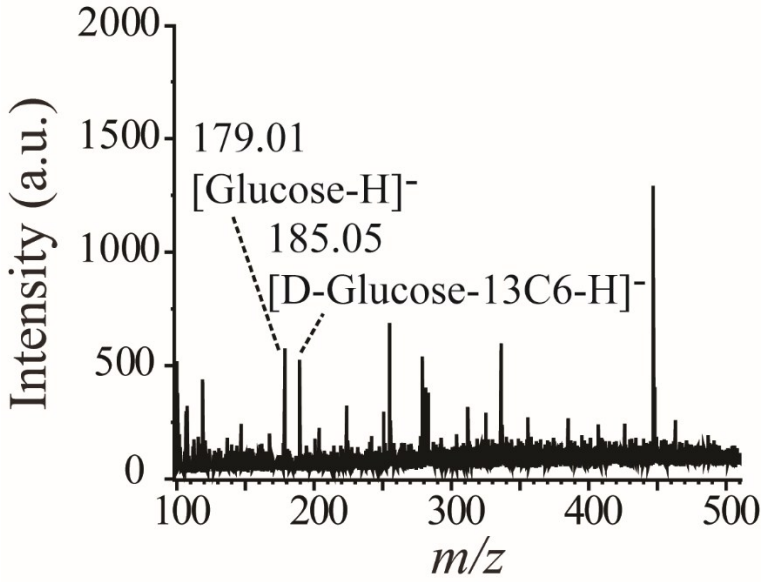

Fig. S15 MALDI-TOF MS spectra of serum with adding 1mM D-Glucose-13C6 using N, S-CDs matrix in negative ion mode.

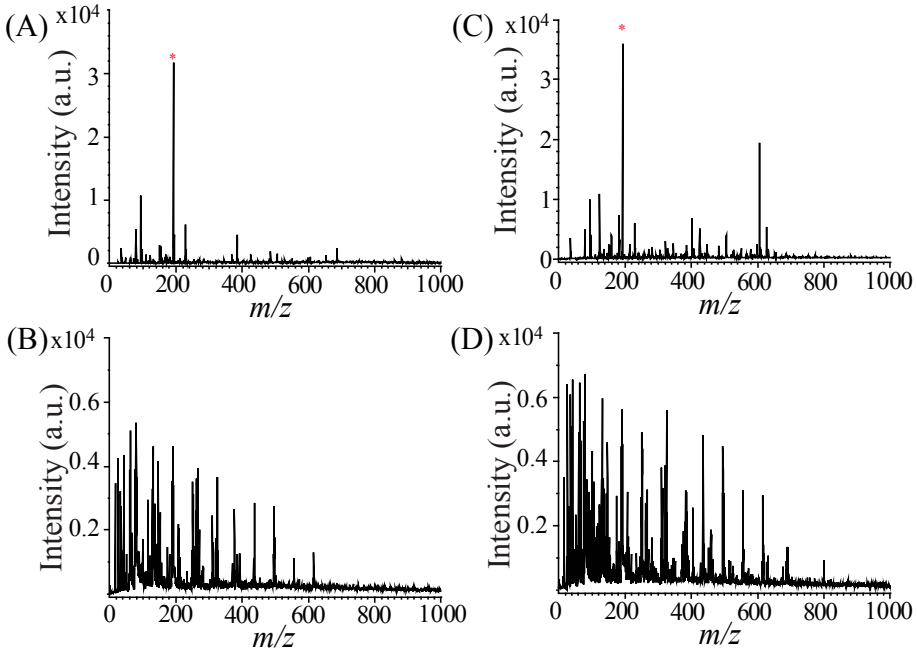

Fig. S16MALDI-TOF MS spectra of BT-549 cell lines by using (A) 9 AA matrix and (B) N,S-CDs; T47-D cell lines by using (C) 9 AA matrix and (D) N,S-CDs matrix in negative ion mode.Matrix-related ions were marked with asterisk.

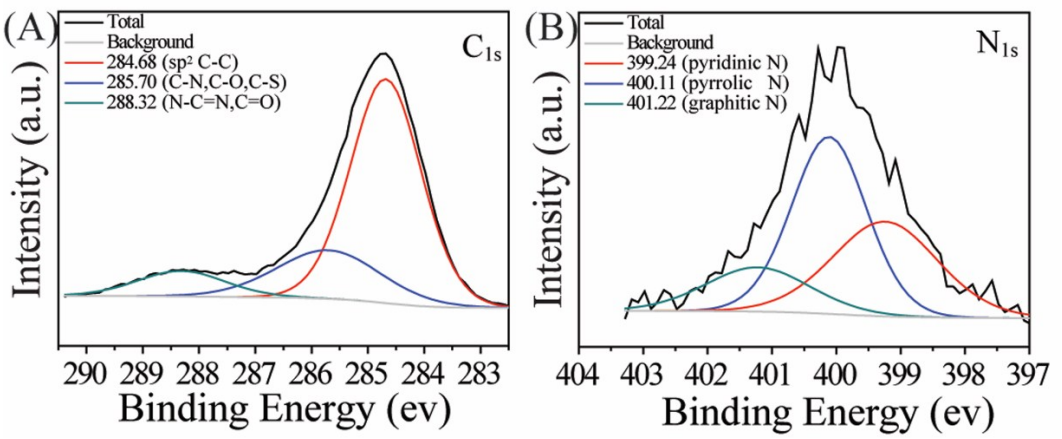

Fig. S17 High-resolution XPS spectra of (A) C1s and (B) N1s peaks of the N, S-CDs.

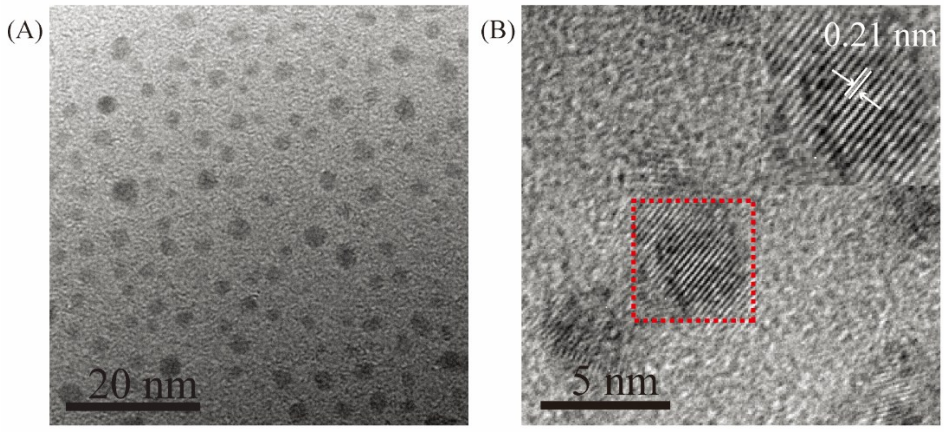

Fig. S18 (A)TEM images and (B) HRTEM images of the obtained N-CDs.

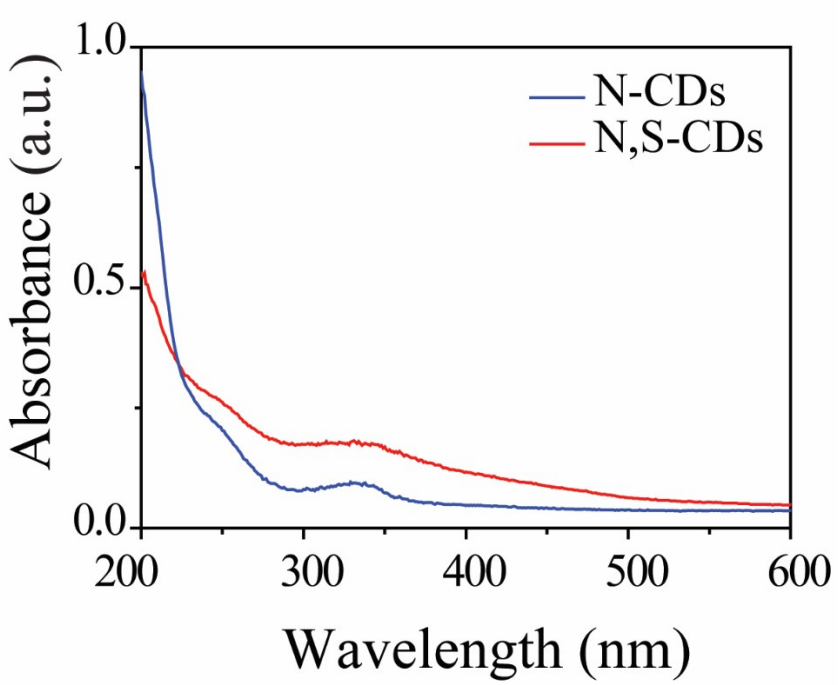

Fig. S19 UV/Vis absorption spectra of N-CDs and N, S-CDs.

Table S1Identification of metabolites in serum by MS/MS fragment analysis.

Compounds	Molecular Formula	Measured mass (Da)	Calculated mass (Da)	Ion forms	Fragment ions
Glucose	$C_6H_{12}O_6$	179.012	180.063	$[M-H]^-$	89.017, 107.200, 124.600, 130.800, 143.000
Murrayanine	$C_{14}H_{11}NO_2$	224.003	225.078	$[M-H]^-$	128.050, 138.034, 166.029, 168.044, 182.024, 190.029
Mukolidine	$C_{14}H_{11}NO_2$	224.003	225.078	$[M-H]^-$	88.259, 138.034, 144.044, 166.029, 168.044, 182.024,
Ethylmyristate	$C_{16}H_{32}O_2$	255.178	256.240	$[M-H]^-$	179.179, 181.195, 199.169, 213.221, 225.185, 227.201, 237.221
Linoleic	$C_{18}H_{32}O_2$	279.185	280.240	$[M-H]^-$	127.068, 221.246, 235.187, 249.185, 261.374, 263.233
Guanosine	$C_{10}H_{13}N_5O_5$	282.211	283.241	$[M-H]^-$	105.933, 124.112, 135.026, 147.257, 150.049, 152.152
S-hydroxymethylglutathione	$C_{11}H_{19}N_3O_7S$	336.349	337.349	$[M-H]^-$	272.088, 288.065, 292.096, 306.075, 319.059
Estradiol glucuronide	$C_{24}H_{32}O_8$	447.219	448.209718	$[M-H]^-$	113.260, 145.049, 193.123, 271.400

Table S2. Elemental atomic percentages of N-CDs and N, S-CDs obtained from XPS spectra.

	C1s (%)	O1s (%)	N1s (%)	S2p (%)
N-CDs	72.98	24.84	2.18	0
N, S-CDs	73.43	17.61	5.85	3.11