Supporting information

Quantitative Profiling and Mapping of Small Molecules by Laser Desorption-Ionization Mass Spectrometry: Combinations of Carbon-based Nano Matrices and Sample Preparation Protocols

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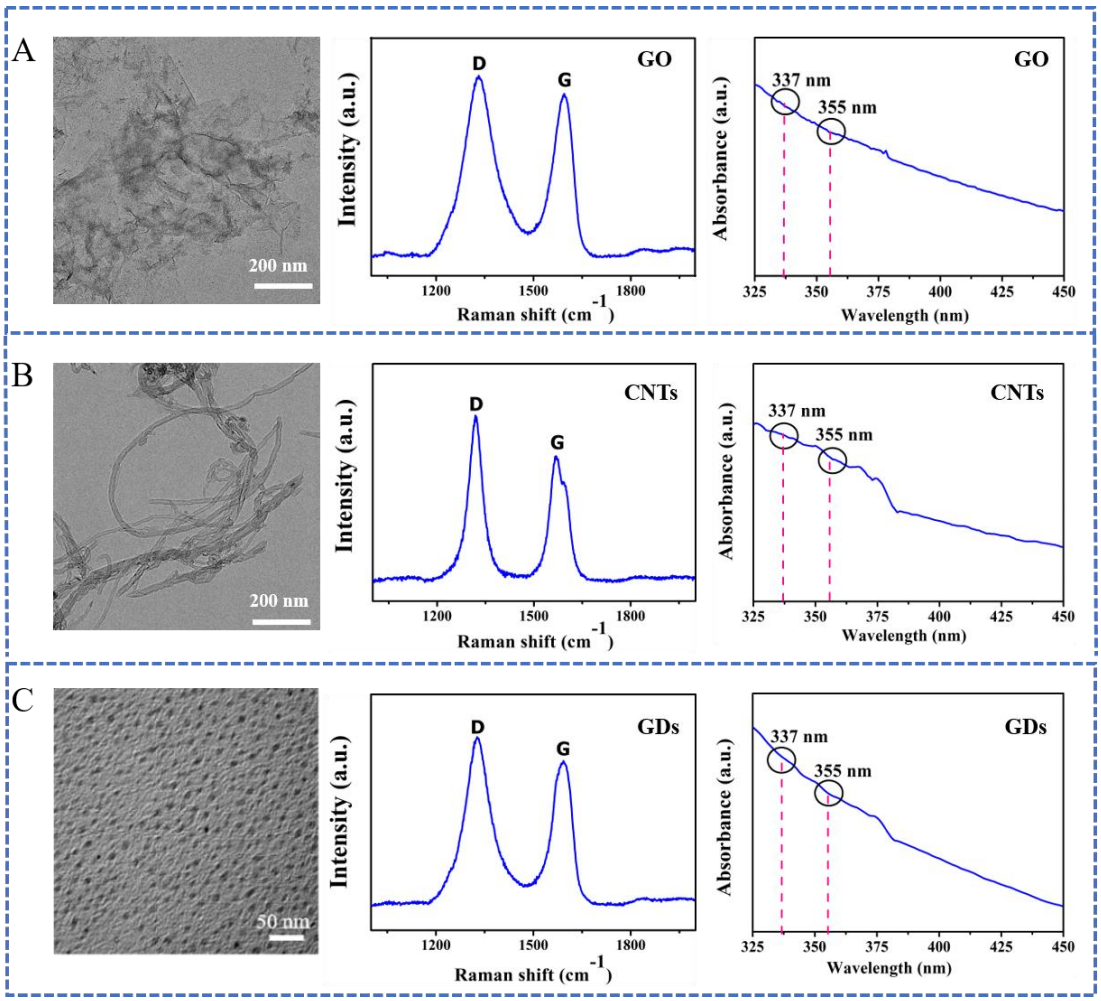


Fig. S1. Characterization of three carbon nano-matrices for MALDI-TOF MS. TEM image, Raman spectrum and UV–Vis absorption spectrum of (A) GO; (B) CNTs; (C) GDs. TEM image: Scale bar of GO, CNTs and GDs are 200 nm, 200 nm and 50 nm, respectively. Raman spectrum: The peak at 1350 cm^{-1} (D band) corresponds to vibration of sp^3-bonded carbon atoms in disordered graphite and the peak at 1595 cm^{-1} (G band) is related to the vibration of sp^2-bonded carbon atoms in a two-dimensional (2D) hexagonal lattice. UV–Vis absorption spectrum with a pink ellipse highlighting the wavelength range (337 nm-355 nm) most widely used in MALDI.

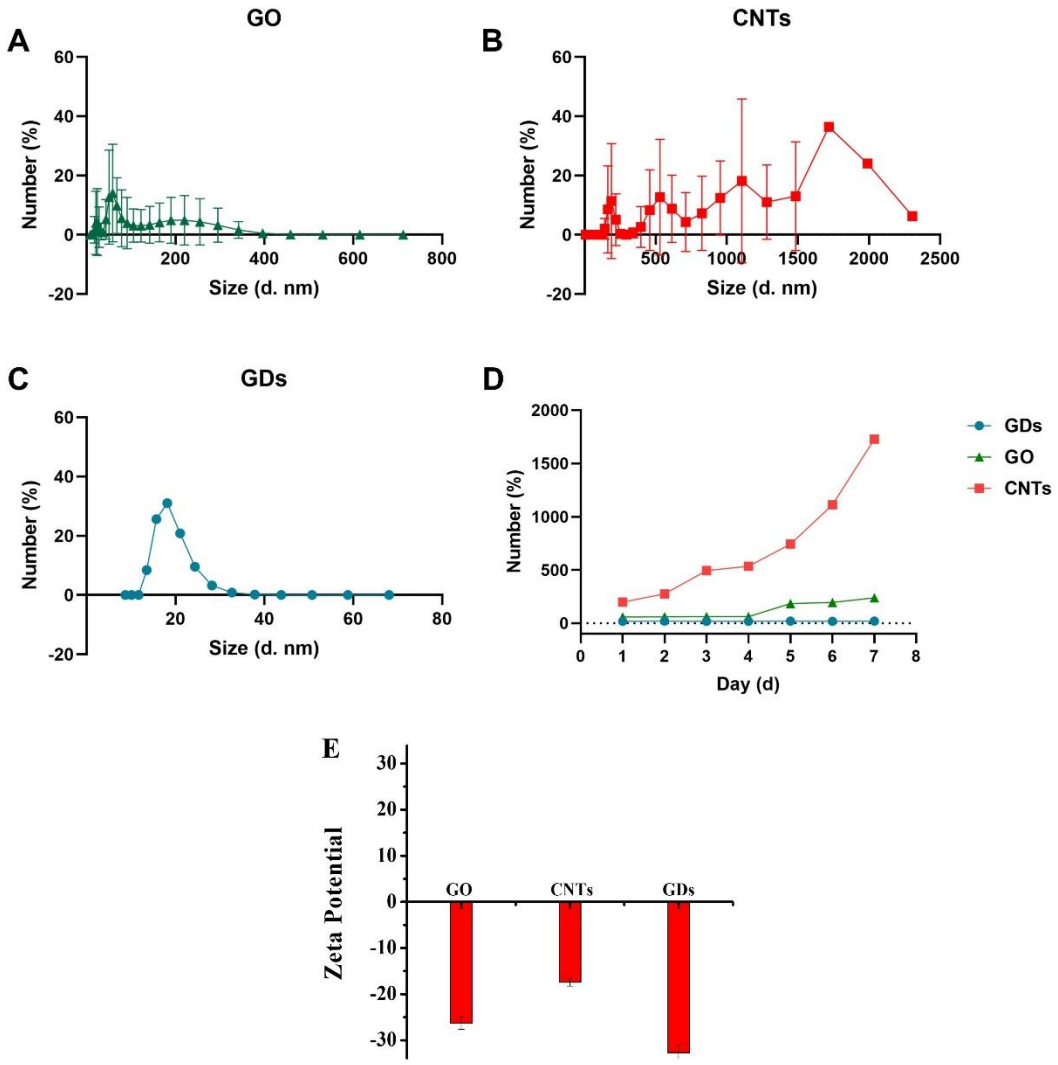


Fig. S2. Size measurements by dynamic light scattering (DLS) of (A) GO, (B) CNTs and (C) GDs in water. (D) Size changes of the above carbon nanomaterials during a stocking period of 7 days.

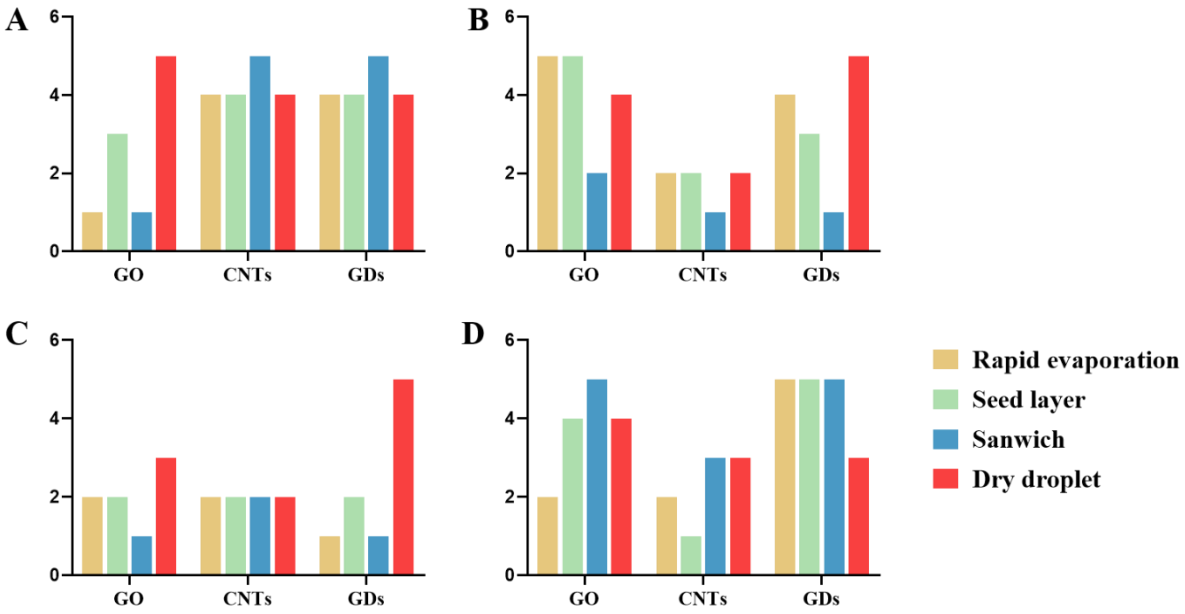


Fig. S3. The RSD assignment level of (A) SEM images, (B) LSCM images, MALDI MSI images for (C) glucose and (D) Ala-Gln.

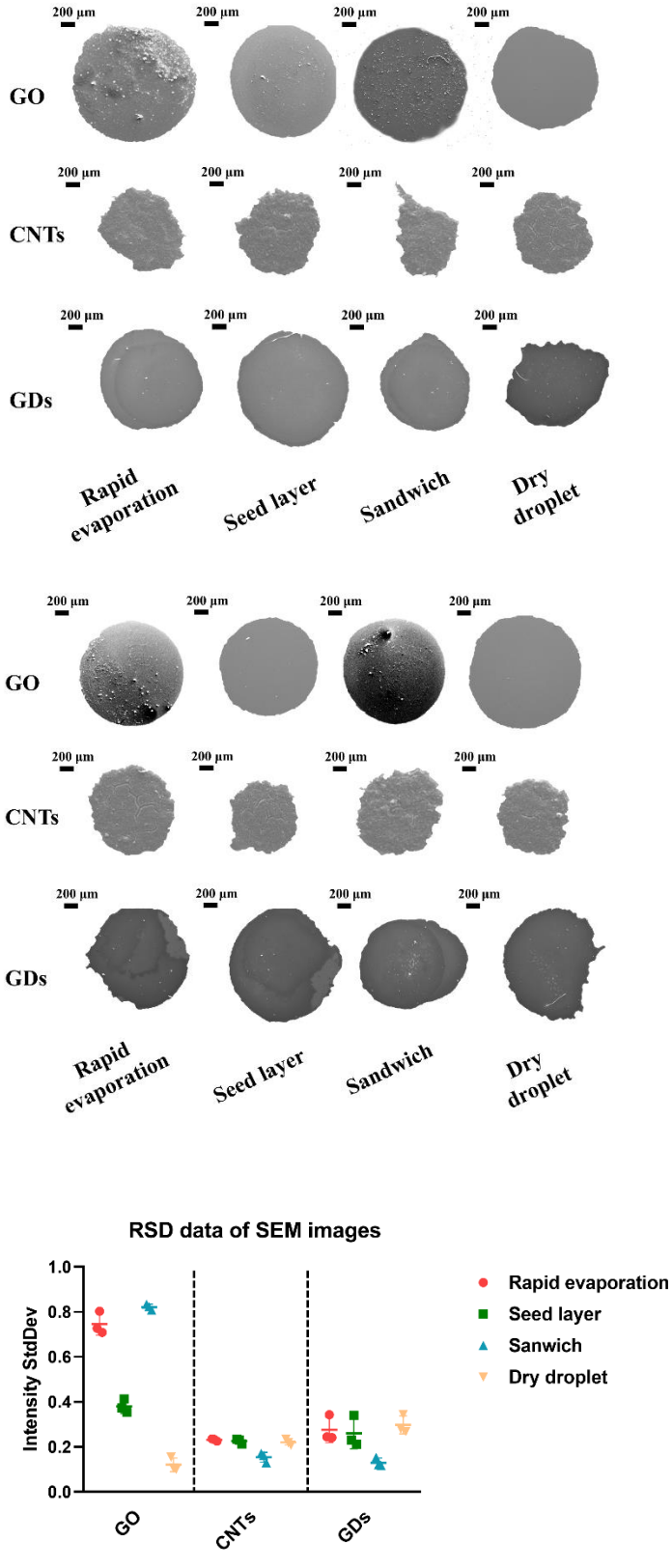


Fig. S4. Two additional panels of SEM images and corresponding RSD values of three carbon nanomaterials (GO, CNTs and GDs) combined with four sample deposition methods (Rapid evaporation, Seed layer, Sandwich, and Dry droplet method), respectively. EHT = 15kV. (Scale bar = 200 µm for GO, CNTs, and GDs).

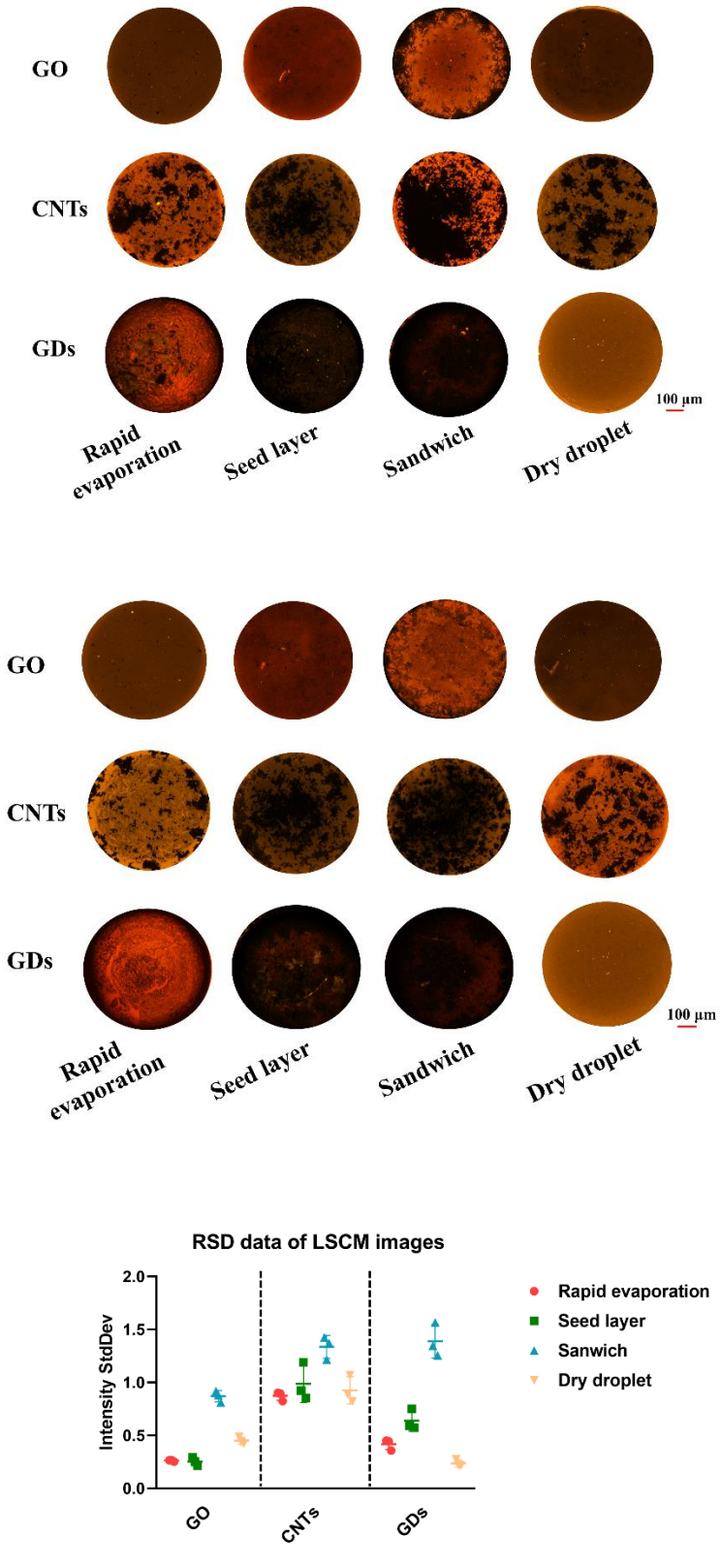
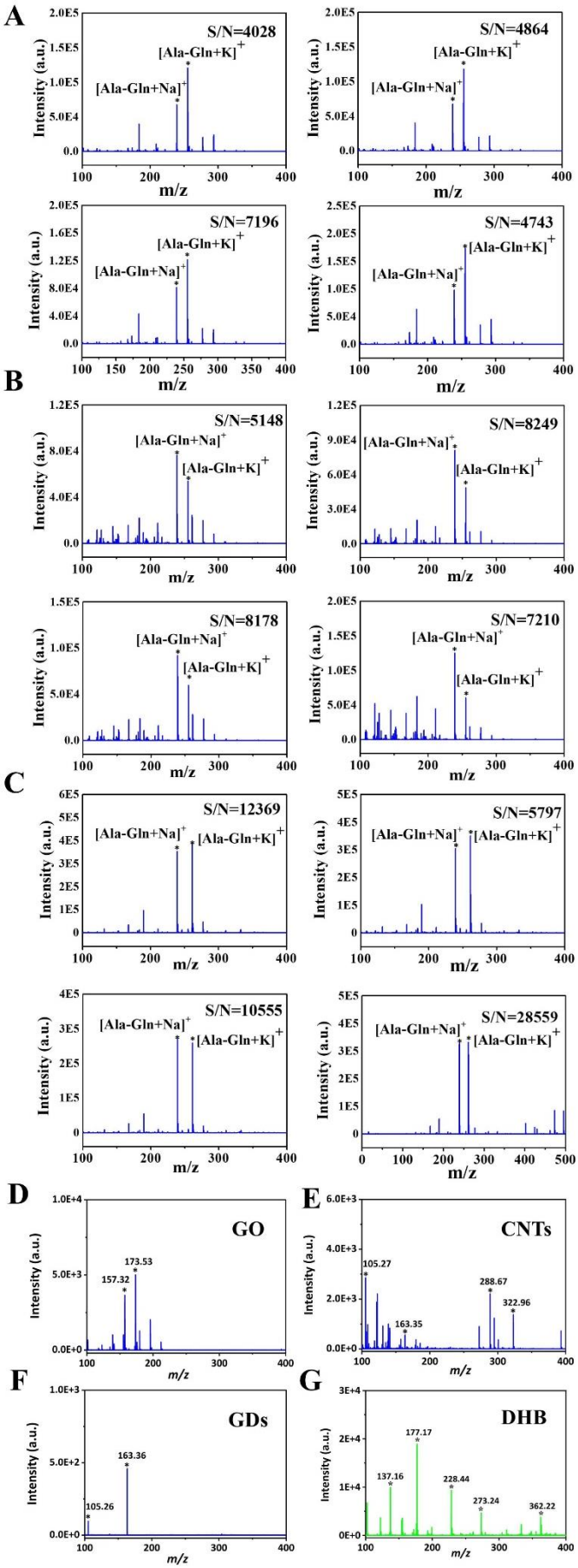


Fig. S5. Two additional panels of LSCM images and corresponding RSD values of the dried spots containing DIL dye (1 mg/mL) using three carbon matrices (GO, CNTs and GDs) combined with four sample deposition methods (Rapid evaporation, Seed layer, Sandwich, and Dry droplet method), respectively. Substrate: ITO glass. (Scale bar = 100 µm for GO, CNTs, and GDs).


A
S/N=4028
[Ala-Gln+K]+
[Ala-Gln+Na]+
S/N=4864
[Ala-Gln+K]+
[Ala-Gln+Na]+
S/N=7196
[Ala-Gln+K]+
[Ala-Gln+Na]+
S/N=4743
[Ala-Gln+K]+
[Ala-Gln+Na]+
B
S/N=5148
[Ala-Gln+Na]+
[Ala-Gln+K]+
S/N=8249
[Ala-Gln+Na]+
[Ala-Gln+K]+
S/N=8178
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S/N=7210
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C
S/N=12369
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[Ala-Gln+K]+
S/N=5797
[Ala-Gln+Na]+
[Ala-Gln+K]+
S/N=10555
[Ala-Gln+Na]+
[Ala-Gln+K]+
S/N=28559
[Ala-Gln+Na]+
[Ala-Gln+K]+
Intensity (a.u.)
m/z
D
GO
173.53
157.32
E
CNTs
105.27
288.67
322.96
163.35
F
GDs
163.36
105.26
G
DHB
177.17
137.16
228.44
273.24
362.22

Fig. S6. The mass spectra of the dipeptide Ala-Gln (1 mg/mL) respectively using matrices of (A) GO, (B) CNTs and (C) GDs combined with four sample deposition methods (Rapid evaporation, Seed layer Sandwich and Dry droplet method) in the positive mode. The baselines of different matrices in MALDI MS spectra of (D) GO, (E) CNTs, (F) GDs and (G) DHB in the positive ion mode. Laser intensity: 61 μJ.

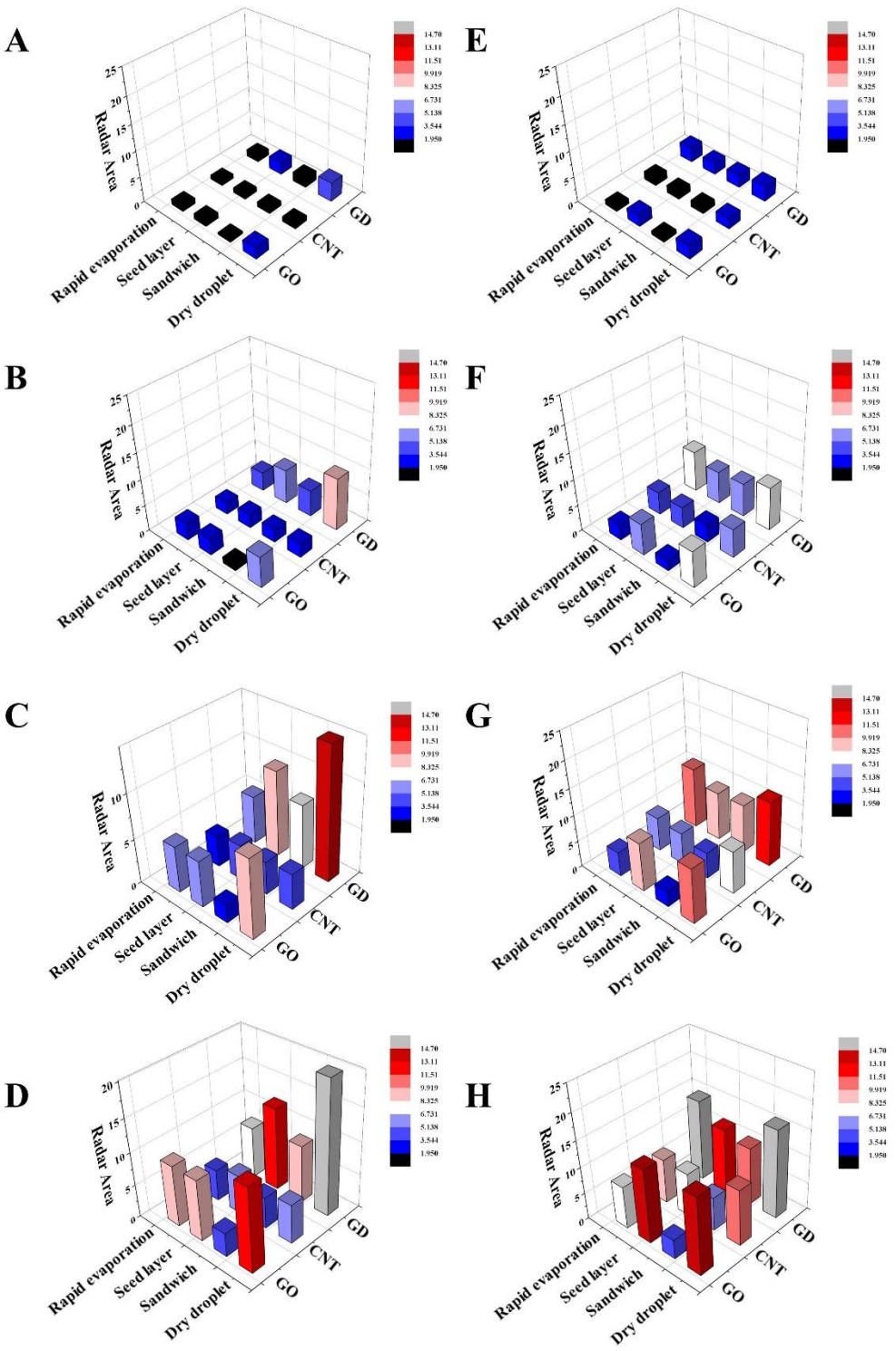

Fig. S7. 3D histograms of the radar areas of glucose (A-D) or dipeptide (E-H) using matrices of GO, CNTs and GDs combined with four sample deposition methods (Rapid evaporation, Seed layer, Sandwich, and Dry droplet method), with different settings of the weight factors for the five dimensions of the radar charts. (A&E) 2% for SEM, LSCM, and CV; 47% for S/N and MSI. (B&F) 6% for SEM, LSCM, and CV; 41% for S/N and MSI. (C&G) 10% for SEM, LSCM, and CV; 35% for S/N and MSI. (D&H) 20% for SEM, LSCM, and CV; 20% for S/N and MSI.

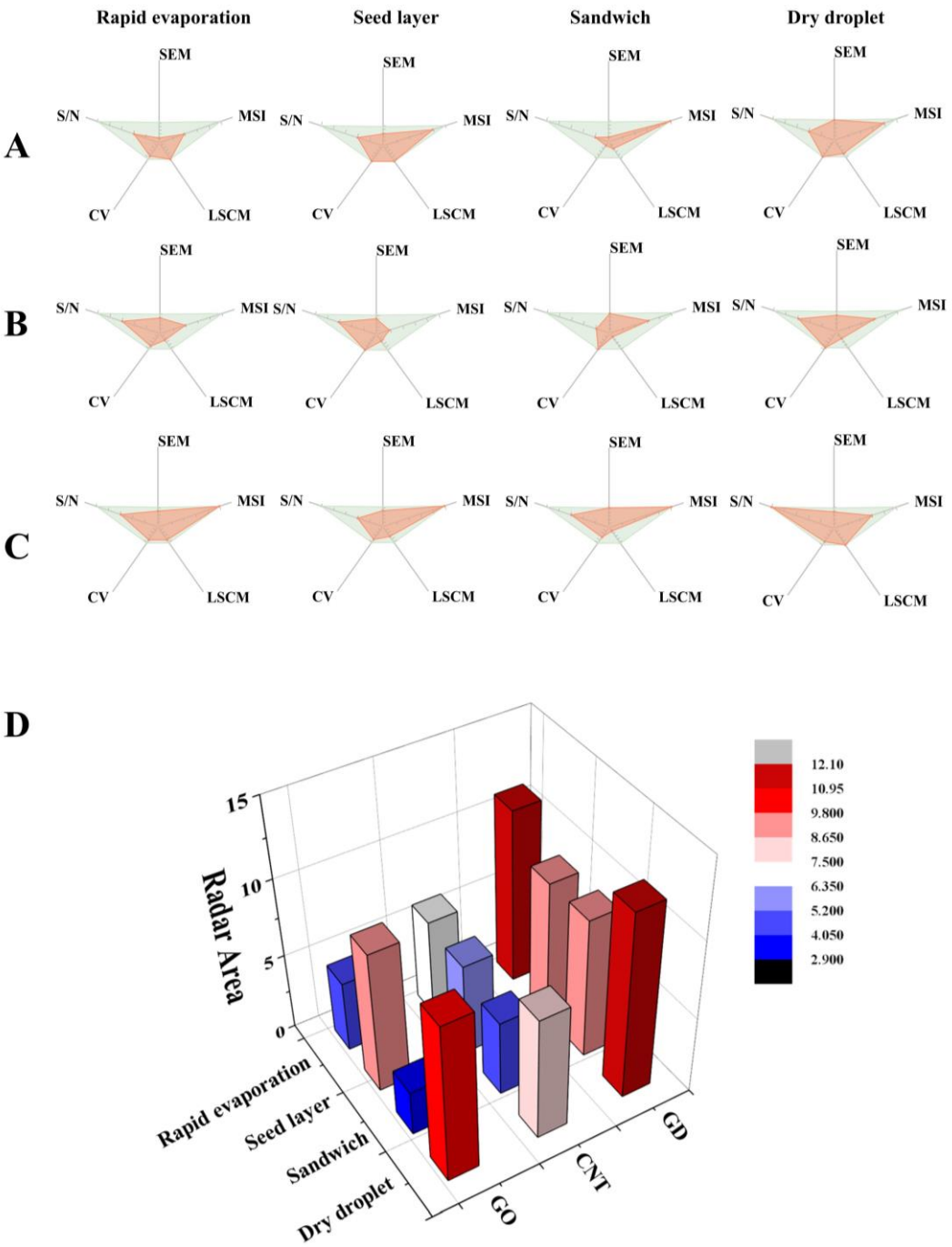

Fig. S8. Radar maps of the dipeptide Ala-Gln (1 mg/mL) using matrices of (A) GO, (B) CNTs and (C) GDs combined with four sample deposition methods (Rapid evaporation, Seed layer, Sandwich, and Dry droplet method) in positive mode. Lightcyan color: a visual guide of the full area; Pink color: the practical metric evaluation of individual matrix/protocol within the five dimensional radar chart. (D) 3D histogram of the radar areas of dipeptide Ala-Gln using matrices of GO, CNTs and GDs combined with four sample deposition methods (Rapid evaporation, Seed layer, Sandwich, and Dry droplet method).

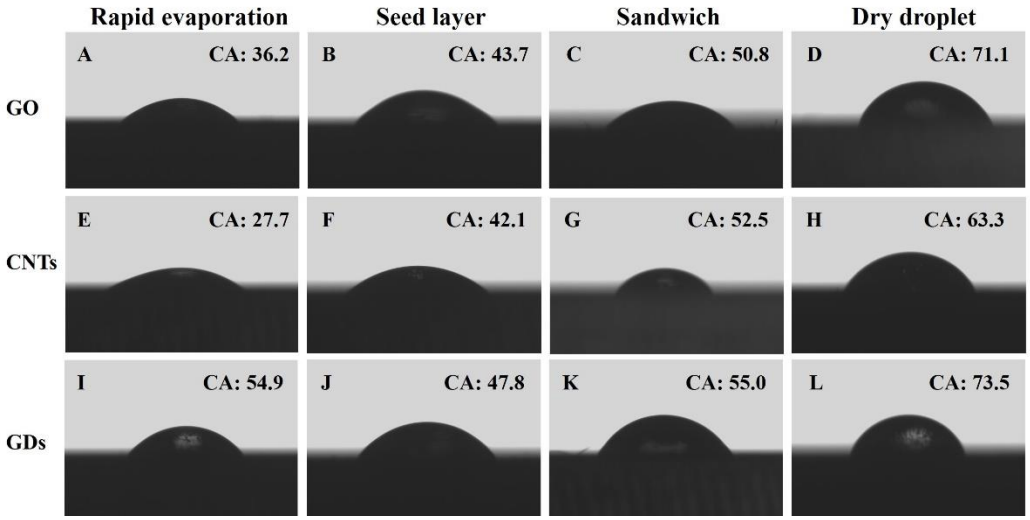

Fig. S9. Comparison of the contact angle (°) measurement results of glucose using matrices of GO, CNTs and GDs combined with four deposition methods of (A) (E) (I) Rapid evaporation, (B) (F) (J) Seed layer, (C) (G) (K) Sandwich, and (D) (H) (L) Dry droplet methods, respectively.

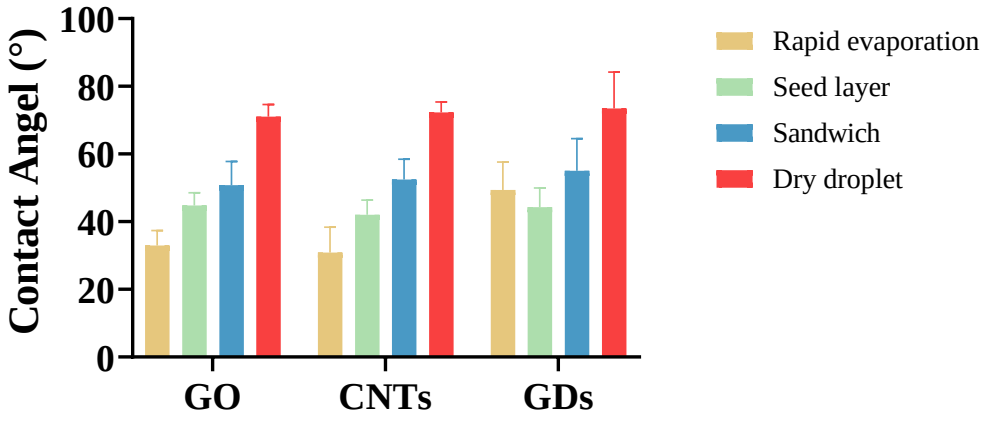

Fig. S10. Histogram of the contact angle (°) measurement results of glucose using matrices of GO, CNTs and GDs combined with four deposition methods (Rapid evaporation, Seed layer, Sandwich, and Dry droplet method).

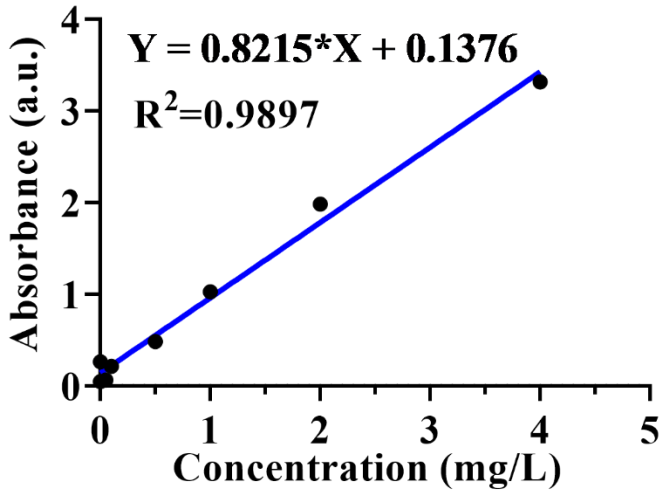

Fig. S11 The calibration curve of glucose in mouse serum samples assayed by using the commercial Glucose Assay Kit (Beyotime, S0201M).

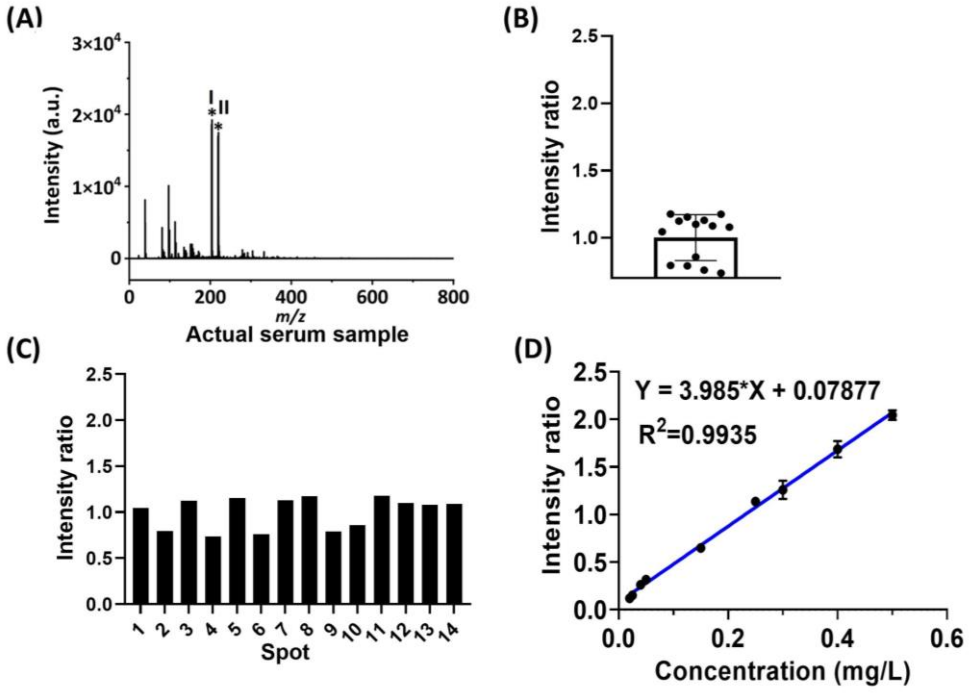

Fig. S12 Detection of glucose in mouse serum samples using matrix of GDs with the dry droplet method in MALDI MS. (A) MALDI MS spectrum of glucose using matrix of GDs combined with the dry droplet method. Concentration of Isotopic internal standard (glucose-C^{13}): 0. 14 mg/mL. The characteristic peaks by label I: [Glucose+

Na]$^+$, m/z 203.04; and label II: [glucose-C^{13}+Na]$^+$, m/z 204.05. (B-C) The signal intensity ratios of the two characteristic peaks at m/z203.04 and m/z 204.05. Repetition spots (n=14). (D) The calibration curve of glucose in mouse serum samples by MALDI MS using the matrix of GDs combined with the dry droplet method. Error bar: standard deviation (n=14).

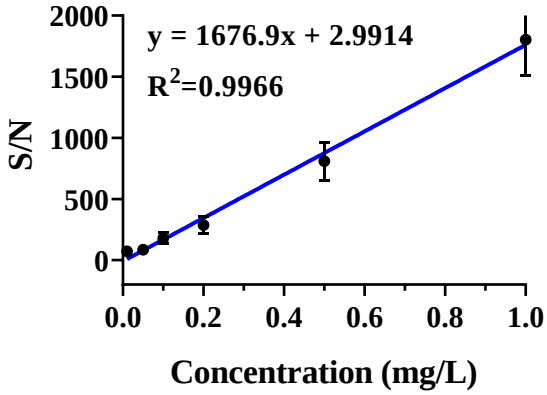

Fig. S13 Limit of detection (LOD) for the measurements of glucose in mouse serum samples using the matrix of GDs combined with the dry droplet method by MALDI MS. Mode: the positive-ion mode. LOD = 1.85 nmol.

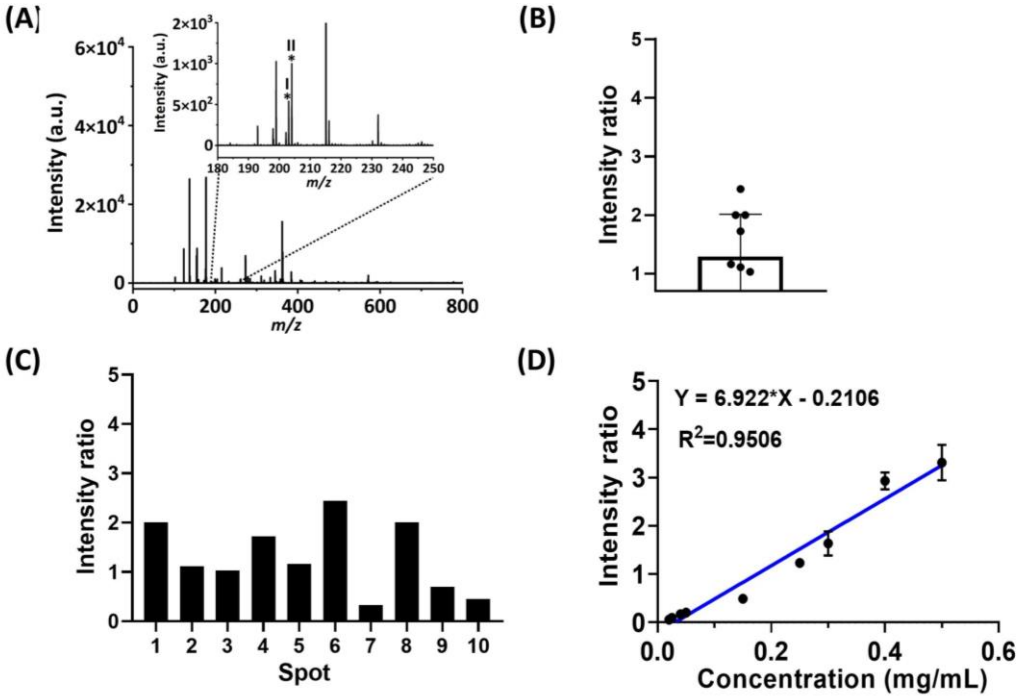

Fig. S14 Detection of glucose in mouse serum samples using the conventional organic matrix (DHB) combined with the rapid evaporation method in MALDI MS. (A) MALDI MS spectrum of glucose using the conventional organic matrix (DHB) combined with the rapid evaporation method. Concentration of Isotopic internal standard (glucose-C^{13}): 0. 14 mg/mL. The characteristic peaks by label I: [Glucose+ $Na]^+$, m/z 203.04; and label II: [glucose-C^{13}+$Na]^+$, m/z 204.05. (B-C) The signal intensity ratios of the two characteristic peaks at m/z203.04 and m/z 204.05. Repetition spots (n=14). (D) The calibration curve of glucose in mouse serum samples by MALDI MS using conventional organic matrix (DHB) combined with the rapid evaporation method. Error bar: standard deviation (n=14).

Table S1. Range assignment of the SEM, LSCM, S/N, CV, MSI (Glucose)

Level	SEM	LSCM	CV	S/N	MSI (Glu)
5	(0.1, 0.2]	(0.2, 0.3]	(0.03, 0.184]	(25000, 30000]	(0.2, 0.3]
4	(0.2, 0.35]	(0.3, 0.5]	(0.184, 0.338]	(20000, 25000]	(0.3, 0.4]
3	(0.35, 0.5]	(0.5, 0.8]	(0.338, 0.492]	(15000, 20000]	(0.4, 0.5]
2	(0.5, 0.7]	(0.8, 1.2]	(0.492, 0.646]	(10000, 15000]	(0.5, 0.65]
1	(0.7, 0.85]	(1.2, 1.6]	(0.646, 0.8]	(5000, 10000]	(0.65, 0.8]

Table S2. Range assignment of the SEM, LSCM, S/N, CV, MSI (Dipeptide: Ala-Gln)

Level	SEM	LSCM	CV	S/N	MSI (Pep)
5	(0.1, 0.2]	(0.2, 0.3]	(0.03, 0.184]	(25000, 30000]	(0.1, 0.35]
4	(0.2, 0.35]	(0.3, 0.5]	(0.184, 0.338]	(20000, 25000]	(0.35, 0.6]
3	(0.35, 0.5]	(0.5, 0.8]	(0.338, 0.492]	(15000, 20000]	(0.6, 0.85]
2	(0.5, 0.7]	(0.8, 1.2]	(0.492, 0.646]	(10000, 15000]	(0.85, 1.15]
1	(0.7, 0.85]	(1.2, 1.6]	(0.646, 0.8]	(5000, 10000]	(1.15, 1. 5]

Table S3. The RSD data of SEM images

	Rapid evaporation	Seed layer	Sandwich	Dry droplet
GO	0.80 (1)	0.37 (3)	0.82 (1)	0.15 (5)
CNTs	0.23 (4)	0.23(4)	0.16 (5)	0.21 (4)
GDs	0.24 (4)	0.21 (4)	0.12 (5)	0.27 (4)

Table S4. The RSD data of LSCM images

	Rapid evaporation	Seed layer	Sandwich	Dry droplet
GO	0.24 (5)	0.29 (5)	0.87 (2)	0.37 (4)
CNTs	0.88 (2)	1.11 (2)	1.54 (1)	1.07 (2)
GDs	0.35 (4)	0.67 (3)	1.27 (1)	0.28 (5)

Table S5. The MALDI MS S/N average values (AVG) and coefficient values (CV) of glucose (white) and dipeptide (orange).

		Analyte	Method	1	2	3	4	5	AVG	CV	LEVEL	
S/N	GO	Glucose	Rapid Evaporation	12198	11570	11793	11018	11469	11609.6	0.03	2	5
			Seed Layer	8944	11440	7695	8490	8534	9020.6	0.14	1	5
			Sandwich	7936	7702	6018	5832	6228	6743.2	0.13	1	5
			Dry Droplet	8727	9847	11705	10072	9922	10054.6	0.09	2	5
		Dipeptide	Rapid Evaporation	4028	7597	5123	5702	5135	5517	0.21	2	4
			Seed Layer	4864	4313	4910	7348	4533	5193.6	0.21	2	5
			Sandwich	7196	1587	1756	1497	1720	2751.2	0.8	1	1
			Dry Droplet	4743	4660	5117	4711	3263	4498.8	0.14	2	5
	CNT	Glucose	Rapid Evaporation	5717	10075	4587	4023	5301	5940.6	0.36	1	3
			Seed Layer	7634	10594	8102	6559	7378	8053.4	0.17	1	5
			Sandwich	6211	8049	7038	6118	4066	6296.4	0.21	1	4
			Dry Droplet	7145	7814	7332	6156	4661	6621.6	0.17	1	5
		Dipeptide	Rapid Evaporation	5148	4710	7804	7406	5138	6041.2	0.21	3	4
			Seed Layer	8249	10193	9316	7708	7845	8662.2	0.11	3	5
			Sandwich	8178	8784	7418	5088	8373	7568.2	0.17	1	5
			Dry Droplet	7210	5808	5191	6193	6479	6176.2	0.11	3	5
	GD	Glucose	Rapid Evaporation	27528	15934	12489	18207	11149	17061.4	0.34	3	3
			Seed Layer	30016	31884	18200	28193	23842	26427	0.19	5	4
			Sandwich	30782	30932	15084	18748	21898	23488.8	0.27	4	4
			Dry Droplet	25772	22518	19758	21379	20905	22066.4	0.09	4	5
		Dipeptide	Rapid Evaporation	12369	19609	22724	21674	17356	18746.4	0.19	3	4
			Seed Layer	4420	2744	3119	3468	5797	3909.6	0.28	2	4
			Sandwich	6155	10555	3916	5299	4924	6169.8	0.37	3	3
			Dry Droplet	15829	26706	18160	14029	28559	20656.6	0.28	5	4

Glucose Ala-Gln

* Repetition number (n=5). Color bar as a visual guide: the highest S/N AVG and the lowest CV of glucose (Red); or the highest S/N AVG and the lowest CV of dipeptide (Pink).

Table S6. The RSD data of MALDI MSI images (glucose)

	Rapid evaporation	Seed layer	Sandwich	Dry droplet
GO	0.54 (2)	0.53 (2)	0.67 (1)	0.48 (3)
CNTs	0.50 (2)	0.60 (2)	0.60 (2)	0.55 (2)
GDs	0.74 (1)	0.60 (2)	0.69 (1)	0.24 (5)

Table S7. The RSD data of MALDI MSI images (Ala-Gln)

	Rapid evaporation	Seed layer	Sandwich	Dry droplet
GO	0.91 (2)	0.49 (4)	0.15 (5)	0.39 (4)
CNTs	0.92 (2)	1.30 (1)	0.62 (3)	0.68 (3)
GDs	0.35 (5)	0.28 (5)	0.25 (5)	0.63 (3)

Table S8 Comparison of Conventional MS signal-based model and Weighted multivariate model

	Conventional MS signal-based model			**Weighted multivariate model**
Deposition methods	Three-layer method [31]	Seed layer method	spray gun, automatic sprayer, sublimation substrate	four typical deposition methods (rapid evaporation, seed layer, sandwich, and dry droplet methods) combined with three carbon-based nanomaterials (2-D GO, 1-D CNTs, and 0-D GDs)
Critical parameters	MS signals [32]	sizes of sample crystallization domains	MSI signals	a) planar dispersity grading b) planar fluorescence homogeneity grading of nano matrix/dye molecules c) CVs of MS signals in repeats d) S/N ratio grading e) planar MSI signal heatmap grading
Characterization protocol	MALDI MS [33]	TEM	MALDI MSI	SEM, LSCM, MALDI MS, MALDI MSI
Advantages	improved tolerance to high concentrations of salts and non-ionic detergents than traditional methods	demonstrated good quantitation linearity over a range of 50-2000 ng mL-1 with reduced signal variation (RSD<10.0%)	an optimized procedure to map small molecules and their metabolites	a) five parameters; b) quantitative; c) widely-used nano and organic matrices; d) systemic guidance and useful clues for MALDI MS.
Disadvantages	a) no more than three parameters; b) non-quantitative; c) without new types of matrices such as nano materials.			