Supporting Information

Synergistic Effect of Graphene Oxide/MWCNT Films in Laser Desorption/Ionization Mass Spectrometry of Small Molecules and Tissue Imaging

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APTES modification of a glass substrate

The glass cover slip was cleaned in Piranha solution (sulfuric acid: hydrogen peroxide (30 %) = 3:1, WARNING: Piranha solution is explosive and corrosive.) for 10 min at 125 °C, washed with water and ethanol, and dried under a stream of nitrogen. The cleaned substrate was immersed in a 10 mM anhydrous toluene solution of APTES for 30 min, sonicated in anhydrous toluene for 2 min, rinsed with ethanol and water and dried under a stream of nitrogen. This process is applicable to any kind of SiO_2 substrates.

Preparation of graphene oxide (GO) suspension

1.5 g of graphite powder and 0.5 g of sodium nitrate were added in 23 mL of sulfuric acid and stirred in an ice bath. Then, 3 g of potassium permanganate was gradually added to the mixture with stirring and kept the temperature below 20 °C. After addition, the temperature of the reaction mixture was raised to 35 °C and the mixture was stirred for an hour. The mixture was diluted with 40 mL of water, stirred for 30 min and further diluted with 100 mL of water in an ice bath to prevent rapid boiling of the reaction mixture because the temperature of the mixture rapidly increased up to 95 °C. Finally, 3 mL of hydrogen peroxide (30 %) was slowly added to the mixture with bright yellow coloration. The product solution was filtered and washed with copious water until the filtrate was neutralized. The filter cake was dried under reduced pressure for 48 h. The prepared graphite oxide powder was analyzed by FT-IR spectroscopy with a KBr pellet method.

The certain amount of graphite oxide was exfoliated in water by bath sonication for an hour to prepare suspension of GO and the GO suspension was centrifuged at 8000 RPM for 30 minutes to remove large aggregates of GO sheets. To confirm the exfoliation of graphite oxide, an APTES-functionalized silicon substrate was immersed in the GO suspension (0.1 mg/mL) for an hour, washed with water and ethanol and dried under a stream of nitrogen. This process resulted in the

electrostatic adsorption of grapheme oxide sheets on the silicon substrate and the adsorbed graphene oxide was scanned by atomic force microscopy.

Preparation of MWCNT-NH_2 suspensions

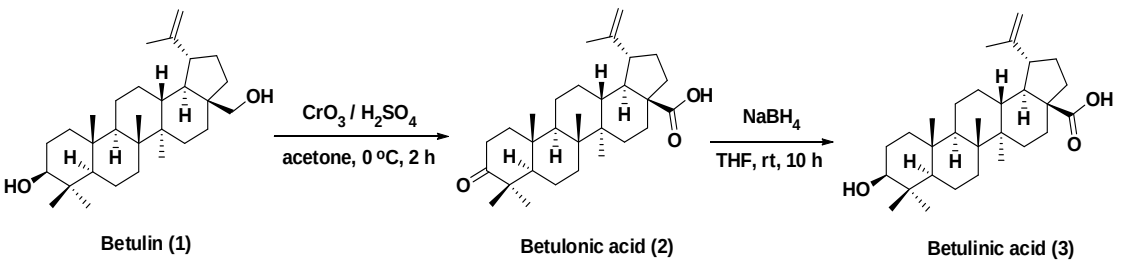
40 mg of MWCNT was sonicated in 40 mL of a mixture of nitric acid and sulfuric acid (1 : 3) for 10 h at 60 ^oC to introduce carboxylic acid groups on the side wall of MWCNT, filtered through an polycarbonate membrane (400 nm in pore size), washed with a plenty of water until the filtrate was neutralized. The filtered cake was washed with ethanol, dried under reduced pressure, re-washed with 40 ml of anhydrous DMF, dispersed in 20 mL of thionyl chloride containing 1 mL of anhydrous DMF by sonication and refluxed at 70 °C for 24 h. After reaction, the acyl chloride functionalized MWCNT was obtained from the mixture by rotary evaporation, suspended in 40 mL of ethylene diamine by sonication and refluxed at 125 °C for five days. Finally, the reaction mixture was filtered through an anodic alumina membrane (200 nm in pore size), washed with ethanol and water, and dispersed in water at 120 μg/mL by sonication.

The shape and chemical structure of surface modified MWCNTs was determined by SEM, XPS and FT-IR measurements.

Synthesis of benzyl pyridinium salt

12 mL of pyridine was mixed with benzyl chloride at a molar ratio 20 : 1 (pyridine/benzyl chloride), refluxed at 60 ^oC for 6 h. The benzyl pyridinium salt was obtained by removal of excess pyridine with rotary evaporation and used to make stock solution at 1 mM concentration in methanol.

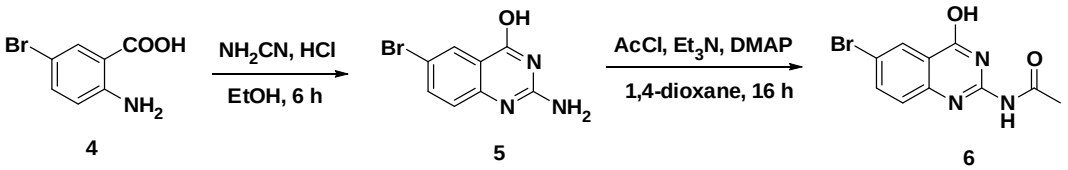
The synthesis of betulin derivatives used as model for monitoring organic reactions



Betulonic acid (2): To a solution of Betulin (**1**) (400 mg, 0.9 mmol) in acetone (20 mL) was added dropwise Jones reagent [freshly prepared from CrO_3 (7.0 g), sulfuric acid (98%, 6.1 mL), and water (30 mL)] at 0 ^oC. The reaction mixture was stirred for 2h at 0 ^oC, and then quenched with methanol (25 mL), stirred for 15 min. The organic solvent was concentrated *in vacuo* and residue was extracted with ethyl acetate (3 X 20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, concentrated *in vacuo.* The residue was purified by flash column chromatography (EtOAc:hexanes, 1:5) to give Betulonic acid (**2**) as white solid (263 mg , 64%). ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (s, 3H), 0.96 (d, *J* = 4.0, 6H), 1.00 (s, 3H), 1.10 (s, 3H), 1.24-1.58 (m, 16H), 1.68 (s, 3H), 1.97 (m, 4H), 1.99 (m, 2H), 2.42 (m, 2H), 2.98 (m, 1H), 4.60 (s, 1H), 4.73 (s, 1H).

Betulinic acid (3): To a solution of Betulonic acid (**2**) (170 mg, 0.37 mmol) in THF (15 mL) was added $NaBH_4$ (141 mg, 3.7 mmol) at 0 ^oC. The solution was stirred at rt for 10 h, then quenched with 1N HCl (4 mL) solution and THF was concentrated under reduced pressure down to 50% volume. The solution was diluted with ethyl acetate (60 mL) and washed with H_2O (5 mL) and brine (5 mL). The combined organic extracts were dried over $MgSO_4$, and concentrated *in vacuo*. Recrystallization of the residue from MeOH afforded Betulinic acid (**3**) as white solid (103 mg , 60%). ^1H NMR (300 MHz, $CDCl_3$) δ 0.73 (s, 3H), 0.80 (s, 3H), 0.92 (s, 3H), 0.95 (d, *J* = 2.9, 6H), 1.19-1.63 (m, 20H), 1.95 (m, 2H), 2.22 (m, 2H), 1.67 (s, 3H), 2.99 (m, 1H), 3.18 (m, 1H), 4.59 (s, 1H), 4.72 (s, 1H).

The synthesis of small conjugated molecules used as model for monitoring organic reactions



4 **5** **6**

2-Amino-4-hydroxy-6-bromo-quinazoline (5): To a solution of 2-amino-5-boromobenzoic acid (**4**) (1000 mg, 4.63 mmol) and cyanamide (233 mg, 5.55 mmol) in EtOH (10 mL) was added concentrated hydrochloric acid (0.5 mL). The solution was refluxed for 6 h. After cooling to rt, the precipitate was collected by filtration, washed with EtOH (10 mL) to afford 2-amino-4-hydroxy-6-

bromo-quinazoline (**5**) as yellowish white solid (418 mg, 37%). ^{1}H NMR (300 MHz, DMSO-D_6) δ 7. 28 (d, *J* = 8.8, 1H), 7.46 (S, broad, 2H), 7.81 (dd, *J* = 2.5, 8.7, 1H), 7.99 (d, *J* = 2.4, 1H).

2-Acetamido-6-bromo-4-hydroxy-quinazoline (6): A solution of 2-amino-4-hydroxy-6-bromo-quinazoline (**5**) (80 mg, 0.33 mmol), acetyl chloride (0.03 mL), triethylamine (0.14 mL) and 4-Dimethylaminopyridine (16 mg, 0.13 mmol) in 1,4-dioxane (2 mL) was refluxed for 16 h. The organic solvent was concentrated *in vacuo*. Dichloromethane (10 mL) was added, then insoluble solid was filtered and washed with Dichloromethane and H_2O to afford 2-acetamido-6-bromo-4-hydroxy-quinazoline (**6**) as yellowish white solid (84 mg, 89%). ^{1}H NMR (300 MHz, DMSO-D_6) δ 2.16(s, 3H), 6.48 (s, broad, 1H), 7.13 (d, *J* = 8.7, 1H), 7.66 (dd, *J* = 2.4, 9.0, 1H), 7.90 (d, *J* = 2.5, 1H).

Supporting figure

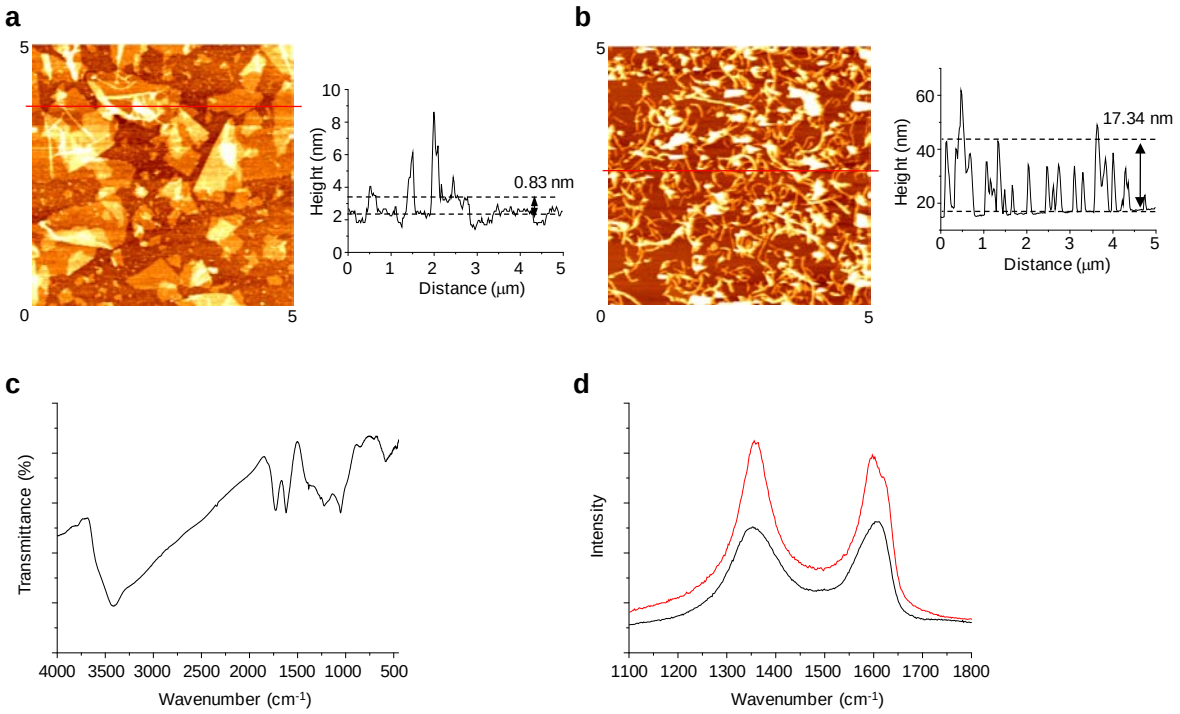


Figure S1. The exfoliation and oxidation of graphite were confirmed by AFM (a) and FT-IR (c) analysis. The thickness of GO sheets was about 0.83 nm corresponding to single layer and FT-IR spectrum showed characteristic peaks of oxygen containing functional groups at 3415 cm^{-1} from O-H vibrations, 1716 cm^{-1} from C=O stretching, 1627 cm^{-1} from C=C skeletal vibrations of the oxidized graphitic domain, 1400 cm^{-1} from O-H deformation, and 1079 cm^{-1} from C-O stretching. The aminated MWCNT used in this study was also analyzed by AFM and its diameter was about 17 nm (b). The reduction of GO/MWCNT double layer was proved by change of relative ratio of D/G peaks in Raman spectra from 0.95 to 1.07 (d).

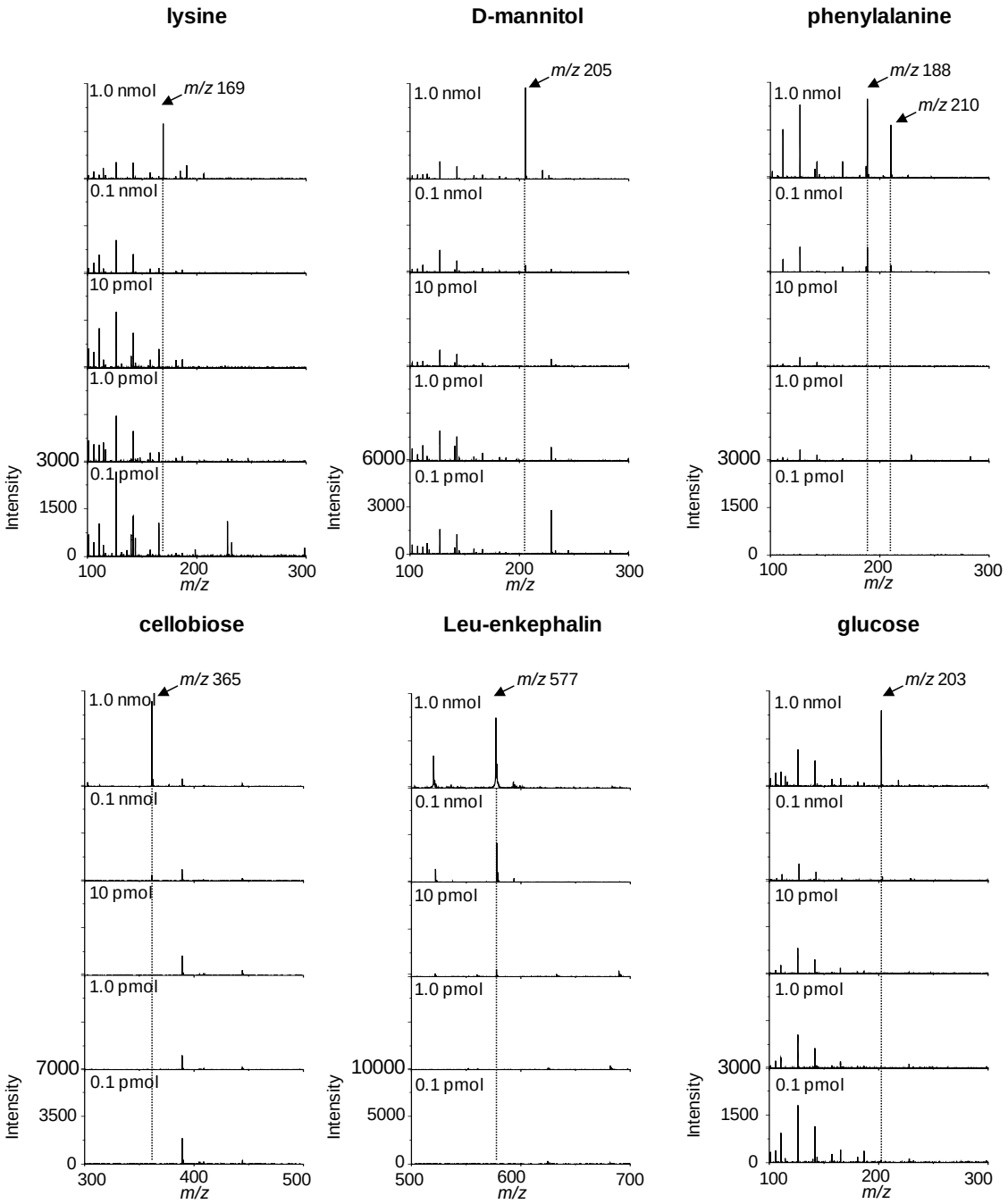


Figure S2. The detection limit of small molecules on GO/MWCNT-NH_2 double layer platform was determined by applying serially diluted small molecule solutions on GO/MWCNT-NH_2 double layer for LDI MS analysis.

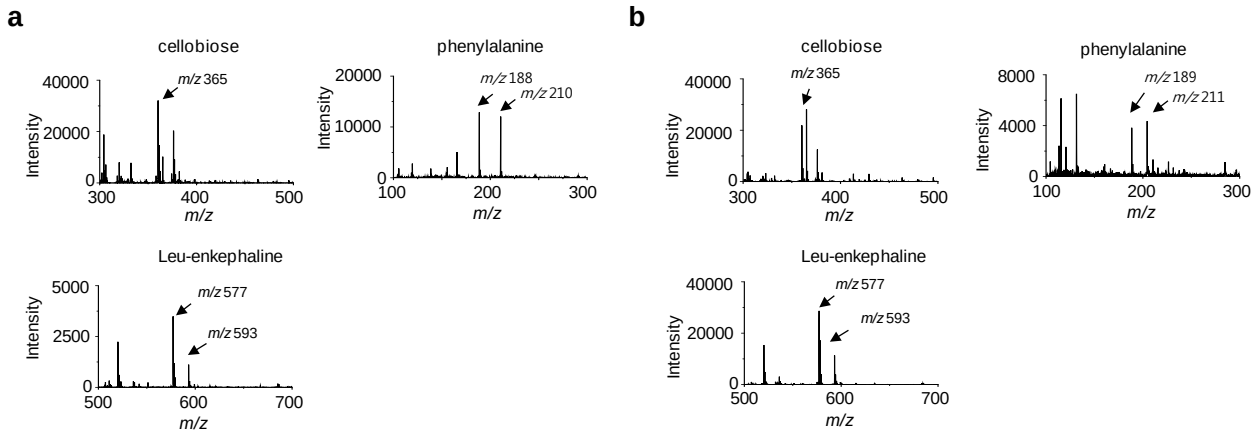


Figure S3. Mass spectra of cellobiose (*m/z* 365 $[M+Na]^+$), phenylalanine (*m/z* 188 $[M+Na]^+$ and *m/z* 210$[M+2Na]^+$) and Leu-enkephaline (*m/z* 577 $[M+Na]^+$ and *m/z* 593 $[M+K]^+$) obtained with GO (a) and RGO (b) dispersed in water.

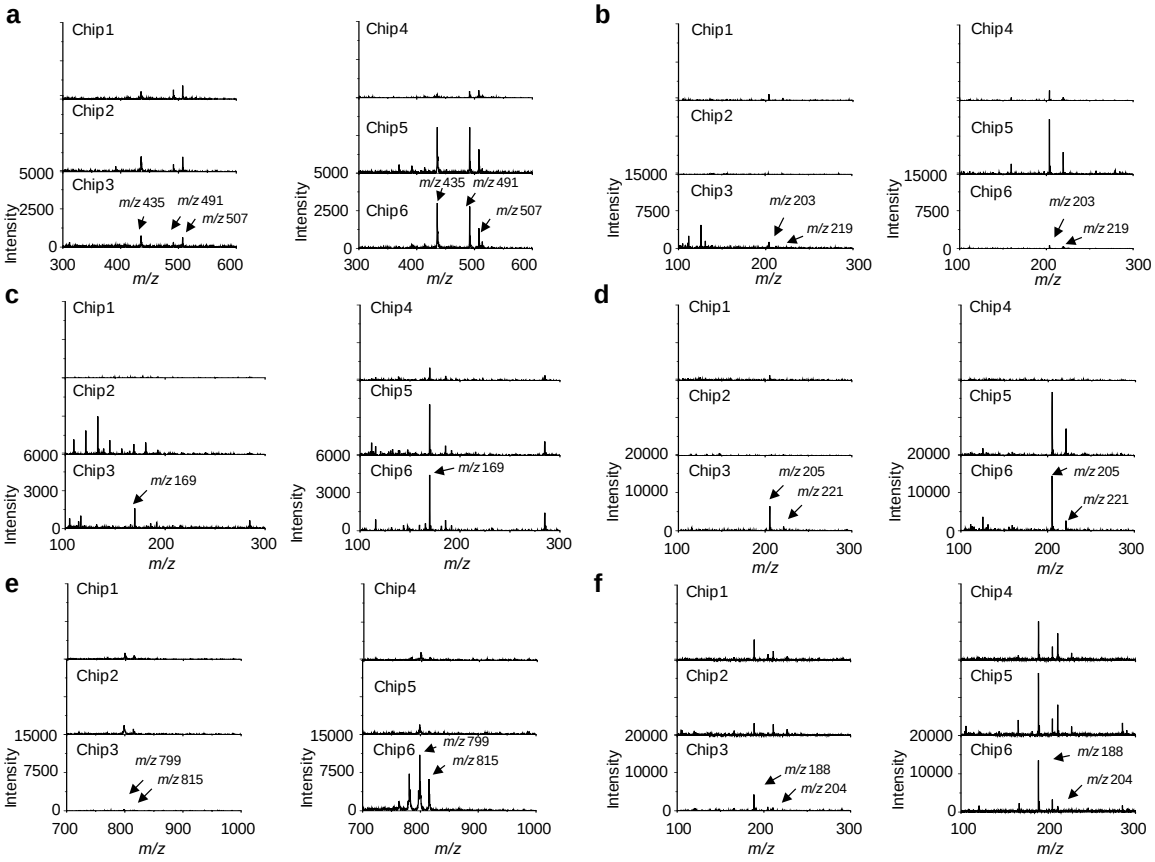


Figure S4. MS spectra of Fmoc-Lys(Boc)-OH (*m/z* 491 $[M+Na]^+$) and (*m/z* 507 $[M+K]^+$) (a), glucose (*m/z* 203 $[M+Na]^+$) and (*m/z* 219 $[M+K]^+$) (b), lysine (*m/z* 169 $[M+Na]^+$) (c), D-mannitol (*m/z* 205 $[M+Na]^+$) and (*m/z* 221 $[M+K]^+$) (d), GGDEVDSG (*m/z* [M+Na]+) and (*m/z* $[M+K]^+$) (e) and phenylalanine (*m/z* 188 $[M+Na]^+$) and (*m/z* 204 $[M+K]^+$) (f).

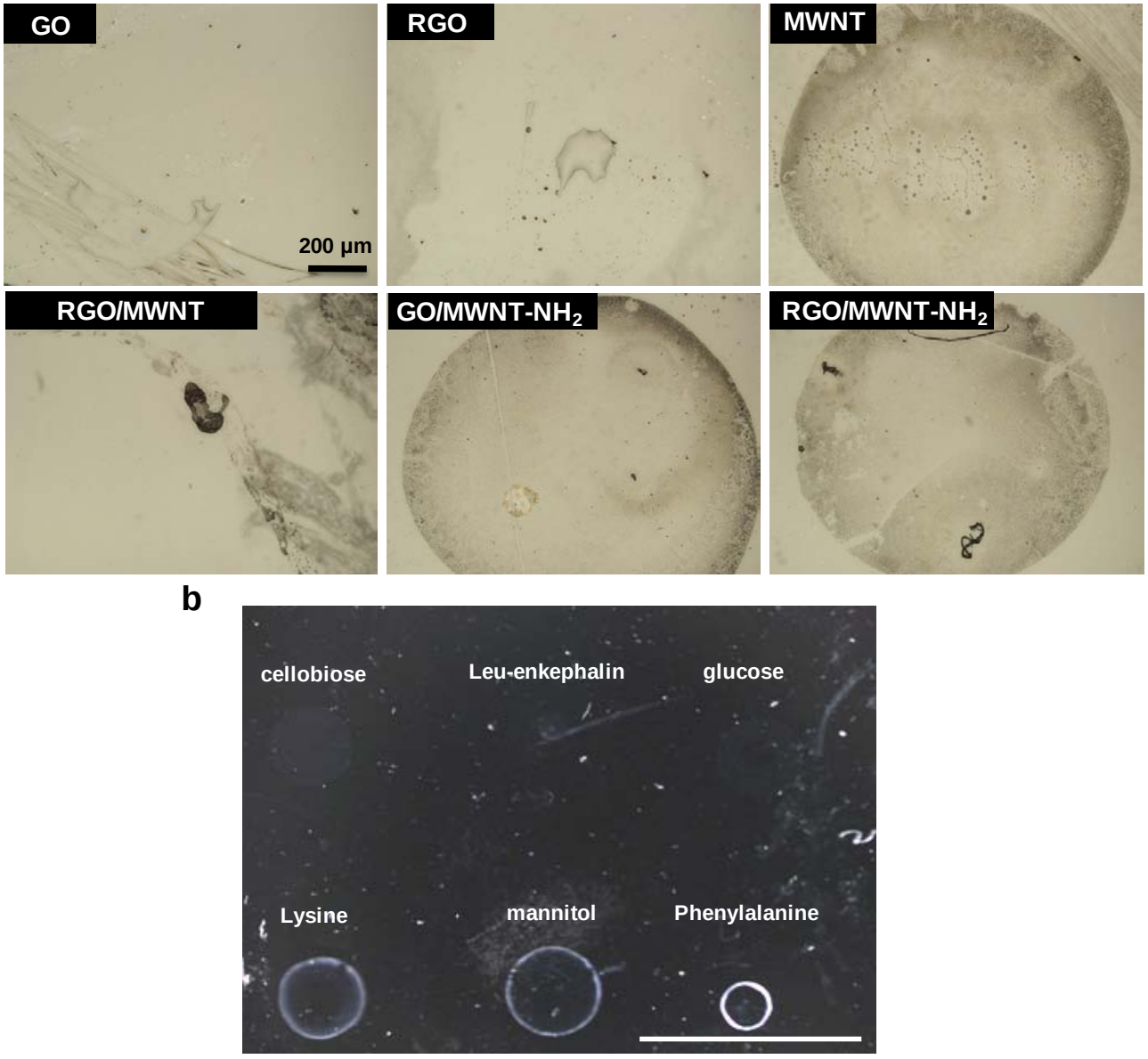
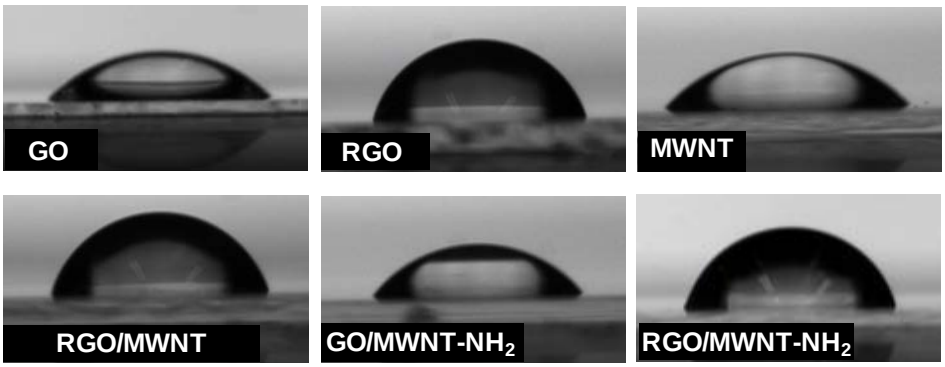


Figure S5. a) The photographs of mannitol spots on carbon nanomaterial chips prepared by spotting 1 µL of 1 mM aqueous solution of mannitol. b) Photograph of cellobiose, Leu-enkephalin, glucose, lysine, mannitol and phenylalanine spots on GO/MWCNT-NH_2 double layer prepared by spotting 1 µL of each aqueous solution (1 µmol/ml) of small molecules (scale bar=2 mm).

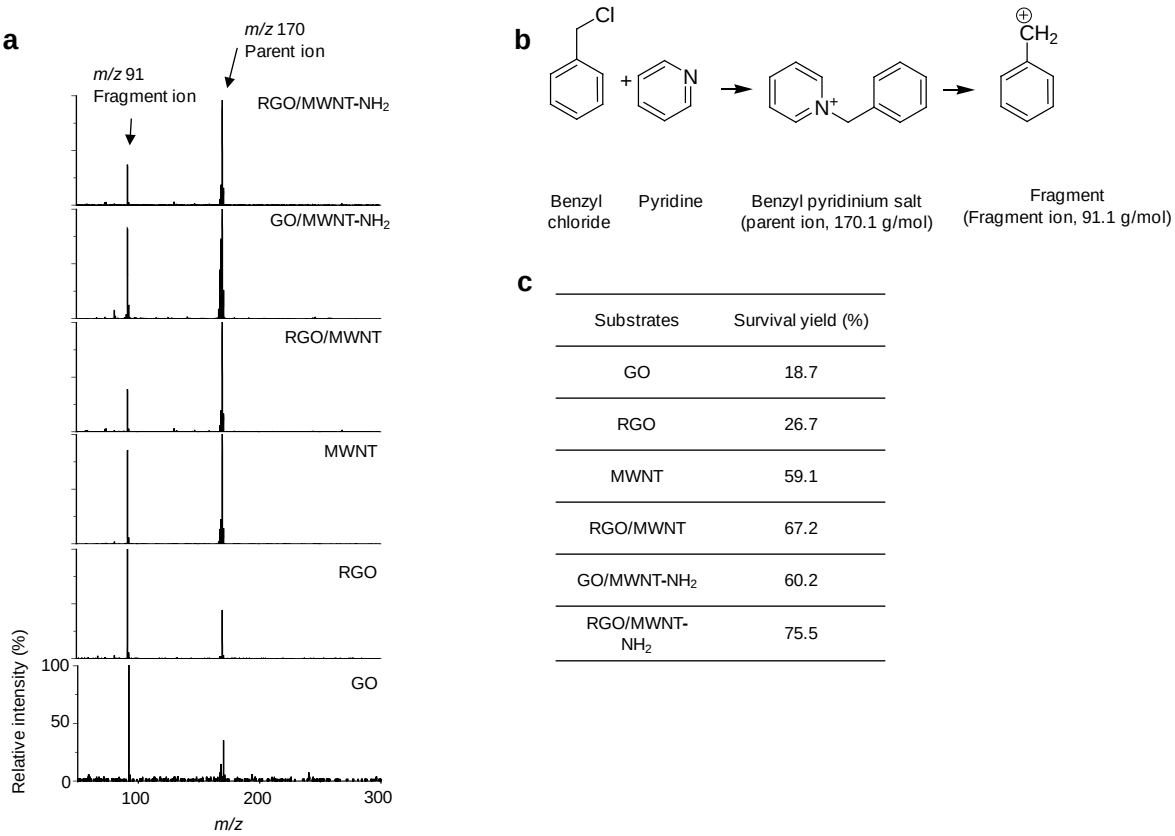
a



b

	GO	RGO	MWNT	RGO/MWNT	GO/MWNT-NH_2	RGO/MWNT-NH_2
Contact angle	41	72	52	75	58	69

Figure S6. The photographs of water droplets on carbon nanomaterial chips (a) and their corresponding water contact angles (b).

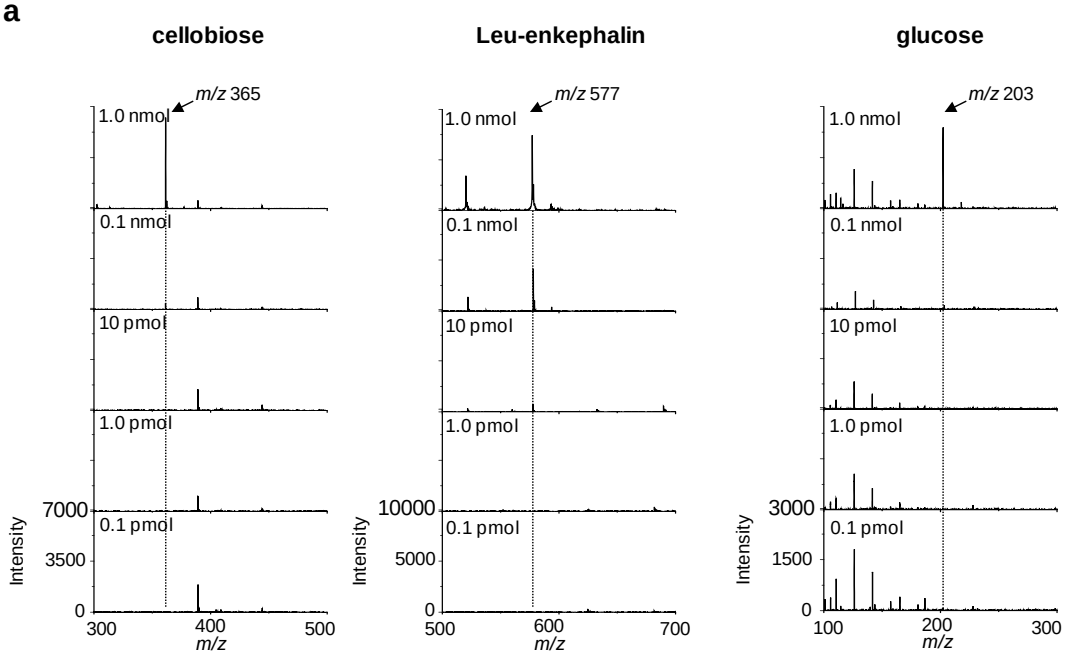


c

Substrates	Survival yield (%)
GO	18.7
RGO	26.7
MWNT	59.1
RGO/MWNT	67.2
GO/MWNT-NH_2	60.2
RGO/MWNT-NH_2	75.5

Figure S7. a) Mass spectra of benzyl pyridinium salt on various carbon nanomaterial chips, b) chemical structures of benzyl pyridinium salt (*m/z* 170) and its fragmentation pattern (*m/z* 91) and c) calculated survival yields of benzyl pyridinium salt on various carbon nanomaterial chips.

a



b

Analyte	Cellobiose	Leu-enkephalin	Glucose	Lysine	Mannitol	Phenylalanine
Detection limit (nmol)	0.10	0.01	0.10	1.00	1.00	0.10

Figure S8. a) The detection limit of small molecules dissolved in PBS on GO/MWCNT-NH_2 double layer platform was determined by LDI MS analysis of serially diluted small molecule solutions on GO/MWCNT-NH_2 double layer. b) The detection limit of small molecules was summarized in a table.

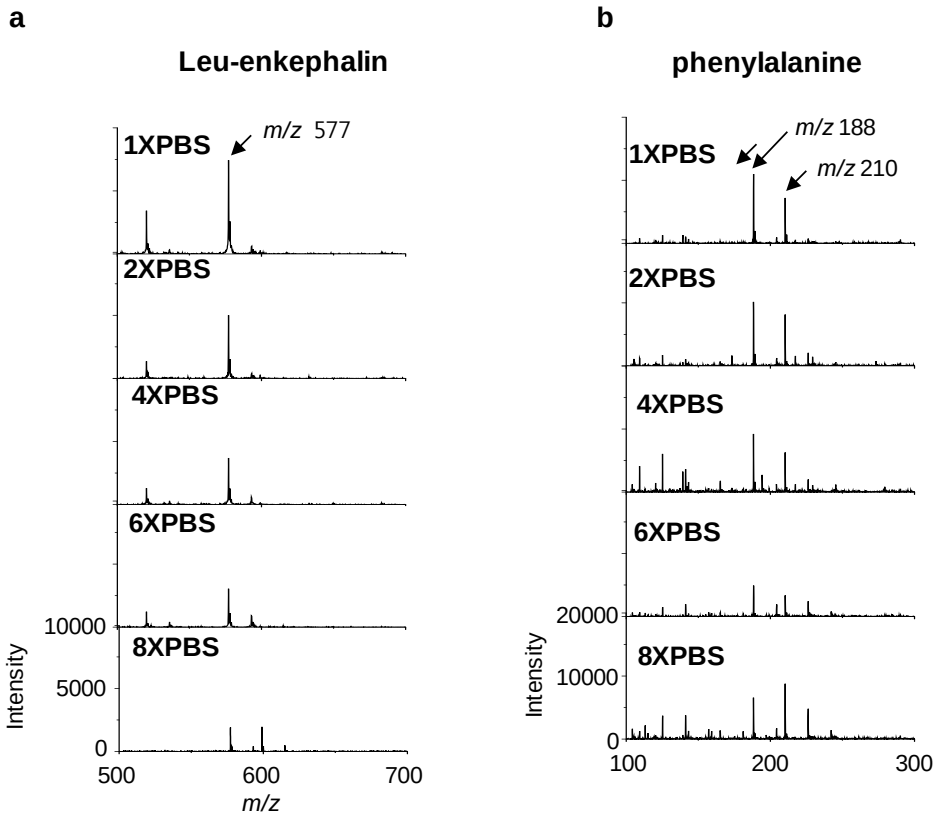


Figure S9. a) Mass spectra of enkephalin (*m/z* 577 $[M+Na]^+$) and b) phenylalanine (*m/z* 188 $[M+Na]^+$, *m/z* 210 $[M+2Na]^+$) dissolved in concentrated PBS buffers obtained on GO/MWNT-NH_2 double layer platform. 1 X PBS contained salts such as 0.21 g/L of KH_2PO_4, 9 g/L of NaCl and 0.72 g/L of $NaHPO_4$.

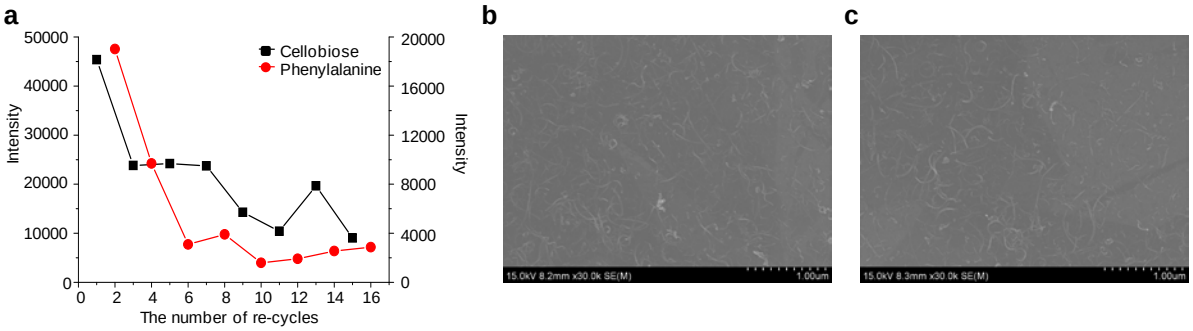


Figure S10. a) The changes in mass signal intensities of cellobiose and phenylalanine on GO/MWCNT-NH_2 double layer platforms were plotted as a function of the number of repeated usages for LDI experiments. The SEM images of GO/MWCNT-NH_2 double layer b) before and c) after 15 cycles of small molecules analysis were shown. The GO/MWCNT-NH_2 double layer was not significantly damaged by repeated irradiation of UV laser.

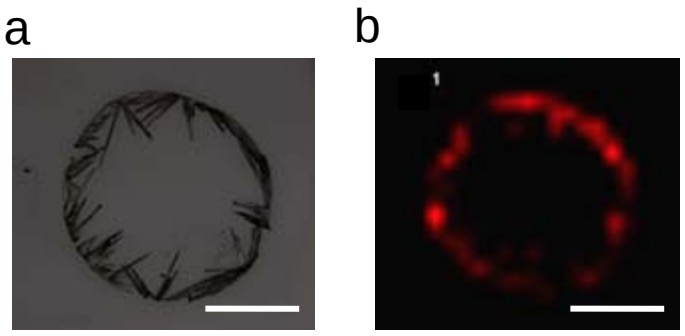


Figure S11. a) Optical and b) mass image of crystal composed of cellobiose and DHB which is frequently used as a matrix in conventional MALDI-ToF MS. The raster width and scale bar were 100 μm and 1 mm, respectively.

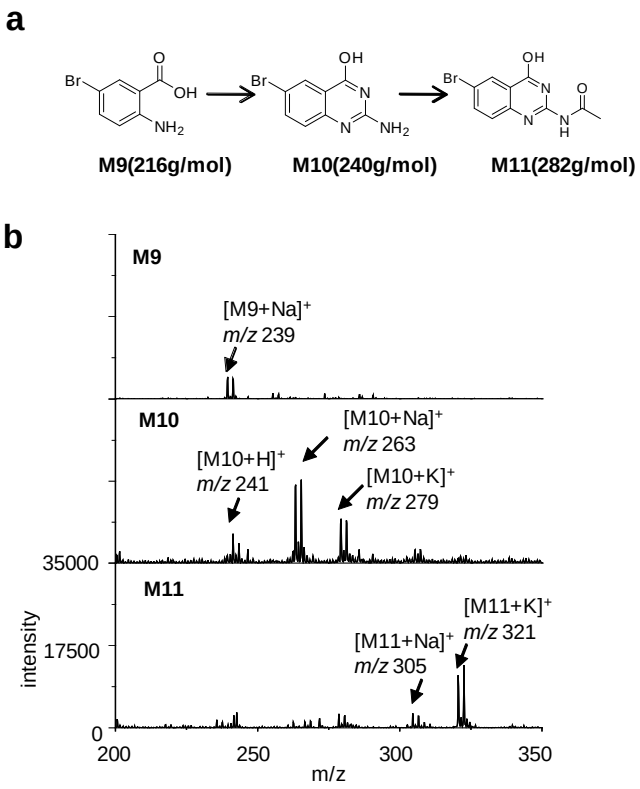


Figure S12. The molecular structures of the synthesized molecules (M7, M8 and M9) (a) and their corresponding mass spectra obtained on GO/MWCNT-NH_2 chip (b)

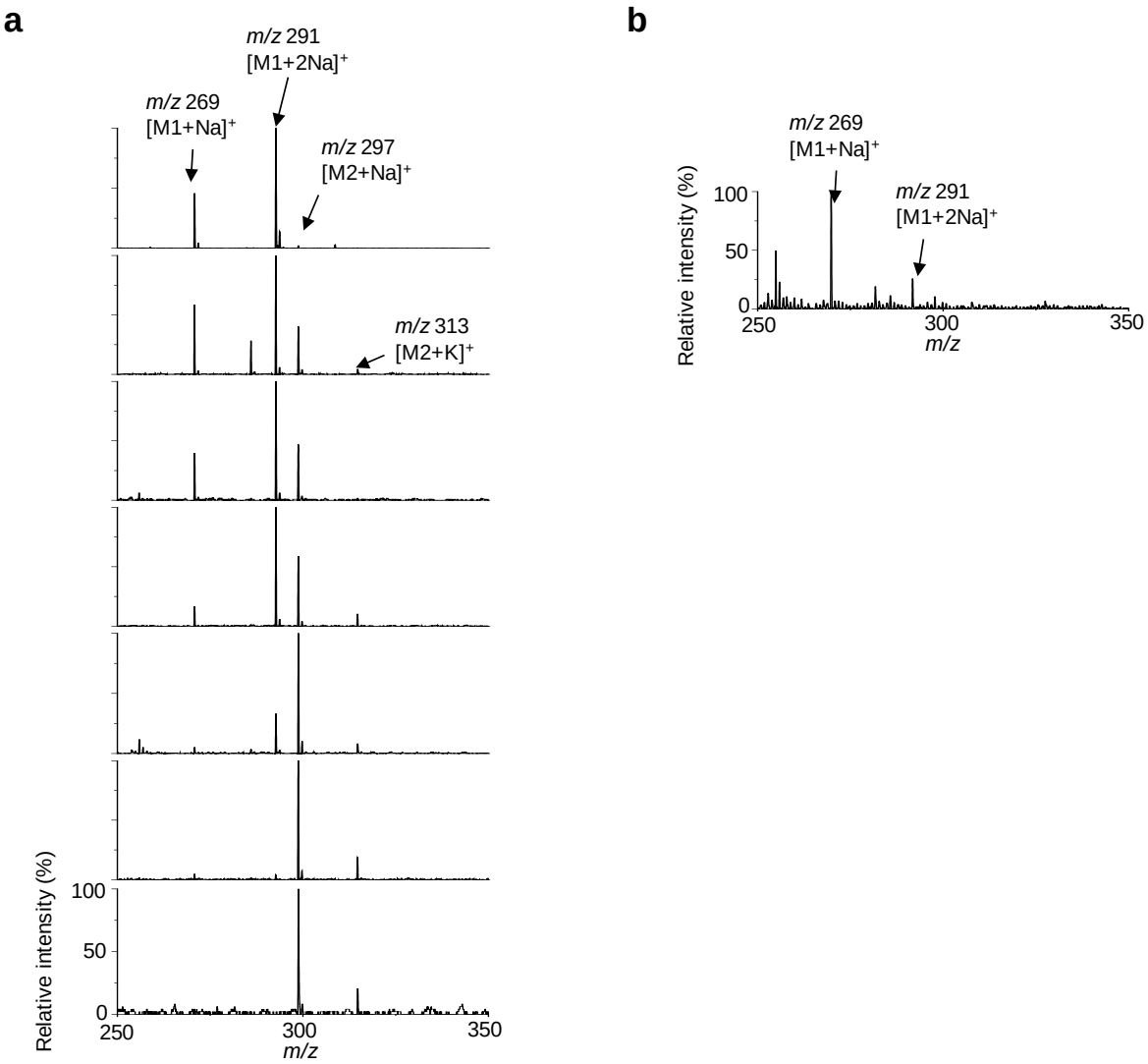


Figure S13. a) Mass spectra obtained from the enzyme reaction mixture for 180 min reaction time and b) a mass spectrum of control sample in which the substrate was incubated for 180 min excluding enzyme.