

## Electronic Supplementary Information

for

### **Graphene oxide embedded sol-gel (GOSG) film as a SALDI MS substrate for robust metabolite fingerprinting**

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## I. Experimental Section.

**Chemicals and Materials.** Graphite (powder, <20  $\mu\text{m}$ ), NaCl,  $\text{H}_2\text{SO}_4$ ,  $\text{K}_2\text{S}_2\text{O}_8$ ,  $\text{P}_2\text{O}_5$ ,  $\text{KMnO}_4$ ,  $\text{H}_2\text{O}_2$ , HCl, ethanol,  $\beta$ -estradiol, 2,5-dihydroxybenzoic acid (DHB), and tetraethyl orthosilicate (TEOS) were obtained from Sigma-Aldrich. (St. Louis, MO, USA). All other organic solvents were purchased from Fisher Scientific (Fairlawn, NJ, USA). Total porcine brain lipid extract in chloroform was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Methanolic extract of tomato (*Lycopersicon esculentum*) metabolites was obtained from Korea Plant Extract Bank (Daejeon, Korea) and a culture of *Chlorella vulgaris* was obtained from Korea Marine Microalgae Culture Center (Busan, Korea).

**Physical Measurements.** Powder X-ray diffraction (PXRD) patterns were recorded on a Bruker D8 Advance diffractometer (40 kV, 40 mA, step size =  $0.02^\circ$ ). Topographic atomic force microscopy (AFM) images were obtained by using a XE-Bio atomic force microscope (Park Systems) in a tapping mode with a scan rate of 0.33 Hz. FE-SEM images were recorded on a Hitachi Ultra-High-Resolution Analytical FE-SEM SU-70 (accelerating voltage = 5 kV) equipped with an EDAX for the EDS spectrum.

**Preparation of graphene oxide (GO).** GO was synthesized by a modified Hummers' method.<sup>1</sup> The mixture of 1.0 g of graphite and 50 g of NaCl was finely ground using a mortar and pestle. The fine powder was washed with deionized water several times and ethanol. The powder was then dried at 80  $^\circ\text{C}$ . The dry powder was mixed with concentrated  $\text{H}_2\text{SO}_4$  (4 mL),  $\text{K}_2\text{S}_2\text{O}_8$  (0.84 g), and  $\text{P}_2\text{O}_5$  (0.84 g) and heated at 80  $^\circ\text{C}$  for 4.5 h. Deionized water (167 mL) was added after cooling and the suspension was further stirred overnight at room temperature. The solid product was filtered and washed with deionized water several times. The powder was dried at 80  $^\circ\text{C}$  for 1 hr. The solids were treated with  $\text{H}_2\text{SO}_4$  (40 mL) and  $\text{KMnO}_4$  (5 g) in an ice bath with gentle stirring. Deionized water was then added and stirred at 35 $^\circ\text{C}$  for 2 hrs. Deionized water (167 mL) and 30%  $\text{H}_2\text{O}_2$  (10 mL) were added after 2 hr incubation and stirred further for 0.5 hr in an ice bath. The final GO product was retrieved by filtration and washed with deionized water until the pH of filtrate was 7.0.

**Preparation of GO-embedded sol-gel (GOSG) films.** The sol-gel film solution was prepared as follows. Fifty milligrams of GO was suspended in 2.1 mL ethanol/ $\text{H}_2\text{O}$ /2M HCl (10:10:1, v/v/v)

and sonicated for 10 min. After sonication, 100  $\mu\text{L}$  of TEOS was added and the final solution was gently stirred for 30 min. A sol-gel film substrate, small pieces of silicon wafer (5 mm  $\times$  5 mm) were washed with Piranha solution to remove organic impurities and then further rinsed with ethanol. The rinsed silicon wafer was dried at 150  $^{\circ}\text{C}$  for 20 min. A total of five drops (10 s between drop application) of the sol-gel solution was applied onto the prepared silicon wafer substrate by spin coating. The spin coated wafer was first dried for 3 hrs at ambient temperature and further dried overnight in the 150 $^{\circ}\text{C}$  oven. In order to remove GO particles on the film surface, the film coated wafers were gently sonicated for 30 min in  $\text{H}_2\text{O}$  and air-dried.

**Sample preparation for GOSG-SALDI mass spectrometry (MS).** For SALDI MS analysis, the silicon wafers coated with the GO-embedded sol-gel film were attached onto the MALDI target plate base by conductive double-sided tape (3M). 0.5  $\mu\text{L}$  of sample solution was applied directly onto the sol-gel film surface and dried. Each sample had at least three replicates in order to examine spot-to-spot reproducibility.

**Sample preparation for GO particle SALDI MS.** For GO particle SALDI MS analysis, 0.5  $\mu\text{L}$  of aqueous GO solution (0.05 mg/mL) was first spotted onto a 384-well MALDI target plate (ASTA Inc., Suwon, Korea) and dried. Sample solution (0.5  $\mu\text{L}$ ) was then spotted onto the GO-dried spot and air-dried.

**LDI MS.** SALDI MS analysis was performed using ABI 4800 Plus MALDI-TOF/TOF analyzer (Applied Biosystems, Foster City, CA) controlled by the 4000 Series Explorer<sup>TM</sup> software. The mass spectrometer was operated in the positive or negative reflectron mode with a 20 kV acceleration voltage. For a given sample spot, a mass spectrum was produced from ions generated by 800 laser shots. In order to obtain tandem mass spectra, collision induced dissociation was performed using air as the collision gas at 1 kV of collision energy. The obtained mass spectra were further processed by DataExplorer V4.8 (Applied Biosystems) and mMass (<http://www.mmass.org>).

## II. Supplementary Tables

**Supplementary Table S1.** Tentative peak assignments for the bare sol-gel and GOSG SALDI mass spectra of a total brain lipid extract presented in Figure 3 and Supplementary Figure S3.

$m/z^a$	Species <sup>b</sup>	$m/z^a$	Species <sup>b</sup>
750	[Cer d18:1/18:0 <sup>b</sup> + Na] <sup>+</sup>	848	[Cer d18:1/24:1h + Na] <sup>+</sup> , [Cer d18:1/25:0 + Na] <sup>+</sup> , [Cer d18:1/24:1 + K] <sup>+</sup>
766	[Cer d18:1/18:0h <sup>c</sup> + Na] <sup>+</sup> , [Cer d18:1/18:0 + K] <sup>+</sup>	850	[Cer d18:1/24:0h + Na] <sup>+</sup>
804	[Cer d18:1/22:1 + Na] <sup>+</sup>	852	[Cer d18:0/24:0h + Na] <sup>+</sup> , [Cer d18:1/23:0h + Na] <sup>+</sup>
806	[Cer d18:1/22:0 + Na] <sup>+</sup>	860	[Cer d18:1/26:1 + Na] <sup>+</sup>
808	[Cer d18:0/22:0 + Na] <sup>+</sup>	862	[Cer d18:1/25:1h + Na] <sup>+</sup>
820	[Cer d18:1/23:0 + Na] <sup>+</sup> , [Cer d18:1/22:1 + K] <sup>+</sup>	864	[Cer d18:1/25:0h + Na] <sup>+</sup> , [Cer d18:1/24:1h + K] <sup>+</sup> , [Cer d18:1/25:0 + K] <sup>+</sup>
822	[Cer d18:1/22:0h + Na] <sup>+</sup> , [Cer d18:1/22:0 + K] <sup>+</sup>	866	[Cer d18:0/25:0h + Na] <sup>+</sup> , [Cer d18:1/24:0h + K] <sup>+</sup>
824	[Cer d18:0/22:0h + Na] <sup>+</sup> , [Cer d18:0/22:0 + K] <sup>+</sup>	876	[Cer d18:1/26:1h + Na] <sup>+</sup> , [Cer d18:1/26:1 + K] <sup>+</sup>
832	[Cer d18:1/24:1 + Na] <sup>+</sup>	878	[Cer d18:1/26:0h + Na] <sup>+</sup> , [Cer d18:1/25:1h + K] <sup>+</sup>
836	[Cer d18:1/23:0h + Na] <sup>+</sup> , [Cer d18:1/23:0 + K] <sup>+</sup>	880	[Cer d18:0/26:0h + Na] <sup>+</sup> , [Cer d18:1/25:0h + K] <sup>+</sup>
838	[Cer d18:0/23:0h + Na] <sup>+</sup> , [Cer d18:1/22:0h + K] <sup>+</sup>	892	[Cer d18:1/26:1h + K] <sup>+</sup>

<sup>a</sup>Indicated  $m/z$ s are nominal  $m/z$ s. <sup>b</sup>Peak assignments were based on previously reported identifications and MALDI MS/MS experiments.<sup>2</sup> <sup>c</sup>Cer A/B corresponds to galactosylceramide sphingoid long chain base (A)/amide-linked fatty acid (B). <sup>h</sup> corresponds to the hydroxyl group at the C2 position of the amide-linked fatty acid.

**Supplementary Table S2.** Tentative peak identifications for the GOSG and GO particle mass spectra of a *Lycopersicon esculentum* (tomato) extract presented in Figure 4 and 5.<sup>a,b</sup>

(a) Positive ion mode

<i>m/z</i>	Species	<i>m/z</i>	Species
202.79	[Glucose + Na] <sup>+</sup>	698.74	[PC 28:1 + Na] <sup>+</sup>
230.66	[Traumatic acid + H] <sup>+</sup>	714.75	[Tomato cerebroside + H] <sup>+</sup>
260.76	[Flavone + K] <sup>+</sup>	716.69	[PE 31:0 + K] <sup>+</sup>
286.98	[Kaempferol + H] <sup>+</sup>	735.52	[Tomato cerebroside + Na] <sup>+</sup>
303.10	[Quercetin + H] <sup>+</sup>	751.43	[Tomato cerebroside + K] <sup>+</sup>
316.86	[Isorhamnetin + H] <sup>+</sup>	770.51	[PC 32:1 + K] <sup>+</sup>
319.88	[Myricetin + H] <sup>+</sup>	812.71	[PC 36:0 + Na] <sup>+</sup>
332.83	[Malvidin + H] <sup>+</sup>	866.37	[PC 40:9 + K] <sup>+</sup>
346.84	[Caffeoyl spermidine + K] <sup>+</sup>	892.57	[TAG 54:4 + Na] <sup>+</sup>
364.77	[Sucrose + Na] <sup>+</sup>	910.68	[PC 42:1 + K] <sup>+</sup>
380.72	[Sucrose + K] <sup>+</sup>	924.59	[β1-tomatidine + Na] <sup>+</sup>
402.81	[Cyananin + Na] <sup>+</sup>	936.64	[PE 48:1 + Na] <sup>+</sup>
416.81	[Tomatidine + H] <sup>+</sup>	954.12	[TAG 58:1 + Na] <sup>+</sup>
438.79	[Tomatidine + Na] <sup>+</sup>	974.05	[TAG 60:5 + Na] <sup>+</sup>
440.91	[Isoliquiritin + Na] <sup>+</sup>	1030.70	[Dehydrotomatine + H] <sup>+</sup>
452.77	[Tomatidenol + K] <sup>+</sup>	1055.96	[α-tomatine + Na] <sup>+</sup>
456.87	[Tomatidine + K] <sup>+</sup>	1071.97	[α-tomatine + K] <sup>+</sup>
632.61	[Ceramide 38:1 + Na] <sup>+</sup>	1087.40	[Lycoperoside H + H] <sup>+</sup>
648.54	[PE 30:0 + H] <sup>+</sup>	1113.51	[Lycoperoside A + Na] <sup>+</sup>
662.63	[LPC 28:1 + H] <sup>+</sup>	1129.36	[Lycoperoside A + K] <sup>+</sup>
674.67	[LPC 26:0 + K] <sup>+</sup>		

a. Peak assignments were performed based on supplementary references 3-7.<sup>3-7</sup>

b. Abbreviations: PE-phosphatidylethanolamine; LPC-lysophosphatidylcholine; PC-phosphatidylcholine; TAG-triacylglycerol.

**Supplementary Table S2. (Continued)**

(b) Negative ion mode

<i>m/z</i>	Species	<i>m/z</i>	Species
193.02	[Ferulic acid – H] <sup>-</sup>	555.41	[zeta-carotene epoxide – H] <sup>-</sup>
205.06	[α-lipoic acid – H] <sup>-</sup>	577.41	[kaempferitin – H] <sup>-</sup>
224.95	[FA C14:1 – H] <sup>-</sup>	609.34	[Rutin – H] <sup>-</sup>
249.14	[Caffeoyl putrescine – H] <sup>-</sup>	623.39	[Quercetin 7-glucuronide 3-rhamnoside – H] <sup>-</sup>
255.16	[FA C16:0 – H] <sup>-</sup>	645.38	[DAG 38:3 – H] <sup>-</sup>
277.26	[FA 18:3 – H] <sup>-</sup>	653.11	[Spinacetin 3-rutinoside – H] <sup>-</sup>
299.10	[FA C20:6 – H] <sup>-</sup>	661.43	[DAG 40:9 – H] <sup>-</sup>
301.10	[Quercetin – H] <sup>-</sup>	713.43	[Tomato cerebroside A – H] <sup>-</sup>
312.19	[FA C20:0 – H] <sup>-</sup>	720.71	[PE 36:5 – H] <sup>-</sup>
320.16	[Feruoyl spermidine – H] <sup>-</sup>	793.70	[TAG 48:6 – H] <sup>-</sup>
334.94	[Caffeoyl shikimate – H] <sup>-</sup>	815.64	[TAG 50:9 – H] <sup>-</sup>
341.18	[Sucrose – H] <sup>-</sup>	827.59	[TAG 50:3 – H] <sup>-</sup>
350.17	[Sinapoyl spermidine – H] <sup>-</sup>	843.60	[TAG 52:9 – H] <sup>-</sup>
367.19	[Feruloylquinic acid – H] <sup>-</sup>	857.60	[TAG 53:9 – H] <sup>-</sup>
377.30	[MAG 20:4 – H] <sup>-</sup>	871.66	[TAG 54:9 – H] <sup>-</sup>
393.22	[LPA 16:0 – H] <sup>-</sup>	887.68	[TAG 55:8 – H] <sup>-</sup>
447.49	[Quercitrin – H] <sup>-</sup>	900.69	[β1-tomatidine – H] <sup>-</sup>
460.31	[Cyanidin 3-O-(6-acetyl-arabinoside) – H] <sup>-</sup>	915.67	[TAG 56:8 – H] <sup>-</sup>
473.42	[Apigenin acetylhexoside – H] <sup>-</sup>	917.73	[TAG 56:7 – H] <sup>-</sup>
490.32	[Isorhamnetin 4-O-glucoside – H] <sup>-</sup>	931.73	[TAG 58:7 – H] <sup>-</sup>

a. Peak assignments were performed based on supplementary references 3~7.

b. Abbreviations: FA-fatty acid; MAG-monoacylglycerol; LPA-lysophosphatidic acid; DAG-diacylglycerol; PE- phosphatidylethanolamine; TAG-triacylglycerol.

**Supplementary Table S3.** Tentative peak identifications for the negative-ion mode mass spectra of *Chlorella Vulgaris* presented in Figure 6 and Supplementary Figure S6.<sup>a,b</sup>

<i>m/z</i>	Species	<i>m/z</i>	Species
224.93	[FA C14:1 – H] <sup>-</sup>	506.14	[ATP – H] <sup>-</sup>
240.94	[FA C15:0 – H] <sup>-</sup>	509.14	[LPG 18:1 – H] <sup>-</sup>
255.16	[FA C16:0 – H] <sup>-</sup>	526.14	[LPE 22:5 – H] <sup>-</sup>
258.95	[FA C17:5 – H] <sup>-</sup>	540.14	[LPC 20:5 – H] <sup>-</sup>
269.08	[FA C17:0 – H] <sup>-</sup>	554.16	[TAG 30:0 – H] <sup>-</sup>
277.14	[FA C18:3 – H] <sup>-</sup>	567.16	[Lutein – H] <sup>-</sup>
279.15	[FA C18:2 – H] <sup>-</sup>	577.24	[DAG 32:2 – H] <sup>-</sup>
281.18	[FA C18:1 – H] <sup>-</sup>	587.18	[DAG 34:4 – H] <sup>-</sup>
298.97	[FA C20:6 – H] <sup>-</sup>	613.23	[DAG 36:5 – H] <sup>-</sup>
311.55	[FA C20:0 – H] <sup>-</sup>	627.22	[DAG 37:5 – H] <sup>-</sup>
316.97	[FA C21:4 – H] <sup>-</sup>	743.47	[PG 34:3 – H] <sup>-</sup>
325.61	[FA C21:0 – H] <sup>-</sup>	787.50	[TAG 47:1 – H] <sup>-</sup>
339.66	[FA C22:0 – H] <sup>-</sup>	793.55	[PG 38:6 – H] <sup>-</sup>
409.91	[dADP – H] <sup>-</sup>	815.54	[PG 40:9 – H] <sup>-</sup>
453.11	[LPE 16:0 – H] <sup>-</sup>	837.53	[PI 34:0 – H] <sup>-</sup>
467.10	[LPA 22:4 –H <sub>2</sub> O – H] <sup>-</sup>	930.79	[Guanosine pentaphosphate adenosine – H] <sup>-</sup>
481.09	[LPA 22:6 – H] <sup>-</sup>	1052.51	[CDP-DAG 40:6 – H] <sup>-</sup>
495.10	[LPS 16:0 – H] <sup>-</sup>	1065.69	[TAG 68:10 – H] <sup>-</sup>

a. Peak assignments were performed based on metabolite exact mass data deposited in <http://www.hmdb.ca/> and supplementary reference 8.<sup>8</sup>

b. Abbreviations: FA-fatty acid; dADP- deoxyadenosine diphosphate; LPE-lysophosphatidylethanolamine; LPA- lysophosphatidic acid; LPS-lysophosphatidylserine; ATP-adenosine triphosphate; LPG-lysophosphatidylglycerol; LPC-lysophosphatidylcholine; TAG-triacylglycerol; DAG-diacylglycerol; PG-phosphatidylglycerol; PI-phosphatidylinositol; CDP-DAG-cytidine diphosphate diacylglycerol.

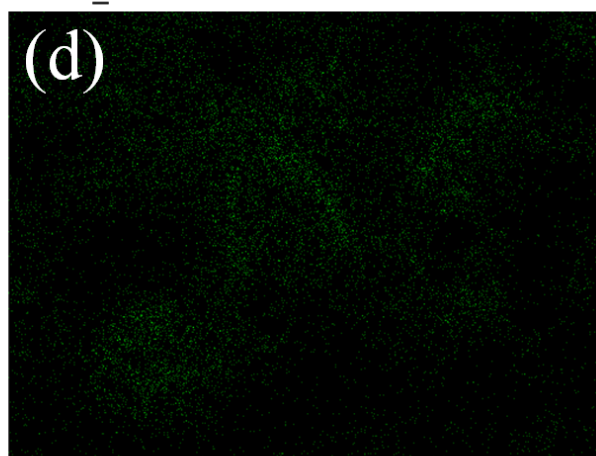
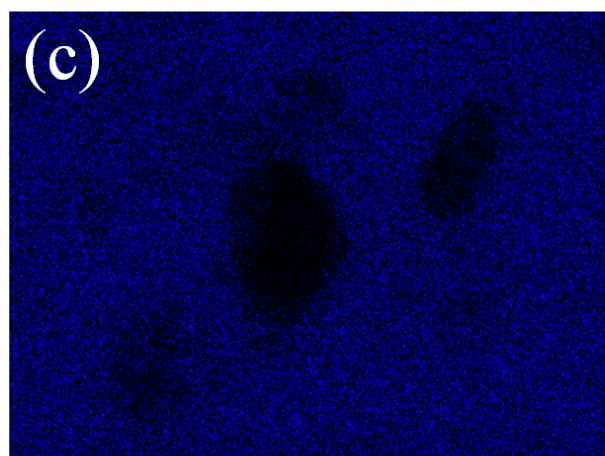
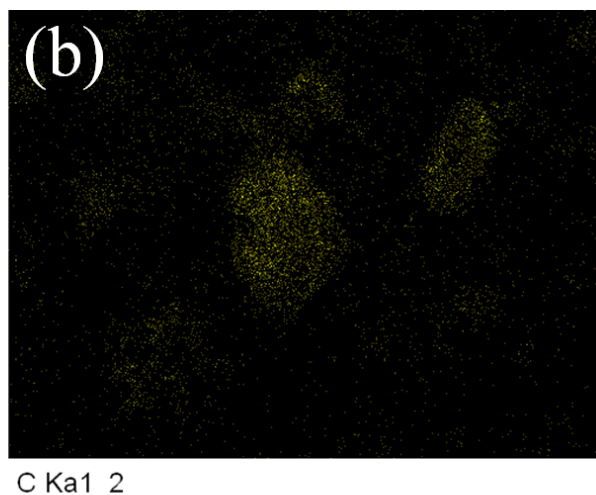
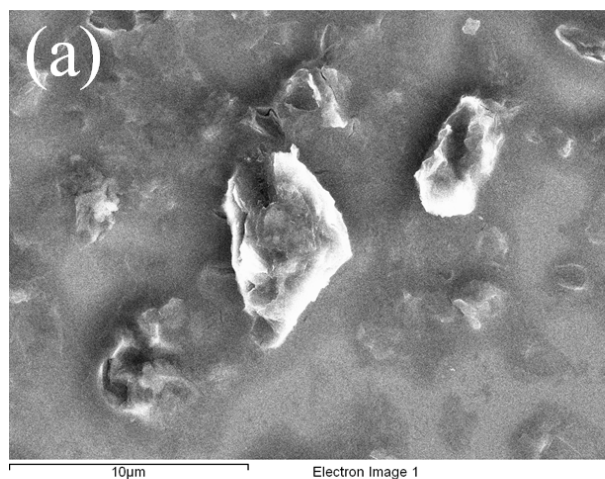
## II. Supplementary Figures

**Supplementary Fig. S1** FE-SEM image of the top surface of the GOSG film after sonication ( $\times 500$ ).

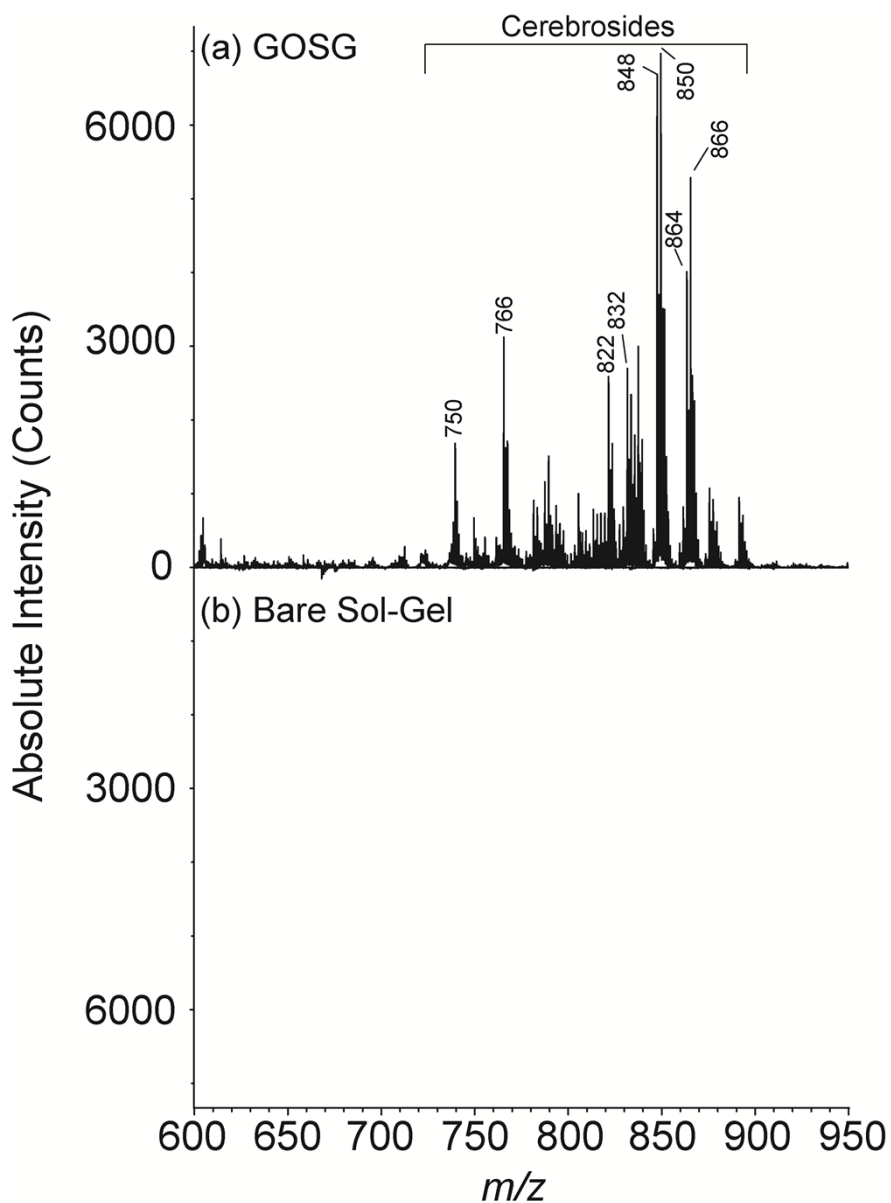




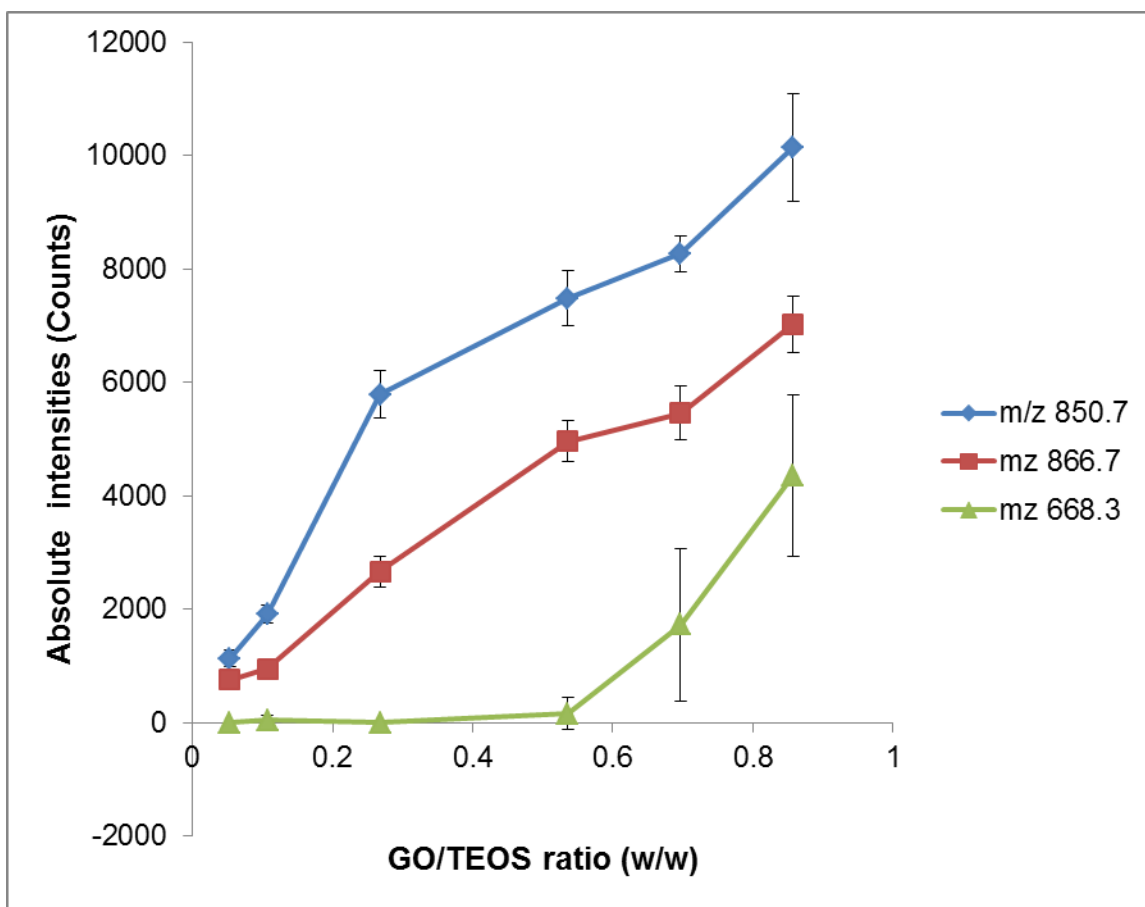
**Supplementary Fig. S2** EDS elemental mapping of the GOSG film. SEM image (a) and the corresponding elemental maps for C (b), Si (c) and O (d).



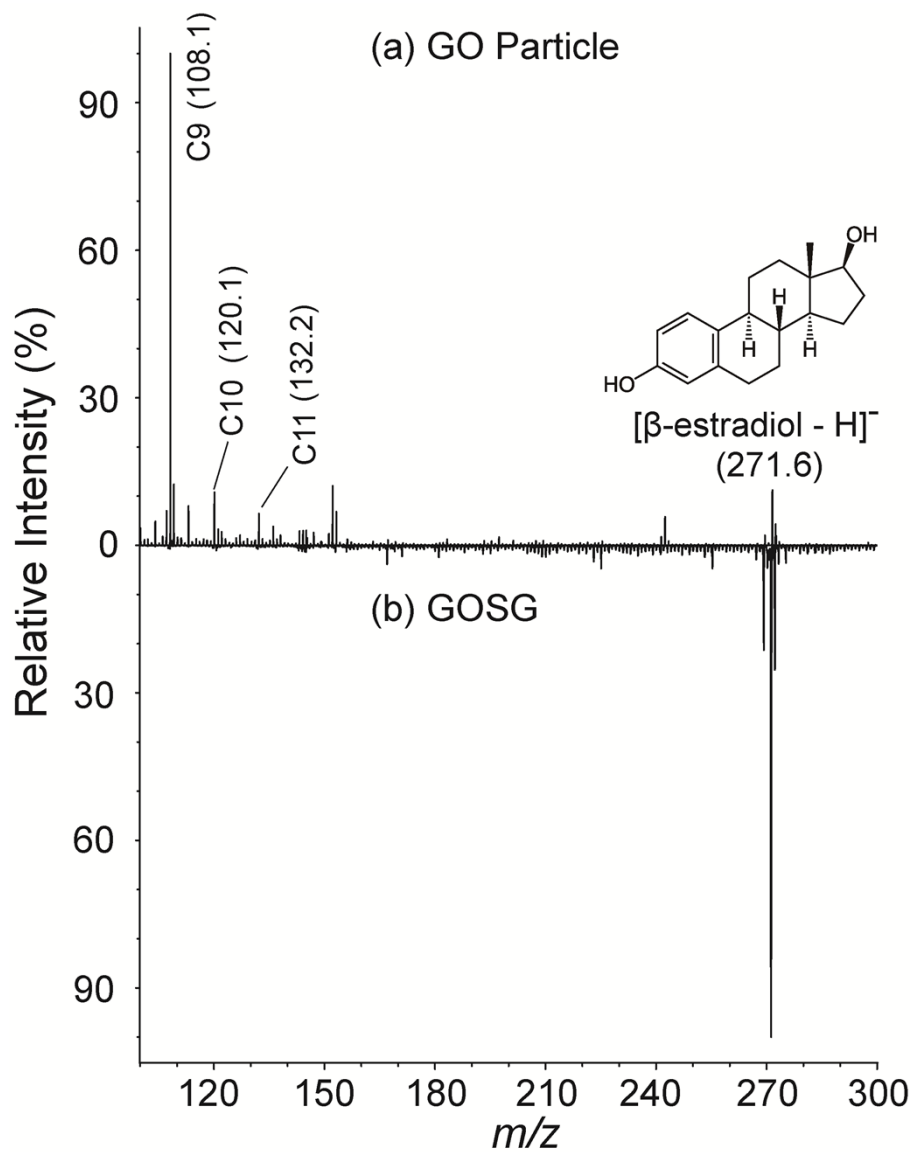
**Supplementary Fig. S3** (a) GOSG and (b) bare sol-gel SALDI mass spectra of total brain lipid extract (0.5  $\mu\text{g}/\text{spot}$ ) in the positive ion mode. GOSG produced a cerebroside-focused profile from the total brain lipid extract while a bare sol-gel film did not produce any signal from the same sample (b). This result demonstrated that the GO embedded in a sol-gel film is mainly responsible for the ionization since carbon material-based LDI matrices have shown to enhance ionization of glycosphingolipids such as cerebrosides but suppress ionization of phospholipids in the previous studies.<sup>2,9</sup> For detailed peak assignments, see supplementary Table S1.



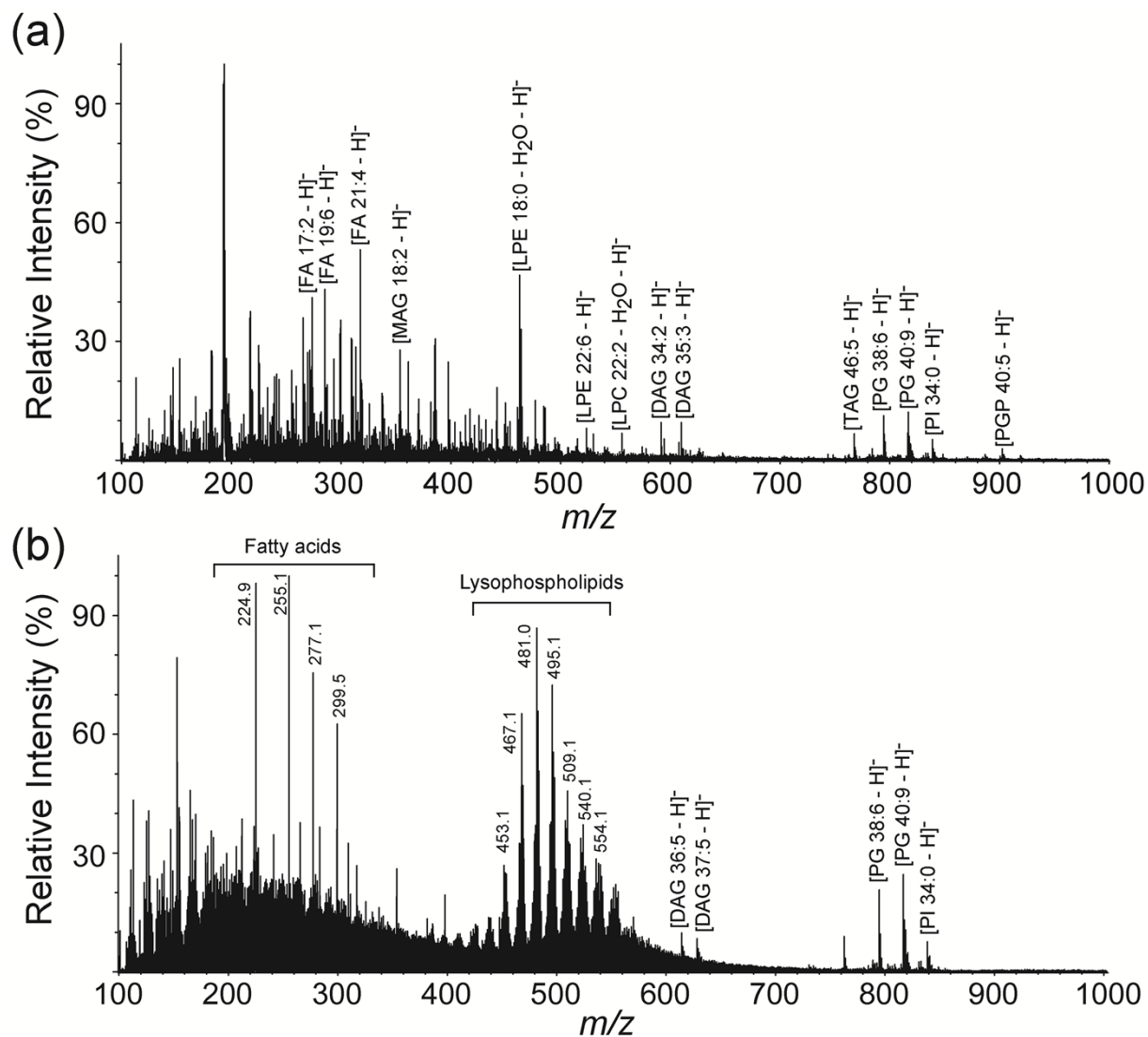
**Supplementary Fig. S4** Spectral quality of GOSG SALDI MS depending on the proportion of GO in a GOSG film. GOSG films with various ratios of GO and TEOS were tested against the total brain lipid extract sample (0.5  $\mu\text{g}/\text{spot}$ ). All GOSG films were sonicated in water for 30 min before use. The signals at  $m/z$  850.7 and 866.7 were from cerebroside (Table S1 and Fig. 3) and the signals at  $m/z$  668.3 were unknown background signals originated from surface-exposed GO particles (Fig. 3, top). For GOSG films with the GO/TEOS ratios (w/w) of 0.70 and 0.85, significant background signals frequently appeared primarily because it was very hard to eliminate surface-exposed GO particles completely by the sonication procedure.



**Supplementary Fig. S5** (a) GO particle and (b) GOSG SALDI mass spectra of  $\beta$ -estradiol (0.5  $\mu\text{g}/\text{spot}$ ) in the negative ion mode. Absolute intensities in counts for the peaks  $[\beta\text{-estradiol} - \text{H}]^-$  were (a) 5900 and (b) 5800 counts, respectively. This result demonstrated that GOSG produced much cleaner background in the low  $m/z$  region than GO particle matrix without losing sensitivity.



**Supplementary Fig. S6** (a) MALDI and (b) Matrix-free, LDI mass spectra of *Chlorella Vulgaris* in the negative ion mode. In case of (a), 9-aminoacridine was used as the MALDI matrix. For detailed peak assignments, see Supplementary Table S3.



#### IV. Supplementary References

1. N. I. Kovtyukhova, P. J. Ollivier, B. R. Martin, T. E. Mallouk, S. A. Chizhik, E. V. Buzaneva and A. D. Gorchinskiy, *Chem. Mater.*, 1999, **11**, 771-778.
2. S. W. Cha and E. S. Yeung, *Anal. Chem.*, 2007, **79**, 2373-2385.
3. M. Stobiecki, *Phytochemistry*, 2000, **54**, 237-256.
4. S. Mintz-Oron, T. Mandel, I. Rogachev, L. Feldberg, O. Lotan, M. Yativ, Z. Wang, R. Jetter, I. Venger, A. Adato and A. Aharoni, *Plant Physiol.*, 2008, **147**, 823-851.
5. S. Moco, R. J. Bino, O. Vorst, H. A. Verhoeven, J. de Groot, T. A. van Beek, J. Vervoort and C. H. R. de Vos, *Plant Physiol.*, 2006, **141**, 1205-1218.
6. M. Gómez-Romero, A. Segura-Carretero and A. Fernández-Gutiérrez, *Phytochemistry*, 2010, **71**, 1848-1864.
7. P. D. Fraser, E. M. A. Enfissi, M. Goodfellow, T. Eguchi and P. M. Bramley, *Plant J.*, 2007, **49**, 552-564.
8. P. L. Urban, T. Schmid, A. Amantonico and R. Zenobi, *Anal. Chem.*, 2011, **83**, 1843-1849.
9. G. Lee, J. Son and S. Cha, *B. Korean Chem Soc.*, 2013, **34**, 2143-2147.