Supplemental Text

Methods

Imaging Mass Spectrometry

Imaging experiments were performed using a reflectron geometry MALDI TOF-TOF mass spectrometer (Ultraflextreme, Bruker Daltonics, Bremen, Germany). For all analyses, the Nd:YAG/355 nm laser spot size was focused to a 8.40 ± 0.84 µm spot size by adjusting laser focus settings to a value of 98% within 100% of the focus range. For negative ion mode imaging, the laser attenuator offset was adjusted to 77% and laser fluency was operated at ≤ 20%, producing an effective laser fluency of 35.6 µJ. Detection limits were from 440 – 1620 *m/z*, matrix suppression set to suppress molecules < 350 Da. Data was collected at a 2.0 Gs sample rate. For negative ion mode, pulsed ion extraction was set to 60 ns, and the source was set to an accelerating voltage of (-) 20.00 kV with an extraction voltage of (-) 17.55 kV, and a lens voltage of 6.00 kV. For positive ion mode, pulsed ion extraction was set to 80 ns, accelerating voltage was set to 21.15 kV and extraction voltage set to 10.75kV, lens voltage at 6.00 kV. Negative ion mode imaging data were acquired by summing up 100 shots per array position without intraspot rastering with a laser repetition rate of 1000 Hz, offsetting x and y by 40 µm and collecting imaging data in the positive ion mode. Calibration of the instrument in negative and positive ion mode was performed using quadratic fit on odd numbered red phosphate clusters from P_{15}-P_{51} (*m/z* 464.607-1579.662).[1] On-tissue signal was optimized using the abundant lipids glycerophosphocholine 34:1, m/z 760.585082 (+) or glycerophosphoinositol 38:4, m/z 885.549853 (-).

Image Processing and Data Analysis

Imaging data were processed using FlexAnalysis v3.3. Images were visualized using the FlexImaging software v2.1. ClinProTools v2.2 was used to calculate individual peak intensities and standard deviations from total ion current. Spectral settings for ClinProTools used a resolution of 2000, baseline flatness of 0.4, enabled noise exclusion at 3.0, and Savitsky-Golay smoothing enabled at 0.1 width/1 cycle over a mass range 450-1600. Peaks with a signal to noise intensity of ≥ 5 were used to calculate total ion current.

Fragmentation

Negative and positive mode lipid fragmentation was performed on imaged tissue sections using a MALDI-LTQ-XL hybrid linear ion trap instrument (Thermo Scientific,Waltham, MA, USA). For positive ion mode fragmentation, matrix solution (25 mg DHB in 50/50 150 mM LiCl/ethanol) was manually spotted onto tissue

sections. Negative ion mode fragmentation was performed on areas coated by sublimated matrix. In both modes, precursors were selected within a 0.4-1.0 Da window using a laser energy of 20 µJ, collecting 3 microscans with 10 laser shots/microscan. Collision energy was set at 25% normalized collision energy and stepped through increasing collision energy to yield production of lysophosphatidic acids (LPA) and fatty acids (FA). For glycerophosphoserines and gangliosides, ions were further fragmented by MS^n to obtain fatty acid information. Sulfatides were fragmented by pulsed q-dissociation with a normalized collision energy set at 35% and stepped through an activation Q of 0.700-0.730, and an activation time of 0.100-0.150 to yield production of sulfate and dehydrated galactose sulfate fragments (*m/z* 97 and 241, respectively). Lipid fragmentation patterns were manually interpreted using tools available at lipidmaps.org.

Accurate Mass Measurements

Accurate masses were determined using a 9.4 Tesla Apex-Qe FT-ICR MS (Bruker Daltonics, Bremen, Germany) equipped with a MALDI source. Accurate mass profiling of tissue sections was performed on same sections that were previously used to produce high spatial resolution images. Data were collected at a 200 µm lateral resolution using 50 shots by an Nd:YAG laser operating at 200 Hz, offsetting by 100 µm for positive ion mode imaging. The instrument was tuned to obtain high mass accuracy for broadband measurement over *m/z* 450-1600 with a 1 M transient (~ 200,00 resolution) averaging four scans per spectra. A ≤ 0.1 ppm accuracy was obtained by calibrating using odd numbered red phosphorous clusters[1] from P_{15}-P_{51} (*m/z* 464.606971-1579.662386 (-), or 1579.661288 (+).

(1) Sládková, K.; Houška, J.; Havel, J. *Rapid Commun Mass Spectrom*, **2009**, 23, 3114-3118.