SUPPLEMENTAL INFORMATION for

A MALDI Imaging Mass Spectrometry Workflow for Spatial Profiling Analysis of N-linked Glycan Expression in Tissues

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Supplementary Methods -1

Recombinant Peptide N-Glycosidase F Expression: The entire Peptide N-Glycosidase F (PNGase F) gene (P21163.2) from the genome of *Flavobacterium meningosepticum* was cloned, expressed and purified into the T7 expression vectors pET 29-b (Novagen) and pQE-T7 (Qiagen). This insertion results in the addition of the sequence for an in frame histidine tag at the C-terminal end of the PNGase F gene sequence. The recombinant PNGase F HIS-tagged construct (rPNGase F) was transformed into bacterial strain BL21 Star (DE3) that carries the gene for the T7 RNA polymerase under control of the *lac*UV5 promoter and allows for high level isopropyl-beta-D-thiogalactopyranoside (IPTG) inducible expression of gene products from T7 expression vectors such as pET and pQE. Bacterial transformation and cell culture growth conditions were done following manufacturers protocol (Invitrogen). Cells were harvested by centrifugation and cell pellets were washed with 1X phosphate buffered saline (PBS) containing protease inhibitors (Sigma*Fast* EDTA-free). Total cellular protein lysates were made using an Avestin C5 high pressure homogenizer. FPLC purification methods for the rPNGasE F histidine tagged protein used Ni-NTA (Qiagen) and IMAC HisTrap HP (GE Healthcare) columns. Bacterial cell lysate from IPTG induced cultures was loaded onto to the column in binding buffer; 50 mM $NaHPO_4$, 1M NaCl (pH 7.0) and bound histidine tagged rPNGasE F was washed and eluted using an imidazole step gradient (250 mM to 800 mM imidazole) in binding buffer. Purified enzyme was dialyzed and stored in PBS buffer at a concentration of 1 mg/mL, analogous to concentrations found in commercial sources of this enzyme.

Supplementary Methods-2

Analysis of asialofetuin and glycan standards in mouse brain tissues.
A frozen mouse brain was cored with a disposable 1.5 mm tissue coring tool. A quartile of a different brain from a littermate, which contained the same region of tissue cored in the other sample, was homogenized in a glass Dounce homogenizer in 0.5 mL water. The goal was to maintain a highly viscous preparation, which was then supplemented with 0.02 mL of water containing 1mg purified asialofetuin glycoprotein standard. This solution was added back to the cored brain, and refrozen at -80^{o}C. This brain was subsequently prepped for MALDI imaging as described for other brain samples.

On-tissue extraction and tissue homogenization for permethylation of N-glycans. PNGaseF (0.02 mg) sprayed mouse brain tissue slides were incubated for 2 hr at 37°C; 50uL water was applied on top of the tissue and incubated for 20 minutes to remove the released glycans. The water was removed and concentrated under vacuum by centrifugation. Permethylation was performed by dissolving the dried glycans in 0.2 uL H_2O, 22uL iodomethane and 50uL dimethylsulfoxide and applied over a sodium hydroxide microspin column as described[21]. The permethylated glycans were dissolved in 5uL 50% MeOH/water and spotted with DHB (10mg in 1mL 50% MeOH). N-glycans were detected by averaging 10 MALDI scans at 25 um laser width, averaging 1000 shots in each acquired scan between m/z = 690-5000. Masses detected in the permethylation experiments were loaded into DataAnalysis 4.0 and searched against the permethylated glycan database provided by the Consortium for Functional Glycomics[24] (see Figure S-2b and S-3a).

Normal Phase HPLC. Tissue extracted PNGaseF released N-glycans (as above) were prepared for rapid glycan sequencing using previously optimized procedures[22,23]. Briefly, the concentrated N-glycans were labeled with 2-aminobenzamide (2-AB) for subsequent normal phase HPLC analysis[23]. Glycan identification was made through sequential exoglycosidase digestion as previously described by Guile et al.[23], as well as bovine kidney fucosidase. The resulting peaks, separated by time of appearance, correspond to specific glycan structures on the basis of glucose unit values (data not shown). All HPLC analyses were performed using a Waters Alliance HPLC System and quantified using the Millennium Chromatography Manager (Waters Corporation, Milford, MA). Glycan structures were identified by the calculation of the glucose unit value, as previously described, as well as through the comparison to known standards and sequential exoglycosidase digestion[22,23] (see Figure S2a).

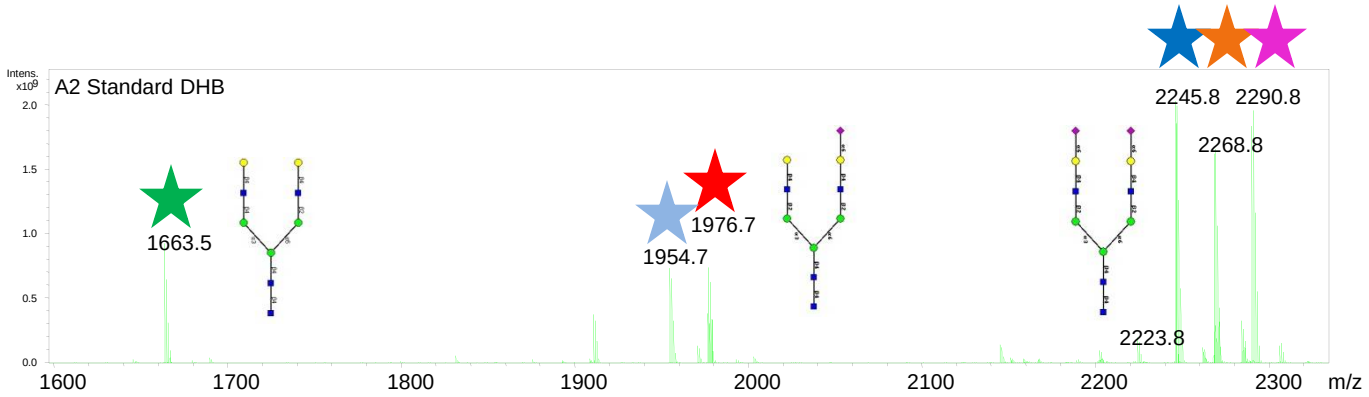
Literature Citations and Supplementary Figure 1 Legend

References:

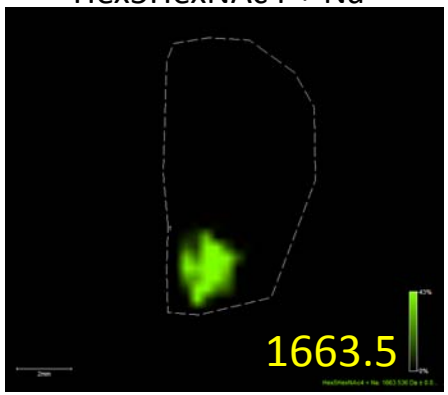
21. Mechref, Y.; Kang, P.; Novotny, M.V. Methods Mol. Biol. 2009, 534, 53-64.
22. Mehta, A.; Carrouee, S.; Conyers, B.; Jordan, R.; Butters, T.; Dwek, R.A.; Block, T.M. Hepatology. 2001, 33, 1488-1495.
23. Guile, G. R.; Rudd, P. M.; Wing, D. R.; Prime, S. B.; Dwek, R.A. Anal. Biochem. 1996, 240, 210-226.
24. Consortium for Functional Glycomics; http://www.functionalglycomics.org

Figure S-1. Native N-glycan Standards Detected by MALDI-IMS. An A2 Standard (Hex5HexNAc4NeuAc2; 0.1 ug) was spotted directly on a ground steel plate (a.), or 0.1 ug/ul standard was spotted onto a mouse brain tissue slice prior to matrix application (**b-g**). The MALDI mass spectrum demonstrates the spectral complexity of non-derivatized native sialic acid N-glycans due to loss of sialic acid residues and different numbers of sodium adducts. Representative images correspond to the ions detected in the mass spectrum localized to the tissue area where they were spotted (b-g). The glycan composition and number of sodium adducts per analyte are indicated above each image panel. The colored stars in the spectra correspond to the colors in the image panel.

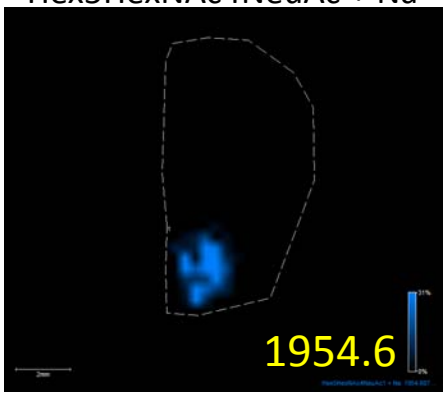
Supplemental
Figure S-1



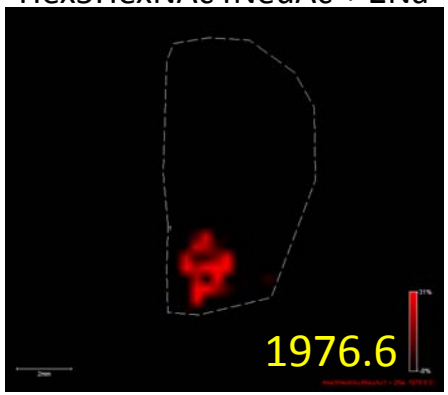
Hex5HexNAc4 + Na



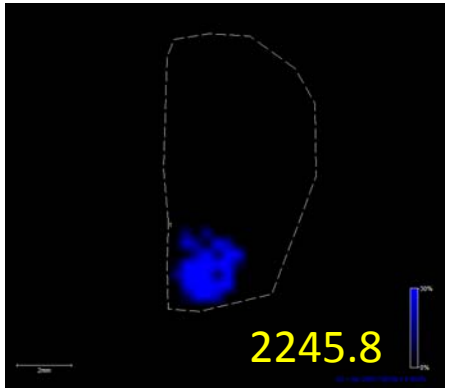
Hex5HexNAc4NeuAc + Na



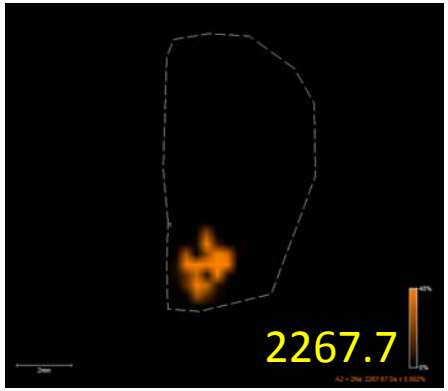
Hex5HexNAc4NeuAc + 2Na



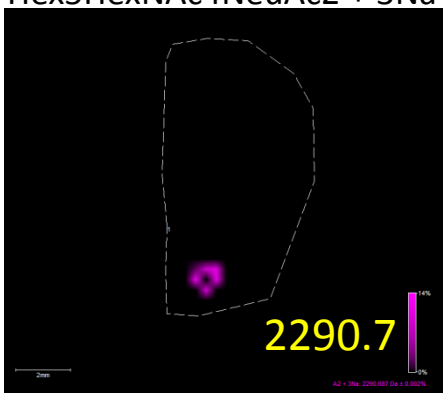
Hex5HexNAc4NeuAc2 + 1Na



Hex5HexNAc4NeuAc2 + 2Na

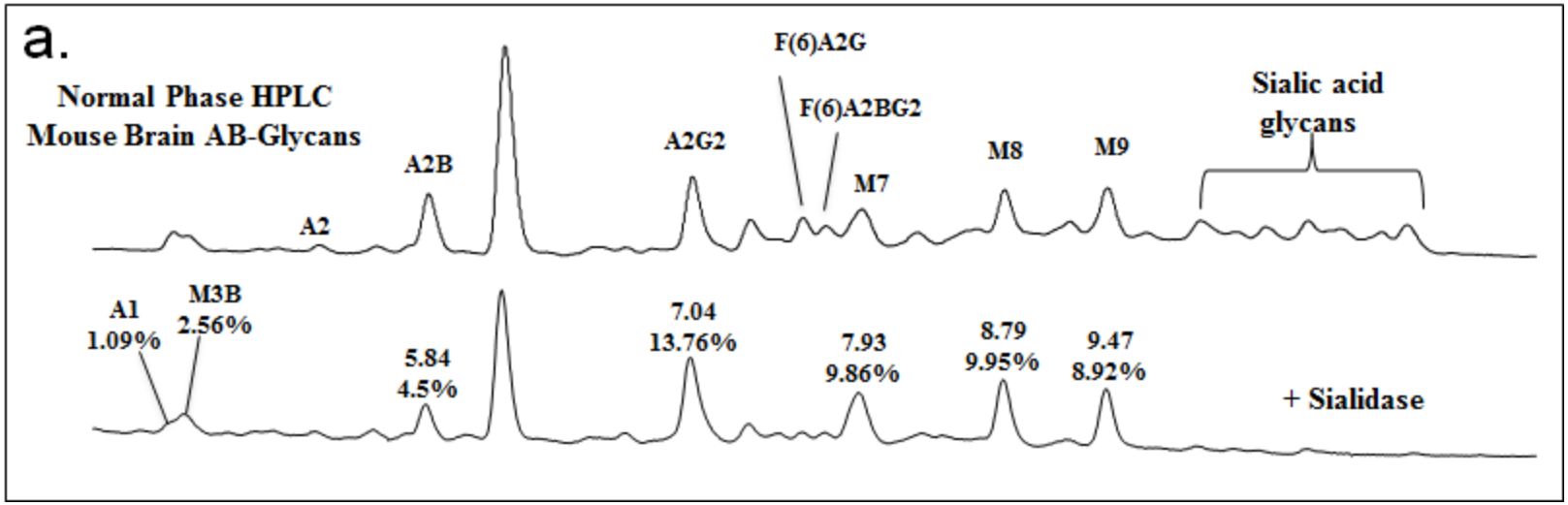


Hex5HexNAc4NeuAc2 + 3Na



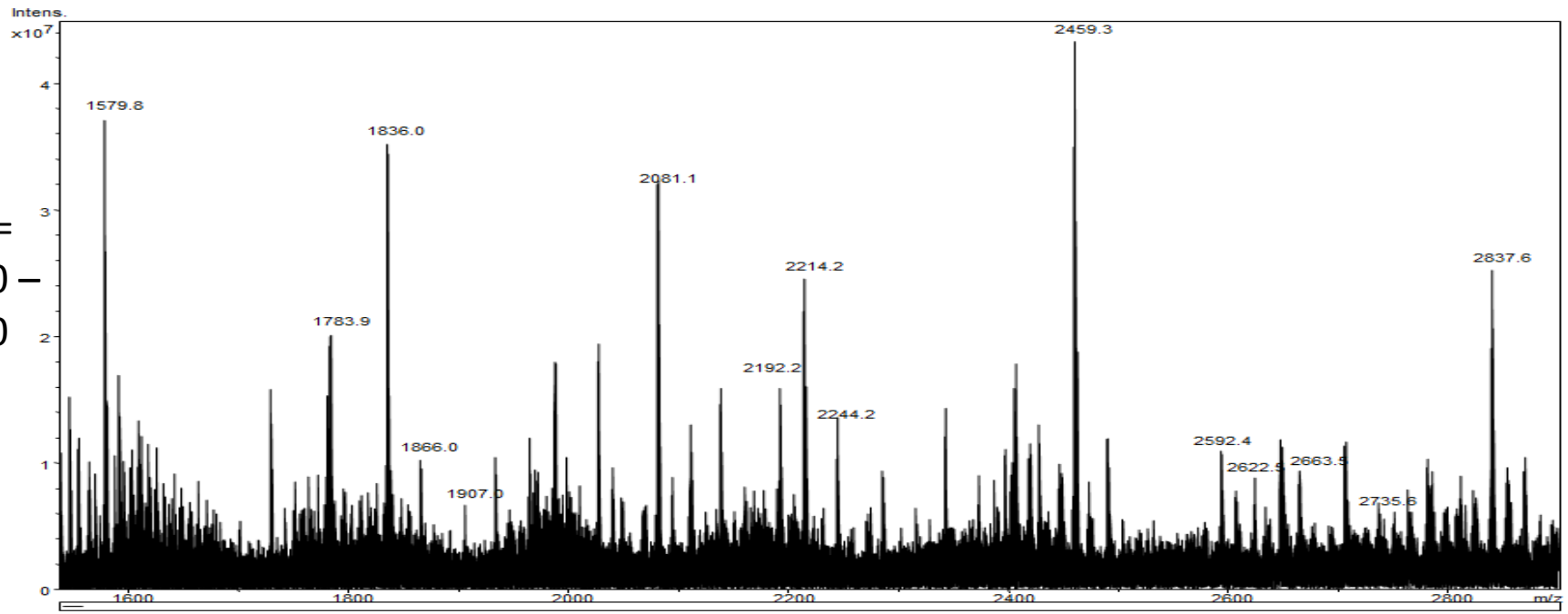
Supplemental Figure S-2. Off-tissue Mouse Brain Tissue PNGaseF Digestions. Mouse brain tissues (10 um slices) were digested on-slide with PNGaseF, extracted with water then concentrated for **A.** derivation with 2-aminobenzoate (2AB) and separated by normal phase HPLC. Sialidase digestion was done in solution with the 2AB modified glycans, prior to HPLC separation. The structure key for indicated normal phase peaks in panel A below. **B.** Permethylation analysis of the same water extract, with MALDI spectra split into two overlapping mass ranges: (top panel) m/z = 1550-2900; (bottom panel) m/z = 2700-4550

A1 – Hex3HexNAc3
A2 – Hex3HexNAc4
A2G2 – Hex5HexNAc4
F(6)A2BG2- Hex5dHex1HexNAc5
M8 - Hex8HexNAc2
M3B – Hex5HexNAc3
A2B – Hex3HexNAc5
F(6)A2G – Hex4dHex1HexNAc4
M7 - Hex7HexNAc2
M9 - Hex9HexNAc2

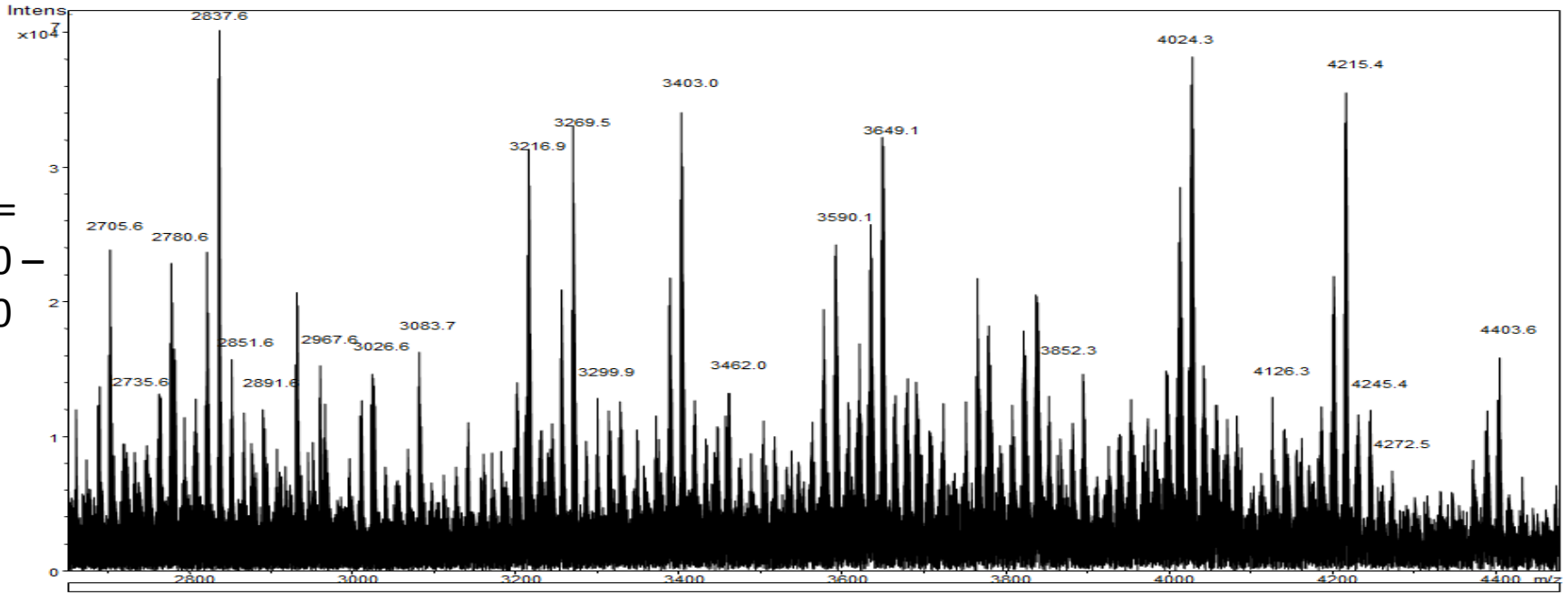


Supplemental Figure S-2b; Permethylated Mouse Brain Glycans

b.



M/z= 1550 – 2900

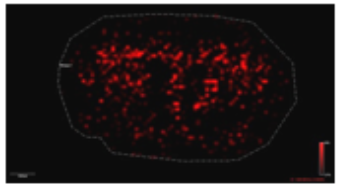


M/z= 2700 – 4450

Supplemental Figure S-3. Correlative Table of Individual Glycan Masses, Structures and Brain Image Distribution – m/z = 1500-2650 S-4a; m/z = 2660-4050 S-4b. Listed for each glycan detected by MALDI-IMS of mouse brain slices is the glycan composition, the ion distribution image, the predicted permethylated mass determined from MALDI-MS mouse brain data in the Consortium for Functional Glycomics database, the observed (detected) permethylated mass after the off-tissue extraction, the predicted native mass, the predicted mass and the observed (detected) native mass in the MALDI-IMS analysis. Sodium adducts are also included, where applicable. Indicated images and masses are representative of MALDI-IMS mouse brain glycan imaging of 5 independent mouse brains and 18 respective imaging analyses.

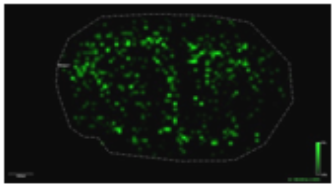
Supplemental Figure S-3a

Hex5HexNAc2 + Na



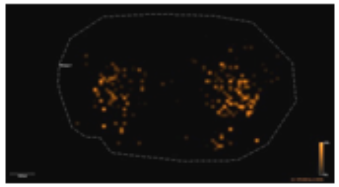
Permeth DB: 1580
Observed Permeth: 1579.8
Native DB: 1235.1
Native DB +Na: 1257.1
Observed Native:1256.6

Hex6HexNAc2



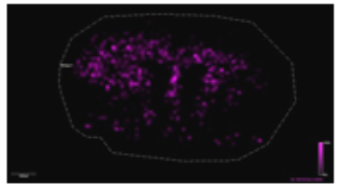
Permeth DB: 1784
Observed Permeth: 1783.9
Native DB: 1397.25
Observed Native: 1398.7

Hex4HexNAc4



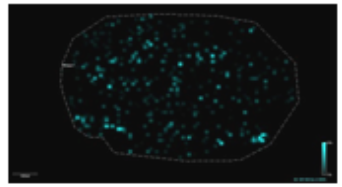
Permeth DB: 1866
Observed Permeth: 1866.1
Native DB: 1479.35
Observed Native: 1479.8

Hex3HexNAc5 + Na



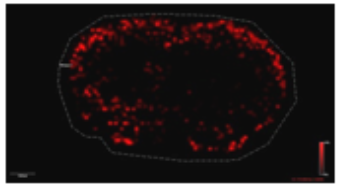
Permeth DB: 1907
Observed Permeth: 1907.0
Native DB: 1520.4
Native DB + Na 1542.4
Observed Native: 1543.8

Hex4dHex1HexNAc4 + Na



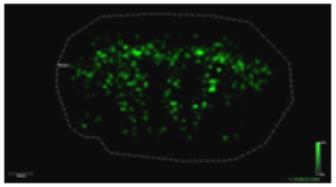
Permeth DB: 2040
Observed Permeth: 2040.1
Native DB: 1625.5
Native DB + Na: 1647.5
Observed Native: 1647.9

Hex3dHex1HexNAc5 + 2Na



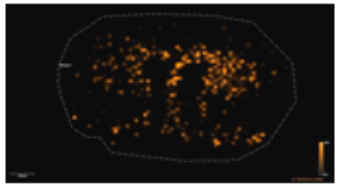
Permeth DB: 2081
Observed Permeth: 2081.0
Native DB: 1666.5
Native DB + 2Na: 1710.5
Observed Native: 1710.9

Hex8HexNAc2 + Na



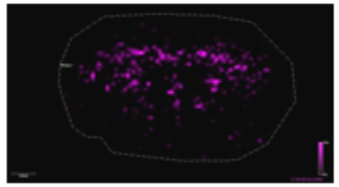
Permeth DB: 2192
Observed Permeth: 2192.1
Native DB: 1721.5
Native DB + Na: 1743.5
Observed Native: 1743.9

Hex8HexNAc2 + 2Na



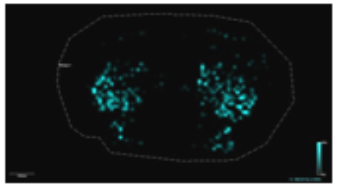
Permeth DB: 2192
Observed Permeth: 2192.1
Native DB: 1721.5
Native DB + 2Na: 1765.5
Observed Native 1766.8

Hex4dHex2HexNAc4 + 2Na



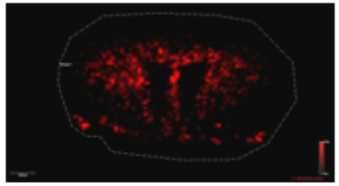
Permeth DB: 2214
Observed Permeth: 2214.1
Native DB: 1771.6
Native DB + 2Na: 1815.6
Observed Native: 1814.9

Hex5dHex1HexNAc4 + Na



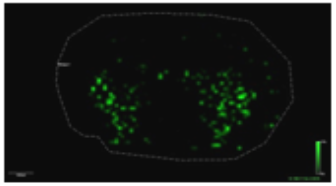
Permeth DB: 2244
Observed Permeth: 2244.2
Native DB: 1787.6
Native DB + Na: 1809.6
Observed Native: 1807.0

Hex5dHex2HexNAc4 + Na



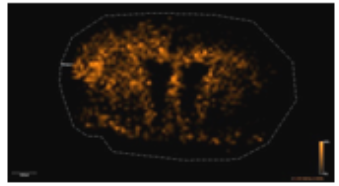
Permeth DB: 2418
Observed Permeth: 2418.3
Native DB: 1933.8
Native DB + Na: 1955.8
Observed Native: 1953.9

Hex4dHex2HexNAc5 + Na



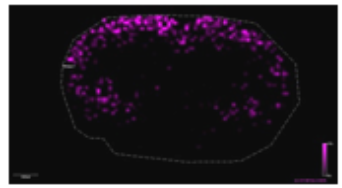
Permeth DB: 2459
Observed Permeth: 2459.3
Native DB: 1974.83
Native DB + Na: 1996.8
Observed Native : 1996.1

Hex5dHex3HexNAc4 + Na



Permeth DB: 2592
Observed Permeth: 2592.4
Native DB: 2079.9
Native DB + Na: 2101.9
Observed Native: 2101.1

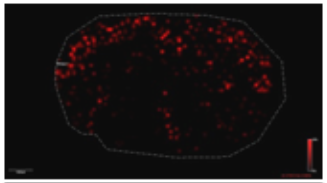
Hex6dHex2HexNAc4 + Na



Permeth DB: 2622
Observed Permeth: 2622.5
Native DB: 2095.9
Native DB + Na: 2117.9
Observed Native: 2118.0

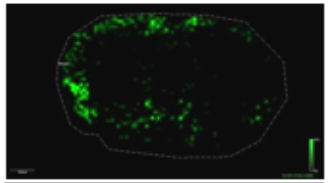
Supplemental Figure S-3b

Hex5dHex2HexNAc5 + 2Na



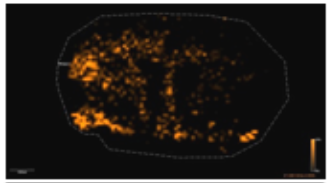
Permeth DB: 2663
Observed Permeth: 2663.5
Native DB: 2137
Native DB + 2Na: 2181.0
Observed Native: 2180.1

Hex5dHex1HexNAc6 + Na



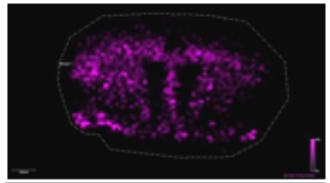
Permeth DB: 2735
Observed Permeth: 2735.6
Native DB: 2194.0
Native DB + Na: 2216.0
Observed Native: 2216.2

Hex5dHex1NeuAc1
HexNAc5 + Na



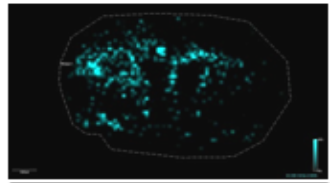
Permeth DB: 2851
Observed Permeth: 2851.4
Native DB: 2282.1
Native DB + Na: 2304.1
Observed Native: 2304.1

Hex5dHex3HexNAc5 + Na



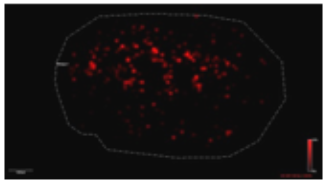
Permeth DB: 2837.1
Observed Permeth: 2837.6
Native DB: 2283.1
Native DB + Na: 2305.1
Observed Native: 2306.1

Hex5dHex1NeuAc1
HexNAc6 + Na



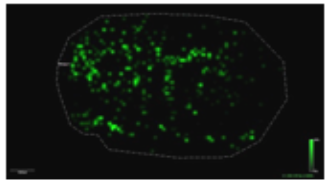
Permeth DB: 2892
Observed Permeth: 2891.6
Native DB: 2323.1
Native DB + Na: 2345.1
Observed Native: 2345.1

Hex5dHex1NeuAc1
HexNAc6 + 2Na



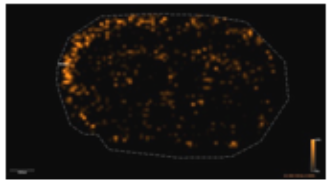
Permeth DB: 2892
Observed Permeth: 2891.5
Native DB: 2367.1
Native DB + 2Na: 2366.2
Observed Native: 2366.2

Hex5dHex1NeuAc2
HexNAc4 + 3Na



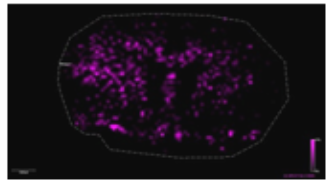
Permeth DB: 2967
Observed Permeth: 2967.6
Native DB: 2370.1
Native DB + Na: 2436.1
Observed Native: 2432.1

Hex5dHex1NeuGc2
HexNAc4 + Na



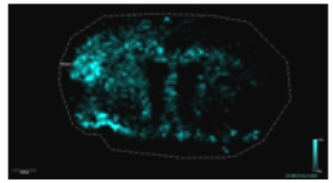
Permeth DB: 3027
Observed Permeth: 3026.6
Native DB: 2402.2
Native DB + Na: 2424.2
Observed Native: 2421.2

Hex5dHex2NeuAc1
HexNAc6 + 2Na



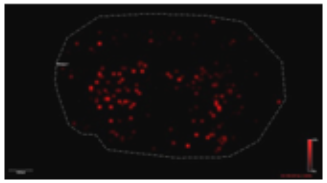
Permeth DB: 3270
Observed Permeth: 3269.5
Native DB: 2631.4
Native DB + 2Na: 2675.4
Observed Native: 2675.3

Hex7NeuAc1HexNAc6



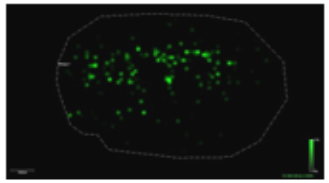
PermethDB: 3300
Observed Permeth: 3299.8
Native DB: 2663.5
Observed Native: 2661.5

Hex6dHex3NeuAc1
HexNAc5 + 2Na



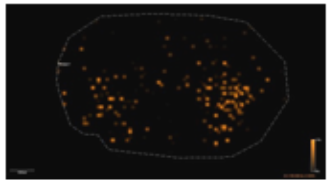
Permeth DB: 3403
Observed Permeth: 3402.2
Native DB: 2736.5
Native DB + 2Na: 2780.5
Observed Native: 2780.3

Hex6dHex2NeuAc2
HexNAc5 + 2Na



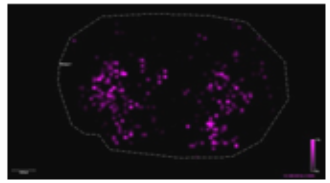
Permeth DB: 3590
Observed Permeth: 3589.9
Native DB: 2881.6
Native DB + 2Na: 2925.6
Observed Native: 2924.4

Hex7dHex3NeuAc1
HexNAc6 + 2Na



Permeth DB: 3852
Observed Permeth: 3852.1
Native DB: 3101.8
Native DB + Na: 3145..8
Observed Native: 3146.6

Hex7dHex4NeuAc1
HexNAc6 + Na



Permeth DB: 4027
Observed Permeth: 4026.5
Native DB: 3248.0
Native DB + Na: 3270.0
Observed Native: 3269.4

Supplemental Figure S-4. Native glycan spectra plus and minus PNGaseF of human kidney. Normal human kidney slices were digested on-tissue and processed for MALDI-IMS. Shown are the averaged spectra of a representative region of interest, processed in FlexImaging 4.0. Representative monoisotopic ion peaks were determined from the averaged image spectra and manually annotated to the spectra profiles below. Top panel, native analytes from non-PNGaseF treated tissue; Bottom panel, native analytes and released N-glycans (in blue font) in PNGaseF treated tissues. Peaks with colored stars match the images shown in Figure 5.