Supplementary Information (SI) for Analyst.

Deep Profiling of Porcine Brain Gangliosides Enabled by TiO_2 Magnetic Nanoparticle-Based Enrichment and Advanced Lipidomic Analysis

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SUPPORTING INFORMATION

Methods

Materials and chemicals

HPLC-grade acetonitrile (ACN), methanol (MeOH), isopropanol (IPA), and deionized water were supplied by Fisher Scientific (Ottawa, ON, Canada). Anhydrous ethanol, ethylene glycol, chloroform, and ammonium hydroxide were obtained from Guoyao Chemical Reagent Co., Ltd. (Shanghai, China). Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was purchased from 3A Materials Technology Co., Ltd. Titanium(IV) butoxide, ammonium acetate, acetic acid, and formic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA). N,N-Dimethylformamide (DMF) was supplied by Aladdin Industrial Corporation (Shanghai, China). Hydrophobic polytetrafluoroethylene (PTFE) membranes (pore size 0.22 μm, diameter 13 mm) were obtained from Welch Materials, Inc. (Jinhua, Zhejiang, China).

Synthesis of TiO_2 MNPs

The synthesis of TiO_2 MNPs was adapted from a previously reported procedure[1]. Magnetic Fe_3O_4 cores were first prepared by dissolving 7.2 g sodium acetate and 2.7 g $FeCl_3 \cdot 6H_2O$ in 150 mL ethylene glycol under stirring. The homogeneous solution was transferred into a Teflon-lined autoclave and maintained at 200 °C for 16 h. The resulting Fe_3O_4 nanoparticles were isolated, rinsed thoroughly with deionized water and ethanol, and dried at 50 °C.

Subsequently, 160 mg of the obtained Fe_3O_4 nanoparticles were dispersed in 395 mL ethanol via ultrasonication for 60 min. Following the addition of 1.44 mL ammonia solution (28 wt%), a mixture of 3.2 mL titanium(IV) butoxide and 5 mL ethanol was introduced dropwise into the suspension. The reaction proceeded at 45 °C for 24 h. The resulting TiO_2-coated particles were washed sequentially with water and ethanol, dried under vacuum, calcined at 400 °C for 2 h, and finally stored at room temperature.

Supplementary Figures

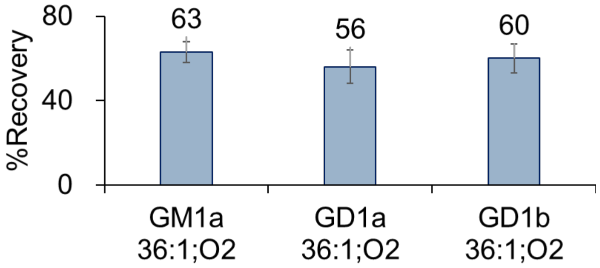

Fig. S1. %Recovery from 100 µg of porcine brain total lipids after enrichment using 800 µg of TiO_2 MNPs.

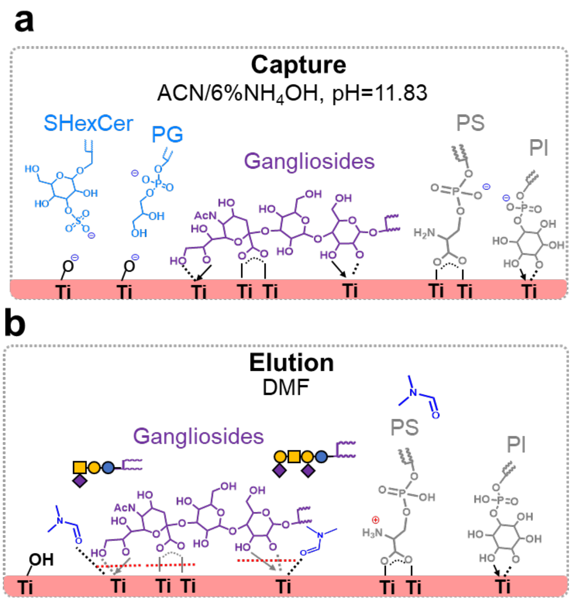

Fig. S2. Schematic illustration of the lipid enrichment workflow using TiO_2 MNPs. (a) Capture and (b) elution processes.

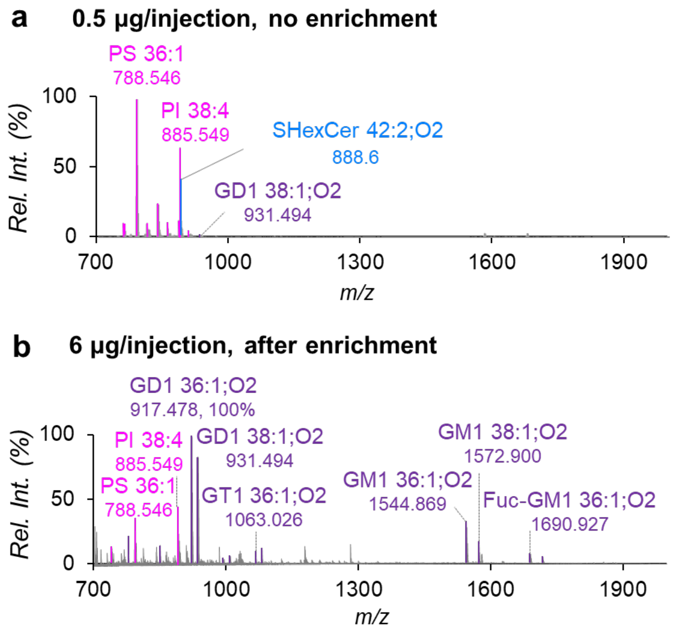

Fig. S3. Negative ion mode MS^1 spectra of gangliosides acquired via amide-HILIC (recorded over retention time of 0–12 min) from (a) 0.5 µg of porcine brain total lipids per injection before enrichment, and (b) 6 µg of porcine brain total lipids per injection after enrichment.

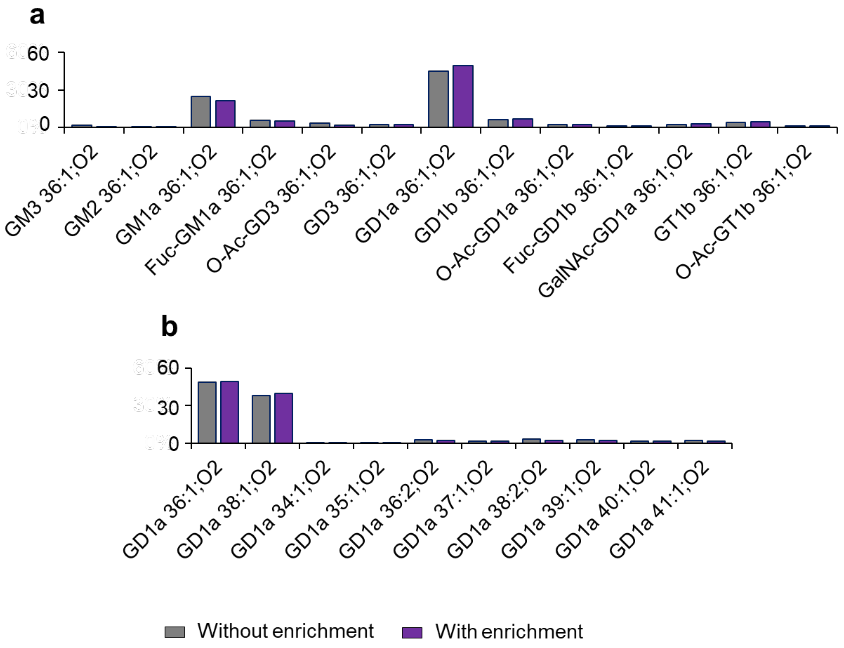

Fig. S4. Comparison of ganglioside distribution profiles. (a) Relative abundance of 13 ganglioside subclasses, with and without enrichment. (b) Relative abundance of 10 GD1a species with different ceramide chain lengths, with and without enrichment.

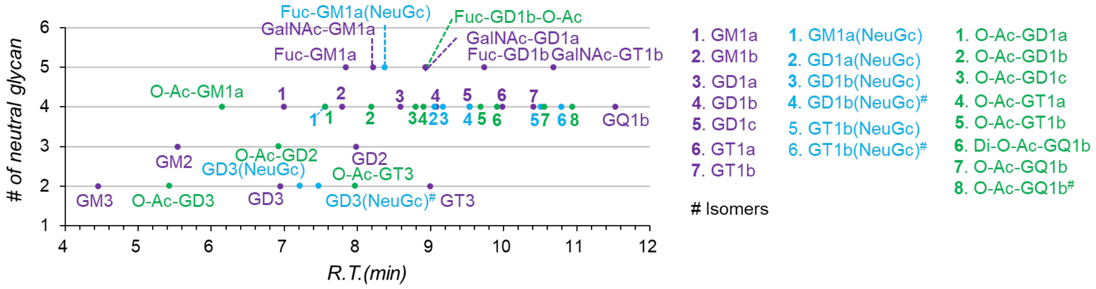

Fig. S5. A plot of the number (#) of neutral glycans vs. retention time (R.T.) from the use of amide HILIC-MS

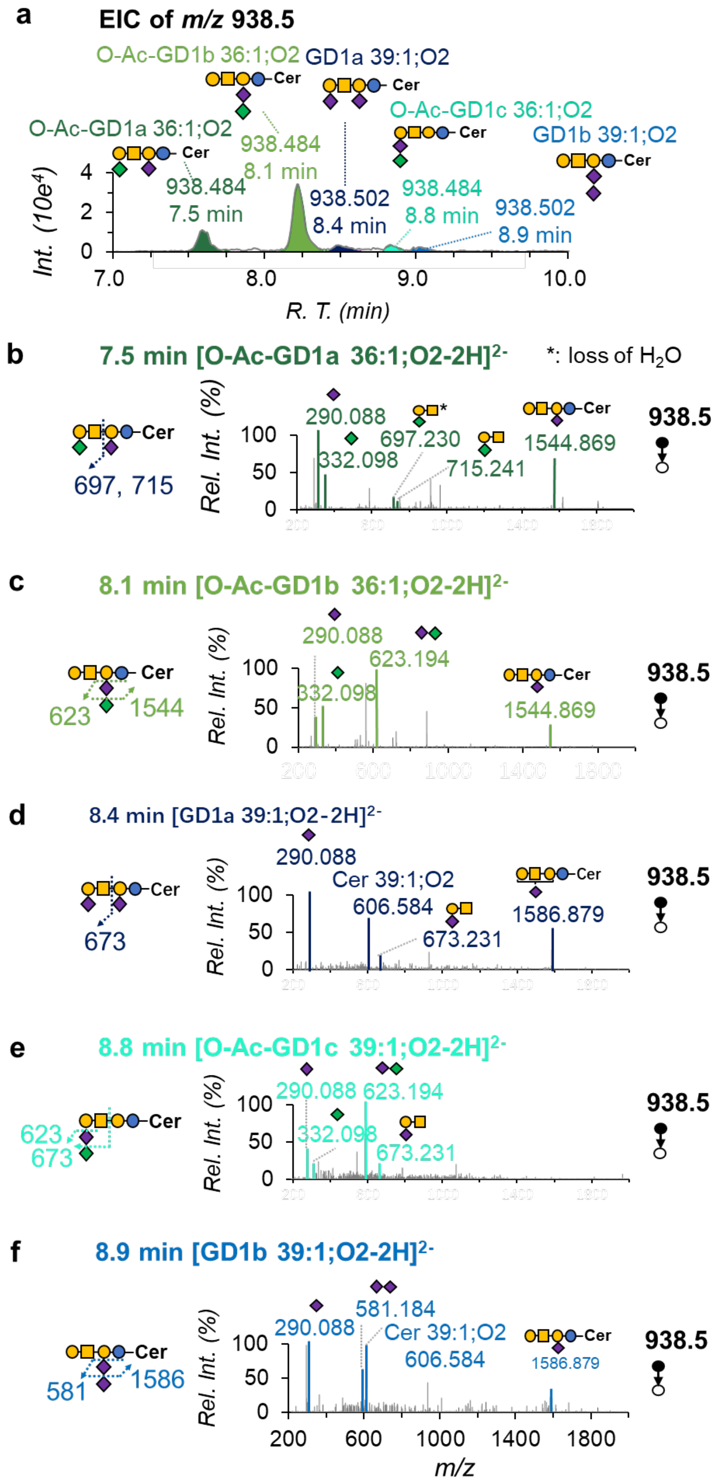

Fig. S6. Separation and identification of glycan isobars caused by NeuAc$_2$. (a) Extracted ion chromatogram (EIC) of *m/z* 938.5, [M - 2H]2-. Negative ion mode MS2 CID spectrum of (b) O-Ac-GD1a 36:1;O2, (c) O-Ac-GD1b 36:1;O2, (d) GD1a 39:1;O2, (e) O-Ac-GD1c 39:1;O2, (f) GD1b 39:1;O2. Characteristic fragment ions used for annotating sialic acid variants and position as well as chain length in ceramide are highlighted.

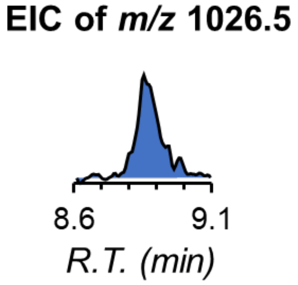

Fig. S7. Extracted ion chromatogram (EIC) of *m/z* 1206.5

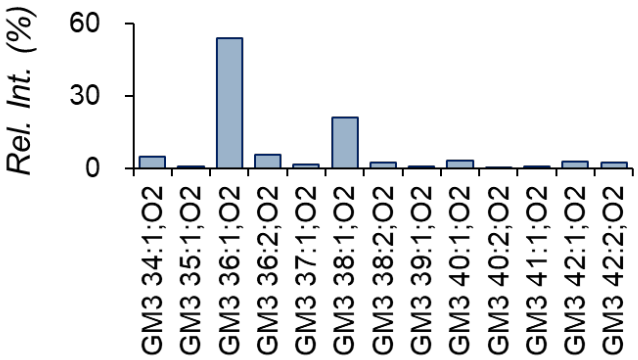

Fig. S8. Distribution of the ceramide chain length in the GM3 subclass from porcine brain

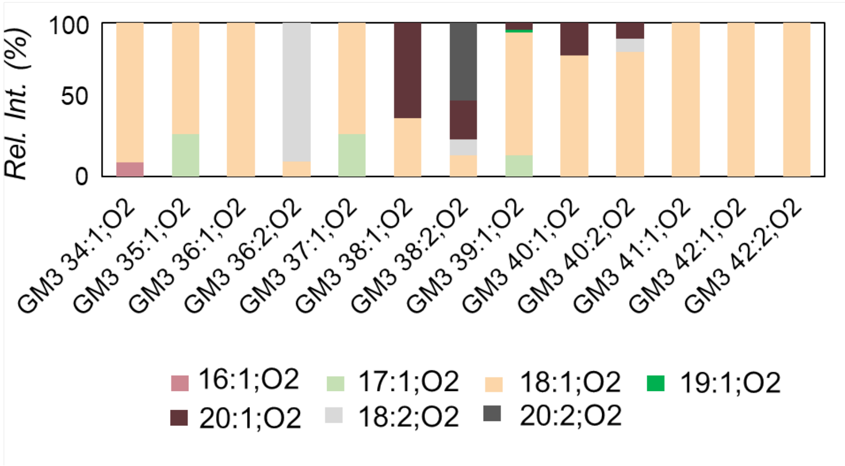

Fig. S9. Distribution of chain composition isomers in the GM3 subclass from porcine brain

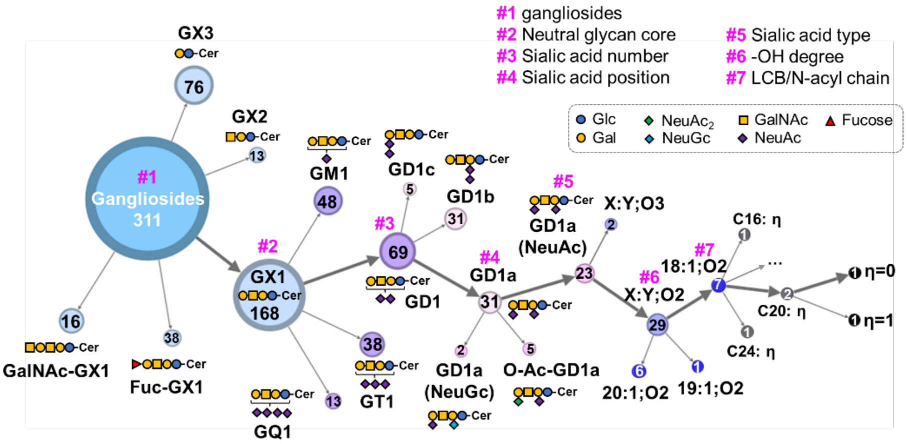

Fig. S10. Structural atlas of gangliosides from porcine brain.

REFERENCES

1. Z. Wang, D. Zhang, J. Wu, W. Zhang and Y. Xia, *Nat. Commun.*, 2024, **15**, 5627.