Supporting Information

Presentation of Membrane-Anchored Glycosphingolipids Determined from Molecular Dynamics Simulations and NMR Paramagnetic Relaxation Rate Enhancement

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Methods

NMR Spectroscpoy. Typical acquisition parameters for the zero-quantum filtered, selective TOCSY experiments were listed in Table S1. The TOCSY mixing time is 30 ms with 4.5 kHz RF field.

Protons	Typical NMR acquisition parameters
a	A g3 pulse with a bandwith of 250 Hz at 5.5 ppm for selective excitation. 1D spectra collected with a spectral width of 10 kHz, 128 scans and 8 s recycle delay.
b, d, h, f	A g3 pulse with a bandwith of 440 Hz at 4.58 ppm for selective excitation. 2D spectra collected with a spectral width of 10 kHz and 460 Hz along direct and indirect dimension respectively, 16 complex points along indirect dimension, 16 scans per t_1 point and 8 s recycle delay.
c, e, i, k	A g3 pulse with a bandwith of 200 Hz at 3.379 ppm for selective excitation. 2D spectra collected with a spectral width of 10 kHz and 600 Hz along direct and indirect dimension respectively, 12 complex points along indirect dimension, 32 scans per t_1 point and 8 s recycle delay.
g	A g3 pulse with a bandwith of 200 Hz at 4.05 ppm for selective excitation. 1D spectra collected with a spectral width of 10 kHz, 128 scans and 8 s recycle delay.
j	A g3 pulse with a bandwith of 200 Hz at 2.62 ppm for selective excitation. 2D spectra collected with a spectral width of 10 kHz and 500 Hz along direct and indirect dimension respectively, 8 complex points along indirect dimension, 8 scans per t_1 point and 8 s recycle delay.

MD simulations. The initial structure for the carbohydrate head group of GM1 was generated using GLYCAM Web.[1] This structure was attached to a ceramide moiety (generated via energy minimization of β-Glc-(1-1)-ceramide structure), as previously described for ganglioside GM3.[2] GM1 was placed in a DMPC bilayer, containing 96 molecules with an area per DMPC molecule of 78.4 $Å^2$ and a bilayer thickness of 41.9 Å, by replacing one DMPC molecule with GM1. The initial insertion depth of GM1 within the DMPC bilayer was set by aligning its hydrophobic (ceramide) and hydrophilic (carbohydrate) regions to the corresponding hydrophobic and hydrophilic domains of the outer-leaflet of the bilayer. GLYCAM06 parameters were used for DMPC and GM1.[2-4] Using the PTRAJ program in AMBER, a 34 Å layer of TIP3P water molecules (with 1 Å spacing) was added to the upper and lower surfaces of the

bilayer, as well as a Na^+ counterion to neutralize the system. The solvent layer contracted to a solvent depth of at least 16 Å from any GM1 or DMPC atom, after the equilibration steps outlined below.

System equilibration was performed using the AMBER 9 version of SANDER.[5] First, water molecules were energy minimized (500 steps of steepest decent, 1500 steps of conjugate gradient) and then subjected to 10 ps of molecular dynamics in the isothermal-isobaric (NPT) ensemble at 1 atm with anisotropic pressure scaling. The entire system was then energy minimized (500 steps of steepest decent, 500 steps of conjugate gradient). Only DMPC molecules were then subjected to 5 ps of MD using the canonical (NVT) ensemble. The entire system was then minimized again for 1000 steps (500 steps of steepest decent, 500 steps of conjugate gradient). In order to adjust the density of the system to correspond with the desired temperature for the production run, the water molecules were subjected to 10 ps of dynamics at 300 K in the NPT ensemble at 1 atm with anisotropic pressure scaling. The entire system was then energy minimized (500 steps of steepest decent, 500 steps of conjugate gradient). Finally, in the NVT ensemble, the entire system was brought to the desired temperature of 300 K over 50 ps. The production MD simulation of GM1 was run for 30 ns at 300 K in the NVT ensemble. Scaling of non-bonded 1-4 van der Waals and electrostatic interactions was not employed (SCEE = SCNB = 1), as is standard when using only GLYCAM force field parameters. A 2 fs time-step was employed throughout for integrating the equations of motion. Hydrogen-containing bonds were constrained with the SHAKE algorithm[6] and long range electrostatics were treated using the particle mesh Ewald method.[7]

In addition, a simulation of the carbohydrate fragment of GM1 was run for comparison. A structure of β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-OH, constructed using GLYCAM Web,[1] was solvated with a pre-equilibrate box of TIP3P waters to a solvent depth of at least 8 Å and a Na^+ counterion was added to neutralize the system. The system was minimized for 10000 steps (5000 steps of steepest decent, 5000 steps of conjugate gradient). With the exception of using the NPT ensemble, the subsequent heating step (50 ps) and production run (20 ns) were run with the same protocol as the GM1 simulation.

Graphics. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).[8]

Results

Table S1. Reference <ASA> for the glycosyl residues of the carbohydrate fragment of GM1 in solution and, for comparison, the comparable values for GM1 bound to a bilayer. Values were determined for water (r_{probe} = 1.4 Å) and Gd(DTPA) (r_{probe} = 4.5 Å) from the average of individual snapshots from the 20 ns simulations. For each snapshot, an ASA was determined using the path traced out by the center of the probe rolling over the surface of the GM1/DMPC system.

	Carbohydrate Fragment <ASA> (Å^2)		Membrane-Bound GM1 <ASA> (Å^2)	
Residue	**Water**	**Gd(DTPA)**	**Water**	**Gd(DTPA)**
Glc	209.45 ± 6.6	380.97 ± 18	52.830 ± 25	6.4644 ± 9.9
Gal	104.00 ± 7.4	113.61 ± 15	40.666 ± 14	2.5130 ± 7.2
GalNAc	192.44 ± 10	284.84 ± 27	155.88 ± 15	132.17 ± 37
Gal'	228.75 ± 8.4	466.50 ± 19	220.31 ± 17	401.11 ± 62
Neu5Ac	338.83 ± 13	592.49 ± 30	283.46 ± 36	257.60 ± 83

Figure S1. Examples of differential PREs of the longitudinal relaxation rates of GM1 protons, with and without the Gd(DTPA) probe. (a) Proton *h,* distant from the membrane surface, showing significant PRE effects in the presence of the probe is compared to (b) proton *b* near the bilayer surface, having little PRE effects. Filled triangles and circles represent the measured values with and without Gd(DTPA), respectively. Lines represent exponential fittings to the experimental data. The quality of the curve-fittings indicates that, in this system, spin diffusion had little effect on the measurements of the longitudinal relaxation rates.

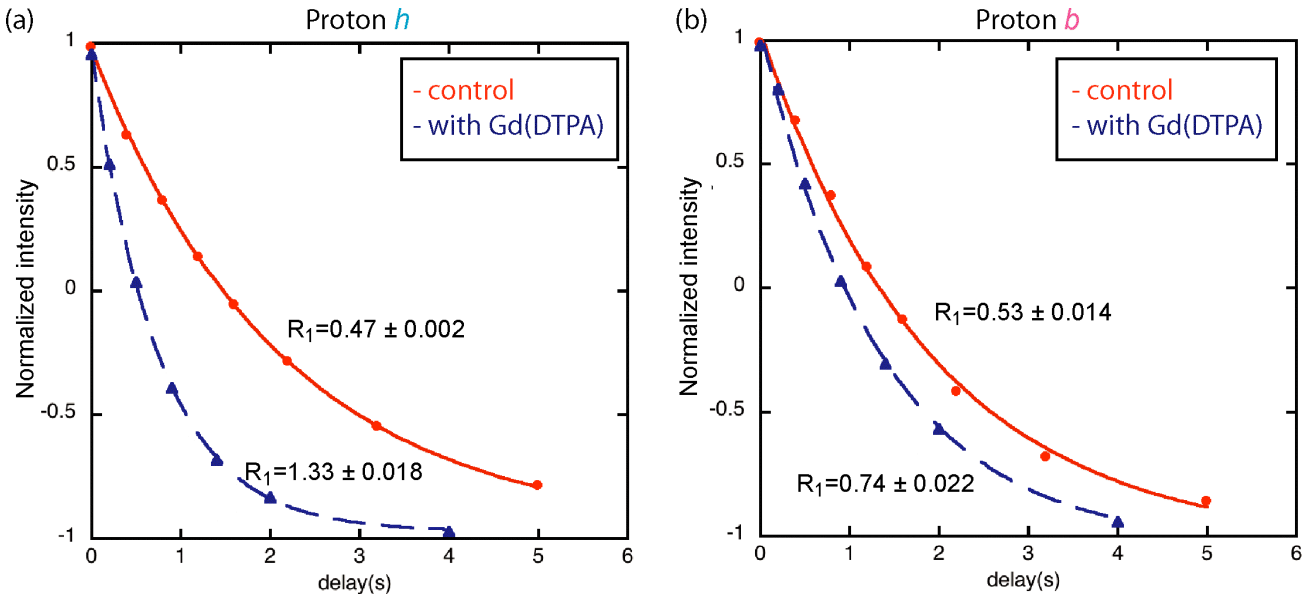
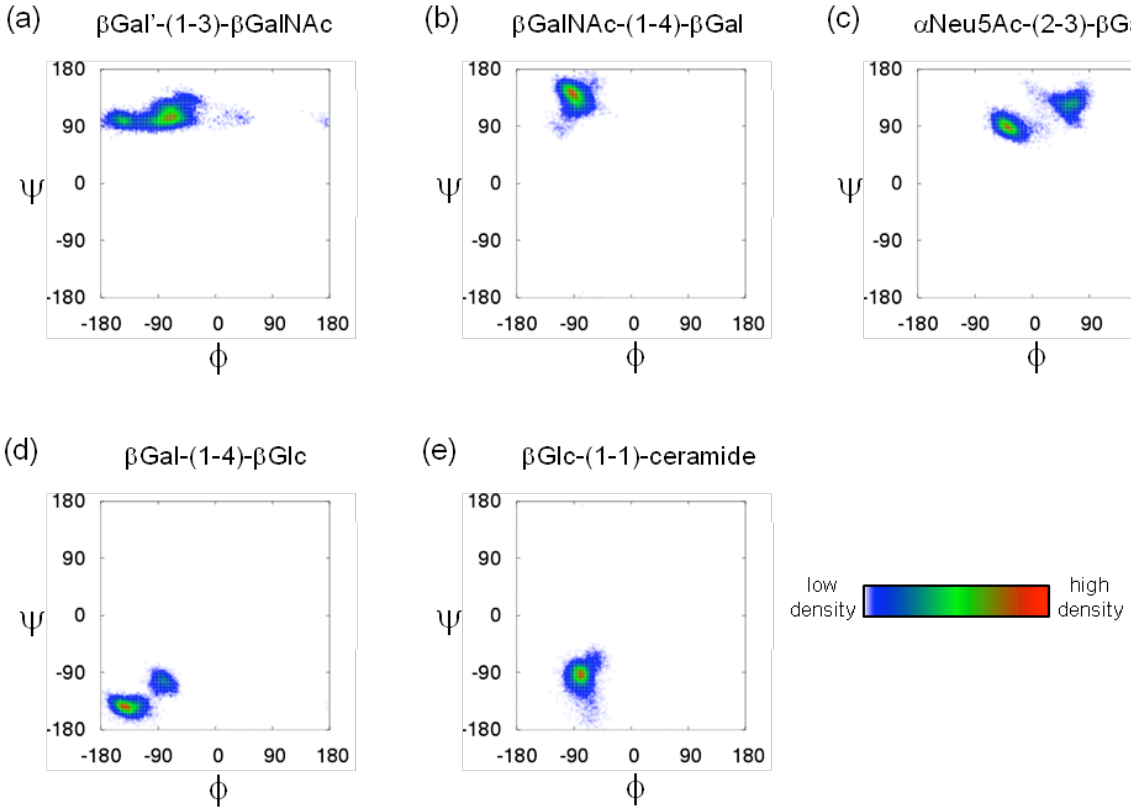


Figure S2. The population density of glycosidic torsion angle pairs (crystallographic definitions: ϕ = $O'_{5/6}$-C'_1-O'_1-C_X and ψ = C'_1-O'_1-C_X-C_{X+1}) from the 20 ns MD simulation of GM1 (β-Gal'-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-ceramide) in a phospholipid bilayer.



References

(1) Woods Group. *GLYCAM Web*. Complex Carbohydrate Research Center, University of Georgia: Athens, GA, 2005-2009.
(2) DeMarco, M. L.; Woods, R. J. *Glycobiology* **2009**, *19*, 344-355.
(3) Kirschner, K. N.; Yongye, A. B.; Tschampel, S. M.; González-Outeiriño, J.; Daniels, C. R.; Foley, B. L.; Woods, R. J. *J. Comput. Chem.* **2008**, *29*, 622-655.
(4) Tessier, M. B.; DeMarco, M. L.; Yongye, A.; Woods, R. J. *Mol. Simul.* **2008**, *34*, 349-364.
(5) Case, D. A.; Cheatham, T. E., 3rd; Darden, T.; Gohlke, H.; Luo, R.; Merz, K. M., Jr.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. J. *J. Comput. Chem.* **2005**, *26*, 1668-88.
(6) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327-341.
(7) Darden, T.; York, D.; Pedersen, L. *J. Chem. Phys.* **1993**, *98*, 10089-10092.
(8) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605-1612.