SUPPORTING INFORMATION

Molecular Dynamics Simulations of Ceramide and Ceramide-Phosphatidylcholine Bilayers

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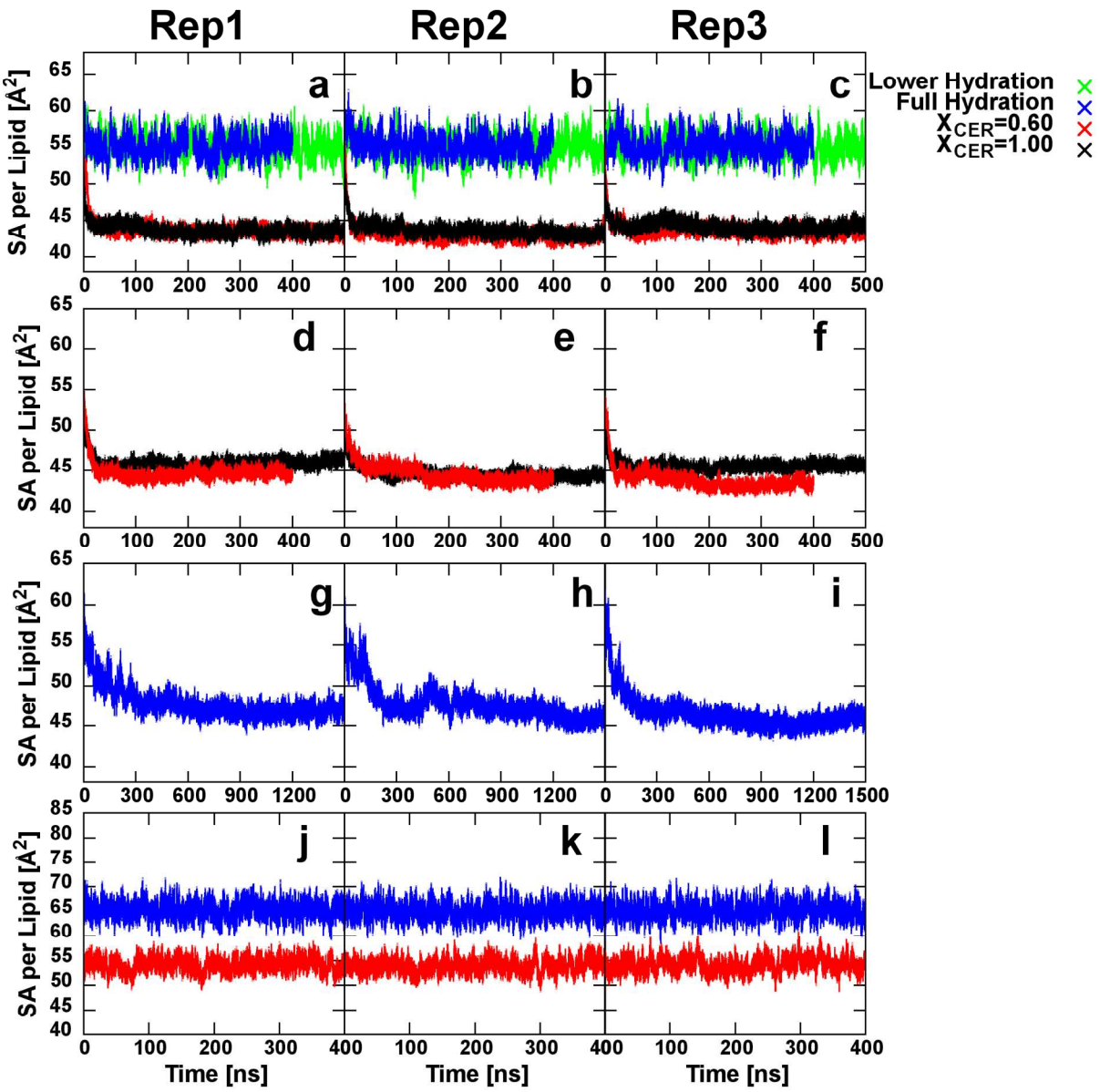

Figure S1. Comparison of overall SA/lip as a function of time for various systems using triplicate replicas for a-c) DMPC/CER16 d-f) DMPC/CER[AP] at X_{CER}=0.60 and pure CER[AP], g-i) DMPC/CER[AP] at X_{CER}=0.14, and j-l) POPC/CER16. X_{CER} concentrations vary among full hydration systems and can be found in Table 1.

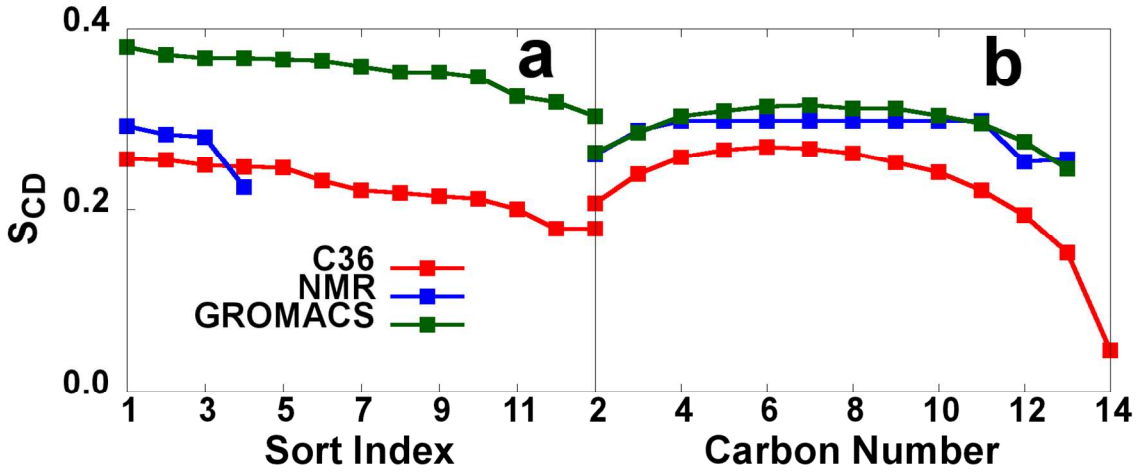

Figure S2. Comparison of S_{CD} between C36, NMR, and GROMOS[1] data for a) CER16 and b) DMPC. Data was taken from DMPC/CER16 at X_{CER}=0.20. Computational values are taken as the average between both chains as done in Dutagaci et al.

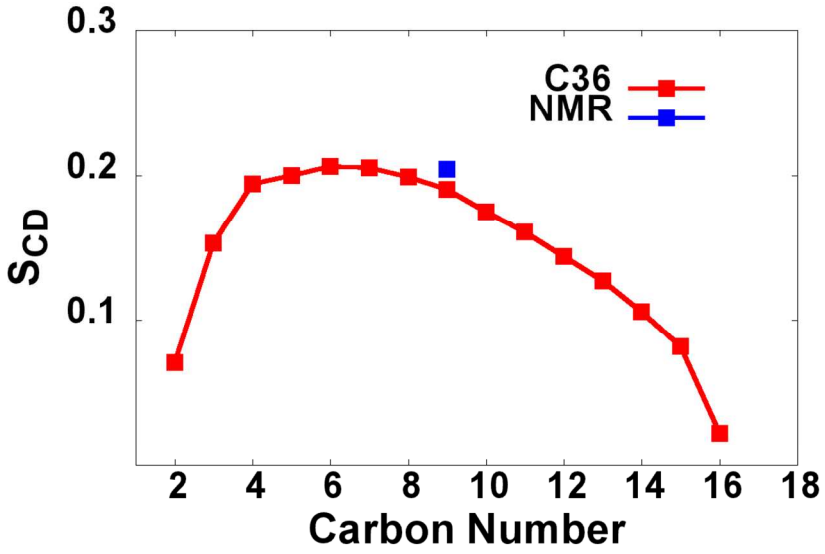

Figure S3. Comparison of S_{CD} between C36 and NMR data[2] for the N-linked chain of CER16 in POPC/CER16 at X_{CER}=0.10.

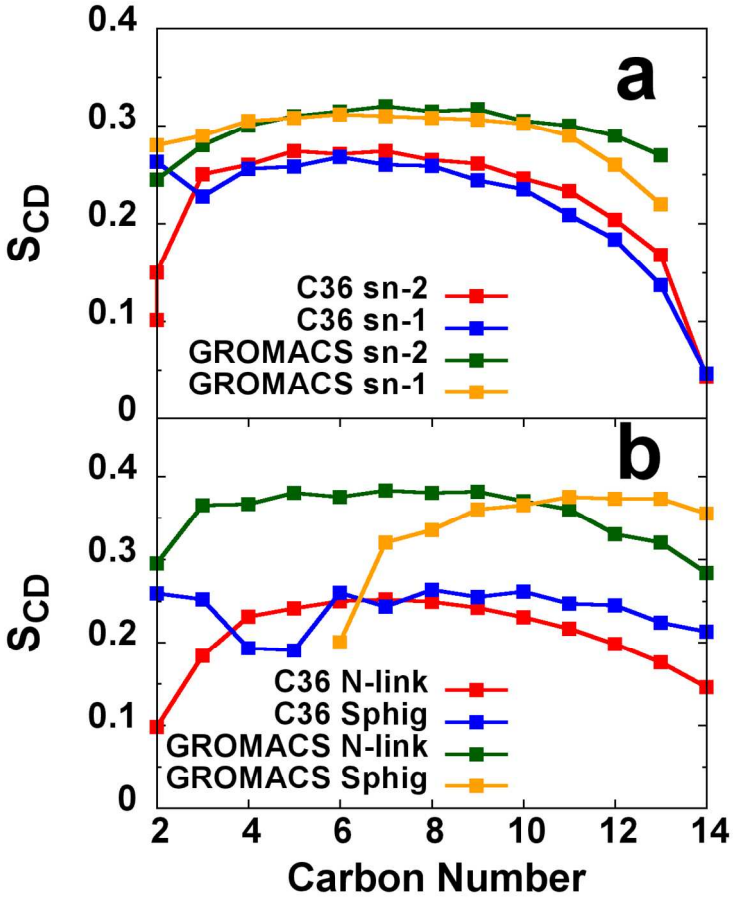

Figure S4. Comparison of S_{CD} between C36 and GROMOS[1] for a) DMPC sn-2 and sn-1 chains and b) CER N-linked and sphingosine (Sphig) chains. Data was taken from DMPC/CER16 at X_{CER}=0.20.

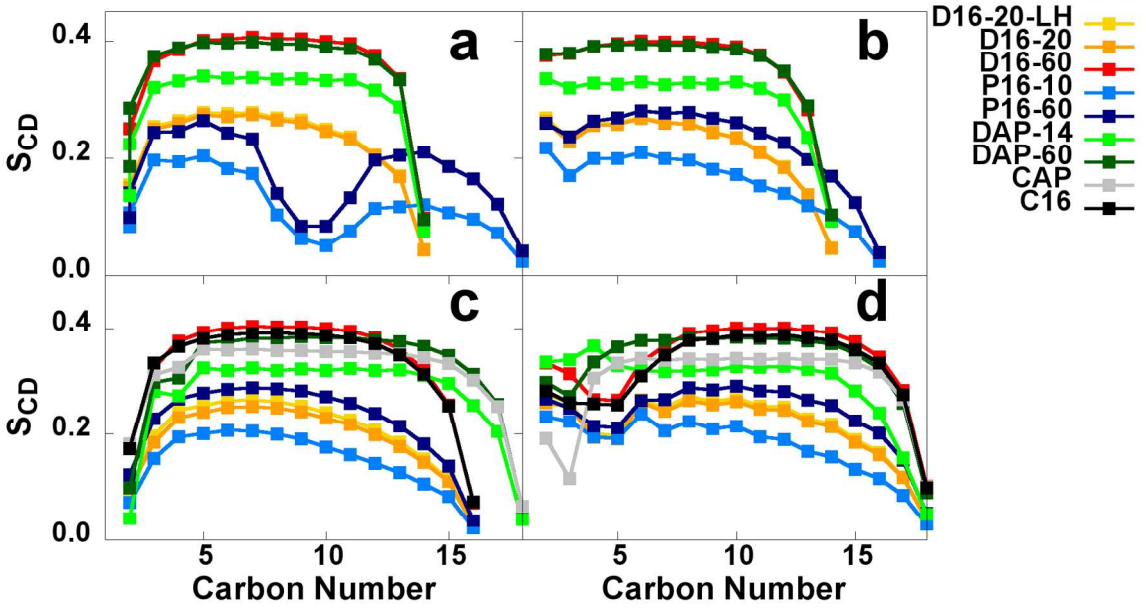

Figure S5. Deuterium order parameters (S_{CD}) of various systems for a) sn-2 chain of PC b) sn-1 chain of PC c) N-linked chain of CER and d) sphingosine chain of CER. Systems represented are DMPC/CER16 at X_{CER}=0.20 and low hydration (D16-20-LH), DMPC/CER16 at X_{CER}=0.20 and full (standard) hydration (D16-20), DMPC/CER16 at X_{CER}=0.60 (D16-60), POPC/CER16 at X_{CER}=0.10 (P16-10), POPC/CER16 at X_{CER}=0.60 (P16-60), DMPC/CER[AP] at X_{CER}=0.14 (DAP-14), DMPC/CER[AP] at X_{CER}=0.60 (DAP-60), CER[AP] (CAP), and CER16 (C16).

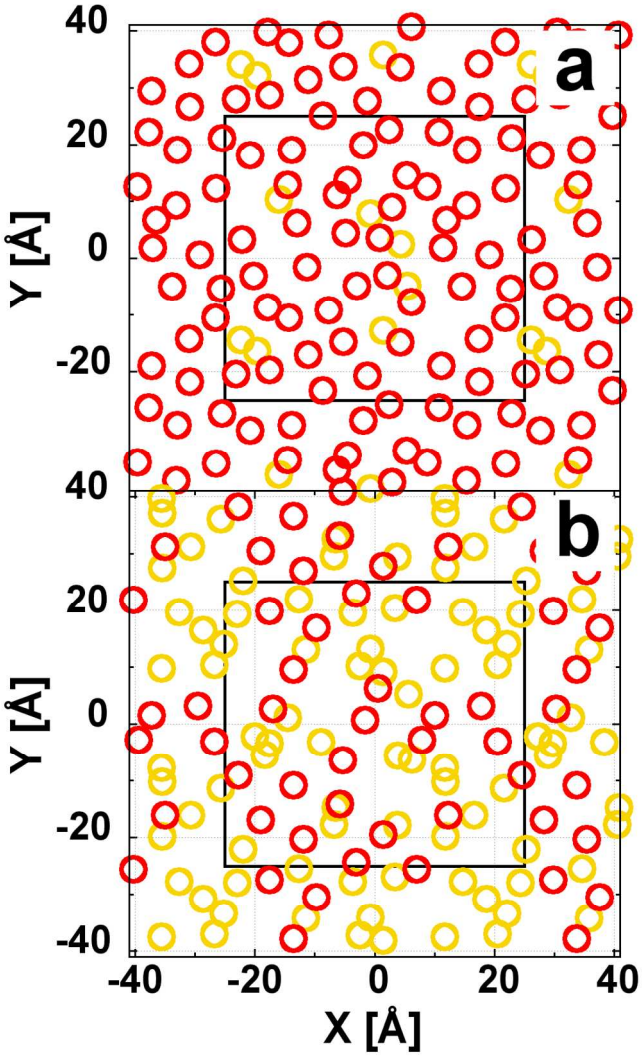

Figure S6. Lateral ordering in a) DMPC/CER[AP] at X_{CER}=0.14 and b) DMPC/CER[AP] at X_{CER}=0.60. Red circles represent DMPC and yellow circles represent CER.

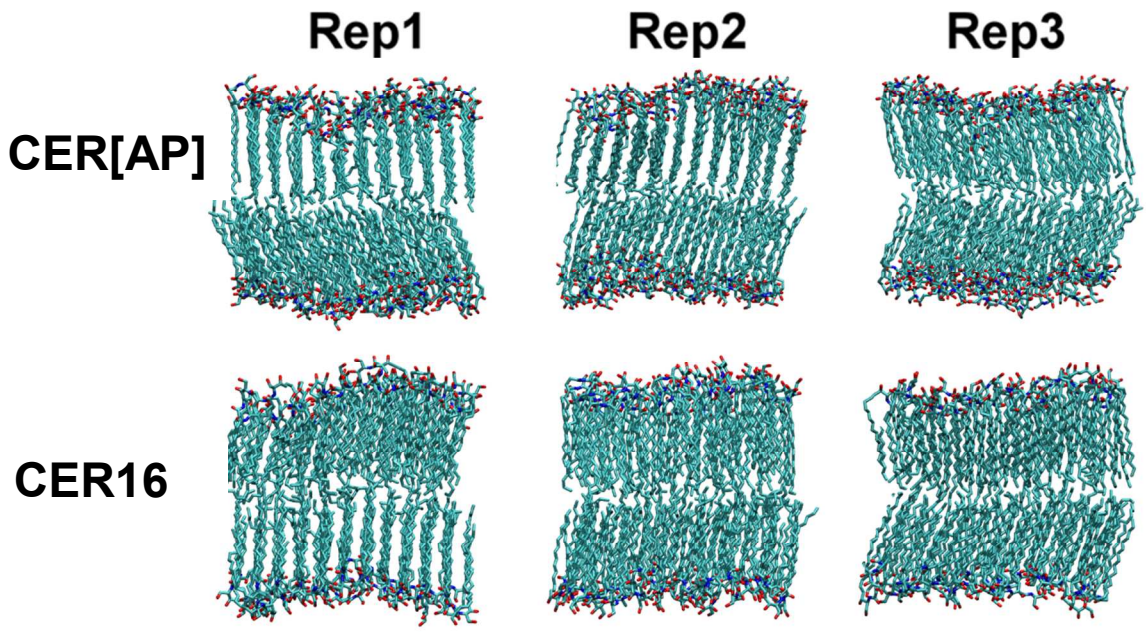

Figure S7. Snapshots of pure CER bilayers taken from the end of simulation.

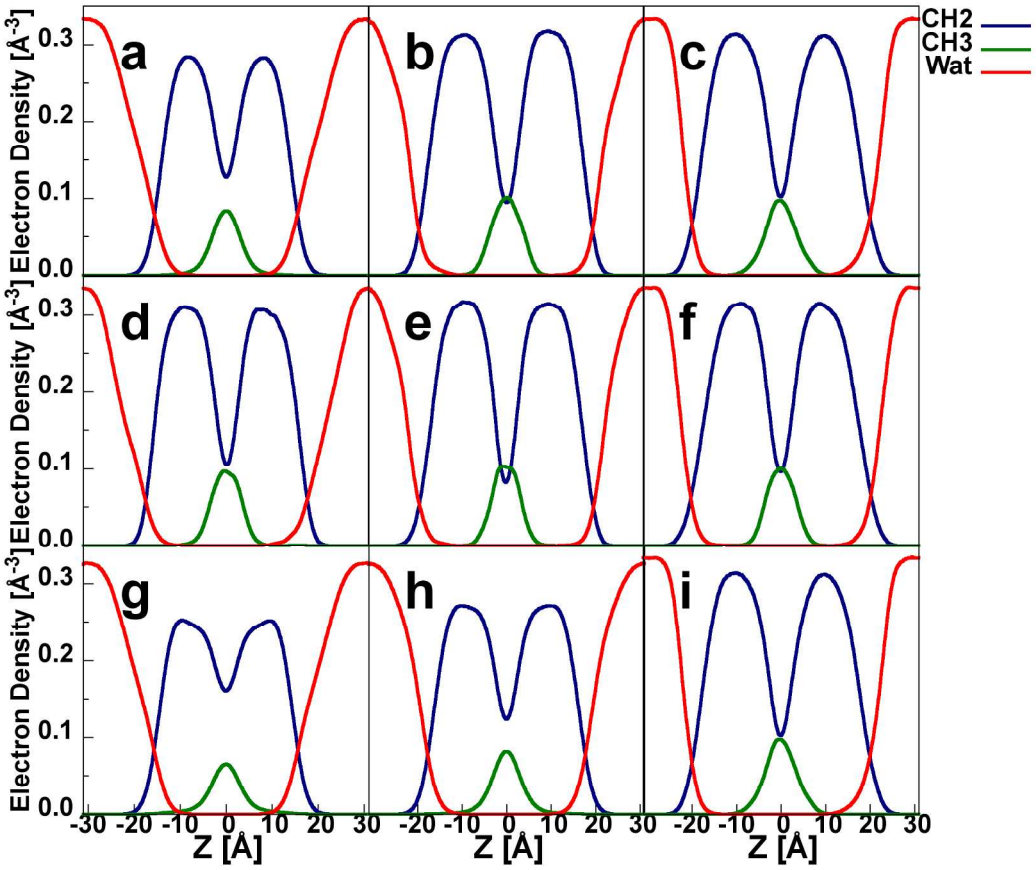

Figure S8. Comparison of component EDPs for a) DMPC/CER16 at X_{CER}=0.20, b) DMPC/CER16 at X_{CER}=0.60, c) pure CER16, d) DMPC/CER[AP] at X_{CER}=0.14, e) DMPC/CER[AP] at X_{CER}=0.60, f) pure CER[AP], g) POPC/CER16 at X_{CER}=0.10, h) POPC/CER16 at X_{CER}=0.60, i) pure CER16. Groups represented are methylene (CH2), methyl (CH3), and water (Wat). Note that c) and i) are identical and are shown twice for simpler comparison against PC/CER mixtures.

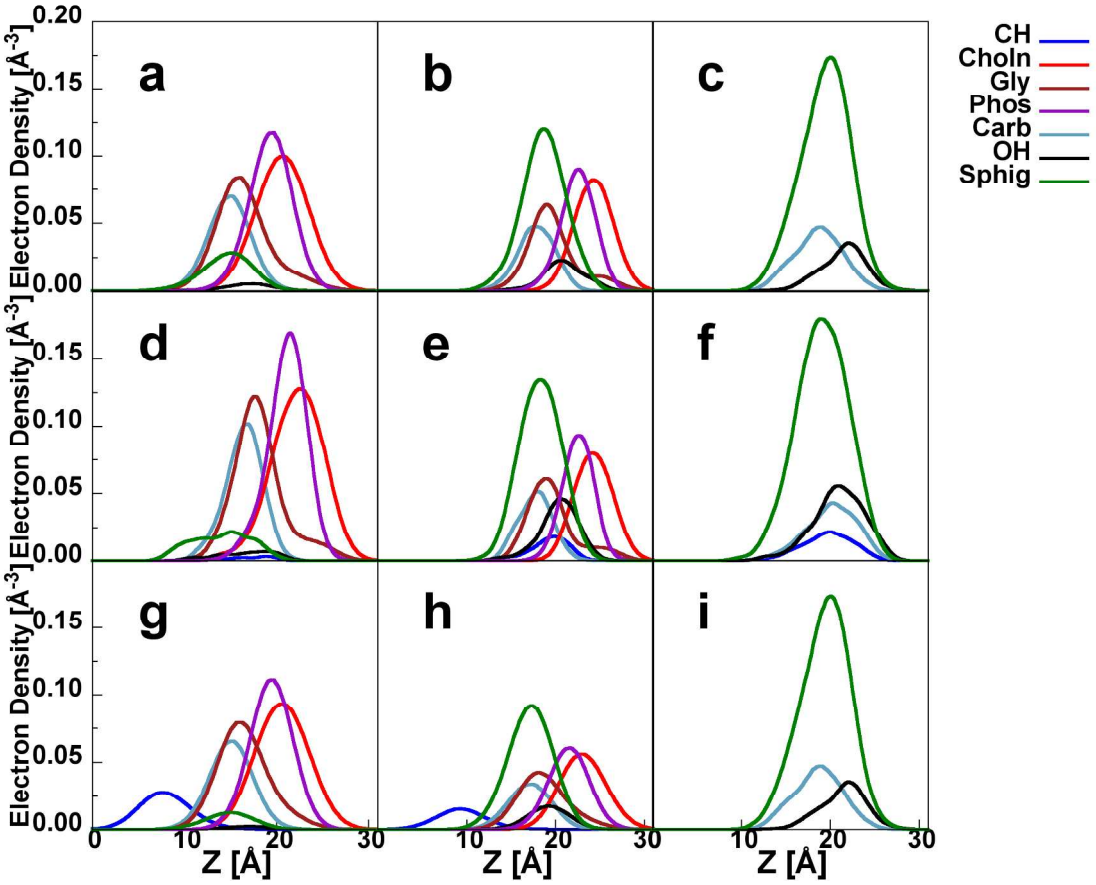

Figure S9. Comparison of symmetrized component EDPs for a) DMPC/CER16 at X_{CER}=0.20, b) DMPC/CER16 at X_{CER}=0.60, c) pure CER16, d) DMPC/CER[AP] at X_{CER}=0.14, e) DMPC/CER[AP] at X_{CER}=0.60, f) pure CER[AP], g) POPC/CER16 at X_{CER}=0.10, h) POPC/CER16 at X_{CER}=0.60, i) pure CER16. Groups represented are methine (CH), carbonyl (Carb), choline (Choln), glycerol (Gly), hydroxyl (OH), phosphate (Phos), and sphingosine/phytosphingosine (Sphig). Note that c) and i) are identical and are shown twice for simpler comparison against PC/CER mixtures.

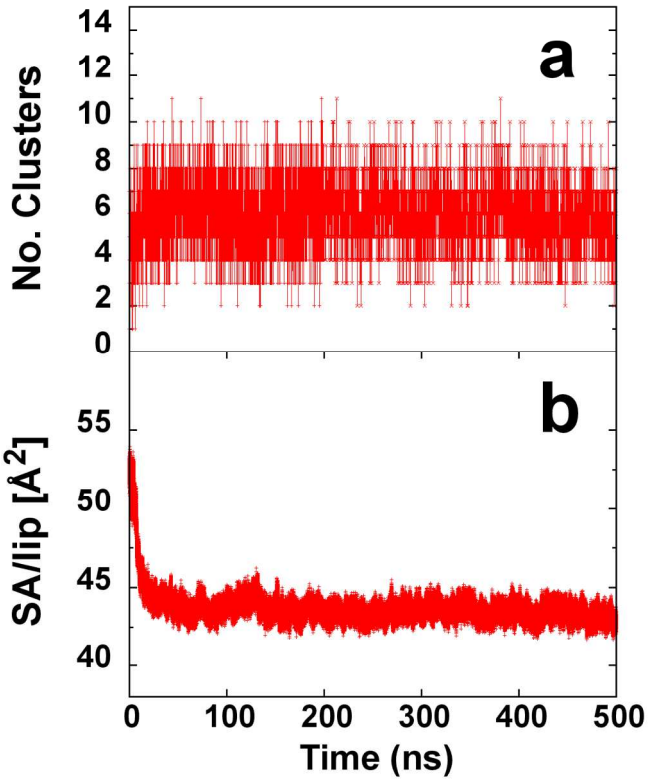

Figure S10. Comparison between a) total number of clusters and b) SA/lip as a function of time for DMPC/CER16 at X_{CER}=0.60.

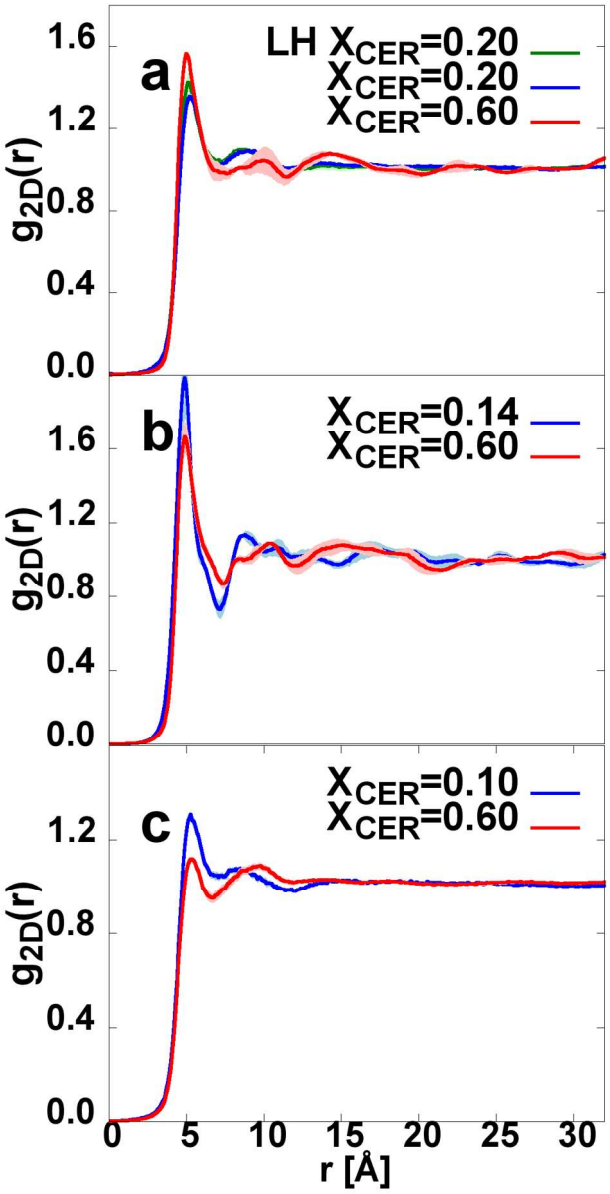

Figure S11. Comparison of PC-CER (C2-C2S) 2D RDFs at different CER concentrations for a) DMPC/CER16 systems b) DMPC/CER[AP] systems and c) POPC/CER16 systems. Lighter shades of each color represent the standard error interval. LH refers to the low hydration system.

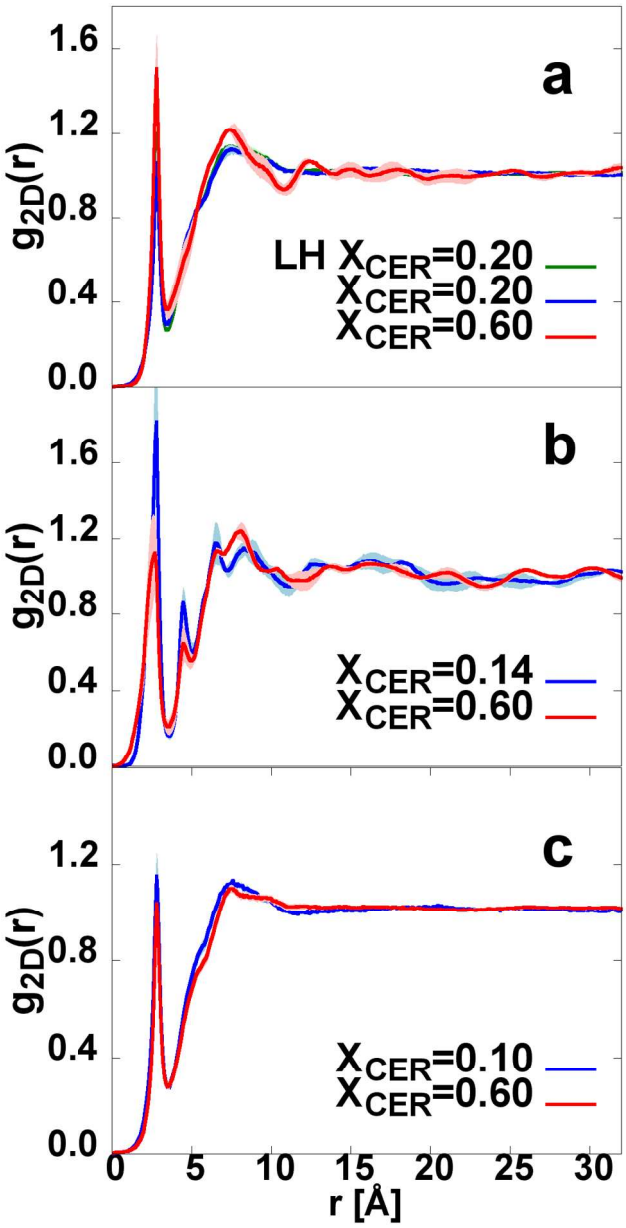

Figure S12. Comparison of 2D RDFs for CER-PC amide-carbonyl (NF-O22) H bonds at different CER concentrations for a) DMPC/CER16 systems b) DMPC/CER[AP] systems and c) POPC/CER16 systems. Lighter shades of each color represent the standard error interval. LH refers to the low hydration system.

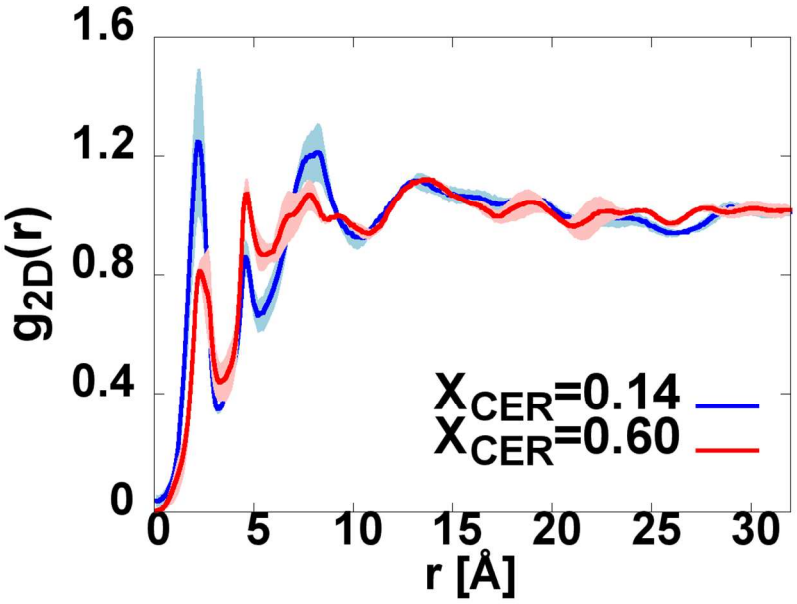

Figure S13. Comparison of 2D RDFs for CER-PC hydroxyl-carbonyl (O4-O22) H bonds at different CER concentrations for DMPC/CER[AP] systems. Lighter shades of each color represent the standard error interval.

Table S1. Average tilt angle of CER bilayers for each leaflet

	Bottom Leaflet Tilt (°)	Top Leaflet Tilt (°)
CER[AP] Rep1	20.3	21.5
CER[AP] Rep2	12.3	15.5
CER[AP] Rep3	21.2	16.8
CER16 Rep1	16.6	12.3
CER16 Rep2	14.4	14.1
CER16 Rep3	16.9	19.0

Table S2. Average P-N vector orientation for all PC systems and for pure DMPC[3]. Subscript LH refers to the low hydration system.

System	X_{CER}	Tilt (°)
DMPC	0.00	109.8*
DMPC/CER16	0.20	111.7±0.1
DMPC/CER16$_{LH}$	0.20	111.7±0.2
DMPC/CER16	0.60	120.1±0.6
DMPC/CER[AP]	0.14	109.5±0.2
DMPC/CER[AP]	0.60	119.2±0.3
POPC/CER16	0.10	110.2±0.04
POPC/CER16	0.60	117.4±0.1

* Value is based on a single replicate

Table S3. Intermolecular hydrogen bond (defined as when the distance between donor and acceptor is less than 2.4 Å and the angle is greater than 150º) count for significant groups/ hydrogen bond pairs between CER lipids. Subscript LH refers to the low hydration system.

Group	System	X_{CER}	N_{HB}	N_{HB}/lip
N-H:::O=C	DMPC/CER16	0.20	1.47±0.19	0.074±0.01
	DMPC/CER16$_{LH}$	0.20	1.55±0.40	0.077±0.02
	DMPC/CER16	0.60	11.95±0.23	0.199±0.004
	CER16	1.00	33.18±0.84	0.332±0.008
	DMPC/CER[AP]	0.14	0*	0*
	DMPC/CER[AP]	0.60	6.14±0.41	0.102±0.007
	CER[AP]	1.00	12.64±0.87	0.126±0.009
	POPC/CER16	0.10	0.40±0.11	0.04±0.011
	POPC/CER16	0.60	12.46±0.13	0.208±0.002
O4-H:::O=C	DMPC/CER[AP]	0.14	1.48±0.38	0.106±0.027
	DMPC/CER[AP]	0.60	2.03±1.25	0.034±0.021
	CER[AP]	1.00	0*	0*
O4-H:::O3	DMPC/CER[AP]	0.14	0.26±0.26	0.018±0.018
	DMPC/CER[AP]	0.60	0.96±0.29	0.016±0.005
	CER[AP]	1.00	4.94±0.90	0.049±0.009
O2-H:::O3	DMPC/CER[AP]	0.14	0*	0*
	DMPC/CER[AP]	0.60	1.30±0.36	0.022±0.006
	CER[AP]	1.00	4.15±0.57	0.041±0.006
O4-H:::O1	DMPC/CER[AP]	0.14	0.51±0.23	0.036±0.017
	DMPC/CER[AP]	0.60	2.63±1.00	0.044±0.017
	CER[AP]	1.00	8.19±1.52	0.082±0.015
O2-H:::O1	DMPC/CER[AP]	0.14	0.04±0.01	0.003±0.001
	DMPC/CER[AP]	0.60	0.76±0.20	0.013±0.003
	CER[AP]	1.00	1.24±0.10	0.012±0.001
O4-H:::O2	DMPC/CER[AP]	0.14	0.23±0.23	0.017±0.017
	DMPC/CER[AP]	0.60	2.09±0.46	0.035±0.008
	CER[AP]	1.00	6.14±0.84	0.061±0.008
O2-H:::O2	DMPC/CER[AP]	0.14	0*	0*
	DMPC/CER[AP]	0.60	1.48±0.36	0.025±0.006
	CER[AP]	1.00	3.55±0.52	0.036±0.005

*Standard error is 0

Table S4. Intermolecular hydrogen bond (defined as when the distance between donor and acceptor is less than 2.4 Å and the angle is greater than 150º) count for significant groups/ hydrogen bond pairs between PC and CER. Subscript LH refers to the low hydration system. Hydroxyls are numbered per chemical structure in Figure 1.

Group	System	X_{CER}	N_{HB}	N_{HB}/(#PC)	N_{HB}/(#CER)
N-H:::O=C	DMPC/CER16	0.20	6.23±0.28	0.078±0.004	0.312±0.014
	DMPC/CER16$_{LH}$	0.20	6.59±0.57	0.082±0.007	0.330±0.029
	DMPC/CER16	0.60	15.59±0.67	0.390±0.017	0.260±0.011
	DMPC/CER[AP]	0.14	8.22±0.74	0.096±0.009	0.587±0.053
	DMPC/CER[AP]	0.60	13.24±0.88	0.331±0.022	0.221±0.015
	POPC/CER16	0.10	2.69±0.08	0.030±0.001	0.269±0.008
	POPC/CER16	0.60	7.83±0.08	0.196±0.002	0.131±0.001
O1-H:::O=P	DMPC/CER16	0.20	1.75±0.04	0.022±0.001	0.088±0.002
	DMPC/CER16$_{LH}$	0.20	1.84±0.01	0.023±0.0001	0.092±0.001
	DMPC/CER16	0.60	4.17±0.15	0.104±0.004	0.070±0.003
	DMPC/CER[AP]	0.14	1.86±0.18	0.022±0.002	0.133±0.013
	DMPC/CER[AP]	0.60	3.91±0.18	0.098±0.005	0.065±0.003
	POPC/CER16	0.10	0.78±0.02	0.009±0.0002	0.078±0.002
	POPC/CER16	0.60	2.18±0.02	0.055±0.001	0.036±0.0003
O3-H:::O=C	DMPC/CER16	0.20	0.03±0.002	0.0004±0.00003	0.002±0.0001
	DMPC/CER16$_{LH}$	0.20	0.03±0.001	0.0004±0.00001	0.002±0.0001
	DMPC/CER16	0.60	0.08±0.02	0.002±0.001	0.001±0.0003
	DMPC/CER[AP]	0.14	1.19±0.18	0.014±0.002	0.085±0.013
	DMPC/CER[AP]	0.60	2.96±0.31	0.074±0.008	0.049±0.005
	POPC/CER16	0.10	0.01±0.001	0.0001±0.00001	0.001±0.0001
	POPC/CER16	0.60	0.03±0.001	0.001±0.00003	0.001±0.00002
N-H:::O=P	DMPC/CER16	0.20	0.44±0.02	0.006±0.0003	0.022±0.001
	DMPC/CER16$_{LH}$	0.20	0.32±0.05	0.004±0.001	0.016±0.003
	DMPC/CER16	0.60	1.23±0.31	0.031±0.008	0.021±0.005
	DMPC/CER[AP]	0.14	0.19±0.14	0.002±0.002	0.014±0.010
	DMPC/CER[AP]	0.60	1.57±0.23	0.039±0.006	0.026±0.004
	POPC/CER16	0.10	0.24±0.04	0.003±0.0004	0.024±0.004
	POPC/CER16	0.60	0.74±0.02	0.019±0.001	0.012±0.0003
O4-H:::O=C	DMPC/CER[AP]	0.14	5.07±0.73	0.059±0.008	0.362±0.052
	DMPC/CER[AP]	0.60	9.15±0.11	0.229±0.003	0.153±0.002
O4-H:::O=P	DMPC/CER[AP]	0.14	0.32±0.16	0.004±0.002	0.023±0.011
	DMPC/CER[AP]	0.60	1.28±0.15	0.032±0.004	0.021±0.003
O2-H:::O=C	DMPC/CER[AP]	0.14	1.74±0.20	0.020±0.002	0.124±0.014
	DMPC/CER[AP]	0.60	2.59±0.40	0.065±0.01	0.043±0.007
O2-H:::O=P	DMPC/CER[AP]	0.14	2.39±0.43	0.028±0.005	0.171±0.031
	DMPC/CER[AP]	0.60	5.39±0.09	0.135±0.002	0.090±0.002

Table S5. Intramolecular hydrogen bond (defined as when the distance between donor and acceptor is less than 2.4 Å and the angle is greater than 150º) count for significant groups/ hydrogen bond pairs between CER lipids. Subscript LH refers to the low hydration system.

Group	System	X_{CER}	N_{HB}	N_{HB}/lip
O3-H:::O=C	DMPC/CER16	0.20	0.10±0.05	0.005±0.003
	DMPC/CER16$_{LH}$	0.20	0.03±0.02	0.001±0.001
	DMPC/CER16	0.60	0.21±0.05	0.004±0.001
	CER16	1.00	0.47±0.08	0.005±0.001
	DMPC/CER[AP]	0.14	0.01±0.01	0.001±0.001
	DMPC/CER[AP]	0.60	1.03±0.42	0.017±0.007
	CER[AP]	1.00	2.59±0.41	0.026±0.004
	POPC/CER16	0.10	0.04±0.01	0.004±0.001
	POPC/CER16	0.60	0.26±0.04	0.004±0.001
O4-H:::O=C	DMPC/CER[AP]	0.14	0.37±0.37	0.027±0.027
	DMPC/CER[AP]	0.60	1.88±1.07	0.031±0.018
	CER[AP]	1.00	5.30±0.24	0.053±0.002

Table S6. Clustering fractions for PC and CER. N_C is the number of lipids per cluster, R_C is the fraction of the particular lipid in clusters, and R_N is the fraction of the particular lipid in the membrane. Subscript LH refers to the low hydration system.

Lipid	System	X_{CER}	N_C	R_C	R_N	R_C-R_N
PC	DMPC/CER16	0.20	1.67±0.01	0.51*	0.80*	-0.29*
	DMPC/CER16$_{LH}$	0.20	1.72±0.05	0.53±0.02	0.80*	-0.27±0.02
	DMPC/CER16	0.60	1.04±0.03	0.29±0.01	0.40*	-0.11±0.01
	DMPC/CER[AP]	0.14	2.03±0.10	0.62±0.05	0.86*	-0.24±0.05
	DMPC/CER[AP]	0.60	1.05±0.06	0.29±0.01	0.40*	-0.11±0.01
	POPC/CER16	0.10	1.89±0.04	0.60±0.02	0.90*	-0.30±0.02
	POPC/CER16	0.60	0.90±0.01	0.27*	0.40*	-0.13*
CER	DMPC/CER16	0.20	1.60±0.02	0.49*	0.20*	0.29*
	DMPC/CER16$_{LH}$	0.20	1.53±0.07	0.47±0.02	0.20*	0.27±0.02
	DMPC/CER16	0.60	2.52±0.08	0.71±0.01	0.60*	0.11±0.01
	CER16	1.00	4.68±0.12	--	--	--
	DMPC/CER[AP]	0.14	1.28±0.23	0.38±0.05	0.14*	0.24±0.05
	DMPC/CER[AP]	0.60	2.55±0.08	0.71±0.01	0.60*	0.11±0.01
	CER[AP]	1.00	4.61±0.17	--	--	--
	POPC/CER16	0.10	1.28±0.07	0.40±0.02	0.10*	0.30±0.02
	POPC/CER16	0.60	2.45±0.02	0.73*	0.60*	0.13*

* Standard error is less than 0.01

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3. Zhuang, X.; Davila-Contreras, E. M.; Beaven, A. H.; Im, W.; Klauda, J. B., An extensive simulation study of lipid bilayer properties with different head groups, acyl chain lengths, and chain saturations. *Biochimica Et Biophysica Acta-Biomembranes* **2016,** *1858* (12), 3093-3104.