Supporting Information

Characterization of Lipid Chain Order and Dynamics in Asymmetric Membranes by Solid-State NMR Spectroscopy

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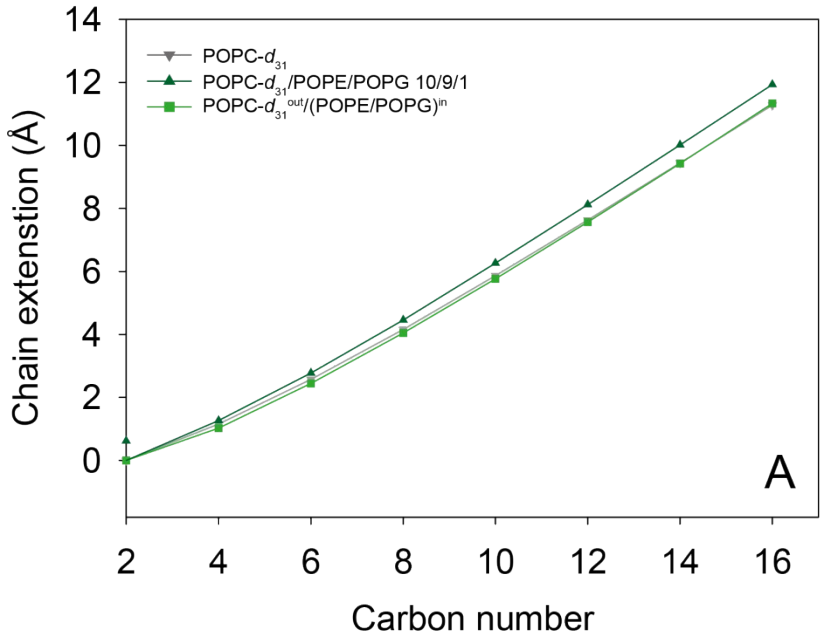

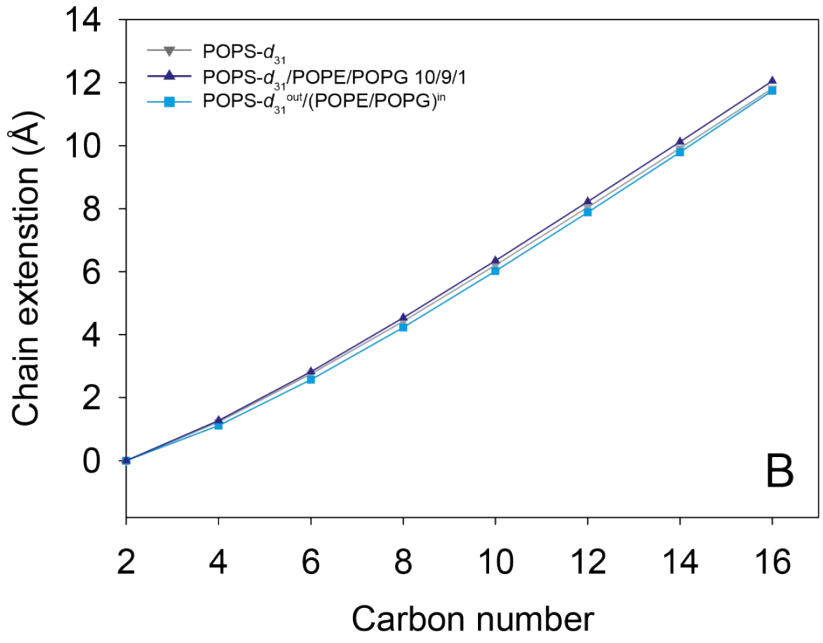

Figure S1. Chain extension plots derived from the ^{2}H NMR spectra of (A) POPC-d_{31}^{out}/(POPE/POPG)^{in} membranes (light green squares), symmetric POPC-d_{31}/POPE/POPG bilayers (dark green triangles), and pure POPC-d_{31} membranes (gray upside down triangles) and (B) POPS-d_{31}^{out}/(POPE/POPG)^{in} membranes (light blue squares), symmetric POPS-d_{31}/POPE/POPG bilayers (dark blue triangles), and pure POPS-d_{31} membranes (gray upside down triangles). Chain extension plots are calculated according to Petrache et al.[1]

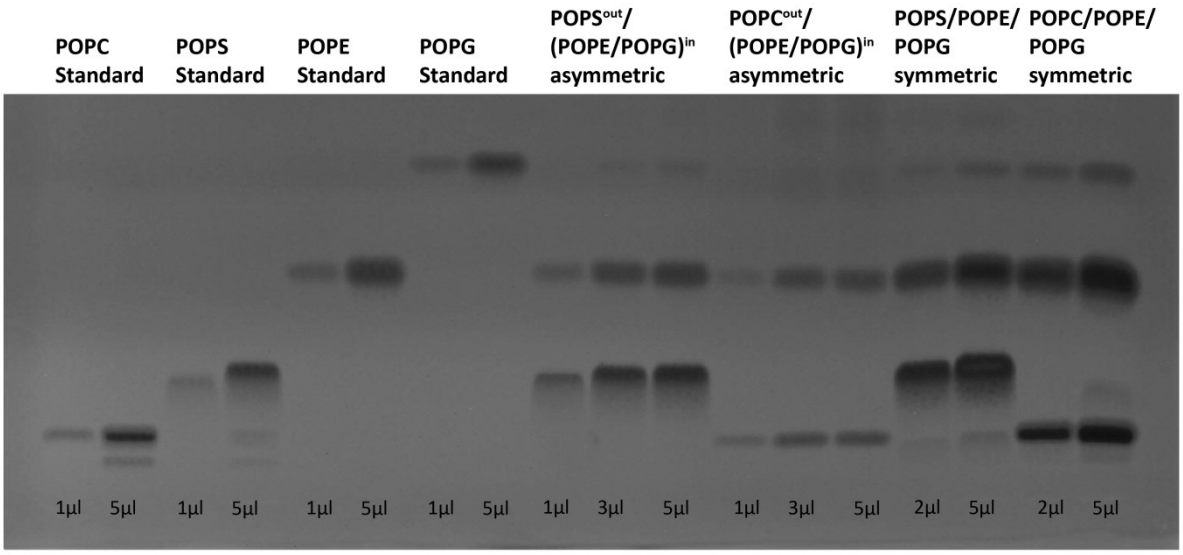

Figure S2. Representative TLC showing all samples for overview. This was used for determining pipetting amounts for the individual quantitative TLC plates. Different volumes of standard lipids and samples were sprayed automatically onto a normal-phase TLC plate. The solvent mixture chloroform/ethanol/water/trieathylamine (30:35:7:35, by vol.) was used as the mobile phase. Lipids were stained by dipping the plate into a primulin solution (100 mg in acetone/water 80:20, by vol.) and visualized under UV light (366 nm).

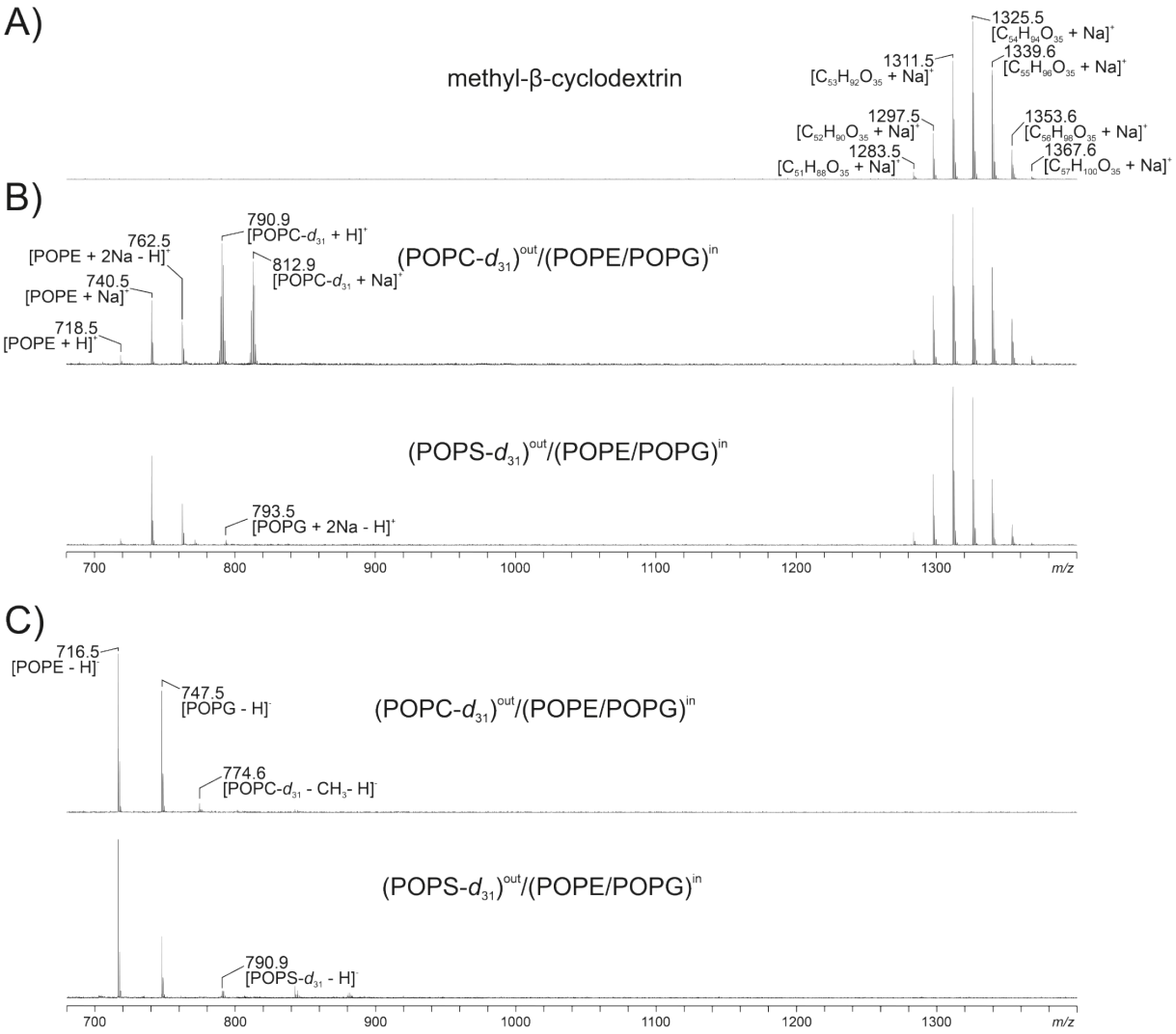

Figure S3: Mass spectra of asymmetric MLVs to analyse the lipid composition and check for residual methyl-β-cyclodextrin. A) Pure methyl-β-cyclodextrin was dissolved in water at a concentration of 1 mg/ml. B) Positive ion mode MALDI-TOF spectra of the asymmetric MLVs in 2,5-dihydroxybenzoic acid (0.5 M in methanol) as the matrix. C) Negative ion mode MALDI-TOF spectra of the asymmetric MLVs in 9-aminoacridine (10 mg/ml in 2-propanol/acetonitrile (3:2, v/v) as the matrix. These mass spectra are not quantitative.

Table S1. Order parameter as a function of carbon position in the *sn*-1 chain of POPC in the individual membrane preparations membranes determined from dePakeing of the ^{2}H NMR spectra.

Carbon number	Pure POPC-d_{31}	POPC-d_{31}/POPE/POPG	POPC-d_{31}^{out}/(POPE/POPG)^{in}
2	0.201	0.224	0.229
3	0.197	0.221	0.219
4	0.193	0.219	0.212
5	0.190	0.216	0.204
6	0.185	0.210	0.198
7	0.180	0.203	0.189
8	0.173	0.197	0.180
9	0.165	0.191	0.168
10	0.153	0.173	0.155
11	0.137	0.158	0.140
12	0.123	0.141	0.124
13	0.106	0.122	0.106
14	0.089	0.102	0.084
15	0.067	0.082	0.052
16	0.017	0.021	0.022

Table S2. Order parameter as a function of carbon position in the *sn*-1 chain of POPS in the individual membrane preparations membranes determined from dePakeing of the ^{2}H NMR spectra.

Carbon number	Pure POPS-d_{31}	POPS-d_{31}/POPE/POPG	POPS-d_{31}^{out}/(POPE/POPG)^{in}
2	0.218	0.233	0.243
3	0.214	0.226	0.233
4	0.212	0.220	0.227
5	0.210	0.216	0.220
6	0.206	0.212	0.214
7	0.198	0.208	0.206
8	0.192	0.200	0.198
9	0.185	0.192	0.186
10	0.172	0.182	0.171
11	0.156	0.166	0.153
12	0.143	0.149	0.134
13	0.124	0.130	0.112
14	0.103	0.110	0.090
15	0.077	0.083	0.062
16	0.022	0.021	0.022

Table S3. Squared order parameters and relaxation rates for the various preparations as plotted in the R_{1Z} vs. S^2 diagrams.

pure POPC-d_{31}		pure POPS-d_{31}	
S^2	R_{1Z} / s^{-1}	S^2	R_{1Z} / s^{-1}
0.04121	26.85	0.048	23.80
0.0392	26.70	0.047	23.12
0.03803	26.36	0.047	22.53
0.03686	25.66	0.046	22.37
0.03572	24.38	0.045	23.10
0.0346	23.22	0.044	26.46
0.03168	23.66	0.038	23.78
0.02822	23.39	0.036	23.96
0.02403	20.32	0.032	19.31
0.01904	19.30	0.026	17.50
0.01538	16.71	0.021	15.34
0.01102	13.88	0.015	12.87
7.57E-03	10.41	0.011	9.910
4.22E-03	7.10	5.93E-03	6.90
3.61E-04	2.51	4.84E-04	2.49

POPC-d_{31}/POPE/POPG		POPS-d_{31}/POPE/POPG	
S^2	R_{1Z} / s^{-1}	S^2	R_{1Z} / s^{-1}
0.049	24.83	0.054	24.44
0.048	24.21	0.052	24.12
0.048	23.65	0.049	24.04
0.048	23.15	0.047	24.10
0.047	22.81	0.046	23.85
0.041	23.94	0.044	23.71
0.040	23.58	0.042	22.99
0.037	22.52	0.038	22.12
0.036	21.28	0.034	20.73
0.031	16.43	0.028	15.63
0.024	13.94	0.021	13.46
0.017	11.75	0.015	11.68
0.012	9.06	0.015	8.22
6.40E-03	6.10	6.24E-03	6.38
5.29E-04	2.43	5.76E-04	2.49

POPC-d_{31}^{out}/(POPE/POPG)^{in}		POPS-d_{31}^{out}/(POPE/POPG)^{in}	
S^2	R_{1Z} / s^{-1}	S^2	R_{1Z} / s^{-1}
0.060	23.62	0.056	24.75
0.052	24.44	0.053	23.49
0.047	25.16	0.049	22.55
0.040	15.38	0.048	21.28
0.033	18.03	0.046	20.17
0.028	13.37	0.044	18.78
0.023	12.98	0.040	18.37
0.018	10.22	0.036	16.1
0.014	8.87	0.027	11.45
0.010	6.63	0.021	9.24
7.40E-03	5.27	0.017	7.37
4.90E-03	4.23	0.013	4.26
3.03E-03	3.27	8.46E-03	3.32
4.00E-04	1.89	4.84E-04	1.89

1. Petrache, H. I.; Dodd, S. W.; Brown, M. F. Area per lipid and acyl length distributions in fluid phosphatidylcholines determined by ^{2}H NMR spectroscopy. *Biophys. J.* **2000**, *79*, 3172-3192.