Electronic Supplementary Information

Structure and Dynamics of the Aliphatic Cholesterol Side Chain in Membranes as Studied by ^{2}H NMR Spectroscopy and Molecular Dynamics Simulation

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Table S1. Order parameters determined for the aliphatic cholesterol chain in various environments (25 mol% cholesterol relative to all other lipids) and at several temperatures from experimental ^{2}H NMR spectra and MD simulations. The first 500 ns were considered as equilibration of the MD simulation and omitted for data evaluation.

	DPPC			PSM			POPC/PSM 1:1	
	37°C	50°C		37°C	50°C		37°C	50°C
	NMR	NMR	MD	NMR	NMR	MD	NMR	NMR
H20	n.d.[a]	n.d.	0.4401	n.d.	n.d.	0.4453	n.d.	n.d.
H21A	n.d.	n.d.	0.1244	n.d.	n.d.	0.1225	n.d.	n.d.
H21B	n.d.	n.d.	0.1226	n.d.	n.d.	0.1244	n.d.	n.d.
H21C	n.d.	n.d.	0.1245	n.d.	n.d.	0.1233	n.d.	n.d.
H22A	n.d.	n.d.	0.3875	n.d.	n.d.	0.3817	n.d.	n.d.
H22B	n.d.	n.d.	0.4205	n.d.	n.d.	0.4215	n.d.	n.d.
H23A	0.2675	0.2339	0.3309	0.1916	0.2156	0.3191	0.2268	0.1996
H23B	0.4272	0.4032	0.4118	0.4295	0.4192	0.4104	0.4112	0.3912
H24A	0.2675	0.2339	0.3206	0.2435	0.2515	0.3033	0.2451	0.2180
H24B	0.3433	0.3114	0.3724	0.3353	0.3162	0.3648	0.3178	0.2930
H25	0.3234	0.2794	0.2864	0.2978	0.2978	0.2601	0.2874	0.2571
H26A	0.0208	0.0190	0.0170	0.0216	0.0224	0.0202	0.0216	0.0200
H26B			0.0187			0.0199		
H26C			0.0184			0.0188		
H27A	0.0224	0.0192	0.0196	0.0240	0.0224	0.0206	0.0200	0.0176
H27B			0.0191			0.0190		
H27C			0.0187			0.0206		

[a] not determined due to the lack of deuteration

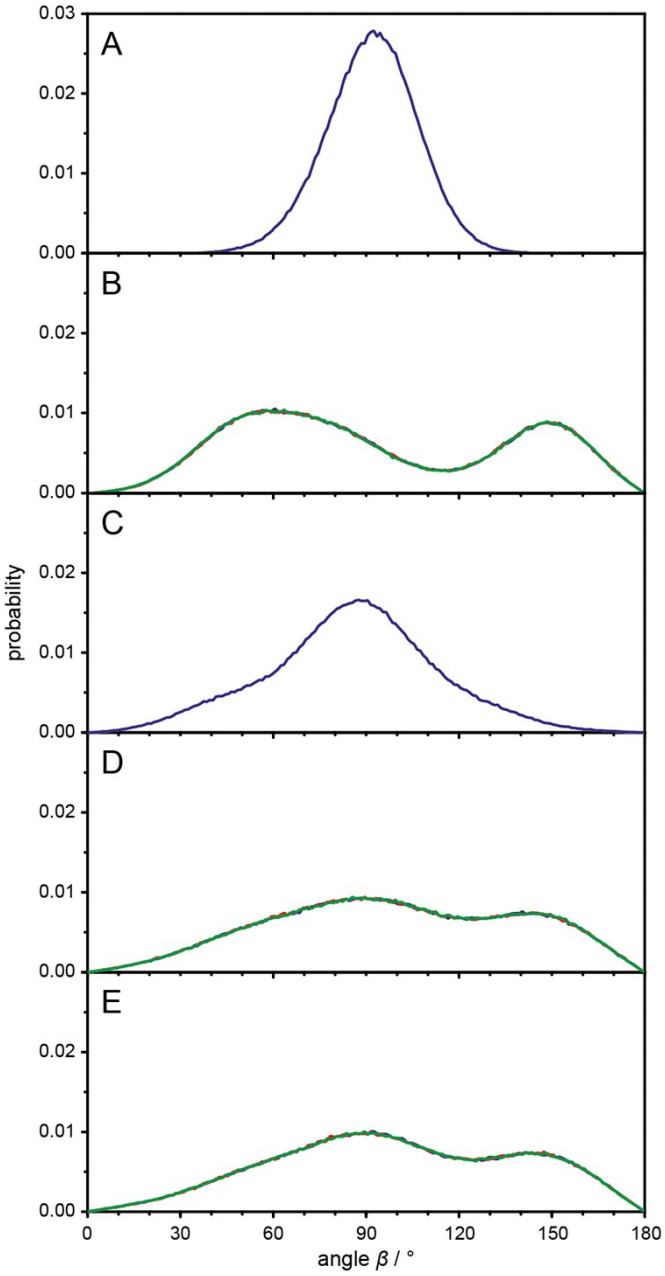

Figure S1. (A) Histogram of the angle β between the carbon hydrogen bond attached to C20 and the membrane normal as obtained from the MD simulation of POPC/cholesterol (3:1 mol/mol) at 37°C. (B), (C), (D), and (E). The same histograms are shown for C21, C25, C26, and C27, respectively. The data for hydrogen A is shown in blue, for hydrogen B in red, and for hydrogen C in green.

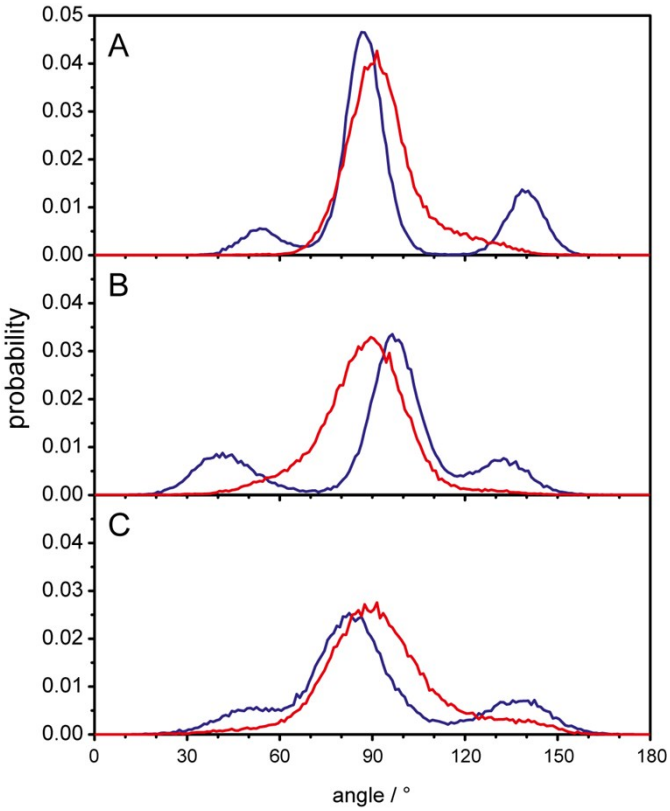

Figure S2. Histograms of the angle between the two carbon hydrogen bonds attached to C22 (A), C23 (B) and C24 (C) and the mean rotational axis of cholesterol as obtained from the MD simulation of POPC/cholesterol (3:1 mol/mol) at 37°C. The data for hydrogen A is shown in blue and for hydrogen B in red.

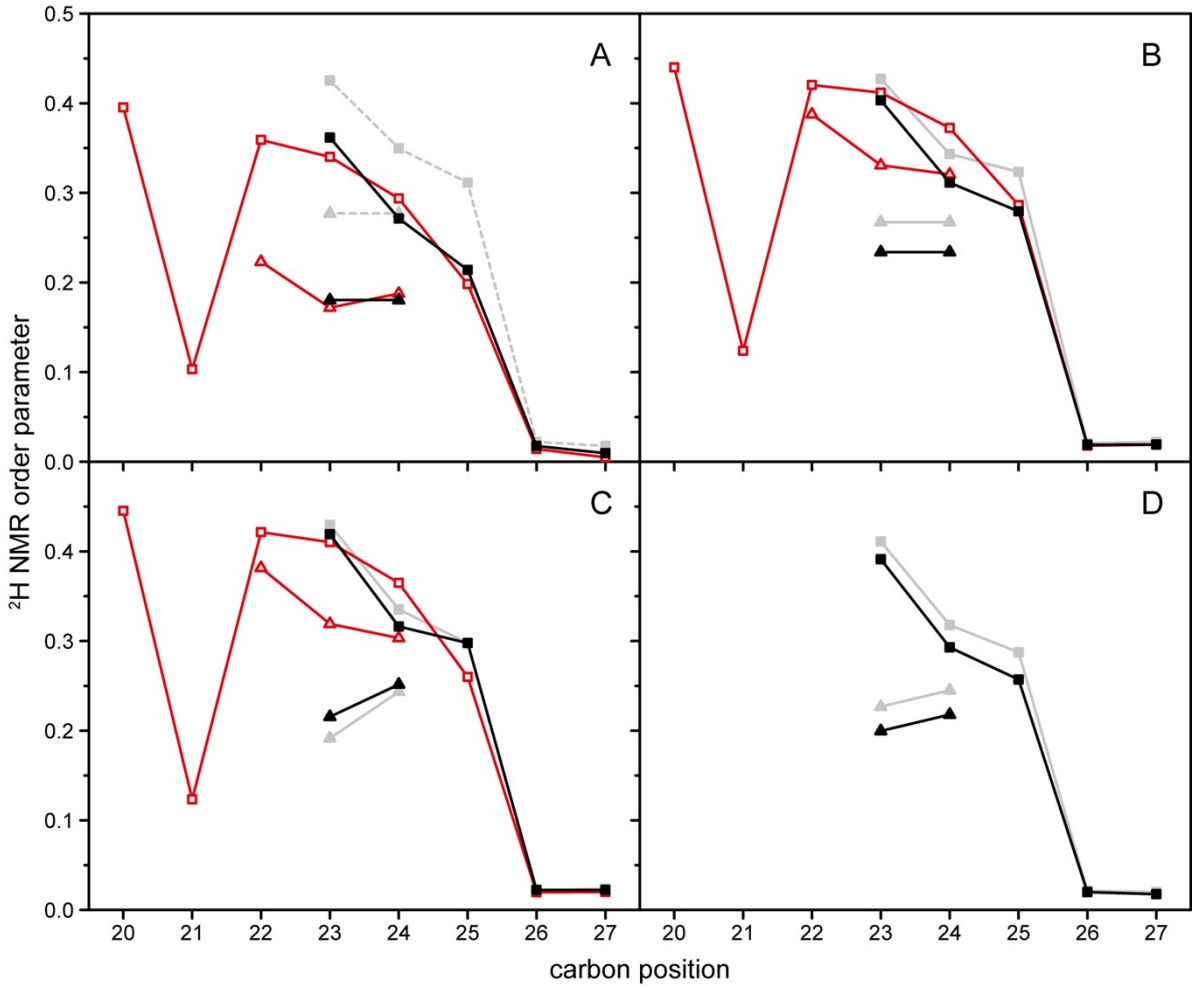

Figure S3. ^{2}H NMR order parameters of the aliphatic cholesterol side chain (25 mol% cholesterol relative to all other lipids) in (A) POPC, (B) DPPC, (C) PSM, and (D) POPC/PSM (1:1 mol/mol). MD simulation order parameters at 50°C, which are shown in red, were calculated for the whole chain while the corresponding experimental data is shown in black (50°C) for all deuterated carbon positions of cholesterol-d_{11}. Experimental data at other temperatures is shown in grey (solid: 37°C, dashed: 5°C). Data for C22, C23, and C24 shows two values because A (triangles) and B (squares) hydrogens are not equivalent at these positions. In the MD simulation data the three hydrogens of the CH_3 groups at C21, C26, and C27 are averaged because their order parameters differ by less than 0.002. The first 500 ns were considered as equilibration of the MD simulation and omitted for data evaluation.

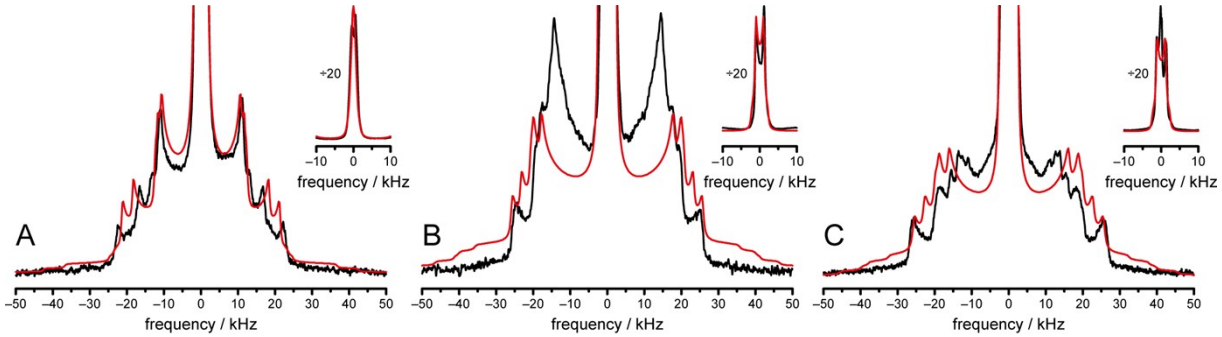

Figure S4. ^{2}H NMR spectra of cholesterol-d_{11} in (A) POPC, (B) DPPC, and (C) PSM (all 1:3 mol/mol) at 50°C. The experimentally obtained spectrum is shown in black while the spectrum calculated from the MD simulation is shown in red. The inset shows the same spectrum vertically divided by a factor of 20 to show the intense Pake doublets from the two terminal CH_3 groups. The first 500 ns were considered as equilibration of the MD simulation and omitted for data evaluation.