Electronic Supplementary Material (ESI) for Soft Matter.

Supporting information for:

Quantitative relationship between cholesterol distribution and ordering of lipids in asymmetric lipid bilayers

Mohammadreza Aghaaminiha[a], Amir M. Farnoud[a], Sumit Sharma[a,*]

[a]Department of Chemical and Biomolecular Engineering, Russ College of Engineering and Technology, Ohio University, Athens, OH 45701, USA

To whom correspondence should be addressed: Dr. Sumit Sharma, Ohio University, Department of Chemical and Biomolecular Engineering, 181 Stocker Center, Athens, OH, 45701 USA, Tel.: (740) 593-1425; E-mail: sharmas@ohio.edu

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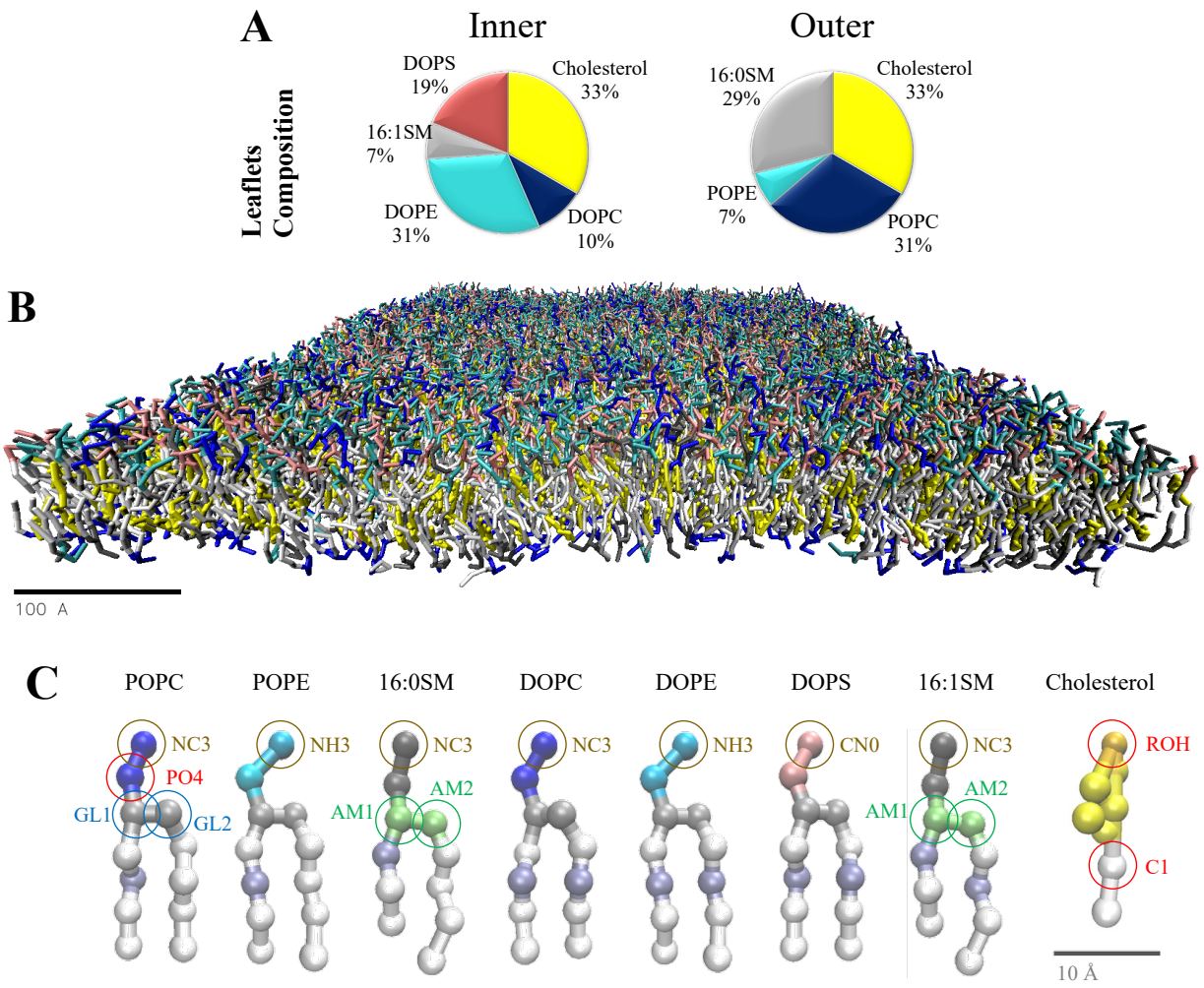

Figure S1. A) Composition of the asymmetric lipid bilayer studied in this work. **B)** A snapshot of equilibrium configuration of the asymmetric lipid bilayer at 37 ºC. The top-view shown in the Figure S1B corresponds to the inner-leaflet while the bottom view (not shown) corresponds to the outer-leaflet. Ions and water molecules are omitted for the sake of clarity. The colors of headgroups shown in Figure S1B match the colors shown in the pie-chart of Figure S1A. Lipid chains are shown in white. **C)** The stucture of lipids in the CG Martini force field representation. The second bead of the headgroups for all phospholipids is phosphate (PO4). The glycerol backbone (GL1 and Gl2) are colored light silver while the green beads show the sphingosine backbone (AM1 and AM2). The beads with the unsaturation are colored in purple. Bead types are as follows: (choline NC3: Q0, ethanolamine NH3: Qd, serine CN0: P5, phosphate PO4: Qa, glycerol GL1: Na, glycerol GL2: Na, amide AM1: P1, amide AM2: P5, and cholesterol-hydroxyl ROH: SP1). GL1, GL2, and AM2 contain a carbonyl oxygen group while AM1 contains a hydroxyl group. The definition of Q0, Qd, Qa. Na, P1, P5, and SP1 are presented in the methodology section while the full explanation is available in the original paper.[1]

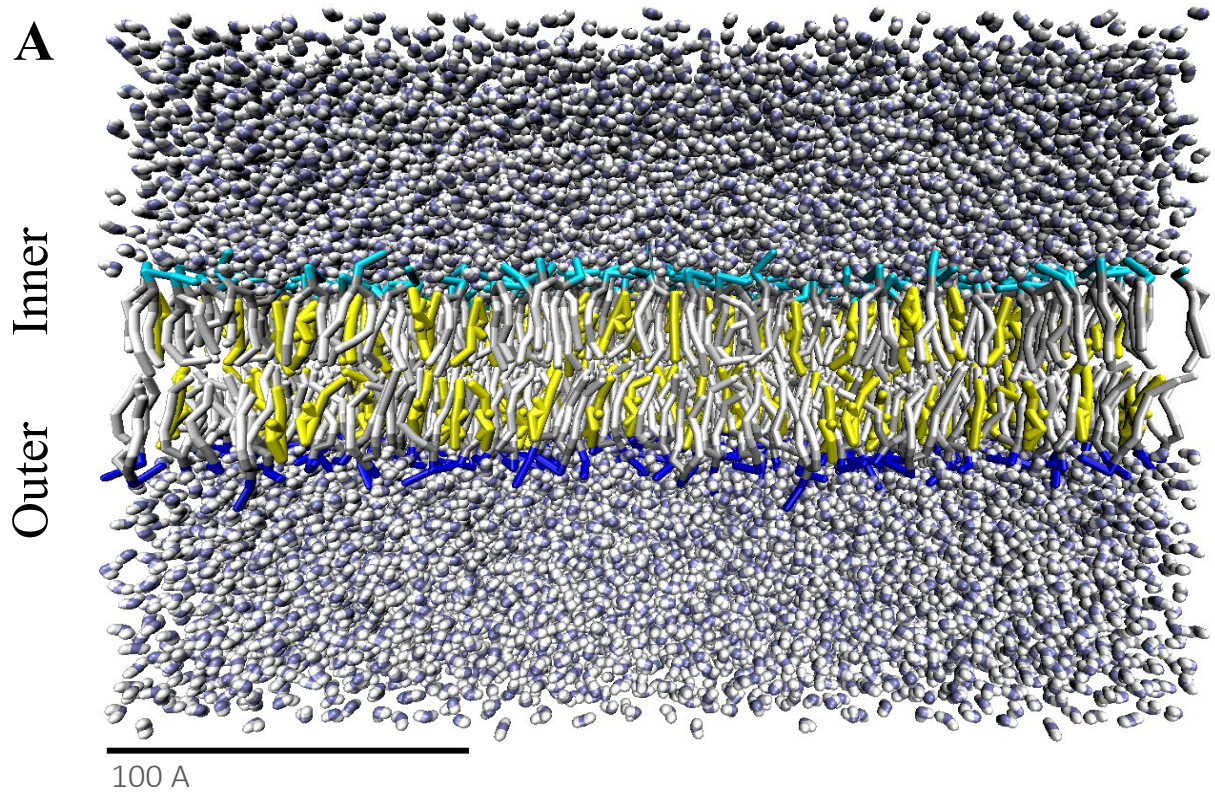

Figure S2. A) A snapshot of the equilibrated configuration of the DPPEi/DPPCo/CHOL system at 37 ºC. **B)** Structure of lipids with CG Martini force field representation to understand the effects of the backbone (16:0SM), headgroup type (DPPE and DPPS), acyl chain saturation (DOPC and POPC), and acyl chain length (12:0PC and 24:0PC). The second bead of the headgroups of all phospholipids is phosphate (PO4). The glycerol backbone (GL1 and Gl2) are colored light silver while the green beads show the sphingosine backbone (AM1 and AM2). The beads with unsaturation are colored in purple. Bead types are as follows: (choline NC3: Q0, ethanolamine NH3: Qd, serine CN0: P5, phosphate PO4: Qa, glycerol GL1: Na, glycerol GL2: Na, amide AM1: P1, amide AM2: P5, and cholesterol-hydroxyl ROH: SP1). According to the molecular structure, GL1, GL2, and AM2 contain a carbonyl oxygen group while AM1 contains a hydroxyl group. The definition of Q0, Qd, Qa. Na, P1, P5, and SP1 are presented in the methodology section while the full explanation is available in the original paper.[1]

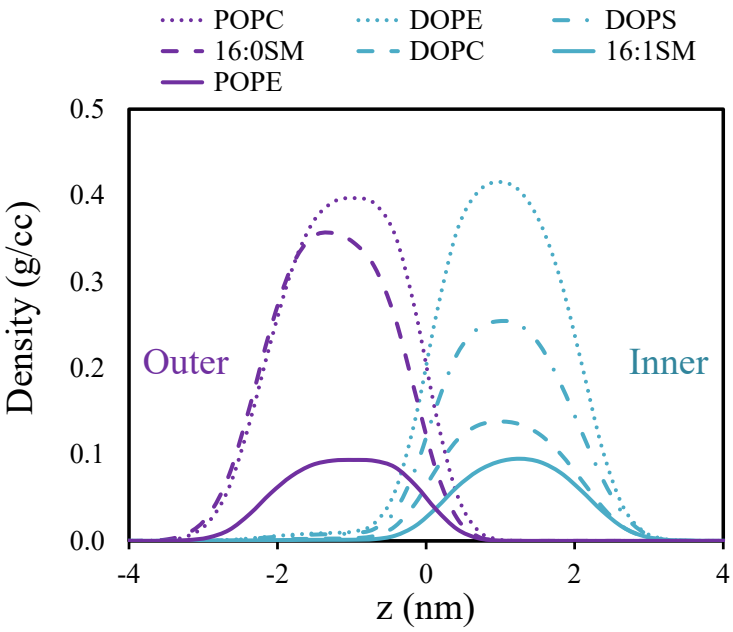

Figure S3. Density profile of phospholipids in the asymmetric system after 10 μs simulation. Negative values of z correspond to the outer leaflet while the positive values represent the inner leaflet.

Table S1. Various properties of the three studied bilayers. Cholesterol was present in all the bilayers in the molar ratio of 1:2 with respect to the total number of lipids. The values of area per lipid is smaller compared to the usually reported values (~ 0.5 to 0.7 nm^2) for the bilayers where no cholesterol present.[2]

Lipid bilayer	System (box) area (nm^2)	Thickness (Å)	Area per lipid (nm^2)		Average order parameter (S_{chain})	
			Inner	Outer	Inner	Outer
Asymmetric	507.63 ± 0.07	41.24 ± 0.14	0.52 ± 0.01	0.48 ± 0.01	0.33 ± 0.02	0.48 ± 0.02
Cyto-symmetric	530.43 ± 0.10	40.28 ± 0.14	0.52 ± 0.01	0.52 ± 0.01	0.32 ± 0.02	0.32 ± 0.02
Exo-symmetric	484.77 ± 0.12	42.25 ± 0.14	0.47 ± 0.01	0.47 ± 0.01	0.48 ± 0.02	0.48 ± 0.02

Table S2. Properties of equimolar asymmetric bilayer systems with DPPC as the outer-leaflet. Cholesterol was present in all the bilayers in the molar ratio of 1:2 with respect to the total number of lipids. The "i" and "o" subscripts represent the inner and outer leaflets, respectively.

Lipid bilayer	System (box) area (nm^2)	Thickness (Å)	Flip-flop rate 1×10^6 1/s	Area per lipid (nm^2)	
				Inner	Outer
DPPCi/DPPCo/CHOL	114.71 ± 0.84	45.11 ± 0.25	0.26 ± 0.06	0.45 ± 0.01	0.45 ± 0.01
16:0SMi/DPPCo/CHOL	115.28 ± 0.93	43.52 ± 0.25	0.36 ± 0.06	0.44 ± 0.01	0.46 ± 0.01
DPPEi/DPPCo/CHOL	114.39 ± 0.80	45.17 ± 0.24	0.22 ± 0.04	0.45 ± 0.01	0.45 ± 0.01
DPPSi/DPPCo/CHOL	115.59 ± 0.90	44.97 ± 0.25	0.29 ± 0.05	0.45 ± 0.01	0.46 ± 0.01
DOPCi/DPPCo/CHOL	124.60 ± 1.07	42.62 ± 0.28	2.40 ± 0.32	0.53 ± 0.01	0.45 ± 0.01
POPCi/DPPCo/CHOL	120.50 ±0.98	43.60 ± 0.25	1.30 ± 0.17	0.50 ± 0.01	0.44 ± 0.01
12:0PCi/DPPCo/CHOL	115.36 ± 0.86	41.81 ± 0.21	0.52 ± 0.07	0.45 ± 0.01	0.45 ± 0.01
24:0PCi/DPPCo/CHOL	114.10 ± 0.93	51.69 ± 0.27	2.10 ± 0.18	0.43 ± 0.01	0.46 ± 0.01
DOPEi/DPPCo/CHOL	123.61 ± 1.04	42.78 ± 0.26	2.10 ± 0.24	0.53 ± 0.01	0.45 ± 0.01
POPEi/DPPCo/CHOL	119.64 ± 0.93	43.75 ± 0.25	1.10 ± 0.17	0.49 ± 0.01	0.45 ± 0.01
DOPSi/DPPCo/CHOL	125.58 ± 1.11	42.42 ± 0.29	2.20 ± 0.25	0.53 ± 0.01	0.46 ± 0.01
POPSi/DPPCo/CHOL	121.77 ± 1.06	43.39 ± 0.26	1.30 ± 0.15	0.49 ± 0.01	0.46 ± 0.01

Table S3. Average S_{chain} of lipids in the equimolar asymmetric bilayers in the presence and absence of cholesterol. The "i" and "o" subscripts represent the inner and outer leaflets, respectively.

	0% Cholesterol		33% Cholesterol		
	S_{Chain}		S_{Chain}		Cholesterol Abundance
Lipid bilayer	Inner	Outer	Inner	Outer	
DPPCi/DPPCo/CHOL	0.40 ± 0.02	0.40 ± 0.02	0.69 ± 0.02	0.68 ± 0.02	Equal
16:0SMi/DPPCo/CHOL	0.40 ± 0.02	0.41 ± 0.02	0.69 ± 0.02	0.62 ± 0.02	Inner
DPPEi/DPPCo/CHOL	0.42 ± 0.02	0.42 ± 0.02	0.71 ± 0.02	0.69 ± 0.02	Inner
DPPSi/DPPCo/CHOL	0.38 ± 0.02	0.37 ± 0.02	0.69 ± 0.02	0.66 ± 0.02	Inner
DOPCi/DPPCo/CHOL	0.27 ± 0.02	0.32 ± 0.02	0.34 ± 0.02	0.64 ± 0.02	Outer
POPCi/DPPCo/CHOL	0.32 ± 0.02	0.35 ± 0.02	0.45 ± 0.02	0.67 ± 0.02	Outer
12:0PCi/DPPCo/CHOL	0.39 ± 0.02	0.40 ± 0.02	0.69 ± 0.02	0.69 ± 0.02	Outer
24:0PCi/DPPCo/CHOL	0.42 ± 0.02	0.40 ± 0.02	0.66 ± 0.02	0.65 ± 0.02	Inner
DOPEi/DPPCo/CHOL	0.28 ± 0.02	0.33 ± 0.02	0.35 ± 0.02	0.64 ± 0.02	Outer
POPEi/DPPCo/CHOL	0.34 ± 0.02	0.37 ± 0.02	0.48 ± 0.02	0.66 ± 0.02	Outer
DOPSi/DPPCo/CHOL	0.26 ± 0.02	0.30 ± 0.02	0.35 ± 0.02	0.60 ± 0.02	Outer
POPSi/DPPCo/CHOL	0.31 ± 0.02	0.33 ± 0.02	0.47 ± 0.02	0.61 ± 0.02	Outer

Table S4. Comparative data of lipid bilayers: we have studied symmetric bilayers, equimolar asymmetric bilayers (denoted by **) and asymmetric bilayers with no build-in stress (denoted by *).

	Initial distribution				Final distribution				Chol%		sys area	$area/\ lipid$		S_{Chain}	
	# lipids		# chol		# lipids		# chol		± 1%		± 1 nm^2	± 0.01 nm^2		± 0.02	
Lipid bilayers	inner	outer	inner	outer	inner	outer	inner	outer	inner	outer		inner	outer	inner	outer
DOPCi/DOPCo/CHOL	170	170	85	85	170	170	85	85	50	50	135	0.53	0.53	0.31	0.31
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
DOPCi/DPPCo/CHOL (*)	192	240	96	120	192	240	102	114	47	53	159	0.54	0.45	0.28	0.69
DOPCi/DPPCo/CHOL (**)	171	171	84	84	170	170	62	106	37	63	125	0.53	0.45	0.34	0.64
POPCi/POPCo/CHOL	170	170	85	85	170	170	86	84	50	50	127	0.50	0.50	0.43	0.43
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
POPCi/DPPCo/CHOL (*)	216	240	108	120	216	240	107	121	47	53	162	0.50	0.45	0.43	0.69
POPCi/DPPCo/CHOL (**)	170	170	85	85	170	170	69	101	41	59	120	0.50	0.44	0.45	0.67
DOPEi/DOPEo/CHOL	170	170	85	85	170	170	85	85	50	50	133	0.52	0.52	0.33	0.33
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
DOPEi/DPPCo/CHOL (*)	192	240	96	120	197	235	101	115	47	53	157	0.52	0.45	0.32	0.69
DOPEi/DPPCo/CHOL (**)	171	171	84	84	171	171	64	104	38	62	124	0.53	0.45	0.35	0.64
POPEi/POPEo/CHOL	170	170	85	85	170	170	87	83	51	49	125	0.49	0.49	0.47	0.45
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
POPEi/DPPCo/CHOL (*)	216	240	108	120	216	240	111	117	49	51	160	0.49	0.45	0.46	0.69
POPEi/DPPCo/CHOL (**)	170	170	85	85	170	170	73	97	43	57	120	0.49	0.45	0.48	0.66
DPPEi/DPPEo/CHOL	170	170	85	85	170	170	82	88	48	52	114	0.45	0.44	0.70	0.73
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
DPPEi/DPPCo/CHOL (*)	240	240	120	120	240	240	124	116	52	48	161	0.44	0.45	0.72	0.69
DPPEi/DPPCo/CHOL (**)	171	171	84	84	171	171	86	82	51	49	114	0.45	0.45	0.71	0.69
16:0SMi/16:0SMo/CHOL	170	170	85	85	171	169	87	83	51	49	114	0.44	0.45	0.69	0.66
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
16:0SMi/DPPCo/CHOL (*)	240	240	120	120	240	240	129	111	54	46	162	0.44	0.46	0.68	0.65
16:0SMi/DPPCo/CHOL (**)	171	171	84	84	171	171	93	75	56	44	116	0.44	0.46	0.69	0.62
12:0PCi/12:0PCo/CHOL	170	170	85	85	175	165	79	91	47	53	115	0.45	0.45	0.70	0.69
DPPCi/DPPCo//CHOL	170	170	85	85	170	170	87	83	51	49	115	0.45	0.45	0.70	0.68
12:0PCi/DPPCo/CHOL (*)	240	240	120	120	240	240	117	123	49	51	162	0.46	0.45	0.68	0.69
12:0PCi/DPPCo/CHOL (**)	171	171	84	84	171	171	83	85	49	51	115	0.45	0.45	0.69	0.69

Table S5. Partial molar area of cholesterol and two-chain lipids in symmetric DOPC/CHOL and DPPC/CHOL bilayers. The $a(x)$ for the DOPC/CHOL bilayer varies linearly with x implying that the $a_{DOPC}(x)$ and $a_{chol}(x)$ are constants. On the other hand, the $a(x)$ for the DPPC/CHOL bilayer shows some curvature implying that the partial molar areas are not constants for this bilayer.

Lipid Bilayer	Cholesterol mole fraction (x)	System area (nm^2)	$a(x), nm^2$	$a_{chol}(x), nm^2$	$a_{lipid}(x), nm^2$	S_{Chain}
			± 0.01	± 0.01	± 0.01	± 0.02
DOPC/CHOL	0	177.68 ± 1.59	0.69		0.69	0.23
	10	164.25 ± 1.60	0.64	0.21	0.69	0.25
	20	151.16 ± 1.42	0.59	0.21	0.69	0.27
	30	139.03 ± 1.33	0.55	0.21	0.69	0.30
	33	134.92 ± 1.35	0.53	0.21	0.69	0.31
	40	127.09 ± 1.21	0.50	0.21	0.69	0.33
	50	115.96 ± 1.12	0.45	0.21	0.69	0.37
DPPC/CHOL	0	157.78 ± 1.52	0.62		0.62	0.40
	10	143.32 ± 1.49	0.56	0.07	0.61	0.47
	20	129.40 ± 1.24	0.51	0.13	0.60	0.56
	30	118.09 ± 0.93	0.46	0.18	0.58	0.66
	33	114.72 ± 0.85	0.45	0.20	0.58	0.69
	40	109.50 ± 0.67	0.43	0.23	0.56	0.72
	50	101.85 ± 0.42	0.40	0.27	0.53	0.79

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