An Upside Down View of Cholesterol's Condensing Effect: Does Surface Occupancy Play a Role?

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S u p p o r t i n g I n f o r m a t i o n

1. General Information

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker Avance 500 spectrometer at 500 MHz. The chemical shifts are given in parts per million (ppm) on the delta scale (δ). The solvent peak was used as the reference value. For ^{1}H-NMR: $CDCl_3$ = 7.27 ppm. For the proton data: s = singlet, d = doublet, t = triplet, and m = multiplet. Mass spectra were recorded either on an Agilent LC-TOF Mass spectrometer (Model 6210) in mixed ESI-APCI mode or on a Waters GCT coupled to a ChromassSpec LIFDI interface (FD-MS). Fluorescence measurements were made using a Perkin Elmer LS50B Luminescence Spectrometer employing a temperature controlled cell holder. Determination of the exchangeable dimer content in NNR reactions was made by HPLC analysis using a 5 μ, 80 Å, 4.6 x 250 mm Ultrasphere ODS C18 column (Beckman-Coulter) and a Waters Breeze HPLC system consisting of a Waters 717plus Autosampler, Waters 1515 Binary Pump, and Waters 2187 Dual λ Absorbance Detector. The column was placed in an oven (Waters Column Heater

Module, SN: CHM008075 controlled through a Waters 2410 refractive index detector) and the temperature was maintained at 31 °C. The analysis was done in isocratic mode using a mobile phase consisting of 760 ml of EtOH, 120 ml of deionized H_2O, 100 ml of hexane, and 10 ml 1 M *aq*. Bu_4NOAc. The flow-rate was 0.9 ml/min and detection was done at 203 nm. The effect of NNR reactions and 25-OH' content on vesicle size was investigated using a Nicomp Model 270 Submicron Particle Sizer. Typically, 50 μl aliquots were taken from each vessel before and after the exchange reaction. These aliquots were diluted with 300 μl of Tris buffer (pH = 7.4) and analyzed at 45 °C assuming a sample viscosity of 0.5960 centipoise. The photopulse rate was adjusted to ~300 kHz. Vesicle size was evaluated through Gaussian analysis. At least 100000 scans were performed.

The exchangeable dimer **AB** (henceforth referred to as {Ch-16}) was prepared as described previously (see refs. 11b and 27 in the main text). 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG, sodium salt) were obtained from Avanti Polar Lipids (Alabaster, AL, USA). 25-hydroxy-5-cholestene-3β-ol was obtained from Steraloids, Inc. (Newport, RI, USA). Cholesterol was obtained from Aldrich (St. Louis, MO, USA). Other chemicals and solvents used in this study were obtained commercially from various vendors and used without further purification.

25-Hydroxy-5-cholestene-3β-tosylate. To a stirred solution of 95 mg (0.236 mmol) of 25-hydroxy-5-cholestene-3β-ol (Steraloids, Inc., Newport, RI) and 1.5 mg (0.012 mmol) of 4-(N,N-dimethylamino) pyridine in a mixture of 0.45 mL of dry $CHCl_3$ plus 0.45 mL of dry pyridine was added 109 mg (0.552 mmol) of *p*-toluenesulfonylchloride at 0° C. After stirring for 4 h at 0° C, the reaction mixture was stirred for an additional 10 h at room temperature. Subsequent dilution with 10 mL of $CHCl_3$, washing with 5 × 5 mL of aqueous 1M HCl and 5 × 5 mL of H_2O, drying over anhydrous Na_2SO_4, concentration under reduced pressure, followed by purification via preparative thin layer chromatograph [SiO_2, hexanes/$CHCl_3$/ethylacetate, (2/10/2, v/v/v)] afforded 96 mg (72%) of 25-hydroxy-5-cholestene-3β-tosylate having ^{1}H NMR ($CDCl_3$, ppm): 7.80(d,2H); 7.31(d,2H); 5.30(d,1H); 4.30(m,1H); 2.44(s,3H); 2.43 (m,1H); 2.26(m,1H); 0.85-2.00(m,34H); 1.16(s,6H); 0.65(s,3H). TOF MS FD$^+$ (M^+): expected: 579.3479; found 579.3470.

25-Hydroxy-5-cholestene (25-OH'). To a solution of 42.0 mg (0.075 mmol) of 25-hydroxy-5-cholestene-3β-tosylate in 1.5 mL of 1,2-dimethoxyethane was added 120 mg (1.83 mmol) of Zn powder, 120 mg (0.80 mmol) of NaI and 0.100 mL of H_2O. The reaction mixture was stirred under reflux for 5 h, cooled to room temperature, diluted with 20 mL of diethylether and washed with 2 × 3 mL 0.5 M aqueous HCl, 2 × 3 mL of 5% aqueous $NaHCO_3$, 2 × 3 mL of 5% $Na_2S_2O_3$ and 4 × 3 mL of H_2O. The organic phase was dried over anhydrous Na_2SO_3, concentrated under reduced pressure, and the crude product purified by repeated column chromatography [SiO_2, hexane/ethylacetate, (15/1,v/v)] to yield 19 mg (66%) of the title compound having ^{1}H NMR ($CDCl_3$, ppm):

5.25(d,1H); 2.20(t,1H); 1.96(m,3H); 1.82(m,2H); 0.70-1.75(m,30H); 1.21(s,6H); 0.67(s,3H). TOF MS FD^+ (M^+): expected: 386.4; found 386.0.

Monolayer Measurements. All surface pressure-area isotherms were recorded using a Nima 612 D film balance (Nima Technologies, Coventry England) with a subphase containing Tris buffer (10 mM Tris·HCl, 150 mM NaCl, 2 mM NaN_3 and 1 mM EDTA, pH 7.4) that was maintained at 25°C. Mixtures of DMPC/cholesterol and DMPC/25-OH' were made from stock solutions of each lipid dissolved in chloroform. The typical concentration of total lipid that was deposited onto the air/water interface was 1 mg/mL of $CHCl_3/CH_3OH$ (2/1, v/v). Experimental procedures that were used for monolayer measurements were similar to those previously described.[13]

Fluorescence experiments. Liposomes made of 2.5/0/97.5/2.5, 40/0/57.5/2.5, 30/10/57.5/2.5, 20/20/57.5/2.5, and 10/30/57.5/2.5 Chol/25-OH'/DPPC/DPPG (mol/mol/mol/mol, 4.17 μmol total lipid each) plus Laurdan (0.5 mol% with respect to total lipid) were prepared from thin lipid films using methods similar to those used in NNR experiments (see below). Liposomal dispersions were placed in sealed fluorescence cuvettes and the fluorescence of each sample then measured as a function of temperature using a Perkin Elmer LS50B Luminescence Spectrometer employing a temperature controlled cell holder. An excitation wavelength of 350 nm was used, along with an excitation slit width of 7.5 nm. Fluorescence emissions were recorded from 350 to 600 nm using an emission slit width of 7.5 nm. To correct for light scattering, a vertical polarizer was placed on the excitation beam and a horizontal polarizer was placed on the emission beam. Generalized Polarization (GP) values were calculated using the

equation: $GP=(I_{440} - I_{490})/(I_{440} + I_{490})$, where I_{440} and I_{490} are fluorescence emission intensities at 440 and 490 nm respectively.

Nearest-Neighbor Recognition Analysis. Thin films of lipid were prepared by evaporating a chloroform solution containing 0.30 μmol **AB** and 6.9 μmol of DPPC plus 4.5 μmol of a mixture of sterols (Chol/25-OH' being 1/0, 3/1, 1/1, or 1/3 mol/mol) under a stream of argon. After drying the thin film overnight under reduced pressure (0.4 mm Hg), 2.0 mL of a 10mM Tris-HCl buffer (10 mM Tris, 150 mM NaCl, 2 mM NaN_3, 1 mM EDTA, pH = 7.4) was added to each of the dried films. The mixtures were then vortexed for 30 s, incubated for 5 min at 60 °C, vortexed for an additional 30 s, and incubated for an additional 30 min at 60°C with intermittent vortexing. The dispersions were then subjected to six freeze/thaw cycles (liquid nitrogen/60°C water bath) and extruded 20 times through a 200 nm pore diameter polycarbonate (Nuclepore, Whatman Inc.) filter at using Argon at a pressure of ~100 psi.

The vesicle dispersions (1700 µl) were heated to 45 °C, oxygen was removed from them by purging with argon, thiolate-disulfide interchange reactions were then initiated by adding *threo*-dithiothreitol (2 eq. with respect to disulfide content) and sufficient amounts of 0.1 M NaOH to bring the pH to 7.4 at 45 °C. Aliquots (250 μl) were withdrawn as a function of time. The exchange reactions were stopped by adding 25 μL 8.3 M acetic acid to the test tubes containing these aliquots, vortexing, and quickly freezing them using liquid nitrogen. Aliquots handled thus were stored at -20 °C until HPLC analysis. To each thawed aliquot was added 1000 μl of $CHCl_3$/MeOH (2/1, v/v), the tubes were vortexed, centrifuged, and the aqueous phases removed using Pasteur pipettes. Volatiles were removed using a Savant SVC-100 SpeedVac concentrator

equipped with a cold trap and vacuum pump (~1 hr at ~ 0.4 torr). The remaining lipids were dissolved in 20 μl $CHCl_3$ and 80 μl HPLC mobile phase. These samples were then analyzed by HPLC using a C18 reversed phase column and a mobile phase that was composed of 10 mM Bu_4NOAc in ethanol/water/hexane (76/13/10, v/v/v) using a flow rate of 0.9 mL/min. The column was maintained at 31°C and the components were monitored at 203 nm. Values of K (K=[**AB**]^2/([**AA**]×[**BB**])) were calculated from peak areas obtained from the HPLC chromatograms using appropriate calibration curves.

2. NMR Spectra

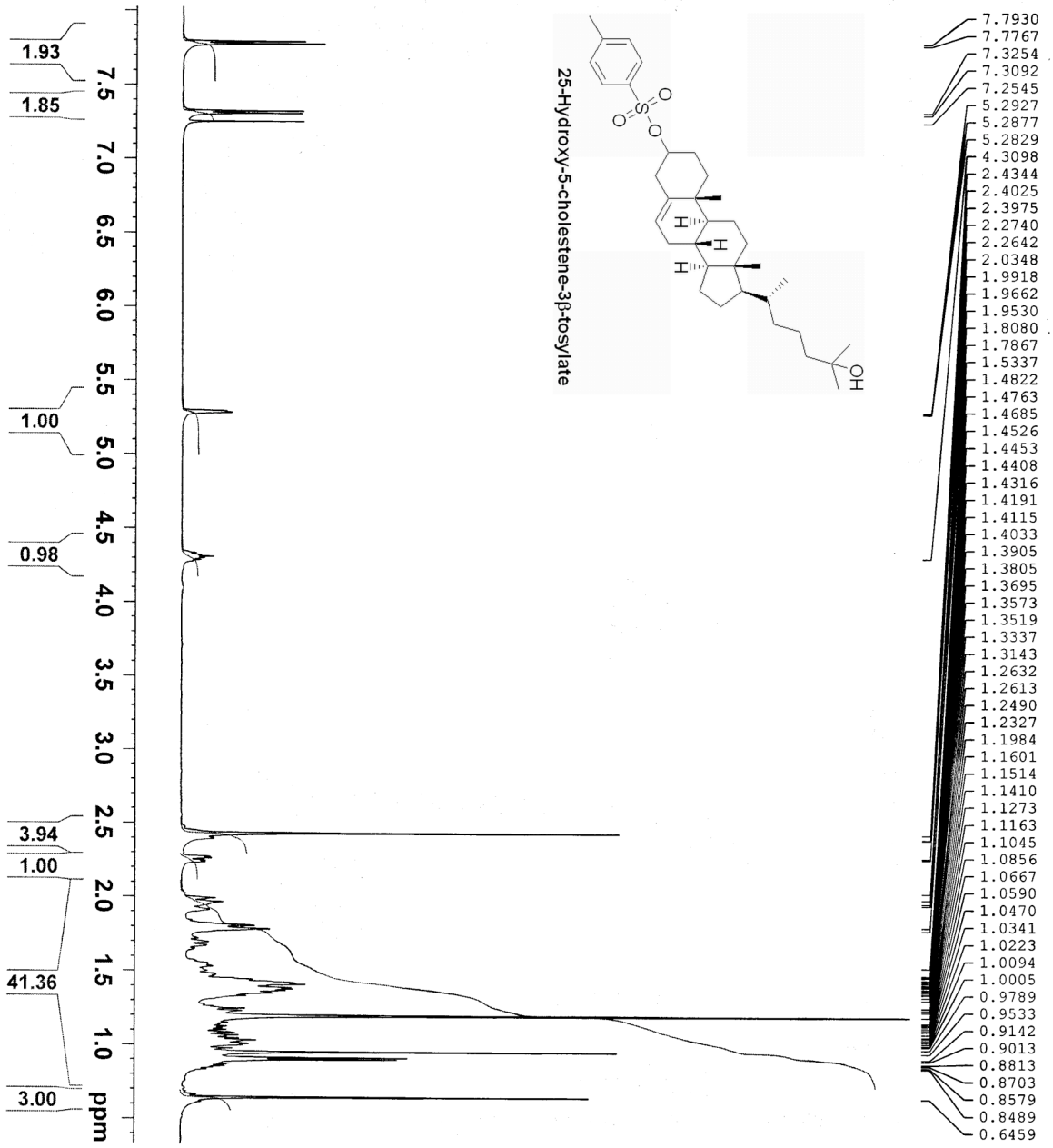

Figure SI-1: ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) for 25-hydroxy-5-cholestene-3β-tosylate.

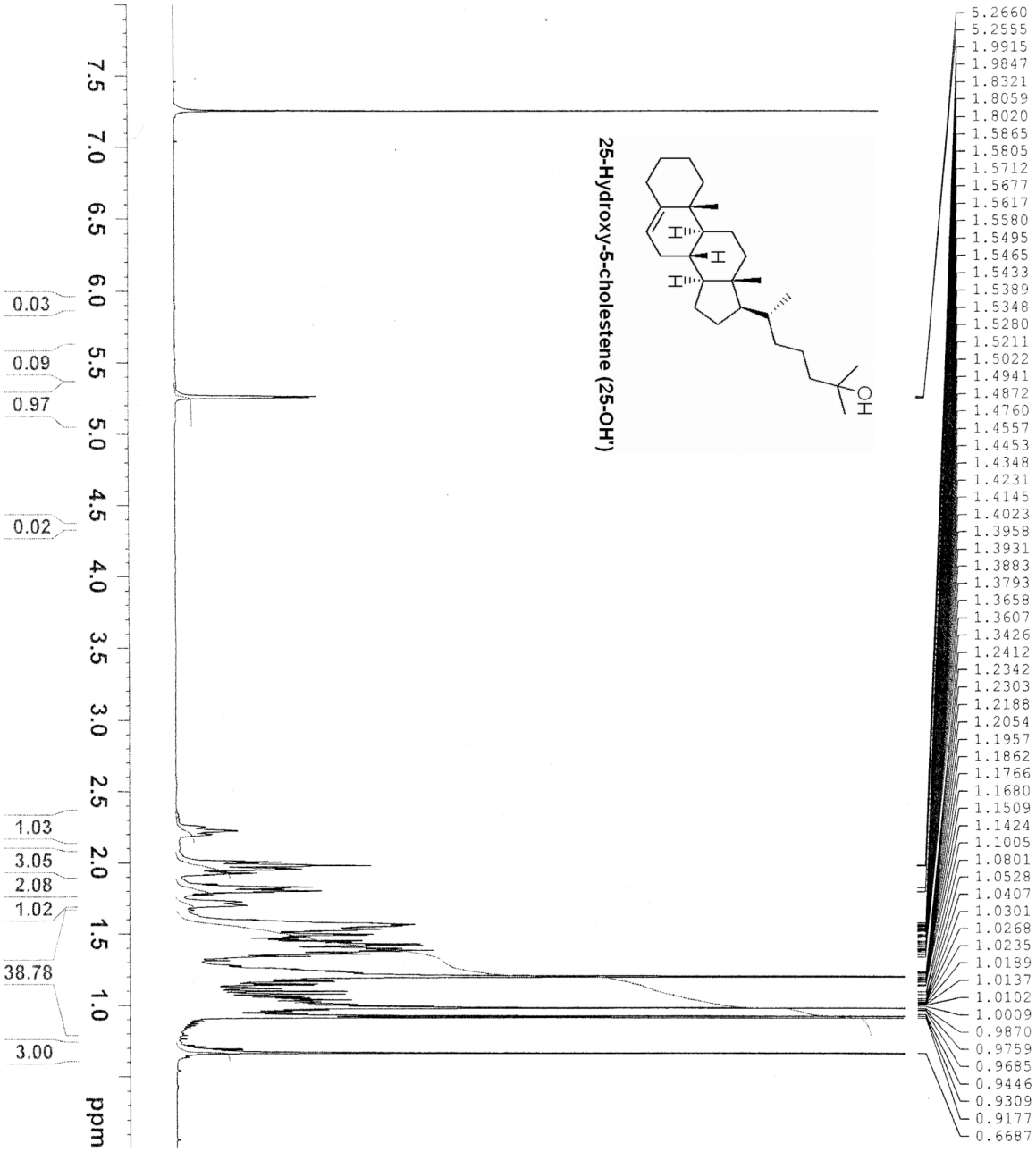

Figure SI-2: ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) for 25-hydroxy-5-cholestene (25-OH').

3. Data for Laurdan General Polarization (GP) Measurements

3.1. Emission Spectra

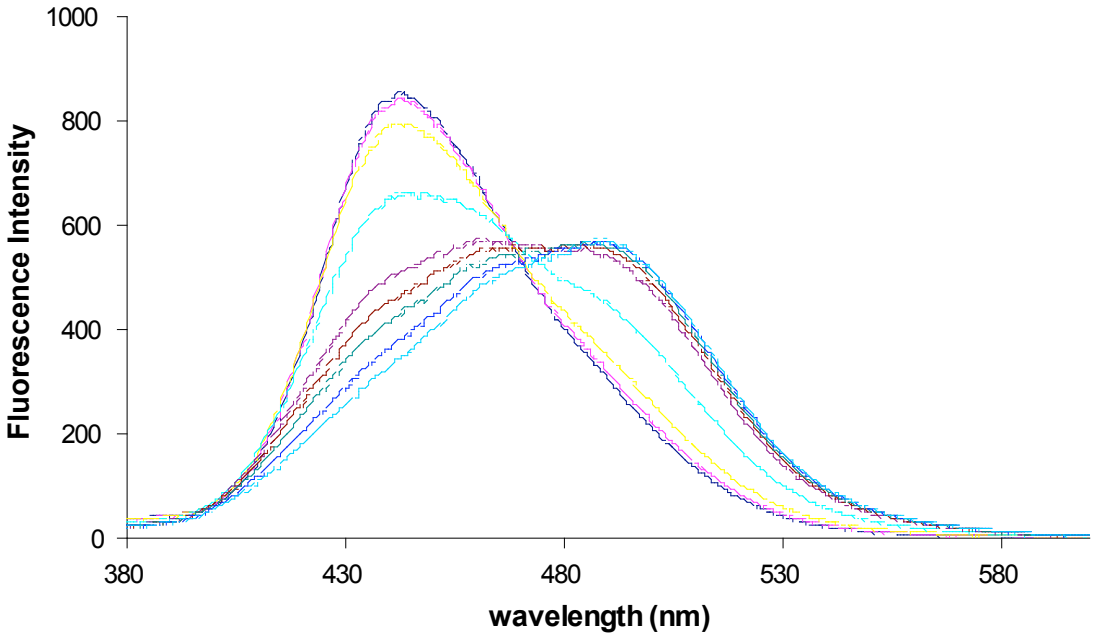

Figure SI-3: Emission spectra for 2.5/95/2.5 (mol/mol/mol) Chol/DPPC/DPPG membranes.

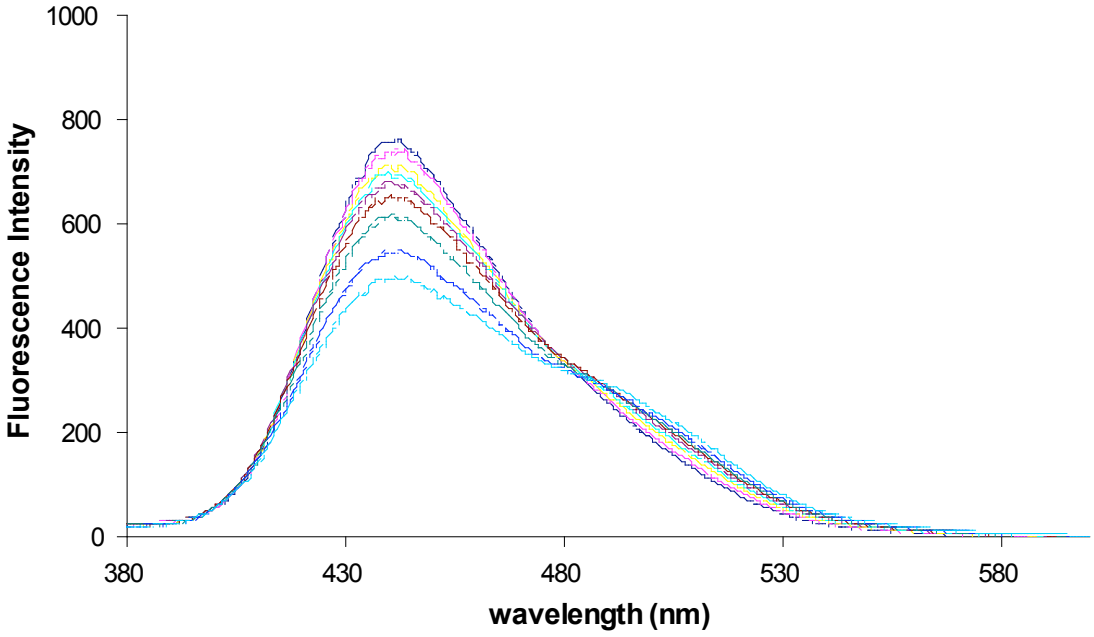

Figure SI-4: Emission spectra for 40/57.5/2.5 (mol/mol/mol) Chol/DPPC/DPPG membranes.

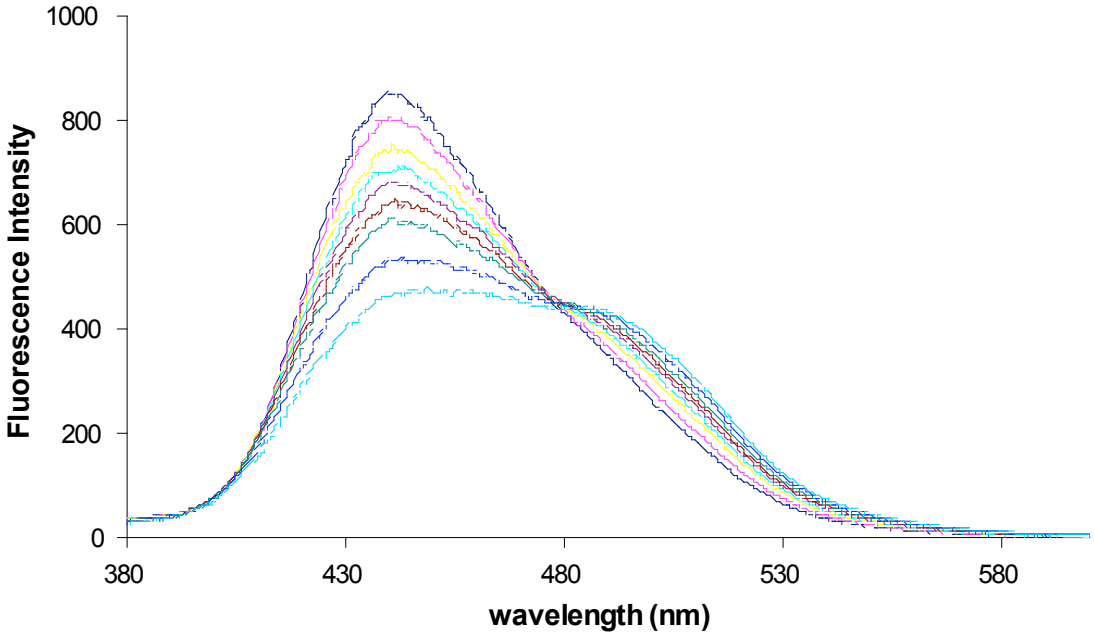

Figure SI-5: Emission spectra for 30/10/57.5/2.5 (mol/mol/mol/mol) 25-OH'/Chol/DPPC/DPPG membranes.

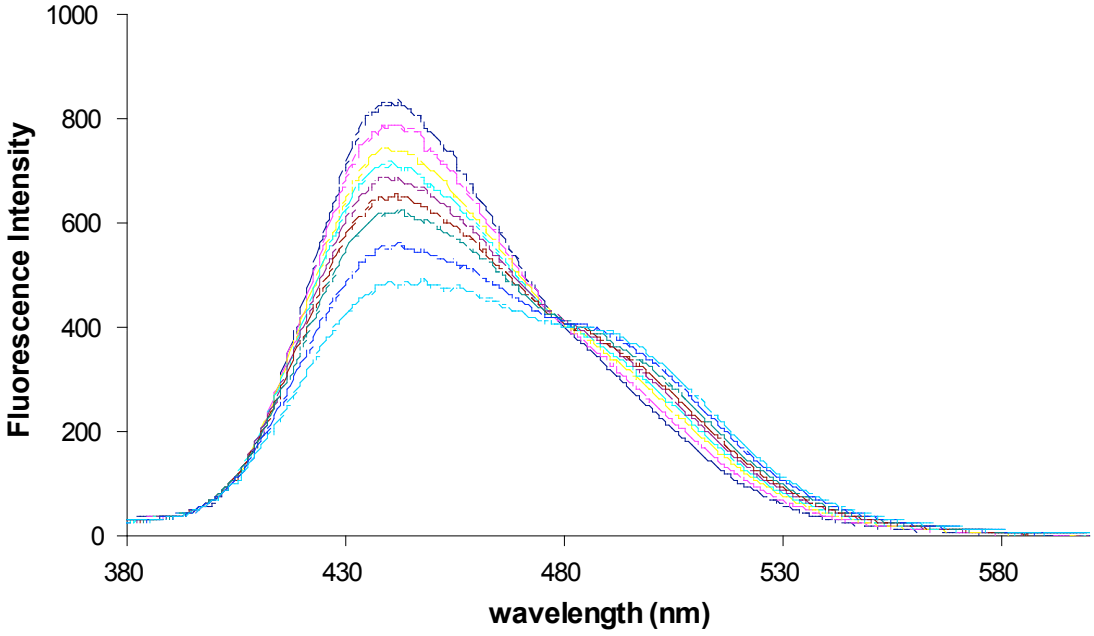

Figure SI-6: Emission spectra for 20/20/57.5/2.5 (mol/mol/mol/mol) 25-OH'/Chol/DPPC/DPPG membranes.

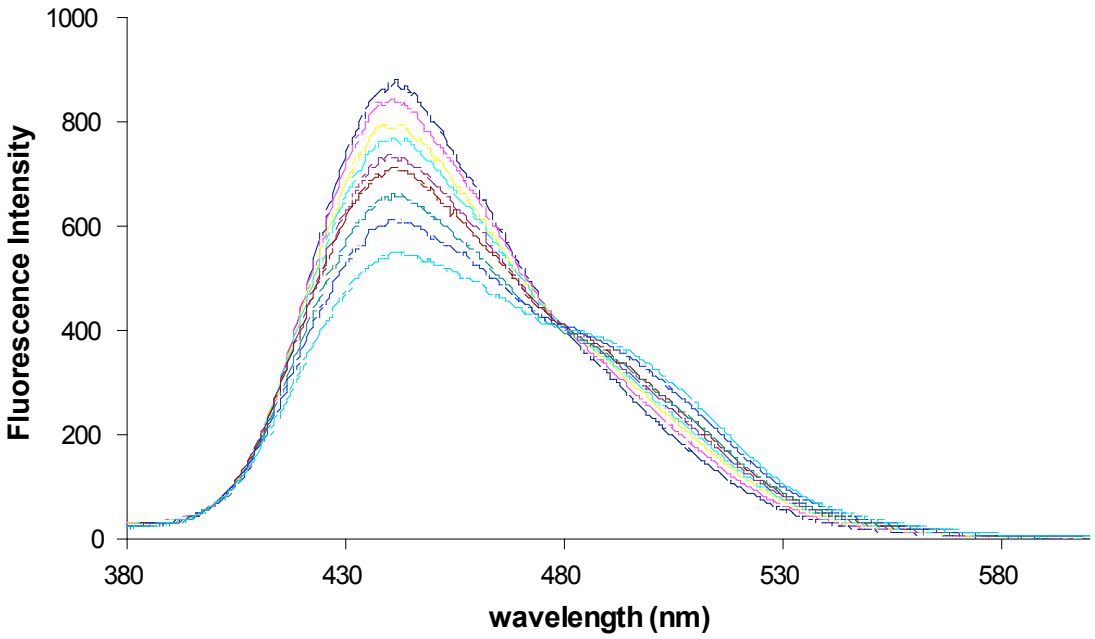

Figure SI-7: Emission spectra for 10/30/57.5/2.5 (mol/mol/mol/mol) 25-OH'/Chol/DPPC/DPPG membranes.

3.2. GP Values

Table SI-1: Laurdan GP values for all liposomes investigated in this study.

2.5 mol% Chol. 95 mol% DPPC 2.5 mol% DPPG		**40 mol% Chol. 57.5 mol% DPPC 2.5 mol% DPPG**		**30 mol% 25-OH' 10 mol% Chol. 57.5 mol% DPPC 2.5 mol% DPPG**		**20 mol% 25-OH' 20 mol% Chol. 57.5 mol% DPPC 2.5 mol% DPPG**		**10 mol% 25-OH' 30 mol% Chol. 57.5 mol% DPPC 2.5 mol% DPPG**	
T (°C)	**GP**	**T (°C)**	**GP**	**T (°C)**	**GP**	**T (°C)**	**GP**	**T (°C)**	**GP**
31.3	0.47	31.3	0.49	29.7	0.42	29.7	0.44	29.6	0.47
34.4	0.45	34.4	0.47	34.6	0.37	34.6	0.40	33.8	0.43
38.4	0.39	38.4	0.45	38.6	0.32	38.6	0.36	37.5	0.40
40.3	0.18	40.3	0.42	40.6	0.28	40.6	0.33	39.6	0.38
42.5	-0.04	42.5	0.41	42.8	0.26	42.8	0.30	41.7	0.35
44.3	-0.10	44.3	0.39	44.8	0.22	44.8	0.28	43.6	0.33
46.5	-0.14	46.5	0.36	46.4	0.18	46.4	0.24	46.1	0.30
50.2	-0.22	50.2	0.31	50.3	0.11	50.3	0.17	49.5	0.24
54.3	-0.27	54.3	0.26	54.6	0.04	54.6	0.11	53.6	0.18

4. NNR Data

Table SI-2: Data for {CH-16} equilibration in 40 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 1). ***K* = 9.47 ± 0.11**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
18	{16-16}	13.03	1017833	2.13	**9.35**
	{Ch-16}	19.95	3620588	6.81	
	{Ch-Ch}	36.39	1469731	2.33	
20	{16-16}	13.04	980262	2.05	**9.45**
	{Ch-16}	19.96	3511656	6.61	
	{Ch-Ch}	36.36	1422642	2.25	
22	{16-16}	13.06	881868	1.85	**9.64**
	{Ch-16}	20.00	3229241	6.08	
	{Ch-Ch}	36.46	1319516	2.08	
24	{16-16}	13.07	973666	2.04	**9.38**
	{Ch-16}	20.01	3488644	6.56	
	{Ch-Ch}	36.50	1425370	2.25	
26	{16-16}	13.07	876909	1.84	**9.44**
	{Ch-16}	20.03	3180427	5.98	
	{Ch-Ch}	36.42	1314855	2.07	
28	{16-16}	13.13	889843	1.86	**9.55**
	{Ch-16}	20.09	3245487	6.11	
	{Ch-Ch}	36.54	1332592	2.10	

Table SI-3: Data for {CH-16} equilibration in 40 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 2). ***K* = 9.36 ± 0.08**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
18	{16-16}	13.13	1066227	2.23	**9.20**
	{Ch-16}	20.11	3812812	7.17	
	{Ch-Ch}	36.61	1573632	2.51	
20	{16-16}	13.20	993768	2.08	**9.39**
	{Ch-16}	20.20	3599097	6.77	
	{Ch-Ch}	36.74	1480071	2.35	
22	{16-16}	13.24	998930	2.09	**9.33**
	{Ch-16}	20.25	3593315	6.76	
	{Ch-Ch}	36.84	1477214	2.34	
24	{16-16}	13.31	985664	2.06	**9.43**
	{Ch-16}	20.35	3543856	6.67	
	{Ch-Ch}	36.97	1443636	2.29	
26	{16-16}	13.37	954918	2.00	**9.39**
	{Ch-16}	20.47	3441893	6.47	
	{Ch-Ch}	37.13	1414060	2.24	
28	{16-16}	13.44	958212	2.01	**9.40**
	{Ch-16}	20.58	3451523	6.49	
	{Ch-Ch}	37.28	1414674	2.24	

Table SI-4: Data for {CH-16} equilibration in 10 mol% 25-OH' + 30 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 1). ***K*** **= 8.90 ± 0.11**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
	{16-16}	12.89	1438701	3.01	
18	{Ch-16}	19.69	4961363	9.32	**9.11**
	{Ch-Ch}	36.29	1965986	3.17	
	{16-16}	12.90	1560656	3.27	
20	{Ch-16}	19.69	5298171	9.95	**8.90**
	{Ch-Ch}	36.36	2109955	3.41	
	{16-16}	12.94	1535518	3.21	
22	{Ch-16}	19.76	5176618	9.73	**8.86**
	{Ch-Ch}	36.46	2058295	3.32	
	{16-16}	13.03	1565588	3.28	
24	{Ch-16}	19.85	5298586	9.96	**8.86**
	{Ch-Ch}	36.61	2112999	3.42	
	{16-16}	12.98	1540104	3.22	
26	{Ch-16}	19.81	5220414	9.81	**8.84**
	{Ch-Ch}	36.54	2089264	3.38	
	{16-16}	12.98	1653077	3.46	
28	{Ch-16}	19.80	5572910	10.47	**8.84**
	{Ch-Ch}	36.53	2212046	3.58	

Table SI-5: Data for {CH-16} equilibration in 10 mol% 25-OH' + 30 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 2). ***K*** **= 8.85 ± 0.03**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
	{16-16}	13.20	1690878	3.54	
18	{Ch-16}	20.16	5764325	10.83	**8.87**
	{Ch-Ch}	37.16	2302319	3.74	
	{16-16}	13.30	1606891	3.36	
20	{Ch-16}	20.30	5445599	10.23	**8.83**
	{Ch-Ch}	37.28	2178090	3.53	
	{16-16}	13.31	1771254	3.71	
22	{Ch-16}	20.30	6000204	11.27	**8.82**
	{Ch-Ch}	37.36	2392206	3.89	
	{16-16}	13.34	1518782	3.18	
24	{Ch-16}	20.38	5160571	9.70	**8.86**
	{Ch-Ch}	37.38	2068345	3.34	
	{16-16}	13.36	1631023	3.41	
26	{Ch-16}	20.41	5509877	10.35	**8.89**
	{Ch-Ch}	37.46	2182126	3.53	
	{16-16}	13.41	1659590	3.47	
28	{Ch-16}	20.49	5624532	10.57	**8.82**
	{Ch-Ch}	37.64	2249206	3.65	

Table SI-6: Data for {CH-16} equilibration in 20 mol% 25-OH' + 20 mol% sterol LUVs at 45 °C using 1.5 equivalents of DTT (Reaction 1). ***K* = 8.37 ± 0.04**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
	{16-16}	12.87	2072937	4.34	
18	{Ch-16}	19.69	6767502	12.71	**8.41**
	{Ch-Ch}	36.63	2713275	4.43	
	{16-16}	12.90	2127861	4.45	
20	{Ch-16}	19.72	6907854	12.97	**8.34**
	{Ch-Ch}	36.68	2774247	4.53	
	{16-16}	12.91	2011166	4.21	
22	{Ch-16}	19.75	6511817	12.23	**8.39**
	{Ch-Ch}	36.70	2599280	4.24	
	{16-16}	12.92	2092222	4.38	
24.5	{Ch-16}	19.76	6827967	12.82	**8.39**
	{Ch-Ch}	36.77	2740044	4.48	
	{16-16}	12.93	1948493	4.08	
27	{Ch-16}	19.78	6320361	11.87	**8.38**
	{Ch-Ch}	36.72	2532031	4.12	
	{16-16}	12.95	2116360	4.43	
28.5	{Ch-16}	19.82	6834866	12.83	**8.29**
	{Ch-Ch}	36.83	2748325	4.49	

Table SI-7: Data for {CH-16} equilibration in 20 mol% 25-OH' + 20 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 2). ***K* = 8.36 ± 0.03**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
	{16-16}	12.96	1935852	4.05	
18	{Ch-16}	19.77	6373976	11.97	**8.34**
	{Ch-Ch}	36.61	2601038	4.24	
	{16-16}	12.97	1862525	3.90	
20	{Ch-16}	19.79	6002537	11.27	**8.32**
	{Ch-Ch}	36.60	2412225	3.92	
	{16-16}	13.01	1909445	3.99	
22	{Ch-16}	19.84	6171420	11.59	**8.37**
	{Ch-Ch}	36.67	2468400	4.02	
	{16-16}	13.03	1885255	3.94	
24	{Ch-16}	19.90	6084264	11.43	**8.37**
	{Ch-Ch}	36.77	2431540	3.95	
	{16-16}	13.04	1912242	4.00	
26	{Ch-16}	19.90	6201653	11.65	**8.40**
	{Ch-Ch}	36.80	2481503	4.04	
	{16-16}	13.07	1858677	3.89	
28	{Ch-16}	19.96	6026741	11.32	**8.38**
	{Ch-Ch}	36.87	2419439	3.93	

Table SI-8: Data for {CH-16} equilibration in 30 mol% 25-OH' + 10 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 1). ***K* = 7.85 ± 0.07**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
	{16-16}	13.09	1844513	3.86	
18	{Ch-16}	20.00	5756950	10.81	**7.75**
	{Ch-Ch}	36.91	2405710	3.91	
	{16-16}	13.12	1714380	3.59	
20	{Ch-16}	20.04	5417535	10.18	**7.91**
	{Ch-Ch}	36.94	2252910	3.65	
	{16-16}	13.14	1717586	3.59	
22	{Ch-16}	20.07	5428949	10.20	**7.87**
	{Ch-Ch}	36.98	2267309	3.68	
	{16-16}	13.16	1574507	3.29	
24	{Ch-16}	20.11	4971541	9.34	**7.92**
	{Ch-Ch}	36.95	2071120	3.35	
	{16-16}	13.14	1723274	3.61	
26	{Ch-16}	20.09	5403483	10.15	**7.79**
	{Ch-Ch}	36.97	2263654	3.67	
	{16-16}	13.18	1664187	3.48	
28	{Ch-16}	20.14	5237549	9.84	**7.85**
	{Ch-Ch}	37.05	2188857	3.54	

Table SI-9: Data for {CH-16} equilibration in 30 mol% 25-OH' + 10 mol% sterol LUVs at 45 °C using 2 equivalents of DTT (Reaction 2). ***K* = 7.83 ± 0.04**.

Reaction Time (h)	Dimer	t_R (min)	Area	N (nmols)	K
	{16-16}	13.50	1947587	4.07	
18	{Ch-16}	20.62	6121917	11.50	**7.83**
	{Ch-Ch}	37.91	2545279	4.15	
	{16-16}	13.61	1857768	3.89	
20	{Ch-16}	20.77	5835198	10.96	**7.86**
	{Ch-Ch}	38.06	2419573	3.93	
	{16-16}	13.62	1860710	3.89	
22	{Ch-16}	20.81	5781927	10.86	**7.76**
	{Ch-Ch}	38.13	2402532	3.91	
	{16-16}	13.71	1878953	3.93	
24	{Ch-16}	20.94	5847325	10.98	**7.84**
	{Ch-Ch}	38.33	2407172	3.91	
	{16-16}	13.79	1903328	3.98	
26	{Ch-16}	21.06	5951101	11.18	**7.83**
	{Ch-Ch}	38.53	2461482	4.01	
	{16-16}	13.86	2012474	4.21	
28	{Ch-16}	21.17	6295210	11.82	**7.85**
	{Ch-Ch}	38.73	2594725	4.23	

Table SI-10: Cumulative *K* and ω_{AB} (cal/mol) values for all NNR experiments.

mol% 25-OH'	mol% Sterol	*K*	ω_{AB} (cal/mol)
0	40	**9.41 ± 0.11**	**-270.15 ± 3.66**
10	30	**8.87 ± 0.08**	**-251.62 ± 2.81**
20	20	**8.36 ± 0.04**	**-232.94 ± 1.34**
30	10	**7.84 ± 0.05**	**-212.39 ± 2.11**

5. DLS Data

Table SI-11: DLS data for vesicles before (t=0 h) and after (t=28 h) NNR reactions. Monomodal size distribution was observed in all cases.

mol% 25-OH'	mol% sterol	Time (h)	Diam. (nm)
0	40	0	181.3 ± 69.6
0	40	28	184.1 ± 54.5
10	30	0	179.9 ± 57.6
10	30	28	180.8 ± 64.5
20	20	0	183.5 ± 61.7
20	20	28.5	180.2 ± 62.1
30	10	0	184.2 ± 65.0
30	10	28	182.9 ± 55.1

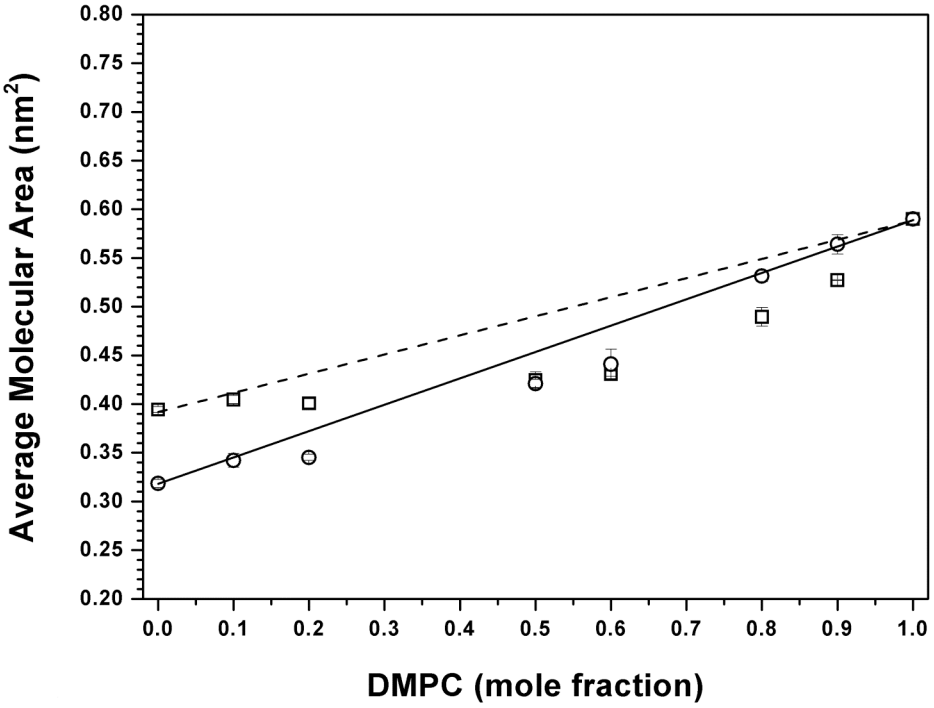

Figure SI-7. Area-additivity curves for (□) DMPC/cholesterol and (○) DMPC/25-OH' with a surface pressure of 25 mN/m. Ideal additivities are shown for (---) DMPC/cholesterol and (—) DMPC/25-OH'.