Loosening and Reorganization of Fluid Phospholipid Bilayers by Chloroform

Serhan Turkyilmaz, Wen-Hua Chen, Hideyuki Mitomo and Steven L. Regen
Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania 18015

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

1. Nearest Neighbor Recognition (NNR) Experiments

1.1. Methods

1.1.1. Preparation of Liposomal Dispersions

The lipsomes (large unilamellar vesicles, LUVs) used in all NNR experiments consisted of 97.5 mol % DPPC/cholesterol and 2.5 mol % of either exchangeable homodimers **AA** and **BB** (at a 1:1 mol ratio, henceforth **AA** will be referred to as {16-16} and **BB** will referred to as {Ch-Ch}) or 2.5 mol % exchangeable homodimer **AB** (henceforth **AB** will be referred to as {Ch-16}). The molecular structures of these lipids are given in Figure SI-1. All of the LUVs having 2.5, 20, 32, and 40 mol % sterol have been prepared using the method detailed below:

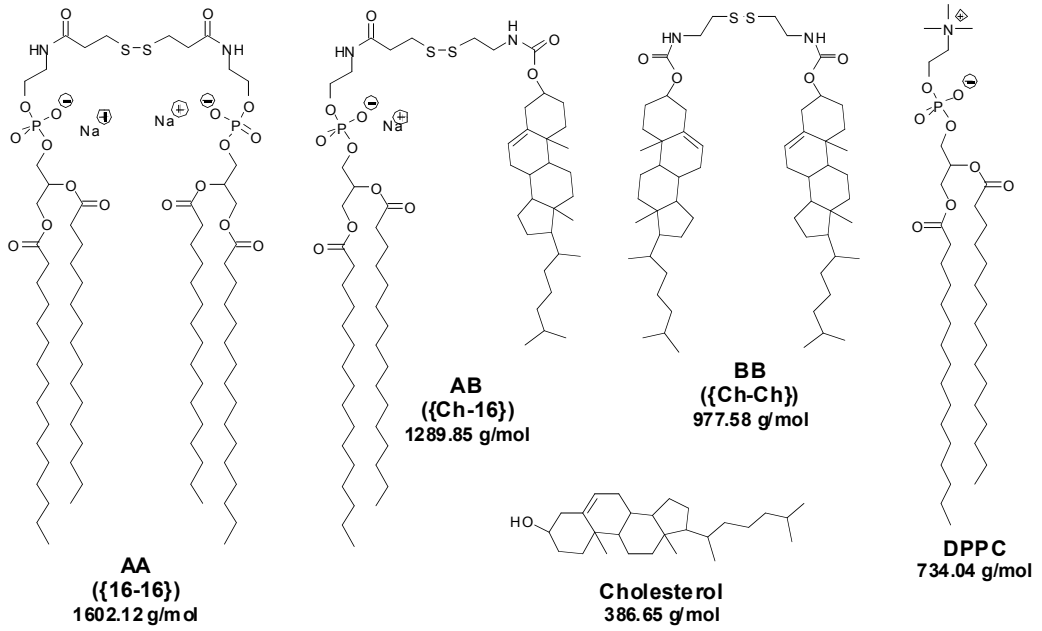

Figure SI-1: Lipids used in NNR experiments.

The amounts of lipids (as stock solutions in $CHCl_3$) given in Table SI-1 were used for film preparation. The given volumes of stock solutions were placed in a 13 x 100 mm test tube (VWR, P.N.: 47729-572), and ~300 µl MeOH was added to the solutions. The solvent was evaporated under a stream of Argon while the test tube was rapidly rotated at an angle of approximately 10° with respect to a flat horizontal surface at ~ 30 °C. A thin film was obtained which covered the inner surface of the test tube up to 1/2 of the height of the tube. A silicone septum was used to seal the mouth of the tube and the tube was wrapped with aluminum foil. The assembly was connected to a vacuum pump via a needle and kept under vacuum (~0.2 torr) overnight (~14 h).

Table SI-1: Lipids used in film preparation for NNR experiments.

	2.5 % Sterol		20 % Sterol		32 % Sterol		40 % Sterol	
	Hetero	**Homo**	**Hetero**	**Homo**	**Hetero**	**Homo**	**Hetero**	**Homo**
{16-16} 1.37 mM	-	110 µl 0.15 µmol	-	110 µl 0.15 µmol	-	110 µl 0.15 µmol	-	110 µl 0.15 µmol
{Ch-16} 1.36 mM	221 µl 0.3 µmol	-	221 µl 0.3 µmol	-	221 µl 0.3 µmol	-	221 µl 0.3 µmol	-
{Ch-Ch} 1.46 mM	-	103 µl 0.15 µmol	-	103 µl 0.15 µmol	-	103 µl 0.15 µmol	-	103 µl 0.15 µmol
DPPC 27.2 mM	430 µl 11.7 µmol	430 µl 11.7 µmol	351 µl 9.54 µmol	351 µl 9.54 µmol	305 µl 8.31 µmol	305 µl 8.31 µmol	261 µl 7.08 µmol	261 µl 7.08 µmol
Cholesterol 20.0 mM	-	-	108 µl 2.16 µmol	108 µl 2.16 µmol	185 µl 3.69 µmol	185 µl 3.69 µmol	231 µl 4.62 µmol	231 µl 4.62 µmol

The lipid film was hydrated with 2 ml Tris-HCl buffer (10 mM Tris, 150 mM NaCl, 2 mM NaN_3, 1 mM EDTA, pH = 7.4) at 60 °C and the mixture was vortexed for 30 s. The mixture was then incubated for 5 min in a 60 °C water bath. The mixture was again vortexed for 30 s followed by a 30 min incubation in a 60 °C water bath. The mixture was then subjected to six freeze/thaw cycles as follows: The test tube was repeatedly immersed in liquid N_2 and then vortexed until a homogeneous thin layer of ice covered the inner surface of the tube. When all of the liquid was frozen the tube was immersed in liquid N_2 until rapid bubble evolution (N_2) in the bath ceased. The tube was then immersed in a 60°C water bath until the frozen multilamellar vesicle (MLV) mixture melted and heated to 60 °C. This freeze/thaw cycle was repeated five more times. The MLVs were then extruded using a Lipex extruder with an argon pressure of 100 psi. Extrusion was done at 60 °C. The MLVs were extruded 20 times through 0.2 µm polycarbonate filters.

1.1.2. Thiolate-Disulfide Exchange Reactions

Thiolate-disulfide exchange reactions were carried out for 2.5, 20, 32, and 40 mol % sterol containing LUVs. For each sterol concentration thiolate-disulfide exchange reactions were done both from the heterodimer side and the homodimer side in the presence and absence of $CHCl_3$. Each reaction was done at least twice. Thus for any given sterol concentration at least eight reactions were performed.

For liquid phase NNR reactions typically 120 µl of 0.84 µM Monensin was added to a 2 ml LUV dispersion. Then, 100 µl of this dispersion was withdrawn and added to 10 µl AcOH/H_2O (1/1, v/v) in a 10 x 75 mm test tube (VWR, P.N.: 60825-402). The tube was rapidly vortexed and the contents were frozen using liquid nitrogen. This was saved as the sample for t = 0 min. A 50 µl aliquot was removed and used for dynamic light scattering (DLS) analysis (see section 1.3.). The remaining dispersion was divided into two portions (950-1000 µl each) and placed into reaction vessels (Wheaton V-Vial, 3 ml volume, PN: 986287) equipped with a small magnetic stir bar and capped with a serrated silicone septum (Aldrich, PN: Z512893). For reactions involving $CHCl_3$ a small test tube (Fisher, P.N.: 14-958A, 0.6 x 50 mm, cut to a height of 26 mm) was also placed in the reaction vessel (Figure SI-2). These reaction vessels were heated to 45 °C and degassed by bubbling Ar through them for 10 min. The argon and bubbler lines were removed and for the reaction involving $CHCl_3$ 250 µl $CHCl_3$ was added into the small test tube within the

reaction vessel. Then, 21 µl of 0.1 M NaOH was added to each dispersion to bring the pH to 7.4 at 45 °C. Thiolate-disulfide exchange was initiated by adding a given volume of 10 mM DTT solution (in Tris buffer) to the reaction. The amount of DTT added (equivalents with respect to exchangeable dimer) depended on the sterol content of the LUVs: 0.8 equivalents for 40 %, 1.2 equivalents for 32 %, 1.2 equivalents for 20 %, and 1.6 equivalents for 2.5 % sterol containing LUVs. An additional 200 µl of $CHCl_3$ was added to the appropriate vessel six hours after the reactions started. The reactions were allowed to proceed at 45 °C for a total of 12 hours after which a 50 µl aliquot was removed and used for DLS analysis (see section 1.3.). The exchange reactions were stopped by adding 100 µl of AcOH/H_2O (1/1, v/v) to each reaction vessel. Four 225 µl aliquots were then withdrawn from each reaction vessel, placed in 10 x 75 mm test tubes (VWR, P.N.: 60825-402), and frozen with liquid N_2.

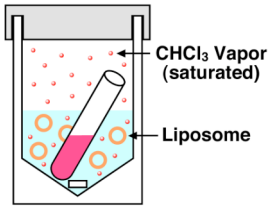

Figure SI-2: Reaction vessel for thiolate-disulfide exchange reactions involving $CHCl_3$.

One set of NNR experiments was carried out in the gel phase for 2.5 mol % sterol membranes. These NNR reactions were performed in a manner similar to above, but the reaction temperature was kept at 35 °C and 0.8 equivalents of DTT (with respect to exchangeable lipid dimers) was used. Gel phase reactions were much slower and needed longer times (24-72 h) to equilibrate.

1.1.3. Determination of Vesicle Size by Dynamic Light Scattering

The effect of the exchange reaction and $CHCl_3$ 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 (in the presence or absence of $CHCl_3$). 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.

1.1.4. HPLC Analysis

1.1.4.1. Chromatographic System

A determination of the dimer content 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 peaks were manually integrated. For a representative chromatogram see Figure SI-3.

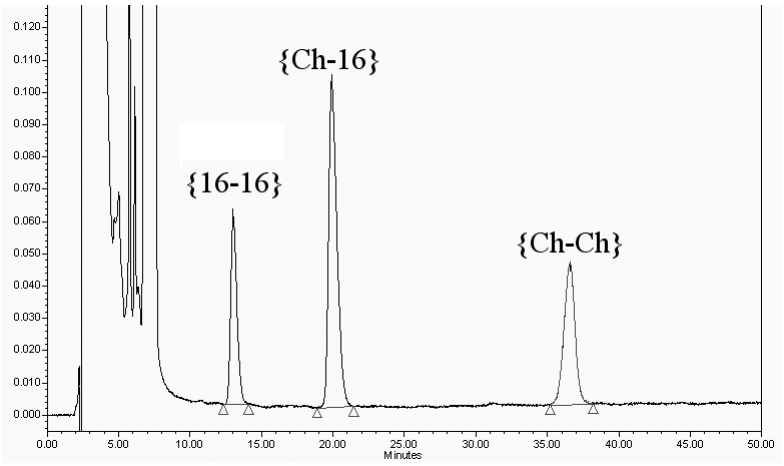

Figure SI-3: A sample chromatogram.

1.1.4.2. Calibration of the Chromatographic System

The chromatographic system was calibrated and the system was found to respond as follows: i. For {16-16} $Signal = 478140 \times n_{\{16-16\}} - 518$ (R^2= 0.9984); ii. For {Ch-16} $Signal = 533520 \times n_{\{Ch-16\}} - 12634$ (R^2 = 0.9988); iii. For {Ch-Ch} $Signal = 591890 \times n_{\{Ch-Ch\}} + 90867$ (R^2 = 0.9988). Calibration data are given in Table SI-2 and the calibration graph is reproduced in Figure SI-4.

Table SI-2: Calibration data for the chromatographic system

	{16-16}			{Ch-16}			{Ch-Ch}	
#	N (nmol)	Area	#	N (nmol)	Area	#	N (nmol)	Area
1	1.37	625466	**20**	1.51	816433	**38**	1.49	940206
2	1.37	659662	**21**	1.51	803229	**39**	1.49	962204
3	1.37	644010	**22**	1.51	807251	**40**	2.98	1844004
4	2.74	1292293	**23**	3.02	1639721	**41**	2.98	1844319
5	2.74	1292591	**24**	3.02	1620989	**42**	4.47	2721145
6	2.74	1355413	**25**	3.02	1528560	**43**	4.47	2804604
7	4.11	1947551	**26**	4.53	2402247	**44**	5.96	3702264
8	4.11	1970380	**27**	4.53	2402983	**45**	5.96	3717866
9	4.11	1987015	**28**	4.53	2405859	**46**	8.94	5153003
10	5.48	2577517	**29**	6.04	3293724	**47**	8.94	5384288
11	5.48	2550765	**30**	6.04	3125143	**48**	14.90	8834895
12	5.48	2668974	**31**	6.04	3146901	**49**	14.90	9041042
13	8.22	3958951	**32**	9.04	4822184			
14	8.22	4084058	**33**	9.06	5004200			
15	8.22	3873902	**34**	9.06	4629127			
16	13.70	6549891	**35**	15.10	7910375			
17	13.70	6336164	**36**	15.10	8122740			
18	13.70	6709740	**37**	15.10	8117872			

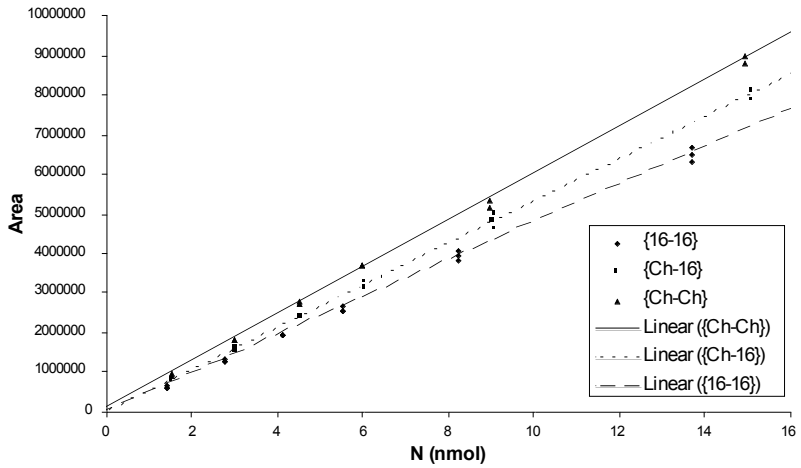

Figure SI-4: Calibration graph for this chromatographic system.

1.1.4.3. HPLC Analysis of Thiolate-Disulfide Exchange Reaction Products

To each sample obtained at the end of section 1.1.2. 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. The organic solvents 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 eluent. Typically, 75 μl injections were done for each sample.

1.2. Results

1.2.1. Vesicle Size

Table SI-3: Mean vesicle diameters from DLS measurements using Gaussian analysis.

	Mean Diameter (nm)					
	Before Reaction		After Reaction without $CHCl_3$		After Reaction with $CHCl_3$	
% Sterol	Hetero	Homo	Hetero	Homo	Hetero	Homo
2.5	180.0	165.5	178.8	167.1	194.5	176.3
	± 50.4	± 51.3	± 57.2	± 51.8	± 52.5	± 58.2
20	194.1	181.7	208.3	183.1	210.0	198.7
	± 69.9	± 61.8	± 83.3	± 60.4	± 73.5	± 43.7
32	211.2	165.7	202.0	181.4	210.3	190.9
	± 76.0	± 54.4	± 72.7	± 66.6	± 67.3	± 44.7
40	195.3	200.4	201.5	197.6	193.0	193.9
	± 66.4	± 66.1	± 74.6	± 63.2	± 67.6	± 62.0

1.2.2. *K* Values

Table SI-4: Summary of *K* values for this study.

Entry	% Sterol	T (°C)	*K* (without $CHCl_3$)	*K* (with $CHCl_3$)
1	2.5	45	3.45 ± 0.66	5.80 ± 0.35
2	20	45	4.21 ± 0.24	5.94 ± 0.27
3	32	45	7.80 ± 0.43	6.06 ± 0.11
4	40	45	9.02 ± 0.48	5.97 ± 0.22
5	2.5	35	0.78 ± 0.03	5.26 ± 0.09

1.2.2.1. *K* values for 2.5 mol % Sterol LUVs at 45 °C

Table SI-5: *K* values for heterodimer equilibration # 1 in 2.5 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	13.16	1482502	3.10	3.82
		{Ch-16}	20.16	3214958	6.05	
		{Ch-Ch}	36.89	1920709	3.09	
2	**720**	{16-16}	13.18	1577429	3.30	3.94
		{Ch-16}	20.19	3450280	6.49	
		{Ch-Ch}	36.96	2007013	3.24	
3	**720**	{16-16}	13.17	1611076	3.37	3.96
		{Ch-16}	20.15	3524892	6.63	
		{Ch-Ch}	36.88	2040211	3.29	
4	**720**	{16-16}	13.16	1564821	3.27	3.91
		{Ch-16}	20.16	3421696	6.44	
		{Ch-Ch}	36.90	2005101	3.23	

Table SI-6: *K* values for heterodimer equilibration # 1 in 2.5 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	13.26	712903	1.49	6.04
		{Ch-16}	20.32	1863807	3.52	
		{Ch-Ch}	36.91	903788	1.37	
2	**720**	{16-16}	13.31	744934	1.56	6.08
		{Ch-16}	20.39	1971021	3.72	
		{Ch-Ch}	37.02	953581	1.46	
3	**720**	{16-16}	13.33	727929	1.52	6.17
		{Ch-16}	20.42	1928755	3.64	
		{Ch-Ch}	37.30	923982	1.41	
4	**720**	{16-16}	13.41	679468	1.42	6.31
		{Ch-16}	20.51	1807467	3.41	
		{Ch-Ch}	37.16	858042	1.30	

Table SI-7: *K* values for heterodimer equilibration # 2 in 2.5 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.02	1441318	3.02	
1	**720**	{Ch-16}	19.80	3545486	6.67	5.16
		{Ch-Ch}	35.04	1781905	2.86	
		{16-16}	13.04	1374531	2.88	
2	**720**	{Ch-16}	19.58	2867855	5.40	3.71
		{Ch-Ch}	34.75	1706540	2.73	
		{16-16}	13.31	1370471	2.87	
3	**720**	{Ch-16}	19.94	2884254	5.43	3.79
		{Ch-Ch}	35.24	1695573	2.71	
		{16-16}	13.51	1426976	2.99	
4	**720**	{Ch-16}	20.29	2974946	5.60	3.79
		{Ch-Ch}	35.84	1731705	2.77	

Table SI-8: *K* values for heterodimer equilibration # 2 in 2.5 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	12.95	552468	1.16	
1	**720**	{Ch-16}	19.43	1358671	2.57	5.73
		{Ch-Ch}	34.37	681150	1.00	
		{16-16}	13.27	551632	1.15	
2	**720**	{Ch-16}	19.86	1330788	2.52	6.11
		{Ch-Ch}	34.85	622650	0.90	
		{16-16}	13.34	550001	1.15	
3	**720**	{Ch-16}	20.02	1328483	2.51	5.91
		{Ch-Ch}	35.19	640140	0.93	
		{16-16}	13.72	567344	1.19	
4	**720**	{Ch-16}	20.64	1349842	2.55	5.98
		{Ch-Ch}	36.05	634444	0.92	

Table SI-9: *K* values for homodimer equilibration # 1 in 2.5 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	12.90	1383132	2.89	
1	**720**	{Ch-16}	19.41	2493696	4.70	2.86
		{Ch-Ch}	34.43	1670228	2.67	
		{16-16}	13.11	1357872	2.84	
2	**720**	{Ch-16}	19.74	2478667	4.67	2.97
		{Ch-Ch}	34.96	1618343	2.58	
		{16-16}	13.35	1386333	2.90	
3	**720**	{Ch-16}	20.09	2475879	4.66	2.83
		{Ch-Ch}	35.45	1661722	2.65	
		{16-16}	13.85	1382674	2.89	
4	**720**	{Ch-16}	20.90	2469979	4.65	2.87
		{Ch-Ch}	36.66	1632927	2.61	

Table SI-10: *K* values for homodimer equilibration # 1 in 2.5 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.05	590947	1.24	
1	**720**	{Ch-16}	19.62	1346779	2.55	4.88
		{Ch-Ch}	34.60	727463	1.08	
		{16-16}	13.53	635594	1.33	
2	**720**	{Ch-16}	20.17	1446993	2.74	5.45
		{Ch-Ch}	35.27	701970	1.03	
		{16-16}	13.54	566900	1.19	
3	**720**	{Ch-16}	20.32	1312841	2.48	5.61
		{Ch-Ch}	35.57	639125	0.93	
		{16-16}	14.34	613650	1.28	
4	**720**	{Ch-16}	21.58	1434707	2.71	5.93
		{Ch-Ch}	37.49	662850	0.97	

Table SI-11: *K* values for homodimer equilibration # 2 in 2.5 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.51	1476118	3.09	
1	**720**	{Ch-16}	22.15	2711061	5.11	2.90
		{Ch-Ch}	39.03	1810590	2.91	
		{16-16}	14.43	1540043	3.22	
2	**720**	{Ch-16}	22.05	2809665	5.29	2.92
		{Ch-Ch}	38.94	1849159	2.97	
		{16-16}	14.46	1462956	3.06	
3	**720**	{Ch-16}	22.10	2640730	4.97	2.84
		{Ch-Ch}	39.07	1777849	2.85	
		{16-16}	14.51	1428418	2.99	
4	**720**	{Ch-16}	22.18	2653750	5.00	2.95
		{Ch-Ch}	39.15	1767435	2.83	

Table SI-12: *K* values for homodimer equilibration # 2 in 2.5 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.66	569399	1.19	
1	**720**	{Ch-16}	22.33	1370444	2.59	5.42
		{Ch-Ch}	39.15	706642	1.04	
		{16-16}	14.66	573064	1.20	
2	**720**	{Ch-16}	22.35	1388626	2.63	5.77
		{Ch-Ch}	39.19	681151	1.00	
		{16-16}	14.67	571572	1.20	
3	**720**	{Ch-16}	22.39	1373561	2.60	5.71
		{Ch-Ch}	39.22	676200	0.99	
		{16-16}	14.71	596310	1.25	
4	**720**	{Ch-16}	22.42	1414434	2.67	5.71
		{Ch-Ch}	39.27	684767	1.00	

Table SI-13: Overall *K* values for 2.5 mol % sterol LUVs (45 °C).

	K values		
	Hetero	**Homo**	**Cumulative**
No $CHCl_3$	4.01 ± 0.47	2.89 ± 0.05	3.45 ± 0.66
With $CHCl_3$	6.04 ± 0.18	5.56 ± 0.32	5.80 ± 0.35

1.2.2.2. *K* values for 20 mol % Sterol LUVs at 45 °C

Table SI-14: *K* values for heterodimer equilibration # 1 in 20 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	12.82	1776252	3.72	4.51
		{Ch-16}	19.58	4171973	7.84	
		{Ch-Ch}	36.12	2263322	3.67	
2	**720**	{16-16}	12.79	1772557	3.71	4.41
		{Ch-16}	19.54	4191395	7.88	
		{Ch-Ch}	36.10	2339333	3.80	
3	**720**	{16-16}	12.91	1781386	3.73	4.48
		{Ch-16}	19.72	4133527	7.77	
		{Ch-Ch}	36.35	2234248	3.62	
4	**720**	{16-16}	12.99	1828864	3.83	4.52
		{Ch-16}	19.86	4351047	8.18	
		{Ch-Ch}	36.57	2382634	3.87	

Table SI-15: *K* values for heterodimer equilibration # 1 in 20 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	12.92	813120	1.70	6.17
		{Ch-16}	19.73	2175206	4.10	
		{Ch-Ch}	36.10	1038674	1.60	
2	**720**	{16-16}	12.88	771511	1.61	5.95
		{Ch-16}	19.72	2093633	3.95	
		{Ch-Ch}	36.04	1050472	1.62	
3	**720**	{16-16}	13.00	850341	1.78	6.14
		{Ch-16}	19.86	2258103	4.26	
		{Ch-Ch}	36.28	1071584	1.66	
4	**720**	{16-16}	13.12	704522	1.47	6.37
		{Ch-16}	20.07	1909197	3.60	
		{Ch-Ch}	36.51	908049	1.38	

Table SI-16: *K* values for heterodimer equilibration # 2 in 20 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	12.95	1592478	3.33	
1	**720**	{Ch-16}	19.83	3668034	6.90	4.35
		{Ch-Ch}	36.50	2034729	3.28	
		{16-16}	12.98	1729399	3.62	
2	**720**	{Ch-16}	19.86	3974317	7.47	4.33
		{Ch-Ch}	36.56	2199372	3.56	
		{16-16}	13.00	1677591	3.51	
3	**720**	{Ch-16}	19.88	3874950	7.29	4.32
		{Ch-Ch}	36.57	2165369	3.50	
		{16-16}	13.00	1504335	3.15	
4	**720**	{Ch-16}	19.92	3444705	6.48	4.40
		{Ch-Ch}	36.57	1887144	3.03	

Table SI-17: *K* values for heterodimer equilibration # 2 in 20 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.05	993886	2.08	
1	**720**	{Ch-16}	19.98	2658138	5.01	6.05
		{Ch-Ch}	36.54	1270329	1.99	
		{16-16}	13.08	948797	1.99	
2	**720**	{Ch-16}	20.03	2524125	4.75	6.04
		{Ch-Ch}	36.50	1207496	1.89	
		{16-16}	13.09	1041706	2.18	
3	**720**	{Ch-16}	20.04	2740669	5.16	5.86
		{Ch-Ch}	36.65	1323949	2.08	
		{16-16}	13.13	1021227	2.14	
4	**720**	{Ch-16}	20.10	2685498	5.06	6.11
		{Ch-Ch}	36.74	1250879	1.96	

Table SI-18: *K* values for homodimer equilibration # 1 in 20 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	12.81	1774505	3.71	
1	**720**	{Ch-16}	19.59	4070730	7.65	4.12
		{Ch-Ch}	36.12	2356991	3.83	
		{16-16}	12.86	1789207	3.74	
2	**720**	{Ch-16}	19.45	4087590	7.69	4.14
		{Ch-Ch}	36.20	2345616	3.81	
		{16-16}	12.95	1721616	3.60	
3	**720**	{Ch-16}	19.81	3950005	7.43	4.11
		{Ch-Ch}	36.43	2297548	3.73	
		{16-16}	13.02	1802736	3.77	
4	**720**	{Ch-16}	19.90	4112778	7.73	4.11
		{Ch-Ch}	36.57	2374582	3.86	

Table SI-19: *K* values for homodimer equilibration # 1 in 20 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	12.88	724445	1.52	
1	**720**	{Ch-16}	19.70	1914298	3.61	5.88
		{Ch-Ch}	35.95	956594	1.46	
		{16-16}	12.98	708531	1.48	
2	**720**	{Ch-16}	19.85	1910866	3.61	6.25
		{Ch-Ch}	36.22	921522	1.40	
		{16-16}	13.07	759867	1.59	
3	**720**	{Ch-16}	19.99	1972263	3.72	5.95
		{Ch-Ch}	36.43	956569	1.46	
		{16-16}	13.11	729807	1.53	
4	**720**	{Ch-16}	20.05	1904353	3.59	6.10
		{Ch-Ch}	36.51	911487	1.39	

Table SI-20: *K* values for homodimer equilibration # 2 in 20 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.13	1710274	3.58	
1	**720**	{Ch-16}	20.09	3762554	7.08	3.89
		{Ch-Ch}	36.76	2219756	3.60	
		{16-16}	13.12	1744164	3.65	
2	**720**	{Ch-16}	20.94	3883779	7.30	3.88
		{Ch-Ch}	38.82	2321722	3.77	
		{16-16}	13.40	1558221	3.26	
3	**720**	{Ch-16}	20.42	3469063	6.53	3.94
		{Ch-Ch}	37.12	2055544	3.32	
		{16-16}	13.27	1458968	3.05	
4	**720**	{Ch-16}	20.32	3199385	6.02	3.83
		{Ch-Ch}	37.09	1926250	3.10	

Table SI-21: *K* values for homodimer equilibration # 2 in 20 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.43	1109374	2.32	
1	**720**	{Ch-16}	20.52	2905389	5.47	5.46
		{Ch-Ch}	37.31	1486750	2.36	
		{16-16}	13.43	1068771	2.24	
2	**720**	{Ch-16}	20.53	2800763	5.27	5.62
		{Ch-Ch}	37.29	1399339	2.21	
		{16-16}	13.49	1158903	2.42	
3	**720**	{Ch-16}	20.62	2899944	5.46	5.57
		{Ch-Ch}	37.46	1397343	2.21	
		{16-16}	13.58	1144019	2.39	
4	**720**	{Ch-16}	20.73	2918796	5.49	5.51
		{Ch-Ch}	37.57	1445491	2.29	

Table SI-22: Overall *K* values for 20 mol % sterol LUVs (45 °C)

	K values		
	Hetero	**Homo**	**Cumulative**
No $CHCl_3$	4.41 ± 0.08	4.00 ± 0.13	4.21 ± 0.24
With $CHCl_3$	6.09 ± 0.15	5.79 ± 0.29	5.94 ± 0.27

1.2.2.3. *K* values for 32 mol % Sterol LUVs at 45 °C

Table SI-23: *K* values for heterodimer equilibration # 1 in 32 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	13.44	1157861	2.42	8.18
		{Ch-16}	20.53	3715856	6.99	
		{Ch-Ch}	37.47	1550326	2.47	
2	**720**	{16-16}	13.43	1254727	2.63	8.16
		{Ch-16}	20.51	4119796	7.75	
		{Ch-Ch}	37.44	1748146	2.80	
3	**720**	{16-16}	13.47	1275164	2.67	8.19
		{Ch-16}	20.57	4093439	7.70	
		{Ch-Ch}	37.58	1695499	2.71	
4	**720**	{16-16}	13.55	1251730	2.62	8.14
		{Ch-16}	20.69	4005941	7.53	
		{Ch-Ch}	37.74	1666676	2.66	

Table SI-24: *K* values for heterodimer equilibration # 1 in 32 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	13.64	972596	2.04	6.08
		{Ch-16}	20.90	2610665	4.92	
		{Ch-Ch}	37.86	1248039	1.96	
2	**720**	{16-16}	13.73	904898	1.89	6.13
		{Ch-16}	21.04	2461862	4.64	
		{Ch-Ch}	38.01	1187787	1.85	
3	**720**	{16-16}	13.76	955543	2.00	6.01
		{Ch-16}	21.08	2533537	4.77	
		{Ch-Ch}	38.13	1211783	1.89	
4	**720**	{16-16}	13.90	871084	1.82	6.07
		{Ch-16}	21.29	2371516	4.47	
		{Ch-Ch}	38.38	1159931	1.81	

Table SI-25: *K* values for heterodimer equilibration # 2 in 32 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.93	1231955	2.58	
1	**720**	{Ch-16}	21.28	3982094	7.49	8.23
		{Ch-Ch}	38.64	1655775	2.64	
		{16-16}	14.03	1346664	2.82	
2	**720**	{Ch-16}	21.43	4411760	8.29	8.26
		{Ch-Ch}	38.90	1840540	2.96	
		{16-16}	14.13	1257021	2.63	
3	**720**	{Ch-16}	21.59	4075910	7.66	8.15
		{Ch-Ch}	39.10	1711528	2.74	
		{16-16}	14.27	1365686	2.86	
4	**720**	{Ch-16}	21.78	4402506	8.28	8.23
		{Ch-Ch}	39.36	1814396	2.91	

Table SI-26: *K* values for heterodimer equilibration # 2 in 32 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.44	882904	1.85	
1	**720**	{Ch-16}	22.14	2379747	4.48	6.13
		{Ch-Ch}	39.67	1142491	1.78	
		{16-16}	14.71	879110	1.84	
2	**720**	{Ch-16}	22.57	2328950	4.39	6.12
		{Ch-Ch}	40.24	1102994	1.71	
		{16-16}	15.01	870498	1.82	
3	**720**	{Ch-16}	23.01	2346874	4.42	6.13
		{Ch-Ch}	40.95	1126970	1.75	
		{16-16}	15.36	879464	1.84	
4	**720**	{Ch-16}	23.57	2341272	4.41	5.92
		{Ch-Ch}	41.81	1147608	1.79	

Table SI-27: *K* values for homodimer equilibration # 1 in 32 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.02	1232709	2.58	
1	**720**	{Ch-16}	19.87	3836960	7.22	7.19
		{Ch-Ch}	36.83	1752291	2.81	
		{16-16}	13.06	1231887	2.58	
2	**720**	{Ch-16}	19.93	3837145	7.22	7.39
		{Ch-Ch}	36.95	1708567	2.73	
		{16-16}	13.05	1219953	2.55	
3	**720**	{Ch-16}	19.96	3773792	7.10	7.22
		{Ch-Ch}	36.96	1709524	2.73	
		{16-16}	13.14	1161464	2.43	
4	**720**	{Ch-16}	20.05	3658865	6.88	7.50
		{Ch-Ch}	37.08	1628866	2.60	

Table SI-28: *K* values for homodimer equilibration # 1 in 32 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.51	734195	1.54	
1	**720**	{Ch-16}	20.67	1971606	3.72	5.85
		{Ch-Ch}	37.73	1001304	1.54	
		{16-16}	13.51	758561	1.59	
2	**720**	{Ch-16}	20.71	2066349	3.90	6.20
		{Ch-Ch}	37.76	1003425	1.54	
		{16-16}	13.59	752450	1.57	
3	**720**	{Ch-16}	20.80	2083062	3.93	6.06
		{Ch-Ch}	37.95	1048032	1.62	
		{16-16}	13.66	766647	1.60	
4	**720**	{Ch-16}	20.88	2083556	3.93	6.04
		{Ch-Ch}	38.04	1034413	1.59	

Table SI-29: *K* values for homodimer equilibration # 2 in 32 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.16	1215616	2.54	
1	**720**	{Ch-16}	20.09	3821953	7.19	7.35
		{Ch-Ch}	37.11	1727053	2.76	
		{16-16}	13.22	1200796	2.51	
2	**720**	{Ch-16}	20.16	3730361	7.02	7.36
		{Ch-Ch}	37.22	1665505	2.66	
		{16-16}	13.24	1163811	2.44	
3	**720**	{Ch-16}	20.23	3753720	7.06	7.75
		{Ch-Ch}	37.32	1654765	2.64	
		{16-16}	13.27	1213555	2.54	
4	**720**	{Ch-16}	20.25	3832851	7.21	7.46
		{Ch-Ch}	37.40	1714661	2.74	

Table SI-30: *K* values for homodimer equilibration # 2 in 32 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.80	717393	1.50	
1	**720**	{Ch-16}	21.11	1948631	3.68	6.17
		{Ch-Ch}	38.35	953608	1.46	
		{16-16}	13.68	728273	1.52	
2	**720**	{Ch-16}	20.94	1950143	3.68	5.87
		{Ch-Ch}	38.10	986841	1.51	
		{16-16}	13.82	771011	1.61	
3	**720**	{Ch-16}	21.16	2112253	3.98	6.19
		{Ch-Ch}	38.49	1031402	1.59	
		{16-16}	13.93	824919	1.73	
4	**720**	{Ch-16}	21.30	2179289	4.11	5.92
		{Ch-Ch}	38.67	1067759	1.65	

Table SI-31: Overall *K* values for 32 mol % sterol LUVs (45 °C).

	K values		
	Hetero	**Homo**	**Cumulative**
No $CHCl_3$	8.19 ± 0.04	7.40 ± 0.18	7.80 ± 0.43
With $CHCl_3$	6.07 ± 0.07	6.04 ± 0.14	6.06 ± 0.11

1.2.2.4. *K* values for 40 mol % Sterol LUVs at 45 °C

Table SI-32: *K* values for heterodimer equilibration # 1 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	14.60	1110311	2.32	9.36
		{Ch-16}	22.18	4052154	7.62	
		{Ch-Ch}	39.41	1671336	2.67	
2	**720**	{16-16}	15.12	1180893	2.47	9.53
		{Ch-16}	22.82	4152471	7.81	
		{Ch-Ch}	40.23	1622083	2.59	
3	**720**	{16-16}	14.96	1101632	2.31	9.48
		{Ch-16}	22.75	4031768	7.58	
		{Ch-Ch}	40.32	1647841	2.63	
4	**720**	{16-16}	15.41	1167786	2.44	8.85
		{Ch-16}	23.45	4128400	7.76	
		{Ch-Ch}	41.19	1740641	2.79	

Table SI-33: *K* values for heterodimer equilibration # 1 in 40 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**720**	{16-16}	14.68	1077535	2.25	6.33
		{Ch-16}	22.35	2955536	5.56	
		{Ch-Ch}	39.46	1374368	2.17	
2	**720**	{16-16}	15.05	1177869	2.46	5.77
		{Ch-16}	22.97	2867616	5.40	
		{Ch-Ch}	40.30	1304322	2.05	
3	**720**	{16-16}	15.03	1053514	2.20	6.20
		{Ch-16}	22.93	2961160	5.57	
		{Ch-Ch}	40.93	1437084	2.27	
4	**720**	{16-16}	15.37	1073088	2.25	6.21
		{Ch-16}	23.47	2835775	5.34	
		{Ch-Ch}	41.15	1300123	2.04	

Table SI-34: *K* values for heterodimer equilibration # 2 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	18.94	1683884	3.52	
1	**450**	{Ch-16}	28.07	5506522	10.34	9.29
		{Ch-Ch}	46.73	2027043	3.27	
		{16-16}	19.03	1541859	3.23	
2	**450**	{Ch-16}	28.21	5066611	9.52	9.01
		{Ch-Ch}	46.95	1936759	3.12	

Table SI-35: *K* values for heterodimer equilibration # 2 in 40 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	18.86	1920776	4.02	
1	**360**	{Ch-16}	28.00	5112772	9.61	6.13
		{Ch-Ch}	46.55	2307982	3.75	
		{16-16}	18.83	1897491	3.97	
2	**435**	{Ch-16}	27.97	4977865	9.35	5.87
		{Ch-Ch}	46.47	2311919	3.75	
		{16-16}	18.98	1792450	3.75	
3	**540**	{Ch-16}	28.22	4820544	9.06	5.79
		{Ch-Ch}	47.38	2327549	3.78	
		{16-16}	19.21	2024841	4.24	
4	**450**	{Ch-16}	28.46	5384412	10.12	5.81
		{Ch-Ch}	47.49	2553085	4.16	
		{16-16}	19.93	2012461	4.21	
5	**450**	{Ch-16}	29.66	5125794	9.63	5.85
		{Ch-Ch}	48.98	2321232	3.77	
		{16-16}	20.14	1803540	3.77	
6	**450**	{Ch-16}	30.06	5075595	9.54	6.50
		{Ch-Ch}	49.44	2284794	3.71	
		{16-16}	20.00	1680343	3.52	
7	**450**	{Ch-16}	29.56	4257157	8.00	5.59
		{Ch-Ch}	48.51	2021665	3.26	

Table SI-36: *K* values for heterodimer equilibration # 3 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.20	1798323	3.76	
1	**300**	{Ch-16}	21.35	5904167	11.09	9.04
		{Ch-Ch}	37.92	2231473	3.62	
		{16-16}	15.76	1961614	4.10	
2	**360**	{Ch-16}	23.54	6513669	12.23	8.94
		{Ch-Ch}	40.46	2504919	4.08	
		{16-16}	15.53	1820075	3.81	
3	**420**	{Ch-16}	23.22	6012837	11.29	8.89
		{Ch-Ch}	39.82	2322319	3.77	
		{16-16}	15.27	1917942	4.01	
4	**480**	{Ch-16}	22.85	6351716	11.93	8.95
		{Ch-Ch}	39.37	2436421	3.96	

Table SI-37: *K* values for heterodimer equilibration # 4 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.25	1790184	3.75	
1	**450**	{Ch-16}	21.37	5840794	10.97	8.80
		{Ch-Ch}	38.14	2251925	3.65	
		{16-16}	14.26	1896290	3.97	
2	**450**	{Ch-16}	21.45	6775121	12.72	9.91
		{Ch-Ch}	38.43	2527679	4.12	
		{16-16}	14.44	1899243	3.97	
3	**450**	{Ch-16}	21.67	6689352	12.56	9.28
		{Ch-Ch}	38.64	2624705	4.28	
		{16-16}	14.12	1257535	2.63	
4	**450**	{Ch-16}	21.22	4250280	7.99	9.49
		{Ch-Ch}	37.51	1603545	2.56	

Table SI-38: *K* values for homodimer equilibration # 1 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.36	973998	2.04	
1	**720**	{Ch-16}	20.41	3133204	5.90	8.40
		{Ch-Ch}	37.52	1292417	2.03	
		{16-16}	13.34	1006472	2.11	
2	**720**	{Ch-16}	20.36	3213465	6.05	8.43
		{Ch-Ch}	37.46	1310497	2.06	
		{16-16}	13.38	1096218	2.29	
3	**720**	{Ch-16}	20.43	3580212	6.73	8.47
		{Ch-Ch}	37.62	1471865	2.33	
		{16-16}	13.45	1078783	2.26	
4	**720**	{Ch-16}	20.54	3469637	6.53	8.27
		{Ch-Ch}	37.77	1440867	2.28	

Table SI-39: *K* values for homodimer equilibration # 1 in 40 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.68	1259493	2.64	
1	**720**	{Ch-16}	22.39	3463465	6.52	5.93
		{Ch-Ch}	39.70	1699037	2.72	
		{16-16}	14.88	1254939	2.63	
2	**720**	{Ch-16}	22.69	3395610	6.39	5.66
		{Ch-Ch}	40.14	1716449	2.75	
		{16-16}	15.40	1378367	2.88	
3	**720**	{Ch-16}	23.45	3678879	6.92	5.92
		{Ch-Ch}	41.21	1751651	2.81	
		{16-16}	15.79	1258054	2.63	
4	**720**	{Ch-16}	24.11	3338577	6.28	5.74
		{Ch-Ch}	42.30	1635436	2.61	

Table SI-40: *K* values for homodimer equilibration # 2 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.94	2661152	5.57	
1	**300**	{Ch-16}	20.94	8696933	16.32	8.45
		{Ch-Ch}	37.59	3443437	5.66	
		{16-16}	15.44	3402200	7.12	
2	**360**	{Ch-16}	23.01	11229126	21.07	8.36
		{Ch-Ch}	40.15	4509120	7.46	
		{16-16}	15.29	3220798	6.74	
3	**420**	{Ch-16}	22.79	10569988	19.84	8.25
		{Ch-Ch}	39.75	4278438	7.07	
		{16-16}	15.08	3554702	7.44	
4	**480**	{Ch-16}	22.47	12017000	22.55	9.08
		{Ch-Ch}	39.38	4545486	7.53	

Table SI-41: *K* values for homodimer equilibration # 3 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	15.34	1785841	3.74	
1	**450**	{Ch-16}	22.81	5939933	11.16	9.06
		{Ch-Ch}	39.51	2266932	3.68	
		{16-16}	15.51	1894637	3.96	
2	**450**	{Ch-16}	23.06	6373075	11.97	8.85
		{Ch-Ch}	39.95	2507024	4.08	
		{16-16}	15.73	1889115	3.95	
3	**450**	{Ch-16}	23.39	6252907	11.74	8.64
		{Ch-Ch}	40.40	2481698	4.04	

Table SI-42: *K* values for homodimer equilibration # 4 in 40 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	14.95	1098575	2.30	
1	**300**	{Ch-16}	22.48	3833808	7.21	9.73
		{Ch-Ch}	39.27	1466796	2.32	
		{16-16}	14.99	1093952	2.29	
2	**360**	{Ch-16}	22.48	3780171	7.11	9.58
		{Ch-Ch}	39.30	1455672	2.31	
		{16-16}	15.06	1045646	2.19	
3	**420**	{Ch-16}	22.57	3717346	6.99	9.36
		{Ch-Ch}	39.42	1503153	2.39	
		{16-16}	15.09	894389	1.87	
4	**480**	{Ch-16}	22.65	3204475	6.03	9.70
		{Ch-Ch}	39.45	1276001	2.00	

Table SI-43: *K* values for homodimer equilibration # 2 in 40 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.51	734195	1.54	
1	**720**	{Ch-16}	20.67	1971606	3.72	5.85
		{Ch-Ch}	37.73	1001304	1.54	
		{16-16}	13.51	758561	1.59	
2	**720**	{Ch-16}	20.71	2066349	3.90	6.20
		{Ch-Ch}	37.76	1003425	1.54	
		{16-16}	13.59	752450	1.57	
3	**720**	{Ch-16}	20.80	2083062	3.93	6.06
		{Ch-Ch}	37.95	1048032	1.62	
		{16-16}	13.66	766647	1.60	
4	**720**	{Ch-16}	20.88	2083556	3.93	6.04
		{Ch-Ch}	38.04	1034413	1.59	

Table SI-44: *K* values for homodimer equilibration # 3 in 40 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 45 °C.

Sample #	Time (min)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	13.80	717393	1.50	
1	**720**	{Ch-16}	21.11	1948631	3.68	6.17
		{Ch-Ch}	38.35	953608	1.46	
		{16-16}	13.68	728273	1.52	
2	**720**	{Ch-16}	20.94	1950143	3.68	5.87
		{Ch-Ch}	38.10	986841	1.51	
		{16-16}	13.82	771011	1.61	
3	**720**	{Ch-16}	21.16	2112253	3.98	6.19
		{Ch-Ch}	38.49	1031402	1.59	
		{16-16}	13.93	824919	1.73	
4	**720**	{Ch-16}	21.30	2179289	4.11	5.92
		{Ch-Ch}	38.67	1067759	1.65	

Table SI-45: Overall *K* values for 40 mol % sterol LUVs (45 °C).

	K values		
	Hetero	**Homo**	**Cumulative**
No $CHCl_3$	9.20 ± 0.33	8.84 ± 0.54	9.02 ± 0.48
With $CHCl_3$	6.02 ± 0.28	5.91 ± 0.13	5.97 ± 0.22

1.2.2.5. *K* values for 2.5 mol % Sterol LUVs at 35 °C

Table SI-46: *K* values for heterodimer equilibration in 2.5 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 35 °C.

Sample #	Time (h)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**23.5**	{16-16}	11.70	2304761	4.82	1.11
		{Ch-16}	18.10	3223298	6.04	
		{Ch-Ch}	34.20	4047989	6.84	
2	**36**	{16-16}	11.70	2532808	5.30	0.83
		{Ch-16}	18.20	2975023	5.58	
		{Ch-Ch}	34.30	4183737	7.07	
3	**48**	{16-16}	11.70	2718838	5.69	0.77
		{Ch-16}	18.20	3104934	5.82	
		{Ch-Ch}	34.30	4561352	7.71	
4	**60.5**	{16-16}	11.70	2736919	5.72	0.81
		{Ch-16}	18.20	3210261	6.02	
		{Ch-Ch}	34.30	4597255	7.77	
5	**71**	{16-16}	11.80	2330710	4.87	0.80
		{Ch-16}	18.30	2633809	4.94	
		{Ch-Ch}	34.30	3702102	6.25	

Table SI-47: *K* values for homodimer equilibration in 2.5 mol % sterol LUVs in the absence of $CHCl_3$. The reaction was carried out at 35 °C.

Sample #	Time (h)	Dimer	t_R (min)	Area	N (nmols)	*K*
1	**23.5**	{16-16}	11.70	3498259	7.32	0.71
		{Ch-16}	18.20	3503905	6.57	
		{Ch-Ch}	34.40	4913367	8.30	
2	**36**	{16-16}	11.70	3480692	7.28	0.73
		{Ch-16}	18.30	3544185	6.64	
		{Ch-Ch}	34.40	4910501	8.30	
3	**48**	{16-16}	11.80	3766461	7.88	0.75
		{Ch-16}	18.30	3852253	7.22	
		{Ch-Ch}	34.50	5197423	8.78	
4	**60.5**	{16-16}	11.80	3514012	7.35	0.76
		{Ch-16}	18.30	3647295	6.84	
		{Ch-Ch}	34.60	4939747	8.35	
5	**71**	{16-16}	11.80	3372182	7.05	0.75
		{Ch-16}	18.40	3498658	6.56	
		{Ch-Ch}	34.60	4801447	8.11	

Table SI-48: *K* values for heterodimer equilibration in 2.5 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 35 °C.

Sample #	Time (h)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	11.50	1734778	3.63	
1	**24**	{Ch-16}	17.70	4478747	8.39	5.30
		{Ch-Ch}	33.70	2167626	3.66	
		{16-16}	11.40	1798808	3.76	
2	**24**	{Ch-16}	17.60	4699242	8.81	5.28
		{Ch-Ch}	33.60	2311252	3.90	
		{16-16}	11.50	1887749	3.95	
3	**24**	{Ch-16}	17.70	4935550	9.25	5.32
		{Ch-Ch}	37.70	2412717	4.08	

Table SI-49: *K* values for homodimer equilibration in 2.5 mol % sterol LUVs in the presence of $CHCl_3$. The reaction was carried out at 35 °C.

Sample #	Time (h)	Dimer	t_R (min)	Area	N (nmols)	*K*
		{16-16}	11.60	2060895	4.31	
1	**24**	{Ch-16}	17.80	5299859	9.93	5.15
		{Ch-Ch}	34.00	2633420	4.45	
		{16-16}	11.60	2041855	4.27	
2	**24**	{Ch-16}	17.90	5451047	10.22	5.35
		{Ch-Ch}	34.10	2705757	4.57	
		{16-16}	11.70	2060632	4.31	
3	**24**	{Ch-16}	18.00	5335069	10.00	5.16
		{Ch-Ch}	34.20	2662763	4.50	

Table SI-50: Overall *K* values for 2.5 mol % sterol LUVs (35 °C).

	***K* values**		
	Hetero	**Homo**	**Cumulative**
No $CHCl_3$	0.80 ± 0.02	0.75 ± 0.01	0.78 ± 0.03
With $CHCl_3$	5.30 ± 0.02	5.22 ± 0.11	5.26 ± 0.09

2. Estimation of $CHCl_3$ Binding to 200 nm LUVs with Varying Sterol Content

In these experiments, the binding of $CHCl_3$ at 45 °C to 2.5, 20, and 40 mol % sterol containing 200 nm LUVs was determined using gas chromatography (GC).

2.1. Calibration for $CHCl_3$ Determination

The GC analysis was done using a Hewlett-Packard 5890 series gas chromatograph equipped with a thermal conductivity detector and a Supelco Vocol capillary fused silica GC column (30 m X 0.53 mm X 3 μl film thickness). The GC was controlled using a PC running HP ChemStation. The following GC

parameters were employed: Detector: 230 °C (TCD); Injection Port: 230 °C; Oven: 35 °C (isothermal); Flow rate: 5 ml/min; Eluent Gas: Helium; Injection Volume: 0.2-0.5 μl; Analysis Time Per Sample: 8 minutes. **Standard Solution A:** 0.2 ml of $CHCl_3$, 10 ml of EtOH, and 39.8 ml of Tris buffer (pH 7.4). **Standard Solution B:** 0.1 ml of $CHCl_3$, 10 ml of EtOH, and 39.9 ml of Tris buffer (pH 7.4).. Calibration data are given in Table SI-51 and the calibration graph is reproduced in Figure SI-5. The data fit the following equation: $y = 149.46x + 120.93$ ($R^2 = 0.9926$)

Table SI-51: GC calibration data for $CHCl_3$ determination.

#	Sln.	Inj. Vol. (μl)	N_{CHCl3} (nmol)	t_R (min)	Area	Av. Area	St. Dev. (±)	% St. Dev. (±)
1	**A**	0.5	25	5.91	3837.9			
2	**A**	0.5	25	5.92	3831.0			
3	**A**	0.5	25	5.90	3899.6	3852.8	35.42	0.92
4	**A**	0.5	25	5.85	3815.5			
5	**A**	0.5	25	5.89	3880.0			
6	**A**	0.4	20	5.89	3259.5			
7	**A**	0.4	20	5.88	2974.0			
8	**A**	0.4	20	5.88	3008.7	3106.0	117.88	3.80
9	**A**	0.4	20	5.90	3177.5			
10	**A**	0.4	20	5.90	3110.5			
11	**A**	0.3	15	5.93	2224.1			
12	**A**	0.3	15	5.94	2236.7			
13	**A**	0.3	15	5.92	2460.0	2405.1	165.43	6.88
14	**A**	0.3	15	5.90	2521.0			
15	**A**	0.3	15	5.92	2583.7			
16	**B**	0.4	10	5.94	1581.8			
17	**B**	0.4	10	5.95	1518.9			
18	**B**	0.4	10	5.96	1657.4	1566.5	67.17	4.29
19	**B**	0.4	10	5.96	1484.4			
20	**B**	0.4	10	5.96	1589.8			
21	**B**	0.3	7.5	6.00	1089.6			
22	**B**	0.3	7.5	6.00	1298.6			
23	**B**	0.3	7.5	5.99	1262.7	1234.1	85.85	6.96
24	**B**	0.3	7.5	6.01	1293.7			
25	**B**	0.3	7.5	6.06	1225.8			
26	**B**	0.2	5	6.06	900.3			
27	**B**	0.2	5	6.12	848.2			
28	**B**	0.2	5	6.01	869.8	891.9	37.66	4.22
29	**B**	0.2	5	6.09	948.5			
30	**B**	0.2	5	6.12	892.5			

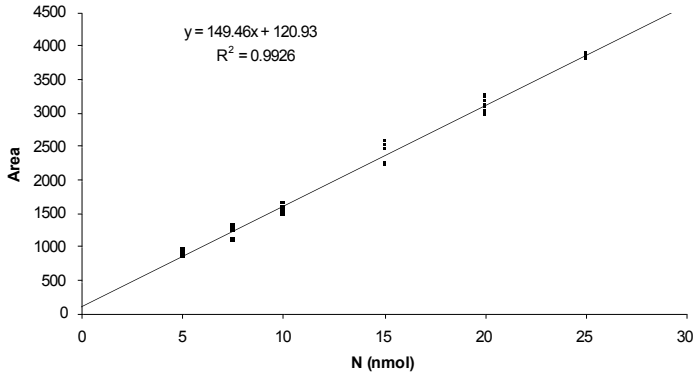

Figure SI-5: Linear regression data for GC calibration.

2.2. Determination of $CHCl_3$ Binding to LUVs

2.2.1. Vesicle Preparation

The amounts of lipids in Table SI-52 were used for film preparation. The given volumes of DPPC, DPPG, and cholesterol solutions were placed in a 13 x 100 mm test tube. The solvent was evaporated under a stream of Argon while the test tube was rapidly rotated at an angle of approximately 10° with respect to a flat horizontal surface at ~ 40 °C. A thin film was obtained which covered the inner surface of the test tube up to 1/2 of the height of the tube. A silicone septum was used to cover the mouth of the tube. The tube was sealed with parafilm, and covered with aluminum foil. The assembly was connected to a vacuum pump via a needle and kept under vacuum (~0.2 torr) overnight (~14 h).

Table 52: Lipids used in film preparation

		40% Sterol		20% Sterol		2.5% Sterol	
Lipid	**Conc. (mM)**	**Vol. (µl)**	**Moles (µmol)**	**Vol. (µl)**	**Moles (µmol)**	**Vol. (µl)**	**Moles (µmol)**
DPPC	27.2	846	23	1140	31	1397	38
DPPG	13.6	74	1	74	1	74	1
Cholesterol	20	800	16	400	8	50	1

The lipid film was hydrated with 2 ml pH 7.4 Tris buffer at 60 °C and the mixture was vortexed for 30 s. The mixture was then rested 5 min in a 60 °C water bath. The mixture was again vortexed for 30 s followed by a 30 min rest in a 60 °C water bath. The mixture was then subjected to six freeze/thaw cycles as follows: The test tube was repeatedly immersed in liquid N_2 and then vortexed until a homogeneous thin layer of ice covered the inner surface of the tube. When all of the liquid was frozen the tube was immersed in liquid N_2 until rapid bubble evolution (N_2) in the bath ceased. The tube was then immersed in a 60°C water bath until the frozen MLV mixture melted and heated to 60°C. This freeze/thaw cycle was repeated five more times. The MLVs were extruded using a Lipex extruder with an argon pressure of 100 psi. Extrusion was done at 60°C. The MLVs were extruded 10 times through 0.2 µm polycarbonate filters.

2.2.2. Determination of the Extend of $CHCl_3$ Binding

A given dispersion (1 ml) was placed in a 3 ml reaction vessel (Wheaton PN: 986287) equipped with a small magnetic stir bar, silicone septum (Aldrich, PN: Z512893), and a small test tube for $CHCl_3$ (Figure SI-2). A reaction similarly configured containing only buffer acted as the control. The reaction vessels were heated to 45 °C and 21 µl 0.1 M NaOH was added to each vessel. 250 µl $CHCl_3$ was placed into the test tube within each reaction vessel. An additional 200 µl $CHCl_3$ was added to each vessel six hours after the experiment started. 0.3 µl aliquots were withdrawn from each reaction vessel after 12-14 h and analyzed using GC.

Table SI-53: N_{CHCl3} found in 0.3 µl buffer.

Entry #	**1**	**2**	**3**	**4**	**5**
Reaction #	1	1	1	2	2
t_R (min)	6.27	6.25	6.28	6.26	6.32
Area	3776.9	3437.2	3205.0	3288.8	3958.8
N_{CHCl3} (nmol)	24.46	22.19	20.63	21.20	25.68
Average N_{CHCl3}			22.83 ± 2.16		

Table SI-54: N_{CHCl3} found in 0.3 µl 40% sterol LUV dispersion.

Entry #	1	2	3	4	5
Reaction #	1	1	1	2	2
t_R (min)	6.28	6.29	6.23	6.32	6.31
Area	4674.4	4811.3	4757.4	4806.6	5255.2
N_{CHCl3} (nmol)	30.47	31.38	31.02	31.35	34.35
Average N_{CHCl3}			31.71 ± 1.52		

Table SI-55: N_{CHCl3} found in 0.3 µl 20% sterol LUV dispersion.

Entry #	1	2	3	4	5
Reaction #	1	1	1	2	2
t_R (min)	6.25	6.26	6.23	6.25	6.33
Area	5478.4	5734.6	5694.9	5823.8	5792.2
N_{CHCl3} (nmol)	35.85	37.56	37.29	38.16	37.95
Average N_{CHCl3}			37.36 ± 0.91		

Table SI-56: N_{CHCl3} found in 0.3 µl 2.5% sterol LUV dispersion.

Entry #	1	2	3	4	5
Reaction #	1	1	1	2	2
t_R (min)	6.26	6.27	6.26	6.24	6.33
Area	6650.7	6539.5	6218.7	5745.1	6211.7
N_{CHCl3} (nmol)	43.69	42.95	40.80	37.63	40.75
Average N_{CHCl3}			41.16 ± 2.36		

Table SI-57: Binding of $CHCl_3$ to LUVs with varying sterol levels[a]

LUV	ΔN_{CHCl3} (nmol)	[CHCl3] (mM)	[Lipid] (mM)	[DPPC] (mM)	$\frac{[CHCl_3]}{[Lipid]}$	$\frac{[CHCl_3]}{[DPPC]}$
40% Sterol	8.88	29.61	20.00	12.00	1.48	2.47
20% Sterol	14.53	48.43	20.00	16.00	2.42	3.03
2.5 % Sterol	18.33	61.10	20.00	19.75	3.06	3.09

[a]ΔN_{CHCl3} is the difference of the $CHCl_3$ amounts found in the corresponding LUV aliquots and buffer aliquots; [$CHCl_3$] is $CHCl_3$ concentration within or directly associated the lipid bilayer.

2.3. Effect of $CHCl_3$ on Vesicle Size

The effect of $CHCl_3$ on vesicle size was investigated using a Nicomp DLS submicron sizer. Aliquots (15 µl) were taken from each dispersion before and after treatment with $CHCl_3$ (at ~ 14 h). These aliquots were diluted with 335 µl tris buffer and analyzed at 45 °C. A sample viscosity of 0.5960 centipoise was assumed. Relevant data are given in Table SI-58. A broad monomodal distribution was observed in all cases.

Table SI-58: Vesicle size before and after treatment with $CHCl_3$ as determined by DLS.

LUV	t = 0 (no $CHCl_3$)		T = 12-14 h (with $CHCl_3$)	
	Average Diameter (nm)	Standard Deviation (%)	Average Diameter (nm)	Standard Deviation (%)
40% Sterol	186.7	42	194.2	37
20% Sterol	190.2	28	211.1	33
2.5% Sterol	179.6	26	202.8	30

3. General Polarization Experiments

3.1. Method

2.5 (95:2.5:2.5 DPPC:DPPG:Cholesterol) and 40 (57.5:2.5:40 DPPC:DPPG:Cholesterol) mol% sterol liposomes (~200 nm, 1.2 mM total lipid concentration) containing or lacking laurdan (ANASpec, 0.5 mol%, added during lipid film preparation) were prepared as described in section 1.1.1. The pH of the dispersions was adjusted to 7.4 at 45 °C and the vesicles were incubated in the presence and absence of $CHCl_3$ for 12 hours at 45 °C (Figure SI-2). The vesicle dispersions were placed in capped fluorescence cuvettes and the fluorescence of each sample was measured as a function of temperature using a Perkin Elmer LS50B Luminescence Spectrometer employing a temperature controlled cell holder. λ_{ex} was 350 nm and a 2.5 nm slit width was used. Fluorescence emissions were recorded from 350 to 600 nm. The effect of scattering due to liposomes was corrected for by subtracting the fluorescence spectra of liposomes lacking laurdan from those that contained laurdan. General Polarization (GP) was calculated using the following equation:

$$GP = \frac{(I_{440} - I_{490})}{(I_{440} + I_{490})}$$

where I_{440} and I_{490} are fluorescence emission intensities at 440 and 490 nm respectively. In addition to fluorescence experiments involving liposomes, the fluorescence of laurdan in a number of organic solvents was also measured.

3.2. Results

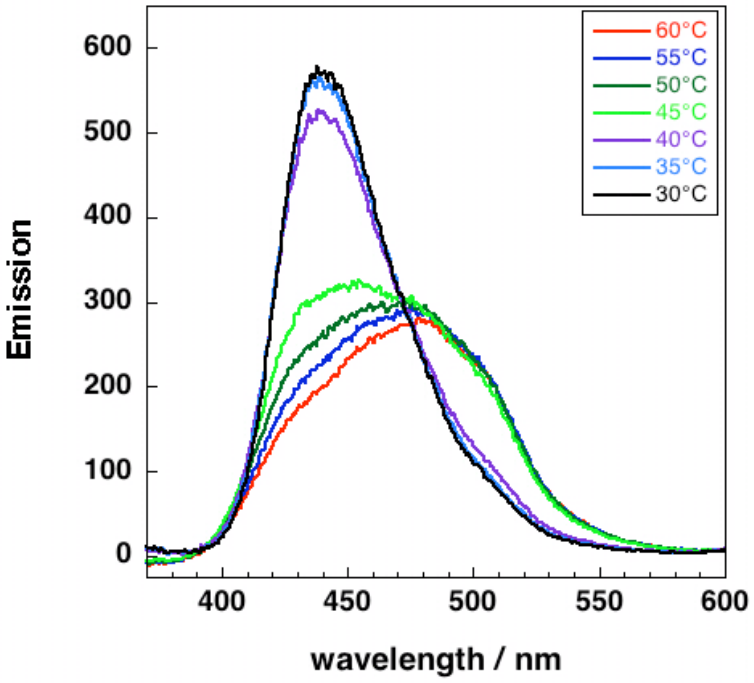

Figure SI-6: Fluorescence spectra for 2.5 mol% sterol liposomes in the absence of $CHCl_3$.

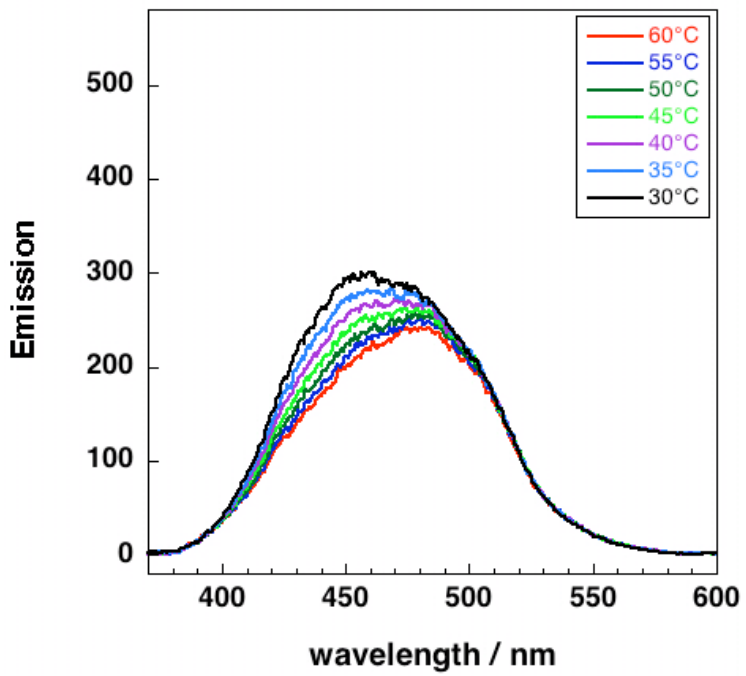

Figure SI-7: Fluorescence spectra for 2.5 mol% sterol liposomes in the presence of $CHCl_3$.

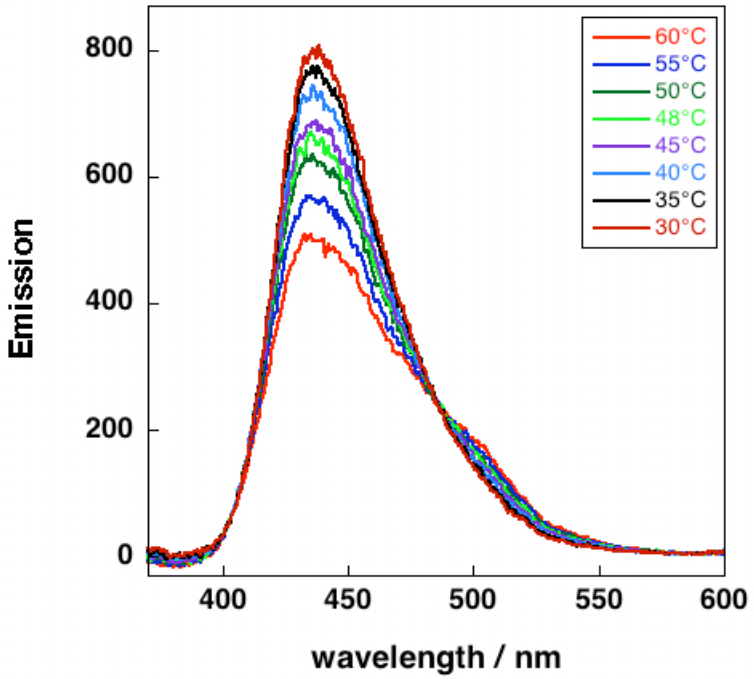

Figure SI-8: Fluorescence spectra for 40 mol% sterol liposomes in the absence of $CHCl_3$.

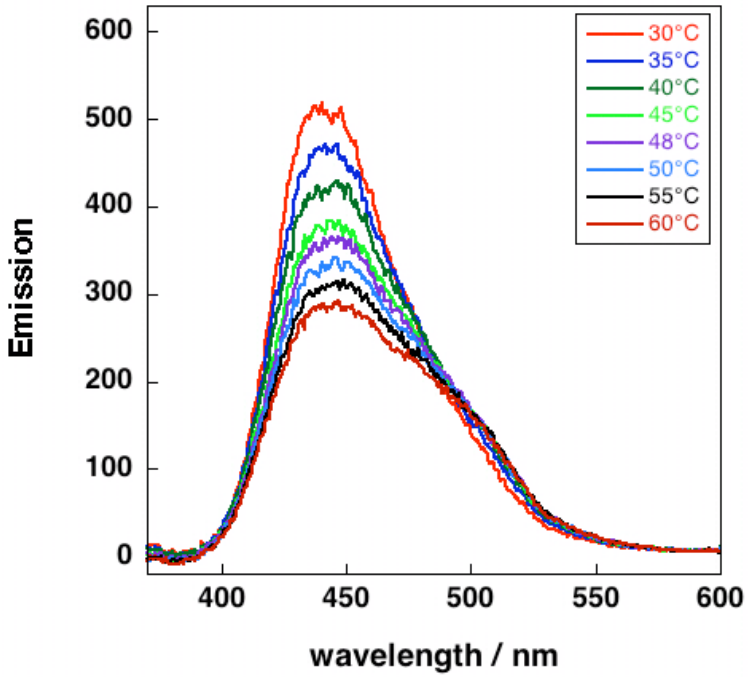

Figure SI-9: Fluorescence spectra for 40 mol% sterol liposomes in the presence of $CHCl_3$.

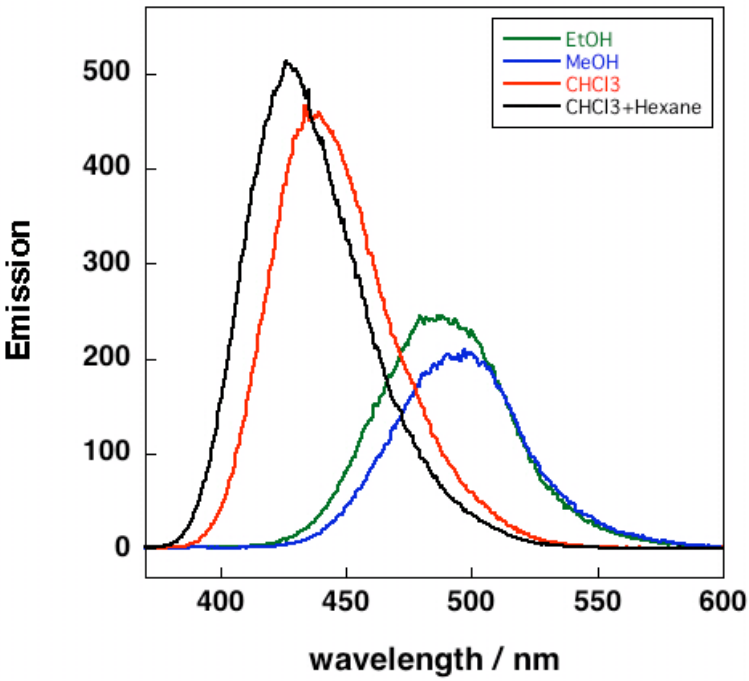

Figure SI-10: Fluorescence spectra for laurdan in various organic solvents.

Table SI-59: GP values for 2.5 and 40 mol% sterol liposomes in the absence and presence of $CHCl_3$.

	General PolarIzatIon			
	2.5 mol% Sterol		40 mol% Sterol	
Temperature (°C)	without $CHCl_3$	with $CHCl_3$	without $CHCl_3$	with $CHCl_3$
30.0	0.569	0.018	0.574	0.472
34.4	0.554	-0.019	0.554	0.397
38.5	0.503	-0.046	0.540	0.330
43.0	0.086	-0.087	0.501	0.287
45.0	-	-0.086	0.490	0.262
46.8	-0.002	-0.096	0.468	0.232
50.9	-0.076	-0.142	0.434	0.233
55.1	-0.118	-0.142	0.391	0.190

Table SI-60: GP values for laurdan in various organic solvents.

Entry	Solvent	GP
1	MeOH	-0.737
2	EtOH	-0.838
3	$CHCl_3$	0.661
4	$CHCl_3$:Hexane (1:1)	0.760

4. Raman Spectra

4.1. Method

Lipid films for 2.5 (204.35 μmol DPPC, 5.38 μmol DPPG, 5.38 μmol cholesterol-95:2.5:2.5 mole ratio) and 40 (123.69 μmol DPPC, 5.38 μmol DPPG, 86.04 μmol cholesterol-57.5:2.5:40 mole ratio) mol% sterol vesicles were prepared. These films were hydrated using 8 ml pH 7.4 Tris buffer and subjected to manipulations outlined in section 1.1.1. The resulting LUV dispersions were placed in centrifuge tubes (Nalgene, PN:3139-0016) and were centrifuged at 48000g (20000 rpm) using a Beckman Avanti J-25 centrifuge equipped with a JA-20 rotor for 105 min at 23 °C. The supernatant for each tube was decanted and the precipitate in each tube was resuspended in sufficient Tris buffer to give a final volume of ~ 1ml. The pH was adjusted to 7.4 at 45 °C. The dispersions were placed in stoppered quartz cuvettes (Starna Cells, PN 29/SOG/5). Raman spectra of these dispersions were taken at 48 °C using a HORIBA Jobin Yvon LabRAM-IR Raman Microscope equipped with a CCD detector. Excitation was done at 442 nm using a tunable laser and spectra were taken from 500 to 3000 cm^{-1} (each spectrum an average of 20 15-second scans). The dispersions were then removed from the cuvettes and placed into reaction vessels (Figure SI-2) and incubated with $CHCl_3$ for 12 h at 45 °C. After this the vesicles were transferred back into the quartz cuvettes and Raman spectra were taken at 48 °C. Vesicle size was checked using DLS after each manipulation and the values found are given in Table SI-61.

Table SI-61: Effect of various manipulations on vesicle mean diameters.

		Mean Diameter (nm)	
Entry	Process	2.5 mol% Sterol	40 mol% Sterol
1	Extrusion	175.4 ± 61.4	172.1 ± 62.0
2	Centrifugation/Resuspension	185.2 ± 57.4	182.5 ± 52.9
3	Treatment with $CHCl_3$	182.0 ± 69.2	189.7 ± 64.5

4.2. Spectra

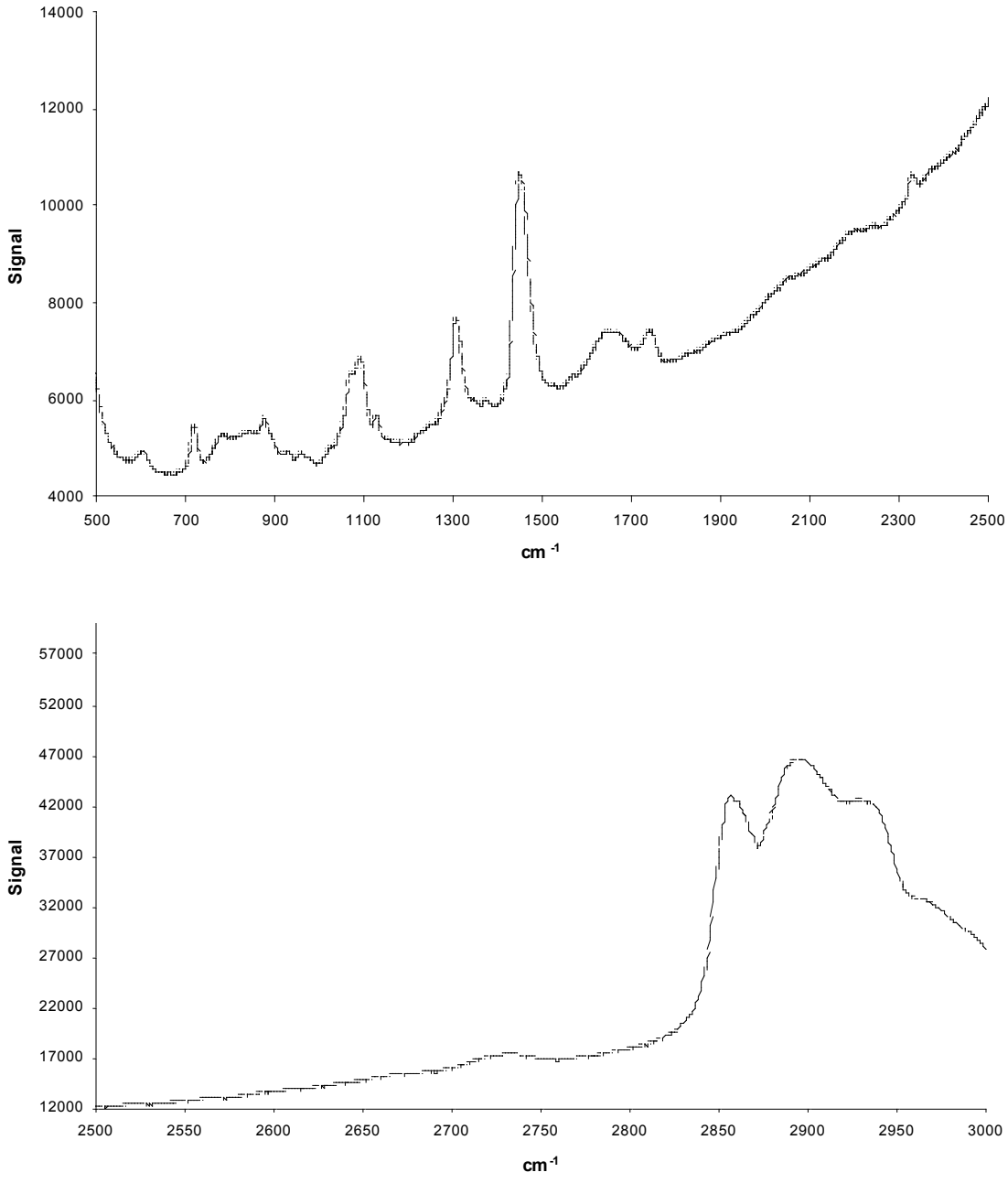

Figure SI-11: Raman spectrum for 2.5 mol% sterol LUVs in the absence of $CHCl_3$ at 48 °C (500-2500 cm^{-1} upper-hand, 2500-3000 cm^{-1} lower-hand).

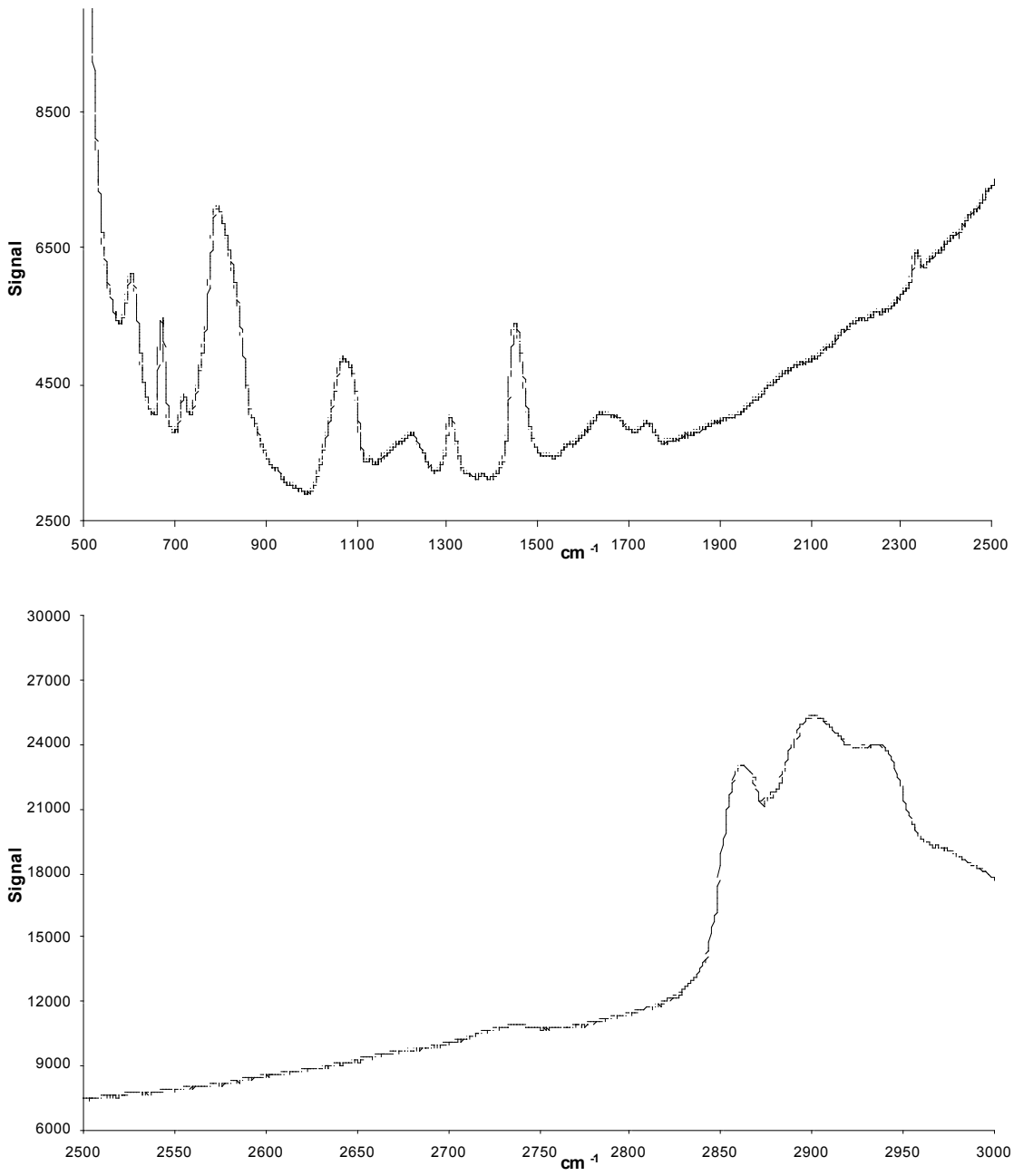

Figure SI-12: Raman spectrum for 2.5 mol% sterol LUVs in the presence of $CHCl_3$ at 48 °C (500-2500 cm^{-1} upper-hand, 2500-3000 cm^{-1} lower-hand).

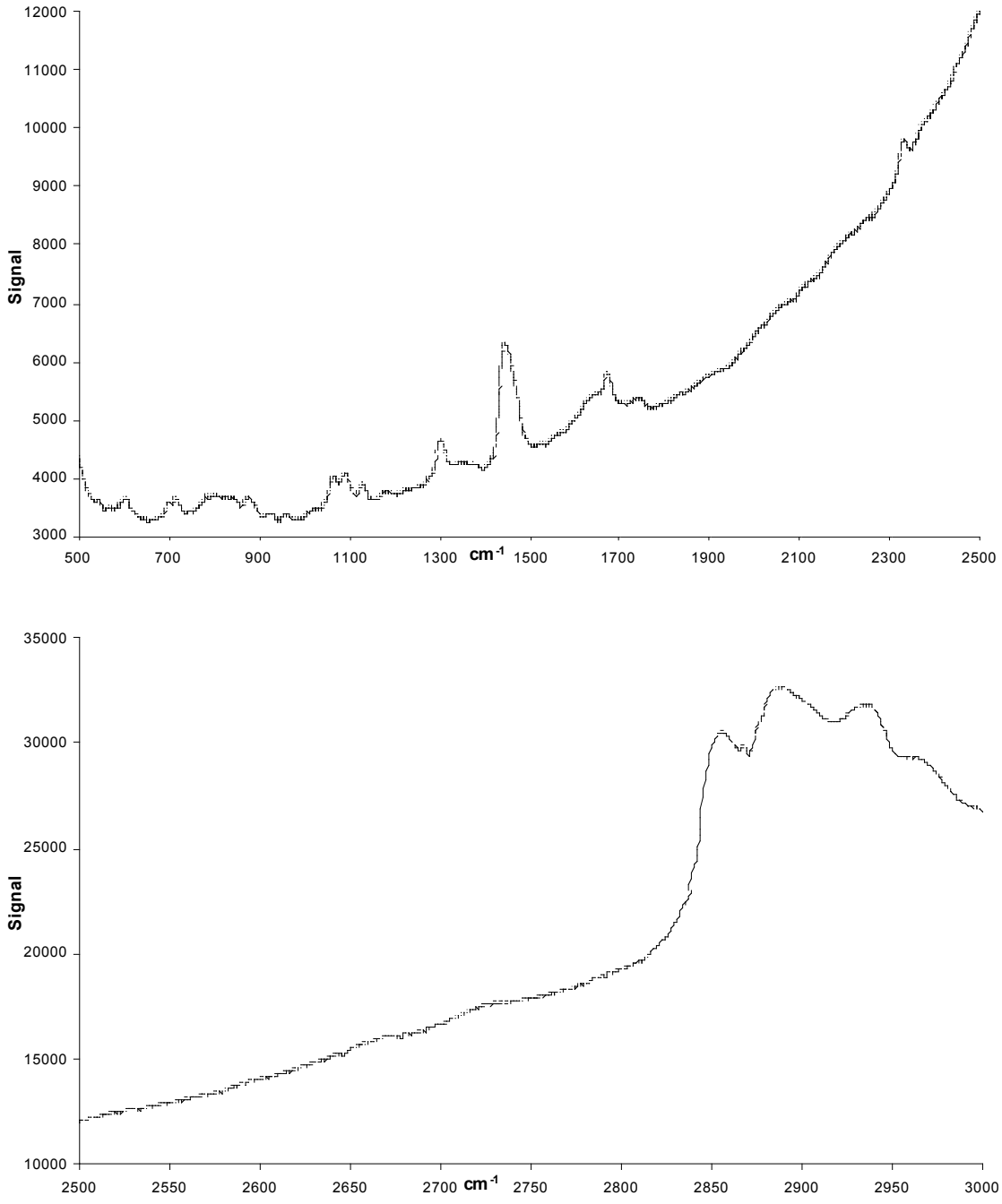

Figure SI-13: Raman spectrum for 40 mol% sterol LUVs in the absence of $CHCl_3$ at 48°C (500-2500 cm^{-1} upper-hand, 2500-3000 cm^{-1} lower-hand).

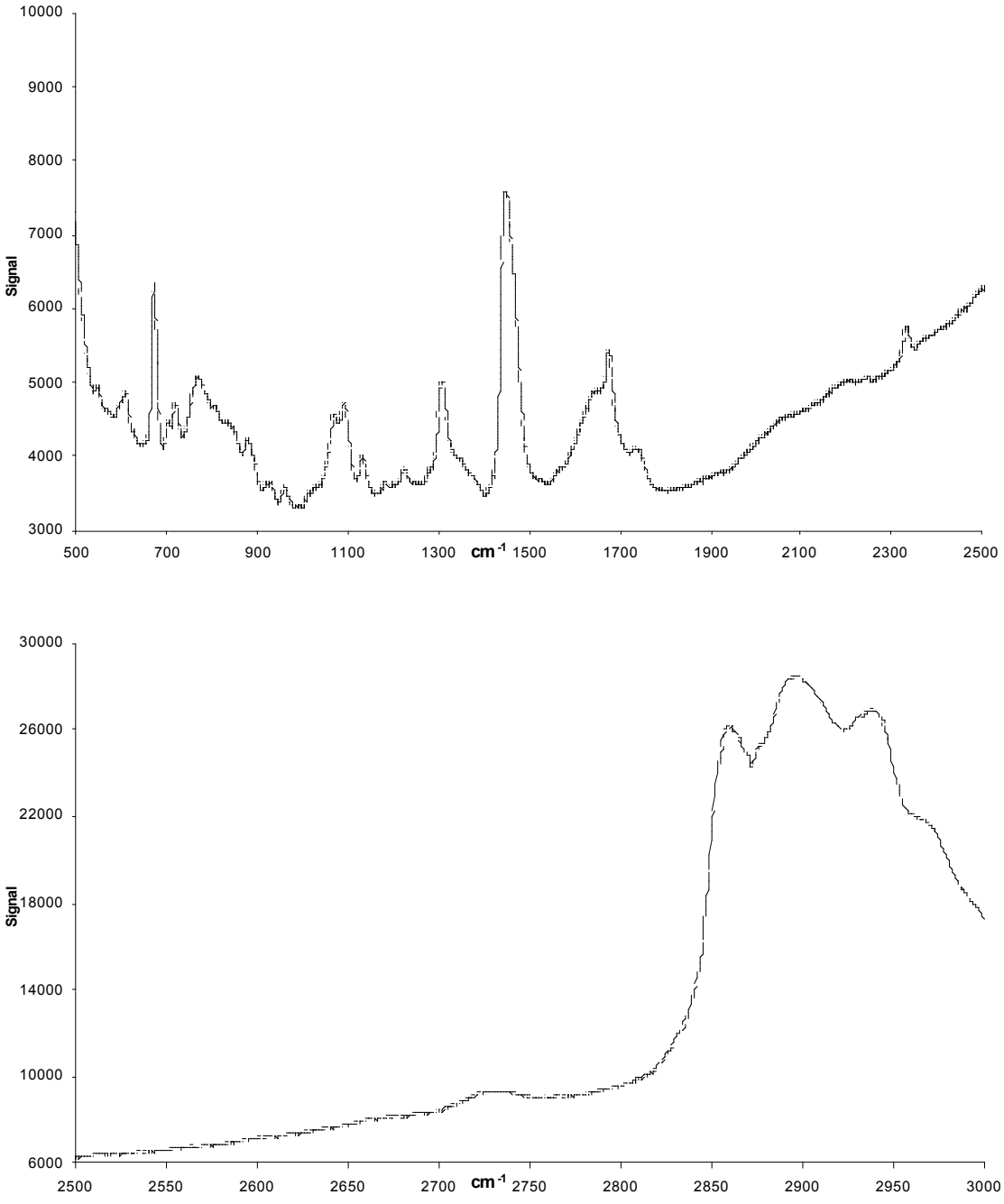

Figure SI-14: Raman spectrum for 40 mol% sterol LUVs in the presence of $CHCl_3$ at 48 °C (500-2500 cm^{-1} upper-hand, 2500-3000 cm^{-1} lower-hand).