

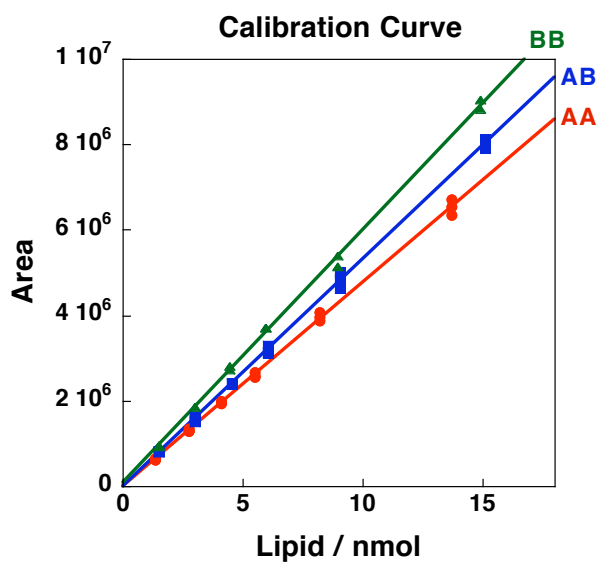
Reduced Sterol-Phospholipid Recognition in Curved Fluid Bilayers

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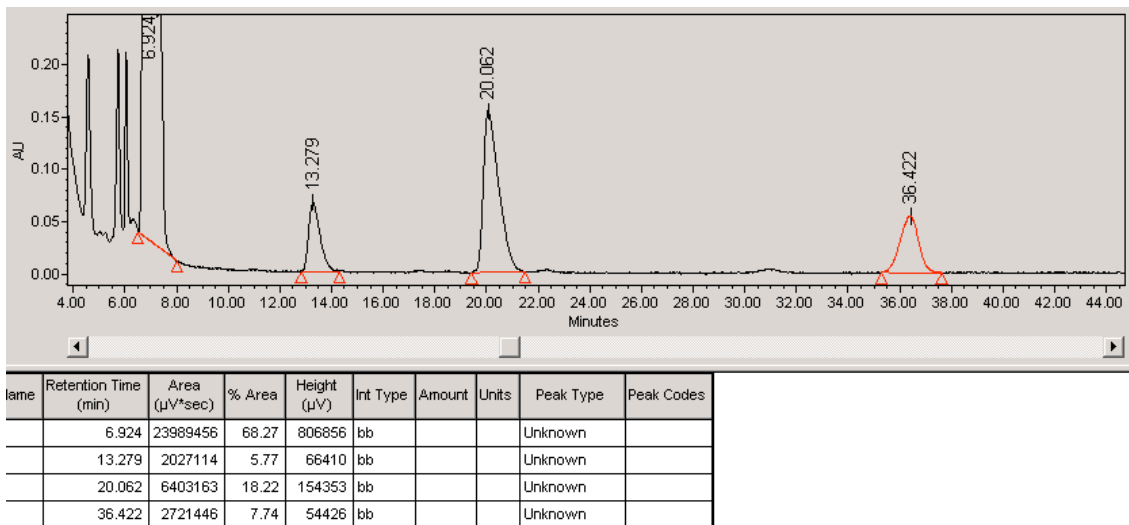
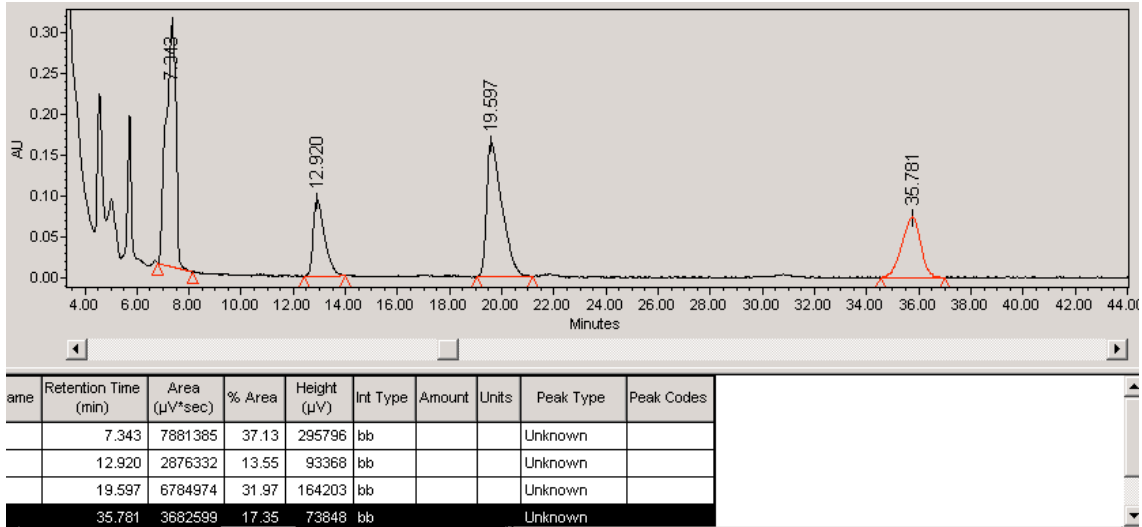
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SUPPORTING INFORMATION

Calibration Curves. The exchangeable lipid dimers, **AA**, **AB**, and **BB**, were calibrated by HPLC. Varying amounts of exchangeable lipids were mixed with the HPLC mobile phase and injected to HPLC system. Peak areas were then plotted against the amount of lipid that was injected.



HPLC Chromatogram. Typical HPLC chromatograms are shown below. The upper figure shows the chromatogram for the lipids used in cholesterol-poor, 200 nm liposomes. The bottom figure shows the chromatogram for the lipids used in cholesterol-rich 200 nm liposomes. The retention times for dimers of **AA**, **AB** and **BB** were ca. 13 min, 20 min and 36 min, respectively.



Values of K . The quantity of each dimer was calculated from peak areas from the HPLC chromatograms using calibration curves; K values were calculated from the following equation: $K=[AB]^2/([AA] \times [BB])$.

Heterodimer

	2.5% cholesterol		40% cholesterol	
	Size (nm)	K	Size (nm)	K
Reverse-phase	1910 ± 1110	4.56 ± 0.26	4700 ± 2770	7.79 ± 0.20
Evaporation methods	1630 ± 990	4.64 ± 0.10	3990 ± 1860	7.76 ± 0.15
Extrusion 400nm filter	288 ± 118	4.37 ± 0.11	301 ± 132	7.59 ± 0.32
200nm filter	183 ± 68	4.32 ± 0.03	194 ± 68	7.37 ± 0.14
100nm filter	104 ± 31	4.35 ± 0.06	117 ± 30	7.05 ± 0.07
50nm filter	65 ± 23	4.54 ± 0.12	88 ± 34	6.35 ± 0.11

Homodimer

	2.5% cholesterol		40% cholesterol	
	Size (nm)	K	Size (nm)	K
Reverse-phase	2610 ± 1020	3.65 ± 0.13	4330 ± 2470	7.29 ± 0.31
Evaporation methods	1490 ± 640	3.78 ± 0.28	3690 ± 2020	7.20 ± 0.14
Extrusion 400nm filter	287 ± 109	3.88 ± 0.05	325 ± 159	7.01 ± 0.19
200nm filter	172 ± 53	3.98 ± 0.08	200 ± 70	6.83 ± 0.23
100nm filter	100 ± 17	4.03 ± 0.15	121 ± 35	6.27 ± 0.22
50nm filter	79 ± 22	4.09 ± 0.02	103 ± 37	5.38 ± 0.33

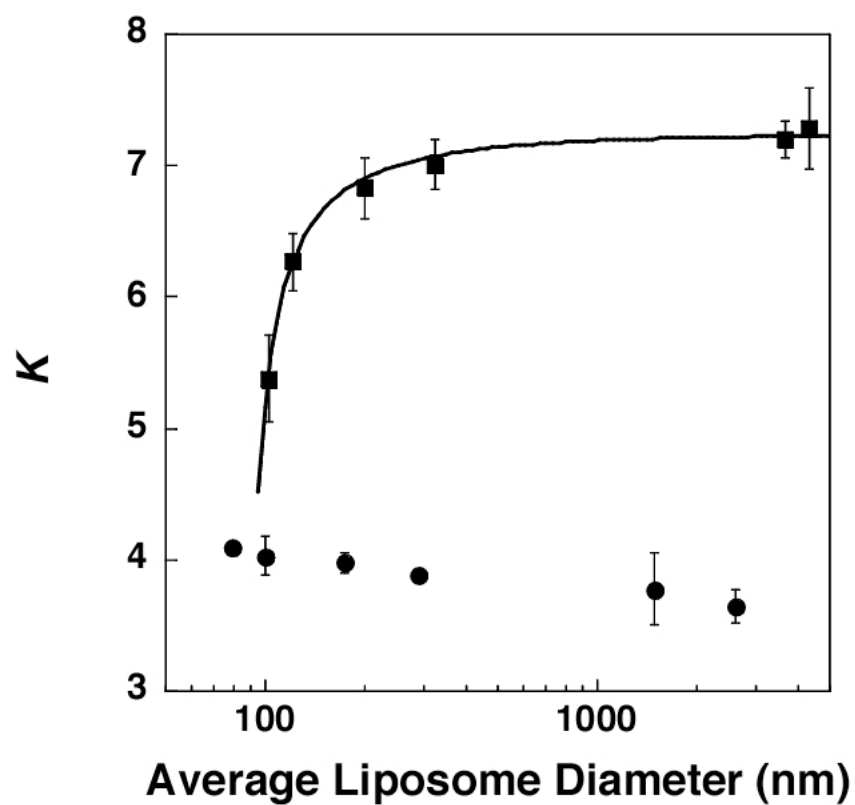


Figure S1. Plot of K versus the average diameter of liposomes that were cholesterol-rich (40 mol%; ■) and cholesterol-poor (2.5 mol%; ●). All liposomes were made using an equimolar mixture of **AA** and **BB** as the source of **A** and **B**; raw data are given in the accompanying tables.

Raw data from NNR experiments; sizes that are indicated were determined before NNR reactions:

2.5% cholesterol (extruded through 400nm pore filter)					
	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homodimer	0	8.75	0	11.12	–
	4	3.49	8.29	5.10	3.86
	4.5	3.64	8.69	5.43	3.82
	5	3.45	8.13	4.93	3.88
	5.5	3.55	8.40	5.03	3.95
				average	3.88
			std	0.05	
from heterodimer	0	0	25.32	0	–
	4	5.21	11.08	5.47	4.31
	4.5	5.25	11.05	5.26	4.42
	5	5.27	11.37	5.46	4.49
	5.5	5.15	10.87	5.40	4.25
				average	4.37
			std	0.11	

2.5% cholesterol (extruded through 200nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homodimer	0	12.82	0	12.45	–
	3.5	6.38	12.77	6.54	3.91
	4	6.76	13.62	6.77	4.05
	4.5	6.48	13.10	6.55	4.04
	5	6.47	12.89	6.56	3.92
			average	3.98	
			std	0.08	
from heterodimer	0	0	25.03	0	–
	4	5.55	11.76	5.74	4.34
	4.5	5.65	11.87	5.82	4.28
	5	6.02	12.72	6.22	4.32
	5.5	5.34	11.20	5.45	4.32
			average	4.32	
			std	0.03	

2.5% cholesterol (extruded through 100nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homo dimer	0	8.50	0	11.50	–
	4	3.82	9.79	6.29	3.98
	4.5	3.74	9.95	6.29	4.21
	5	3.84	9.65	6.28	3.86
	5.5	3.98	10.18	6.40	4.07
			average	4.03	
			std	0.15	
from hetero dimer	0	0	24.33	0	–
	4	5.47	11.55	5.49	4.44
	4.5	5.37	11.30	5.50	4.33
	5	5.60	11.86	5.82	4.32
	5.5	5.63	11.72	5.64	4.33
			average	4.35	
			std	0.06	

2.5% cholesterol (extruded through 50nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homo dimer	0	11.03	0	11.11	–
	3.5	5.50	11.24	5.64	4.08
	4	5.29	10.84	5.44	4.09
	4.5	5.33	11.10	5.60	4.12
	5	5.37	11.15	5.69	4.07
	5.5	5.23	10.76	5.41	4.10
			average	4.09	
			std	0.02	
from hetero dimer	0	0	20.74	0	–
	4	4.69	10.10	4.75	4.58
	4.5	4.55	9.88	4.66	4.59
	5	4.56	9.59	4.63	4.36
	5.5	4.45	9.69	4.57	4.62
			average	4.54	
			std	0.12	

40% cholesterol (extruded through 400nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homodimer	0	9.54	0	12.15	–
	4	3.22	10.50	5.05	6.77
	4.5	3.29	10.66	4.97	6.95
	5	3.28	10.96	5.08	7.22
	5.5	3.15	10.55	4.97	7.11
			average	7.01	
			std	0.19	
from heterodimer	0	0	23.37	0	–
	4	4.06	11.46	4.44	7.29
	4.5	4.01	11.75	4.30	8.00
	5	4.10	11.81	4.43	7.68
	5.5	4.07	11.72	4.57	7.39
			average	7.59	
			std	0.32	

40% cholesterol (extruded through 200nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homodimer	0	8.16	0	10.53	–
	4	3.15	9.98	4.80	6.60
	4.5	3.02	9.92	4.68	6.96
	5.5	2.91	9.64	4.35	7.33
	0	12.33	0	12.45	–
	3.5	4.99	13.43	5.40	6.69
	4	5.05	13.52	5.33	6.78
	4.5	5.03	13.52	5.35	6.79
	5	5.05	13.55	5.42	6.72
	5.5	4.51	11.95	4.70	6.74
			average	6.83	
			std	0.23	
from heterodimer	0	0	23.49	0	–
	3.5	4.37	12.37	4.69	7.47
	4	4.36	12.35	4.73	7.41
	4.5	4.24	12.11	4.59	7.54
	5	4.24	12.00	4.60	7.39
	5.5	4.14	11.70	4.39	7.53
	0	0	26.24	0	–
	4	5.05	13.94	5.23	7.36
	4.5	5.02	14.01	5.38	7.26
	5	5.10	14.25	5.47	7.28
5.5	5.14	14.16	5.49	7.11	
			average	7.37	
			std	0.14	

40% cholesterol (extruded through 100nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homodimer	0	10.17	0	10.78	–
	4	4.47	11.61	4.91	6.13
	4.5	4.32	11.69	4.78	6.63
	5	4.46	11.46	4.73	6.22
	5.5	4.34	11.82	4.88	6.60
	0	9.55	0	13.39	–
	4	3.89	12.81	6.90	6.11
	4.5	3.80	12.67	6.76	6.26
	5	3.77	12.22	6.44	6.15
	5.5	4.05	13.18	7.09	6.05
			average	6.27	
			std	0.22	
from heterodimer	0	0	21.98	0	–
	4	4.28	11.67	4.48	7.09
	4.5	4.30	11.81	4.53	7.17
	5	4.47	12.26	4.73	7.11
	5.5	4.51	12.38	4.83	7.05
	0	0	22.91	0	–
	4	4.81	12.93	4.98	6.98
	4.5	4.94	13.27	5.08	7.02
	5	4.73	12.88	5.03	6.97
	5.5	4.91	13.25	5.09	7.02
			average	7.05	
			std	0.07	

40% cholesterol (extruded through 50nm pore filter)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homodimer	0	8.54	0	11.18	–
	4	3.42	9.71	5.05	5.45
	4.5	3.42	9.91	5.09	5.64
	5	3.37	9.97	5.17	5.69
	5.5	3.44	10.05	5.12	5.74
	0	9.21	0	9.86	–
	3.5	4.45	10.38	4.90	4.95
	4	4.57	10.45	5.01	4.77
	4.5	4.29	10.53	4.81	5.38
	5	4.40	10.99	5.04	5.44
	5.5	4.22	10.50	4.84	5.38
				average	5.38
				std	0.33
from heterodimer	0	0	23.17	0	–
	4	4.63	12.17	4.98	6.42
	4.5	4.65	12.20	5.04	6.36
	5	4.54	12.07	4.95	6.49
	5.5	4.75	12.19	5.01	6.25
	0	0	22.74	0	–
	4	4.61	12.31	5.07	6.47
	4.5	4.64	12.15	5.12	6.21
	5	4.74	12.28	5.07	6.27
	5.5	4.79	12.50	5.17	6.31
				average	6.35
				std	0.11

2.5% cholesterol (Reverse-phase Extruded Vesicle)
: 1490 nm , 43% error (from DLS)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homodimer	0	8.92	0	8.32	–
	4.5	3.38	6.42	3.49	3.49
	5	3.11	6.22	3.25	3.82
	5.5	2.96	5.77	3.08	3.66
	6	2.67	5.74	2.98	4.14
				average	3.78
				std	0.28

2.5% cholesterol (Reverse-phase Extruded Vesicle)
: 2610 nm , 39% error (from DLS)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from homodimer	0	10.91	0	10.81	–
	7	4.18	8.16	4.52	3.53
	8	3.95	7.92	4.39	3.62
	9	3.58	7.38	3.96	3.84
	10	3.83	7.66	4.07	3.76
	11	4.17	8.18	4.42	3.62
	12	2.62	4.90	2.59	3.53
					average
				std	0.13

40% cholesterol (Reverse-phase Extruded Vesicle)
: 4330 nm , 57% error (from DLS)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homodimer	0	11.85	0	12.28	–
	8	3.82	10.78	4.36	6.97
	9	4.02	11.48	4.56	7.20
	10	3.89	11.06	4.46	7.04
	11	3.77	11.13	4.30	7.64
	12	3.54	10.44	4.06	7.59
				average	7.29
				std	0.31

40% cholesterol (Reverse-phase Extruded Vesicle)
: 3960 nm , 51% error (from DLS)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homodimer	0	12.74	0	13.26	–
	10	4.22	11.71	4.61	7.05
	11	4.57	13.19	5.34	7.13
	12	3.36	9.64	3.81	7.25
	12.1	2.23	6.60	2.65	7.37
				average	7.20
				std	0.14

**2.5% cholesterol (Reverse-phase Extruded Vesicle)
: 1910 nm , 59% error (from DLS)**

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from heterodimer	0	0	21.07	0	–
	4.5	3.86	8.46	3.92	4.72
	5	3.87	8.38	3.85	4.72
	5.5	3.81	8.30	3.91	4.63
	6	3.82	7.81	3.83	4.17
				average	4.56
				std	0.26

**2.5% cholesterol (Reverse-phase Extruded Vesicle)
: 1630 nm , 61% error (from DLS)**

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from heterodimer	0	0	20.23	0	–
	8	3.24	7.22	3.45	4.66
	9	3.24	7.31	3.47	4.74
	10	3.09	6.98	3.35	4.71
	11	3.03	6.81	3.34	4.58
	12	3.05	6.85	3.42	4.50
				average	4.64
				std	0.10

40% cholesterol (Reverse-phase Extruded Vesicle)
: 4770 nm , 59% error (from DLS)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from heterodimer	0	0	23.33	0	–
	4	4.13	12.00	4.57	7.62
	4.5	3.97	11.82	4.35	8.10
	5	3.94	11.33	4.27	7.63
	5.5	3.80	10.91	4.06	7.72
	6	3.45	10.09	3.76	7.86
				average	7.79
				std	0.20

40% cholesterol (Reverse-phase Extruded Vesicle)
: 3390 nm , 55% error (from DLS)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
from heterodimer	0	0	23.80	0	–
	8	3.55	10.39	3.83	7.94
	9	3.48	10.24	3.93	7.68
	10	3.57	10.51	3.99	7.75
	11	3.34	9.70	3.74	7.52
	12	3.22	9.47	3.55	7.85
	12.1	2.92	8.63	3.28	7.79
				average	7.76
				std	0.15

50% cholesterol (from pure hetero dimer)

extruded through 200nm pore filter : 178 nm , 23% error (from DLS)

Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
0	0	20.43	0	–
4	4.43	12.74	4.34	8.44
4.5	4.34	12.43	4.31	8.25
5	4.51	12.79	4.51	8.04
5.5	4.18	11.89	4.12	8.22
0	0	24.10	0	–
4	5.19	14.41	4.89	8.17
4.5	5.07	14.23	4.76	8.38
5	5.23	14.21	4.82	8.02

average 8.22

std 0.16

50% cholesterol (from pure hetero dimer)

extruded through 50nm pore filter : 85 nm , 40% error (from DLS)

Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	<i>K</i>
0	0	18.54	0	–
4	3.99	11.07	4.02	7.63
4.5	4.19	11.09	4.21	6.97
5	3.91	10.68	3.96	7.37
5.5	4.18	11.12	4.05	7.30
0	0	18.93	0	–
4	4.46	11.40	4.02	7.25
4.5	4.41	11.34	3.97	7.34
5	4.34	11.38	3.99	7.46
5.5	4.50	11.33	3.98	7.17

average 7.31

std 0.20