Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplemental Data for:

Determining the Lipid Specificity of Insoluble Protein Transmembrane Domains

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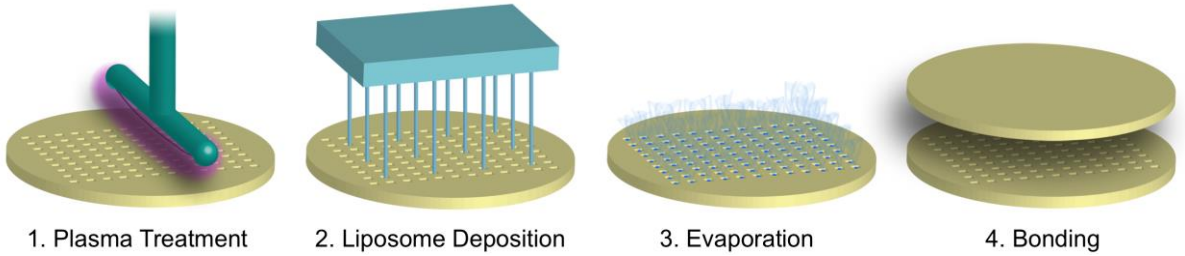
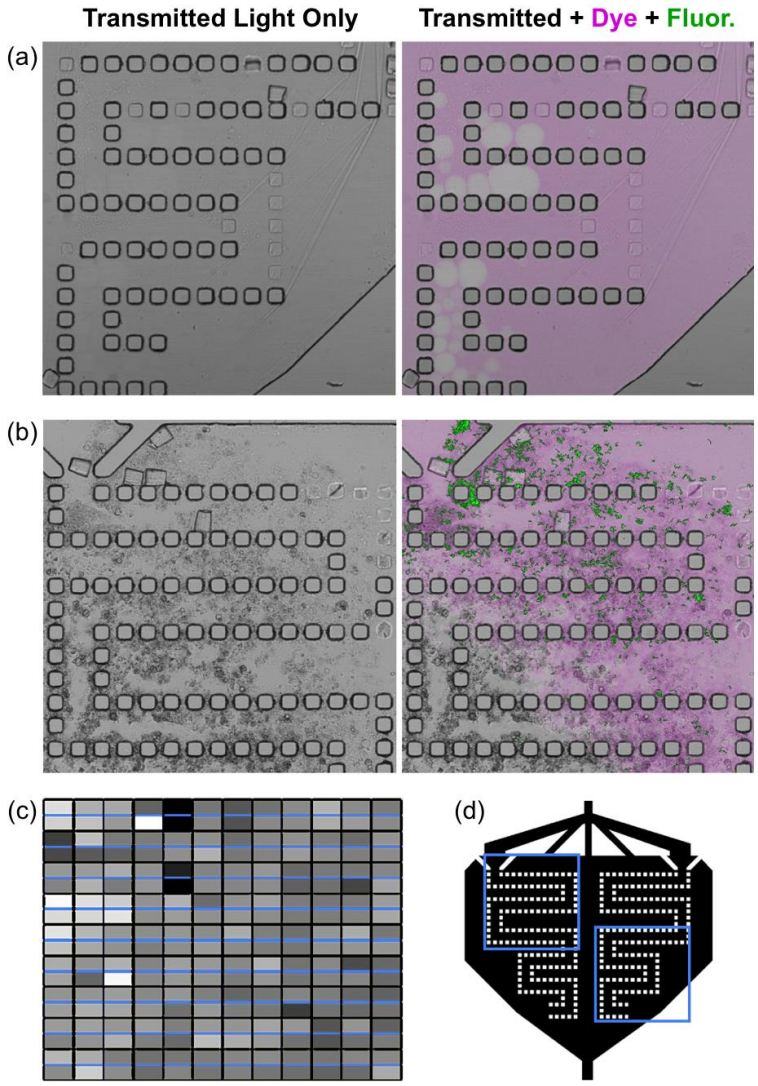
Construction of GFP-TMD and SNAP-TMD DNA: The TMDs used in this study were chosen based on previous work that demonstrated their specificity to local lipid environments. The human TMD genes for transmembrane emp23 domain-containing protein 10 (residues 186-206) (p23), transmembrane emp24 domian-containing protein 2 (residues 169-189) (p24), tumor necrosis factor receptor superfamily member 5 (residues 194-215) (CD40), receptor type tyrosine-protein phosphatase C (residues 576-597) (CD45), and human PD-1 transmembrane helix (residue 171-191) (PD1) were synthesized by IDT (Integrated DNA Technologies, Coralville, IA).

The TMD genes were amplified by PCR and cloned into the C-terminus of GFP or SNAP with a glycine-serine linker in a pUCA105T7 GFP or SNAP vector to create the DNA templates: GFP-TMD or SNAP-TMD. pUCA105T7 GFP and SNAP vectors were derived from pUC19 modified with a T7 promoter.(1) The peptide sequences encoded by the TMD genes are shown in Figure 4a.

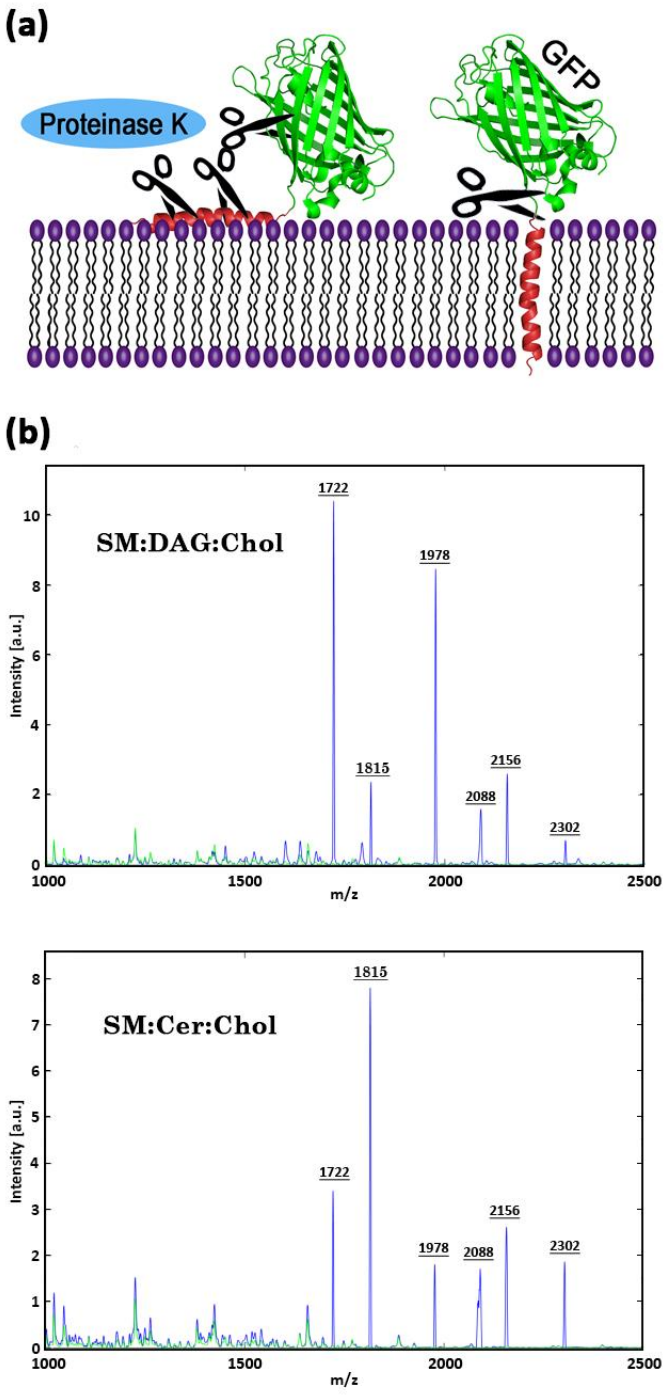
To determine whether lipid-bound TMDs were inclined on the membrane surface or embedded in the membrane, a **protease protection assay** was performed as follows: The lipid formulation of SM:DAG:Cholesterol:POPC (Supplementary table S1) was dissolved in chloroform, placed in a tube, and left under vacuum overnight, leaving dry lipid films. Liposomes were made by hydrating these films and heating to 60°C for 1 hour, followed by bath sonication for 10 minutes. Each GFP-TMD was synthesized by adding the IVTT reagents to the GFP-PD1 DNA and incubating at 37°C for 3 hours. Following insertion, Proteinase K was added at a final concentration of 0.5 mg/mL and incubated for 30 minutes on ice. Proteinase K was deactivated by heating to 90°C for 5 minutes and doubling the sample volume by adding 1:1 chloroform: methanol. A similar process is performed on a control, containing liposomes and the IVTT reagents, without GFP-PD1 DNA. Three peaks at Mw= 1977, 1793 and 1722 gr/mole were identified by BRUKER Ultraflextreme MALDI-TOF, corresponding to 20, 18, 17 AA fragments of the PD1 transmembrane domain, which were not apparent in the control sample. (Figure S3)



Supplementary figure 1: Preparation of the PDMS microfluidic device. 1. The PDMS device with channels was plasma-treated using a hand held corona-discharge unit. 2. A 3D printed capillary brush (blue) was used to simultaneously collect the lipid solutions from 12 glass vials and the lipid mixtures were seeded onto the PDMS device. This process was repeated 9 times for the seeding of all 108 wells. 3. After seeding, the solvent was allowed to evaporate, leaving behind the dried lipid films. 4. A flat PDMS slab was then used to seal the treated PDMS device.



Supplementary figure 2:In the final step of each experiment, and after measuring fluorescence from each of the individual wells, the microfluidic chip was filled with a fluorescent dye (purple). This step was performed to determine how much fluorophore (green) each well was exposed to during initial loading. (a) Addition of the dye was also used to identify liposomes which were difficult to observe from the transmitted light image. (b) Mapping the dye could also be used to explain localization of fluorescence and visualize the actual flow field to which the liposomes were exposed. The total fluorescence measured from the liposomes could then be normalized to the actual concentration of dye molecules to which it was exposed. Note that the lipid compositions in (a) and (b) were different and each were taken from different wells. (c) Total dye as measured from each well; there were two read outs per well, resulting in an 18x12 matrix of total reads. (d) A sample well, marked in black, with read areas delimited by blue lines, that correlate to positions shown in (a) and (b). As can be seen, there were variations in flow to each well, due to liposome clogging, which was concentrated at the inlet channels.



Supplementary figure 3: (a) Schematics of a GFP-TMD inclined on (left) or embedded (right) in a lipid bilayer. The embedded TMD was protected from proteinase K degradation. (b) MALDI mass spectrum of SM:DAG:Cholesterol and SM:Cer:Chol membranes. PD1, P24, and P23 were expressed using IVTT with each membrane. The resulting spectra show TMD-PD1 fragments measuring 17 and 20 amino-acids length and TMD-P24 fragments measuring 16, 18 and 20 amino acids in length (Blue). No residues of P23 were detected. Membrane samples with IVTT that do not include DNA for TMD-GFP expression were used as a control (Green).

Supplemental Table 1: (Separated into 4 Parts):

(1 of 4)

Position	Bar	Lipid Group 1		Lipid Group 2		Lipid 3	Lipid 4	Protein Transmembrane Domains					
								PD1	CD40	CD45	P24	P23	CTb
								Competitor					
Y	X	Name	Mol Ratio %	Name	Mol Ratio %	Cholesterol %	POPC %	P24 P23	PD1	PD1	P23 PD1	P24 PD1	
7	L	PC-he	30	DGPP	55	15		9.6	0	0	0.7	0	0
7	I	PC-he	16	PC-s	69	15		0	0	1.8	13	0	0
7	F	PC-he	25	PE-s	25	15	35	0	6.4	75	14	2.7	0
7	C	PC-he	23	PG	23	15	39	6.7	0	0	4	0.3	0
4	L	PC-he	22	PS	63	15		14	11	5.9	1.6	2.5	0
4	I	PC-he	22	PI	22	15	41	10	0	0	2.3	0	0
4	F	PC-he	21	PA	21	15	44	23	0	0	2	4.8	0
4	C	PC-he	65		0	35		14	0	2	38	0	0
1	L	PC-he	17	SM	17	15	51	73	0	0	8.1	1.8	0
1	I	PC-he	15	Gang	15	15	55	60	0	0	7.1	0	13
1	F	PC-he	16	Cerb	16	15	53	66	0	0	55	1.6	0
1	C	PC-he	15	Cer	15	15	55	7	5.3	2.2	55	1.1	0
7	K	PC-he	15	LyPC	15	15	54	34	0	0	31	3.2	5.4
7	H	PC-he	15	NPG	15	15	55	28	7.4	0	9	0.4	0
7	E	PC-he	19	PE-ec	19	15	46	64	0	0	21	21	0
7	B	PC-he	15	DAG	15	15	55	74	0	0	92	0	4.4
4	K	PE-ec	53	DGPP	32	15		34	47	5.4	0.6	0	4
4	H	PE-ec	36	PC-s	49	15		5.8	0	0	3.2	10	0
4	E	PE-ec	33	PE-s	52	15		9.4	0	0	24	45	0
4	B	PE-ec	17	PG	68	15		13	0	1.6	1.6	1	0
1	K	PE-ec	43	PS	42	15		18	12	10	0.7	3.6	0
1	H	PE-ec	32	PI	32	15	22	22	4.7	2.5	2	1.5	0
1	E	PE-ec	29	PA	29	15	26	6.6	0	0	1.4	4.5	0
1	B	PE-ec	77		0	23		25	5.3	1.3	1.9	2.2	0.5
7	J	PE-ec	22	SM	22	15	41	29	12	3.6	1	6	7.7
7	G	PE-ec	19	Gang	19	15	47	17	0	2.6	5.4	0.4	100
7	D	PE-ec	20	Cerb	20	15	44	49	0	0	4.1	2.6	0
7	A	PE-ec	19	Cer	19	15	47	68	0	2	15	11	7.5
4	J	PE-ec	20	LyPC	20	15	45	40	0	3.4	10	3.3	0
4	G	PE-ec	19	NPG	19	15	47	24	19	24	2.3	5.8	4.7

Position		Lipid Group 1		Lipid Group 2		Lipid 3	Lipid 4	Protein Transmembrane Domains					
Bar								PD1	CD40	CD45	P24	P23	CTb
								Competitor					
Y	X	Name	Mol Ratio %	Name	Mol Ratio %	Cholesterol %	POPC %	P24 P23	PD1	PD1	P23 PD1	P24 PD1	
4	D	PE-ec	19	DAG	19	15	47	32	0	0	18	11	0
4	A	DAG	30	DGPP	55	15		6.7	0	27	2.6	0	0
1	J	DAG	16	PC-s	69	15		15	17	14	2.2	51	0
1	G	DAG	25	PE-s	25	15	36	32	0	2.3	39	6.3	0
1	D	DAG	23	PG	23	15	39	17	0	0	2.1	0	0
1	A	DAG	22	PS	63	15		13	0	2.8	1.6	0	0
8	L	DAG	22	PI	22	15	41	10	0	0	0.5	0	4.4
8	I	DAG	21	PA	21	15	44	15	3.6	6.7	1.9	7.3	2.2
8	F	DAG	64		0	36		24	11	2.9	3.8	0	0
8	C	DAG	17	SM	17	15	51	95	0	6.3	87	2.3	0.7
5	L	DAG	15	Gang	15	15	55	44	4	0	2.1	1.2	92
5	I	DAG	16	Cerb	16	15	54	68	3.9	0	22	2.4	5.3
5	F	DAG	15	Cer	15	15	55	100	21	8.2	59	17	0
5	C	DAG	15	LyPC	15	15	54	41	0	0	99	31	0
2	L	DAG	15	NPG	15	15	55	26	0	0	3	7	5.9
2	I	SM	38	DGPP	47	15		12	0	0	2.2	0	0
2	F	SM	22	PC-s	63	15		3.6	0	0	1.1	1.2	0
2	C	SM	20	PE-s	65	15		0	0	11	2.1	1	0
8	K	SM	28	PG	28	15	30	16	0	0	0.5	0	0
8	H	SM	29	PS	56	15		10	0	1.5	4	0	3.8
8	E	SM	26	PI	26	15	34	0	0	0	2.9	0	0
8	B	SM	24	PA	24	15	37	16	0	0	2.8	0	3.6
5	K	SM	70			30		60	81	49	8.4	6.6	0
5	H	SM	17	LyPC	17	15	50	33	11	22	100	7.5	0
5	E	SM	17	NPG	17	15	51	38	13	41	14	0.7	0
5	B	SM	38			15	47	26	12	13	4.9	8.1	0
2	K	SM	17	Gang	17	15	51	4.5	0	0	0.4	0	33
2	H	SM	18	Cerb	18	15	50	18	0	5.3	32	5	0
2	E	SM	17	Cer	17	15	51	48	11	4.2	59	3.4	3.9
2	B	Gang	30	DGPP	55	15		16	0	0	1.1	1.4	11
8	J	Gang	16	PC-s	69	15		0	6.6	2.9	1	56	37

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Position		Lipid Group 1		Lipid Group 2		Lipid 3	Lipid 4	Protein Transmembrane Domains					
								PD1	CD40	CD45	P24	P23	CTb
Bar								Competitor					
Y	X	Name	Mol Ratio %	Name	Mol Ratio %	Cholesterol %	POPC %	P24 P23	PD1	PD1	P23 PD1	P24 PD1	
8	G	Gang	25	PE-s	25	15	36	5.8	0	4.1	4	0.5	39
8	D	Gang	23	PG	23	15	39	0	0	0	2.7	0	44
8	A	Gang	22	PS	63	15		0	0	0	3.6	0	59
5	J	Gang	22	PI	22	15	41	10	0	0	1.6	0	36
5	G	Gang	21	PA	21	15	44	3.8	0	0	2.8	0	64
5	D	Gang	64		0	36		7.4	0	1.7	2.3	0	57
5	A	Gang	15	LyPC	15	15	54	0	0	0	1.6	0.3	26
2	J	Gang	15	NPG	15	15	55	33	0	0	0.7	0.3	37
2	G	Gang	15	Gang	15	15	55	9.7	15	6.1	2.2	0.3	68
2	D	Gang	16	Cerb	16	15	54	29	5.4	5.8	2	1.1	28
2	A	Gang	15	Cer	15	15	55	21	0	12	4.8	0.8	97
9	L	Cerb	33	DGPP	52	15		23	66	0	2	3	0
9	I	Cerb	18	PC-s	67	15		4.7	0	2.9	3.1	50	0
9	F	Cerb	16	PE-s	69	15		8.5	6.8	12	18	60	5
9	C	Cerb	25	PG	25	15	35	9.3	0	3.2	6.7	0.5	3.6
6	L	Cerb	24	PS	61	15		0	100	46	1.1	0	0
6	I	Cerb	23	PI	23	15	38	4.6	0	0	1.8	8.6	0
6	F	Cerb	22	PA	22	15	41	11	0	4.8	1.4	0.5	0
6	C	Cerb	67		0	33		27	0	1.5	2.9	5	0
3	L	Cerb	16	LyPC	16	15	53	64	0	6.5	23	0.8	0
3	I	Cerb	16	NPG	16	15	54	9.4	0	2.7	35	0.3	0
3	F	Cerb	16	Cerb	16	15	52	86	0	0	28	1.5	0
3	C	Cerb	16	Cer	16	15	54	96	0	4.2	14	1.6	2.8
9	K	Cer	30	DGPP	55	15		0	28	9.8	2.7	12	3.2
9	H	Cer	16	PC-s	69	15		8.5	0	60	16	4	0
9	E	Cer	25	PE-s	25	15	36	31	13	85	30	25	0
9	B	Cer	23	PG	23	15	39	34	0	6	47	12	5
6	K	Cer	22	PS	63	15		17	38	6.9	2.9	1.3	0
6	H	Cer	22	PI	22	15	41	53	46	29	21	15	0
6	E	Cer	21	PA	21	15	44	65	27	4.8	23	19	3.9
6	B	Cer	64		0	36		30	15	0	8.2	0	3.5

Position		Lipid Group 1		Lipid Group 2		Lipid 3	Lipid 4	Protein Transmembrane Domains					
Bar								PD1	CD40	CD45	P24	P23	CTb
								Competitor					
Y	X	Name	Mol Ratio %	Name	Mol Ratio %	Cholesterol %	POPC %	P24 P23	PD1	PD1	P23 PD1	P24 PD1	
3	K	Cer	15	LyPC	15	15	54	0	0	3.3	6.4	5	0
3	H	Cer	15	NPG	15	15	55	36	0	0	14	0	0
3	E	LyPC	32	DGPP	53	15		14	0	0	2.2	0	0
3	B	LyPC	17	PC-s	68	15		20	0	12	4	100	0
9	J	LyPC	16	PE-s	69	15		8.6	4.5	10			0
9	G	LyPC	24	PG	24	15	37	11	5.5	5.9			1.1
9	D	LyPC	23	PS	62	15		19	0	0			3.1
9	A	LyPC	23	PI	23	15	40	15	0	0			4
6	J	LyPC	21	PA	21	15	42	6.4	0	1.6			0
6	G	NPG	30	DGPP	55	15		31	15	31			0
6	D	NPG	16	PC-s	69	15		34	4.9	12			0
6	A	NPG	25	PE-s	25	15	36	21	0	3.7			0
3	J	NPG	23	PG	23	15	39	46	61	59			0
3	G	NPG	22	PS	63	15		0	0	5.9			0
3	D	NPG	22	PI	22	15	41	50	18	100			0
3	A	NPG	21	PA	21	15	44	28	0	0			0
9	J	BMP	32	PE-ec	53	15					2.2	1.3	
9	G	BMP	55	DAG	30	15					5.3	0	
9	D	BMP	47	SM	38	15					3.7	0	
9	A	BMP	55	Cer	30	15					8.5	1	
6	J	BMP	43	DGPP	43	15					3.1	0	
6	G	BMP	43	PC-s	43	15					5.1	6.8	
6	D	BMP	43	PG	43	15					3.9	0	
6	A	BMP	43	PS	43	15					5.5	0	
3	J	BMP	43	PI	43	15					1.3	0.8	
3	G	BMP	43	PA	43	15					2.3	0	
3	D	BMP	85		0	15					2.2	0	
3	A	BMP	100		0	0					2.2	0	

Supplemental Table 1: Each of the matrices was normalized to its highest intensity. Comparison of the highest labeling positions between all TMDs showed a ratio of: 11:4:3:7:1 for PD1:CD40:CD45:P24:P23, respectively. However, it is risky to directly compare these absolute intensities as the IVTT is highly sensitive to minor changes in its environment and is likely to vary from one experiment to another. Position bars X,Y correspond to the locations in the matrices as they appear in figures 3 and 4.

Supplemental Table 2: (Separated into 2 Parts): (1 of 2)

		Lipid As designated in Table 1 (in text)	Designation in Table S1		Avanti Polar Catalog #
Saturated	Glycerol	**Hydro Egg PC**	PC-he	L-α-phosphatidylcholine, hydrogenated (Egg, Chicken)	840059
		E. coli PE	PE-ec	L-α-phosphatidylethanolamine *(E. coli)*	840027
		DAG	DAG	1,2-dipalmitoyl-*sn*-glycerol	800816
		Egg Lyso PC	LyPC	L-α-lysophosphatidylcholine (Egg, Chicken)	830071
	Sphingo	**Sphingomyelin (egg)**	SM	Sphingomyelin (Egg, Chicken)	860061
		Ganglioside total extract Brain	Gang	Total Ganglioside Extract (Brain, Porcine-Ammonium Salt)	860053
		Cerebrosides (Total Brain)	Cerb	Total Cerebrosides (Brain, Porcine)	131303
		Ceramide (Egg)	Cer	Ceramide (Brain, Porcine)	860051
		N-palmitoylglycine	NPG	N-palmitoylglycine	870817

		Lipid As designated in Table 1 (in text)	Designation in Table S1		Avanti Polar Catalog #
Unsaturated	Glycerol	**DGPP**	DGPP	dioleoylglycerol pyrophosphate (ammonium salt)	810811
		Soy PC	PC-s	L-α-phosphatidylcholine (Soy)	840054
		Soy PE	PE-s	L-α-phosphatidylethanolamine (Soy)	840024
		Egg PG	PG	L-α-phosphatidylglycerol (Egg, Chicken) (sodium salt)	841138
		Soy PS	PS	L-α-phosphatidylserine (Soy, 99%) (sodium salt)	870336
		Soy PI	PI	L-α-phosphatidylinositol (Soy) (sodium salt)	840044
		Egg PA	PA	L-α-phosphatidic acid (Egg, Chicken) (sodium salt)	840101
		BMP (S,R) 18:1	BMP	bis(monooleoylglycero)phosphate (S,R Isomer) (ammonium salt)	857133
		POPC		1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine	850457
Sterols		**Cholesterol**		cholesterol (ovine wool, >98%)	700000

Supplemental table 2: Detailed information of all lipid products and their acronyms as they appear in Table 1 and Supplementary Table 1.

1. Asahara H & Chong SR (2010) In vitro genetic reconstruction of bacterial transcription initiation by coupled synthesis and detection of RNA polymerase holoenzyme. *Nucleic Acids Res* 38(13).