SUPPLEMENTAL MATERIALS

A. ***Double labeling of RBL-2H3 cells with BODIPY-GM1 and 10PC to determine co-localization***

Fluorescence microscopy shows that when RBL-2H3 mast cells are labeled with fluorescently labeled lipid probes such as BODIPY-GM1 at low temperature, the label is retained in the plasma membrane for an hour or more (P. Sengupta, unpublished results). On the other hand, in the ESR experiments, we observe that the spin label intensity undergoes a steady decay even at low temperature (Fig. 11).

To confirm that the spin labels remain in the plasma membrane until they decay due to reducing agents in the plasma membrane or in the cell interior, we performed double labeling experiments. In these experiments RBL-2H3 cells were labeled at 5°C with BODIPY-GM1, and 10PC was added either at the same time or after the BODIPY-GM1 had equilibrated. The cells were washed after the BODIPY-GM1 (or both labels) were added, resuspended in BSS/BSA at 10^6cells/ml, and the fluorescence (ex: 500 nm; em 520 nm) was monitored as a function of time at 15°C. In one experiment 10PC was added to the BODIPY-GM1 labeled and washed cells. 35% fluorescence quenching was observed immediately upon 10PC addition; no quenching was observed with the same amount of the methanol vehicle (data not shown). These results show that 10PC effectively quenches BODIPY-GM1 when both are localized in the plasma membrane

In a second experiment BODIPY-GM1 labeled cells were co-labeled, or not, with 10PC and washed (Fig. S1). Cells labeled with BODIPY-GM1 alone exhibited constant fluorescence (Trace 1) consistent with its stable localization in the plasma membrane. Consistent with this interpretation, fluorescence microscopy of the cells after 50 min showed little or no internalization of the fluorescence label. The cells co-labeled with BODIPY-GM1 and 10PC and washed showed a steady increase in fluorescence corresponding to relief of quenching that occurs as the spin label intensity decays (Trace 2). These results support the view that most of the 10PC stays in the plasma membrane together with the BODIPY-GM1 during the time course of these experiments.

B. ***Fig. S1***

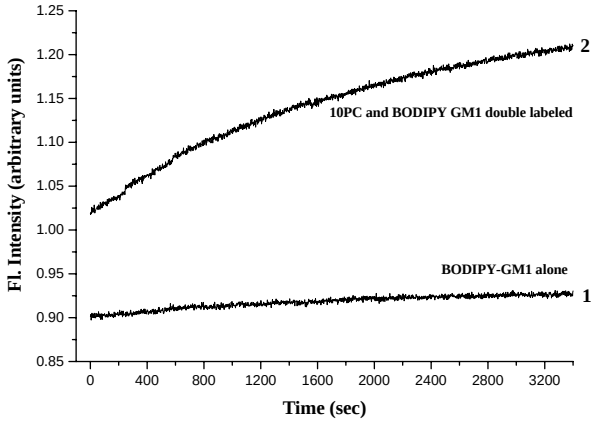

Fig. S1. Fluorescence of BODIPY GM1 in RBL-2H3 cells is quenched in the presence of 10PC. Cells were labeled on ice, washed with cold buffer and then fluorescence intensity was recorded at 15^{o}C. Trace 1 corresponds to RBL-2H3 cells labeled with BODIPY-GM1 alone. Trace 2 corresponds to RBL-2H3 cells that were double labeled with BODIPY-GM1 and 10PC. The samples shown were labeled with somewhat different amounts of BODIPY-GM1 resulting in different initial levels of fluorescence.

C. ***Tables S1 – S6***

Table S1. Parameters obtained from NLLS fitting of ESR spectra of spin labels in plasma membrane of live RBL cells

spin label	T (^oC)	C*	$R_\perp(s^{-1})$	S_0	P*
	37	Ld	1.22×10^8	0.19	0.22
		Lo	5.30×10^7	0.34	0.78
5PC	25	Ld	6.56×10^7	0.21	0.37
		Lo	3.77×10^7	0.38	0.63
	15	Ld	5.46×10^7	0.22	0.26
		Lo	2.85×10^7	0.38	0.74
	5	Ld	4.90×10^7	0.31	0.19
		Lo	2.67×10^7	0.55	0.81
	37	Ld	9.77×10^7	0.14	0.29
		Lo	5.90×10^7	0.34	0.71
7PC	25	Ld	6.67×10^7	0.23	0.25
		Lo	3.79×10^7	0.32	0.75
	15	Ld	6.58×10^7	0.22	0.21
		Lo	3.17×10^7	0.35	0.79
	5	Ld	4.94×10^7	0.31	0.28
		Lo	1.52×10^7	0.43	0.72
	37	Ld	9.71×10^7	0.16	0.32
		Lo	5.30×10^7	0.33	0.68
10PC	25	Ld	6.58×10^7	0.19	0.32
		Lo	3.66×10^7	0.32	0.68
	15	Ld	6.19×10^7	0.25	0.27
		Lo	3.81×10^7	0.38	0.73
	5	Ld	4.58×10^7	0.28	0.28
		Lo	2.12×10^7	0.46	0.72
	37	Ld	1.03×10^8	0.10	0.49
		Lo	5.24×10^7	0.14	0.51
12PC	25	Ld	1.00×10^8	0.14	0.34
		Lo	5.94×10^7	0.30	0.66
	15	Ld	7.50×10^7	0.22	0.25
		Lo	4.10×10^7	0.33	0.75
	5	Ld	6.01×10^7	0.27	0.39
		Lo	2.00×10^7	0.48	0.61

$*$ C = component, P = population.

Table S2. Parameters obtained from NLLS fitting of ESR spectra of spin labels in plasma membrane of live COS7 cells

spin label	T (^oC)	C*	$R_\perp(s^{-1})$	S_0	P*
	37	Ld	1.62×10^8	0.12	0.22
		Lo	5.89×10^7	0.35	0.78
5PC	25	Ld	1.26×10^8	0.15	0.26
		Lo	4.46×10^7	0.36	0.74
	15	Ld	7.31×10^7	0.17	0.25
		Lo	3.23×10^7	0.37	0.75
	5	Ld	4.83×10^7	0.20	0.31
		Lo	2.30×10^7	0.43	0.69
	37	Ld	2.26×10^8	0.09	0.44
		Lo	8.61×10^7	0.28	0.56
7PC	25	Ld	1.16×10^8	0.11	0.53
		Lo	5.01×10^7	0.33	0.47
	15	Ld	6.82×10^7	0.12	0.44
		Lo	6.08×10^7	0.38	0.56
	5	Ld	4.92×10^7	0.15	0.44
		Lo	3.85×10^7	0.38	0.56
	37	Ld	2.16×10^8	0.13	0.33
		Lo	7.43×10^7	0.29	0.67
10PC	25	Ld	1.37×10^8	0.14	0.32
		Lo	5.06×10^7	0.31	0.68
	15	Ld	7.87×10^7	0.14	0.48
		Lo	4.69×10^7	0.34	0.52
	5	Ld	4.98×10^7	0.18	0.53
		Lo	4.47×10^7	0.39	0.47
	37	Ld	4.93×10^8	0.08	0.46
		Lo	7.78×10^7	0.20	0.54
12PC	25	Ld	3.21×10^8	0.13	0.43
		Lo	5.87×10^7	0.23	0.57
	15	Ld	1.69×10^8	0.15	0.39
		Lo	4.93×10^7	0.23	0.61
	5	Ld	7.74×10^7	0.17	0.54
		Lo	4.08×10^7	0.35	0.46

$*$ C = component, P = population.

Table S3. Parameters obtained from NLLS fitting of ESR spectra of spin labels in plasma membrane of live 3T3 cells

spin label	T (^oC)	C*	$R_\perp(s^{-1})$	S_0	P*
	37	Ld	1.94×10^8	0.10	0.39
		Lo	6.43×10^7	0.34	0.61
5PC	25	Ld	1.09×10^8	0.11	0.30
		Lo	5.08×10^7	0.34	0.70
	15	Ld	6.31×10^7	0.18	0.38
		Lo	3.52×10^7	0.36	0.62
	5	Ld	4.95×10^7	0.25	0.41
		Lo	2.72×10^6	0.48	0.59
	37	Ld	1.82×10^8	0.09	0.60
		Lo	7.38×10^7	0.30	0.40
7PC	25	Ld	8.77×10^7	0.11	0.35
		Lo	5.60×10^7	0.33	0.65
	15	Ld	7.55×10^7	0.18	0.33
		Lo	5.01×10^7	0.36	0.67
	5	Ld	5.12×10^7	0.16	0.42
		Lo	4.79×10^7	0.39	0.58
	37	Ld	1.06×10^8	0.05	0.46
		Lo	7.06×10^7	0.24	0.54
10PC	25	Ld	8.99×10^7	0.11	0.50
		Lo	5.22×10^7	0.28	0.50
	15	Ld	6.92×10^7	0.11	0.43
		Lo	4.74×10^7	0.30	0.57
	5	Ld	6.41×10^7	0.16	0.50
		Lo	3.44×10^7	0.27	0.50
	37	Ld	1.25×10^8	0.06	0.59
		Lo	7.01×10^7	0.26	0.41
12PC	25	Ld	1.26×10^8	0.12	0.36
		Lo	8.95×10^7	0.29	0.64
	15	Ld	1.14×10^8	0.14	0.35
		Lo	6.18×10^7	0.29	0.65
	5	Ld	6.52×10^7	0.14	0.50
		Lo	4.02×10^7	0.34	0.50

$*$ C = component, P = population.

Table S4. Parameters obtained from NLLS fitting of ESR spectra of spin labels in plasma membrane of live CHO cells

spin label	T (^oC)	C*	$R_\perp(s^{-1})$	S_0	P*
7PC	37	Ld	1.61×10^8	0.13	0.27
		Lo	1.00×10^8	0.36	0.73
	25	Ld	8.11×10^7	0.19	0.37
		Lo	5.18×10^7	0.37	0.65
	15	Ld	6.87×10^7	0.24	0.14
		Lo	4.26×10^7	0.36	0.86
	5	Ld	5.04×10^7	0.22	0.22
		Lo	4.50×10^7	0.44	0.78
10PC	37	Ld	8.51×10^7	0.09	0.36
		Lo	8.32×10^7	0.29	0.64
	25	Ld	7.28×10^7	0.16	0.39
		Lo	8.02×10^7	0.45	0.61
	15	Ld	5.38×10^7	0.17	0.36
		Lo	7.10×10^7	0.47	0.64
	5	Ld	4.46×10^7	0.19	0.41
		Lo	3.98×10^7	0.48	0.59
12PC	37	Ld	1.41×10^8	0.13	0.33
		Lo	9.55×10^7	0.34	0.67
	25	Ld	1.00×10^8	0.14	0.56
		Lo	6.21×10^7	0.43	0.44
	15	Ld	7.46×10^7	0.18	0.39
		Lo	4.98×10^7	0.41	0.61
	5	Ld	6.35×10^7	0.23	0.34
		Lo	4.51×10^7	0.46	0.66

$*$ C = component, P = population.

Table S5. Parameters obtained from NLLS fitting of ESR spectra of 16PC in plasma membrane of live RBL, COS7 and CHO cells

T (^oC)	$R_\perp(s^{-1})$	S_0	P*
	RBL		
37	1.65×10^8	0.05	
25	1.34×10^8	0.08	
15	1.08×10^8	0.12	
5	9.82×10^7	0.16	0.44
	COS7		
37	1.62×10^8	0.16	0.25
25	1.07×10^8	0.17	0.14
15	1.12×10^8	0.19	0.10
5	9.55×10^7	0.20	0.13
	CHO		
37	1.51×10^8	0.14	0.14
25	1.15×10^8	0.18	0.09
15	8.51×10^7	0.17	0.07
5	8.32×10^7	0.20	0.06

$*$ P: the population of the well-resloved component. For RBL cells, the 16PC spectra at temperatures $15^o, 25^o, 37^o$C were fit with only a single component. The spectrum at 5^oC was fit with two well-resolved components. The values of $R_\perp$ and S_0 of the Lo-like component (second component) are $2.80 \times 10^7 s^{-1}$ and 0.25, respectively.

Table 6. Parameters obtained from NLLS fitting of ESR spectra of spin labels in model membranes consisting of sphingomyelin, DOPC and cholesterol at 22^oC*

sample	spin label	$R_\perp(s^{-1})$	S_0
	5PC	$1.78\times^7$	0.43
	7PC	$2.34\times^7$	0.38
Lo[hc]	10PC	$4.07\times^7$	0.33
	12PC	$5.31\times^7$	0.34
	14PC	$1.17\times^8$	0.35
	16PC	$3.07\times^8$	0.27
	5PC	$2.95\times^7$	0.48
	7PC	$3.43\times^7$	0.32
Lo[mc]	10PC	$4.44\times^7$	0.26
	12PC	$6.12\times^7$	0.21
	14PC	$1.05\times^8$	0.27
	16PC	$2.18\times^8$	0.20
	5PC	$4.37\times^7$	0.43
	7PC	$4.57\times^7$	0.28
Ld[lc]	10PC	$5.82\times^7$	0.15
	12PC	$7.62\times^7$	0.11
	14PC	$1.00\times^8$	0.10
	16PC	$2.08\times^8$	0.09

$*$ Compositions of model membranes are: sphingomyelin/DOPC/Chol = 0.43/0.11/0.46, 0.24/0.42/0.34 and 0.30/0.63/0.07 for high chol (Lo), moderate chol (Lo) and low chol (Ld), respectively.