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Supporting Information

Transmembrane Helix Induces Membrane Fusion through Lipid Binding and Splay

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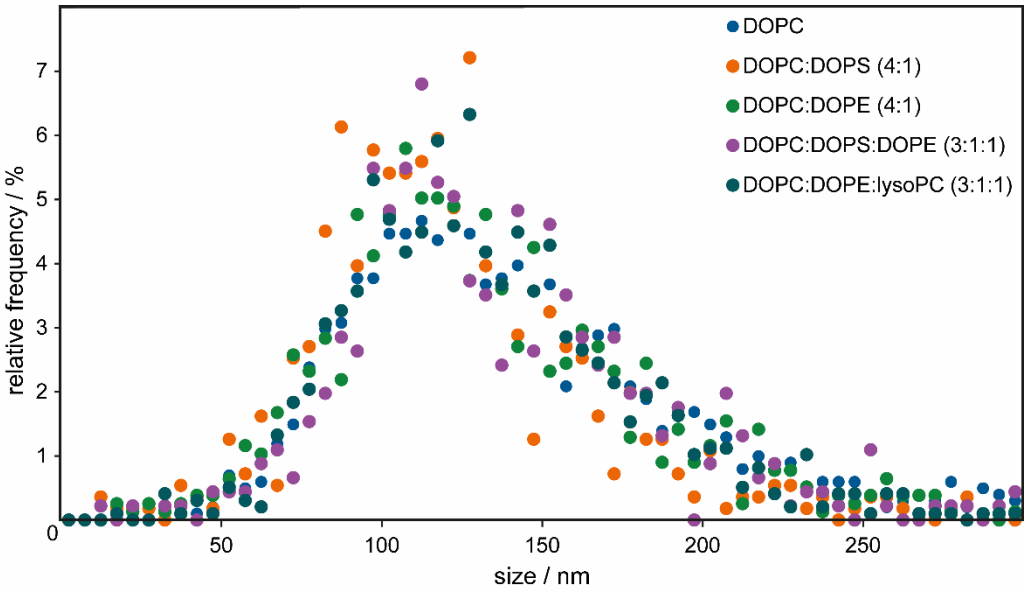

Figure S1. Liposome size distribution of blank liposomes prepared by sonication of lipid mixtures. The measurements were performed at 28 °C in buffer with an appropriate dilution to track a minimum 500 particles. Each data point describes the percentage of particles within a certain size resulting in an area under the curve (AUC) = 100%. The median size for all compositions is 124 nm ± SD (= 6.1 nm).

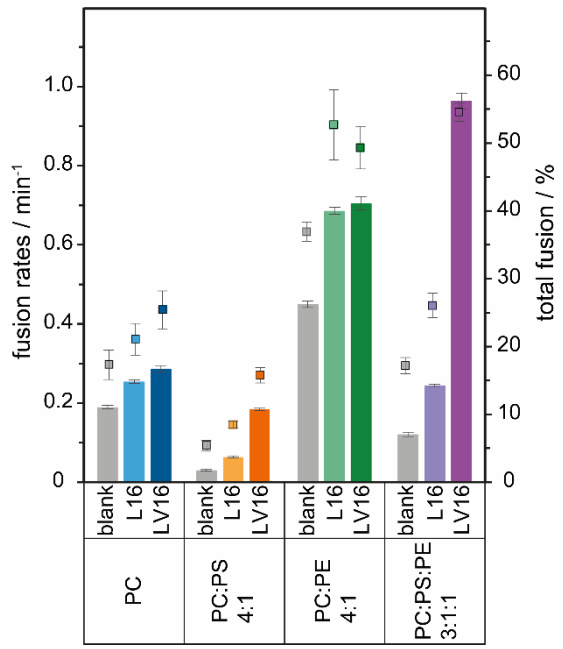

Figure S2. A comparison of initial fusion rates (bars) and extents of fusion, as seen after 3 h (symbols). The rank orders of the different metrics match for all lipid mixtures examined here thus qualifying the fusion extent as a valid metric of fusion. n = 4, means ± SEM.

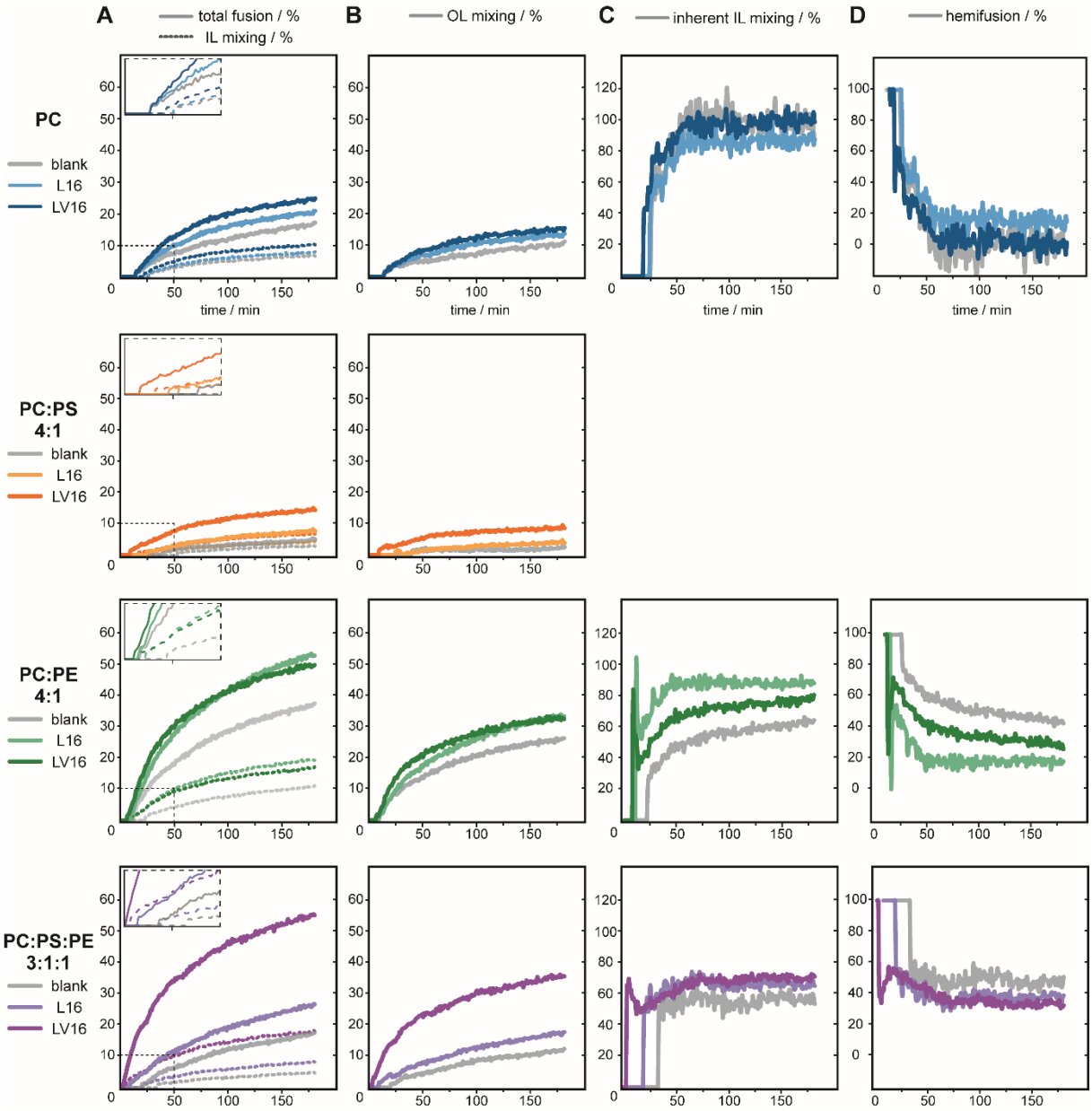

Figure S3. Impact of TMDs on lipid mixing of blank liposomes or liposomes containing L16 or LV16 at a peptide/lipid ratio = 0.005. (A) Average fluorescence dequenching kinetics representing total fusion (in the absence of dithionite) or IL mixing (after bleaching NBD fluorescence by dithionite). Note that dithionite silences OL mixing by converting the fluorescence donor of the OL to a non-fluorescent derivative such that only IL mixing results in fluorescence dequenching.[1] (B) Average OL mixing kinetics obtained by subtracting IL mixing from total lipid mixing. (C) Average inherent IL mixing kinetics, obtained by normalizing the measured IL mixing to OL mixing (=100%). (D) Percentage of hemifusion as a function of reaction time. Experiments were performed at 37°C; n = 4, means ± SEM.

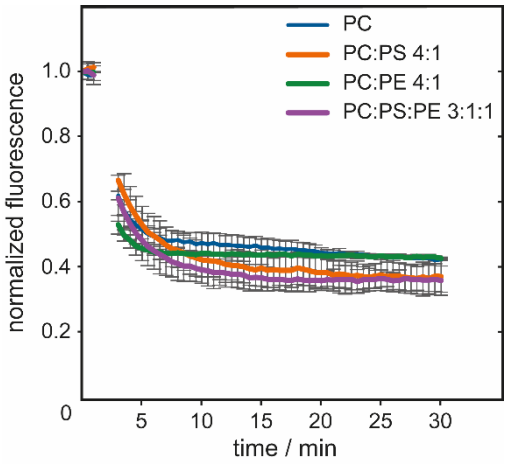

Figure S4. Control experiment demonstrating dithionite bleaching of blank liposomes containing 1.5 mol% of the fluorescent 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE). Note that NBD fluorescence decreases by only up to ~60%. Since this percentage corresponds to the fraction of OL lipids in small unilamellar liposomes [2], this control experiment demonstrates selective silencing of the OL.

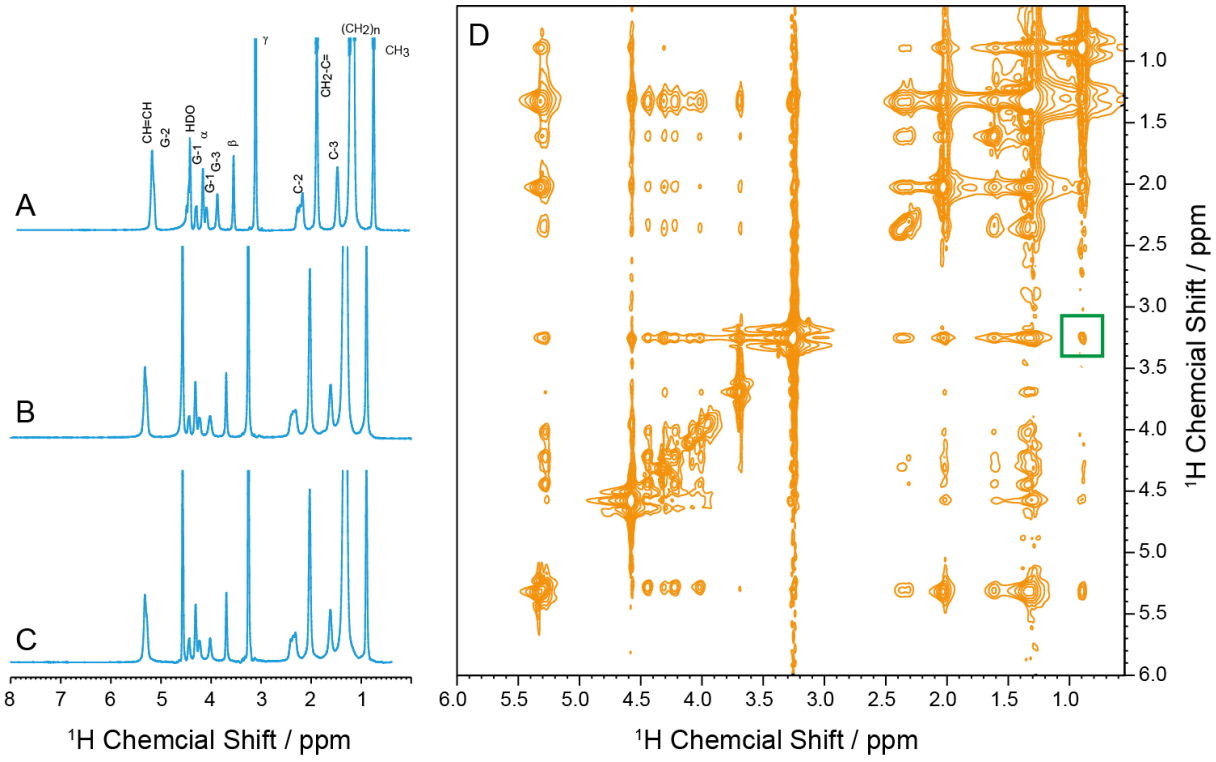

Figure S5. Typical ^{1}H MAS NMR spectra of DOPC membranes in the absence (A) and in the presence of L16 (B) or LV16 (C). Assignment of the lipid peaks is indicated. Panel D shows a ^{1}H-^{1}H NOESY NMR spectrum of DOPC/DOPS/DOPE (3:1:1) at a mixing time of 300 ms. The cross peak between the terminal methyl groups of the lipid chains (CH_3) and the choline γ group of the lipid headgroup that was used in the analysis is indicated by a green box.

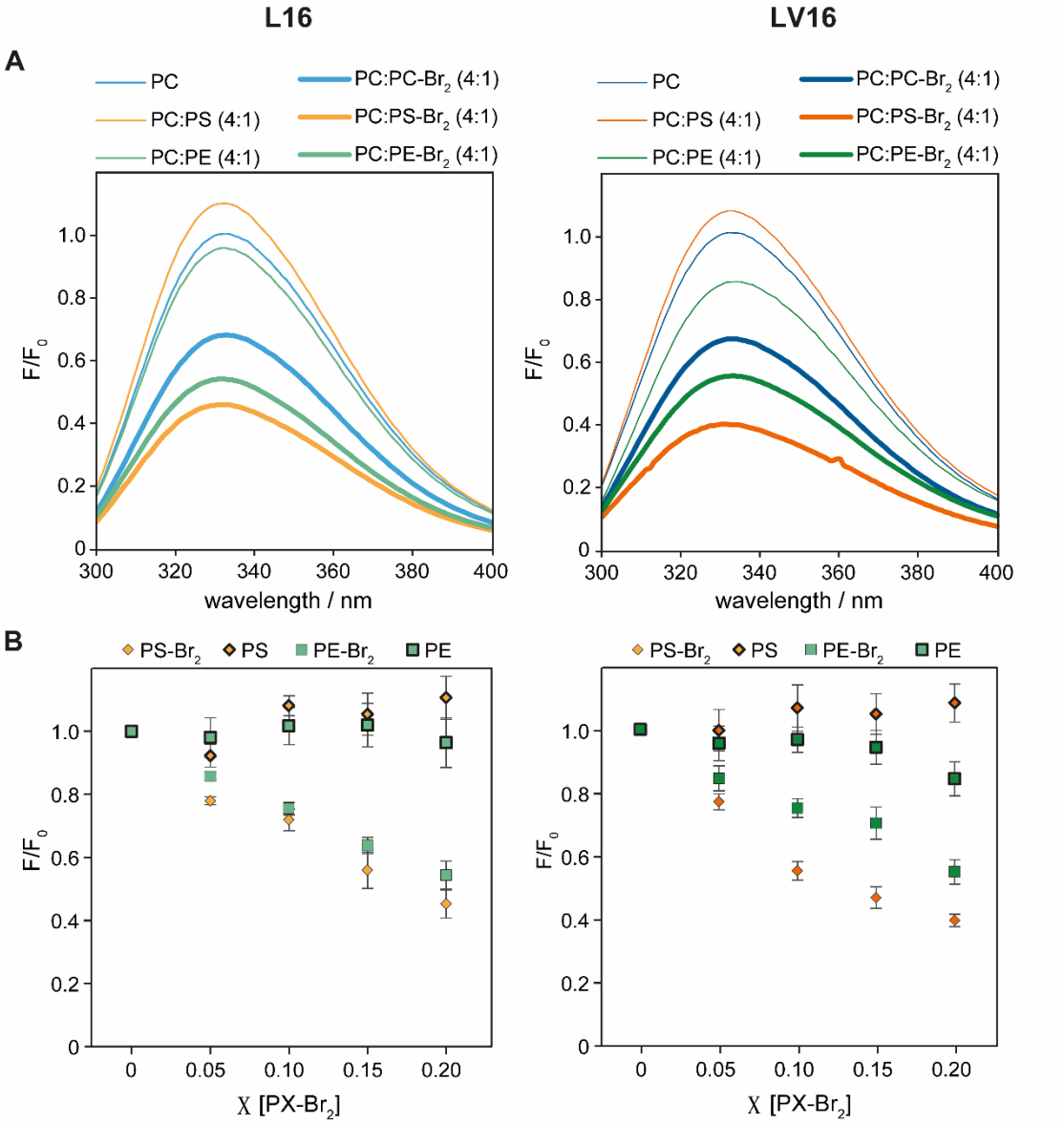

Figure S6. Lipid binding to the TMDs assessed by a Trp quenching assay. (A) Averaged Trp fluorescence spectra (λ_{ex} = 280 nm) with or without 20 mol% of non-brominated DOPS or DOPE (thin lines) or of PC-Br_2, PS-Br_2, or PE-Br_2 (bold lines) in POPC host lipids. All spectra were normalized to the one recorded in pure DOPC (F/F_0 = 1.0). Spectra recorded with other mole fractions of added lipids are ommited for clarity. (B) Normalized maximal Trp fluorescence plotted against the mole fraction χ of the different PS and PE lipids given in the legend above the graphs (left panel L16; right panel LV16). Data for PC lipids are omitted for clarity but given in Table S5. Means ± SEM, n = 4-5.

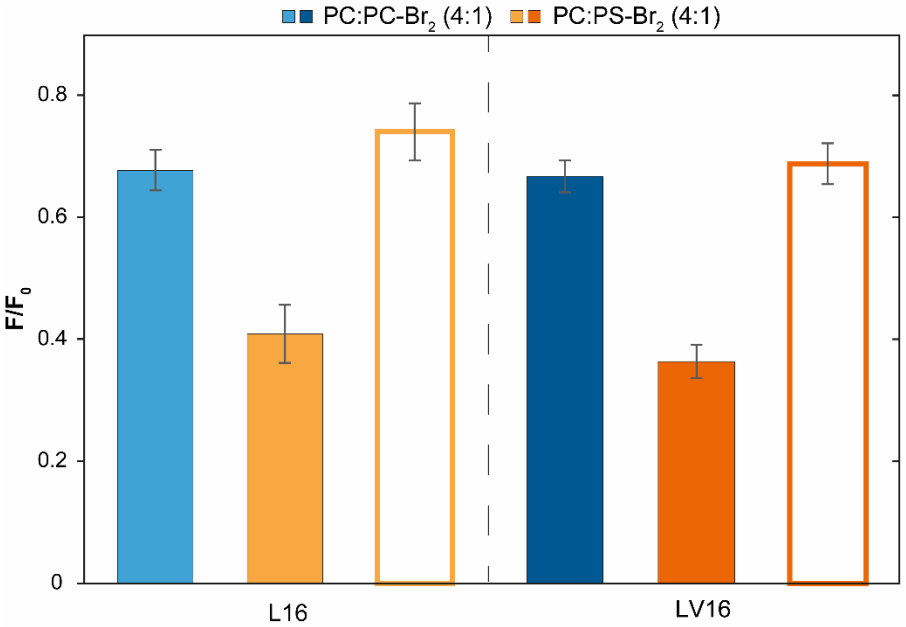

Figure S7. Effect of ionic strength on peptide/lipid interaction. A strong quenching of Trp fluorescence is seen in liposomal membranes composed of DOPC/PC-Br_2 upon exchanging PC-Br_2 for PS-Br_2 in 0.15 M NaCl (filled bars), but not in 1.0 M NaCl (empty bars). This indicates that the Trp fluorescence quench by PS-Br_2 depends on ionic strength.

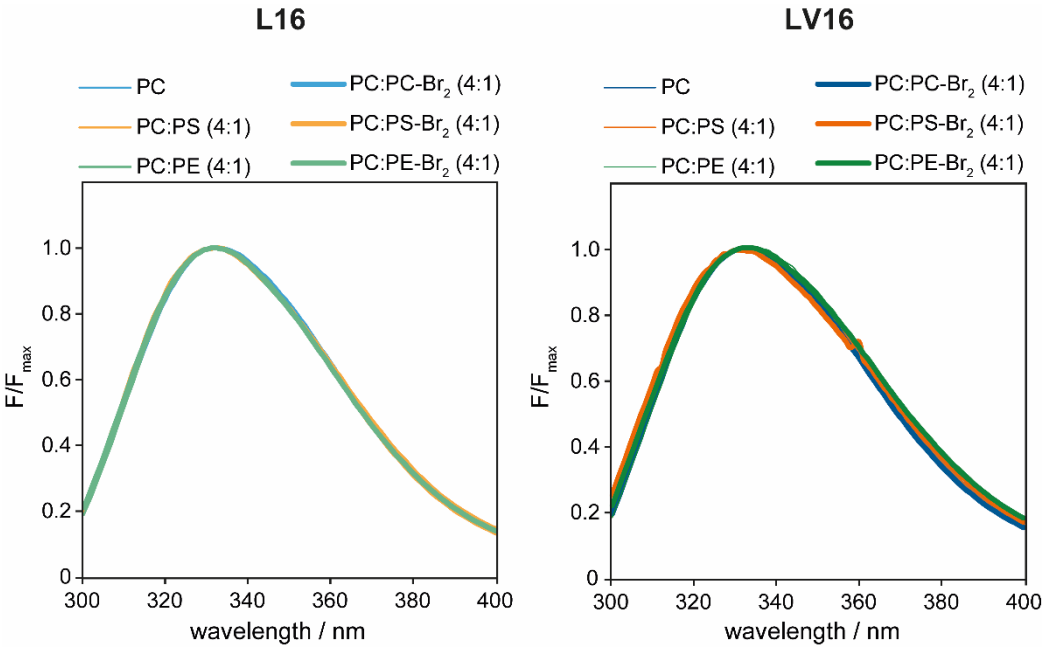

Figure S8. Dependence of membrane integration on lipid environment. Averaged Trp fluorescence spectra (λ_{ex} = 280 nm) with or without 20 mol% of non-brominated DOPS or DOPE (thin lines) or of PC-Br_2, PS-Br_2, or PE-Br_2 (bold lines). All spectra correspond to the ones shown in Figure S6 but were normalized (at λ_{max}: F/F_0 = 1.0) for comparability. The similarity of all spectra reflects similar hydrophobicities around the Trp residues in all lipid mixtures.

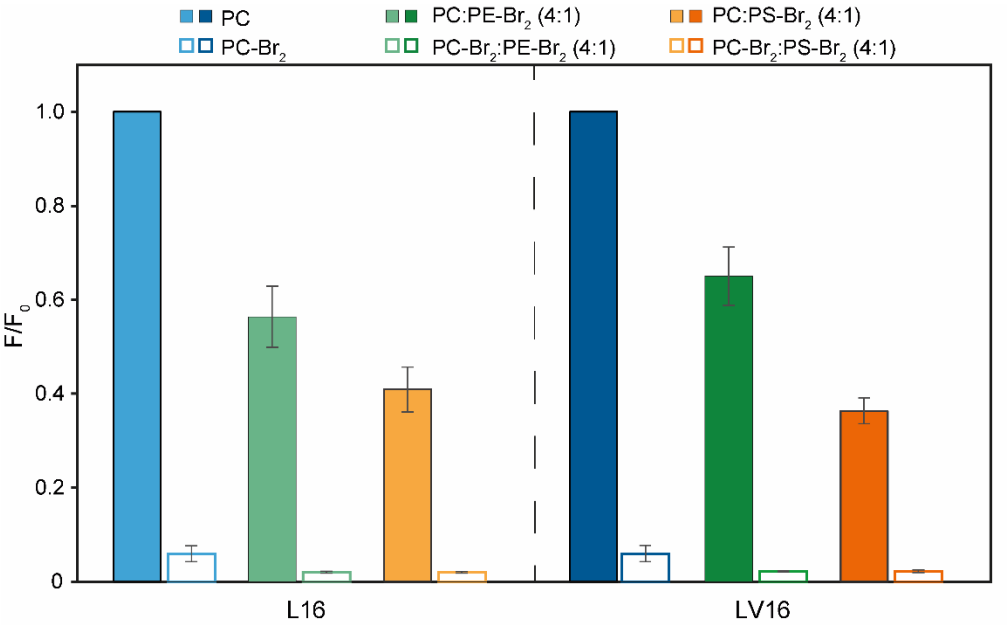

Figure S9. Lipid accessibility of membrane-embedded TMDs. Incorporation of peptides into liposomes formed from fully brominated lipids leads to complete quenching of their Trp fluorescence suggesting accessibility of ≥95% of the peptides to lipids.

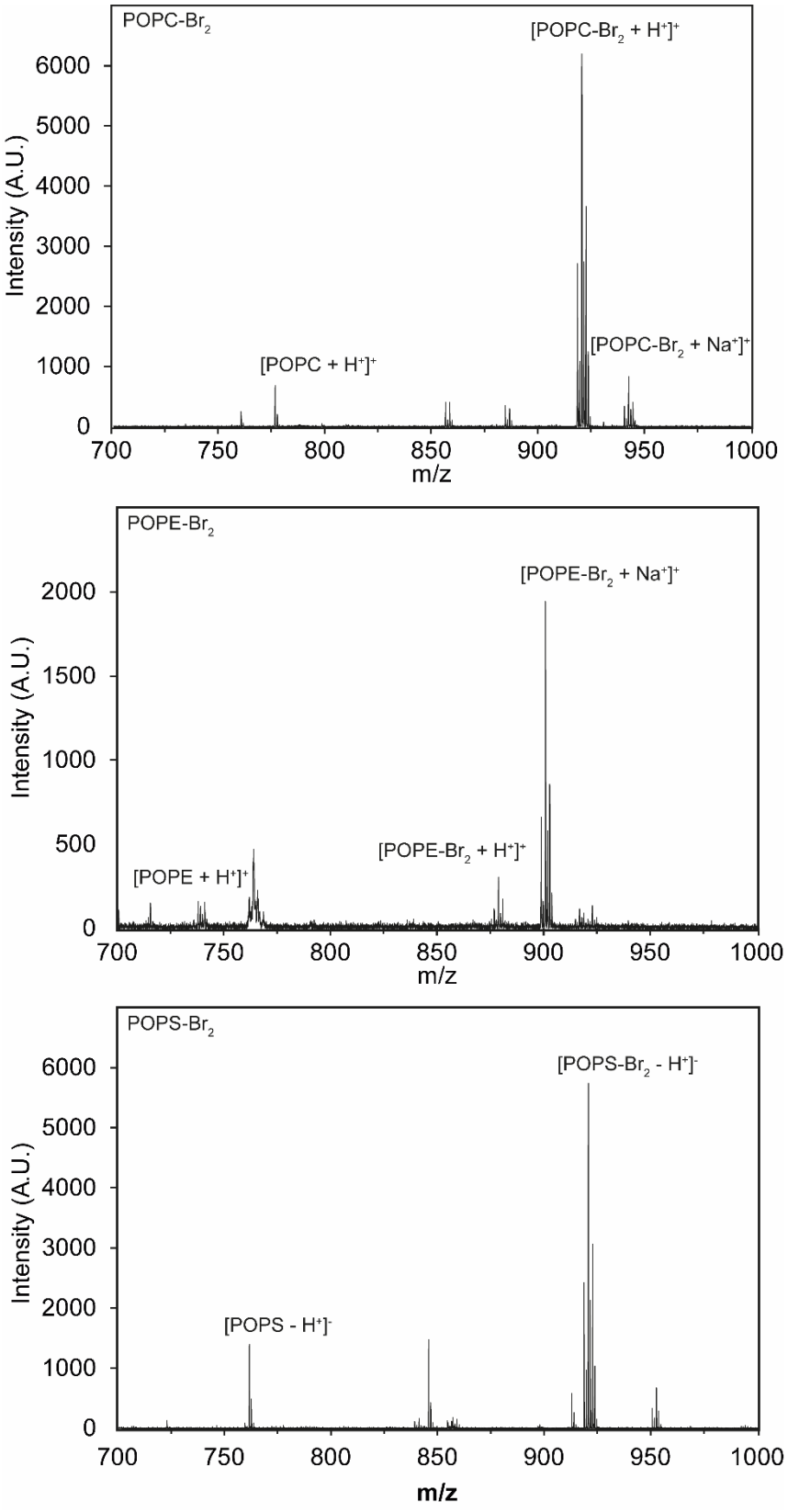

Figure S10. MALDI mass spectra of the different brominated lipids used here for Trp quenching assays.

Table S1. Numerical values of liposome fusion experiments.

Membrane composition	Extent of total fusion [%][1]			Initial rate of total fusion [min^{-1}][2]		
	blank	L16[3]	LV16[3]	blank	L16[3]	LV16[3]
DOPC	17.3 ± 2.2	21.1 ± 2.4	25.4 ± 2.8	0.1870 ± 0.0041	0.2522 ± 0.0042	0.2841 ± 0.0082
DOPC:DOPS (4:1)[4]	5.4 ± 0.8	8.5 ± 0.5	15.8 ± 1.1	0.0274 ± 0.0022	0.0621 ± 0.0028	0.1822 ± 0.0030
DOPC:DOPE (4:1)[4]	36.9 ± 1.4	52.7 ± 5.2	49.3 ± 3.1	0.4474 ± 0.0080	0.6837 ± 0.0090	0.7038 ± 0.0167
DOPC:DOPS:DOPE (3:1:1)[4]	17.1 ± 1.2	26.0 ± 1.8	54.5 ± 1.3	0.1188 ± 0.0057	0.2434 ± 0.0031	0.9627 ± 0.0206

[1] values correspond to extents of fusion seen after 3 h of reaction time, means±SEM, n=4

[2] values represent means±SEM, n=4

[3] Peptide/lipid ratios of ~0.005 were used

[4] molar ratios are given

Table S2. Numerical values of IL mixing fusion experiments.

Membrane composition	Extent of IL mixing [%][1]		
	blank	L16[2]	LV16[2]
DOPC	6.9 ± 1.1	8.0 ± 0.8	10.4 ± 1.2
DOPC:DOPS (4:1)[3]	3.1 ± 0.6	4.5 ± 0.4	7.3 ± 1.0
DOPC:DOPE (4:1)[3]	10.8 ± 0.9	19.3 ± 2.1	16.7 ± 1.8
DOPC:DOPS:DOPE (3:1:1)[3]	4.8 ± 0.9	8.2 ± 1.0	17.9 ± 1.7

[1] values correspond to extents of fusion seen after 3 h of reaction time, means±SEM, n=4

[2] Peptide/lipid ratios of ~0.005 were used

[3] molar ratios are given

Table S3. Surface potential of liposomes.

Membrane composition	Zeta potential [mV] [1]		
	blank	L16 [2]	LV16 [2]
DOPC	-24.7 ± 5.6	-22.9 ± 4.7	-29.5 ± 6.6
DOPC:DOPS (4:1) [3]	-56.4 ± 1.3	-56.3 ± 0.5	-58.0 ± 1.6
DOPC:DOPE (4:1) [3]	-33.0 ± 2.3	-24.1 ± 5.4	-28.2 ± 9.4
DOPC:DOPS:DOPE (3:1:1) [3]	-57.7 ± 3.4	-53.0 ± 6.0	-55.9 ± 3.5

[1] values represent means±SEM, n=3

[2] Peptide/lipid ratios of ~0.005 were used

[3] molar ratios are given

Table S4. Numerical values of cross relaxation rates.

Membrane composition	Cross relaxation rates [s^{-1}] [1]		
	blank	L16 [2]	LV16 [2]
DOPC	0.0239 ± 0.0026	0.0283 ± 0.0030	0.0510 ± 0.0038
DOPC:DOPS (4:1) [3]	0.0150 ± 0.0013	0.0178 ± 0.0043	0.0272 ± 0.0049
DOPC:DOPE (4:1) [3]	0.0372 ± 0.0050	0.0407 ± 0.0138	0.0370 ± 0.0081
DOPC:DOPS:DOPE (3:1:1) [3]	0.0147 ± 0.0026	0.0251 ± 0.0069	0.0374 ± 0.0115

[1] values represent means±error that denote the uncertainty determined from fitting experimental crosspeak volumes, n=2

[2] Peptide/lipid ratio = 0.02

[3] molar ratios are given

Table S5. Corrected Trp fluorescence values.

Mole fraction of brominated lipid[1]	L16[2]			LV16[2]		
	PC-Br_2[3]	PS-Br_2[4]	PE-Br_2[4]	PC-Br_2[3]	PS-Br_2[4]	PE-Br_2[4]
0.00	1.000 -	1.000 -	1.000 -	1.000 -	1.000 -	1.000 -
0.05	0.933 ± 0.006	0.846 ± 0.035	0.874 ± 0.057	0.894 ± 0.018	0.773 ± 0.057	0.883 ± 0.065
0.10	0.787 ± 0.031	0.665 ± 0.037	0.743 ± 0.048	0.785 ± 0.029	0.516 ± 0.045	0.776 ± 0.045
0.15	0.760 ± 0.035	0.530 ± 0.064	0.626 ± 0.049	0.739 ± 0.011	0.445 ± 0.042	0.745 ± 0.068
0.20	0.677 ± 0.033	0.409 ± 0.048	0.564 ± 0.065	0.667 ± 0.026	0.364 ± 0.028	0.650 ± 0.062
1.00	0.046 ± 0.035	nd nd	nd nd	0.059 ± 0.043	nd nd	nd nd

[1] mole fraction of brominated lipid given as $\frac{[\mathrm{PX{-}Br_2}]}{[\mathrm{PC}]+[\mathrm{PX{-}Br_2}]}$

[2] peptide/lipid ratios of 0.01 were used

[3] normalized degree of Trp quench, $\frac{F}{F_0}$, values represent mean±SEM, n=4-5

[4] normalized degree of Trp quench corrected using values measured for corresponding unlabeled lipids, $\frac{F}{F_0}$(corrected). Values represent mean±SEM, n=4-5

nd: not determined

Experimental Section

Materials

The lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh-PE) were purchased from Avanti Polar Lipids. Chloroform ($CHCl_3$), tetrafluoroethanol (TFE), sodium dithionite (DTN), bromine (Br_2), 2,5-dihydroxybenzoic acid (2,5-DHB), 9-aminoacridine hydrochloride monohydrate (9-AA), trifluoroacetic acid (TFA) were purchased from Sigma Aldrich. The solvent tert-butanol (*t*-BuOH) was purchased from Roth. 3-[(3-Cholamidopropyl)-Dimethylammonio]-1-Propanesulfonate (CHAPS), 2-Amino-2-Hydroxymethyl-1,3-Propanodiol Hydrochloride (TRIS-HCl), Ethylenediaminotetraacetic Acid Disodium Salt (EDTA) and Sodium Chloride (NaCl) were purchased from Applichem. Silica gel 60 0.04-0.063 mm (230-400 mesh) was purchased from Roth.

Peptide synthesis

Peptides were synthesized by Fmoc chemistry (PSL, Heidelberg, Germany) and were >90 % pure as confirmed by mass spectrometry. Concentrations were determined using a tryptophan extinction coefficient of 5600 $M^{-1}cm^{-1}$.

Synthesis of brominated lipids

1-Palmitoyl-2-(9,10-dibromostearoyl) lipids (PC-Br_2, PS-Br_2 and PE-Br_2) used in the peptide-lipid interaction assay were synthesized by adding a molar excess of Br_2 drop-wise to POPC, POPS, or POPE in $CHCl_3$ on ice.[3] The reaction was allowed to proceed for 3 h at -20 °C. The excess unreacted Br_2 was separated from the brominated lipid product with a gradient of $CHCl_3$:MeOH from 0-80 % MeOH using a silica 60 gel column. The fractions containing the product were identified by mass spectrometry (MS). The relevant fractions were pooled and concentrated by vacuum centrifugation. The final concentration of brominated lipid was quantified using the Bartlett assay [4], and the lipids stored at -20 °C in $CHCl_3$.

MALDI-TOF MS of brominated lipids

Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS of the synthesis products was performed using an Autoflex MALDI-TOF mass spectrometer (Bruker Daltonics). Lipids were dried and resuspended in MeOH (final concentration 50-100 µM) by vortexing, and mixed 1:1 (v/v) with either 2,5-DHB (10 mg/ml in MeOH with 0.1% TFA) or 9-AA (10 mg/ml in MeOH). 2,5-DHB was used for detecting PC and PE lipids in the positive ion mode, while 9-AA was used to detect PS in the negative ion mode. For both conditions 0.5 µl lipid-matrix solution was spotted onto an MTP anchor-target (Bruker Daltonics) and dried under vacuum. Mass spectra were acquired from 500-3000 m/z. The laser power was set

to a value slightly above the ionization threshold for each sample, with higher laser powers required for 9-AA, and the laser frequency was set to 10 Hz (Figure S10).

Preparation of small unilamellar liposomes

Lipids dissolved in $CHCl_3$ were mixed with either peptide (L16 or LV16) dissolved in TFE, or NBD-PE and Rh-PE (1:1) dissolved in $CHCl_3$. The solutions were diluted with three volumes of *t*-BuOH and lyophilized to receive a powder. Rehydration in buffer (25 mM TRIS-HCl, 0.1 mM EDTA, 150 mM NaCl, pH 7.4) was supported by shaking (50 °C, 1 h, 1400 rpm for fusion assays or 37 °C, 2 h, 1400 rpm for peptide-lipid interaction assays) which resulted in multilamellar liposomes. Volumes were chosen such that the liposome suspension contained 3 mM lipid with or without peptide at a 0.005 molar peptide/lipid ratio or 1.5 mol% of NBD-PE and Rh-PE, each for fusion assays. For the peptide-lipid interaction assay, a peptide/lipid ratio = 0.01 was chosen. Small unilamellar vesicles were formed by sonication for 2 -3 x 10 min (Branson Sonifier 450) under external ice cooling and debris was removed by centrifugation (12000 rpm, 10 min, 4 °C). Samples for the IL mixing fusion assay were prepared by extinguishing NBD fluorescence of the OL during the centrifugation mentioned above. Therefore, a freshly prepared DTN solution (final concentration = 20 mM) was added to the sonicated donor liposomes, centrifuged and excess DTN was removed after centrifugation on a Sepharose G-50 spin column.

Determining outer leaflet NBD-quench kinetics

OL NBD-quench kinetics were recorded by measuring NBD fluorescence at intervals of 30 s over 60 cycles at 4 °C by a fluorescence spectrometer (Cary Eclipse Varian). The initial fluorescence was determined after adding 80 µL of buffer to 20 µL 1.5 mol% NBD-PE-containing liposomes and performing 3 cycles of measurement. Subsequently, DTN was added (final concentration = 20 mM) and the kinetics of NBD fluorescence bleaching were recorded. The fluorescence values were normalized to the mean fluorescence seen prior to DTN addition.

Fusion assay

A standard lipid-mixing assay [5] was used to determine total lipid and IL mixing. To this end, donor liposomes containing 1.5 mol% NBD-PE and Rh-PE were mixed in a 96-well plate (black NUNC) on ice at a ratio of 1+9 (v + v) with peptide-containing or peptide-free (=blank) acceptor liposomes. NBD fluorescence (λ_{ex} 460 nm, λ_{em} 520 nm) was measured at 37 °C for 180 cycles (1 min / cycle) in a plate reader (BMG Lab Technologies FluoStar). A control was measured in triplicate by mixing donor liposomes with buffer 1+9 to detect fusion-independent changes in fluorescence over time (‘drift control’). To determine the maximally dequenched NBD-PE-fluorescence expected after 100% fusion, samples where donor liposomes (with or without prior treatment with dithionite, see below) had been mixed with buffer 1+9 and lysed by adding CHAPS (final concentration = 11 mM) for 2 min were run in parallel. Experiments were done in triplicate.

The lysis of donor liposomes resulted in an increase of fluorescence ΔF_{max} [Eq. (2)] that was independent of lipid composition; thus, fusion experiments with different lipids are comparable.

Results were evaluated using the following formalism:

ΔF_t, the corrected NBD fluorescence at time t, was calculated by Eq. (1)

$$\Delta F_t = F_t - F_0 \tag{1}$$

where F_t represents NBD fluorescence at time t and F_0 represents NBD fluorescence at t = 0 min.

100 % Fusion is defined by ΔF_{max} as calculated by Eq. (2)

$$\Delta F_{max} = F_0(CHAPS\ lysis) - F_0(drift\ control) \tag{2}$$

where F_0 (drift control) is the fluorescence of donor liposomes in the absence of acceptor liposomes at t = 0 min (where NBD is maximally quenched) and F_0 (CHAPS lysis) is the fluorescence obtained by CHAPS lysis of donor liposomes (where NBD is maximally dequenched).

The kinetics of total fusion [%] or IL mixing [%] were then calculated by drift-correcting the fluorescence at each time point ΔF_t using the correction factor f_t derived from Eq. (3), ΔF_t, and normalizing the corrected values to ΔF_{max} [Eqns. (4) and (5)].

$$f_t(drift\ control \pm DTN) = \frac{F_t(drift\ control \pm DTN)}{F_0(drift\ control \pm DTN)} \tag{3}$$

$$Total\ fusion\ [\%] = \frac{\Delta F_t(-DTN) \cdot f_t\,(drift\ control - DTN)}{\Delta F_{max}} \cdot 100 \tag{4}$$

$$IL\ mixing\ [\%] = \frac{\Delta F_t(+DTN) \cdot f_t\,(drift\ control + DTN)}{\Delta F_{max}} \cdot 100 \tag{5}$$

OL mixing corresponds to the difference between total fusion and IL mixing [Eq. (6)].

$$OL\ mixing\ [\%] = Total\ fusion\ [\%] - IL\ mixing\ [\%] \tag{6}$$

The ratio of NBD residing in the OL (fluorescence NBD_{OL}) or the IL (fluorescence NBD_{IL}) of a liposome was determined in an independent measurement (see chapter: Determining outer leaflet NBD-quench kinetics) and equals a factor of 1.5 [Eq. (7)].

$$\frac{fluorescence\ NBD_{OL}}{fluorescence\ NBD_{IL}} = \frac{60\ \%}{40\ \%} = 1.5 \tag{7}$$

After multiplying IL mixing [%] by the factor 1.5, we normalized IL mixing to OL mixing. This results in inherent IL mixing as a measure of the efficiency of the hemifusion-to-fusion transition, independent of the efficiency of prior OL mixing [Eq. (8)].

$$Inherent\ IL\ mixing\ [\%]\ = \frac{IL\ mixing\ [\%] \cdot 1.5}{OL\ mixing\ [\%]} \cdot 100 \qquad (8)$$

The prevalence of liposomes in a hemifused state was quantitated according to Eq. (9)

$$hemifused\ states\ [\%] = OL\ mixing\ [\%] - 1.5\ \cdot IL\ mixing [\%] \qquad (9)$$

The prevalence of liposomes in a fully fused state was quantitated according to Eq. (10)

$$fully\ fused\ states\ [\%] = \frac{Total\ fusion\ [\%] - (OL\ mixing\ [\%] - 1.5 \cdot IL\ mixing [\%])}{2} \qquad (10)$$

For better comparability, the percentage of hemifusion is then calculated by Eq. (11)

$$Hemifusion\ [\%] = \frac{hemifused\ states\ [\%]}{hemifused\ states\ [\%] + fully\ fused\ states\ [\%]} \cdot 100 \qquad (11)$$

Initial fusion rates were obtained as the slope of the first 30 min of fluorescence increase as fit by linear regression, a linear model was found to be adequate to fit the initial phase of fluorescence dequenching (Origin Pro 9.1G).

Measuring the Zeta potential and liposome size

Zeta potential and liposome size were measured with a ZetaView apparatus (Particle Metrix). Size measurements were performed with liposomes prepared in buffer as described above, using an appropriate dilution to reach a minimum of 500 traced particles. The instrument was set to a 11-position readout, measuring two cycles using a frame rate of 30 1/s, a trace length of 15, and a shutter set to 70. Zeta potential measurements were performed with liposomes diluted 1:20,000 by adding 2.5 mM TRIS-HCl, 0.01 mM EDTA, 15 mM NaCl, pH 7.4 to lower the conductivity to approximately 1800 µS/cm. Measurements were performed immediately after the dilution. Liposome sizes and Zeta potentials were assessed using the software ZetaView 8.04.02.

Peptide-lipid interaction assay

The quenching of peptide Trp fluorescence as a function of increasing mole fractions of brominated lipid was used to quantify peptide-lipid interaction [6]. Liposomes prepared in buffer containing 0.15 or 1.0 M NaCl (as indicated in the Figure legends) were added to a 10 mm quartz cuvette (Hellma Analytics) and the Trp fluorescence (λ_{ex} 280 nm, λ_{em} 300-400 nm) was measured by a fluorescence spectrometer (Cary Eclipse, Varian) at room temperature. Three accumulations were averaged for each measurement.

The background fluorescence of corresponding peptide-free blank liposomes was subtracted from each measurement according to Eq. (12)

$$F_0 = F(peptide + PC) - F(PC) \quad (12)$$

where F(peptide + PC) equals the fluorescence of a given TMD in DOPC, and F(PC) equals the background fluorescence of blank DOPC liposomes.

The blank-corrected fluorescence F_0 was used to normalize fluorescence values obtained after adding brominated lipids using Eq. (13)

$$\frac{F}{F_0} = \frac{F(peptide+PC+Xlipid)-F(PC+Xlipid)}{F_0} \quad (13)$$

Where F(peptide+PC+χlipid) equals Trp fluorescence of a given peptide in DOPC including different mole fractions of brominated lipids and F(PC+χlipid) equals the corresponding blank.

In order to correct for effects of the PS or PE headgroups on Trp fluorescence, corresponding spectra with POPS or POPE in place of their brominated derivatives were also recorded. In doing so, we ensure comparability of Trp quenching data by the brominated lipid analogs. The correction was factored into the normalized Trp fluorescence using Eq. 14

$$\frac{F}{F_0}(corrected) = \frac{\frac{F}{F_0}(mean\ PX-Br_2)}{\frac{F}{F_0}(mean\ PX)} \quad (14)$$

Where (meanPX-Br_2) and (meanPX) correspond to averaged values (n=4-5) obtained in the presence of brominated or un-brominated lipids, respectively.

As described in [7], the data were fit to a model [Eq. (15)] where the degree of quenching is a function of the mole fraction of brominated lipid

$$\frac{F}{F_0}(corrected) = F_{min} + (1 - F_{min})\left(1 - \frac{\chi_{Br}}{\chi_{Br}+K_{rel}(1-\chi_{Br})}\right)^n \quad (15)$$

where χ_{Br} is the mole fraction of brominated lipid, F_{min} is the residual fluorescence when χ_{Br}=1, n is the stoichiometry of brominated lipids close enough to the Trp to cause quenching, and K_{rel} is the binding constant relative to PC with a K_{rel}<1 implying a stronger affinity relative to PC.

All fits were performed using the nonlinear least squares fit in KaleidaGraph (Synergy Software).

In binary DOPC/PC-Br_2 membranes, it was assumed that binding of both PC derivatives is equal (K_{rel}=1), allowing n to be obtained (L16: n = 1.79±0.10; LV16: n = 1.92±0.07). As the

F_{min} measured in DOPC was very close to 0, this parameter was omitted from Eq. (15) which results in Eq. (16)

$$\frac{F}{F_0}(corrected) = (1 - \chi_{Br})^n \quad (16)$$

For binary DOPC/PS-Br and PC/PE-Br membranes, it was not practical to directly measure F_{min} due to the inability to form pure PS or PE liposomes holding our TMDs. It was assumed that F_{min} would be similar to that measured in PC-Br and thus again set as F_{min}=0. The n determined from fitting PC:PC-Br data to Eq. (16) was inserted and the data fitted to Eq. (17)

$$\frac{F}{F_0}(corrected) = \left(1 - \frac{\chi_{Br}}{K_{rel}(1-\chi_{Br})}\right)^n \quad (17)$$

K_{rel} was then obtained from the fits.

Statistical significance of differences in K_{rel} was confirmed through calculating the x% confidence intervals (CI) according to Eq. (18) using the SEM of the parameter estimate as reported by KaleidaGraph.

$$CI\ 95\% = K_{rel} \pm t(x\%, df = n - 1) \cdot SEM \quad (18)$$

where t is the t-value , n the number of data points fitted, and df the degrees of freedom. Non-overlapping CI indicates significant differences at the x% confidence level.

Sample preparation for NMR experiments

The peptides were dissolved in TFE and mixed with the respective phospholipids (DOPC; DOPC/DOPS (4:1); DOPC/DOPE (4:1); or DOPC/DOPS/DOPE (3:1:1) that were dissolved in $CHCl_3$ at a molar peptide/lipid ratio = 0.02. After evaporation of the solvent using a rotary evaporator, the samples were redissolved in cyclohexane and lyophilized overnight at high vacuum. The obtained fluffy powder was hydrated with 40 wt% D_2O and equilibrated by freeze-thaw cycles. After gentle centrifugation, samples were transferred 4 mm HR MAS rotors with spherical Kel-F inserts.

^{1}H MAS NMR Spectroscopy

^{1}H MAS NMR measurements were carried out on a Bruker Avance III 600 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany) using a 4 mm HR MAS probe at a MAS frequency of 5 kHz. The proton $\pi/2$ pulse length was 4 µs. A ^{2}H lock was used for field stability. All measurements were conducted at a temperature of 45°C. Two-dimensional ^{1}H MAS NOESY spectra [8] were acquired at five mixing times (between 0.1 ms and 500 ms) with a relaxation delay of 3.5 s. The spectra width was adjust to 5 kHz. The volume of the diagonal and cross peaks of the terminal methyl group of the lipid chains and the choline group of the lipid headgroup were integrated using the Bruker Topspin 3.5 software package.

NOE build-up curves were fitted in Origin (OriginLab Cooperation, Northampton, MA) to the spin pair model yielding cross-relaxation rates (σ_{ij}) according to Eq. (19) [9]:

$$A_{ij}(t_m) = \frac{A_{jj}(0)}{2} \cdot (1 - \exp(-2\sigma_{ij} t_m)) \exp(-t_m / T_{ij}) \quad (19)$$

where A_{ij} (t_m) represents the cross peak volume at mixing time t_m and A_{jj} (0) the diagonal peak volume at mixing time zero. The value $1/T_{ij}$ defines a rate of magnetization leakage towards the lattice.

Supplementary References

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