

**Supplementary Table 1:** Using Image Lab software (Bio-rad), the PE band intensities of MscS samples in Lanes (1-3) of the TLC chromatogram (Fig 2C) were quantified using PE standard (Lane 5). The Quantity toolbox was used to carefully define the PE bands, including the reference band (Lane 5) and to enable for background correction. The signal intensities obtained were used to quantify the PE content in each band. Molar ratios of PE to protein were then calculated.

<b>Lane no.</b>	<b>Sample</b>	<b>Protein content (nmoles)</b>	<b>Volume (Intensity)</b>	<b>PE (nmoles)</b>	<b>Relative Quantity</b>	<b>Molar ratio of PE: MscS monomer</b>
1	238 µg MscS-DDM	7.3	2366980	3.86	2.314035	0.53:1
2*	280 µg MscS-DDM*	8.7	757619	1.24	0.740672	0.14:1
3	305 µg MscS-Fos-14	9.5	3042969	4.96	2.974903	0.52:1
4	-	-	-	-	-	-
5	1.2 µg PE	-	1022880	1.67	1.000000	-
6	8.0 µg <i>E. coli</i> lipids	-	4050540	6.61	3.959937	-

**Supplementary Table 2:** Fractional quenching of tryptophan fluorescence. MscS mutants with tryptophan residues at different positions in a tryptophan-free background were reconstituted into either 100% DOPC or 100% BrPC. Fluorescence spectra were recorded and the fractional quenching was calculated from the fluorescence intensities at 340 nm as  $FrQ=(F_0-F)/F_0$  where  $F_0$  is the intensity for the DOPC sample and  $F$  for the BrPC sample. Data were obtained in the same way for reconstitution in 80% DOPE/20% DOPG or their brominated equivalents (right column). Mean values are given with standard deviations

MscS mutant	Fractional DOPC	quenching Fractional PE/PG	quenching
A103W	0.75 ± 0.08		
V107W	0.57 ± 0.06		
L111W	0.68 ± 0.21	0.80 ± 0.06	
A119W	0.88 ± 0.04	0.90 ± 0.02	
L123W	0.49 ± 0.07	0.56 ± 0.05	
<b>Controls</b>			
M47W	0.64 ± 0.09		
L105W	0.32 ± 0.04		
Q203W	-0.01 ± 0.12		
W240	0.01 ± 0.01		

**Supplementary Table 3:** Calculated lipid volumes for existing PDB x-ray structures, containing resolved lipids, using MolSpace, a VMD software plug-in

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Lipid headgroup (PDB code)	Total number of carbons	Volume (Å <sup>3</sup> )
LHG(1JB0)_5001	32	1599
PE (1P84)_710	32	1480
PE(1P84)_711	25	1241
PE(3M9I)_301	13	849
PE(3M9I)_302	22	1205
PE(3M9I)_303	27	1318
PE(3M9I)_304	25	1208
PE(3M9I)_305	23	1180
PE(3M9I)_306	33	1496
PE(3M9I)_307	25	1230

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