Supporting Information

NMR Determination of Protein Partitioning into Membrane Domains with Different Curvatures and Application to the Influenza M2 Peptide

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Estimation of isotropic vesicle sizes from ^{31}P NMR linewidths

Static ^{31}P NMR spectra were simulated by Traikia et al for vesicles of different sizes (1). The model considers both isotropic tumbling of the vesicles and lipid reorientation induced by lateral diffusion over the curved surface of the vesicle. The correlation time of motion depends on the vesicle radius according to:

$$\frac{1}{\tau} = \frac{1}{\tau_t} + \frac{1}{\tau_l} = \frac{3kT}{4\pi\eta r^3} + \frac{6D_L}{r^2} \quad (1)$$

In the simulations, a value of 7.808 x 10^{-4} Poise was used for η, the membrane viscosity. The temperature T was set at 298 K. The lateral diffusion coefficient D_L was set to 10^{-7} cm^2/s. Using these parameters, a vesicle diameter of 100 nm (r = 50 nm) has a correlation time τ = 8 μs, while a vesicle diameter of 30 nm (r = 15 nm) gives a τ of 0.25 μs. The calculated ^{31}P NMR spectra for these two diameters give an isotropic peak linewidth of 7.9 ppm and 1.6 ppm, respectively.

In 40% hydrated membrane samples, isotropic tumbling is much slower $\tau_t >> \tau_l$, thus we can ignore the first term and calculate τ using lipid lateral diffusion alone, $\tau = r^2/6D_L$. The value of D_L can be estimated from the literature. Fluorescence recovery after photobleaching (2), pulsed-field gradient and relaxation NMR experiments (3-5) indicate that a D_L range of 3 – 10 x 10^{-8} cm^2/s encompasses both low-viscosity lipid membranes and protein-containing or cholesterol-rich membranes. Using this range, we find that to obtain a τ of 8 μs, for an isotropic linewidth of 7.9 ppm, the vesicle diameter is 24-44 nm, which is reduced from 100 nm due to the lack of tumbling. To obtain an isotropic linewidth of 1.6 ppm or τ of 0.25 μs, the vesicle diameter ranges from 4.2 to 8.0 nm.

Interpolating these diameters for the isotropic linewidths obtained for DMPC and VM membranes, we find that the 6.0 ppm linewidth of the DMPC isotropic peak corresponds to a vesicle diameter of 18-33 nm (mean value = 26 nm), while the 2.2 ppm linewidth of the VM isotropic peak translates to a vesicle diameter of 7-13 nm (mean value = 10 nm). The latter is about the minimum diameter for a unilamellar vesicle. On the other hand, the weak isotropic peak of the M2TM-DMPC sample has a linewidth of ~11 ppm, which translates to a vesicle diameter of ~50 nm, suggesting 4-5 bilayers.

These semi-quantitative estimates support the qualitative conclusion that M2(21-61) exerts strong curvature to cholesterol-rich virus-mimetic membranes and moderate curvature to DMPC bilayers. In the smallest isotropic vesicle, M2 does not bind drug, indicating that the conformation of the TM helical bundle is perturbed by the high curvature of the membrane.

M2 and amantadine contents of the various lipid membranes

For M2-containing membranes, we used peptide : lipid molar ratios of 1:8 for M2TM and 1:15 for M2(21-61). The lipid molar amounts do not include cholesterol for the VM membrane. Amantadine (Amt) was added at a mole ratio of 5:1 drug : tetramer. These quantities translate to very low mass content of the drug in the membranes. The Amt, M2(22-46) and M2(21-61) molecular weights are about 166, 2750, and 4720 g/mol, respectively, after taking into account the isotopic labels. The DMPC molar mass is 678 g/mol. The average molecular weight of the VM membrane, without including cholesterol, is 712 g/mol. Based on these values, the M2TM : Amt : DMPC membrane at a molar ratio of 1 : 1.25 : 8 corresponds to amantadine at 2.5 wt% of the entire proteoliposome and 3.8 wt% of the lipids. For the VM samples, the Amt mass percentage is even lower due to the higher molecular weight of the lipids. For the M2(21-61) : Amt : DMPC sample with a molar ratio of 1 : 1.25 : 15, Amt constitutes 1.4 wt% of the entire proteoliposome and 2.0 wt% of the lipids. Membrane samples without M2 but with amantadine used the same drug : lipid molar ratio as the M2-containing samples. Thus, the amantadine mass percentage is low (2.0-3.8%) in all drug-containing membrane samples, and the significant isotropic peaks observed in the static ^{31}P spectra cannot be attributed solely to the effects of amantadine.

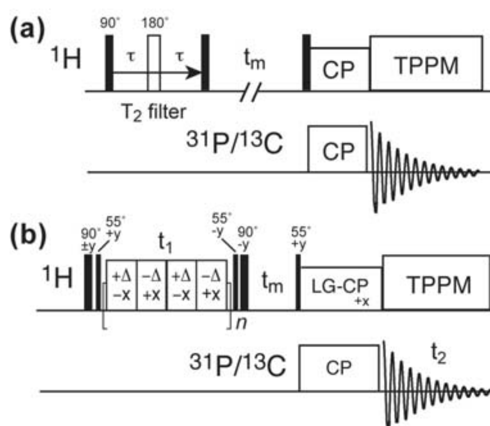

Fig. S1. Pulse sequences for determining protein localization in membrane domains with different curvatures. (a) ^{31}P or ^{13}C-detected ^{1}H T_2 relaxation experiment without ^{1}H homonuclear decoupling and with ^{1}H spin diffusion (t_m). (b) ^{1}H-^{31}P or ^{1}H-^{13}C 2D heteronuclear correlation experiment with ^{1}H homonuclear decoupling.

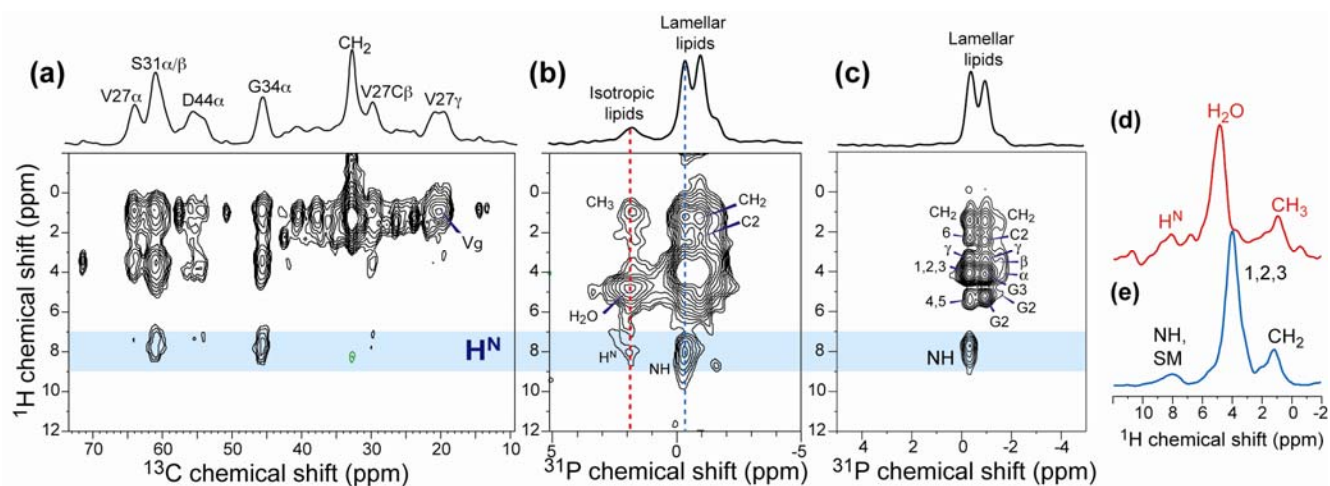

Fig. S2. 2D ^{1}H-^{13}C and ^{1}H-^{31}P HETCOR spectra of VM membrane with and without M2(21-61). (a) ^{1}H-^{13}C HETCOR spectrum of M2(21-61) with 200 μs ^{1}H spin diffusion and 300 μs LG-CP. (b) ^{1}H-^{31}P HETCOR spectrum of M2(21-61)-VM membrane with 250 μs ^{1}H spin diffusion and 3 ms LG-CP. (c) ^{1}H-^{31}P 2D spectrum of peptide-free control VM membrane with 500 μs ^{1}H spin diffusion and 3 ms LG-CP. An intramolecular H^{N}-^{31}P cross peak is detected for sphingomyelin. (d) ^{1}H cross section of the +1.8 ppm ^{31}P peak in (b). (e) ^{1}H cross section of the -0.4 ppm ^{31}P peak in (b). All spectra were measured at 297 K under 7.5 kHz MAS.

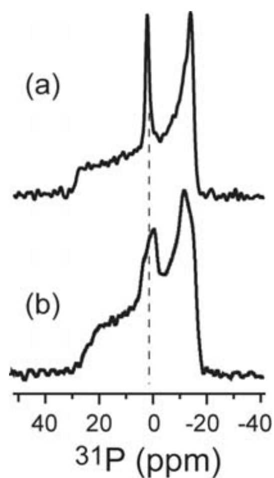

Fig. S3. Static ^{31}P spectra of the M2(21-61)-VM membrane when freshly prepared (a) and after several months (b). The isotropic peak broadened with time, suggesting fusion of small isotropic vesicles to larger aggregates.

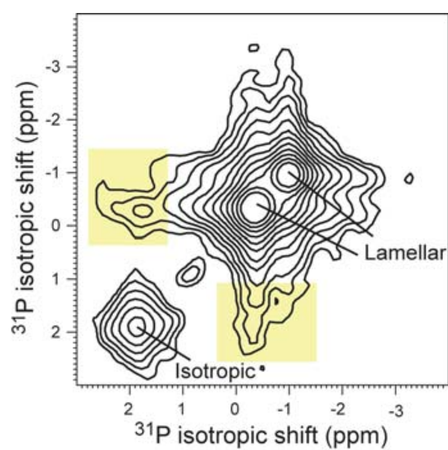

Fig. S4. 2D ^{31}P-^{31}P correlation spectra of M2(21-61)-VM with 50 ms spin diffusion, measured at 296 K under 4 kHz MAS. The ^{31}P magnetization was directly excited by a 90˚ pulse. The isotropic lipid and lamellar lipid peaks exhibit cross peaks, indicating that the two membrane domains are not well separated from each other.

Table S1. Quantification of the amount of OG in the membrane pellet after ultracentrifugation.

Sample	Normalized integrals	OG mass	Pellet mass	Lipid mass	Water in pellet	OG mass in pellets	OG/lipid mass ratio
OG control	1.000	28.2 mg	-	-	-	-	-
VM, 4℃ dialysis	0.015	0.4 mg in 4 ml	61.3 mg	16.3 mg	45.0 mg (45 uL)	~4.5ug	0.028%
VM, RT dialysis	0.007	0.2 mg in 4 ml	67.1 mg	16.2 mg	50.9 mg (50.9 uL)	~2.5ug	0.015%

Table S2. ^{31}P-detected ^{1}H T_2 relaxation times (ms) of DMPC membranes.

M2(21-61) bound DMPC membrane				
Mixing Time	Isotropic phase		Lamellar phase	
25 ms	0.12±0.04 (43%)	6±1 (57%)	0.3±0.1 (15%)	14±3 (85%)
100 ms	0.10±0.20 (38%)	6±2 (62%)	0.25±0.02 (11%)	13.0±0.3 (89%)
225 ms	0.12±0.06 (33%)	4±1 (67%)	0.28±0.05 (16%)	8.9±0.7 (84%)
DMPC membranes				
Mixing Time	Isotropic vesicles		Lamellar bilayers	
25 ms	0.1±0.1 (33%)	3.3±0.6 (67%)	0±5 (15%)	34±2 (85%)

References

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