Supporting Information

Membrane-Dependent Effects of a Cytoplasmic Helix on the Structure and Drug Binding of the Influenza Virus M2 Protein

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Table S1. Conditions of ^{13}C-^{2}H REDOR experiments of d_{15}-Amt complexed M2(21-61) in DMPC bilayers.

Amt : Tetramer	Spectral region	^{13}C carrier (ppm)	REDOR type	Mixing time (ms)	Number of scans [a]
1 : 1	S31, D44 Cα	61.4	Multiple ^{2}H pulses	15.1 ms	125,952
	G34 Cα	45.0	Multiple ^{2}H pulses	15.1 ms	189,440
	Sidechains	23.8	Single ^{2}H pulse	8 ms	69,632
5 : 1	S31, D44 Cα	55.8	Multiple ^{2}H pulses	15.1 ms	74,752
	G34 Cα	44.8	Multiple ^{2}H pulses	15.1 ms	18,432

Table S2. Fractions of bound drug estimated from 2D correlation spectra of the M2 peptides and ^{2}H spectra of d_{15}-Amt.

Amt : Tetramer	Membrane	M2(22-46)			M2(21-61)		
		2D NC	^{2}H	^{2}H splitting (kHz)	2D NC	^{2}H	^{2}H splitting (kHz)
1 : 1	DMPC	65%	76%	36	50-70%	47%	37
	VM	60%	75%	39	0%	0%	0
	VM+	96%	82%	37	0%	16%	36
5 : 1 or 4 : 1	DMPC	>95%	-	-	50-70%	-	-
	VM	60-70%	-	-	0%	-	-
	VM+	99%	-	-	0%	-	-

Table S3. ^{31}P chemical shifts of VM and VM+ membranes at 303 K based on Herzfeld-Berger analysis of the MAS sideband intensities.

Sample	Expt	δ_{iso} (ppm)	δ_{11} (ppm)	δ_{22} (ppm)	δ_{33} (ppm)	δ (ppm)	η
VM	CP	-0.36	17	9	-27	-27	0.28
		-1.0*	45*	13*	-61*	-60*	0.54*
		-1.6	82	20	-107	-105	0.59
VM+	DP, CP	-0.38	41	-20	-22	42	0.05
		-0.96	49	-26	-26	50	0

* The sideband fitting for the -1.0 ppm peak is not satisfactory, indicating that at least two CSAs are present for this peak. This means that the peak results from lipids in multiple phases of the membrane.

Table S4. Summary of short protein-drug distances and chemical shift (CS) perturbations by amantadine for different M2 constructs in different lipid membranes.

Drug : Tetramer	Lipids	M2(22-46)		M2(21-61)	
		Distance	CS changes	Distance	CS changes
1 : 1	DMPC	S31	Yes	S31	Yes
	VM	-	Yes	-	No
	VM+	-	Yes	-	No
5 : 1 or 4 : 1	DMPC	S31, D44	Yes	S31	Yes
	VM	-	Yes	-	No
	VM+	-	Yes	-	No

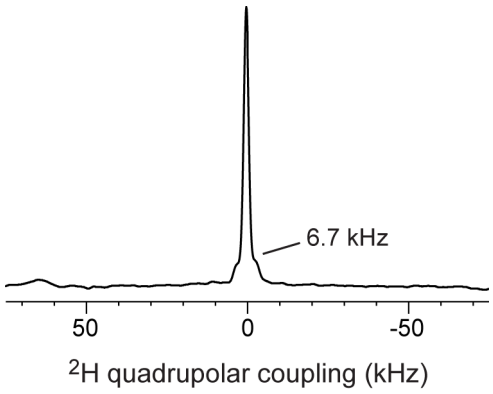

Figure S1. Static ^{2}H spectrum of d_{15}-Amt bound to M2(21-61) in the VM membrane at a P/L mole ratio of 1:30. The spectrum is still dominated by an isotropic peak, similar to the spectrum of the 1:15 sample shown in **Fig. 4c**, indicating that drug exclusion from the membrane is due to the high viscosity of the VM membrane rather than the high protein concentration.

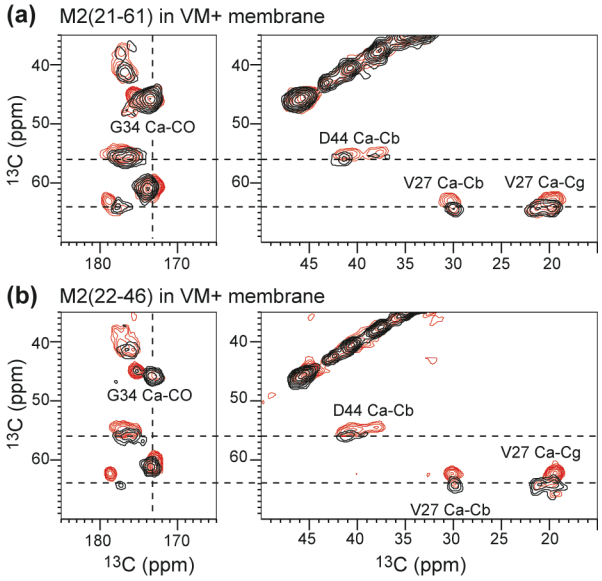

Figure S2. 2D ^{13}C-^{13}C correlation spectra of M2 peptides in VM+ membranes without (black) and with (red) Amt (1:1 ratio), measured at 273 K. (a) M2(21-61) in VM+ membranes. (b) M2TM(22-46) in VM+ membranes. Note the significant chemical shift changes of V27, G34 and D44 in M2TM, while M2(21-61) shows weaker chemical shift perturbations.