Conformation and Trimer Association of the Transmembrane Domain of the Parainfluenza Virus Fusion Protein in Lipid Bilayers From Solid-State NMR: Insights Into the Sequence Determinants of Trimer Structure and Fusion Activity

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Table S1. Fractions of α-helical cross peak intensities out of the total intensities in the PIV5 TMD obtained from low-temperature 2D ^{13}C-^{13}C correlation spectra of all samples measured in this study and in a previous study [1].

Residue	Peak	POPC/Chol	POPE
I488	Cα-Cγ2/δ	100%	22%
A490	Cα-Cβ	91%	38%
I491 [a]	Cα-Cγ2/δ	66%	41%
A492	Cα-Cβ	64%	49%
L493 [b]	Cα-CO	82%	57%
G494	Cα-CO	91%	81%
S495	Cα/β-CO	100%	100%
G497	Cα-CO	94%	70%
L498	Cα-CO	100%	57%
I499 [a]	Cα- Cγ2/δ	77%	54%
L500 [b]	Cα-CO	82%	57%
I501	Cα- Cγ2/δ	100%	88%
I502	Cα- Cγ2/δ	79%	70%
L503	Cα-CO	74%	69%
S505	Cα/β-CO	79%	61%
V506	Cα-Cγ	59%	39%
V507	Cα-Cγ	56%	44%
V508	Cα-Cγ	35%	25%
Average		79%	57%

[a] The I491 and I499 Cα-Cγ2/δ cross peaks in the ILSILV-labeled peptide are overlapped. The helicity values reported here are interpolated from the helicity of their neighboring residues and averaged to satisfy the observed helicity. The I491 helicity is interpolated from the A490 and A492 helicity values, while the I499 helicity is interpolated from the L498 and L500 values. For the POPC/cholesterol-bound peptide, the observed overlapped I491/I499 Cα-Cγ2/δ helicity is 72%, while the POPE-bound sample has an overlapped helicity of 47%.

[b] The reported helicity of the overlapped L493/L500 Cα-CO cross peaks in the ILSILV-labeled peptide was the directly measured value without interpolation, since these residues both reside within the central α-helical domain.

Table S2. ^{13}C and ^{15}N chemical shifts (ppm) of the PIV5 TMD in POPC/cholesterol and POPE membranes. The ^{13}C chemical shifts are referenced to TMS and the ^{15}N chemical shifts are referenced to liquid ammonia. Chemical shift values in bold are those of the major conformer.

Residue	POPC/Chol						POPE					
	N	CO	Cα	Cβ	Cγ	Cδ	N	CO	Cα	Cβ	Cγ	Cδ
[1]I488	**-**	**175.6**	**63.4**	**35.7**	**27.3/15.7**	**11.9**	**124.0**	**172.2**	**57.9**	**39.8**	**25.9/15.1**	**12.4**
							121.3	175.5	62.4	35.2	26.9/15.6	11.5
[1]A490	**-**	**177.3**	**53.8**	**16.8**			**128.8**	**173.1**	**48.0**	**20.2**		
	-	172.6	47.8	19.9			120.6	177.4	53.4	16.5		
[3]I491	**117.8**	**176.5**	**63.2**	**35.6**	**27.7/15.3**	**11.8**	**121.8**	**171.5**	**57.2**	**39.9**	**25.5/15.0**	**12.3**
	122.2	171.5	57.2	39.9	25.5/15.1	12.3	117.7	176.5	62.8	35.6	27.6/15.3	11.6
[4]A492	**121.9**	**176.8**	**54.0**	**16.4**			**-**	**173.5**	**48.1**	**20.8**		
	124.2	173.2	48.1	20.8			-	176.8	53.8	16.4		
[3]L493	**117.4**	**176.5**	**55.7**	**39.5**	**24.4**	**20.8**	**117.4**	**176.6**	**55.7**	**39.5**	**24.4**	**20.7**
	123.1	172.2	51.0	43.6	24.9	23.6	123.1	172.5	51.1	43.6	24.9	23.5
[4]G494	**104.0**	**172.7**	**45.7**				**-**	**172.8**	**45.6**			
	-	169.3	43.1				-	169.3	42.9			
[3]S495	**118.4**	**173.0**	**61.1**	**61.1**			**118.2**	**173.0**	**60.9**	**60.9**		
[1]G497	**-**	**172.2**	**45.7**				**105.5**	**172.2**	**45.4**			
							-	169.0	42.9			
[1]L498	**-**	**176.3**	**56.1**	**39.5**	**24.9**	**21.5**	**120.8**	**176.1**	**55.8**	**39.6**	**24.6**	**21.4**
							-	172.4	51.9	44.4	25.1	24.3
[3]I499	**118.2**	**175.0**	**63.8**	**35.4**	**27.7/15.0**	**11.4**	**117.9**	**175.0**	**63.7**	**35.4**	**27.6/15.0**	**11.5**
	122.2	171.5	57.2	39.9	25.5/15.1	12.3	121.8	171.5	57.2	39.9	25.5/15.0	12.3
[3]L500	**118.9**	**176.5**	**55.7**	**39.5**	**24.4**	**20.8**	**119.0**	**176.6**	**55.7**	**39.5**	**24.4**	**20.7**
	123.1	172.2	51.0	43.6	24.9	23.6	123.1	172.5	51.1	43.6	24.9	23.5
[1]I501	**-**	**176.8**	**63.8**	**35.8**	**27.9/15.3**	**11.9**	**-**	**176.8**	**63.5**	**35.4**	**27.4/15.2**	**11.7**
							-	-	58.0	38.4	25.3/15.3	12.2
[4]I502	**118.9**	**175.3**	**64.4**	**35.8**	**27.6/15.6**	**12.3**	**-**	**175.5**	**64.3**	**35.8**	**27.6/15.5**	**12.2**
	122.7	172.0	57.6	39.8	25.8/15.4	12.7	-	172.1	57.9	39.7	25.8/15.4	12.7
[4]L503	**115.4**	**176.5**	**56.6**	**39.9**	**24.7**	**21.0**	**-**	**176.8**	**56.6**	**39.8**	**24.7**	**21.0**
	123.8	172.2	51.5	43.7	25.7	24.2	-	172.4	51.6	43.8	25.7	23.9
[1]S505	**-**	**173.4**	**61.3**	**61.3**			**119.0**	**171.2**	**54.4**	**64.5**		
	-	171.1	54.3	64.3			115.6	173.3	60.9	60.9		
[1]V506	**-**	**175.3**	**64.9**	**29.4**	**21.0/20.2**		**125.0**	**172.4**	**58.5**	**33.1**	**19.1**	
	-	172.0	58.5	33.3	19.0		121.6	175.4	64.3	29.4	20.8/19.9	
[4]V507	**115.8**	**176.9**	**65.1**	**29.5**	**21.3/19.9**		**-**	**172.7**	**58.7**	**33.3**	**19.4**	
	122.7	172.3	58.7	33.2	19.3		-	176.9	64.9	29.5	21.2/20.0	
[3]V508	**126.3**	**171.8**	**58.5**	**32.5**	**18.9**		**126.1**	**171.9**	**58.4**	**32.5**	**18.9**	
	119.9	175.5	64.0	29.4	21.0/20.0		119.4	175.3	63.9	29.3	20.7/19.7	

Chemical shifts were obtained from 2D ^{13}C-^{13}C and ^{15}N-^{13}C correlation spectra measured at 253-263 K. [1]From the IAGLSV sample. [2]From the mixed GV and IS sample. [3] From the ILSILV sample. [4] From the AGILV (L493F) sample.

Table S3. ^{19}F CODEX experimental conditions for membrane-bound PIV5 fusion protein TMD.

Sample	CSA recoupling time (ms)	t_{mix} (ms)	Number of scans for S_0 and S each	Total Experimental times (hrs)
L493F, POPC/Chol membrane	0.5 ms	1	11264	10
		10	11264	10
		100	11264	11
		250	11264	12
		600	16384	20
		1000	16384	24
		1500	49152	85
		2000	47104	94
L493F, POPE membrane	0.5 ms	1	10240	9
		10	10240	9
		100	10240	10
		250	10240	11
		500	11264	13
		1000	31744	46
		1500	48128	83
L500F, POPC/Chol membrane	0.25 ms	100	9216	10
		250	9216	11
		500	17408	23
		1000	28160	47
		1500	60928	115
L504F, POPC/Chol membrane	0.5 ms	10	9216	9
		50	11264	12
		100	10240	11
		250	10240	12
		350	11264	14
		500	10240	13
		1000	25600	40
		1500	32768	60

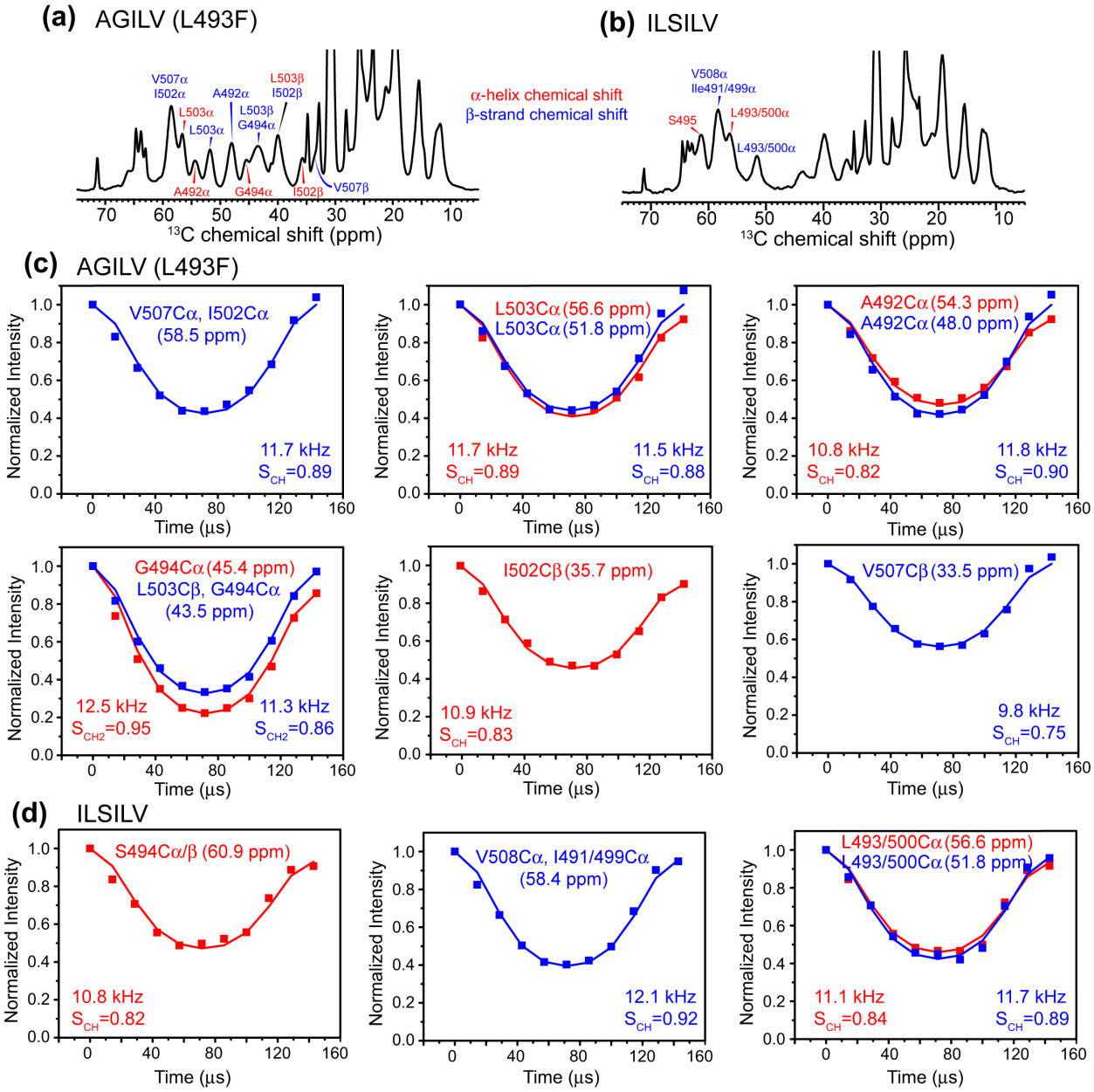

Figure S1. 2D ^{13}C-^{1}H DIPSHIFT data to determine the mobility of membrane-bound PIV5 TMD. ^{13}C spectra of the (a) AGILV (L493F) and (b) ILSILV samples in the POPE membrane, measured at 303 K under 7 kHz MAS. (c-d) ^{1}H-^{13}C dipolar dephasing curves for (c) the AGILV (L493F) sample and (d) the ILSILV sample. Best-fit C-H dipolar couplings are scaled from the rigid-limit value by the FSLG scaling factor of 0.577 [2]. The C-H order parameters are given for each panel. Both α-helical and β-strand conformations of the TMD show large C-H dipolar couplings, with backbone Cα-Hα order parameters of 0.82-0.95 for the α-helical TMD and 0.86-0.92 for the β-sheet TMD, indicating that the peptide backbone is largely immobilized in the lipid membrane.

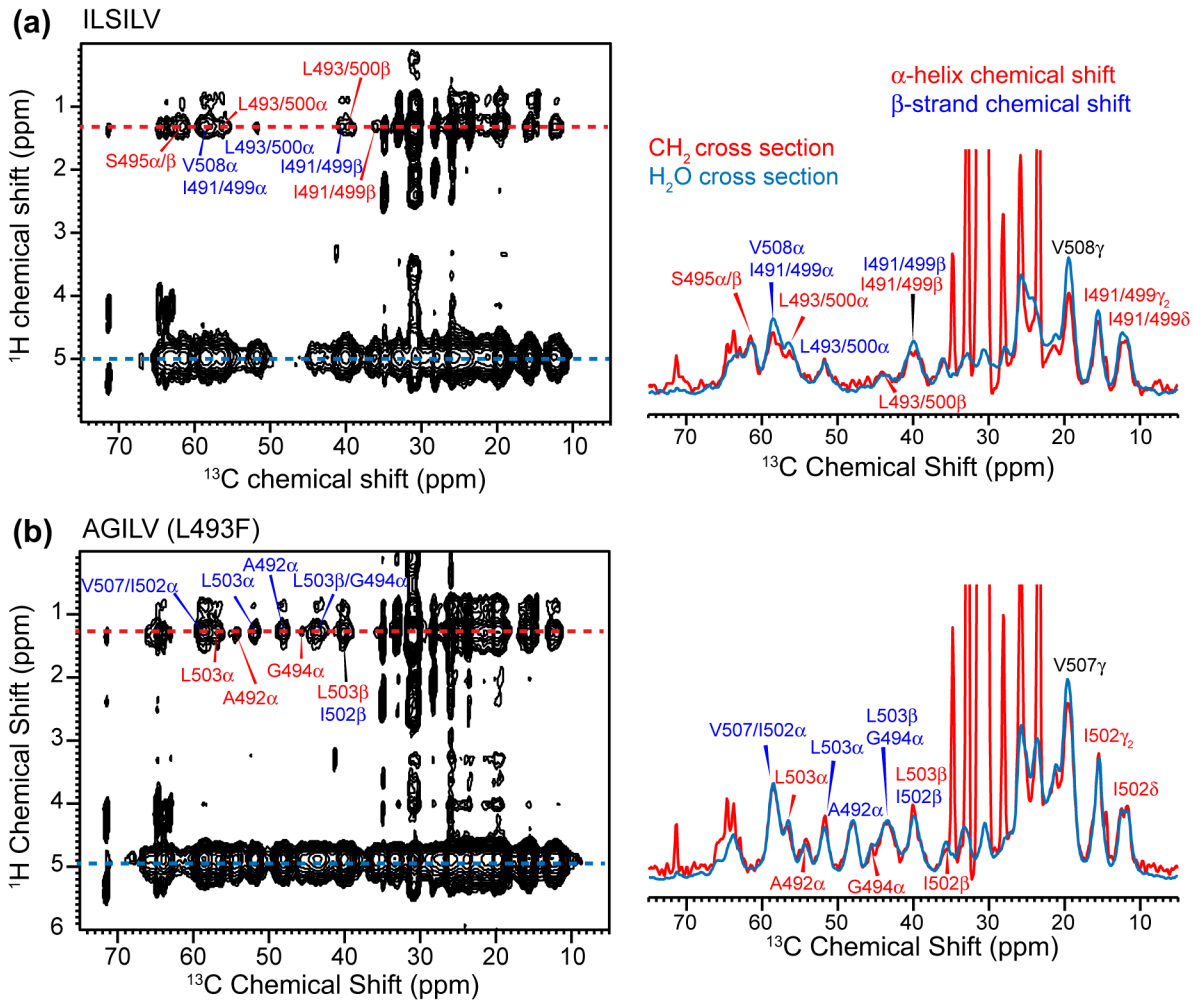

Figure S2. 2D ^{1}H-^{13}C correlation spectra of (a) ILSILV-TMD and (b) AGILV (L493F)-TMD in liquid-crystalline POPE membranes. Clear cross peaks with lipid CH_2 were observed from α-helical and β-strand conformations at 100 ms of ^{1}H spin diffusion, indicating that both conformations are well inserted into the membrane.

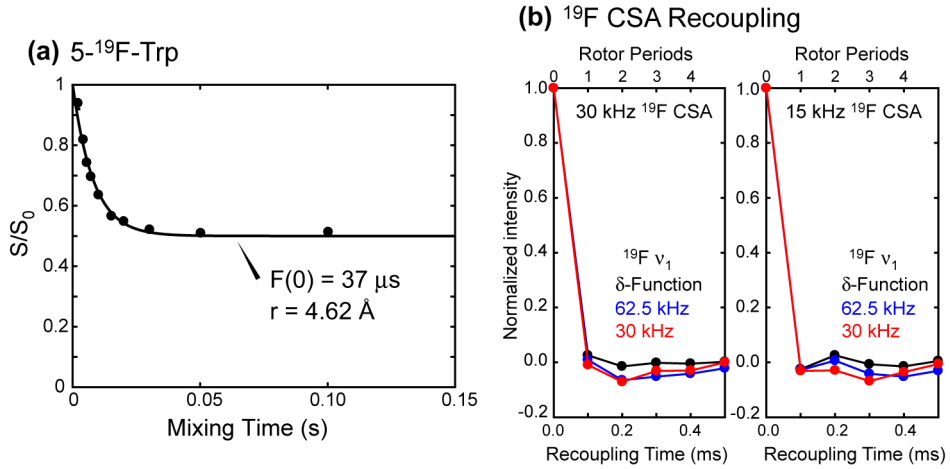

Figure S3. Optimization of ^{19}F CODEX experiments. (a) ^{19}F CODEX data of 5-^{19}F-Trp measured under 8 kHz MAS using a total CSA recoupling time of 0.25 ms. The S/S_0 values equilibrated to 0.5, consistent with the $P2_1$ space group of Trp. Best fit for a nearest-neighbor ^{19}F-^{19}F distance of 4.62 Å was obtained using an $F(0)$ value of 37 µs. (b) Simulated ^{19}F CSA dephasing as a function of the length of the π-pulse train. Simulation used a ^{19}F Larmor frequency of 376 MHz (9.4 Tesla), an MAS frequency of 10 kHz, variable ^{19}F CSAs of 30 kHz (80 ppm) and 15 kHz (40 ppm), and ^{19}F rf field strengths from infinitely strong to 62.5 kHz and 30 kHz. Under these conditions, the recoupled ^{19}F CSAs fully dephased the intensities by 0.4 ms.

References

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[2] Bielecki A, Kolbert AC, Levitt MH. Frequency-Switched Pulse Sequences - Homonuclear Decoupling and Dilute Spin Nmr in Solids. Chem. Phys. Lett. 1989;155:341-346.