# Support information

# Conformation and Lipid Interaction of the Fusion Peptide of the Paramyxovirus PIV5 in Anionic and Negative-Curvature Membranes From Solid-State NMR

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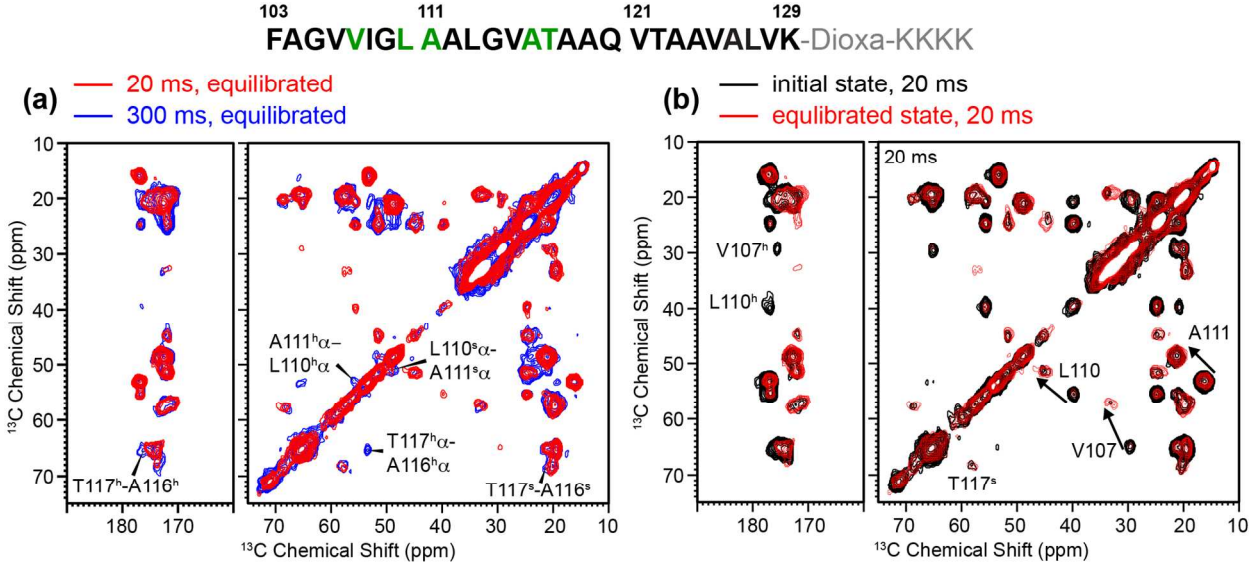


**Figure S1.** 2D $^{13}C$-$^{13}C$ correlation spectra of VLAAT-FPK4 in the DOPC/DOPG (4 : 1) membrane in the gel phase. (a) 2D spectra of the equilibrated sample at 20 ms (red) and 300 ms (blue) mixing. Inter-residue cross peaks are observed only for the same secondary structure and not between different secondary structures. For A116 and T117, the α-helical inter-residue cross peaks are stronger than the β-strand ones, whereas for L110 and A111, the β-strand inter-residue cross peaks are stronger than the α-helix ones. (b) Overlay of the initial (black) and equilibrated (red) spectra. The initial spectrum shows mainly α-helical chemical shifts for all labeled residues, while the equilibrated spectrum shows primarily β-strand chemical shifts for N-terminal residues (V107, L110, and A111).

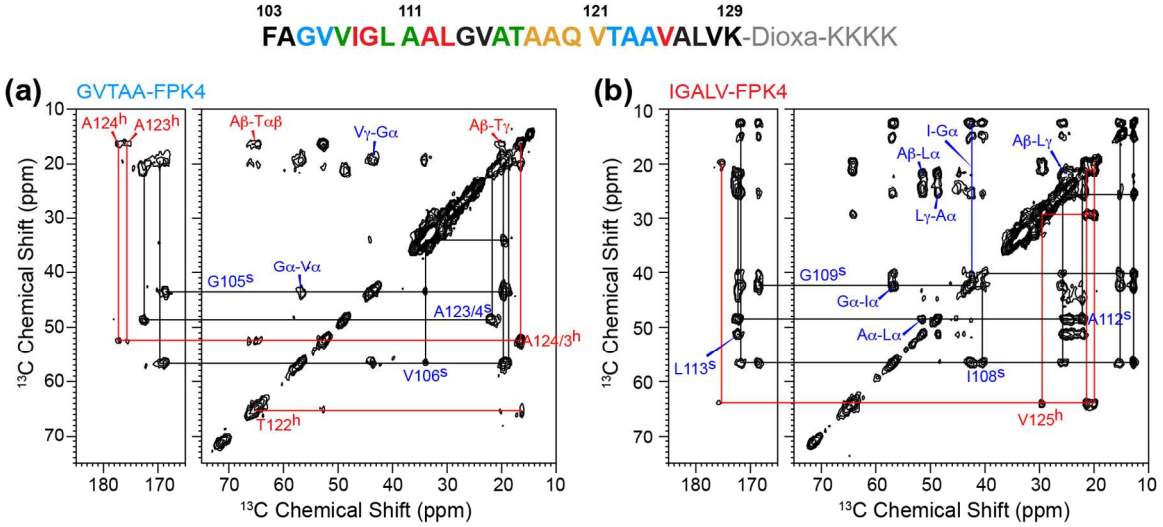


**Figure S2.** 2D $^{13}C$-$^{13}C$ correlation spectra of FPK4 in the gel-phase DOPC/DOPG (4 : 1) membrane (246 K). (a) GVTAA-FPK4 spectrum with 500 ms mixing. (b) IGALV-FPK4 spectrum with 500 ms mixing. Inter-residue cross peaks were observed in both spectra, but no cross peak between N-terminal and C-terminal residues is seen. Thus, if the peptide is oligomerized in the DOPC/DOPG bilayer, then the packing is parallel.

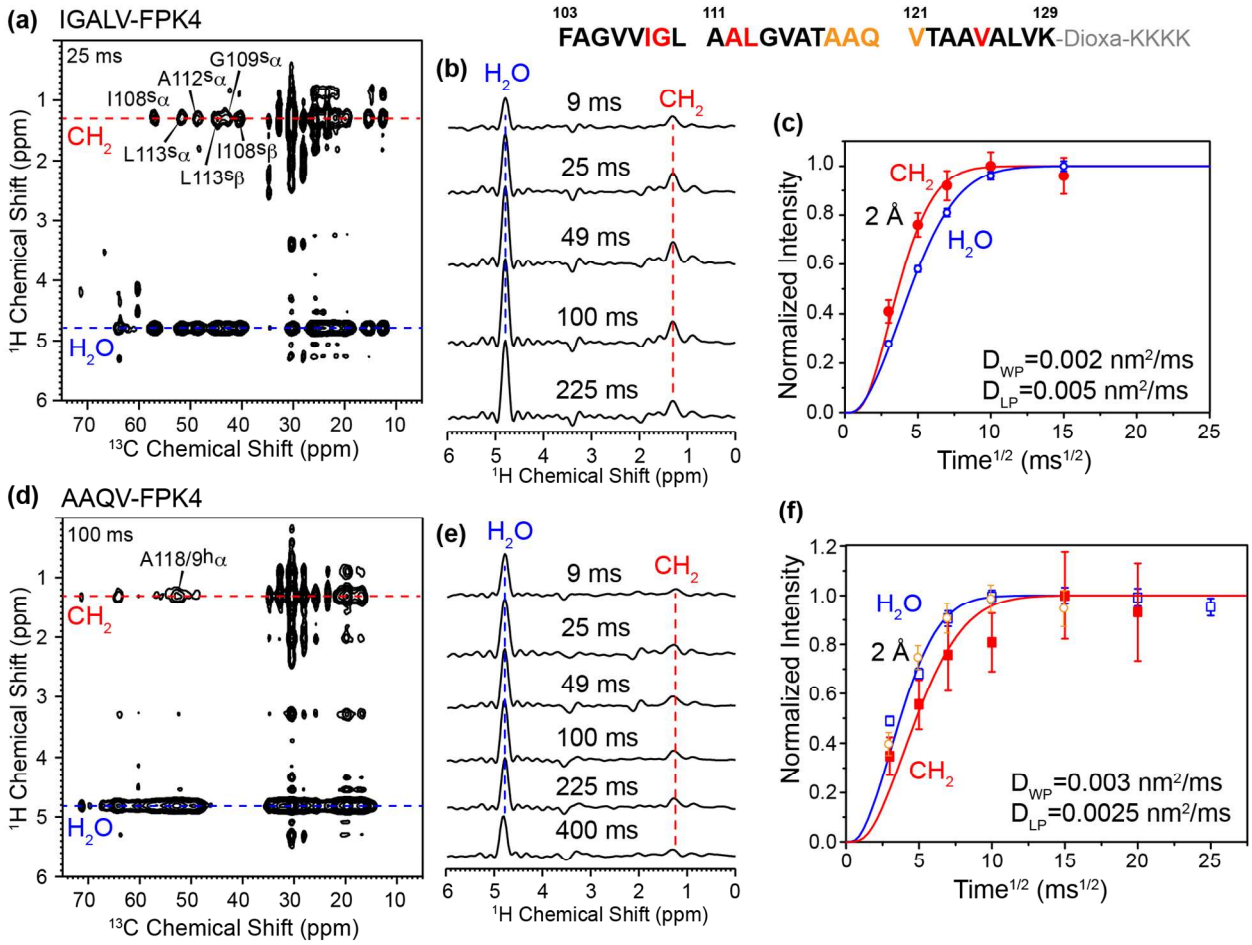


**Fig. S3**. 2D $^{13}$C-$^{1}$H spin-diffusion correlation spectra of FPK4 in the DOPC/DOPG membrane at 293 K. (a) 2D spectrum of IGALV-FPK4 with 25 ms $^{1}$H spin diffusion. (b) $^{1}$H cross sections of the 2D spectra with mixing times from 9-225 ms. The cross sections were integrated from $^{13}$C chemical shifts of 37.8-59.0 ppm, corresponding to the β-strand Cα and Cβ signals. (c) Measured and best-fit buildup curves of water-peptide (blue) and lipid-peptide (red) spin diffusion. (d) 2D spectrum of AAQV-FPK4 with 100 ms $^{1}$H spin diffusion. (e) $^{1}$H cross sections with mixing times of 9-400 ms. The cross sections were integrated from $^{13}$C chemical shifts of 51.5 to 54.0 ppm, corresponding to the α-helical Cα signals. (f) Measured and best-fit buildup curves of water-peptide (blue) and lipid-peptide (red) spin diffusion. Clear lipid-peptide cross peaks are observed for both conformations of the peptide, indicating that FPK4 is well inserted into the DOPC/DOPG membrane.

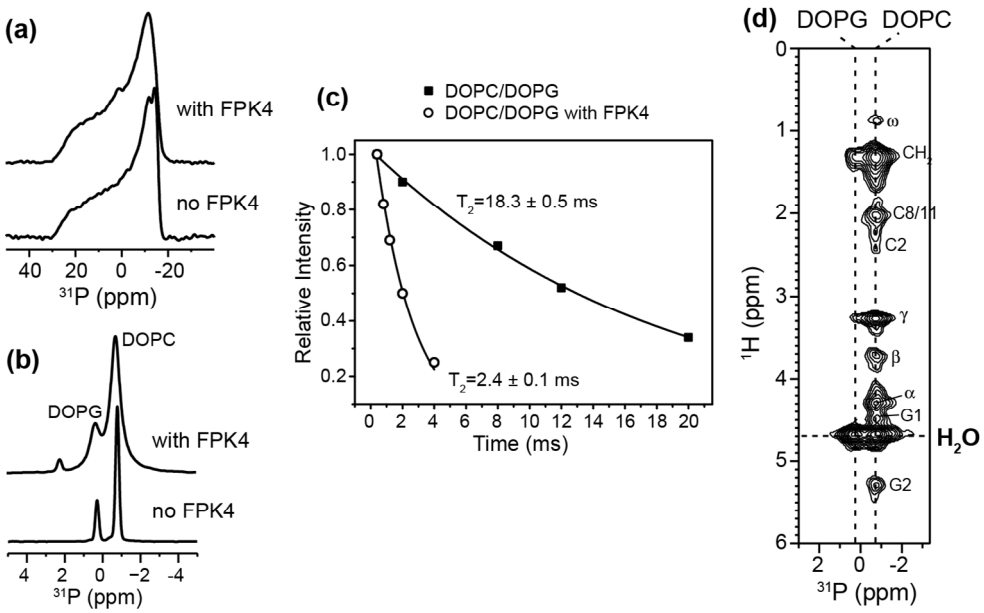


**Fig. S4**. FPK4 interaction with the DOPC/DOPG membrane. (a) 1D $^{31}$P static spectra at 293 K with and without FPK4. (b) $^{31}$P MAS spectra with and without FPK4. The apparent linewidths are ~30 Hz for the peptide-free membrane and ~130 Hz for the peptide-bound membrane. (c) $^{31}$P transverse relaxation times ($T_2$) of DOPC in the DOPC/DOPG membrane without and with the peptide. The DOPG $T_2$ values are similar to the DOPC values in the same sample. The $T_2$ values were measured using a Hahn-echo pulse sequence with a $^{1}$H decoupling field strength of 50 kHz. (d) 2D $^{31}$P-$^{1}$H correlation spectrum of the peptide-bound sample at 303 K, measured with a $^{1}$H spin diffusion mixing time of 225 ms.

**Table S1**. Residue-specific helicity (%) of FPK4 in DOPC/DOPG membranes based on the NMR spectral intensities.

| Residue | Cross peak | Helicity | Residue | Cross peak | Helicity |
|---|---|---|---|---|---|
| G105 | Cα-CO | 0 | A116 | Cα-Cβ | 54% |
| V106 | Cα-Cβ | 0 | T117 | CO-Cα | 53% |
| V107 | Cβ-Cγ | 34% | A118 | Cα-Cβ | 83% |
| I108 | Cα-Cβ | 0 | A119 | Cα-Cβ | 83% |
| G109 | Cα-CO | 0 | Q120 | Cα-Cβ | 100% |
| L110 | Cα-Cβ | 26% | V121 | Cα-Cγ | 85% |
| A111 | Cα-Cβ | 22% | T122 | Cα/β-Cγ | 66% |
| A112 | Cα-Cβ | 18% | A123 | Cα-Cβ | 57% |
| L113 | Cα-CO | 17% | A124 | Cα-Cβ | 57% |
| G114 | ND | ND | V125 | Cα-Cβ | 100% |
| V115 | ND | ND | | | |

For each residue, resolved cross peaks in the 20 ms 2D $^{13}C$-$^{13}C$ correlation spectra were selected for volume integration. A111 and A116 Cα-Cβ cross peaks overlap and indicate a total helical content of 38%. We interpolated the helicities of L110 and A112 to obtain the A111 helicity, and the A116 helicity was the difference between the total helicity and the A111 helicity.