

## **Supplementary Material**

### **High-Resolution Orientation and Depth of Insertion of the Voltage-Sensing S4 Helix of a Potassium Channel in Lipid Bilayers**

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**Table S1.**  $^{13}\text{C}$  isotropic chemical shifts (ppm) of labeled residues in KvAP S4 in DMPC/DMPG bilayers. Secondary chemical shifts were calculated by subtracting the experimental shifts by the random coil (rc) chemical shifts <sup>1</sup>. The chemical shifts are relative to TMS.

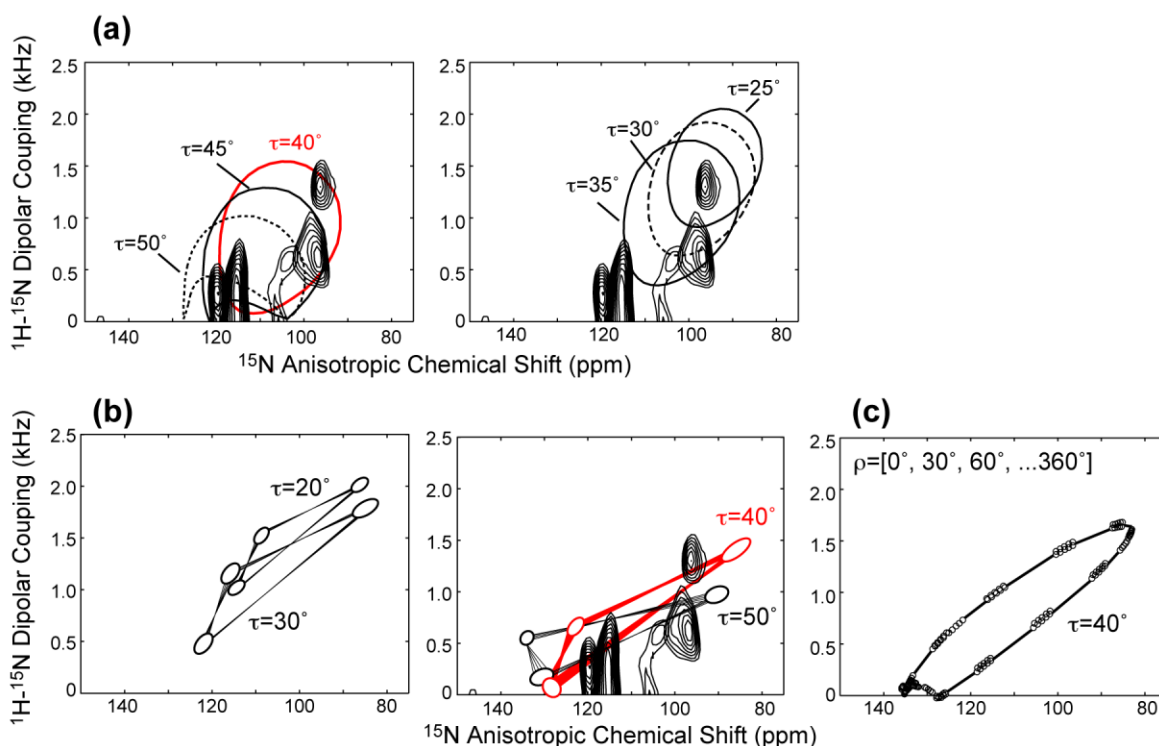
Residue	Site	$\delta_{expt}^{KvAP}$	$\delta^{rc}$	$\delta_{expt}^{KvAP} - \delta^{rc}$
G2	CO	171.9	172.6	-0.7
	C $\alpha$	42.6	43.6	-1.0
L6	CO	176.3	174.9	1.4
	C $\alpha$	55.9	53.2	2.7
	C $\beta$	39.4	40.2	-0.8
V7	CO	176.0	174.1	1.9
	C $\alpha$	64.9	60.1	4.8
	C $\beta$	29.3	31.0	-1.7
R8	CO	177.2	174.2	3.0
	C $\alpha$	57.4	54.3	3.1
	C $\beta$	28.5	28.8	-0.3
L9	CO	176	174.9	1.1
	C $\alpha$	56.0	53.2	2.8
	C $\beta$	39.7	40.2	-0.5
I15	CO	175.9	173.8	2.1
	C $\alpha$	61.9	58.9	3.0
	C $\beta$	36.1	36.6	-0.5

**Table S2.** TALOS<sup>2</sup> predicted ( $\phi$ ,  $\psi$ ) torsion angles for Val<sub>7</sub> and Arg<sub>8</sub> of the S4 helix, obtained using DSS-referenced <sup>13</sup>C chemical shifts.

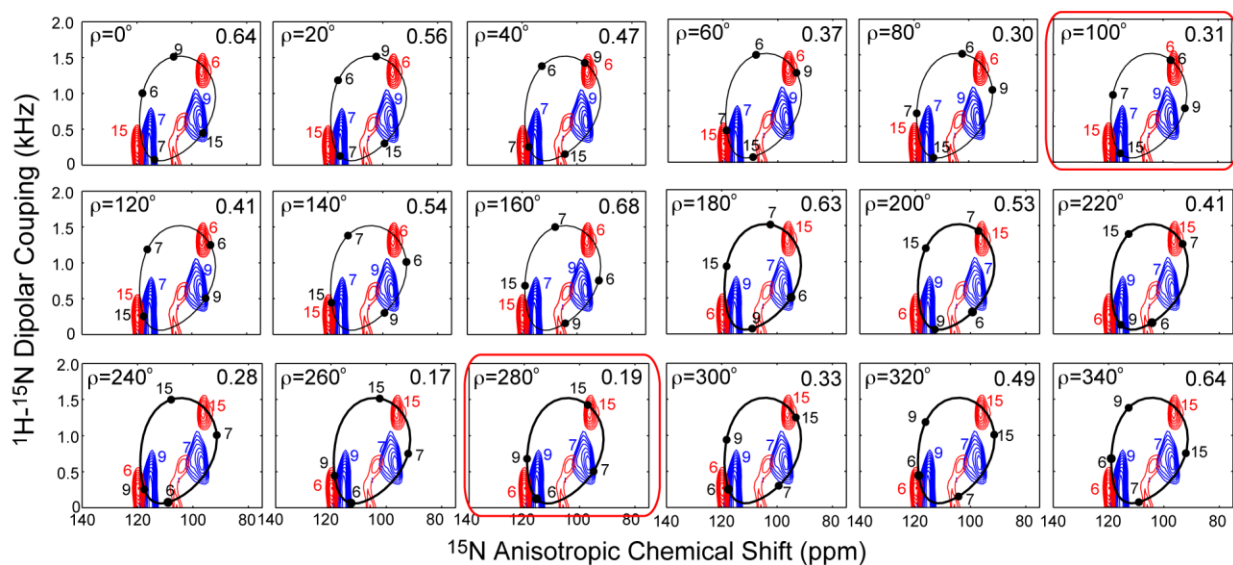
Residue	( $\phi$ , $\psi$ )
Val <sub>7</sub>	(-61±5°, -48±9°)
Arg <sub>8</sub>	(-61±4°, -43±8°)

**Table S3.** Observed <sup>31</sup>P anisotropic chemical shift  $\delta_{obs}$  of DMPC lipids in DMPC/6-O-PC bicelles containing the KvAP S4 peptide. The order parameter was calculated using  $S_{bicelle} = (\delta_{obs} - \delta_{iso}) / (\delta_{90P} - \delta_{iso})^3$ , where the input chemical shifts were  $\delta_{iso} = -0.8$  ppm and  $\delta_{90P} = -14.9$  ppm.

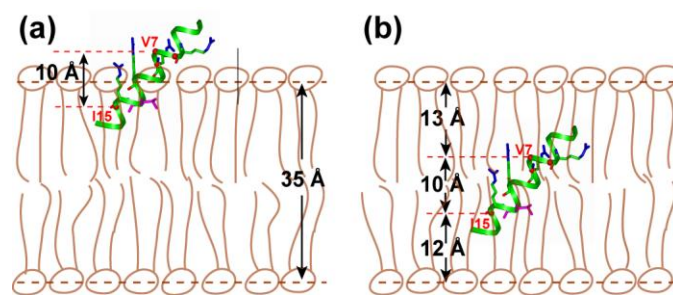
Sample	$\delta_{obs}$ (ppm)	$S_{bicelle}$
G2, L6, I15-labeled S4	-12.4	0.82
V7, L9-labeled S4	-13.2	0.88



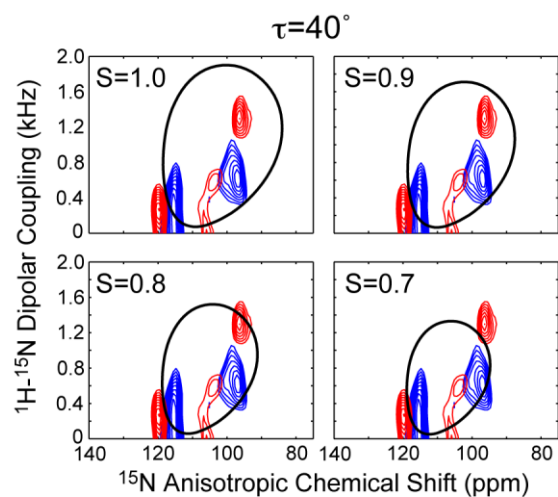
**Fig. S1.** Simulated 2D  $^{15}\text{N}$ - $^1\text{H}$  dipolar and  $^{15}\text{N}$  chemical shift correlation spectra to estimate the precision and accuracy of the S4 helix orientation. (a) Simulated spectra for  $\alpha$ -helices with tilt angles of  $25^\circ$  to  $50^\circ$  at  $5^\circ$  steps. An 18-residue ideal  $\alpha$ -helix with  $(\phi, \psi)$  angles of  $(-64^\circ, -40^\circ)$  was used as the input. The  $40^\circ$  PISA wheel (red) best fit the experimental spectrum, with a precision of better than  $5^\circ$ . (b) Simulated 2D  $^{15}\text{N}$  spectra for  $3_{10}$  helices with tilt angles from  $20^\circ$  to  $50^\circ$  at  $10^\circ$  steps and an arbitrary rotation angle. A 15-residue  $3_{10}$  helix with  $(\phi, \psi)$  angles of  $(-49^\circ, -26^\circ)$  were used as the input. Circles indicate the simulated peak positions. Lines connect the peak positions of consecutive residues but do not indicate possible peak positions. Due to the 3-residue per turn geometry of the  $3_{10}$  helix, the spectral patterns have an approximate 3-residue periodicity, and the peaks for each unique  $(\tau, \rho)$  pair are clustered at three positions, leaving most of the spectral region empty. (c) When multiple rotation angles (at  $30^\circ$  steps) are combined for a tilt angle ( $\tau=40^\circ$  as an example), an elliptical pattern emerges. Since a functional transmembrane helix has unique rotation angles (within  $< 10^\circ$  uncertainty), most of the positions of each ellipse are not populated, in contrast to an  $\alpha$ -helix.



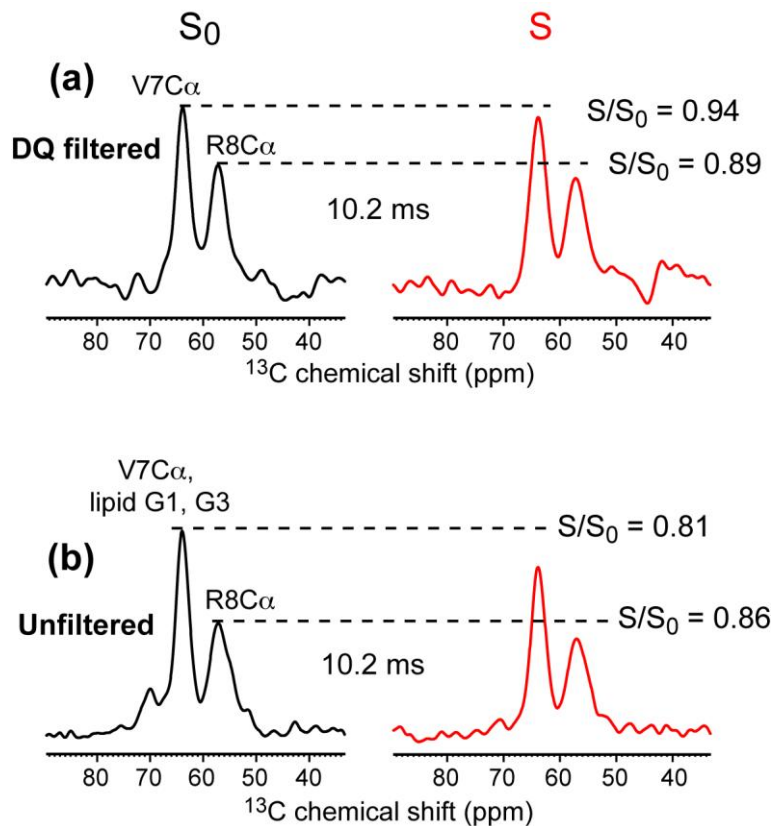
**Fig. S2.** Simulated 2D  $^{15}\text{N}$  anisotropic correlation spectra for  $\tau = 40^\circ$  and  $\rho$  from  $0^\circ$  to  $340^\circ$ . The assignment for each simulation is indicated in black and the closest experimental assignment is indicated in blue and red. Two degenerate best fits to the experimental spectrum are found at  $\rho = 100^\circ \pm 20^\circ$  and  $280^\circ \pm 20^\circ$ .



**Fig. S3.** Two alternative membrane topology models of KvAP S4 that can be ruled out based on solid-state NMR constraints. The S4 peptide is shown to scale with a 35 Å thick DMPC bilayer<sup>4</sup>. The vertical separation between V7 C $\alpha$  and I15 C $\alpha$ , which depends on the helical conformation and the peptide orientation, is 10 Å. (a) Half insertion model. If this model were correct, then the sum of the Val<sub>7</sub> and Ile<sub>15</sub> <sup>13</sup>C $\alpha$ -<sup>31</sup>P distance should be equal to 10 Å. However, the measured sum of <sup>13</sup>C $\alpha$ -<sup>31</sup>P distances is 16 Å, thus ruling out this model. (b) Full insertion model without membrane thinning. This model would require Val<sub>7</sub> and Ile<sub>15</sub> C $\alpha$  to be 12-13 Å from the <sup>31</sup>P plane, which does not agree with the measured distance of 7.5 Å and 8.5 Å.

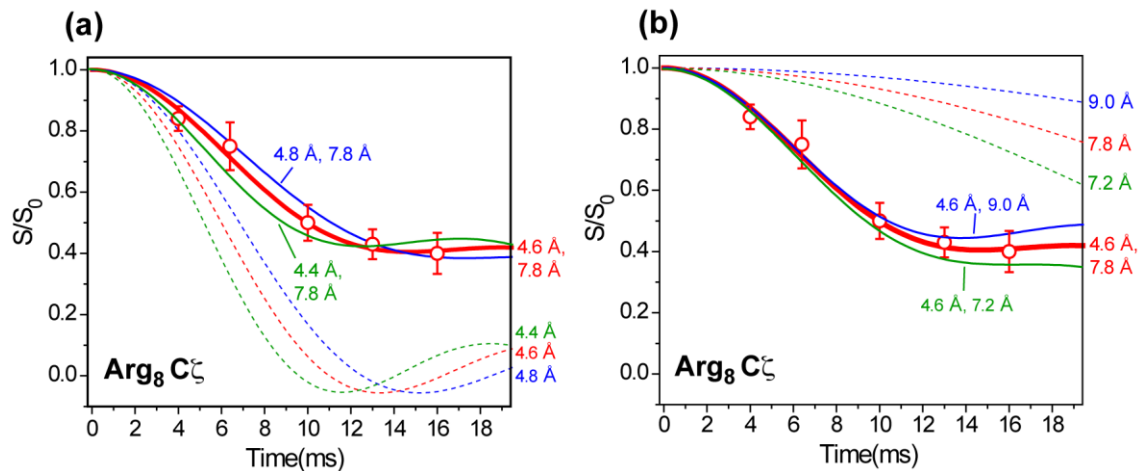


**Fig. S4.** Simulated 2D  $^{15}\text{N}$  anisotropic correlation spectra to verify the bicelle wobble order parameter. A tilt angle of  $\tau = 40^\circ$  was simulated using a generalized order parameter  $S$  from 1.0 to 0.7. Best fit was found for  $S = 0.8$ , consistent with the  $^{31}\text{P}$  spectra and previous literature evidence for this bicelle composition<sup>5-7</sup>.



**Fig. S5.**  $^{13}\text{C}$  double-quantum filtered  $^{13}\text{C}$ - $^{31}\text{P}$  REDOR data of  $\text{Val}_7 \text{C}\alpha$  and  $\text{Arg}_8 \text{C}\alpha$  in DMPC/DMPG-bound S4 helix. The lipid natural-abundance glycerol G1 and G3 peaks, which overlap with the  $\text{Val}_7 \text{C}\alpha$  peak, were removed by the DQ filter. (a) Double-quantum filtered REDOR  $S_0$  and  $S$  spectra at a mixing time of 10.2 ms. (b) Unfiltered  $^{13}\text{C}$ - $^{31}\text{P}$  REDOR spectra at the same mixing time. The regular REDOR spectra show lower  $S/S_0$  values for the  $\text{Val}_7 \text{C}\alpha$  peak due to the influence of the glycerol G3 and G1 carbons, which are close to the phosphate group.





**Figure S6.** Distance uncertainty for the Arg<sub>8</sub> C $\zeta$ -<sup>31</sup>P REDOR data. (a) Uncertainty of the short component of the dual-distance fit. The 4.6 Å fit is distinguishably better than 4.4 Å and 4.8 Å, indicating an error bar of 0.2 Å. (b) Uncertainty of the long component of the dual-distance fit. The 7.8 Å has an uncertainty of +1.2 Å and -0.6 Å, consistent with the larger difficulty of measuring longer distances with high precision.

## References

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