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Supporting Information

Impact of PIP2 Lipids, Force Field Parameters, and Mutational Analysis on the Binding of Osh4's α_6-α_7 Domain

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Table S1. Displays the distance in (Å) between the peptide and the phosphate component of the membrane with "avg" being the average distance and "min" as the minimum distance, and the different initial peptide orientations that were horizontal (H) or vertical (V) to the membrane.

Distance from Membrane Phosphate to Peptide Center of Mass	H1		H2		H3		V1		V2	
	Min	Avg	Min	Avg	Min	Avg	Min	Avg	Min	Avg
non-PIP2	8	16	8	16	17	19	6	22	3	28
PIP2	15	21	14	21	16	21	13	30	>25	36

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Table S2: Details of the peptide binding: the time at which the peptide crossed the main phosphate plane, distance that the deepest residues travel beneath the phosphate with ±standard error (z_{min}) and average center of mass of peptide relative to the phosphate plane with ±standard error (z_{COM}).

		H1a	H2a	H3a	V1a	V2a
Non-PIP2	Time (ns)	25	20	110	11	--
	zmin (Å)	-4.2 ± 2.3	-4.8 ± 1.8	-4.4 ± 2.0	-3.3 ± 1.9	--
	z_{COM} (Å)	5.6 ± 1.4	4.2 ± 1.3	4.9 ± 1.4	7.1 ± 1.5	--
PIP2	Time (ns)	73	108	67	45	42
	zmin (Å)	-6.3 ± 2.3	-5.3 ± 1.9	-4.9 ± 2.1	-7.0 ± 2.2	-5.1 ± 3.1
	z_{COM} (Å)	6.4 ± 4.0	6.1 ± 1.2	5.7 ± 1.5	2.6 ± 2.3	7.5 ± 1.7
		H1b	H2b	H3b	V1b	V2b
Non-PIP2 set 2	Time (ns)	10	40	30	50	25
	zmin (Å)	-4.2 ± 2.3	-4.8 ± 1.8	-4.4 ± 2.0	-3.3 ± 1.9	--
	z_{COM} (Å)	5.6 ± 1.4	4.2 ± 1.3	4.9 ± 1.4	7.1 ± 1.5	--
PIP2 set 2	Time (ns)	30	135	95	55	50
	zmin (Å)	-6.3 ± 2.3	-5.3 ± 1.9	-4.9 ± 2.1	-7.0 ± 2.2	-5.1 ± 3.1
	z_{COM} (Å)	6.4 ± 4.0	6.1 ± 1.2	5.7 ± 1.5	2.6 ± 2.3	7.5 ± 1.7

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Table S3) This is the peptide-peptide interaction summary for the non-PIP2 HMMM data

HMMM Non-PIP2	D319-K315	K315-E338	E338-K334
H1a	0.9797	0.1649	0.3553
H2a	0	0	0.3511
H3a	0.0001	0.0005	0.4291
V1a	0.0001	0.0003	0.2773
V2a	0.5249	0.0012	0.3883
H1b	0.7215	0.0068	0.2839
H2b	0.8774	0.0001	0.3235
H3b	0.6053	0.0008	0.3159
V1b	0.9463	0.0069	0.4049
V2b	0.7451	0.0091	0.1936
Total Average	0.54004	0.01906	0.33229

Table S4) This is the summary of the full length summary of the peptide-peptide salt bridge breakdown indicating that H1a was the only simulation with high interaction across 315-338, but that it is still possible in other simulations but only briefly.

Full Length	D319-K315	K315-E338	E338-K334
H1a	0.5229	0.3462	0.1565
H2a	0.7858	0.0001	0.2746
H1b	0	0.0911	0.2474
H2b	0	0.0003	0.2670
H1a PIP2	0.4279	0	0.3329
V2a PIP2	0	0.0012	0.3312
V1b PIP2	0.3042	0	0.2903
V2b PIP2	0	0.0012	0.2302
Total Average	0.2551	0.055013	0.2662625

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Table S5) This table shows the total fractional breakdown of the peptide-peptide salt bridges between residues that may help to stabilize the hydrophobic region and also stabilize the charged amino acids that could enhance the dwell time on the liposome surface when they are not interacting with charged headgroups

HMMM PIP2	D319-K315	K315-E338	E338-K334
H1a PIP2	0.3204	0.0005	0.1678
H2a PIP2	0.6671	0.0315	0.3156
H3a PIP2	0	0.0573	0.4054
V1a PIP2	0	0.0113	0.5021
V2a PIP2	0.2250	0.0183	0.3141
H1b PIP2	0.1099	0.0043	0.4530
H2b PIP2	0.3748	0.0014	0.4916
H3b PIP2	0	0.0042	0.2299
V1b PIP2	0	0.0047	0.3954
V2b PIP2	0.4004	0.0135	0.4139
Total Average	0.20976	0.0147	0.36888

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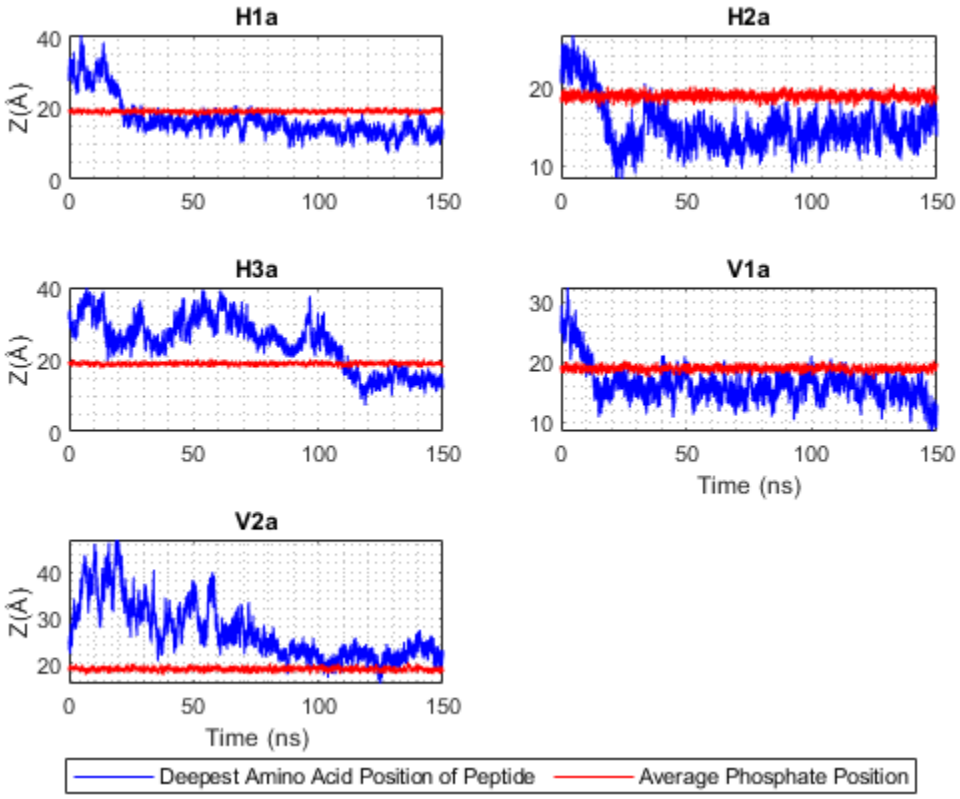

Figure S1. Time series of deepest (when bound) or closest (before binding) residues to the membrane. Position is relative to the phosphates and the red line is the main phosphate position. The main point is that the peptide starts detached from the membrane and makes a deep connection to the membrane beneath the main phosphate layer in all trials except V2. There is a natural fluctuation that is expected and there may be some variation in the average position as well but nothing significant. This is defined as the center of mass of the deepest residue in the membrane.

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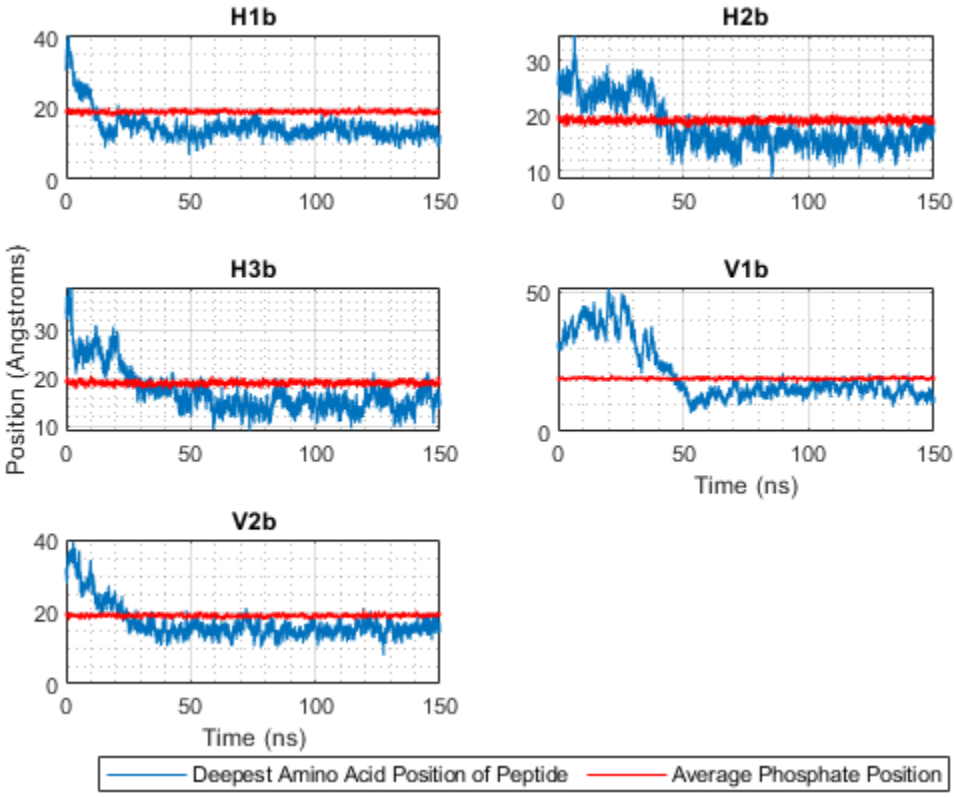

Figure S2. Showing the deepest amino acid residue COM for the second set of HMMM trials. These show that all of the simulations bound within roughly the same amount of time.

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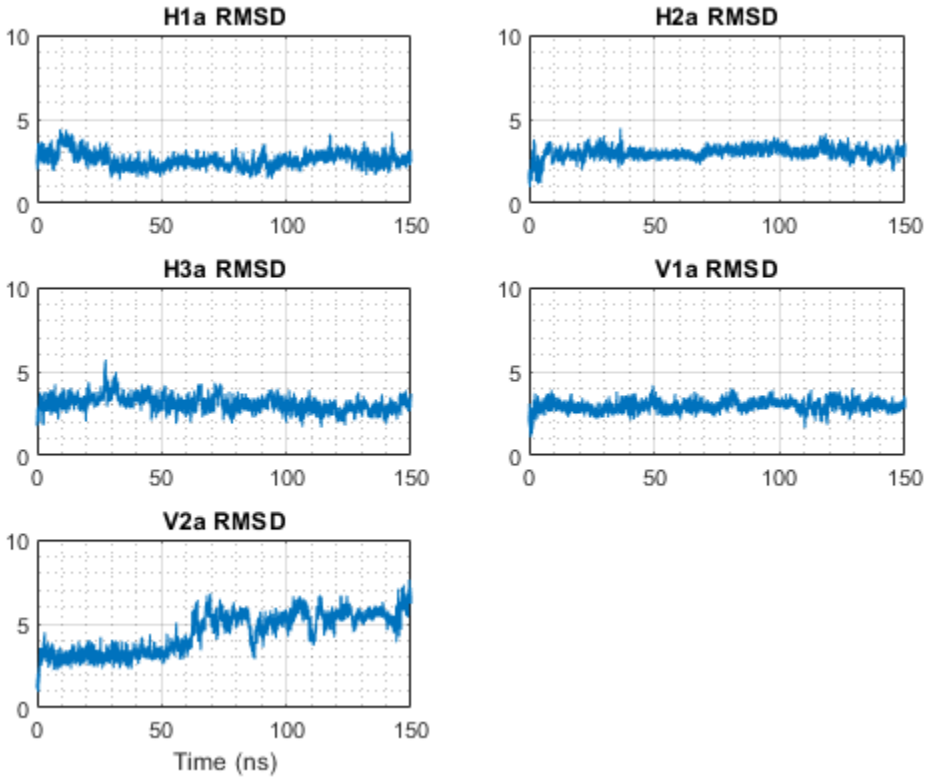

Figure S3. RMSD of the HMMM section of the non-pip simulations with an average value of RMSD around 3-4. The main point of the figure is that all of the simulations in this series converged to a stable state except V2 which has a higher RMSD compared to the others due to some deformations on the α_7 helix.

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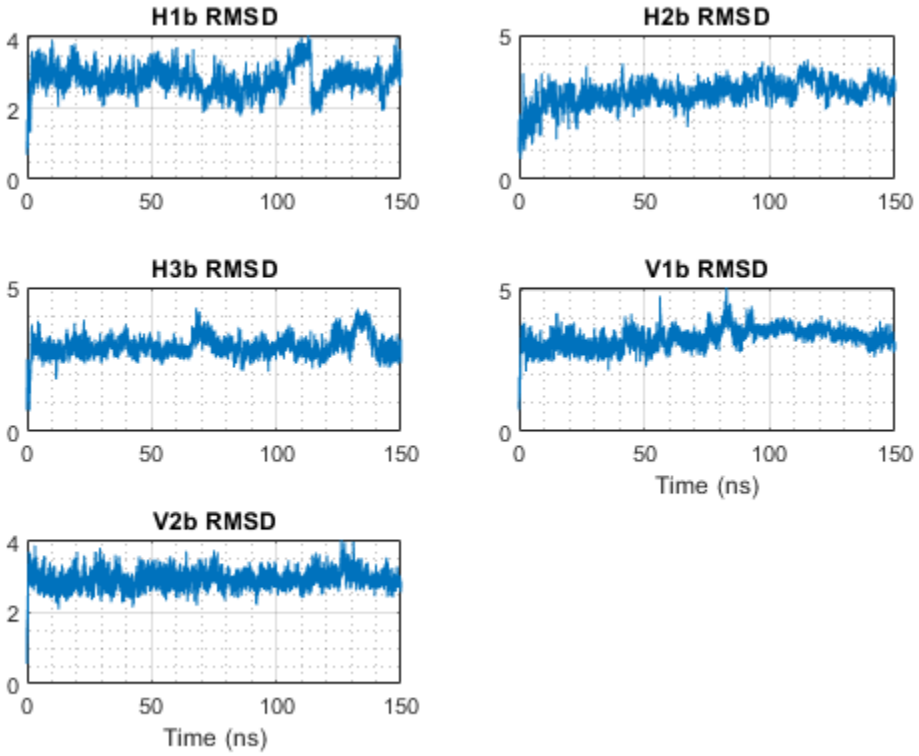

Figure S4. Showing the RMSD of the second set of HMMM trials. The main point of the figure is that all of the simulations in this set have approximately the same RMSD showing convergence to a stable state.

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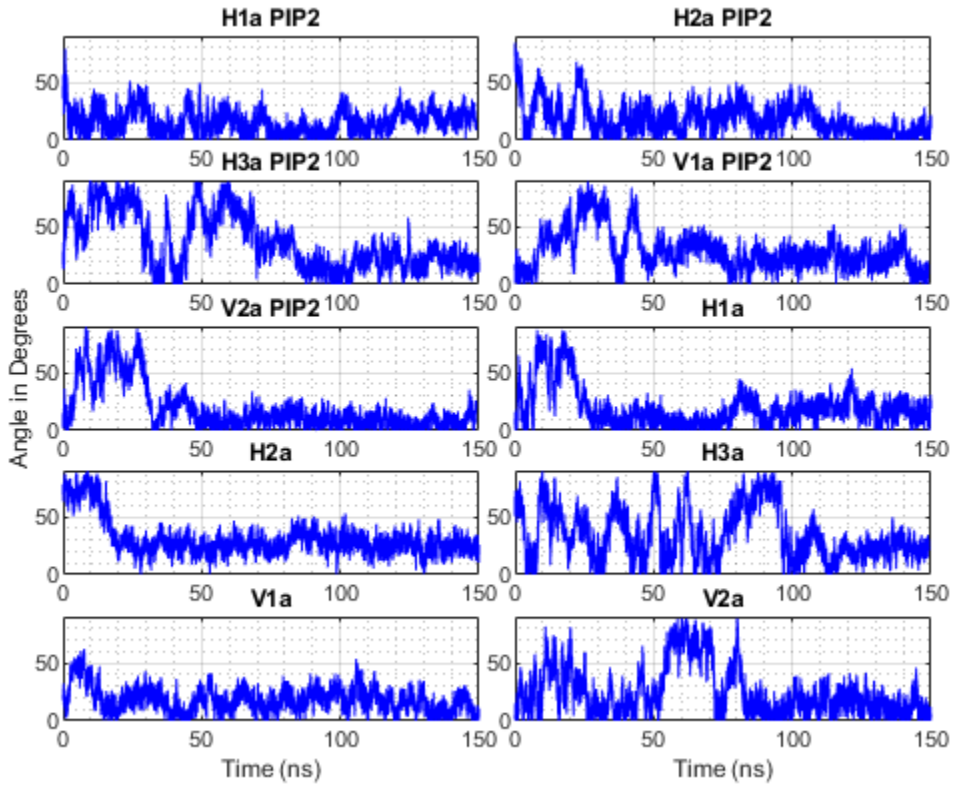

Figure S5. Showing the ϕ angle of the HMMM trials over the course of the simulations. The main point is that these trials converge to a stable low angle and that before that moment there is lots of rearrangement occurring.

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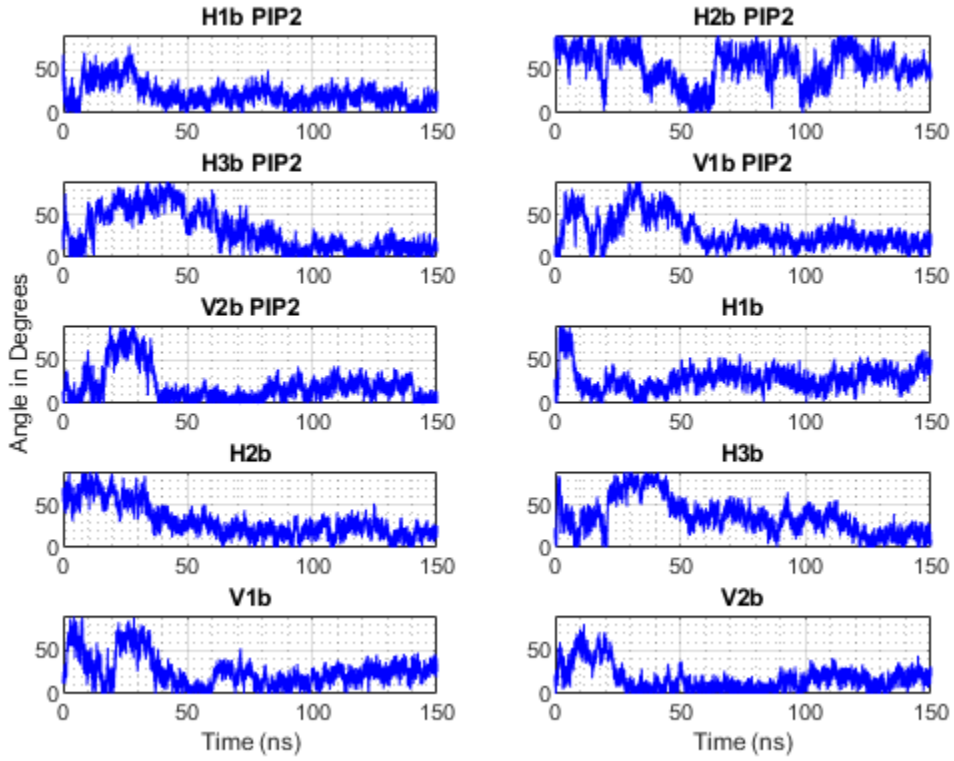

Figure S6. This is the second set of trials for the ϕ angle. The main point of this figure is to show that there are a variety of different angles that exist at binding.

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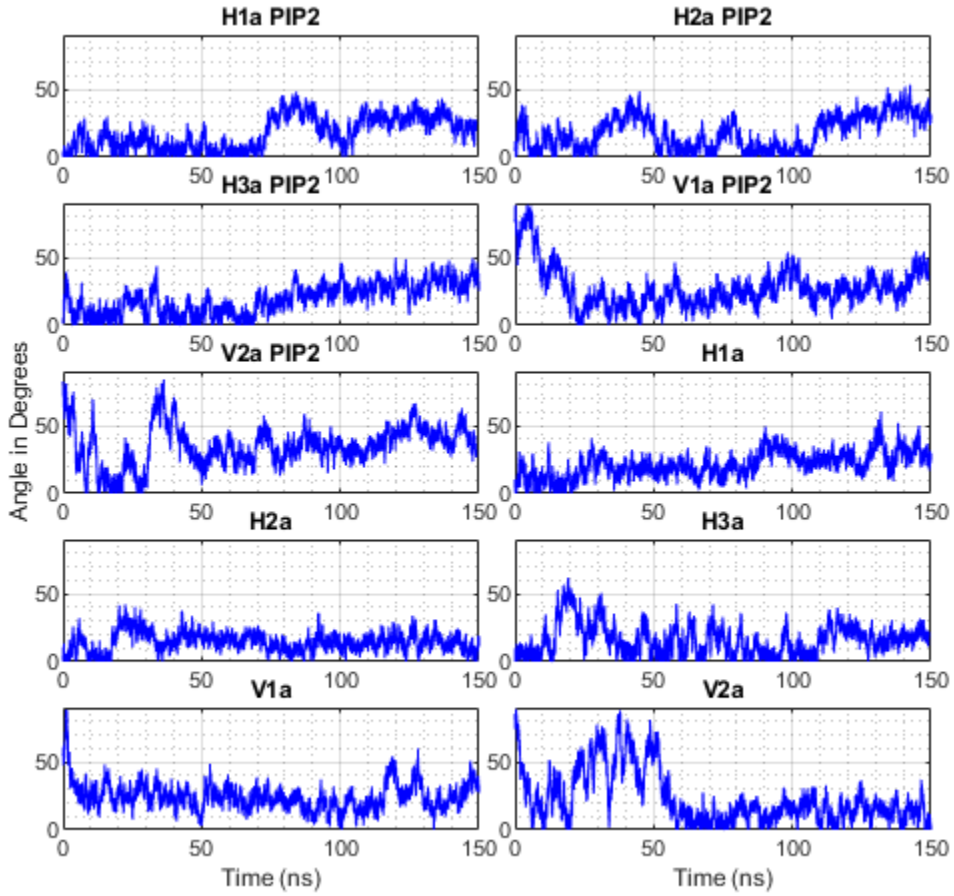

Figure S7. The individual θ_2 which is defined in figure 1, showing the individual angles formed between the α_6 and helix and the membrane over the course of the non-PIP membrane simulations. There are two states that were identified with this angle, a high and low anlge the low ange can be seen whenever the angle frequently reaches 0 degrees and the high angle is observed when the angle never touches 0 degrees for a period of time.

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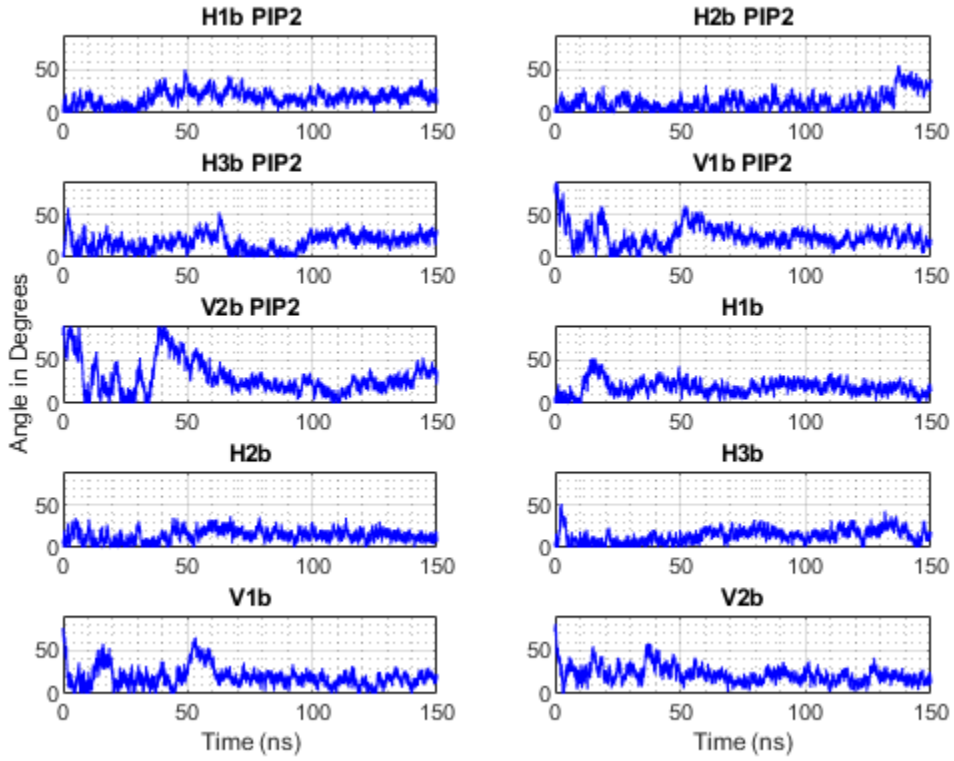

Figure S8. Showing the individual θ_2 angles for the second set of trials. It was noticed that some of the simulaiotns touched down to zero and others didn't such as H2b touching to zero and H1b not reaching zero as frequently after binding.

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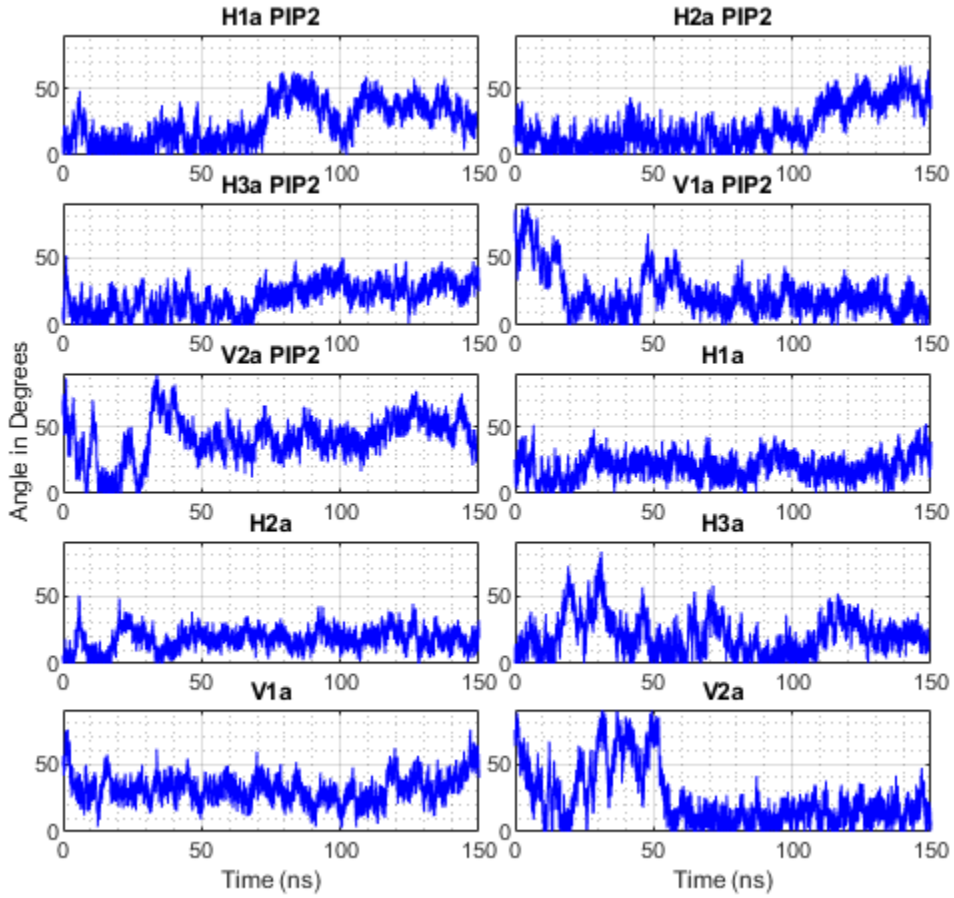

Figure S9. The individual θ_1 which is defined in figure 1, showing the individual angles formed between the α_6 helix and the membrane over the course of the first round of membrane simulations. This variable shows a difference in high and low angles too, but is not expected to impact the interaction energy because the α_6 is shorter and remains in better contact with the membrane compared to the α_6 helix.

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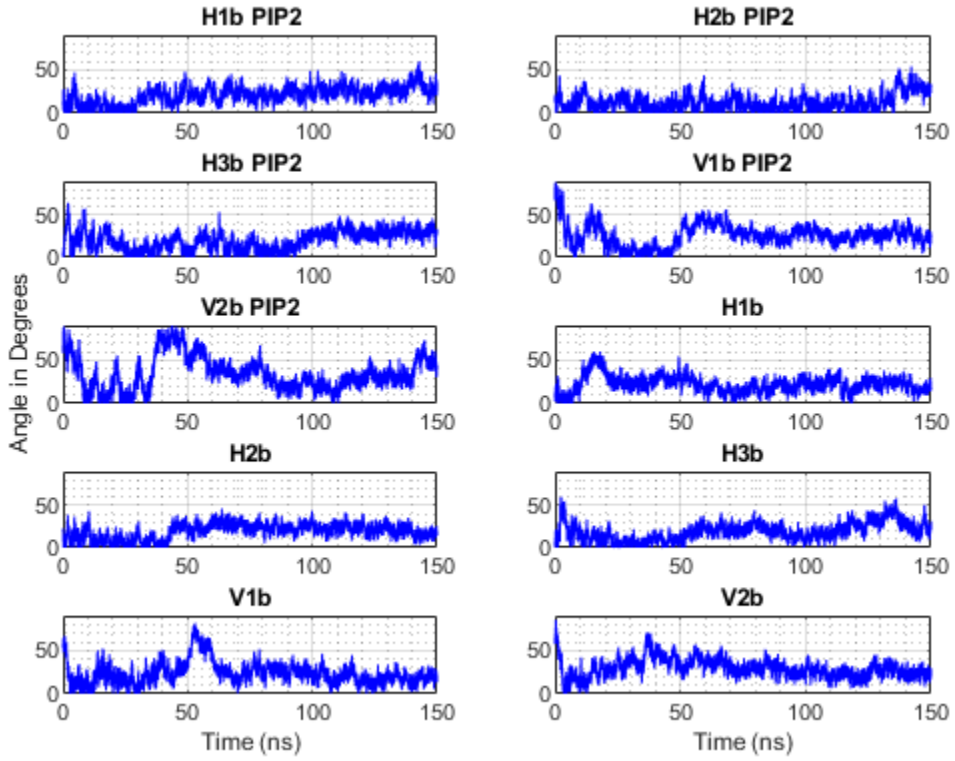

Figure S10. Showing the individual θ_1 angles. There were differences in this angle as well and they were calculated to help visualize the simulations.

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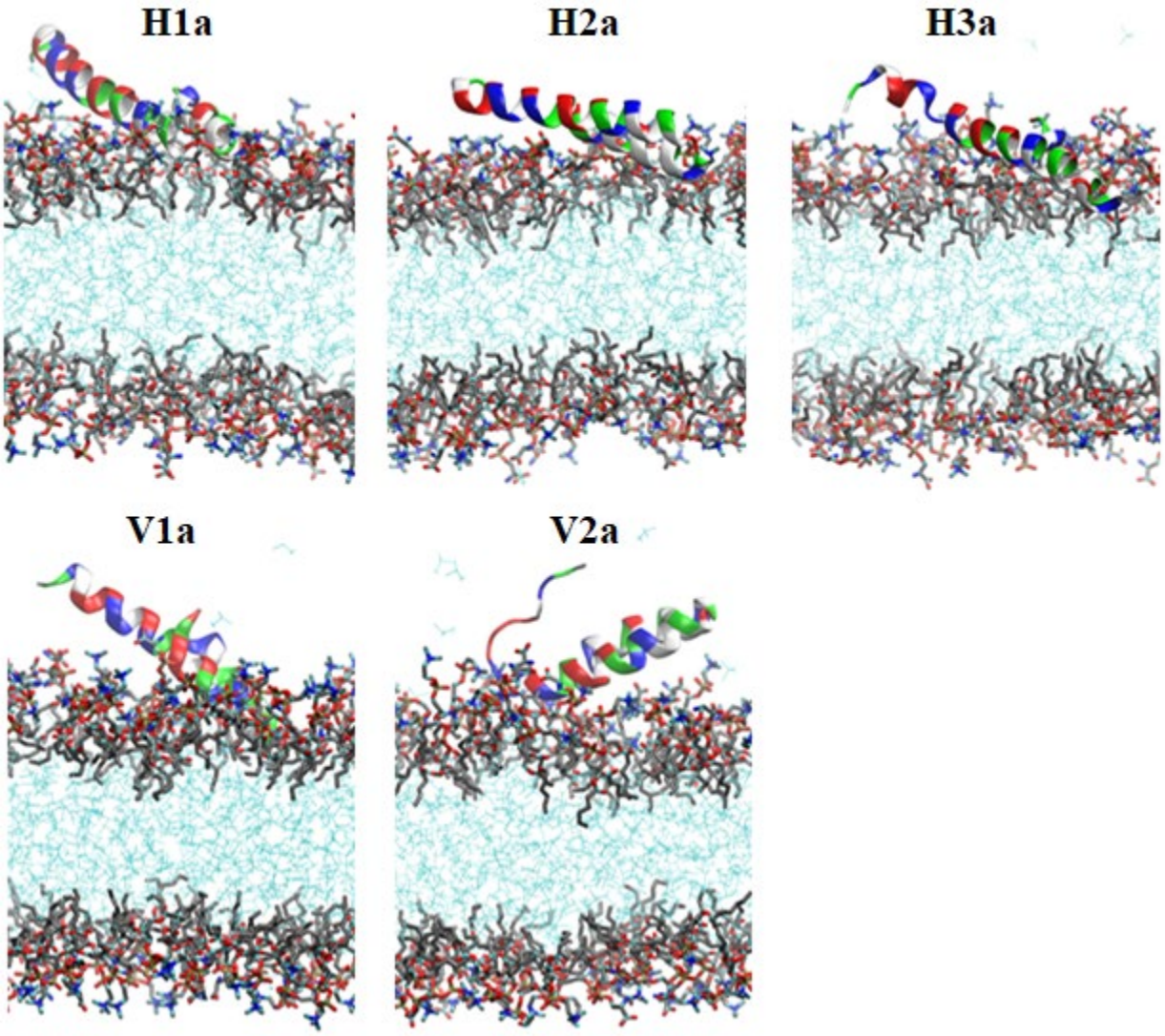

Figure S11. This shows the final bound states of the HMMM simulations. It is possible to see a slight difference in the angle of the α_7 helix (the longer helix) between H1 and H2 where the higher angle is on H1 and the lower angle is viewed on H2, and simulation V2 didn't bind properly.

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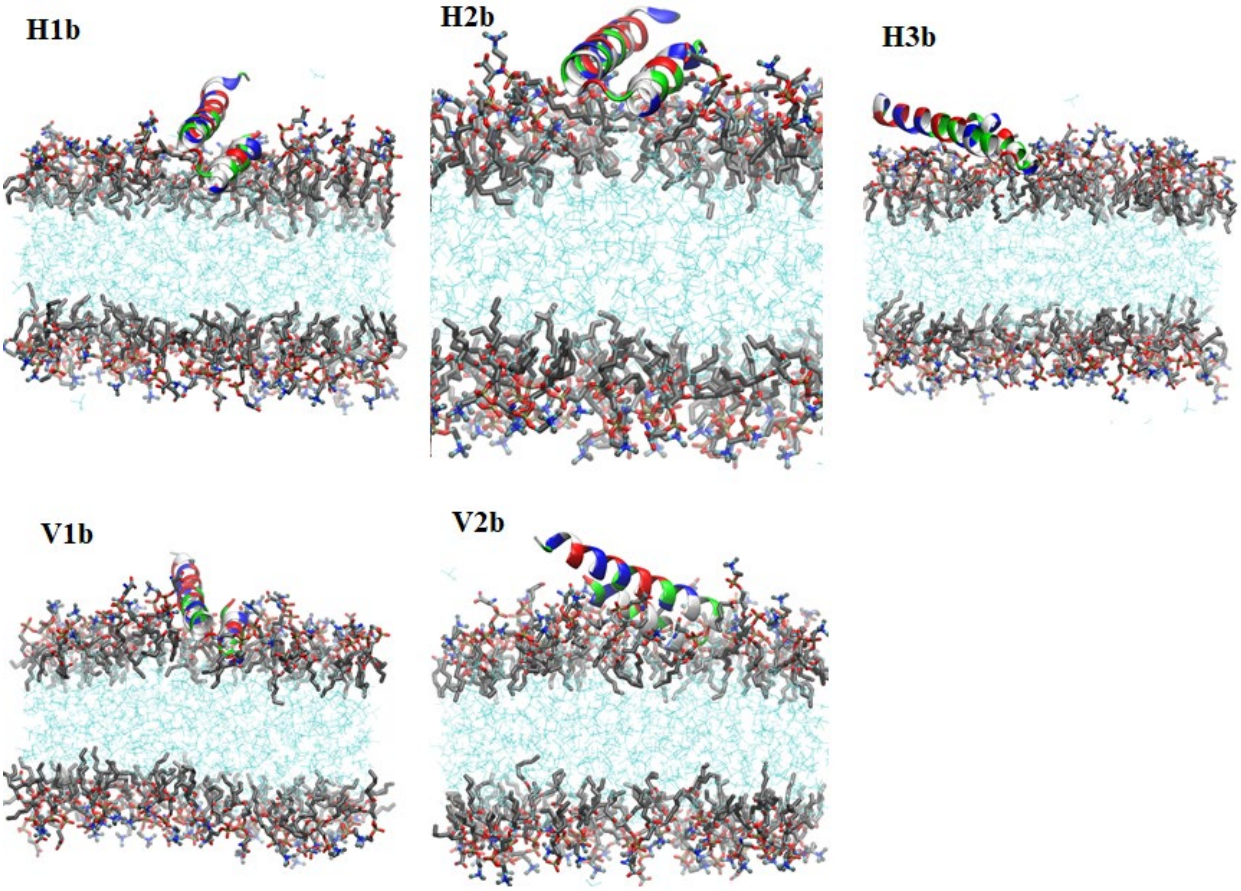

Figure S12. These are the images of the second set of simulations and show different views of the final bound states. The main point is to show that all of these simulations bound to the membrane and to show the orientation of the peptide in the membrane.

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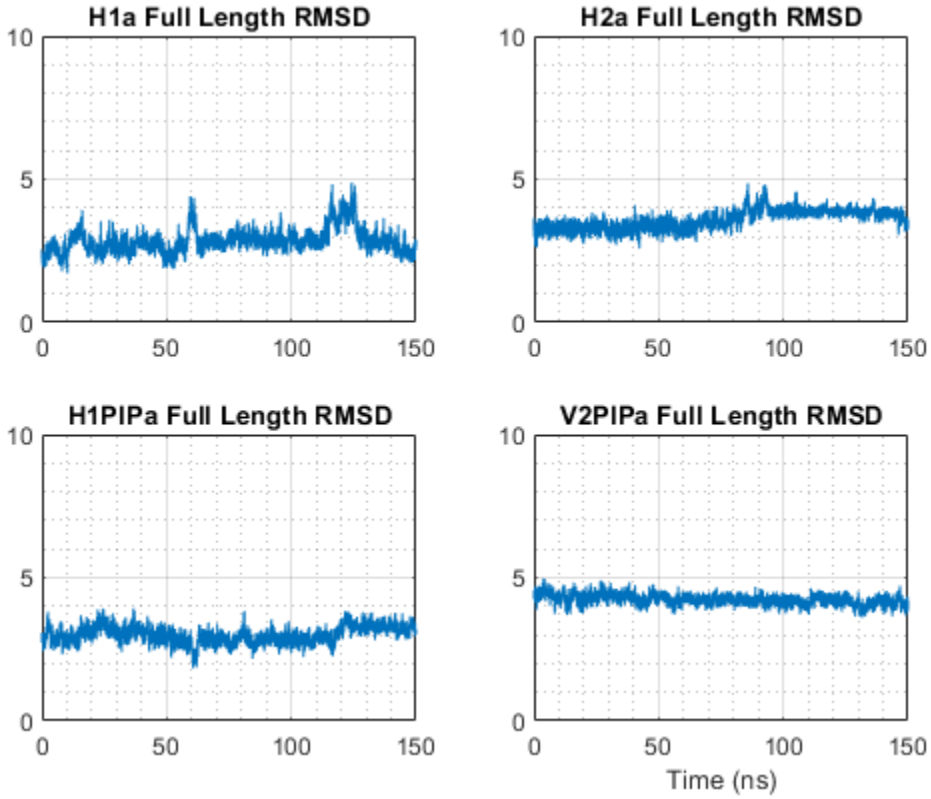

Figure S13. RMSD of the all atom section of the simulations. This indicates that the full length simulations had RMSDs that fell in an expected range.

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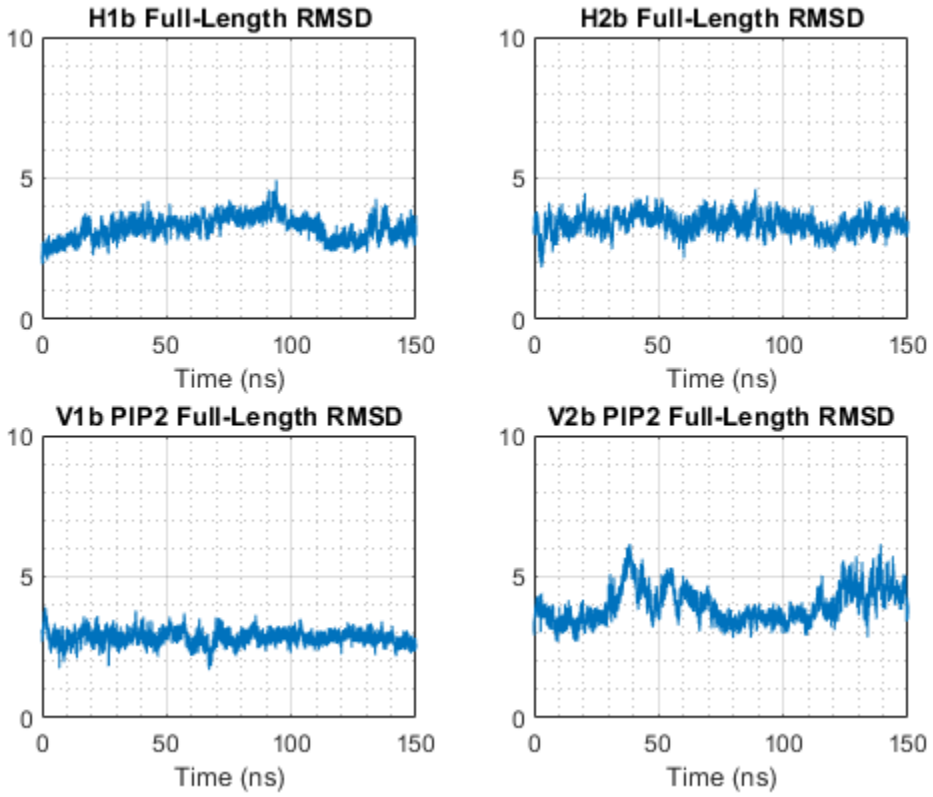

Figure S14. RMSD of the second set of full length trials and shows that the simulations also fell into an expected range and that none of these simulations deviated much above 5 angstroms.

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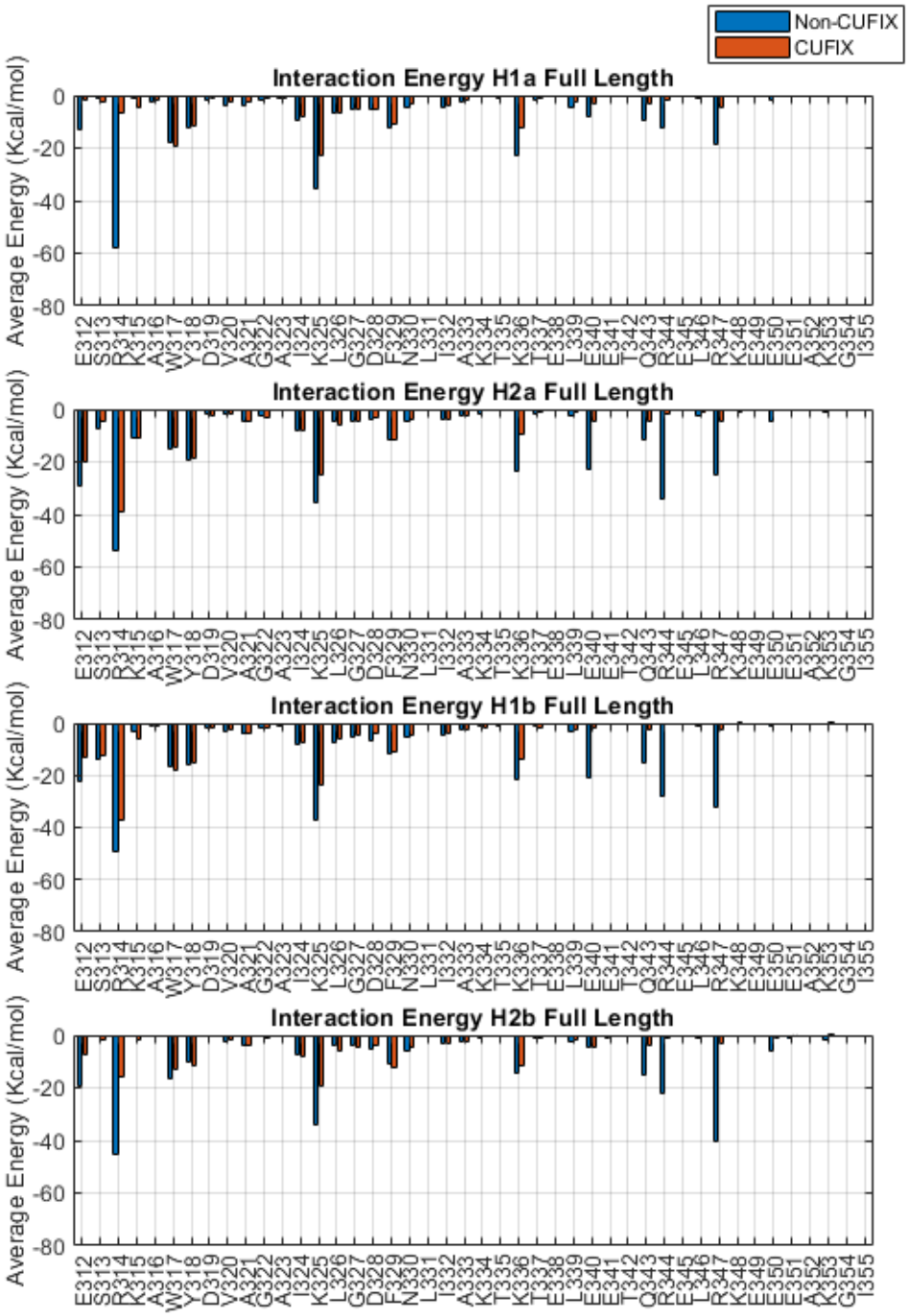

Figure S15. Displays the energy per residue for full length membrane simulations; interestingly, R314, E312, and K325 have much higher energies in H2 than the ones seen in H1, additionally residues R344 and R347 may have higher energy because of closer proximity to the membrane. It can also be seen that the residues on the α_7 helix (beyond K325) of H1 have lower interaction energy compared to the interaction energy of H2 due to the higher angle.

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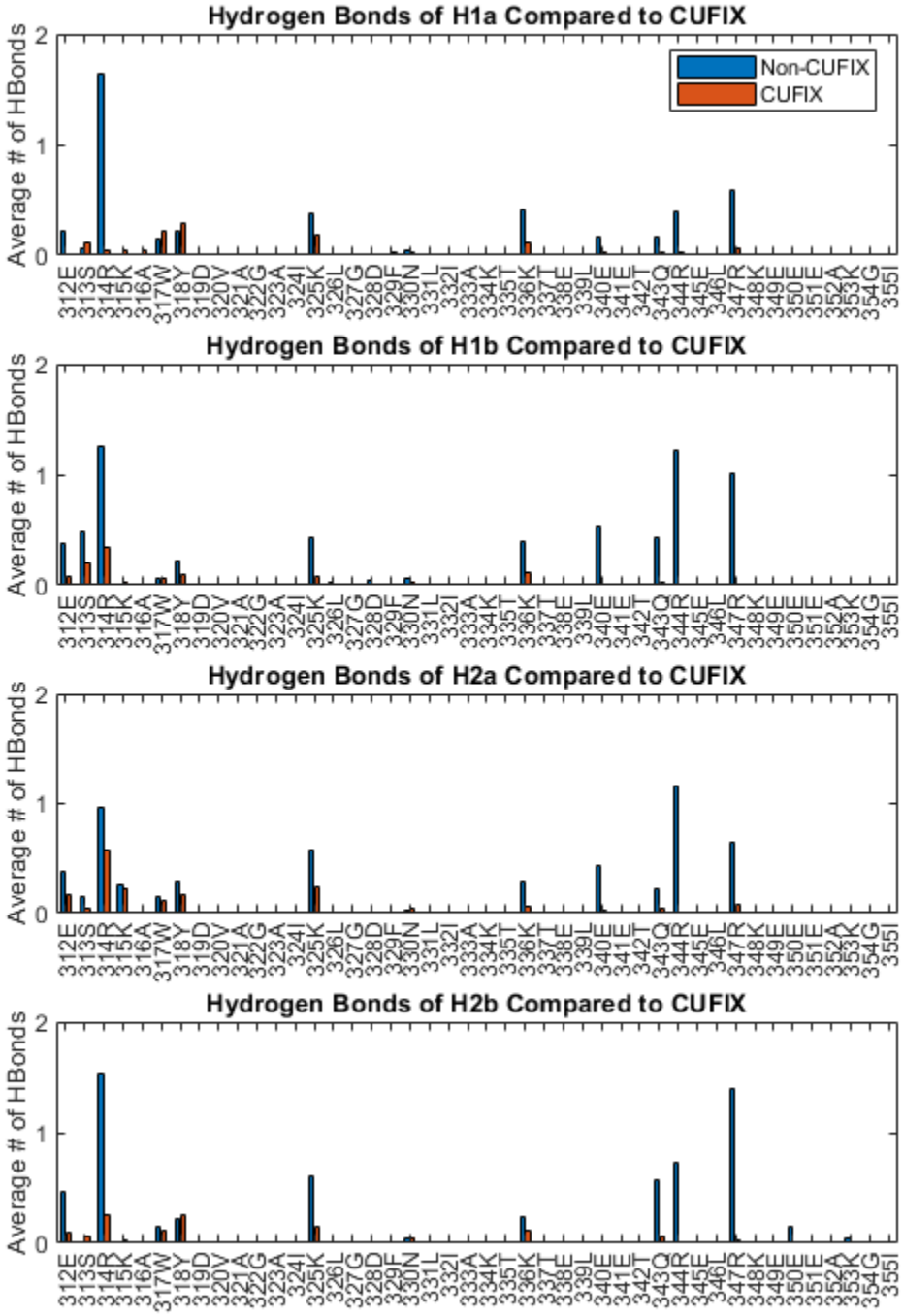

Figure S16. This shows the hydrogen bonding of the two non-PIP Full Length simulations.

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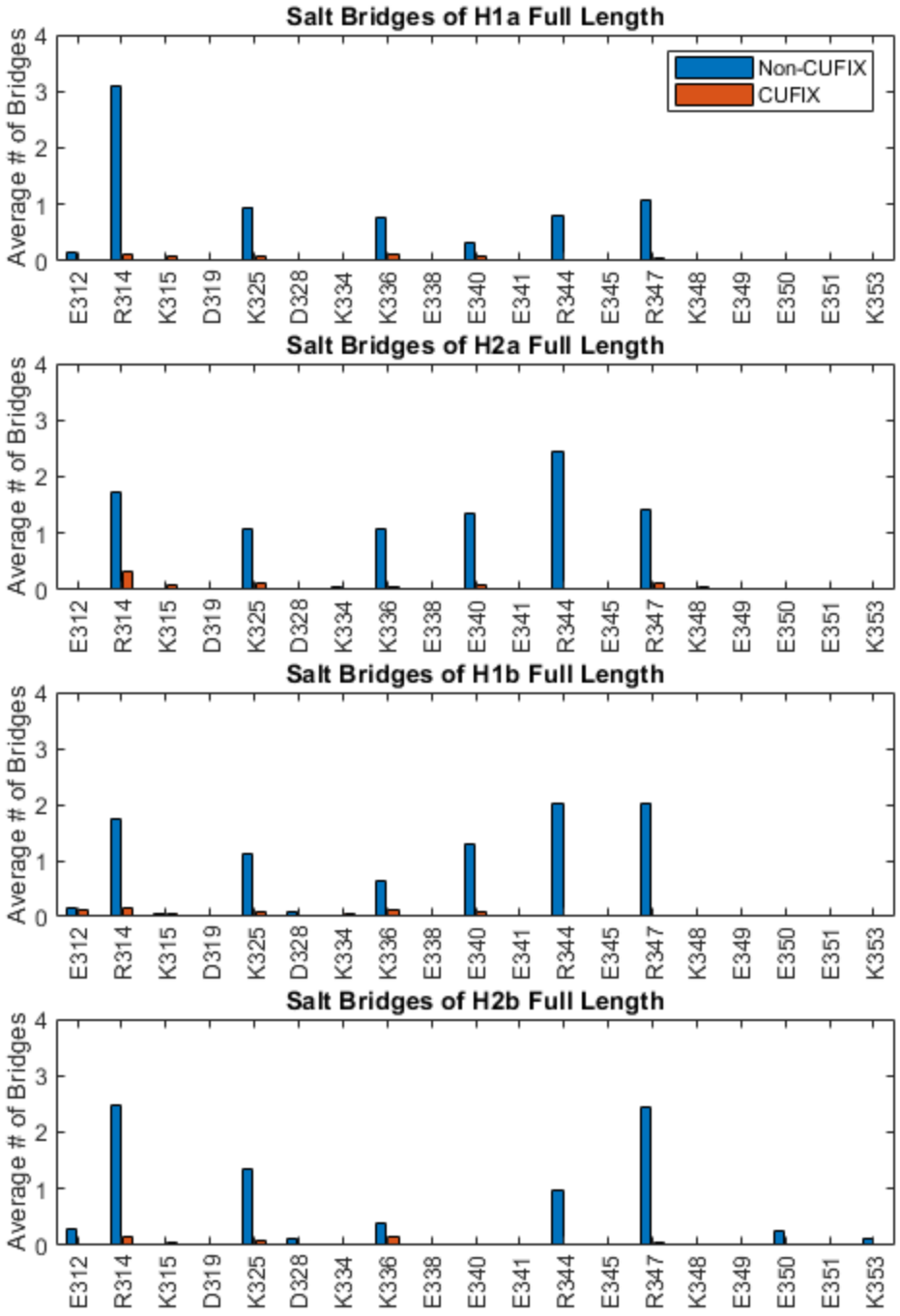

Figure S17. Displays the number of salt bridges for the full length non-PIP2 simulations, simulation H1 had fewer salt bridges compared to simulation H2; additionally, supplementary data in Figure S4 shows that the hydrogen bonds cause the lower angle.

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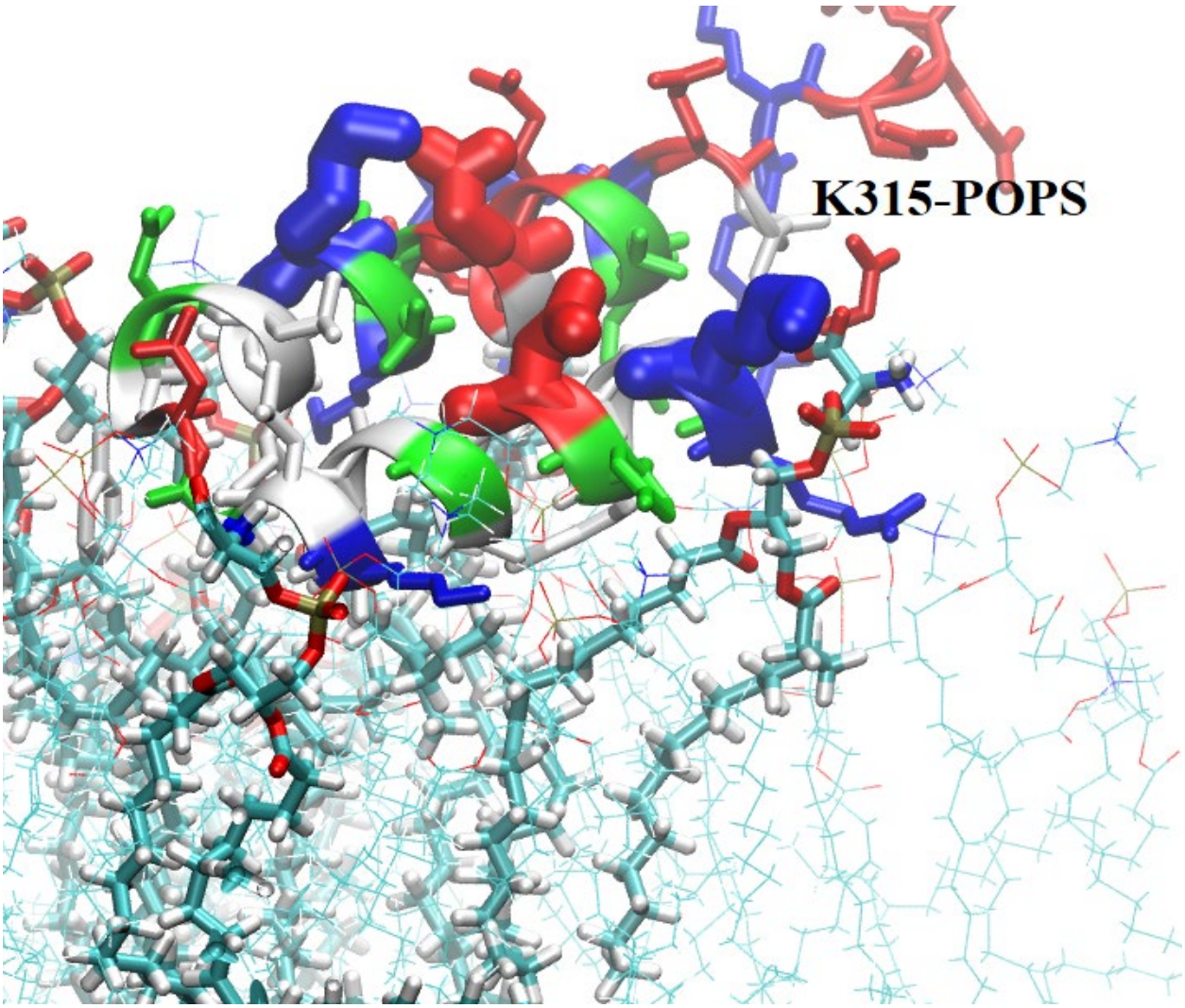

Figure S18. H2a showing the residue K315 interacting with a POPS lipid, much of the time this was visualized it does not appear to be in contact with the POPS lipid, but the salt bridges indicates that there were high numbers for both force fields. H1a was observed to have some interaction with POPS in the second force field consistent with the salt bridges results. While there was a POPS lipid close by in simulation H1b it was not detected in the salt bridges, something similar happened with H2b where there were POPS lipids around but the K315 didn't interact with them, at least in H1a and H2b residue K315 was higher above the membrane from the placement graphs maybe placing them out of reach.

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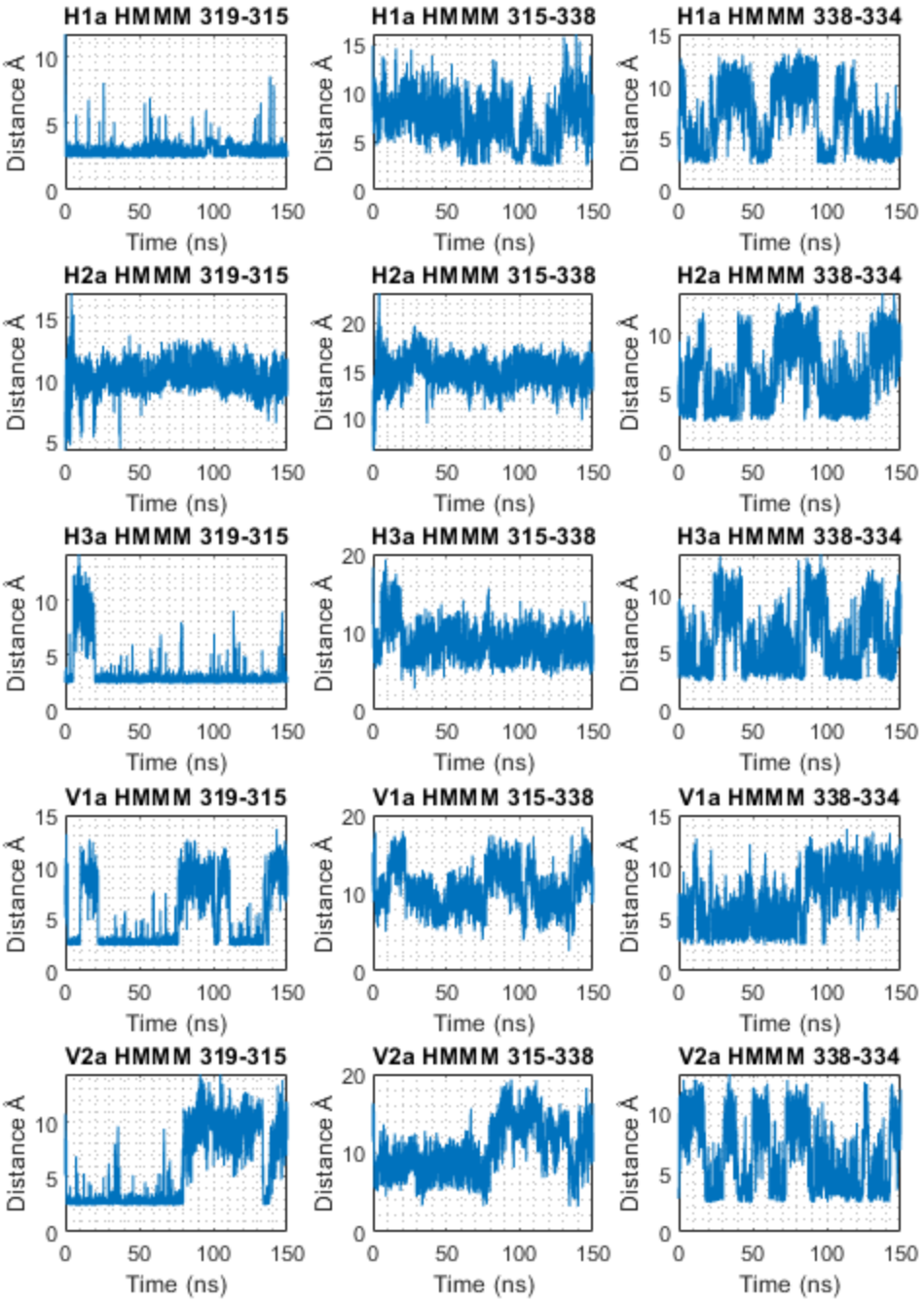

Figure S19. Image of the distance between residues in HMMM simulations that form salt bridges. These are important because these long range water mediated interactions help to stabilize the α_6-α_7 helices, and they are in the range to be important

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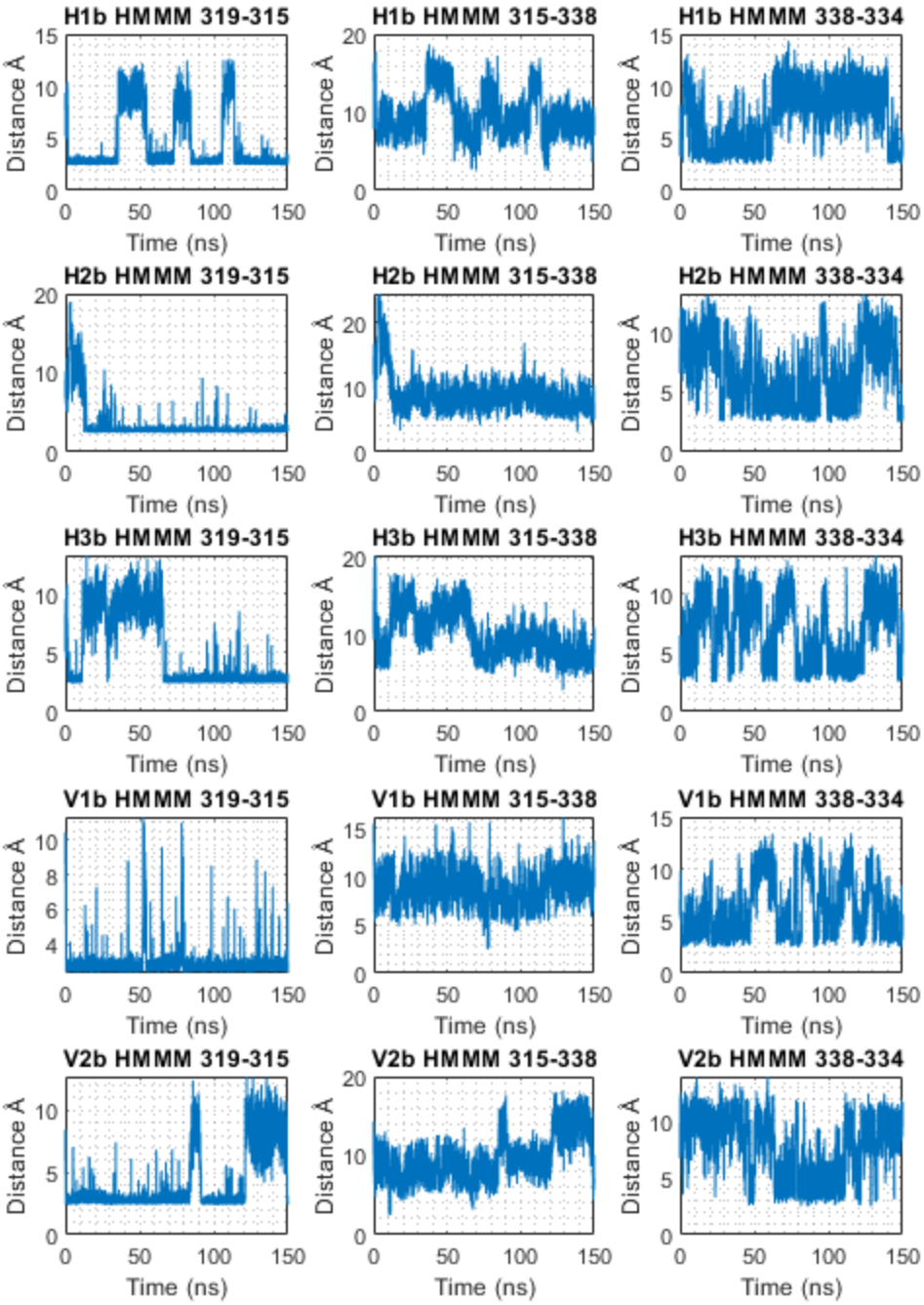

Figure S20. Image showing the second set of distances between many of these simulations were closer than 4 angstroms, but not frequently observed for the bridge between 315-338 that connects the two helices.

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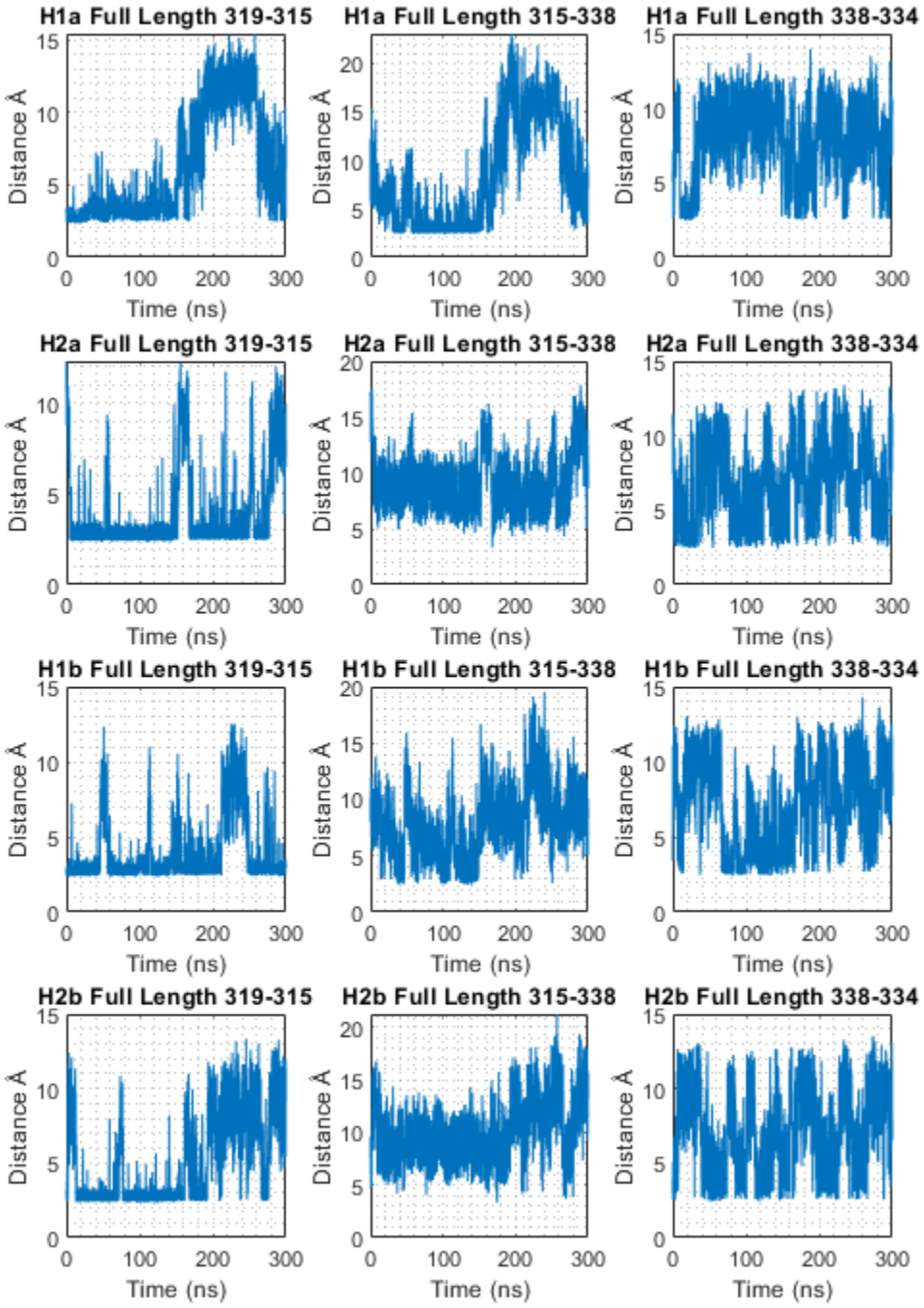

Figure 21. Image showing the full length simulation distances vs time. Again showing a high number of salt bridges for 319-315 and 338-334 and for 315-338 but only in simulation H1a.

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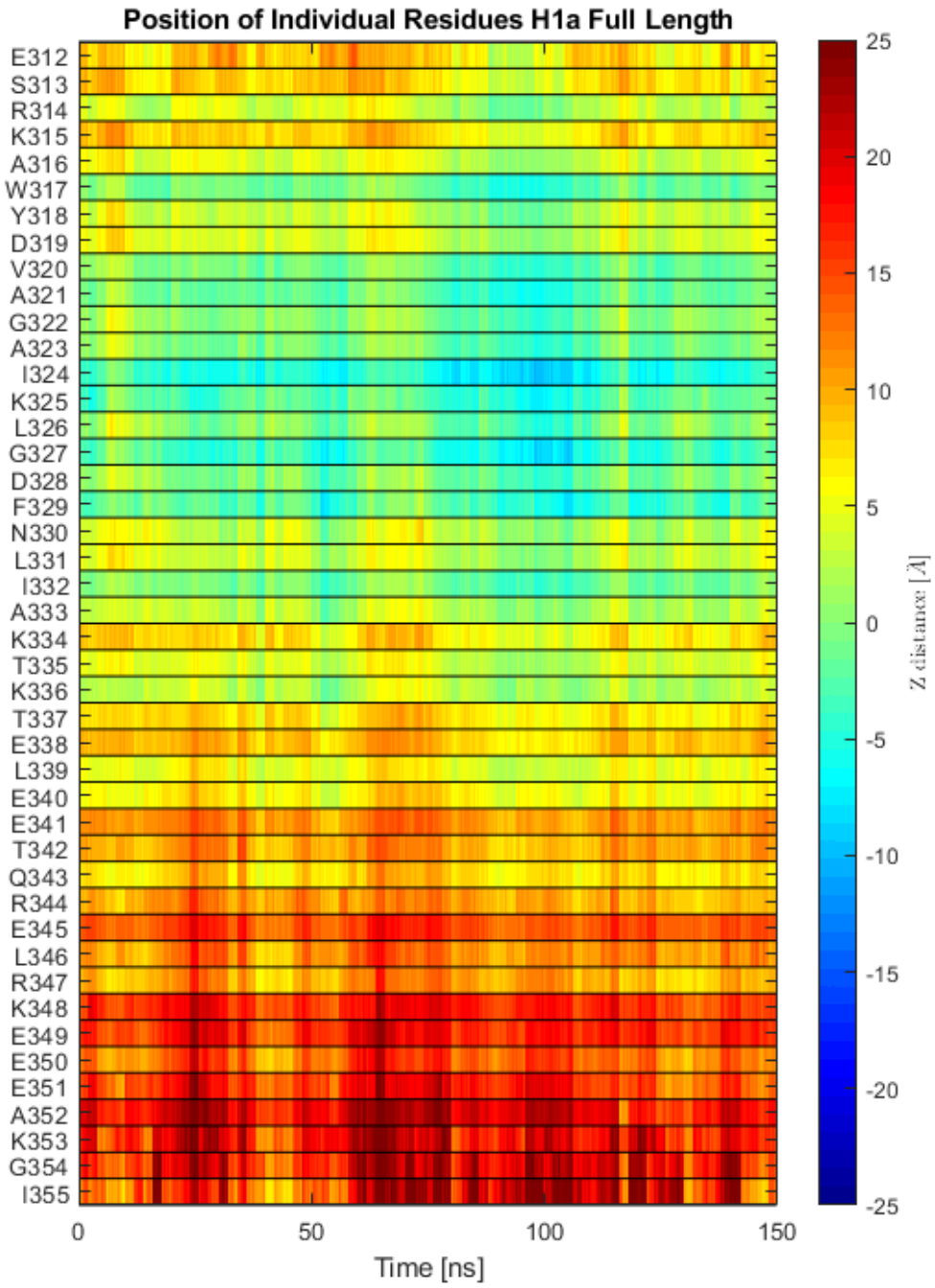

Figure S22. Image describing the average position of individual peptide residues location relative to the average membrane phosphate. It is seen that the peptide begins bound and remains bound throughout the course of the simulaiton, and that the resiudes in the middle are the deepest below the phosphate.

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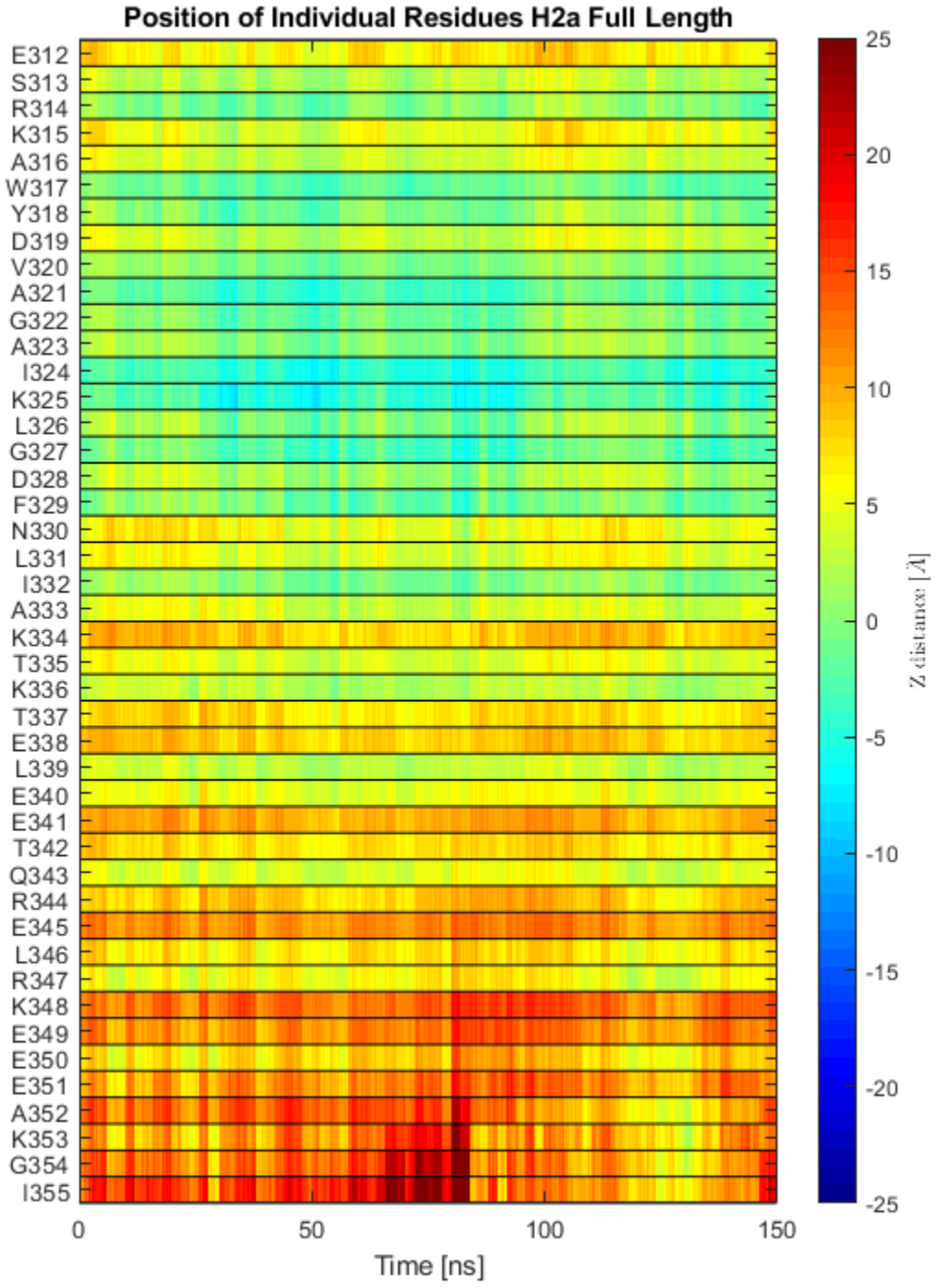

Figure S23. Image describing the average position of individual peptide residues location relative to the average membrane phosphate. Residues in middle of the peptide are deepest.

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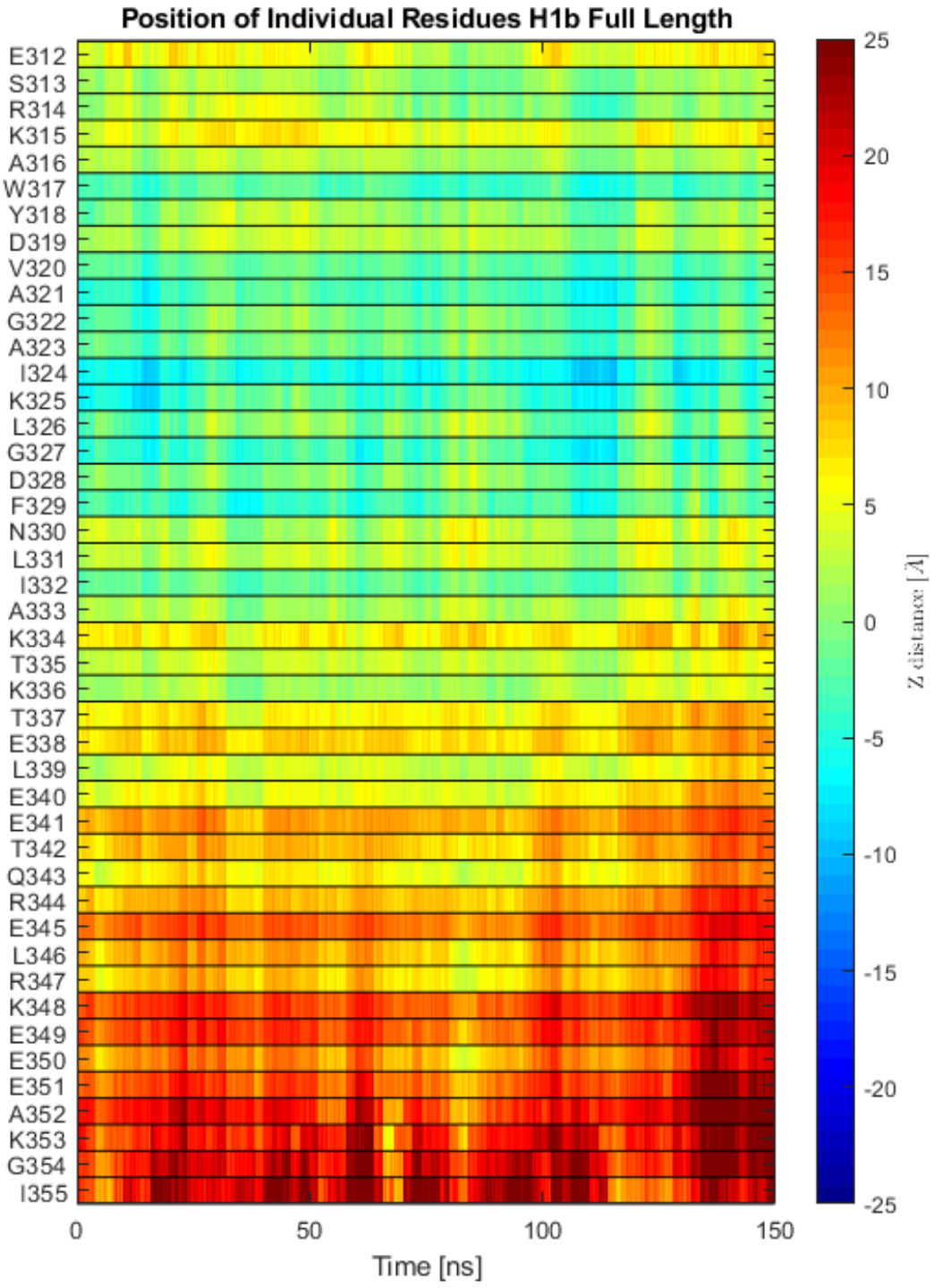

Figure S24. Image showing the placement of H1b, this shows follows the same trends as the other simulaitons except that K315 is a little deeper than in other simulations.

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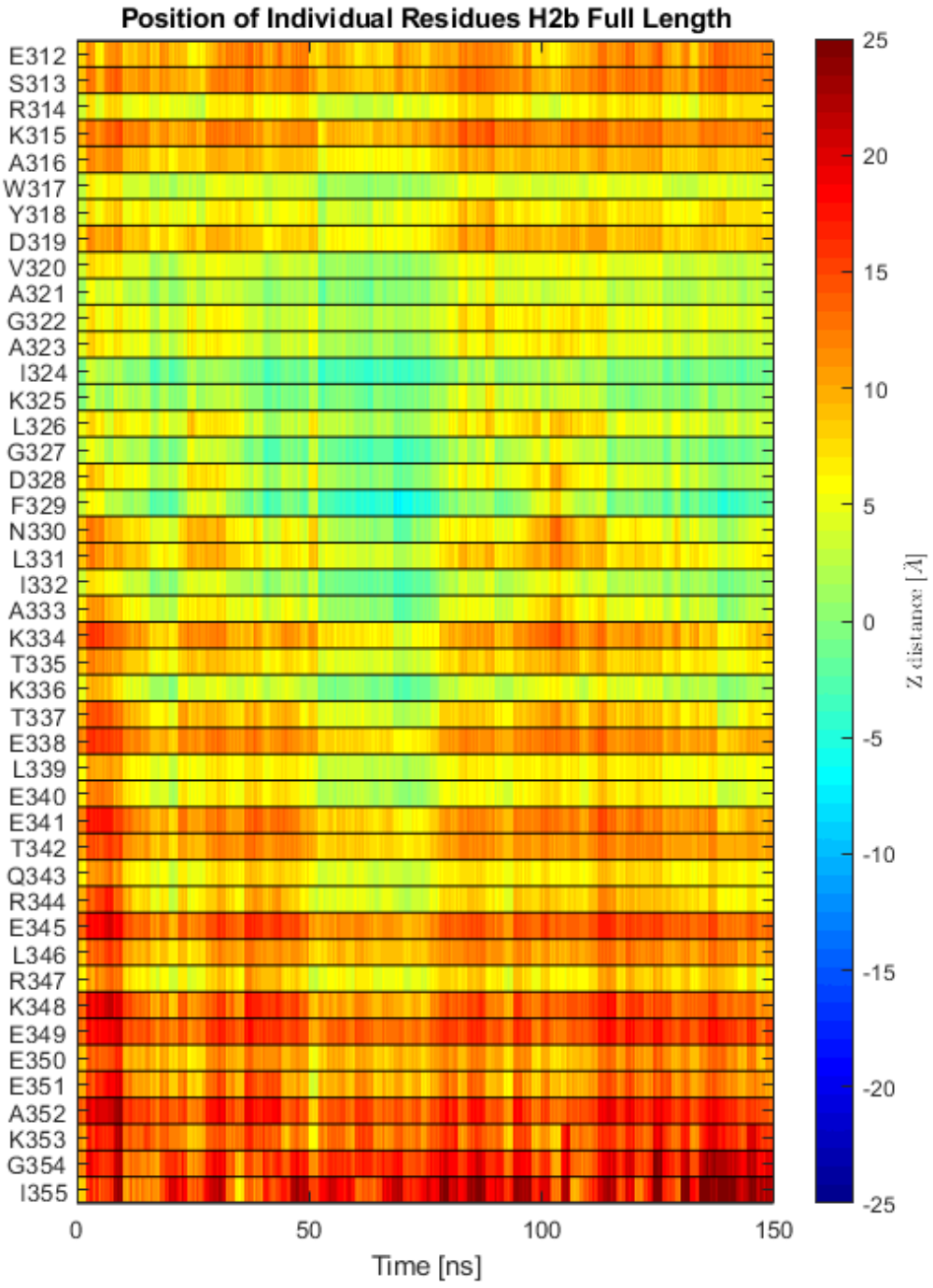

Figure S25. Image showing the placement of the H2b simulation following the same trends as the other simualtions except that k315 is not as deep possibly explaining why this simualitons had less interaction eneryg on residue K315 even with POPS lipids close by to interact with.

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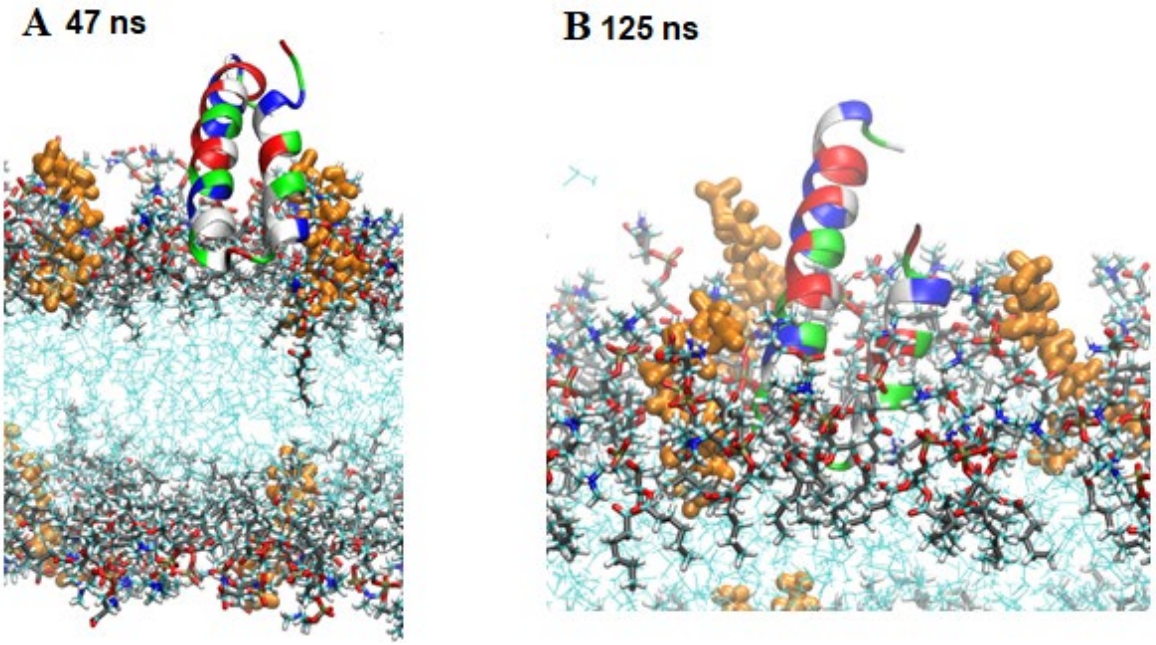

Figure S26. (a) simulation V2a, the only simulation that bound with the α_7 helix deepest is depicted moments before binding, (b) simulation H1a, shows the bound state with PIP lipids interacting with the exposed residues that are inaccessible to the main membrane lipids.

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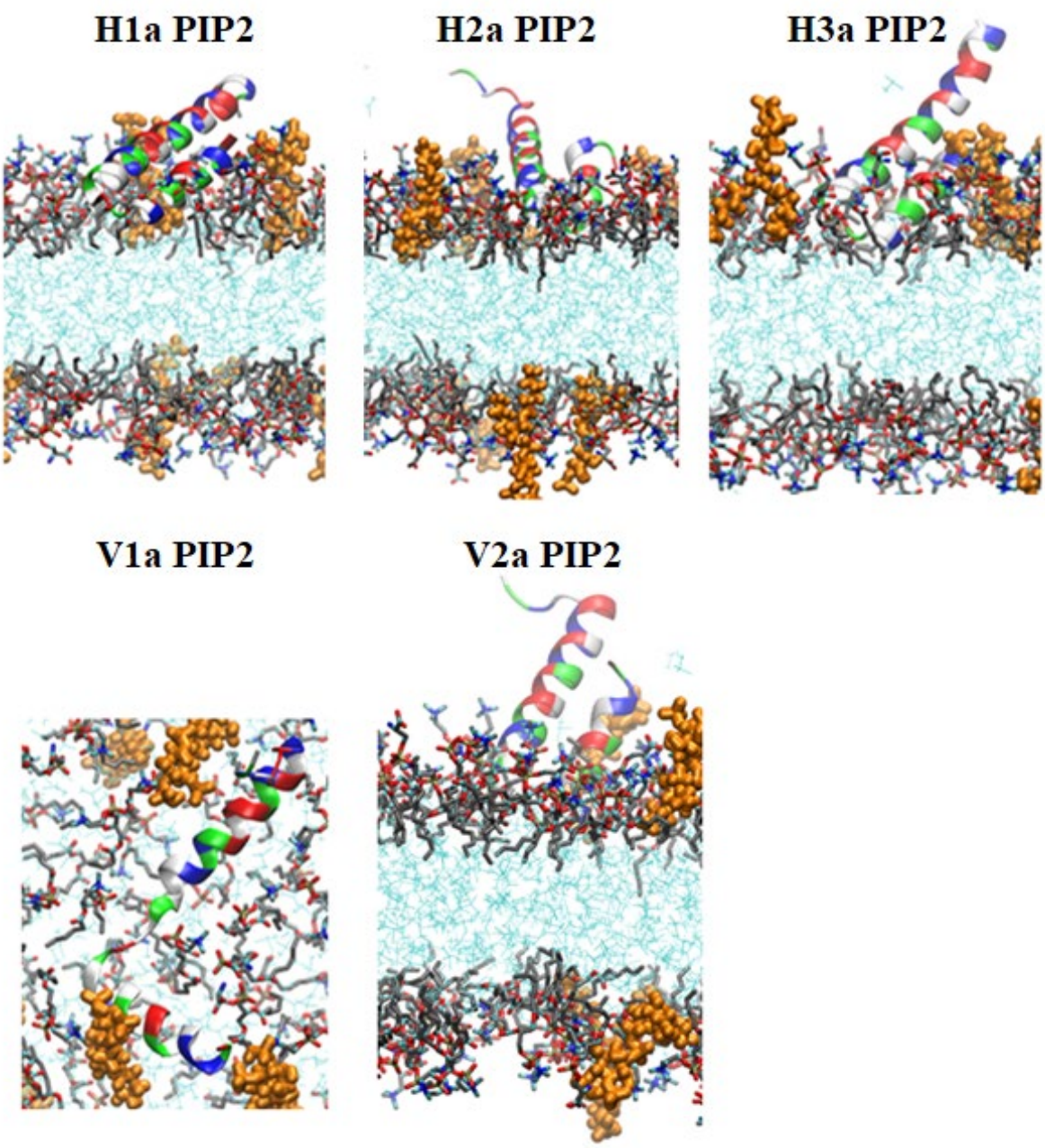

Figure S27. Shows the final bound states for the HMMM simulations of PIP2 lipids. These generally have higher angles compared to the lower angles seen in the non-pip trials, likely due to the charges of the PIP2 lipids. The trial V1 PIP2 had the α_6 and α_7 helices split apart.

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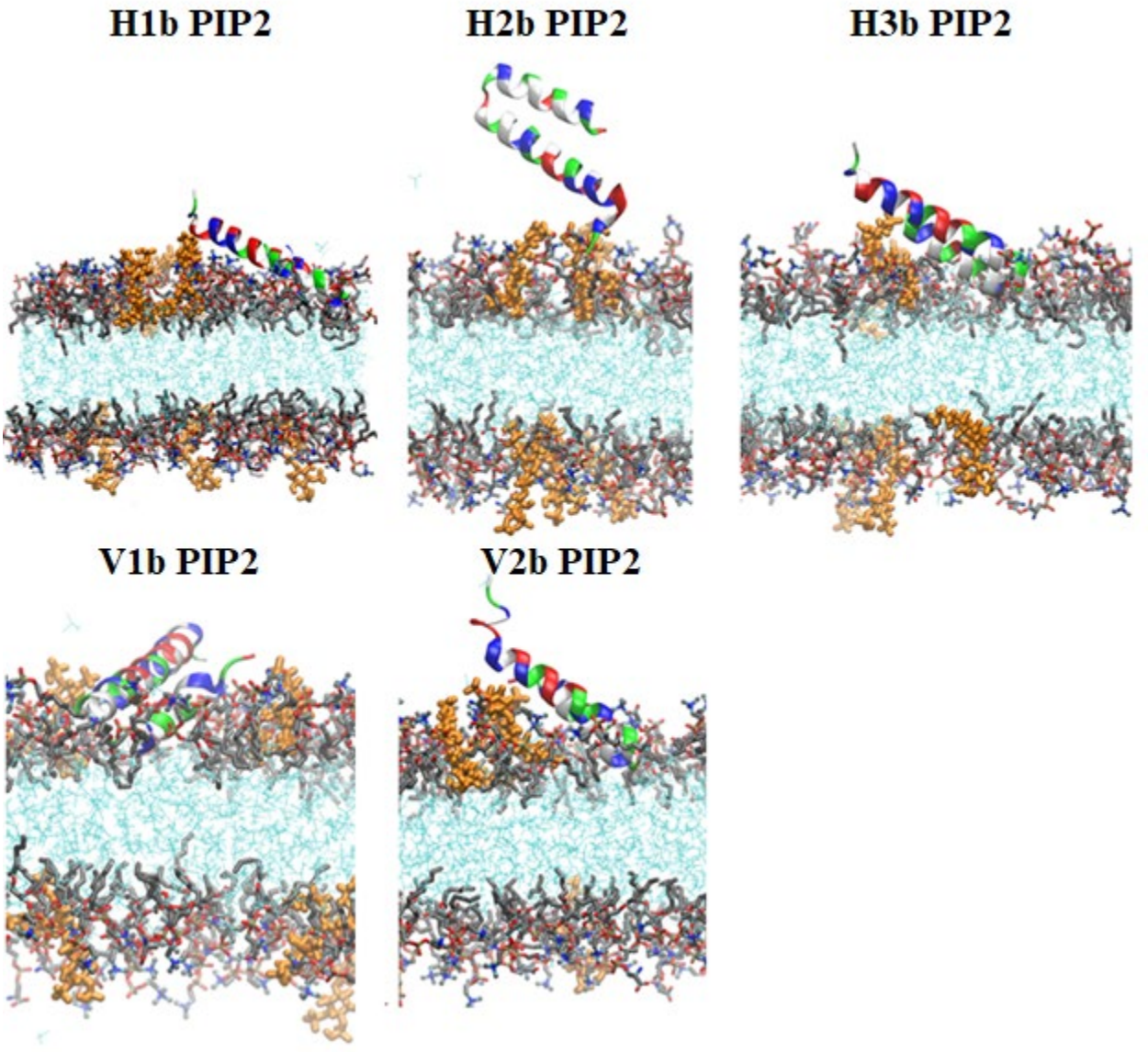

Figure S28. Image showing the final bound states of the PIP2 b trials. The results indicate that all of the simulations bound to the membrane except one of them which was H2b.

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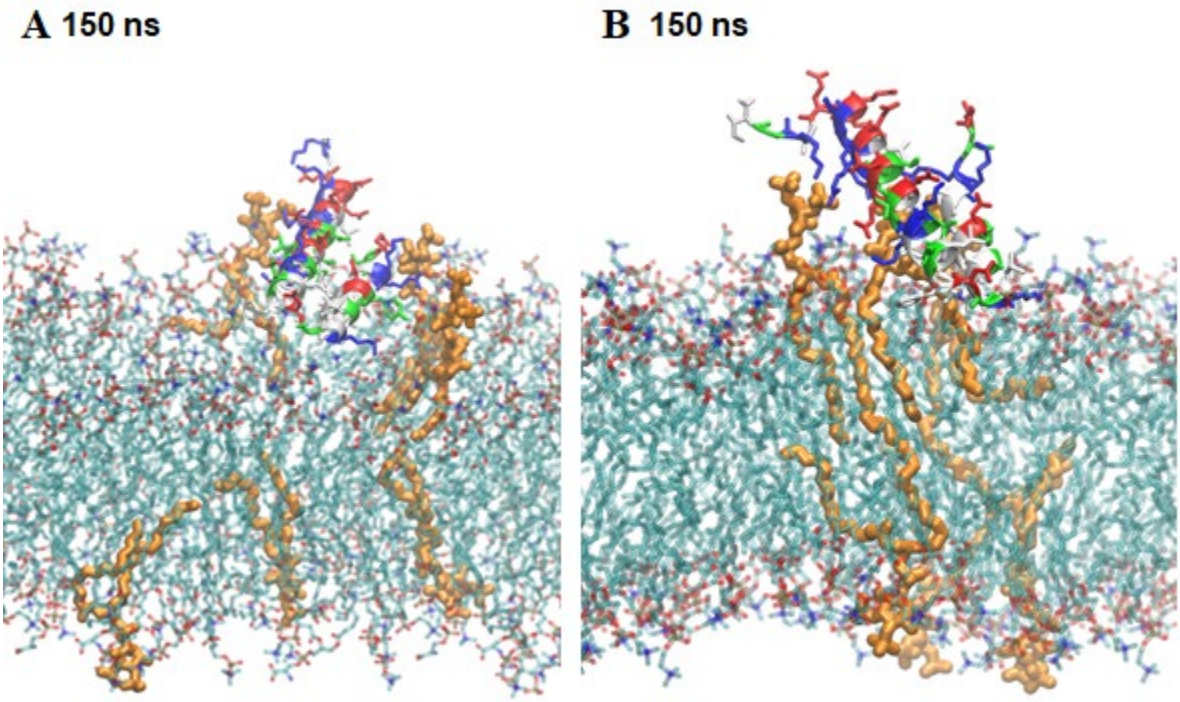

Figure S29. Left shows the placement of the peptide during the H1a simulation with PIP lipids on both sides of the peptide, and the right shows the peptide placement during simulation V2a with PIP lipids directly underneath the peptide. These are the images of the equilibrated full length membranes with PIP2 peptide interactions.

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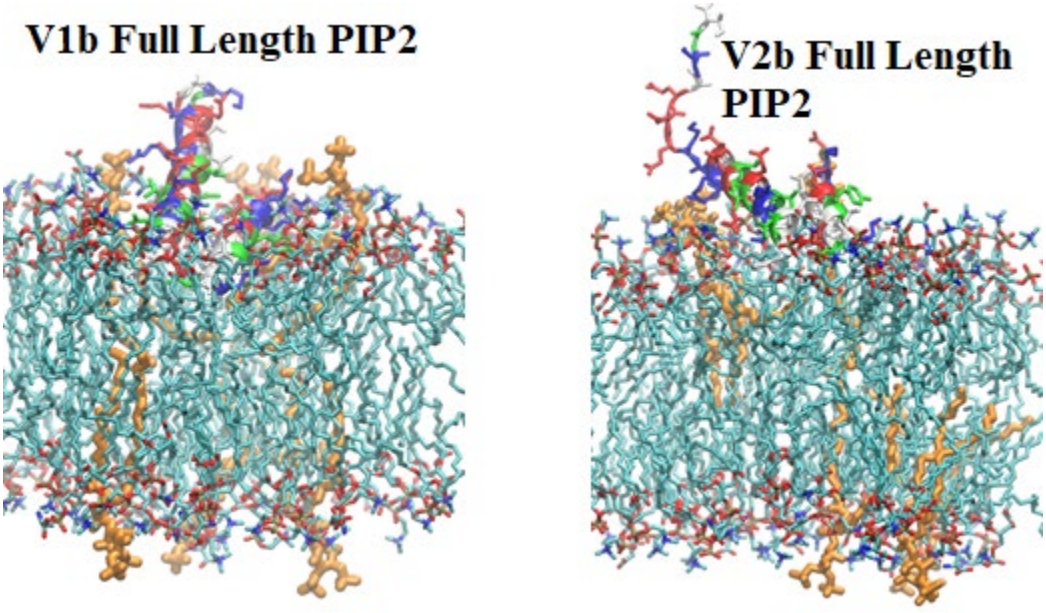

Figure S30. This image shows the two PIP2 simulations with the PIP2 lipids interacting on the sides of the membrane. The main point is that these simulations were chosen because they had PIP2 lipids interacting with a portion of the membrane.

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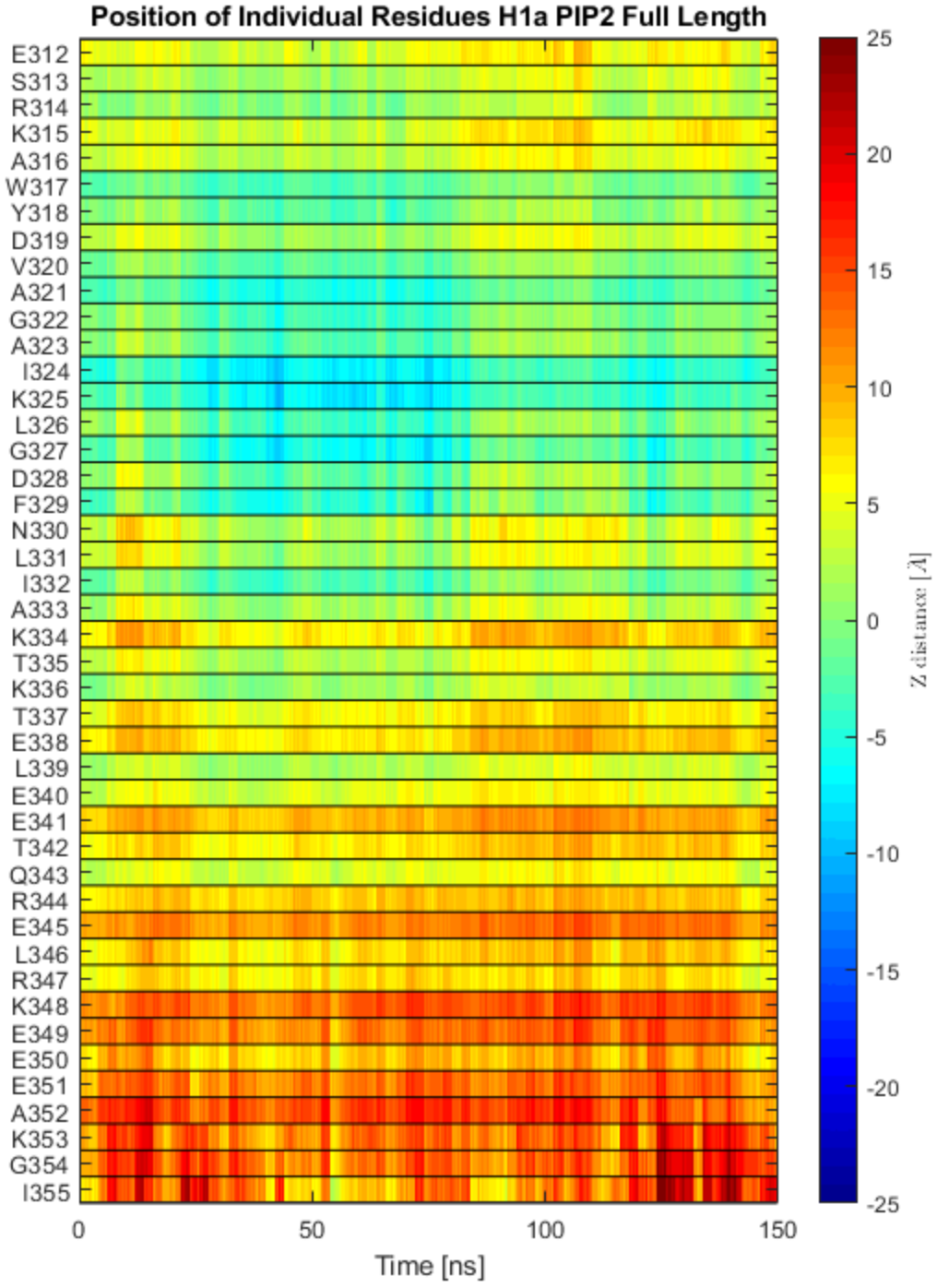

Figure S31. Image describing the average position of individual peptide residues location relative to the average membrane phosphate. The peptide begins in the full length membrane fully bound to the membrane. The deepest residues on the peptide are between V320 to F329.

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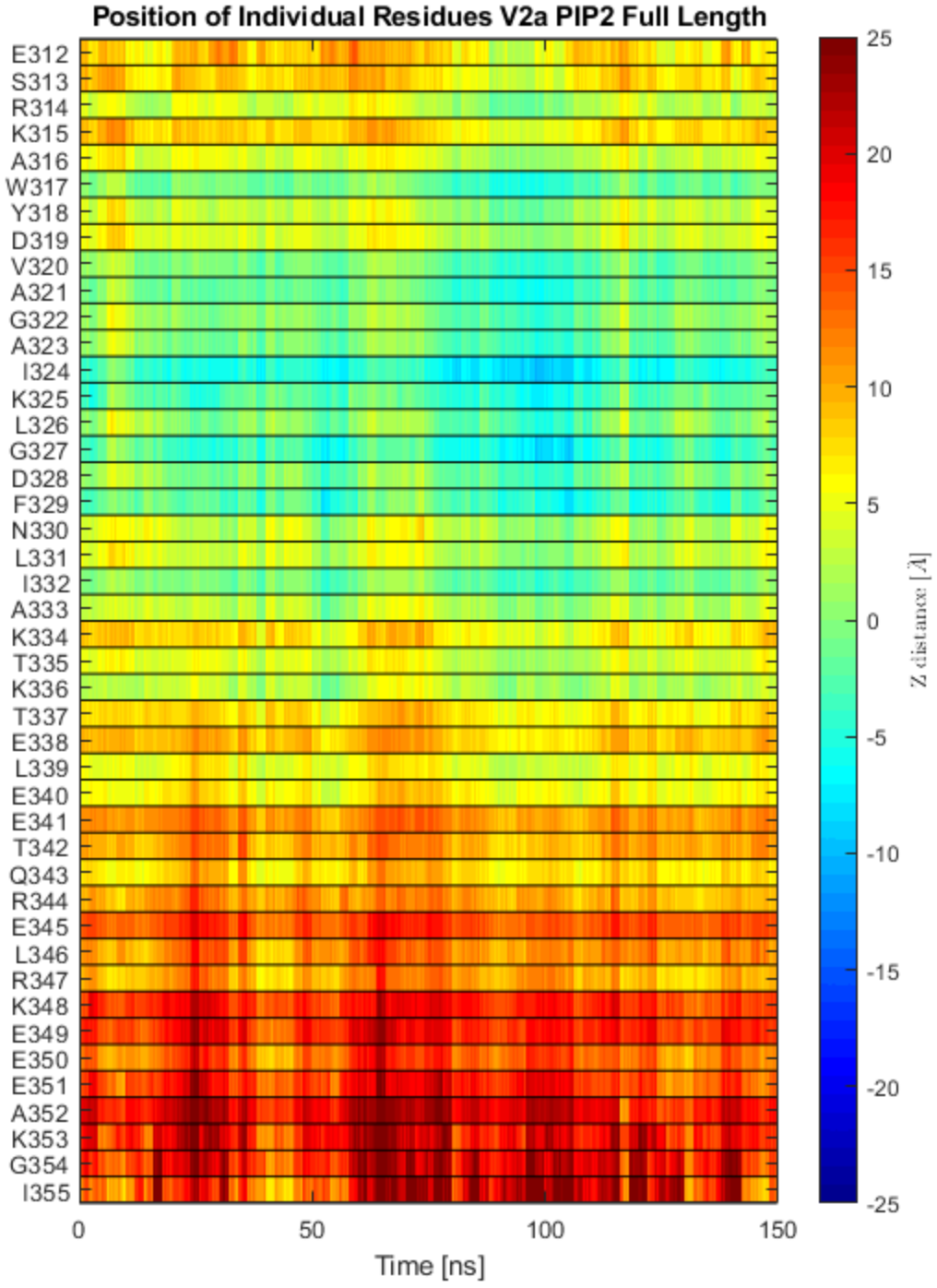

Figure S32. Image describing the average position of individual peptide residues location relative to the average membrane phosphate. This shows thtat the Full length simulation begins with the peptide bound to the membrane and that the deepest residues on the peptide are between V320 to F329.

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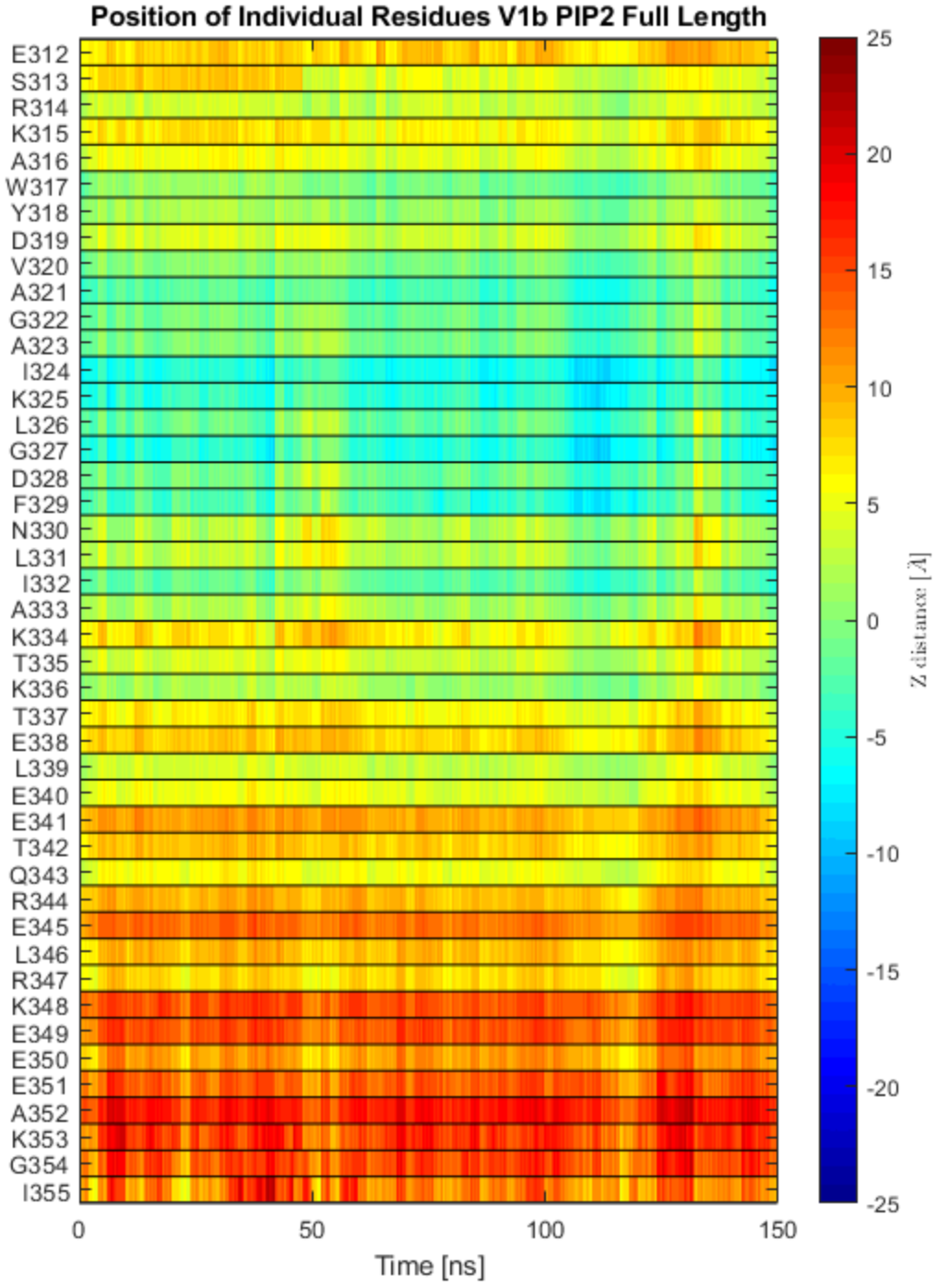

Figure S33. Image showing the placement of all the amino acids of the peptide over time, and how there is a range of residues that are deeply imbeded into the memrbane and some that fluctuate in position such as K315 that is deeper in this simulaiont but higher in other simulaoints showing some variability in plaement by different bound states.

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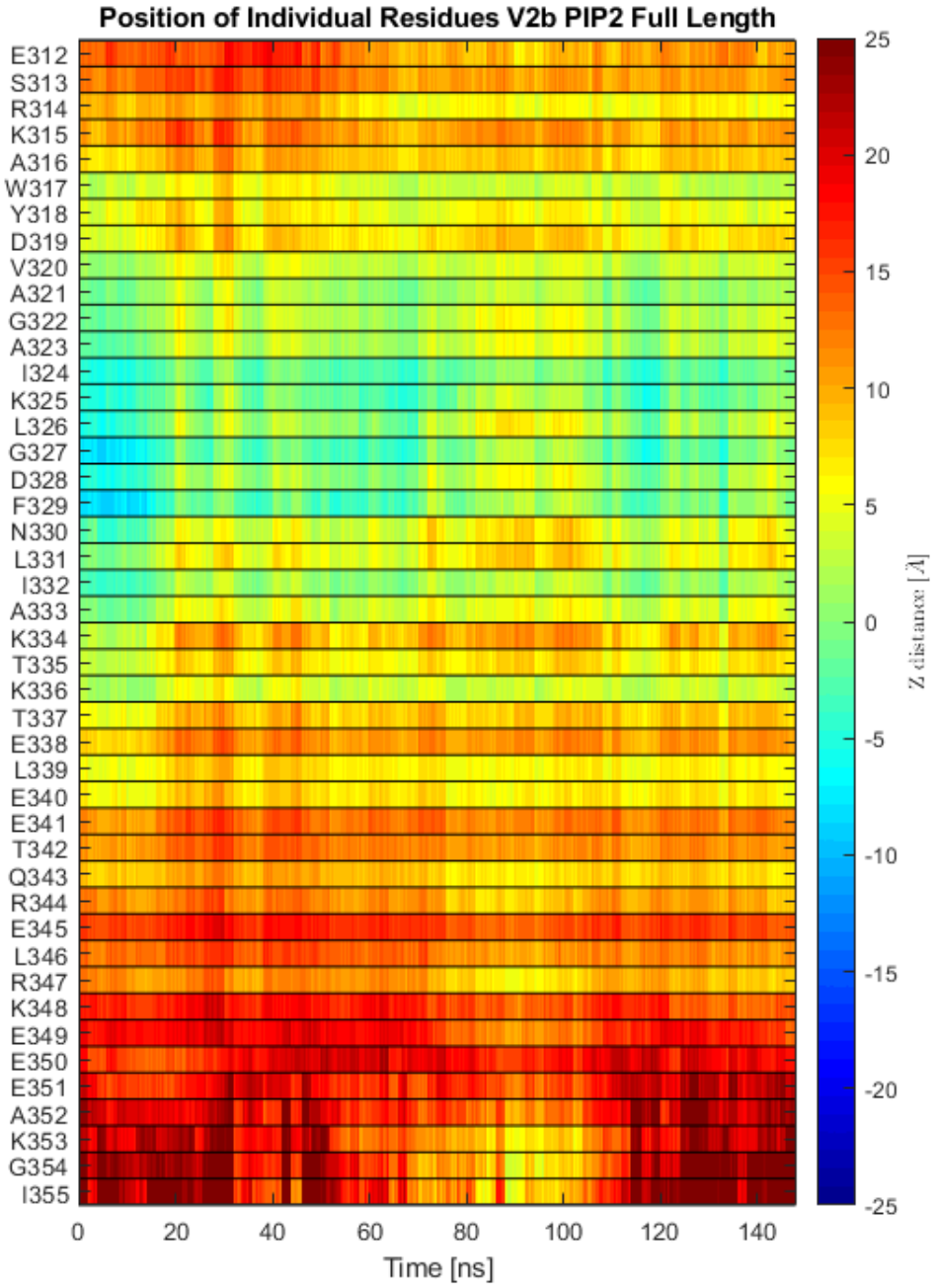

Figure S34. This image shows the placement of different amino acid residues over time, and shows that the amino acid residue K315 is higher than it is observed to be in the simulaion V1b PIP2 and this may account for the differeence in interaction energy of this amino acid residue.

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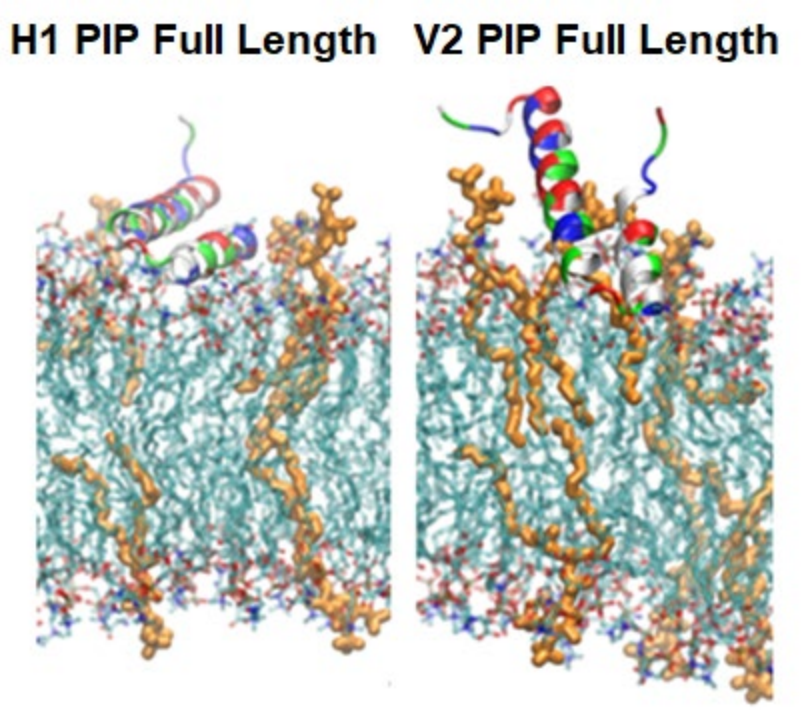

Figure S35. This image shows the final bound states of the all atom simulations, and the main point is that the PIP2 lipids are interacting with the peptide in the upper region of the peptide.

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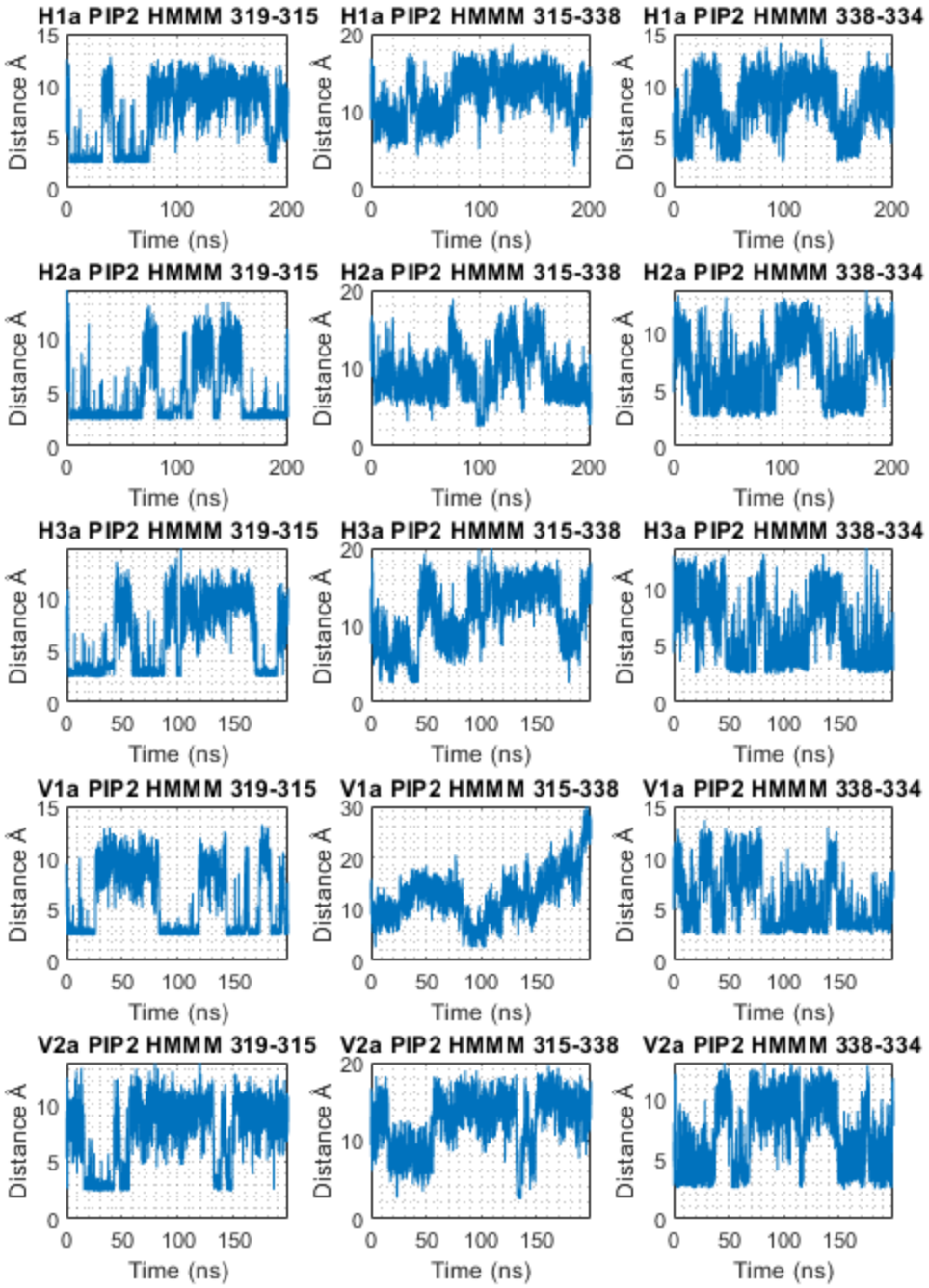

Figure S36. This figure shows the distances between the key amino acid residues on the top of the peptide, and shows no interaction between 315-338 but there is certainly some interaction below 4 angstroms for the other bridges, additionally V1a split apart between the α_6-α_7 helices.

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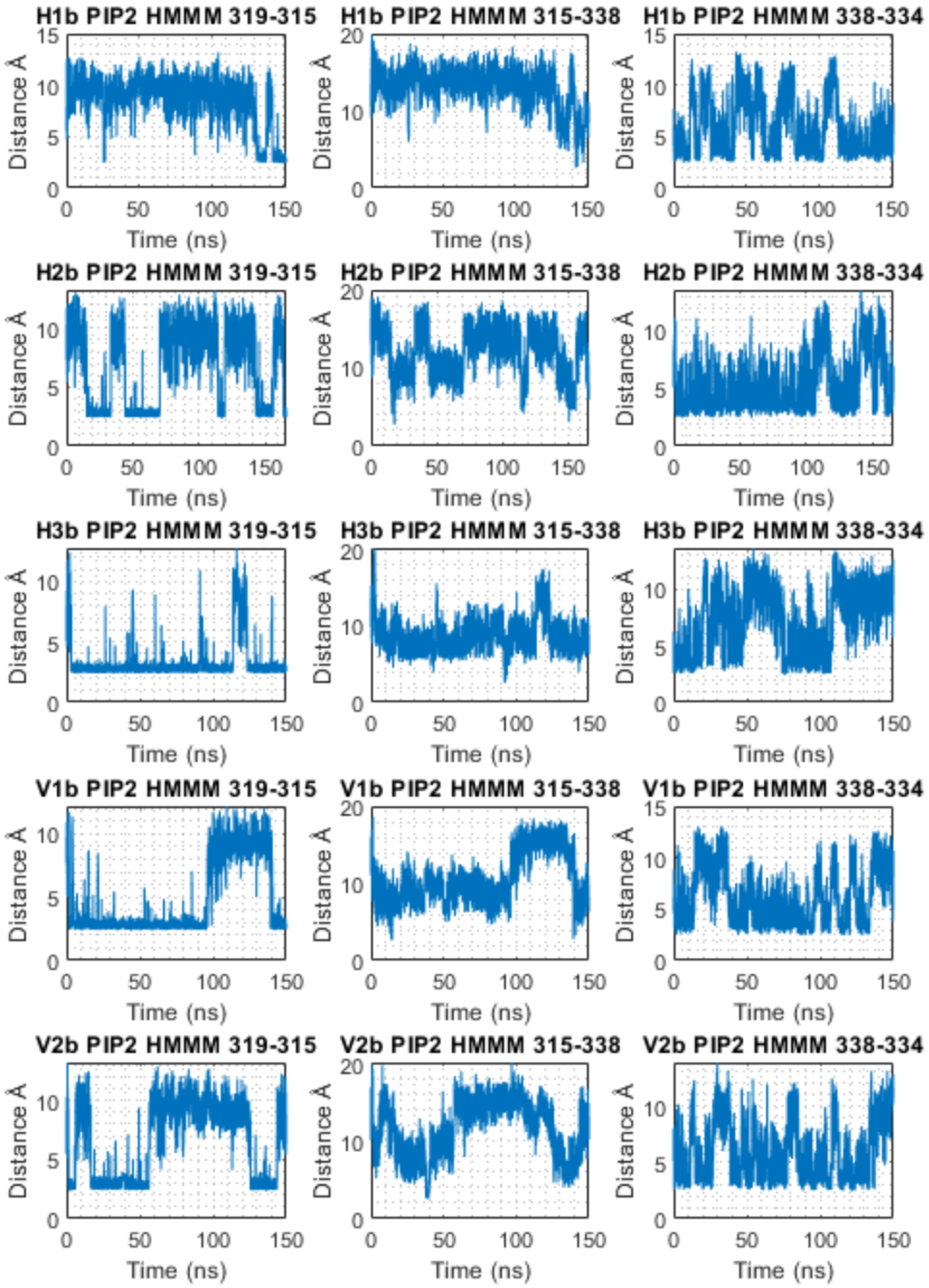

Figure 37. Image of the distance between the key residues that stabalize the membrane binding region from HMMM simulation. These are important because these long range water mediated interactions help to stabilize the α_6-α_7 helices, and they are in the range to be important.

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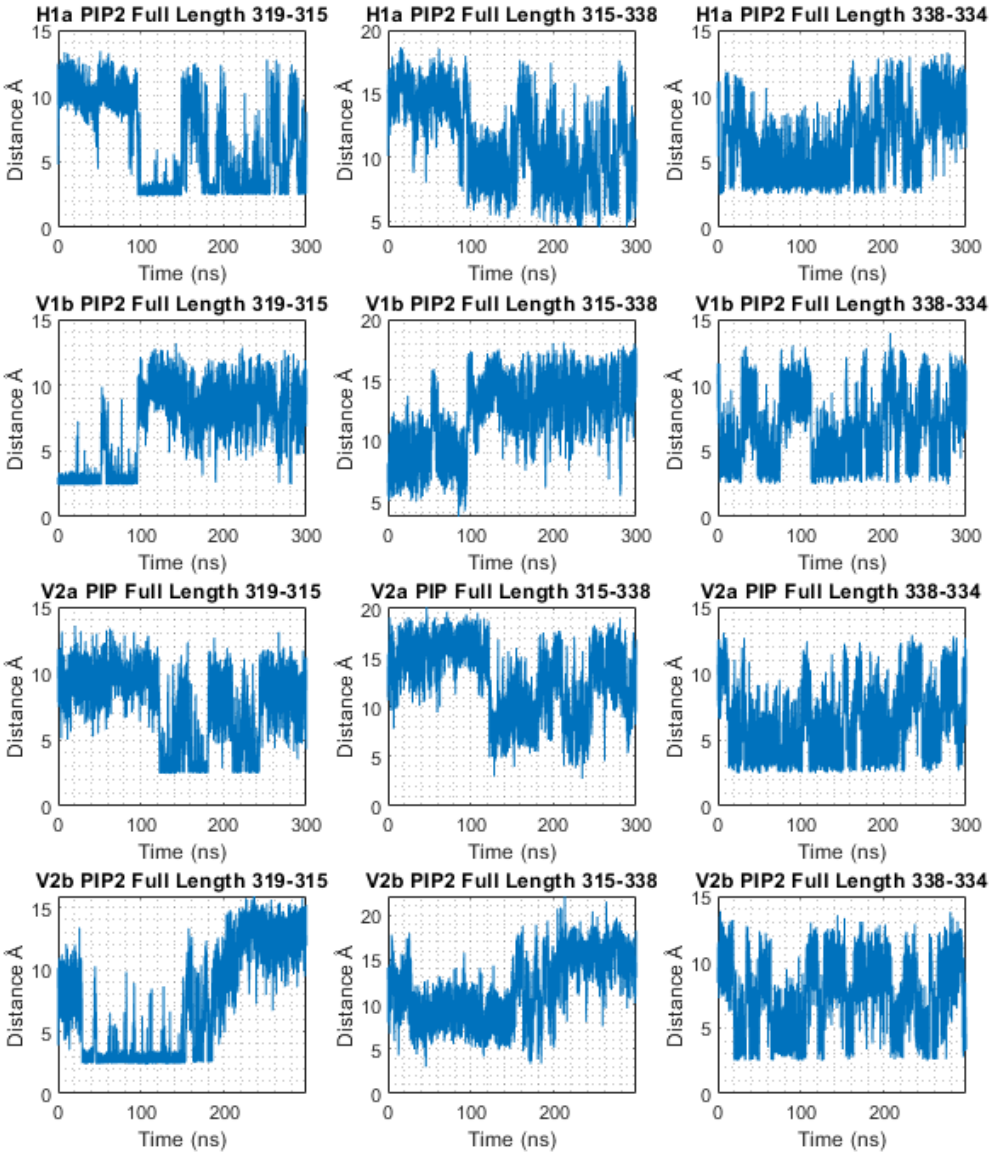

Figure S38. This is the full length simulations showing similar results with no interaction between the 315-338 bridge. The main point was to quantify how often the different residues were in contact, and it is clear that the other bridges are frequently close enough for interaction except for 315-338.

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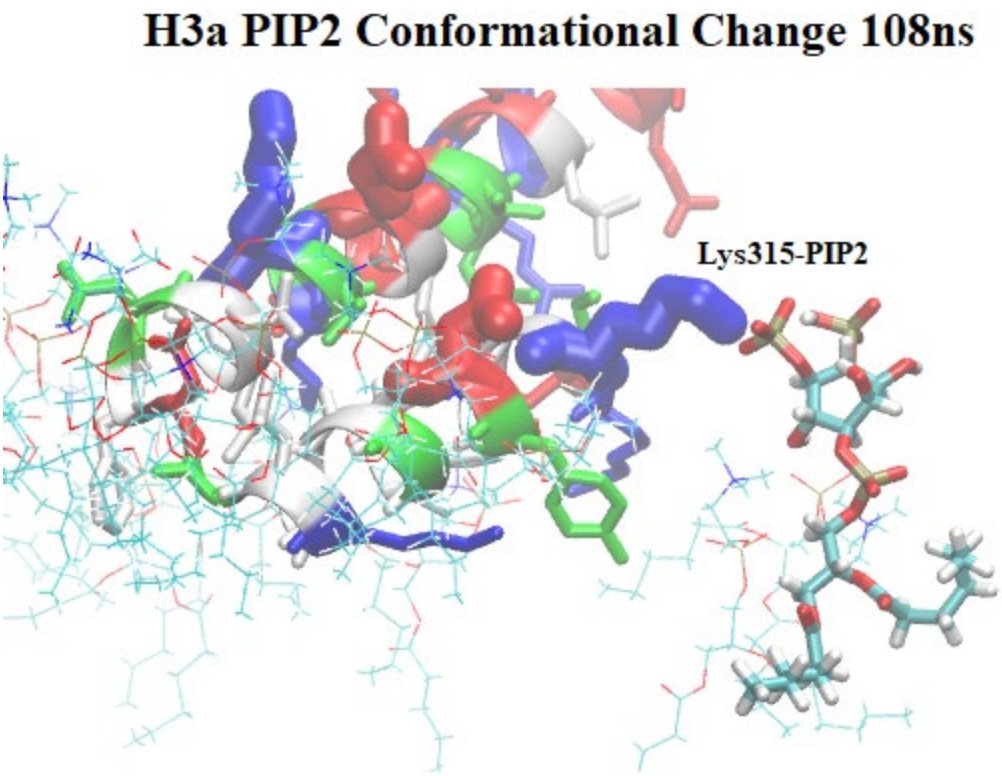

Figure S39. This is an image of the third conformation change of the side chain showing that the PIP2 lipids have enough charge to displace the Lys315 from interacting with D319, and shows preference for the PIP2 lipid. A similar result is expected for the other enlarged lysine residue K334

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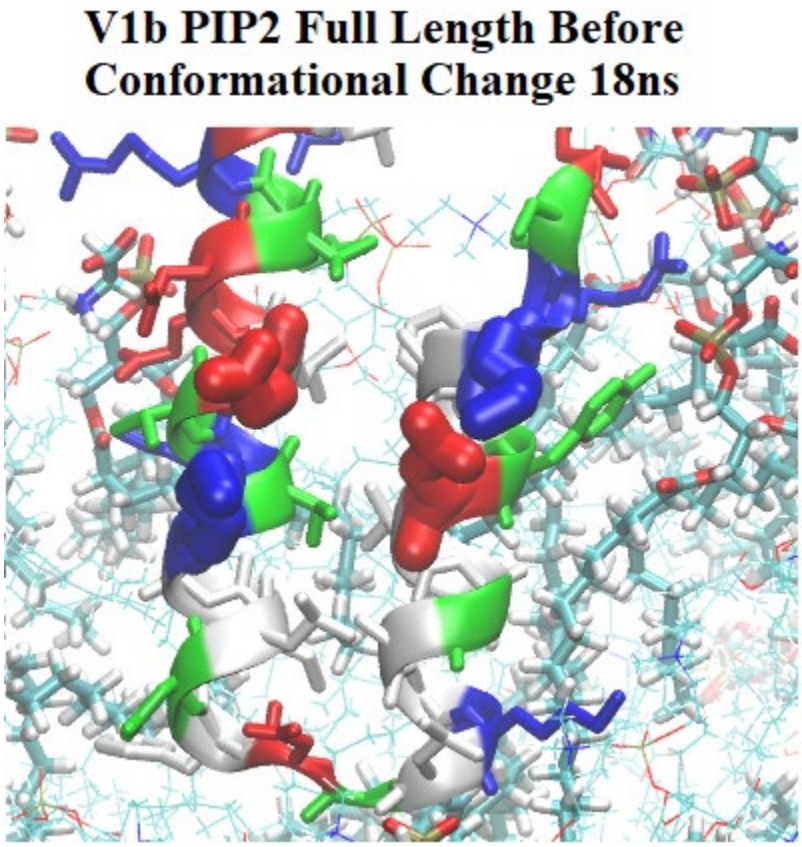

Figure S40. This is another image of the peptide before the conformaitonal change, and the main point is that K315-D319 is close enough to interact and K334-E338 are close enough to interact even if the bridge between K315-E338 is not close enough to interact.

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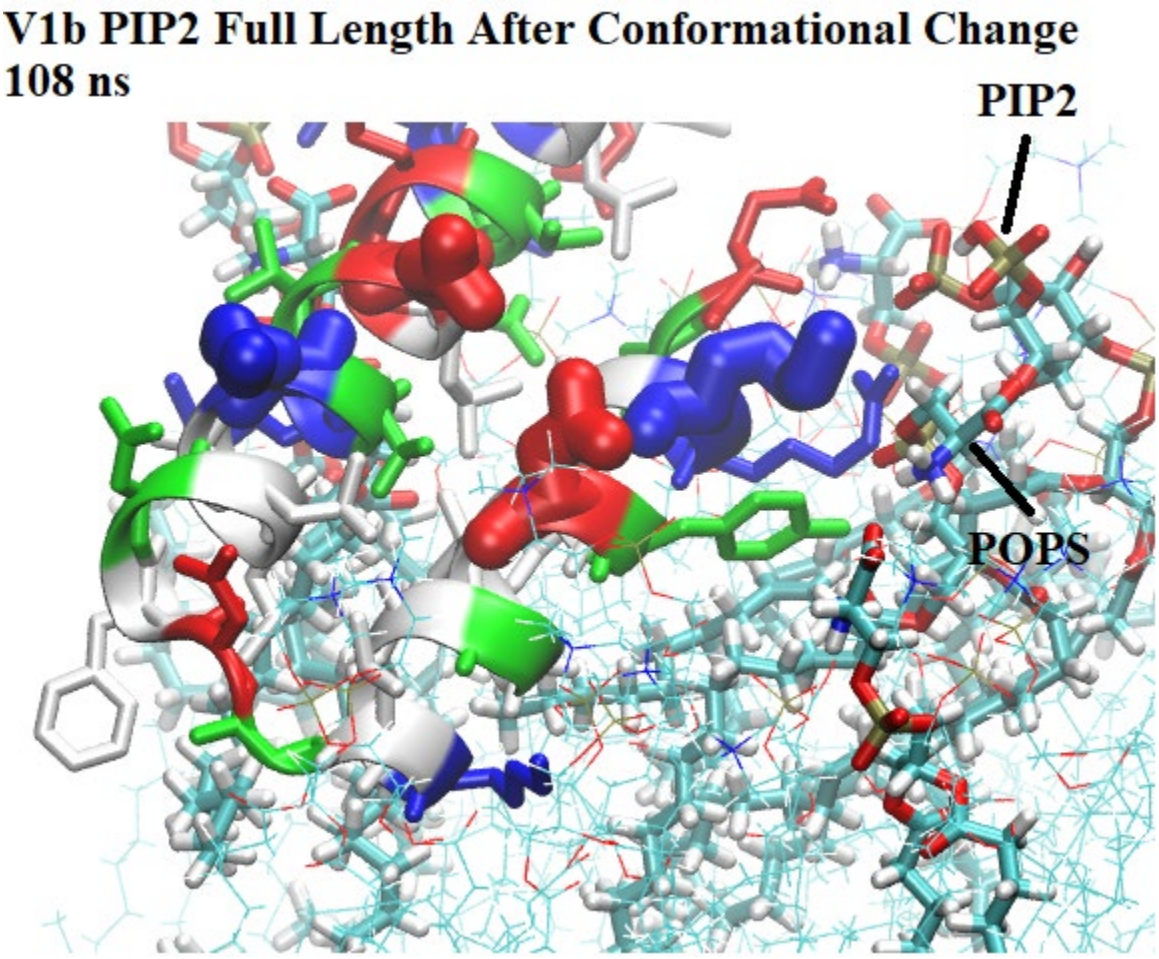

Figure S41. Here in this case the peptide is interacting with the POPS and the PIP2 simultaneously, and this shows that there can be synergy between the two lipids.

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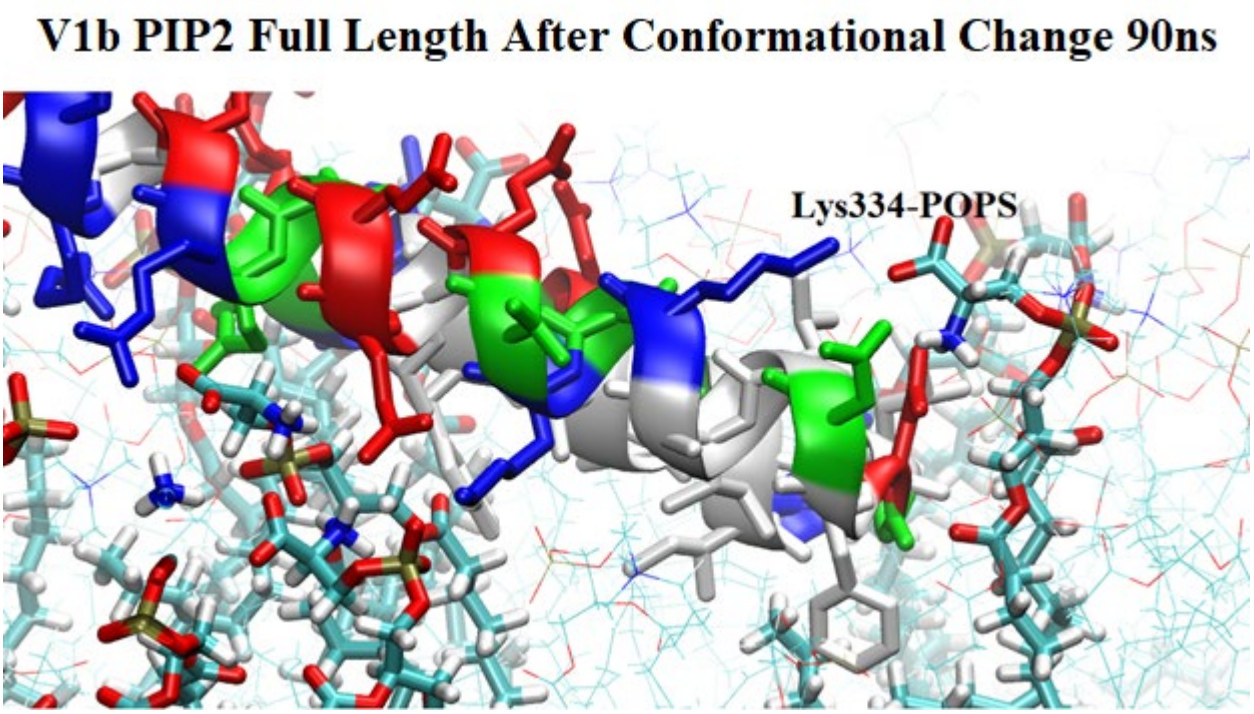

Figure S42. This image of the peptide shows the conformational change with Lys334 that was much less frequently observed, but proves that it is possible for the conformational change to take place without disrupting the peptide structure and indicates that it may be stabilized with POPS lipids.

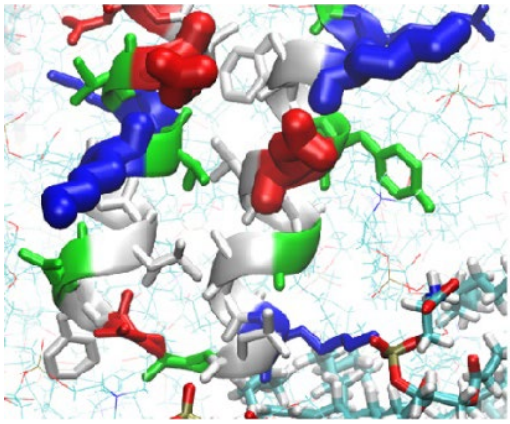

Figure S43. Image shown on the left is taken from simulation H1a full length at 22ns showing the long lived and spontaneously formed peptide-peptide salt bridges. On the right is H1a PIP2 full length showing both amino acids in the conformational change although K344 is not stabilized by a PIP2 or POPS lipid so it quickly moves back to form peptide-peptide salt bridges.

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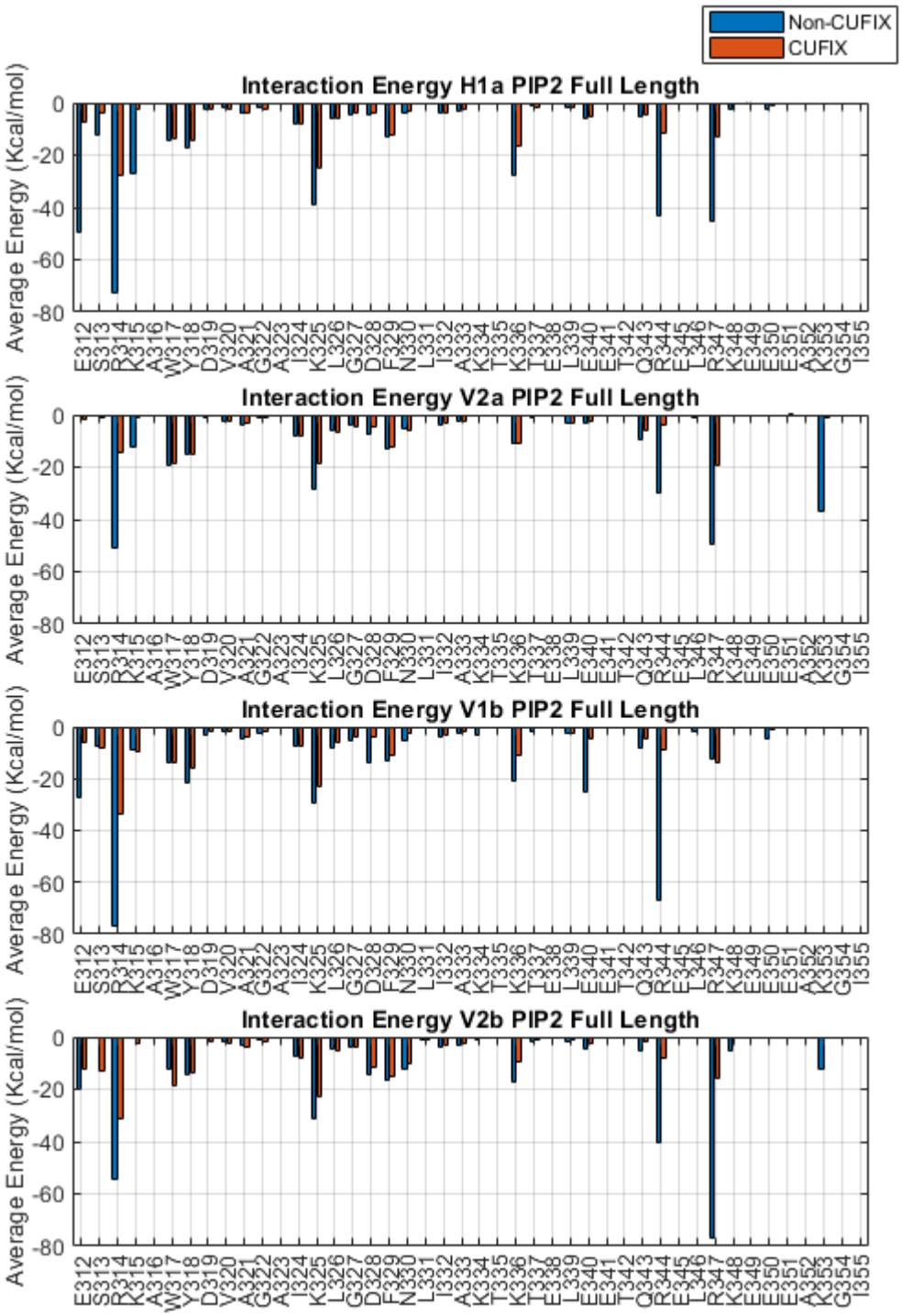

Figure S44. The per residue interaction energy of full length simulations H1PIP and VFPIP, and the higher energy residues are on the two sides such as E312, R314, R344, and R347, and V2PIP has lower energy than H1PIP because of the PIP2 lipids being directly underneath the peptide pushing the peptide somewhat away from the membrane.

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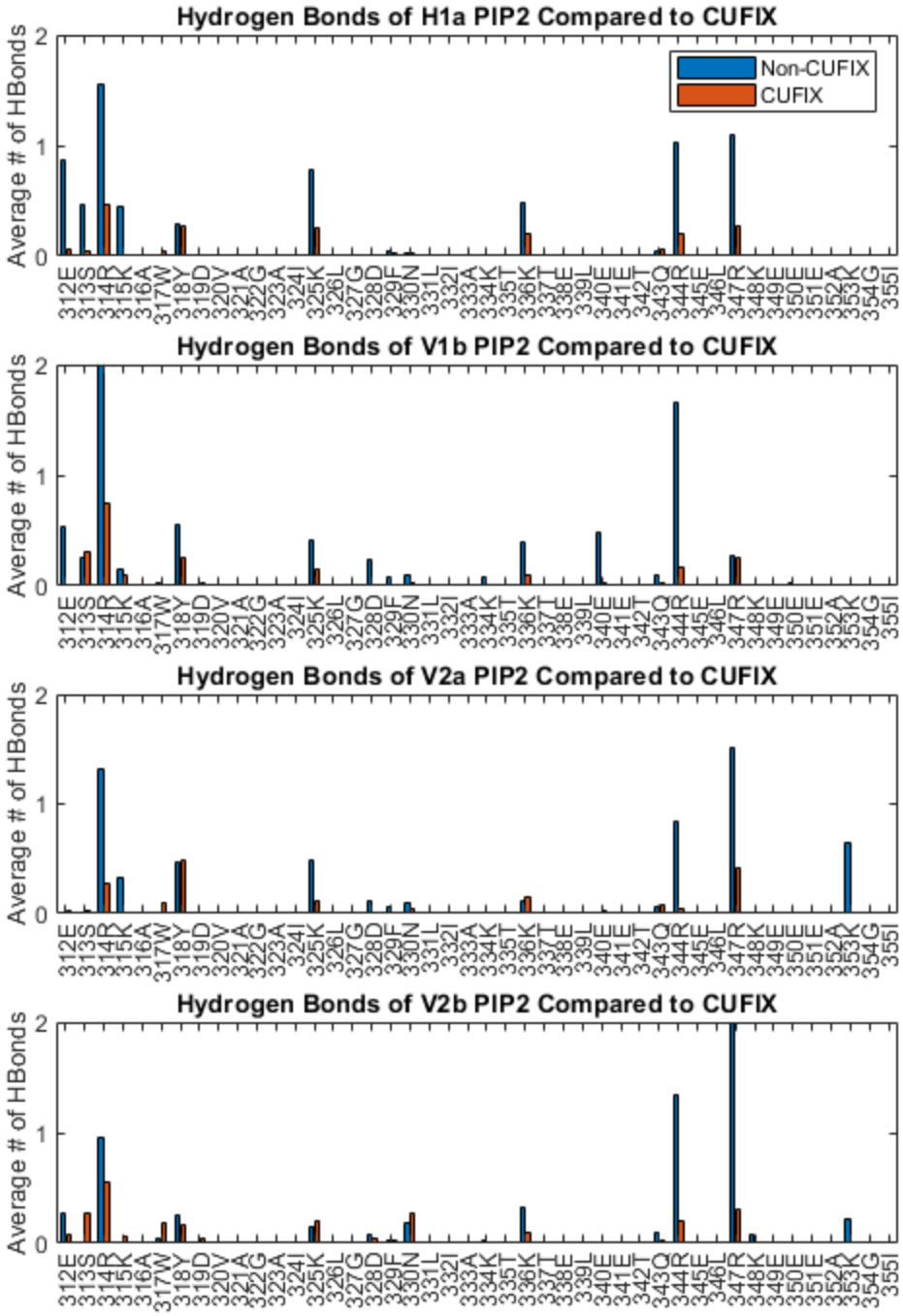

Figure S45. Shows the number of hydrogen bonds between the membrane and the peptide. It can be seen that the NBFIX parameters do drastically reduce the values of the hydrogen bonding for many residues, and there are slightly more hydrogen bonds expected in H1 PIP compared to V2 PIP.

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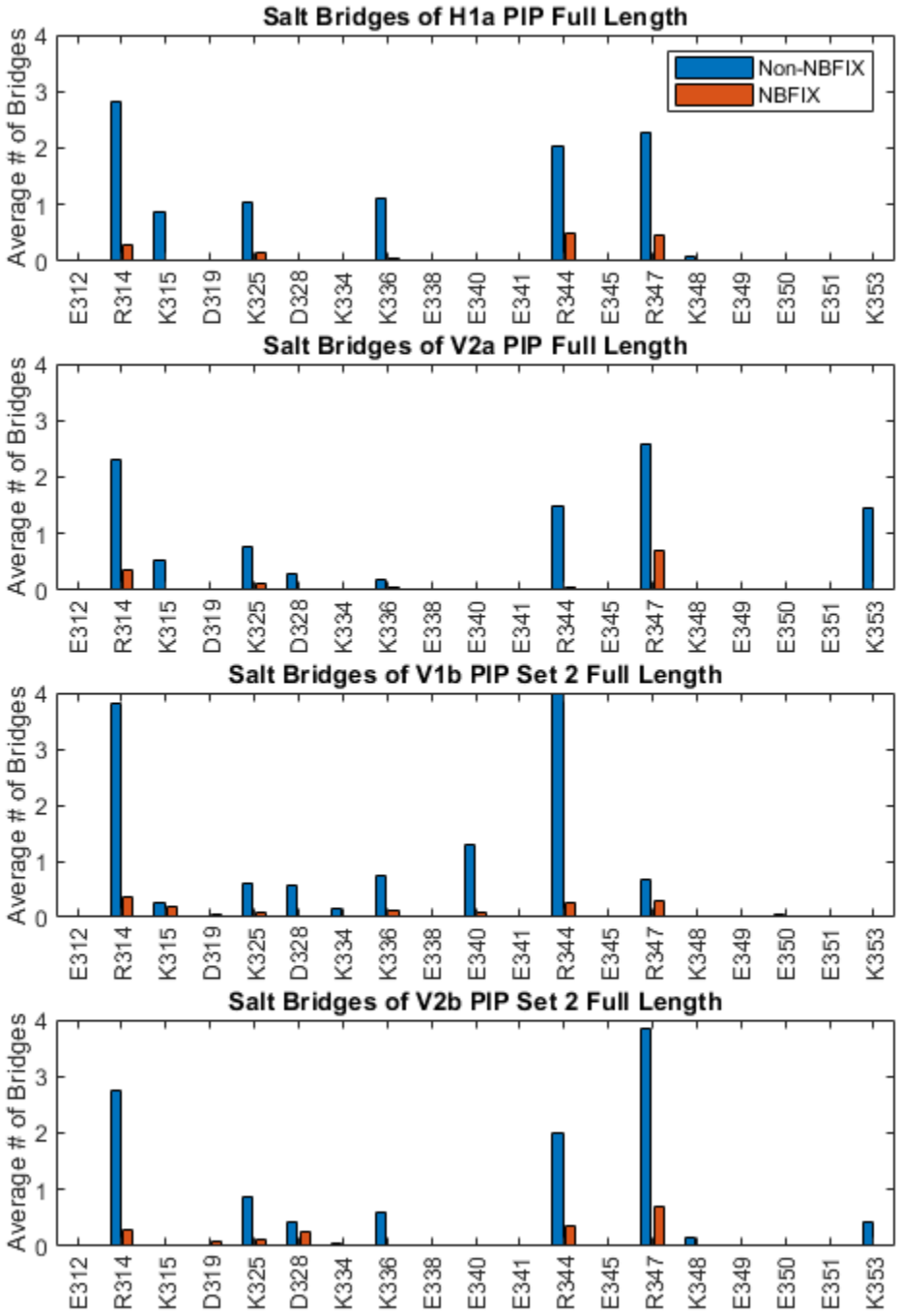

Figure S46. Displays the number of salt bridges per residue for full length membranes, showing that there are a higher number of hydrogen bonds for residue R347 in both simulations. R347 is the residue that is accessible to the PIP2 lipid head groups.

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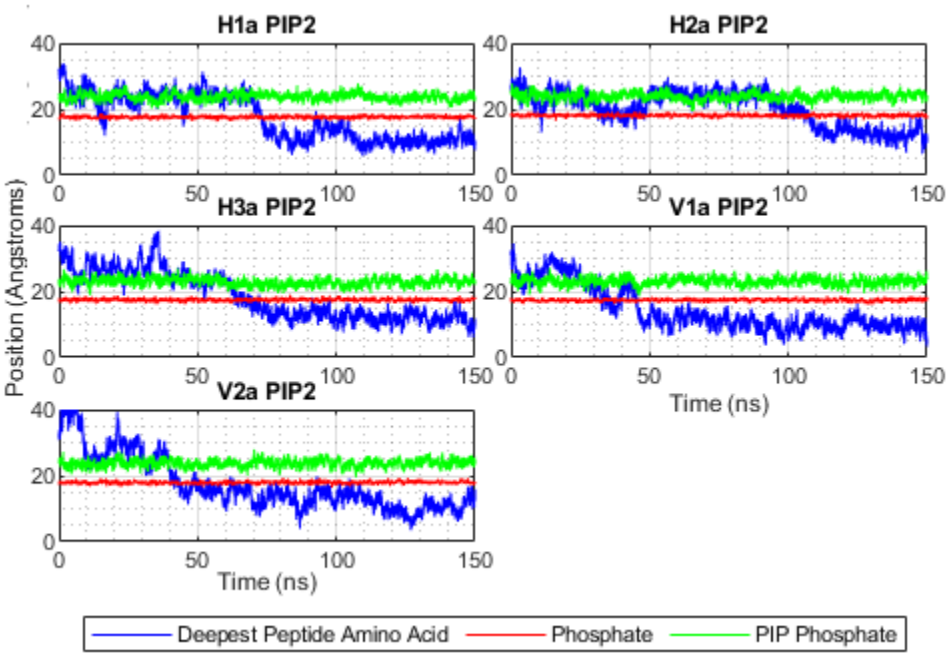

Figure S47. Image shows the position of the deepest residue for the PIP trials vs time along with the position of the PIP phosphate, and the main phosphate groups. This is similar to the other image of position of deepest residue vs time showing that the peptide starts off away from the membrane and approaches to the membrane except that the PIP2 lipids head groups stick up higher than the main membrane phosphates. Defined as the center of mass of the deepest residue.

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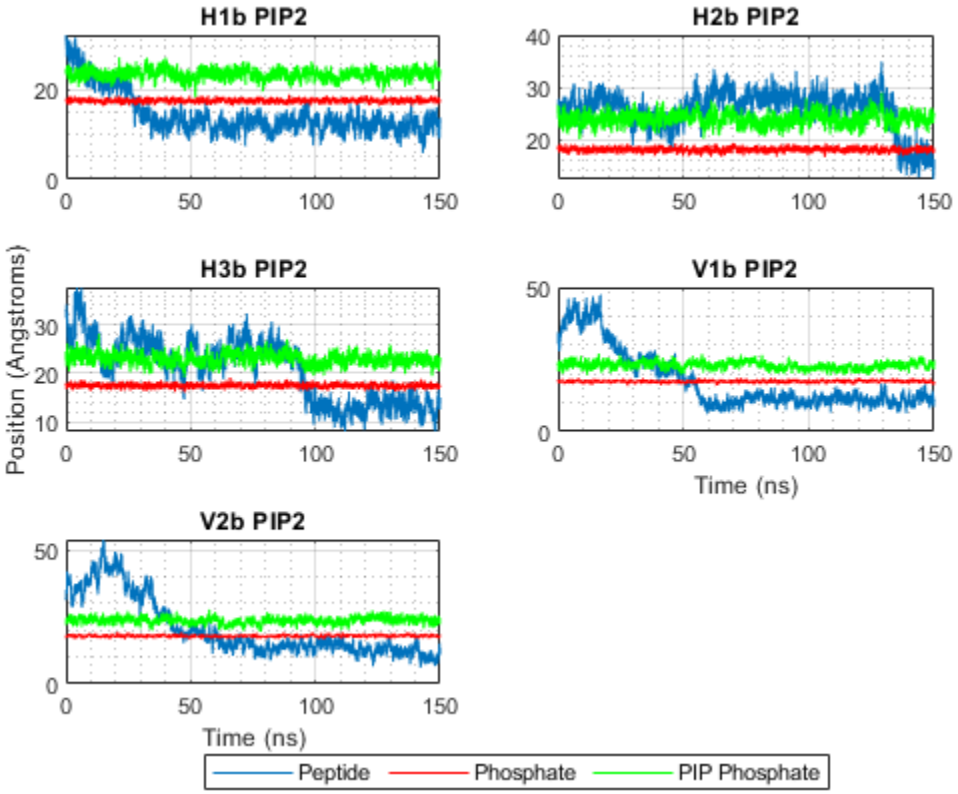

Figure S48. Image of the positoin of the COM of the deepest amion acid residue vs time.

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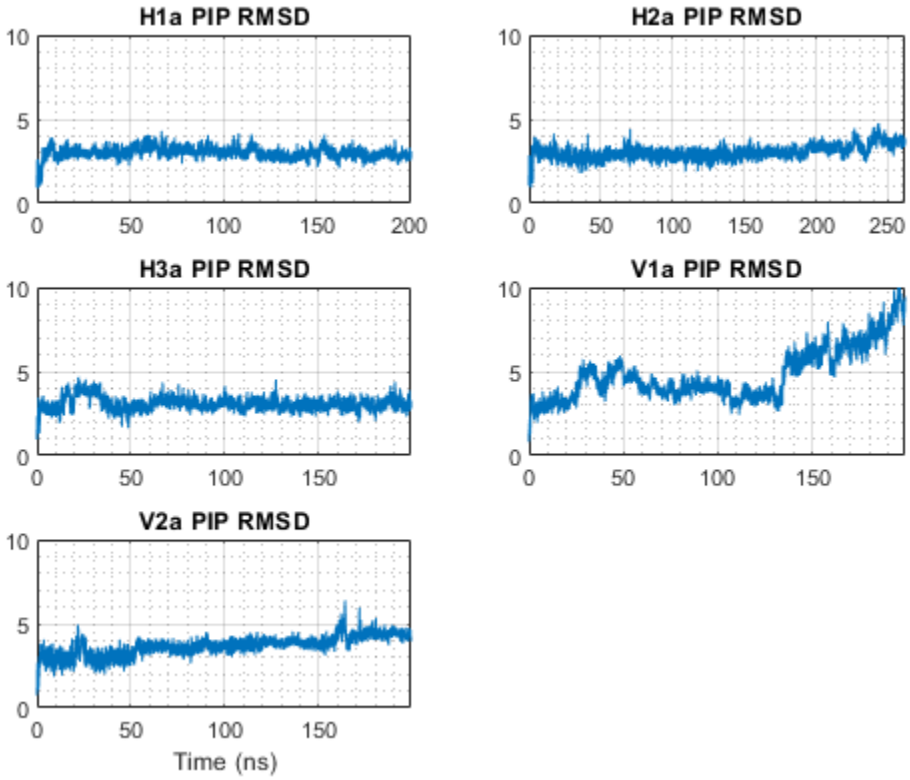

Figure S49. RMSD of the HMMM simulations, it is interesting in simulation V1 that the two helices were broken apart causing the higher RMSD. All of the simulations show stability except for the V1 PIP trial where the two helices split apart.

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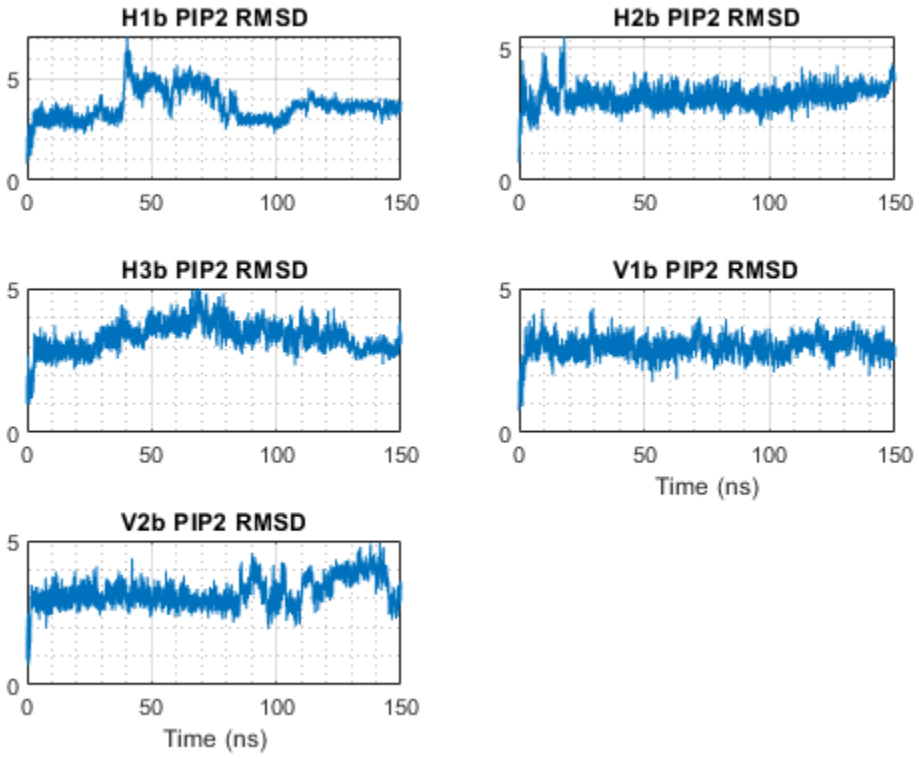

Figure S50. Image showing the RMSD of the HMMM simualations over time, the mian point is that all of the simulations had stable RMSD values and that the helices never split apart.

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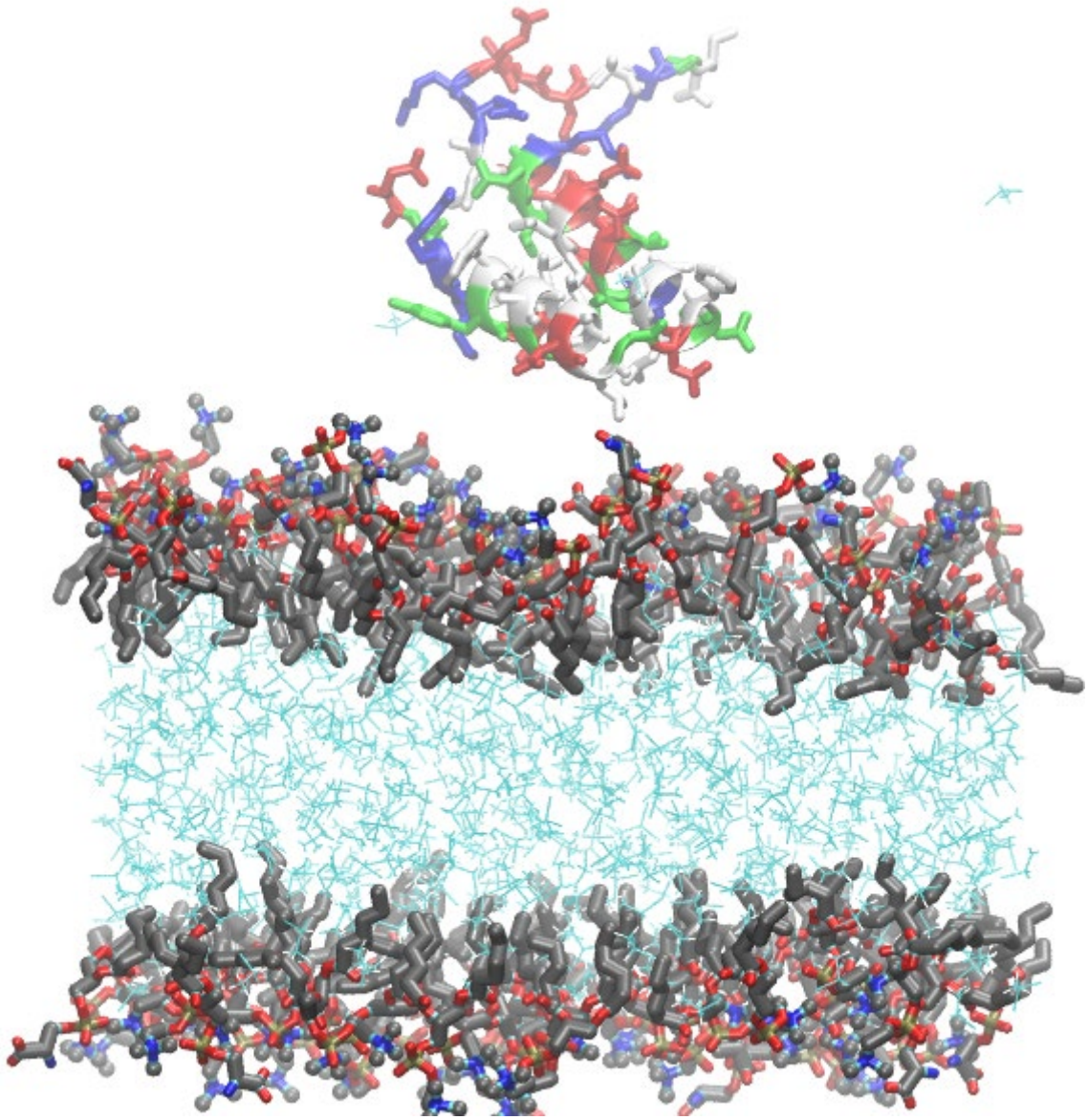

Figure S51. Image of the peptide unable to bind to the membrane without the two amino acids that were found to be essential. At the end of the simulation, 200ns, there was no binding.