The disordered plant dehydrin Lti30 protects the membrane during water-related stress by cross-linking lipids

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Running title: *Modulation of membrane fluidity by Lti30*

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System	Sequence	pH	Overall peptide charge	Lipid Bilayer	Simulation time (µs)
K-segment	EKKGMTEKVMEQLPG	5.8-9	0	DOPC	1.4
				DOPC:DOPS (4:1)	1.4
				DOPC:DPPC (1:1)	1.4
His-K-segment	VHEKKGMTEKVMEQLPGHHG	5.8	+3	DOPC	1.4
				DOPC:DOPS (4:1)	1.4
				DOPC:DPPC (1:1)	1.4
		9	0	DOPC	1.4
				DOPC:DOPS (4:1)	1.4
				DOPC:DPPC (1:1)	1.4

Table S1. Summary of peptide-membrane molecular dynamics simulations performed at pH values of 5.8 or 9.

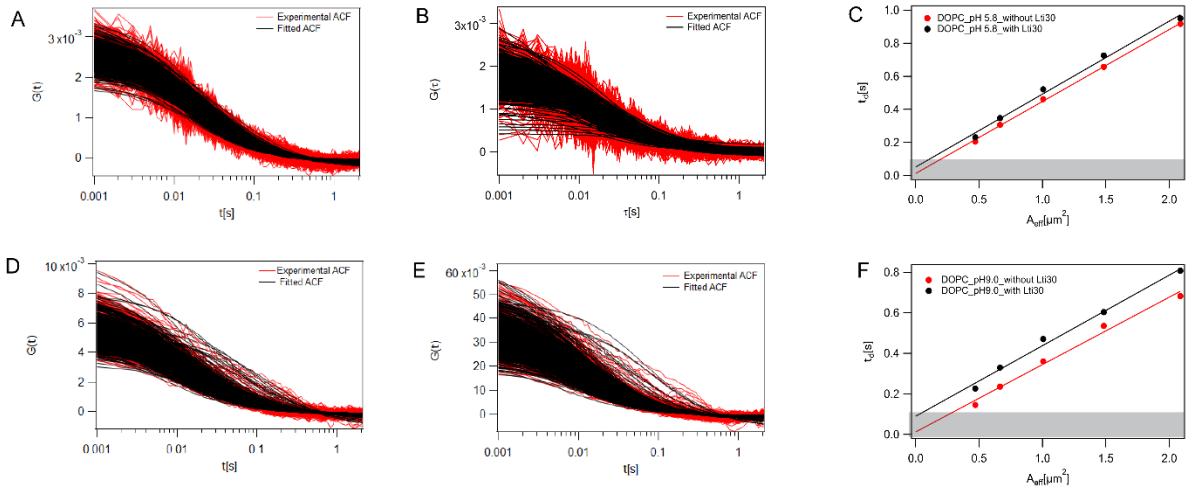

Figure S1: Representative raw data (autocorrelation function) for ITIR-FCS experiments to examine Lti30 binding to rhodamine-PE labelled DOPC bilayer at pH 5.8 and pH 9.0. A) ACF for DOPC membrane without Lti30 binding at pH 5.8 B) ACF for DOPC membrane with bound Lti30 at pH 5.8 C) FCS diffusion law plots for DOPC membrane lipid diffusion at pH 5.8 with and without Lti30 D) ACF for DOPC membrane without Lti30 binding at pH 9.0 E) ACF for DOPC membrane with bound Lti30 at pH 9.0 F) FCS diffusion law plots for DOPC membrane lipid diffusion at pH 9.0 with and without Lti30.

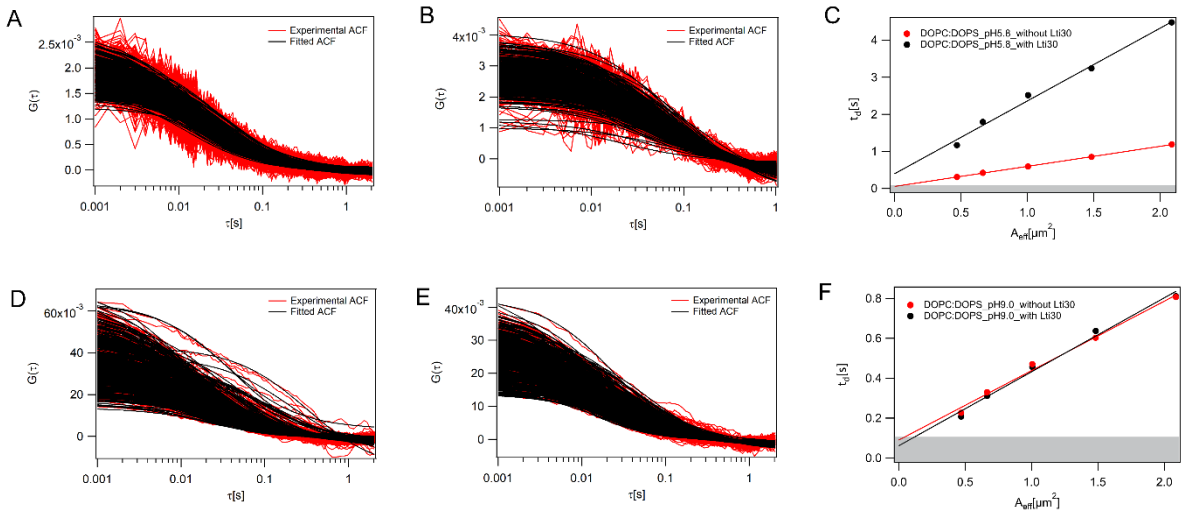

Figure S2: Representative raw data for ITIR-FCS experiments to examine Lti30 binding to rhodamine-PE labelled DOPC:DOPS (4:1) bilayer at pH 5.8 and pH 9.0. A) ACF for DOPC:DOPS (4:1) membrane without Lti30 binding at pH 5.8 B) ACF for DOPC:DOPS (4:1) membrane with bound Lti30 at pH 5.8 C) FCS diffusion law plots for DOPC:DOPS (4:1) membrane lipid diffusion at pH 5.8 with and without Lti30 D) ACF for DOPC:DOPS (4:1) membrane without Lti30 binding at pH 9.0 E) ACF for DOPC:DOPS (4:1) membrane with bound Lti30 at pH 9.0 F) FCS diffusion law plots for DOPC:DOPS (4:1) membrane lipid diffusion at pH 9.0 with and without Lti30. Grey shaded area in the FCS diffusion law plots corresponds to the region in which probe shows free diffusion.

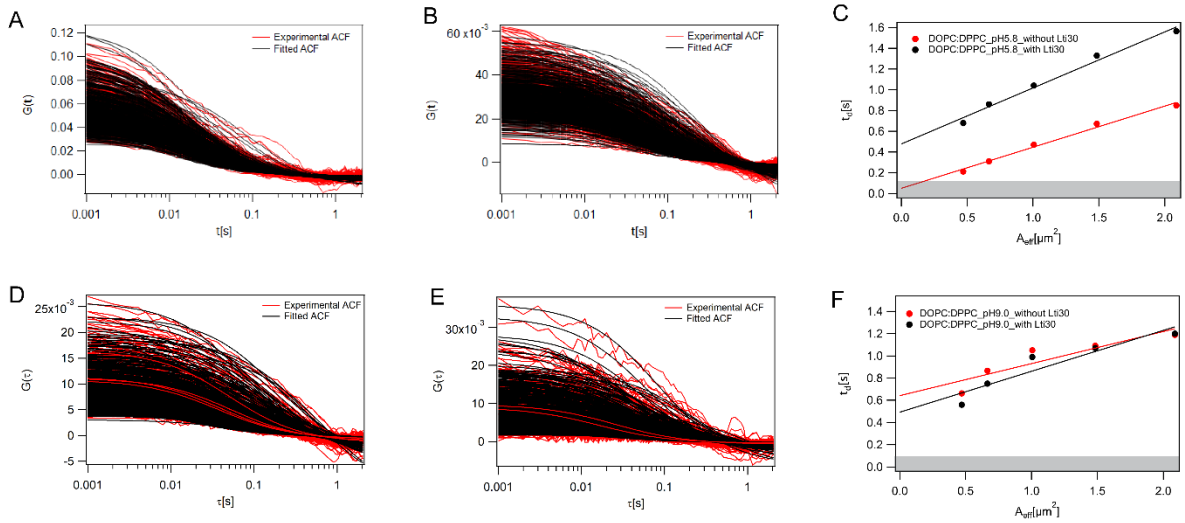

Figure S3: Representative raw data for ITIR-FCS experiments to examine Lti30 binding to rhodamine-PE labelled DOPC:DPPC (1:1) bilayer at pH 5.8 and pH 9.0. A) ACF for DOPC:DPPC (1:1) membrane without Lti30 binding at pH 5.8 B) ACF for DOPC:DPPC (1:1) membrane with bound Lti30 at pH 5.8 C) FCS diffusion law plots for DOPC:DPPC (1:1) membrane lipid diffusion at pH 5.8 with and without Lti30 D) ACF for DOPC:DPPC (1:1) membrane without Lti30 binding at pH 9.0 E) ACF for DOPC:DPPC (1:1) membrane with bound Lti30 at pH 9.0 F) FCS diffusion law plots for DOPC:DPPC (1:1) membrane lipid diffusion at pH 9.0 with and without Lti30. Grey shaded area in the FCS diffusion law plots corresponds to the region in which probe shows free diffusion.

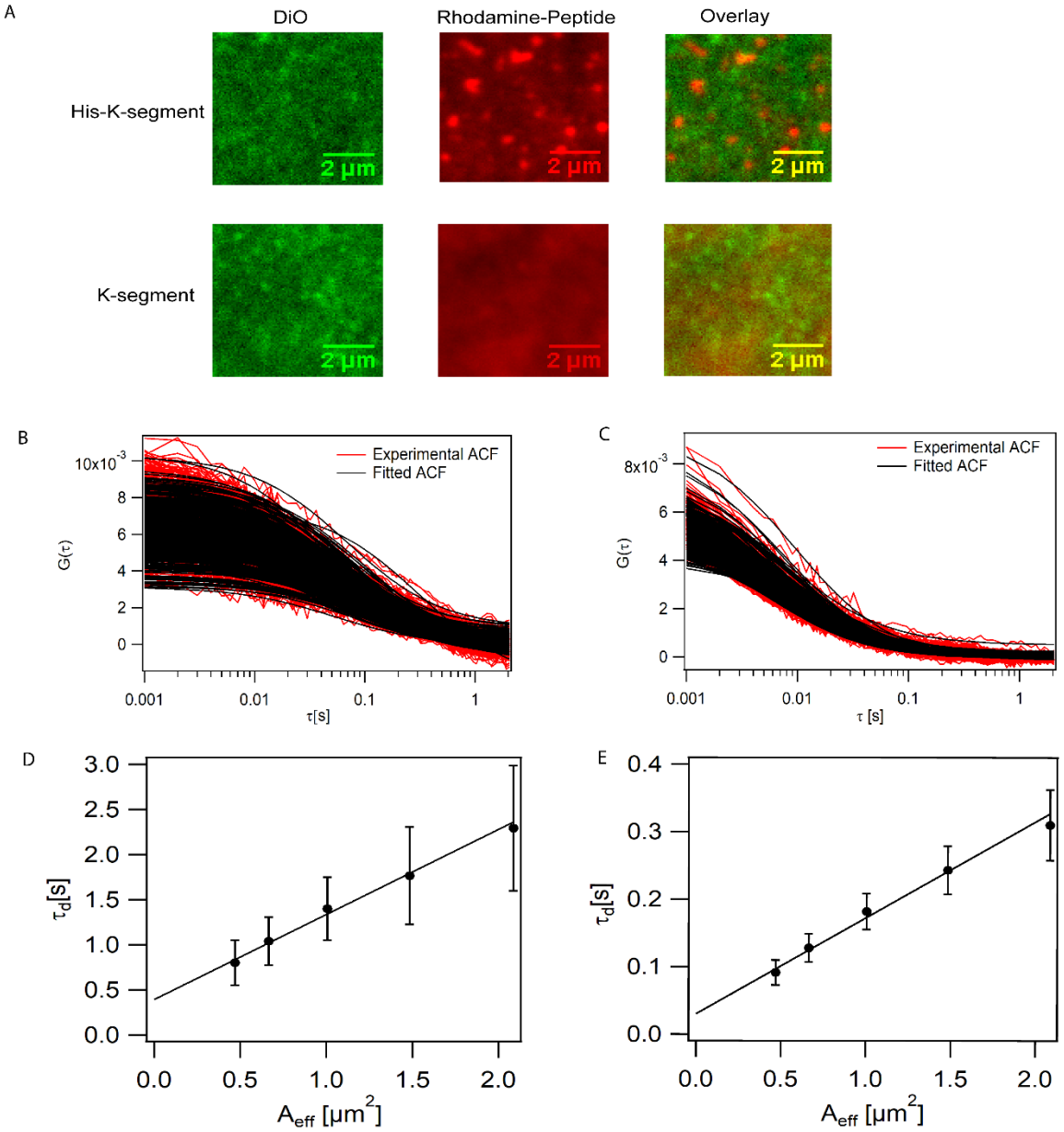

Figure S4: Representative raw data for the diffusion of rhodamine labelled individual K-segment and His-K-segment on DOPC:DOPS (4:1) bilayer measured by ITIR-FCS. A) Images of DiO labelled DOPC:DOPS (4:1) bilayer interacting with rhodamine labelled peptides at pH 5.8 recorded using a TIRF microscope. DiO is a lipophilic dye. His-K-segment form aggregates in the membrane environment while K-segments do not show membrane localization. Image overlay is performed using ImageJ (64 bit). B) ACF for the diffusion of His-K-segment on the membrane at pH 6.3 C) ACF for the diffusion of K-segment on the membrane at pH 6.3. D) FCS diffusion law plot for His-K-segment diffusing on the membrane E) FCS diffusion law plot for K-segment diffusing on the membrane.

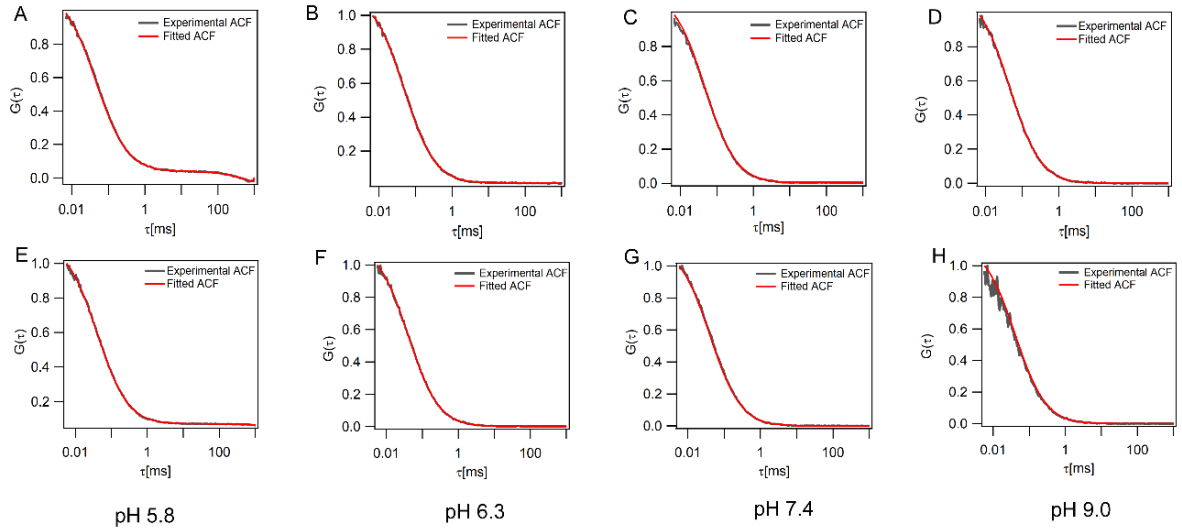

Figure S5: Representative raw data for diffusion of rhodamine labelled individual His-K-segment (A, B, C, D) and K-segment (E, F, G, H) in solution at pH 5.8, 6.3, 7.4 and 9.0. Measurements are performed on a confocal microscope.

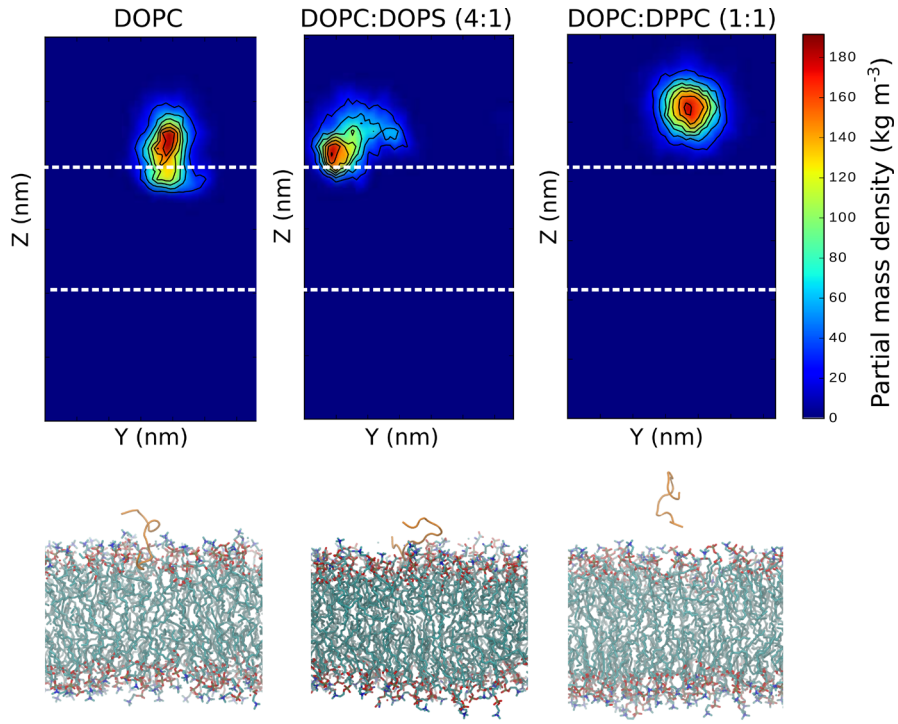

Figure S6: K-segment-membrane interactions during simulations at pH 5.8-9.0. Each panel corresponds to a particular membrane system: DOPC, DOPC:DOPS (4:1), or DOPC:DPPC (1:1). On the top of each panel, the final 0.2 μs averaged partial mass density of the peptide is shown (with respect to Z-normal and Y-axis), with the outer leaflet phosphates group indicated by a dashed line. The final snapshot of the simulation is shown on the bottom of each panel (peptide shown in cartoon representation in orange, lipids shown in licorice representation, with carbons colored cyan, oxygens red, and nitrogens blue).

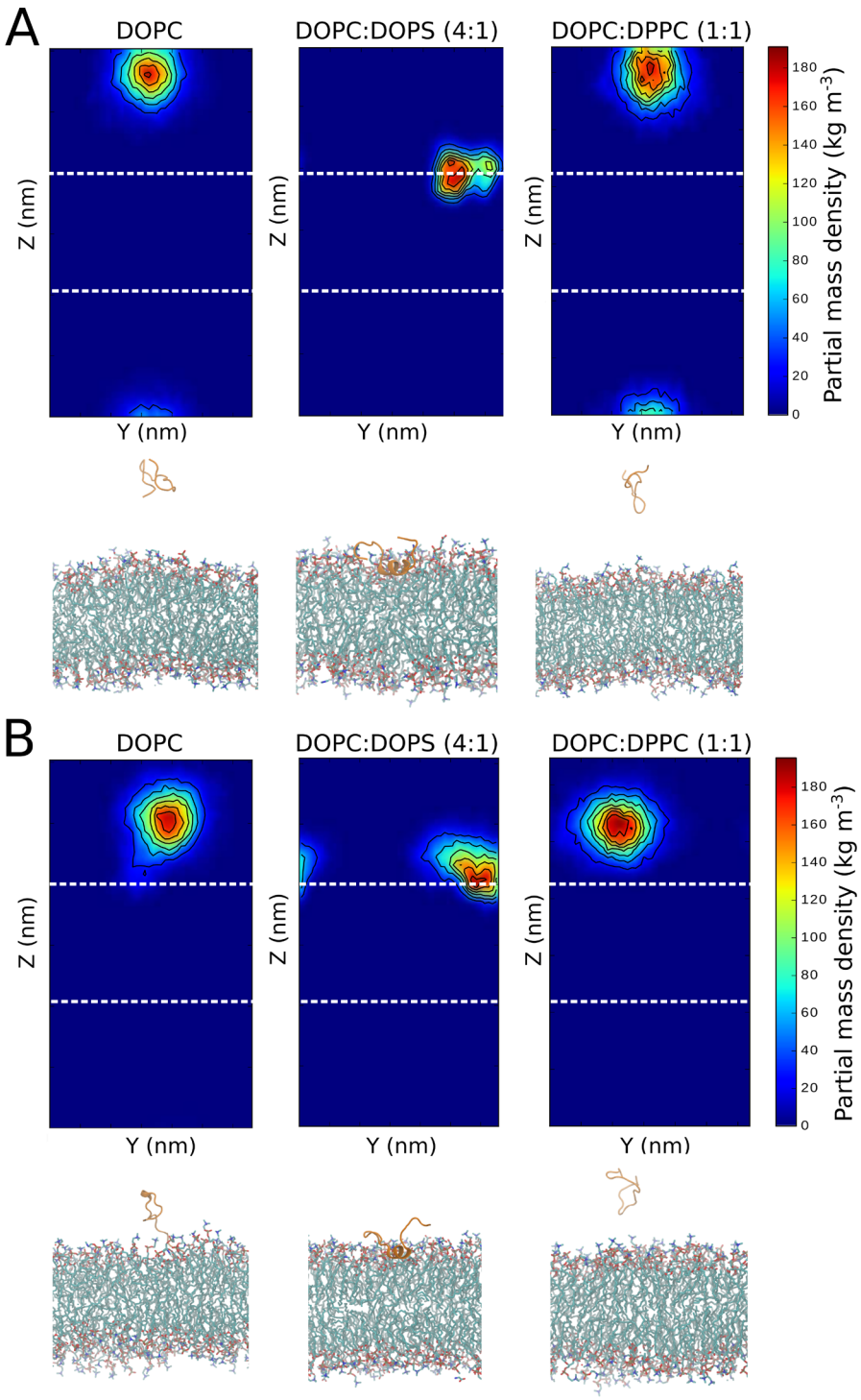

Figure S7: His-K-segment - membrane interactions during simulations. (A) pH 5.8 (protonated histidines) and **(B)** pH 9 (deprotonated histidines). Each panel corresponds to a particular membrane system: DOPC, DOPC:DOPS (4:1), or DOPC:DPPC (1:1). On the top of each panel, the final 0.2 μs averaged partial mass density of the peptide is shown (with respect to Z-normal and Y-axis), with the outer leaflet phosphates groups indicated by a dashed line. The final snapshot of the simulation is shown on the bottom of each panel (peptide shown in cartoon representation in orange, lipids shown in licorice representation, with carbons colored cyan, oxygens red, and nitrogens blue).

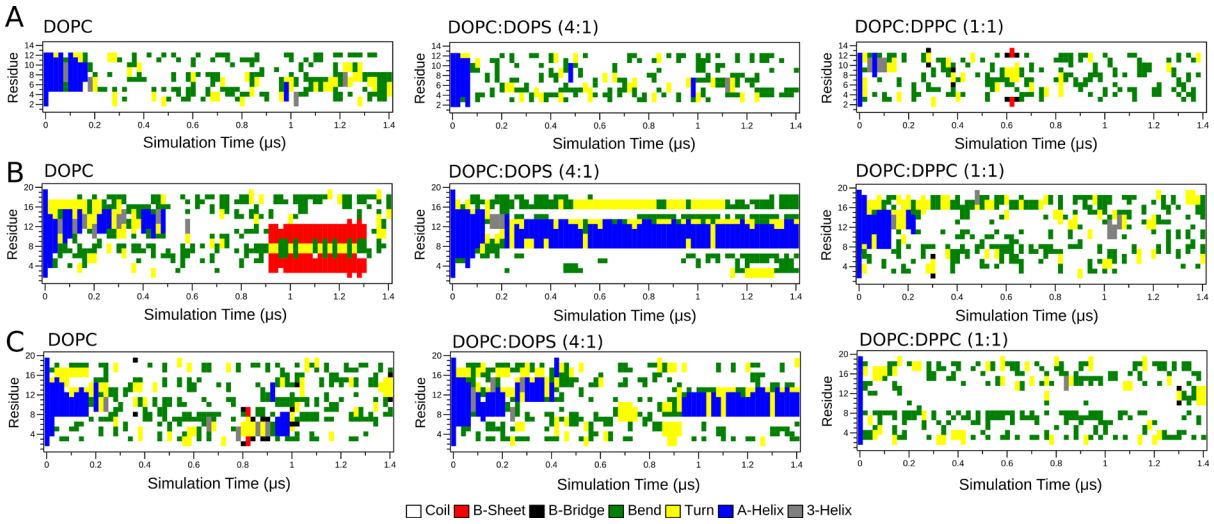

Figure S8. Per-residue peptide secondary structure propensity over the simulation time in lipid membranes. (A) K-segment at pH 5.8-9.0, **(B)** His-K-segment at pH 5.8, **(C)** His-K-segment at pH 9.0.