Supporting Information

pH-Responsive Peptide Nanopores are Stabilized by Lipid and Water-Mediated Hydrogen Bonding Networks

Ana-Nicoleta Bondar,*[,1,2] Samo Lesnik,[3,4] and Kalina Hristova[5], William C. Wimley*[,6]

[1]University of Bucharest, Faculty of Physics, Atomiștilor 405, Măgurele 077125, Romania

[2]Forschungszentrum Jülich, Institute of Computational Biomedicine, IAS-5/INM-9, Wilhelm-Johnen Straße, 5428 Jülich, Germany

[3]University of Maribor, Faculty of Chemistry and Chemical Engineering, SI-2000 Maribor, Slovenia

[4]Institute for Environmental Protection and Sensors, Beloruska ulica 7, SI-2000, Slovenia

[5]Johns Hopkins University, Department of Materials Science and Engineering, Institute for NanoBio Technology, Baltimore, Maryland, USA

[6]Tulane University School of Medicine, Department of Biochemistry and Molecular Biology, New Orleans, Louisiana, USA

*To whom correspondence should be addressed.

Supporting Information contains:

- **Supporting Information Figures S1-S23**

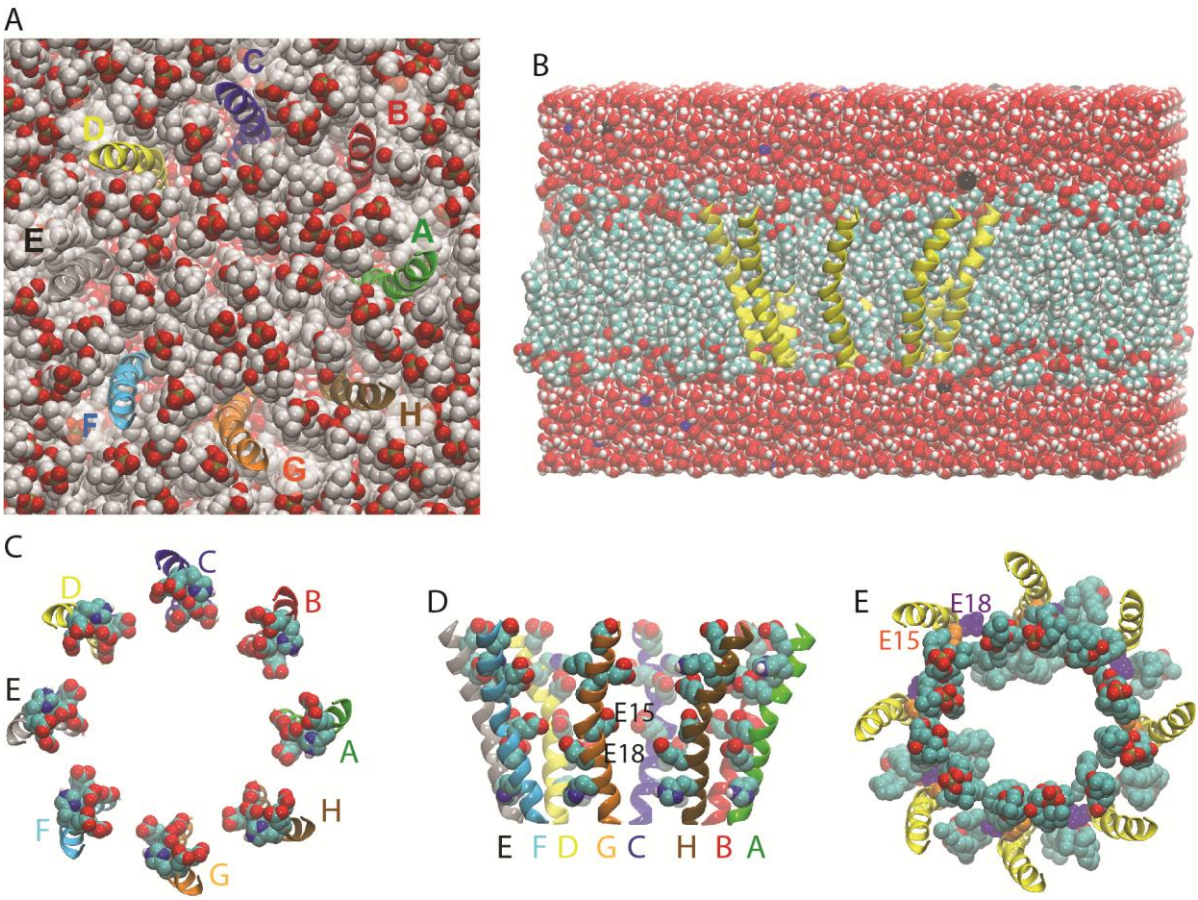

Figure S1. Starting geometry for the 8-pHD108 peptide systems with lipids in the inner region delineated by the peptides. (A, B) Cut-away view of the 8 peptides in a hydrated lipid membrane environment, viewed from the surface of the membrane (panel A) vs. across the membrane (panel B). Peptides are shown as ribbons, lipid, water molecules, and ions, as van der Waals spheres. (C, D) Peptides with their Glu and His sidechains shown as van der Waals spheres, viewed from the surface of the bilayer (panel C) vs. laterally (panel D). For clarity, only the H atoms bound to His-Nε2 are shown. Note that this image was prepared from the starting geometry of the simulation with all Glu sidechains negatively charged, and all His in the -Nε2 tautomeric state. (E) View from the membrane surface of the peptides in the starting geometry, with the E15 and E18 sidechains shown as van der Waals spheres colored orange and violet, respectively. Lipids shown have any atom within 3.5Å of the E15 and E18 carboxylic atoms Oε1 or Oε2.

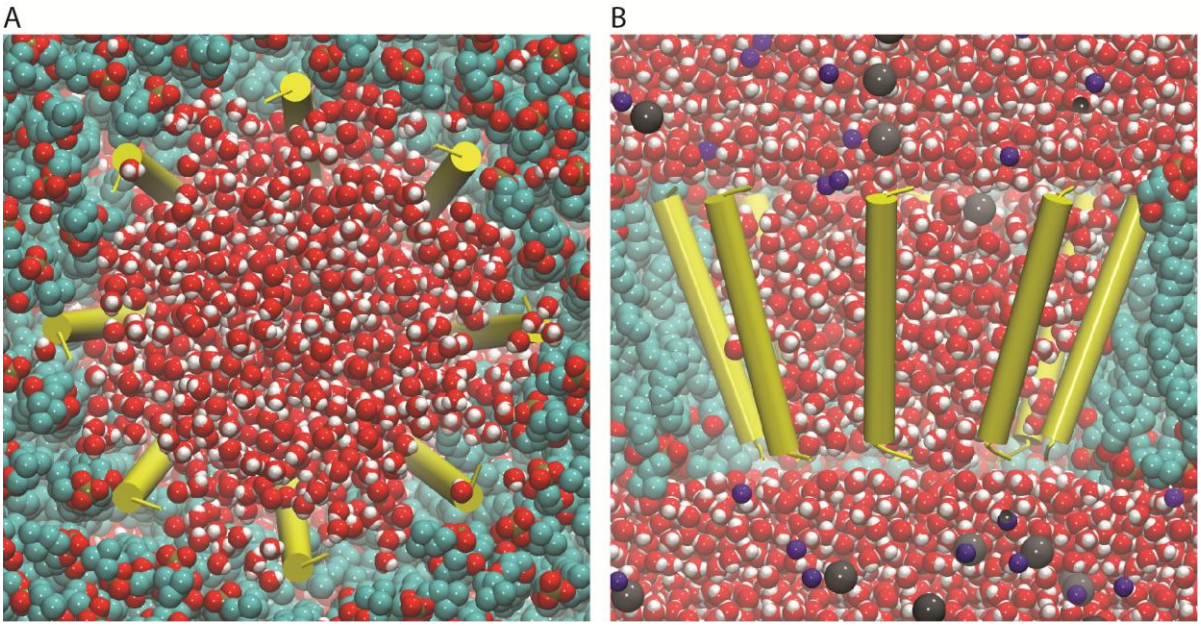

Figure S2. Starting coordinates of an 8-mer with water filling the inner region delineated by the peptides. Peptides are shown as cartoons colored yellow. (A, B) Cut-away views from the membrane surface (panel A) vs. side view (panel B).

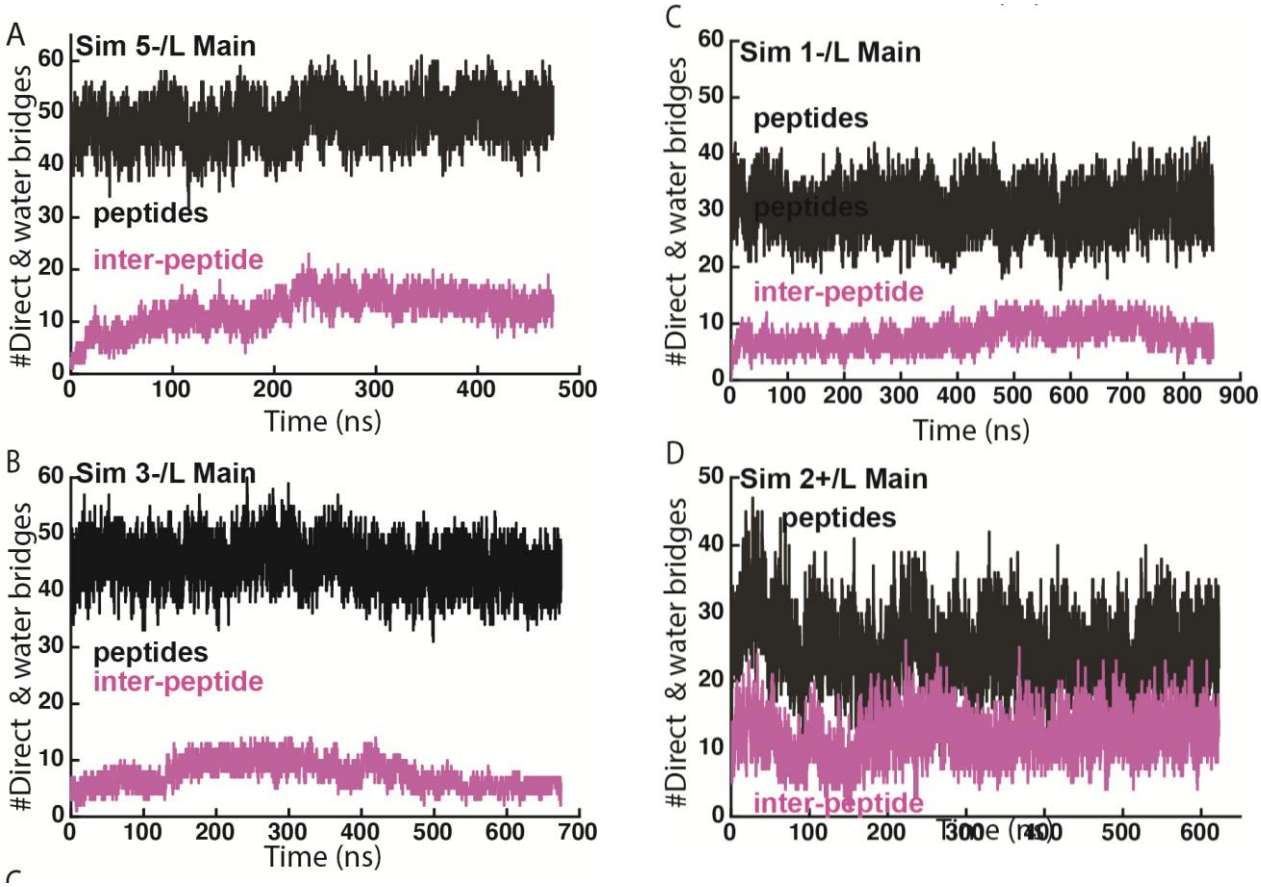

Figure S3. Time series of the number of H-bonds sampled during the main simulations. We used the complete production runs to compute the H-bond graphs for the direct H-bonds between sidechains and three-water bridges. We summed up all intra-peptide H-bonds ('peptides', black) vs. all inter-peptide H-bonds ('inter-peptide', magenta). For clarity, we plot values with a timestep of 100ps. **A-D.** Numbers of direct and water-mediated bridges computed for intra- and inter-peptide interactions sampled in each of the main simulations.

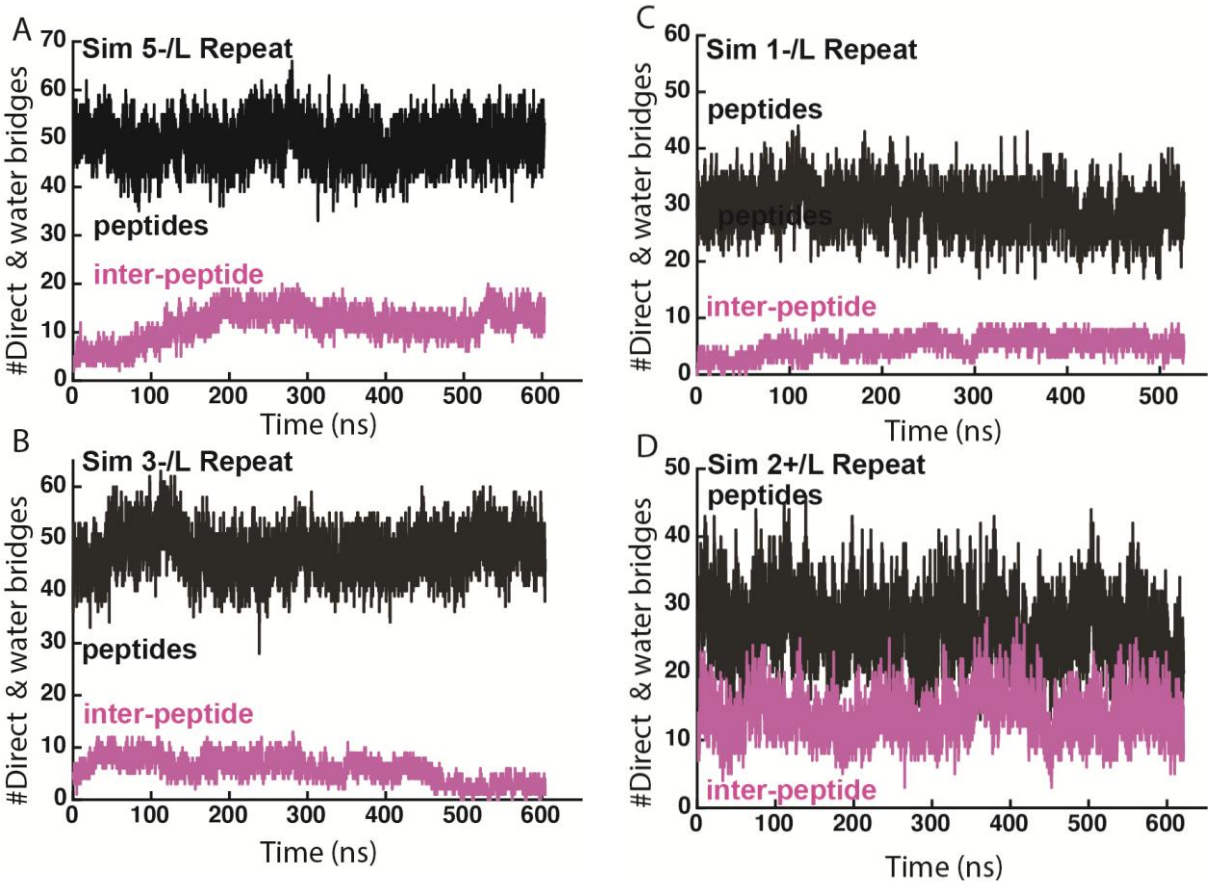

Figure S4. Time series of the number of H-bonds sampled during the repeat simulations. **A-D**. Number of direct and water-mediated H-bonds sampled during each of the repeat simulations.

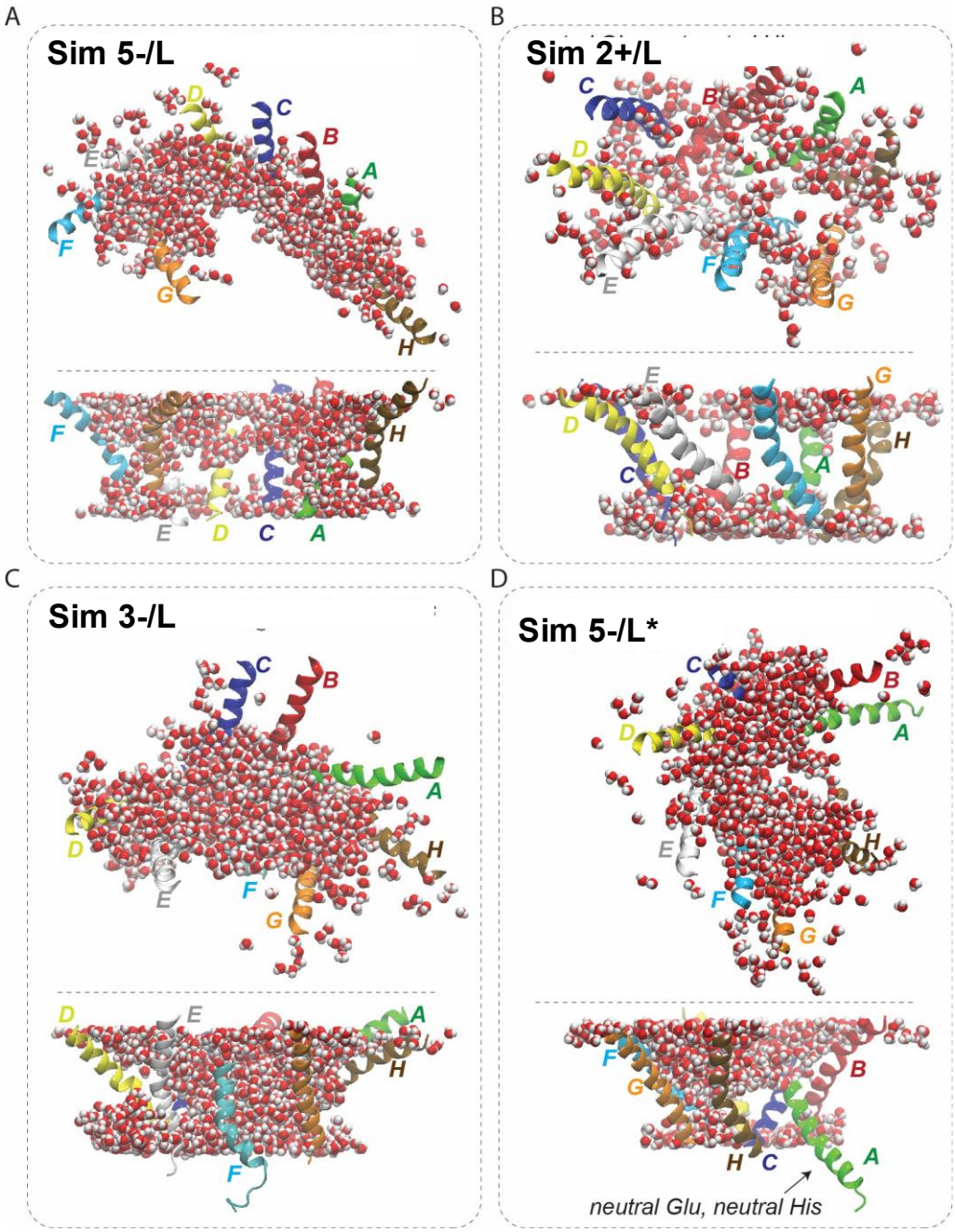

Figure S5. Water in main simulations that were initiated with lipids in the areas delineated by the peptides.

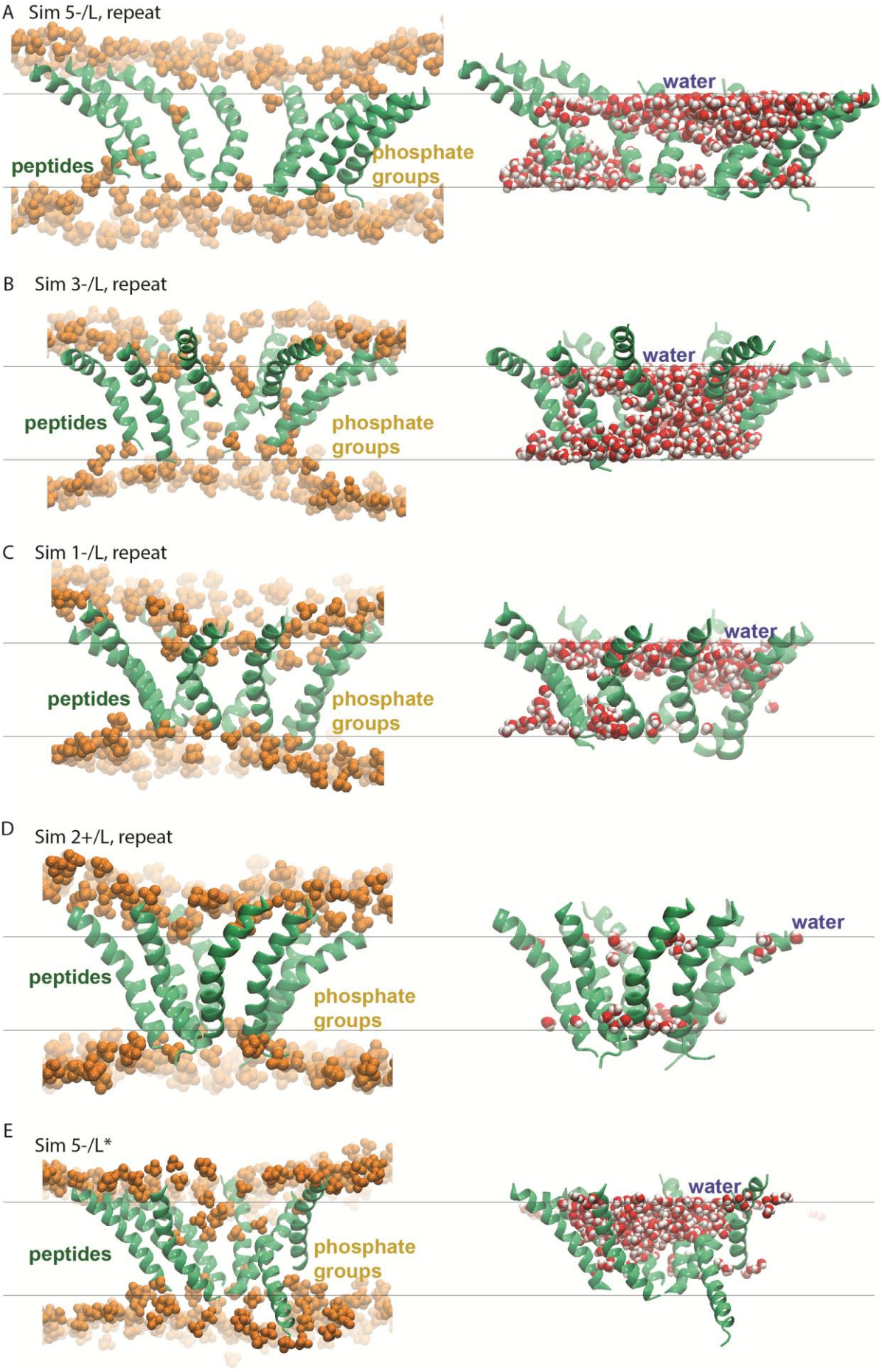
A Sim 5-/L, repeat
peptides
phosphate groups
water
B Sim 3-/L, repeat
peptides
phosphate groups
water
C Sim 1-/L, repeat
peptides
phosphate groups
water
D Sim 2+/L, repeat
peptides
phosphate groups
water
E Sim 5-/L*
peptides
phosphate groups
water

Figure S6. Illustration of the pore interactions sampled in the repeat simulations. **A-D**. Molecular graphics prepared from the repeat simulations. **E.** For comparison, we also show images from the Sim5-/L* simulation.

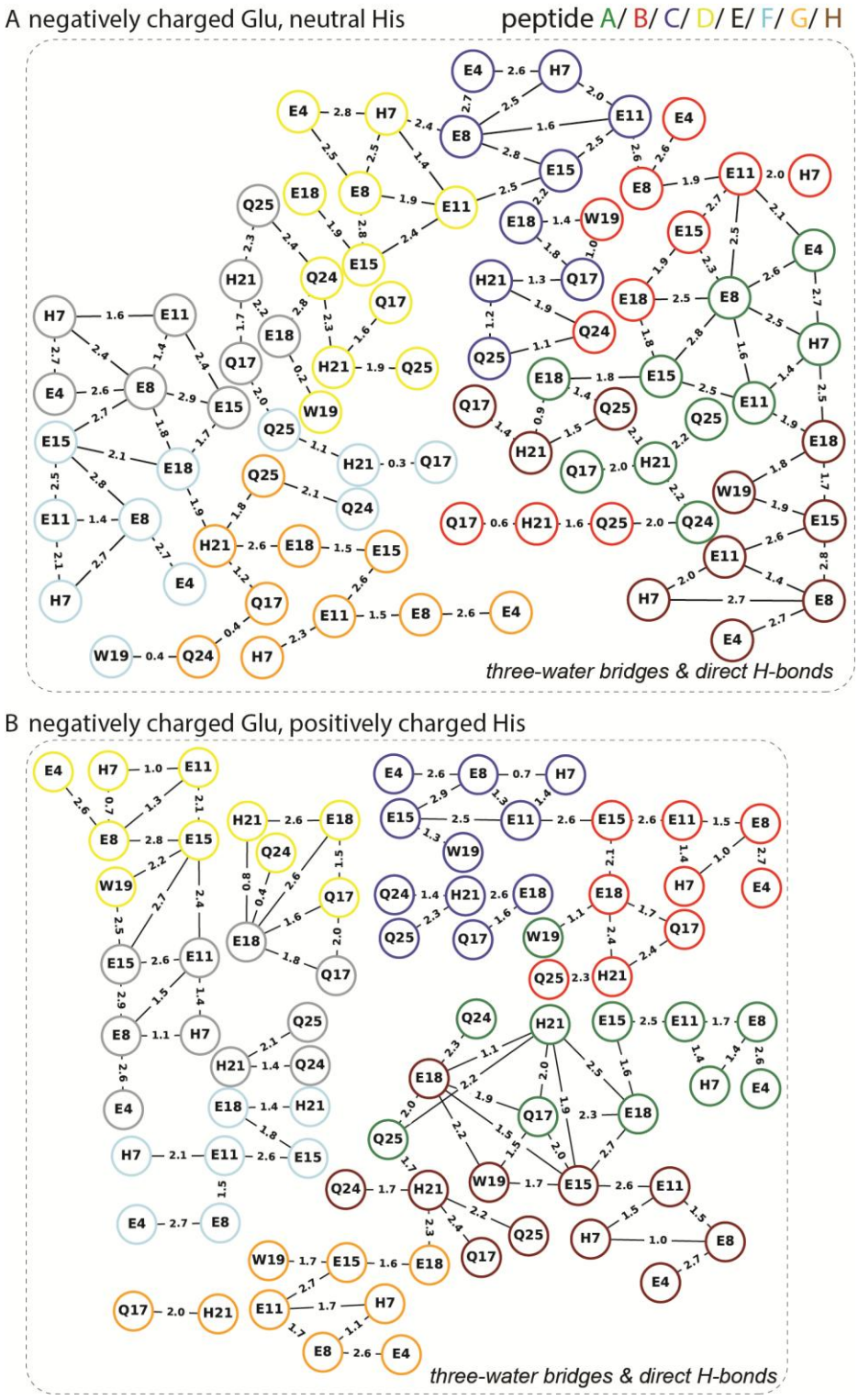

Figure S7. **Sim 5-/L and Sim 3-/L** Average number of water molecules in water-mediated bridges computed for the direct and 3-water mediated bridges between sidechains. A. **Sim 5-/L** with all Glu and His residues deprotonated. **B**. **Sim 3-/L** with all Glu sidechains negatively charged, and all His, positively charged. The initial configurations had lipids inside the region delineated by the peptides, **Fig. S1**.

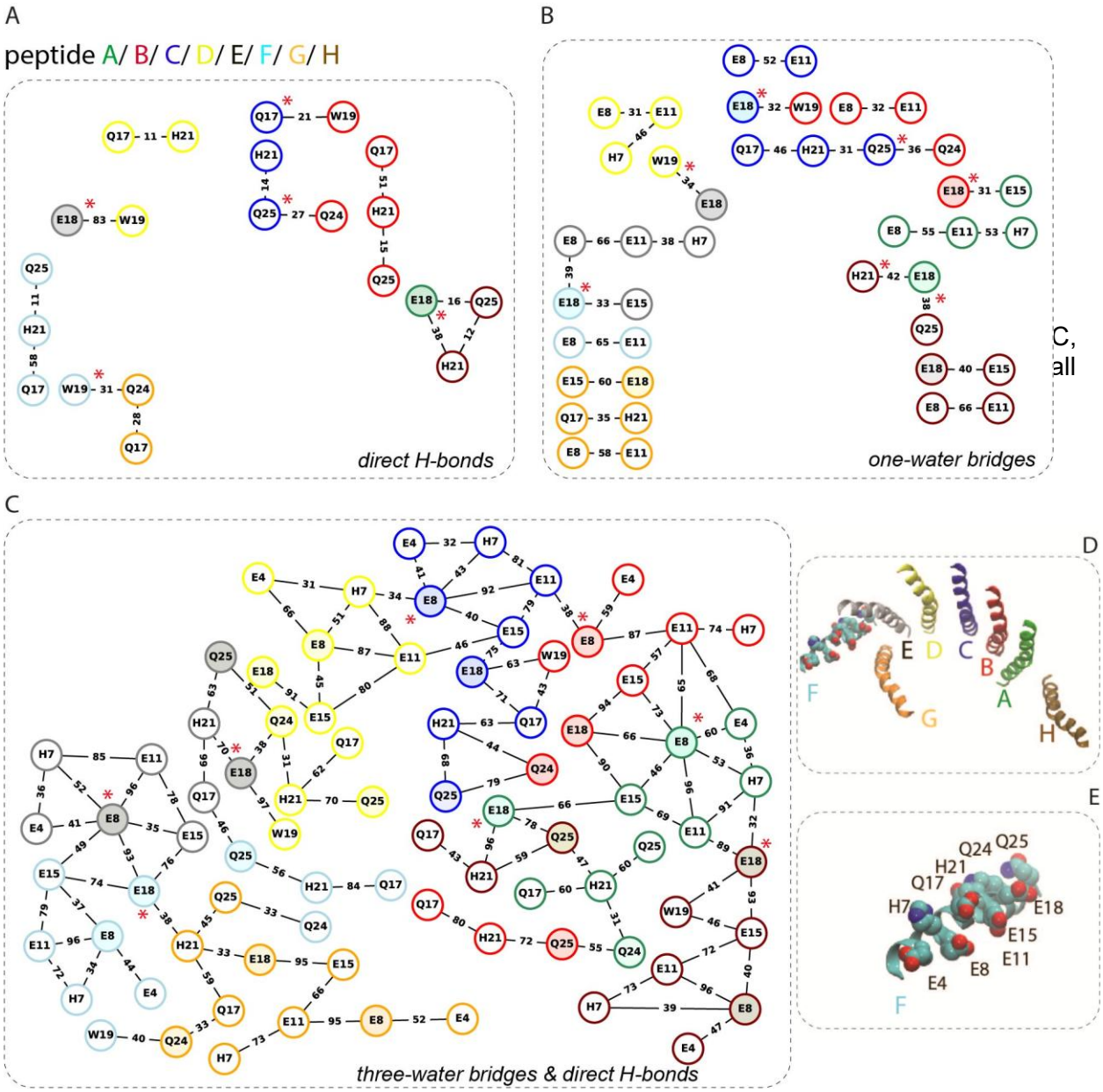

Figure S8. H-bond graphs computed from **Sim 5-/L**. The red asterisks in panels A and C indicate sites where peptides inter-connect. Values on the connecting lines are % occupancy for that interaction over the last 200 ns of the simulation. **A.** Graph of the direct H-bonds between peptide sidechains. **B.** Graph of the one-water-mediated bridges between peptide sidechains. **C.** Graph of the direct and water-mediated H-bonds with three or fewer waters. The minimum H-bond occupancy shown is 10% in panel A, and 30% in panels B-C. **D.** View, from the membrane surface, of the eight peptides color coded to match the nodes in panels A, B and C. Glu, Gln, His, and Trp sidechains of peptide G are shown as van der Waals spheres. **E**. Close view of peptide A with labels for selected sidechains.

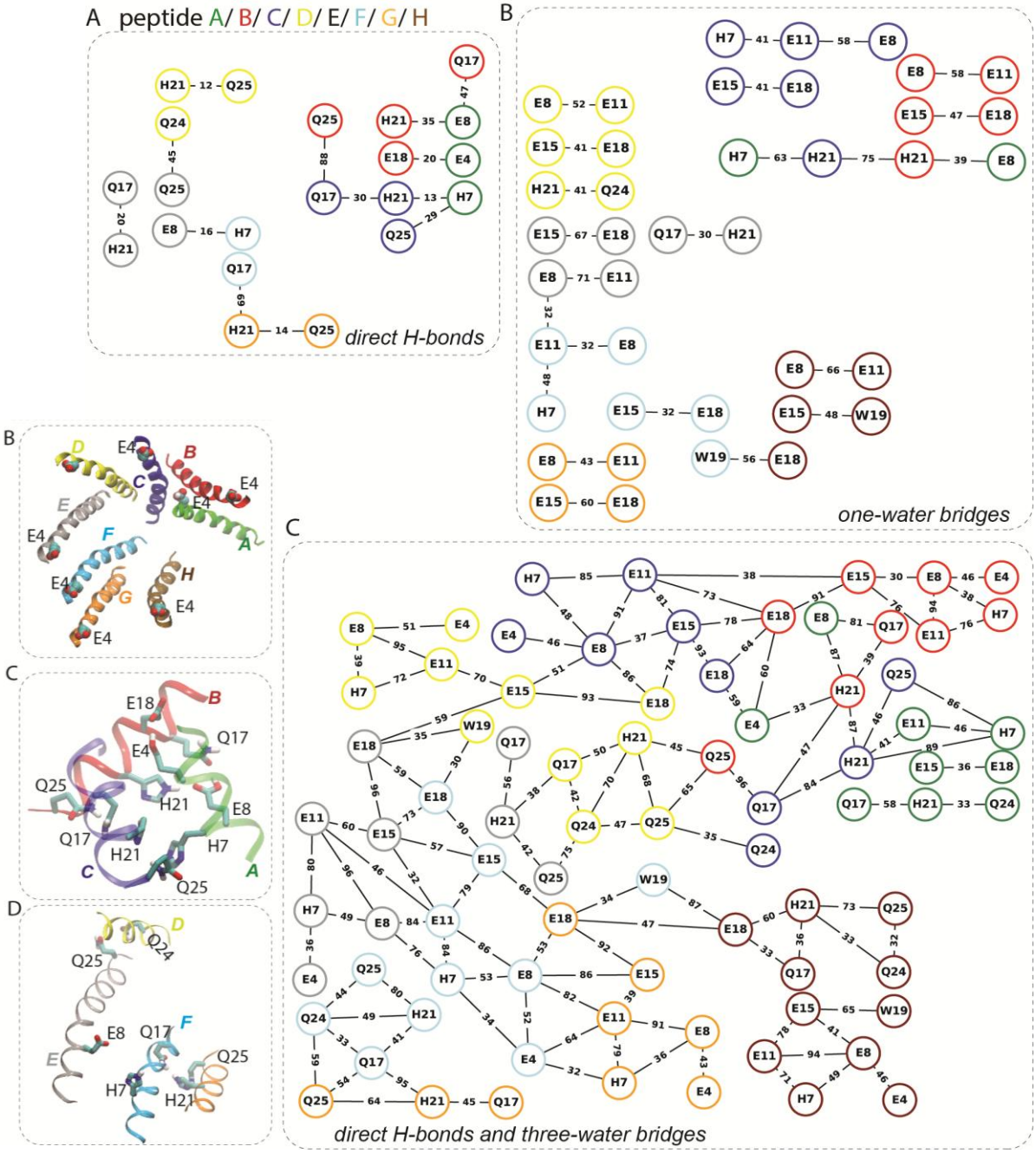

Figure S9. H-bond graphs computed from **Sim 5-/L*** in which peptides B-H have deptotonated Glu and His residues and peptide A has protonated Glu and deprotonated His. The red asterisks in panels A and C indicate sites where peptides inter-connect. Values on the connecting lines are % occupancy for that interaction over the last 200 ns of the simulation. **A.** Graph of the direct H-bonds between peptide sidechains. **B.** Graph of the one-water-mediated bridges between peptide sidechains. **C.** Graph of the direct and water-mediated H-bonds with three or fewer waters. The minimum H-bond occupancy shown is 10% in panel A, and 30% in panels B-C. **D.** View, from the membrane surface, of the eight peptides color coded to match the nodes in panels A, B and C. **E&F.** Individual views of individual peptides in the pore.

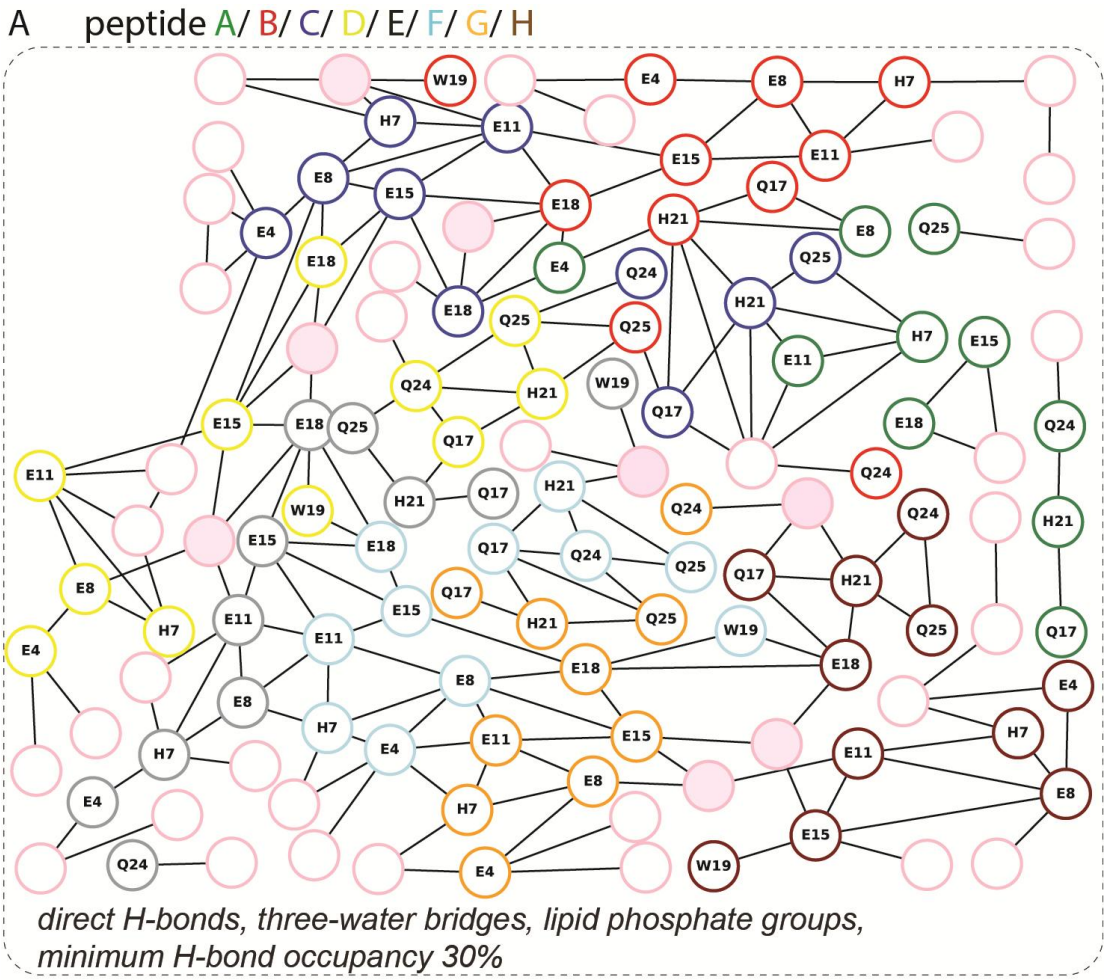

Figure S10. **Sim 5-*/L** Lipid, water, and peptides H-bond network computed from simulations with peptides B-H in deprotonated states, and peptide A, with neutral Glu sidechains. The initial configuration had lipids inside the region delineated by the peptides (Figure S1). Lipid nodes that inter-connect sidechains from two distinct peptides are shown as color-filled circles. H-bond graphs shown at H-bond occupancies of at least 30% (panel A), vs. at least 50% (panel B).

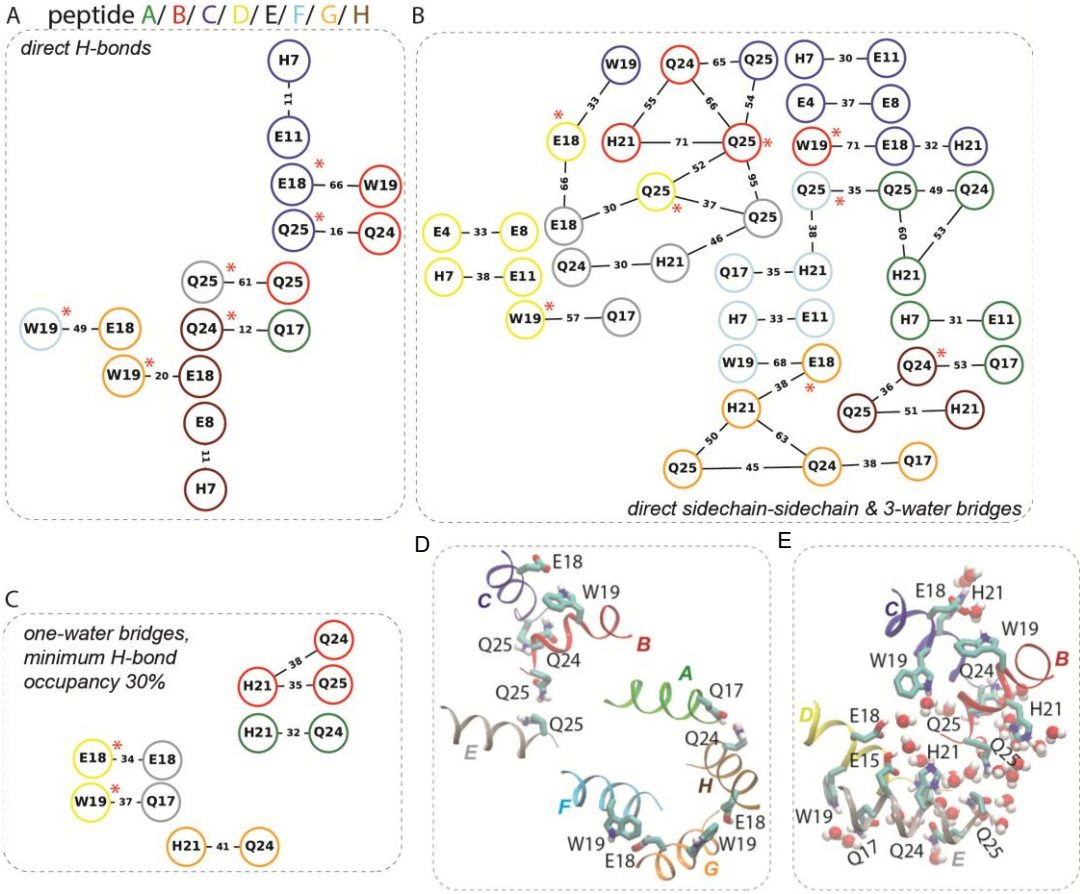

Figure S11. H-bond graphs computed from **Sim 2+/L** in which peptides have protonated Glu and deprotonated His and are thus uncharged. The red asterisks in panels A and C indicate sites where peptides inter-connect. Values on the connecting lines are % occupancy for that interaction over the last 200 ns of the simulation. **A.** Graph of the direct H-bonds between peptide sidechains. **B.** Graph of the direct and water-mediated H-bonds with three or fewer waters. **C.** Graph of the one-water-mediated bridges between peptide sidechains. The minimum H-bond occupancy shown is 10% in panel A, and 30% in panels B-C. **D&E.** View, from the membrane surface, of the eight peptides color coded to match the nodes in panels A, B and C.

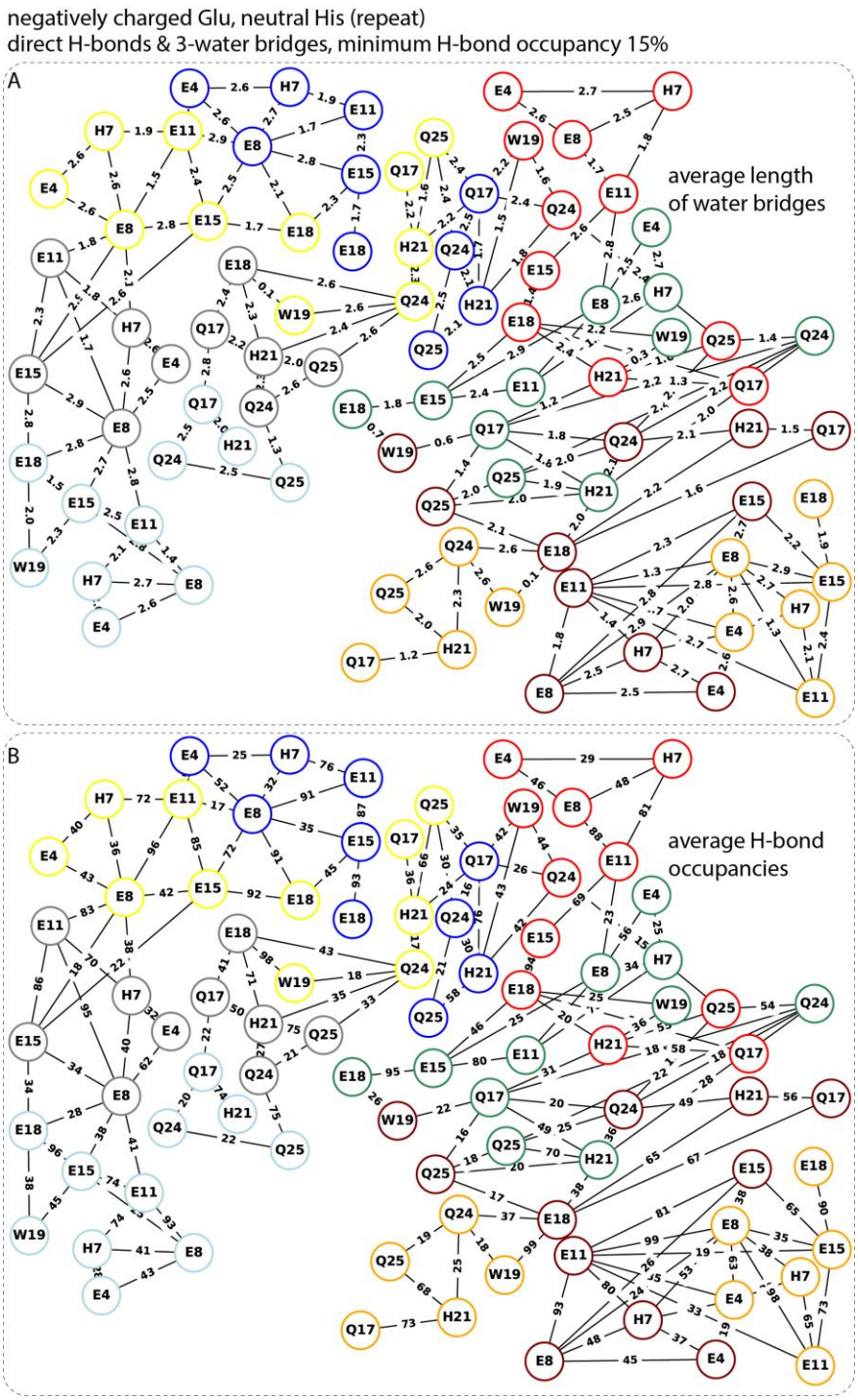

Figure S12. **Sim 5-/L repeat.** H-bond graph for direct H-bonds between sidechains and water-mediated bridges with up to 3 H-bonded waters computed from the repeat simulation with standard protonation states for all Glu and His sidechains. The minimum H-bond occupancy shown is 15%. (A, B) H-bond graphs with edges marked according to the average water bridge (panel A) vs. average H-bond occupancy (panel B).

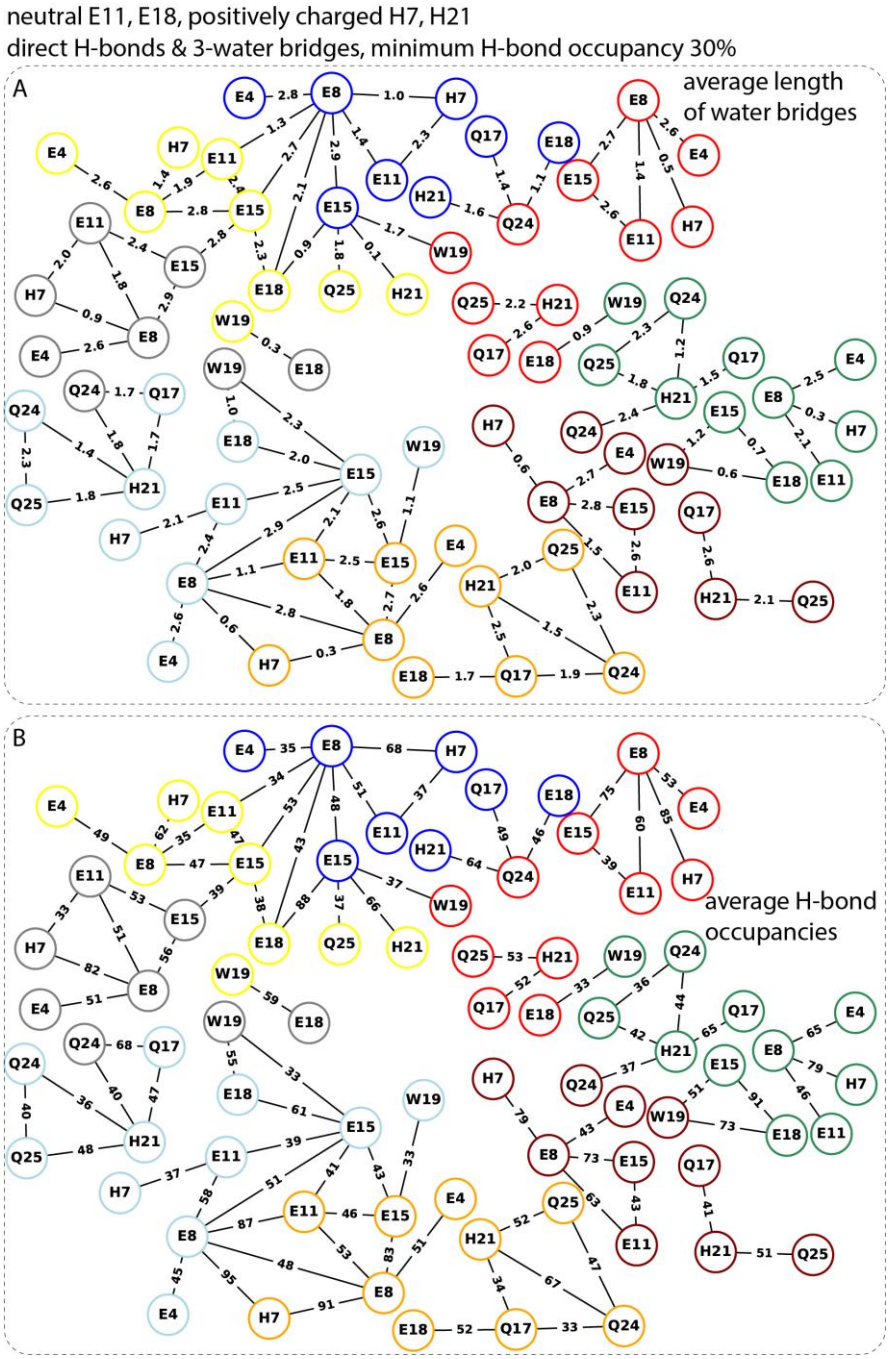

Figure S13. **Sim 3-/L repeat.** H-bond graph for direct H-bonds between sidechains and water-mediated bridges with up to 3 H-bonded waters computed from the with H-bond graph for direct H-bonds between sidechains and water-mediated bridges with up to 3 H-bonded waters computed from the repeat simulation with Glu charged and The minimum H-bond occupancy shown is 30%. (A, B) H-bond graphs with edges marked according to the average water bridge (panel A) vs. average H-bond occupancy (panel B).

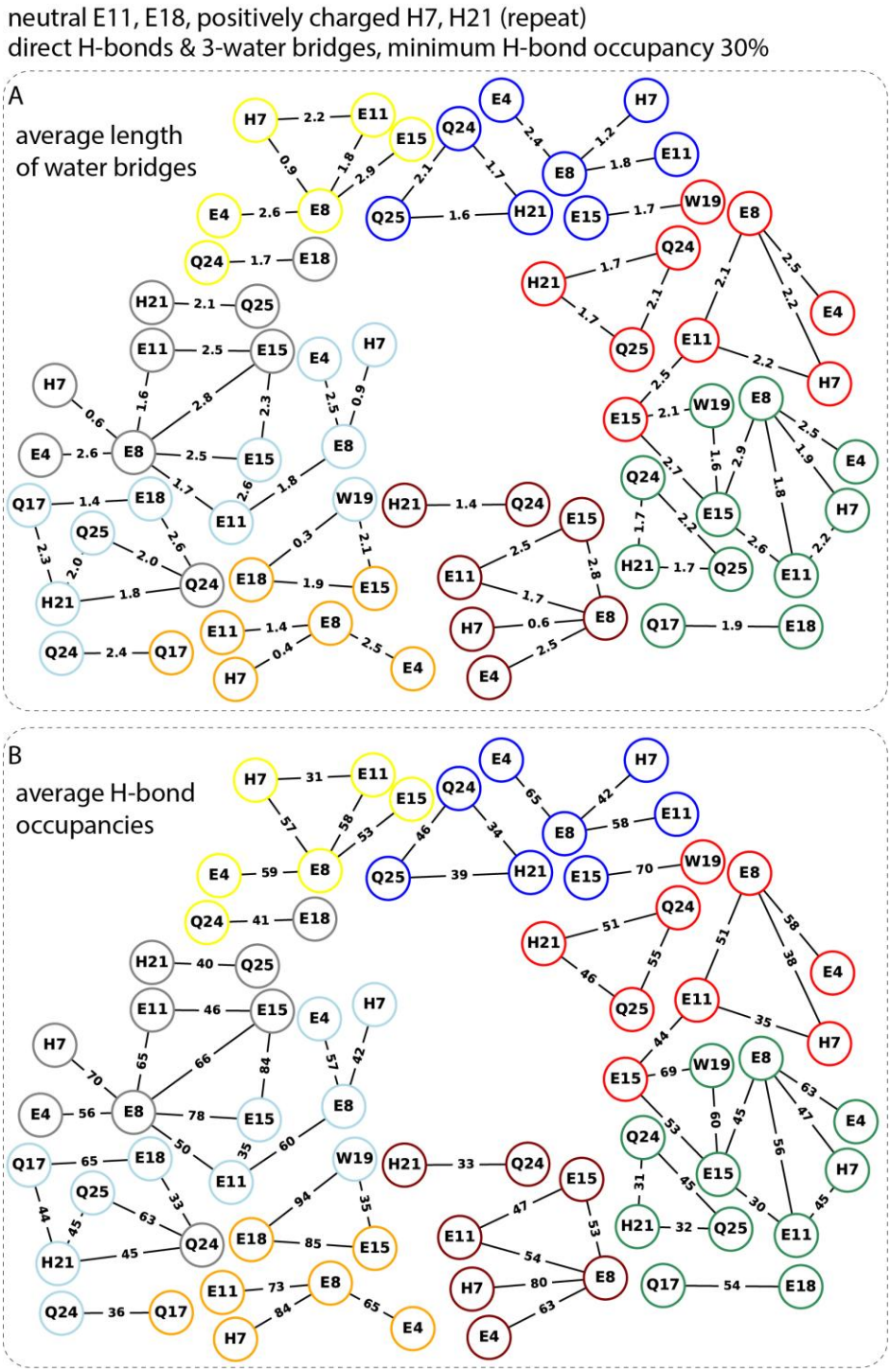

Figure S14. Sim 1-/L repeat. H-bond graph for direct H-bonds between sidechains and water-mediated bridges with up to 3 H-bonded waters computed from the repeat simulation with E11 and E18 neutral, and H7 and H21, positively charged. The minimum H-bond occupancy shown is 30%. (A, B) H-bond graphs with edges marked according to the average water bridge (panel A) vs. average H-bond occupancy (panel B).

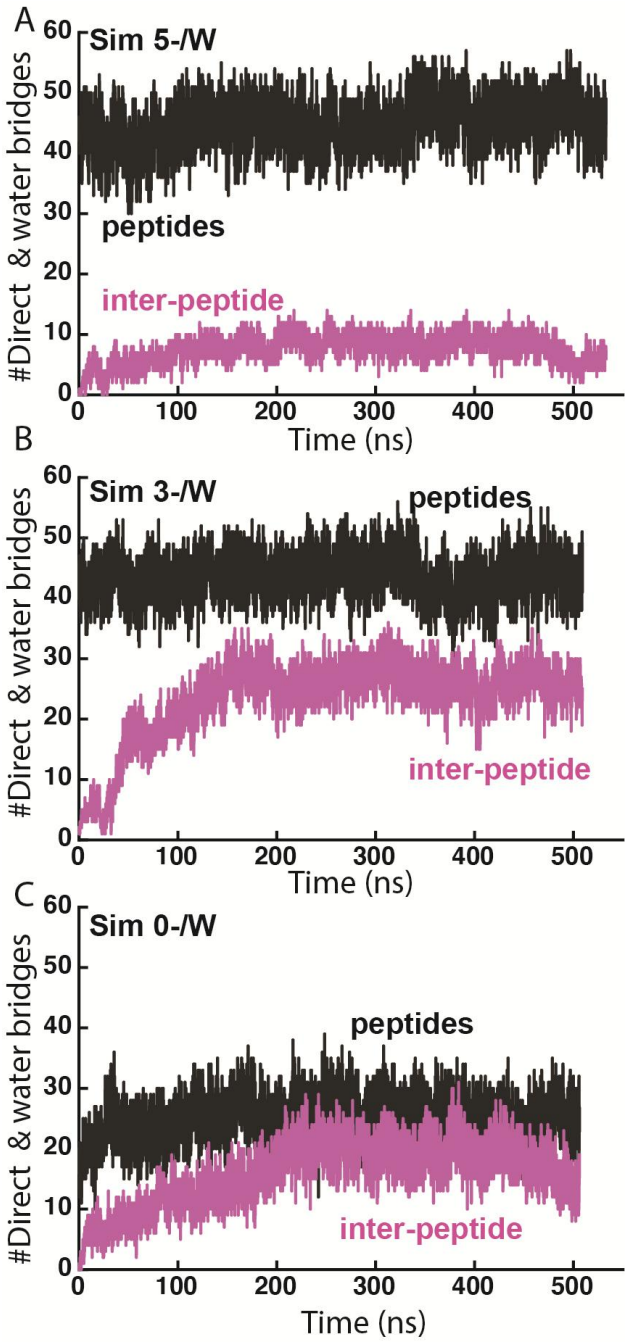

Figure S15. Time series of the number of H-bonds sampled during simulations started with water inside the region delineated by the pores.

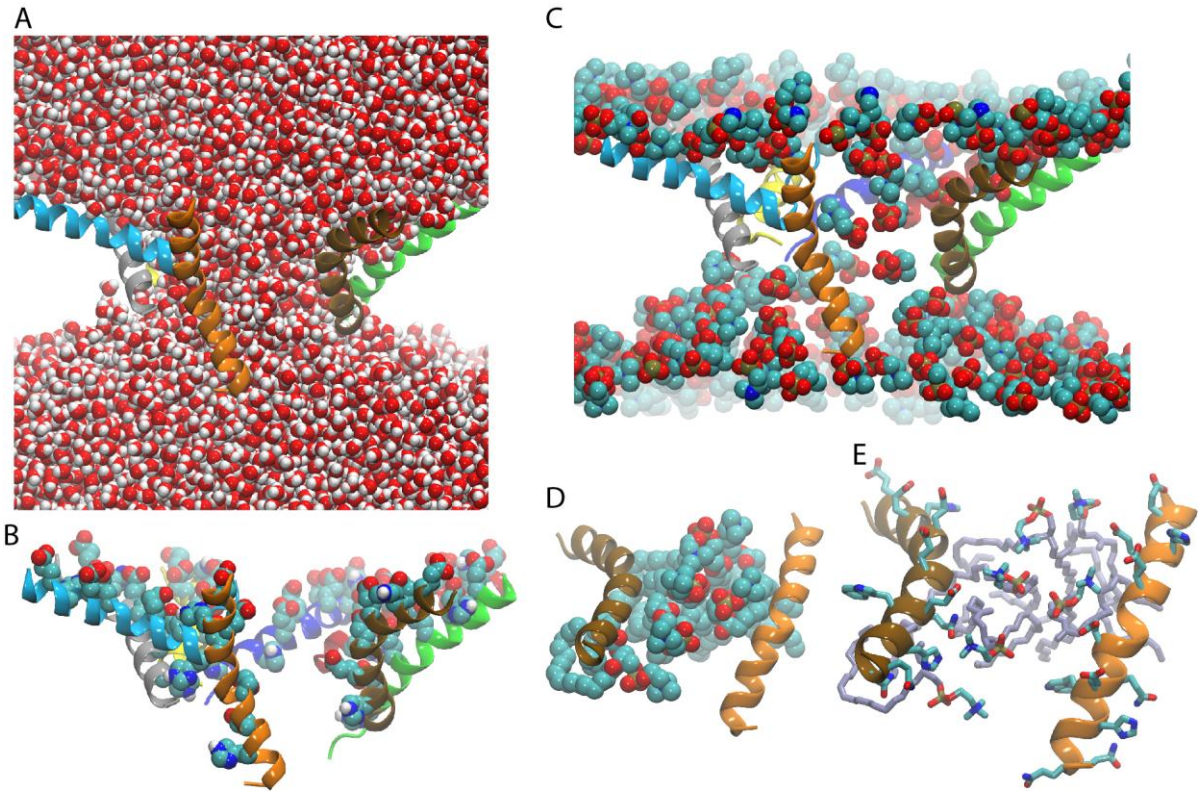

Figure S16. Final structures from **Sim 5-/W** in which peptides have deprotonated Glu and deprotonated His. Initial structure had water in the areas delineated by peptide. (A, B) Cut-away view of the peptides showing water inside the region delineated by the peptides (panel A) and lipids (panel B). Note that the peptide colored green has partially de-inserted. (C, D) Close view of the peptides viewed laterally along the membrane normal (panel C) vs. from the membrane interface (panel D). (E) The same representation of the peptides as in panel D, now with the Glu, His, Gln, and Trp sidechains shown as van der Waals spheres. Glu and His are in atom colors, Gln, violet, and Trp, iceblue.

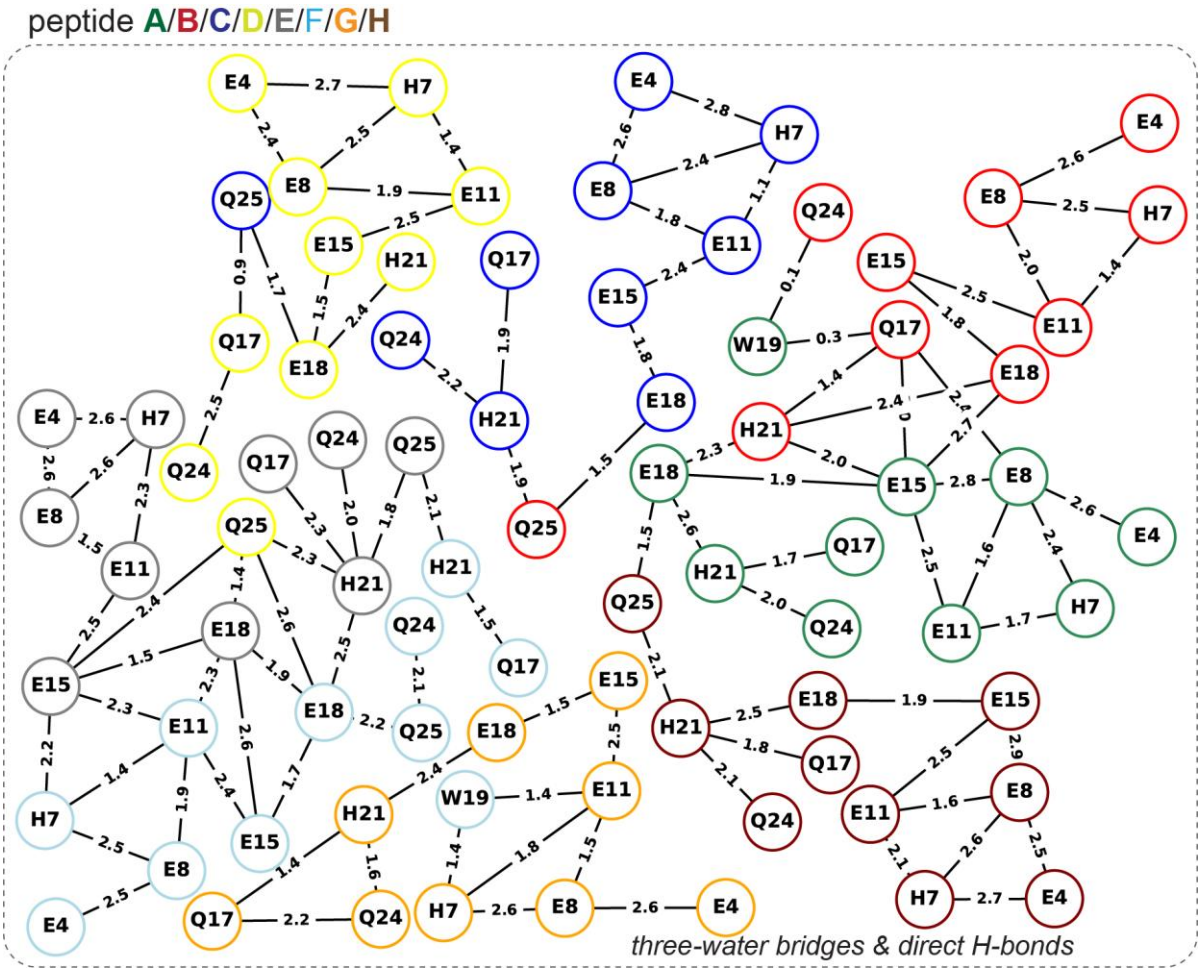

Figure S17. H-bond graph for Sim 5-/W in which peptides have deprotonated Glu and deprotonated His. Initial structure had water in the areas delineated by peptide. Direct H-bonds between sidechains and water-mediated bridges with up to 3 H-bonded waters The minimum H-bond occupancy shown is 30%. Values on the edges indicate the average number of water molecules bridging the interactions.

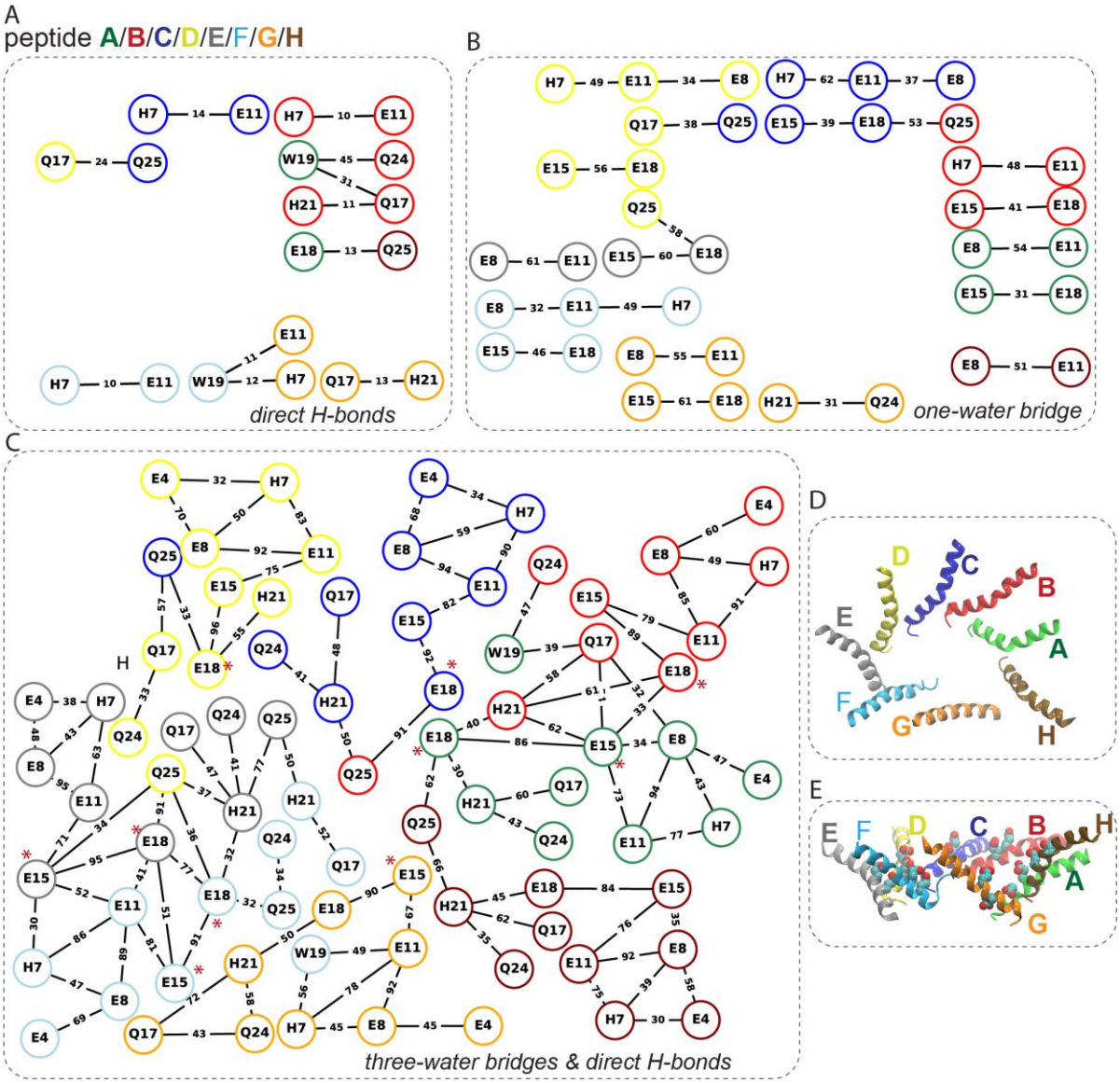

Figure S18. H-bond graphs computed from **Sim 5-/W** in which peptides have deprotonated Glu and deprotonated His. Initial structure had water in the areas delineated by peptide. The red asterisks in panels A and C indicate sites where peptides inter-connect. Values on the connecting lines are % occupancy for that interaction over the last 200 ns of the simulation. **A.** Graph of the direct H-bonds between peptide sidechains. **B.** Graph of the one-water-mediated bridges between peptide sidechains. **C.** Graph of the direct and water-mediated H-bonds with three or fewer waters. The minimum H-bond occupancy shown is 10% in panel A, and 30% in panels B-C. **D&E.** View, from the membrane surface, of the eight peptides color coded to match the nodes in panels A, B and C.

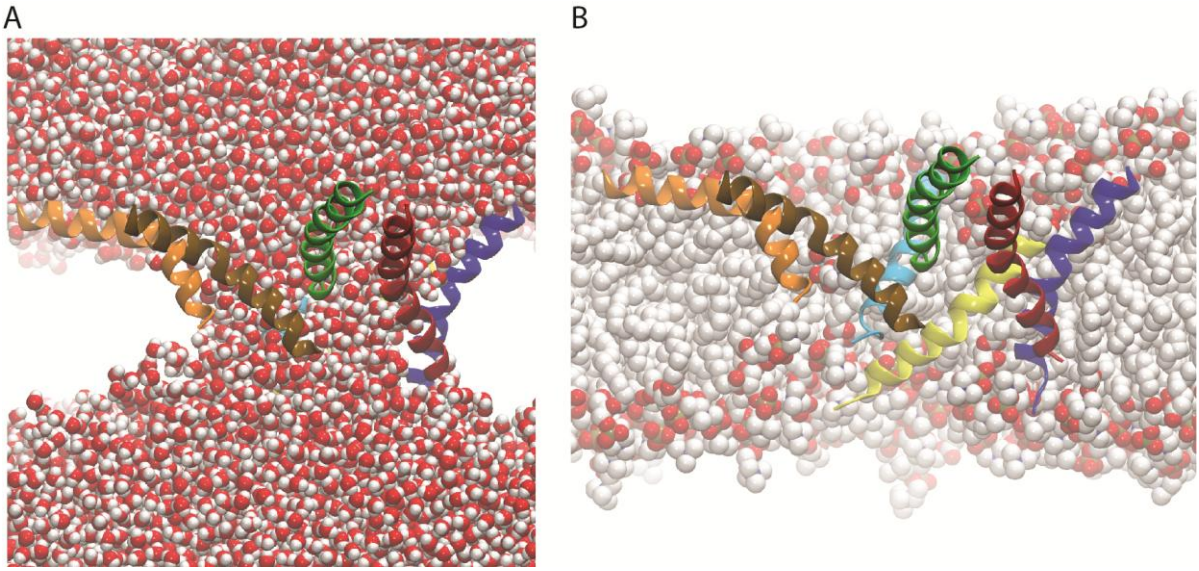

Figure S19. Final structures from **Sim 3-/W** in which peptides have deprotonated Glu and protonated His. Initial structure had water in the areas delineated by peptide. (A, B) Cut-away view of the peptides showing water inside the region delineated by the peptides (panel A) and lipids (panel B). Note that the peptide colored green has partially de-inserted. (C, D) Close view of the peptides viewed laterally along the membrane normal (panel C) vs. from the membrane interface (panel D). (E) The same representation of the peptides as in panel D, now with the Glu, His, Gln, and Trp sidechains shown as van der Waals spheres. Glu and His are in atom colors, Gln, violet, and Trp, iceblue.

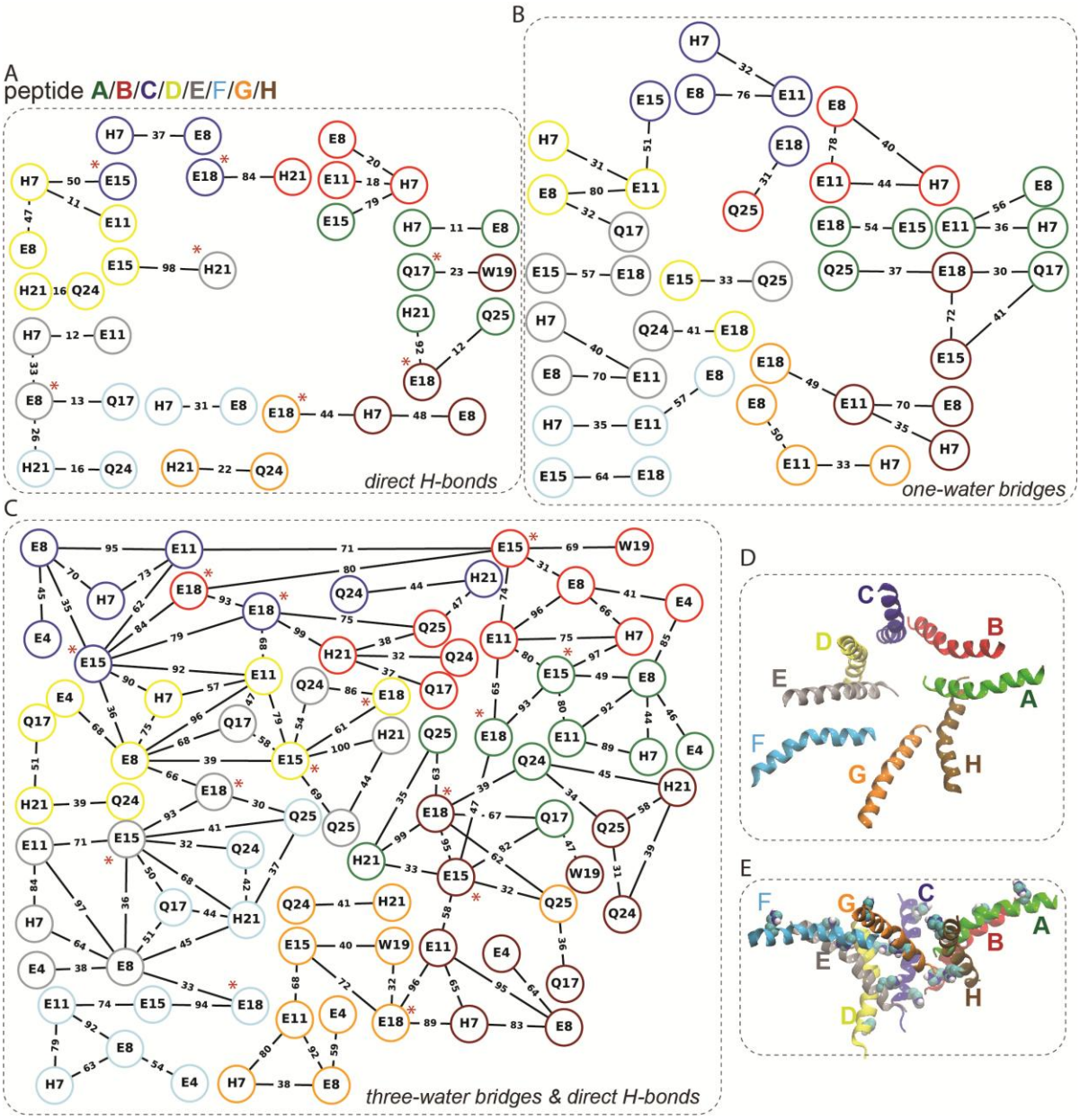

Figure S20 H-bond graphs computed from **Sim 3-/W** in which peptides have deprotonated Glu and protonated His. Initial structure had water in the areas delineated by peptide. The red asterisks in panels A and C indicate sites where peptides inter-connect. Values on the connecting lines are % occupancy for that interaction over the last 200 ns of the simulation. **A.** Graph of the direct H-bonds between peptide sidechains. **B.** Graph of the one-water-mediated bridges between peptide sidechains. **C.** Graph of the direct and water-mediated H-bonds with three or fewer waters. The minimum H-bond occupancy shown is 10% in panel A, and 30% in panels B-C. **D&E.** View, from the membrane surface, of the eight peptides color coded to match the nodes in panels A, B and C.

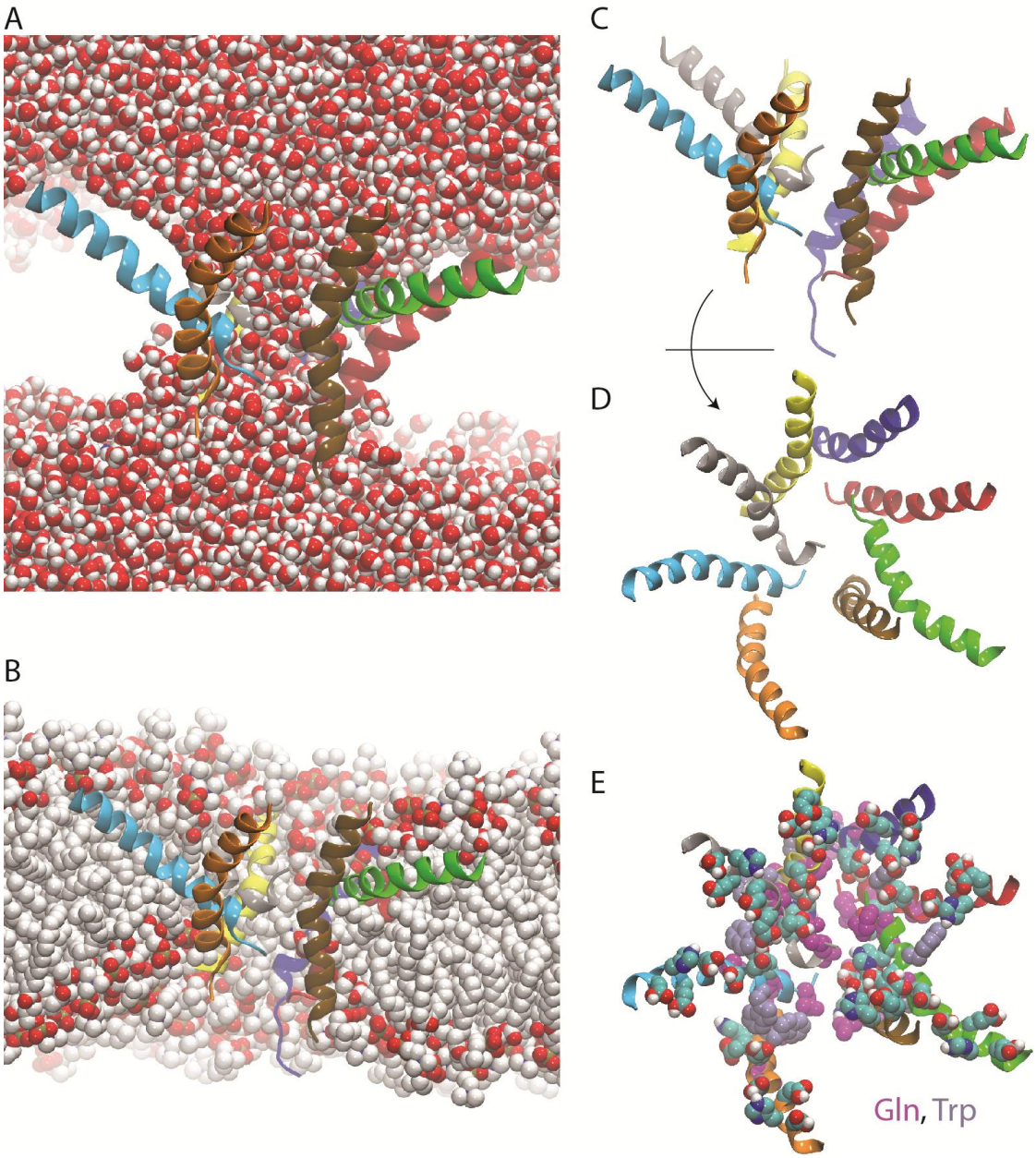

Figure S21. Final structures from **Sim 0-/W** in which peptides have protonated Glu and deprotonated His and are thus uncharged. Initial structure had water in the areas delineated by peptide. (A, B) Cut-away view of the peptides showing water inside the region delineated by the peptides (panel A) and lipids (panel B). Note that the peptide colored green has partially de-inserted. (C, D) Close view of the peptides viewed laterally along the membrane normal (panel C) vs. from the membrane interface (panel D). (E) The same representation of the peptides as in panel D, now with the Glu, His, Gln, and Trp sidechains shown as van der Waals spheres. Glu and His are in atom colors, Gln, violet, and Trp, iceblue.

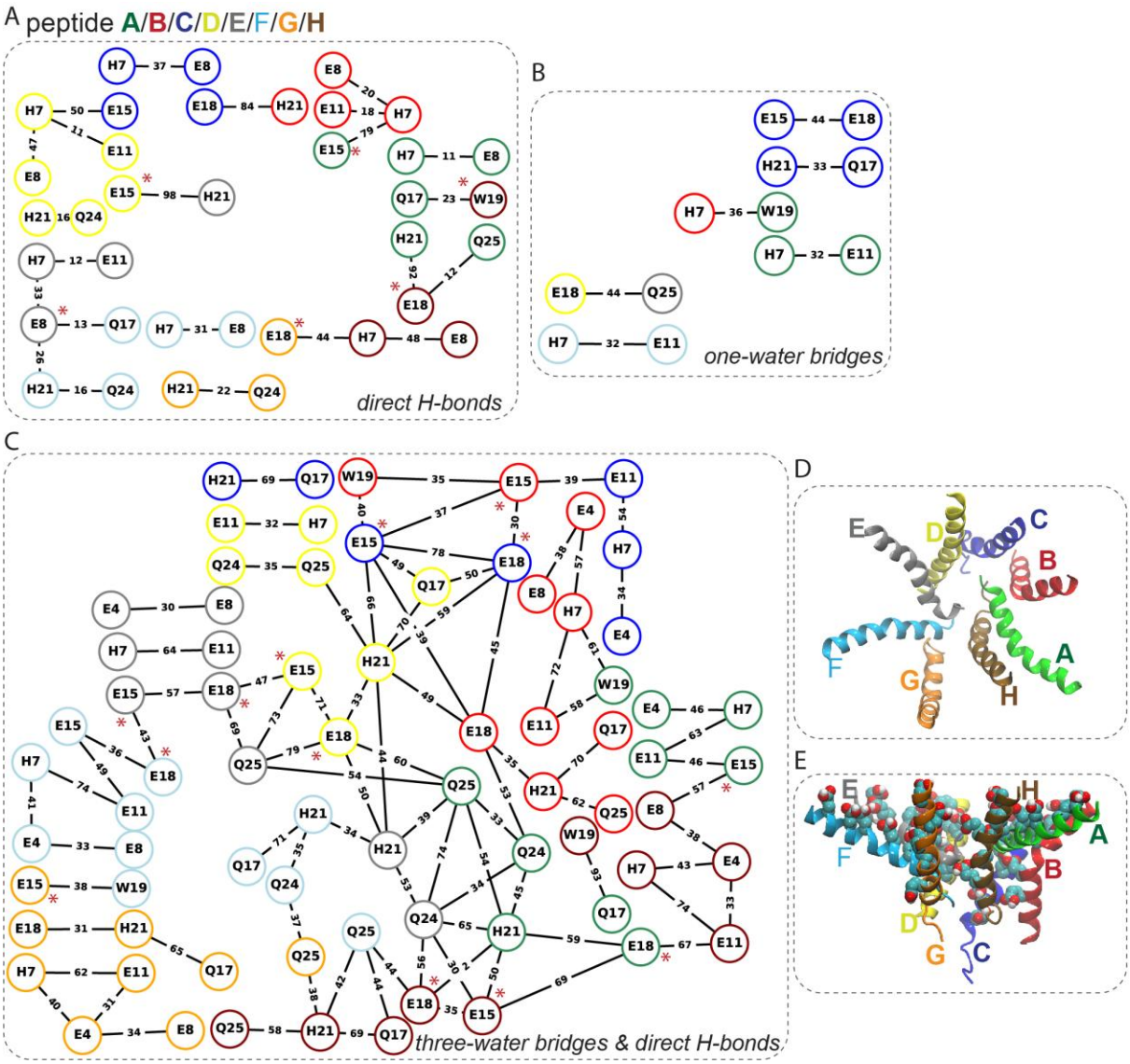

Figure S22. H-bond graphs computed from **Sim 0-/W** in which peptides have protonated Glu and deprotonated His and are thus uncharged. Initial structure had water in the areas delineated by peptide. The red asterisks in panels A and C indicate sites where peptides inter-connect. Values on the connecting lines are % occupancy for that interaction over the last 200 ns of the simulation. **A.** Graph of the direct H-bonds between peptide sidechains. **B.** Graph of the one-water-mediated bridges between peptide sidechains. **C.** Graph of the direct and water-mediated H-bonds with three or fewer waters. The minimum H-bond occupancy shown is 10% in panel A, and 30% in panels B-C. **D&E.** View, from the membrane surface, of the eight peptides color coded to match the nodes in panels A, B and C.

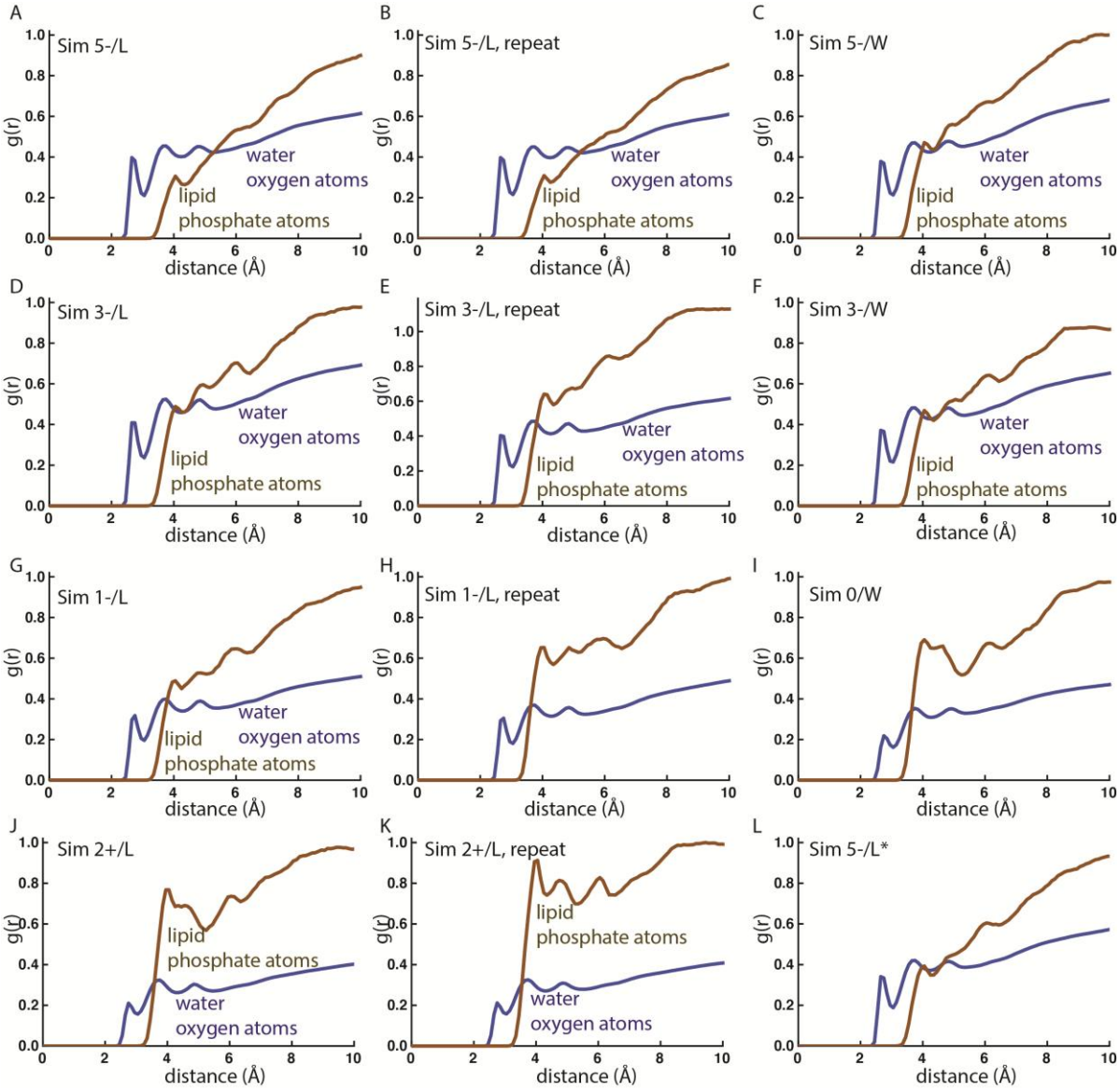

Figure S23. Radial distribution functions of the water oxygen atoms (blue) and lipid phosphate atoms (brown) within 10 Å of the peptide hetero-atoms. A-L: Results from all simulations are shown. Radial distribution functions computed from each of the simulations using the Radial Pair Distribution Function plugin in VMD. We used ~4500 equally spaced coordinate snapshots from the last about 45 ns of each simulation.