Supplementary Information

Title: Revealing the Interaction between Peptide Drugs and Permeation Enhancers in the Presence of Intestinal Bile Salts

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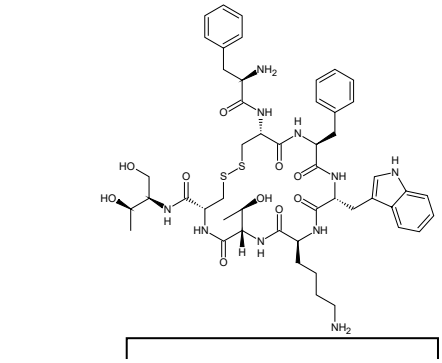
Table of contents:

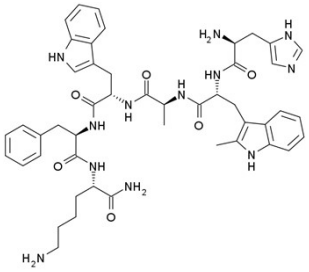
Supplementary Figures

(a) Octreotide



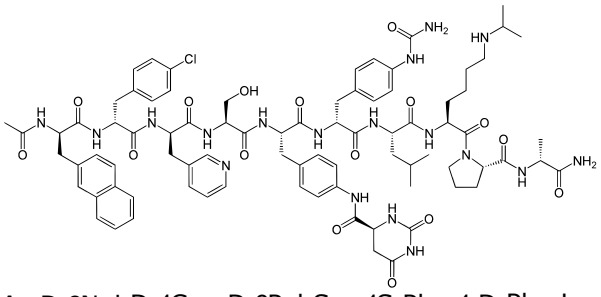
H—DPhe—Cys—Phe—DTrp—Lys—Thr—Cys—Thr—ol

(b) Hexarelin



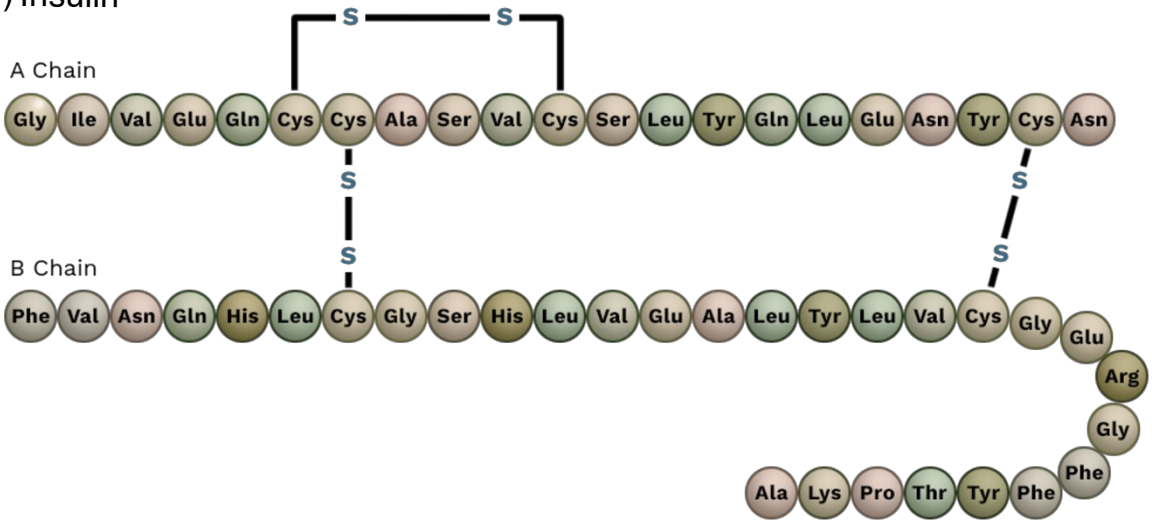
H- His-D-2-Methyl-Trp-Ala-Trp-D-Phe-Lys-NH2

(c) Degarelix



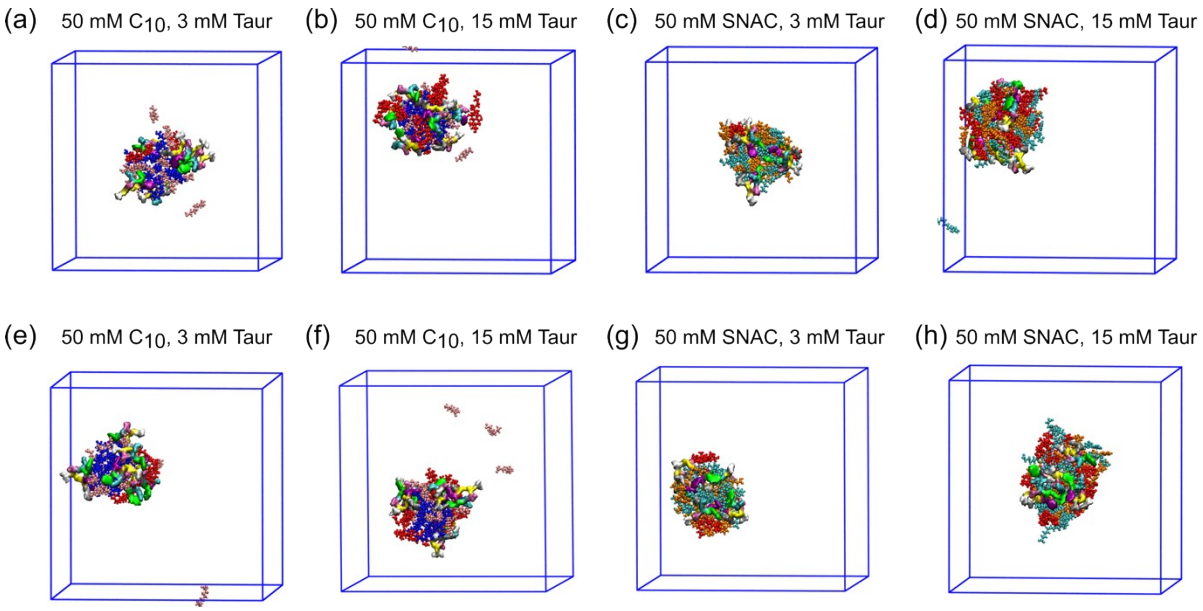
Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4S-Phe-4-D-Phe-Leu-N6-Lys-Pro-D-Ala-NH2

(d) Insulin

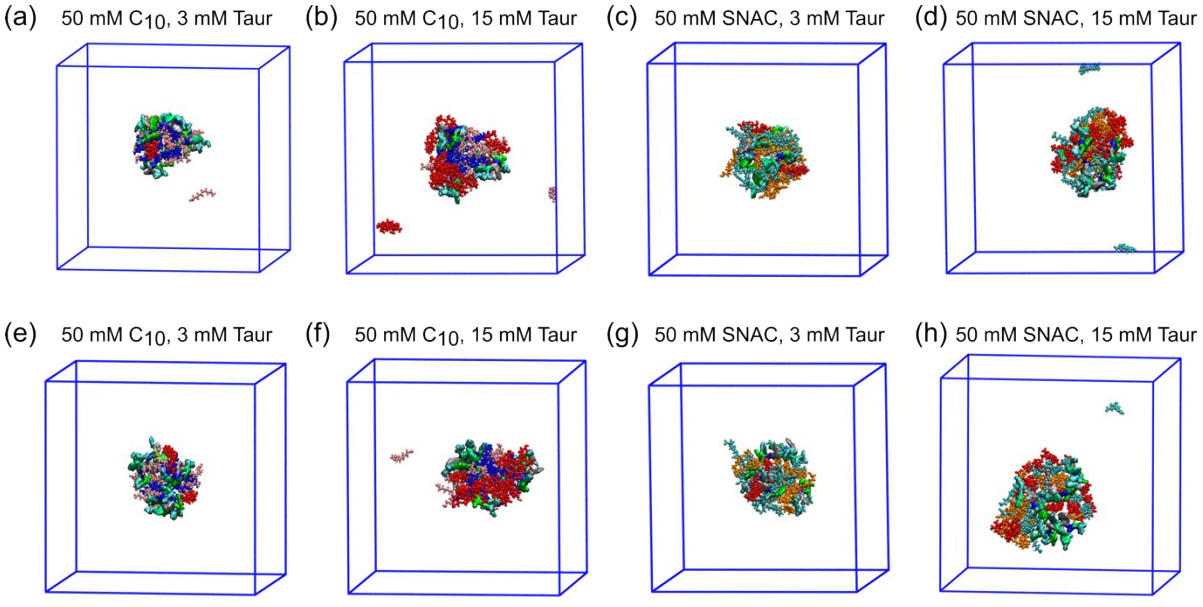


Supplementary Figure 1: Molecular structures and amino acid sequence of the investigated peptides.

Note that, the complete name for degarelix is as follows- N-Acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-4-[[[(4S)-hexahydro-2,6-dioxo-4-pyrimidinyl]carbonyl]amino]-L-phenylalanyl-4-[(aminocarbonyl)amino]-D-phenylalanyl-L-leucyl-N6-(1-methylethyl)-L-lysyl-L-prolyl-D-alaninamide

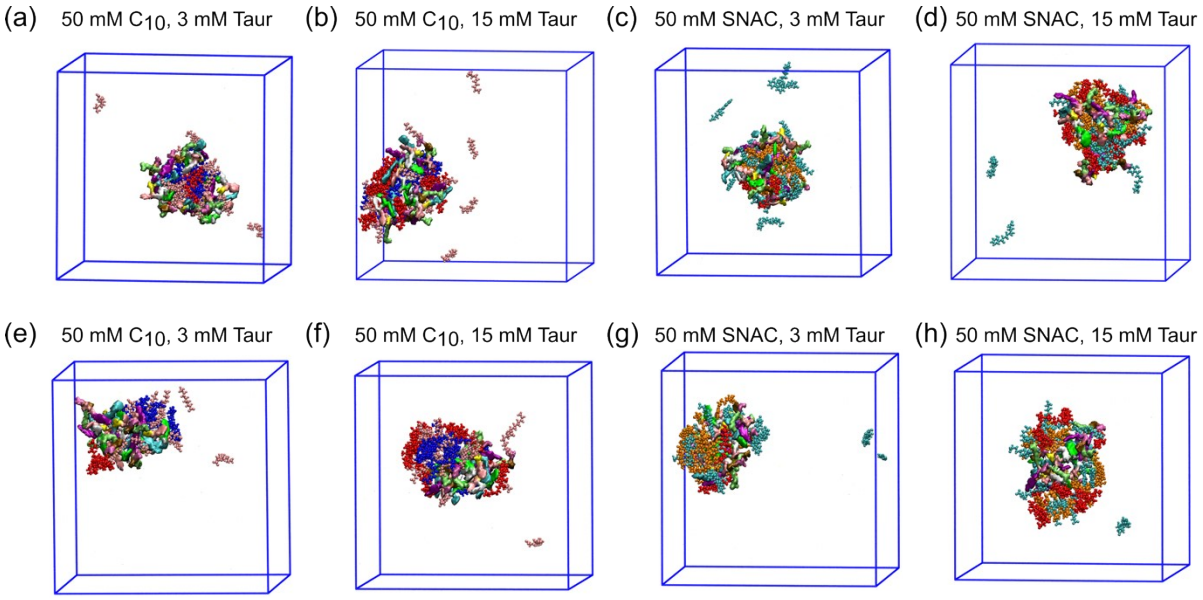


Supplementary Figure 2: Representative snapshots at the end of the simulations for different systems containing Octreotide. Panels (a-d) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. Peptides are shown by the surface representation. Caprate, SNAC, and taurocholate molecules are presented by CPK. The neutral and negatively charged caprates are represented by blue and pink, respectively. The neutral and negatively charged SNAC are represented by orange and cyan, respectively. Taurocholate molecules are represented by red color.

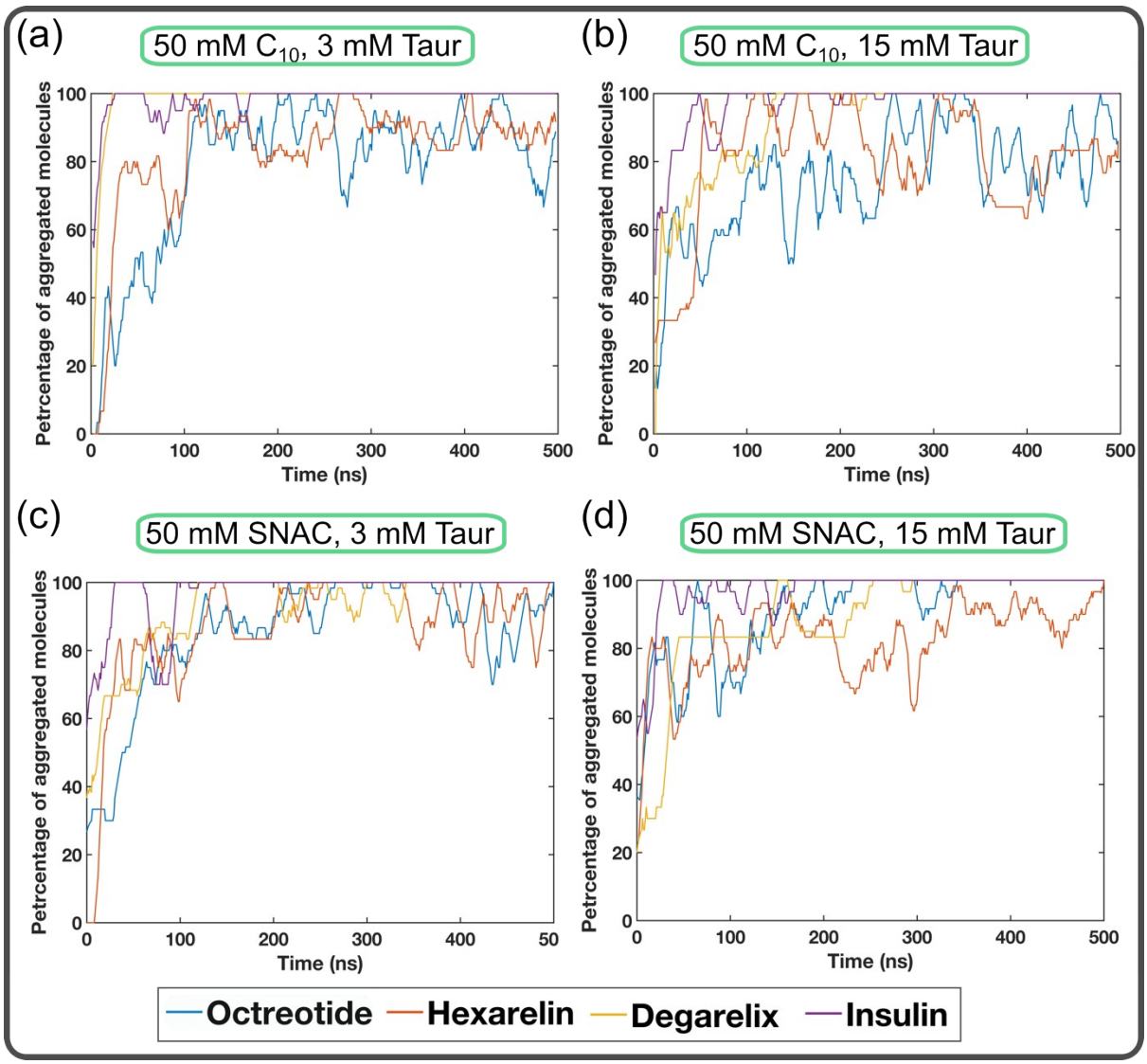


Supplementary Figure 3: Representative snapshots at the end of the simulations for different systems containing Hexarelin. Panels (a-d) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. Peptides are shown by the surface representation. Caprate, SNAC, and taurocholate molecules are presented by CPK. The

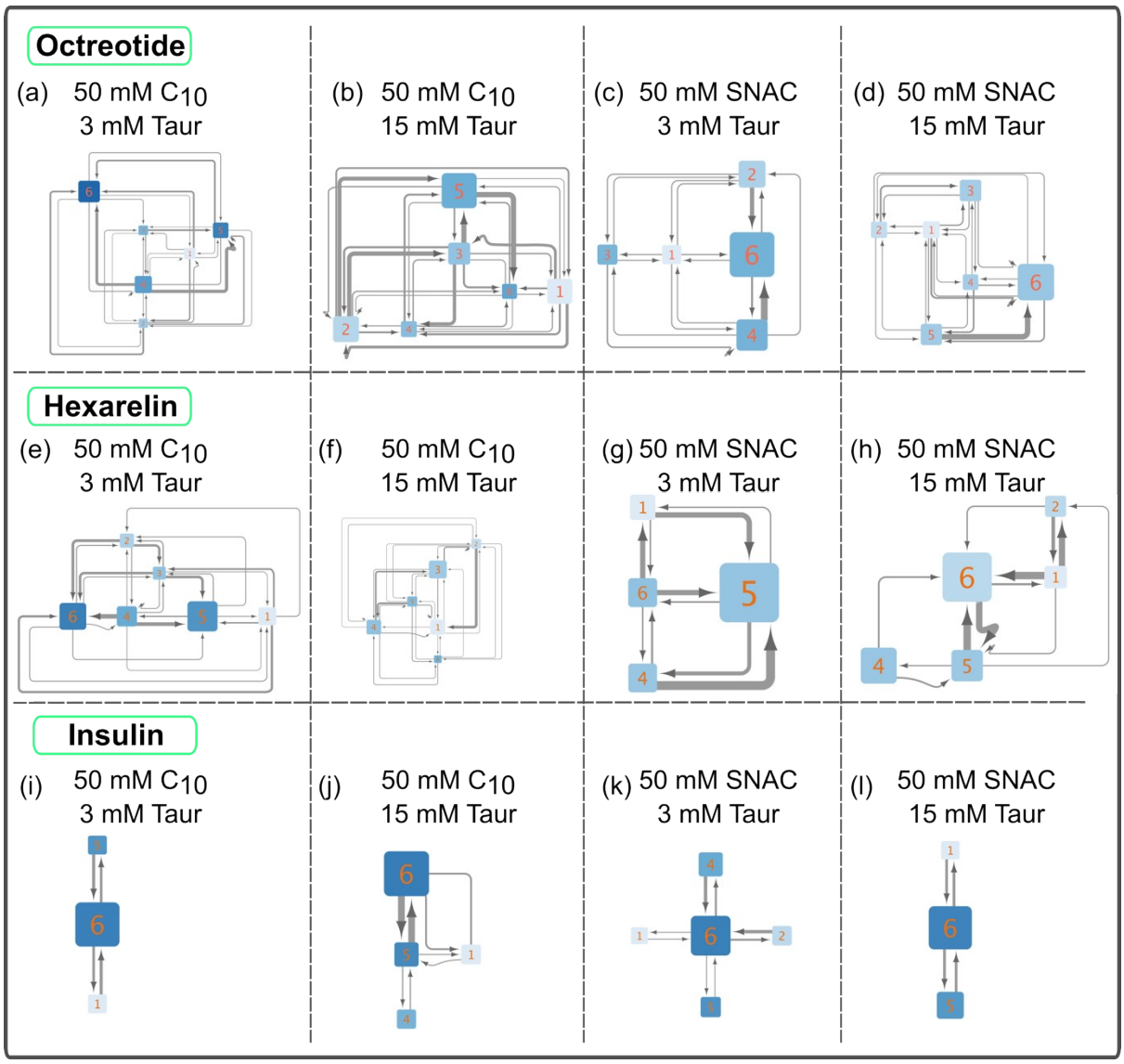
neutral and negatively charged caprates are represented by blue and pink, respectively. The neutral and negatively charged SNAC are represented by orange and cyan, respectively. Taurocholate molecules are represented by red color.



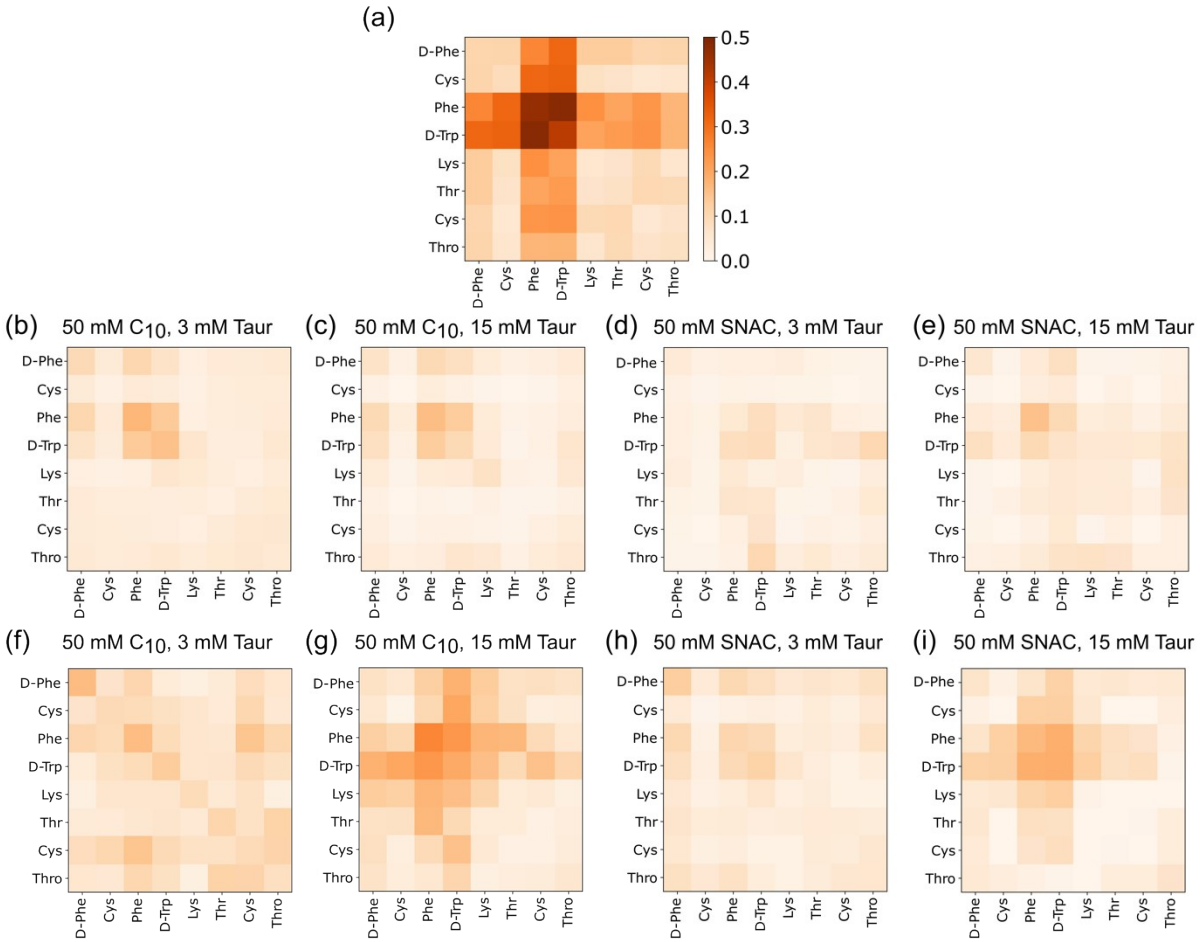
Supplementary Figure 4: Representative snapshots at the end of the simulations for different systems containing Degarelix. Panels (a-d) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. Peptides are shown by the surface representation. Caprate, SNAC, and taurocholate molecules are presented by CPK. The neutral and negatively charged caprates are represented by blue and pink, respectively. The neutral and negatively charged SNAC are represented by orange and cyan, respectively. Taurocholate molecules are represented by red color.



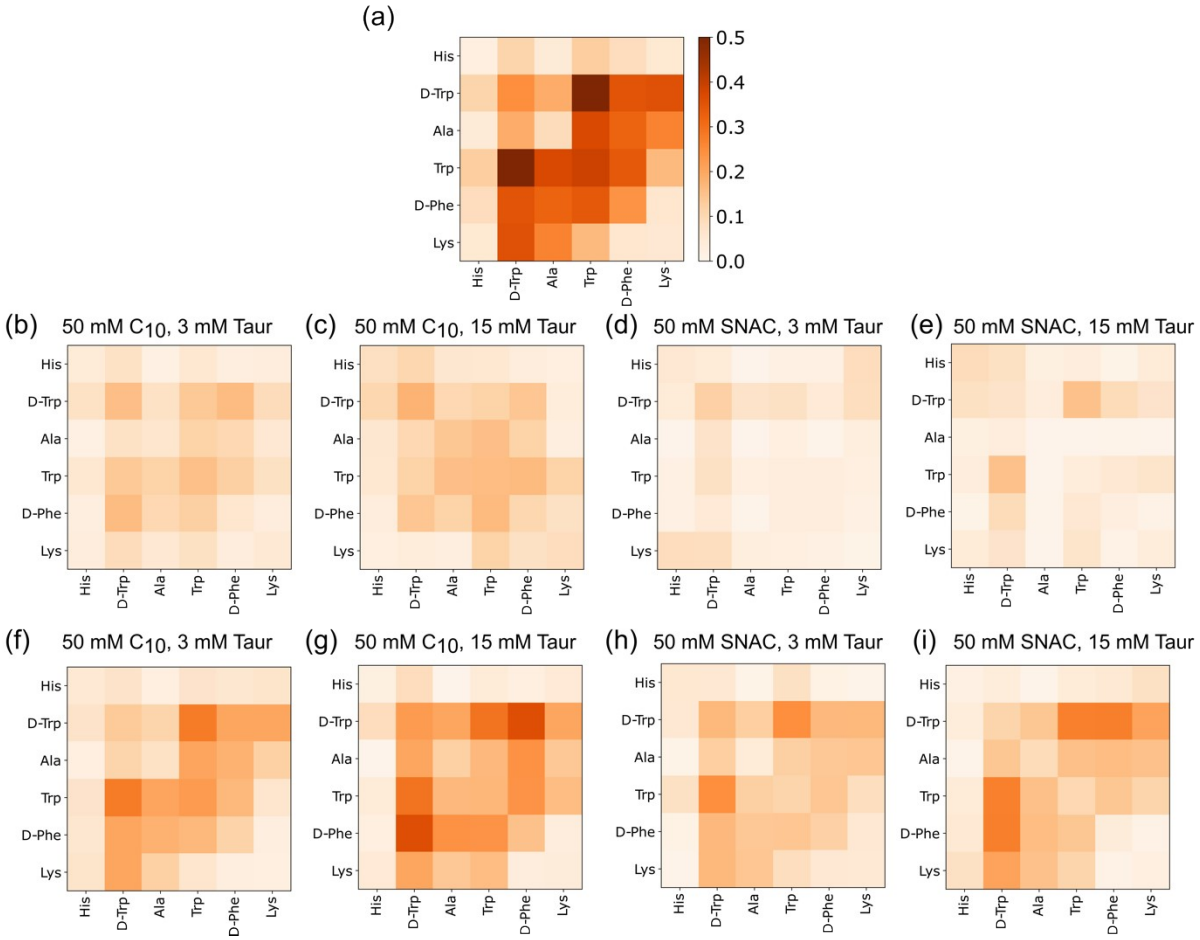
Supplementary Figure 5: The variation in the percentage of aggregated molecules for systems with 10 mM peptides and (a) 50 mM C_{10}, and 3 mM Taurocholate, (b) 50 mM C_{10}, and 15 mM Taurocholate, (c) 50 mM SNAC, and 3 mM Taurocholate, and (d) 50 mM SNAC, and 15 mM Taurocholate. The simulations' initial setup featured a starting configuration where all molecules, including the peptides, were randomly placed within the simulation box.



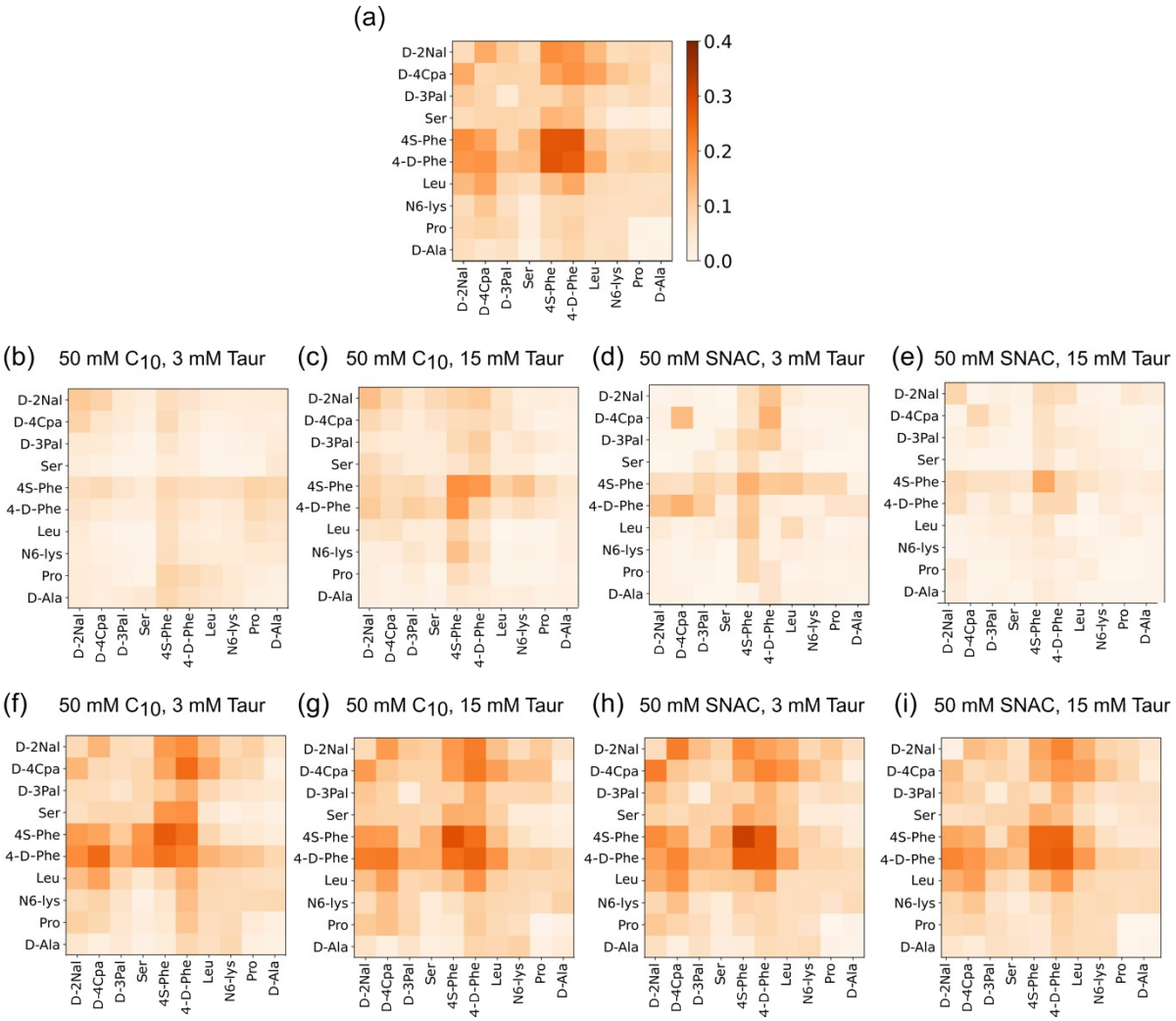
Supplementary Figure 6: Illustrations of peptide aggregation transition networks for systems containing 10 mM peptides, including octreotide (panels a-e), hexarelin (f-j), insulin (k-o), and degarelix (p-t). The simulations' initial setup featured a starting configuration where peptides were in the pre-aggregated form, and PEs and taurocholates were randomly placed within the simulation box. The peptide aggregates were taken from the end of the simulations shown in Figure 1, and are thus of different sizes for each peptide.



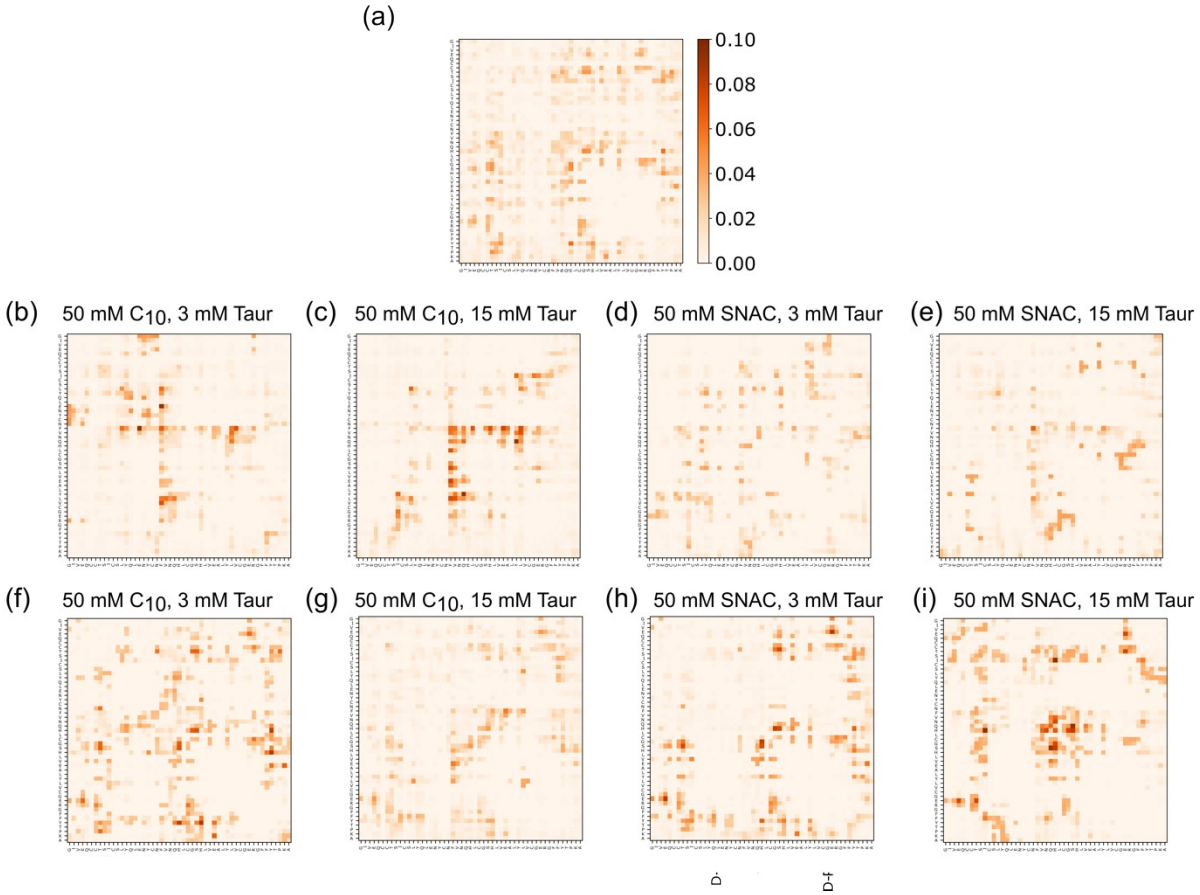
Supplementary Figure 7: Fractional residue-residue contact maps for different systems containing octreotide. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly.



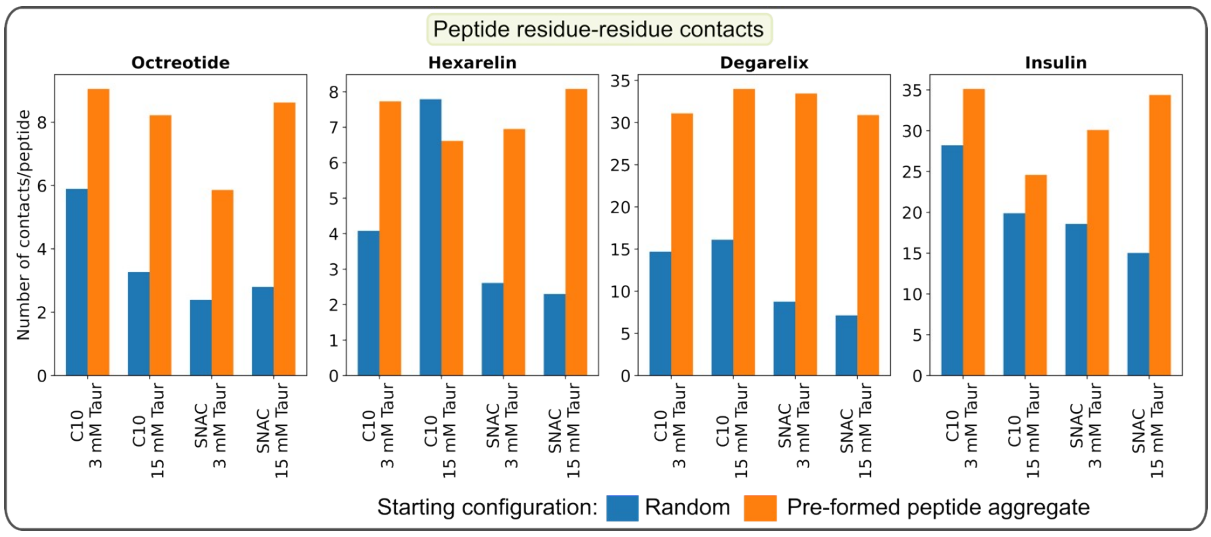
Supplementary Figure 8: Fractional residue-residue contact maps for different systems containing hexarelin. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly.



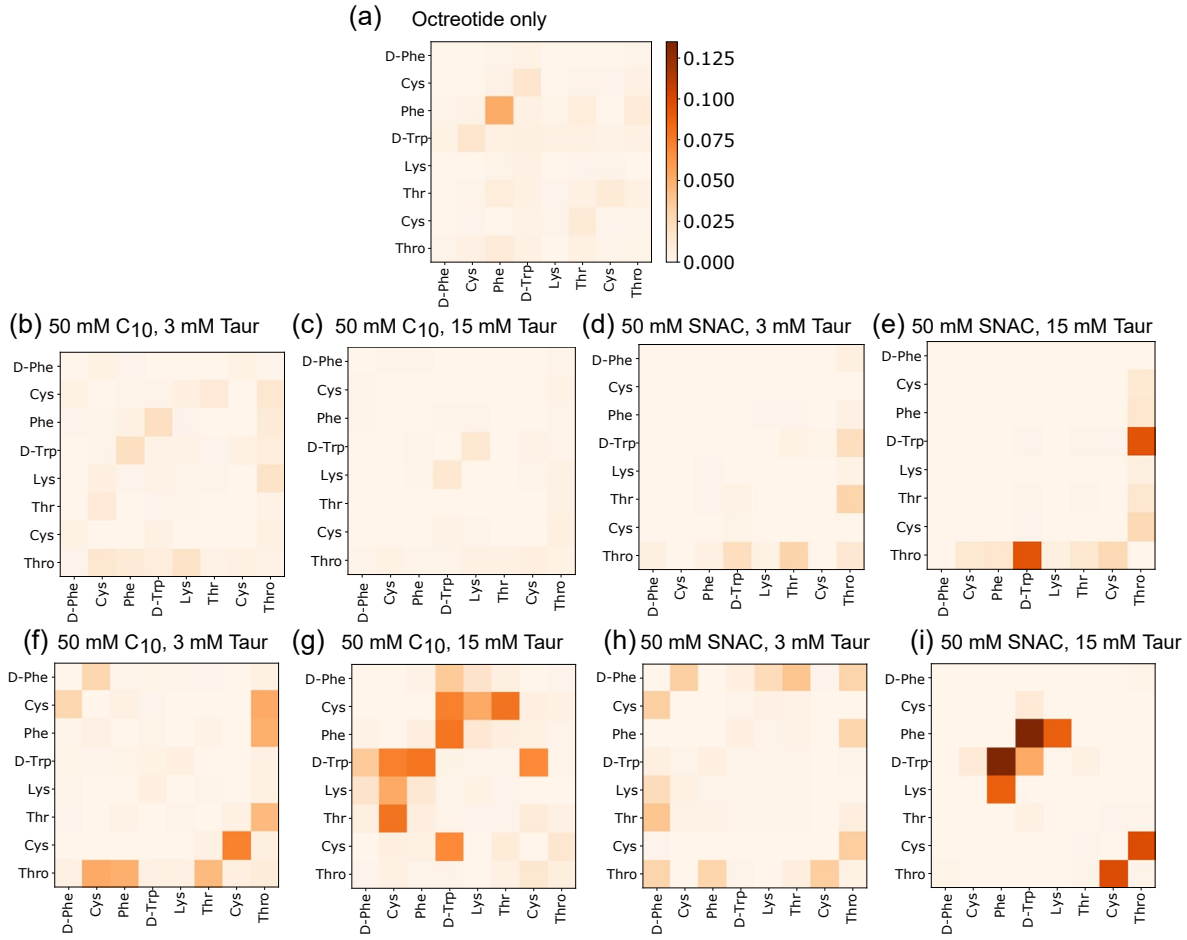
Supplementary Figure 9: Fractional residue-residue contact maps for different systems containing degarelix. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly.



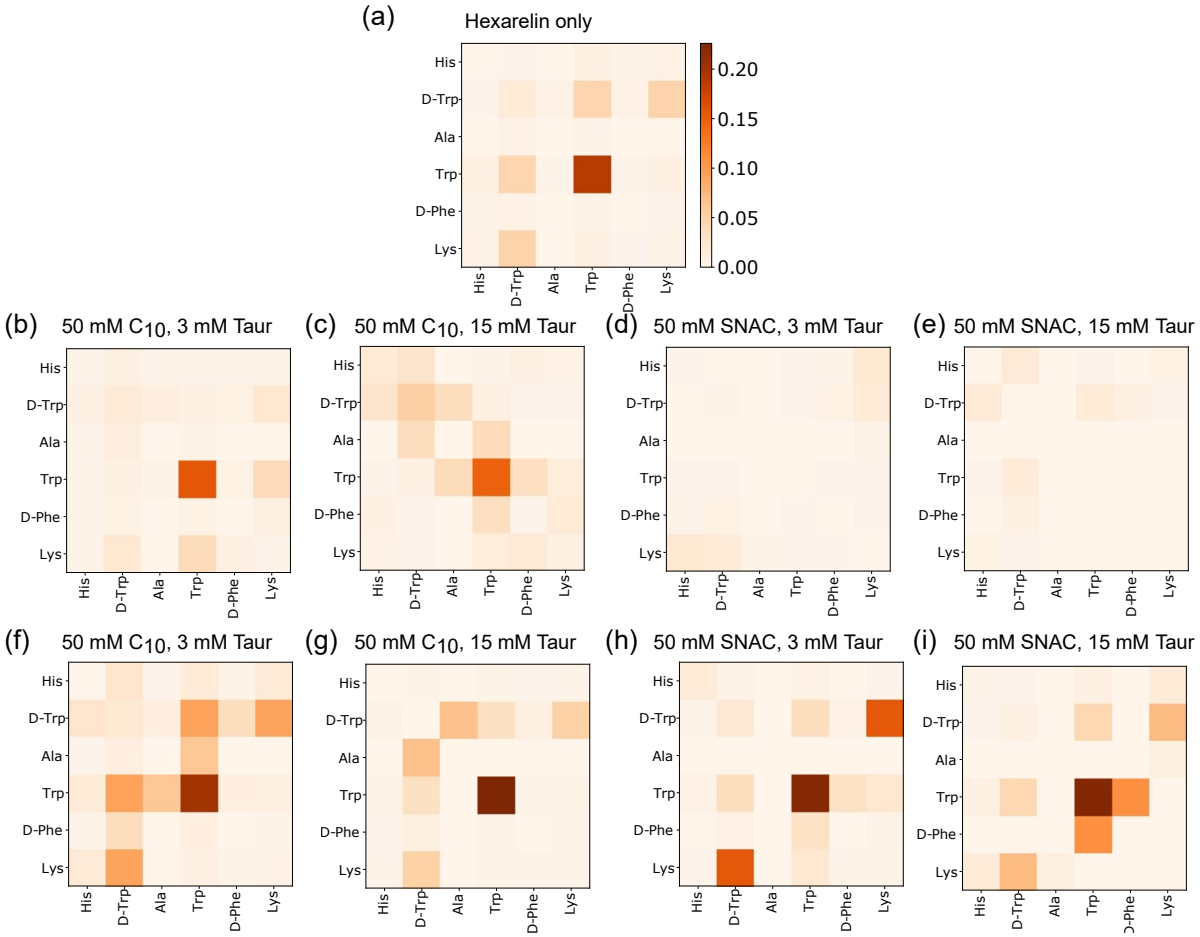
Supplementary Figure 10: Fractional residue-residue contact maps for different systems containing insulin. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly.



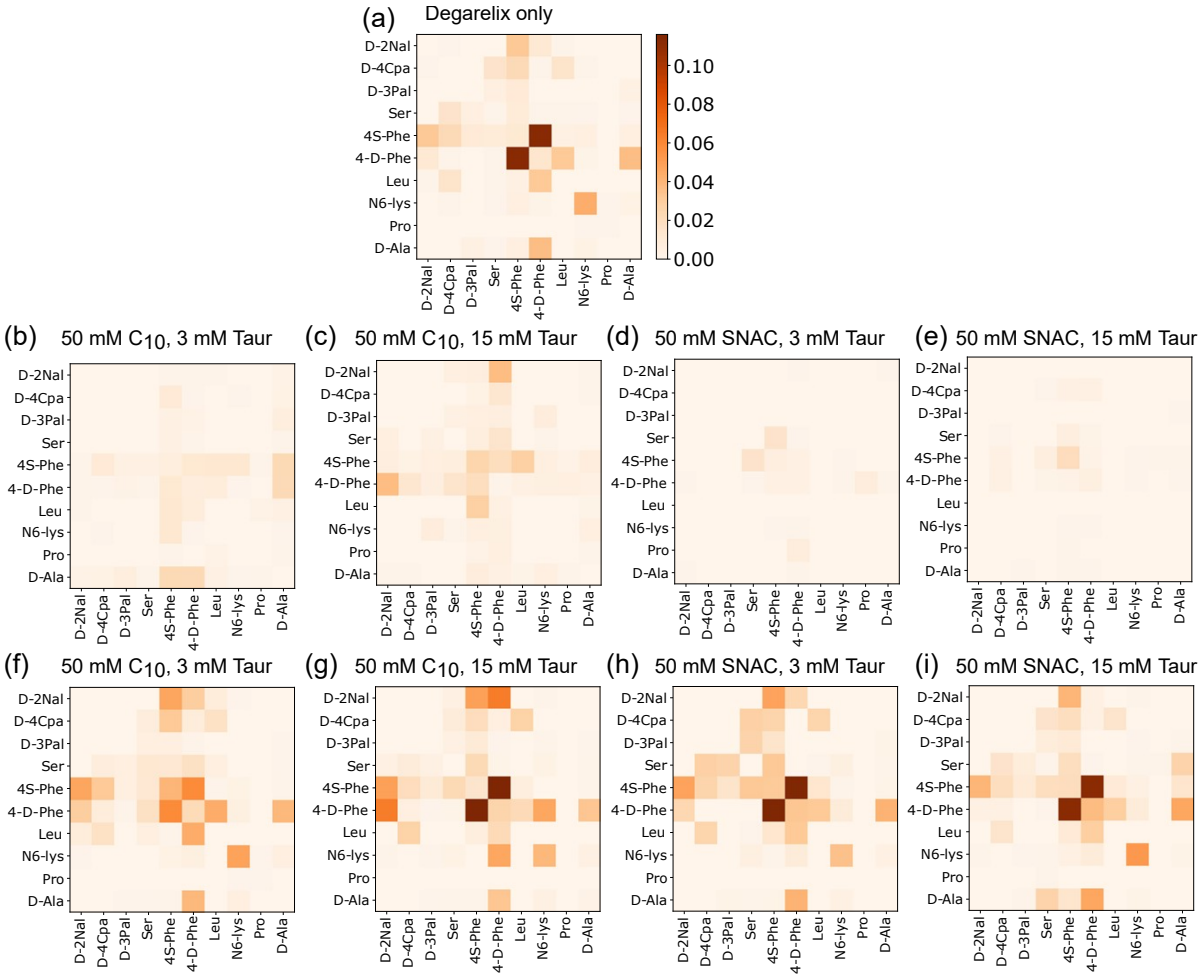
Supplementary Figure 11: Average number of peptide residue-residue contacts per peptide calculated over the final 100 ns of the simulation. The data represents the systems with 10 mM peptide concentration.



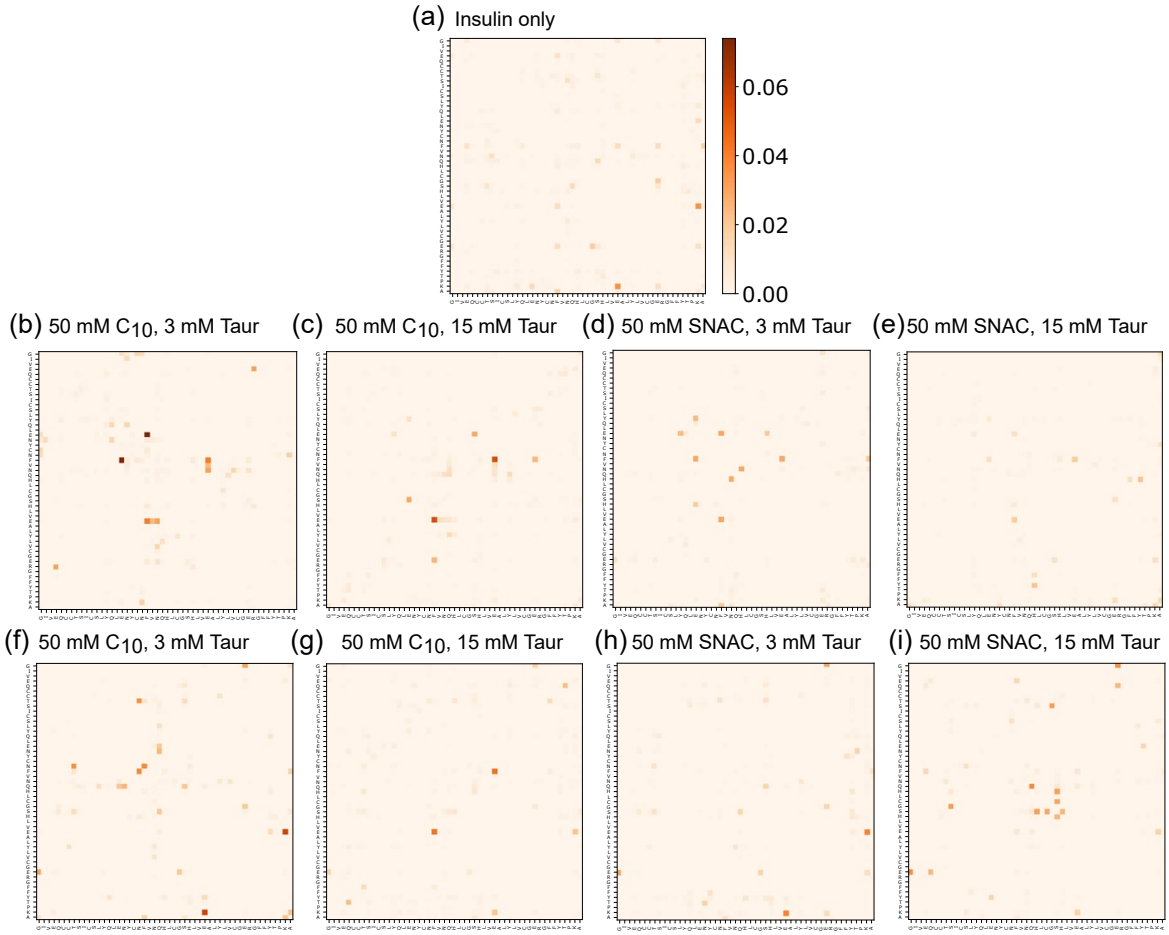
Supplementary Figure 12: Hydrogen bond maps between peptide residues for systems containing Octreotide. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. The values are normalized by the maximum total hydrogen bonds among these systems.



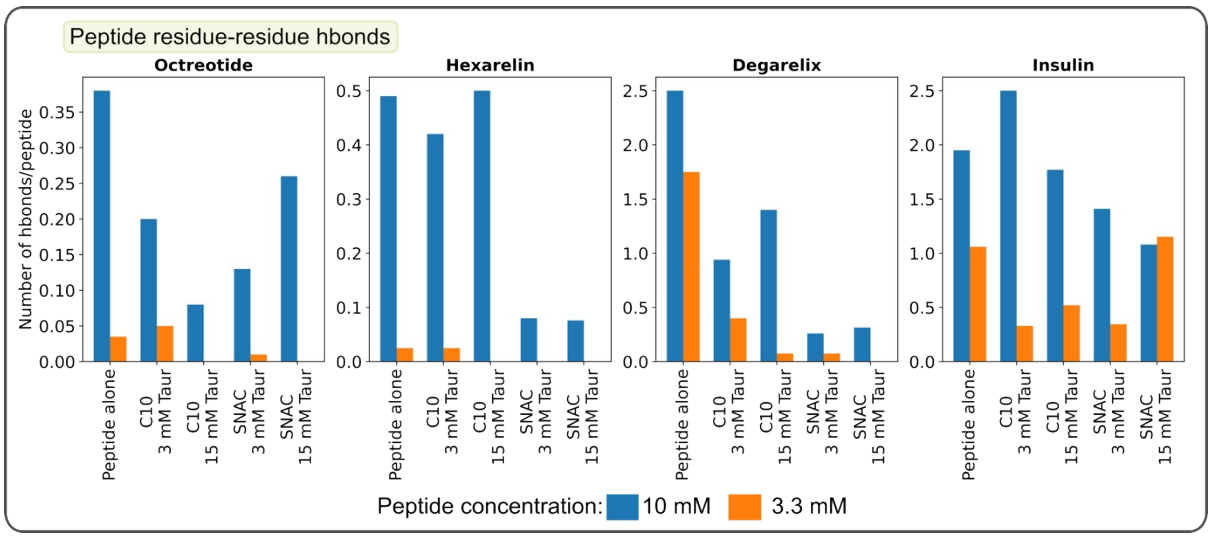
Supplementary Figure 13: Hydrogen bond maps between peptide residues for systems containing Hexarelin. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. The values are normalized by the maximum total hydrogen bonds among these systems.



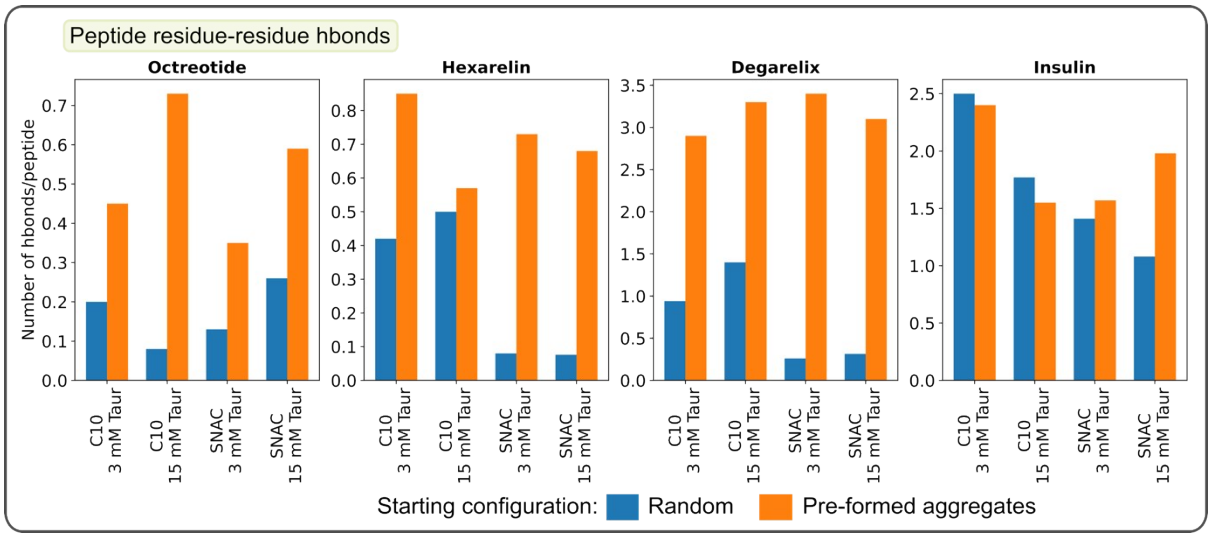
Supplementary Figure 14: Hydrogen bond maps between peptide residues for systems containing Degarelix. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. The values are normalized by the maximum total hydrogen bonds among these systems.



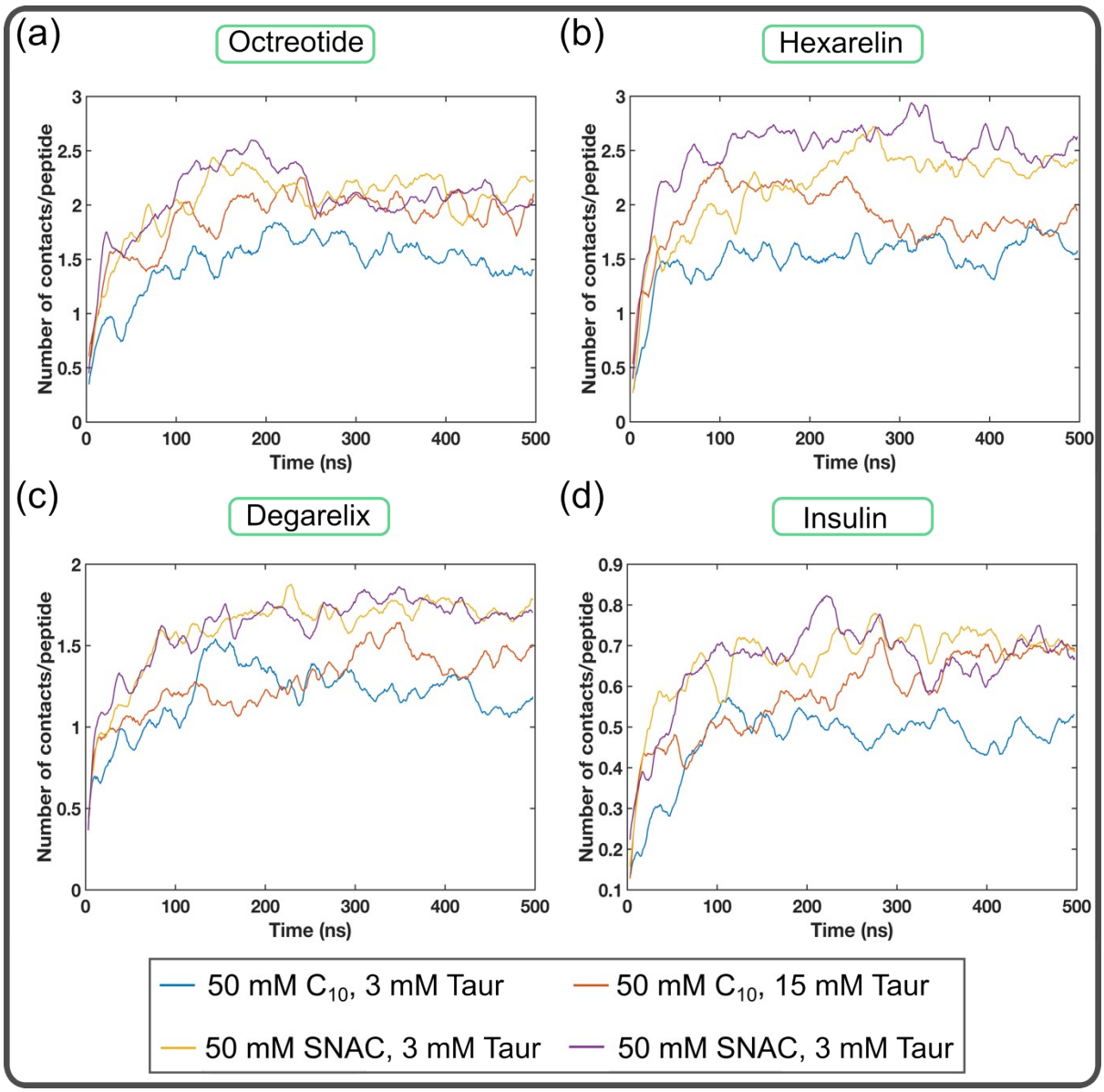
Supplementary Figure 15: Hydrogen bond maps between peptide residues for systems containing Insulin. (a) System containing the octreotide molecules alone, panels (b-e) represent the starting configuration where all the molecules (including the peptides) were randomly placed in the simulation box, and panels (e-h) represent the starting configuration where the peptides are in a pre-aggregated state, but with PE and taurocholate molecules placed randomly. The values are normalized by the maximum total hydrogen bonds among these systems.



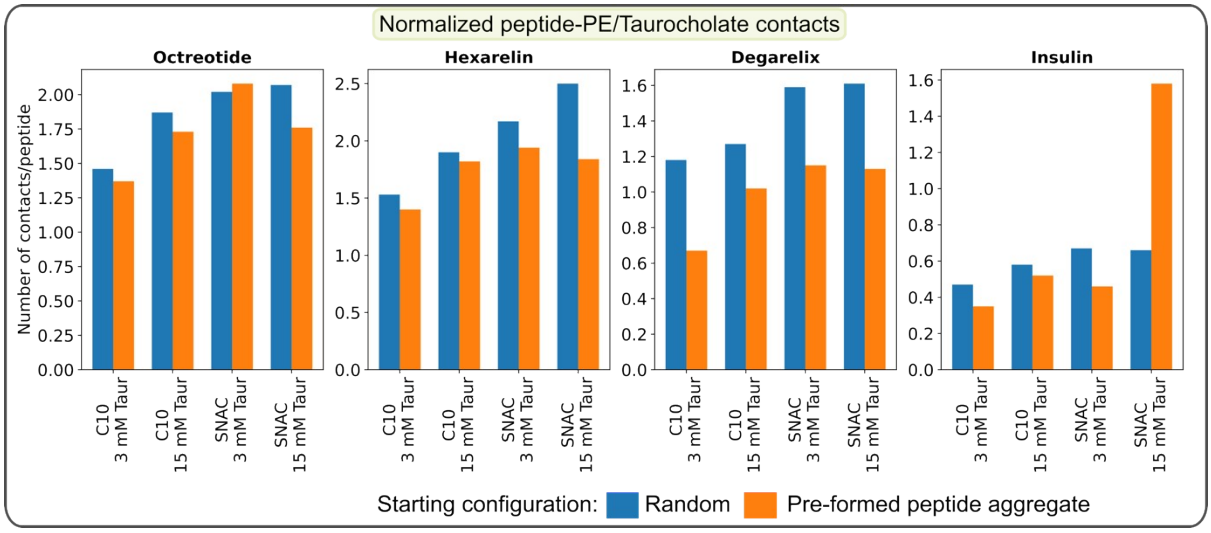
Supplementary Figure 16: Average number of peptide residue-residue hbonds per peptide calculated over the final 100 ns of the simulation. The data represents the systems initiated with a starting configuration where all molecules, including the peptides, were randomly placed within the simulation box.



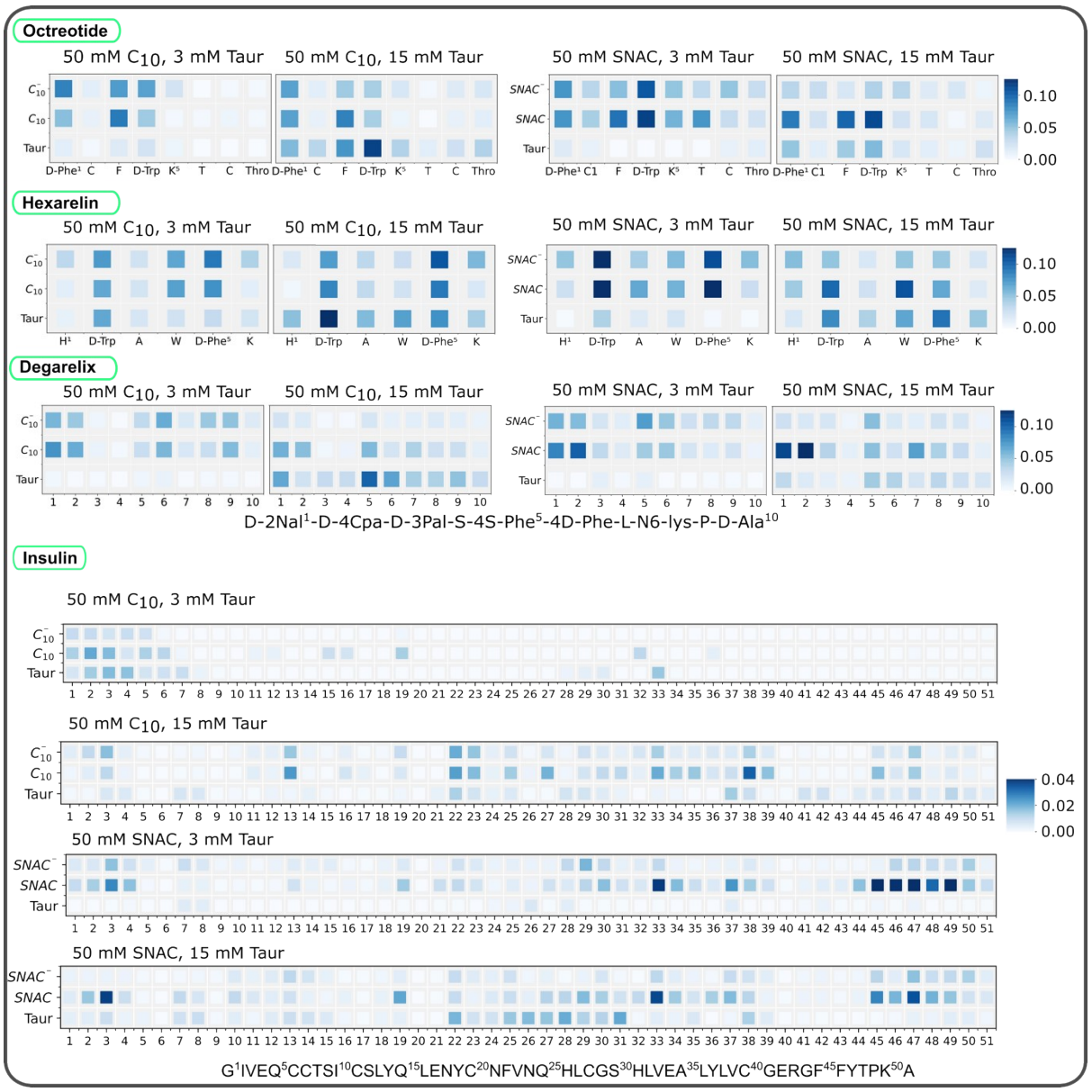
Supplementary Figure 17: Average number of peptide residue-residue hbonds per peptide calculated over the final 100 ns of the simulation. The data represents the systems with 10 mM peptide concentrations.



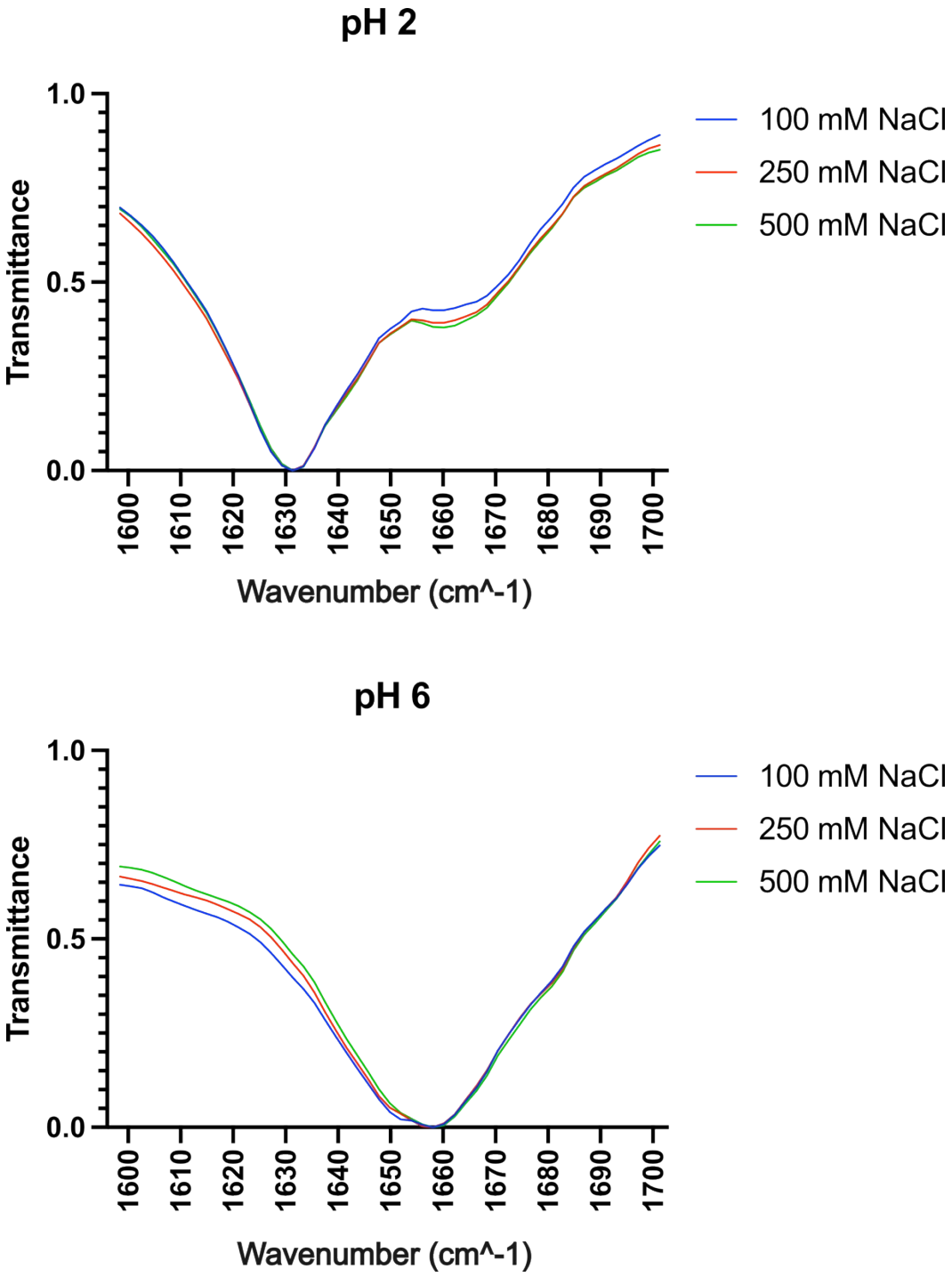
Supplementary Figure 18: Time evolution of the average number of contacts per peptides for the systems containing 10 mM peptides and starting configuration with random positioning of the peptides initially.



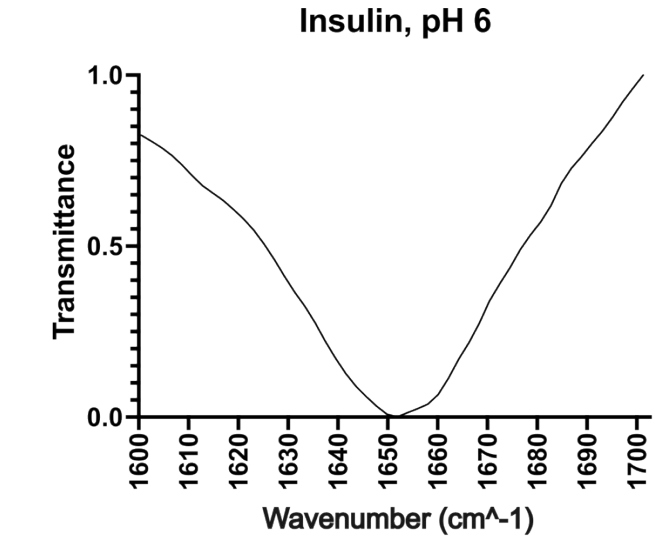
Supplementary Figure 19: Average number of contacts between peptides and PEs/taurocholates per peptide calculated over the final 100 ns of the simulation and normalized by the number of atoms for each peptide. The data represents the systems initiated with 10 mM of peptides.

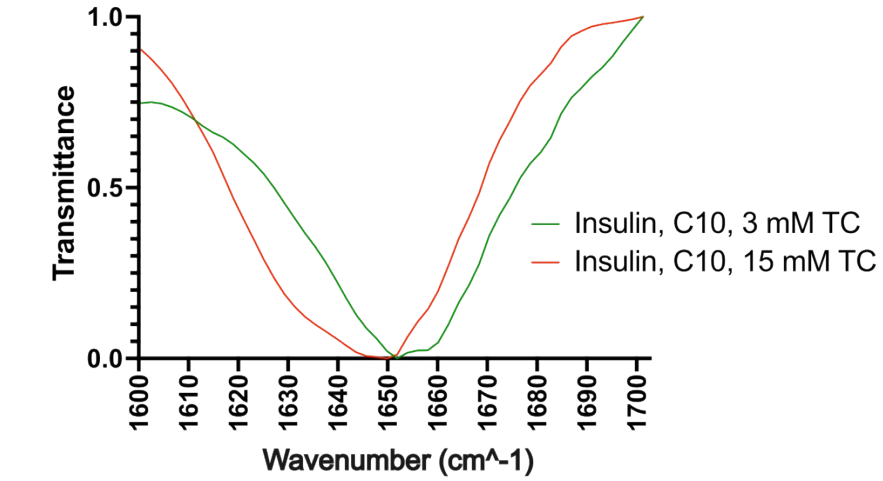


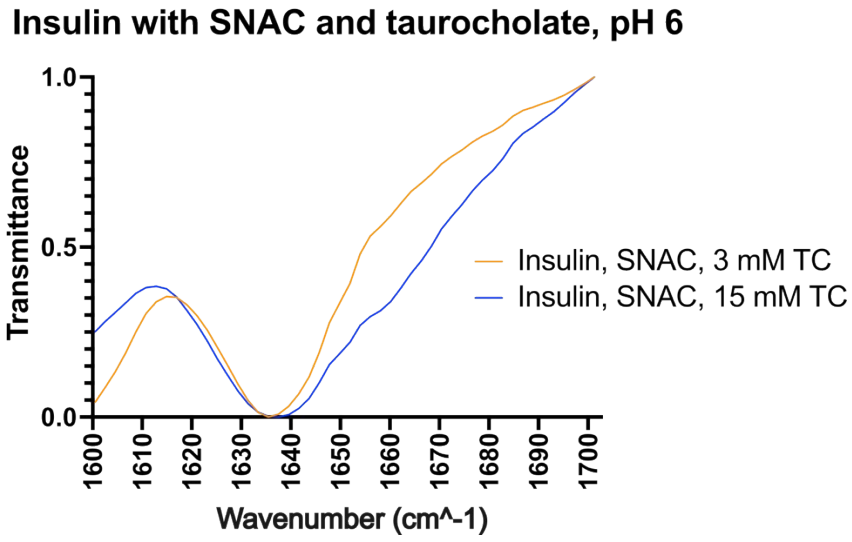
Supplementary Figure 20: **Normalized contact between peptide residues, PEs, and taurocholates for different systems** containing 3.3 mM of octreotide, hexarelin, degarelix and insulin. For systems containing a specific peptide, the contact values are normalized using the maximum number of contacts found in each case. The data here represents the starting configuration where all the molecules including the peptides were randomly placed in the box. C_{10} and C_{10}^- represent neutral and negatively charged sodium caprate molecules, and *SNAC* and $SNAC^-$ represent neutral and negatively charged SNAC molecules. Taur represents taurocholate molecules.



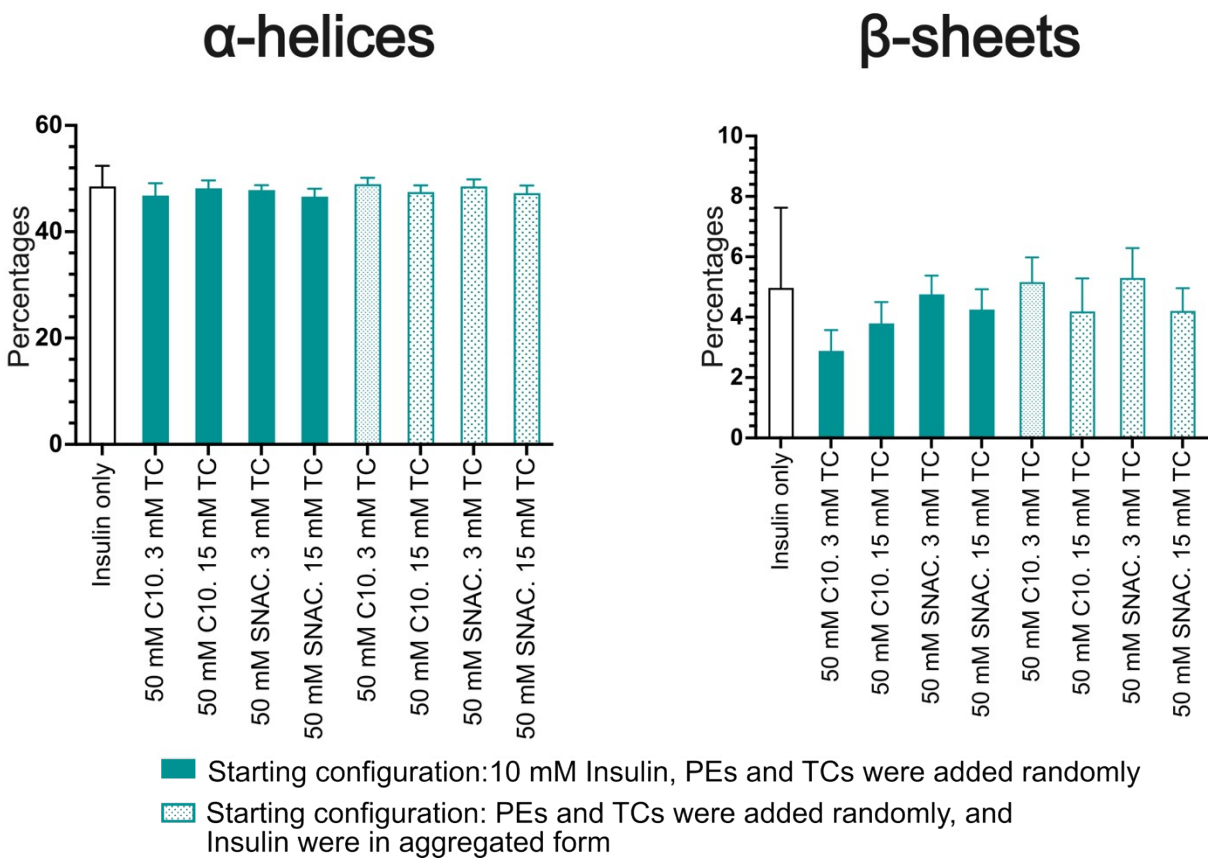
Supplementary Figure 21: Second derivatives of FTIR spectra for Insulin in the presence of different NaCl concentration at two different pH values.







Supplementary Figure 22: Second derivatives of FTIR spectra for Insulin in the presence/absence of PEs and different concentration of taurocholates



Supplementary Figure 23: Secondary structure propensity (DSSP) analysis for Insulin from the simulations for systems consisting different PEs and different concentration of taurocholates.