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Coarse-grained molecular dynamics of tetrameric transmembrane peptide bundles within a lipid bilayer

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Abstract

The conformations of model transmembrane peptides are studied to understand the structural and dynamical aspects of tetrameric bundles using a series of coarse grain (CG) molecular dynamics (MD) simulations since membrane proteins play a crucial role in cell function. In this work, two different amphipathic models have been constructed using similar hydrophobic/hydrophilic characteristics with two structurally distinct morphologies to evaluate the effect of roughness and hydrophilic topology on the structure of tetrameric bundles, one class that forms an ion-channel and one class that does not. Free energy calculations of typical amphipathic peptide topologies show that using a relatively smooth surface morphology allows for a stable conformation of the tetramer bundle in a diamond formation. However, the model with side chains attached to the core in order to roughen the surface has a stable square tetramer bundle which is consistent with experimental data and all-atom (AA) MD simulations. Comparisons of the CG simulations with AA MD simulations are in reasonable agreement with the formation of tetrameric homo-oligomers, partitioning within the lipid bilayer and tilt angle with respect to the bilayer normal. We concluded that a square or diamond shape tetrameric homo-oligomers could be stabilized by rational design of the peptide morphology and topology of the surface, thus allowing us to tune the permeability of the bundle or channel.

Keywords

Ion-Channel; LS₂ peptide; All-Atom; Tetramer bundle; WALP peptide; DMPC bilayer

1. Introduction

Membranes and their embedded proteins play a crucial role in numerous cell processes such as signaling, energy conversion, and ion conductance (Estes, et al., 2008). In particular, ion-channels regulate the ionic concentration by selectively allowing certain species of ion, such as Na⁺, Ca²⁺, and K⁺, to be transported across the membrane (Colin G. Nichols and Thomas P. Flag,). They are ubiquitous in processes involving basic cell function as well as intercellular communications. In many cases, ion-channels are homo-oligomeric with a hydrophilic lumen,

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which assemble from separate monomers (Torres, et al., 2001, Yang, et al., 2001). A common motif found in monomers forming these ion-channels is the amphipathic nature of the α -helices in the transmembrane domain (Lopez, et al., 2005). The polar residues of the peptide are lined up to form a hydrophilic region or patch, which form the lumen, leaving the rest of the helix hydrophobic with non-polar residues that interact with the bilayer hydrophobic layer. Various experimental techniques such as NMR spectroscopy and X-ray crystallography have been used to determine the structures of various ion-channels with this motif (Jiang, et al., 2002, Marassi and Opella, 1998, Oiki, et al., 1988a, Oiki, et al., 1988b, Stevens, 2000, Unger, 2001). Properties such as gating, conductivity, and ion selectivity of some simple ion-channels including the influenza M2 channels (Duff and Ashley, 1992, Husslein, et al., 1998, Sugrue and Hay, 1991, Yin, et al., 2007, Zhong, et al., 1998b), synthetic LS₂ (Howard, et al., 2002, Lear, et al., 1988, Lear, et al., 2001, Slovic, et al., 2004), LS₃ channels (Zhong, et al., 1998a, Zhong, et al., 1998d), WALP (peptide bundle) (Bond, et al., 2007, de Planque, et al., 2003, Killian, 2003), and HIV1 VPU (Lopez, et al., 2002a, Moore, et al., 1998) have been extensively studied both experimentally (Bond and Sansom, 2004, Li, et al., 2003, Wang, et al., 1994) and theoretically (de Vries, et al., 2004, Newns, et al., 2000, Sansom, et al., 2000, Tieleman, et al., 2001). Here in this study, we investigated model ion-channels (LS₂) and WALP like transmembrane peptides. WALP peptides form similar transmembrane oligomers as ion-channels but they do not conduct ions.

Molecular Dynamics (MD) simulations have been used extensively to study a variety of dynamical events ranging from self-assembly of a lipid bilayer to insertion of helical peptides into a membrane (Newns, et al., 2000, Zhong, et al., 1998a, Zhong, et al., 1998b, Zhong, et al., 1998d). However, conventional all-atom (AA) MD simulations can only access small temporal (<100ns) and spatial scales (<10nm) (Richard B. Ross and Sanat Mohanty, 2008) which is too short to see homo-oligomerization. In complex systems such as the formation of ion-channels in lipid bilayers, Coarse Grain (CG) techniques can be employed resulting in the removal of certain structural details (Thogersen, et al., 2008) which allows for simulations in spatial and temporal ranges beyond AA MD simulations into the microsecond and micrometer range.

Due to the reduction in the number degrees of freedom, CG modeling usually incorporates some of the entropy of fine detail into the potentials of the enthalpy term which allows for the correct free energy by compensating the entropy loss with enthalpy, thus describing the ΔG more accurately. Recent CG models developed by Klein et al. (Lopez, et al., 2002a, Lopez, et al., 2004, Shelley, et al., 2001), Voth et al. (Chang, et al., 2005), Marrink et al. (de Vries, et al., 2004, Marrink and Mark, 2003), and Scheraga et al. (Makowski, et al., 2007, Nianias, et al., 2006) retain specific chemical identities and specific interactions. The model-building scheme had been shown to properly describe specific biological materials in amphipathic systems (Chang, et al., 2005, Shelley, et al., 2001). By using the CG model developed by Klein et al. (Lopez, et al., 2004, Lopez, et al., 2002c, Shelley, et al., 2001), pore insertion into a lipid bilayer has been observed (Lopez, et al., 2004, Lopez, et al., 2005). The same model has also been used to study lipid bilayer perturbation by a transmembrane nanotubes model (Nielsen, et al., 2004).

It has been shown by Hummer et al. that nanotubes architecture may be used to represent homo-oligomeric peptides that assemble to form an ion-channel for water and protons (Hummer, et al., 2001). Previous works done by Fernandez-lopez involves forming synthetic structures from naturally occurring units in the assembly of tubular peptides (Fernandez-Lopez, et al., 2001). Motivated by Hummer and Fernandez-lopez researches, we constructed two nanotube models where one model can assemble into a tetrameric bundle that can form an ion-channel with a pore large enough to allow water to pass through, while the other model does not.

The initial representation of the nanotube peptide model while simplistic captures some of the essential features of peptides forming an ion-channel; it is however overly schematic compared to realistic homo-oligomeric ion-channels. The structural criteria necessary for helices to assemble into a tetramer bundle have been studied for many years, at least since the de-novo designed of LS₂ synthetic peptides (Degrado, et al., 1989). The LS₂ peptide has a simple sequence of (LSLLSL)₃ and is known to form a square tetrameric ion-channel (Degrado, et al., 1989). The small polar serine residues are designed to line the lumen of the channel in order to facilitate ion transport. The hydrophobic leucine residues are chosen to allow partition of the peptide into the membrane, and are able to sustain a vertical transmembrane orientation (Degrado, et al., 1989). In addition to the amphipathic nature of the peptide, the length of the peptide is designed to be comparable to the thickness of the hydrocarbon portions of the lipid bilayer minimizing the hydrophobic mismatch (Horia I. Petrache, et al., 2002). The topology of LS₂ can be seen in Figure 1A. For comparison purposes, the model peptides that had sample hydrophobic-hydrophilic topology as the WALP peptides were also used (Bond, et al., 2007, de Planque, et al., 2003, Killian, 2003) (See Figure 1D). Although the WALP peptides do not form an ion-channel, it does form a similar ion bundle but without any ion conductance. The simplicity of the LS₂ and WALP peptides have been used in AA MD to further understand the structural and functional properties of channel forming peptides (Newns, et al., 2000, Zhong, et al., 1998c).

Our overall goal is to identify the salient structural and dynamics features of homo-oligomeric tetrameric bundles as well as their assembly that can provide us further insights into designing future ion-channel and transmembrane helical packing. Specifically, we wanted to quantify the packing arrangements of peptides that assemble into ion-channels or peptide bundles. By understanding the packing of proteins into these homo-oligomers within a lipid bilayer, we could control the properties and the function of the channel. If the peptides assemble into a close-packed geometry, it would prevent water or ions to travel through the channel. However, if the peptides form an open geometry, it would allow the bundle to be “open” so water or ions can travel through the bundle. Therefore, if we could understand the dynamics features as well as the structural features in the geometry packing of the peptides assembling to form tetrameric homo-oligomers, we would have the ability to tune the permeability of ion-channels or the packing arrangement and control the properties of the bundle.

In addition to MD simulations, we also ran metadynamics simulations in order to explore the free-energy of the assembled homo-oligomers, specifically one class that can form an ion-channel and another class that can assemble into a WALP like bundle. Metadynamics are capable of exploring free-energies in the space defined by a manifold of collective coordinates that characterizes the reaction process. As has been shown elsewhere, meaningful results can be obtained in a relatively short simulation time, if the coordinates can discriminate between the initial and final state and includes all the relevant reaction coordinate motions. This method has already been successfully applied to the study of the mechanism of oxidation of DNA (Ensing and Baerends, 2002) and to clarify the translocation of antibiotic molecules through a porin (Ensing, et al., 2006), as well as the peptide backbone (Takahashi, et al., 2002). With our metadynamics simulations, we quantify the differences between the packing of the peptide models forming tetrameric bundles in a lipid bilayer, which has not been shown before to our knowledge.

For this study, we constructed CG LS₂ and WALP tetramer bundles, where each α -helix is modeled using the CG model developed by Klein et al. (Klein and Shinoda, 2008). This construction scheme is appropriate for a homo-oligomeric tetramer bundle. In addition, we modified the smooth nanotube model to capture a rough topology of the monomers which are found in ion-channels. By designing different topologies (rough surface and smooth surface),

we would have the ability to control the properties of the tetramer bundle which would allow us to tune the permeability of ion-channels and peptide bundles.

2. Methodology

Construction of the CG model

CG models of the phospholipid dimyristoylphosphatidylcholine (DMPC) and water developed by Klein et al. were used in this study (Lopez, et al., 2002a, Lopez, et al., 2002b, Nielsen, et al., 2003, Shelley, et al., 2001). These CG models have been tested extensively in previous computational studies of lipid bilayers (Lopez, et al., 2002a, Lopez, et al., 2002b, Lopez, et al., 2005, Nielsen, et al., 2003, Nielsen, et al., 2004, Nielsen, et al., 2005, Shelley, et al., 2001) and were able to reproduce experimental bulk densities as well as surface tension (Lopez, et al., 2002b). In this study, four tubes are used for each system to model a four-helical bundle of transmembrane peptides. We constructed two types of models, a smooth tube (Figure 1B and 1E), which we labeled type I, and a rough tube that more closely resembles an α -helical peptide (Figure 1C and 1F), which we labeled type II. In addition, two different subtypes of each type were also modeled, subtype *a* (Figure 1B and 1C) has the same hydrophilic/hydrophobic topology as LS₂ and subtype *b* (Figure 1E and 1F) has the same hydrophobic/hydrophilic topologies as the WALP peptides (Bond, et al., 2007). All types have hydrophilic caps that act as anchors at the ends, which are solvated by water and keep the model in a transmembrane orientation (Lopez, et al., 2005). Subtype *a* also contained a vertical hydrophilic patch to model the amphipathic nature of LS₂ peptide, whereas subtype *b* is entirely hydrophobic between the caps (See Figure 1) which models the peptide bundles.

Type I Model (smooth, Figure 1B and 1E): Type I model was first used by Lopez et. al. (Lopez, et al., 2005, Nielsen, et al., 2005), in which the helical protein was modeled as a cylindrical object similar to a nanotube with eight 8-bead rings (64 beads total) that were stacked vertically and linked with bonds and bends between neighboring beads. The equilibrium bond distance was 4.65 Å for all bonds. The intramolecular morphology of type I CG peptide model (Figure 1B and 1E) was constructed as follows: each individual ring was a perfect octahedral that had 8 bonds and 8 bends of 45° angle in the plane of the ring. Eight individual rings were stacked on top of each other, with every adjacent ring offset from by a 22.5° rotation, which was then connected by 16 bonds for a total of 112 bonds in the TM peptide model. There are a total of 176 bonds and 752 bends per tube model. For the subtype *a* CG model, two out of the eight beads are hydrophilic (Figure 1B), which is very similar to the LS₂ peptides. As for the subtype *b* CG model, there are only hydrophilic caps and the ends of the tube, leaving the rest of the tube hydrophobic (Figure 1E). This model is very similar to the WALP peptides (Killian, 2003). The harmonic bonds and bends were included in the model with force constants of 10,000 K/rad² (19.86 kcal/Å²) and 10,000 K/rad² (19.86 kcal/Å²), respectively, which keeps the structure fairly rigid.

Type II Models (rough, Figure 1C and 1F): For the second model (type II), two subtypes were constructed. The differences between the two subtypes are in the hydrophilic patches. In subtype *a* there is a vertical patch of hydrophilic beads to line the lumen of the ion-channel (Figure 1C). This mimics the LS₂ peptide topology (Figure 1A). In subtype *b* there is no hydrophilic patch (Figure 1F), which is similar to the WALP peptides (Figure 1D). The diameter of both type I and II models are ~14.00 Å which is consistent with the peptide interactions distances.

The type II models were constructed similar to the type I models, however the eight levels of CG atoms was rotated by 45° rather than 22.5°. Each level consisted of 4-beads in a perfect square, where each core bead has a CG group (side-residue) attached to it extending away from the center of the core. This construction mimics the hydrophilic core of a peptide (the peptide

backbone) with the core beads, and amino acids side chains with the residues attached to the core. There are 4 bonds (32 total) and 4 bends (32 total) in the plane of the ring for each ring. All bonds have an equilibrium distance of 5.00 Å or 2.5 Å for rough and intermediate cases. 56 bonds and 168 bends connected the adjacent rings together with an equilibrium angle of 60°. There are 112 bends involved in the adjacent rings. The harmonic force constants used for bonds and bends are 8000 K/rad² (15.89 kcal/Å²) and 8000 K/rad² (15.89 kcal/Å²), respectively. Both ends of the tube are capped with one hydrophilic ring. The cylindrical center core of the tube is hydrophilic in order to mimic the polar backbone of a peptide.

For the type II CG tube models, the length of the “side chains” was 5.00 Å, which is longer than the side chain length of leucine and serine residues. Since the purpose of this research is to understand the physical properties of TM peptides that lead to ion-channel stability, structure and dynamics, we extended the existing type I CG tube models with a rough topology; but without the difference in side chain length and our type I tubes are shorter in length, and the tube opening is less flexible compared to the model presented by Smeijers et al. Our type II tubes have similar features as the scramblases model presented by Smeijers et al. (Smeijers, et al., 2006). However, the diameter of the core tubes in our type II models had been reduced so that the overall diameter of the type II tubes remains the same as the type I tubes.

Simulation Details

We investigated four different systems in this study, with four proteins embedded within the bilayer of each type and subtype. In each system, four tubes were placed 15 Å apart (the approximate van der Waals radius for nanotube-nanotube interactions) in a square arrangement (Figure 2). A cavity large enough to accommodate the homo-oligomeric bundle was then created by removing lipids from the center of a pre-equilibrated hydrated lipid bilayer. Each system consists of four peptides, 190 lipids, and 4640 water beads. A constant temperature (300 K) and pressure (bilayer plane in which 85 × 85 box sides are constrained to change in the same proportion, and bilayer normal (z) pressure was separately set to 1 atm) ensemble was used with Nose-Hoover chains of length 3 (Evans and Holian, 1985). The systems were allowed to equilibrate until the volume of the system and each component of energy term fluctuate around equilibrium values. The average box size was approximately 83 Å × 83 Å × 97 Å. After equilibration, 15 ns trajectories for each system were collected at 1 ps intervals and were used for analysis. Longer trajectories were not needed for each system since the pressure, volume, and potential energy of the systems fluctuated around a constant value. This can be observed by the distance measurements shown in Figure 5, where drifting or irregular movements were not observed. All simulations were carried out using the Code for Molecular Modeling and Molecular Dynamics (CM3D) designed by Moore et. al. (Moore, 2005).

Free Energy Methods

Initial simulations indicated that there are two stable conformations, a diamond (close packed) and a square arrangement (open packed). Both structures are observed to be stable for type II models for the duration of the simulations with no conversion between the two structures. We thus employed “Metadynamics” (Ensing, et al., 2006, Laio and Parrinello, 2002) in order to explore the free energy profile of conversion between these two arrangements. Metadynamics is a validated method that aims to enhance the exploration of free energy surface (Ensing, et al., 2006, Laio and Parrinello, 2002). Using metadynamics, the free energy of conversion from diamond arrangement to square arrangement, and back to diamond arrangement was explored for both type I and type II CG tetramer systems. The collective variable is defined as the diagonal distance of the tetramer bundle, measured from the centers of mass (COM) of two tubes at opposite corners, ranging from 14 Å to 30 Å. There were no other potentials employed in the systems, thus the peptide models were free to rotate or shift with respect to the bilayer. Only two collective variables were placed between the two different cornered peptides center

of mass which leaves the other two peptides to move according to Newton's equations. The width of each Gaussian was at 0.5 Å, and the hill depth was 100 K (0.1986 kcal/mol). For each system, a diamond arrangement of tetramer bundle was constructed and equilibrated. The equilibrated structure was then used as an initial conformation for 15 ns of metadynamics simulations, which is long enough to explore the conformations of space.

3. Results and Discussions

We hypothesized that a rough surface topology model of the LS₂ peptide would prefer the ion-channel to have a stable conformation in a diamond and a square orientation, and a smooth surface topology would prefer the ion-channel to be in a diamond orientation. This information is critical because a diamond orientation would be close-packed which would not allow water or ions to travel through the ion-channel whereas a square or rectangular orientation would allow the ion-channel to be "open" so water or ions can travel through the channel. By having the ability to control the properties of the ion-channel, one can also control the function of the channel as well. For example, when using the rough surface topology model, the square or diamond orientation can be made to be stable which means that the pore size and dynamics of the channel can be influenced. The opening and closing of the ion-channel will be slowed in the square conformation due to peptide-lipid and peptide-peptide interactions, which will allow larger ions to transverse through the channel. Thus, using different topology models would allow one to control the overall properties and functions of the ion-channel.

Simulation of Type Ia CG tube tetramer

Four type Ia CG tubes were initially placed in the center of an equilibrated CG DMPC bilayer and water in a square arrangement. The coordinates of the tubes were initially fixed in place, allowing lipids and water to equilibrate around the tetramer for 1 ns. The tubes were then allowed to move and the system was equilibrated for another 0.5 ns. Within 100 ps of simulation, the tubes rearranged to form a diamond shaped tetramer (Figure 2), instead of the initial square configuration as previously reported in both experimental (Degrado, et al., 1989, Howard, et al., 2002, Lear, et al., 2001) and computational studies (Newns, et al., 2000, Zhong, et al., 1998c). The observed diamond configuration was allowed to further evolve for 15 ns. During which no change of configuration was observed. To test the hypothesis that the hydrophilic interactions were the primary driving force between tubes, the width of the vertical patch was widened to 4 beads per ring instead of 2 beads per ring allowing for more interaction of the pore residues. Again, diamond arrangements of the tetramer were observed within the first 100 ps during the equilibration, thus indicating that it was not the hydrophilic interactions, but more likely the smooth morphology of the peptide model. In all cases, the diamond arrangement was stable throughout the production run following the equilibration process.

Topologically, the diamond orientation is of a close contact configuration; therefore it is preferred over the square configuration on packing considerations. However, experimentally, LS₂ peptides are observed to form a tetramer bundle in a slightly distorted square configuration (Degrado, et al., 1989, Howard, et al., 2002, Lear, et al., 2001). Further examination of the structure suggested that the smoothness of the tube surface and the rigidity of the type Ia CG tube model may not be able to withhold the lateral pressure exerted by the surrounding lipid, thus collapsing to a diamond configuration (Figure 2). The side chains of leucine and serine in the AA model are able to interact with the surrounding lipid through steric interactions, therefore sustaining the square tetramer bundle structure. Experimental crystallographic data have shown that rigid transmembrane α -helices, such as those in rhodopsin, forms a close packed heptamer bundle suggesting that the stiffness of the helices play an important role in determining if the square or diamond arrangement is preferred (Krzysztof Palczewski, et al.,

2000). Therefore, we modified the type Ia model to be able to simulate a square conformation as observed in the AA simulations and in experiments by allowing for roughness and flexible side chains.

Simulation of Type Ib CG model

Research done by Lopez et. al. (Lopez, et al., 2005) using the type Ib model was compared with our type Ia model. From this research, it was determined that the type Ib model in a tetramer bundle was more stable in a diamond conformation. We initially thought that the type Ib tetramer model would be stable in a diamond conformation based on this research, and that the type Ia tetramer model would be slightly more stable in a square conformation due to the increase in polarity between the peptides. However, based on the simulations of the type Ia that was conducted, it was determined that both type I models are more stable in a diamond conformation. We conclude that the smooth model topology will lead the tetramer peptide bundle to be more stable in a diamond conformation than a square conformation (Figure 2).

Simulation of Type IIa CG model

Type IIa model (Figure 1C) was constructed based on the original concept of type Ia CG (Figure 1B) tube model, a cylindrical shape with a hydrophilic patch running the length of the peptide. The core tube consists of only hydrophilic beads to resemble the backbones of LS₂ peptide. For each bead on the core tube, a “side chain” bead was attached to resemble side chains in the atomistic model. In addition, the harmonic force constant for all bonds and bends was reduced from 10,000 K/rad² to 8,000 K/rad² in order to introduce more flexibility to the model. For each ring on the tube, only one side chain bead was designated hydrophilic. Since the rings are stacked in a zigzag fashion, the resulting hydrophilic arrangement resembles the original design of the LS₂ peptide (Degrado, et al., 1989), in which the idealized packing diagram shows a square type interaction among the serine residues at the same level, and is also staggered from the adjacent levels. The length of the side chain was 5.00 Å, which is the equilibrium distance between two CG beads and is longer than the AA model.

The CG MD simulation of type IIa CG tube tetramer was similar to that of type Ia CG tube tetramer, in which four tubes are placed in the center of a CG lipid bilayer in a square formation. Following similar simulation protocols, the tube coordinates were initially fixed to allow lipid and water to equilibrate around the tetramer for 1ns. The constraints on the tubes were removed and the system was allowed to further equilibrate for another 0.5 ns again. Within 100 ps, the monomers congregate together in a square arrangement. The observed square arrangement of tetramer remained stable during the 15 ns production run. We conclude that the roughness in the model allows for interactions that stabilize the square conformation over the diamond conformation (Figure 3).

All-Atom Comparison

Comparing our CG models with AA models from previous research, we found that our CG model gives similar results to the AA models (Bond and Sansom, 2006, Lopez, et al., 2005). The dimension of the atomistic model is comparable with the CG models by design. The length of the AA LS₂ model is 32.85 Å in length measured from cap to cap. Both type I and II CG models are 32 Å in length that also included the hydrophilic caps on both end (Lopez, et al., 2005). Since both types of CG models are capped with one ring of hydrophilic beads on each end of the tube, the hydrophobic tube body length is actually 24.20 Å, which is similar to the lipid hydrophobic layer. These two hydrophilic caps are necessary to anchor the models into the bilayer in a transmembrane orientation (Lopez, et al., 2004). This has been seen in early simulation of tube insertion into a lipid bilayer indicates that without hydrophilic caps, the CG tube model would not stay embedded in the bilayer (Lopez, et al., 2004). Previous CG simulation of DMPC lipid bilayer indicates that the average height of lipid head group from

the center of the bilayer is around 15.5 Å (Lopez, et al., 2005), making the average thickness between top and bottom layer lipid head groups to be around 31 Å. The length of the CG tubes chosen here ensured that the hydrophilic caps anchor the tube so that the hydrophobic portions of the tube and lipid bilayer match. The widths of the hydrophilic vertical patch on both type I and type II CG models were around 9 Å. The hydrophilic patch width is chosen to ensure hydrophilic interactions between CG tubes resembling that of all atom simulations (Newns, et al., 2000, Zhong, et al., 1998c)(Figure 1).

The structural details of the square tetramer bundle were analyzed using data collected during the production run. The distances between COM of two tubes are plotted in Figure 4. The figure indicated that the formation is slightly rectangular rather than a perfect square where the average distances of the four sides are 13.7 Å, 14.2 Å, 13.6 Å, and 14.2 Å (instead of all at 14Å). The rectangular shape results from the preferential bonding of the hydrophilic beads. These residues interlace with each other forming a pair, these pairs then form a rectangular tetrameric bundle. Previous AA MD simulation reported that the pair-wise distances between two helices are around 8.8 Å and 10.0 Å, a dimerization of the tetramer bundle (Lopez, et al., 2005). Dimerization of the tetramer was observed in the present CG simulations; however, the pair distance in the CG model is significantly larger than that of the AA model. Such difference is due to the force field that was employed in this study. Former AA MD simulations have reported the average pore radius size for a LS₂ tetramer bundle to be around 2 Å, which allows for a single water molecule to pass through the channel (Lopez, et al., 2005).

The square tetramer bundle of type IIa CG tubes was observed to tilt collectively with respect to the bilayer normal. The average tilt angle is around $10.4 \pm 3.4^\circ$. In addition, the average “helix crossing angle”, defined by the angle between two tubes, is measured to be less than 2 degrees over the course of the simulation, indicating that there is no twisting of the tube in contrast to previous AA simulations (Newns, et al., 2000, Zhong, et al., 1998c). The lack of twisting indicates that the present model is most likely too stiff compared to the AA. In order to more closely match the AA results, further parameterization of the CG beads used as side chains would be required and is beyond the scope of this research, and we believe a more flexible model would not change the structural results reported herein.

Upon further examination of the trajectory, the side chains are observed to “interlace”, meaning that the side-chains from neighboring tubes are stacked one on top of each other (Figure 5). The type IIa CG model is constructed by stacking rings on top of each other and 45° out of phase from the nearest neighbor. As a result, the distance between the two closest in phase rings on a type II CG tube is about 8.5 Å, which provides ample room for nearby hydrophilic side chain beads to interact with the polar CG core. The interlaced side chain beads are positioned at equal distances from the hydrophilic core beads and the nearby side chain beads to maximize the interaction. Energetically, these “interlacing” hydrophilic interactions stabilize the tetramer bundle into a square formation, and resemble the strong serine-serine-water and serine-water-backbone interactions observed in AA simulations.

Simulation of Type IIb CG model

To investigate the hydrophilic interactions between the type IIa CG tubes, a simulations of type IIa CG tubes with no hydrophilic patches (type IIb) were carried out. Similar to the WALP peptides these models will not have the strong intermolecular interactions of type IIa model from the capping residues. During the equilibration stage, a square arrangement of the tetramer bundle was again observed. The square arrangement again remained stable throughout the 15 ns simulation. The structural property of the tetramer was analyzed in a similar fashion as the type IIa CG tubes with hydrophilic patch. The distances between two neighboring tubes are 14.13 Å, 14.11 Å, 14.12 Å, and 14.15 Å (Figure 4), which is very close to a perfect square conformation. Unlike type IIa CG tubes, no dimerization was observed. This is expected as

the type *b* models are cylindrically symmetric and have no directional preference. This is in contrast to the type *a* models, which have a hydrophilic patch running the length of the model, which allows for dimerization. Therefore, by tuning the strength of the interaction, one should be able to control how square the conformation is. Such investigations are beyond the scope of the present paper but are ongoing.

Similar to type *Ia* CG simulation, the “helix crossing angle” deviates very little from zero, indicating that here is no twisting of the tubes (coil-coil). However, although the tubes were collectively tilted with respect to the bilayer norm, the average tilt angle of $17.5 \pm 3.2^\circ$ is much larger than the observed value in type *Ia* system. Compared to previous research done by Bond et al., our type *Ib* model is representative of the WALP peptide, whose average tilt angle was reported as $14.0 \pm 8.0^\circ$ (Bond, et al., 2007). Since the bonds and bends force constants of the model does not allow twisting of the tube, hydrophobic interactions between the tubes and lipid bilayer tail can only be achieved by tilting the tubes with respect to the bilayer normal orientation, resulting in large tilt angles as observed during the simulation. Similar to type *Ia* tetramer, “interlacing” effect is also observed in type *Ib* tetramer. This interlacing is similar to the interactions that can occur between protein hydrophobic side-chains within the interior of proteins or between proteins in lipid bilayers.

Metadynamics Simulation

Using metadynamics (Ensing, et al., 2006, Laio and Parrinello, 2002), the free energy of conversion from diamond arrangement to square arrangement, and back to diamond arrangement was explored for both type I and type II CG tetramer systems. For each system, a diamond arrangement was constructed and equilibrated. The collective variable was defined as the distance between the COM of two tubes diagonally located in the tetramer. This choice of collective variable restricts the distance between two of the peptides but makes no constraints on the orientation, tilt, height, or other structures of the peptides. Further, there is no constraints place on the other peptides or the lipids. Thus as the distance varies between the peptides many conformations can result. For example, a T-shape or dissociation of the bundle as well as lipid penetration into the interior of the ion-channel did results when we allowed distance between the peptides to increase beyond 30 Å, which corresponds to dissociation of the bundle. The metadynamics simulation for each system was carried out for 15 ns. The energy profiles are plotted in Figure 6. In all three cases, at least two energy minima were located, corresponding to the two diamond arrangements i.e. close packed.

Energy minimum corresponding to the square arrangements were located in type II simulations and not in type I. A slight asymmetric nature of the free energy profile was observed which is most likely due to the incomplete description of the reaction coordinate by the collective variables (Ensing, et al., 2006) and sampling issues. Since the reaction coordinate is symmetric, we accepted the two diamond shape free energies to have the same free energy. Higher order metadynamics simulations could be use to fully explore the free energy profiles but were not preformed due to limited computational resources. There are a lot of variables within the reaction coordinate that would be needed to be included within the simulations, such as the interlacing, rotation, height, packing, and solvation of the helices for a complete description of the energetics of the bundle which would require enormous computational resources to explore. However, both the symmetric and slight asymmetric profiles gave the same calculations and barrier heights. Therefore, the free energy profiles support the conclusion that type II models forms both diamond and square formations. Interlacing and sliding of the models relative each other, are part of the transition between stable minimum.

In type *Ia* or type *Ib* CG tubes, there is no energy minimum corresponding to the square arrangement, meaning that type *Ia* CG model will only formed a diamond arrangement. The energy barrier between one diamond configuration and the symmetric other diamond

configuration is greater than 30 kcal/mol, which would not be crossed in typical MD simulations. Although it would be interesting to see the transformation of a diamond arrangement to a square arrangement, the probability of finding the configuration on top of the energy barrier is too low (1.39×10^{-22}). Hence, the tubes will be constrained to that region of space. It is possible to design a peptide model that would have a smaller energy barrier which would enable us to use observe a transition between the diamond and square geometry, but that is out of scope of this paper and a topic of ongoing research.

In the free energy profile of type II peptides, there are three energy minimums, which correspond to the structural arrangement of diamond, square, and then diamond again. It is observed that significant energy e.g. greater than 20 kcal/mol is required to convert a diamond tetramer to the square tetramer. Research done by Lopez et. al. have shown a similar free-energy profile for the type *Ib* model, where the diamond orientation is more stable for the tetramer (Lopez, et al., 2005). For the type *IIa* CG tubes, the relative free energy difference between the square arrangement and the diamond arrangement is ~9 kcal/mol, whereas the difference in type *IIb* is less than ~3 kcal/mol. The differences in energy between the type *IIa* and *b* model can be attributed to the hydrophilic patch in the type *IIa* CG model. This patch favors hydrophilic-hydrophilic interactions, in turn, creating a more stable structure. Hence, it would lower the energy of the square or rectangular conformation thus by tuning the hydrophilic interactions within the peptide, the relative stabilities of the square and diamond configuration can be modified.

Although the free energy profiles support the observation of a stable square tetramer for type II CG tubes, it also suggested that the diamond tetramer for type II is stable, but was not observed in our initial simulations. To confirm this finding, 15 ns of CG MD simulations were carried out using the initial diamond tetramers of both type *IIa* and type *IIb* CG models. During the simulations, each tetramer remained in the diamond formation as expected. Although type *IIa* and type *IIb* have identical geometries (a rough surface), their energy-profiles have different energy barriers and energy minimums. This means that not only does geometry have significance on the peptides packing arrangement, but the peptides molecular interactions sequence also contribute to their packing arrangements.

Finally to test the hypothesis that the stabilization can be tuned via the topology of the peptide, we constructed a type *IIa* model with a short bonding to the hydrophilic backbone core. We set the bond length at 2.5 Å, which is ½ of the original bond length. This result in a roughness between the smooth type *Ia* model and the rough type *IIa* models discussed above. As expected the square configuration was destabilized, but was still at a local minimum.

4. Conclusion

We performed a series of CG MD and metadynamics simulations of four LS_2 and WALP peptide bundle using two different types of CG model transmembrane peptides. A summary of the various tubes simulated and their preferred orientation can be found in Table 1. Hydrophilic and hydrophobic characteristics of the LS_2 and WALP peptides were taken into consideration when constructing the models. The main difference between the two models is that the LS_2 model can form an ion-channel while the WALP model cannot. In all of our simulations, we found reasonable agreement between the CG and AA results. Our free energy metadynamics calculations show that using a smooth surface nanotube type model (type I) will lead a diamond formation for the tetramer bundle instead of a square formation. Our simulations indicated that by attaching side chains to the tube surface (type II) in order to make the model rougher, a square formation is observed. This is due mostly to the increase roughness of the model peptides and not the hydrophilic interactions.

The free energy calculations support the observation from the MD simulations, in which diamond tetramer is the most stable formation for type I CG tubes, whereas both diamond and square tetramers are stable for type II CG tubes. Energy barriers of further than 30 kcal/mol to convert a diamond formation into a square formation were observed indicating stable structure for these models. These simulations suggest that particular topologies (square of diamond shaped) are designable with appropriate interactions. We verified this by changing the roughness of the peptide and destabilizing the square conformation. In particular, the relative energies and barrier heights between the square and diamond shape can be tuned by the strength of the hydrophilic interactions. It is possible that different ion-channel and peptide bundle similar to our CG models or peptide topologies can be constructed with particular tetrameric topologies.

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Figure 1.

Hydrophilic residues shown in red and hydrophobic residues shown in green;

(A) LS₂ synthetic peptide;

(B, type Ia) a smooth surface peptide model of the LS₂ synthetic peptide;

(C, type IIa) a rough surface peptide model of the LS₂ synthetic peptide. The cylindrical hydrophilic core (pink) serves as the backbone of the helical peptide and hydrophobic and hydrophilic beads are attached onto the surface, which act as “side-chains”;

(D) the WALP peptide;

(E, type Ib) a smooth surface peptide model of the WALP peptide;

(F, type II*b*) a modified rough surface peptide model. Other than the two hydrophilic caps, there is no hydrophilic patch on the peptide similar to the WALP peptide.

Figure 2.
The smooth surface peptide model cannot support a void with the bundle. The initial square arrangement of peptides (left) collapsed into a diamond shape (right).

Figure 3.

The type IIa model was able to support either a void within the bundle or a bundle filled with water. The initial square tetramer arrangement (left) was retained through out the simulation (right).

Figure 4.

Distance between COM of two CG tubes in the tetramer. (left) type *IIa*. Two sets of (right) type *IIb*. A slight dimerization is observed for type *IIa* CG tetramer in which the tetramer is more rectangular in shape instead of square. On the other hand, the distance of four sides in the type *IIb* tetramer are nearly identical. The significant difference in the diagonal distances results from type *IIb* tetramer in a diamond shaped configuration.

Figure 5. The final square arrangement of the type IIa CG tetramer has an interlacing effect. The side-chain beads stack themselves one on top another to maximize hydrophilic interactions. A similar effect was observed in type IIb CG tetramer.

Figure 6.

Free energy profiles of converting from a diamond tetramer bundle to a square tetramer bundle from metadynamics simulations. The collective variable is defined as the distance between the COM of two diagonally located CG tubes. We have zeroed the energy minimum to put the free energy surface on the same scale and averaged the two diamond configurations. Type II Short has a shortened side-chain bond, and as expected can be used to tune the square conformation stabilization.

Table 1

Summary of all CG systems and their preferred orientation based on the MD simulations. A diamond orientation represents a close-packed tetrameric helical bundle, and a square orientation represents an open-packed tetrameric helical bundle.

Coarse-Grain Models	Orientation
Smooth (typeI)	
LS ₂	Diamond
WALP	Diamond
Rough (typeII)	
LS ₂	Square
WALP	Square
Modified LS ₂	Square