Published in final edited form as:

J Comput Chem. 2019 June 15; 40(16): 1570–1577. doi:10.1002/jcc.25807.

U-shaped Caveolin-1 Conformations are Tightly Regulated by Hydrogen Bonds with Lipids

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Abstract

The structure and dynamics of a truncated (residues 82–136) caveolin-1 (Cav1) construct having a helix-break-helix motif are explored by both all-atom free energy and molecular dynamics (MD) simulations in an explicit bilayer membrane. Two stable Cav1 conformations with small (LB-Cav1) and large hinge angles (RB-Cav1) between two helices are identified although their relative free energy cannot be reliably estimated due to the sampling issues. RB-Cav1s contain one or two lipids residing in-between the helices that are hydrogen bonded (h-bonded) to both in a multi-dentate fashion. LB-Cav1s show the contacting helices with mono-dentate lipid h-bond interactions or multi-dentate interactions limited to a single helix at most. The two conformational states of Cav1 remain their initial state during 2- μ s MD simulation, which suggests that there is likely a significant hidden barrier (other than the insertion depth of Cav1 and its hinge angle) and the Cav1 conformational states are tightly regulated by the h-bonds between Cav1 and lipids along with the associated lipid rearrangement during the course of Cav1 conformational changes.

Graphical Abstract

Two stable conformational states of a truncated (residues 82–136) caveolin-1 (Cav1) with small and large hinge angles between two helices are identified although their relative free energy cannot be reliably estimated due to the sampling issues. These Cav1 conformation states remain their initial state during 2- μ s MD simulation, which suggests that the Cav1 conformational states are tightly regulated by the h-bonds between Cav1 and lipids along with the associated lipid rearrangement during Cav1 conformational changes.

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Keywords

Caveolae; Membrane protein conformation and orientation; Protein-lipid interactions; Hydrogen bond

Introduction

Caveolin is the main structural protein of caveolae and is essential for caveolae formation,^[1,2] which are plasma membrane invaginations involved in a variety of cellular processes such as endocytosis, signal transduction, and mechano-protection.^[3–7] Numerous human diseases are caused by misregulation and mutant forms of caveolae, such as infection, muscular dystrophy, heart disease, Alzheimer's disease, and cancer.^[8–11] Among the three isoforms of caveolin, i.e., caveolin-1, -2, and -3, caveolin-1 is the most ubiquitous^[12] and is expressed abundantly in endothelial, fibrous, and adipose tissue.^[12,13]

Caveolin-1 is an integral membrane protein having a unique topology where both N- and C-termini are exposed to the cytoplasmic side of the plasma membrane, and are not accessible from the extracellular side (Fig. 1a).^[14] Figure 1b schematically illustrates the domains and secondary structure of caveolin-1, where caveolin-1 is composed of four domains: the N-terminal, the scaffolding, the intramembrane, and the C-terminal. In a recent NMR studies,^[15,16] the secondary structure of a functional construct of caveolin-1 (residues 62–178) was determined to have three helices (H1, H2, and H3) located in the scaffolding, intramembrane, and C-terminal domains (residues 87–175) linked by short breaks, G108-P110 (H1-H2) and A129-P132 (H2-H3). The dynamic, unstructured N-terminal domain is connected to H1 by an unstructured break, residues 80–88.

In our recent study,^[17] we characterized the structure and dynamics of a truncated caveolin-1 construct (D82-S136, Cav1) that encompassed the putative intramembrane turn which is the key structural feature of the protein. The MD simulations were carried out in a DMPC (dimyristoylphosphatidylcholine) bilayer by defining the degrees of freedom necessary to describe the conformation and orientation of Cav1 in a membrane bilayer (Fig. 2). The conformation and orientation of Cav1 are defined using the hinge angle (θ) between H1 and H2, tilt angles of H1 (α) and H2 (β) in the Cav1 molecular plane, the tilt angle (ϕ) between Cav1 molecular plane and the membrane normal, and insertion depth of the G108-

P110 break region (Z_{COM}). Cav1 in a DMPC bilayer was observed to adopt a broad range of U-shaped conformations that were consistent with tryptophan fluorescence measurements.

In this study, we aim to better characterize the stable Cav1 conformations and its interactions with lipids in a bilayer by a series of all-atom free energy and molecular dynamics (MD) simulations of Cav1 in a DMPC bilayer. The free energy surface of Cav1 conformations were calculated from a two-dimensional window exchange umbrella sampling MD (2D-WEUSMD) simulation along θ and Z_{COM} (160 ns for each of 80 windows), where two similar free energy basins separated by an apparently low energy barrier of $\sim 2 k_{\text{B}} T$ were observed; k_{B} is the Boltzmann constant and T is temperature. However, the sampling was challenging during the simulation time and only several replicas sampled both basins, making the barrier and the free energy difference between the two conformations unreliable. The dynamics and stability of Cav1 in these basins were further examined by a subsequent 2- μ s MD simulation for each of 10 representative configurations taken from each of these basins. In the MD simulations, the Cav1 conformations in each basin remained stable during the simulation time except for one system.

These results strongly suggest the existence of a high hidden barrier (other than θ and Z_{COM}) that were not captured in free energy simulations. The detailed analysis of the Cav1 conformations and its interactions with lipids revealed that Cav1 in the larger θ basin adopted more “locked” conformations due to the increased hydrogen bonds (h-bonds) with bottom leaflet and the extensive h-bonds between both helices and one or two lipids compared to Cav1 in the smaller θ basin. From the results, we infer the hidden barrier appears to originate from the h-bonds between Cav1 and lipids and associated lipid rearrangements in the course of the conformational changes. We conclude with a speculation on the roles of C-terminal domain in the structure and orientation of caveolin-1 in a bilayer.

Methods

Free energy simulations

The sequence of Cav1 model was chosen to be the same as in our previous study (residues 82–136).^[17] Using a conformation of Cav1 from our previous study,^[17] a simulation system was generated, which contains Cav1 embedded in a bilayer of 303 DMPC molecules (146 in upper and 157 in lower leaflet, respectively) with 12,700 bulk water molecules and 0.15 M KCl. We chose a reaction coordinate θ between H1 and H2 to sample Cav1 conformations and performed a 30-ns preliminary WEUSMD simulation whose initial configurations for each window were generated by a sequence of umbrella sampling MD simulations by gradually changing target θ values. We chose another reaction coordinate Z_{COM} to sample Cav1 orientations in a bilayer membrane and chose initial configurations for 2D-WEUSMD from the trajectories of the preliminary WEUSMD. A total of 80 windows were uniformly distributed over 6–120° along θ with $\Delta\theta = 6^\circ$ and -10 –2 Å along Z_{COM} with $\Delta Z_{\text{COM}} = 4$ Å.

Then, a 160-ns 2D-WEUSMD simulation for each window was performed using CHARMM^[18] with the CHARMM36 protein^[19] and lipid^[20] force field, TIP3P water model,^[21–23] and an integration time-step of 2 fs using SHAKE algorithm.^[24] Simulations were run under constant temperature ($T = 310$ K) and pressure ($p = 1$ atm) conditions, where

temperature and pressure were controlled by a Hoover thermostat^[25] and Langevin-piston.^[26] A force-switching function^[27] was used to smoothly switch off the van der Waals interactions over 10–12 Å and the particle-mesh Ewald method^[28] was used to calculate the long-range electrostatic interactions with a mesh size of ~1 Å for fast Fourier transformation and a sixth-order B-spline interpolation. Window exchanges were attempted every 1 ps and controlled by the REPDSTR module^[29] in CHARMM. The bias force constants for the restraint potentials to restrain θ ^[30,31] and Z_{COM} were determined using a relation, $k^{1/2}d = 0.8643 (2k_{\text{B}}T)^{1/2}$, where d is the window spacing (θ or Z_{COM}).^[32]

Two-dimensional potential of mean force (2D-PMF) along θ and Z_{COM} were calculated by weighted histogram analysis method^[33] using the last 80-ns trajectories of 2D-WEUSMD, during which the 40-ns block PMFs show well separated basins ($< k_{\text{B}}T$) at small and large hinge angles (LB and RB, respectively). The conformations of Cav1 in these basins were analyzed by cluster analysis as follows. Among the configurations sampled every 0.8 ns for all windows, we collected the configurations whose Cav1 conformations fall in the two basins. These configurations were then clustered based on the pairwise C α root mean square deviation (RMSD) for residues 87–129 with a cutoff value of 4 Å to obtain representative sets of Cav1 conformations. From the LB, we obtained 3 clusters of Cav1 conformations; cluster 0 (74%), 1 (16%), and 2 (9%), and four clusters from the RB; cluster 3 (41%), 4 (17%), 5 (26%), and 6 (16%). Note that the cluster index of the Cav1 conformations in the RB starts from 3 to continue from the LB for easy analysis of transition of Cav1 conformations between clusters (see below). Hereinafter, these clusters are referred to as the 2D-PMF clusters and the conformations belonging to clusters 0–2 and 3–6 to as LB-Cav1 and RB-Cav1, respectively. The fraction of configurations in the obtained clusters are summarized in Table 1.

Molecular Dynamics Simulations

From each of two basins LB and RB, 10 representative configurations were chosen for subsequent 2- μ s MD simulations from the 2D-WEUSMD clusters proportionally to their size (Table 1). For the first set (MD-LB), we chose 7, 2, and 1 initial configurations from clusters 0, 1, and 2. For the other set (MD-RB), we chose 4, 1, 1, and 1 initial configurations from clusters 3, 4, 5, and 6 at $Z_{\text{COM}} < -4$ Å and, to include those with Cav1 at shallower Z_{COM} (> -4 Å), 2 and 1 configurations from clusters 3 and 5. For both sets (MD-LB and MD-RB), the restraint free MD simulations were carried out using OpenMM^[34] under the same simulation conditions used in the 2D-WEUSMD simulation. The temperature and the pressure was controlled by Langevin dynamics with a friction coefficient 1 ps^{-1} and a semi-isotropic Monte Carlo barostat^[35,36] with a pressure coupling frequency of 100 steps. We used the OpenMM input scripts generated by CHARMM-GUI *Membrane Builder*.^[37–41]

Before the trajectory analysis, we re-centered each frame to have the bilayer center at $Z = 0$ and the XY -COM of Cav1 at the origin of the XY -plane by aligning the vector connecting the centers of H1 and H2 along the X -axis (H2 on the left). Then, we calculated the hinge angle between H1 and H2 (θ), the degrees of freedom for the orientation of Cav1 (ϕ , α , β , and Z_{COM}), and the insertion depth of Trp residues (W85, W98, W115, and W128) in a DMPC bilayer (Fig. 2). In addition to the Cav1 conformation and orientation, we calculated

the positions of DMPC P atoms (X , Y , and Z) and analyzed the h-bonds between Cav1 residues and DMPC molecules. For the cluster-wise analysis, we also determined the nearest cluster to the Cav1 conformation by comparing its RMSD from the centroids of 2D-PMF clusters.

Except the time series, the analyses were performed for the last 1.2- μ s trajectories. The 1.2- μ s time range for the analyses was chosen based on the observations of time series of the data (Fig. S1 in the Supporting Material), during which the data were stable. The distributions of θ and Z_{COM} of Cav1 were calculated for each simulation set (MD-LB and MD-RB) and for each 2D-PMF cluster. The distributions of the degrees of freedom for Cav1 orientation and the insertion depth of Trp residue, the membrane thickness, and h-bond occupancy were calculated with respect to each 2D-PMF cluster. Also, the liquid crystal (P2) order parameter of lipid tail defined as $P2 = 3 \langle \cos^2 \phi \rangle - 1 / 2$, was calculated for each 2D-PMF clusters, where ϕ is the angle between the end-to-end vector of each lipid tail (vector connecting C22/C32 and C214/C314) and the membrane normal (the Z -axis). The h-bonds between a pair of Cav1 residue and DMPC lipid was monitored for each simulation system. Independent cluster analysis of MD simulations based on C α -RMSD for residues 87–129 with the same cutoff value of 4 Å was also performed to verify that the obtained clusters are consistent with 2D-PMF clusters.

Results and Discussion

This section is organized as follows. First, the 2D-PMF from the 2D-WEUSMD and sampling issues are presented. Then, the results of sampled conformations of Cav1 from the MD simulations are given and discussed followed by analyses of the conformational change and h-bonds between Cav1 and lipid.

The energetics of Cav1 conformations in a DMPC bilayer is shown in the 2D-PMF (Fig. 3) as a function of θ and Z_{COM} . Two basins at small (left basin, LB) and large (right basin, RB) hinge angles (minima at 19° and 76°, respectively) were observed, which are separated by an apparently low barrier ($\sim 2k_B T$) that could be overcome by thermal motion. However, careful examination revealed that the sampling was challenging during the simulations time. The replicas traveled partially across the window space (Fig. S2) and the majority of replicas sampled only one basin (Fig. S3). Although 12 out of 80 replicas sampled both basins (Figs. 4 and S4), single transitions were observed for all these replicas (that could not provide reliable relative weights between the basins). Thus, the relative free energy between the two basins from the 2D-PMF would not be quantitative, although the existence of two basins is clear.

The sampling results suggest the existence of high barriers along hidden degrees of freedom (DOF) other than θ and Z_{COM} , which are likely to be related to Cav1-lipid interactions (including h-bonds). To better sample the hidden DOF, one has to either extend the free energy simulation at least several times longer for multiple round trip between two basins (e.g., see Fig. 4), or run a high-dimensional (>3) free energy simulation by introducing additional reaction coordinates to describe the hidden DOF. Considering the required computational resources, both are practically prohibitive. In addition to the computational

cost, the DOF to properly describe these Cav1-lipid interactions are challenging to define. For example, multiple h-bonds between Cav1 and many lipids cannot be well-described by a small number of reaction coordinates to our best knowledge. Thus, we did not pursue the free energy simulations further. Instead, we probed the stability and dynamics of Cav1 conformations in the two basins and their interactions with lipids using unrestrained MD simulations as described below.

For the investigation of the stability of Cav1 conformations in the two basins, we set up two sets of MD simulations, MD-LB and MD-RB. For each of the two sets, 10 configurations of Cav1 were chosen from the 2D-PMF clusters obtained for LB (clusters 0, 1, and 2) and RB (clusters 3, 4, 5, and 6), respectively (Table 1). One configuration from each cluster represented the centroid while the other configurations were chosen at random. For example, for cluster 0, which has 7 configurations, 1 configuration was the centroid while the other 6 were randomly chosen. Figure 5 shows the distributions of sampled θ and Z_{COM} from the 2- μs MD simulation of each set of simulations. The majority (75% for MD-LB and 77% for MD-RB) of sampled conformations of Cav1 were populated within the $2k_{\text{B}}T$ contour of the 2D-PMF (red and green regions). Also, the distributions for each set did not show any significant cross-sampling between the left and right basins except a single system (MD-LB8: one LB to RB transition during 2- μs , see below and Fig. 7). These observations strongly support the existence of high barriers between them.

To explore the dynamics of Cav1 conformations more closely, we monitored the nearest cluster index (NCI) of the 2D-PMF clusters. The NCI for a given MD Cav1 conformation is defined as the cluster index where its conformation is closest to the centroid of that particular cluster index (i.e. minimum RMSD). In order to ensure that the NCI analysis would be useful and valid, we first examined the RMSD data obtained from this analysis (Fig. S5). It is clear from the data that the RMSD of the Cav1 conformation from the centroid of the nearest cluster (CNC) remains mostly below 4 Å, the cut off value for the 2D-PMF clusters. For MD-LB systems, RMSD of all Cav1 conformations are below ~ 4 Å. However, for a few MD-RB systems, larger RMSD values were observed. In MD-RB6 and MD-RB7, these conformations appeared only briefly, whereas, in MD-RB10, these conformations appeared over 0.8–1.3 μs . These Cav1 conformations have larger hinge angles between H1 and H2 with more extended break (G108-P110) than that of the CNC.

As shown in the time series of the NCI for each system (Fig. S5), in 19 out of 20 systems, the final NCI at 2 μs belonged to the same basin (i.e. LB or RB) indicating that Cav1 conformations in both basins are stable within our simulation time. However, intra-basin transitions more frequently occurred in MD-LB than MD-RB, suggesting that clusters in the RB are less dynamic. In 2 of the 20 systems, transient transitions (between NCIs 1 and 3 and 1 and 5) were observed between NCIs belonging to different basins (MD-LB8 and MD-RB10). In these transitions ($\theta \sim 40$ – 60°), the changes in θ and Z_{COM} were marginal ($\theta \sim 10^\circ$ and $Z_{\text{COM}} \sim 1$ Å) (Fig. S1) which shows that the Cav1 conformations and orientations are similar. This is supported by Figure 6 which shows that the outer limits of the two clusters are close.

Interestingly, in one of the MB-LB systems (MB-LB8), the Cav1 conformation shows a transition from LB to RB (NCI 1 to 4); its NCI and associated RMSD time series are shown in Figure 7. During the course of the 2- μ s simulation, the tilt initially increased ($t < 0.5 \mu$ s, α from 60° to 100° and β from 40° to 60°) while the hinge angle remained around 40° . It was followed by widening of the hinge angle ($t < 1.0 \mu$ s, θ from 40° to 70°), and finally, it settled into a new conformation (NCI 4 after 1 μ s; Fig. S5 and Movie S1).

Since there is strong agreement between the MD and 2D-PMF clusters, we classified the Cav1 conformations from MD simulations according to the cluster index of 2D-PMF clusters (i.e., total 7 clusters: 0–2 from LB and 3–6 from RB). From a cluster-wise analysis, the obtained distributions of θ , Z_{COM} , and Cav1 orientations (α , β , ϕ) are consistent with the corresponding 2D-PMF clusters (Figs. S6–S7 and Table S1). Hereinafter, we will describe the structure of Cav1 and its interactions with lipids using these results.

The representative configurations of Cav1 in a DMPC bilayer for the largest clusters in the two basins (cluster 0 for LB and cluster 3 for RB, Table 1) obtained from the cluster-wise analysis of the MD trajectories are shown in Figures 8a–b. In cluster 0 (LB, Fig. 8a), H1 and H2 are in contact with each other and tilted, so that these helices interact primarily with different leaflets; H1 (red) interacts mostly with top leaflet while H2 (green) interacts with the bottom leaflet. In cluster 3 (RB, Fig. 8b), however, H1 and H2 interact with both leaflets. Importantly, the insertion depths of Trp residues of these configurations are consistent with the fluorescence measurement data^[17] (Fig. S6). As shown in Figure 8c, both Cav1 conformations induce membrane thinning primarily through interactions with the bottom leaflet, although cluster 3 induces more pronounced membrane thinning than cluster 0 (Fig. 8c). The P2 order parameter for leaflets are also consistent with the membrane deformation such that the effects are shown more clearly in P2 profile for lower leaflet and the lower leaflet for cluster 3 are more ordered than that for cluster 0 (Fig. S8). These data indicate these Cav1 conformations influence differently on membrane deformation and lipid packing.

To characterize how these Cav1 conformations interact with the lipid molecules, we analyzed the average number of h-bonds between each residue and DMPC molecules for residues 89–128 (consisting of H1-break-H2, Figs. S9–S10). As shown in Figure 9, both clusters show a significant number of Cav1-lipid h-bonds although cluster 3 has additional h-bonds (13.6 ± 0.1) when compared to cluster 0 (12.0 ± 0.1). Cluster 0 shows a preference for h-bonds with the top leaflet (9.2 ± 0.1 versus 2.8 ± 0.1) while cluster 3 shows a more equal distribution (7.7 ± 0.1 versus 5.9 ± 0.1). Although clusters 0 and 3 contain a similar number of direct h-bonds in the break region (residues 104–110), cluster 3 shows a marked increase in the number of bridged h-bonds which is likely due to its deeper penetration in the bottom leaflet (Fig. 8b). Interestingly, there are a number of residues which interact with the top leaflet (R101, Y97, and W98) or the bottom leaflet (W115 and Y118) in cluster 0, but interact with the opposite leaflet in cluster 3. This change gives cluster 3 significant h-bond interactions between both helices and both leaflets, and is consistent with the observed membrane thinning of the bottom leaflet in Figure 8c.

The Cav1-lipid interactions were further investigated by analyzing the frequency of specific DMPC molecules interacting with Cav1 (Table S2). The analysis was performed for

simulation systems whose dominant NCI was 0 or 3 during the last 1.2 μ s of simulation time. The apparent difference between cluster 3 and cluster 0 is the existence of one or two DMPC molecules that interact strongly with the residues in both H1 and H2: this is not observed in cluster 0 which has DMPC molecules interacting in a monovalent fashion or those interacting only with a single helix at most. These distinct h-bond patterns imply that RB-Cav1 conformations (cluster 3) are more tightly “locked” by extensive h-bonding with a couple of lipids situated in between both helices. This interpretation is consistent with the observed dynamics of the NCI, i.e., much less NCI transitions in the RB compared to those in LB (Fig. S5). Considering the membrane thickness profiles (Fig. 8c), the increased hydrogen bonds between RB-Cav1 conformations and DMPC appeared to compensate the energetic penalty due to the membrane deformation.

When all data are taken together, we can speculate on the transition between the two free energy basins, LB and RB. The two basins appear to be separated by a high energy barrier from both basins ($> \sim 16 k_B T$ as a rough estimate from the transition state theory^[42] for their lifetimes $> 2 \mu$ s), whose in-between intermediary states, in principle, can be determined unambiguously from the accurate PMF along a properly-defined set of progress variables. However, in the presence of such high barriers, it is not generally probable to directly observe the intermediary states during transitions between the basins in unrestrained MD simulations^[43], which could be determined from targeted MD simulations with careful analyses of the progress variables^[44]. Although the calculated 2D-PMF along Z_{COM} and θ (Fig. 3) were not able to quantitatively determine the relative free energies between LB and RB Cav1 conformations, one can still obtain useful information on a probable pathway (a low free energy path) of the interconversion between LB and RB by considering the samplings along Z_{COM} and θ for the clusters from multiple unrestrained MD simulations (Fig. S6A). In this context, the transitions between both basins would likely follow the sequence of cluster 0 (LB) $\leftrightarrow$ 1 $\leftrightarrow$ 5/4 $\leftrightarrow$ 3 (RB) conformations along the low energy valley ($< 2 k_B T$) in the 2D-PMF.

Along this pathway, a transition from RB to LB can be described as follows. As a few polar/charged H1 residues in RB Cav1 (Y97, W98, R101, and S104, see Fig. 9 and Fig. S9) lose their interactions with bottom leaflet DMPC molecules, the insertion of Cav1 becomes shallower (cluster 5/4). It follows that the breakage of multiple h-bonds between K96, Y100, W115, and Y118 with a couple of lipids in between the two helices (Table S2) that must take place prior to Cav1 conformational change. Because these h-bonds must break simultaneously, this step is energetically costly. Then, Cav1 undergoes a conformational change to cluster 1 with associated lipid rearrangements, without significant changes in Z_{COM} and Cav1-lipid h-bond interactions. Lastly, W115 and Y118 in H2 of Cav1 form interactions with the bottom leaflet DMPC molecules, resulting in stable LB Cav1 conformations (cluster 0). Considering that typical energy of h-bonds for N-H $\cdots$ O and O-H $\cdots$ O ranges over 4 – 6 kcal/mol ($6 - 10 k_B T$ at room temperature)^[45], the h-bonds seem not to be enough for the observed stability of the Cav1 conformations in two basins (lifetime $> 2 \mu$ s). Therefore, the associated lipid rearrangements would also be a major contributor to the free energy barrier.

Conclusions

In the present study, we have examined the structure and dynamics of a truncated construct of caveolin-1, Cav1 (residues 82–136), by both all-atom free energy and MD simulations in a DMPC bilayer. From the free energy simulations, we identified two free energy basins of U-shaped Cav1 conformations at small and large hinge angles. Although the two free energy basins of Cav1 conformations are separated by an apparently low barrier ($\sim 2k_B T$) along θ and Z_{COM} , sampling was challenging in the free energy simulations. Only a few replicas were able to sample both basins, which was consistent with the results of 2- μ s MD simulations (no transition between basins except in the case of one system out of 20 systems). The results strongly suggest the existence of a high energy barrier ($> 16 k_B T$) between them and thus the stability of these basins. Detailed h-bond analysis reveals that Cav1 conformations with larger hinge angles are more tightly “locked” by one or two lipids making strong h-bonds with both H1 and H2, which is absent for Cav1 with small hinge angles. From the results, we infer that h-bond interactions between Cav1 and lipids compensate for the penalty from membrane deformation, and that the required h-bond rearrangements associated with lipid diffusion during conformational changes hinder fast interconversion between these basins.

Both of these models (LB- and RB-Cav1) have been postulated by our lab and now we have data which suggest that both are possible. Therefore, the results bring us one step closer to unraveling the mystery of the conformation of caveolin-1 and how its conformation facilitates its function of curving membranes. However, care must be taken because we simulated a truncated model (residues 82–136) without the C-terminal helix H3 (residues 133–175).^[15] H3 could further stabilize the orientation of H2 or participate actively in the regulation of Cav1 conformations by interacting with scaffolding and/or N-terminal domain(s). These effects due to presence of H3 could have marked consequences on the free energy landscape of caveolin-1 conformations. Also, the H3 can be a useful model in understanding the regulation of membrane protein structure by a parallel helix to membrane. To our best knowledge, there is no available information on the orientations of H3 relative to H1 and H2. Thus, the modeling of Cav1 construct containing all H1, H2, and H3 would be computationally even more intensive compared to the current truncated Cav1 (residues 82–136) and deserves to be a separate future study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH R01-GM092950, U54-GM087519, XSEDE MCB070009 (to W.L.) and NIH R01-GM093258 (to K.J.G.).

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Figure 1.
(A) Topology and (B) proposed domains and secondary structure of caveolin-1.

Figure 2.

Degrees of freedom used to define the conformation and orientation of Cav1. (A) The hinge angle (θ) between H1 and H2 and (B) tilt angles of H1 (α) and H2 (β) in the Cav1 molecular plane, the tilt angle (ϕ) between Cav1 molecular plane and the membrane normal, and insertion depth of the break region (Z_{COM}) describing the orientation of Cav1 with respect to a membrane bilayer are shown. The membrane is centered at $Z = 0$. H1 and H2 are shown as cylinders (red and green, respectively) and the residues in the break region (G108-P110) and Trp residues are indicated by colored spheres.

Figure 3.

Two-dimensional potential of mean force of Cav1 as a function of hinge angle (θ) and Z center of mass of three residues G108-P110 (Z_{COM}). The bin sizes were set to 1.125° and $0.5 \text{ \AA}$ along θ and Z_{COM} respectively in the weighted histogram analysis for 2D-PMF calculation, and the contour lines are shown at every $k_B T$.

Figure 4. Scattered plots of sampled Z_{COM} and θ for replica 32 with $k_B T$ contour of the 2D-PMF (black lines). In scattered plots, sampled times are shown in different colors.

Figure 5.

Distributions of sampled θ and Z_{COM} for each set of MD simulations over the last 1.2 μs overlaid with the 2D-PMF contours. The histograms were calculated using bin sizes 1.2° and $0.4 \text{ \AA}$ along θ and Z_{COM} , respectively. The frequency of each bin was normalized by the peak value. The black circles represent the 10 initial conformations for each set.

Figure 6.

Overlay of distributions of sampled θ and Z_{COM} for NCIs (A) 1 and 3 and (B) 1 and 5 from MD simulations over the last 1.2 μs . The frequency of each bin was normalized by the peak value with the same bin sizes to those for Figure 5.

Figure 7.

(A) Time series of the nearest cluster index (see main text for its definition) for MD-LB8 and (B) that of Cav1 RMSD from the centroid of the corresponding cluster. The snapshots of Cav1 before (left, at $t = 0 \mu\text{s}$) and after (right, at $t = 1.7 \mu\text{s}$) transition are also shown in (A), where H1 and H2 are shown in red and green respectively. Data were plotted every 1 ns.

Figure 8.

Representative configurations of Cav1 in a DMPC bilayer for (A) cluster 0 (LB) and (B) cluster 3 (RB), and (C) membrane thickness profiles (Z -positions of P atoms) along the XY -dimensions for bilayer and leaflets for cluster 0 (top) and cluster 3 (bottom). (A-B) H1 and H2 are shown in a cartoon representation (red and green, respectively). Side chains of polar/charged residues and those of the break region (G108-P110) are shown in a stick representation, among which the residues h-bonding to lipid (either direct or water-bridged) are marked with blue color. The head groups (P atom) of lipids are shown as orange spheres and the other components are omitted for clarity. The black dashed lines represent the bilayer center ($Z=0$). (C) Before calculation, the bilayers were re-centered at $Z=0$ and the vector connecting the centers of H1 and H2 was aligned along the X -axis (H2 on the left). The Cav1 density contours at 0.25 and 0.5 are shown for the region occupied by Cav1.

Figure 9.

(A) Number of h-bonds between residues 89–128 and lipids. (B) The difference of the number of h-bonds between clusters 3 and 0. The number of h-bonds between lipids in each leaflet are shown in different colors: top leaflet is red and bottom leaflet is blue. The sum of the number of h-bonds between residues 89–128 and lipids are shown in each panel of (A). The error bars are the standard errors from 200-ns block averages for (A) and the estimated error for (B).

Table 1.

Population of the clusters from each basin, f_i on the 2D-PMF and the number of initial configurations, N_i for MD simulations.

	Basin									
	LB			RB						
Index ^[a]	0	1	2	3	3'	4	5	5'	6	
$f_i^{[b]}$	0.74	0.16	0.09	0.29	0.11	0.17	0.18	0.08	0.16	
$N_i^{[c]}$	7	2	1	4	2	1	1	1	1	

^[a] Cluster index. Population of clusters from RB-Cav1 at deeper $Z_{\text{COM}} < -4 \text{ \AA}$ and shallower $Z_{\text{COM}} > -4 \text{ \AA}$ (indicated by primed cluster index) were separately given.

^[b] Normalized by the total number of sample conformations from each basin.

^[c] A total of 10 initial configurations from each basin.