Molecular dynamics simulation studies on binding of activator and inhibitor to Munc13-1 C1 in the presence of membrane

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Abstract

Munc13-1 is a presynaptic active zone protein that plays a critical role in priming the synaptic vesicle and releasing neurotransmitters in the brain. Munc13-1 acts as a scaffold and is activated when diacylglycerol (DAG)/phorbol ester binds to its C1 domain in the plasma membrane. Our previous studies showed that bryostatin 1 activated the Munc13-1, but resveratrol inhibited the phorbol ester-induced Munc13-1 activity. To gain structural insights into the binding of the ligand into Munc13-1 C1 in the membrane, we conducted 1.0 μs molecular dynamics (MD) simulation on Munc13-1 C1–ligand–lipid ternary system using phorbol 13-acetate, bryostatin 1 and resveratrol as ligands. Munc13-1 C1 shows higher conformational stability and less mobility along membrane with phorbol 13-acetate and bryostatin 1 than with resveratrol. Bryostatin 1 and phorbol ester remained in the protein active site, but resveratrol moved out of Munc13-1 C1 during the MD simulation. While bryostatin 1-bound Munc13-1 C1 showed two different positioning in the membrane, phorbol 13-acetate and resveratrol-bound Munc13-1 C1 only showed one positioning. Phorbol 13-acetate formed hydrogen bond with Ala-574 and Gly-589. Bryostatin 1 had more hydrogen bonds with Trp-588 and Arg-592 than with other residues. Resveratrol formed hydrogen bond with Ile-590. This study suggests that different ligands control Munc13-1 C1’s mobility and positioning in the membrane differently. Ligand also has a critical role in the interaction between Munc13-1 C1 and lipid membrane. Our results provide structural basis of the pharmacological activity of the ligands and highlight the importance of membrane in Munc13-1 activity.

Introduction

Munc13-1 is a member of the Munc13 family of proteins that play role in synaptic vesicle priming and neurotransmitter release at the presynaptic active zone (Betz et al., 1997; Quade et al., 2019; Sassa et al., 1999). Munc13-2, Munc13-3, and Munc13-4 are the other members of the family known till date (Augustin, Betz, et al., 1999; Chen et al., 2013). Munc13-1 is expressed primarily in the hippocampus, cortex, cerebellum, and striatum regions of rat brain (Augustin, Betz, et al., 1999; Augustin, Rosenmund, et al., 1999). It regulates short-term presynaptic plasticity and long-term potentiation by interacting with an active zone protein, Rab3 Interacting Molecules alpha (RIMα) (Lipstein et al., 2013; Yang & Calakos, 2011; Zarebidaki et al., 2020). Double-knockout mice of Munc13-1 and Munc13-2 die within a few hours of birth suggesting the critical role of Munc13 proteins in neurons (Augustin, Rosenmund, et al., 1999). The deletion of Munc13-1 results in complete abolishment of neurotransmitter release (Aravamudan et al., 1999; Richmond et al., 1999; Varoqueaux et al., 2002). Munc13-1’s role has been implicated in neurodegenerative disorders (Lipstein et al., 2017). In an Alzheimer’s disease model, Munc13-1 was shown to involve in Aβ-induced neurotoxicity (Hartlage-Rübsamen et al., 2013; Ikin et al., 2007; Rossner, 2004) and regulate the metabolism of secretory amyloid precursor protein (APP) that generates the β-amyloid peptides, the main constituents of senile plaques in the brain of Alzheimer’s disease patients (Rossner et al., 2004). A single-nucleotide polymorphism in Munc13-1 gene is involved in fronto-temporal dementia and amyotrophic lateral sclerosis (Bosco & Landers, 2010; Diekstra et al., 2014; Finsterer & Burgunder, 2014; Su et al., 2014). In addition, Munc13-1 is associated with insulin release (Kwan et al., 2006). Munc13-1 is expressed in islet β-cells, and the expression level of Munc13-1 is reduced in islets of type 2 diabetic rodent and human models. Furthermore, Munc13-1 has binding site(s) for alcohols, and deficiency in the level of Munc13-1 causes defects in alcohol sensitivity, tolerance, and self-administration in Drosophila (Das et al., 2013; Xu et al., 2018) suggesting its role in alcohol addiction.

Munc13-1 is a large peripheral membrane protein that consists of five domains, three C2 domains (C2A, C2B, and C2C), one C1 domain, and one MUN domain (Aravamudan et al., 1999; Ma et al., 2011; Shin et al., 2010). The C2A domain is at the N-terminus, followed by the C1 domain, C2B, MUN and C2C. The C1 domain binds to diacylglycerol
(DAG) and phorbol ester like other homologous typical C1 domains, such as conventional (α, β and γ) and novel (δ, ε and η) protein kinase C (PKC). The C2B domain binds plasma membrane where phosphatidylinositol phosphate (PIP) or phosphatidylinositol 4,5-bisphosphate (PIP2) is and also has binding sites for two calcium ions (Shin et al., 2010). The residues 859-1531 form the MUN domain which is between the C2B and C2C domains (Basu et al., 2005). The C2C domain is located at the C-terminal. C1-C2B-MUN of Munc13-1 forms a rod-shaped structure as revealed by X-ray crystallography (PDB: 5UE8) (Xu et al., 2017).

The C1 domain of Munc13-1 plays a critical role in the activity of the Munc13-1. Binding of endogenous DAG to the C1 domain stimulates translocation of Munc13-1 from cytoplasm to lipid membrane like the homologous C1 domains (Figure 1(A)) (Das & Rahman, 2014). Translocation of Munc13-1 to the plasma membrane triggers assembling of Munc18-1 and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex for releasing neurotransmitters (Rizo & Xu, 2015). The structure of Munc13-1 C1 (PDB: 1Y8F) (Figure 1(B)) (Shen et al., 2005) is very similar to the C1 domain of protein kinase C (PKC) isozymes (Das & Rahman, 2014). It has two β-sheets (β12 and β34), a short C-terminal α-helix, and two Zn2+ finger motifs like PKCδ C1B and PKCζ C1B (Shen et al., 2005; Zhang et al., 1995). The side chain of Trp-588 (Trp-22 in the C1 domain) occludes the DAG/phorbol ester binding site, but the homologous tryptophan residues do not do so in the C1B domain of PKCδ and PKCζ (Rahman et al., 2013; Shen et al., 2005; Zhang et al., 1995), suggesting that ligand binding site of Munc13-1 C1 domain might be different from that of the homologous C1 domains. Indeed, the binding affinity of phorbol ester to the Munc13-1 C1 domain is lower than that of PKCδ C1B (Ananthanarayanan et al., 2003; Betz et al., 1998; Wang et al., 2001).

Several natural and synthetic compounds have been studied for their binding affinity, isofom selectivity, and the membrane translocation property for the C1 domain-containing proteins (Blumberg et al., 2008; Boije af Gennås et al., 2011; Das & Rahman, 2014; Dries et al., 2007; Giorgione et al., 2006; Stewart & Igumenova, 2017). In addition to the biochemical studies, molecular docking and molecular dynamics studies have been conducted to understand the interaction between the C1 domain and ligands (Katti & Igumenova, 2021). One of the compounds, ingenol-3-angelate (Picato) that targets PKC C1 domain, has been approved by the Food and Drug Administration (FDA) and European Medicine Agency (EMA) in 2012 for the treatment of actinic keratosis (Heron & Feldman, 2021; Kedei et al., 2004; Kostovic et al., 2017) showing the feasibility of discovering drug targeting C1 domains.

In order to develop modulators of Munc13-1 for regulating the related disease states, we studied various ligands for their effects on Munc13-1 activity (Blanco et al., 2019; Das et al., 2013; Pany et al., 2017). We showed that bryostatin 1, an oxygenated macrocyclic lactone isolated from the marine bryozoan Bugula neritina, activates the translocation of Munc13-1 through its binding to Munc13-1 C1 like phorbol ester (Blanco et al., 2019). The neuroprotective effects of bryostatin 1 have been studied up to phase II clinical trials on AD patients (Clinicaltrials.gov identifier NCT02431468). We also studied the effect of resveratrol, a natural polyphenol isolated from grapes, red wine, cranberries, olive oil, peanuts, etc. (Pervaiz, 2003; Wang et al., 2002) on Munc13-1 activity. Several studies reported the role of resveratrol in neuroprotection (Rege et al., 2014; Richard et al., 2011). We found that resveratrol binds to the C1 domain and inhibits phorbol ester-induced activity of Munc13-1 (Pany et al., 2017).

Here, we investigated the structural basis of the pharmacological activity of the three ligands, phorbol 13-acetate, bryostatin 1, and resveratrol in the ternary complex, Munc13-1 C1-ligand–membrane, using molecular docking and molecular dynamics simulation. We found that these ligands interact with different residues at the active site of Munc13-1 C1. We also showed that ligand controls the positioning and movement of Munc13-1 C1 in the membrane.

Material and methods

Molecular docking

Phorbol 13-acetate, bryostatin 1, and resveratrol were docked into the NMR structure of Munc13-1 C1 domain (PDB: 1Y8F) (Shen et al., 2005) using AUTODOCK 4.2 software. Munc13-1 C1 is composed with 50 residues and the total charge of the C1 domain with two zinc ions is −5. The 6 cysteine residues coordinating the two zinc ions were deprotonated. The molecular docking of the ligands was prepared and analyzed using AutoDockTools (ADT). The structure of phorbol 13-acetate and resveratrol was drawn using ChemBioDraw version 12.0 (PerkinElmer) and the crystal structure of bryostatin 1 was used from the Cambridge Structural Database (Pettit et al., 1982). The total charge of the three ligands were 0. The energy of the ligands was minimized with 0.1 RMS kcal/mol/Å2 gradient level using MOE 2018 (MOE; Chemical Computing Group). For the charge, Gasteiger charges and Kollman charges were assigned to the ligand and the 3D crystal structures were visualized using Discovery Studio Visualizer 4.5 (DS, Biovia Inc., San Diego, CA).

Building a complex of ligand and Munc13-1 C1 in phospholipid membrane

The tilt angle and depth of insertion of the Munc13-1 C1-ligand complex in the lipid membrane were determined using
PPM server (Lomize et al., 2012). The conformation of ligand in the complex that had the lowest calculated binding energy in the largest cluster from the best twenty conformers was used in model building. The transfer energy of the ligand-bound Munc13-1 C1 to the lipid bilayer is calculated in the PPM server. The transfer energy is the sum of two factors. One factor is the solvent accessible surface area accounting hydrogen bonding solvent-solute interactions, entropy of solvent molecules, and Van der Waals in the first solvation shell. The second factor is electrostatic properties including deionization penalty of ionizable groups and solvation energy of dipoles and ions in non-polar environment (Lomize et al., 2004). The transfer energy in PPM server was expressed as:

$$
\Delta G_{\text{transf}} = \sum_i A S A_i \sigma_{i,W-M} f(z_i) \tag{1}
$$

where $ASA_i$ is the accessible surface area of atom (Chothia, 1975) and $\sigma_{i,W-M}$ is the solvation parameter of atom (Lomize et al., 2004). A sigmoid water concentration profile $f(z_i)$, as determined in EPR studies on spin-labeled phospholipids, was used (Marsh, 2001). The model of the ternary complex, lipid membrane-ligand-Munc13-1 C1, in the rectangle box was built using the CHARMM-GUI Bilayer Builder (Jo et al., 2008). N-terminus of the C1 domain was terminated with acetylation and C-terminus was terminated with methyl amimation. The initial size of the simulation box was set at $X = 61$ Å and $Y = 61$ Å and Z-length was set based on water thickness. The system box was filled by TIP3P water molecules and the minimum height of water on top and bottom was set to 20 Å. Potassium ions were added into the system to neutralize the net charge of the system. C1 domain-containing proteins show specificity for phosphatidylyserine (PS) of lipid membrane in upper and lower leaflets were 64 and 58, respectively. The charmm36 force field was applied to describe the system (Lee et al., 2016).

**Molecular dynamics simulation**

Molecular dynamics (MD) simulations of the ternary complex, Munc13-1 C1–ligand–lipid membrane (PS), were carried out using GROMACS 2018.3 package (Hess et al., 2008). Energy minimization was performed until the maximum force was below 500 kJ/mol/nm. The steep descent method was used to eliminate steric clashes generated during solvating the ternary complex in the simulation box. Equilibration of the energy minimized system was conducted for maintaining the temperature and pressure of systems (300 K, 1 bar). To equilibrate, the systems were gradually relaxed according to the position and angle restraints which CHARMM-GUI provided. NVT optimization was performed for 25 ps in the first and second phases with harmonic restraints (kcal/mol Å²) of protein backbone, protein sidechain, lipid, and lipid angle. The harmonic restraints were 4.0, 2.0, 1.0, 1.0 for phase 1 and 2.0, 1.0, 1.0, 0.4 for phase 2. And then, NPT optimization was performed for 25 ps, 100 ps, 100 ps, and 10.1 ns in the 3rd to 6th phases, respectively. The restraints were 1.0, 0.5, 0.4, 0.2 for phase 3; 0.5, 0.2, 0.2, 0.2 for phase 4; 0.2, 0.2, 0.04, 0.1 for phase 5; and 0.05, 0, 0, 0 for phase 6. The equilibrated systems were used for the MD production with the Parrinello-Rahman barostat algorithm and Nose-Hoover thermostat algorithm. The time step of MD production was 2 fs and the time constant was kept at 0.5 ps and 5 ps for the temperature and the pressure coupling, respectively (Desikan et al., 2021; Velasco-Bolom & Garduño-Juárez, 2021). Electrostatic

**Figure 1.** Simplified scheme showing the function of Munc13-1 for synaptic vesicle fusion and structure of its C1 domain. (A) Munc13-1 is translocated from the cytosol to the plasma membrane through the interaction between the C1 domain and its ligand, such as diacylglycerol (DAG) and phorbol ester (represented by the red triangle). The ligand binding C1 domain and Ca²⁺ binding C2B domain anchor to the plasma membrane. The C2C domain binds to the synaptic vesicle. Munc13-1 initiates assembling of the other proteins, including SNARE proteins and Munc18-1. It regulates the docking and fusion of the neurotransmitter loaded synaptic vesicle with the plasma membrane. (B) NMR structure of Munc13-1 C1 (PDB: 1Y8F). Trp-588 and Zn²⁺ are represented by red colored stick and gray CPK, respectively.
interactions of the system were calculated by the particle mesh Ewald (PME) method with 1.6 Å grid spacing and fourth-order cubic interpolation (Essmann et al., 1995). A 12 Å cutoff was applied for the calculation of Coulombic, Van der Waals, and electrostatic interactions. The parallel LINCS method was used for constraining all bonds. For analysis of MD simulation, the atomic coordinates were saved every 2 ps during 1.0 μs simulation time.

**Analysis of MD simulation and statistics**

The MD trajectories were analyzed using GROMACS analysis tools, including gmx rmsf, gmx rms, gmx distance, gmx hbond, and gmx bundle. The gmx_rmsf computes the root mean square fluctuation (RMSF) of atomic positions in the trajectory based on the positions at 0 ns. The gmx rms calculates the root mean square deviation (RMSD) to compare structures to the initial structure at 0 ns. The distance between two molecules was calculated based on the center of geometry of the molecules by using gmx distance. Hydrogen bonds were determined based on cutoffs of the angle of the hydrogen – donor atom – acceptor atom and the distance of between the acceptor and donor using gmx hbond. The cut-off angle and distance were 30° and 3.5 Å, respectively. To calculate the tilt angle of the C1 domain toward the lipid membrane, an axis of the C1 domain was determined by selecting the center of two loops including the ligand binding site and the center of Met-595 and Cys-604 which are at the center of the C1 domain, and then the tilt angle of the axis from the Z-axis of the lipid membrane was monitored by using gmx bundle. The binding energy was calculated from 100 ps or 5 ns interval MD trajectories by Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA) method (Kollman et al., 2000) using g_mmpbsa package (Kumari et al., 2014). The binding energy for the binary complex (ligand-protein) was calculated by deducting the free energy of the ligand-protein complex from the sum of the free energies of the ligand and the protein.

$$\Delta G_{\text{bind}} = G_{\text{binary complex}} - (G_{\text{Munc13-1 C1}} + G_{\text{ligand}})$$  \hspace{1cm} (2)

For the ternary complex (ligand-protein-membrane), the binding energy was calculated by deducting the free energy of the ligand-protein-membrane complex from the sum of the free energies of the ligand-bound protein and the membrane.

$$\Delta G_{\text{bind}} = G_{\text{ternary complex}} - (G_{\text{ligand-bound Munc13-1 C1}} + G_{\text{membrane}})$$  \hspace{1cm} (3)

For the polar contributions, a box was generated with 0.5 Å grid spacing and the coarse grid-box (cfac) was set as 1.5 and the finer grid-box (fadd) was set as 5. The values for solvent (sdie) and the vacuum (vdie) dielectric constants were set as 1 and 80, respectively. The solute (pdie) dielectric constant was taken as 2 for ligand-Munc13-1 C1 complex and as 50 for the ligand-Munc13-1 C1-membrane complex. For the non-polar solvation contributions, solvent accessible surface area (SASA)-only model was used. The binding energy was calculated from 100 ps interval MD trajectories for the C1 domain-ligand complex and 5 ns interval MD trajectories for the ligand-bound C1-membrane complex using g_mmpbsa (Kumari et al., 2014). The graphs were plotted with QrGrace. Data were analyzed using Prism 9.0 software (GraphPad Software, Inc., San Diego, CA). All statistical analyses were conducted on the basis of three independent simulations. Each simulation was reproduced for the MD production using the same equilibrated system in temperature and pressure. Standard error of the mean (SEM) of three independent simulations were used for the statistics of tilt angle, moving area, number of hydrogen bond, and calculated binding energy. The structures and trajectories were visualized using DS 4.5 and PyMol version 1.7 (Schrodinger, LLC).

**Results and discussion**

In the present study we performed molecular dynamics simulations of ligand-bound Munc13-1 C1 in the presence of membrane. We used three ligands, phorbol 13-acetate, bryostatin 1 and resveratrol of which the former two are activators and the latter is an inhibitor. Previously we characterized the biochemical effects of these ligands on Munc13-1 (Blanco et al., 2019; Das et al., 2018; Pany et al., 2017; You et al., 2021). Munc13-1 bridges the plasma membrane and vesicular membrane and acts as a master regulator of neurotransmitter release process in the brain (Betz et al., 1997; Sassa et al., 1999). Binding of the C1 domain with the membrane is critical to the activity of Munc13-1. As such, structural studies of the membrane associated proteins are scarce, even more scarce is the studies on the protein-ligand-membrane interactions of peripheral membrane proteins. Understanding the ligand-Munc13-1 C1–membrane interaction will aid in understanding the mechanism of actions of these ligand and in the development of potent Munc13-1 modulators for neurodegenerative diseases and alcohol use disorder as this protein is involved in the regulation of these disease states.

Munc13-1 is a ~200 kDa protein having multiple domains with distinct functions. During the formation of SNARE complex it underdoes massive conformational change enabling optimal protein-protein interactions among its partner proteins. The binding affinity of phorbol ester for the C1 domain and the full-length Munc13-1 is not significantly different (Das et al., 2018) and this piecemeal approach is very common for studying large modulatory proteins like Munc13-1.

**Docking mode of phorbol 13-acetate, bryostatin 1, and resveratrol into Munc13-1 C1**

To understand the structural basis of ligand-induced activity of Munc13-1, we docked three ligands, phorbol ester, bryostatin 1, and resveratrol, into Munc13-1 C1 and studied the molecular dynamics in the presence of membrane (Figure 2). Phorbol ester (PMA) and bryostatin 1 bind to the C1 domain of Munc13-1 and activate the protein by translocating it from the cytosol to the plasma membrane (Blanco et al., 2019). However, resveratrol did not activate the translocation of Munc13-1. On the contrary, it inhibited the phorbol ester-induced activity of Munc13-1 (Pany et al., 2017). As Munc13-
1 C1 is a structural homologue of PKCδ C1B, we used the phorbol ester-binding region/residues of PKCδ C1B for the molecular docking of the ligand into Munc13-1 C1. Based on the binding and activity measurement studies, the ligands were docked into the phorbol ester/DAG binding site of Munc13-1. The docking parameters were verified by comparing the docking results of phorbol ester in PKCδ C1B and the co-crystal structure of PKCδ C1B with phorbol ester (PDB: 1PTR). Ligands were docked into the C1 domain and a docking pose which has the lowest binding energy in the largest cluster was selected for molecular dynamics simulation (Figure 3). The selected docking poses showed that the essential pharmacophoric elements, C20-OH in phorbol 13-acetate and C26-OH in bryostatin 1 for binding to PKCδ C1B, are placed in the active site of Munc13-1 C1 and are able to form hydrogen bonds (Ashida et al., 2016; Nelson & Alkon, 2009). Phorbol 13-acetate formed three hydrogen bonds with Trp-588, Ile-590, and Arg-592 (Figure 3(A) and Table 1). The C21 carboxyl group of phorbol ester forms a hydrogen bond with the amino group of the indole of Trp-588. The C20 hydroxyl group of phorbol ester is slightly buried in the shallow pocket and interacts with the backbone carbonyl group of Ile-590 and the backbone amino group of Arg-592. It also showed Pi-Sigma hydrophobic interaction with Trp-588. In the case of bryostatin 1, it formed four hydrogen bonds with backbone amino group and side chain amino group of Arg-592, backbone carboxyl group of Ile-590, and backbone carboxyl group of Gly-589 (Figure 3(B)). It also showed a Pi-Alkyl hydrophobic interaction with Trp-588. Resveratrol showed similar docking pose with phorbol 13-acetate by forming hydrogen bonds with backbone amino group and side chain amino group of Arg-592 and backbone carboxyl group of Ile-590, but it did not form any bonds with Trp-588 (Figure 3(C)). The phorbol 13-acetate and bryostatin 1 were located close to the hydrophilic region at the ligand binding site rather than hydrophobic region (Figure 3). Covering the hydrophilic region at the rim of the two loops enhances the interaction between C1 domain and plasma membrane (Rahman & Das, 2015). The calculated binding energy of phorbol 13-acetate (-2.64 kcal/mol) was less than bryostatin 1 (0.68 kcal/mol) even though bryostatin 1 formed more hydrogen bonds than phorbol 13-acetate (Table 1). In the case of resveratrol, it showed a slightly less calculated binding energy (-2.86 kcal/mol) than phorbol 13-acetate.

DAG is the endogenous ligand that binds to Munc13-1 C1 and lowers the energy barrier for vesicle fusion (Basu et al., 2007). Phorbol ester mimics the actions of DAG (Geiger et al., 2003; Goel et al., 2007) and binds to the C1 domain with higher affinity than DAG (Kazanietz et al., 1992; Sharkey & Blumberg, 1986). The lower binding affinity of DAG has been attributed to its flexible long chains as compared to the phorbol ester which has a rigid structure (Kazanietz et al., 1992; Sharkey & Blumberg, 1986). We previously reported that the neuroprotective agent bryostatin 1 binds to the C1 domain and activates Munc13-1 (Blanco et al., 2019). On the other hand, plant-derived polyphenol resveratrol binds to the C1 domain but inhibits the phorbol ester-induced activation of Munc13-1 (Pany et al., 2017). Recently we characterized the ligand binding of Munc13-1 at its C1 domain (You et al., 2021) and this site has been used to dock phorbol ester, bryostatin 1 and resveratrol. In our docking model phorbol 13-acetate bound to the region surrounded with Trp-588, Ile-590, and Arg-592 (You et al., 2021). These residues are somewhat different than the homologous residues in the phorbol 13-acetate binding residues in PKCδ C1B. These differences arise because Trp-588 in Munc13-1 blocked the ligand binding site, but the homologous Trp-252 in PKCδ does not do so. In the phorbol 13-acetate-bound PKCδ C1B, the C20 hydroxyl group of phorbol 13-acetate formed hydrogen bonds with Thr-242 and Leu-251. In Munc13-1 C1, however, Ile-590 and Arg-592 formed hydrogen bonds with the C20 hydroxyl group (Figure 3(A)). The pharmacophoric core of bryostatin 1, the C26 hydroxyl group, which is equivalent to the C20 hydroxyl group of phorbol 13-acetate also formed hydrogen bond with Ile-590 and Arg-592 of Munc13-1 C1 (Figure 3(B)). In the bryostatin 1-PKCδ C1B complex, however, the C26 hydroxyl group of bryostatin 1 formed hydrogen bonds with Thr-242 and Leu-251 of PKCδ C1B (Keck et al., 2010). In both Munc13-1 C1 and PKCδ C1B, the A and B rings of bryostatin 1 lied above the docking site in the plane of the lipid membrane, although the binding site of bryostatin 1 in Munc13-1 C1 is slightly different from the binding site in PKCδ C1B.

**Embedding the ligand-bound Munc13-1 C1 into the plasma membrane**

To understand the influence of the lipid membrane on the protein-ligand complex, we embedded the ligand-bound Munc13-1 C1 to the PS membrane and performed 1.0 μs MD simulations of the ligand–Munc13-1 C1–PS ternary complex. Using PPM server, we calculated the orientation of Munc13-1 C1 domain which directly binds to the plasma membrane. We found that in the absence of ligand, Munc13-1 C1 did not embed into the membrane and was oriented in parallel to the membrane with a high tilt angle, 68° (Figure S1(A)). In contrast, ligand-bound Munc13-1 C1 embedded into the membrane over 3.9 Å of depth. Among ligands, the activators, phorbol 13-acetate and bryostatin 1, showed less tilt angle and greater depth of penetration in the membrane than the inhibitor, resveratrol (Figure S1(B–D)). Bryostatin 1-bound Munc13-1 C1 embedded more to the membrane than phorbol 13-acetate bound Munc13-1 C1 with lower tilt angle and higher depth of penetration (Table 1). The bryostatin 1-bound Munc13-1 C1 showed less transfer energy to the membrane than phorbol 13-acetate. In contrast to the two activators, resveratrol embedded into the membrane with high tilt angle and shorter depth of penetration. Only β34 loop of the protein in the resveratrol complex interacted with the membrane (Figure S1(D)). The number of embedded residues was also different for different ligands. In the resveratrol complex, only Gly-589 and Ile-590 on the loop β34 bound to the hydrocarbon core of membrane because the resveratrol did not cover the hydrophilic site on the loop β12 of Munc13-1 C1 domain which is electrostatically unfavorable for interacting with the hydrophobic
membrane (Figure S1(D)). But, in the bryostatin 1 complex, five residues, Pro-577, Trp-588, Gly-589, Ile-590, and Arg-592 were embedded into the hydrocarbon core of the membrane. Bryostatin 1 covered multiple residues on the rim of the C1 domain including hydrophilic residues by stretching out its structure (Figure S1(C)). In the phorbol 13-acetate complex, Trp-588, Gly-589, and Ile-590 were embedded into the hydrocarbon core of membrane. The membrane-embedded models suggest that the interactions between Munc13-1 C1 and plasma membrane are greatly influenced by the ligand.

Table 1. Molecular docking and parameters for building the membrane system.

|                     | No ligand | Phorbol 13-acetate | Bryostatin 1 | Resveratrol |
|---------------------|-----------|--------------------|--------------|-------------|
| Molecular docking   |           |                    |              |             |
| Binding energy (kcal/mol) | 2.64      | 0.68               | 2.86         |             |
| Number of hydrogen bonds | 3         | 4                  | 3            |             |
| Parameters for membrane embedded model | | | | |
| Tilt Angle (°)      | 68 ± 15   | 20 ± 4             | 17 ± 11      | 26 ± 16     |
| Depth/Hydrophobic Thickness (Å) | 0.7 ± 2.3 | 6.6 ± 0.7         | 8.7 ± 0.7   | 3.9 ± 1.5   |
| ΔGtransfer (kcal/mol) | -2.5      | -6.6               | -7.5         | -3.0        |
| Membrane embedded residues (in the hydrocarbon core) | His-576   | Trp-588            | Gly-589      | Ile-590     |
|                     | Gly-589   | Trp-588            | Gly-589      | Ile-590     |
|                     | Ile-590   | Ile-590            | Arg-592      |             |
Effect of ligands on the mobility of Munc13-1 C1 in the lipid membrane

To understand the movements of the ligand-bound Munc13-1 C1 along the membrane, we monitored the changes in the tilt angle to the lipid membrane and the distance between the center of geometry (cog) of Munc13-1 C1 and the cog of the membrane along X-, Y-, and Z- axes. The orientation of the ligand-bound Munc13-1 C1 in the lipid membrane varied with ligand (Figure 4). In the phorbol 13-acetate-bound Munc13-1 C1, both the hydrophobic region including Ile-590 and the hydrophilic region where the phorbol ester bound were embedded in the hydrocarbon core of the membrane causing low tilt angle of Munc13-1 C1 to the lipid membrane (Figure 4(A)). On the other hand, Ile-590 in the bryostatin 1-bound Munc13-1 C1 did not interact with the hydrocarbon core. In the bryostatin 1 complex, bryostatin 1 intermediated between the C1 domain and lipid membrane by covering hydrophilic region of the rim of the C1 domain (Figures 3(B) and 4(B)). Bryostatin 1-bound Munc13-1 C1 also showed low tilt angle position like the phorbol ester complex (Figure S2(A)). In contrast to either phorbol ester or bryostatin 1 complex, Ile-590 of the resveratrol complex served as the pivot of binding between Munc13-1 C1 to the lipid membrane by interacting with the hydrocarbon of the lipid membrane (Figure 4(C) and Figure S1(D)). Both phorbol 13-acetate (17.52 ± 2.41°) and bryostatin 1 (20.13 ± 8.22°) complex showed two times less tilt angle to the lipid membrane than the resveratrol complex (49.29 ± 4.18°) during the simulation time (Figure 4(D)). The bryostatin 1 complex had slightly higher tilt angle than the phorbol ester complex, but bryostatin 1 showed two phases, a low tilt angle in the replica 3 and the replica 2 from 150 ns to 600 ns and a high tilt angle in the replica 1 and the replica 2 from 700 ns to 1000 ns (Figures S2 and S3).

The area of horizontal movement of the Munc13-1–ligand complex along the membrane is shown as scattered dots where each dot represents a X-Y coordinate of the complex in the membrane (Figure S4(B)). The total area traversed by the phorbol 13-acetate and bryostatin 1 complex in the XY plane were 3.789 ± 0.457 nm² and 5.876 ± 0.236 nm², respectively, suggesting that the phorbol 13-acetate complex moved significantly less than the bryostatin 1 complex. In the case of resveratrol, the total area was higher (6.107 ± 1.25 nm²) than either the phorbol 13-acetate or the bryostatin 1 complex (Figure S4(C)). The vertical movement of the Munc13-1–ligand complexes in the membrane was also monitored (Figure S5(A)). The phorbol 13-acetate and bryostatin 1 complexes maintained their initial depth of insertion to the membrane at ~20Å as measured by the distance between the cog of Munc13-1 C1 and the cog of the membrane. One replicate of bryostatin 1 complex showed slightly higher distance between the complex and the membrane (lower depth) than phorbol 13-acetate complex, but it remained inserted in the membrane till the end of the simulation. However, for the resveratrol complex the distance was always more than 20Å during the simulation time. Even the resveratrol complex was found to eject out of the membrane at 900 ns in one replicate. It means that ligand controls the mobility of Munc13-1 C1 along the membrane.

Effect of ligands on the conformational dynamics of Munc13-1 C1 in lipid membrane

In addition to the effect of the ligand on the orientation and movement of Munc13-1 C1 along the membrane, we calculated its changes in the root-mean-square deviations (RMSD) during the simulation to understand whether ligands could affect the conformational dynamics of Munc13-1 C1 in the presence of the lipid membrane (Figure 6). The phorbol 13-acetate complex showed stable plots of the RMSD of Munc13-1 C1 in all the three replicates during 1.0 μs simulation time. In the bryostatin 1 complex, the RMSD plots showed higher fluctuations except in one replicate. This replicate showed two conformational states: one with a RMSD value of 4.48 Å (0 to 250 ns and 800 to 1,000 ns) and the other with a RMSD value of 3.52 Å (200 to 800 ns). Resveratrol complex showed stable plots of the RMSD in all three replicates by 700 ns, but in one replicate it changed its conformation from 700 ns to the end of the simulation.

To find out which residues contribute to the high conformational dynamics in C1 domain (Figure 6), the root-mean-square fluctuation (RMSF) was calculated. In the bryostatin 1 and resveratrol-bound Munc13-1 C1, the C-terminal residues, Cys-608 to Cys-616, which form α-helix in C-terminal showed higher RMSF values than other residues (Figure 7). It indicates that the C-terminal residues increased the RMSD value of bryostatin 1 and resveratrol-bound Munc13-1 C1 as compared to the other residues. In the phorbol 13-acetate-bound Munc13-1 C1, however, the C-terminal residues did not show high fluctuation and its RMSD value was also steady in consistent with RMSF during the simulation (Figures 6 and 7). It suggests that the ligand influences the structure of the α-helix which plays a role in partitioning of C1 domain to the membrane (Stewart et al., 2014). To find out if ligand could change the structure of the ligand binding site, we recalculated the RMSD of the active site residues of the ligand-bound Munc13-1 C1 (Figure S5). The phorbol 13-acetate showed similar stable plots of RMSD with the plots for all residues of Munc13-1 C1. However, the plots of the active site residue RMSD of bryostatin 1 or resveratrol
complexes did not show much fluctuation as seen for the all residues RMSD plots.

The comparison of RMSF values for the three ligands showed that the overall residues of bryostatin 1 complex fluctuated more than that of phorbol 13-acetate complex. Thr-575 at the loop \( \beta \)12 of the phorbol 13-acetate-complex fluctuated less as compared to the Thr-575 in the other two groups. In the bryostatin 1-complex, Ala-591 and Arg-592 in the loop \( \beta \)34 showed higher fluctuations in the RMSF values. Moreover, residues from Ala-596 to Arg-603 showed high RMSF values in the bryostatin 1 complex as compared to these residues in the phorbol ester and resveratrol complexes. The RMSF of Glu-599 in bryostatin 1 complex was 1.3-fold higher than this residue in the other two complexes. The Glu-593 in the resveratrol complex fluctuated more than the Glu-593 in the phorbol 13-acetate complex. These comparisons suggest that the ligands exert differential conformational dynamics not only at the ligand binding site but also at other regions including the C-terminal \( \alpha \)-helix of Munc13-1 C1.

Interaction between ligand and Munc13-1 C1 in the lipid membrane

We previously showed that lipid membrane could affect the binding of ligands to Munc13-1 C1 (Blanco et al., 2019). To gain structural insights into how these interactions could vary for different ligands, we monitored the binding of phorbol 13-acetate, bryostatin 1, and resveratrol to Munc13-1 C1 in the membrane. Both the distance between the ligand and Munc13-1 C1 and between Munc13-1 C1 and the membrane were measured during 1.0 \( \mu \)s simulation time (Figure 5). The phorbol 13-acetate maintained its initial binding site in the two of three replicates (Figure 5(B)). In one replicate, phorbol
13-acetate left the active site of Munc13-1 C1 and moved inside the membrane at around 600 ns, but the protein did remain in the membrane. Bryostatin 1, in all the replicates, remained at the active site of the protein till the end of the simulation. These results suggest that bryostatin 1 has higher affinity to Munc13-1 C1 in the lipid membrane than phorbol 13-acetate, which was shown in the ligand affinity assays in the presence of lipid (Blanco et al., 2019). In contrast, resveratrol left the protein active site in two of the three replicates, one at 425 ns and the other at 800 ns. In the resveratrol complex the Munc13-1 C1 left the membrane after the departure of resveratrol from its active site in one replicate (Figure 5(A)), a feature not seen in the case of either bryostatin 1 or phorbol 13-acetate. These results suggest that resveratrol inhibits the interaction between the protein and the membrane which is consistent with our biochemical data that resveratrol acts as an inhibitor of Munc13-1 (Pany et al., 2017).

Phorbol 13-acetate, bryostatin 1, and resveratrol interact with Munc13-1 C1 and lipid membrane differently. Phorbol 13-acetate caps the hydrophilic region of Munc13-1 C1 (Figure 3(A)) and facilitates the two loops to embed into the hydrophobic core of the membrane (Figure 4(A)). Binding of phorbol 13-acetate reduced the fluctuation of Thr-575, Ile-590, Ala-591, and Arg-592 (Figure 7). On the other hand, bryostatin 1 intermediated between Munc13-1 C1 and the lipid membrane during the simulation, instead of existing as a compact complex like phorbol 13-acetate. The amphiphilic chain of C39 in bryostatin 1 is buried in the hydrophobic core of lipid membrane while the C1-acetate group, OH
group on C3, and OH group on C26 bound to the active site of Munc13-1 C1 (Figures 2 and 4(B)). The rings A and C lied above the binding site in the plane of the lipid membrane. This conformation of bryostatin 1-bound Munc13-1 C1 in membrane is similar with the model of bryostatin 1-bound PKCδ C1B as reported earlier (Keck et al., 2010; Nelson & Alkon, 2009; Wender et al., 1988). The active site residues located in the two loops showed higher RMSF values suggesting high fluctuations. These fluctuations may contribute to the formation of two different positions of the bryostatin 1 complex in the membrane (Figure 7). In the case of resveratrol complex, resveratrol bound to Munc13-1 C1 in the close proximity of Ile-590 (Figure 3(C)) unlike both phorbol 13-acetate and bryostatin 1. The resveratrol complex did not maintain one dominant position and conformation since the hydrophilic region of the loops could not embed into the hydrophobic core of lipid membrane (Figure 4(C)).

**Hydrogen bond formation between Munc13-1 C1 and ligand**

To understand how the ligand interacts with the Munc13-1 C1 in the lipid membrane, hydrogen bond formation between the residues of Munc13-1 C1 and ligand was monitored during the 1.0 μs simulation time (Figure 8). Phorbol 13-acetate formed two hydrogen bonds during most of the simulation time except in one replicate wherein the phorbol 13-acetate was found to leave the binding pocket at 600 ns (Figure 8(A)). This loss of the hydrogen bonds might be the reason why it could not remain stable at the protein active site. Bryostatin 1 maintained one hydrogen bond during most of simulation time in all three replicates, although the plots showed fluctuation continually (Figure 8(B)). Unlike the two activators, resveratrol formed fewer hydrogen bonds than the other two ligands and did not maintain the hydrogen bond formation continuously (Figure 8(C)). The resveratrol molecule lost hydrogen bonds at 325 ns and 700 ns in two of the three replicates and left the binding site within 100 ns from these time points (Figure 5(B)), suggesting the importance of these hydrogen bonds in stabilizing the protein-ligand complex. All the donor and acceptor of the hydrogen bonds between the ligand and the protein (Figure 9). Phorbol 13-acetate formed hydrogen bonds predominantly with Ala-574, Trp-588, Gly-589, Ile-590, and Arg-592, are critical for the ligand-Munc13-1 C1 interactions (Figure 8(D)). The involvements of these residues for each ligand were compared for the formation of hydrogen bond between the ligand and the protein (Figure 9). Phorbol 13-acetate formed hydrogen bonds predominantly with Ala-574, Trp-588, Gly-589, Ile-590, and Arg-592 in all the three replicates. While bryostatin 1 formed hydrogen bond predominantly with Trp-588 and Arg-592 in all three replicates, resveratrol formed hydrogen bonds with Trp-588 and Ile-590 predominantly.

Hydrogen bond between the ligand and the protein plays a critical role for the stability of the ligand–protein–lipid ternary complex. Phorbol 13-acetate in one replicate and resveratrol in two replicates moved out of the active site during simulation in several replicates once they lost their hydrogen bonds with the protein (Figures 5(B) and 8(A,C)). This was not observed in bryostatin 1 complex, although bryostatin 1 formed less number of hydrogen bonds than phorbol 13-acetate during simulation time (Figure 8(B)), suggesting that maintaining the hydrogen bonds for longer duration is more important.
The core residues forming hydrogen bonds with a ligand were different for different ligands (Figure 9). Ala-574 and Gly-589 were involved in hydrogen bonds between C1 and phorbol 13-acetate, but not bryostatin 1. The residues involved in the formation of hydrogen bonds to the ligands. The five residues, Ala-574, Trp-588, Gly-589, Ile-590 and Arg-592 formed hydrogen bonds with each ligand prominently. The five residues are represented by line structure in different colors.

Figure 9. Number of hydrogen bond formation between the ligand and the five residues of Munc13-1 C1 during 1 μs MD simulation. Plot of the number of hydrogen bonds between the ligand and the single residue during MD simulation for three different systems: Munc13-1 C1 + phorbol 13-acetate (left); Munc13-1 C1 + bryostatin 1 (middle); and Munc13-1 C1 + resveratrol (right). Three independent simulations are shown in black, magenta, and cyan color for each system. In phorbol 13-acetate-bound Munc13-1 C1, Ala-574 and Gly-589 formed and maintained more number of the hydrogen bonds than the other residues. Trp-588 and Arg-592 are critical residues for forming hydrogen bonds with bryostatin 1. In resveratrol-bound Munc13-1 C1, Trp-588, Ile-590, and Arg-592 formed more hydrogen bonds with resveratrol than Ala-574 and Gly-589.

Figure 8. Hydrogen bond formation between the ligand and Munc13-1 C1 during 1 μs MD simulation. Plot of the number of hydrogen bonds between the ligand and Munc13-1 C1 during MD simulation for three different systems: Munc13-1 C1 + phorbol 13-acetate (A); Munc13-1 C1 + bryostatin 1 (B); and Munc13-1 C1 + resveratrol (C). Three independent simulations are shown in black, magenta, and cyan color for each system. The plot is the 30 ps running averages of the number of hydrogen bonds. Phorbol 13-acetate formed and maintained about two hydrogen bonds during the simulation time, except in one replica. Bryostatin 1 formed and maintained about one hydrogen bond during the simulation time. Resveratrol formed less number of hydrogen bonds than the other two ligands. (D) The residues involved in the formation of hydrogen bonds to the ligands. The five residues, Ala-574, Trp-588, Gly-589, Ile-590 and Arg-592 formed hydrogen bonds with each ligand prominently. The five residues are represented by line structure in different colors.

The higher number of the hydrogen bond formation for shorter duration for the stabilization of the ternary complex. The core residues forming hydrogen bonds with a ligand were different for different ligands (Figure 9). Ala-574 and Gly-589 were involved in hydrogen bonds between C1 and phorbol 13-acetate, but not bryostatin 1
and resveratrol. For bryostatin 1, Trp-588 and Arg-592 were critical for forming hydrogen bonds. Hydrophobic interactions can also play a major role on the ligand-protein interactions. In the phorbol 13-acetate complex, Trp-588 and Ile-590 formed hydrophobic interaction with C19 of the phorbol 13-acetate. In the bryostatin 1 complex, Pro-577 and Leu-586, as well as Trp-588 and Ile-590 were involved in the hydrophobic interaction. Trp-588 formed 2\textsuperscript{2}/3\textsuperscript{pi}-alkyl bonds with multiple atoms of bryostatin 1. Resveratrol also formed hydrophobic interactions with various residues, such as Leu-586, Trp-588, Gly-589, Ile-590, and Ala-59, of the \textit{b\textsubscript{34}} loop.

**Effect of ligand on the interaction between Munc13-1 C1 and lipid membrane**

In addition to the interaction between Munc13-1 C1 and ligand, the interaction between Munc13-1 C1 and lipid membrane was also monitored during the entire simulation time. Phorbol 13-acetate allowed Munc13-1 C1 to move to hydrophobic core of the lipid membrane, and thus the PS covered its two loops by forming about 12 hydrogen bonds during the simulation time (Figure 10(A,D)). In the bryostatin 1 complex, PS interacted with bryostatin 1 more than the protein, but the head group of the lipid membrane also binds to the protein by forming about 8 hydrogen bonds (Figure 10(B,D)). The number of hydrogen bond between the bryostatin 1 complex and the membrane fluctuated more than the phorbol ester complex during the simulation (Figure S6). Resveratrol-bound Munc13-1 C1 formed about 6 hydrogen bonds with PS which is 2 and 1.3 times less than for the phorbol 13-acetate-bound C1 and bryostatin 1-bound C1, respectively (Figure 10(D) and Figure S6). PS surrounded and interacted with the loop \textit{b\textsubscript{34}} more than the loop \textit{b\textsubscript{12}} and Ile-590 of the loop \textit{b\textsubscript{34}} is the major residue for such interaction (Figure 10(C)). The calculated relative binding energy of C1 domain to the lipid membrane showed that phorbol 13-acetate-bound Munc13-1 C1 has significantly lower binding energy than the others (Figure S7(A)). In all three ligands, the complex of Munc13-1 C1 and ligand showed lower binding energy to lipid membrane than the C1 domain only (Figure S7). However, the binding energy of bryostatin 1 complex decreased 2.8 times than that of phorbol 13-acetate and resveratrol complex. These results suggest that the three different ligands influenced the interaction between C1 domain and lipid membrane differently.

Association with the membrane is a hallmark of the activation of Munc13-1 and other C1-domain containing proteins (Das & Rahman, 2014; Newton, 2001). The binding affinity of the ligands to the C1 domain increases several fold in the presence of lipid membrane and the affinity is also
dependent on the lipophilicity of the ligand and the percent of the PS in the membrane. Our previous study showed that the binding affinity of bryostatin 1 was 3-fold higher in 20% PS and 15-fold higher in 100% PS than phorbol 13-acetate under the same experimental condition (Blanco et al., 2019). It means that the binding affinity of bryostatin 1 to C1 domain was more influenced by the presence of PS in the lipid membrane than that of phorbol 13-acetate. In MD simulation, the average calculated binding energy of bryostatin 1 without consideration of membrane trajectories was higher than that of phorbol 13-acetate (Figure S8). But, the binding affinity (lower $K_i$) of bryostatin 1 measured by radioligand binding in the presence of phospholipids was higher than that of phorbol 13-acetate (Blanco et al., 2019) highlighting the role of lipids in the binding of the ligand to the protein. Also, in all the replicates, bryostatin 1 resided at the active site of C1 domain until the end of simulation, but in one of the replicates phorbol 13-acetate was found to move out of Munc13-1 C1. Because of the lower penetration of Munc13-1 C1 into the membrane in the bryostatin 1 complex, bryostatin 1 interacted more with lipid membrane than did Munc13-1 C1 (Figure 10(B)). In phorbol 13-acetate complex, however, the lipid membrane covered all two loops of the C1 including the phorbol 13-acetate with higher penetration of Munc13-1 C1 into the membrane (Figure 10(A)), suggesting a more prominent role of the membrane for the bryostatin 1 complex than the phorbol 13-acetate complex. Resveratrol, on the other hand, showed the lowest calculated binding energy in molecular docking (Table 1) and had lower calculated binding energy than bryostatin 1 before it left the active site during the MD simulation (Figure S8). However, the resveratrol-bound Munc13-1 C1 did not penetrate deep into the membrane unlike phorbol 13-acetate complex (Table 1, Figures 5(A) and 10(C)). Moreover, resveratrol moved out of the protein binding pocket to the hydrophobic core of the membrane in two replicates (Figure 5(B)) resulting in the dissociation of the Munc13-1 C1 from the lipid membrane in one of the replicates (Figure 5(A)). It suggests that resveratrol does not facilitate membrane binding of Munc13-1 C1. The binding affinity of resveratrol with Munc13-1 C1 in membrane has not been determined experimentally, but most likely it inhibits Munc13-1 activity by competing with the phorbol ester and preventing its association of the protein with the membrane.

In the initial membrane embedded model, the bryostatin 1-bound Munc13-1 C1 showed significantly less tilt angle and lower transfer energy to the lipid membrane and greater depth of penetration into the membrane than either phorbol 13-acetate or resveratrol (Table 1 and Figure S1). The bryostatin 1-bound Munc13-1 C1 showed more dynamic mobility and conformational change in the lipid membrane than the phorbol 13-acetate complex during 1.0 μs MD simulation (Figures 4(D) and 6 and Figures S3 and S4). When the ligand binding energy data were plotted against both the distance between the Munc13-1 C1 and the membrane and the tilt angle between them, we found two low ligand-binding energy basins (represented by population of red colored dots) with regard to the tilt angle and depth of the bryostatin 1 complex to the lipid membrane (Figure 11). In one of the low energy basins, bryostatin 1 complex showed low tilt angle and high depth of penetration into the membrane (Figure S2(A)) and the other shows high tilt angle and low depth to the membrane (Figure S2(B)). However, phorbol 13-
acetate-bound Munc13-1 C1 showed only one low energy basin reflecting one dominant position to the membrane (Figures 4(A) and 11(A)). These two states of bryostatin 1 complex and one state of phorbol 13-acetate complex were also reported while studying the binding of these ligands with PKCδ C1B (Ryckbosch et al., 2017). The resveratrol complex showed one lower energy basin like the phorbol 13-acetate complex (Figure 11(C)), but was more scattered, indicating the lack of a clear dominant position. In addition to the orientation and depth of Munc13-1 C1, both bryostatin 1 and resveratrol complex showed significant higher mobility along the membrane than the phorbol 13-acetate complex (Figure S4). These results suggest that ligand controls the position and the mobility of the Munc13-1 C1 in the lipid membrane generating multiple states of Munc13-1 that may exert different interactions with its partner proteins in orchestrating the process of vesicle fusion.

Conclusion

Our study finds distinctive structural features of Munc13-1 C1–ligand–membrane interactions using a long time scale molecular dynamics simulation. The core residues of Munc13-1 C1 for forming hydrogen bonds to ligand are different for different ligands. Ala-574 and Gly-589 formed hydrogen bonds with phorbol 13-acetate, but not with bryostatin 1 and resveratrol. Trp-588 and Arg-59 were more critical for forming hydrogen bonds with bryostatin 1 than the other ligands. Resveratrol did not form strong hydrogen bonds with C1 domain. In addition, phorbol 13-acetate, bryostatin 1, and resveratrol differentially controlled the positioning and mobility of the Munc13-1 in the membrane. Phorbol 13-acetate allowed the C1 domain to embed deep into the lipid membrane by covering the hydrophilic region of the loops of the C1 domain. Bryostatin 1 intermediated between C1 domain and lipid membrane on the surface of the membrane by interacting both C1 and membrane. Resveratrol-protein complex shows less membrane penetration and the resveratrol-protein-membrane complex is less stable as compared to the corresponding complexes of phorbol 13-acetate and bryostatin 1. The small molecules obscure the hydrophilic region of the C1 domain thereby embedding deep into the lipid membrane or maintaining its position in the membrane. The study provides structural insights of small molecule binding to Munc13-1 and highlights the importance of membrane in explaining their pharmacological actions.

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JD designed and YY performed the study. JD and YY wrote the paper.

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