CHARMM-GUI Nanodisc Builder for Modeling and Simulation of Various Nanodisc Systems

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Abstract

Nanodiscs are discoidal protein-lipid complexes that have wide applications in membrane protein studies. Modeling and simulation of nanodiscs is challenging due to the absence of structures of many membrane scaffold proteins that wrap around the membrane bilayer. We have developed the CHARMM-GUI Nanodisc Builder (http://www.charmm-gui.org/input/nanodisc) to facilitate the setup of nanodisc simulation systems by modeling the membrane scaffold proteins with defined size and known structural features. A total of 11 different nanodiscs with a diameter from 80 Å to 180 Å are made available in both the all-atom CHARMM and two coarse-grained (PACE and Martini) force fields. The usage of the Nanodisc Builder is demonstrated with various simulation systems. The structures and dynamics of proteins and lipids in these systems were analyzed, showing similar behaviors to those from previous all-atom and coarse-grained nanodisc simulations. We expect the Nanodisc Builder to be a convenient and reliable tool for modeling and simulation of nanodisc systems.
Introduction

Nanodiscs are discoidal protein-lipid complexes where the lipid bilayer is stabilized by the encircling membrane scaffold proteins (MSPs). The MSP, initially derived from the human ApoA-1 protein, is amphipathic and wraps around the bilayer in an antiparallel way to form a double belt structure. A variety of MSPs has been engineered by truncation or repetition of the constituent helices to assemble different sizes of nanodiscs. Due to the close reassembly of physiologically relevant membrane environments, nanodiscs have found remarkable applications in membrane biochemistry and biophysics. Although the crystal structure of the lipid-free ApoA-1 has long been known, the structure of an intact nanodisc had remained elusive until the NMR structure of the MSPΔH5 nanodisc was very recently determined, revealing key stabilizing structural features and structural rearrangement after loading of lipids. Notably, Nasr et al. covalently linked the N- and C-termini of MSPs to form circularized nanodiscs that show enhanced stability, well-defined diameter sizes, and tunable shapes.

From the computational and modeling perspective, simulations of nanodiscs using both all-atom and coarse-grained force field reveal many important features of nanodisc structure and dynamics. The focuses of molecular dynamics (MD) simulations include structural models of ApoA-1-lipid complex, mechanisms of nanodisc assembly and disassembly, behaviors of lipids in a nanodisc compared to that in a pure bilayer, and effects of the embedded protein on the structure of the nanodisc. More detailed descriptions of previous computational studies are provided in two recent publications. Notably, Siuda et al. developed a computational method to build arbitrary nanodisc structures for MD simulations. This method first built a coarse-grained Martini model by adding additional long elastic bonds to the ApoA-I crystal structure, where two MSP chains show a twisted conformation. Simulations were performed to build the discoidal MSP structures. The all-atom structure was then obtained using the backward approach on the coarse-grained model. Simulations of 36 coarse-grained and all-atom nanodiscs generated using this method show similar properties with those from experiments and previous computational studies.
Despite all these simulation studies, setting up a nanodisc simulation system is not a trivial task especially when the nanodisc structure is unknown. To surmount this difficulty, we have developed the Nanodisc Builder in CHARMM-GUI (http://www.charmm-gui.org/input/nanodisc) to provide users ready-to-go nanodisc systems for MD simulation by taking advantage of the existing framework of CHARMM-GUI modules.\textsuperscript{18} It provides a total of 11 different sizes of nanodiscs in the CHARMM all-atom\textsuperscript{19,20} and PACE and Martini coarse-grained representations,\textsuperscript{21-23} covering many commonly used nanodisc assemblies in experiments.

**Methods**

**Construction of Nanodisc Structures**

An extended helical structure of a single MSP was first built. The N- and C-termini were then slowly pulled together during MD simulations in an implicit solvent model\textsuperscript{24} and with the backbone dihedrals restrained in the helical regions. A pseudo hydrophobic atom with a van der Waals radius that is equal to the nanodisc radius was fixed at the center of the nanodisc to provide a hydrophobic environment in the interior and make the MSP circular. Fig. 1 shows the snapshots during the modeling of the MSP1D1 structure, whose amino acid sequence was derived without residue 1-11 of the MSP1TEV MSP.\textsuperscript{25} The CHARMM scripts to build the MSP monomer are available in Supplementary Materials. Two MSPs were then stacked together with the 5/5 registry (the helix 5 from each MSP is juxtaposed with each other in antiparallel direction) to make the nanodisc structure. The dimer structure was further relaxed in a 2-ns simulation using implicit solvent\textsuperscript{24} and the pseudo atom.
Figure 1. Sequential snapshots of MSP1D1 during modeling of the monomer structure using the CHARMM scripts in Supplementary Materials. The pseudo atom that provides the hydrophobic environment in the interior is shown as a yellow sphere.

Once the MSP dimer structure is constructed, lipids are packed by following the general workflow of the CHARMM-GUI Membrane Builder.[26,27] The number of lipids are determined using $n = \frac{\pi(r - 5.5)^2 - p}{s}$, where $r$ is the nanodisc radius, $p$ is the protein area if included by the user, and $s$ is the per-lipid area, which can be adjusted by the user to add different number of lipids. Following the Membrane Builder workflow, lipid-like pseudo atoms are randomly placed in the nanodisc with the $Z$ coordinates being fixed and a short vacuum simulation is carried out to evenly distribute them on the $X$-$Y$ plane, while keeping the MSP atoms fixed. All-atom lipids are then placed following the $X$-$Y$ positions of the pseudo atoms. Then, following the workflow in Micelle Builder,[28] water molecules and ions are added to complete a nanodisc building.

**Molecular Dynamics Simulations and Trajectory Analysis**

All-atom simulations were performed with the CHARMM36 force field[19,20] using NAMD 2.10,[29] following the default protocol for solution systems in CHARMM-GUI.[30] Non-bonded interactions were smoothly switched off at 10-12 Å with a forceswitch function.[31] Long-range electrostatic forces were calculated with the particle mesh Ewald algorithm. Temperature was maintained at 303.15 K using Langevin dynamics with a friction coefficient of 1 ps$^{-1}$. Pressure was controlled at 1 bar using a Langevin-piston method[32] with a piston period of 50 fs and a decay of 25 fs. Martini systems were simulated using Gromacs 5.0.7[33] with the protocol provided by Martini Maker.[34] Lennard-Jones interactions were switched off at 9-12 Å and columbic interactions were shifted off at 0-12 Å. Dielectric constant was set to 15 and time step was 20 fs. Pressure was maintained at 1 bar using the Berendsen barostat[35] with a coupling constant of 5 ps and a compressibility of $4.5 \times 10^{-5}$ bar$^{-1}$. Temperature was maintained at 303.15 K using the velocity rescaling method.[36] PACE systems were simulated using a modified NAMD package.[21] The simulation protocol was identical to that described previously.[37]

Trajectories were analyzed using CHARMM,[38] VMD,[39] and MDAnalysis.[40] Lipid order parameters were calculated as $S_{CD} = \frac{1}{2} \langle 3 \cos^2 \theta - 1 \rangle$, where $\theta$ is the angle
between the C-H bond vector and the membrane normal. The lipid area was calculated using the Voronoi tessellation method described in our previous works. The nanodiscs were aligned to the initial structures to remove the movement of the MSPs before calculation of lipid diffusion.

Results and Discussion

*Nanodisc Builder* provides 11 different MSPs for construction of different diameters of nanodiscs from ~80 Å to ~180 Å (Table 1: all MPS sequences in Table S1). Among them, two covalently circularized MSPs (cNW9 and cNW11) in which the N- and C-termini are covalently connected are made available for all-atom systems. In addition, a recently published NMR structure of the nanodisc MSPΔH5 (apoA-1 with residues 1-54 and 121-142 removed, PDB ID 2N5E) is also included. Because the structures of the nanodiscs are unknown except for 2N5E, we used CHARMM to model the initial structure of the MSPs (see Methods). To demonstrate the function of the Nanodisc Builder and test the quality of the assembled systems, we simulated 11 all-atom and coarse-grained nanodisc systems and analyzed their properties (see Table S2 for system information).

<table>
<thead>
<tr>
<th>Name</th>
<th>Diameter (Å)</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>2N5E</td>
<td>81</td>
<td>From PDB ID 2N5E</td>
</tr>
<tr>
<td>cNW9</td>
<td>85</td>
<td>Covalently circularized nanodiscs cNW9</td>
</tr>
<tr>
<td>MSP1D1-44</td>
<td>86</td>
<td>Deletion 1-44 of MSP1TEV</td>
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<tr>
<td>MSP1D1-33</td>
<td>90</td>
<td>Deletion 1-33 of MSP1TEV</td>
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<tr>
<td>MSP1D1-22</td>
<td>94</td>
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<tr>
<td>MSP1D1</td>
<td>95</td>
<td>Deletion 1-11 of MSP1TEV</td>
</tr>
<tr>
<td>cNW11</td>
<td>102</td>
<td>Covalently circularized nanodiscs cNW11</td>
</tr>
<tr>
<td>MSP1E1D1</td>
<td>105</td>
<td>Extended MSP1D1, helix 4 repeated</td>
</tr>
<tr>
<td>MSP1E2D1</td>
<td>111</td>
<td>Extended MSP1D1, helix 4 and 5 repeated</td>
</tr>
<tr>
<td>MSP1E3D1</td>
<td>121</td>
<td>Extended MSP1D1, helix 4, 5, and 6 repeated</td>
</tr>
<tr>
<td>MSP2N2</td>
<td>184</td>
<td>Fusion of MSP1D1 and MSP1D1–22</td>
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</tbody>
</table>

† The sequences for cNW9 and cNW11 are from reference and others are from reference. cNW9 and cNW11 are not available in Martini and PACE due to the lack of force field parameters.
All-atom systems

For all-atom nanodisc-only systems (i.e., nanodiscs without embedded membrane proteins), we have tested 1-palmitoyl-2-oleoyl-phosphocholine (POPC) lipids in four different nanodiscs, namely 2N5E, MSP1D1-44, MSP1D1, and MSP1E3D1. The initial MSP structure of the 2N5E nanodisc comes from a NMR structure, and could be used as a baseline to compare the quality of our nanodisc models. The structures of the 2N5E, MSP1D1-44, and MSP1D1 are overall stable, with root mean square deviation (RMSD) values of MSP dimer less than 5 Å (Fig. 2, 3A). However, the second largest MSP1E3D1 nanodisc reaches a much larger RMSD of ~10 Å. Comparison of the first and last snapshots shows that the MSP1E3D1 nanodisc is still discoidal after simulation, but the shape becomes elliptical (Fig. S1). To quantitatively characterize the shape of the nanodiscs, we aligned the first and second principal axes of the MSPs to the X- and Y-axis and calculated the $R_x/R_y$ ratio, where $R_x$ and $R_y$ are the radii of gyration of the MSPs along the X- and Y-axis. For a circular nanodiscs, the ratio is close to one. The time series of the ratio shows that the 2N5E, MSP1D1-44 and MSP1D1 nanodiscs remain mostly circular while the MSP1E3D1 nanodisc reaches a ratio of ~1.3 (Fig. S2). Interestingly, Arleth et al. reported that, based on small-angle scattering, the MSP1D1 nanodiscs possess intrinsically an elliptical shape, and becomes more circular with increasing temperature.[43] The secondary structures of the MSPs show helical structures, indicating the overall stability of the simulations (Fig. S3). Because the initial MSP structures except 2N5E were modeled using an implicit solvent model, to characterize the rearrangement of the MSP residues before and after the simulations, we have calculated the total solvent accessible surface area (SASA) of the hydrophobic and non-hydrophobic residues in the initial, equilibrated, and final structures (Table 2). In all systems, the SASA decrease rapidly from the initial structure to the equilibrated one and further relax to smaller values during the simulations. This is not surprising because the lipids in the initial systems were placed by simple geometric packing, leaving many spaces that can be filled during equilibration and simulations.
Table 2. Total SASA of hydrophobic and non-hydrophobic residues in four nanodiscs before and after simulations†.

<table>
<thead>
<tr>
<th>System</th>
<th>SASA hydrophobic (Å²)</th>
<th>SASA non-hydrophobic (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2N5E</td>
<td>4804</td>
<td>4140</td>
</tr>
<tr>
<td>MSP1D1-44</td>
<td>3925</td>
<td>2868</td>
</tr>
<tr>
<td>MSP1D1</td>
<td>5066</td>
<td>3884</td>
</tr>
<tr>
<td>MSP1E3D1</td>
<td>7252</td>
<td>5230</td>
</tr>
</tbody>
</table>

†Surface area was calculated using a probe radius of 1.4 Å in the presence of the lipid atoms.

Figure 2. Top and side views of the simulated MSP1D1-44 nanodisc at (A) 0 ns and (B) 200 ns.

We next examined the properties of the lipids in these nanodiscs. Similar to the findings in previous studies, the diffusion of POPC is slower than that in a bilayer due to confinement of the MSPs, i.e., the smaller nanodiscs have smaller mean square displacement (MSD) than the larger ones (Fig. 3B). The thickness of the membrane, calculated from the $z$ positions of lipid head groups as a function of distance from the nanodisc center (Fig. S4), is between 40 and 50 Å at the center of a nanodisc (Fig. 3C), close to the 46 Å thickness estimated from SAXS experiments,[25] but thicker than that of a POPC bilayer (~38.5 Å). It decreases substantially toward the edge because the
Peripheral lipids tilt outward from the nanodisc center. As a result, the per-lipid area on the $X$-$Y$ plane also decreases at the edge due to overlapping of the lipid atoms on the $X$-$Y$ plane (Fig. 3D), whereas at the nanodisc center, the area is around 62 to 67 Å$^2$, close to that in a simulated POPC bilayer (~65.9 Å$^2$) and the experimental value of 69 Å$^2$.\[25\] Moreover, the area increases slightly before its value drops, which is attributed to the tilt of the peripheral lipids that leaves more space for the lipids in-between the central and the peripheral lipids. Order parameters are an important measure that characterizes the flexibility of lipids. We have calculated the order parameters for each tail carbon atom as a function of radial distance from the nanodisc center, using the MSP1D1 nanodisc as an example (Fig. S5). The order parameter decreases as a function of distance, indicating that the central lipids are more ordered than the peripheral lipids. To compare with a pure bilayer, we calculated the average order parameter for the central lipids (distance < 15 Å) and found that the central lipids are more ordered than lipids in a pure bilayer (Fig. 4).

**Figure 3.** Simulation of POPC in the 2N5E, MSP1D1-44, MSP1D1, and MSP1E3D1 nanodiscs. (A) RMSD of the MSPs. (B) MSD of POPC in nanodiscs and a pure bilayer.
(C) Thickness of the POPC bilayer as a function of distance from the nanodisc center. (D) Per-lipid area as a function of distance from the nanodisc center.

**Figure 4.** Comparison of the POPC order parameters in the center of MSP1D1 nanodisc (distance < 15 Å from the nanodisc center) and a pure bilayer.

In *Nanodisc Builder*, the number of lipids can be manually adjusted by changing the default per-lipid area for lipid number calculation. To determine the effects of excessive and inadequate lipids in a nanodisc, we have simulated the MSP1D1 nanodisc with 15% more (15%+) and 15% fewer (15%-) POPC lipids than the default one described above (Fig. 5). The default system shows smaller RMSD in the second half of the simulation with an average value of 3.91±0.57 Å, whereas the 15%+ and 15%- systems have average values of 5.04±0.57 Å and 5.61±0.73 Å. The diffusions of the lipids have very similar slopes with the 15%- system showing slightly larger MSD value (Fig. 6B). The variation of thickness and per-lipid area as a function of distance from the nanodisc center is as expected (Fig. 5C, D), i.e., the 15%+ system has thicker bilayer and smaller per-lipid area than the default one, and the opposite for the 15%- system. The average order parameters of the central lipids in the 15%- system are smaller than those in the default system but still larger than those in a pure bilayer (Fig. S6). These results suggest that the default number of POPC lipids is acceptable, however users may adjust the lipid number if needed.
Figure 5. Simulations of MSP1D1 nanodiscs with different numbers of POPC lipids. (A) RMSD of the MSPs. (B) MSD of the lipids. (C) Thickness and (D) per-lipid area as a function of distance from the nanodisc center. 15%+: 15% more POPC than the default number, 15%–: 15% fewer POPC than the default number.

*Nanodisc Builder* allows users to embed a protein in a nanodisc much easier by following the protocol from CHARMM-GUI *Membrane Builder*. To demonstrate this function, we have simulated two proteins, namely a voltage-dependent anion channels (VDAC, PDB ID 2K4T[8]) and the transmembrane region of a B2-adrenergic G protein–coupled receptor (GPCR, PDB ID 2RH1[44]) in MSP1D1 nanodiscs (*Fig. 6AB*). The RMSD of the embedded proteins shows a value of ~3 Å (VDAC) and 2 Å (GPCR), respectively, and the MSP dimer reaches a RMSD value of ~4 Å, comparable to that in the protein-free MSP1D1 nanodisc with POPC lipids (*Fig. 6C*). Due to the interactions between the embedded proteins and the lipids, the diffusion of the POPC shows a MSD of 90-120 Å² at 100 ns, smaller than that in the protein-free nanodiscs (*Fig. 6D*). The radii of the MSPs in both systems change slightly after the simulations, and the secondary structures of the MSPs remain mostly helical, suggesting the embedded proteins do not have significant impacts on the MSP conformation (*Fig. S7*).
Figure 6. Simulations of protein-embedded nanodiscs. (A, B) Top and side views of the initial structures of the (A) VDAC and (B) GPCR systems. (C) RMSD of the MSP dimers and the embedded proteins. (D) MSD of the POPC lipids.

In addition to conventional nanodiscs, Nanodisc Builder provides two covalently circularized nanodiscs, cNW9 and cNW11. We have simulated the cNW9 nanodisc with POPC lipids (Fig. 7A). The RMSD of the MSPs reaches ~7 Å (Fig. 7B), larger than those for 2N5E, MSP1D1-44, and MSP1D1 in Fig. 3. The radius of gyration is 41.47 Å with a standard deviation of 0.30 Å, which is comparable to those of 0.27, 0.26, and 0.22 Å for 2N5E, MSP1D1-44, and MSP1D1. In negative-stain electron microscope observations, the non-circularized cNW11 has much wider diameter distribution than cNW11, likely due to large conformational change of the MSPs and different amount of lipid loading, which cannot be simulated in our 200-ns simulations. The MSD, thickness, and per-lipid area of the lipids are similar to those in non-circularized ones (Fig. 3).
Figure 7. Simulation of the cNW9 nanodisc. (A) Top and side views of the initial structure. (B-E) RMSD, MSD, thickness, and per-lipid area.

Coarse-grained systems

In addition to all-atom systems, Nanodisc Builder also provides CG simulation systems using the PACE\textsuperscript{[21]} and Martini force fields.\textsuperscript{[22,23]} We have simulated the MSP1D1 nanodisc with POPC lipids in both force fields (Fig. 8). The nanodiscs in both force fields show RMSD values around 4-6 Å, comparable to those in all-atom simulations. The Martini nanodisc has a larger RMSD because the system was setup without the elastic network model, which adds additional elastic bonds between residue pairs within a certain distance and essentially makes the MSPs more rigid. The secondary structure of the PACE MSPs remain mostly helical, suggesting the MSPs are stable in the PACE force field (Fig. S8). The diffusion of POPC lipids reaches a MSD of \( \sim 800 \) Å\(^2\) at 100 ns, much larger than that in the all-atom systems, which is expected for CG lipids. The thickness of the bilayer is comparable to that in all-atom nanodiscs (Fig. S9A, Fig. 3C), but the Martini system shows slightly smaller thickness than PACE, which we attribute to the increased radius of the Martini nanodisc (Fig. S9C).
Conclusions

Tests of various nanodisc systems show that Nanodisc Builder is a reliable tool for building nanodisc simulation systems. The secondary structures of the MSPs remain helical mostly and the RMSD values are around 5 Å except for largest nanodisc MDP1E3D1, where an elliptical shape was formed during the simulation. Such a deformation highlights the difficulty of making a nanodisc model and suggests potential flexibility of the large nanodiscs perhaps without an embedded membrane protein. The lipids in a nanodisc show slower diffusion, larger thickness, and higher order parameters at the center compared to those in a pure bilayer. These features are due to the confinement of the nanodisc shape, and have been observed in previous computational studies. Although we only used POPC in our simulations, it should be noted that users can use all lipid types available in Membrane Builder. Since its release, Nanodisc Builder has already been used by other research groups.\textsuperscript{16} We hope that it continue to be a valuable tool for simulating various nanodisc systems.

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References

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