Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Methods Mol Biol. Author manuscript; available in PMC 2022 January 01.

Published in final edited form as:

Methods Mol Biol. 2021 ; 2302: 237–251. doi:10.1007/978-1-0716-1394-8_13.

Preparing membrane proteins for simulation using CHARMM-GUI

Yupeng Li[1,2], **Jinchan Liu**[1,2], **James C. Gumbart**[2]

[1]Department of Chemistry, Jilin University, Changchun, Jilin, China

[2]School of Physics and School of Chemistry & Biochemistry, Georgia Institute of Technology, GA, USA

Abstract

Molecular dynamics simulations of membrane proteins have grown dramatically in the last 20 years. Running these simulations first requires embedding the protein's three-dimensional structure in a lipid bilayer of a suitable composition, one that resembles its native environment. This step is far from trivial, especially for modeling heterogeneous mixtures of lipids. CHARMM-GUI, a webserver for simulation system preparation greatly simplifies this step, allowing for the construction of complex heterogeneous and/or asymmetric membranes. Here, we demonstrate how to use CHARMM-GUI to build the membrane for the outer-membrane protein BamA.

1 Introduction

Membrane proteins perform multiple functions and are vital to the survival of all organisms [1]. It is estimated that the genes coding for membrane proteins make up 20–30 % of the genomes of organisms [2]. They serve as channels [3,4], transporters [5–7], receptors [8], enzymes [9] and function in cell signaling [10,11], translocation of substrates [12–14], energy transduction [15,16] and cell-cell recognition [17–19]. Due to their vital significance, advanced technological methods such as NMR [20], cryogenic electron microscopy (cryo-EM) [21] and X-ray crystallography [22] have been developed to determine structures of membrane proteins. However, these experimental methods in most cases only provide a static state of proteins while molecular dynamics (MD) simulations are capable of probing their dynamic behavior [23]. Therefore, preparing a membrane-protein system at atomic resolution is a major concern of simulators.

Previous studies have emphasized the importance of building a native membrane: not only are the lipid-protein interactions responsible for regulating or stabilizing the conformation of membrane proteins [24–27], but also the composition of the membrane will influence their structure and function [28,29]. Consequently, one should be especially careful to select the appropriate membrane for a given membrane protein.

To simplify and automate the building process for a native membrane-protein system for MD simulations, CHARMM-GUI (http://www.charmm-gui.org) [19,30] provides a graphical user interface (GUI) of multiple modules for the biomolecular simulation program CHARMM [31]. Membrane Builder [19] is one of the modules in CHARMM-GUI, which offers users a relatively easy way to build complicated membranes with all types of lipids

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

through both user-specified and automated steps, including PDB loading, protein orientation, system size determination, generation for lipids, pore water, bulk water as well as ions, and assembly of components [19].

In this chapter, we will go through the process of preparing a membrane-protein system step by step in CHARMM-GUI using BamA as an example (Figure 1). BamA is the central component of the BAM complex [32–35]. It is an outer membrane protein (OMP) of Gram-negative bacteria, which is responsible for the folding and insertion of other OMPs [36]. It contains a transmembrane β-barrel of 16 strands along with five periplasmic polypeptide-transport-associated (POTRA) domains (Figure 2). We use the structure from *E. coli* (PDB ID: 5AYW [33]), which includes all 5 POTRA domains.

2. Materials

2.1 Programs

1. In order to access CHARMM-GUI, a web browser such as Chrome, Firefox, etc. is required.

2.2 Hardware

1. A computer or laptop with any modern operating system (Windows, Linux, Mac OS).

3. Methods

3.1 Read Protein Coordinates and Manipulate Structure

The process of building a membrane-protein system via CHARMM-GUI starts with the loading of protein coordinates, followed by several alternative manipulation options, and finally generating a Protein Structure File (PSF) and PDB file containing the entire system. Users can upload a pre-oriented protein structure or specify a Protein Data Bank (PDB) ID to download PDB files directly from either the Research Collaboratory for Structural Bioinformatics (RCSB) database [37,38] or the Orientations of Proteins in Membranes (OPM) database [39].

3.1.1 Load PDB File

1. Open CHARMM-GUI (http://www.charmm-gui.org) in a web browser. Select the menu item **Input Generator → Membrane Builder** on the leftmost part of the website.
2. Drag the scroll bar to the middle. Two options will appear on the screen: **Protein/Membrane System** and **Membrane Only System**. Choose the former one.
3. Enter **5ayw** (PDB ID of one conformation of BamABCDE complex [33]) into the **Download PDB File** blank, meanwhile, selecting **OPM** as the **Download Source** (*see* Note 1). Then, click on the **Next Step: Select/Model Chain** button in the lower right corner.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. View **Model/Chain Selection Option**. This PDB file contains five proteins. Information such as type, segID, PDB ID, first and last residue ID of chains and engineered residues are listed here as well.

Users are able to view the constitutive segments already present in the PDB file, which mainly include protein chains, substrates, crystallographic water molecules, ions and crystallization detergents. They can also select whatever segments they want to use as well.

5. Here, we will focus on BamA alone. Check on the box of **PROA** only. Then, click on the **Next Step: Manipulate PDB** button in the lower right corner.

Beyond deciding which segments to include in their system, users also have the ability to select a subset of residues of a chain, rename every segment and remove engineered residues. These operations will not be used in this chapter.

3.1.2 Manipulate PDB file—

In order to generate a system suitable for simulation, additional manipulation is required. CHARMM-GUI provides users with diverse options for manipulation to meet multiple demands, including terminal group patching, modeling missing residues, mutation, protonation, disulfide bonds, add lipidation, etc. Here, we will focus on terminal group patching and disulfide bonds manipulation options only. Readers can explore other options on their own.

1. Check the box labeled **Terminal group patching**. Select **NTER** for **First** and **CTER** for **Last.**
2. Check the box labeled **Disulfide bonds**. Set Pair 1 Residue ID to **690** while set Pair 2 Residue ID to **700** (*see* Note 2).
3. Click on the **Next Step: Generate PDB and Orient Molecule** button in the lower right corner. Users can view the loading structure (Figure 4) in the next step by clicking on the **view structure** button on the top of the website.

You may notice another option called **Symmetry Operation Options** when you scroll down to the end of the page. This option is only supported when the PDB file contains the information about oligomerization, in which the protein oligomer is composed of two or more associating monomers with different or identical structure [40,41].

3.2 Orient the Protein

After PDB loading and manipulation, the protein needs to be oriented and positioned properly relative to the membrane bilayer. This step consists of two subsections, i.e., orient and position protein and generate pore water.

3.2.1 Orient and Position Protein—

CHARMM-GUI's Membrane Builder defines the Z axis as the membrane normal and $Z = 0$ Å as the center of membrane bilayer [19,42]. Therefore, to build a system with the proper protein orientation and position, it must be aligned with the Z axis and its hydrophobic region centered on $Z = 0$ Å. Since we use a pre-oriented protein from OPM, orientation and positioning are not necessary here.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. Locate **Orientation Options**. Four options are provided here. Each option is labeled with the situation it is intended for (*see* Note 3).
2. Subsequently, select **Use PDB Orientation**. Users can see the orientation file (Figure 5) in the next step by clicking on the **view structure** button on the top of the website.

Usually, proper orientation information is not available for PDB files from the RCSB database, such that proteins most likely need to be reoriented and repositioned in this step. Users can select **Use PDB Orientation** if they use PDB files from the OPM database (*see* Note 4).

3.2.2 Generate Pore Water—

In general, proteins with pores, such as ion channels, transporters and porins, can accommodate water molecules inside their internal cavity. CHARMM-GUI provides a general approach for pore water generation.

1. Locate the **Area Calculation Options**.
2. Click on the box of **Generate Pore Water** (*see* Note 5**) and Measure Pore Size.**
3. Select **Using protein geometry**.
4. Click on the **Next Step: Calculate Cross-Sectional Area** button in the lower right corner.

Note that the cross-sectional area of the protein will be calculated in this subsection to help determine the system size in the next step.

3.3 Determine the System Size

According to the cross-sectional area of the protein calculated in the previous step and lipid surface areas from experiments, the system size in the XY-plane and along the Z axis can be determined by multiple user-specified parameters in **System Size Determination Options**, including lipid types, system shape, water thickness along the Z axis on the top and bottom of the membrane, and numbers or ratios of lipid components.

Since we are building the membrane for BamA in *E. coli*, we will use an *E. coli* membrane. *E. coli* is a Gram-negative bacteria enveloped by two membranes, an inner membrane (IM) and outer membrane (OM). BamA resides in the OM. In Gram-negative bacteria, there is a special outer membrane component, lipopolysaccharide (LPS), that consists of a lipid A and a polysaccharide on the upper leaflet. The lower leaflet of the OM is a mixture of phospholipids. Here, we will use LPS for the upper leaflet while using PVCL2, PMPE, PMPG, PVPE and PVPG for the lower leaflet, with a ratio of 2:8:1:8:2 [43,44] (*see* Note 6).

1. Locate **System Size Determination Options**.
2. Select the **Heterogeneous Lipid** option.

Presently, the **Homogeneous Lipid** option is not supported, but users can select one type of lipid when using the **Heterogeneous Lipid** option to generate a homogeneous lipid bilayer.

3. Select **Rectangular** as the **Box Type**.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. In the **Length of Z based on** option, select **Water thickness**. Change its initial parameter from 22.5 Å to 30 Å (*see* Note 7).

5. In the **Length of XY based on** option, select **Ratios of lipid components**.

Membrane Builder gives users two options to determine the system size in the *XY*-plane: **Ratio of lipid components**, which corresponds to the **Length of X and Y**, and **Numbers of lipid components**, which corresponds to the **XY dimension ratio**.

6. Go to **Lipid Type** column. In **CL (cardiolipin) Lipids**, set **PVCL2**'s **Lowerleaflet Ratio** as **2** and **Upperleaflet Ratio** as **0**. In **Bacterial Lipids**, set the **Lowerleaflet Ratio** of **PMPE**, **PMPG**, **PVPE** and **PVPG** as **8**, **1, 8** and **2**, respectively, while keeping the **Upperleaflet Ratio** of all of them as 0.

7. Locate **LPS (lipopolysaccharides)**. Set the **Upperleaflet Ratio** as 1 and the **Lowerleaflet Ratio** as 0.

8. Click on **LPSA** button. In the pop-up, set all the parameters to match those shown in Figure 8 Then click on the **Next Step: Update LPS** button in the lower right corner.

9. Returning to the **Length of XY based on** option, enter **135** in the **Length of X and Y** box as an initial guess. Then click on the **Show the system info** button and you should *see* the information shown in Figure 9 (a).

This situation is caused by the difference in areas between the upper leaflet and the lower leaflet of the membrane. Generally, in order to solve it, we will use the **Ratio of lipid components** option first to determine the numbers of every membrane component under a certain initial guess. Then, use the **Numbers of lipid components** option to fine tune the number of lipids according to the feedback.

10. Select the **Numbers of lipid components** option. Change the upperleaflet lipid number of LPS from 92 to 93. Click on **Show the system info** button and you will *see* the information in Figure 9 (b).

11. Click on the **Next Step: Determine the System Size** button.

3.4 Build the Components

On the basis of the system size, the generation of individual components for the system, including the membrane, bulk water, and counter ions will be completed in this step.

1. Locate the **System Building Options**. Then select **Replacement method** (*see* Note 8).
2. Move on to **Component Building Options**. Check the **Include Ions** box.
3. Use **KCl** as neutralizing species with a concentration of **0.15 M**. Choose **Mg^{2+}** as the counter ions for both lipid A and core. Keep the **Ion Placing Method** as **Distance** (*see* Note 9).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. Go to **Pore Water Options**. Inappropriately placed water molecules can be removed here (*see* Note 10). Usually, there are no extra water molecules that need to be removed and this step can be skipped.

5. Click on the **Next Step: Build Components** button in the lower right corner. The lipid bilayer will be generated first in this step.

6. To generate water molecules and ions, click on the **Next Step: Assemble Components** button in the lower right corner.

3.5 Assemble the Components

Components generated in the previous steps will be assembled in this step.

1. Check carefully to ensure the system is built as intended. If no problem exists, then click on the **Next Step: Assemble Components** button in the lower right corner to complete the assembly. Otherwise, go back to rebuild the system.

2. Download all the output files by click on **download.tgz**.

At this point, the entire system containing protein, lipid bilayer, bulk water, and ions has been generated through users-specified parameters and options in CHARMM-GUI. Users can load the system into a molecular visualization program such as VMD for further inspection. If desired, simulation input files can also be generated by CHARMM-GUI.

4. Notes

1. Users can also select **RCSB** or upload their own pre-oriented PDB file. Options for **PDB Format** need to be chosen when using your own PDB file. Note that PDB files obtained from the OPM database have already been pre-oriented with respect to the membrane normal (Z axis by definition) while those from RCSB database need to be oriented manually by users themselves using **VMD** or in the subsequent step through CHARMM-GUI.

2. Generally, CHARMM-GUI detects structural information automatically, such as missing residues, disulfide bonds and others if indicated by remarks in PDB files (Figure 3). However, depending on the source of the PDB file, these remarks may have been written inadequately or even lost altogether. If that occurs, CHARMM-GUI cannot load those kinds of structure information, requiring users to add them manually in this step.

3. In CHARMM-GUI, the protein can be placed appropriately in the membrane by reorienting it via the alignment of its principal axis or a vector between two residues with the Z axis in **Orientation Options**, and repositioning it by means of the rotation with respect to the X or Y axis, or translation along the Z axis in **Positioning Options**. Users can also just utilize the original orientation and position information contained in the PDB file.

4. Users can use move and rotate commands in the software program VMD to write a pre-oriented PDB file and then upload it to CHARMM-GUI.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

5. During the pore water generation process, CHARMM-GUI solvates the transmembrane region of protein with a water box and runs high temperature dynamics with the protein fixed and water restrained in the transmembrane region. Water molecules inside the pore will remain while water molecules outside the pore will evaporate [19] (Figure 6). Water staying close to the protein exterior due to strong interactions, can be removed by a refinement step in **3.4**.

6. Ideally, the types and numbers of lipids are chosen to match the native membrane. Users should search the literature to determine which species the protein is from as well as the composition of its membrane in advance.

7. The scale of the entire system along the Z axis is determined by the height of the protein in Z and the thickness of the added water slabs (Figure 7). In general, the default water thickness of 22.5 Å, is sufficient. For a membrane-only system, users can select the **Hydration number** (number of water molecules per one lipid molecule) option to define the total number of water molecules [19].

8. Replacement method (Figure 10) distributes lipid-like pseudo atoms around the protein first, and then replaces them with lipid molecules selected randomly from a lipid molecule library, which contains 2,000 different conformations of lipids from MD simulations of pure bilayers [19]. Note that **Insertion method** is no longer supported in CHARMM-GUI.

9. In order to neutralize the system, Membrane Builder creates an appropriate number of ions based on the user-specified ion concentration and type. The initial configuration of ions is then determined through Monte Carlo simulations using a simplified model, i.e., van der Waals and scaled Coulombic interactions [19].

10. Pore water generated in **3.2.2** can be refined in this step, to ensure that no water molecules are left outside of the protein in the membrane hydrophobic core region. Users can download the structure file to verify whether those water molecules are removed and select the residue numbers of water molecules needing to be removed on the website.

Acknowledgement

This work was supported by the National Institutes of Health (R01-GM123169). Computational resources were provided through the Extreme Science and Engineering Discovery Environment (XSEDE; TG-MCB130173), which is supported by NSF Grant ACI-1548562.

References

1. Almen MS, Nordstrom KJ, Fredriksson R, Schioth HB (2009) Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biol 7:50 [PubMed: 19678920]
2. Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305 (3):567–580 [PubMed: 11152613]
3. Hidalgo P, MacKinnon R (1995) Revealing the Architecture of a K’ Channel Pore Through Mutant Cycles with a Peptide Inhibitor. Science 268:307–310 [PubMed: 7716527]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S (2002) Aquaporin water channels--from atomic structure to clinical medicine. J Physiol 542 (Pt 1):3–16 [PubMed: 12096044]
5. Rahman KS, Cui G, Harvey SC, McCarty NA (2013) Modeling the conformational changes underlying channel opening in CFTR. PLoS One 8 (9):e74574
6. Fairman JW, Noinaj N, Buchanan SK (2011) The structural biology of beta-barrel membrane proteins: a summary of recent reports. Curr Opin Struct Biol 21 (4):523–531 [PubMed: 21719274]
7. Gadsby DC (2007) Ion pumps made crystal clear. Nature 450:957–959 [PubMed: 18075569]
8. Shukla AK, Manglik A, Kruse AC, Xiao K, Reis RI, Tseng WC, Staus DP, Hilger D, Uysal S, Huang LY, Paduch M, Tripathi-Shukla P, Koide A, Koide S, Weis WI, Kossiakoff AA, Kobilka BK, Lefkowitz RJ (2013) Structure of active beta-arrestin-1 bound to a G-protein-coupled receptor phosphopeptide. Nature 497 (7447):137–141 [PubMed: 23604254]
9. Cojocaru V, Balali-Mood K, Sansom MS, Wade RC (2011) Structure and dynamics of the membrane-bound cytochrome P450 2C9. PLoS Comput Biol 7 (8):e1002152
10. Jordan JD, Landau EM, Iyengar R (2000) Signaling networks: the origins of cellular multitasking. Cell 103:193–200 [PubMed: 11057893]
11. Hunter T (2000) Signaling–2000 and beyond. Cell 100:113–127 [PubMed: 10647936]
12. Fu D, Libson A, Miercke LJ, Weitzman C, Nollert P, Krucinski J, Stroud RM (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. Science 290 (5491):481–486 [PubMed: 11039922]
13. Khademi S, O'Connell J 3rd, Remis J, Robles-Colmenares Y, Miercke LJ, Stroud RM (2004) Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 A. Science 305 (5690):1587–1594 [PubMed: 15361618]
14. Yellen G (2002) The voltage-gated potassium channels and their relatives. Nature 419:35–42 [PubMed: 12214225]
15. Dong J, Yang G, McHaourab HS (2005) Structural basis of energy transduction in the transport cycle of MsbA. Science 308:1023–1028 [PubMed: 15890883]
16. Elston T, Wang H, Oster G (1998) Energy transduction in ATP synthase. Nature 391:510–513 [PubMed: 9461222]
17. Medley QG, Kedersha N, O'Brien S, Tian Q, Schlossman SF, Streuli M, Anderson P (1996) Characterization of GMP-17, a granule membrane protein that moves to the plasma membrane of natural killer cells following target cell recognition. Proc Natl Acad Sci 93:685–689 [PubMed: 8570616]
18. Brooks JM, Lee SP, Leese AM, Thomas WA, Rowe M, Rickinson AB (2009) Cyclical expression of EBV latent membrane protein 1 in EBV-transformed B cells underpins heterogeneity of epitope presentation and CD8+ T cell recognition. J Immunol 182 (4):1919–1928 [PubMed: 19201845]
19. Jo S, Kim T, Im W (2007) Automated builder and database of protein/membrane complexes for molecular dynamics simulations. PLoS One 2 (9):e880 [PubMed: 17849009]
20. Hong M, Zhang Y, Hu F (2012) Membrane protein structure and dynamics from NMR spectroscopy. Annu Rev Phys Chem 63:1–24 [PubMed: 22136620]
21. Earl LA, Falconieri V, Milne JL, Subramaniam S (2017) Cryo-EM: beyond the microscope. Curr Opin Struct Biol 46:71–78 [PubMed: 28646653]
22. Garavito RM, Picot D, Loll PJ (1996) Strategies for Crystallizing Membrane Proteins. J Bioenerg Biomembr 28:13–27 [PubMed: 8786233]
23. Gumbart J, Wang Y, Aksimentiev A, Tajkhorshid E, Schulten K (2005) Molecular dynamics simulations of proteins in lipid bilayers. Curr Opin Struct Biol 15 (4):423–431 [PubMed: 16043343]
24. Lee AG (2011) Biological membranes: the importance of molecular detail. Trends Biochem Sci 36 (9):493–500 [PubMed: 21855348]
25. Landreh M, Marklund EG, Uzdavinys P, Degiacomi MT, Coincon M, Gault J, Gupta K, Liko I, Benesch JL, Drew D, Robinson CV (2017) Integrating mass spectrometry with MD simulations reveals the role of lipids in Na(+)/H(+) antiporters. Nat Commun 8:13993 [PubMed: 28071645]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

26. Dawaliby R, Trubbia C, Delporte C, Masureel M, Antwerpen PV, Kobilka BK, Govaerts C (2016) Allosteric regulation of G protein–coupled receptor activity by phospholipids. Nat Chem Biol 12:35–39 [PubMed: 26571351]
27. Dowhan W, Bogdanov M (2011) Lipid-protein interactions as determinants of membrane protein structure and function. Biochem Soc Trans 39 (3):767–774 [PubMed: 21599647]
28. Vigh L, Escriba PV, Sonnleitner A, Sonnleitner M, Piotto S, Maresca B, Horvath I, Harwood JL (2005) The significance of lipid composition for membrane activity: new concepts and ways of assessing function. Prog Lipid Res 44 (5):303–344 [PubMed: 16214218]
29. Saeedimasine M, Montanino A, Kleiven S, Villa A (2019) Role of lipid composition on the structural and mechanical features of axonal membranes: a molecular simulation study. Sci Rep 9 (1):8000 [PubMed: 31142762]
30. Jo S, Kim T, Iyer VG, Im W (2008) CHARMM-GUI: a web-based graphical user interface for CHARMM. J Comput Chem 29 (11):1859–1865 [PubMed: 18351591]
31. Bernard R. Brooks REB, Olafson Barry D., States David J., Swaminathan S. and Martin Karplus (1983) CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. Journal of Computational Chemistry 4 (2):187–217
32. Gu Y, Li H, Dong H, Zeng Y, Zhang Z, Paterson NG, Stansfeld PJ, Wang Z, Zhang Y, Wang W, Dong C (2016) Structural basis of outer membrane protein insertion by the BAM complex. Nature 531 (7592):64–69 [PubMed: 26901871]
33. Han L, Zheng J, Wang Y, Yang X, Liu Y, Sun C, Cao B, Zhou H, Ni D, Lou J, Zhao Y, Huang Y (2016) Structure of the BAM complex and its implications for biogenesis of outer-membrane proteins. Nat Struct Mol Biol 23 (3):192–196 [PubMed: 26900875]
34. Iadanza MG, Higgins AJ, Schiffrin B, Calabrese AN, Brockwell DJ, Ashcroft AE, Radford SE, Ranson NA (2016) Lateral opening in the intact beta-barrel assembly machinery captured by cryo-EM. Nat Commun 7:12865 [PubMed: 27686148]
35. Bakelar J, Buchanan SK, Noinaj N (2016) The structure of the β-barrel assembly machinery complex. Science 351 (6269):180 [PubMed: 26744406]
36. Noinaj N, Kuszak AJ, Gumbart JC, Lukacik P, Chang H, Easley NC, Lithgow T, Buchanan SK (2013) Structural insight into the biogenesis of beta-barrel membrane proteins. Nature 501 (7467):385–390 [PubMed: 23995689]
37. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. Nucleic Acids Res 28:235–242 [PubMed: 10592235]
38. Rose PW, Prlic A, Bi C, Bluhm WF, Christie CH, Dutta S, Green RK, Goodsell DS, Westbrook JD, Woo J, Young J, Zardecki C, Berman HM, Bourne PE, Burley SK (2015) The RCSB Protein Data Bank: views of structural biology for basic and applied research and education. Nucleic Acids Res 43 (Database issue):D345–356 [PubMed: 25428375]
39. Lomize MA, Lomize AL, Pogozheva ID, Mosberg HI (2006) OPM: orientations of proteins in membranes database. Bioinformatics 22 (5):623–625 [PubMed: 16397007]
40. Ali MH, Imperiali B (2005) Protein oligomerization: how and why. Bioorg Med Chem 13 (17):5013–5020 [PubMed: 15993087]
41. Protein Complex Oligomerization. https://www.ebi.ac.uk/QuickGO/term/GO:0051259 Accessed 14 Oct 2004
42. Jo S, Lim JB, Klauda JB, Im W (2009) CHARMM-GUI Membrane Builder for mixed bilayers and its application to yeast membranes. Biophys J 97 (1):50–58 [PubMed: 19580743]
43. Pandit KR, Klauda JB (2012) Membrane models of E. coli containing cyclic moieties in the aliphatic lipid chain. Biochim Biophys Acta 1818 (5):1205–1210 [PubMed: 22274566]
44. Lind TK, Wacklin H, Schiller J, Moulin M, Haertlein M, Pomorski TG, Cardenas M (2015) Formation and Characterization of Supported Lipid Bilayers Composed of Hydrogenated and Deuterated Escherichia coli Lipids. PLoS One 10 (12):e0144671

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

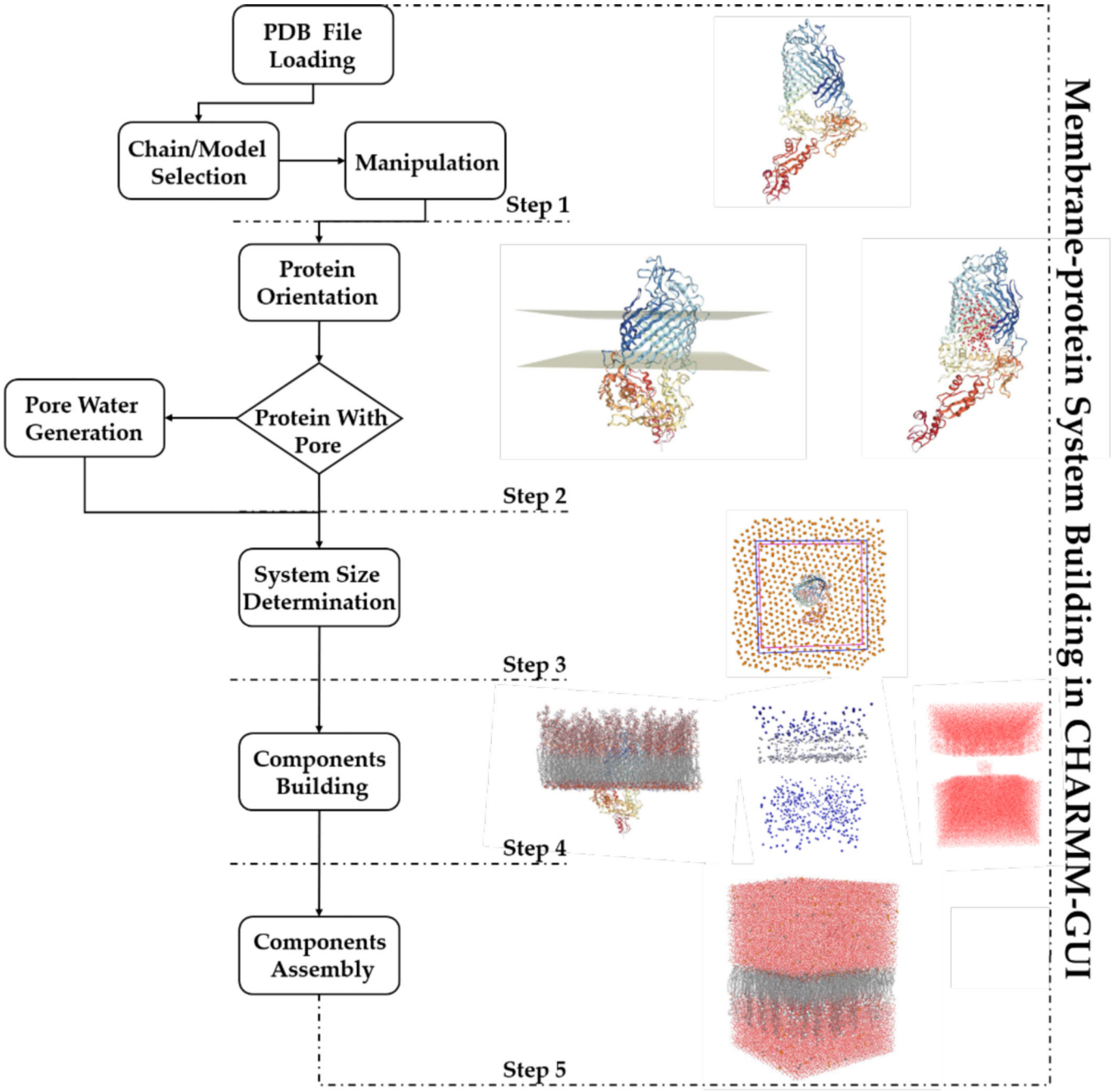

Figure 1.
Membrane-protein-system building process in CHARMM-GUI.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

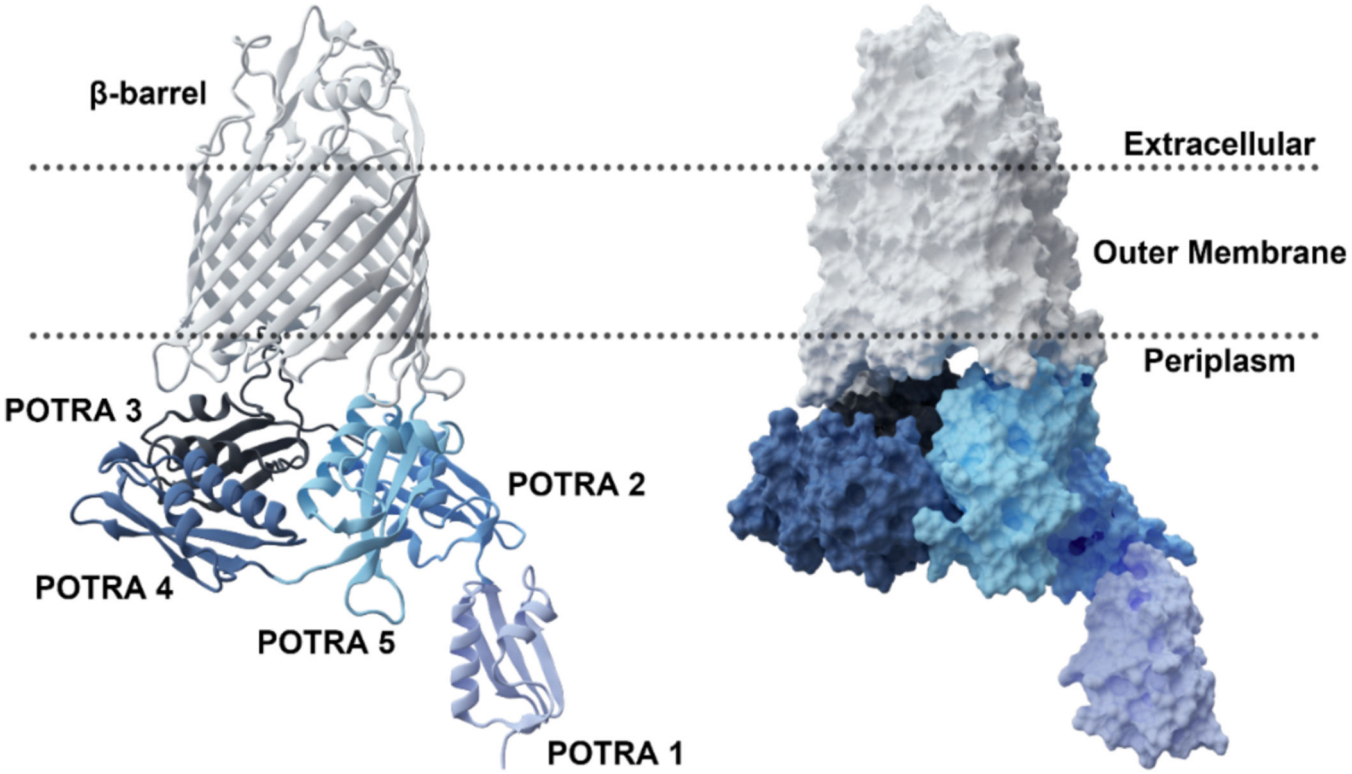

Figure 2.
Structure of *E. coli* BamA.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

```
SHEET   1 AA1 3 ASP A  28  GLU A  32  0
SHEET   2 AA1 3 THR A  83  GLU A  90  1  O  VAL A  88   N  GLU A  32
SHEET   3 AA1 3 PHE A  72  ARG A  79 -1  N  ARG A  76   O  GLN A  87
SHEET   1 AA2 3 THR A  93  SER A 100  0
SHEET   2 AA2 3 ARG A 162  VAL A 168  1  O  VAL A 163   N  ALA A  95
SHEET   3 AA2 3 LYS A 152  PRO A 157 -1  N  LYS A 152   O  VAL A 168
SHEET   1 AA3 4 GLU A 176  VAL A 183  0
SHEET   2 AA3 4 GLY A 253  THR A 261  1  O  ILE A 254   N  GLN A 178
SHEET   3 AA3 4 ASN A 239  LEU A 247 -1  N  GLN A 244   O  THR A 257
SHEET   4 AA3 4 SER B 193  LEU B 194  1  O  SER B 193   N  VAL A 245
SHEET   1 AA4 4 GLU A 294  LEU A 295  0
SHEET   2 AA4 4 LYS A 267  ASN A 276 -1  N  LEU A 268   O  GLU A 294
SHEET   3 AA4 4 THR A 334  ASP A 342  1  O  VAL A 335   N  LYS A 267
SHEET   4 AA4 4 ARG A 321  ASN A 329 -1  N  MET A 325   O  ARG A 338
SHEET   1 AA5 3 TYR A 348  GLU A 355  0
SHEET   2 AA5 3 GLN A 411  GLU A 420  1  O  VAL A 412   N  TYR A 348
SHEET   3 AA5 3 PHE A 395  ARG A 404 -1  N  GLU A 396   O  LYS A 419
```

Figure 3.
Part of the structural information in the original PDB file.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

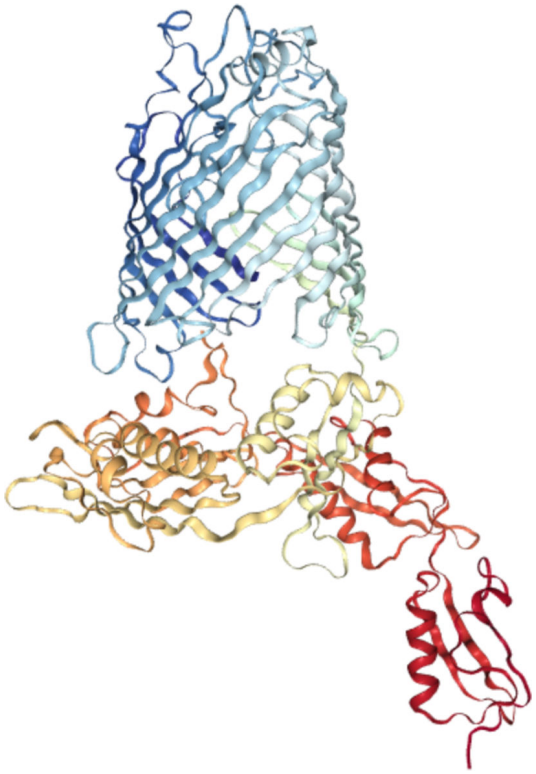

Figure 4.
Structure of *E. coli* BamA as seen in CHARMM-GUI.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

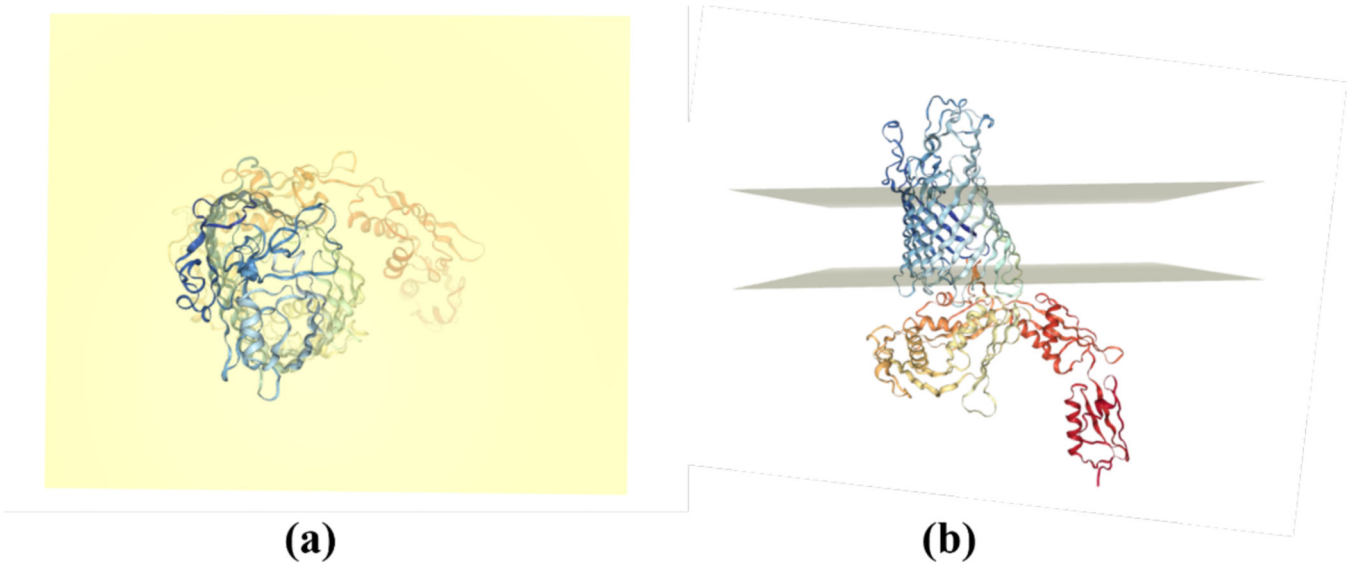

Figure 5.
Protein orientation. The yellow sheets are the XY-planes of membrane.(a) Top view. (b) Side view.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

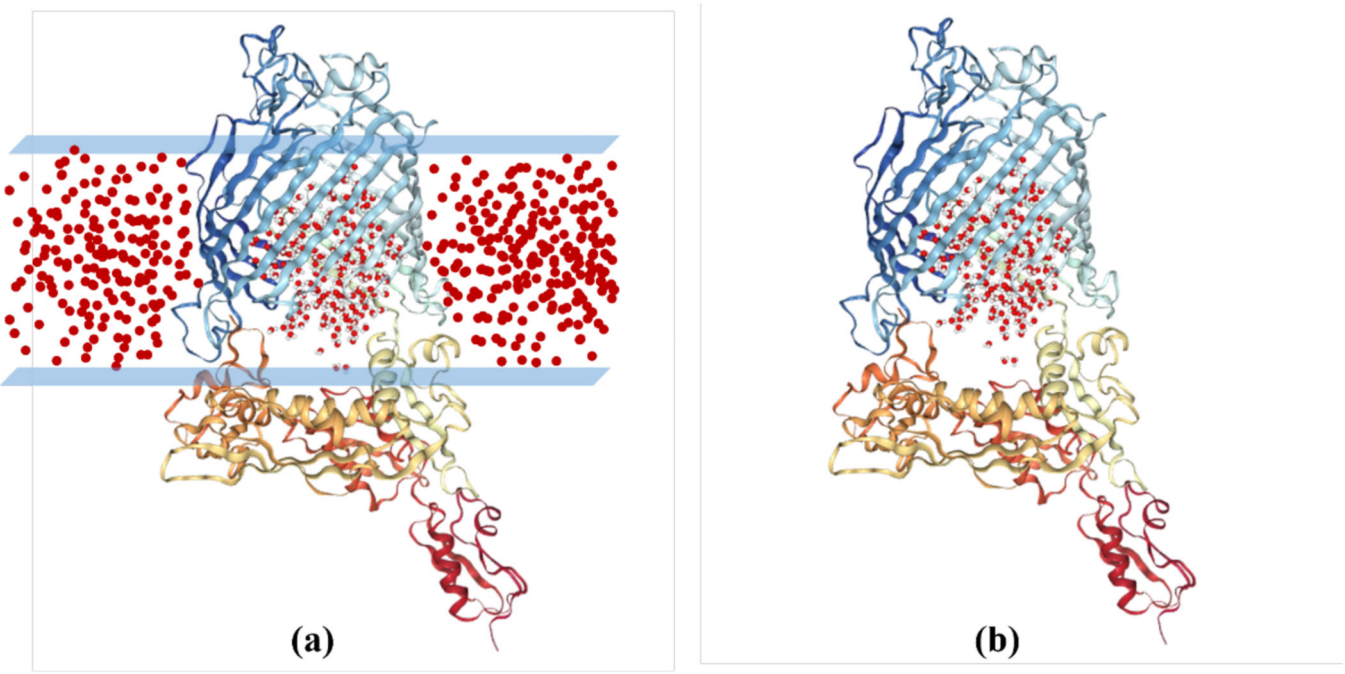

Figure 6.
Pore water generation. (a) Solvating the transmembrane region with water. (b) Pore water remains after high-temperature dynamics.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

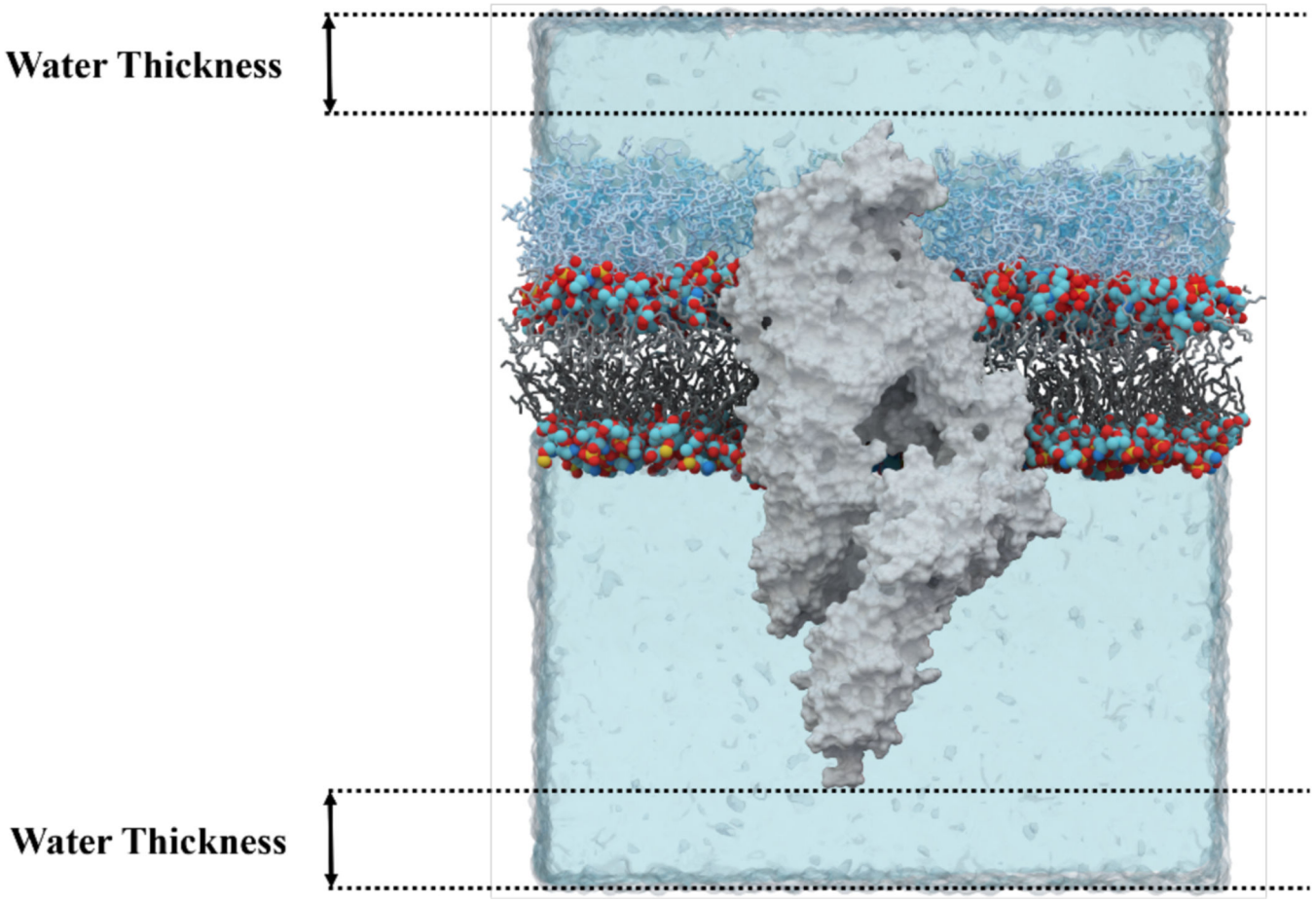

Figure 7.
Water thickness above and below the protein.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Figure 8.
LPS type and core sequence.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Calculated Number of Lipids:

Lipid Type	Upperleaflet Number	Lowerleaflet Number
PVCL2	0	24
PMPE	0	96
PMPG	0	12
PVPE	0	96
PVPG	0	24
LPSA	92	0

Calculated XY System Size:

	Upperleaflet	Lowerleaflet
Protein Area	1655.20977	1960.7824
Lipid Area	17480	17306.4
# of Lipids	92	252
Total Area	19135.20977	19267.1824
Protein X Extent	35.19	
Protein Y Extent	52.57	
Average Area	19201.20	
A	138.57	
B	138.57	

The upperleaflet can have more lipids

(a)

Calculated XY System Size:

	Upperleaflet	Lowerleaflet
Protein Area	1655.20977	1960.7824
Lipid Area	17670	17306.4
# of Lipids	93	252
Total Area	19325.20977	19267.1824
Protein X Extent	35.19	
Protein Y Extent	52.57	
Average Area	19296.20	
A	138.91	
B	138.91	

(b)

Figure 9.

Feedback information for determining the membrane size. (a) Only using ratio to determine the membrane size may lead to one leaflet having too few lipids. (b) Adjusting the lipd numbers slightly will eliminate this problem.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

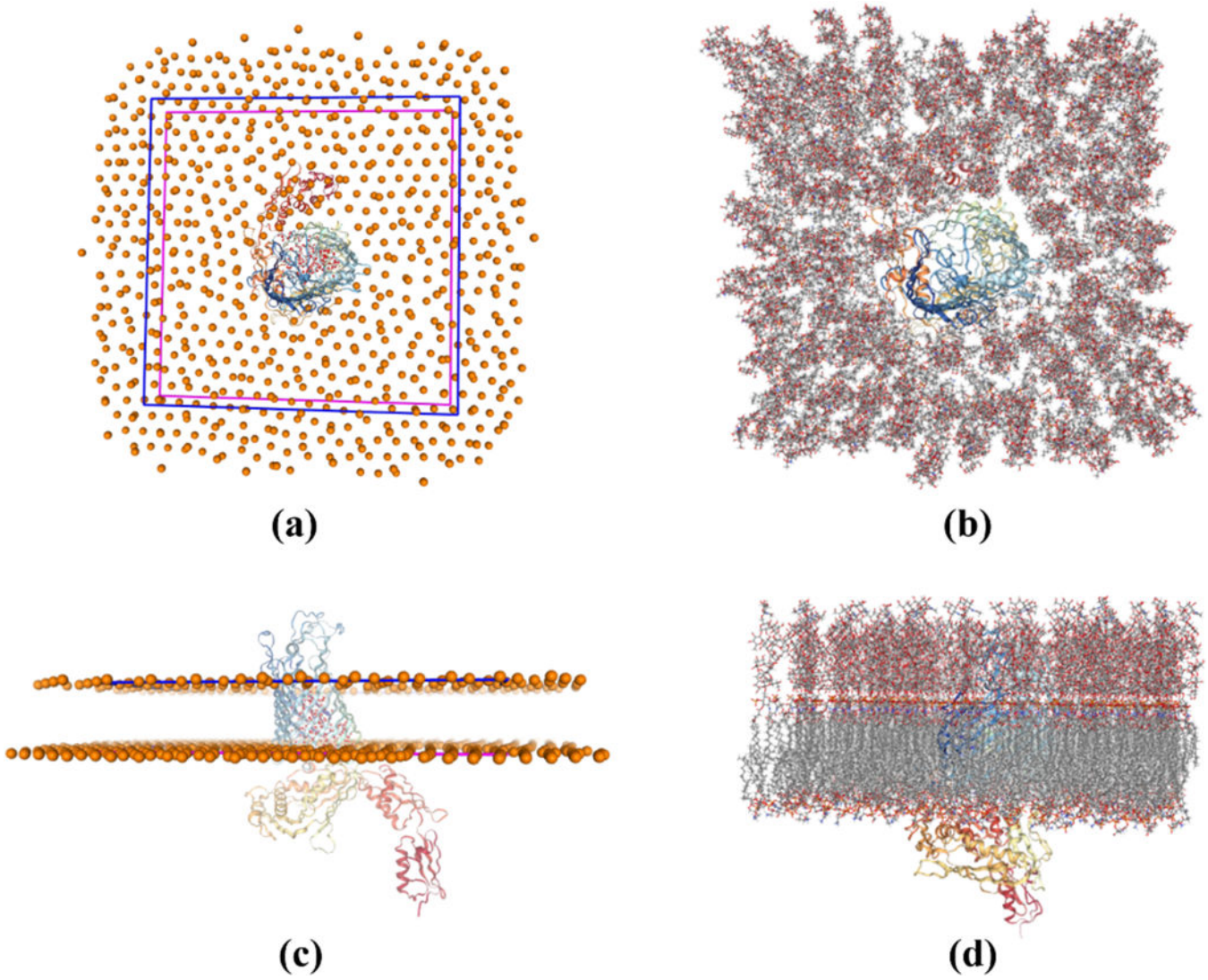

Figure 10.
The replacement method uses lipid-like pseudo-atoms to build lipids around the protein.