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CHARMM-GUI MDFF/xMDFF Utilizer for Molecular Dynamics Flexible Fitting Simulations in Various Environments

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Abstract

X-ray crystallography and cryo-electron microscopy are two popular methods for the structure determination of biological molecules. Atomic structures are derived through the fitting and refinement of an initial model into electron density maps constructed by both experiments. Two computational approaches, MDFF and xMDFF, have been developed to facilitate this process by integrating the experimental data with molecular dynamics simulation. However, the set-up of an MDFF/xMDFF simulation requires knowledge of both experimental and computational methods, which is not straightforward for non-expert users. In addition, sometimes it is desirable to include realistic environments such as explicit solvent and lipid bilayers during the simulation, which poses another challenge even for expert users. To alleviate these difficulties, we have developed *MDFF/xMDFF Utilizer* in CHARMM-GUI that helps users to set up an MDFF/xMDFF simulation. The capability of *MDFF/xMDFF Utilizer* is greatly enhanced by integration with other CHARMM-GUI modules, including protein structure manipulation, a diverse set of lipid types, and all-atom CHARMM and coarse-grained PACE force fields. With this integration, various simulation environments are available for *MDFF Utilizer* (vacuum, implicit/explicit solvent, and bilayers) and *xMDFF Utilizer* (vacuum and solution). In this work, three examples are shown to demonstrate the usage of *MDFF/xMDFF Utilizer* (<http://www.charmm-gui.org/input/mdff> and <http://www.charmm-gui.org/input/xmdff>).

Graphical Abstract

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Supporting Information

Five figures. Figure S1: Flowchart of *MDFF Utilizer* using *Membrane Builder*. Figure S2: Comparison of the final YidC structure from vacuum simulation and the target structure. Figure S3: RMSF of YidC in different MDFF simulations. Figure S4: Comparison of final HTL structural models from MDFF simulations. Figure S5: Comparison of the initial and final HTL structures from the periplasm side. Figure S6: RMSD of the backbone and heavy atoms in RBP xMDFF simulations.

INTRODUCTION

X-ray crystallography remains the most dominant method for solving atomic structures. An increasing number of structures are submitted each year to the Protein Data Bank,¹ with over 90% of the current entries coming from X-ray crystal structures. However, for relatively large systems, the availability of only medium-to-low-resolution diffraction data often limits the determination of all-atom details. Rivaling X-ray crystallography, cryo-electron microscopy (cryo-EM) has evolved into one of the most effective structure determination tools in recent years.² The cryo-EM based structure determination overcomes two major bottlenecks faced in traditional X-ray crystallography, namely, the arduous task of preparing well-ordered crystals of macromolecules,³ and the more fundamental problem with capturing these molecules in unphysiological states as a result of crystal contacts.³ Consequently, cryo-EM provides a natural way of resolving the structures of large macromolecular complexes.

Both crystallographic and cryo-EM experiments provide structural data in the form of electron density maps. EM directly delivers the density maps, which can be interpreted with rigid-body⁴ or flexible fitting^{5–9} algorithms to derive atomic models. In contrast, banded patterns measured in crystallography provide only amplitude of the diffracted X-ray beam. Reconstruction of the crystallographic phase through techniques such as molecular replacement or anomalous scattering, together with the stated diffraction amplitudes yields an initial guess density, which is further refined with fitting methods to derive the final atomic models.

Molecular dynamics (MD) simulation has been employed as an effective technique for structure determination from the aforementioned density data.^{10–11} Starting with cryo-EM density or initially-phased crystallographic density, MD simulations can flexibly fit an initial guess model into the density map until certain map-model validation criteria are met, indicating accurate structural refinement. To achieve this goal, two methods have been developed, namely molecular dynamics flexible fitting (MDFF) for cryo-EM maps,^{12–13} and its successor xMDFF directed towards the refinement of crystallographic density.¹⁴ In MDFF, an initial atomic structure is subjected to an MD simulation biased by an additional potential energy term that is proportional to the sign-inverse of the EM map (see supplementary material Section 1).^{12–13, 15} Through this biasing potential, steering forces locally guide atoms towards high-density regions, thereby fitting the structure to the map.

xMDFF, on the other hand, fits an initial model into an iteratively updating electron-density map until the theoretical and experimental diffraction patterns match.

Both MDFF and xMDFF have been used extensively for resolving the structure of biological systems. While MDFF has been successfully employed to resolve the structure of respiratory complexes,^{16–17} ribosomal translocation machinery,^{18–20} and a host of viruses,^{21–22} xMDFF resolved the structures of ion transporters,²³ voltage sensor channels,²⁴ and organic macrocycles.^{25–26} The set-up and running of the MDFF and xMDFF refinements require knowledge of MD simulations parameters, in addition to that of the experimental data. Thus, performing MDFF/xMDFF refinements can become a nontrivial task for non-expert users, such as young experimental or computational scientists. To address this barrier, a graphical user interface (GUI) has been created on the visualization and analysis software VMD.²⁷ This interface provides a robust platform for the set-up, execution, and preliminary analysis of MDFF and xMDFF refinements in vacuum, standard implicit or explicit solvent environment.²⁸ However, often the environment under which a macromolecule is experimentally determined includes more exotic molecules such as lipids, detergents, or non-aqueous solvents, which should be accounted for in MDFF refinements to achieve accurate results.^{29–32} Setting up of realistic environments such as complex bilayers requires a comprehensive modeling endeavor, far more than a single “click”, and often incurs a considerable amount of human time, which is an important issue for non-expert and experts alike. To address this imminent challenge, we have developed *MDFF/xMDFF Utilizer* in CHARMM-GUI to facilitate MDFF/xMDFF simulation system preparation in different environments including vacuum, implicit solvent, explicit solvent, and membranes with an all-atom or coarse-grained (CG) model: <http://www.charmm-gui.org/input/mdff> and <http://www.charmm-gui.org/input/xmdff>.

METHODS

User Uploads and Inputs

MDFF Utilizer requires a PDB file that contains the protein structure, and a density map from cryo-EM experiment. It is suggested that the uploaded protein structure is rigidly fitted to the map using fitting tools such as Situs.⁴ For MDFF refinement within an explicit bilayer, a second PDB file is required that contains the same protein structure but properly oriented with respect to the membrane normal. This structure is used as a reference to translate and orient the density map to the correct position in the membrane. The OPM database (<http://opm.phar.umich.edu>)³³ provides pre-oriented protein structures and is available by typing the PDB ID during file uploading. In the case of *xMDFF Utilizer*, a user needs to upload a PDB file and a MTZ diffraction data file.

System Setup

The building and assembly of a simulation system in different environments in *MDFF/xMDFF Utilizer* follow the same procedures in the corresponding CHARMM-GUI modules,³⁴ i.e., vacuum, GB, and solution systems use the same modules from *PDB Reader*³⁵ and *Quick MD Simulator*,³⁶ bilayer systems use *Membrane Builder*,^{37–39} and the systems using the PACE force field (FF) are prepared through *PACE CG Builder*.⁴⁰ In

MDFF Utilizer, the uploaded cryo-EM density map is converted to a grid force file using the MDFF plugin^{12–13, 15} in VMD,²⁷ which is then used to provide additional biasing potentials during simulations. As *Membrane Builder* assumes that the membrane center is located at $z = 0$, the grid force is translated and oriented based on the second PDB structure that is properly oriented in a bilayer (see Figure S1 for a flowchart of the building process). In *xMDFF Utilizer*, the user-uploaded MTZ data file is used without any processing.

Simulation Protocol

All simulations presented in this study were carried out with NAMD2.10⁴¹ using the CHARMM36 FF⁴² for all-atom systems in vacuum, with explicit TIP3P water^{43–44} and lipids, or with the generalized Born implicit solvent (GBIS) model³⁰ in NAMD, and the PACE FF⁴⁵ for CG systems. In the simulations with the CHARMM FF, the non-bonded interactions were smoothly switched off at 10–12 Å by a force-switching function.⁴⁶ The particle mesh Ewald algorithm⁴⁷ was applied to calculate electrostatic forces in explicit solvent systems. Temperature was maintained using Langevin dynamics with a coupling coefficient of 5 ps⁻¹ for vacuum systems and 1 ps⁻¹ for explicit solvent systems. Nosé–Hoover Langevin–piston method^{48–49} was used to maintain constant pressure (1 bar) with a piston period of 50 fs and a piston decay of 25 fs, and no pressure control was applied in vacuum systems. A dielectric constant of 80 for electrostatic interactions was used in vacuum systems. In simulations with the GB model, a 15–16 Å force-switching function was used for non-bonded energy calculations. Ion concentration was set to 0.15 M, and a surface tension of 0.006 kcal/mol/Å² was used to calculate the nonpolar solvation energy using SASA (solvent-accessible surface area). Temperature control was the same as in the vacuum simulations, and pressure control was not applied. In simulations with the PACE FF, a 9–12 Å force-switching function was used. The dielectric constant was set to 15, and the integration time-step was 5 fs. Temperature was controlled with the Langevin dynamics method and pressure was controlled with the Hoover Langevin–piston method.^{48–49} The fitting process consisted of three separate intervals of simulations, each of which applied biasing forces to different atoms and with different scaling factors. The coupled atoms in the three steps were C α atoms, backbone atoms, and non-hydrogen atoms, and the grid force scales were 0.3, 0.5, and 0.7, respectively. The atomic mass of the coupled atoms was used for the atom-dependent weight. Additional restraints prepared using the MDFF plugin in VMD²⁷ were applied in all simulations to maintain the correct chiral centers, peptide bond conformations, and secondary structures of each protein. These simulation protocols are all included in the NAMD input files from *MDFF/xMDFF Utilizer*.

RESULTS AND DISCUSSIONS

MDFF/xMDFF Utilizer provides a number of environments for setting up the MDFF/*xMDFF* simulation system and NAMD input files. Specifically, vacuum, solution, and bilayer system generations are available for *MDFF Utilizer*, and vacuum and solution system generations are available in *xMDFF Utilizer*. The set-up of various simulation systems takes advantage of existing CHARMM-GUI modules, namely *PDB Reader & Manipulator*,³⁵ *Quick MD Simulator*,³⁶ *Membrane Builder*,^{37–39} and *PACE CG Builder*,⁴⁰ which enable full integration of CHARMM-GUI features including various PDB structure processing and a

large number of lipid types in bilayer systems. In *MDFF Utilizer*, a density map and an initial protein structure that is roughly docked, e.g., using Situs, to the map are required. For bilayer systems, another protein structure that is oriented with respect to the membrane center is also required. In *xMDFF Utilizer*, users need to upload a protein structure and a MTZ reflection data file (see Methods for details). In the following sections, we show two examples of *MDFF Utilizer* using CHARMM FF and PACE FF, and one example of *xMDFF Utilizer*.

MDFF Example: YidC in Vacuum, GB, Solution, and Bilayer

The bacteria YidC is a conserved protein that contains five transmembrane (TM) helices and inserts proteins into the plasma membrane of bacterial cells.⁵⁰ Beckmann et al. have proposed a structural model of this protein in the ribosome-bound state during co-translocation of the substrate F₀c using evolutionary co-variation analysis, lipid-versus-protein-exposure, and MD simulations.²⁰ The model was docked to an 8-Å cryo-EM map and suggests a mechanism for the co-translational mode of YidC-mediated membrane protein insertion. Here, we try to fit an artificial initial structure of YidC to the cryo-EM map in different environments using *MDFF Utilizer* (Figure 1). The initial structure of YidC was obtained by subjecting the reported structure²⁰ to a 20-ns MD simulation at a temperature of 350 K in vacuum with secondary structure restraints. Trajectory frames recorded at 2-ps intervals were evaluated for backbone RMSDs with respect to the reported structure. A frame with an RMSD value of 4.5 Å and the lowest global cross-correlation with respect to the reported map was selected as an initial structure. The MDFF simulations were performed in vacuum, GB implicit solvent, explicit solution, and a POPC bilayer. The TM helix of the nascent chain F₀c was placed next to the YidC structure in the simulation to account for prominent densities in the map. Each simulation consisted of two 2-ns steps followed by one 4-ns step, in which the coupling atoms are C α atoms, backbone atoms, and all heavy atoms, and the coupling strength (i.e., grid force scale) is 0.3, 0.5, and 0.7, respectively. The overall structures from four MDFF simulations are similar to each other. The C α RMSD of the simulation structures is ~2.5 Å in vacuum, and ~2.0 Å in GB, solution, and bilayer to the structural model from Beckmann et al. (Figure 2). The larger RMSD value in vacuum simulation is due to rotation and tilt of TM5 (Figure S2). To obtain insight into the biological relevance of applying correct environmental conditions during MDFF simulations, we calculated the root mean square fluctuations (RMSF) of YidC in different MDFF simulations. The RMSF value per each residue was computed by aligning the last 2-ns trajectories to the last snapshot in each simulation using the C α atoms of TM helices. Noting that, as the RMSF directly implies the flexibility of a protein segment, the explicit solvent conditions appear to unnecessarily overestimate protein flexibility relative to that in a solvated membrane environment (Figure S3). Such considerations are relevant because MDFF refinements can accurately assess B-factors required for the sharpening of EM maps.¹³ Employing the relationship $B=8\pi^2(\text{RMSF})^2/3$, the simulated RMSF values provide a measure of these factors. Noting that the RMSF values are very sensitive to correct choice of environment, this result underlines the importance of choosing proper environments in computing RMSF values, which can provide MDFF users a guideline for assessing the quality of B-factors.

MDFF Example: Holo-translocon in Vacuum, GB, and PACE Bilayer

In the second example, the holo-translocon (HTL) structure was fitted to a 13-Å cryo-EM map. HTL is a membrane protein complex that consists of SecYEG, SecDF, YajC, and YidC, and functions for protein secretion and membrane protein insertion.^{51–54} The initial model was built by placement of existing x-ray structures of the individual HTL components. The MDFF simulation was performed in vacuum, GB implicit solvent, PACE solution, and PACE bilayer, which consists of 1,007 POPC at the top leaflet and 1,000 POPC at the bottom leaflet. The total number of atom in the PACE bilayer system is ~190,000 (Figure 3A), which is 10 times smaller than that in a corresponding all-atom system. The simulation consisted of three 10-ns steps. The coupling atoms and the grid force scale in each step are the same as those in the all-atom MDFF simulation. The cross-correction coefficient (CCC) values in all simulations increased from ~0.6 to 0.8–0.9, with the PACE bilayer simulation showing a slightly higher CCC value (Figure 3B). The overall architecture of the complex from three simulations is similar to each other (Figure S4). The major conformational change during the simulation was opening of the translocation channel (Figure S5). A more comprehensive modeling of the HTL atomic structure using cryo-EM, small-angle neutron scattering, and a biochemical analysis will be published elsewhere.

xMDFF Example: Ribose Binding Protein

As the final example, the open-state structure of ribose binding protein (RBP) was fitted to the closed state using *xMDFF Utilizer*. An *in silico* 5-Å X-ray diffraction data of the closed state was calculated based on a crystal structure (PDB ID: 2DRI)⁵⁵. The diffraction data and the open structure (PDB ID: 1URP)⁵⁶ were used as inputs in xMDFF simulations in vacuum, GB implicit solvent, and explicit solvent environments. Each simulation consists of two 2-ns steps followed by one 4-ns step, and the coupled atoms and coupling strength in each step were the same as those in the above MDFF simulations. All three simulations converged to structures with ~0.5 Å RMSD (C α atoms) to the target structure (Figure 4). The R_{free} and R_{work} values show that the solution structure is slightly improved in comparison to vacuum and GB structures (Table 1). The structure from the solution simulation has a slightly lower RMSD value when the sidechain heavy atoms are included in the RMSD calculation (Figure S6). To explore the reason for this difference, the average sidechain χ_1 dihedral of each residue from the last 2-ns simulations is plotted against that from the crystal structure (Figure 5). The polar residues show better agreement with crystal structure in explicit solution, whereas the nonpolar residues show similar deviations in three environments. To show the difference quantitatively, the average difference of χ_1 dihedrals was calculated for polar and nonpolar residues (Table 2). Polar residues show decreasing difference in vacuum, GB, and explicit solvent systems, while nonpolar residues show similar average difference in three systems. These results suggest that the polar sidechain atoms are modeled better when explicit solvent is included in the simulation, and show the benefits of including the environment explicitly when xMDFF simulation is carried out with medium or low-resolution data.

CONCLUSIONS

In this study, we have described the development of *MDFF/xMDFF Utilizer* in CHARMM-GUI to set-up MDFF and xMDFF simulations in various environments and force fields. A few examples are tested to show the capability of the module. These test cases suggest that sometimes it is beneficial to perform the fitting in realistic environments that include explicit solvent and lipids.³¹ The advantage of *MDFF/xMDFF Utilizer* is its seamless integration with CHARMM-GUI functionality including protein structure manipulation, various lipid type selection, and all-atom or coarse-grained force fields. Note that the standard protocol provided in *MDFF/xMDFF Utilizer* does not always guarantee a best result. Users are encouraged to explore more on simulation parameters to obtain a good fitting. More complex control of the parameters including symmetric restrains and density mask, as well as results analysis tools are available in VMD.²⁷

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Initial and target structure of YiDC. (A) Side view with cytoplasmic side on top and (B) top view. The initial and target structures are colored in cyan and green.

Figure 2. MDFF simulation of YiDC in different environments. (A) RMSD of YiDC to the target structure. The RMSD value was calculated using C α atoms of TM2–6. (B) Comparison of the final simulation structures. Only the TM helices are shown for clarity. The target structure is colored in green and the simulated structures are in cyan.

Figure 3.

MDFF simulation of the Holo-translocon. (A) Final structure of HTL in PACE bilayer simulation. The periplasmic side is on top. Lipid head groups are shown in red spheres, and lipid tails are shown in gray sticks. The density map is shown as cyan mesh. SecYEG is colored in green, SecDF in red, YajC cyan, and YidC in blue. (B) Time-series of cross-correction coefficient (CCC) between the simulation structures and density map.

Figure 4. xMDFF simulation of RBP. (A) The initial (cyan, PDB ID 1URP) and target structure (green, PDB ID 2DRI) of RBP. (B) RMSD of the Ca atoms in simulations with different environments.

Figure 5. Comparison of sidechain χ_1 dihedral from xMDFF simulations to that in the crystal structure. (A) Vacuum, (B) GB implicit solvent, and (C) explicit solution. The sidechain dihedral of each residue from the simulated structures is plotted against the corresponding dihedral from a crystal structure (PDB ID: 2DRI). Nonpolar residues are shown as blue dots and polar residues are shown as red dots.

Table 1

R_{free} and R_{work} of the final structures from three simulations.

Simulation Environment	Vacuum	GB	Solution
R_{free}	0.332	0.317	0.308
R_{work}	0.266	0.257	0.254

Table 2

Average difference of sidechain χ_1 dihedral between xMDFF simulation and the target crystal structure for nonpolar and polar residues.

Simulation Environment	Vacuum	GB	Solution
Polar residues	33.48°	26.55°	20.73°
Nonpolar residues	14.86°	12.90°	13.89°