

SUPPORTING INFORMATION FOR

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

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Section 1. Incorporating EM data into simulation

In MDFF simulation, the biasing potential converted from the density map has the following form

$$U_{EM}(\mathbf{R}) = \sum_j w_j V_{EM}(\mathbf{r}_j)$$

where

$$V_{EM}(\mathbf{r}) = \begin{cases} \xi [1 - (\Phi(\mathbf{r}) - \Phi_{thr}) / (\Phi_{max} - \Phi_{thr})] & \text{if } \Phi(\mathbf{r}) \geq \Phi_{thr} \\ \xi & \text{if } \Phi(\mathbf{r}) < \Phi_{thr} \end{cases}$$

Here w_j is a per-atom weight, typically set to the atomic mass, ξ is a force scaling, $\Phi(\mathbf{r})$ is the EM density at position \mathbf{r} , Φ_{max} is the maximum value of EM density, and Φ_{thr} is a density threshold (often chosen to remove background noise from the map, such as due to solvation). An atom placed in this external potential feels a force of

$$\mathbf{f}_i^{EM} = -\frac{\partial}{\partial \mathbf{r}_i} U_{EM}(\mathbf{R}) = -w_i \frac{\partial}{\partial \mathbf{r}_i} V_{EM}(\mathbf{r}_j)$$

The details of the biasing potential can be found in ref¹⁻³.

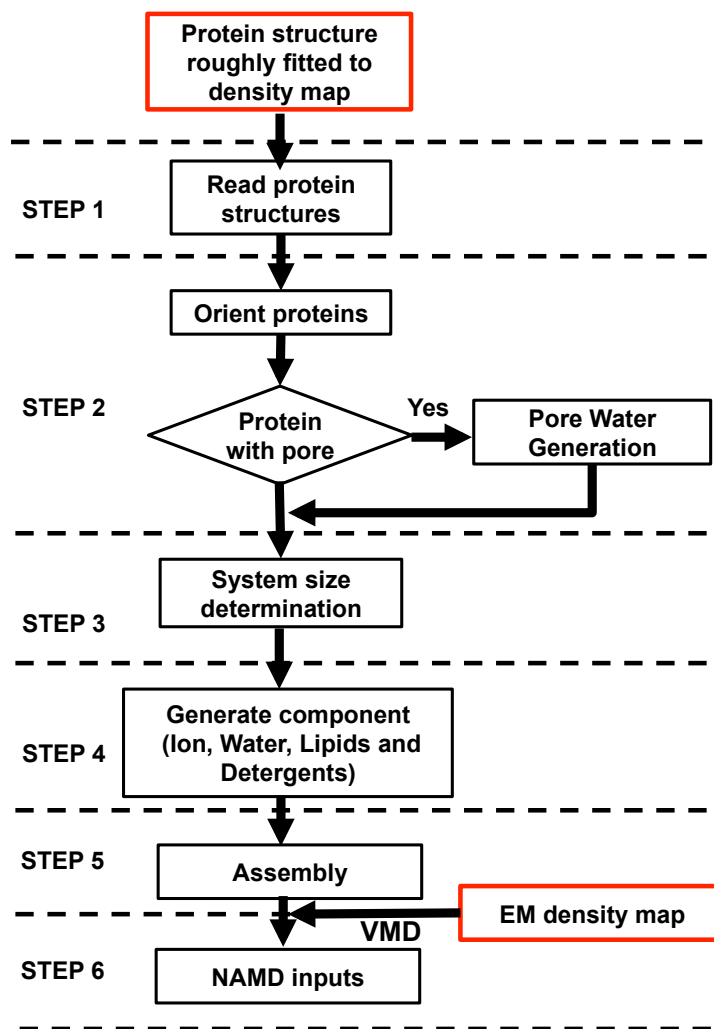


Figure S1. Flowchart of *MDFFF Utilizer* using *Membrane Builder*, showing the process of building a MDFFF simulation system with a membrane protein in a bilayer. The user inputs are in red boxes.

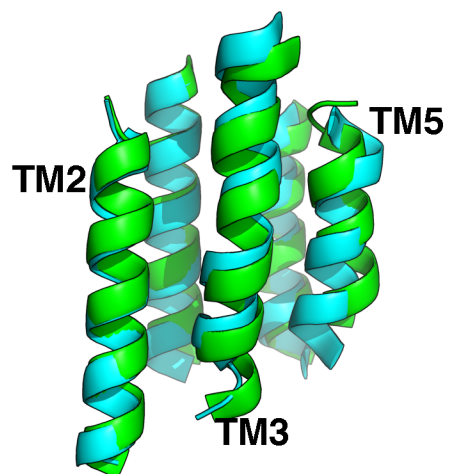


Figure S2. Comparison of the final YiDC structure from vacuum simulation and the target structure. Only the TM helices are shown for clarity. The target structure is colored in green and the simulated structure is in cyan.

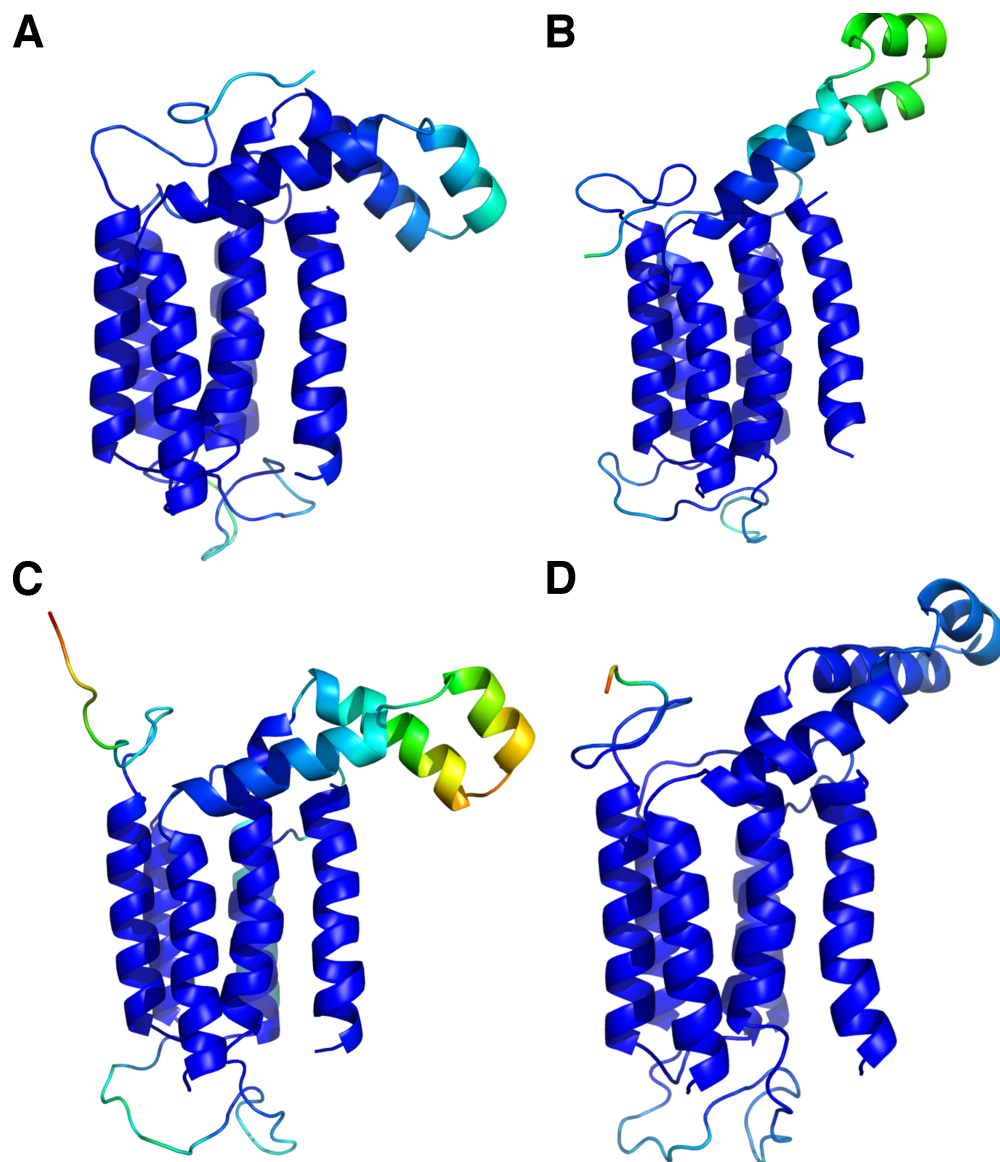


Figure S3. The root mean square fluctuations (RMSF) of YidC in different MDFF simulations: (A) vacuum, (B) GB, (C) solution, and (D) bilayer. The RMSF value per each residue was calculated by aligning the C α atoms of the TMs from the last 2-ns trajectories to the last snapshot in each simulation. Residues are colored based on their RMSF values from blue (0.25 Å) to red (7.5 Å).

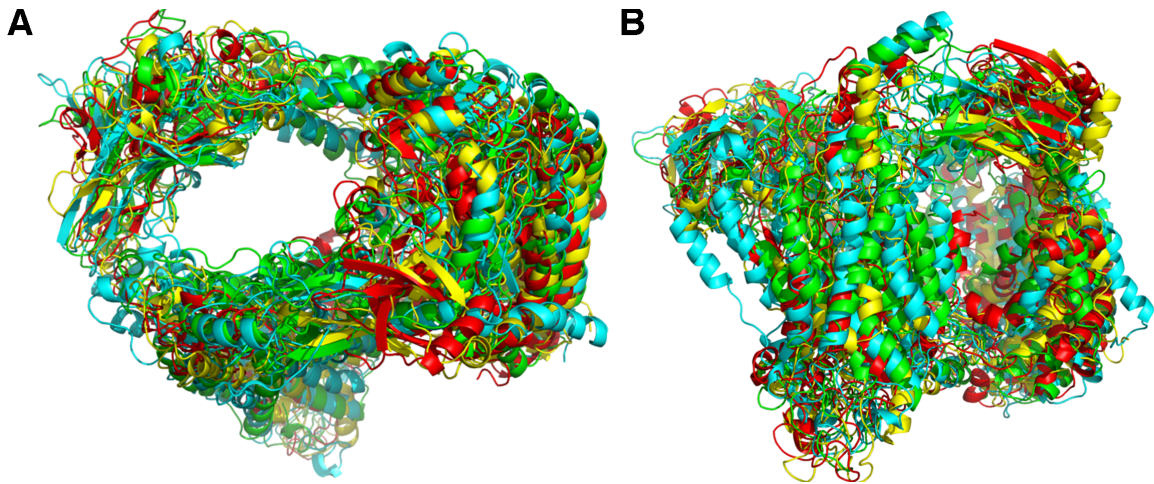


Figure S4. Comparison of final HTL structural models from MDFF simulations. (A) Top view from the periplasmic side. (B) Side view with the periplasmic side on top. Vacuum structure is colored in green, GB structure in cyan, PACE bilayer structure in red, and PACE solution in yellow.

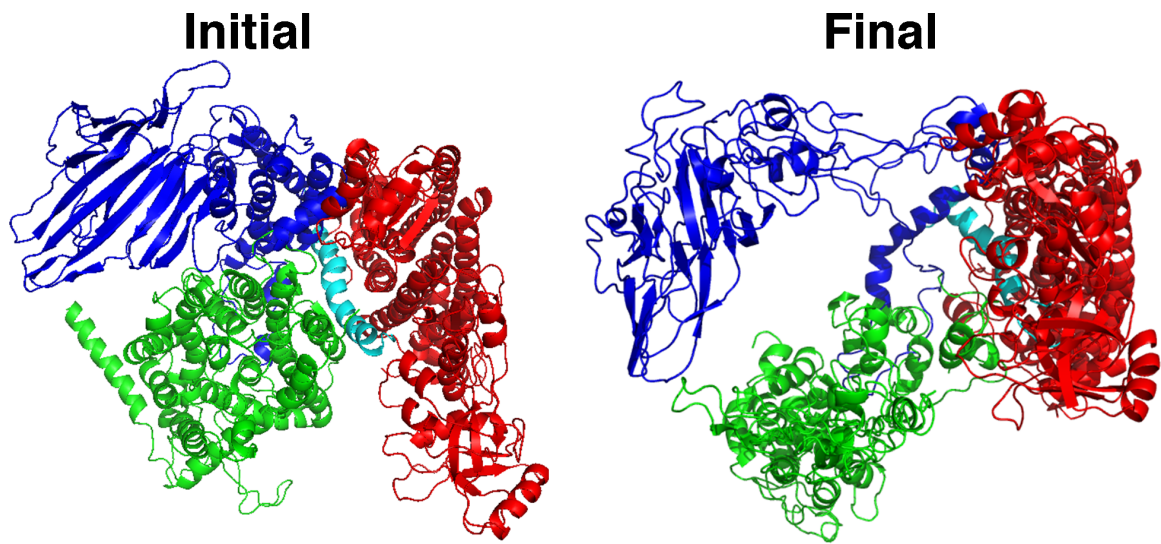


Figure S5. Comparison of the initial and final HTL structures from the periplasmic side. The final HTL structure is taken from the PACE bilayer simulation. SecYEG is colored in green, SecDF in red, YajC cyan, and YidC in blue.

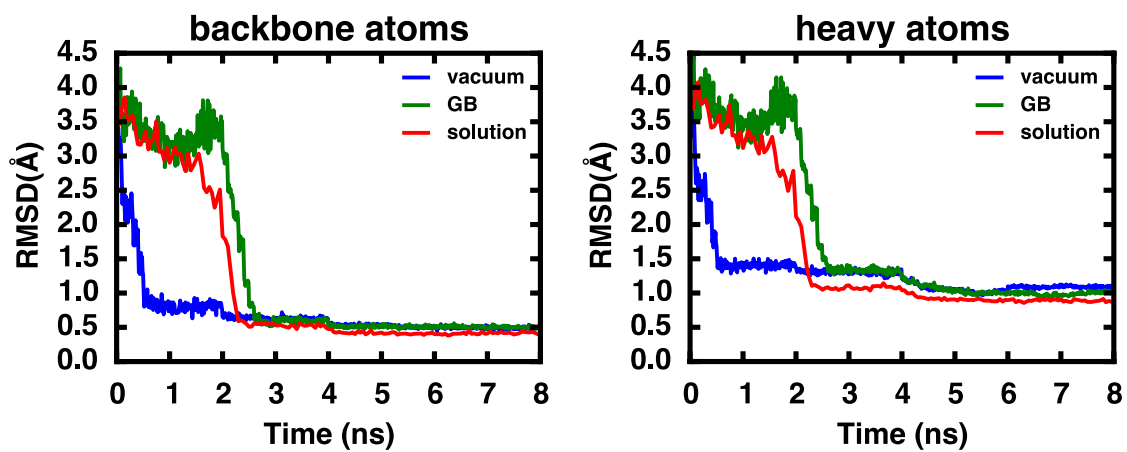


Figure S6. RMSD of the backbone and heavy atoms in RBP xMDFF simulations.

REFERENCES

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2. Trabuco, L. G.; Villa, E.; Schreiner, E.; Harrison, C. B.; Schulten, K. Molecular dynamics flexible fitting: A practical guide to combine cryo-electron microscopy and x-ray crystallography. *Methods* **2009**, *49*, 174-180.
3. Singharoy, A.; Teo, I.; McGreevy, R.; Stone, J. E.; Zhao, J.; Schulten, K. Molecular dynamics-based refinement and validation for sub-5 Å cryo-electron microscopy maps. *Elife* **2016**, *5*.