

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

This research has been conducted using research facilities provided by Computational Chemistry Unit Cell, Department of Chemistry, Faculty of Science, Chulalongkorn University.

3.1.1 Hardware

The high-performance computing cluster “Phoenix” Workstation, personal computer, notebook or laptop

3.1.2 Software

NAMD

NAMD program is a molecular dynamic (MD)³⁶ simulations package for studying macromolecular systems. NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign.

CHARMM

The CHARMM program allows generation and analysis of a wide range of molecular simulations. The most basic kinds of simulation are minimization of a given structure and production runs of a molecular dynamics trajectory. More advanced features include free energy perturbation (FEP), quasi-harmonic entropy estimation, correlation analysis and combined quantum, and molecular mechanics (QM/MM) methods.

Visual molecular dynamics (VMD)

VMD is a 3D molecular visualization program for biological systems.³⁷ The program also provides a built-in function that can be used within scripts for analyzing MD trajectories, produced by the program NAMD. The VMD program developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign.



SSH Secure Shell

Secure Shell (SSH) is a program used for logging into a server or transferring data between machines via secure network.

APBS (Adaptive Poisson-Boltzmann Solver)

APBS (Adaptive Poisson-Boltzmann Solver) is a software package for the numerical solution of the Poisson-Boltzmann equation (PBE) for modeling bio-molecular solvation.

APBS program uses a popular continuum model for describing electrostatic interactions between molecular solutes over a wide range of length scales. The program is available with free of charge (<http://www.poissonboltzmann.org/apbs>).

wordom

Wordom program is a program for efficient analysis of molecular dynamics simulations. Wordom aims at fast manipulation and analysis of individual molecular structures and molecular conformation ensembles. The program is used to analyze secondary structure and distance analysis. Wordom is maintained and developed mainly in the Fanelli Lab at the University of Modena and Reggio Emilia (IT). The program is free software under the GPL license (<http://wordom.sourceforge.net>).

DSSP (Define Secondary Structure of Proteins)

The DSSP (Define Secondary Structure of Proteins) algorithm is the method for assigning secondary structure information to amino acids of protein that given the atomic-resolution coordinates of the protein.³⁸

The DSSP program was designed by Wolfgang Kabsch and Chris Sander. DSSP is free of charge (<http://swift.cmbi.ru.nl/gv/dssp>).

HOLE program

HOLE is a program has been used for visualizing and analysis of the dimension of the ion channel pore.³⁹ The algorithm uses a Monte Carlo simulated annealing procedure to find the best route for a sphere with variable radii to squeeze through the channel. Results can be displayed in a graphical fashion or visualized with most common molecular graphics packages.



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3.2. Methodology.

3.2.1 Model building of the MscL in its closed conformation

A homology model of E.coli MscL channel in its closed conformation (cl-ecoMscL) was constructed based on the crystal structure of M.tuberculosis MscL (PDB code: 2OAR). The cl-ecoMscL model was further refined using PaDSAR approach. The EPR dataset were taken from ΠO_2 and ΠNiEDDA data (Figure 1.10) of 66 SL cysteine mutants obtained in 18:1 dioleoyl-phosphatidylcholine (PC18). The solvent accessibility data of the SL mutants comprising TM1 (residues 14-43) and TM2 (residues 72-107) helices were translated into the pseudoatom EP-types. A list of residue number of EcoMscL associated with an assignment of the EP-types for structure modeling in the closed state conformation is summarized in Table 3.1.

TABLE 3.1 Residue number of EcoMscL with an assignment of the EP-types for close state modeling. The assignment was done by an analysis of ΠO_2 , ΠNiEDDA and ΔH_0^{-1} profiles.

Type* Residue number

Type*	Residue number
EP1	15 16 17 18 19 20 21 22 23 24 25 26 27 28 30 31 34 39 41 43 72 73 76 80 81 84 85 88 89 92 93 94 96 97 98 101 102 107 (n=38)
EP2	14 35 38 74 77 78 104 106 (n=6)
EP3	29 32 33 36 37 40 42 75 79 82 83 86 87 90 91 95 99 100 103 (n=19)
EP4	105 (n=1)

Note: * EP1 = Buried, EP2 = Water-exposed, EP3 = Lipid-exposed, EP4= water-lipid interface

Then the assigned EP- pseudo-atoms were attached to the alpha carbon of the cl-ecoMscL model and 300 oxygen pseudoatom (denoted as OXY) and 300 NiEDDA pseudoatom (denoted as NIC) were added to the system at pre-generated coordinates (Figure 3.1) obtained by MD simulation using CHARMM command scripts.

In the simulation, OXY particles were positioned within the membrane slab whereas NIC particles were in aqueous regions locating both intracellular and extracellular sides of membranes. The united atom CHARMM PARAM19 force field was employed for calculating interaction between protein atoms. The restraint potential for pseudoatom interactions was based on the Lennard-Jones-like potential function as previously described in the literature. During the calculation, the secondary structure of N-terminal, TM1, TM2 and C-terminal helices were restrained. Using the miscellaneous mean field potential in CHARMM, one can control the motion of OXY and NIC particles within a defined boundary as follows

For OXY: $80 \leq x \leq 80$, $80 \leq y \leq 80$ and $-12 \leq z \leq 12$

For NIC: $80 \leq x \leq 80$, $80 \leq y \leq 80$, and either $z \leq -12$ or $z \geq 12$

In addition, PaDSAR treated each model as a single subunit, and refined it using the image facility within the CHARMM program to maintain the five-fold symmetry of the MscL pentamer. In PaDSAR run, several cycles of energy minimization and molecular dynamics simulations with EPR-derived restraints were performed to generate an ensemble of structures.



* EP1 = Buried, EP2 = Water-exposed, EP3 = Lipid-exposed, EP4= water-lipid interface

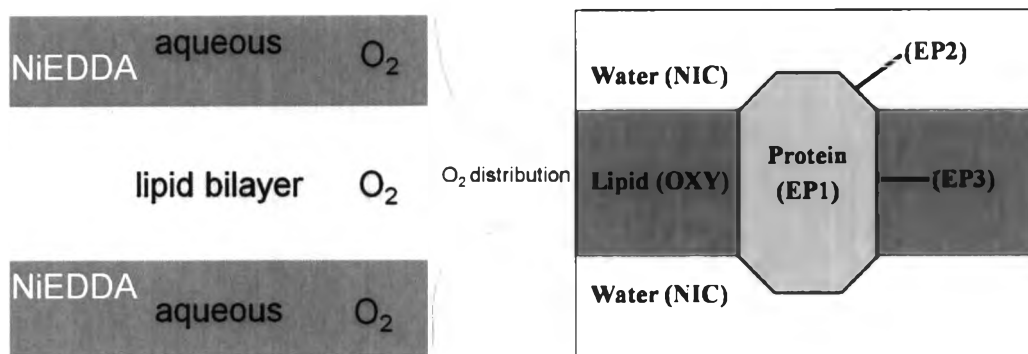


FIGURE 3.1 (Left) Show that the distribution properties of NiEDDA and O₂ in bilayer (NiEDDA, nickel ethylene diamine diacetate is hydrophilic. It cannot partition in lipid membrane and O₂ dissolves in membrane). (Right) Schematic representation for definition of pseudo-atoms used by PaDSAR.



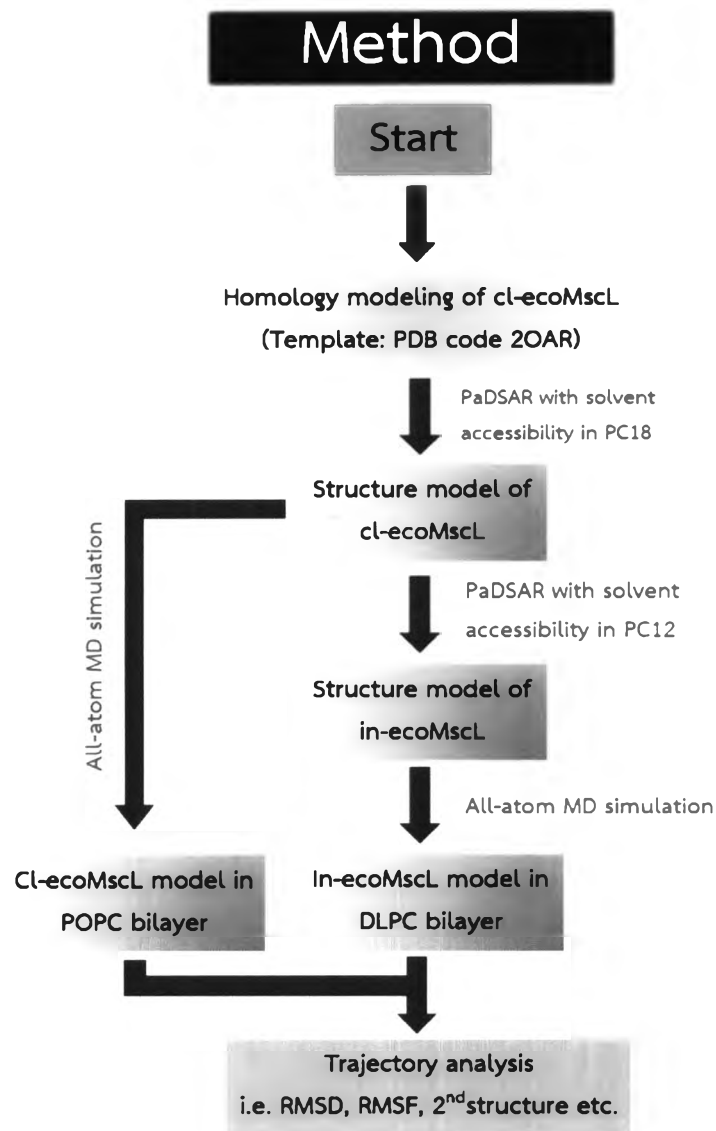


FIGURE 3.2 Flowchart of the method used to generate structural models of EcoMscL in Close and intermediate state respectively and to MD simulation.

3.2.2 Model building of the MscL in an intermediate conformation

The cl-ecoMscL model was taken as a starting conformation for modeling at an intermediate conformation of ecoMscL (in-ecoMscL). The present EPR dataset used for structure modeling of in-ecoMscL were taken from ΠO_2 and $\Pi NiEDDA$ data of the SL cysteine mutants obtained in 12:1 dilauroyl-phosphatidylcholine (PC12). The PC12 type has a shorter acyl chain length compared to PC18 (Figure 3.3). Structure modeling of the in-ecoMscL was performed with the same PaDSAR procedure as described in model building of the cl-ecoMscL. A list of residue number of EcoMscL associated with an assignment of the EP-types for modeling the intermediate conformation is summarized in Table 3.2.

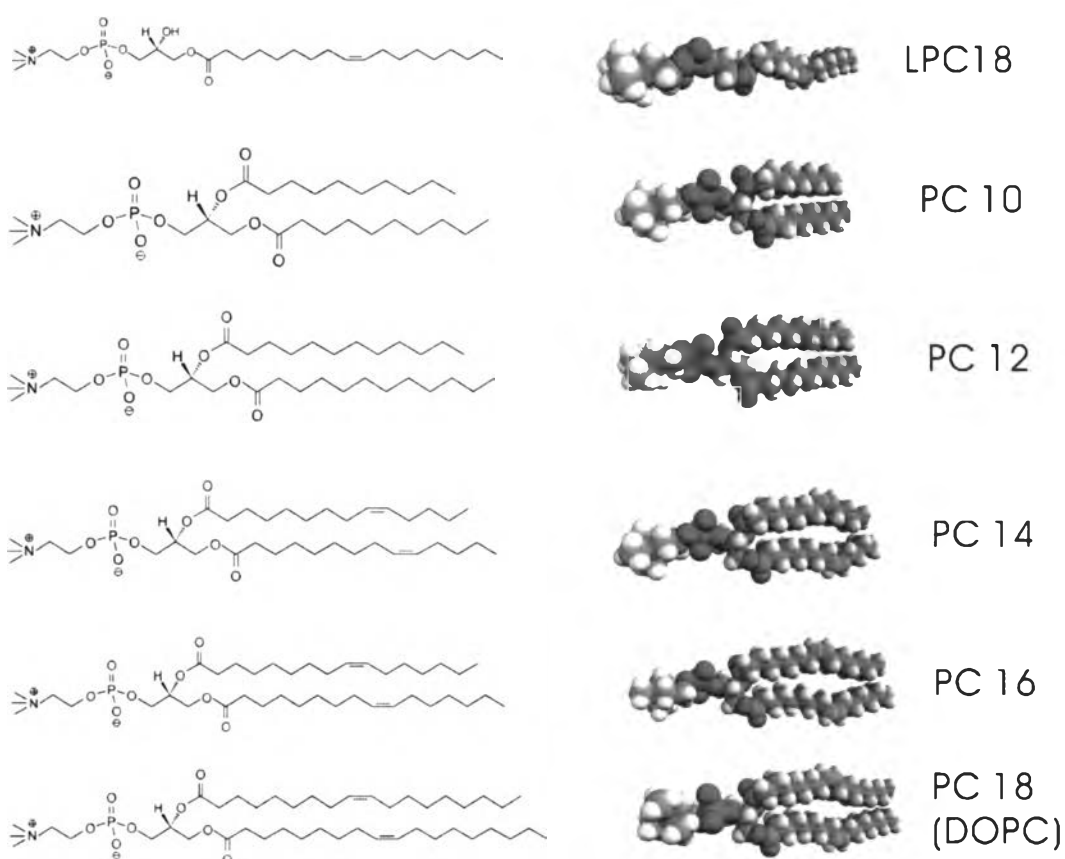


FIGURE 3.3 Structures of Some Lipids

TABLE 3.2 Residue number of EcoMscL with an assignment of the EP-types for the intermediate state modeling. The assignment was done by an analysis of ΠO_2 , $\Pi NiEDDA$ and ΔH_0^{-1} profiles.

Note: * EP1 = Buried, EP2 = Water-exposed, EP3 = Lipid-exposed, EP4= water-lipid interface

Type*	Residue number
EP1	14 15 17 19 21 23 25 27 28 31 32 33 34 35 36 37 40 41 42 43 73 81 85 86 89 90 91 92 93 94 95 96 97 98 100 101 102 107 (n=38)
EP2	26 30 39 74 80 99 104 105 106 (n=9)
EP3	16 18 20 22 24 29 72 75 76 78 79 82 83 84 87 88 103(n=17)
EP4	38 (n=1)

Note: * EP1 = Buried, EP2 = Water-exposed, EP3 = Lipid-exposed, EP4= water-lipid interface



3.2.3 Molecular dynamics simulations

To investigate structure and dynamics properties of the cl-ecoMscL and in-ecoMscL models, molecular dynamics simulations of the protein-membrane system were performed. The structure model of cl-ecoMscL was embedded in a palmitoyl oleoyl phosphatidyl cholines (POPC) membrane bilayer and TIP3P waters. For in-ecoMscL, DLPC (dilauroylglycero-phosphocholines), the lipid model for PC12 was used in the simulation. Side-chain ionization states that were expected at pH 7 were assigned based on pKa calculations using PROPKA. The pre-equilibrated patch of POPC membrane was built from VMD membrane builder plugin whereas DLPC membrane was initially built from Membrane Builder Module available at www.charmm-gui.org. The protein was inserted into membrane patch by removing the lipid and water molecule that overlap with protein.

MD simulation of two models were carried out using the NAMD2.7b program. CHARMM 22 and CHARMM 27 were used as a force field parameter for proteins and lipids, respectively. MD simulation were carried out with a periodic cell (Figure 3.4). The two systems was run at constant temperature of 300 K and constant pressure of 1 atm. Langevin dynamics (LD) was used for controlling the temperature of the system and the system was controlled the pressure by using a No se-Hoove Langevin Piston respectively. Energy minimizations and restrained MD simulations were employed to relax geometric, angle, torsional and other structural strains of the two models systems. Finally, each MD simulation was performed for 0.5ns with an integration time step of 2fs. The preparation protocol as described above was processed in relax the conformation, remove bad contacts due to steric overlap, and permit a proper assembly of lipids, water, ions and the protein. The MD simulations of the two systems were performed for 100 ns with an integration time steps of 2 fs.



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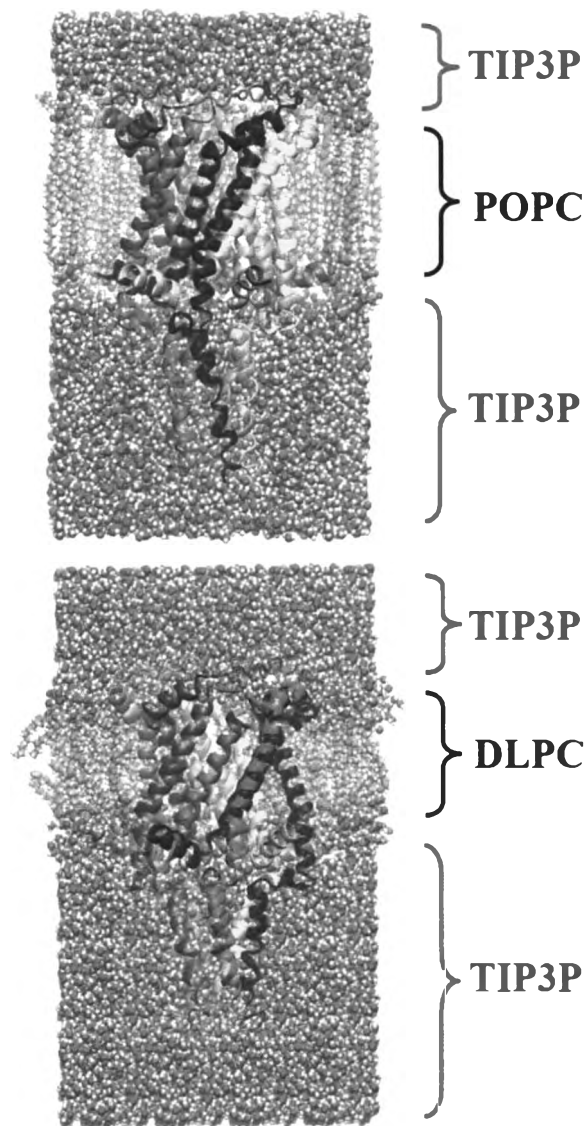


FIGURE 3.4 The membrane proteins of two system. (Up), Palmitoyl oleoyl phosphatidyl Choline (POPC) are membrane bilayer for PC18 and (Down), Dilauroylglycero-phosphocholine (DLPC) for PC12 and TIP3P are force field parameter for water molecules.

3.2.4 Analysis of MD trajectory

Production run of the simulations was analyzed to examine structure and dynamics properties of the simulated systems. The result of all MD simulation were analyzed including Root Mean Square Deviation (RMSD), RMSF, secondary structure analysis, inter-atomic distances, helix-tilting angle etc. RMSD is used to monitor to stability of the structure of protein during MD simulation times. RMSF allows to evaluate the flexibility of the protein. Secondary structure element of protein structure was characterized based on DSSP algorithm. Distances between residues located near the pore were extracted. Analyzing the trajectories of MD simulation and graphical representation of protein structures and membrane protein systems was primarily obtained using the VMD program.

