CHAPTER IV
CONCLUSION

Pseudoatoms Driven Solvent Accessibility Refinement or PaDSAR is the method incorporating the molecular dynamics simulation with the experimental EPR/SDSL data for refine membrane protein structures. In this work the PaDSAR approach has been implemented on the program NAMD. A test of NAMD-PaDSAR for its computational efficiency has been conducted by refolding the known 3D membrane protein structure from the KcsA potassium channel. It has demonstrated that the modified NAMD-PaDSAR gave the results comparable to those of original CHARMM-PaDSAR version.

NAMD-PaDSAR requires only two initial script files *run.tcl* and *res.tcl*. All simulation parameters were assigned in *run.tcl* once at first and execute *run.tcl* to generate basic files needed for running simulation. These script commands can be applied to a variety of membrane protein systems which contains either single or multiple subunits.

Additionally, in NAMD-PaDSAR, the extra command "fix atoms" may be given in the NAMD configuration file (input parameters for MD) to restrain the motion of some atoms or residues within desired positions during the simulation. In addition, it is used to constraint the secondary structure element as rigid helix or beta-sheet. Lastly, *tclBC* command is employed to maintain the distribution of OXY and NIC pseudoatoms within the water or membrane boundary. It was found that the modification of tclBC by adding powerful commands *cleardrops* and *dropatoms* to update the atom list for calculating the interactions between atom pairs can effectively reduce the simulation time. The results show for KcsA protein a total number of 300 OXY and 300 NIC atoms presents the optimum results in terms of CPU time and RMSD values. Analysis of the pseudoatom RDF plots revealed that the simulation with NAMD-PaDSAR are capable of maintaining membrane and non-membrane parts of protein structure with the orientation of the pseudoatom being accessible by appropriate solvent environment. By taking into account the flexibility of the nitroxide sidechain, this study also shows that the modification of the force constant for the improper dihedral angle of pseudoatom (K_{imp}) can improve the quality of structure refinement. It was found that K_{imp} = 10 kcal mol^{-1} rad^{-2} appears to give the best results for refolding the decoys back to the native KcsA conformation.

For the large decoy data set, Steered MD was used to generate 524 decoys of distorted KcsA conformation having RMSD 3 – 13Å. It was found that 433 out of 524

(82%) decoys can be refolded with RMSD < 3Å relative to the native structure. It is worth nothing that in NAMD-PaDSAR the percent of the correct folds increases if the decoy has the initial RMSD < 6Å away from the target structure. However, this study also demonstrates that NAMD-PaDSAR is able to refine decoy structure with RMSD as large as 10Å to the correct fold with considerable rate of success.

To make the NAMD-PaDSAR more reliable. Increasing number of samples and testing different kind of proteins are needed. Using NAMD-PaDSAR to refine the proteins structure without the knowing the native from is a challenge to be studied in the near future.