# Molecular dynamics simulation of membrane proteins

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## ABSTRACT

Membrane proteins including transporters, channels and receptors, play crucial roles in biological systems by controlling the traffic across cell membranes and mediating signal transduction. Determining the structure and function mechanism of these proteins has always been a challenge. Molecular dynamics simulation techniques with recent significant improvements can capture the behavior of biological molecules in atomic detail, shedding light on the biomolecular mechanisms which are difficult to observe experimentally. Despite the increasing application of molecular dynamics simulations on this subject, there are some concerning drawbacks in these techniques including inadequate sampling timescales and required approximations which can lead to results that are inconsistent with experimental data. Here, with specific examples, molecular dynamics studies on membrane proteins and the accompanying challenges will be discussed.

## INTRODUCTION

Membrane proteins including transporters, channels and receptors, play crucial roles in biological systems by controlling the traffic across cell membranes and mediating signal transduction. Determining the structure and function mechanism of these proteins has always been a challenge [1, 2].

Ion channels are water filled holes in the membrane which provide a pathway for passive diffusion of ions through membrane. Transporters on the other hand, use energy for transporting ions and other substrates by more complex mechanisms [1]. Receptor proteins mediate the signal transduction through cell membrane mostly by conformational changes [2].

Some experimental techniques can provide information about the dynamics of proteins but they are usually limited in terms of space and time resolutions. An interesting alternative way is to computationally model atoms and molecules motions. The common computational method to study biological macromolecules is all-atom molecular dynamics (MD) simulation [3].

In MD simulations, positions and velocities of atoms are computed using Newton's laws of motion. Although MD simulation suffers from some limitations, it can provide a sufficiently close approximation to capture a broad range of biochemical processes which makes it possible to explore structure, dynamics and function of biomolecules [3].

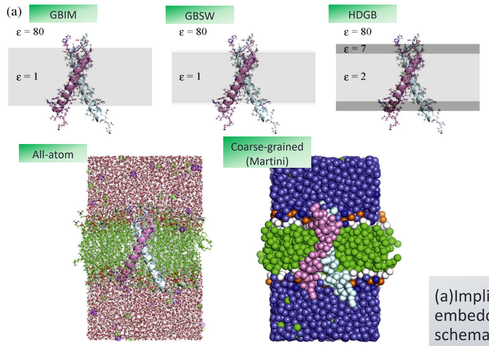
After conducting a simulation with new results, a question arises as to how accurate these results are and whether they can be trusted. As a case, membrane-active antimicrobial peptides have been subjected to countless simulation studies, because of challenges accompanying the experimental studies on peptides in membrane. Diverse results and variety of proposed mechanisms, arises the question about the precision. Interestingly, to this date, little efforts have been made to clarify this issue [4].

## DISCUSSION

### MD simulation of membrane systems

MD simulation is a widely used computational tool for investigating membranes and membrane proteins. Despite of ongoing advances, because of limited time scales, many biomembrane phenomena are still challenging to be simulated by all-atom simulations. The transition time between functional states of proteins in membrane are too long to be simulated. To overcome this issue two main approaches have been implemented: (1) using simpler molecular models like implicit models for membrane system. (2) using efficient conformational sampling methods such as Temperature replica-exchange molecular dynamics [5].

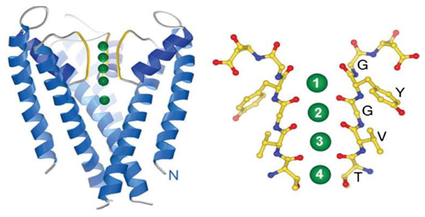
## DISCUSSION




(a)Implicit and explicit models of membrane with embedded glycophorine A as an example. (b) schematic illustration of replica-exchange (Temperature-REMD)

### Ion channels

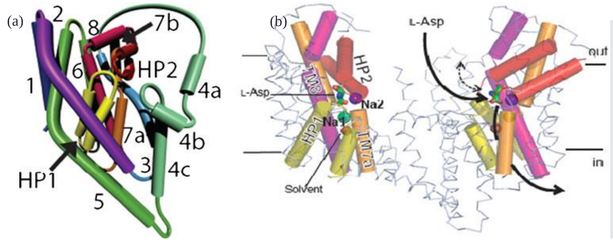
Low dielectric field of membrane environment makes it very difficult to crystallize membrane-embedded proteins. Bacterial KcsA potassium channel was the first crystallized channel (1998). Therefore little work has been conducted on modeling of ion channels before 1998. Since then structure of some other channels have been determined. Many groups performed MD simulations on potassium channels. Quantitative description of channel selectivity and permeation features are the main challenges. With the help of new developed force fields some groups were able to obtain quantitative results for KcsA and mammalian K v1.2 channels which were consistent with experimental data [1].




Crystal structure of KcsA potassium channel(only 2 monomers of the tetramer structure). Carbonyl oxygens (red) form $K^+$ binding sites (green).

### Transporters

Structure and function of transporters are much more complex than ion channels which have been reflected in the progress made in their field research. After determination of crystal structures several groups worked on MD simulation of transporters like BtuCD (Vitamin B12 ABC transporter) and $Glt_{ph}$ (aspartate secondary active transporter). For each substrate $Glt_{ph}$ transports three $Na^+$ but only 2 ion binding site were revealed in the crystal structure. After conducting MD simulations, several binding sites were suggested. Mutagenesis experiment confirmed the site formed by N310, D312 on TM7 and Y89, T92, S93 on TM8 is the third binding site. Furthermore MD simulations were able to the binding free energies and order of ion and Asp [1].

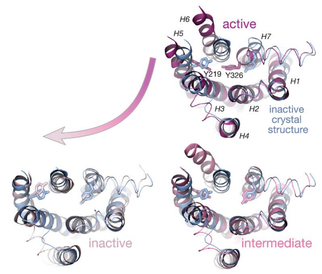



(a)Topology illustration of a single monomer $Glt_{ph}$ (b) Two monomers of in $Glt_{ph}$ the membrane. HP2 (cylinder) serves as a gate. $Na^+$ serves as a lock to this gate.

## DISCUSSION

### GPCRs

Until a few years ago there was not much data on structures of GPCRs. Since then many groups performed MD simulation to investigate the structure and function of GPCRs. Because of their importance and complexity, many of the longest simulations have been performed on GPCRs. Combination of experiments and MD simulations on GPCRs can provide a better understanding of their biology and function. For example, this techniques makes it possible to observe intermediate states which are not easy to capture by experimental methods only. As a case, Dror *et al.* studied the activation mechanism of β2 adrenergic receptor (β-2AR) and revealed that G protein is necessary to achieve the fully active receptor. Their results were confirmed by NMR and further MD studies [2].




The β-2AR G-protein-binding site adopts three major conformations: active, inactive and a previously unobserved intermediates. Each conformations is superimposed on the inactive crystal structure

### How reliable MD simulations are?

Studies indicate that simulation results are highly depend on force field parameters, therefore experimental verification is necessary. On the other hand, conformational fluctuations and changes, ion permeation and other activities in biomembrane environment happen at multi-micro second time scales which are not easily accessible using all-atom MD simulations by conventional hardware. In a recent study, MD simulations were performed on membrane active antimicrobial peptides using different force fields which yielded quite different results. These results emphasize on the necessity of validation for MD simulations [4].

## CONCLUSIONS

By providing an atomic level microscope, and combined with experiments MD simulation can serve as powerful tool to study complex dynamics of biomembrane environment. Although there are still challenges in application, molecular dynamics tools have provided undeniable help in understanding behaviour and structure of membrane proteins and by continues improvement of hardware, software and algorithms, they can play a more substantial role in the future.

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