Modeling and Molecular Dynamics of Membrane Proteins

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Why Do Living Cells Need Membrane

- Living cells also need to exchange materials and information with the outside world

... however, in a highly selective manner.
Phospholipid Bilayers Are Excellent Materials For Cell Membranes

- Hydrophobic interaction is the driving force
- Self-assembly in water
- Tendency to close on themselves
- Self-sealing (a hole is unfavorable)
- Extensive: up to millimeters
Lipid Diffusion in a Membrane

Lateral diffusion

\[ D_{\text{lip}} = 10^{-8} \text{ cm}^2 \text{ s}^{-1} \]

(50 Å in \( \sim 5 \times 10^{-6} \) s)

\[ D_{\text{wat}} = 2.5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \]

Modeling mixed lipid bilayers!

Tranverse diffusion (flip-flop)

Once in several hours!

(\( \sim 50 \) Å in \( \sim 10^4 \) s)

\~9 orders of magnitude slower ensuring bilayer asymmetry
Fluid Mosaic Model of Membrane

Lateral Diffusion Allowed

Flip-flop Forbidden

Ensuring the conservation of membrane asymmetric structure
Technical difficulties in Simulations of Biological Membranes

- **Time scale**
- **Heterogeneity of biological membranes 😞**

60 x 60 Å
Pure POPE
5 ns
~100,000 atoms
Coarse-grained modeling of lipids

150 particles

9 particles!

Also, increasing the time step by orders of magnitude.
by: J. Siewert-Jan Marrink and Alan E. Mark, University of Groningen, The Netherlands
Protein/Lipid ratio

- Pure lipid: insulation (neuronal cells)
- Other membranes: on average 50%
- Energy transduction membranes (75%)
  Membranes of mitochondria and chloroplast
  Purple membrane of halobacteria

- Different functions = different protein composition
Protein / Lipid Composition

The purple membrane of halobacteria
Gramicidin A
Might be very sensitive to the lipid head group electrostatic and membrane potential
Analysis of Molecular Dynamics Simulations of Biomolecules

• A very complicated arrangement of hundreds of groups interacting with each other
• Where to start to look at?
• What to analyze?
• How much can we learn from simulations?

It is very important to get acquainted with your system
Aquaporins of known structure:

- **GLpF** - E. coli glycerol channel (aquaglycerolporin)
- **AQP1** - Mammalian aquaporin-1 (pure water channel)

AqpZ and AQP0 (2004)
Functionally Important Features

- Tetrameric architecture
- Amphipatic channel interior
- Water and glycerol transport
- Protons, and other ions are excluded
- Conserved asparagine-proline-alanine residues; NPA motif
- Characteristic half-membrane spanning structure

~100% conserved -NPA- signature sequence
A Semi-hydrophobic channel
Molecular Dynamics Simulations

Protein: ~ 15,000 atoms
Lipids (POPE): ~ 40,000 atoms
Water: ~ 51,000 atoms
Total: ~ 106,000 atoms

NAMD, CHARMM27, PME
NpT ensemble at 310 K
1ns equilibration, 4ns production
10 days/ns - 32-proc Linux cluster
3.5 days/ns - 128 O2000 CPUs
0.35 days/ns - 512 LeMieux CPUs
Protein Embedding in Membrane

Hydrophobic surface of the protein

Ring of Tyr and Trp
Embedding GlpF in Membrane

77 Å
A Recipe for Membrane Protein Simulations

• Align the protein along the z-axis (membrane normal): OPM, Orient.

• Decide on the lipid type and generate a large enough patch (MEMBRANE plugin in VMD, other sources). Size, area/lipid, shrinking.

• Overlay the protein with a hydrated lipid bilayer. Adjust the depth/height to maximize hydrophobic overlap and matching of aromatic side chains (Trp/Tyr) with the interfacial region

• Remove lipids/water that overlap with the protein. Better to keep as many lipids as you can, so try to remove clashes if they are not too many by playing with the lipids. Add more water and ions to the two sides of the membrane (SOLVATE / AUTOIONIZE in VMD)

• **Constrain** (not **FIX**) the protein (we are still modeling, let’s preserve the crystal structure; fix the lipid head groups and water/ion and minimize/simulate the lipid tails using a short simulation.)
A Recipe for Membrane Protein Simulations

• Continue to constrain the protein (heavy atoms), but release everything else; minimize/simulate using a short “constant-pressure” MD (NPT) to “pack” lipids and water against the protein and fill the gaps introduced after removal of protein-overlapping lipids.

• Watch water molecules; They normally stay out of the hydrophobic cleft. If necessary apply constraints to prevent them from penetrating into the open cleft between the lipids and the protein.

• Monitor the volume of your simulation box until the steep phase of the volume change is complete (.xst and .xsc files). Do not run the system for too long during this phase (over-shrinking; sometimes difficult to judge).

• Now release the protein, minimize the whole system, and start another short NPT simulation of the whole system.

• Switch to an NP_nAT or an NVT simulation, when the system reaches a stable volume. Using the new CHARMM force field, you can stay with NPT.
Lipid-Protein Packing During the Initial NpT Simulation
Adjustment of Membrane Thickness to the Protein Hydrophobic Surface
Glycerol-Saturated GlpF
Description of full conduction pathway
Complete description of the conduction pathway

Constriction region

Selectivity filter
Channel Hydrogen Bonding Sites

... 

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animate goto $frame
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lappend [$donor get index] list1
set acceptor [atomselect top "rename GCL and name O and within 2 of (protein and name HN HO)]
lappend [$acceptor get index] list2

} 

...
## Channel Hydrogen Bonding Sites

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Note: The table lists the atoms involved in hydrogen bonding and their respective positions in the molecules.
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The Substrate Pathway is formed by \( C=O \) groups
Non-helical motifs are stabilized by two glutamate residues.

The Substrate Pathway is formed by $\text{C=O}$ groups.
Conservation of Glutamate Residue in Human Aquaporins
Glycerol – water competition for hydrogen bonding sites
Revealing the Functional Role of Reentrant Loops
AqpZ vs. GlpF

- Both from *E. coli*
- AqpZ is a pure water channel
- GlpF is a glycerol channel
- We have high resolution structures for both channels
Steered Molecular Dynamics is a non-equilibrium method by nature

- A wide variety of events that are inaccessible to conventional molecular dynamics simulations can be probed.

- The system will be driven, however, away from equilibrium, resulting in problems in describing the energy landscape associated with the event of interest.

\[ W \geq \Delta G \]  

Second law of thermodynamics
In principle, it is possible to obtain free energy surfaces from repeated non-equilibrium experiments.
Steered Molecular Dynamics

**constant force**
(250 pN)

**constant velocity**
(30 Å/ns)
SMD Simulation of Glycerol Passage

Trajectory of glycerol pulled by constant force
Constructing the Potential of Mean Force

4 trajectories

\( v = 0.03, 0.015 \, \text{Å/ps} \)

\( k = 150 \, \text{pN/Å} \)

\[
f(t) = -k[z(t) - z_0 - vt]
\]

\[
W(t) = \int_0^t dt' \, vf(t')
\]
- Captures major features of the channel
- The largest barrier ≈ 7.3 kcal/mol; exp.: 9.6±1.5 kcal/mol

Features of the Potential of Mean Force

$e^{-\Delta G/k_B T} = \langle e^{-W/k_B T} \rangle$

Asymmetric Profile in the Vestibules

Artificial induction of glycerol conduction through AqpZ

Three fold higher barriers

AqpZ  22.8 kcal/mol
GlpF    7.3 kcal/mol

Could it be simply the size?

It is probably just the size that matters!

Water permeation

18 water conducted
In 4 monomers in 4 ns
1.125 water/monomer/ns
Exp. = ~ 1-2 /ns

5 nanosecond Simulation

7-8 water molecules in each channel
The single file of water molecules is maintained.
Diffusion of Water in the channel

One dimensional diffusion: \[ 2Dt = \langle (z_t - z_0)^2 \rangle \]

Experimental value for AQP1: 0.4-0.8 e-5

D = \frac{\text{slope}}{2} = 0.046 \text{ Å}^2/\text{ps} = 0.46 \times 10^{-5} \text{ cm}^2/\text{s}
Diffusion of Water in the channel

\[ 2Dt = \left\langle (z_t - z_0)^2 \right\rangle \]

Graph showing the relation between MSD (A^2) and time (ps), with the diffusion coefficient \( D \) calculated as \( D = \frac{\text{slope}}{2} = 0.046 \text{ Å}^2/\text{ps} = 0.46 \times 10^{-5} \text{ cm}^2/\text{s} \).

Improvement of statistics
Water Bipolar Configuration in Aquaporins
Water Bipolar Configuration in Aquaporins
One of the most useful advantages of simulations over experiments is that you can modify the system as you wish: You can do modifications that are not even possible at all in reality!

This is a powerful technique to test hypotheses developed during your simulations. Use it!
Electrostatic Stabilization of Water Bipolar Arrangement
Proton transfer through water
**Cl⁻ channel**

Anti-parallel

**K⁺ channel**

Parallel (barrel stave)

**Aquaporins**
"Surprising and clearly not a hydrophobic channel"

A Repulsive Electrostatic Force at the Center of the Channel

QM/MM MD of the behavior of an excessive proton