Lipid Bilayers Are Excellent 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 Membrane

\[ D_{\text{lip}} = 10^{-8} \text{ cm}^2\cdot\text{s}^{-1} \]
\[ D_{\text{wat}} = 2.5 \times 10^{-5} \text{ cm}^2\cdot\text{s}^{-1} \]

Lateral diffusion
\[ D = 1 \mu\text{m}^2\cdot\text{s}^{-1} \]
50 Å in \( \sim 2.5 \times 10^{-5} \text{ s} \)

~9 orders of magnitude difference

Once in several hours!
(\(10^4 \text{ s}\))
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 grain 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
Lipid Bilayers Are Excellent 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 Membrane

Lateral diffusion

\[ D = 1 \ \mu m^2.s^{-1} \]

50 Å in \( \sim 2.5 \times 10^{-5} \) s

\(~9\) orders of magnitude difference

Tranverse diffusion (flip-flop)

\[ D_{\text{lip}} = 10^{-8} \ cm^2.s^{-1} \]
\[ D_{\text{wat}} = 2.5 \times 10^{-5} \ cm^2.s^{-1} \]

Once in several hours! \((10^4 \ s)\)
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 grain 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
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
Membrane water channels
Lipid Bilayer Permeability

Water is an exception:

- Small size
- Lack of charge
- Its high concentration
Water Transport Across Cell Membrane

Always passive; bidirectional; osmosis-driven

• Diffusion through lipid bilayers

  slower, but enough for many purposes

• Channel-mediated

  Large volumes of water needed to be transported
  (kidneys).

  Fast adjustment of water concentration is necessary
  (RBC, brain, lung).
# Aquaporins in Human Body

<table>
<thead>
<tr>
<th>Aquaporin</th>
<th>Location/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquaporin-0</td>
<td>Eye: lens fiber cells</td>
</tr>
<tr>
<td>Aquaporin-1</td>
<td>Red blood cells, Kidney: proximal tubules, Eye: ciliary epithelium, Brain: choroid plexus, Lung: alveolar</td>
</tr>
<tr>
<td>Aquaporin-2</td>
<td>Epithelial cells, Kidney: collecting ducts</td>
</tr>
<tr>
<td>Aquaporin-3</td>
<td>Kidney: collecting ducts, Trachea: epithelial cells</td>
</tr>
<tr>
<td>Aquaporin-5</td>
<td>Epithelium, Salivary glands, Lacrimal glands</td>
</tr>
<tr>
<td>Aquaporin-6</td>
<td>Kidney</td>
</tr>
<tr>
<td>Aquaporin-7</td>
<td>Testis and sperm</td>
</tr>
<tr>
<td>Aquaporin-8</td>
<td>Testis, pancreas, liver</td>
</tr>
<tr>
<td>Aquaporin-9</td>
<td>Leukocytes</td>
</tr>
</tbody>
</table>

Additional members are suspected to exist.
High Permeation to Water

>200 Liters Water Everyday!
Monomeric pores
Water, glycerol, ...

Tetrameric pore
Perhaps ions???

Aquaporins of known structure:

GlpF - E. coli glycerol channel (aquaglyceroporin)
AQP1 - Mammalian aquaporin-1 (pure water channel)
AqpZ and AQPO (2004)
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
GlpF in VMD
A Recipe for Membrane Protein Simulations

• Insert your protein into a hydrated lipid bilayer.

• Fix the protein; minimize the rest and run a short “constant-pressure” MD to bring lipids closer to the protein and fill the gap between the protein and lipids.

• Watch water molecules; if necessary apply constraints to prevent them from penetrating into the open gaps between lipids and the protein.

• Monitor the volume of your simulation box until it is almost constant. Do not run the system for too long during this phase.

• Now release the protein, minimize the whole system, and start an NpT simulation of the whole system.

• If desired, you may switch to an NVT simulation, when the system reaches a stable volume.
Lipid-Protein Packing During the Initial NpT Simulation
Adjustment of Membrane Thickness to the Protein Hydrophobic Surface
An extremely stable protein
Glycerol-Saturated GlpF
Complete description of the conduction pathway

Constriction region

Selectivity filter
Details of Protein-Substrate Interaction Are Always Important

- Identify those groups of the protein that are directly involved in the main function of the protein.
- Look at the interaction of these primary residues with other groups in the protein.
- Look at buried charged residues inside the protein; they must have an important role.
- Backbone hydrogen bonds are mainly responsible for stabilization of secondary structure elements in the protein; side chain hydrogen bonds could be functionally important.
Channel Hydrogen Bonding Sites

```bash
{set frame 0}{frame < 100}{incr frame}{
    animate goto $frame
    set donor [atomselect top "name O N and within 2 of (resname GCL and name HO)"]
lappend [$donor get index] list1
    set acceptor [atomselect top "resname GCL and name O and within 2 of (protein and name HN HO)"]
lappend [$acceptor get index] list2
}
```

...
## Channel Hydrogen Bonding Sites

<table>
<thead>
<tr>
<th>Residue</th>
<th>Bonding Partner</th>
<th>Atom</th>
<th>Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN</td>
<td>OE1 NE2</td>
<td>LEU</td>
<td>197</td>
</tr>
<tr>
<td>TRP</td>
<td>O NE1</td>
<td>THR</td>
<td>198</td>
</tr>
<tr>
<td>GLY</td>
<td>O</td>
<td>GLY</td>
<td>199</td>
</tr>
<tr>
<td>ALA</td>
<td>O</td>
<td>PHE</td>
<td>200</td>
</tr>
<tr>
<td>HIS</td>
<td>O ND1</td>
<td>ALA</td>
<td>201</td>
</tr>
<tr>
<td>LEU</td>
<td>O</td>
<td>ASN</td>
<td>203</td>
</tr>
<tr>
<td>ASN</td>
<td>ND2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP</td>
<td>OD1</td>
<td>LYS</td>
<td>33</td>
</tr>
<tr>
<td>GLY</td>
<td>O</td>
<td>GLN</td>
<td>41</td>
</tr>
<tr>
<td>SER</td>
<td>O</td>
<td>TRP</td>
<td>48</td>
</tr>
<tr>
<td>TYR</td>
<td>O</td>
<td>HIS</td>
<td>66</td>
</tr>
<tr>
<td>PRO</td>
<td>O N</td>
<td>ASN</td>
<td>68</td>
</tr>
<tr>
<td>ASN</td>
<td>OD1 ND2</td>
<td>TYR</td>
<td>138</td>
</tr>
<tr>
<td>HIS</td>
<td>ND1</td>
<td>ASN</td>
<td>140</td>
</tr>
<tr>
<td>THR</td>
<td>OG1</td>
<td>HIS</td>
<td>142</td>
</tr>
<tr>
<td>GLY</td>
<td>O</td>
<td>GLY</td>
<td>199</td>
</tr>
<tr>
<td>PRO</td>
<td>O</td>
<td>ASN</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ARG</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Channel Hydrogen Bonding Sites

<table>
<thead>
<tr>
<th>Residue</th>
<th>Position</th>
<th>Hydrogen Bonding Partner</th>
<th>Residue</th>
<th>Position</th>
<th>Hydrogen Bonding Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN</td>
<td>41</td>
<td>OE1 NE2</td>
<td>LEU</td>
<td>197</td>
<td>O</td>
</tr>
<tr>
<td>TRP</td>
<td>48</td>
<td>O NE1</td>
<td>THR</td>
<td>198</td>
<td>O</td>
</tr>
<tr>
<td>GLY</td>
<td>64</td>
<td>O</td>
<td>GLY</td>
<td>199</td>
<td>O</td>
</tr>
<tr>
<td>ALA</td>
<td>65</td>
<td>O</td>
<td>PHE</td>
<td>200</td>
<td>O</td>
</tr>
<tr>
<td>HIS</td>
<td>66</td>
<td>O ND1</td>
<td>ALA</td>
<td>201</td>
<td>O</td>
</tr>
<tr>
<td>LEU</td>
<td>67</td>
<td>O</td>
<td>ASN</td>
<td>203</td>
<td>ND2</td>
</tr>
<tr>
<td>ASN</td>
<td>68</td>
<td>ND2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP</td>
<td>130</td>
<td>OD1</td>
<td>LYS</td>
<td>33</td>
<td>HZ1 HZ3</td>
</tr>
<tr>
<td>GLY</td>
<td>133</td>
<td>O</td>
<td>GLN</td>
<td>41</td>
<td>HE21</td>
</tr>
<tr>
<td>SER</td>
<td>136</td>
<td>O</td>
<td>TRP</td>
<td>48</td>
<td>HE1</td>
</tr>
<tr>
<td>TYR</td>
<td>138</td>
<td>O</td>
<td>HIS</td>
<td>66</td>
<td>HD1</td>
</tr>
<tr>
<td>PRO</td>
<td>139</td>
<td>O N</td>
<td>ASN</td>
<td>68</td>
<td>HD22</td>
</tr>
<tr>
<td>ASN</td>
<td>140</td>
<td>OD1 ND2</td>
<td>TYR</td>
<td>138</td>
<td>HN</td>
</tr>
<tr>
<td>HIS</td>
<td>142</td>
<td>ND1</td>
<td>ASN</td>
<td>140</td>
<td>HN HD21 HD22</td>
</tr>
<tr>
<td>THR</td>
<td>167</td>
<td>OG1</td>
<td>HIS</td>
<td>142</td>
<td>HD1</td>
</tr>
<tr>
<td>GLY</td>
<td>195</td>
<td>O</td>
<td>GLY</td>
<td>199</td>
<td>HN</td>
</tr>
<tr>
<td>PRO</td>
<td>196</td>
<td>O</td>
<td>ASN</td>
<td>203</td>
<td>HN HD21 HD22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ARG</td>
<td>206</td>
<td>HE HH21 HH22</td>
</tr>
</tbody>
</table>
The Substrate Pathway is formed by $C=O$ groups.
Non-helical motifs are stabilized by two glutamate residues.

The Substrate Pathway is formed by $C=O$ groups.
Single Glycerol per channel
Note that glycerols moved, but not as extensively as earlier! We need to enforce an entire conduction event.
Steered Molecular Dynamics

constant force
(250 pN)

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

Trajectory of glycerol pulled by constant force
Evidence for **Stereoselectivity of Glycerol**

Cannot be verified by experimental measurements
Free Energy Calculation in SMD

**SMD simulation**

a non-equilibrium process

\[ \Delta G \leq \left\langle W \right\rangle \]

One needs to discount irreversible work

\[ e^{-\Delta G / k_BT} = \left\langle e^{-W / k_BT} \right\rangle \]

Jarzynski, *PRL* 1997
Hummer, *PNAS, JCP* 2001
Liphardt, et al., *Science* 2002
Constructing the Potential of Mean Force

4 trajectories

\( v = 0.03, \ 0.015 \ \text{Å/ps} \)

\( k = 150 \ \text{pN/Å} \)

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

\[
W(t) = \int_0^t dt' \ v f(t')
\]
• Captures major features of the channel
• The largest barrier $\approx 7.3$ kcal/mol; exp.: $9.6\pm1.5$ kcal/mol
Asymmetry of the Potential of Mean Force

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

Asymmetric Profile in the Vestibules

Theoretical Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign

phosphorylation
Glycerol-Free GlpF
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
**Diffusion of Water in the channel**

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

Experimental value for AQP1: $0.4-0.8 \text{ e}^{-5}$
channel region (20 Å)

Selectivity Filter

NPA
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