Molecular Dynamics Simulation of Membrane Channels

Part II. Structure-Function Relationship and Transport in Aquaporins

Emad Tajkhorshid
Beckman Institute, UIUC

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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).
The Aquaporin Superfamily

GLP cluster

Glycerol transport

Water transport

Discovered in 1992

## Aquaporins in Human Body

<table>
<thead>
<tr>
<th>Aquaporin</th>
<th>Organs/Cells</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquaporin-0</td>
<td>Eye: lens fiber cells</td>
<td>Fluid balance of the lens</td>
</tr>
<tr>
<td>Aquaporin-1</td>
<td>Red blood cells, Kidney: proximal tubules, Eye: ciliary epithelium, Brain: choroid plexus, Lung: alveolar epithelial cells</td>
<td>Osmotic protection, Concentration of urine, Aqueous humor, Production of CSF, Alveolar hydration</td>
</tr>
<tr>
<td>Aquaporin-2</td>
<td>Kidney: collecting ducts</td>
<td>ADH hormone activity</td>
</tr>
<tr>
<td>Aquaporin-3</td>
<td>Kidney: collecting ducts, Trachea: epithelial cells</td>
<td>Reabsorption of water, Secretion of water</td>
</tr>
<tr>
<td>Aquaporin-5</td>
<td>Salivary glands, Lacrimal glands</td>
<td>Production of saliva, Production of tears</td>
</tr>
<tr>
<td>Aquaporin-6</td>
<td>Kidney</td>
<td>Very low water permeability!</td>
</tr>
<tr>
<td>Aquaporin-7</td>
<td>Testis and sperm</td>
<td></td>
</tr>
<tr>
<td>Aquaporin-8</td>
<td>Testis, pancreas, liver</td>
<td></td>
</tr>
<tr>
<td>Aquaporin-9</td>
<td>Leukocytes</td>
<td></td>
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<tr>
<td>Aquaporin-10</td>
<td></td>
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</tbody>
</table>

Additional members are suspected to exist.
Aquaporins in the Kidney

- Urine concentration
- Reabsorption of water into blood
- ADH activity

Kidney → Cortex → Nephron → Proximal tubules → Collecting duct

Aquaporins (AQP0, AQP2, AQP3)

Extracellular fluid → Proximal tubule lumen → Collecting duct lumen

ADH activity increases AQP2 expression, enhancing water reabsorption.
High Permeation to Water

Nephrogenic diabetes insipidus

>200 Liters Water Everyday!
Aquaporins of known structure:

- **GlpF** - E. coli glycerol channel (aquaglycerolporin)
- **AQP1** - Mammalian aquaporin-1 (pure water channel)
Architecture of the Channel

Channel Fold


RMSD 1.3 Å

Internal gene duplication
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

A Semi-hydrophobic channel
Complementarity

glycerol molecule ↔ channel

acceptor

hydrophobic
donor
Tight Packing in the Selectivity Filter
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

1 ns equilibration, 4 ns 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 aromatic side chains, specially tyrosines
Embedding GlpF in Membrane

77 Å
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.
Lipid-Protein Packing During the Initial NpT Simulation
Adjustment of Membrane Thickness to the Protein Hydrophobic Surface
An extremely stable protein

Stability of NPA – NPA Interaction

RMSD (Å)

[Graph showing RMSD values for different protein conformations and distances between specific amino acid pairs]
Glycerol-Saturated GlpF
Description of full conduction pathway
Complete description of the conduction pathway
Details of Protein-Substrate Interaction are 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

...{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

GLN  41  OE1 NE2  LEU  197  O
TRP  48  O NE1  THR  198  O
GLY  64  O  GLY  199  O
ALA  65  O  PHE  200  O
HIS  66  O ND1  ALA  201  O
LEU  67  O  ASN  203  ND2
ASN  68  ND2
ASP  130  OD1  LYS  33   HZ1 HZ3
GLY  133  O  GLN  41   HE21
SER  136  O  TRP  48   HE1
TYR  138  O  HIS  66   HD1
PRO  139  O N  ASN  68   HD22
ASN  140  OD1 ND2  TYR  138   HN
HIS  142  ND1  ASN  140  HN HD21 HD22
THR  167  OG1  HIS  142   HD1
GLY  195  O  GLY  199   HN
PRO  196  O  ASN  203  HN HD21HD22
          ARG  206  HE HH21HH22
## Channel Hydrogen Bonding Sites

<table>
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<tr>
<th>Residue</th>
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<th>Atom 2</th>
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<th>Atom 4</th>
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The Substrate Pathway is formed by $\text{C}=\text{O}$ groups
Non-helical motifs are stabilized by two glutamate residues.

The Substrate Pathway is formed by C=O groups.
Conservation of Glutamate Residue in Human Aquaporins

AQP0_HUMAN  --LNLIPAVSVAVQAVATIVVEIFLILQVLQFVLCIFATYDE-RRNQQLG
AQP1_HUMAN  --RNLADGQVNGQGIGIEGTLGQVLQVLATTDGR-RRDLDGG
AQP2_HUMAN  --VNLNSSTTIGAVATVELFLTLOVLCLIFASTDE-RGGNEPG
AQP3_HUMAN  GIVATYPSGSLDINGVPQFTGTAASLVCLVAIVDPYNNPVRG
AQP4_HUMAN  --VTVHGNLITAGHGVLITLPQLVPTIFASCDS-KRTDVTC
AQP5_HUMAN  --VNLNNNTTIOAMTYELTLPOLALCIFASTDS-RRTSPVG
AQP6_HUMAN  --INVVRNSVSTGAAVVELILNLQVLQVFASTDS-RQTS--G
AQP7_HUMAN  GIVATYLPDHMTLWRFGANEAVLTGMLQ-CLFAITQDENPAPL
AQP8_HUMAN  -AAAVTVQEQQVAGALVAETILLVLAIVCHQVIN-—EKTGP
AQP9_HUMAN  HIFATYPAVSLANAFADQVATMILLIVFAIFLSPNLGAPGC
GLPF_ECOLI  GTFSVYPNHINEVQAFAVEMVITAQMLILALTDDGNGYPRG
  ruler ...180......190......200......210......22
Glycerol - water competition for hydrogen bonding sites
Revealing the Functional Role of Reentrant Loops
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

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

One needs to discount irreversible work

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

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

4 trajectories

\( v = 0.03, \ 0.015 \ \text{Å/ps} \)

\( k = 150 \ \text{pN/Å} \)

\[
\begin{align*}
 f(t) &= -k[z(t) - z_0 - vt] \\
 W(t) &= \int_0^t dt' \ v f(t')
\end{align*}
\]
Features of the Potential of Mean Force

- Captures major features of the channel
- The largest barrier ≈ 7.3 kcal/mol; exp.: 9.6±1.5 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
Assymetric structure; biological implication?
Asymmetric structure of maltoporin
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
Correlated Motion of Water in the Channel

Water pair correlation

\[ C(z) = \frac{\langle dz_i \cdot dz_j \rangle}{\langle dz_i \cdot dz_j \rangle^{1/2}}; \]
\[ dz_i = z_i(t) - z_i(t+dt); \]
\[ dt = 10 \text{ ps} \]

The single file of water molecules is maintained.
Correlated Motion of Water in the Channel

The single file of water molecules is maintained.
Diffusion of Water in the channel

One dimensional diffusion:

Experimental value for AQP1: 0.4-0.8 e-5
Water Distribution in Aquaporins

- Relative atomic density (10^-3)
- W90: Selectivity Filter
- W84, W441, W86, W349
- O199, O200, O201, O202, O67, O66, O65
- Hδ203, 4.7±0.4
- Hδ68, 6.1±0.3
Water Bipolar Configuration in Aquaporins
Water Bipolar Configuration in Aquaporins

![Diagram showing water bipolar configuration in Aquaporins with various atomic and molecular interactions depicted.]
REMEMBER:

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
Cl$^{-}$ channel

Anti-parallel

K$^{+}$ channel

Parallel (barrel stave)

Aquaporins
Proton Blocking by a Global Orientation Mechanism