Molecular Dynamics Simulation of Membrane Channels

Part II. Structure-Function Relationship and Transport in Aquaporins

Emad Tajkhorshid
Beckman Institute, UIUC

Summer School on Theoretical and Computational Biophysics
June 2004, University of Western Australia
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

Discovered in 1992

Water transport

### Aquaporins in Human Body

<table>
<thead>
<tr>
<th>Aquaporin</th>
<th>Tissues/Cells</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquaporin-o</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>
</tr>
<tr>
<td>Aquaporin-10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional members are suspected to exist.
Aquaporins in the Kidney

- reabsorption of water into blood
- ADH activity

Kidney

Cortex

Nephron

Proximal tubules

Collecting duct

Extracellular fluid

Proximal tubule lumen

Urine concentration

AQP0

AQP3

AQP2

ADH

AQP0

AQP0
High Permeation to Water

Proximal tubule lumen
Extracellular fluid
AQP1
AQP1

Collecting duct lumen
Extracellular fluid
AQP3
AQP2
ADH

>200 Liters Water Everyday!

Nephrogenic diabetes insipidus
Monomeric pores
Water, glycerol, ...

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

Channel Fold

periplasm

cytoplasm

Internal gene duplication

RMSD 1.3 Å

**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
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 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
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

<table>
<thead>
<tr>
<th>Residue</th>
<th>Number</th>
<th>Atom 1</th>
<th>Atom 2</th>
<th>Residue</th>
<th>Number</th>
<th>Atom 1</th>
<th>Atom 2</th>
<th>Atom 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN</td>
<td>41</td>
<td>OE1</td>
<td>NE2</td>
<td>LEU</td>
<td>197</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP</td>
<td>48</td>
<td>O</td>
<td>NE1</td>
<td>THR</td>
<td>198</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLY</td>
<td>64</td>
<td>O</td>
<td></td>
<td>GLY</td>
<td>199</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA</td>
<td>65</td>
<td>O</td>
<td></td>
<td>PHE</td>
<td>200</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIS</td>
<td>66</td>
<td>O</td>
<td>ND1</td>
<td>ALA</td>
<td>201</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEU</td>
<td>67</td>
<td>O</td>
<td></td>
<td>ASN</td>
<td>203</td>
<td>ND2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASN</td>
<td>68</td>
<td>ND2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP</td>
<td>130</td>
<td>OD1</td>
<td></td>
<td>LYS</td>
<td>33</td>
<td>HZ1</td>
<td>HZ3</td>
<td></td>
</tr>
<tr>
<td>GLY</td>
<td>133</td>
<td>O</td>
<td></td>
<td>GLN</td>
<td>41</td>
<td>HE21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SER</td>
<td>136</td>
<td>O</td>
<td></td>
<td>TRP</td>
<td>48</td>
<td>HE1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYR</td>
<td>138</td>
<td>O</td>
<td></td>
<td>HIS</td>
<td>66</td>
<td>HD1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRO</td>
<td>139</td>
<td>O</td>
<td>N</td>
<td>ASN</td>
<td>68</td>
<td>HD22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASN</td>
<td>140</td>
<td>OD1</td>
<td>ND2</td>
<td>TYR</td>
<td>138</td>
<td>HN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIS</td>
<td>142</td>
<td>ND1</td>
<td></td>
<td>ASN</td>
<td>140</td>
<td>HN</td>
<td>HD21</td>
<td>HD22</td>
</tr>
<tr>
<td>THR</td>
<td>167</td>
<td>OG1</td>
<td></td>
<td>HIS</td>
<td>142</td>
<td>HD1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLY</td>
<td>195</td>
<td>O</td>
<td></td>
<td>GLY</td>
<td>199</td>
<td>HN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRO</td>
<td>196</td>
<td>O</td>
<td></td>
<td>ASN</td>
<td>203</td>
<td>HN</td>
<td>HD21</td>
<td>HD22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ARG</td>
<td>206</td>
<td>HE</td>
<td>HH21</td>
<td>HH22</td>
</tr>
</tbody>
</table>
Channel Hydrogen Bonding Sites

<table>
<thead>
<tr>
<th>Residue</th>
<th>Position</th>
<th>Hydrogen Bond Donor</th>
<th>Hydrogen Bond Acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN</td>
<td>41</td>
<td>OE1 NE2</td>
<td>LEU 197 O</td>
</tr>
<tr>
<td>TRP</td>
<td>48</td>
<td>O NE1</td>
<td>THR 198 O</td>
</tr>
<tr>
<td>GLY</td>
<td>64</td>
<td>O</td>
<td>GLY 199 O</td>
</tr>
<tr>
<td>ALA</td>
<td>65</td>
<td>O</td>
<td>PHE 200 O</td>
</tr>
<tr>
<td>HIS</td>
<td>66</td>
<td>O ND1</td>
<td>ALA 201 O</td>
</tr>
<tr>
<td>LEU</td>
<td>67</td>
<td>O</td>
<td>ASN 203 ND2</td>
</tr>
<tr>
<td>ASN</td>
<td>68</td>
<td>ND2</td>
<td></td>
</tr>
<tr>
<td>ASP</td>
<td>130</td>
<td>OD1</td>
<td>LYS 33 HZ1 HZ3</td>
</tr>
<tr>
<td>GLY</td>
<td>133</td>
<td>O</td>
<td>GLN 41 HE21</td>
</tr>
<tr>
<td>SER</td>
<td>136</td>
<td>O</td>
<td>TRP 48 HE1</td>
</tr>
<tr>
<td>TYR</td>
<td>138</td>
<td>O</td>
<td>HIS 66 HD1</td>
</tr>
<tr>
<td>PRO</td>
<td>139</td>
<td>O N</td>
<td>ASN 68 HD22</td>
</tr>
<tr>
<td>ASN</td>
<td>140</td>
<td>OD1 ND2</td>
<td>TYR 138 HN</td>
</tr>
<tr>
<td>HIS</td>
<td>142</td>
<td>ND1</td>
<td>ASN 140 HN HD21 HD22</td>
</tr>
<tr>
<td>THR</td>
<td>167</td>
<td>OG1</td>
<td>HIS 142 HD1</td>
</tr>
<tr>
<td>GLY</td>
<td>195</td>
<td>O</td>
<td>GLY 199 HN</td>
</tr>
<tr>
<td>PRO</td>
<td>196</td>
<td>O</td>
<td>ASN 203 HN HD21 HD22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ARG 206 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 $\text{C}=\text{O}$ groups.
Conservation of Glutamate Residue in Human Aquaporins

| AQP0_HUMAN | LNLHPAVSVGQATTVEIFLTLLCVLCLFAFYEDERRNQOLG |
| AQP1_HUMAN | RNDLADCVGSQLGLGIEGETQQLVCVLATDDRSPRLGG |
| AQP2_HUMAN | VNALSNSTTAGQAVTELPTLLQLVLCLIFASTDERRGEPGC |
| AQP3_HUMAN | GIFATYPSCHLDMINGFDQFGTASLIVCVLAVDPPYNNFVPRG |
| AQP4_HUMAN | VTMVHGNLTAHGLLIVELQITPQLVFTIFASCDS-KRTDVTG |
| AQP5_HUMAN | VNALNNITOGIAMVEELINPOLALCLIFASTDS-RRTSPVG |
| AQP6_HUMAN | INVVRNVSSTQGAVAVELLNLQOLCVFASTDS-RQTS-G |
| AQP7_HUMAN | GIFATYLPDHMTLWRGELNEAVLICMLQ-CMFAIDQENFFPALS |
| AQP8_HUMAN | AAFFTVQEQQQVAGALVAEEILLLALAVCNAG--EKTKGP |
| AQP9_HUMAN | HIFATYPAPYSLANAPADQVATMLLIVFAIFPLRNLAGPRC |
| GLPF_ECOLI  | GFSTYPNPHEFVQAFVEMVTAHLMGLILALTDGNGVPRPG |

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

\( v = 0.03, \quad 0.015 \ \text{Å/ps} \)

\( k = 150 \ \text{pN/Å} \)

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

\[ W(t) = \int_0^t dt' \ v f(t') \]
• 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

7-8 water molecules in each channel
Correlated Motion of Water in the Channel

The single file of water molecules is maintained.

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

Anti-parallel

K⁺ channel

Parallel (barrel stave)

Aquaporins
Proton Blocking by a Global Orientation Mechanism