The behaviour of water in hydrophobic nanopores

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Outline

1. Introduction: Hydrophobic pores in ion channels

2. Simplified model pore: Molecular dynamics

3. Water in hydrophobic pores: temporal oscillations liquid $\rightleftharpoons$ vapour
   - thermodynamics (equilibrium)
   - simple thermodynamic model
   - dynamics (diffusion, flux, permeability coefficient)

4. Water in the nicotinic receptor nAChR
nAChR*: A hydrophobic gate to ion permeation?

closed  \( R \approx 3.1 \text{ Å} \\ hydrophobic girdle (Leu, Val, Phe) \\
open  \( R \approx 6.5 \text{ Å} \\ polar pore lining

* nAChR: nicotinic acetylcholine receptor, a cation selective, ligand-gated ion channel
Putative gates are lined by **hydrophobic amino acids** (Leu, Val, Ile, Phe, ...).
Model system

**Molecular Dynamics**

- Hydrophobic “atoms”: unified methane molecules
- Hydrophilic: two parallel dipoles (backbone charges): surface character
- Harmonically restrained CH₄: flexibility
- Water model: SPC
- GROMACS 3, ffgmx
- Electrostatics: PME; \( T = 300 \text{ K}, P = 1 \text{ bar} \) (weak coupling)
- \( 52 \text{ ns} \leq T_{\text{sim}} \leq 120 \text{ ns} \)

**Dimensions** (≈ nAChR gate)
- Pore radius: \( 1.5 \text{ Å} \leq R \leq 10 \text{ Å} \)
- Pore length: \( L_P = 8 \text{ Å} \)
- Mouth
  - \( R_M = 10 \text{ Å} \)
  - \( L_M = 8 \text{ Å} \)
$z$-averaged and radially averaged water density in units of SPC bulk water at 300 K and 1 bar. Grid spacing 0.5 Å.
Oscillations in the pore occupancy*

Strong dependence of the pore state on the radius $R$.

Analysis in terms of

1. density
2. free energy $f(T,n;R)$
3. state (“openness” $\langle \omega \rangle$)
4. kinetics ($\text{liquid} \rightleftharpoons \text{vapour}$)
5. dynamical properties

*O. Beckstein and M. S. P. Sansom, *PNAS* **100** (2003), 7063
Free energy

Helmholtz free energy $F(T,V,N)$ from the pore occupancy distribution $p(N)$ ($T = 300$ K, $V = V_P = \text{const}$)

$$\beta F = -\ln p(N) + C$$

Free energy density and chemical potential ($n$ as order parameter)

$$f(T,n) = \frac{F}{V}$$

$$\mu(T,n) = \frac{\partial f}{\partial n}$$
Characterization of the pore state

- **water-filled** ⇒ ion conducting ['"open", \( \omega(t) = 1 \)] if
  \[
  \frac{n_{\text{pore}}}{n_{\text{bulk}}} \geq \eta,
  \]

- empty ⇒ ions cannot permeate ['"closed", \( \omega(t) = 0 \)]

- state-detection:
  Schmitt-trigger with \( \eta = 0.4 \pm 0.25 \)

- openness:
  \[
  \langle \omega \rangle = \frac{T_{\text{open}}}{T_{\text{sim}}}
  \]

\( T_{\text{open}} \): time during which the pore is in the open state
Equilibrium liquid ⇌ vapour: Openness

Openness/probability for liquid state:

$$\langle \omega \rangle = \frac{1}{T_{\text{sim}}} \int_{0}^{T_{\text{sim}}} dt \, \omega(t) = \frac{T_o}{T_{\text{sim}}}$$

Equilibrium constant $K$:

$$K = \frac{T_c}{T_o} = \frac{T_{\text{sim}} - T_o}{T_o} = \langle \omega \rangle^{-1} - 1$$

(open = liquid state, closed = vapour state)
Equilibrium liquid $\rightleftharpoons$ vapour: Free energy difference

\[
\beta \Delta \Omega(R) = \beta [\Omega_c(R) - \Omega_o(R)] = -\ln K(R) = -\ln \left( \langle \omega(R) \rangle^{-1} - 1 \right)
\]

\[
K(R) = \frac{T_c(R)}{T_o(R)} \\
= \frac{T_{\text{sim}} - T_o(R)}{T_o(R)} \\
= \langle \omega(R) \rangle^{-1} - 1
\]
Equilibrium liquid $\rightleftharpoons$ vapour: Theoretical model


\[
\Delta \Omega(R) = \Omega_v(R) - \Omega_l(R) = -p_v(\mu) L \pi R^2 + 2\pi R L \gamma_{vw} + 2\pi R^2 \gamma_{lv} - ( - p_l(\mu) L \pi R^2 + 2\pi R L \gamma_{lw})
\]

\[
p(T, \mu) = p(T, \mu_{sat}) + (\mu - \mu_{sat}) \frac{\partial p(T, \mu)}{\partial \mu} \bigg|_{\mu = \mu_{sat}} + \cdots,
\]

At saturation \( p_l(T, \mu_{sat}) = p_v(T, \mu_{sat}) \)

\[
\Delta \Omega(R) = \left[ 2\gamma_{lv} - (\mu - \mu_{sat}) (n_v(T, \mu_{sat}) - n_l(T, \mu_{sat})) L \right] \pi R^2 + 2\pi L (\gamma_{vw} - \gamma_{lw}) R
\]

\[
= \left[ 2\gamma_{lv} + \Delta \mu \Delta n_v L \right] \pi R^2 + 2\pi L \Delta \gamma_w R = a_2 R^2 + a_1 R
\]
Equilibrium \( \text{liquid} \rightleftharpoons \text{vapour} \): MD and model

\[
\langle \omega(R) \rangle = \frac{1}{1 + \exp[-\beta \Delta \Omega(R)]}
\]

- thermodynamic model based on surface energy arguments seems to hold even at atomic dimensions (?!)

- **hydrophobic gating**

  (change in radius or more polar pore lining can open a hydrophobic gate)

<table>
<thead>
<tr>
<th></th>
<th>( 2\gamma_{lv} + \Delta \mu \Delta n_{vl} L )</th>
<th>( \Delta \gamma_w )</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydrophobic</td>
<td>( 0.21 \pm 0.01 ) ( \text{[}k_B T/\text{Å}^2\text{]} )</td>
<td>(-7.2 \pm 0.2) ( \text{[}10^{-2} \times k_B T/\text{Å}^2\text{]} )</td>
</tr>
<tr>
<td>two dipoles</td>
<td>( 0.16 \pm 0.01 )</td>
<td>(-3.0 \pm 0.0)</td>
</tr>
</tbody>
</table>

expt.: \( \gamma_{lv}(\text{water}) = 0.17 k_B T/\text{Å}^2 \), \( \gamma_{lw}(\text{water}/n\text{-octane}) = 0.12 k_B T/\text{Å}^2 \)

Lifetimes

Average open and closed time $\tau_o = \langle t_o \rangle$ and $\tau_c = \langle t_c \rangle$

![Graph showing lifetime vs. radius](image)

$\text{Prob}(\text{filling}) \propto 1/\tau_c$

constant $\Rightarrow$ evaporation

$\text{Prob}(\text{emptying}) \propto 1/\tau_o$ exponential $\Rightarrow$ cavitation

Probability $p(\lambda, n \leq n_{\text{max}})$ for the formation of a cavity of given radius $\lambda$ and density below $n_{\text{max}}$ is an exponential*

*G. Hummer et al, *PNAS* 93 (1996), 8951
Dynamics

- multi-pass (no single-filing)
- transition time $\approx 30$ ps

$\langle \tau_p \rangle$ mean permeation time

$j_0$ equilibrium current density

$D_z$ diffusion coefficient in $z$

$\langle \tau_{p,\text{bulk}} \rangle = 29.9 \pm 0.1$ ps

$j_{0,\text{bulk}} = 320 \pm 3$ ns$^{-1}$ nm$^{-2}$

$D_{\text{bulk}} = 4.34 \pm 0.01$ nm$^2$ ns$^{-1}$
### Water transport in experiment and simulation

<table>
<thead>
<tr>
<th></th>
<th>Ref.</th>
<th>$p_f \times 10^{14}$ [cm$^3$s$^{-1}$]</th>
<th>$\Phi_0$ [ns$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqp1</td>
<td>(1)</td>
<td>4.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Aqp4</td>
<td>(1)</td>
<td>15</td>
<td>9.7</td>
</tr>
<tr>
<td>AqpZ</td>
<td>(2)</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>gA</td>
<td>(3)</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>desformyl gA</td>
<td>(4)</td>
<td>110</td>
<td>71</td>
</tr>
<tr>
<td>$R = 0.20$ nm</td>
<td></td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>$R = 0.30$ nm</td>
<td></td>
<td>1.9</td>
<td>1.2</td>
</tr>
<tr>
<td>$R = 0.35$ nm</td>
<td></td>
<td>4.0</td>
<td>2.6</td>
</tr>
<tr>
<td>$R = 0.40$ nm</td>
<td></td>
<td>5.7</td>
<td>3.7</td>
</tr>
<tr>
<td>$R = 0.45$ nm</td>
<td></td>
<td>30.0</td>
<td>19.4</td>
</tr>
<tr>
<td>$R = 0.50$ nm</td>
<td></td>
<td>66.5</td>
<td>43.0</td>
</tr>
<tr>
<td>$R = 0.55$ nm</td>
<td></td>
<td>117</td>
<td>75.8</td>
</tr>
<tr>
<td>$R = 0.60$ nm</td>
<td></td>
<td>363</td>
<td>235</td>
</tr>
<tr>
<td>$R = 0.70$ nm</td>
<td></td>
<td>700</td>
<td>453</td>
</tr>
<tr>
<td>$R = 1.0$ nm</td>
<td></td>
<td>1480</td>
<td>956</td>
</tr>
<tr>
<td>carbon nanotube</td>
<td>(5)</td>
<td>26.2</td>
<td>16.9</td>
</tr>
<tr>
<td>desformyl gA (DH)</td>
<td>(6)</td>
<td>10</td>
<td>5.8</td>
</tr>
</tbody>
</table>

**non-equilibrium flux**

$$\Phi = \beta p_f (\Delta P - \Delta \Pi)$$

**equilibrium flux**

$$\Phi_0 = \nu_x / T_{\text{sim}}$$

**osmotic permeability coefficient**

$$p_f = \frac{1}{2} \Phi_0 v_l$$

**Water translocation occurs in bursts**

Influence of wall flexibility: Squidgy pores

<table>
<thead>
<tr>
<th></th>
<th>restraint force constant $k$ [kJ mol$^{-1}$ Å$^{-2}$]</th>
<th>RMSD $\rho_P$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>2</td>
<td>1.70 ± 0.31</td>
</tr>
<tr>
<td>Middle</td>
<td>10</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>Bottom</td>
<td>50</td>
<td>0.35 ± 0.01</td>
</tr>
</tbody>
</table>

• greater flexibility shifts equilibrium towards *vapour* state
• more than just decreasing effective pore radius $R - \rho_P(k)$
Water in a carbon nanotube\textsuperscript{1} and a mean field model\textsuperscript{2}

<table>
<thead>
<tr>
<th>Ref.</th>
<th>$\rho_w$</th>
<th>$\varepsilon_{fw}$</th>
<th>$\varepsilon_{\text{eff}}$</th>
<th>$R_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>this work</td>
<td>8</td>
<td>0.906493</td>
<td>7</td>
<td>$\approx 0.55$</td>
</tr>
<tr>
<td>(1)</td>
<td>50</td>
<td>0.478689</td>
<td>24</td>
<td>$&lt; 0.24$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.272937</td>
<td>14</td>
<td>$&gt; 0.24$</td>
</tr>
<tr>
<td>(2)</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4</td>
<td>154</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.45</td>
<td>160</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>220</td>
<td>0</td>
</tr>
</tbody>
</table>

$\rho_w$ density of wall atoms (nm\textsuperscript{-3})
$\varepsilon_{fw}$ fluid-wall interaction (kJ mol\textsuperscript{-1})
$\varepsilon_{\text{eff}}$ effective interaction strength
$\rho_w \varepsilon_{fw}$ (kJ mol\textsuperscript{-1} nm\textsuperscript{-3})
$R_c$ critical/coexistence pore radius (nm)

$\Rightarrow$ wall-density/surface roughness influences behaviour strongly

Water in the nicotinic receptor

MD with Cα harmonically restrained; membrane mimetic. $T_{\text{sim}} = 60$ ns.

$z$-averaged and radially averaged water density in units of SPC bulk water at 300 K and 1 bar. Grid spacing 0.5 Å. Minimum pore radius 3.1 Å.
Water in nAChR

Water density in units of SPC water at 1 bar, 300K

Lower constriction, S248 (6')

Gate, V255 (13')

Cavity, L251:O (9')
Summary

- oscillations liquid ⇌ vapour explained in a simple thermodynamic model
  - surface tension $\gamma_{vw} - \gamma_{lw}$ determines “hydrophobicity”
  - wall-water interaction strength (packing and well-depth) is crucial

- hydrophobic gating

- hydrophobic environment
  - induces collective water movements (bursts)
  - accelerates water transport
  - strategically placed hydrophilic groups mimic bulk water environment in Aqps

- flexible (“jelly”) pores favour vapour
Additional slides

- water structure and radial PMF
- capillary condensations
- Temperature dependence of the openness
- H-bonds in nAChR
Water structure

<table>
<thead>
<tr>
<th>state</th>
<th>phase</th>
<th>(n/n_{\text{bulk}})</th>
<th>pore saturated, expt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>closed</td>
<td>vapour</td>
<td>0.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>open</td>
<td>liquid</td>
<td>0.82</td>
<td>1.0</td>
</tr>
</tbody>
</table>

[see also Brovchenko, Geiger & Oleinikova, Phys. Chem. Chem. Phys. 3 (2001)]

radial PMF of a water molecule:

\[
\beta F(r) = -\ln \frac{n(r)}{n_{\text{bulk}}} + C
\]
Capillary condensation and evaporation

Kelvin’s equation

\[ \ln \frac{p}{p_0} = -\frac{\beta \gamma_{lv} v_l}{r} \]

Young’s equation

\[ \gamma_{wv} = \gamma_{wl} + \gamma_{lv} \cos \theta \]

and geometry

\[ R = r \cos \theta \]

Kelvin’s equation for cylindrical pores:

\[ \ln \frac{p(R)}{p_0} = -\frac{\beta(\gamma_{wv} - \gamma_{wl}) v_l}{R} \]

... with hydrophobic walls

\[ \Rightarrow \gamma_{wv} < \gamma_{wl} \Rightarrow \gamma_{wv} - \gamma_{wl} < 0 \]

\[ \Rightarrow \ln p(R)/p_0 > 0 \Rightarrow p(R) > p_0 \]

\[ \Rightarrow \text{if actual vapour pressure} > p(R) > p_0 \Rightarrow \text{condensation} \]
Temperature dependence of the openness

$$F = k_B T$$

Pore radius $R = 5.5$ Å.
Hydrogen bonds in nAChR

Snapshot after 60 ns of molecular dynamics, showing an interrupted network of H-bonds above the hydrophobic girdle (α-V255) near α-V259 and at the lower constriction α-S248.