Ion selectivity of a biological channel at high concentration ratio:
insights on small ion diffusion and binding

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Outline

- Introduction:
  - Ion selectity
  - OmpF porin
  - Previous results in OmpF selectivity

- Reversal potential results

- Modelling

- Conclusions
• **Selectivity** in ionic channels:

**Partitioning**: equilibrium, measures ionic exclusion.

**Diffusion**: non equilibrium, measures relative mobility of different ions.
Outer membrane protein F in *E. Coli*

Reconstituted into planar lipid membranes
OmpF porin forms *trimeric* channels

Each monomer has hourglass-like shape, and it is slightly *cation selective* at neutral pH
The selectivity is not a number but depends on:

- **pH**

**Figure:**
- Cation selective
- Anion selective

**Graph Details:**
- Cis = 1M
- Trans = 0.1M

**Reference:**
The selectivity is not a number but depends on:
Salt concentration and concentration gradient

The selectivity is not a number but depends on:
Type of electrolyte

Through ion channel reconstitution in planar lipid bilayers

Ag/AgCl with salt bridges, 2M KCl

Cis: 0.09 M – 6M

Trans: 0.03 M

pH = 6

Electrolytes: LiCl, NaCl, KCl, CsCl
**Selectivity** in channels:

**Partitioning**: equilibrium, measures ionic **exclusion**

**Diffusion**: non equilibrium, measures relative ionic **mobility**

\[ RP = 2 \text{ Donnan} + \text{ diffusion} \]
Reversal Potential Model II

\[ \Delta \phi_{\text{exclusion}} = \Delta \phi_{\text{Donnan cis}} - \Delta \phi_{\text{Donnan trans}} \]

\[ = \frac{k_B T}{e} \ln \left( \frac{-X / 2c_{\text{trans}}}{-X / 2c_{\text{cis}}} + \sqrt{\frac{(-X / 2c_{\text{trans}})^2 + 1}{(-X / 2c_{\text{cis}})^2 + 1}} \right) \]

Diffusion potential: Electroneutrality Approximation

\[ \Delta \phi_{\text{Diffusion}} = \frac{k_B T}{e} \ln \left( \frac{D_- - D_+}{D_- + D_+} \right) \frac{D_+ \bar{c}_{\text{cis}} + D_- (\bar{c}_{\text{cis}} - X)}{D_+ \bar{c}_{\text{trans}} + D_- (\bar{c}_{\text{trans}} - X)} \]
Exclusion and diffusion

Diffusion goes with ln r at high r
Slope information D+/D−

Exclusion saturates at high r

Why high concentration gradients?
- At high concentration exclusion is saturated
- We can separate different contributions
LiCl and NaCl RP results I

Concentration Ratio ($\frac{\text{cis}}{\text{trans}}$)

Reversal Potential (mV)

-40
-30
-20
-10
0

LiCl

NaCl

Cationic selectivity ⇒ Anionic selectivity

**LiCl and NaCl RP results II**

**Graphs:**
- **LiCl** and **NaCl**

**Concentration Ratio (cis/trans):**
- 1
- 10
- 100

**Reversal Potential (mV):**
- -60
- -40
- -20
- 0
- 20

**Diffusion Conditions:**
- Diffusion $D_+ < D_-$
- Diffusion $D_+ = D_-$
- Diffusion $D_+ > D_-$
- Exclusion

**Exclusion + diffusion can explain the results but........ slopes 50% higher aprox.**
### CsCl and KCl RP results

<table>
<thead>
<tr>
<th>Ion</th>
<th>Li⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cs⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>D(cm²/s)</td>
<td>1.03</td>
<td>1.33</td>
<td>1.96</td>
<td>2.06</td>
<td>2.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration Ratio (cis/trans)</th>
<th>Reversal Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-40</td>
</tr>
<tr>
<td>10</td>
<td>-30</td>
</tr>
<tr>
<td>100</td>
<td>-20</td>
</tr>
</tbody>
</table>

**Exclusion** + diffusion cannot explain the results. There is another phenomenon involved.

**Binding could influence selectivity??**

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Cation binding in OmpF I


Fluorescence exp,
Dissociation constant 3 mM

- Noise: Glu117
- MD: Asp113

Free energy calculations

Push-out mechanism slows down cation diffusion
Cation binding in OmpF II

MD : 10 nS

Binding: Glu117, Asp113
Push-out mechanism slows down cation diffusion
RP Results at high gradients

Linear behaviour with Ln r ⇒ diffusional behaviour

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>CsCl</th>
<th>KCl</th>
<th>NaCl</th>
<th>LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>((D_+/D_-)<em>\text{eff}/(D</em>+/D_-)_{\text{bulk}})</td>
<td>0.48 ± 0.06</td>
<td>0.50 ± 0.08</td>
<td>0.58 ± 0.01</td>
<td>0.56 ± 0.05</td>
</tr>
</tbody>
</table>

Diffusion is reduced in a common pattern (50%) 

Binding slows down cation
Molecular model for binding I

Aim of the model: obtain fraction of unbound acid, $x$

Binding to two specific acids: Glu117, Asp113

Statistical mechanics: Hill 1956


Binding of antibiotic to OmpF

Possible states in grand canonical ensemble of the two acids
Molecular model for binding II

\[ q = \exp\left(-\frac{u}{k_B T}\right) + 2z_H \lambda_H + 2z_K \lambda_K + 2z_H \lambda_H z_K \lambda_K + z_H^2 \lambda_H^2 + z_K^2 \lambda_K^2 = \]
\[ = \exp\left(-\frac{u}{k_B T}\right) + 2 \left(10^{pK_a-pH}\right) + 2 \left(10^{pK_a-pH} 10^{pK_c} c\right) + 10^{2(pK_a-pH)} + (10^{pK_c} c)^2 \]

\[ 1 - x = \frac{1}{2} \lambda_K \frac{\partial \ln q}{\partial \lambda_K} \]
Modelling the reversal potential I

Modelling reduced diffusion

\[ D_{\text{eff}} = x D_{\text{free}} + (1 - x) D_{\text{bound}} \]

If we assume at high c, \( x = 0 \), \( (D_{\text{eff}})_{\text{high concentration}} = D_{\text{bound}} \)

\( (D_{\text{eff}})_{\text{high concentration}} = D_{\text{bulk}} / 2 \)

\[ D_{\text{bound}} = D_{\text{bulk}} / 2 \]
Modelling the reversal potential II

Fluorescence exp. Dissociation constant = 3mM ($pK_c = 2.5$)
Dissociation constant = 0.3 M ($pK_c = 0.5$)

Theory versus experiments

\[ \text{RP} = 2 \text{ Donnan} + \text{ diffusion} \]

**Graph a:**
- Concentration Ratio (cis/trans)
- Reversal Potential (mV)
- LiCl
- NaCl
- KCl

**Graph b:**
- Concentration Ratio (cis/trans)
Conclusions

• **RP** measurements at large concentration ⇒ useful information.

• At high concentration ratios diffusion is hampered.

• The reduction of the cation diffusion is the same for all the electrolytes and is consistent with a cation binding site.

• The theoretical model explains qualitatively RP results.
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