Dynamical View of Energy Coupling Mechanisms in Active Membrane Transporters

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25th Molecular Modeling Workshop
April 2011, Erlangen, Germany

Proton-Driven Sugar Transport in LacY

ADP/ATP Exchange in Mitochondria (AAC)

Mechanically(?) Driven Transporters of the Outer Membrane

ATP-Driven Transport in ABC Transporters

Na⁺-Driven Neurotransmitter Uptake Glutamate Transporter
Molecular Dynamics Simulations

Solving the Newtonian equations of motion for all particles at every time step

Major limitations:
- Time scale / sampling
- Force field approximations

Major advantage:
- Unparalleled spatial and temporal resolutions, simultaneously

SPEED LIMIT
1 fs
Access to More Computational Power

HP 735 cluster
12 processors (1993)

SGI Origin 2000
128 processors (1997)

PSC LeMieux AlphaServer SC
3000 processors (2002)

Ranger/Kraken
~60,000 processors (2007)

Blue Waters (UIUC)
200,000+ processors (2012)

Anton/DESHAW/PSC
512 processors (2010)
Complexity of Transporter Function

• Active transport is **coupled** to an energy source in the cell

• Transporters Function on **µs and longer** time scales

• Protein conformational changes of various forms and magnitudes coupled to **step-wise** vectorial translocation of the substrate and co-transported materials

• The **sequence** of molecular events is largely unknown
In situ Molecular Dynamics Simulations

Atom count: 100-500k
~10 ns/day on 128-1024 processors
100-500 ns for each system
Alternating Access Model

Hard to define the number of (sub)states involved?
Glutamate Transporter

Amara and Fontana, Neurochemistry International
41:313-318 (2002)

Glutamate Transporter (Glt_{ph})

Sequence and Coupling of Events in an Ion-Coupled Transporter

<table>
<thead>
<tr>
<th>monomer</th>
<th>substrate</th>
<th>Na1</th>
<th>Na2</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S1B</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S1C</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S2A</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S2B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S2C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S3A</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S3B</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>S3C</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Glutamate Transporter (Glt\text{ph})


Z. Huang and E. Tajkhorshid, Biophysical Journal 2008
Dynamics of the **Extracellular Gate**

Ion-Substrate Coupling on the Extracellular Side

Substrate binding forms the Na2 binding site

Na2 seals the binding site

Inward-Facing, Occluded Glt_{ph}

Transport mechanism of a bacterial homologue of glutamate transporters

Nicolas Reyes¹, Christopher Ginter¹ & Olga Boudker¹

Glutamate transporters are integral membrane proteins that catalyse a thermodynamically uphill uptake of the neurotransmitter glutamate from the synaptic cleft into the cytoplasm of glia and neuronal cells by harnessing the energy of pre-existing electrochemical gradients of ions. Crucial to the reaction is the conformational transition of the transporters between outward and inward facing states, in which the substrate binding sites are accessible from the extracellular space and the cytoplasm, respectively. Here we describe the crystal structure of a double cysteine mutant of a glutamate transporter homologue from Pyrococcus horikoshii, Glt_{ph}, which is trapped in the inward facing state by cysteine crosslinking. Together with the previously determined crystal structures of Glt_{ph} in the outward facing state, the structure of the crosslinked mutant allows us to propose a molecular mechanism by which Glt_{ph} and, by analogy, mammalian glutamate transporters mediate sodium-coupled substrate uptake.

Reyes, et al., Nature 2009
Different Modes of Cytoplasmic (HP1) and Extracellular (HP2) Gating

Coupled to substrate release

Na1-controlled

inward-facing gate

Leighton, et al., JBC (2006)

outward-facing gate

Substrate-controlled

Coupled to Na2 binding

periplasm

cytoplasm
Na1 Dependence of the Cytoplasmic Gate

Inward-facing, occluded with Na1/substrate

Occluded to open transition after Na1 release
vSGLT: A Secondary Membrane Transporter in the **Occluded** Inward-Facing State

Na⁺ was modeled based on LeuT

Faham et al., Science, 810-814, 2008
Spontaneous Na\(^+\) Unbinding in Multiple Simulations

- Several independent simulations, all resulting in Na\(^+\) unbinding
- The crystal structure is not an occluded state, rather an open inward-facing state.

Comparison of the Na$^+$ Binding Sites in vSGLT, LeuT and Mhp1

<table>
<thead>
<tr>
<th>bond</th>
<th>distance (Å)</th>
<th>bond</th>
<th>distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G20(O)–Na</td>
<td>2.23</td>
<td>A62(O)–Na</td>
<td>3.64</td>
</tr>
<tr>
<td>V23(O)–Na</td>
<td>2.15</td>
<td>I65(O)–Na</td>
<td>3.32</td>
</tr>
<tr>
<td>A351(O)–Na</td>
<td>2.29</td>
<td>A361(O)–Na</td>
<td>3.23</td>
</tr>
<tr>
<td>T354(O$_{\gamma}$)–Na</td>
<td>2.25</td>
<td>S364(O$_{\gamma}$)–Na</td>
<td>3.13</td>
</tr>
<tr>
<td>S355(O$_{\gamma}$)–Na</td>
<td>2.35</td>
<td>S365(O$_{\gamma}$)–Na</td>
<td>3.68</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>bond angle</th>
<th>angle (degree)</th>
<th>bond angle</th>
<th>angle (degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V23(O)–Na–S355(O$_{\gamma}$)</td>
<td>99.3</td>
<td>I65(O)–Na–S365(O$_{\gamma}$)</td>
<td>60.5</td>
</tr>
<tr>
<td>V23(O)–Na–T354(O$_{\gamma}$)</td>
<td>112.8</td>
<td>I65(O)–Na–S364(O$_{\gamma}$)</td>
<td>133.0</td>
</tr>
<tr>
<td>T354(O$<em>{\gamma}$)–Na–S355(O$</em>{\gamma}$)</td>
<td>147.9</td>
<td>S364(O$<em>{\gamma}$)–Na–S365(O$</em>{\gamma}$)</td>
<td>73.5</td>
</tr>
<tr>
<td>G20(O)–Na–A351(O)</td>
<td>166.5</td>
<td>A62(O)–Na–A361(O)</td>
<td>150.6</td>
</tr>
</tbody>
</table>
Artificially Recovering the Occluded State
Cytoplasmic Gate?

Substrate-bound state → substrate-free state?
Early Stage of Substrate Release Captured by Free MD

A

Set 1

Set 2

B

crystal structure
Transporter Dogma: Alternating Access Mechanism

- Transporters switch the substrate access between the two sides.
- The central binding site should **never** be exposed to both sides **simultaneously**.
ATP-Driven Transport in ABC Transporters

- **Architecture**
  - 2 NBDs
    - Conserved domains
    - ATPase activity
  - 2 TMDs
    - Diverse sequence and structure
    - Substrate transport
- 1 BP
  - ABC importers only
  - Substrate recognition and binding
Nucleotide-Dependent State of NBDs

Lu et al., PNAS, (2005)
Simulation Systems

• MalK dimer (1Q12.PDB)
• Placing Mg$^{2+}$
• Solvate (80,000 atoms)
• Equilibrium MD - 75 ns
• 4 simulation systems
  – ATP / ATP
  – ADP-P$_{i}$ / ATP
  – ATP / ADP-P$_{i}$
  – ADP-P$_{i}$ / ADP-P$_{i}$

1 or 2 ATP hydrolysis?
Hydrolysis or release of products?
Simulating the Immediate Effect of ATP Hydrolysis

ADP/Pi-Bound

ATP-Bound

P. Wen and E. Tajkhorshid, Biophysical Journal 2008
ATP/ATP 2 Hydrolysis

1 hydrolysis - top

2 Hydrolysis

1 hydrolysis - bottom

P. Wen and E. Tajkhorshid, Biophysical Journal 2008
Pinpointing the Mechanism

Nucleotide Binding Domains

- Two subdomains
  - RecA-like subdomain
    - Majority of ATP binding site
    - Walker A motif
  - Helical subdomain
    - Complimentary to ATP binding
    - Signature “LSGGQ” motif

- Two nucleotide binding sites
  - Both at the dimer interface
  - “Nucleotide-sandwiched” dimer

ATP binding $\rightarrow$ dimerization
Hydrolysis $\rightarrow$ dimer opening

Why?
Discovery of Buried Charges in the maltose transporter

MalEFGK, ATP-bound
- 320,000 atoms
- averaged between $t = 70-80$ ns

MalEFGK, Nucleotide-Free
- 320,000 atoms
- averaged between $t = 70-80$ ns

+500 mV isosurface
Discovery of Buried Charges in the molybdate/tungstate transporter

ModABC Nucleotide-Free
~220,000 atoms
averaged between
$t = 0-10$ ns

ModABC, ADP docked
~220,000 atoms
averaged between
$t = 0-10$ ns

+500 mV isosurface
Discovery of Buried Charges
in other ABC transporters

MsbA ATP-bound
~200,000 atoms
averaged between
\( t = 0-10 \text{ ns} \)

BtuCDF, Nucleotide-Free
~220,000 atoms
averaged between
\( t = 0-10 \text{ ns} \)
Charge Conservation at the Core of the Helical Subdomain

Analyzed from all 185 representative sequences in NCBI CDD #cd00267
Conserved Arginines in the Helical Subdomain
Key Role of Buried Charges in NBD Dimerization

MalK dimers
All ATP Bound
\( t = 50 \text{ ns} \)
Recovery of NBD Dimerization in the Mutants

Arg^{139}-Arg^{140}-Gln^{141} \quad \text{(wt = Arg^{139}-Gln^{140}-Arg^{141})}
Recovery of NBD Dimerization in the Mutants

Conclusion: **Buried** positive charges in the helical subdomains are essential for NBD dimerization.
So Why/How Do NBDs Dimerize?

• Three factors determine the NBD dimerization
  – Binding
    • Walker A motif binds ATP
  – Attracting
    • Positive charged residues in the helical subdomain
    • Optimal: 2 positive charges near the LSGGQ motif
    • Location, location, location!
  – Locking
    • Network of hydrogen bonds between the ATP and the LSGGQ motif
Unusually Strong Electrostatic Potential

• Very strong (~1.4V) positive potential at the AAC basin provides the driving force for ADP binding.

Y. Wang and E. Tajkhorshid, *PNAS 2008*
Almost all MCF members are strongly charged (positive).

Most substrates of MCFs are negatively charged.
  - Substrate recruitment
  - Anchoring the proteins into the negatively charged inner mitochondrial membrane.
Capturing Substrate Binding - Case II

GlpT

N-terminal domain (6 TMH)

C-terminal domain (6 TMH)

Putative binding site

R45

H165

R269

Inter-domain loop
(not resolved in the crystal)

phosphate/organic phosphate antiporter

Capturing Substrate Binding - Case II


G. Enkavi and E. Tajkhorshid, *Biochemistry* 2010
Characterizing Substrate Selectivity

Phosphate Binding Site

<table>
<thead>
<tr>
<th>GlpT protein</th>
<th>$K_d$ P$_i$ binding (µM)</th>
<th>$K_d$ G3P binding (µM)</th>
<th>$K_d$ phosphomycin binding (µM)</th>
<th>G3P-P$_i$ exchange transport activity?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>7.4 ± 0.4</td>
<td>0.8 ± 0.2</td>
<td>0.18 ± 0.02</td>
<td>Yes</td>
</tr>
<tr>
<td>N162A</td>
<td>4.4 ± 1.4</td>
<td>No binding</td>
<td>No binding</td>
<td>n.d.</td>
</tr>
<tr>
<td>Y266F</td>
<td>1.7 ± 0.4</td>
<td>No binding</td>
<td>No binding</td>
<td>No</td>
</tr>
<tr>
<td>Y393F</td>
<td>8.6 ± 0.7</td>
<td>No binding</td>
<td>43 ± 6</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Glycero-phosphate Binding Site

Capturing the Initial Steps of the Rocker-Switch Mechanism

Substrate induced straightening of Helices 5 and 11

Normal Mode Analysis

Cytoplasmic View

N-term                   C-term

H5                   H11

G. Enkavi and E. Tajkhorshid, Biochemistry 2010