Biology 5357: Chemistry & Physics of Biomolecules

Lecture 6: Active & Facilitated Transport

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Lecture Outline

1. Active transport
2. Facilitated transport
3. Methods of measuring transport
Active transport

• How is the concentration gradient set in the first place?

\[ E_K = \frac{RT}{zF} \ln \left( \frac{K_o}{K_i} \right) \]

At 25°C, \( \frac{RT}{F} \approx 25 \text{ mV} \)

\[ \Rightarrow E_K \approx -70 \text{ mV} \]
The Na+/K+ ATPase

Figure 1. The molecular structure of Na⁺,K⁺-ATPase. The figure is based upon the crystal structure of the homologous Na⁺,K⁺-ATPase in the E₂P₂K conformation (Shinoda et al., 2009) and drawn by Flemming Cornelius (Aarhus University, Aarhus, Denmark). The Na⁺,K⁺ pump comprises an α and a β subunit, a glycoprotein that participates in the translocation of the molecule from the cell interior to its correct position in the lipid bilayer of the plasma membrane. A regulatory subunit (FXYD) is also shown. During each transport cycle of the Na⁺,K⁺ pump, one ATP molecule is bound to the cytoplasmic site of the α subunit; its hydrolysis provides energy for the active transport of Na⁺ and K⁺. The transmembrane domain consists of 10 transmembrane helices and contains the binding sites for three Na⁺ or two K⁺ ions, respectively, which pass sequentially through the same cavity in the molecule during each transport cycle.
The proton motive force

\[ \Delta G = \Delta \mu_{H^+} = F \Delta \Psi + RT \ln \left( \frac{[H^+]_i}{[H^+]_o} \right) \]

\[ \rho_{\text{mf}} = \Delta \rho = - \frac{\Delta \mu_{H^+}}{F} = - \Delta \Psi + (59.1 \text{ mV}) \Delta \Phi \]
ATP Synthases

A. F$_1$-ATP synthase

OUTSIDE BACTERIAL CELL OR INTERMEMBRANE SPACE

B. Mechanism of ATP synthesis

CROSS SECTION

ADP + $P_i$ binding

ATP formed from ADP + $P_i$

ATP release

BACTERIAL CYTOPLASM OR MITOCHONDRIAL MATRIX

C. V$_1$-ATPase

VACUOLE LUMEN OR OUTSIDE CELL

ATP

ADP + $P_i$

ATP

ATP release

CYTOPLASM
Glucose transporters manage the traffic of glucose across the cell's outer membrane. They act by alternating between two states. First, the transporter has an opening facing the outside of the cell, and it picks up a molecule of glucose. Then it shifts shape, and opens towards the inside, releasing glucose into the cell. Glucose transporters generally act passively: since glucose is rapidly phosphorylated by hexokinase, the concentration of free glucose in the cytoplasm is generally very low, and the higher concentration of glucose in the blood drives transport of glucose into the cell.
Passive transport/Facilitative diffusion

e.g. semi-sweet sugar transporter

Occluded states distinguishes “transporter” from “channel” mechanism
Passive transport/Facilitative diffusion

\[ V_0 = \frac{V_{\text{max}}[S]}{K_M} \]

Active membrane transport without ATP: the alternating-access hypothesis

Conformational transitions are spontaneous and reversible

Preferred cycle directionality results from imbalance in electrochemical gradients

Mitchell, Nature '57; Jardetsky, Nature '66

Explains how uphill transport of one substrate can be powered by a transmembrane gradient in the electrochemical potential of another

Antiport/symport
Major Facilitators

The GLUT family is part of a larger group of transporters, collectively termed the major facilitator superfamily. They share a similar mechanism, with two domains that rock back and forth to transport molecules across membranes. Many, however, link transport of two different molecules, harnessing a large concentration gradient of one to power transport of the other. For instance, the bacterial lactose permease LacY (PDB entry 1pv6) uses a hydrogen ion gradient to power the transport of lactose. Both molecules move in the same direction into the cell, so LacY is named a symporter. On the other hand, GlpT (PDB entry 1pw4) is an antiporter that uses a gradient of phosphate ions to pump glycerol-3-phosphate in the opposite direction.
Active membrane transport without ATP: the alternating-access hypothesis


Explains how uphill transport of one substrate can be powered by a transmembrane gradient in the electrochemical potential of another.

- Facilitated transport
- Fully thermodynamically reversible. Directionality is set by the driving substrate
- Indirectly connected to ATPases, using Na+, K+, H+ concentration gradients as driving force

Conformational transitions are spontaneous and reversible. Preferred cycle directionality results from imbalance in electrochemical gradients.

(From J. Faraldo-Gomez http://www.faraldolab.org/)
Secondary active transport

Alternating-access mechanism: what we don’t know

Same fold & function, different stoichiometry

Same fold, different function

High-affinity ligands often inhibit, are not transported

The functional specificity of a transporter, i.e. its biological activity cannot be trivially inferred from static structures or binding properties

(From J. Faraldo-Gomez http://www.faraldolab.org/)
Secondary active transport

The DOs and DON’Ts of a symporter

- Neither the substrate or driving ions are transported alone!
  - Binding can be cooperative
  - Binding of both is required for conformational change

(From J. Faraldo-Gomez http://www.faraldolab.org/)
Inverted topology repeats in transporters

https://doi.org/10.3389/fphar.2015.00183

Vergara-Jaque et al., 2015
Fig. 1. Kinetic model of the transport cycle. (A) Three known conformations in the transport cycle. \textit{Left} show close-up views of the ion-transport pathway of the E148Q mutant of EcCLC (\textit{Top}), WT EcCLC (\textit{Middle}), and WT CmCLC (\textit{Bottom}), respectively. Selected residues are shown as sticks and Cl\textsuperscript{−} as red spheres. Right panels show schematics of different ion transport pathway conformations corresponding to structures shown on the \textit{left}.

Bacteria have developed many different types of multidrug resistance transporters to protect themselves from natural and therapeutic antibiotics. Some use the ATP-powered scissoring motion like Sav1866, which is similar to transporters that move other molecules, such as lipids and vitamins, across cell membranes. Some are simpler, such as EmrD from *Escherichia coli* (PDB entry 2gfp) which forms a small membrane pump that is powered by the flow of hydrogen ions through the protein. Some are very much more complex. The AcrB transporter (PDB entry 1iwg) pumps drugs out of the inner membrane of *Escherichia coli* and into a tube formed by TolC (PDB entry 1ek9), which directs the drugs all the way out through the outer membrane of the cell. The protein AcrA (PDB entry 2f1m) is thought to form a ring that connects AcrB and TolC, linking the entire complex into a closed tube.
(A) Simplified cartoon model of single cross-linked GltPh protomer in outward- (top) and inward-facing states (bottom). Trimerization scaffold domain is colored wheat; transport domain is blue with gating hairpins (HP1 and HP2) colored yellow and red, respectively. Substrate is represented as a blue box and ions are show as green filled circles. (B) Single GltPh protomers constrained in outward- (top) inward-facing (bottom) states. Trimerization scaffold domains are shown in surface representation and interfacial regions of the transport domains are shown as ribbons, with the remainder of the transport domain omitted for clarity. Color scheme is as in (A). Bound substrate is shown as sticks and ions as spheres with Na⁺ colored green and cross-linking Hg²⁺ black. (C) Examples of ITC binding isotherms for outward- (top) and inward-facing (bottom) states. Integrated heats are shown with baseline-subtracted inverted power data in the insets. Solid lines through the data are fits to the independent binding site model with $K_D$ of 220 and 210 nM, respectively; $n$ value of 0.8 for both; and $\Delta H$ of $-16.4$ and $-17.8$ kcal M⁻¹, respectively.
Radioactive concentrative uptake

Maduke et al., 1999. DOI: 10.1085/jgp.114.5.713

$^{36}$Cl$^-$ flux through reconstituted EriC. Concentrative uptake of $^{36}$Cl$^-$ was followed as in materials and methods. (A) Time course of accumulation of $^{36}$Cl$^-$ in vesicles reconstituted with 4.5 µg EriC/mg lipid (•, mean ± SEM, n = 4) or without protein (○, n = 1). $^{36}$Cl$^-$ release was measured after addition of valinomycin at 21 min (○, n = 1). (B) Protein concentration-dependent accumulation of $^{36}$Cl$^-$ into vesicles reconstituted with EriC (○, •) or KcsA (•). Uptake was measured at 20 min (mean ± SEM, n = 3).
Cl−-driven H+ pumping by Y445 mutants. Proton uptake against a pH gradient, driven by outwardly directed Cl− gradient was assayed in liposomes reconstituted with the indicated CLC-ec1 variants. Traces of external pH are shown. Uptake was initiated by Vln addition and reversed by FCCP.

The “Cl−-dump” experiment: raw traces. Liposomes reconstituted with CLC-ec1 at the indicated protein density (μg/mg) and loaded with 300 mM Cl− were suspended in 1 mM Cl− medium, and external Cl− concentration was monitored. Efflux was initiated by addition of Vln + FCCP. After most of the transporting liposomes had dumped their Cl−, detergent was added to release Cl− from the entire population of liposomes. Released Cl− is shown normalized to the fully dumped value. Experimental time courses (black traces) are fit with exponentials (red) as described in Materials and methods.
Reversal potential measurements of stoichiometry
**Single-molecule observation of proton pumping reveals active and inactive states.** (A) Typical examples of pH changes inside individual AHA2<sup>∞</sup> reconstituted vesicles. ATP and Mg<sup>2+</sup> (2 mM) were added to initiate proton pumping, and CCCP (5 μM) was added to collapse the pH gradients. Traces show −ΔpH defined as a difference between the initial and final pH. Images of each respective liposome at different time points are shown below each trace. At the right-hand side of the traces, we plotted histograms of pH plateaus numbered to indicate the number of active pumps per vesicle. The pH inside the majority of vesicles showed no changes indicating the absence of functional transporter molecules (top panel). For the majority of active vesicles, we observed intermittent H<sup>+</sup> pumping, indicating the presence of single molecules (middle panels). The observation of two discrete steady-state pH plateaus in single-vesicle traces indicated the occasional presence of two active pumps per single vesicle (bottom panel). (B) Population histogram of pH plateaus for AHA2<sup>∞</sup>-reconstituted vesicles (n = 3, where hereafter n is the number of independent experiments). (C and D) Same as in (A) and (B) but for full-length AHA2. For (D), n = 2. Labeling of AHA2 with Alexa Fluor 647 enabled counting on the same vesicles of both the number of labeled AHA2 proteins (E) and of the respective activity dynamics (C). (F) The histogram of active proteins per vesicle was calculated from step-bleaching analysis of the data in (E) that was corrected for labeling efficiency and the probability that a proton pump is active (12). The two independent methods for estimating the number of active molecules agreed that ~70% of vesicles containing a protein have one active proton pump.

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**Veshaguri et al., 2016. DOI: 10.1126/science.aad6429**

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(A) Illustration of AHA2 reconstituted vesicles tethered to a passivated glass surface and imaged on an individual basis with TIRF microscopy. Zoom: Extravesicular addition of both ATP and Mg<sup>2+</sup> activated exclusively outward-facing AHA2 molecules, triggering H<sup>+</sup> pumping in the vesicle lumen. We quantified changes in the vesicular H<sup>+</sup> concentration by calibrating the response of the lipid-conjugated pH-sensitive fluorophore pHrodo. Valinomycin was always present to mediate K<sup>+</sup>/H<sup>+</sup> exchange and prevent the buildup of a transmembrane electrical potential. (B) TIRF image of single vesicles tethered on a passivated glass slide. (C) Acidification kinetics of single vesicles upon addition of ATP and Mg<sup>2+</sup>. Red traces highlight three representative signals from single vesicles, showcasing the absence of transport activity, the continuous pumping of protons, and fluctuations in proton-transport activity. The black trace is the average of ≈600 single-vesicle traces. As expected, addition of the protonophore CCCP collapsed the proton gradient established by AHA2<sup>∞</sup>.
Shown are two Gltn protomers (top), labeled with the donor and acceptor fluorescent dyes, when both are in the outward-facing orientations (left), and when one of them is in an inward-facing orientation (right). The relative motions of the protomers are detected by smFRET (bottom). Such recordings reveal that protomers alternate between periods of rapid transitions and periods of quiescence, marked by black and red lines, respectively.