Lecture 7: Membrane Proteins: Direct & Secondary Active Transport

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Reading for this week:


Mechanisms of membrane transport

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

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

At 25°C, \( RT/F \sim 25 \text{ mV} \)

\[ \Rightarrow E_K \sim -70 \text{ mV} \]

- How do cells obtain key nutrients, or expel unwanted substances?
Mechanisms of membrane transport

Passive diffusion

\[ J_A = P(A_{out} - A_{in}); \quad P = \frac{D}{x} \]

From Fick’s 1st law
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**

$$J_A = k_3 [RA]; v_0 = k_{cat} [ES]$$

At $t = 0$, $A_{in} = 0$, $A = A_{out}$

$$K_d = \frac{[A][R]}{[RA]}; \quad K_M = \frac{[E][S]}{[ES]}$$

$$R_{total} = [R] + [RA]; \quad E_{total} = [E] + [ES]$$

$$K_d = \frac{[A](R_{total} - [RA])}{[RA]}$$

$$[RA] = \frac{R_{total}[A]}{K_d + [A]}$$

$$v_0 = J_A = k_3 \frac{R_{total}[A]}{K_d + [A]}$$

$$V_{max} = k_3 R_{total}$$

$$v_0 = \frac{V_{max}[A]}{K_d + [A]}$$

**Initial flux ($J_A$ or $v_0$):**

- $J_A = k_3 [RA]$; $v_0 = k_{cat} [ES]$

- $A_{in} = 0$, $A = A_{out}$

- $K_d = \frac{[A][R]}{[RA]}; \quad K_M = \frac{[E][S]}{[ES]}$

- $R_{total} = [R] + [RA]; \quad E_{total} = [E] + [ES]$

**Graph:**

- $V_{max}$
- $1/2 V_{max}$
- Facilitated transport
- Passive diffusion

**Equation:**

$$J_A = P(A_{out} - A_{in}); \quad P = \frac{D}{x}$$

**Diagram:**

- OUT
- $A_{OUT}$
- IN
- $A_{IN}$
- $R + A_{OUT} \xleftrightarrow{k_1} RA \xrightarrow{k_3} R + A_{IN}$
- $R_{total} = [R] + [RA]; E_{total} = [E] + [ES]$
Passive transport/Facilitative diffusion

Facilitated Diffusion of Glucose

Asymmetric glucose transporter showing the proportions of states with 1 mM glucose present in both inside and outside solutions.

- $K_D^{\text{out}} = 1 \text{ mM}$
- $K_D^{\text{in}} = 10 \text{ mM}$
- $K_D^{\text{out}} = 1 \text{ mM}$
- $K_D^{\text{in}} = 10 \text{ mM}$

- $k_{12} = 1$
- $k_{14} = 0.1$
- $k_{21} = 1$
- $k_{31} = 0.1$
- $k_{32} = 0.1$
- $k_{41} = 0.01$
- $k_{42} = 0.1$

Glucose in:
- $G_{\text{in}}$ (in)
- $C_{\text{in}}$ (in)

Glucose out:
- $G_{\text{out}}$ (out)
- $C_{\text{out}}$ (out)
Passive transport/Facilitative diffusion

e.g. semi-sweet sugar transporter

Occluded states distinguish “transporter” from “channel” mechanism

(A) Simplified cartoon model of single cross-linked Glts 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 shown as green filled circles. (B) Single Glts 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.
Mechanisms of membrane transport

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.
Secondary active transport

Active membrane transport without ATP: the alternating-access hypothesis

*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

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

(From J. Faraldo-Gomez http://www.faraldolab.org/)
Symport mechanisms

The DOs and DON'Ts of a symporter

(From J. Faraldo-Gomez http://www.faraldolab.org/)
Antiport mechanisms

https://www.biorxiv.org/content/10.1101/141937v1.full
CLC - antiport with minimal movement

Inverted topology repeats in transporters

https://doi.org/10.3389/fphar.2015.00183
Vergara-Jaque et al., 2015
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/)
The Na+/Ca2+ exchanger
Mechanisms of membrane transport

ABC transporters

Procko et al., 2009
P-type ATPases

The molecular structure of Na\(^+\),K\(^+\)-ATPase. The figure is based upon the crystal structure of the homologous Na\(^+\),K\(^+\)-ATPase in the E\(_2\)P\(_2\)K 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...
P-type ATPases cycle between two main conformational states E1 and E2. Hydrolysis of one ATP molecule fuels one transport cycle of ions against counter ions through the membrane.

Generic cycle exemplified by a transport stoichiometry of 2:2 ions
The chemiosmosis theory

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

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

A. $F_1$-ATP synthase
- Outside bacterial cell or intermembrane space
- $\text{ADP} + P_i$ binding
- ATP formed from $\text{ADP} + P_i$
- ATP release

B. Mechanism of ATP synthesis
- Cross section
- $\beta$ binding
- $\beta$ subunit
- ATP synthesis
- ATP release

C. $V_1$-ATPase
- Vacuole lumen or outside cell
- $\text{ADP} + P_i$
- ATP synthesis
- ATP release

D. Bacterial cytoplasm or mitochondrial matrix
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.
Radioactive concentrative uptake

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

A

\[ \text{\^{36}Cl}^\text{- flux through reconstituted EriC} \]

Concentrative uptake of \( \text{\^{36}Cl}^- \) was followed as in materials and methods. (A) Time course of accumulation of \( \text{\^{36}Cl}^- \) in vesicles reconstituted with 4.5 \( \mu \)g EriC/mg lipid (\( \bullet \), mean \( \pm \) SEM, \( n = 4 \)) or without protein (\( \bigcirc \), \( n = 1 \)). \( \text{\^{36}Cl}^- \) release was measured after addition of valinomycin at 21 min (\( \bigcirc \), \( n = 1 \)).

B

\[ \text{\^{36}Cl}^\text{- protein concentration-dependent accumulation of \( \text{\^{36}Cl}^- \) into vesicles reconstituted with EriC (\( \bigcirc \), \( \bullet \)) or KcsA (\( \bigcirc \)). Uptake was measured at 20 min (mean \( \pm \) SEM, \( n = 3 \)). \]}
**Electrical measurement of transport**

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.

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.
Reversal potential measurements of stoichiometry
Single-vesicle observation of proton pumping reveals active and inactive states. (A) Typical examples of pH changes inside individual AHA2\textsuperscript{R} reconstituted vesicles. ATP and Mg\textsuperscript{2+} (2 mM) were added to initiate proton pumping, and CCCP (5 \mu M) was added to collapse the pH gradients. Traces show \(\Delta pH\) 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\textsuperscript{+} 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\textsuperscript{R}-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 (F) and of the respective activity dynamics (C). (E) The extravesicular addition of both ATP and Mg\textsuperscript{2+} activated exclusively outward-facing AHA2 molecules, triggering H\textsuperscript{+} pumping in the vesicle lumen. We quantified changes in the vesicular H\textsuperscript{+} concentration by calibrating the response of the lipid-conjugated pH-sensitive fluorophore pHrodo. Valinomycin was always present to mediate K\textsuperscript{+}/H\textsuperscript{+} 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\textsuperscript{2+}. 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 \(\approx 600\) single-vesicle traces. As expected, addition of the protonophore CCCP collapsed the transmembrane proton gradient. 

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