Kinetic modelling of coupled transport across biological membranes

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In this report, we have modelled a secondary active co-transporter (symport and antiport), based on the classical kinetics model. Michaelis-Menten model of enzyme kinetics for a single substrate, single intermediate enzyme catalyzed reaction was proposed more than a hundred years ago. However, no single model for the kinetics of co-transport of molecules across a membrane is available in the literature. We have made several simplifying assumptions and have followed the basic Michaelis-Menten approach. The results have been simulated using GNU Octave. The results will be useful in general kinetic simulations and modelling.

Keywords: Antiport, Symport, Simulation, Octave, Michaelis-Menten kinetics

Living cells maintain different concentrations of ions and metabolites across biological membranes, so as to maintain the functionality and compartmentalization by accumulating high concentration of molecules that the cell needs, such as ions, glucose and amino acids etc. Such a strategy helps the cell to maintain intracellular environment different from the extracellular environment\(^1\). Cells have evolved several transport systems, including co-transport systems, which can transport molecules against their concentration gradient to cater to the personalised needs of various compartments. However, movement of molecules against the concentration gradient requires energy and is termed as active transport, which can be further classified as primary and secondary active transport.

Primary active transport mainly includes transmembrane ATPases and uses ATP to transport ions across the membrane, while other transporters use redox energy or light energy to facilitate the transport. In secondary active transport or coupled transport (co-transport), the energy is derived from electrochemical potential difference (free energy) created by the ion pumps, instead of directly using ATPs. The structural basis of secondary active transport mechanism is widely studied and is reviewed by Forrest\(^2\). Co-transport (or secondary active transport) can be further classified into symport and antiport, depending on the ligands movement in the same or opposite directions. Cells facilitate the transport of some substances against a concentration gradient by using energy already stored in molecular concentration gradients across the membrane, such as proton, sodium or other charge/concentration gradients via membrane proteins called transporters\(^3\).

Even after hundred years since Michaelis-Menten kinetics model was proposed, none could explain the kinetics behind co-transport of molecules across a membrane in a satisfactory, usable manner. Various earlier approaches in this direction have resulted in overly complicated models\(^4,5\).

Sometimes, the system facilitates the transport of a species by transporting another species across the membrane, where the two species may move in same or opposite direction, termed as symport and antiport, respectively (Fig. 1). The movement of one species across the membrane along its concentration gradient supplies the free energy for the movement of second species, irrespective of its concentration gradient and, therefore, the second species may or may not move along its concentration gradient\(^6\). These transports are usually tightly coupled, so that the transport is strictly 1:1 ratio of ligands across the membrane\(^7\).

In many cases, the substrates being transported are electrically charged. For such molecules, any electric field across the membrane shall cause a force acting on the molecule and this will be in addition to the force due to the concentration gradient. Depending on the charge on the substrate molecule and the electric
field, it is to be added to the concentration gradient
and the computations may be modified accordingly.

Extending Michaelis-Menten equation to a process,
where no active biochemical reaction takes place, has
not been widely used. In the present study, the
secondary active transport across membrane is
considered as a rate process and the equivalent
biochemical reaction is the transport of the molecule
across the membrane. The present approach is
intuitive, simple and conceptually elegant.

**Methodology**

The possible intermediates for a system with a
transporter (protein) E and two species A and B
located on sides 1 and 2 of a membrane and labelled
as A₁, A₂, B₁ and B₂ are shown in Fig. 1.

The protein has two binding sites on both sides of
the membrane — one for A and B each, so that the
transporter can function in a reversible manner. The
possible intermediates are shown in Fig. 2 in a
simplified form. After binding the correct number of
ligands, for example, EA₁₁B₁₁̅, EA₁₂B₂₁̅, EA₂₁B₁₂̅ for symport or
EA₁₂B₁₂̅, EA₂₁B₂₁̅ for antiport, the protein undergoes a
conformational change, resulting in the transport of
ligands to the opposite side (Fig. 2).

All the binding sites of transporter are considered
independent and equivalent. Therefore, all the binding
constants (equilibrium constants in the above
equations, k) are considered equal. This simplifies the
subsequent equations and is also a very reasonable
assumption. Looking at the symmetry of equations,
one can immediately write down the concentrations of
various intermediate species (Table 1). Flux is derived
using Michaelis-Menten approach.

In case when one or both the ligands are
electrically charged, the membrane potential i.e.
potential difference existing between the two sides of
the transporter will also play an important role. However, in the present study, we have not included
the role of electric field on the transport to maintain
costual simplicity. The electric potential can be
added to the total free energy and the computations
be carried out without loss of generality.

Following the Michaelis-Menten approach, we
calculate the total enzyme concentration:

\[
\left[E^0\right] = [E] + [EA_1] + [EA_2] + [EB_1] + [EB_2] + [EA_1A_2] + [EA_1B_1] + [EA_1B_2] + [EA_2B_1] + [EA_2B_2] + [EA_1A_2B_1] + [EA_1A_2B_2] + [EA_1B_1B_2] + [EA_2B_1B_2]
\]

where [E] is the free enzyme concentration and \[E^0\]
is the total enzyme concentration. Substituting values
from the rate equation given above, we get.

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Fig. 1 — Diagrammatic representation of two types of coupled transport: antiport and symport [A₁, A₂ represent the concentrations of ligand A in sides 1 and 2 of the membrane, respectively. Similarly, B₁ and B₂ represent the concentrations of ligand B in sides 1 and 2 of the membrane, respectively. White opaque circles indicate the ligand binding sites and it is expected that at any given point of time two of these will be occupied, giving rise to six such possibilities in both the cases. Of these six possibilities, only two will result in transportation for a given set. The arrow shown in the centre of the figure indicates the positive direction of flux and has been fixed arbitrarily for the sake of uniformity during scripting.

Fig. 2 — Intermediate reactions and the products formed during transport [Reactions involved in co-transport are not perfect bisubstrate reactions, but are essentially carried forward via two substrate binding and the substrates can bind in any order. The outline shown here represents possible combinations and thus the possible intermediates and their formation route. Of the six bisubstrate intermediates formed, two are suitable for symport and two for antiport and the rest two will block one of the channels and no transport can take place. After transport, the ligands enter the other side and thus the changes in subscript (1→2 and 2→1) indicate the respective change of side. The intermediates with three and four ligands bound are not shown for the sake of clarity because they do not give rise to effective transport and are inhibitory in nature].
Table 1—Intermediate reactions taking place during coupled transport and their rate equations [The states of transporter with three and four ligands bound to it are not shown for sake of clarity. The complete set has been included in the equations]

\[
E + A_1 \rightleftharpoons EA_1 \; k = \frac{[EA_1]}{[E][A_1]} = k[E][A_1]
\]

\[
E + A_2 \rightleftharpoons EA_2 \; k = \frac{[EA_2]}{[E][A_2]} = k[E][A_2]
\]

\[
E + B_1 \rightleftharpoons EB_1 \; k = \frac{[EB_1]}{[E][B_1]} = k[E][B_1]
\]

\[
E + B_2 \rightleftharpoons EB_2 \; k = \frac{[EB_2]}{[E][B_2]} = k[E][B_2]
\]

\[
EA_1 + A_2 \rightleftharpoons EA_1A_2 \; k = \frac{[EA_1A_2]}{[EA_1][A_2]} = k[E][A_1][A_2]
\]

\[
EA_1 + B_1 \rightleftharpoons EA_1B_1 \; k = \frac{[EA_1B_1]}{[EA_1][B_1]} = k[E][A_1][B_1]
\]

\[
EA_1 + B_2 \rightleftharpoons EA_1B_2 \; k = \frac{[EA_1B_2]}{[EA_1][B_2]} = k[E][A_1][B_2]
\]

\[
EA_2 + B_1 \rightleftharpoons EA_2B_1 \; k = \frac{[EA_2B_1]}{[EA_2][B_1]} = k[E][A_2][B_1]
\]

\[
EA_2 + B_2 \rightleftharpoons EA_2B_2 \; k = \frac{[EA_2B_2]}{[EA_2][B_2]} = k[E][A_2][B_2]
\]

\[
EB_1 + B_2 \rightleftharpoons EB_1B_2 \; k = \frac{[EB_1B_2]}{[EB_1][B_2]} = k[E][B_1][B_2]
\]

\[
\left[ E^o \right] = [E] + k[E][A_1] + k[E][A_2] + k[E][B_1] + k[E][B_2] + k^2[E][A_1][A_2] + k^2[E][A_1][B_2] + k^2[E][A_2][B_1] + k^2[E][B_1][B_2] + k'[E][B_1][B_2] + k'[E][A_1][B_2] + k'[E][A_2][B_1] + k'[E][A_1][A_2] + k'[E][A_1][A_2] + k'[E][A_2][B_1] + k'[E][A_2][B_1] + k'[E][A_2][B_1] + k'[E][A_2][B_1] + k'[E][A_2][B_1] + k'[E][A_2][B_1]
\]

\[
\frac{\left[ E^o \right]}{[E]} = S, \text{ where } S \text{ depends on } A_1, A_2, B_1, \text{ and } B_2, \text{ the expression on the right hand side (RHS) in the equation above.}
\]

The concentration of free enzyme, E can be given as

\[
[E] = \left[ E^o \right]/S
\]

This value of E has been further used for equation of symport and antiport.

Sympor

For symport, the rate of transport will be given by

\[
symport\; flux = k \cdot \left[ EA_1B_1 \right] - k'' \cdot \left[ EA_2B_2 \right]
\]

Since only A_1B_1 and A_2B_2 are the correct conformations to facilitate symport (See Figs 1 and 2). The fluxes in the two terms of the above equation are in opposite directions.
The enzyme function must be symmetric and, therefore, binding constants of the enzyme for A and B are expected to be same on both sides of the membrane. So, we assume, $k' = k''$ for all practical purposes.

From Table 1 we have,

$$[EA_1B_1] = k^2[E][A_1][B_1]$$
and
$$[EA_2B_2] = k^2[E][A_2][B_2]$$

substituting values of E, we get

$$[EA_1B_1] = (k^2[E^0])[A_1][B_1] / S$$
and
$$[EA_2B_2] = (k^2[E^0])[A_2][B_2] / S$$

substituting these values in the equation for symport flux, we have

$$\text{symport flux} = (k'k^2[E^0])([A_1][B_2] - [A_2][B_1]) / S$$

**Antiport**

For antiport, the rate of transport will be given by

$$\text{antiport flux} = k'[EA_2B_2] - k''[EA_2B_1]$$

($k'$ and $k''$ are forward and reverse rate constants, respectively) since only $A_1B_2$ and $A_2B_1$ are the correct conformations to facilitate antiport.

The enzyme function must be symmetric and we expect $k'$ and $k''$ to be equal, i.e. $k' = k''$.

From Table 1 we have,

$$[EA_2B_2] = k^2[E][A_2][B_2]$$
and
$$[EA_1B_1] = k^2[E][A_1][B_1]$$

substituting value of E, we get

$$[EA_1B_1] = (k^2[E^0])[A_1][B_1] / S$$
and
$$[EA_2B_2] = (k^2[E^0])[A_2][B_2] / S$$

substituting these values in the equation for antiport flux, we have

$$\text{antiport flux} = (k'k^2[E^0])([A_1][B_2] - [A_2][B_1]) / S$$

We have used Octave for simulation of the transporters using ODE (ordinary differential equation) solver. In our earlier works, we have used similar approach to simulate Krebs cycle, oxidative phosphorylation and mitochondrial translation\(^12\).

Octave scripts written for simulation of coupled transport process are provided in Table 2. The initial values can be varied and system behaviour can be studied accordingly.

### Table 2—Octave script used for simulation of symport and antiport

Script for symport simulation:

```octave
1;
% solve symport
% Equations for symport
% the rate equations follow:
[ds]=zeros(4,1);
s(1)=0.4; % a1
s(2)=0.3; % a2
s(3)=0.1; % b1
s(4)=0.6; % b2
f1=0.1*(s(1)*s(3)-s(2)*s(4))/t1;
alpha=(0.1*(s(1)*s(3)-s(2)*s(4)))/t1;
ds(1)=-alpha;
ds(2)=alpha;
ds(3)=-alpha;
ds(4)=alpha;
return
end
% use lsode to solve this set...
lsode_options("relative tolerance",1e-4);
lsode_options("absolute tolerance",1e-3);
t=0:0.1:50;
% supply the initial concentrations ...
s0=s;
[s,T,MSG]=lsode(@sp,s0,t);
T
(MSG
plot(t,s(:,1),"linewidth",5,t,s(:,2),"linewidth",5,t,s(:,3),"linewidth",5,t,s(:,4),"linewidth",5);
grid on;
set (gca,'FontSize', 20);
axis([0,50,-0.1,1.01]);
set (gca,'FontSize', 20)
set(get(gcf,'children'), 'linewidth', 4 )
legend("A1","A2","B1","B2", "location", "northeast")
```

We have used these equations for calculating the flux of each ligand for both systems, viz. antiport and symport. GNU Octave is a high-level interpreted language, primarily intended for numerical computations. It provides the numerical solution of linear and non-linear problems and performs other numerical experiments. It also provides extensive graphics capabilities for data visualization and manipulation\(^11\).
The simulations have been carried out with GNU Octave and the graphs have been made using gnuplot.

Results and Discussion

We plotted a curve for \([EA_1] vs. [A_1] \) to see the binding behaviour of one of the ligand, when the other ligands are present in different concentrations (Fig. 3). The presence of other ligands in higher concentrations shows an inhibitory effect on the formation of \(EA_1\) intermediate. When compared with the standard Michaelis-Menten curve, the other curves do not show the hyperbolic nature. This is most likely due to the additive nature of several parallel reactions (transports in the present study), giving rise to same product (transport of the molecule concerned being the product). Individually, each reaction will give a hyperbolic behaviour, when studied in isolation, but the sum of several such processes is not necessarily going to reflect in a hyperbolic graph.

The results of flux simulation are represented graphically for symport and antiport (Fig. 4).

Symport

The simulation curves in Fig. 4a indicate the behaviour of two species, depending on their concentration gradient across a membrane in a symport system. Here, the concentration gradient of A and B across the membrane is 0.1 and -0.5. The gradient of B is higher and hence it will move along its concentration gradient and will drive the movement of A against the concentration gradient. Here, the blue and green curves form a pair \((A_1-A_2)\) and A moves against the concentration gradient. The
free energy of the concentration gradient of B is partly used up in building the free energy of the concentration gradient of A, while the overall free energy of the combined process decreases as a spontaneous reaction.

**Antiport**

The simulation curves in Fig. 4b indicate the behaviour of two species A and B, depending on their concentration gradient across a membrane in an antiport system. Here, the concentration gradient of A and B across the membrane is 0.1 and -0.5. The gradient of B is higher and hence it will move along its concentration gradient and will drive the movement of A in the opposite direction being coupled in an antiport. As seen in the figure, A moves along the concentration gradient initially, but continues to move in the same direction even after attaining same concentration on both sides of the membrane and this movement against the concentration gradient is driven by the movement of B along its concentration gradient.

A surface plot is plotted by keeping the concentration of \(A_2\) and \(B_2\) constant and varying the concentration of \(A_1\) and \(B_1\) in the range of 1 to 10 (Fig. 5).

**Allosteric effect**

The binding of ligands to the transporter complex resembles the allosteric binding process, first proposed by Monod et al\(^{13}\). This involves co-operativity, leading to strong binding of subsequent ligand with a change in conformation of different subunits. It is indeed important to include interactions between different binding sites on the transporter. The substrate binding to the transporter is accordingly affected by this internal interaction and this can be modelled according to the Monod's allosteric model\(^{13}\). We have avoided this approach basically to keep the transporter model simple and avoiding additional computational complexities. It is in fact quite straight forward to include any arbitrary binding process in our present model.

Many transporters are also made up of multiple subunits, which is an important requirement to attain the symmetry of the transporter on either side of the membrane. It is highly likely that a mechanism similar to cooperative binding will be operative in transporters in membranes. Co-transport of ligands will then rapidly take place when the required conformational conditions are achieved\(^{14}\). However,
the experimental work in this direction is scant, because of the difficulties in working with the membrane-bound proteins. Experimental detection of conformational changes in a protein due to binding of multiple ligands is also very tricky.

Conclusion
We have simulated co-transport across a transporter. This approach is based on Michaelis-Menten approximation and can be used for kinetic simulation of shuttles operating across the membranes, such as malate-aspartate shuttle, citrate-pyruvate shuttle etc.

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References
6 Krupka R M (1993) *Biochim Biophys Acta (BBA)-Bioenergetics* 1183, 105-113
8 Geck P & Heinz E (1976) *Biochim Biophys Acta (BBA)-Biomembranes* 443, 49-63
9 Michaelis L & Menten M L (1913) *Biochem Z* 49, 333-369
10 Heinz E, Geck P & Wilbrandt W (1972) *Biochim Biophys Acta (BBA)-Biomembranes* 255, 442-461
11 GNU Octave: http://www.gnu.org/software/octave/
14 Jardetzky O (1966) *Nature* 211, 969-970