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Modeling Transport Kinetics with StarLogo

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Abstract—**StarLogo, an agent-based modeling and simulation platform, was used to simulate adsorption-mediated transcytosis of a molecule from the lumen side of a cell membrane to the abluminal extra-cellular fluid (ECF). The model contains small non-diffusible substrate molecules, transporters, and substrate-transporter agents. The "reaction" is a transporter combining with the substrate which then crosses the cell cytoplasm. The substrate that is deposited on the ECF side becomes the "product." Results showed characteristics consistent with Michaelis-Menten enzyme kinetics. The model can serve as an example of agent-based modeling and simulation.**

Keywords—**membrane transport, agent-based model (ABM), Michaelis-Menten kinetics**

I. INTRODUCTION

Systems biology involves the interaction of individual units at different biological levels. Intracellular enzymes, cells, tissues and organs, interacting to form a dynamic structure [1]. Modeling the interactions that take place in one biological system, a cell membrane (e.g., gastrointestinal tract or blood brain barrier) allows the different experimental conditions that affect drug or nutrient membrane transport to be simulated and studied prior to, or in place of, wet lab experiments [3].

One approach to representing complex systems is agent-based modeling (ABM). Agents, implemented in object-oriented programming, are objects capable of sensing and modifying the states of other agents as well as that of their environment [4]. There can be multiple types of agents in an ABM program—each species possessing different parameters and functions. This feature of ABM makes it appealing to applications in biological systems because of their high degree of complexity and hetereogenity. Additionally, the agent structure offers a modular alternative to large sets of complex equations, such as ordinary or partial differential equations, which have traditionally been used to describe a non-linear system. [5-8]. One example of the use of ABM as a complex systems modeling tool in biology is modeling kinetics of cell membrane transport.

We present an ABM representation of one transport process, adsorption-mediated transcytosis. To evaluate the appropriateness of this modeling technique, we test whether the ABM system can be redefined in terms of Michealis-Menten kinetics, a standard kinetic model in cell membrane transport. Students who wish to understand the basics of systems biology modeling can use this as an example to study and build their own StarLogo programs.

A. Adsorption-Mediated Transcytosis

Adsorption-mediated transcytosis involves substrate uptake into the cell cytoplasm by adsorption or plasmalemmal vesicles, and transport across to the abluminal side. Initial luminal substrate attachment may occur because of cationic-anionic attraction at the membrane [2], or binding of the substrate to a fluid-borne transporter on the luminal side.

Examples of adsorption-mediated transcytosis are the transport of polyclonal bovine immunoglobulin and heptapeptide E-2078 across the blood-brain barrier [2, 9]; albumin-ligand transport via vesicles [9]; albondin-mediated lung capillary permeability to albumin [11]; and high-density lipoprotein scavenger receptor transport in kidney cells [12]. Beyond basic science interest, modeling adsoprtion-mediated transcytosis is of relevance because of its applications in drug delivery [13]. Peptide vectors are currently being designed and tested to transport drugs via adsorption-mediated transcytosis [2].

B. Michealis-Menten

Michealis-Menten kinetics is used in biology to represent enzyme and substrate binding. In particular, for our purposes, Michaelis-Menten has been used to model transport of solutes and drugs across membranes (e.g., glucose across the blood-brain barrier). The Michaelis-Menten equation is where initial velocity is a function of initial substrate concentration:

$$V_0 = (V_{max}\,[S]) / (K_m + [S]) \qquad (1)$$

where V_0 is the initial rate of reaction (mol/g-min), V_{max} is the maximal rate of transport (mol/g-min), S is the substrate concentration (mM) and K_m is the Michealis-Menten constant (half-saturation constant, mM). For ease of graphical representation, the Lineweaver-Burk equation transforms (1) so that the relationship between $1/V_0$ and 1/S is linear [14]. The constants V_{max} and K_m can be calculated from the slope and y-intercepts:

$$1/V_0 = (K_m/V_{max})(1/[S]) + 1/V_{max} \qquad (2)$$

When applying Michealis-Menten to the agent-based model presented here, the enzyme is represented by a transporter, and the substrate is the molecule being transported across the cell membrane. The model design and parameters as well as simulation results are presented. The goal of this experiment is to simulate membrane transport from a stochastic, even-based perspective using

ABM programming software that is simple enough to be used by non-expert programmers.

C. StarLogo Software Background

StarLogo was the software of choice, for simplicity and basic ABM capability. Based on Logo, the StarLogo application was created by the Media Lab group at MIT using Java. StarLogo is a "programmable modeling environment for exploring the workings of decentralized systems" [15]. Some of the suggested uses are modeling populations of organisms or movements of molecules. The StarLogo user can create independent agents that move on their own accord; in StarLogo lingo, these agents are called turtles. When there is more than one type of agent—in our case, substrate and transporters—each population is called a breed. Each unit of space in the two-dimensional grid environment is referred to as a "patch" and the "observer" is allowed to change patches and turtles [16].

One characteristic feature of ABM is stochastic representation. As opposed to continuous, deterministic systems, there is a randomness associated with agent movement and rules. The random number generator in StarLogo automatically reseeds every time a simulation is run such that each trial results in a somewhat different outcome. This is good for consistent randomization. A disadvantage is that this automatic reseeding also prevents the user from regenerating earlier results or being able to make manipulations in the code and seeing the effects on the same trial.

The StarLogo software is simple enough for novices to learn quickly yet powerful enough to model and simulate complicated biomedical phenomena [17]. Students can easily build a model that generates analyzable data.

II. METHODOLOGY

A. System Environment Parameters

Three regions needed to be represented: the luminal, cell membrane, and abluminal side. To simulate the effects of blood flow in capillaries, molecules are allowed to move left to right [18]—molecules that run off the right side of the screen wrap to the left side, so that the initial representation would be analogous to placing molecules into a cell culture well for transport studies [11]. An impenetrable border is set at the top and bottom of the environment.

When the program is started, the substrate randomly disperses itself from the center of the luminal side's channel. There is a negligible bolus effect since the arterial channel is nearly square and the substrate is evenly distributed. The transporters (10 or 20) are dispersed from the center as well. The dimensions of Version 1 and 2 are discussed in Table 1.

TABLE 1 Patch dimensions of the environment

version	width	luminal	membrane	abluminal
1	28	25	7	18
2	28	25	14	11

B. Agent Parameters

There are three agents: the substrate, the transporter, and the substrate-transporter (Fig. 1). Since substrates are impenetrable to the cell membrane, they can only move in the luminal and abluminal sides. Transporters and substrate-transporter agents move in all three regions. Molecules in the lumen tend to move right to left with blood flow and downwards (towards the walls) due to simulated laminar flow. Once a substrate joins with a transporter, the unit diffuses across the cell cytoplasm. The substrate-transporter agent then moves to the abluminal side and they separate, freeing the substrate. The transporter is free to return to the lumen side and combine with another substrate. Equilibrium is reached when the number of substrate on both sides of the membrane are equal. Substrate-transporters and transporter cease moving with specific direction and begin to move randomly.

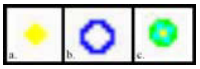

Fig. 1. Breeds in model: a) substrate; b) transporters; and c) substrate-transporter units.

Agents move on a nine-neighbor square grid [19] (Fig. 4). There are eight options of movement. Each has a different movement rule based on location. Fig. 2 and Table 2 describe the agents' movement rules.

Fig. 2. Turtles move on a grid environment. Molecules in this model can only move to adjacent spaces.

TABLE 2 Grid movement allowed for agent (see Fig. 2)

	Substrate	Transporter	Substrate-Transporter
Luminal	2, 3, 4, 5	2, 3, 4, 5	3, 4, 5
Membrane	NA	7, 8, 1, 2, 3	3, 4, 5, 6, 7
Abluminal	1-8	7, 8, 1, 2, 3	1-8

C. Initialization, Simulation, and Output

Duration of an experiment in StarLogo depends on the processor speed and the number of active agents [15]. For simulations that need to be run for designated amounts of time, the number of data entries during an experiment serve as a measure of time (e.g. data from the *n*th entry at time *t* can be considered to be at the same time as the *n*th entry of another set of data).

Initialization, as mentioned above, begins with *s* substrates and *tr* transporters randomly distributed from the center of the luminal-side fluid (e.g., blood in capillaries of the blood-brain barrier, or outer intestinal fluid in the GI tract). For this experiment, the simulation was run until

more than 500 data points were collected (values for the luminal and abluminal sides as well as the number of free and joined transporter units). The *t*th data point is the measure of time. This process was repeated for initial values of *s* = 25-300 in increments of 25. Both versions were simulated with *tr* = 10 and *tr* = 20. Ten simulations of each condition (i.e. s = 100, tr = 10) were run. An example result is presented in Fig. 6. Data from the simulations were extracted and averaged, and a Lineweaver-Burk plot is created to calculate kinetic constants.

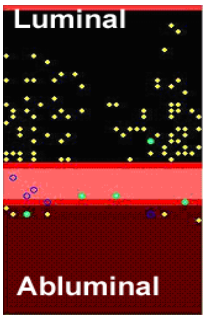

Fig. 3. Example of Version 1, with 100 substrate, 10 transporters, and at time 150.

III. RESULTS

To represent the ABM model in Michealis-Menten terminology, the units for V_m are (molecules/time unit) and K_m are molecules. The constants were derived from the Lineweaver-Burk linear regression equations. The times t = 150 and t = 200 were used because at those points, 10% or less of the substrate had been transported to the across the abluminal side, which allows for the calculation of V_{max} [20].

TABLE 3
V_{max} and K_m values using 10 and 20 transporters. Time ranges from t_0 = 0 to t = 150.

	tr = 10			tr = 20		
version	V_{max}	K_m	R^2	V_{max}	K_m	R^2
1	0.083	13.99	0.95	0.172	20.41	0.98
2	0.068	6.29	0.96	0.139	11.32	0.98

TABLE 4
V_{max} and K_m values using 10 and 20 transporters. Time ranges from t_0 = 0 to t = 200.

	tr = 10			tr = 20		
version	Vm	Km	R2	Vm	Km	R2
1	0.098	21.96	0.99	0.204	31.00	0.99
2	0.053	4.78	0.84	0.108	7.81	0.96

Results from all the simulations show Michaelis-Menten-like behavior. Figure 4 graphs the initial number of plasma substrate found on the luminal side against the number of abluminal substrate at time *t* and suggests transporter saturation at high substrate concentrations, a characteristic of Michaelis-Menten [21].

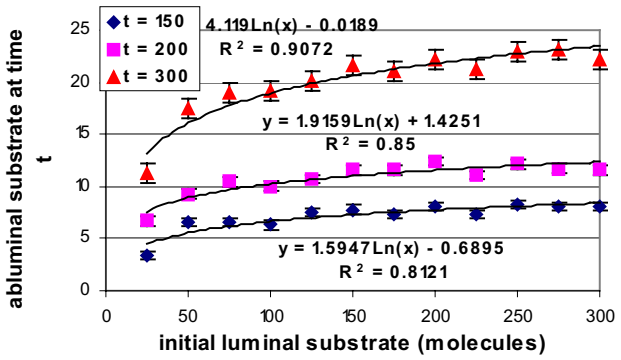

Fig. 4. Version 1, with 10 transporters. Graph of initial substrate count vs. abluminal substrate count at time *t* = 150, 200 and 300.

Two other graphs, the Lineweaver-Burk and Michaelis-Menten, were created for *tr* = 10 and *tr* = 20. The Michaelis-Menten (Fig. 5) plots initial luminal substrate (V_o) against initial velocity (dC/dt). The data are consistent with a logarithmic relationship. Lineweaver-Burk plots are presented in Fig. 6.

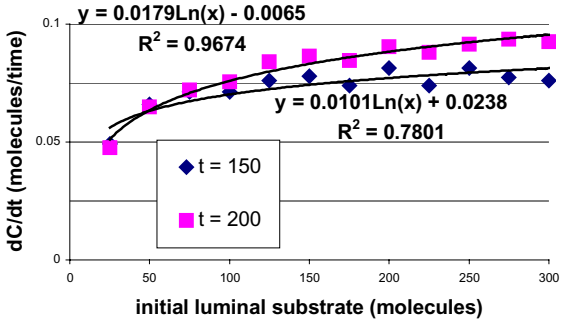

Fig. 5. Michaelis-Menten graph for Version 1, 10 transporters and 100 substrate.

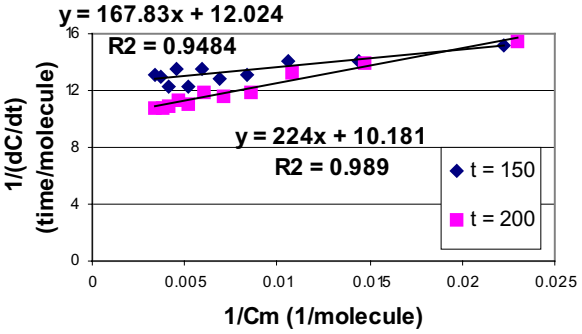

Fig. 6. Lineweaver-Burk graph for Version 1, 10 transporters and 100 substrate.

IV. DISCUSSION AND CONCLUSION

Adsorption-mediated transcytosis, which is an otherwise difficult biological process to study at the molecular level, has been modeled with discrete events and time that can sufficiently emulate membrane transport. The analysis shows the results from StarLogo model exhibit Michaelis-Menten properties. Both the initial luminal substrate versus abluminal substrate and the Michaelis-Menten graphs have indicative logarithmic curves; and the high R^2 values in the Lineweaver-Burk graphs illustrate the expected linear relationship. The expected relationship is verified when the V_{max} values for 20 transporters are double that of the value for 10 transporters. When the width of the membrane was doubled, V_{max} increased, although not linearly. This pattern also underlines Michaelis-Menten kinetics.

In the ABM model, changing properties of either the agents or the environment allows the user to simulate other possible scenarios whose data can also be statistically analyzed. This model is only one of many membrane transport processes that can be modeled and students can apply these methodologies to other mathematical models or biological systems.

As a particular ABM tool, StarLogo is simple with limited flexibility compared with more advanced ABM programming software [22] like Swarm [23] or Repast [24]. However, it has advantages. StarLogo can help those not familiar with agent based programming understand the concepts involved as well as let those who are not adept at programming participate in model making. A plausible classroom assignment could consist of students, individually or in pairs, building their own programs in StarLogo to simulate a phenomenon of their choice and analyzing their generated data using a variety of mathematical models. From this StarLogo example we conclude that accessible agent-based model building is one way to build an understanding of the mechanistic details in membrane transport. The model can serve an example for the future use of StarLogo and agent-based model building applied to biological processes.

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