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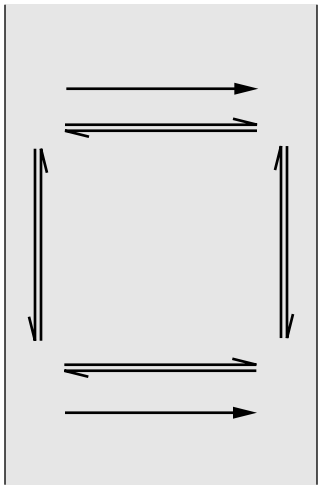
Chapter 4

STEADY-STATE CHEMICALLY MEDIATED TRANSPORT



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4.1 INTRODUCTION

Transport of many solutes including important metabolites (e.g. simple sugars or amino acids) through cellular membranes is accomplished by membrane-bound carrier molecules (transporters) that combine with the solute molecule on one face of the membrane, then translocate in the membrane and uncombine at the other face. Thus transport involves binding and unbinding chemical reactions at a site on the transporter. Different molecules compete for this site; for example, glucose and sorbose (two sugars) compete for the sugar transporter site. Hence, transport of one sugar can inhibit transport of another simply by occupying a site to which both can bind. This type of transport is called *chemically-mediated transport*.

There are canonical models of chemically-mediated transport that capture important properties of the transport of metabolites. It is important to understand these canonical models in order to understand how metabolites are transported across membranes. Derivations of predictions of these models are not particularly difficult to follow; the individual steps are simple. However, the models typically result in messy algebraic expressions that relate flux to concentration and transport parameters. Thus it is easy to get lost in algebraic manipulation as well as in a sea of parameters so that an intuitive grasp of the models can be missed. The simulation of these equations is intended to develop intuition for these models.

4.2 DESCRIPTION OF THE MODEL

Descriptions of chemically-mediated transport as well as models of such transport processes can be found elsewhere [Weiss, 1996]. Here we consider two models, one a special case of the other, and list both the assumptions and important results. First we consider a transporter that binds only one solute; then we consider a transporter that binds two solutes with different affinities. Because the resulting equations for equilibrium of the transporter with solute are analogous to those of the binding of an enzyme to its substrate we refer to the transporter as an enzyme.

4.2.1 Single solute

We assume that the membrane contains N_{ET} moles of enzyme per cm^2 of membrane. Each of these enzymes exist in one of four states which we label EA^i, EA^o, E^i, and E^o (Figure 4.1). In the EA states, the solute A is bound to the enzyme E; in the E state the enzyme is unbound. In the EA^i and E^i states, the enzyme, bound and unbound, communicates with the solution on the inner side of the membrane. In the EA^o and E^o states, the enzyme, bound and unbound, communicates with the solution on the outer side of the membrane. The concentrations of enzyme in the four states are n^i_{EA}, n^o_{EA}, n^i_E, and n^o_E moles/cm^2. The fluxes of bound and unbound enzyme are ϕ_{EA} and ϕ_E and the flux of solute is ϕ_A. The flux is defined as positive when the flux is in the outward direction; the units are in moles/cm^2-sec. The model is defined by the following assumptions:

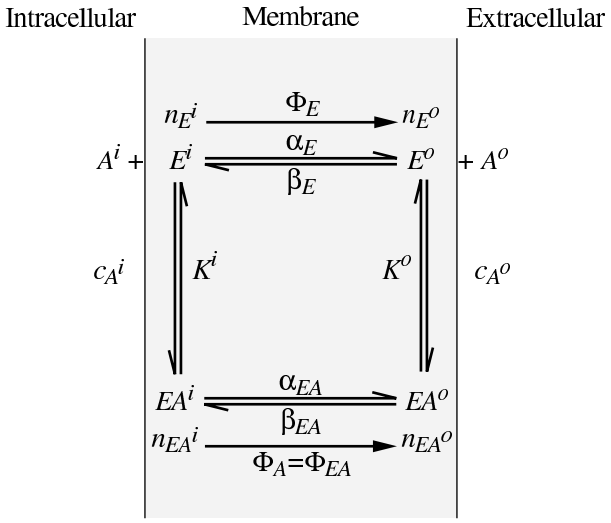

Figure 4.1: Kinetic diagram of a chemically-mediated transport model in which the carrier binds a single solute.

- The total amount of enzyme, bound and unbound, is constant, i.e. the sum of the concentration of enzyme over all of its states equals the total concentration of enzyme

$$N_{ET} = n^i_{EA} + n^o_{EA} + n^i_E + n^o_E \, . \tag{4.1}$$

- Since the enzyme resides permanently in the membrane, the total flux of enzyme must be zero, i.e.

$$\phi_{EA} + \phi_E = 0 \tag{4.2}$$

- The only way the solute can cross the membrane is when it is bound to the enzyme; EA^o is assumed to undergo a reversible change in conformation to the form EA^i. We assume that the unbound enzyme undergoes a similar reaction between the two conformations E^i and E^o. These two reactions are assumed to be first-order reactions with forward and reverse rate constants, α_E, α_{EA}, β_E, and β_{EA}. Therefore,

$$\frac{dn^o_{EA}}{dt} = \alpha_{EA} n^i_{EA} - \beta_{EA} n^o_{EA} \, , \tag{4.3}$$

and

$$\frac{dn^o_E}{dt} = \alpha_E n^i_E - \beta_E n^o_E \, , \tag{4.4}$$

- The fluxes equal the rates of change of enzyme concentration, so that

$$\phi_{EA} = \phi_A = \frac{dn^o_{EA}}{dt} = \alpha_{EA} n^i_{EA} - \beta_{EA} n^o_{EA} \, , \tag{4.5}$$

Similarly,

$$\phi_E = \frac{dn^o_E}{dt} = \alpha_E n^i_E - \beta_E n^o_E \, . \tag{4.6}$$

- The binding reactions at the membrane interfaces are assumed to be rapid compared to the rate of transport of solute across the membrane so that the membrane interface reactions are assumed to be at equilibrium, i.e.,

$$\frac{c_A^o n_E^o}{n_{EA}^o} = K_A^o \text{ and } \frac{c_A^i n_E^i}{n_{EA}^i} = K_A^i \,, \tag{4.7}$$

  where K_A^o and K_A^i are the dissociation constants on the two membrane interfaces.

We wish to sove these equations to find the n's and the ϕ's as a function of the concentrations of solute A and of the transport parameters. One way to obtain these solutions is to solve for the n's in terms of the concentrations and transport parameters and then to use Equations 4.5 and 4.6 to find the ϕ's. By combining Equations 4.2, 4.5, and 4.6, we obtain

$$(\alpha_{EA} n_{EA}^i - \beta_{EA} n_{EA}^o) + (\alpha_E n_E^i - \beta_E n_E^o) = 0 \,. \tag{4.8}$$

We can express Equations 4.7, 4.8, and 4.1 as a matrix equation as follows:

$$\begin{bmatrix} K_A^i & 0 & -c_A^i & 0 \\ 0 & K_A^o & 0 & -c_A^o \\ \alpha_{EA} & -\beta_{EA} & \alpha_E & -\beta_E \\ 1 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} n_{EA}^i \\ n_{EA}^o \\ n_E^i \\ n_E^o \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ N_{ET} \end{bmatrix} . \tag{4.9}$$

The first two rows correspond to the two relations in Equation 4.7. The third row results from Equation 4.8, and the fourth row corresponds to Equation 4.1. This set of simultaneous equations has the following solutions:

$$n_{EA}^i = N_{ET} \frac{c_A^i(\beta_E K_A^o + \beta_{EA} c_A^o)}{D_1} \,, \tag{4.10}$$

$$n_{EA}^o = N_{ET} \frac{c_A^o(\alpha_E K_A^i + \alpha_{EA} c_A^i)}{D_1} \,, \tag{4.11}$$

$$n_E^i = N_{ET} \frac{K_A^i(\beta_E K_A^o + \beta_{EA} c_A^o)}{D_1} \,, \tag{4.12}$$

$$n_E^o = N_{ET} \frac{K_A^o(\alpha_E K_A^i + \alpha_{EA} c_A^i)}{D_1} \,, \tag{4.13}$$

where

$$D_1 = (\beta_E K_A^o + \beta_{EA} c_A^o)(K_A^i + c_A^i) + (\alpha_E K_A^i + \alpha_{EA} c_A^i)(K_A^o + c_A^o) \,. \tag{4.14}$$

ϕ_A and ϕ_E can be obtained from Equations 4.5 and 4.6.

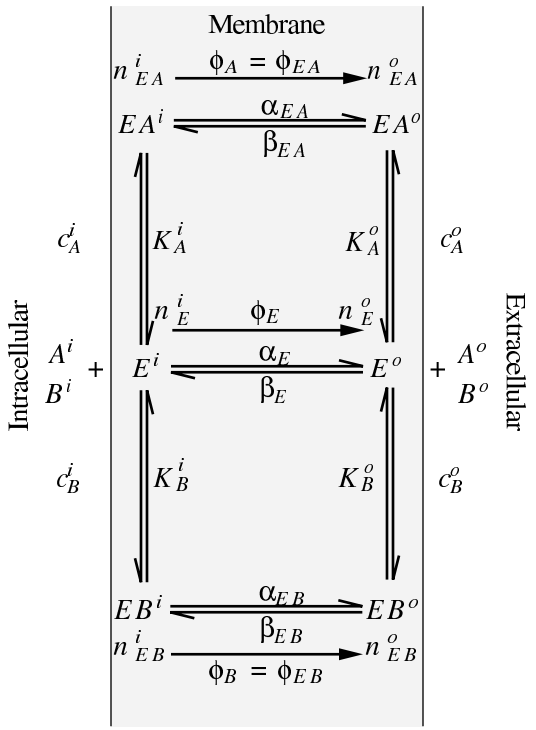

Figure 4.2: Kinetic diagram of a chemically-mediated transport model in which the carrier can bind one of two solute.

4.2.2 Two solutes

The single-solute model shown in Figure 4.1 can be extended to account for the binding of two solutes that compete competitively for binding sites on the enzyme (Figure 4.2). In this scheme, solutes A and B can combine with enzyme E but with different affinities. The binding to solute A has dissociation constants K_A^i and K_A^o and the binding to B has dissociation constants K_B^i and K_B^o.

The kinetic equations are analogous to those derived for the single-solute case except that the enzyme now has 6 states:

- We assume that the total amount of enzyme, free and complexed, is constant,

$$N_{ET} = n_{EA}^i + n_{EA}^o + n_{EB}^i + n_{EB}^o + n_E^i + n_E^o \,, \tag{4.15}$$

  where N_{ET} is the total concentration of enzyme in the membrane.

- Since the enzyme remains in the membrane, the net flux of enzyme must be zero

$$\phi_{EA} + \phi_{EB} + \phi_E = 0 \tag{4.16}$$

- The fluxes equal the rate of change of enzyme concentration so that for solute A

$$\phi_{EA} = \phi_A = \frac{dn_{EA}^o}{dt} = \left(\alpha_{EA} n_{EA}^i - \beta_{EA} n_{EA}^o\right) . \tag{4.17}$$

For solute B

$$\phi_{EB} = \phi_B = \frac{dn^o_{EB}}{dt} = (\alpha_{EB} n^i_{EB} - \beta_{EB} n^o_{EB}) \,. \tag{4.18}$$

Also

$$\phi_E = \frac{dn^o_E}{dt} = (\alpha_E n^i_E - \beta_E n^o_E) \,. \tag{4.19}$$

- The reactions at the membrane interfaces are assumed to take place so rapidly compared to the rate of transport of solute across the membrane that the membrane interface reactions are assumed to be at equilibrium, i.e.,

$$\frac{c^o_A n^o_E}{n^o_{EA}} = K^o_A \,, \frac{c^i_A n^i_E}{n^i_{EA}} = K^i_A \text{ and } \frac{c^o_B n^o_E}{n^o_{EB}} = K^o_B \,, \frac{c^i_B n^i_E}{n^i_{EB}} = K^i_B \,, \tag{4.20}$$

  where K^o_A, K^i_A, K^o_B and K^i_B are the dissociation constants for solutes A and B, respectively at the two membrane interfaces.

By combining Equations 4.16 and 4.17, 4.18, and 4.19, we obtain

$$(\alpha_{EA} n^i_{EA} - \beta_{EA} n^o_{EA}) + (\alpha_{EB} n^i_{EB} - \beta_{EB} n^o_{EB}) + (\alpha_E n^i_E - \beta_E n^o_E) = 0 \,. \tag{4.21}$$

We wish to obtain the flux of solutes A and B as a function of both concentrations and the transport parameters K^i_A, K^o_A, K^i_B, K^o_B, α_{EA}, α_{EB}, α_E, β_{EA}, β_{EB}, β_E, and N_{ET}. Therefore, it is useful to regard the system of algebraic equations given by Equations 4.16 through 4.21 as a set of 6 equations in the 6 unknowns n^i_{EA}, n^o_{EA}, n^i_{EB}, n^o_{EB}, n^i_E, and n^o_E. With this in mind we can rewrite the equations in matrix form as follows:

$$\begin{bmatrix} K^i_A & 0 & 0 & 0 & -c^i_A & 0 \\ 0 & K^o_A & 0 & 0 & 0 & -c^o_A \\ 0 & 0 & K^i_B & 0 & -c^i_B & 0 \\ 0 & 0 & 0 & K^o_B & 0 & -c^o_B \\ \alpha_{EA} & -\beta_{EA} & \alpha_{EB} & -\beta_{EB} & \alpha_E & -\beta_E \\ 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} n^i_{EA} \\ n^o_{EA} \\ n^i_{EB} \\ n^o_{EB} \\ n^i_E \\ n^o_E \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ N_{ET} \end{bmatrix} \,. \tag{4.22}$$

The first four rows correspond to the four relations in Equation 4.20. The fifth row results from Equation 4.21, and the sixth row corresponds to Equation 4.15. This set of simultaneous equations has solutions:[1]

$$n^i_{EA} = N_{ET} \frac{c^i_A K^i_B (\beta_E K^o_A K^o_B + \beta_{EA} c^o_A K^o_B + \beta_{EB} K^o_A c^o_B)}{D_2} \,, \tag{4.23}$$

$$n^o_{EA} = N_{ET} \frac{c^o_A K^o_B (\alpha_E K^i_A K^i_B + \alpha_{EA} c^i_A K^i_B + \alpha_{EB} K^i_A c^i_B)}{D_2} \,, \tag{4.24}$$

$$n^i_{EB} = N_{ET} \frac{K^i_A c^i_B (\beta_E K^o_A K^o_B + \beta_{EA} c^o_A K^o_B + \beta_{EB} K^o_A c^o_B)}{D_2} \,, \tag{4.25}$$

[1]Solutions were obtained using the symbolic mathematics software packages *MACSYMA* and *MATHEMATICA*. Equations 4.10-4.13 were obtained by setting $c^i_B = c^o_B = 0$ in Equations 4.23-4.28.

$$n^o_{EB} = N_{ET}\frac{c^o_B K^o_A(\alpha_E K^i_A K^i_B + \alpha_{EA} c^i_A K^i_B + \alpha_{EB} K^i_A c^i_B)}{D_2}\,, \tag{4.26}$$

$$n^i_E = N_{ET}\frac{K^i_A K^i_B(\beta_E K^o_A K^o_B + \beta_{EA} c^o_A K^o_B + \beta_{EB} K^o_A c^o_B)}{D_2}\,, \tag{4.27}$$

$$n^o_E = N_{ET}\frac{K^o_A K^o_B(\alpha_E K^i_A K^i_B + \alpha_{EA} c^i_A K^i_B + \alpha_{EB} K^i_A c^i_B)}{D_2}\,, \tag{4.28}$$

where

$$\begin{aligned} D_2 \;=\; & K^i_A(c^i_B\alpha_{EB} + K^i_B\alpha_E)(K^o_A K^o_B + c^o_A K^o_B + K^o_A c^o_B) + \\ & K^o_A(c^o_B\beta_{EB} + K^o_B\beta_E)(K^i_A K^i_B + c^i_A K^i_B + K^i_A c^i_B) + \\ & c^i_A K^i_B\alpha_{EA}(K^o_A K^o_B + c^o_A K^o_B + K^o_A c^o_B) + \\ & c^o_A K^o_B\beta_{EA}(K^i_A K^i_B + c^i_A K^i_B + K^i_A c^i_B)\,. \end{aligned} \tag{4.29}$$

4.2.3 Choice of numerical parameters

The software enables the user to compute the n's and ϕ's for any values of the parameters, the α's, β's, and K's, and the concentrations of solute, the c's. The software is initiated with default parameters chosen to approximate hexose transport in human erythrocytes [Carruthers, 1984, Stein, 1986] assuming a symmetric transport scheme. The density of transporters was set to $N_{ET} = 10$ pmoles/cm^2. The dissociation constant of solute A was set to approximate that of D-glucose, $K^i_A = K^o_A = 2$ μmoles/cm^2, and that of B was set to that of a solute to which the transporter binds with lower affinity, approximating that of D-xylose, so that $K^i_B = K^o_B = 200$ μmoles/cm^2. All the rate constants were set equal with a value that made the maximum flux 100 pmoles/cm^2-s so that $\alpha_{EA} = \beta_{EA} = \alpha_{EB} = \beta_{EB} = \alpha_E = \beta_E = 20\,\text{s}^{-1}$. The concentrations of solute were chosen arbitrarily as follows: $C^i_A = 2$, $C^0_A = 1$, $C^i_B = 2$, $C^o_B = 1$ μmoles/cm^3.

4.3 USER'S GUIDE

The software has two environments. In the *interactive environment*, the user can change the independent variables also called the parameters (the rate constants, the α's and β's; the dissociation constants, the K's; and the concentrations, the c's) and observe changes in the dependent variables (the enzyme concentrations, the n's; and the fluxes, the ϕ's). In the *graphing environment* the user can plot any dependent variable versus any independent variable over a specified range of the independent variable.

4.3.1 Interactive environment

When the software is initiated, it is in the interactive environment. The display (Figure 4.3) contains a large window with a menubar and two panels. One panel is labelled *Transport Parameters* (left) and we shall refer to it as the Parameters panel. The other is labelled

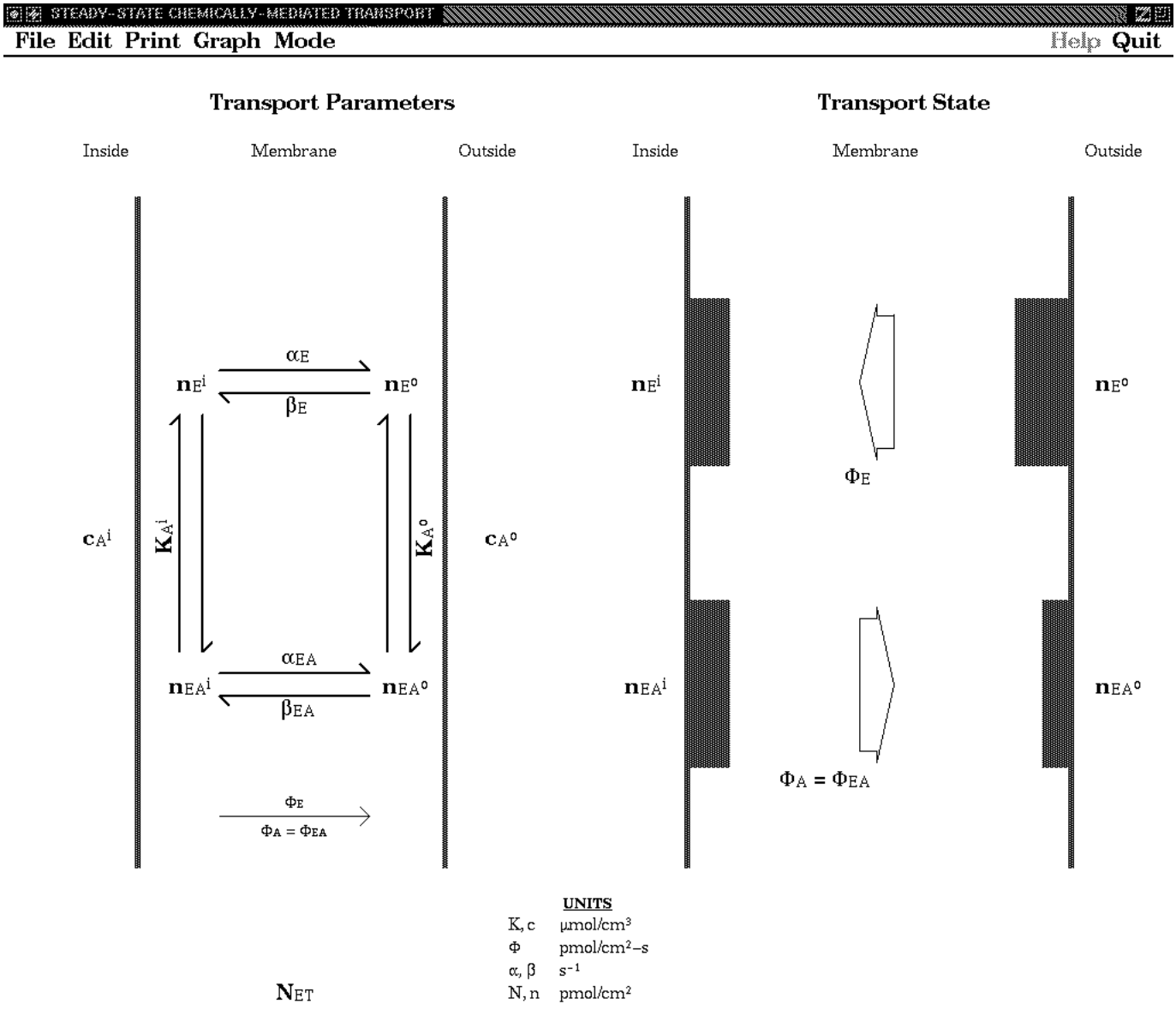

Figure 4.3: Display when software is initiated. The software is in the Interactive Environment with the Single Solute mode selected.

Transport State (right) and we shall refer to it as the State panel. The Parameters panel shows a schematic diagram of the transport mechanism with both the independent and dependent variables shown. The State panel, shows the state of the transport mechanism, i.e. the values of all the dependent variables, for the chosen (initially the default) values of the independent variables. The lengths of the dark bars are proportional to the concentrations of the enzyme in its four permissible states. The arrows indicate the directions and the relative magnitudes of the fluxes; the thickness of the arrow is proportional to the relative magnitude of the flux. The menubar contains **File, Edit, Print, Graph, Mode, Help and Quit** options.

File

File handling is the same as described in the chapter on Random Walk Model of Diffusion. The only difference is that file extensions in the Chemically Mediated Transported package are **.cmt**. The **File** menu has three choices: read in data from file, write to file, delete a file. All three choices allow the user to search their directory tree to find the file to be read, written or deleted. When any of the three options is selected, a scroll bar is displayed showing all the filenames in the current directory. Selecting any filename or typing a filename into the text edit window results in one of two possibilities. If the selected filename is the name of a directory, then that directory can be selected by clicking on **Set Directory**. If the filename is the name of a file then that file can be read, written or deleted. Selecting the **Read in data from file...** entry reads a file containing all the parameter values but does not change simulation modes. The order in which the values are read and stored in the file is as follows: n_E^i, n_E^o, n_{EA}^i, n_{EA}^o, n_{EB}^i, n_{EB}^o, ϕ_E, ϕ_{EA}, ϕ_{EB}, α_E, β_E, α_{EA}, β_{EA}, α_{EB}, β_{EB}, c_A^i, c_A^o, c_B^i, c_B^o, K_A^i, K_A^o, K_B^i, K_B^o, and N_{ET}. The **Write to file...** entry writes the values of all the parameters to the chosen file. If the file already exists, it is overwritten. The **Delete a file...** entry is used to delete a file from the directory.

Edit

Selection of **Edit** allows modification of the parameters of the simulation. When the software is initiated, all parameters have their default values and those values are not displayed. The **Edit** menu has three options.

If **Show Parameters** is selected, then a rectangle containing the value of a parameter is displayed next to each parameter (Figure 4.4). Clicking on a value allows the user to change that parameter. The parameter can be changed by typing into the rectangle or by changing the value using the mouse. Clicking on the $>$ or $<$ symbols allows the value to be increased/decreased by a fixed amount. Clicking on $\gg$ or $\ll$ increases/decreases the parameter at a fixed rate. Clicking on the $\circ$ stops the increasing/decreasing process. As the parameter is changed, the bar graph of enzyme states and the flux arrows are changed appropriately.

Selecting **Reset Defaults** resets all parameters to their default values.

Selecting **Increment Value** allows the user to set the value of increment used to change parameter values — the increment is initially set to 1.

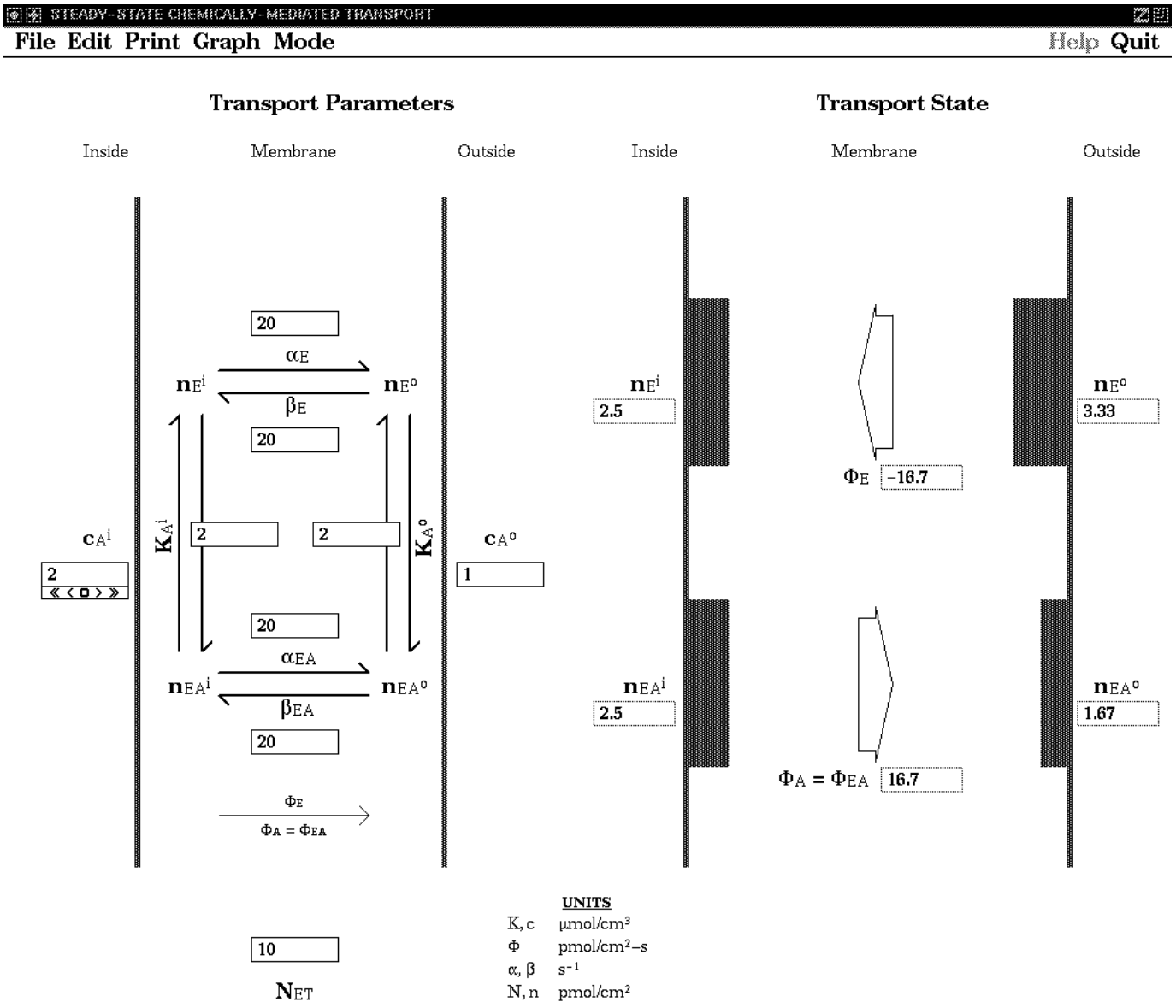

Figure 4.4: Display after **Show Parameters** is selected.

Print

Print allows selection of a printer for printing the contents of the screen. This option is described fully in the description of the Random Walk Model of Diffusion.

Graph

Selecting **Graph** transfers control to the Graph Environment.

Mode

Their are currently two simulation modes, Single Solute and Two Solutes, which can be selected with this menu. When the Two Solutes mode is selected the Parameters and State panels are changed to that shown in Figure 4.5.

Help

Help is only partially implemented at this time.

Quit

Quit transfers control out of the chemically mediated transport software to the main menu which allows access to other software.

4.3.2 Graph environment

To switch to the Graph Environment, click on Graph. The screen now contains both the Parameters and State panels in the upper half of the screen. Several options are available after selecting **Graph**.

File, Print, Help and Quit

See above sections.

Graph

Selecting **Graph** allows several options. Selecting **Setup Graph...** allows the user to choose to plot several vertical (dependent) variables versus one horizontal (independent) variable. The range of the chosen independent variable is shown in two text edit boxes and can be changed by the user. Each axis can be linear or logarithmic. The logarithm of the magnitude is used for the ordinate; if the ordinate is zero, the value is not plotted. The title of the graph can also be chosen. The user can **Start Graph** or **Cancel** the choices by clicking on the appropriate button (see Figure 4.6). If **Start Graph** is chosen, then the relation between the dependent variables and the independent variable are computed. When

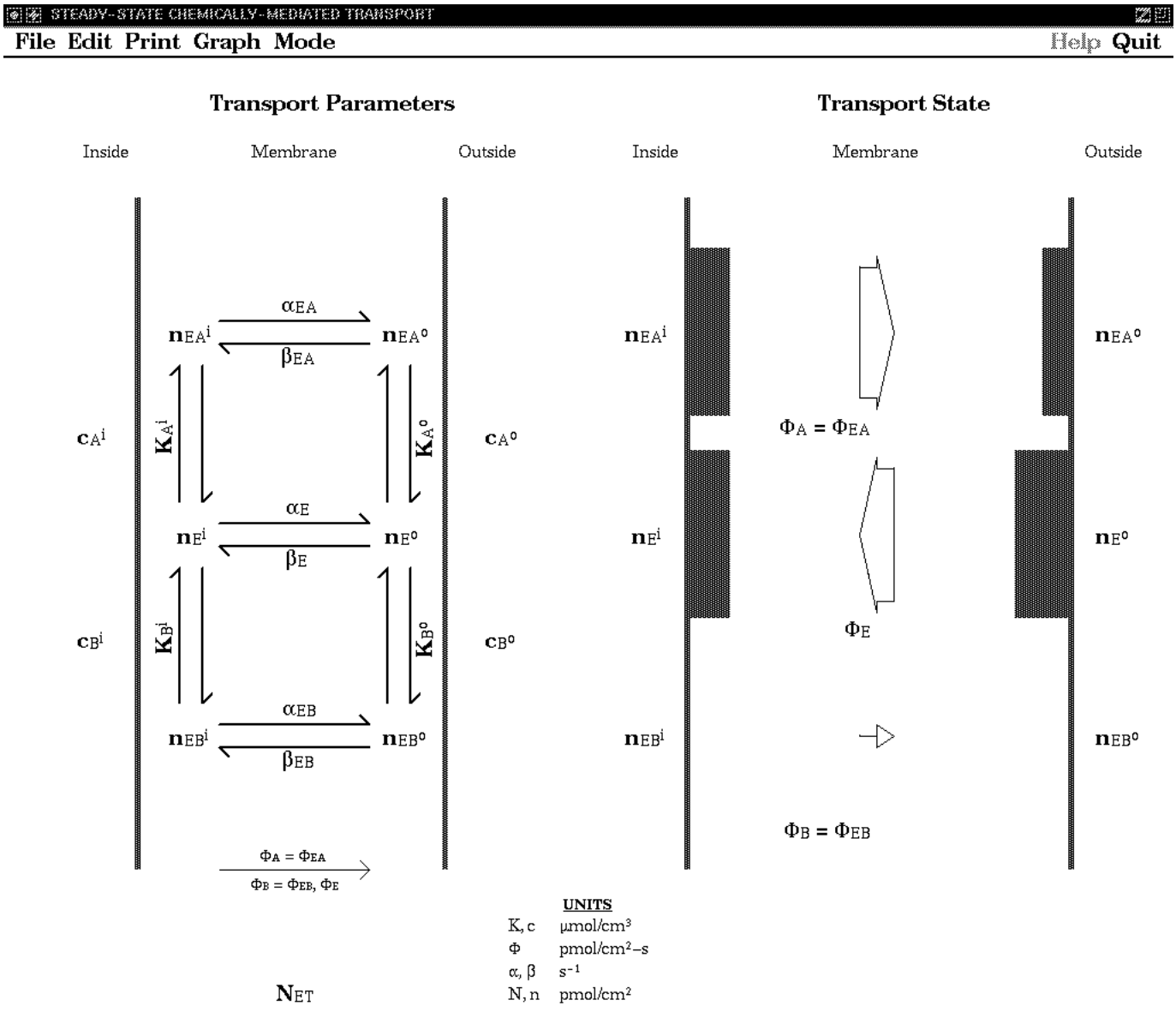

Figure 4.5: Display when the Two Solutes Mode is chosen.

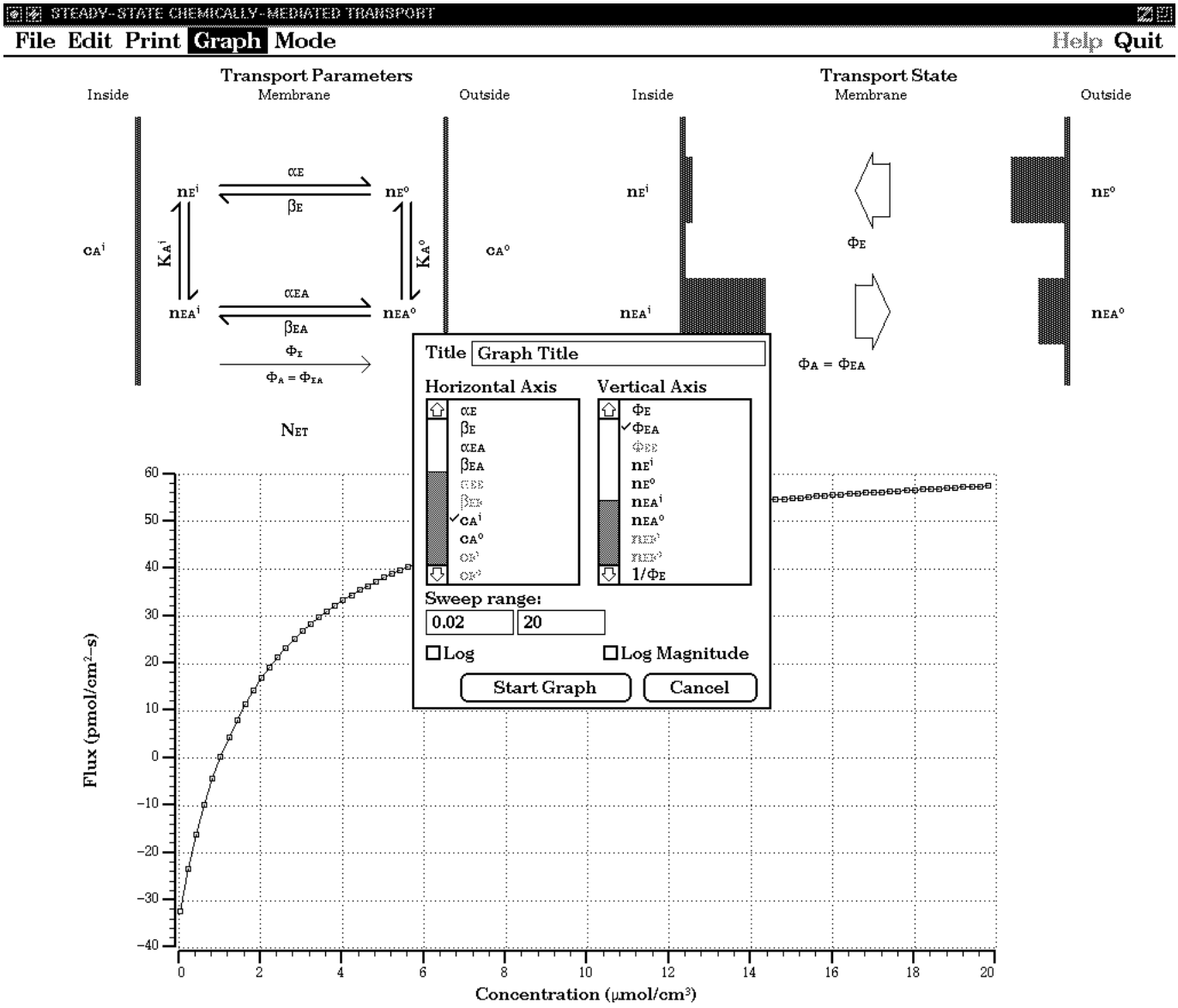

Figure 4.6: Choosing graph variables. The display shows dialog box used to choose variables for plotting after a graph has been completed. The graph is shown in the background window.

the computation is completed, bar graphs of enzyme state and flux arrows are updated continuously as the graph is plotted.

Zoom allows the user to zoom in on a small part of the graph to look at details of the graph. To return to the original scale, click on **Unzoom**.

Annotate allows the user to type and place a text string on the graph area.

Edit

The user can select the **Number of Samples** of the independent variable at which the dependent variables are computed for the specified range of the independent variable. The rate at which the curves are displayed can be decreased by increasing the **Plotting Interval**.

Mode

If in the graph environment, choice of one of the allowable mode transfers control to the interactive environment.

4.4 PROBLEMS

Problem 4.1 This problem is intended to develop your intuition for the chemically-mediated transport model for a single solute. You will observe the effects of changes in parameters on both the flux and the enzyme concentrations. Use the Single Solute mode and start with all the parameters at their default values.

a) What is the relation of ϕ_A and ϕ_E? Explain.

b) What is the relation of the direction of ϕ_A to the sign of $c_A^i - c_A^o$? Explain.

c) How does the state of the enzyme depend on concentration?

i) Do changes in c_A^i change n_{EA}^i and n_E^i? If so, how? If not, why not?

ii) Do changes in c_A^i change n_{EA}^o and n_E^o? If so, how? If not, why not?

iii) Do changes in c_A^o change n_{EA}^i and n_E^i? If so, how? If not, why not?

iv) Do changes in c_A^o change n_{EA}^o and n_E^o? If so, how? If not, why not?

Reset all the parameters to their default values, except set $\alpha_{EA} = 100\ \text{s}^{-1}$.

d) What is the relation of the direction of ϕ_A to the sign of $c_A^i - c_A^o$? Explain.

Reset all the parameters to their default values, except set $\alpha_E = \beta_E = 0\ \text{s}^{-1}$.

e) What is the relation of ϕ_A to $c_A^i - c_A^o$? Explain.

Problem 4.2 This problem is designed to explore the functional relation between flux and concentration when expressed in different coordinates. Use the Single Solute mode and start with all the parameters at their default values, except set $c_A^o = 0$. In parts a) through c) your job is to estimate the values of $(\phi_A)_{max}$ and K_A^i from the graphs specified, where $(\phi_A)_{max}$ is the maximum flux of A with $c_A^o = 0$.

a) Obtain a graph of ϕ_A versus c_A^i in linear coordinates.

b) Obtain a graph of ϕ_A versus c_A^i in double logarithmic coordinates.

c) Obtain a graph of $1/\phi_A$ versus $1/c_A^i$.

d) Determine the values of $(\phi_A)_{max}$ and K_A^i from the model parameters.

e) Compare the 4 sets of values you have obtained for $(\phi_A)_{max}$ and K_A^i.

Problem 4.3 Use the Single Solute mode and start with all the parameters at their default values, except set $c_A^o = 0$. Obtain a graph of all four enzyme states as a function of c_A^i. Some of the these n's increase, others decrease, while others remain constant. Summarize and explain the results you found.

Problem 4.4 Use the Two Solute mode and start with all the parameters at their default values. Set the concentrations of A and B to zero on both sides of the membrane.

a) Explain the initial enzyme states and flux values.

b) Increase c_A^i and observe both the enzyme states and the flux. What is the relation of the flux of A to c_A^i?

c) Now set $c_A^i = 10$ and increase c_B^i. How do the fluxes of A and B depend upon c_B^i?

d) Set $c_A^i = 10$ and $c_A^o = 5$. Now increase c_B^i from an initial value of 0. How do the fluxes of A and B depend upon c_B^i? Pay particular attention to the direction of the flux of A.

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