SUPPLEMENTAL MATERIAL

Derivation of 1D analytical concentration profiles

Steady-state post-enrichment, 1D model

The steady-state equations of change for diffusing species *A*, *B*, and *C* are:

$$\frac{\partial A}{\partial t} = 0 = D_A \frac{\partial^2 A}{\partial x^2} \tag{1}$$

$$\frac{\partial B}{\partial t} = 0 = D_B \frac{\partial^2 B}{\partial x^2} - k_B B \tag{2}$$

$$\frac{\partial C}{\partial t} = 0 = D_C \frac{\partial^2 C}{\partial x^2} + k_B B \tag{3}$$

The boundary conditions are:

$$J_A\big|_0 = J_A\big|_L = J \tag{4}$$

$$J_B\big|_0 = -J_A\big|_0 = J \tag{5}$$

$$J_B\big|_L = 0 \tag{6}$$

$$J_C\big|_0 = 0 \tag{7}$$

$$J_C\big|_L = -J_A\big|_0 = J \tag{8}$$

where J is the flux. These conditions state that the flux from the reaction surfaces are diffusion-limited (hence they do not contain rate constants and do not depend on reactant concentration) and constant at steady-state. As a basis of calculation, we allow the surface reaction of B, $B(0)$, to reach an arbitrary constant value of B_0 at steady-state, which will ultimately depend on system parameters such as the reaction rate constants and total amount of protein present in the embryo (see below).

Solving for A:

$$\frac{\partial^2 A}{\partial x^2} = 0$$

$$\frac{\partial A}{\partial x} = m$$

$$A = mx + A_0 \tag{9}$$

where A_0 is the concentration of A at x=0. We see that A is linear, and the flux of A is constant over all x from 0 to L.

$$J_A = -D_A \frac{dA}{dx} = -D_A m$$

Solving for B:

$$\frac{\partial^2 B}{\partial x^2} = \frac{k_B}{D_B} B$$

With steady flux at x=0 and zero flux at x=L, we expect the concentration of B to achieve an arbitrary steady value we will denote by the constant B_0, such that

$$B = B_0 \frac{\cosh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1 - \frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}} \tag{10}$$

We see that the flux of B is

$$J_B = -D_B \frac{dB}{dx} = B_0 \sqrt{k_B D_B} \frac{\sinh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1 - \frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}}$$

so the flux of *B* at 0 is

$$J_B\big|_0 = B_0\sqrt{k_B D_B}\,\frac{\sinh\sqrt{\dfrac{k_B L^2}{D_B}}}{\cosh\sqrt{\dfrac{k_B L^2}{D_B}}} = B_0\sqrt{k_B D_B}\,\tanh\sqrt{\frac{k_B L^2}{D_B}}$$

and the flux of *B* at L is

$$J_B\big|_L = B_0\sqrt{k_B D_B}\,\frac{\sinh(0)}{\cosh\sqrt{\dfrac{k_B L^2}{D_B}}} = 0$$

Solving for *C*:

$$\frac{\partial^2 C}{\partial x^2} = -\frac{k_B}{D_B}B = -\frac{k_B}{D_B}B_0\,\frac{\cosh\left[\sqrt{\dfrac{k_B L^2}{D_B}}\left(1-\dfrac{x}{L}\right)\right]}{\cosh\sqrt{\dfrac{k_B L^2}{D_B}}}$$

$$\frac{\partial C}{\partial x} = \frac{B_0}{D_C}\sqrt{D_B k_B}\,\frac{\sinh\left[\sqrt{\dfrac{k_B L^2}{D_B}}\left(1-\dfrac{x}{L}\right)\right]}{\cosh\sqrt{\dfrac{k_B L^2}{D_B}}} + c_1$$

$$C = -B_0\left(\frac{D_B}{D_C}\right)\frac{\cosh\left[\sqrt{\dfrac{k_B L^2}{D_B}}\left(1-\dfrac{x}{L}\right)\right]}{\cosh\sqrt{\dfrac{k_B L^2}{D_B}}} + c_1 x + c_2$$

We can solve for c_1 by considering the zero flux condition at *x*=0:

$$J_C\big|_0 = 0 = -D_C\left.\frac{\partial C}{\partial x}\right|_0$$

$$\left.\frac{\partial C}{\partial x}\right|_0 = 0 = \frac{B_0}{D_C}\sqrt{D_B k_B}\tanh\sqrt{\frac{k_B L^2}{D_B}} + c_1$$

$$c_1 = -\frac{B_0}{D_C}\sqrt{D_B k_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}$$

Plugging in for c_1, we obtain

$$C = -B_0\left(\frac{D_B}{D_C}\right)\frac{\cosh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1-\frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}} - \frac{B_0}{D_C}\sqrt{D_B k_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}x + c_2$$

The flux of *C* is

$$\frac{\partial C}{\partial x} = \frac{B_0}{D_C}\sqrt{D_B k_B}\,\frac{\sinh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1-\frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}} - \frac{B_0}{D_C}\sqrt{D_B k_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}$$

Similarly to the case for *B*, with a steady flux of *C* at x=L, we expect the concentration of *C* to attain a steady value at x=0 that we will denote C_0, such that

$$C(0) = C_0 = -B_0\left(\frac{D_B}{D_C}\right) + c_2$$

$$C_2 = C_0 + B_0\left(\frac{D_B}{D_C}\right)$$

Plugging in for c_2, we obtain

$$C = C_0 + B_0\left(\frac{D_B}{D_C}\right)\left[1-\frac{\cosh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1-\frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}}\right] - \frac{B_0}{D_C}\sqrt{k_B D_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}x$$

(11)

The flux of *C* is

$$J_C = -D_C \frac{\partial C}{\partial x} = -B_0 \sqrt{D_B k_B} \frac{\sinh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1 - \frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}} + B_0 \sqrt{D_B k_B} \tanh\sqrt{\frac{k_B L^2}{D_B}}$$

So the flux at *x*=0 is

$$J_C\big|_0 = -D_C \frac{\partial C}{\partial x}\bigg|_0 = -B_0 \sqrt{D_B k_B} \tanh\sqrt{\frac{k_B L^2}{D_B}} + B_0 \sqrt{D_B k_B} \tanh\sqrt{\frac{k_B L^2}{D_B}} = 0$$

And the flux at *x*=L is

$$J_C\big|_L = -D_C \frac{\partial C}{\partial x}\bigg|_L = B_0 \sqrt{D_B k_B} \tanh\sqrt{\frac{k_B L^2}{D_B}}$$

which, as expected, is equal to the flux of *B* at *x*=0. Of course, the flux of *B* at *x*=0 must be equal and opposite of the flux of *A* at *x*=0. Likewise, the flux of *C* at *x*=L must be equal and opposite to the flux of *A* at *x*=L. Hence,

$$J_B\big|_0 = B_0 \sqrt{k_B D_B} \tanh\sqrt{\frac{k_B L^2}{D_B}} = -J_A\big|_0 = D_A m$$

So

$$m = \frac{B_0}{D_A} \sqrt{k_B D_B} \tanh\sqrt{\frac{k_B L^2}{D_B}}$$

We can check the flux at x=L…

$$J_C\big|_L = B_0 \sqrt{D_B k_B} \tanh\sqrt{\frac{k_B L^2}{D_B}} = J_A\big|_L = D_A m$$

And we again find that

$$m = \frac{B_0}{D_A}\sqrt{D_B k_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}$$

Hence,

$$A = mx + A_0 = \left(\frac{B_0}{D_A}\sqrt{D_B k_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}\right)x + A_0 \qquad (13)$$

A_0, which determines the relative concentration of A in the system, is left as a tunable parameter.

The total concentration can then be calculated by

$$Total = A + B + C$$

$$\begin{aligned} Total &= A_0 + C_0 + B_0\left(\frac{D_B}{D_C}\right) \\ &+ \left(\frac{1}{D_A} - \frac{1}{D_C}\right)B_0\sqrt{k_B D_B}\tanh\sqrt{\frac{k_B L^2}{D_B}}x \\ &+ \left(1 - \frac{D_B}{D_C}\right)B_0\frac{\cosh\left[\sqrt{\frac{k_B L^2}{D_B}}\left(1 - \frac{x}{L}\right)\right]}{\cosh\sqrt{\frac{k_B L^2}{D_B}}} \end{aligned} \qquad (14)$$

In order to test the ability of our model to correctly predict the overall concentration gradient, we measured fluorescence intensity along the A/P axis of embryos expressing MEX-5::GFP (with background fluorescence subtracted) and used data from the central 40um of the A/P axis (L), and used values for the diffusion coefficients (D_A, D_B, D_C) measured with FCS. Species intensity (A_0, B_0, C_0) and reaction (k_B) parameters were tuned to minimize squared error. Diffusion coefficients for slow system components (B and C) were constants equal to 0.4 $\mu m^2/s$ and 1.0 $\mu m^2/s$, respectively, while the diffusion coefficient for the fast species (A) was constant and equal to 15 $\mu m^2/s$ (all of which are within experimental error of our FCS measurements). As a basis for calculation, the total intensity at the anterior pole (x=0μm) was normalized to $Total(0) = 5$ (a.u.).

The rate constants used in our modeling are modified such that $k_{cytoplasmic} = k_{phys} k_I [P]$ and $k_{surface} = k_{phys} k_I \frac{E_{tot}}{K_M}$ (assuming first order surface reactions), where k_{phys} is the physiological rate constant, $k_I = \frac{[S]}{S}$ is an (unknown) constant relating fluorescence intensity (S) to cytoplasmic concentration ([S]), [P] is the (unknown) concentration of the putative cytoplasmic phosphatase, E_{tot} is the (unknown) total surface concentration of enzyme on the reaction surface, K_M is the Michaelis constant, and k_{phys} is the physiological rate constant. The modified rate constants estimated from the modeling were k_A=0.065 μm/s, k_B = 0.0025 s^{-1}, and k_C = 0.2 μm/s, and the concentration parameters (a.u.) were estimated to be A_0 = 1, B_0 = 2.1, C_0 = 1.9.

FEMLAB

Multi-dimensional solutions to the partial differential equations were found using the finite element method (FEM) via COMSOL 3.5a (FEMLAB) software. FEM analysis is a useful tool for calculating solutions to complicated systems of equations over complex geometries including intricate biological problems (Reddy, 1993; Sun et al., 2009). Briefly, the finite element method calculates the solution to complicated systems by breaking the geometry of a given system into subdomains, or finite elements. Solutions to each finite element are calculated by approximating solutions to the partial differential equations over that element as a linear sum of algebraic polynomials where the undetermined coefficients of these polynomials are valued according to the governing partial differential equations. The finite elements themselves are not fixed throughout solving the governing system of equations but may change to reduce the error in approximation over a given element and maintain continuity of the solution over all elements (Reddy, 1993).

Here, we used a simple oval geometry (major and minor axes of 50µm and 30µm, respectively) centered about the origin, consisting of two spatially identical and opposite reactive surfaces, the anterior and posterior, to represent the dividing embryo. The *A* to *B* reactions were allowed to occur only on the anterior surface, the *B* to *C* reaction again occurred everywhere within the cytoplasmic region and the *C* to *A* reaction was allowed to occur only on the posterior surface. All reactions rates used to calculate the multi-dimensional solutions were first order. Diffusion coefficients for slow system components (*B* and *C*) were constants equal to 0.4 $\mu m^2/s$ and 1.0 $\mu m^2/s$, respectively, while the diffusion coefficient for the fast species (*A*) was constant and equal to 15 $\mu m^2/s$ (all of which are within experimental error of our FCS measurements). As a basis for calculation, the concentration of *A* at the anterior pole (far left anterior node) was considered a known quantity and set equal to 1 (a.u.). The modified rate constants (see above) used in the modeling were k_A=0.02 µm/s, k_B = 0.001 s^{-1} and k_C = 0.5 µm/s.

Analytical concentration profiles with reactive species *B*

The governing equations are identical to those for the non-reacting *B* species, however the zero-flux boundary condition at *x*= *L* must be allowed to take some finite value, *β*. Thus, the general equation for species *B* becomes

$$B = a\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) + b\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

We specify that *B* attains for steady-state value at *x*=0 such that

$$B(0) = B_0 = a + b$$

$$b = B_0 - a$$

$$B = a\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) + (B_0 - a)\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

$$B = a\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) - a\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right) + B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

$$B = a\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) - \exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right] + B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

and

$$\frac{dB}{dx} = \sqrt{\frac{k_B}{D_B}}a\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) - \sqrt{\frac{k_B}{D_B}}(B_0 - a)\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

$$\frac{dB}{dx} = \sqrt{\frac{k_B}{D_B}}a\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) + \sqrt{\frac{k_B}{D_B}}a\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right) - \sqrt{\frac{k_B}{D_B}}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

$$\frac{dB}{dx} = a\sqrt{\frac{k_B}{D_B}}\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right] - \sqrt{\frac{k_B}{D_B}}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

We allow the flux of *B* to attain some finite value, *β*, at steady-state such that

$$J_B = -D_B \left.\frac{dB}{dx}\right|_{x=L} = \beta = -\sqrt{k_B D_B}\, a \exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \sqrt{k_B D_B}\left(B_0 - a\right)\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)$$

$$\beta = B_0\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - a\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - a\sqrt{k_B D_B}\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)$$

$$\beta = B_0\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - a\sqrt{k_B D_B}\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]$$

$$a = \frac{B_0\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta}{\sqrt{k_B D_B}\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}$$

$$B = \frac{\left[B_0\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta\right]}{\sqrt{k_B D_B}} \frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) - \exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} + B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

$$\frac{dB}{dx} = \frac{\left[B_0\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta\right]}{D_B} \frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} - \sqrt{\frac{k_B}{D_B}}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

where β is a constant. Thus, the flux of B is given by

$$J_B = -D_B\frac{dB}{dx} = \left[\beta - B_0\sqrt{k_B D_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right] \frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} + \sqrt{k_B D_B}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

We can use the explicit expression for B to solve for C by integration

$$\frac{d^2C}{dx^2} = -\frac{k_B}{D_C}B = -\frac{k_B}{D_C}\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\sqrt{k_BD_B}}\frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right)-\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} - \frac{k_B}{D_C}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$

$$\frac{dC}{dx} = -\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{D_C}\frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} + \frac{\sqrt{k_BD_B}}{D_C}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right) + c_1$$

The flux of C at x=0 is specified to equal 0 such that

$$\left.\frac{dC}{dx}\right|_{x=0} = 0 = -\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}\frac{2}{D_C} + \frac{\sqrt{k_BD_B}}{D_C}B_0 + c_1$$

$$c_1 = \frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}\frac{2}{D_C} - \frac{\sqrt{k_BD_B}}{D_C}B_0$$

$$\frac{dC}{dx} = -\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{D_C}\frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} + \frac{\sqrt{k_BD_B}}{D_C}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)$$
$$+\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}\frac{2}{D_C} - \frac{\sqrt{k_BD_B}}{D_C}B_0$$

$$C = c_2 - \sqrt{\frac{D_B}{k_B}} \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{D_C} \frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} x \right) - \exp\left(-\sqrt{\frac{k_B}{D_B}} x \right) \right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} L \right) + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]}$$

$$- \frac{D_B}{D_C} B_0 \exp\left(-\sqrt{\frac{k_B}{D_B}} x \right) + \left\{ \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{\left[\exp \sqrt{\frac{k_B}{D_B}} L + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]} \frac{2}{D_C} - \frac{\sqrt{k_B D_B}}{D_C} B_0 \right\} x$$

Finally, we specify that C attains a constant value, C_0, at x=0:

$$C(0) = C_0 = c_2 - \frac{D_B}{D_C} B_0$$

$$c_2 = C_0 + \frac{D_B}{D_C} B_0$$

such that

$$C = C_0 + \frac{D_B}{D_C} B_0 - \sqrt{\frac{D_B}{k_B}} \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{D_C} \frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} x \right) - \exp\left(-\sqrt{\frac{k_B}{D_B}} x \right) \right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} L \right) + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]}$$

$$- \frac{D_B}{D_C} B_0 \exp\left(-\sqrt{\frac{k_B}{D_B}} x \right) + \left\{ \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{\left[\exp \sqrt{\frac{k_B}{D_B}} L + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]} \frac{2}{D_C} - \frac{\sqrt{k_B D_B}}{D_C} B_0 \right\} x$$

and thus

$$\frac{dC}{dx}=-\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{D_C}\frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}$$

$$+\frac{\sqrt{k_BD_B}}{D_C}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)+\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\left[\exp\sqrt{\frac{k_B}{D_B}}L+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}\frac{2}{D_C}-\frac{\sqrt{k_BD_B}}{D_C}B_0$$

Therefore, the flux of C is given by

$$J_C=-D_C\frac{dC}{dx}=\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]\frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}x\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}$$

$$-\sqrt{k_BD_B}B_0\exp\left(-\sqrt{\frac{k_B}{D_B}}x\right)-2\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\left[\exp\sqrt{\frac{k_B}{D_B}}L+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}+\sqrt{k_BD_B}B_0$$

As expected, the flux of C at x=0 is

$$J_C\big|_{x=0}=-D_C\frac{dC}{dx}\bigg|_{x=0}=\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]\frac{[1+1]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right)+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}$$

$$-\sqrt{k_BD_B}B_0-2\frac{\left[B_0\sqrt{k_BD_B}\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)-\beta\right]}{\left[\exp\sqrt{\frac{k_B}{D_B}}L+\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}+\sqrt{k_BD_B}B_0=0$$

Similarly, the flux of C at x=L is

$$J_C\Big|_{x=L} = -D_C \frac{dC}{dx}\bigg|_{x=L} = \sqrt{k_B D_B}\, B_0 - 2\frac{\left[B_0\sqrt{k_B D_B}\,\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta\right]}{\left[\exp\sqrt{\frac{k_B}{D_B}}L + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} - \beta$$

We can verify that the flux of *B* at *x*=0 equals the sum of the flux of *C* at *x*=*L* and the flux of *B* at *x*=*L*:

$$J_B(0) = \sqrt{k_B D_B}\, B_0 - 2\frac{\left[B_0\sqrt{k_B D_B}\,\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}$$

$$J_B(L) = \beta$$

Finally, the flux of *A* at *x*=0 must be equal and opposite to the flux of *B* at *x*=0, such that

$$= \sqrt{k_B D_B}\, B_0 - 2\frac{\left[B_0\sqrt{k_B D_B}\,\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]} = D_A m$$

$$m = \frac{\sqrt{k_B D_B}}{D_A} B_0 - \frac{2}{D_A}\frac{\left[B_0\sqrt{k_B D_B}\,\exp\left(-\sqrt{\frac{k_B}{D_B}}L\right) - \beta\right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}}L\right) + \exp\left(-\sqrt{\frac{k_B}{D_B}}L\right)\right]}$$

And the concentration profile of *A* is given by

$$A = A_0 + \left\{ \frac{\sqrt{k_B D_B}}{D_A} B_0 - \frac{2}{D_A} \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} L \right) + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]} \right\} x$$

The (constant) flux of A is given by

$$J_A = -D_A \frac{dA}{dx} = \frac{\sqrt{k_B D_B}}{D_A} B_0 - \frac{2}{D_A} \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} L \right) + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]}$$

The total concentration profile is then given by

$$\begin{aligned} Total &= A + B + C \\ &= A_0 + C_0 + \frac{D_B}{D_C} B_0 + \left(1 - \frac{D_B}{D_C} \right) B_0 \exp\left(-\sqrt{\frac{k_B}{D_B}} x \right) \\ &+ \left(1 - \frac{D_B}{D_C} \right) \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{\sqrt{k_B D_B}} \frac{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} x \right) - \exp\left(-\sqrt{\frac{k_B}{D_B}} x \right) \right]}{\left[\exp\left(\sqrt{\frac{k_B}{D_B}} L \right) + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]} \qquad (15) \\ &+ \left(\frac{1}{D_C} - \frac{1}{D_A} \right) \left\{ 2 \frac{\left[B_0 \sqrt{k_B D_B} \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) - \beta \right]}{\left[\exp\sqrt{\frac{k_B}{D_B}} L + \exp\left(-\sqrt{\frac{k_B}{D_B}} L \right) \right]} - \sqrt{k_B D_B} B_0 \right\} x \end{aligned}$$