MATHEMATICAL MODEL

A simple model was developed to analyze the transport of Aβ through the blood-brain-barrier (BBB) under constant flux conditions with fully developed concentration profiles. This model is likely to be valid immediately following initial transient development of Aβ concentration profiles across the BBB endothelium. Experimentally, this timescale is relevant when we observe a linear dependence of Aβ concentration with time for data such as that shown in Figure S1; the linear regime for Figure S1 is between 2 and 10 min. While this model depends on parameters which are not easily experimentally accessible, it will provide a basis for understanding differences in behavior observed for different Aβ variants as well as highlight departures from model predictions and experimental observations. The experimentally measureable quantities are C_A, C_L, F, $k_{EL,A\beta}$, and $k_{EL,DutchA\beta}$. These quantities are the abluminal Aβ concentration, the luminal Aβ concentration, and the Aβ flux across the BBB, Aβ40 plasma/BBB partition coefficient, and DutchAβ40 plasma/BBB partition coefficient, respectively.

Figure 5 illustrates the geometry of our simple model for the BBB. As shown, the abluminal (brain) compartment is in direct contact with the basement membrane. A layer of endothelial cells with tight junctions exists between the BM and the luminal (blood) compartment. Numerical values for each parameter will be discussed in a later section of the supplemental text.

Below are the assumptions of our transport model:

1) The Aβ concentrations in the luminal and abluminal compartments are assumed to be constant in time. Although, Aβ concentrations in the plasma and brain extracellular space increase with the age of the patient, the change is gradual and spans over several years. Hence, during the time-frame pertinent to Aβ transcytosis at the BBB, the Aβ concentrations are expected to remain constant.
2) Concentration profiles within the basement membrane and endothelial cells are time-independent (fully developed), so that this model will predict "steady-state" fluxes.
3) Transport of Aβ within the basement membrane is governed by Fick's law.
4) Transport of Aβ into the endothelial cells occurs via receptor-mediated endocytosis and is governed by Michaelis-Menten kinetics. Since Aβ concentrations are well below the saturation levels[1], the Michaelis-Menten kinetics would predict linear behavior.
5) Vesicular transport of Aβ through the endothelial cells is governed by Fick's Law[2-3].
6) Aβ is transported out of endothelial cells by vesicular fusion with the inside surface of the abluminal membrane. The rate of Aβ transport in this direction across the cell membrane is assumed to be directly proportional to the concentration of Aβ inside the cell.

The following sections will detail the derivation of the BBB transport model, which incorporates the 6 assumptions outlined above.

Transport within the basement membrane

Fick's Law with constant flux:

$$-D_C \frac{d^2C}{dz^2} = 0 \tag{1}$$

$$F = -D_C \frac{dC}{dz}, \text{ for } 0 \leq z < L_C \tag{2a}$$

$$F = -D_E \frac{dC}{dz} \text{ for } L_C < z < L_C + L_E \tag{2b}$$

Where D_C is the diffusivity of Aβ within the basement membrane, D_E is the diffusivity of Aβ within the endothelial cell, z is the position along the z-axis, L_C is the thickness of the basement membrane, and L_E is the thickness of the endothelial cell. The following boundary conditions exist for the basement membrane compartment.

$$C|_{z=0} = k_{CA}C_A \tag{3}$$

$$C|_{z=L_C^-} = C_{L_C^-} \tag{4}$$

The concentration C_A is a known quantity describing the concentration of Aβ in the abluminal compartment, the concentration $C_{L_C^-}$ is an unknown quantity describing the local concentration of Aβ at the outside surface of the cell in contact with the basement membrane, andk_{CA} is the partition coefficient for Aβ between the abluminal compartment and the basement membrane. In subsequent steps, we will eliminate the variable $C_{L_C^-}$ in terms of experimentally accessible quantities. Note that flux is a measurable quantity; this quantity, as described in the model assumptions, is constant throughout the BBB. Combining Equations 1through 4 provide an equation for flux in the basement membrane.

$$F = \frac{D_c}{L_C}\left(k_{CA}C_A - C_{L_C^-}\right) \tag{5}$$

Flux at the basement membrane-endothelial cell interface

The following boundary condition exists for the endothelial cell.

$$C|_{x=L_C^+} = C_{L_C^+} \tag{6}$$

The concentration $C_{L_C^+}$ is a second unknown quantity which describes the local concentration of Aβ at the inner surface of the cell in contact with the basement membrane. An equation for flux at the interface between the basement membrane and endothelial cell can be developed which balances the effect of Michaelis-Menton kinetics with that of vesicular recombination. Equations 4 and 6 provide the boundary conditions for the following equation.

$$F = \left(\frac{1}{A_{cs}}\right)\left[\left(\frac{k_{CE}V_{max,3}}{k_{m,3}+k_{CE}C_{L_C^-}}C_{L_C^-}\right) - k_2C_{L_C^+}\right] \cong \left(\frac{1}{A_{cs}}\right)\left[\left(\frac{k_{CE}V_{max,3}}{k_{m,3}}C_{L_C^-}\right) - k_2C_{L_C^+}\right] \tag{7}$$

Where A_{cs} is cross-sectional area,k_{CE} is the partition coefficient for Aβ between the basement membrane and the outer surface of the endothelial cell in contact with the basement membrane, $V_{max,3}$ is the rate at which Aβ receptors are bound on the abluminal side of the endothelial membrane, $k_{m,3}$ is the Michaelis constant at the interface between the basement membrane and the endothelial cell, and k_2 is the rate constant for transport of Aβ due to vesicular fusion with the endothelial membrane at the interface with the basement membrane. Experimental evidence suggests that the term $k_{m,3}$ is significantly larger than the term$k_{CE}C_{L_C^-}$ allowing for further simplification of Equation 7 as shown[1].

Transport within the endothelial cell

A second boundary condition exists for the endothelial cell.

$$C|_{x=(L_C+L_E)^-} = C_{(L_C+L_E)^-} \tag{8}$$

Our third unknown is the quantity $C_{(L_C+L_E)^-}$ which describes the local concentration of Aβ at the inner surface of the cell in contact with the luminal cavity. By combining Equations 1, 2, 6, and 8, we can derive an equation for flux within the endothelial cell.

$$F = \frac{D_E}{L_E}\left(C_{L_C^+} - C_{(L_C+L_E)^-}\right) \tag{9}$$

Flux at the endothelial cell-luminal interface

The following boundary condition exists for the luminal compartment.

$$C|_{x=(L_C+L_E)^+} = k_{EL}C_L \tag{10}$$

The concentration C_L is a known quantity describing the concentration of Aβ in the luminal compartment and k_{EL} is the partition coefficient for Aβ between the luminal compartment and the outer surface of the endothelial cell in contact with the luminal space. Similar to the flux at the interface between the basement membrane and the endothelial cell, an equation for the flux at the interface between the endothelial cell and luminal compartment can be developed. Equations 8 and 10 provide the boundary conditions for the following equation.

$$F = \left(\frac{1}{A_{cs}}\right)\left[\left(k_4 C_{(L_C+L_E)^-}\right) - C_L\left(\frac{k_{EL}V_{max,1}}{k_{m,1}+k_{EL}C_L}\right)\right] \cong \left(\frac{1}{A_{cs}}\right)\left[\left(k_4 C_{(L_C+L_E)^-}\right) - C_L\left(\frac{k_{EL}V_{max,1}}{k_{m,1}}\right)\right] \tag{11}$$

Where k_4 is the rate constant for the transport of Aβ due to vesicular fusion with the endothelial membrane at the luminal interface, $V_{max,1}$ is the rate at which the Aβ receptor is bound on the luminal side of the endothelial membrane, and $k_{m,1}$ is the Michaelis constant at the interface between the endothelial cell and the luminal compartment. Experimental evidence suggests that the term $k_{m,1}$ is significantly larger than the term $k_{EL}C_L$ allowing for further simplification of Equation 11 as shown[1].

Total Flux

At this point, we have developed 4 equations for flux, each of which contains at least one unknown concentration. Based on our second assumption of "steady-state" fluxes, equations 5, 7, 9, and 11 are all equivalent. It is possible to solve for a total flux in terms of known variables through a series of substitutions. First, we can solve Equation 5 for $C_{L_C}{}^-$. We can then substitute the equation for $C_{L_C}{}^-$ into Equation 7 and solve for $C_{L_C}{}^+$. In the same fashion, we can substitute the equation for $C_{L_C}{}^+$ into Equation 9 and solve for $C_{(L_C+L_E)^-}$. This equation can then be substituted into Equation 11 and we are left with an equation for flux, which is expressed in terms of C_A and C_L, the two known concentrations. This total flux, F_T, is shown below in Equation 12.

$$F_T = \frac{D_C D_E(-C_L k_2 k_{EL} k_{m.3} V_{max,1} + C_A k_4 k_{CA} k_{CE} k_{m.1} V_{max,3})}{D_C k_{m.1} k_{m.3}(A_{CS} D_E(k_2+k_4) + k_2 k_4 L_E) + D_E k_4 k_{CE} k_{m.1} L_C V_{max,3})} \tag{12}$$

Equation 12 can be compared to experimental results where both C_A and C_L are nonzero, meaning Aβ is present in both the luminal and abluminal cavities. We can also obtain "unidirectional fluxes."In this case, unidirectional fluxes describe the experimental condition where Aβ is present on only one side of the model, either the abluminal side or the luminal side. Our "abluminal flux" is defined by setting C_A to a nonzero value and C_L to a value of zero. In the opposite fashion, we have defined "luminal flux" by settingC_A to a value of zero and C_L to a nonzero value. The equations for abluminal flux, F_A, and luminal flux,F_L, are shown in Equations 13 and 14.

$$F_A = \frac{C_A D_C D_E k_4 k_{CA} k_{CE} V_{max,3}}{A_{CS} D_C D_E k_2 k_{m,3} + A_{CS} D_C D_E k_4 k_{m,3} + D_C k_2 k_4 k_{m,3} L_E + D_E k_4 k_{CE} L_C V_{max,3}} \tag{13}$$

$$F_L = \frac{-C_L D_C D_E k_2 k_{EL} k_{m.3} V_{max,1}}{k_{m.1}(A_{CS} D_C D_E k_2 k_{m,3} + A_{CS} D_C D_E k_4 k_{m,3} + D_C k_2 k_4 k_{m,3} L_E + D_E k_4 k_{CE} L_C V_{max,3})} \tag{14}$$

Since these equations are linear, the total flux, or bidirectional flux, should be the sum of the unidirectional fluxes.

$$F_T = F_A + F_L \tag{15}$$

Model Parameters

To improve correlation with experimental results, we used experimentally accessible parameters (normal text) such as: C_A = 12 nM, C_L = 4 nM, $F_{T,DutchA\beta}$ = 66.6ng/cm^2/min, $F_{T,A\beta}$ = 60.3 ng/cm^2/min, and $k_{CA} = k_{CE}$ =2, $k_{EL,A\beta}$ = 2, $k_{EL,DutchA\beta}$ = 5; previously recorded values from relevant literature sources[4] (**indicated in bold**) such as: $\boldsymbol{A_{cs}}$ **= 1 dm^2,** $\boldsymbol{L_C}$ **= 40 nm,** $\boldsymbol{L_E}$ **=300 nm**; and the literature values that have been slightly modified to fit the experimental data better (***indicated in bold italics***): $\boldsymbol{v_{max,1,A\beta} = v_{max,3,A\beta}}$ ***= 15.94 pM/min*** [5]***,*** $\boldsymbol{v_{max,1,DutchA\beta} = v_{max,3,DutchA\beta}}$ ***= 25.23 pM/min*** [5]***,*** $\boldsymbol{k_{m,1} = k_{m,3}}$ ***= 45 nM*** [5]***, ,*** $\boldsymbol{D_C = 6.0 \cdot 10^{-8}}$ ***cm^2/s*** [3]***,*** $\boldsymbol{D_E = 1.3 \cdot 10^{-9}}$ ***cm^2/s*** [3]***,*** $\boldsymbol{k_2}$ ***= 0.01 1/min*** [6]***, and*** $\boldsymbol{k_4}$ ***= 0.01 1/min*** [6].

Model Predictions

We suggest that the endothelial accumulation of different Aβ variants could be understood in terms of the effects of changes in model parameters on steady state concentration profiles and total flux. The Aβ proteins are known to interact with lipid bilayers, and hence may affect vesicles' ability to recombine with the inner surface of the cell membrane[7]. Although Figure S1 shows predicted effects when k_2 and k_4 vary simultaneously, it is also possible that these parameters may vary independently.

We have also considered the extent of endothelial accumulation under different flux conditions: bidirectional, where both C_A and C_L are non-negligible, abluminal to luminal (A-L) unidirectional flux, where only C_A is non-negligible, and luminal to abluminal (L-A) unidirectional flux, where only C_L is non-negligible. The bidirectional model corresponds to experimental conditions where Aβ is initially present in both the abluminal and luminal compartment, A-L unidirectional model corresponds to experimental conditions in which Aβ is initially present only in the abluminal compartment, and L-A unidirectional model corresponds to experimental conditions where Aβ is initially present in only the luminal compartment. We also note that accumulation of Aβ inside endothelial cell could also result from increases in $v_{max,1}$ or $v_{max,3}$. We do not consider this possibility, because the receptors tend to diminish in their efficacy and/or usually down-regulate under disease conditions.

The impact of the rate of vesicular fusion on Aβ accumulation in the endothelial cells can be represented by arbitrarily varying the values of k_2 and $k_4$concurrently between 0.006 and 0.02 min^{-1}. The bidirectional case (Fig. S1-A) indicates that a decrease in the rate of vesicular fusion at each interface, corresponding to lower values for k_2 and k_4, results in a higher level of Aβ accumulation within the endothelial cell, particularly at the interface with the basement membrane. The A-L flux (Fig. S1-B) and the L-A flux (Fig. S1-C) also illustrate accumulation of Aβ within the endothelial cell with higher cellular concentrations at distances closer to the Aβ reservoir; in these cases, changes in intra-cellular accumulation of Aβ due to changes in k_2 and k_4 would be accompanied by significant effects of overall flux across the BBB.

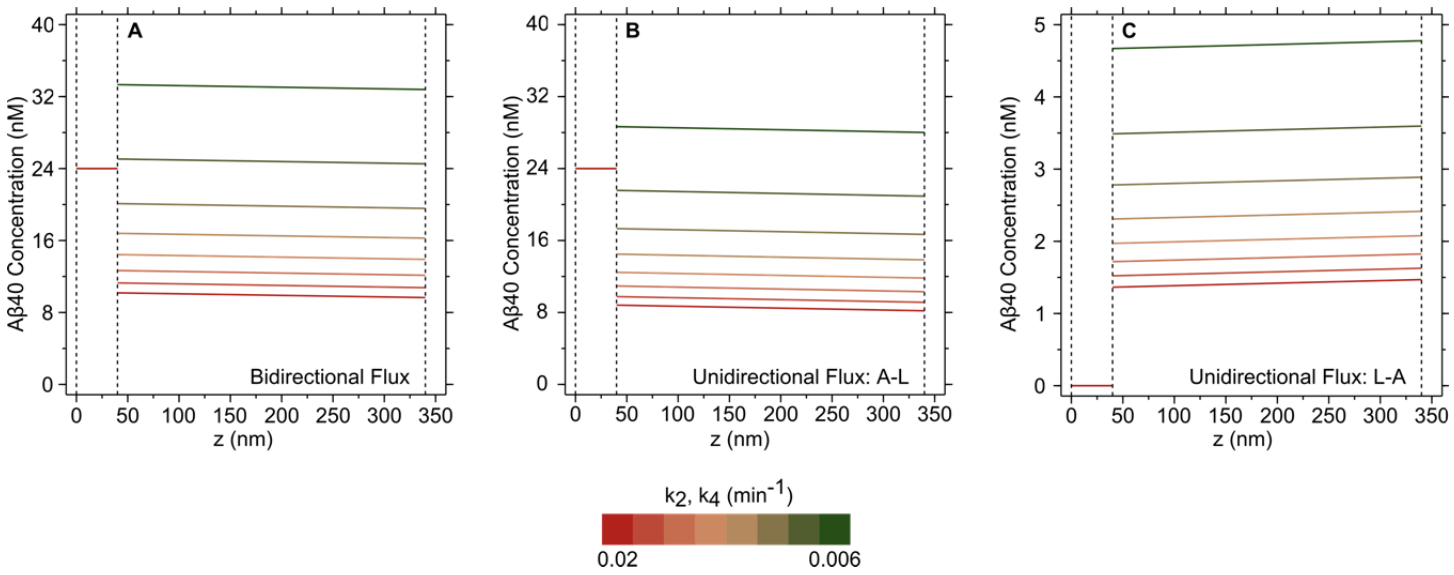

Figure S1. Predicted concentration profiles of Aβ40 in the basement membrane (0 – 40 nm) and endothelial cell (40 – 340 nm). Figures A, B, and C illustrate the effect of simultaneously varying values of k_2 and k_4 (from 0.006 to 0.02min^{-1}, increment of 0.002) for bidirectional flux, A-L flux, and L-A flux, respectively.

References

(1) Kandimalla, K. K.; Curran, G. L.; Holasek, S. S.; Gilles, E. J.; Wengenack, T. M.; Poduslo, J. F. *J Pharmacol Exp Ther* **2005**, *313*, 1370.

(2) Shea, S. M.; Bossert, W. H. *Microvascular Research* **1973**, *6*, 305.

(3) Tomlin, S. G. *Biochim Biophys Acta* **1969**, *183*, 559.

(4) Shea, S. M.; Karnovsky, M. J.; Bossert, W. H. *J Theor Biol* **1969**, *24*, 30.

(5) Zlokovic, B. V.; Ghiso, J.; Mackic, J. B.; McComb, J. G.; Weiss, M. H.; Frangione, B. *Biochem Biophys Res Commun* **1993**, *197*, 1034.

(6) Liu, T.; Wang, T.; Chapman, E. R.; Weisshaar, J. C. *Biophys J* **2008**, *94*, 1303.

(7) Uemura, E.; Greenlee, H. W. *Exp Neurol* **2001**, *170*, 270.