

## Appendix A. Michaelis-Menten derivation

Adopting Michaelis-Menten kinetics to the endothelial serotonin transporter requires deriving  $V_{\max}$  from the individual components that make up the transporter complex. The basic first order reactions between the transporter (T), substrate (S), and transporter-substrate complex (TS) are given by:

$$\frac{dTS}{dt} = k_{on} \cdot T \cdot S - k_{off} \cdot TS, \quad (A1)$$

$$\frac{dS}{dt} = -k_{on} \cdot T \cdot S + k_{off} \cdot TS, \quad (A2)$$

$$\frac{dT}{dt} = -\frac{dTS}{dt}, \quad \text{and} \quad (A3)$$

$$k_d = \frac{T \cdot S}{TS}, \quad (A4)$$

where T, S, and TS are in mmol/ml and  $k_{off}$ ,  $k_{on}$ , and  $k_d$  are reaction rate constants and it is assumed that the reactions reach a local equilibrium on each side of the membrane. The total concentration of the transporter ( $T_{tot}$ ) within the membrane is assumed constant and given by:

$$T_{tot} = T_{ec} + TC_{ec} + T_p + TC_p, \quad (A5)$$

where  $T_{ec}$  and  $TC_{ec}$  are the concentrations of the transporter and complexes on the EC side of the membrane and  $T_p$  and  $TC_p$  are the concentrations on plasma side.

If  $P_T$  and  $P_{TS}$ , with units of 1/s and are the permeability rate constants, is defined as the permeability rate per volume for the transporter and transporter complex respectively between the two sides of the membrane, then  $P_T$  and  $P_{TS}$  are the rates at which the transport flips between the plasma and EC side of the membrane.  $k_{dec}$   $k_{dp}$ , in mmol/ml, are the disassociation constants for the EC and plasma sides of the membrane.  $P_T$ ,  $P_{TS}$ ,  $T_{tot}$ ,  $k_{dec}$ , and  $k_{dp}$  are related through the total flux ( $J_{T_{ec}}$ ) for the transporter from EC to the plasma side, and  $J_{T_p}$ , the total flux from the plasma to EC side of the membrane. This flux includes both T and its complexes ( $TC_{ec}$  and  $TC_p$ ):

$$J_{T_{ec}} = P_T T_{ec} + P_{TS} \cdot TC_{ec} \quad (A6a)$$

$$J_{T_p} = P_T T_p + P_{TS} \cdot TC_p \quad (A6b)$$

The total transporter flux within the membrane is assumed to be in equilibrium:

$$J_{Tec} + J_{Tp} = 0, \text{ and with:} \quad (A7)$$

$TC_{ec} = T_{ec} \cdot \frac{C_{ec}}{k_{dec}}$  combined with eq (A6a) and (A6b), we have

$$T_{ec} = \frac{(P_T + P_{TS} \cdot \frac{C_p}{k_{dec}})}{(P_T + P_{TS} \cdot \frac{C_{ec}}{k_{dec}})} \cdot T_p \quad (A8)$$

For a Michaelis-Menten like reaction,  $P_{TS}$  and  $P_T$  are equal and the binding-unbinding of the transporter to the substrate is considered infinitely fast,  $k_{dec}$  is equal to  $k_{dp}$  making  $K_m$  equal to disassociation constant  $k_d$ . This reduces Eq. (A8) to:

$$T_{ec} = \frac{k_d + C_p}{k_d + C_{ec}} \cdot T_p \quad (A9)$$

And with Eqs. A4 and A5 we can now solve  $T_p$  and  $TC_p$  in terms of  $T_{tot}$ :

$$T_p = \frac{T_{tot} k_d}{2(k_d + C_p)}, \text{ and} \quad (A10)$$

$$TC_p = \frac{T_{tot} \cdot C_p}{2(k_d + C_p)} \quad (A11)$$

Given that the fluxes for the transporter complex are the same as for the solute, we have the flux  $J_p$  and  $J_{ec}$  given by:

$$J_p = P_{TS} \cdot TC_p = P_{TS} \cdot \frac{T_{tot} \cdot C_p}{2(k_d + C_p)}, \text{ and} \quad (A12)$$

$$J_{ec} = P_{TS} \cdot TC_{ec} = P_{TS} \cdot \frac{T_{tot} \cdot C_{ec}}{2(k_d + C_{ec})} \quad (A13)$$

This reduces to the one-way flux described in eq (A) where the Michaelis-Menten constant and the maximum reaction rate are then given by:

$$Km_{ecl} = k_d \quad (A14)$$

$$\text{And } V_{\max\_ecl} = \frac{1}{2} T_{tot} P_{TS} \quad (A15)$$