Supporting Information

Leary et al. 10.1073/pnas.1108785108

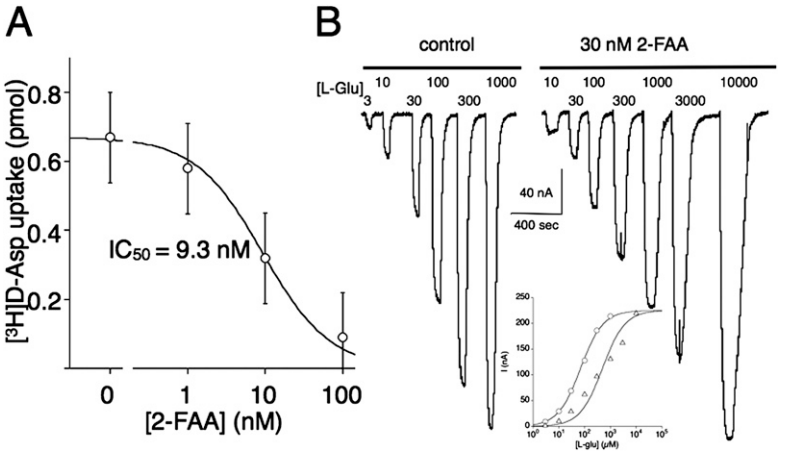


Fig. S1. (*A*) 2-FAA concentration dependence of block of D-[^{3}H]aspartate (1 μM) uptake into oocytes expressing excitatory amino acid transporter (EAAT)3. Langmuir fit of mean data gave an IC_{50} value of 9.3 nM (n = 6 oocytes). (*B*) [L-Glu] dependence of currents in representative voltage-clamped oocyte (−60 mV) in control and in the presence of 30 nM 2-FAA showing that 2-FAA inhibition is overcome by increasing [L-Glu]. Langmuir isotherms (*Inset*) have $K_{0.5}$ values of 69 μM (control; circles) and 491 μM (with 30 nM 2-FAA; triangles). The shift is consistent with competitive 2-FAA inhibition with a K_D of 4.9 nM based on the equation $K_{0.5\ (2\text{-FAA})} = K_{0.5\ (con)}\ (1 + 30\ \text{nM}/K_D)$.

A

$$B + U_0 \underset{\frac{b_1}{(3\frac{f_2}{b_2}+1)}}{\overset{\frac{3f_1\frac{f_2}{b_2}}{(3\frac{f_2}{b_2}+1)}}{\rightleftharpoons}} D_1 + B \underset{\frac{2b_1}{(2\frac{f_2}{b_2}+1)}}{\overset{\frac{2f_1\frac{f_2}{b_2}}{(2\frac{f_2}{b_2}+1)}}{\rightleftharpoons}} D_2 + B \underset{\frac{3b_1}{(\frac{f_2}{b_2}+1)}}{\overset{\frac{f_1\frac{f_2}{b_2}}{(\frac{f_2}{b_2}+1)}}{\rightleftharpoons}} D_3$$

B

$$\frac{d[D_3]}{dt} = \frac{-3b_1}{(\frac{f_2}{b_2}+1)}[D_3] + \frac{[B]f_1\frac{f_2}{b_2}}{(\frac{f_2}{b_2}+1)}[D_2]$$

$$\frac{d[D_2]}{dt} = -(\frac{[B]f_1\frac{f_2}{b_2}}{(\frac{f_2}{b_2}+1)} + \frac{2b_1}{(2\frac{f_2}{b_2}+1)})[D_2] + \frac{[B]f_1 2\frac{f_2}{b_2}}{(2\frac{f_2}{b_2}+1)}[D_1] + \frac{3b_1}{(\frac{f_2}{b_2}+1)}[D_3]$$

$$\frac{d[D_1]}{dt} = -(\frac{2[B]f_1\frac{f_2}{b_2}}{(2\frac{f_2}{b_2}+1)} + \frac{b_1}{(3\frac{f_2}{b_2}+1)})[D_1] + \frac{2b_1}{(2\frac{f_2}{b_2}+1)}[D_2] + \frac{3[B]f_1\frac{f_2}{b_2}}{(3\frac{f_2}{b_2}+1)}[U_0]$$

$$\frac{d[U_0]}{dt} = -\frac{3[B]f_1\frac{f_2}{b_2}}{(3\frac{f_2}{b_2}+1)}[U_0] + \frac{b_1}{(3\frac{f_2}{b_2}+1)}[D_1]$$

C

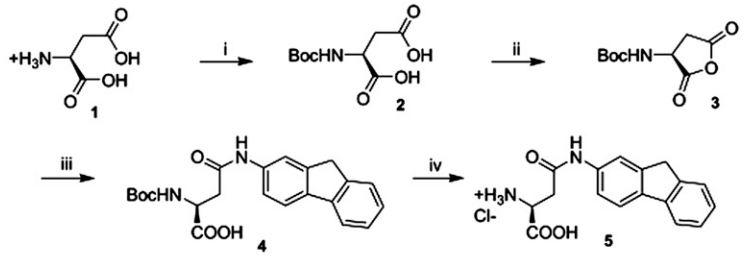
$$f_1 = 9.41x10^5 M^{-1}s^{-1}\_CI_{95\%} = (9.10x10^5, 9.97x10^5)$$

$$b_1 = 0.0076s^{-1}$$

$$\frac{f_2}{b_2} = 2.91\_CI_{95\%} = (2.80, 3.02)$$

Fig. S2. Diffusion barrier reaction mechanism reduction and analysis. The full model (Fig. 7*C*) involves seven differential equations with four parameters, two of which cannot be independently estimated (f_2, b_2). These are therefore collapsed into the ratio f_2/b_2 shown in the asymptotically reduced model (*A*) using the boundary function method (1). U_0, unoccupied transporter; B, blocker; D_n, transporter bound with n molecules of blocker. (*B*) The reduced-model differential equations with three parameters. The solution of the reduced model was a good approximation of the original model solution using the parameter values estimated from experimental data. (*C*) Summary results of reduced-model fitting of experimental data were performed using the MATLAB software package (v. 7.9.0.529) and MATLAB Optimization and Statistics Toolboxes (The MathWorks). Ninety-five percent confidence intervals are given for f_1 (9.1×10^5 $M^{-1}s^{-1}$ to 9.9×10^5 $M^{-1}s^{-1}$) and f_2/b_2 (2.80–3.02); f_1 was fixed to 0.0076 s^{-1}, the value of the recovery rate from β-2-fluorenyl-aspartylamide (2-FAA) block in the presence of 100 μM L-Glu (Fig. 4).

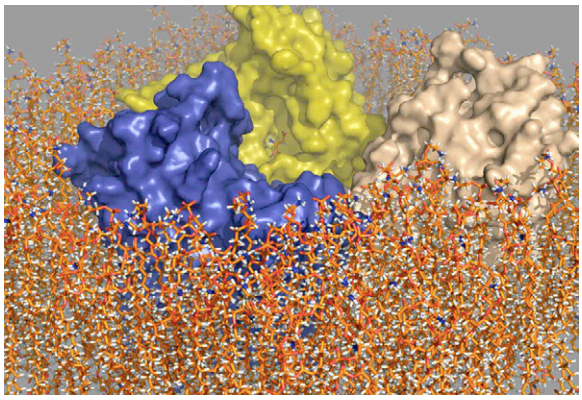
1. Vasil'eva AB, Butuzov VF, Kalachev LV (1995) *The Boundary Function Method for Singular Perturbation Problems* (SIAM, Philadephia).



Synthesis of β-2-fluorenyl-aspartylamide (2-FAA) 5: (i) Boc anhydride, 50/50 TEA / methanol at 22°C; (ii) acetic anhydride, 40°C; (iii) 2-amino-fluorene in DMSO, 60° C; (iv). 1.) 1:1 TFA and CH_2Cl_2 at 22° C. 2.) 1.2 eq 0.01 M HCl, lyophilize.

2-FAA ^{1}H-NMR (400 MHz, d-DMSO at 2.5 ppm) ppm 10.65 (s, 1H), 8.47 (s, 3 H), 7.95 (s, 1H), 7.805 (d, J = 3.88 Hz, 1H), 7.78 (d, J = 2.60 Hz, 1H), 7.59 (d, J = 8.41 Hz, 1H), 7.52 (d, J = 7.76 Hz, 1H), 7.33 (dd, J = 14.24Σ, 7.12 Hz, 1H), 7.24 (dd, J = 14.88Σ, 7.44 Hz, 1H), 4.24 (dd, J = 10.35Σ, 5.18 Hz, 1H), 3.87 (s, 2H), 3.12 (dd, J = 22.00Σ, 17.16, 4.86 Hz, 1Ha), 3.05 (dd, J = 22.65Σ, 17.15, 5.50 Hz, 1Hb). ^{13}C-NMR (400 MHz, d-DMSO at 39.5 ppm) ppm 170.3, 167.4, 143.7, 142.8, 141.0, 137.9, 136.5, 126.8, 126.2, 125.0, 120.2, 119.6, 118.0, 116.1, 48.8, 36.5, 36.0. CHN analysis for $C_{17}H_{19}ClN_2O_3 \cdot H_2O$ calc. C, 58.21; H, 5.46; N, 7.99; O, 18.24; Cl, 10.11. Found: C, 59.34; H, 5.02; N, 7.78. [α]$_{Na}$ = + 20° (c = .28, DMSO)

Fig. S3. Synthesis of 2-FAA. (*i*) tBoc anhydride, 50/50 triethylamine/methanol at 22 °C. (*ii*) Acetic anhydride at 60 °C. (*iii*) 2-Aminofluorene (0.90 eq) in DMSO (0.05 M) at 60 °C. (*iv*) (1) TFA:CH_2Cl_2 (1:1) at 22 °C; (2) 0.01 M HCl (1.2 eq), freeze dry. An NMR spectra summary is shown below.



Movie S1. Movie illustrating the binding site of L-aspartate, including cognate EAAT3 residues R447 and D440 in Glt_{Ph} and the relationship of the binding sites to the central cavity. The movie illustrates the motion of the HP2 loop (dark blue), which transitions between an open and closed conformation to occlude the bound ligand between the HP2 and HP1 (light blue) loops.

Movie S1