Supporting Information (SI)

Containing derivation of the apparent rate constant of Zn competing with Cu to bind Aβ, details of coupled reaction-diffusion simulations and supplementary figures (Figs S1-S3).

Derivation of the apparent rate constant of Zn competing with Cu to bind Aβ

We have shown in our previous publication\(^1\) that the binding of Cu\(^{2+}\) to Aβ is nearly diffusion limited. The expected fast binding of Aβ to Zn\(^{2+}\), a high \(K_d\) of approximately 10 µM and no quenching of labelled fluorophore on Aβ by Zn\(^{2+}\) prevent direct methodologies being applied to investigate the kinetics of the Aβ reaction with Zn\(^{2+}\). Therefore, indirect competition experiments must be used. There are two forms of competition experiments possible: a mixture of Aβ and a Zn\(^{2+}\) indicator competing for Zn\(^{2+}\), and Zn\(^{2+}\) competing with Cu\(^{2+}\) for labelled Aβ.

The problem with the Aβ competing against a zinc indicator for Zn\(^{2+}\) is that the concentration of Aβ and zinc indicator must be in a 20 fold excess of the concentration of Zn\(^{2+}\) for the experiment to be under pseudo-first-order conditions. Given that the literature \(K_d\) is in the range of 1 µM to 100 µM, even stoichiometric concentrations of Aβ would cause rapid Aβ aggregation, more so with a 20 fold excess. Therefore we measured Zn\(^{2+}\) competing with Cu\(^{2+}\) for labelled Aβ.

We take a simplified reaction model to describe the kinetic competition experiments as shown below

\[
\begin{align*}
\text{Aβ} + \text{Cu}^{2+} + \text{Zn}^{2+} & \rightleftharpoons \text{Aβ} \cdot \text{Cu} + \text{Zn}^{2+} \\
\text{Aβ} \cdot \text{Cu} + \text{Zn}^{2+} & \rightleftharpoons \text{Aβ} \cdot \text{Zn} + \text{Cu}^{2+}
\end{align*}
\]

The reaction of Aβ-Cu → Aβ + Cu\(^{2+}\) is ignored as the rate is approximately 0.5 s\(^{-1}\), much slower than the observed binding rates (20 s\(^{-1}\) to 100 s\(^{-1}\)). The reaction Aβ-Cu + Zn\(^{2+}\) → Zn·Aβ·Cu is also ignored as the rate constant is 3×10\(^3\) M\(^{-1}\)s\(^{-1}\) (determined in main text; Kinetics of Zn binding to Aβ-Cu) which gives a rate of 0.6 s\(^{-1}\) at the highest Zn\(^{2+}\) concentration used in the experiments. The reaction Aβ·Zn + Cu\(^{2+}\) → Zn·Aβ·Cu is ignored because in order for the reaction to have a rate of at least 1 s\(^{-1}\), a rate constant of 2×10\(^6\) M\(^{-1}\)s\(^{-1}\) or greater is required. This is deemed unlikely given that the rate constants for the reaction Aβ·Cu + Zn\(^{2+}\) → Zn·Aβ·Cu and Aβ-Cu + Cu\(^{2+}\) → Cu·Aβ-Cu are 3×10\(^3\) M\(^{-1}\)s\(^{-1}\) and 1×10\(^5\) M\(^{-1}\)s\(^{-1}\) respectively. The later reaction is therefore also too slow to participate (0.05 s\(^{-1}\)). By the same reasoning further reaction of Zn\(^{2+}\) with Aβ-Zn is also ignored.

If the experiment is conducted under pseudo-first-order conditions, such that \([\text{Zn}^{2+}]\gg[\text{Aβ}]\) and \([\text{Cu}^{2+}]\gg[\text{Aβ}]\), the solution for Aβ-Cu is of the form

\[
[Aβ \cdot Cu] = \frac{[Aβ][Cu^{2+}]}{2\beta^2}
\begin{pmatrix}
2\beta^2 \\
+\beta (k_{Cu}[Cu^{2+}] - k_{Zn}[Zn^{2+}] - k_{Zn} - \beta) e^{-\alpha(\alpha - \beta)} \\
-\beta (k_{Cu}[Cu^{2+}] - k_{Zn}[Zn^{2+}] - k_{Zn} + \beta) e^{-\alpha(\alpha + \beta)}
\end{pmatrix}
\]

where

\[
\alpha = k_{Cu}[Cu^{2+}] + k_{Zn}[Zn^{2+}] + k_{Zn}
\]

(1)
\[ \beta = \sqrt{\alpha^2 - 4k_{Zn}k_{Cu}[Cu^{2+}]} \]  

(3)

Applying the initial conditions \([A\beta \cdot Cu]_{t=0} = [A\beta \cdot Zn]_{t=0} = 0\), the solution is a double exponential with the rates

\[ k = \frac{1}{2}(\alpha \pm \beta) = \]

\[ \frac{1}{2} \left( k_{Cu}[Cu^{2+}] + k_{Zn}[Zn^{2+}] + k_{Zn} \pm \sqrt{(k_{Cu}[Cu^{2+}] + k_{Zn}[Zn^{2+}] + k_{Zn}k_{d})^2 - 4k_{Cu}k_{Zn}[Cu^{2+}]} \right) \]  

(4)

The fast phase of the curves obtained from the stopped flow measurements were fitted to

\[ k = \]

\[ \frac{1}{2} \left( k_{Cu}[Cu^{2+}] + k_{Zn}[Zn^{2+}] + k_{Zn}K_d + \sqrt{(k_{Cu}[Cu^{2+}] + k_{Zn}[Zn^{2+}] + k_{Zn}K_d)^2 - 4k_{Cu}[Cu^{2+}]k_{Zn}K_d} \right) \]  

(5)

where \( K_d \) is the equilibrium dissociation constant \((K_d = k_{zn}/k_{zn})\) of A\(\beta\)-Zn complex.

Simulation of the reactions between metal ions and A\(\beta\) in the synaptic cleft by coupled reaction-diffusion equations

The simulation was based on a simplified model of the synaptic cleft with a height of 20 nm. It is technically a 3D simulation, but we assume that the cleft width is infinite so that the diffusion of metal ions released is not restricted to the typical synaptic width of a few hundred of nanometers. Simulation in confined space is not appropriate as metal ions would not be able to escape. We also assume that the space is both translationally and rotationally symmetric, so only 1D is needed in polar coordinates. Metal ions (30 \(\mu\)M Cu\(^{2+}\) or 300 \(\mu\)M Zn\(^{2+}\)) were assumed to release into the centre of the synapse via 40 nm diameter vesicles and react with 3 nM A\(\beta\) in the cleft. The diffusion coefficients used are \(D_{Zn} = D_{Cu} = 650\) \(nm^2/\mu s\) \(\times 2\) \(nm^2/\mu s\) \(^{-1}\) \(^{-2}\) \(D_{A\beta} = 304\) \(nm^2/\mu s\) \(^{-1}\) and \(D_{HSA} = 61\) \(nm^2/\mu s\) \(^{-1}\). The synapse height adopted is in line with the literature. \(^{4,5}\)

To simulate the diffusion of metal ions and A\(\beta\) in the synapse, Fick’s second law with constant diffusion coefficient was used,

\[ \partial_t \varphi = D \nabla^2 \varphi \]  

(6)

where \( \varphi \) is a scalar field of the concentration, \( t \) is the time and \( D \) is the diffusion coefficient. In cylindrical coordinates, this equation can be simplified to

\[ \partial_t \varphi = D (\partial_r^2 + r^{-1} \partial_r) \varphi \]  

(7)

To solve this equation numerically, it needs to be discretized. The forward Euler method was used for the temporal term; while the central finite difference and the backwards Euler method were applied to \( \partial_r^2 \) and \( \partial_r \) respectively. This gives

\[ \frac{\varphi(r,t+\delta t) - \varphi_r^t}{\delta t} = D \left( \frac{\varphi(r+\delta r,t+\delta t) - 2\varphi_r^t + \varphi(r-\delta r,t+\delta t)}{(\delta r)^2} + \frac{\varphi(r+\delta r,t-\delta t) - \varphi(r-\delta r,t-\delta t)}{2r^2} \right) \]  

(8)
Backward methods were used because the forward method is stable only for very small $\delta r$. Eqn. 7 can be rearranged to give

$$\phi_r^t = \phi_{r-\delta r}^t \frac{D \delta t}{\delta r} \left( \frac{1}{2r} - \frac{1}{2r+\delta r} \right) + \phi_r^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right) - \phi_{\delta r}^{t+\delta t} \frac{D \delta t}{\delta r} \left( \frac{1}{2r} + \frac{1}{2r+\delta r} \right)$$  

(9)

which forms a set of equations for the next time step. Due to a pole at $r = 0$, the function needs to be moved off the axis by mapping $r$ to $r + \delta r/2$ in the implementation, thus Eqn 8 becoming

$$\phi_r^t = \phi_{r-\delta r}^t \frac{D \delta t}{\delta r} \left( \frac{1}{2r} - \frac{1}{2r+\delta r} \right) + \phi_r^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right) - \phi_{\delta r}^{t+\delta t} \frac{D \delta t}{\delta r} \left( \frac{1}{2r} + \frac{1}{2r+\delta r} \right)$$  

(10)

The boundary condition at $r = 0$ is

$$\phi_0^t = \phi_0^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right) - \phi_{\delta r}^{t+\delta t} \frac{2D \delta t}{(\delta r)^2}$$  

(11)

At the edge of the disc ($r = R$), there are two options. The first option is to set the concentration of the ring outside the disc to 0, that is

$$\phi_R^t = \phi_{R-\delta r}^t \frac{D \delta t}{\delta r} \left( \frac{1}{2R} - \frac{1}{2R+\delta r} \right) + \phi_R^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right)$$  

(12)

This will cause the molecules to “leak” off the edge in the simulation. Although this is suitable for metal ions diffusing from the centre, it is not suitable for a homogeneous concentration of Aβ as Aβ will diffuse off the disc. To counteract this, the edge of the disc was connected to itself by mapping $\phi_{R+\delta r}^{t+\delta t}$ to $\phi_{R-\delta r}^{t+\delta t}$, making the boundary conditions

$$\phi_R^t = -\phi_{R-\delta r}^{t+\delta t} \frac{2D \delta t}{(\delta r)^2} + \phi_R^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right)$$  

(13)

To incorporate reactions into the model, the rate of the change of the concentration due to reaction $R(\phi)$ is added to the diffusion Eqn 5, thus becoming

$$\partial_\phi \phi = D \nabla^2 \phi + R(\phi)$$  

(14)

For a simple reaction, $A + B \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} C$

if the scalar fields of concentrations of A, B & C are $\phi$, $\psi$ and $\chi$ respectively, the changes in concentration due to the reaction will be

$$R(\phi) = k_{\text{on}}\chi - k_{\text{off}}\phi\psi, R(\psi) = k_{\text{off}}\chi - k_{\text{on}}\phi\psi \quad \text{and} \quad R(\chi) = k_{\text{on}}\phi\psi - k_{\text{off}}\chi$$  

(15)

Hence discretising these and applying the Euler method, the equations to be solved are

$$\phi_r^t = -\delta t (k_{\text{off}}\phi_{\delta r}^t - k_{\text{on}}\phi_r^{t+\delta t}) + \phi_{\delta r}^{t+\delta t} \frac{D \delta t}{\delta r} \left( \frac{1}{2r} - \frac{1}{2r+\delta r} \right) + \phi_r^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right) - \phi_{\delta r}^{t+\delta t} \frac{D \delta t}{\delta r} \left( \frac{1}{2r} + \frac{1}{2r+\delta r} \right)$$  

(16)

$$\psi_r^t = -\delta t (k_{\text{off}}\psi_{\delta r}^t - k_{\text{on}}\psi_r^{t+\delta t}) + \psi_{\delta r}^{t+\delta t} \frac{D \delta t}{\delta r} \left( \frac{1}{2r} - \frac{1}{2r+\delta r} \right) + \psi_r^{t+\delta t} \left( 1 + \frac{2D \delta t}{(\delta r)^2} \right) - \psi_{\delta r}^{t+\delta t} \frac{D \delta t}{\delta r} \left( \frac{1}{2r} + \frac{1}{2r+\delta r} \right)$$  

(17)
\[ \chi^t_r = -\delta t (k \phi^t_r \psi^t_r - k_{off} \chi^t_r) + \chi^{t+\delta t}_{r-\delta r} \frac{\Delta x^t}{\Delta r} \left( \frac{1}{2r+\delta r} - \frac{1}{2r} \right) + \chi^{t+\delta t}_r \left( 1 + \frac{2D \delta t}{(2r+\delta r)^2} \right) - \chi^{t+\delta t}_r \frac{\Delta x^t}{\Delta r} \left( \frac{1}{2r+\delta r} + \frac{1}{2r} \right) \quad (18) \]

Boundary conditions can be produced similarly as above.

In the reaction-diffusion equations (Eqn. 14-18) the reaction and diffusion parts may be treated separately in each time step simplifying the implementation, as the reaction only depends on the concentrations at a particular \( r \) and the diffusion depends on all \( r \) but only for the concentrations in one field. It can therefore be easily parallelized using OpenMP.\(^6\)

The discretized solution for \( \phi \) is of the matrix form

\[ \phi^t + \delta t R(\phi, \psi, \chi) = M \phi^{t+1} \quad (19) \]

where \( M \) is a matrix of coefficients related to diffusion (similarly for \( \psi \) and \( \chi \)). This gives the order of the procedure as the following: get the concentration fields, perform the reactions, and then calculate the concentrations at the next time step from diffusion. The concentrations of the next time step can be obtained from

\[ \phi^{t+1} = M^{-1} (\phi^t + \delta t R(\phi, \psi, \chi)) \quad (20) \]

\( M \) is tridiagonal, therefore solving for \( \phi^{t+1} \) may be done more efficiently than via matrix inversion. The gsl_linalg_solv_tridiag from the GSL\(^7\) was then used.

For the simulation, the time steps were chosen to increase exponentially after the second time step \( t_1 (t_0 = 0) \), defined by

\[ t_{i+1} = t_1 \times 10^{\log(T/t_1) \times i/(N_t - 1)} \quad (21) \]

where \( T \) is the maximum time of the simulation, \( t \) is the first non-zero time step, \( N_t \) is the number of time steps, and \( i \) is an integer from 0 to \( N_t \). Logarithmic time steps allow the long timescale behaviour to be seen without exponentially increasing processing power. For the space steps, linear spacing is used as the central finite difference method requires \( \delta r \) to be constant across the simulation.

To simulate the case of periodic pulsed release of metal ions during neurotransmission, the concentration of metal ions at the centre (20 nm radius) of each release was reset to initial concentration. The time step was set to

\[ t_{i+1} = T_p \left\lfloor \frac{i}{N_s} \right\rfloor + t_1 \times 10^{\log(T_p/t_1) \times (i \text{ mod } N_s)/(N_t - 1)} \quad (22) \]

where \( T_p \) is the pulse period, \( N_s \) is the number of steps per pulse, and mod is the modulo operator. Then when \( i \text{ mod } N_s = 0 \), the central 20 nm of the simulation is reset to the initial concentration of metal ions.

The simulation code was written in C++, using the GSL and the OpenMP API, and complied with GCC.\(^8\)
References

1. Branch T., Girvan P., Barahona M., Ying L. M. (2015) Introduction of a fluorescent probe to amyloid-β to reveal kinetic insights into its interactions with copper(II). Angew. Chem. Int. Ed. 54, 1227–1230.
2. Kariuki, S., and Dewald, H. D. (1996) Evaluation of diffusion coefficients of metallic ions in aqueous solutions. Electroanalysis 8, 307–313.
3. Steven Vogel (1988) Life’s Devices, Princeton University Press, ISBN 0691024189.
4. Qu L., Akbergenova Y., Hu Y. M., Schikorski T. (2009) Synapse-to-synapse variation in mean synaptic vesicle size and its relationship with synaptic morphology and function. J. Comp. Neurol. 514, 343–352.
5. Savtchenko L. P., Rusakov D. A. (2007) The optimal height of the synaptic cleft. Proc. Natl. Acad. Sci. USA 104, 1823–1828.
6. Open Multiprocessing API (2.5) at http://openmp.org.
7. GNU Scientific Library (1.16) at http://www.gnu.org/software/gsl/.
8. GNU Compiler Collection (4.9.1) at https://gcc.gnu.org/.
Figure S1. Temporal profiles of (Aβ·Cu)$_i$ concentration at indicated distance from point of release. Release frequency indicated in top right hand corner of each panel.
Figure S2. Temporal profiles of (Aβ-Cu)$_n$ concentration at indicated distance from point of release. Release frequency indicated in top right hand corner of each panel. All lines nearly overlap.
Figure S3. Temporal profiles of Aβ•Zn concentration at indicated distance from point of release. Release frequency indicated in bottom right hand corner of each panel.