Supporting materials, methods & Figs S1, S2 & S3

Materials

The sequences of the various amyloid beta peptides used in this work are shown below.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ16(H6R)</td>
<td>DAEFR RDSGY EVHHQ K*</td>
</tr>
<tr>
<td>Aβ16(D7N)</td>
<td>DAEFR HNSGY EVHHQ K*</td>
</tr>
<tr>
<td>Aβ16(pS8)</td>
<td>DAEFR HDS†GY EVHHQ K*</td>
</tr>
<tr>
<td>Aβ40(H6R)</td>
<td>DAEFR RDSGY EVHHQ KLVFC AEDVG SNKGA IIGLM VGGVV</td>
</tr>
<tr>
<td>Aβ40(D7N)</td>
<td>DAEFR HNSGY EVHHQ KLVFC AEDVG SNKGA IIGLM VGGVV</td>
</tr>
<tr>
<td>wt-Aβ40</td>
<td>DAEFR HDGYS EVHHQ KLVFC AEDVG SNKGA IIGLM VGGVV</td>
</tr>
</tbody>
</table>

* HiLyte 488  † phosphorylated serine

Coordination environments for Aβ·Cu²⁺ complexes

Figure S1 Coordination of Cu²⁺ bound to A) wt-Aβ and B) Aβ(H6R) showing both major components at physiological pH. Aβ(D7N) is expected to bind in a similar manner to the wild type. [1]
Association rate constant of copper to Aβ

When determining the association rate of Aβ with Cu²⁺ independent of HEPES in the buffer, the HEPES concentration was varied from 10 mM to 100 mM, with 20 nM of Aβ and 400 nM of CuCl₂ in 100 mM NaCl. The apparent rate was obtained by fitting the fluorescence quenching data (Figure S2) with multi-exponentials, taking the major, fast phase. To determine the HEPES independent reaction rate, an empirically chosen parabola centred on zero was used to fit a plot of inverse apparent rate, against HEPES concentration, where the inverse of the intercept corresponds to the HEPES independent rate.

Figure S2 Kinetic traces showing quenching of fluorescence as Cu(II) binds to Aβ16 A) H6R, B) D7N and C) pS8 at various concentrations of HEPES. D) Fluorescence titration of 133 nM Hilyte 488 labelled Aβ16 by Cu²⁺ in 50 mM HEPES and 100 mM NaCl buffer solution (pH 7.5).
Interconversion of mutated (H6R & D7N) and phosphorelated pS8 Aβ·Cu complexes

The methods used were described in detail in the supporting information of our recent publication (Ref 2). Briefly, the minimum symmetric reaction scheme shown below was used to account for the spontaneous dissociations of the two Aβ·Cu species (Component I and II), their interconversion, and the reactions of the ligand (EDTA), with the two species.

This model gives the following differential equations.

\[
\frac{d[(Aβ·Cu)_I]}{dt} = -[(Aβ·Cu)_I](k_{10ff} + k_{1Loff}[L] + k_{1→2}) + [(Aβ·Cu)_II]k_{2→1} \quad (1)
\]

\[
\frac{d[(Aβ·Cu)_II]}{dt} = -[(Aβ·Cu)_II](k_{20ff} + k_{2Loff}[L] + k_{2→1}) + [(Aβ·Cu)_I]k_{1→2} \quad (2)
\]

As the experiments here were performed under pseudo-first-order conditions we can assume

\[
\frac{d[L]}{dt} = 0 \quad (3)
\]

An analytical solution can be found for the concentration of the two Aβ·Cu species as a function of time. The solutions are double exponential functions with amplitudes \(A_1, A_2\) and rate constants \(k_1, k_2\) as the following.

\[
A_1 = \frac{1}{2\delta}((\beta + \gamma)[(Aβ·Cu)_I]|_{t=0} - 2k_{2→1}[(Aβ·Cu)_II]|_{t=0}
+ (\beta - \gamma)[(Aβ·Cu)_II]|_{t=0} - 2k_{1→2}[(Aβ·Cu)_I]|_{t=0}) \quad (4)
\]

\[
k_1 = \frac{\alpha + \beta}{2} \quad (5)
\]

\[
A_2 = \frac{1}{2\delta}((\beta - \gamma)[(Aβ·Cu)_I]|_{t=0} + 2k_{2→1}[(Aβ·Cu)_II]|_{t=0}
+ (\beta + \gamma)[(Aβ·Cu)_II]|_{t=0} + 2k_{1→2}[(Aβ·Cu)_I]|_{t=0}) \quad (6)
\]

\[
k_2 = \frac{\alpha - \beta}{2} \quad (7)
\]
Where

\[ \alpha = k_{1\to2} + k_{2\to1} + k_{1\text{off}} + k_{2\text{off}} + (k_{1\text{Loff}} + k_{2\text{Loff}})[L] \]  

(8)

\[ \beta = (k_{1\to2}^2 + 2k_{1\to2}(k_{2\to1} + k_{1\text{off}} - k_{2\text{off}} + (k_{1\text{Loff}} - k_{2\text{Loff}})[L])) + (k_{2\to1} - k_{1\text{off}} + k_{2\text{off}} - (k_{1\text{Loff}} - k_{2\text{Loff}})[L])^2 \]  

(9)

\[ \gamma = k_{1\to2} - k_{2\to1} + k_{1\text{off}} - k_{2\text{off}} + (k_{1\text{Loff}} - k_{2\text{Loff}})[L] \]  

(10)

\[ \delta = \sqrt{-4(k_{2\to1}(k_{1\text{off}} + k_{1\text{Loff}}[L]) + (k_{1\to2} + k_{1\text{off}} + k_{1\text{Loff}}[L])(k_{2\text{off}} + k_{2\text{Loff}}[L])) + \alpha^2} \]  

(11)

These solutions were used to fit the experimentally obtained data (Figure S3) and extract the parameters shown in the scheme above.

**Figure S3a** Raw kinetic traces showing recovery of fluorescence as Cu is removed from the Aβ(H6R)-Cu complex by various concentrations of EDTA. Fitted lines are shown in red.
Figure S3b Raw kinetic traces showing recovery of fluorescence as Cu is removed from the Aβ(D7N)-Cu complex by various concentrations of EDTA. Fitted lines are shown in red.

Figure S3c Raw kinetic traces showing recovery of fluorescence as Cu is removed from the Aβ(pS8)-Cu complex by various concentrations of EDTA. Fitted lines are shown in red.
Determining $pK_a$ of mutated (H6R & D7N) and phosphorylated (pS8) Aβ·Cu complexes

The interconversion of Components I & II of the Aβ-Cu complex involves the removal of a proton. Therefore by using the rates of interconversion $k_{1\rightarrow2}$ & $k_{2\rightarrow1}$ determined from fitting the data to our model along with the Henderson–Hasselbalch equation the $pK_a$ of Aβ-Cu complex can be determined.

\[
(A\beta\cdot Cu)_I \xrightleftharpoons[k_{1\rightarrow2}]{k_{2\rightarrow1}} (A\beta\cdot Cu)_II + H^+
\]

\[
pH = pK_a + \log_{10} \frac{k_{1\rightarrow2}}{k_{2\rightarrow1}} \quad (12)
\]

**Derivation of apparent equilibrium dissociation constant of Aβ·Cu complex from kinetic rate constants**

\[
A\beta + Cu^{2+} \xrightleftharpoons[k_{on}]{k_{off}} (A\beta\cdot Cu)_I \xrightleftharpoons[k_{2\rightarrow1}]{k_{1\rightarrow2}} (A\beta\cdot Cu)_II
\]

As the dissociation rate constant of Component II is much slower compared to that of the Component I and the interconversion rates, we can use the scheme shown above to work out the apparent equilibrium dissociation constant of Aβ-Cu complex.

\[
K_A = \frac{[(A\beta\cdot Cu)_I] + [(A\beta\cdot Cu)_II]}{[A\beta][Cu]} = \frac{k_{on}}{k_{off}} \left(1 + \frac{k_{1\rightarrow2}}{k_{2\rightarrow1}}\right) \quad (13)
\]

\[
K_D = \frac{1}{K_A} \quad (14)
\]

**Reference**
