Supplemental Material

Human serum albumin can regulate amyloid-beta fiber growth in the brain interstitium. Implications for Alzheimer's Disease

Helen F. Stanyon and John H. Viles*

Including:
Affinity Calculations:
Supplemental Figure 1. Aβ(1-40) fibril growth in the presence of HSA.
Supplemental Figure 2 : Kinetic parameters for mean fibril growth of Aβ(1-42) in the presence of varying concentrations of HSA.

Affinity Calculations:
The albumin-Aβ complex is in equilibrium with free Aβ with a dissociation constant of 5 μM.

$$\begin{bmatrix}\text{Albumin-A}\beta \\ \text{1:1 complex}\end{bmatrix} \rightleftharpoons \begin{bmatrix} \text{A}\beta \text{ (free)} \\ (\text{A}\beta \text{ monomers} \rightleftharpoons \text{A}\beta \text{ fibrils}) \end{bmatrix} + \begin{bmatrix}\text{HSA (free)}\end{bmatrix}$$

$$K_d = \frac{[\text{HSA free}]\left[\text{A}\beta \text{ free}_{\text{(monomeric or fibrillar)}}\right]}{[\text{HSA-A}\beta \text{ complex}]}$$

This can be rewritten as:

$$K_d = \frac{([\text{Total HSA}]-[\text{Bound HSA}])([\text{Total A}\beta]-[\text{Bound A}\beta])}{[\text{Bound HSA-A}\beta]}$$

As the bound concentration is unknown, this becomes x:

$$K_d = \frac{([\text{Total HSA}]-x)\ ([\text{Total A}\beta]-x)}{x}$$

When the appropriate values are submitted within the above equation, a quadratic equation is produced. Therefore, x is solved using the following equation:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

The K_d for the complex is 5 μM.

Predicted percentage of Aβ bound to HSA with different concentrations of albumin can be calculated as follows:

For total Aβ concentration of 10 μM and total HSA concentration of 10 μM.:

$$5\ \mu\text{M} = \frac{(10\ \mu\text{M} - x)(10\ \mu\text{M} - x)}{x}$$

$$5x = x^2 - 20x + 100$$

$$x^2 - 25x + 100 = 0$$

$$x = \frac{25 \pm \sqrt{25^2 - (4\times1\times100)}}{2\times1}$$

x = 5, x= 20.

However, as the concentration bound must be lower than the total concentration,
so x = 5 μM.

$$\textbf{\% A}\boldsymbol{\beta}_{\text{bound}} = \frac{5\ \mu\text{M}\times 100}{10\ \mu\text{M}} = \textbf{50 \%}$$

A similar calculation can be carried out for 5, 3 and 1 μM total HSA. The proportion of free and HSA bound Aβ is calculated based upon a K_d of 5 μM and is tabulated below.

Total HSA (μM)	**Aβ Bound to HSA (μM)**	**Aβ Free (μM)**	**Aβ Free (%)**
10	5.00	5.00	50.0
5	2.93	7.07	70.7
3	1.86	8.14	81.4
1	0.65	9.35	93.5

These values are correlated with percentage fraction of Aβ fibers generated in the presence of increasing levels of albumin in Figure 3b.

Similarly, the predicted percentage of Aβ bound to HSA in the CSF based on a K_d of 5 μM with HSA concentration of 3 μM and Aβ levels of 1 nM can be calculated, giving the result that 37.5 % of Aβ will be bound to HSA in the CSF.

$$5\ \mu\text{M} = \frac{(3\ \mu\text{M} - x)(0.001\ \mu\text{M} - x)}{x}$$

$$5x = x^2 - 3.001x + 0.003$$

$$x^2 - 8.001x + 0.003 = 0$$

$$x = \frac{8.001 \pm \sqrt{8.001^2 - (4 \times 1 \times 0.003)}}{2 \times 1}$$

x = 0.375, x = 7.997

However, as the concentration bound must be lower than the total concentration, x = 0.375 μM.

$$\textbf{\% A}\boldsymbol{\beta}_{\text{bound}} = \frac{0.375\ \mu\text{M} \times 100}{10\ \mu\text{M}} = \textbf{37.5 \%}$$

With a decrease in HSA concentration to 1 μM and Aβ remaining at 1 nM, the percentage of Aβ bound to albumin will be only 16.7 %.

$$5\ \mu\text{M} = \frac{(1\ \mu\text{M} - x)(0.001\ \mu\text{M} - x)}{x}$$

$5x = x^2 - 1.001x + 0.001$

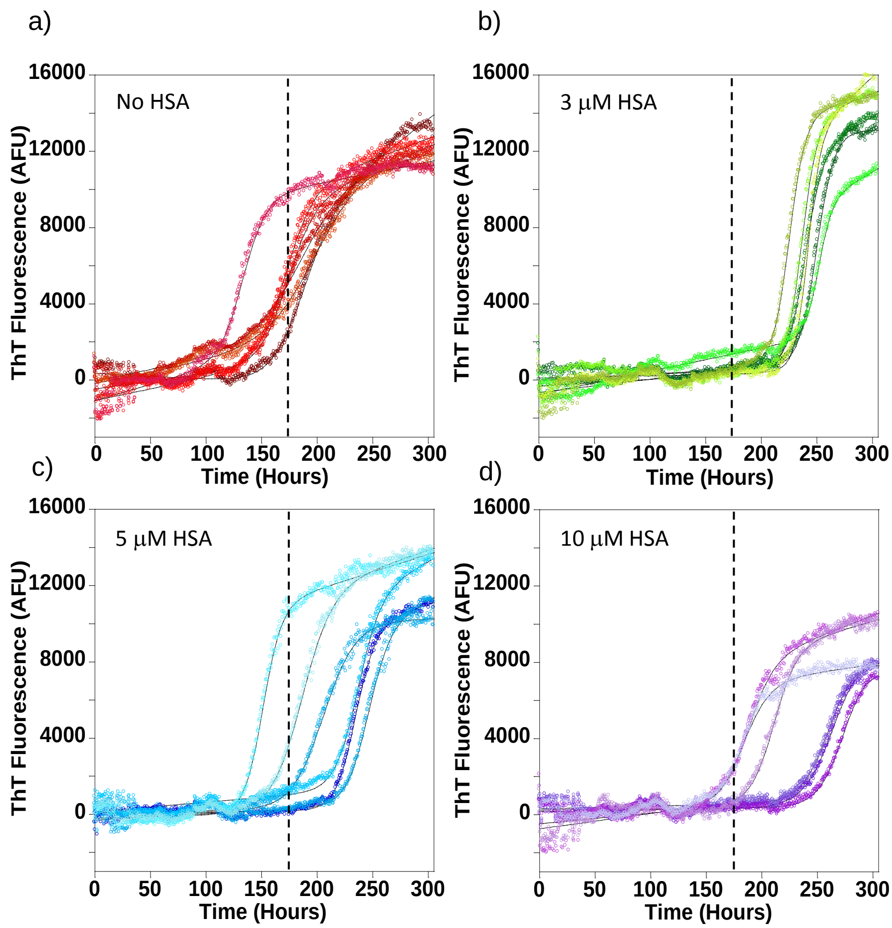
$x^2 - 6.001x + 0.001 = 0$

$$x = \frac{6.001 \pm \sqrt{6.001^2 - (4 \times 1 \times 0.001)}}{2 \times 1}$$

$x = 1.667 \times 10^{-4}$, x = 6.001

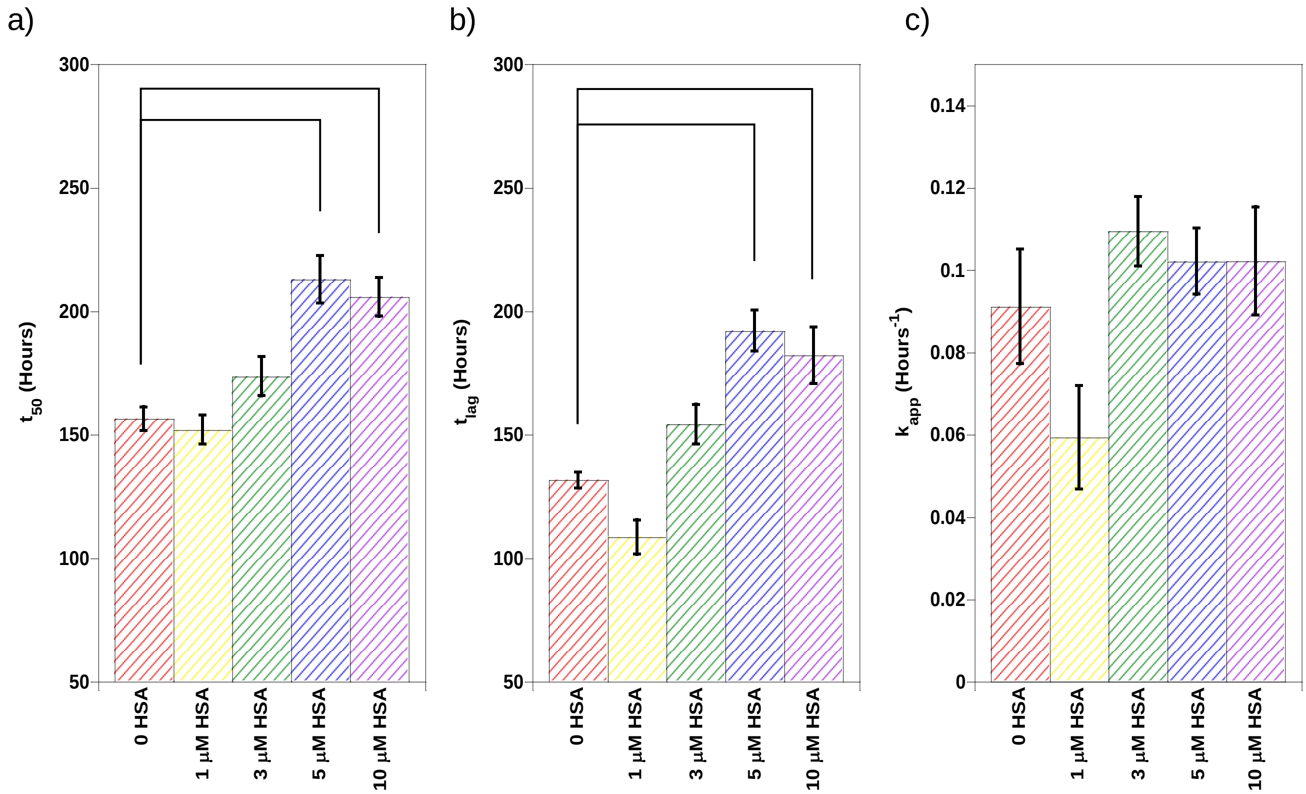
As the concentration bound can't be higher than the total concentration, x = 0.167 nM.

$$\textbf{\% A}\boldsymbol{\beta}_{\text{bound}} = \frac{0.167\ \text{nM} \times 100}{1\ \text{nM}} = \textbf{16.7 \%}$$



Supplemental Figure 1. Aβ(1-40) fibril growth in the presence of HSA. (One of 4 repeat experiments)

Fibrillisation of 10 μM Aβ(1-40) in 30 mM HEPES and 160 mM NaCl at pH 7.4 at 30 °C was monitored using ThT fluorescence. Here individual traces of Aβ alone (a) and Aβ in the presence of 3 μM (b), 5 μM (c) and 10 μM (d) HSA are shown. The average t_{50} for Aβ alone is shown on traces a-d as a dashed line.



Supplemental Figure 2 : Kinetic parameters for mean fibril growth of Aβ(1-42) in the presence of varying concentrations of HSA.

Fibrillization of 10 µM Aβ(1-42) in 30 mM HEPES and 160 mM NaCl at pH 7.4 at 30 °C was monitored using ThT fluorescence. Here the t_{50} (A), lag times (t_{lag}) (B) and average apparent growth rates (k_{app}) (C), with their standard errors are shown. Significant differences between Aβ alone and albumin concentrations are shown by connecting lines, typically observed for 5 and 10 µM HSA.