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Supplementary Data

Structural and Functional Alterations in Amyloid-β Precursor Protein Induced by Amyloid-β Peptides

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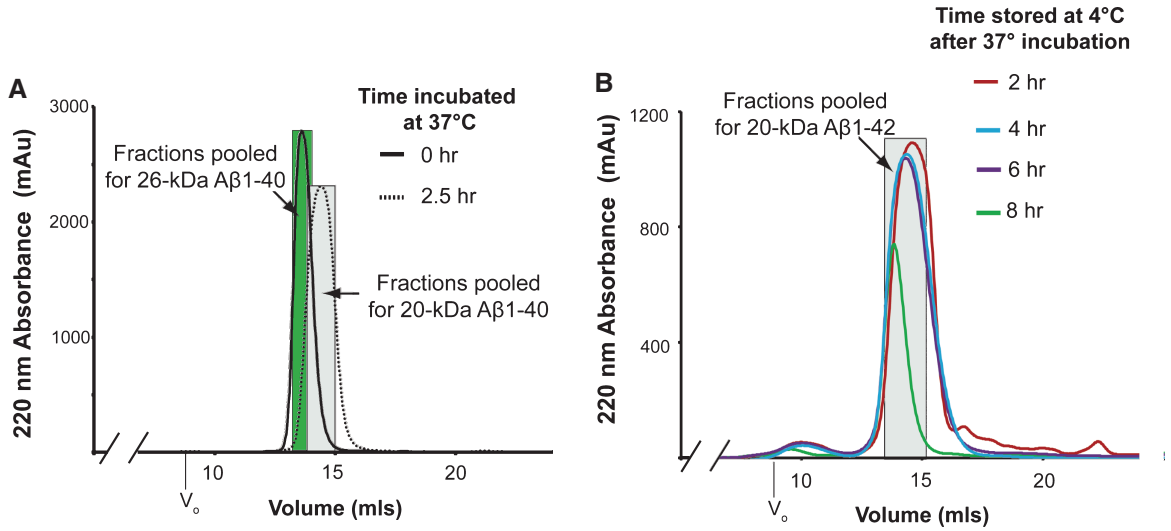

Supplementary Figure 1. Changes in the Aβ oligomers with incubation. A) Superdex S-75 profile demonstrating the decrease in size of $A\beta_{1–40}$ after incubation at 37°C. B) Superdex S-75 profiles demonstrating the change in the shape of the 20-kDa $A\beta_{1–42}$ peak with increasing time incubated at 4°C. The primary change observed is an increase in species too large to enter the column and a gradual decrease in the amount of $A\beta_{1–42}$ oligomers of all sizes eluting from the column over time. The starting material was $A\beta_{1–42}$ incubated at 37°C for 2 hours before being moved to 4°C. The incubation conditions and methods for column purification are the same as in Fig. 1A. Experiments were planned so that all the $A\beta_{1–42}$ oligomers required could be generated within 4–6 h in order to conserve $A\beta_{1–42}$ peptide. The Aβ peptides that accumulated on the column frits and top were removed with regular cleanings using 200 mM NaOH. Columns were thoroughly cleaned between $A\beta_{1–42}$ and $A\beta_{1–40}$ purifications to avoid cross-contamination.

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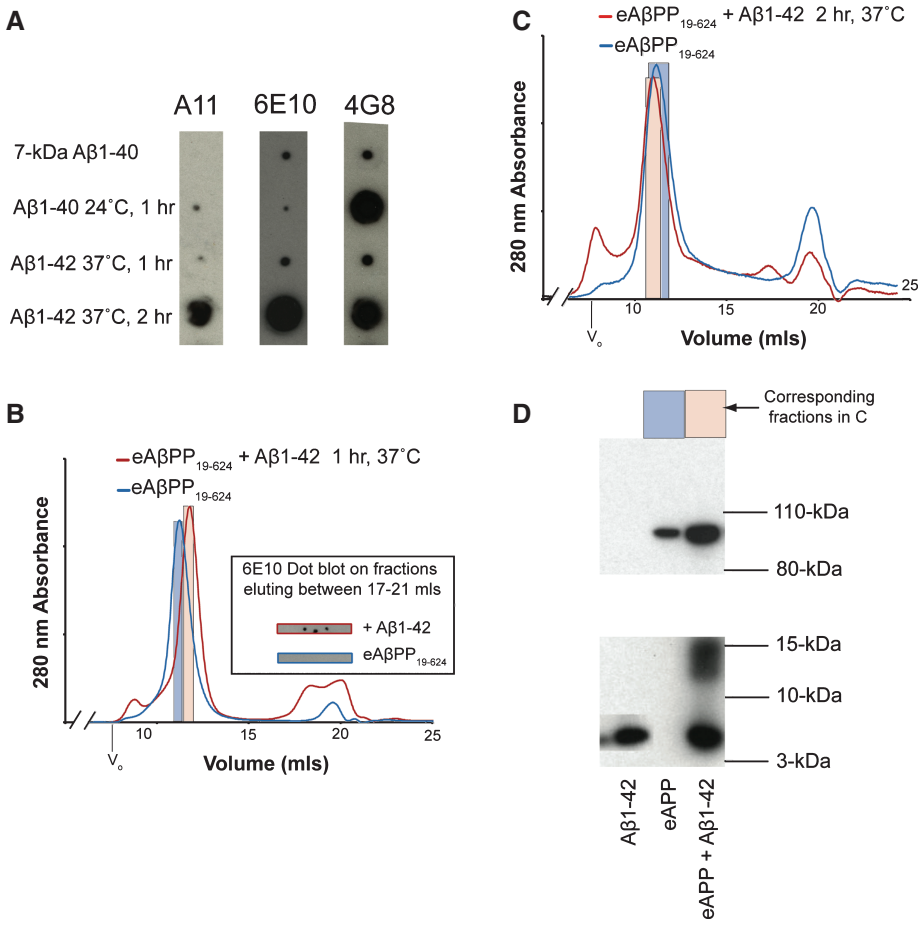
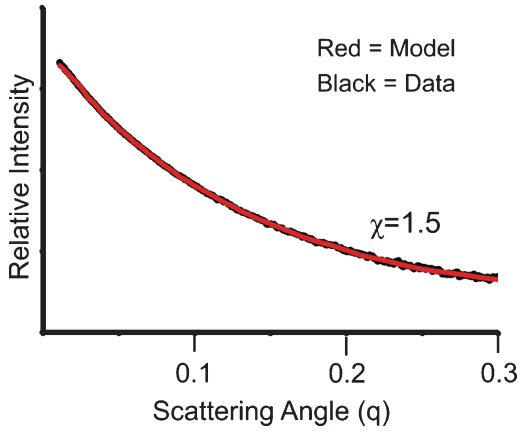


Supplementary Figure 2. Purification of $A\beta_{1-42}$ is not required for binding. A) Dot blots demonstrating the A-11 reactivity of purified 7-kDa $A\beta_{1-40}$, $A\beta_{1-40}$ incubated at 24°C for 1 h prior to purification for the 26-kDa $A\beta_{1-40}$ (see Supplementary Fig. 1A), $A\beta_{1-42}$ incubated for 1 h and 2 h at 37°C. Although all dots contain 0.67 μg Aβ (Pierce Coomassie Plus Protein Assay), the reactivity to all three antibodies varies with time points because of the change in the Aβ species present in the sample. Our data replicates the pattern seen in Kayed et al. [3]. For our samples, the A-11 reactivity develops within 2 h rather than 6 h suggesting a shorter lag time for the aggregation of the $A\beta_{1-42}$. This is consistent with the fact that the $A\beta_{1-42}$ is not filtered prior to incubation in these experiments in order to reduce loss of the Aβ peptides. B) Superdex S-200 profile of $eA\beta PP_{19-624}$ incubated on ice for 1 hour with A-11 negative unpurified $A\beta_{1-42}$. The $A\beta_{1-42}$ in this experiment was the sample incubated at 37°C for 1 h. Its reactivity pattern is shown in A. The molar ratio is 1:20 $eA\beta PP_{19-624}$ assuming the Aβ is monomeric. The complex elutes from the Superdex S-200 column at 11.7 ml, close to the elution volume of monomeric $eA\beta PP_{19-624}$ (12 ml) suggesting that in the absence of A-11 reactivity, the 7-kDa oligomers in the sample are dominating. The SEC methods are the same as was used for the data in Fig. 4. C) Superdex S-200 profile of $eA\beta PP_{19-624}$ incubated on ice for 1 hour with A-11 positive unpurified $A\beta_{1-42}$. The $A\beta_{1-42}$ in this experiment was the sample incubated at 37°C for 2 h. Its reactivity pattern is shown in A. The parameters for this complex are tabulated in Table 1 and are within the experimental error of the parameters observed for the A-11 positive 20-kDa $A\beta_{1-40}$. D) Western blot analysis of the $A\beta_{1-42}$ $eA\beta PP_{19-624}$ complex purified in C. The lanes are from a LDS-PAGE gel transferred and probed with 6E10. From right to left, lane 1 contains the A-11 positive unpurified $A\beta_{1-42}$. Lane 2 is the indicated fraction from the SEC purification of $eA\beta PP_{19-624}$ in part C (blue). Lane 3 is the indicated fraction from the SEC purification of the complex in part C (pink). LDS-insoluble trimers and monomer $A\beta_{1-42}$ are observed in the complex lane, but no other bands. Western blots for $eA\beta PP_{19-624}$ complexes for which there were no significant changes such as the A-11 negative 70-kDa $A\beta_{1-40}$ and 26-kDa $A\beta_{1-40}$ (Figs. 5A and B.) had no detectable Aβ under the same Western blot conditions.



Supplementary Figure 3. Fit of the homodimer model of $eA\beta PP_{19–624}$. The SAXS data for $eA\beta PP_{19–624}$ is plotted in black. The error bars for the data points are less than the width of the line. The predicted curve for the scattering by the homodimer model of $eA\beta PP_{19–624}$ is shown in red. X is a measure of the agreement between the two curves.