Supporting information for

The binding of apoE with oligomers and fibrils of amyloid-β alters kinetics of amyloid aggregation

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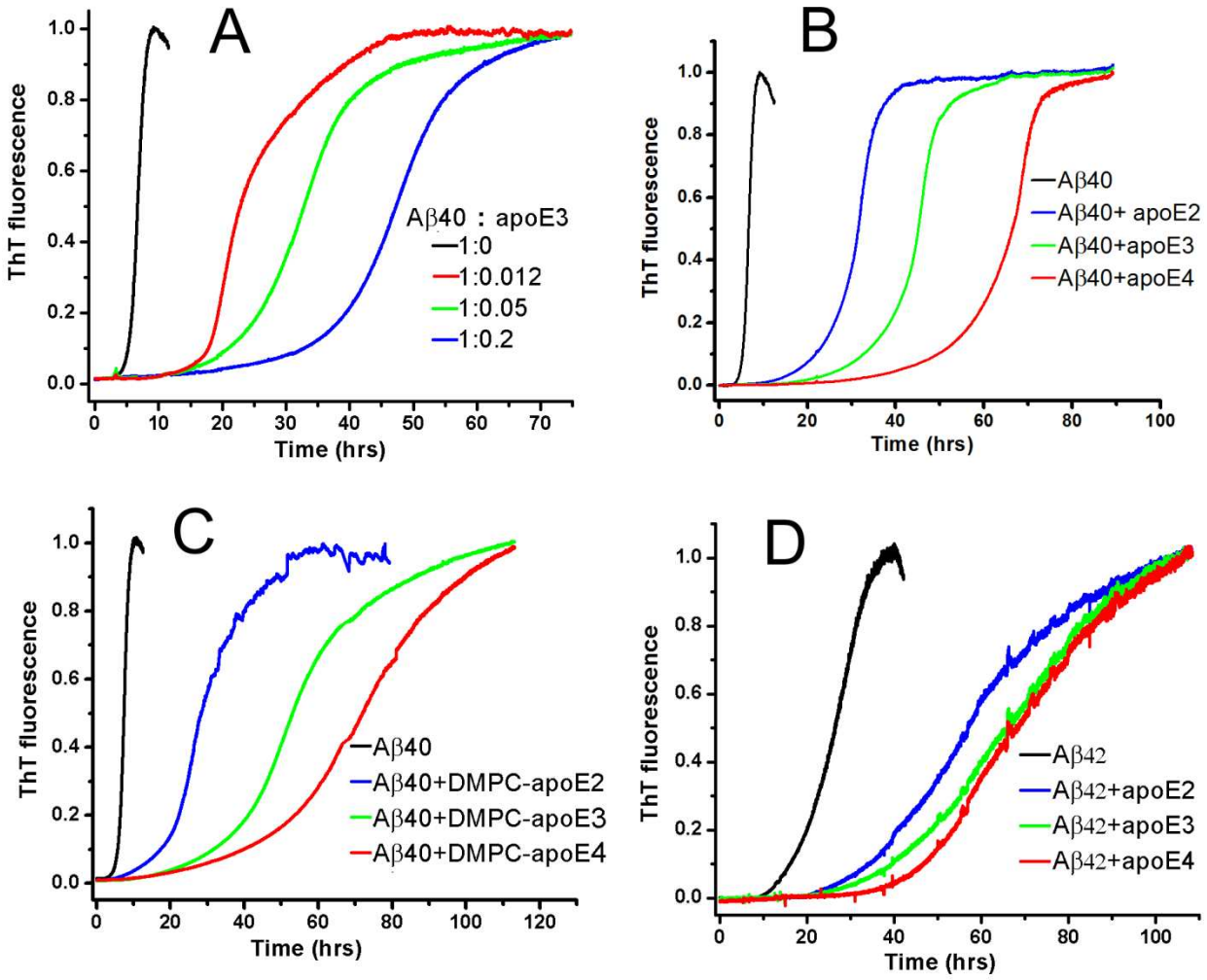


Figure S1: Time course of ThT fluorescence of WT-Aβ_{1-40} and Aβ_{1-42} in presence of the apoE isoforms. The black lines in all 4 graphs correspond to the time course of aggregation of Aβ_{1-40} or Aβ_{1-42} in absence of any apoE. A) aggregation of Aβ_{1-40} (7 μM) is presence of recombinant lipid-free apoE3 (0, 0.085, 0.34 and 1.37 μM). B-C) aggregation of Aβ_{1-40} (11 μM) in presence of the recombinant isoforms of lipid-free apoE or DMPC apoE. Concentration of all the apoE isoforms in B and C is 0.5 μM. D) aggregation of Aβ_{1-42} (5 μM) in presence of the recombinant isoforms of lipid-free apoE (0.2 μM). Clearly both lipid-free and lipidated apoE proteins strongly influences aggregation of Aβ. The data in B and C indicate that effects of apoE is isoform dependent with apoE4 having strongest and apoE2 the weakest effects. The data in D indicate that differences between the apoE isoforms on the aggregation time course of Aβ_{1-42} are small.

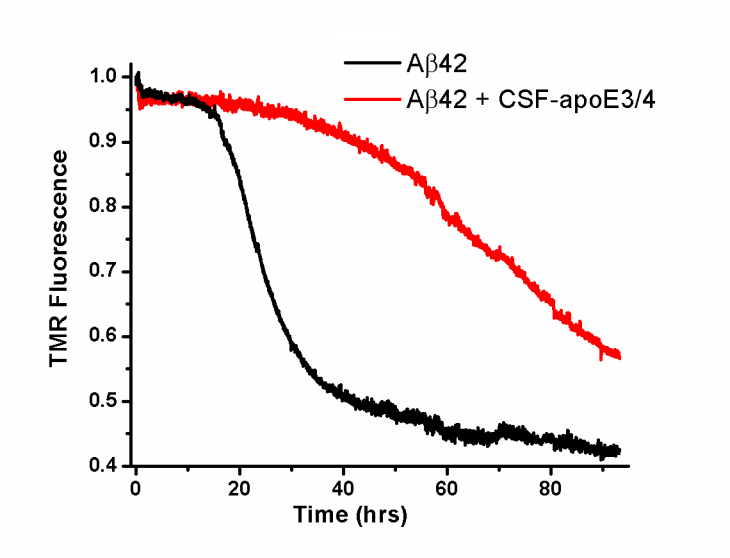


Figure S2: Effect of apoE lipoproteins derived from human Cerebrospinal fluid (CSF) on the aggregation of TMR-A$\beta_{1\text{-}42}$. The kinetics of aggregation of 2 μM TMR-A$\beta_{1\text{-}42}$ in absence (black line) and in presence (red line) of 10 nM apoE associated lipoproteins derived from the CSF of apoe3/4 human brain. All experiments are carried out at RT in pH 7.4 PBS buffer containing 5 mM βMe and 1 mM EDTA in the presence of continuous stirring. CSF derived apoE lipoproteins were purified from apoE3/4 human CSF samples using size exclusion chromatography as described in .[1]

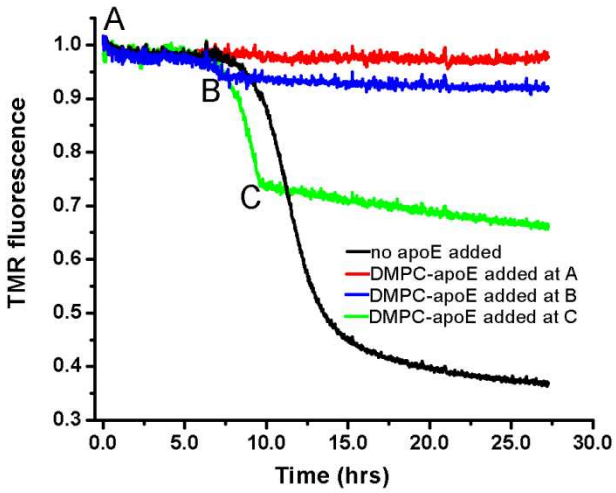


Figure S3: Effect of DMPC-apoE on the growth of the Aβ fibrils. The black line represents time course of aggregation of TMR- $A\beta_{1\text{-}40}$ (3 μM) in absence of apoE. The red, blue and green lines represent the time course if 500 nM DMPC-apoE4 is added at time points A, B and C respectively. A, B and C represents aggregation stages of Aβ corresponding loss of TMR fluorescence by 0.0%, 8% and 23% respectively. Evidently, addition of DMPC-apoE4 in the growth phase alters the mechanism of growth of the Aβ fibrils (blue and green curves).

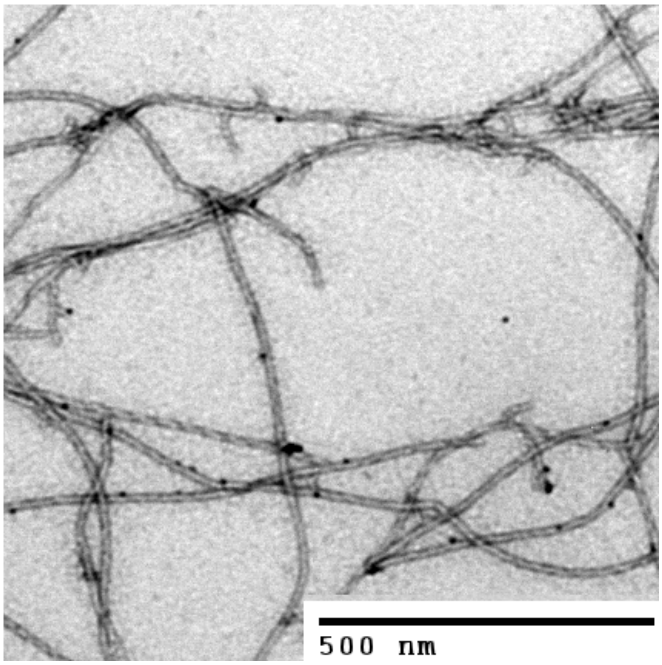


Figure S4: Control image for examination of non-specific labeling of the $A\beta_{1-42}$ fibrils with the Ni-NTA gold nano particles. A 12 µl aliquot of $A\beta_{1-42}$ solution from the growth phase in absence of apoE was used for the EM measurements. The image obtained using the same $A\beta_{1-42}$ sample incubated with 6x-his-apoE4 is shown in Figure 3B in the main manuscript. The details of the experimental protocol are described in the materials and methods section the main manuscript. The black dots in this image correspond to gold nano particles. Unlike the images presented in Figure 3 the gold nano particles are sparse in this image indicating minimal non-specific labeling of the Aβ fibrils by the nano particles.

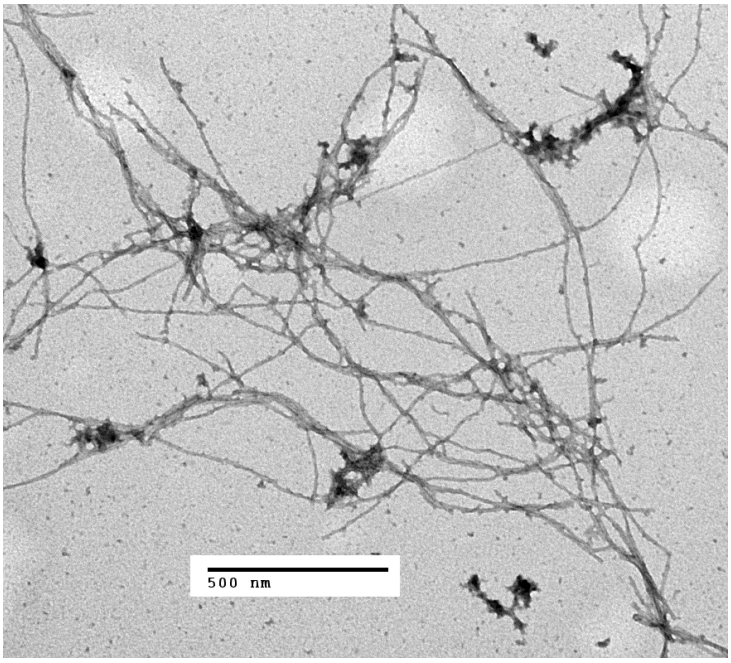
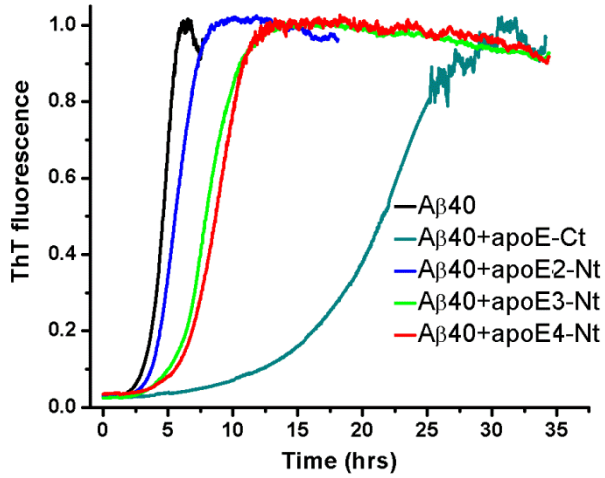


Figure S5: EM images of the WT-Aβ_{1-42} fibrils before sonication. Clearly, the fibrils appear long and unbroken before sonication. The images after taken after sonication are shown in the Figure 4 in the main manuscript.



FigureS6: Comparison of the effects of the N- and C-terminal fragments of the apoE isoforms on aggregation of Aβ. Time courses of aggregation of WT- $A\beta_{1\text{-}40}$ (12 μM) in absence (black line) or in presence 400 nM N-terminal fragment of apoE4 (red), apoE3 (green) or apoE2 (blue) or 400 nM of C-terminal fragment of apoE (cyan). The fragments of apoE are generated by cleavage of the full-length apoE proteins by thrombin as described earlier by Garai et al.[2] The aggregation of WT-$A\beta_{1\text{-}40}$ aggregation is monitored by fluorescence of thioflavin T.

References

1. Verghese, P. B., Castellano, J. M., Garai, K., Wang, Y., Jiang, H., Shah, A., Bu, G., Frieden, C., and Holtzman, D. M. (2013) ApoE influences amyloid-beta (Abeta) clearance despite minimal apoE/Abeta association in physiological conditions, *Proc Natl Acad Sci U S A 110*, E1807-1816.
2. Garai, K., Mustafi, S. M., Baban, B., and Frieden, C. (2010) Structural differences between apolipoprotein E3 and E4 as measured by ^{19}F NMR, *Protein Sci 19*, 66-74.