Charge dependent retardation of amyloid β aggregation by hydrophilic proteins

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Supplementary figures

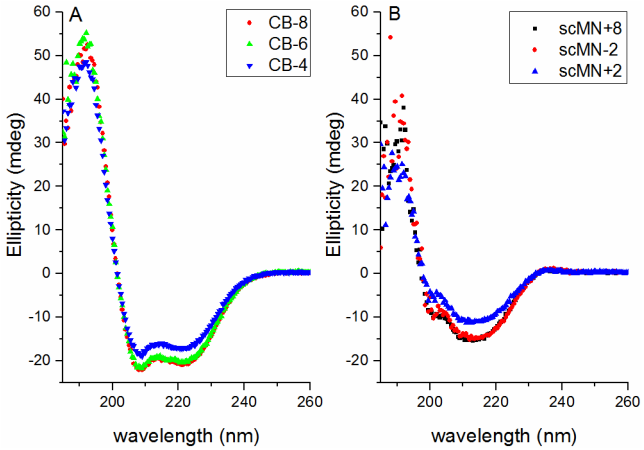

Figure S1. CD spectra of 0.2 mg/ml CB (A) and scMN (B) mutants in 10 mM phosphate buffer, pH 7.4, with 30 µM EDTA, reveal no structural difference between the charge variants of CB and scMN. The minor difference between the samples is assumed to originate from concentration variations.

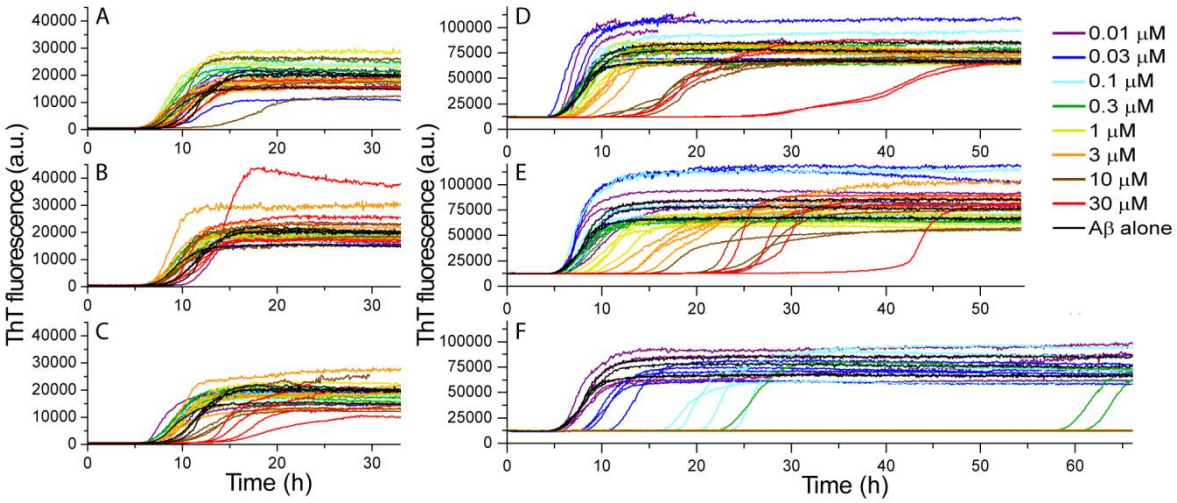

Figure S2. ThT fluorescence from Aβ40 aggregation in the absence and presence of CB-8 (A), CB-6 (B), CB-4 (C), scMN-2 (D), scMN+2 (E), and scMN+8 (F). Each protein was mixed with 10 µM Aβ40 at seven or eight concentrations ranging from 0.01 to 30 µM. There are four technical replicates for each concentration. Some curves end before the completion of the experiment for technical reasons. These are the same data as in Figure 1 but are not normalized to display the lack of variation in maximal fluorescence upon the presence of added protein.

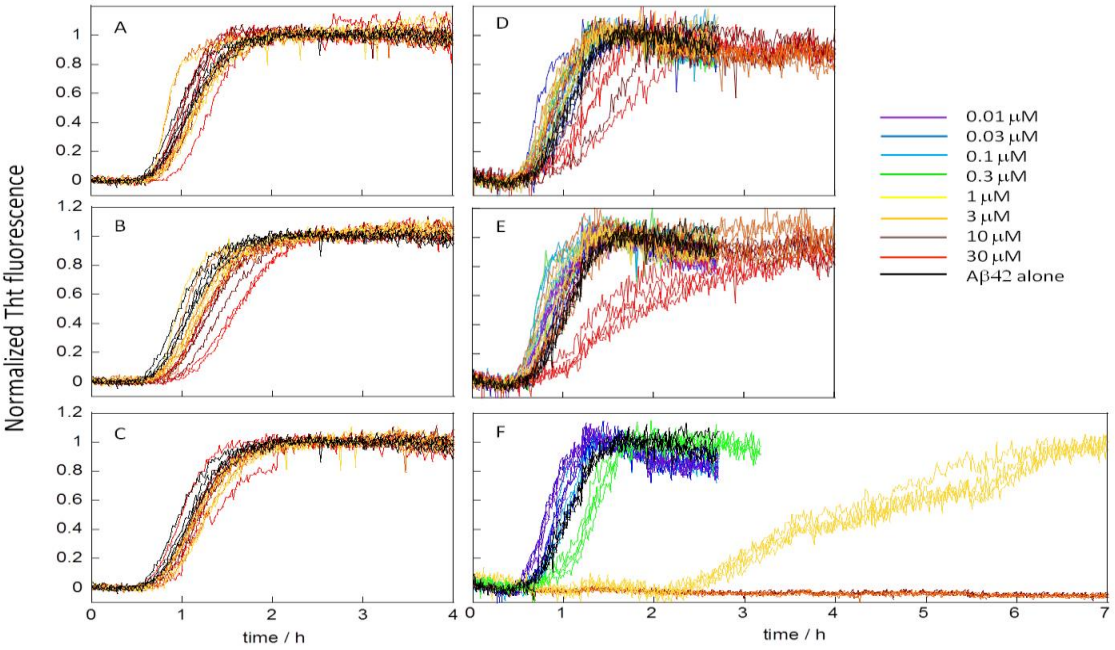

Figure S3. Normalized ThT fluorescence from Aβ42 aggregation in the absence and presence of CB-8 (A), CB-6 (B), CB-4 (C), scMN-2 (D), scMN+2 (E), and scMN+8 (F). Each protein was mixed with 3 μM Aβ42 at seven or eight concentrations ranging from 0.01 to 30 μM.

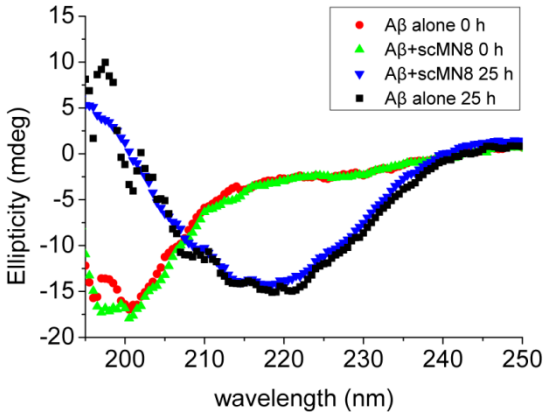

Figure S4. CD spectra of 4 μM Aβ40 in the absence and presence of a 1:0.01 molar ratio of scMN+8 before and after aggregation. The samples were incubated at 37°C and stirred at 450 rpm during the measurements. Both samples transformed from a random coil structure to beta sheet structure during the 25 h of the experiment. The presence of scMN+8 has no effect on the Aβ40 structure. Spectra of buffer alone were recorded separately in the same cuvettes and subtracted from the spectra shown.

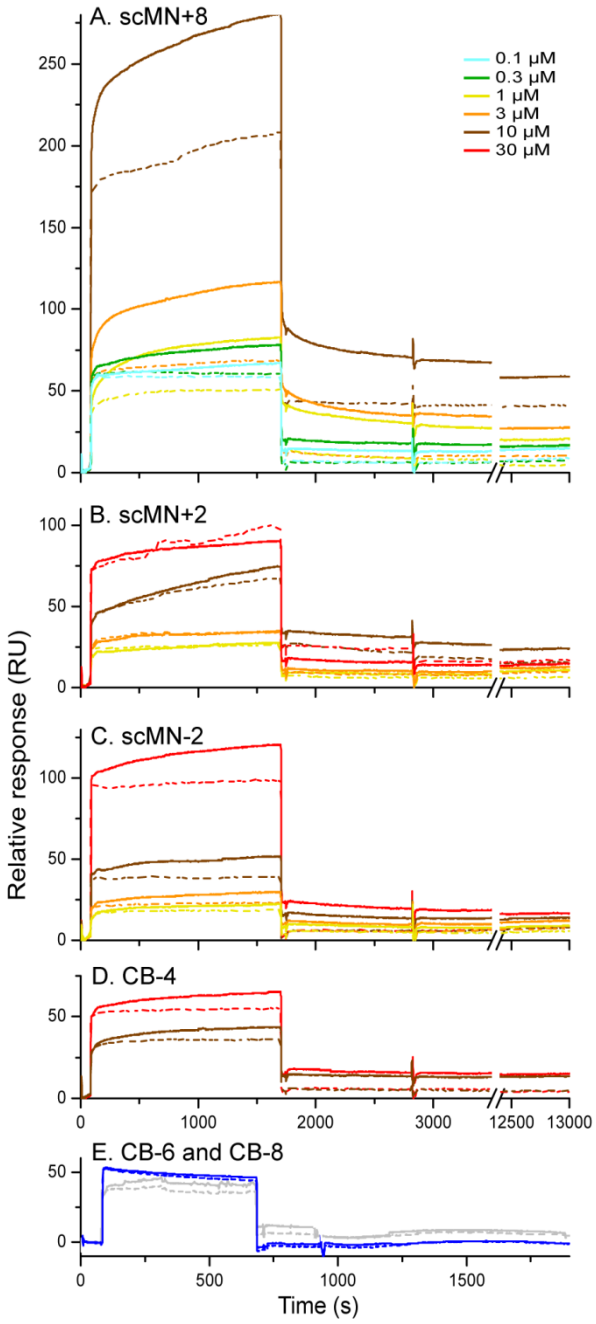

Figure S5. SPR signals from the blank flow cell (dashed) and the flow cell with immobilized Aβ40 (solid) with the protein variants indicated flowing over the cells. The average of the signal before the injection has been subtracted from all curves to facilitate comparison. For panels A-D, binding is seen for several protein concentrations as indicated by the legend. scMN+2 (B) binds to the surface of the reference cell, as well as the cell with Aβ40, which gives no information about the interaction. In panel E, data for 30 µM CB-6 (blue) and CB-8 (grey) display lack of binding between Aβ40 and these mutants. The flow rate is 10 µl/min in panel A-D and 30 µl/min in panel E. Data for scMN+8, scMN-2 and CB-4 were used for further characterization of the Aβ40 interaction.

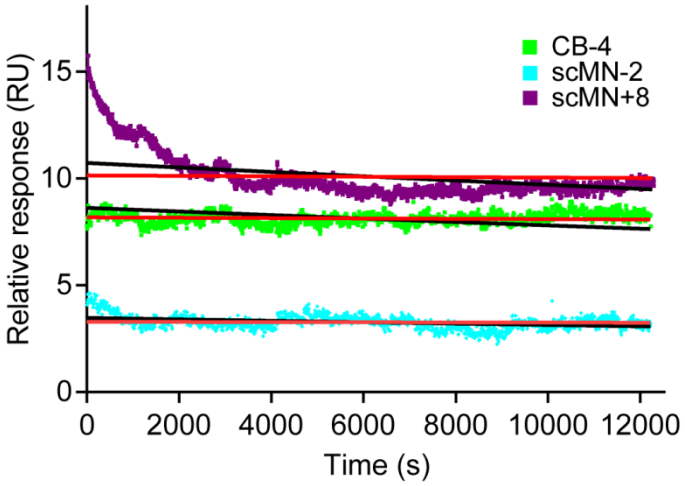

Figure S6. The dissociation phase of the SPR experiment in Figure 6 with 300 nM scMN+8, 1 µM scMN-2, and 10 µM CB-4. Complete dissociation is not reached within the experiment; therefor the initial fast decrease in signal seen for the two scMN mutants is a different faster process than the extremely slow dissociation. The curves are fitted with the dissociation rate constant (k_{off}) being 10^{-5} s^{-1}(black) or 10^{-6} s^{-1} (red). Because both fit the flat part of the curve we are conservative in the estimate of k_{off} and select 10^{-5} s^{-1} as the maximal value even if the real rate might be orders of magnitude slower.