Supporting Information

Intracellular and extracellular chaperones sequester amyloid-β oligomers and inhibit their interactions with neuronal membranes

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Methods

Aggregation and disaggregation reactions

Prior to each of the aggregation reactions, the desired quantities of each peptide were brought to a pH of 7.4 by diluting into SSPE buffer (150 mM NaCl, 10 mM $Na_2H_2PO_4 \times H_2O$, 10 mM Na_2EDTA, pH 7.4) to obtain the desired concentration and then were placed under the desired conditions (e.g. 37 °C, agitation). The concentration of all labeled samples was measured prior to mixing using cTCCD as described previously (1).

For the aggregation experiments, Aβ40 at bulk concentrations (2 μM) was allowed to aggregate in SSPE buffer (defined above) at 37 °C with agitation (200 rpm on a rotary shaker). For disaggregation experiments, Aβ40 fibrils were first formed from an 8 μM solution of Aβ40 for 72 h at 37 °C. Repeated pelleting and washing were carried out by centrifugation at ~10,000×g for 30 min followed by removal of the supernatant in order to isolate the insoluble species. cTCCD data for both aggregation and disaggregation experiments and TIRFM images of monomers, oligomers and fibrils were acquired using previously described protocols (9). Oligomer distributions were analyzed by isolating bursts coincident in both detection channels from non-coincident bursts and the brightness of these bursts were compared to the brightness of monomeric bursts to yield an apparent oligomer size.

TIRFM image registration

Good image registration was achieved using a grid consisting of regularly spaced ion-beam etched holes in gold on a glass coverslip was used. Dual-View™ optics were adjusted to maximize the overlap between two detection channel images of the grid under white-light illumination, resulting in a measured mean image registrations in the range of 50 nm.

Data and discussion

Experimental design

In order to study the aggregation of Aβ40 and the interaction of Aβ40 with chaperones, two experimental setups were used. In the first, a mixture of HiLyteFluor488-labeled and HiLyteFluor647-labeled Aβ40 is combined with unlabeled chaperone. This mode allows for the observation of Aβ40 oligomer formation and the effects of the unlabeled chaperone on this process. The second mode involves a mixture of HiLyteFluor488-labeled Aβ40 and AlexaFluor647-labeled chaperones which allows us to probe any direct association between the chaperone and the Aβ molecules.

No complex formation is observed between monomeric Aβ40 and αB-crystallin

To investigate the mechanism by which these chaperones prevent the growth and dissociation of oligomers, the Aβ40 aggregation reaction was sampled at various times and these samples were incubated with labeled chaperones. cTCCD was then used to detect any association between the chaperones and Aβ40. Concurrent characterization of a two-color Aβ40 aggregation in the absence of chaperones was used to determine the fraction of Aβ in oligomeric species.

When this experiment was performed with clusterin, throughout the reaction, the fraction of Aβ40 peptides in oligomers corresponded well to the fraction of Aβ40 peptides in clusterin:Aβ complexes, indicating that even in the presence of an excess of monomeric Aβ, it is the oligomeric fraction of the Aβ species that binds to clusterin. It was not possible to detect such complex formation in similar experiments with αB-crystallin (see Figure S1).

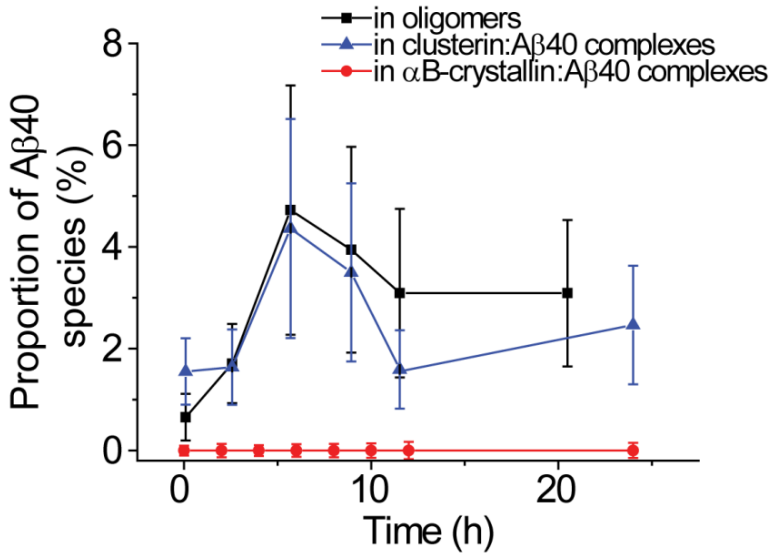

Figure S 1 The proportion of Aβ40 species in oligomers, in complexes with clusterin and in complexes with αB-crystallin during the course of an aggregation reaction, Aβ40 concentration is 2 μM, chaperones are added at a 1:1 molar ratio to each aliquot and incubated for 30 min at 21 °C prior to measurement (N=3, error bars are SEM).

Fluorescent labeling of αB-crystallin does not affect its activity

The fluorescent labeling of the chaperones with AlexaFluor647 was found to not affect the ability of either chaperone to inhibit fibril formation. It was especially important to ensure this for αB-crystallin as labeling is likely to occur at the C-terminal extension of αB-crystallin, which contains lysine residues at positions 166, 174 and 175 and has been shown to be involved in the chaperone action of αB-crystallin (2, 3). This was accomplished by measuring the rate and extent of fibril formation of the Aβ40 peptide in the absence of chaperones and in the presence of either labeled or unlabeled chaperones (see Figure S 2). From these measurements both labeled and unlabeled chaperones were found to inhibit fibril formation suggesting that the labeling with AlexaFluor647 does not alter either chaperone's functionality.

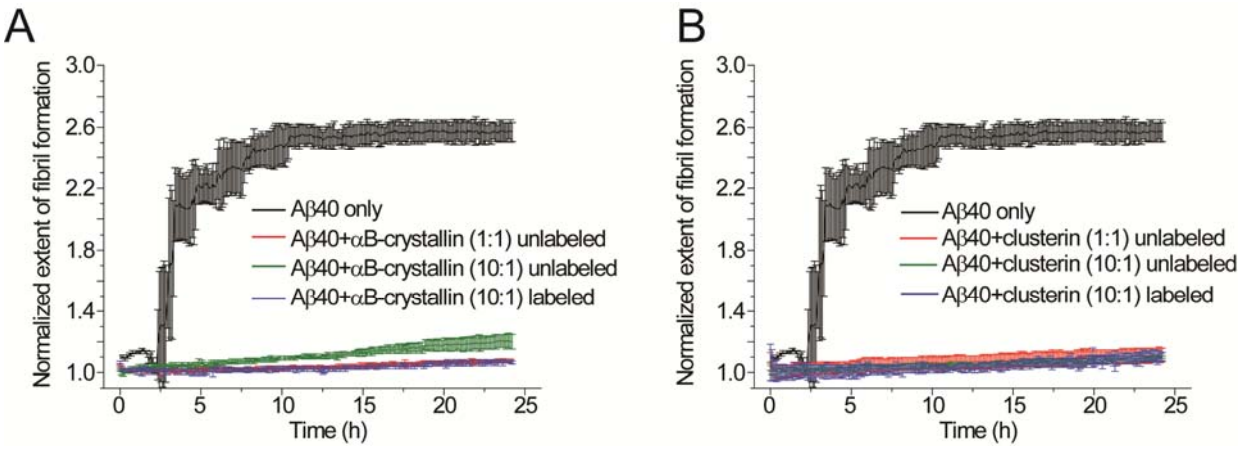

Figure S 2 Fluorescent labeling of the chaperones does not affect their ability to inhibit Aβ fibril formation. Normalized extent of fibril formation with time as monitored by Thioflavin-T fluorescence for (A) 5 μM unlabeled Aβ40 in the presence of 5 μM unlabeled αB-crystallin, 500 nM unlabeled αB-crystallin or 500 nM AlexaFluor647-labeled αB-crystallin or (B) 5 μM unlabeled Aβ40 in the presence of 5 μM unlabeled clusterin, 500 nM unlabeled clusterin or 500 nM AlexaFluor647-labeled clusterin (N=3, error bars are SD).

In order to confirm that labeling αB-crystallin with AlexaFluor647 did not affect its behavior during the disaggregation reaction, we compared the effects labeled and unlabeled αB-crystallin had on the disaggregation. The rate of disaggregation of Aβ40 fibrils was very similar in the presence of the unlabeled and labeled forms of either chaperone suggesting that the labeling does not affect the chaperone function in this context as well (see Figure S3).

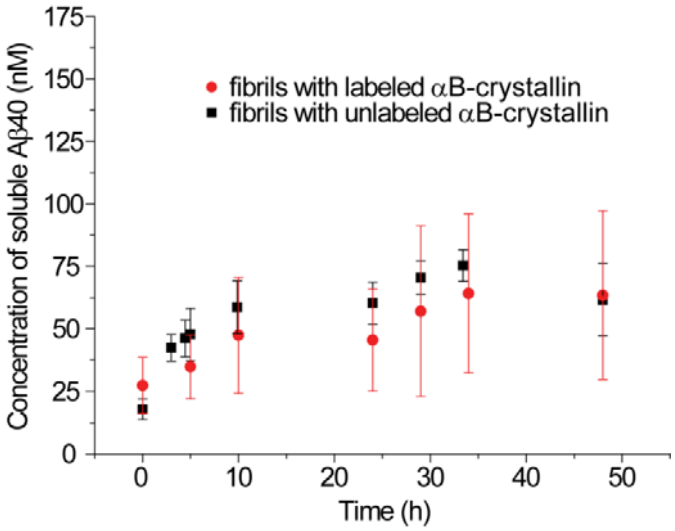

Figure S 3 Fibrils incubated with labeled and unlabeled αB-crystallin disaggregate at similar rates, N=5 (unlabeled), N=3 (labeled), error bars are SEM.

Stoichiometry of αB-crystallin:Aβ40 complexes

In each of the chaperone-oligomer complexes, the median ratio of chaperone molecules to Aβ (peptides) was 1.2 ± 1.1 (median ± IQR) in the case of clusterin and 0.99 ± 0.94 (median ± IQR) for αB-crystallin (see Figure S4). This suggests that there is a direct correlation between the number of chaperone molecules and the number of Aβ peptides in each complex.

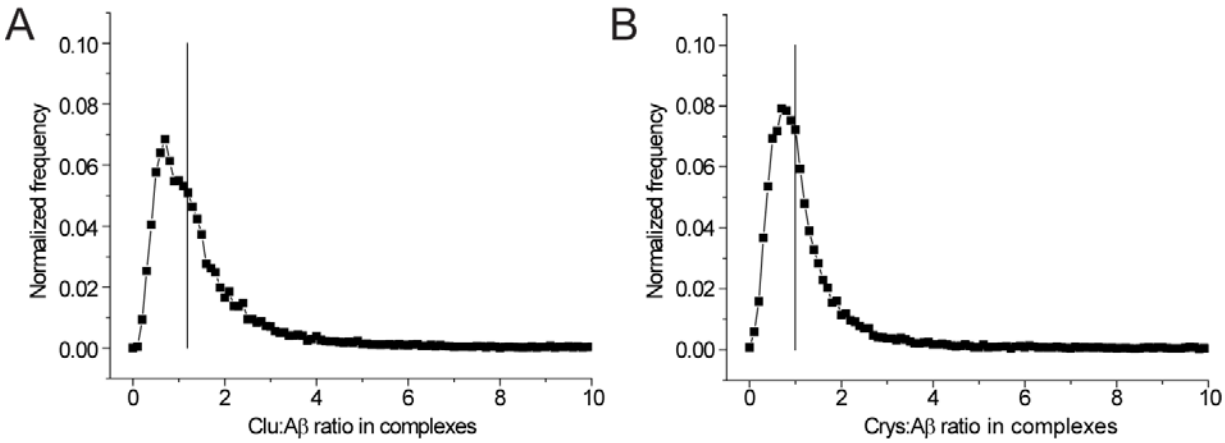

Figure S 4 Distribution of ratios of αB-crystallin:Aβ40 within complexes, median is denoted by a vertical line.

References

1. Narayan P, *et al.* (2012) The extracellular chaperone clusterin sequesters oligomeric forms of the amyloid-β1−40 peptide. *Nat. Struct. Mol. Biol.* 19(1):79-83.

2. Treweek TM, *et al.* (2007) Site-directed mutations in the C-terminal extension of human αB-crystallin affect chaperone function and block amyloid fibril formation. *PLoS ONE* 2(10):e1046.

3. Treweek TM, Rekas A, Walker MJ, & Carver JA (2010) A quantitative NMR spectroscopic examination of the flexibility of the C-terminal extensions of the molecular chaperones, αA- and αB-crystallin. *Exp. Eye Res.* 91(5):691-699.