SUPPLEMENTARY INFORMATION

ANS Binding Reveals Common Features of Cytotoxic Amyloid Species.

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Peptide and Protein Preparation

Aβ_{1-42} and E22G Aβ_{1-42}, synthesized through solid-phase synthesis, were purchased from Bachem. The peptide was prepared by dissolving it at 1 mg/ml in trifluoroacetic acid (TFA) followed by subsequent sonication for 30 seconds on ice. The TFA was removed by lyophilisation and the powder then dissolved in 1,1,1,3,3,3-hexafluoro-2- propanol (HFIP) and aliquoted, the HFIP was then removed by rotary evaporation at room temperature. This method disrupts preformed aggregates initially present and gives a high yield of monomeric material (See Teplow, D.B., (2006) Preparation of amyloid beta-protein for structural and functional studies. *Methods Enzymol*, *413*, 20-33. And AC. Brorsson, *et al.* (2010) Intrinisc Determinants of Neurotoxic Aggregate Formation by the Amyloid Beta Peptide *Biophys J.* 98). The quantity of peptide in the aliquots was determined by quantitative amino acid analysis.
I59T human lysozyme was recombinantly expressed in *P. pastoris* and purified as previously described (Kumita et al., 2006). Stock solutions of lyophilized material were made in Milli-Q water and passed through 0.22 mm pore size filters and concentrations were determined by UV-spectroscopy using an extinction coefficient of 36,940 M^{-1} cm^{-1} at 280 nm. SH3 was expressed in E. coli, and purified as previously described (Yerbury et al., 2007).

Protein Aggregation and Thioflavin T (ThT) Fluorescence

For the *ex situ* ThT measurements the peptides were dissolved in 50mM NaH_2PO_4 at pH 7.4 to a final concentration of 30 μM, shaken for 1 minute and allowed to aggregate at 29°C. At each time point a 10 μl aliquot of the sample was added to 50 μl of 240 μM ThT 50 mM NaH_2PO_4 in a fluorescence cuvette. A Cary Eclipse spectrofluorimeter (Variant Ltd., Oxford UK) was used to measure the fluorescence intensity of ThT at 480 nm upon excitation at 440 nm . The I59T lysozyme variant (6.8 ∝M, 0.1M sodium citrate buffer pH 5.0) was incubated with stirring at 60 °C in a Cary Eclipse spectrofluorimeter over a time course of 500 minutes while monitoring the static light scattering from the sample with the excitation and emission monochromators set at 500 nm. Aliquots (30 μL) were removed at indicated time points for ThT and ANS fluorescence measurements (also performed in a Cary Eclipse). ThT was excited at 440 nm and emission spectra were recorded from 450-600 nm and ANS was excited at 350 nm and emission spectra were recorded from 400-600 nm. The SH3 domain of PI3 (250 μM) was incubated in phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8 mM Na_2HPO_4, pH 7.5) heated to 60 °C whilst shaking at 500 rpm for 72 h and ThT (50 μM) was added to aliquots of samples taken at specific time points following

the initiation of fibril formation. The ThT fluorescence was measured on a FLUOstar OPTIMA fluorescence plate reader using excitation and emission filters of 440 +/-10 and 480 +/-10 nm, respectively.

Sample Preparation for Transmission Electron Microscopy

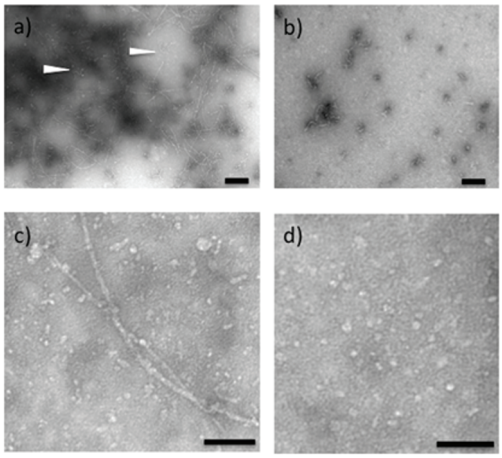
Preparations of aggregates were adsorbed on formvar-coated 400 Mesh copper grids (Agar,Scientific) for two minutes. The samples were then blotted and the grid washed twice with distilled water. The samples were then negatively stained using 2% (w/v) uranyl acetate. The grid was allowed to stand for 2 minutes, blotted again with filter paper and left to dry in air. Samples were imaged with a Philips CEM100 transmission electron microscope.

Circular Dichroism

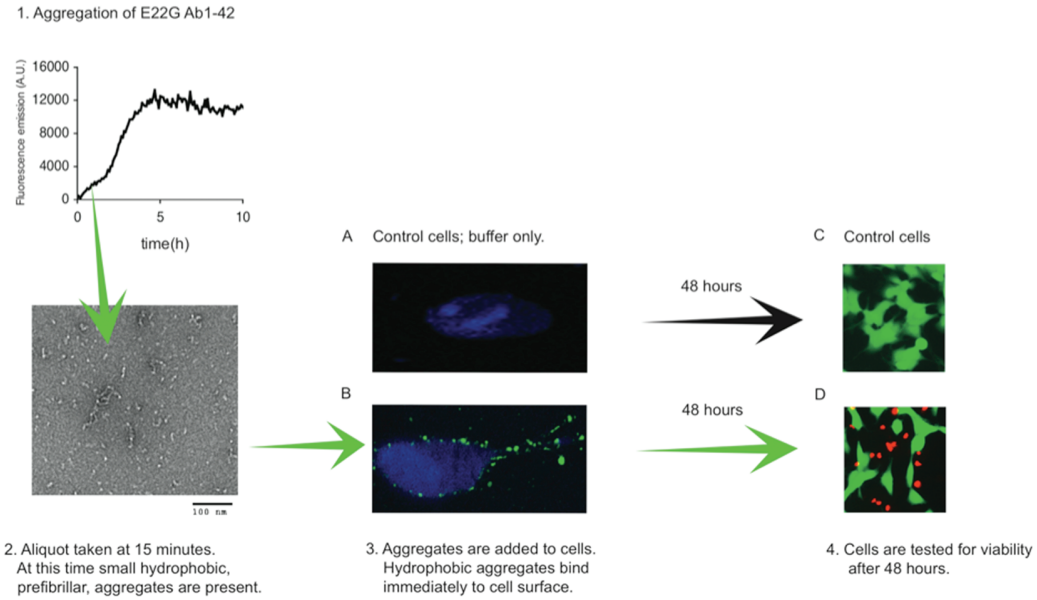
The $A\beta_{1-42}$ peptides were allowed to aggregate at a concentration of 30 μM in 50 mM NaH_2PO_4 pH 7.4 at 29 ^oC. At various time intervals aliquots of the peptide solutions were taken and diluted to give a 5 $\propto$M concentration. Circular dichroism spectra were recorded in quartz cuvettes with a 1 mm path length on a AP Chirascan spectropolarimeter (Applied Photophysics). At least 4 scans (195-260 nm) were collected and averaged. Spectra were background corrected by subtraction of appropriate blanks.

Cell Imaging

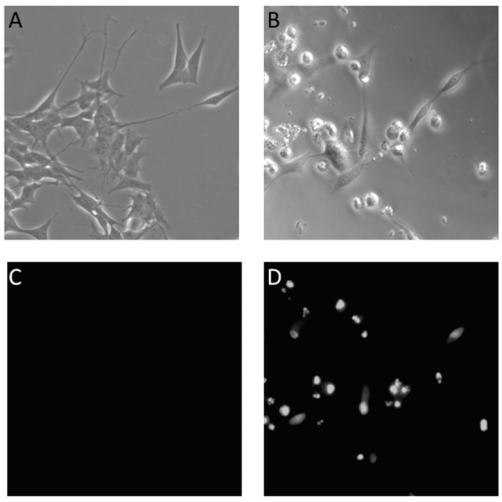
SH SY5Y Cells were cultured in a LabTek II 8 well chamber slide for 24 hours. The cells were then washed three times with PBS before adding aggregates at a concentration of 10 μM in PBS. After 5 minutes of incubation the supernatant was removed and the cells were washed twice with PBS. The ALEXA 488-Labeled Abeta-specific antibody 6E10 (Covance) was incubated with the cells for 30 minutes, on ice, at a 1:1000 dilution. A 1:1000 dilution of TOTO-3 dye was also used to stain nucleic acids (no RNAse treatment was performed and TOTO-3 thus stained both the cytoplasm and the nucleus). The samples were then fixed with 4% PFA and mounting media was added. The slide was imaged through a Nikon 90i Eclipse confocal microscope.
The fluorescence images of live/dead stained SH SY5Y cells stained with calcein-AM and propidium iodide (SI. fig 3) were taken on an Olympus IX71 Fluorescence microscope using a F-View II camera, composite images were constructed in the Cell*F imaging software.



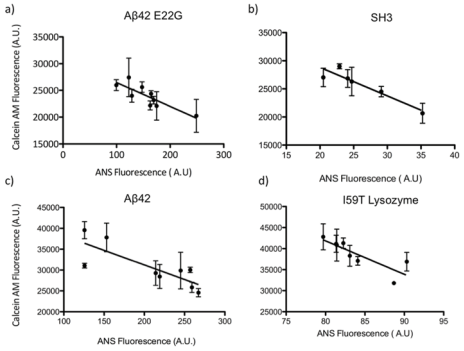
Supp. Fig 1. Oligomeric aggregates persist during Aβ fibril formation. TEM images of persistent prefibrillar aggregates in E22G and WT $A\beta_{1-42}$ aggregation reactions. Samples were taken at 500 min and 1440 min for E22G $A\beta_{1-42}$ and $A\beta_{1-42}$ respectively when Thioflavin T measurements had reached a plateau. Images shown are representative of at least 3 independent experiments.



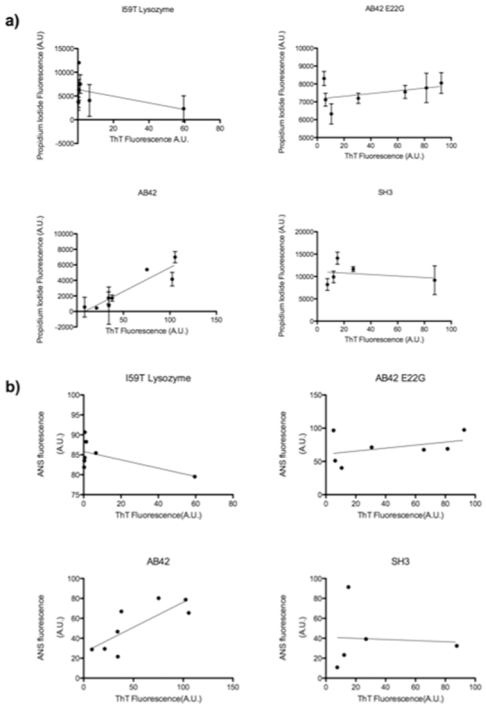
Supp. Fig 2. E22G Aβ1-42 Aggregates bind rapidly to the cell surface and are toxic. During the aggregation process, aliquots were taken at various time points. The sample containing aggregates taken at 15 minutes of E22G $A\beta_{1\text{-}42}$ during aggregation reactions contained prefibrillar oligomers, including worm-like structures. These aggregates were immediately added to SH SY5Y cells and incubated for 5 minutes. We then imaged the cells to look for E22G $A\beta_{1\text{-}42}$ binding to the plasma membrane (B), and compared Aβ treated cells to those treated only with buffer (A) (Green: amyloid beta aggregates as detected with Alexa 488-labeled 6E10 antibody- Blue: SH SY5Y cell as stained with TOTO3). Although the aggregates bound immediately to the cell surface significant differences in toxicity (D) compared to control cells (C) were measurable only after 48 hours (Green: healthy SH SY5Y cells- calcein-AM stained, Red: permeabilised SH SY5Y cells, PI stained).



Supp. Fig 3. Cell morphology of cells incubated with E22G Aβ1-42 aggregates. SH SY5Y cells were incubated with 15 minute E22G Aβ1-42 aggregates for 48 hours (see methods for details). Cells were stained with either Propidium iodide to measure membrane permeability. Cells were also examined for morphological changes. Healthy SH-SY5Y cells exhibited flattened morphology, extended neurites (A) and no PI staining (C). SH-SY5Y cells incubated with toxic aggregates were physically smaller, partially lost the ability to adhere to the plastic, exhibit retracted processes (B) and stain with PI (C) typical of dead or dying cells. Images shown are representative of at least 3 independent experiments.



Supp. Fig 4. ANS binding of prefibrillar aggregates negatively correlates with cell viability. Correlation graphs of the increment in ANS fluorescence signal and the corresponding decrease in cell viability, as measured by calcein-AM fluorescence, of the aggregates at different time points for a) E22G $A\beta_{1\text{-}42}$, b) SH3-PI3, c) I59T Lysozyme, and c) $A\beta_{1\text{-}42}$. ANS data points are single measurements, while calcein-AM fluorescence data points are means of triplicate determinations with SE bars. All results shown are representative of at least three independent experiments.



Supp. Fig 5. **Relationship between ThT and toxicity or ANS**. a) The correlation between ThT signal and toxicity of amyloid forming aggregates. b) The correlation between ANS signal and ThT signal of amyloid forming aggregates. In the case of WT Aß1-42, we observe a significant correlation between both ThT and toxicity and ThT and ANS binding. This correlation remains regardless of the increase in ANS prior to ThT (see Fig. 2) and is likely to be a result of the proximity in appearance of ANS binding and subsequent ThT binding aggregates. ThT and ANS data points are single measurements, while PI fluorescence data points are means of triplicate determinations with SE bars. All results shown are representative of at least three independent experiments.