Supporting, Methods and Figures for "A Rational Conformational Epitope Defines a Distinct Subclass of Toxic Amyloid-Beta Oligomers"

Supporting Methods

Atomic Force Microscopy of Aβ species

Diluted Aβ42 monomer, oligomer, and fibril preparations were spotted on mica, washed and dried. In tapping mode, images were acquired using a Cypher AFM (Asylum Research, Santa Barbara, CA, USA) with silicon tips (AC160TS from Olympus; nominal spring constant of 40 N/m). To determine the height distribution of the various species, section analyses were performed using the AFM software.

Dot blotting

Stated amounts of $A\beta_{42}$ oligomers and prion protein oligomers (a gift from Dr. Ojer) were dotted directly onto 0.45μm nitrocellulose membranes (Pall). The membranes were probed pan Aβ antibody 6E10 (Covance, 1:1000), A11 anti-sera (Milipore, 1:500), and anti-$A\beta O^{cSNK}$ (10μg/ml).

Immunohistochemistry of mouse brain sections

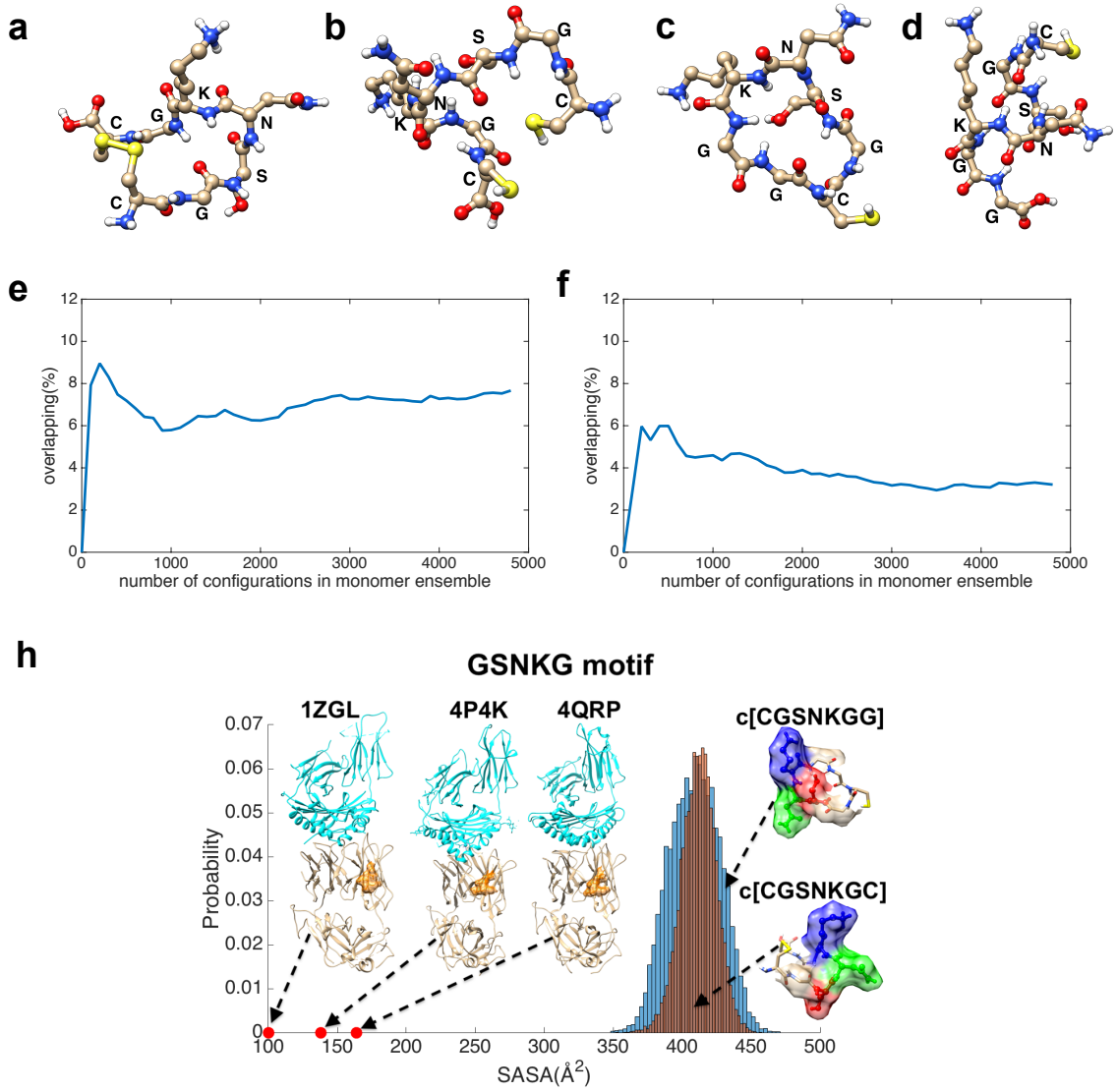
Mice were cardiac perfused by PBS and posed fixed in 4% PFA for 24-48 h. Tissues were dehydrated in 30% sucrose for 48h, cryosectioned into 25-30 μm thick sections and mounted on Superfrost plus slides. Endogenous peroxidase activity was quenched using 0.5% hydrogen peroxide in methanol for 20 min. After washing 3 x 15 min in TBS, samples were put in a humidified chamber, and non-specific staining was blocked by incubation with serum-free protein blocking reagent (Dako Canada Inc., Mississauga, ON, Canada) for 1 h. For immunostaining, anti-$A\beta O^{cSNK}$ (1 μg/ml), 4G8 (1 μg/ml, Santa Cruz Biotechnology), and Iba1 (1 μg/ml, Wako Chemicals, Neuss Germany,) were used as primary antibodies. Sections were incubated overnight at 4 ºC, and washed 3 x 5 min in TBS-T. Anti-Mouse IgG Horseradish Peroxidase conjugated (1:1000, ECL) was applied to sections and incubated 45 min, then washed 3 x 5 min in TBS-T. DAB chromogen reagent (Vector Laboratories, Burlington ON, Canada) was applied and sections rinsed with distilled water when the desired level of target to background staining was achieved. Sections were counterstained with Mayer's haematoxylin, dehydrated, and cover slips were applied. Slides were examined under a light microscope (Zeiss Axiovert 200M, Carl Zeiss Canada, Toronto ON, Canada) and representative images captured at 50, 200 and 400X magnification using a Leica DC300 digital camera and software (Leica Microsystems Canada Inc., Richmond Hill, ON).

Immunoblotting and TEM of Aβ aggregation assay

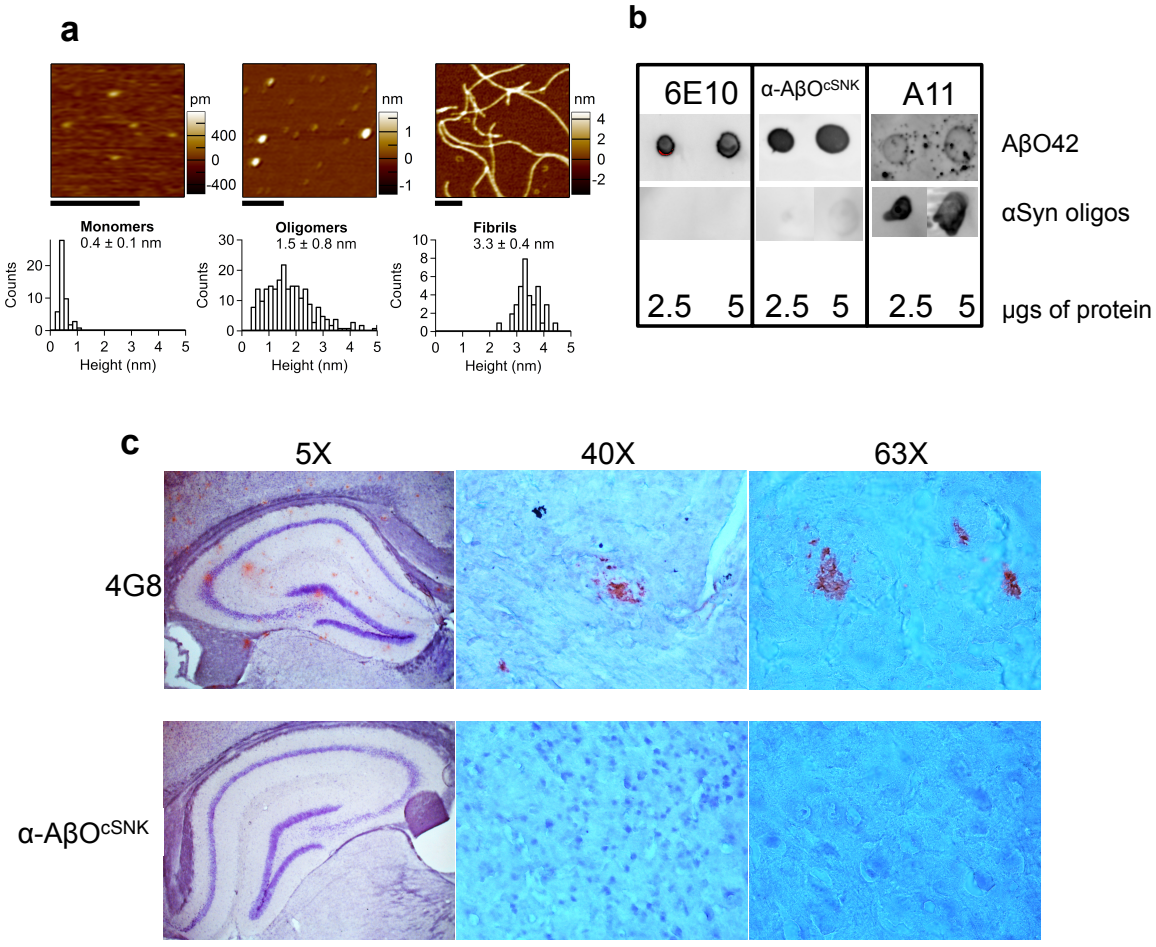
HFIP-treated Amyloid beta (Aβ) peptides (BACHEM, catalogue #H-7442) were resuspended in 10 mM NaOH to a concentration of 500 μM, sonicated for 10

min, then diluted to 50 μM in 1 mM EDTA Tris-HCL buffer. They were then immediately added to a black walled 96-well plate with either buffer alone, 5 μM IgG1 isotype control, or 5 μM anti-AβO^{cSNK}, for a final volume of 100 μL and an antibody:Aβ molar ratio of 1:10. Assay controls included buffer alone, and antibody only, and treatment groups were tested in duplicates. Thioflavin T (10 μM final) was incubated with sample at room temperature for 6, 18, 30, 48 and 72 hours, readings taken every hour at 440 nm excitation and 485 nm emission.
For every time-point, samples were collected and the wells washed with 10 mM NaOH. The collected samples were centrifuged at 18,000g for 14 min at 4C, and pellets were resuspended in 10 mM NaOH. Supernatants and pellets were boiled and resolved on SDS-PAGE before immunoblotting with a pan-Aβ antibody (Rabbit anti-Aβ1-14, 1 ug/mL, Abcam). Duplicates of samples for measurement of ThioT by [platereader] were prepared in parallel, and frozen at each timepoint studied (6, 30, 48 and 72 hours). These samples were then thawed on ice for analysis by TEM. Samples were diluted 2:3 in filtered distilled water, and 5μl spotted on each carbon/formvar-coated Ni grid for 10 minutes; excess liquid was blotted away, and the grids allowed to air dry. Grids were stained with 1% aqueous uranyl acetate for 1 min, and air dried again. TEM was performed as described above.

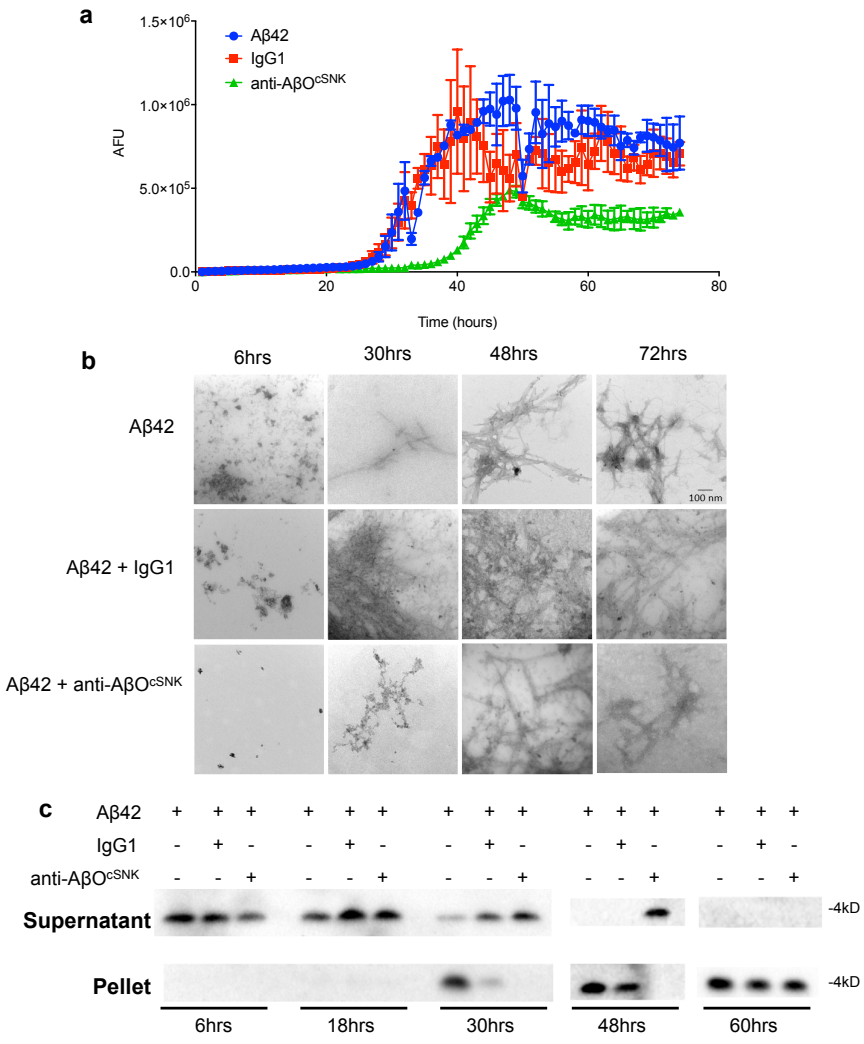
Supporting Figures



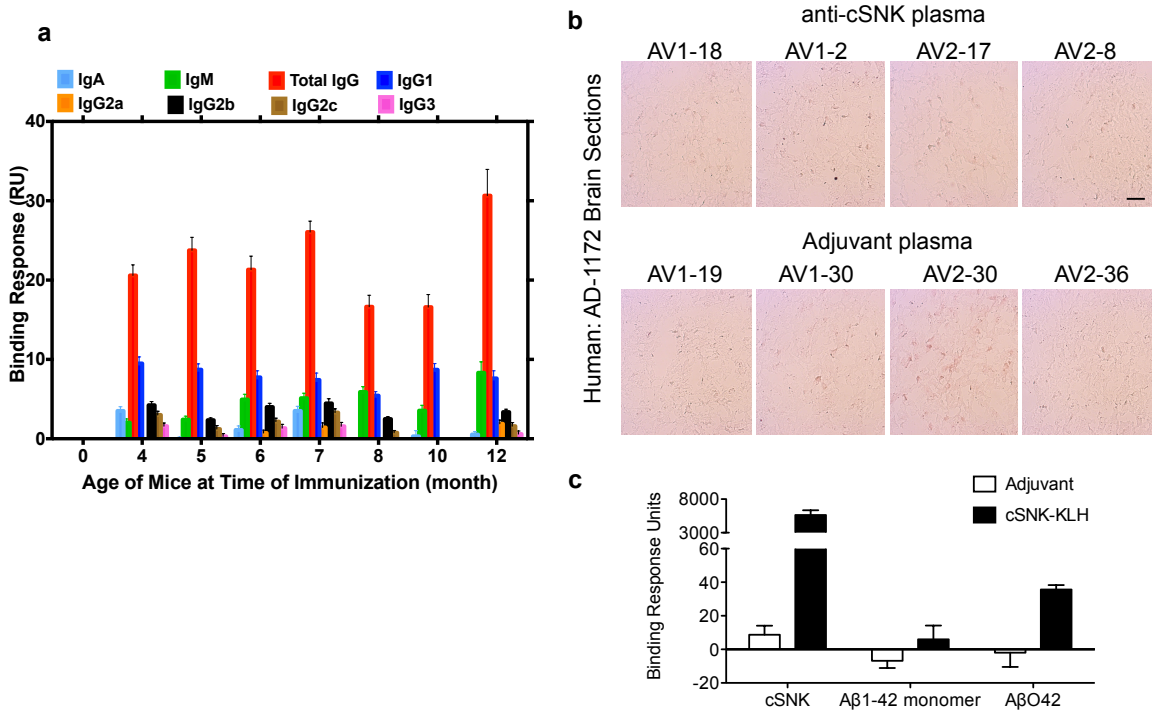
Supporting Figure 1. **cSNK has a unique structure distinct from Aβ monomers and fibrils. a-d**) Peptides synthesized for experiments. **a**) c[CGSNKGC] was cyclized via disulfide oxidation. **b**) Unstructured CGSNKGC. **c**) c[CGSNKGG] was cyclized head-to-tail with a peptide bond. **d**) Unstructured CGSNKGG. **e**,**f**) Percentage of overlap in conformation ensembles between $A\beta_{42}$ monomer and cSNK peptides c[CGSNKGC] (**e**) and c[CGSNKGG] (**f**). **h**) The solvent accessible surface area (SASA) of the GSNKG motif in three structures of the alpha chain of the variable domain of T-cell receptor protein and for the conformational ensembles of the cyclic peptide constructs c[CGSNKGG] and c[CGSNKGC]. The solvent-accessible surface of the GSNKG motif is highlighted in orange in the T-cell receptor structures (PDB: 1ZGL, 4P4K, 4QRP). Schematics of representative centroid structures for the cyclic peptides are also shown.



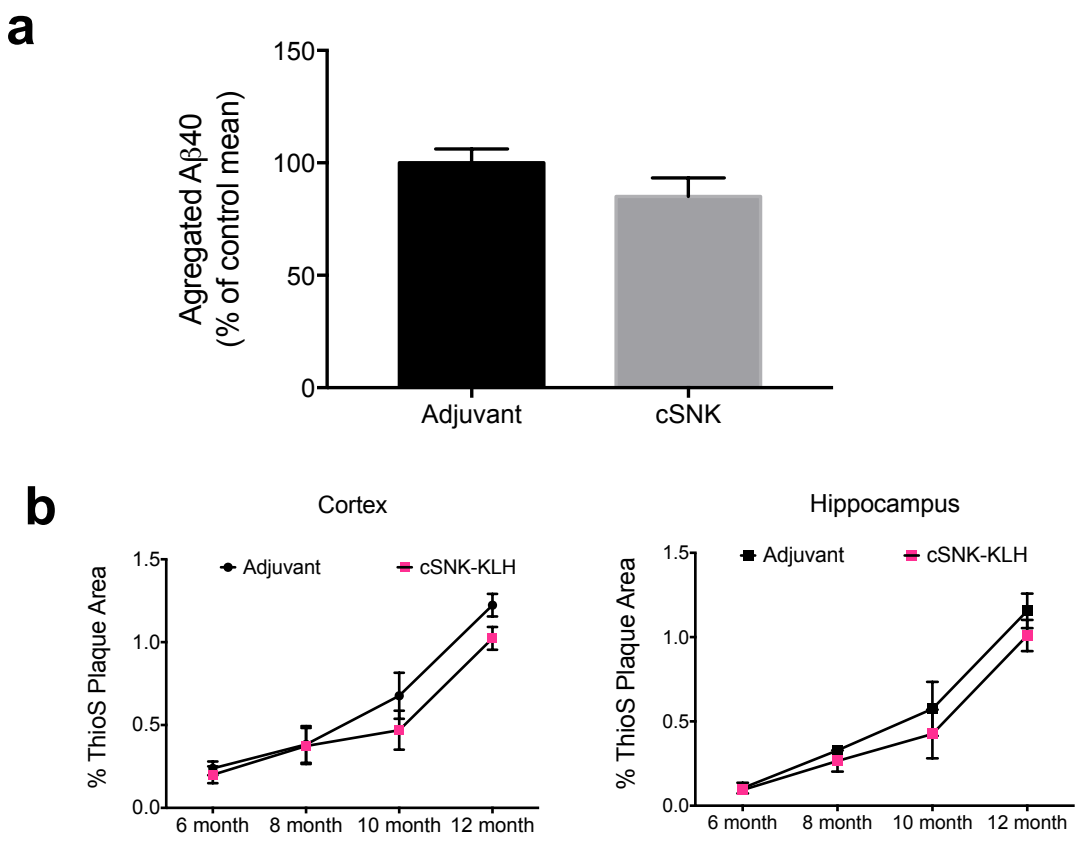
Supporting Figure 2. **Anti-AβO^{cSNK} is specific to AβO42 and does not bind Aβ plaque.** **a**) Atomic force microscopy images and height distributions of Aβ42 monomers, oligomers (AβO42), and fibrils generated at 4°C after 7 days incubation. Scale bar: 200 nm, for all images. **b**) Dot blots of AβO42 and α-synuclein oligomers (αSyn oligos) probed with 6E10, anti-AβO^{cSNK} (α-AβO^{cSNK}) and the oligomer specific antibody A11. **c**) Immunohistochemistry of the hippocampus of a male 13 month old APP/PS1 mouse. Sections were stained with 1 μg/mL 4G8 and 50 μg/mL anti-AβO^{cSNK}. Images of the hippocampus collected from the same area under increasing optical magnification. For **a-c**) data are from one of three identical experiments.



Supporting Figure 3. **Anti-AβO^{cSNK} markedly delays the aggregation of Aβ into an insoluble aggregate. a**) Beta-sheet formation was tracked for 6, 18, 30, 48 and 72 hours *in vitro* by a Thioflavin-T fluorescent assay after addition of Aβ monomers alone, or in the presence of either anti-AβO^{cSNK} or a non-specific IgG1 isotope control at a molar ratio of 10:1 Aβ:antibody. Error bars are SEM. **b**) Samples were collected at representative time points and imaged by TEM. Scale bar is applicable to all images and is 100nm. **c**) Samples were collected at representative time points and fractionated into soluble (Supernatant) or insoluble (Pellet) Aβ by centrifugation, then resolved on SDS-PAGE for immunoblotting with pan-Aβ antibody. Aβ gradually converts to its insoluble form starting at 30 h in the absence of antibody, while anti-AβO^{cSNK} retains it in its soluble form for a longer time (> 48 h). IgG1 seems to have an effect, but the delay is less pronounced than with anti-AβO^{cSNK} (between 30 and 48 h). Shown are representative data from one of three identical experiments.



Supporting Figure 4. **No antigenic drift occurs upon repeated vaccination with cSNK. a**) Immunoglobulin isotyping of cSNK induced polyclonal response was determined by surface plasmon resonance imaging. IgG is the dominant class with IgG1 in the majority. At endpoint, IgM is approximately equal to IgG1 levels. **b**) Human AD brain sections from the frontal cortex Brodmann 9 region were probed with plasma, collected after 8 months of injections, from cSNK and adjuvant treated animals. Based on the titer data (see Fig. 5), sections were probed with approximately 1 µg/ml of anti-cSNK IgG. Since no anti-cSNK response was detected in adjuvant treated animals, we tested an equivalent volume of anti-adjuvant plasma as the anti-cSNK plasma. Scale applies to all images = 50 µm. **c**) Baseline and 8 months post-vaccination plasmas from Adjuvant and cSNK-KLH treated animals were analyzed by SPR for binding to cSNK-BSA peptide, monomeric Aβ1-42, and AβO42. Binding of the 8 month plasma to the antigens was normalized to the baseline values for each animal. SEM is shown throughout.



Supporting Figure 5. **Total aggregated Aβ burdens were largely unaffected by cSNK vaccination. a)** AβO levels in the brain were not affected by cSNK vaccination (p=0.0782 Mann-Whitney two-tailed, unpaired T-test; p=0.290 regression model ANOVA see Table 2). **b)** Small differences in percent of ThioS positive plaque area were found in the animals treated to endpoint (12 month) in the cortex (p=0.077), but not in the hippocampus (p=0.484). Both p values are reporting from a regression model with categorical covariates (ANOVA), see Table 2. SEM is shown throughout.