Supporting Information

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SI Materials and Methods

Transgenic Mice. Tg(APP23) mice expressing Swedish mutant human APP (751-amino acid isoform) under the control of the Thy-1.2 promoter (1) were a gift from Matthias Staufenbiel (Novartis, Basel) and were maintained on a C57BL/6 background. Tg(βgal-luc) mice expressing firefly luciferase under the control of the murine βgal promoter (2) were a gift from Caliper Life Sciences (Alameda, CA) and were maintained on an FVB/N background. Homozygous Tg(βgal-luc) mice were generated by intercrossing hemizygous animals and were confirmed by backcrossing. To create bimice mice, Tg(APP23) mice were crossed with homozygous Tg(βgal-luc) animals. For inoculations, 30 μL of 0.4 mg/mL synthetic amyloid β (Aβ) aggregates were used. Weanling (~2 mo old) Tg(APP23;βgal-luc) mice were inoculated in the right cerebral hemisphere using a 27-gauge syringe. In all bioluminescence imaging (BLI) experiments, equal numbers of male and female mice were used. All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at the University of California at San Francisco.

Electron Microscopy. Negative-stain electron microscopy was carried out as follows: 5 μL of either proteinase K-treated or untreated synthetic Aβ aggregates were incubated on freshly glow-discharged, 200-mesh formvar/carbon-coated copper grids (Ted Pella) for 60 s. Following adsorption, the grids were washed with 0.1 and 0.01 M ammonium acetate and then stained with a solution of 2% (wt/vol) ammonium molybdate, and excessive fluid was removed with filter paper. After air drying, the samples were examined using a Philips/FEI Tecnai F20 electron microscope at 80 kV.

Kinetic Measurements Using Thioflavin T. For kinetic measurements, 100-μL aliquots of synthetic Aβ were adjusted to a final Thioflavin T concentration of 10 μM and added to 96-well plates (BD Biosciences). Plates were sealed with a clear film (Nunc) and then incubated in a Spectramax M2 plate reader ( Molecular Devices) set at 37 °C. Samples were subjected to repeated rounds of 1-min rest and 4-min shaking, and top-read fluorescence measurements (444-nm excitation and 485-nm emission filters) were taken every 5 min over the course of aggregation.

BLI. BLI was performed as described previously (3). Briefly, bimice Tg(APP23;βgal-luc) mice were imaged using an IVIS Lumina II imaging system (PerkinElmer) after being given an i.p. injection of β-luciferin potassium salt solution (Gold BIotech) prepared in PBS, pH 7.4 (Invitrogen). Each mouse received 50 μL of the 30 mg/mL luciferin solution. Before BLI measurements, mice were anesthetized using an isoflurane/oxygen mixture. Black paper cutouts were placed over the ears to minimize extraneous signals. Bioluminescence values were quantified from images displaying surface radiance using circular regions of interest and then converted to total flux of photons (photons per second) using Living Image 4.1 software (PerkinElmer).

SDS/PAGE, Immunoblotting, and Silver Staining. SDS/PAGE was performed by using 4–12% Bis-Tris gradient gels (Invitrogen). Silver staining was carried out according to the protocol of Merrill et al. (4).

For immunoblotting following SDS/PAGE, gels were transferred to PVDF membranes and then blocked for 2 h at room temperature with blocking buffer [5% (wt/vol) nonfat milk in Tris-buffered saline containing 0.05% Tween-20 (TBST)]. Membranes were incubated with primary antibody at 4 °C overnight in blocking buffer. Blots were rinsed three times with TBST, incubated with HRP-conjugated secondary antibody (BioRad) or HRP-conjugated streptavidin (Pierce) for 1–2 h, rinsed three times with TBST, and then developed using the enhanced chemiluminescence detection system (Amersham). The following primary antibodies were used: biotinylated anti-human APP/Aβ6E10 (Covance) and anti-actin 20–33 (Sigma). All analyses used brains from age- and sex-matched animals.

Statistical Methods Used for Data Analysis. Statistical analysis of Aβ40 and Aβ42 levels were performed using Prism 6 software (GraphPad Software). Statistical differences between groups were assessed using unpaired, two-tailed t tests or by performing one-way ANOVA with a Tukey’s multiple comparison posttest. A significance threshold of P = 0.05 was used for all experiments.

Quantification of Aβ40 and Aβ42 by ELISA. Brain homogenates (10% wt/vol) were normalized for 500 μg total brain protein by bichinonic acid assays (Thermo Fisher). Two volumes of cold formic acid were added to one volume of brain homogenate or purified Aβ preparations, followed by a 5-min sonication. Samples were then centrifuged at 100,000 × g for 1 h at 4 °C, and the supernatants were neutralized by the addition of 19 volumes of 1 M Tris-HCl and 500 mM NaP (pH unadjusted). Following further dilution, Aβ40 and Aβ42 levels were quantified using sandwich ELISAs specific for each peptide according to the manufacturer’s instructions (In-vitrogen), and the values expressed as micrometers per milliliter of normalized brain homogenate.

Neuropathology. All comparisons were conducted on brains from age- and sex-matched animals. Samples were immersion-fixed in 10% (vol/vol) buffered formalin and then embedded in paraffin using standard procedures. Sections (8 μm) were cut, deparaffinized, and processed for immunohistochemistry. Endogenous peroxidase activity was blocked by incubation in 3% (vol/vol) hydrogen peroxide (in methanol), and then sections to be stained with anti-Aβ antibodies were pretreated with formic acid for 5 min. Following blocking with 10% normal goat serum, sections were incubated with primary antibody overnight at 4 °C. The following antibodies were used: anti-Aβ 4G8 (Covance) and anti-GFAP (DAKO) to detect astrocytic gliosis. Antibody binding was detected using a Vectastain ABC peroxidase kit (Vector Laboratories) and visualized using 3,3′-diaminobenzidine (DAB). Slides were photographed using a Zeiss AxioImager.A1 microscope.

For ThioS staining, slides were baked at 50 °C overnight and then deparaffinized with xylene and alcohols to water. Sections were stained with 0.05% (vol/vol) ThioS (Sigma-Aldrich) in 50% (vol/vol) ethanol in the dark for 8 min, and residual ThioS was removed by a rapid rinse in distilled water. Slides were mounted with Vectashield with DAPI (Vector Laboratories) aqueous mount and viewed with a Leica DM-IRB microscope.

For immunofluorescence double staining with specific antibodies targeting Aβ40 (Millipore AB5074) and Aβ42 (Covance SIG-39142), brain sections were pretreated with formic acid for 5 min. Sections were blocked with 10% (vol/vol) normal goat serum and then incubated with primary antibodies at a 1:200 dilution overnight at 4 °C. The following day, sections were thoroughly washed and incubated with the secondary antibodies goat anti-mouse AlexaFluor 488 (A-11029; Life Technologies)
and goat anti-rabbit AlexaFluor 647 (A-21245; Life Technologies). Sections were stained with 0.05% (wt/vol) ThioS (Sigma-Aldrich) in 50% (wt/vol) ethanol and washed before mounting. Samples were visualized using a 63× water-immersion lens (1.2 NA) in sequential scan mode on a Leica SP8 confocal microscope equipped with three HyD detectors and an acousto-optical beam splitter (AOBS). Using control (spontaneous disease) brains to establish standardized acquisition settings for all experimental groups, eight-bit image z-stacks (1.5-μm steps) were collected at 1,024 × 1,024-pixel resolution with the full dynamic range of fluorescence intensity.

Quantification of Amyloid Plaque Aβ40:Aβ42 Fluorescence Ratio. Aβ40 and Aβ42 fluorescence intensities of individual amyloid plaques were imaged with a 63× water-immersion lens (1.2 NA) in sequential scan mode and analyzed with National Institutes of Health (NIH) ImageJ software. A z-projection of three confocal optical slices through the center of the plaque core was prepared from each image stack and used for analysis. A standard region of interest was used to measure the intensities of Aβ40 and Aβ42 fluorescence along the plaque core perimeter as demarcated by ThioS labeling. Multiple regions of interest were acquired per individual plaque and used to calculate the average ratio of Aβ40/Aβ42 fluorescence. Brain slices from three to five animals per experimental or control group were used for quantification.

Quantification of Amyloid Plaque Number and Size. ThioS-labeled plaques were imaged by confocal microscopy using a 40× water-immersion lens (1.1 NA) and analyzed with NIH ImageJ software. A z-projection of three confocal optical slices through the center of the plaque core was prepared from each image stack and used for analysis. A standard fluorescence intensity threshold was used to create a binarized image. Using the Analyze Particles function in NIH ImageJ, plaques were automatically selected and measured. Brain slices from three to five animals per experimental or control group were used for quantification.


Fig. S1. Formation of synthetic Aβ40 amyloids in the absence and presence of SDS. Aβ40 (0.05 mg/mL) was adjusted to 10 mM sodium phosphate (NaP) (pH 7.4) in the absence (black line) or presence of 0.87 (green line), 1.74 (red line), 3.47 (blue line), and 8.67 mM SDS (gray line). The aggregation kinetics were monitored using Thioflavin T (10 μM) fluorescence measurements (arbitrary units).
Fig. S2. Representative electron micrographs of synthetic Aβ prions formed in the presence of NaP (first and third rows) and NaP/SDS (second and fourth rows). Reactions were performed with 3.47 mM SDS and/or 10 mM NaP (pH 7.4). Fibrillar aggregates of Aβ40 and Aβ42 formed in NaP or NaP/SDS buffer were structurally intact after treatment with 50 μg/mL proteinase K for 1 h at 37 °C (Right). (Scale bars, 100 nm.)
Fig. S3. Distribution and accumulation of Aβ in the brains of Tg(APP23:Gfap-luc) mice inoculated with synthetic Aβ fibrillized in NaP (Left) or NaP/SDS (Right). (A and B) Aβ40 and Aβ42 levels in the brains of mice at 390 d after inoculation were significantly increased compared with age-matched (405 d) uninoculated controls, as quantified by ELISA (*P < 0.05; **P < 0.01; ***P < 0.001). The inocula were synthetic Aβ40 and Aβ42 prepared in NaP (A) or NaP/SDS (B). Values are mean ± SEM from four to six mice. (C–F) Total Aβ deposits, stained with the 4G8 antibody, were found along the corpus callosum and CA1 layer of the hippocampus in the brains of inoculated mice.
Fig. S4. In the brains of Tg(APP23:Gfap-luc) killed 330 d after inoculation with either Aβ40 (A, B, E, and F) or Aβ42 (C, D, G, and H), amyloid deposition and brain histopathology were assessed by Thioflavin S (ThioS; Left) and H&E (Right). Independent of the synthetic Aβ prion preparation used for inoculation, all Aβ prions induced a continuous band of ThioS-positive deposition in the corpus callosum, representing a pathologic signature of natural and synthetic Aβ prions.

Fig. S5. Hippocampal sections from Tg(APP23) mice inoculated with synthetic Aβ42(NaP) prions and euthanized at 30 dpi. Staining with Aβ42-specific antibody (A), H&E (B), or ThioS (C) did not show any traces of residual inoculum.
Fig. 56. Reactive astroglization associated with the induced deposition patterns of synthetic Aβ prions. GFAP-immunolabeled astrocytes (red) and ThioS-positive amyloid plaques (cyan) were visualized by confocal microscopy. Z-projection images show plaques and associated reactive astrocytes in the corpus callosum of Tg(APP23:Gfap-luc) mice inoculated with synthetic Aβ40 (A, B, E, and F) or Aβ42 (C, D, G, and H) formed with NaP (A–D) or NaP/SDS (E–H). B, D, F, and H are magnified high-resolution confocal projections of the corpus callosum (40× 1.1 NA lens). (Scale bar in A applies to C, E, and G, 1 mm; scale bar in B applies to D, F, and H, 50 μm.)
Fig. S7. Differential distribution of induced Aβ deposition in Tg(APP23:Gfap-luc) mice following inoculation with synthetic Aβ40 (A and B) or Aβ42 (C and D) formed in NaP (A and B) or NaP/SDS (C and D). Fixed brain slices of the CA1 region were immunolabeled with Aβ40-specific antibody (red) and Aβ42-specific antibody (green) and stained with ThioS (blue). Images were taken in sequential scan mode on a Leica SP8 confocal microscope using the automated tiling and stitching features to acquire a large field of view (20x 0.75 NA lens). Brain slices from spontaneous ill, age-matched (E) and 2-y-old (F) mice were similarly labeled and imaged for comparison. (Scale bars, 500 μm.)
Fig. S8. Aβ42 prions formed in the absence of SDS accelerates the appearance of diffuse plaques composed exclusively of Aβ42. (A–F) Immunolabeling for Aβ40 (red) and Aβ42 (green) of fixed brain slices from mice inoculated with Aβ42(NaP) and imaged by confocal microscopy (63× 1.2 NA lens; zoom 3). Z-projections of confocal cross sections show plaques in the cerebral cortex of mice killed at 330 d after inoculation. (Scale bars in A and D apply to B and C and E and F, respectively, 10 μm.) (G) Quantification of the total number of diffuse plaques labeled only by the Aβ42-specific antibody in a hemicoronal brain slice. Among the four inocula, only injection with Aβ42(NaP) resulted in a significant increase in the amount of Aβ42-exclusive diffuse plaques in the cortex (***, **P < 0.001). The number of Aβ42-exclusive plaques was not significantly different with other inocula compared with age-matched spontaneous control mice (ns, not significant). Data shown as mean ± SEM acquired from three to five animals per experimental group.