Supporting Information

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SI Text

$A\beta_{1-42}$ Synthesis and Sample Preparation. Depsi-peptide $A\beta_{1-42}$ (1) was synthesized in a mainly automated manner using an ABI 433A synthesiser (Applied Biosystems) on TentaGel-resin (Novabiochem) with a 10-fold excess of Fmoc-protected L-amino acids and 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluoro-phosphate as coupling reagent (2). Only the depsi-peptide Boc-Ser(Fmoc-Gly) (Novabiochem) was manually introduced after position 27, using a filter fritted syringe (3). Afterward, the amino acids were again coupled using the automated synthesizer. The peptide was cleaved from the resin with 0.3 g phenol in a H2O/THS/EDT/TFA (water/thioanisol/ethanedithiol/trifluoroacidic acid) (0.2 mL/0.2 mL/0.1 mL/4 mL) solution for 3 h, poured into ice-cold diethylether, filtered, washed three times with ice-cold diethylether, and dried under vacuum for 20 min. The peptide was purified by HPLC equipped with a C4 column (Water Symmetry 19 × 150 mm). The molecular mass of the final product was determined by MALDI-TOF spectrometry (Bruker).

Depsi-$A\beta_{1-42}$ was dissolved in an acidic solution (TFA 0.02%, pH <3) at a concentration of ~1 mg/mL and centrifuged through a 10-kDa cut-off filter (YM-10, Millipore) at 14,000 × *g*. The filter was washed twice by addition of same acidic solution, and the centrifugation was repeated. This stock solution was flash-frozen in dry ice/ethanol and stored at −80 °C. Samples were used within 2 weeks. The native sequence was obtained by adding 0.5 M NaOH:ammonium (1:3) solution on ice to pH >10.5. This solution was kept for 15 min on ice. The concentration of the native peptide in the final, switched, alkaline stock solution was 300 μM. This was diluted to 1 μM Aβ in PBS and used immediately ("initial state solution"). To obtain $A\beta_{1-42}$ oligomers, the stock solution was diluted to 100 μM Aβ in 50 mM phosphate buffer and 150 mM NaCl, pH 7.4, and incubated for 24 h at either 4 °C (4) or 22 °C (5). To prepare $A\beta_{1-42}$ fibrils, the stock solution was diluted with water to 100 μM Aβ, acidified to pH 2.0 with 1 M HCl, and left for 24 h at 37 °C (6).

Size Exclusion Chromatography. Size exclusion chromatography (SEC) was performed on an FPLC apparatus (Biologic FPLC system, Biorad) equipped with a precision column prepacked with Superdex 75 resin, with a separation range of 3–70 kDa (GE Healthcare). The mobile phase flow rate was set at 0.5 mL/min, and the elution peaks were detected at 214 and 280 nm UV absorbance. The mobile phase was 25 mM phosphate buffer (PBS, pH 7.4). The column was calibrated using insulin chain B (3.5 kDa), ubiquitin (8.5 kDa), ribonuclease A (13.7 kDa), carbonic anhydrase (29.0 kDa), ovalbumin (43.0 kDa), and BSA (67.0 kDa). The void volume was determined with Blue dextran 2000 (2,000 kDa), and each peptide solution was injected at a final concentration of 20 μM in a volume of 100 μL.

Surface Plasmon Resonance and $A\beta_{1-42}$ Oligomer Binding to PrP^C. Binding studies were done with the ProteOn XPR36 Protein Interaction Array system (Bio-Rad), based on surface plasmon resonance (SPR) technology. The system contains six parallel-flow channels, which can uniformly immobilize up to six strips of ligands on the sensor surface (7). Two anti-PrP antibodies, 3F4 (8) against epitopes 108–111, and 94B4 (9) against epitopes 187–194 (mouse PrP numbering) were immobilized in two parallel lanes, using amine-coupling chemistry, with final immobilization levels of ~6,000 resonance units (RU, 1 RU = 1 pg protein/mm^2) for both. "Reference" surfaces were prepared in parallel following the same immobilization procedure but without antibodies. After 90° rotation of the fluid system, a brain homogenate from Tg(WT-E1) mice overexpressing mouse PrP carrying an epitope tag for the monoclonal antibody 3F4 (10) (0.5 mg protein/mL in PBS containing 0.5% Nonidet P-40 and 0.5% Na-deoxycholate) was injected in three parallel flow channels. The signal was negligible in the three reference surfaces (31 ± 1 RU, mean ± SD) but reached 454 ± 12 in the three 3F4-coated surfaces and 310 ± 3 in the three 94B4-coated surfaces, confirming that these antibodies specifically capture PrP^C. This signal decayed very slowly, indicating very slow dissociation of PrP^C from the antibody.

The $A\beta_{1-42}$ initial state, oligomers, and fibrils were then injected simultaneously over the different surfaces (coated with PrP^C captured by either 3F4 or 94B4, with 3F4 or 94B4 alone, or empty "reference" surfaces). Experiments were repeated at least twice. All of these assays were performed at 25 °C. The Aβ-dependent signals on the surfaces immobilizing PrP were obtained by double referencing, subtracting the response observed on surfaces immobilizing the antibodies alone, and the signal observed injecting the vehicle alone (which allows correction for binding-independent responses, e.g., drift effects). The resulting sensorgrams were fitted by the simplest 1:1 interaction model (ProteOn analysis software), to obtain the corresponding association and dissociation rate constants (K_{on} and K_{off}).

Animals. C57BL/6 mice were obtained from Charles River-Italy. Zürich I $Prnp^{0/0}$ mice (11) maintained on a pure C57BL/6 background were obtained from the European Mouse Mutant Archive (strain EM01723). Animals were 7–8 weeks old. All animals were handled for 2 days before the experiments. Based on the previous experience, we used only males (12). All procedures involving animals and their care were conducted according to European Union (EEC Council Directive 86/609, OJ L 358,1; 12 December 1987) and Italian (D.L. n.116, G.U. suppl. 40, 18 February 1992) laws and policies, and in accordance with the United States Department of Agriculture Animal Welfare Act and the National Institute of Health (Bethesda, MD) policy on Humane Care and Use of Laboratory Animals.

Intracerebroventricular Incannulation. Mice were anesthetized with Forane (Abbott) using stereotaxic apparatus (model 900, David Kopf) a 7 mm-long guide cannula was implanted into the cerebral lateral ventricle (L ± 1.0 and DV -3.0 from dura with incisor bar at 0°) and secured to the skull with two stainless steel screws and dental cement. To avoid infections the animals received i.p.injections of 150 mg/kg/day Amplital (Pfizer) for 3 consecutive days after surgery. Mice were allowed 10–15 days to recover from surgery before the experiment.

$A\beta_{1-42}$ Treatment. The $A\beta_{1-42}$ preparations, checked by AFM, were infused into the lateral cerebral ventricle using an injection unit inserted into the guide cannula. The $A\beta_{1-42}$ were diluted to 1 μM in 5 mM PBS, pH 7.4, and 7.5 μL were infused using a Hamilton syringe in a total time of 5 min. The injection unit was left in place for 2 min more to allow the liquid to diffuse.

Object Recognition Task. Mice were tested in an open-square gray arena (40 × 40 cm), 30 cm high, with the floor divided into 25 squares by black lines. The following objects were used: a black plastic cylinder (4 × 5 cm), a glass vial with a white cup (3 × 6 cm), and a metal cube (3 × 5 cm). The task started with a habituation trial during which the animals were placed in the empty arena for 5 min, and their movements were recorded as the

number of line crossings. The next day, mice were again placed in the same arena containing two identical objects (familiarization phase). Exploration was recorded in a 10-min trial by an investigator (C.B.) blinded to the strain and treatment. Sniffing, touching, and stretching the head toward the object at a distance not more than 2 cm were scored as object investigation.

Twenty-four hours later (test phase) mice were again placed in the arena containing two objects: one identical to one of the objects presented during the familiarization phase (familiar object), and a new, different one (novel object), and the time spent exploring the two objects was recorded for 10 min. Memory was expressed as a discrimination index, i.e., (seconds on novel – seconds on familiar)/(seconds on novel + seconds on familiar). Animals with no memory impairment spent longer investigating the novel object, giving a higher discrimination index. Mice were injected with $A\beta_{1-42}$ samples or the vehicle (PBS) alone 2 h before the familiarization and test phases. At the end of the experiment the mice were killed and their brains were taken for histological analysis to verify the correct placement of the cannula.

Hippocampal Neuron Cultures and Determination of $A\beta_{1-42}$ Oligomer Toxicity. Primary hippocampal cultures were prepared from mice 2 days of age. The dissected hippocampus was incubated with 200 units of papain (Sigma Aldrich) for 30 min at 34 °C, and with trypsin inhibitor (Sigma Aldrich) for 45 min at 34 °C before mechanical dissociation. Neurons were plated on 96-well plates ($\sim 5 \times 10^4$ cells/well) precoated with 25 μg/mL poly-D-lysine (Sigma Aldrich). The plating medium was B27/neurobasal (Life Technologies) supplemented with 0.5 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Twelve days from the plating date, the neurons were treated with either 1 or 3 μM synthetic $A\beta_{1-42}$ oligomers prepared at both 4 °C and 22 °C. After 72 h of Aβ treatment, cell survival was measured by MTT assay.

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