Supplementary Methods

Materials

Uniformly ^{15}N,^{13}C-labelled recombinant Aβ1-40 (Aβ40) was produced as follows. Aβ40 was expressed as a fusion protein using a modified pET28a vector (Novagen). Protein expression was performed in *E. coli* strain BL21(DE3) at 37 °C in minimal medium with ^{15}N-ammonium chloride as nitrogen source and $^{13}C_6$-β-D-glucose as carbon source. After purification on a nickel column (Macherey-Nagel) the fusion protein was digested overnight on ice with TEV protease. Final purification was performed on a C4 reversed phase Vydac HPLC column. The released Aβ40 peptide eluted in a linear (0-100%) acetonitrile gradient from this column as a single peak. The purified peptide was lyophilized before use. To disaggregate the pre-formed aggregates, the lyophilized Aβ40 peptide powders were dissolved in 20 mM NaOH at a concentration of 2 mg/mL (~ 460 μM). The monomerized Aβ40 solutions were then aliquoted, flash frozen in liquid nitrogen and stored at -80 °C until use.

Aggregation experiments

Aβ40 samples were prepared at 0.4 mg/mL (~ 92 μM) concentration and pH 7.4, buffered with 25 mM HEPES and 50 mM sodium chloride. The Aβ40 samples were incubated at 37 °C and gently agitated using tiny magnetic rods. After 6 hours, 24 hours, 48 hours, one week and two months of incubation in the above-mentioned condition, aliquots were taken out for electron microscopy and high-pressure NMR experiments. For electron microscopy, the 20-μL Aβ40 aliquots were diluted with the buffer, deposited onto carbon-coated copper mesh grids and negatively stained with 2% (w/v) uranyl acetate. After washing out the excess stain and drying the sample grids under air, the samples were visualized using a Philips CM 120 BioTwin transmission electron microscope (Philips Inc.). Quantification of individual amyloid fibril diameters in electron micrographs was carried out using ImageJ (version 1.53t), with 2-3 fibrils analyzed per image resulting in 20 fibril diameters per incubation time. For high-pressure NMR experiments, the 250-μL Aβ40 aliquots were diluted with the same buffer to a final volume of 670 μL, needed to completely fill the high-pressure ceramic NMR tube.

High-pressure NMR experiments

High-pressure NMR experiments were conducted at a 701.13 MHz Bruker spectrometer equipped with a cryogenic probe and a high-pressure device (Daedalus Innovations LLC, PA). The Aβ40 samples incubated in the aggregation-promoting condition for 6 hours, 24 hours, 48 hours, one week or two months were transferred to a ceramic high-pressure NMR tube and subjected to a temperature of 278 K and ambient or high pressure level of 2000 bar. Pressure-induced monomer dissociation from Aβ aggregates was monitored through real-time 1D ^1H and 2D ^{15}N,^1H HSQC experiments, measured after a rapid elevation of pressure from 1 to 2000 bar. Due to the need for pressure equilibration and technical set-ups, there was ~ 0.5 hour delay between pressure elevation and the first NMR experiment measured at the higher pressure. The NMR signal intensities were normalized with respect to NMR signal intensities of a reference non-aggregated Aβ40 sample. The monomer release curves were analyzed through global fitting of the time-dependent HSQC peak intensity (I) data to a mono-exponential equation:

$$I = I_\infty + (I_0 - I_\infty)e^{-t/T} \qquad \text{(eq. S1)}$$

where I_0 and I_∞ are normalized initial and final intensities and T is a global fitting parameter, representing the characteristic time of Aβ40 monomer release from aggregates. Assuming a two-state model:

$$F \rightleftharpoons m$$

for the conversion between NMR-invisible fibrillar (F) and monomeric (m) state, the rate constants k_{off} and $k_{on,app}$ governing the dissociation and back-association of monomeric peptide were obtained through the following equations:

$$I_\infty = \frac{k_{off}}{k_{off}+k_{on,app}} \qquad \text{(eq. S2)}$$

$$\frac{1}{T} = k_{off} + k_{on,app} \qquad \text{(eq. S3)}$$