Supporting Information

Materials and Methods

Preparation of Aβ samples:

Unlabeled samples of $A\beta_{1-40}$ were purchased either from Genscript Inc. (Piscataway, NJ, USA) or GL Biochem (Shanghai, China) with >95 % purity. Uniformly ^{15}N-labeled $A\beta_{1-40}$ was purchased from rPeptide (Bogart, GA). The uniformly $^{15}N/^{13}C$-labeled $A\beta_{1-40}$ peptide was recombinantly expressed and purified as described elsewhere.[1] To remove preformed aggregates, the purified peptide was dissolved in 1% ammonium hydroxide (v/v) at a concentration of 1 mg/ml followed by removal of the solvent by lyophilization for 24 hours in aliquots of 0.1 mg. The aliqouted peptide was then stored at -20 ^{O}C and only used once.

The purity of Aβ peptides were checked by high-performance liquid chromatography (HPLC) and mass spectrometry analysis. A reverse phase HPLC system (Shimadzu, Japan) with Phenomenix C18 column (dimension 250 × 10 mm, 5-μm particle size, pore size 100 Å) and UV detector (SPD-10A) were used. A linear gradient elution method using a dual solvent system (water/methanol or water/acetonitrile) was applied.

Preparation of a primarily monomeric $A\beta_{1-40}$ sample was performed as described previously.[2] Briefly, 0.1 mg of the lyophilized peptide was first dissolved in 3 μL of DMSO and sonicated until the peptide was solubilized. The peptide solution was then dissolved in H_2O (or 10% D_2O for NMR measurements), 20 mM phosphate buffer, pH 7.4, 50 mM NaCl and diluted to a final peptide concentration of 80 μM. The peptide concentration was confirmed by UV absorbance at 280 nm (ε_{280} = 1490 M^{-1} cm^{-1}).

For seeded NMR experiments, a fibrillar $A\beta_{1-40}$ sample was prepared as described previously.[2] The peptide was dissolved directly into a solution of 50 mM NaCl, 20 mM phosphate buffer with pH 7.4, and 0.01% NaN_3. The peptide concentration was adjusted to 150 μM (confirmed by UV absorbance). The sample was then kept at 37 ºC with agitation to produce samples to be used as preformed fiber seeds.

NMR experiments:

NMR experiments were performed on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm cryogenic probe or on a Bruker Avance III 500 MHz NMR spectrometer equipped with a 5 mm SMART probe or on a Bruker Avance 800 MHz spectrometer equipped with a 5 mm cryogenic probe. NMR experiments were performed at 10 °C or 25 °C. Prior to adding a fibril seed to the NMR tube containing monomeric $A\beta_{1-40}$, a 2D band-Selective Optimized-Flip-Angle Short-Transient (SOFAST) heteronuclear multiple quantum coherence (HMQC) spectrum was acquired to compare differences immediately before and after titration with fibrillar Aβ. Resonance assignments of the monomer 2D $^{1}H/^{15}N$ HMQC spectra were taken from the literature.[3]

To monitor $A\beta_{1-40}$ aggregation with atomic resolution, real-time 2D NMR experiments were performed by consecutively acquiring ^{1}H-^{15}N SOFAST-HMQC spectra. Each ^{1}H-^{15}N SOFAST-HMQC spectrum was obtained from 64 t1 experiments, 48 scans, 8 dummy scans, and a 0.1 s recycle delay. The spectral widths were 12 and 26 ppm, and the offsets were 4.7 and 118 ppm for the proton and nitrogen dimensions, respectively. For seeded real-time 2D NMR experiments, $A\beta_{1-40}$ fibril seeds were added to monomer solutions at concentrations of 5% up to 30% of the total peptide concentration. At 1:10 molar ratios of fiber to monomer or lower and at a temperature of 25 °C or lower, real-time 2D NMR experiments could be used to track the time-course intensity depletion of monomer peaks in a residue-specific manner.

Transmission electron microscopy:

Fibrillar samples of $A\beta_{1-40}$ for transmission electron microscopy (TEM) analysis were deposited on continuous films on copper rhodium 100 mesh grids (Electron Microscopy Services, EMS, Hartfield, PA). Prior to adding samples, the grids were charged using a glow discharger for 15 s at 30 mA negative discharge. Fibrillar samples at a concentration of 150 μM were adsorbed

to the grids for 2 minutes prior to rinsing with a 10 µL drop of freshly filtered 2% uranyl acetate (EMS) for 15 s before blotting excess stain. Samples were analyzed using a Philips CM-100 microscope operating at 80 kV.

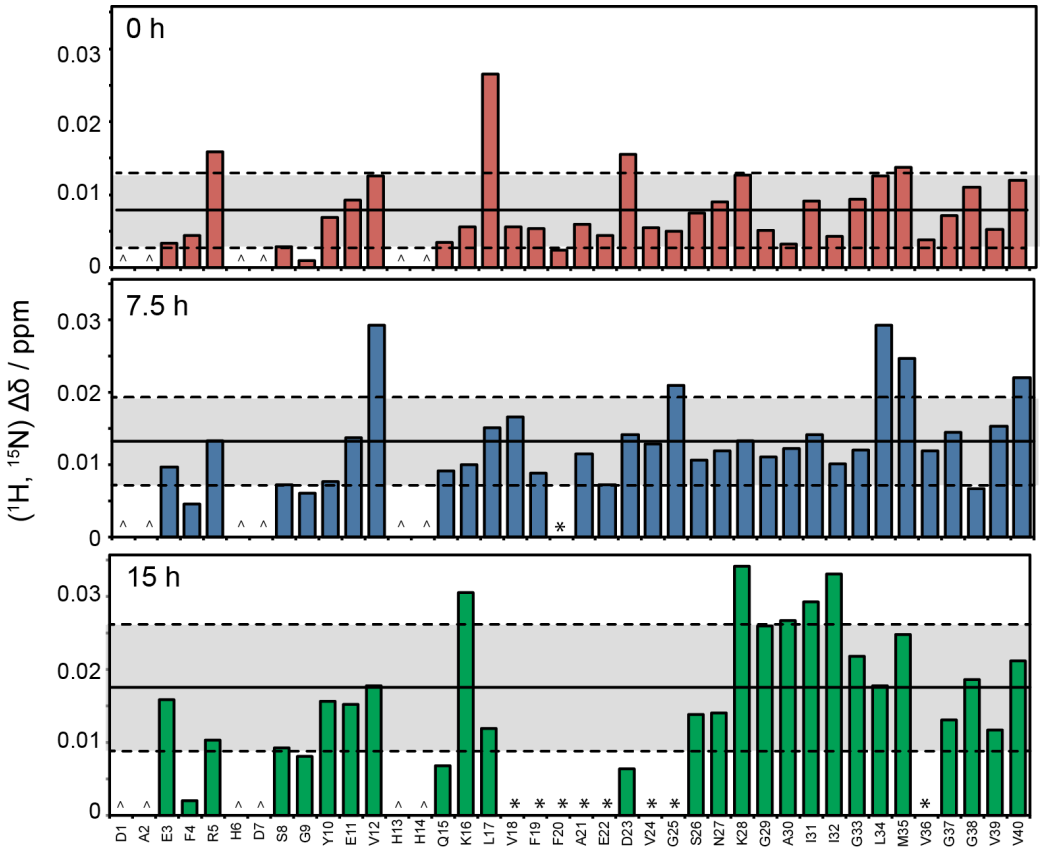

Figure S1. Chemical shift perturbations of $A\beta_{1\text{-}40}$ over time following the addition of preformed $A\beta_{1\text{-}40}$ fibers. The chemical shift perturbation was taken as the difference between the time point before and after fiber addition using the relation $\Delta\delta = \sqrt{(\Delta\delta N \times 0.2)^2 + (\Delta\delta H)^2}$. Solid horizontal lines are the average chemical shift perturbations and dashed lines are the average ± one standard deviation. The symbols * and ^ denote residues whose resonances disappeared from SOFAST-HMQC spectra or did not display discernible resonances, respectively. The data was obtained from the 2D spectra represented in Fig.S2. SOFAST-HMQC were acquired at 500 MHz and 10 ºC in 50 mM NaCl, 20 mM phosphate buffer, pH 7.4, at an $A\beta_{1\text{-}40}$ concentration of 80 µM and with a 5 mol % preformed fiber seed. Representative chemical shift perturbations were determined at 0 h (red), 7.5 h (blue), and 15 h (green). The top-most 0 hour data were measured from the 2D SOFAST-HMQC spectrum collected right after the addition of the fiber seed to the monomeric $A\beta_{1\text{-}40}$ sample.

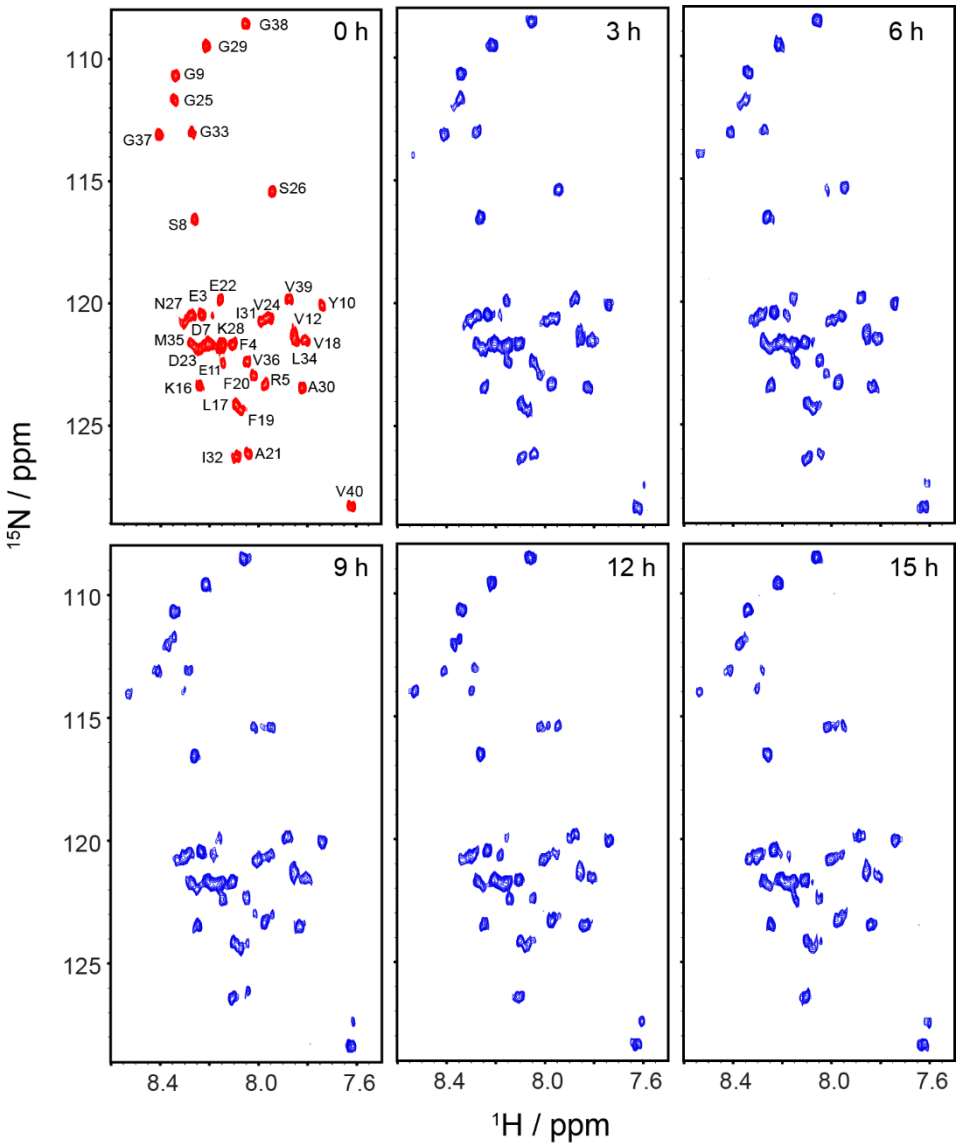

Figure S2. Representative real-time SOFAST-HMQC spectra of $A\beta_{1\text{-}40}$ aggregation seeded with a 5 mol% preformed fiber. SOFAST-HMQC were acquired sequentially at 500 MHz over a 15 hour period at 10 ºC in 50 mM NaCl, 20 mM phosphate buffer, pH 7.4 and an $A\beta_{1\text{-}40}$ concentration of 80 µM. The red spectrum at the 0 hr time point corresponds to the point at which the fiber seed was added and corresponds to the reference for the other time points. Blue spectra were acquired at the indicated times.

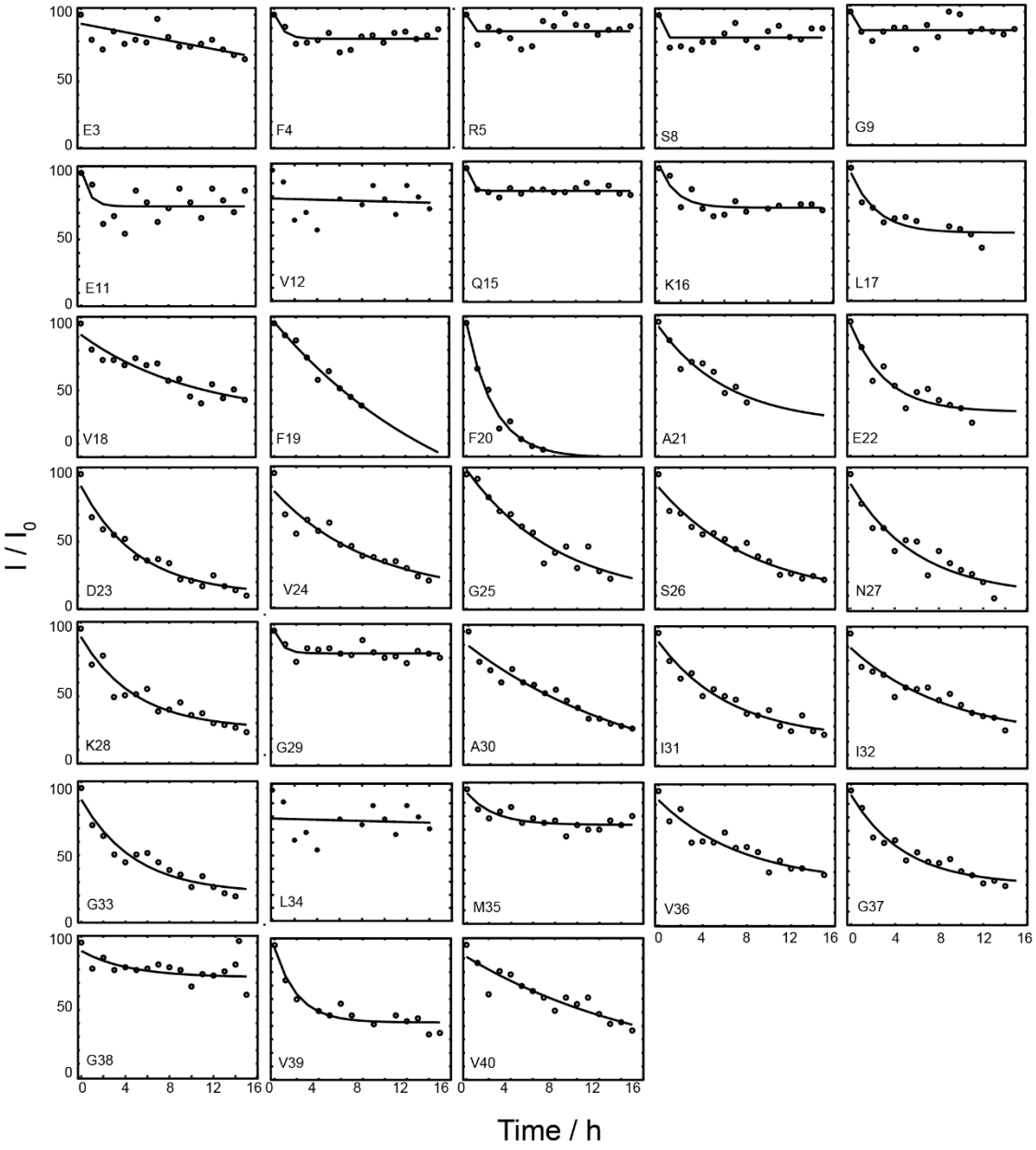

Figure S3. Time-course of the intensity changes measured from the ^{1}H-^{15}N SOFAST-HMQC spectra of a fiber-seeded $A\beta_{1-40}$ sample at 10 ºC. All data were fit to a single exponential function. The seed concentration was 5 mol % of the total $A\beta_{1-40}$ concentration. The data was analyzed and processed from the same datasets shown in figures S1-2.

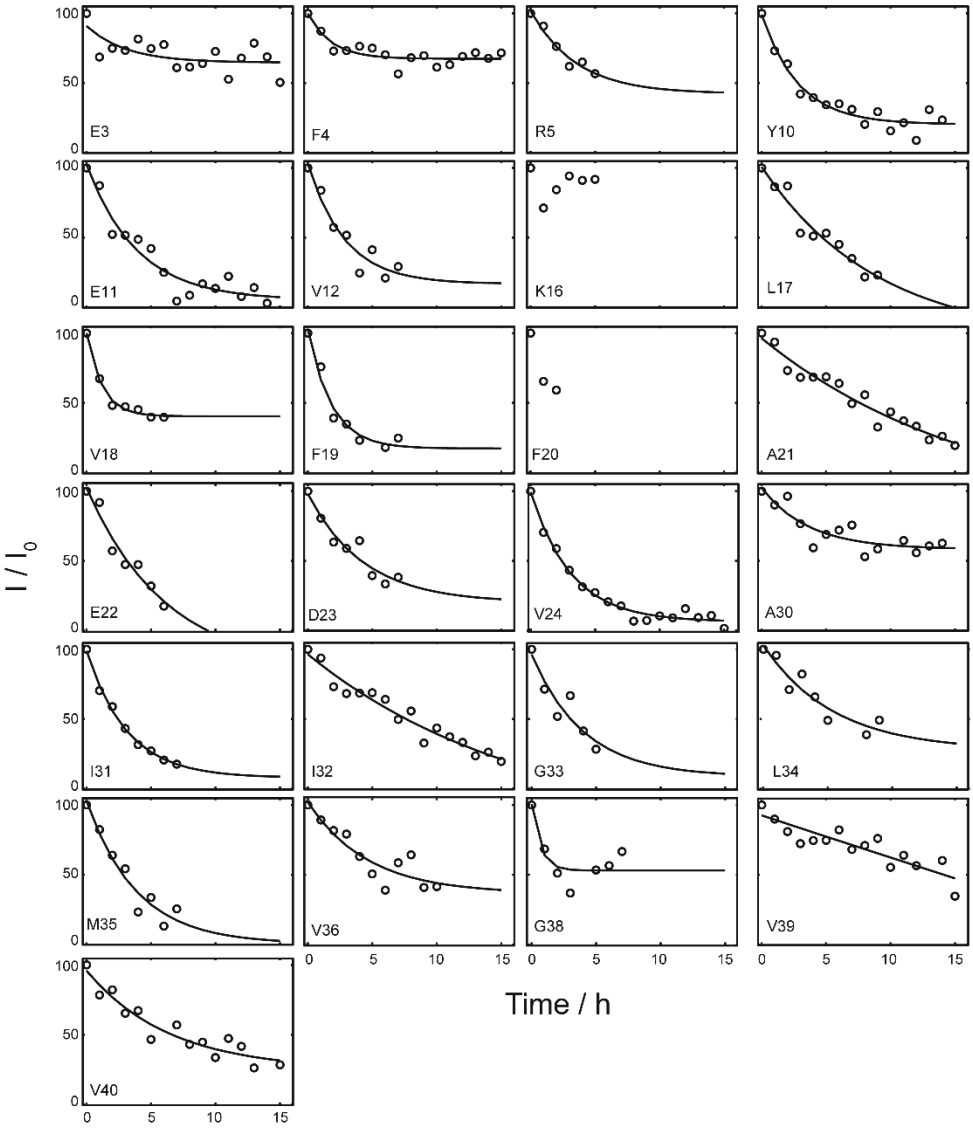

Figure S4. Time-course intensity changes measured from ^{1}H-^{15}N SOFAST-HMQC spectra of a fiber-seeded $A\beta_{1\text{-}40}$ sample at 25 ºC. SOFAST-HMQC were acquired at 500 MHz in 50 mM NaCl, 20 mM phosphate buffer, pH 7.4, an $A\beta_{1\text{-}40}$ concentration of 80 μM, and with a 7 mol % preformed fiber seed. All data were fit to a single exponential function. Fits for residues K16 and F20 are not provided due to the limited number of points available for fitting.

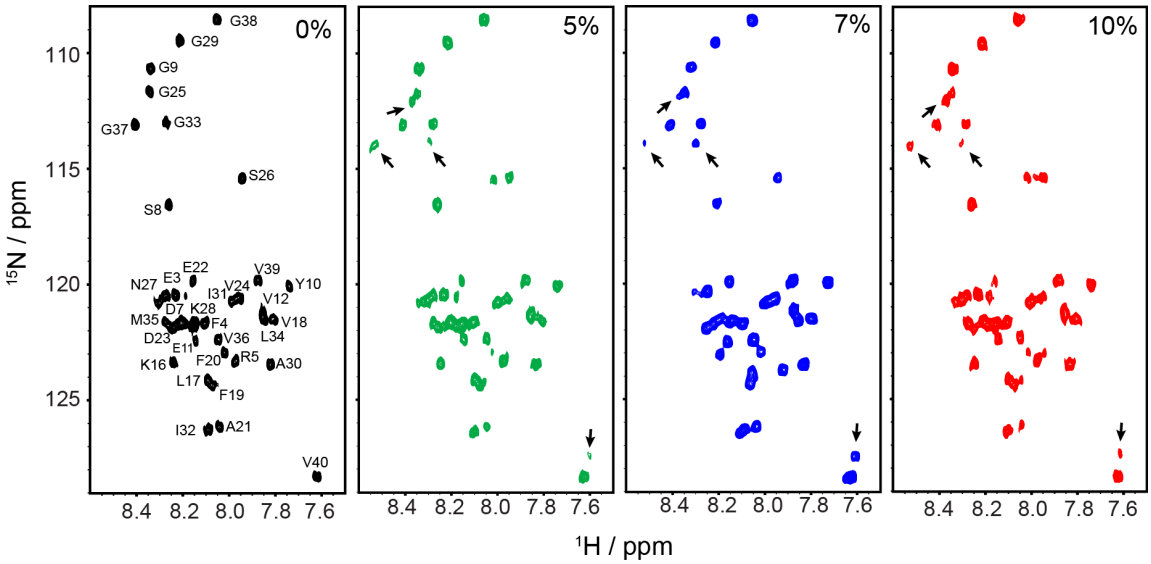

Figure S5. New peaks appear reproducibly in ^{1}H-^{15}N SOFAST-HMQC spectra obtained at different concentrations of preformed fiber seeds. Each of the freshly dissolved $A\beta_{1-40}$ sample was seeded with a different amount of $A\beta_{1-40}$ fibers (0, 5, 7 and 10 mol %). Spectra were recorded from each sample at 10 ºC in 50 mM NaCl, 20 mM phosphate buffer, pH 7.4. The resonances for monomeric $A\beta_{1-40}$ were assigned and given in the spectrum without fiber seeds (black spectrum). The representative spectra were obtained from different real-time NMR experiments after 12 hours from the addition of the fiber seed. The arrows indicate some of the new peaks that appeared due to fiber-seeded aggregation of the peptide.

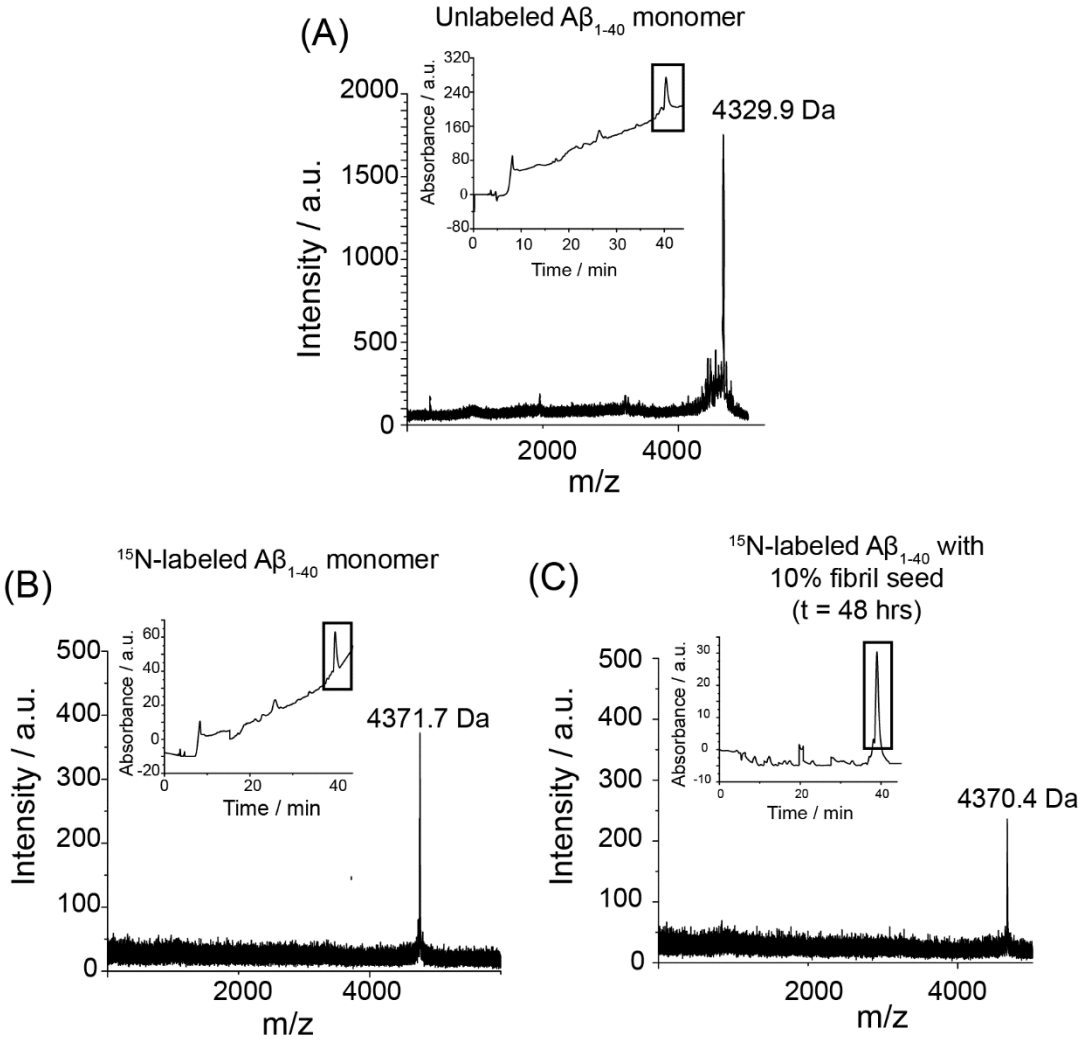

Figure S6. HLPC (insets) and mass spectrometry (MS) results demonstrate the $A\beta_{1\text{-}40}$ does not degrade over the time-course of the real-time SOFAST-HMQC NMR experiments. The peptide dissolved in initial buffer conditions showed no impurities in both unlabeled (A) and ^{15}N-labeled (B) $A\beta_{1\text{-}40}$ monomers. Furthermore, no degraded peptide product was observed for the uniformly ^{15}N-labeled $A\beta_{1\text{-}40}$ monomer after 48 hours of (C) fiber addition. It should be noted that new peaks in 2D SOFAST-HMQC spectrum of $A\beta_{1\text{-}40}$ solution appeared 48 hours after the addition of fibers (see Fig. S7), ruling out the possibility of contributions from any impurities or fragments in the observed SOFAST-HMQC spectra in our study (Fig.S7).

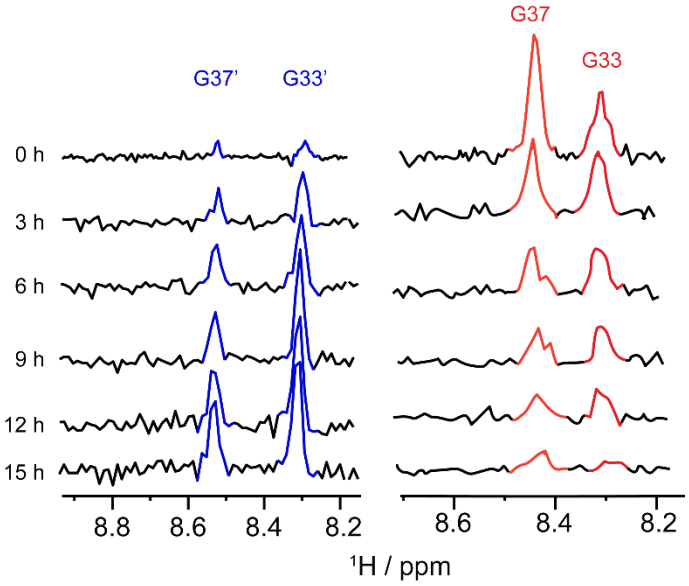

Figure S7. One-dimensional ^{1}H slices taken from the glycine region of ^{1}H-^{15}N SOFAST-HMQC spectra in Figs. 4 and S2. The corresponding decay (red peaks) and build-up (blue peaks) of these peaks strongly suggest that the new peaks (blue) originate from residues G33 and G37.

References:

1. Lopez del Amo, J. M.; Schmidt, M.; Fink, U.; Dasari, M.; Fandrich, M.; Reif, B., An Asymmetric Dimer as the Basic Subunit in Alzheimer's Disease Amyloid beta Fibrils. *Angew Chem Int Edit* **2012,** *51* (25), 6136-6139.
2. Kotler, S. A.; Brender, J. R.; Vivekanandan, S.; Suzuki, Y.; Yamamoto, K.; Monette, M.; Krishnamoorthy, J.; Walsh, P.; Cauble, M.; Holl, M. M.; Marsh, E. N.; Ramamoorthy, A., High-resolution NMR characterization of low abundance oligomers of amyloid-beta without purification. *Sci Rep* **2015,** *5*, 11811.
3. Vivekanandan, S.; Brender, J. R.; Lee, S. Y.; Ramamoorthy, A., A partially folded structure of amyloid-beta(1-40) in an aqueous environment. *Biochem Bioph Res Co* **2011,** *411* (2), 312-316.