Supporting Information

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SI Materials and Methods

Pulse-Labeling HD Exchange Experiment. An important parameter in the design of a pulse-labeling exchange experiment is the pulse intensity, I_p, defined as $I_p = \Delta t_{label} \times k_{ch}$ where Δt_{label} is the pulse length and k_{ch} is the chemical exchange rate. To trap species that form during amyloid fibril formation, two aspects are important. One is that Δt_{label} must be short relative to the timescale of the conformational changes occurring during aggregation. PI3-SH3 aggregation occurs within 21 days whereas the Δt_{label} we use in our experiments is either 30 min or 2 h (see *Materials and Methods* in main article). Another aspect is that k_{ch} must be large for significant isotope exchange of unprotected regions to occur during this interval. Aggregation takes place at pH 1.5 and 2, which are not optimal because they are close to pH 2.7 where exchange is minimum. Despite this, the pH was kept the same as that used during aggregation because changing the pH may significantly affect the conformation of the trapped species (e.g., at neutral pH, PI3-SH3 folds in its native conformation).

Another aspect of the pulse-labeling exchange experiment is the use of either dialysis or dilution since each of them has advantages and inconvenience. Dialysis requires longer times than dilution, which may fail to detect low protected or structurally dynamic species. On the other hand, in the dilution experiment, protein samples get diluted that could promote dissolution of the aggregates. Finally, dilution experiments have larger volumes to lyophilize; if nonvolatile salts are present they get concentrated during lyophilization giving rise to adduct peaks during MS analysis. Despite the differences between the two methods similar results are obtained when dialysis and dilution are used at the same pH.

Preservation of HD Exchange Information. The use of 95% DMSO-d_6 (CIL) /5% D_2O (Euriso-top) as the solvent to solubilize the fibrils is based on the fact that it preserves the deuterium content of the protein molecules because amide hydrogen exchange with solvent is very slow (1, 2). We have previously used this protocol to solubilize PI3-SH3 fibrils into monomers and shown that only the very labile side chain and chain termini hydrogens exchange within the time of the NMR and MS experiments (3).

NMR Spectroscopy and Assignments. Spectra of PI3-SH3 were recorded at 25 °C on an 800-MHz spectrometer (Bruker Avance 800) equipped with a triple-axis gradient triple-resonance cryoprobe ($^1H/^{13}C/^{15}N$). The sample conditions were 0.1 mM protein in 95% DMSO-d_6 (Euriso-top), 5% H_2O at pH* 4.6. The pH was adjusted with dichloroacetic acid-d_3 (Aldrich) as described in refs. 1 and 2. The field-frequency lock was referenced to the signal from DMSO-d_6. Triple resonance experiments, CBCANH, CBCA(CO)NH, HNCO were recorded for assignment of the PI3-SH3 resonances in DMSO. The assignments were confirmed by analyzing the 3D ^{15}N TOCSY-HSQC (τ_m = 65 ms) and 3D ^{15}N NOESY-HSQC (τ_m = 200 ms). ^1H chemical shifts were referenced with respect to the 2.5 ppm methyl signal of DMSO-d_6. ^{15}N and ^{13}C chemical shifts were indirectly referenced by using the frequency ratio ω_N/ω_H = 0.10132905 and ω_C/ω_H = 0.25144952, respectively. Data were processed and analyzed by using the programs NMRPipe (4) and NMRView (5).

HD Exchange Analyzed by NMR. Pulse-labeled samples obtained at different Δt_{agg} periods were dissolved in 300 μL of 95% DMSO-d_6, 5% D_2O at pH* 4.6 (2). After dissolution and mixing for 1.5 min, the sample solution was immediately transferred into an NMR tube (Shigemi) and ^1H-^{15}N HSQC spectra recorded at 25 °C. For all of the samples analyzed, the same times for parameter adjustment (5.5 min) and spectrum acquisition (11 min) were used.

Although 95% DMSO-d_6/5% D_2O at pH* 4.6 was used as the solvent to solubilize the samples because it preserves the deuterium content acquired during the exchange reaction, some exchange of amide protons does occur. To eliminate the effect of this behavior on the experimental measurement of peak volumes, we report proton occupancies. These are obtained by measuring the relative peak volumes, $V_{\Delta tagg = X}/V_{\Delta tagg = 0}$, where $V_{\Delta tagg = X}$ and $V_{\Delta tagg = 0}$ correspond, respectively, to the peak volumes in the ^1H-^{15}N HSQC spectra of the samples incubated for Δt_{agg} equal to X days and for Δt_{agg} equal to 0 days. For the samples obtained at pH 1.5, PF conditions, since amide protection is observed for Δt_{agg} = 0 days, the peak volumes in the ^1H-^{15}N HSQC spectra are normalized by using a sample prepared as that of Δt_{agg} equal to 0 days but initially dissolved in H_2O instead of D_2O. The ratio $V_{\Delta tagg = X}/V_{\Delta tagg = 0}$ after a given time in the solubilizing solvent can be expressed as:

$$V_{\Delta tagg=X}(t)/V_{\Delta tagg=0}(t) = (V_{\Delta tagg=X}(0)\ \exp(-k_{ex\text{-}s}t)/(V_{\Delta tagg=0}(0)\ \exp(-k_{ex\text{-}s}t))$$

where $k_{ex\text{-}s}$ is the exchange rate in the solubilizing solvent and $V_{\Delta tagg = X}(0)$ and $V_{\Delta tagg = 0}(0)$ are the volumes at zero time when no exchange with the solubilizing solvent has occurred. Because the same mixing times and dead times for parameter adjustment and spectrum acquisition were used, $V_{\Delta tagg = X}(t)/V_{\Delta tagg = 0}(t)$ is equal to $V_{\Delta tagg = X}(0)/V_{\Delta tagg = 0}(0)$, where exchange with the DMSO-based buffer has not taken place. The relative total protein concentration of the solution was determined from integration of the nonexchangeable hydrogens in the methyl region of a 1D proton NMR spectrum recorded directly before each individual ^1H-^{15}N HSQC spectrum.

HD Exchange Analyzed by MS. Mass spectrometry was carried out on an LCT mass spectrometer (Waters) fitted with a modified in-house nano-ESI closed source. Samples were electrosprayed from gold-coated glass capillaries prepared in-house as described un ref. 6, with an applied capillary voltage of 1,600 kV and a cone voltage of 200 V. Pulse-labeled samples exposed to HD exchange and processed as described above were dissolved in 20 μL of 95% DMSO-d_6/5% D_2O at pH* 4.2 (0.4 mM) under an IR lamp. After dissolution, the sample solution was immediately introduced into the mass spectrometer. The same dead time for parameter adjustment (5 min) was used for all measurements. Mass spectra of the samples in the DMSO-d_6/D_2O buffer were also recorded after ≈2 h. Over time, the peak corresponding to S_{mon}-U_{agg} species in AM samples fully converted into the one corresponding to the F_{agg} species, and the peaks corresponding to D_{agg} and O_{agg} species in PF samples fully converted into those corresponding to the F_{agg} species (Fig. S7). The spectra were analyzed by using MassLynx v 4.1 (Micromass). The intensity of each peak detected for PI3-SH3 aggregation under PF conditions was measured by simulating the ESI-MS spectra at each Δt_{agg} by using the program SigmaPlot.

The theoretical number of exchangeable hydrogens in the PI3-SH3 monomer was calculated by taking into consideration all labile hydrogens in the side chains and in the main chain.

These hydrogens are those that are covalently bound to nitrogen, oxygen, or sulfur atoms but not to carbon atoms. Consequently, for the PI3-SH3 monomer, there are 85 labile hydrogens from the main-chain amide groups (allowing for the 3 proline residues and the additional hydrogens at the chain termini). Addition of the 72 labile hydrogens from the side chains gives the total number of exchangeable hydrogens as 157. The number of amides protected for each of the detected peaks is calculated by subtracting the number of labile side-chain and termini hydrogens, from the observed average mass increase of the protein.

1. Zhang YZ, Paterson Y, Roder H (1995) Rapid amide proton exchange rates in peptides and proteins measured by solvent quenching and two-dimensional NMR. *Protein Sci* 4:804–814.
2. Hoshino M, et al. (2002) Mapping the core of the beta(2)-microglobulin amyloid fibril by H/D exchange. *Nat Struct Biol* 9:332–336.
3. Carulla N, et al. (2005) Molecular recycling within amyloid fibrils. *Nature* 436:554–558.
4. Delaglio F, et al. (1995) NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* 6:277–293.
5. Johnson BA (2004) Using NMRView to visualize and analyze the NMR spectra of macromolecules. *Methods Mol Biol* 278:313–352.
6. Nettleton EJ, et al. (1998) Protein subunit interactions and structural integrity of amyloidogenic transthyretins: evidence from electrospray mass spectrometry. *J Mol Biol* 281:553–564.

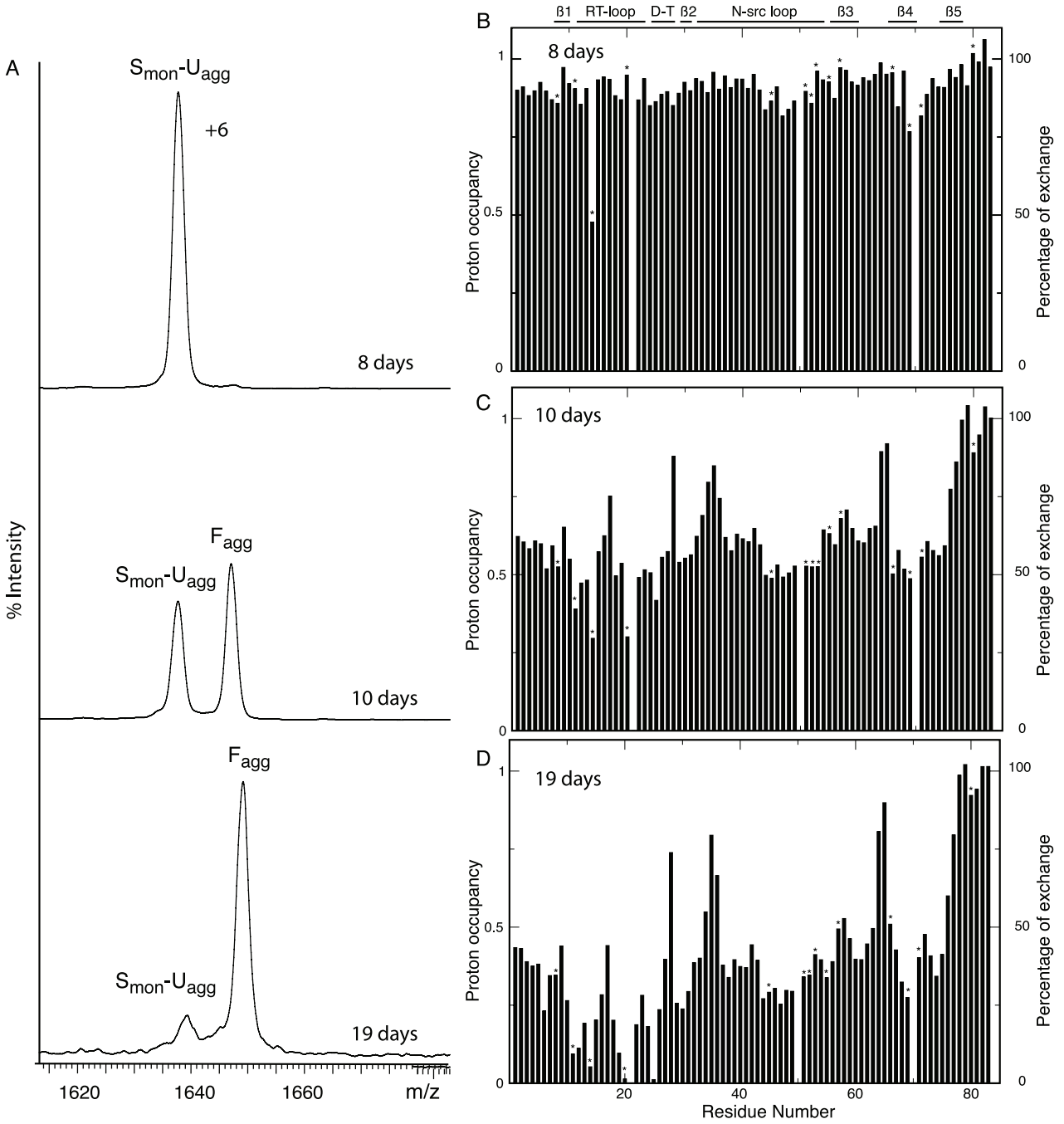

Fig. S1. ESI-MS and NMR analysis of PI3-SH3 pulse-labeled samples prepared under AM conditions. (*A*) ESI mass spectra (+6 charge state) showing the relative populations of S_{mon}-U_{agg} and F_{agg} species at the indicated Δt_{agg} times. Peak intensities are normalized to he overall species population. NMR analysis of PI3-SH3 pulse-labeled samples obtained at Δt_{agg} of 8 (*B*), 10 (*C*), and19 days (*D*). The bars represent the proton occupancy and percentage of exchange for each residue. Bars are calculated as the relative peak volumes, $V_{\Delta tagg = X}/V_{\Delta tagg = 0}$, where $V_{\Delta tagg = X}$ and $V_{\Delta tagg = 0}$ correspond, respectively, to the peak volumes in the ^{1}H-^{15}N HSQC spectra of the samples incubated for Δt_{agg} equal to *X* days and for Δt_{agg} equal to 0 days. The locations of the β-sheet strands, turns, and loops in the native state of PI3-SH3 are indicated above the graph. An asterisk above a bar indicates a residue whose resonance is not fully resolved; the absence of a bar indicates that the resonance of the residue is not detectable except for residues 50, 70, and 84 that are Pro.

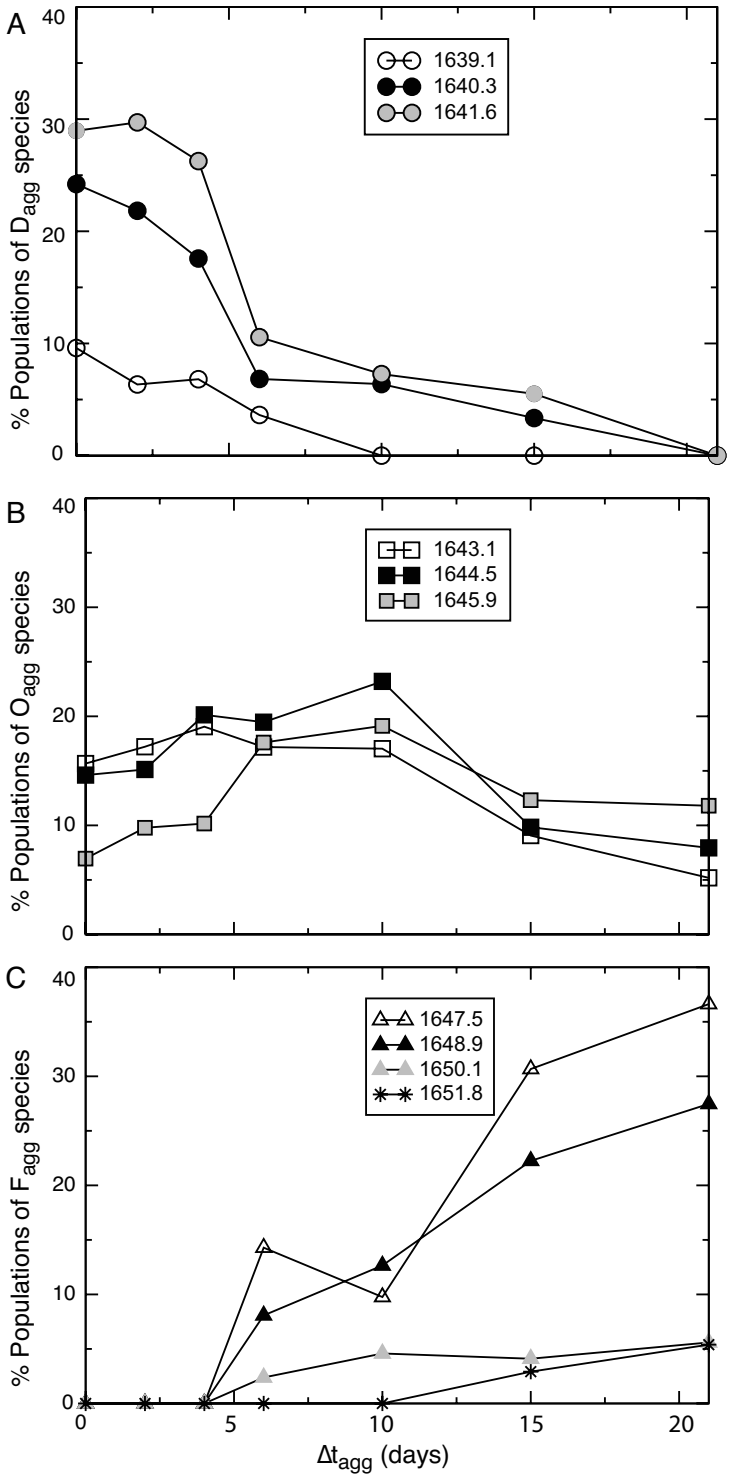

Fig. S2. Population percentage of each detected peak in the mass spectra as a function of Δt_{agg} for pulse-labeled samples prepared under PF conditions D_{agg} (*A*), O_{agg} (*B*), and F_{agg} (*C*) species.

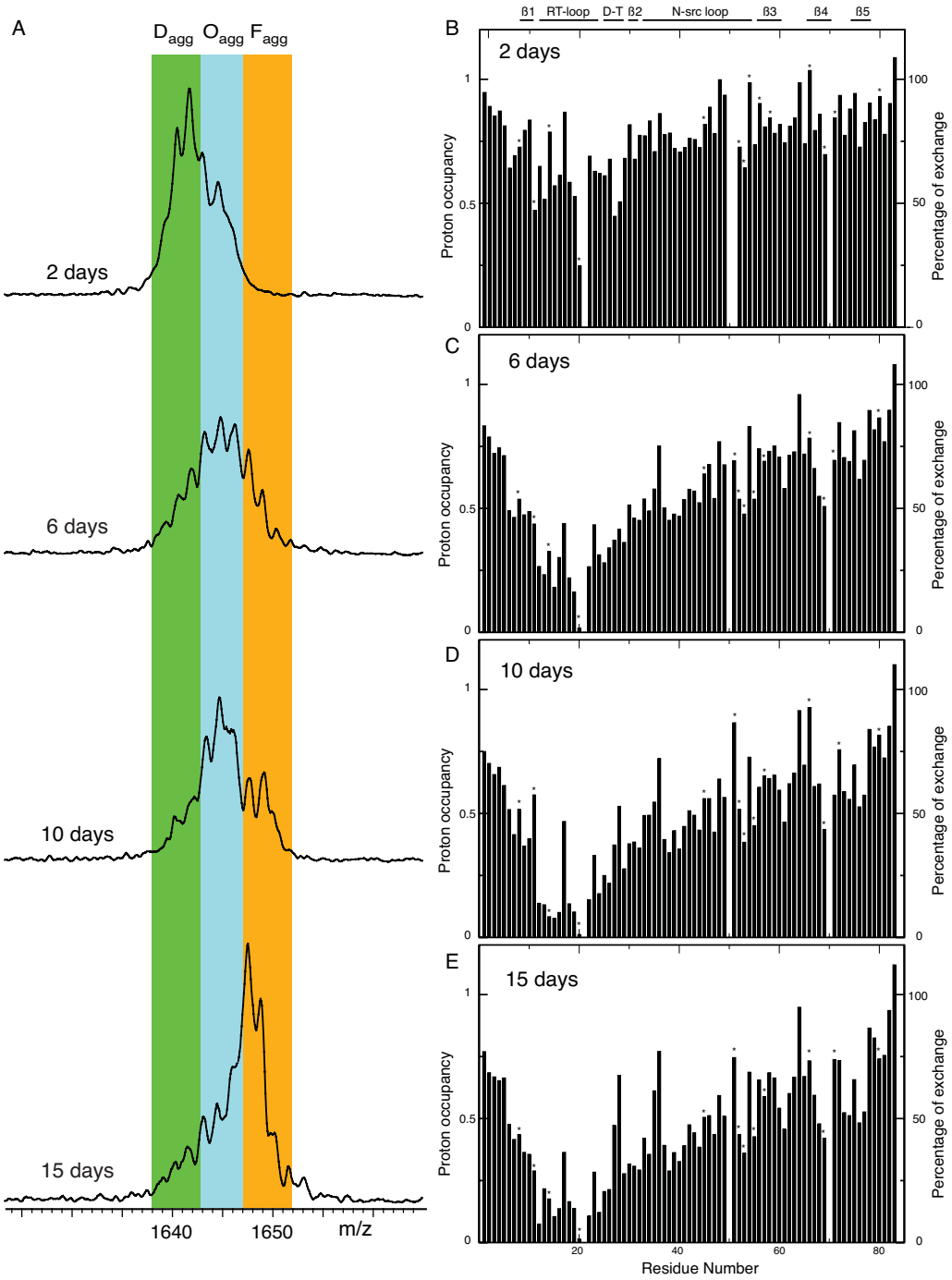

Fig. S3. ESI-MS and NMR analysis of PI3-SH3 pulse-labeled samples obtained at different Δt_{agg} times while being incubated under PF conditions. (*A*) ESI mass spectra (+6 charge state) showing the relative populations of D_{agg} (green band), O_{agg} (blue band), and F_{agg} (orange band) species at the indicated Δt_{agg} times. NMR analysis of samples obtained after 2 (*B*), 6 (*C*), 10 (*D*), and 15 (*E*) days of aggregation. The bars represent the proton occupancy and percentage of exchange for each residue. Bars are calculated as the relative peak volumes $V_{\Delta tagg = X}/V_{\Delta tagg = 0}$, where $V_{\Delta tagg = X}$ correspond to the peak volumes in the ^{1}H-^{15}N HSQC spectra of the samples incubated for Δt_{agg} equal to *X* days and $V_{\Delta tagg = 0}$ correspond to a sample prepared as that of Δt_{agg} equal to 0 days but initially dissolved in H_2O instead of D_2O. The locations of the β-sheet strands, turns, and loops in the native state of PI3-SH3 are indicated above the graph. An asterisk above a bar indicates a residue whose resonance is not fully resolved; the absence of a bar indicates that the resonance of the residue is not detectable except for residues 50, 70, and 84 that are Pro.

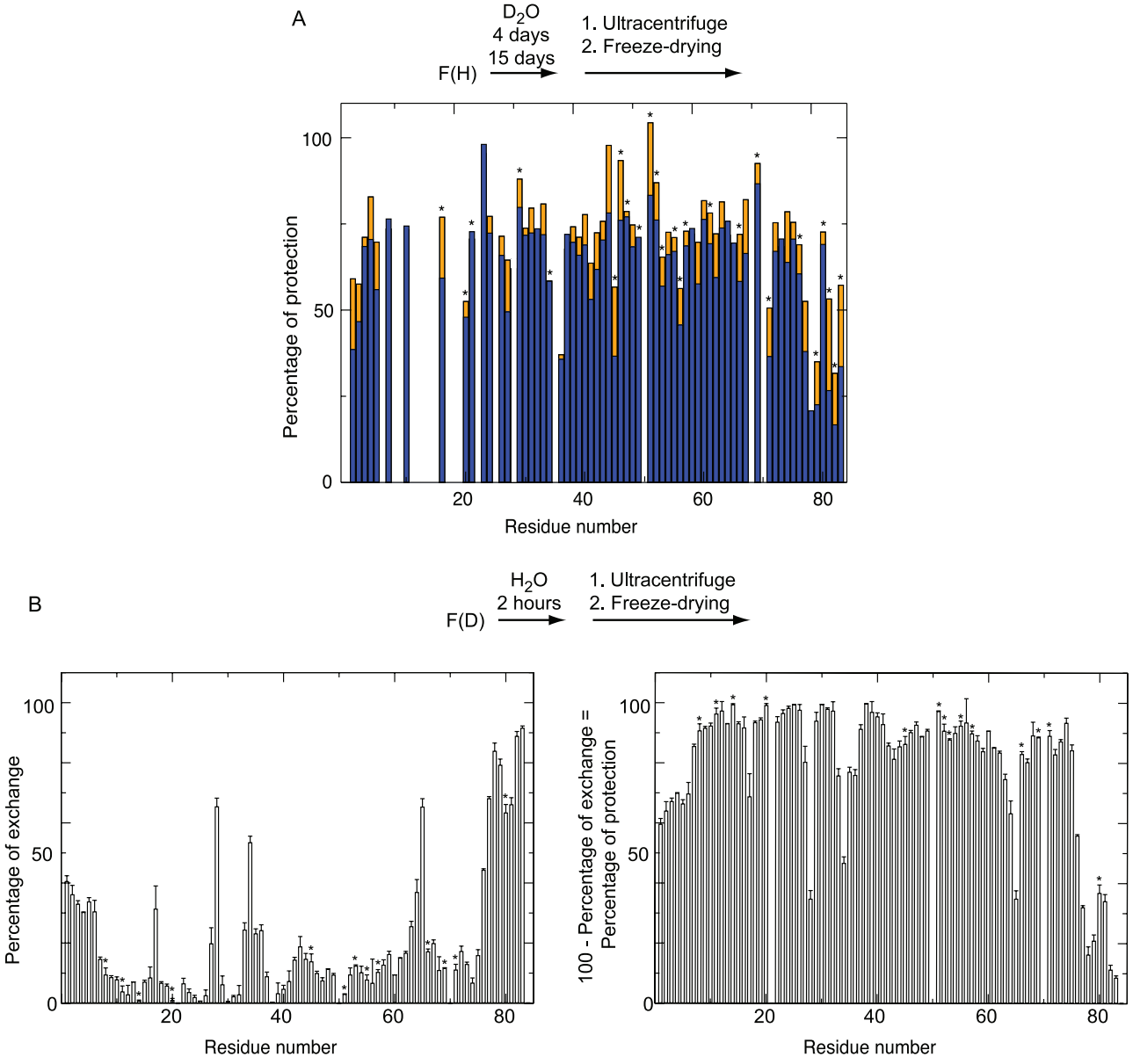

Fig. S4. HD exchange experiments on PI3-SH3 amyloid fibrils. (*A*) Use of long exchange times (4 days and 15 days). Exchange dominated by the recycling mechanism. (*B*) Use of short exchange times (2 h). Exchange is no longer dominated by the recycling mechanism and an exchange pattern for the PI3-SH3 molecules incorporated within the fibril can be obtained. The exchange pattern for PI3-SH3 molecules indicated here is somewhat lower than the one shown in Fig. 5*B* because of the protocol used. Ultracentrifugation followed by freeze drying in Fig. S4 versus only freeze drying in Fig. 5*B*.

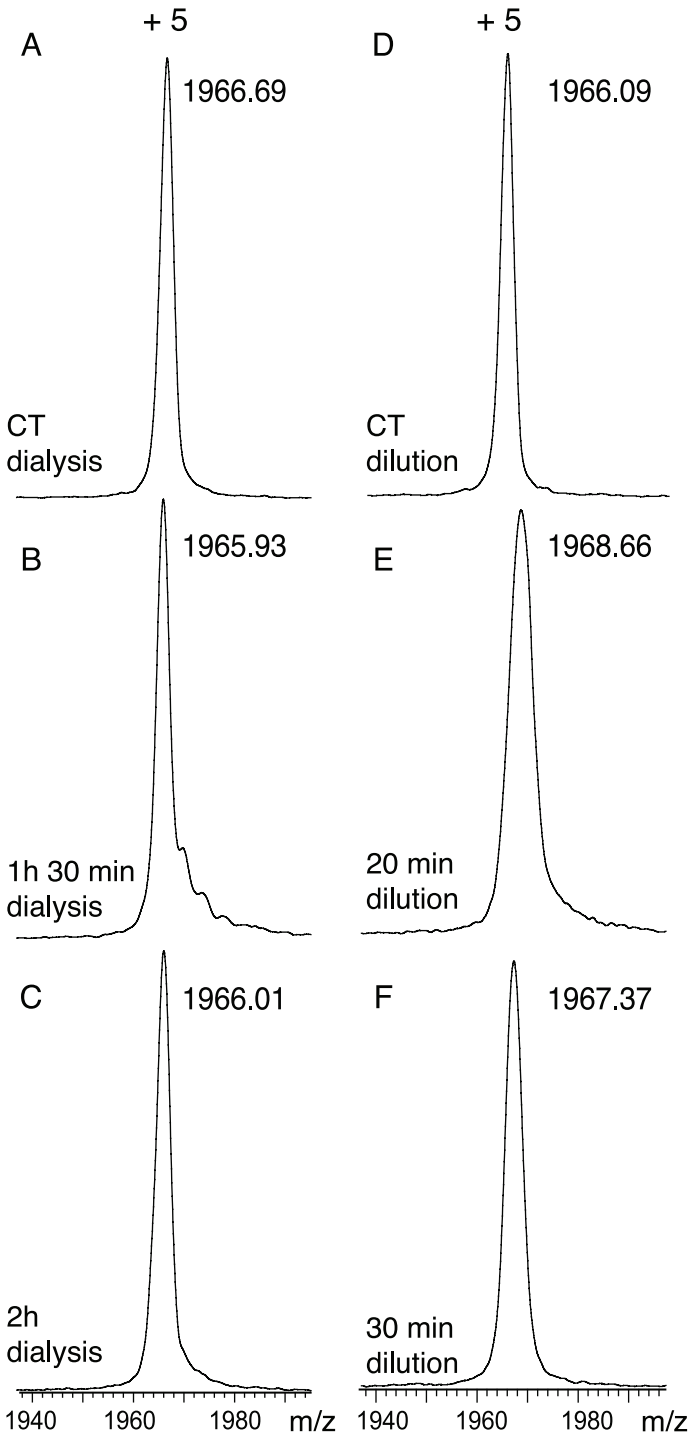

Fig. S5. Control experiments to determine the minimum time required for unprotected deuterons to exchange completely with protons during the labeling pulse achieved by dialysis or dilution. Lyophilized PI3-SH3 is dissolved in D_2O at pH* 1.6 and after an hour, an aliquot is taken and it is lyophilized and resuspended in the same volume by using H_2O at pH 2.0 for 1 h (*A*); it is dialyzed against H_2O at pH 2.0 for 1 h and 30 min (*B*); it is dialyzed against H_2O at pH 2.0 for 2 h (*C*); it is lyophilized, diluted 1/15 by using H_2O at pH 2.0 during 1 h (*D*); it is diluted 1/15 by using H_2O at pH 2.0 during 20 min (*E*); it is diluted 1/15 by using H_2O at pH 2.0 during 30 min (*F*). After this, all samples are lyophilized, resuspended in 95%DMSO-d_6/5% D_2O at pH*4.2 and analyzed by ESI-MS. The + 5 charge state is shown for all of the spectra shown in the figure.

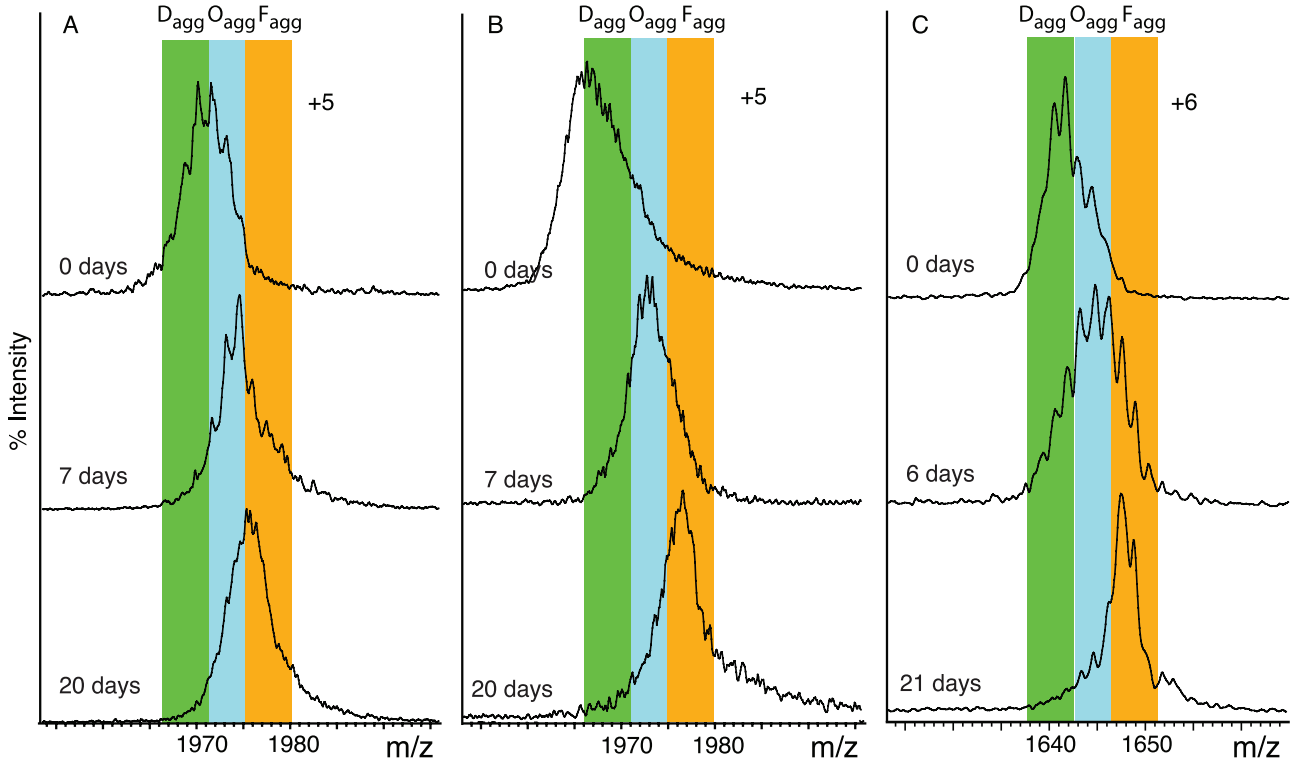

Fig. S6. Pulse-labeling experiments carried out during PI3-SH3 aggregation under PF conditions. ESI-MS analysis of pulse-labeled samples obtained by means of dialysis and using HCl to adjust the pH of the dialysis buffer (*A*), dilution and using HCl to adjust the pH of the dilution buffer (*B*), and dilution and using formic acid to adjust the pH of the dilution buffer at the indicated Δt_{agg} times (*C*). The most intense charge state is shown for each condition, +5, for *A* and *B*, and +6 for *C*.

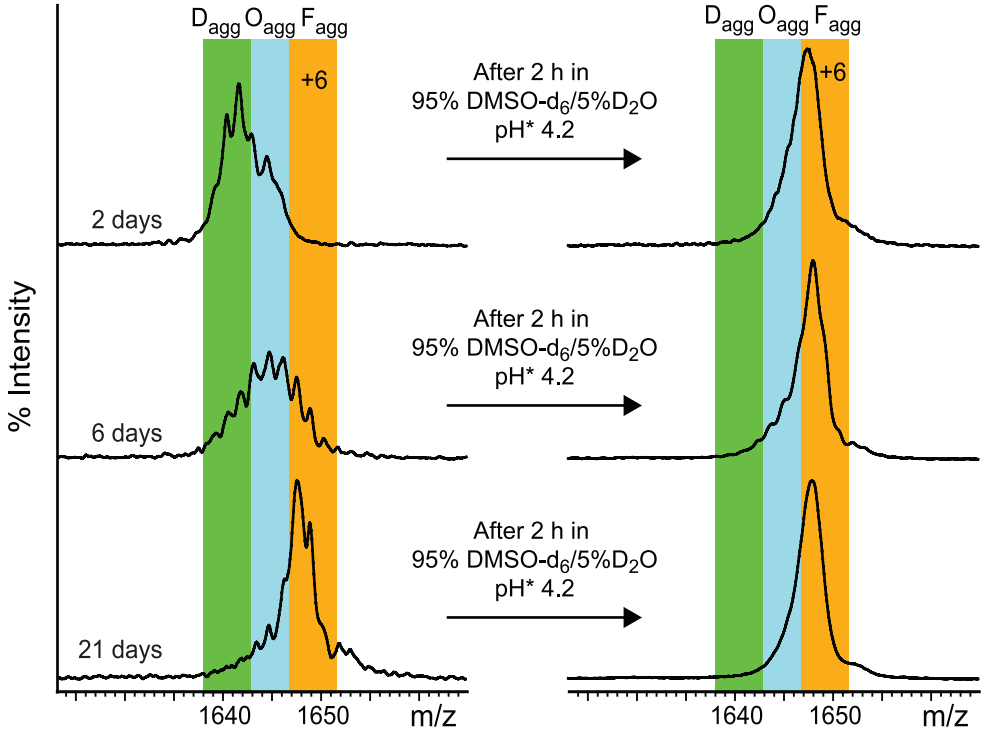

Fig. S7. ESI-MS mass spectra (+6 charge state) showing the relative populations of D_{agg} (green band), O_{agg} (blue band), and F_{agg} (orange band) at Δt_{agg} times of 2, 6, and 21 days and after being dissolved in the DMSO-d_6/D_2O buffer for 5 min (*Left*) and for 2 h (*Right*).