

# Supporting Information

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## SI Materials and Methods

**Chemicals.** All chemicals and reagents were purchased from Sigma-Aldrich unless stated otherwise. All gels, membranes, and reagents for Western blotting were purchased from Invitrogen. D<sub>2</sub>O buffers were prepared by dissolving PBS in D<sub>2</sub>O (Cambridge Isotope Laboratories Inc.).

**Details: Protein Preparation.** Both purchased A $\beta_{40}$  and A $\beta_{42}$  were treated to remove any aggregated species before storage by dissolving the material in hexafluoroisopropyl alcohol (HFIP) at 1 mM. The protein solution was aliquoted in low-binding tubes (Eppendorf), and the solvent was evaporated overnight. The samples were vacuum-centrifuged (Eppendorf) to remove any existing HFIP, leaving an amyloid beta (A $\beta$ ) film in the tubes. This A $\beta$  film was then stored at  $-80^{\circ}\text{C}$  for future use.

**Details: A $\beta$  Aggregates and Preformed Fibril Preparation.** A $\beta$  aggregation experiments (at  $25^{\circ}\text{C}$ ) were investigated at nine time points for A $\beta_{40}$  and 20 time points for A $\beta_{42}$ . Soluble aggregates were enriched by centrifugation ( $16,000 \times g$ , 5 min at  $4^{\circ}\text{C}$ ). The centrifuged sample was then carefully divided into two equal-volume parts (i.e., 10  $\mu\text{L}$  each), referred to in all contexts as “top half” and “bottom half.” Only the bottom-half solution was submitted to MS analysis to obtain the plots shown in the various figures. For some samples, the bottom half was also submitted to native gel and Western blotting.

To study the effect of various parameters on A $\beta_{42}$  aggregation, a procedure similar to that described above was followed except for those involving Cu<sup>2+</sup>. CuCl<sub>2</sub> was dissolved in a PBS buffer to give a Cu<sup>2+</sup> concentration equal to the protein concentration. The stages of aggregation were interrogated at 13 time points by varying the incubation time from 1 min to 48 h. Before MS analysis, the sample was submitted to centrifugation under the same conditions as described above, and only the bottom-half solution was collected.

For a comparison, the process of aggregation was also monitored by varying the temperature (i.e.,  $37^{\circ}\text{C}$  vs.  $25^{\circ}\text{C}$ ) and agitation (i.e., incubator rotating horizontally at 150 rpm). Experiments were investigated for an average of 15 time points, in the absence or presence of Cu<sup>2+</sup>.

A $\beta_{42}$  fibrils were formed following a similar procedure, but incubating at  $37^{\circ}\text{C}$  for 3 wk. After a white precipitate formed, the solution was centrifuged, and the pellet was redissolved in PBS buffer and analyzed by pulsed hydrogen–deuterium exchange (HDX).

**Transmission EM.** The A $\beta_{42}$  fibrils used for the transmission EM (TEM) study were formed by diluting monomeric A $\beta_{42}$  1:19 (vol/vol) and incubating at  $37^{\circ}\text{C}$  for 24 h. The sample was centrifuged at  $16,000 \times g$  for 5 min at  $4^{\circ}\text{C}$ . The sample from the bottom-half solution was submitted to TEM analysis, as previously described (1). To examine the effect of quenching solution on fibrils, both 3 M urea and 3 M urea with 1% TFA were mixed with the protein solution in a 1:1 volume ratio.

**Pulsed HDX.** HDX experiments were carried out by mixing the protein and D<sub>2</sub>O buffer. We chose a 1:1 (vol/vol) dilution over, for example, 1:10, to minimize dilution and consequent shifts in the equilibrium of aggregation/disaggregation. The quenched exchanged sample was then digested by passing through a custom-packed pepsin column at 200  $\mu\text{L}/\text{min}$ , and peptic peptides were captured on a C<sub>8</sub> trap column (2 mm  $\times$  1 cm; Agilent Inc.) and

desalted (total time for digestion and desalting was 3 min). Peptides were then separated with a linear gradient of 4–40% (vol/vol) CH<sub>3</sub>CN and 0.1% formic acid over 5 min. Both protein digestion and peptide separation were performed at  $0^{\circ}\text{C}$  to minimize back-exchange. The eluted peptides were analyzed by a MaXis quadrupole time-of-flight (Bruker Daltonics Inc.) in the positive-ion electrospray ionization mode. All analyses were done in triplicate.

**A $\beta_{40}$  Back-Exchange.** We evaluated the extent of back-exchange by incubating A $\beta_{40}$  (same concentration as described above, starting by diluting from stock solution in d<sub>6</sub>-DMSO) in D<sub>2</sub>O buffer, with 4.5 M GdnCl (GdnHCl already exchanged with D<sub>2</sub>O) to fully unfold the protein. The solution was allowed to stand for 2 d at  $25^{\circ}\text{C}$  without agitation, based on a published procedure (2). After incubation, the sample was treated exactly the same as described above.

**Peptide Identification, Data Processing, and Modeling.** Peptic peptides identification was performed as described previously (3). The centroid mass of the peptides was converted by MagTran v1.03. The percent protection was calculated by using Eq. S1:

$$\% \text{protection} = 100\% - \%D = \left(1 - \frac{m_{\text{HDX}} - m_{\text{control}}}{(N - 2) \times 0.5}\right) \times 100\%, \quad [\text{S1}]$$

where  $m_{\text{HDX}}$  is the centroid mass of the deuterated peptides,  $m_{\text{control}}$  was the centroid mass of nondeuterated peptides,  $(N - 2)$  is the number of exchangeable amide hydrogens, and 0.5 is the final D<sub>2</sub>O content of the buffer system.

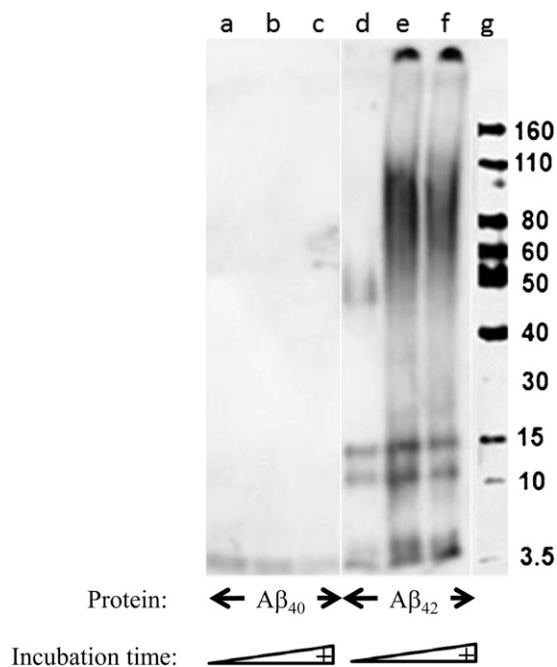
The modeling was carried out by Mathcad 14.0 M020 (Parametric Technology Corp.). The least-squares fit was implemented with the “Minimize” function in the nonlinear quasi-Newton mode. In each trial of the minimization process, the determined rate constants specified an ordinary differential equation, which was solved with the adaptive step-size fourth-order Runge–Kutta Rkadapt function in Mathcad. Each reaction species accounted for a time-varying fraction of the total original monomer molecules in solution. Each fraction was weighted by a postulated percentage of protection for that species. The postulated rate constants and protection weights were changed for each trial of the search to minimize the difference between the data curve and the sum of weighted species fractions. The modified Finke–Watzky (F–W) modeling was applied to all experiments except experiments done at  $37^{\circ}\text{C}$ , with agitation at 150 rpm and in the absence of Cu<sup>2+</sup>, which shows no lag phase and does not need a modified F–W modeling.

In the bootstrap strategy, we constructed trial datasets by randomly resampling with replacement at each time point. The number of times for resampling equals the number of replicates at that time point. The model was refitted to each of the 104 trial datasets. Analysis of the accumulated results gave a mean and SD.

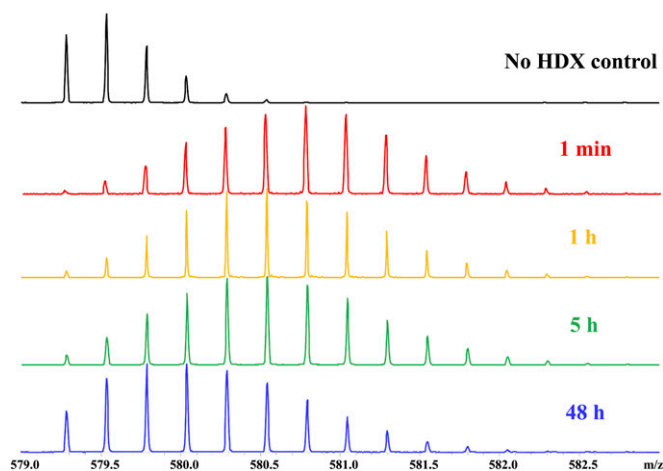
## SI Results and Discussion

**A $\beta_{40}$  Back-Exchange.** The considerable protection seen for A $\beta_{40}$  controls is due to the back-exchange and to the short time for the HDX pulse, during which full exchange did not occur. To evaluate the back-exchange, after quenching the HDX, the solution was subjected to online-pepsin digestion, column trapping, and eluting. During these processes, all of the solvents are H<sub>2</sub>O-based, which means the protein undergoes back-exchange. We found only  $\sim 60\%$  of deuterium uptake (an average based on the peptide level). This result shows that there is  $\sim 40\%$  back-exchange for this highly disordered protein on our HDX platform.

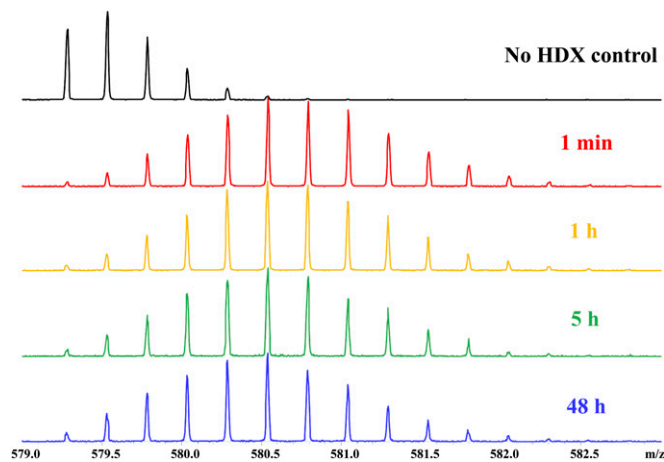
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- Zhang Z, Zhang A, Xiao G (2012) Improved protein hydrogen/deuterium exchange mass spectrometry platform with fully automated data processing. *Anal Chem* 84(11): 4942–4949.
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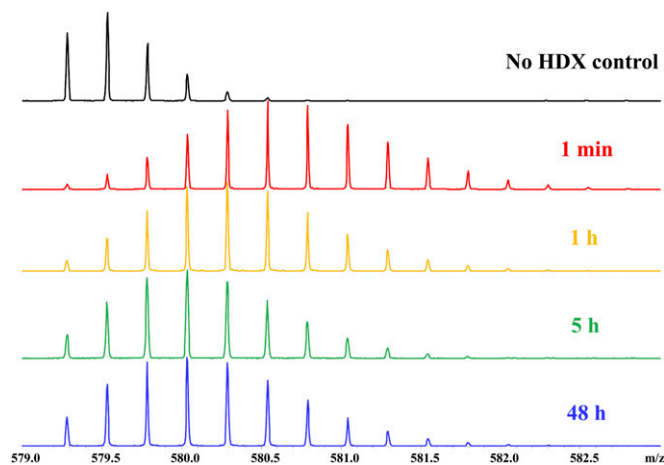
**Fig. S1.** Western blotting for both  $A\beta_{40}$  (a–c) and  $A\beta_{42}$  (d–f), and MW marker (g). The sample film was dissolved in DMSO to maintain monomeric form (1 mM). The sample was diluted by 20-fold in PBS buffer to initiate aggregation. Aggregation was at 25 °C, no agitation, and in the absence of  $Cu^{2+}$ . The sample was then centrifuged at  $16,000 \times g$  for 5 min at 4 °C. Aggregation time points were 1 min (a and d), 24 h (b and e), and 48 h (c and f), respectively.



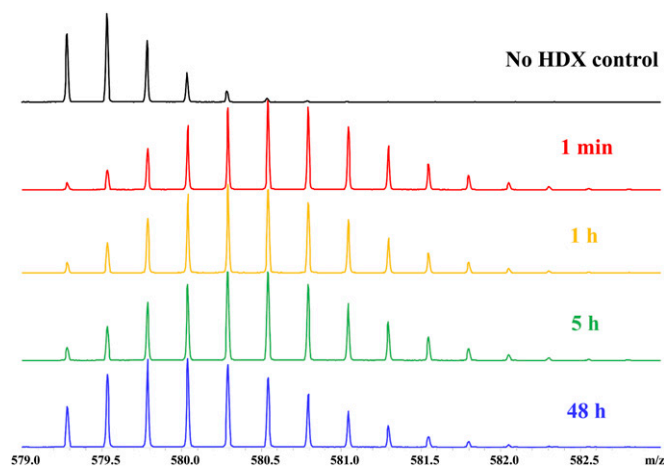
**Fig. S2.** Raw data for HDX experiments, shown only for the peptide 1–19 (+4 charge). Experimental conditions: 25 °C, no agitation, in the absence of  $Cu^{2+}$ . The black trace is the spectrum showing isotopic distribution with no deuterium uptake, and the rest of the spectra show isotopic distribution with deuterium uptake at different incubation times.



**Fig. 53.** Raw data for HDX experiments, shown only for the peptide 1–19 (+4 charge). Experimental conditions: 25 °C, no agitation, in the presence of  $\text{Cu}^{2+}$ . The black trace is the spectrum showing isotopic distribution with no deuterium uptake, and the rest of the spectra show isotopic distribution with deuterium uptake at different incubation times.

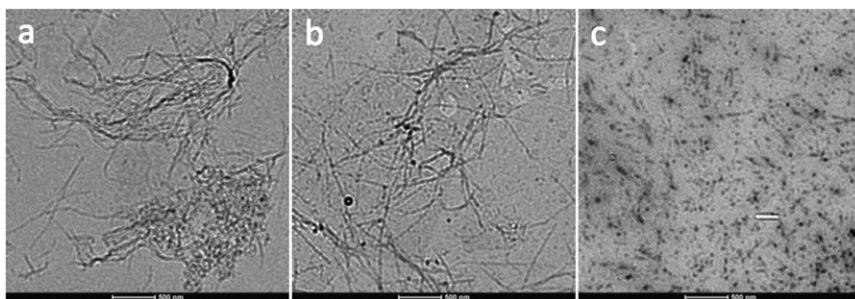


**Fig. 54.** Raw data for HDX experiments, shown only for the peptide 1–19 (+4 charge). Experimental conditions: 37 °C, 150 rpm agitation, in the absence of  $\text{Cu}^{2+}$ . The black trace is the spectrum showing isotopic distribution with no deuterium uptake, and the rest of the spectra show isotopic distribution with deuterium uptake at different incubation times.



**Fig. 55.** Raw data for HDX experiments, shown only for the peptide 1–19 (+4 charge). Experimental conditions: 37 °C, 150 rpm agitation, in the presence of  $\text{Cu}^{2+}$ . The black trace is the spectrum showing isotopic distribution with no deuterium uptake, and the rest of the spectra show isotopic distribution with deuterium uptake at different incubation times.





**Fig. S9.** Transmission EM images for effect of quenching solution on A $\beta_{42}$  fibrils. (A) A $\beta_{42}$  fibrils, (B) A $\beta_{42}$  fibrils with 3 M urea, and (C) A $\beta_{42}$  fibrils with 3 M urea and 1% TFA. (Scale bars, 500 nm.)