Supporting Information

Characterizing the dynamics of alpha-synuclein oligomers using hydrogen/deuterium exchange monitored by mass spectrometry

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Figure S1: Comparison of deuterium uptake in Day 0 and Day 2 oligomers after 30s exchange-in.

Figure S2: Analysis αSN oligomer dissociation using gel filtration chromatography.

Figure S3: SDS-PAGE analysis of in-solution digestion of αSN monomers and oligomers.

Figure S4-S6: Representative spectra illustrating deuterium uptake in the 5-17, 18-38 and 125-140 peptides from monomer and oligomer preparations.

Figure S7: Deuterium uptake plots for each individual peptide identified in the monomeric and oligomeric αSN preparations.

Figure S8: An expanded heat map of the local exchange patterns observed in peptides derived from deuterium labeled αSN oligomers.
Figure S1. Mass spectra of peptides derived from two preparations of deuterated oligomeric αSN, one labeled 3 hours (Day 0) and one 2 days (Day 2) after their initial enrichment. Samples were subjected to exchange-in for 30 seconds. Qualitatively, the deuteration patterns observed in the two labelings appeared to be identical. The fully deuterated isotopic distribution, corresponding to monomeric αSN, is slightly more abundant in the Day 0 labeling, possibly due to batch-to-batch variability. The slightly lower deuterium content observed in the Day 2 spectra are caused by increase in back-exchange due to instrumental changes between analyses, not changes in protein dynamics.
Figure S2. Analysis αSN oligomer dissociation using gel filtration chromatography.
Preparations of αSN monomers and oligomers were subjected to gel filtration chromatography, with and without having been treated with 35% formic acid for 2 minutes. Untreated samples had elution patterns similar to those previously observed (see Figure 1), with the oligomer preparation giving rise to a peak at 21 minutes, and the monomer preparation giving rise to a peak at 37 minutes. Following treatment, the contents of the monomer and oligomer preparations eluted after 39 minutes, an elution time similar to that of the untreated monomer preparation.
Figure S3. SDS-PAGE analysis of in-solution digestion of αSN monomers and oligomers. Preparations of αSN monomers and oligomers were analyzed in PBS (Lane 1+2), under our quench conditions, 0.5% formic acid PBS (Lane 3+4) and after being subjected to in-solution digestion on ice for 2 minutes (Lane 5+6). A reference lane containing pepsin was also included (Lane 7). The αSN bands from the monomer and oligomer preparations prepared under quench conditions showed very clear signs of degradation, with a second lower-mass band appearing. Following 2 minutes of in-solution pepsin digestion, all bands belonging to the monomer had completely disappeared. A single low-mass band could however be observed in lane 6, indicating that a small subset of αSN from the oligomer preparation had not been fully digested. Dashed lines indicate places where the image was cropped.
Figure S4. Representative spectra illustrating deuterium uptake in the 5-17 peptide from monomer and oligomer preparations. Blue ion sticks were used to quantify deuterium uptake in the peptide. In the monomer preparation the peptide was observed to exchange completely after 0.5 seconds. In the oligomer preparation two isotopic distributions could be observed, one matching the fully exchanged conformer and the other corresponding to a conformer which was very highly protected in this region. As these didn’t overlap to a significant degree, the deuterium content of the protected conformer was quantified according to the average mass of its isotopic distribution.
**Figure S5.** Representative spectra illustrating deuterium uptake in the 18-38 peptide from monomer and oligomer preparations. The blue fitted gaussian distributions and blue ion sticks were used to quantify deuterium uptake in the peptide. In the monomer preparation the peptide was observed to exchange completely after 0.5 seconds. In the oligomer preparation two isotopic distributions could be observed, one matching the fully exchanged conformer and the other corresponding to a conformer which was very highly protected in this region. As these overlapped, two gaussian peaks were fitted to the isotopic distributions and the deuterium content of the protected conformer was quantified based on the lower-mass distribution.
Figure S6. Representative spectra illustrating deuterium uptake in the 125-140 peptide from monomer and oligomer preparations. Blue ion sticks were used to quantify deuterium uptake in the peptide. In the monomer preparation the peptide was observed to exchange completely after 0.5 seconds. In the oligomer preparation the peptide also only exhibited a single isotopic distribution, corresponding to the fully exchanged peptide observed in the monomer preparation.
Figure S7. Deuterium uptake plots for each individual peptide identified in the monomeric (blue) and oligomeric (red) αSN preparations. Samples were exposed to HDX for 0.5, 2, 8, 32, 128 and 256 minutes. The numbers in the top right corner of each plot denotes the identity of the peptide. The oligomer samples were analyzed in triplicate, and standard deviations for deuterium uptake in the oligomer preparation are given at each time point. Deuterium uptake in the 70-89 peptide after 256 minutes of exchange is omitted, as the signal was very weak in these samples.
**Figure S8.** Expanded heat map of the local exchange patterns observed in peptides derived from deuterium labeled αSN oligomers. Peptides are color coded according to fractional deuterium uptake, relative to peptides derived from the fully exchanged α-syn monomers. The coloring of each peptide, from top to bottom, represents deuterium uptake after 0.5, 2, 8, 32, 128 and 256 minutes of exchange-in.