O-GlcNAcylation of α-synuclein at serine 87 reduces aggregation without affecting membrane binding

Yuka E. Lewis,[a] Ana Galesic,[a] Paul M. Levine,[a] Cesar A. De Leon,[a] Natalie Lamiri,[a] Caroline K. Brennan,[a] and Matthew R. Pratt[a,b,*]

[a]Department of Chemistry, [b]Department of Molecular and Computational Biology
University of Southern California, Los Angeles, CA 90089
*Correspondence should be addressed to matthew.pratt@usc.edu

Supplementary Information

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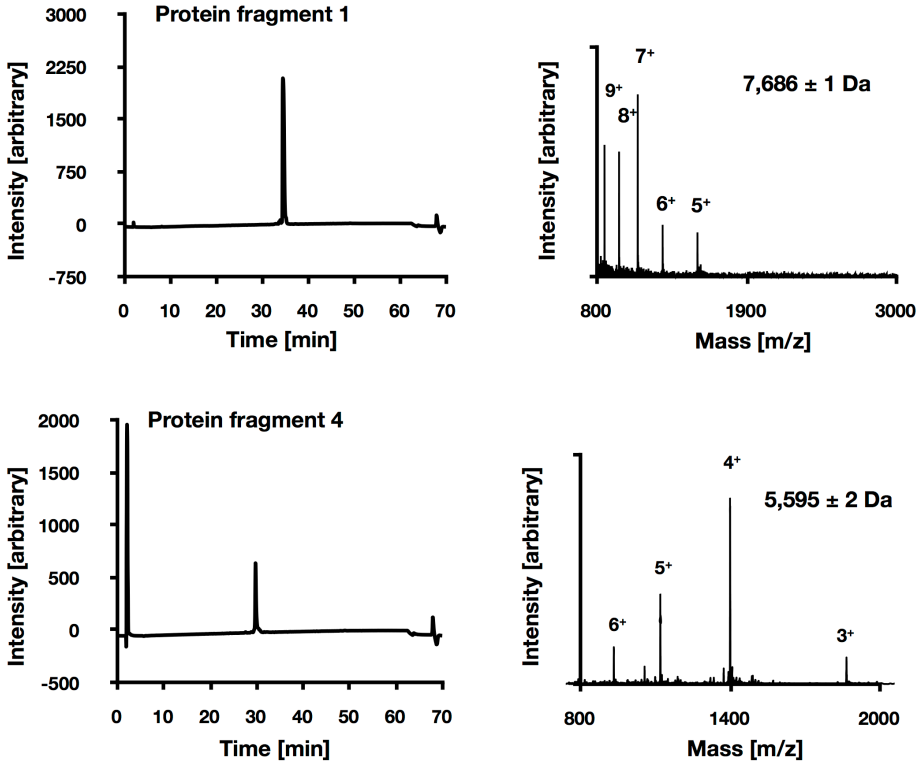

Figure S1. Characterization of α-synuclein protein fragments. Recombinant protein-thioester (**1**) and the recombinant C-terminus (**4**) were characterized by RP-HPLC and ESI-MS. **1** expected mass is 7,686 Da, and the observed mass was 7,686 ± 1 Da; **4** expected mass is 5,593 Da, and the observed mass was 5,595 ± 2 Da.

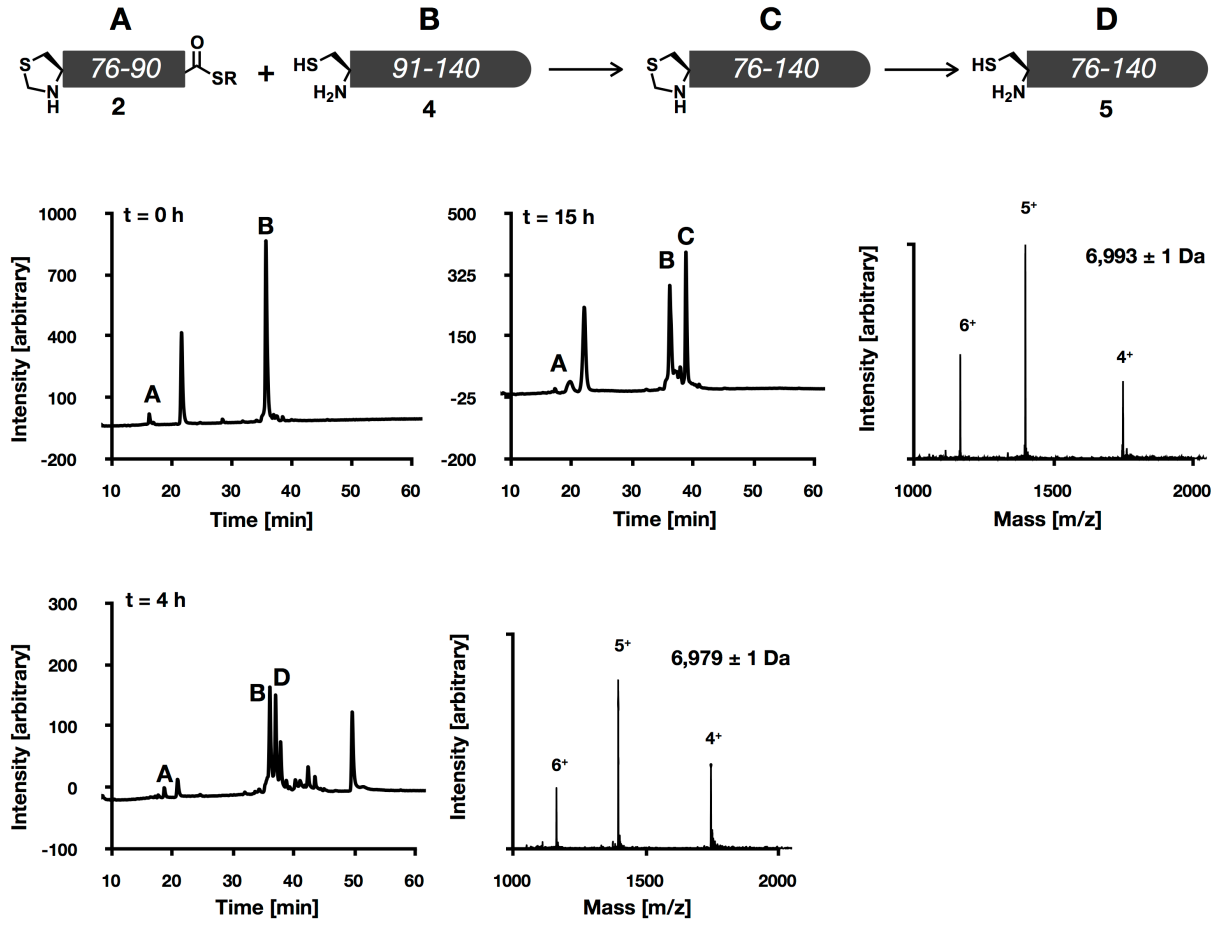

Figure S2. Ligation of peptide 2 and protein 4 and the subsequent deprotection to give protein fragment 5. The ligation reaction was followed by RP-HPLC and the identity of intermediate product **C** was confirmed by ESI-MS. This was followed by deprotection in the same pot to give the product **D**, which again was confirmed by ESI-MS. **C** expected mass is 6,992 Da, and the observed mass was 6,993 ± 1 Da; **D** expected mass is 6,980 Da, and the observed mass was 6,979 ± 1 Da.

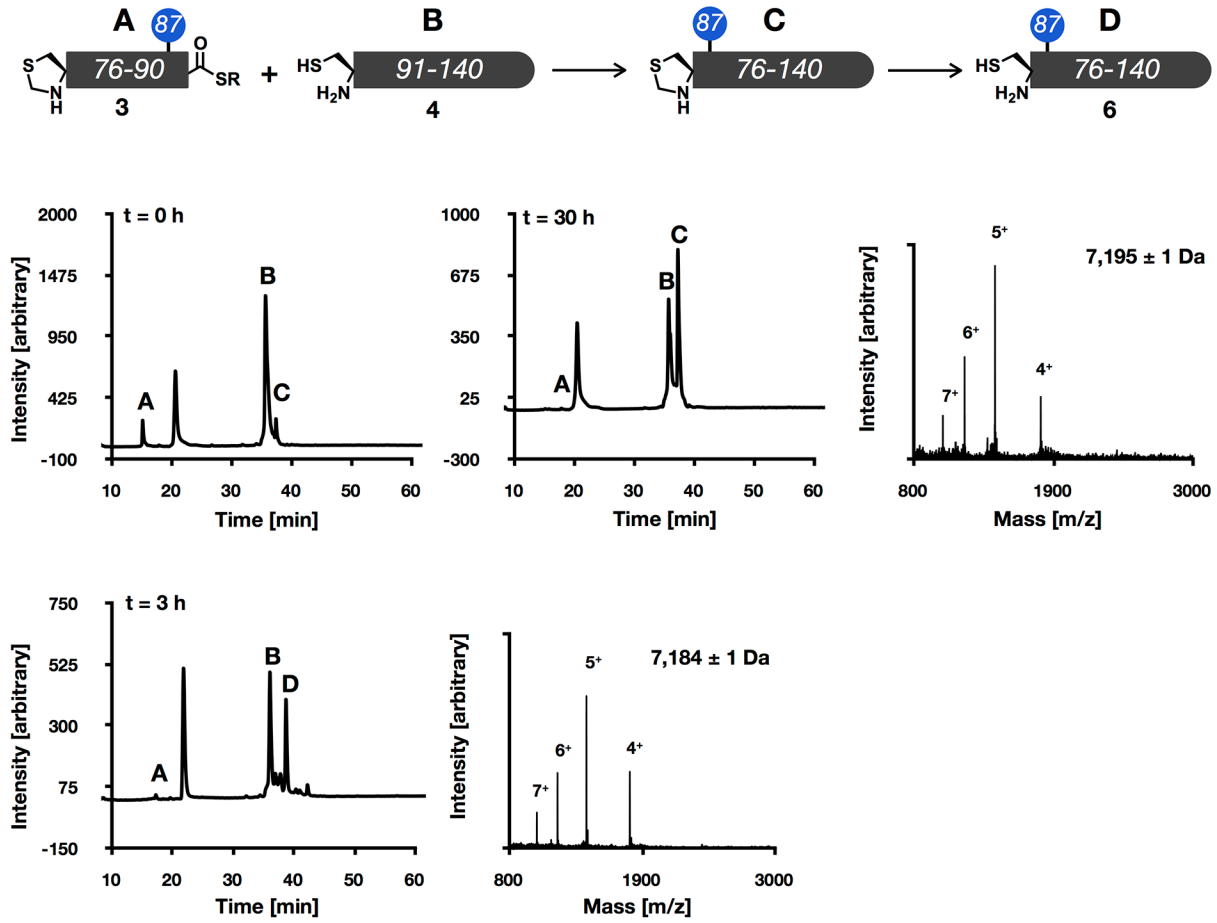

Figure S3. Ligation of peptide 3 and protein 4 and the subsequent deprotection to give protein fragment 6. The ligation reaction was followed by RP-HPLC and the identity of intermediate product **C** was confirmed by ESI-MS. This was followed by deprotection in the same pot to give the product **D**, which again was confirmed by ESI-MS. **C** expected mass is 7,195 Da, and the observed mass was 7,195 ± 1 Da; **D** expected mass is 7,184 Da, and the observed mass was 7,184 ± 1 Da.

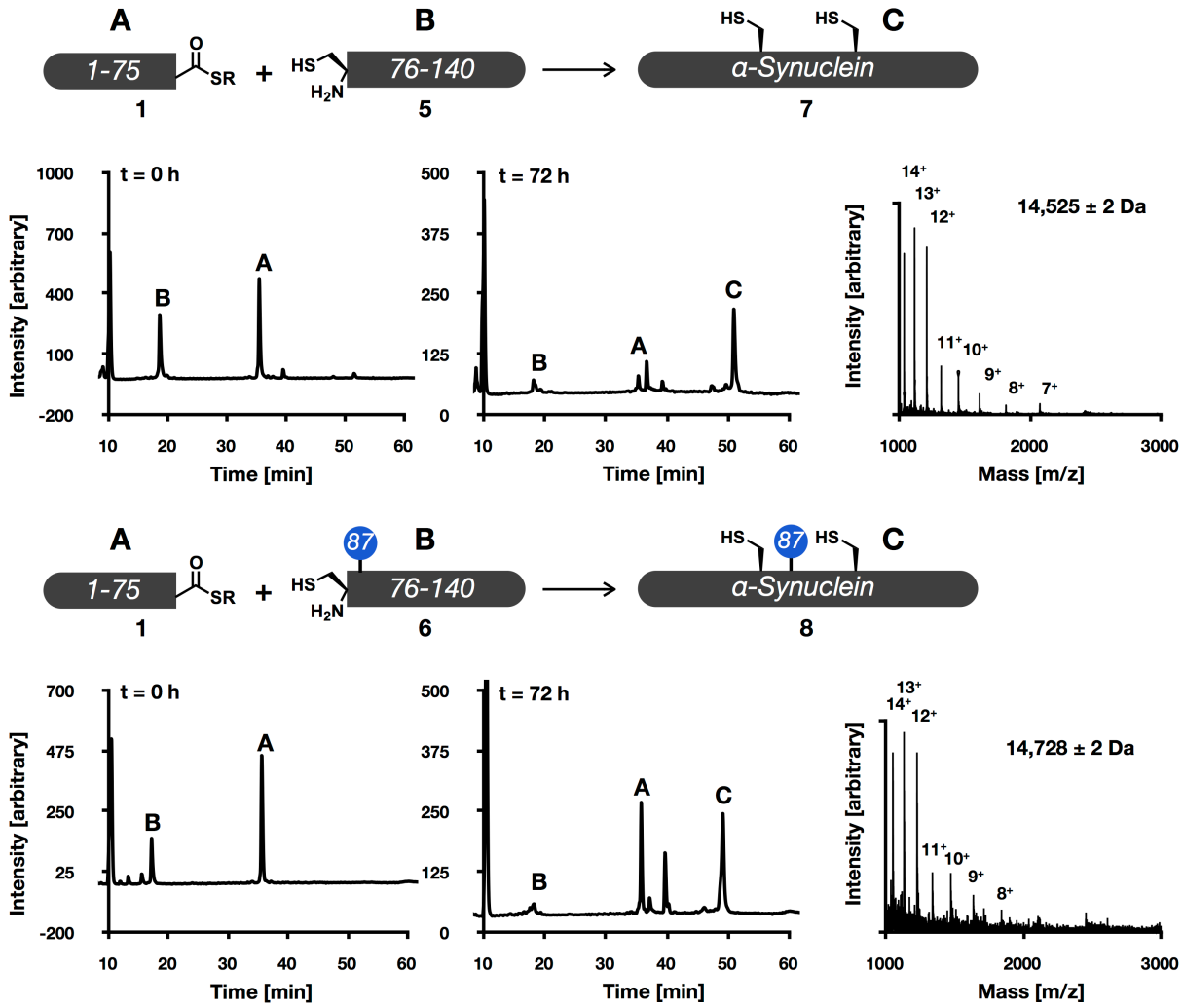

Figure S4. Ligation of proteins 5 or 6 and protein-thioester 1 to give proteins 7 or 8. The ligation reactions were followed by RP-HPLC and the identities of the products **C** were confirmed by ESI-MS. Unmodified **C** expected mass is 15,524 Da, and the observed mass was 15,525 ± 2 Da; O-GlcNAcylated **C** expected mass is 14,728 ± 2 Da, and the observed mass was 14,728 ± 2 Da.

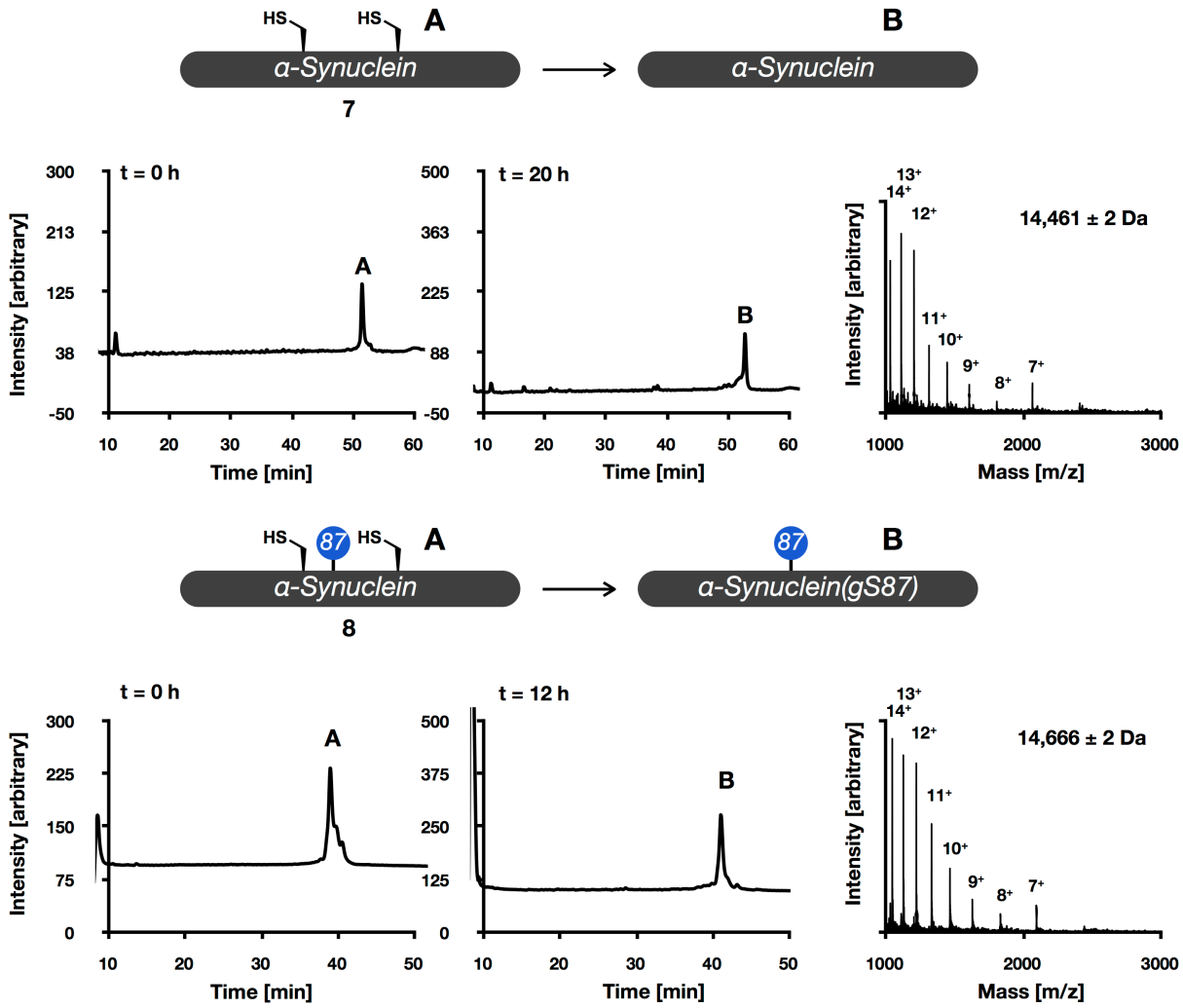

Figure S5. Desulfurization of proteins 7 or 8. The desulfurization reactions were followed by RP-HPLC and the identities of the products **B** were confirmed by ESI-MS. Unmodified **B** expected mass is 14,460 Da, and the observed mass was 14,461 ± 1 Da; O-GlcNAcylated **B** expected mass is 14,663 Da, and the observed mass was 14,666 ± 2 Da.

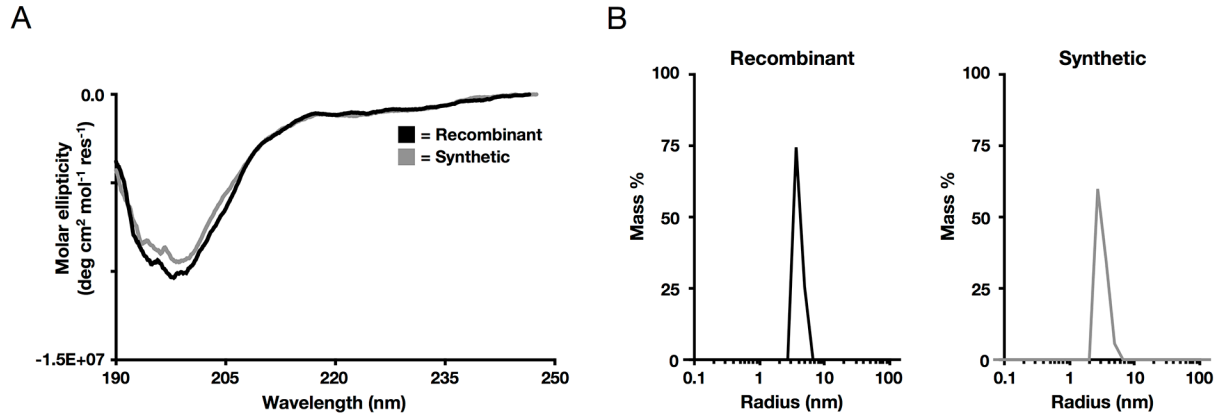

Figure S6. Structural characterization of α-synuclein(gS87) and α-synuclein(S87E) using circular dichroism (CD) and dynamic light scattering (DLS). (A) α-Synuclein(gS87) and α-synuclein(S87E) are unstructured in solution. CD spectra were collected for freshly dissolved proteins at 7.5 μM concentration. (B) Both proteins were monomeric in nature with Stoke's Radii of approximately 4 nm, and no significant, larger peaks. The indicated proteins were analyzed using DLS at 50 μM concentration.

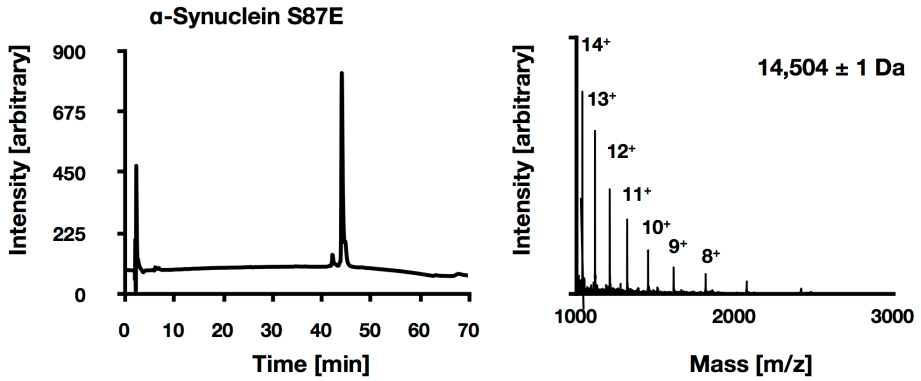

Figure S7. Expression and characterization of α-synuclein(S87E). Recombinant α-synuclein(S87E) was characterized by RP-HPLC and ESI-MS. Expected mass is 14,502 Da and the observed mass was 14,504 ± 1 Da.

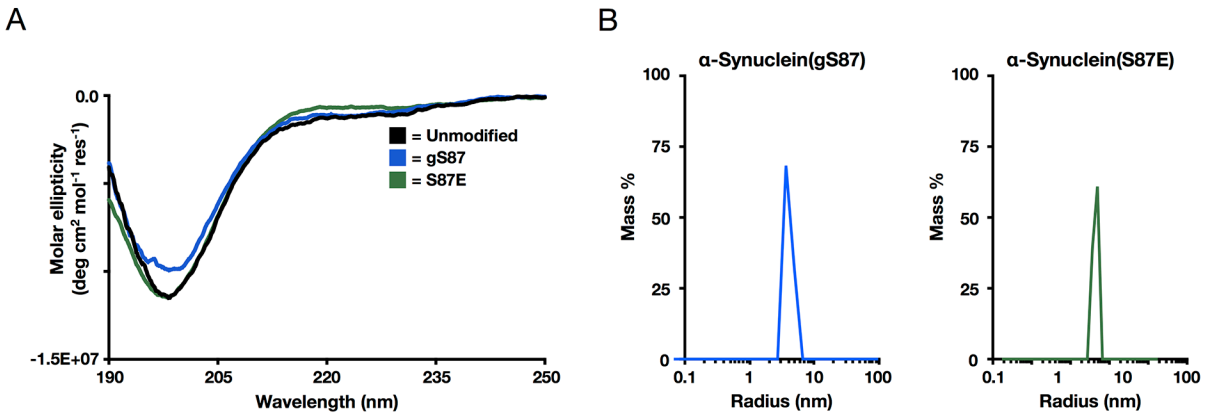

Figure S8. Structural characterization of synthetic and recombinant unmodified α-synuclein using circular dichroism (CD) and dynamic light scattering (DLS). (A) Both recombinant and synthetic α-synuclein are unstructured in solution. CD spectra were collected for freshly dissolved proteins at 7.5 μM concentration. (B) Both proteins were monomeric in nature with Stoke's Radii of approximately 4 nm, and no significant, larger peaks. The indicated proteins were analyzed using DLS at 50 μM concentration.

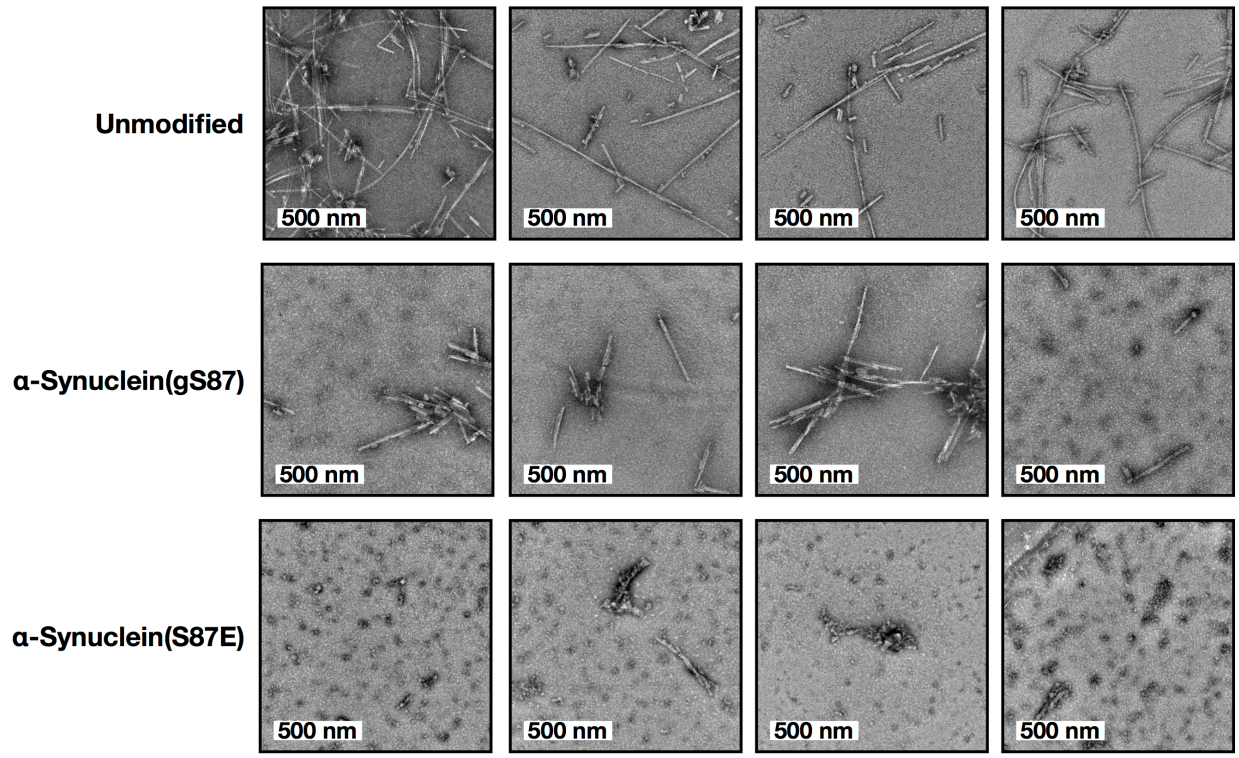

Figure S9. Transmission electron microscopy (TEM) images of the from the α-synuclein aggregation reactions. Unmodified α-synuclein, α-synuclein(gS87), or α-synuclein(S87E) were subjected to aggregation conditions (50 μM concentration and agitation at 37 °C) for 168 h before visualization of any aggregate structures by TEM. Scale bars, 500 nm.

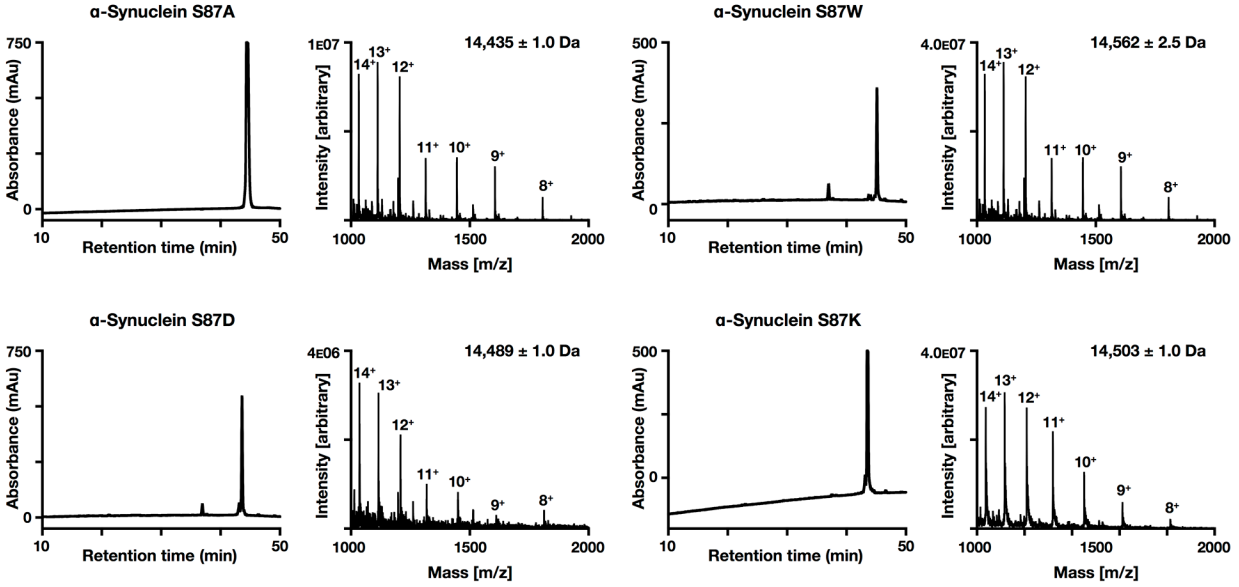

Figure S10. Characterization of the additional α-synuclein mutants S87A, S87D, S87W, and S87K. The indicated recombinant α-synuclein mutants were characterized by RP-HPLC and ESI-MS.Expected mass of α-synuclein(S87A) is 14,435 Da, and observed mass was 14,435 ± 1 Da. Expected mass of α-synuclein(S87D) is 14,488 Da, and observed mass was 14,489 ± 1 Da. Expected mass of α-synuclein(S87W) is 14,560 Da, and observed mass was 14,562 ± 2.5 Da. Expected mass of α-synuclein(S87K) is 14,501 Da, and observed mass was 14,503 ± 1 Da.

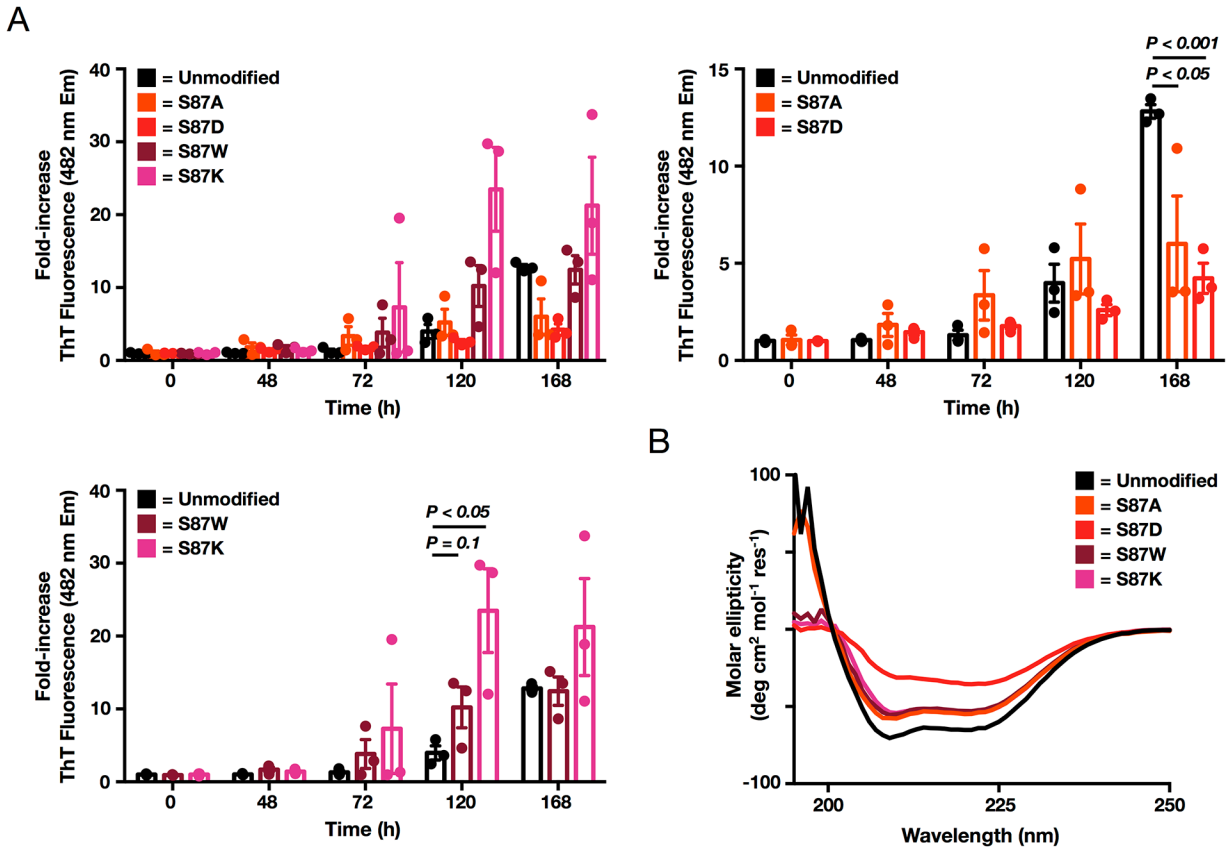

Figure S11. Analysis of mutant α-synuclein aggregation and membrane binding. (A) Mutations of α-synuclein at S87 have different effects on protein aggregation. Recombinant α-synuclein or the indicated α-synuclein mutants were individually subjected to aggregation conditions (25 μM concentration and agitation at 37 °C) for the indicated lengths of time before analysis by ThT fluorescence (λ_{ex} = 450 nm, λ_{em} = 482 nm). The y-axis shows the fold-increase of fluorescence compared with the corresponding protein at t = 0. Error bars represent ±s.e.m from the mean of biological replicates (n = 3), and statistical significance was calculated using a two-tailed Student's t-test. (B) Mutation of S87 to D inhibits α-synuclein membrane binding. Recombinant α-synuclein or the indicated α-synuclein mutants were incubated with an 100-fold excess of the indicated, preformed vesicles and analyzed using circular dichroism (CD). In the presence of negatively charged vesicles (POPG), most of the proteins gave essentially indistinguishable CD spectra consistent with the formation of an extended α-helix. However, α-synuclein(S87D) showed diminished signal corresponding to less membrane binding and α-helix formation. POPG = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-RAC-(1-glycerol)].