



*Citation for published version:*

Meade, R, Morris, K, Watt, K, Williams, RJ & Mason, J 2020, 'The Library Derived 4554W Peptide Inhibits Primary Nucleation of -Synuclein', *Journal of Molecular Biology*, vol. 432, no. 24, 166706.  
<https://doi.org/10.1016/j.jmb.2020.11.005>

*DOI:*

[10.1016/j.jmb.2020.11.005](https://doi.org/10.1016/j.jmb.2020.11.005)

*Publication date:*

2020

*Document Version*

Peer reviewed version

[Link to publication](#)

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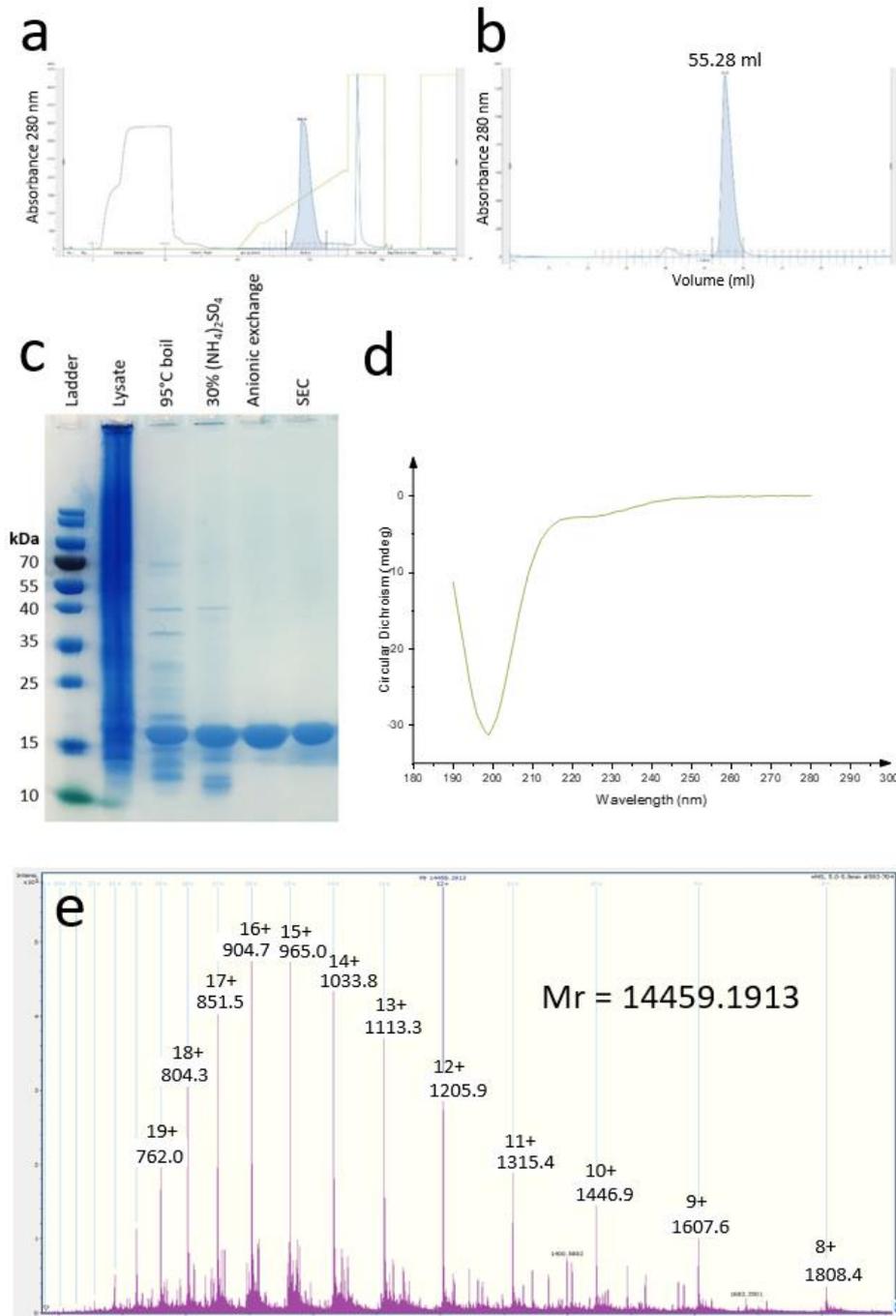
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## Supporting Information

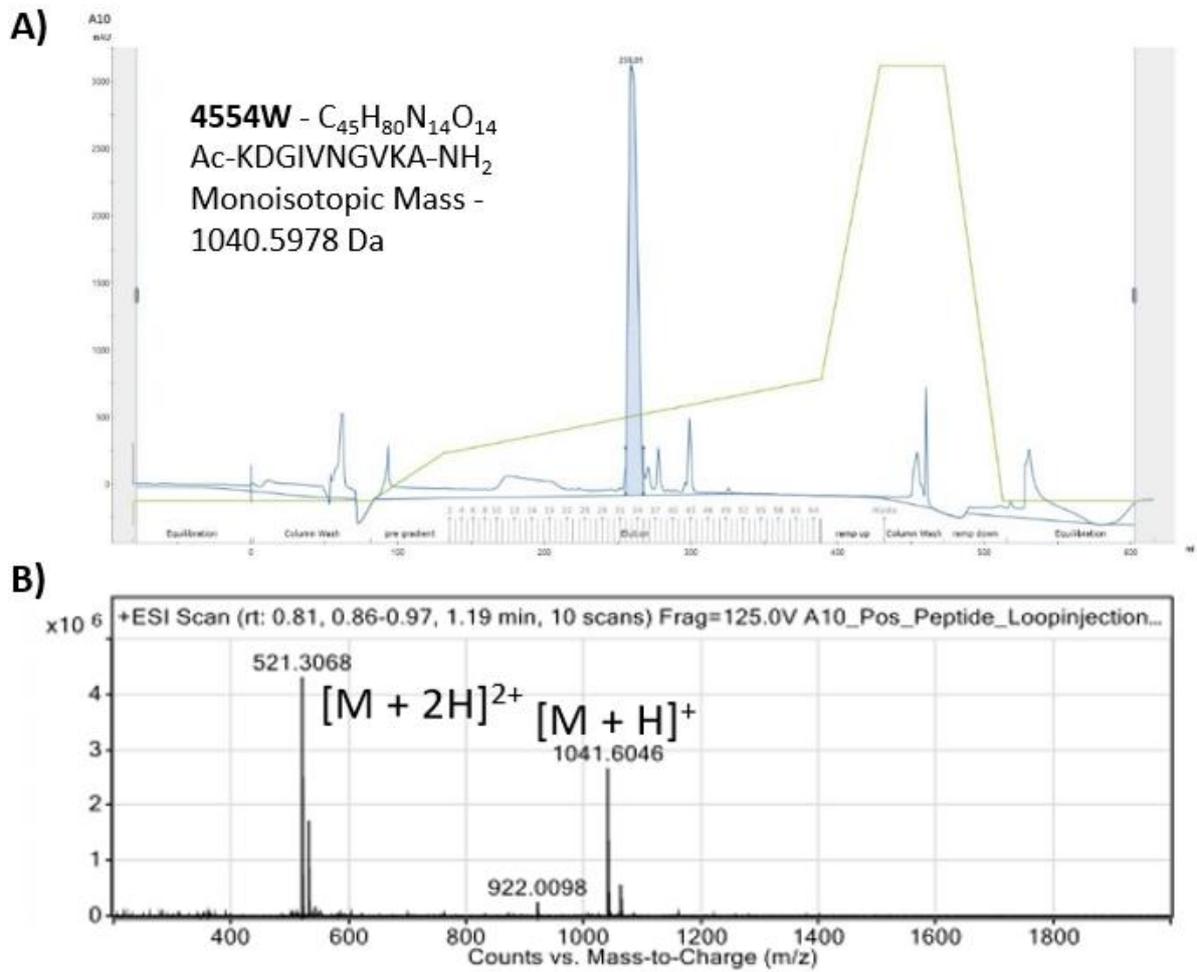
### The 4554W Peptide Inhibits $\alpha$ S Lipid-induced Primary Nucleation

#### Production and of Purification of Human wt $\alpha$ S (140)

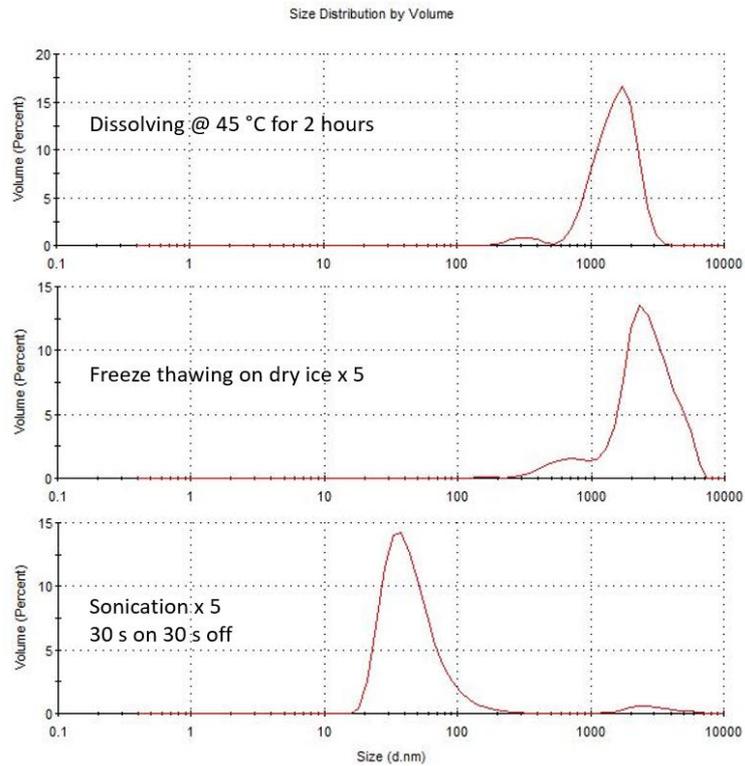


**Supplementary Figure 1:** Overview of the purification of  $\alpha$ S used for the experiments. a) Chromatograph of the Hi-Q anionic exchange purification. b) Chromatograph of the Size exclusion chromatography and buffer exchange. c) SDS page gel showing an overview of the entire purification protocol. d) Far-UV circular dichroism spectra of the purified  $\alpha$ S showing that the

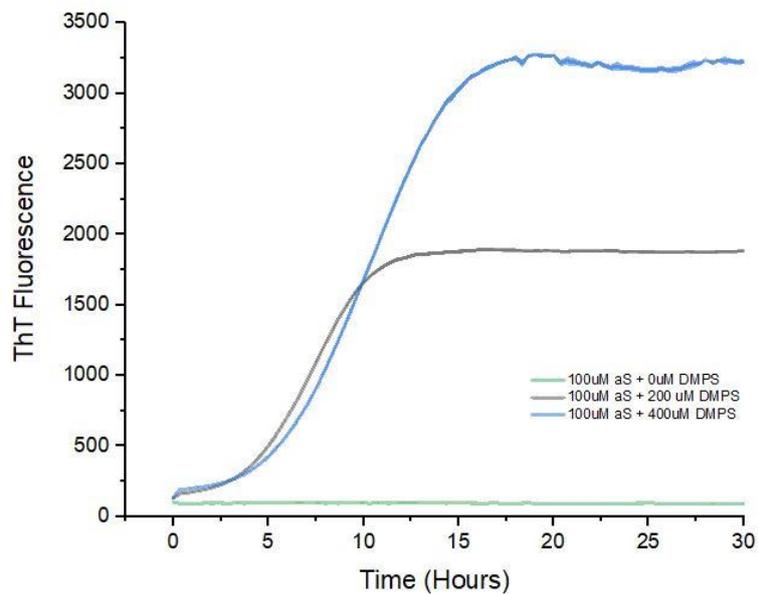
monomeric  $\alpha$ S is in a random coil conformation. e) De-convoluted mass spectrum, showing a mass of the protein of 14459 m/z, representing the mass of wt Human  $\alpha$ S (1-140).



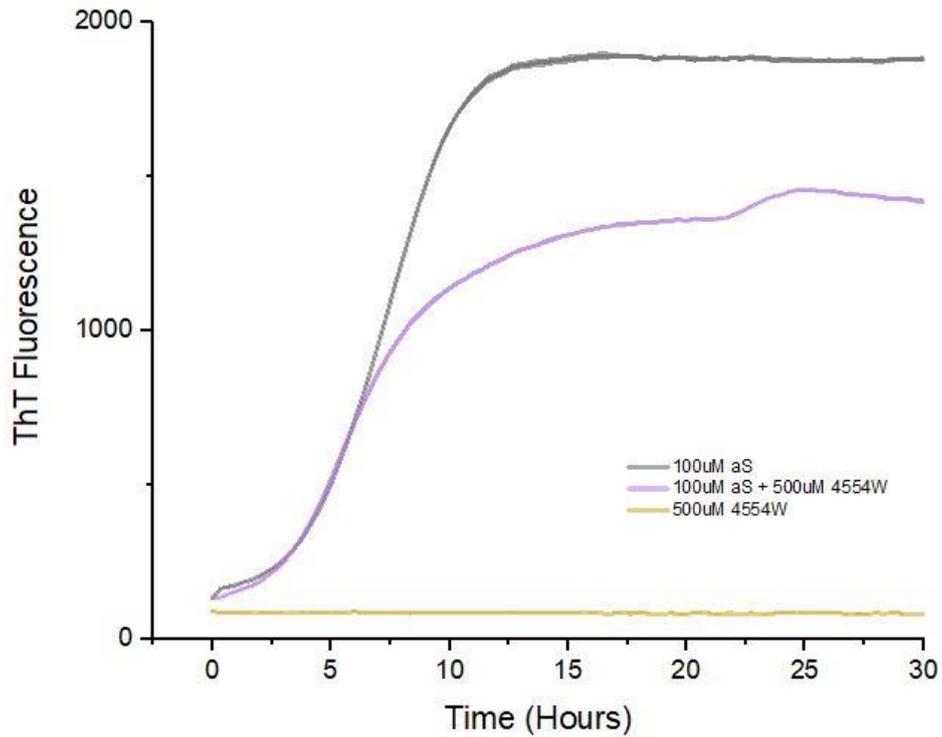
**Supplementary Figure 2:** Production and purification of the 4554W peptide. A) HPLC purification trace measured at 215 nm for 4554W. B) Mass spectrometry profile measured by time of flight spectroscopy confirming 4554W mass to be 1040.6 Da, as expected.



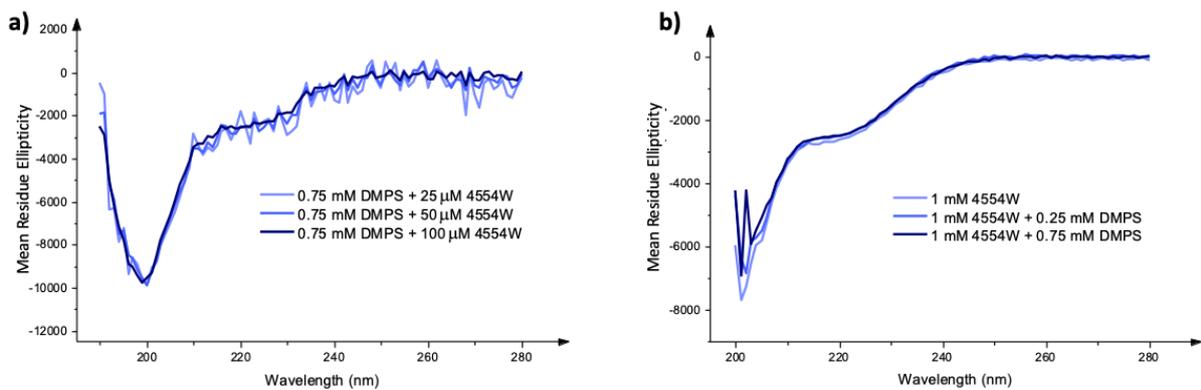
**Supplementary Figure 3:** Dynamic light scattering size distribution of the DMPS small unilamellar vesicles (SUVs) used for lipid induced nucleation assays, showing a size distribution centred around 30nm post sonication.



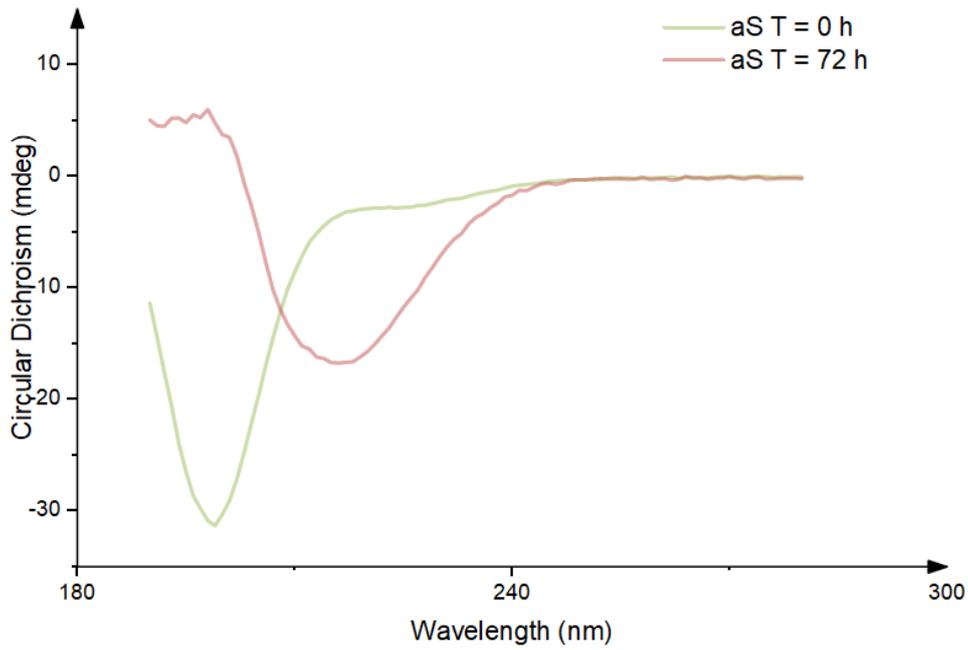
**Supplementary Figure 4:** ThT fluorescence intensity when 100  $\mu\text{M}$   $\alpha\text{S}$  is incubated with 0  $\mu\text{M}$ , 200  $\mu\text{M}$ , or 400  $\mu\text{M}$  DMPS vesicles and 50  $\mu\text{M}$  Thioflavin T in 20 mM phosphate buffer (pH 6.5) under quiescent conditions at 30 °C.



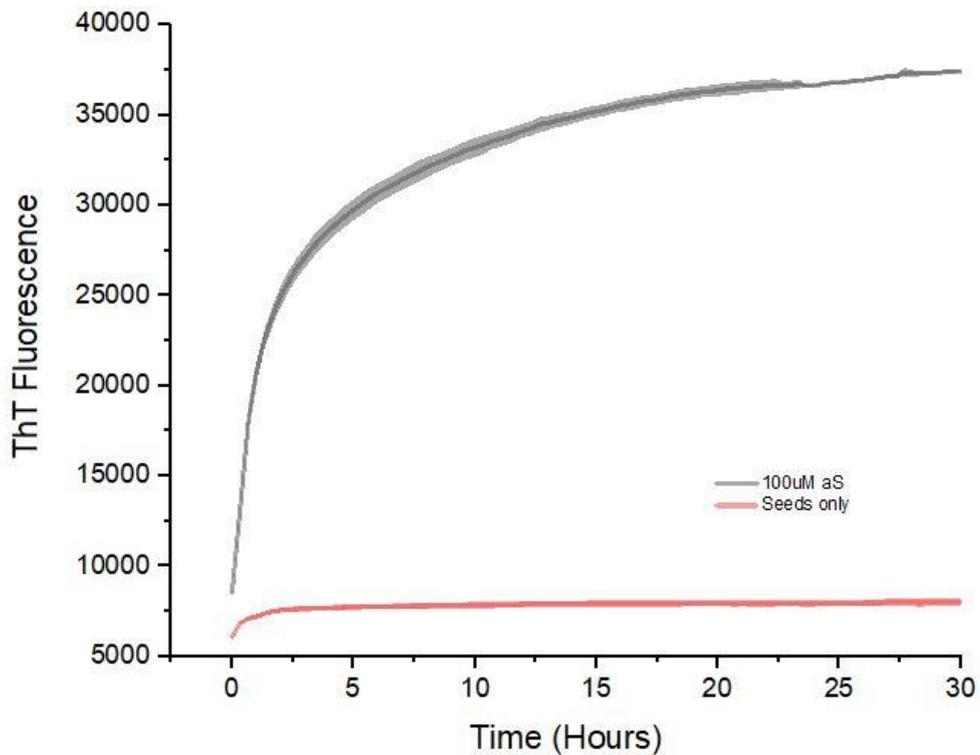
**Supplementary Figure 5:** ThT fluorescence intensity when 100  $\mu\text{M}$   $\alpha\text{S}$  is incubated with 200  $\mu\text{M}$  DMPS vesicles and 50  $\mu\text{M}$  Thioflavin T in 20 mM phosphate buffer (pH 6.5) under quiescent conditions at 30  $^{\circ}\text{C}$ . It can be seen that the 4554W peptide has an inhibitory effect on lipid induced primary nucleation, and no aggregation is observed for 4554W peptide in the absence of  $\alpha\text{S}$ .



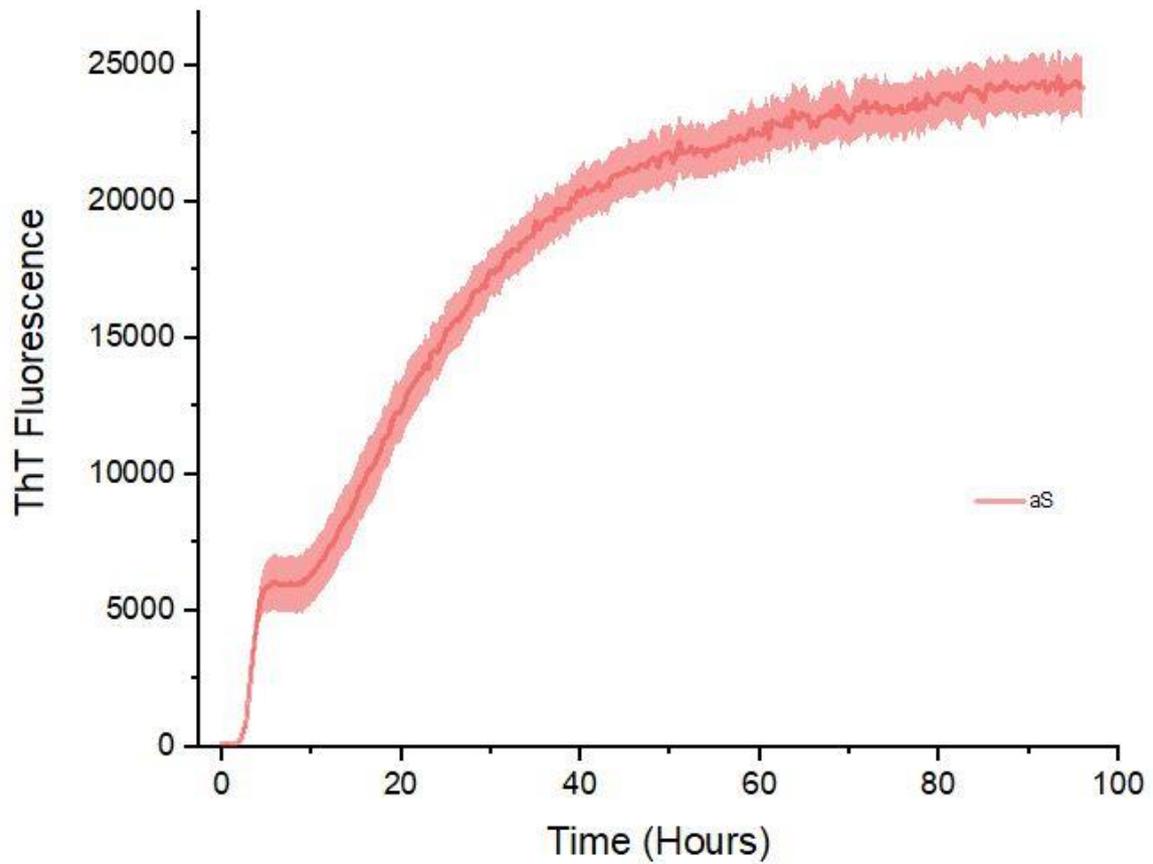
**Supplementary Figure 6:** Circular dichroism spectra of a) increasing concentrations (25-100  $\mu\text{M}$ ) of 4554W incubated at 30  $^{\circ}\text{C}$  with 0.75 mM DMPS vesicles, and b) increasing concentrations (0 - 0.75 mM) of DMPS SUVs incubated at 30  $^{\circ}\text{C}$  with 1 mM 4554W, showing that the peptide retains a random coil structure in the presence of lipid vesicles, suggesting that the peptide does not bind independently to lipid vesicles. All spectra were recorded as an average of 3 scans, blanked against corresponding DMPS concentration in 20mM sodium phosphate buffer (pH 6.5), and presented as mean residue ellipticity.



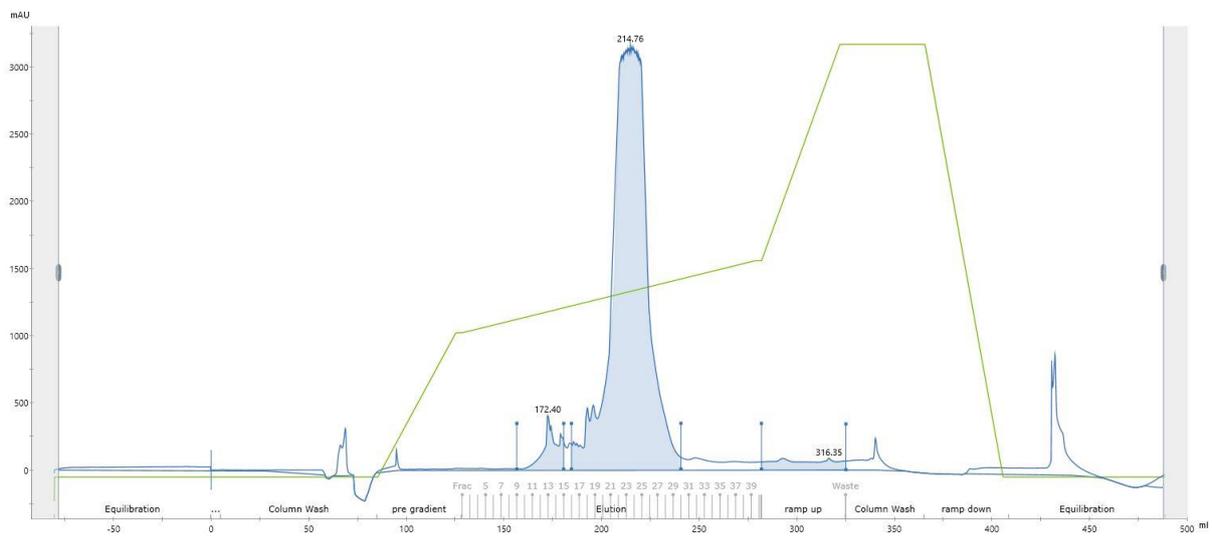
**Supplementary Figure 7:** Circular dichroism spectra of 10  $\mu\text{M}$  seeds formed for ThT assay to probe fibril elongation rates. The  $\alpha\text{S}$  monomer before aggregation shows a random coil spectra (green). The  $\alpha\text{S}$  is seen to be fully converted to beta-sheet fibril seeds (red) after 400  $\mu\text{M}$   $\alpha\text{S}$  was stirring at 40°C with a teflon bar at 1500rpm for 48 hours, followed by 3 rounds of freeze-thawing in liquid  $\text{N}_2$  and 3 rounds of sonication for 10 seconds on 10 seconds off.



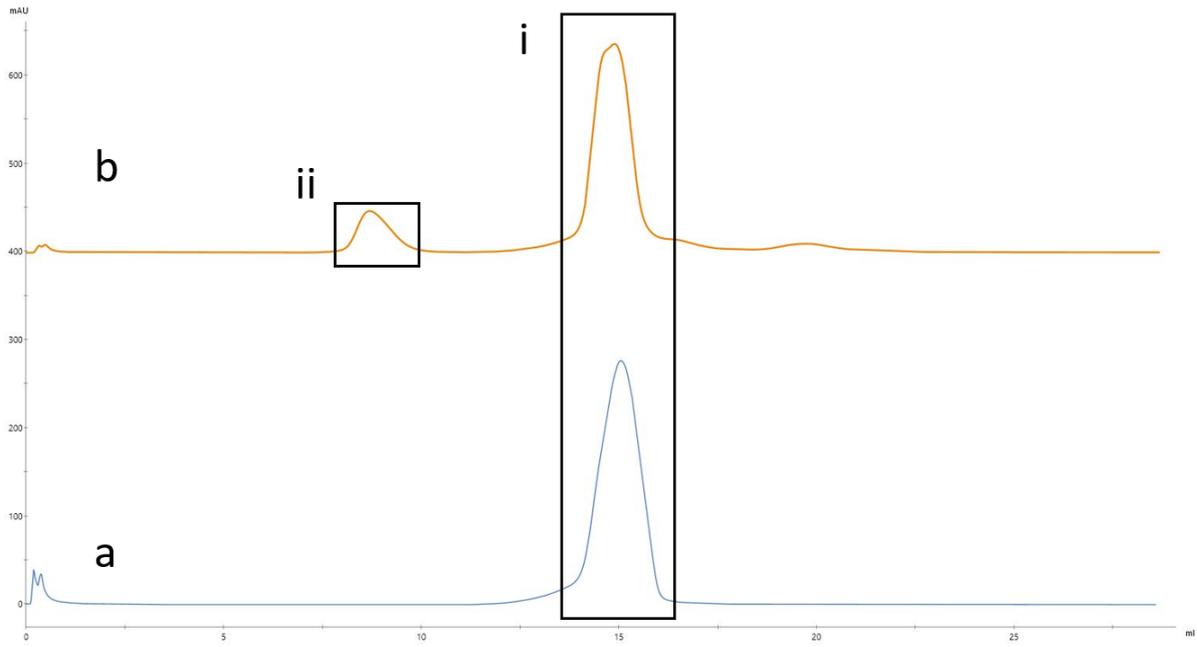
**Supplementary Figure 8:** ThT fluorescence experiment measuring seeds only vs seeds and monomer to show seed stability. 100  $\mu\text{M}$  in the presence of 15 $\mu\text{M}$  seeds (grey) vs 15  $\mu\text{M}$  seeds without addition of monomer (red), showing that the seeds remained stable.



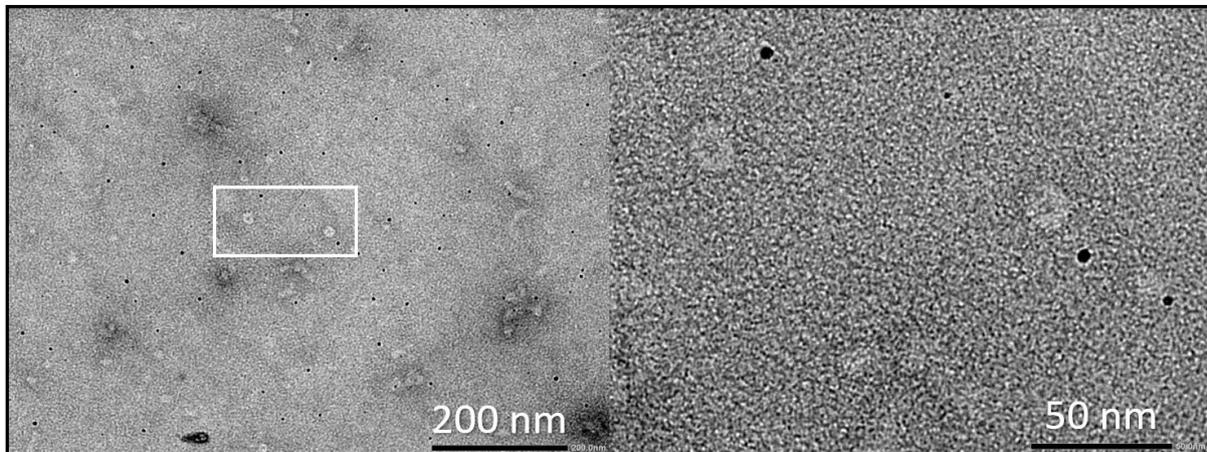
**Supplementary Figure 9:** ThT curve following seed creation for secondary nucleation studies, created in 96 well plates containing glass beads in the wells, with shaking at 500 rpm at a constant temperature of 37 °C.



**Supplementary Figure 10:** HPLC purification of Pre-purified  $\alpha$ S by affinity to C18 column to remove buffer prior to lyophilisation for cell toxicity assays. Absorption at 280 nm used to follow the elution of  $\alpha$ S from the c18 column (blue), by increasing concentrations of acetonitrile + 0.1% TFA (green).



**Supplementary Figure 11:** SEC elution chromatogram when 100  $\mu$ l of 800  $\mu$ M  $\alpha$ S sample is loaded onto a Superdex 200 Increase 10/300 column (GE lifesciences), and run at 0.5 ml/min in PBS buffer pH 7.4, showing absorbance at 280 nm. **a)** Monomeric  $\alpha$ S showing a clean monomer peak (i) at 15ml elution volume. **b)** Lyophilised  $\alpha$ S resuspended in PBS pH 7.4 and incubated at 37  $^{\circ}$ C for 24hrs and filtered through a 0.22  $\mu$ M filter showing a monomer peak (i) at 15ml elution volume, and an additional oligomer peak (ii) eluted at 9ml run volume.



**Supplementary Figure 12:** Negative stain TEM images taken from the oligomer SEC Peak (supplementary figure 11ii), that appear to show doughnut like oligomer structures similar in shape and diameter to those previously reported.