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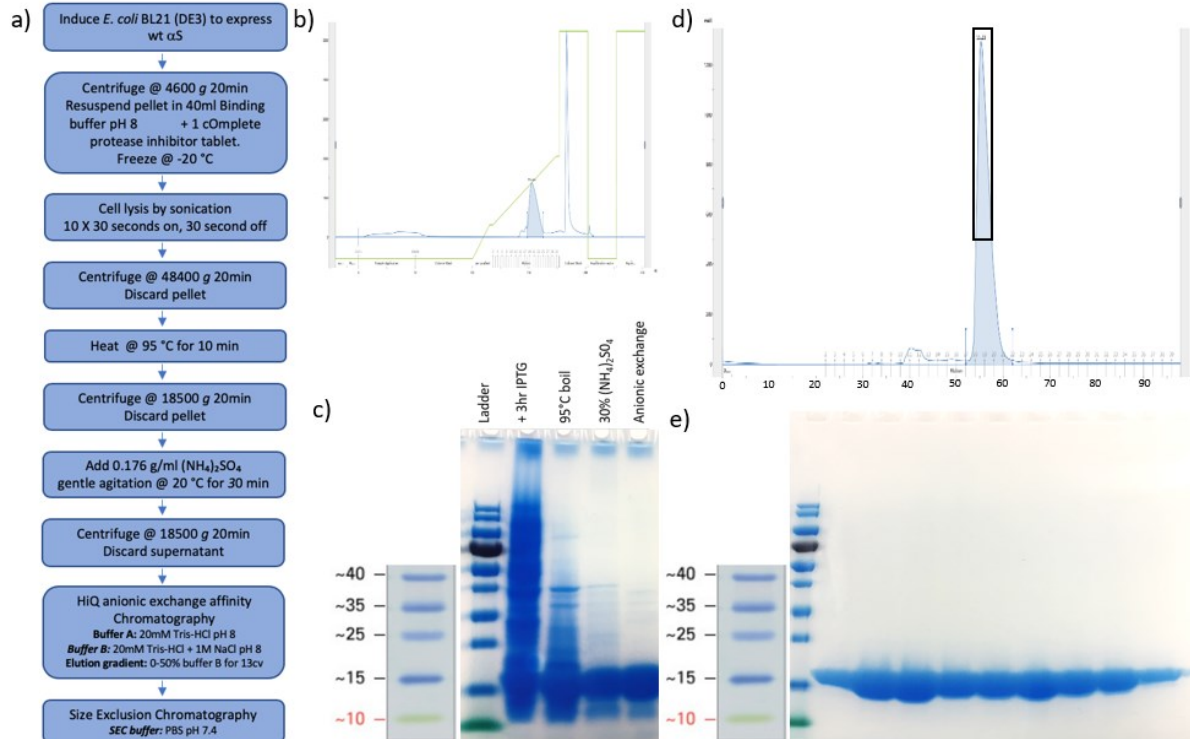
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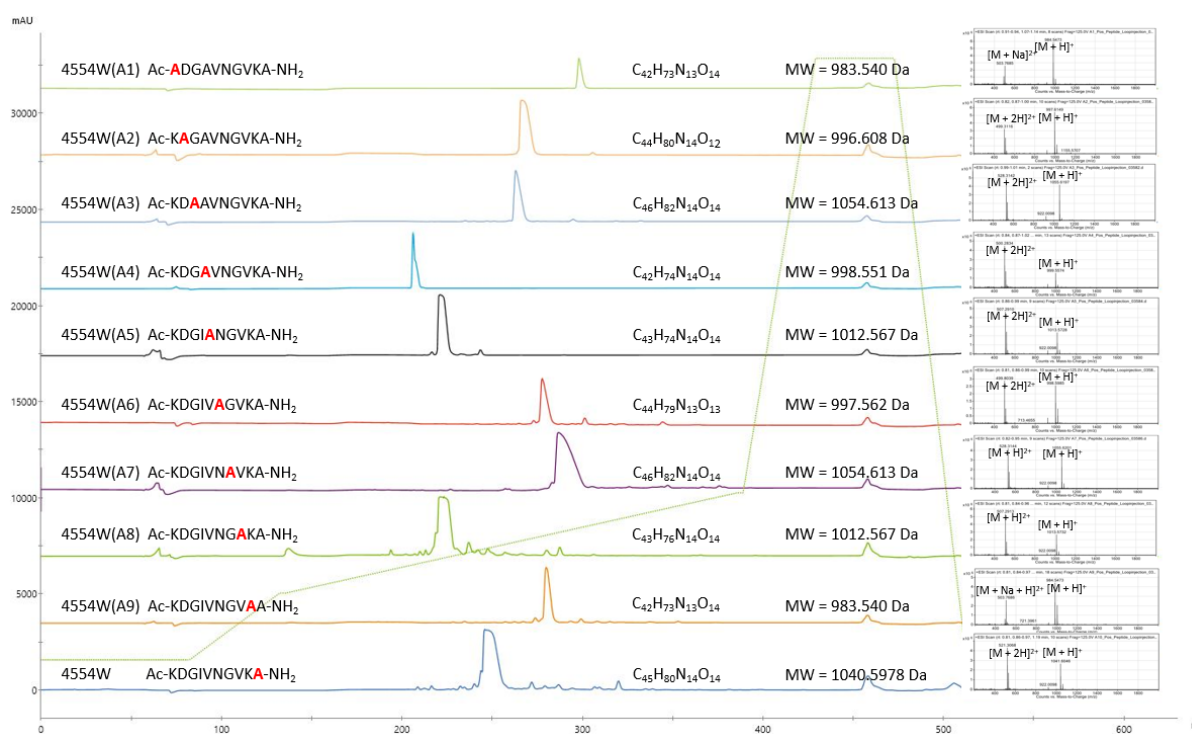
Supporting information



Supplementary Figure 1: **a)** Overview of the purification of α S used for the experiments. **b)** Chromatogram of the Hi-Q anionic exchange purification. **d)** SDS page gel showing an overview of the entire purification protocol before SEC. **d)** Chromatogram of the size exclusion chromatography and buffer exchange, and the area of the peak (black box) collected and used for the experiments). **e)** SDS page gel showing the purity of the final purified α S.

Peptide	Peptide Sequence	Formula	Monoisotopic mass (Da)
4554W(K1A)	Ac-ADGIVNGVKA-NH ₂	C ₄₂ H ₇₃ N ₁₃ O ₁₄	983.540
4554W(D2A)	Ac-KAGIVNGVKA-NH ₂	C ₄₄ H ₈₀ N ₁₄ O ₁₂	996.608
4554W(G3A)	Ac-KDAIVNGVKA-NH ₂	C ₄₆ H ₈₂ N ₁₄ O ₁₄	1054.613
4554W(I4A)	Ac-KDGAIVNGVKA-NH ₂	C ₄₂ H ₇₄ N ₁₄ O ₁₄	998.551
4554W(V5A)	Ac-KDGIANGVKA-NH ₂	C ₄₃ H ₇₆ N ₁₄ O ₁₄	1012.567
4554W(N6A)	Ac-KDGIVAGVKA-NH ₂	C ₄₄ H ₇₉ N ₁₃ O ₁₃	997.562
4554W(G7A)	Ac-KDGIVNAVKA-NH ₂	C ₄₆ H ₈₂ N ₁₄ O ₁₄	1054.613
4554W(V8A)	Ac-KDGIVNGAKA-NH ₂	C ₄₃ H ₇₆ N ₁₄ O ₁₄	1012.567
4554W(K9A)	Ac-KDGIVNGVAA-NH ₂	C ₄₂ H ₇₃ N ₁₃ O ₁₄	983.540
4554W	Ac-KDGIVNGVKA-NH ₂	C ₄₅ H ₈₀ N ₁₄ O ₁₄	1040.5978

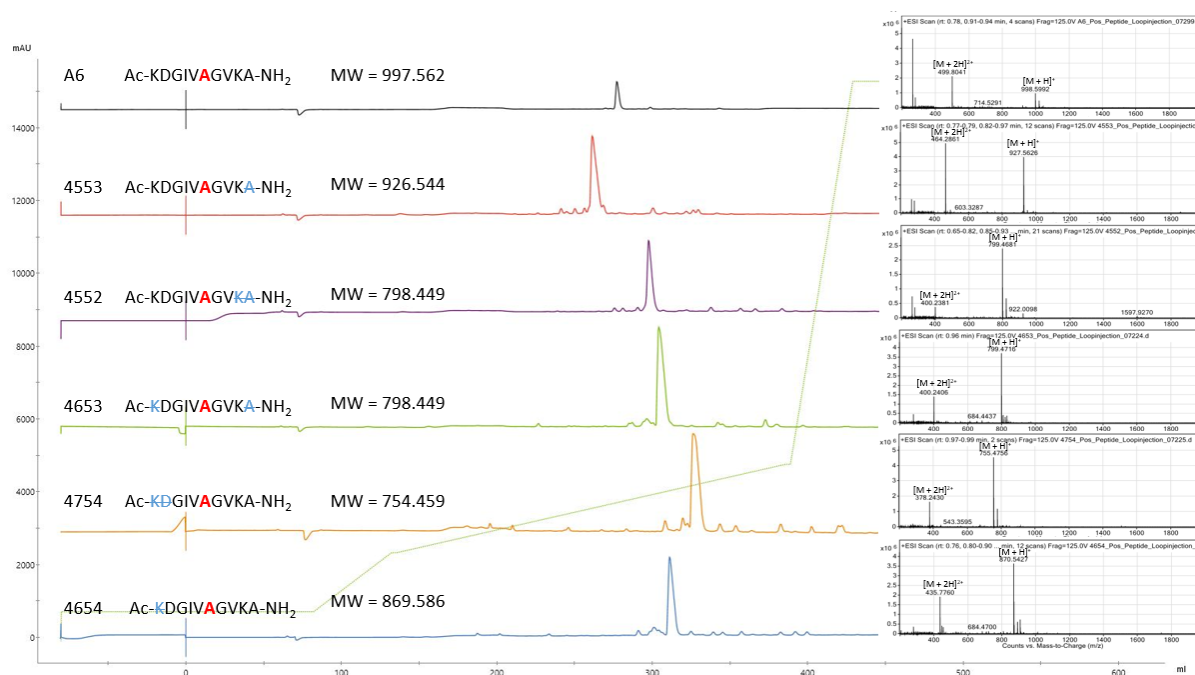
Supplementary Table 1: Sequence and monoisotopic masses for the alanine scan variant peptides.



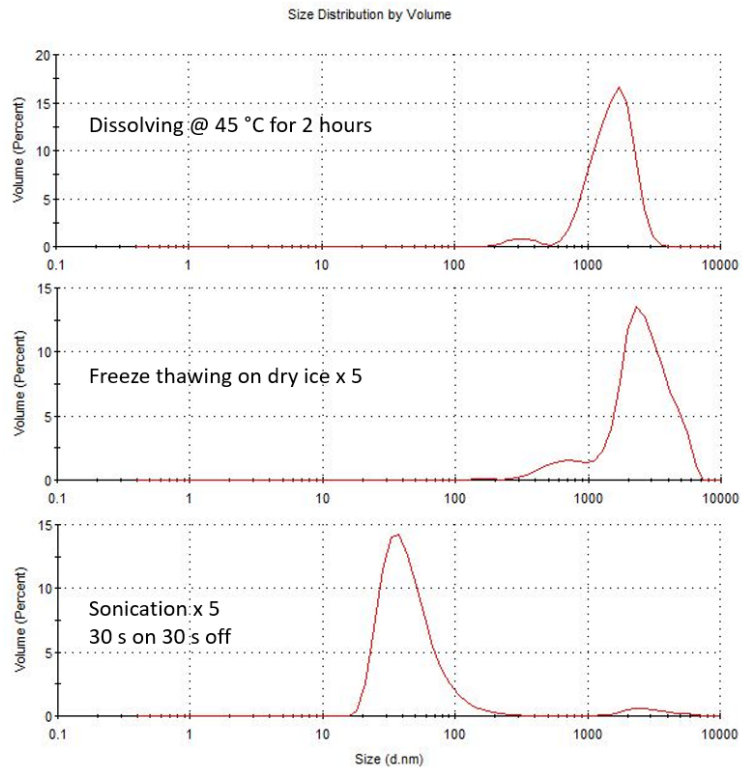
Supplementary Figure 2: HPLC traces and corresponding mass spectrometry for alanine scan variant peptides.

Peptide	Peptide Sequence	Formula	Monoisotopic mass (Da)
4554W(N6A)	Ac-KDGIVAGVKA-NH ₂	C ₄₄ H ₇₉ N ₁₃ O ₁₃	997.562
4654W(N6A)	Ac-DGIVAGVKA-NH ₂	C ₃₈ H ₆₇ N ₁₁ O ₁₂	869.486
4754W(N6A)	Ac-GIVAGVKA-NH ₂	C ₃₄ H ₆₂ N ₁₀ O ₉	754.459
4553W(N6A)	Ac-KDGIVAGVK-NH ₂	C ₄₁ H ₇₄ N ₁₂ O ₁₂	926.544
4552W(N6A)	Ac-KDGIVAGV-NH ₂	C ₃₅ H ₆₂ N ₁₀ O ₁₁	798.449
4653W(N6A)	Ac-DGIVAGVK-NH ₂	C ₃₅ H ₆₂ N ₁₀ O ₁₁	798.449

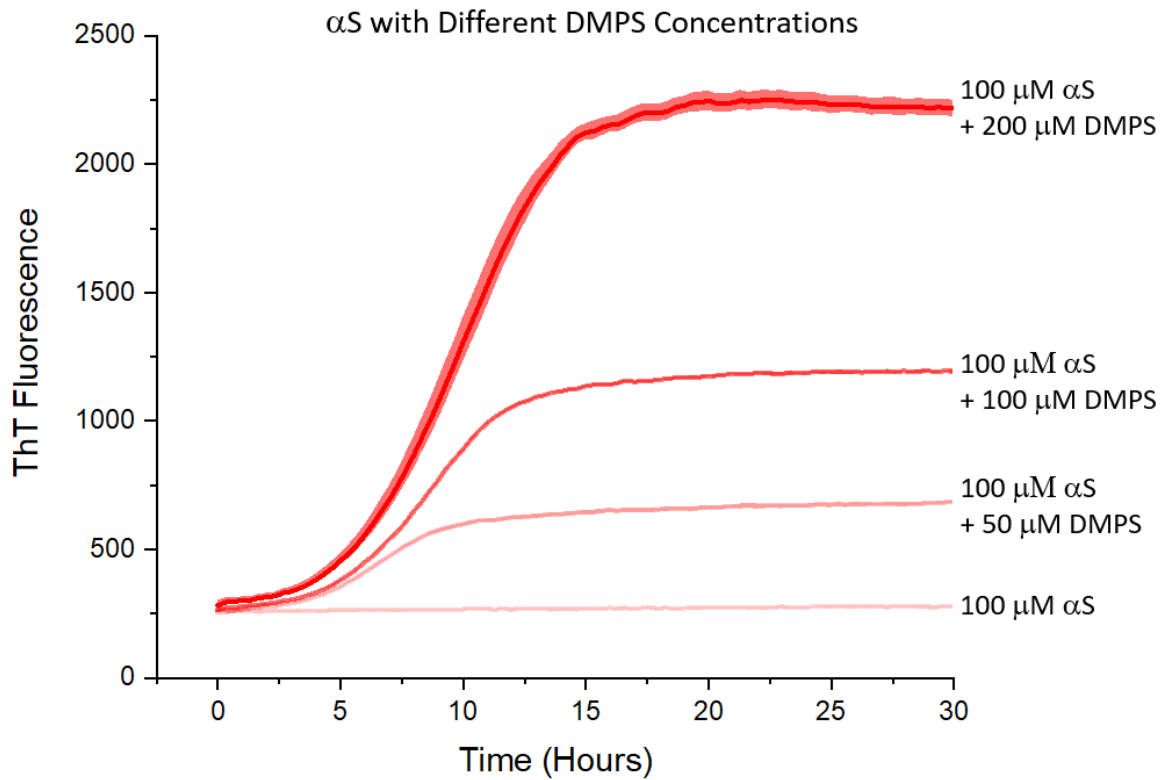
Supplementary Table 2: Sequence and monoisotopic masses for the truncated variant peptides.



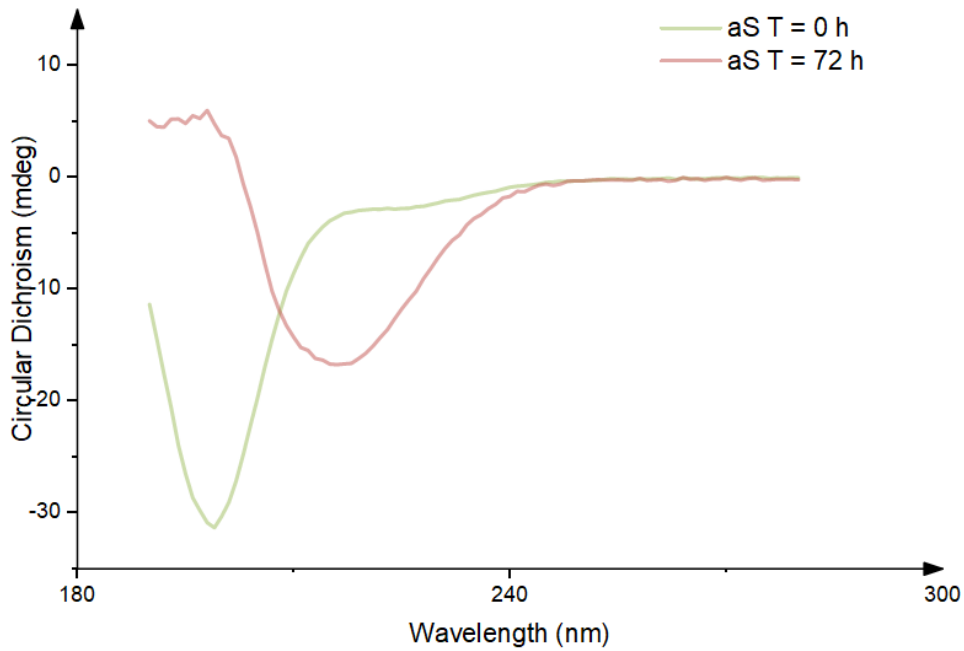
Supplementary Figure 3: HPLC traces and corresponding mass spectrometry for truncated variant peptides.



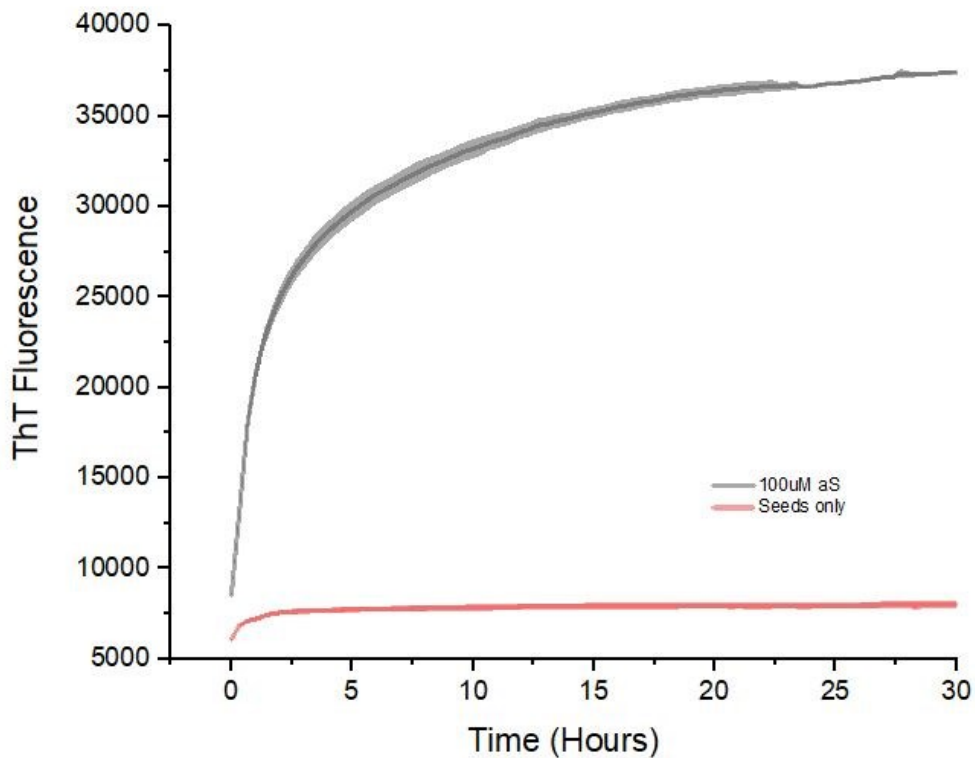
Supplementary Figure 4: Dynamic light scattering size distribution of the DMPS small unilamellar vesicles (SUVs) used for lipid induced nucleation assays, showing a size distribution centred around 30 - 40 nm post sonication.



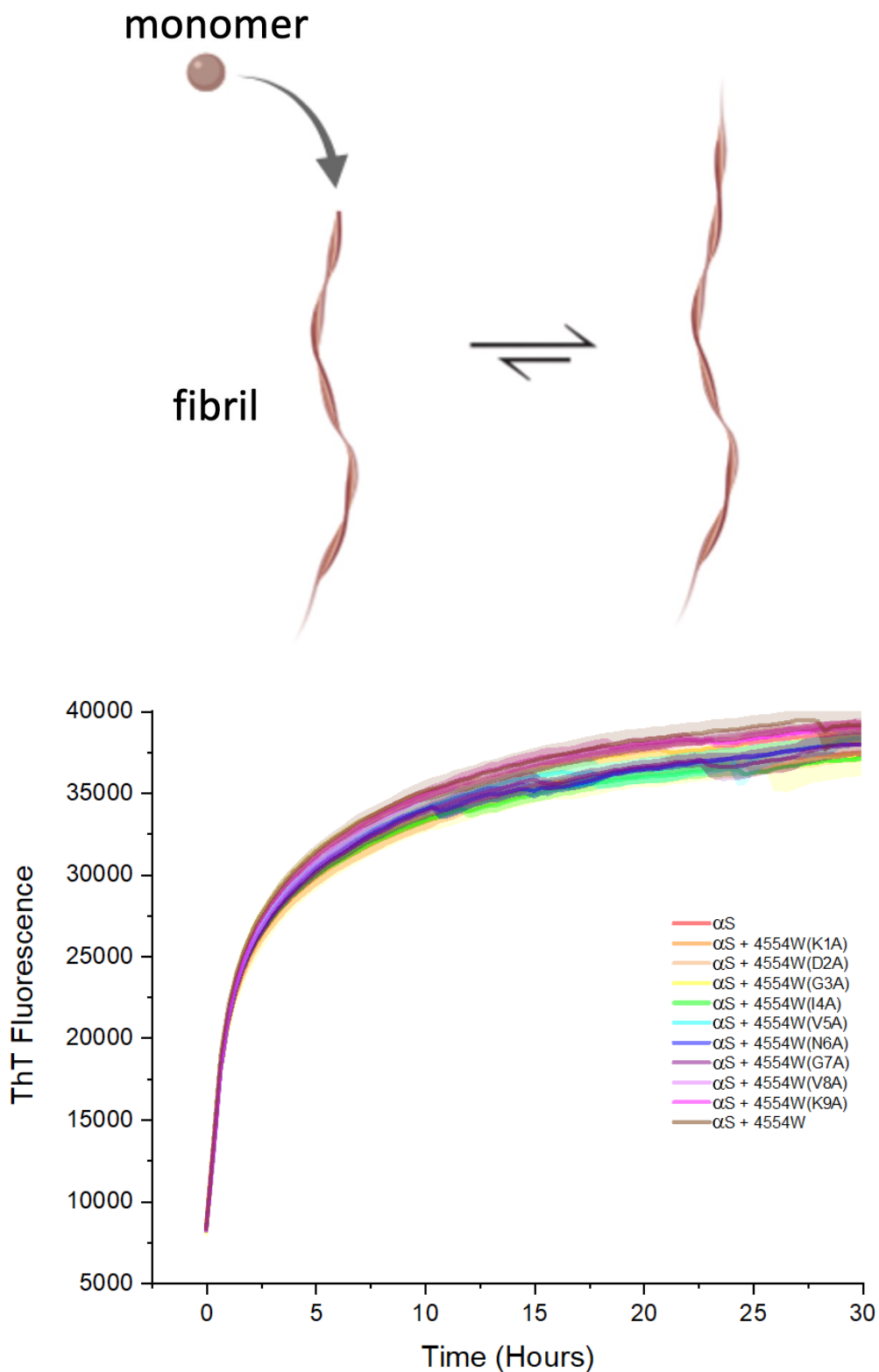
Supplementary Figure 5: ThT fluorescence intensity when 100 μ M α S is incubated with 0 μ M, 50 μ M, 100 μ M or 200 μ M, DMPS vesicles and 50 μ M ThT in 20 mM phosphate buffer (pH 6.5) under quiescent conditions at 30 °C.



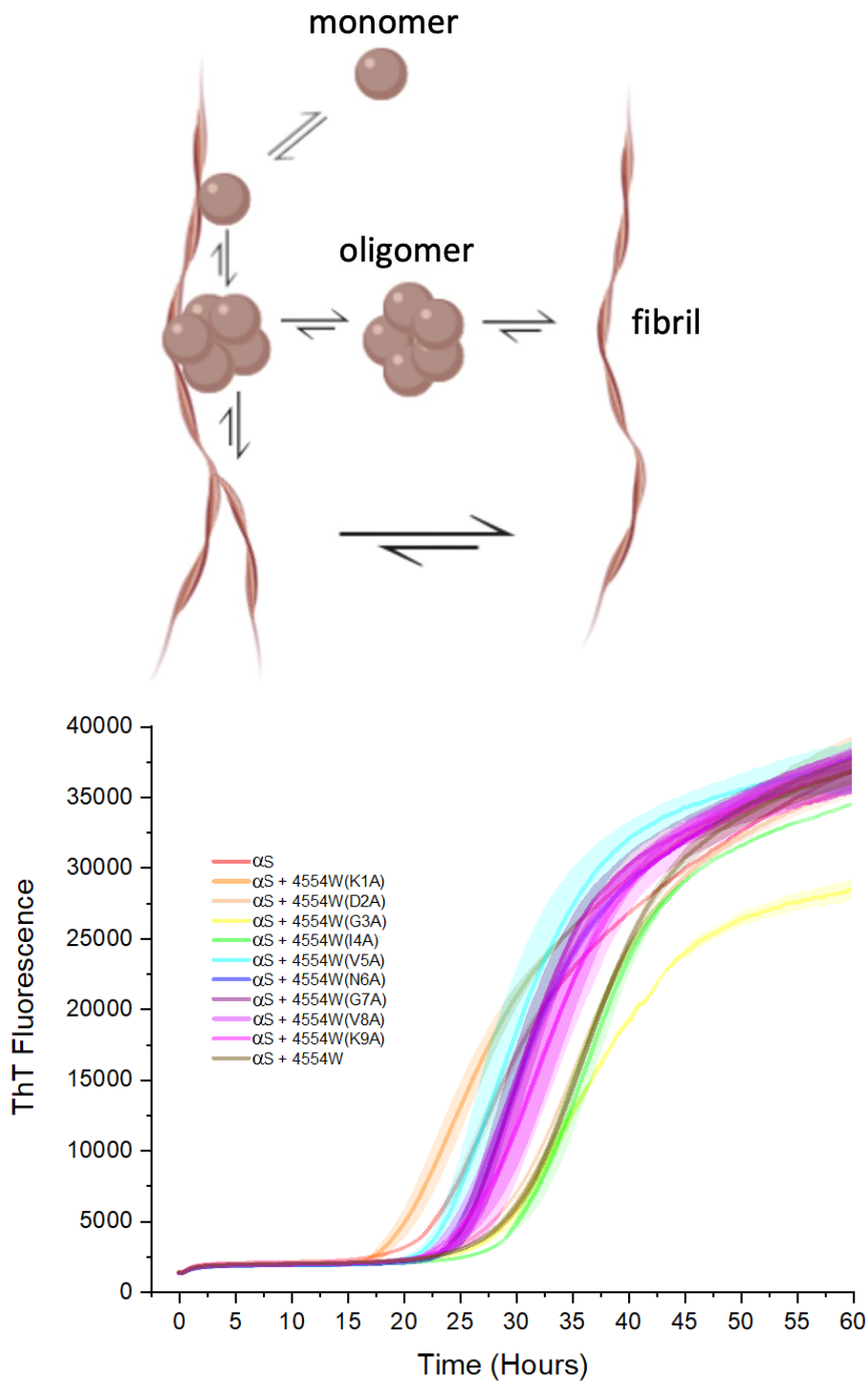
Supplementary Figure 6: Circular dichroism spectra of 10 μM seeds formed for ThT assay to probe fibril elongation rates. The αS monomer before aggregation shows a random coil spectra (green). The αS is seen to be fully converted to β -sheet fibril seeds (red) after 400 μM αS was stirring at 40 $^{\circ}\text{C}$ with a Teflon bar at 1500 rpm for 48 hours, followed by 3 rounds of freeze-thawing in liquid N_2 and 3 rounds of sonication for 10 seconds on 10 seconds off.



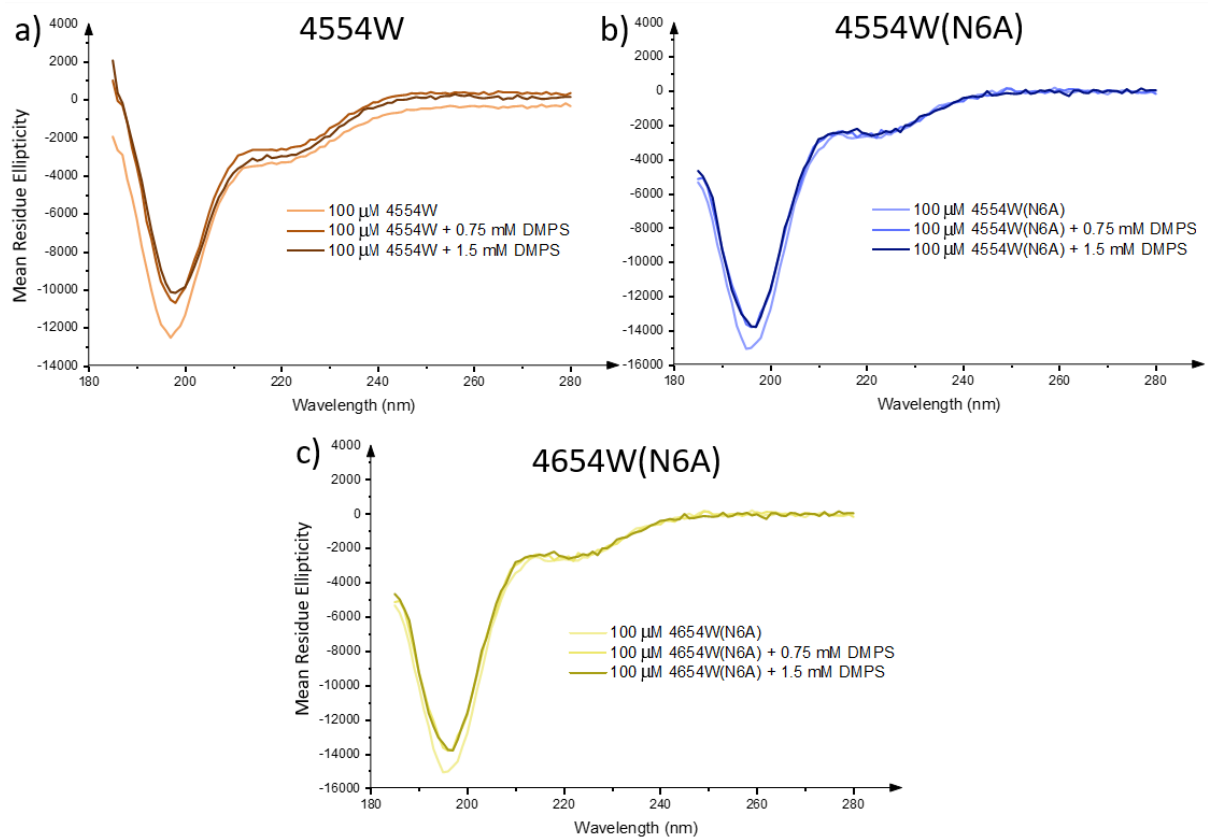
Supplementary Figure 7: ThT fluorescence experiment measuring seeds only vs seeds and monomer to show seed stability. 100 μM in the presence of 15 μM seeds (grey) vs 15 μM seeds without addition of monomer (red), showing that the seeds remained stable.



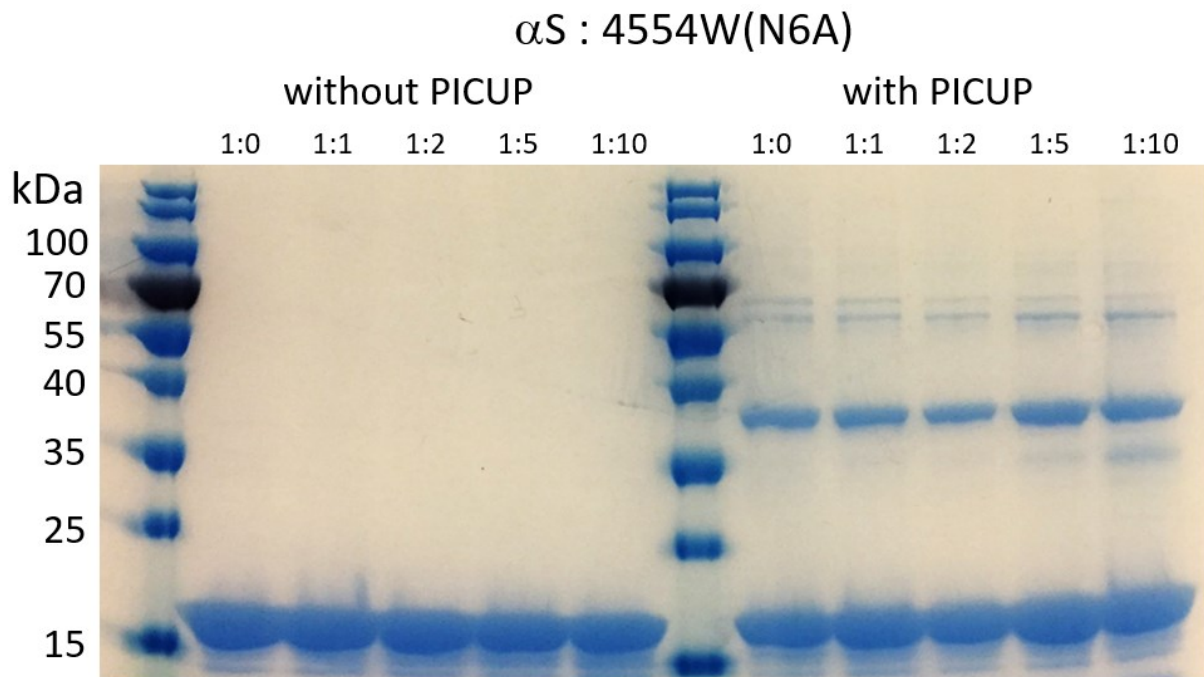
Supplementary Figure 8: Effect of 4554W and the alanine scan variants on Fibril elongation. Top) Elongation is caused by the addition of soluble random coil α S monomers to the ends of preformed insoluble β -sheet fibrils, a more energetically stable configuration. **Bottom)** Fibril Elongation measured by the change in ThT fluorescence intensity when 100 μ M monomeric α S was incubated in the presence of 15 μ M preformed fibrils and 50 μ M ThT in 20 mM sodium phosphate buffer pH 6.5 under quiescent conditions at 37 $^{\circ}$ C, with 1000 μ M of the different 4554W peptide alanine scan variants. No effect on the ThT trace was observed by the addition of the peptides.



Supplementary Figure 9: Effect of 4554W and the alanine scan variants on fibril amplification/secondary nucleation. Top) Secondary nucleation of amyloid fibrils through surface catalysed nucleation, whereby single monomeric α S aggregates to the side of preformed fibrils which can then cause formation and release of toxic oligomers or branching of the amyloid fibril. Branch points can shear off leading to fibril amplification. **Bottom)** change in ThT fluorescence intensity when 100 μ M monomeric α S was incubated in the presence of 1 μ M preformed fibril seeds and 50 μ M ThT in 20 mM sodium acetate buffer pH 5 under quiescent conditions at 37 $^{\circ}$ C, with 1000 μ M of the different 4554W peptide alanine scan variants. No effect on the ThT trace was observed by the addition of the peptides.



Supplementary Figure 10: Effect of DMPS on the structure of the peptides. 100 μ M of a) 4554W, b) 4554W(N6A), c) 4654W(N6A), were incubated in 20 mM phosphate buffer (pH 6.5) in the presence of DMPS SUVs (0 mM, 0.75 mM and 1.5 mM) at 30 $^{\circ}$ C for 1 hour and circular dichroism spectra measured. Spectra show that the peptides remain in random coil configuration in the presence of excess SUVs, therefore remain unbound. All spectra are shown as an average of three repeats and blanked against solutions containing the relevant DMPS concentrations.



Supplementary Figure 12: Repeat of PICUP crosslinking of α S aggregates, in the presence of DMPS lipid vesicles and increasing concentrations of 4554W(N6A). In the absence of PICUP crosslinking (left) no visible oligomeric species are seen. When the same aggregation mixtures are subjected to PICUP crosslinking (right) the oligomeric bands become visible on the gel.