An Intracellular Cyclisation Screen Generates Short Helical Peptide Inhibitor of Alpha Synuclein Aggregation

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1. Introduction and Previous Work

- Alpha-Synuclein (αS) is heavily associated with Parkinson's Disease (PD) pathology [1]. αS aggregates into toxic oligomers and fibrils within neuronal cells, ultimately resulting in cell death [1], and PD symptoms (Fig 1A).
- αS adopts an α-helical conformation in the presence of lipids, which is required for its proposed role in synaptic transmission[2]. Key residues involved in lipid binding are found in the N-terminal region (Fig 1B, Purple Region).
- Short peptides have been shown to reduce αS aggregation and associated toxicity [3, 4].
- We have previously shown that helical, N-terminal based peptides can effectively inhibit αS aggregation, resulting in the formation of the potent inhibitor, $\alpha S_{2\text{-}12}$ (Fig 1C and D).
- Here, we utilised a novel intracellular cyclisation screening method, to further improve $\alpha S_{2\text{-}12}$.

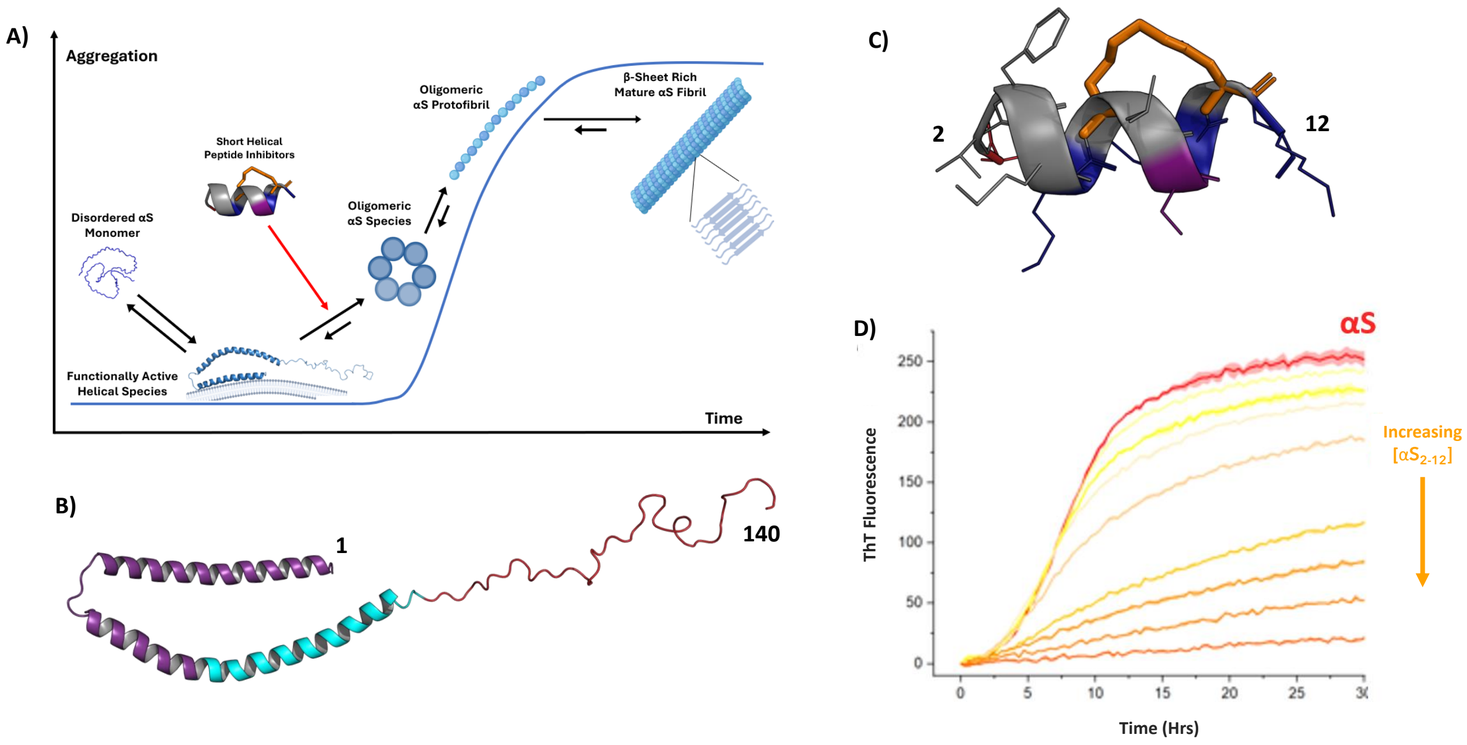


Figure 1. A) Schematic of αS aggregation. B) αS contains three domains: The N-terminal (Purple), The NAC region (Blue) and the C-terminal (Red) (PDB=1XQ8). C) Solution NMR structure of $\alpha S_{2\text{-}12}$ peptide (PDB 80L8). D) ThT assay showing dose dependent inhibition of αS aggregation by $\alpha S_{2\text{-}12}$.

2. Protein Fragment Complementation Assay

- We screened a peptide library using the Protein-fragment Complementation Assay (PCA).
- PCA selects interactions between a peptide and a target protein by recovering bacterial survival in the presence of trimethoprim (TMP). TMP selectively inhibits bacterial Dihydrofolate Reductase (DHFR). Bacterial survival is recovered by supplementing the bacterial cells with a split, exogenous murine DHFR (mDHFR).
- Peptides that bind αS recombine mDHFR resulting in cell survival. A winning peptide hit is selected as the fasting growing colony in liquid passaging.

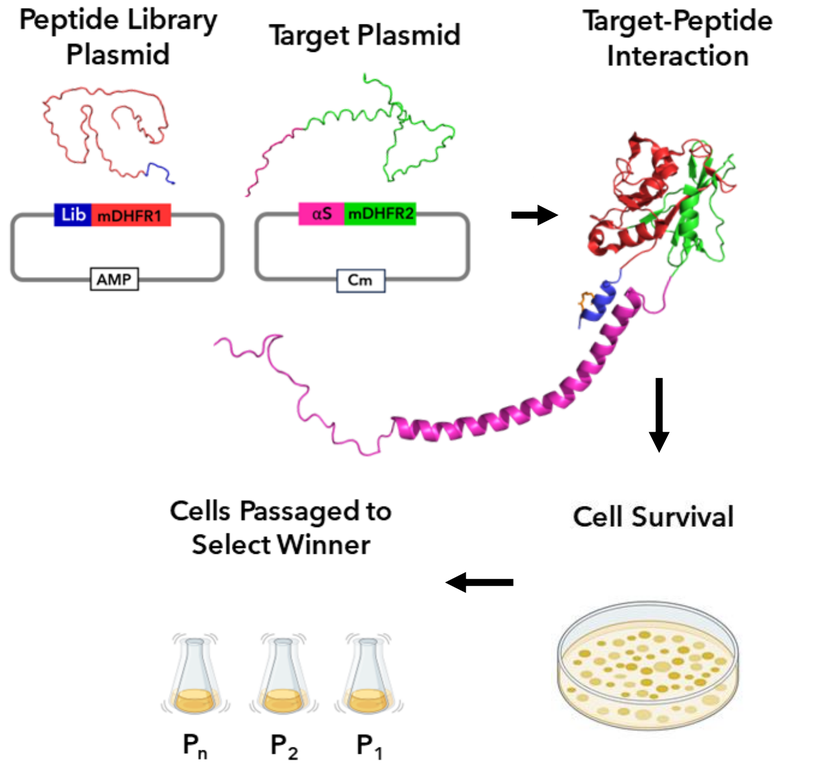


Figure 2. Protein-Fragment Complementation Assay used to screen peptide library.

3. Library Design and Selection of $\alpha S_{2\text{-}12}W$

- A 20-million-member library was designed to generate a charged, polar, and hydrophobic residue options at each position (Fig 3A).
- The library design permits intracellular i→i+4 cyclisation, locking the peptide into an α-helical arrangement by the addition of a peptide stapling agent.

A)

Residues 6 and 10 stapled

	1	2	3	4	5		7	8	9		11
$\alpha S_{2\text{-}12}$ Sequence	D	V	F	M	K		L	S	K		K
Peptide Library Options	D	V	F	M	K		L	S	K		K
	V	I	L	V	I		V	I	I		I
	I	G	V	G	G		M	L	G		G
	N	E	Y	E	V		Q	V	V		V
	E	K	D	R	E		E	G	E		E
	K	R	H	K	R		K	N	R		R
								D			
								R			
								H			
								K			
20,155,392 Library Members								E			
								Q			

- A winning peptide ($\alpha S_{2\text{-}12}W$) was selected after 4 liquid passages, corresponding to the clean pool sequence readout (Fig 3B).

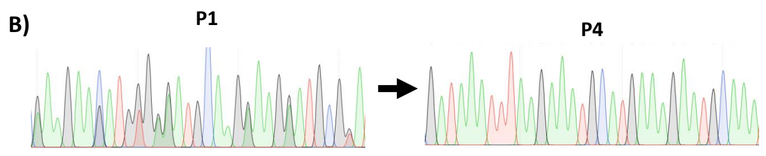
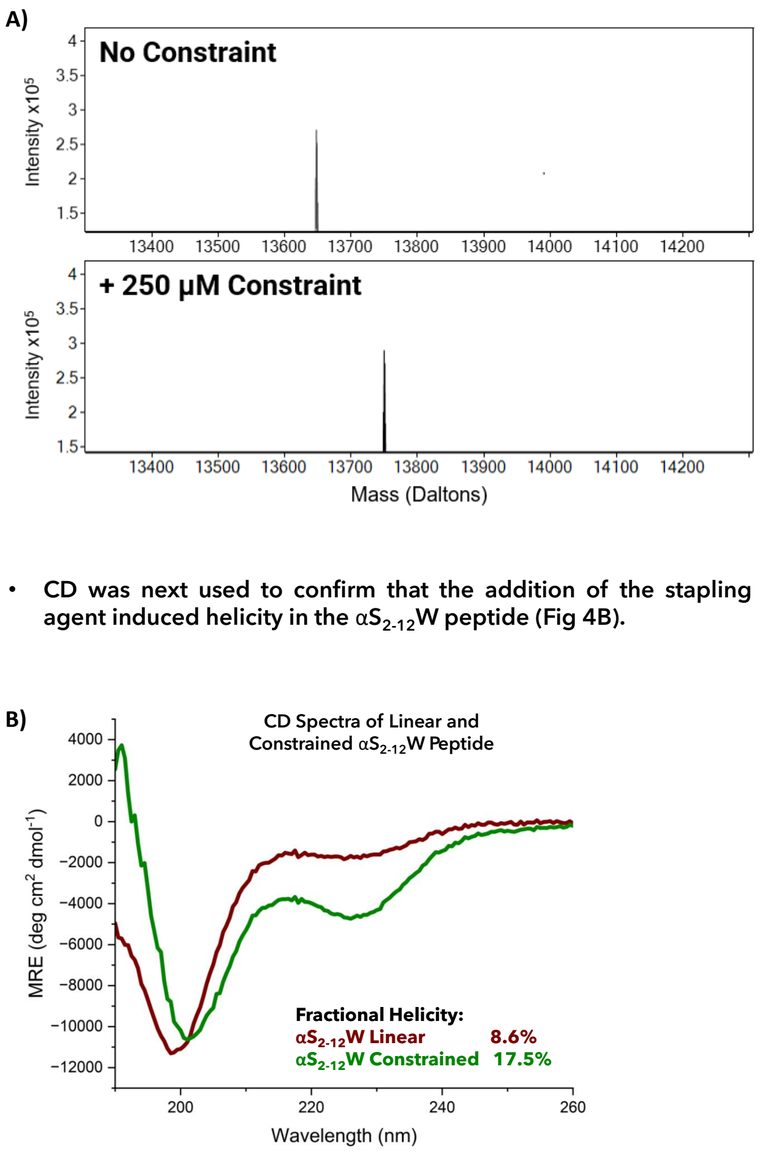


Figure 3. A) Peptide library generated based on the $\alpha S_{2\text{-}12}$ peptide. B) Sequencing shows a clean readout in the fourth passage, indicating a single peptide sequence in the screen.

4. Biophysical Analysis Confirms Intracellular Stapling Induces Helicity

- Addition of an N-terminal SUMO tag facilitated purification of $\alpha S_{2\text{-}12}W$ following bacterial expression in the presence or absence of a constraining agent.
- A mass increase corresponding to the constraint was observed, thus confirming the intracellular cyclisation of $\alpha S_{2\text{-}12}W$ (Fig 4A).



- CD was next used to confirm that the addition of the stapling agent induced helicity in the $\alpha S_{2\text{-}12}W$ peptide (Fig 4B).

Figure 4. A) Intact mass spectrum shows a mass increase equivalent to the addition of the constraint, indicating successful *in vivo* cyclisation of the peptide sequence. B) CD Spectrum confirming induction of helicity in $\alpha S_{2\text{-}12}W$ by the stapling agent.

5. $\alpha S_{2\text{-}12}W$ Inhibits αS aggregation

- ThT fluorescence studies were used to analyse the inhibition of αS aggregation by $\alpha S_{2\text{-}12}W$.
- $\alpha S_{2\text{-}12}W$ is more potent than $\alpha S_{2\text{-}12}$ at substoichiometric concentrations (Fig 5A).
- TEM shows addition of the $\alpha S_{2\text{-}12}W$ peptide significantly reduces the formation of αS fibrils (Fig 5B).

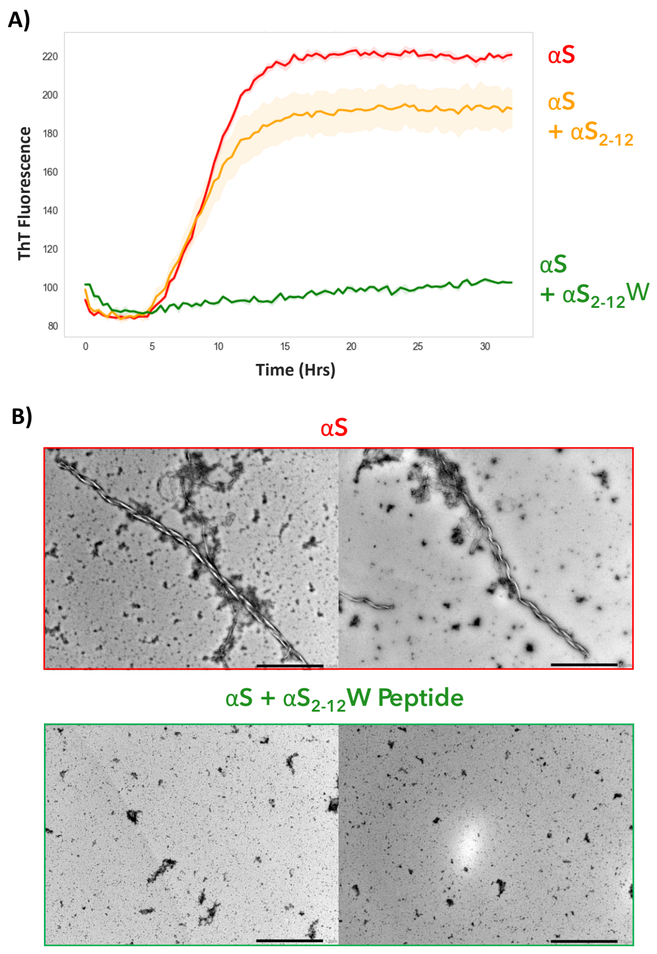


Figure 5. A) ThT assay shows the $\alpha S_{2\text{-}12}W$ peptide hit further improves inhibition from the initial $\alpha S_{2\text{-}12}$ peptide. B) TEM shows reduction in αS fibril formation in the presence of $\alpha S_{2\text{-}12}W$.

6. Conclusions and Next Steps

- We have developed a novel intracellular cyclisation method, utilising the PCA peptide screening platform, to generate a potent inhibitor of αS aggregation.
- The peptide, $\alpha S_{2\text{-}12}W$, inhibits αS aggregation at substoichiometric concentrations.
- Further characterisation for the specific mode of action of $\alpha S_{2\text{-}12}W$ inhibition is currently ongoing using several biophysical techniques.
- We next plan to study the impact of different constraints on the activity of $\alpha S_{2\text{-}12}W$ and collect cytotoxicity data.
- We aim to publish this work in the coming months.

LinkedIn, Contact Details, and Digital Poster:

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7. References

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Background Work