

Supporting information for

The small aromatic compound SynuClean-D inhibits the aggregation and seeded polymerization of multiple α -synuclein strains

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Supplementary Figure 1. SynuClean-D. (A) Chemical structure and (B) absorption spectra of SynuClean-D (5-nitro-6-(3-nitrophenyl)-2-oxo-4-(trifluoromethyl)-1*H*-pyridine-3-carbonitrile) in different buffer conditions. Red area indicates the used excitation and emission wavelength of Th-T in aggregation assays.

Supplementary Figure 2. Fibril morphology. (A-F) Representative TEM images of strain A (A and B), strain B (C and D) and strain C (E and F) end-point aggregates. Magnification is 1500X for images A, C and E, and 10000X for images B, D and F. Scale bars corresponds to 2 μ m (A, C and E) and 200 nm (B, D and F), respectively. Discontinuous line delimited squares in low magnification images A, C and E indicate the grid area selected for medium magnification images in B, D and F. Discontinuous line delimited squares in B, D and F indicate the grid area selected for the high magnification images shown in Figure 1D-F (15000X).

Supplementary Figure 3. Denaturation of strain B and C. Chemical denaturation of mature fibrils of strains B (blue) and C (red) in presence of 4 M urea followed by Th-T fluorescence.

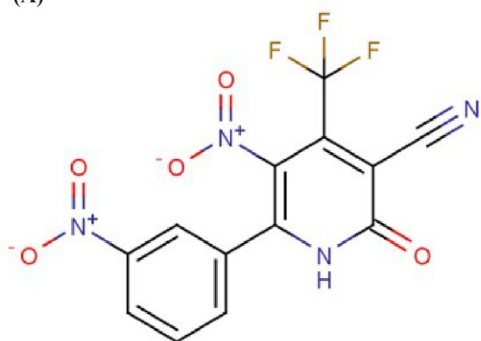
Supplementary Figure 4. Seeds characterisation. (A) Th-T fluorescence measurements of strain B (dotted lines) and strain C (straight lines) fibrils after 0s, 15s, 30s and 60s of sonication. (B) Th-T fluorescence measurements of strain A, strain B and strain C fibrils before (straight lines) and after (dotted lines) 4 rounds of washing with Milli Q water. (C) PK digestion of mature fibrils before and after 4 rounds of washing with Milli Q water. (D) TEM images of mature fibrils before and after 4 rounds of washing with Milli Q water. Scale bars correspond to 200 nm.

Supplementary Figure 5. Effect of different α -Syn strains in aggregation of endogenously expressed α -Syn-EGFP. Representative images from α -Syn-EGFP expressing stable cell line after treatment with buffers A, B and C (-) or the aggregates (+) generated in these solutions, respectively. Scale bar corresponds to 30 μ m.

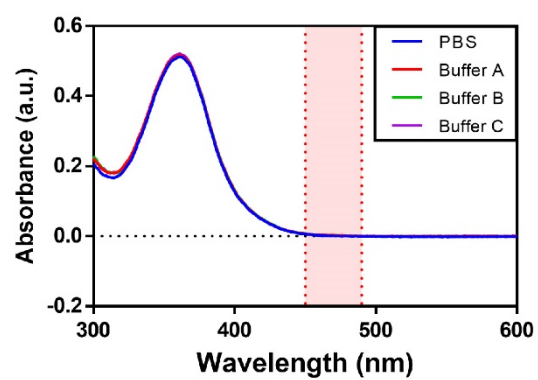
Supplementary Figure 6. SynuClean-D interaction with α -Syn fibrillar structures. General (A) and close-up views (B) of the most favourable SC-D binding with fibrils previously predicted by molecular docking (30). NAC domain is labelled in pink and interacting residues are coloured in blue.

Supplementary figure 1.

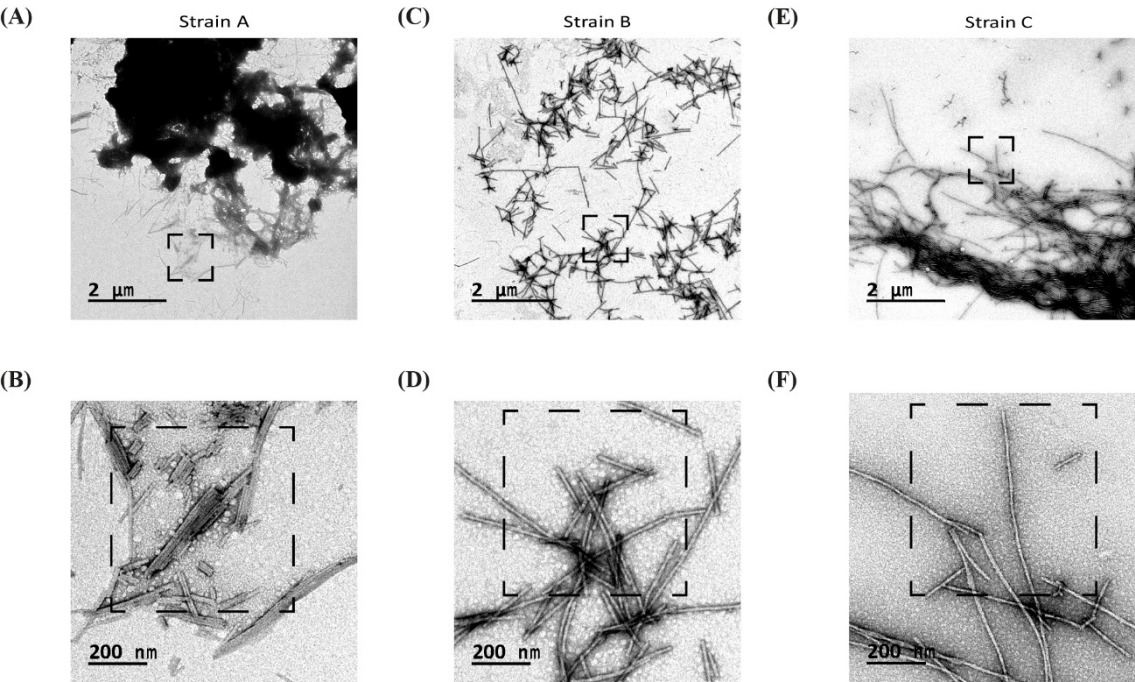
(A)



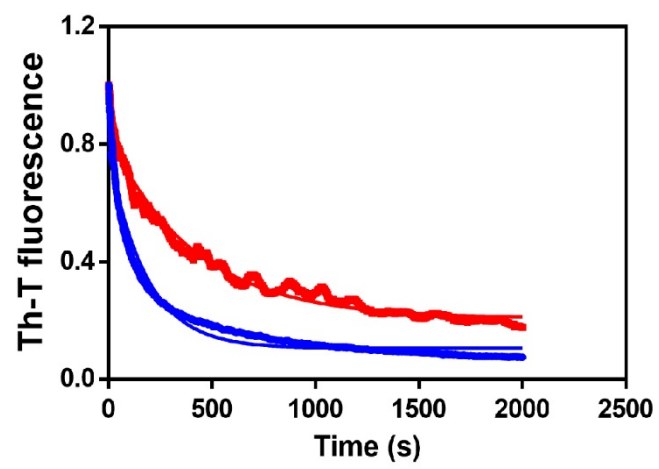
(B)



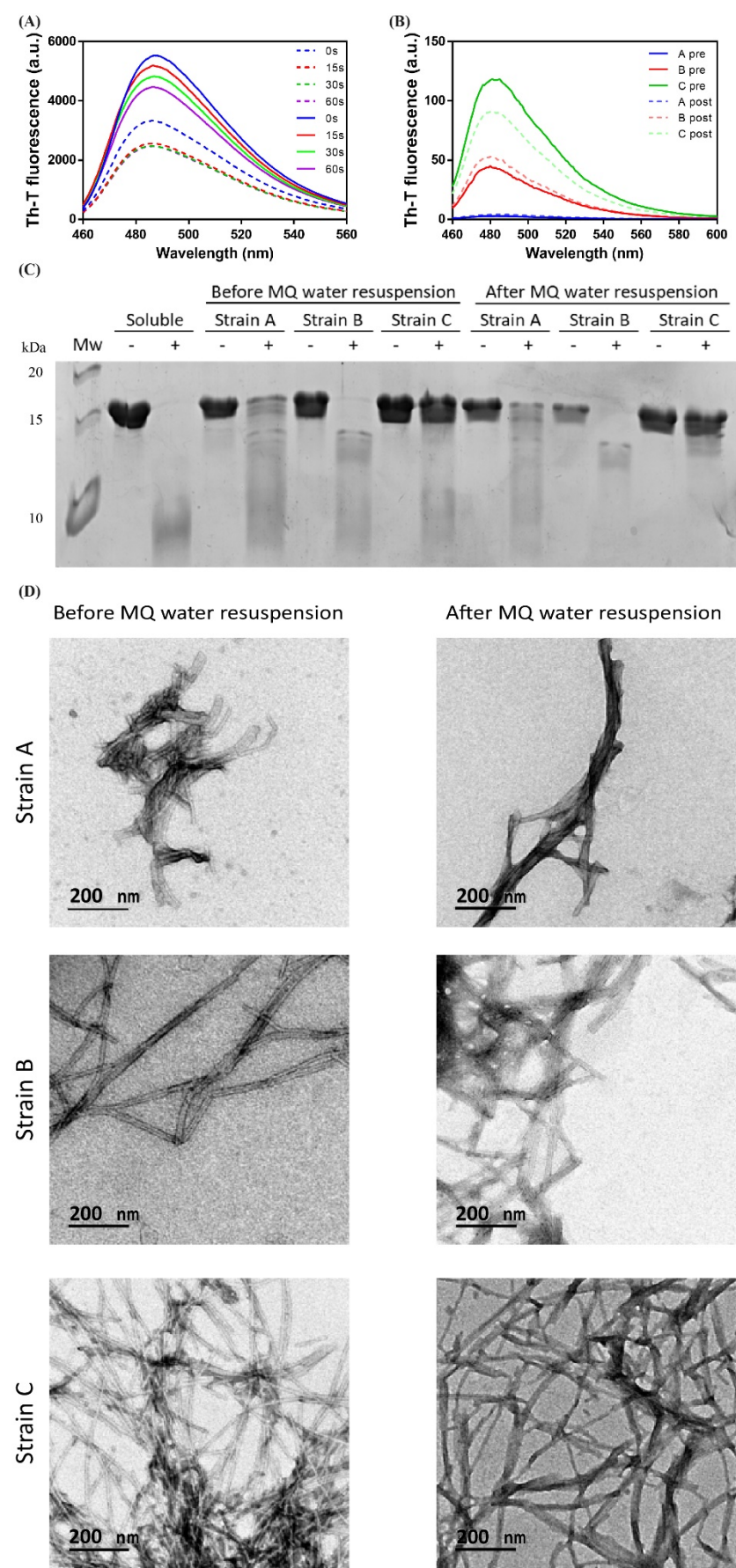
Supplementary figure 2.



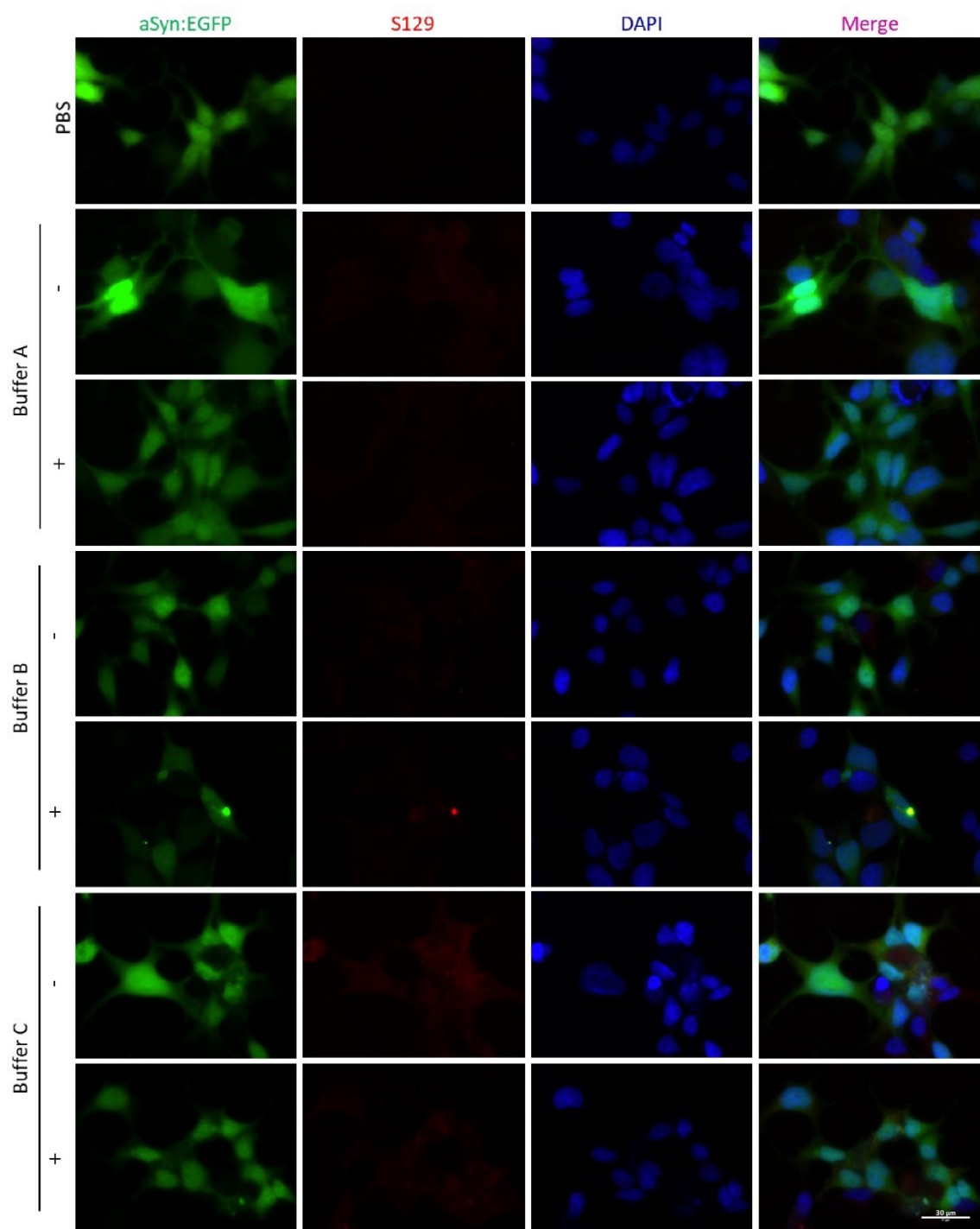
Supplementary figure 3.



Supplementary figure 4.

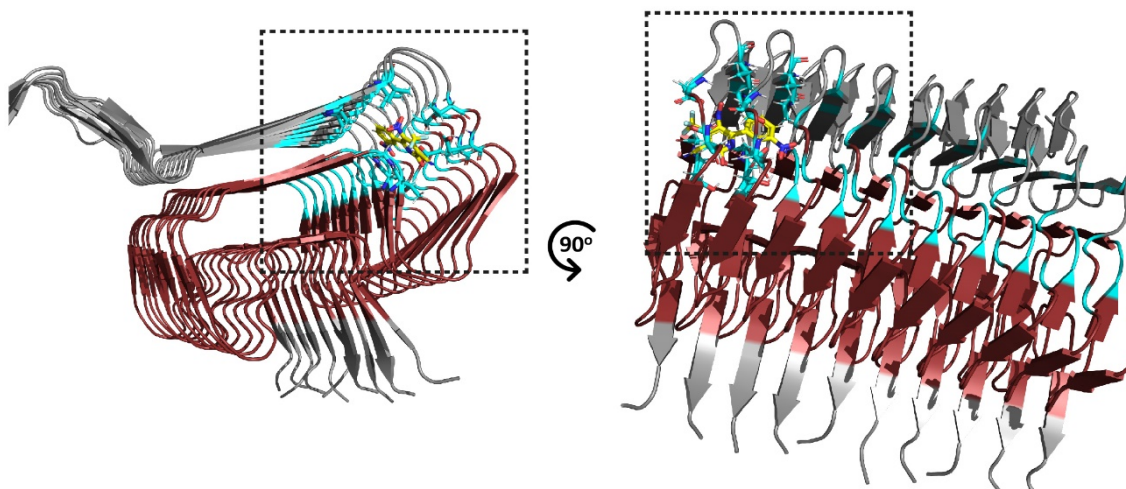


Supplementary figure 5.

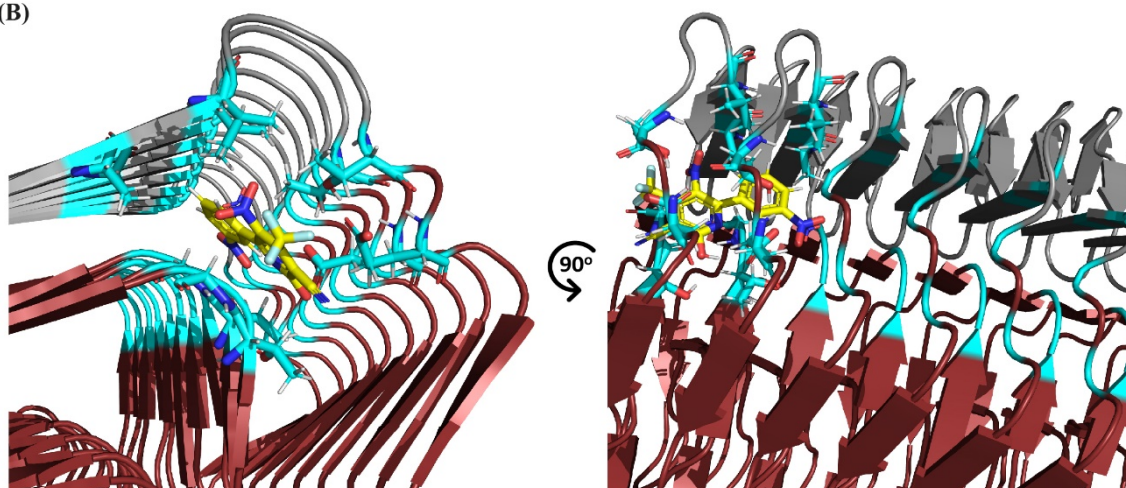


Supplementary figure 6.

(A)



(B)



Supplementary Table 1. Assignment of the secondary structure of aggregated strains. The secondary structure of strains A, B and C aggregates at final point was obtained by measuring ATR-FTIR absorbance in the amide I region, which was acquired and the fitted individual bands after Gaussian deconvolution are indicated

Strain A			Strain B			Strain C		
Band (cm ⁻¹)	Area (%)	Structure	Band (cm ⁻¹)	Area (%)	Structure	Band (cm ⁻¹)	Area (%)	Structure
1628	33	β-sheet (inter)	1627	39	β-sheet (inter)	1624	43	β-sheet (inter)
1645	28	Unordered	1647	28	Unordered	1643	23	Unordered
1666	32	Loop/β-turn	1666	25	Loop/β-turn	1662	25	Loop/β-turn
1686	8	β-turn	1683	8	β-turn	1681	9	β-turn