Reducing the amyloidogenicity of functional amyloid protein FapC increases its ability to inhibit α-synuclein fibrillation

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Supplementary Information S1
**Figure S1**

**FapC and FapC ΔR1R2R3 protein sequences.** In both sequences the 24 residues signal peptide has been removed and a C-terminal 6xHis-tag has been included (shown in bold). The three imperfect repeats in the wild-type FapC protein are colored blue (R1), green (R2) and orange (R3), respectively. The Cys residues of the C-terminal CXXC motif are colored red.

| Protein          | Amino acid sequence                                                                 | MW     |
|------------------|-------------------------------------------------------------------------------------|--------|
| FapC             | GPAEKWKPTP10APGTVAAV20TDTQVSDKNK30FDDTALKNN40GANGLSLNSK50GNLGANIAAG60SGNQQDNAA70TSSAGDAAT80VF| 23.6 kDa|
|                  | AVADIYQAV50SKDNKFTNKG100TQNNALLNS110ANNSSGNVG120NVAAGQGNNQQ130KNNLAIVTAD140GKNVAAASNT150EQVSLDNHFL160NEASSKHSYK170PQYVNNAGL180LNSANNASG190IGVNVAAGAG195NQQSNLTLAG210SGCTVCAAGT220GSKLAFLLEHHHHHH |
| FapC ΔR1R2R3     | GPAEKWKPTP10APGTVAAV20TDTQVSDKNK30FDDTTLTSS40AGDAATVFAVA50ADYQESKDN60KFTNKGTVQVT70ADGKNVAAAS80NTEQVSLDNH90FLNEASSKHS100YPQYVVGLS110GCTVCAAGT120GSKLAFLLEHHHHHH |
|                  |                                                                                     | 13.9 kDa|
**Figure S2**

**Dot blot setup.** Baits of $\alpha$-SN monomers (M), oligomers (O) and fibrils (F) (orange spheres) were immobilized on nitrocellulose membranes in decreasing concentrations and allowed to dry. Then FapC/FapC ΔR1R2R3 (blue sphere) labelled with Alexa 546 (pink star) was added to detect binding of these proteins to the baits.

| BAIT: | $\alpha$-SN |
|-------|-------------|
| 2 µg  | M O M       |
| 400 ng| M O M       |
| 200 ng| M O M       |
| 100 ng| M O M       |
| 50 ng | M O M       |
| 25 ng | M O M       |

- BSA background
- Bait protein
- Alexa 546-label
- Protein probe
Both FapC and FapC ΔR1R2R3 recognizes its own and the other protein’s monomers. Decreasing concentrations of FapC and FapC ΔR1R2R3 monomers (M) and fibrils (F) were immobilized on nitrocellulose membranes and the amounts of membrane-bound protein were visualized using Ponceau S before further analysis.
No inhibitory effect on $\alpha$-SN fibrillation is seen when $\alpha$-SN is incubated with FapC. Fibrillation of 1 mg/mL $\alpha$-SN (69 µM) was followed with ThT in the presence of FapC concentrations ranging from 0.004 mg/mL (0.2 µM) to 1 mg/mL (42 µM). The experiment was repeated three times.
**Figure S5**

FapC ΔR1R2R3 forms disulfide-bonded higher-order species that increase in size during incubation. **A**) 2 mg/mL FapC ΔR1R2R3 (145 μM) was run on SEC right after desalting (no shaking, black curve) and after shaking (4.25 hours, 37°C, 700 rpm) either with (blue) or without DTT present (grey). **B**) 4 mg/mL α-SN incubated with FapC ΔR1R2R3 in a 1:0.5 ratio in the presence of DTT (light green) had no effect on α-SN oligomer formation and simply resulted in an elution profile that was a combination of the individual spectra for α-SN (black) and reduced FapC ΔR1R2R3 (dark green). Numbers represent the precise elution volumes (in ml) obtained from Gaussian fitting to the elution profiles.
FapC ΔR1R2R3 fibrils formed with or without DTT present are equally stable towards urea. FapC ΔR1R2R3 and FapC fibrils were A) investigated with FTIR and B) incubated with different concentrations of urea (0 M or 8 M) to test differences in fibril stability.

Lane 1: protein ladder, lanes 2-3: 1 mg/mL freshly desalted FapC ΔR1R2R3, lanes 4-7: FapC ΔR1R2R3 fibrils, lanes 8-11: FapC ΔR1R2R3 fibrils formed in the presence of DTT, lanes 12-15: FapC fibrils.
Both the three repeats and the two linker regions contain conserved Asn, Gln and Ala residues. A) Comparison of protein, repeat and linker lengths and B) sequence alignment of different Pseudomonas species. Parentheses represent accession numbers. The three repeats are shown with black boxes and fully conserved linker residues are shown in red boxes. Abbreviations correspond to: P. putida (Pput), Pseudomonas sp. UK4 (UK4), Pseudomonas sp. FH1 (FH1), P. aeruginosa PAO1 (PAO1), P. fragi (Pfra) and P. protegens (Ppro).