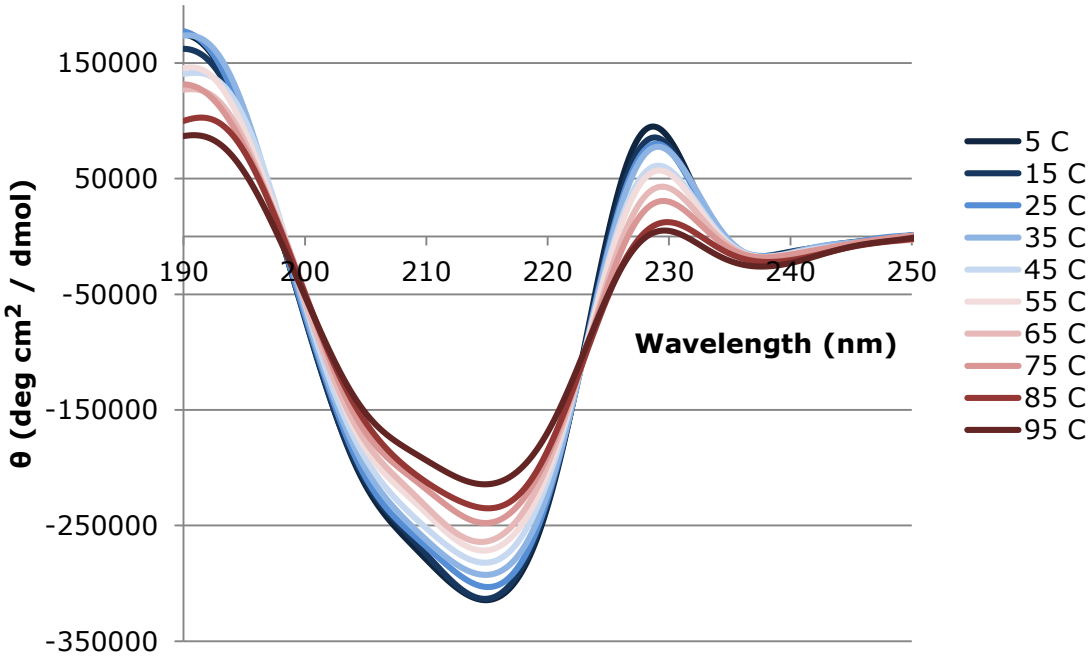
Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING MATERIAL

Characterization of cp-WW2

The cp-WW2 sequence also forms a hairpin which was characterised by both NMR and CD. The CD displays the expected exciton couplet about 223 nm, with a ~228 nm CD maximum (Figure S1, immediately below).



The 2D NMR indicated that this hairpin had some interesting characteristics. The Trp pair, located near the termini, is acting as a β-cap with the C-termini Trp being the edge in the EtF (edge-to-face) interaction. This is indicated by the large upfield shift of the C-termini's Trp Hε3. The shift at this probe site is even more upfield that what is observed for WW2 and for cyclo WW2 (Figure S2).

The larger upfield shift for the Hε3 proton in cp-WW2 in comparison to cyclo WW2 may be a reflection of the cyclised hairpin being in a very rigid conformation. In cp-WW2, the Trp/Trp pair has some room to 'wiggle', finding the optimal position for the Trp/Trp interaction to occur.

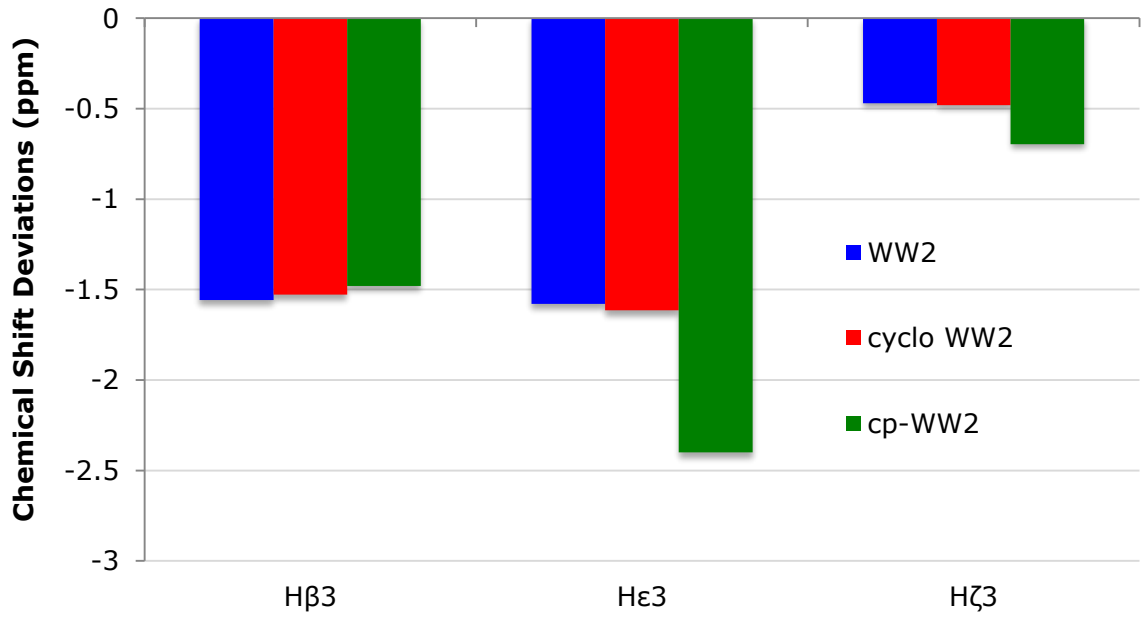


Figure S2. Diagnostic ring current shifts for the edge Trp in the WW2 series.

Additional examples of CD spectral monitoring of the inhibition of β-structuring of α-syn.

These appear as panels of Figures S3. Figure S3 shows partial inhibition of β-structuring by 2 molar equivalents of EGCG in panel **B**. The absence of significant inhibiton by El-Agnaf's peptide inhibitor (RGAVVTGR) at both 2 (panel **A1**) and 4 (panel **A2**) molar equivalents is also shown in Figure S3. In each case, the CD spectrum recorded immediately after HFIP addition (t = 0), shortly thereafter and after the usual 14 – 18 h incubation that afford the full β CD signal in uninhibited control experiments are show.

Part C of Figure S3 shows the time course of CD changes in the presence of 2 molar equivalents of RW-HCH-WE, and in the lower panel, one equivalent of YY-μPro. In both panels, the CD spectrum before and immediately (t=0) after HFIP addition is shown as well as time points during and late in the incubation.

Figure S3.

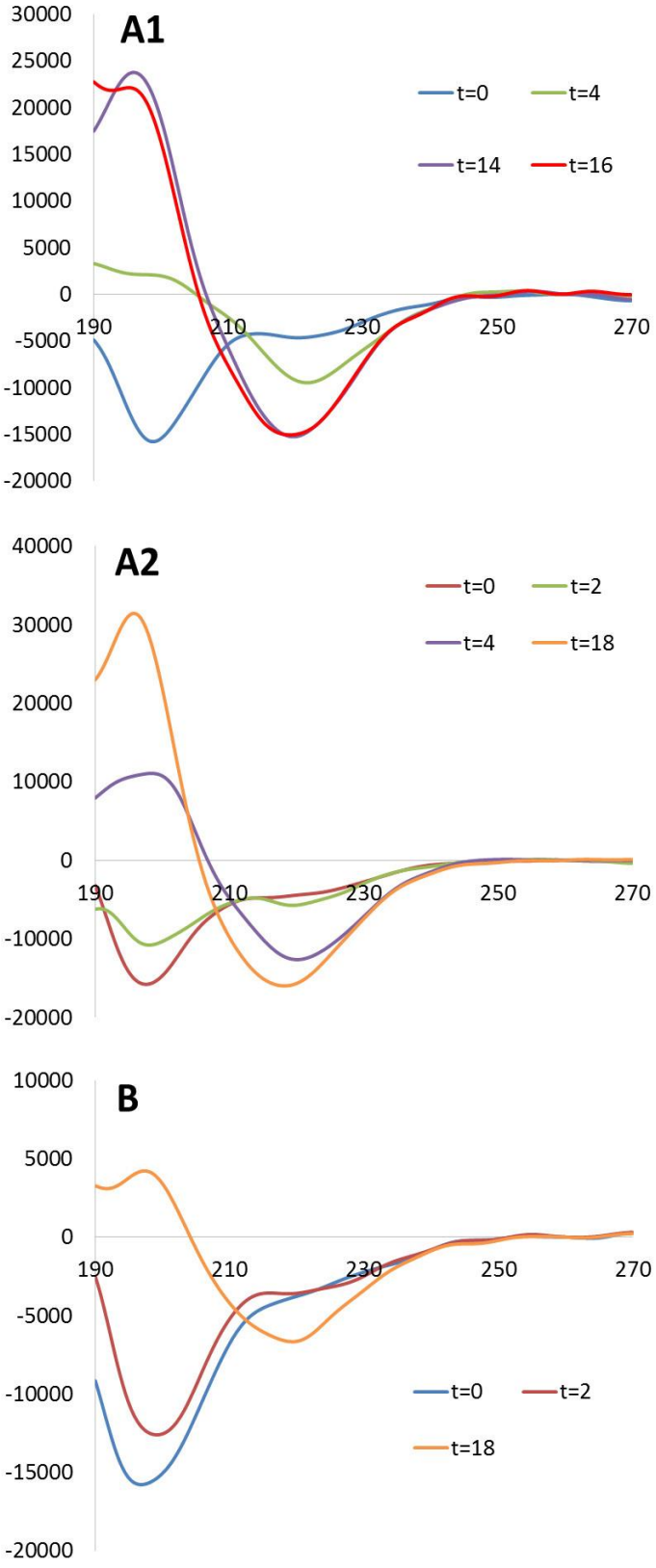


Fig. S3. Additional CD assays of β-structure formation inhibition, in each case the time course is shown. Very little inhibition is observed in panels **A1** and **A2**.

Panel **A1** – 2 equivalents of RGAVVTGR.

Panel **A2** – 4 equivalents of RGAVVTGR.

Panel **B** – 2 equivalents of EGCG.

Panels **A1** and **A2** of **Fig. S3** show initial formation of β-structure 4 hours after the pulse of HFIP. This was also the case for the "uninhibited control" spectrum in panel **A** of **Figure 2**. The time course of enhanced ThT fluorescence at 482 nm (Figure **S4** panel **A**) also shows this temporal feature. It would appear that even at the earliest detected stage of β-structure formation, long before the appearance of visible fibril structures, the β-oligomers have the cross-β structure that produces the red-shift and enhanced ThT fluorescence of bound ThT that is diagnostic of amyloid fibrils.

Figure S3 C.

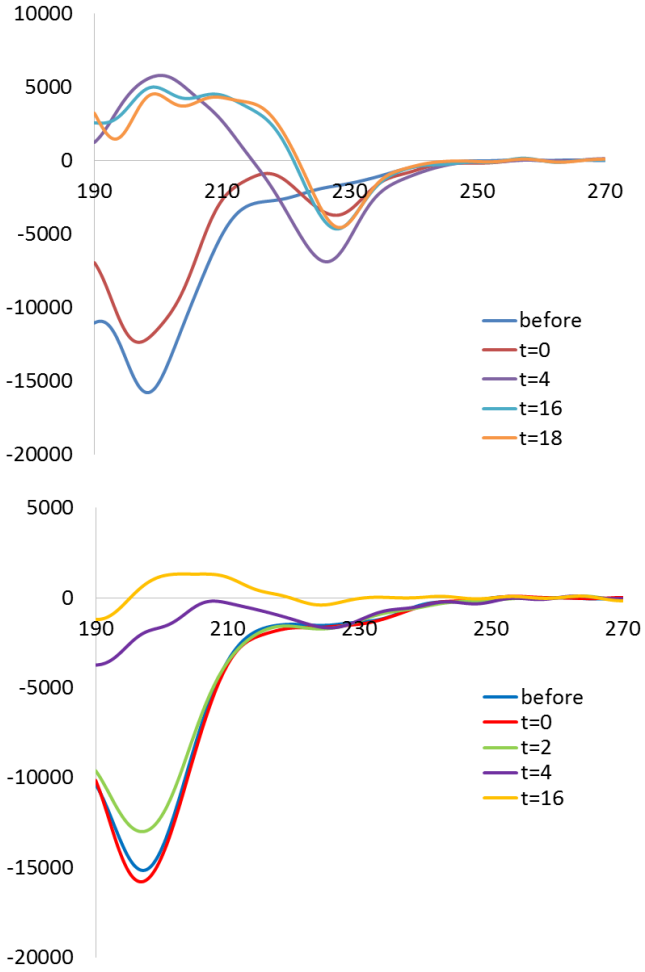


Fig. S3C. Additional CD assays of β-structure formation inhibition, in each case the time course is shown. The top panel shows RW-DS-WE as the inhibitor. The spectrum at the end shows that the RW-DS-WE has also precipitated from the solution. The lower panel shows the CD time course with 1 molar equivalents of YY-μPro added.

Figure S4 A, B. The time course of ThT fluorescence for ThT/α-syn mixtures (32 μM ThT and 4.5 μM α-syn initially) with and without the addition of HFIP to a 2-vol % level. Left panel (**A**) – a comparison of the time course of A_{482} values with a without the addition of HFIP. Right panel (**B**) – the emission spectrum (with 450 nm excitation) recorded at the t = 0, 2, 4, 6, 16 and 18 hour points for the HFIP-containing sample in panel **A**.

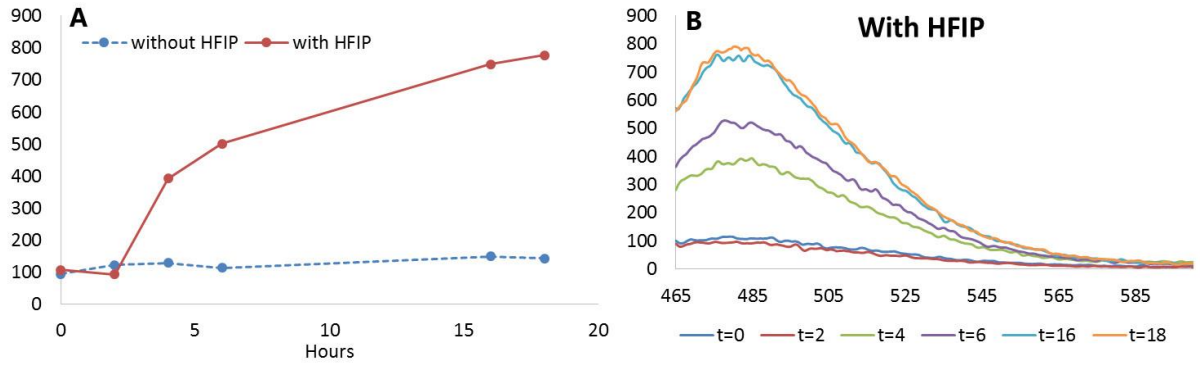
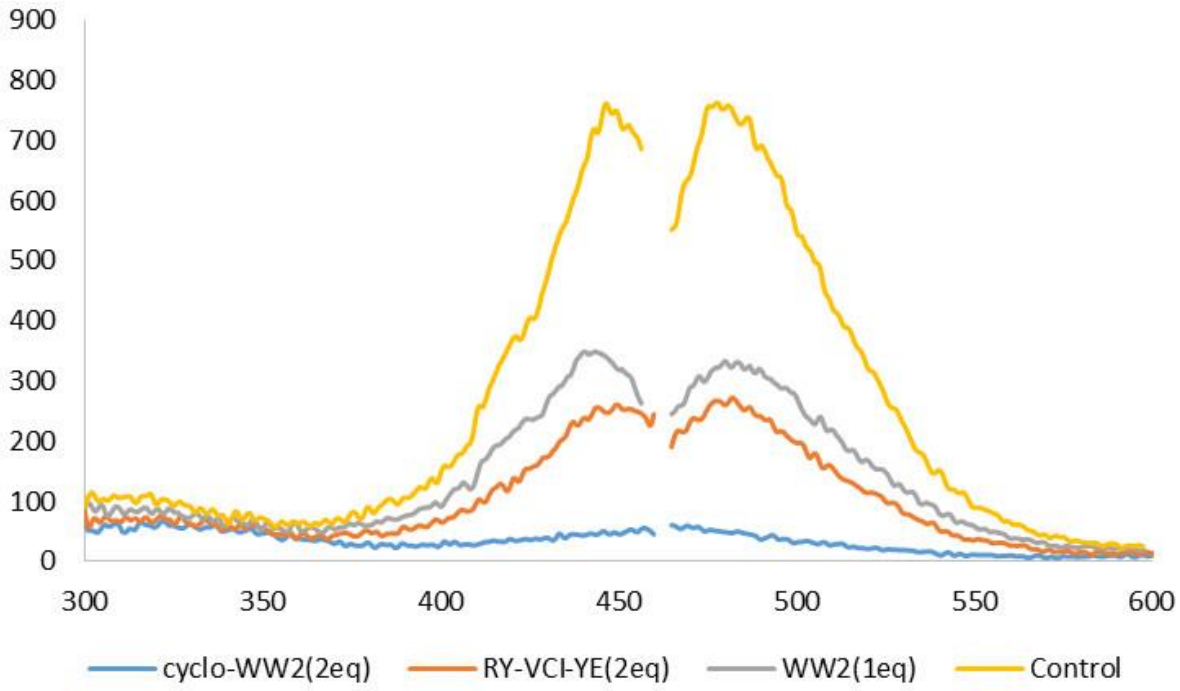


Figure S4 C. Representative ThT excitation and emission spectra at the 18-h post HFIP-addition point of aggregation assays without (the control) or with the addition of 1 or 2 molar equivalents (versus α-syn) peptide inhibitor as specified in the internal figure legend.



Additional spectra illustrating peptide binding effects (or the lack of them) on the spectrum of α-syn.

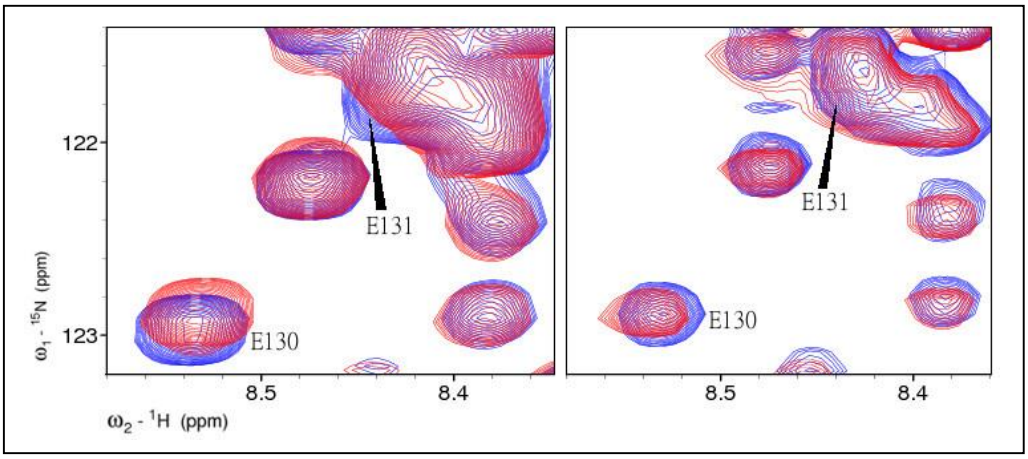


Figure S5. A comparison of the shifts in the ^{15}N-HSQC spectral segment of 400 μM α-syn including the E130 and E131 resonances: the left panel shows the data for peptide WW2 addition, **blue** with 0.6 equivalents of WW2, **red** with 1.5 equivalents of the peptide, the right panel shows cp-WW2 addition effects (**blue**, prior to peptide addition; **red**, 0.6 equivalents of peptide added).

Addition of YY-μPro to 200 μM α-syn at 303K. The sequence of additions and incubation times was: adding 0.5 molar equivalents of peptide, after 2 h the peptide:α-syn ratio was brought to 1.5:1. Spectra recorded every 2 hours over a 10 h incubation at 303K. At this point, HFIP was added to a final 1.5 vol-% composition. Spectra were recorded immediately and after an additional 24 h of incubation.

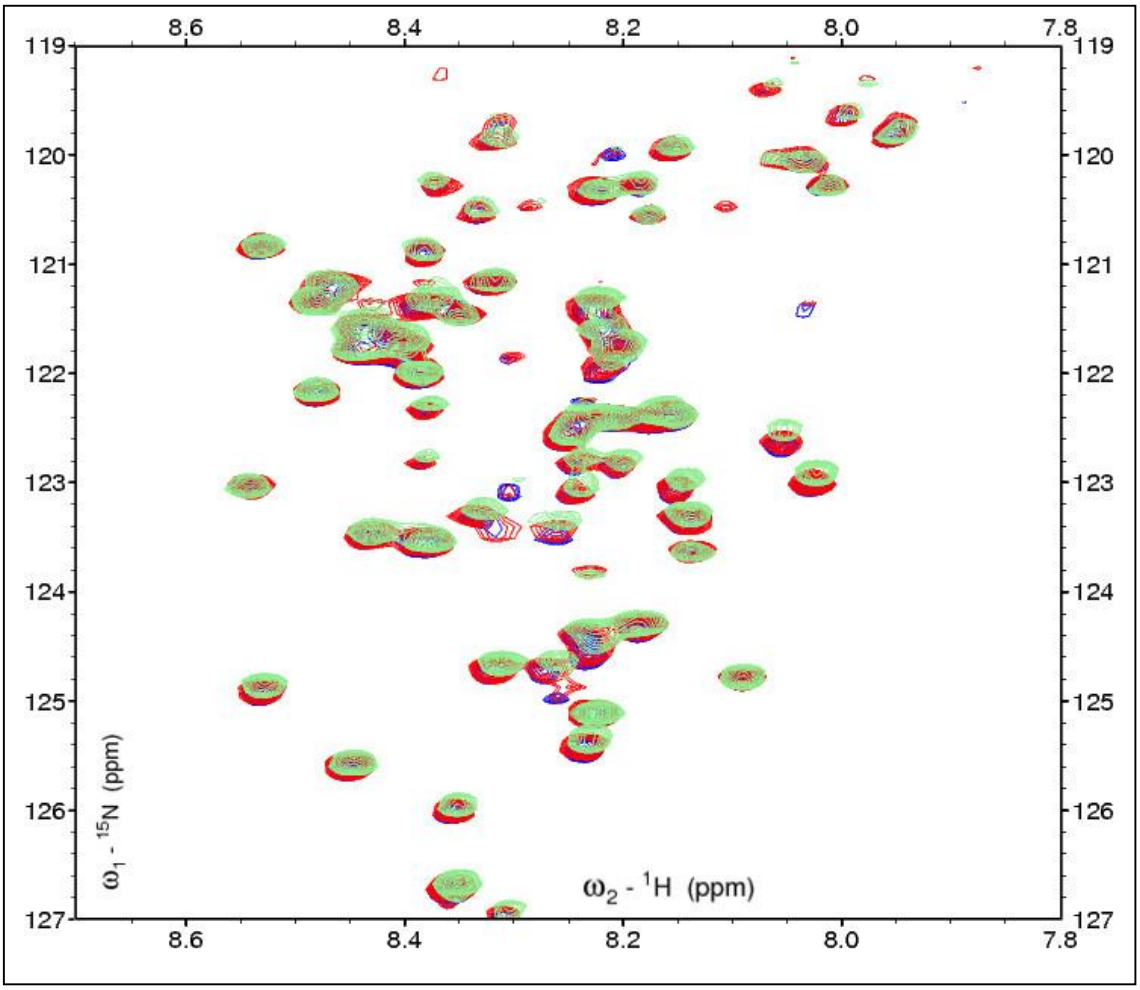
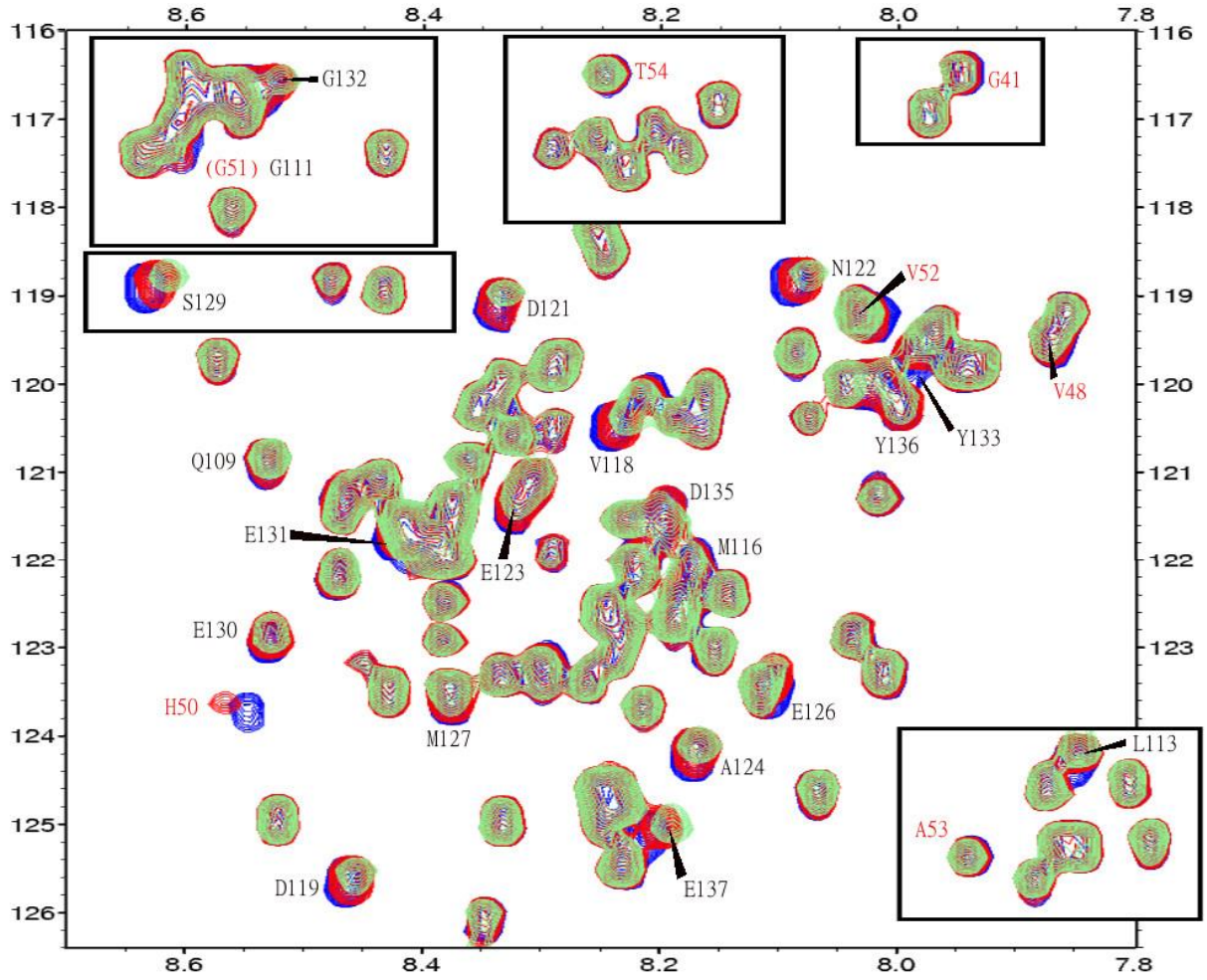


Figure S6. The central section of the ^{15}N-HSQC spectra recorded prior to YY-μPro addition (**blue**), at a 1.5:1 peptide:α-syn ratio (**red**), and immediately after HFIP addition (**green**) are overlaid with the A140 resonances perfectly aligned.

There were no significant titration shifts (**blue** vs **red**). While some peaks were absent from the onset (notably H50 and S9), incubation with YY-μPro led to the partial reappearance of some of these rather than further peak losses due to β-oligomerization. Of note, there was also no further peak attenuation over the 24 h period after HFIP addition. Both of these observations imply inhibition of the usual β-oligomerization pathway.

Additional studies of Trp-bearing β-peptides

Figure S7, panel **A**. The 800 MHz HSQC spectra of 200 μM α-syn prior to (blue) and after the addition of 0.6 (red) and 1.2 molar equivalents (green) of cyclo-WW2 at 293 K. The enboxed segments are from spectral regions outside of that shown by the axis labels.



All of the large shifts observed in the Q109 – E137 span are labelled in black and are very similar, in both relative magnitude and direction, to those observed with WW2 (see Fig. 6 and Fig. S5). At this temperature the **H50** signal is retained and displays a large titration shift (and significant signal diminution) for the first cyclo-WW2 addition. With further addition of cyclo-WW2 this peak disappears from the spectrum (Fig. S7B). Smaller titration shifts were observed for other signals in the G41 – A53 residue span; these are labelled in red and suggest an additional binding locus or, less likely, long-range interactions with the peptide bound in the C-terminal site.

The continuation of this experiment, titration to 2.2 equivalents of added cyclo-WW2 and the effects of HFIP addition, appears in Figure 7B.

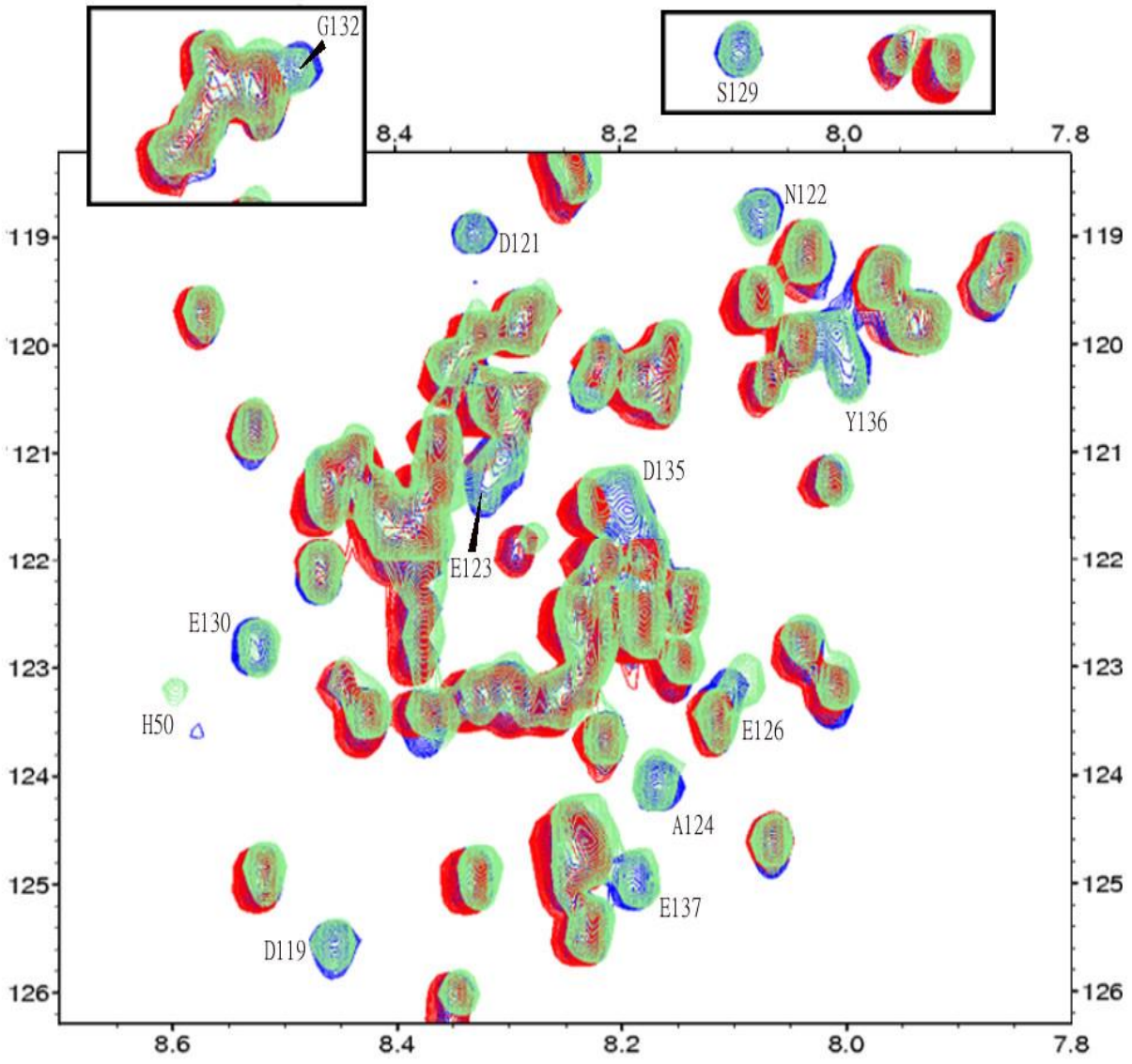


Figure S7B. Blue signals are the last spectrum from Fig. S7A (with 1.2 equivalents of cyclo-WW2. The red spectrum was recorded with 2.2 equivalents of added peptide; the green spectrum is the same sample after adjusting the HFIP content to 1.5 vol-%. Signals that are absent at 2.2 equivalents of cyclo-WW2 that reappear upon HFIP addition, blue and green peaks but no red peaks, are labelled.

All of peaks that had displayed large shifts in the presence of 1.2 equivalents of cyclo-WW2 were attenuated to the point of disappearing from the spectrum when the amount of cyclo-WW2 was increased to 2.2 equivalents. These peaks re-appeared at their prior locations upon HFIP addition.

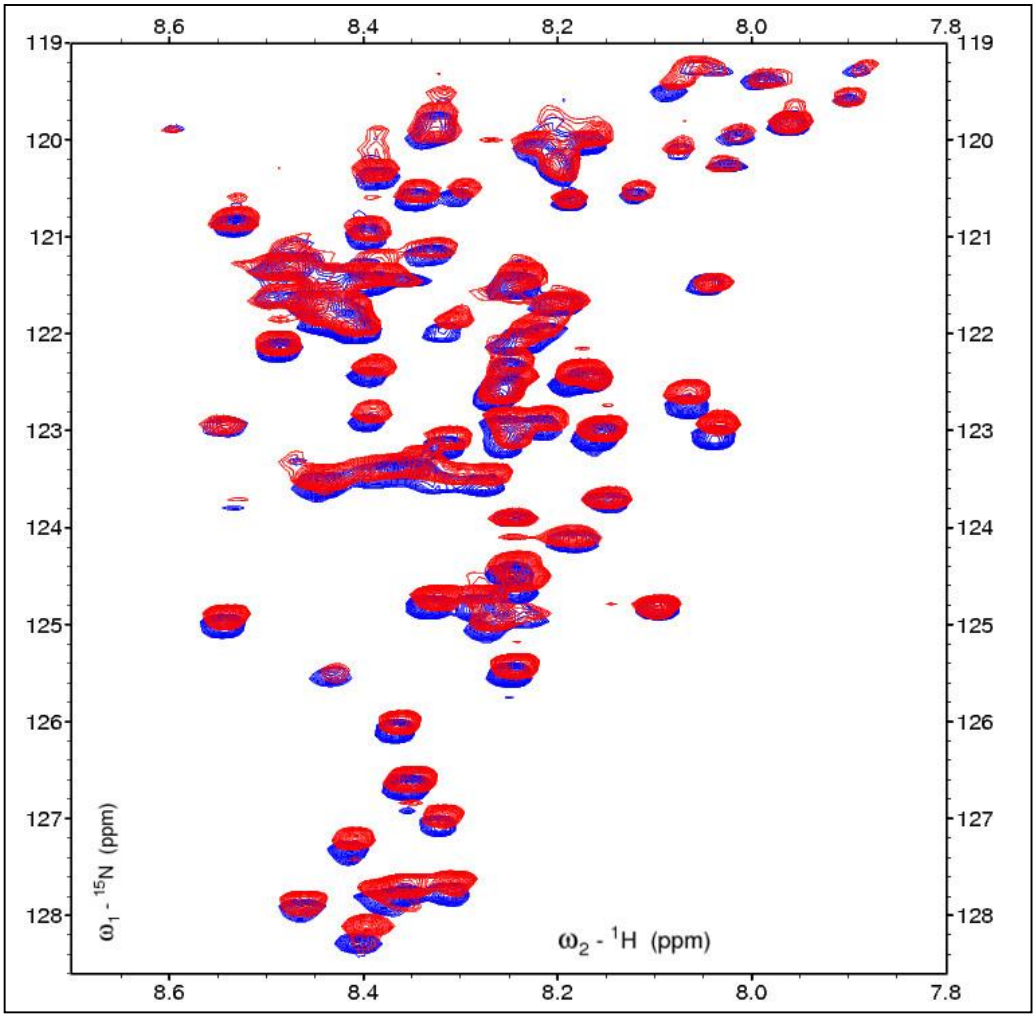


Figure S8. HFIP-induced (addition to 1.5 vol-% solvent composition) shifts observed for the 1.5:1 RW-HCH-WE/α-syn NMR sample: blue just prior to HFIP addition, red, within an hour after addition.

The largest shifts upon HFIP addition appear to be associated with alanine and valine residues; but we could discern no sequence location pattern regards to HFIP induced shifts and there total absence for some signals. In the majority of the cases, the shifts are similar to those observed for α-syn samples containing added WW2-related peptides or YY-μPro (Fig. S6). While transient helix formation in the presence of HFIP is a possibility, the lack of sequence selectivity for the HFIP-induced shifts would argue against this hypothesis. The partial reversal of inhibitor-binding associated shifts upon HFIP addition observed in a number of cases could represent a conformational shift, but can also be explained as a decrease in binding affinity as the medium becomes more solubilizing for the hydrophobic sidechains of the peptide inhibitors.

Studies of EGCG binding

At 200 μM α-syn (50mM NaCl in 50mM Potassium Phosphate buffer, pH 6.5) with 5 molar equivalents of EGCG added, the time window prior to precipitation and β-oligomer formation was sufficient to allow the collection of ^{15}N HSQC data. An overlay of that spectrum with an α-syn spectrum collected on another day (as the starting point of a titration experiment, *vide infra*), appears in Figure S9. We also observe many peaks that move upfield, but these shifts ($\Delta\delta(^1H)$ = 0.007 - 0.010 ppm) were much smaller than those in the prior literature spectra[11]. These shifts are based on aligning the C-terminal A140 peak perfectly (as shown); with this cross-calibration there were also no significant shifts, $\Delta\delta(^1H) < 0.004$ ppm and $\Delta\delta(^{15}N) < 0.04$ ppm, for the sidechain $CONH_2$ resonances (one is illustrated) and for circa 15 backbone sites.

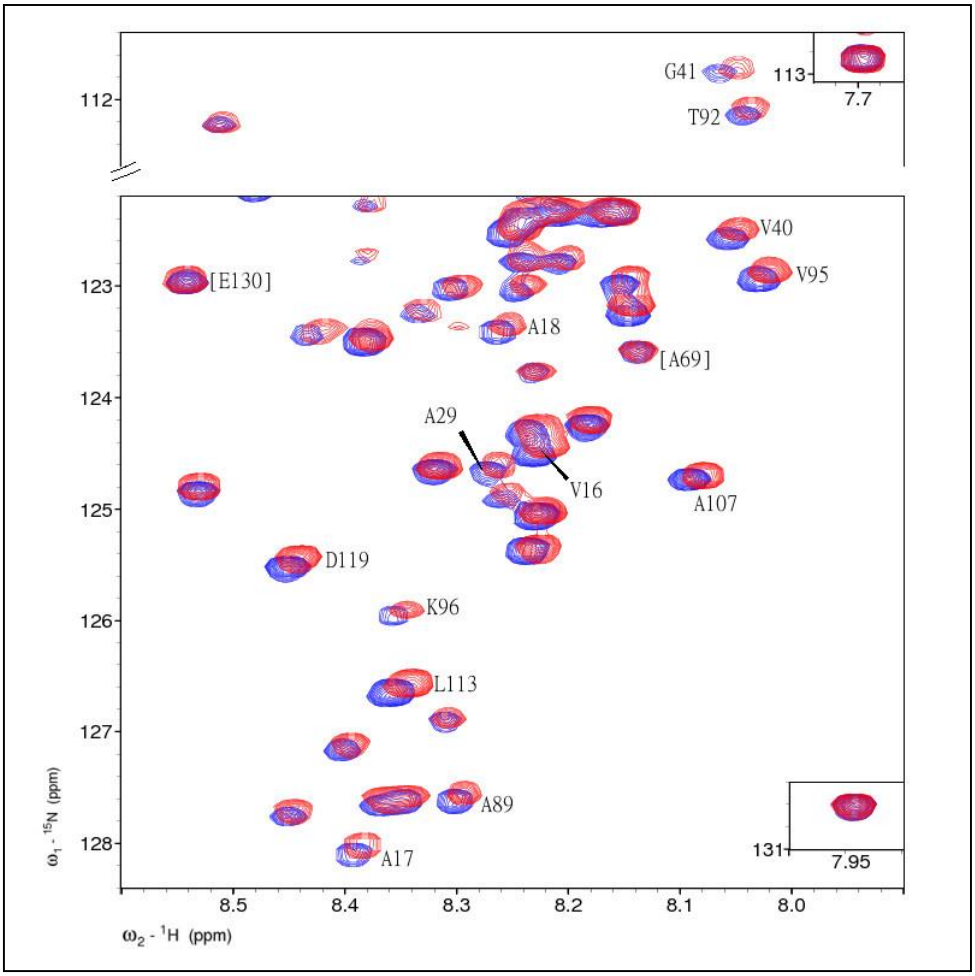


Figure S9. Segments of the HSQC spectrum of ^{15}N-α-syn in the absence (**blue**) presence of 5 molar equivalents of EGCG (**red**).

We turned to a direct titration at lower EGCG/α-syn ratios to ascertain whether there were loci of higher affinity binding for EGCG (Figure S10). None of the titration shifts at 1.5:1 EGCG/α-syn were as large as those we observed for 0.6:1 "WW2-peptide"/α-syn mixtures: the larger shifts (maximally ~ 0.008 ppm in the ^{1}H dimension, and/or ~ 0.07 ppm in the ^{15}N

dimension), indicated by underlining in the shifted resonance list that follows: A17, A18, T22, A27, E28, G31, T33, V37, L38, V40, G41, T44, V48, A53, T54, V82?, E83, T92, G93, K96, K97, G101, Q109, E110, I112, L113, D115, D119, D121, A124, Y125, E126, S129 and E137. These were not clustered in a single sequence fragment. The comparison, and the determination of whether titration shifts are occurring, is complicated by the serial nature of the experiment. With increasing time since dissolution of the α-syn sample, a number of peaks display some intensity attenuation; this and minor changes in peak shape can appear as a titration shift for the smaller peaks; G93 in the figure below illustrates this.

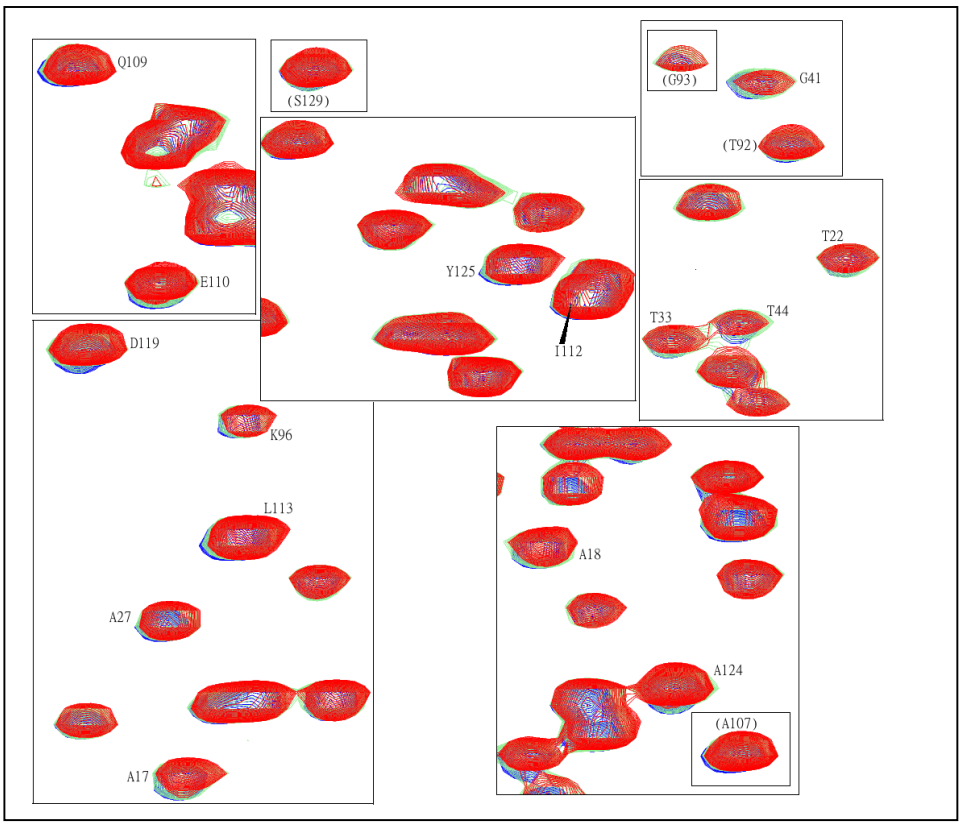


Figure S10. Alpha-synuclein shift changes upon EGCG addition: 200μM α-syn with the color code - blue (no added EGCG), cyan (1 equiv. added), red (1.5 equiv. added).

Virtually all of the α-syn HSQC peaks were still present in the 1.5:1 EGCG/α-syn sample 2 hours after preparation (the exception were the S9, K10, A11, K43, and H50 signals); although a number of the peaks that display diminished intensity in Panel C of Fig. 4 were also somewhat attenuated at this point. We followed the course of further changes upon addition of HFIP to a 1.5 vol-% level. No precipitation occurred over the next 96 hours; a comparison of segments of the spectra obtained immediately after HFIP addition and 96 hours later appear in Figure S11. Peaks that had "completely disappeared" by 96 hours are labelled without parentheses in Figure S11. The complete disappearance of the G31,68,86,93 as well as

T22,33,44 and A17 signals was evident in other spectral segments. Many of the peaks of other residues situated in the A17 – K102 span were still observed at the level cut-off in Figure S11 but had reduced intensity; examples of this in Figure S11 are shown by parenthetic residue labels.

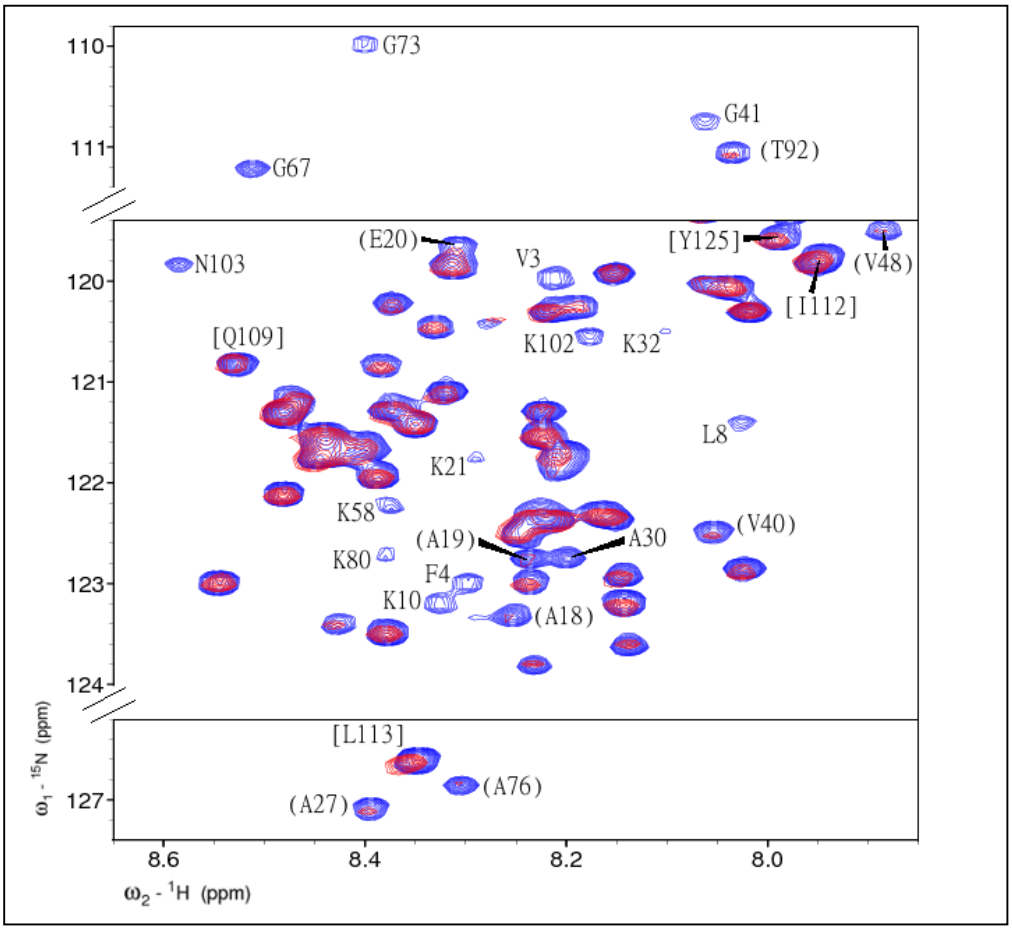


Figure S11. Peak attenuation observed during a 96 h incubation in 2 vol-% HFIP of 200 μM α-syn with 1.5 molar equivalents of EGCG present. The spectrum at 96 h post HFIP addition (red) is compared to spectrum taken directly after the addition of HFIP.

Without exception the peaks of attenuated intensity both at the early time point and at the last time point in Figure S11, corresponded to peaks that disappeared early and somewhat later with HFIP-induced α-syn β-structuring in the absence of an added peptide or EGCG.

In addition, there were time dependent changes in chemical shifts for some residues in the C-terminal segment of α-syn after HFIP addition. These were the same residues that had displayed relatively large titration shifts on EGCG addition in the absence of HFIP (see Figures S9 and S10). In each of these cases, Q109/I112/L113/Y125 are illustrated in Figure S11, the shift change upon standing after HFIP addition was in the opposite direction of the α-syn shift changes observed upon EGCG addition. Two possible rationales for this observation can be suggested: that the binding avidity decreases upon HFIP addition, and that the spectrum for C-terminal residues increasingly reflects the flexible tail region of the β-oligomers or protofibrils.