

Supplementary Material

The pathological G51D mutation in alpha-synuclein oligomers confers distinct structural attributes and cellular toxicity

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[†] Dedicated to the memory of Prof. Sir Christopher Dobson FRS, who helped and inspired us over many years and who is missed greatly by all who knew him.

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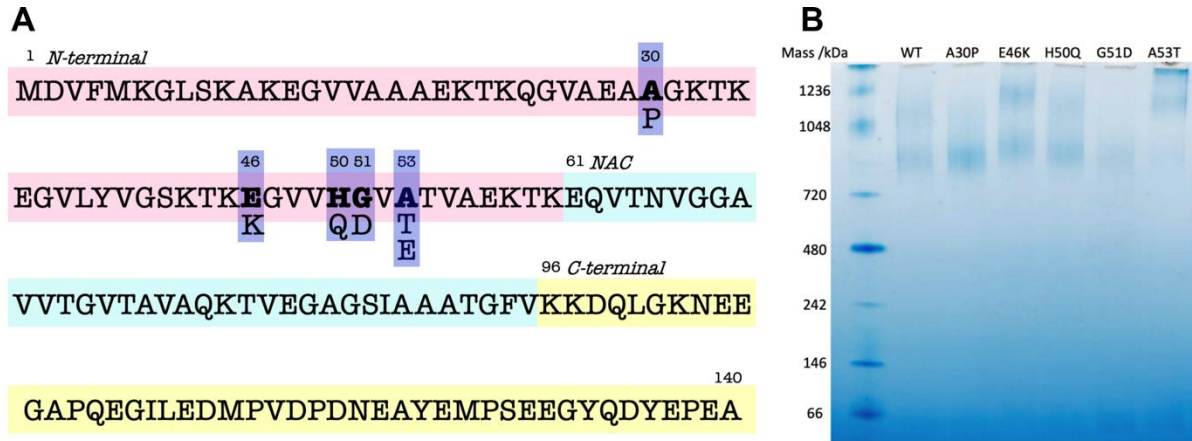


Figure S1. (A) Primary structure of α -synuclein, showing the locations of the disease-associated mutations (highlighted in dark blue). (B) Native-PAGE analysis of variant oligomers, showing the same size distributions as determined by AUC, demonstrating that native-PAGE is able to resolve oligomer size populations.

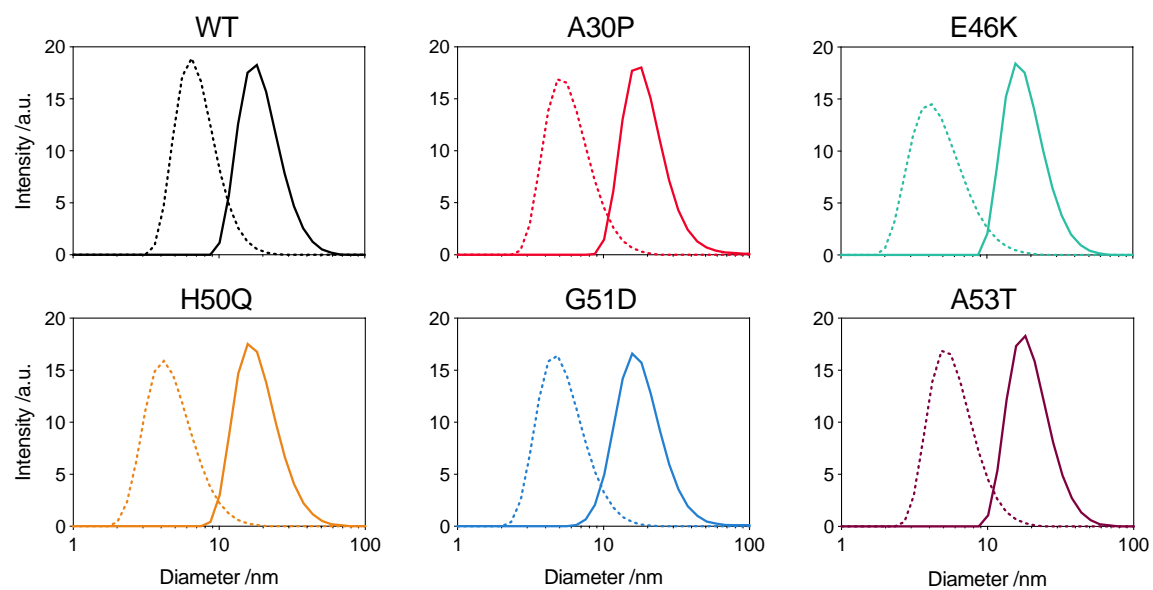


Figure S2. Dynamic light scattering (DLS) analysis of oligomers (solid) and monomers (dotted); all variants exhibit almost identical size distributions at this resolution, with monomer and oligomer populations being distinct from each other.

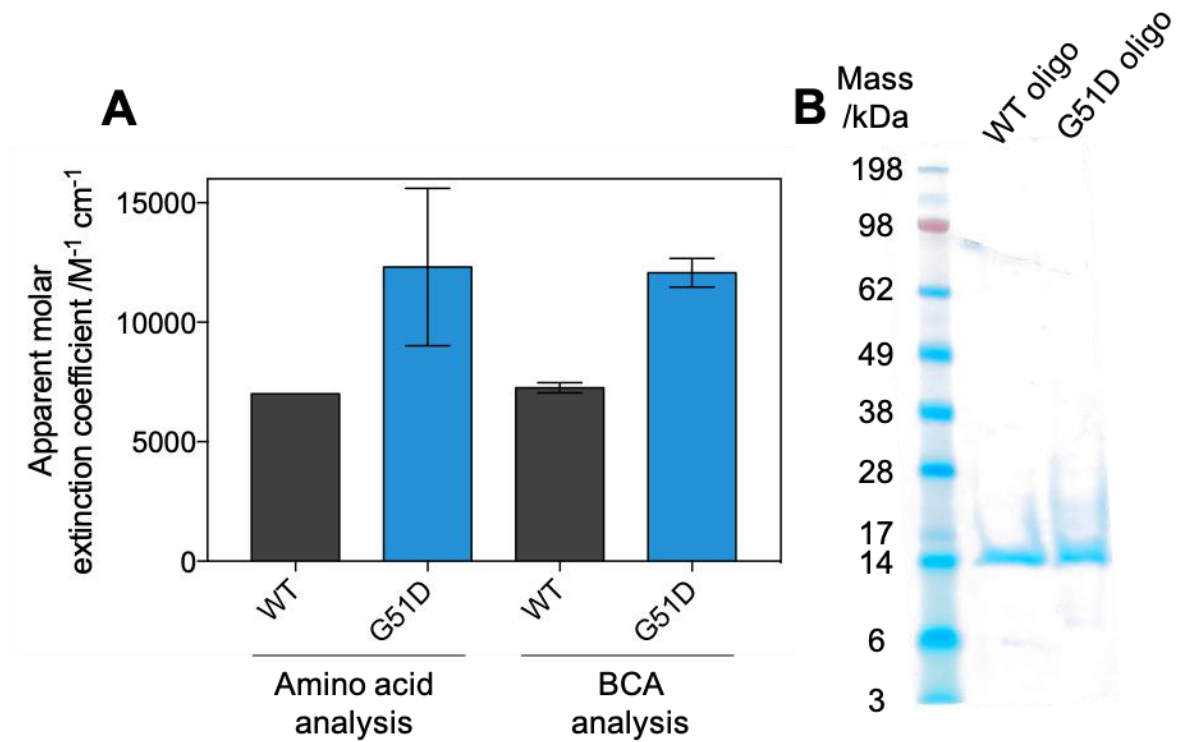


Figure S3. WT and G51D oligomers exhibit different molar extinction coefficients. **(A)** Using UV-vis spectroscopy, absorption at 275 nm of both oligomeric species was compared to protein concentration determined through amino acid analysis (WT: $n = 1$, G51D: $n = 2$) and BCA analysis ($n = 4$), normalised against the corresponding variant monomeric α -synuclein, and the apparent molar extinction coefficient determined (error bars for standard deviation). The two methods yielded almost identical results. We further confirmed these extinction coefficients by incubating WT and G51D oligomers, whose concentrations were determined using molar extinction coefficients of $7000 M^{-1} cm^{-1}$ and $12444 M^{-1} cm^{-1}$, respectively, with 6 M guanidinium thiocyanate. The resulting monomeric samples were then analysed by SDS-PAGE **(B)**, and resulted in similar band intensities, indicating that these extinction coefficients are accurate.

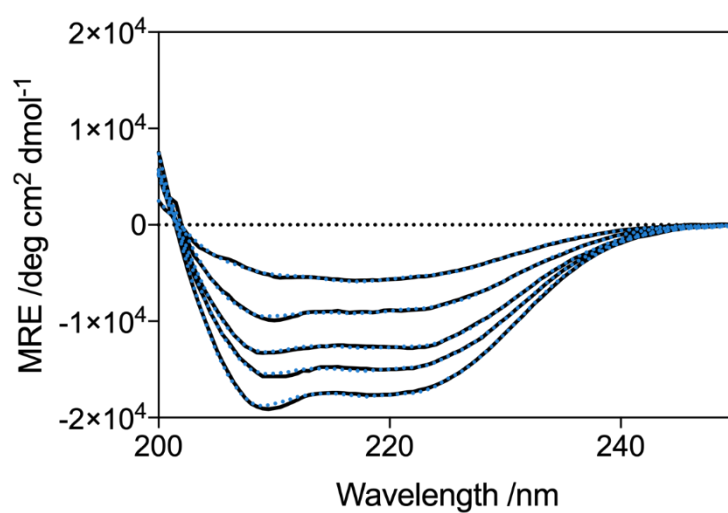


Figure S4. Fitted G51D CD spectra recapitulate the experimentally obtained spectra. CD spectra of G51D oligomers displaying different degrees of α -helical structure (black solid), shown alongside the fitted spectra (blue dotted), obtained using the BestSel software (3; 4)

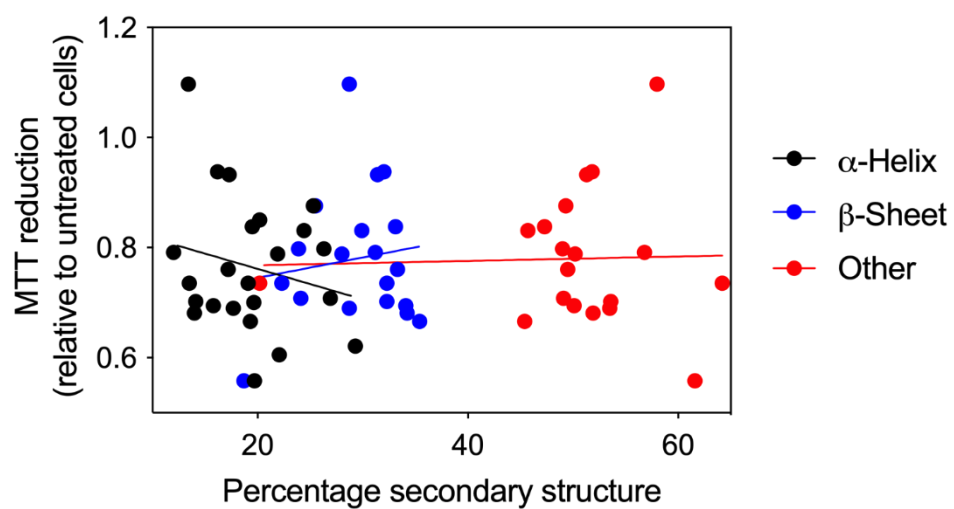


Figure S5. MTT reduction correlates only with α -helical content of oligomers; no correlation is observed with β -sheet content or random coil/other structures.

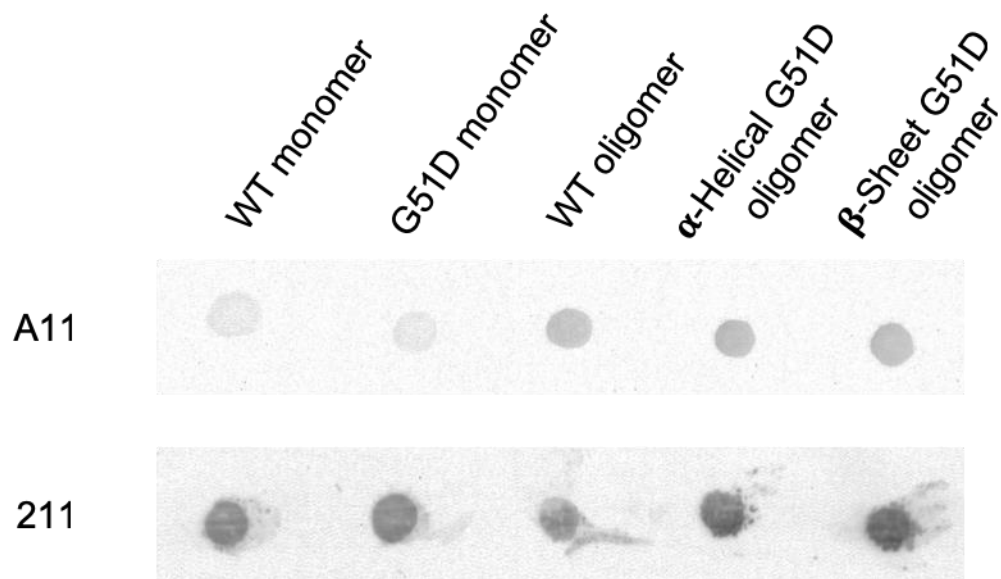


Figure S6. Both WT and isoforms of G51D oligomers bind the A11 antibody. Dot blots of WT and G51D monomers and oligomers. The A11 antibody, proposed to bind toxic amyloid oligomers, binds both WT and G51D oligomers with different conformations, with similar affinity (2). The 211 antibody has been found to bind the C-terminus of α -synuclein, and was therefore used as a control to confirm that the difference in binding of A11 to monomers and oligomers was not due to differences in α -synuclein concentration (1).

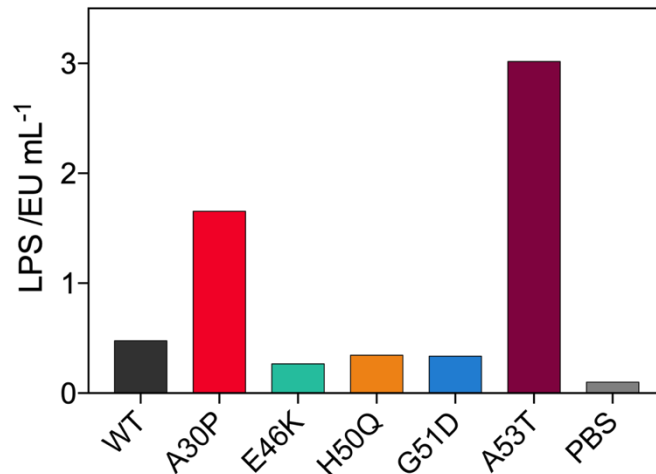


Figure S7. Quantification of LPS concentrations in α -synuclein monomer stocks. The LPS content of samples does not correlate with MTT reduction, indicating that LPS does not contribute to the observed cytotoxicity.

References:

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