Supplementary information:

Early stages of aggregation of engineered α-synuclein monomers and oligomers in solution

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Supplementary figures and tables

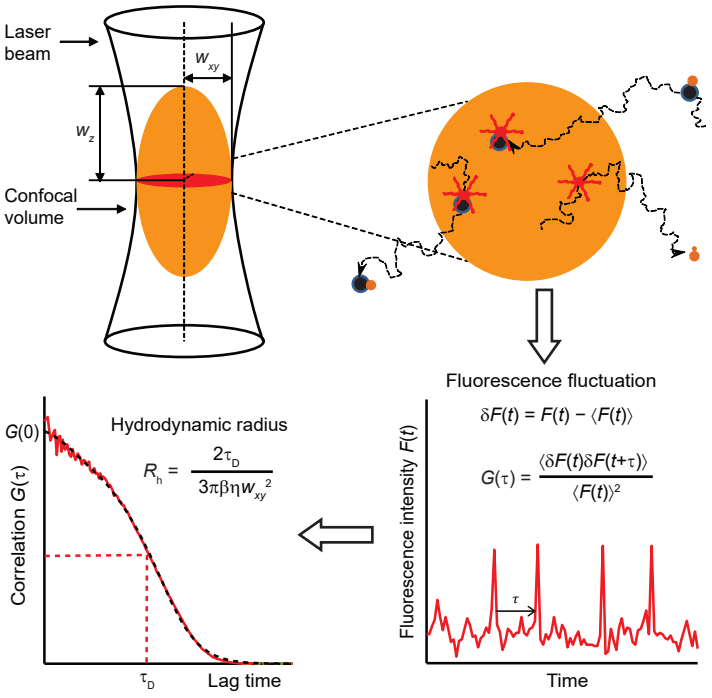

Figure S1: FCS assay. Schematic representation of the measurement and analysis of FCS data. Protein molecules labelled with a single dye passing through the confocal volume emit bursts of fluorescence. The decay time of the autocorrelation of the fluorescence intensity yields the hydrodynamic radius.

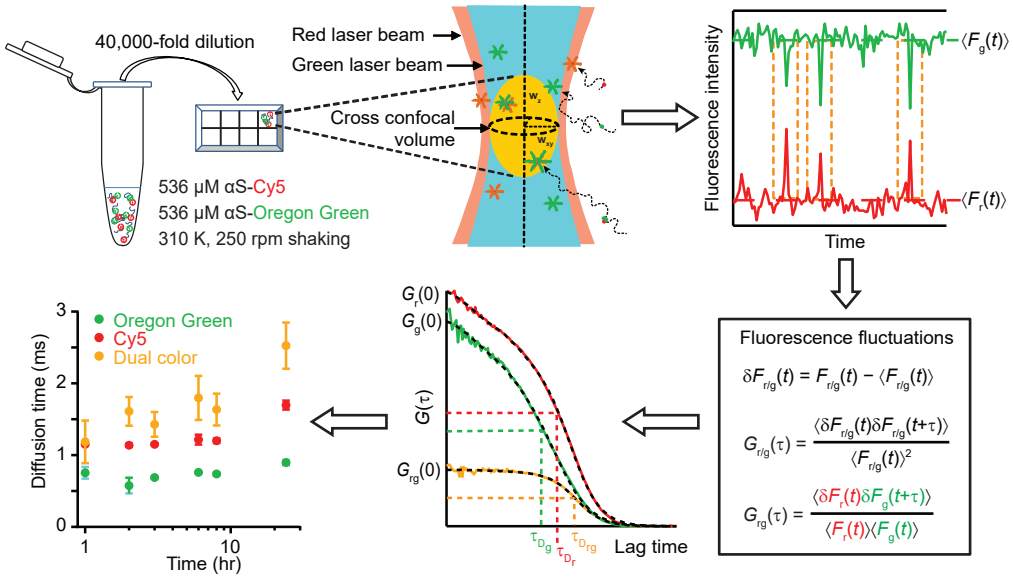

Figure S2: FCS/FCCS assay. Schematic representation of the measurement and analysis of FCCS data. Proteins labelled with Cy5 were incubated with other proteins labelled with Oregon green in microfuge tubes. At specific time-points, a sample was diluted for measurement. Assemblies containing red-labelled, green-labelled, or dual-labelled proteins that passed through the overlapping confocal volume (yellow) generated fluorescence fluctuations. The two auto-correlations and the cross-correlation were analyzed to determine diffusion times.

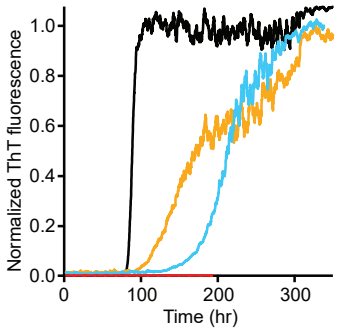

Figure S3: ThT fluorescence assay of aggregation performed in a microplate. Fluorescence from monomers (black), dimers (orange), tetramers (cyan), and octamers (red).

Table S1: The diffusion time of labeled αS is independent of protein auto-fluorescence.

Concentration of green- labelled αS-4 (nM)	Concentration of unlabelled αS-4 (nM)	G(0)	τ_D (μs)
34	10898	0.100±0.002	464±25
34	5449	0.099±0.002	459±21
34	2724	0.104±0.002	440±32
34	1362	0.092±0.002	450±28
34	681	0.133±0.003	473±34
34	341	0.104±0.002	456±28
34	170	0.151±0.003	460±27
34	85	0.129±0.004	476±37

Table S2: Dual-colour FCCS experiment codes.

	Oregon-green labelled α-synuclein			
Cy-5 labelled α-synuclein	**αS-1 (536 μM)**	**αS-2 (268 μM)**	**αS-4 (134 μM)**	**αS-8 (67 μM)**
αS-1 (536 μM)	$(\alpha S\text{-}1)_r(\alpha S\text{-}1)_g$	$(\alpha S\text{-}1)_r(\alpha S\text{-}2)_g$	$(\alpha S\text{-}1)_r(\alpha S\text{-}4)_g$	$(\alpha S\text{-}1)_r(\alpha S\text{-}8)_g$
αS-2 (268 μM)	$(\alpha S\text{-}2)_r(\alpha S\text{-}1)_g$	$(\alpha S\text{-}2)_r(\alpha S\text{-}2)_g$	-	-
αS-4 (134 μM)	$(\alpha S\text{-}4)_r(\alpha S\text{-}1)_g$	-	$(\alpha S\text{-}4)_r(\alpha S\text{-}4)_g$	-
αS-8 (67 μM)	$(\alpha S\text{-}8)_r(\alpha S\text{-}1)_g$	-	-	$(\alpha S\text{-}8)_r(\alpha S\text{-}8)_g$