# Supporting Information

## Alpha-synuclein Oligomers and Fibrils may originate in Two Distinct Conformer Pools: A Small Angle X-ray Scattering and Ensemble Optimisation Modelling Study

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### Table S1

Parameters derived from ensemble optimisation modelling using Advanced EOM 2.0* for wild-type and mutant α-syn in the absence and presence of $Cu^{2+}$ and the anti-fibril agent VK7

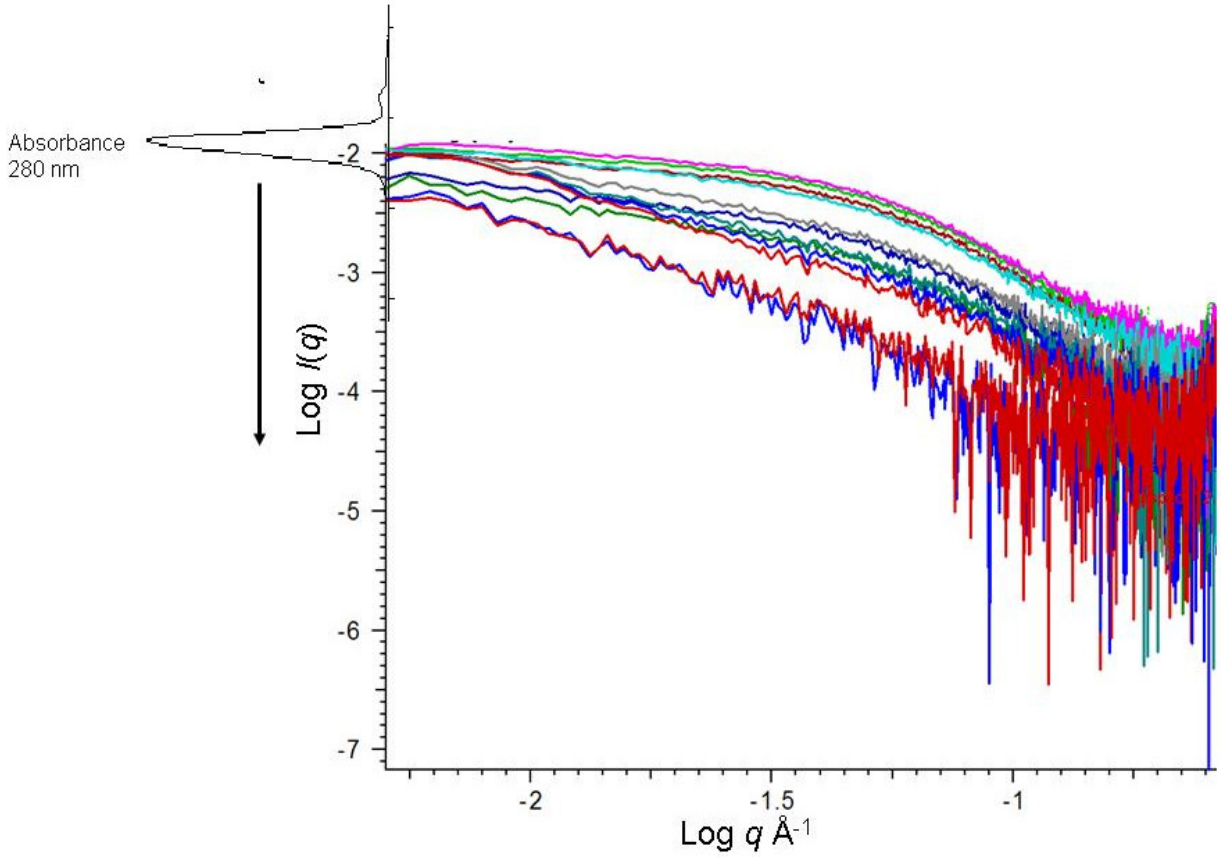
| **Protein** | **Average $R_g$ Å of ensemble** | **Average $D_{max}$ Å of ensemble** | **χ** |
|---|---|---|---|
| Wild-type | 44.34 | 129.13 | 1.1 |
| A30P | 28.30 | 84.69 | 0.94 |
| E46K | 39.46 | 115.23 | 0.93 |
| A53T | 41.75 | 124.16 | 1.1 |
| | | | |
| Wild-type + $Cu^{2+}$ | 28.81 | 79.94 | 1.2 |
| A30P + $Cu^{2}$ | 26.88 | 81.06 | 1.2 |
| A53T + $Cu^{2}$ | 34.03 | 101.85 | 0.97 |
| | | | |
| 4M4A** | 37.97 | 111.26 | 1.1 |
| 4M4A + $Cu^{2}$ | 25.52 | 75.48 | 1.1 |
| Wild-type + VK7 | 36.00 | 106.49 | 1.2 |
| | | | |

*Each run generated 1000 ensembles and fitted 50 curves

**The M1A/M5A/M116A/M127A substituted protein

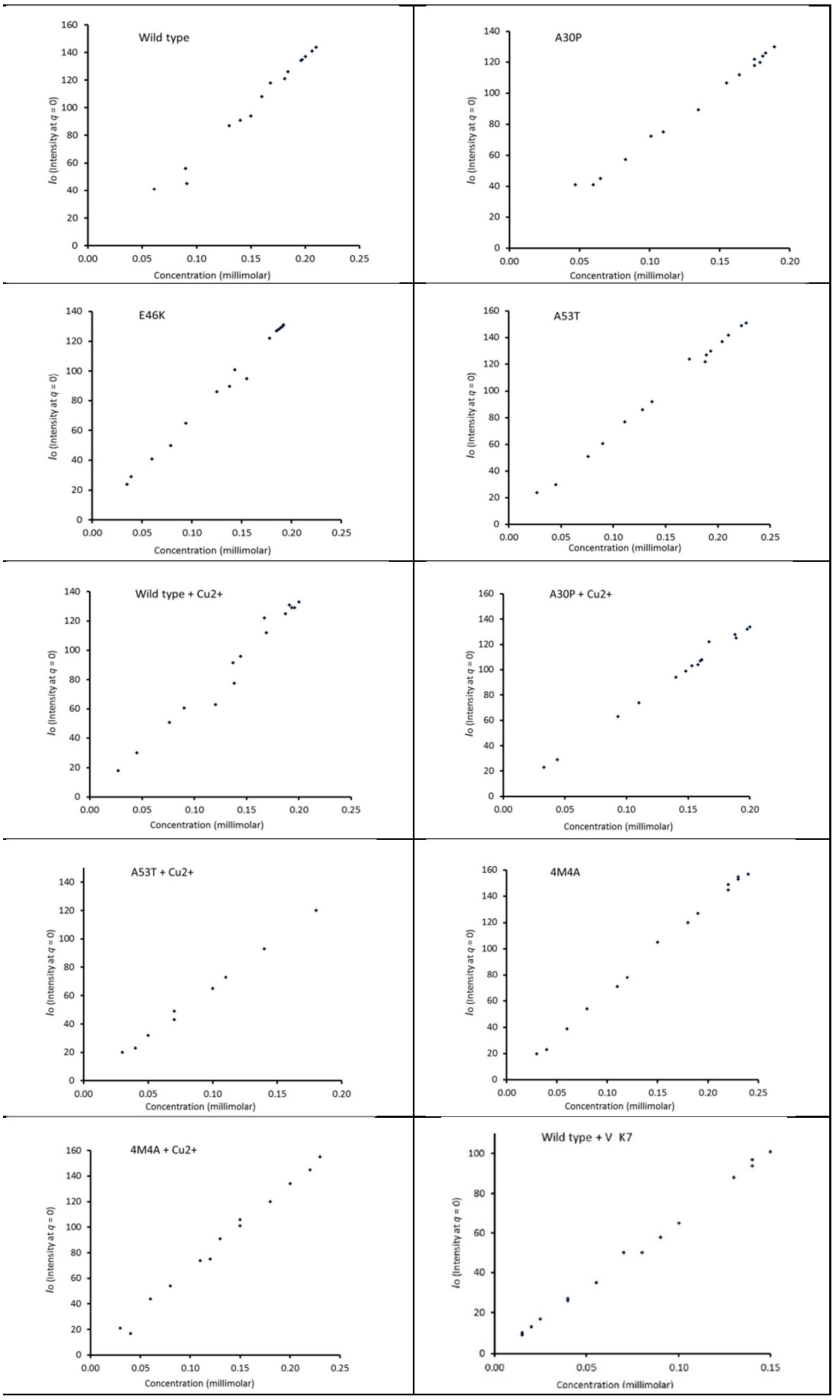
**Figure S1**

Scattering profiles of wild type α-syn taken across size-exclusion elution absorbance peak to illustrate need to examine each individual profile.



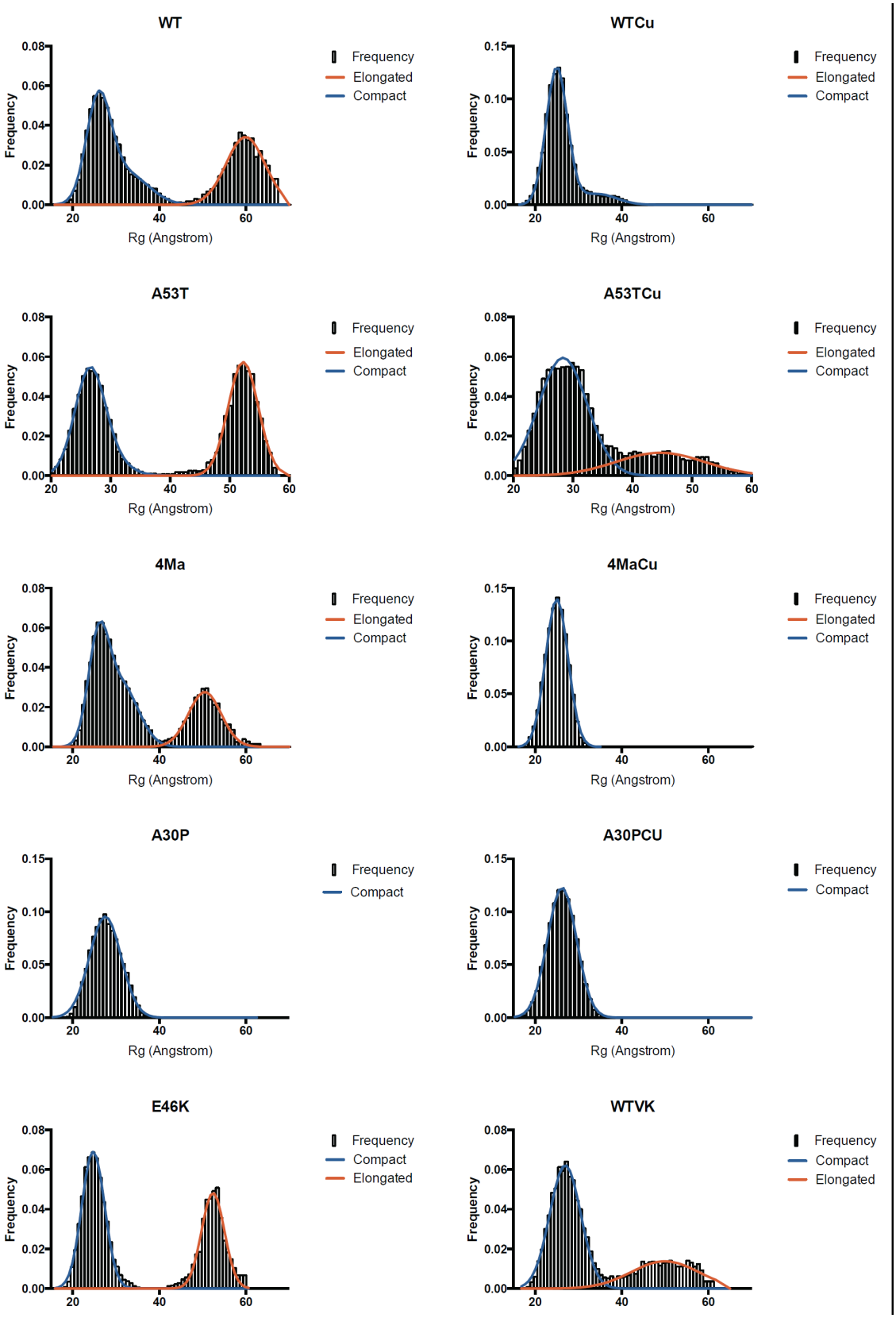
## Figure S2

Plots of intensity at $q$= 0 ($I_0$) against concentration of protein (millimolar) as estimated from the 280 nm absorption of the SEC peak. Each point is a 2.1 sec snapshot.



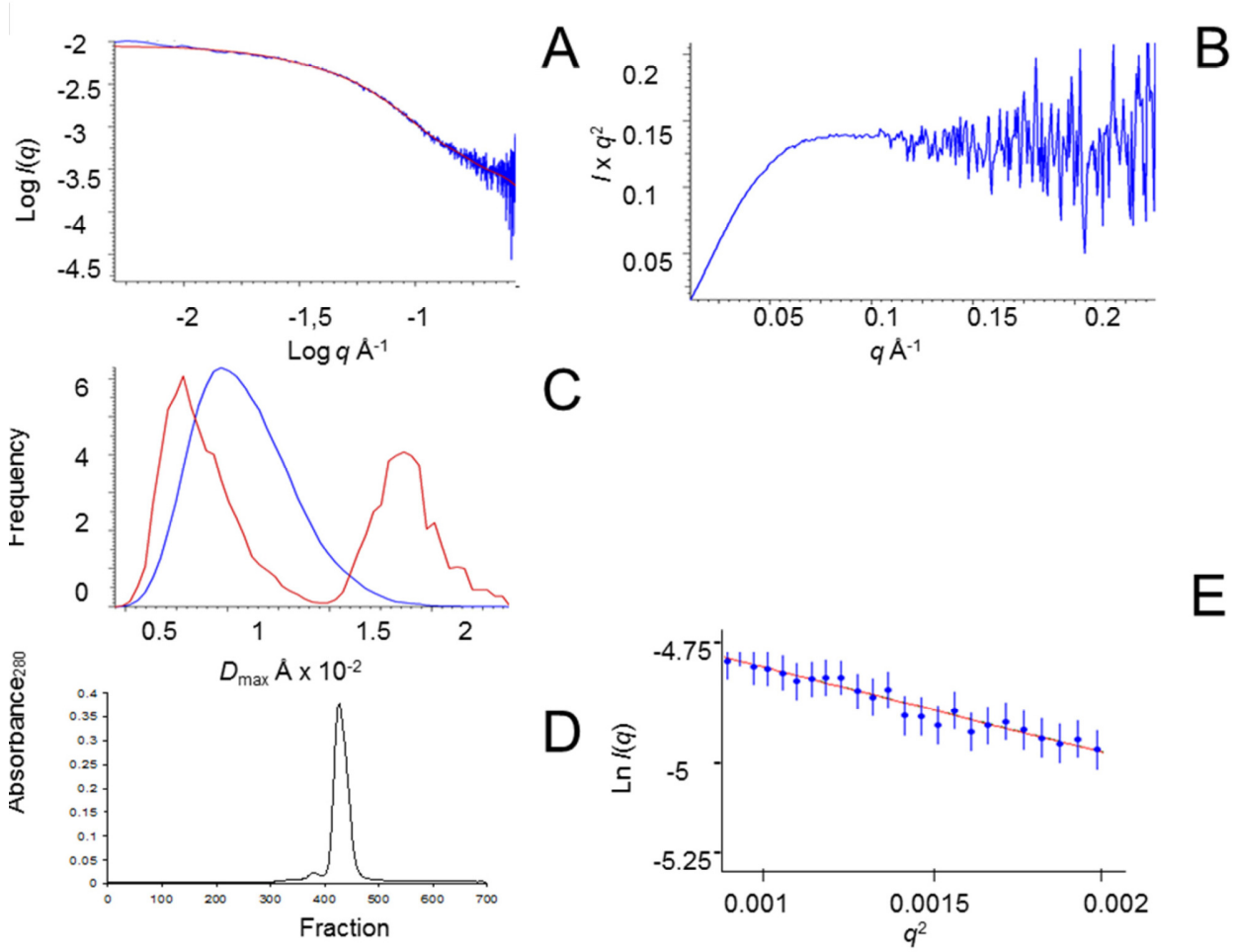
## Figure S3

Gaussian deconvolutions and cut-offs of $R_g$ distributions for WT and mutant α-syn in buffer, + $Cu^{2+}$ and WT + VK7

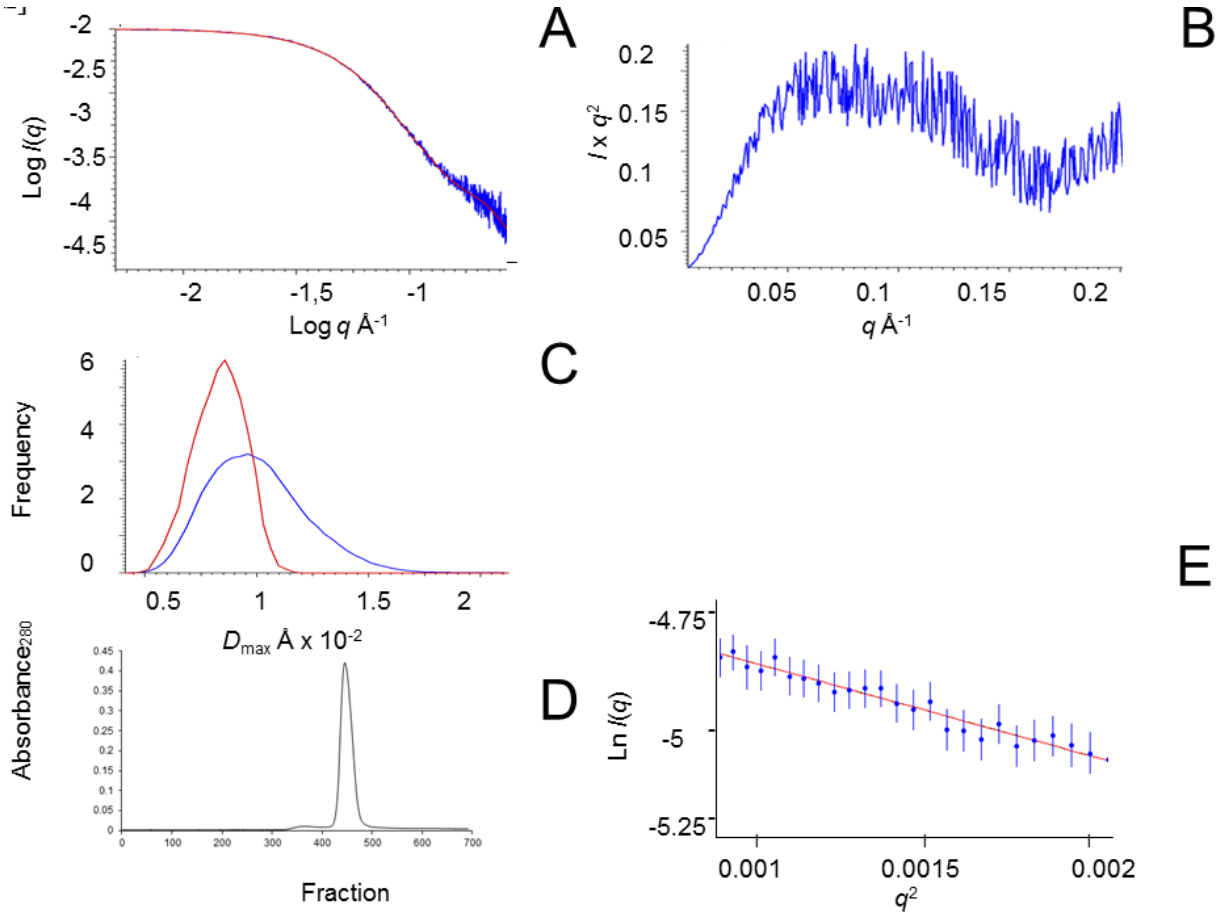


**Figures S4**

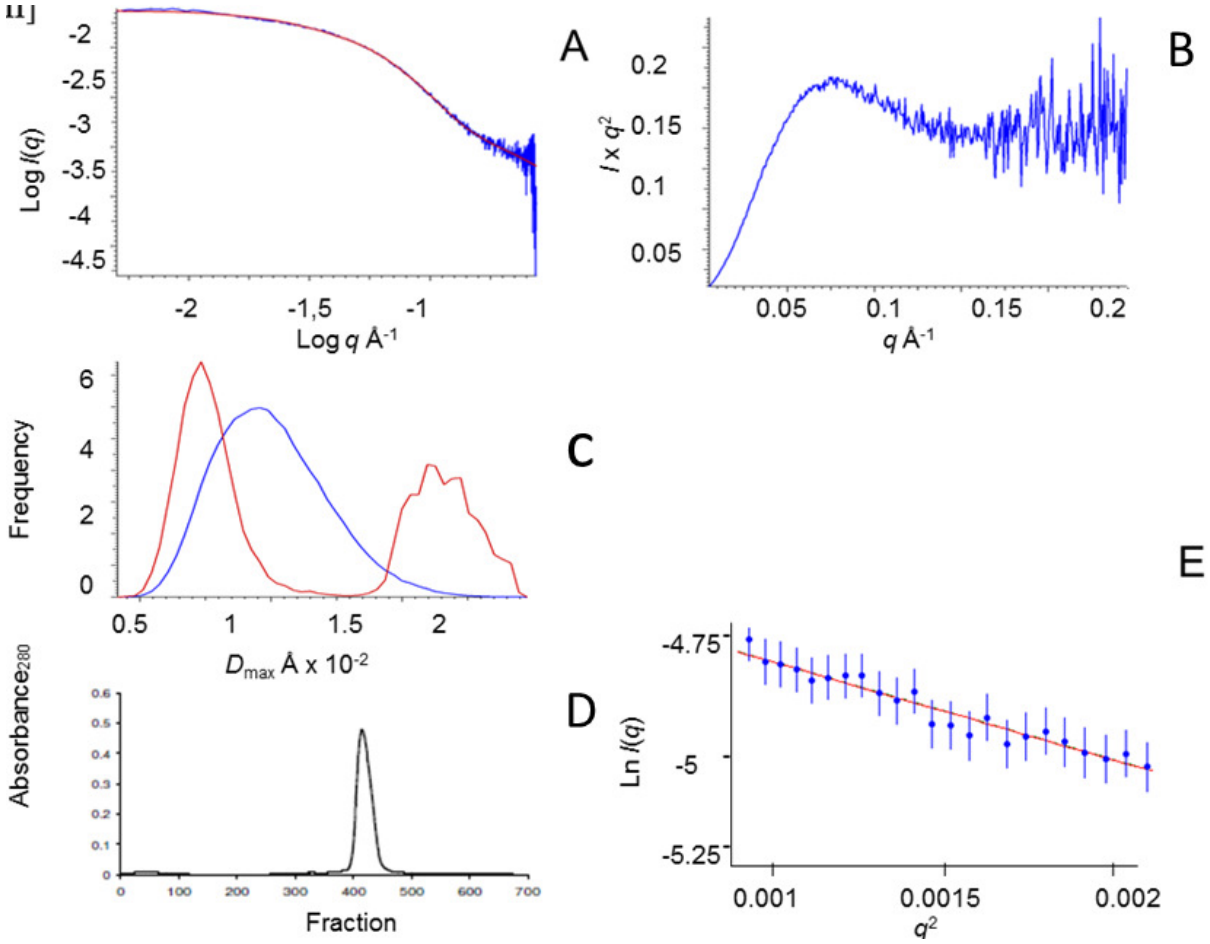
S4( I) A, Fitted scattering profile of wild-type α-syn; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.



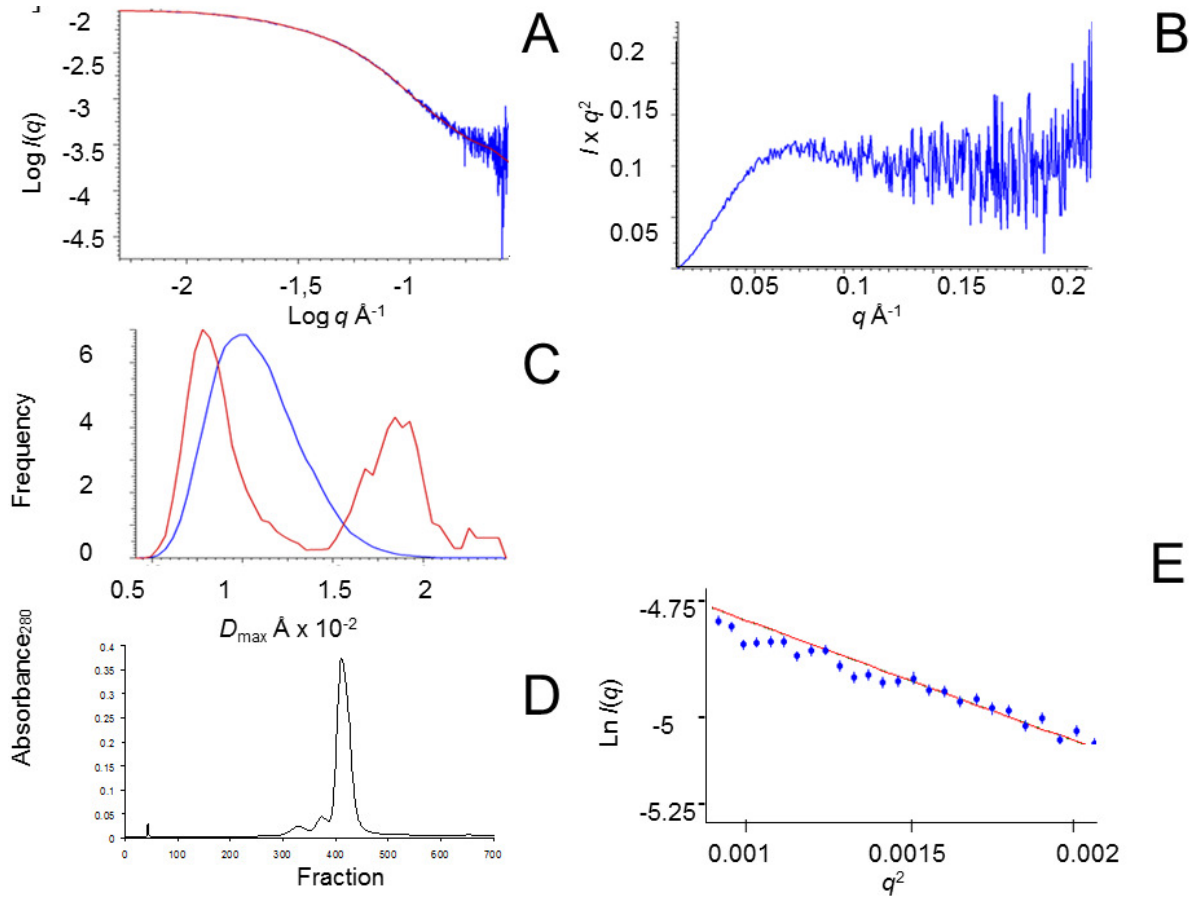
S4 (II) A, Fitted scattering profile of A30P mutant α-syn; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.



S4 (III) A, Fitted scattering profile of E46K mutant α-syn; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.

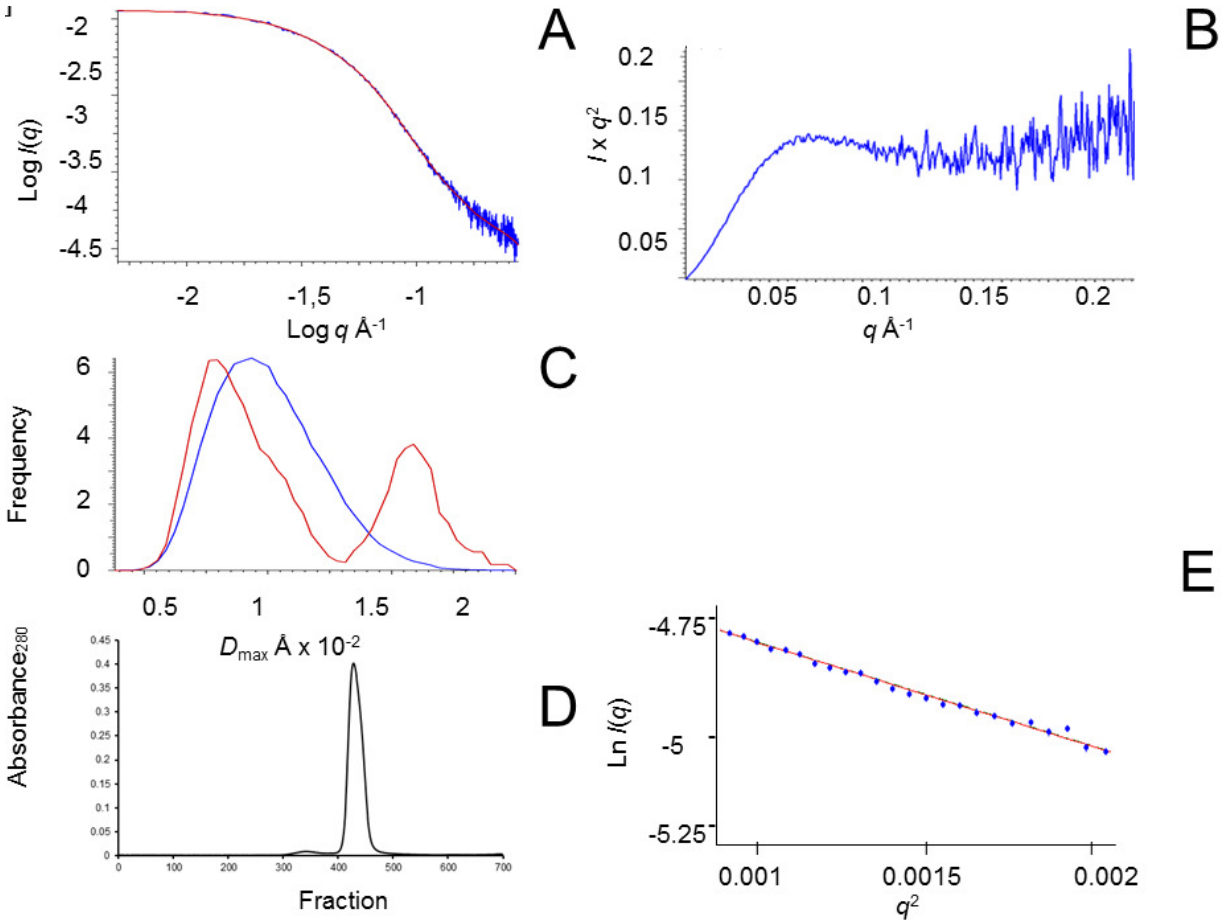


S4 (IV) A, Fitted scattering profile of A53T mutant α-syn; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.

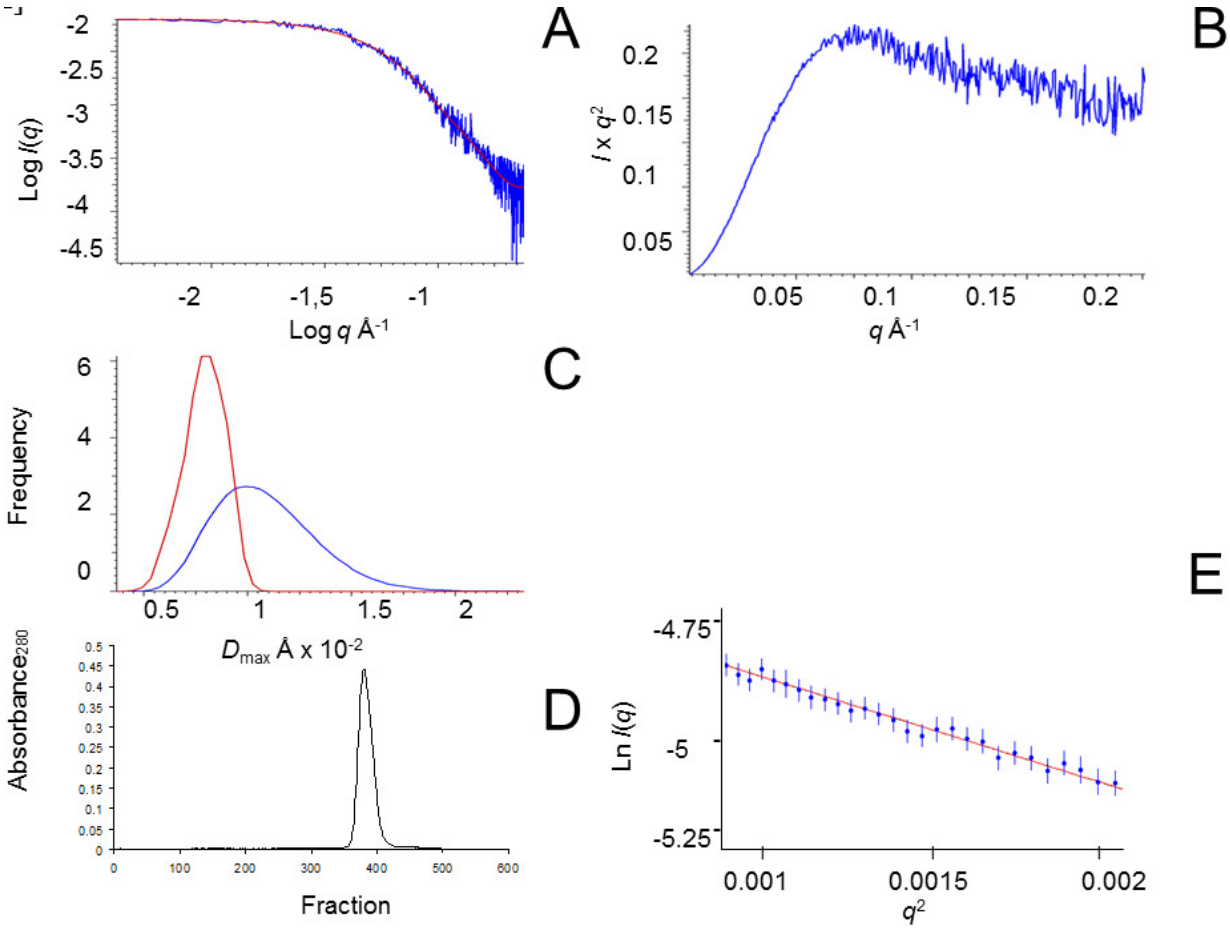


**Figures S5**

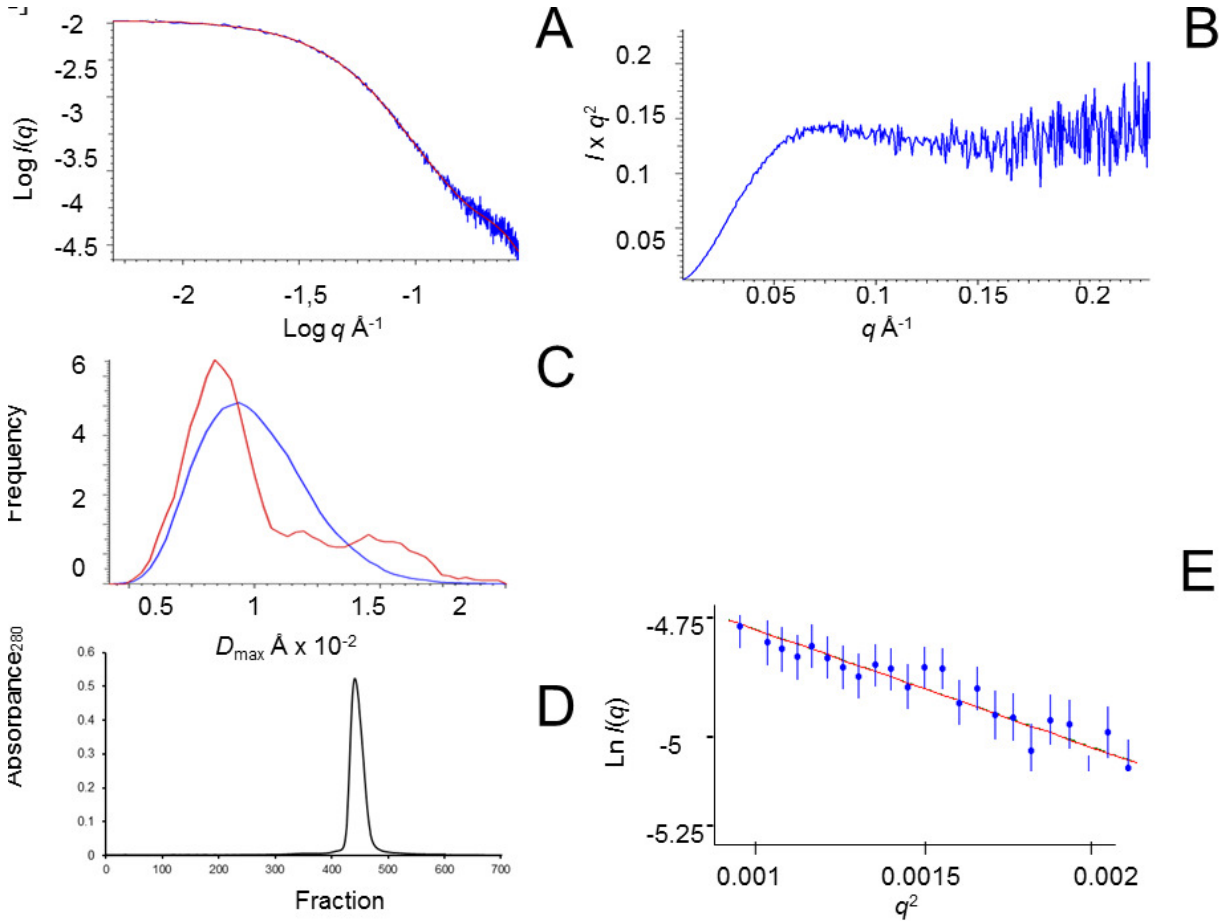
S5 (I) A, Fitted scattering profile of wild type α-syn + $Cu^{2+}$; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.



S5 (II) A, Fitted scattering profile of A30P mutant α-syn + $Cu^{2+}$; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.

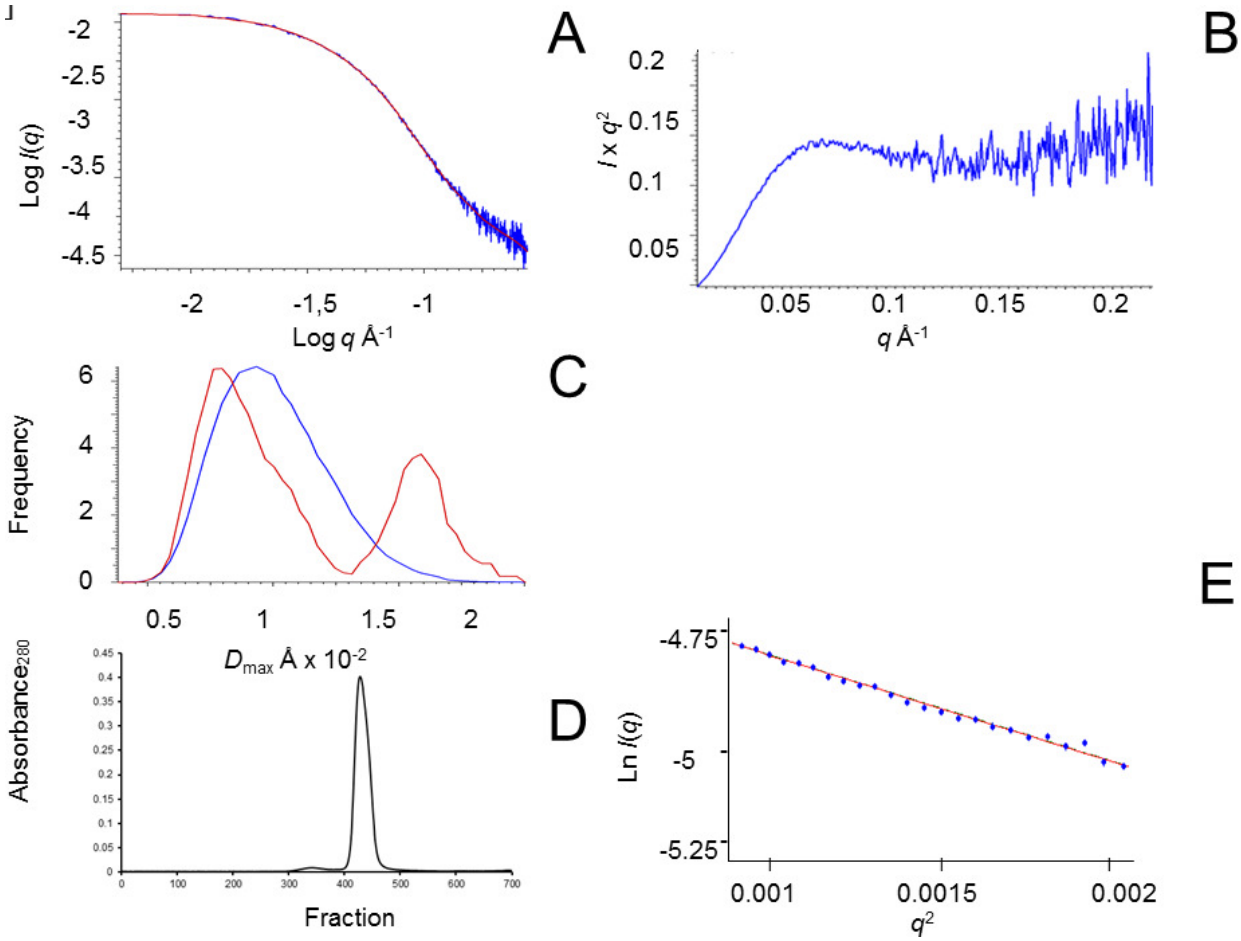


S5(III) A, Fitted scattering profile of A56T mutant α-syn + $Cu^{2+}$; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.

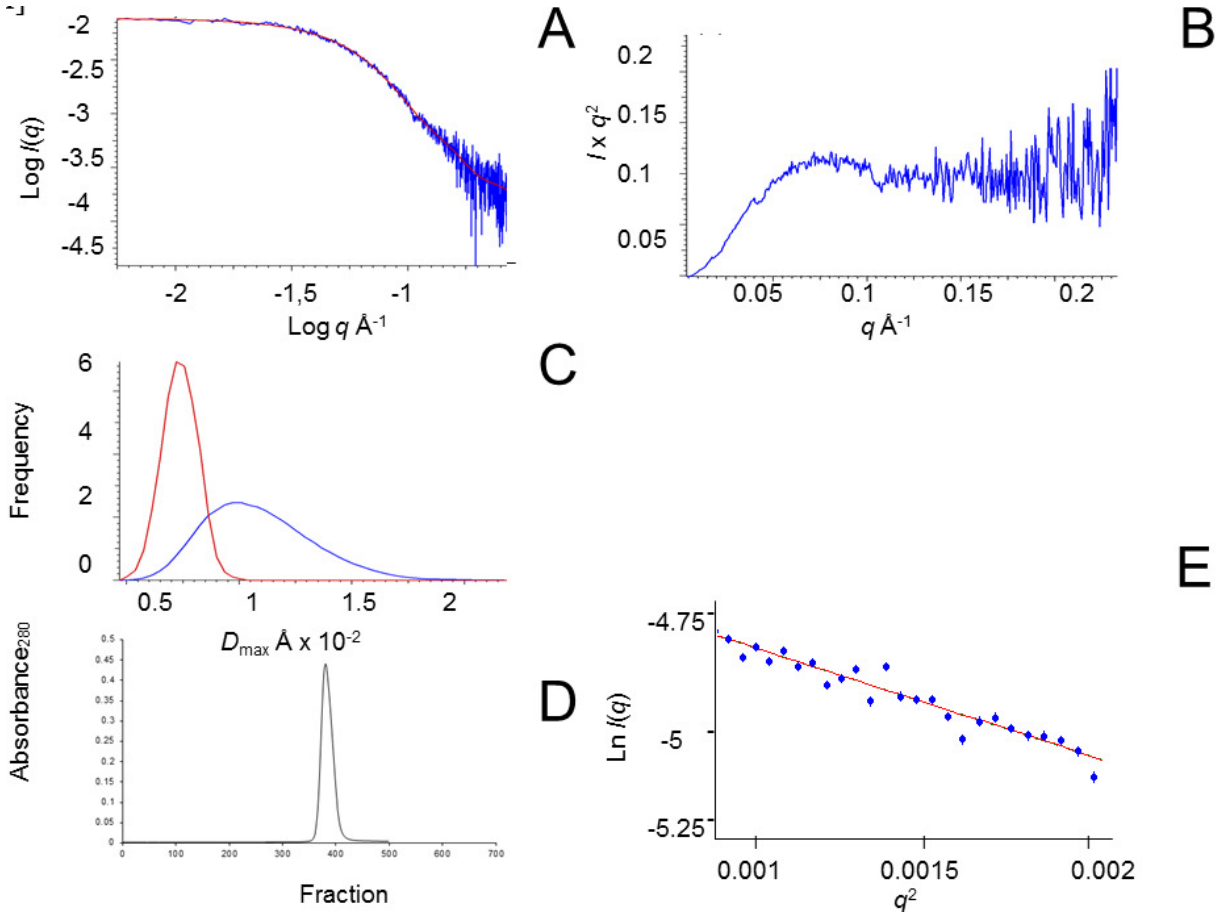


**Figures S6**

S6 (I) A, Fitted scattering profile of M1A/M5A/M116A/M127A substituted α-syn; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.



S6 (II) A, Fitted scattering profile of M1A/M5A/M116A/M127A substituted α-syn + $Cu^{2+}$; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.



S6 (III) A, Fitted scattering profile of wild-type α-syn + anti-fibril agent VK7; B, Kratky plot; C, $D_{max}$ distribution; D, SE column elution profile; E, Guinier plot.