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Article

Shear Stress Induces α-Synuclein Aggregation Due to a Less Strained Protein Backbone and Protein Tyrosyl Groups Do Not Intervene in the Aggregation

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Supplementary Information

SI. 1 SDS-PAGE of the α-synuclein purification

The corresponding SDS-PAGE of the α-synuclein purification is presented in Figure S1. The protein is almost pure after anion-exchange chromatography revealing mostly a band at *ca*. 18 kDa in the SDS-PAGE, which corresponds to α-synuclein (Figure S1A). In order to remove high molecular weight aggregates of α-synuclein, the resulting pooled fractions of anion-exchange chromatography were submitted to size exclusion chromatography. The protein is pure after size exclusion chromatography, revealing a band in the SDS-PAGE at *ca*. 18 kDa (Figure S1B).

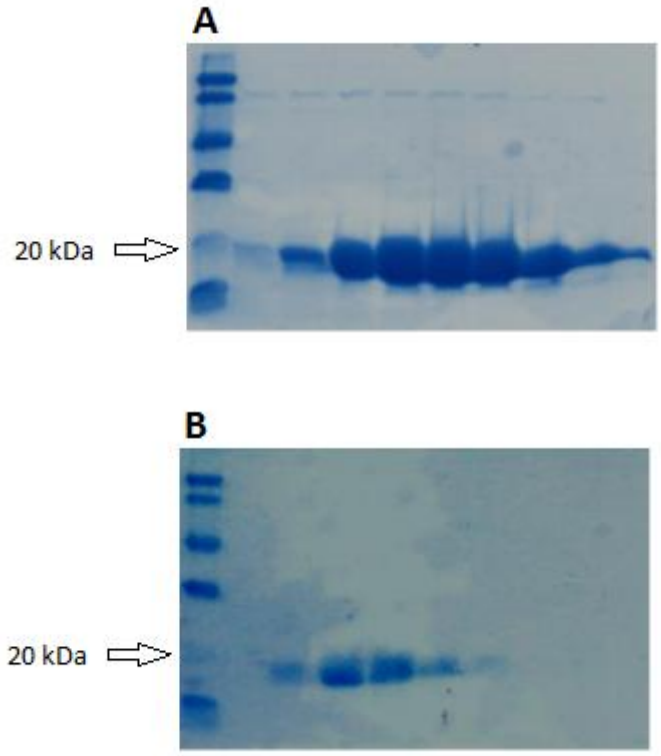

Figure S1. Some of the collected fractions were applied to a SDS-PAGE (13 % acrylamide) (denaturing conditions) (**A**) from anion-exchange chromatography and (**B**) from size exclusion chromatography. α-Synuclein appeared as a band at *ca*. 18 kDa.

SI. 2 Size exclusion chromatography (SEC) of α-synuclein

In Figure S2, the blue line represents the recorded absorbance at 280 nm and the red line represents the recorded conductivity. Absorbance measurements at 280 nm reveal

three peaks; the first peak at ca. 43 mL of the elution corresponds to α-synuclein aggregates (as observed in the SDS-PAGE in Figure S1A), the second peak at 60-80 mL of the elution corresponds to pure α-synuclein (as observed in the SDS-PAGE in Figure S1B, and these fractions were pooled, concentrated and used in the biophysical studies, i.e. after dialysis against water overnight) and the second peak at 80-110 mL of the elution corresponds to a low molecular weight impurity that was not detected in the SDS-PAGE in Figure S1A,B. Conductivity measurements reveal one peak at 95-110 mL of the elution, which corresponds to the ion species present in the SEC buffer used (50 mM Tris-HCl + 150 mM NaCl at pH 7.5). According to the conductivity measurements (red line), the maximum of the peak (at ca. 100 mL of the elution) corresponding to the referred ion species present in the SEC buffer used is very close to the maximum of the peak corresponding to the mentioned low molecular weight impurity (at ca. 95 mL of the elution) (blue line). This retrieves that the low molecular weight impurity observed possesses a molecular weight far below the 10 kDa (first bottom mark in the SDS-PAGE in Figure S1A,B), and therefore this impurity is indeed not possible to be observed in the SDS-PAGE presented in Figure S1A,B.

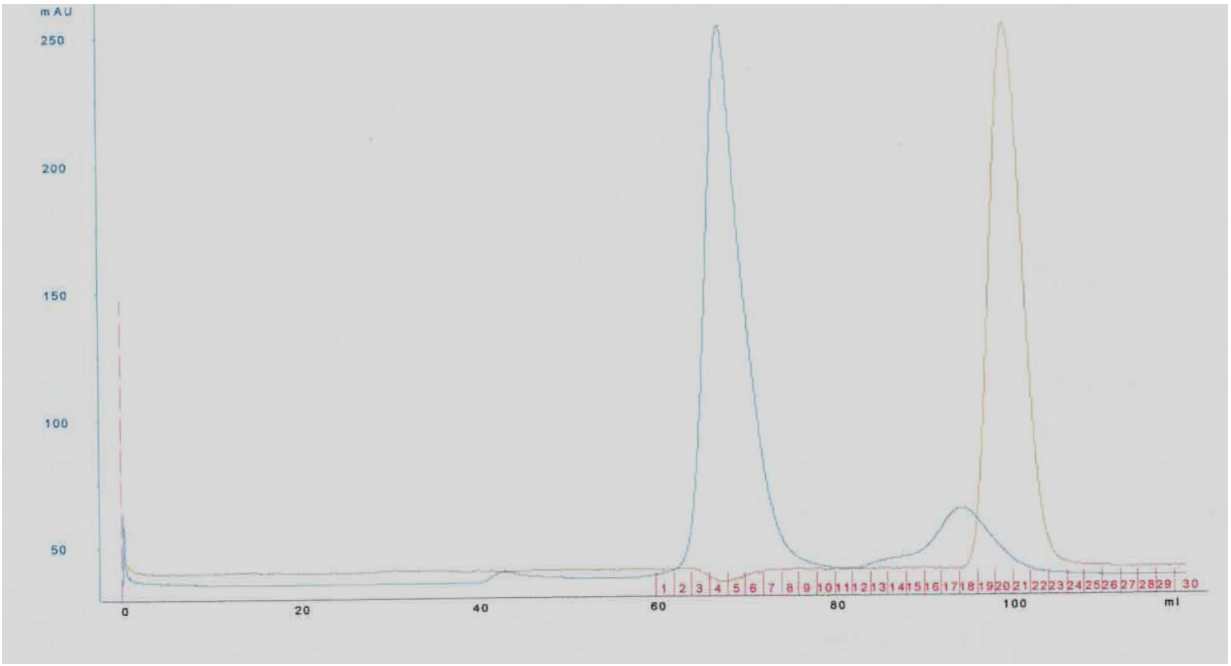

Figure S2. Original size exclusion chromatogram obtained of α-synuclein samples, after anion-exchange chromatography. .