Electronic Supplementary Material (ESI) for Nanoscale.

SUPPORTING INFORMATION

SARS-CoV-2 N-protein induces the formation of composite α-synuclein/N-protein fibrils that transform into a strain of α-synuclein fibrils

Slav A. Semerdzhiev[1], Ine Segers-Nolten[1], Paul van der Schoot[2], Christian Blum[1], Mireille M.A.E. Claessens[1]

[1]Nanobiophysics, Faculty of Science and Technology, MESA + Institute for Nanotechnology and

Technical Medical Centre, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

[2]Theory of Polymers and Soft Matter, Eindhoven University of Technology,

P.O. Box 513, 5600 MB Eindhoven, The Netherlands

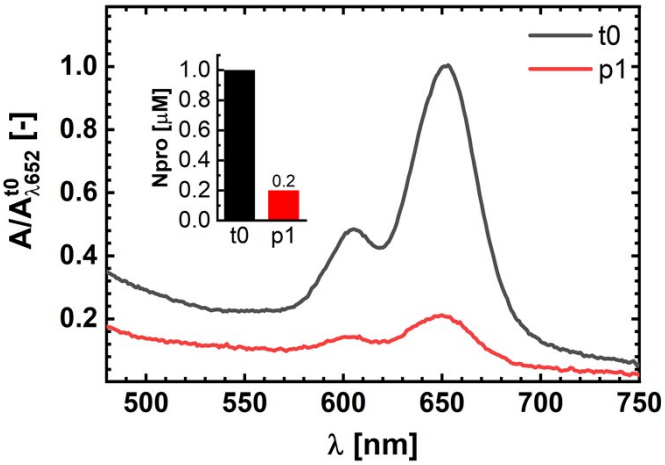

Figure S1. Residual N-protein monomer concentration in p1 at high ionic strength. The absorbance of N-protein-AT647 before aggregation at t0 is shown in black. The p1 absorbance spectrum for N-protein-AT647 in the supernatant (red) was taken after increasing the NaCl concentration up to 500 mM and then spinning down the fibrils from the solution. The data are peak-normalized to the t0-stage maximum absorbance. The inset shows the derived total residual N-protein monomer concentrations at the different stages of the aggregation process.

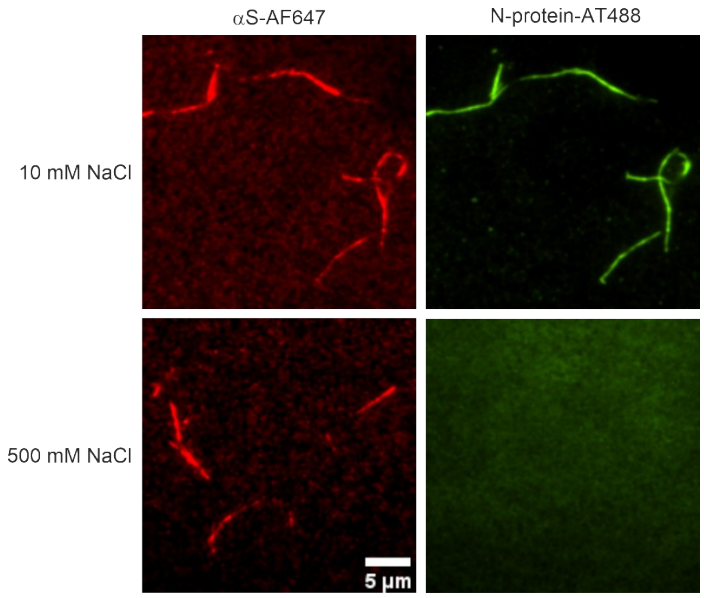

Figure S2. Electrostatically-driven adsorption of N-protein on αS fibrils. TIRFM images sowing N-protein-AT647 (red) and N-protein-AT488 (green). Preformed αS fibrils containing a fraction of αS-AF647 were incubated with N-protein-AT488 at 10 mM NaCl (top row) and at 500 mM NaCl. The colocalization of fibrils and N-protein at low ionic strength (top row) and the absence of such at high ionic strength (bottom row) indicates adsorption of the N-protein onto the fibrils mediated by electrostatic interaction between the net negatively charged αS and net positively charged N-protein.

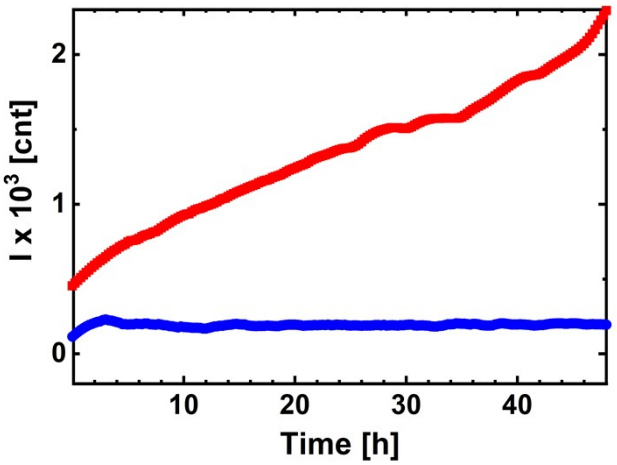

Figure S3. Seeded aggregations with s2 fibrils. ThT assay of resuspended isolated s2 fibrils under quiescent conditions after αS monomer (red) and N-protein (blue) addition. Addition of αS to the s2 fibrils results in a linear increase of the ThT whereas the addition of N-protein does not induce any notable changes in THT emission.

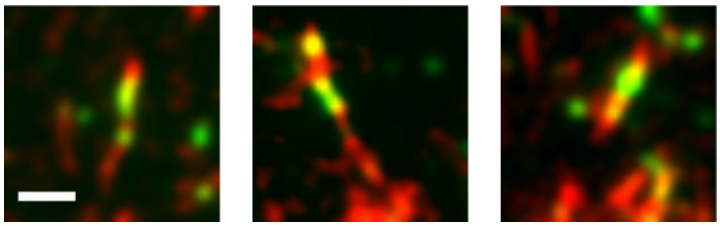

Figure S4. Visualizing the two steps of αS aggregation in the presence of N-protein. The starting αS/N-protein aggregation mixture in stage t0 contains a fraction of αS-AF647 (green). Once the first plateau p1 is reached, αS-AF568 is added (red). After p2 was reached, the resulting fibrils were imaged using TIRF microscopy. The fibrils clearly show segments of different color. This evidences that the s1 fibril strains extends and converts into the s2 fibril strain. The presence of one color fibrils we attribute to fibril breaking. Scale bar is 2 µm.

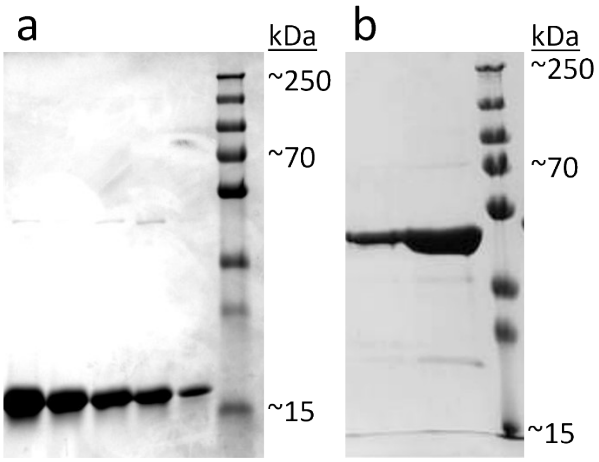

Figure S5. SDS-page analysis of the recombinantly produced purified proteins. **a)** The α-synuclein fractions obtained after purification over a resource Q column. The fractions shown in the different lanes were pooled for further studies. **b**) The lanes on the gel show the SARS-CoV-2 N-protein after the 2nd gel filtration column (left) and the final concentrated N-protein sample. Both gels are shown with a PageRuler Plus prestained protein ladder.