Electronic Supplementary Material (ESI) for Nanoscale.

Strong Interactions with Polyethylenimine-Coated Human Serum Albumin Nanoparticles (PEI-HSA NPs) Alter *α*- Synuclein Conformation and Aggregation Kinetics

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Reagents and chemicals

Ployethylenimine (PEI, 10 kDa) were obtained from Alfa Aesar. Penicillin–streptomycin, fetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibco BRL (Gaithersberg, MD, USA). 1,2-dioleoyl-sn-3-phosphatidyl-glycerol (DOPG) were from Avanti Polar Lipids (Alabaster, AL). All other chemicals were from Sigma Aldrich (St. Louis, MO).

Experimental procedures

Protein production and purification

*α*SN was expressed in *Escherichia coli* BL21(DE3) strain with a plasmid vector pET11-D using auto-induction according to the protocol described by Lorenzen *et al*.[1] Briefly, cells were pelleted by centrifugation (3500 rpm, 4 °C, and 20 min). The pellet from 1 L culture was resuspended in 100 mL osmotic shock buffer (30 mM Tris-HCl, 40 % sucrose, 2 mM EDTA, pH 7.2), incubation for 10 min followed by centrifugation (9000 g, 20 °C, 30 min). The resulting pellet was resuspended in 90 mL ice-cold deionized water and 40 μL of saturated $MgCl_2$ was added, followed by incubation on ice for 3 min. The supernatant was collected after centrifugation (9000 g, 4 °C, and 20 min). The supernatant was precipitated by titration with 1 M HCl to pH 3.5 and then incubated for 5 min. The supernatant was collected by centrifugation (9000 g, 4 °C, and 20 min) and immediately titrated to pH 7.5 with drop-wise addition of 1 M NaOH. Before loading on a Q-Sepharose column (HiTrap Q H P) pre-equilibrated with 20 mM Tris-HCl pH 7.5, the protein extract was filtered (0.45 μm). Then, the column was washed with three column volumes of 0.1 M NaCl in buffer and *α*SN was eluted with a NaCl gradient from 0.1-0.5 M. The fractions were analyzed using SDS-PAGE and the collected purified *α*SN was dialyzed exhaustively against deionized water, lyophilized and stored at -20 °C.

Fibril elongations assays

The fibril elongation assays were performed on a platereader setup, using the same setting as for the fibrillation assay in the presence of 0.05 mg/mL seeds. For the experiments, the snapfrozen mature fibrils were thawed, diluted to the desired concentration and fragmented by sonication for 2 minutes on ice (pulse 5 sec. on and 5 sec. off) with an amplitude of 20 % on a QSonica Sonicators (Q500, Newtown, CT, USA) to obtain short fibrils, which were employed as seeds.

Fibril disaggregation assays

70 μM aggregated αSN (monomer equivalents) was incubated either alone or with the NPs, using the same setting for the fibrillation assay.

Preparation of oligomers

αSN oligomers were prepared as previously described [2]. Briefly, 12 mg/mL αSN was incubated in PBS buffer for 5 h at 37 °C and 900 rpm on a Eppendorf thermoshaker, TS-100, BioSan, Latvia. The sample was then centrifuged (13400 rpm, 10 min) to remove insoluble material and the supernatant was loaded on an a Superose 6 Prep Grade column, GE healthcare Life Sciences, Sweden, in PBS at 2.5 mL/min. Small oligomers were collected and were concentrated with 15 mL Amicon ultracentrifugal filters (Merck).

Transmission Electron Microscopy (TEM)

5 μL of αSN sample in PBS buffer was transferred to a carbon-coated, glow-discharged 400-mesh grid for 30 s. The grids were washed using 2 drops of doubly distilled water, stained with 1% phosphotungstic acid (pH 6.8), and blotted dry. The samples were viewed in a microscope (JEM-1010; JEOL, Tokyo, Japan) operating at 60 kV. Images were obtained using an Olympus KeenView G2 camera.

Atomic Force Microscopy (AFM)

2 μL sample was deposited on a freshly cleaved mica surface and incubated for 5 minutes before gently rinsing with deionized water and drying under a N_2 flow. All images were captured using a commercial Nanoscope VIII

MultiMode SPM instrument (Bruker, Santa Barbara, CA) in tapping mode under ambient conditions. Ultrasharp silicon cantilevers (triangular, OMCL-AC160TS-R3, Olympus) were used with a typical resonance frequency of 300 kHz, a spring constant of 26 N/m, and a nominal tip radius of 7 nm. The original resolution of all AFM images is 512 × 512 pixels per image. The images were flattened and analysed using the Scanning Probe Image Processor software (SPIP, version 6.0.13, Image Metrology ApS, Lyngby, Denmark).

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The secondary structure of fibrils made from the ThT fibrillation assay were analyzed with a Tensor 27 FTIR (Bruker Optics, Billerica, MA). Two μL of sample was carefully dried with nitrogen gas on the crystal. Spectra were accumulations of 68 scans, measured with a resolution of 2 cm^{-1} in the range from 1000 to 3998 cm^{-1}. Data processing were performed within the software OPUS version 5.5 (http://www.stsci.edu/software/OPUS/kona2.html). The amide I (1600-1700 cm^{-1}) band of the spectra was fitted using a Gaussian species model.

Supplementary Results

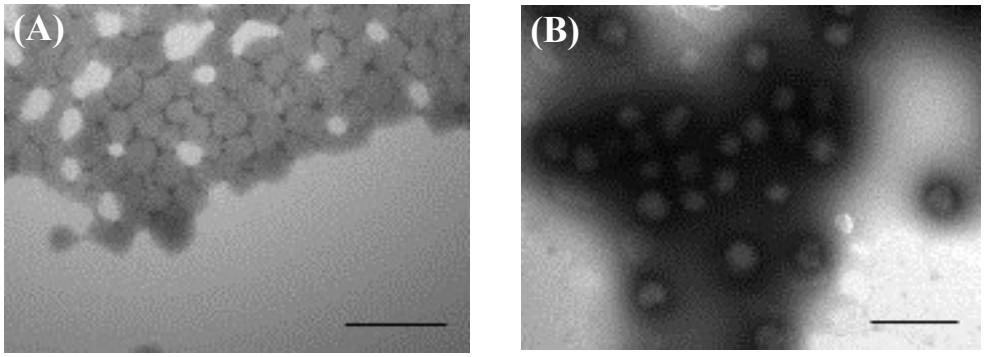

Fig. S1. TEM images of (A) HSA NPs and (B) PEI-HSA NPs. Scale bar, 200 nm.

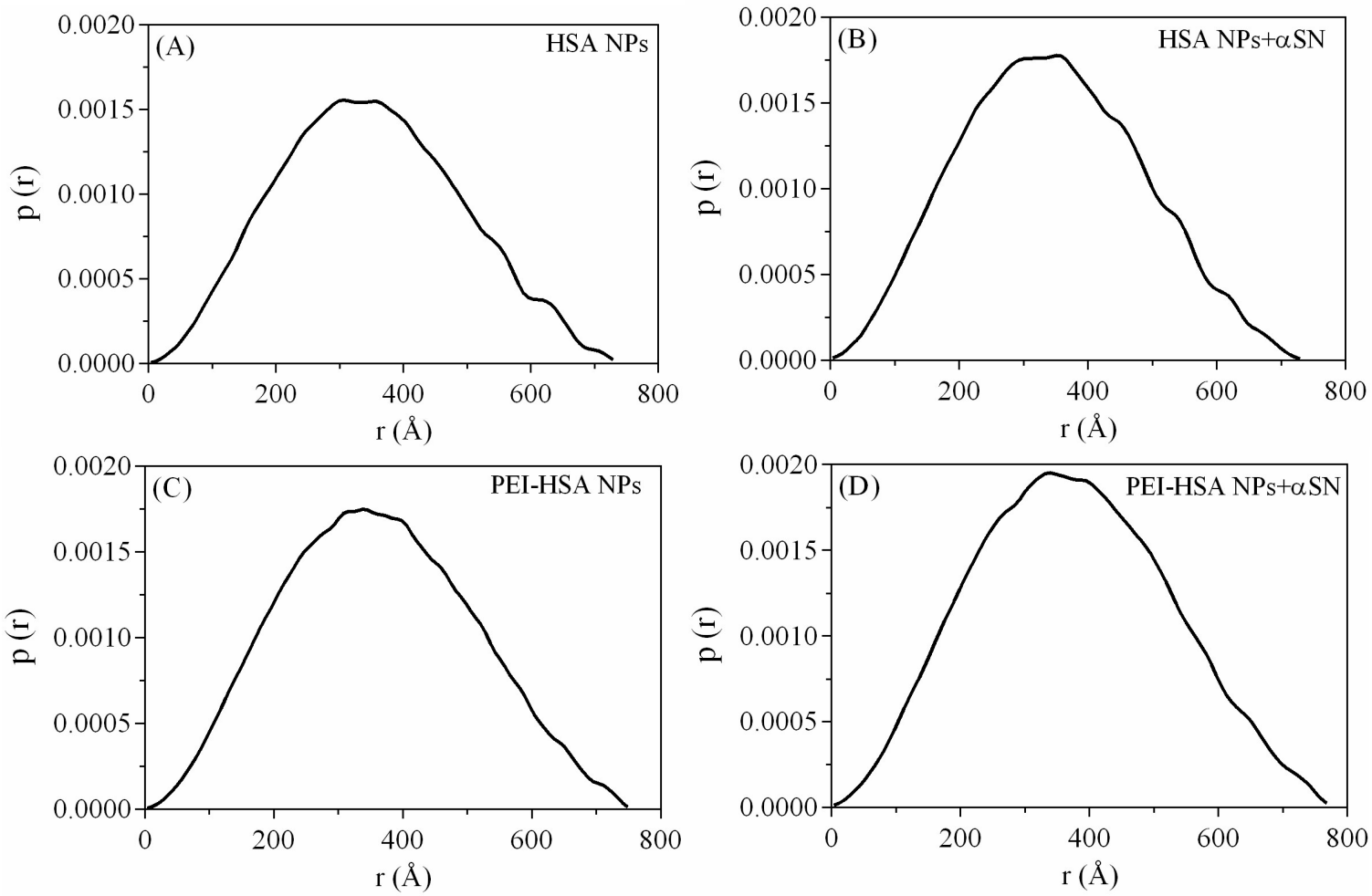

Fig. S2. Pair-distance distribution function for (A) HSA NPs, (B) HSA NPs + *α*SN, (C) PEI-HSA NPs, and (D) PEI-HSA NPs + *α*SN.

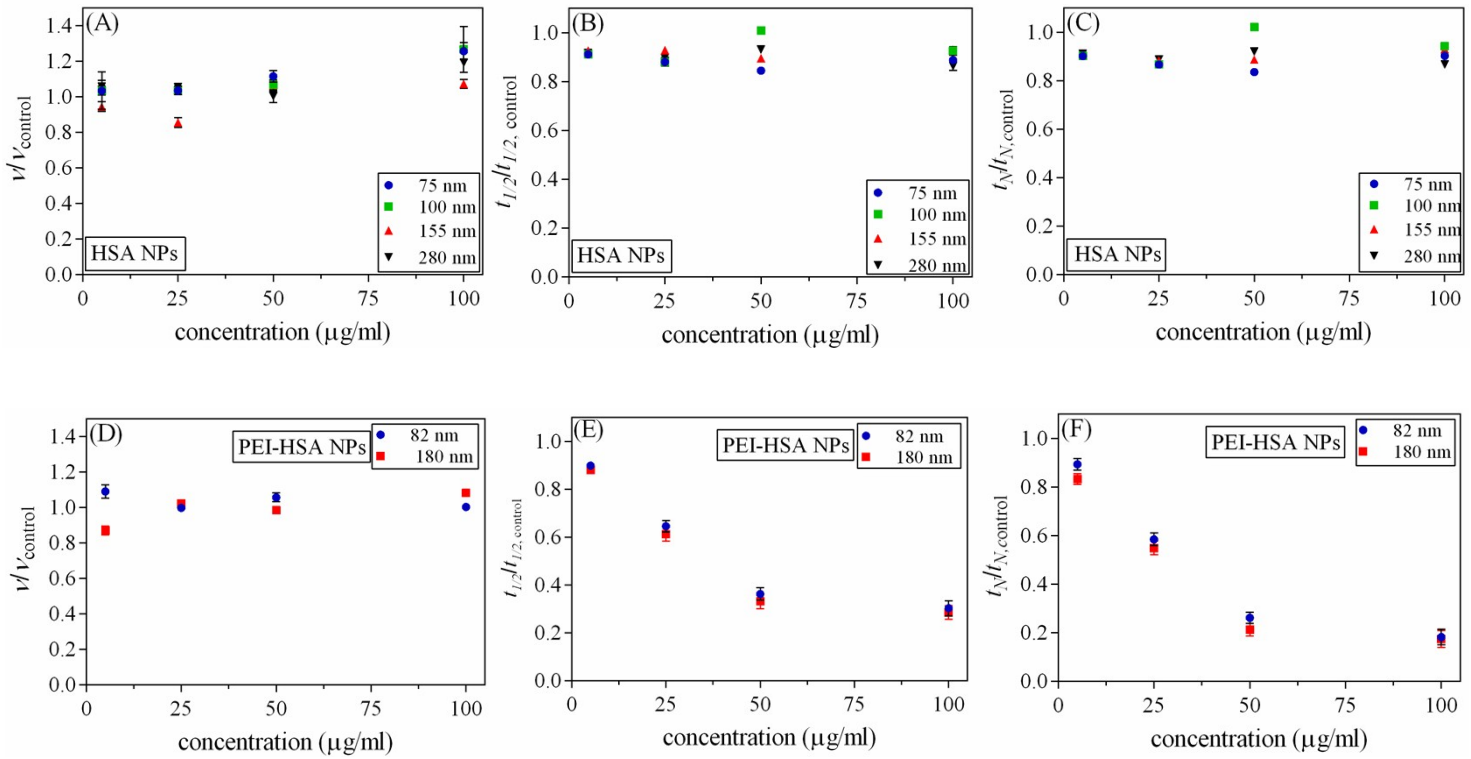

Fig. S3. Kinetic parameters of αSN fibrillation as a function of various concentrations of HSA NPs of different sizes ((A) relative growth rate ($v/v_{control}$), (B) relative half time ($t_{1/2}/t_{1/2,control}$), and (C) relative lag time ($t_N/t_{N,control}$)) and PEI-HSA NPs of different sizes ((D) relative growth rate ($v/v_{control}$), (E) relative half time ($t_{1/2}/t_{1/2,control}$), and (F) relative lag time ($t_N/t_{N,control}$)).

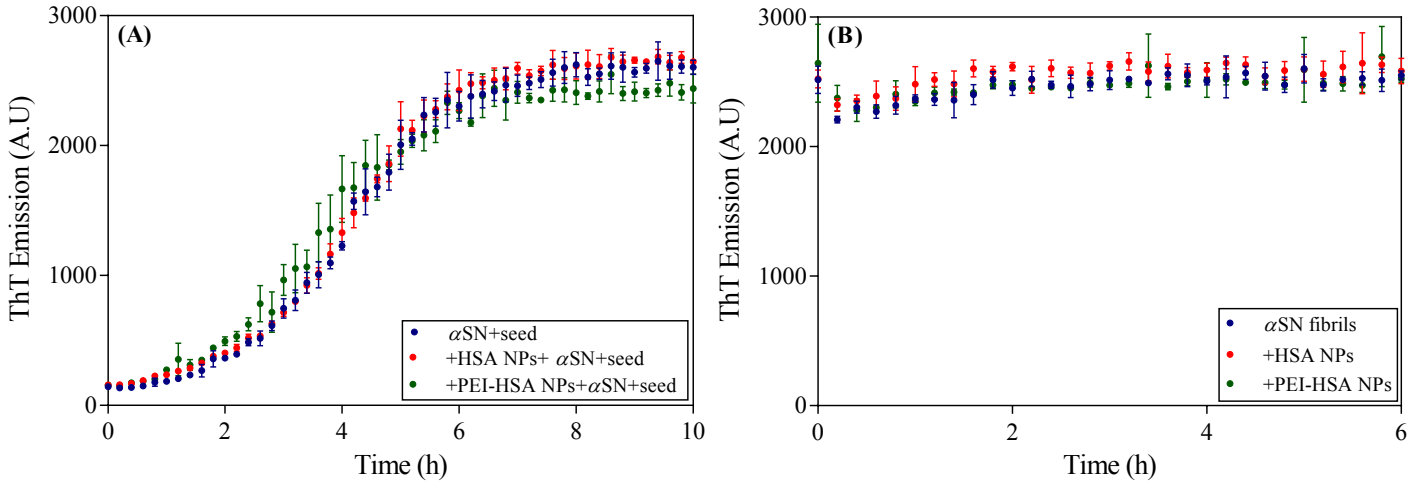

Fig. S4. The effect of NPs and on the (A) seeding of αSN aggregation and (B) disaggregation of αSN fibrils.

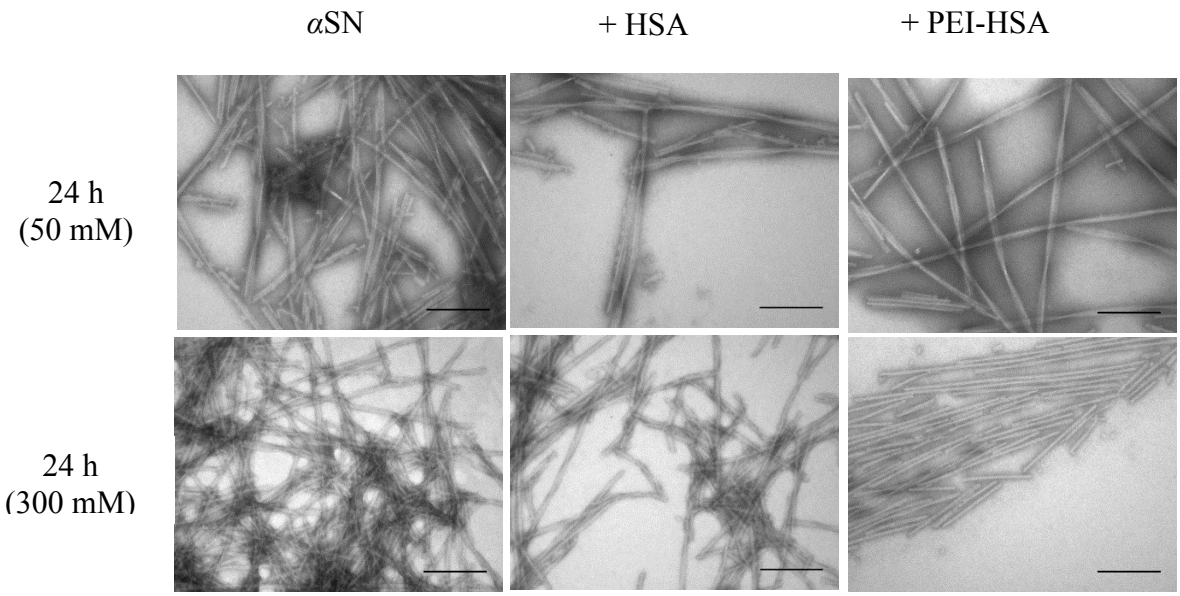

Fig. S5. Electron microscopy images of *α*SN incubated alone or in the presence of either HSA or PEI-HSA NPs after 24 h (at 50 and 300 mM NaCl concentration). Scale bar, 200 nm.

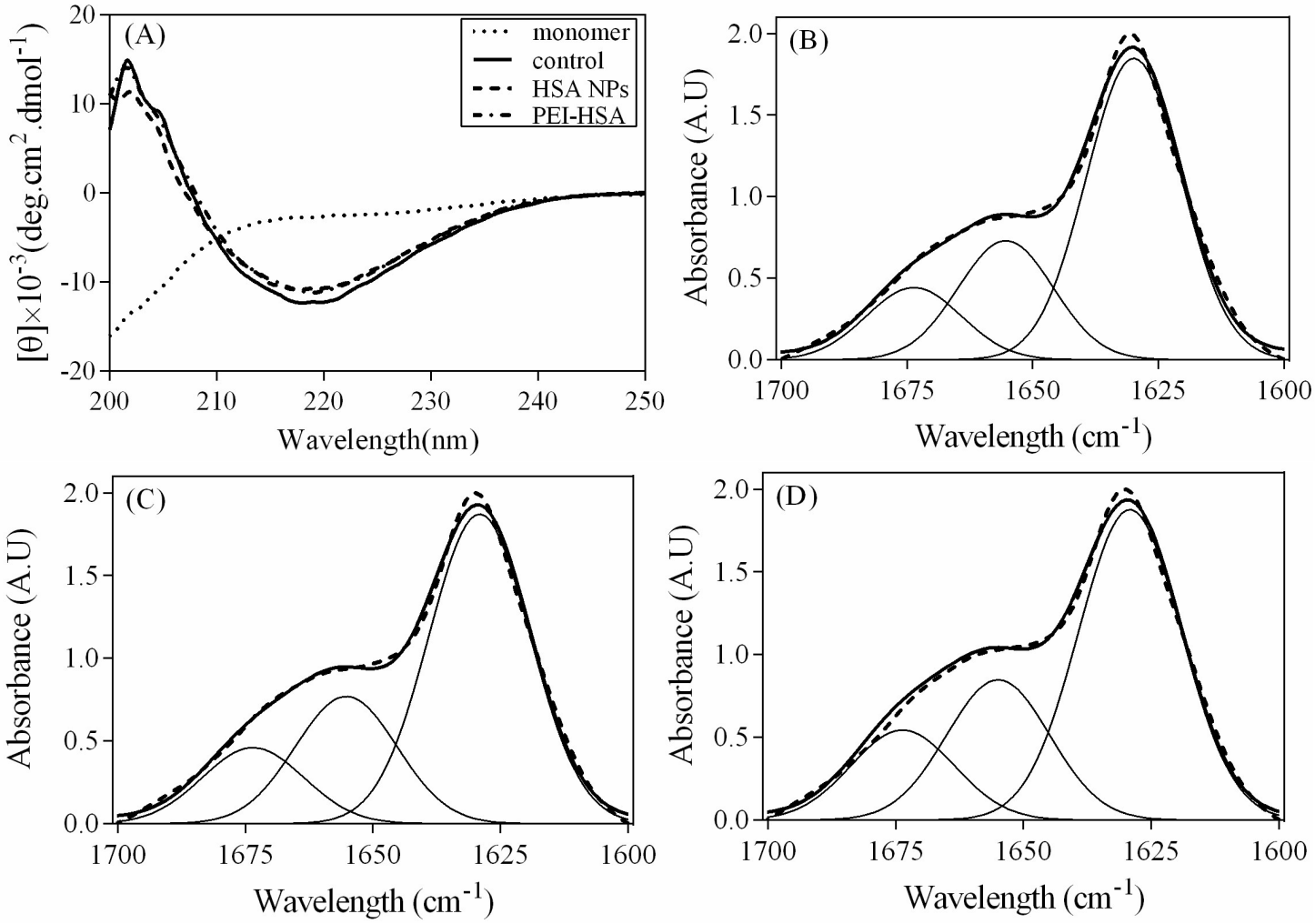

Fig. S6. Structural analysis of the fibrils formed alone or in the presence of either HSA or PEI-HSA NPs. (A) Far-UV CD spectra. ATR-FTIR analysis of fibrils formed alone (B), in the presence of HSA NPs (C), and in the presence of PEI-HSA NPs (D).

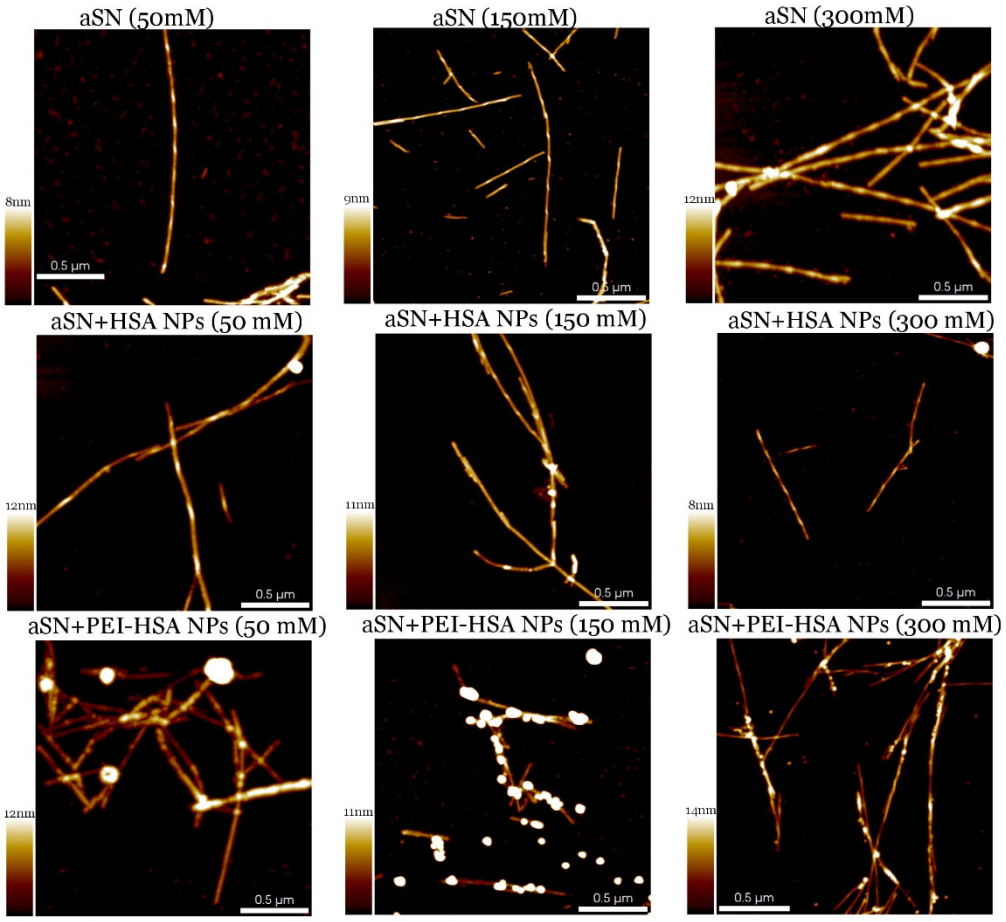

Fig. S7. AFM images of the *α*SN fibril morphology formed after incubation of 70 µM *α*SN alone and in the presence of either HSA or PEI-HSA NPs (at 50, 150, and 300 mM NaCl concentrations). Scale bar, 500 nm.

REFERENCES

1. N. Lorenzen, L. Lemminger, J. N. Pedersen, S. B. Nielsen, and D. E. Otzen, *FEBS letters*, 2014, **588**, 497–502.

2. N. Lorenzen, S. B. Nielsen, Y. Yoshimura, B. S. Vad, C. B. Andersen, C. Betzer, J. D. Kaspersen, G. Christiansen, J. S. Pedersen, P. H. Jensen, and others, *Journal of Biological Chemistry*, 2014, **289**, 21299–21310.