

Electronic Supplementary Information for:

Conducting microhelices from self-assembly of protein fibrils

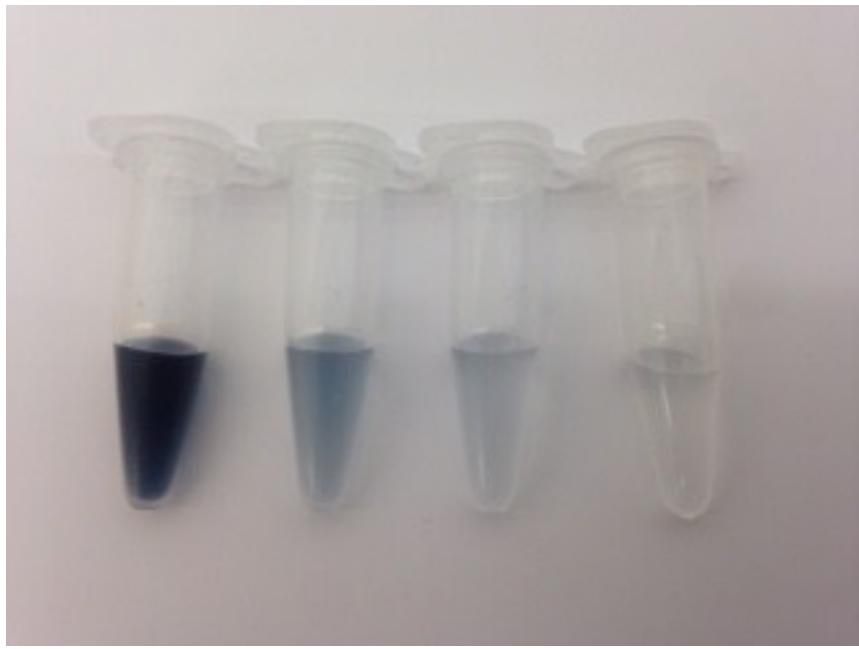
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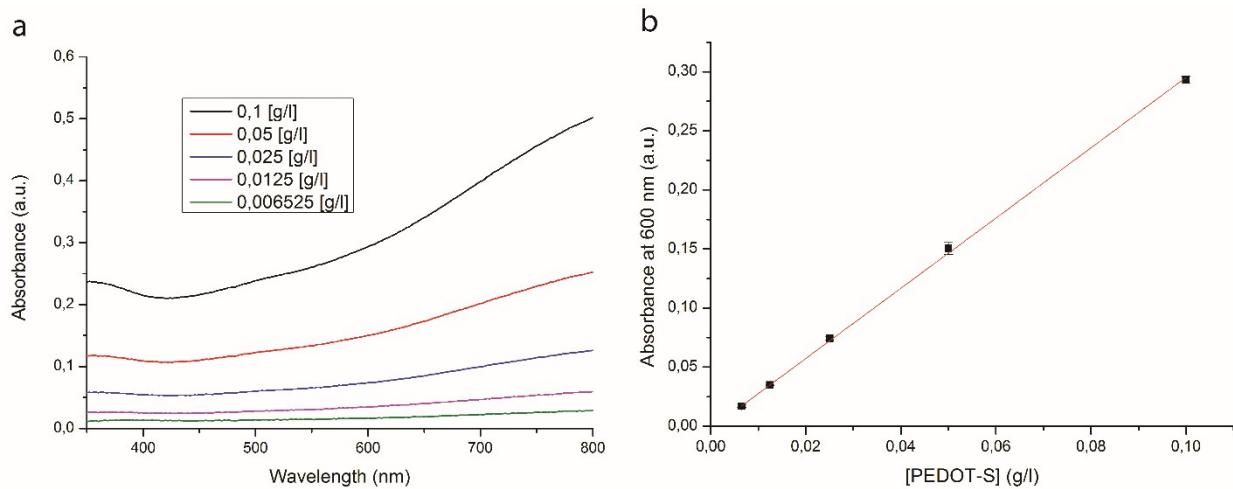
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Supplementary Figure 1. Photo of pelleted structures without (left) and with absorbed PEDOT-S (right)

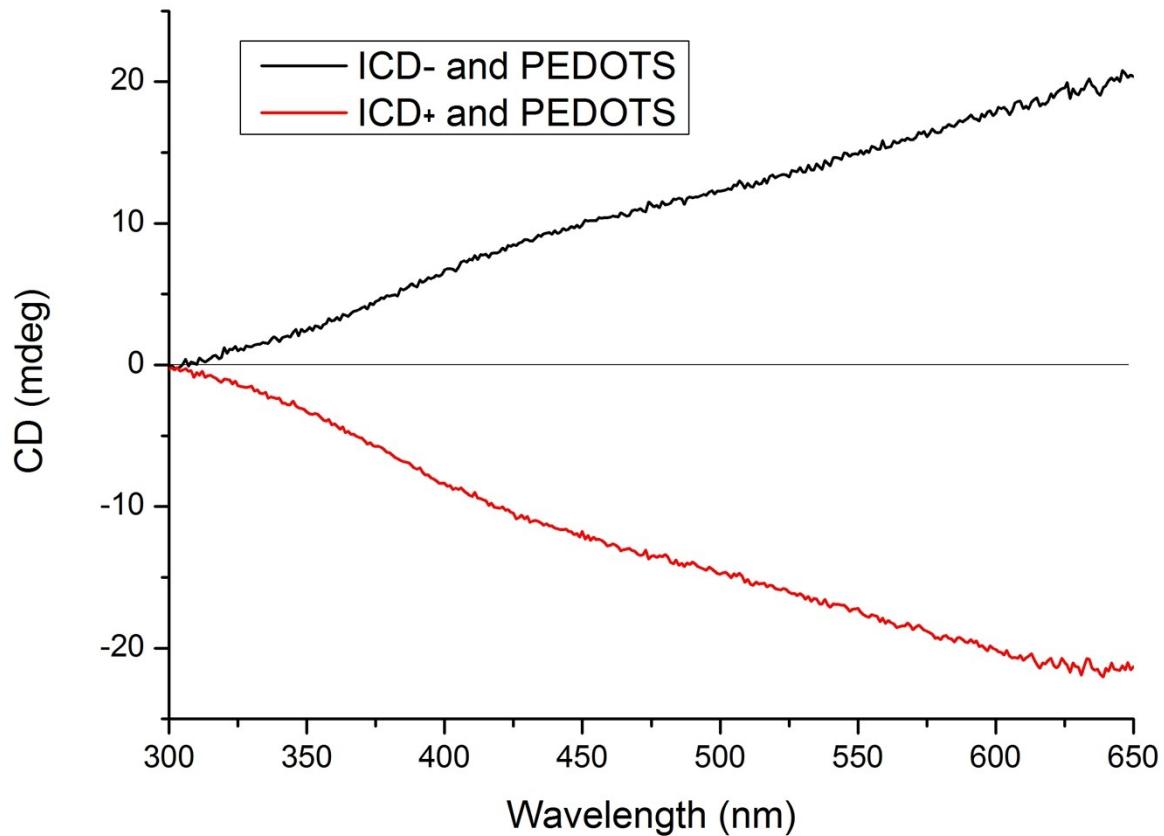


Supplementary Figure S2. Photo of the discarded supernatant from the washing procedure after successive washing steps. From left; 1) discarded supernatant after the first spin. 2)-4) discarded supernatant after three successive washing steps.



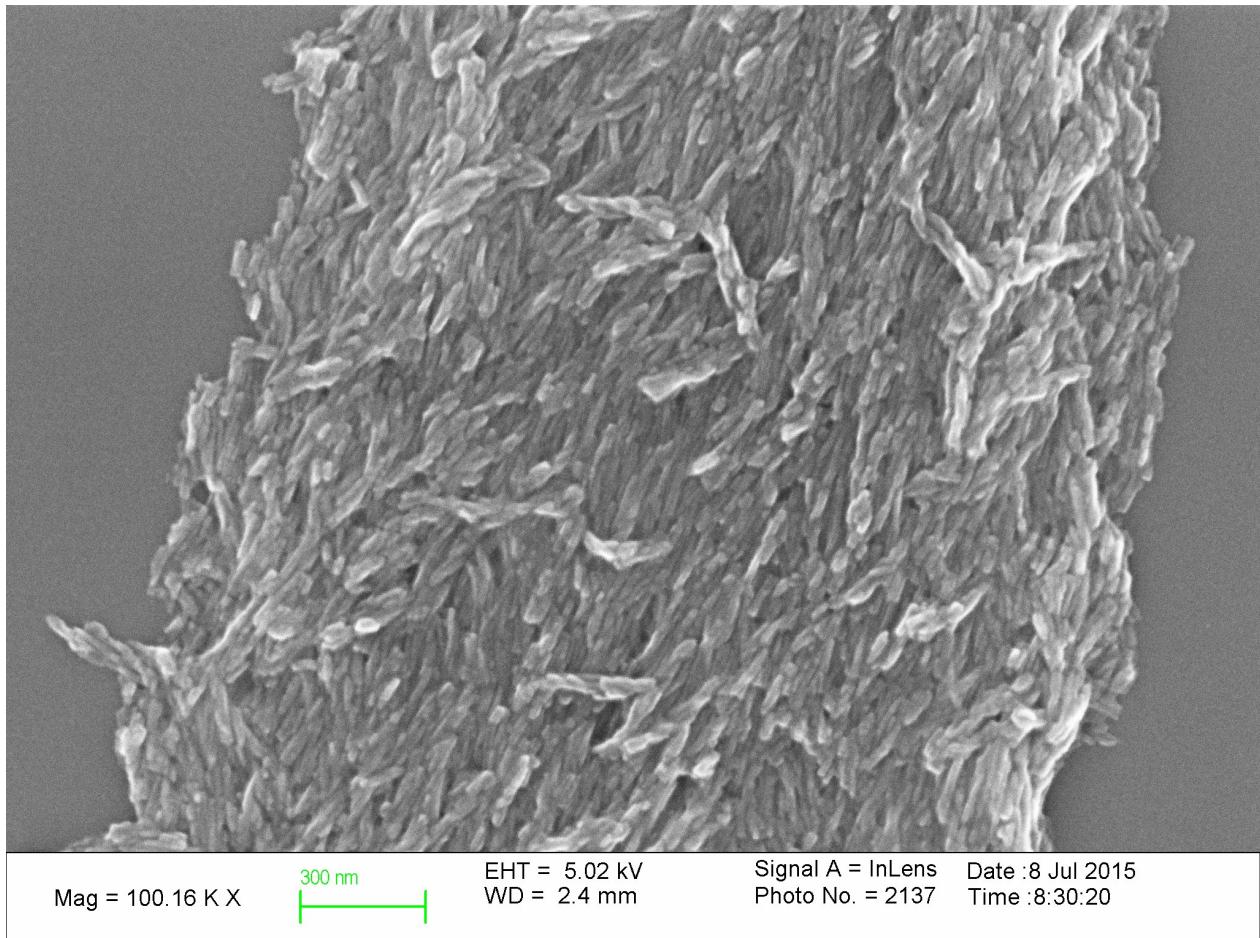
Supplementary Figure S3. **a**, Absorbance curves of PEDOT-S dissolved in water at various concentrations. **b**, PEDOT-S calibration data. In red: linear regression fit of the recorded absorbance yielding a linear relation between absorbance and PEDOT-S concentration; $\text{Absorbance} = 2,97 \cdot [\text{PEDOT-S}](\text{g/L})$.

Supplementary video V1. Video of a dispersion of PEDOT-S covered superstructures. As the structures tumble and rotate due to Brownian motion their axis vis-à-vis the crossed polarizers will change and they will go from light (45 degree versus the polarizers) to dark (<45 degree versus the polarizers).

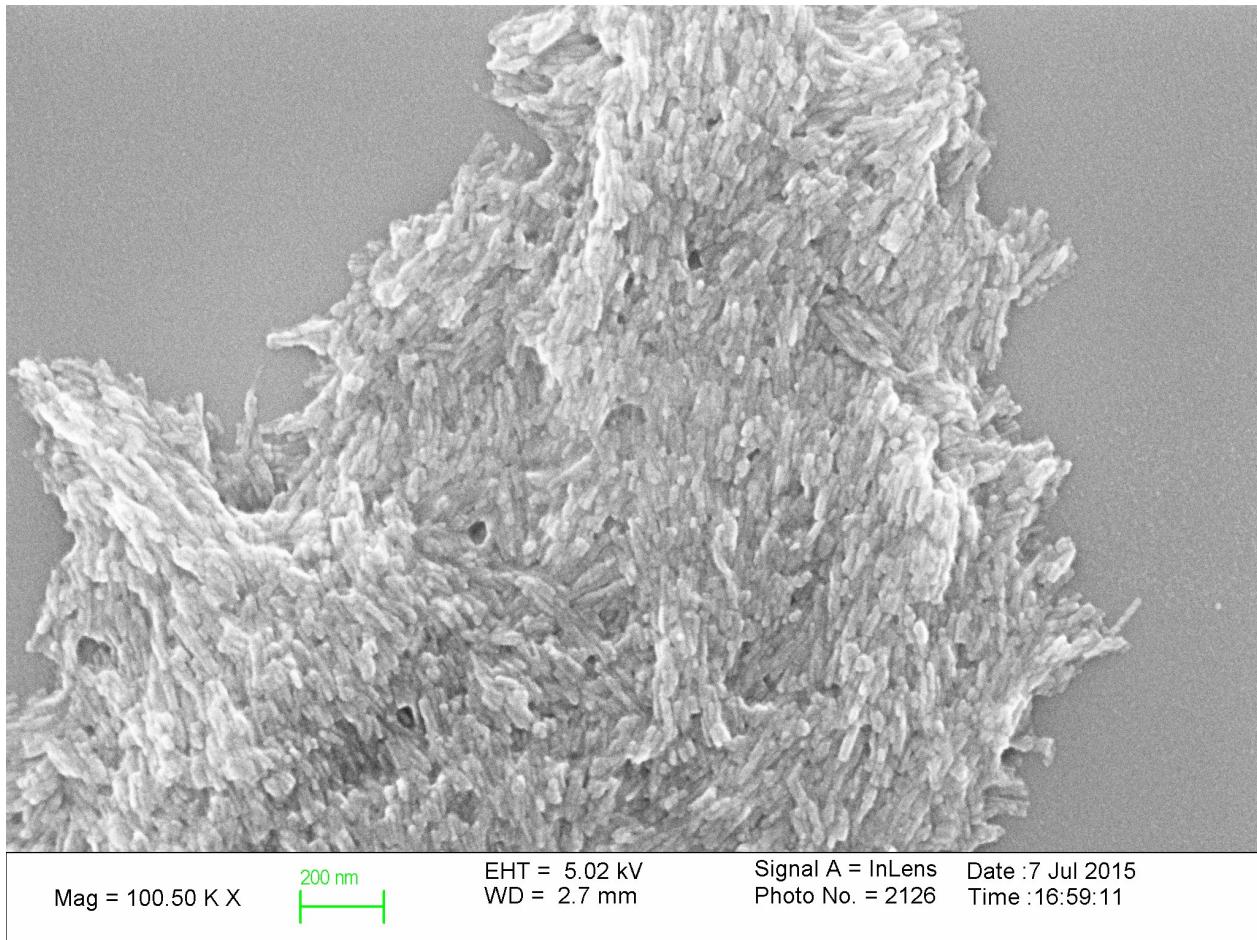


Supplementary Figure S4: CD spectra on PEDOT-S covered superstructures drop-casted onto glass.

a



b



Supplementary Figure S5. Close up SEM image of PEDOT-S covered superstructures. The scale bar represents 200nm. a) minus b) plus

Experimental details:

Materials: Bovine insulin was purchased from Sigma-Aldrich, (cat. No. I6634) was used without further purification.

+ICD/-ICD insulin fibril superstructures were prepared following published procedures.¹ Namely, 1 wt% (10 mg/ml) bovine insulin solution in 100 mM NaCl in D₂O (pD 1.9, adjusted with 20% DCl/D₂O – according to uncorrected pH-meter readout) was partitioned into several Eppendorf tubes and incubated at 40 °C with agitation (1400 rpm) for 48 hours. Both + and -ICD fibril forms were selected from individual tubes based on the sign and amplitude of their induced ThT CD. The deuterated environment was employed in order to permit quality-control of amyloid samples which includes analysis of infrared amide I' band (which is normally obscured by H₂O bending vibrations). It must be stressed, however, that the H₂O/D₂O solvent exchange is not necessary for preparation of the chiral insulin amyloid superstructures. Thioflavin T (ThT) was obtained from Sigma-Aldrich, and dimethyl

sulfoxide ($\geq 99.7\%$) and sodium chloride from Fisher Scientific. Deuterium oxide and deuterium chloride (20% w/w solution in D₂O) were purchased from Cambridge Isotope Laboratories, Inc.

PEDOT-S was synthesized according to previous published procedure. In short: The synthesis of PEDOT-S is based on an oxidative polymerization of EDOT-S (Heraeus) in water using as oxidants a catalytic amount of iron (III) chloride and sodium persulfate (both were purchased from Sigma Aldrich) (monomer to oxidant ratio of 0.5) at room temperature for 4 hours. Instantaneously upon addition of the oxidants the EDOT-S solution turns to deep blue.

After 4h the reaction is quenched and the iron is removed with the ion exchange resin Dowex (Sigma Aldrich). Finally, the obtained product was dialyzed with deionized water for 48 hours using a 3000 g/mol cutoff membrane and freeze-dried. Previous GPC characterization has PEDOT-S gave the values Mn=1700 (g/mol), Mw=5500 (g/mol) and Mz=10000 (g/mol) and an estimated degree of polymerization of 16 monomer.²

Complexation/Washing procedure: 0.1 g/l helical fibrils was incubated with 1 g/l PEDOT-S in 0.1 M NaCl and 25Mm HCl for 10 minutes. 1 ml of the resulting PEDOT-S-insulin superstructure dispersion was then centrifuged at 2000g whereafter 900 μ l of the supernatant was removed and the pellet was subsequently re-suspended in 900 μ l of MilliQ water. This centrifugation washing process was repeated three times to achieve a clear supernatant. The pellet was re-suspended a final time in the remaining 100 μ l volume after a final withdrawal of 900 μ l supernatant. After a subsequent 20 times dilution the sample was investigated by CD-spectroscopy.

Determination of the amount of PEDOT-S bound to the protein superstructure: In order to estimate the ratio of PEDOT-S relative to protein after the washing procedure the combined supernatant fractions obtained during the washing procedure was pooled. In order to calculate the PEDOT-S concentration a calibration curve for PEDOT-S was recorded. A stock solution of 10 g/l PEDOT-S was diluted in pure water to various concentrations. The calibration curve is shown in Figure S1.

Assuming that all protein structures pelleted during the washing procedure, thus leaving a supernatant free from proteins and containing only non-bound (free) PEDOT-S, and given that the initial amount of PEDOT-S and protein respectively was known, it is possible to get a rough estimation of the bound PEDOT-S to protein ratio. Starting with 2 mg of PEDOT-S and 0.2 mg of protein, the pooled supernatant from a washing procedure was diluted 10 times and the amount of PEDOT-S in the sample was calculated using the calibration curve shown in Figure S2. The amount of PEDOT-S remaining in the supernatant was then determined to be 1.78 mg. Thus, 0.22 mg of PEDOT-S appears to have associated with 0.2 mg of protein. In other words approximately a 1:1 weight ratio between bound PEDOT-S and insulin fibril superstructures is achieved after washing.

Circular dichroism (CD) spectroscopy: Measurements were performed on a Chirascan CD spectrometer from Applied Photophysics. Samples from a 1 wt% stock solution of insulin fibril superstructures was diluted 150 times in 0.1 M NaCl or alternatively pretreated according to the described washing

procedure. All measurements on solution were performed using a Hellma 1 mm pathlength quartz cuvette. Background measurements were performed with the same settings on a cuvette containing only solvent and subtracted from the sample spectra. Measurements on film were performed with the same setup as for the measurements on solution but with a cut glass slide as film substrate and a cut plain glass substrate as background.

Polarized optical microscopy: The polarized light microscopy images were recorded using an Olympus BH-2 microscope equipped with crossed polarizers with a top mounted digital camera.

UV-Vis spectroscopy: Absorption data was recorded on an Infinite M1000 PRO microplate reader from TECAN.

Scanning electron microscopy (SEM): SEM images were recorded on a 1550 Gemini SEM microscope from LEO. The water incubated sample in Figure 3 c was diluted 100 times in water from the 1 wt % stock solution and incubated for 10 minutes prior to drop casting on a silica substrate. Prior to imaging a 10 Å layer of W was sputtered onto the sample using a Leica EM SCD500 sputter coater.

C-AFM: C-AFM measurements were performed on a Dimension3100/NanoScope IVa system equipped with a C-AFM module (Veeco, 1 nA/V sensitivity). Commercial (Budget Sensors) Pt/Cr coated silicon probes with a nominal spring constant of 0.2 N/m were used for electrical characterization in contact mode at constant load force of 5 nN. Current maps were obtained by applying a voltage of 100 mV to the ITO electrode while keeping the C-AFM probe at ground.

- 1 A. Loksztejn and W. Dzwolak, *J. Mol. Biol.*, 2008, **379**, 9–16.
- 2 K. M. Persson, R. Karlsson, K. Svennersten, S. Löfller, E. W. H. Jager, A. Richter-Dahlfors, P. Konradsson and M. Berggren, *Adv. Mater.*, 2011, **23**, 4403–8.