Supporting Information

Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis

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Phosphoric acid hydrolysis of cotton pulp. Different reaction temperatures, concentrations and reaction times were employed until optimal parameters were determined.

Sample	[H₃PO₄] (M)	T (°C)	Time (min)	Result
PC111	6.2	50	180	Pulp
PC112	6.2	50	240	Pulp
PC113	6.2	50	300	Pulp
PC211	7.8	50	180	Pulp
PC212	7.8	50	240	Pulp
PC213	7.8	50	300	Pulp
PC311	9	50	180	Pulp
PC312	9	50	240	Pulp
PC313	9	50	300	Pulp
PC221	7.8	100	180	Fibers
PC222	7.8	100	240	Thick CNCs + agglomerates
PC223	7.8	100	300	Small CNCs + fibers + agglomerates
PC321	9	100	120	Pulp
PC322	9	100	180	Thick CNCs + agglomerates
PC323	9	100	240	Thick CNCs + fibers + agglomerates
PC421	10.7	100	30	Fibers
PC422	10.7	100	60	Thick CNCs + fibesr
PCNC90	10.7	100	90	CNCs
PC424	10.7	100	120	Small CNCs
PC425	10.7	100	150	Small CNCs + round CNCs

Table S1. Products obtained through the hydrolysis of cotton pulp under various reaction conditions. Reaction conditions of PCNC90 were determined to be optimal. (Bold)

Transmission Electron Microscopy of Reference CNCs

TEM micrographs were recorded in a Philips CM 100 microscope with an accelerating voltage of 80 kV. Samples were prepared by dropping 50 µL of an aqueous CNC dispersion (CNC content 0.1 mg/mL) onto a carbon supported copper grid (Electron Microscopy Science, Hatfield, Pennsylvania, USA.); the sample was subsequently dried in an oven at 70 °C for 1 h.
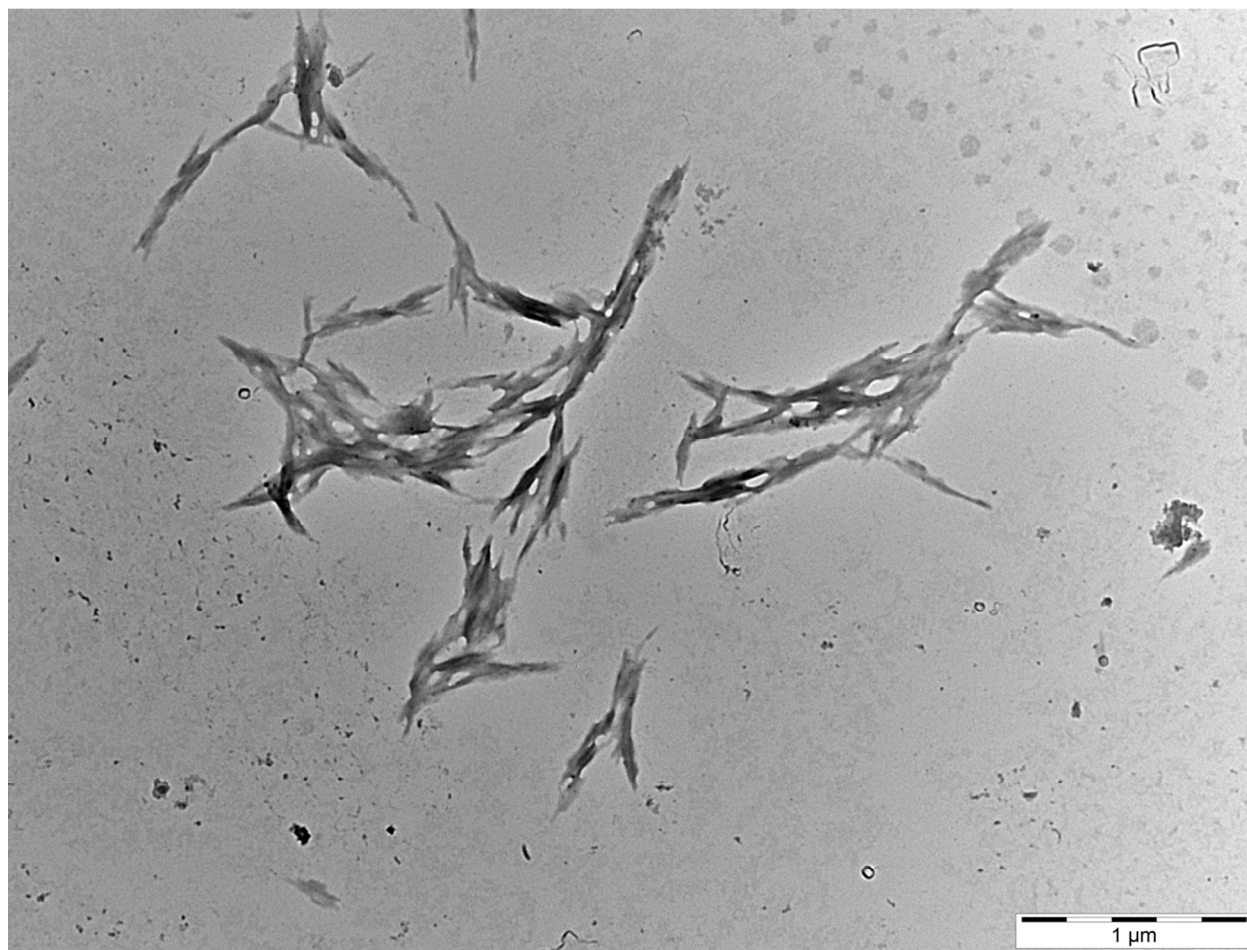
Figure S1: TEM image of CNCs isolated by hydrolysis with HCl (H-CNCs).
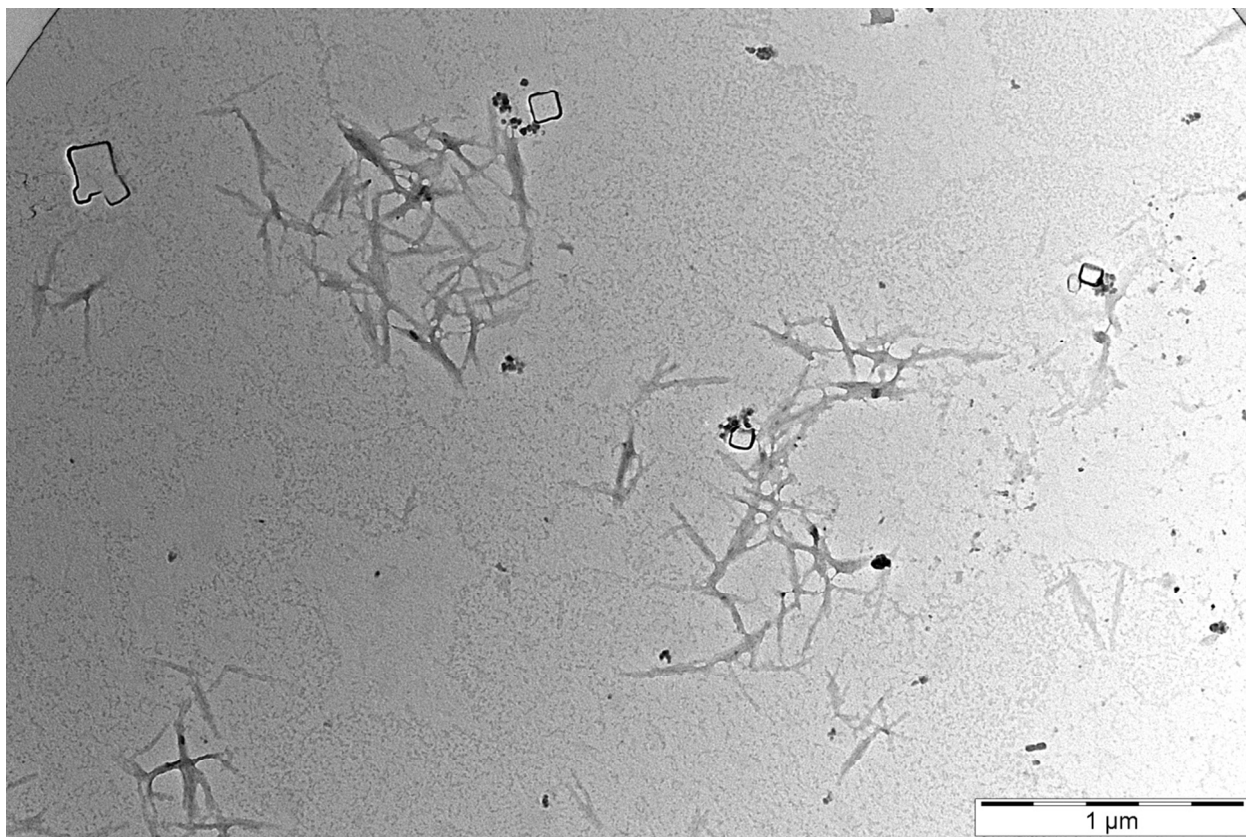
Figure S2: TEM image of CNCs isolated by hydrolysis with sulfuric acid (S-CNCs).

X-Ray Diffraction (XRD)

Diffractograms were recorded with a Rigaku Ultima IV equipped with a CuKα radiation source (λ = 1540 Å) operating at 40kV. CNC samples (~ 150 mg) were grounded, dissolved in 1 mL of water and dried out in the oven at 100 °C for 30 minutes. These more compact CNC tablets were later regrounded in a mortar and pestle and transferred to quartz holders. Samples were scanned over an angular 2ϴ range of 8 - 70°.

Diffractograms were fitted using free software (www.magicplot.com) by placing Gaussian shaped peaks after background subtraction. Peaks were let free for intensity, position and

FWMH with exception of the non-crystalline peak which was locked at position. Data was fitted taking into account only cellulose type I.[1-2]
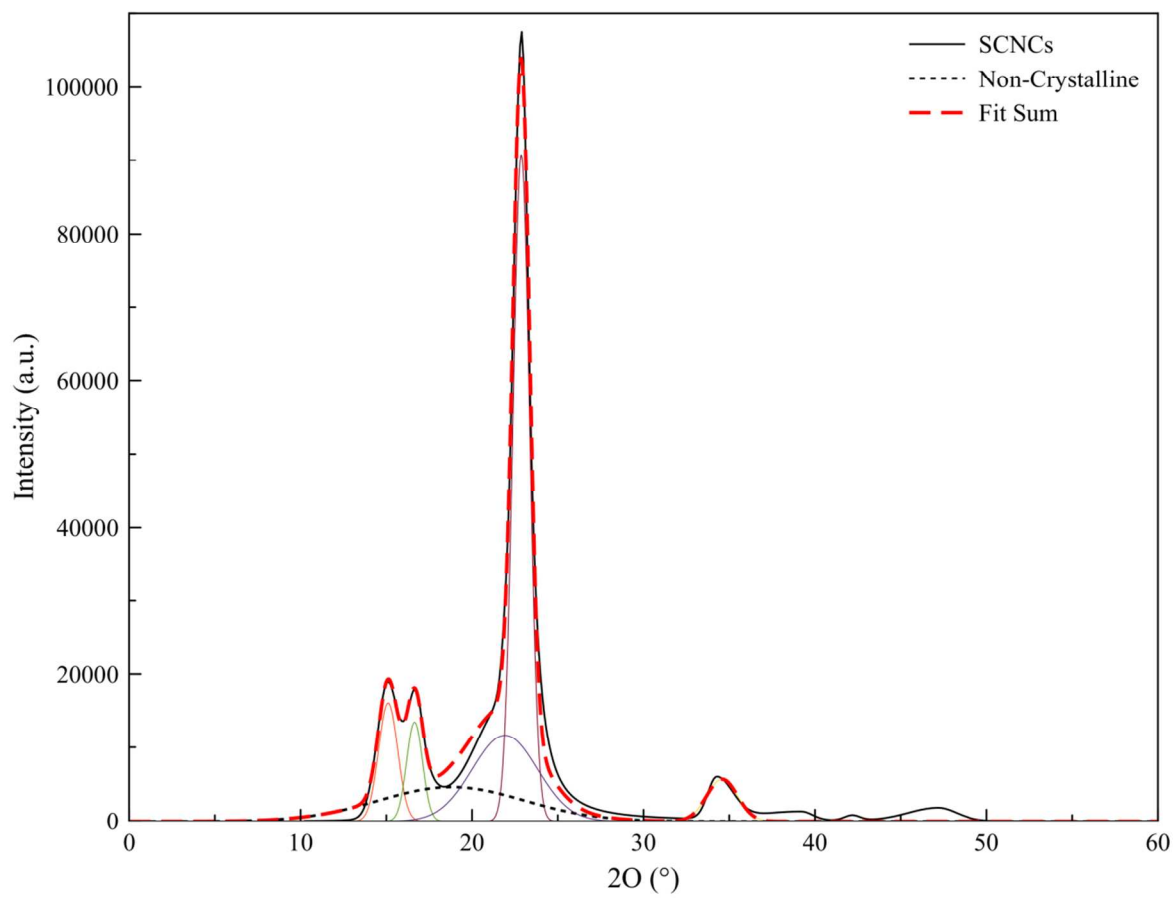
(101): 14.7 °   (10I): 16.8 °   (021): 20.6 °   (002): 22.5 °   (040): 34.7 °
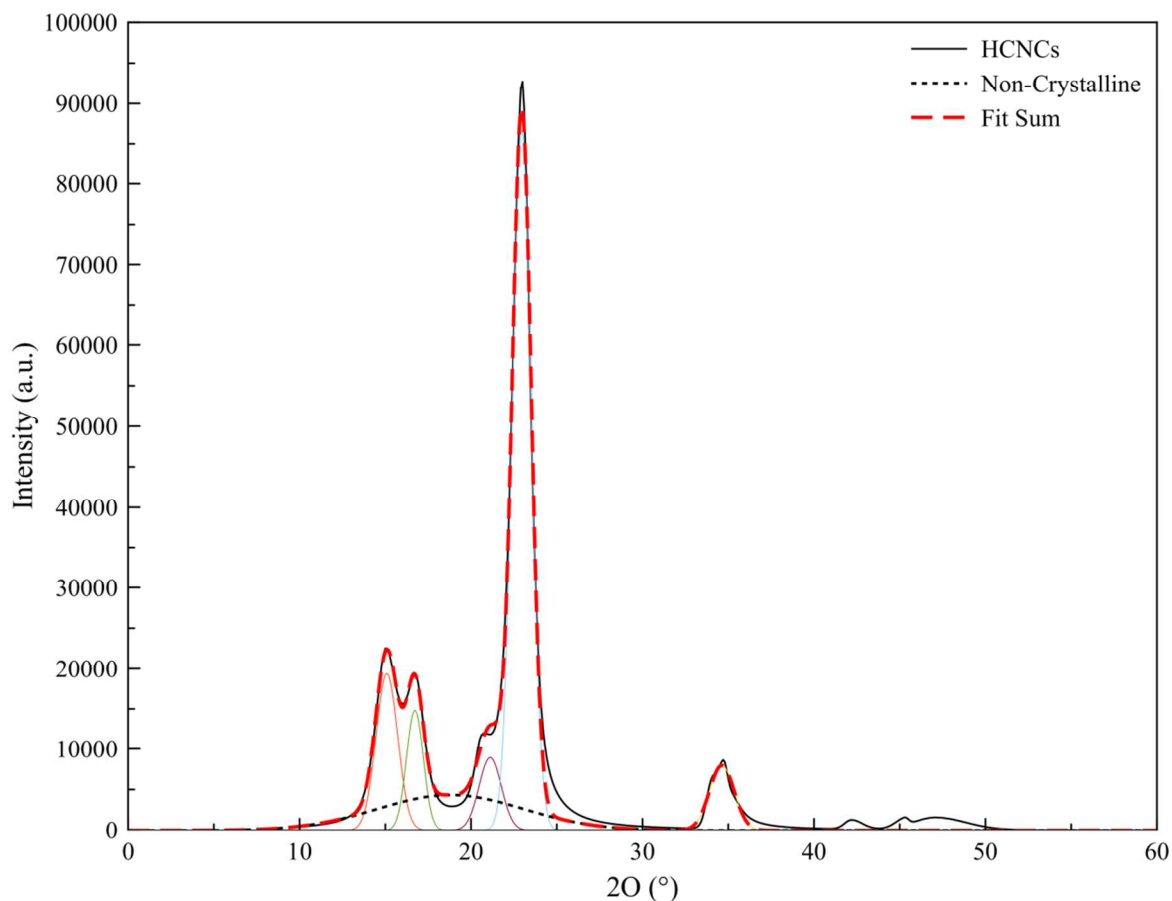
Apparent crystallinity was calculated from the formula:

$$\text{Apparent crystallinity (\%)} = \frac{[int(101) + int(10I) + int(021) + int(002) + int(040)]x100}{[int(101) + int(10I) + int(021) + int(002) + int(040) + int(non-crystalline)]}$$
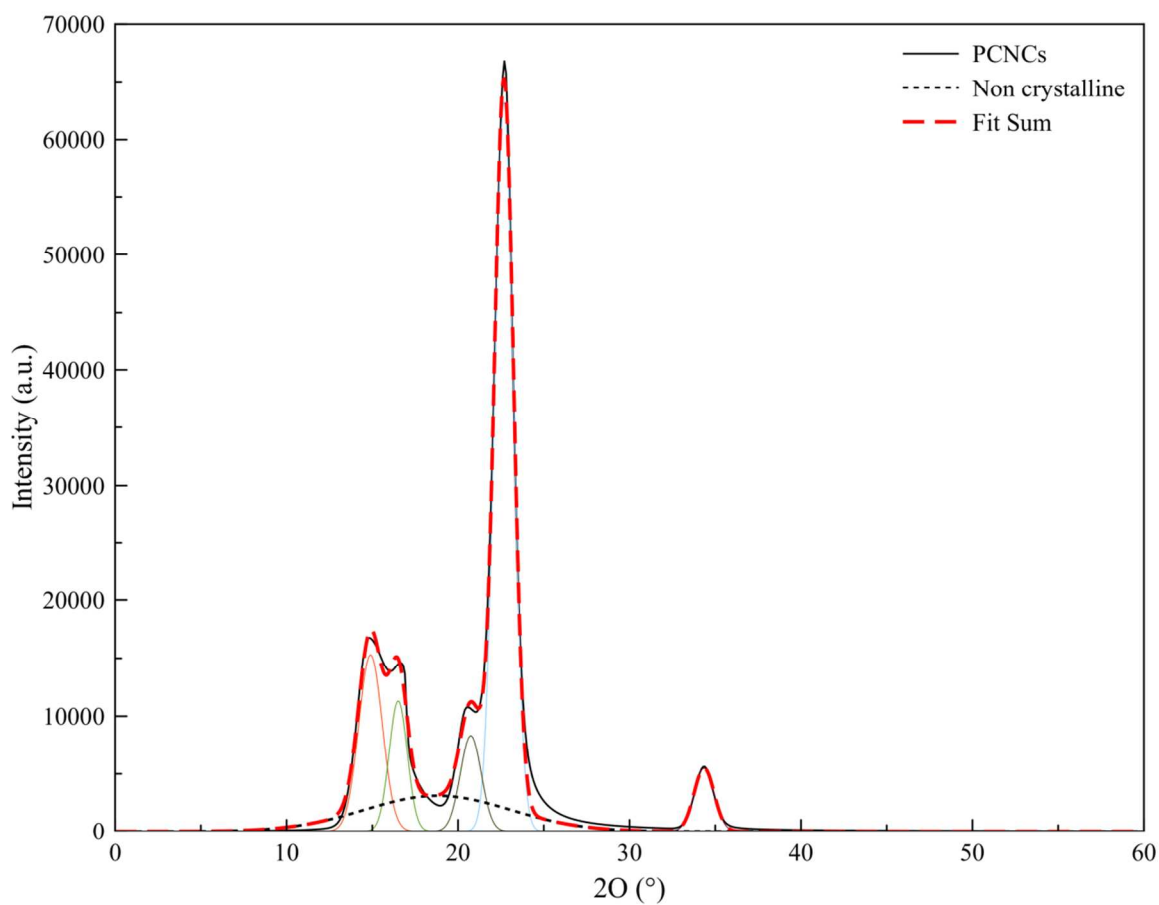
Figure S3. XRD diffractogram of P-CNCs.

Figure S4. XRD diffractogram of S-CNCs.

Figure S5. XRD diffractogram of H-CNCs.

Conductometric titration of CNCs. To determine the surface charge density, conductometric titrations were carried out. CNCs ($\approx$ 50 mg) were dispersed in a mixture of 10 mL of 10 mM aqueous HCl (Sigma Aldrich, 37%) and 25 mL H_2O (Sartorius Stedim, Arium 611VF) in a glass beaker by 10 min sonication in a horn sonicator (Branson Digital Sonifier 102). The dispersion was subsequently titrated with 10 mM aqueous NaOH (Sigma Aldrich, 98% reagent grade). In order to increase the accuracy of the titration, the neat medium (mixture of 10 mL of 10 mM HCl and 25 mL H_2O which had also been sonicated), was also titrated with 10 mM aqueous NaOH. The titration volume was determined by plotting the data and fitting it to three lines; the first one

corresponding to the titration of the HCl, the second one corresponding to the sample or acidity of the medium, and the third one to the excess of base. The three different series of data were separated by evaluating the slope. Intersection of the line fitted to the sample titration series with the other two series was calculated and the volume comprised between the two intersecting points was calculated.

The volume required for titration as well as concentrations are an average of three titration measurements. Thus, an average volume of 0.507 mL of NaOH was calculated to be required for titration of the neat medium. This volume was then subtracted to the volume required to titrate each of the samples.

The net volume required for sample titration was employed to calculate the concentration of sulfate and phosphate groups on S-CNCs and P-CNCs, respectively.

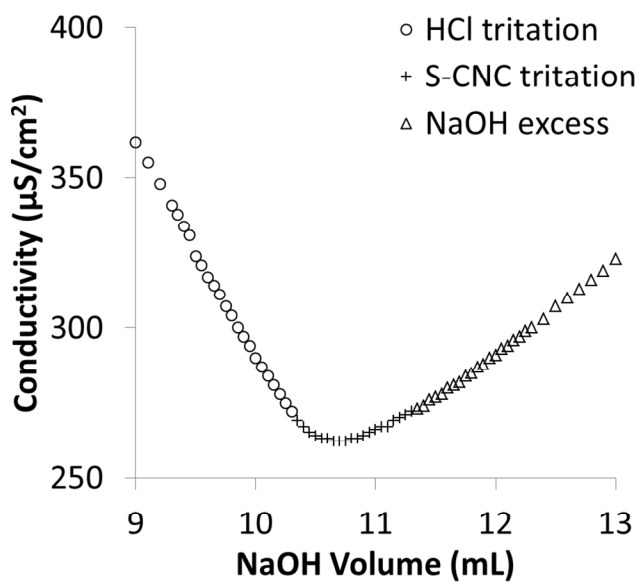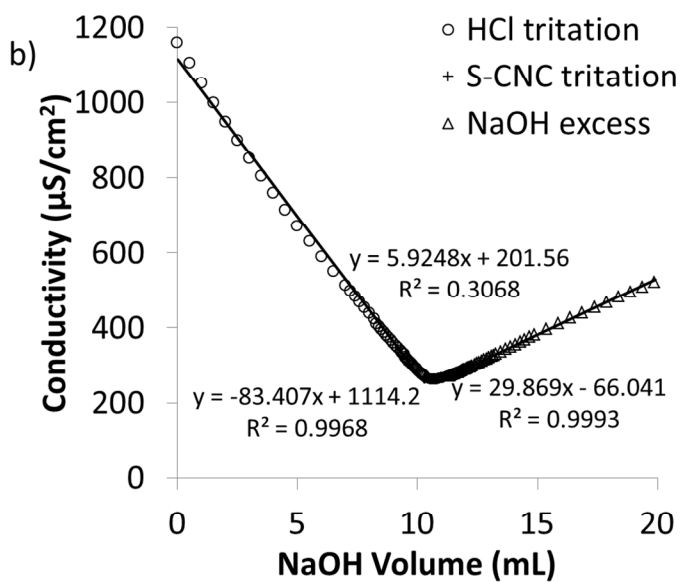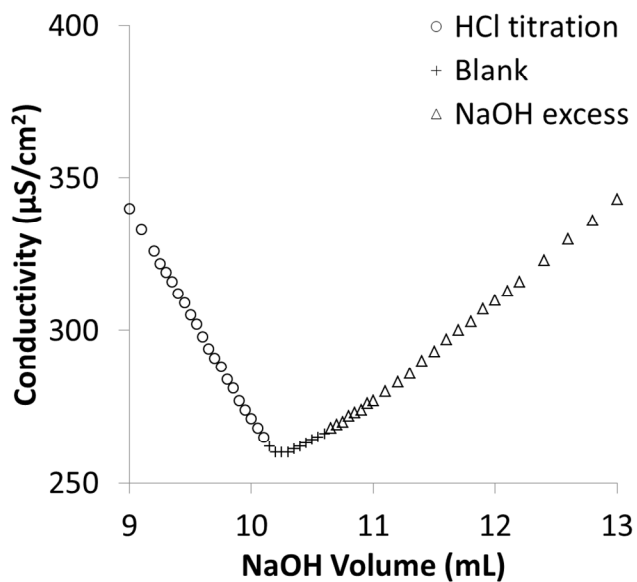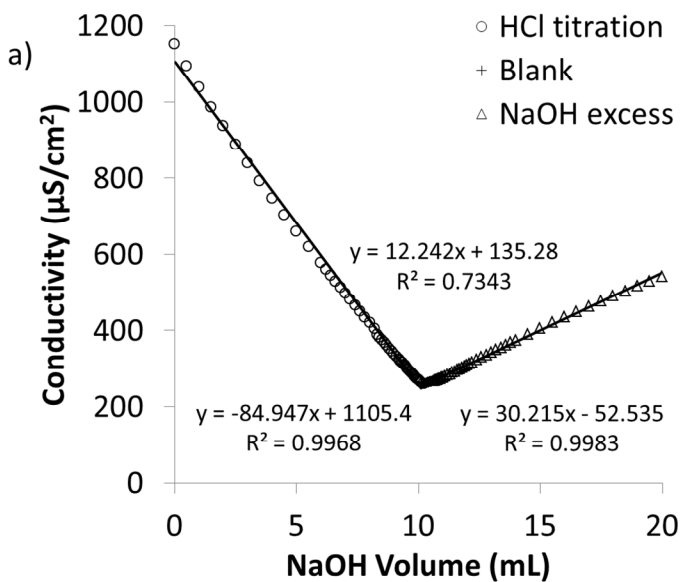The concentration of sulfate groups was calculated following the equation :

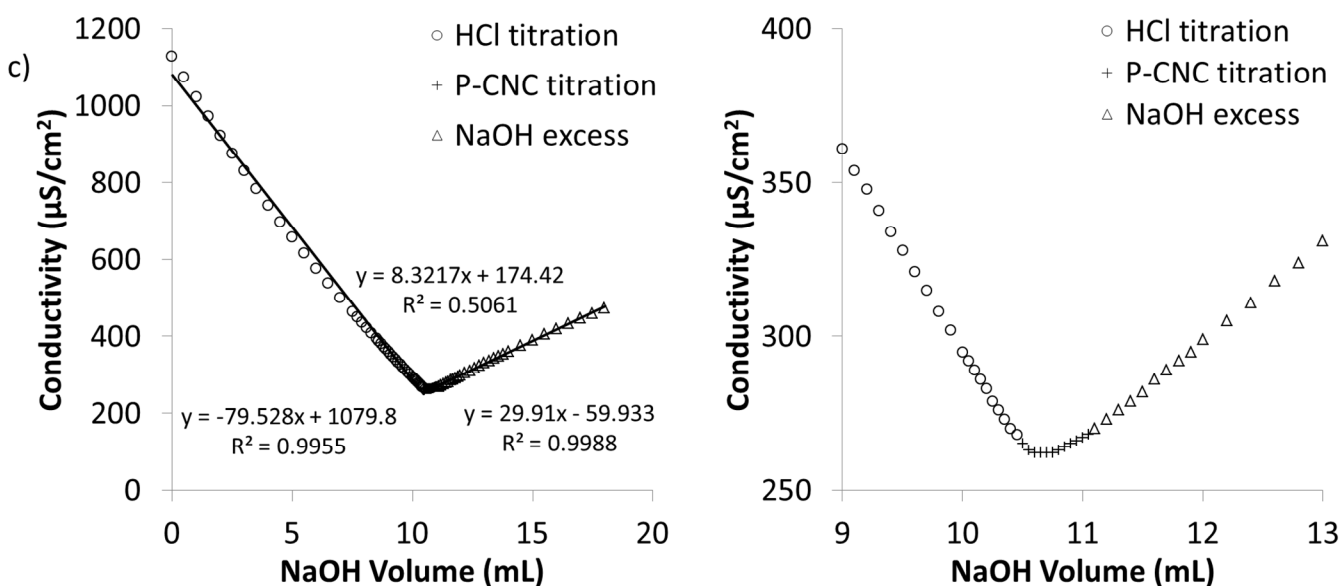$$\frac{mmol\ SO_4^-}{kg\ cellulose} = \frac{C_{NaOH} * V_{NaOH}}{W_{CNC}} * 10^6$$

where, C_{NaOH} is the concentration of the base used for titration (M), V_{NaOH} is the volume (L), and W_{CNC} is the weight of S-CNCs employed for the measurement (g).

Or, in the case of P-CNCs:

$$\frac{mmol\ HPO_4^-}{kg\ cellulose} = \frac{C_{NaOH} * V_{NaOH}}{W_{CNC}} * 10^6$$

where W_{CNC} is the weight of P-CNCs titrated (g) and V_{NaOH} corresponds to the volume of NaOH required to reach the first equivalence point (between pKa1 ≈ 2.12 and pKa2 ≈ 7), that is to say, to titrate one proton.

a)

b)

c)

y = 8.3217x + 174.42
R² = 0.5061

y = -79.528x + 1079.8
R² = 0.9955

y = 29.91x - 59.933
R² = 0.9988

Figure S6. Representative conductometric titration curves of a) neat medium b) S-CNCs and c) P-CNCs.

NMR studies. ^{31}P NMR spectra (300MHz, 10000 scans) were acquired on a Bruker Avance III 300 MHz spectrometer. Samples were prepared by dissolving 150 mg of material in a bi-solvent system of perdeuterated pyridinium chloride and d6-DMSO at 80 °C under stirring for 90 min. The ionic liquid was prepared by deuteration of d5-pyridine with d4-methanol and acetyl chloride in dry ether as described previously.[3] The P-CNC solution in the ionic liquid was added to a solution of triphenyl phosphine (reference) in DMSO-d6 and ^{31}P-NMR spectra were recorded (Figure S4c and S4d). For reference purposes, ^{31}P-NMR spectra were also recorded of a mixture of phosphoric acid, triphenyl phosphine, and DMSO-d6 (Figure Sa and S4b)

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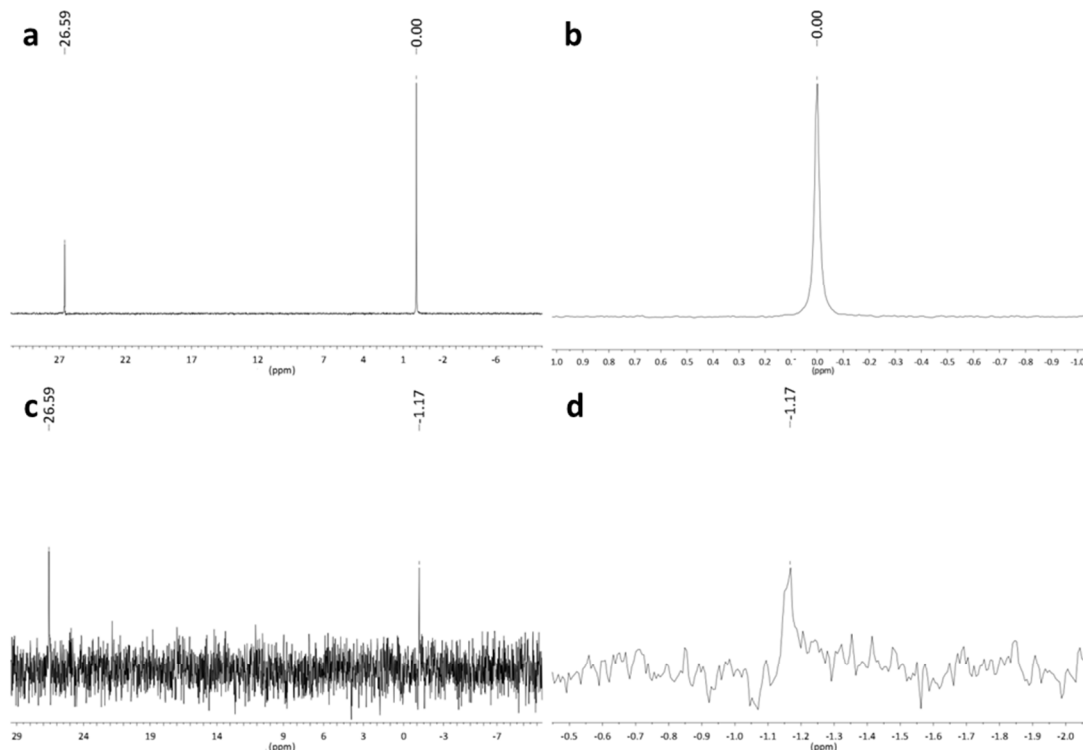
Figure S7. a) ^{31}P-NMR spectra of a) phosphoric acid and triphenyl phosphine as a reference; b) magnification of the spectrum shown in (a) around the phosphoric acid peak; c) P-CNCs and triphenyl phosphine as reference; d) magnification of the spectrum shown in (c) around the P-CNC peak.

Energy dispersive X-ray spectroscopy (EDS). The presence of phosphorous in the P-CNC samples was also determined by EDS. Experiments were performed in a JEOL JSM-6400 scanning electron microscope working at an accelerating voltage of 20 kV. Samples were analyzed at a working distance of 25 mm with a system based on Oxford ISIS equipped with a SDD detector with a resolution higher than 133 eV. Samples were prepared by casting drops of a 4 mg/mL aqueous P-CNC dispersion, drying at 70 °C for 1 h, and repeating this cycle until ca. 250 µL of the dispersion had been applied and a thin P-CNC film had formed on top of a Si single crystal held by a stainless steel holder. No coating was employed.

EDS spectra of P-CNCs and S-CNCs show the presence of phosphorous and sulfur, respectively, but also the presence of silicon, carbon and oxygen. The presence of silicon is attributed to the substrate in which sample was casted. Carbon and oxygen correspond to the cellulose. In each case, spectra were recorded on several particles of the same sample, getting the same composition for all the particles. Images of P-CNCs and S-CNCs were the spectra were recorded are also shown. As in the recorded NMR spectra, the intensity of the phosphorous and sulfur signals was very weak and further quantification was not possible.
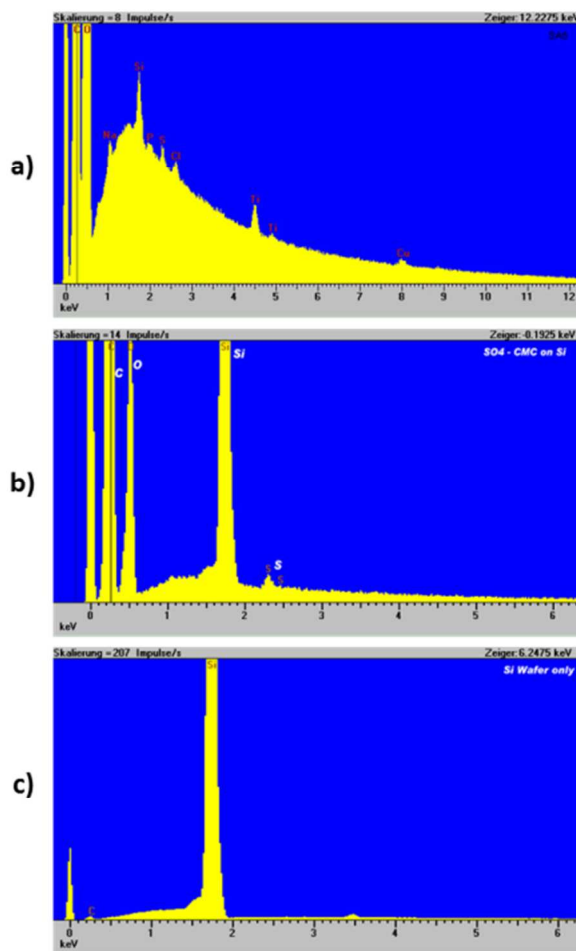
Figure S8. EDS spectra of a) P-CNCs, b) S-CNCs and c), the substrate where the presence of silicon can be seen.

Microdetermination of phosphorous. The phosphor content of the P-CNCs was also determined by microdetermination according to the protocol developed by Fiske *et al.*[4] The organic phosphorous was digested by sulfuric acid and then complexed with ammonium molybdate. The resulting phosphomolybdate was reacted with L-ascorbic acid, producing a blue complex in which absorbance at 820 nm was measured. A 0.65 mM phosphorous standard solution (Sigma-Aldrich) was employed to create a calibration. The phosphorous content was determined to be 3.95 ± 0.5 mmol/kg cellulose. All the reagents were from Sigma-Aldrich.
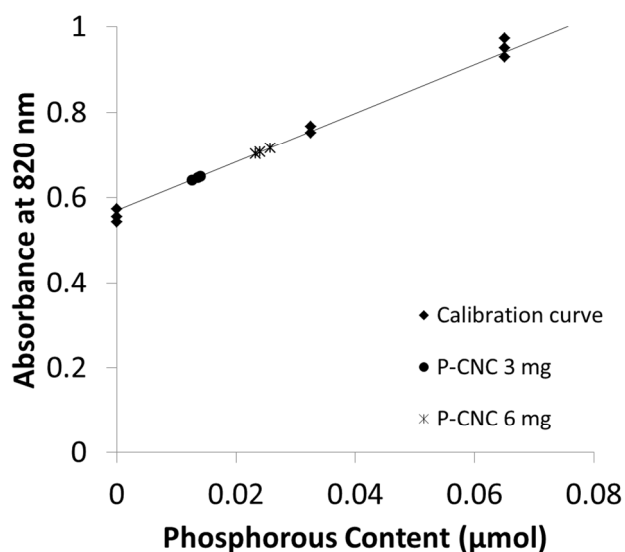
Figure S9. Calibration curve and absorbance data of phosphorous on P-CNCs at 820 nm.

Dispersibility of different CNCs

The dispersibility of S-CNCs, P-CNCs and H-CNCs in H_2O, DMSO, DMF and THF was further probed by combining the components (5 mL of solvent and CNCs at a concentration of 9 mg/mL) in a vial, sonication for 10 min in a horn ultrasonicator (Branson Digital Sonifier 102 C), letting the dispersions stand two months, diluting them to a concentration of 0.1 mg/mL, casting 50 µL of the dispersions onto a carbon supported carbon grid and drying at 70 °C, and taking electron microscopy images according to the procedure detailed above.
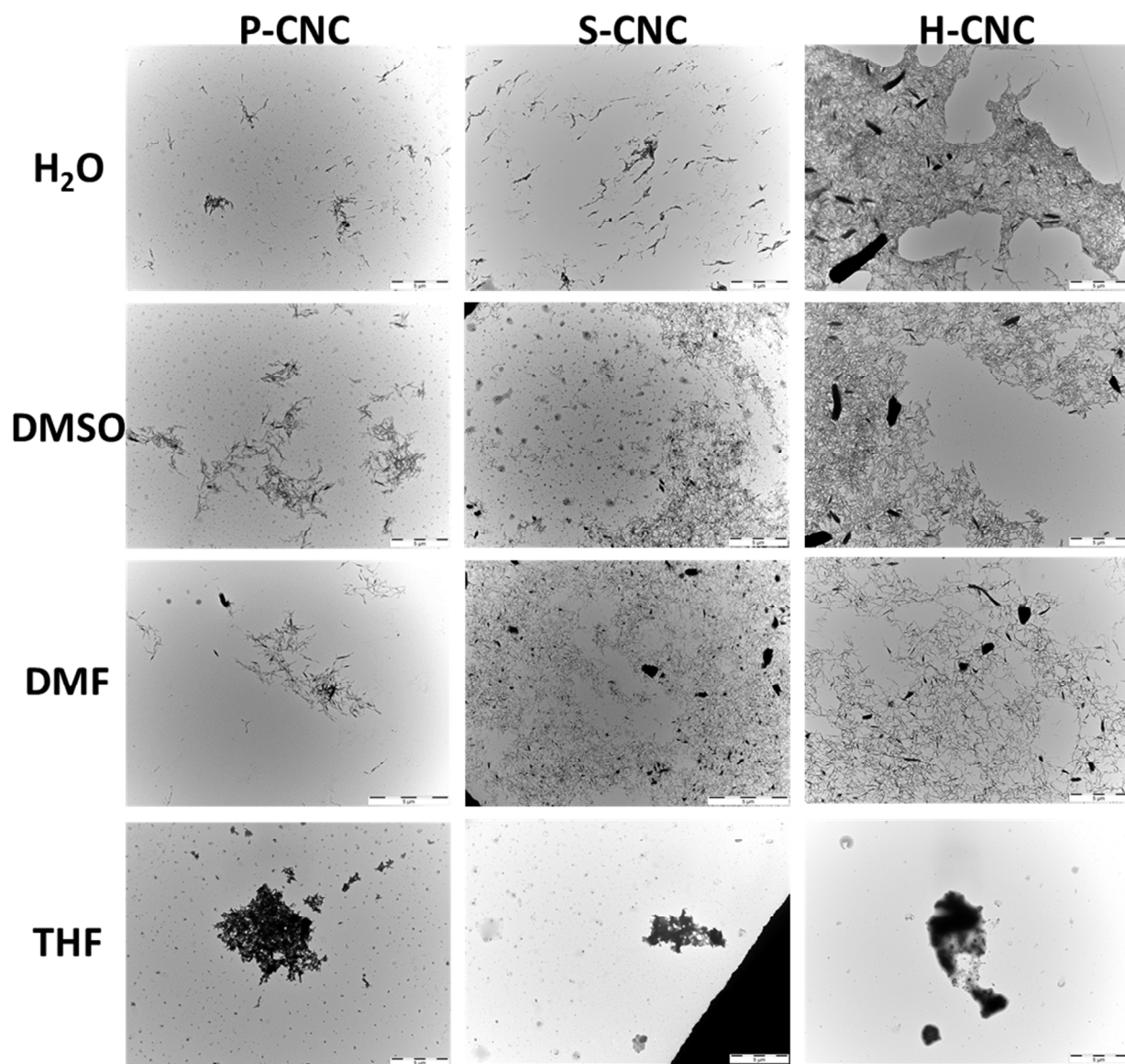
Figure S10. TEM images of CNCs deposited from dispersions (0.1 mg/mL) in the solvents indicated.
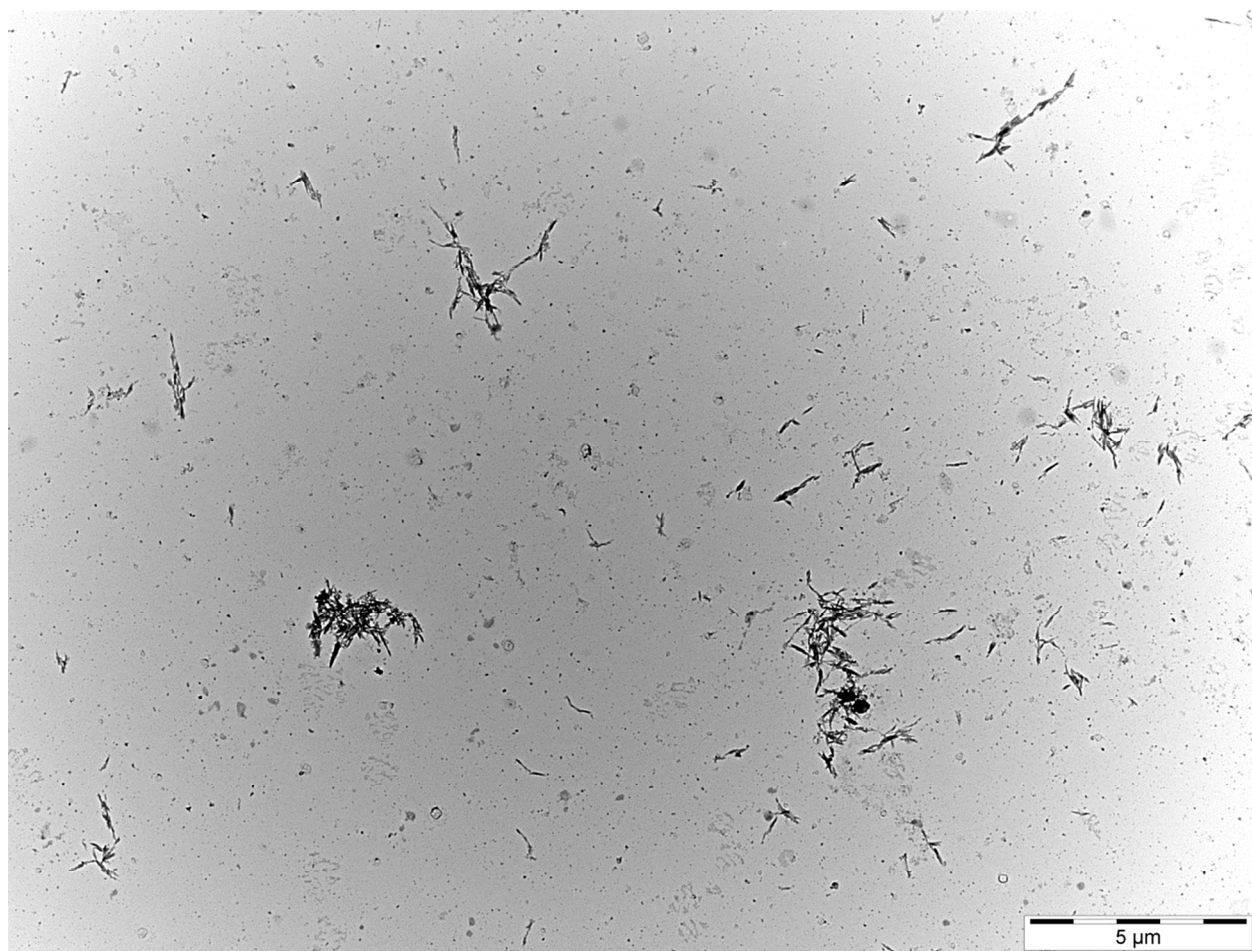
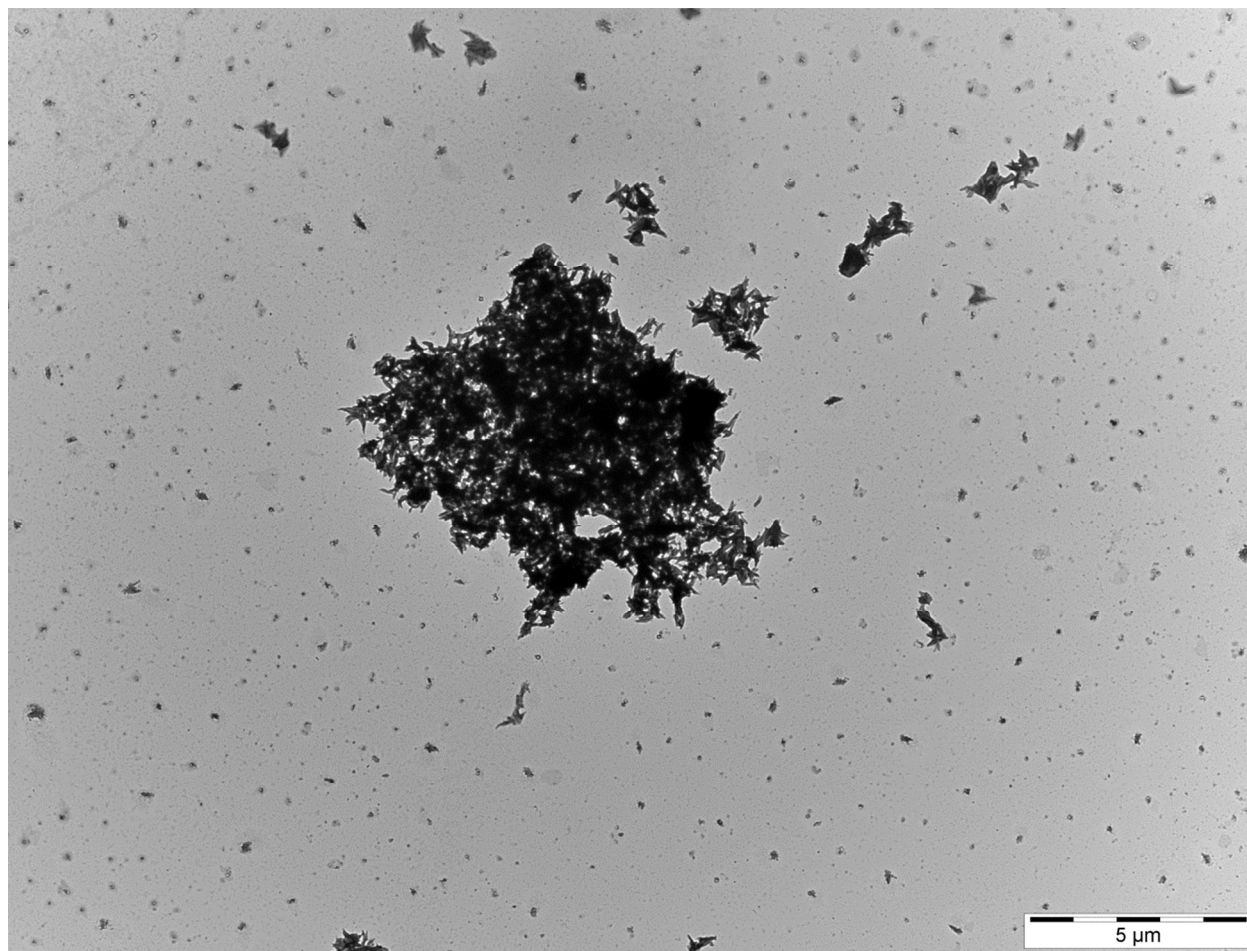**Figure S11.** TEM image of P-CNCs deposited from a dispersion (0.1 mg/mL) in water.
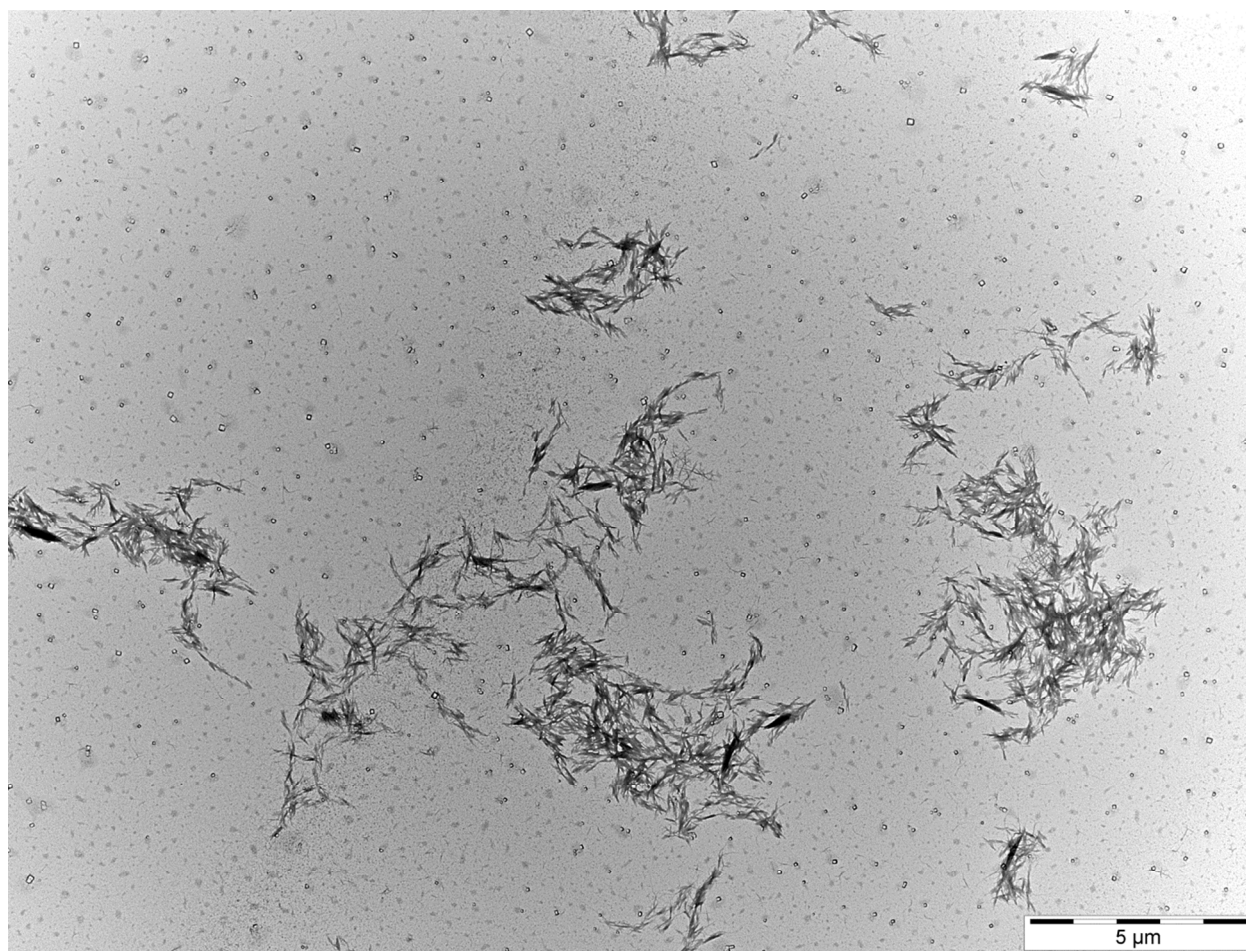
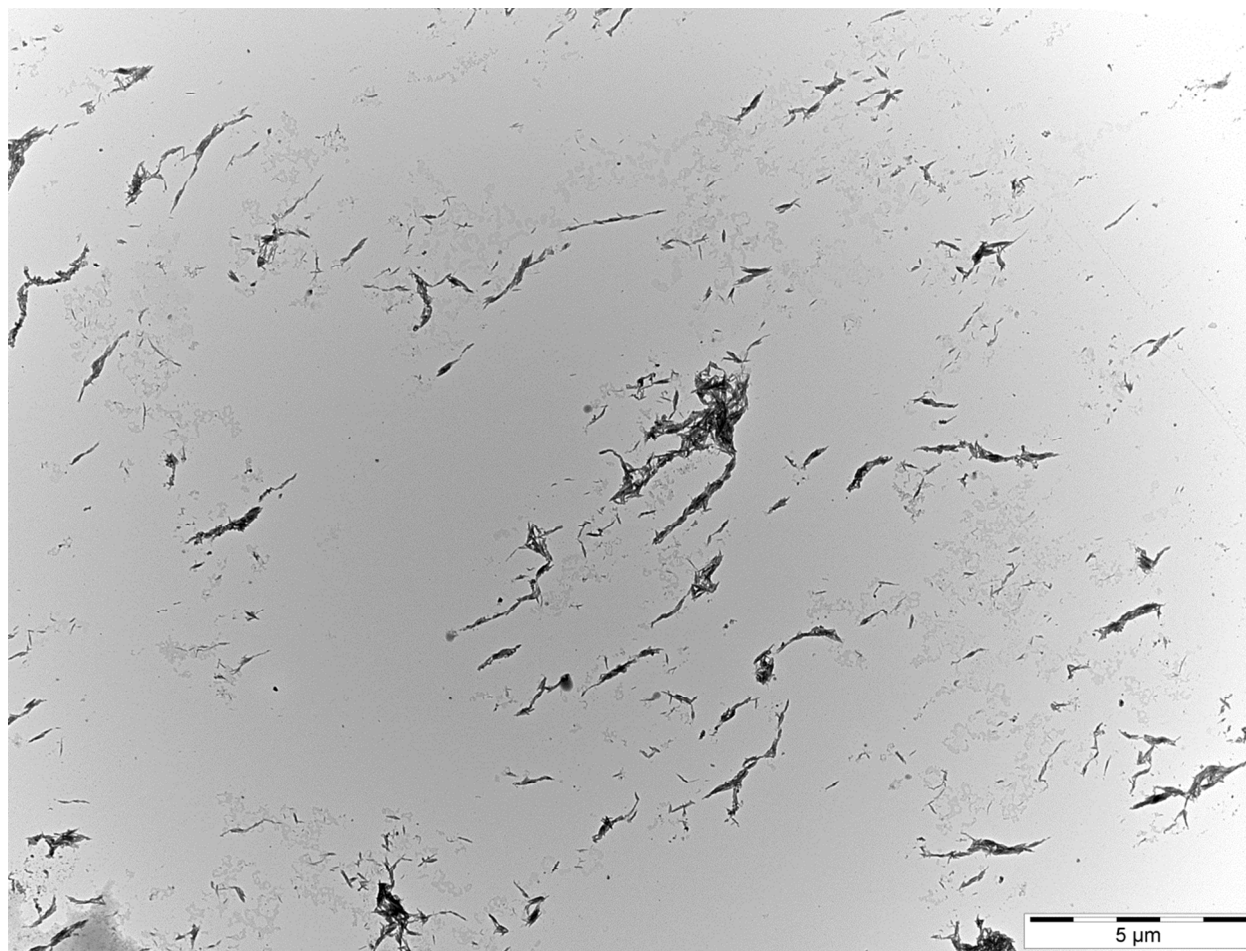**Figure S12.** TEM image of P-CNCs deposited from a dispersion (0.1 mg/mL) in DMSO.
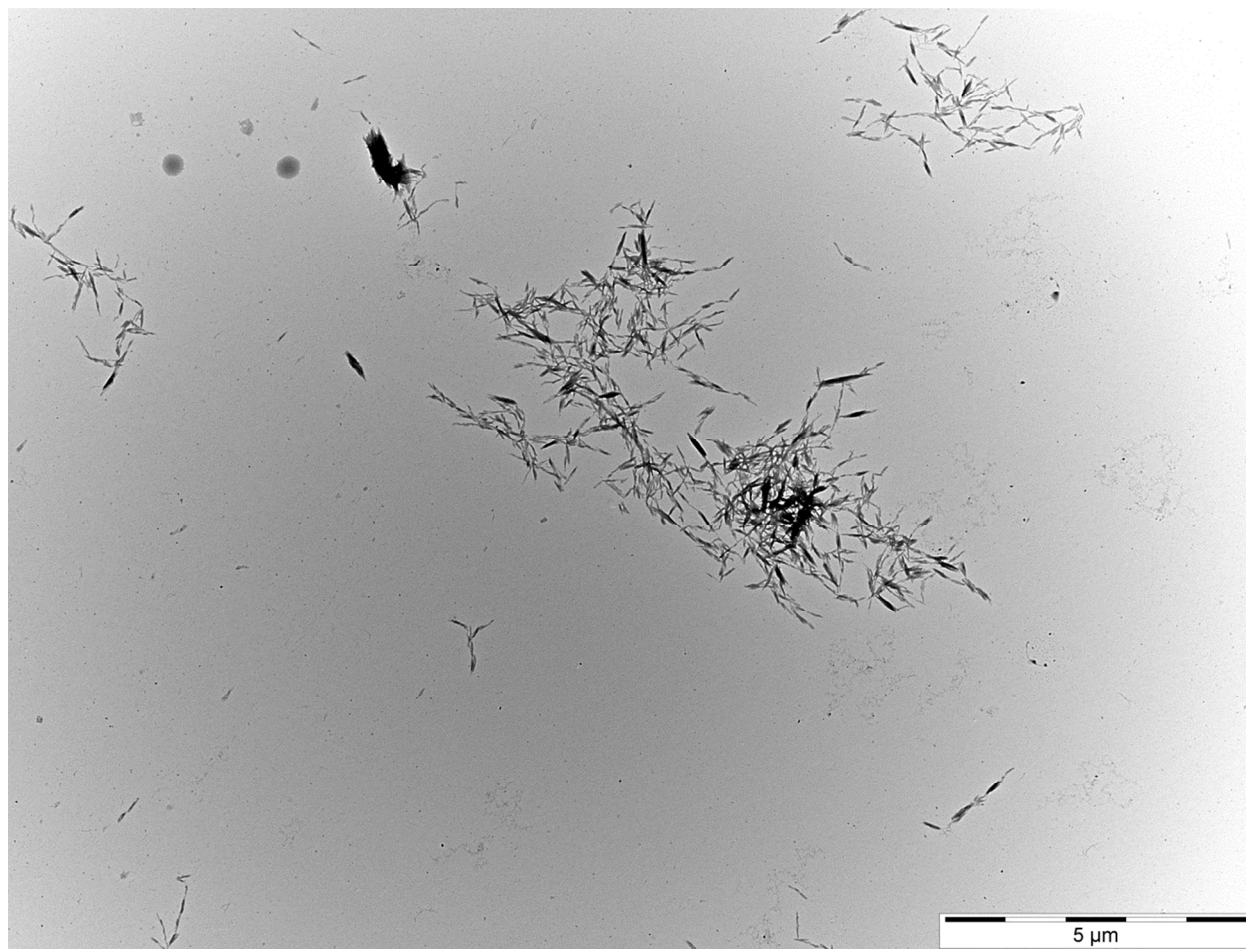
Figure S13. TEM image of P-CNCs deposited from a dispersion (0.1 mg/mL) in DMF.
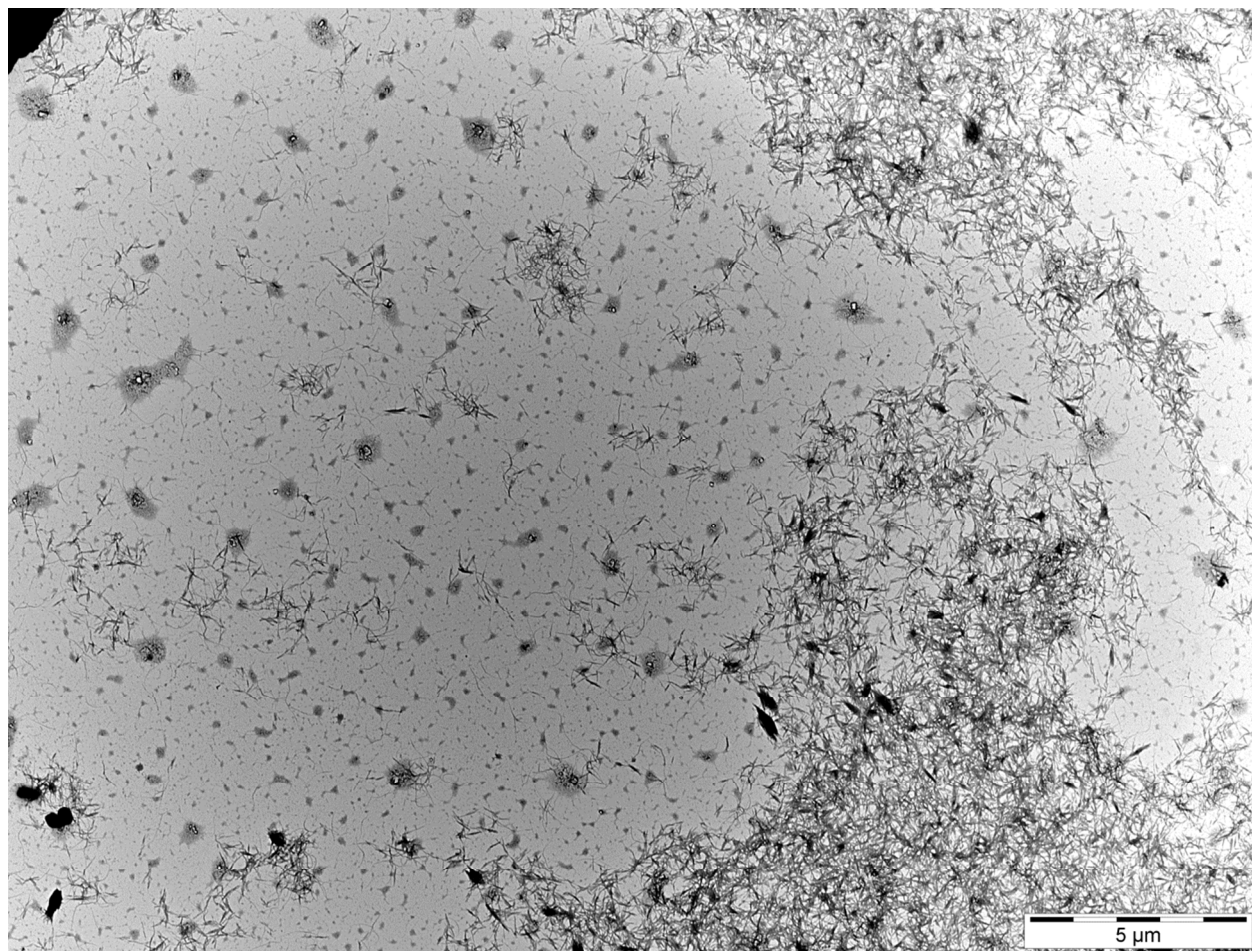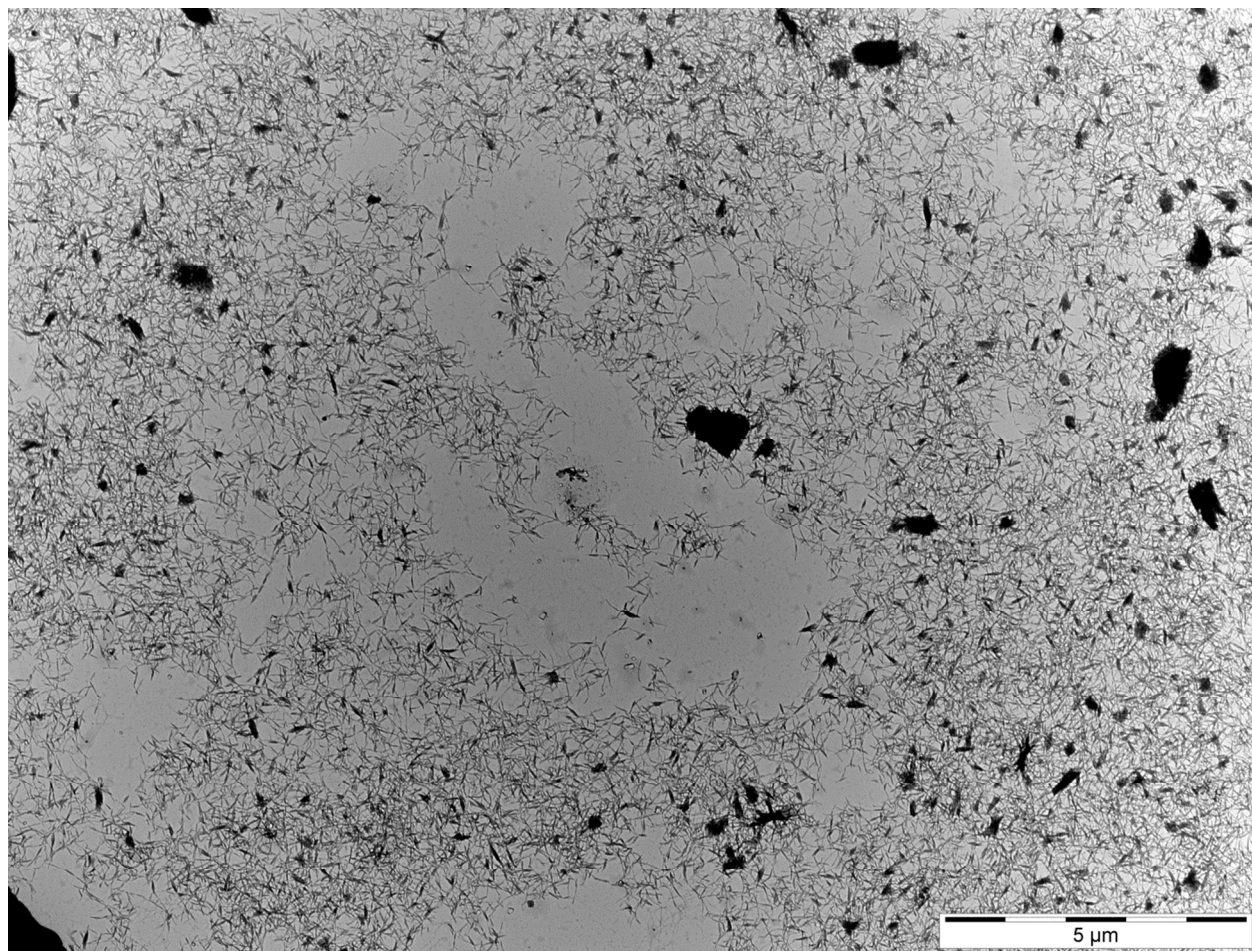
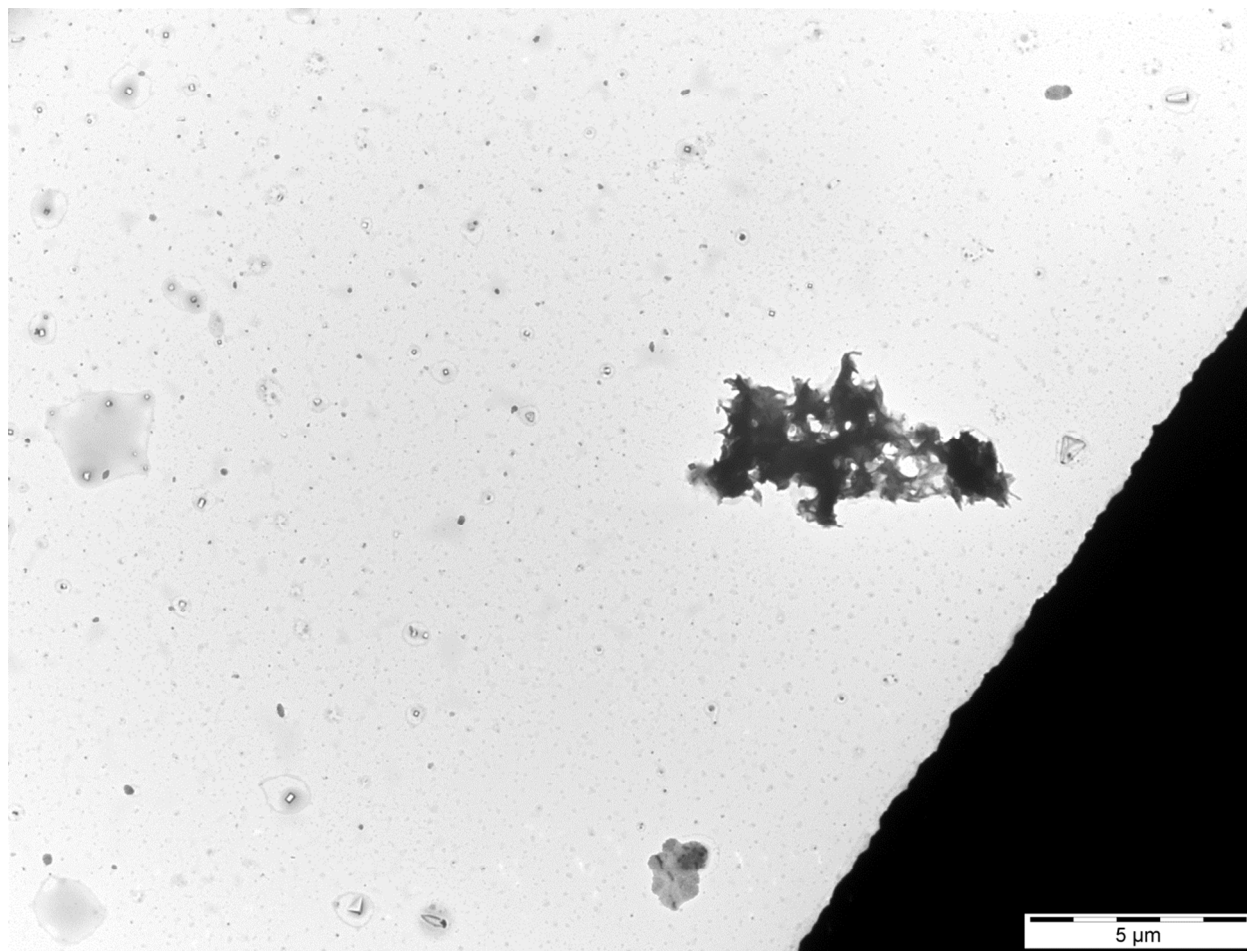**Figure S14.** TEM image of P-CNCs deposited from a dispersion (0.1 mg/mL) in THF.

Figure S15. TEM image of S-CNCs deposited from a dispersion (0.1 mg/mL) in water.

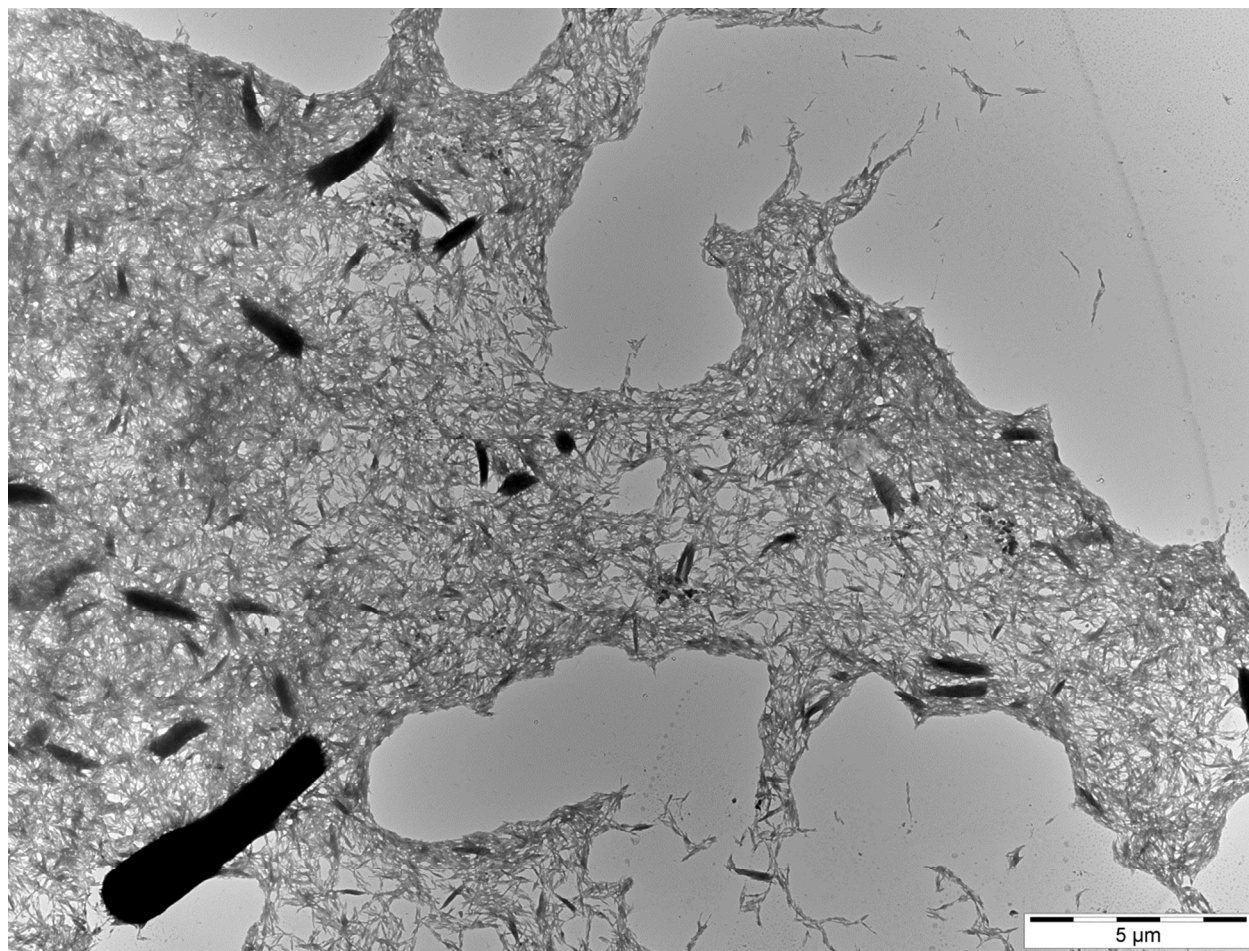**Figure S16.** TEM image of S-CNCs deposited from a dispersion (0.1 mg/mL) in DMSO.

Figure S17. TEM image of S-CNCs deposited from a dispersion (0.1 mg/mL) in DMF.
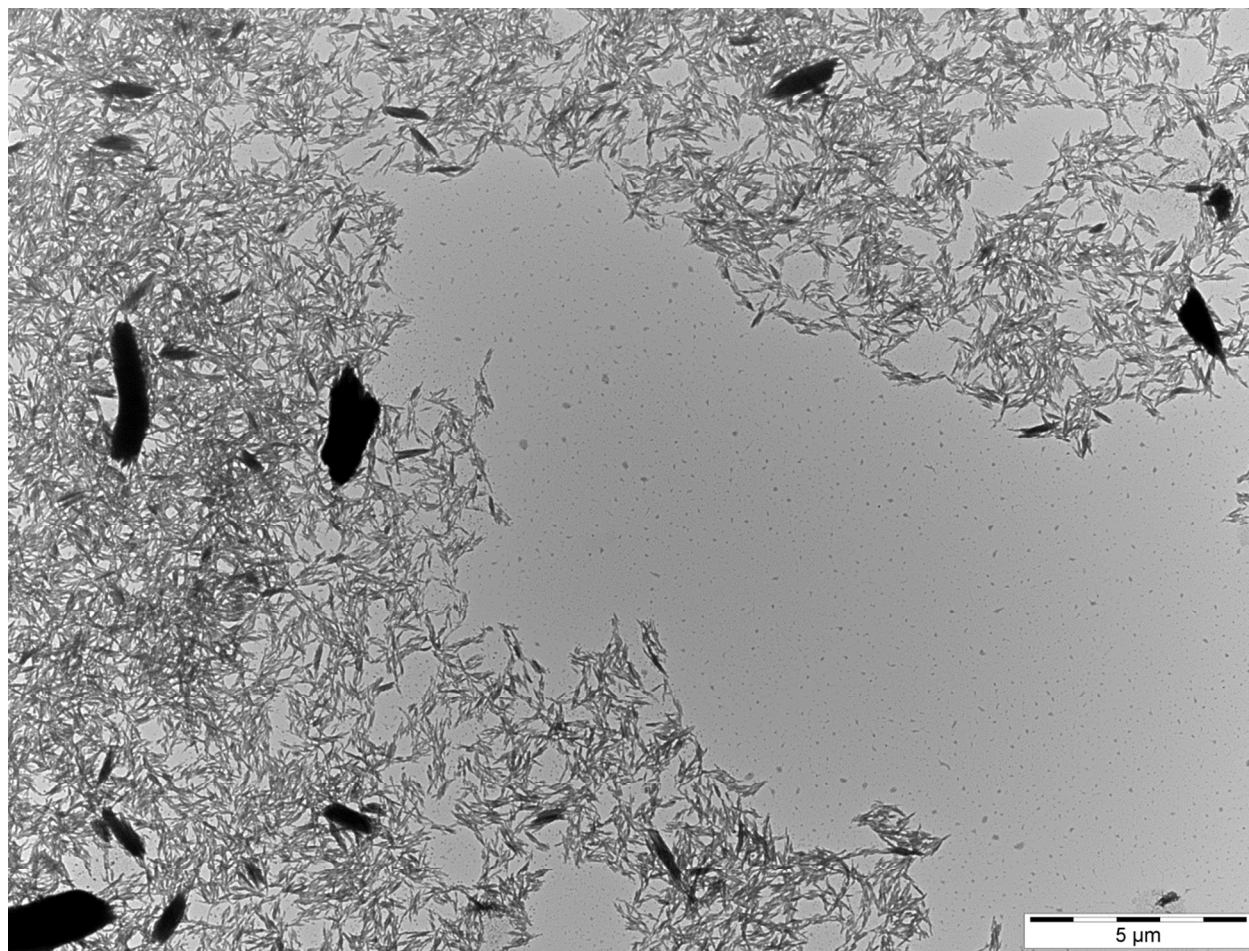
Figure S18. TEM image of S-CNCs deposited from a dispersion (0.1 mg/mL) in THF.
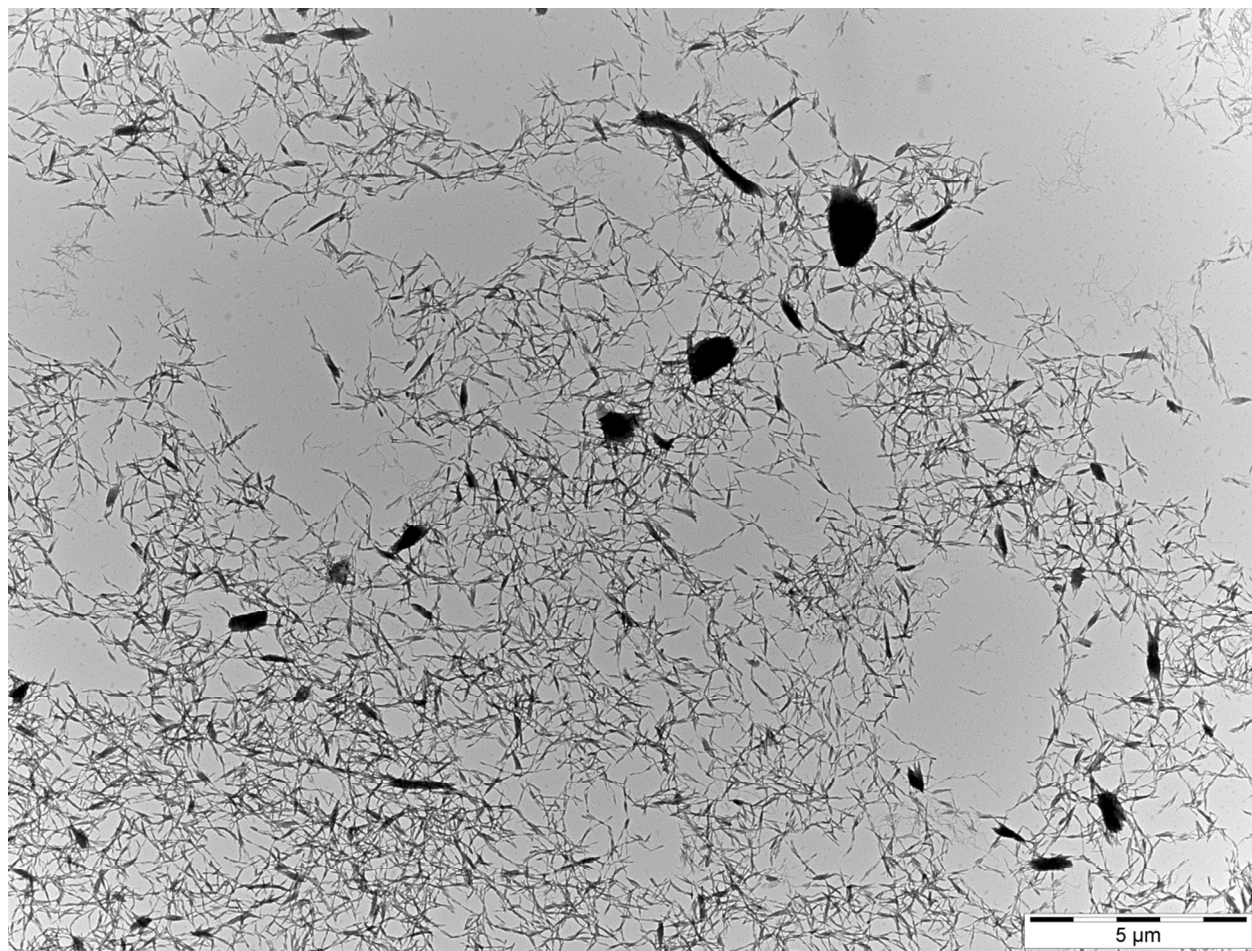
Figure S19. TEM image of H-CNCs deposited from a dispersion (0.1 mg/mL) in water.
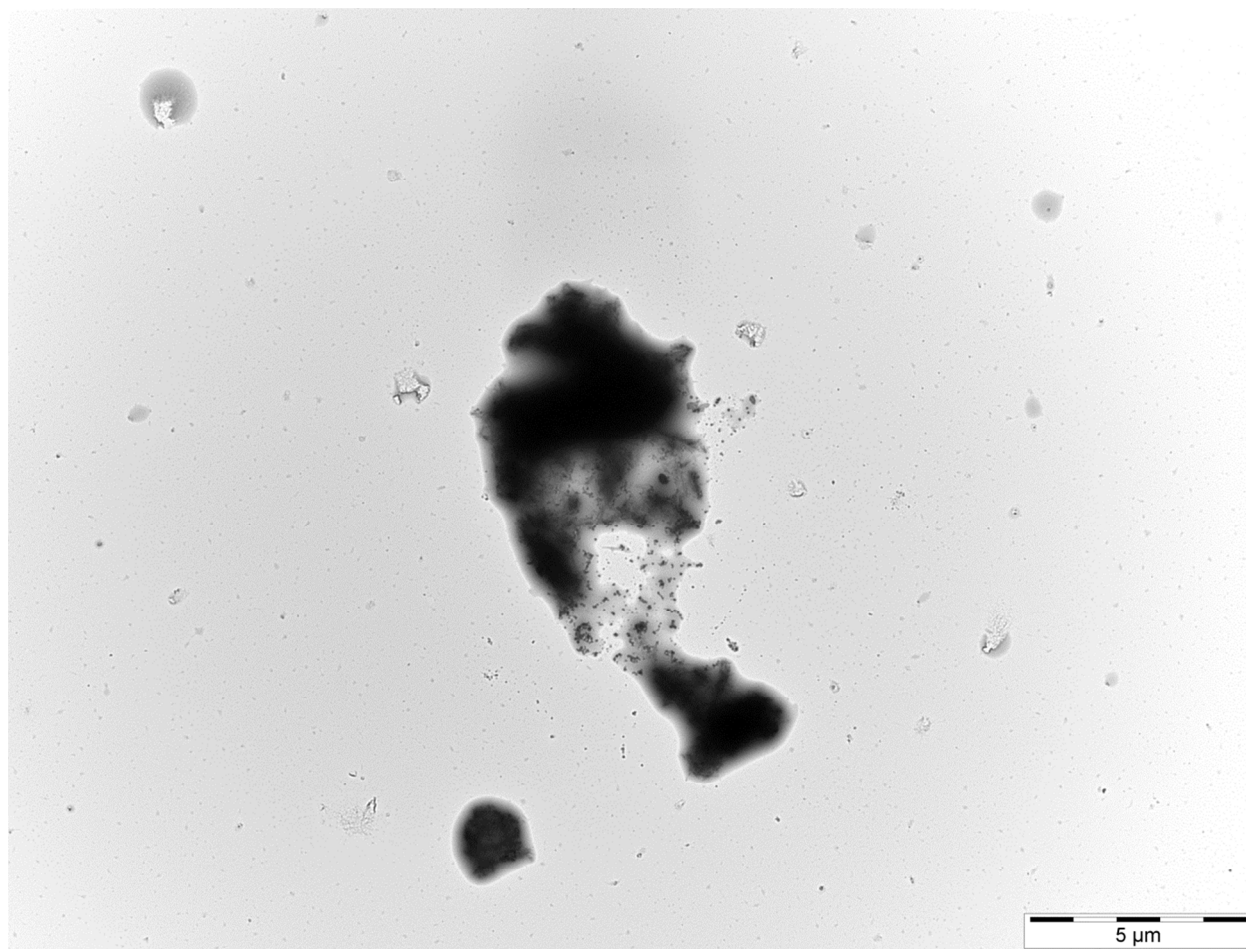
Figure S20. TEM image of H-CNCs deposited from a dispersion (0.1 mg/mL) in DMSO.

Figure S21. TEM image of H-CNCs deposited from a dispersion (0.1 mg/mL) in DMF.

Figure S22. TEM image of H-CNCs deposited from a dispersion (0.1 mg/mL) in THF.

References

1. Johnson Ford, E. N., *Journal of Engineered Fibers and Fabrics* **2010,** *5,* 10-20.
2. Correa, A. C.; Teixeira, E. d. M.; Pessan, L. A.; Capparelli Mattoso, L. H., *Cellulose* **2010,** *17,* 1183-1192.
3. Jiang, N.; Pu, Y.; Samuel, R.; Ragauskas, A. J., *Green Chem.* **2009,** *11,* 1762-1766.
4. Fiske, C. H.; Subbarow, Y., *J. Biol. Chem* **1925,** *66,* 375-400.