

Supplementary information for

Cellulose Nanocrystalline- Porphyrin Hybrid: Supramolecular, and Singlet-Oxygen Production Properties

by

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1. General information: All reagents were purchased from commercial sources and used without further purification. Microcrystalline cellulose starting material was from Sigma-Aldrich with a crystallinity degree of 80%.^{R1} TSPP-NH₂ was synthesized as previously reported by us.^{R2} IR was carried out in KBr on a Nicolet 5700 FT-IR instrument. UV-vis and fluorescence measurements were performed on a Cary 100 Scan Varian spectrophotometer and on Cary Eclipse Varian or Perkin Elmer LS55 fluorimeters, respectively. ¹H-NMR spectra were collected on a NMR Bruker Advance at 300 MHz. Dynamic light scattering and z-potential experiments were conducted on a Malvern Instrument Zetasizer Nanoseries. Thermal analyses were performed on TgaQ5000 TA instrument (Temperature program: 10°C/min until 80°C, maintained at 80°C for 2 mins then increased the temperature to 700°C at 5°C/min, maintained at 700°C for 2 mins). Centrifugations were carried out with a Thermo scientific SL16 centrifuge. TEM analysis was performed on a Tecnai G² Fei – 100kV microscope and the images were analysed with the help of Image-J package for size measurements and the data were averaged and elaborated graphically with Origin 7.0 software.

2. NCC synthesis: Microcrystalline cellulose (5.0 g) was added to a pre-heated solution (T=45°C) of sulfuric acid (63% w/w, made by mixing 21.5 mL of 95-98% H₂SO₄ with 22.5 mL of water) and the suspension was stirred for 2 hrs. Hydrolysis was stopped by dilution with water (900 mL). Most of the supernatant was decanted then the remaining suspension was divided in 4 centrifuge tubes (100 mL) and centrifuged at 4000 rpm for 5 minutes in order to remove most of the acidic supernatant solution. The solids were collected together, washed once by suspension in water and recovered by centrifugation at 8000 rpm for 5 mins. The suspension was transferred in dialysis tubes (12 kD cut-off) and dialysed against tap water (6 days) then milliQ-water (2 days). The final product was recovered by lyophilization. Yield 2.94 g (58%).

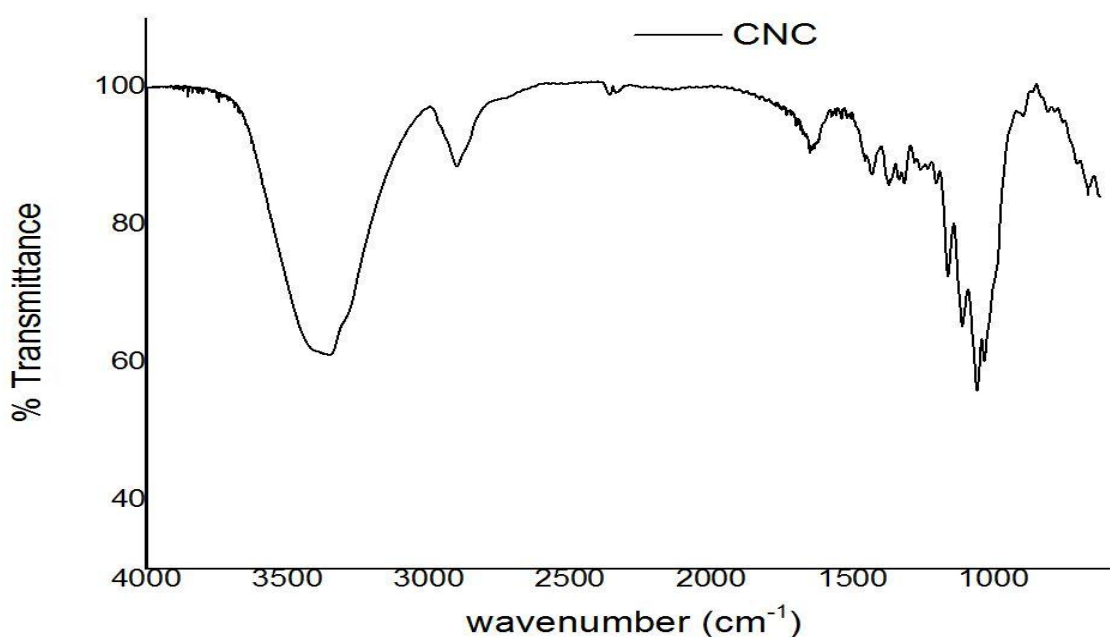


Figure S1: FTIR-spectrum of NCC (KBr)

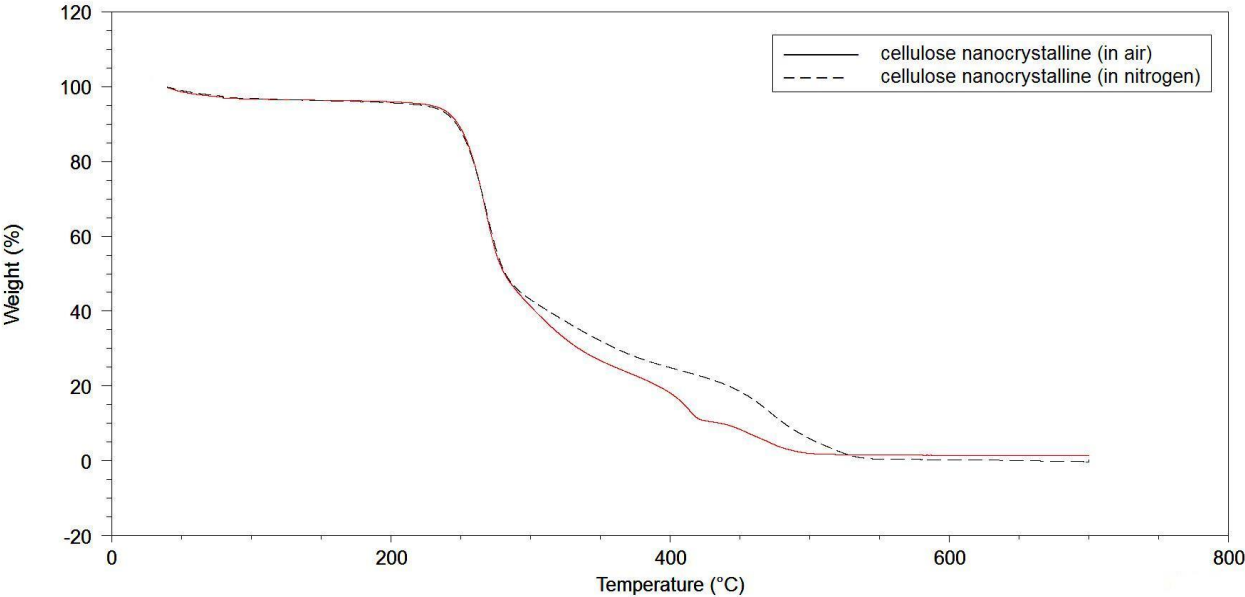


Figure S2. TGA plots for NCC carried out in air (continuous line) or under nitrogen (dotted line)

Cellulose nanocrystalline	composition (%)
C	39,75
H	6,47
N	/
S	0,54

Table S1. Elemental analysis of cellulose nanocrystalline

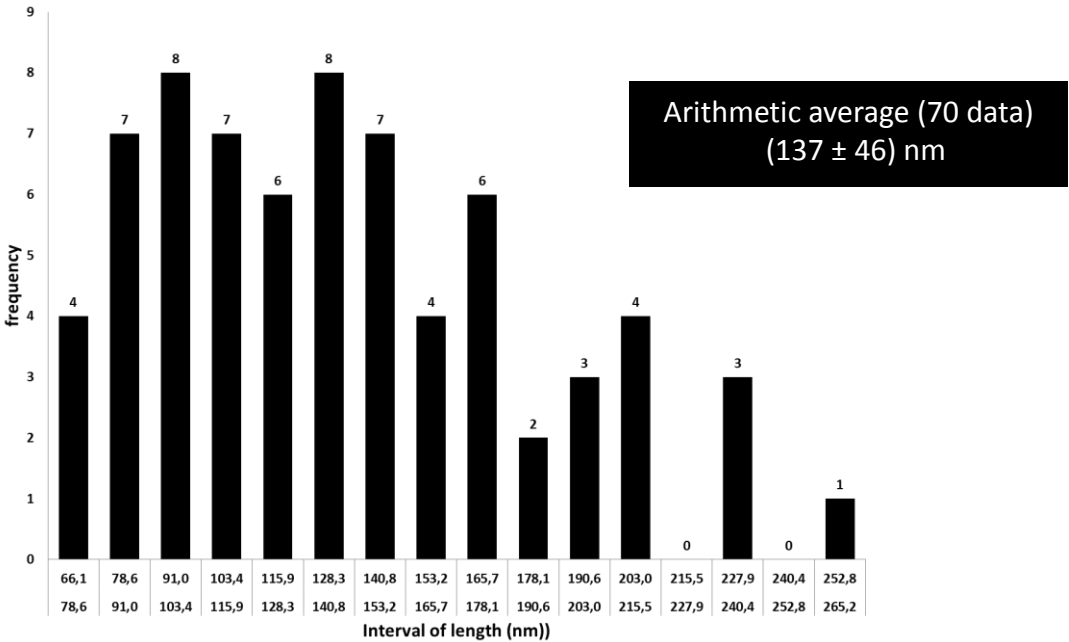


Figure S3. Distribution histogram of NCC lengths from TEM analysis

2. NCC-COOH synthesis: NCC (0.5 g) was suspended water (50 mL) and subjected to sonication in order to obtain a stable suspension. An aqueous solution (50 mL) of TEMPO (14.83 mg, 0.095 mmol) and NaBr (162.9 mg, 1.58 mmol) was added together with a solution of NaClO 2.73 M (1.78 mL). pH was adjusted to 10 with 1M NaOH. The mixture was stirred at room temperature for 3 hrs. The reaction was stopped adding ethanol (1 mL) then adding 0.5 M HCl to pH=7. The supernatant was removed by centrifugation at 13000 rpm for 10 mins then washed 3 times by suspension in water and centrifugation. The final product was lyophilized obtaining 0.250 g of **NCC-COOH** (yield: 50%).

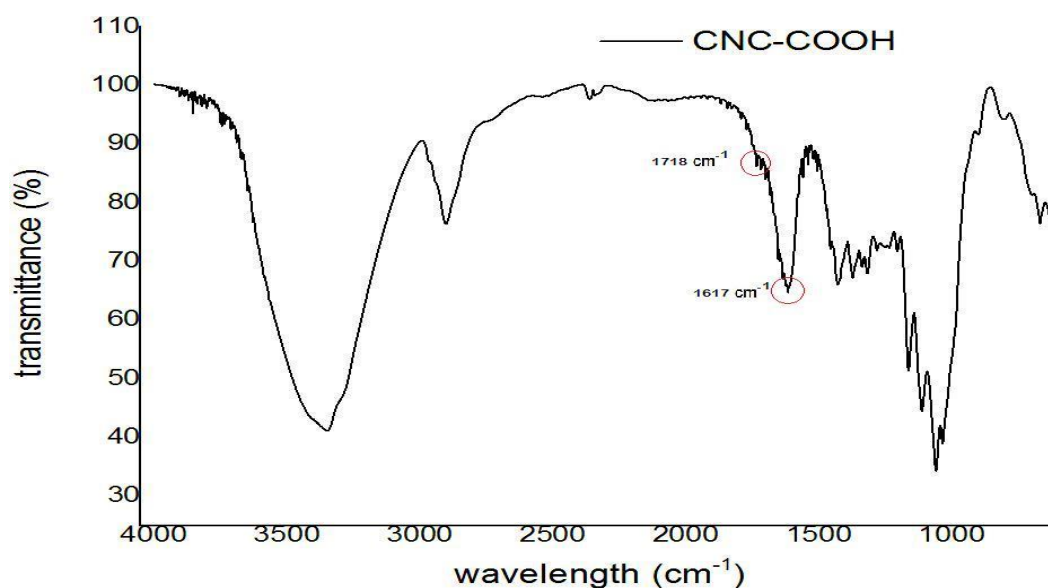


Figure S4. FTIR-Spectrum of **NCC-COOH** (KBr)

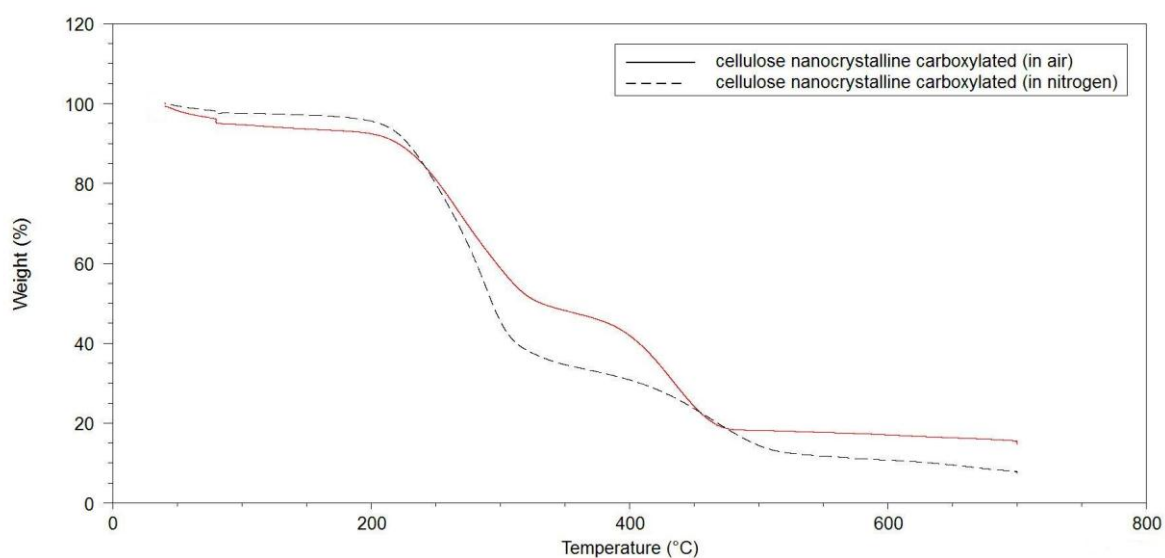


Figure S5. TGA plots for **NCC-COOH** carried out in air (continuous line) or under nitrogen (dotted line)

Cellulose nanocrystalline carboxylated (0,54mg/mL aqueous suspension)	Zeta Potential (mV)
1° measurement	-32,1
2° measurement	-32,6
3° measurement	-32,4
4° measurement	-34,7
arithmetic average	-33
standard deviation	1

Table S2. Zeta potential measurements for NCC-COOH

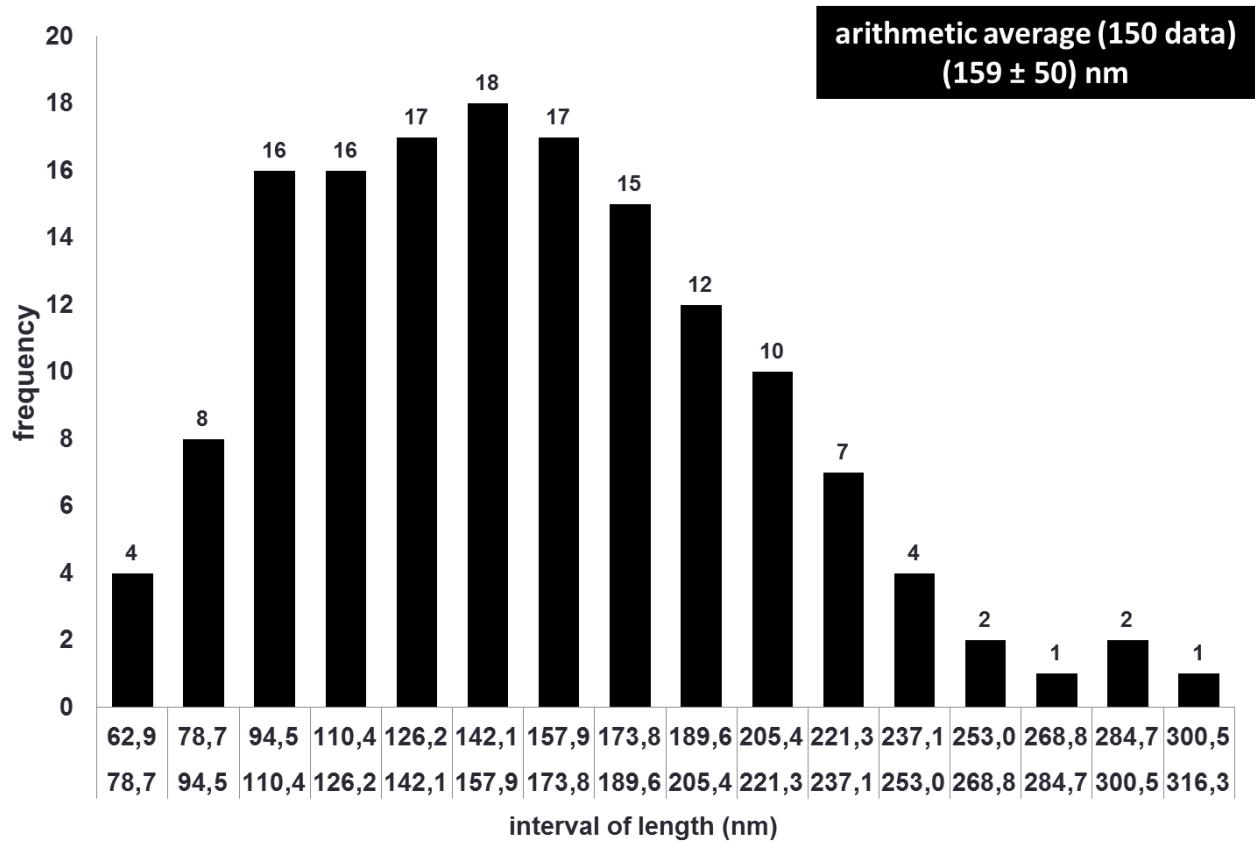


Figure S6. Distribution histogram of NCC-COOH lengths from TEM analysis

3. NCC-TSPP synthesis: NCC-COOH (0.153 g, 0.126 mmol of COOH groups) was suspended in a solution of 50 mM MES buffer (18 mL) at pH = 4. The suspension was sonicated for 5 mins then EDC·HCl (0.242 g, 1.26 mmol) and NHS (0.149 g, 1.29 mmol) were added. After 5 minutes the TPPS-NH₂ (0.072 gr, 0.0827 mmol) was added. The mixture was stirred for 4 days at room temperature. The solid was recovered by centrifugation at 13000 rpm for 10 minutes and re-suspended in water and centrifuged again as above until the supernatant was colorless. The suspension was transferred in a dialysis tube and dialyzed against brine for one day then against milliQ-water for 6 days. The product was recovered by lyophilization obtaining 68.7 mg of **NCC-TSPP** as a green solid (Yield: 44.8%).

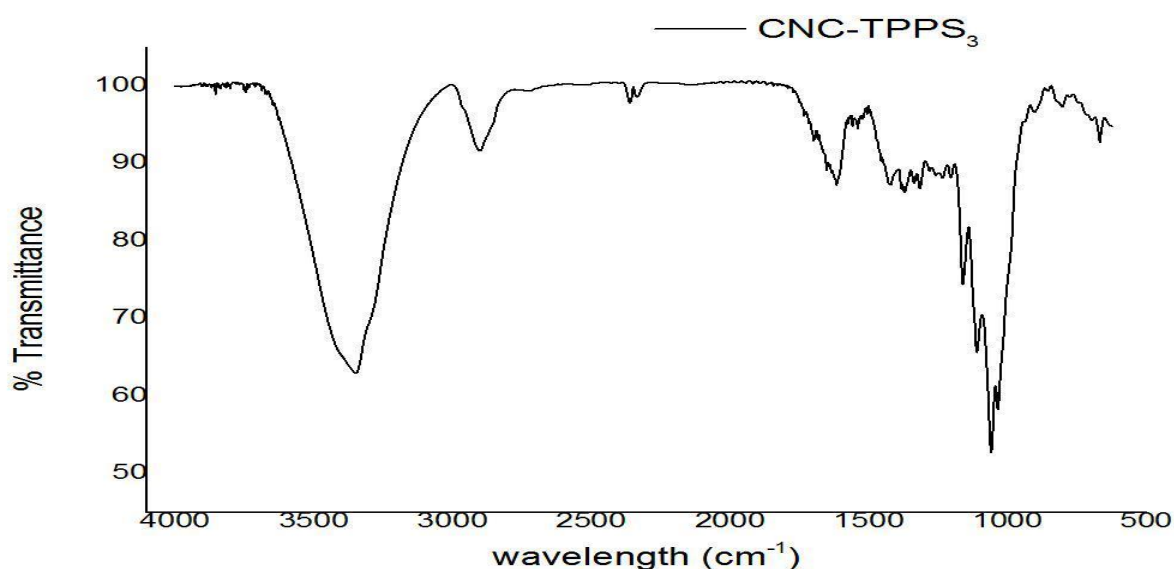


Figure S7. FTIR-Spectrum of **NCC-TSPP** (KBr)

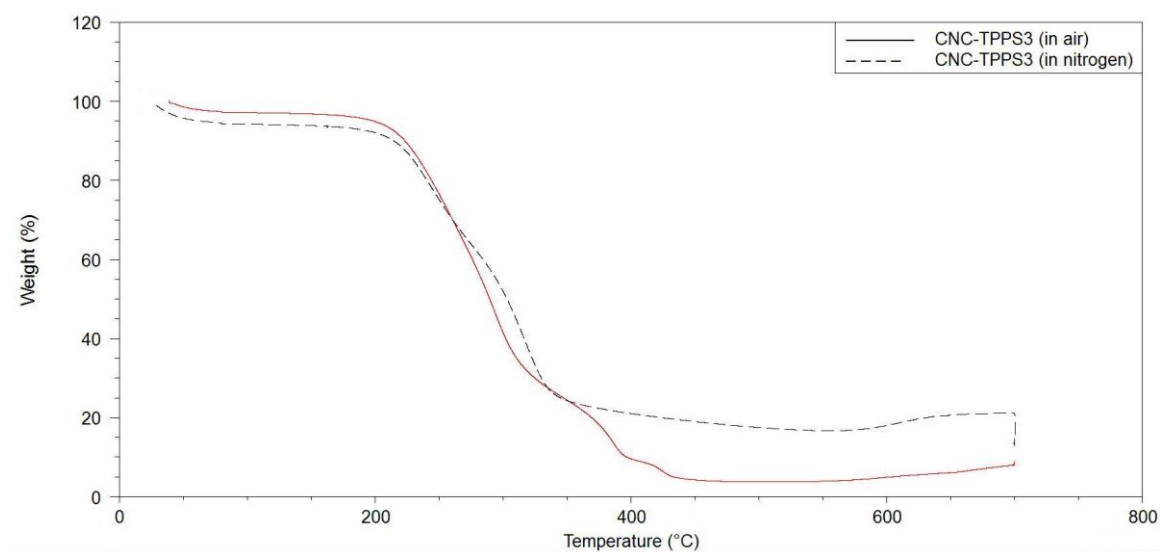


Figure S8. TGA plots for **NCC-TSPP** carried out in air (continuous line) or under nitrogen (dotted line)

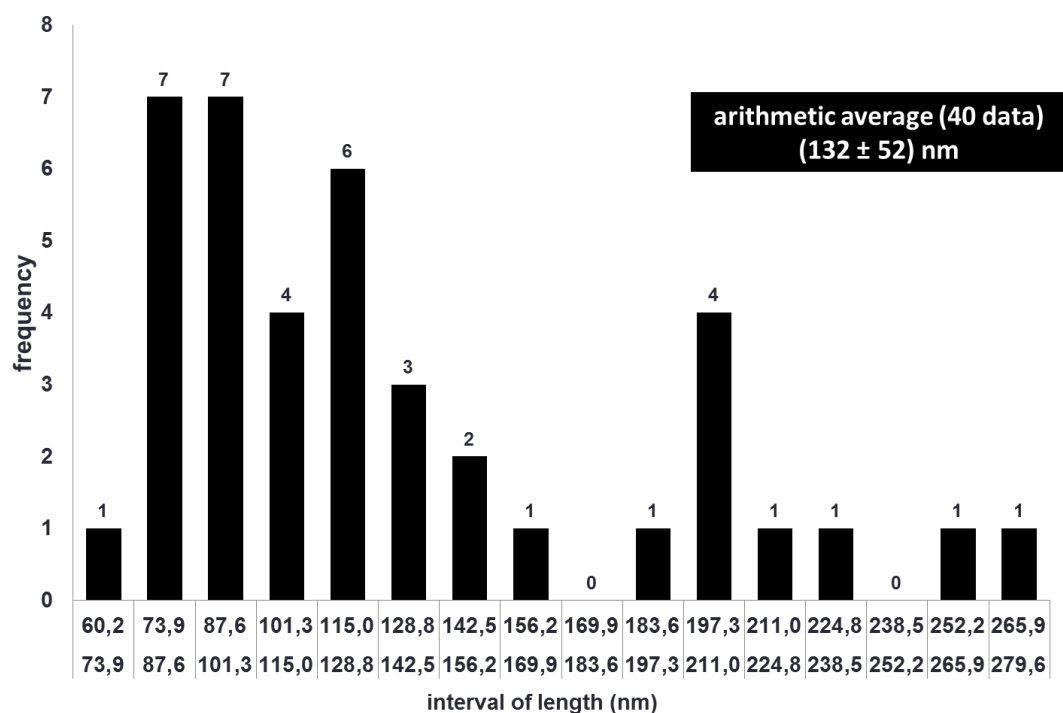


Figure S9. Distribution histogram of NCC-TSPP lengths from TEM analysis

4. NCC-TSPP(Zn) synthesis: NCC-TSPP (25.3 mg) was suspended in methanol (3 mL). Added a solution of Zn(OAc)₂ (11.4 mg in 2 mL of methanol) and stirred at 30°C for 3 hrs. The solid was recovered by centrifugation at 12000 rpm for 5 mins then washed first with methanol (3 times) then with water (2 times) by re-dispersion followed by centrifugation at 12000 rpm for 5 mins. The product was recovered by liophilization obtaining 21.7 mg of a green solid (yield: 85.8 %).

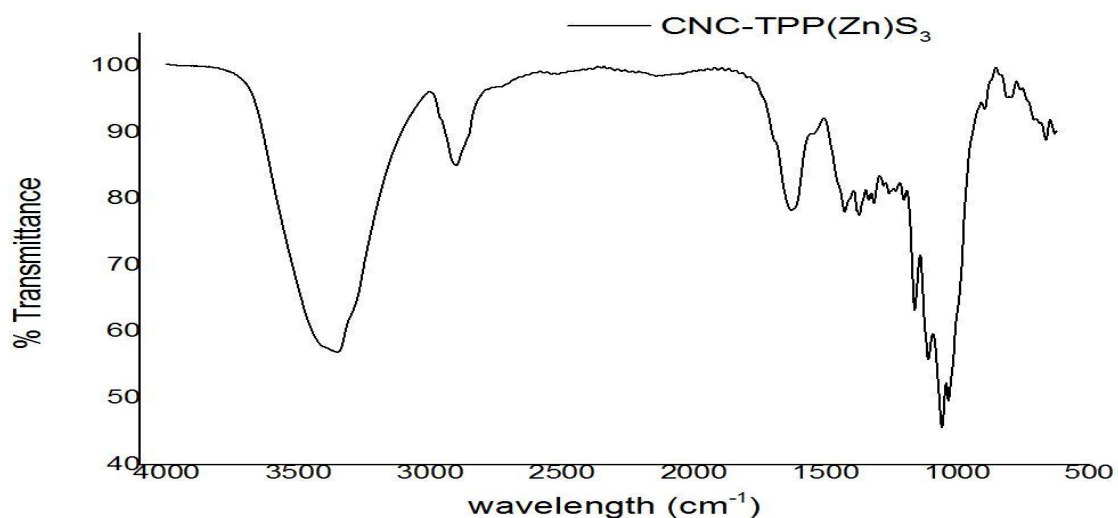


Figure S10. FTIR-Spectrum of NCC-TSPP(Zn) (KBr)

Figure

5. Determination of carboxylic group content of NCC-COOH via conductimetric titration: A known amount of **NCC-COOH** (range: 25÷50 mg) was suspended in 0.01N HCl (10 mL) and sonicated for 10 mins. A conductimetry probe (Metrohm CH-9100HERISAU) was immersed in the suspension and connected to a conductimetry apparatus (Metrohm 660). The suspension was titrated with 0.01N NaOH while recording the conductance. Graph of the conductivity vs. volume of NaOH added (Figure 1S) showed two inflection points. The first one corresponded to the volume of NaOH needed to titrate the free strong acid (V1), whereas the second one (V2) represented the volume of NaOH required to neutralize the carboxylic groups of **NCC-COOH**. The Degree of Oxidation (DO) can be calculated according the following formula:

$$DO = \frac{162(V_2 - V_1)C}{w - 14(V_2 - V_1)C}$$

in which w = weight of the sample (in grams) and C the concentration of NaOH (0.01 N in our case). This value can be expressed as mmol of COOH groups for gram of CNC.

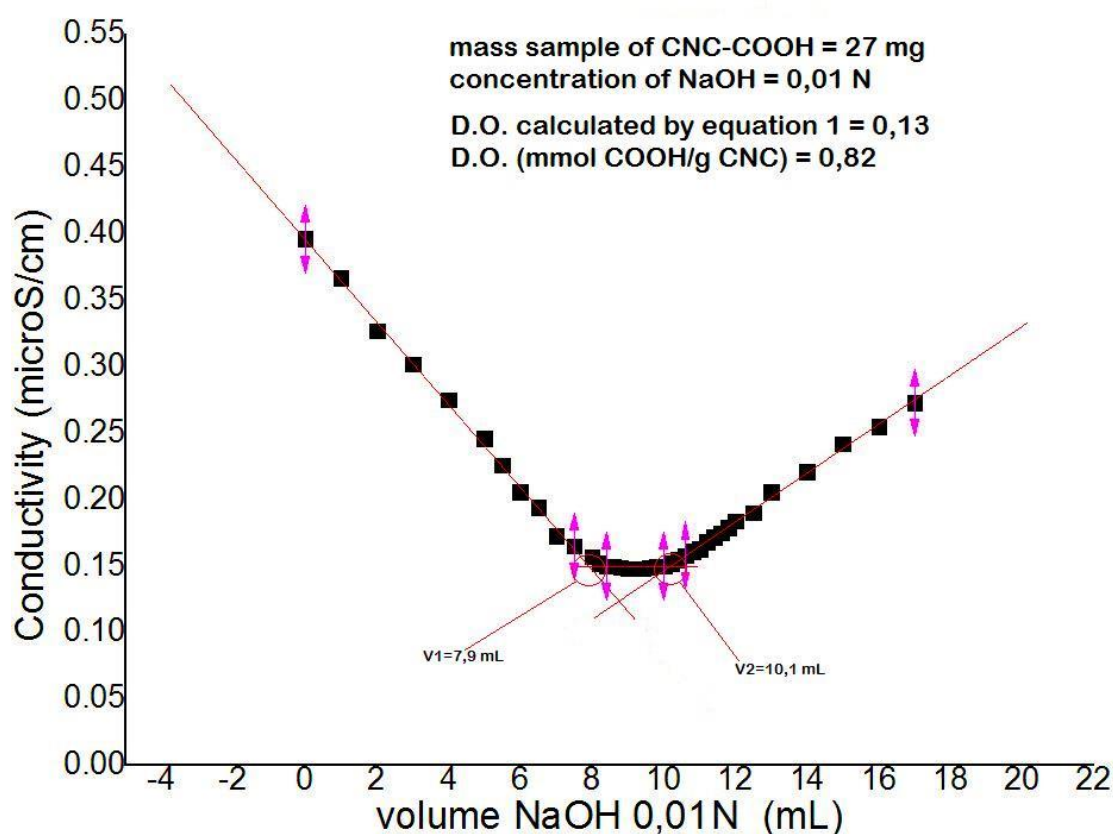


Figure S11. Conductimetric titration of cellulose nanocrystalline carboxylated

6. Determination of the binding constant between NCC-TSPP and TM β CD by fluorescence

spectroscopy: A known amount of **NCC-TSPP** was suspended in water and sonicated to obtain a stable suspension. The suspension was transferred in a quartz cuvette and the fluorescence spectrum recorded upon excitation at 420 nm (Soret band of **NCC-TSPP**). The initial fluorescence at 653 nm represented the F_0 value. Small portions of a solution of **TM β CD** were added and each time the fluorescence spectrum was recorded. The value of fluorescence at 653 nm, F , was notated after each addition of **TM β CD**. The addition of **TM β CD** was continued until a constant value (plateau) of fluorescence was observed. This value was the so-called F_∞ . The degree of binding, θ , was calculated according the formula:

$$\theta = \frac{F - F_0}{F_\infty - F_0}$$

Values of θ in the range 0.2–0.8 were plotted versus [**TM β CD**] (concentration was corrected to account for dilution) and subjected to non linear fitting using the typical binding isotherm for a 1:1 binding model in the form:

$$\theta = \frac{B_{max} \cdot [TM\beta CD]}{K_d + [TM\beta CD]}$$

where B_{max} is the maximum specific binding parameter and K_d is the equilibrium dissociation constant (i.e. the reciprocal of the binding constant) defined as:

$$K_d = \frac{[TM\beta CD] \cdot [CNC - TSPP]}{[TM\beta CD - CNC - TSPP]}$$

The binding model assumes that only a small fraction of **TM β CD** binds to **NCC-TSPP** so the [**TM β CD**] at the equilibrium can be considered identical to the total concentration of **TM β CD**.

7. Effect of α -cyclodextrin on fluorescence properties of NCC-TSPP: The fluorescence enhancement observed above could be, in principle, the result of a non-specific interaction of **TM β CD** with the **NCC** itself without involving the porphyrin moiety. To rule-out this event, we carried out a control experiment using α -cyclodextrin instead, which is supposed to interact only weakly with the porphyrin since its hydrophobic cavity is smaller than the cavity of a β -cyclodextrin derivative. In Figure S12 we report the results of this experiment. The effect of α -cyclodextrin on the fluorescence is negligible.

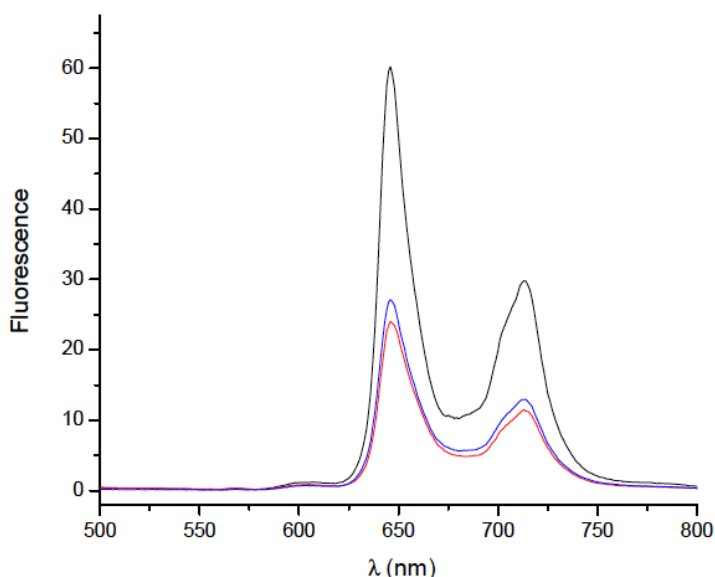


Figure S12: Effect on fluorescence of **NCC-TSPP** (blue curve, 0.192mg/mL of NCC-TSPP, corresponding to 5.6×10^{-7} M porphyrin) upon addition of **TMBCD** (black curve, 114 equivalents with respect to porphyrin) and α -cyclodextrin (red curve, 116 equivalents with respect to porphyrin).

7. L-methionine methyl ester photooxidation: **NCC-TSPP** (1.5 mg) was suspended in D_2O (1.5 mL) and sonicated to obtain a stable suspension then added the L-methionine methyl ester (54 mg). The suspension was divided in two identical portions and introduced into two quartz cuvettes. In one cuvette (herein referred as “1”) a solution of **TM β CD** in D_2O (5.8×10^{-2} mM, 153 μ L) was added whereas an equal amount of D_2O (153 μ L) was added to the second cuvette (herein referred as “2”). Both cuvettes were capped with a septum and saturated with oxygen then they are exposed for 75 mins to a 500 W Xe-Hg lamp through a filter that cuts off wavelengths <365 nm. The suspension was then centrifuged to remove **NCC-TSPP** and analyzed by 1H -NMR spectroscopy. L-methionine methyl ester and the corresponding sulfoxide were identified according our previous report.^{R2}

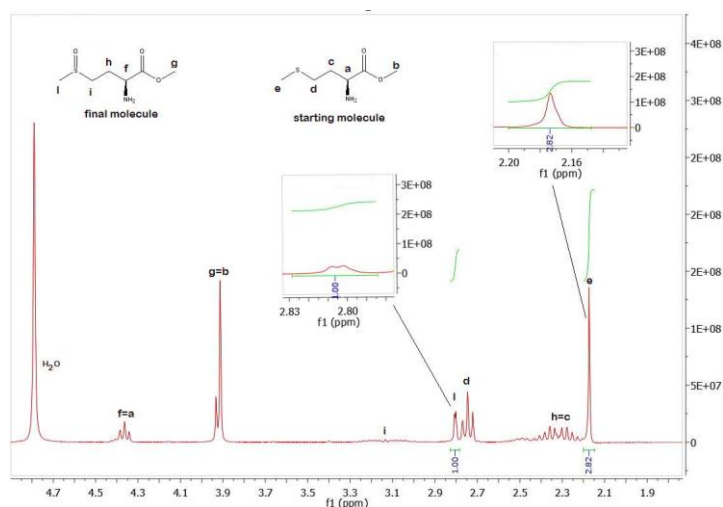


Figure S13: 1H -NMR spectrum (D_2O) for photooxygenation carried out in cuvette 1 (in the presence of **TM β CD**)

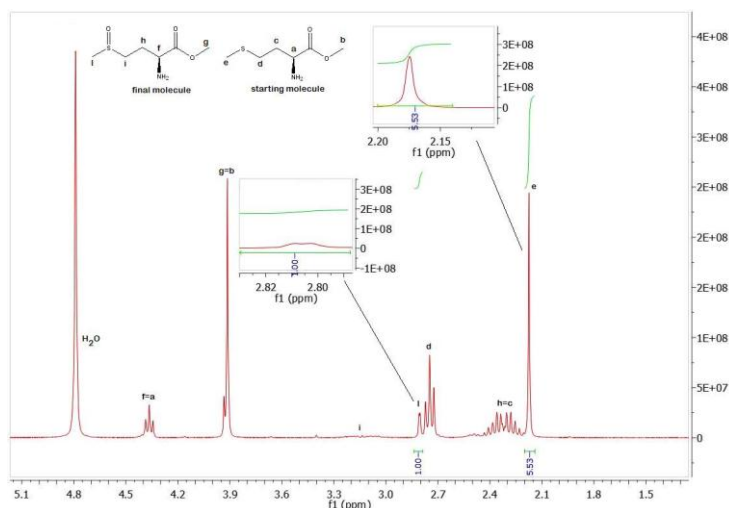


Figure S14: ^1H -NMR spectrum (D_2O) for photooxygenation carried out in cuvette 2 (i.e in the absence of **TM β CD**)

8. Singlet oxygen detection with ABDA probe: 9,10-Anthracenediyl-bis(methylene)dimalonic acid, **ABDA**, can be used as a singlet oxygen probe due to the formation of the corresponding endoperoxide. To this purpose a 0.13 mM solution (in D_2O) of **ABDA** in phosphate buffer (0.01M, pH=7) was saturated with oxygen then illuminated with white LEDs in a previously reported apparatus^{R2} for 12 minutes. Under these conditions the UV-Vis spectrum of **ABDA** was not affected (Figure S14).

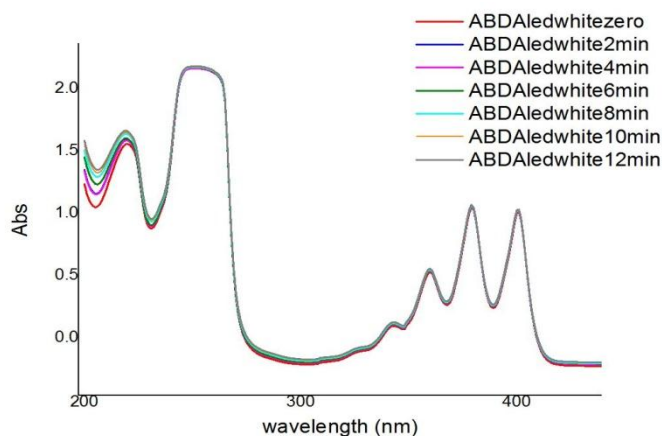


Figure S15: Light stability test for **ABDA** probe in the absence of **NCC-TSPP(Zn)**

On the other hand, the same experiment carried out in the presence of **NCC-TSPP(Zn)** (0.5 mg) led to a 22% decreasing (Figure S15) of the **ABDA** spectrum thus confirming the production of singlet oxygen under illumination conditions.

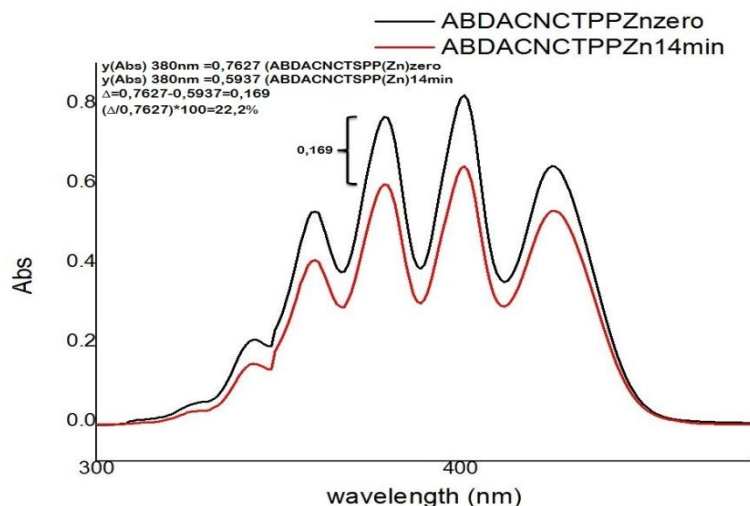


Figure S16: Changements of **ABDA** spectrum upon illumination with white LEDs in the presence of **NCC-TSPP(Zn)** for 14 mins.

10. **XPS analysis of NCC-TSPP(Zn):** Figure S17 reports the XPS survey scan for **NCC-TSPP(Zn)**. The signals for nitrogen, sulfur and zinc are present, together with the oxygen and carbon signals which belong both to NCC and porphyrin ring. This results should be considered only qualitative since the escape depth for XPS experiment is typically many times the thickness of a single nanocrystal, so the analysis is not that of the true nanocrystal surface. Nevertheless, it should be reasonable to compare Zn (1.26%) and N (1.57%) since they come only from the porphyrin moiety. Thus, after considering that %N contains also the contribute from the amide linking to NCC, it is possible to calculate a N/Zn ratio of 4.62, which is only slightly larger than the theoretical that would be 4.

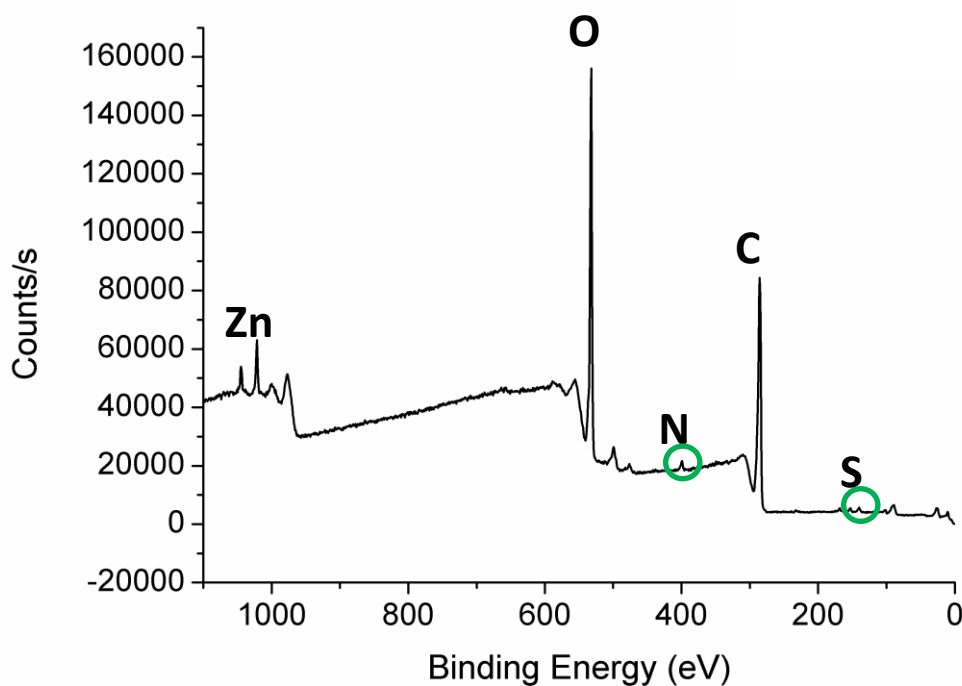


Figure S17: XPS survey scan for **NCC-TSPP(Zn)**

11. References

R1: L. Szczesniak, A. Rachocki, and J. Tritt-Goc, *Cellulose*, 2008, *15*, 445.

R2: T. Carofiglio, P. Donnola, M. Maggini, M. Rossetto, and E. Rossi, *Adv. Synth. Catal.*, 2008, **350**, 2815.