Supplementary information for

A Nanocellulose-Dye Conjugate for Multi-Format Optical pH-Sensing

by

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1. General information

Chemicals: 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] Dye **1** was synthesized as previously reported.[R2]

Instrumentation:

- **UV-vis measurements** were performed on a Cary 100 Scan Varian.
- **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.
- **Scanning electron microscopy** (SEM, JEOL model JSM-6490, Japan) was used to characterize the morphology of the samples, which were gold coated in advance.
- **The X-ray photoelectron spectroscopy (XPS)** analyses were performed with a VGMicrotech ESCA 3000Multilab, equipped with a dual Mg/Al anode. The spectra were excited by the unmonochromatized Al Kα source (1486.6 eV) run at 14 kV and 15 mA. The analyser was operated in the constant analyser energy (CAE) mode. For the individual peak energy regions, a pass energy of 20 eV set across the hemispheres was used. Survey spectra were measured at 50 eV pass energy. The pressure in the analysis chamber was in the range of 10^{-8} Torr during data collection. The constant charging of the samples was removed by referencing all the energies to the C 1s set at 285.1 eV, arising from the C-C bond. The invariance of the peak shapes and widths at the beginning and at the end of the analyses ensured absence of differential charging. Analyses of the peaks were performed with the software provided by VG, based on non-linear least squares fitting program using a weighted sum of Lorentzian and Gaussian component curves after background subtraction according to Shirley[R3] and Sherwood[R4]. Atomic concentrations were calculated from peak intensity using the sensitivity factors provided with the software. The binding energy values are quoted with a precision of ± 0.15 eV and the atomic percentage with a precision of ± 10%.
- **AFM imaging** was carried out on an Agilent Technologies 5500 scanning probe microscope, operating in acoustic AC AFM mode, with silicon AppNano ACTA cantilever (resonance frequency of 350 kHz, declared radius of curvature < 10 nm). WSxM [R5] software was used to process the images. For the preparation of the samples, freshly cleaved mica was used as substrate, after washing with a 0.1%wt aqueous solution of poly(allylamine) and rinsing with DI water. Few drops of a 0.001%wt water suspension of **NCC** material were deposited onto the surface, followed by washing with DI water after a few seconds. The samples were left to dry in air.

2. NCC preparation: 29.6 gr of microcrystalline cellulose were added to 222 mL of 64%wt sulfuric acid pre-heated at 45°C and the suspension was stirred for 45 minutes. Hydrolysis was stopped by dilution with water (1.5 L). The suspension was left to settle down overnight. The surnatant was decanted off and the remaining suspension centrifuged at 12000 rpm for 30 minutes in order to remove most of the acidic solution. The **NCC** pad was re-suspended in water and centrifuged again at 12000 rpm for 30 minutes. Washing with water was repeated further two times then the pad was re-suspended in about 250 mL of water and transferred in dialysis tubes (12 kD cut-off) and dialysed against deionized water for 6 days. The suspension was transferred in a beaker and a dialysis tube filled with ion exchange resin (Dowex Marathon MR-30 hydrogen and hydroxide form, 20-50 mesh) was immersed in the suspension and stirred overnight. The suspension was then fractionated by centrifugation at 6000 rpm for 30 hr. The sediment was discarded and subjected to a further centrifugation at 12000 rpm for 60 minutes. The sediment was discarded and the surnatant was centrifuged at 12000 rpm for 5 hrs to collect the final **NCC** (Yield 2.2 gr, 7.4%). This fraction was dispersed in water at a final concentration of 4.6%wt.

3. NCC-dye 4 preparation: Dye **1** (58.5 mg) was treated with 96% sulfuric acid (1,78 gr) for 30 minutes. Then it was diluted with water (35 mL) and pH set to 10 with NaOH 1M (ca 50 mL). To this intense purple solution a 4.6%$_{wt}$ suspension of **NCC** (41,3 gr, corresponding to 1.8 gr of dry **NCC**) was added and stirred for 2 hours obtaining a purple, gel-like material. The suspension was centrifuged at 12000 rpm for 2 hrs. The deposited solid was re-suspended in water a centrifuged for 2 hrs. The solid was suspended in water, transferred in dialysis tubes (12 kD cut-off) and dialysed against deionized water for 6 days. The color of the suspension turned orange, i.e. the color of the neutral form of **4**. The suspension was centrifuged at 12000 rpm for 5 hours and the solid was suspended in a minimum amount of water. The concentration of the suspension was determined to be 2.3%wt by gravimetry corresponding to 0.7 gr of **4** (yield 37%).

4. AFM imaging

Unfunctionalized cellulose nanocrystals (NCC): Figure 1 shows, on the bottom-left, the amplitude signal recorded on a 5 µm-side square area, where elongated structures are sparsely distributed over the flat mica surface. In the closed-in image (top-right corner), the single objects can be better appreciated. Figure 2 shows both the amplitude signal and the topographical reconstruction of another portion of the surface (1.5 µm-side square area) together with four height profiles along selected paths. As typically reported, cellulose nanocrystals tend to aggregate along their longer axis, albeit small, isolated crystals can be seen as well. The observed length of most isolated objects is in the 170 nm, while their height is around 5 nm. All the structures profiled in figure 2 display very similar widths at their bases, of about 50 nm.

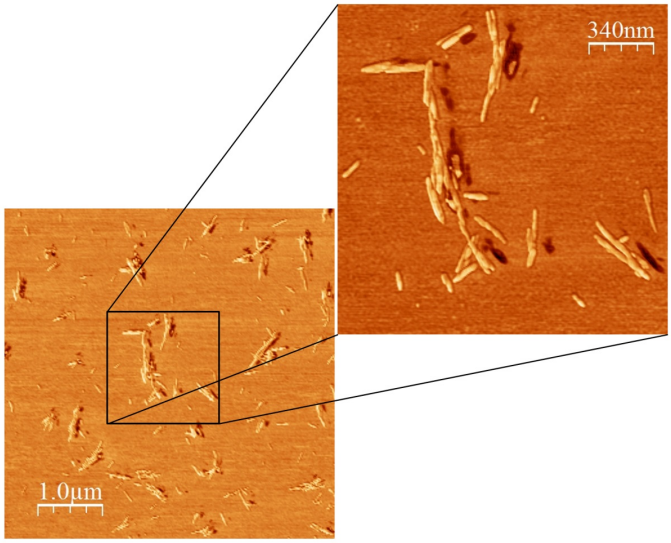


Figure 1. (bottom-left) Wide-field amplitude image of the sample surface and (top-right) close-in image highlighting the single cellulose nanocrystals.

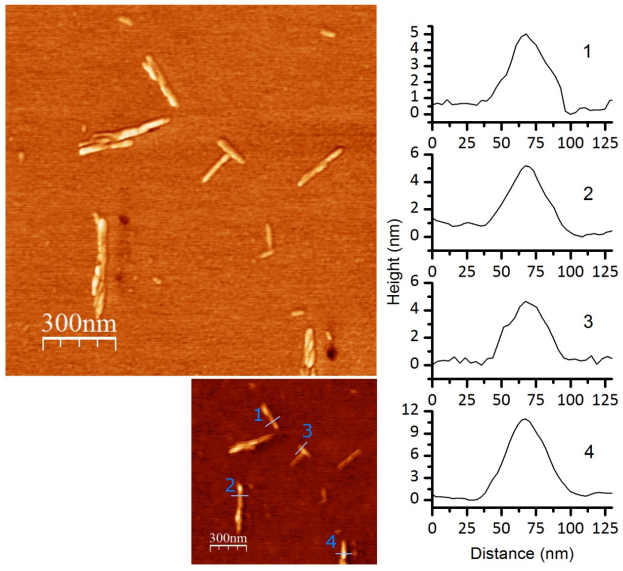


Figure 2. (top) Amplitude signal and (bottom) topography of **NCC**. Graphs on the right report the profiles along the four paths highlighted in the topography image.

Functionalized cellulose nanocrystals (NCC-dye 4). A wide field image of the functionalized sample is reported in Figure 3, together with a close up showing the cellulose nanocrystals in sharper detail.

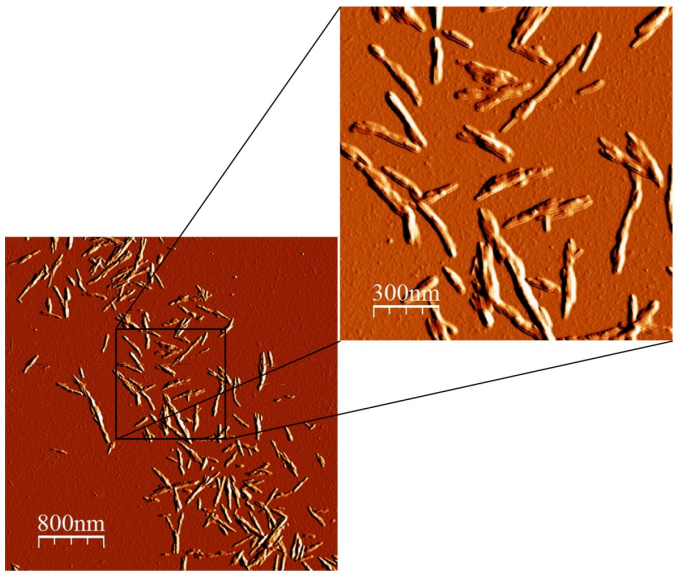


Figure 3. (top) Amplitude signal and (bottom) topography of **NCC-dye 4** nanocrystals.

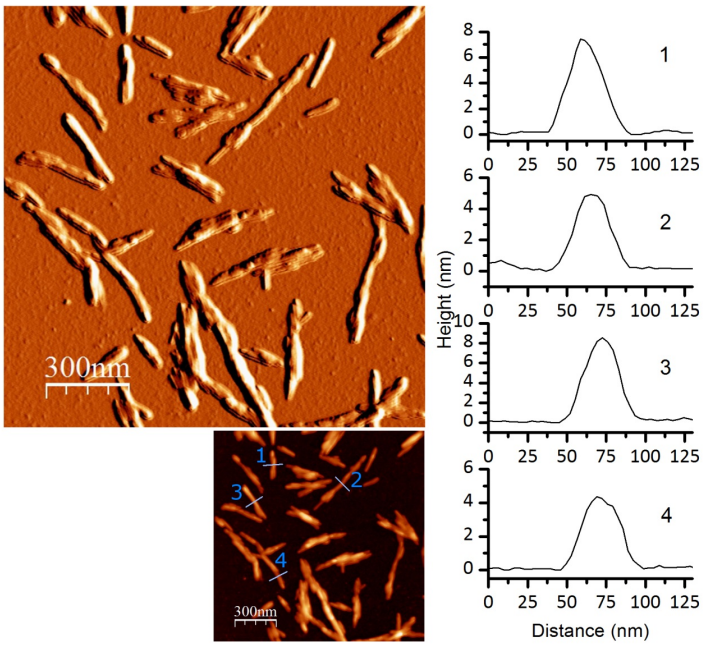


Figure 4. (top) Amplitude signal and (bottom) topography of NCC-dye **4** nanocrystals. Graphs on the right report the profiles along the four paths highlighted in the topography image.

5. XPS analysis

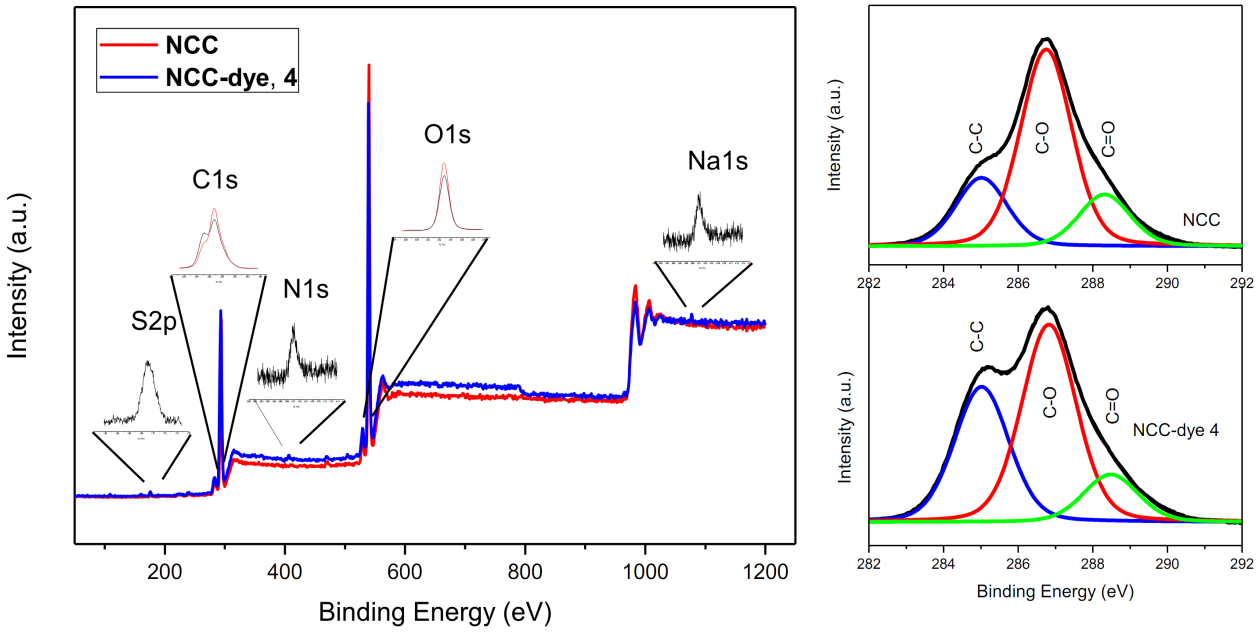


Figure 5: Survey spectra of **NCC** and **NCC-dye 4** (left) and C1s high resolution region (right)

	C1s B.E.(eV) (%)	**O1s B.E. (eV) [O/C]**	**S2p B.E.**(eV) **[S/C]**	**N1s B.E.**(eV) **[N/C]**
NCC	285.0 (22%) 286.7 (62%) 288.3 (16%)	532.7 [0.66]	-	-
NCC-dye 4	285.0 (35%) 286.8 (52%) 288.4 (13%)	532.8 [0.56]	168.8 [0.01]	400.0 [0.007]

Table: C1s, O1s, S2p and N1s binding energies

XPS analysis confirms the covalent bonding of the dye onto the surface of **NCC**. As evidenced by the survey spectra, the **NCC-dye 4** sample shows, along with the C and O photoemission peaks, the peaks relative to sulphur, nitrogen and sodium. The analysis of the high resolution C1s region confirms the presence of the dye by the increasing of the aromatic carbon at 285 eV with respect to the oxygen bonded carbon.

6. Thermal analysis (TGA)

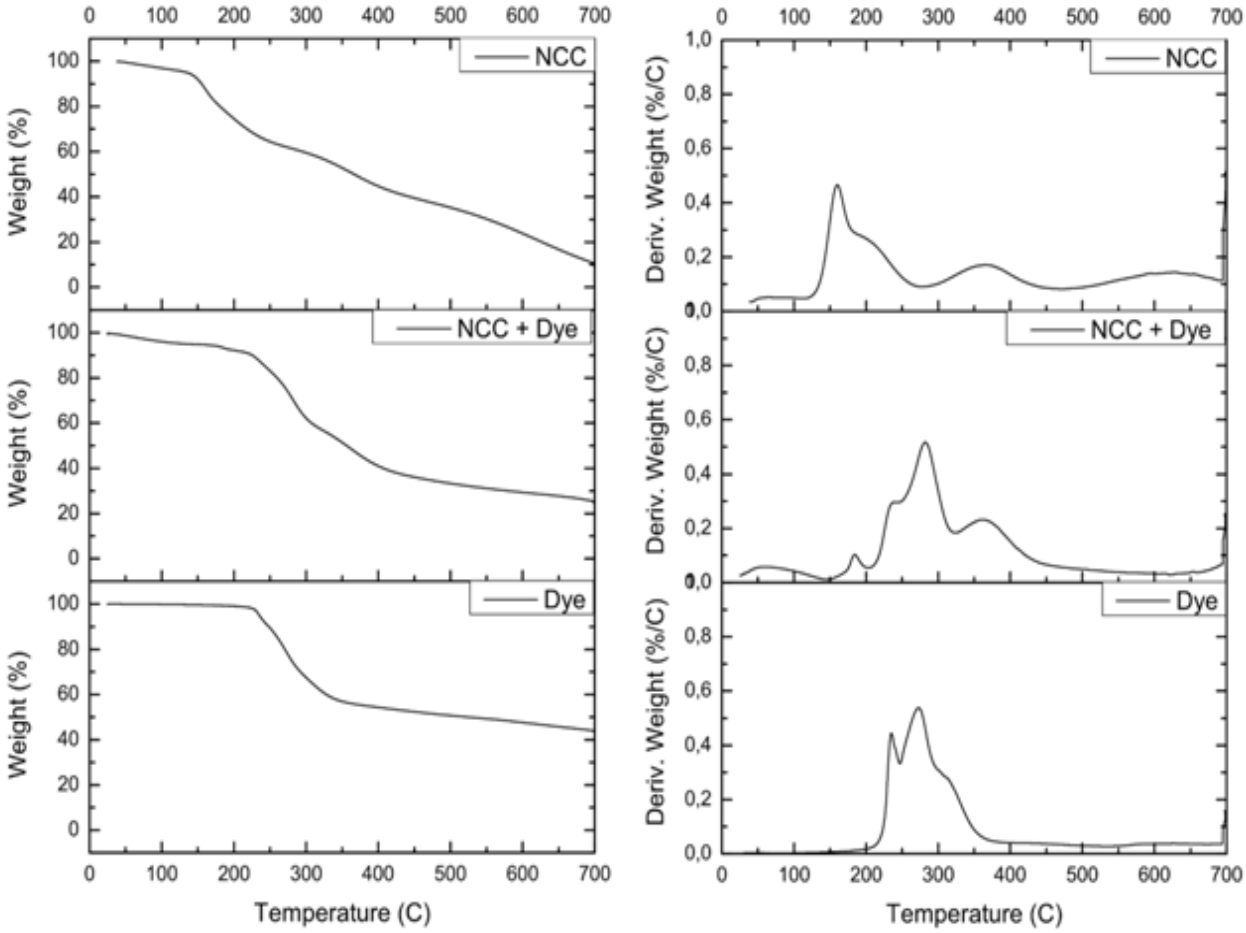
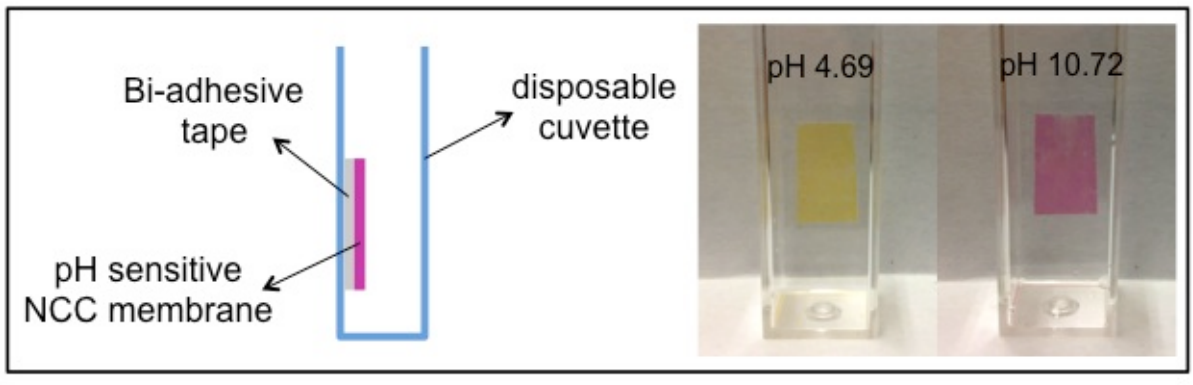


Figure 6: *Left*: TGA analysis of dye **1** (bottom) and of membranes made with **NCC** (top) and **NCC-dye 4** (middle). *Right*: Corresponding thermogram derivatives.

7. Attaching NCC-dye membrane inside a disposable cuvette



11. References

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R2: T. Carofiglio, C. Fregonese, G.J. Mohr, F. Rastrelli, U. Tonellato, *Tetrahedron*, 2006, **62**, 1502.

R3: D.A. Shirley, *Phys. Rev. B*, 1972, **5**, 4709.

R4: P.M.A. Sherwood, in: D. Briggs, M.P. Seah (Eds.), *Practical Surface Analysis*, Wiley, New York, 1990, p. 181.

R5: Horcas et al. *Rev. Sci. Instrum*. 2007, **78**, 13705.