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# 1                    **Adsorption versus Grafting of Poly(N-** 2                    **Isopropylacrylamide) in aqueous conditions on the** 3                    **Surface of Cellulose Nanocrystals**

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## 13                    **ABSTRACT**

14                  This study proposes a grafting strategy of thermo-sensitive amine-terminated oligomers of  
15                  Poly(N-**Isopropylacrylamide**) (Pnipam<sub>2500</sub>) onto the surface of Cellulose Nanocrystals (CNCs).  
16                  Pnipam<sub>2500</sub> grafting in aqueous condition via peptidic coupling was explored to obtain CNC  
17                  hydrogel with thermo-reversible aggregation and new colloidal properties. A discussion  
18                  between grafting vs adsorption /presence of the Pnipam<sub>2500</sub> is proposed. A large range of  
19                  experimental techniques was used to investigate the properties of the CNC decorated with  
20                  polymer and to confirm the grafting. Elemental analysis, infrared spectroscopy, solid state  
21                  NMR and conductometric titration of washed CNC-g-Pnipam<sub>2500</sub> demonstrate that at least a  
22                  part of Pnipam<sub>2500</sub> was covalently bonded with CNC. A thermo-reversible aggregation was  
23                  observed by Dynamic Light Scattering experiments and thermo-sensitive behavior is  
24                  observed by rheological experiments. **For grafted polymer the viscosity increases from 0.008**  
25                  **to 40 Pa·s at low shear rate when the LCST is reached, whereas, in the case of polymer**  
26                  **adsorption, the viscosity increases only from 0.002 to 0.3 Pa·s.** This thermo-reversible, bio-  
27                  based and biocompatible system paves the way for the design of injectable hydrogel and  
28                  biomedical nanocomposite materials.

29                  **Keywords:** Cellulose nanocrystals; Poly(N-**Isopropylacrylamide**); surface functionalization;  
30                  thermo-sensitive

## 31        **1. Introduction**

32        The increasing interest to use biodegradable, renewable, non-toxic and sustainable material  
33        has led to the increasing use of nanoscale cellulose in advanced materials. Among such  
34        nanoscale cellulose, cellulose nanocrystals (CNCs) are obtained by acid hydrolysis and are  
35        rigid rod-shaped particles with dimensions ranging from 3 to 30 nm in cross-section and  
36        from 100 to 500 nm in length. Known since the 50's (Rånby, 1951), they exhibit several  
37        interesting properties including excellent mechanical strength, high aspect ratio, low density  
38        and an ability to self-organize into liquid crystalline phases (Habibi, 2014; Klemm et al., 2011;  
39        Moon et al., 2011; Revol et al., 1992). Besides, this treatment also introduces sulfate ester  
40        groups on the surface of CNC which induces a colloidal stability in aqueous media. Owing to  
41        their specific physical properties and high aspect ratio (depending on the source), CNC are  
42        used in several domains (Oksman et al., 2014) like environmentally friendly nanocomposites  
43        (Dufresne, 2013; Eichhorn et al., 2010; Siqueira et al., 2010a), biomedical applications  
44        (Camarero Espinosa et al., 2016; Domingues et al., 2014; Endes et al., 2016; Jorfi and Foster,  
45        2015; Klemm et al., 2011; Lin and Dufresne, 2014; Naseri et al., 2016), coatings and printed  
46        electronics (Gicquel et al., 2017; Hoeng et al., 2016; Li et al., 2013), hydrogels and aerogels  
47        (De France et al., 2016, 2017; Eichhorn et al., 2010). Furthermore, CNCs are not only  
48        produced at laboratory scale but are now industrially available in high quantities (Chauve  
49        and Bras, 2014).

50        New properties can even be implemented to CNCs by modifying the numerous hydroxyl  
51        groups at their surface and several reviews focus on the chemical surface modification of  
52        CNC with molecule or polymers (Eyley and Thielemans, 2014; Habibi, 2014; Tang et al.,  
53        2017). Two main approaches can be used to covalently attach polymer chains on the surface  
54        of CNCs, the "grafting-from" or the "grafting-to" methods. The "grafting-from" allows  
55        highest grafting polymers length chains on CNCs surfaces and highest grafted polymer  
56        diversity. For the grafting from approach, the surface is first functionalized with an initiator  
57        monolayer. Then, the polymerization is carried out on the surface-initiated, resulting on the  
58        growth of the polymer brush directly from the surface (Alosmanov et al., 2017; Dong and  
59        Roman, 2007; Follain et al., 2010; Kan et al., 2013; Wu et al., 2015; Zoppe et al., 2010).  
60        However, the "grafting-from" method induces the presence of free homopolymers and  
61        generally uses toxic solvents or reagents.

62 In the “grafting-onto” strategy, a greener process can be tested. Presynthesized and well  
63 characterized polymer chains are reacted with cellulose hydroxyl groups or surface  
64 carboxylate groups generated by TEMPO oxidation. Nonetheless, steric hindrance can  
65 prevent optimal grafting and limit the grafts surface density. As examples of the “grafting-  
66 onto” process, Araki et al. and Kloser et al. respectively grafted epoxy-terminated  
67 polyethylene glycol (PEG) and polyethylene oxide (PEO) on CNC to create sterically stabilized  
68 system (Araki et al., 2001; Kloser and Gray, 2010; Mangalam et al., 2009).

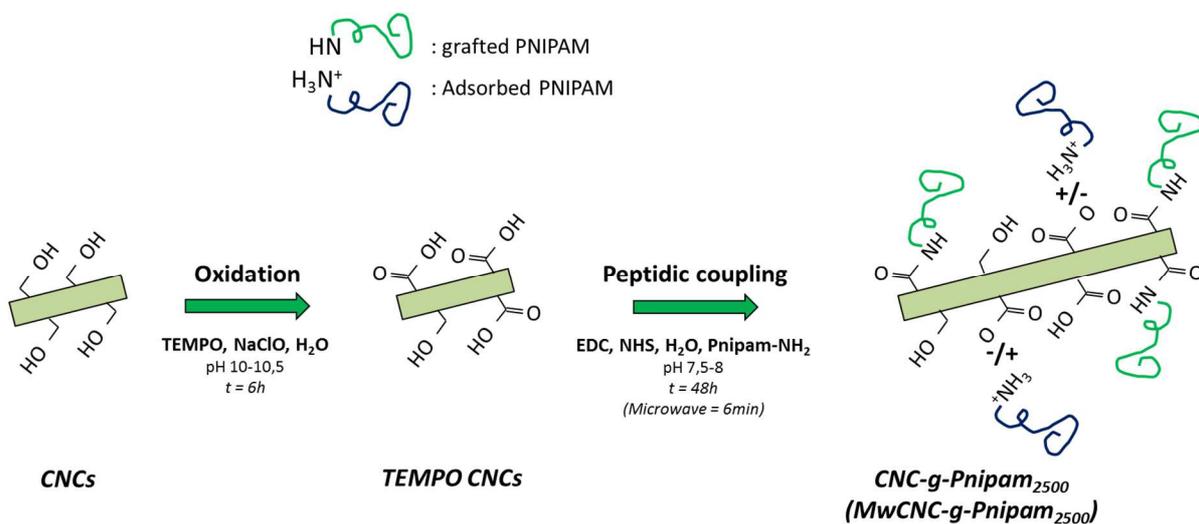
69 In this study, the “grafting-onto” strategy is used. The aim is to create a covalent amide bond  
70 between a primary amine-terminated thermo-sensitive polymer and carboxylated CNC as  
71 shown in **Figure 1**. In the literature, this approach has been successfully applied by coauthors  
72 to the grafting of copolymers of ethylene oxide and propylene oxide , referred to as  
73 Jeffamine polyetheramines (Huntsman Corporation) on TEMPO oxidized CNC (Azzam et al.,  
74 2010, 2016). First, CNC are oxidized and then reacted with amine-terminated polymers using  
75 a carbodiimide as amidation agent. Such reactions can be performed in an organic solvent or  
76 in aqueous media (Azzam et al., 2010, 2016) but requires long reaction times and washing  
77 steps. As reported in the literature, the realization of stimuli-sensitive CNC complex  
78 represents an interesting way to design smart hydrogel and materials, in particular in the  
79 field biomedical applications (Jorfi and Foster, 2015; Naseri et al., 2016).

80 In this work, we have focused on the peptidic grafting of one of the most common thermo-  
81 sensitive polymer Poly(N-**isopropylacrylamide**) also called PNIPAM. This polymer exhibits a  
82 lower critical solution temperature (LCST) at 32°C. Below the LCST, the polymer chains are in  
83 good solvent conditions and adopt a swollen coil conformation with highly hydrated chains.  
84 Above the LCST, the polymer becomes hydrophobic and turns into a collapsed globule (Lai et  
85 al., 2013; Pelton, 2010; Plunkett et al., 2006; Schild, 1992). PNIPAM shows a LCST close to  
86 physiological temperature whatever almost irrespective of its molecular weight (Okahata et  
87 al., 1986), and has a huge potential in biomedical applications (Alosmanov et al., 2017; Chen  
88 et al., 2014; Hebeish et al., 2014). **Its bio-compatibility and non-toxicity were discussed in a  
89 recent review by Lanzalaco et al. (Lanzalaco and Armelin, 2017) and detailed in vivo  
90 experiments with cartilage formation (Cho et al., 2004), ocular integration (Cao et al., 2007;  
91 Lima et al., 2016), subcutaneous tissues (Ohya et al., 2004), cell sheets (Lin et al., 2012) or  
92 drug release (Das et al., 2015).**

93 In the literature, the “grafting-from” method has mainly been used to graft this thermo-  
 94 sensitive chains (Alosmanov et al., 2017; Hemraz et al., 2014; Zoppe et al., 2010, 2011; Zubik  
 95 et al., 2017). Zoppe and co-workers (Zoppe et al., 2010, 2011) grafted PNIPAM brushes from  
 96 CNC via SET-LRP, in which they control exactly the density and the length of brushes  
 97 depending on the ratio of initiator and molecular ratio of NiPAAm monomer. However, such  
 98 strategy needs toxic solvent and to the best of our knowledge, no previous works propose  
 99 “grafting-onto” of PNIPAM on CNCs.

100 Meanwhile, more and more studies **prove** the possibility of high adsorption of poly-  
 101 electrolyte or oligomers on the surface of CNC using various techniques like QCM or SPR  
 102 (Reid et al, 2017; Bensefelt et al, 2016). In some case, it seems even possible to have  
 103 irreversible adsorption and no desorption is observed in spite of multiple washing steps.

104 In this paper, we compare the surface functionalization of CNCs with amine-terminated  
 105 thermosensitive PNIPAM chains using either simple adsorption or peptidic coupling. The new  
 106 systems are then characterized by conductometric titration, infrared spectroscopy, solid  
 107 state NMR and elemental analysis. Dynamic light scattering (DLS) and transmission electron  
 108 microscopy (TEM) are used to describe the structural modification of the nanoparticles.



109

110 **Figure 1:** Schematic representation of chemical modification on CNCs presents in this work

## 111 2. Materials and Methods

### 112 2.1. Materials

113 Colloidal suspension of ~12 wt.% Commercial Cellulose Nanocrystals (CNCs) was purchased  
114 from UMaine process Development Center (University of Maine, USA). They have been  
115 produced from wood pulp using sulfuric acid hydrolysis process and have been never freeze-  
116 dried. The dry matter was measured using a moisture analyzer (Ohaus® MB-35, Sigma-  
117 Aldrich, USA). Poly(N-Isopropylacrylamide) with amine terminated was purchased from  
118 Sigma-Aldrich (USA). The molecular weight is about 2500 g·mol<sup>-1</sup>, and the LCST is close to  
119 32°C. For the TEMPO oxidation, following chemicals are purchased from Sigma-Aldrich:  
120 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO, 2564-83-2), Sodium hypochlorite (NaClO, 12  
121 wt.%, 7681-52-9) and Sodium bromide (NaBr, 7647-15-6). For the peptidic grafting,  
122 chemicals are purchased from Sigma-Aldrich: N-(3-Dimethylaminopropyl)-N'-  
123 ethylcarbodiimide hydrochloride (EDC, 25952-53-8) and N-Hydroxysuccinimide (NHS, 6066-  
124 82-6). Other chemicals were purchased from Sigma-Aldrich. Distilled water was used for all  
125 experiments.

### 126 2.2. Carboxylation of cellulose nanocrystals by TEMPO oxidation

127 CNCs were subjected to TEMPO-mediation oxidation using a previously reported procedure  
128 (Habibi et al., 2006). 11 g of CNC were dispersed in 730mL of distilled water. Then, this  
129 suspension was exposed to an ultrasonic dispersive energy of 5 kJ per gram of dry CNC using  
130 a 250 Watt sonication probe (Sonifier S-250A, Branson, USA) at 50% of maximum energy  
131 during 15 minutes in this case. To prevent burning of CNC during the dispersion, the  
132 suspension was placed in a crystallizer full of ice. TEMPO (323 mg, 2.07 mmol) and NaBr  
133 (3.564 mg, 34.61 mmol) were dissolved in 250mL of deionized water by magnetic stirring  
134 and slowly added to the CNC suspension. Then, 66 g (0.12M) of NaClO suspension was added  
135 dropwise to the suspension to start the oxidation. The mixture was stirred for 3 h at room  
136 temperature. The pH condition of the suspension was maintained between 10 and 10.5 by  
137 addition of sodium hydroxide solution (NaOH) at 0.5M during the reaction. Reaction was  
138 then quenched by the addition of ethanol (40 ml), the suspension color turned from yellow  
139 to white. The resulting CNC were washed with hydrochloric acid (HCl) at 0.5M, to decrease  
140 the pH to 1-2, at least three times by centrifugation (10 000 rpm, 30 min). After the last

141 centrifugation, the oxidized CNC were re-dispersed in distilled water using the minimum  
142 volume to recover all CNC. This suspension was dialyzed against distilled water at least one  
143 week until a neutral pH was obtained (membrane 6-8 kDa). TEMPO CNCs were stored in the  
144 fridge at constant neutral pH to allow the CNC to be in their carboxylate form.

### 145 **2.3. Polymer grafting by peptidic coupling in water**

146 Grafting of amine-terminated PNIPAM<sub>2500</sub> on TEMPO CNCs was performed by peptidic  
147 coupling in water according to protocol of Bulpitt and Aeschlimann (Bulpitt and  
148 Aeschlimann, 1999). The reaction was performed at ambient temperature, and started with  
149 a ~1 wt.% TEMPO CNCs suspension,  $N_{COOH}$  is the mole of carboxyl unit measured by  
150 conductometry. This suspension was exposed to an ultrasonic dispersive energy using a  
151 250 Watt sonication probe (Sonifier S-250A, Branson, USA) at 50% of maximum energy  
152 during 80 seconds per dry grams of CNC to remove any aggregates. The pH was adjusted to  
153 4.5 (CNC-COOH configuration) with 0.5M HCl. Then, 2mL of EDC solution was added to the  
154 suspension ( $N_{EDC}$ : mol per carboxyl group) and stirred during 30 min. Then, 2mL of NHS  
155 solution was added ( $N_{NHS}$ : mol per carboxyl group) and stirred during 30 min. The pH was  
156 then adjusted to 7.5-8.0 before the addition of 10mL of an aqueous suspension containing  
157 PNIPAM-NH<sub>2</sub> ( $N_P$ : mol per carboxyl group). In accordance with the protocol of Azzam et al.  
158 (Azzam et al., 2010), all results were obtained with  $N_{EDC} = N_{NHS} = N_P = 4 \times N_{COOH}$ . The reaction  
159 lasted 48 h at room temperature under stirring while maintaining the pH at 7.5-8.0 by  
160 addition of 0.5M NaOH or 0.5M HCl. The reaction was quenched by the decreasing of the pH  
161 at 1-2 by addition of 0.5M HCl and the resulting suspension was dialyzed against distilled  
162 water to remove excess of reagents at least seven days with exchange of water regularly  
163 until neutral pH is obtained. PNIPAM<sub>2500</sub> grafted CNC will be referred to as CNC-g-Pnipam<sub>2500</sub>.

### 164 **2.4. Conductometric and pH-metric titrations: Carboxyl content**

165 The carboxyl content of oxidized CNC and grafted CNC were determined by conductometric  
166 titration coupled with pH-metric titration. About 15 mg of CNC was suspended in 200 mL of  
167 distilled water and treated by ultrasonic bath for 5min to remove gas and increase the  
168 dispersion. The pH of the suspension is adjusted to acidic condition (pH = 3) with 0.1 M HCl  
169 to replace the sodium counter-ions by protons. The suspension was then titrated with  
170 0.2 mL increment of 0.01 M NaOH. The titration curves exhibit two turns: the first one

171 corresponds to the NaOH neutralization of the strong acid due to the excess of HCl, and then  
172 follows by the neutralization of the weak acid related to the carboxyl content.

173 Degree of Oxidation (DO) of oxidized CNC is the number of primary hydroxyl groups that  
174 have been oxidized into carboxyl groups per AnhydroGlucose Unit (AGU). It was calculated  
175 according to the following **Equation 1** from Da Silva Perez et al. (Da Silva Perez et al., 2003):

$$176 \quad DO = \frac{162 \times C \times (V_{eq2} - V_{eq1})}{m - 36 \times C \times (V_{eq2} - V_{eq1})} \quad (1)$$

177 Where 162 (g·mol<sup>-1</sup>) corresponds to the molar mass of an AGU,  $C$  (mol·L<sup>-1</sup>) is the exact  
178 concentration of the NaOH solution,  $m$  (g) is the weight of the oven-dried sample, 36 in  
179 g·mol<sup>-1</sup> corresponds to the difference between the molecular weight of an AGU (162 g·mol<sup>-1</sup>)  
180 and that of the sodium salt of a glucuronic acid moiety (198 g·mol<sup>-1</sup>), and  $V_{eq1}$  and  $V_{eq2}$  are  
181 the equivalent volumes of NaOH on the bends during the titration. To minimize errors,  
182 titrations were reproduced at least three times and an average value was used for the  
183 discussion.

184 **Equation 2** gives the calculation of the oxidation charge concentration ( $X_{ox}$ ), in  $\mu\text{mol}\cdot\text{g}^{-1}$ :

$$185 \quad X_{ox} = \frac{C \times (V_{eq2} - V_{eq1})}{m} \quad (2)$$

186 Where  $C$  (mol·L<sup>-1</sup>),  $m$  (g),  $V_{eq1}$  and  $V_{eq2}$  are the same as previously.

187 After grafting by peptidic coupling, a part of the carboxyl groups is consumed in the reaction  
188 with the amine-terminated polymer. A new oxidation rate  $X_{res}$  (in  $\mu\text{mol}\cdot\text{g}^{-1}$ ), corresponded to  
189 the residual carboxyl content, was calculated after conductometric titration as given by the  
190 following **Equation 3**:

$$191 \quad X_{res} = \frac{C \times (V_{eq4} - V_{eq3})}{w} \quad (3)$$

192 Where  $C$  (mol·L<sup>-1</sup>) is the exact concentration of the NaOH solution,  $w$  (g) is the pure dry CNC  
193 weight in total samples weight of the oven-dried sample and  $V_{eq3}$  and  $V_{eq4}$  are the equivalent  
194 volumes of NaOH on the post-grafting titration.

195 Finally, the percentage of carboxyl groups which has been substituted ( $\%COOH_{\text{grafted}}$ ) was  
196 calculated according to the equation **Equation 4**, and represents the peptidic coupling yield:

$$197 \quad \%COOH_{\text{grafted}} = \frac{X_{ox} - X_{res}}{X_{ox}} \times 100 \quad (4)$$

## 198 **2.5. Fourier transform infra-red spectroscopy (FTIR)**

199 Infrared spectra of oxidized CNC and grafted CNC were performed using a Perkin-Elmer  
200 spectrum 65 (PerkinElmer, USA). Prior to avoid the superposition of the carbonyl band with  
201 the one of the water, CNC suspensions were acidified to pH 3. Samples were dried during 2 h  
202 in an oven at 80°C and reduced in powder form prior to analysis. KBr pellets containing 1  
203 wt.% solid cellulose sample were prepared. At least spectra of two samples (to check the  
204 reproducibility) per type of CNC were recorded in transmission mode with 16 scans in the  
205 400-4000  $\text{cm}^{-1}$  wavenumber range.

## 206 **2.6. Elemental analysis (EA)**

207 Elemental analysis was carried out by the Analysis science institute of the “centre national  
208 de la recherche scientifique”, Lyon, France. The Carbon, Hydrogen, Oxygen, Sulfur and  
209 Nitrogen contents for CNC, TEMPO CNC, CNC-g-Pnipam<sub>2500</sub> and MwCNC-g-Pnipam<sub>2500</sub> were  
210 measured independently. The obtained results from elemental analysis were used to  
211 determine the Degree of Oxidation after TEMPO oxidation ( $DO_{EA}$ ) (**Equation 5**):

$$212 \quad DO_{EA} = \frac{72.07 - \%C1 \times 162.14}{\%C1 \times 35.93} \quad (5)$$

213 Where %C1 corresponds to the relative carbon content in oxidized sample and 72.07,  
214 162.14, 35.93 are respectively the molecular weight of carbon in AGU, molecular weight of  
215 an AGU, the difference between the molecular weight of an AGU and an oxidized AGU with  
216 sodium salt.

## 217 **2.7. Solid-State NMR**

218 NMR experiments were performed with a Bruker Avance DSX 400 MHz spectrometer  
219 operating at 100.6 MHz for  $^{13}\text{C}$ , using the combination of cross-polarization, high-power  
220 proton decoupling and magic angle spinning (CP/MAS) methods. The spinning speed was set  
221 at 12 kHz. The  $^1\text{H}$  radio frequency field strength was set to give a 90° pulse duration at 2.5 $\mu\text{s}$ .

222 The  $^{13}\text{C}$  radio frequency field strength was obtained by matching the Hartman-Hahn  
223 conditions at 60 kHz. Recording at least 2000 transients with contact time and recycle delay,  
224 respectively, of 2 ms and 2 s represented standard conditions. The acquisition time was set  
225 at 30 ms and the sweep width at 29400 Hz. The position and width of the lines were  
226 maintained constant throughout a series of samples. The area corresponding to the  
227 integration of the  $\text{C}_1$  signal was used as an internal standard and set to one.

## 228 **2.8. Transmission Electron Microscopy (TEM)**

229 Drops of about 0.001 wt.% of CNC suspension were deposited onto glow-discharged carbon-  
230 coated TEM grids. After 2 minutes, the liquid in excess was absorbed with filter paper, and  
231 prior to drying, a drop of Urany-Less (Delta Microscopies, France) was deposited on the  
232 specimen. Then, after 2 minutes, the solution in excess was adsorbed and the grid was dried  
233 under room temperature. The sampling was observed using a Philips CM200 (FEI, USA)  
234 operating at 200 kV.

235 This protocol was adapted to account for the LCST of the grafted thermo-sensitive polymer  
236 ( $\sim 32^\circ\text{C}$ ). In a first case, TEM specimens were prepared at room temperature. In a second  
237 case, TEM specimens were prepared with CNC-g-Pnipam<sub>2500</sub> suspension and Urany-Less  
238 solution both preheated at  $50^\circ\text{C}$ .

## 239 **2.9. Dynamic light scattering (DLS)**

240 DLS measurements were performed on Malvern Nano ZS instrument (Malvern instruments,  
241 United Kingdom). Samples were diluted in deionized water at  $10^{-2}$  wt.% and the conductivity  
242 was adjusted to  $500\ \mu\text{S}\cdot\text{cm}^{-1}$  by addition of NaCl solution. All measurements were made at a  
243 well-controlled ( $\pm 0.05^\circ\text{C}$ ) temperature at a backscattering detection angle of  $173^\circ$ . Non-  
244 negative least squares analysis (NNLS) was performed to achieve the intensity size  
245 distribution (corresponding to hydrodynamic diameter  $z^*$ ) from the analysis of the  
246 correlation function on the Malvern DTS software. For each samples, the final data in this  
247 paper represent an average of at least 3 acquisitions with 10 measurements.

## 248 **2.10. Atomic Forces Microscopy (AFM)**

249 CNCs were imaged using AFM (Dimension icon<sup>®</sup>, Bruker, USA). All suspensions were  
250 previously diluted to  $10^{-4}$  wt.% and a drop of 0.2 mL was deposited onto a freshly cleaved

251 mica plate. Samples were dried overnight at room conditions. The samples were  
252 characterized in tapping mode using a silica coated cantilever (OTESPA® 300 kHz - 42 N/m,  
253 Bruker, USA). Scans of 10x10  $\mu\text{m}^2$  and 3.3x3.3  $\mu\text{m}^2$  were performed to analyze the  
254 dimensions of CNC. At least 4 images per sample were obtained. In order to extract the  
255 length and height dimensions, measurements of about 200 particles were performed by  
256 using the ImageJ software to obtain a representative average size.

257

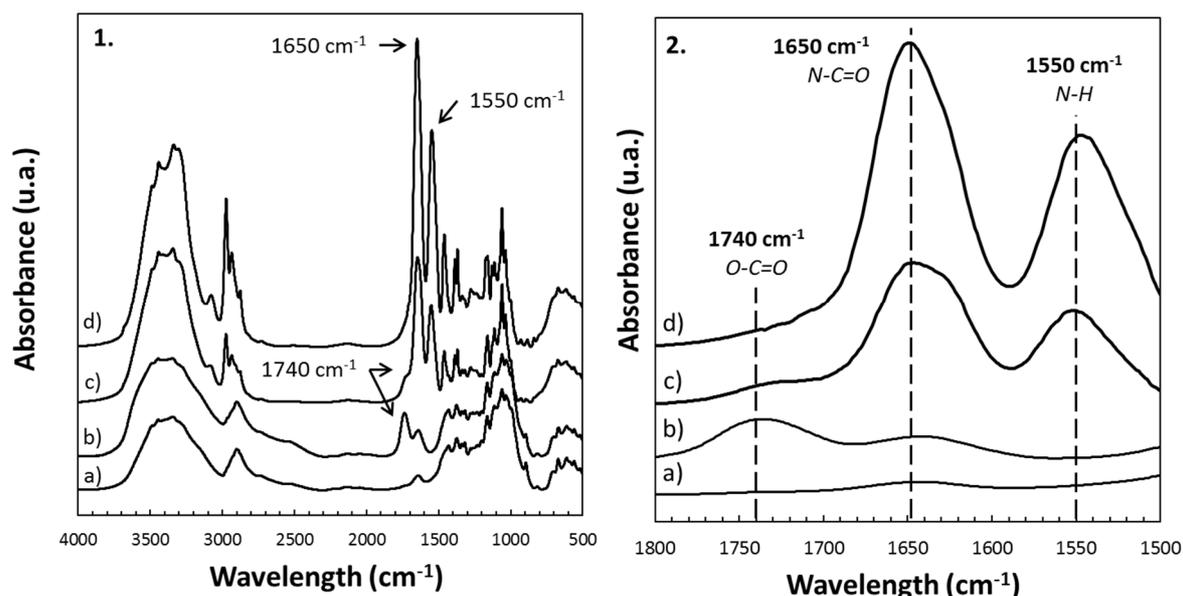
## 258 3. Results and Discussion

### 259 3.1. Grafting of amine terminated Pnipam<sub>2500</sub> onto TEMPO CNCs

260 Aqueous commercial CNC suspensions were previously carboxylated using TEMPO oxidation  
261 process. By conductometric titration (**Figure S1a – Support information**) and **Equation 1**, a  
262 degree of surface oxidation DO of 0.25 (mol/mol of anhydroglucose unit) and an oxidation  
263 rate  $X_{ox}$  of 1450 ( $\mu\text{mol}\cdot\text{g}^{-1}$ ) were obtained.

264 Aqueous TEMPO CNCs were then grafted with amine terminated Pnipam<sub>2500</sub> via peptidic  
265 coupling. **Figure S1b (Support information)** reports conductometric titration. After  
266 calculation with **Equation 2** and **Equation 3**, the residual carboxyl content ( $X_{res}$ ) is 1010  
267 ( $\mu\text{mol}\cdot\text{g}^{-1}$ ) and the percentage of carboxyl groups which has been substituted by amine  
268 groups ( $\%COOH_{grafted}$ ) is 30 %.

269 Nevertheless, these results are indirect proof of the grafting and would be similar in case of  
270 adsorption. FTIR spectroscopy and Elemental Analysis were performed to confirm the  
271 presence of the polymer chains onto the CNC. **Figure 2** shows FTIR spectra of (**Figure 2a**)  
272 regular CNC, (**Figure 2b**) TEMPO CNCs, (**Figure 2c**) TEMPO CNCs mixed with Pnipam<sub>2500</sub> and  
273 (**Figure 2d**) CNC-g-Pnipam<sub>2500</sub>. All samples have been extensively washed before any  
274 characterization using dialysis.



275

276 **Figure 2:** 1. Infrared spectra of a) sulfated commercial CNCs, b) TEMPO CNCs c) TEMPO CNCs mixed  
277 with Pnipam<sub>2500</sub> and d) Pnipam<sub>2500</sub> grafted onto CNCs: CNC-g-Pnipam<sub>2500</sub>. Each spectrum is normalized  
278 at 1110 cm<sup>-1</sup>. 2. Infrared Spectra zoom on characteristic peaks.

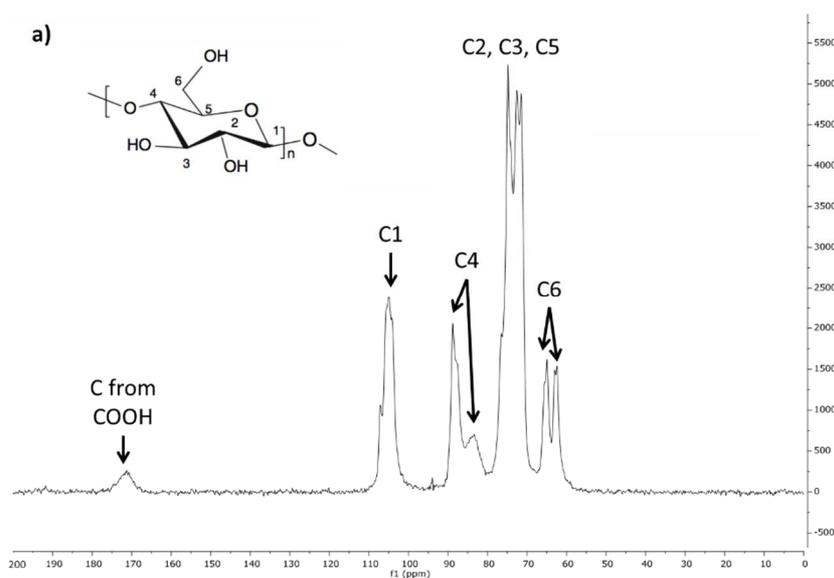
279 Clear changes occur in each steps of the chemical modification. By comparing to the  
280 commercial CNCs, the spectra of the TEMPO CNCs exhibits a high absorption band at  
281 1740 cm<sup>-1</sup>. This peak is characteristic to the acidic form of the carboxylic acid function  
282 (COOH), which validated the oxidation of CNCs. In the case of a peptidic grafting, peaks  
283 characteristic of the amide bonding appear in two absorption bands. Unfortunately, in our  
284 case such amide bonds are also present in the polymer and cannot prove the grafting but  
285 mainly the presence or not of the polymer. The first band at 1650 cm<sup>-1</sup> can be attributed to  
286 the amide bond (amide I absorption band) but also to the residual water adsorbs on the  
287 cellulose (or polymer). Furthermore, at 1550 cm<sup>-1</sup>, another absorption peak characteristic of  
288 the N-H bond exists (called amide II band). On the **Figure 2c**, referring to Pnipam<sub>2500</sub> only  
289 mixed with TEMPO CNCs without coupling agent and following similar washing steps, the  
290 two characteristic peaks of amine are present (1650 and 1550 cm<sup>-1</sup>). They prove the  
291 presence of Pnipam<sub>2500</sub> in the suspension of CNCs even without grafting and after the  
292 washing steps. This result was surprising and we have first checked the efficiency of our  
293 washing procedure. By dialysis of pure Pnipam<sub>2500</sub> during 7 days, we have checked that most  
294 of the polymer (ab. 80-85%) pass through the membrane and is eliminated during the  
295 dialysis. The 15-20% still present seems to be non-solubilized / aggregated polymer or  
296 impurities nanoparticles (like catalyst) present in this commercial grades, as proved by some  
297 DLS measurement showing some aggregates or impurities of about 200nm dimensions. So as  
298 most of the Pnipam<sub>2500</sub> is eliminated, the **Figure 2c** confirms the presence of oligomer in the  
299 suspension. We can suppose this due to the aggregate and to the physically adsorbed  
300 oligomer onto the CNC surface. This adsorption might be due to the hydrogen bonds and has  
301 been investigated more in details in recent study ([Gicquel, 2017](#)).

302 However, the presence of a small peak at 1740 cm<sup>-1</sup> (e.g. COOH characteristic pic) indicates  
303 that still some carboxylic ends are available after adsorption and washing. In the case of the  
304 grafted sample (**Figure 2d**), the two characteristic peaks of the amine bond are present  
305 (1650 and 1550 cm<sup>-1</sup>). Even if it is not quantitative proof, the intensities of these peaks are  
306 largely superior to those one of Pnipam<sub>2500</sub> adsorbed on CNCs. Besides, as previously said  
307 PNIPAM-NH<sub>2</sub> contains N-H bonds in the repeating NIPAM monomer. The molecular weight

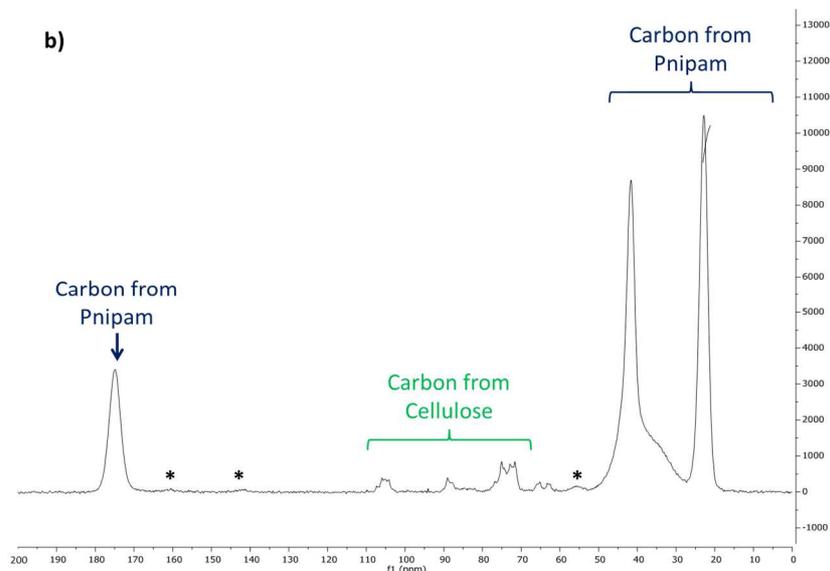
308  $\sim 2500 \text{ g}\cdot\text{mol}^{-1}$  corresponds to  $\sim 22$  time of monomer ( $\sim 22$  amine groups) and one amine on  
309 the termination. So, with a superior intensity compared to **Figure 2c**, more Pnipam<sub>2500</sub> are  
310 present on the CNCs' surface which might be due to a different polymer configuration on the  
311 surface. In this case, the grafting efficiency is also revealed thanks to the disappearance of  
312 the  $1740 \text{ cm}^{-1}$  peak which probably reveals the consumption of CNC's carboxyl groups in the  
313 reaction with the amine-terminated polymers. This disappearance might be due also to  
314 overlapping with peak at  $1650 \text{ cm}^{-1}$ . Probably one part of Pnipam<sub>2500</sub> is adsorbed and one  
315 part is grafted in this case but it is difficult to separate the amount of grafted and adsorbed  
316 polymer on CNCs.

317 So this FTIR analyses proves the presence of Pnipam<sub>2500</sub> into oxidized CNCs suspension. Such  
318 oligomer **stays** in the suspension in spite of washing steps but also that when grafting is  
319 performed a higher quantity seems present and all carboxylic group disappears.

320



321



322  
 323 **Figure 3:**  $^{13}\text{C}$  Solid-state NMR spectra of A. TEMPO CNCs and B. CNC-g-Pnipam<sub>2500</sub>. Stars (\*) correspond  
 324 to the spinning side band of Pnipam<sub>2500</sub> at 10 kHz (results not shown). *Insert figure shows chemical*  
 325 *structure related to C<sub>1</sub>-C<sub>6</sub> of cellulose.*

326 **Figure 3** shows the  $^{13}\text{C}$  solid-state NMR of TEMPO CNCs and CNC-g-Pnipam<sub>2500</sub>. In the case of  
 327 TEMPO CNCs (**Figure 3a**), carbon contribution of the cellulose were clearly identified (Atalla  
 328 and VanderHart, 1999; Montanari et al., 2005). The region between 60 and 70 ppm  
 329 correspond to the C<sub>6</sub> carbon of the AGU, and between 70 and 80 ppm to the carbon  
 330 contribution of the C<sub>2</sub>, C<sub>3</sub> and C<sub>5</sub>. C<sub>4</sub> contribution is the region between 83 and 88 ppm. The  
 331 C<sub>1</sub> carbon appears at 105 ppm. The area corresponding to the integration of the C<sub>1</sub> signal  
 332 was used as an internal standard and set to one. The contribution of the carboxyl group is  
 333 located at 174 ppm (Montanari et al., 2005). The **Figure 3b** represents the NMR spectra after  
 334 the grafting of Pnipam<sub>2500</sub> on CNCs (after dialysis washing steps). On this spectrum, the  
 335 contribution of the carbon from the polymer is very high related to the internal standard on  
 336 C<sub>1</sub> (from 20 to 36 times the integration of the C<sub>1</sub> signal). The peptidic grafting creates a new  
 337 link between carbon and nitrogen (i.e. a new amide). As already said, this link is also present  
 338 inside the NIPAM monomer of the polymer chain, so it is difficult to separate the  
 339 contribution of the carbon amide occurring after the grafting from the carbon amide in  
 340 polymer contribution. The disappearance of the peak at 174 ppm (related to COOH) replaced  
 341 by a strong peak at 176 ppm (related to -CONH-) could be due to the grafting but also to  
 342 solid “dilution” due to the presence of Pnipam<sub>2500</sub> and high amount of amide bounds. The  
 343 **Figure 3b** reveals indeed the presence in huge amount of PNIPAM on the CNCs surface after  
 344 several washing steps. These values are in accordance with a presence of Pnipam<sub>2500</sub> on

345 TEMPO CNCs surface, but cannot confirm if it is grafted or adsorbed. A comparison with only  
 346 mixed Pnipam<sub>2500</sub> would not help due to this dilution but could give us more quantitative  
 347 information. For such quantitative data, we have preferred using elemental analysis.

### 348 3.2. Quantitative analysis of Pnipam<sub>2500</sub> onto CNCs

349 Further evidence of the grafting of Pnipam<sub>2500</sub> from CNCs was supported by the elemental  
 350 analysis of extensively washed CNC and used to quantify the amount of polymer present on  
 351 the surface. **Table 1** shows the experimental atomic composition (C, H, N, O and S) of CNCs,  
 352 TEMPO CNCs, CNC-g-Pnipam<sub>2500</sub> and CNCs mixed with Pnipam<sub>2500</sub>.

353 **Table 1:** Atomic composition determined by Elemental Analysis (standard deviation is considered to  
 354 be related to equipment at 0.1 % for each value).

	<i>Experimental values</i>					<i>Corrected values</i>	
	% C	% H	% N	% O	% S	% C	%O
CNCs	40.2	6.0	< 0.1	49.7	0.9	44.44	49.37
TEMPO CNCs	37.0	5.9	< 0.1	52.2	0.8	40.91	51.80
CNC-g-Pnipam <sub>2500</sub>	53.8	8.9	8.9	24.3	0.3	59.50	27.11
TEMPO CNCs + Pnipam <sub>2500</sub>	53.9	8.8	9.1	24.2	0.2	59.69	24.04

355 In the case of pure cellulose, theoretical values of the weight fraction of carbon are 44.44%  
 356 and 49.37% for oxygen, and theoretical ratio of oxygen-to-carbon is close to 1.11. Therefore,  
 357 experimental value presents a ratio about 1.24 for CNC. It is well known that the difference  
 358 between the theoretical and the experimental values in the case of the carbon content is  
 359 probably due to sulfate content, the impurities present in the sample and experimental  
 360 error. To incorporate this difference, the experimental values reported in **Table 1** are  
 361 corrected with the product between the experimental value obtained for a given material  
 362 and the ratio of the theoretical to experimental value for non-grafted nanoparticles. This  
 363 strategy has already been used to determine the degree of substitution (Missoum et al.,  
 364 2012; Siqueira et al., 2010b).

365 In the case of TEMPO CNCs, the DO<sub>EA</sub> calculated from the **Equation 5** gives an oxidation rate  
 366 of about 0.38 which is close and in the same range than conductometry titration value  
 367 (i.e. DO = 0.25) if we consider the standard deviation of E.A. From these results, it is possible

368 to estimate the surface density of the carboxyl content and to calculate the maximum  
 369 amount possible of polymers which can be grafted. After a TEMPO oxidation, carboxylic  
 370 groups are mainly on the surface of CNCs (Isogai et al., 2011). With a DO of about 0.25 and  
 371 assuming that the surface of an AGU is 1 nm<sup>2</sup>, it corresponds to a density ~25 COOH every  
 372 100 nm<sup>2</sup>, assuming that most of oxidation occurs at the surface of CNC. **CNC dimensions**  
 373 **a x b x c are 150 x 10 x 10 nm<sup>3</sup> (Table 2).** As the surface of CNC is 6000 nm<sup>2</sup>, 1500 COOH ends  
 374 are available on the surface. The maximum grafting molecule will be with 1500 polymer  
 375 chains per CNC. Referring to the conductometric titration, ~30 % carboxyl groups were  
 376 substituted by amine groups. It corresponds to ~450 polymer chains grafted on the surface  
 377 of CNCs.

378 The presence of a high amount of nitrogen content in the CNC-g-Pnipam<sub>2500</sub>, extensively  
 379 washed confirms that Pnipam<sub>2500</sub> is still present after grafting on TEMPO CNCs. This value is  
 380 equivalent to the quantity of Pnipam<sub>2500</sub> of 5 wt.% using ratio of %C/%N with following  
 381 Equation 6 and 7.

$$382 \quad \%C = \frac{[(1 - \%Pnipam_{2500}) \times M_C \times 6] + [\%Pnipam_{2500} \times (M_C \times 134)]}{M_{tot}} \quad (6)$$

$$383 \quad \%N = \frac{\%Pnipam_{2500} \times M_N \times 23}{M_{tot}} \quad (7)$$

384 Where (1 - %Pnipam<sub>2500</sub>), M<sub>C</sub>, 6, %Pnipam<sub>2500</sub>, 134, M<sub>N</sub>, 23 and M<sub>tot</sub> are respectively the  
 385 CNC mass, carbon molecular weight, number of carbon in AGU, mass of Pnipam<sub>2500</sub>, number  
 386 of carbon in Pnipam<sub>2500</sub>, Nitrogen molecular weight, number of nitrogen in Pnipam<sub>2500</sub> and  
 387 total mass. To evaluate the number of CNCs in a suspension, the density of cellulose (1.59)  
 388 and dimension of CNC (a x b x c = 150 x 10 x 10 nm<sup>3</sup>) were used.

389 Based on the presence of ~4 E<sup>16</sup> CNCs in 0.95 g of suspension (1g - 5% of Pnipam<sub>2500</sub>) and  
 390 ~1200 E<sup>16</sup> Pnipam<sub>2500</sub> chains in 0.05 g of suspension, this value of 5 wt.% means ~300  
 391 polymer chains on the surface of TEMPO CNCs. This value shows that all the COOH have not  
 392 been grafted. But it is difficult to say if it is grafted or adsorbed. Indeed, unfortunately similar  
 393 amount of Pnipam<sub>2500</sub> is present when only adsorption and washing occurs.

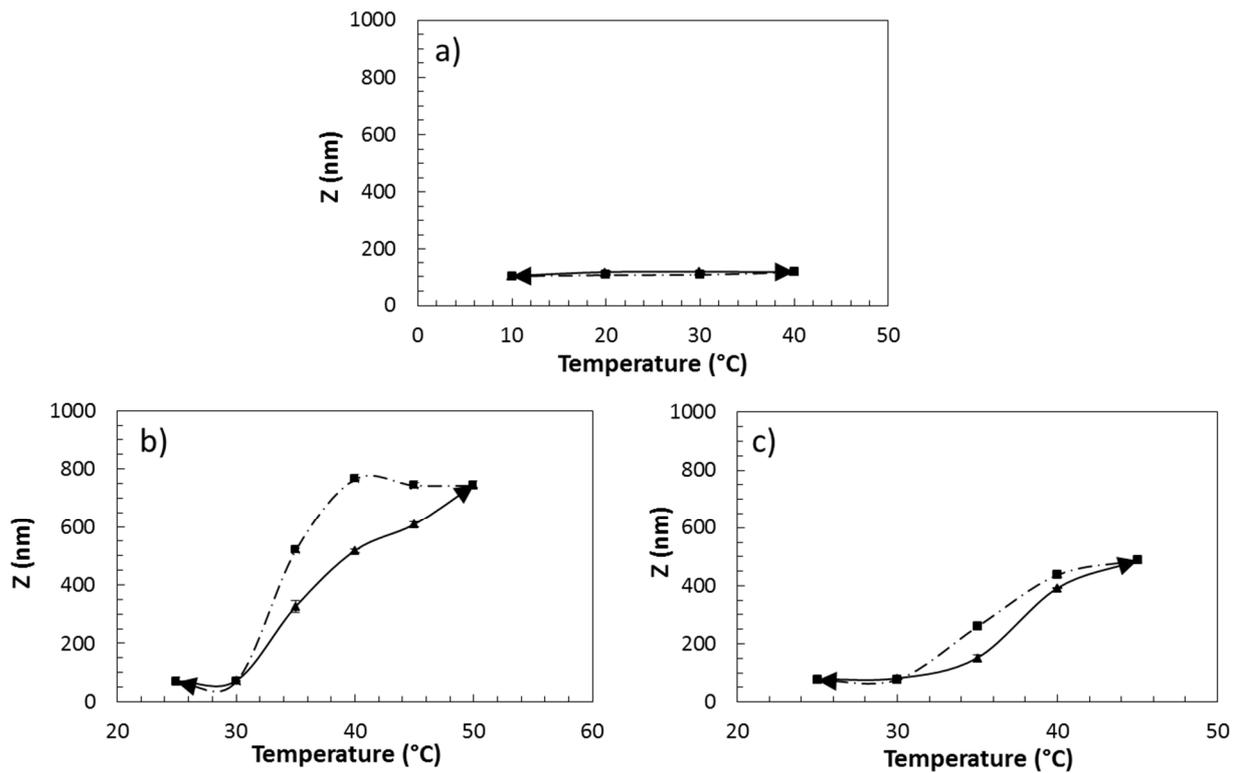
394 If we consider only adsorption, the surface of adsorbed polymer chains should be  
 395 characterized by a mushroom regime, in which they do not interact together, in the case of

396 moderate polymer densities. The size of the polymer is in the order of the gyration radius,  
397 which is 1.5 nm, given by  $R_g = 0.022 \times M_w^{0.54}$  (Kubota et al., 1990) where  $M_w$  is the  
398 polymer molecular weight, *i.e.* 2500 g·mol<sup>-1</sup>. To be in mushroom regime, each polymer needs  
399 to be at a distance higher than twice time the  $R_g$ . The CNC size  $a \times b \times c$  are  
400 150 x 10 x 10 nm<sup>3</sup>, equivalent to an area about 6000 nm<sup>2</sup>. The surface occupied by a polymer  
401 is 7 nm<sup>2</sup> ( $\pi \times R_g^2$ ), so on a CNC the maximum of adsorbed polymer is ~860 polymer chains.  
402 This value is in the range of grafting calculation. So in both case, adsorption and grafting,  
403 similar amount of polymer chain can be present on CNC surface.

404 As a conclusion, it is difficult to have a direct proof of the grafting vs adsorption/presence  
405 with techniques we have used. Our assumption is that similar amount of Pnipam<sub>2500</sub> is at the  
406 surface of CNC but in the case of adsorbed polymer, all polymer is present as aggregate in  
407 the suspension or at the surface whereas when it is grafted, one part is grafted on the  
408 surface and another part is adsorbed or present as aggregate.

409 Indirect methods have then been performed to check if we have a difference between  
410 grafted and adsorbed system. Furthermore, the elemental analysis gave us an idea of the  
411 ratio of Pnipam<sub>2500</sub> and CNC. This has been confirmed by weight measurement of  
412 suspensions. Indeed, after grafting, all samples are washed directly with dialysis, so we are  
413 sure that CNCs stays (does not pass through dialysis membrane). So, knowing the initial CNCs  
414 mass and just by measuring the final mass, we can calculate the quantity of Pnipam<sub>2500</sub> which  
415 is still present (adsorbed or grafted or both). In our case this weight measurement gives a  
416 ratio of 25% of CNC and 75% of PNIPAM after adsorption and after grafting. This value will  
417 be considered in DLS characterization (Figure 4).

418 **3.3. Thermosensitive behavior of CNCs decorated with Pnipam<sub>2500</sub>**



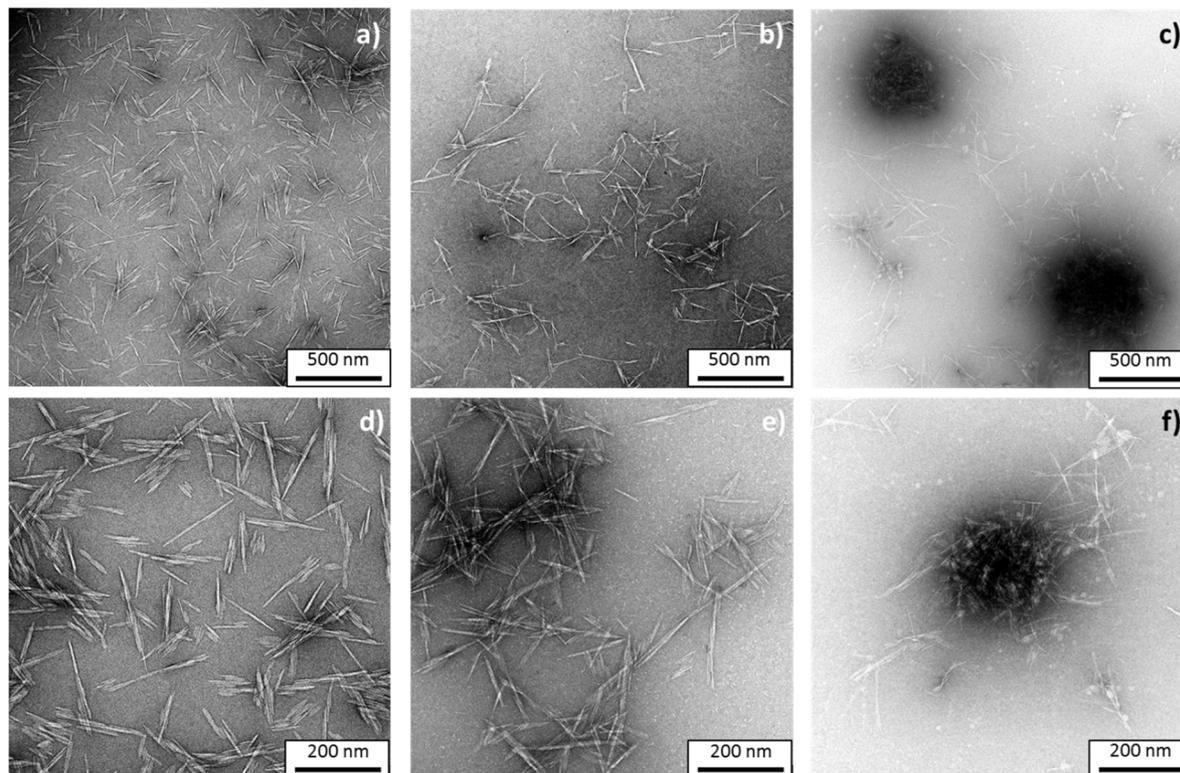
419 **Figure 4:** DLS curve for (a) TEMPO CNCs at 0.1 wt.%, (b) CNC-g-Pnipam<sub>2500</sub> at 0.1 wt.% (ratio  
 420 estimated at 25% CNC and 75% Pnipam<sub>2500</sub>) and (c) TEMPO CNCs + Pnipam<sub>2500</sub> (ratio at 25% TEMPO  
 421 CNCs and 75% Pnipam<sub>2500</sub>). Full line: temperature increases and dot line: Temperature decreases.  
 422

423 In **Figure 4**, the hydrodynamic diameter of CNC-g-Pnipam<sub>2500</sub> and TEMPO CNCs + Pnipam<sub>2500</sub>  
 424 suspension and CNCs raw materials were measured as function of temperature. For  
 425 TEMPO CNCs (**Figure 4a**), temperature has no clear influence on the size of the particles.  
 426 Referring to the literature (Fujishige et al., 1989; Wu, 1998), PNIPAM shows sensitivity to the  
 427 temperature. In works of Kubota (Fujishige et al., 1989; Kubota et al., 1990; Senff and  
 428 Richtering, 1999; Wu, 1998) and Wu (Wang and Wu, 1999; Wu, 1998), under the LCST  
 429 (~34°C), PNIPAM polymer presents coil conformation with hydrodynamic diameter about  
 430 100 nm. In our case the measurement of DLS was difficult due to the presence of impurities  
 431 (catalyst nanoparticles or other) detected with an initial DLS value of 500nm which is not  
 432 possible for a so short oligomer. Then above the LCST, the polymer turns in globule  
 433 conformation and presents a hydrodynamic diameter about 20 nm. For the grafting sample  
 434 CNC-g-Pnipam<sub>2500</sub> (**Figure 4b**), under the LCST the hydrodynamic diameter is low and  
 435 constant (close to the CNC size as it is a short oligomer). After 30°C, an increase in size was  
 436 observed (750 nm), probably due to aggregates formation. When the sample is cooling back  
 437 to 20°C, the hydrodynamic diameter decreases and, at T < 30°C the system recovers a low

438 and constant value similar to the one measured during the heating process. This  
439 phenomenon is considered reversible, even if a 5°C hysteresis is observed. This result is very  
440 positive and confirms indirectly that at least a part of CNC is grafted mainly when it is  
441 compared with only adsorbed Pnipam<sub>2500</sub> (**Figure 4c**). In this case, the aggregation is limited  
442 compared to grafted molecule, and the maximum collapse system size is close to 480 nm (vs  
443 610 nm for the grafted CNC at same Temperature). The difference is even higher when  
444 temperature decrease, i.e. 480 for adsorbed and 750nm for grafted CNC. Nonetheless, the  
445 thermo-reversible behavior is similar in the case of grafted or adsorbed Pnipam<sub>2500</sub>. So the  
446 thermo-stimuli properties of CNC-g-Pnipam<sub>2500</sub> and TEMPO CNCs + Pnipam<sub>2500</sub> are different.  
447 This confirms indirectly that we don't have only presence or adsorption in the first case.

448 The grafting of Pnipam<sub>2500</sub> polymer chain on the surface of CNCs gives a thermo-reversible  
449 behavior to the suspension, as proved by the thermo-reversible aggregation in DLS data. The  
450 behavior of a PNIPAM polymer was previously explained in detail (Lai et al., 2013). In this  
451 study, when the temperature is below the LCST of Pnipam<sub>2500</sub>, grafted chains are under good  
452 solvent conditions. Steric repulsion forces between particles are maximal, and dispersion  
453 with individual objects appears. For temperature higher than the LCST, the polymer is in  
454 poor solvent condition in water, resulting in individual collapsed and CNC-g-Pnipam<sub>2500</sub> are  
455 aggregated together. This process is reversible and generates a hysteresis effect. Similar  
456 behavior **was** observed with another thermosensitive polymer grafted onto CNC (Azzam et al  
457 2016).

458 **3.4. CNCs and Pnipam<sub>2500</sub> grafted onto CNCs (CNC-g-Pnipam<sub>2500</sub>)**  
 459 **characterization**



460  
 461 **Figure 5:** TEM images of a)-d) Cellulose Nanocrystals (CNCs) before any treatment and b)-e) CNC-g-  
 462 Pnipam<sub>2500</sub> deposited on TEM grid at 20°C and c)-f) CNC-g-Pnipam<sub>2500</sub> deposited at 50°C. Pictures are  
 463 representatives of each sample.

464 As confirmed in **Figure 5a** and **5d**, CNC have a rod-like shape. Size measurements on TEM  
 465 images show an average length of  $150 \pm 30$  nm and a section of  $10 \pm 5$  nm. These values are  
 466 comparable with the literature data (Habibi et al., 2010).

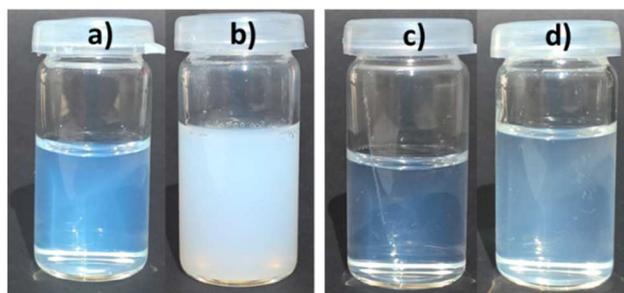
467 **Table 2:** Physical and chemical properties of CNCs from university of Maine.

Properties	Tools	Value	S.D.
Morphology	AFM & TEM	Length (nm)	150 (30)
		Thickness (nm)	10 (5)
		Aspect ratio	15 -
Chemical	EA	Sulfur content ( $\mu\text{mol}/g_{\text{CNC}}$ )	305 (15)
Physicochemical	Calculation based on EA & TEM	Surface charge density ( $e/\text{nm}^2$ )	0.46 (0.02)

468

469 In details in **Table 2**, the sulfur content estimated by elemental analysis is 305  $\mu\text{mol}/\text{g}_{\text{CNC}}$ .  
470 The surface charge density is approximately 0.46  $\text{e}/\text{nm}^2$ , as calculated using TEM and EA  
471 data.

472 **Figure 5** presents TEM images of negatively stained CNC (**Figure 5a** and **5d**), CNCs decorated  
473 with Pnipam<sub>2500</sub> under LCST condition (**Figure 5b** and **5e**) and CNC-g-Pnipam<sub>2500</sub> after LCST  
474 (**Figure 5c** and **5f**). By comparison between **Figure 5d** and **Figure 5e**, the morphology of the  
475 rod-like particles is unchanged after peptidic grafting. Under LCST, Pnipam<sub>2500</sub> grafted CNCs  
476 seems to be well-dispersed. The individualization of nanocrystals can be attributed to the  
477 presence of polymer chains on the surface, which generate entropic repulsion forces  
478 between nanoparticles. Nonetheless, polymer chains are not visible on TEM images, due to  
479 their tiny size and a low surface density (hinder a detectable contrast in TEM stained grid).  
480 After LCST (**Figure 5c** and **5f**), CNC-g-Pnipam<sub>2500</sub> seems to be packed together and shows a  
481 'bird-nest' configuration, due to the collapse Pnipam<sub>2500</sub> on the surface of TEMPO CNCs.  
482 These pictures are in agreement with Pnipam<sub>2500</sub> grafting and thermal aggregation of the  
483 system when the temperature is above the polymer LCST. Nonetheless, the system does not  
484 present CNC-g-Pnipam<sub>2500</sub> packed by 3-4 CNCs (as previously published by Azzam et al.  
485 (Azzam et al., 2016)) but a huge aggregate without well-defined size.

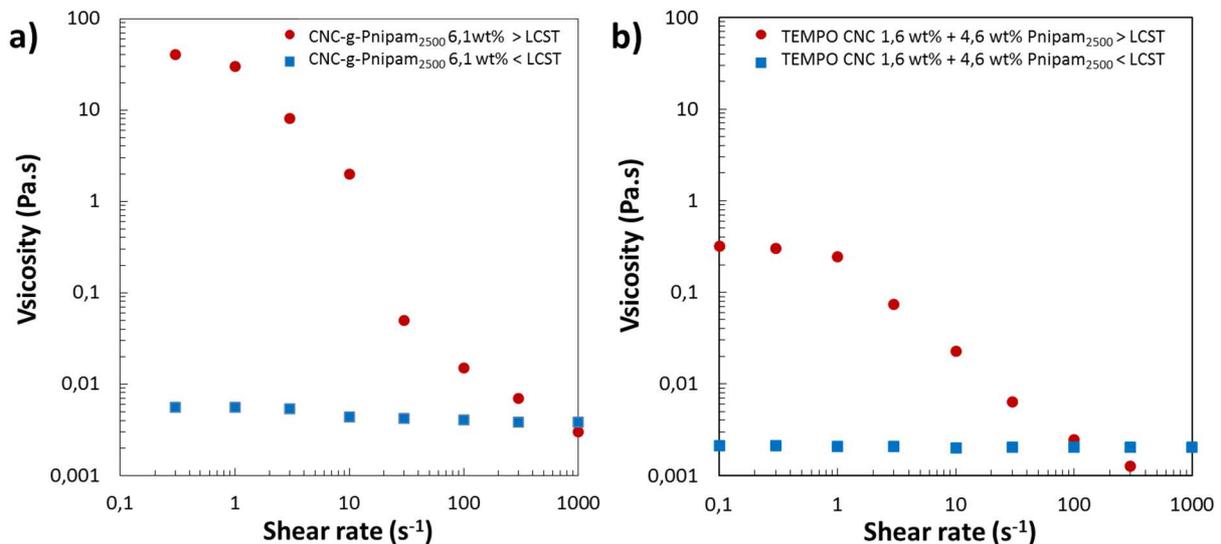


486  
487 **Figure 6:** Pictures of cellulose nanocrystals suspension: a) oxidized CNC without salt, b) TEMPO CNCs  
488 with 1M NaCl, c) CNC-g-Pnipam<sub>2500</sub> without salt and d) CNC-g-Pnipam<sub>2500</sub> with 1M NaCl. Each  
489 suspension has a concentration of 2 wt.%.

490 An interesting property acquired by the presence of polymer chains (adsorbed or grafted) on  
491 the CNC surface is the colloidal stability with presence of electrolyte. **Figure 6** shows the  
492 behavior of TEMPO CNCs and polymer grafted CNCs when NaCl is added. Colloidal  
493 suspension of TEMPO CNCs (**Figure 6a**) immediately precipitates after addition of 1 M NaCl  
494 and forms a turbid suspension (**Figure 6b**). At the opposite, Pnipam<sub>2500</sub> grafted on CNCs  
495 suspension (**Figure 6c**) prevent any precipitation and remain stable the suspension in

496 presence of electrolyte (**Figure 6d**). Steric stabilization caused by the presence of polymer  
497 (grafted or adsorbed) was already reported in literature (Araki et al., 2001; Azzam et al.,  
498 2010; Kloser and Gray, 2010).

499 **Figure 7** presents the rheological behavior of CNC-g-Pnipam<sub>2500</sub> and TEMPO  
500 CNCs + Pnipam<sub>2500</sub> under and above the PNIPAM LCST. System concentrations are 6.1 wt.%  
501 (estimated ratio is 25% of CNCs and 75% of Pnipam<sub>2500</sub>). For grafted polymer (**Figure 7a**) the  
502 viscosity increases from 0.008 to 40 Pa·s at low shear rate when the LCST is reached.  
503 Nonetheless, in the case of polymer adsorption exclusively (**Figure 7b**), the viscosity  
504 increases only from 0.002 to 0.3 Pa·s when the LCST is reached. This observation shows a  
505 difference in kinetics of aggregation whereas similar amount of polymer is present. This  
506 confirms once again indirectly that there is a difference between these two CNCs materials.



507  
508 **Figure 7:** flow curves of a) CNC-g-Pnipam<sub>2500</sub> at 6.1 wt.% and b) TEMPO CNCs at 1.6 wt.% +  
509 Pnipam<sub>2500</sub> at 4.6 wt.% (final concentration 6.2 wt.%). Each graph presents the system flow  
510 curve under (blue square) and above (red dot) the PNIPAM LCST.

511 This peptidic grafting is promising and can modify CNCs suspension properties by using  
512 water based grafting or adsorption. One drawback could be attributed to the low quantity of  
513 grafting (similar to adsorption) and to the time duration of such treatment.

514

## 515 **4. Conclusions**

516 In this study, thermo-sensitive Pnipam<sub>2500</sub> were successfully adsorbed and grafted (or both)  
517 onto oxidized cellulose nanocrystals by a green peptidic coupling. Results demonstrate the  
518 presence and the formation of the polymer onto the nanocrystals after multiple washings.  
519 The polymer density was sufficient to create a steric stabilization of the CNC-g-Pnipam<sub>2500</sub> in  
520 high ionic strength which prevents the flocculation. In spite of several methods, it has been  
521 difficult to distinguish the adsorbed from the grafted CNCs. However indirect methods show  
522 clearly a difference between the two samples indicating that of CNC-g-Pnipam<sub>2500</sub> has at  
523 least one part which is grafted. Thanks to this sufficient density, the thermo-reversible  
524 behavior of the polymer allows us to realize a thermo-reversible suspension composed with  
525 nanoparticles, CNCs, and biocompatible polymers, Pnipam<sub>2500</sub>. CNCs give percolation  
526 network properties to the system and the polymer, a thermosensitive behavior, observed in  
527 rheology **measurement**. These results open the road to biomedical applications such as  
528 injectable hydrogel for reparative surgery and esthetic surgery.

529

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540

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