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1 Asymmetric modification of cellulose nanocrystals
2 with PAMAM dendrimers for the preparation of pH-
3 responsive hairy surfaces

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11

12 **KEYWORDS:** tunicate cellulose nanocrystals; reducing end; dendrimer; PAMAM; quartz crystal
13 microbalance with dissipation (QCM-D); surface plasmon resonance (SPR).

14

15 **ABSTRACT**

16 In this work, we present a straightforward method to attach a globular dendrimer at the
17 reducing end of cellulose nanocrystals obtained from tunicates (t-CNC). We investigated the
18 first four generations of poly(amidoamine) dendrimers (PAMAMs G0 to G3) to obtain hybrid t-
19 CNCs. Taking advantage of the inherent chemical asymmetry resulting from the parallel
20 organization of cellulose chains in CNCs, we prepared asymmetric nanocrystals by selectively
21 modifying their reducing end. The aggregation behavior of hybrid t-CNCs was studied by
22 dynamic light scattering (DLS) and scanning transmission electron microscopy (STEM); and
23 interactions of these asymmetric nanoparticles with gold surface were elucidated using quartz
24 crystal microbalance with dissipation (QCM-D) and surface plasmon resonance (SPR). Hybrid t-
25 CNCs formed hairy layers onto gold surface combining the properties of rigid rod-like
26 nanocrystals with globular and flexible PAMAM dendrimers. Moreover, the presence of amino
27 groups provided pH-responsive properties to hybrid t-CNCs, and QCM results revealed
28 reversible swelling/deswelling behavior. Thereby, we achieved to synthesize tree-shaped
29 functional bio-based materials that adsorbed on gold and formed pH-responsive hairy surfaces.

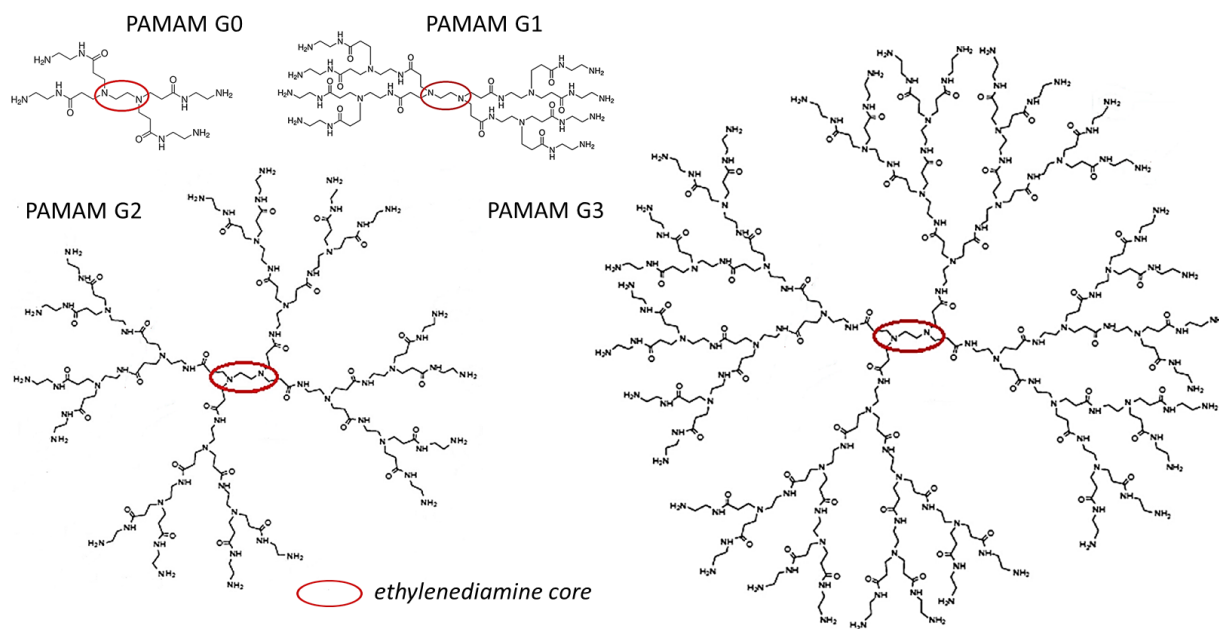
30

31 **1. INTRODUCTION**

32 Cellulose nanocrystals (CNCs) have arisen as promising candidates for high-end materials
33 applications. Besides their high abundance, renewability and low environmental impact, CNCs
34 have outstanding material characteristics, including high tensile strength and barrier properties
35 suitable for composite reinforcement (Habibi, Lucia, & Rojas, 2010), surface properties for
36 emulsion stabilization (Capron, Rojas, & Bordes, 2017), chiral nematic liquid crystal behavior for

37 optically active materials (Shopsowitz, Qi, Hamad, & MacLachlan, 2010) and colloidal stability
38 for the formation of gels (Lewis, Hatzikiriakos, Hamad, & Maclachlan, 2019; Talantikite, Beury,
39 Moreau, & Cathala, 2019). CNCs are produced from cellulose fibers after removal of the
40 disordered cellulose domains by acid hydrolysis. This leads to rigid, high crystalline nanorods
41 bearing hydroxyl groups on their surface. The particularity of CNCs is the presence of two
42 differentiated chemical ends resulting from the parallel alignment of cellulose I chains (Hieta,
43 Kuga, & Usuda, 1984; Koyama, Helbert, Imai, Sugiyama, & Henrissat, 1997). Thus, the
44 hemiacetal groups allow the asymmetric introduction of non-cellulosic functionalities at the
45 reducing end, enabling novel interactions, self-assembly capacity and new properties. Up to
46 now, only few studies take advantage of the parallel arrangement of cellulose chains to
47 functionalize CNCs in an asymmetric way (Tao, Lavoine, Jiang, Tang, & Lin, 2020). For instance,
48 research has been focused on the grafting of synthetic polymers, such as polyacrylamide, or
49 natural ones, like β -casein, at the reducing end of cellulose nanocrystals (Karaaslan, Gao, &
50 Kadla, 2013; Risteen et al., 2018; Sipahi-Saglam, Gelbrich, & Gruber, 2003; Zoppe et al., 2017).
51 Modification of reducing ends was also implemented to drive the self-assembly of hybrid CNCs.
52 In this field, the introduction of thermoresponsive polyetheramines results in thermally
53 switchable star-like supramolecular assemblies (Lin, Cousin, Putaux, & Jean, 2019). 2D surface
54 adsorption was also achieved using specific interactions, such as biotin/streptavidin (Villares,
55 Moreau, & Cathala, 2018) or thiol/gold (Arcot, Lundahl, Rojas, & Laine, 2014; Arcot et al., 2013).
56 In these cases, hybrid CNCs were found to be adsorbed in an upright orientation leading to cilia-
57 mimetic hairy surfaces. Additionally, recent works used thiol functionalized cellulose
58 nanocrystals for composite reinforcement (Li et al., 2018; Tao, Dufresne, & Lin, 2019).

59 In this work, we study the functionalization of cellulose nanocrystals from tunicate (t-CNC) at
60 their reducing ends by the introduction of poly(amidoamine) dendrimers (PAMAM). PAMAMs
61 are globular and flexible dendrimers with an ethylenediamine core as illustrated on Fig. 1. They
62 display several primary amine groups on their surface as well as tertiary amine and amide
63 groups in their core whose number increases with generation. They are then responsive to pH
64 changes thanks to the protonation/deprotonation of the amine groups (Lee, Athey, Wetzel,
65 Meixner, & Baker, 2002). Thanks to their pH-responsive properties PAMAM dendrimers have
66 found promising applications in the biomedical field, mostly as molecular vectors and
67 nanoreactors (Crooks, Zhao, Sun, Chechik, & Yeung, 2001; Esfand & Tomalia, 2001; Venditto,
68 Regino, & Brechbiel, 2005).



69
70 **Fig. 1.** PAMAM dendrimers chemical structures from generation 0 to generation 3

71
72 To the best of our knowledge, few reports on the functionalization of CNCs with PAMAMs are
73 reported. Dadkhah Tehrani and Basiryani studied the chemical surface modification of CNCs

74 from cotton to synthesize step-by-step PAMAM dendrimers up to the 3rd generation (Dadkhah
75 Tehrani & Basiryani, 2015). They showed that the rod-like shape of CNCs was preserved, and
76 that the presence of grafted PAMAMs, as small globular residues covering the CNC surface, was
77 detectable. Chen et al. grafted PAMAM of 6th generation on wood CNCs surface and showed
78 that their aggregation behavior depended on pH (L. Chen, Cao, Grishkewich, Berry, & Tam,
79 2015). Indeed, stable dispersions were obtained at very low or very high pH only to allow
80 electrostatic repulsions from positive or negative charges respectively. They further showed the
81 possibility to load gold nanoparticles in the PAMAMs grafted on CNCs leading to improved gold
82 catalysis activity (Li Chen, Cao, Quinlan, Berry, & Tam, 2015). Very recently, Tavakolian et al.
83 functionalized electrosterically stabilized nanocrystalline cellulose produced from softwood
84 kraft pulp at their both ends using PAMAM 5th generation (Tavakolian et al., 2019). They
85 showed that this system formed 3D structures whose shape depended on the PAMAM
86 concentration.

87 The goal of this work relies on the selective introduction of PAMAM functionalities at the
88 reducing ends of t-CNCs. By **selectively** grafting this functional dendrimer at the reducing end of
89 t-CNCs, we aim at combining the stretch-compress properties of PAMAMs with the surface
90 properties of cellulose nanorods. We focused on the first four generations of PAMAM
91 dendrimers, from a small molecule (G0) to a globular dendrimer (G3) in order to monitor the
92 properties as a function of **the** PAMAM generation. We **studied** the behavior (interactions and
93 assembly) of the obtained hybrid nanoparticles by **dynamic light scattering (DLS)**, quartz crystal
94 microbalance with dissipation (QCM-D) and surface plasmon resonance (SPR). **The pH-**

95 responsive properties of the PAMAM-modified t-CNCs adsorbed on the gold surface was
96 evaluated by QCM-D.

97

98 **2. EXPERIMENTAL**

99 *2.1. Materials*

100 Poly(amidoamine) dendrimers (PAMAM Dendrimer Kit, generations 0-3), N-(3-
101 dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS),
102 sodium chlorite (NaClO₂), potassium chloride (KCl), potassium hydroxide (KOH), sodium
103 hydroxide (NaOH), hydrochloric acid (HCl 37%), sulfuric acid (H₂SO₄) and acetic acid were
104 purchased from Sigma-Aldrich (France) and were used without further purification. Dialysis
105 membrane, molar mass cut off 12-14 kDa, was purchased from Spectrum Laboratories Inc.
106 Water was purified by Millipore Milli-Q purification system (18.2 MΩ).

107 *2.2. Methods*

108 *2.2.1. Cellulose nanocrystals preparation*

109 Tunicate cellulose nanocrystals (t-CNC) were obtained accordingly to our previous work
110 (Villares et al., 2018). Briefly, cellulose pulp from tunicate (*Styela clava*) was boiled in 1% NaOH
111 for 10 h and washed with milli-Q water. The pulp was further purified by a 0.3% NaClO₂
112 treatment at pH 4.9 for 2 h at 70 °C, and washed thoroughly in milli-Q water, similarly to
113 previously described procedures (Favier, Chanzy, & Cavaillé, 1995; Sugiyama, Persson, &
114 Chanzy, 1991). t-CNCs were then obtained by sulfuric acid hydrolysis. To a cooled suspension of
115 tunicate cellulose pulp in water, sulfuric acid was slowly added under vigorous mechanical
116 stirring to reach a final concentration of 65%. The dispersion was kept at 40 °C for 90 min under

117 stirring. The dispersion was cooled to 0°C, filtered over a 0.5 µm fritted glass filter, and washed
118 with milli-Q water until neutrality was reached. Dialysis (molar mass cut off 12-14 kDa) was
119 performed for 15 days. Note that t-CNCs are negatively charged due to sulfate groups at their
120 surface ($22\pm 1 \mu\text{mol g}^{-1}$ (Villares et al., 2018)) which gives a stable colloidal suspension.

121 *2.2.2. Introduction of PAMAM dendrimer at the reducing end of t-CNCs*

122 t-CNCs were functionalized at their reducing end in aqueous medium, following the procedure
123 previously described with minor modifications (Arcot et al., 2014). The aldehyde groups on the
124 reducing end of t-CNCs (50 mL at 6.9 g L^{-1}) were oxidized to carboxyl groups by the addition of
125 8.7 mmol of NaClO_2 , and the pH was adjusted to 3.5 using acetic acid, followed by stirring for 20
126 h at room temperature. The reaction mixture was purified by centrifugation (20000 g, 20°C, 30
127 min) to remove most of the chloric derivatives and then by dialysis (molar mass cut off 12-14
128 kDa) against milli-Q water for 6 days.

129 A 2 g L^{-1} suspension of t-CNC-COOH (5.8 mL) was degassed by bubbling nitrogen for 20 min, and
130 0.72 µmol of NHS were added, followed by 7.2 µmol of EDC, and the pH was checked to be
131 close to 6.5. Appropriate amounts of KCl were added such that the final suspension was 0.3 M.
132 PAMAM dendrimers were added in large excess to avoid the grafting of several t-CNCs on the
133 same dendrimer. Since the number of amine functions are increasing with the dendrimer
134 generation, decreasing amounts of PAMAMs were added, *i.e.* 72, 36, 18 and 9 µmol for
135 generations 0, 1, 2 and 3, respectively. Then, pH was checked to be more than 9.2. Note that
136 the addition of salt in the suspension would screen negative charges at the t-CNCs surface and
137 PAMAM dendrimers are not positively charged at pH above 9, which must limit PAMAM
138 unspecific adsorption on t-CNC surface. The reaction was incubated under stirring at room

139 temperature for 24 h. PAMAM-functionalized t-CNCs were purified by centrifugation (20000 g,
140 60 min, 20 °C) and then dialyzed (molar mass cut off 12-14 kDa) against Milli-Q water for 14
141 days. After coupling, hybrid nanoparticles are denoted t-CNC-Gx, x being the PAMAM
142 generation number.

143 *2.3 Characterization*

144 *2.3.1. Zeta potential (ρZ) and dynamic light scattering (DLS)*

145 ρZ and DLS experiments were performed with a Malvern NanoZS instrument. All measurements
146 were made at 20 °C with a detection angle of 12.8° and 173° for ρZ and DLS, respectively. The
147 correlogramms were recorded using Malvern DTS software. t-CNC and hybrid t-CNC-Gx
148 suspensions were diluted to 0.1 g L⁻¹ in pure water or in KOH 1 mM and then filtered through 5
149 μm .

150 *2.3.2. Scanning Transmission Electron Microscopy (STEM)*

151 t-CNC and hybrid t-CNC-Gx suspensions were diluted to 0.2 g L⁻¹ in pure water or in KOH 1 mM
152 and deposited on freshly glow-discharged carbon-coated electron microscope grids (200 mesh,
153 Delta Microscopies, France). After 1 min, the excess of solvent was removed by blotting
154 (Whatman filter paper). The grids were dried overnight in air at ambient temperature and then
155 coated with platinum layer by an ion-sputter coater (thickness = 0.5 nm). The grids were
156 observed with a Quattro scanning electron microscope (Thermo Scientific) with a STEM
157 detector, working at 10 kV.

158 *2.3.3. Quartz crystal microbalance with dissipation (QCM-D)*

159 The QCM-D measurements were performed with a Q-Sense E4 instrument (AB, Sweden) using a
160 piezoelectric AT-cut quartz crystal coated with gold electrodes on each side (QSX301, Q-Sense).

161 All measurements were carried out at 20 °C using the QCM flow cell modules. A baseline was
162 first established by continuously flowing KOH 1 mM solution on the quartz crystal surface, then
163 frequency and dissipation signals were off-set to zero just before injection of dispersions in a
164 continuous mode at a flow rate of 100 $\mu\text{L min}^{-1}$. t-CNC and t-CNC-Gx dispersions (0.3 g L^{-1} in
165 KOH 1 mM) and PAMAM G3 solution (0.001 g L^{-1} in KOH 1 mM) were injected into the QCM-D
166 cell until a plateau value of frequency and dissipation signals was reached. Then, a rinsing step
167 of the surface with KOH 1 mM was performed. For the study of the pH-response, after the
168 adsorption of t-CNC-Gx on the gold surface followed by the rinsing step, the pH was cycled
169 between pH 11 and pH 2 by the successive injection of aqueous solutions of KOH 1mM and HCl
170 10 mM, respectively. QCM-D experiments were repeated at least twice and average results of
171 two measurements are given.

172 Frequency ($\Delta f_n/n$) and dissipation (ΔD_n) changes were simultaneously registered at 5 MHz
173 fundamental resonance frequency and its several overtones as a function of time. Any material
174 adsorbed on the crystal surface induces a decrease of the resonance frequency $\Delta f_n/n$.
175 Dissipation signals (ΔD_n) were recorded simultaneously to frequency shifts and they provided a
176 measure of the frictional losses due to the viscoelastic properties of the adsorbed layer. The
177 overtone $n = 5$ was used for comparing the adsorption of unmodified and modified t-CNCs. If
178 the adsorbed mass is evenly distributed, rigidly attached and small compared to the mass of the
179 crystal, $\Delta f_n/n$ is directly proportional to the adsorbed mass per surface unit (Γ) using the
180 Sauerbrey's equation (Sauerbrey, 1959) :

181
$$\Gamma_{\text{QCM}} = - C \frac{\Delta f_n}{n} \quad (\text{Equation 1})$$

182 where C is the constant for the mass sensitivity of the quartz crystal ($0.177 \text{ mg m}^{-2} \text{ Hz}^{-1}$ at $f_0 = 5$
183 MHz) and n is the overtone number.

184 Sauerbrey's equation was used to estimate the deposited mass of t-CNC-Gx on the gold surface.
185 Indeed, the very low values of ΔD_n recorded combined to almost no separation of all overtones
186 signals (Fig. S2 to S5) suggested the formation of rigid layers on the gold surface, and validated
187 the use of the Sauerbrey's equation.

188

189 2.3.4. Surface Plasmon Resonance (SPR)

190 The SPR measurements were performed with a Biacore x100 (GE Healthcare, Sweden) using
191 gold substrates. All measurements were carried out at $20 \text{ }^\circ\text{C}$ with a 0.3 g L^{-1} suspension in KOH
192 1mM at $10 \text{ } \mu\text{L min}^{-1}$. Results are expressed as the mean of two experiments. Molecules
193 adsorbing on the surface change the solute and solvent concentration profiles and thus the
194 refractive index within the evanescent wave range, and the resonance angle increases ($\Delta\theta$).
195 From this angle, one can calculate the adsorbed mass per surface unit using the following
196 equation (Orelma, Filpponen, Johansson, Laine, & Rojas, 2011; Tulpar & Ducker, 2004;
197 Wintgens & Amiel, 2005):

$$198 \quad \Gamma_{\text{SPR}} = \frac{l_d}{2} \cdot \frac{\Delta\theta}{m \left(\frac{dn}{dc} \right)} \quad (\text{Equation 2})$$

199 Where l_d is the decay length of the evanescent electromagnetic field estimated as 0.37 of the
200 light wavelength ($\lambda_d = 760 \text{ nm}$), m is the calibration coefficient of the sensor ($m = 103.3^\circ$) and
201 the refractive index increment, dn/dc , is 0.15 mL g^{-1} for polysaccharides in water (Potthast et
202 al., 2015).

203

204 3. RESULTS AND DISCUSSION

205 3.1. Introduction of PAMAM dendrimers at reducing end of cellulose nanocrystals.

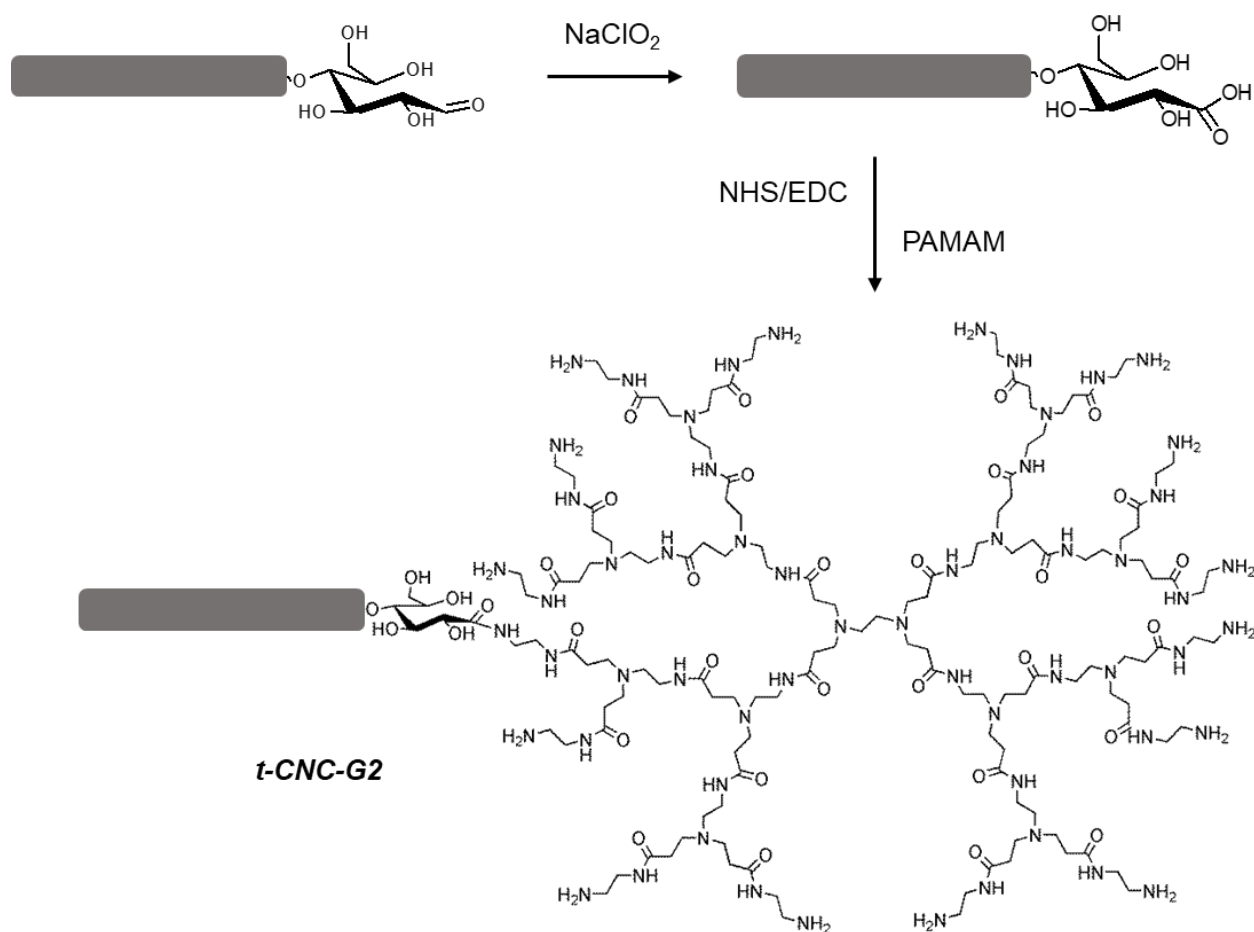
206 In this work, we focused on tunicate cellulose nanocrystals (t-CNC) as model nanorods because
207 of their high aspect ratio and rather homogeneous length distribution (Habibi et al., 2010).

208 Moreover, in contrast to cotton or wood CNCs, nanocrystals from tunicate are composed of a
209 single crystal displaying parallel arrangement of cellulose chains, which is of interest when
210 aiming to functionalize the reducing end. t-CNCs appeared as long rod-like nanocrystals, with an
211 average length of 1360 ± 630 nm and an average width of 11 ± 3 nm (Villares et al., 2018). They
212 were well individualized in water and there was no evidence of large-scale aggregation, as
213 previously described (Sacui et al., 2014; Zhao, Zhang, Lindström, & Li, 2015). The colloidal
214 suspension was stable in water because t-CNCs were negatively charged due to the sulfate
215 groups introduced during acid hydrolysis as confirmed by **their value of zeta potential around -**
216 **27 mV.**

217 t-CNCs were asymmetrically functionalized by introducing poly(amidoamine) dendrimers
218 (PAMAM) at their reducing ends. PAMAMs of 4 generations were compared, from a very small
219 molecule (G0) to a globular dendrimer (G3) as shown in Fig. 1. They had an ethylenediamine
220 core and multiple amine groups at their surface, whose number increases exponentially with
221 the PAMAM generation. Dendrimers structural parameters are summed up in Table S1.

222
223 The reactivity of PAMAM amine groups was used for coupling them to the oxidized reducing
224 ends of t-CNCs by amide bonds as illustrated in Fig. 2. Thus, at first, aldehyde groups of t-CNC
225 reducing ends were oxidized to carboxylic acid groups and, then, the PAMAM amine groups

226 reacted with the NHS/EDC activated carboxylic acid groups. Arcot *et al.* have also used this
227 strategy to introduce thiol moieties at the reducing ends of CNCs by using 6-amino-1-
228 hexanethiol. They showed that this method is a mild way for the synthesis of asymmetric CNCs
229 preventing their aggregation during the reaction in contrast to reductive amination (Arcot *et al.*,
230 2014). Unspecific adsorption of PAMAMs onto the t-CNC surface was **limited** since the reaction
231 was carried out in a high ionic strength media (0.3 M) and at basic pH over 9.2 to screen the
232 electrostatic interactions between the sulfate groups of t-CNCs and the amine groups of
233 PAMAMs.



234
235 **Fig. 2.** Schematic illustration of the synthetic protocol used to graft PAMAM G2 at the reducing end of t-
236 CNC, involving a first step of oxidation and a second step of carbodiimide coupling. Dimensions of t-CNC
237 (grey rectangles) and chemical structures are not at scale.

238

239 By specifically functionalizing the t-CNC reducing end, the number of PAMAM functional groups
240 is small compared to the number of glucose units in the cellulose nanocrystal, and common
241 techniques such as elementary analysis or infrared spectroscopy are not sensible enough to
242 detect the introduction of PAMAM functionalities (Kaldéus, Nordenström, Carlmark, Wågberg,
243 & Malmström, 2018; Villares et al., 2018; Zoppe et al., 2017). In a recent study, Heise *et al.*
244 employed advanced liquid-state NMR techniques to characterize CNCs after the introduction of
245 a β -diketon functionality at their reducing end (Heise et al., 2019). Nevertheless, the detection
246 of the reducing end modification required complete dissolution of CNCs in ionic liquids to
247 facilitate NMR signal resolution. This study demonstrated that the elucidation of the chemical
248 structure, and therefore, the detection of functionalities at the reducing ends of cellulose
249 nanocrystals is a real analytical challenge to consider. In our work, we demonstrated the
250 introduction of PAMAM functionalities at the reducing ends of t-CNCs by studying the
251 suspension stability and the behavior of the nanocrystals before and after PAMAM
252 functionalization in contact to gold surfaces as indirect proofs of successful PAMAM
253 hybridation.

254

255 *3.2. Stability of PAMAM functionalized t-CNC suspensions*

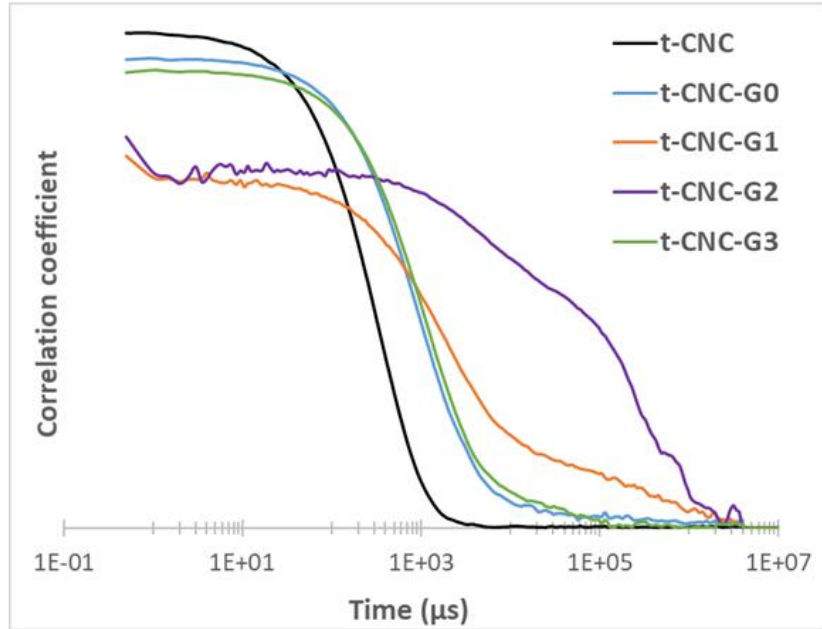
256 Regardless of pH, t-CNCs are negatively charged due to sulfate groups present on their surface.
257 Differently, the charge of PAMAMs is pH dependent since both primary and tertiary amine
258 groups are assumed to be unprotonated above pH 10 whereas they are fully protonated below
259 pH 4, and therefore, positively charged (Lee et al., 2002). In pure water, PAMAMs are then

260 partially positively charged and may interact with negatively charged t-CNCs leading to
261 aggregation (L. Chen et al., 2015). Therefore, to stabilize the suspension, it may be interesting
262 to work at basic pH to ensure the PAMAM neutrality. Indeed, the stability of the colloidal
263 suspensions of t-CNC-Gx was investigated in pure water and in KOH 1 mM (pH 11) by zeta
264 potential measurements and dynamic light scattering (DLS). DLS measures the diffusion of
265 nanoparticles due to the Brownian motion and thus the rate at which the intensity of scattered
266 light fluctuates in time is monitored. Then, the correlation function of the scattered intensity is
267 used to illustrate the sample state in terms of mean size, dispersion and aggregation state.
268 Indeed, scattered signal of aggregates fluctuates slower than dispersed particles, and the
269 suspensions can be characterized by plotting the autocorrelation function as a function of time
270 (correlogram). Then, longer delay times indicate larger particles and a steeper slope indicates a
271 more monodisperse sample. Thus, comparison of the correlograms is a qualitative mean to
272 compare the aggregation state of the different modified nanorods. The zeta potentials (pZ) and
273 the corresponding correlograms of t-CNC-Gx suspensions, as well as photographs of t-CNC-G2
274 suspensions, either in water or in KOH 1mM are displayed in Fig. 3. **Photographs of the four t-**
275 **CNC-Gx suspensions either in water or in KOH 1mM are displayed in Fig. S1.**

276

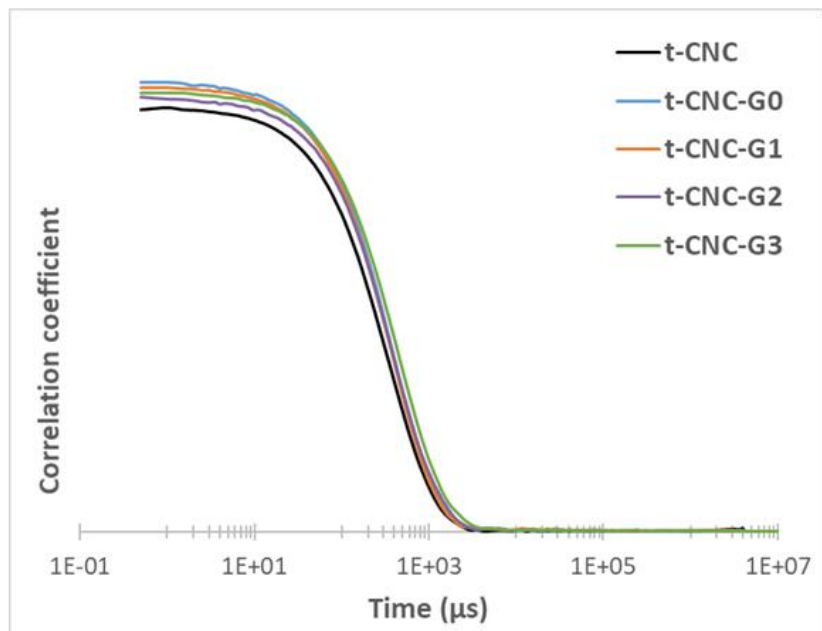
A	pZ (mV)
t-CNC	-27.4 ± 2.5
t-CNC-G0	-16.5 ± 0.8
t-CNC-G1	-18.2 ± 1.0
t-CNC-G2	15.1 ± 1.0
t-CNC-G3	32.1 ± 0.7

t-CNC-G2 at 0.3 g L⁻¹



B	pZ (mV)
t-CNC	-26.8 ± 2.1
t-CNC-G0	-30.4 ± 1.0
t-CNC-G1	-31.8 ± 1.3
t-CNC-G2	-29.0 ± 1.2
t-CNC-G3	-22.9 ± 1.8

t-CNC-G2 at 0.3 g L⁻¹



277

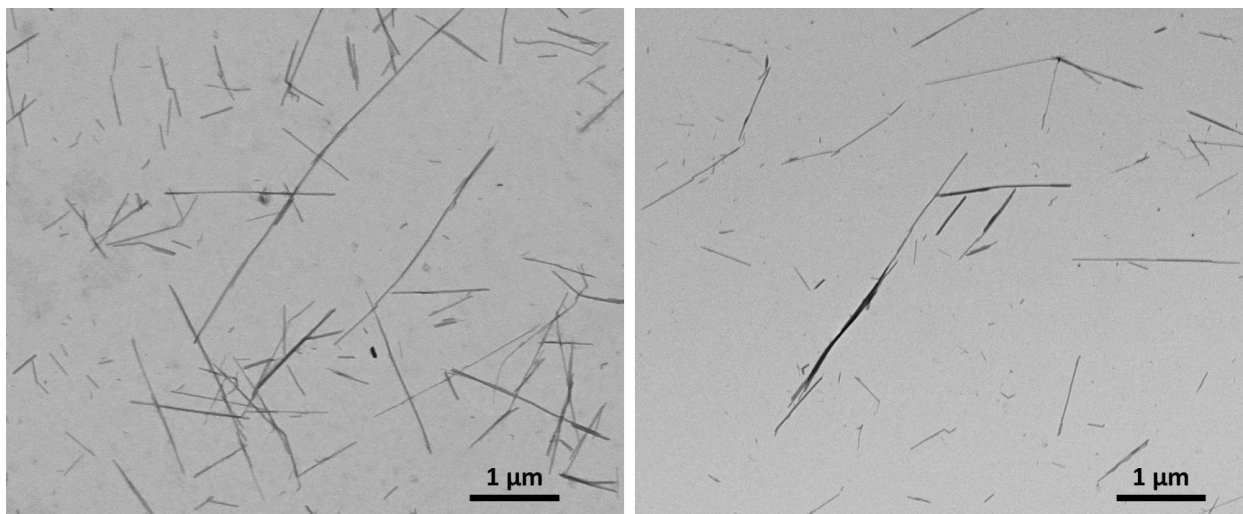
278 **Fig. 3.** Zeta potential (pZ) values and DLS correlograms for t-CNCs before and after PAMAM
 279 functionalization at 0.1 g L^{-1} in A. water and B. KOH 1 mM. Additionally **photographs of tubes containing**
 280 t-CNC-G2 **suspensions** at 0.3 g L^{-1} in A. water and B. KOH 1mM are shown.

281

282 In the case of t-CNC and t-CNC-Gx in pure water (Fig. 3A), the introduction of PAMAM at the
 283 reducing end produced a remarkable effect on correlation coefficients. Correlograms showed

284 three different aggregation states. The far most aggregated sample was t-CNC-G2 followed by
285 the t-CNC-G1 hybrids, which were significantly more aggregated than t-CNC-G0 and t-CNC-G3.
286 Thus, the aggregation is likely due to the interaction between positively charged amine groups
287 of PAMAMs and the negatively charged t-CNCs. Therefore, the aggregation state depends on
288 the PAMAM generation, which determines the number of amine groups and therefore the
289 balance between negative and positive charges. In order to give more insight into the charge
290 balance, the zeta potential of the t-CNC-Gx suspensions was determined. *pZ* provides an
291 indirect measurement of the “net charges” on the aggregated particles surface by measuring
292 the zeta potential at the interface between the electrical double layer of particles and water.
293 For t-CNC-G0 and t-CNC-G3, the lower extent of aggregation can be justified by the charge
294 imbalance. Thus, in the case of t-CNC-G0, the number of amine groups from PAMAMs is so
295 small that the sulfate groups from t-CNCs ensure the suspension stabilization. This assumption
296 was demonstrated by the negative values of zeta potential. Differently, for t-CNC-G3, the
297 positive values of zeta potential suggested that the number of amine groups from PAMAMs is
298 high compared to the number of sulfate groups from t-CNCs, which ensures colloidal
299 stabilization by the positive charges. In the case of t-CNC-G1 and t-CNC-G2 there was a drastic
300 change towards a more pronounced aggregation state, as illustrated **on the photograph of the**
301 **t-CNC-G2 suspension in water (Fig. 3A)**. A fair hypothesis would be that PAMAMs G1 and G2
302 had an intermediate charge density whose value promotes a significant aggregation through
303 electrostatic interactions. Moreover, some of the PAMAM charges could be screened inside the
304 aggregates, which explains a non-linear increase of *pZ* values with PAMAM generation.

305 To ensure that these aggregation states were due to charged PAMAMs, we then studied the
306 suspensions of t-CNC and t-CNC-Gx in KOH 1 mM at 0.1 g L^{-1} (Fig. 3B). At pH 11 amine groups
307 from PAMAMs are not charged and no electrostatic attraction would occur between PAMAMs
308 and t-CNCs. The correlograms of all t-CNC-Gx were very similar to the one of unmodified t-CNC,
309 which confirmed that hybrid nanoparticles were well-dispersed in KOH 1 mM (no aggregation)
310 as seen on the photograph of the t-CNC-G2 suspension in KOH 1 mM (Fig. 3B). The pZ values of
311 all suspensions were then close to the one of unmodified t-CNCs confirming that amino groups
312 were not protonated. The t-CNC-Gx suspensions were therefore stabilized by electrostatic
313 repulsions between nanocrystals, **as shown by the visual examination of the suspensions**
314 **(photographs on Fig. S1)**. The individualization of t-CNC-Gx in KOH 1 mM was confirmed by
315 scanning transmission electron microscopy (STEM). Representative images of every t-CNC-Gx
316 suspension either in pure water or in KOH 1 mM are presented on Fig. S2. The morphology
317 comparison between t-CNC before and after PAMAM coupling confirmed first that t-CNC-Gx
318 were well dispersed in KOH 1 mM and then that the PAMAM functionalization did not affect
319 the t-CNC morphology. Representative STEM images of hybrid t-CNC-PAMAM (t-CNC-G2)
320 compared to native t-CNC in KOH 1 mM are shown in Fig. 4. Chen *et al.* introduced PAMAM
321 dendrimers at the surface of CNCs and showed that their suspensions was stable either at acidic
322 or basic pH (L. Chen et al., 2015). In our case, it is interesting to notice that despite the very
323 small amount of introduced amine groups at the **nanocrystal end**, the aggregation state
324 depends on pH. Therefore, we decided to work in KOH 1 mM to have individualized hybrid CNC-
325 PAMAM particles and to study their adsorption behavior on gold surface. This aqueous solvent
326 have the advantage to be fully compatible with QCM-D and SPR measurements.

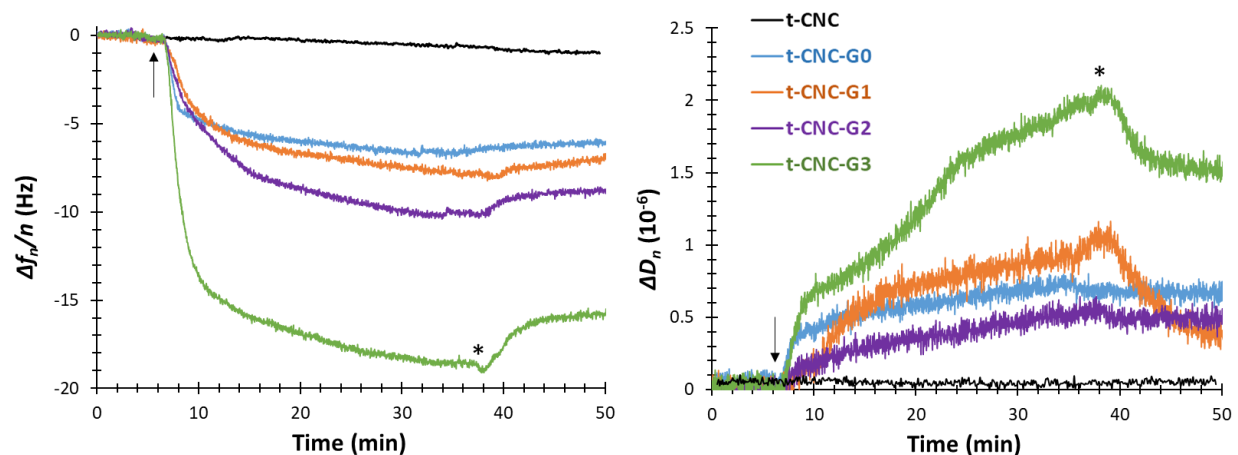


327
328 **Fig. 4.** Representative STEM images of native t-CNC (left) and t-CNC-G2 (right) at 0.2 g L^{-1} in KOH 1 mM
329

330 3.3. Adsorption of PAMAM functionalized t-CNCs on gold surface

331 The affinity of amine groups for gold was used to adsorb t-CNC-Gx on gold surface. Their
332 adsorption behavior was investigated by quartz crystal microbalance with dissipation (QCM-D)
333 and surface plasmon resonance (SPR). The objective of this set of experiments was to
334 demonstrate the selective binding of PAMAM functionalized t-CNCs on gold and to further
335 study their adsorption behavior. Moreover, the comparison between QCM-D and SPR allows
336 the calculation of the amount of water trapped in the deposited layer (Eronen, Junka, Laine, &
337 Österberg, 2011; Hedin, Löfroth, & Nydén, 2007; C. Wang et al., 2012).

338 Firstly, t-CNC-Gx suspensions in KOH 1 mM were studied by QCM-D and the changes in
339 frequency and dissipation were monitored upon time (Fig. 5). The changes of $\Delta f_n/n$ and ΔD_n
340 signals for overtone numbers $n = 3, 5, 7, 9, 11$ and 13 for t-CNC-G0, t-CNC-G1, t-CNC-G2 and t-
341 CNC-G3 upon time are depicted on Fig. S3, S4, S5 and S6, respectively.



342
 343 **Fig. 5.** Frequency ($\Delta f_n/n$) and dissipation (ΔD_n) changes for the overtone $n = 5$ monitored by QCM-D
 344 during adsorption of t-CNC and t-CNC-Gx ($x = 0, 1, 2, 3$) at 0.3 g L^{-1} in KOH 1 mM. The arrow indicates the
 345 injection of t-CNCs and the asterisk the rinsing step with KOH 1 mM.
 346

347 When t-CNC-Gx were injected, changes in frequency and dissipation were observed, indicating
 348 adsorption onto the gold surface. Since unmodified tunicate nanocrystals did not adsorb
 349 onto gold substrates due to the repulsion between the anionic sulfate groups and the
 350 negatively charged gold surfaces (black line on Fig. 5), then the adsorption of t-CNC-Gx was due
 351 to PAMAM amine moieties. The irreversibility of the binding was assessed by rinsing with KOH 1
 352 mM, which did not induce significant changes nor in frequency nor in dissipation. QCM-D
 353 results showed that the frequency decrease and dissipation increase values are in agreement
 354 with Arcot *et al.* who described the chemisorption of CNCs on gold by their thiol-functionalized
 355 reducing end (Arcot *et al.*, 2014, 2013). Moreover, the changes observed with t-CNC-Gx
 356 adsorption increased with PAMAM generation. More specifically, a gap was observed between
 357 generations G2 and G3. For $x = 0, 1, 2$, the changes in frequency were very close, between -6
 358 and -9 Hz, as well as the changes in dissipation between 0.4×10^{-6} and 0.7×10^{-6} , while for
 359 **generation** $x = 3$ the changes in frequency and dissipation were much higher, about -16 Hz and
 360 $1.5 \cdot 10^{-6}$ respectively. This non-linearity in frequency and dissipation changes may not only

361 suggest a higher adsorbed mass for t-CNC-G3 but also a difference in the adsorption process.

362 The very low dissipation values obtained for $x = 0, 1$ and 2 may also indicate that the amount of

363 adsorbed nanoparticles is too low for completely covering the gold surface. For t-CNC-G3, the

364 amount of adsorbed nanoparticles may be high enough to form a continuous layer that would

365 lead to the increase in dissipation change, in line with the slight separation of overtones for

366 dissipation signals observed only for t-CNC-G3 (Fig. S3 to S6).

367 Deeper QCM-D results analysis can lead to the determination of the kinetic constant of the

368 adsorption process. In a previous study (Chemin, Moreau, Cathala, & Villares, 2020), we

369 detailed a kinetic modeling to determine kinetic constants based on the collision between a

370 particle and a surface (supplementary material), which is valid independently of the particles

371 content in the system (Alinec, Petlicki, & van de Ven, 1991). Briefly, the frequency values

372 obtained by QCM-D were transformed onto surface coverage by using the Sauerbrey's

373 expression (Equation 1 in section 2.3.3.). The experimental fractional coverage values as a

374 function of time are then plotted as shown in Fig. 6. The fit of these data allowed the

375 calculation of the apparent kinetic constant given by $\alpha_0 k_{12} S$ where α_0 is the collision rate

376 efficiency factor, k_{12} is the rate constant and S is the initial number of surfaces per unit volume.

377 Plots on Fig. 6 showed a good fit between the theoretical prediction (Equation S3) and the

378 measured data for all the t-CNC-Gx studied. Values obtained for the kinetic constant $\alpha_0 k_{12} S$

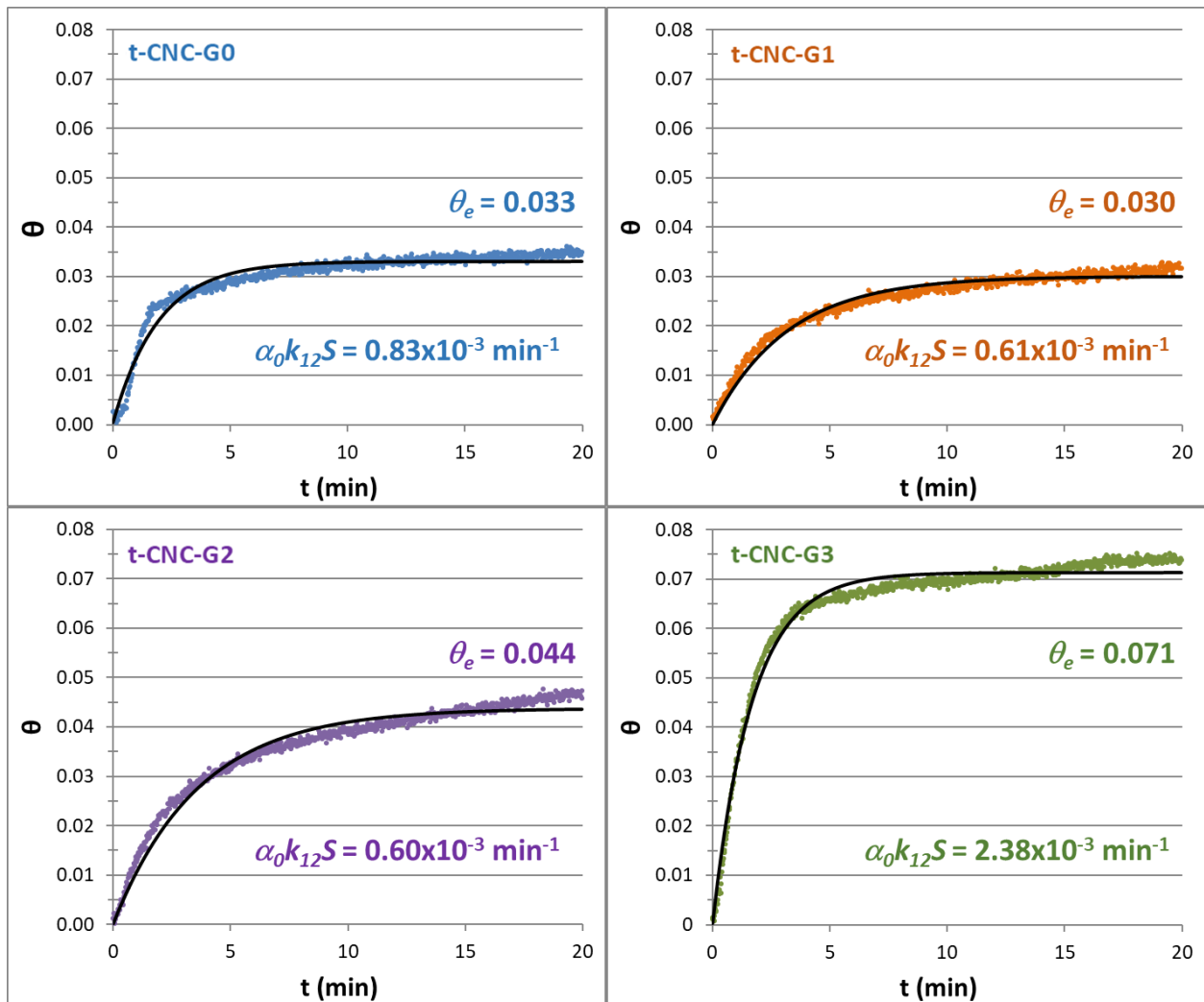
379 confirmed a different adsorption behavior between the three first t-CNC-Gx ($x = 0, 1, 2$) and t-

380 CNC-G3 with a drastic change in the kinetic constant increasing from $0.6/0.8 \cdot 10^{-3} \text{ min}^{-1}$ to

381 $2.4 \cdot 10^{-3} \text{ min}^{-1}$ (Fig. 6). PAMAM G3 is the biggest globular dendrimer used that has 32 amino

382 groups with most of them accessible to adsorb on the gold surface, so that the collision rate

383 efficiency factor may be higher. QCM-D results showed a breaking point in the adsorption
 384 behavior that occurred between t-CNC-G2 and t-CNC-G3. Results suggested that PAMAM G3
 385 had a critical size, which marked a change in properties.



386
 387 **Fig. 6.** Fractional surface coverage for t-CNC-Gx as a function of time. The colored dots correspond to
 388 experimental data fitted by the Sauerbrey model and the solid black lines represent the fit to Equation
 389 S3, allowing the calculation of the **apparent kinetic constant $\alpha_0 k_{12} S$** and the **equilibrium fractional**
 390 **surface coverage θ_e .**

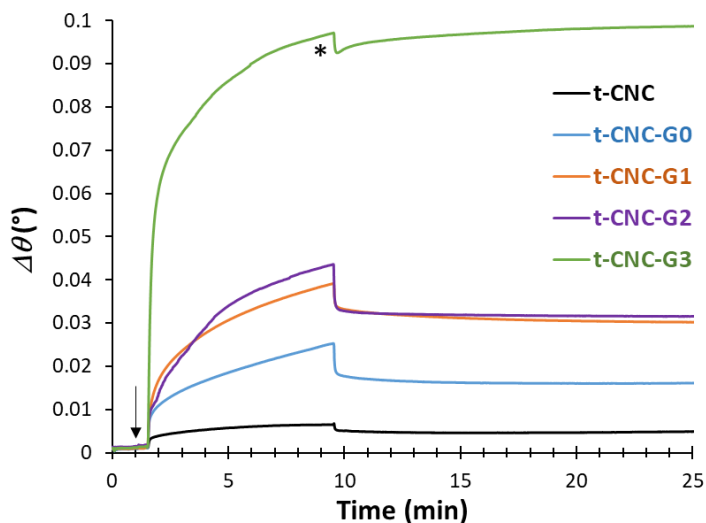
391
 392 In an attempt to obtain more insight into the adsorption process of t-CNC-Gx, their adsorption
 393 onto gold surfaces was then studied by SPR. The SPR technique is based on the resonant

394 oscillation of electrons occurring at the interface between a conductive metal and dielectric
395 fluid when stimulated by incident light. As these oscillations are very sensitive to any change at
396 the interface, the adsorption of molecules onto the conducting surface can be monitored. The
397 mechanism of detection is based on changes in the local index of refraction by the adsorbing
398 molecules, changing the resonance conditions of the surface plasmon waves and the angle of
399 minimum reflection ($\Delta\theta$) (Ahola, Myllytie, Österberg, Teerinen, & Laine, 2008). The angle shift
400 was then monitored upon time (Fig. 7) and allowed to calculate the deposited mass according
401 to calculations detailed in the experimental section (Equation 2 in section 2.3.4.). The
402 adsorption of t-CNC-Gx monitored by SPR gave similar trends than QCM-D, the amount of
403 adsorbed t-CNC-Gx increased with PAMAM generation. The same gap was observed between
404 generations G2 and G3 with a much higher adsorbed mass of 88 ng cm^{-2} on gold surface for t-
405 CNC-G3. For a molecular layer, the shift in the SPR angle is usually referred as the “optical
406 thickness” and does not take the water mass into account (Schoch & Lim, 2013). Therefore,
407 combining SPR and QCM experiments, the amount of trapped water in the adsorbed layer can
408 then be calculated as (C. Wang et al., 2012) (see results in Fig. 7) :

$$409 \quad \% \text{ water} = \left[1 - \left(\frac{\Gamma_{\text{SPR}}}{\Gamma_{\text{QCM}}} \right) \right] \times 100 \quad (\text{Equation 3})$$

410 For all t-CNC-Gx, the amount of trapped water was high, between 68% and 87%. The lowest
411 water amount was obtained with t-CNC-G3. In fact, the SPR results indicated that the mass
412 adsorption of t-CNC-G3 was 6 times higher than t-CNC-G0, and 3 times higher than t-CNC-G1
413 and t-CNC-G2. The increase in PAMAM generation seemed to favor the adsorption of PAMAM-
414 functionalized t-CNC, and a higher number of hybrid t-CNC were adsorbed onto the gold

415 surface. This fact could be justified by the increasing number of anchoring groups, so the
 416 interaction of the amino groups with the gold surface could result in a rather flat conformation
 417 of PAMAM, which may favor water expulsion.

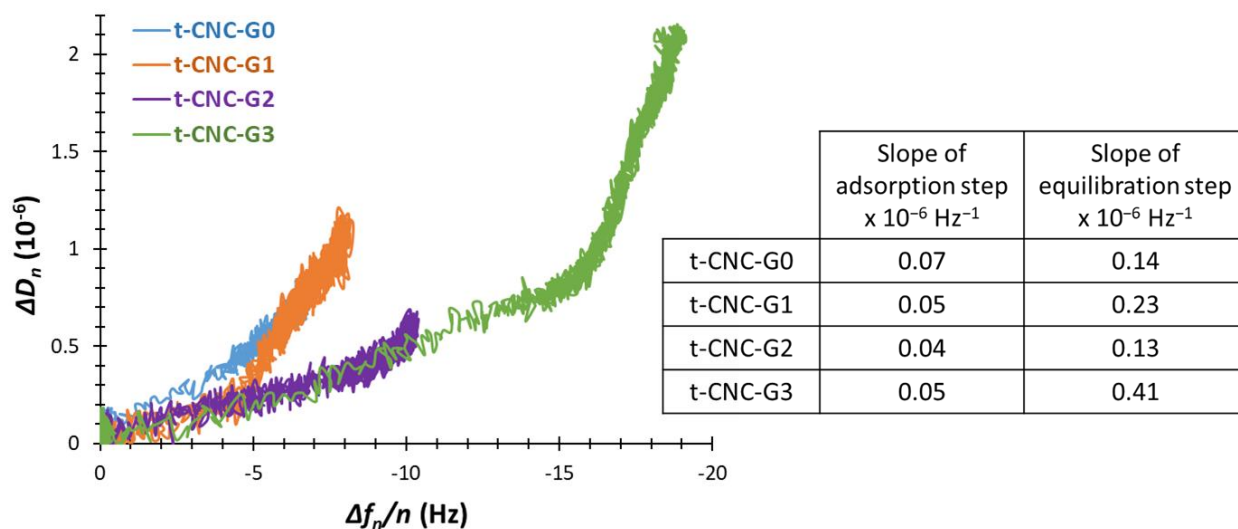


	Γ_{SPR} (ng cm ⁻²)	Γ_{QCM} (ng cm ⁻²)	% water
t-CNC-G0	13.7	105.2	87.0
t-CNC-G1	26.5	117.6	77.5
t-CNC-G2	27.1	154.6	82.5
t-CNC-G3	88.4	276.4	68.0

418
 419 **Fig. 7.** Angle changes ($\Delta\theta$) monitored by SPR during adsorption of t-CNC and t-CNC-Gx (x = 0, 1, 2, 3) at
 420 0.3 g L⁻¹ in KOH 1 mM. The arrow indicates the injection and asterisk the rinsing step; and the respective
 421 calculated masses adsorbed using SPR and QCM experiments to obtain the amount of trapped water
 422

423 3.4. Viscoelastic properties of t-CNC-Gx adsorbed on gold surfaces

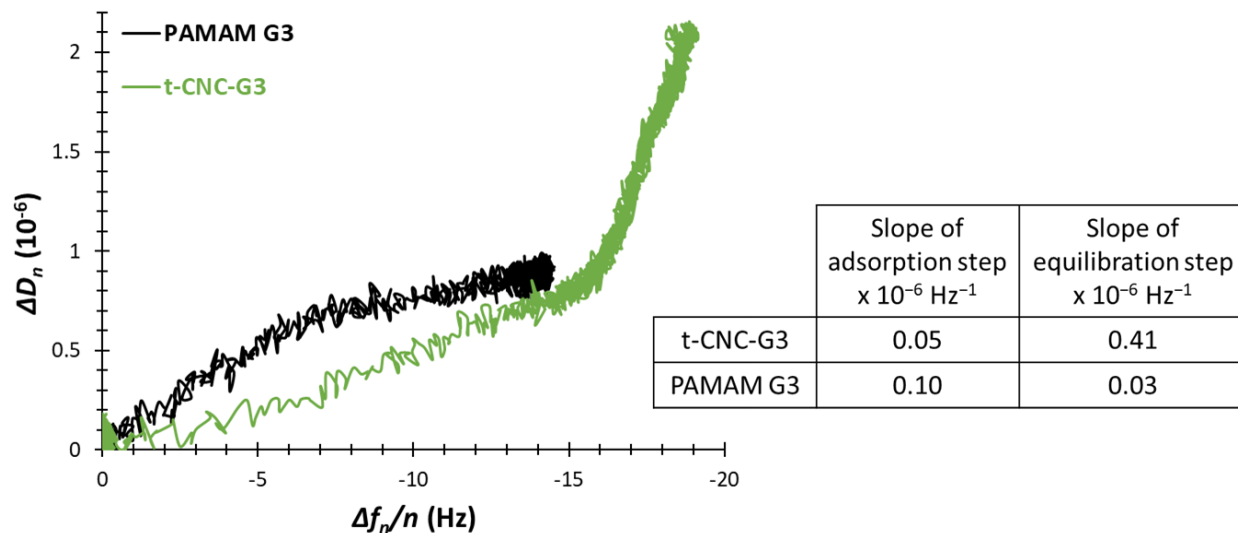
424 QCM-D allows investigating the arrangement of hybrid t-CNCs onto the solid surfaces as well as
 425 the properties of the adsorbed layers. For that purpose, the changes in ΔD_n were plotted as a
 426 function of $\Delta f_n/n$ (Fig. 8). The slope of the ΔD_n vs $\Delta f_n/n$ plot reveals at any point of the curve
 427 how much dissipation is induced by frequency unit allowing a comparison of the viscoelasticity
 428 characteristics of the deposited layers (Ahola, Salmi, Johansson, Laine, & Österberg, 2008;
 429 Rodahl et al., 1997). Thus, the absolute slope and changes in the slope in the plots provide
 430 information about the adsorption regimes and conformation changes (Amirkhani, Volden, Zhu,
 431 Glomm, & Nyström, 2008).



432
 433 **Fig. 8.** Change in dissipation as a function of the change in frequency ($\Delta D_n - \Delta f_n/n$) for the overtone $n = 5$
 434 corresponding to t-CNC-Gx at 0.3 g L^{-1} in KOH 1 mM adsorbed onto gold surfaces; and the respective
 435 calculated slopes ($\times 10^{-6} \text{ Hz}^{-1}$)
 436

437 For the adsorption of t-CNC-Gx on gold, ΔD_n increased linearly with increasing frequency but
 438 two different slopes occurred, which suggested that nanocrystals did undergo orientation
 439 changes in the course of the adsorption process (Amirkhani et al., 2008). After the adsorption
 440 step - which exhibit a very small slope between 0.04×10^{-6} and $0.07 \times 10^{-6} \text{ Hz}^{-1}$ according to the
 441 $\Delta D_n - \Delta f_n/n$ plot - occurred an equilibration step having a higher slope. For the three first hybrids
 442 having the PAMAM from G0 to G2, the slope of the equilibrium step was between 0.13×10^{-6}
 443 and $0.23 \times 10^{-6} \text{ Hz}^{-1}$. The obtained values were in agreement with the work of Arcot *et al.*, which
 444 described the chemisorption of thiol-functionalized CNCs in an upright orientation **by the**
 445 **increase in the $\Delta D_n - \Delta f_n/n$ slope compared to the adsorption in a flat orientation (Arcot et al.,**
 446 **2013). The increase of the $\Delta D_n - \Delta f_n/n$ slope up to $0.41 \times 10^{-6} \text{ Hz}^{-1}$ was then more marked in the**
 447 **case of t-CNC-G3.** PAMAM G3 is the largest dendrimer studied, and the size and flexibility of
 448 poly(amidoamine) branches could provide enough freedom to the hybrid nanorods to
 449 straighten. Therefore, hybrid t-CNCs would bond to the gold surface in a rather upright

450 orientation with the non-reducing end facing up. In order to support this hypothesis, the
451 adsorption of PAMAM G3 dendrimer on gold was also monitored by QCM-D and the changes in
452 ΔD_n were plotted as a function of $\Delta f_n/n$ (Fig. 9). The adsorption behavior of PAMAM G3 was
453 very different compared to t-CNC-G3. Two regimes were also observed but the slope of the
454 equilibration step ($0.03 \times 10^{-6} \text{ Hz}^{-1}$) was smaller than the one of the adsorption step (0.10×10^{-6}
455 Hz^{-1}). During adsorption, the $\Delta D_n - \Delta f_n/n$ slope of PAMAM G3 was slightly higher than the slope
456 of t-CNC-G3 because nanocrystals may have a stiffening effect. After the adsorption, the
457 decrease of the slope for PAMAM G3 indicated a conformation change that may induce a
458 thinner and/or more rigid layer (X. Wang, Ruengruglikit, Wang, & Huang, 2007; Zhou et al.,
459 2004). PAMAM dendrimers may maximize their interactions with the surface leading to a
460 flattening effect. Indeed, Mecke *et al.* studied the deformation of PAMAMs from G2 to G5 on
461 mica surfaces and showed that flattening of dendrimers occurred at the liquid-solid interface
462 with multiple interaction sites between the branched ends and the substrate (Mecke, Lee,
463 Baker, Holl, & Orr, 2004). These data could explain that the increase of the slopes during the
464 equilibrium step of t-CNC-Gx adsorption was due to the cellulose nanorods, which must
465 reorganize as a hairy layer. Indeed, hybrid t-CNCs benefited enough freedom thanks to PAMAM
466 dendrimer as attachment point to adopt an upright orientation and repelled each other
467 electrostatically due to the presence of sulfate groups along the nanocrystal surface. This
468 phenomenon was more noticeable in the case of t-CNC-G3 thanks to the higher PAMAM size
469 and flexibility.
470

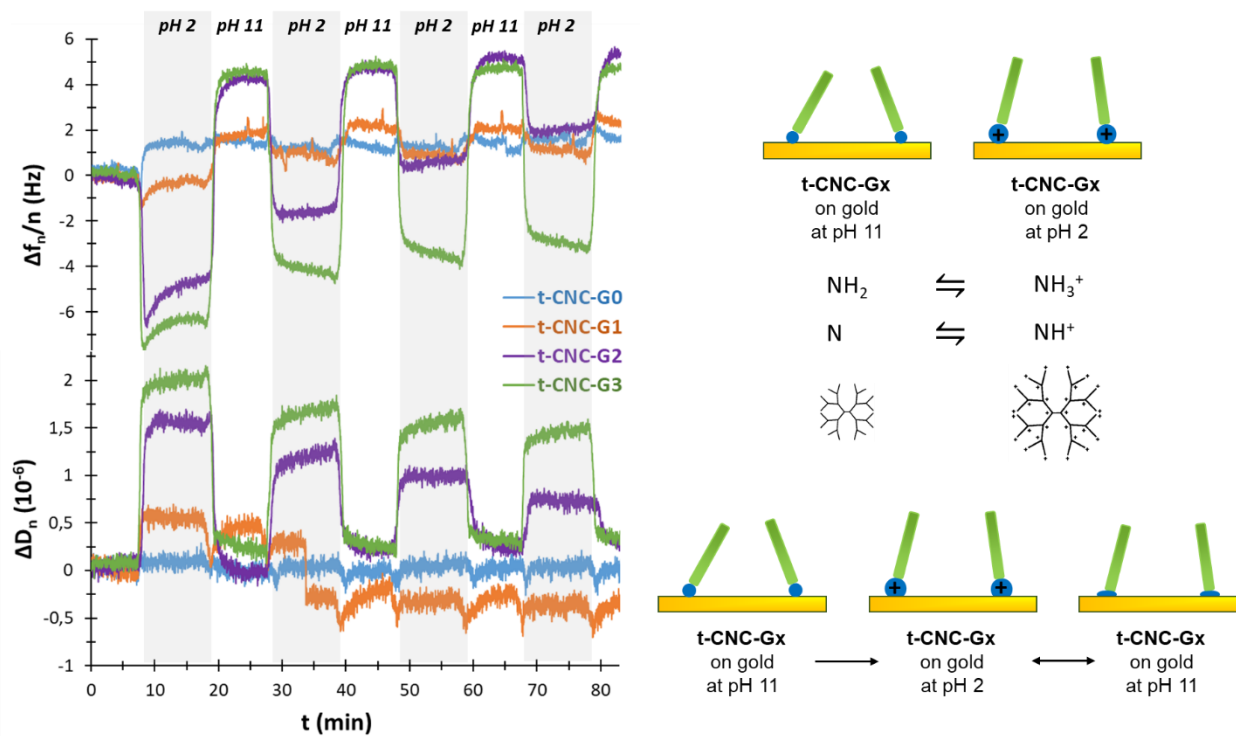


471
 472 **Fig. 9.** Change in dissipation as a function of the change in frequency ($\Delta D_n - \Delta f_n/n$) for the overtone $n = 5$
 473 corresponding to t-CNC-G3 at 0.3 g L^{-1} in KOH 1 mM (green) and to PAMAM G3 at 0.001 g L^{-1} in KOH 1
 474 mM (black) adsorbed onto gold surfaces; and the respective calculated slopes ($\times 10^{-6} \text{ Hz}^{-1}$) which are
 475 indicated with dashed lines.

476

477 3.5. pH response of t-CNC-Gx adsorbed layers on gold surfaces

478 One of the particularities of anchoring PAMAM at the reducing end of t-CNC was the
 479 introduction of multiple amine groups that are sensitive to pH. The protonation of the amine
 480 groups at low pH leads to electrostatic repulsions between charged groups and their
 481 subsequent separation, which results in water uptake. The swelling behavior of t-CNC-Gx layers
 482 at different pHs was investigated by QCM-D as is was shown to inform about polymers
 483 conformational modifications upon pH changes (Howard, Craig, Fitzgerald, & Wanless, 2010).
 484 After the adsorption of t-CNC-Gx as previously described, four solvent exchange – from KOH 1
 485 mM to HCl 10 mM – were performed and the changes in frequency and dissipation were
 486 recorded (Fig. 10).



487

488 **Fig. 10.** Change in frequency and in dissipation as a function of time for the overtone $n = 5$
 489 corresponding to t-CNC-Gx adsorbed on gold surfaces, submitted to solvent exchange from KOH 1 mM
 490 to HCl 10 mM (4 consecutive times) showing swelling as illustrated on the right.

491

492 In the case of t-CNC-G0, the grafted PAMAM G0 was too small to clearly respond to pH
 493 changes. Both frequency and dissipation showed only slight variations. Differently, starting
 494 from PAMAM G1 the response to pH increased with the PAMAM generation-Gx. When the solvent
 495 was changed from alkaline to acidic pH, from KOH 1 mM to HCl 10 mM respectively, a
 496 spectacular decrease in frequency concomitant to an increase in dissipation occurred, which
 497 was consistent with swelling. Indeed, PAMAM move from a globular and loosely compact
 498 structure at pH 11 to an highly extended one at pH 2 with amine groups protonation and water
 499 uptake (Lee et al., 2002). This was confirmed by the separation of the overtones only at pH 2 (t-
 500 CNC-G3 on Fig. S7) showing a more viscoelastic behavior thanks to swelling. When the pH was
 501 changed back to alkaline, an increase in frequency concomitant to a decrease in dissipation

502 occurred, showing reversibility, and the swelling and the deswelling processes occurred at
503 similar rate. Comparing t-CNC-G2 and t-CNC-G3, it is very interesting to note that their $\Delta f_n/n$
504 and ΔD_n values in KOH 1 mM were similar while they differed in HCl 10 mM. This phenomenon
505 corroborated the Lee's et al. study, who explained using computer simulations that at high pH,
506 the atom density distribution is conserved across generations while at low pH, the interior
507 hollow spaces of PAMAM increased with generation grows (Lee et al., 2002).

508 After the first solvent exchange, it seemed that even more water was expelled from the layer
509 since during the second cycle in KOH 1 mM, the frequency plateau value was higher than at the
510 beginning of the experiment. The changes in ΔD_n were plotted as a function of $\Delta f_n/n$ for each
511 solvent exchange (Fig. S8). Interestingly, these plots revealed that the first solvent exchange
512 (orange dots on Fig. S8) was shifted from all the others (gray dots on Fig. S8), showing that the
513 first change in conformation was non-reversible. The swelling occurring in HCl 10 mM must
514 increase the PAMAM area in contact with the gold surface, and therefore the interactions
515 between charged amine moieties of PAMAM and gold increased. These new interactions may
516 be strong enough to resist to the next solvent exchange. Back in KOH 1 mM, the PAMAM
517 deswelling may then lead to a flatter and more rigid PAMAM anchoring as illustrated on Fig. 10,
518 explaining the higher frequency plateau value in KOH 1 mM for the following cycles. The
519 reversibility of this phenomenon was evaluated by repeating cycles between alkaline and acidic
520 pH several times. After the first solvent exchange, this phenomenon is remarkably reversible
521 and reproducible (within instrumental drift) but the intensity of the t-CNC-Gx response to pH
522 was decreased. There might be a reinforcement of the PAMAM interactions with gold during

523 multiple cycles explaining the decreasing PAMAM response to pH. The PAMAM generation
524 used could then allow tuning the pH response of these functional materials.

525

526 **4. CONCLUSION**

527 In this work, bio-based materials were obtained by grafting PAMAM dendrimers at the reducing
528 end of tunicate cellulose nanocrystals in mild conditions. These hybrid asymmetric
529 nanoparticles showed that the PAMAM functionalities led to a reducing end-driven adsorption
530 on gold. We succeeded in the formation of hairy layers combining the properties of rigid rod-
531 like nanocrystals to globular and flexible PAMAM dendrimers. Multiple solvent exchange from
532 pH 11 to pH 2 showed a remarkable reversible response to pH of these functional materials.

533 The size of the PAMAM dendrimer determined the arrangement of the adsorbed layer, and the
534 change from PAMAM G2 to PAMAM G3 clearly modified the adsorbed mass, water content and
535 pH-responsive properties.

536 This work may bring new opportunities for the development of pH-responsive hairy surfaces,
537 since it demonstrates that properties can be tuned by changing the PAMAM generation.

538

539 **APPENDIX. Supplementary Material**

540

541 **Author Contributions**

542 AV and MC designed the experimental plan and conducted project development. MC
543 completed the experimental work and data analyses. AV, BC, and CM conceived the project and

544 contributed to data analysis, especially the QCM-D. MC prepared the manuscript and all
545 authors read, revised and approved the submitted version.

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548 **Conflict of Interest**

549 The authors declare that the research was conducted in the absence of any commercial or
550 financial relationships that could be construed as a potential conflict of interest.

551

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558

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