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1 **Ice-templated freeze-dried cryogels from tunicate cellulose nanocrystals with**
2 **high specific surface area and anisotropic morphological and mechanical**
3 **properties**

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10

11 **Abstract**

12 High aspect ratio cellulose nanocrystals (CNCs) extracted from tunicate were used to create so-
13 called cryogels from an ice-templating directional freeze-drying process. The structure of the
14 resulting solid foam was investigated at the micro- and nanoscales by scanning electron microscopy
15 and nitrogen adsorption measurements were used to extract the specific surface area. The
16 mechanical properties were probed by compression tests. To highlight the specificities of tunicate
17 CNC-based cryogels, results were compared with the one obtained from two other types of
18 nanocellulose, namely cellulose nanofibrils (CNFs) from wood and cellulose nanocrystals (CNCs) from
19 cotton, which exhibit different dimensions, aspect ratio, flexibility and crystallinity. While CNF- and
20 cotton CNC-based cryogels exhibited a classical morphology characterized by a sheet-like structure, a
21 particular honeycomb organization with individual particles was obtained in the case of tunicate CNC
22 cryogels. The latter cryogels presented a very high specific surface area of about 122 m²/g, which is
23 unexpected for foams prepared from a water-based process and much higher than what was
24 obtained for CNF and cotton CNC cryogels (25 and 4 m²/g, respectively). High mechanical resistance
25 and stiffness were also obtained with such tunicate CNC cryogels. These results are explained by the
26 high crystallinity, aspect ratio and rigidity of the tunicate CNCs combined with the particular
27 honeycomb architecture of the cryogel.

28 **Keywords:**

29 cryogel, freeze-drying, nanocellulose, tunicate CNCs

30 **Abbreviations:**

31 CNFs: cellulose nanofibrils

32 CNCs: cellulose nanocrystals

33 SEM: scanning electron microscopy

34 TEM: transmission electron microscopy
35 BET: Brunauer, Emmett, Teller
36 DLS: dynamic light scattering
37 NMR: nuclear magnetic resonance
38 NLDFT: nonlocal density functional theory
39 AFM: atomic force microscopy

40

41 **Introduction**

42 During the last decade, there has been increasing interest for nanoscale celluloses which can be
43 extracted from plants, bacteria, fungi, algae and one marine animal, tunicate (Sacui et al. 2014), for
44 which cellulose is present in the epidermal layer (Klemm et al. 2018). Two main types of
45 nanocelluloses are classically described (Klemm et al. 2011; Dufresne 2012): cellulose nanofibrils
46 (CNFs) and cellulose nanocrystals (CNCs). CNFs are a few micrometers long and 5 to 60 nm in
47 diameter fibrils (Klemm et al. 2011; Lavoine et al. 2012; Nechyporchuk et al. 2016) comprising
48 amorphous and crystalline regions leading to flexibility and possible entanglements. They are most
49 commonly produced from a combination of a chemical or enzymatic pre-treatment followed by
50 mechanical disintegration. CNCs are rigid rod-shaped particles usually produced from the acid
51 hydrolysis of any cellulose source. They have dimensions of 100 nm to a few μm in length, and 4 to
52 70 nm in cross-section (Habibi et al. 2010; Klemm et al. 2011; Trache et al. 2017), which depend on
53 the cellulose source and, to a lesser extent, on the hydrolysis conditions (Chauve et al. 2014). Owing
54 to an extended range of properties (lightweight, natural abundance and renewable origin,
55 biocompatibility, mechanical strength, tailorable surface chemistry), nanocelluloses are ideal building
56 blocks for a wide range of applications (Abitbol et al. 2016) such as photonics (CNC chiral nematic
57 properties for the production of iridescent films), nanocomposites (Wei et al. 2014), paper and
58 packaging (Bardet and Bras 2013), electronics (Hoeng et al. 2016), biomedical applications (Jorfi and
59 Foster 2015), or as rheology modifiers for cosmetics, paintings, etc.

60 Specifically, among the different nanocellulose-based materials and as discussed in recent
61 reviews (Lavoine and Bergström 2017; De France et al. 2017), foams represent a very interesting
62 category, which allows targeting a wide range of applications. Low-density materials with very high
63 specific surface areas and a tunable architecture can be produced and these biosourced solid foams
64 are able to compete with fragile inorganic aerogels.

65 These porous structures can be obtained from different processes. Solvent removal and
66 formation of pores can be achieved by freeze-drying of the nanocellulose suspension with or without
67 prior solvent exchange. Alternatively, supercritical conditions can be used, leading to a more fibrillary
68 structure. Although both resulting structures are sometimes called “aerogels”, for a better
69 understanding of the process accomplished and because the final structures are very different, the
70 materials produced here via a freeze-drying process will be called “cryogels”. Solvent exchange of the
71 nanocellulose suspensions from water to another solvent (commonly *tert*-butyl alcohol, *t*-BuOH) has
72 been used to increase the specific surface area of the prepared porous materials. For example,
73 Sehaqui et al. (2011) obtained CNF-cryogels with a surface area of 153 m²/g that increased to
74 249 m²/g after solvent exchange with *t*-BuOH. Similar results have also been reported by Fumagalli et
75 al. (2013)b who discussed the influence of the water/*t*-BuOH ratio on the CNF cryogels morphology.
76 Alternatively, a spray freeze-drying technique (Jiménez-Saelices et al. 2017) has been developed to
77 prepare nanocellulose cryogels with reduced pore size and specific surface area of 100 m²/g for
78 insulating applications.

79 Despite the potential benefits that could be expected from these near-perfect high aspect ratio
80 nanocrystals, the preparation of tunicate CNC (tuCNC) cryogels is poorly documented. Indeed, only
81 Ishida et al. very briefly reported on the use of tuCNCs to prepare solid foams by freeze-drying with
82 or without prior solvent exchange with *t*-BuOH (Ishida et al. 2004), while other authors reported the
83 use of tuCNCs in the design of composite cryogels in combination with clay, polyvinylalcohol or
84 polyimide (Gawryla et al. 2009; Mueller et al. 2015; Nguyen et al. 2016). In the present work, we aim
85 to investigate the potential specificities of pure tuCNC-based cryogels. Therefore, porous structures
86 were produced from the initial aqueous suspensions by an ice-templating freeze-drying procedure
87 and the structural and physical properties of the resulting tuCNC-based materials were measured.
88 Additionally, cryogels were also prepared with a similar process from commercial wood CNFs and
89 CNCs derived from cotton (cCNCs) to investigate the influence of the starting nanocellulose type on
90 the morphology and physical properties of the produced cryogels. This strategy aims at establishing
91 the nanocellulose type-cryogels properties relationship to better adapt the nanocellulose source to a
92 specific application. When of interest, properties of cryogels prepared from a suspension in water
93 were compared to cryogels prepared from a suspension in *tert*-butyl alcohol.

94

95 **Materials and methods**

96 **Materials**

97 *Chemicals*

98 Hydrochloric acid (CAS Number: 7647-01-0, 37%), sodium hydroxide ($\geq 97.0\%$, pellets, CAS
99 number: 1310-73-2), sodium chlorite (technical grade, 80%, CAS number: 7758-19-2), sulfuric acid
100 (98%, CAS number: 7664-93-9) were supplied by Sigma Aldrich and directly used as received and *tert*-
101 butyl alcohol ($\geq 99.0\%$, CAS number: 75-65-0) was purchased from Fisher Scientific and used without
102 any further purification.

103 *Tunicate CNCs*

104 Mantles of tunicate (*Halocynthia roretzi*) were purified with 1N NaOH and 0.3% NaClO₂
105 treatments. Before hydrolysis, they were rinsed thoroughly, cut in small pieces and mixed with a
106 blender. They were then partially dried over a Büchner membrane. Acid hydrolysis was carried out in
107 50 wt% sulfuric acid at 50°C during 20 hours under stirring. After hydrolysis, the acid was removed
108 via centrifugation-dispersion cycles (centrifugation at 5000 to 7000 rpm during 30 min to 1 hour at
109 25°C) followed by dialysis at room temperature during 1 week. A suspension at 1.2 wt% was
110 obtained.

111 *Commercial CNFs*

112 Cellulose nanofibrils from wood called Exilva® (type P) were provided by Borregaard at 2 wt% or
113 10 wt% solid content and were redispersed at 1 wt% solid content in distilled water using an Ultra
114 Turrax IKA T25 stirrer at room temperature at 8 000 rpm during 5 minutes before use. The
115 hemicellulose content was measured through alditol acetates preparation for monosaccharide
116 analysis according to the procedure described by Blakeney et al (Blakeney et al., 1983) followed by
117 analysis by GC-MS (Agilent GC6850-MS5975) using injection on a SP 2380 column (Supelco) with
118 helium as carrier gas (1.1 ml.min⁻¹). A hemicellulose content of 2.8 % was obtained.

119 *Cotton CNCs*

120 Cotton linters from Buckeye Cellulose Corporation were cut in small pieces and mixed with a
121 blender. Acid hydrolysis was carried out in 65 wt% sulfuric acid at 60°C during 30 min under
122 mechanical stirring according to the method described by Revol et al. (1992). The acid was removed
123 via centrifugation-dispersion cycles (centrifugation at 20000g for 30 minutes) followed by dialysis
124 against distilled water until the conductivity reached the value of deionized water. The suspension
125 was then ultra-sonicated using a Branson sonifier during 4 min in an ice-bath to prevent any
126 temperature increase. The suspension was then filtered through a 1 µm Whatman membrane with a
127 Sartorius filtration equipment to remove large aggregates. A suspension at 2.2 wt% was finally
128 obtained.

129

130 Sample preparation

131 *Solvent exchange*

132 Nanocellulose particles were solvent exchange via centrifugation-dispersion cycles. A
133 suspension of nanocellulose was centrifuged at 11200 rpm during 30 minutes at room temperature.
134 The supernatant was discarded and the pellet was redispersed in tert-butyl alcohol at 30°C with Ultra
135 Turrax IKA T25 stirrer at highest speed. Following centrifugations took place at 30°C to avoid freezing
136 of tert-butyl alcohol. Four cycles of centrifugation-dispersion were necessary and the last pellet was
137 dispersed in t-BuOH/H₂O 80:20 (V/V). For cotton CNCs, the addition of salt was necessary to increase
138 the first centrifugation efficiency.

139 *Freeze-drying*

140 Nanocellulose cryogels were prepared from freeze-drying in a Cryotec® shelves freeze-drier. The
141 nanocellulose suspension at specific solid content was poured in polystyrene cylindrical molds where
142 the suspension was in direct contact with the freeze-drier shelves at -50°C. Directional freezing
143 occurred at -50°C for 2 hours. The average freezing rate was of -10°C.min⁻¹ and differences between
144 the bottom and top part of the cryogel were observed, with a faster freezing rate occurring at the
145 bottom than at the top. The primary freeze-drying step took place at 0.1 mbar with increasing
146 temperature from -50°C to 20°C in 24 hours. It was followed by a secondary freeze-drying step at
147 20°C and 0.01 mbar for 30 minutes, after which cryogels were recovered. They were stored in a
148 nitrogen cabinet to avoid humidity uptake.

149 Characterizations

150 *Elemental analysis*

151 C, O, H, and S elemental analysis was carried out by Institut des Sciences Analytiques (UMR
152 5280, Villeurbanne) on freeze-dried samples. At least duplicates were performed.

153 *Dynamic light scattering*

154 A suspension of nanocellulose at 0.1 %, was analyzed in a temperature-controlled chamber at
155 20 °C with a Malvern NanoZS instrument equipped with a 2 mW HeNe laser at a wavelength of
156 632.8 nm and detection was performed at an angle of 173°. Data analysis was performed with the
157 multiple narrow mode algorithm from Malvern DTS software and the reported hydrodynamic
158 diameter was averaged from 3 measurements of 15 runs each.

159 *Titration*

160 CNCs were prepared from sulfuric acid hydrolysis and their surface chemistry present OSO_3^-
161 groups. A suspension of CNCs containing about 0.1 g dry CNCs was titrated at least twice with 0.01 M
162 NaOH in order to evaluate the sulfur content. The degree of substitution was calculated from the
163 following formula:

$$DS = \frac{V_{eq} \times C_{NaOH} \times M_{mean}}{m_{cellulose}}$$
$$M_{mean} = \frac{162}{1 - 80 \times (V_{eq} \times C_{NaOH} / m_{cellulose})}$$
$$\text{Sulfur amount} = \frac{32 \times DS}{M_{mean}}$$

164 Where V_{eq} is the volume in mL of NaOH necessary for the titration of the CNC suspension,
165 C_{NaOH} the concentration of NaOH for the titration is of 0.01M, M_{mean} is the mean molecular weight,
166 $m_{cellulose}$ is the mass of titrated CNCs in g.

167 *Cellulose nanofibrils nanoscale and microscale properties*

168 The nanoscale and microscale particles fractions were evaluated by determining the
169 homogeneity and the nano-sized fraction as explained in a quality index specifically developed for
170 cellulose nanofibrils (Desmaisons et al. 2017). The microscopic part of the suspension was
171 characterized from optical images obtained from 0.1% diluted suspensions. CNFs were sorted into
172 three classes of dimensions: lower than 5 μm , from 5 to 10 μm and larger than 10 μm . Homogeneity
173 was defined as the highest percentage of CNFs in any of the three dimensions' classes.

174 The nanoscale particles were also isolated from the CNF suspension after centrifugation of a
175 0.02% suspension at 1000 rpm during 15 minutes (Naderi et al. 2015). The supernatant was
176 recovered and considered to be composed of nanoscale particles only. At least triplicates were
177 performed.

178 The nanoscale fraction (%) is defined as $\frac{\text{Concentration after centrifugation (wt\%)}}{\text{Concentration before centrifugation (wt\%)}} \times 100$

179 *NMR analysis*

180 ^{13}C solid-state NMR spectra were recorded with a Bruker Avance DSX 400 MHz spectrometer, in
181 a cross-polarization and magic angle spinning conditions (CP-MAS). The ^{13}C radio frequency field
182 strength was obtained by matching the Hartman–Hahn conditions at 60 kHz. The spinning speed was

12000 Hz, the operating condition was set at 100.6 MHz and a minimum number of 10 000 scans were acquired with a contact time and recycle delay, respectively, of 2 ms and 2 s representing standard conditions. The acquisition time was set at 35 ms and the sweep width at 29 400 Hz. Spectra were corrected with the scaling factor obtained from a reference spectrum of glycine. The spectra were normalized with cellulose C1 peak at 100 to 110 ppm. Crystallinity was calculated from the integration of the crystalline (87 to 93 ppm) and amorphous (80 to 87 ppm) form of carbon C4.

189 *Electron microscopy*

The cellulose nanocrystal suspensions diluted 100 to 1000 times were observed with a Philips CM200 CRYO transmission electron microscope (TEM) operating at 80 kV. One drop of suspension was deposited on a carbon grid and excess liquid was removed. A 2% uranyl acetate stain was deposited on top of the samples, excess liquid was removed after 2 minutes and the sample was allowed to dry before observation.

Nanocellulose cryogels images were recorded with a FEI Quanta 250 scanning electron microscope (SEM) equipped with a field emission gun and operating at 2 kV, after sample coating with gold/palladium. At least ten images were recorded and the most representative were selected for discussion.

199 *Atomic force microscopy (AFM)*

Cellulose nanocrystal suspensions were diluted to 10^{-4} wt% and a drop was deposited on cleaved mica plate. The plate was left to dry overnight at room temperature. Analysis of the nanoparticles was carried out using a Dimension icon[®] atomic force microscope (Bruker, USA) in tapping mode with a silicon cantilever (OTESPA, Bruker, USA). At least 10 images were recorded and used for image treatment.

205 *Image treatment*

Particle sizes were measured from electron, optical and atomic force microscopy images using the ImageJ software. Reported average values of length and width correspond to at least 150 measurements.

Pore sizes of large pores ($> 1\mu\text{m}$) were measured from SEM images at magnifications from 200 to 1000 times. At least 100 replicates were recorded and pore size distribution was analyzed.

211 *Density and porosity*

The volume of the produced cryogels was determined from height and diameter measurements using a digital caliper. The density is given as the mass of cryogel for a given volume and an average

214 value of ten measurements was taken. The porosity, P , was calculated from the following equation:
215 $P (\%) = (1 - \frac{\rho}{\rho_c}) \times 100$, where ρ the density of the cryogel and ρ_c the density of cellulose (1.60
216 g.cm⁻³)

217 *Nitrogen adsorption*

218 Specific surface area and pore size distribution were obtained using a Micromeritics ASAP 2420
219 Surface Area and Porosity Analyzer. Cryogels samples (around 100 mg) were degassed at 105°C
220 during at least 12 hours. Analysis was performed with nitrogen adsorption and desorption at -196°C,
221 at relative pressures from 0.01 to 0.03 for BET specific surface area and from 0.01 to 0.995 for pore
222 size distribution. The specific surface area measurement was performed three times for each sample
223 and for a given set of parameters two different batches were characterized. The specific surface area
224 was calculated by assuming that nitrogen is adsorbed at the surface of the sample as a monolayer.

225 A nonlocal density functional theory (NLDFT) method was applied to obtain the pore size
226 distribution of cryogels after pore geometry adjustment (Ravikovitch and Neimark 2001). The
227 MicroActive software was used for the calculations.

228 *Compression test*

229 Compression tests were performed on a Shimadzu (autograph) AGS-X with load cell capacities of
230 20 N and 500 N (depending on the cryogel resistance) at 24°C and 50 (± 3) % relative humidity after
231 at least 24 hours stabilization storage of cryogel in same conditions. Cryogels of cylindrical shape of
232 15 mm in diameter and 20 to 30 mm in height were compressed at 1 mm/min. Triplicates were
233 performed. The compression moduli were calculated in the elastic region at strains (± 2.5%)
234 corresponding to half the strain of the beginning of the plateau region.

235 **Results and Discussion**

236 *Nanocellulose morphology and properties*

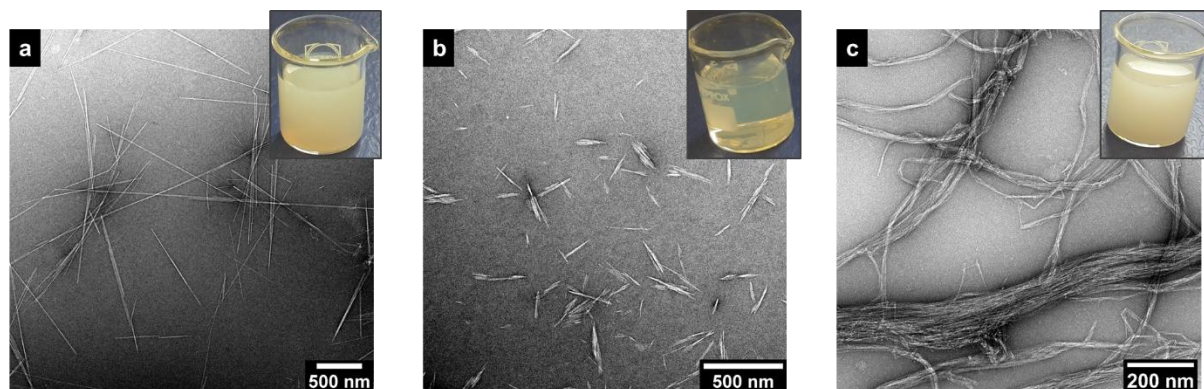
237 Cellulose nanocrystal and cellulose nanofibril suspensions exhibit very different macroscopic
238 features, as shown in the insets in **Fig.1**. For both types of CNCs, the preparation yields homogeneous
239 and stable colloidal suspensions with a low viscosity. In the case of tunicate, some temporary
240 iridescence can be observed when shearing is applied, due to the easy alignment of these high aspect
241 ratio particles. The CNF suspensions appear more viscous, gel-like and non-transparent due to
242 entanglements between the very long objects and the presence of poorly disaggregated elements.
243 Characterization of cellulose nanomaterials is still a challenge and requires adapted technique or
244 specific preparations as recently reviewed by the community (Foster et al. 2018). To investigate the

245 particles morphology, TEM images were acquired and are shown in **Fig.1**. Cotton CNCs have a rod-
246 like structure with a moderate aspect ratio and are often observed as bundles of 3 to 4 laterally
247 associated elementary crystallites, which the acid hydrolysis did not separate. This is in agreement
248 with previously reported data (Elazzouzi-Hafraoui et al. 2008). Tunicate CNCs exhibit a much higher
249 aspect ratio and a needle-like structure, due to a higher crystallinity when compared to cotton.
250 TuCNC production rather yields isolated elementary crystallites with a lower degree of polydispersity
251 than cCNCs. Regarding CNF material, a much more heterogeneous morphology is observed when
252 multiscale tools like optical microscopy, SEM and TEM are used. The finest fraction in the CNF sample
253 observed by TEM appears as long and flexible fibrils with a varying degree of defibrillation. The
254 particles sizes distributions are recorded in supplementary materials, in figure SM1.

255 **Table 1** summarizes the intrinsic properties of the three types of nanocelluloses in terms of
256 dimensions and crystallinity. Sizes and aspect ratio of the different nanoparticles were deduced from
257 TEM and AFM images analysis. Cotton CNCs have an average length of 150 nm and an average width
258 of 19 nm and a height of 8 nm, giving an aspect ratio of 14, in agreement with literature values
259 (Elazzouzi-Hafraoui et al. 2009; Cherhal et al. 2015). Tunicate CNCs have an average length of 1120
260 nm and a width of about 21 nm on average and a height of 12 nm giving an aspect ratio of 63, which
261 is much bigger than the value for cCNCs. Dynamic light scattering (DLS) was used to measure the
262 hydrodynamic diameter of CNCs only, since this technique is not suitable for micrometer-sized CNFs.
263 Values of 129 and 381 nm were obtained for cCNCs and tuCNCs, respectively. It has here to be
264 recalled that these values do not correspond to the length nor to the cross-section of the particles
265 but to the size of the sphere with the same diffusion coefficient as the considered particle.

266 CNFs are much longer and entangled flexible fibers, as the defibrillation process mainly
267 separates the fibrils and reduce their length to a much lesser extent than the acid hydrolysis used to
268 prepare CNCs. Disordered regions along the fibrils provide flexibility to the CNFs. The cellulose
269 nanofibril suspension is heterogeneous in size with nano, micro, and macroscale dimensions and
270 were evaluated with the recently defined quality index (Desmaisons et al. 2017). The homogeneity of
271 the suspension was determined from optical microscopy images. The various measured dimensions
272 are sorted into three classes of different sizes. The homogeneity was defined as the highest
273 percentage of CNF sizes in a same class and was of 37%, expressing a great heterogeneity. The
274 micron-sized class dimensions were measured from optical microscopy images and are of 9 μm in
275 length and 0.9 μm in diameter, on average. The nanoscale components were analyzed after
276 centrifugation of a highly diluted suspension without entangled particles. The quantity of nanofibrils
277 is defined from the nano-sized fraction, and was equal to 34 % of the total suspension. The nano-
278 sized fraction defines the part of nanoparticles in a suspension composed of nanofibrils and also CNF

279 aggregates and large objects. Their dimensions are estimated from electron microscopy images and
 280 objects with an average diameter of 25 nm and length higher than 1.7 μm are observed. It should be
 281 noted that the full length of individual nanofibrils can usually not be observed as they do not fit in
 282 the image size, therefore the aspect ratio of the cellulose nanofibrils is higher than 68.



283

284 **Fig.1** TEM images and corresponding photographs of suspensions of tunicate CNCs (a), cotton CNCs
 285 (b), and CNFs (c).

286 **Table 1** Nanocellulose dimensions and crystallinity. ^a Extracted form TEM micrographs. ^b Obtained
 287 from AFM measurements. ^c Calculated from the ratio of the length to the average cross-section

	tuCNCs	cCNCs	CNFs (nanosize fraction)
length ^a (nm)	1120 \pm 473	150 \pm 56	> 1702
width ^a (nm)	21 \pm 6	19 \pm 8	25 \pm 14
height ^b (nm)	12	8	n/a
Aspect ratio ^c	63	14	> 68
Hydrodynamic diameter ^d (nm)	381 \pm 172	129 \pm 76	n/a
Crystallinity ^e	94 %	75 %	49 %

288 dimension. ^d Obtained from DLS measurements. ^e Assesed from ¹³C NMR measurements.

289

290 The crystallinity was assessed by ¹³C NMR analysis. It is commonly calculated from the signals
 291 related to carbon C4, which appears at 83 ppm in its amorphous form and at 86 ppm in its crystalline
 292 form. Cotton CNCs have a degree of crystallinity of 75 %. Tunicate CNCs exhibit near-perfect
 293 crystallinity with a degree of crystallinity of 94 %. This is explained by the high degree of crystallinity
 294 of tunicate cellulose that plays a structural role for this marine organism, although this value seems
 295 to vary a lot with the tunicate species (Zhao and Li 2014). CNFs appears to be semi-crystalline with 49
 296 % crystallinity. This is explained by the low crystallinity of cellulose in wood and the extraction
 297 process used which does not imply acid hydrolysis but enzymatic treatment (commonly

298 endoglucanase enzymes attack parts of the cellulose) and a mechanical treatment to defibrillate the
299 fibrils.

300 Cryogels morphology and structure

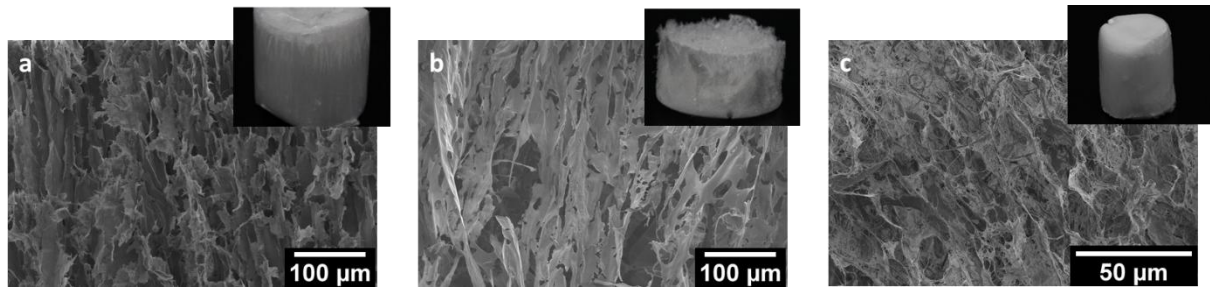
301 The morphological and structural properties of nanocellulose cryogels obtained from ice-
302 templating and freeze-drying were investigated. The macro- and microscales morphologies are
303 shown in **Fig.2**. The cylindrical shaped cryogels appear as non-transparent white foams. Cotton CNC
304 cryogels are crumbly and brittle, however, CNF and tuCNC cryogels have a better holding and more
305 homogeneous structure. It is clearly possible to observe an upright orientation of the structure for
306 tuCNC cryogels at the macroscopic scale (**Fig.2a**)

307 Firstly, for each nanocellulose cryogel, a flat and sheet-like structure seems to be observed.
308 However, it is worth noting that when walls of tuCNC cryogels are zoomed-in, a fine organization
309 emerges and appears as “interlaced” or “netted” sheets, with isolated nanoparticles as observed in
310 **Fig3c**. On the contrary, cCNC and CNF cryogels sheets appear thick and dense. Tunicate CNC cryogels
311 present a morphology similar to previously observed cross-linked short CNCs. Indeed, cross-linking
312 appears to result in cryogels that do not present fully packed walls and with a microporosity as
313 observed by Yang and Cranston (2014) and Osorio et al. (2018). Such results suggest that tunicate
314 CNCs are naturally forming a network, whereas for cCNCs it is necessary to chemically cross-link the
315 nanocrystals to obtain an analogous cryogel morphology.

316 The sheet-like structure is explained by the growth of ice-crystals during the freezing step that
317 pushes the nanocellulose particles away and together. This was not observed when cryogels are
318 obtained from a suspension of nanocellulose in *tert*-butyl alcohol (*t*-BuOH/H₂O, 80:20), which appear
319 to have a finest and more fibrillary structure (Supplementary material, Figure SM2). Such a behavior
320 has been observed before, for example by Jin et al. (2004) where they studied the effect of the
321 solvent exchange on the final cryogel structure.

322 Cryogels prepared from tunicate CNCs are anisotropic and present a completely different
323 architecture depending on whether the longitudinal section or the cross-section is considered, as
324 shown in **Fig.3a** and **b**. An alveolar structure is observed for cross-section images, resulting from the
325 growth of ice in a specific direction. The freezing is induced from the bottom part of the hydrogel
326 that is in contact with the frozen shelf of the freeze-drier. Instead of growing in random directions,
327 the crystals grow towards the upper part of the hydrogel. The high aspect ratio and great rigidity of
328 the tunicate CNCs prevent the arbitrary expansion of water, hence favor the resulting honeycomb
329 structure. Similar organization was observed for TEMPO-oxidized CNF cryogels by Chen et al. (2019)
330 with honeycomb pores in the cross section and directional tunnels in the longitudinal section. Munier

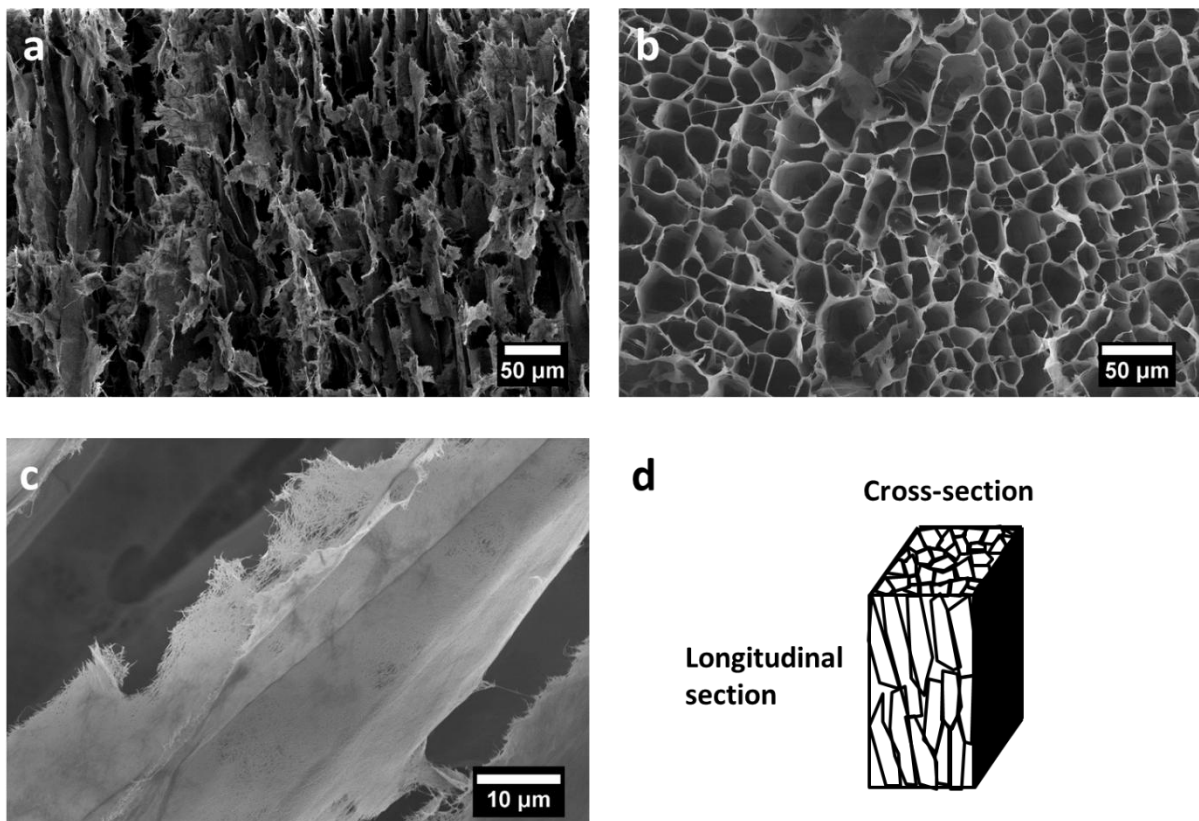
331 et al. (2016) have observed orientation of the particles induced by unidirectional freezing, that leads
 332 to the formation of cellular structures, as observed here for tuCNC cryogels. They quantified the
 333 degree of orientation with X-ray diffraction and confirmed the highest orientation for freeze-casted
 334 cryogels compared to the freeze-crashing ones. The lower orientation of CNF than CNC cryogels is
 335 even more pronounced due to CNF flexibility and aggregates, the presence of which explains why
 336 very little orientation is observed for the ice-templated heterogeneous CNF suspensions.



337

338 **Fig.2** Longitudinal section SEM images of tunicate CNC (a), cotton CNC (b), CNF (c) cryogels prepared
 339 from a suspension in water.

340



341

342 **Fig.3** SEM images of tunicate CNC cryogels from a longitudinal section (a) and cross-section (b),
 343 zoomed-in image of tuCNC cryogels wall (c), cross and longitudinal section schematic representation
 344 (d).

345 Various properties, namely specific surface area, density and pore size of the prepared cryogels,
346 are summarized in **Table 2**.

347 The value of density for tuCNC cryogels and CNF cryogels are close to 10 mg/cm^3 , close to the
348 initial concentration (1 wt%). This indicates that there is little shrinkage and pore collapse. However,
349 the density measurement was not accurate for cCNC cryogels because of their very brittle behavior
350 and an estimated value was reported here for this sample. The samples exhibit very high porosity,
351 with calculated values of $99.3 (\pm 0.1) \%$ for tuCNC cryogels and 99.4% for CNF cryogels. For cotton
352 CNC cryogels, a value of $99.3 (\pm 0.1) \%$ of porosity was estimated, assuming that the cryogels exhibit
353 between 0 and 33 % shrinkage.

354 The specific surface areas of each cryogel were determined from nitrogen adsorption and
355 application of the Brunauer–Emmett–Teller (BET) theory, estimating the amount of nitrogen
356 molecules adsorbed as a monolayer at the surface of the cryogels. Full adsorption and desorption
357 isotherms were acquired and type IV isotherms, corresponding to mesoporous samples, were
358 obtained according to IUPAC classification.

359 Cryogels prepared from cotton CNCs exhibit a very low specific surface area of $4 \pm 2 \text{ m}^2/\text{g}$, which
360 is in the lower range compared to what has been published, with values of $17 \text{ m}^2/\text{g}$ observed for
361 cotton CNC cryogels (Fumagalli et al. 2013), although few cryogels have been produced with sulfate
362 half-ester CNCs using the freeze-drying process. CNF cryogels exhibit a specific surface area of 25 ± 2
363 m^2/g , in fair agreement with results reported by Cervin et al. (2012) for CNF porous cryogels
364 prepared under similar conditions. Such low specific surface area values are usually attributed to the
365 growth of large ice crystals during the freezing step of the freeze-drying process, which pushes the
366 nanocellulose elements towards the outer limits of the crystals, inducing their aggregation as sheets
367 located between neighboring ice crystals. However, for tuCNC-based samples and despite the use of
368 water-based suspensions, an unexpectedly high surface area of $122 \pm 10 \text{ m}^2/\text{g}$ was measured. Such a
369 high value would in fact have been expected for cryogels prepared from suspensions in *tert*-butyl
370 alcohol. It has indeed been shown in literature that solvent exchange from water to *tert*-butyl alcohol
371 is highly beneficial to a strong increase of the specific surface area of CNF or CNC-based cryogels up
372 to $249 \text{ m}^2/\text{g}$ (Sehaqui et al. 2011). While we could verify that such a solvent exchange results in a very
373 significant increase of the specific surface area from 25 ± 2 to $97 \pm 4 \text{ m}^2/\text{g}$ for CNF-based cryogels, in
374 agreement with literature data (with surface areas after solvent exchange and freeze-drying twice
375 higher than for regular cryogels (Jin et al. 2004)), only a slight increase to $150 \pm 21 \text{ m}^2/\text{g}$ could be
376 obtained for tuCNC-based cryogels prepared from suspensions in *tert*-butyl alcohol (Supplementary
377 material Table SM1). This result emphasizes the very peculiar behavior of tuCNCs, which enable the

378 preparation of cryogels with high specific surface area directly from water-based suspensions.
379 Surprisingly, using tunicate CNCs, Ishida et al. (2004) reported specific surface area values of 25 and
380 130 m²/g for cryogels prepared by freeze-drying without solvent exchange and after solvent
381 exchange with t-BuOH, respectively, but no details on the freeze-drying process or further
382 characterizations were provided to allow for a deeper comparison.

383 The theoretical value of the specific surface area of isolated tuCNCs is of 165 m²/g, considering
384 average dimensions of 1120 x 21 x 12 nm³ and a density of 1.6 g.cm⁻³. This value is very close to the
385 specific surface area obtained for tuCNC cryogels using the experimental nitrogen adsorption BET
386 method. This suggests that almost every nanocrystal is individualized and contributes to the specific
387 surface area. Such an hypothesis is supported by SEM observations (Fig.3a and c), which show that
388 the tunicate nanocrystals forming the solid foam remain well separated and not aggregated as in the
389 case of cCNC and CNF cryogels. This high degree of individualization of the particles in the final
390 cryogel material enables to preserve the accessibility of the tuCNC surface. It has also to be noted
391 that, even if not easily detected by TEM, thin particles with widths lower than 20 nm were not taken
392 into account in the theoretical surface area calculation but would strongly contribute to it. The high
393 specific surface area of tunicate CNC cryogels therefore arises from a distinct morphology resulting
394 from the high rigidity and crystallinity of the tuCNCs, in addition to their high aspect ratio. This
395 characteristic morphology limits the random growth of ice crystals. This behavior can tentatively be
396 explained by the formation upon drying of a rigid yet sparse tuCNC network that was formed through
397 the creation of a limited number of hydrogen bonds along the particle's long axis. This situation is
398 reminiscent of the use of tuCNCs in nanocellulose/polymer layer-by-layer films, where the long and
399 stiff tuCNCs allowed the formation of a "porous matchsticks pile", which led to strong antireflective
400 properties, while the use of cCNCs rather resulted in very dense layers, preventing antireflection
401 (Podsiadlo et al. 2007; Jean et al. 2008; Martin and Jean 2014).

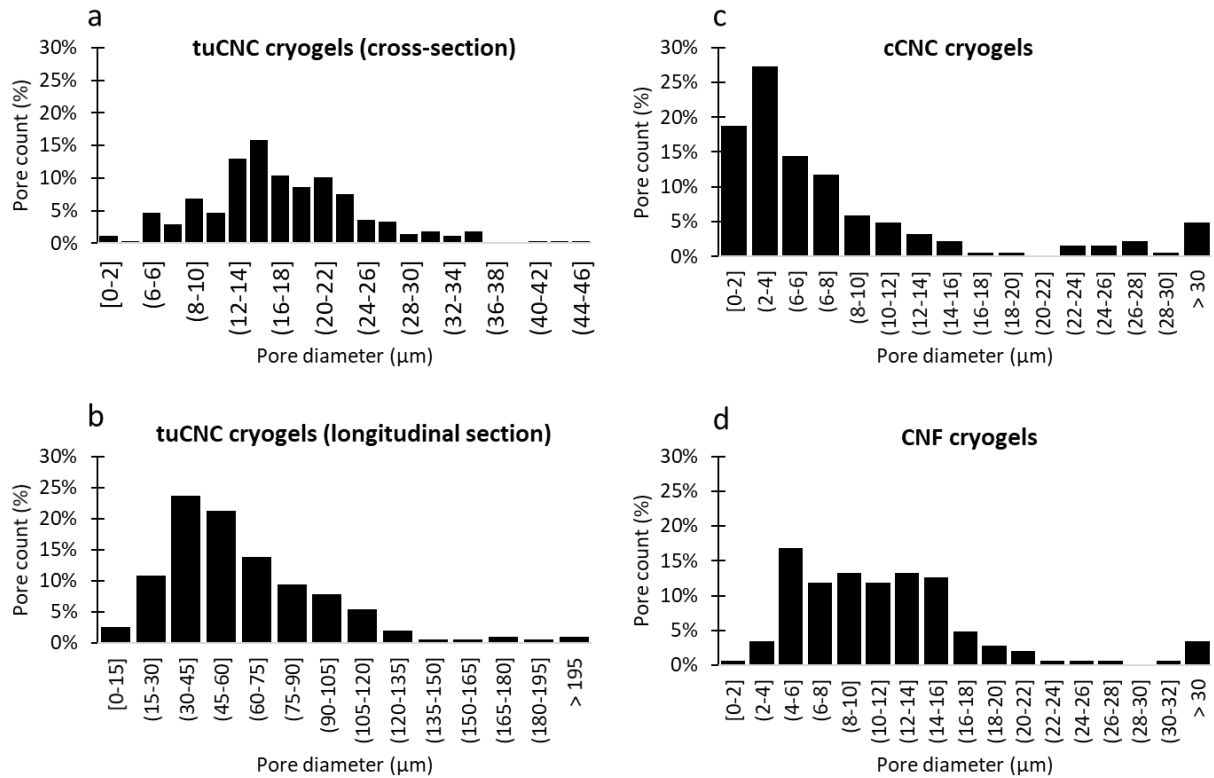
402 The sulfur content was determined by conductometric titration and a value of 0.73 % was obtained
403 for cCNCs. This method was not accurate for tuCNC sulfur amount determination as the amount is
404 too low. Elemental analysis complements the titration results, with 0.61% S for cotton CNCs and a
405 value inferior to 0.1 % for tunicate CNCs. The charge density being lower for tuCNCs compared to
406 cCNCs, tuCNCs would be expected to be more aggregated. Therefore, the great individualization of
407 the tuCNCs is not due to the surface charge present on the surface of the CNCs and must be
408 explained with the intrinsic organization of cellulose inside the tunicate mantles and their high
409 rigidity and crystallinity.

410 **Table 2** Specific surface areas (SSA) calculated from BET method and average pore sizes measured
 411 from SEM images, for cryogels prepared from tunicate CNCs, cotton CNCs and CNFs from a
 412 suspension in water

	SSA (m ² /g)	Density (mg.cm ⁻³)	Average pore size (μm) ^a
tuCNCs	122 ± 10	11.5 ± 1.0	17.6 ± 5.7 (<i>cross-section</i>) 66.2 ± 30.4 (<i>longitudinal section</i>)
cCNCs	4 ± 2	10 (<i>estimated</i>)	8.7 ± 7.5
CNFs	25 ± 2	10.2 ± 0.2	17.8 ± 13.8

413

414 The pore sizes were determined from the treatment of SEM images and the resulting pore size
 415 distribution histograms are plotted in **Fig.4**. Because of their singular organization, the pore size of
 416 tunicate CNC cryogels were measured for both longitudinal and cross-section directions. They have
 417 an average diameter of 66 μm in the longitudinal section and 18 μm in cross-section. Cotton CNC
 418 cryogels have 9 μm pores. CNF cryogels have a mean pores diameter of 18 μm.



419

420 **Fig.4** Pore size distribution extracted from SEM images of tunicate CNC (a: cross-section, b:
 421 longitudinal section), cotton CNC (c) and CNF (d) cryogels prepared from a suspension in water

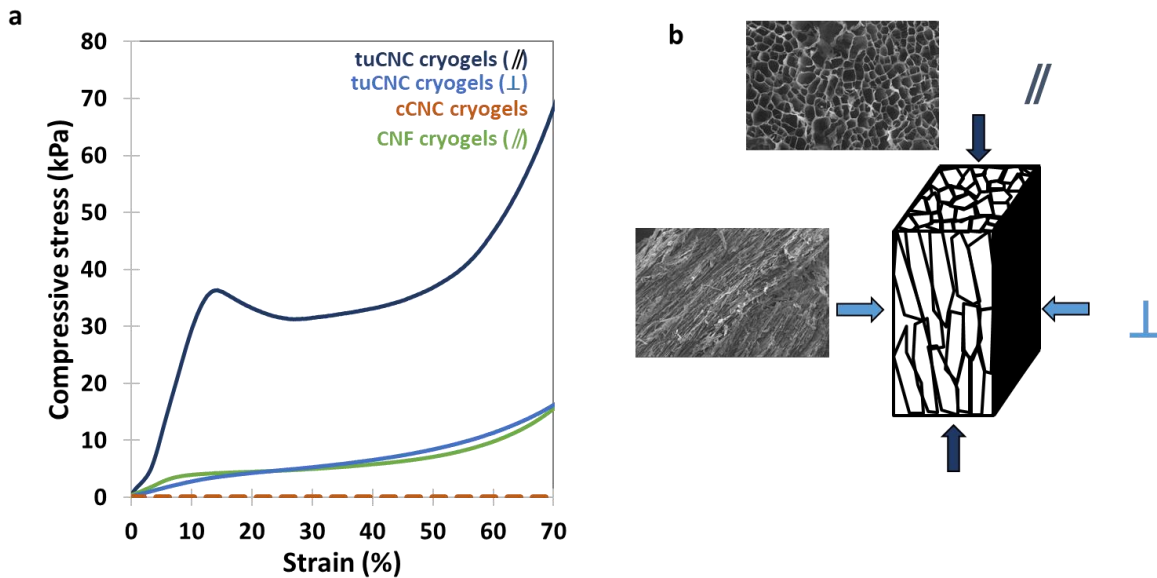
422 In order to perform a multiscale pore size analysis, the mesoporosity was calculated from
 423 nitrogen adsorption and after applying nonlocal density functional theory (NLDFT) and adjusting the
 424 pore geometry. The pore size distributions are presented in supplementary material (Fig. SM4).
 425 However, as discussed by Rudaz et al. (2014), nitrogen adsorption technique only considers a very
 426 small portion of pores with width lower than 60 nm.

427 The combination of the two techniques brings to light the presence of both micron-sized pores
 428 and mesopores in each cryogel. Large pores, observed from microscopy images, are introduced
 429 during the freezing process and the pore size distribution profiles are very different for the three
 430 types of nanocellulose. The amount of small pores is influenced by the starting nanocellulose
 431 particles, as observed from nitrogen adsorption experiments. However, the distribution profiles of
 432 small pores are very similar, with analogous well-defined pore sizes observed for each structure. The
 433 pores of size inferior to 60 nm are resulting from contact point between the nanocellulose particles,
 434 as suggested by Robitzer et al. (2011).

435 Cryogels mechanical properties

436 Compression tests were carried out on the cylindrical shaped cryogels and the obtained stress-
 437 strain curves are shown in **Fig.5a**. In each case, two different regions are observed: the first part of

438 the curve corresponds to the elastic domain with a deformation below 10%-20 % depending on the
 439 cryogels and after a yield point the plastic region is reached. Martoia et al. (2016) observed these
 440 typical regimes for CNF and TEMPO-oxidized CNF cryogels. At early stage of elastic deformation,
 441 bending of the pore walls was observed. After increasing strain, collapse and severe deformation of
 442 the pore occurs, as well as tearing. After 70% deformation (not represented here), the densification
 443 of the material occurs and the pores collapsed and packed together. These regimes are similarly
 444 observed here for tunicate CNC and CNF cryogels.



445

446 **Fig.5** Stress-strain compression curves of tunicate CNC, cotton CNC and CNF cryogels (a). For tunicate
 447 CNC cryogels, measurements were carried out both parallel and perpendicular to the direction of
 448 freezing as illustrated in (b).

449 The modulus was calculated in the elastic region. Compression moduli and maximum stress at
 450 70% deformation are summarized in **Table 3**. Cotton CNC cryogels were very fragile and crumbly,
 451 therefore cryogels with the appropriate shape could not be prepared and accurate compression
 452 curves could not be obtained. Compression was performed both parallel and perpendicular to the
 453 ice-templating direction, as presented in **Fig.5b** for tunicate CNC cryogels. The perpendicular
 454 direction corresponds to the longitudinal section compression and the parallel direction corresponds
 455 to the cross-section compression. CNF cryogels compression curves are presented for the
 456 compression direction parallel to the direction of freezing only due to the difficulty to cut cryogels
 457 with suitable and defined shape for compression testing in the perpendicular direction.

458 **Table 3** Compression modulus, normalized modulus and maximum stress at 70% deformation for
 459 tuCNC and CNF cryogels.

Cryogel	Modulus (kPa)	Normalized modulus (kPa. mg ⁻¹ . cm ³)	Maximum stress at 70% deformation (kPa)
---------	---------------	------------------------------------------------------------------	--------------------------------------------

tuCNCs //	138 ± 44	12 ± 3.8	57 ± 9
tuCNCs ⊥	36 ± 13	3.1 ± 1.1	17 ± 3
cCNCs	<i>Not suitable</i>	<i>Not suitable</i>	<i>Not suitable</i>
CNFs //	28 ± 4	2.7 ± 0.4	17 ± 2

460

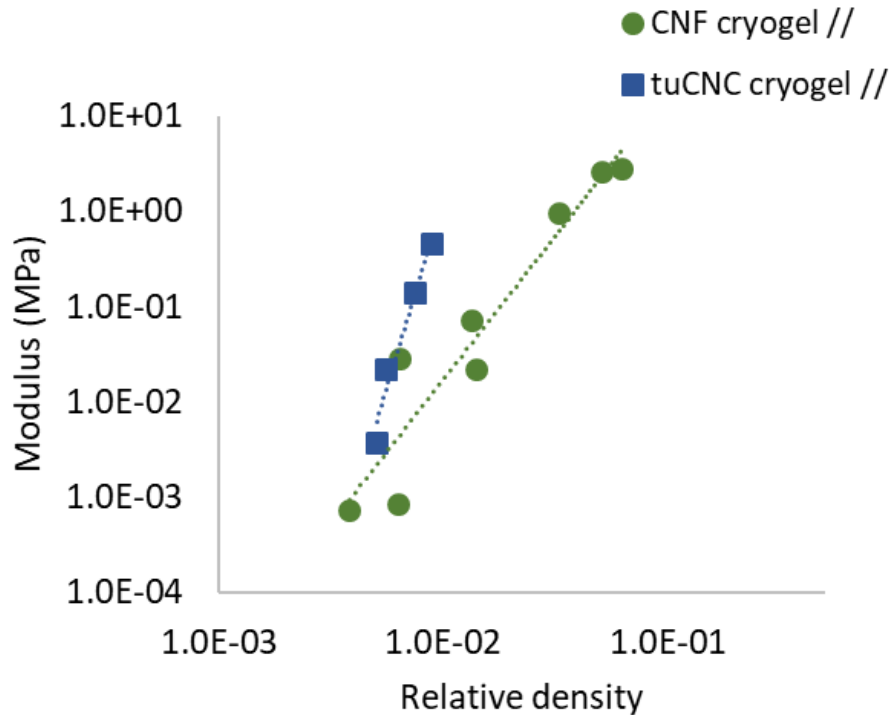
461 A striking difference is observed for the parallel compression curves of tuCNC and CNF cryogels.
462 While the compression modulus of CNF cryogels was of 28 kPa, a much higher value of 138 kPa was
463 measured for tuCNC cryogels. For the latter, a very clear region of pore collapse characterized by a
464 decrease of stress with increasing strain is observed from 15 to 25% of compression strain, followed
465 by a plateau and finally a steep rise after 55% strain reflecting a densification of the material. The
466 cryogel morphology, with directional and elongated pores, can explain the high resistance of the
467 material until a certain degree of compression after which the pores collapse. This compressive
468 behavior is specific to lattice materials, for which the plateau region is due to elastic buckling (Ashby
469 2006). These materials are also said to present a “cellular” organization and are designed to prepare
470 stiff and resistant materials for applications that require a light material or when as little material as
471 possible is to be used. The observed mechanical behavior is qualitatively in close agreement with
472 results recently reported by Chen et al. (2019) on TEMPO-oxidized CNF aerogels exhibiting the same
473 type of structural anisotropy.

474 The tunicate CNC morphology and properties, *i.e.* their high aspect ratio, high crystallinity and
475 rigidity, are also responsible for the stiffness of the cryogels. When tuCNC cryogels are compressed in
476 the perpendicular direction, the compression modulus drops to 36 kPa, and the curve profile and
477 modulus are similar to those of parallel CNF cryogels. Martoia et al. (2016) observed that the
478 compression modulus of TEMPO-oxidized CNFs with high aspect ratio was higher than for enzymatic
479 heterogeneous CNFs. They studied the effect of the CNF type and morphology on the structural
480 organization and observed that TEMPO-oxidized CNFs lead to more regular anisotropic pore
481 structures with unimodal size. However, CNF suspensions that are more heterogeneous lead to
482 reduced anisotropy. By comparing classical freezing to sorbet freezing, the role of a cellular
483 organisation on the increase of mechanical properties was emphasized. The effect of the
484 nanocellulose morphology on the mechanical properties was also investigated by Kriechbaum et al.
485 (2018). They especially studied the effect of the energy consumption during CNF production on the
486 CNF diameters and on the compression modulus of the prepared cryogels. A decrease in the
487 diameter of the CNFs resulted in an increase of compression modulus (26-fold), until a plateau is

488 reached. The authors suggested that when the amount of CNFs of small diameters increases, more
489 bonds and entanglements are formed which increases the mechanical properties. Such a mechanism
490 could account for the higher moduli measured for tuCNCs than for CNFs since tuCNCs only comprise
491 thin rods while CNFs also contain thicker objects, which were not defibrillated.

492 Compression moduli have been normalized with the cryogel density. Mechanical behavior
493 reported for CNF materials are similar to what is observed in this paper, with normalized moduli of
494 2.5 kPa.mg⁻¹.cm³ (Sehaqui et al. 2011), 3.8 and 6.3 kPa.mg⁻¹.cm³ (Gupta et al. 2018). For TEMPO-
495 oxidized CNF cryogels moduli of 6.7 and 5.9 kPa.mg⁻¹.cm³ were reported by Jiang and Hsieh (2014)
496 and Martoïa et al. (2016). Fewer literature is available on the mechanical properties of CNC-only
497 cryogels. A modulus of 9.4 kPa.mg⁻¹.cm³ was obtained by Osorio et al. (2018) for cross-linked CNC
498 cryo-templated aerogels, obtained by supercritical drying. Tripathi et al. (2019) reported the highest
499 values of compression modulus with values up to 21.5 kPa.mg⁻¹.cm³ for CNC aerogels. However, the
500 materials differ from the tuCNC cryogels presented in this study because they are prepared from
501 supercritical drying and have 10-fold higher densities (171 mg.cm⁻³).

502 A power-law relationship has been described by Gibson and Ashby (1999) for cellular materials.
503 The compression modulus plotted as a function of the relative cryogel density gives a curve of the
504 type $E \propto (\rho / \rho_{\text{cellulose}})^m$. The scaling exponent, m , was described to be dependent on the type of foam
505 (Budtova 2019). Cryogels at various densities were prepared and the moduli calculated from the
506 compression stress-strain curves. **Fig.6** illustrates the power-law relationship between the modulus
507 and the density for tuCNC and CNF cryogels.



508

509 **Fig.6** Compression modulus of tuCNC and CNF cryogels as a function of relative density.

510 A scaling exponent of 3 was observed for CNF cryogel, close to what has been previously
 511 described for enzymatic CNF cryogels by Martoia et al. (2016), who reported a scaling exponent of
 512 3.1. However, for tuCNC cryogels, a value of 7.8 was unexpectedly obtained. As mentioned by Zhao
 513 et al. (2018) in their review, the scaling exponent of biopolymer foams usually varies from 1 to 4. At
 514 increasing solid content, the cellulose nanocrystals present more contact points and interactions and
 515 this results in very high mechanical properties. Although the experiment was performed on a small
 516 range of densities, the mechanical behavior of tuCNC cryogels is outstanding and promising for the
 517 design of highly resistant porous materials.

518 **Conclusion**

519 In this work, different types of nanocelluloses obtained from different sources and different
 520 extraction processes were compared. Cellulose nanocrystals from cotton and tunicate both have high
 521 crystallinities and tuCNCs present an aspect ratio 5 times higher than cCNCs. Semi-crystalline
 522 cellulose nanofibrils are derived from wood sources. From these different cellulose nanoparticles,
 523 cryogels were prepared by an ice-templating freeze-drying process, and morphological observations
 524 were made on the lightweight and highly porous materials. While CNF and cotton CNC cryogels
 525 displayed a classical sheet-like structure and a random organization, tunicate CNC cryogels revealed
 526 an eminently organized honeycomb structure.

527 The nitrogen adsorption analysis demonstrates the very high specific surface area of 122 m²/g
528 for the tunicate CNC cryogel prepared directly from a water suspension. This result is particularly
529 interesting as such high values of specific surface area are usually obtained after time-consuming
530 solvent exchange steps. In a second step, the mechanical properties of the cryogels were assessed by
531 compression experiments. Cotton CNC cryogels were too weak to produce cryogels of the desired
532 shape for compression tests. CNF cryogels displayed a weak resistance and a low Young's modulus of
533 28 kPa. Tunicate CNC cryogels displayed a different behavior depending on the direction of
534 compression. This anisotropic behavior had not been observed before. Indeed, while tuCNC cryogels
535 present a similar compressive behavior to CNF cryogel for perpendicular compression, when the
536 compression direction was out of plane they displayed a high mechanical resistance to compression
537 and a Young's modulus of 138 kPa. This result is remarkable for a 1% solid content material. Such
538 unexpected and outstanding properties of high specific surface area and mechanical properties are
539 explained by the specific honeycomb configuration of the tuCNC cryogel, itself explained by the
540 directional freezing process and the near-perfect crystallinity and high aspect ratio of the tunicate
541 cellulose nanocrystals. This work illustrates how the near-perfect tuCNCs can be used to yield
542 materials with enhanced and new properties.

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553

554 **References**

- 555 Abitbol T, Rivkin A, Cao Y, et al (2016) Nanocellulose, a tiny fiber with huge applications. *Curr Opin*
556 *Biotechnol* 39:76–88. doi: 10.1016/j.copbio.2016.01.002
- 557 Ashby MF (2006) The properties of foams and lattices. *Philos Trans R Soc Lond Math Phys Eng Sci*
558 364:15–30. doi: 10.1098/rsta.2005.1678

- 559 Bardet R, Bras J (2013) Cellulose Nanofibers and Their Use in Paper Industry. In: Handbook of Green
560 Materials. WORLD SCIENTIFIC, pp 207–232
- 561 Blakeney AB, Harris PJ, Henry RJ, Stone BA (1983). A simple and rapid preparation of alditol
562 acetates for monosaccharide analysis. Carbohydr Res 113(2), 291-299. doi:
563 10.1016/0008-6215(83)88244-5.
- 564 Budtova T (2019) Cellulose II aerogels: a review. Cellulose 26:81–121. doi: 10.1007/s10570-018-2189-
565 1
- 566 Cervin NT, Aulin C, Larsson PT, Wågberg L (2012) Ultra porous nanocellulose aerogels as separation
567 medium for mixtures of oil/water liquids. Cellulose 19:401–410. doi: 10.1007/s10570-011-
568 9629-5
- 569 Chauve G, Fraschini C, Jean B (2014) Separation of Cellulose Nanocrystals. In: Materials and Energy.
570 WORLD SCIENTIFIC, Singapore, pp 73–87
- 571 Chen Y, Zhou L, Chen L, et al (2019) Anisotropic nanocellulose aerogels with ordered structures
572 fabricated by directional freeze-drying for fast liquid transport. Cellulose 26:6653–6667. doi:
573 10.1007/s10570-019-02557-z
- 574 Cherhal F, Cousin F, Capron I (2015) Influence of Charge Density and Ionic Strength on the
575 Aggregation Process of Cellulose Nanocrystals in Aqueous Suspension, as Revealed by Small-
576 Angle Neutron Scattering. Langmuir 31:5596–5602. doi: 10.1021/acs.langmuir.5b00851
- 577 De France KJ, Hoare T, Cranston ED (2017) Review of Hydrogels and Aerogels Containing
578 Nanocellulose. Chem Mater. doi: 10.1021/acs.chemmater.7b00531
- 579 Desmaisons J, Boutonnet E, Rueff M, et al (2017) A new quality index for benchmarking of different
580 cellulose nanofibrils. Carbohydr Polym 174:318–329. doi: 10.1016/j.carbpol.2017.06.032
- 581 Dufresne A (2012) Nanocellulose: From Nature to High Performance Tailored Materials. Walter de
582 Gruyter, Berlin
- 583 Elazzouzi-Hafraoui S, Nishiyama Y, Putaux J-L, et al (2008) The Shape and Size Distribution of
584 Crystalline Nanoparticles Prepared by Acid Hydrolysis of Native Cellulose. Biomacromolecules
585 9:57–65. doi: 10.1021/bm700769p
- 586 Elazzouzi-Hafraoui S, Putaux J-L, Heux L (2009) Self-assembling and Chiral Nematic Properties of
587 Organophilic Cellulose Nanocrystals. J Phys Chem B 113:11069–11075. doi:
588 10.1021/jp900122t
- 589 Foster EJ, Moon RJ, Agarwal UP, et al (2018) Current characterization methods for cellulose
590 nanomaterials. Chem Soc Rev 47:2609–2679. doi: 10.1039/C6CS00895J
- 591 Fumagalli M, Sanchez F, Boisseau SM, Heux L (2013) Gas-phase esterification of cellulose nanocrystal
592 aerogels for colloidal dispersion in apolar solvents. Soft Matter 9:11309. doi:
593 10.1039/c3sm52062e
- 594 Gawryla MD, van den Berg O, Weder C, Schiraldi DA (2009) Clay aerogel/cellulose whisker
595 nanocomposites: a nanoscale wattle and daub. J Mater Chem 19:2118. doi:
596 10.1039/b823218k

- 597 Gibson LJ, Ashby MF (1999) Cellular Solids: Structure and Properties. Cambridge University Press,
598 Cambridge
- 599 Gupta P, Singh B, Agrawal AK, Maji PK (2018) Low density and high strength nanofibrillated cellulose
600 aerogel for thermal insulation application. *Mater Des* 158:224–236. doi:
601 10.1016/j.matdes.2018.08.031
- 602 Habibi Y, Lucia LA, Rojas OJ (2010) Cellulose Nanocrystals: Chemistry, Self-Assembly, and
603 Applications. *Chem Rev* 110:3479–3500. doi: 10.1021/cr900339w
- 604 Hoeng F, Denneulin A, Bras J (2016) Use of nanocellulose in printed electronics: a review. *Nanoscale*
605 8:13131–13154. doi: 10.1039/C6NR03054H
- 606 Ishida O, Kim D-Y, Kuga S, et al (2004) Microfibrillar carbon from native cellulose. *Cellulose* 11:475–
607 480. doi: 10.1023/B:CELL.0000046410.31007.0b
- 608 Jean B, Dubreuil F, Heux L, Cousin F (2008) Structural Details of Cellulose
609 Nanocrystals/Polyelectrolytes Multilayers Probed by Neutron Reflectivity and AFM. *Langmuir*
610 24:3452–3458. doi: 10.1021/la703045f
- 611 Jiang F, Hsieh Y-L (2014) Amphiphilic superabsorbent cellulose nanofibril aerogels. *J Mater Chem A*
612 2:6337–6342. doi: 10.1039/C4TA00743C
- 613 Jiménez-Saelices C, Seantier B, Cathala B, Grohens Y (2017) Spray freeze-dried nanofibrillated
614 cellulose aerogels with thermal superinsulating properties. *Carbohydr Polym* 157:105–113.
615 doi: 10.1016/j.carbpol.2016.09.068
- 616 Jin H, Nishiyama Y, Wada M, Kuga S (2004) Nanofibrillar cellulose aerogels. *Colloids Surf Physicochem*
617 *Eng Asp* 240:63–67. doi: 10.1016/j.colsurfa.2004.03.007
- 618 Jorfi M, Foster EJ (2015) Recent advances in nanocellulose for biomedical applications. *J Appl Polym*
619 *Sci* 132:n/a-n/a. doi: 10.1002/app.41719
- 620 Klemm D, Cranston ED, Fischer D, et al (2018) Nanocellulose as a natural source for groundbreaking
621 applications in materials science: Today's state. *Mater Today* 21:720–748. doi:
622 10.1016/j.mattod.2018.02.001
- 623 Klemm D, Kramer F, Moritz S, et al (2011) Nanocelluloses: A New Family of Nature-Based Materials.
624 *Angew Chem Int Ed* 50:5438–5466. doi: 10.1002/anie.201001273
- 625 Kriechbaum K, Munier P, Apostolopoulou-Kalkavoura V, Lavoine N (2018) Analysis of the Porous
626 Architecture and Properties of Anisotropic Nanocellulose Foams: A Novel Approach to Assess
627 the Quality of Cellulose Nanofibrils (CNFs). *ACS Sustain Chem Eng* 6:11959–11967. doi:
628 10.1021/acssuschemeng.8b02278
- 629 Lavoine N, Bergström L (2017) Nanocellulose-based foams and aerogels: processing, properties, and
630 applications. *J Mater Chem A* 5:16105–16117. doi: 10.1039/C7TA02807E
- 631 Lavoine N, Desloges I, Dufresne A, Bras J (2012) Microfibrillated cellulose – Its barrier properties and
632 applications in cellulosic materials: A review. *Carbohydr Polym* 90:735–764. doi:
633 10.1016/j.carbpol.2012.05.026

- 634 Martin C, Jean B (2014) Nanocellulose/polymer multilayered thin films: tunable architectures
635 towards tailored physical properties. *Nord Pulp Pap Res J* 29:19–30. doi: 10.3183/npprj-2014-
636 29-01-p019-030
- 637 Martoia F, Cochereau T, Dumont PJJ, et al (2016) Cellulose nanofibril foams: Links between ice-
638 templating conditions, microstructures and mechanical properties. *Mater Des* 104:376–391.
639 doi: 10.1016/j.matdes.2016.04.088
- 640 Mueller S, Sapkota J, Nicharat A, et al (2015) Influence of the nanofiber dimensions on the properties
641 of nanocellulose/poly(vinyl alcohol) aerogels. *J Appl Polym Sci* 132:. doi: 10.1002/app.41740
- 642 Munier P, Gordeyeva K, Bergström L, Fall AB (2016) Directional Freezing of Nanocellulose Dispersions
643 Aligns the Rod-Like Particles and Produces Low-Density and Robust Particle Networks.
644 *Biomacromolecules* 17:1875–1881. doi: 10.1021/acs.biomac.6b00304
- 645 Naderi A, Lindström T, Sundström J (2015) Repeated homogenization, a route for decreasing the
646 energy consumption in the manufacturing process of carboxymethylated nanofibrillated
647 cellulose? *Cellulose* 22:1147–1157. doi: 10.1007/s10570-015-0576-4
- 648 Nechyporchuk O, Belgacem MN, Bras J (2016) Production of cellulose nanofibrils: A review of recent
649 advances. *Ind Crops Prod* 93:2–25. doi: 10.1016/j.indcrop.2016.02.016
- 650 Nguyen BN, Cudjoe E, Douglas A, et al (2016) Polyimide Cellulose Nanocrystal Composite Aerogels.
651 *Macromolecules* 49:1692–1703. doi: 10.1021/acs.macromol.5b01573
- 652 Osorio DA, Seifried B, Moquin P, et al (2018) Morphology of cross-linked cellulose nanocrystal
653 aerogels: cryo-templating versus pressurized gas expansion processing. *J Mater Sci* 53:9842–
654 9860. doi: 10.1007/s10853-018-2235-2
- 655 Podsiadlo P, Sui L, Elkasabi Y, et al (2007) Layer-by-Layer Assembled Films of Cellulose Nanowires
656 with Antireflective Properties. *Langmuir* 23:7901–7906. doi: 10.1021/la700772a
- 657 Ravikovitch PI, Neimark AV (2001) Characterization of Micro- and Mesoporosity in SBA-15 Materials
658 from Adsorption Data by the NLDFT Method. *J Phys Chem B* 105:6817–6823. doi:
659 10.1021/jp010621u
- 660 Revol J-F, Bradford H, Giasson J, et al (1992) Helicoidal self-ordering of cellulose microfibrils in
661 aqueous suspension. *Int J Biol Macromol* 14:170–172. doi: 10.1016/S0141-8130(05)80008-X
- 662 Robitzer M, Renzo FD, Quignard F (2011) Natural materials with high surface area. Physisorption
663 methods for the characterization of the texture and surface of polysaccharide aerogels.
664 *Microporous Mesoporous Mater* 140:9–16. doi: 10.1016/j.micromeso.2010.10.006
- 665 Rudaz C, Courson R, Bonnet L, et al (2014) Aeropectin: Fully Biomass-Based Mechanically Strong and
666 Thermal Superinsulating Aerogel. *Biomacromolecules* 15:2188–2195. doi:
667 10.1021/bm500345u
- 668 Sacui IA, Nieuwendaal RC, Burnett DJ, et al (2014) Comparison of the Properties of Cellulose
669 Nanocrystals and Cellulose Nanofibrils Isolated from Bacteria, Tunicate, and Wood Processed
670 Using Acid, Enzymatic, Mechanical, and Oxidative Methods. *ACS Appl Mater Interfaces*
671 6:6127–6138. doi: 10.1021/am500359f

- 672 Sehaqui H, Zhou Q, Berglund LA (2011) High-porosity aerogels of high specific surface area prepared
673 from nanofibrillated cellulose (NFC). *Compos Sci Technol* 71:1593–1599. doi:
674 10.1016/j.compscitech.2011.07.003
- 675 Trache D, Hussin MH, Haafiz MKM, Thakur VK (2017) Recent progress in cellulose nanocrystals:
676 sources and production. *Nanoscale* 9:1763–1786. doi: 10.1039/C6NR09494E
- 677 Tripathi A, Tardy BL, Khan SA, et al (2019) Expanding the upper limits of robustness of cellulose
678 nanocrystal aerogels: outstanding mechanical performance and associated pore compression
679 response of chiral-nematic architectures. *J Mater Chem A*. doi: 10.1039/C9TA03950C
- 680 Wei H, Rodriguez K, Renneckar S, J. Vikesland P (2014) Environmental science and engineering
681 applications of nanocellulose-based nanocomposites. *Environ Sci Nano* 1:302–316. doi:
682 10.1039/C4EN00059E
- 683 Yang X, Cranston ED (2014) Chemically Cross-Linked Cellulose Nanocrystal Aerogels with Shape
684 Recovery and Superabsorbent Properties. *Chem Mater* 26:6016–6025. doi:
685 10.1021/cm502873c
- 686 Zhao S, Malfait WJ, Guerrero-Alburquerque N, et al (2018) Biopolymer Aerogels and Foams:
687 Chemistry, Properties, and Applications. *Angew Chem Int Ed* 57:7580–7608. doi:
688 10.1002/anie.201709014
- 689 Zhao Y, Li J (2014) Excellent chemical and material cellulose from tunicates: diversity in cellulose
690 production yield and chemical and morphological structures from different tunicate species.
691 *Cellulose* 21:3427–3441. doi: 10.1007/s10570-014-0348-6
- 692