Supplementary information of

Elastic Constants of Biological Filamentous Colloids: Estimation and Implications on Nematic and Cholesteric Tactoid Morphologies

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**Figures S1** Length distributions of amyloid fibrils. The histograms show the length distributions of amyloid in the isotropic phase (left), in the isotropic-nematic mixture (center) and in the nematic phase (right). The solid lines represent the log-normal fits.

**Figures S2** Length distributions of sulfated cellulose nanocrystals. The histograms show the
length distributions of SCNCid in the isotropic phase (left), in the isotropic-nematic mixture (center) and in the nematic phase (right). The solid lines represent the log-normal fits.

**Figure S3** Length distributions of carboxylated cellulose nanocrystals. The histograms show the length distributions of CCNC in the isotropic-nematic mixture. The solid line represents the log-normal fit.

**Figure S4** Height distributions of BLG amyloid fibrils (A), SCNC (B) and CCNC (C). The histograms show the height distributions for the three systems extracted from AFM analysis. The solid lines represent the normal distribution fit. The average height, corresponding to the rods diameter (D), resulted 3.75±0.83 nm for BLG, 4.6±1.00 nm for SCNC and 3.75±1.18 nm for CCNC.

**Effective diameter**

In order to evaluate the elastic constant behavior of the three system studied, the effect of electrostatic interactions on the effective diameter of the rods is investigated. The effective diameter proposed by Onsager, when taking into account the changes become:
\[ D_{\text{eff}} = D + k^{-1}(\ln A + C + \ln 2 - \frac{1}{2}) \]

where \( D \) is the diameter of the rod, \( k^{-1} \) the Debye length, \( C \) the Euler's constant \((C = 0.577)\) and \( A \), for weakly charged rod in anisotropic phase, is given by eq. (5.1) in ref.\(^1\) and is equal to:

\[ A = \frac{8\pi Q e^{-kD}}{v_{\text{eff}} k^3 D^2 K_1^2 \left( \frac{1}{2} k D \right)} \]

Where \( Q \) is the Bjerrum length \((0.71 \text{ nm})\), \( K_1 \) the modified Bessel function \( v_{\text{eff}} \) the linear charge density.

For BLG we have a linear charge density of \(~0.43 \text{ e/nm}\), at ionic strength of \(13.4 \text{ mmol/L}\), Debye 2.6 nm\(^2\). For CCNC we have from ionic strength of \(~0.56 \text{ mmol/L}\) \((\text{Gustav acs nano})\), surface charge \(0.038 \text{ e/nm}^2\), lin. Charge 0.19 e/nm, Debye Length 12.9 nm\(^3\). For SCNC we have an ionic strength 4.2 mM/L, lin. Charge 0.66 e/nm, Debye length 4.7 nm\(^4\).

**Persistence length measurements**

Beta-lactoglobulin amyloid fibrils are characterized by a persistence length of 1.98 \(\mu\)m\(^5\). For the other two systems SCNC and CCNC, AFM measurements were performed and persistence length values extracted from MS end-to-end distance \(<R^2>\) plots in fig. S2. In case of SCNC and CCNC the persistence length estimated was 3.3 and 1.3 \(\mu\)m respectively. Due to the crystalline nature of cellulose rods, it is intuitive to have persistence length higher compared to amyloid, such as SCNC. However, CCNC seems show more flexible behavior due to the numerous kinks formed during sample preparation\(^6\).
**Figure S5** Persistence length measurements. The blue dots are experimental points and the red line is the fit. The persistence length of SCNC and CCNC measurements obtained by AFM analysis are plotted in panel (A) and (B) respectively.

<table>
<thead>
<tr>
<th>System</th>
<th>D (nm)</th>
<th>D_{eff} (nm)</th>
<th>&lt;L&gt; (nm)</th>
<th>L_p (nm)</th>
<th>L_p/&lt;L&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLG</td>
<td>3.75±0.8</td>
<td>5.5±0.8</td>
<td>652±400</td>
<td>1980</td>
<td>3.0±1.8</td>
</tr>
<tr>
<td>SCNC</td>
<td>4.6±1.0</td>
<td>15.7±1.0</td>
<td>325±168</td>
<td>3319</td>
<td>10.2±5.3</td>
</tr>
<tr>
<td>CCNC</td>
<td>3.75±1.1</td>
<td>20.0±1.1</td>
<td>465±281</td>
<td>1370</td>
<td>2.9±1.8</td>
</tr>
</tbody>
</table>

*Table S1* Summary table of the characteristic parameters for the three different systems extracted from AFM analysis of 486, 426 and 400 particles for BLG, SCNC and CCNC respectively.

**Molecular Theory**

Following the theory proposed in Ref.\(^1\), the elastic contributions \( F_{el} \) to the free energy of a nematic/cholesteric phase formed by a polydisperse mixture of fibrils can be expressed as a functional of the contour length distribution of fibrils \( \nu(L) \), where \( L \) is the length of a fibril. The contour length distribution obeys the normalization condition \( \int_0^\infty \nu(L) = 1 \). By truncating the Taylor expansion of \( F_{el} \) with respect to the nematic director displacements, we can write:
\[ \frac{F_{el}}{V} = \frac{1}{2} K_1 q_1^2 + \frac{1}{2} K_2 q_2^2 + \frac{1}{2} K_3 q_3^2 \quad (1) \]

where \( q_1, q_2 \) and \( q_3 \) are the wavelengths of the distortion associated to splay \( (K_1) \), twist \( (K_2) \) and bend elastic constants, respectively.

Following Ref.7, segments (fibrils) shorter than \( L_0 \) are considered as rigid, while those longer than or equal to \( L_0 \) are decomposed into effective rigid segments of length \( L_0 \), so that the elastic constants can be written as follows:

\[ K_l = \frac{k_B T \eta(\Phi) A^2}{2} \left[ \int_0^{L_0} \int_0^{L_0} dL \, dL' \, \nu(L)\nu(L') \, \overline{v}_{ll}(L,L') + 2 \int_0^{L_0} \int_0^{\infty} dL \, dL' \, \nu(L)\nu(L') \, \frac{L'}{L_0} \, \overline{v}_{ll}(L,L_0) + \int_0^{\infty} \int_0^{\infty} dL \, dL' \, \nu(L)\nu(L') \, \frac{L \, L'}{L_0^2} \, \overline{v}_{ll}(L_0,L_0) \right] \quad (2) \]

where \( A = \frac{c_m}{\mu_0 L_c} \) with \( c_m \) mass concentration of fibrils, \( \mu_0 \) the mass of a fibril per unit length and \( L_c \) the average length of the fibrils (the values of these parameters used in the calculations are reported in Table 1), \( T \) is the temperature, \( \overline{v}_{ll} \) are the generalized excluded volumes between two fibrils 1 and 2 of length \( L \) and \( L' \) respectively and \( \eta(\Phi) \) is the Parsons–Lee which is introduced to account for higher order terms in the virial expansion. Here, \( \Phi = \xi \phi \), with \( \phi \) the volume fraction of fibrils is an effective volume fraction and \( \xi \) a scaling factor which should be intended to an adjustable parameter which is introduced to account for electrostatic repulsion. In our calculations we set \( \xi = 2.7 \), so that the value of the splay elastic constant \( (K_1) \) of betalactoglobuline fibrils is roughly in line with the other estimates discussed in the main text (i.e. \( K_1 \approx 1 \text{pN} \)).

The generalized excluded volumes \( \overline{v}_{ll} \) can be expressed as follows:
\[
\vec{v}_{ii}(L, L', \alpha_N) = - \int \left( \vec{R}'_{12} \right)^2 e_{12}^{LL'} \left( \vec{R}_{12}, \vec{\Omega}_1, \vec{\Omega}_2 \right) \left( \vec{u}_1 \cdot \vec{\hat{X}} \right) \left( \vec{u}_2 \cdot \vec{\hat{X}} \right) \left. \left( \hat{f}_o(x) \right) \right|_{x=\vec{u}_1 \cdot \vec{\hat{Z}}} \left. \left( \hat{f}_o(x) \right) \right|_{x=\vec{u}_2 \cdot \vec{\hat{Z}}}
\]

\[d \vec{R}_{12} d \vec{\Omega}_1 d \vec{\Omega}_2 \]  

(3)

where \( \vec{u}_i \) is the unit vector parallel to the axis of the fibril \( i \) (with \( i = 1, 2 \)) which is treated as an

hard cylinder , \( \vec{\Omega}_i \) are the Euler angles that define its orientation in the laboratory frame, \( \vec{R}'_{12} \) is a
cartesian component of vector \( \vec{R}_{12} = (X_{12}, Y_{12}, Z_{12}) \), which is the center-of-mass position of fibril

2 relative to fibril 1 in a reference frame \((X, Y, Z)\) with \( Z \) parallel to the local director at the position

of fibril 1 \( (\vec{R}'_{12} \) is equal to \( X_{12} \) for \( i = 1 \), \( Y_{12} \) for \( i = 2 \), \( Z_{12} \) for \( i = 3 \) ), \( \vec{\hat{X}} \) and \( \vec{\hat{Z}} \) are the unit vectors

parallel to the \( X \) -axis and \( Z \) -axis respectively, \( f_o(x) \) is the Onsager trial function, \( i.e. \)

\[ f_o(x) = \frac{\alpha_N}{4\pi \sinh \alpha_N} \cosh(\alpha_N \cos x), \]  

(4)

\( \hat{f}_o(x) \) is its first derivative with respect to its argument and \( e_{12}^{LL'} \) is the Mayer function\(^{12} \), \( i.e. \)

\[ e_{12}^{LL'} \left( \vec{R}_{12}, \vec{\Omega}_1, \vec{\Omega}_2 \right) = \exp \left\{ - \frac{U_h(\vec{R}_{12}, \vec{\Omega}_1, \vec{\Omega}_2)}{k_BT} \right\} - 1 \]  

(5)

where \( U_h(\vec{R}_{12}, \vec{\Omega}_1, \vec{\Omega}_2) \) is the hard core interaction potential between two fibrils:

\[ U_h(\vec{R}_{12}, \vec{\Omega}_1, \vec{\Omega}_2) = \begin{cases} 
\infty, & \text{if } 1,2 \text{ overlap} \\
0, & \text{if } 1,2 \text{ do not overlap} 
\end{cases} \]  

(6)

The parameter \( \alpha_N \) in Eq. (4) is related to the degree of orientational order \( (\alpha_N = 0 \text{ in the isotropic}

phased and \( \alpha_N > 0 \text{ for calamitics}) \). From \( \alpha_N \) the order parameter \( S \) can be calculated through the

following relation:

\[ S = \int_0^\pi f_o(\theta) P_2(\cos \theta) 2\pi \sin \theta d\theta \]  

(7)
where $P_2(x)$ is the second order Legendre polynomial.

Concerning $L_0$, according to Ref.\(^\text{13}\), it has to be related to the deflection length $\lambda = \frac{L_p}{a_N}$ rather than the bare persistence length $L_p$ and thus we set $L_0 = \lambda = \frac{L_p}{a_N}$ (see Table 1 for the experimental values of $L_p$ used in the calculations).

As in Ref.\(^\text{7}\) the contour length distribution $\nu(L)$ of amyloid fibrils in Eq. (2) is conveniently modelled by a log-normal distribution, i.e.

$$
\nu(L) = \frac{A}{L \sigma \sqrt{2\pi}} e^{-\frac{\left(\ln(L/L_0)\right)^2}{2\sigma^2}}
$$

where $A$ is a normalization constant and $\sigma$ and $L_0$ are two parameters. The values of $\sigma$ and $L_0$, which we used in the calculations, are obtained from the experimental contour length distribution and they are shown in Table 1.

The generalized excluded volumes $V_{ii}$, which enter into Eq. (2), are calculated for several values of $\alpha_N$, ranging from 3.62 ($S=0.4$) to 50.0 ($S=0.94$), by employing the same procedure based on Monte Carlo integration which is discussed in the SI of Ref.\(^\text{1}\). In these calculations fibrils are modelled as hard cylinders of effective diameter $D_{eff}$, whose values, for the different fibrils investigated here, are those discussed in the main text and which are shown in Table 1.
Table S2 Values of the parameters \(c_m\), \(\mu_0\), \(L_c\) (see Eq. 2), \(\sigma\) and \(L\) (see Eq. 8) and \(D_{eff}\) used in the theoretical calculations of the elastic constants for betalactoglobuline (BLG), CCNC and SCNC amyloid fibrils.

**SCNC radial cholesteric formation.** Equilibrated radial cholesteric configuration has not been observed in SCNC based tactoids. However, it was possible observing droplet characterized by high volumes where the director field configuration starts to bend, suggesting that the droplet is evolving to radial cholesteric configuration\(^7\). The droplet diameter of these droplets approaches 200 µm, which is equal to the couvette inner diameter, and so the droplet cannot further grow in size and complete the transition at even higher volumes..

Figure S6 Uniaxial to radial cholesteric transition. On the left, PolScope color map image of a S-CNC tactoid where the director field starts to bend, suggesting that the droplet might undergo...
radial cholesteric configuration at higher volumes. On the right, another droplet with director field bending following the interface of the droplet, viewed under cross-polarized microscope. This non-equilibrium configuration is assumed by tactoids undergoing uniaxial to radial cholesteric transition.

**Order Parameter estimation from birefringence**

Polscope analysis provides the quantitative evaluation of the optical retardance of the specimen pixel by pixel, allowing the estimation of the orientational order parameter $S^{10,11}$. In particular, the optical retardance $r$ is equal to $r = d\Delta n$, where $\Delta n$ is the specific birefringence of the specimen and $d$ his thickness. The orientational order parameter $S = \Delta n / \Delta n_0$ where $\Delta n_0$ is the birefringence corresponding to a perfectly aligned nematic phase ($S=1$). The exact value of $\Delta n_0$ is experimentally elusive since the order parameter is often well below the unity for lyotropic liquid crystals. To overcome this limitation we assumed as $\Delta n_0$ the highest birefringence value measured for each system. In particular, these strongly birefringence domains were found at the bulk nematic phase in macroscopically phase separated systems (BLG and SCNC), or at the edge of the couvette (for CCNC liquid-glue interface) where slow evaporation induces strong fibril alignment.

The order parameter was calculated for several tactoids by measuring thickness and specific retardance at the center of each tactoid in the same couvette used for the other experiments (inner $D = 200 \ \mu m$). Figure S4 show the order parameter plotted as function of tactoid volume for tactoids with different director field configurations.

The average order parameter measured in BLG cholesteric tactoids is equal to 0.54, which is in good agreement with previously reported value of $0.49\pm0.03$, extracted with fluorescence analysis$^7$. In case of CCNC and SCNC the average order parameter measured in cholesteric tactoids is equal to $0.58\pm0.06$ and $0.89\pm0.06$ respectively.
Figure S7 Order Parameter estimation from birefringence measurements: Estimation of the order parameter $S$ for tactoids of BLG (A), CCNC (B) and SCNC (C). The points in orange correspond to homogeneous tactoids, in yellow bipolar and blue uniaxial cholesteric. The average order parameter $S$ in uniaxial cholesteric droplets for the three system is equal to $0.54\pm0.03$ for BLG, $0.58\pm0.06$ for CCNC and $0.89\pm0.06$ for SCNC.
References


9. Oldenbourg, R., Salmon, E. D. & Tran, P. T. Birefringence of single and bundled


