A study across scales to unveil microstructural regimes in the multivalent metal driven self-assembly of cellulose nanocrystals

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Understanding the behaviour of self-assembled systems, from nanoscale building blocks to bulk materials, is a central theme for the rational design of high-performance materials. Herein, we unraveled, at different length scales, how the self-assembly of TEMPO-oxidised cellulose nanocrystals (TOCNC) into rod fractal gels is directed by the complexation of Fe³⁺ ions on the surface of the colloidal particles. Different specificities in Fe³⁺ binding on the TOCNC surface and conformational changes of the nanocellulose chain were unveiled by paramagnetic NMR spectroscopy. The macroscopic properties of systems presenting different concentrations of TOCNC and Fe³⁺ ions were investigated by rheology and microscopy, demonstrating the tunability of the self-assembly of cellulose nanorods driven by Fe³⁺ complexation. Near-atomic coarse-grained molecular dynamics simulations were developed to get microscopic insight into the behaviour of this colloidal system. We found that the formation of different self-assembled architectures is driven by metal-nanocellulose complexation combined with the attenuation of electrostatic repulsion and water structuration around cellulose, leading to different microstructural regimes, from isolated nanorods to disconnected rod fractal clusters and rod fractal gels. These findings lay the foundation to unlock the full potential of cellulose nanocrystals as sustainable building blocks to develop assembled materials with defined structural control for a range of advanced applications.

Introduction

The hallmark of life is the assembly of individual and simple units into bigger and complex structures. Examples are DNA strands that pair, proteins that form quaternary structures, lipids that assemble into membranes and cells that form tissues. Importantly, a pronounced difference can exist between the individual and the collective behaviour of these units which, by acting in concert, are able to produce final complex architectures over various length scales. Colloids are interesting systems with biomimetic behaviour in terms of self-aggregation. Particularly, the self-assembly of polyelectrolytic colloids in the presence of ions in water solutions has been attributed to different mechanisms such as the formation of ion-colloid complexes, ion-ion correlations, and gradients of ionic strength or pH. In addition to polyelectrolyte-ion interactions, a fine balance of polyelectrolyte-polyelectrolyte and polyelectrolyte-water intermolecular forces needs to be considered to fully understand the self-assembly of these systems. Therefore, to achieve insights into the structure-property correlation of these assembled systems, it is pivotal to conduct investigations that link atomistic, molecular, macromolecular and supramolecular level perspectives to the final material properties.

To understand the complex self-assembly landscape of polyelectrolytic nanocolloids in the presence of salts, we selected to study the interaction between 2,2,6,6-tetramethylpiperidin-1-y1-oxyl (TEMPO)-oxidized cellulose nanocrystals (TOCNCs) and the trivalent transition metal Fe³⁺ ions as a model system (Fig. 1). Cellulose nanocrystals (CNCs), are affordable, renewable, and biocompatible nanomaterials attractive for a wide range of applications given their outstanding mechanical and optical properties. CNCs have a needle-like nanorod structure and, thanks to the presence of negative charges, form stable colloidal suspensions in water. The negative charges on the CNCs surface can either derive from the harsh hydroslysis of cellulose with sulfuric acid or from other chemical treatments as, for example, with the TEMPO oxidant. In the last case, TOCNCs present a nearly regioselective modification of hydroxymethyl groups into carboxylic functional groups, that was employed for the functionalisation with different biomolecules and polymers. The negative charges on the CNCs surface and on other TEMPO-oxidised nanocelluloses, such as the TEMPO-oxidised cellulose nanofibers, have shown binding affinity to alkali and transition metal ions leading to the formation of hydrogels. The sol-gel transition of aqueous CNCs suspension in the presence of metal ions has been investigated by rheology and scattering techniques and the mechanism of formation of the 3D network has been proposed. At first, the negative repulsive forces generated by the carboxylate or sulphate surface charges of cellulose are screened by the metals present in solutions.

Sufficient screening enables then the particles to come close together to form an interconnected network.
enough to establish van der Waals and other cohesive interactions, i.e., coordination complexes with metal ions, and hydrogen bonding, that result into gelation. This behaviour has been generally investigated in the presence of monovalent ions but deeper insight on the assembly mechanism in the presence of multivalent ions, able to establish specific interactions, is still missing.

Herein, the mechanism by which TOCNC nanorods form a 3D network in the presence of Fe$^{3+}$ ions is proposed by using a combination of experimental investigations and computational modelling, offering a study across scales spanning from molecular interactions to macromolecular properties. In particular, the presence of Fe$^{3+}$ ions allowed the use of paramagnetic NMR approaches unveiling the specific interaction between Fe$^{3+}$ ions and surface exposed carboxylic groups of TOCNC and the cooperative contribution of the surrounding hydroxyl groups in the coordination of metal ions. The redox behaviour of the coordination complex involving Fe$^{3+}$ ions and TOCNC has been investigated by cyclic voltammetry, showing the facile modulation of the complexation of the colloidal network by redox stimuli. Changes in macroscopic properties, finely tuned by different concentrations of cellulose and metal ion, have been fully characterized by rheology and scanning electron microscopy (SEM). Finally, the molecular mechanism behind the nanocellulose rods self-assembly in the presence of Fe$^{3+}$ ions, including the transition from isolated nanorods to disconnected rod fractal clusters and to rod fractal gels (Fig. 1), has been unveiled by molecular dynamics simulation with the MARTINI force-field. Even though initially developed for biomolecular simulations, the MARTINI force-field finds nowadays extensive applications in the soft matter field.

Experimental Section

Materials. TOCNC 1wt% dispersion with a degree of oxidation of 1.9–2.0 mmol/g was purchased from Cellulose Lab (Canada). TOCNC nanorod dimensions were 8-15 nm in wideness and 100-150 nm in length. Iron(III) nitrate nonahydrate [Fe(NO$_3$)$_3$]·9H$_2$O; 98%] and deuterium oxide (D$_2$O, 99.9% atom D) were purchased from Sigma-Aldrich.

Samples preparation. TOCNC 1wt% dispersion was freezer-dried and re-dispersed in Milli-Q water or D$_2$O. For NMR experiments, samples were prepared by adding defined aliquots of a Fe$^{3+}$ stock solution (10 mM) on a 2wt% dispersion of TOCNC in D$_2$O under stirring. For rheology and SEM analysis, samples containing 1wt% or 3wt% of TOCNC were prepared in Milli-Q water, followed by addition of a Fe$^{3+}$ stock solution (100 mM) under stirring.

Nuclear Magnetic Resonance. All NMR experiments were performed using a Bruker DMS 600 MHz cryoprobe spectrometer operating at 600.13 MHz for $^1$H experiments and 150.90 MHz for $^{13}$C experiments.
Oscillatory rheology was performed with a parallel plate geometry on gels of TOCNC 3 wt% with Fe$^{3+}$ 50 mM. The linear viscoelastic region was determined by amplitude strain sweeps. Following this, dynamic frequency sweeps were performed with oscillatory frequencies between 0.1 and 100 Hz at a constant strain of 0.1%.

**Scanning Electron Microscopy.** Scanning electron microscopy (SEM) images were taken on a Zeiss Sigma HD microscope (5kV), equipped with a Schottky FEG source, one detector for backscattered electrons, and two detectors for secondary electrons (InLens and Everhart Thornley). SEM samples were prepared by supercritical point drying and analysed without coating.

**MD Simulations.** Coarse-grained molecular dynamics simulation using MARTINI_v2.3P force-field have been developed, starting from an all-atom model, to investigate the self-assembly of TOCNC nanorods in the presence of different concentrations of Fe$^{3+}$ ions in water (see Scheme S1 for an outline of the simulations). Simulations were performed using GROMACS2020.1 simulation package with CG martini_v2.3P force field to which we added the PX atom type and the related self- and cross-term interactions.15

**Results and Discussion**

**Molecular interaction of Fe$^{3+}$ ions with TOCNC.** At first, we recorded 1D $^1$H and 2D $^{13}$C HSQC NMR spectra of TOCNC 1wt% dispersions in the absence and in the presence of Fe$^{3+}$ ions and monitored the disappearance of signals mediated by the paramagnetic relaxation enhancement (PRE) effect of Fe$^{3+}$ ions. Acquisition of $^1$H NMR spectrum of TOCNC revealed four different spin systems, namely the reducing (r), the non-reducing (nr), the central (c) and the oxidised (ox) spin systems (Fig. 1a), which could be unambiguously assigned (Fig. 2a, top projection in the $^1$H-$^{13}$C HSQC spectrum). As shown in Fig. 2a, the $^1$H-$^{13}$C HSQC spectra recorded in the presence of Fe$^{3+}$ ions 5 mM show disappearance of the peaks assigned to C3,5 of the oxidised ring, the C3, C4, and C5 of the non-reducing ring, the C2, C3, C4, and C5 of the reducing ring, and the C6 of the unfunctionalized central TOCNC residues. In addition, a reduction of cross-peak volume was recorded for all the other assigned carbons (C1c, C1r, C2c, C4c and C3,5c). As the PRE effect is distance-dependent, these data indicate a spatial proximity between the distinct TOCNC spin systems and the Fe$^{3+}$ ions in the bound state. To monitor the reduction in the cross-peaks’ volume, titration experiments with Fe$^{3+}$ concentrations from 0 to 5 mM were performed. The decay of the signal of each isolated peak in the $^1$H-$^{13}$C HSQC spectra was measured as the ratio of the cross-peak volume between the samples in the presence and in the absence of Fe$^{3+}$ ions. All the reported ratios show values lower than one (Table S1) and a linear decay (Fig. S1), which could be ascribed to an increase of the fraction of TOCNC-bound Fe$^{3+}$ ions upon increasing of the Fe$^{3+}$ concentration in the sample. Analysis of the slope of the lines shows a faster decay for C3,5ox, indicating a stronger binding of Fe$^{3+}$ ions to the carboxyl residues. The second fastest decay was observed for the cross-peaks of the non-reducing and reducing residues, followed by the decay of the signals assigned to the C6 in unfunctionalized glucohexopyranose rings.
Finally, the carbons from C6 in the central spin system showed the slowest decay. The different behaviour observed for the nanorods extremities could be attributed to either a more intimate interaction with the Fe$^{3+}$ ions, or their higher exposition to the surrounding solvent and ions. To investigate the effect of Fe$^{3+}$ ions on the conformation of residues on the TOCNC surface, 2D $^1$H-$^1$H NOESY spectra in the absence and in the presence of Fe$^{3+}$ ions (1 and 2 mM) were acquired at increasing mixing times (Fig. S2, S3 and S4). Significant changes in the normalised NOE build-up curve rates in the presence of Fe$^{3+}$ ions were recorded for the H1 to H6, H6' and H3,5r (Fig. 2b), which might indicate a conformational rearrangement of the hydroxymethyl group and of the interglycosidic linkage at that reducing ring following TOCNC-Fe$^{3+}$ interaction. Protons H3,5ox, H3,4nr and H3,4,5c, on the contrary, showed less pronounced changes (Fig. S4a and S4b) or no changes at all (Fig. S4c), inferring that, even upon binding of the Fe$^{3+}$ ions to the carboxylic function, no significant conformational changes occurred. These NMR based investigations allowed us to obtain a detailed map of the interaction between TOCNC and the Fe$^{3+}$ ions, as represented in Fig. 2c. In particular, Fe$^{3+}$ ions interact with the carboxylic groups on the TOCNC surface, as well as with the diols of the glucohexopyranose units at the cellulose chain extremities and the hydroxymethyl group in the unfunctionalized glucohexopyranose residues. This is not surprising given their higher exposition to the environment and their ability to bind Fe$^{3+}$, as previously demonstrated for different carbohydrates and also for catechol-Fe$^{3+}$ complexes.

The role of water on TOCNC-Fe$^{3+}$ interaction. To probe the properties of water at the TOCNC interface upon introduction of the metal crosslinker we performed STD$^{33, 34}$ and SDTD NMR experiments. First, we collected STD build-up curves from TOCNC to HDO at increasing saturation times (Fig. S5), then we applied the SDTD NMR protocol. From the SDTD NMR characterisation, we observed a stiffer slope of the SDTD curves upon addition of Fe$^{3+}$ ions (Fig. 2d and Table S2), which can be ascribed to a faster-magnetization transfer from the gelator to the bound HDO and, therefore, to an enhanced water structuration. The sudden increase in water structuration, observed upon the first addition of 1 mM Fe$^{3+}$ ions, appears to gradually level out for higher Fe$^{3+}$ concentrations, from 3 to 5 mM, which indicates the saturation of the effect between TOCNC and HDO. From these results, it is clear that the interaction between TOCNC and Fe$^{3+}$ ions induces a tighter binding of HDO to TOCNC surface or an increase in the water confined among TOCNC nanorods.

The redox behaviour of the TOCNC-Fe$^{3+}$ complex. To understand the change of the redox properties of Fe$^{3+}$ ions in complex with TOCNC, we performed cyclic voltammetry (CV) measurements. Solutions of Fe$^{3+}$ ions at different concentrations show a quasi-reversible behaviour, with the anodic and cathodic peaks at 0.61 V and 0.29 V vs Ag/AgCl, respectively (Fig. S6). When CV of Fe$^{3+}$ ions is performed in the presence of TOCNC 1wt%, the Fe$^{3+}$/Fe$^{2+}$ redox couple show a more reversible electrochemical behaviour (Fig. 3a), with a shift of the anodic peak potential towards more negative values (0.49 V) compared to Fe$^{3+}$ ions free in solution (0.61 V).
Fig. 3 (a) Cyclic voltammograms of TOCNC 1wt% in the presence of various Fe$^{3+}$ concentrations in a solution of Na$_2$SO$_4$ 0.1 M (scan rate of 0.01 V/s). The arrow indicates the scanning direction. Dashed lines and solid lines refer to the left and right y-axis, respectively. (b) Comparison of the anodic and cathodic peaks potential in the absence (gray) and in the presence (red) of TOCNC for increasing concentrations of Fe$^{3+}$ ions.

The peak-to-peak separation ($\Delta E_p$, Fig. 3b) revealed that, for low concentrations of Fe$^{3+}$ ions, the presence of TOCNC facilitates the electron transfer at the electrode-solution interface. On the contrary, at a concentration of Fe$^{3+}$ 10 mM and above in TOCNC 1wt% a larger peak separation is measured in comparison with Fe$^{3+}$ free in solution (Fig. 3b). The larger variation in potential required with higher Fe$^{3+}$ ions could be attributed to the Fe$^{3+}$ ions non complexed with TOCNC and the initial formation of aggregates of TOCNC and Fe$^{3+}$ ions at high concentrations. In comparison, the more positive oxidation potential of the 10 mM solution of Fe$^{3+}$ ions without TOCNC, indicates a slower diffusion of the Fe$^{3+}$ ions in the viscous TOCNC-Fe$^{3+}$ solution. These results indicate that the oxidation/reduction reactions of Fe$^{3+}$/Fe$^{2+}$ redox couple occurred at lower potential and is more reversible in the presence of TOCNC for low concentration of Fe$^{3+}$ ions, which can be attributed to a complexation-induced stabilization of the metal ions by TOCNC.

The transition from sol to gel of TOCNC in the presence of Fe$^{3+}$. To probe the TOCNC ability to form hydrogels in the presence of Fe$^{3+}$ ions, we performed experiments on 1wt% (TOCNC1) and 3wt% (TOCNC3) TOCNC suspensions at different Fe$^{3+}$ concentrations, 0 (Fe0), 5 (Fe5) and 50 (Fe50) mM. The six resulting systems are henceforth labelled as TOCNC1Fe0, TOCNC1Fe5, TOCNC1Fe50, TOCNC3Fe0, TOCNC3Fe5 and TOCNC3Fe50. The formation of hydrogel occurred only for TOCNC3Fe50, while at lower TOCNC or lower Fe$^{3+}$ concentrations we always observed a sol form. Changes in solution properties have been characterized by viscometry experiments. TOCNC 1wt% and 3wt% alone or in the presence of a low concentration of Fe$^{3+}$ ions show a typical shear thinning behaviour at low shear rate, attributed to the alignment of the

Fig. 4 Effect of TOCNC and Fe$^{3+}$ concentrations on sol and gel formation. (a) Viscometry experiments of TOCNC1Fe0, TOCNC1Fe5, TOCNC1Fe50, TOCNC3Fe0 and TOCNC3Fe5. (b) Dynamic frequency sweeps for TOCNC3Fe50 system. The variations in the storage moduli, $G'$, and loss moduli, $G''$, are shown with closed and open symbols, respectively. (c) State diagrams for Fe$^{3+}$ solutions of various concentrations added to TOCNC dispersions of 1wt% or 3wt% concentrations. The low viscosity sol and gel states are indicated as triangles and squares, respectively. The star indicates the high viscosity sol. (d) SEM images (scale bar 100 μm) of (i) TOCNC1Fe50, (ii) TOCNC3Fe0, (iii) TOCNC3Fe5 and iv) TOCNC3Fe50 systems. The images are labelled on top with the corresponding symbols in the state diagram.
n nanocrystals along the shear direction (Fig. 4a), and a Newtonian plateau at high shear rates, dictated by the complete alignment of the particles.\textsuperscript{36} This result agrees with the electrochemical behaviour observed at low Fe\textsuperscript{3+} concentrations in TOCNC dispersions, where a higher reversibility of the CVs is obtained due to the formation of homogeneous TOCNC-Fe\textsuperscript{3+} complexes. Interestingly, a change to high viscosity shear thinning behaviour over the whole range of investigated shear rates has been observed for TOCNC1Fe50 (Fig. 4a). This transition suggests the presence of aggregates within the system,\textsuperscript{36} which is further supported by the electrochemical experiments performed on TOCNC dispersions at high Fe\textsuperscript{3+} concentrations. The properties of TOCNC3Fe50 gel, instead, were measured by oscillation rheology. In the investigated frequency range, G' values about one order of magnitude greater than G" were measured, confirming the gel-like properties of the sample (Fig. 4b). In addition, both moduli showed little dependence on frequency in the studied range with a loss factor (\(\tan \delta = G''/G'\)) significantly smaller than unity, suggesting the formation of a stable gel with a dominant elastic behaviour and with minimal defects that would contribute to viscous energy loss.\textsuperscript{21, 22} A summary of the changes of TOCNC physical states upon introduction of Fe\textsuperscript{3+} ions is reported in Fig. 4c.

To gain further insight in the sol-gel structures, SEM images of the TOCNC-Fe\textsuperscript{3+} sols and hydrogel have been acquired (Fig. 4d). SEM images revealed a three-dimensional highly porous network created by the interconnection of the nanorods. In the case of TOCNC1Fe50, the large distribution of the area of the pores (Fig. S7) is indicative of the reorganization of the nanorods into microstructures, even without reaching the degree of particle interpenetration required to create a gel structure. This finding aligns with the highly viscous rheological behaviour of the systems described above. SEM images obtained for TOCNC 3wt% in the absence and in the presence of Fe\textsuperscript{3+} (5 and 50 mM) show a decrease in the pore diameter from 43 \(\mu\)m for TOCNC3Fe5 to 29 \(\mu\)m for TOCNC3Fe50. The inverse correlation between the concentration of Fe\textsuperscript{3+} ions and the system porosity is in good agreement with the change from sol to gel observed by rheological investigation for the TOCNC3Fe50 system. This trend is consistent with previously reported behaviour of CNC in the presence of monovalent ions, showing that suspensions with low concentration of CNC (1wt%) and salt (NaCl 10 mM) form isolated clusters, while networks with densely aggregated rods resulted from higher concentrations of CNC and salt (CNC 5wt% and NaCl 100 mM).\textsuperscript{37} In a recent study, Amini et al.\textsuperscript{38} investigated the rheological behaviour of CNC hydrogels with different salts and found that the addition of divalent and trivalent ions enhances their viscoelastic properties, leading to a denser structure with smaller pores, which is in agreement with our results. In particular, for CNC 5wt% in the presence of Fe\textsuperscript{3+} 100 mM, a uniform structure with pore sizes ranging between 31 and 47 \(\mu\)m was reported. The smaller pore size observed for our TOCNC3Fe50 system could be due to the stronger coordination of Fe\textsuperscript{3+} ions to the carboxylic groups present on the TOCNC surface.

**Modelling the Fe\textsuperscript{3+}-driven self-assembly of TOCNC.** To gain further insight into the molecular mechanism behind self-assembly of nanocellulose rods in the presence of Fe\textsuperscript{3+} ions, we carried out molecular dynamics (MD) simulations, using a coarse-grained approach based on the MARTINI 2 force-field.\textsuperscript{39} Our cellulose nanocrystal model was built starting from all-atom coordinates of cellulose I\(\beta\) fibrils.\textsuperscript{40} In order to maintain the physical properties of the TOCNC used in our experimental work (i.e., crystal structure, aspect ratio, and charge density), we constructed TOCNC nanorods formed by 9 chains of cellulose (Fig. 5b, top), each composed of 20 repeating cellobiose units (Table S4) and with a total number of 100 negative charges on...
their surface (Fig. 5b, bottom). Hence, cellulose chains were constructed by repetition of cellobiose units, each one described by 6 different beads (B1, B2, B3, B4, B5 and B6), and assigned to different types of beads (P1, P4 and PX) with distinct polar character (Fig. 5a and Table S3), following the scheme proposed by Lopez et al.25 In addition, we introduced another kind of bead (BX, of bead type QA), bearing a negative off-centred charge to represent the carboxyl groups (Fig. 5a and Table S4). We performed MD simulations for three systems: i) 2 rods of TOCNC with 600 Fe³⁺ cations (TOCNC1Fe50); ii) 6 rods of TOCNC with 60 Fe³⁺ and 420 Na⁺ cations (TOCNC3Fe5); iii) 6 rods of TOCNC with 600 Fe³⁺ cations (TOCNC3Fe50) (see Supporting Information Table S5 and S6). The CG model, despite some inherent approximations, was able to capture differences in the ions-TOCNC interaction between the three systems. In all systems, cations were adsorbed on the surface of the rods, near the negative charges. Therefore, we calculated the average number of positive charges as a function of the distance from the centre of the 8X beads (Fig. 5b, Fig. S8 and Table S7). In both TOCNC1Fe50 and TOCNC3Fe50 this number is higher than 0.8, meaning that almost all charges on TOCNC are screened and no electrostatic repulsion exists between the rods. In the system with lower Fe³⁺ concentration (TOCNC3Fe5), on the contrary, the average number of positive charges, coming from both Fe³⁺ and Na⁺ cations, amounts to about 0.6. Compared to Na⁺, Fe³⁺ ions tend to stay slightly closer to the carboxyl groups (see Fig. S9) and for longer times, as shows by the decay of the probability to remain in their proximity (Fig. 5c(iii)). Figure 5c(ii) also shows some differences in the average survival probability of water near TOCNC at high and low Fe³⁺ concentrations. This effect could be related to the increase of water structuring upon increase of Fe³⁺ concentration as observed from the SSTD NMR experiments. MD simulations confirmed distinct self-assembly regimes for different combinations of TOCNC and Fe³⁺ concentrations, consistent with the experimental findings from rheology and SEM characterisation. In particular, the TOCNC3Fe5 and TOCNC3Fe50 show different behaviours that reflect their rheological properties. In both cases, the same starting configuration was assumed, with the six rods randomly oriented (Fig. S10 b and c). In TOCNC3Fe5 during the trajectory the rods stay apart from each other and, even though they occasionally approach, then they move apart (Fig. 6a and Fig. S11a). In TOCNC3Fe50, on the contrary, their random motion rods can get closer and, when close enough, they form persistent contacts, which in most cases are bridged by Fe³⁺ ions (Fig. 5b and Fig. S11b). Crossings in the system predominantly occurs between the highly polar B1 and B5 beads, which correspond to the hydroxyl groups at positions C2 and C3 of the gluconohexopyranose ring located at the ends of the nanorods. This observation aligns with the results of our H-13C HSQC and H-1H NOESY experiments. In TOCNC1Fe50 the two rods, initially nearly parallel at a distance of 100 Å from each other (Fig. S11b), during the trajectory approach and reach a distance of around 40 Å. However, the formation of persistent contacts was not observed, which can be ascribed to the low collision probability at low density of rods. Overall, MD simulations offer a microscopic perspective on the findings from rheology and SEM experiments. At low Fe³⁺ concentration, electrostatic repulsion between the rods hinders their approach, while at higher Fe³⁺ concentration the negative surface charges of TOCNC are effectively screened, allowing the rods to come into contact. Consequently, persistent connections are established through the Fe³⁺ ions leading to the formation of a physically crosslinked 3D network when the rod density is high enough (Fig. S12). However, if the rod density falls within the dilute regime (volume fraction much smaller than the square of the aspect ratio),42 only local contacts can be established, resulting in the formation of clusters comprised of crosslinked rods.

Conclusions
We established a model system to study the self-assembly behaviour of polyelectrolytic nanocellulose in the presence of multivalent transition metal ions across different length scales. We demonstrated that by varying the concentration of TOCNC and Fe³⁺ ions, it is possible to tune the macroscale properties of the investigated systems from sol to gel. By NMR spectroscopy we showed that Fe³⁺ ions do not exclusively interact with the carboxylic function on the TOCNC surface, but also with the...
dioxane on the glucohexopyranose unit and, specifically, with the dioxane at the cellulose chain extremities. This result is in agreement with near-atomistic coarse-grained molecular dynamics simulations, which demonstrate the ability of TOCNC to cross-link by means of the extremities through complexion with Fe³⁺ ions. In addition, we found a good correlation between the MD simulations and the changes in water structuration revealed by NMR spectroscopy. Furthermore, the combination of these simulations with rheological and microscopic (SEM) data allowed us to translate supramolecular associative interactions into the behaviour of a larger network, obtaining mechanistic insights into gel formation. Hence, we have understood how these rods progressively change microstructural regimes, from isolated nanorods to disconnected rod fractal clusters and rod fractal gels, with a consequent transition from sol to high viscous sol to gel. Fe³⁺ ions play a twofold specific role: (i) they bind to the carboxylate groups, so screening the negative charges on the TOCNC surface and suppressing the electrostatic repulsion that stabilise the colloidal suspension, and (ii) they drive the formation of a crosslinked network bridging pairs of rods. In addition, we demonstrated the electrochemical modulation of the properties of this TOCNC-Fe³⁺ system, which could pave the way for the development of new stimuli-responsive materials based on these metal-coordinated colloidal networks. To the best of our knowledge, this is the first time that such a detailed description of the mechanism of rod fractal gel formation, driven by the interaction between polyelectrolytic nanocellulose and multivalent transition metal ions, has been achieved. We have demonstrated that the application of the innovative toolkit herein proposed enables to achieve unique insights and to correlate molecular interactions, supramolecular architectures and material properties, expanding the toolbox for a study across scales of colloidal and, more generally, self-assembling systems.

Conflicts of interest
There are no conflicts to declare.

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Notes and references