Abstract: Hybrid nanoparticles involving 10-nm silver nanoparticles (AgNPs) nucleated on
unmodified rod-like cellulose nanocrystals (CNCs) were prepared by chemical reduction. H₂O₂ used
as a post-treatment induced a size-shape transition following a redox mechanism, passing from
10-nm spherical AgNPs to 300-nm triangular or prismatic NPs (AgNPrisms), where CNCs are the
only stabilizers for AgNPs and AgNPrisms. We investigated the role of the H₂O₂/AgNP mass
ratio (α) on AgNPs. At α values above 0.20, the large amount of H₂O₂ led to extensive oxidation
that produced numerous nucleation points for AgNPrisms on CNCs. On the contrary, for α below
0.20, primary AgNPs are only partially oxidized, releasing a reduced amount of Ag⁺ ions and thus
preventing the formation of AgNPrisms and reforming spherical AgNPs. While XRD and EXAFS
reveal that the AgNP fcc crystal structure is unaffected by the H₂O₂ treatment, the XANES spectra
proved that the AgNP–AgNPrism transition is always associated with an increase in the metallic Ag
fraction (Ag₀). In contrast, the formation of new 15-nm spherical AgNPs keeps the initial Ag₀/Ag⁺
ratio unmodified. For the first time, we introduce a complete guide map for the fully-controlled
preparation of aqueous dispersed AgNPs using CNC as a template.

Keywords: CNC/AgNP hybrids; H₂O₂ redox post-treatment; H₂O₂/AgNP mass ratio; oxidation state;
XANES-EXAFS

1. Introduction

In recent decades, silver nanoparticles (AgNPs) have attracted considerable attention because of
their tunable properties, allowing them to be used in various application fields such as sterilization [1,2],
catalysis [3,4], electronics [5–7], and optics [8,9]. Indeed, AgNP surface plasmon resonance (SPR)
properties strongly depend on nanoparticle size and shape (e.g., spheres, rods, cubes, bipyramids,
prisms, triangles, and hexagons) [10–12], making them suitable for biolabeling [13] or surface-enhanced
Raman spectroscopy (SERS) [14,15]. In this context, two-dimensional plate-like nanostructures
(also referred to as silver nanoprisms (AgNPrisms) [12]) emerged because of their typical anisotropy
(i.e., a lateral dimension larger than the thickness), which allows the tuning of their localized surface
plasmon resonance (LSPR) linked to the aspect ratio [12,16,17]. Several approaches have made it possible
to synthesize AgNPrisms. Lithographic techniques [17,18] are often used to obtain surface-confined
AgNP and AgNPrism arrays with a good size-shape control, but they are not adapted to solution-based
applications. Light-mediated methods are based on the use of visible light to orient pre-formed NPs.
(i.e., photo-induced aggregation [19]). As proposed by Jin et al. [20], monodispersed AgNPrisms with an edge length of 30–120 nm can be obtained via a dual-beam illumination. In addition, photo-induced growth treatment can also be performed on Ag seeds in the presence of Ag$^+$ ions that are reduced at the seed surface [21]. Callegari et al. [22] indicate that irradiance conditions can transform AgNPs in suspension into larger NPs with different shapes, demonstrating that the photochemical growth of metallic NPs can be controlled by selecting the light color. Other methods to synthesize AgNPs and AgNPrisms are based on the chemical reduction of silver ions [23–25]. Several studies [10,23,25–27] propose the use of a reducing agent to produce the Ag seeds (e.g., sodium borohydride, NaBH$_4$) and the addition of a capping agent to promote AgNP stabilization (e.g., trisodium citrate (TSC); polyvinylpirrolidine (PVP); and dextran). For example, Sun et al. [28] indicated that Ag triangular nanoplates can be produced from spherical 3.5-nm AgNPs previously synthesized by reducing Ag$^+$ ions in the presence of NaBH$_4$, PVP, and sodium citrate. The authors underline that PVP provided stable Ag seeds smaller than the critical size necessary for morphological transformation, whereas citrate effectively induced nanoplate formation. However, the extensive use of stabilizers could prevent AgNP and AgNP Prism functionalization, preventing them from being used for sensing applications [29]. In addition to capping agents, other authors [10,11,30] propose the introduction of hydrogen peroxide (H$_2$O$_2$) to promote the formation of AgNPrisms. Indeed, the addition of H$_2$O$_2$ to the suspension where AgNPs are already synthesized induces the oxidative dissolution of unstable Ag seeds while preserving those with twin defects or stacking defaults [11,30,31]. Moreover, under a neutral environment, H$_2$O$_2$ is able to reduce Ag$^+$ ions into Ag atoms, which then aggregate into seeds necessary for the formation of AgNPrisms. In their pioneering work, Métraux et al. [23] proposed a simple procedure to prepare AgNPrisms in aqueous solution using AgNO$_3$/NaBH$_4$/PVP/TSC/H$_2$O$_2$, controlling the AgNPrisms thickness. In 2011, an approach similar to the one proposed by Métraux et al. was adopted by Zhang et al. [31] to identify the role of each agent involved in AgNP Prism formation. They pointed out the critical role of H$_2$O$_2$ instead of citrate in the well-known chemical reduction route to AgNPrisms. Parnklang et al. [32] developed a novel approach for AgNP Prism synthesis with a tunable localized surface plasmon reference, focusing on the chemical shape transformation of AgNPs by the addition of H$_2$O$_2$ and proving that the H$_2$O$_2$ injection rate and mixing efficiency are the key parameters to control the LSPR wavelengths.

Finally, biopolymeric templates such as cellulose nanocrystals (CNCs) and nanofibers (CNFs) can also be used as stabilizers in the synthesis of AgNPs and AgNPrisms, allowing the production of new hybrid nanomaterials. These studies [33–36] used modified CNC and CNF surface chemistry. In this context, 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO)-oxidized CNFs appear to be an excellent substrate for the stabilization of AgNPs synthesized by NaBH$_4$ reduction since most of the surface primary C6 hydroxyls can be converted to carboxylates by TEMPO oxidation [37]. The introduction of H$_2$O$_2$ as redox post-treatment allows the conversion from AgNPs to AgNPrisms in this hybrid system as well. To our knowledge, all the post-treatments reported on AgNPs nucleated on CNCs required preliminary surface treatment.

In this study, for the first time, nanocellulose with unmodified surface is used to investigate the impact of a H$_2$O$_2$ redox post-treatment on the morphology, physicochemical properties, and structural organization of grafted AgNPs. We followed a two-step process: First, AgNP nucleation is initiated by chemical reduction on native CNCs to form a CNC/AgNP hybrid without any additional capping agent and second, a H$_2$O$_2$ redox post-treatment convert AgNPs into AgNPrisms. We elucidate the role of the initial H$_2$O$_2$/AgNP mass ratio ($\alpha$), proposing a detailed characterization of the AgNP-AgNP Prism conversion. In particular, we correlate the size-shape variations of AgNPs to their oxidation state evaluated by X-ray absorption near-edge structure (XANES) and to their crystallographic structure using X-ray powder diffraction (XRD) and extended X-ray absorption fine structure (EXAFS). Our approach allows a complete control of the properties of AgNPs in hybrid nanomaterials, opening the way to new application fields.
2. Experimental Section

Chemicals. Cellulose nanocrystals were purchased from CelluForce (Windsor, Canada, product number 2015-009). They were obtained from bleached Kraft pulp by acid hydrolysis and then neutralized to sodium form and spray-dried (length = 183 ± 88 nm; cross-section = 6 ± 2 nm; aspect ratio = 31) [38]. Silver nitrate (AgNO$_3$ ≥ 99%), sodium borohydride (NaBH$_4$ ≥ 96%), and hydrogen peroxide (H$_2$O$_2$) were purchased from Sigma-Aldrich (France) and used without further purification. All of the aqueous suspensions and solutions were prepared using ultra-pure water.

Synthesis of CNC/AgNP hybrid suspensions and H$_2$O$_2$ post-reaction. To produce well-dispersed CNC/AgNP hybrid suspensions, CNC aqueous suspension (2 g/L) was dialyzed against water for 3 days (dialysis bath volume to sample volume = 10:1). Then, 10 mL were mixed at room temperature for 1 min with various amounts of AgNO$_3$ aqueous solution (50 mM, from 15 to 700 µL). A quantity of 500 µL of freshly-prepared NaBH$_4$ aqueous solution (100 mM) was then added to reduce Ag$^+$ ions, obtaining AgNPs. NaBH$_4$ aqueous solution was placed in ice to minimize its decomposition. AgNP formation induced a variation of color suspension (i.e., from translucent to yellow). The final suspension was covered with aluminum foil to prevent AgNP oxidation by light, mixed at room temperature for 24 h and then dialyzed against water for 24 h. For the H$_2$O$_2$ redox post-treatment, various amounts of H$_2$O$_2$ (0, 40, 80, 120, 160, and 250 µL) were added under stirring to the hybrid suspension immediately after the introduction of NaBH$_4$. An exothermic reaction took place, leading to the formation of gas bubbles resulting from the H$_2$O$_2$ decomposition [10]. Finally, the suspension was dialyzed against water for 24 h to remove unreacted reagents (dialysis bath volume to sample volume = 10:1).

Characterization. A Mettler-Toledo UV7 spectrophotometer (Columbus, OH, USA) equipped with a 10-mm quartz cell was used to record the light-visible absorbance of hybrid suspensions in the 300–900 nm range. All the samples were diluted (1:10) and ultra-pure water was used as a blank reference.

The AgNP content in CNC/AgNP hybrid suspensions was determined by digesting 1 mL of sample with 40 mL water/aqua regia mixture (i.e., 30% v aqua regia; HCl/HNO$_3$: 3/1) and then analyzing it by atomic absorption spectroscopy (AAS) (ICE 3300 AAS, Thermo Fisher, Waltham, MA, USA). A calibration curve was obtained using a silver standard solution (1000 µg/mL, Chem-Lab NV, Zedelgem, Belgium) at different concentrations, from 0.25 to 10 ppm. Two independent measurements were repeated for each sample. The final AgNP content was expressed in mg of AgNP per g of sample (wt%).

To obtain scanning transmission electron microscopy (STEM) acquisitions, hybrid suspensions were diluted with water at 0.5 g/L in CNC content. Then, 10 µL were deposited on glow-discharged carbon coated grids (200 meshes, Delta Microscopies, Maurensac, France) for two minutes and the excess was removed using Whatman filter paper. The grids were dried overnight in air and then coated with a 0.5-nm platinum layer by an ion-sputter coater (LEICA AM ACE600, Wetzlar, Germany). Images were recorded with a Quattro S field emission gun scanning microscope (Thermo Fisher Scientific, Waltham, MA, USA) at 10 kV using a STEM detector. The acquired STEM images were analyzed by ImageJ software to determine the mean AgNP Feret diameter (i.e., the largest distance between two tangents to the contour of the measured particle), averaged over the largest possible number of particles (from 20 to 100, depending on the sample).

XANES and EXAFS measurements were performed to study the AgNP oxidation state and bulk atomic structure (e.g., bond length, interatomic distance), respectively. XANES-EXAFS spectra were simultaneously recorded in transmission mode at the Ag K-edge from 25,250 to 27,750 eV on a SAMBA beamline at the SOLEIL synchrotron (Saint Aubin, France). The Si (220) monochromator was calibrated to 25,515.6 eV at the first inflection point of the Ag foil XANES spectrum. The freeze-dried hybrid samples were pressed to obtain circular pellets with a diameter of 6 mm with a controlled amount of AgNPs to reach an absorption edge jump close to 1. The pellets were placed on a sample rod and immersed in a liquid nitrogen bath before being introduced into the He cryostat (T = 20 K). Silver foil (Agfoil) and AgNO$_3$ aqueous solution with 1 wt% glycerol (AgH$_2$O) were used as standards. For each
sample, one scan was collected in transmission and in continuous scan mode along the 25,250 to 27,750 eV energy range with 5 eV/s monochromator velocity and 0.08 s/point integration time. Scans were normalized and background-subtracted using the Athena software package [39]. XANES data were analyzed by a linear combination fitting (LCF) procedure using the fit range \([E_0 - 20 \text{ eV}, E_0 + 50 \text{ eV}]\) with \(E_0\) set to 25,514 eV, and using Agfoil and AgH\(_2\) standards as components. All component weights were forced to be positive, and the relative proportions of the components were forced to add up to 100%. The EXAFS oscillations were background-subtracted using an autobk algorithm (Rbkb = 1, k-weight = 3) and the Fourier transform of the \(k^3\)-weighted EXAFS spectra was calculated over a \(k\) range of 2.5–18 Å\(^{-1}\) using a Hanning apodization window (width of the transition region window parameter = 1). \(k^3\) EXAFS fitting was performed in the 2.35–7.7 Å distance range (R) with the Artemis [39] interface to IFEFFIT library, using least-squares refinements. Paths used for fitting standards and samples were obtained from a metallic silver crystallographic model [40] using the FEFF6 algorithm included in the Artemis interface. Only paths with a rank higher than 7% were considered. \(E_0\) was fixed to 25520 eV. For sample fitting, the amplitude reduction factor \(S_0^2\) was fixed to 0.978 after being determined by fitting the first coordination sphere of the Agfoil spectrum over a range of 2.30–2.83 Å. Degeneracy of the paths, energy shift \(\Delta E_0\), R shift \(\Delta R\), and thermal and static disorder \(\sigma^2\) were fitted for each of the selected paths for a total of 52 independent points and 19 variables. All R-factors were lower than 0.05.

A Bruker D8 Discover diffractometer was used to record XRD diffractograms. Cu-K\(_{\alpha1}\) radiation (Cu K\(_{\alpha1}\)1.5405 Å) was produced in a sealed tube at 40 kV and 40 mA, parallelized using a Gobèl mirror parallel optic system and then collimated to produce a 500-mm beam diameter. The data were collected in a 2\(\theta\) angle range from 3\(^\circ\) to 70\(^\circ\) (10 min of acquisition). The AgNP crystallite size (CS) was determined using Scherrer’s equation [41]:

\[
CS = \frac{K\lambda}{\beta \cos \theta}
\]

where \(K\) is the shape factor (0.9), \(\lambda\) is the X-ray wavelength (1.54 Å), \(\beta\) is the full-width at half-maximum (FWHM), and \(\theta\) is the angle of the diffraction peak of the crystalline phase (Bragg’s angle). The FWHM was determined considering the AgNP characteristic peak at 2\(\theta\) = 38\(^\circ\).

3. Results and Discussion

The H\(_2\)O\(_2\) redox reaction is known to lead to the conversion of spherical AgNPs into AgNPrisms. To shed light on the role of the H\(_2\)O\(_2\)/AgNP mass ratio (\(\alpha\)) on the AgNP-AgNPrism transition, we proposed a two-step approach. We first synthesized CNC/AgNP hybrid aqueous suspensions where hydrophilic CNCs are used as substrate to easily disperse and stabilize AgNPs of about 10 nm in water. Indeed, the good CNC dispersion is ensured by the negative surface charges (SO\(_3^–\) groups), and the hydroxyl groups on the CNC surface act as nucleation points that allow the growth of well-dispersed AgNPs on the CNC surface [42]. In a second step, the H\(_2\)O\(_2\) redox post-treatment is performed adding various H\(_2\)O\(_2\) volumes to the primary hybrid suspension. The following notations will be used from now on:

(i) AgNPs: the primary 10-nm spherical NPs nucleated on the CNC surface in initial CNC/AgNP hybrid suspensions;
(ii) AgNPs\(_{H_2O_2}\): AgNPs after the addition of H\(_2\)O\(_2\);
(iii) AgNPrism: AgNPs\(_{H_2O_2}\) for which the H\(_2\)O\(_2\) post-treatment leads to the formation of triangular shaped NPs [12,34].

Such an experimental approach made it possible to investigate the AgNP size-shape transition as a function of \(\alpha\).

Firstly, we focused on a CNC/AgNP hybrid aqueous suspension at 8.7 wt% AgNP treated with different volumes of H\(_2\)O\(_2\) (0, 40, 80, 120, 160, and 250 \(\mu\)L), thus varying the \(\alpha\) parameter from 0 up
to 0.42 (Figure 1a). After the addition of H$_2$O$_2$, the samples were dialyzed and the average amount of AgNP (AgNPs$_{\text{H}_2\text{O}_2}$) was estimated at 6.9 ± 1.2 wt% for the six samples, proving the efficiency of the H$_2$O$_2$ reduction. In Figure 1a, the reference hybrid suspension (i.e., no H$_2$O$_2$) displays the typical yellow color of aqueous suspensions with well-dispersed and stabilized AgNPs. The increasing addition of H$_2$O$_2$ enshrines a variation of the color of the suspension from yellow to blue. In the UV-Vis spectra (Figure 1b), the $\lambda_{\text{max}}$ value of the in-plane dipole surface peak was shifted from 400 nm for the reference sample, to 450 nm and 495 nm for samples at $\alpha$ equal to 0.07 and 0.13, respectively. This shift is associated with an increase in the average diameter of AgNPs$_{\text{H}_2\text{O}_2}$ from 10 nm for the reference, to 34 nm and 52 nm for $\alpha$ of 0.07 and 0.13, respectively, as measured on STEM images (Figure 1c). Furthermore, the presence of two shoulders around 340 and 380 nm in the UV-Vis spectra reveal the beginning of a morphological modification of the primary AgNPs, even if the AgNPs$_{\text{H}_2\text{O}_2}$ still displayed a quite well-defined spherical shape. At $\alpha = 0.20$, a sharp low intensity peak is present at $\lambda_{\text{max}} = 335$ nm (red line in Figure 1b), which is generally referred to as the out-of-plane quadrupole resonance peak, representing a good indicator of the AgNP architectural modification related to the aspect ratio [19]. Indeed, the AgNPs$_{\text{H}_2\text{O}_2}$ lost their spherical shape, assuming an irregular triangular shape with an increase in their average size (i.e., shift of the in-plane peak to a higher wavelength). The low-intensity peak at 335 nm was also identified in the UV-Vis spectra of hybrids at $\alpha$ equal to 0.27 and 0.42, for which the in-plane dipole plasmon peak was outside of the measurement window. At these high $\alpha$ values, the AgNPs$_{\text{H}_2\text{O}_2}$ presented a well-defined triangular shape with an average diameter of up to 324 nm. Such a size-shape transition clearly identified the passage from 10-nm spherical NPs to 300-nm AgNPrisms. We emphasize here that the synthesis of primary AgNPs and the subsequent production of AgNPrisms by H$_2$O$_2$ redox treatment can be achieved using unmodified native CNCs as stabilizers, without the introduction of additional capping agents, since hydroxyl groups on the CNC surface act as perfect nucleation points [42].

Since silver can exist in various forms (e.g., metallic Ag$_0$, ionic Ag$^+$, and Ag$_2$O oxide), the oxidation state of AgNPs and AgNPs$_{\text{H}_2\text{O}_2}$ in hybrid samples were characterized by XANES. An example of fitting of spectra in the XANES region by the LCF procedure was reported in Figure S1. The analysis of the XANES spectra of CNC/AgNP hybrid suspensions at 8.7 wt% AgNP where the $\alpha$ parameter varied from 0 up to 0.42 (Figure 2a) made it possible to reveal the increase in the variation of the Ag$_0$ content from 65% to 94% with the increase in the $\alpha$ value. The R-factor and the Chi-square values of the fits of the XANES spectra are reported in Table S1.

As for the reference hybrid, the XRD diffractograms of all the CNC/AgNP$_{\text{H}_2\text{O}_2}$ (Figure 2b) were characterized by peaks at 38.1°, 44.0°, and 64.2°, corresponding to (111), (200), and (220) planes, respectively. This clearly defined a face-centered cubic (fcc) silver structure, characterized by the isotropic nature of the crystals [34] (JCPDS Card No. 89-3722). In addition to XANES, the EXAFS spectra of the same samples were recorded and analyzed. All Fourier transform spectra of the CNC/AgNP hybrid containing 8.7 wt% AgNP (Figure S2a,b) were fitted with the same crystallographic structure of metallic silver (Ag$_0$), with an R-factor systematically lower than 0.016. The overall variation in interatomic distance (R) values obtained from the fits with increasing $\alpha$ from 0 to 0.47 (Table S2) were systematically negligible as the error bars associated with the R values always partly overlapped with each other. This indicated that the interatomic distances in the hybrids did not significantly change in comparison to the metallic silver distances and that the space group of the AgNPs still corresponded to the fcc silver structure, as suggested by XRD. It therefore follows that the initial and final crystal structural organizations were not affected by the H$_2$O$_2$ redox post-reaction. These experimental results seemed to suggest that the size-shape transition from AgNPs to AgNPrisms could be achieved only above a critical $\alpha$ value of 0.20, and that the H$_2$O$_2$ post-reduction did not affect the crystalline structure while partially modifying the oxidation state of the AgNPs$_{\text{H}_2\text{O}_2}$.
To better shed light on the impact of the \( \alpha \) parameter on the efficiency of the \( \text{H}_2\text{O}_2 \) redox post-treatment and on the morphological and physicochemical properties of AgNPs/\( \text{H}_2\text{O}_2 \) hybrid suspensions at a fixed concentration but containing 12.5, 18.6, or 24.7 wt\% AgNP were also mixed with various \( \text{H}_2\text{O}_2 \) volumes (from 40 to 250 \( \mu \text{L} \)). All the \( \alpha \) values considered in the study are summarized in Table 1. After introducing \( \text{H}_2\text{O}_2 \), the Ag contents were found to be equal to 9.3, 17.1, and 24.1 wt\%, respectively, confirming once again that this redox post-treatment occurs at a very high yield, close to 100%. In Figure 3a, we propose STEM images of hybrids at various initial AgNP
contents mixed with 160 µL of H$_2$O$_2$, thus varying $\alpha$ from 0.09 to 0.27. It appeared that the formation of AgNPrisms with a size of 150–300 nm was obtained only at $\alpha$ equal to 0.20 and 0.27. In contrast, AgNPs_H$_2$O$_2$ produced at $\alpha$ equal to 0.09 and 0.12 maintained a spherical shape with an average diameter of 15–20 nm. AgNP_H$_2$O$_2$ size distributions are reported in Figure S3a,b, and the NP average diameters are summarized in Table S3. These last results agreed with those at $\alpha$ equal to 0.12 (18.6 wt% AgNP) and 0.09 (24.7 wt% AgNP), we found the same spherical morphology as that obtained for hybrids at $\alpha$ equal to 0.13 and 0.07 (8.7 wt% and 12.5 wt% AgNP, respectively). On the other hand, AgNPrisms were formed for higher $\alpha$, as shown at 0.20 and 0.27. Furthermore, it could be observed that smaller satellite AgNPs (between 10 and 35 nm) were formed near AgNPrisms, which were not observed before the H$_2$O$_2$ reduction step (Figures 1c and 3a). Even if the average diameter of these smaller AgNPs was reported in Figure S3a, they were not considered to determine the average size of the AgNPrisms.

### Table 1. Characteristics of CNC/AgNP hybrids at different AgNP contents with a H$_2$O$_2$ redox post-treatment (CNC/AgNP$_{H_2O_2}$).

| Ag Content in CNC/AgNP Hybrid (wt%) | H$_2$O$_2$ Vol. (µL) | Ag Content in CNC/AgNP$_{H_2O_2}$ Hybrid (wt%): $\alpha$ (H$_2$O$_2$/AgNP Mass Ratio) | AgNP$_{H_2O_2}$ Diameter (nm): 1 | CS Crystallite Size (nm): 2 | Ag Content (%): 3 |
|-----------------------------------|---------------------|---------------------------------------------------------------------------------|-----------------|------------------------|-----------------|
| 8.7 ± 0.05                        | 0                   | -                                                                               | 10 ± 7          | 7.4                    | 65 ± 2          |
|                                  | 40                  | 0.07                                                                            | 34 ± 20         | 7.0                    | 77 ± 2          |
|                                  | 80                  | 0.13                                                                            | 52 ± 21         | 7.4                    | 82 ± 2          |
|                                  | 120                 | 0.20                                                                            | 121 ± 68        | -                      | -               |
|                                  | 160                 | 0.27                                                                            | 296 ± 70        | 6.5                    | 95 ± 3          |
|                                  | 250                 | 0.42                                                                            | 325 ± 100       | 7.0                    | 94 ± 3          |
| 12.5 ± 0.08                      | 0                   | -                                                                               | 11 ± 6          | 3.2                    | 57 ± 2          |
|                                  | 40                  | 0.05                                                                            | 49 ± 23         | -                      | 62 ± 2          |
|                                  | 80                  | 0.09                                                                            | 49 ± 18         | -                      | 55 ± 2          |
|                                  | 120                 | 0.14                                                                            | 77 ± 29         | -                      | 63 ± 2          |
|                                  | 160                 | 0.20                                                                            | 145 ± 51        | 6.7                    | 97 ± 3          |
|                                  | 250                 | 0.29                                                                            | -               | -                      | 100 ± 3         |
| 18.6 ± 0.1                        | 0                   | -                                                                               | 12 ± 9          | -                      | 32 ± 1          |
|                                  | 40                  | 0.03                                                                            | 12 ± 10         | -                      | 47 ± 1          |
|                                  | 80                  | 0.06                                                                            | 17 ± 7          | -                      | 49 ± 1          |
|                                  | 120                 | 0.09                                                                            | 20 ± 9          | -                      | 43 ± 1          |
|                                  | 160                 | 0.12                                                                            | 14 ± 9          | 6.2                    | 50 ± 2          |
|                                  | 250                 | 0.18                                                                            | -               | -                      | 41 ± 1          |
| 24.7 ± 0.2                        | 0                   | -                                                                               | 11 ± 9          | 3.0                    | 34 ± 1          |
|                                  | 40                  | 0.02                                                                            | 14 ± 10         | -                      | 25 ± 1          |
|                                  | 80                  | 0.05                                                                            | 16 ± 8          | -                      | 33 ± 1          |
|                                  | 120                 | 0.07                                                                            | 17 ± 8          | -                      | 36 ± 1          |
|                                  | 160                 | 0.09                                                                            | 17 ± 6          | 7.3                    | 29 ± 1          |
|                                  | 250                 | 0.15                                                                            | 16 ± 7          | -                      | 32 ± 1          |

- Not measured, 1 by STEM analysis, 2 by XRD, 3 by XANES, and the standard error as 3% of the measured value.

The UV-Vis spectra (Figure 3b) showed the shift of the main peak outside of the measurement window and the appearance of the low-intensity peak at $\lambda_{\text{max}} = 335$ nm only for hybrids at $\alpha$ of 0.20 and 0.27, indicating a size-shape transition of 10-nm spherical AgNPs to 150–350 nm AgNPrisms. For other hybrids, the in-plane peak remained at around $\lambda_{\text{max}} = 445$ nm, with a wide low-intensity shoulder between 345 nm and 375 nm. In this case, AgNP$_{H_2O_2}$ maintained a spherical shape grafted on CNC with a slight increase in the average diameter from 10 nm to 15–20 nm.
Figure 3. (a) STEM images and (b) UV-Vis spectra of CNC/AgNP-H$_2$O$_2$ hybrids at various AgNP-H$_2$O$_2$ contents where 160 µL of H$_2$O$_2$ were added. In STEM images, bar scales of 500 nm are indicated. (c) Schematic representation of the H$_2$O$_2$ oxidation-reduction mechanism in hybrids at low and high AgNP contents (i.e., $\alpha$ parameter above and below the critical value of 0.20).

On the basis of these results, we propose a H$_2$O$_2$ redox mechanism where H$_2$O$_2$ first induced the oxidative dissolution of primary AgNPs, generating Ag$^+$ ions. After that, two scenarios are occurring; at $\alpha$ equal to or greater than 0.20 (i.e., hybrids at lower initial AgNP contents), the H$_2$O$_2$
oxidation affected quickly most of the AgNPs, except the contact location where AgNPs are effectively grafted onto the CNC surface. Indeed, H₂O₂ etching usually works on the most unstable NPs, while maintaining parts with high stability intact [30,31]. These residual sites on the CNC surface could actually work as nucleation sites for the formation of newly formed AgNPrisms. We assumed that the appearance of the bigger AgNPrisms was promoted by the limited number of Ag residual sites on the CNC surface. On the other hand, for α below 0.20 (i.e., hybrids at higher initial AgNP contents), even the introduction of the largest H₂O₂ volume probably induced only a partial etching of most of the primary AgNPs, due to the higher number of NPs with respect to the amount of H₂O₂ introduced. Thus, it appeared that the reduction step did not allow the synthesis of AgNPrisms, as in the previous case. The higher number of residual sites available on the CNC surface from the oxidative dissolution could prevent the formation of 300-nm AgNPrisms. A schematic representation of this H₂O₂ oxidation-reduction process is proposed in Figure 3c. It could thus be concluded that the mechanism that controlled the size-shape transition of primary AgNPs in a CNC/AgNP hybrid suspension was mainly governed by the H₂O₂/AgNP ratio. As for the primary AgNPs, the AgNP Prism stabilization was provided by CNCs without any additional stabilizer since, otherwise, AgNP and AgNP Prism aggregation and sedimentation occurred.

Concerning the structure, the XRD diffractograms showed the persistence of a fcc Ag crystalline structure for all the samples (Figure S4), independently of the α value. Hybrids formulated with the introduction of H₂O₂ still showed a well-defined (111) peak, indicating that even bigger AgNPrisms with a triangular shape had a fcc structure and that they were preferentially oriented parallel to the substrate during the acquisition. However, the crystallite size increased from 3.2 nm up to 7 nm with the progressive addition of H₂O₂, which confirmed that oxidation/reduction steps occurred and maintained the fcc structure intact, with a slight variation of the CS.

The EXAFS Fourier transform spectra (Figure S5a,b) of the CNC/AgNP hybrid were fitted with the crystallographic structure of metallic silver, with an R-factor systematically lower than 0.048 (Table S5). The overall variation in interatomic distance values, R, obtained from the fits were systematically negligible as the error bars associated to the R values always partly overlapped with each other.

This result showed that the interatomic distances in the CNC/AgNP_H₂O₂ hybrids do not significantly change in comparison to the metallic silver distances and that the space group of the samples still corresponds to the fcc silver structure, as shown by XRD. As previously shown, the final crystal structural organization was neither affected by the H₂O₂ redox post-reaction, nor by the initial Ag content in CNC/AgNP hybrids.
Finally, we checked the influence of the NaBH₄/AgNO₃ initial molar ratio on the H₂O₂ redox post-treatment. CNC/AgNP suspensions at the highest silver content (i.e., the lowest NaBH₄/AgNO₃ ratio equal to 1.5) were prepared to obtain the NaBH₄/AgNO₃ molar ratio of the sample at the lowest AgNP content (i.e., NaBH₄/AgNO₃ ratio = 30) and then mixed with 160 μL of H₂O₂ (α = 0.09). Even in these conditions, the UV-Vis spectra of such suspensions at an NaBH₄/AgNO₃ ratio of 30 overlapped the one of the same sample prepared at a NaBH₄/AgNO₃ ratio of 1.5 (Figure S6), thus proving that this parameter did not affect the H₂O₂ post-reduction in our experimental conditions.

4. Conclusions

In this study, we investigated the impact of the H₂O₂ redox post-treatment on the morphology, physicochemical properties, and structural organization of CNC/AgNP aqueous hybrid suspensions formulated using unmodified CNCs as bio-based support to stabilize AgNPs without the addition of any other capping agent. Hybrids at various AgNP contents (i.e., 8.7, 12.5, 18.6, and 24.7 wt%) were mixed with different H₂O₂ volumes (i.e., 0, 40, 80, 120, 160, and 250 μL) to obtain various H₂O₂/AgNP mass ratios (α) up to 0.42.

We demonstrated that a critical α value of 0.20 had to be overcome to achieve a size-shape transition from 10-nm spherical NPs (AgNPs) to 300-nm triangular or prismatic NPs (AgNPPrisms). Furthermore, we proposed an H₂O₂ redox mechanism that considered the CNCs as stabilizers for AgNP_H₂O₂ and AgNPPrisms. We speculated that at large amounts of H₂O₂, for α values higher than 0.20, the H₂O₂ oxidative action concerned most of the NP except its part effectively grafted onto the CNC surface, which acted as a nucleation seed for AgNPPrism formation. On the other hand, at α lower than 0.20, primary AgNPs were only partially oxidized and spherical AgNPs-H₂O₂ of about 15–20 nm were formed again.

We proved that the transition from 10-nm spherical AgNPs to 300-nm triangular AgNPPrisms (i.e., α ≥ 0.20) was associated with an increase in the Ag₀ content up to 100%. However, the oxidation state was slightly affected when the H₂O₂ post-reaction did not modify the size and shape of AgNPs_H₂O₂ (i.e., α < 0.20). Finally, the AgNP_H₂O₂ structure was not affected by the H₂O₂ redox reaction since a fcc model structure was maintained, regardless of the α value.

The present results make it possible to create a guide map to fully control AgNP properties in hybrid NPs where CNCs serve as substrate, making these hybrid NPs suitable for new applications fields.

Figure 4. (a) XANES spectra of hybrids at different initial AgNP content treated with 160 μL of H₂O₂ (i.e., α values above and below critical value of 0.20) (b) evolution of the average diameter of AgNPs_H₂O₂ in CNC/AgNP_H₂O₂ hybrids as a function of their Ag₀ content estimated from XANES spectra.
**Supplementary Materials:** The following are available online at [http://www.mdpi.com/2079-4991/10/8/1559/s1](http://www.mdpi.com/2079-4991/10/8/1559/s1), Figure S1: Example of a XANES spectrum of CNC/AgNP hybrid and its corresponding linear combination fit (LCF) using Agfoil and AgNO₃ aqueous solution as components, Table S1: R-factor and Chi-square values for the linear combination fitting procedure applied to the XANES spectra of CNC/AgNP hybrid suspension at 8.7 wt% mixed with various H₂O₂ volumes (i.e., various H₂O₂/AgNP mass ratios, α), Figure S2: (a) EXAFS spectra Fourier transform (solid gray lines) and fit (dotted lines); (b) magnitude and imaginary part (solid gray lines) and fit (dotted lines) of the Fourier transform; CNC/AgNP hybrid suspensions at 8.7 wt% AgNP treated with various H₂O₂ volumes (i.e., different α values), Table S2: EXAFS fit results for CNC/AgNP hybrid suspensions at 8.7 wt% AgNP treated with various H₂O₂ volumes (i.e., different α values), Figure S3: AgNP size distributions of CNC/AgNP-H₂O₂ hybrids at (a) 8.7 wt% and 12.5 wt% AgNP and (b) 18.6 wt% and 24.7 wt% AgNP, mixed with the addition of 160 µL of H₂O₂, thus varying α from 0.09 to 0.27, Table S3: Average diameter of AgNPs-H₂O₂ in CNC/AgNP hybrids at 8.7 wt%, 12.5 wt%, 18.6 wt% and 24.7 wt% AgNP, mixed with the addition of 160 µL of H₂O₂ to reach various α values, Figure S4: XRD diffractograms of hybrids at different initial AgNP content treated with 160 µL of H₂O₂ (i.e., α values above and below critical value of 0.20), Table S4: R-factor and Chi-square values for the linear combination fitting procedure applied to the XANES region of CNC/AgNP hybrid suspension at various AgNP contents mixed with 160 µL H₂O₂, Figure S5: (a) EXAFS spectra Fourier transform (solid gray lines) and fit (dotted lines); (b) magnitude and imaginary part (solid gray lines) and fit (dotted lines) of the Fourier transform of CNC/AgNP hybrid suspensions at various AgNP contents treated with 160 µL of H₂O₂ (i.e., α values from 0.09 to 0.27), Table S5: EXAFS fit results for CNC/AgNP hybrid suspensions at CNC/AgNP_H₂O₂ hybrids at various AgNP, H₂O₂ contents, treated with 160 µL of H₂O₂ (i.e., α above and below the critical value of 0.20), Figure S6: UV-Vis spectra of CNC/AgNP hybrid suspension prepared at 24.7 %wt at two different NaBH₄/AgNO₃ molar ratios (i.e., 1.5 and 30) and then mixed with 160 µL H₂O₂ (i.e., α = 0.09). In our experimental conditions, such a parameter did not affect the H₂O₂ redox post-treatment.

**Author Contributions:** Conceptualization, methodology, data curation and original draft writing: I.C. and D.M.; Microscopy acquisition analysis: D.M. and B.N.; XANES-EXAFS measurements, data analysis and discussion: C.R., G.L., D.M. and J.C.; Supervision, project administration, funding acquisition: I.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work is a contribution to the Labex SERENADE (n° ANR-11-LABX-0064) funded by the “Investissement d’Avenir” French government program of the French National Research Agency (ANR) through the A*MIDEX project (n° ANR-11-IDEX-0001-02).

**Acknowledgments:** We acknowledge SOLEIL for providing synchrotron radiation facilities on the SAMBA beamline. We are grateful to S. Durand (BIA-Nantes) for her support with FTIR measurements, F. X. Leefvre and N. Guichard (Université de Nantes) for support with AAS experiments, B. Pontoire (BIA-Nantes) for performing XRD acquisitions, and S. Haouache (BIA-Nantes) for her assistance during SAMBA beam time.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Xiu, Z.M.; Zhang, Q.B.; Puppala, H.L.; Colvin, V.L.; Alvarez, P.J.J. Negligible particle-specific antibacterial activity of silver nanoparticles. *Nanot. Lett.* 2012, 12, 4271–4275. [CrossRef]

2. Jain, P.; Pradeep, T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnol. Bioeng.* 2005, 90, 59–63. [CrossRef]

3. Aadil, K.R.; Pandey, N.; Mussatto, S.I.; Jha, H. Green synthesis of silver nanoparticles using acacia lignin, their cytotoxicity, catalytic, metal ion sensing capability and antibacterial activity. *J. Environ. Chem. Eng.* 2019, 7, 103296. [CrossRef]

4. Christopher, P.; Xin, H.; Linic, S. Visible-light-enhanced catalytic oxidation reactions on plasmonic silver nanostructures. *Nat. Chem.* 2011, 3, 467–472. [CrossRef]

5. Williams, N.X.; Noyce, S.; Cardenas, J.A.; Catenacci, M.; Wiley, B.J.; Franklin, A.D. Silver nanowire inks for direct-write electronic tattoo applications. *Nanoscale* 2019, 11, 14294–14302. [CrossRef]

6. Darroudi, M.; Ahmad, M.B.; Mashreghi, M. Gelatinous silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. *J. Optoelectron. Adv. Mater.* 2014, 16, 182–187.

7. Haes, A.J.; Van Duyne, R.P. A nanoscale optical biosensor: Sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles. *J. Am. Chem. Soc.* 2002, 124, 10596–10604. [CrossRef]

8. Rithesh Raj, D.; Prasanth, S.; Vineeshkumar, T.V.; Sudarsanakumar, C. Surface plasmon resonance based fiber optic dopamine sensor using green synthesized silver nanoparticles. *Sens. Actuators B Chem.* 2015, 224, 600–606. [CrossRef]
9. Kulkarni, A.P.; Noone, K.M.; Munechika, K.; Guyer, S.R.; Ginger, D.S. Plasmon-enhanced charge carrier generation in organic photovoltaic films using silver nanoprisms. *Nano Lett.* 2010, 10, 1501–1505. [CrossRef]

10. Tsuji, M.; Gomi, S.; Maeda, Y.; Matsunaga, M.; Hikino, S.; Uto, K.; Tsuji, T.; Kawazumi, H. Rapid transformation from spherical nanoparticles, nanorods, cubes, or bipyramids to triangular prisms of silver with PVP, citrate, and $\text{H}_2\text{O}_2$. *Langmuir* 2012, 28, 8845–8861. [CrossRef]

11. Parmaklang, T.; Lamlua, B.; Gatementa, H.; Thammacharoen, C.; Kuimalee, S.; Lohwongwatana, B.; Egkasit, S. Shape transformation of silver nanoshapers to silver nanoplates induced by redox reaction of hydrogen peroxide. *Mater. Chem. Phys.* 2015, 153, 127–134. [CrossRef]

12. Millstone, J.E.; Hurst, S.J.; Métraux, G.S.; Cutler, J.I.; Mirkin, C.A. Colloidal gold and silver triangular nanoprisms. *Small* 2009, 5, 646–664. [CrossRef]

13. Wiley, B.J.; Im, S.H.; Li, Z.Y.; McLellan, J.; Siekkinen, A.; Xia, Y. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. *J. Phys. Chem. B* 2006, 110, 15666–15675. [CrossRef]

14. Li, X.; Zhang, J.; Xu, W.; Jia, H.; Wang, X.; Yang, B.; Zhao, B.; Li, B.; Ozaki, Y. Mercaptoacetic acid-capped silver nanoparticles colloid: Formation, morphology, and SERS activity. *Langmuir* 2003, 19, 4285–4290. [CrossRef]

15. Chaudhari, K.; Ahuja, T.; Murugesan, V.; Subramanian, V.; Ganayee, M.A.; Thundat, T.; Pradeep, T. Appearance of SERS activity in single silver nanoparticles by laser-induced reshaping. *Nanoscale* 2019, 11, 321–330. [CrossRef]

16. Rycegna, M.; Cobley, C.M.; Zeng, J.; Li, W.; Moran, C.H.; Zhang, Q.; Qin, D.; Xia, Y. Controlling the synthesis and assembly of silver nanostructures for plasmonic applications. *Chem. Rev.* 2011, 111, 3669–3712. [CrossRef]

17. Pastoriza-Santos, I.; Liz-Marzán, L.M. Colloidal silver nanoplates. State of the art and future challenges. *J. Mater. Chem.* 2008, 18, 1724–1737. [CrossRef]

18. Shin, D.O.; Jeong, J.R.; Han, T.H.; Koo, C.M.; Park, H.J.; Lim, Y.T.; Kim, S.O. A plasmonic biosensor array by block copolymer lithography. *J. Mater. Chem.* 2010, 20, 7241–7247. [CrossRef]

19. Jin, R.; Cao, Y.; Mirkin, C.; Kelly, K.; Schatz, G.; Zheng, J. Photoinduced Conversion of Silver Nanospheres to Nanoprisms. *Science* 2013, 1901, 1901–1904. [CrossRef]

20. Jin, R.; Cao, Y.C.; Hao, E.; Métraux, G.S.; Schatz, G.C.; Mirkin, C.A. Controlling anisotropic nanoparticle growth through plasmon excitation. *Nature* 2003, 425, 487–490. [CrossRef]

21. Maillard, M.; Huang, P.; Brus, L. Silver Nanodisk Growth by Surface Plasmon Enhanced Photoreduction of Adsorbed Ag1+. *Nano Lett.* 2003, 3, 1611–1615. [CrossRef]

22. Callegari, A.; Tonti, D.; Chergui, M. Photochemically Grown Silver Nanoparticles with Wavelength-Controlled Size and Shape. *Nano Lett.* 2003, 3, 1565–1568. [CrossRef]

23. Métraux, G.S.; Mirkin, C.A. Rapid thermal synthesis of silver nanoprisms with chemically tailorable thickness. *Adv. Mater.* 2005, 17, 412–415. [CrossRef]

24. Dong, X.; Ji, X.; Jing, J.; Li, M.; Li, Y.; Yang, W. Synthesis of triangular silver nanoprisms by stepwise reduction of sodium borohydride and trisodium citrate. *J. Phys. Chem. C* 2010, 114, 2070–2074. [CrossRef]

25. Haber, J.; Sokolov, K. Synthesis of stable citrate-capped silver nanoprisms. *Langmuir* 2017, 33, 10525–10530. [CrossRef]

26. Zhang, Q.; Hu, Y.; Guo, S.; Goebel, J.; Yin, Y. Seeded growth of uniform Ag nanoplates with high aspect ratio and widely tunable surface plasmon bands. *Nano Lett.* 2010, 10, 5037–5042. [CrossRef]

27. Lokanathan, A.R.; Uddin, K.M.A.; Rojas, O.J.; Laine, J. Cellulose nanocrystal-mediated synthesis of silver nanoparticles: Role of sulfate groups in nucleation phenomena. *Biomacromolecules* 2014, 15, 373–379. [CrossRef]

28. Sun, Y.; Mayers, B.; Xia, Y. Transformation of silver nanospheres into nanobelts and triangular nanoplates through a thermal process. *Nano Lett.* 2003, 3, 675–679. [CrossRef]

29. Sherry, L.J.; Jin, R.; Mirkin, C.A.; Schatz, G.C.; Van Duyne, R.P. Localized surface plasmon resonance spectroscopy of single silver triangular nanoprisms. *Nano Lett.* 2006, 6, 2060–2065. [CrossRef]

30. Li, N.; Zhang, Q.; Quinlivan, S.; Goebel, J.; Gan, Y.; Yin, Y. $\text{H}_2\text{O}_2$-aided seed-mediated synthesis of silver nanoplates with improved yield and efficiency. *Chem. Phys. Chem.* 2012, 13, 2526–2530. [CrossRef]

31. Zhang, Q.; Li, N.; Goebel, J.; Lu, Z.; Yin, Y. A systematic study of the synthesis of silver nanoplates: Is citrate a “magic” reagent? *J. Am. Chem. Soc.* 2011, 133, 18931–18939. [CrossRef] [PubMed]
32. Parnklang, T.; Lertvachirapaiboon, C.; Pienpinijtham, P.; Wongravee, K.; Thammacharoen, C.; Ekgasit, S. \( \text{H}_2\text{O}_2 \)-triggered shape transformation of silver nanospheres to nanoprisms with controllable longitudinal LSPR wavelengths. *RSC Adv.* 2013, 3, 12886–12894. [CrossRef]

33. Gu, J.; Dichiara, A. Hybridization between cellulose nanofibrils and faceted silver nanoparticles used with surface enhanced Raman scattering for trace dye detection. *Int. J. Biol. Macromol.* 2020, 143, 85–92. [CrossRef] [PubMed]

34. Jiang, F.; Hsieh, Y. Lo Synthesis of cellulose nanofibril bound silver nanoprism for surface enhanced raman scattering. *Biomacromolecules* 2014, 15, 3608–3616. [CrossRef] [PubMed]

35. Ifuku, S.; Tsuji, M.; Morimoto, M.; Saimoto, H.; Yano, H. Synthesis of silver nanoparticles templated by TEMPO-mediated oxidized bacterial cellulose nanofibers. *Biomacromolecules* 2009, 10, 2714–2717. [CrossRef] [PubMed]

36. Chook, S.W.; Yau, S.X.; Chia, C.H.; Chin, S.X.; Zakaria, S. Carboxylated-nanoncellulose as a template for the synthesis of silver nanoprism. *Appl. Surf. Sci.* 2017, 422, 32–38. [CrossRef]

37. Reid, M.S.; Villalobos, M.; Cranston, E.D. Benchmarking Cellulose Nanocrystals: From the Laboratory to Industrial Production. *Langmuir* 2017, 33, 1583–1598. [CrossRef]

38. Suh, I.K.; Ohta, H.; Waseda, Y. High-temperature thermal expansion of six metallic elements measured by dilatation method and X-ray diffraction. *J. Mater. Sci.* 1988, 23, 757–760. [CrossRef]

39. Singhsa, P.; Narain, R.; Manuspiya, H. Bacterial Cellulose Nanocrystals (BCNC) Preparation and Characterization from Three Bacterial Cellulose Sources and Development of Functionalized BCNCs as Nucleic Acid Delivery Systems. *ACS Appl. Nano Mater.* 2018, 1, 209–221. [CrossRef]

40. Musino, D.; Rivard, C.; Novales, B.; Landrot, G.; Rabilloud, T.; Capron, I. Hydroxyl Groups on Cellulose Nanocrystal Surfaces Form Nucleation Points for Silver Nanoparticles of Varying Shapes and Sizes. *Nanoscale* submitted.