

## Regioselective modification of cellulose nanocrystals: synthesis, assemblies and functional properties

Fangbo Lin

### ▶ To cite this version:

Fangbo Lin. Regioselective modification of cellulose nanocrystals : synthesis, assemblies and functional properties. Other. Université Grenoble Alpes [2020-..], 2020. English. NNT : 2020GRALV029 . tel-03171908

## HAL Id: tel-03171908 https://theses.hal.science/tel-03171908

Submitted on 17 Mar 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## THÈSE

Pour obtenir le grade de

### DOCTEUR DE L'UNIVERSITÉ GRENOBLE ALPES

Spécialité : Sciences des Polymères Arrêté ministériel : 25 mai 2016

Présentée par

## **Fangbo LIN**

Thèse dirigée par Bruno JEAN

préparée au sein du Laboratoire CEntre de Recherche sur les MAcromolécules Végétales dans l'École Doctorale Chimie et Sciences du Vivant

## Modification régiosélective de nanocristaux de cellulose : synthèse, assemblages et propriétés fonctionnelles

## Regioselective modification of cellulose nanocrystals : synthesis, assemblies and functional properties

Thèse soutenue publiquement le **10 novembre 2020**, devant le jury composé de :

#### Madame Isabelle CAPRON Directeur de recherche, INRAE, Rapportrice Monsieur Gilles SEBE Maître de conférences, Université de Bordeaux, Rapporteur Madame Aurélia CHARLOT Maître de conférences, INSA Lyon - IMP, Examinatrice Monsieur Blaise TARDY DOCTEUR EN SCIENCES, Department of bioproducts and biosystems, Aalto University, Examinateur Monsieur Frédéric PIGNON Directeur de recherche, LRP UMR 5520 CNRS, Examinateur Monsieur Guy ROYAL Professeur, Université Grenoble Alpes, Président



#### Remerciements

Ce travail a été effectué au Centre de Recherches sur les Macromolécules Végétales (CERMAV) à Grenoble. Je remercie Anne Imberty de m'y avoir accueilli.

Je tiens tout d'abord à remercier grandement mon directeur de thèse, Bruno Jean, qui a dirigé mes recherches et sans qui je n'aurais pu concrétiser ce projet doctoral. Je lui suis extrêmement reconnaissant pour le temps qu'il m'a accordé et ses précieux conseils, étant toujours disponible, à l'écoute, ayant des qualités scientifiques et humaines exceptionnelles. Merci de m'avoir accompagné, permis de progresser et d'améliorer la qualité de mes travaux. Je suis ravi d'avoir pu travailler à ses côtés.

Je suis très reconnaissant à Jean-Luc Putaux de m'avoir formé à la manipulation du microscope et à l'analyse des images. Je le remercie également pour le temps conséquent qu'il m'a accordé, ses nombreux conseils scientifiques pendant la discussion et la rédaction des articles scientifiques. J'adresse ensuite ma gratitude profonde à Henri Chanzy pour sa grande compétence, sa connaissance inépuisable de la cellulose ainsi que son enthousiasme scientifique qui m'ont toujours impressionné. Un grand merci pour toutes les discussions scientifiques et les lignes directrices qu'il m'a chaleureusement proposées.

Tous mes remerciements s'adressent à Frédéric Pignon qui a participé à mon travail et dirigé la partie rhéologie. Ses compétences et sa passion ont été la force motrice de la finalisation de mon projet. Merci également pour les nombreuses discussions scientifiques et son soutien pendant la soutenance. Je souhaite remercier vivement Fabrice Cousin, qui m'a accueilli deux fois au LLB et aidé pendant les expériences de diffusion des neutrons ou rayons X aux petits angles. Cela constitue une partie importante de mon travail.

Je voudrais remercier Isabelle Capron et Gilles Sèbe qui ont accepté d'être les rapporteurs de ce travail et Aurélia Charlot, Blaise Tardy, Frédéric Pignon et Guy Royal pour leur participation au jury en tant qu'examinateur. J'adresse ma gratitude également à Sébastien Fort et Lionel Bureau pour avoir fait partie de mon comité de suivi individuel.

Je remercie forcément tous les membres de l'équipe SPG pour leur aide pendant ces trois

belles années. Merci à Laurent Heux, Pierre Sailler, Sonia Boisseau, Stéphanie Pradeau, Christine Lancelon-Pin, Yoshiharu Nishiyama, Yu Ogawa, Franck Dahlem et Karim Mazeau pour leur soutien. Merci à toute la jeunesse de l'équipe : Clémentine, Marie-Alix, Maud, François, Maëva, Julien, Yotam, Fabien, Antoine, Axel, Khanh et Wei pour cette bonne ambiance qui régnait au rez-de-chaussée pendant ma thèse.

Merci à mes parents et ma belle-mère pour leur soutien. Même s'ils ne connaissent pas la France, ne parlent pas français et ne font pas de recherche, ils m'ont beaucoup soutenu dans la vie quotidienne pendant ces trois années.

Un grand merci spécial à ma femme, Silun, la plus belle rencontre de ma vie. Je lui exprime ici mes sincères remerciements pour son soutien constant et la motivation qu'elle m'a insufflée. C'est elle qui m'a appris que quoiqu'il se soit passé, le meilleur reste toujours à venir. Merci d'être toujours à mes côtés, de m'avoir supporté dans les moments difficiles durant ces trois années et de m'avoir aidé parfois à traiter des images ! Tout simplement, merci pour ton amour !

Merci à vous tous !

Abbreviations	5
Résumé étendu en français	7
General introduction	21
Chapter I Literature review	29
I.1 Cellulose	31
I.1.1 Chemical structure	31
I.1.2 Cellulose microfibrils and their different allomorphs	33
I.1.2.1 Cellulose microfibrils	33
I.1.2.2 Different cellulose allomorphs	35
I.2 Cellulose nanocrystals	36
I.2.1 Preparation	37
I.2.1.1 Preparation of type I cellulose nanocrystals (CNC-I)	37
I.2.1.2 Preparation of type II cellulose nanocrystals (CNC-II)	38
I.2.2 Morphology	39
I.2.3 Properties	44
I.3 Chemical polarity of CNC-I and chemical symmetry of CNC-II	47
I.3.1 Chemical polarity of CNC-I and identification of reducing end	47
I.3.2 Chemical symmetry of CNC-II	51
I.4 Reducing end modifications of cellulose nanocrystals	53
I.4.1 Brief summary of surface modifications	53
I.4.2 Reducing end modification of native cellulose nanocrystals	55
I.4.2.1 Different modifications with small molecules	55
I.4.2.2 Polymer grafting	62
I.4.2.3 Alternative strategy	68
I.5 Conclusion	69
Chapter II Regioselective labeling of various types of cellulose nanocrystals with g	Jold
nanoparticles	71
II.1 Introduction	73
II.2 Materials and methods	74
II.2.1 Materials	74
II.2.2 Native CNCs from cotton (cCNC-I)	74
II.2.3 Native CNCs from wood (wCNC-I)	74
II.2.4 Cellulose II CNCs from cotton (cCNC-II)	75
II.2.5 Sulfur content	75
II.2.6 Aldehyde content	76
II.2.7 Thioureation	76
II.2.8 Preparation of gold nanoparticles (AuNPs)	77
II.2.9 CNC labeling with gold nanoparticles	77
II.3 Results and discussion	77
II.3.1 Morphology and physicochemical properties of different starting materials	77
II.3.1.1 Characterization of cCNC-I and cCNC-II by WAXS, FTIR and CP/MAS N	IMR

## Table of contents

	77
II.3.1.2 Morphology of the different CNCs	81
II.3.1.3 Gold nanoparticles	82
II.3.2 Nanocrystals prefunctionalization with sulfur atoms	83
II.3.3 Regioselective labeling of cCNC-I and cCNC-II with gold nanoparticles	84
II.3.4 Discussion	93
II.3.4.1 Optimized labeling	93
II.3.4.2 Insights into the production and characteristics of cellulose nanocrysta	ls94
II.4 Conclusion	98
Chapter III Temperature-controlled star-shaped cellulose nanocrystal assemblies result	ing
from asymmetric polymer grafting	99
III.1 Introduction	101
III.2 Materials and methods	102
III.2.1 Materials	102
III.2.2 Treatment of cellulose nanocrystals with N-Methylmorpholine N-Oxide (NMI	NO)
	103
III.2.3 Pinnick oxidation	103
III.2.4 Polymer grafting by peptide coupling	103
III.3 Results and discussion	104
III.3.1 Evaluation of LCST of Jeffamine $^{\circ}$ polyetheramine T5000	104
III.3.2 CNCs and their two-step asymmetric functionalization	105
III.3.3 Temperature-triggered assembly of the end-derivatized cCNC-I	107
III.3.4 Rheological properties of cCNC-I and their star-shaped assemblies	116
III.4 Conclusion	121
Chapter IV Temperature-triggered formation of cellulose II nanocrystal network throu	ıgh
regioselective derivatization	123
IV.1 Introduction	125
IV.2 Materials and methods	126
IV.2.1 Materials	126
IV.2.2 cCNC-II suspension preparation	126
IV.2.3 Pinnick oxidation	126
IV.2.4 Polymer grafting by peptide coupling	127
IV.3 Results and discussion	127
IV.3.1 Physicochemical properties and morphology of cellulose II cotton nanocryst	als127
IV.3.2 Two-step functionalization of cCNC-II	129
IV.3.3 Temperature-triggered assembling properties of polymer grafted cCNC-II	130
IV.3.4 Rheological behavior of regioselectively-derivatized cCNC-II	135
IV.4 Conclusion	142
Chapter V Strategies towards the optimization of polymer grafting onto the reducing e	end
of native cellulose nanocrystals	143
V.1 Optimization of polymer grafting onto the reducing end of CNC-1	145
V.1.1 Introduction	145
V.1.2 Materials and methods	146
V.1.2.1 Materials	146

V.1.2.2 Native CNCs from tunicate (tCNC-I)	
V.1.2.3 Polymer Grafting	147
V.1.3 Results and discussion	147
V.1.3.1 Influence of reaction type	147
V.1.3.2 Influence of the catalysts	
V.1.3.3 Effect of the solvent	151
V.1.3.4 Influence of the polyetheramine	153
V.1.3.5 Influence of the aspect ratio	155
V.1.4 Conclusion	159
V.2 NMMO treatment of cCNC-I to improve the accessibility and reactivity of the	neir reducing
end	
V.2.1 Introduction	
V.2.2 Materials and methods	
V.2.2.1 Materials	
V.2.2.2 Recrystallization of 4-methylmorpholine 4-oxide n (NMMO•H2O)	10nohydrate 164
V.2.2.3 Freeze-drying of cCNC-I	
V.2.2.4 NMMO•H <sub>2</sub> O treatment of cCNC-1	
V.2.3 Results and discussion	
V.2.3.1 Swelling of CNCs end by NMMO treatment	
V.2.3.2 Separation of crystallites of cCNC-I by NMMO treatment	
V.3 Conclusion	
General conclusion and perspectives	
Appendix	
Appendix I Characterization techniques	
A.I.1 Wide-angle X-ray scattering (WAXS)	
A.I.2 Fourier-transform infrared spectroscopy (FTIR)	
A.I.3 Solid-state <sup>13</sup> C nuclear magnetic resonance spectroscopy (CP/MAS N	VMR) 187
A.I.4 Ultraviolet-visible spectroscopy (UV-Vis)	
A.I.5 Dynamic light scattering (DLS)	
A.I.6 Transmission electron microscopy (TEM)	
A.I.7 ζ-potential	
A.I.8 Small-angle X-ray scattering (SAXS)	
A.I.9 Small-angle neutron scattering (SANS)	
A.I.10 Viscosity measurements	
A.I.11 Viscoelasticity measurements	
A.I.12 Differential scanning calorimetry (DSC)	
Appendix II Rheological properties of bare cCNC-II	
A.II.1 Rheological behavior of bare cCNC-II	
A.II.2 Resilience of CNC-II gel and time-dependent behavior under	shear and
successive rest conditions	
References	199

## Abbreviations

#### Materials and chemicals

AGU: D-anhydro glucopyranose unit AuNP: Gold nanoparticles AuNP-e-CNC: Gold nanoparticles end-labeled cellulose nanocrystals BNC: Bacterial cellulose nanocrystals CNC: Cellulose nanocrystals CNC-I: Cellulose nanocrystals prepared from cellulose I allomorph CNC-II: Cellulose nanocrystals prepared from cellulose II allomorph CNC-NMMO-X: Cellulose nanocrystals treated in a NMMO/water solution with a X molar percent of water cCNC-I: Cotton cellulose nanocrystals prepared from cellulose I allomorph cCNC-II: Cotton cellulose nanocrystals prepared from cellulose II allomorph **CNF:** Cellulose nanofibrils LCST: Lower critical solution temperature NMMO: N-methylmorpholine N-oxide Ox-e-CNC: End-carboxylated cellulose nanocrystals polymer-e-CNC: Polymer end-grafted cellulose nanocrystals S-e-CNC: Sulfur atom end-functionalized cellulose nanocrystals tCNC-I: Tunicate cellulose nanocrystals prepared from cellulose I allomorph wCNC-I: Wood cellulose nanocrystals prepared from cellulose I allomorph

#### **Characterization methods**

AFM: Atomic force microscopy

CP/MAS NMR: Cross-polarization and magic angle spinning <sup>13</sup>C solid-state nuclear magnetic resonance spectroscopy

DLS: Dynamic light scattering

DSC: Differential scanning calorimetry

FTIR: Fourier-transformed infrared spectroscopy

SANS: Small-angle neutron scattering

SAXS: Small-angle X-ray scattering

TEM: Transmission electron microscopy

UV-Vis: Ultraviolet-visible spectroscopy

WAXS: Wide-angle X-ray scattering

Résumé étendu en français

La prise de conscience par un large public des nombreux problèmes environnementaux causés par l'utilisation de produits chimiques à base de pétrole fait de la synthèse de matériaux fonctionnels à partir de ressources naturelles une préoccupation majeure. Dans ce contexte général, les nanocristaux de cellulose (NCC) apparaissent comme des briques élémentaires biosourcées prometteuses pour la préparation de matériaux verts en raison de leur origine abondante et renouvelable associée à leurs nombreuses propriétés physico-chimiques intrinsèques exceptionnelles. Obtenus par hydrolyse acide de microfibrilles de cellulose natives, sous forme de suspension colloïdale aqueuse, les NCC (Figure R.1) sont des bâtonnets cristallins de quelques centaines de nanomètre à quelques microns de long pour une section de quelques nanomètres à quelques dizaines de nanomètres, bénéficiant d'un facteur de forme élevé, de 10 à 100, comparable à celui des nanotubes de carbone, et possédant une surface spécifique importante ( $\sim 250 \text{ m}^2/\text{g}$ ). Les NCC présentent par ailleurs des propriétés mécaniques exceptionnelles associées à une faible densité. Ces particules peuvent également s'auto-organiser en phase cristal-liquide chirale nématique permettant l'obtention de propriétés optiques ou la production de structures mésoporeuses nanotexturées. Ainsi, les NCC sont adaptés à la conception d'architectures hiérarchisées et de matériaux adaptés à diverses fonctions et présentent un fort potentiel applicatif dans des domaines variés comme l'emballage, la récupération assistée du pétrole mais également les cosmétiques et le biomédical.

Depuis moins d'une dizaine d'années, un second type de nanocristaux, formé de l'allomorphe II de la cellulose et non plus de l'allomorphe I (natif), suscite un intérêt académique qui va en s'amplifiant. Ces NCC-II sont le plus souvent obtenus par une première étape de mercerisation, qui permet de transformer la cellulose I native en cellulose II, suivie d'une hydrolyse acide. Les NCC-II ainsi préparés sont des bâtonnets cristallins plus courts que les NCC issus de cellulose I mais de section équivalente. Les deux types de nanocristaux possèdent, quand ils ont été obtenus par hydrolyse à l'acide sulfurique, des groupements estersulfates chargés négativement à leur surface.

NCC-I et NCC-II présentent des caractéristiques chimiospécifiques particulières qui n'ont encore été que peu exploitées et sur lesquelles se focalisera ce travail de thèse. En effet, la biosynthèse conduit à des chaînes de cellulose (homopolymère d'unités anhydroglucose) comportant deux extrémités différentes d'un point de vue chimique. L'extrémité non-réductrice comporte un carbone anomérique engagé dans une liaison glycosidique et ne présente que des groupements hydroxyles. L'autre extrémité dite réductrice est en équilibre entre une forme hémiacétale et une forme minoritaire ouverte présentant un aldéhyde réactif. L'assemblage parallèle de ces chaînes de cellulose au sein des NCC-I et antiparallèle dans le cas des NCC-II aboutit à la situation suivante : une extrémité des NCC-I regroupe toutes les extrémités réductrices des chaînes et l'autre toutes les extrémités non-réductrices alors que les deux extrémités des NCC-II sont équivalentes et constituées d'un nombre égal d'extrémités de chaînes réductrices et non-réductrices. Autrement dit, les NCC-I possèdent une polarité chimique alors que les NCC-II ont leurs extrémités équivalentes (symétrie chimique) (Figure R.1). Cette propriété permet d'envisager la fonctionnalisation régiospécifique de l'extrémité réductrice des NCC-I et des deux extrémités des NCC-II par des réactions mettant en jeu la fonction réactive aldéhyde.



*Figure R.1.* Polarité chimique des chaînes de cellulose, production et caractéristiques morphologiques et chimiques des NCC natifs (type I) et des NCC de type II.

Dans ce travail, nous proposons des stratégies pour modifier chimiquement l'extrémité des NCC-I et les deux extrémités des NCC-II. L'intérêt de telles modifications est l'obtention de nanoparticules hybrides bénéficiant à la fois des propriétés des NCC et des 10 particules/macromolécules greffées régiosélectivement. Le projet vise en particulier, par l'introduction de chaînes de polymères stimulables, à contrôler la formation d'assemblages innovants susceptibles de générer des nouvelles propriétés macroscopiques, par exemple rhéologiques. Ce projet impose de relever des défis du côté de la synthèse comme de l'analyse/caractérisation. En effet, la réactivité de l'extrémité réductrice est limitée par le petit nombre de groupements et l'équilibre chimique défavorable De plus, les outils de caractérisation courants n'ont souvent pas la sensibilité suffisante pour détecter une telle modification chimique puisque l'extrémité ne représente que quelques pourcents de la surface totale des nanocristaux de cellulose.

Dans ce manuscrit, Le premier chapitre décrit l'état de l'art sur l'obtention et les propriétés des NCC-I et NCC-II et présente les différentes stratégies mises en œuvre pour la modification chimique de l'extrémité réductrice de NCC-I et les propriétés qui en découlent.

Le deuxième chapitre présente une méthode d'optimisation du marquage des extrémités réactives par des nanoparticules d'or qui permet d'élargir les connaissances fondamentales sur la structure des NCC.

Le troisième et le quatrième chapitre concernent respectivement la fonctionnalisation de l'extrémité réductrice des NCC-I et des deux extrémités des NCC-II par des polymères thermosensibles ainsi que la formation d'assemblages contrôlés par la température et l'évaluation des propriétés rhéologiques associées.

Le cinquième chapitre présente différentes voies d'optimisation du greffage régiosélectif par la comparaison de l'influence de différents paramètres et l'utilisation d'un solvant spécifique de la cellulose.

Les résultats clés des différents chapitres sont décrits ci-après.

# Chapitre II. Marquage régiosélectif de divers types de nanocristaux de cellulose par des nanoparticules d'or

Deux stratégies de fonctionnalisation régiosélective des NCC-I et NCC-II par des nanoparticules d'or ont été mises en œuvre. La première (réaction directe), déjà rapportée dans la littérature, consiste à faire réagir les NCC préfonctionnalisés par des molécules de thiosemicarbazide avec des nanoparticules d'or (AuNP) présynthétisées. La seconde (croissance *in situ*) a consisté à synthétiser *in situ* les AuNPs à partir de dérivés d'or solubles en présence de NCC préfonctionnalisés par des molécules de thiosemicarbazide (Schéma R.1). Les résultats d'imagerie par microscopie électronique en transmission (MET) révèlent que la réaction directe aboutit à un faible taux de marquage et la formation non désirée d'agrégats de nanoparticules d'or. A l'opposé, la croissance *in situ* permet d'obtenir pour les NCC-I comme pour les NCC-II des rendements de marquage jamais atteints, de l'ordre de 80 %. En outre, cette stratégie limite la formation d'agrégats d'AuNPs et génère des AuNPs faiblement polydisperses (Figure R.2).



*Schéma R.1.* Préfonctionnalisation des NCC-I par le thiosemicarbazide (a) et représentation schématique des deux stratégies de marquage régiosélectif par des nanoparticules d'or (réaction directe et croissance in situ) (b). Des modifications équivalentes ont été réalisées pour les NCC-II.



**Figure R.2.** Images MET en coloration négative de NCC-I (a et b) et NCC-II (c-e) fonctionnalisés de façon régiosélective et optimisée par des nanoparticules d'or par la stratégie de croissance in situ.

Ces développements nous ont permis d'approfondir les connaissances fondamentales sur les nanocristaux en confirmant de manière statistiquement convaincante l'arrangement antiparallèle des chaînes de cellulose dans les NCC-II et en montrant que les NCC-I dérivés du coton sont constitués d'un assemblage parallèle de cristallites élémentaires chimiquement polaires. Ce dernier point démontre que lors du protocole de préparation des NCC-I, l'hydrolyse acide isole des fragments de microfibrilles préexistants sous la forme de fagots parallèles.

## Chapitre III Assemblages thermo-induits de nanocristaux de cellulose en étoiles résultant du greffage asymétrique de polymères

La fonctionnalisation de l'extrémité réductrice des NCC-I par des chaînes de polymères thermosensibles a été entreprise par une stratégie en deux étapes. Premièrement, les groupements aldéhydes à l'extrémité réductrice ont été oxydés sélectivement par l'hypochlorite de sodium en acide carboxylique (réaction de Pinnick). Cette oxydation a permis de réaliser dans une deuxième étape une réaction de couplage peptidique impliquant l'acide nouvellement généré et les fonctions amines terminales du polymère à greffer (Schéma R.2). Les polymères greffés étaient des polyétheramines thermosensibles de la famille des Jeffamines de masse molaire et de température critique inférieure de solubilité (LCST) respectivement égales à 2000

g.mol<sup>-1</sup> et 16 °C pour la polyétheramine Jeffamine<sup>®</sup> M2005 et 5000 g.mol<sup>-1</sup> et 12 °C pour la polyétheramine Jeffamine<sup>®</sup> T5000.



Schéma R.2. Fonctionnalisation régiosélective en deux étapes des NCC-I (a) et NCC-II (b) avec des chaînes de polyétheramine thermosensible en milieu aqueux.

Alors que les spectroscopies infra-rouge et RMN du solide ne permettent pas de détecter les chaînes greffées, la diffusion des neutrons aux petits angles (DNPA) prouve non seulement leur présence mais montre qu'elles adoptent une conformation étendue, attribuée à une densité de greffage sur l'extrémité réductrice importante. Les mesures de diffusion dynamique de la lumière (DDL) ont montré clairement que les NCC-I ainsi modifiés présentent un comportement d'agrégation thermoréversible. En accord avec la DDL et la DNPA, les images MET d'échantillons préparés à partir de suspensions à une température inférieure à la LCST prouvent que les nanoparticules modifiées sont individuelles, en accord avec la présence de forces d'interaction uniquement répulsives (répulsions électrostatiques engendrées par les charges de surface et répulsion stérique apportée par les chaînes greffées).

Au-delà de la LCST, la MET révèle la présence d'assemblages en forme d'étoile avec 3 à 6 nanocristaux fixés par leurs extrémités (Figure R.3b). Dans ces conditions les interactions entre chaînes de polymères désolvatées sont attractives alors que les répulsions latérales entre surfaces chargées persistent. Ce phénomène d'agrégation est parfaitement réversible lorsque l'échantillon est refroidi à des températures inférieures à la LCST. Des mesures de viscosité et viscoélasticité indiquent un comportement de gel élastique faible au-delà de la LCST découlant probablement d'associations entre étoiles de NCC plutôt que de gêne stérique entre étoiles

a T T Z00 nm

quand leur fraction volumique augmente.

*Figure R.3. Images TEM par coloration négative de T5000-e-NCC-I préparée à partir de suspensions aqueuses à 4 °C (a) et 40 °C (b).* 

#### Chapitre IV Réseaux de nanocristaux de cellulose II par modification régiosélective

La même voie de greffage de chaînes de polymères thermosensibles a été appliquée aux NCC-II. Pour des températures inférieures à la LCST, la morphologie des NCC-II n'est pas affectée par le greffage et les interactions entre particules sont purement répulsives à l'instar du cas des NCC-I exposé précédemment. Par contre, au-delà de la LCST, une association en réseaux supra-microniques résultant d'un chaînage des NCC-II modifiés (Figure R.4a, b) est dévoilée par les images de MET. Ce nouveau type d'association est cohérent avec la présence de polyetheramines aux deux extrémités qui permet une association bout à bout des particules à haute température. Comme dans le cas des NCC-I modifiés à leurs extrémités, une transition d'un liquide visqueux sous la LCST à un gel élastique au-delà est démontré par les mesures rhéologiques en fonction de la température. Par contre, ce gel est caractérisé par une valeur de tan  $\delta$  dix fois plus élevée que dans le cas des NCC-I, caractérisant un gel fort. Cette différence est logiquement attribuée à la présence d'un réseau connectant un grand nombre de particules dans le cas des NCC-II alors que des interactions plus faibles ou en moins grand nombre sont en jeu pour les NCC-I.



**Figure R.4.** Images TEM par coloration négative de T5000-e-NCC-II préparée à partir de suspensions aqueuses à 4 °C (a) et 40 °C (b). (c) Module de stockage G' (carrés rouges) et module de perte G'' (points bleus) d'un échantillon à 5 % en poids de T5000-e-cNCC-II mesurés pendant un cycle de température de 8 à 24 °C (chauffage : ligne continue, et refroidissement : ligne pointillée) à une déformation de 0,01 et une fréquence angulaire de 1 Hz.

## Chapitre V Stratégies d'optimisation du greffage de polymères sur l'extrémité réductrice de NCC natifs

Cette partie concerne l'optimisation du greffage régiosélectif de polyétheramines sur l'extrémité réductrice des NCC-I afin d'atteindre une fonctionnalisation de tous les NCC présents et de maximiser pour chaque NCC le nombre de chaînes greffées. Pour cela, dans un premier temps, l'influence de différents facteurs du processus chimique sur l'efficacité du greffage et la formation ultérieure d'assemblages en étoile a été étudiée. Cinq paramètres ont été pris en compte : le type de réaction (oxydation Pinnick suivie d'un couplage peptidique ou amination réductrice directe), la combinaison des catalyseurs (DCC/DMAP, DCC/4-PPY ou DCC/DBU), le type de polyétheramine (monoaminé Jeffamine<sup>®</sup> M2005 ou tri-aminé T5000), le solvant (eau ou DMF) et le facteur de forme des NCC-I (environ 12 pour du coton, 20 pour du bois et 110 pour du tunicier). Afin de palier l'impossibilité de quantifier le greffage par des techniques spectroscopiques, nous avons choisi ici d'utiliser des méthodes indirectes telles que la DDL et la MET pour fournir des informations semi-quantitatives. Les données prouvent de façon cohérente que le taux de formation d'étoiles dépend fortement de tous les paramètres testés, à l'exception du type de polyétheramine qui n'a qu'une influence faible (Figure R.5). Ces résultats ont permis d'identifier des conditions optimales permettant à plus de la moitié des NCC-I modifiés de s'assembler sous forme de complexes en étoile quand la température est élevée au-delà de la LCST. Ces conditions (utilisation du couplage peptidique dans le DMF

avec le couple de catalyseurs DCC/DMAP ou DCC/4-PPY sur des NCC-I de faible facteur de forme) favorisent la réaction chimique et limitent la gêne stérique au moment de la formation des étoiles.



*Figure R.5.* Rendement de la formation de complexes en étoile défini comme le nombre de NCC appartenant à des complexes divisé par le nombre total de NCC. Chaque paramètre étudié est représenté par une couleur donnée (par exemple vert pour les catalyseurs). Les barres de deux ou trois couleurs sont comparées deux ou trois fois avec d'autres.

Dans une seconde partie, l'utilisation de la N-oxyde de N-méthylmorpholine (NMMO), un des rares solvants de la cellulose, dans le but d'induire un gonflement des extrémités des NCC-I et de favoriser la réaction a été étudiée. Pour cela, des NCC-I lyophilisés ont été dispersés à 90°C dans des solutions de NMMO à différentes teneurs en eau dans le but d'atteindre des conditions correspondant à des régions du diagramme de phase autorisant un gonflement partiel et localisé. Cependant, aucun gonflement de l'extrémité des NCC-I n'a pu être mis en évidence par MET. En revanche, cette étude a pu mettre en lumière que des traitements dans NMMO à plus forte teneur en eau résultent en une séparation notable des cristallites élémentaires formant les NCC-I dans une gamme précise et réduite de teneur en eau (Figure R.6). Une étude statistique des images a été réalisée en comptant le nombre de cristallites dans un nanocristal. Lorsque la teneur en eau dans NMMO atteint 30 % molaire, la fraction de NCC composée de trois cristallites diminue d'environ 40 à 9 %, tandis que celle correspondant à des NCC sous forme d'une seule cristallite élémentaire passe d'environ 3 à 73 %, soit presque 25 fois plus. A notre connaissance, un tel effet n'avait pas encore été rapporté dans la littérature. Ces résultats laissent penser qu'à 90 °C le solvant NMMO-H<sub>2</sub>O 30 % molaire est capable de pénétrer entre les cristallites élémentaires et de rompre les liaisons hydrogène intercristallites et/ou permet la séparation grâce à la solubilisation des chaînes de surface sulfatées.



*Figure R.6. Images TEM par coloration négative de NCC-NMMO échantillons avec différentes teneurs en eau qui correspondent au chiffre.* 

En conclusion générale, comme les modifications entreprises concernent une fraction très réduite des unités anhydroglucose disponibles, une caractérisation quantitative directe de la modification régiosélective des NCC reste difficile, même si l'utilisation de techniques avancées telles que les méthodes de diffusion donnent des informations fructueuses. Toutefois, les travaux menés montrent qu'une telle fonctionnalisation localisée, associée à l'utilisation de particules biosourcées, permet un contrôle fin de l'assemblage en structures innovantes qui donnent naissance à de nouvelles propriétés macroscopiques, comme démontré par l'obtention de différents gels élastiques, forts ou faibles, par les études rhéologiques. Par ailleurs, une partie des résultats obtenus a permis d'avancer sur la connaissance de certaines propriétés

morphologiques ou chimiques fondamentales des NCC.

Au vu de ces résultats, une série de perspectives peuvent être envisagée.

La stratégie de marquage régiosélectif optimisée ouvre la voie au greffage d'autres nanoparticules métalliques ou magnétiques et/ou à l'utilisation d'autres types de nanocristaux de cellulose provenant de différentes sources. Les assemblages en suspension et matériaux solides incluant ces particules hybrides pourraient bénéficier de synergies entre les propriétés intrinsèques des NCC et celles des nanoparticules attachées. En particulier, le greffage de nanoparticules magnétiques et l'utilisation de champs magnétiques pourrait permettre d'obtenir des structures alignées ou orientées à façon.

Dans ce travail, les NCC marqués par des particules d'or ont été caractérisés à l'état dilué dans des suspensions. Une étude de milieux plus concentrés, en particulier répondant aux conditions de formation de phase cristal liquide de ces nanoparticules hybrides serait particulièrement intéressante. En effet, des effets nouveaux issus de l'association des propriétés optiques des phases concentrés de NCC et des propriétés de résonance plasmonique de surface des particules d'or pourraient être obtenus.

Plusieurs pistes d'optimisation plus avancée du greffage régiosélectif de polymères sur les NCC sont envisageables : utilisation de micro-ondes ou ultrasons, passage de la stratégie de grafting onto à celle du grafting from, utilisation d'enzymes, etc.

L'étude de l'influence de différents paramètres physico-chimiques (concentration, force ionique, etc.) sur l'organisation et les propriétés des assemblages sous forme d'étoiles ou de réseaux est une extension naturelle de ce projet. Par ailleurs, comme révélé par l'état de l'art, l'étude des propriétés des NCC-II nus ou modifiés a encore été peu abordée et représente à elle seule un vaste champ d'exploration.

**General introduction** 

Due to increasing environmental and ecological awareness, the synthesis of fine chemicals and functional materials from natural resources is of great public value. For this reason, a number of bio-based materials benefiting from good sustainability and biocompatibility have attracted interest. Among these, nanocellulose may prove to be one of the most promising green materials of modern times due to its intrinsic properties, renewability, abundance and commercial availability in industrial-scale quantities (Klemm 2005; Thomas 2018; Trache 2017).

The term "nanocellulose" encompasses a number of cellulose-based materials, namely cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and bacterial nanocellulose (BNC), whose chemical and physical properties vary typically as a function of their source and extraction method (Klemm 2018). The two most commonly studied forms of nanocellulose are CNCs and CNFs (Figure 1) (Hao 2020).



*Figure 1.* Cellulose nanocrystals and cellulose nanofibers from plant cellulose fibers (Figure adapted from Hao 2020).

CNCs obtained by acid hydrolysis of native cellulose microfibrils in the form of aqueous colloidal suspension, are particularly attractive biosourced nanoparticles. They are crystalline

rods with a cross section between a few and tens of nanometers and a length from tens of nanometers to a few microns that depends on the cellulose source and preparation method. These nanoparticles possess high aspect ratios from about 10 to 100 comparable to those of carbon nanotubes, and large specific surface areas (150-300 m<sup>2</sup>/g). CNCs exhibit outstanding mechanical properties, such as a high Young's modulus about 150 GPa, accompanied by a low density of 1.6 g/cm<sup>3</sup>. These particles also possess self-organizing properties in chiral nematic phases able to generate optical properties or to produce nanotextured mesoporous structures. All these properties, combined with the abundant and renewable nature of cellulose, make CNCs ideal building blocks for the design of hierarchical architectures and materials tailored to various functions (Dufresne 2012; Lagerwall 2014; Ng 2015; Oun 2016; Revol 1992).

For a considerable lot of envisioned applications, CNCs need to be adapted to various physicochemical environments, which requires their surface modification. Various reports on cellulose nanocrystals surface modifications have been distributed and licensed as of late, including non-covalent surface modifications, small molecule covalent surface modifications and polymer grafting (Kontturi 2018; Tang 2017; Wohlhauser 2018).

For less than a decade, a second type of cellulose nanocrystals, comprising the allomorph II of cellulose instead of the (native) allomorph I, has aroused growing academic interest. These CNC-II are most often obtained by a first mercerization step, which transforms the native cellulose I into cellulose II, followed by acid hydrolysis. Such CNC-II are shorter crystalline rods than the one derived from cellulose I (CNC-I) but of equivalent cross section.<sup>1</sup> Both types of nanocrystals possess, when obtained by sulfuric acid hydrolysis, negatively charged estersulfate groups on their surface.

CNC-I and CNC-II exhibit particular chemospecific characteristics that have not been largely exploited yet. Indeed, the biosynthesis leads to cellulose chains (homopolymer of anhydroglucose units) with two chemically different ends. The non-reducing end has an

<sup>&</sup>lt;sup>1</sup> In the literature, the term cellulose nanocrystal and the abbreviation CNC refer in a vast majority of cases to native cellulose particles. In the present work though, CNCs will designate a family of crystalline cellulosic rods regrouping particles derived from the allomorph I of cellulose and referred to as CNC-I, and particles derived from the allomorph II that will be called CNC-II.

anomeric carbon engaged in a glycosidic bond, and exhibits only hydroxyl groups. The other so-called reducing end is in equilibrium between a hemiacetal form and an open minority form presenting a reactive aldehyde. The parallel assembly of these cellulose chains within CNC-I and antiparallel arrangement in the case of CNC-II results in the following situation: one end of CNC-I gathers all the reducing ends of the chains and the other all the non-reducing ends, while both ends of CNC-II are equivalent and consist of an equal number of reducing and nonreducing chain ends. In other words, CNC-I have a chemical polarity while CNC-II have equivalent ends (chemical symmetry). This property makes it possible to consider the regiospecific functionalization of the reducing end of CNC-I and both ends of CNC-II by reactions involving the aldehyde reactive function. An example of preparation of type I and II cellulose nanocrystals from native cellulose microfibrils and mercerized cellulose microfibrils, respectively, is shown in Figure 2.



**Figure 2.** (a) Schematic representation of the mercerization of cellulose I to cellulose II and the acid hydrolysis of the two cellulose allomorphs. In the mercerization process, the cellulose chains are rearranged into an anti-parallel configuration in which reducing and non-reducing ends are redistributed on both extremities. Sulfuric acid hydrolysis of cellulose I and II respectively yields CNC-I (blue) and CNC-II (orange). Only one reducing end is shown at the end of a CNC for simplicity. (b)WAXS spectra of the two different CNC types and their starting materials (Figure extracted from Delepierre 2020).

Research works devoted to the investigation, labelling and functionalization of the reducing end of CNCs started in 1984 (Hieta 1984) but progressed slowly over the next 20 years (Chanzy 1985; Koyama 1997; Kuga 1988; Sipahi-Saglam 2003). In 2006, this subject came back to our eyes (Kim 2006) and in the last 7 years, a surge of interest for this field was observed (Chemin 2020a, b; Du 2017; Heise 2019; Huang 2013; Imlimthan 2019; Karaaslan 2013; Li 2018a; Lokanathan 2013, 2014; Risteen 2018; Tang 2018; Tao 2019; Tavakolian 2019; Villares 2018; Yang 2016; Zoppe 2017). However, compared to the large and ever-increasing body of literature about CNCs, a very limited number of articles and only two reviews (Heise 2020; Tao 2020) have been published on the reducing end modification of these particles. Therefore, despite a huge innovation potential and far-reaching application prospects, this topic can almost be considered as still in its infancy.

In this work, we propose strategies to chemically modify the reducing end of CNC-I and both extremities of CNC-II. The interest of such modifications is to obtain hybrid nanoparticles benefiting both from the properties of CNCs and the regioselectively-grafted particles/macromolecules. The project especially aims at the end-grafting of sensitive polymer chains, in order to control the formation of innovative assemblies likely to generate new macroscopic properties, for example rheological properties. We expect to contribute to the development of new bio-based materials that could be used in various applications and to gain further fundamental knowledge in this developing field. This project also aims at tackling chemical and analytical challenges. Indeed, the reactivity of the reducing end is limited by the small number of available reactive aldehyde groups compared to the total number anhydroglucose units and the unfavorable chemical equilibrium. Moreover, current characterization tools often do not have sufficient sensitivity to detect such chemical modifications since the surface corresponding to the end(s) of the crystals only represent a few percent of the total surface area of CNCs.

Therefore, the main goals of this PhD work are to provide new chemical strategies focusing on the reducing end of CNCs, to propose methods to generate innovative assemblies and to identify whenever possible the interaction-structure-property relationships. Accordingly, the research work presented in this manuscript involves a wide variety of aspects, from the 26

extraction of raw materials to the design of controlled structures, via the identification and functionalization of the CNCs. The thesis manuscript is divided into five chapters.

Chapter I is a literature review focusing on the current knowledge on cellulose and the CNC-I and CNC-II derived from it, their characteristics, properties, chemospecific features and the different methods used to modify the reducing end of CNC-I.

Chapter II presents an optimized regioselective labeling method of various types of cellulose nanocrystals with gold nanoparticles. An alternative efficient method is described and the resulting gold nanoparticles-labeled CNCs are investigated by different characterization techniques. These developments are finally used to give insight into some fundamental properties of CNC-I and CNC-II.

Chapter III focuses on the grafting of thermosensitive polymer chains at the reducing end of CNC-I. The chemical modification strategy is described and the temperature-triggered assembly of the derivatized CNC-I into star-shaped complexes is investigated. The effect of such a grafting on macroscopic properties is then explored by rheological measurements.

An equivalent strategy applied to CNC-II is investigated in Chapter IV. DLS, TEM and rheology are used as main characterization tools to investigate the effect of the grafting and to characterize the network structure obtained at temperatures higher than the LCST of the grafted polymer.

Chapter V introduces two different ways to optimize the coupling efficiency of thermosensitive polymers at the reducing end of CNC-I. First, a semi-quantitative method is used to identify the effect of various chemical reaction and physico-chemical parameters on the grafting and subsequent formation of star-like assemblies. Second, a treatment of CNC-I by N-methylmorpholine N-oxide was investigated with the intention to favor the accessibility of the reducing end groups through partial swelling.

Chapters I to IV of the manuscript are structured as independent scientific publications. However, to avoid redundancies and facilitate the reading, the introduction part of each chapter only contains specific elements from the literature that were not treated in the bibliographic
study presented in Chapter I. In addition, the experimental section in each chapter only includes the materials and (chemical) methods used in the considered study, while details about the characterization tools and techniques are presented in Appendix I at the end of the manuscript.

With the exception of the rheological study part, results in Chapter III have been published in ACS Macro Letters (Lin, F.; Cousin, F.; Putaux, J.-L.; Jean, B. Temperature-controlled star-shaped cellulose nanocrystal assemblies resulting from asymmetric polymer grafting. *ACS Macro Letters* **2019**, 345-351. DOI: 10.1021/acsmacrolett.8b01005).

# Chapter I

Literature review

Through a literature review, the present chapter is aiming at providing the reader with some basic knowledge about cellulose nanocrystals (CNCs) followed by a focus on their reducing end chemical modification that reveals the opportunities and challenges of the project.

The first part is devoted to general information about cellulose, its chemical structure and different allomorphs. The preparation, morphology and properties of CNCs will be exposed in a second part. In a third part, emphasis will be put on the chemical polarity of CNCs. Finally, the reducing end modifications will be reviewed in detail after a brief summary of CNC surface modification in the fourth part, where the different chemical modification strategies and subsequent new properties will be presented.

# I.1 Cellulose

Cellulose, a linear polysaccharide composed of  $\beta$ -D-anhydroglucopyranose units, is the most abundant natural polymer and a renewable resource for the production of biobased materials and fuels (Dufresne 2012; Eichhorn 2010, 2011; Habibi 2010; Klemm 2005, 2011; Kontturi 2018; Moon 2011). This biodegradable organic biopolymer with an annual world production between  $10^{11}$  and  $10^{12}$  tons (Hon 1994) is the primary structural component of plant cell walls. Cellulose is found in a wide variety of species, such as higher plants, tunicates, algae, fungi, bacteria, although strong variations in cellulose content in these organisms are observed (Klemm 1998, 2018).

## I.1.1 Chemical structure

75 years after the successful isolation of cellulose from different plants in 1838 (Payen 1838), its basic molecular formula  $C_6H_{10}O_5$  was revealed by Willstätter (Willstätter 1913). The molecular structure of cellulose is illustrated in Figure I.1.



Figure I.1. Molecular structure of cellulose (Figure adapted from French 2017).

Cellulose is a linear homo-polysaccharide of D-anhydro glucopyranose units (AGU) linked together by an equatorial glycosidic  $\beta$ -(1-4) linkage around which the AGU units are oriented at 180° from each other (Haworth 1930). AGUs are in chair conformation (Chu 1968) and have 3 hydroxyl functions: 2 secondary alcohols in positions 2 and 3 and a primary alcohol in position 6 (Freudenberg 1928; Irvine 1923). These hydroxyl functions, as well as the glycosidic bonds, are located in the equatorial position relative to the plane of the cycle, which therefore results in the hydrogens of the cycle being in the axial position.

The molecular weight of a cellulose sample is equal to the number of AGUs in a cellulose chain, the so-called degree of polymerization (DP), multiplied by the mass of an AGU (162 g mol<sup>-1</sup>). The molecular weight or DP are expressed as a mean value because a wide distribution of chain length can be observed within the same sample. Cellulose has an average DP ranging between 1000 and 50000 depending on the source. We can quote DPs of 1500 for cellulose of wood pulp, 20000 for cotton secondary wall and 50000 for *Valonia* (Conner 1995).

As shown in Figure I.1, the two ends of the cellulose chains are not chemically equivalent. The so-called non-reducing end consists of a D-glucopyranose whose anomeric carbon is engaged in a glycosidic bond and that has a free secondary alcohol function in position 4. The other end, referred to as the reducing end is a D-glucopyranose unit whose anomeric carbon is free: we therefore have a cyclic function, which is in equilibrium with a minority open aldehyde form. This difference gives the cellulose chain a chemical polarity.

#### I.1.2 Cellulose microfibrils and their different allomorphs

#### I.1.2.1 Cellulose microfibrils

Cellulose is synthesized at the plasma membrane by cellulose synthases which, in higher plants, are organized in rosette (Li 2016; Pear 1996). During the biosynthesis, the cellulose chains combine in parallel via inter- and intra-molecular hydrogen bonds (Figure I.2) to form ordered crystalline structures called microfibrils (Figure I.3 A) (Li 2014; Saxena 2005). It has also been shown that a network of van der Waals links is established between the chain sheets (French 1993). These numerous interactions prevent the dissolution of cellulose microfibrils in most solvents (Lindman 2010).



*Figure I.2. Hydrogen bonding interactions in biosynthesized cellulose chains (Figure extracted from Kang 2013).* 

In nature, cellulose occurs as assemblies of individual cellulose chains forming fibers because of the spinning of individual molecules at the site of biosynthesis. If the biosynthesis is not disturbed or the native microfibrils are never dried, long crystalline cellulose microfibrils are maintained (Horikawa 2018; Lai-Kee-Him 2002). However, on the one hand, cellulose microfibrils are required to push their way during their deposition in the cell wall, which results in the formation of periodically disorganized domains along the microfibril length. On the other hand, heating also generates disorganized domains in native cellulose microfibrils leading alternated crystalline regions and a limited number of disorganized regions along the microfibril (Atalla 2014; Horikawa 2018). Several models have been proposed to describe the arrangement of the chains inside the microfibrils, which agree that they are formed from stretched cellulose

chains and disorganized regions distributed along their major axis following dislocations of certain chains (Figure I.3b) (Rowland 1972).



*Figure I.3.* Schematic representation of the structural hierarchy in a cellulosic fiber constituting a plant cell wall (Figure extracted from Genome management information system/ORNL) (a) and of the alternation of crystalline and amorphous zones along a microfibril (b) (Figure extracted from Rowland 1972).

The existence of the disordered zones has been demonstrated by <sup>13</sup>C-solid nuclear magnetic resonances (Earl 1982), wide- and small-angle X-ray diffraction (Fink 1987), as well as by tensile tests on ramie fibers (Ishikawa 1997). The actual model of cellulose microfibrils considers a very crystalline core surrounded by less organized surface chains whose cohesion with the internal chains is lower. The proportion of surface chains whose nuclear magnetic resonances signal is different from that of the crystalline core, depends directly on the cross-section dimensions of the microfibrils (Heux 1999; Newman 1999). Cellulose microfibrils are the major constituents of plant cell walls where they are embedded in an amorphous matrix of hemicellulose, pectin, lignin and proteins to form a composite structure (Figure I.3a) (Young 1986).

## I.1.2.2 Different cellulose allomorphs

Intra- and interchain hydrogen bonds shown in Figure I.2 allow the formation of highly organized crystalline structures. Since the 1930s, numerous studies have been carried out to elucidate the crystal structure of cellulose (Honjo 1958; Meyer 1937; Sarko 1974). It is now known that cellulose can exist in the form of seven well described allomorphs:  $I_{\alpha}$ ,  $I_{\beta}$ , II, III<sub>I</sub>, III<sub>I</sub>, IV<sub>I</sub> and IV<sub>II</sub> with different possibilities of conversion between them through chemical or thermal treatments (Figure I.4) (Chanzy 1979; Helbert 1997; Isogai 1994).

Native cellulose is type I. <sup>13</sup>C solid-state NMR (Atalla 1984; Vanderhart 1984) and electron diffraction experiments (Sugiyama 1991) have shown that type I cellulose is in fact divided into two crystalline forms: a triclinic  $I_{\alpha}$  phase with one chain per mesh and a monoclinic  $I_{\beta}$  phase with two chains per mesh. The proportion of cellulose  $I_{\alpha}$  and  $I_{\beta}$  differs depending on the origin of the cellulose. Cellulose  $I_{\alpha}$  is mainly found in primitive organisms such as in the wall of certain algae or produced by bacteria, whereas the I<sub> $\beta$ </sub> phase predominates in plants such as cotton or in the walls of tunicates (Belton 1989; Kono 2002). The proportion of each allomorph within a cellulose sample can be determined by infrared spectroscopy or solid-state NMR. Combined neutron diffraction and X-ray measurements on oriented cellulose fibers (Nishiyama 2002, 2003) have allowed the refinement of the mesh parameters, atom positions and intra- and interchain hydrogen bond positions of phases  $I_{\alpha}$  and  $I_{\beta}$ . In these studies, the coordinates of C and O atoms were derived from X-ray spectra, while those of hydrogen atoms engaged in hydrogen bonds were determined from neutron diffraction spectra by analyzing the differences between deuterated and hydrogenated samples. These studies precisely define the intramolecular hydrogen bridges that stiffen the chains, while others, of the intermolecular type, associate the chains in the form of sheets. The cohesion of the sheets within the crystal depends mainly on van der Waals-type interactions.

When cellulose is mercerized or dissolved and then precipitated, a different crystal structure, called cellulose II, is formed. It has an antiparallel arrangement in the monoclinic two-chain crystal lattice. This arrangement, proposed in the 1970s (Kolpak 1976; Sarko 1974), has been confirmed by neutron diffraction studies (Langan 1999, 2001). Moreover, the cellulose

II allomorph is the most thermodynamically stable form. For these reasons, it is possible to convert cellulose I to cellulose II but not *vice versa* (Sarko 1974). In the present study, only the allomorphs I and II were considered and the other allomorphs will not be described further.



*Figure I.4. Relations between the different allomorphs of cellulose (Figure extracted from KroonBatenburg 1996).* 

# I.2 Cellulose nanocrystals

Cellulose nanocrystals (CNCs) are biobased highly crystalline rod-like particles. All industrially produced CNCs and 99 % of the academic studies refer to nanocrystals made of native cellulose, which are isolated from the controlled acid hydrolysis of microfibrils from a huge variety of sources (Brinchi 2013; Domingues 2014; Habibi 2010; Klemm 2011; Mariano 2014; Moon 2011; Ng 2015; Peng 2011). These CNCs, which will be referred to as CNC-I in the present manuscript, have been first prepared in the fifties but the academic and industrial interest for these nanoparticles truly started in the nineties and has attracted a growing attention ever since due to their numerous properties and application potential. The interest for CNCs made of the cellulose II allomorph dates back to less than 10 years and has resulted in a reduced, yet growing number of works. In this section, the preparation methods, morphological features and properties of CNCs constituted of the allomorphs I and II will be presented.

### **I.2.1** Preparation

#### I.2.1.1 Preparation of type I cellulose nanocrystals (CNC-I)

Nickerson and Habrle (Nickerson 1947) initiated the idea of employing acid hydrolysis to isolate crystalline CNC-I upon degradation of the less ordered regions along the microfibrils. This idea was then affirmed by Rånby (Rånby 1949), when he delivered colloidal suspensions of cellulose nanocrystals.

The main process used to obtain cellulose nanocrystals is sulfuric acid hydrolysis based on the seminal protocol described by Revol and coworkers (Revol 1992). Controlled reaction conditions including time, temperature, acid concentration and acid/cellulose ratio, are necessary to prepare stable suspensions of nanocrystals (Chauve 2014). Conditions must be adequate to avoid partial hydrolysis of the fibers, without reaching conversion into D-glucose. Once the nanometric rods are obtained, the hydrolysis reaction is stopped by cooling and dilution in water. The acid is removed by successive centrifugation/redispersions cycles followed by dialysis. Finally, a sonication treatment is applied to obtain individual objects in a stable colloidal suspension.

The sulfuric acid hydrolysis introduces negatively charged sulfate half-ester groups on the surface of the nanocrystals, thus providing colloidal stability by electrostatic repulsion. The charge density, varying between 0.1 and  $0.6 \text{ e}^{-}/\text{nm}^2$ , increases with the acid concentration, the hydrolysis time, the acid/cellulose ratio and the temperature (Beck-Candanedo 2005; Dong 1998; Hamad 2010).

While acid hydrolysis using strong acids, namely sulfuric and hydrochloric acids, is the most frequently utilized procedure to prepare CNC-I, other mineral and organic acids have likewise been used to prepare CNC-I (de Oliveira 2016; Du 2016; Espinosa 2013; Kontturi 2016; Tang 2011; Thakur 2015). Several alternative preparation methods have also been developed, for example, enzymatic hydrolysis (Anderson 2014; Chen 2012; Satyamurthy 2011; Xu 2013), mechanical refining (Amin 2015; Li 2012; Yu 2013), ionic fluid treatment (Abushammala 2015; Anderson 2014; Chen 2012; Lazko 2016; Mao 2015; Satyamurthy 2011;

Tan 2015; Xu 2013), subcritical water hydrolysis (Novo 2015, 2016), oxidation method (Cao 2012; Cheng 2014; Leung 2011; Sun 2015) and combined processes (Beltramino 2016; Chowdhury 2016; Lu 2013; Rohaizu 2017; Tang 2015b; Trache 2017).

I.2.1.2 Preparation of type II cellulose nanocrystals (CNC-II)

Four different routes have been used to produce CNC-II. The most frequent method is the mercerization of cellulose in NaOH followed by acid hydrolysis. Various conditions have been reported. Kim *et al.* (Kim 2006) mercerized ramie cellulose fibers in 3.5 M NaOH followed by thorough washing in distilled water and air-drying. The mercerized fiber was then hydrolyzed by 2.5 M HCl for 1 h. Using cotton fiber (Yue 2012) or wood pulp (Flauzino Neto 2016), the pristine materials were mercerized in a 20 wt. % NaOH aqueous solution at room temperature for 4 to 5 h and the obtained slurry was thoroughly washed with distilled water. The mercerized fiber was dried at 40 °C in a vacuum oven. Acid hydrolysis was subsequently carried out with 64 wt. % sulfuric acid at 45 °C for about 1 h. Microcrystalline cellulose (MCC) was also used by Gong *et al.* (Gong 2017, 2018) and Kuang *et al.* (Kuang 2020). MCC was immersed in 18.5 wt. % NaOH aqueous solution at room temperature for 1.5 h and then washed by distilled water. The mercerized MCC was hydrolyzed by 64 wt. % sulfuric acid at 45 °C for 40 min. Delepierre *et al.* (Delepierre 2020) used cotton filter paper which was mercerized in 17.5 wt. % NaOH for 24 h followed by 64 wt. % sulfuric acid hydrolysis at 55 °C for 25, 20 and 10 minutes to prepare CNC-II.

Another method is the mercerization of CNC-I in 17.5 wt. % NaOH aqueous solution at room temperature for 30 minutes (Jin 2016) or 1.5 h (Gong 2018) which gives the generated CNC-II a good thermal stability but poor colloidal stability, which could be improved by introduction of negative charges via TEMPO-mediated oxidation.

Using mercerized kraft pulp or cotton linters (20 wt. % NaOH at room temperature for 24 h), TEMPO-mediated oxidation has also been used by Hirota *et al.* (Hirota 2010, 2012) to produce CNC-II, with or without an intermediate hydrochloric acid hydrolysis (boiling in 3 M HCl for 1 h).

A last method to obtain CNC-II is the dropwise addition of given amounts of concentrated sulfuric acid hydrolysis on microcrystalline cellulose at 44 °C for given times (Sèbe 2012) or on kraft pulp at 35 °C or 45 °C for 30 minutes (Merlini 2020) without any mercerization.

These different preparation methods lead to different morphologies of CNC-II that will be discussed in the next part. A schematic summary of the different preparation methods for CNC-I and CNC-II is presented in Figure I.5.



*Figure 1.5.* Schematic representation of the different preparation methods for CNC-I and CNC-II (Figure adapted from Delepierre 2020).

#### I.2.2 Morphology

The morphological characteristics of CNCs are commonly studied by transmission electron microscopy (TEM), atomic force microscopy (AFM), field emission gun scanning electron microscopy (SEM-FEG) and depolarized dynamic light scattering (DDLS) (Foster 2018). From a general point of view, CNC-I are rod-like particles with a length between 100 nm and 1  $\mu$ m and a cross-section in the 3-70 nm range. However, the length, width and thickness considerably vary with the cellulose source and to a lesser extent with the hydrolysis conditions (Figure I.6 and Table I.1). Several studies concur to show that the harsher the acid hydrolysis

conditions, the shorter the particles (Beck-Candanedo 2005; Bondeson 2006; Dong 1998; Elazzouzi-Hafraoui 2008; Fan 2012; Filson 2009; Hamad 2010). Consequently, the aspect ratio of these nanocrystals, defined as the length to cross-section ratio, which plays a major role in phenomena like the self-organization of CNC-I into chiral nematic crystal-liquid phases, reaches out over a wide range, typically from 10 to 100.

*In vivo*, the cross-section of the elementary fibrils is determined by the terminal complexes organization (Brown 2004). Consequently, the cross-section of CNC-I, which results from the acid hydrolysis of these elements, mimics the shape and dimensions of their parent fibril. A representation in the form of a parallelepiped or lozenge section is generally admitted, but not demonstrated (Figure I.7). It has been shown experimentally using combinations of structural investigation techniques that CNC-I extracted from cotton, bamboo, eucalyptus, sisal and curauá comprise a few laterally associated elementary crystallites (Brito 2012; Elazzouzi-Hafraoui 2008). These results show that the hydrolysis conditions do not generally permit to break lateral interactions between elementary crystallites that preexist in the parent microfibrils. As a consequence, CNC-I extracted from these cellulose sources have a height that is similar to the size of the parent elementary fibril (e.g. 5-6 nm in the case of cotton as shown in Figure I.6) but a width that is an integer multiple of the latter value (e.g. 4 × 5-6 nm = 20-24 nm for cotton CNCs) (Figure I.8).

Source	L (nm)	W (nm)	Technique	References
Bacterial	100-1000	10-50	TEM	Araki 2001
	100-1000	30-50	TEM	Grunert 2002
Cotton	100-150	5-10	TEM	Araki 2001
	70-170	~7	TEM	Dong 1996
	200-300	8	TEM	Heux 2000
	255	15	DDLS	De Souza Lima 2002
	150-210	5-11	AFM	Miller 2003
Cotton linter	100-200	10-20	SEM-FEG	Roohani 2008
	25-320	6-70	TEM	Elazzouzi-Hafraoui 2008
	300-500	15-30	AFM	Li 2009
Microcrystalline cellulose	35-265	3-48	TEM	Elazzouzi-Hafraoui 2008
	250-270	23	TEM	Pranger2008
	~500	10	AFM	Capadona 2009
Ramie	150-250	6-8	TEM	Habibi 2008
	50-150	5-10	TEM	Junior de Menezes 2009
Sisal	100-500	3-5	TEM	Garcia 2006
	150-280	3.5-6.5	TEM	Siqueira 2009
Tunicate	-	18.2	SANS	Terech 1999
	1160	16	DDLS	De Souza Lima 2002
	500-1000	10	TEM	Angles 2001
	1000-3000	15-30	TEM	Kimura 2005
	100-1000	15	TEM	Heux 2000
	1073	28	TEM	Elazzouzi-Hafraoui 2008
Valonia	>1000	10-20	TEM	Revol 1982
Soft wood	100-200	3-4	TEM	Araki 1998
	100-150	4-5	AFM	Beck-Candanedo 2005
Hard wood	140-150	4-5	AFM	Beck-Candanedo 2005

Table I.1. CNC-I dimensions from different sources (Table extracted from Habibi 2010).



*Figure I.6. TEM images of negatively stained CNC-I obtained from different cellulose sources (Figure extracted from Chauve 2014).* 



Figure I.7. Morphology and dimensions of elementary fibrils from different cellulose sources.

![](_page_51_Figure_1.jpeg)

*Figure I.8. (a)* Schematic representation of a cotton CNC-I comprising three elementary crystallites. (b) *TEM image of negatively stained cotton CNC-I showing their composite nature (Figure extracted from Elazzouzi-Hafraoui 2008).* 

In most cases, type II cellulose nanocrystals are also rod-like nanoparticles whose dimensions depend on the preparation method. Observed by transmission electron microscopy, the typical mercerization followed by acid hydrolysis process results in cellulose II nanoparticles composed of few laterally associated elementary crystallites, as shown in Figure I.9a (Kim 2006; Flauzino Neto 2016; Yue 2012). CNC-II, which were prepared by Sèbe *et al.* (Sèbe 2012) using dropwise addition of specific amounts of concentrated sulfuric acid from cellulose I substrates, were ribbon-like nanoparticles with rounded shape at the tip (Figure I.9b).

![](_page_51_Picture_4.jpeg)

*Figure I.9. (a) TEM image of cotton CNC-II prepared by mercerization followed by sulfuric acid hydrolysis (Figure extracted from Flauzino Neto 2016). (b) AFM phase image of CNC-II prepared by dropwise addition of specific amounts of concentrated sulfuric acid from cellulose I substrate (Figure extracted from Sèbe 2012).* 

However, these nanoparticles are shorter than CNC-I with a nearly identical width and height, indicating a lower aspect ratio of CNC-II when compared to CNC-I (Delepierre 2020; Gong 2017). CNC-II with a larger width than that of CNC-I can be prepared by acid hydrolysis followed by TEMPO-mediated oxidation of mercerized cellulose microfibrils. This preparation method also produced longer CNC-II compared to other methods. TEMPO-mediated oxidation also favors the dispersion of CNC-II in water through the introduction of carboxylates that provide additional electrostatic repulsion forces (Gong 2018; Hirota 2010, 2012). Prepared by a mercerization of CNC-I, Jin *et al.* (Jin 2016) showed that CNC-II tend to aggregate and form granular spheres.

#### I.2.3 Properties

As a material, CNC-I can be extracted by acid hydrolysis from a wide variety of natural sources such as trees, annual plants, tunicates, algae and bacteria. These renewable cellulose-based nanoparticles have a low density of 1.6 g/cm<sup>3</sup> and a high aspect ratio ranging from 10 to 100. CNC-I which can be produced at an industrial scale (Dufresne 2012) have a high Young's modulus about 150 GPa, which is close to that of Kevlar (Habibi 2010; Klemm 2011). Their tensile strength estimated at 7.5 GPa is also relatively high (Ng 2015). As for thermal properties, the coefficient of thermal expansion of CNC-I, about 1 ppm/K, is very low (Bullard 2020). Additionally, CNC-I are a particularly attractive nanoparticle because they have low environmental, health and safety risks, are inherently sustainable, biocompatible and biodegradable like the sources from which they are extracted (Lagerwall 2014; Oun 2016).

In 1992, Revol *et al.* (Revol 1992) showed that above a certain critical concentration, a suspension of CNC-I spontaneously separates into an upper isotropic phase and a lower anisotropic phase. These nanoparticles are organized as nematic liquid crystals whose director rotates at a constant angle with respect to an axis called the cholesteric axis. This self-organization was uncovered by the presence of "fingerprint" patterns observed by polarized optical microscopy on CNC-I suspensions, indicating a chiral nematic ordering (Figure I.10a) (Edgar 2002). When observed between crossed polarizers, the regular change of orientation within the liquid crystal results in alternating fringes of illumination and extinction when the

sample is observed perpendicularly to the cholesteric axis. The figure thus resembles a fingerprint. This helical structure is characterized by the pitch, P, which corresponds to the distance measured after a 360° rotation of the objects (Figure I.10b). Therefore, the distance between two identical bands corresponds to a half pitch (P/2). The pitch can generally vary from a few to tens of microns (Lagerwall 2014).

![](_page_53_Figure_2.jpeg)

*Figure I.10. (a)* Optical micrograph between cross polarizers of an anisotropic, sulfuric acid hydrolyzed CNC-I suspension showing the characteristic fingerprint texture of a chiral nematic phase with few disclinations (scale bar 100 mm) (Figure extracted from Edgar 2002). (b) Schematic representation of the cholesteric phase of CNC-I (Figure extracted from Revol 1992).

The origin of this organization is entropic. The particles are randomly distributed in the isotropic phase. There is no preferred order of position or orientation. In the nematic phase, the particles have random positions but have an order of orientation. The system therefore loses entropy due to the increase in order, but gains translational entropy from a certain particle concentration when organized due to the greater freedom of translational motion of the particles when ordered (Onsager 1949).

Besides, the exposed -OH groups on CNC-I surfaces can be readily chemically modified to tune the colloidal interactions and surface properties, making it possible to adjust CNC-I self-assembly and dispersion in a wide range of solvents and matrix polymers and generally to control interfacial properties in composites, Pickering emulsions, etc. (Eyley 2014; Habibi 2010).

Such remarkable properties are the origin of a wide range of applications in different domains (Azeredo 2017; Buchtová 2016; De France 2017; Domingues 2014; Fang 2016; Ferrer

2017; Gaspar 2014; Habibi 2010; Jorfi 2015; Martin A. Hubbe 2017; Ng 2015; Sacui 2014; Salas 2014; Tang 2017; Thomas 2018). CNC-I can alter the rheology of various media that are used in many industrial applications, such as paints, coatings, adhesives, food, cosmetics, drugs and cements. As a reinforcement for polymer materials, CNC-I alter the mechanical properties of the resulting composites and can be used in the development of robust, flexible, durable, lightweight, transparent and dimensionally stable films which may be used in packaging. CNC-based composites incorporating adapted derivatized CNC-I have attracted interest as barrier films with potential uses in selective filtration and batteries. In addition to these, CNC-I have a broad application prospects in tissue engineering, biosensors, hydrogels and aerogels and templates for electronic devices.

As far as CNC-II are concerned, most research works have focused so far on the preparation methods, which resulted in different morphologies and redispersion properties. Very recently, Delepierre *et al.* (Delepierre 2020) showed that CNC-II can also self-organize into cholesteric phases over much longer periods of time when compared to CNC-I and resulting in large-pitch structures. The authors pointed out that the surface charge characteristics of the two types of particles are rather similar. The observed difference is tentatively attributed to a combination of factors, including the inferred faster rotational diffusion of CNC-II and the different crystal structures of CNC-I and CNC-II, which are responsible for the presence and absence of a giant electric dipole moment, respectively. Kuang *et al.* (Kuang 2020) fabricated porous SiO<sub>2</sub> particles with smaller and narrower pore width distribution using CNC-II as templates, when compared to samples prepared using CNC-I. Gonzalez-Domínguez *et al.* stated that CNC-II could be used as carbon nanotube dispersing agents (Gonzalez-Dominguez 2019).

These early studies show that different properties can be achieved using CNC-II instead of CNC-I, which broadens the potential use of CNCs in various applications. Importantly, CNC-I and CNC-II also exhibit specific and different localizations of the cellulose chains reducing ends. The following section introduces this notion which plays a central role in the present PhD work.

# I.3 Chemical polarity of CNC-I and chemical symmetry of CNC-II

# I.3.1 Chemical polarity of CNC-I and identification of reducing end

As aforementioned, one cellulose chain has two different termini (Figure I.1). On one end, the hemiacetal cyclic group is in equilibrium with a non-cyclic form exhibiting an aldehyde group with reducing properties. As the aldehyde group can reduce Cu (II) into Cu (I) ions in Fehling's solution (Dufresne 2012), this end is consequently called the reducing end. The other extremity of the chains comprises a cyclic form displaying only hydroxyls and is referred to as the non-reducing end.

The equilibrium at the reducing end, so-called tautomeric equilibrium, normally prefers the aldehyde form. While in case of sugar, the -OH groups at C4 or C5 can facilely combine with aldehyde and form a five- or six-membered hemiacetal structure, which in turn leads to a minority aldehyde form. Although the two organizations are possible, almost all of the simple sugars exist in the six-membered ring form (Vollhardt 2014). Thus, the ring-opening becomes the rate limiting step (Capon 1974). The tautomeric equilibrium can be affected by the external chemical environment. The decrease of pH or water activity can promote the generation of aldehyde groups at the reducing end (Fan 2012; Soderberg 2012).

Due to the parallel character of the cellulose chains' arrangement in native cellulose microfibrils, all reducing ends in a microfibril are located on one side, and all non-reducing ends are at the other extremity (Brown 1996, 2004; Williamson 2002). Since the acid hydrolysis procedure does not change the chain direction, CNCs prepared from cellulose I microfibrils by acid hydrolysis (CNC-I) conserve this chemical polarity, which is shown in Figure I.11a.

Due to the low quantity of reducing end groups and consequent low reactivity and difficult characterization, the regioselective modification of the reducing end of CNC-I was ignored for a long time. However, an international renewed interest for the topic has recently emerged, as shown by the publication of recent studies and of two reviews at the early 2020 (Heise 2020; Tao 2020).

![](_page_56_Figure_1.jpeg)

*Figure I.11.* Chemical equilibrium of hemiacetal and aldehyde groups on reducing end of CNC-I (a) and CNC-II (b).

In order to activate the reactive sites, the identification, or verification of the existence of the reducing end is essential. Such a demonstration has been started from 1984 and was pursued and optimized until now due to the difficulty of a chemical reaction on such a small surface.

A first work was reported by Hieta *et al.* (Hieta 1984). *Valonia* cellulose nanocrystals were treated by NaClO<sub>2</sub> so that the reducing ends would be oxidized to carboxyls. These carboxyls were expected to form a silver salt in the silver reagent medium (1.3 % silver nitrate and 2.5 % ammonia) and to serve as nuclei for silver deposition. The resulting labeled nanoparticles were then observed by transmission electron microscopy using negative staining. Even though a very low labeling density was obtained, the TEM image (Figure I.12) still unambiguously showed that the silver particles were attached at only one end of the nanocrystals.

![](_page_56_Picture_5.jpeg)

*Figure I.12.* Negatively stained TEM micrographs of silver-stained Valonia nanocrystals (Figure extracted from Hieta 1984).

In 1985, using the same *Valonia* cellulose nanocrystals and a specific non-reducing end enzyme, 1,4-β-D-glucan cellobiohydrolase II (CBHII), Chanzy *et al.* (Chanzy 1985) 48 demonstrated first the exo-activity of the enzyme and furthermore the parallel arrangement of the cellulose chains from the asymmetric degradation observed by TEM images. Figure I.13a indeed reveals that CBHII erodes only one of the two tips of the nanocrystals. The event of sharp end digested by CBHII showed that the consecutive attack probably begins by stripping off the accessible surface of the non-reducing end. Hidden chains are consequently revealed and get degraded in an ensuing step. These successive attacks yield a pointed end as schematically described in Figure I.13b.

![](_page_57_Figure_2.jpeg)

**Figure I.13.** (a) TEM image of a Valonia nanocrystal after 16 h of digestion with CBHII. R: reducing end; NR: non-reducing end. The scale bar is 250 nm. (b) Schematic representation of the exo-attack giving rise to sharpened nanocrystal ends. In (a) the exoenzyme (•) attacks accessible non-reducing chain-ends (NR) at the crystal surface. In (b) degradation of the outer layer exposes a new surface of NR chain-ends to the enzymes. As this progresses (c), a sharpened tip morphology appears at the NR of the nanocrystal (Figure extracted from Chanzy 1985).

In order to verify the parallel chain structure in other organisms such as bacterial cellulose, which has a morphology between that of *Valonia* and higher plant celluloses, the silver labeling was also used by Kuga *et al.* (Kuga 1988). The aldehyde specific ligation of thiosemicarbazide used in their report took into consideration the asymmetric chemical connection of a silver protein complex giving nucleation loci for silver nanoparticles. This method also demonstrated the parallel packing of cellulose chains for both  $I_{\alpha}$  and  $I_{\beta}$  allomorphs.

Combining the silver staining technique and the selective CBHII enzyme degradation,

Koyama *et al.* (Koyama 1997) successfully achieved these two manipulations on bacterial cellulose nanocrystals. Using microdiffraction-tilting electron crystallographic analysis, they showed that the reducing end of the developing cellulose chains points away from the bacterium. A direct proof that biosynthesis by the cellulose synthase occurs at the non-reducing end was thus provided. A schematic diagram and the TEM image are shown in Figure I.14.

![](_page_58_Figure_2.jpeg)

*Figure I.14.* Schematic diagram of the reducing end labeling and the enzyme treatment of non-reducing end. *R.E.:* reducing end. *N.E.:* non-reducing end. *(a)* TEM micrographs of labeled reducing end and treated non-reducing end bacterial cellulose. Filled arrows: reducing end. Empty arrows: non-reducing end. *(b and c)* (Figure extracted from Koyama 1997).

Lokanathan *et al.* (Lokanathan 2014) functionalized in aqueous media the reducing end of CNC-I by a first oxidation to produce carboxyl groups and second a carbodiimide reaction to introduce thiol groups. The confirmation of the success of the chemical modification was achieved by silver labeling and X-ray photoelectron spectroscopy. The adsorption of thiolated CNC-I onto gold surfaces was investigated by using quartz crystal microbalance and the system was further analyzed for surface density and distribution by using atomic force microscopy.

![](_page_59_Figure_1.jpeg)

**Figure I.15.** Schematic representation of chemical modification at reducing end of CNC-I and frequency as a function of time measured by QCM-D for different CNC-I samples (Figure extracted from Lokanathan 2014).

## I.3.2 Chemical symmetry of CNC-II

The mercerization process (Mercer 1850) that consists in swelling native cellulose in concentrated sodium hydroxide solutions followed by removal of the swelling agent converts cellulose I into cellulose II. In contrast to cellulose I, which has a parallel arrangement of cellulose chains, cellulose II possesses antiparallel chains leading to a more stable structure. An interdigitation mechanism was proposed to explain the transition of chain direction from cellulose I to II. Indeed, the microfibrillar constraints can be overcome by NaOH. Thus, the cellulose chains are independent and allowed to coalesce and interdigitate in an antiparallel way (Figure I.16) (Kolpak 1976; Langan 2001, 2005; Okano 1985). Another illustration proposed by Kim *et al.* (Kim 2006) is shown in Figure I.17.

![](_page_59_Figure_5.jpeg)

*Figure I.16.* Schematic representation of the interdigitation mechanism of mercerization of cellulose by NaOH (Figure extracted from Okano 1985).

Considering the chemical polarity of individual cellulose chains, such an antiparallel

arrangement in the cellulose II allomorph hence results in the presence of both reducing and non-reducing ends at both extremities of the crystals. Thus, cellulose II microfibrils lose their chemical polarity while gaining one more chemical reactive site. As mentioned, the acid hydrolysis process only results in removing disordered regions (and possibly imparting charged groups) but does not influence the cellulose chain direction. Consequently, rod-like CNCs prepared from cellulose II microfibrils (CNC-II) by hydrolysis also exhibit both reducing and non-reducing ends at each of their extremity.

Kim *et al.* (Kim 2006) applied biotin-streptavidin binding followed by gold labeling in order to visualize the reducing end of cellulose nanocrystals from cellulose I, cellulose II and cellulose III<sub>I</sub> allomorphs. Together with the results of electron diffraction, the labeling proved that the chains were organized in a parallel way in cellulose I from ramie and *Valonia* and also in cellulose III<sub>I</sub> from *Valonia*. In cellulose II nanocrystals from mercerized ramie, the labeling method showed that the chains were packed into an antiparallel mode (Figure I.17a), in line with a mercerization process causing an intermingling of the cellulose chains from neighboring microfibrils of opposite polarity (Figure I.17b).

![](_page_60_Figure_3.jpeg)

*Figure I.17. (a) TEM micrographs of mercerized ramie with gold nanoparticles on both nanocrystal ends. (b) Schematic conversion of nanocrystals of ramie I into ramie II. The dark dots correspond to chain ends of "up" polarity whereas the white dots correspond to chain end of "down" polarity (Figure extracted from Kim 2006).* 

# I.4 Reducing end modifications of cellulose nanocrystals

So far, different studies have been carried out to modify the surface of CNCs in a controlled manner to introduce new chemical functionalities that widened the application potential of these particles. However, a very reduced number of these chemical derivatization studies focused on the modification of the reducing end through reactions involving the aldehyde groups, due to the moderately low number of reactive sites. Nevertheless, the research enthusiasm for regioselective modification of CNCs has intensified in the course of recent years. Reported works especially focus on the different chemical pathways that can be envisaged for a successful modification and on potential applications of end-functionalized CNCs. The following section thus reflects the state of the art in the field of the reducing end modification of CNC-I after a brief summary of the non-regioselective surface modification of these biosourced particles. To our knowledge, the chemical modification of CNC-II has been limited to TEMPO-oxidation, as shown in section I.2.2 (Hirota 2010, 2012).

#### I.4.1 Brief summary of surface modifications

In the last decade, different reviews (Eyley 2014; Habibi 2010, 2014; Klemm 2011; Kwok-Mern Chin 2018; Lima 2004; Moon 2011; Peng 2011; Wohlhauser 2018) have elaborated in detail the different surface modifications of CNCs. They can mainly be divided into three categories: non-covalent modifications, covalent modifications by small molecules and grafting of polymer chains.

Non-covalent surface modifications of CNCs are commonly accomplished via adsorption of surfactants, oppositely charged elements or polyelectrolytes, or neutral polymers allowed by interactions guaranteed through hydrophilic affinity, electrostatic attractions, hydrogen bonds or van der Walls forces. This methodology was accounted for first by Heux *et al.* (Heux 2000), and developed in several studies (Bondeson 2007; Kim 2009; Rojas 2009; Salajkova 2012; Villares 2015; Zhou 2009).

Covalent surface modifications of CNCs by small molecules are typically achieved by a number of representative chemical reactions (Figure I.18), such as TEMPO-mediated oxidation

(conversion of primary hydroxyl groups to their carboxylic form) (Araki 2001; Denooy 1994; Habibi 2006; Montanari 2005), esterification (Braun 2009; Sassi 1995; Sobkowicz 2009; Yang 2013; Yuan 2006), etherification (Hasani 2008; Ho 2011; Olszewska 2011), silylation (Raquez 2012; Yang 2010), urethanization (Biyani 2013; Rueda 2011; Shang 2013; Taipina 2013), periodate oxidation (Leguy 2018, 2019) and click chemistry (Eyley 2011; Pahimanolis 2011; Sadeghifar 2011).

![](_page_62_Figure_2.jpeg)

*Figure I.18.* Schematic representation of different chemical reactions for covalent surface modifications of CNCs by small molecules (Figure extracted from Moon 2011).

The grafting of polymer chains on the outer surface of cellulose nanocrystals can be accomplished by "grafting onto" or "grafting from" approaches. The "grafting onto" strategy (Azzam 2010, 2016b; Harrisson 2011; Ljungberg 2005; Mangalam 2009) includes connection of pre-synthesized polymer chains, displaying reactive groups, onto the bare or modified CNC surface. However, steric hindrance can hamper optimal attachment. Instead, the "grafting from" approach (Majoinen 2011; Morandi 2012; Yi 2008, 2009; Zoppe 2010, 2012) was chosen to possibly increase the grafting density on the surface and allow to attach longer chains. In this strategy, polymer chains are synthesized *in situ* from initiator groups at the CNC surface using

controlled polymerization techniques such as ring opening polymerization or atom transfer radical polymerization.

## I.4.2 Reducing end modification of native cellulose nanocrystals

A summary of the different chemical reactions to modify the reducing end of CNC-I and the corresponding main mechanism is shown in Figure I.19 (Heise 2020).

![](_page_63_Figure_4.jpeg)

*Figure I.19.* Schematic representation of different chemical reactions used to target cellulose reducing end groups and their main mechanisms (Figure adapted from Heise 2020).

I.4.2.1 Different modifications with small molecules

## I.4.2.1.a) Wolff-Kishner reduction

The Wolff-Kishner reduction is an organic redox reaction that generates a hydrazone by condensation of hydrazine with a ketone or aldehyde group (Figure I.20).

$$\begin{array}{c} O \\ R \\ \hline H_{2}O \\ \hline H_{2$$

#### Figure I.20. Wolff-Kishner reduction.

The formation of a C=N bond at the reducing end was favored in several studies. Huang *et al.* (Huang 2013) prepared fluorescent CNC-I by labeling their reducing end with fluorophores containing hydrazine or amino groups. These rod-like nanoparticles were inspected by several techniques such as CP/MAS NMR, UV-Vis spectroscopy, fluorescence spectroscopy, TEM, elemental analysis and X-ray photoelectron spectroscopy. Karaaslan *et al.* (Karaaslan 2013) first functionalized CNC-I by 4-hydrazinobenzoic acid in order to introduce azide groups at the reducing end. Then, a click reaction between functionalized CNC-I and  $\beta$ -casein micelles bearing acetylene groups was achieved (Figure I.21). However, the results do not clearly show the designed structure. Furthermore, the AFM images are ambiguous since many CNC-I were functionalized on their lateral surface by casein micelles, shedding doubt on a true regioselective modification

![](_page_64_Figure_4.jpeg)

**Figure I.21.** (a) Schematic representation of the click reaction between azide functionalized CNC-I and acetylene functionalized  $\beta$ -casein, and possible configurations for the bio-conjugated nanoparticles. (b) *AFM* image of assemblies of modified CNC-I and  $\beta$ -casein micelles (Figure extracted from Karaaslan 2013).

Some researches concentrated on emulsion stabilization. By a ligation of hydrazine followed by amidation reaction, Du *et al.* specifically installed hydrophobic  $C_{18}$  alkyl chains at the reducing end of CNC-I (Du 2017). A relatively low content of CNC- $C_{18}$  could stabilize a n-hexane in water Pickering emulsions. Thanks to the possible breakage of the C=N linkage by pH changes, de-emulsification could be triggered by the pH. In addition, PS microspheres with amino-rich surfaces could be generated using this emulsion as a template.

Recently, radiolabeled molecular imaging probes were set up by forming tetraxetan-hydrazide at the reducing end and on the surface of cellulose nanocrystals (Imlimthan 2019). The two modified particles were further radiolabeled by <sup>111</sup>InCl<sub>3</sub> and then examined by single photon emission computed tomography imaging for evaluating their behavior in biological systems *in vitro* and *in vivo*. The results showed the end modified CNC-I had a freer motion in the biolabeling application due to the preservation of the surface -OH groups, which gives more possibilities for subsequent functionalization.

The same hydrazine linkage was used to block reducing end groups by phenylhydrazine, in order to achieve TEMPO-mediated oxidation and a peptide coupling reaction with propargylamine only on the lateral surface of cellulose nanocrystals. The resulting particles were characterized by <sup>1</sup>H NMR and showed a typical chemical shift of aromatic ring (Sadeghifar 2011).

I.4.2.1.b) Pinnick oxidation and reductive amination

The Pinnick oxidation is an organic reaction by which aldehydes can be oxidized into their corresponding carboxylic acids using sodium chlorite (NaClO<sub>2</sub>) under mild acidic conditions (Figure I.22).

![](_page_65_Figure_6.jpeg)

Figure I.22. Pinnick oxidation.

Reductive amination is a form of amination that involves the conversion of a carbonyl group to an amine via an intermediate imine. The carbonyl group is most commonly a ketone or an aldehyde (Figure I.23).

![](_page_66_Figure_2.jpeg)

#### Figure I.23. Reductive amination.

By functionalizing CNC-I with thiol groups using a reductive amination, Lokanathan *et al.* (Lokanathan 2013) showed a straightforward strategy to form nanoscale cilia-like structures through adsorption of end functionalized CNC-I onto a gold surface, permitting their immobilization. Labeled by silver nanoparticles at the reducing end, the functionalization was qualified by observing these tagged nanoparticles using cryo-TEM. X-ray photoelectron spectroscopy was used to quantify the thiol groups content. The formed hairy surface was investigated by AFM and QCM-D (Figure I.24). After a directional drying process, some alignments of adsorbed CNC-I were observed.

![](_page_67_Figure_1.jpeg)

**Figure 1.24.** (a) Schematic representation of chemical modification at reducing end of CNC-I and labeling of silver nanoparticles. (b) AFM topography images of CNC-I (a) and CNC-SHs (b) adsorbed on a gold surface followed by convective flow from an evaporating drop of water. The range of the height scale in both images is -2 to +12 nm, and the image size is 2  $\mu$ m × 2  $\mu$ m (Figure extracted from Lokanathan 2013).

In order to improve the redispersion capacity of CNC-I after the freeze-drying or ovendrying treatment, a triazole molecule was used to chemically modify the reducing end of CNC-I by reductive animation (Figure I.25) (Li 2018a). Without negative influence of electrostatic repulsion provided by sulfate groups on the surface of CNC-I, the end-grafted triazoles added a steric stabilization as an advantage for the redispersion and stability of CNC-I in aqueous suspension. Taking advantage of the thiol groups via a click linkage between CNC-I and natural rubber chains, a reactively enhanced composite was structured. However, TEM images aiming at proving the authors point rather indicate a low grafting yield.

59

![](_page_68_Figure_1.jpeg)

*Figure I.25. (a)* Mechanisms for the dispersion of end-modified cellulose nanocrystals in water and reactive compatibility of end-modified nanocrystals and natural rubber. (b) TEM images of bare CNC-I (A), silver nanoparticles functionalized CNC-I (B and C) (Figure extracted from Li 2018a).

Another report by Tao *et al.* (Tao 2019) was published, in which the CNC reducing end groups were modified by cysteamine through a reductive amination. The selective thiolation was used to covalently incorporate the end-modified CNC-I into a thermoplastic elastomer (styrene-butadiene-styrene copolymer, SBS) by means of an UV-instigated thiol-ene click reaction. The target was to save the nanocrystals surface properties guaranteeing the development of a stable percolation network with strong filler-matrix and filler-filler interactions. The ensuing UV post-curing during the molding procedure framed a twofold system because of the chemical self-crosslinking of the bulk SBS. However, even if a number of results such as mechanical and rheological properties showed a difference between SBS, CNC/SBS composite and end-modified CNC/SBS composite, the TEM image could not fully demonstrate the success of chemical modification at reducing end as the silver nanoparticles were rarely labeled at the end but on the lateral surface of CNC-I (Figure I.26).

![](_page_69_Figure_1.jpeg)

*Figure I.26. (a)* Chemical modification at reducing end of CNC-I and post UV irradiation treatments. *(b)* TEM image of labeling of silver nanoparticles at reducing end of CNC-I (Figure extracted from Tao 2019).

#### I.4.2.1.c) Knoevenagel condensation

A Knoevenagel condensation is a nucleophilic addition of an active hydrogen compound to a carbonyl group followed by a dehydration reaction in which a molecule of water is eliminated (Figure I.27). This active hydrogen compound, noted as  $CH_2Z_2$  in Figure I.27, is such that Z is a powerful electron withdrawing functional group, like -CN, -COOH or -NO<sub>2</sub>.

![](_page_69_Figure_5.jpeg)

# Figure I.27. Knoevenagel condensation.

Heise *et al.* (Heise 2019) recently applied this reaction to target the reducing end groups of CNC-I. In aqueous condition, an enolate anion, emerging from the deprotonation of the acidic

 $\alpha$ -carbon of a dicarbonyl, attacks the reducing end under weakly basic conditions. The underlying condensation is then treated by water elimination with cyclization to a C-glycoside intermediate that finally went through retro-Claisen aldolization forming the new C-glycoside ketone (Figure I.28). Compared to other reactions, Knoevenagel condensations gave excellent yields and stereoselectivity and the mechanism can be transferred and applied to more complex compounds.

![](_page_70_Figure_2.jpeg)

*Figure I.28. Knoevenagel condensations on reducing end of cellulose nanocrystals (Figure extracted from Heise 2019).* 

I.4.2.2 Polymer grafting

I.4.2.2.a) Grafting onto

In 2003, Sipahi-Saglam *et al.* (Sipahi-Saglam 2003) chemically activated aldehyde groups at the reducing end of CNC-I via hydrazine linkages. Macromolecules carrying reactive end groups were subsequently attached to the reactive cellulose nanocrystals by "grafting onto" methods (Figure I.29). The resulting particles, combining with other end-modified CNC-I in their report, formed a block copolymer, which presented a tendency to form film superstructures in the suspension. After freeze-drying, the layer structure is observable by the naked eye.

![](_page_71_Figure_1.jpeg)

*Figure I.29.* Sequence of "grafting onto": (a) preparation of carboxyl-terminated cellulose by 4hydrazinobenzoic acid; (b) grafting of 3-amino-propyl-terminated polyethyleneglycol using carbodiimide as a catalyst (Figure extracted from Sipahi-Saglam 2003).

Through reductive amination, Tang *et al.* (Tang 2018) grafted hydrophobic polystyrene onto the reducing end of CNC-I. Several characterizations such as DLS, FTIR and aldehyde content showed the success of grafting and a temperature of 70 °C favored the grafting, while the use of liquid NMR was not a good thought. The resulting nanoparticles were used as stabilizer to prepare hexadecane or toluene in water Pickering emulsion. The formed emulsion presented a good stability for time. They showed also the increase of molecular weight of polystyrene slightly raised the droplet size. One hypothesis is the longer chains screened the reducing end groups leading to a lower grafting yield.

A series of studies on the reducing end modification of tunicate CNC-I were carried out by Villares *et al.* and Chemin *et al.* (Chemin 2020a, b; Villares 2018). First, tunicate cellulose nanocrystals have been modified by the introduction of a biotin functionality at their reducing ends (Villares 2018). Two biotin derivatives consisting of an amine or hydrazine group were studied. After adding streptavidin, a protein that can bind up to four biotin molecules, a specific and controlled supramolecular assembly of several nanocrystals was formed (Figure I.30). The end modified CNC-I and the assembly were characterized by DLS, QCM-D, AFM and TEM. The QCM-D results showed that the steric effect plays an important role on reducing end modification. Using PEG as a longer linker that decreased the steric effect between CNC-I and biotin, a much higher grafting yield could be observed and verified by AFM images.


*Figure I.30.* Schematic representation of biotin functionalized CNC-I and assembly of CNC-I by biotinstreptavidin interactions (Figure extracted from Villares 2018).

Later, Chemin *et al.* (Chemin 2020b) grafted PAMAM dendrimers at the reducing end of tunicate CNC-I in order to adsorb these end-modified nanoparticles on gold surface with the aid of affinity between gold and NH<sub>2</sub> groups in PAMAM dendrimers. A functional hairy surface was thus formed. Compared to the cilia-like structure formed by Lokanathan *et al.* (Lokanathan 2013), the PAMAM-CNC complex retained the rigid property of CNCs and add more flexibility due to PAMAM dendrimers. A pH reversible response of these functional materials was observed by changing the pH from 11 to 2. The size of the PAMAM dendrimer determined the arrangement of the adsorbed layer, and the change from PAMAM G2 to PAMAM G3 (G: generation that increases with the number of primary, tertiary amine groups on the surface of PAMAM) clearly modified the adsorbed mass, water content and pH-responsive properties.



**Figure I.31.** (a) Reducing end chemical modification of tunicate CNC by PAMAM dendrimer. (b) Change in frequency and in dissipation as a function of time for the overtone n = 5 corresponding to t-CNC-Gx adsorbed on gold surfaces, submitted to solvent exchange from KOH 1 mM to HCl 10 mM (4 consecutive times) showing swelling/deswelling as illustrated on the right (Figure extracted from Chemin 2020b).

Consecutively, Chemin *et al.* (Chemin 2020a) compared the adsorption properties of unmodified CNC-I and CNC-I that were modified by polyamine or biotin at their reducing end. The kinetic investigation by QCM-D showed that unmodified CNC-I were adsorbed on polycationic layer by hydrogen bonds and electrostatic interactions in a random but flat mode which had a high surface extent. However, end modified CNC-I ought to pick a perpendicular

inclination due to the steric hindrance of these nanorods as the adsorbed surface took place only at reducing end. Thus, the surface coverage for end-grafted CNC-I were much lower. For biotin-grafted CNC-I that adsorbed on streptavidin surface with hydrogen bonds and van der Waals interactions, this orientation was more upright than PAMAM dendrimer grafted CNC-I. An explanation is that the PAMAM dendrimer is larger and more flexible, resulting in a freer adsorption inclination. Furthermore, the PAMAM polymer chains could be associated leading to the assembly of several nanocrystals which were adsorbed in groups. This phenomenon prevents the adsorption as the steric hindrance was increased due to the association of nanocrystals.



*Figure 1.32. (a)* Schematic description of chemical modifications at reducing end of CNC-I. (b) Fractional surface coverage for CNC-I as a function of time calculated from the Voight-based model. The squares correspond to experimental data fitted by the Voight's model and the solid lines represent the fit (Figure extracted from Chemin 2020a).

# I.4.2.2.b) Grafting from

In 2003, Sipahi-Saglam *et al.* (Sipahi-Saglam 2003) started a radical polymerization of acrylamide from the cellulose nanocrystals after attaching reactive initiators at the reducing end (Figure I.33). These particles, as mentioned before, combined with other end modified CNC-I in their report, formed a block copolymer, which generated the layer structure in suspensions.



*Figure I.33.* Sequence of "grafting from": (a) selective immobilization of azo initiator on the cellulose reducing end; (b) radical polymerization with acrylamide (Figure extracted from Sipahi-Saglam 2003).

Using the "grafting from" method, Zoppe and coworkers aimed at grafting thermosensitive poly(N-isopropylacrylamide) chains the at the reducing end of CNC-I by atom transfer radical polymerization (Figure I.34) (Risteen 2018; Zoppe 2017). The number of available reducing end groups before and after the modifications were quantified by colorimetric assays. The success of modifications was confirmed by FTIR, DSC and SEC measurements even though the TEM images of these CNC-I labeled by gold nanoparticles are ambiguous. It has to be noted that the pristine CNC-I in their case had a few -COOH groups on the surface which led to "patchy" CNC-I that were functionalized not only at the reducing end but also at different positions along the majority surface of the rods. Furthermore, the authors have shown that both "patchy"-modified CNC-I and surface-only polymer grafted CNC-I formed liquid crystal phases at room temperature yet a distinction of responses to heat was observed. With the influence of the LCST of the polymer, the reducing end modified CNC-I had a lower polymer content and therefore exhibited a different temperature switch of liquid crystal phase. This system could be used as a thermal sensor.



*Figure I.34.* Synthetic protocols for the polymerization from (*a*) reducing end groups and (*b*) surfaces of CNC-I (Figure extracted from Zoppe 2017).

# I.4.2.3 Alternative strategy

All chemical reactions reported in the previous sections were directly performed at the reducing end of CNC-I. Alternatively, the group of van de Ven has reported a different approach leading to symmetrically end-functionalized CNCs, referred to as "hairy" cellulose nanocrystals. According to these works, a periodate oxidation followed by chlorite oxidation or hot water treatment results in CNC comprising an intact crystalline central regions and hairy extremities of derivatized periodate-oxidized or carboxylated chains. Using two different batches of hairy CNCs that were further modified to bear either alkyne or azide groups at both ends, an end-to-end association was reported using a click chemistry reaction, even if AFM images show that each assembly is formed of a limited number of particles (Yang 2016).

In a subsequent work, polyamidoamine (PAMAM) was grafted at the two ends carboxylated hairy CNCs by a bioconjugation reaction, which allowed to generate CNC complexes regrouping particles through end-to-end associations (Figure I.36) (Tavakolian 2019).



*Figure I.35. (a)* Schematic description of the end-to-end assembly of hairy CNCs by azide-alkyne click reaction. (b) AFM image of modified CNC assembly (Figure extracted from Yang 2016).



*Figure I.36. (a)* Schematic description of self-assembled structures of hairy CNCs by bioconjugation between hairy CNCs and PAMAM. (b) AFM height images of the assembled HENCC with different ratio of HENCC:PAMAM (Figure extracted from Tavakolian 2019).

# **I.5** Conclusion

This literature review shows that CNC-I and CNC-II exhibit a specific structure that makes them amenable to a regioselective functionalization targeting only one end of the rods in case of CNC-I and the two extremities of the crystals in the case of CNC-II. As described in the last part, the reducing end modification of CNC-I has already been investigated by different

groups using various chemical pathways. The resulting end-modified CNCs exhibit assembling and colloidal properties that are different from their unmodified or homogeneously modified counterparts. Such results show the interest of the strategy but also reveal some challenges. First, a direct characterization of the success of the derivatization is difficult since the ends of the CNCs only represent a very minor fraction of the surface (a few percent), which makes a quantitative description difficult. Secondly, indirect evidences provided in the reported works often suggest that rather low modification yields are obtained, while total modification of all the objects in the suspension would be preferable. Attempts to optimize the derivatization yield and to use appropriate techniques to prove the achievement of the reactions are thus mandatory. Such issues will be tackled in the following chapters. This bibliographic survey also points out that relatively few research works have focused on CNC-II, and that especially the regioselective derivatization of their ends is a very new and open field. In addition to this chapter, some more specific literature data will be presented in the introduction section of each chapter.

# **Chapter II**

Regioselective labeling of various types of cellulose nanocrystals with gold nanoparticles

# **II.1 Introduction**

The combination of CNCs with metal nanoparticles (NPs) is a way to obtain hybrid organic-inorganic materials benefiting from the properties of both components and expectedly prone to offer new synergistic behaviors. Works have thus been dedicated to the association of both types of particles, including grafting of metal NPs at the reducing end of CNC-I particles. For example, thanks to their large specific surface area and their colloidal stability in water, CNCs have been used as efficient stabilizers for many metal NPs, such as Pd (Wu 2013), Ag (Shi 2015; Tang 2015a) and Au (Chen 2015; Semenikhin 2018; Wu 2014; Yokota 2008; Zhang 2018). Semenikhin et al. (Semenikhin 2018) have prepared individually dispersed gold nanoshell-bearing CNCs by using gold nanoparticles. Different attempts specifically targeting the reducing end of CNCs have also been reported. Hieta et al. (Hieta 1984) stained the reducing ends of Valonia CNCs with silver nanoparticles using a reductive amination reaction. Kuga et al. (Kuga 1988) and Koyama et al. (Koyama 1997) have used a silver-staining technique as well to identify the reducing end of CNCs from Valonia, ramie and bacterial cellulose. The reducing end of CNC-I or CNC-II particles prepared from Valonia and ramie have also been stained with gold nanoparticles to demonstrate the molecular directionality (Kim 2006). Lokanathan et al. (Lokanathan 2013, 2014) have produced cilia-mimetic hairy surfaces, based on endimmobilized nanocellulose at gold surfaces by an oxidation followed by a peptide coupling reaction. More recently, Li et al. (Li 2018a) have used a triazole end-grafting reaction on CNCs for water-redispersion improvement using gold NPs. Nevertheless, judging from the reported transmission electron microscopy (TEM) images shown in the aforementioned works and based on reported low labeling yield values of only 15 %, efficient methods to regioselectively label the reducing end of CNCs still need to be developed.

In the present chapter, we propose to tackle the optimization of the regioselective labeling of CNCs with gold nanoparticles (AuNPs), which stands as a prerequisite to fully benefit from synergistic properties of materials comprising such hybrids. The work is based on the use of an alternative labeling strategy avoiding the use of pre-synthesized AuNPs and favoring high attachment yields. Complementary TEM, dynamic light scattering, small-angle X-ray scattering and UV-Vis spectroscopy analyses were used to characterize the labeled particles. Importantly, two types of native CNCs from different cellulose sources were used and the possibility of both-ends dual labeling of CNC-II particles was considered. Such a strategy additionally allowed us to give some new insight into the basic characteristics of these particles and/or provide statistically-relevant confirmations of debated morphological aspects.

# **II.2 Materials and methods**

# **II.2.1 Materials**

Cotton linters were provided by Buckeye Cellulose Corporation and used as the cellulose source without any further purification. Acetic acid (HAc) was purchased from Carlo Erba Reagents and other chemicals were purchased from Sigma-Aldrich and used as received. Deionized water was used in all experiments.

# **II.2.2** Native CNCs from cotton (cCNC-I)

Cotton linters were treated with 65 wt. % sulfuric acid during 30 min at 63 °C under mechanical stirring following the protocol described by Revol *et al.* (Revol 1992). The resulting suspension was washed by repeated centrifugation/redispersion cycles and dialyzed against distilled water until the conductivity of the dialysis bath reached the conductivity of distilled water. In the following step, the suspension was ultrasonicated for 4 min with a Branson 250 digital sonifier and successively filtered through 8  $\mu$ m and 1  $\mu$ m cellulose nitrate membranes using a Sartorius filtration equipment. These native cellulose CNCs obtained from cotton will be referred to as cCNC-I.

#### II.2.3 Native CNCs from wood (wCNC-I)

A 3 wt. % aqueous dispersion of neutral CNCs was provided by Melodea Ltd (Israel) and used after dilution to 1 wt. % with deionized water. These nanocrystals, that will be referred to as wCNC-I, were produced from wood by the reported sulfuric acid (64 wt. %) hydrolysis method (Bondeson 2006).

## II.2.4 Cellulose II CNCs from cotton (cCNC-II)

cCNC-II suspensions were prepared according to the method described by Flauzino Neto *et al.* (Flauzino Neto 2016). The cotton linters were treated with a 20 wt. % sodium hydroxide aqueous solution under mechanical stirring for 5 h at 25 °C, using 30 mL of solution per gram of material. The material was washed several times with distilled water until complete alkali removal, and dried at 40 °C for 12 h in an air-circulating oven. The mercerized cotton linters were submitted to sulfuric acid hydrolysis. Here, for each gram of mercerized cotton linter, 20 mL of 60 wt. % H<sub>2</sub>SO<sub>4</sub> was used. The hydrolysis was performed at 45 °C for 50 min under vigorous stirring. Sample preparation was then completed by centrifugation/redispersion cycles, dialysis, ultrasonication and filtration using the same conditions as those detailed for cCNC-I.

#### **II.2.5 Sulfur content**

A suspension of CNC containing about 100 mg of cellulose was titrated with a solution of 0.01 M NaOH using a MeterLab<sup>®</sup> CDM 210 conductivity meter. At the equivalence we have:

$$V_{eq} \times C_{NaOH} = n_{cellulose} \times d_S$$
 (Equation 1)

with  $V_{eq}$  the added volume of NaOH,  $n_{cellulose}$  the number of moles of glucose residues and  $d_s$  the degree of substitution representing the number of sulfate groups per unit of glucose. And we know that:

$$n_{cellulose} = \frac{m_{cellulose}}{M_{ave}}$$
 (Equation 2)

where  $m_{cellulose}$  is the dry weight of introduced CNC and  $M_{ave}$  is the average molar mass of a substituted glucose residue which is given by the following relation:

$$M_{ave} = 162 \times (1 - d_S) + 242d_S$$
 (Equation 3)

where 162 g/mol et 242 g/mol are respectively the molar masses of a unit of anhydroglucose and a mono-sulfated unit. The equations 1 and 2 allow us to write:

$$d_{S} = \frac{V_{eq} \times C_{NaOH} \times M_{ave}}{m_{cellulose}}$$
(Equation 4)

After solving the equations 3 and 4,  $M_{ave}$  was calculated from the following relation:

$$M_{ave} = \frac{162}{1 - 80 \times \frac{V_{eq} \times C_{NaOH}}{m_{cellulose}}}$$
(Equation 5)

The sulfur content,  $\tau_s$ , was then calculated using the following relation:

$$\tau_S = \frac{32 \times d_S}{M_{ave}}$$
 (Equation 6)

#### II.2.6 Aldehyde content

The Fehling test was used to measure the aldehyde content (Vollhardt 2014). Two solutions A and B were prepared. For solution A, 69.28 g of CuSO<sub>4</sub>•5H<sub>2</sub>O was dissolved in 1 L of distilled water. Solution B was prepared by dissolving 346 g of KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>•4H<sub>2</sub>O and 120 g of NaOH in 1 L of distilled water. The final solution for the titration was obtained by mixing these two solutions in equal volume. 2 g of freeze dried CNCs were dispersed into 50 mL of deionized water. The suspension was heated to 65 °C with a very gentle stirring and then titrated dropwise with the titration solution using a micropipette until there was no more red precipitate. After each drop, about 2 minutes were allowed for the detection of the red precipitate.

## **II.2.7** Thioureation

Thioureation refers to the reaction that introduces the thiourea group. An aqueous solution containing 10 wt. % HAc and 2 wt. % thiosemicarbazide was prepared. Then, a suspension containing about 2 g of cellulose was mixed with the previous solution in the same volume in a flask surmounted by a condenser. The mixture was heated at 60 °C for 3 h and finally cooled in ice. Then, NaCl was added until its concentration was approximately 0.4 M. The suspension was then centrifuged for 30 minutes at 11200 rpm and the modified CNCs were redispersed in HAc. The latter operation was repeated twice. The final product, hereafter referred to as S-e-CNC, was dialyzed against distilled water until constant conductivity of the dialysis bath and ultrasonicated for 4 min with a Branson 250 digital sonifier.

# **II.2.8** Preparation of gold nanoparticles (AuNPs)

Deionized water (925 mL) was heated to 95 °C and 92.5 mg of chloroauric acid was added under stirring. Once 25 mL of a 1 wt. % trisodium citrate solution was added, the mixture was stirred for 30 min at 95 °C and then cooled down (Haiss 2007).

# **II.2.9 CNC labeling with gold nanoparticles**

Two methods were used to label CNCs with gold nanoparticles.

*Direct reaction method:* 1 L of AuNP suspension containing 54 mg of pure gold was mixed with 2 g of a 1 wt. % S-e-CNC suspension. The mixture was stirred for 3 days away from light.

*In situ growth method:* Two grams of a 1 wt. % S-e-CNC suspension was added into 925 mL deionized water. The suspension was then heated to 95 °C. 92.5 mg of chloroauric acid and 25 mL of a 1 wt. % trisodium citrate aqueous solution were then added under stirring for 30 min at 95 °C. The resulting suspension was then cooled down to room temperature.

# **II.3 Results and discussion**

# **II.3.1** Morphology and physicochemical properties of different starting materials

II.3.1.1 Characterization of cCNC-I and cCNC-II by WAXS, FTIR and CP/MAS NMR

The comparison between cCNC-I and cCNC-II was achieved by WAXS, FTIR and CP/MAS NMR measurements. The X-ray diffraction (WAXS) two-dimensional patterns and profiles of cCNC-I and cCNC-II are shown in Figure II.1. The main diffraction rings/peaks are in good agreement with literature report (French 2014).



Figure II.1. X-ray diffraction pattern (a) and indexed profiles (b) of freeze-dried cCNC-I and cCNC-II.

For cCNC-I, the pattern is typical of cellulose I nanocrystals (Flauzino Neto 2016). Three equatorial curves allotted to  $(1\overline{10})$ , (110) and (200) planes, as well as the trademark slender meridional bend related to the (004) planes could be observed. In Figure II.2b, between 15 and 20 degrees, two small peaks were observed, which are attributed to  $(1\overline{10})$  and (110) crystal planes. At about 22 degrees, a high signal indicates the (200) crystal plane and the (004) plane could be observed at about 35 degrees (French 2014).

The WAXS pattern of cCNC-II is clearly different from the cCNC-I pattern. Three principal arcs on the equator, corresponding to the  $(1\overline{1}0)$ , (110) and (020) planes, which are incarnated in the scattering spectrum at  $12.5^{\circ}$ ,  $20.5^{\circ}$  and  $22.5^{\circ}$ , indicate that the mercerization process resulted in the allomorphic conversion from cellulose I to cellulose II (French 2014; Flauzino Neto 2016).

Fourier-transformed infrared spectroscopy (FTIR) allows to observe the characteristic functional groups of cellulose. In addition, it is a fast method and consumes little sample quantity. Here, attenuated total reflection (ATR) as a sampling technique used in conjunction with FTIR enables samples to be examined directly in the solid or liquid state without further preparation.

Figure II.2 shows typical FTIR spectrum of cCNC-I and cCNC-II. For cCNC-I (Figure II.2a), several characteristic peaks could be identified.

Between 3000 and 3600 cm<sup>-1</sup>, the stretching vibration bands of O-H bonds of primary and secondary alcohol functions were observed. At 1315, 1335, 1430 and 1470 cm<sup>-1</sup>, the in-plane deformation vibrations of O-H bonds of alcohols were detected. At 1110, 1060 and 1035 cm<sup>-1</sup>, vibrations of the C-O bond of carbon 2, 3 and 6 were noticed. The peaks located at 665 and 705 cm<sup>-1</sup> are attributed to the out-of-plane deformation vibrations of the O-H functions of alcohols. The vibration peak around 2900cm<sup>-1</sup> is attributed to stretching vibrations of C-H bond. The asymmetric stretching vibration of C-O-C glycosidic bond appears at 1160 cm<sup>-1</sup>. This peak is normally used to normalize the spectra. All characteristic peaks are in good agreement with literature reports (Marechal 2000).

For cCNC-II, the FTIR spectrum shows the presence of two distinctive peaks at about 3490 and 3450 cm<sup>-1</sup>, that correspond to intramolecular hydrogen bonding in cellulose II. Another evidence for the conversion of cellulose I into cellulose II is provided by the significant decrease of the peak intensity at 1430 cm<sup>-1</sup> due to the conformation change of hydroxymethyl group from *tg* to *gt* form. The stretching vibration of C-O at C6 peak was shifted to 1030 cm<sup>-1</sup> as the torsional angles of  $\beta$ -(1,4)-D-glycosidic linkages changed. Consequently, the C-O-C vibration of this linkage was switched to 895 cm<sup>-1</sup>. These results correspond well to literature reports (Dhar 2015; Han 2013; Oh 2005; Zuluaga 2009).



Figure II.2. FTIR spectra of freeze-dried cCNC-I (a) and cCNC-II (b).

We also used solid-state NMR, which is a powerful technique for analyzing CNCs and their chemical modifications and ultrastructure (Heux 1999). Figure II.3 shows the CP/MAS NMR spectra of cCNC-I and cCNC-II.



Figure II.3. CP/MAS NMR spectra of freeze-dried cCNC-I (a) and cCNC-II (b).

Figure II.3a is typical for cCNC-I (Atalla 1984; Montanari 2005; Vanderhart 1984). The region between 60 and 70 ppm is attributed to carbon C6. The signal is divided into two contributions: the first at 64 ppm which comes from the ordered chains of the crystalline part and the second at 62 ppm which arises from the less ordered surface chains. The region between 70 and 80 ppm is attributed to carbons C2, C3 and C5. Like the C6 signal, the C4 carbon signal is divided into a first contribution of crystalline C4 at 88 ppm and a second at 83 ppm attributed to amorphous C4 of surface chains. The carbon C1 signal appears around 105 ppm and is generally used to normalize the spectra.

For cCNC-II, an increase in the intensity of the peak near 107.3 ppm is attributed to the C1 of the crystalline part of cellulose II. Moreover, the disappearance of the signal at 65 ppm, associated with the crystalline regions of cellulose I, also proved the transition from cellulose I to cellulose II. The presence of the peak at 76 ppm, attributed to C2, C3 and C5, occurs because

of variations in hydrogen bonding patterns resulting from a new molecular conformation of the chains. These results are also in good agreement with the literature reports (Park 2009; Zuluaga 2009).

WAXS, FTIR and solid-state NMR data therefore unambiguously prove the successful allomorphic transition from cellulose I to cellulose II during the mercerization process.

# II.3.1.2 Morphology of the different CNCs

cCNC-I resulting from the sulfuric acid hydrolysis of cotton linters consisted of rodlike nanoparticles with a length between 100 and 300 nm and a width between 10 and 30 nm. These CNCs are composed of a fascicle of laterally associated elementary crystallites (Figure II.4a), in good agreement with literature reports (Elazzouzi-Hafraoui 2008; Lokanathan 2013). The average sulfur content obtained by conductometric titration was about 0.22 mmol g<sup>-1</sup> and a  $\zeta$ -potential of -34 ± 5 mV was measured, confirming that cCNC-I possess negative sulfate half-esters on their surface.



*Figure II.4. TEM micrographs of negatively stained cCNC-I* (*a*), *wCNC-I* (*b*) *and cCNC-II* (*c*) *prepared from aqueous suspensions at room temperature.* 

The TEM micrograph of wCNC-I shown in Figure II.4b reveals that these nanoparticles are morphologically different from cCNC-I. wCNC-I are indeed rodlike but are longer (length between 200 and 400 nm) and thinner (less than 15 nm) than cCNC-I. Their average aspect ratio is about 20, compared to about 10 for cCNC-I. In contrast with cCNC-I, wCNC-I comprise only one or two elementary crystallites. A sulfur content of 0.325 mmol g<sup>-1</sup> has been reported by the manufacturer (Rivkin 2015) and a zeta-potential of  $-33.7 \pm 5$  mV was measured. The

negative surface charges on cCNC-I and wCNC-I generate electrostatic repulsions, which results in a pronounced individualization of the nanorods and the absence of aggregates.

cCNC-II prepared from the sulfuric acid hydrolysis of mercerized cotton linters are shown in Figure II.4c. These CNCs also exhibit a rodlike morphology but with an aspect ratio of about 6, *i.e.* smaller than the one of cCNC-I. Their length is in the 50-150 nm range while their width is  $16 \pm 2$  nm. Like cCNC-I, they are composed of an average of 3-5 laterally-associated elementary crystallites. These morphological features of cCNC-I are in agreement with those reported for cCNC-II prepared from ramie (Kim 2006) and eucalyptus (Flauzino Neto 2016). The sulfur content was about 0.21 mmol g<sup>-1</sup> and the zeta-potential was -32.1 ± 4 mV, showing that cCNC-II also exhibit surface sulfate half-esters and that their charge density is comparable to that of cCNC-I.

#### II.3.1.3 Gold nanoparticles

An aqueous colloidal suspension of gold nanoparticles (AuNP) was prepared from a typical sodium citrate-stabilized method. One minute after addition of Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, the transparent solution turned into wine red upon formation of gold colloids. These nanoparticles are expected to exhibit a size-dependent plasmonic resonance behavior. In our case, the final suspension was characterized by UV-Visible spectroscopy and DLS (Figure II.5). The absorption peak at about 525 nm observed the in UV spectrum indicated a nanoparticle diameter between 10 and 30 nm (Haiss 2007). The DLS measurement gave a hydrodynamic diameter of about  $20 \pm 5$  nm, in good agreement with the UV-Vis spectroscopy results.



*Figure II.5. (a) UV-Vis spectrum of gold nanoparticles. (b) Intensity size distribution of a 0.5 wt. % gold nanoparticle suspension measured by DLS at 20 °C.* 

# **II.3.2** Nanocrystals prefunctionalization with sulfur atoms

In order to promote the binding of AuNPs to CNCs, sulfur atoms were introduced by a one-step thioureation of the cellulose chain reducing ends, as described in Scheme II.1. Samples prefunctionalized with S atoms are designated with the prefix "S-e-" (standing for sulfur at the reducing end). In the presence of acetic acid, the aldehyde groups react with thiosemicarbazide and produce an imine group. As the reducing ends represent only a few percent of the total CNC surface, techniques such as FTIR could not detect such small changes of functional groups due to a limited sensitivity. However, the initial and post-thioureation aldehyde contents of the three different CNCs, *i.e.* the concentration of reducing ends before and after prefunctionalization were measured using the Fehling test and compared. The initial values were  $24.9 \pm 0.4$ ,  $18.8 \pm 0.6$  and  $22.7 \pm 0.5 \,\mu$ mol CHO g<sup>-1</sup> for cCNC-I, wCNC-I, cCNC-II, respectively. After the thioureation step, the aldehyde content decreased to  $9.1 \pm 0.5$ ,  $6.9 \pm 0.6$  and  $8.1 \pm 0.5 \,\mu$ mol g<sup>-1</sup> for S-e-cCNC-I, S-e-wCNC-I and S-e-cCNC-II, respectively. This decrease in the number of aldehyde groups shows that the thioureation reaction was successful. In the three cases, the molar reaction yield was high and of about 64 %.

Figures II.6a, b and c show TEM micrographs of the different S-e-CNCs. These nanocrystals are very similar to their parent particles, which reveals that the thioureation reaction did not significantly alter their morphology.



*Scheme II.1. Asymmetric and symmetric thioureation of the reducing ends of native CNCs (cCNC-I and wCNC-I) and cCNC-II particles where the aldehyde groups are converted into imine groups.* 



*Figure II.6. TEM micrographs of negatively stained S-e-cCNC-I* (*a*), *S-e-wCNC-I* (*b*) and *S-e-cCNC-II* (*c*) prepared from aqueous suspensions at room temperature.

# II.3.3 Regioselective labeling of cCNC-I and cCNC-II with gold nanoparticles

Using the thioureation reaction, sulfur atoms were successfully grafted on the reducing ends of CNCs, as indicated by the very significant decrease in the aldehyde content. Starting from this prefunctionalized nanocrystals, the labeling with AuNPs relied on the well-known spontaneous reaction between sulfur and gold, which occurs through a  $\sigma$ - $\pi$  coordinate bond (Ning 1996; Ulman 1996). Two strategies, namely a direct reaction of S-e-CNCs with presynthesized AuNPs and an *in situ* growth method, consisting in synthesizing the gold colloids from soluble derivatives in the presence of S-e-CNCs were applied to functionalize the various CNCs with AuNPs. The two procedures, described in section II.2.9 are depicted in 84





*Scheme II.2. (a)* Asymmetric and symmetric functionalization of cCNC-I and cCNC-II particles with gold nanoparticles. (b) Schematic representation of two different functionalization strategies (direct reaction and in situ growth) using cCNC-I as an example.

End-functionalized cCNC-I with gold particles, hereafter referred to as AuNP-e-cCNC-I, prepared by direct reaction and *in situ* growth were first investigated by DLS. As shown in Figure II.7a, the hydrodynamic diameter ( $D_h$ ) of presynthesized AuNPs was about 20 nm and that of S-e-cCNC-I was about 91 nm, which corresponds to the value usually reported for bare cotton CNCs prepared by a similar sulfuric acid hydrolysis (Elazzouzi-Hafraoui 2008). This  $D_h$  indicated that the thioureation reaction had no effect on the size of CNCs, which was confirmed by the TEM images in Figure II.6. For AuNP-e-cCNC-I obtained by the direct reaction methods, the intensity size distribution plot shows three peaks. The first one at about 20 nm can be ascribed to the presence of individual AuNPs (not bonded to CNCs). The second one at 98 nm is slightly shifted to larger values when compared to the  $D_h$  corresponding to as-prepared or sulfur-functionalized cCNC-I (91 nm), which might indicate that a fraction of CNCs were labeled with AuNPs. Although it is difficult to fully trust the value corresponding to the third population at about 5000 nm, which is at the upper confidence limit of the technique, this third signal indicates the presence of large size aggregates.

Figure II.7b shows the intensity size distribution of a 0.1 wt. % AuNP-e-cCNC-I aqueous suspension resulting from the *in situ* growth strategy. When compared to the direct reaction case, several differences can be observed. First, a peak at about 17 nm is also observed, but its intensity is considerably lower. This signal can tentatively be attributed to the presence of a limited number of individual AuNPs, which would have a slightly smaller hydrodynamic diameter than the presynthesized ones. A second population with a  $D_h$  of about 116 nm is detected. In contrast with the sample generated by direct reaction, this signal is clearly shifted when compared to the as-prepared cCNC-I peak at 91 nm, revealing that most particles have increased in size but without reaching a value large enough to account for aggregation. Such features are compatible with the sample being constituted of CNCs bearing AuNPs. No further peak is detected in the large size region, showing the absence of aggregates.



*Figure II.7.* Intensity size distribution of a 0.5 wt. % gold nanoparticles suspension (red solid line), 0.1 wt. % S-e-cCNC-I suspension (black solid line) and 0.1 wt. % AuNP-e-cCNC-I suspension (violet dashed line) prepared by direct reaction (a) and in situ growth (b) measured by DLS at 20 °C.

In order to complement the results obtained by DLS, TEM was used to observe the samples prepared by the two different methods. In the TEM images shown in Figure II.8, the AuNPs appear as dark spheres (due to the high electron density of gold) with an average diameter of about 20 nm and a narrow size distribution, irrespective of the method used to synthesize them. Figures II.8a and b show the TEM micrographs of AuNP-e-cCNC-I prepared by direct reaction. These images evidence that the sample comprises non-derivatized CNCs, free AuNPs gathered in clusters and a fraction of CNCs labeled with an AuNP. In the latter case, it is clear from Figure II.8b that the AuNP was attached at only one end of the crystals. Furthermore, in most cases, the AuNP attached at the end of the CNC is located at a specific position in direct alignment with the long axis of symmetry (c-axis) of one of the elementary crystallites constituting it. Rather similar results of relatively low efficiency of the labeling were already reported in the literature using different strategies. Indeed, in these works, observation of TEM images and some estimation indicated an about 15 % labeling yield on the reducing end of cCNC-I, which is also the value that we achieved in the present study using the direct reaction method.



*Figure II.8. TEM micrographs of negatively stained AuNP-e- cCNC-I prepared by direct reaction (a, b)* and in situ growth (*c*, *d*) from aqueous suspensions at room temperature.

In contrast, as shown in Figures II.8c and d, a large number of cCNC-I were

functionalized with AuNPs when using the *in situ* growth strategy. It can indeed clearly be seen that most cCNC-I are labeled with several AuNPs located on one end of the elementary crystallites constituting the cCNC-I. Each elementary crystallite bears no more than one AuNP coaligned along the c-axis. Furthermore, for all hybrid particles, the AuNPs are systematically located on one side of the CNC. These results evidence a remarkable asymmetric functionalization. Moreover, when compared to the direct reaction method, a reduced number of non-functionalized cCNC-I and individual isolated AuNPs can be observed. Based on the observation of 32 TEM micrographs containing about 650 particles, a statistical analysis was performed to quantitatively characterize the grafting yield that reached 81 % (Figure II.9). This yield, 5 to 10 times higher than those previously obtained, shows the very high efficiency of the *in situ* growth method, which can be attributed to the preferential nucleation and growth of AuNPs at the sulfur-derivatized end of cCNC-I acting as nucleating agents.



**Figure II.9.** Number distribution of AuNP-labeled cCNC-I and bare cCNC-I resulting from the in situ growth method in presence S-e-cCNC-I. Particles that could not unambiguously be attributed to one or the other category appear as "unclassified". The histogram results from the analysis of 650 particles.

Moreover, it is clear from the TEM images in Figures II.8c and d that the AuNPs that were synthesized in the presence of S-e-cCNC-I and grown on their end have a very low polydispersity. In order to get more information, SAXS experiments were performed on a 1 wt. % AuNP-e-cCNC-I suspension prepared by *in situ* growth. As shown in Figure II.10, oscillations with scattering minima at  $Q_1 = 0.055$  Å<sup>-1</sup> and  $Q_2 = 0.095$  Å<sup>-1</sup>, can be observed. Such features readily show the presence of well-defined objects. Since, as described in the experimental 88 section, the contrast term between gold nanoparticles and solvent is much higher than between cellulose and solvent, the scattering intensity of AuNP-e-cCNC-I particles in water is considered as arising from AuNPs only. Accordingly, assuming interaction-free particles due to the low concentration used, the experimental data were fitted using the form factor of dense spheres. As shown in Figure II.10, a good quality of the fit was obtained, revealing the presence AuNP spheres of radius  $7.6 \pm 1$  nm. This result thus confirms the presence of AuNPs with a low polydispersity, in full agreement with the TEM images.



*Figure II.10. SAXS* profile of a 1 wt. % AuNP-e-cCNC-I suspension obtained by the in situ growth method. The solid red straight line is a fit to the data using the form-factor of spheres of radius  $7.6 \pm 1$  nm.

UV-Vis spectroscopy experiments were performed on suspensions of AuNP-e-cCNC-I synthesized by the two methods. The results shown in Figure II.11 first evidence that the plasmon resonance signal is the same for a pure suspension of AuNPs as for a suspension of AuNP-e-cCNC-I prepared by the direct reaction method, which demonstrates that this technique did not detect the formation of AuNP clusters. When the *in situ* growth strategy was used, the signal was shifted towards a lower wavelength (from 524 to 508 nm), showing that the AuNPs that were synthesized *in situ* were smaller than the presynthesized ones, in agreement with DLS data and TEM images.

Overall, the different characterization techniques (DLS, TEM, SAXS and UV-Vis spectroscopy) coherently concur to show the strong advantage in using the *in situ* growth approach that makes it possible to functionalize with a very high efficiency the reducing end of cCNC-I with AuNPs exhibiting a low polydispersity, while avoiding the formation of AuNP clusters.



Figure II.11. UV-Vis absorption spectra of the different AuNP-containing CNC samples.

The *in situ* growth method was also applied to wCNC-I. TEM images of AuNP-e-wCNC-I distinctly show a spectacular asymmetric functionalization of single crystallite wCNC-I on one end of the rods (Figure II.12). A quite high attaching yield is observed even if the width, *i.e.* the reducing end surface of these wood CNCs is very low and inferior to that of cCNC-I. The *in situ* growth method can therefore efficiently target the reducing end of CNCs even in the case where the end surface only represents a very small fraction of the whole rod surface. As in the case of cCNC-I functionalization, a statistical analysis showed that about 70 % of the wCNC-I could be labeled with a gold nanoparticle (Figure II.13). The use of wCNC-I constituted of only one or two elementary crystallites results in the majority of cases to the production of matchstick-like CNCs labeled with a single AuNP.

As shown in Figure II.14, DLS data for unlabeled and reducing end-labeled wCNC-I are in agreement with TEM images. These results indeed confirm the presence after the *in situ*  labeling reaction of a reduced amount of individual AuNPs, show that the hydrodynamic diameter of wCNC-I has increased from 140 to 164 nm after the labeling treatment and evidence the absence of aggregates. These data are in qualitative agreement with those obtained with cCNC-I using the *in situ* growth method and confirm the high labeling efficiency achieved by this technique. UV-Vis data for AuNP-e-wCNC-I in Figure II.11 show a plasmon resonance signal with a maximum at 515 nm, in line with the presence of about 20 nm AuNPs.



*Figure II.12. TEM images of negatively stained AuNP-e-wCNC-I prepared by in situ growth from aqueous suspensions at room temperature.* 



**Figure II.13.** Number distribution of AuNP-labeled wCNC-I and bare wCNC-I resulting from the in situ growth method in presence S-e-wCNC-I. Particles that could not unambiguously be attributed to one or the other category appear as "unclassified". The histogram results from the analysis of 348 particles.



*Figure II.14.* Intensity size distribution of a 0.5 wt. % gold nanoparticles suspension (red solid line), 0.1 wt. % S-e-wCNC-I suspension (black solid line) and 0.1 wt. % AuNP-e-wCNC-I suspension (purple dashed line) prepared by in situ growth and measured by DLS at 20 °C.

Finally, the *in situ* labeling strategy was applied to cCNC-II particles. As aforementioned, after a mercerization step, the packing of cellulose chains changed from parallel to antiparallel, which results in the presence of reducing end groups (as well as non-reducing end groups) on both extremities of each nanocrystal. TEM images of AuNP-e-cCNC-II showed the prominent formation of "dumbbells" consisting of one cCNC-II with one AuNP grafted on both ends (Figure II.15). Using mercerized ramie cellulose, Kim *et al.* (Kim 2006) performed a labeling experiment with presynthesized gold nanoparticles using a biotin/streptavidin-gold technique targeting the reducing end of cellulose II nanocrystals. They reported that only about 15 % of ramie mercerized CNCs were labeled at two ends. In contrast here, our method resulted in a very high labeling efficiency since TEM images showed that almost all cCNC-II particles exhibited a single AuNP on both ends, thus proving the antiparallel character of chains constituting the crystals.

As in the other two cases, the DLS data are in line with the TEM observations. Indeed, as shown in Figure II.16, two peaks were observed after the labeling step. The first one around 20 nm should correspond to individual AuNPs in limited abundance and the second one around 154 nm to cCNC-II labeled at both ends. It has to be noted that among the three types of tested nanocrystals, the  $D_h$  increase upon labeling is the highest in the case of cCNC-II (32 nm increase compared to a 24 nm increase for type I CNCs), which is consistent with a double labeling in 92

that case.



*Figure II.15. TEM images of negatively stained AuNP-e-cCNC-II prepared by in situ growth from aqueous suspensions at room temperature.* 



*Figure II.16.* Intensity size distribution of a 0.5 wt. % gold nanoparticles suspension (red solid line), 0.1 wt. % S-e-cCNC-II suspension (black solid line) and 0.1 wt. % AuNP-e-cCNC-II suspension (purple dashed line) prepared by in situ growth and measured by DLS at 20 °C.

# **II.3.4 Discussion**

## II.3.4.1 Optimized labeling

Even if the labeling of the CNC reducing end with gold or silver NPs has already been addressed by different groups based on the grafting of presynthesized particles, the labeling efficiency was significantly improved by using the *in situ* growth strategy described in the present work. While past works indeed reported maximum values of about 15 % labelling efficiency, we achieved here up to 81 % for cCNC-I. In addition, the AuNPs grown *in situ* at

the CNC reducing end exhibit a very narrow size distribution, as especially determined by SAXS. Importantly, the method was applied with a similar success to CNC-I particles of two different origins, including wCNC-I that possess a smaller cross-section than cCNC-I, as well as to cCNC-II particles, demonstrating a high degree of flexibility in the choice of the starting nanocrystals. Thus, the proposed method allowed us to overcome the synthesis challenge associated with the endwise modification of CNCs with their significantly low abundance and the small dimensions of the targeted reducing end sites together with their limited reactivity. Data show that the nucleation and growth of AuNPs on sulfur-bearing reducing ends is an efficient way to increase the labelling efficiency while minimizing the AuNP polydispersity and formation of AuNP clusters. When compared to the grafting of presynthesized particles, the *in situ* growth technique does not suffer from limitations such as steric hindrance and electrostatic repulsions between similarly-charged CNCs and AuNPs but benefits from fast diffusion of the reactant in a water-soluble form. As a result, AuNPs preferentially nucleate and grow directly at the sulfur-derivatized reducing end, optimizing the labeling efficiency and limiting the synthesis of individual AuNPs and the presence of clusters.

#### II.3.4.2 Insights into the production and characteristics of cellulose nanocrystals

Beyond this optimization that paves the way to the use of heavily functionalized CNCs for advanced applications, data also contribute to give additional insight into more fundamental aspects or to confirm with higher statistics conclusions that had been much debated in the cellulose community.

Cellulose is synthesized at the plasma membrane by cellulose synthases that, in higher plants, are organized into rosette-like assemblies, acting as cellulose synthase spinneret (Li 2016; Pear 1996). The cellulose chains in one cellulose microfibril are simultaneously spun by the rosette arrangement of cellulose synthases and due to the lack of solubility of the elongated chains, unidirectional cellulose crystallization occurs very shortly after the extrusion of the chains (Haigler 1991). Due to the enzymatic synthesis, the nascent cellulose nanofibrils are oriented, with the chains aligned in a parallel fashion (Li 2016). Dried cellulose microfibrils used as a source for the production of CNCs display crystalline and more disordered regions.

During the hydrolysis, the acid preferentially etches away these less ordered regions, releasing shorter segments where the parallel packing of the chains is conserved, yielding rodlike particles with a chemical polarity. For CNCs made of a single elementary crystallite, all reducing ends are located on one end of the particle, as shown by crystallographic studies (Koyama 1997; Nishiyama 2002). This phenomenon was verified independently by using the selective cellulase Cel6A which degraded the non-reducing end of *Valonia* cellulose nanocrystals (Chanzy 1985) and by the gold nanoparticles labelling method on the reducing end of *Valonia* cellulose (Kim 2006).

In the present work, we could show that for commercial wCNC-I originating from terrestrial plants and comprising one or two elementary crystallites, our labeling strategy successfully labelled only one end of the rods.

In contrast, the cCNC-I were shown to be formed on average of 3 to 4 elementary subunits, which are laterally associated, forming flat objects having a width 3 to 4 times larger than their height (Elazzouzi-Hafraoui 2008). This feature can easily be observed in Figure II.1a and has been confirmed through the use of advanced techniques such as small-angle X-ray or neutron scattering (Azzam 2020; Cherhal 2015). Such a composite nature of cCNC-I has also been observed for CNC-I extracted from other plants, e.g. bamboo (Brito 2012). However, the respective orientation of these elementary units inside a CNC-I is not trivial and should depend on the way the CNC-I were initially produced. A first possibility is that the acid hydrolysis has isolated microfibril fragments composed of several neighboring crystallites without breaking the transverse hydrogen bonds connecting them, *i.e.* that the elementary crystallites constituting nanocrystals have inherited the chemical polarity of their parent microfibrils and should be formed of chemically polar parallel crystallites, thus forming CNC-I with all reducing ends of each crystallite located on the same side of the nanocrystal. In other words, with this scenario, the chemical polarity of the crystallites would be transferred to the CNC regrouping them.

A second hypothesis is that the acid hydrolysis is not only able to break the glycosidic linkages in the disordered regions along the nanofibrils, releasing shorter and more crystalline elements, but also to laterally separate the subunits, leading to individual single crystals that would subsequently re-aggregate into the composite material observed. Upon re-aggregation, the respective orientation of the subunits within a CNC could be parallel, antiparallel, or random leading in the first case to reducing ends localized on the same side of the CNC and in the two other cases to reducing ends on both sides of the rod.

Figure II.17 shows high-magnification TEM images of AuNP-labeled native cotton cellulose nanocrystals produced using the *in situ* growth method. As seen from these micrographs, AuNPs could successfully be bonded to one end of a majority of elementary subunits even though some elementary crystallites were not functionalized. Furthermore, when a CNC is considered, it is very clear that the AuNPs are all localized on the same side (there is no example of a cCNC-I tagged with AuNPs on both extremities). These results clearly show that elementary crystallites are oriented in a parallel manner in cCNC-I with all reducing ends on the same side.



*Figure II.17. TEM micrographs of negatively stained AuNP-e-cCNC-I prepared by in situ growth from aqueous suspensions at room temperature.* 

These data provide a clear visual proof that cCNC-I formed by multiple laterally-associated crystallites are chemically polar. Based on the analysis of WAXS data recorded from a film made of native CNCs extracted from eucalyptus, Flauzino Neto *et al.* concluded on a random organization of elementary crystallites in native CNCs extracted from eucalyptus (Flauzino Neto 2016). It seems that the case of cotton is different. It has to be noted that this conclusion is in full agreement with results in Chapter III, where we report the grafting

of thermoresponsive polyetheramines at the reducing ends of cCNC-I and the ability of these asymmetrically functionalized particles to assemble into star-shaped complexes (See Chapter III) (Lin 2019). Indeed, since only star like structures were formed and no end-to-end association was detected, the presence of reducing ends on both ends of the CNCs could be ruled out.

The localization of all reducing ends in composite cCNC-I on a single side is consistent with our first hypothesis that the subunits in a CNC were never separated but does not strictly rule out the second hypothesis (re-aggregation of separated elementary crystallites) since even in this case a parallel re-assembly is still possible, though not statistically probable. However, the fact that sulfuric acid hydrolysis not only individualizes the crystalline rods but also converts part of primary hydroxyl groups into sulfate half esters is in favor of the first speculation. Indeed, if individual elementary subunits would have been produced at some point during the hydrolysis, it would be expected that they have been imparted with these negative surface charges, which would have strongly hampered a possible re-association due to electrostatic repulsions. Consequently, we believe that the first CNC production scenario based on never-separated crystallites is more likely to take place than the second one involving separation and subsequent reaggregation of elementary crystallites. Additionally, this conclusion is in line with electron microscopy observations of plant cell walls that distinctly show tight bundles of microfibrils.

CNC-II are usually prepared by a mercerization process followed by acid hydrolysis. One possible mechanism occurring during mercerization and accounting for the transition from cellulose I with a parallel packing of chains to cellulose II with an antiparallel arrangement is the so-called interdigitation mechanism first proposed by Okano and Sarko (Okano 1985). Upon swelling in NaOH, cellulose chains from neighboring fibrils of opposite polarity would intermingle and recrystallize into an antiparallel fashion upon washing and drying (Nishiyama 2000; Okano 1985). Kim *et al.* gave an indication of the antiparallel character of cellulose II nanocrystals by showing one Ramie CNC-II particle labeled at both ends (Kim 2006). Thanks to the much higher labeling yield allowed by the *in situ* labeling method, we provide here additional statistically relevant evidence for this.

# **II.4 Conclusion**

Two different regioselective labeling strategies were applied on different cellulose nanocrystals (native nanocrystals from cotton and wood, and cellulose II nanocrystals from cotton). The first method is a direct reaction in which CNCs prefunctionalized with sulfur atoms at the reducing end of the chains were mixed with pre-synthesized gold nanoparticles. As shown by DLS and TEM, such a strategy results in a low labeling ratio and the presence of gold nanoparticles aggregates. Then, a so-called *in situ* growth functionalization strategy was applied, where AuNPs were synthesized from soluble derivatives in the presence of sulfur-bearing CNCs. In this case, an unprecedently high labeling efficiency could be reached, with the observation of a vast majority of cCNC-I bearing one or several AuNP on one end of the rods and almost all cCNC-II labeled with one AuNP at both ends of the particles. Furthermore, there were nearly no gold nanoparticles aggregates and these in situ-synthesized AuNPs had a very low polydispersity. All complementary techniques used, namely TEM, DLS, UV-Vis spectroscopy and SAXS spectra, also support these. This optimized labeling strategy was subsequently used to address questions about the morphology of CNCs. Our results evidence that in native cotton CNCs made of the lateral assembly of a few elementary crystallites, these crystallites are arranged in a parallel manner with all reducing ends located on the same side of the nanocrystals. This organization shows that the acid hydrolysis applied to generate the nanocrystals isolates preexisting assemblies of parallelly-packed microfibrils, without laterally separating them. The formation of dumbbell structure where almost all cCNC-II particles are labeled with one AuNP at each of their end further confirm the antiparallel arrangement of cellulose chains in cellulose II nanocrystals.

# **Chapter III**

# Temperature-controlled star-shaped cellulose nanocrystal assemblies resulting from asymmetric polymer grafting
### **III.1 Introduction**

The homogeneous (*i.e.* on the whole surface) grafting of thermosensitive polymers onto the surface of CNC-I is a convenient way to tune the colloidal interactions between these biosourced particles by conferring the particles with steric interactions and a temperature-controlled association behavior (Azzam 2010, 2016a, 2016b, 2020; Yi 2008; Zoppe 2010, 2011).

In aqueous solution, thermosensitive polymers exhibit a phase separation behavior characterized by a lower critical solution temperature (LCST). Figure III.1 shows the representative outline of the phase diagram of an LCST system. LCST is characterized as the minimum of the demixing curve, it accordingly corresponds to a precise polymer volume fraction  $\varphi_{\text{LCST}}$ . The transition temperature at any polymeric volume portion,  $\varphi$ , is known as the cloud point.





In a polymer-solution system, the monomer-monomer, monomer-solvent and solvent-solvent interactions influence the polymer behavior and conformation. The interactions between water molecules are especially strong and unique and characterized by a partially ordered structure (Tanford 1991). The thermoresponsiveness of polymers in aqueous solution has both an enthalpic and entropic origin (Schild 1992). At a temperature below the LCST, the 101

formation of hydrogen bonds between the polar part of a thermosensitive polymer and the water molecules ensures its solubility and a swollen coil conformation. This interaction prompts an exothermic enthalpy term. Nevertheless, the water molecules, which cannot generate hydrogen bonds with the non-polar part of the polymer, must reorient around them and tend to develop an ordered structure. This phenomenon known as the hydrophobic effect (Tanford 1991) requires a decrease in the mixing entropy. At such a temperature below the LCST, the unequivocally negative mixing enthalpy will conquer the decrease of the entropy and permit the solubility of the polymer molecules in water.

When the temperature is increased above the LCST, the thermosensitive polymer chains collapse due to poor solvent conditions caused by the increase of enthalpy and entropy and accordingly turn from hydrophilic to hydrophobic. Consequently, in the aqueous solution, the interactions between these collapsed polymer chains become attractive. If the polymer concentration is adequate, the substitution of the polymer-solvent interactions with polymer-polymer and solvent-solvent interactions leads to the precipitation of the polymer chains. In this presenting work, we will benefit this temperature-controlled property and produce new assemblies.

In this chapter, we have used these two polyetheramines for the specific functionalization of the reducing tips of CNCs with the goal of producing a thermosensitive reversible association of these nanocrystals through the derivatized ends. A two-step chemical modification approach was performed. Different characterization techniques, such as DLS, TEM and SANS, were used to investigate the changes that occurred during the reaction and to analyze the temperaturetriggered assembly of the derivatized CNC-I.

### **III.2 Materials and methods**

### **III.2.1 Materials**

Cotton linters were provided by Buckeye Cellulose Corporation and used as the cellulose source without any further purification. Two thermosensitive polyetheramines were used in this study: Jeffamine<sup>®</sup> Polyetheramines M2005 and T5000 (gift from the Huntsman Corporation). 102

Jeffamine<sup>®</sup> polyetheramine M2005 is a statistical amine-terminated polymer of ethylene oxide (EO) and propylene oxide (PO) with a molecular weight about 2000 g mol<sup>-1</sup> (EO / PO monomer composition of 6 / 29). Jeffamine<sup>®</sup> polyetheramine T5000 is a three-branched polymer of PO with amine end groups at each of its branches and a molecular weight of about 5000 g mol<sup>-1</sup>. Other chemicals were purchased from Sigma-Aldrich and used as received. N-methylmorpholine N-oxide monohydrate (NMMO•H2O, Sigma-Aldrich) was recrystallized in dry acetone. Deionized water was used in all experiments.

The descriptions of the preparation of cCNC-I and the measurement of the sulfur and aldehyde contents are given in Chapter II.2.2, II.2.5 and II.2.6.

# III.2.2 Treatment of cellulose nanocrystals with N-Methylmorpholine N-Oxide (NMMO)

0.5 g of freeze-dried cCNC-I was dispersed into a solution of 80 g NMMO•H<sub>2</sub>O and 20 mL water at 80 °C. The mixture was kept at this temperature for 30 min after which the resulting suspension of cCNC-I-NMMO was diluted with water, cooled and washed by repeated centrifugations and finally redispersed in deionized water.

### **III.2.3 Pinnick oxidation**

NaClO<sub>2</sub> (2.826 g, 0.025 mol) was added to a 2.4 wt. % aqueous cCNC-I suspension containing 1 g dry CNC or cCNC-I-NMMO. HAc was then added until the pH reached 3.5 and the suspension was kept for 48 h under magnetic stirring. The reaction was stopped by adding 0.5 M NaOH solution to reach a pH of about 8. The suspension was dialyzed against distilled water until a constant conductivity of the dialysis bath.

### **III.2.4** Polymer grafting by peptide coupling

Jeffamine<sup>®</sup> Polyetheramine M2005 (0.088 g, 0.044 mmol) or T5000 (0.22 g, 0.044 mmol) were added to an (~1 wt. %) Ox-e-cCNC-I suspension, containing 0.5 g dry CNC and stirred until dissolution. The reaction was performed at 4 °C (well below the LCST) to ensure a good solubility of the polymer. The catalysts dicyclohexylcarbodiimide (DCC, 13.65 mg, 0.066

mmol) and 4-pyrrolidinopyridine (4-PPY, 6.52 mg, 0.044 mmol) or 4-dimethylaminopyridine (DMAP, 5.38 mg, 0.044 mmol) were then added. After 24 h under stirring, the resulting suspensions were filtered through 100  $\mu$ m nylon filters to get rid of the catalyst and dialyzed against distilled water to remove excess reagents including non-grafted polymers.

### **III.3 Results and discussion**

### III.3.1 Evaluation of LCST of Jeffamine® polyetheramine T5000

The evaluation of the LCST of Jeffamine<sup>®</sup> polyetheramine was carried out by UV-Vis spectroscopy, through measurements of the absorbance of polymer solutions as a function of temperature. When the temperature is below the LCST, the polymer is in good solvent condition. Thus, the solution is transparent and almost no absorbance can be detected. When the temperature is above the LCST, the polymer is in bad solvent condition and the solution becomes turbid. Consequently, an absorbance of UV can be observed.

Figure III.2 shows the chemical structure of Jeffamine<sup>®</sup> polyetheramine M2005 and T5000 which were described in III.2.1 of this chapter. The LCST of M2005, which is about 16 °C, was determined by Azzam *et al.* (Azzam 2010) using the same method. Figure III.3 presents the UV absorbance spectra of 0.5, 1 and 2 wt. % solutions of T5000. The absorbance increased promptly at about 12 °C, which is, in conclusion, the LCST of T5000.



M2005

T5000

*Figure III.2.* Chemical structure of Jeffamine<sup>®</sup> polyetheramines M2005 and T5000.



*Figure III.3.* UV absorbance spectra of 0.5, 1 and 2 wt. % solutions of Jeffamine<sup>®</sup> polyetheramine T5000 as a function of temperature showing a LCST around 12 °C.

### **III.3.2** CNCs and their two-step asymmetric functionalization

The cCNC-I used in this study were prepared from cotton linters following the sulfuric acid hydrolysis protocol described by Revol et al. in their seminal work (Revol 1992). The resulting particles had average dimensions of 150 nm  $\times$  22 nm  $\times$  6 nm, as revealed by statistical measurements from TEM and AFM images. Conductometric titration gave a sulfur content of 0.69 %, which, combined to the CNC dimensions, corresponds to an average surface charge density of 0.48 e<sup>-nm<sup>-2</sup></sup>. A  $\zeta$ -potential of -34  $\pm$  5 mV was measured from a 0.1 wt. % suspension. Some cCNC-I were used as such, whereas others were treated in a non-swelling dilute aqueous solution of N-methyl morpholine N-oxide (NMMO) to improve the accessibility of the chain ends in the CNC tips. The asymmetric functionalization of the reducing ends of cCNC-I with Jeffamine® polyetheramine M2005 and T5000 chains was achieved in aqueous medium, following the two-step protocol described in Scheme III.1a. First, cCNC-I were end-carboxylated using the Pinnick oxidation method in which the aldehyde groups at the reducing end were transformed to carboxylate moieties. Since in the corresponding samples, hereafter referred to as Ox-e-cCNC-I, the reducing ends only represent a few percent of the total CNC surface, attempts to measure the degree of oxidation of Ox-e-cCNC-I using techniques such as Fourier-transform infrared (FTIR) or <sup>13</sup>C solid-state nuclear magnetic resonance (NMR) spectroscopy were unsuccessful. Even in the case of our polyetheramine grafting of the whole surface of the cCNC-I, the collected signals were weak, leaving no chance to properly identify the chemical modifications in the case of end functionalization (Azzam 2010). However, both the initial and post-oxidation aldehyde contents, *i.e.* the concentration of reducing ends, could be measured. The initial value was  $24.9 \pm 0.4 \mu$ mol CHO g<sup>-1</sup>, in good agreement with the values calculated from the CNC average dimensions, and  $5.3 \pm 0.8 \mu$ mol CHO g<sup>-1</sup> after the oxidation step, which corresponds to an oxidation reaction yield of 78 %.



**Scheme III.1** a) Two-step asymmetric functionalization of cCNC-I with thermosensitive polyetheramine chains in aqueous medium. The first step consists in carboxylating the reducing ends to yield Ox-e-cCNC-I, which are in a second step grafted with polyetheramine chains via a peptide coupling reaction. b) Mechanism of peptide coupling reaction where in our case, DCC acts as  $R_1$ - $N=C=N-R_2$  and DMAP or 4-PPY as X.

In a second step, the polyetheramines were covalently grafted onto Ox-e-cCNC-I by a peptide coupling reaction between the amino groups of the polymer chain end and the previously generated carboxylate groups. Scheme III.1b shows a general mechanism of peptide coupling reaction. Peptide bond formation is a condensation reaction of a nucleophile amino group and a carboxyl acid. Activation of the carboxyl component is achieved by the introduction of electron accepting moieties, such as DCC in our case. Furthermore, in order to avoid the path b, which produces the urea byproduct that is very stable and non-soluble, 4-PPY or DMAP are added to favor the path a (Scheme III.1b). Again, for the aforementioned reason, the degree of substitution and peptide coupling reaction yield could not be measured by FTIR or solid-state NMR.

### III.3.3 Temperature-triggered assembly of the end-derivatized cCNC-I

The effect of the presence of Jeffamine<sup>®</sup> polyetheramine M2005, locally grafted at one end of the cellulose rods was investigated by dynamic light scattering (DLS) as a function of temperature and comparisons with bare as-prepared CNCs and Ox-e-cCNC-I were made. As shown in Figure III.4a, the hydrodynamic diameter ( $D_h$ ) of bare cCNC-I and Ox-e-cCNC-I remained constant at about 96 nm in the 4-40 °C temperature range. This value, which corresponds to what is usually reported for cotton CNCs prepared under conditions similar to ours, shows that the samples consisted of non-aggregated CNCs irrespective of the temperature within the probed range and the same conclusion can be drawn for Ox-e-cCNC-I. For the M2005-e-cCNC-I, when the temperature was below the LCST of the polyetheramine, *i.e.* 16 °C, the hydrodynamic diameter was constant at about 114 nm, indicating that the sample was also composed of individualized M2005-e-cCNC-I without any association. The small increase in hydrodynamic diameter when comparing M2005-e-cCNC-I and Ox-e-cCNC-I was consistent with the occurrence of a lower diffusion coefficient due to the presence of polymer chains at the CNC ends. Thus, the DLS measurements provided an indirect proof of the successful grafting.



**Figure III.4. (a)** Hydrodynamic diameter as a function of a temperature cycle between 4 and 40 °C for cCNC-I (red squares), Ox-e-cCNC-I (blue dots) and M2005-e-cCNC-I (purple triangles) in 0.5 wt. % aqueous suspensions. Heating and cooling are indicated by arrows; **(b)** Hydrodynamic diameter variations for M2005-e-cCNC-I in 0.5 wt. % aqueous suspensions during multiple cycles of temperature increase and decrease between 4 and 40 °C. The dotted lines are only guiding for the eye.

When the temperature was increased from below the LCST to 40 °C, a spectacular

increase in hydrodynamic diameter was observed as D<sub>h</sub> nearly tripled, going from 114 to 326 nm. This  $D_h$  increase is consistent with the formation of finite-sized aggregates. When the heated samples were then cooled to 4 °C, the hydrodynamic diameter decreased until it reverted to the initial value of ~114 nm as soon as the temperature was below 12 °C. This result clearly shows that the temperature-induced aggregation is fully reversible even though a hysteresis related to association-dissociation kinetics was observed. This behavior is very different from what was observed by Risteen et al. (Risteen 2018) for "patchy" thermosensitive polymer-modified CNCs, since, in their case, a very moderate increase in  $D_h$  (29 %) was measured. The remarkable reversibility and reproducibility of the phenomenon was evidenced when temperature cycles between 4 and 40 °C were repeated (Figure III.4b), showing that no degradation or loss of the thermally-induced aggregation occurred when multiple temperature variations were applied and thus evidencing the robustness of the system. As shown in Figures III.5a and b, identical results were obtained for M2005-e-cCNC-I-NMMO and T5000-e-cCNC-I-NMMO samples (as detailed in Chapter V, the NMMO-treated cCNC-I used here are in fact similar to as-prepared cCNC-I). Additionally, as shown in Figure III.6, D<sub>h</sub> was unaffected by temperature for a suspension of Ox-e-cCNC-I that was submitted to the grafting protocol with the M2005 polyetheramines but without the addition of the catalyst, thus preventing the coupling to take place. Such data show that the effect of temperature results from the covalent grafting of the thermosensitive polymer at the reducing end of the CNCs.



*Figure III.5. Hydrodynamic diameter as a function of temperature of 0.5 wt. % suspensions of M2005-e-cCNC-I-NMMO (blue dots) and cCNC-I (red squares) (a) and of T5000-e-cCNC-I-NMMO (blue dots) and cCNC-I (red squares) (b). Heating and cooling are indicated by arrows.* 



**Figure III.6.** Hydrodynamic diameter as a function of temperature of a suspension of a 0.5 wt. % suspensions of M2005-e-cCNC-I (purple triangles) and cCNC-I submitted to the grafting protocol with the M2005 polyetheramine without the addition of the catalyst (orange dots).

In order to further characterize the temperature-triggered aggregation revealed by DLS, TEM was used to observe the samples at 4 and 40 °C. Figure III.7a shows TEM micrographs of cCNC-I prepared by sulfuric acid hydrolysis of cotton linters. In consistence with literature reports, these particles have a length between 100 and 300 nm and a width between 10 and 30 nm (Elazzouzi-Hafraoui 2008). The negative staining clearly reveals that most particles are in fact fascicles of a few parallel elementary subunits. The interparticle distance indicates a significant electrostatic repulsion due to the negative charge of sulfate groups at the surface of the CNCs, resulting from the sulfuric acid hydrolysis.



Figure III.7. TEM micrographs of negatively stained cCNC-I at room temperature (a) and of Ox-e-cCNC-I prepared from aqueous suspensions at 4 (b) and 40 °C (c).

The TEM micrograph of Ox-e-cCNC-I shown in Figure III.7b reveals that these nanoparticles are very similar to the initial cCNC-I and that the chlorite oxidation reaction had

no effect on their morphology. Furthermore, when the temperature was raised from 4 to 40 °C (Figure III.7c), no significant difference could be detected, proving the non-sensitivity of these samples to temperature. TEM images at 4 and 40 °C of M2005-e-cCNC-I, M2005-e-cCNC-I-NMMO and T5000-e-cCNC-I-NMMO are shown in Figure III.8. In Figures III.8a, c and e, the samples prepared from suspensions kept at 4 °C showed rod-like nano-objects that are well-dispersed and similar to the initial cCNC-I shown in Figure III.8a. Thus, at a temperature below the LCST of the polyetheramine, polymer-e-cCNC-I behaved as repulsive individual nanoparticles. The grafted chains are too short to be detected by TEM, but the presence of these chains under good solvent conditions should generate entropic repulsion forces between the nano-objects, which add to the electrostatic interactions provided by the inherent ester sulfate groups at the CNC surface.



**Figure III.8.** TEM images of negatively stained M2005-e-cCNC-I prepared from suspensions at 4 °C (a) and 40 °C (b); M2005-e-cCNC-I-NMMO prepared from suspensions at 4 °C (c) and 40 °C (d) and T5000-e-cCNC-I-NMMO prepared from suspensions at 4 °C (e) and 40 °C (f).

**Chapter III** - Temperature-controlled star-shaped cellulose nanocrystal assemblies resulting from asymmetric polymer grafting



Scheme III.2. Thermoreversible association of polyetheramine-e-cCNC-I into star-shaped assemblies.

TEM images of cCNC-I prepared from the same suspensions at 40 °C clearly showed the spectacular formation of assemblies of cCNC-I organized in a star-shaped aggregation (Scheme III.2) of four-, five- or six- nanocrystals hooked by their ends (Figures III.8b, d and f). The observation of these CNC stars at 40 °C is fully consistent with the DLS data that reveal the presence of large objects showing a close to three-fold higher hydrodynamic diameter at this temperature as compared to the samples prepared at 4 °C. The temperature-induced formation of these star-shaped assemblies can be explained by modifications in the polyetheramine conformation and hydration. Indeed, when the temperature is increased above the LCST, the thermosensitive polymer chains grafted at the reducing end of cCNC-I collapse due to poor solvent conditions and thus turn from hydrophilic to hydrophobic. Thus, in the aqueous environment, the interactions between these collapsed chains become attractive, with the result of the association of the polymer-e-cCNC-I through their reducing ends. In the images shown in Figures III.8b, d, f, besides the star structures that are dominant, a small number of individual polymer-e-cCNC-I is still observed: they can likely be attributed to the dilute conditions required for TEM observations, which limit the probability of polymer-e-cCNC-I to associate. These individual particles might also correspond to end-grafted cCNC-I with a low degree of grafting. It has to be noted that the thermally-induced formation of star-like structures is a proof that the grafting only exclusively occurred at the reducing end of the cCNC-I since we could not detect any CNC strings that would have been formed from associations involving both tips of the nanocrystals.

DLS results have shown that the formation of these CNC stars was fully reversible. Indeed,

when the temperature was decreased below the LCST of the considered polyetheramine, good solvent conditions were recovered, hydrogen bonds were re-established between the polymer chains and water and polymer-polymer interactions became repulsive, leading to the dissociation of the star-shaped assembly into individual particles.

A remarkable characteristic of these star-shaped aggregates is their high degree of symmetry. As shown in Figures III.8b, d, f, the arms of the stars are separated by angular sectors of values close to  $2\pi/n$ , where *n* is the number of CNCs in a star, *i.e.*  $\pi/2$ ,  $2\pi/5$  and  $\pi/3$  for four-, five- and six-nanocrystals stars, respectively. This structure is the result of opposite interaction forces between the polymer-e-cCNC-I. Indeed, while at T>LCST the polymer-polymer interactions are attractive and connect the polymer-e-cCNC-I by their end, electrostatic repulsive forces still persist between the surface length of the non-covered negatively-charged sulfated CNCs.

As a control, a simple mixture of Ox-e-cCNC-I and M2005 suspension was prepared but without catalysts. TEM experiments were carried out on these samples and the images are shown in Figure III.9. Clearly, at 4 or 40 °C, these nanorods are very similar to the initial cCNC-I and no temperature induced aggregation was observed.



**Figure III.9.** TEM micrographs of control samples composed of Ox-e-cCNC-I submitted to the grafting protocol with the M2005 polyetheramine without the addition of the catalyst at 4 (a) and 40 °C (b) showing the absence of thermally-induced aggregation.

To get more insight into the structure of the CNC assemblies in aqueous suspension, small-angle neutron scattering (SANS) experiments were performed. The curve corresponding

to as-prepared cCNC-I could be fitted using the form factor of a parallelepiped with a rectangular cross-section, giving average dimensions of  $150 \times 20 \times 5$  for the initial nanoparticles (Figure III.10).



**Figure III.10.** SANS spectra of a 1 wt. % cCNC-I suspension in  $D_2O$  and fit using the form factor of a parallelepiped with dimensions  $150 \times 20 \times 5$  nm<sup>3</sup>. A Gaussian polydispersity of 0.4 and a lognormal polydispersity of 0.13 were applied on the thickness and width, respectively.

Then, SANS experiments were carried out on a 1 wt. % M2005-e-cCNC-I suspension in D<sub>2</sub>O at different temperatures. The scattering results are shown in Figure III.11a, which also presents the data corresponding to as-prepared CNCs. A plot where the spectra were arbitrarily shifted is available in Figure III.11b. For the two temperatures below the LCST of the polyetheramine, namely 8 and 12 °C, both SANS spectra of the M2005-e-cCNC-I suspension are identical and correspond to individual asymmetrically-modified CNCs. Both spectra exhibit the typical Q<sup>-1</sup> decay of rod-like objects in the low-Q range, as is also the case for bare cCNC-I. However, in the high Q-range, while the spectrum of bare cCNC-I follow a Q<sup>-4</sup> decay corresponding to the sharp interface between the crystalline rods and the solvent, M2005-e-cCNC-I exhibit a decay close to Q<sup>-1</sup>. This scattering behavior in a Q-region where the nanometer length-scale is probed is attributed to the presence of the chains grafted at the end of the CNCs, which are expected to scatter as Q<sup>-1/v</sup>, where v is the Flory exponent. From the slope close to -1 at high Q, it can be deduced that the grafted chains are described by a  $v \sim 1$  Flory exponent, corresponding to an extended chain conformation. Such a conformation reveals that

the grafted chains are in a brush-like regime resulting from a high grafting density allowed by the high density of chain-end carboxylates (about  $4 \text{ COO}^{-} \text{ nm}^{-2}$ ) reached after the oxidation step.



**Figure III.11.** (a) SANS spectra of a 1 wt. % M2005-e-cCNC-I suspension in  $D_2O$  at 8 °C (blue circles), 12 °C (green squares), 16 °C (orange triangles) and 24 °C (red diamonds) and of a 1 wt. % cCNC-I suspension (blue crosses). Solid straight lines show the characteristic decay of the intensity in the high *Q*-region. (b) Curves were arbitrarily vertically shifted.

At 16 °C, a slight increase in the intensity at low-Q is observed but the SANS curve superimposes with the data at 8 and 12 °C for  $Q > 1.10^{-2}$  Å<sup>-1</sup>. These features at a temperature close to the LCST may result from the formation of complexes involving no more than two functionalized particles, since the intensity when Q tends to zero is only about 1.5 times higher at 16 °C than at 8 °C. However, a further increase of the temperature up to 24 °C (8 °C above the LCST) results in a significant change of the scattering signal in the low-Q region. A 10-fold increase of the intensity when Q tends to zero is observed but at the same time the intensity tends to plateau when Q decreases. Such features suggest the formation of finite-sized assemblies of about 10 individual particles corresponding to star-like aggregates with an increased number of branches due to the higher concentration used for the SANS experiments and/or to the association of 5-branches stars. No chain conformation change was observed at 24 °C due to the high grafting density. As shown in Figure III.12, a perfect superimposition of the spectra is obtained when the temperature is decreased back to 8 °C, evidencing a complete disassembly of the star-shaped complexes when the temperature goes below the LCST, which thus confirms the reversibility of the system as already observed with the DLS results.



*Figure III.12.* SANS spectra of a 1 wt. % M2005-e-cCNC-I suspension in  $D_2O$  at 8 °C (red data points) and of the same suspension at 8 °C after cooling from 24 °C (blue data points).

### III.3.4 Rheological properties of cCNC-I and their star-shaped assemblies

In addition to results obtained by TEM and DLS on dilute samples, further experiments aiming at investigating the rheological properties of polyetheramine-e-CNCs were carried out. For comparison purposes, a first series of measurements was performed with as-prepared CNCs.

Flow curves of 1 to 5 wt. % cCNC-I suspensions are shown in Figure III.13a. For each sample, the viscosity decreased when the shear rate increased from 0.1 to 1000 s<sup>-1</sup>, revealing a shear thinning rheological behavior. Furthermore, for 1, 2 and 3 wt. % cCNC-I samples, at high shear rates (> 100 s<sup>-1</sup>), the flow index *n* was close to 1 indicating a partial near-Newtonian liquid behavior. Figure III.13b shows the evolution of viscosity with temperature at a constant shear rate of 30 s<sup>-1</sup>. Clearly, for these 5 samples no temperature effect on the viscosity could be detected.

These results are very similar to the one reported in the literature. (Gicquel 2019; Xu 2017, 2018) cCNC-I suspensions behave as weak shear thinning systems, which turn into near-Newtonian liquids at high shear rates. The behavior of the 5 wt. % sample is somehow different, with a faster decrease of the viscosity for shear rate values higher than 30 s<sup>-1</sup>. This difference might be attributed to the fact that 5 wt. % is close to the onset of appearance of the liquid crystalline phase, which is known to drastically affect the rheological behavior of the samples (Shafiei-Sabet 2012). In the latter article, the authors showed that the viscosity of CNC-116

I suspensions decreased when the temperature increased at a given shear rate. In our case, as the temperature range is relatively narrow, no significant difference could be detected. Moreover, at 30 s<sup>-1</sup> the same value with ours, in a temperature range between 8 and 24 °C, the viscosity of cCNC-I was very close to the one measured by Shafiei-Sabet and coworkers.



*Figure III.13. (a)* Flow curves of cCNC-I samples of concentration from 1 wt. % to 5 wt. % at 15 °C. (b) Viscosity as a function of a temperature in the 8-24 °C range for the same series of samples at a constant shear rate of 30 s<sup>-1</sup>.

Figure III.14a shows the storage modulus G' and loss modulus G'' as a function of concentration. For the samples with a concentration of 1, 2 and 3 wt. %, G' could not be measured as the suspensions were too fluid. As a viscous fluid, the loss modulus G'' increased with the concentration from 1 to 3 wt. %. Obviously, the storage modulus G' was lower than the loss modulus G'' for 4 and 5 wt. % samples, showing that under these concentrations the samples behaved as a viscous fluid prone to higher particle interactions when the concentration increases.

The influence of temperature was then investigated and the result are shown in Figure III.14b and c. Comprehensively, G' and G'' are not influenced by temperature in the 8-24 °C range. It has to be noted that the scattered data points for G' at 4 wt. % are ascribed to the fluidity of the sample and not to a temperature effect.



*Figure III.14. (a)* Storage modulus G' (red point) and loss modulus G" (blue dashed line) as a function of concentration of cCNC-I samples. Experiments were performed at 15 °C at 0.02 strain and at an angular frequency of 1 Hz. (b) Storage modulus G' and (c) loss modulus G" as a function of temperature of cCNC-I samples. Experiments were performed in a temperature range between 8 and 24 °C at 0.02 strain and at an angular frequency of 1 Hz.

To investigate the rheological properties of polyetheramine end-grafted cCNC-I, five T5000-e-cCNC-I suspensions of concentration ranging from 1 to 5 wt. % were prepared at 4 °C. Macroscopically, at such a temperature well below the LCST of the polyetheramine, all samples were transparent and fluid.

Figure III.15 shows the corresponding flow curves at 8 °C in a shear rate range from 0.1 to 1000 s<sup>-1</sup>. Clearly, the viscosity of all five samples decreases when the shear rate increases indicating a shear thinning behavior. Compared with unmodified cCNC-I samples (Figure III.13a), very similar results are obtained. Such a result is in line with an expected limited influence of the presence of short chains grafted at the reducing end.



*Figure III.15.* Flow curves of T5000-e-cCNC-I samples of which the concentration is from 1 wt. % to 5 wt. % at 8 °C (< LCST) in a shear rate range from 0.1 to 1000 s<sup>-1</sup>.

As shown by DLS data and TEM images, T5000-e-cCNC-I formed thermoreversible starshaped complexes. In order to characterize the rheological properties of these suspensions as a function of temperature, the viscosity, storage modulus and loss modulus of five T5000-e-cCNC-I samples of concentrations ranging from 1 to 5 wt. % were measured in a temperature cycle between 8 and 24 °C. For the measurements of viscosity, the experiments were performed at a shear rate of 30 s<sup>-1</sup> after defining strain and the frequency corresponding to the linear domains. A 10 min time interval was applied during the increase of temperature (red straight line) and a 30 min one during cooling (blue dashed line). The viscosity as a function of temperature of a 5 wt. % suspension is shown in Figure III.16a. At 8 °C, below the LCST, the viscosity is about 0.01 Pa.s, which corresponds to the value in the flow curve in Figure III.15. At 12 °C, i.e. at about the LCST, the viscosity increases a little indicating the start of conformation changes of polymer chains. When T > 12 °C, the viscosity raises from 0.5 to about 1.4 Pa.s and tends to plateau showing a significant increase in interparticle or interaggregate interactions, which is consistent with the formation of star-shaped assemblies. A hysteresis effect was observed when the sample was cooled down even though the time interval allowed for temperature equilibration was tripled. When the temperature reached back 8 °C, the viscosity reverted to its initial value, showing a full reversibility allowed by a complete dissociation of the star-shaped complexes. This behavior is consistent with the

temperature-induced association-dissociation of cCNC-I into star-like assemblies revealed by DLS measurements and TEM micrographs. The evolutions of viscosity for the other four samples have the same tendency but different values. The higher the concentration, the more pronounced the effects.



**Figure III.16.** (a) Viscosity of a 5 wt.% T5000-e-cCNC-I suspension as a function of a temperature cycle between 8 and 24 °C via heating (red squares straight line) and cooling (blue squares dashed line) at a shear rate of 30 s<sup>-1</sup>. (b) Storage modulus G' (red squares) and loss modulus G'' (blue dots) of a 5 wt. % T5000-e-cCNC-I sample as a function of a temperature cycle between 8 and 24 °C via heating (straight line) and cooling (dashed line) at 0.02 strain and an angular frequency of 1 Hz. The black arrow shows the LCST~12 °C. A 10 min of time interval was applied between measurements during the increase of temperature and 30 min during the decrease, for equilibration purposes.

Oscillatory measurements were then carried out in the linear domain at 0.02 strain and an angular frequency of 1 Hz to probe the viscoelastic properties. Figure III.16b shows the evolution of the storage and loss moduli *G*' and *G*" of a 5 wt. % T5000-e-cCNC-I suspension as a function of temperature between 8 and 24 °C. At 8 °C, *G*' is lower than *G*" indicating a viscous fluid behavior. As aforementioned, at such a temperature, entropic repulsive interactions prevent the formation of aggregate or connections and the nanoparticles are individual in the suspension. At T=12 °C, *G*' has slightly increased but is still below *G*", showing the persistence of a viscous fluid behavior even though the LCST has been reached. This result shows that for this system either the LCST is shifted towards slightly higher values or the temperature equilibrium had not been reached. At T = 16 °C, a significant increase of *G*' is observed while *G*" only moderately increased. From this temperature *G*' is systematically higher than *G*" indicating the formation of an elastic gel. A tan  $\delta$  ratio of about 5 was measured, 120

which is comparable to what was obtained in the case of gels made of CNCs decorated with Jeffamine<sup>®</sup> M2005 over their entire surface (Azzam 2016b). However, as will be shown in the next chapter, much higher values can be obtained when both extremities of the rods are modified with polyetheramines. A ratio of G'/G'' about 5 was observed showing a transformation from a viscous fluid to an elastic gel. As for the viscosity measurements, a full reversibility is observed when samples are cooled down to 8 °C, even though a pronounced hysteresis is observed in the case of G' when the temperature is around the LCST. These results are consistent with the observations by DLS and TEM but one has to keep in mind that they correspond to samples with a much higher concentration.

The same tendency could be found for the sample at 4 wt. % with lower values in G' and G''. For the samples at 1, 2 and 3 wt. % measured at 8 °C, G' could not be measured. When the temperature increased around the LCST of the polyetheramine, G' could be observed and then follow the same tendency. When the temperature decreased back to 8 °C, G' was not measurable anymore.

However, since the star-shaped assemblies formed by a few nanocrystals actually reduce the concentration of the objects, which give more free space in the suspension, an explanation of the formation of the weak gel is not easy to find. From the TEM images shown in Figure III.8, some connections between CNC stars could be observed under dilute conditions. Therefore, a hypothesis may be formulated that in concentrated conditions, the CNC star-like aggregates partially associate to form a soft elastic phase. Such a behavior is in line with SANS data revealing that aggregates of ~10 CNCs were formed at 24°C, possibly arising from associations of two to three complexes.

### **III.4 Conclusion**

We present here the grafting of thermoresponsive polyetheramines at the reducing ends of cellulose nanocrystals using a two-step protocol involving an end carboxylation followed by a peptide coupling with the primary amine moiety of the polyetheramine. In aqueous suspensions these end-modified cCNC-I became associated by their derivatized tips when the temperature was raised past a lower critical solution temperature (LCST), above which these polyetheramines are known to collapse and become hydrophobic in aqueous environment. The CNC association was reversible when the temperature was lowered and the phenomenon of association/dissociation was totally reproducible in repeated temperature cycles as followed by dynamic light scattering (DLS) data. Small-angle neutron scattering (SANS) data revealed the presence of grafted chains with an extended conformation and showed the assembly into swollen aggregates in suspension at T>LCST. Transmission electron microscopy (TEM) images confirmed that the once dispersed derivatized cCNC-I at low temperature became associated through their reducing-ends above the LCST. At such temperatures, these modified cCNC-I attached themselves in a remarkable fashion, forming the arms of regular four-, five- or sixbranched stars. Moreover, rheological properties of T5000-e-cCNC-I were studied. The results indicated that below the LCST, the unmodified cCNC-I and polymer grafted cCNC-I had a very similar behavior both on viscosity and viscoelasticity. Above the LCST, the viscosity, the storage and the loss moduli of T5000-e-cCNC-I showed cyclic variations with the temperature cycle. Under these conditions, a soft elastic gel is obtained, probably due to a limited number of connections between star-like complexes.

### **Chapter IV**

## Temperature-triggered formation of cellulose II nanocrystal network through regioselective derivatization

### **IV.1 Introduction**

In the last chapter, based on the chemical polarity of cellulose I, a regioselective grafting of thermosensitive polymer chains at the reducing end of native CNCs could be successfully achieved from consecutive oxidation and peptide coupling reactions. The obtained derivatized objects were able to reversibly form star-shaped complexes via a simple control of temperature. In contrast with cellulose I nanocrystals, the presence of aldehyde groups on both ends allows the functionalization of both cellulose II rod extremities, as evidenced by the dual labelling with gold nanoparticles reported by Kim *et al.* (Kim 2006), which we optimized thanks to the *in situ* growth strategy in Chapter II.

So far, most of studies concentrated in cellulose II microfibrils (Miura 2016; Tulos 2019; Yue 2015) or gels containing cellulose II nanospheres (Beaumont 2016, 2019; Budtova 2019) and only a limited number of recent studies focused on cellulose II nanocrystals, as described in Chapter I.

In the present chapter, we aimed at functionalizing the two ends of cellulose II nanocrystals with temperature-responsive polymer chains. From a chemical point of view, the same two-step reaction pathway as the one applied in Chapter III for cCNC-I was applied. The morphological features and assembling properties of these innovative nanoparticles were investigated as a function of temperature (below and above the lower critical solution temperature, LCST, of the thermosensitive polymer) using dynamic light scattering and TEM. In addition, the rheological and viscoelastic properties of these regioselectively-derivatized cellulose II nanocrystals were evaluated. In order to focus on the chemical modification of as-prepared cCNC-II is shown in Appendix II of the manuscript.

### **IV.2 Materials and methods**

### **IV.2.1 Materials**

Cotton linters were provided by Buckeye Cellulose Corporation and used as the cellulose source without any further purification. Jeffamine<sup>®</sup> polyetheramine T5000 (gift from the Huntsman Corporation), with a LCST of ~12 °C is a three-branched polymer of propylene glycol with amine end groups at each of its branches (chemical structure and evaluation of the LCST shown in Chapter III.3.1) and a molecular weight of about 5000 g mol<sup>-1</sup>. Oher chemicals were purchased from Sigma-Aldrich and used as received. Deionized water was used in all experiments.

The descriptions of the preparation of cCNC-II and the measurement of the sulfur and aldehyde contents are given in Chapter II.2.4, II.2.5 and II.2.6.

### **IV.2.2 cCNC-II suspension preparation**

The pH of a 1 wt. % cCNC-II suspension containing 3 g of dry CNCs was adjusted to 7 by adding 0.1 M NaOH solution. The suspension was then freeze-dried and the cCNC-II were redispersed in distilled water to prepare redispersed cCNC-II samples at a given concentration in the 1-5 wt. % range. Sample homogeneity was ensured by ultrasonication three times for 2 min.

### **IV.2.3** Pinnick oxidation

NaClO<sub>2</sub> (5.652 g, 0.05 mol) was added to a 2.4 wt. % aqueous cCNC-II suspension containing 1 g dry CNC. HAc was then added until the pH reached 3.5 and the suspension was kept for 48 h under magnetic stirring. The reaction was stopped by adding 0.5 M NaOH solution to reach a pH of about 8. The suspension was dialyzed against distilled water until a constant conductivity of the dialysis bath.

### **IV.2.4** Polymer grafting by peptide coupling

Jeffamine<sup>®</sup> Polyetheramine T5000 (0.44 g, 0.088 mmol) were added to an (~1 wt. %) end-carboxylated CNC suspension, containing 0.5 g dry CNC and stirred until dissolution. The reaction was performed at 4 °C (well below the LCST) to ensure a good solubility of the polymer. The catalysts dicyclohexylcarbodiimide (DCC, 27.3 mg, 0.132 mmol) and 4-dimethylaminopyridine (DMAP, 10.75 mg, 0.088 mmol) were then added. After 24 h under stirring, the resulting suspensions (T5000-e-cCNC-II) were filtered through 100  $\mu$ m nylon filters to get rid of the catalyst and dialyzed against distilled water to remove excess reagents including non-grafted polymers.

### **IV.3 Results and discussion**

## IV.3.1 Physicochemical properties and morphology of cellulose II cotton nanocrystals

The morphology and dimensions of the cCNC-II were characterized by TEM imaging (Figure IV.1a) and a SANS experiment (Figure IV.1b). As shown in Figure IV.1a, cCNC-II are rodlike particles with a length ranging between 50 and 150 nm and an average width of 16 nm, even if some wider particles can be observed. They are therefore shorter than cCNC-I produced from the same cellulose source, while having a more or less comparable width. CNC-II therefore exhibit a lower aspect ratio than their cellulose I homolog. Like native CNCs, they are composed of a few laterally-associated elementary crystallites. These morphological features are in agreement with those reported for cellulose II nanocrystals obtained from ramie, cotton or eucalyptus (Delepierre 2020; Gong 2017; Kim 2006; Flauzino Neto 2016; Yue 2012). Figure IV.1b shows the SANS spectrum of a 1 wt. % cCNC-II suspension in D<sub>2</sub>O. A very clear  $Q^{-4}$  decay can be observed in the high *Q*-region (from Q~0.05 Å<sup>-1</sup>). Such a Porod law behavior readily shows that the cCNC-II-water interface is sharp and smooth and arises from the crystalline and smooth character of all the facets of the particles. In the low *Q*-region, a plateau is observed and a shoulder can be detected at  $Q^*$ ~6.5 10<sup>-3</sup> Å<sup>-1</sup>. The latter feature can be attributed to the electrostatic repulsion between particles giving rise to some contribution of the

structure factor to the scattered intensity. The origin of this electrostatic interaction is the presence of sulfate half-esters resulting from the sulfuric acid hydrolysis. The position of this peak can be used to calculate the average interparticle distance, d, from  $d=2\pi/Q^*$ . A value of d of 97 nm can thus be extracted. As shown in various studies, SANS data of dilute CNC suspensions have been well-described using a model based on the form factor of a parallelepiped with a rectangular cross-section (Azzam 2020; Cherhal 2015; Mao 2017; Terech 1999). A similar model was used here since literature data and TEM images show that cCNC-I and cCNC-II exhibit the same morphology but with different dimensions. As shown in Figure IV.1b, a good agreement between the fitting and the experimental spectrum was obtained for  $Q>1.10^{-2}$  Å<sup>-1</sup>. Nevertheless, as the fitting process did not take into account the influence of the structure factor whose contribution is observed at low Q-values, the model only fairly fits the data in the low Q-range. The best fit was obtained with values of 74.0, 21.5 and 3.6 nm for the length, width and thickness, respectively, using a polydispersity of 0.05 on the width and 0.32 on the thickness. In addition to the overall good fitting quality, these average length and width values are in good agreement with our TEM data and more generally with dimensions reported in the literature. The height of 3.6 nm is smaller than the one measured by AFM by Delepierre et al.  $(5 \pm 1 \text{ nm})$  (Delepierre 2020). Knowing the dimensions of the rods and the volume fraction, an average distance between the center of mass of the particles can be calculated assuming a close packing. Such a calculation yields a value of 109 nm, which is close to the value independently obtained from the intensity maximum at  $Q^*$  and therefore confirms the validity of the dimensions extracted from the fit.

A sulfur content (S%) of 0.67 % was measured by conductometric titration which is very close to the value of cotton cellulose I nanocrystals (0.69 %). This sulfur content provided by sulfate groups on the surface of the nanorods corresponds to a surface charge density of 0.3  $e^{-}nm^{-2}$  which is a little lower than that of cCNC-I. The zeta potential was -32.1 ± 4 mV. Both the sulfur content and zeta potential values are comparable with values reported for cellulose II nanocrystals extracted from eucalyptus or cotton and confirm that cCNC-I and cCNC-II particles display similar surface characteristics (Flauzino Neto 2016).



**Figure IV.1.** (a) TEM micrographs of negatively stained cCNC-II prepared from aqueous suspension. (b) SANS spectra of a 1 wt. % cCNC-II suspension in  $D_2O$  and fit using the form factor of a parallelepiped with dimensions  $74 \times 21.5 \times 3.6 \text{ nm}^3$ . Gaussian polydispersity of 0.05 and 0.32 were applied on the width and thickness, respectively.

As shown in Chapter III, FTIR, WAXS and <sup>13</sup>C solid-state NMR confirmed the successful allomorphic transition from cellulose I to cellulose II during the mercerization process. As a consequence, the particles characterized here by TEM and SANS are cellulose II nanocrystals.

### **IV.3.2** Two-step functionalization of cCNC-II

In order to graft thermosensitive polymers onto both ends of cCNC-II particles, a two-step protocol described in Scheme IV.1 was followed. First, the aldehyde groups present at both extremities were converted into carboxylates by a chlorite oxidation method and the resulting particles will be referred to as Ox-e-cCNC-II. The aldehyde contents before and after oxidation reaction could be measured by the Fehling test. The initial and post-oxidation values were  $22.7 \pm 0.5 \mu$ mol CHO g<sup>-1</sup> and  $5.6 \pm 0.4 \mu$ mol CHO g<sup>-1</sup>, respectively, proving the success of the oxidation reaction with a yield of about 75 %. The polyetheramine T5000 was then grafted onto Ox-e-cCNC-II by a peptide coupling reaction. In the presence of catalysts, the amino groups of the polymer chains and the carboxylated groups on the rod ends condensed to form an amide bond. Such a regioselective grafting only concerns a very small number of AGU units (only the ones at the ends of the rods, which only represents a few percent of the total AGU units), which made attempts to quantity the functionalization yield by FTIR or solid-state NMR unsuccessful.



**Scheme IV.1.** Two-step functionalization of cCNC-II particles with Jeffamine<sup>®</sup> polyetheramine chains in aqueous medium. After a carboxylation of the reducing ends, the grafting of the polyetheramine chains was achieved via a peptide coupling reaction.

### IV.3.3 Temperature-triggered assembling properties of polymer grafted cCNC-II

In order to investigate the effect of the regioselective grafting of polyetheramine onto the ends of cCNC-II, DLS was used to measure the hydrodynamic diameter  $(D_h)$  as a function of temperature for unmodified cCNC-II, Ox-e-cCNC-II and polymer grafted cCNC-II (T5000-e-cCNC-II). As shown in Figure IV.2a, cCNC-II present a constant  $D_h$  at about 120 nm, which is very close to the hydrodynamic diameter of end-oxidized cCNC-II, showing, as expected, no significant influence of chlorite oxidation. As well, in the temperature range of 4-24 °C, D<sub>h</sub> of bare cCNC-II and Ox-e-cCNC-II remain constant showing no temperature effect on these two nanoparticles. Then, for T5000-e-cCNC-II, when the temperature was below the LCST of the polyetheramine, a constant value of about 150 nm was measured. This value that is significantly higher than the one for cCNC-II and Ox-e-cCNC-II is attributed to the presence of polyetheramine chains on the ends of the rods, which slows the dynamics of the rods, and is therefore an indirect evidence of the success of polymer grafting. It must be noted that this increase of hydrodynamic diameter after polyetheramine could also come from the formation of duplex or triplex particle associations because of the use of the polyetheramine T5000 that is a triamine, which theoretically allows for connecting two or three cCNC-II. However, such a reaction is unlikely due to steric hindrance and no such duplex or triplex structures were observed by TEM (vide supra).

In contrast with the constant value measured below the LCST, a significant increase in hydrodynamic diameter was observed when the sample was heated above this critical temperature. Indeed, an eight-fold increase in  $D_h$ , from 150 to about 1200 nm, was revealed, showing the formation of aggregates. This value is much higher than the one obtained in the 130

case of temperature-induced formation of CNC star-shaped complexes formed by thermosensitive grafted polymer at the reducing end of cCNC-I particles. When the temperature decreased from above the LCST to 4 °C, the  $D_h$  value spectacularly decreased until it reached back the initial diameter of about 150 nm, indicating the complete dissociation of the aggregates. This result reveals a fully thermoreversible behavior characterized by a hysteresis phenomenon related to a faster association than dissociation. In fact, when temperature cycles between 4 and 24 °C were repeated, this thermoreversible behavior was totally reproducible (Figure IV.2b). Even though the suspension experienced numerous temperature variations conditions, the reversible temperature-induced aggregation was preserved.



**Figure IV.2.** (a) Hydrodynamic diameter as a function of a temperature cycle between 4 and 24 °C for cCNC-II (red squares), Ox-e-cCNC-II (cyan dots) and T5000-e-cCNC-II (purple triangles) in 0.1 wt. % aqueous suspensions. Heating (red) and cooling (blue) are indicated by arrows. (b) Hydrodynamic diameter variations for T5000-e-cCNC-II in 0.1 wt. % aqueous suspensions during multiple cycles of temperature increase and decrease between 4 and 24 °C.

To further characterize the temperature-induced aggregation revealed by DLS, the samples prepared at 4 and 24 °C were observed by TEM. Figure IV.3 shows images of Ox-e-cCNC-II after oxidation at 4 and 24 °C. Ox-e-cCNC-II nanoparticles shown in Figure IV.3a are almost identical to their parent cCNC-II, indicating that the chlorite oxidation did not have any effect on the morphology of the particles. When the temperature was increased to 24 °C (Figure IV.3b), similar observations could be made, showing that a temperature increase had no effect on Ox-e-cCNC-II, which confirmed the DLS measurements.



*Figure IV.3. TEM micrographs of negatively stained Ox-e-cCNC-II at 4 °C (a) and 24 °C (b) prepared from an aqueous suspension.* 

The TEM micrograph of the T5000-e-cCNC-II sample prepared from suspensions kept at 4 °C is shown in Figure IV.4. These polymer-grafted type II CNCs are well-dispersed and exhibit the same morphological features as their parent particles. The T5000 chains are not visible, which is expected due to their very reduced size, even though their presence contributes to the hydrodynamic diameter. Additionally, despite the use of triamine, no duplex or triplex structures were observed.



*Figure IV.4. TEM image of negatively stained T5000-e-cCNC-II at 4 °C prepared from aqueous suspension.* 

As shown in Figures IV.5, peculiar assemblies of T5000-e-cCNC-II were formed from the same sample prepared at 24 °C. Upon temperature increase above the LCST, the derivatized cCNC-II particles indeed self-assembled into a network where the particles are hooked by their

ends. Using a lower magnification (Figure IV.5c), the observed network appears to extend to the supra-micron range. This spectacular phenomenon is due to the presence of thermosensitive polymers on the two ends of the cCNC-II rods. When the temperature increases above the LCST of the thermosensitive polymer, the solvent conditions become poor and polymer chains collapse. This switch in conformation is accompanied by a change from entropic repulsions below the LCST to attractive interactions mediated by hydrophobic polymer chains above the LCST. Sulfate charges on the side of cCNC-II are not affected by variations in temperature in that range and lateral repulsive interactions are preserved. In addition to the presence of interconnected loops made of end-to-end linked derivatized cCNC-II, some lateral associations are also observed. The latter assembling could result from connections involving both ends of the particles that overcome the electrostatic lateral repulsions. This organization might also arise from artefacts during TEM sample drying. Accordingly, above the LCST, T5000-e-cCNC-II assemble through their ends and occasionally via their lateral surface to form the observed network structure, as shown in Scheme IV.2.



Scheme IV.2. Thermoreversible association into a network of polyetheramine-e-cCNC-II.



*Figure IV.5. TEM images of negatively stained T5000-e-cCNC-II at 24 °C prepared from aqueous suspensions.* 

It can be seen in Figure IV.5c that the network connects a majority of T5000-e-cCNC-II particles and that only a very small number of CNCs stay individualized. Such a feature is an indirect proof of a successful derivatization of both ends of the particles with a very high yield. When compared to our previous work with native CNCs for which only one end of the rods

was functionalized with thermosensitive polymers, allowing the formation of star-shaped complexes above the LCST, the present regioselective dual derivatization increases the association probability, resulting in the temperature-triggered assembling of a very large number of objects. It must be noted that these networks could spontaneously dissociate when the temperature was decreased below the LCST, as shown by TEM images of a sample first heated to 24 °C and then cooled down to 4 °C prior to TEM observation (Figure IV.6), in good agreement with the reversibility shown by DLS measurements during temperature cycles.



Figure IV.6. TEM image of T5000-e-cCNC-II from aqueous suspension at 4 °C cooled down from 24 °C.

### IV.3.4 Rheological behavior of regioselectively-derivatized cCNC-II

Derivatized CNC-II particles and their assembly into networks were well characterized by DLS and TEM in dilute conditions. However, the samples prepared for TEM observations were thin dry films, which could be affected by drying artefacts. To get more information on these systems from a 3D point of view, a series of rheological experiments including the investigation of the viscosity and viscoelasticity of the suspensions were carried out.

First, flow curves of T5000-e-cCNC-II suspensions at concentrations from 1 to 5 wt. % were recorded at 8 °C, well below the LCST of the polyetheramines (Figure IV.7a). Macroscopically, at such a temperature all samples were fluid and no gel was formed. Clearly, the viscosity of all five samples decreased when the shear rate increased, indicating a shear
thinning behavior. A Newtonian or shear-thinning behavior is well depicted by the power law  $(\tau = K \cdot \dot{\gamma}^n)$  where  $\tau$  represents the stress (Pa),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>), *K* is the consistency (Pa.s<sup>n</sup>) and *n* is the shear-thinning index. Figure IV.7b shows the evolution of the stress as a function of shear rate from which the consistency, *K*, and the shear-thinning index, *n*, could be calculated. The results are shown in Table IV.1.



**Figure IV.7.** (a) Flow curves and (b) fits of stress as a function of shear rate of T5000-e-cCNC-II samples at concentrations of 1, 2, 3, 4 and 5 wt. % at 8 °C (< LCST) in a shear rate range from 1 to  $1000 \text{ s}^{-1}$ . (c) Consistency and shear thinning index as a function of concentration of T5000-e-CNC-II.

*Table IV.1.* Consistency and shear-thinning index values of T5000-e-cCNC-II suspensions at 8 °C derived from the fitting of experimental data with the expression given in the text.

Concentration (wt. %)	1	2	3	4	5
K (Pa.s <sup>n</sup> )	0.0022	0.0032	0.0041	0.0046	0.0052
n	0.95	0.93	0.92	0.91	0.89

In order to easily compare with cCNC-II samples in Appendix II, the scales of consistency and shear thinning index in Figure IV.7c were set to the same value range as in Figure A.II.1c. For all samples, the shear-thinning index was close to 1 indicating a near-Newtonian behavior. Figure IV.7c clearly showed that no significant variation was observed. When compared with cCNC-II samples (Appendix II Figure A.II.1a), 1 and 2 wt. % T5000-e-cCNC-II suspensions behave similarly and no significant difference was observed. However, the viscosity of 3, 4 and 5 wt. % T5000-e-cCNC-II samples is lower than that of unmodified cCNC-II samples at the same concentration, in agreement with macroscopic visual observations (Appendix II Figure A.II.2) showing that as-prepared cCNC-II suspensions at 3, 4 and 5 wt. % are almost gel-like. This result can be ascribed to the presence of polyetheramines chains onto the ends of the rods in the case of T5000-e-cCNC-II samples that generate entropic repulsive forces when the polymer is in good solvent conditions. Such interactions, which are not present in the case of as-prepared cCNC-II particles, contribute to a better individualization of the nanoparticles and prevent connections to take place, favoring a liquid-like behavior.

Temperature effects on the viscosity and viscoelasticity of a 5 wt. % T5000-e-cCNC-II suspensions are shown in Figure IV.8 in the 8-24 °C range. Viscosity measurements were performed at a shear rate of 30 s<sup>-1</sup>. It was first checked that the viscosity behavior of the underivatized cCNC-II suspensions measured in the same conditions is temperature independent, as shown in Appendix II Figure A.II.1d. For the polyetheramine end-grafted samples, below the LCST, at 8 °C, the viscosity was about 0.005 Pa.s, which corresponds to the value in the flow curve in Figure IV.7a. At 12 °C, *i.e.* at the LCST of the T5000 polyetheramine, a moderate increase in viscosity is detected, indicating the start of conformation changes of the polymer chains. When T > 12 °C, the viscosity strongly increased to about 180 Pa.s and tended to reach a plateau, suggesting a formation of connections between the nanocrystals, which is in good agreement with the network structure revealed by TEM images. A hysteresis effect was observed when the sample was cooled down even though the time interval between two measurements was tripled. When the temperature was decreased back to 8 °C, the initial viscosity value was recovered, showing a perfect thermoreversibility and a complete dissociation of the network upon cooling. These data confirm the reversibility observed by DLS

and TEM. The evolution of the viscosity for the other four samples exhibits the same tendency but with different values. The higher the concentration, the higher the viscosity increase with temperature.



**Figure IV.8.** (a) Viscosity of a 5 wt. % T5000-e-cCNC-II sample during an 8-24 °C temperature cycle (heating: red squares and continuous line, and cooling: blue dots and dashed line) at a shear rate of 30  $s^{-1}$ . (b) Storage modulus G' (red squares) and loss modulus G'' (blue dots) of a 5 wt. % T5000-e-cCNC-II sample during an 8-24 °C temperature cycle (heating: red squares and continuous line, and cooling: blue dots and dashed line) at 0.01 strain and an angular frequency of 1 Hz. The black arrow shows the LCST~12 °C.

Figure IV.8b shows the evolution of the storage, G', and loss, G'', moduli of a 5 wt. % T5000-e-cCNC-II suspension as a function of temperature between 8 and 24 °C. Measurements were carried out at 0.01 strain and an angular frequency of 1 Hz after defining the strain and frequency corresponding to the linear domains. In the same way as for the viscosity, the viscoelastic behavior of non-modified cCNC-II suspensions is insensitive to the temperature in the 8-24 °C range (Appendix II Figure A.II.3), in contrast with results reported by Yue *et al.* who could observe some increase of G' when the temperature was increased from 15 to 25 and then 35 °C (Yue 2012). For the T5000-e-cCNC-II suspension, at 8 °C, G' is lower than G'' indicating a viscous fluid behavior. As aforementioned, at such a temperature, entropic repulsive interactions prevent the formation of aggregates or connections and the nanoparticles were individual in the suspension. When the temperature increased toward the LCST of the polyetheramine, G' increased strongly and G'' increased more slowly. For T > 12 °C, G'

continued to increase, while G" almost reached a plateau value at 1 Pa from 16 °C. G' is higher than G" from T=12 °C and the G'/G" ratio increases with the temperature to reach a value of about 30 at 24 °C. A temperature-induced transition from a viscous fluid to an elastic gel is therefore observed when the temperature is increased above the LCST of the polyetheramine. The gel behavior is attributed to attractive hydrophobic interactions between collapsed polymer chains at the extremities of the rods, which result in end-to-end connections of the rods and network formation. Interestingly, when the sample was cooled down from 24 °C to 8 °C, G' and G" decreased and recovered almost their value before heating. A full reversibility is thus observed and the cooling induces a transition from an elastic to a viscous fluid following the breakage of end-to-end associations and the recovery of individual repulsive particles. This result is in full agreement with DLS, TEM and viscosity data. The same tendency could be found for the other 4 concentrations, but the higher the concentration, the higher the effects.

This viscoelastic behavior observed here is different from that of suspensions of polyetheramine-grafted cCNC-I particles, where the thermosensitive chains were decorating the whole surface of the rods, as reported by Azzam et al. (Azzam 2016b). In their case, cCNC-I particles were grafted with the thermosensitive M2005 polymer. When the temperature was above the LCST, these nanocrystals aggregated into bundles through attractive interaction forces between hydrophobic polymer chains on the lateral surfaces of the nanocrystals and the subsequent connection of the bundles resulted in a gelation. The variation of G' and G'' during the increase of temperature from below to above the LCST had a similar tendency to ours. However, the G' / G'' ratio in that case was limited to 3, while it reached 30 in the case of T5000-e-cCNC-II. In fact, the gelation caused by CNCs aggregates in the case of the entire nanocrystal surface modification is due to the hydrophobic interactions between collapsed polymer chains adding interaggregate connection at a high concentration of nanoparticles. In the present work, a greater number of connections can be achieved as the cCNC-II nanorods can associate through their ends to form a supra-micron network. The thermoreversible character of the gelation could be observed in both cases. This comparison shows that the fine tuning of the grafting loci allows controlling the rheological properties and the extent of the gelation phenomenon.

In order to further investigate the T5000-e-cCNC-II network, the evolution of stress as a function of shear rate was shown in Figure IV.9a. Obviously, all five samples had a yield stress at low shear rate. However, a significant increase of yield stress between 4 and 5 wt. % T5000-e-cCNC-II samples was observed, indicating a strong structuration within the samples. Experimental data were fitted with the Herschel-Buckley law  $\tau = \tau_0 + K \cdot \dot{\gamma}^n$ , and values of the various parameters obtained are summarized in Table IV.2. Besides the strong increase of the yield stress, a transition of rheological behavior was also clearly observed. The shear thinning index for 1, 2 and 3 wt. % samples were below 1 indicating a shear thinning behavior. However, *n* increased from 0.49 to 1.1 for a 4 wt. % suspension and turned to above 1, indicating a shear thickening behavior. Figure IV.9b showed the variation more intuitively. The onset of the shear thickening phenomenon is between 3 and 4 wt. % and above this threshold the higher the concentration, the more dramatic the phenomenon. Further investigations will be carried out in the future to well define the transition and to further characterize these new properties.



*Figure IV.9. (a)* Evolution of stress as a function of shear rate of T5000-e-cCNC-II at 24 °C. (b) Consistency and shear thinning index as a function of concentration of T5000-e-cCNC-II at 24 °C.

Table IV.2. Yield stress, consistency and shear-thinning index of T5000-e-cCNC-II suspensions at 24°C.

Concentration (wt. %)	1	2	3	4	5
τ <sub>0</sub> (Pa)	1.14	3.44	10.6	604	2018
K (Pa.s <sup>n</sup> )	0.18	1.2	3.6	5.9	6.5
n	0.63	0.52	0.49	1.1	1.7

The 1, 2 and 3 wt. % T5000-e-cCNC-II samples have an elastoplastic behavior that is characterized by a yield stress and a storage modulus. Based on the TEM images, a fractal behavior of dimension D was considered to characterize the CNC network. Usually, such a fractal dimension is revealed by light, neutron or X-ray scattering from log-log plots of the scattered intensity vs. scattering vector. However, in the case of various colloidal and polymeric systems, such as thixotropic clay suspensions (Pignon 1997), silica-silicone physical gels (Piau 1999) and hydrophobic silica in polyol (Saint-Michel 2003), scaling laws between G' and the volume fraction,  $\varphi$ , and between  $\tau_0$  and  $\varphi$  involving the fractal dimension were extracted from a mathematical modeling of the rheological data. In our case, we have thus used these scaling laws (Equation IV.1) to extract D from the measured rheological data.



 $G' \propto \varphi^{\frac{5}{3-D}}$  and  $\tau_0 \propto \varphi^{\frac{4}{3-D}}$  Equation IV.1

*Figure IV.10.* Evolution of storage modulus and yield stress at different volume fractions of T5000-e-cCNC-II at 24 °C and fits using the scaling laws in Equation IV.1.

Figure IV.10 shows the modeling of G' and  $\tau_0$  with these scaling laws taking into account the 1, 2 and 3 wt. % T5000-e-CNC-II samples, as 4 and 5 wt. % samples exhibited a shear thickening behavior. Two relations,  $G' \propto \varphi^{2.3}$  and  $\tau_0 \propto \varphi^{2.5}$ , could be obtained, respectively resulting in the two values, 0.85 and 1.4 for the fractal dimension D. As the fit was carried out using only three points, a relatively high error could exist but we can conclude that a fractal behavior of dimension D about 1 is revealed by the modeling, indicating an alignment

of the T5000-e-cCNC-II that leads to the formation of a fibrous gel. Generally speaking, the fractal approach gave a new thinking of the gel behavior of CNC network, which is worth going further into detail in the future. Also, the shear thickening behavior of T5000-e-cCNC-II in higher concentration is still waiting for studying.

#### **IV.4 Conclusion**

In this chapter, we took advantage of the presence of reactive groups located at both ends of cCNC-II particles to symmetrically functionalize these biosourced rods with thermosensitive polymer chains on both rod extremities. To achieve such a derivatization, a simple two-step chemical pathway was followed. Thanks to this derivatization, the colloidal behavior of cCNC-II was modified and became temperature-sensitive. Below the LCST of polyetheramine chains, repulsive entropic interactions between swollen chains located at the rod ends add to the electrostatic repulsion provided by the surface sulfate half-esters on the lateral sides of the nanocrystals to yield purely repulsive particles. In these conditions the derivatized particles are individual and the resulting suspensions exhibit a shear-thinning behavior. When the temperature is increased above the LCST of the polymer, attractive interactions between collapsed hydrophobic chains develop, allowing end-to-end associations to take place and leading to the spectacular formation of a supra-micron network, as observed from TEM images. From a macroscopic point of view, rheological measurements evidence the temperatureinduced transition from a viscous fluid to an elastic gel, which is characterized by a high G'/G''ratio. All the experimental techniques used, namely DLS, TEM and viscosity and viscoelasticity measurements, consistently show a full reversibility upon sample cooling due to breakage of end-to-end associations when polymer-polymer interactions are turned to repulsive at low temperature. Overall, the possibility to chemically modify both ends of cCNC-II particles provides new properties when compared to the reducing-end modification of native CNCs, further revealing the interest of the cellulose II allomorph for alternative functionalities that could not be reached with CNC-I particles.

### Chapter V

# Strategies towards the optimization of polymer grafting onto the reducing end of native cellulose nanocrystals

This chapter is divided into two parts. The first part deals with the optimization of the grafting of Jeffamine<sup>®</sup> polyetheramines onto the reducing end of CNC-I based on the study of the effects of different physics-chemical parameters.

In the second part, a treatment of cCNC-I with N-methylmorpholine N-oxide (NMMO) was considered to improve the accessibility and reactivity of the reducing end of cCNC-I.

#### V.1 Optimization of polymer grafting onto the reducing end of CNC-I

#### V.1.1 Introduction

The influence of different factors of the chemical process on the grafting efficiency and subsequent formation of star-like assemblies were investigated. Five parameters were considered: the reaction type (Pinnick oxidation followed peptide coupling or a direct reductive amination), the combination of catalysts (DCC/DMAP, DCC/4-PPY or DCC/DBU), the polyetheramine type (mono-aminated Jeffamine® M2005 or tri-aminated T5000) the solvent (water or DMF) and the aspect ratio of the CNC-I (12 for cotton, 20 for wood and 110 for tunicate). Since, as aforementioned, the FTIR or NMR methods could not quantitatively characterize the grafted polymer at the reducing end due to low modification and low sensitivity of the techniques, we have chosen here to use indirect methods such as DLS and TEM to provide semi-quantitative information. Namely, DLS was used to compare the hydrodynamic diameters and TEM was used to compare the yields of star-shapes complexes' formation, defined as the number of CNCs belonging to such aggregates divided by the total number of CNCs, as a function of the reaction parameters. This yield was obtained from the analysis, for a given set of parameters, of eight TEM images, containing a total of about 150 CNCs, which ensured a fair statistical relevance. All DLS measurements were performed in the exact same conditions (concentration, heating and cooling rates) for the different samples to minimize kinetic effects and avoid thermal history artefacts. A histogram summarizing the effects of these parameters (Figure V.13) on this yield is shown at the end of this part.

In order to clearly show the differences between samples, Table V.1 lists all acronyms with precise investigated experimental conditions.



Table V.1. Detailed conditions corresponding to the different samples used in the optimization study

#### V.1.2 Materials and methods

#### V.1.2.1 Materials

Cotton linters were provided by Buckeye Cellulose Corporation and used as the cellulose source without any further purification. Mantles of tunicate (*Halocynthia roretzi*) were purified with 1M KOH and 0.3 % NaClO<sub>2</sub> treatments. Two thermosensitive polyetheramines were used in this study: Jeffamine<sup>®</sup> Polyetheramines M2005 and T5000 (gift from the Huntsman Corporation). Jeffamine<sup>®</sup> polyetheramine M2005, which has lower critical solution temperature (LCST) ~16 °C, is a statistical amine-terminated polymer of ethylene oxide (EO) and propylene oxide (PO) with a molecular weight about 2000 g mol<sup>-1</sup> (EO / PO monomer composition of 6 / 29). Jeffamine<sup>®</sup> polyetheramine T5000, with a LCST of ~12 °C is a three-branched polymer of PO with amine end groups at each of its branches and a molecular weight of about 5000 g mol<sup>-1</sup>. Other chemicals were purchased from Sigma-Aldrich and used as received.

The descriptions of the preparation of cCNC-I, wCNC-I and the measurement of the sulfur and aldehyde contents are given in Chapter II.2.2, II.2.3, II.2.5 and II.2.6. The conditions for the Pinnick oxidation are given in Chapter III.2.3.

V.1.2.2 Native CNCs from tunicate (tCNC-I)

Mantles of tunicate (*Halocynthia roretzi*) were purified with 1M KOH and 0.3 % NaClO<sub>2</sub> treatments. Before the hydrolysis, they were rinsed thoroughly, cut in small pieces and mixed

with a blender. Tunicate CNC-I were obtained by hydrolysis with 48 wt. % sulfuric acid at 55 °C for 15 h. Acid-free CNC aqueous suspensions were obtained after repeated centrifugations/washing cycles followed by ultrasonication. A suspension at 1.2 wt. % was obtained.

V.1.2.3 Polymer Grafting

#### V.1.2.3.a. Grafting by reductive amination

Jeffamine<sup>®</sup> Polyetheramine M2005 (0.088 g, 0.044 mmol) or T5000 (0.22 g, 0.044 mmol) were added to a (~1 wt. %) CNC suspension, containing 0.5 g dry CNC-I and stirred until dissolution. The reaction was performed at 4 °C (well below the LCST) to ensure a good solubility of the polymer. The catalyst sodium cyanoborohydride (NaBH<sub>3</sub>CN, 2.8 g, 44 mmol) was then added. After 48 h under stirring, the resulting suspensions were dialyzed against distilled water to remove excess reagents including non-grafted polymers.

V.1.2.3.b. Grafting by peptide coupling reaction

Jeffamine<sup>®</sup> Polyetheramine M2005 (0.088 g, 0.044 mmol) or T5000 (0.22 g, 0.044 mmol) were added to an (~1 wt. %) end-carboxylated CNC suspension, containing 0.5 g dry CNC-I and stirred until dissolution. The reaction was performed at 4 °C (well below the LCST) to ensure a good solubility of the polymer. The catalysts dicyclohexylcarbodiimide (DCC, 13.65 mg, 0.066 mmol) and 4-pyrrolidinopyridine (4-PPY, 6.52 mg, 0.044 mmol) or 4-dimethylaminopyridine (DMAP. 5.38 0.044 mmol) mg. or 1.8-diazabicyclo(5.4.0)undec-7-ene (DBU, 6.7 mg, 0.044 mmol) were then added. After 24 h under stirring, the resulting suspensions were filtered through 100  $\mu$ m nylon filters to get rid of the catalyst and dialyzed against distilled water to remove excess reagents including non-grafted polymers.

#### V.1.3 Results and discussion

#### V.1.3.1 Influence of reaction type

Reductive amination (RA) that directly transforms aldehyde groups into amine groups 147

was carried out on cCNC-I as an alternative to the two step Pinnick oxidation and peptide coupling process (PC). NaBH<sub>3</sub>CN was used as a catalyst and the reaction was performed in water using polyetheramine M2005. As shown in Figure V.1, the hydrodynamic diameter of M2005-e-cCNC prepared by reductive amination in water (M-e-cCNC-I-RA/N/H) and by peptide coupling in water using DCC/DMAP as catalysts (M-e- cCNC-I-PC/DD/H) was measured as a function of temperature by DLS.

When the temperature was below the LCST (16 °C), the hydrodynamic diameter of both samples kept stable. However, a difference of about 30 nm between the two values was observed. Based on the assumption that the higher the grafting density, the lower the diffusion coefficient, we can conclude from these results that the samples with the highest hydrodynamic diameter corresponds to the highest grafting yield. Accordingly, DLS data tend to show that, in the reaction conditions probed, peptide coupling is a more efficient reaction than reductive amination for the grafting of M2005 polyetheramine on the reducing end of cCNC-I.



**Figure V.1.** Hydrodynamic diameter as a function of temperature of 0.5 wt. % suspensions of *M*-e-cCNC-I-RA/N/H and *M*-e-cCNC-I-PC/DD/H. Heating and cooling are indicated by red and blue arrows, respectively.

When the temperature is increased above the LCST of M2005,  $D_h$  of the two samples increased but a difference of growth rate was observed. Clearly, the increase rate and value at 40 °C are higher for PC than for RA, suggesting that more aggregates were formed or that bigger aggregates comprising a higher number of CNCs were formed for PC than for RA. When the temperature reverted back to its initial value,  $D_h$  decreased until it recovered to its initial low temperature value, indicating a good thermoreversibility of CNCs aggregates for both two samples.

TEM observations were performed on these two samples from suspensions heated at 40 °C. In both cases, star-shaped CNC assemblies could be observed, as shown in Figure V.2. The yield of star-shaped assemblies' formation was about 7.5 % and 22 % for M-e-cCNC-I-RA/N/H and M-e-cCNC-I-PC/DD/H, respectively, indicating that more stars could be observed after the peptide coupling reaction.



*Figure V.2. TEM images of negatively stained M-e-cCNC-I-PC/DD/H (a) and M-e-cCNC-I-RA/N/H (b)* prepared from suspensions at 40 °C.

Overall, these results tend to indicate that the peptide coupling reaction is more efficient than the reductive amination for the grafting of Jeffamine<sup>®</sup> polyetheramine at the reducing end of cCNC-I. Based on that conclusion and in order to limit the number experiments, the peptide coupling reaction was chosen to test the influence of the other parameters and the same characterization protocol was followed.

#### V.1.3.2 Influence of the catalysts

Chemical modifications targeting the grafting of polyetheramines M2005 onto the reducing end of cCNC-I via the peptide coupling reaction in water were carried out using three different couples of catalysts, DCC/DMAP, DCC/4-PPY and DCC/DBU.

Figure V.3 shows the evolution of the hydrodynamic diameter of samples prepared using

DCC/DMAP (M-e-cCNC-I-PC/DD/H), DCC/4-PPY (M-e-cCNC-I-PC/DP/H) and DCC/DBU (M-e-cCNC-I-PC/DU/H) as a function of temperature measured by DLS. Obviously, at a temperature lower than the LCST of polyetheramine M2005,  $D_h$  of M-e-cCNC-I-PC/DD/H and M-e-cCNC-I-PC/DP/H remain constant at about 120 nm, which is 23 nm higher than the value for bare CNCs. However, the hydrodynamic diameter of M-e-cCNC-I-PC/DU/H was close to that of unmodified CNCs.

When the temperature increased above the LCST, the hydrodynamic diameter of M-e-cCNC-I-PC/DD/H and M-e-cCNC-I-PC/DP/H samples increased with nearly the same rate, indicating the formation of aggregates. The rate and the nearly indistinguishable  $D_h$  values at 40 °C showed the almost identical catalytic capacity of these two couples of catalysts. However, no temperature effect was observed for the M-e-cCNC-I-PC/DU/H sample. When the temperature decreased below the LCST, hydrodynamic diameter of M-e-cCNC-I-PC/DD/H and M-e-cCNC-I-PC/DD/H reverted to their initial values indicating a good thermoreversibility.



*Figure V.3. Hydrodynamic diameter as a function of temperature of 0.5 wt. % suspensions of M-e-cCNC-I-PC/DD/H, M-e-cCNC-I-PC/DP/H and M-e-cCNC-I-PC/DU/H. Heating and cooling are indicated by red and blue arrows, respectively.* 

Figure V.4 shows TEM micrographs of M-e-cCNC-I-PC/DD/H, M-e-cCNC-I-PC/DP/H and M-e-cCNC-I-PC/DU/H samples prepared from aqueous suspension kept at 40 °C. The star-shaped assemblies of CNCs could be observed for M-e-cCNC-I-PC/DD/H and M-e-cCNC-I-PC/DP/H samples, which showed the success of the grafting reaction catalyzed

by DCC/DMAP and DCC/4-PPY. Comparable yields of CNCs stars' formation, about 22 % and 25.3 % could be deduced for both samples, indicating a comparable catalytic capacity of these two couples. No complexes could be observed for M-e-cCNC-I-PC/DU/H at 40 °C among the eight analyzed images.



*Figure V.4. TEM images of negatively stained M-e-cCNC-I-PC/DD/H (a), M-e-cCNC-I-PC/DP/H (b)* and *M-e-cCNC-I-PC/DU/H (c) prepared from suspensions at 40* °C.

In conclusion, DLS and TEM results consistently show that the catalyst couples DCC/DMAP and DCC/4-PPY are effective for peptide coupling reaction of polyetheramine at the reducing end of CNCs and that their catalytic capacities are almost identical, whereas the DCC/DBU couple proved to result in the absence of grafting.

#### V.1.3.3 Effect of the solvent

The influence of the solvent was investigated by comparing the reducing end derivatization of CNC-I with the M2005 polyetheramine using the peptide coupling reaction and DCC/DMAP as catalysts in water and in DMF. The choice of DMF was motivated by the easy redispersion of cCNC-I in this solvent that was demonstrated in a previous work. Furthermore, it was shown that the homogeneous grafting of the M2005 polyetheramine onto the whole surface of cCNC-I by peptide coupling yields higher degrees of substitution when the reaction is performed in DMF than in water. This effect was attributed to the fact that, as observed for peptide synthesis (Vollhardt 2014), DMF prevents competition between the solvent and the polymer for the reaction with the CNCs. (Azzam 2016b) Since H<sub>2</sub>O is produced during the PC reaction the use of a non-aqueous solvent tends to move the chemical equilibrium towards the formation of the amide bond.

The reaction in DMF was carried out after the redispersion of Ox-e-cCNC-I in this solvent, which was achieved by repeated dialysis of an initial aqueous suspension in pure DMF. The final water content was negligible. The peptide coupling reaction was subsequently performed without any adjustment of pH at 4 °C.

Figure V.5 shows the evolution with temperature of samples prepared from reactions in water and in DMF. In both cases, at a temperature below the LCST,  $D_h$  is quite constant around 120 nm, which is 23 nm higher than the value for bare CNCs, suggesting a successful modification of the particles.

When the temperature is increased above the LCST,  $D_h$  of both samples increased showing the formation of temperature induced aggregates. However, a steeper increase is observed for the sample prepared in DMF, which also reaches a higher  $D_h$  values at 40 °C than the sample prepared in water. A perfect reversibility accompanied by a hysteresis effect is obtained in both cases.



*Figure V.5. Hydrodynamic diameter as a function of temperature of 0.5 wt. % suspensions of M-e-cCNC-I-PC/DD/H and M-e-cCNC-I-PC/DD/D. Heating and cooling are indicated by red and blue* arrows, respectively.

TEM images obtained from suspensions prepared at 40 °C (Figure V.6) show star-shaped assemblies in the two conditions probed but the estimated complex formation is almost doubled (43 %) when DMF is used instead of water (22 %).



*Figure V.6. TEM images of negatively stained M-e-cCNC-I-PC/DD/H (a) and M-e-cCNC-I-PC/DD/D* (b) prepared from suspensions at 40 °C.

All data consistently show that the reaction yield is drastically improved when DMF is used. In addition to the favorable shift of the chemical equilibrium, it can also be hypothesized that the catalysts used are better dissolved in DMF than in water.

#### V.1.3.4 Influence of the polyetheramine

As shown in the previous sections, the peptide coupling reaction with DCC/DMAP in DMF can lead to a higher grafting yield. Using these conditions, the effect of the type of polyetheramine was considered by comparing the use of the mono-aminated M2005 polymer and the tri-aminated T5000 polyetheramine.

Hydrodynamic diameter of two samples prepared with M2005 (M-e-cCNC-I-PC/DD/D) and T5000 (T-e-cCNC-I-PC/DD/D) were measured as a function of temperature by DLS (Figure V.7). When the temperature was below the LCST (16 °C for M2005 and 12 °C for T5000),  $D_h$  of two suspensions remain constant at about 140 nm, which is higher than bare CNCs. This observation may be caused by the successful grafting of polyetheramines.

When the temperature was increased above the LCST of T5000, the  $D_h$  of the T-e-cCNC-I-PC/DD/D suspension raised very quickly and at 24 °C, the value was about five times higher than the low-temperature value. A temperature-induced aggregation is also observed for the M-e-cCNC-I-PC/DD/D sample when the sample is heated above the LCST of the M2005 chains, but the growth rate is slower than in the T5000 case. This result shows that

both modified CNCs can form temperature-induced aggregates above a temperature onset that can be tuned by the choice of the polymer's LCST. Furthermore, DLS data tend to evidence more pronounced effects when T5000 is used when compared to M2005. Again, thermoreversibility could be confirmed by cooling down the samples and the hydrodynamic diameter recovered its initial low-temperature value.



*Figure V.7. Hydrodynamic diameter as a function of temperature of 0.5 wt. % suspensions of M-e-cCNC-I-PC/DD/D and T-e-cCNC-I-PC/DD/D. Heating and cooling are indicated by arrows.* 

As for the other parameters, TEM observations of samples prepared at 40 °C were carried out (Figure V.8). Clearly, star-shaped CNC assemblies could be found for each sample. However, the formation yield of these complexes was about 53 % when T5000 was used and 43 % when M2005 was considered. Besides, the number of individual nanorods involved in the complexes, *i.e.* the number of branches for each star for these two samples is different. At 40 °C, most stars are composed of 3 to 4 individual nanoparticles in the case of M2005, while in T-e-cCNC-I-PC/DD/D suspensions, star-shapes assemblies with 4, 5 and up to 5 arms can be observed. These results obviously show that the use of polyetheramine T5000 favors the formation of temperature-triggered star-shaped complexes. In the case of the formation of star-like CNC complexes through streptavidin-induced gathering of biotin end-derivatized CNCs, Villares and coworkers (Villares 2018) evidenced that the length of the linker between the biotin moiety and the CNC significantly favors the assembly probability by reducing steric hindrance effects. In the present case, it is not that obvious to invoke a similar effect. Even if the molecular weight of the T5000 polymer is higher than that of the M2005 chains, the T5000 polyetheramine has a three-branches configuration (Figure III.2) that makes its length quite comparable to the one of M2005 polymers. The trivalent character of the T5000 macromolecules could have theoretically made possible the connection during the synthesis step of two or three CNCs, *i.e.* the formation of aggregates containing two or three CNCs attached by their end on each branch of a single T5000 polymer at temperatures below the LCST. However, such duplexes or triplexes were never observed at low temperature and the high temperature structures observed can therefore not be the result of association of several of duplexes.



*Figure V.8. TEM images of negatively stained M-e-cCNC-I-PC/DD/D (a) and T-e-cCNC-I-PC/DD/D* (b) prepared from suspensions at 40 °C.

#### V.1.3.5 Influence of the aspect ratio

The question of possible steric hindrance effects on the formation of star-like complexes was raised by the comparison between T5000 and M2005 end-modified CNC-I. To further investigate such effects, comparisons were made using CNCs of various dimensions and aspect ratio. Namely, in addition to cotton CNC-I considered so far, wood CNC-I provided by the Melodea (wCNC-I) company and lab-made tunicate CNC-I (tCNC-I) were used as alternative CNCs. wCNCs were already presented in Chapter II but we recall here their basic morphological characteristics. wCNC-I nanoparticles are different with cotton CNCs. based on the analysis of 20 TEM images containing a total of about 500 particles, it can be concluded that most of wCNC-I are composed of only one or two elementary subunits, in contrast with cCNC-I consisting in bundles of 3-4 laterally associated subunits. With a length between 200

and 400 nm and a width below 15 nm, wCNC-I are both longer and thinner than cCNC-I and consequently possess a larger aspect ratio of about 20.

Figure V.9 shows Ox-e-wCNC-I prepared from aqueous suspensions kept at 4 and 40 °C. The images show that these particles are very similar to initial unmodified wCNC-I and no significant aggregates were observed, indicating that no morphological effect was caused by the chlorite oxidation and that the particles were insensitive to temperature.



*Figure V.9. TEM images of negatively stained wCNC-I* (a) *Ox-e-wCNC-I prepared from suspensions at* 4 (b) and 40  $^{\circ}$ C (c).

TEM images of T5000 polymer end-grafted wCNC-I (T-e-wCNC-I-PC/DD/D) obtained using optimized conditions (peptide coupling in DMF using DCC/DMAP as catalysts) are shown in Figure V.10. When the preparation temperature was 4 °C, these nanorods appeared as individual bare wCNC-I. Here both repulsive electrostatic and steric forces add to ensure colloidal stability and particles separation. When the temperature reached 40 °C, star-shaped assemblies with the same characteristics as the one obtained with cCNC-I were observed. However, using the larger aspect ratio wCNCs the star-like assemblies formation yield decreased to about 12.7 %, compared to about 54 % for cCNC-I. Consequently, a primitive conclusion is that a larger aspect ratio drastically reduces the formation of assemblies as the molecular collision probability decreases with aspect ratio.



*Figure V.10. TEM images of negatively stained T-e-wCNC-I-PC/DD/D prepared from suspensions at 4* (a) and 40  $^{\circ}$ C (b).

In order to further confirm this influence, the same modification was carried using tunicate CNCs that were prepared as described in the experimental section part by mechanical treatment followed by acid hydrolysis. The resulting suspension was used directly and submitted to the two step-functionalization procedure to graft T5000 polymer at their reducing end (peptide coupling in DMF using DCC/DMAP as catalysts). Figure V.11 shows the TEM images of as-prepared tCNC-I and Ox-e-tCNC-I prepared from suspensions at 4 °C and 40 °C. Clearly, tunicate CNCs whose length is between 1 and 4  $\mu$ m and width is between 20 and 30 nm are much longer than Melodea CNCs and cotton CNCs. Besides, most tCNC-I were composed of only 1 or 2 elementary crystallites. The observation showed a much larger aspect ratio compared to the other two cellulose nanocrystals, which can be estimated to about 110.



*Figure V.11. TEM images of negatively stained tCNC-I* (a) *Ox-e-tCNC-I prepared from suspensions at* 4 (b) and 40  $^{\circ}$ C (c).

Images of Ox-e-tCNC-I nanoparticles shown in Figure V.11 reveal that these nanocrystals are almost identical to as-prepared tCNC-I, indicating that no morphological effect was observed after chlorite oxidation reaction. When the temperature increased to 40 °C (Figure V.11c), these nano-objects were similar to the low-temperature sample and no significant aggregation was noticed. Consequently, temperature had no effect on Ox-e-tCNC-I nanoparticles.

The TEM micrograph of T-e-tCNC-I-PC/DD/D sample prepared from suspensions kept at 4 °C is shown in Figure V.12a. These polymer grafted tCNC-I cannot be distinguished from as prepared tCNC-I.

As shown in Figure V.12b, only one CNC star could be observed from the same sample prepared at 40 °C giving a yield of stars' formation of about 3 %. These CNCs self-organized in a relatively huge star hooked via their ends by attractive forces between end-grafted polyetheramine T5000 in bad solvent conditions. Interestingly, a close to 120 ° angle between the branches is observed, which is attributed to repulsions between the negatively-charged lateral sides of the tCNC-I. It has to be noted that no stars could be observed in other samples using M2005 polyetheramine or using water as a solvent. This result confirmed one more time the optimized process conditions.



*Figure V.12. TEM images of negatively stained T-e-tCNC-I-PC/DD/D prepared from suspensions at 4* (a) and 40  $^{\circ}$ C (b).

#### V.1.4 Conclusion



*Figure V.13.* Yield of star-shaped complexes formation defined as the number of CNCs belonging to complexes divided by the total number of CNCs. Each studied parameter is represented by a given color (e.g. green for the catalysts). Bars with two or three colors are compared twice or three times with others. See text for details about the analysis procedure.

Figure V.13 summarizes the effect of the different parameters probed to optimize the grafting of polyetheramines at the reducing end of CNC-I. Even though a complete metrical comparison was not undertaken and despite the semiquantitative and not fully quantitative character of the approach, the combination of DLS measurements and TEM observations allowed us to identify optimized conditions. All parameters investigated have a crucial role on the ability of the derivatized CNCs to form star-like assemblies, with the exception of the polyetheramine type that only induces rather small changes. An optimum yield of about 54 % was reached when grafting Jeffamine<sup>®</sup> polyetheramine T5000 onto the end of cCNC-I using peptide coupling in DMF and the catalysts DCC/DMAP or DCC/4-PPY.

## V.2 NMMO treatment of cCNC-I to improve the accessibility and reactivity of their reducing end

#### **V.2.1 Introduction**

Discovered by Johnson (Johnson 1966), NMMO (Figure V.14) is a direct solvent of cellulose that is industrially used in the so-called lyocell process as an environmentally friendly treatment for fiber making (Adorjan 2004). This polar aprotic solvent is thought to behave somewhat similar to ionic liquids. More specifically, it is believed that the high polarity of N-O bonds in NMMO causes the hydrogen bond network in cellulose to break and form new stronger hydrogen bonds between the cellulosic hydroxyl groups and the N-O groups (Igarashi 2007), allowing cellulose to dissolve.



#### Figure V.14. Chemical structure of NMMO.

So far, most of the reported works focused on the treatment of cellulose fibers by NMMO and their applications. To our knowledge, only one paper (Zhang 2020) has reported the use of NMMO to modify cellulose nanocrystals photonic films. In this work, CNC films were swollen by NMMO, resulting in red-shifted reflected colors (Figure V.15). The swelling effect is supposed to come from NMMO permeation into the crystalline regions of individual CNCs and intercalating in between CNC particles. When NMMO was removed upon rinsing with water and drying, the reflected colors of CNC films blue shifted because of the reduced helical pitches. Interestingly, NMMO-treated CNC films could rapidly capture/release water molecules in air, resulting in responsive coloration due to expansion/contraction of the helical pitch in the chiral nematic structure.



**Figure V.15.** (a) Photographs and polarized optical microscopy images where all the scale bars are the same as shown in CNC films swelled in NMMO without further washing and drying. (b) UV-Vis reflection spectra and circular dichroism spectra of CNC film and  $N_x$ -CNC films (x = wt. % of NMMO) (Figure extracted from Zhang 2020).

The swelling of cellulose fibers by NMMO treatment was investigated in the 1980s by Chanzy and coworkers (Chanzy 1984). The water content in NMMO was found to play a crucial role and a phase diagram of the NMMO-H<sub>2</sub>O system showing the swelling and dissolution regions as a function of temperature and water content was established (Chanzy 1982). The behavior of NMMO/water/cellulose system was further investigated and a complete ternary phase diagram was set up (Eckelt 2009).

Figure V.16 shows the phase diagram of cellulose in NMMO as a function of temperature and water content resulting from the work of Chanzy *et al.* (Chanzy 1984). Three characteristic zones have been delimited by A, B and C, respectively corresponding to conditions resulting in dissolution, swelling and no effect.



*Figure V.16.* Phase diagram of NMMO-cellulose as a function of water content and temperature. Zone *A*: dissolution zone. Zone *B*: swelling zone. Zone *C*: no deformation effect (Figure extracted from Chanzy 1982).

As aforementioned and emphasized in the recent reviews, the reducing ends in a CNC only account for a very small fraction of the order of a few percent of the total number of AGUs, making their chemical modification both difficult to achieve and characterize. Especially, the proximity of the reactive aldehyde groups concentrated at the CNC ends prevents to reach high functionalization yields with bulky molecules for steric hindrance reasons. In this sense, it was shown by Villares *et al.* (Villares 2018) that a linker of a certain length between the biotin moiety and the end of the nanocrystal was necessary for the supramolecular assembly of several cellulose nanocrystals by streptavidin. To circumvent this issue, Henri Chanzy suggested us to use NMMO to achieve a partial dissolution of the CNC-I that should preferentially take place at the end of the rod, as suggested by results showing the swelling of *Valonia* cellulose microfibrils and ramie cellulose fiber from one end under the treatment with NMMO at 80 °C (Figure V.17).



*Figure V.17.* Swelling of Valonia cellulose microfibrils (*left*) before (*a*) and after (*b*-*d*) the treatment of NMMO monohydrate at 80 °C. Swelling of ramie cellulose fiber (*right*) from one end under the treatment of NMMO monohydrate at 80 °C (*Figure extracted from Noe 2008 and Chanzy 1984*).

The idea was therefore to induce a local swelling of CNC-I at their reducing ends to obtain sea anemone-like particles comprising swollen reducing ends while the rest of the CNC would keep its structural integrity. The swollen reducing ends should greatly favor the reaction with bulky groups. This section is therefore dedicated to the investigation of NMMO treatments on CNC-I to achieve such a modification. Practically, CNC-I were subjected to treatments with NMMO in different regions of the phase diagram in order to identify the conditions for partial swelling of the particles.

#### V.2.2 Materials and methods

#### V.2.2.1 Materials

Cotton linters were provided by Buckeye Cellulose Corporation and used as the cellulose source without any further purification. N-methylmorpholine N-oxide monohydrate (NMMO•H<sub>2</sub>O, Sigma-Aldrich) was recrystallized in dry acetone. Deionized water was used in all experiments. Other chemicals were purchased from Sigma-Aldrich and used as received.

The description of the preparation of cCNC-I is given in Chapter II.2.2.

V.2.2.2 Recrystallization of 4-methylmorpholine 4-oxide monohydrate (NMMO•H<sub>2</sub>O)

Following the protocol of recrystallization of NMMO•H<sub>2</sub>O described by Chanzy *et al.* (Chanzy 1982), purchased NMMO was dissolved in anhydrous acetone at 90 °C in a flask surmounted by a condenser. For 1 g of NMMO, 2.5 mL of acetone were used. The mixture was heated until complete dissolution of the solid sample. Afterward, the hot solution was rapidly transferred into a beaker and then placed in an oven at 60 °C to evaporate the acetone. The resulting recrystallized NMMO was put into a plastic reagent bottle and sealed with a parafilm.

V.2.2.3 Freeze-drying of cCNC-I

The pH of a 2 wt. % cCNC-I suspension containing 10 g dry cCNC-I was adjusted to 7 by addition of a NaOH solution after which the suspension was transferred into a 1 L flask and freeze-dried.

V.2.2.4 NMMO•H<sub>2</sub>O treatment of cCNC-I

The monohydrate form of NMMO (NMMO•H<sub>2</sub>O) was obtained from the recrystallization procedure described above. The corresponding molar fraction of H<sub>2</sub>O of such a solvent is about 13 %. By accurate addition of water, NMMO solvents of increasing H<sub>2</sub>O molar fractions from 14 to 36 % were prepared. Freeze-dried cCNC-I (0.1 g) were added into 4 g of each of the prepared NMMO solvents. The samples were heated at 90 °C in a flask surmounted by a condenser for 30 min after which the resulting suspension was diluted with water, cooled and washed by repeated centrifugations and finally redispersed in deionized water (Zhang 2020). The resulting aqueous suspension (about 0.5 wt. %) that were treated with the different NMMO solvents will be referred to as CNC-NMMO-water molar fraction in % (e.g. CNC-NMMO-28 for a cCNC-I suspension treated with the NMMO solvent containing 28 % H<sub>2</sub>O molar fraction).

#### V.2.3 Results and discussion

#### V.2.3.1 Swelling of CNCs end by NMMO treatment

As shown in Figure V.16, the water content plays an important role in treatments with NMMO. In order to confirm the water content of as prepared recrystallized NMMO, DSC was used to measure the melting point. Figure V.18 shows the heating thermogram of recrystallized NMMO with a scan speed of 5 °C/min. Clearly, this curve shows a single melting endotherm at a peak temperature of 74 °C, which is close to the value from literature (78 °C) for NMMO monohydrate. Consequently, here the recrystallized NMMO was considered as NMMO monohydrate.



Figure V.18. Heating thermogram of recrystallized NMMO with a scan speed of 5 °C/min.



*Figure V.19.* Conditions used for the preparation of the different samples on the phase diagram of NMMO-cellulose as a function of water content and temperature (Figure adapted from Chanzy 1984).

To take into account water content errors caused by solvent preparation or heating, the NMMO treatments were carried out every 1 mol. % of  $H_2O$  from 13 to 25 mol. %, a range that covers the three zones in the phase diagram including the swelling region (Figure V.19). It is expected that samples corresponding to violet, red and orange points respectively belong to the dissolution, swelling and no effect zones.

Figure V.20 shows the hydrodynamic diameter,  $D_h$ , of as-prepared cCNC-I and the different NMMO-treated samples as a function of the water content. As-prepared particles obtained from redispersion of freeze-dried cCNC-I had a  $D_h$  of 97 nm. For CNC-NMMO samples whose water content is in the 13- 16 mol. % range, no size data could be measured by DLS suggesting that the samples may no longer exist as a suspension containing cellulose particles but as a solution containing dissolved cellulose. For samples treated by NMMO with a water content from 17 to 25 mol. %, no significant difference was observed compared with as-prepared cCNC-I.



**Figure V.20.** Hydrodynamic diameter of untreated cCNC-I (blue line) and different NMMO-treated cCNC-I samples with different water contents in NMMO solution. Color coding refers to sample preparation conditions shown in Figure V.19.

According to the phase diagram in Figure V.19, the dissolution region should extend until 19 mol % water content. However, Figure V.20 suggests that particles are present from a value of the water content of 17 mol. % onwards. The difference in the melting point temperature corresponding to NMMO monohydrate and that of our recrystallized solvent might explain this observation. For a hydrated compound, the melting point is lower when more water is combined. In our case, the measured melting point of 74 °C, which is lower than reported value of 78 °C, indicated that our NMMO is not exactly monohydrate. As a result, the calculated water content of 17 % may be 19 % in reality, which is the lower limit of the swelling zone. One hypothesis to explain a more or less constant hydrodynamic diameter for samples treated with NMMO with water contents in the 17-25 mol.% range is that the treatments had no effect or only a minor effect, which does not alter the diffusion coefficient of the particles.

To further characterize these samples, small-angle neutron scattering (SANS) experiments were carried out. Figure V.21 shows the SANS spectra of untreated and NMMO-treated cCNC-I suspensions in D<sub>2</sub>O. All NMMO-treated samples gave the same scattering signal, which also superimposes the signal from the untreated sample for Q> $5.10^{-3}$  Å<sup>-1</sup>. The only difference between treated and untreated samples is in the very low-Q region

where treated samples scatter more than the as-prepared one. This last feature could be attributed to the presence of a small fraction aggregated CNCs in the treated samples that were formed during the sample preparation process.

All samples exhibit a  $Q^{-4}$  decay in the high-Q range, which corresponds to a sharp interface between the crystalline rods and the solvent. If swelling had occurred and resulted in the presence of swollen chains, the corresponding signal would have appeared in the high-Q region as a  $Q^{-2}$  or  $Q^{-1.7}$  decay, as was for example the case for polyetheramines grafted at the reducing end of CNCs (see Chapter II). The absence of such a scattering signal and the relatively little difference with as-prepared cCNC-I spectrum suggests that the NMMO treatments probed here had no or very little effect on the cCNC-I.



*Figure V.21.* SANS spectra of untreated cCNC-I and different NMMO-treated cCNC-I samples with different water contents in NMMO solution. The concentration of all samples is 1 wt. %.

In order to investigate these samples more intuitively, transmission electron microscopy (TEM) was used to obtain more morphological information.

**Chapter V** - Strategies towards the optimization of polymer grafting onto the reducing end of native cellulose nanocrystals



*Figure V.22. TEM images freeze-dried cCNC-I and NMMO-treatment cCNC-I samples with different water contents in NMMO solution. The number in the upper left corner is the water content (mol. %).* 

TEM micrographs of as-prepared cCNC-I and samples treated by NMMO whose water content was from 13 to 25 mol. % are shown in Figure V.22. Freeze-dried redispersed cCNC-I are very similar to the initial cCNC-I and appear as individual particles. The electrostatic repulsive forces provided by the sulfate groups on the surface of the nanocrystals contribute to the good dispersion of CNCs. For samples treated with NMMO with a water content between 13 and 16 mol. %, no cellulose nanocrystals could be seen in the TEM images. This result clearly shows that dissolution occurred, in fair agreement with the phase diagram and the DLS measurements. Samples treated with NMMO containing higher H<sub>2</sub>O molar ratio in the range 17-25 mol. % exhibit a morphology that is identical to the one of as-prepared and freeze-dried and redispersed cCNC-I. No obvious effect on the morphology could be detected, even for 17, 18 and 19 mol. % ratio that should correspond to the swelling conditions.

Treatments of cCNC-I with NMMO containing low amounts of water in the 13-16 mol. % range successfully resulted in the dissolution of the sample, in agreement with the phase diagram (Chanzy 1982). However, the structural investigation tools used here (DLS, SANS and TEM) did not provide any evidence of morphological changes upon treatments with NMMO-xH<sub>2</sub>O with x in the 17-25 mol. % range even if conditions for swelling should have been reached at some point (*i.e.* just after the dissolution region, *i.e.* for  $16 < H_2O$  mol. % < 20). Such a result indicates that the treatment conditions used were not suitable for an efficient swelling or that the changes were not preserved when NMMO was removed and the CNCs redispersed in water.

#### V.2.3.2 Separation of crystallites of cCNC-I by NMMO treatment

In second series of experiments, new samples corresponding to 26, 27, 28, 29, 30, 32, 34 and 36 mol. % water contents were prepared as described in the methods part and freeze-dried for FTIR, NMR and WAXS experiments. Never-dried samples were used for DLS,  $\zeta$ -potential measurements and TEM observations.

In order to have a global view of nanoparticles size after NMMO treatment, the hydrodynamic diameter of 0.1 wt. % non-treated cCNC-I and treated cCNC-I was measured by DLS. Figure V.23 shows the variation tendency in which the blue line corresponds to the  $D_h$  170

value of non-treated cCNC-I at 97 nm. From 26 to 30 mol. % H<sub>2</sub>O, the value of  $D_h$  decreased but subsequently increased until it recovered its initial value for the sample treated with NMMO containing 34 mol. % H<sub>2</sub>O. Such a result shows that the NMMO treatment had an effect which increased the diffusion coefficient of the nanoparticles, thus decreased the hydrodynamic diameter in the range of water content from 25 to 30 mol. %. Furthermore, water content plays an important role in this effect. The increase of water content first reinforced then weakened the phenomenon.



*Figure V.23. Hydrodynamic diameter (black dot) and*  $\zeta$ *-potential (red square) of untreated CNCs (blue line) and NMMO treated CNCs samples as a function of water contents in NMMO solution.* 

 $\zeta$ -potential of these nine samples was measured and results are shown in Figure V.23 in which the blue line represents the value for non-treated cCNC-I. The  $\zeta$ -potential values moderately increased when the water content increased from 25 to 30 mol. % and then decreased for higher water contents. 30 mol. % corresponds to the lowest absolute  $\zeta$ -potential value and therefore seems to be a critical value. Higher absolute  $\zeta$ -potential values were measured when the water content was strictly higher than 30 mol. % when compared to the untreated sample.

Fourier-transformed infrared spectroscopy (FTIR) allows to observe the characteristic
functional groups of cellulose. In addition, it is a fast method and consumes little sample quantity. Here, attenuated total reflection (ATR) as a sampling technique used in conjunction with FTIR enables samples to be examined directly in the solid or liquid state without further preparation.

FTIR spectra of cCNC-I treated by NMMO whose water contents were 28, 29, 30, 32 and 34 mol. % were superimposed with non-treated cCNC-I as shown in Figure V.24. All of the characteristic peaks of cotton cellulose nanocrystals could be observed for all samples. Overall, no significant difference was detected except that for example, between 3000 and 3600 cm<sup>-1</sup>, the absorbance intensity of CNC-NMMO-30 was a little lower than other samples and at about 2900 cm<sup>-1</sup> as well.



Figure V.24. FTIR spectra of untreated cCNC-I and CNC-NMMO samples with different water contents.

Figure V.25 shows superimposed normalized spectra of CNC-NMMO treated by NMMO whose water content were 28, 29, 30, 32 and 34 mol. % with non-treated cCNC-I. In order to clearly show the differences, the spectra were separated into four parts which show the carbons C1, C4 and C6, respectively. As the curves were normalized, the peak area represents the atom quantity. From these spectra, the iconic peaks which represent C1, C4 and C6 could be observed for all NMMO-treated CNC samples. No significant difference of chemical shift was detected. However, when increasing the water content from 28 to 30 mol. %, a small effect of peak 172

refining is observed. A broadening and return to the untreated sample situation are seen from 30 to 34 mol. %.

FTIR and CP/MAS NMR spectra evidence that the CNC-NMMO-30 exhibits the greatest difference compared with the non-treated sample. These two samples were then characterized by X-ray diffraction scattering measurements (Figure V.26) Non-treated cCNC-I displayed a typical spectrum of cotton cellulose nanocrystals (French 2014). Compared with non-treated cCNC-I, the spectrum of CNC-NMMO-30 was very similar and no significant difference was detected.



*Figure V.25. CP/MAS NMR spectra of non-treated cCNC-I and CNC-NMMO samples with different water contents (upper left); The spectra in detail for C1 (upper right), C4 (lower left) and C6 (lower right).* 



Figure V.26. WAXS spectra of untreated cCNC-I and CNC-NMMO-30.

In order to further investigate the effect provided by NMMO treatment, TEM was used to observe the samples (Figure V.27). Non-treated cCNC-I and the treated samples whose water content of NMMO was 26, 27, 28, 29, 30, 32, 34 and 36 mol. % were prepared from aqueous suspensions at room temperature.

Non-treated cCNC-I prepared by sulfuric acid hydrolysis have a length between 100 and 300 nm and a width between 10 and 30 nm, which is consistent with literature reports (Elazzouzi-Hafraoui 2008). The negative staining clearly reveals that most particles are in fact fascicles of three or more than three parallel elementary subunits, *i.e.* crystallites. The interparticle distance indicates a significant electrostatic repulsion due to the negative charge of sulfate groups at the surface of the CNCs, resulting from the sulfuric acid hydrolysis.

When the water content of NMMO increased from 26 to 30 mol. %, by visual observation, more and more nanocrystals composed of two or only one crystallite could be observed. Nevertheless, further increase of water content did not amplify the phenomenon but, contrariwise, more and more nanorods composed of three or even more crystallites are seen. Such a tunable result corresponds to what we have observed from DLS and  $\zeta$ -potential experiments. Perceptibly, NMMO treatment had a tunable effect of separation of crystallites and the water content of 30 mol. % could maximize the effect.



**Figure V.27.** TEM images of negatively stained freeze-dried redispersed cCNC-I and NMMO treated CNCs samples with different water contents in NMMO solution. The number in upper left corner is the water content.

A statistical study was performed by counting for each sample the number of crystallites in one CNC from about 700 nanocrystals, yielding the histogram shown in Figure V.28. Clearly, when the water content increased from 26 to 30 mol. %, the percentage cCNC-I comprising 3 elementary crystallites decreased from about 40 to 9 % while the percentage of CNCs made of a single crystallite drastically increased from about 3 to 73 %. At the same time, the number of nanocrystals composed of more than three crystallites also strongly decreased or even went to zero. These quantitative data distinctly show an effect of separation of crystallites of cotton cellulose nanocrystals by NMMO treatment.

A mirror effect was detected the water content was increased from 30 to 36 mol. %: the percentage of single crystallites decreased from 73 back to about 6 %, while for objects made up of three crystallites, the value increased from 9 up to about 35 %. Simultaneously, the number of nanocrystals composed of more than 3 crystallites slowly rose again. The situation at 36 mol. % is close to the one for untreated samples (Figure V.29).

All the results above showed a significant separation of elementary crystallites of cotton cellulose nanocrystals by NMMO treatment. In fact, cCNC-I and their crystallites can be observed by negatively stained TEM images since the dye can penetrate into the crevices between crystallites, which are linked by hydrogen bonds. Consequently, one hypothesis is that even if 30 mol. % of water is out of the dissolution or swelling region in the phase diagram it is probable that such a solvent is able to penetrate between crystallites, cut off hydrogen bonds and release confined water molecules. Further work is needed to investigate in greater details the mechanism behind this interesting effect.

**Chapter V** - Strategies towards the optimization of polymer grafting onto the reducing end of native cellulose nanocrystals



*Figure V.28. Histograms of percentage of different numbers of crystallites for untreated cCNC-I and NMMO treated CNCs samples with different water content in NMMO solution.* 



*Figure V.29. Histogram of percentage of the crystals composed of 3 crystallites (a) and 1 crystallite (b) with different water content in NMMO solution.* 

# V.3 Conclusion

The optimization of the regioselective grafting of polyetheramines at the reducing end of CNC-I was investigated. Optimal conditions allowing more than half of the modified CNC-I to assemble as star complexes when the temperature is elevated beyond the LCST were defined. These conditions (use of peptide coupling in DMF with the catalyst couple DCC/DMAP or DCC/4-PPY on low form factor CNC-I) promote the chemical reaction and limit the steric hindrance when end-functionalized CNC-I gather into star-like complexes.

NMMO solutions with different water contents were used to treat cotton CNC-I. The expected swelling of the reducing end of the nanocrystals when samples were exposed to treatment conditions likely to generate this effect could not be detected. However, during a further study, we could evidence that treatments of the cCNC-I from cotton in an NMMO solution with a water content equal to 30 mol. % at 90 °C induces a strong morphological effect. As shown by different techniques, such a treatment indeed resulted in the disassembling of the initial composite cCNC-I structure made of the lateral assembly of a few elementary crystallites into cCNC-I mainly consisting in single crystallites. The underlying mechanism is still not clear and further investigations are needed.

General conclusion and perspectives

## **Conclusions**

This Ph.D. work has focused on regioselective modifications of cellulose nanocrystals, regrouping in that terminology both the widely-studied native cellulose nanocrystals and the developing cellulose II nanocrystals. These modifications aimed, on the one hand, at identifying the reducing end of different types of cellulose nanocrystals and optimize a regioselective labeling method, which is a prerequisite to fully benefit from synergistic properties of the resulting hybrid particles. On the other hand, the derivatizations aimed at providing temperature-responsiveness to control the interactions between the nanoparticles and form innovative assemblies. Throughout this project, a constant concern was the investigation of the interaction-structure-property relationships.

In the course of this project, *in situ* growth was developed as an optimized regioselective labeling method of the two types of CNCs with gold nanoparticles that is characterized by an unprecedented high functionalization yield. This optimized labeling strategy allowed us to have a deeper look at the morphology of CNCs. The high magnification TEM images evidenced that the laterally assembled elementary crystallites, which make up native cotton CNCs, are arranged in a parallel way, indicating a single side location of all reducing ends. Such an organization shows that no lateral separation of preexisting assemblies of parallel microfibrils has occurred during the acid hydrolysis to fabricate cellulose nanocrystals. A dumbbell structure was formed with one AuNP attached at each end of cCNC-II, which confirmed the antiparallel arrangement of cellulose chains in cellulose II nanocrystals.

Then, we presented the grafting of thermoresponsive polyetheramines at the reducing ends of native cotton cellulose nanocrystals. These end-grafted cCNC-I can associate by their modified ends when the temperature passes the lower critical solution temperature of the grafted thermoresponsive polymers. The thermoreversibility and reproducibility of the association/dissociation of end-grafted CNCs was confirmed by DLS measurements. Furthermore, SANS data demonstrated the success of the grafting and also showed that the attached chains had an extended conformation. Especially, remarkable star-shaped assemblies of CNCs were observed from TEM images. The rheological study on these end-derivatized CNCs showed a cyclic variation of the viscosity, storage and loss moduli with the temperature. At T > LCST, a soft elastic gel is obtained and attributed to a limited number of connections between star-like complexes.

The same modification strategy was applied on cCNC-II by taking advantage of the existence of reducing groups at both ends. The derivatization was successfully achieved and these end-grafted cCNC-II became thermosensitive. While keeping the reversibility observed with CNC-I, an end-to-end association of the derivatized CNC-II occurred at T>LCST, which led to the dramatic formation of a supra-micron CNC network, as observed from TEM images. The rheological measurements revealed a significant variation of macroscopic properties. Compared to the case of the homogeneous surface grafting or the end-grafting of CNC-I with thermosensitive polymers, the modification of cCNC-II gives a network that behaves as a strong elastic gel with a high tan  $\delta$  about 30. Such a remarkable thermoreversible gel is totally provided by the grafting of thermosensitive polyetheramine at reducing ends, which gives extensive research value and application prospects.

This manuscript provides the first example of a systematic comparison of the chemospecific properties of CNC-I and CNC-II. In contrast with cCNC-I, which are Janus nanoparticles possessing a chemical polarity, cCNC-II are shorter but chemically symmetric nanorods due to the presence of reducing ends at both extremities. However, in both cases, the surface corresponding to the end(s) of the nanocrystals only represent few percent of the total surface area of CNCs. Therefore, current characterization tools, such as spectroscopy, often do not have sufficient sensitivity to detect chemical modifications at their reducing end(s). In this project, a combination of different techniques of characterization was used and played an important role during the investigation of the newly synthesized biosourced nanoparticles and assemblies. Although FTIR or CP/MAS NMR could not give sufficient information to prove the success of end modifications, the scattering methods such as DLS, SANS or SAXS could provide indirect evidences. Additionally, TEM is always a good intuitive tool to observe the samples. However, when artefacts from drying are suspected to become an interference factor, the scattering methods and also the rheological measurements, which are adapted to a wide range of concentration, can give supplementary information. Accordingly, the difficult stage of 182

characterization can be overcome by combining multiple manners and integrated analysis.

Some benefits of using CNC-I instead of CNC-II were highlighted in this work. In fact, one more reducing end in cCNC-II does not only supply one more reactive site, but provides new strategies, which can lead to the design complex structures. These architectures, in some cases, can generate greater differences in macroscopic properties when compared to CNC-I. In our case, the introduction of thermosensitive polymers made possible to build thermosensitive asymmetric or symmetric cellulose nanocrystals. Such different structures could associate into star-shaped assemblies or network by the precisely controlled temperature. These organizations thus resulted in new rheological properties and maybe more new characteristics to investigated. The regioselective chemical modifications can introduce molecules, polymer chains or functional motifs with their intrinsic properties, such as thermal, magnetic, optical, mechanical or biological features, to cellulose nanocrystals. A Janus structure for CNC-I or a symmetric structure for CNC-II is therefore constructed. New properties then naturally arise or are provided through precise modifications. These properties will provide again new ideas of regiospecific derivatizations at reducing end(s) of cellulose nanocrystals, which keep the cycle going.

In our case, all of the challenges and results pushed us to find a way in order to increase the accessibility and optimize the reactivity of reducing ends. Thus, two different pathways were carried out. First, from the point of view of the chemical reaction, several physicochemical parameters were studied and optimized conditions then came to the surface. Second, a treatment of cCNC-I by NMMO was investigated with the intention to favor the accessibility of the reducing end groups through partial swelling. Even though the swelling phenomenon was not discovered, the separation of elementary crystallites highlighted a new route and gave novel research topics.

As a general conclusion, as the modifications undertaken concern a very small fraction of the available anhydroglucose units, a direct quantitative characterization of the regioselective modification of CNCs remains difficult, even if the use of advanced techniques such as diffusion methods gives fruitful information. However, the work carried out shows that such localized functionalization, associated with the use of biosourced particles, allows a fine control of the assembly into innovative structures that gives rise to new macroscopic properties, as demonstrated by the preparation different elastic gels, strong or weak. In addition, some of the results obtained have led to advances in the knowledge of certain fundamental morphological or chemical properties of CNCs.

#### **Perspectives**

In view of these results, a series of perspectives can be envisaged.

The optimized regioselective labeling strategy gives a pathway to the grafting of other sulfur-sensitive metal or magnetic nanoparticles and the use of other types of cellulose nanocrystals from different sources. The assemblies in suspension and new materials including these hybrid particles could benefit from synergies between the intrinsic properties of CNCs and those of the attached nanoparticles. In particular, the grafting of magnetic nanoparticles and the use of magnetic fields could lead to aligned or custom oriented structures.

In this work, gold-labeled CNCs were characterized as dilute suspensions. A study of more concentrated media, in particular reaching the liquid crystal phase formation conditions of these gold-tagged CNCs would be particularly interesting. Indeed, novel effects resulting from the combination of the optical properties of liquid-crystal CNC phases and the plasmon resonance properties of gold particles could be obtained.

Several avenues for more advanced optimization of regioselective grafting of polymers on CNCs are possible: use of microwaves or ultrasound, switching from the strategy of grafting onto to grafting from, use of enzymes, etc...

The study of the influence of different physico-chemical parameters (concentration, ionic strength, etc.) on the organization and properties of star-shaped or network assemblies is worth investigating. Moreover, as revealed by the literature review, the study of the properties of bare or modified CNC-II has not yet been much exploited, which will be a vast field of exploration.

Appendix

## **Appendix I Characterization techniques**

## A.I.1 Wide-angle X-ray scattering (WAXS)

Lyophilized CNC powders were pressed into a thin film and X-rayed in a Warhus vacuum chamber using a Philips PW3830 generator operating at 30 kV and 20 mA (Ni-filtered CuK $\alpha$  radiation,  $\lambda = 0.1542$  nm). Two-dimensional diffraction patterns were recorded on Fujifilm imaging plates, read offline with a Fujifilm BAS 1800-II bioimaging analyzer. Profiles were calculated by rotational averaging of the 2D patterns.

## A.I.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of cCNC-I and cCNC-II were recorded on a Perkin-Elmer spectrum 65 (PerkinElmer, USA) between 400 and 4000 cm<sup>-1</sup> with a resolution of 1 cm<sup>-1</sup> and 16 scans were acquired. Attenuated total reflectance (ATR) mode was used on all samples and baseline correction was applied. The spectra were normalized at 1160 cm<sup>-1</sup>.

## A.I.3 Solid-state <sup>13</sup>C nuclear magnetic resonance spectroscopy (CP/MAS NMR)

Solid-state <sup>13</sup>C NMR spectra were recorded with a Bruker Avance DSX 400 MHz spectrometer, in cross-polarization and magic angle spinning conditions (CP-MAS). The <sup>13</sup>C radio frequency field 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 2000 of scans were integrated with a contact time of 2 ms using a ramp CP protocol and a recycle delay of 2 s. The acquisition time was 35 ms and the sweep width set at 29400 Hz. The chemical shifts were calibrated with respect to the carbonyl peak of glycine (176.03 ppm). The spectra were normalized with cellulose C1 peak at 100 to 110 ppm.

## A.I.4 Ultraviolet-visible spectroscopy (UV-Vis)

The measurements of suspensions of gold nanoparticles or AuNP-e-CNCs were performed on a CARY 50 spectrophotometer using 0.5 wt. % samples and the wavelength scan

mode (from 400 to 800 nm) at 25 °C. The measurements of Jeffamine<sup>®</sup> polyetheramine T5000 were performed on a CARY 50 spectrophotometer at 560 nm using temperature scan mode from 5 to 42 °C.

## A.I.5 Dynamic light scattering (DLS)

DLS experiments were carried out with a Malvern NanoZS instrument. All measurements were made at a well-controlled ( $\pm 0.05$  °C) temperature with a 632.8 nm laser at a backscattering detection angle of 173°. The intensity size distribution was obtained from the analysis of the correlation function using the multiple narrow mode algorithm of the Malvern DTS software. Five measurements were done and averaged to yield the reported value.

## A.I.6 Transmission electron microscopy (TEM)

Drops of about 0.001 wt. % CNC aqueous suspensions were deposited onto carboncoated TEM grids freshly glow-discharged in an easiGlow plasma cleaning system (Pelco). After 2 min, the liquid in excess was blotted away with a filter paper and, prior to drying, a drop of a 2 wt. % uranyl acetate aqueous solution was deposited onto the specimen. After 2 min, the stain in excess was wicked off, and the remaining thin liquid film allowed to dry. The specimens were observed either with a Philips CM200 'Cryo' or a JEOL JEM 2100-Plus microscope operated at 200 kV. The images were recorded with TVIPS F216 TemCam and Gatan Rio 16 digital cameras, respectively. After labeling with AuNPs, a statistical analysis of the labeling yield was performed using the ImageJ software based on the observation of a statistically relevant number of objects (between 200 and 600).

#### A.I.7 ζ-potential

The  $\zeta$ -potential of CNCs was measured from 0.1 wt. % suspensions by electrophoresis coupled with laser Doppler velocimetry using a Malvern NanoZS instrument. Samples contained 10 mM NaCl. Data were averaged over 3 measurements, each measurement comprising 10 subruns.

## A.I.8 Small-angle X-ray scattering (SAXS)

Samples were measured using a Xeuss 3.0 apparatus (SAXS Lab, Saclay, France) equipped with a micro-focused Cu K $\alpha$  source (wavelength of 1.54 Å, 8 keV) and a Pilatus detector (Dectris, Switzerland). The distance between the sample and detector was 2.48 m, which corresponds to a Q range from 0.0045 to 0.25 Å<sup>-1</sup>. An X-ray flux of about 7 × 106 photons per second was provided by a collimated beam size of 0.5 × 0.6 mm<sup>2</sup>. 1.5 mm external diameter glass capillaries (0.01 mm wall thickness) were used as sample containers. Independent measurements of solvent, empty capillary and dark field were carried out for data subtraction purposes. The incoming data were normalized to absolute units.

The scattering intensity as a function of Q generally follows.

$$I(Q) = c \cdot \Delta \rho^2 \cdot P(Q) \cdot S(Q)$$

where I(Q) the scattering intensity, c the sample concentration, P(Q) the form factor, S(Q) the structure factor,  $\Delta \rho^2$  the contrast between sample and solvent,  $Q = \frac{4\pi \sin \theta}{\lambda}$  the norm of the scattering vector with  $\theta$  the scattering angle and  $\lambda$  the wavelength of incoming X-ray.

The contrast term is defined as the square of the scattering length density difference between the sample and the solvent, *i.e.* 

$$\Delta \rho^2 = \left(\rho_{sample} - \rho_{solvent}\right)^2$$

Scattering length densities of cellulose, gold nanoparticles and water are equal to 14.5  $\times 10^{-6}$ Å<sup>-2</sup> and 12.5  $\times 10^{-5}$ Å<sup>-2</sup>, and 9.47 $\times 10^{-6}$ Å<sup>-2</sup>, respectively, leading to

$$\Delta \rho_{AuNP/solvent}^{2} = 1.33 \times 10^{-8} \text{\AA}^{-4} \gg \Delta \rho_{CNC/solvent}^{2} = 2.5 \times 10^{-11} \text{\AA}^{-4}$$

Consequently, the SAXS intensity arising from an aqueous suspension containing comparable concentrations of CNCs and AuNPs will mostly be attributed to gold nanoparticles.

SAXS data were fitted by assuming that S(Q) = 1 for the dilute suspensions probed and using the form factor of spheres given by

$$P(Q) = \left[\frac{\sin(QR) - QR\cos(QR)}{QR^3}\right]^2$$

The fit was achieved using the open source Sasview software (http://www.sasview.org/).

#### A.I.9 Small-angle neutron scattering (SANS)

SANS experiments were performed at Laboratoire Léon Brillouin (CEA-CNRS, Saclay, France) on the instrument PAXY. Four wavelength/sample-detector distance configurations (4 Å at 1m, 6 Å at 3 m, 8.5 Å at 5 m and 15 Å at 6.7 m) were used to access a momentum transfer range  $\Delta Q$  from 2.10<sup>-3</sup> Å<sup>-1</sup> to 0.6 Å<sup>-1</sup>. The radially averaged spectra were corrected for solvent, empty cell, incoherent scattering and background noise to yield intensity, *I*, in cm<sup>-1</sup> vs scattering vector, *Q*, in Å<sup>-1</sup> plots.

To extract the dimensions of the particles, the curve corresponding to the as-prepared CNCs was fitted using the form factor of a parallelepiped with a rectangular section, averaged over all space orientations. The procedure used was the same as the one described by Cherhal *et al.* (Cherhal 2015).

#### A.I.10 Viscosity measurements

The rheological behavior measurements were carried out in the flow mode with a DHR3 rheometer from TA Instruments using a cone and plate geometry mode (angle 0°59'42", diameter 60.0 mm, gap 29  $\mu$ m). Viscosity measurements were performed in a shear rate range from 0.01 to 1000 s<sup>-1</sup>. The ionic strength was controlled by adding NaCl solution until a 1 mM concentration was reached. The evolution of the viscosity as a function of temperature was investigated by varying the temperature from 8 to 24 °C (1 °C min<sup>-1</sup> temperature ramp) on a Peltier plate at a shear rate of 30 s<sup>-1</sup>. Distilled water was used to saturate the atmosphere around the sample in order to avoid evaporation during the temperature ramp.

#### A.I.11 Viscoelasticity measurements

The DHR3 rheometer from TA Instruments was used to measure the viscoelastic

properties. The ionic strength was controlled by adding NaCl solution until a 1 mM concentration was reached. Experiments were carried out in the oscillatory mode with a cone and plate geometry mode (angle 0°59'42", diameter 60.0 mm, gap 29  $\mu$ m). In order to determine the evolutions of the storage and loss moduli *G*' and *G*" in a temperature range from 8 to 24 °C, a Peltier plate that allowed varying the temperature was used and the same setup as for the viscosity measurements was used to avoid evaporation. The temperature ramp was 3 °C min<sup>-1</sup> and wait times of 10 min or 30 min were applied after reaching the set point upon temperature increase or decrease, respectively. Strain sweep procedures were applied in the range of 0.001 to 3 at an angular frequency of 1Hz to define the strain range corresponding to the linear domains of deformation. Then, angular frequency sweep procedures of 0.1 - 10 Hz were performed at a strain of 0.1 or 0.2 according to the samples to define the frequency value. For the tests of temperature effect, the sweep procedures were carried out at 8 and 24 °C to determine average values of strain and frequency corresponding to the linear domains for each sample. These values were used along the temperature range.

## A.I.12 Differential scanning calorimetry (DSC)

The DSC measurements were made with a Q200 apparatus from TA instruments. About 6  $\mu$ g of recrystallized NMMO was put in a 40  $\mu$ L pan with a hole in the lid and heated at 5 °C/min from 30 to 130 °C under a 50 mL/min nitrogen flow.

# Appendix II Rheological properties of bare cCNC-II

## A.II.1 Rheological behavior of bare cCNC-II

Derivatized CNC-II and their assemblies into networks were well characterized by DLS and TEM in dilute conditions. However, the samples prepared in TEM experiments were thin dry films. To get more information on these systems from a 3D point of view, a series of rheological experiments including the investigation of the viscosity and viscoelasticity of the suspensions were carried out.

Figure A.II.1 shows aqueous cCNC-II suspensions of increasing concentrations from 1 to 5 wt.%. Clearly, the 1 and 2 wt.% suspensions are liquid while from 3 wt. % a viscous sample reminiscent of a gel-phase is observed. Flow curves of the same series of samples were measured and results are shown in Figure A.II.1a. A Newtonian or shear-thinning behavior is well depicted by the power law  $\tau = K \cdot \dot{\gamma}^n$  where  $\tau$  represents the stress (Pa),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>), *K* is the consistency (Pa.s<sup>n</sup>) and *n* is the shear-thinning index. Figure A.II.1b shows the evolution of the stress as a function of shear rate from which the consistency *K* and the shear-thinning index *n* could be calculated. The results are shown in Table A.II.1 and presented in Figure. A.II.1c.

**Table A.II.1.** Yield stress, consistency and shear-thinning index of cCNC-II suspensions at concentrations from 1 to 5 wt. % resulting from the fit of the data with the power law described in the text.

Concentration (wt. %)	1	2	3	4	5
$ au_0$ (Pa)			2.6	3.7	11.1
K (Pa.s <sup>n</sup> )	0.0015	0.0084	0.46	1.6	5.8
n	0.978	0.841	0.406	0.346	0.200

For 1 and 2 wt. % samples, the viscosity decreased from about 0.06 to 0.002 Pa.s when the shear rate increased from 0.1 to 1000 s<sup>-1</sup>, revealing a moderate shear thinning behavior. Furthermore, at high shear rates (> 10 s<sup>-1</sup>), the flow index *n* was close to 1 indicating a partial near-Newtonian liquid behavior. The viscosity of 3, 4 and 5 wt. % cCNC-II suspensions is much 192 higher than at 1 and 2 wt. %, in agreement with the macroscopic observation of the samples (Figure A.II.2). For these samples, a significant decrease of the viscosity is obtained when the shear rate is increased, revealing that these cCNC-II suspensions behave as a shear thinning fluid with shear thinning index of 0.4, 0.35 and 0.2 for 3, 4 and 5 wt. % cCNC-II suspensions, respectively. This phenomenon could be clearly observed from Figure A.II.1c in which the shear-thinning index decreased strongly between 2 and 3 wt. %. Also, the 3, 4 and 5 wt. % cCNC-II suspensions possess a yield stress whose values are summarized in Table A.II.1. Figure A.II.1d shows the evolution of viscosity of the samples with temperature in the 8-24 °C at a fixed shear rate of 30 s<sup>-1</sup>. Constant values were clearly measured, indicating that the viscosity behavior of the CNC-II suspensions is temperature independent.



*Figure A.II.1. (a)* Flow curves and (b) stress as a function of shear rate of cCNC-II aqueous suspensions at concentrations 1, 2, 3, 4 and 5 wt. %. (c) Consistency and shear thinning index as a function of concentration of cCNC-II. (d) Viscosity as a function of temperature in the 8-24 °C range for cCNC-II aqueous suspensions at concentrations 1, 2, 3, 4 and 5 wt. % and at a shear rate of 30 s<sup>-1</sup>.



*Figure A.II.2 Photographs of cCNC-II samples of which the concentration is from 1 wt. % to 5 wt. % at room temperature.* 

These results reveal significant differences with the case of CNC-I aqueous suspensions (Gicquel 2019; Xu 2017, 2018). At low concentrations of 1 and 2 wt. %, CNC-I suspensions are reported to behave as near-Newtonian liquids while cCNC-II suspensions were shear thinning fluids even though the flow index n was close to 1 at high shear rates. Such a difference is due to the smaller dimensions of cCNC-II when compared with CNC-I, which results at a given concentration in a higher number density in the case of cCNC-II, increasing the particle interactions. Furthermore, the viscosity of cCNC-II is higher than CNC-I suspensions at the same concentration and the same shear rate (Gicquel 2019). This phenomenon is more evident when the concentration overtakes 3 wt. %. In fact, for cotton CNC-I nanoparticles, a significant increase of viscosity began at a concentration range between 6 and 8 wt. %, corresponding to the appearance of the liquid crystalline phase and the rheological behavior changes to a shear thinning fluid at high shear rate. The characteristic concentration of the occurrence of liquid crystalline phase depends on aspect ratio of CNCs prepared from different cellulose resources. At higher concentrations which correspond to the anisotropic phase, cotton CNC-I suspensions exhibit an obvious shear thinning behavior. Here in our case, 3 wt. % is the characteristic concentration of cCNC-II gelation. The viscosity suddenly increases at such a concentration and a strong shear thinning behavior could be observed.

At 2 wt. %, Yue *et al.* (Yue 2012) reported for cCNC-II suspensions prepared by 7 high-pressure homogenizer processes after mercerization and acid hydrolysis a viscosity value at  $0.1 \text{ s}^{-1}$  which is 1000 times higher than ours. Such a difference points out that CNC-II suspensions might be very sensitive to post-preparation homogenization treatments, as is also the case for CNC-I suspensions (Gicquel 2019).

Figure A.II.3a shows the storage modulus, G', and loss modulus, G'', as a function of concentration for as-prepared CNC-II suspensions. Obviously, the storage modulus G' is lower than the loss modulus G'' for 1 and 2 wt. % concentrations, showing that under these conditions,

the samples behaved as a viscous fluid. Nevertheless, when the concentration of nanocrystals increased to 3 wt. %, *G* increased strongly and became higher than *G* ". The loss modulus *G* " also increased showing that the viscous response was strengthened by interparticle interactions. These interactions had a greater influence on the storage modulus *G* as the ratio of G'/G" > 1 and for the sample of 5 wt. %, this value became nearly 10. The result indicates distinctly that the sample turns from a viscous fluid to an elastic gel when the concentration reaches 3 wt. %. The influence of temperature was then investigated and the results are reported in Figures A.II.3b and c. Comprehensively, *G* and *G* " kept a constant value in the 8-24 °C temperature range, showing no temperature effect on the viscoelasticity of the as-prepared cCNC-II suspensions. It has to be noted that the scattered data points of *G* at 1 wt. % were observed due to its fluidity and a lower accuracy in the measurements as the measured torque is near the lower limit of the sensor range. It is therefore not a temperature effect.

Comparing with cotton CNC-II after 7 high-pressure homogenizer processes (Yue 2012), a significant difference is observed one more time besides the rheological properties. In the publication, G' and G'' of a 2 wt. % CNC-II suspension were measured at 15, 25 and 35 °C. First, a huge variation of strain was applied. At 1 Hz using 0.5 strain, the values of G' and G''for 2 wt. % CNC-II were about 0.1 and 0.3 Pa at 15 °C which are 100 and 10 times more than our values, respectively. As for temperature effect, in their report, a temperature induced gelation could be observed for bare CNC-II nanorods as G' increased from below to above G''at 1 Hz and 0.5 strain when the temperature increased from 15 to 35 °C. However, for us, at least in a temperature range from 8 to 24 °C, no significant effect could be noticed.



**Figure A.II.3. (a)** Storage modulus G' (red straight line) and loss modulus G" (blue dashed line) as a function of concentration of cCNC-II samples. Experiments were performed at 15 °C at 0.01 strain and at an angular frequency of 1 Hz for 2 to 5 wt. % suspensions and at 0.02 strain for 1 wt. % suspension owing to its fluidity. **(b)** Storage modulus G' and **(c)** loss modulus G" as a function of temperature of cCNC-II samples. Experiments were performed in a temperature range between 8 and 24 °C at 0.01 strain and at an angular frequency of 1 Hz for 2 to 5 wt. % suspensions and at 0.02 strain for 1 wt. % suspension owing to its fluidity.

# A.II.2 Resilience of CNC-II gel and time-dependent behavior under shear and successive rest conditions.

In order to get more insight into the resilient capacity of the cCNC-II gel, further measurements were performed on a 5 wt. % suspension. Figure A.II.4 shows the repeated measurements of G' and G'' in a strain range from 0.001 to 3 at an angular frequency of 1 Hz after defining strain and the frequency corresponding to the linear domains. No time interval was applied between the first and the second measurements and 1 min for the second and the third, respectively. In the beginning, the values of G' and G'' were constant when the strain was below 10, showing a stable elastic gel behavior. After that, above a critical strain, G' decreased 196

strongly and became smaller than G'' indicating that the molecular connectivity was lost owing to the high strain and the sample became a viscous fluid. Immediately, the same measurement was achieved one more time. Comparing to the first measurement, G' and G'' were only a little smaller than the initial value. This result shows that even after high shear rates, cCNC-II gel only lost a little elastic capacity and could regain the gel properties rapidly. The significant decrease of G' was observed at a same critical strain showing a good stability of the CNC-II gel. The third experiment was performed 1 min after the second. The result indicates that G'and G'' were nearly the same as their initial values and an identical tendency was noticed. The three experiments give an obvious evidence that the cCNC-II gels have a good resilience after high shear rates. In other words, the time dependent effect induced by a break-down build-up behavior is slower than 1 min. This kind of break-down build-up behavior would be better associated to a gel structural organization where repulsive forces and excluded volume effects induce the viscoelasticity of the system more than attractive interaction between cCNC-II which could lead to a kind of fractal organization associated to longer time-dependent phenomena.



**Figure A.II.4.** Repeated measurements of storage modulus G' (red square) and loss modulus G'' (blue dots) of a 5 wt. % cCNC-II sample as a function of strain. Experiments were performed at 15 °C in a strain range between 0.001 and 3 and at an angular frequency of 1 Hz. Dashed black lines are only for comparison of values.

References

Abushammala, H.; Krossing, I.; Laborie, M. P. Ionic liquid-mediated technology to produce cellulose nanocrystals directly from wood. Carbohydr. Polym. 2015. 134, 609-616.

Adorjan, I.; Sjoberg, J.; Rosenau, T.; Hofinger, A.; Kosma, P. Kinetic and chemical studies on the isomerization of monosaccharides in N-methylmorpholine-N-oxide (NMMO) under lyocell conditions. Carbohydr. Res. 2004. 339 (11), 1899-1906.

Amin, K. N. M.; Annamalai, P. K.; Morrow, I. C.; Martin, D. Production of cellulose nanocrystals via a scalable mechanical method. RSC Adv. 2015. 5 (70), 57133-57140.

Esposito, D.; Gillette, W.; Anderson, S. R.; Zhu, J. Y.; Baxa, U.; McNeil, S. E. Enzymatic preparation of nanocrystalline and microcrystalline cellulose. Tappi J. 2014. 13 (5), 35-42.

Angles, M. N.; Dufresne, A. Plasticized starch/tunicin whiskers nanocomposite materials. 2. Mechanical behavior. Macromolecules 2001, 34, 2921

Araki, J.; Wada, M.; Kuga, S.; Okano, T. Flow properties of microcrystalline cellulose suspension prepared by acid treatment of native cellulose. Colloids Surfaces, A 1998, 142, 75.

Araki, J.; Wada, M.; Kuga, S. Steric stabilization of a cellulose microcrystal suspension by poly(ethylene glycol) grafting. Langmuir 2001. 17 (1), 21-27.

Atalla, R. H.; Vanderhart, D. L. Native cellulose - a composite of 2 distinct crystalline forms. Science 1984. 223 (4633), 283-285.

Atalla, R. S.; Crowley, M. F.; Himmel, M. E.; Atalla, R. H. Irreversible transformations of native celluloses, upon exposure to elevated temperatures. Carbohydr. Polym. 2014. 100, 2-8.

Rosa, M. F.; Mattoso, L. H. C. Nanocellulose in bio-based food packaging Azeredo, H. M. C.; applications. Ind. Crop. Prod. 2017. 97, 664-671.

Heux, L.; Putaux, J. L.; Jean, B. Preparation by grafting onto, characterization, and Azzam, F.; properties of thermally responsive polymer-decorated cellulose nanocrystals. Biomacromolecules 2010. 11 (12), 3652-3659.

Azzam, F.; Heux, L.; Jean, B. Adjustment of the chiral nematic phase properties of cellulose nanocrystals by polymer grafting. Langmuir 2016a. 32 (17), 4305-4312.

Azzam, F.; Siqueira, E.; Fort, S.; Hassaini, R.; Pignon, F.; Travelet, C.; Putaux, J. L.; Jean, B. Tunable aggregation and gelation of thermoresponsive suspensions of polymer-grafted cellulose nanocrystals. Biomacromolecules 2016b. 17 (6), 2112-2119.

Azzam, F.; Frka-Petesic, B.; Semeraro, E. F.; Cousin, F.; Jean, B. Small-angle neutron scattering reveals the structural details of thermosensitive polymer-grafted cellulose nanocrystal suspensions. Langmuir 2020. 36 (29), 8511-8519.

Beaumont, M.; Rennhofer, H.; Opietnik, M.; Lichtenegger, H. C.; Potthast, A.; Rosenau, T. Nanostructured cellulose II gel consisting of spherical particles. ACS Sustain. Chem. Eng. 2016. 4 (8), 4424-4432.

Beaumont, M.; Rosenfeldt, S.; Tardy, B. L.; Gusenbauer, C.; Khakalo, A.; Nonappa; Opietnik, M.; Potthast, A.; Rojas, O. J.; Rosenau, T. Soft cellulose II nanospheres: Sol-gel behaviour, swelling and material synthesis. Nanoscale 2019. 11 (38), 17773-17781.

Beck-Candanedo, S.; Roman, M.; Gray, D. G. Effect of reaction conditions on the properties and behavior

## References

of wood cellulose nanocrystal suspensions. Biomacromolecules 2005. 6 (2), 1048-1054.

Belton, P. S.; Tanner, S. F.; Cartier, N.; Chanzy, H. High-resolution solid-state carbon-13 nuclear magnetic resonance spectroscopy of tunicin, an animal cellulose. *Macromolecules* **1989**. *22*, 1615-1617.

Beltramino, F.; Roncero, M. B.; Torres, A. L.; Vidal, T.; Valls, C. Optimization of sulfuric acid hydrolysis conditions for preparation of nanocrystalline cellulose from enzymatically pretreated fibers. *Cellulose* **2016**. *23* (3), 1777-1789.

Biyani, M. V.; Foster, E. J.; Weder, C. Light-healable supramolecular nanocomposites based on modified cellulose nanocrystals. *ACS Macro Lett.* **2013.** *2* (3), 236-240.

Bondeson, D.; Mathew, A.; Oksman, K. Optimization of the isolation of nanocrystals from microcrystalline cellulose by acid hydrolysis. *Cellulose* **2006.** *13* (2), 171-180.

Bondeson, D.; Oksman, K. Dispersion and characteristics of surfactant modified cellulose whiskers nanocomposites. *Compos. Interfaces* **2007.** *14* (7-9), 617-630.

Braun, B.; Dorgan, J. R. Single-step method for the isolation and surface functionalization of cellulosic nanowhiskers. *Biomacromolecules* **2009.** *10* (2), 334-341.

Brinchi, L.; Cotana, F.; Fortunati, E.; Kenny, J. M. Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications. *Carbohydr. Polym.* **2013.** *94* (1), 154-169.

Brito, B. S. L.; Pereira, F. V.; Putaux, J. L.; Jean, B. Preparation, morphology and structure of cellulose nanocrystals from bamboo fibers. *Cellulose* **2012.** *19* (5), 1527-1536.

Brown, R. M. The biosynthesis of cellulose. J. Macromol. Sci.-Pure Appl. Chem. 1996. A33 (10), 1345-1373.

Brown, R. M. Cellulose structure and biosynthesis: What is in store for the 21st century? *J. Polym. Sci. Pol. Chem.* **2004.** *42* (3), 487-495.

Buchtová, N.; Budtova, T. Cellulose aero-, cryo- and xerogels: Towards understanding of morphology control. *Cellulose* **2016**. *23* (4), 2585-2595.

Budtova, T. Cellulose II aerogels: A review. Cellulose 2019. 26 (1), 81-121.

Bullard, K. K.; Srinivasarao, M.; Gutekunst, W. R. Modification of cellulose nanocrystal surface chemistry with diverse nucleophiles for materials integration. *J. Mater. Chem. A* **2020.** 

Cao, X. W.; Ding, B.; Yu, J. Y.; Al-Deyab, S. S. Cellulose nanowhiskers extracted from TEMPOoxidized jute fibers. *Carbohydr. Polym.* **2012.** *90* (2), 1075-1080.

Capadona, J. R.; Shanmuganathan, K.; Trittschuh, S.; Seidel, S.; Rowan, S. J.; Weder, C. Polymer nanocomposites with nanowhiskers isolated from microcrystalline cellulose. *Biomacromolecules* **2009**, *10*, 712-716.

Capon, B.; Walker, R. B. Kinetics and mechanism of the mutarotation of aldoses. *Journal of the Chemical Society, Perkin Transactions 2* **1974.** 1600-1610.

Chanzy, H.; Imada, K.; Mollard, A.; Vuong, R.; Barnoud, F. Crystallographic aspects of subelementary cellulose fibrils occurring in the wall of rose cells cultured *in vitro*. *Protoplasma* **1979.** *100* (3-4), 303-316.

Chanzy, H.; Nawrot, S.; Peguy, A.; Smith, P.; Chevalier, J. Phase-behavior of the quasi-ternary system 202

N-methylmorpholine-N-oxide, water, and cellulose. J. Polym. Sci. Pt. B-Polym. Phys. 1982. 20 (10), 1909-1924.

Chanzy, H. Nouveaux solvants de la cellulose. Image de la chimie 1984. 56 (Suppl.), 67-71.

Chanzy, H.; Henrissat, B. Undirectional degradation of *Valonia* cellulose microcrystals subjected to cellulase action. *FEBS Lett.* **1985.** *184* (2), 285-288.

Chauve, G.; Fraschini, C.; Jean, B. Handbook of green materials. World Sci. Pub. Co.: 2014.

Chemin, M.; Moreau, C.; Cathala, B.; Villares, A. Adsorption behavior of reducing end-modified cellulose nanocrystals: A kinetic study using quartz crystal microbalance. *J. Renew. Mater.* **2020a.** *8* (1), 29-43.

Chemin, M.; Moreau, C.; Cathala, B.; Villares, A. Asymmetric modification of cellulose nanocrystals with PAMAM dendrimers for the preparation of pH-responsive hairy surfaces. *Carbohydr. Polym.* **2020b.** *249*.

Chen, L.; Cao, W. J.; Quinlan, P. J.; Berry, R. M.; Tam, K. C. Sustainable catalysts from gold-loaded polyamidoamine dendrimer-cellulose nanocrystals. *ACS Sustain. Chem. Eng.* **2015.** *3* (5), 978-985.

Chen, X. Q.; Deng, X. Y.; Shen, W. H.; Jiang, L. L. Controlled enzymolysis preparation of nanocrystalline cellulose from pretreated cotton fibers. *BioResources* **2012.** *7* (3), 4237-4248.

Cheng, M.; Qin, Z. Y.; Liu, Y. N.; Qin, Y. F.; Li, T.; Chen, L.; Zhu, M. F. Efficient extraction of carboxylated spherical cellulose nanocrystals with narrow distribution through hydrolysis of lyocell fibers by using ammonium persulfate as an oxidant. *J. Mater. Chem. A* **2014.** *2* (1), 251-258.

Cherhal, F.; Cousin, F.; Capron, I. Influence of charge density and ionic strength on the aggregation process of cellulose nanocrystals in aqueous suspension, as revealed by small-angle neutron scattering. *Langmuir* **2015.** *31* (20), 5596-5602.

Chowdhury, Z. Z.; Abd Hamid, S. B. Preparation and characterization of nanocrystalline cellulose using ultrasonication combined with a microwave-assisted pretreatment process. *BioResources* **2016.** *11* (2), 3397-3415.

Chu, S. S. C.; Jeffrey, G. A. The refinement of the crystal structure of  $\beta$ -D-glucose and cellobiose. *Acta Crystallographica Section B* **1968**. *24*, 830-838.

Conner, A. H. Handbook of size exclusion chormatography. Marcel Dekker: New York, 1995.

De France, K. J.; Hoare, T.; Cranston, E. D. Review of hydrogels and aerogels containing nanocellulose. *Chemistry of Materials* **2017.** *29* (11), 4609-4631.

de Oliveira, F. B.; Bras, J.; Pimenta, M. T. B.; Curvelo, A. A. D.; Belgacem, M. N. Production of cellulose nanocrystals from sugarcane bagasse fibers and pith. *Ind. Crop. Prod.* **2016.** *93*, 48-57.

De Souza Lima, M. M.; Borsali, R. Static and dynamic light scattering from polyelectrolyte microcrystal cellulose. *Langmuir* **2002**. *18* (4), 992-996.

Delepierre, G.; Eyley, S.; Thielemans, W.; Weder, C.; Cranston, E. D.; Zoppe, J. O. Patience is a virtue: Self-assembly and physico-chemical properties of cellulose nanocrystal allomorphs. *Nanoscale* **2020.** *12*, 17480-17493.

Denooy, A. E. J.; Besemer, A. C.; Vanbekkum, H. Highly selective TEMPO mediated oxidation of

## References

primary alcohol groups in polysaccharides. *Recl. Trav. Chim. Pays-Bas-J. Roy. Neth. Chem. Soc.* 1994. 113 (3), 165-166.

Dhar, P.; Tarafder, D.; Kumar, A.; Katiyar, V. Effect of cellulose nanocrystal polymorphs on mechanical, barrier and thermal properties of poly(lactic acid) based bionanocomposites. *RSC Adv.* **2015.** *5* (74), 60426-60440.

Domingues, R. M.; Gomes, M. E.; Reis, R. L. The potential of cellulose nanocrystals in tissue engineering strategies. *Biomacromolecules* **2014.** *15* (7), 2327-46.

Dong, X. M.; Revol, J. F.; Gray, D. G. Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose. *Cellulose* **1998.** *5* (1), 19-32.

Dong, X. M.; Kimura, T.; Revol, J. F.; Gray, D. G. Effects of ionic strength on the isotropic-chiral nematic phase transition of suspensions of cellulose crystallites. *Langmuir* **1996**. *12* (8), 2076-2082.

Du, H. S.; Liu, C.; Mu, X. D.; Gong, W. B.; Lv, D.; Hong, Y. M.; Si, C. L.; Li, B. Preparation and characterization of thermally stable cellulose nanocrystals via a sustainable approach of FeCl<sub>3</sub>-catalyzed formic acid hydrolysis. *Cellulose* **2016**. *23* (4), 2389-2407.

Du, W. B.; Guo, J.; Li, H. M.; Gao, Y. Heterogeneously modified cellulose nanocrystals-stabilized pickering emulsion: Preparation and their template application for the creation of PS microspheres with amino-rich surfaces. *ACS Sustain. Chem. Eng.* **2017.** *5* (9), 7514-7523.

Dufresne, A. *Nanocellulose: From nature to high performance tailored materials*. Walter de Gruyter GmbH: Berlin, **2012**.

Earl, W. L.; Vanderhart, D. L. Measurement of C-13 chemical-shifts in solids. J. Magn. Reson. 1982. 48 (1), 35-54.

Eckelt, J.; Eich, T.; Roder, T.; Ruf, H.; Sixta, H.; Wolf, B. A. Phase diagram of the ternary system NMMO/water/cellulose. *Cellulose* **2009**. *16* (3), 373-379.

Edgar, C. D.; Gray, D. G. Influence of dextran on the phase behavior of suspensions of cellulose nanocrystals. *Macromolecules* **2002.** *35* (19), 7400-7406.

Eichhorn, S. J.; Dufresne, A.; Aranguren, M.; Marcovich, N. E.; Capadona, J. R.; Rowan, S. J.; Weder, C.; Thielemans, W.; Roman, M.; Renneckar, S.; Gindl, W.; Veigel, S.; Keckes, J.; Yano, H.; Abe, K.; Nogi, M.; Nakagaito, A. N.; Mangalam, A.; Simonsen, J.; Benight, A. S.; Bismarck, A.; Berglund, L. A.; Peijs, T. Review: Current international research into cellulose nanofibres and nanocomposites. *J. Mater. Sci.* **2010.** *45* (1), 1-33.

Eichhorn, S. J. Cellulose nanowhiskers: Promising materials for advanced applications. *Soft Matter* **2011.** *7* (2), 303-315.

Elazzouzi-Hafraoui, S.; Nishiyama, Y.; Putaux, J. L.; Heux, L.; Dubreuil, F.; Rochas, C. The shape and size distribution of crystalline nanoparticles prepared by acid hydrolysis of native cellulose. *Biomacromolecules* **2008.** *9* (1), 57-65.

Espinosa, S. C.; Kuhnt, T.; Foster, E. J.; Weder, C. Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis. *Biomacromolecules* **2013.** *14* (4), 1223-1230.

Eyley, S.; Thielemans, W. Imidazolium grafted cellulose nanocrystals for ion exchange applications. *Chem. Commun.* **2011.** *47* (14), 4177-4179.

Eyley, S.; Thielemans, W. Surface modification of cellulose nanocrystals. *Nanoscale* **2014.** *6* (14), 7764-7779.

Fan, L. T.; Gharpuray, M. M.; Lee, Y. H. Cellulose hydrolysis. Springer Science & Business Media: 2012.

Fang, W. W.; Arola, S.; Malho, J. M.; Kontturi, E.; Linder, M. B.; Laaksonen, P. Noncovalent dispersion and functionalization of cellulose nanocrystals with proteins and polysaccharides. *Biomacromolecules* **2016**. *17* (4), 1458-1465.

Ferrer, A.; Pal, L.; Hubbe, M. Nanocellulose in packaging: Advances in barrier layer technologies. *Ind. Crop. Prod.* **2017.** *95*, 574-582.

Filson, P. B.; Dawson-Andoh, B. E.; Schwegler-Berry, D. Enzymatic-mediated production of cellulose nanocrystals from recycled pulp. *Green Chem.* **2009.** *11* (11), 1808-1814.

Fink, H. P.; Philipp, B.; Paul, D.; Serimaa, R.; Paakkari, T. The structure of amorphous cellulose as revealed by wide-angle X-ray-scattering. *Polymer* **1987.** *28* (8), 1265-1270.

Foster, E. J.; Moon, R. J.; Agarwal, U. P.; Bortner, M. J.; Bras, J.; Camarero-Espinosa, S.; Chan, K. J.; Clift, M. J. D.; Cranston, E. D.; Eichhorn, S. J.; Fox, D. M.; Hamad, W. Y.; Heux, L.; Jean, B.; Korey, M.; Nieh, W.; Ong, K. J.; Reid, M. S.; Renneckar, S.; Roberts, R.; Shatkin, J. A.; Simonsen, J.; Stinson-Bagby, K.; Wanasekara, N.; Youngblood, J. Current characterization methods for cellulose nanomaterials. *Chem. Soc. Rev.* **2018.** *47* (8), 2609-2679.

French, A. D.; Miller, D. P.; Aabloo, A. Miniature crystal models of cellulose polymorphs and other carbohydrates. *Int. J. Biol. Macromol.* **1993.** *15* (1), 30-36.

French, A. D. Idealized powder diffraction patterns for cellulose polymorphs. *Cellulose* **2014.** *21* (2), 885-896.

French, A. D. Glucose, not cellobiose, is the repeating unit of cellulose and why that is important. *Cellulose* **2017.** *24* (11), 4605-4609.

Freudenberg, K.; Braun, E. Methylcellulose 5. *Mitteilung über Lignin und Cellulose Ann.* **1928.** *460* (1), 288-304.

Garcia, d. R. N. L.; Thielemans, W.; Dufresne, A. Sisal cellulose whiskers reinforced polyvinyl acetate nanocomposites. *Cellulose* **2006**, *13*, 261.

Gaspar, D.; Fernandes, S. N.; de Oliveira, A. G.; Fernandes, J. G.; Grey, P.; Pontes, R. V.; Pereira, L.; Martins, R.; Godinho, M. H.; Fortunato, E. Nanocrystalline cellulose applied simultaneously as the gate dielectric and the substrate in flexible field effect transistors. *Nanotechnology* **2014.** *25* (9), 11.

Gicquel, E.; Bras, J.; Rey, C.; Putaux, J. L.; Pignon, F.; Jean, B.; Martin, C. Impact of sonication on the rheological and colloidal properties of highly concentrated cellulose nanocrystal suspensions. *Cellulose* **2019.** *26* (13-14), 7619-7634.

Gong, J.; Li, J.; Xu, J.; Xian, Z. Y.; Mo, L. H. Research on cellulose nanocrystals produced from cellulose sources with various polymorphs. *RSC Adv.* **2017.** *7* (53), 33486-33493.

Gong, J.; Mo, L. H.; Li, J. A comparative study on the preparation and characterization of cellulose nanocrystals with various polymorphs. *Carbohydr. Polym.* **2018.** *195*, 18-28.

Gonzalez-Dominguez, J. M.; Anson-Casaos, A.; Grasa, L.; Abenia, L.; Salvador, A.; Colom, E.; 205

## References

Mesonero, J. E.; Garcia-Bordeje, J. E.; Benito, A. M.; Maser, W. K. Unique properties and behavior of nonmercerized type-II cellulose nanocrystals as carbon nanotube biocompatible dispersants. *Biomacromolecules* **2019**. *20* (8), 3147-3160.

Grunert, M.; Winter, W. T. Nanocomposites of cellulose acetate butyrate reinforced with cellulose nanocrystals. *Journal of Polymers and the Environment* **2002**. *10* (1-2), 27-30.

Habibi, Y.; Chanzy, H.; Vignon, M. R. TEMPO-mediated surface oxidation of cellulose whiskers. *Cellulose* **2006.** *13* (6), 679-687.

Habibi, Y.; Goffin, A.-L.; Schiltz, N.; Duquesne, E.; Dubois, P.; Dufresne, A. Bionanocomposites based on poly(epsilon-caprolactone)-grafted cellulose nanocrystals by ring-opening polymerization. *Journal of Materials Chemistry* **2008**, *18*, 5002-5010.

Habibi, Y.; Lucia, L. A.; Rojas, O. J. Cellulose nanocrystals: Chemistry, self-assembly, and applications. *Chem. Rev.* **2010.** *110* (6), 3479-3500.

Habibi, Y. Key advances in the chemical modification of nanocelluloses. *Chem Soc Rev* **2014.** *43* (5), 1519-1542.

Haigler, C. H. Biosynthesis and biodegradation of cellulose. Marcel Dekker: New York, 1991.

Haiss, W.; Thanh, N. T. K.; Aveyard, J.; Fernig, D. G. Determination of size and concentration of gold nanoparticles from UV-Vis spectra. *Anal. Chem.* **2007.** *79* (11), 4215-4221.

Hamad, W. Y.; Hu, T. Q. Structure-process-yield interrelations in nanocrystalline cellulose extraction. *Can. J. Chem. Eng.* **2010.** *88* (3), 392-402.

Han, J. Q.; Zhou, C. J.; French, A. D.; Han, G. P.; Wu, Q. L. Characterization of cellulose II nanoparticles regenerated from 1-butyl-3-methylimidazolium chloride. *Carbohydr. Polym.* **2013.** *94* (2), 773-781.

Hao, W. S.; Wang, M. Z.; Zhou, F. S.; Luo, H. Z.; Xie, X.; Luo, F. L.; Cha, R. T. A review on nanocellulose as a lightweight filler of polyolefin composites. *Carbohydr. Polym.* **2020**. *243*, 15.

Harrisson, S.; Drisko, G. L.; Malmstrom, E.; Hult, A.; Wooley, K. L. Hybrid rigid/soft and biologic/synthetic materials: Polymers grafted onto cellulose microcrystals. *Biomacromolecules* **2011**. *12* (4), 1214-1223.

Hasani, M.; Cranston, E. D.; Westman, G.; Gray, D. G. Cationic surface functionalization of cellulose nanocrystals. *Soft Matter* **2008.** *4* (11), 2238-2244.

Haworth, W. N.; Hirst, E. L.; Thomas, H. A. The existence of the cellobiose residue in cellulose. *Nature* **1930.** *126* (3177), 438.

Heise, K.; Koso, T.; Pitkanen, L.; Potthast, A.; King, A. W. T.; Kostiainen, M. A.; Kontturi, E. Knoevenagel condensation for modifying the reducing end groups of cellulose nanocrystals. *ACS Macro Lett.* **2019.** *8* (12), 1642-1647.

Heise, K.; Delepierre, G.; King, A. W. T.; Kostiainen, M. A.; Zoppe, J. O.; Weder, C.; Kontturi, E. Chemical modification of cellulose nanocrystal reducing end groups. *Angew. Chem.-Int. Edit.* **2020.** 

Helbert, W.; Sugiyama, J.; Ishihara, M.; Yamanaka, S. Characterization of native crystalline cellulose in the cell walls of oomycota. *J. Biotechnol.* **1997.** *57* (1-3), 29-37.

Heux, L.; Dinand, E.; Vignon, M. R. Structural aspects in ultrathin cellulose microfibrils followed by C-13 CP-MAS NMR. *Carbohydr. Polym.* **1999.** *40* (2), 115-124.

Heux, L.; Chauve, G.; Bonini, C. Nonflocculating and chiral-nematic self-ordering of cellulose microcrystals suspensions in nonpolar solvents. *Langmuir* **2000**. *16* (21), 8210-8212.

Hieta, K.; Kuga, S.; Usuda, M. Electron staining of reducing ends evidences a parallel-chain structure in *Valonia* cellulose. *Biopolymers* **1984.** *23* (10), 1807-1810.

Hirota, M.; Tamura, N.; Saito, T.; Isogai, A. Water dispersion of cellulose II nanocrystals prepared by TEMPO-mediated oxidation of mercerized cellulose at pH 4.8. *Cellulose* **2010.** *17* (2), 279-288.

Hirota, M.; Tamura, N.; Saito, T.; Isogai, A. Cellulose II nanoelements prepared from fully mercerized, partially mercerized and regenerated celluloses by 4-acetamido-TEMPO/NaClO/NaClO<sub>2</sub> oxidation. *Cellulose* **2012.** *19* (2), 435-442.

Ho, T. T. T.; Zimmermann, T.; Hauert, R.; Caseri, W. Preparation and characterization of cationic nanofibrillated cellulose from etherification and high-shear disintegration processes. *Cellulose* **2011**. *18* (6), 1391-1406.

Hon, D. N. S. Cellulose - a random-walk along its historical path. Cellulose 1994. 1 (1), 1-25.

Honjo, G.; Watanabe, M. Examination of cellulose fibre by the low-temperature specimen method of electron diffraction and electron microscopy. *Nature* **1958.** *181*, 326-328.

Horikawa, Y.; Shimizu, M.; Saito, T.; Isogai, A.; Imai, T.; Sugiyama, J. Influence of drying of chara cellulose on length/length distribution of microfibrils after acid hydrolysis. *Int. J. Biol. Macromol.* **2018.** *109*, 569-575.

Huang, J. L.; Li, C. J.; Gray, D. G. Cellulose nanocrystals incorporating fluorescent methylcoumarin groups. *ACS Sustain. Chem. Eng.* **2013.** *1* (9), 1160-1164.

Igarashi, K.; Wada, M.; Samejima, M. Activation of crystalline cellulose to cellulose III<sub>I</sub> results in efficient hydrolysis by cellobiohydrolase. *Febs J.* **2007.** *274* (7), 1785-1792.

Imlimthan, S.; Otaru, S.; Keinanen, O.; Correia, A.; Lintinen, K.; Santos, H. A.; Airaksinen, A. J.; Kostiainen, M. A.; Sarparanta, M. Radiolabeled molecular imaging probes for the *in vivo* evaluation of cellulose nanocrystals for biomedical applications. *Biomacromolecules* **2019**. *20* (2), 674-683.

Irvine, J. C.; Hirst, E. L. LXIV.-The constitution of polysaccharides. Part VI. The molecular structure of cotton cellulose. *Journal of the Chemical Society, Transactions* **1923**. *123*, 518-532.

Ishikawa, A.; Okano, T.; Sugiyama, J. Fine structure and tensile properties of ramie fibres in the crystalline form of cellulose I, II, III<sub>I</sub> and IV<sub>I</sub>. *Polymer* **1997.** *38* (2), 463-468.

Isogai, A. Cellulosic polymers: Blends and composites. Hanser: Germany, 1994; Vol. p.1.

Jin, E.; Guo, J. Q.; Yang, F.; Zhu, Y. Y.; Song, J. L.; Jin, Y. C.; Rojas, O. J. On the polymorphic and morphological changes of cellulose nanocrystals (CNC-I) upon mercerization and conversion to CNC-II. *Carbohydr. Polym.* **2016.** *143*, 327-335.

Johnson, D. L. Compounds dissolved in cyclic amine oxides. US. US3447939A, 1966.

Jorfi, M.; Foster, E. J. Recent advances in nanocellulose for biomedical applications. *J. Appl. Polym. Sci.* **2015.** *132* (14).
Junior de Menezes, A.; Siqueira, G.; Curvelo, A. A. S.; Dufresne, A. Extrusion and characterization of functionalized cellulose whiskers reinforced polyethylene nanocomposites. *Polymer* **2009**, *50*, 4552-4563.

Kang, H. L.; Liu, R. G.; Huang, Y. Cellulose derivatives and graft copolymers as blocks for functional materials. *Polym. Int.* **2013.** *62* (3), 338-344.

Karaaslan, M. A.; Gao, G. Z.; Kadla, J. F. Nanocrystalline cellulose/beta-casein conjugated nanoparticles prepared by click chemistry. *Cellulose* **2013**. *20* (6), 2655-2665.

Kim, J.; Montero, G.; Habibi, Y.; Hinestroza, J. P.; Genzer, J.; Argyropoulos, D. S.; Rojas, O. J. Dispersion of cellulose crystallites by nonionic surfactants in a hydrophobic polymer matrix. *Polym. Eng. Sci.* **2009.** *49* (10), 2054-2061.

Kim, N. H.; Imai, T.; Wada, M.; Sugiyama, J. Molecular directionality in cellulose polymorphs. *Biomacromolecules* **2006.** *7* (1), 274-280.

Kimura, F.; Kimura, T.; Tamura, M.; Hirai, A.; Ikuno, M.; Horii, F. Magnetic alignment of the chiral nematic phase of a cellulose microfibril suspension. *Langmuir* **2005**, *21*, 2034.

Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. Comprehensive cellulose chemistry, volume 1: General principles & analytical methods. Wiley: **1998**.

Klemm, D.; Heublein, B.; Fink, H. P.; Bohn, A. Cellulose: Fascinating biopolymer and sustainable raw material. *Angew. Chem.-Int. Edit.* **2005.** *44* (22), 3358-3393.

Klemm, D.; Kramer, F.; Moritz, S.; Lindstrom, T.; Ankerfors, M.; Gray, D.; Dorris, A. Nanocelluloses: A new family of nature-based materials. *Angew. Chem.-Int. Edit.* **2011.** *50* (24), 5438-5466.

Klemm, D.; Cranston, E. D.; Fischer, D.; Gama, M.; Kedzior, S. A.; Kralisch, D.; Kramer, F.; Kondo, T.; Lindstrom, T.; Nietzsche, S.; Petzold-Welcke, K.; Rauchfuss, F. Nanocellulose as a natural source for groundbreaking applications in materials science: Today's state. *Mater. Today* **2018.** *21* (7), 720-748.

Kolpak, F. J.; Blackwell, J. Determination of the structure of cellulose II. *Macromolecules* **1976.** *9* (2), 273-278.

Kono, H.; Yunoki, S.; Shikano, T.; Fujiwara, M.; Erata, T.; Takai, M. CP/MAS <sup>13</sup>C NMR study of cellulose and cellulose derivatives. 1. Complete assignment of the CP/MAS <sup>13</sup>C NMR spectrum of the native cellulose. *J. Am. Chem. Soc.* **2002.** *124*, 7506-7511.

Kontturi, E.; Meriluoto, A.; Penttila, P. A.; Baccile, N.; Malho, J. M.; Potthast, A.; Rosenau, T.; Ruokolainen, J.; Serimaa, R.; Laine, J.; Sixta, H. Degradation and crystallization of cellulose in hydrogen chloride vapor for high-yield isolation of cellulose nanocrystals. *Angew. Chem.-Int. Edit.* **2016**. *55* (46), 14453-14456.

Kontturi, E.; Laaksonen, P.; Linder, M. B.; Nonappa; Groschel, A. H.; Rojas, O. J.; Ikkala, O. Advanced materials through assembly of nanocelluloses. *Adv. Mater.* **2018.** *30* (24), 39.

Koyama, M.; Helbert, W.; Imai, T.; Sugiyama, J.; Henrissat, B. Parallel-up structure evidences the molecular directionality during biosynthesis of bacterial cellulose. *Proc. Natl. Acad. Sci. U. S. A.* **1997.** *94* (17), 9091-9095.

KroonBatenburg, L. M. J.; Bouma, B.; Kroon, J. Stability of cellulose structures studied by MD simulations. Could mercerized cellulose II be parallel? *Macromolecules* **1996.** *29* (17), 5695-5699.

Kuang, Y. S.; Li, X.; Luan, P. C.; Zhang, X.; Xu, J.; Mo, L. H.; Gong, J.; Li, J. Cellulose II nanocrystal: A promising bio-template for porous or hollow nano SiO<sub>2</sub> fabrication. *Cellulose* **2020.** *27* (6), 3167-3179.

Kuga, S.; Brown, R. M. Silver labeling of the reducing ends of bacterial cellulose. *Carbohydr. Res.* **1988.** *180* (2), 345-350.

Chin, K-M.; Ting, S.S.; Ong, H. L.; Omar, Mf. Surface functionalized nanocellulose as a veritable inclusionary material in contemporary bioinspired applications: A review. *J. Appl. Polym. Sci.* **2018.** *135* (13), 46065-46084.

Lagerwall, J. P. F.; Schütz, C.; Salajkova, M.; Noh, J.; Hyun Park, J.; Scalia, G.; Bergström, L. Cellulose nanocrystal-based materials: From liquid crystal self-assembly and glass formation to multifunctional thin films. *NPG Asia Materials* **2014.** *6* (1), e80-e80.

Lai-Kee-Him, J.; Chanzy, H.; Muller, M.; Putaux, J. L.; Imai, T.; Bulone, V. *In vitro* versus *in vivo* cellulose microfibrils from plant primary wall synthases: Structural differences. *J. Biol. Chem.* **2002.** *277* (40), 36931-36939.

Langan, P.; Nishiyama, Y.; Chanzy, H. A revised structure and hydrogen-bonding system in cellulose II from a neutron fiber diffraction analysis. *J. Am. Chem. Soc.* **1999.** *121*, 9940-9946.

Langan, P.; Nishiyama, Y.; Chanzy, H. X-ray structure of mercerized cellulose II at 1 angstrom resolution. *Biomacromolecules* **2001.** *2* (2), 410-416.

Langan, P.; Sukumar, N.; Nishiyama, Y.; Chanzy, H. Synchrotron X-ray structures of cellulose I-beta and regenerated cellulose II at ambient temperature and 100 K. *Cellulose* **2005.** *12* (6), 551-562.

Lazko, J.; Sénéchal, T.; Bouchut, A.; Paint, Y.; Dangreau, L.; Fradet, A.; Tessier, M.; Raquez, J. M.; Dubois, P. Acid-free extraction of cellulose type I nanocrystals using brønsted acid-type ionic liquids. *Nanocomposites* **2016.** *2* (2), 65-75.

Leguy, J.; Diallo, A.; Putaux, J. L.; Nishiyama, Y.; Heux, L.; Jean, B. Periodate oxidation followed by NaBH<sub>4</sub> reduction converts microfibrillated cellulose into sterically stabilized neutral cellulose nanocrystal suspensions. *Langmuir* **2018.** *34* (37), 11066-11075.

Leguy, J.; Nishiyama, Y.; Jean, B.; Heux, L. Ultrastructural characterization of the core-shell structure of a wide range of periodate-oxidized cellulose from different native sources by solid-state C-13 CP-MAS NMR. *ACS Sustain. Chem. Eng.* **2019.** *7* (1), 412-420.

Leung, A. C. W.; Hrapovic, S.; Lam, E.; Liu, Y. L.; Male, K. B.; Mahmoud, K. A.; Luong, J. H. T. Characteristics and properties of carboxylated cellulose nanocrystals prepared from a novel one-step procedure. *Small* **2011.** *7* (3), 302-305.

Li, L.; Tao, H.; Wu, B. L.; Zhu, G.; Li, K.; Lin, N. Triazole end-grafting on cellulose nanocrystals for water-redispersion improvement and reactive enhancement to nanocomposites. *ACS Sustain. Chem. Eng.* **2018a.** *6* (11), 14888-14900.

Li, L.; Zhu, Y. F.; Yang, J. H. 3D bioprinting of cellulose with controlled porous structures from NMMO. *Mater. Lett.* **2018b.** *210*, 136-138.

Li, Q.; Zhou, J. P.; Zhang, L. N. Structure and properties of the nanocomposite films of chitosan reinforced with cellulose whiskers. *Journal of Polymer Science Part B-Polymer Physics* **2009**.47 (11), 1069-1077.

Li, S. D.; Bashline, L.; Lei, L.; Gu, Y. Cellulose synthesis and its regulation. Arabidopsis Book: 2014.

Li, S. D.; Bashline, L.; Zheng, Y. Z.; Xin, X. R.; Huang, S. X.; Kong, Z. S.; Kim, S. H.; Cosgrove, D. J.; Gu, Y. Cellulose synthase complexes act in a concerted fashion to synthesize highly aggregated cellulose in secondary cell walls of plants. *Proc. Natl. Acad. Sci. U. S. A.* **2016.** *113* (40), 11348-11353.

Li, W.; Yue, J. Q.; Liu, S. X. Preparation of nanocrystalline cellulose via ultrasound and its reinforcement capability for poly(vinyl alcohol) composites. *Ultrason. Sonochem.* **2012.** *19* (3), 479-485.

Lima, M. M. D.; Borsali, R. Rodlike cellulose microcrystals: Structure, properties, and applications. *Macromol. Rapid Commun.* **2004.** *25* (7), 771-787.

Lin, F. B.; Cousin, F.; Putaux, J. L.; Jean, B. Temperature-controlled star-shaped cellulose nanocrystal assemblies resulting from asymmetric polymer grafting. *ACS Macro Lett.* **2019.** *8* (4), 345-351.

Lindman, B.; Karlstrom, G.; Stigsson, L. On the mechanism of dissolution of cellulose. *J. Mol. Liq.* **2010.** *156* (1), 76-81.

Ljungberg, N.; Bonini, C.; Bortolussi, F.; Boisson, C.; Heux, L.; Cavaille, J. Y. New nanocomposite materials reinforced with cellulose whiskers in atactic polypropylene: Effect of surface and dispersion characteristics. *Biomacromolecules* **2005.** *6* (5), 2732-2739.

Lokanathan, A. R.; Nykanen, A.; Seitsonen, J.; Johansson, L. S.; Campbell, J.; Rojas, O. J.; Ikkala, O.; Laine, J. Cilia-mimetic hairy surfaces based on end-immobilized nanocellulose colloidal rods. *Biomacromolecules* **2013**. *14* (8), 2807-2813.

Lokanathan, A. R.; Lundahl, M.; Rojas, O. J.; Laine, J. Asymmetric cellulose nanocrystals: Thiolation of reducing end groups via NHS-EDC coupling. *Cellulose* **2014.** *21* (6), 4209-4218.

Lu, Z. X.; Fan, L. W.; Zheng, H. Y.; Lu, Q. L.; Liao, Y. Q.; Huang, B. A. Preparation, characterization and optimization of nanocellulose whiskers by simultaneously ultrasonic wave and microwave assisted. *Bioresour. Technol.* **2013.** *146*, 82-88.

Ma, X. J.; Zhang, X. J. Nano-Fe<sub>3</sub>O<sub>4</sub>/NMMO-cellulose composite membrane prepared by the in-situ coprecipitation method. *Wood Res.* **2018.** *63* (1), 25-33.

Majoinen, J.; Walther, A.; McKee, J. R.; Kontturi, E.; Aseyev, V.; Malho, J. M.; Ruokolainen, J.; Ikkala, O. Polyelectrolyte brushes grafted from cellulose nanocrystals using Cu-mediated surfaceinitiated controlled radical polymerization. *Biomacromolecules* **2011**. *12* (8), 2997-3006.

Mangalam, A. P.; Simonsen, J.; Benight, A. S. Cellulose/DNA hybrid nanomaterials. *Biomacromolecules* **2009.** *10* (3), 497-504.

Mao, J.; Heck, B.; Reiter, G.; Laborie, M. P. Cellulose nanocrystals' production in near theoretical yields by 1-butyl-3-methylimidazolium hydrogen sulfate (BMIM HSO<sub>4</sub>) - mediated hydrolysis. *Carbohydr. Polym.* **2015.** *117*, 443-451.

Mao, Y. M.; Liu, K.; Zhan, C. B.; Geng, L. H.; Chu, B.; Hsiao, B. S. Characterization of nanocellulose using small-angle neutron, X-ray, and dynamic light scattering techniques. *J. Phys. Chem. B* **2017.** *121* (6), 1340-1351.

Marechal, Y.; Chanzy, H. The hydrogen bond network in I-beta cellulose as observed by infrared spectrometry. J. Mol. Struct. 2000. 523, 183-196.

Mariano, M.; El Kissi, N.; Dufresne, A. Cellulose nanocrystals and related nanocomposites: Review of some properties and challenges. *J. Polym. Sci. Pt. B-Polym. Phys.* **2014.** *52* (12), 791-806.

Martin A. Hubbe, A. F., Preeti Tyagi, Yuanyuan Yin, Carlos Salas, Lokendra Pal, and Orlando J. Rojas Nanocellulose in thin films, coatings, and plies for packaging applications: A review. *BioResources* **2017**.

Mercer, J. Improvements in the preparation of cotton and other fabrics and other fibrous materials. Br. Patent 13296, **1850**.

Merlini, A.; Claumann, C.; Zibetti, A. W.; Coirolo, A.; Rieg, T.; Machado, R. A. F. Kinetic study of the thermal decomposition of cellulose nanocrystals with different crystal structures and morphologies. *Industrial & Engineering Chemistry Research* **2020**. *59* (30), 13428-13439.

Meyer, K. H. e. M. Positions des atomes dans le nouveau modèle spacial de la cellulose. *Helvetica Chimica Acta* **1937.** *20*, 232-244.

Miller, A. F.; Donaol, A. M. Imaging of anisotropic cellulose suspensions using environmental scanning electron microscopy. *Biomacromolecules* **2003**. *4* (3), 510-517.

Miura, K.; Nakano, T. Analysis of the effects of restriction of longitudinal contraction on the conversion from cellulose I to cellulose II using CP/MAS C-13 NMR: Mercerization of ramie fibers under noncooling and cooling conditions. *J. Mater. Sci.* **2016.** *51* (13), 6331-6340.

Montanari, S.; Rountani, M.; Heux, L.; Vignon, M. R. Topochemistry of carboxylated cellulose nanocrystals resulting from TEMPO-mediated oxidation. *Macromolecules* **2005.** *38* (5), 1665-1671.

Moon, R. J.; Martini, A.; Nairn, J.; Simonsen, J.; Youngblood, J. Cellulose nanomaterials review: Structure, properties and nanocomposites. *Chem. Soc. Rev.* **2011.** *40* (7), 3941-3994.

Morandi, G.; Thielemans, W. Synthesis of cellulose nanocrystals bearing photocleavable grafts by ATRP. *Polym. Chem.* **2012.** *3* (6), 1402-1407.

Flauzino Neto, W. P.; Putaux, J. L.; Mariano, M.; Ogawa, Y.; Otaguro, H.; Pasquini, D.; Dufresne, A. Comprehensive morphological and structural investigation of cellulose I and II nanocrystals prepared by sulphuric acid hydrolysis. *RSC Adv.* **2016.** *6* (79), 76017-76027.

Newman, R. H. Estimation of the lateral dimensions of cellulose crystallites using C-13 NMR signal strengths. *Solid State Nucl. Magn. Reson.* **1999.** *15* (1), 21-29.

Ng, H. M.; Sin, L. T.; Tee, T. T.; Bee, S. T.; Hui, D.; Low, C. Y.; Rahmat, A. R. Extraction of cellulose nanocrystals from plant sources for application as reinforcing agent in polymers. *Compos. Pt. B-Eng.* **2015.** *75*, 176-200.

Nickerson, R. F.; Habrle, J. A. Cellulose intercrystalline structure. *Industrial & Engineering Chemistry Research* **1947.** *39*, 1507-1512.

Ning, Y. G.; Xie, H.; Xing, H. T.; Deng, W. L.; Yang, D. B. Comparison of self-assembled monolayers of n-alkanethiols and phenylthioureas on the surface of gold. *Surf. Interface Anal.* **1996.** *24* (9), 667-670.

Nishiyama, Y.; Kuga, S.; Okano, T. Mechanism of mercerization revealed by X-ray diffraction. *J. Wood Sci.* **2000.** *46* (6), 452-457.

Nishiyama, Y.; Langan, P.; Chanzy, H. Crystal structure and hydrogen-bonding system in cellulose I beta from synchrotron X-ray and neutron fiber diffraction. *J. Am. Chem. Soc.* **2002.** *124* (31), 9074-9082.

Nishiyama, Y.; Sugiyama, J.; Chanzy, H.; Langan, P. Crystal structure and hydrogen bonding system in cellulose I(alpha), from synchrotron X-ray and neutron fiber diffraction. *J. Am. Chem. Soc.* **2003.** *125* (47), 14300-14306.

Noe, P.; Chanzy, H. Swelling of *Valonia* cellulose microfibrils in amine oxide systems. *Can. J. Chem.-Rev. Can. Chim.* **2008.** *86* (6), 520-524.

Novo, L. P.; Bras, J.; Garcia, A.; Belgacem, N.; Curvelo, A. A. S. Subcritical water: A method for green production of cellulose nanocrystals. *ACS Sustain. Chem. Eng.* **2015.** *3* (11), 2839-2846.

Novo, L. P.; Bras, J.; Garcia, A.; Belgacem, N.; Curvelo, A. A. D. A study of the production of cellulose nanocrystals through subcritical water hydrolysis. *Ind. Crop. Prod.* **2016.** *93*, 88-95.

Oh, S. Y.; Yoo, D. I.; Shin, Y.; Kim, H. C.; Kim, H. Y.; Chung, Y. S.; Park, W. H.; Youk, J. H. Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and ftir spectroscopy. *Carbohydr. Res.* **2005.** *340* (15), 2376-2391.

Okano, T.; Sarko, A. Mercerization of cellulose .2. Alkali cellulose intermediates and a possible mercerization mechanism. *J. Appl. Polym. Sci.* **1985.** *30* (1), 325-332.

Olszewska, A.; Eronen, P.; Johansson, L. S.; Malho, J. M.; Ankerfors, M.; Lindstrom, T.; Ruokolainen, J.; Laine, J.; Osterberg, M. The behaviour of cationic nanofibrillar cellulose in aqueous media. *Cellulose* **2011**. *18* (5), 1213-1226.

Onsager, L. The effects of shape on the interaction of colloidal particles. *Annals New York Academy of Sciences* **1949.** *51*, 627-659.

Oun, A. A.; Rhim, J. W. Isolation of cellulose nanocrystals from grain straws and their use for the preparation of carboxymethyl cellulose-based nanocomposite films. *Carbohydr. Polym.* **2016.** *150*, 187-200.

Pahimanolis, N.; Hippi, U.; Johansson, L. S.; Saarinen, T.; Houbenov, N.; Ruokolainen, J.; Seppala, J. Surface functionalization of nanofibrillated cellulose using click-chemistry approach in aqueous media. *Cellulose* **2011**. *18* (5), 1201-1212.

Park, S.; Johnson, D. K.; Ishizawa, C. I.; Parilla, P. A.; Davis, M. F. Measuring the crystallinity index of cellulose by solid state C-13 nuclear magnetic resonance. *Cellulose* **2009**. *16* (4), 641-647.

Payen, A. Mémoire sur la composition du tissu propre des plantes et du ligneux. (memoir on the composition of the tissue of plants and of woody material.) *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* **1838.** 7, 1052-1056.

Pear, J. R.; Kawagoe, Y.; Schreckengost, W. E.; Delmer, D. P.; Stalker, D. M. Higher plants contain homologs of the bacterial CelA genes encoding the catalytic subunit of cellulose synthase. *Proc. Natl. Acad. Sci. U. S. A.* **1996.** *93* (22), 12637-12642.

Peng, B. L.; Dhar, N.; Liu, H. L.; Tam, K. C. Chemistry and applications of nanocrystalline cellulose and its derivatives: A nanotechnology perspective. *Can. J. Chem. Eng.* **2011.** *89* (5), 1191-1206.

Piau, J. M.; Dorget, M.; Palierne, J. F. Shear elasticity and yield stress of silica-silicone physical gels: Fractal approach. *J. Rheol.* **1999.** *43* (2), 305-314.

Pignon, F.; Magnin, A.; Piau, J. M.; Cabane, B.; Lindner, P.; Diat, O. Yield stress thixotropic clay suspension: Investigation of structure by light, neutron, and X-ray scattering. *Phys. Rev. E* 1997. 56 (3),

# 3281-3289.

Pranger, L.; Tannenbaum, R. Biobased nanocomposites prepared by in situ polymerization of furfuryl alcohol with cellulose whiskers or montmorillonite clay. *Macromolecules* **2008**, *41*, 8682-8687.

Rac-Rumijowska, O.; Fiedot, M.; Karbownik, I.; Suchorska-Wozniak, P.; Teterycz, H. Synthesis of silver nanoparticles in NMMO and their *in situ* doping into cellulose fibers. *Cellulose* **2017.** *24* (3), 1355-1370.

Rånby, B. G. Aqueous colloidal solutions of cellulose micelles. *Acta Chemica Scandinavica* **1949.** *3*, 649-650.

Raquez, J. M.; Murena, Y.; Goffin, A. L.; Habibi, Y.; Ruelle, B.; DeBuyl, F.; Dubois, P. Surfacemodification of cellulose nanowhiskers and their use as nanoreinforcers into polylactide: A sustainablyintegrated approach. *Compos. Sci. Technol.* **2012.** *72* (5), 544-549.

Revol, J. F. On the cross-sectional shape of cellulose crystallites in Valonia ventricosa. *Carbohydrate Polymers* **1982**, *2*, 123.

Revol, J. F.; Bradford, H.; Giasson, J.; Marchessault, R. H.; Gray, D. G. Helicoidal self-ordering of cellulose microfibrils in aqueous suspension. *Int. J. Biol. Macromol.* **1992.** *14* (3), 170-172.

Risteen, B.; Delepierre, G.; Srinivasarao, M.; Weder, C.; Russo, P.; Reichmanis, E.; Zoppe, J. Thermally switchable liquid crystals based on cellulose nanocrystals with patchy polymer grafts. *Small* **2018.** *14* (46), 10.

Rivkin, A.; Abitbol, T.; Nevo, Y.; Verker, R.; Lapidot, S.; Komarov, A.; Veldhuis, S. C.; Ziberman, G.; Reches, M.; Cranston, E. D.; Shoseyov, O. Bionanocomposite films from resilin-CBD bound to cellulose nanocrystals. *Industrial Biotechnology* **2015**. *11* (1), 44-58.

Rohaizu, R.; Wanrosli, W. D. Sono-assisted TEMPO oxidation of oil palm lignocellulosic biomass for isolation of nanocrystalline cellulose. *Ultrason. Sonochem.* **2017.** *34*, 631-639.

Rojas, O. J.; Montero, G. A.; Habibi, Y. Electrospun nanocomposites from polystyrene loaded with cellulose nanowhiskers. *J. Appl. Polym. Sci.* **2009.** *113* (2), 927-935.

Roohani, M.; Habibi, Y.; Belgacem, N. M.; Ebrahim, G.; Karimi, A. N.; Dufresne, A. Cellulose whiskers reinforced polyvinyl alcohol copolymers nanocomposites. *European Polymer Journal* **2008**. *44* (8), 2489-2498.

Rowland, S. P.; Roberts, E. J. The nature of accessible surfaces in the microstructure of cotton cellulose. *Journal of Polymer Science Part A-1: Polymer Chemistry* **1972.** *10* (8), 2447-2461.

Rueda, L.; d'Arlas, B. F.; Zhou, Q.; Berglund, L. A.; Corcuera, M. A.; Mondragon, I.; Eceiza, A. Isocyanate-rich cellulose nanocrystals and their selective insertion in elastomeric polyurethane. *Compos. Sci. Technol.* **2011.** *71* (16), 1953-1960.

Sacui, I. A.; Nieuwendaal, R. C.; Burnett, D. J.; Stranick, S. J.; Jorfi, M.; Weder, C.; Foster, E. J.; Olsson, R. T.; Gilman, J. W. Comparison of the properties of cellulose nanocrystals and cellulose nanofibrils isolated from bacteria, tunicate, and wood processed using acid, enzymatic, mechanical, and oxidative methods. *ACS Appl. Mater. Interfaces* **2014.** *6* (9), 6127-6138.

Sadeghifar, H.; Filpponen, I.; Clarke, S. P.; Brougham, D. F.; Argyropoulos, D. S. Production of cellulose nanocrystals using hydrobromic acid and click reactions on their surface. *J. Mater. Sci.* 2011. 46

(22), 7344-7355.

Saint-Michel, F.; Pignon, F.; Magnin, A. Fractal behavior and scaling law of hydrophobic silica in polyol. *J. Colloid Interface Sci.* **2003.** *267* (2), 314-319.

Salajkova, M.; Berglund, L. A.; Zhou, Q. Hydrophobic cellulose nanocrystals modified with quaternary ammonium salts. *J. Mater. Chem.* **2012.** *22* (37), 19798-19805.

Salas, C.; Nypelö, T.; Rodriguez-Abreu, C.; Carrillo, C.; Rojas, O. J. Nanocellulose properties and applications in colloids and interfaces. *Current Opinion in Colloid & Interface Science* **2014.** *19* (5), 383-396.

Sarko, A.; Muggli, R. Packing analysis of carbohydrates and polysaccharides. III. *Valonia* cellulose and cellulose II. *Macromolecules* **1974.** *7*, 486-494.

Sassi, J. F.; Chanzy, H. Ultrastructural aspects of the acetylation of cellulose. *Cellulose* **1995.** *2* (2), 111-127.

Satyamurthy, P.; Jain, P.; Balasubramanya, R. H.; Vigneshwaran, N. Preparation and characterization of cellulose nanowhiskers from cotton fibres by controlled microbial hydrolysis. *Carbohydr. Polym.* **2011.** *83* (1), 122-129.

Saxena, I. M.; Brown, R. M. Cellulose biosynthesis: Current views and evolving concepts. *Ann. Bot.* 2005. *96* (1), 9-21.

Schild, H. G. Poly (N-isopropylacrylamide) - experiment, theory and application. *Prog. Polym. Sci.* **1992.** *17* (2), 163-249.

Sèbe, G.; Ham-Pichavant, F.; Ibarboure, E.; Koffi, A. L. C.; Tingaut, P. Supramolecular structure characterization of cellulose II nanowhiskers produced by acid hydrolysis of cellulose I substrates. *Biomacromolecules* **2012**. *13* (2), 570-578.

Semenikhin, N. S.; Kadasala, N. R.; Moon, R. J.; Perry, J. W.; Sandhage, K. H. Individually dispersed gold nanoshell-bearing cellulose nanocrystals with tailorable plasmon resonance. *Langmuir* **2018.** *34* (15), 4427-4436.

Shafiei-Sabet, S.; Hamad, W. Y.; Hatzikiriakos, S. G. Rheology of nanocrystalline cellulose aqueous suspensions. *Langmuir* **2012.** *28* (49), 17124-17133.

Shang, W. L.; Huang, J.; Luo, H.; Chang, P. R.; Feng, J. W.; Xie, G. Y. Hydrophobic modification of cellulose nanocrystal via covalently grafting of castor oil. *Cellulose* **2013.** *20* (1), 179-190.

Shi, Z. Q.; Tang, J. T.; Chen, L.; Yan, C. R.; Tanvir, S.; Anderson, W. A.; Berry, R. M.; Tam, K. C. Enhanced colloidal stability and antibacterial performance of silver nanoparticles/cellulose nanocrystal hybrids. *J. Mat. Chem. B* **2015.** *3* (4), 603-611.

Sipahi-Saglam, E.; Gelbrich, M.; Gruber, E. Topochemically modified cellulose. *Cellulose* **2003.** *10* (3), 237-250.

Siqueira, G.; Bras, J.; Dufresne, A. New process of chemical grafting of cellulose nanoparticles with a long chain isocyanate. *Langmuir* **2009**, *26*, 402.

Sobkowicz, M. J.; Braun, B.; Dorgan, J. R. Decorating in green: Surface esterification of carbon and cellulosic nanoparticles. *Green Chem.* **2009.** *11* (5), 680-682.

Soderberg, T. *Organic chemistry with a biological emphasis*. University of Minnesota Morris Digital Well: Morris, **2012**.

Sugiyama, J.; Vuong, R.; Chanzy, H. Electron-diffraction study on the 2 crystalline phases occurring in native cellulose from an algal cell-wall. *Macromolecules* **1991.** *24* (14), 4168-4175.

Sun, B.; Hou, Q. X.; Liu, Z. H.; Ni, Y. H. Sodium periodate oxidation of cellulose nanocrystal and its application as a paper wet strength additive. *Cellulose* **2015.** *22* (2), 1135-1146.

Taipina, M. D.; Ferrarezi, M. M. F.; Yoshida, I. V. P.; Goncalves, M. D. Surface modification of cotton nanocrystals with a silane agent. *Cellulose* **2013**. *20* (1), 217-226.

Tan, X. Y.; Hamid, S. B. A.; Lai, C. W. Preparation of high crystallinity cellulose nanocrystals (CNCs) by ionic liquid solvolysis. *Biomass Bioenerg.* **2015.** *81*, 584-591.

Tanford, C. *The hydrophobic effect: Formatino of micelles and biological membranes*. 2 ed.; Krieger Publishing Company: Malabar, **1991**.

Tang, C. X.; Spinney, S.; Shi, Z. Q.; Tang, J. T.; Peng, B. L.; Luo, J. H.; Tam, K. C. Amphiphilic cellulose nanocrystals for enhanced pickering emulsion stabilization. *Langmuir* **2018.** *34* (43), 12897-12905.

Tang, J.; Sisler, J.; Grishkewich, N.; Tam, K. C. Functionalization of cellulose nanocrystals for advanced applications. *J Colloid Interface Sci* **2017.** *494*, 397-409.

Tang, J. T.; Shi, Z. Q.; Berry, R. M.; Tam, K. C. Mussel-inspired green metallization of silver nanoparticles on cellulose nanocrystals and their enhanced catalytic reduction of 4-nitrophenol in the presence of beta-cyclodextrin. *Industrial & Engineering Chemistry Research* **2015a.** *54* (13), 3299-3308.

Tang, L. R.; Huang, B.; Ou, W.; Chen, X. R.; Chen, Y. D. Manufacture of cellulose nanocrystals by cation exchange resin-catalyzed hydrolysis of cellulose. *Bioresour. Technol.* **2011.** *102* (23), 10973-10977.

Tang, Y. J.; Shen, X. C.; Zhang, J. H.; Guo, D. L.; Kong, F. G.; Zhang, N. Extraction of cellulose nano-crystals from old corrugated container fiber using phosphoric acid and enzymatic hydrolysis followed by sonication. *Carbohydr. Polym.* **2015b.** *125*, 360-366.

Tao, H.; Dufresne, A.; Lin, N. Double-network formation and mechanical enhancement of reducing endmodified cellulose nanocrystals to the thermoplastic elastomer based on click reaction and bulk crosslinking. *Macromolecules* **2019.** *52* (15), 5894-5906.

Tao, H.; Lavoine, N.; Jiang, F.; Tang, J. T.; Lin, N. Reducing end modification on cellulose nanocrystals: Strategy, characterization, applications and challenges. *Nanoscale Horiz.* **2020.** *5* (4), 607-627.

Tavakolian, M.; Lerner, J.; Tovar, F. M.; Frances, J.; van de Ven, T. G. M.; Kakkar, A. Dendrimer directed assembly of dicarboxylated hairy nanocellulose. *J. Colloid Interface Sci.* **2019**. *541*, 444-453.

Terech, P.; Chazeau, L.; Cavaille, J. Y. A small-angle scattering study of cellulose whiskers in aqueous suspensions. *Macromolecules* **1999.** *32* (6), 1872-1875.

Thakur, V. K. *Nanocellulose polymer nanocomposites: Fundamentals and applications*. John Wiley & Sons: **2015**.

Thomas, B.; Raj, M. C.; B, A. K.; H, R. M.; Joy, J.; Moores, A.; Drisko, G. L.; Sanchez, C. Nanocellulose, a versatile green platform: From biosources to materials and their applications. *Chem Rev* 

**2018.** *118* (24), 11575-11625.

Trache, D.; Hussin, M. H.; Haafiz, M. K. M.; Thakur, V. K. Recent progress in cellulose nanocrystals: Sources and production. *Nanoscale* **2017**. *9* (5), 1763-1786.

Tulos, N.; Harbottle, D.; Hebden, A.; Goswami, P.; Blackburn, R. S. Kinetic analysis of cellulose acetate/cellulose II hybrid fiber formation by alkaline hydrolysis. *ACS Omega* **2019.** *4* (3), 4936-4942.

Ulman, A. Formation and structure of self-assembled monolayers. Chem. Rev. 1996. 96 (4), 1533-1554.

Vanderhart, D. L.; Atalla, R. H. Studies of microstructure in native celluloses using solid-state C-13 NMR. *Macromolecules* **1984.** *17* (8), 1465-1472.

Villares, A.; Moreau, C.; Dammak, A.; Capron, I.; Cathala, B. Kinetic aspects of the adsorption of xyloglucan onto cellulose nanocrystals. *Soft Matter* **2015.** *11* (32), 6472-6481.

Villares, A.; Moreau, C.; Cathala, B. Star-like supramolecular complexes of reducing-end-functionalized cellulose nanocrystals. *ACS Omega* **2018**. *3* (11), 16203-16211.

Vollhardt, K. P. C.; Schore, N. E. Organic chemistry. Macmillan International Higher Education: 2014.

Williamson, R. E.; Burn, J. E.; Hocart, C. H. Towards the mechanism of cellulose synthesis. *Trends Plant Sci.* **2002.** *7* (10), 461-467.

Willstätter, R.; Zechmeister, L. Zur kenntnis der hydrolyse von cellulose I. . Berichte der deutschen chemischen Gesellschaft 1913. 46, 2401-2412.

Wohlhauser, S.; Delepierre, G.; Labet, M.; Morandi, G.; Thielemans, W.; Weder, C.; Zoppe, J. O. Grafting polymers from cellulose nanocrystals: Synthesis, properties, and applications. *Macromolecules* **2018.** *51* (16), 6157-6189.

Wu, X. D.; Lu, C. H.; Zhang, W.; Yuan, G. P.; Xiong, R.; Zhang, X. X. A novel reagentless approach for synthesizing cellulose nanocrystal-supported palladium nanoparticles with enhanced catalytic performance. *J. Mater. Chem. A* **2013.** *l* (30), 8645-8652.

Wu, X. D.; Lu, C. H.; Zhou, Z. H.; Yuan, G. P.; Xiong, R.; Zhang, X. X. Green synthesis and formation mechanism of cellulose nanocrystal-supported gold nanoparticles with enhanced catalytic performance. *Environ.-Sci. Nano* **2014.** *1* (1), 71-79.

Xu, Y.; Atrens, A. D.; Stokes, J. R. Rheology and microstructure of aqueous suspensions of nanocrystalline cellulose rods. *J. Colloid Interface Sci.* **2017.** *496*, 130-140.

Xu, Y.; Atrens, A. D.; Stokes, J. R. "Liquid, gel and soft glass" phase transitions and rheology of nanocrystalline cellulose suspensions as a function of concentration and salinity. *Soft Matter* **2018.** *14* (10), 1953-1963.

Xu, Y. L.; Salmi, J.; Kloser, E.; Perrin, F.; Grosse, S.; Denault, J.; Lau, P. C. K. Feasibility of nanocrystalline cellulose production by endoglucanase treatment of natural bast fibers. *Ind. Crop. Prod.* **2013.** *51*, 381-384.

Yang, H.; van de Ven, T. G. M. A bottom-up route to a chemically end-to-end assembly of nanocellulose fibers. *Biomacromolecules* **2016.** *17* (6), 2240-2247.

Yang, Q. A.; Pan, X. J. A facile approach for fabricating fluorescent cellulose. J. Appl. Polym. Sci. 2010. 117 (6), 3639-3644.

Yang, Z. Y.; Wang, W. J.; Shao, Z. Q.; Zhu, H. D.; Li, Y. H.; Wang, F. J. The transparency and mechanical properties of cellulose acetate nanocomposites using cellulose nanowhiskers as fillers. *Cellulose* **2013**. *20* (1), 159-168.

Yi, J.; Xu, Q. X.; Zhang, X. F.; Zhang, H. L. Chiral-nematic self-ordering of rodlike cellulose nanocrystals grafted with poly(styrene) in both thermotropic and lyotropic states. *Polymer* **2008.** *49* (20), 4406-4412.

Yi, J.; Xu, Q. X.; Zhang, X. F.; Zhang, H. L. Temperature-induced chiral nematic phase changes of suspensions of poly(N,N-dimethylaminoethyl methacrylate)-grafted cellulose nanocrystals. *Cellulose* **2009.** *16* (6), 989-997.

Yokota, S.; Kitaoka, T.; Opietnik, M.; Rosenau, T.; Wariishi, H. Synthesis of gold nanoparticles for *in situ* conjugation with structural carbohydrates. *Angew. Chem.-Int. Edit.* **2008.** 47 (51), 9866-9869.

Young, R. A.; Rowell, R. M. Cellulose: Structure, modification, and hydrolysis. John Wiley & Sons: 1986.

Yu, H. Y.; Qin, Z. Y.; Liang, B. L.; Liu, N.; Zhou, Z.; Chen, L. Facile extraction of thermally stable cellulose nanocrystals with a high yield of 93 % through hydrochloric acid hydrolysis under hydrothermal conditions. *J. Mater. Chem. A* **2013.** *1* (12), 3938-3944.

Yuan, H. H.; Nishiyama, Y.; Wada, M.; Kuga, S. Surface acylation of cellulose whiskers by drying aqueous emulsion. *Biomacromolecules* **2006.** *7* (3), 696-700.

Yue, Y. Y.; Zhou, C. J.; French, A. D.; Xia, G.; Han, G. P.; Wang, Q. W.; Wu, Q. L. Comparative properties of cellulose nano-crystals from native and mercerized cotton fibers. *Cellulose* **2012**. *19* (4), 1173-1187.

Yue, Y. Y.; Han, J. Q.; Han, G. P.; Zhang, Q. G.; French, A. D.; Wu, Q. L. Characterization of cellulose I/II hybrid fibers isolated from energycane bagasse during the delignification process.: Morphology, crystallinity and percentage estimation. *Carbohydr. Polym.* **2015**. *133*, 438-447.

Zhang, Y.; Tian, Z. J.; Fu, Y. J.; Wang, Z. J.; Qin, M. H.; Yuan, Z. W. Responsive and patterned cellulose nanocrystal films modified by N-methylmorpholine-N-oxide. *Carbohydr. Polym.* **2020.** *228*, 8.

Zhang, Z.; Sebe, G.; Wang, X. S.; Tam, K. C. Gold nanoparticles stabilized by poly(4-vinylpyridine) grafted cellulose nanocrystals as efficient and recyclable catalysts. *Carbohydr. Polym.* **2018**. *182*, 61-68.

Zhou, Q.; Brumer, H.; Teeri, T. T. Self-organization of cellulose nanocrystals adsorbed with xyloglucan oligosaccharide-poly(ethylene glycol)-polystyrene triblock copolymer. *Macromolecules* **2009.** *42* (15), 5430-5432.

Zimmermann, R.; Muller, Y.; Freudenberg, U.; Jehnichen, D.; Potthast, A.; Rosenau, T.; Werner, C. Oxidation and structural changes in NMMO-regenerated cellulose films. *Cellulose* **2016.** *23* (6), 3535-3541.

Zoppe, J. O.; Habibi, Y.; Rojas, O. J.; Venditti, R. A.; Johansson, L. S.; Efimenko, K.; Osterberg, M.; Laine, J. Poly(N-isopropylacrylamide) brushes grafted from cellulose nanocrystals via surface-initiated single-electron transfer living radical polymerization. *Biomacromolecules* **2010**. *11* (10), 2683-2691.

Zoppe, J. O.; Osterberg, M.; Venditti, R. A.; Laine, J.; Rojas, O. J. Surface interaction forces of cellulose nanocrystals grafted with thermoresponsive polymer brushes. *Biomacromolecules* **2011.** *12* (7), 2788-2796.

Zoppe, J. O.; Venditti, R. A.; Rojas, O. J. Pickering emulsions stabilized by cellulose nanocrystals grafted with thermo-responsive polymer brushes. *J. Colloid Interface Sci.* **2012.** *369*, 202-209.

Zoppe, J. O.; Dupire, A. V. M.; Lachat, T. G. G.; Lemal, P.; Rodriguez-Lorenzo, L.; Petri-Fink, A.; Weder, C.; Klok, H. A. Cellulose nanocrystals with tethered polymer chains: Chemically patchy versus uniform decoration. *ACS Macro Lett.* **2017.** *6* (9), 892-897.

Zuluaga, R.; Putaux, J. L.; Cruz, J.; Velez, J.; Mondragon, I.; Ganan, P. Cellulose microfibrils from banana rachis: Effect of alkaline treatments on structural and morphological features. *Carbohydr. Polym.* **2009.** *76* (1), 51-59.

#### Résumé en français

Les nanocristaux de cellulose (NCC) ont été identifiés comme des briques de base particulièrement attractives pour la conception de matériaux biosourcés innovants. Ces nanoparticules sont en effet issues d'une source abondante et renouvelable, les fibres de cellulose, et possèdent des propriétés exceptionnelles : très grande surface spécifique, faible densité, non-toxicité, biocompatibilité et propriétés mécaniques comparables à celles du Kevlar. Une caractéristique encore peu exploitée de ces nanoobjets est leur polarité chimique. En effet, la biosynthèse conduit à des extrémités différentes des bâtonnets d'un point de vue chimique, ce qui permet de générer des NCC fonctionnalisés de manière asymétrique. En plus de l'utilisation de la cellulose native, il est possible de produire des NCC constitués de l'allomorphe II de la cellulose (NCC-II). Les NCC-II présentent une géométrie assez similaire à celle des NCC, mais les deux extrémités des bâtonnets peuvent être modifiées chimiquement. Ce projet de thèse s'est donc concentré sur de nouvelles stratégies pour modifier efficacement de manière régiosélective les NCC et les NCC-II afin de générer des assemblages innovants et fonctionnels. Tout d'abord, une stratégie de croissance in situ a été développée pour optimiser de manière drastique le marquage régiosélectif des NCC et NCC-II avec des nanoparticules d'or par rapport aux données de la littérature (le rendement du marquage est passé d'environ 15 à 80%). Ce développement nous a permis d'approfondir les connaissances fondamentales sur les nanocristaux en confirmant l'arrangement antiparallèle des chaînes de cellulose dans les NCC-II et en montrant que les NCC dérivés du coton sont constitués d'un assemblage parallèle de cristallites élémentaires chimiquement polaires. Deuxièmement, les deux types de particules ont été fonctionnalisées de manière régiosélective avec des chaînes de polymères thermosensibles en utilisant une stratégie en deux étapes d'oxydation suivie d'un couplage peptidique. Dans le cas des NCC, les particules hybrides résultantes s'assemblent sous l'effet d'une augmentation de la température en agrégats sous forme d'étoile composés de 3 à 6 nanocristaux fixés par leurs extrémités. En utilisant les NCC-II, une association réversible déclenchée par la température en réseaux supra-microniques est obtenue par chaînage des NCC-II modifiés. Les caractéristiques structurales de ces nouveaux objets et de leurs assemblages ont été étudiées par microscopie électronique à transmission, diffusion dynamique de la lumière et la diffusion des rayons X ou des neutrons aux petits angles. Des mesures de rhéologie ont démontré que dans les deux cas, au-dessus de la LCST des chaînes de polymères greffés, on obtient un comportement de type gel mais que la structure du réseau conduit à des effets plus importants que les complexes en forme d'étoile. Enfin, l'optimisation du processus de greffage a été étudiée et l'utilisation de DCC/DMAP ou de 4-PPY comme catalyseurs et du DMF comme solvant s'est avérée la plus efficace. L'utilisation du N-oxyde de N-Méthylmorpholine (NMMO) dans le but d'induire un gonflement des extrémités des NCC et de favoriser la réaction a également été étudiée. Cependant, aucun gonflement n'a pu être détecté mais le traitement avec NMMO a eu un effet notable sur la séparation des cristallites élémentaires formant les NCC. Comme les modifications entreprises concernent une fraction très réduite des unités anhydroglucose disponibles, une caractérisation quantitative directe de la modification régiosélective des NCC reste difficile, même si l'utilisation de techniques avancées telles que les méthodes de diffusion donnent des informations fructueuses. Toutefois, les travaux menés montrent qu'une telle fonctionnalisation localisée, associée à l'utilisation de particules biosourcées, permet un contrôle fin de l'assemblage en structures innovantes qui donnent naissance à de nouvelles propriétés macroscopiques.

#### English abstract

Cellulose nanocrystals (CNCs) have been identified as highly attractive building blocks for the design of innovative biosourced materials. These nanoparticles are indeed derived from an abundant and renewable source, cellulose fibers, and possess exceptional properties such as a very large surface area, a low density, non-toxicity, biocompatibility and mechanical properties comparable to those of Kevlar. An interesting feature of these nano-objects, which has not been widely exploited yet, is their chemical polarity. Indeed, the biosynthesis leads to different extremities of the rods from a chemical point of view, which makes it possible to generate asymmetrically functionalized CNCs. Alternatively, CNCs made of the allomorph II of cellulose (CNC-II) can also be produced. CNC-II exhibit a rather similar geometry as CNCs but both rod ends are amenable to chemical modification. This thesis project has thus focused on new strategies to efficiently modify in a regioselective manner CNCs and CNC-II particles in order to generate innovative and functional assemblies. First, an in situ growth strategy was developed to drastically optimize the regioselective labelling of CNCs and CNC-II with gold nanoparticles when compared to literature data (labelling yield increased from about 15 to 80%). This development allowed us to get insight into fundamental morphological features by confirming the antiparallel packing of cellulose chains in CNC-II and by showing that CNCs derived from cotton are made of a parallel assembling of chemically polar elementary crystallites. Secondly, both types of nanocellulose particles were successfully regioselectively functionalized with thermosensitive polymer chains using a two-step oxidation and peptide coupling strategy. In the case of CNCs, the resulting hybrid particles underwent a thermally induced-aggregation into star-shaped aggregates composed of 3 to 6 nanocrystals attached by their ends. Using CNC-II particles, a reversible temperature-triggered association into supra-micronic networks could be obtained through end-to-end attachment of the cellulose rods. The structural features of these new objects and their assemblies were characterized by transmission electron microscopy, dynamic light scattering and small angle X-ray or neutron scattering. Rheology measurements were used to show that in both cases, above the LCST of the grafted polymer chains, a gel-like behavior is obtained but the network structure led to stronger effects than the star-shaped complexes. Finally, the optimization of the grafting process was investigated and the use of DCC/DMAP or 4-PPY as catalysts of the peptide coupling and DMF as the solvent turned out to be the best conditions. The use of N-Methylmorpholine N-oxide (NMMO) to induce a swelling of the CNC ends and favor the reaction was also studied. However, no swelling could be detected but the treatment with NMMO had a noticeable effect of separating the elementary crystallites forming the CNCs. Since the undertaken modifications concern a very reduced fraction of the available anhydroglucose units, a quantitative direct characterization of the regioselective derivatization of CNCs remains challenging, even if the use of advanced techniques such as scattering methods give fruitful information. However, the present work shows that such a site-selective functionalization coupled with the use of biosourced particles allows a fine tuning of stimuli-sensitive assembling into innovative structures that give rise to new macroscopic properties.