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Active, Multi-functional Biopolymer Interfacial Constructs: Beyond Structural Nanocomposites

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14. ABSTRACT

Naturally derived polymers, produced by plants, animals, and micro-organisms as the main components of their bodies, have the ability to self-organize into complex structures and function as systems possessing hierarchical and long-range order. These structures demonstrate good mechanical properties, lightweight, renewability, and prospective low-cost combined with prospective biocompatibility and biodegradability. The overall goal of this project is to leverage the exceptional properties of bioderived materials in order to create novel multifunctional nanocomposites based on the integration of synthetic and bioderived materials. We consider a combination of assembly approaches such as guided assembly, spontaneous selfassembly, 2D printing and lithographic techniques to fabricate bionanocomposites with patterned electrical conductivity for the generation of micro- and nanoscale organized functional bio-enabled materials with matched biological and synthetic materials. In the course of this study, we have demonstrated how biopolymeric matrix of cellulose nanocrystals (CNCs) can improve photonic properties of carbon quantum dots; how robust hybrid composites can be fabricated by directed assembly; how CNCs can be carefully organized in stack with cellulose nanofibers, as well as other amorphous polymers for improved mechanical strength and toughness, all while maintaining their vivid structural color and chiral nematic morphology. For mixed bundles, stress–strain plots show significant plastic deformation, and a higher resistance was observed for the mixed samples. Overall, the resulting strength of co-assembled composites is much higher than that of regular CNCs, and there is significantly higher strain energy for limited bundles. Furthermore, we considered the integration of synthetic optically active nanocomponents into organized hierarchical biopolymer frameworks for added optical functionalities, such as enhanced iridescence and chiral photoluminescence for emerging photonic applications.

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Final Report
Annual Performance Report
FA9550-17-1-0297: Active Multifunctional Biopolymer Nanocomposites - Beyond Structural Materials

July 2019 - June 2020

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Project Objectives

Naturally derived polymers, produced by plants, animals, and micro-organisms as the main components of their bodies, have the ability to self-organize into complex structures and function as systems possessing hierarchical and long-range order. These structures demonstrate good mechanical properties, lightweight, renewability, and prospective low-cost combined with prospective biocompatibility and biodegradability. Therefore, they are attractive candidates for high-performance and functional bionanocomposites thanks their abovementioned properties, flexibility, and versatile surface chemistry resulting in availability of multiple reactive sites for introducing novel functionalities.

The materials in this project- *bio-derived nanocomposites- have advanced characteristics to meet the current and future needs of the Department of Defense and United States Air Force.* Their advanced characteristics combine the strength and functionality of constituent materials yielding in enhanced functional utilities. This array of unique properties offers an opportunity to create devices that are not only strong and flexible, but that better assure mission success through chemical sensing, actuating, and smart-shielding. Because of their abundant functional groups, multi-leveled organization, and easily reconfigured secondary structure, biopolymers can produce complementary interfaces with various synthetic components with tailored functionalities. This arises *the overall goal of this project*- to leverage the exceptional properties of **bioderived materials** in order to **create novel multifunctional nanocomposites based on the integration of synthetic and bio-derived materials.**

Specifically, we focused on:

- Studying *organization and fundamental mechanisms driving bio-derived materials' assembly* in various bio-polymers such as cellulose nanocrystals, silk fibroin and marine structural protein- suckerin-12.

- a *critical* ***understanding of fundamental mechanisms*** underlying the strengthening, toughening, and reinforcement between constituent biotic/synthetic components in organized bionanocomposites **with added functionalities**.
- Implementing *heterogeneous surface chemistry of bio-derived components, their topology* and global geometry for their tuned assembly, interfacial behavior and phase enhancement in **functional organized bionanocomposites**.
- *a combination of assembly approaches* such as guided assembly, spontaneous self-assembly, 2D printing and lithographic techniques to fabricate bionanocomposites with patterned electrical conductivity for the generation of micro- and nanoscale **organized functional bio-enabled materials with matched biological and synthetic materials**.
- ***Integrating bio-polymers with synthetic materials*** such as carbon quantum dots or 2D MXenes for enhanced performance, dispersion stability, while studying their effect on the overall assembly, and critical functional properties.

Over the past year, we have demonstrated how biopolymeric matrix of cellulose nanocrystals (CNCs) can improve photonic properties of carbon quantum dots; how CNCs can be carefully organized in stack with cellulose nanofibers, as well as other amorphous polymers for improved mechanical strength and toughness, all while maintaining their vivid structural color; and studied how silk bioencapsulation protects 2D $Ti_3C_2T_x$ MXene flakes from surface oxidation.

This cross-disciplinary project further enhances the ongoing collaboration with AFRL researchers from Functional Materials (T. Bunning) and Human Signature Branch (N. Kelley-Loughnane). The collaborative efforts are facilitated by multiple personnel visits, student exchange program, participation of AFRL researchers as co-advisors for GT students, summer student internships at AFRL, joint presentations and publications, and the mutually beneficial transfer of technologies relevant to long-term fundamental research interests at USAF. Students who participate in the project constantly interact with AFRL researchers, and will mature as many-sided scientists deeply involved in USAF-relevant fundamental studies.

Ongoing collaboration with academic labs brings to the PI's lab novel biomolecules such as silk fibroin from D. Kaplan, Bioengineering Department, Tufts. Additionally, collaboration with Dr. Y. Yingling at the North Carolina State University provides input from large molecular dynamic simulations of the cellulose molecules within different systems and conditions.

The current project provides or have provided full or partial financial support for one postdoc and three graduate students. A postdoc, Dr. Rui Xiong worked on the assembly of high-performance nanocellulose nanocomposites with superior robustness and multi-functionalities (added conductivity, photoluminescence, and photonic ability of cellulose-based nanocomposites). Katarina Adstedt (2018-present) investigated the assembly behavior of cellulose nanocrystals in combination with amorphous bio-polymers; Michelle Krecker is working on the advanced mechanical characterization and assembly of natural polymer nanocomposites with 2D materials; Daria Bukharina's task is in understanding the fundamentals of the cellulose nanocrystals' assembly and the origin of the chirality in polysaccharides nanocrystals.

Summary of Major Results for the past year is presented below.

Biopolymeric photonic structures: design, fabrication, and emerging applications. A comprehensive review on how biological photonic structures can precisely control light propagation, scattering, and emission via hierarchical structures and diverse chemistry, therefore, enabling biophotonic applications for transparency, camouflaging, protection, mimicking and signaling, was published. In this review, we provided a summary of the light phenomena in biophotonic structures found in nature, the selection of corresponding biopolymers for synthetic photonic structures, the fabrication strategies for flexible photonics, and corresponding emerging photonic-related applications (Figure 1). We introduced various photonic structures, including multi-layered, opal, and chiral structures, as well as photonic networks in contrast to traditionally considered light absorption and structural photonics. The bottom-up and top-down fabrication approaches and physical properties of organized biopolymers were summarized and the advantages of biopolymers as building blocks for realizing unique bioenabled photonic structures-highlighted. Furthermore, we considered the integration of synthetic optically active nanocomponents into organized hierarchical biopolymer frameworks for added optical functionalities, such as enhanced iridescence and chiral photoluminescence. Finally, an outlook on current trends in biophotonic materials design and fabrication, current issues, critical needs, as well as promising emerging photonic applications were presented.

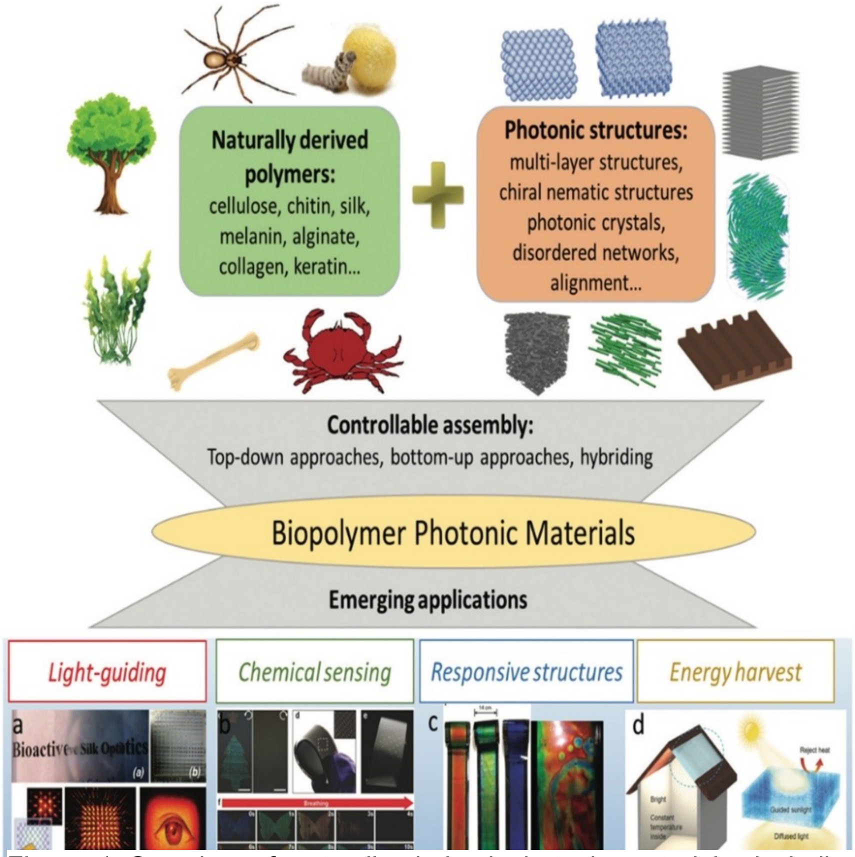

Figure 1 Overview of naturally derived photonic materials, including biopolymer selection, photonic structures, construction strategies, and emerging applications. (a) Silk optical elements. Reprinted with permission from the American Chemical Society, copyright 2008. (b) Cellulose photonic sensors. Reprinted with permission from Wiley, copyright 2018. (c) Responsive cellulose nanocrystal photonics. Reprinted with permission from Springer Nature, copyright 2018. (d) Transparent wood for light harvesting. Wiley, copyright 2016.

Marine Structural Protein Stability Induced by Hofmeister Salt Annealing and Enzymatic Cross-Linking. Natural materials including cellulose, silk, and chitin produced by plants, insects, and crustaceans find vast utilization due to their mechanical tunability, susceptibility to chemical modification, and bioabundance. In this work we focused on the popular suckerin-12 isoform to understand what makes the secondary structure of this biopolymer different in water and the potential role of diverse physical and chemical cross-linkings. By choosing a salt post-treatment, in accordance with the Hofmeister series, we achieved film stability with salt annealing that is comparable to chemical cross-links (Figure 2). By correlating the film morphology with the protein secondary structure changes, suckerin-12 films were shown to contract upon treatment with kosmotropic salts and exhibited increased stability in water. These changes are related to the rearrangement of suckerin-12 secondary structure from random coils and helices to β-sheets. Overall, understanding secondary structure changes caused by aqueous and ionic environments are necessary for the tuning of the suckerin film sclerotization, its conversion to a tough biological material, and ultimately to produce the natural squid sucker ring teeth.

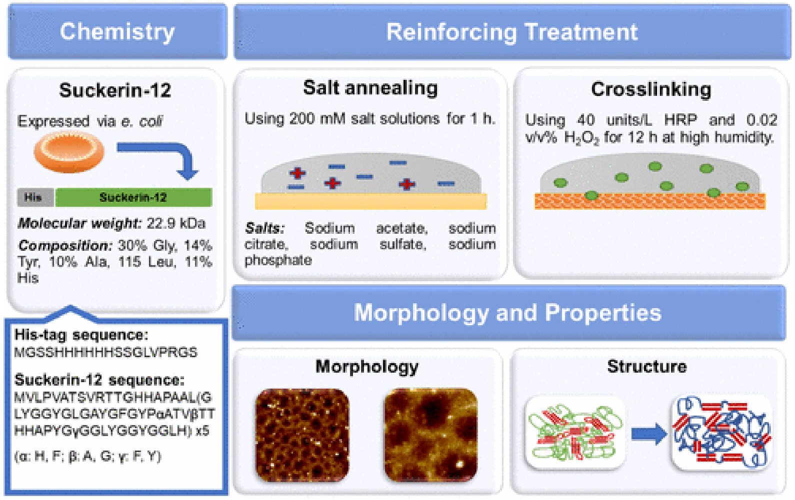

Figure 2. Scheme detailing suckerin-12 protein sequence and composition, how sodium salt annealing and enzymatic cross-linking are done on top of deposited protein, AFM images showing morphological dewetting patterns due to spin assisted deposition, and suggested molecular structure changes in response to

Large and Emissive Crystals from Carbon Quantum Dots onto Interfacial Organized Templates. Carbon quantum dots (CQDs) have several advantages compared to inorganic quantum dots- they are water soluble, have diverse surface chemistry due to the presence of both hydrophobic C-C/C=C and hydrophilic OH/NH groups, and demonstrate low toxicity. The challenge in CQDs' application is that their organized structures length scale is usually limited to nanoscale. In this work, we reported template-assisted assembly of CQDs nanocrystals on organized cellulose nanocrystals templates at the liquid-air interface.

The hierarchical crystal growth of the CQDs is proposed to be concluded in three stages. In the initial stage, negatively charged CNC surface facets stabilize CQDs to prevent aggregation and form homogeneous dispersion supported by hydrogen bonding, Columbic, and hydrophobic interactions. Then, CNCs play role of organized cargo carrier to control and guide quantum dots' adsorption onto the liquid-air interface. And finally, in the last stage crystals stack in layered manner, with each new CQD selectively stack on the hydrophobic regions of pre-formed monolayers via $\pi - \pi$ interactions and on hydrophilic facets via hydrogen bonding, resulting in growth in z-direction. This packing mode results in highly ordered, dense crystals that can hinder quenching phenomenon common for random CQD aggregation. Further confocal fluorescent laser scanning microscopy of the resulting CQDs unveils high and uniform light emission- the CQDs crystals exhibit more than 20 times higher photoluminescent (PL) intensity than the aggregates (Figure 3). We suggested that this is due to the resulting packing of the nanocrystals as they are bound to different facets within monolayers resulting in suppression of molecular rotation and restriction of the intermolecular energy transfer which allows photons to be constantly emitted. Moreover, by implementing the amphiphilic nature of the nanocrystals, we introduced different amphiphilic ligands which resulted in growth of diverse CQDs crystals, including long fibers, microplates and micro-flowers (Figure 3). To our best knowledge, this is the first example of growing large-scale highly ordered highly emissive microcrystals from amphiphilic CQDs.

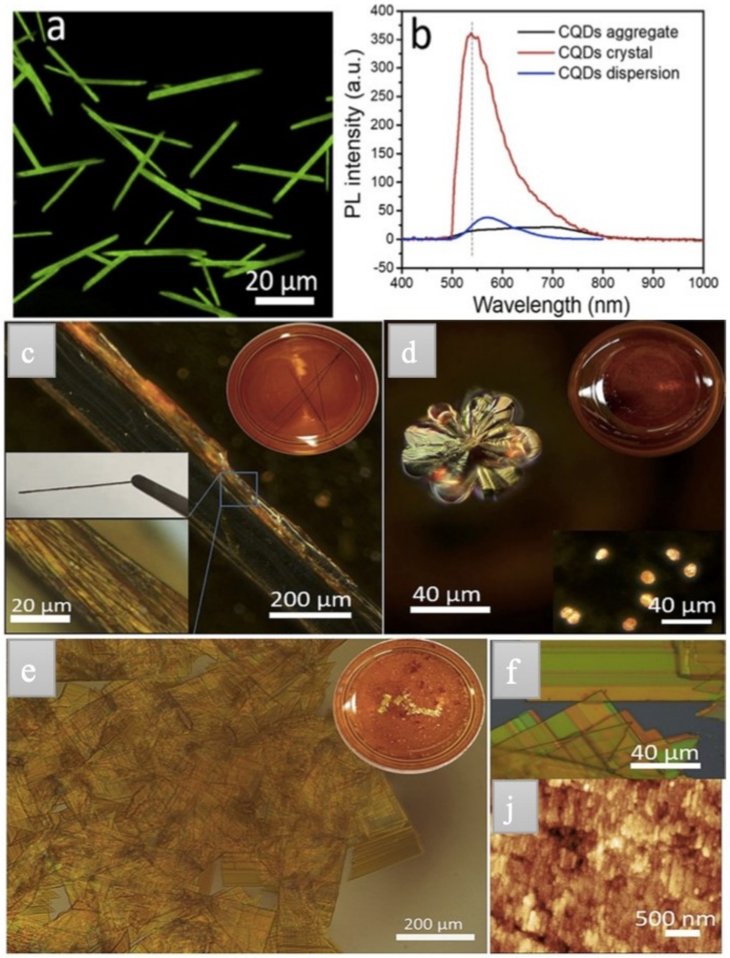

Figure 3 a) The CLSM image of CQDs crystals at excitation wavelength of 488 nm. b) The PL spectra of CQDs dispersions, CQDs crystals, and CQDs aggregates as collected from different locations. The optical microscopy image of the ultralong CQD crystal with tween 60 (c), the CQD microflower with PVP (d), and the CQD microplates with CTAB (e). f) The magnified optical microscopy image of CQD microplates. j) The AFM image of CQD microplate surface, demonstrating layering of CQD nanocrystals. Inset (top right) in (c), (d), and (e): the photos of the responding crystals dispersions. Inset (bottom left) in (c): the photo of a freestanding ultralong crystal handed by a regular tweezer. Inset (bottom right) in (d): the optical microscopy image of CQD discs formed with PVP after 1 day.

Alternating Stacking of Nanocrystals and Nanofibers into Ultrastrong Chiral Biocomposite Laminates. Design and fabrication of hierarchical laminated CNC-based composite films with alternating cellulose nanocrystals (CNC) and cellulose nanofibers (CNF) layers were reported. Due to such alternating stacking the resulted films adapt a combination of improved mechanical characteristics while preserving structural coloration despite high CNF content up to 23 wt.%, never achieved before. For example, C3F2 stacked film which consists of 3 layers of CNC and 2 layers of CNF, demonstrated enhanced compliance with maximum strain value of 2.6±0.14% and a high ultimate strength at 152±9.4 MPa. Notably, toughness of the same film increased dramatically to 2.7±0.06 MJm^{-3} from pristine CNC film's 0.07±.012MJm^{-3} – 40-fold (Figure 4). We suggest that in the demonstrated alternating CNC-CNF stacks, the CNF layer acts as the bridging ductile component as well as mechanical buffer preventing cracks originating within the brittle CNC phase.

The CNC layers, in their turn, ensure efficient binding and loading transfer between the two phases resulting in improved toughness and strength. Simultaneously attained enhanced mechanical performance and vivid iridescence in the realm of randomized CNC-based chiral nematic composite materials is rarely achieved. Furthermore, our films demonstrated outstanding out-of-plane stiffness and in-plane compliance- can be folded 180° repeatedly, coiled into rolls, and even tied into a knot, without any discernible damage (Figure 4).

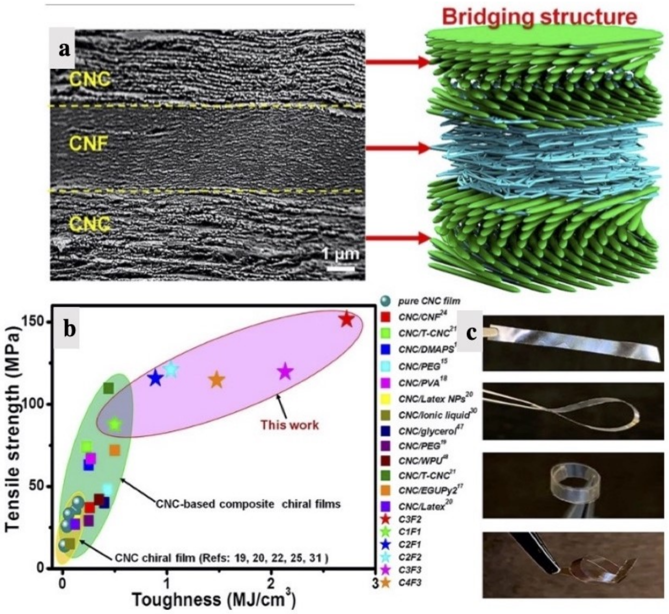

Figure 4 (a) SEM image of stacked morphology and the corresponding organization of the composite film. (b) Ashby plot of tensile strength *vs* toughness of the stacked CNC/CNF films and the previously reported CNC-based chiral nematic films (yellow, green, and pink regions represent the pristine CNC films, CNC-based composite films, and multilayered CNC/CNF films, respectively; (c) Out-of-plane ductility tests for the 5-layer C3F2 film (twisting and bending).

Self-Assembly of Emissive Nanocellulose/Quantum Dot Nanostructures for Chiral Fluorescent Materials. Self-assembled emissive CQD - decorated CNC nanostructure for flexible robust chiral fluorescent materials is reported. Nanostructures resulting spontaneous decoration of polymer-stabilized CQD around the CNC nanocrystals, can self-organize into the fluorescent chiral liquid crystalline phase and further preserve this chiral morphology in flexible solid films. The films, achieved via solvent-evaporation-induced self-assembly in ambient environment, demonstrate characteristic fluorescent fingerprint that has never been observed for claimed chiral films. Those films demonstrate right-handed chiral fluorescence with asymmetric factor of - 0.2 (Figure 5).

The fluorescence of these robust free-standing films is 2 orders higher than that for pure CQD films, as well as for reported CQD/CNC films, which we attribute to the formation of chiral morphologies rather than random distribution of the core/shell nanostructures. Increase in ultimate strength is reported [62 ± 5 MPa (CQD/CNC film) vs 40 ± 2 MPa (control CNC film)] and ultimate strain [1.2% (CQD/CNC film) vs 0.6% (control CNC film)], and resulted in a 3-fold higher toughness of ~0.50 ± 0.06 MJ/m^3 with 10 wt % CQD loading, all in combination with the increased elastic modulus of 10 ± 2 GPa (Figure 5). Enhanced structural colors with selective light reflection and emissive fluorescent patterns were further realized via soft lithography and 2D chemical printing.

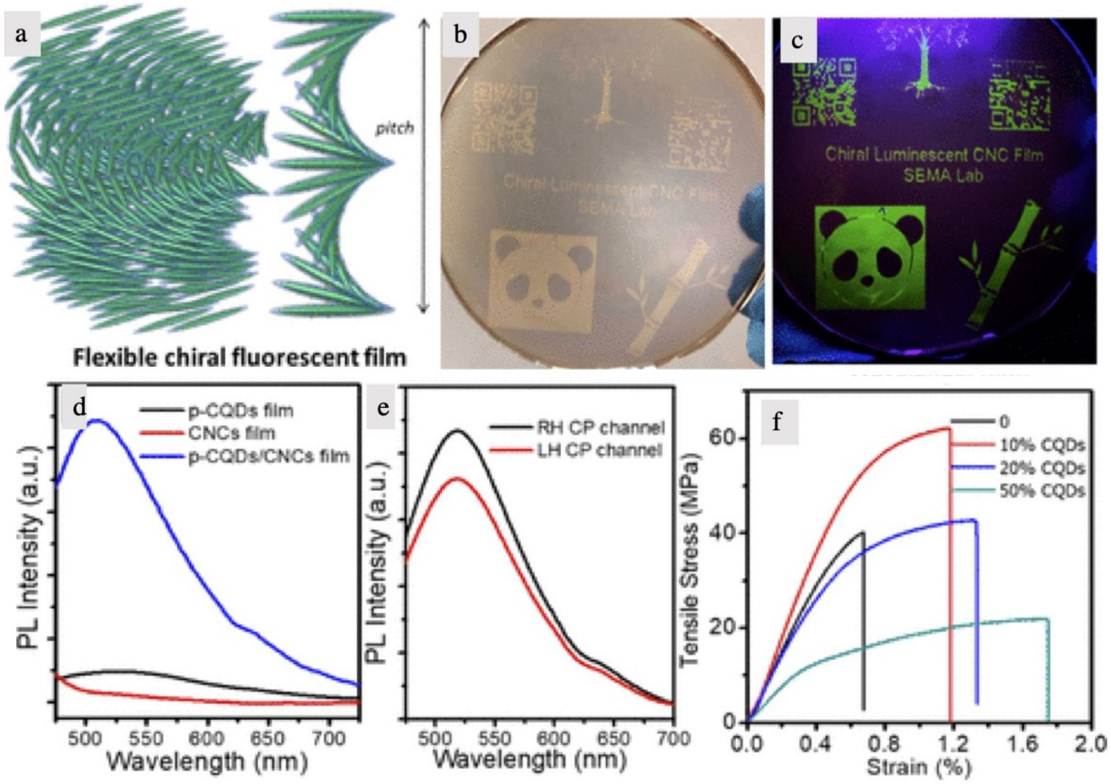

Figure 5. (a) Self-Assembly of Chiral Fluorescent CQD/CNC Nanostructure. Patterned chiral luminescent CNC films with 10 cm diameter under natural light (b) and at 365 nm UV light (c). (Patterns are designed by the authors.) (d) PL spectra of *p*-CQDs, CNC, and *p*-CQD/CNC films. (e) PL spectra of CQD/CNC solid films under left- and right-hand (RH and LH) circular polarizers (CP). (f) Representative stress–strain curves of *p*-CQD/CNC films with different CQD loading.

Integration of Optical Surface Structures with Chiral Nanocellulose for Enhanced Chiroptical Properties. Facile and efficient surface patterning approach to integrate various photonic surface elements onto freestanding chiral CNC films is reported. This approach enables the construction of photonic CNC structures with various surface patterns with critical dimensions of individual elements down to 300 nm (Figure 6). In these integrated surface engineered CNC films, strong left CP light is generated from the undisturbed chiral structure within the film interior, while the surface gratings facilitate the high asymmetry of the circular dichroism that is unachievable in traditional unpatterned CNC films.

The resulting freestanding surface-patterned CNC films exhibit a combination of unique chiroptical properties in comparison with traditional uniform CNC films that include much narrower selective reflection width, controllable view-angle color appearance with very different color appearance from the same films, and high CP light (Figure 6). Furthermore, significantly enhanced CP light with an asymmetric factor reaching a record value of 0.9 was demonstrated in CNC films integrated with microlens surface arrays.

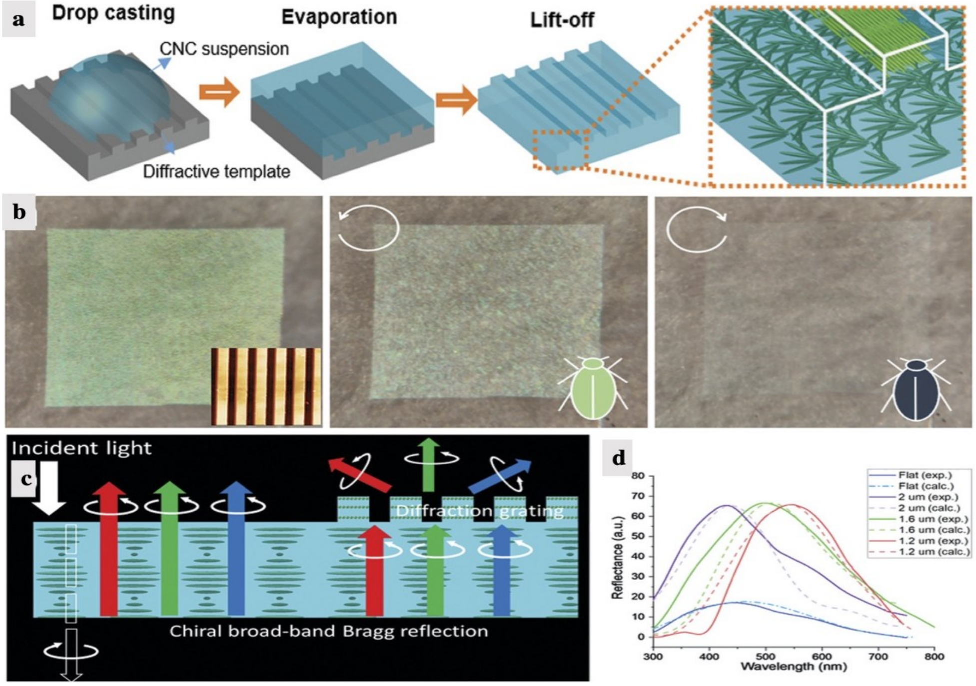

Figure 6. a) Assembly of chiral CNC photonic structures with surface gratings. Chiroptical properties of the patterned CNC films. b) Images of CNC film patterned with grating with 1.6 μm periodicity, taken with no polarizers (left), with left-hand circular polarizer (middle), and with right-hand circular polarizer (right). Size of the patterned area is 1 × 1 mm. Inset is AFM topography image (10 × 10 μm) of the corresponding grating structure. c) Schematics of enhanced CP light from CNC films with gratings structures. d) Measured reflectance spectra (solid line) and simulated spectra (dashed line) of CNC film with grating structures of different periodicities

Co-assembling Polysaccharide Nanocrystals and Nanofibers for Robust Chiral Iridescent Films. We demonstrated nontraditional reinforcing mechanism which is based upon the added molecular entanglement and dynamic hydrogen bonding between rigid and flexible nanostructures that gives rise to dramatic enhancement of the interfacial shearing strength. Hierarchical polysaccharide materials co-assembled from rigid nanocrystals and long flexible nanofibers (CNC−CNF bundles) have been prepared via ultrasonication assisted exfoliation of the weakly hydrolyzed wood pulp residues.

The distinctive mixed hierarchical morphology of tightly bundled rigid needle-like nanocrystals and embedded long flexible nanofibers enable mechanical reinforcement while maintaining a twisted-controlled helical organization and, therefore, structural coloration (Figure 7).

With help from our collaborators from North Carolina State University's Dr. Yingling group, large-scale molecular dynamics (MD) simulations were conducted for mixed bundles in order to understand the molecular reinforcing mechanism within these hierarchical CNC/CNF films. The MD simulations show that bundles of short rCNCs retain their composition and shape with minimal bundle deformations showing not much pathways for energy dissipation. In contrast, mixed bundles of hNC with embedded longer CNFs displayed significant shearing transformation under shear stresses, including disentanglement and defibrillation (Figure 7). For mixed bundles, stress–strain plots show significant plastic deformation, and a higher resistance was observed for the hNC samples. Overall, the resulting strength of hNC is much higher than that of rCNC, and there is significantly higher strain energy (area under the curve) for hNC bundles.

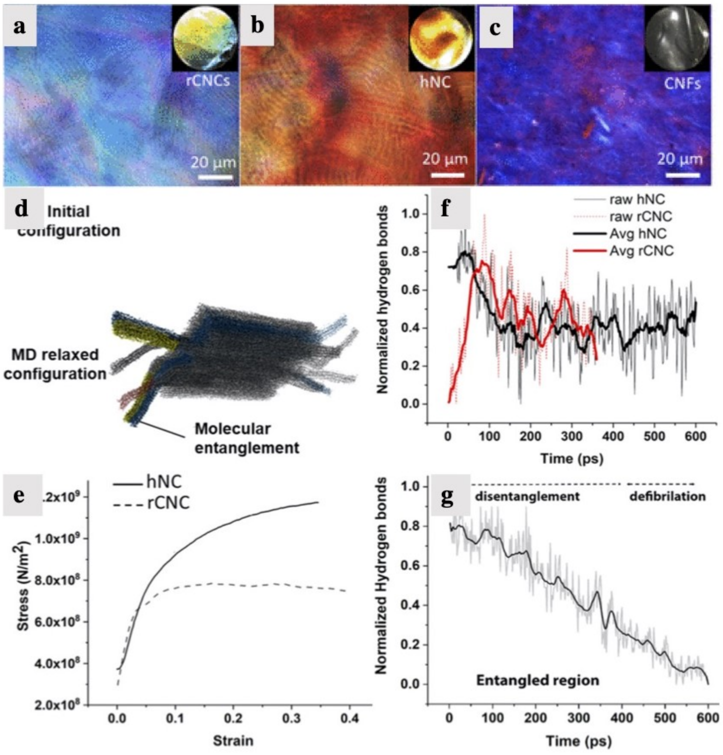

Figure 7. Optical microscopy of rCNCs (a) and hNC films (b); (c) polarized optical microscope images of CNFs film; the right-top insets in (a−c) are the photos of rCNC, hNC, and CNF films with 6 cm diameter. MD simulations of the mechanical deformation of hierarchical assemblies. (d) Initial configuration and MD relaxed configuration of the hNC organization (different coloration is for better identification of different nanocellulose fibrils). (e) Stress vs strain plot under uniaxial shear. Hydrogen bond network as a function of time, (f) comparing sandwiched bundles of rCNC and hNC materials, and (g) molecular entanglement network of hNC.

Peer-reviewed publications (published in 2019-2020):

1. R. Xiong, J. Luan, S. Kang, C. Ye, S. Singamaneni, V. V. Tsukruk, Natural Biopolymers for Organized Photonic Structures, *Chem. Soc. Review,* **2020**, *49*, 983-1031. *Invited Review*
2. R. Xiong, A. Singh, S. Yu, S. Zhang, Y. G. Yingling, D. Nepal, T. J. Bunning, V. V. Tsukruk, Co-assembling Polysaccharide Nanocrystals and Nanofibers for Robust Chiral Iridescent Films, *ACS Appl. Mater. Interfaces*, **2020**, *12*, 35345–35353.
3. R. Xiong, X. Zhang, M. Krecker, S. Kang, M. J. Smith, V. V. Tsukruk, Large and Highly Emissive Crystals from Carbon Quantum Dots onto Interfacial Organized Templates, *Angew. Chem. Int. Ed.* **2020**, in print
4. Grant, M. Krecker, M. Gupta, P. Dennis, M. Crosby, V. Tsukruk, Marine protein stability via Hofmeister Salt Annealing and Enzymatic Crosslinking, *ACS Biomater. Sci. Eng.* **2020,** in print
5. X. Zhang, R. Xiong, S. Kang, Y. Yang, V. V. Tsukruk, Stacking Nanocrystals and Nanofibers into Ultra-Strong Chiral Nematic Biocomposite Laminates, *ACS Nano*, **2020,** in print
6. R. Xiong, S. Yu, S. Kang, K. M. Adstedt, D. Nepal, T. J. Bunning, V. V. Tsukruk, Integration of Optical Surface Structures with Chiral Nanocelluloses for Enhanced Chiroptical Properties, *Adv. Mater.*, **2019**, 1905600.
7. R. Xiong, S. Yu, M. J. Smith, J. Zhou, M. Krecker, L. Zhang, D. Nepal, T. J. Bunning, V. V. Tsukruk, Assembling Carbon Quantum Dots on Cellulose Nanocrystals for Chiral Luminescent Biophotonic Materials, *ACS Nano*, **2019**,*13,* 9074-9081.

Total, 3 presentations (poster and oral presentations, invited talks and seminars) have been delivered by the PI and his students at professional conferences and seminars. Many 2020 presentations have been cancelled because of pandemic situation.

Personnel training and collaboration with AFRL researchers

The PI has a long history of fruitful education and training of graduate students and post-docs, involving them in collaboration with AFRL researchers, and enhancing USAF workforce (see summary of collaborations and student training below).

Unfortunately, student summer internships at AFRL have been cancelled because of the pandemic, all communications moved to on-line.

Current and recent MSE post-doc and graduate students, who participated in the project during 2019-2020 are:

- Dr. Rui Xiong (2015-2019) worked as a visiting student and then post-doctoral researcher on the assembly of high-performance nanocellulose and silk-based nanocomposites with superior robustness and multifunctionalities.
- Michelle Krecker (2016-present) is a PhD student studying the assembly and characterization of LbL structures, participated in an internship in AFRL’s Materials Research Directorate (MRD) with Dr. Patrick Dennis in July 2018.

- Katarina Adstedt (2018-present), graduate student, studied nanocellulose assembly with bio-derived amorphous polymers and the resulting nanostructures' mechanical and optical properties.
- Daria Bukharina (2018-present), graduate student, is involved in studying nanocellulose components, their assembly behavior, and interactions with synthetic materials.

Honors, fellowships, awards, and professional services by participating personnel

Students:

- Dr. Rui Xiong received Humboldt Foundation Post-doctoral Fellowship and moved to U. Konstanz, Germany for post-doc position.

PI:

- Fulbright Visiting Professor, Technical University of Graz, Austria (2020, suspended because of the pandemic, PI was expatriated back home in the middle of term).
- 2020 Georgia Tech Sigma Xi Sustained Research Award.