High Humidity Shaker Aging to Access Chitin and Cellulose Nanocrystals

Tony Jin[a], Tracy Liu[a], Faezeh Hajiali[a], Madison Santos[a,b], Yali Liu[c], Davis Kurdyla[c], Sophie Régnier[c], Sabahudin Hrapovic[c], Edmond Lam[a,c*] and Audrey Moores[a,d*]

[a]Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, Quebec, H3A 0B8 Canada
[b]Department of Bioengineering, McGill University, 3480 University St. #350, Montreal, Quebec, H3A 0E9, Canada
[c]Aquatic and Crop Resource Development Research Centre, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec, H4P 2R2 Canada
[d]Department of Materials Engineering, McGill University, 3610 University Street, Montreal, Quebec H3A 0C5, Canada
*audrey.moores@mcgill.ca; Edmond.Lam@cnrc-nrc.gc.ca

KEYWORDS: Chitin, cellulose, biomass, nanocrystal, mechanochemistry, aging, nanomaterial, solid-state

Abstract: To unlock nature's potential for functional biomaterials, many efforts have been devoted to isolating the nanocrystalline domains within the supramolecular structure of polysaccharides. Yet, low reactivity and yield in aqueous systems along with excessive solvent usage hinders its development. In this report, the first solvent-free pathway to access carboxylated chitin and cellulose nanocrystals with excellent mass balance is described, relying on a new method coined high humidity shaker-aging. The method involves a mild grinding of the polysaccharide with ammonium persulfate followed by an aging phase under high humidity and on a shaker plate. Insights into the mechanism were uncovered, which highlighted the unique role of high humidity to afford a gradual uptake of water by the material up to deliquescence when the reaction is complete. This process was then validated for direct synthesis of nanocrystals from biomass sources including crab and soft wood pulp.

Introduction:

Nanomaterials coming from polysaccharides such as cellulose and chitin have received exceptional attention in the past couple of decades owing to their properties such as high aqueous colloidal dispersibility, tensile and mechanical strength, gelling ability, high aspect ratio, biocompatibility, as well as liquid crystalline self-assembly.[1] They have found a number of applications in the general fields of catalysis, pharmaceuticals, templating, textiles, adhesives, and much more.[1a, 1b] Cellulose nanocrystals (CNCs) coming from woody cellulosic biomass for example are produced industrially up to the ton scale per day by 10 organization worldwide,[2] while chitin nanocrystals (ChNCs) from crustacean shells have yet to be developed on the industrial scale, but proven to have great promise in initial applications.[3] They both exist within biomass as organized domains, with chitin organizing in the so-called "Bouligand structure" surrounded by a matrix of proteins and calcium carbonate (**Figure 1a**).[4] To liberate the nanocrystals within the biopolymer, the amorphous regions must be selectively cleaved *via* acidic or oxidative pathways (**Figure 1b**). Usually, solutions of mineral acid (HCl, H_2SO_4) or oxidants such as 2,2,6,6-Tetramethylpiperidyl-1-Oxyl (TEMPO) or ammonium persulfate (APS) are used to induce partial depolymerization of the polysaccharide into nanocrystals (**Figure 1c**).[5] The challenge in these methods relies on the fact dilute concentrations are required to preserve the delicate crystalline structure while the low aqueous solubility of bulk cellulose and chitin drastically reduces reactivity, all resulting in waste generation.

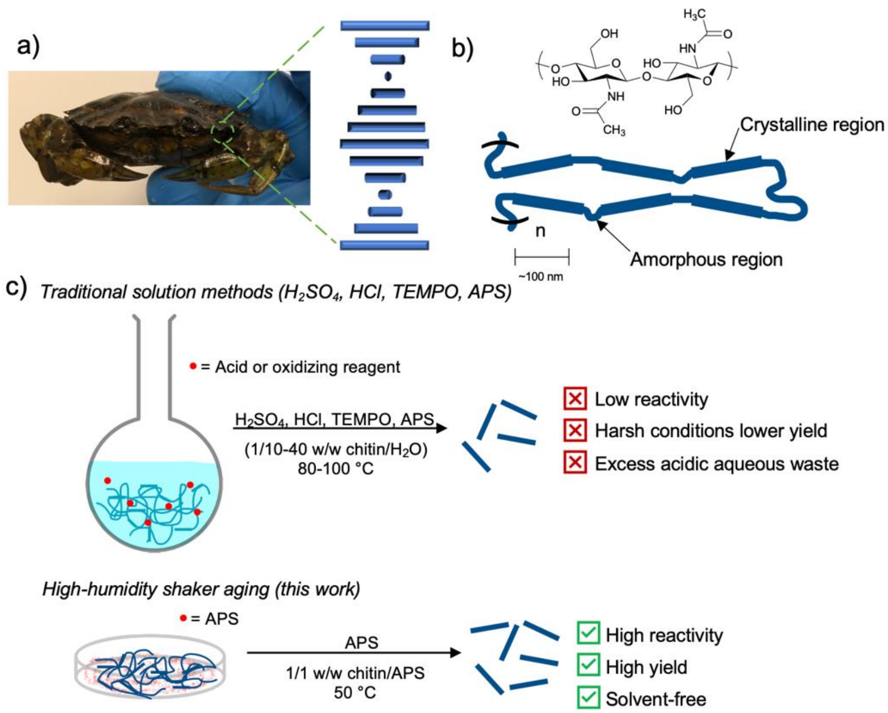

Figure 1: (a) Natural self-assembly of chitin fibers into the Bouligand structure as existed within the shells of the pictured green crab. (b) The chitin polysaccharide chemical structure as well as its macromolecular depiction divided into amorphous and crystalline regions, with a scale-bar to show the reader the approximate dimensions of the crystalline regions. (c) Schematic comparing the classic solution-based method using various reagents with the high-humidity shaker aging method in this work.

Mechanochemistry is rapidly establishing itself as an effective tool to cut on solvent/aqueous waste,[6] and it has been explored for the valorization of biomass, in the context of biopolymers deconstruction[7] and functional materials fabrication.[8] It is commonly used as pre-treatment to solution-based chemical methods and thus seen essentially as a physical method to break bonds. For example, cellulose nanocrystals (CNCs) were made from bamboo pulp by Huang and coworkers, in a sequence where ball-milling was used as a minor pre-treatment, followed by the solution-based synthesis with phosphotungstic acid.[9] More recently, mechanochemistry has been used as a reactive medium, such as in the work of van de Ven and coworkers who produced CNCs via cryogrinding cellulose with alkaline chloroacetate.[10] Yet such mechanochemical methods cause a loss of crystallinity in polysaccharides, which limits the ability to form high quality nanocrystals with high yield.[11] Herein we report the exploration of a spectrum of mechanochemistry and aging-based techniques to achieve high yield/low impact synthesis of excellent quality chitin and cellulose nanocrystals and the discovery a novel way to perform mechanochemistry via high humidity shaker-aging.

Results and discussion:

Initially, we ball milled dry powders of chitin and APS for 30 min at 29.5 Hz and attempted to suspend the resulting product in water. APS was selected as a mild, cheap and environmentally benign oxidant, able to afford carboxylated nanocrystals, which are more colloidally stable as compared to mineral acid-based nanocrystals.[12] Large sediments were observed at the bottom of the vial, indicative of untransformed bulk chitin even after ultrasonication and vigorous stirring (**Figure S1**). Analysis *via* dynamic light scattering (DLS) of the same suspension after brief ultrasonication confirmed particles were too large to be ChNC. An increase in reaction time to 90 min milling did not afford any significant ChNC liberation. XRD patterns of the resulting powders revealed the crystallinity remained high after 30 min of milling with APS (crystallinity index (CrI)=66%) but dropped rapidly after 90 min (CrI=49%, reference 66% for bulk chitin), (see supplementary info and **Figure S2**), ruling out increased milling time as a viable path.

Aside from mechanochemistry, which focuses on shearing and grinding to induce mechanical force needed to break bonds, accelerated aging has emerged as another powerful and low-energy technique to produce high quality materials in the solid phase, as exemplified by Friščić and coworkers who produced zeolitic imidazolate frameworks in this fashion.[13] We recently used this technique to deacetylate chitin and ChNCs into chitosan and chitosan nanocrystals (ChsNCs) respectively, in settings where pure mechanochemistry was too harsh on the materials.[14] This idea has also been explored for the production of CNCs by Konturri and coworkers who used HCl vapor to induce hydrolysis at the surface of cellulose-based cotton fibers, [15] although in this case vigorous sonochemical treatments were still necessary to liberate the CNCs, and the demonstration was only done qualitatively on a small scale. Spurred by this, we investigated whether accelerated aging could be the solid-state method to achieve high reactivity for the liberation of ChNCs. After initial APS and chitin grinding for 30 minutes, the mixed solid samples were placed into a controlled relative humidity chamber at slightly elevated temperature of 50 °C for 3 days (**Figure S3**, **Supplemental Information**). While the resulting product yielded an opaque suspension indicative of ChNC formation, both DLS (**Table S1**, **Entry 1**) and transmission electron microscopy (TEM) confirmed that the reaction was still largely incomplete with aggregates still present within the sample (see supplementary information - **Figure S3**). A control experiment was also done where we substituted the 30 min ball milling pre-treatment with just mortar and pestle mixing for 3 minutes before 3 days aging, which also incurred similar aggregation in the product (**Table S1**, **Entry 2**). The XRD of the mortar and pestle sample also indicated that this mixing technique did not affect the crystallinity value, displayed by the CrI of the sample having no change from the initial chitin CrI (**Figure S2**). Importantly, we noticed that water adsorbed on the material from the humidity chamber was essential for oxidation to take place. This was reminiscent of the conditions of Konturri who used HCl vapours to trigger hydrolysis in a filter paper,[15] in which is it critical the vapour reaches all the sample for the reaction to proceed. In our system, we reasoned diffusion to layers deeper than the surface could be a limiting factor. To aid in this, we sought a gentle agitation method, in the form of a shaker incubator chamber. With this modification, we used mortar and pestle to mix PG chitin and APS together before proceeding to the proposed high humidity shaker aging (HHSA) at 50 °C and 98 % relative humidity **(Figure 2a**). This strategy was very successful, as after 1 day, both the Z-average value from DLS data as well as the polydispersity index (PDI) had dropped to 267.2 nm and 0.436, respectively (**Figure 2b**, **Table S1 Entry 3**). After 3 days of aging, DLS analysis displays the lowest Z-average value of 156.7 nm, with a PDI of 0.268, which is similar to the ChNCs produced in solution (**Table S1**, **Entry 5**).[16] In parallel, conductometric titration experiments were used to deduce the amount of carboxylate functionalities found on the ChNCs after HHSA (**Figure 2c**). The sample with 3 days of aging showed a peak of a degree of oxidation (DO) of 0.25, which is higher than the solution based method.[16] This high DO leads to high dispersity and colloidal stability as demonstrated by a representative TEM image of the 3 day-aged sample, which has a measured length of 173 ± 35 nm (**Figure 2d**), as well as a ζ-potential value of -31.2 mV. By ^{13}C cross polarization (CP) magic-angle spinning (MAS) nuclear magnetic resonance (NMR) and Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy, we observed minimal to no change to the polysaccharide backbone structure throughout the HHSA process (**Figure S4, S5**). Quantitative NMR also confirmed no deacetylation took place in this process. The FTIR carboxyl C=O stretch peak usually at around 1720 cm^{-1} is very low in signal, which is a common feature for carboxylated ChNCs.[12, 17] Overall, these experiments show that ChNCs are effectively produced from chitin by HHSA, as a critical method, with ball milling being merely a mixing component.

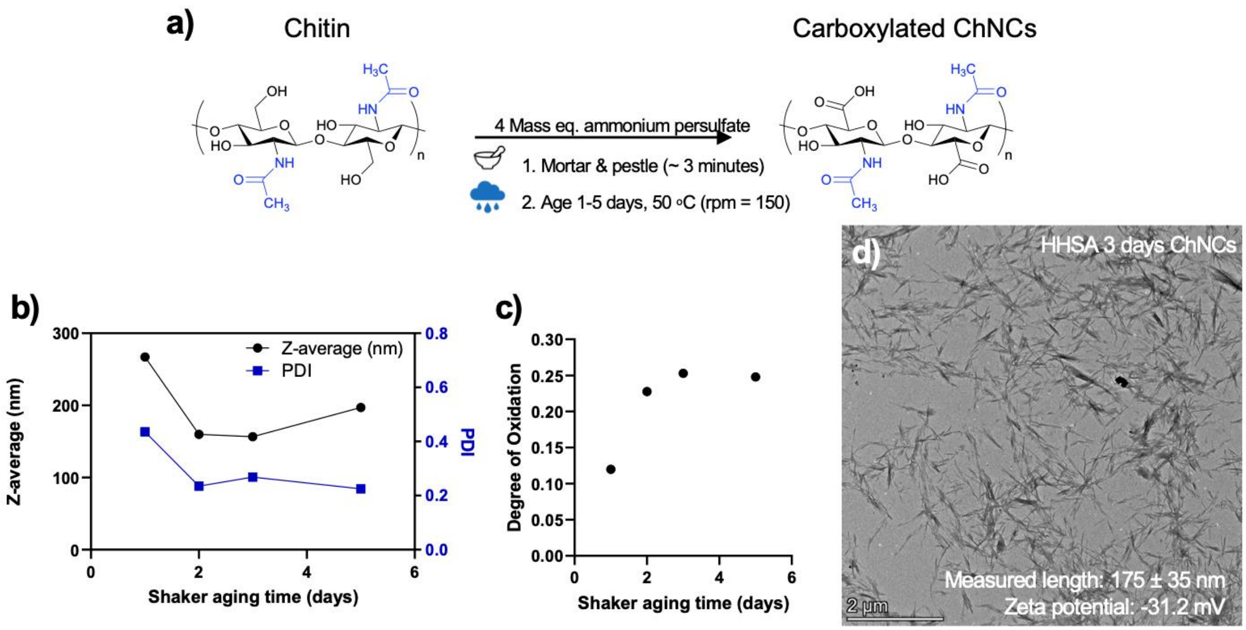

Figure 2: (a) Scheme for shaker aging under humidity to make ChNCs. (b) Results from dynamic light scattering showing the Z-average value (black dots and lines) and polydispersity index (blue dots and lines) of the resulting ChNC suspension in relation to the aging time. (d) Representative TEM micrograph of carboxylated ChNCs with 3 days of HHSA.

We then explored the mechanism of APS oxidation within our solid-state system, which has been little studied.[18] From negative control experiments, it is determined that both the formation of radicals as well as the need for water is essential in this reaction to afford the oxidation of chitin (See **Supplemental Information** for more details). An important observation that is seen within all HHSA reactions is the uptake of water within the solid mixture of APS and chitin. To better understand the role of water adsorption in this reaction, we experimentally determined the kinetics of water adsorption within our solid mixture by following its content within the solid sample over the course of 7 days under HHSA (**Figure 3**) using thermogravimetric analysis (TGA). Almost no water sorption happened up to 6 hr of HHSA until a "threshold" was reached, and rapid, autocatalytic adsorption of water into the solid mixture took place between 1 day and 2 days (up to 60% of all water adsorption happened between days 1 and 2). After 2 days, the rate of water adsorption decreased, deliquescence was achieved at 3 days and a plateau was reached between the timepoints at days 4 and 7. Upon analyzing this curve, we wondered if keeping the water constant within the reaction vessel could favor the reaction. This is in tune with efforts in the area of mechanochemistry to provide catalytic amounts of liquid which in some cases can drastically catalyze or even chemically alter the reaction, dubbed the term liquid-assisted grinding (LAG).[19] We investigated two different values of LAG: η = 0.2 and = 0.4, in order to simulate the initial and final stages of the rapid water sorption progress in the water content experiment **(Figure 3**). Resulting material morphology was analyzed by DLS (**Table S1**) and TEM micrographs (**Figure S7**), and revealed aggregates of ChNCs for η = 0.4, with a high Z-average value of 736.7 nm with PDI = 0.285 (**Table S1**, **Entry 7**), while the η = 0.2 sample shows smaller sizes of 274.2 nm with PDI 0.268 (**Table S1**, **Entry 8**), although still higher than HHSA samples. This comparison reveals that LAG was not successful in offering the high quality ChNCs seen with HHSA. Specifically, η = 0.2 LAG aging, with water content equivalent to 1-day HHSA, seems to provide good initial results, but the particle size and dispersity remained high, as if the reaction was not completed. On the other hand, η = 0.4 LAG aging, with water content equivalent to 2-day HHSA, the Z-average value was more than 3 times higher than the 2-day HHSA sample they both have relatively similar water contents of 21 % and 26 %, respectively, hinting that starting off at high water content is not efficient, possibly because APS radical formation is too fast at high water content. A further control experiment was done to mimic the slurry condition found after day 3 aging, in which deliquescence is reached ("slurry" synthesis). While the reaction proceeded under these conditions, both the DLS Z-average (240.0 nm) and PDI values (0.313) were higher than that of the sample after 3 days HHSA (Z-average = 156 nm, PDI = 0.268). This outlines that it is not simply the amount of water that is present within the sample that matters, but the gradual increase in water content while the reaction is proceeding that is the catalyzing factor deciding the controlled depolymerization of chitin into ChNCs. This is the first time this observation has been reported for aging as a solid-state reaction pathway depending on gradual water adsorption.

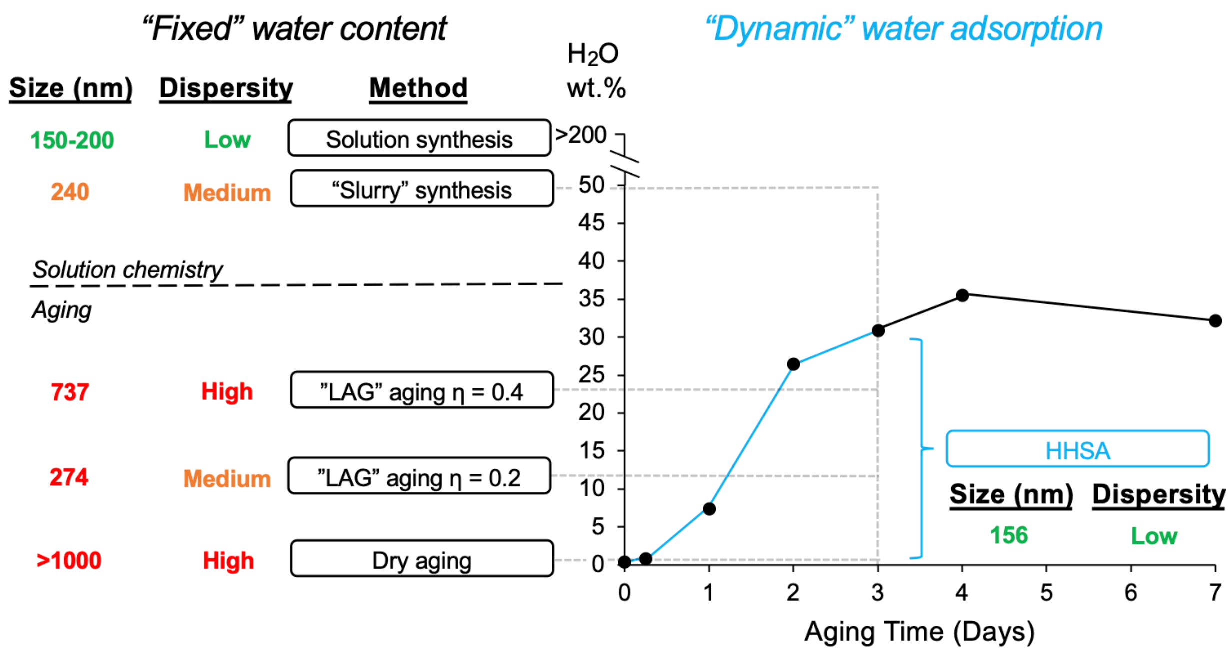

Figure 3 Varying methods with a "fixed" water content value throughout the time of reaction, with comparison between solution chemistry and aging with general figure of merits in size and yield. All reactions were conducted under 50 °C and 3 days, while "solution synthesis" represents general solution-based hydrolysis methods using APS as an oxidant. "Dynamic" water adsorption over time monitored by TGA for the HHSA reaction found in the solid mixture of chitin (300 mg) and APS (1.2 g) under 98 % relative humidity and 50 °C.

In HHSA, it can be envisioned that slow water adsorption is occurring from the APS and chitin solid mixture within a high humidity environment that is allowing for ultra-high aqueous concentrations of persulfate radical and hydrogen peroxide to be generated in *situ* within the solid mixture (**Figure S8**). In this case, as water slowly starts to penetrate the solid mixture, ultra-high concentrations of sulfate radicals and peroxides may form which can effectively cleave the amorphous regions of chitin and liberating space for more water to permeate through and react with local APS. The timing here seems crucial so that radical formation, gradual hydrolysis, and water permeation take place at similar pace to afford a homogeneous material in the end. This is the first time that ChNCs can be made from chitin starting from the solid-state, which is as comparable to traditional solution-based synthesis techniques in terms of making highly disperse nanocrystals with no aggregation.

We validated the versatility of this new method by producing high quality carboxylated CNCs from microcrystalline cellulose, in 3 days, with a ζ-potential = -44.3 mV, Z-average = 202.1 nm and PDI = 0.192 determined by DLS. Fully dispersity of CNCs was achieved, while TEM confirmed the length of the crystallites at 220 ± 40 nm (**Figure 4**). CNC produced by this novel method have the same properties as the ones made through other methods in terms of size and polydispersity.[20] Importantly, we obtained CNC in high yield of 61 % up to 1 g reaction scale, better than the solution based method . Furthermore, a process mass intensity (PMI) calculation was done comparing the HHSA method to a standard solution-based method with APS as the oxidizing agent, which verifies that the amount of water and reagent needed to make CNCs using HHSA is an order of magnitude below that of the solution-based method, with PMI values of 12.5 compared to 203 for the traditional method (**Supporting Information**). Finally, the resulting CNC are also superior in that they are easily suspendable, with suspensions stable over the course of 3 months, with similar ζ-potential values before and after. At a concentration range of 5 wt. %, the carboxylated CNC suspension depict gel-like behavior, as shown in the inset of **Figure 4**. Interestingly, their ζ-potential -44.3 mV are at least 10 mV more negative than CNCs from industrial sources.[20]

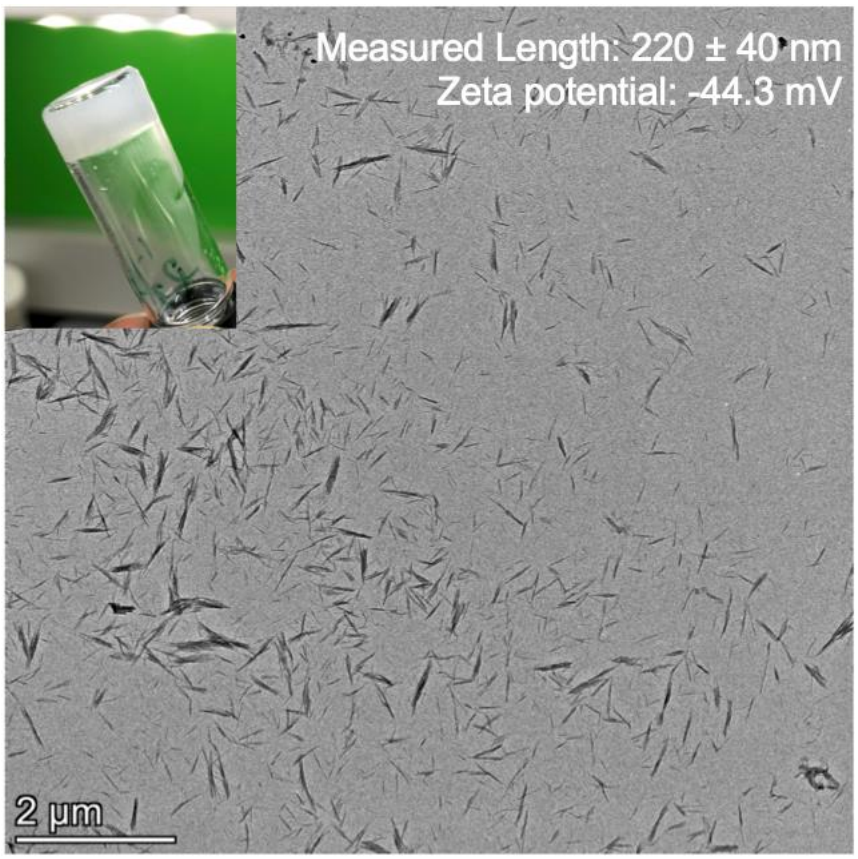

Figure 4: Unstained TEM image of carboxylated CNCs made from microcrystalline cellulose using HHSA method. Inset shows of a 5.75 wt. % suspension of carboxylated CNCs.

To further streamline polysaccharide nanocrystals fabrication, we used raw chitinous biomass - powdered green crab shell- and soft wood pulp, to directly access ChNCs and CNC respectively. Soft wood pulp CNCs afforded excellent dispersions and whiskers similar to the one made from cellulose (**Figure S9**). With green crab shells, TEM revealed that ChNCs are isolated as into individual whiskers (**Figure S10**), while minerals from the shell were also present in the form of small black dots, identified to be calcium-rich by energy dispersive X-ray spectroscopy (EDX) (**Figure S11**). Interestingly, this provides context that the APS oxidation of green crab shells does not get rid of the calcium carbonate present within it, but still selectively depolymerizing chitin. This work has the potential to afford a new valorisation route for crustacean shell, as an important biowaste stream.

Conclusion

In conclusion, HHSA is a new method to achieve solid-state reactivity for the formation of ChNCs and CNCs. In contrast to mechanochemistry, which focuses on shearing and grinding to induce mobility, HHSA is a "softer" form of mechanochemistry focusing on the progression between pure solid mixtures and slurries, in which control of relative humidity is the key in promoting gradual water adsorption and thereby radical-based oxidation reactivity. The as-made ChNCs and CNCs have comparable properties to traditional solution-made ones, while their synthesis has a higher yield and a much improved PMI to the solution based methods.

Acknowledgements

We thank the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant, Discovery Accelerator Supplement, the Canada Foundation for Innovation (CFI), the Fonds de Recherche du Quebec - Nature et Technology (FRQNT), the Centre for Green Chemistry and Catalysis (CGCC) of McGill University and the NRC Industrial Biotechnology program for their financial support. TJ was supported by the Lawrence Light Sustainability Fellowship, the Walter

Sumner Fellowship, and the NSERC Postgraduate Scholarship-Doctoral award. We are grateful to Dr. Robin Stein for help in acquiring solid-state NMR and to Dr. Hatem Titi for his help in acquiring the pXRD data.

References

[1] aT. Jin, T. Liu, E. Lam, A. Moores, *Nanoscale Horiz.* **2021**, *6*, 505-542; bY. Habibi, L. A. Lucia, O. J. Rojas, *Chem. Rev.* **2010**, *110*, 3479-3500; cB. Thomas, M. C. Raj, A. K. B, R. M. H, J. Joy, A. Moores, G. L. Drisko, C. Sanchez, *Chem. Rev.* **2018**, *118*, 11575-11625.

[2] O. M. Vanderfleet, E. D. Cranston, *Nat. Rev. Mater.* **2021**, *6*, 124-144.

[3] aX. Chen, H. Yang, N. Yan, *Chem. Eur. J.* **2016**, *22*, 13402-13421; bM. J. Hülsey, *Green Energy Environ.* **2018**, *3*, 318-327; cC. Chen, Q. Wu, Z. Wan, Q. Yang, Z. Xu, D. Li, Y. Jin, O. J. Rojas, *Chem. Eng. J.* **2022**, *442*, 136173; dF. M. Kerton, Y. Liu, K. W. Omari, K. Hawboldt, *Green Chem.* **2013**, *15*, 860-871.

[4] L. Bai, T. Kämäräinen, W. Xiang, J. Majoinen, J. Seitsonen, R. Grande, S. Huan, L. Liu, Y. Fan, O. J. Rojas, *ACS Nano* **2020**, *14*, 6921-6930.

[5] aZ. Fang, H. Zhu, C. Preston, L. Hu, *Translational Materials Research* **2014**, *1*, 015004; bB. O'Connor, R. Berry, R. Goguen, in *Nanotechnology environmental health and safety*, Elsevier, **2014**, pp. 225-246.

[6] aJ.-L. Do, T. Friščić, *ACS Cent. Sci.* **2017**, *3*, 13-19; bS. L. James, C. J. Adams, C. Bolm, D. Braga, P. Collier, T. Friščić, F. Grepioni, K. D. M. Harris, G. Hyett, W. Jones, A. Krebs, J. Mack, L. Maini, A. G. Orpen, I. P. Parkin, W. C. Shearouse, J. W. Steed, D. C. Waddell, *Chem. Soc. Rev.* **2012**, *41*, 413-447.

[7] E. Calcio Gaudino, G. Cravotto, M. Manzoli, S. Tabasso, *Chem. Soc. Rev.* **2021**, *50*, 1785-1812.

[8] aG. Margoutidis, F. M. Kerton, in *Biomass Valorization*, **2021**, pp. 343-365; bF. Hajiali, T. Jin, G. Yang, M. Santos, E. Lam, A. Moores, *ChemSusChem* **2022**, *15*, e202102535.

[9] Q. Lu, Z. Cai, F. Lin, L. Tang, S. Wang, B. Huang, *ACS Sustain. Chem. Eng.* **2016**, *4*, 2165-2172.

[10] F. Mahrous, R. Koshani, M. Tavakolian, K. Conley, T. G. M. van de Ven, *Cellulose* **2021**, *28*, 8387-8403.

[11] aT. Di Nardo, A. Moores, *Beilstein J. Org. Chem.* **2019**, *15*, 1217-1225; bS. Kuga, M. Wu, *Cellulose* **2019**, *26*, 215-225.

[12] A. C. W. Leung, S. Hrapovic, E. Lam, Y. Liu, K. B. Male, K. A. Mahmoud, J. H. T. Luong, *Small* **2011**, *7*, 302-305.

[13] M. J. Cliffe, C. Mottillo, R. S. Stein, D.-K. Bučar, T. Friščić, *Chem. Sci.* **2012**, *3*, 2495-2500.

[14] aT. Jin, T. Liu, S. Jiang, D. Kurdyla, B. A. Klein, V. K. Michaelis, E. Lam, J. Li, A. Moores, *Green Chem.* **2021**, *23*, 6527-6537; bT. Di Nardo, C. Hadad, A. Nguyen Van Nhien, A. Moores, *Green Chem.* **2019**, *21*, 3276-3285.

[15] E. Kontturi, A. Meriluoto, P. A. Penttilä, N. Baccile, J.-M. Malho, A. Potthast, T. Rosenau, J. Ruokolainen, R. Serimaa, J. Laine, H. Sixta, *Angew. Chem. Int. Ed.* **2016**, *55*, 14455-14458.
[16] J. H. Luong, E. Lam, C. W. Leung, S. Hrapovic, K. B. Male, United States **2019**.
[17] M. Liu, Q. Peng, B. Luo, C. Zhou, *Eur. Polym. J.* **2015**, *68*, 190-206.
[18] A. Shrotri, H. Kobayashi, A. Fukuoka, *ChemCatChem* **2016**, *8*, 1059-1064.
[19] G. A. Bowmaker, *Chem. Commun.* **2013**, *49*, 334-348.
[20] G. Delepierre, O. M. Vanderfleet, E. Niinivaara, B. Zakani, E. D. Cranston, *Langmuir* **2021**, *37*, 8393-8409.