

Expandable and scalable production of cellulose nanofibrils via highly efficient swelling-modification in deep eutectic solvent

Corresponding Author: Professor Zhiguo Wang

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The manuscript proposed a swelling-modification strategy using a hydrated DES ($\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$), moreover, modified DES was further designed by using different kinds of cyclic anhydrides to achieve highly efficient introduction of carboxyls and nanofibrillation of cellulose. The noteworthy points include that the CNFs can be prepared in a high yield and efficiency (yield higher than 90%, nanofibrillation can be completed within 5 min by using a kitchen blender) with high quality (DP higher than 600, aspect ratio higher than 1500), moreover, the process can be expandable and scalable with recyclability of the DES. The manuscript contributes to not only an idea of DES based “multifunctional operating platform”, but also to overcome the gap from lab to scaling-up production of CNF, which is a quite promising biomass-based nanomaterials. The followings are specific comments to help the further improvement of the manuscript.

1. It is recommended to keep no more than 5 keywords.
2. P6, Line 92, “DESS” should be “DESS”.
3. P7, Line 101, it is recommended to replace “smaller diameter” with “ultrathin diameter”, since “-er” should be used in a comparison scene.
4. In some batches the diameter of MAH-CNF is 3.47 nm, while, when comparing four kinds of cyclic anhydrides, the diameter of MAH-CNF is 3.72, it is normal that the diameter varies in a reasonable range, therefore it is recommended to describe the diameter of CNF as 3-4 nm in abstract or other corresponding parts.
4. Fig.1a, “Nnanofibril” should be “Nanofibril”, “Non-crystalline” should be “Amorphous”.
5. Fig. 1b shows both CNF dispersion (suspension) and hydrogel? But the text only mentions CNF suspension.
6. Fig. 1d, the volume of the bottle should be noted in the figure, as well as in Fig. 5d.
7. Both “diameter” and “width” are used to describe the size of CNF in the manuscript, please choose one to unify it.
8. Table S5, S6 was not cited in the manuscript.
9. The authors prepared four kinds of CNF (MAH-CNF, SAH-CNF, PAH-CNF and EAH-CNF). While, in Table S1, only one line was used to describe the CNF prepared by the authors. The 2.024 mmol/g of the carboxyl content is corresponding to SAH-CNF, how about other parameters, also corresponds to SAH-CNF or other kinds of CNF? It is recommended to make it clear. Or, to include the information of all four kinds of CNF in the table.

Reviewer #2

(Remarks to the Author)

This manuscript reports a swelling–modification strategy for the scalable production of cellulose nanofibrils (CNFs) using a hydrated $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ deep eutectic solvent system. By integrating controlled cellulose swelling, cyclic anhydride esterification, and low-energy mechanical processing, the authors achieve CNFs with simultaneously high carboxyl content, high aspect ratio, preserved degree of polymerization, and excellent solvent recyclability.

First of all, this study recalls a déjà-vu image, as shown in a recently published paper, as cited in the manuscript ‘25 Shi, X. et al. Scalable production of carboxylated cellulose nanofibres using a green and recyclable solvent. Nat. Sustain. 7, 315-325 (2024).’

The major experiments, even the presentation style, is quite similar :) This is probably not a problem, but the problem would be the innovation point. What is the major innovation of this study compared to the cited work? What is the conceptual

advance relative to existing DES-based CNF preparation methods?

Several other issues require clarification.

1. Lines 124–126: The authors claim that the $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ system enhances the accessibility of surface hydroxyl groups at elevated temperatures, which is attributed to cellulose swelling. However, the definition of “swelling” in this context is not sufficiently clear. The authors are encouraged to provide more direct evidence of cellulose swelling, such as changes in fiber diameter, crystallinity, or quantitative indicators related to accessible $-\text{OH}$ groups (e.g., specific surface area or related measurements).
2. The buffering function of $\text{Zn}(\text{OAc})_2$ is central to the proposed mechanism for suppressing acid hydrolysis and preserving DP. Although indirect evidence is provided, a more explicit discussion of how the buffering capacity compares with other acetate salts or DES systems would strengthen this argument. If available, quantitative pH or acidity-related data during the modification process should be briefly discussed.
3. The demonstration of four cyclic anhydrides effectively supports the idea of a “multifunctional operating platform.” A short discussion on the criteria for selecting these anhydrides (e.g., reactivity, melting point, industrial relevance) would further reinforce the generality and expandability of the approach.
4. The reported aspect ratio (~ 1800) is notably high and represents an important advantage of the method. To improve confidence in this result, the authors are encouraged to clarify the statistical basis of the measurement (e.g., number of fibrils analyzed, distribution range, and potential projection effects in TEM).
5. The authors are encouraged to include additional control experiments to clarify the respective roles of each component in the system, such as cyclic anhydride modification without $\text{Zn}(\text{OAc})_2$, $\text{Zn}(\text{OAc})_2$ -containing DES without cyclic anhydride, and DES swelling followed by mechanical processing without cyclic anhydride.
6. The comparison with TOCN is important for positioning the method. The authors may consider clarifying potential differences in surface chemistry (e.g., ester-linked versus oxidized carboxyl groups) and how these differences might affect downstream applications, beyond carboxyl content alone.
7. The manuscript attributes the disruption of hydrogen bonding primarily to DES-induced swelling. However, it remains unclear whether temperature or water content may play a dominant role in this process. It is helpful to clarify whether a $\text{ChCl}/\text{H}_2\text{O}$ system at the same temperature would exhibit comparable swelling behavior.
8. Lines 200–202: The authors claim that $\text{Zn}(\text{OAc})_2$ acts as an effective pH buffer during nanofibrillation, but the source of protons (H^+) is not clearly identified. Please clarify whether H^+ originates from $-\text{COOH}$ dissociation, hydrolytic side reactions, or other processes, and whether $\text{Zn}(\text{OAc})_2$ truly functions as a buffer rather than a neutral salt.
9. The manuscript emphasizes hydrogen-bond competition as the dominant swelling mechanism in the $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ system. Please clarify whether coordination interactions and ionic effects (e.g., involving Zn^{2+} or acetate) also contribute to cellulose swelling.
10. The mechanical performance of CNFSAH films (Supplementary Fig. S23) should be compared with representative CNF films from the literature under similar testing conditions would help readers better contextualize the performance improvements.
11. The large-scale simulation using conventional industrial equipment is convincing. To further enhance industrial relevance, the authors could briefly discuss potential challenges in such processing, such as DES viscosity control, water content management, or long-term accumulation of impurities during solvent recycling.
12. Regarding the statement that water “enhancing viscosity” (Page 14, Lines 242–244): this claim is incorrect and contradicts your own data (Supplementary Fig. S8, which shows viscosity decreases with added water). The effect (between viscosity, hydrogen-bond network, swelling and mobility) should be accurately explained and revised.
13. The combined SAXS, LF-NMR, and MD simulation results convincingly show the regulatory role of water in the DES. The mechanistic discussion could be further streamlined to emphasize a single coherent physical picture, highlighting how water indirectly enhances cellulose swelling by modulating DES component mobility and interactions.
14. Line 329, the unit for the diffusion coefficient is cm^2/S , not cm/S .
15. Line 336–339, how is the interaction energy calculated and normalized (e.g., per molecule, per cellulose chain, or per simulation box).

Reviewer #3

(Remarks to the Author)

The manuscript presents a highly efficient swelling-modification strategy for producing carboxylated cellulose nanofibrils (CNFs) using a hydrated deep eutectic solvent (DES) system composed of $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$. The authors demonstrate production of CNFs with high carboxyl content (2.024 mmol/g), exceptional aspect ratio (~ 1800), high yield (93%), and excellent solvent recyclability (10 cycles). The work establishes a “multifunctional operating platform” enabling diverse chemical modifications and demonstrates scalability with reduced environmental impact compared to TEMPO oxidation methods. This manuscript represents a significant advancement in sustainable CNF production with clear improvements over existing methods. The work is comprehensive, well-executed, and addresses critical limitations in the field. However, several areas require clarification and improvement before acceptance in Nature Communications.

1 While your work uses $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ vs. $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$ system, What fundamental limitations of the $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$ system your approach addresses? How your “swelling-modification strategy” differs mechanistically from the $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$ approach. o The dual role of $\text{Zn}(\text{OAc})_2$ (pH buffering + swelling enhancement) is not fully resolved? Reference 25 Line 704 : Shi, X. et al. Scalable production of carboxylated cellulose nanofibres using a 704 green and recyclable solvent. Nat. Sustain. 7, 315–325 (2024)

2 The reference paper (Shi et al.) achieved: Carboxyl content: 1.5 mmol/g; Aspect ratio: $\sim 2,500$; Yield: 90.12%; Your work achieves: Carboxyl content: 2.024 mmol/g (35% improvement); Aspect ratio: $\sim 1,800$ (28% lower); Yield: 93% (3% improvement). Please add a dedicated paragraph discussing why the trade-off of lower aspect ratio for higher carboxyl

content represents an advancement. Is this deliberate tuning or an inherent limitation? Is the lower aspect ratio due to: o Deliberate tuning via pH buffering (preserving DP but limiting swelling)? Inherent limitation of Zn(OAc)₂ system? Different mechanical treatment intensity?

3 The work attributes success to "Zn(OAc)₂ acted as a pH buffering", but the evidence was Insufficient? How about the pH of Zn(OAc)₂/ChCl/H₂O systems comparison with unbuffered controls?

4 What happens to Zn content in CNF? ICP-MS quantification of Zn in final CNF is required?

5 Why SAH is so much more efficient which showed 2.024 mmol/g carboxyl content for CNFSAH, while CNFMAH showed only 1.412 mmol/g carboxyl content?

6 Statement: "aspect ratio ~1800" (Line 202), How were lengths measured? (From TEM - manual or automated?)

7 "Exceptionally high aspect ratio (~1800)" which is lower than the reference paper's 2,500? "Scalable and expandable production" - Demonstrated at pilot scale (colloid mill), not true industrial scale?

8 Figure 1 o Panel (a): Schematic is conceptually clear but lacks molecular-level detail showing Zn²⁺ role; 3. Figure 3: Panel (c): Low-field NMR - axes labels too small? Figure 4: Panels (f-i): TEM images -add diameter distribution histograms as insets will be better

9 Minor Grammar and Writing Style: Inconsistent use of "cellulose nanofibril" vs. "cellulose nanofibrils", CNF or CNFs throughout the manuscript ? L125 " through a ring-opening reaction" should be "a ring-opening esterification reaction" L92 DESS?

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

The authors could address my major concerns.

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Reviewer #1 (Remarks to the Author):

The manuscript proposed a swelling-modification strategy using a hydrated DES ($\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$), moreover, modified DES was further designed by using different kinds of cyclic anhydrides to achieve highly efficient introduction of carboxyls and nanofibrillation of cellulose. The noteworthy points include that the CNFs can be prepared in a high yield and efficiency (yield higher than 90%, nanofibrillation can be completed within 5 min by using a kitchen blender) with high quality (DP higher than 600, aspect ratio higher than 1500), moreover, the process can be expandable and scalable with recyclability of the DES. The manuscript contributes to not only an idea of DES based “multifunctional operating platform”, but also to overcome the gap from lab to scaling-up production of CNF, which is a quite promising biomass-based nanomaterials. The followings are specific comments to help the further improvement of the manuscript.

Response: We are thankful for the critical and detailed evaluation of our manuscript and giving your insightful comments and useful suggestions. Your comments and suggestions were constructive and helped us improve the quality of our manuscript. We have carefully addressed all queries and revised the manuscript in light of your valuable comments and suggestions. We hope that the responses to your comments and subsequent amendments in the revised manuscript are satisfactory to recommend it for publication in ‘Nature Communications’.

Comment #1

It is recommended to keep no more than 5 keywords.

Author’s Response: We appreciate your valuable suggestion. In accordance with your recommendation, we have reduced the number of keywords by removing two, and we now retain the following five keywords at line 48-49:

Keywords: Cellulose nanofibrils; Deep eutectic solvent; Expandable functional modification; Scalable production; Multifunctional operating platform

Comment #2

P6, Line 92, “DESS” should be “DESS”.

Author’s Response: Thank you for your helpful suggestions. We have corrected the term ‘DESS’ to ‘DESS’ as per your recommendation and have highlighted the change at line 87 of the revised manuscript.

Comment #3

P7, Line 101, it is recommended to replace “smaller diameter” with “ultrathin diameter”, since “-er” should be used in a comparison scene.

Author’s Response: We appreciate your insightful suggestion. In line with your recommendation, we have replaced ‘smaller diameter’ with ‘ultrathin diameter’ and have marked the change at line 96 of the revised manuscript.

Comment #4

In some batches the diameter of MAH-CNF is 3.47 nm, while, when comparing four kinds of cyclic anhydrides, the diameter of MAH-CNF is 3.72, it is normal that the diameter varies in a reasonable range, therefore it is recommended to describe the diameter of CNF as 3-4 nm in abstract or other corresponding parts.

Author’s Response: Thank you for your thoughtful suggestion. We agree with your observation and have revised the manuscript to describe the diameter of MAH-CNF as ranging from 3 to 4 nm in the abstract and other relevant sections. The changes have been highlighted in the revised manuscript.

Comment #5

Fig.1a, “Nnanofibril” should be “Nanofibril”, “Non-crystalline” should be “Amorphous”.

Author’s Response: Thank you for your valuable suggestions. We have made the necessary corrections, replacing the ‘Nnanofibril’ with ‘Nanofibril’ and ‘Non-crystalline’ with "Amorphous" in Fig. 1a. The updated figure has been provided in the revised manuscript.

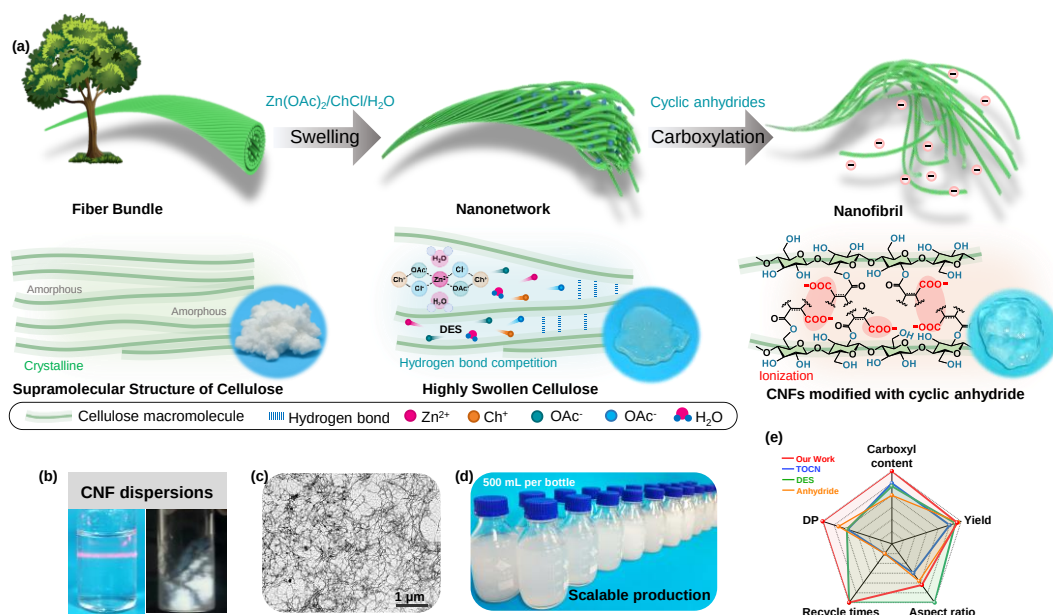


Fig. 1 | Method and advantages of expandable and scalable cellulose nanofibrils (CNFs) production in a DES system using a highly efficient swelling-modification strategy.

Comment #6

Fig. 1b shows both CNF dispersion (suspension) and hydrogel? But the text only mentions CNF suspension.

Author's Response: Thank you for your insightful comment. The Fig. 1b shown is the CNF suspensions. To avoid unnecessary misunderstanding, we have replaced the Fig. 1b and clarified this description in the manuscript.

Line 130-134: After neutral washing, the modified cellulose was mechanically dispersed using a simple kitchen blender, forming CNF dispersions in just 5 min. The CNF dispersions exhibited the Tyndall effect by showing an obvious laser line, and birefringence when observed under polarized light (Fig. 1b, Supplementary Video 1).

Comment #7

Fig. 1d, the volume of the bottle should be noted in the figure, as well as in Fig. 5d.

Author's Response: Thank you for your helpful suggestion. We have added the volume of the bottle in both Fig. 1 and Fig. 5, as recommended. The revised figures have been provided in the manuscript.

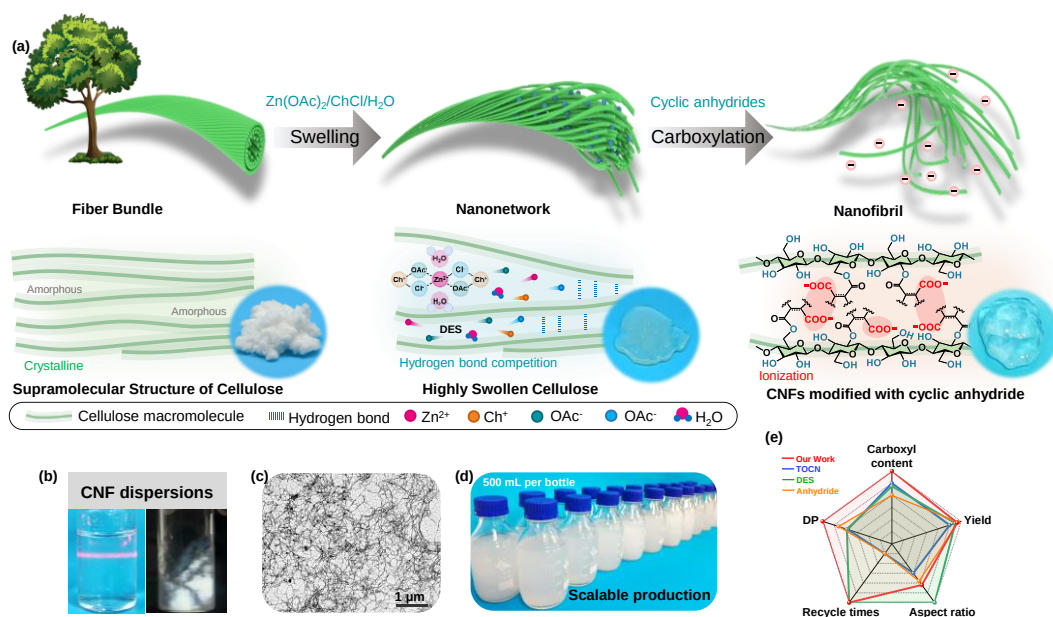


Fig. 1 | Method and advantages of expandable and scalable cellulose nanofibrils (CNFs) production in a DES system using a highly efficient swelling-modification strategy.

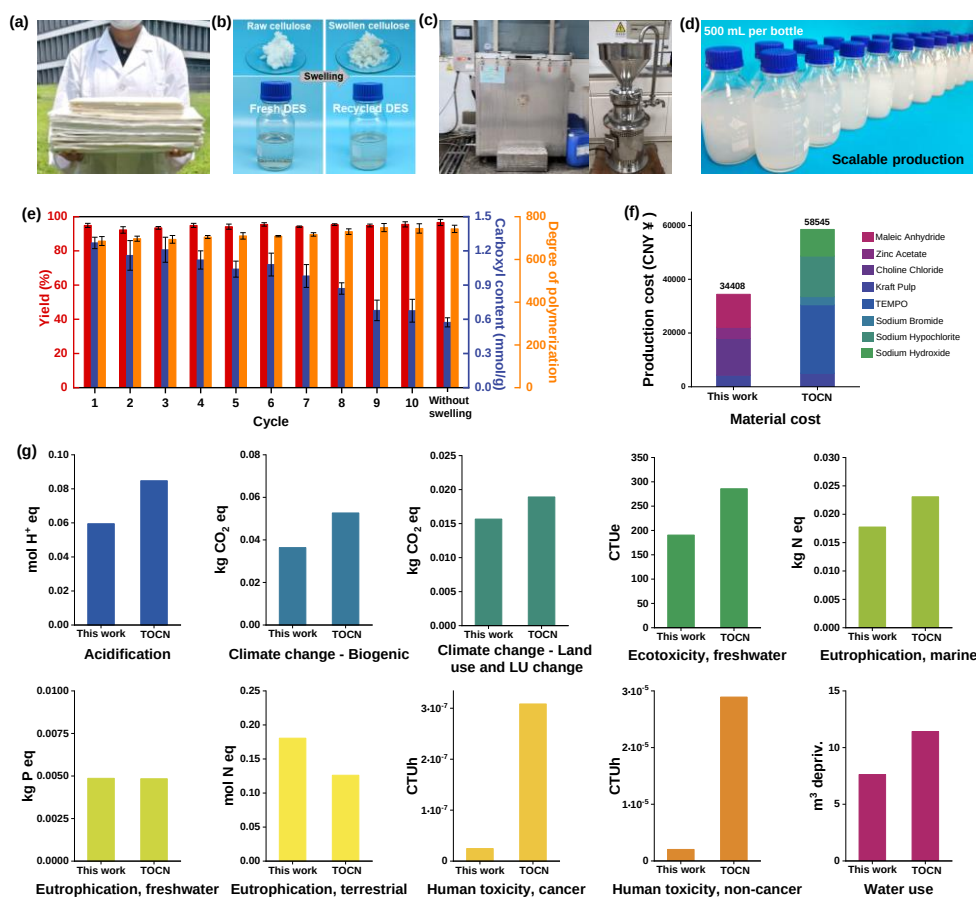


Fig. 5 | Scalable production of CNFs under the highly efficient swelling-modification strategy and life cycle assessment (LCA) for environmental impact evaluation.

Comment #8

Both “diameter” and “width” are used to describe the size of CNF in the manuscript, please choose one to unify it.

Author’s Response: Thank you for your valuable suggestion. In response, we have standardized the terminology by replacing “width” with “diameter” throughout the revised manuscript.

Comment #9

8. Table S5, S6 was not cited in the manuscript.

Author’s Response: Thank you for pointing this out. We have now cited Table S5 and Table S6 at their respective places in the revised manuscript, as shown below.

Line 482-484: The surface degree of substitution (DS) calculated by XPS was positively correlated with carboxyl content, with CNF_{SAH} having the highest DS of 0.303 (Supplementary Table S5).

Line 582-583: However, even after multiple cycles, the initial CNF still exhibited a higher DP of 686 (Supplementary Table S6).

Comment #10

The authors prepared four kinds of CNF (MAH-CNF, SAH-CNF, PAH-CNF and EAH-CNF). While, in Table S1, only one line was used to describe the CNF prepared by the authors. The 2.024 mmol/g of the carboxyl content is corresponding to SAH-CNF, how about other parameters, also corresponds to SAH-CNF or other kinds of CNF? It is recommended to make it clear. Or, to include the information of all four kinds of CNF in the table.

Author’s Response: Thank you for your valuable suggestion. We initially selected the most representative SAH-CNF parameters for comparison in the original Table S1, as they are central to our study. However, in response to your comment, we have now included the detailed parameters for all four types of CNF (MAH-CNF, SAH-CNF, PAH-CNF, and EAH-CNF) and have updated Table S1 accordingly, as shown below.

131 [Supplementary information Lines 212-213:](#)

132 **Supplementary Table S1.** Comparison of different preparation methods for CNFs and their corresponding data.

Type	COOH (mmol/g)	Diameter (nm)	Length (μm)	Aspect ratio	Yield (%)	DP	Cycle	Ref.
Zn(OAc) ₂ /ChCl/H ₂ O+SAH	2.024	3.61	5-8	1800	90-93	690	10	Our work
Zn(OAc) ₂ /ChCl/H ₂ O+MAH	1.412	3.72		1800		680		
Zn(OAc) ₂ /ChCl/H ₂ O+EAH	1.08	4.52		1200		730	-	
Zn(OAc) ₂ /ChCl/H ₂ O+PAH	0.51	5.19		900		780		
TOCN	1.5	4	0.87	217	82	-	-	1
TOCN	1.25	4	0.93	233	20-65	380		2
TOCN	1.74	3.5	0.23	65	-	370		3
OA/ChCl/p-TA	0.43	5.9	0.5	85	6			4
OA/ChCl	0.29	6.9	0.35	50	39			4
OA/ChCl/AlCl ₃ ·6H ₂ O	0.74	15.34	0.475	31	56			5
CA/ChCl	1.5	3.4	10.4	2500	90.12		10	6
OA/ChCl	0.27	15.7	0.337	21	76			7
OA/ChCl/FeCl ₃ ·6H ₂ O	0.2	5-20	0.05-0.3		72		3	8
CA/ChCl	0.31	8			76			9
OA/ChCl	0.12-0.17	4-34		5.1-7.9	14-33	223-282	5	10
MAH	1.6	5		390	95			11
Sulfamic acid/Glycerol	0.18-0.7	10-25			80-95	412-638		12
Cyclic anhydride	0.5-1.2	3-5		1400	98			13

133

Reviewer #2 (Remarks to the Author):

This manuscript reports a swelling–modification strategy for the scalable production of cellulose nanofibrils (CNFs) using a hydrated $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ deep eutectic solvent system. By integrating controlled cellulose swelling, cyclic anhydride esterification, and low-energy mechanical processing, the authors achieve CNFs with simultaneously high carboxyl content, high aspect ratio, preserved degree of polymerization, and excellent solvent recyclability.

Response: We are thankful for your critical and detailed evaluation of our manuscript.

Major comment

First of all, this study recalls a déjà-vu image, as shown in a recently published paper, as cited in the manuscript ‘25 Shi, X. et al. Scalable production of carboxylated cellulose nanofibres using a green and recyclable solvent. *Nat. Sustain.* 7, 315-325 (2024).’ The major experiments, even the presentation style, is quite similar :) This is probably not a problem, but the problem would be the innovation point. What is the major innovation of this study compared to the cited work? What is the conceptual advance relative to existing DES-based CNF preparation methods?

Author’s Response: We sincerely thank the reviewer for raising this critical point and for recognizing the significance of the referred study (*Nat. Sustain.* 7, 315-325, 2024). We agree that both works share the goal of advancing scalable CNFs production via DES, and we acknowledge the inspiring contributions of that study. However, the **conceptual advances** of our work are fundamentally distinct, which we summarize in three core dimensions:

(1) From "single-function" to "multi-function" platform. The referred work employed $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$, a system in which the carboxylic acid serves simultaneously as both swelling agent and esterification modifier. This intrinsic coupling restricts each acidic DES (e.g., LA/ChCl , OA/ChCl , CA/ChCl) to producing **only one type of carboxyl-modified CNF**. In contrast, our work **decouples swelling from modification** through a non-acidic $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ pretreatment, establishing a

"multifunctional operating platform" that accommodates diverse cyclic anhydrides (SAH, MAH, EAH, PAH). This enables CNFs functionalized not only with -COOH, but also with **C=C double bonds, bridged bicyclic, and aromatic rings**, all from a single DES framework (Fig. 4).

(2) Different mechanism: dual role of Zn(OAc)₂ (pH buffering + swelling enhancement). Acidic DESs are inherently a "double-edged sword"—their acid hydrolysis simultaneously promotes nanofibrillation and degrades the cellulose backbone, often limiting the aspect ratio. Our Zn(OAc)₂ uniquely provides both: (i) effective pH buffering via $\text{OAc}^- + \text{H}^+ \rightleftharpoons \text{HOAc}$, suppressing glycosidic bond cleavage; and (ii) swelling enhancement via $\text{Zn}^{2+}/\text{OAc}^-$ coordination with cellulose hydroxyl oxygens (RDF: 2.05 Å and 1.47 Å, Fig. 3h). This synergy preserves a high DP (600-900) while achieving a high aspect ratio (~1800) and high carboxyl content (up to 2.024 mmol/g).

(3) From "specific characteristics" to "customization platform". As CNFs increasingly serve as fundamental nanomaterials across diverse fields, the demand has shifted from optimizing a single property to enabling **tailored functionalization for downstream applications**. Our strategy delivers this customization while maintaining scalability (10 recycles, 93% yield) and industrial compatibility (kitchen blender / colloid mill).

The similarity in presentation style reflects shared challenges in translating DES-based CNFs from laboratory to factory; however, the **scientific question we address—how to expand a single DES system into a versatile modification platform**—is fundamentally different from optimizing a specific acidic DES system.

Several other issues require clarification.

Comment #1

1. Lines 124-126: The authors claim that the Zn(OAc)₂/ChCl/H₂O system enhances the accessibility of surface hydroxyl groups at elevated temperatures, which is attributed to cellulose swelling. However, the definition of "swelling" in this context is not

sufficiently clear. The authors are encouraged to provide more direct evidence of cellulose swelling, such as changes in fiber diameter, crystallinity, or quantitative indicators related to accessible -OH groups (e.g., specific surface area or related measurements).

Author's Response: Thanks for your valuable feedback. We agree that the definition of "swelling" in this context should be clarified. To address this, we have provided additional details on the swelling behavior of cellulose in the $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ system. Because the raw cellulose we used was hardwood bleached kraft pulp, whose fiber diameter was not uniform, we could not quantitatively demonstrate direct evidence of cellulose swelling from the change in cellulose fiber diameter before and after swelling. To more directly explain the change in crystallinity caused by swelling, we compared the difference in crystallinity of the raw cellulose before and after swelling in our previous data (Supplementary Fig. 4). The crystallinity of cellulose increased slightly after swelling, which is attributed to the hydrolysis and destruction of amorphous regions during the swelling process of DES. More direct evidence is the obvious etching and brooming phenomenon on the surface of cellulose after swelling in SEM images. This is significantly different from the smooth and dense surface morphology of the raw cellulose (Supplementary Fig. 1). We acknowledge that quantitative indicators such as BET surface area would provide additional confidence. However, given that BET requires dried samples (which would collapse the highly swollen state), we relied on direct morphological observation (broom-like fibrillation), elemental penetration (EDS cross-section, 6.4% Zn^{2+}), and SAXS analysis (Fig. 3a-b, where inter-fibrillar correlation length increased from 3.81 nm to 5.62 nm) as complementary evidence of cellulose swelling. In summary, we believe that the data provided can directly prove the swelling of cellulose in DES. We hope these data will help to better illustrate the extent of cellulose swelling and its role in enhancing the accessibility of surface hydroxyl groups at elevated temperatures. The manuscript also added and marked related discussions.

Lines 123-126: The swelling of raw cellulose typically results in nearly all fibers being

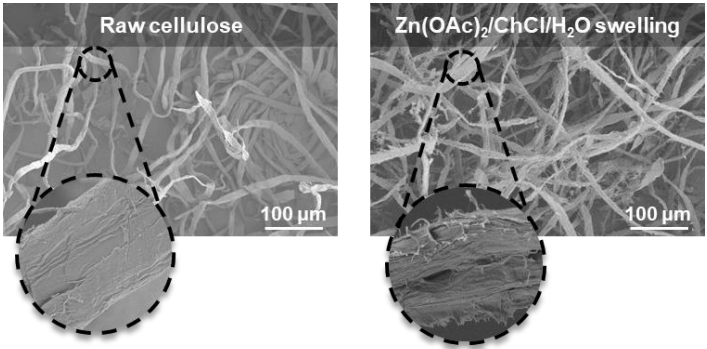
etched and exhibiting a distinctive broom-like fibrillation. The $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ DES system exhibited strong swelling ability (Supplementary Fig. S1).

Lines 182-186: As shown in Supplementary Fig. S4, the cellulose pretreated with both anhydrous DES and hydrated DES systems retained high crystallinity (73-76%), which showed a slight increase compared to the raw cellulose. This was attributed to the hydrolysis and destruction of amorphous regions during the swelling process.

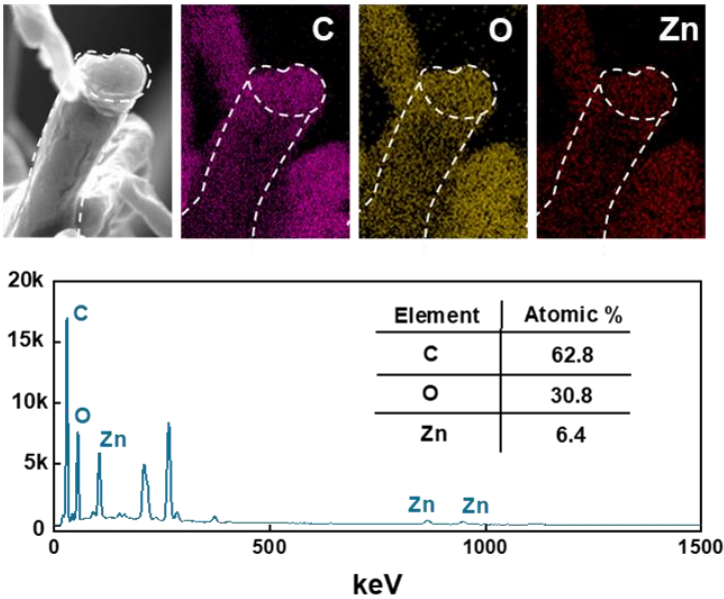
Lines 224-250: Furthermore, direct evidence of cellulose swelling was observed through EDS-SEM analysis of cross-sections of swollen cellulose (Supplementary Fig. S11). The swollen cellulose cross-sections exhibited a high Zn^{2+} content (6.4%). This indicates that during the swelling process, DES can penetrate the raw cellulose, achieving a high degree of swelling.

Additionally, the effect of each component in the DES on the swelling effect of raw cellulose was investigated by comparing the swelling ability of the $\text{ChCl}/\text{H}_2\text{O}$ system without $\text{Zn}(\text{OAc})_2$ (Supplementary Fig. S12). After pretreatment with $\text{ChCl}/\text{H}_2\text{O}$, the surface of the raw cellulose showed no obvious etching or broom-like fibrillation, maintaining a smooth and dense surface structure similar to the original raw cellulose. The differences between direct mechanical treatment of cellulose in DES without MAH modification and direct MAH modification/mechanical treatment without DES swelling were explored. The cellulose treated with both DES swelling and MAH modification achieved the best nanofibrillation effect. This is due to the highly efficient carboxylation modification, which generates enough $-\text{COO}^-$ groups and produces electrostatic repulsion, thereby facilitating cellulose nanofibrillation. The cellulose after DES swelling, followed by mechanical processing without MAH modification, still contained many micron-sized fibers. Digital images of the dispersion during mechanical processing showed a white dispersion rather than a typical gel-like morphology. This indicates that the nanofibrillation effect of cellulose without MAH modification is weak due to the lack of MAH carboxylation modification of cellulose (Supplementary Fig. S13). The carboxyl content of raw cellulose directly modified by

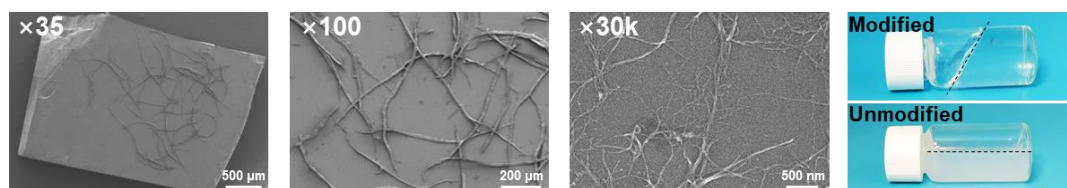
MAH without DES swelling was as low as 0.573 mmol/g, and many larger cellulose fibers were not completely nanofibrillated (Supplementary Fig. S14). The dispersion also exhibited significant sedimentation within 24 h, indicating low accessibility of cellulose-OH and reduced modification efficiency during the MAH modification process without DES pretreatment, which directly affected the nanofibrillation.



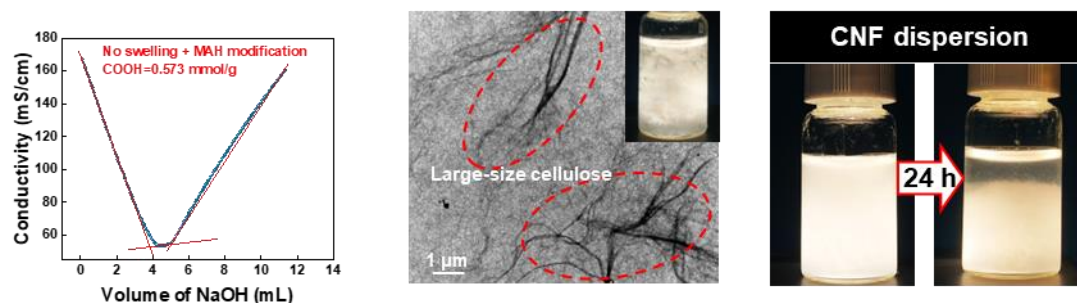
Supplementary Fig. S1. Comparison of SEM images of raw cellulose and highly swollen cellulose in the Zn(OAc)₂/ChCl/H₂O system.



Supplementary Fig. S11. EDS-SEM images of cellulose swollen cross-section with Zn(OAc)₂/ChCl/H₂O DES systems.



Supplementary Fig. S12. SEM images and digital photographs of the cellulose dispersion prepared by direct mechanical treatment of cellulose raw material after DES swelling.



Supplementary Fig. S13. Carboxyl content, TEM image and photo of cellulose raw material after MAH modification without DES swelling (blank control).

Comment #2

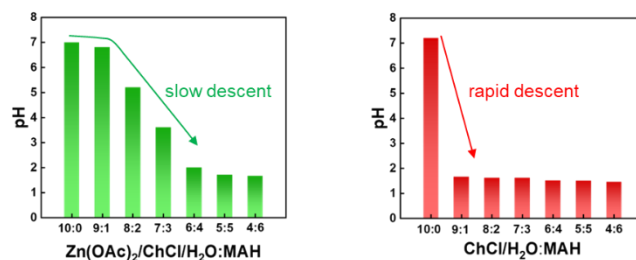
The buffering function of $\text{Zn}(\text{OAc})_2$ is central to the proposed mechanism for suppressing acid hydrolysis and preserving DP. Although indirect evidence is provided, a more explicit discussion of how the buffering capacity compares with other acetate salts or DES systems would strengthen this argument. If available, quantitative pH or acidity-related data during the modification process should be briefly discussed.

Author's Response: Thank you for your thoughtful suggestions. In response, we have added more detailed data on the pH changes in both the $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ and $\text{ChCl}/\text{H}_2\text{O}$ systems with the same MAH mass ratio, as well as titration curves of $\text{Zn}(\text{OAc})_2$ solution to demonstrate its buffering capacity. These data, along with a brief discussion, have been incorporated into the revised manuscript and highlighted in Supplementary Fig. S6-S8. Given that research on metal acetate salts as buffers in DES systems is limited, our attempts to prepare a DES system with potassium acetate and sodium acetate were unsuccessful. As a result, we chose to investigate a traditional organic acid-based DES system for comparison. The inclusion of $\text{Zn}(\text{OAc})_2$ as a buffer

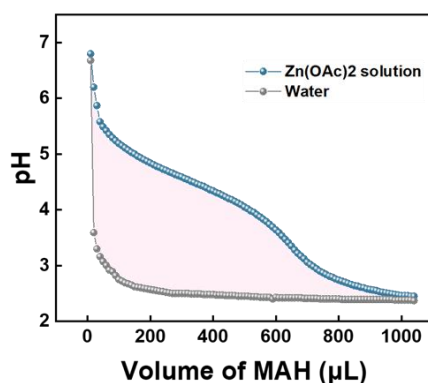
allowed us to compare the effect of its presence on the DP of cellulose after swelling. Additionally, we compared the classic formic acid/choline chloride (FA/ChCl), acetic acid/choline chloride (AA/ChCl), and lactic acid/choline chloride (LA/ChCl) pretreatment systems with and without Zn(OAc)₂. The results clearly showed that the pretreatment systems with Zn(OAc)₂ significantly increased the DP of swollen cellulose (Supplementary Fig. S13), supporting the hypothesis that Zn(OAc)₂ acts as a buffer, balancing H⁺ and mitigating acid hydrolysis. Please see the revised text as follow.

Line 207-218: The pH differences between the Zn(OAc)₂/ChCl/H₂O and ChCl/H₂O systems with the same MAH ratio are shown in Supplementary Fig. S6. The presence of Zn(OAc)₂ slowed down the rate of pH decrease, whereas the blank control group (ChCl/H₂O) without Zn(OAc)₂ experienced a significant pH decrease upon the addition of a small amount of MAH. This trend mirrors the pH titration curve of the Zn(OAc)₂ solution (Supplementary Fig. S7). Furthermore, the DP of the swollen raw cellulose in the ChCl/H₂O system dropped sharply to around 220 after MAH modification, and the resulting CNFs exhibited a spindle-shaped structure similar to cellulose nanocrystals (Supplementary Fig. S8). The recovered solution displayed a distinct yellow color compared to the Zn(OAc)₂/ChCl/H₂O system, indicating the presence of degraded fragments caused by vigorous acid hydrolysis.

Lines 251-258: Moreover, the effect of the buffering capacity of Zn(OAc)₂ on the pretreatment process of raw cellulose in an acidic DES system was investigated. The morphology and DP of cellulose in the classic acidic DES systems of lactic acid/choline chloride (LA/ChCl), formic acid/choline chloride (FA/ChCl), and acetic acid/choline chloride (AA/ChCl) were compared with and without Zn(OAc)₂ during the pretreatment stage (Supplementary Fig. S14). In all acidic DES systems containing Zn(OAc)₂, cellulose maintained a high DP and lighter morphological deconstruction, demonstrating the positive role of Zn(OAc)₂ as a buffer during cellulose pretreatment.



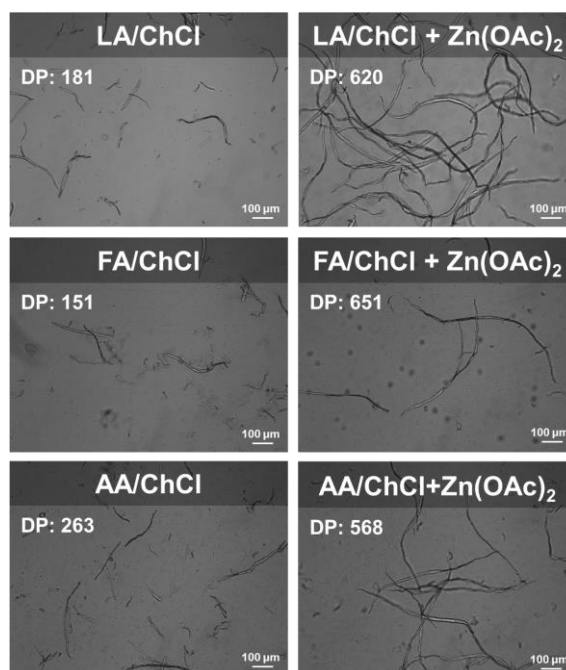
Supplementary Fig. S6. pH changes in $\text{Zn(OAc)}_2/\text{ChCl}/\text{H}_2\text{O}$ and $\text{ChCl}/\text{H}_2\text{O}$ with the same MAH mass ratio.



Supplementary Fig. S7. Comparison of titration curves of Zn(OAc)_2 solution and water with MAH solution.



Supplementary Fig. S8. SEM images of CNF under the $\text{ChCl}/\text{H}_2\text{O}$ system and digital photos of its recovered solution.



Supplementary Fig. S15. Microscopic images and DP comparison of cellulose with and without $\text{Zn}(\text{OAc})_2$ pretreatment in the LA/ChCl, FA/ChCl, and AA/ChCl systems. (Supplementary Note: Attempts to substitute $\text{Zn}(\text{OAc})_2$ with KOAc or NaOAc in the DES formulation failed to form a homogeneous eutectic mixture under identical conditions (1:2:2 molar ratio, 80 °C), highlighting the unique role of $\text{Zn}(\text{OAc})_2$ not only as a pH buffer but also in maintaining the hydrogen-bonding network essential for DES formation.)

Comment #3

The demonstration of four cyclic anhydrides effectively supports the idea of a “multifunctional operating platform.” A short discussion on the criteria for selecting these anhydrides (e.g., reactivity, melting point, industrial relevance) would further reinforce the generality and expandability of the approach.

Author’s Response: Thank you for your valuable suggestion. In response, we have added a brief discussion on the criteria for selecting the four cyclic anhydrides, including factors such as reactivity, melting point, and industrial relevance at lines 502-515 of the revised manuscript, as follows. This additional explanation further supports the generality and expandability of the “multifunctional operating platform”.

Line 524-540: The four acid anhydrides (SAH, MAH, EAH, and PAH) selected in this study are all common, cost-effective chemical reagents widely used in existing industrial production (such as synthetic resins, surfactants, adhesives and plasticizers), which enhances their potential for large-scale production. It is worth noting that the four anhydrides selected for this work can all form modified DES with $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ at temperatures below 100 °C. This process maintains a low viscosity and facilitates the esterification reaction between the four acid anhydrides and the swollen cellulose, which is crucial for maintaining high modification efficiency. Furthermore, these four classic acid anhydrides impart representative active functional groups to CNFs, including carboxyl groups, double bonds, benzene rings, and bridged bicyclic rings. MAH, with its extremely low melting point (~55 °C), facilitates convenient processing. SAH, known for its high reactivity and low cost, has been widely recognized in the literature²⁷. The benzene ring and bridged bicyclic structures of PAH and EAH offer hydrophobicity and UV shielding ability for the functionalization of CNFs in downstream applications⁵⁶. Therefore, this study utilizes these four classic acid anhydrides as active modifiers, supporting the concept of a "multifunctional operating platform", and it further demonstrates the generality and expandability of the strategy.

Comment #4

The reported aspect ratio (~1800) is notably high and represents an important advantage of the method. To improve confidence in this result, the authors are encouraged to clarify the statistical basis of the measurement (e.g., number of fibrils analyzed, distribution range, and potential projection effects in TEM).

Author's Response: Thank you for the constructive suggestion. To improve clarity and confidence in the reported aspect ratio, we have added a detailed description of the statistical basis for the measurement.

Line 706-709: The diameter and length of at least 100 fibers were randomly measured from the TEM images, and their diameter, length, and aspect ratio distributions were

calculated using the software Nano Measurer 1.2.5 (developed using the Visual Basic 6.0 programming language).

Comment #5

The authors are encouraged to include additional control experiments to clarify the respective roles of each component in the system, such as cyclic anhydride modification without Zn(OAc)₂, Zn(OAc)₂-containing DES without cyclic anhydride, and DES swelling followed by mechanical processing without cyclic anhydride.

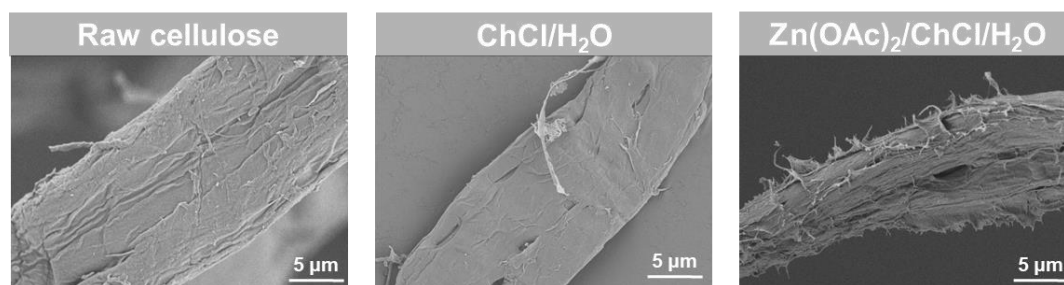
Author's Response: Thank you for your thoughtful suggestion. In response, we have conducted additional control experiments to clarify the roles of each component in the system. Specifically, we have included the following:

- ChCl/H₂O swelling without Zn(OAc)₂, presented in Supplementary Fig. S12.
- DES swelling followed by mechanical processing without MAH, shown in Supplementary Fig. S13.
- MAH modification without the DES system and without Zn(OAc)₂ swelling, illustrated in Supplementary Fig. S14.

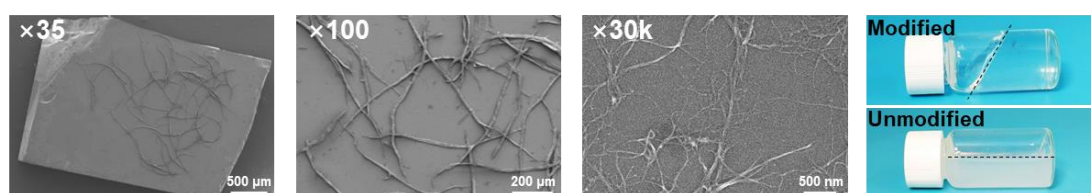
A detailed discussion of these control experiments has been added at the revised manuscript, as follows.

Lines 229-250: Additionally, the effect of each component in the DES on the swelling effect of raw cellulose was investigated by comparing the swelling ability of the ChCl/H₂O system without Zn(OAc)₂ (Supplementary Fig. S12). After pretreatment with ChCl/H₂O, the surface of the raw cellulose showed no obvious etching or broom-like fibrillation, maintaining a smooth and dense surface structure similar to the original raw cellulose. The differences between direct mechanical treatment of cellulose in DES without MAH modification and direct MAH modification/mechanical treatment without DES swelling were explored. The cellulose treated with both DES swelling and MAH modification achieved the best nanofibrillation effect. This is due to the highly efficient carboxylation modification, which generates enough -COO⁻ groups and produces electrostatic repulsion, thereby facilitating cellulose nanofibrillation. The

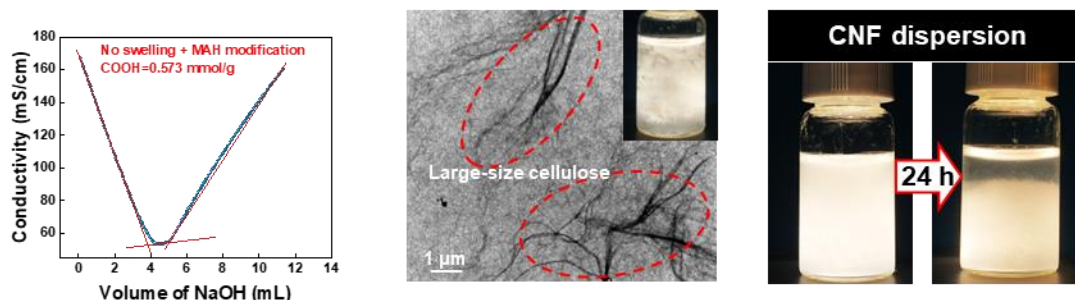
cellulose after DES swelling, followed by mechanical processing without MAH modification, still contained many micron-sized fibers. Digital images of the dispersion during mechanical processing showed a white dispersion rather than a typical gel-like morphology. This indicates that the nanofibrillation effect of cellulose without MAH modification is weak due to the lack of MAH carboxylation modification of cellulose (Supplementary Fig. S13). The carboxyl content of raw cellulose directly modified by MAH without DES swelling was as low as 0.573 mmol/g, and many larger cellulose fibers were not completely nanofibrillated (Supplementary Fig. S14). The dispersion also exhibited significant sedimentation within 24 h, indicating low accessibility of cellulose-OH and reduced modification efficiency during the MAH modification process without DES pretreatment, which directly affected the nanofibrillation.



Supplementary Fig. S12. Comparison of SEM images of cellulose and raw cellulose after ChCl/H₂O and DES swelling.



Supplementary Fig. S13. SEM images and digital photographs of the cellulose dispersion prepared by direct mechanical treatment of raw cellulose after DES swelling.



Supplementary Fig. S14. Carboxyl content, TEM image and photo of raw cellulose after MAH modification without DES swelling (blank control).

Comment #6

The comparison with TOCN is important for positioning the method. The authors may consider clarifying potential differences in surface chemistry (e.g., ester-linked versus oxidized carboxyl groups) and how these differences might affect downstream applications, beyond carboxyl content alone.

Author's Response: Thank you for your valuable suggestion. We appreciate the opportunity to clarify the differences in surface chemistry and their potential impact on downstream applications. In the revised manuscript, we have emphasized that the modified CNFs exhibit varied surface functionalization depending on the type of cyclic anhydride used. Specifically, the esterification reaction resulted in ester-linked functional groups (-COOR) and carboxyl groups (-COO⁻) on the CNF surface, as confirmed by FT-IR analysis (Fig. 4c). We recognize that surface chemistry, beyond just carboxyl content, plays a critical role in determining the performance of modified CNFs in various applications. For example, CNF_{SAH} can introduce a higher carboxyl content (~2.0 mmol/g), while the carboxyl content of currently reported TOCN is approximately 1.2-1.7 mmol/g. It also exhibits lower degradation and shows a significantly higher aspect ratio and DP than TOCN. This is attributed to the dual mechanism of pH buffering + swelling enhancement in this study. In addition to having a carboxyl content comparable to traditional TOCN, CNF_{MAH} has surface modification with double bond functional groups. This facilitates chemical crosslinking and derivatization with other polymers in downstream applications, which has attracted widespread attention in the preparation of CNF gels and composite materials. Although CNF_{EAH} and CNF_{PAH} have lower carboxyl content than TOCN, they possess unique bicyclic and benzene rings functional groups. This will help improve the hydrophobicity and UV shielding ability of CNF, respectively. Considering the limitations of TOCN in chemical modification, our work did not solely pursue the preparation of CNFs with high carboxyl content, but rather focused on the modification

of flexible functional groups in CNFs under the DES system and its potential expansion for downstream applications. In summary, the features of CNFs proposed in this work have unique advantages over TOCN in downstream applications. These clarifications have been included in the revised manuscript to provide a more comprehensive understanding of the impact of modifications beyond carboxyl content. Please see below revised text.

Lines 473-481: Compared to the oxidized carboxyl group of TOCN, CNFs modified by flexible esterification with DES can not only produce CNF_{SAH} with a much higher carboxyl content than TOCN¹², but also produce CNF_{MAH}, CNF_{EAH}, and CNF_{PAH} containing chemical structures by changing different types of anhydrides. Among these, the double bond of CNF_{MAH} provides active sites for chemical crosslinking and derivatization modification, while CNF_{EAH} and CNF_{PAH} feature bicyclic and benzene rings. These will help improve the hydrophobicity and UV shielding ability of CNFs, respectively. These differences give the CNFs of this work a unique advantage over TOCN for downstream applications.

Comment #7

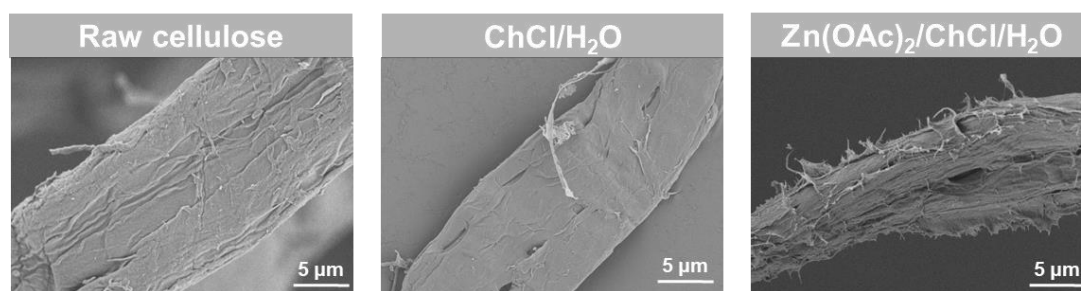
The manuscript attributes the disruption of hydrogen bonding primarily to DES-induced swelling. However, it remains unclear whether temperature or water content may play a dominant role in this process. It is helpful to clarify whether a ChCl/H₂O system at the same temperature would exhibit comparable swelling behavior.

Author's Response: Thank you for your thoughtful suggestions. In our previous investigations, we found that water content is the most dominant factor influencing the swelling process (Fig. 2 and Supplementary Fig. S19-22). Specifically, the addition of water helps to reduce the viscosity of the DES, balancing protons, and improving hydrogen bonding network. To clarify the role of the DES system, we have added a comparison in Supplementary Fig. S11, which shows the swelling behavior of the ChCl/H₂O system and DES at the same temperature. The results demonstrate that the ChCl/H₂O system exhibits almost no swelling effect under the same conditions,

highlighting the critical role of the DES system and $\text{Zn}(\text{OAc})_2$ in cellulose pretreatment.
Please see revised text below.

Lines 229-234: Additionally, the effect of each component in the DES on the swelling effect of raw cellulose was investigated by comparing the swelling ability of the $\text{ChCl}/\text{H}_2\text{O}$ system without $\text{Zn}(\text{OAc})_2$ (Supplementary Fig. S12). After pretreatment with $\text{ChCl}/\text{H}_2\text{O}$, the surface of the raw cellulose showed no obvious etching or broom-like fibrillation, maintaining a smooth and dense surface structure similar to the original raw cellulose.

Lines 338-348: In summary, the regulatory role of water in DES can be outlined in the following key aspects: (i) Water reduces the viscosity of DES and increases its ionic conductivity, which enhances solvent penetration into the cellulose fibrils and improves mass transfer during pretreatment³⁸. (ii) Water modulates the proton activity and acid–base equilibrium of DES, thereby influencing the catalytic ability and the interaction strength between DES components and cellulose²⁵. (iii) A small amount of bound water can participate in hydrogen-bond bridging, facilitating the disruption of hydrogen bonds within cellulose and promoting better interaction between DES components and cellulose³⁹. (iv) Water dilution lowers the cost of DES and alleviates processing challenges associated with high viscosity⁴⁰.



Supplementary Fig. S12. Comparison of SEM images of cellulose and raw cellulose after $\text{ChCl}/\text{H}_2\text{O}$ and DES swelling.

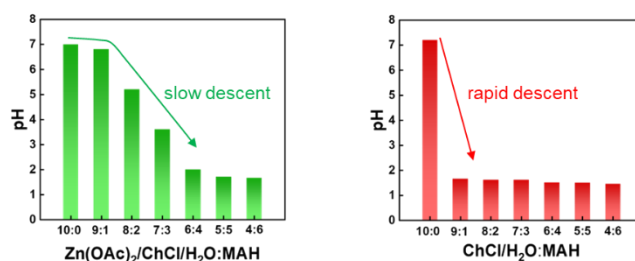
Comment #8

Lines 200-202: The authors claim that $\text{Zn}(\text{OAc})_2$ acts as an effective pH buffer during

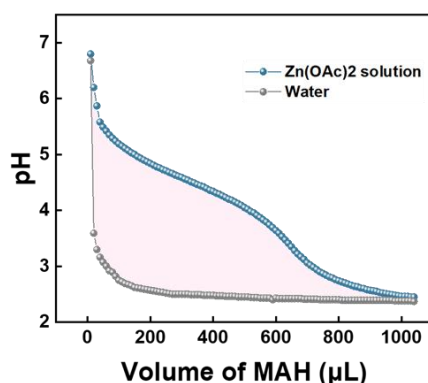
nanofibrillation, but the source of protons (H^+) is not clearly identified. Please clarify whether H^+ originates from $-COOH$ dissociation, hydrolytic side reactions, or other processes, and whether $Zn(OAc)_2$ truly functions as a buffer rather than a neutral salt.

Author's Response: Thank you for your insightful suggestion. The protons (H^+) involved in the process primarily originate from the hydrolytic side reactions of the acid anhydrides. To clarify this, we have included pH trends at different ratios of DES to MAH and provided SEM images of CNF prepared using the blank control ($ChCl/H_2O$) without $Zn(OAc)_2$. These additions highlight the irreplaceable role of $Zn(OAc)_2$ in the DES system, further demonstrating its function as a pH buffer rather than merely acting as a neutral salt. Please see the explanation below in the revised manuscript.

Line 207-218: The pH differences between the $Zn(OAc)_2/ChCl/H_2O$ and $ChCl/H_2O$ systems with the same MAH ratio are shown in Supplementary Fig. S6. The presence of $Zn(OAc)_2$ slowed down the rate of pH decrease, whereas the blank control group ($ChCl/H_2O$) without $Zn(OAc)_2$ experienced a significant pH decrease upon the addition of a small amount of MAH. This trend mirrors the pH titration curve of the $Zn(OAc)_2$ solution (Supplementary Fig. S7). Furthermore, the DP of the swollen raw cellulose in the $ChCl/H_2O$ system dropped sharply to around 220 after MAH modification, and the resulting CNFs exhibited a spindle-shaped structure similar to cellulose nanocrystals (Supplementary Fig. S8). The recovered solution displayed a distinct yellow color compared to the $Zn(OAc)_2/ChCl/H_2O$ system, indicating the presence of degraded fragments caused by vigorous acid hydrolysis.



Supplementary Fig. S6. pH changes in $Zn(OAc)_2/ChCl/H_2O$ and $ChCl/H_2O$ with the same MAH mass ratio.



Supplementary Fig. S7. Comparison of titration curves of Zn(OAc)₂ solution and water with MAH solution.



Supplementary Fig. S8. SEM images of CNF under the ChCl/H₂O system and digital photos of its recovered solution.

Comment #9

The manuscript emphasizes hydrogen-bond competition as the dominant swelling mechanism in the Zn(OAc)₂/ChCl/H₂O system. Please clarify whether coordination interactions and ionic effects (e.g., involving Zn²⁺ or acetate) also contribute to cellulose swelling.

Author's Response: Thank you for your thoughtful suggestion. We acknowledge that coordination interactions and ionic effects, particularly involving Zn²⁺ or acetate, can also contribute to cellulose swelling, as reported in previous studies (*Green Chem.*, 2023, 25, 5086; *ACS Nano* 2025, 19, 20991-21006). In response, we have added a comparative analysis of the radial distribution function (RDF) and coordination number for the two DES systems in Supplementary Fig. S25. We have also modified the

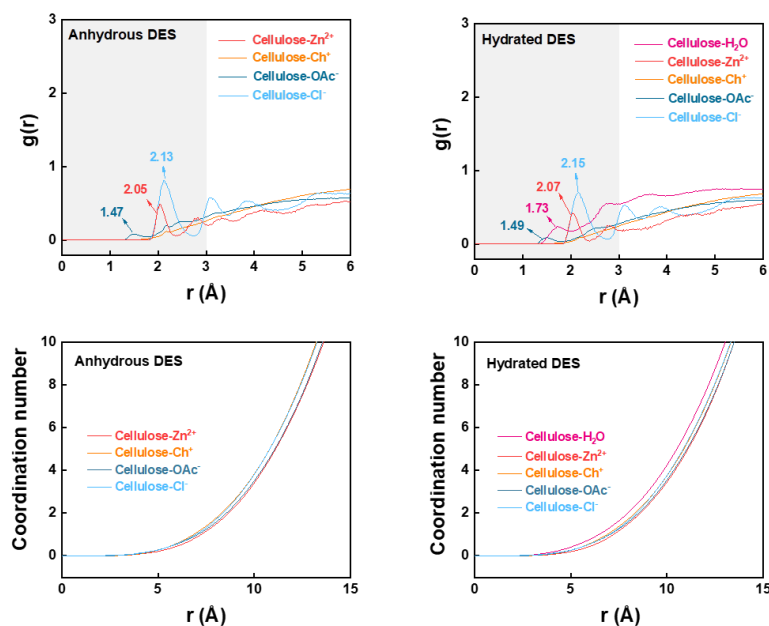
manuscript to include a discussion on the role of these interactions, and the relevant changes have been marked at lines 393-401 and 711-716, as follows.

Lines 406-416: The RDF data revealed that the distances between cellulose and OAc⁻, Zn²⁺, and Cl⁻ were 1.47, 2.05, and 2.13 Å, respectively. These results indicate that strong interactions were established between cellulose and the DES system, contributing to the high swelling of cellulose in the DES system (Supplementary Fig. S26.)⁴⁶. However, compared to the hydrated DES system, while H₂O maintained a relatively small distance of 1.73 Å between cellulose molecules, the distances between the ions and cellulose in both DES systems did not change significantly. Although DES interacts with cellulose, the DES system does not exhibit a significant coordination number with cellulose. This may be attributed to the fact that contributions of coordination interactions and ionic effects are not the primary mechanisms driving cellulose swelling in this research⁴⁷.

Lines 740-745: The coordination number of cellulose with each component was calculated based on the RDF curve using the following formula:

$$N = 4\pi\rho \int_0^r r^2 g(r) dr$$

Where N is the coordination number, ρ represents the density of the system, r is the radial distance, and g(r) is the radial distribution function (pair correlation function) representing the probability of finding a particle at distance r relative to an ideal gas distribution.



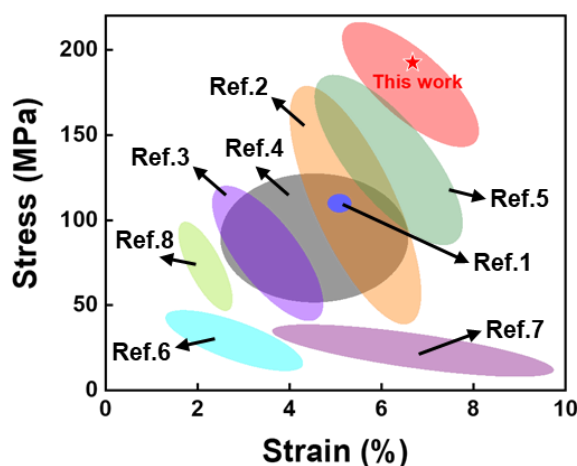
Supplementary Fig. S26. RDF and coordination number comparison of raw cellulose during the swelling process of anhydrous and hydrated DES systems.

Comment #10

The mechanical performance of CNFSAH films (Supplementary Fig. S23) should be compared with representative CNF films from the literature under similar testing conditions would help readers better contextualize the performance improvements.

Author's Response: Thanks for your valuable suggestion. In response, we have added a comparison of the stress and strain data for the four types of expandable functionalized CNFs in this study with similar CNF films reported in the literature. This comparison is presented in Supplementary Fig. S31. Additionally, we have included a discussion in the revised manuscript to help contextualize the performance improvements, as follows.

Lines 518-520: The tensile stress and Young's modulus of CNFSAH films reached 210 MPa and 3.64 GPa, respectively (Supplementary Fig. S31), significantly surpassing the values of traditional CNF films (Supplementary Fig. S32)^{54,55}.



Supplementary Fig. S32. Comparison of stress and strain of four types of four expandable functionalized CNFs in this work with similar works¹⁻⁸.

Comment #11

The large-scale simulation using conventional industrial equipment is convincing. To further enhance industrial relevance, the authors could briefly discuss potential challenges in such processing, such as DES viscosity control, water content management, or long-term accumulation of impurities during solvent recycling.

Author's Response: Thank you for your thoughtful suggestion. To further enhance the industrial relevance of our study, we have included a discussion on potential challenges in large-scale processing, as follows.

Lines 615-624: While research on the preparation of CNFs with thin diameters, high aspect ratios, and various modifications using DESs has become increasingly advanced^{40,58}, there is still limited focus on the low-cost, recyclable, and large-scale production of CNFs using DESs. While ensuring the chemical structure and microstructure of CNFs, future research should also prioritize the cost, efficiency, and recyclability of DES in large-scale production. This includes considering factors such as the control of DES viscosity, water content management, and the presence of low-molecular-weight or small-molecule residues after multiple solvent cycles, which can

affect the recycling efficiency of DES. Ultimately, the goal is to transition CNFs production using DES systems from the "laboratory" to the "factory".

Comment #12

Regarding the statement that water “enhancing viscosity” (Page 14, Lines 242-244): this claim is incorrect and contradicts your own data (Supplementary Fig. S8, which shows viscosity decreases with added water). The effect (between viscosity, hydrogen-bond network, swelling and mobility) should be accurately explained and revised.

Author’s Response: Thank you for pointing out this discrepancy. We apologize for the incorrect expression. The statement regarding water “enhancing viscosity” has been corrected to “reduce viscosity” in line with the data presented in Supplementary Fig. S8. Please see the revised text as follow.

Lines 302-304: Water has been recognized as an effective DES modifier, reducing viscosity, improving the hydrogen bonding network, and increasing conductivity, thereby promoting cellulose nanofibrillation¹⁴.

Comment #13

The combined SAXS, LF-NMR, and MD simulation results convincingly show the regulatory role of water in the DES. The mechanistic discussion could be further streamlined to emphasize a single coherent physical picture, highlighting how water indirectly enhances cellulose swelling by modulating DES component mobility and interactions.

Author’s Response: Thank you for your valuable suggestion. In response, we have streamlined the mechanistic discussion to present a more coherent physical picture. We now emphasize how water indirectly enhances cellulose swelling by modulating the mobility and interactions of the DES components. Please see revised text below.

Lines 338-348: In summary, the regulatory role of water in DES can be outlined in the following key aspects: (i) Water reduces the viscosity of DES and increases its ionic

conductivity, which enhances solvent penetration into the cellulose fibrils and improves mass transfer during pretreatment³⁸. (ii) Water modulates the proton activity and acid–base equilibrium of DES, thereby influencing the catalytic ability and the interaction strength between DES components and cellulose²⁵. (iii) A small amount of bound water can participate in hydrogen-bond bridging, facilitating the disruption of hydrogen bonds within cellulose and promoting better interaction between DES components and cellulose³⁹. (iv) Water dilution lowers the cost of DES and alleviates processing challenges associated with high viscosity⁴⁰.

Comment #14

Line 329, the unit for the diffusion coefficient is cm²/S, not cm/S.

Author's Response: Thank you for your helpful suggestion. We have corrected the unit for the diffusion coefficient to cm²/S, as follow.

Line 392-395: Specifically, the diffusion coefficient of Ch⁺ in the hydrated DES system increased significantly from 5.37×10⁻⁴ cm²/S to 7.36×10⁻⁴ cm²/S, confirming that water enhanced the diffusion capacity of Ch⁺, which is consistent with the increased interaction energy.

Comment #15

Line 336-339, how is the interaction energy calculated and normalized (e.g., per molecule, per cellulose chain, or per simulation box).

Author's Response: Thank you for your thoughtful suggestion. We have added a detailed explanation of how the interaction energy is calculated and normalized. Please see revised text below.

Line 733-738: The interaction energies between cellulose and the DES system were calculated. In the A-B system, component A refers to the cellulose, while component B refers to the entire DES system or a single component (H₂O, Zn²⁺, Ch⁺, OAc⁻). The interaction energy (ΔE) was determined using the following formula:

$$\Delta E = E_{AB} - (E_A + E_B)$$

640 Where E_{AB} is the total energy of the system, E_A is the energy of component A, and E_B
641 is the energy of component B^{43,46}.

Reviewer #3 (Remarks to the Author):

The manuscript presents a highly efficient swelling-modification strategy for producing carboxylated cellulose nanofibrils (CNFs) using a hydrated deep eutectic solvent (DES) system composed of $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$. The authors demonstrate production of CNFs with high carboxyl content (2.024 mmol/g), exceptional aspect ratio (~1800), high yield (93%), and excellent solvent recyclability (10 cycles). The work establishes a "multifunctional operating platform" enabling diverse chemical modifications and demonstrates scalability with reduced environmental impact compared to TEMPO oxidation methods. This manuscript represents a significant advancement in sustainable CNF production with clear improvements over existing methods. The work is comprehensive, well-executed, and addresses critical limitations in the field. However, several areas require clarification and improvement before acceptance in Nature Communications.

Response: We are thankful for the critical and detailed evaluation of our manuscript and giving your feedback in the form of insightful comments and useful suggestions. Your comments and suggestions were constructive and helped us improve the quality of our manuscript. We have carefully addressed all queries and revised the manuscript in light of your valuable comments and suggestions. We hope that the responses to your comments and subsequent amendments in the revised manuscript are satisfactory to recommend it for publication in 'Nature Communications'.

Comment #1

While your work uses $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ vs. $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$ system, What fundamental limitations of the $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$ system your approach addresses? How your "swelling-modification strategy" differs mechanistically from the $\text{CA}/\text{ChCl}/\text{H}_2\text{O}$ approach. The dual role of $\text{Zn}(\text{OAc})_2$ (pH buffering + swelling enhancement) is not fully resolved? Reference 25 Line 704: Shi, X. et al. Scalable production of carboxylated cellulose nanofibres using a green and recyclable solvent. Nat. Sustain. 7, 315-325 (2024).

Author's Response: We greatly appreciate the reviewer's incisive question, which

gives us the opportunity to clarify the conceptual and mechanistic distinctions of our work. We address this in three points:

(1) Limitation of CA/ChCl/H₂O addressed by our approach. In the CA/ChCl/H₂O system, citric acid plays a dual role as both swelling agent and esterification modifier. This **structural coupling** confines each DES system to a single modification type. Our Zn(OAc)₂/ChCl/H₂O system **decouples swelling from modification**, allowing the same swelling platform to accommodate multiple anhydride modifiers (SAH, MAH, EAH, PAH), thereby producing CNFs with diverse functional groups (-COOH, C=C, bridged bicyclic, aromatic rings) within a unified framework.

(2) Mechanistic distinction. Acidic DES systems leverage acid hydrolysis to assist nanofibrillation, but excessive hydrolysis sacrifices DP and aspect ratio (e.g., OA/ChCl yields CNCs rather than CNFs). Our strategy proceeds through **two independent and controllable stages**: (i) high-temperature DES swelling to enhance hydroxyl accessibility (broom-like fibrillation, EDS-confirmed Zn²⁺ penetration of 6.4%, SAXS-confirmed dc increase from 3.81 to 5.62 nm); and (ii) cyclic anhydride esterification on the highly accessible cellulose surface. This sequential design avoids the "double-edged sword" of acidic DES.

(3) Dual role of Zn(OAc)₂—both pH buffering AND swelling enhancement. This is supported by **complementary experimental and computational evidence**:

- **pH buffering:** pH titration shows Zn(OAc)₂/ChCl/H₂O resists pH drop upon MAH addition, whereas ChCl/H₂O drops sharply (Supplementary Fig. S6, S7). The mechanism follows $\text{OAc}^- + \text{H}^+ \rightleftharpoons \text{HOAc}$ ($\text{pK}_a \approx 4.76$). Without Zn(OAc)₂, DP drops to ~220 with severe hydrolytic browning (Supplementary Fig. S8).
- **Swelling enhancement:** MD simulations reveal Zn²⁺/OAc⁻ coordinate with cellulose at 2.05 Å and 1.47 Å (Fig. 3h), and EDS-SEM confirms internal Zn²⁺ penetration of 6.4% (Supplementary Fig. S11). Removal of Zn(OAc)₂ eliminates broom-like fibrillation and yields a smooth, dense surface (Supplementary Fig. S12).

- **Synergy validated in acidic DES systems:** Adding $\text{Zn}(\text{OAc})_2$ to LA/ChCl, FA/ChCl, and AA/ChCl significantly preserves cellulose DP and morphology (Supplementary Fig. S15), confirming that the buffering role of $\text{Zn}(\text{OAc})_2$ is **transferable** and not merely a property of the specific system studied here.

In summary, the conceptual advance of our work lies in **transitioning DES-based CNFs production from "specific characteristics" to a "customization platform"**, enabled by the unique dual function of $\text{Zn}(\text{OAc})_2$. We have revised the manuscript (Lines 203-213, 246-253, 402-412) and added supporting evidence (Supplementary Fig. S6-S8, S12, S15) to strengthen this argument.

Comment #2

The reference paper (Shi et al.) achieved: Carboxyl content: 1.5 mmol/g; Aspect ratio: ~2,500; Yield: 90.12%; Your work achieves: Carboxyl content: 2.024 mmol/g (35% improvement); Aspect ratio: ~1,800 (28% lower); Yield: 93% (3% improvement). Please add a dedicated paragraph discussing why the trade-off of lower aspect ratio for higher carboxyl content represents an advancement. Is this deliberate tuning or an inherent limitation? Is the lower aspect ratio due to: o Deliberate tuning via pH buffering (preserving DP but limiting swelling)? Inherent limitation of $\text{Zn}(\text{OAc})_2$ system? Different mechanical treatment intensity?

Author's Response: Thank you for your insightful suggestions. As you noted, our work demonstrates a significant increase in carboxyl content compared to the reference study (*Nat. Sustain.* 7, 315-325, 2024), although the aspect ratio is slightly lower. First, we noticed that the calculation method of aspect ratio is different between the reference and ours. The average aspect ratio used in *Nat. Sustain.* 7, 315-325, 2024 is calculated by the ratio of the average length to the average diameter of CNFs. However, our calculation method uses the average aspect ratio of multiple individual CNFs. Using the calculation method described in *Nat. Sustain.* 7, 315-325, 2024, the average aspect ratio of CNF_{SAH} and CNF_{MAH} in this work can reach 2300-3000. This difference in aspect ratio value is due to the different calculation methods. However, in the manuscript, we believe that the average aspect ratio of multiple individual fibers more

accurately reflects the microstructure of CNFs. Therefore, the aspect ratio in the manuscript is seemingly lower than 2500 in *Nat. Sustain.* 7, 315-325, 2024.

Secondly, the relationship between microstructure (such as aspect ratio) and degree of modification in our study is a deliberate design choice to prioritize flexibility in modification and scalability. pH buffering mechanism using $\text{Zn}(\text{OAc})_2$ prevents excessive acid hydrolysis during pretreatment, thus preserving the structural integrity of cellulose while still allowing for efficient modification and nanofibrillation. Furthermore, our approach employed commonly used industrial equipment, such as oil-bath digesters and colloid mills, which are more compatible with large-scale production processes compared to high-energy techniques like high-pressure homogenization or ultrafine grinding. This makes our method more adaptable to industrial settings, enhancing its potential for scalable production. Thus, the slight sacrifice in aspect ratio is not an inherent limitation but a trade-off designed to optimize flexibility in modification and scalability for large-scale production.

Therefore, the focus of our work is not solely on improving specific performance parameters of CNFs (such as nanoscale size, degree of modification, and yield) as the main innovation. Instead, we further aim to develop a more flexible and efficient DES system that enables modification of CNFs with multiple functional groups (as we demonstrate with four different CNFs). This strategy offers a new approach for low-cost, reusable, large-scale production, and a cutting across from specific characteristics to customization platform of CNFs.

In summary, the seemingly decrease in aspect ratio compared to the referred study (*Nat. Sustain.* 7, 315-325, 2024) is a difference arising from calculation methods, and deliberate optimization that enables the core innovation of this work: flexible modification and scalable production. This approach allows for a broader range of CNF applications, making it a more versatile and industrially relevant method.

Comment #3

The work attributes success to “ $\text{Zn}(\text{OAc})_2$ acted as a pH buffering” , but the evidence

was Insufficient? How about the pH of Zn(OAc)₂/ChCl/H₂O systems comparison with unbuffered controls?

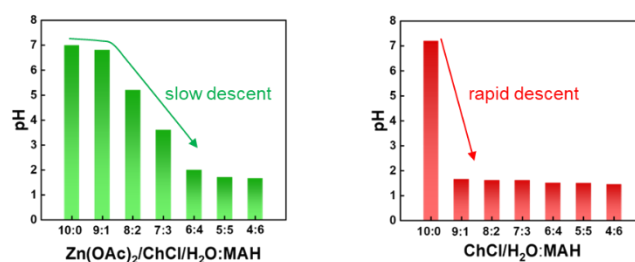
Author's Response: Thank you for your valuable suggestion. In response, we have included a comparison of the pH trends at different ratios of DES to MAH, as well as SEM images of CNF obtained using the blank control (ChCl/H₂O) without Zn(OAc)₂. These additions help to demonstrate the irreplaceable role of Zn(OAc)₂ in the DES system and provide stronger evidence of its buffering effect. A blank control sample was set up, which was modified with MAH only and did not undergo Zn(OAc)₂/ChCl/H₂O swelling, to highlight the key role of DES swelling in CNFs modification and preparation. Additionally, we compared the classic formic acid/choline chloride (FA/ChCl), acetic acid/choline chloride (AA/ChCl), and lactic acid/choline chloride (LA/ChCl) pretreatment systems with and without Zn(OAc)₂. The results clearly showed that the pretreatment systems with Zn(OAc)₂ significantly increased the DP of swollen cellulose, supporting the hypothesis that Zn(OAc)₂ acts as a buffer, balancing H⁺ and mitigating acid hydrolysis. Please see below revised text.

Line 207-218: The pH differences between the Zn(OAc)₂/ChCl/H₂O and ChCl/H₂O systems with the same MAH ratio are shown in Supplementary Fig. S6. The presence of Zn(OAc)₂ slowed down the rate of pH decrease, whereas the blank control group (ChCl/H₂O) without Zn(OAc)₂ experienced a significant pH decrease upon the addition of a small amount of MAH. This trend mirrors the pH titration curve of the Zn(OAc)₂ solution (Supplementary Fig. S7). Furthermore, the DP of the swollen raw cellulose in the ChCl/H₂O system dropped sharply to around 220 after MAH modification, and the resulting CNFs exhibited a spindle-shaped structure similar to cellulose nanocrystals (Supplementary Fig. S8). The recovered solution displayed a distinct yellow color compared to the Zn(OAc)₂/ChCl/H₂O system, indicating the presence of degraded fragments caused by vigorous acid hydrolysis.

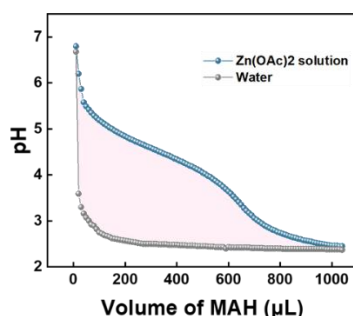
Lines 245-258: The carboxyl content of raw cellulose directly modified by MAH without DES swelling was as low as 0.573 mmol/g, and many larger cellulose fibers

were not completely nanofibrillated (Supplementary Fig. S14). The dispersion also exhibited significant sedimentation within 24 h, indicating low accessibility of cellulose-OH and reduced modification efficiency during the MAH modification process without DES pretreatment, which directly affected the nanofibrillation.

Moreover, the effect of the buffering capacity of $\text{Zn}(\text{OAc})_2$ on the pretreatment process of raw cellulose in an acidic DES system was investigated. The morphology and DP of cellulose in the classic acidic DES systems of lactic acid/choline chloride (LA/ChCl), formic acid/choline chloride (FA/ChCl), and acetic acid/choline chloride (AA/ChCl) were compared with and without $\text{Zn}(\text{OAc})_2$ during the pretreatment stage (Supplementary Fig. S15). In all acidic DES systems containing $\text{Zn}(\text{OAc})_2$, cellulose maintained a high DP and less severe morphological deconstruction, demonstrating the positive role of $\text{Zn}(\text{OAc})_2$ as a buffer during cellulose pretreatment.



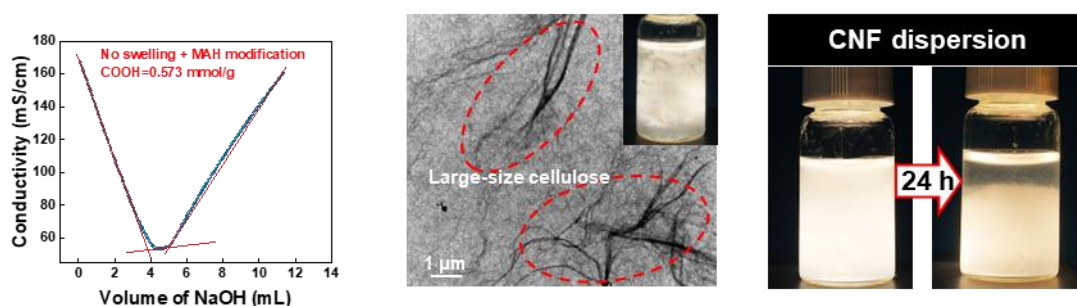
Supplementary Fig. S6. pH changes in $\text{Zn}(\text{OAc})_2/\text{ChCl}/\text{H}_2\text{O}$ and $\text{ChCl}/\text{H}_2\text{O}$ with the same MAH mass ratio.



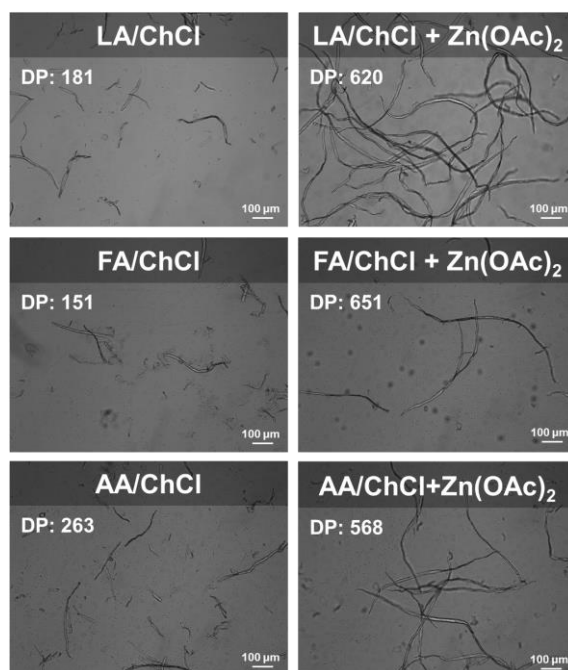
Supplementary Fig. S7. Comparison of titration curves of $\text{Zn}(\text{OAc})_2$ solution and water with MAH solution.



Supplementary Fig. S8. SEM images of CNF under the ChCl/H₂O system and digital photos of its recovered solution.



Supplementary Fig. S14. Carboxyl content, TEM image and photo of raw cellulose after MAH modification without DES swelling (blank control).



Supplementary Fig. S15. Microscopic images and DP comparison of cellulose with and without Zn(OAc)₂ pretreatment in the LA/ChCl, FA/ChCl, and AA/ChCl systems. . (Supplementary Note: Attempts to substitute Zn(OAc)₂ with KOAc or NaOAc in the DES formulation failed to form a homogeneous eutectic mixture under identical

conditions (1:2:2 molar ratio, 80 °C), highlighting the unique role of Zn(OAc)₂ not only as a pH buffer but also in maintaining the hydrogen-bonding network essential for DES formation.)

Comment #4

What happens to Zn content in CNF? ICP-MS quantification of Zn in final CNF is required?

Author's Response: Thank you for your valuable suggestion. We have used ICP-MS analysis to quantify the Zn content in the CNF, and the results are shown here for your check. The content of Zn in the four CNF samples ranged from 0.028% to 0.014% by weight. With extremely low levels of elemental residues. Please see below details.

Sample	Sample quality (g)	Element content (wt%)
CNF _{MAH}	0.0589	0.021
CNF _{SAH}	0.0522	0.028
CNF _{EAH}	0.0592	0.020
CNF _{PAH}	0.0576	0.014

An inductively coupled plasma mass spectrometer, Agilent ICP-MS7850 was used for all measurements. The instrument was equipped with standard nickel sampling and skimmer cones, a standard glass concentric nebulizer, quartz spray chamber, and a quartz torch with a 2.5 mm id injector. Operating conditions were as follows: nebulizer flow 1.08 L/min; auxiliary gas: 0.9 L/min; sample flush time: 35 s; RF power 1550 W.

Comment #5

Why SAH is so much more efficient which showed 2.024 mmol/g carboxyl content for CNF_{SAH}, while CNF_{MAH} showed only 1.412 mmol/g carboxyl content?

Author's Response: Thank you for your insightful suggestion. The observed difference in carboxyl content between CNF_{SAH} (2.024 mmol/g) and CNF_{MAH} (1.412 mmol/g) can be attributed to several factors that influence the efficiency of ring-opening

esterification with acid anhydrides. As highlighted in previous studies (*Adv. Mater.* 2024, 2312220; *Green Chem.*, 2023, 25, 4696), the efficiency of esterification varies significantly depending on the acid anhydride used. Even within the same pretreatment system, several factors influence the esterification process, including steric hindrance due to the molecular weight and functional groups of the acid anhydride, the diffusion capacity and mass transfer efficiency of the DES/acid anhydride system, the reactivity of different acid anhydrides, and their compatibility with the DES system. These factors contribute to the differences in carboxyl content observed for the CNF_{SAH} and CNF_{MAH} samples. We have expanded on these points and included a discussion of the relevant factors in the revised manuscript as follows.

Lines 484-495: Although the high swelling of raw cellulose in the DES system significantly improved the esterification efficiency between CNFs and acid anhydrides, the ring-opening esterification modification of the acid anhydride is still affected by several factors, including the steric hindrance of acid anhydride molecules⁴⁹, the mass transfer limitations due to the diffusion ability of the acid anhydride and DES system⁵⁰, the reactivity of different acid anhydrides⁵¹, and the compatibility of the acid anhydride with the DES⁵². Therefore, although all four modifiers used in this work are cyclic anhydrides, the differences in their chemical structures lead to variations in steric hindrance and reaction efficiency. Examples include the double bonds in MAH, the bicyclic ring structure EAH, and the benzene rings in PAH. These factors collectively influence the accessibility of cellulose hydroxyl groups and ultimately determine the DS.

Comment #6

Statement: "aspect ratio ~1800" (Line 202), How were lengths measured? (From TEM - manual or automated?)

Author's Response: Thank you for your helpful suggestion. The length of CNFs was measured as a result of statistical analysis of complete single fibers in TEM images at lower magnification (as shown in **Supplementary Fig. S9**). To clarify, the length and

diameter at least 100 fibers were measured manually from the TEM images. These measurements, along with the diameter, length, and aspect ratio distributions, were then analyzed using Nano Measurer software. We have added these details in the revised manuscript, as follows.

Line 706-709: The diameter and length of at least 100 fibers were randomly measured from the TEM images, and their diameter, length, and aspect ratio distributions were calculated using the software Nano Measurer 1.2.5 (developed using the Visual Basic 6.0 programming language).

Comment #7

"Exceptionally high aspect ratio (~1800)" which is lower than the reference paper's 2,500? "Scalable and expandable production" - Demonstrated at pilot scale (colloid mill), not true industrial scale?

Author's Response: Thank you for your valuable suggestion. First, as addressed in the response to your comment #2, we noticed that the calculation method of aspect ratio is different between the reference and ours. The average aspect ratio used in *Nat. Sustain.* 7, 315-325, 2024 is calculated by the ratio of the average length to the average diameter of CNFs. However, our calculation method uses the average aspect ratio of multiple individual CNFs. Using the calculation method described in *Nat. Sustain.* 7, 315-325, 2024, the average aspect ratio of CNFSAH and CNFMAH in this work can reach 2300-3000. This difference in aspect ratio value is due to the different calculation methods. However, in the manuscript, we believe that the average aspect ratio of multiple individual fibers more accurately reflects the microstructure of CNFs. Therefore, the aspect ratio in the manuscript is seemingly lower than 2500 in *Nat. Sustain.* 7, 315-325, 2024. Furthermore, although the aspect ratio of CNF in this work is seemingly slightly lower than that reported in *Nat. Sustain.* 7, 315-325 (2024) (2500), it remains superior to many similar studies. While, according to your comment, for more accurate expression, we have revised the description to "remarkably high aspect ratio (~1800)". Regarding the statement on "scalable and expandable production", we have revised it

to "pilot scale" and "potential for scalable and expandable production" to more accurately reflect the scope of our work. These changes have been highlighted in the revised manuscript as follow.

Line 541-542: Potential for scalable production of CNFs under the highly efficient swelling-modification strategy

Line 566-567: utilizing widely available industrial equipment and processing methods to ensure the accuracy and authenticity of the pilot-scale simulation.

Line 613-614: This makes it a promising approach for scalable, sustainable CNFs production.

Line 626-629: In this study, a hydrated DES system composed of Zn(OAc)₂/ChCl/H₂O demonstrated the potential for scalable and expandable production of CNFs at a pilot scale, achieving a high carboxyl content (2.024 mmol/g) and a remarkably high aspect ratio (~1800) through a highly efficient swelling-modification strategy.

Comment #8

Figure 1 o Panel (a): Schematic is conceptually clear but lacks molecular-level detail showing Zn²⁺ role; 3. Figure 3: Panel (c): Low-field NMR - axes labels too small? Figure 4: Panels (f-i): TEM images -add diameter distribution histograms as insets will be better.

Author's Response: Thank you for your helpful suggestions. In response, we have made the following revisions.

- Fig. 1a: We have added molecular-level details to better illustrate the role of Zn²⁺ in the schematic, as per your suggestion.
- Fig. 3c: We have enlarged the axis labels in the Low-field NMR to improve readability.
- Fig. 4f-i: We have added diameter distribution histograms as insets to the TEM images for better clarity and comparison.

These changes have been incorporated and marked in the revised manuscript.

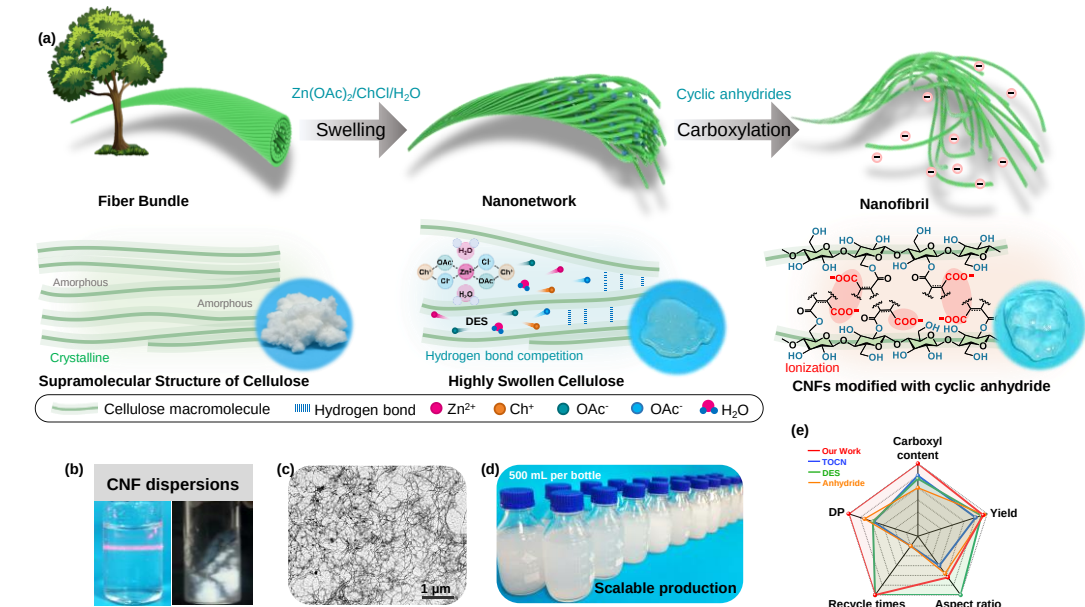


Fig. 1 | Method and advantages of expandable and scalable cellulose nanofibrils (CNFs) production in a DES system using a highly efficient swelling-modification strategy.

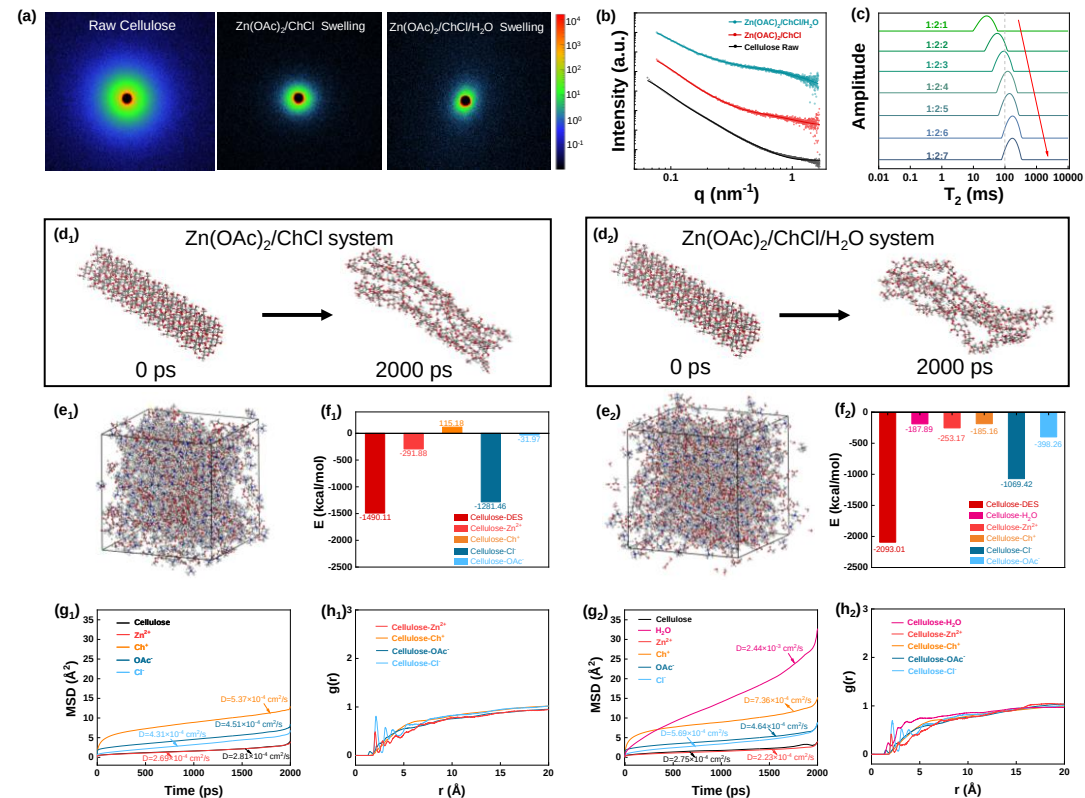


Fig. 3 | Potential mechanism and simulation analysis of cellulose swelling in the DES system.

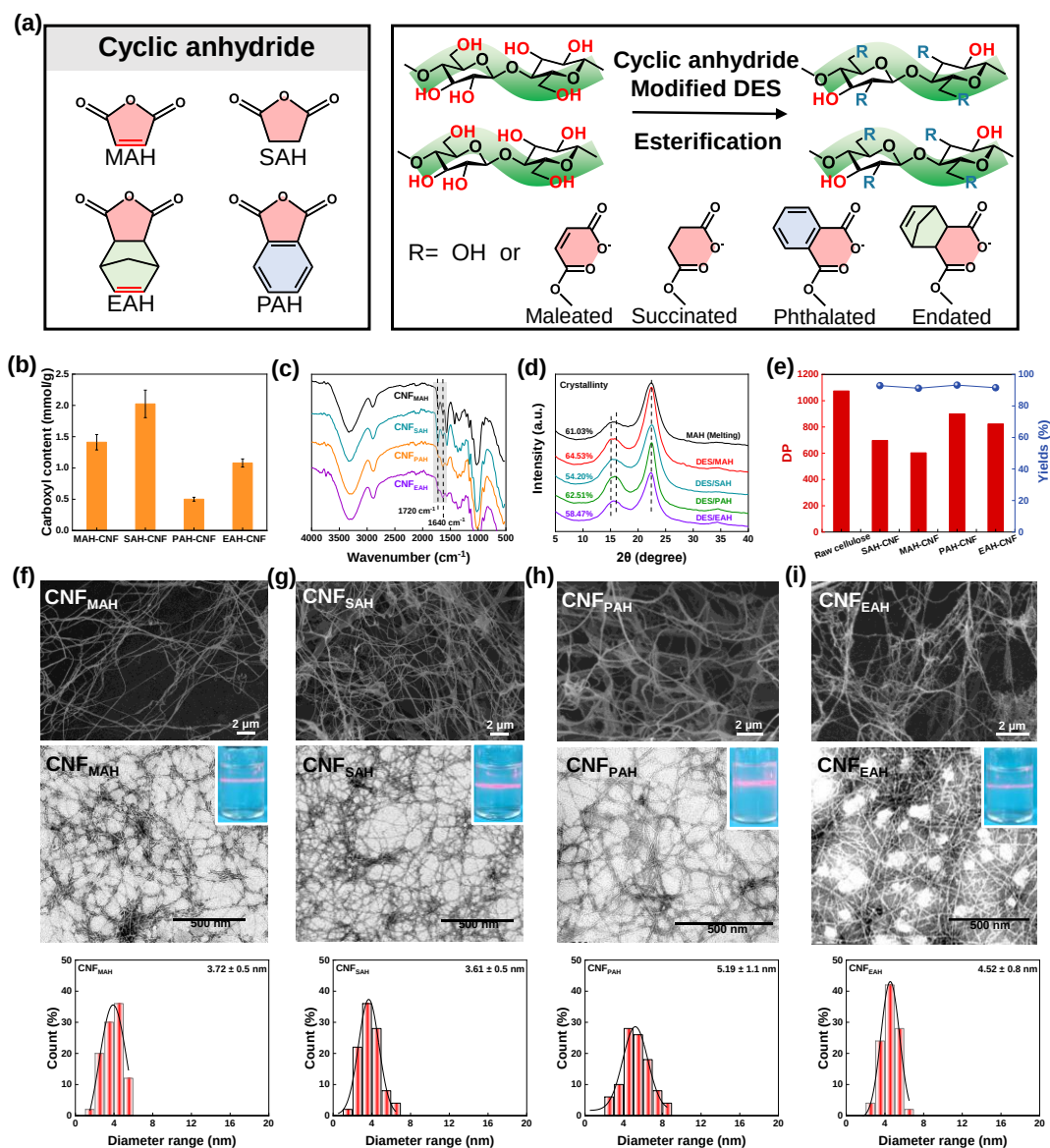


Fig. 4 | Flexible preparation of expandable functionalized CNFs using the highly efficient swelling-modification strategy.

Comment #9

Minor Grammar and Writing Style: Inconsistent use of "cellulose nanofibril" vs. "cellulose nanofibrils", CNF or CNFs throughout the manuscript? L125 "through a ring-opening reaction" should be "a ring-opening esterification reaction" L92 DESS?

Author's Response: Thank you for your valuable suggestions. We have thoroughly reviewed the manuscripts and standardized the terminologies, ensuring consistent use of "cellulose nanofibrils" and "CNFs" throughout. Additionally, we have revised

934 “through a ring-opening reaction” to “a ring-opening esterification reaction” , and
935 clarified the use of “DESS” for consistency. Furthermore, the manuscript was proofread
936 for other grammatical errors, typos, punctuation, etc. These revisions have been marked
937 in the revised manuscript.