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COMMUNICATION

The Unexpected Flexibility of Natural Cellulose at the Single-chain Level and Its Implications to the Design of Nano Materials †

Yu Bao,[a] Hujun Qian,[b] Zhongyuan Lu,*[,b] Shuxun Cui*[,a]

At the crystal or larger scale, natural cellulose was generally recognized as a rigid material. Our single-chain mechanical measurements, however, reveal that natural cellulose is as flexible as common synthetic polymers at the single-chain
10 **level, casting new light on the design of nano materials.**

Cellulose is a fantastic material from nature, which serves as the main building material for plants.[1-4] Recent studies show that cellulose is very useful in fabricating nano materials.[5] It has been reported that the Young's modulus of a cellulose crystal is similar
15 to steel, which is much higher than most polymer materials.[6-8] Therefore, it is generally believed that an individual cellulose chain should be rigid (or semi-rigid).[1, 3] However, since natural cellulose (NC) can be hardly dissolved in any common solvents, the measurement of the Young's modulus of a single chain of NC
20 has not been realized yet. In the previous studies, water soluble cellulose derivatives were used instead.[9-12] It is worth noting that those derivatives are polyelectrolytes actually.[9, 11, 12] The strong repulsion among the charges from the polyelectrolyte chain will greatly influence the chain's behavior, including the single-chain
25 Young's modulus (elasticity).[12, 13]

Recently, several kinds of ionic liquids (IL) have been found to be non-derivatizing solvents for NC.[14] The ^{13}C NMR spectrum of NC dissolved in IL has shown that the IL are true solvents, in which NC can be molecularly dispersed.[15] More recently, the
30 individual NC chains have been observed by AFM imaging.[16] Here in this letter, we attempt to investigate the single-chain elasticity of NC by measuring the single-molecule sample prepared from a natural cellulose/IL (1-allyl-3-methylimidazolium chloride, AMIMCl)[15] solution by atomic
35 force microscope-based single-molecule force spectroscopy (AFM-SMFS, Nanowizard II, JPK Instruments).[9, 17-21] Surprisingly, we find that the single chain Young's modulus of natural cellulose is very close to that of common flexible polymers with carbon-carbon backbone (such as polyethylene
40 and polyacrylamide). This result is supported by theoretical single-chain elasticity obtained by the quantum mechanical (QM) *ab-initio* calculations. This interesting finding implies that the high rigidity of cellulose crystal/fiber is a consequence of the macromolecular self-assembly, which casts new light on the
45 design of nano materials.

On the molecularly dispersed sample, the single-chain force-extension curves of NC are obtained in an organic solvent, octane (Figure 1A). After the beginning part, the force rises
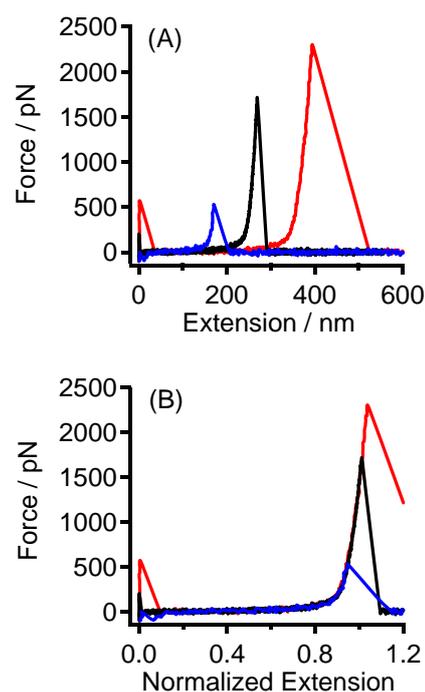
50 **Fig. 1.** (A) Typical force curves of NC obtained in octane, and (B) the normalized force curves shown in A.

monotonically with extension, corresponding to the increasing restoring force during the elastic elongation. Due to both the
55 polydisperse nature of polymer and the random position where the AFM tip picks, the apparent contour lengths of the stretched polymer chains vary (Figure 1A). It has been reported that in this environment, inherent single-chain elasticity can be obtained, since the interactions between the nonpolar organic solvent
60 molecules and the solute molecules are dominated by van der Waals forces in general, which can be ignored in many cases.[18, 22] The superposition of the normalized force curves (Figure 1B) indicates that the elastic force signals present the single-chain elasticity of NC.[9]

65 It has been reported that the freely joint-chain (FJC) model and the modified ones can be used to describe the single-chain behavior of polysaccharides.[9, 10, 17] The FJC model, which is based on the Langevin function, treats a macromolecule as a chain of statistically independent elastic segments (Kuhn
70 segments). In the force range relevant for this study here, the
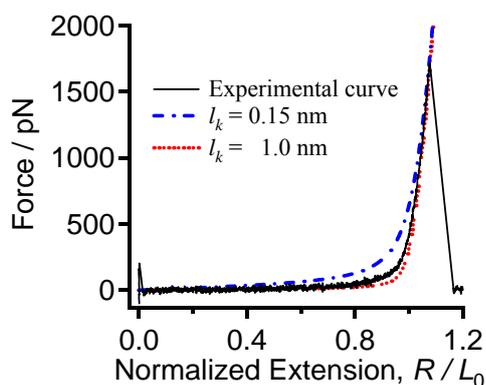
Fig. 2 Normalized single-molecule force curve of NC (solid line) vs QM-FJC fitting curves with various l_k values (dotted lines).
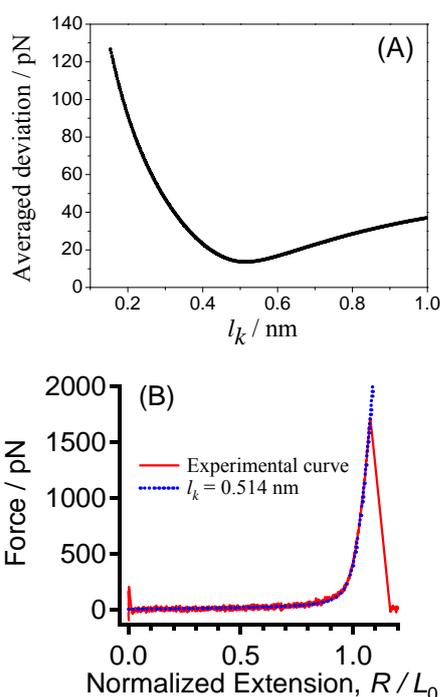
Fig. 3 (A) Averaged deviation of force between the experimental curve of NC and the QM-FJC fitting curve as a function of l_k. The minimum deviation is obtained at $l_k = 0.514$ nm. (B) QM-FJC fitting curve with $l_k = 0.514$ nm vs the normalized experimental force curve of NC obtained in octane.

relationship between the extension (R) of a polymer chain with FJC model and the stretching force (F) can be written in a good approximation as:

$$R = L[F] \cdot \{\coth[(F \cdot l_k)/(k_B \cdot T)] - (k_B \cdot T)/(F \cdot l_k)\} \tag{1}$$

where $L[F]$ is contour length at a given F, l_k is the Kuhn length of the polymer chain, k_B is the Boltzmann constant, and T is the temperature on the Kelvin scale.

Note that the contour length $L[F]$ is force dependent. The relationship between the contour length at a given F ($L[F]$) and that at zero force (L_0) is obtained by our QM calculations (see ESI for details).[22-24] The chain elasticity is non-linear, which can be expressed in a polynomial expansion to provide the basis for a
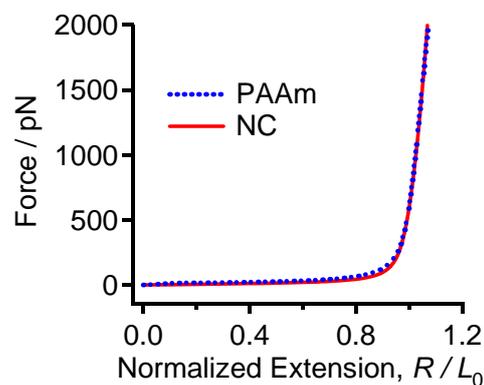
Fig. 4 Normalized theoretical fitting curves of NC and polyacrylamide (PAAm).

numerical fit of the experimental force curves, see Eq 2:

$$F = \sum_{i=1}^{3} \gamma_n (L[F]/L_0 - 1)^n \tag{2}$$

where γ_1 is the linear elastic modulus, and the other two coefficients are nonlinear corrections which become important at higher force range. By fitting we obtain $\gamma_1 = 10.57$ nN, $\gamma_2 = 90.23$ nN, and $\gamma_3 = -44.12$ nN.

As shown in Eq 1, the FJC model has two free parameters. One is the contour length of the polymer chain $L[F]$, the other is the Kuhn length l_k. By introducing L_0 into the model, we can normalize Eq 2 as:

$$R/L_0 = (L[F]/L_0) \cdot \{\coth[(F \cdot l_k)/(k_B \cdot T)] - (k_B \cdot T)/(F \cdot l_k)\} \tag{3}$$

where R/L_0 is the normalized extension of a polymer chain.

Note that for any reasonable value of $L[F]/L_0$, the corresponding F can be calculated by Eq 2 (see ESI for details). Thus, there is only one free parameter left (l_k) in Eq 3. The new FJC model (Eq 3), which is integrated with the results from QM calculations, is called QM-FJC model.

One can find from Figure 2 that the lower value of l_k, the higher force is needed in the low force regime. This is reasonable since lower value of l_k means more rotating units in the chain, which will lead to a larger entropic elasticity upon stretching. At high force regime, both of the fitting curves tend to coincide with each other. This is because that the high force regime is mainly governed by the enthalpic elasticity of the polymer chain, which is fixed by the results of QM calculations (Eq 2). One can find that at high force regime (e.g. larger than 1000 pN), there is no remarkable deviation between the experimental curve and QM-FJC fitting curves (Figure 2). This result validates that the non-linear elasticity obtained from the QM calculations can be used to describe the real enthalpic elasticity of NC.

As shown in Figure 2, although various l_k values can lead to satisfactory fitting curves for the high force regime, there is a remarkable deviation in the low force regime (e.g. lower than 500 pN). However, Figure 2 implies that the inherent value of l_k for NC is in the range of 0.15-1.0 nm. To find out the optimum value of l_k for curve fitting, a detailed analysis on the force deviation between the experimental and fitting curves is carried out, in which l_k is varied from 0.15 to 1.0 nm with a small increment of 0.002 nm. In the analysis, each point of the experimental force

curve in the range from $R/L_0 = 0.7$ to the topmost point of the curve is compared with the QM-FJC fitting curve.[24, 25] The averaged deviation force between the experimental and fitting curve for all points in the compared range, namely

$(\sum_{i=1}^{n} |F_{exp} - F_{fit}|)/n$, where n is the number of points in the

force curve, is plotted against l_k in Figure 3A. There is a minimum of force deviation at $l_k = 0.514$ nm, where the value of averaged deviation (13.5 pN) is very close to the standard deviation of the noise in the experimental curve (11.3 pN).[22, 24] In this case, the fitting curve is so close to the experimental curve that the deviation in between is negligible, see Figure 3B. We notice that when l_k is in the range of 0.514 ± 0.004 nm, the resulted fitting curves show the same performance for the fitting. Similar analyses also show that the optimum value for l_k is 0.514 $\pm$ 0.004 nm for different experimental force curves. Therefore, we determine that for NC, $l_k = 0.514$ nm. In this way, there is no free parameter left in Eq 3. Note that the value of l_k is virtually equal to the length of a repeating unit of NC, indicating that each repeating unit of NC corresponds to a Kuhn segment.[1, 26-28] The excellent fitting result in the entire force regime indicates that the QM calculations reflect the inherent single-chain elasticity of NC and the QM-FJC model is appropriate for NC. With a single pyranose ring as the Kuhn segment and the single-chain elasticity obtained from QM calculations, the QM-FJC model has been developed into a parameter free and structure relevant single-chain model, which can quantitatively describe the single-chain behavior of NC.

Worm-like chain (WLC) model and the modified ones are also popularly employed to fit the single-chain force curves.[13, 24] However, the fitting results indicate that the QM-WLC model is not suitable for NC (see ESI for details).

Recently, we studied the single-chain elasticities of three kinds of polymers with carbon-carbon (C-C) backbone and found that all the polymers have the same single-chain elasticity, which can be fitted well by a theoretical model that integrated by the results from QM calculations.[22] It is very interesting to compare the single-chain elasticity of a polymer with C-C backbone (taking polyacrylamide as an example) and that of NC.

Since the single-chain elasticities obtained by QM calculations is non-linear (Table S1, ESI), the comparison of the single-chain elasticity between two kinds of polymers is not straightforward. Yet, the normalized fitting curves of NC and polyacrylamide can provide a direct comparison (Figure 4). Surprisingly, we find that the two force curves have little difference. It was generally believed that NC is rigid or semi-rigid.[3, 29] However, our new result on the single-chain level reveals that NC is as flexible as the common polymers with C-C backbone, though they have totally different primary structures and l_k.

Then, why NC was previously believed to be rigid? The main reason is that NC is not soluble in any common solvents. All the previous experimental results were obtained on the NC crystals or even larger structures, or cellulose derivatives of polyelectrolytes. To form more H-bonds, the cellulose chains are almost fully stretched in the crystal.[1, 3, 4, 7] The strong H-bonding network in the cellulose crystal will greatly toughen the material further. With these two reasons, the modulus of cellulose crystals is dramatically enhanced.[7, 8] However, the flexibility of single-chain NC should be favorable for the NC assembly process from single chains to fibrils in plants,[4] since it would be easier for a flexible chain to find the binding sites of H-bonds, which is a directional interaction. The QM-FJC model fitting reveals that each pyranose ring of NC is a Kuhn segment in the chain. This implies that each pyranose ring can perform a random walk before it is merged into the crystal. With the fine flexibility, each of the pyranose rings can find its best binding site on the crystal surface, which perfectly lowers the energy of the self-assembled structures.

In summary, we find, for the first time, that although natural cellulose is much more rigid than common synthetic polymers at the material scale, it is as flexible as those polymers at the single-chain level. This interesting finding implies that the high rigidity of cellulose crystal is a consequence of the macromolecular self-assembly,[30] which perfectly demonstrates that the whole is more than the sum of its parts. The H-bonding is a kind of weak intermolecular interaction. However, due to the highly oriented chains (parallel or anti-parallel) in the crystal, these numerous weak interactions (H-bonding network) will work synergistically, which effectively reinforce the material. Via the H-bonding directed self-assembly, the very flexible NC chains are fabricated into a very rigid material. It is expected that via complexation with proper molecules, the H-bonding network in the cellulose nanocomposites can be adjusted effectively, by which the properties of the nanocomposites can be tuned in a broad range. Cellulose is undoubtedly another miracle in mother nature. From the viewpoint of evolution, our finding may explain why NC was selected by plants during the long time evolution. More importantly, our finding casts new light on the design of novel nano materials.

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Notes and references

[a] *Key Lab of Advanced Technologies of Materials, Ministry of Education of China, Southwest Jiaotong University, Chengdu 610031, China. E-mail: cuishuxun@swjtu.edu.cn*
[b] *State Key Laboratory of Theoretical and Computational Chemistry, Institute of Theoretical Chemistry, Jilin University, Changchun 130023, China. E-mail: luzhy@jlu.edu.cn*
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1. D. Klemm, B. Philipp, T. Heinze and U. Heinze, in *Comprehensive Cellulose Chemistry, Fundamentals and Analytical Methods*, Wiley-VCH, New York, 1998, vol. 1, pp. 9-29.
2. M. S. Doblin, I. Kurek, D. Jacob-Wilk and D. P. Delmer, *Plant Cell Physiol.*, 2002, **43**, 1407-1420.
3. D. Klemm, B. Heublein, H. P. Fink and A. Bohn, *Angew. Chem.-Int. Edit.*, 2005, **44**, 3358-3393.
4. C. Somerville, *Annu. Rev. Cell Dev. Biol.*, 2006, **22**, 53-78.

5. R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Youngblood, *Chem. Soc. Rev.*, 2011, **40**, 3941-3994.

6. I. Sakurada, Y. Nukushina and T. Ito, *J. Polym. Sci.*, 1962, **57**, 651-660.

7. S. J. Eichhorn and R. J. Young, *Cellulose* 2001, **8**, 197-207.

8. M. A. S. Azizi Samir, F. Alloin and A. Dufresne, *Biomacromolecules* 2005, **6**, 612-626.

9. H. B. Li, M. Rief, F. Oesterhelt, H. E. Gaub, X. Zhang and S. Jiacong, *Chem. Phys. Lett.*, 1999, **305**, 197-201.

10. S. Zou, W. K. Zhang, X. Zhang and B. Z. Jiang, *Langmuir*, 2001, **17**, 4799-4808.

11. A. Scherer, C. Q. Zhou, J. Michaelis, C. Brauchle and A. Zumbusch, *Macromolecules*, 2005, **38**, 9821-9825.

12. S. M. Notley, *ACS Appl. Mater. Interfaces*, 2009, **1**, 1218-1223.

13. S. X. Cui, C. J. Liu, Z. Q. Wang and X. Zhang, *Macromolecules*, 2004, **37**, 946-953.

14. H. Wang, G. Gurau and R. D. Rogers, *Chem. Soc. Rev.*, 2012, **41**, 1519-1537.

15. H. Zhang, J. Wu, J. Zhang and J. S. He, *Macromolecules*, 2005, **38**, 8272-8277.

16. Z. Z. Wan, L. Li and S. X. Cui, *Biopolymers*, 2008, **89**, 1170-1173.

17. M. Rief, H. E. Gaub, F. Oesterhelt and B. Heymann, *Science*, 1997, **275**, 1295-1297.

18. S. X. Cui, C. Albrecht, F. Kuhner and H. E. Gaub, *J. Am. Chem. Soc.*, 2006, **128**, 6636-6639.

19. W. Zhao, M. Cai, H. Xu, J. Jiang and H. Wang, *Nanoscale*, 2013, **5**, 3226-3229

20. Y. Pan, F. Wang, Y. Liu, J. Jiang, Y.-G. Yang and H. Wang, *Nanoscale*, 2014, **6**, 9951-9954

21. X. Tan, Y. Yu, K. Liu, H. Xu, D. Liu, Z. Wang and X. Zhang, *Langmuir*, 2012, **28**, 9601-9605.

22. K. F. Wang, X. C. Pang and S. X. Cui, *Langmuir*, 2013, **29**, 4315-4319.

23. T. Hugel, M. Rief, M. Seitz, H. E. Gaub and R. R. Netz, *Phys. Rev. Lett.*, 2005, **94**, 048301.

24. S. X. Cui, Y. Yu and Z. B. Lin, *Polymer*, 2009, **50**, 930-935.

25. M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann and H. E. Gaub, *Science*, 1999, **283**, 1727-1730.

26. P. E. Marszalek, A. F. Oberhauser, Y. P. Pang and J. M. Fernandez, *Nature*, 1998, **396**, 661-664.

27. P. O'Donoghue and Z. A. Luthey-Schulten, *J. Phys. Chem. B*, 2000, **104**, 10398-10405.

28. A. D. French and G. P. Johnson, *Cellulose* 2009, **16**, 959-973.

29. J. Cai, Y. T. Liu and L. N. Zhang, *J. Polym. Sci. Pt. B-Polym. Phys.*, 2006, **44**, 3093-3101.

30. X. Yan, D. Xu, X. Chi, J. Chen, S. Dong, X. Ding, Y. Yu and F. Huang, *Adv. Mater.*, 2012, **24**, 362-369.

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