Unveiling the Intricate Beauty of Trees

A new study reveals the nanostructure of the world's most abundant organic material—wood cellulose fibers, the microfibrils of which contain 24 sugar chains.

The cellulose in wood cell walls is the most abundant organic material on earth, constituting one-fourth of global biomass. Cellulose is made up of basic units called cellulose microfibrils (CMFs). However, deciphering CMF nanostructure has been a century-old challenge in wood research. A team of scientists led by Hwan-Ching Tai of National Taiwan University (NTU; Tai is currently affiliated with Xiamen University, China) has determined that wood CMFs contain 24 sugar chains arranged in a core–shell configuration, as opposed to the 36-chain model proposed in many textbooks (**Fig. 1**, see next page). The study of wood cell walls is closely connected with the development of modern science. In 1665, Hooke observed cork and wood cell walls under the microscope and coined the term "cell." In 1837, Payen proposed that wood cellulose was an isomer of starch, which marked the beginning of carbohydrate chemistry. Wood cellulose was also one of the first materials to have its nanostructure probed by X-ray. Nishikawa showed its fibrous nature in 1913 using small-angle X-ray scattering (SAXS), and Herzog showed its crystalline nature in 1920 using X-ray diffraction (XRD). However, more than a century later, the nanostructure of CMFs remains elusive, as we still do not understand how it is both partly crystalline and partly flexible. Furthermore, the number of sugar chains in each wood CMF is highly debated. Each CMF is synthesized by an enzyme complex with sixfold rosette symmetry, implying that chain numbers should be multiples of six. Despite the resolving power of modern electron microscopes and atomic force microscopes, these techniques cannot differentiate cellulose against surrounding hemicellulose chains, which means that directly counting the chains is not feasible.

Previously, CMF diameters were underestimated in SAXS studies because of the scattering signals being incorrectly attributed to the whole CMF and the 2D-disc fitting model being oversimplified. In this new study, Cheng-Si Tsao (NTU) and Tai used the ultra-bright synchrotron X-ray beam from the **TPS 13A** and **TLS 23A1** at the NSRRC to probe the internal structure of CMFs, revealing a high-density crystalline core and a low-density semi-disordered shell. The researchers showed that scattering signals originated from the core zone of CMFs and developed 3D-cylinder fitting models that included length-scattering effects. The model allowed them to determine the cross-section aspect ratio and the chain number of the core zones.

To estimate the CMF chain number, a ratio measurement between core/shell glucans is required. This was obtained by developing a novel solid-state nuclear magnetic resonance (NMR) technique called Global Iterative Fitting of T1ρ-Edited Decay (by Jerry Chun Chung Chan of NTU). This new technique can separate the complex NMR spectra of wood into different components that represent molecules with different mobilities. It shows that shell glucans are more mobile than core glucans, and that their ratios could be determined from the extrapolated cellulose spectra.

Combining SAXS and NMR data, the researchers calculated the average CMF chain numbers for several gymnosperm and angiosperm tree species, and the results were always around 24. The basic structure of wood CMFs appears to have been conserved over 300 million years of evolution. Approximately half of the glucans were found to be located in the crystalline core, while the shell glucans are semi-disordered and somewhat flexible. This observation explains how CMFs combine the strength of crystalline materials with the flexibility of fibrous materials to meet the functional demands of plant growth.

Recently, an alternative CMF model has been proposed, in which 18-chain CMFs are initially synthesized and undergo crystalline fusion to increase their crystallite widths. According to this hypothesis, naturally aged wood should undergo further fusion with spontaneous decomposition of hemicellulose chains over time. When the research team examined wood samples aged 500–2000 years old from antique Chinese guqin zithers, their XRD crystallite widths remained unchanged (measured at **TLS 01C2**). Instead, the altered SAXS profiles of aged wood revealed that CMFs formed dimeric aggregates through lateral contact without crystalline fusion. It argues against the 18-chain fusion model and suggests that 24-chain CMFs in normal wood remain segregated unless hemicellulose becomes substantially degraded (**Fig. 1**).

By solving the century-old puzzle of wood cellulose nanostructure, the new CMF model provided by the research team could be beneficial for predicting the physical, chemical, and biological properties of wood cellulose. These properties are also critical to several applications in sustainable bio-economies, such as biofuel production, pulp and paper manufacturing, genetic engineering of wood, and new cellulosic composite materials. The novel SAXS and NMR methods developed by the research team are also helpful in analyzing cellulose nanostructures in various plant materials such as primary cell walls, reaction wood, or flax fibers, further providing

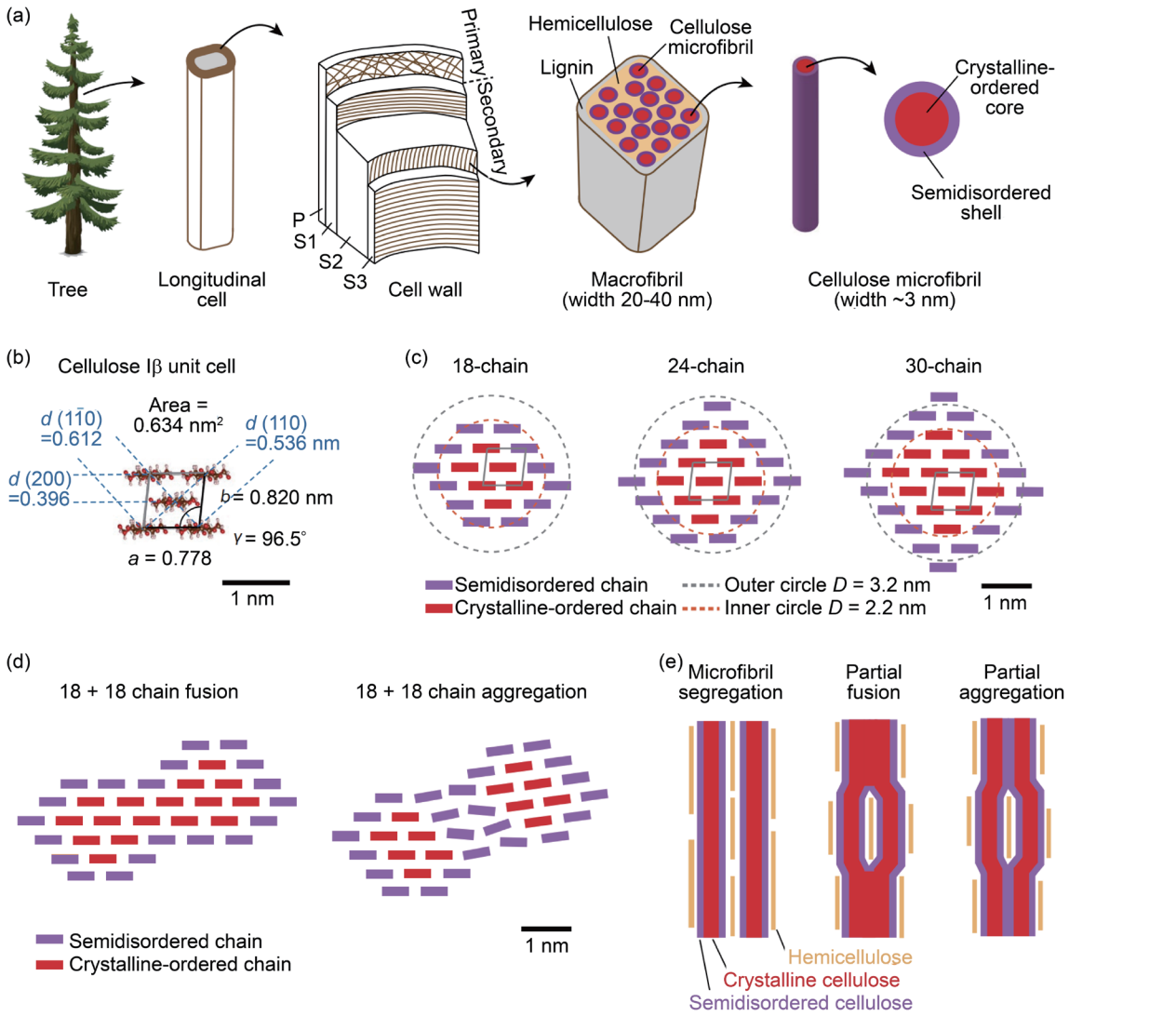

Fig. 1: Proposed structural models of the hierarchical CMF structure in wood cell walls. (a) Most wood CMFs originate from the S2 layer of longitudinal cells with a core–shell structure. (b) A monoclinic unit cell of cellulose Iβ. (c) CMF models with 18, 24, and 30 chains, each showing just one of many possible configurations. (d) Horizontal cross-section of a CMF pair in fusion and aggregation states. (e) Vertical cross-section of a CMF pair in segregation, partial fusion, and partial aggregation states. [Reproduced from Ref. 1]

information on how structural variations of cell walls are tailored for different biological functions.

Furthermore, this study also solved a thousand-year-old mystery regarding the most valuable musical instrument in Chinese culture—the guqin zither. Back in the Tang dynasty, a famous guqin maker once said, "Five centuries of aging is required for optimal acoustic properties." For more than a millennium, guqin makers have wondered how aging promoted wood transformations. The research team showed that as hemicellulose spontaneously degrades over time, CMFs would form dimeric aggregates, accompanied by increases in SAXS cross-section areas but not XRD crystallite widths. The breakdown of hemicellulose matrixes and formation of CMF dimers would alter how sound waves propagate through wood fibers, giving antique guqin a rounded tone and rich harmonics. (Reported by Cheng-Si Tsao, National Taiwan University, and Hwan-Ching Tai, Xiamen University, China)

This report features the work of Cheng-Si Tsao, Hwan-Ching Tai, and their collaborators published in Nat. Plants ***9****, 1154 (2023).*

TPS 13A Biological Small-angle X-ray Scattering
TLS 23A Small/Wide Angle X-ray Scattering
TLS 01C2 X-ray Powder Diffraction

- GIWAXS, GISAXS, WAXS, SAXS, and XRD
- Chemistry, Materials Science

Reference

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