The 2024 MWP Award – Full motivation

Citation

Title: Understanding lignin biosynthesis and structural diversity for novel lignin applications.

The 2024 Marcus Wallenberg Prize is awarded to Professors Wout Boerjan and John Ralph for their groundbreaking research leading to a fuller understanding of lignin biosynthesis and structural diversity. Both scientists developed and innovatively applied advanced analytical techniques in ways that greatly enhance our view of lignin biosynthesis and structure in trees. Their research provides a basis for development of wood deconstruction processes and new applications for lignin, the most abundant source of natural aromatics in the biosphere.

Background and Prize Motivation

Lignin, cellulose, and hemicelluloses are the three main components of wood. Typically, around one quarter of the tree is lignin, a complex and variable aromatic polymer that is crucial for many important wood properties such as strength and resistance to microbial decay. Lignin and its building blocks are also interesting feedstocks for different chemical and material applications.

When wood is processed into fibres, lignin is modified and removed through deconstruction processes. Currently the most prevalent industrial pulping process is the kraft process, in which the chemically modified lignin is solubilized in a liquor and further burnt for energy and chemical recovery, affecting the CO_2 balance. As industry and societies move toward carbon neutrality and the sustainable use of forest resources to replace fossil-carbon feedstocks, there is a need to reduce CO_2 emissions and to maximize the use of wood or wood components in new materials. In this context, the development of new uses for the extracted lignin and its components is essential. Key to this transition is a full understanding of the biosynthesis of lignin, its composition, natural variation, and structure (1).

The biosynthesis of lignin occurs through oxidative radical coupling reactions between monolignol monomers and the growing polymer. Natural softwood lignins are mostly composed of guaiacyl units, and hardwoods also have syringyl units; these units are biosynthesized by polymerization of coniferyl and sinapyl alcohols, respectively. As a result of the monomer unit diversity in plants, and the different linkages generated by the coupling reactions, lignin structure is highly variable and depends on the plant source. Thorough analysis of the macromolecular lignin has been hampered by this complexity, the difficulty in isolation of the lignin, and the limited analytical methods available for chemical and structural elucidation. Understanding lignin structure also requires an understanding of the monolignol biosynthetic mechanisms and polymerisation of lignin from its building blocks.

Professors Wout Boerjan and John Ralph have made seminal contributions to the analysis and understanding of lignin biosynthesis and how the lignin polymer is built from its monomers. The research of Prof. Boerjan has provided deep fundamental insights into the pathways determining the synthesis of lignin monomers using systems biology and mass-spectrometry-based metabolite profiling as a tool. His innovative genomic approach to studying lignin biosynthesis led to the discovery of the Caffeoyl Shikimate Esterase (CSE) enzyme and its corresponding gene, providing an unexpected new aspect to lignin biosynthesis, without which the process of wood formation could not be adequately understood (2,3). He has effectively used both the model plant Arabidopsis together with studies on poplar for testing modified lignin structures and compositions and has taken research from the lab to experimental field trials (4).

The research of Prof. John Ralph has provided deep fundamental insights into the subunit structure, linkages, and composition of lignin, especially using advanced Nuclear Magnetic Resonance (NMR) methods. His whole wood analysis through solution-state NMR of partially solubilized samples is underpinned by rigorous assignment of the lignin structures in the spectra. He has characterized a large number of transgenic and naturally diverse plants, finding large variation and novelty in the composition of monolignol subunits and other metabolites that could be incorporated into lignin (5,6). As examples, a polymer of caffeyl alcohol in plant seeds (C-lignin) was found in diverse plant species, and with others he showed that the flavone tricin is also widely incorporated into lignin. He has also made major contributions to our understanding of how lignin is polymerized and led the demonstration of the enormous plasticity in the process (7,8).

Professors Boerjan and Ralph have provided a conceptual framework for lignin biosynthesis, polymerization, and manipulation, and they have also demonstrated the potential for flexibility in lignin structure. Many of these advances are described in a large body of papers published together describing their joint collaborative work. They have interacted with many other key players in the lignin field, such as Chapple, Chiang, Dixon, Halpin, Lapierre, Mansfield, Sederoff, and many others, sometimes resulting in joint publications, extending our understanding of lignin in trees and also other important plants. The methodological and conceptual advances provided by Boerjan and Ralph have widely influenced the characterization and generation of different lignins in native or transgenic plants, and in tailoring lignins for improved processability. This is exemplified by the concept of ZIP-lignins which incorporate easily cleavable bonds (9). Their work has therefore provided a solid foundation to tailor lignin structure in wood with great potential for cost-effective chemical pulping or valorization of lignin for different applications. The conceptual and practical advances of these two researchers are paving way for improved processing concepts of woody biomass and the development of new applications for lignin and its constituents.

Wout Boerjan

Wout Boerjan was born in 1963, he completed his undergraduate studies in Ghent University, Belgium in 1985 and his PhD in Plant Biotechnology in Ghent under the supervision of Prof. Marc Van Montagu and Prof. Dirk Inzé in 1993. In January 1994, he became a group leader in the Lab of Genetics at Ghent University, and from June 1996, group leader in the Department of Plant Systems Biology, VIB, Ghent University. He is also a professor at Ghent University. In 2009, Wout Boerjan was named "Forest Biotechnologist of the Year". He is an elected EMBO member and visiting professor at Tokyo University of Agriculture and Technology, Japan. In 2019, he received an ERC-Advanced-Grant to discover metabolites and their biosynthetic pathways in the model tree poplar. In 2020, he received the 'Excellence Prize in Applied Sciences' from the Fund for Scientific Research Flanders (FWO).

John Ralph

John Ralph was born in 1954 and studied Chemistry at Canterbury University, New Zealand graduating in 1976. In 1982 he earned his Ph.D. in the topic of Chemistry/Forestry, University of Wisconsin-Madison, USA. During this time, he already studied lignins using NMR under the supervision of Professor Raymond A. Young and Dr. Larry L. Landucci. He was a Research Scientist at the Forest Research Institute, Rotorua, New Zealand before moving to the USA. After a brief period as Scientific Head of the Research Laboratory for Nuclear Magnetic Resonance Spectroscopy, Chemistry

Department, University of California Berkeley he continued his career in Madison, Wisconsin, USA. From 1988 to 2008 he was a Research Chemist at the USDA-ARS, U.S. Dairy Forage Research Center. He then moved to University of Wisconsin-Madison as a Full Professor in the Departments of Biochemistry and Biological Systems Engineering and was the Plants Area lead in the US Department of Energy's (DoE) Great Lakes Bioenergy Research Center from 2008 to 2017. John Ralph is an elected Fellow of the American Association for the Advancement of Science (AAAS) and was recipient of the 2023 Lifetime Achievement Award from the International Symposium of Wood, Fiber, and Pulping Chemistry. He is a Distinguished Professor, Tokyo University of Agriculture and Technology, Japan.

References

(1) Ralph J., Lapierre C., and Boerjan W. (2019) Lignin Structure and its Engineering. Current Opinion in Biotechnology 56: 240–249.

(2) Vanholme R., Cesarino I., Rataj K., Xiao Y., Sundin L., Goeminne G., Kim H., Cross J., Morreel K., Araujo P., Welsh L., Haustraete J., McClellan C., Vanholme B., Ralph J., Simpson G., Halpin C., and Boerjan W. (2013) Caffeoyl Shikimate Esterase (CSE), a Newly Discovered Gene in the Lignin Biosynthetic Pathway. Science, 341(6150): 1103-1106.

(3) Saleme M.L.S., Cesarino I, Vargas L, Kim H, Vanholme R, Goeminne G, Van Acker R, Fonseca FCA, Pallidis A, Voorend W, Junior JN, Padmakshan D, Van Doorsselaere J, Ralph J, and Boerjan W. (2017). Silencing *CAFFEOYL SHIKIMATE ESTERASE* Affects Lignification and Improves Saccharification in Poplar. Plant Physiology 175(3):1040-1057.

(4) De Meester B., Vanholme R., Mota T. and Boerjan W. (2022). Lignin engineering in forest trees: from gene discovery to field trials. Plant Communications 3: 100465.

(5) del Río JC., Rencoret J., Gutiérrez A., Elder T., Kim H., and Ralph J. (2020) Lignin Monomers from beyond the Canonical Monolignol Biosynthetic Pathway: Another Brick in the Wall. ACS Sustainable Chemistry & Engineering 8(13): 4997–5012.

(6) Karlen, S. D., Smith, R. A., Kim, H., Padmakshan, D., Bartuce, A., Mobley, J. K., Free, H. C. A., Smith, B. G., Harris, P. J., and Ralph, J. (2017) Highly Decorated Lignins in Leaf Tissues of the Canary Island Date Palm *Phoenix canariensis*. Plant Physiology 175, 1058–1067.

(7) Boerjan W., Ralph J., and Baucher M. (2003) Lignin Biosynthesis. Annual Reviews in Plant Biology 54: 519-546

(8) Ralph J., Lundquist K., Brunow G., Lu F., Kim H., Schatz P.F., Marita J.M., Hatfield R.D., Ralph S.A., Christensen J.H., and Boerjan W. (2004) Lignins: Natural Polymers from Oxidative Coupling of 4-Hydroxyphenylpropanoids. Phytochemistry Reviews 3: 29–60.

(9) Wilkerson C.G., Mansfield S.D., Lu F., Withers S., Park J.-Y., Karlen S.D., Gonzales-Vigil E., Padmakshan D., Unda F., Rencoret J., and Ralph J. (2014) Monolignol Ferulate Transferase Introduces Chemically Labile Linkages into the Lignin Backbone. Science 344(6179): 90–93.