



## BLISS: Shining a light on lignification in plants

Clémence Simon, Cedric Lion, Brigitte Huss, Anne-Sophie Blervacq, Corentin Spriet, Yann Guérardel, Christophe Biot & Simon Hawkins

To cite this article: Clémence Simon, Cedric Lion, Brigitte Huss, Anne-Sophie Blervacq, Corentin Spriet, Yann Guérardel, Christophe Biot & Simon Hawkins (2017) BLISS: Shining a light on lignification in plants, *Plant Signaling & Behavior*, 12:8, e1359366, DOI: [10.1080/15592324.2017.1359366](https://doi.org/10.1080/15592324.2017.1359366)

To link to this article: <https://doi.org/10.1080/15592324.2017.1359366>



© 2017 The Author(s). Published with license by Taylor & Francis Group, LLC © Clémence Simon, Cedric Lion, Brigitte Huss, Anne-Sophie Blervacq, Corentin Spriet, Yann Guérardel, Christophe Biot, and Simon Hawkins



Published online: 18 Sep 2017.



Submit your article to this journal [↗](#)



Article views: 718



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 2 View citing articles [↗](#)

## BLISS: Shining a light on lignification in plants

Clémence Simon<sup>†</sup>, Cedric Lion <sup>†</sup>, Brigitte Huss, Anne-Sophie Blervacq, Corentin Spriet, Yann Guéardel, Christophe Biot, and Simon Hawkins

Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, Lille, France

### ABSTRACT

Lignin is a polyphenolic polymer of the plant cell wall formed by the oxidative polymerization of 3 main monomers called monolignols that give rise to the lignin H-, G- and S-units. Together with cellulose and hemicelluloses, lignin is a major component of plant biomass that is widely exploited by humans in numerous industrial processes. Despite recent advances in our understanding of monolignol biosynthesis, our current understanding of the spatio-temporal regulation of their transport and polymerization is more limited. In a recent publication, we have reported the development of an original Bioorthogonal Labeling Imaging Sequential Strategy (BLISS) that allows us to visualize the simultaneous incorporation dynamics of H and G monolignol reporters into lignifying cell walls of the flax stem.<sup>11</sup> Here, we extend the application of this strategy to other plant organs such as roots and rapidly discuss some of the contributions and perspectives of this new technique for improving our understanding of the lignification process in plants.

### ARTICLE HISTORY

Received 5 July 2017  
Accepted 20 July 2017

### KEYWORDS

Lignin; monolignol; metabolic engineering; chemical probes; dual labeling; click chemistry; bioorthogonal chemistry; SPAAC; CuAAC; cell wall; fibers; flax

Together with chloroplasts and vacuoles, the presence of cell walls is one of the distinctive structural features that distinguish plant cells from animal cells. Cell walls of land plants are composed of cellulose, hemicellulose and pectins.<sup>1</sup> In certain specialized tissues (e.g., xylem, sclerenchyma) the cell walls become reinforced by a 3-dimensional phenolic polymer called lignin that can constitute up to 25% of the cell wall dry weight.<sup>2</sup> The lignin polymer is essential for plants as it plays a major role in mechanical support, water transport, wound healing and defense against pathogens, and has contributed to their successful colonization of the terrestrial habitat.<sup>3</sup> It is also an important parameter that influences the quality of plant-based bio-resources in many industrial areas including timber, paper and textile fiber production, animal feed, bio-fuels etc.<sup>4,5</sup>

Lignin is progressively assembled in the cell wall by an oxidative polymerization process in which the monomeric units (monolignols) are first enzymatically oxidized by peroxidases and/or laccases to form free radicals that then undergo spontaneous polymerization. In angiosperms, three main monolignols (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) are oxidized giving rise to the H (hydroxyphenyl) units, G (guaiacyl) units and S (syringyl) units of the lignin polymer, respectively. In gymnosperm lignin, S units are absent although recent work has shown that they can be induced by targeted genetic engineering.<sup>2,6,7</sup>

Given the biologic and industrial importance of lignin, its biosynthesis has long been studied by a combination of

biochemical, molecular and genetic approaches. For imaging studies, lignin can be visualized *in situ* by a variety of different techniques including histochemistry, UV-autofluorescence, immunolocalisation, and spectroscopy (e.g., Infra-red, Raman, X-ray). While certain of these techniques can produce high-resolution images, they all present the disadvantage of not being able to distinguish newly deposited lignin from pre-existing lignin thereby making detailed studies of lignification dynamics complicated. However, thanks to the expansion of the chemical toolbox for biomolecular imaging applications, the plant biologist can now study this process quite easily.<sup>8</sup> Methods using bioorthogonal chemical reporters have been successfully developed and applied *in vivo*.<sup>9,10,11</sup> In these approaches chimeric monolignols bearing an azide or an alkyne are metabolically introduced into lignifying cell walls and specifically derivatized with a fluorescent dye by bioorthogonal click chemistry allowing incorporation to be followed *in vivo*. The facility of this technique promises to simplify cell wall studies compared with classical methods such as radioisotope labeling or indirect profiling using antibodies. In a recent article, we reported the dual labeling BLISS sequential combination of two well-known click reactions: the strain-promoted alkyne-azide cycloaddition (SPAAC) reaction and the copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction.<sup>11</sup> The major advantage of SPAAC and CuAAC is their operational simplicity, making the application of these bioorthogonal couplings extremely straightforward.

**CONTACT** Cedric Lion  [cedric.lion@univ-lille1.fr](mailto:cedric.lion@univ-lille1.fr)  UMR8576 - UGSF, Université de Lille, bat. C9, Cité Scientifique, Villeneuve d'Ascq, Nord, F-59000, France.

<sup>†</sup> These authors contributed equally to this work and are listed as co-first authors.

Addendum to: Lion C, Simon C, Huss B, Blervacq A-S, Tirot L, Toybou D, Spriet C, Slomianny C, Guéardel Y, Hawkins S, Biot C. BLISS: A Bioorthogonal Dual-Labeling Strategy to Unravel Lignification Dynamics in Plants. *Cell Chem Bio.* 2017;24(3):326–338. doi:10.1016/j.chembiol.2017.02.009.

© 2017 Clémence Simon, Cedric Lion, Brigitte Huss, Anne-Sophie Blervacq, Corentin Spriet, Yann Guéardel, Christophe Biot, and Simon Hawkins. Published with license by Taylor & Francis Group, LLC This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

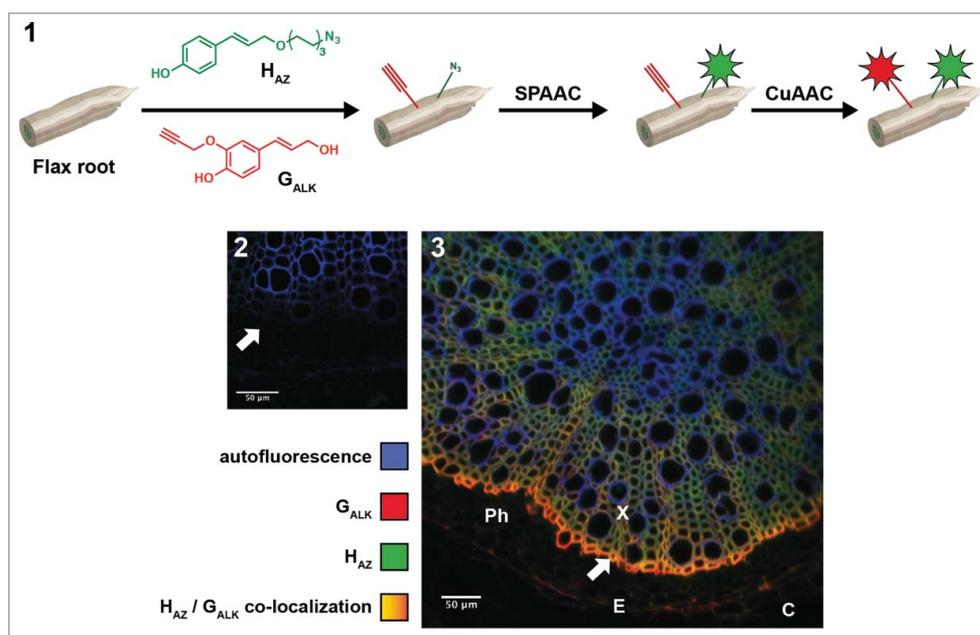
Using this approach we incorporated the two different chimeric monolignols  $H_{AZ}$  and  $G_{ALK}$  (tagged with an azide and alkyne chemical handle, respectively) into lignifying cell walls of the flax stem. The BLISS methodology generates precise 3-color localization maps reflecting the differential incorporation of  $H_{AZ}$  and/or  $G_{ALK}$  chemical reporters into freshly synthesized lignin vs. preexisting lignin - the latter being observed by autofluorescence. It thus provides spatial information on the presence or absence of active lignification machinery between different tissues, between different cell types and within different wall layers of the same cell. Although lacking many of the genetic resources and tools that are available for Arabidopsis, flax is an economically relevant species and represents an interesting biologic model for evaluating the dual bioorthogonal lignin labeling strategy as the stem of this plant contains two distinct populations of cells with contrasted cell wall composition.<sup>12</sup> Xylem cells present in inner-stem tissues have walls that contain approximately 25% lignin whereas bast fibers, present in outer-stem tissues contain much lower amounts (typically 2–4%).<sup>13,14</sup>

As well as a proof of concept paper demonstrating the robustness of this technique, this work generated results that are of interest for the whole scientific community studying lignification,<sup>11</sup> as well as for researchers working on bast fiber cell wall biology in flax and other related species such as hemp, jute and ramie. Similar amounts of both  $H_{AZ}$  and  $G_{ALK}$  chemical reporters were incorporated into lignifying xylem cell walls when supplied in equal quantities despite the fact that ‘natural’ flax lignin, like that of most angiosperms, only contains low amounts of H-lignin. Furthermore, the relative incorporation of each reporter could

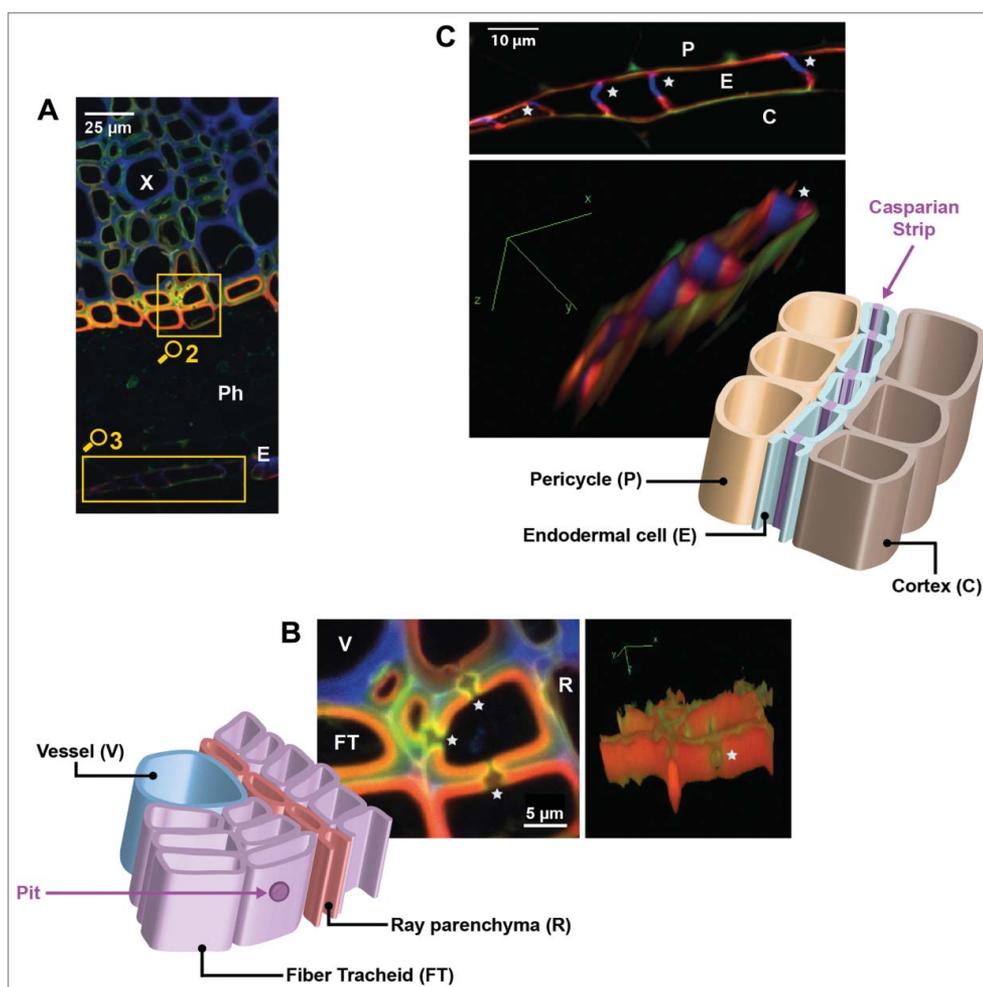
be simply modified by changing the  $H_{AZ}/G_{ALK}$  ratio in the feeding medium. These observations indicate that lignin monomer composition is most likely regulated by monomer supply and not by the specificity of cell wall oxidizing enzymes for certain monolignol substrates. This is in agreement with numerous observations showing that targeted downregulation of specific monolignol biosynthesis genes modifies lignin structure. Nevertheless, the fact that  $H_{AZ}$ - and  $G_{ALK}$ -units did not always co-localize to the same sub-domain of the cell wall would suggest that other factors such as the nature of the polysaccharide matrix and/or localization of the oxidizing enzymes could also play a part in regulating monolignol incorporation into lignin.<sup>15,16</sup>

The observation that  $H_{AZ}$ - and  $G_{ALK}$ -unit chemical reporter incorporation into bast fiber cell walls remains limited under the same conditions that allow substantial incorporation into xylem cell walls strongly suggests that bast fiber hypolignification results from an absence of cell wall machinery capable of oxidizing monolignols. Certainly, this hypothesis is in agreement with the observation that ectopic lignification in the flax *lbf1* mutant is associated with a significant increase in the accumulation of lignin-related peroxidase transcripts.<sup>17</sup>

In this Article Addendum we report that BLISS is also suitable for investigating lignification dynamics in other plant organs such as roots (Fig. 1) and discuss the new possibilities that this methodology opens up for the fine study of lignification dynamics. As previously observed in stems, the fluorescent signal in flax roots is most intense in the first few layers of the secondary xylem actively undergoing lignification. As shown by a more detailed analysis (Fig. 2),



**Figure 1.** Bioorthogonal Labeling Imaging Sequential Strategy (BLISS) illustrating incorporation of monolignol chemical reporters into cell walls in 2-month-old flax roots. (1) Dual labeling strategy consisting of monolignol feeding followed by specific click ligation of fluorescent probes to incorporated  $H_{AZ}$ -units (SPAAC) and  $G_{ALK}$ -units (CuAAC) in freshly synthesized lignin. (2,3) View of BLISS-labeled hand section of flax roots previously incubated with native *p*-coumaryl and coniferyl alcohols as negative control (2), or with azide-labeled *p*-coumaryl alcohol ( $H_{AZ}$ ) and alkyne-labeled coniferyl alcohol ( $G_{ALK}$ ) monolignol reporters (3) and observed by confocal microscopy.<sup>11</sup> Blue channel: lignin autofluorescence (405 nm); green channel:  $H_{AZ}$ -unit fluorescence (526 nm); red channel:  $G_{ALK}$ -unit fluorescence (565 nm). Xylem (X), phloem (Ph), endodermis (E), cortex (C), differentiating xylem (arrow).



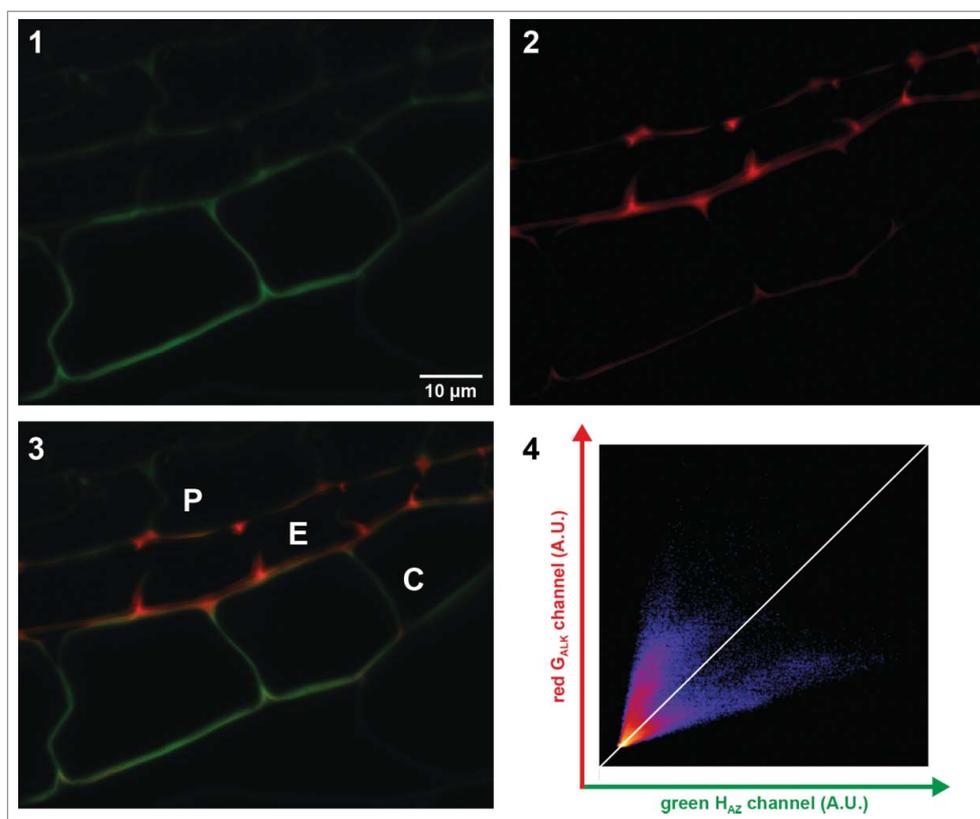
**Figure 2.** Confocal microscope imaging of monolignol chemical reporter incorporation into cell walls of 2-month-old flax roots. (1) View of BLISS-labeled root secondary xylem (X), phloem (Ph) and endodermis (E) with merged lignin autofluorescence channel (blue, 405 nm),  $H_{AZ}$  fluorescence (green, 526 nm) channel and  $G_{ALK}$  (red, 565 nm) channel. (2) 2D slice and 3D reconstruction of confocal z-stack zoom of a secondary xylem fiber-tracheid and neighboring cells in xylem differentiation zone. Note differential incorporation of chemical reporters vs autofluorescence highlighting cell wall layers of fiber tracheids (FT), vessels (V) or ray cells (R) as well as cell wall substructures such as pits (\*). (3) 2D slice and 3D reconstruction of confocal z-stack zoom of root endodermis region showing incorporation of monolignol chemical reporters into radial and tangential cell walls of endodermal cells. The Casparian strip (\*) only displays autofluorescence and does not incorporate  $H_{AZ}$  or  $G_{ALK}$ .

BLISS also provided evidence for the existence of specific (i.e.,  $H_{AZ}$  vs  $G_{ALK}$ ) monolignol-incorporation capacity in different wall domains of the same cell. For example, the bordered pits connecting adjacent cells in the differentiating xylem zone depicted in Fig. 2B can be clearly distinguished from the rest of the cell wall by their yellow color indicating  $H_{AZ}$  and  $G_{ALK}$  co-localization, whereas the orange/red color of the S2 secondary cell wall layer indicates that less  $H_{AZ}$  reporter is incorporated under the same conditions in this cell wall region.

Of interest is the fact that chemical reporters are also incorporated into the walls of cells in the endodermis (Fig. 2C). This cell layer forms an apoplastic transport barrier contributing to the selective uptake capacities of plant roots as well as restricting radial oxygen loss and microorganism access.<sup>18</sup> During the early stages of development the endodermal cell wall is characterized by the presence of a Casparian strip (CS) on the radial and transverse walls that can later spread to other cell walls. The  $H_{AZ}$  and  $G_{ALK}$  reporters are clearly incorporated into tangential walls and

the parts of the radial walls where the CS is absent, but not at all in the CS itself. This indicates that the CS is mature at this stage of plant development while the other walls are still undergoing further polymer deposition. Endodermal cell walls contain both suberin and lignin and have been shown to possess the enzymatic machinery capable of oxidizing monolignols.<sup>19,20,21</sup>

Labeling is also observed (Fig. 3) in the walls of certain cells in the cortical layer immediately exterior to the endodermis (as well as in cell wall corners of certain pericycle cells) suggesting that the flax root cortex may well contain additional lignified/suberized cell layers as previously observed in rice.<sup>22</sup> The comparison of endodermal and cortical cell wall labeling in this region shows that these two cell types show very different patterns of monolignol reporter incorporation. Cortical cells show a marked preference for  $H_{AZ}$  reporters while endodermal cells preferentially incorporate  $G_{ALK}$  reporters suggesting the existence of cell-specific monolignol-oxidizing machinery and/or cell wall structure in these two neighboring cell types.



**Figure 3.** Monolignol chemical reporter incorporation into endodermal and inner cortical cell walls of 2-month-old flax roots. (1)  $H_{AZ}$  channel, green, 526 nm; (2)  $G_{ALK}$  channel, red, 565 nm; (3) merged  $H_{AZ}$  and  $G_{ALK}$  channels; (4) associated fluorogram illustrating the anti-correlation between  $G_{ALK}$  and  $H_{AZ}$  incorporation in this region. Indeed, few pixels are found on the co-localization diagonal except for the background while high green fluorescence is associated to low red signal (cortex) and vice-versa (endodermis). Pericycle (P), Endodermis (E), Cortex (C).

## Conclusion

In conclusion, the development of a dual labeling technique that allows co-visualization by 2-color fluorescence detection at subcellular levels opens up exciting new perspectives for studying lignin biosynthesis in different plant organs and tissues. BLISS is capable of not only revealing differences in monolignol incorporation between different cell types, but is also able to detect differences between different cell wall layers/sub-structures within the same cell. Furthermore, such an approach can also be used for studying the dynamics of sugar incorporation into cell wall polysaccharides.<sup>23,24,25</sup> Such knowledge should ultimately contribute to better engineering and/or exploitation of lignocellulose biomass.

## Acknowledgments

We thank the TisBio (Univ. Lille) for access to instruments and technical advice. We are indebted to the Research Federation FRABio (Univ. Lille, CNRS, FR 3688, FRABio, Biochimie Structurale et Fonctionnelle des Assemblages Biomoléculaires) for providing the technical environment necessary for this work. CS gratefully acknowledges the financial support (PhD grant) of the Université de Lille.

## ORCID

Cedric Lion  <http://orcid.org/0000-0001-6591-4573>

## References

1. Carpita NC, Gibeaut, DM. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 1993;3(1):1–30. doi:10.1111/j.1365-313X.1993.tb00007.x.
2. Sarkanen KV, & Ludwig CH. *Lignins. Occurrence, formation, structure, and reactions.* New York (NY): Wiley-Interscience. 1971.
3. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. *Annu Rev Plant Biol.* 2003;54(1):519–546. doi:10.1146/annurev.arplant.54.031902.134938.
4. Boudet A M, Kajita S, Grima-Pettenati J, & Goffner D. Lignins and lignocellulosics: a better control of synthesis for new and improved uses. *Trends Plant Sci.* 2003;8(12):576–581. doi:10.1016/j.tplants.2003.10.001.
5. Vanholme R, Morreel K, Darrah C, Oyarce P, Grabber JH, Ralph J, & Boerjan W. Metabolic engineering of novel lignin in biomass crops. *New Phytol.* 2012;196(4):978–1000. doi:10.1111/j.1469-8137.2012.04337.x.
6. Ralph J, Brunow G, Boerjan W. Lignins. *Encyclopedia of Life Sciences (eLS).* Wiley; 2007. doi:10.1002/9780470015902.a0020104.
7. Wagner A, Tobimatsu Y, Phillips L, Flint H, Geddes B, Lu F, & Ralph J. Syringyl lignin production in conifers: Proof of concept in a Pine tracheary element system. *Proc Natl Acad Sci U S A.* 2015;112(19):6218–6223. doi:10.1073/pnas.1411926112.
8. Sletten EM, Bertozzi CR. From mechanism to mouse: A tale of two bioorthogonal reactions. *Acc Chem Res.* 2011;44(9):666–676. doi:10.1021/ar200148z.
9. Bukowski N, Pandey JL, Doyle L, Richard TL, Anderson CT, Zhu Y. Development of a Clickable Designer Monolignol for Interrogation of Lignification in Plant Cell Walls. *Bio- Conjug Chem.* 2014;25:2189–2196.

10. Tobimatsu Y., Van de Wouwer D, Allen E, Kumpf R, Vanholme B, Boerjan W, Ralph J. A click chemistry strategy for visualization of plant cell wall lignification. *Chem Commun (Camb.)*. 2014;50:12262–12265.
11. Lion C, Simon C, Huss B., Blervacq AS, Tiroit L, Toybou D, Biot C. BLISS: A Bioorthogonal dual-labeling strategy to unravel lignification dynamics in plants. *Cell Chem Biol*. 2017;24(3):326–338. doi:10.1016/j.chembiol.2017.02.009.
12. Huis R, Morreel K, Fliniaux O, Lucau-Danila A, Fénart S, Grec S, Hawkins S Natural hypolignification is associated with extensive oligolignol accumulation in flax stems. *Plant Physiol*. 2012;158(4):1893–1915. doi:10.1104/pp.111.192328.
13. Day A, Ruel K, Neutelings G, Crônier D, David H, Hawkins S, & Chabbert B. Lignification in the flax stem: evidence for an unusual lignin in bast fibers. *Planta*. 2005;222(2):234–245. doi:10.1007/s00425-005-1537-1.
14. del Río JC, Rencoret J, Gutiérrez A, Nieto L, Jiménez-Barbero J, Martínez ÁT. Structural characterization of guaiacyl-rich lignins in flax (*Linum usitatissimum*) fibers and shives. *J Agric Food Chem*. 2011;59(20):11088–11099. doi:10.1021/jf201222r.
15. Wi SG, Singh AP, Lee KH, & Kim YS. The pattern of distribution of pectin, peroxidase and lignin in the middle lamella of secondary xylem fibres in alfalfa (*Medicago sativa*). *Ann Bot*. 2005;95(5):863–868. doi:10.1093/aob/mci092.
16. Fernández-Pérez F, Vivar T, Pomar F, Pedreño MA, Novo-Uzal E. Peroxidase 4 is involved in syringyl lignin formation in *Arabidopsis thaliana*. *J Plant Physiol*. 2015;175:86–94. doi:10.1016/j.jplph.2014.11.006.
17. Chantreau M, Portelet A, Dauwe R, Kiyoto S, Crônier D, Morreel K, Yoshinaga A. Ectopic lignification in the flax lignified bast fiber1 mutant stem is associated with tissue-specific modifications in gene expression and cell wall composition. *Plant Cell*. 2014;26(11):4462–4482. doi:10.1105/tpc.114.130443.
18. Schreiber L, Franke RB. Endodermis and exodermis in roots. *eLS*. Wiley. 2011. doi:10.1002/9780470015902.a0002086.pub2.
19. Zeier J, Goll A, Yokoyama M, Karahara I, Schreiber L. Structure and chemical composition of endodermal and rhizodermal/hypodermal walls of several species. *Plant, Cell & Environment*, 1999;22(3):271–279. doi:10.1046/j.1365-3040.1999.00401.x.
20. Lee Y, Rubio MC, Alassimone J, Geldner N. A mechanism for localized lignin deposition in the endodermis. *Cell*. 2013;153(2):402–412. doi:10.1016/j.cell.2013.02.045.
21. Graça J. Suberin: the biopolyester at the frontier of plants. *Front Chem*. 2015;3:62. doi:10.3389/fchem.2015.00062.
22. Henry S, Divol F, Bettembourg M, Bureau C, Guiderdoni E, Périn C, Diévert A. Immunoprofiling of rice root cortex reveals two cortical subdomains. *Frontiers in plant science*, 2015;6:1139. doi:10.3389/fpls.2015.01139.
23. Anderson CT, Wallace IS, Somerville CR. Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in *Arabidopsis* cell walls. *Proc Natl Acad Sci U S A*. 2012;109(4):1329–1334. doi:10.1073/pnas.1120429109.
24. Dumont M, Lehner A, Vauzeilles B, Malassis J, Marchant A, Smyth K, Schapman D. Plant cell wall imaging by metabolic click-mediated labelling of rhamnogalacturonan II using azido 3- deoxy- d- manno- oct- 2- ulosonic acid. *Plant J*. 2016;85(3):437–447. doi:10.1111/tbj.13104.
25. Zhu Y, Wu J, Chen X. Metabolic labeling and imaging of N- Linked Glycans in *Arabidopsis Thaliana*. *Angewandte Chemie*. 2016;128(32):9447–9451. doi:10.1002/ange.201603032.