

#### Studying the dynamics of cell wall polysaccharides by metabolic click-mediated labeling of pectins

Marc Ropitaux, Quentin Hays, Aurélie Baron, Laura Fourmois, Isabelle Boulogne, Boris Vauzeilles, Jean-Claude Mollet, Patrice Lerouge, Arnaud Lehner

#### ▶ To cite this version:

Marc Ropitaux, Quentin Hays, Aurélie Baron, Laura Fourmois, Isabelle Boulogne, et al.. Studying the dynamics of cell wall polysaccharides by metabolic click-mediated labeling of pectins. 5ème Journées Scientifiques de la Fédération de Recherche Normandie-Végétal- FED4277, Jul 2023, Rouen, France. hal-04153829

#### HAL Id: hal-04153829 https://normandie-univ.hal.science/hal-04153829v1

Submitted on 6 Jul 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.









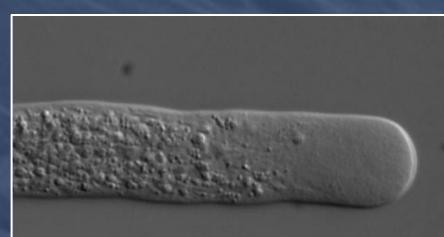












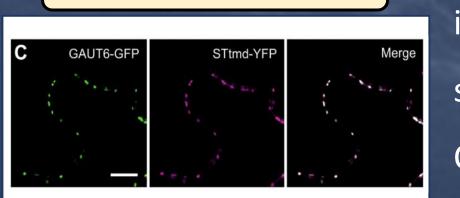
# Studying the dynamics of cell wall polysaccharides by metabolic click-mediated labeling of pectins.

Ropitaux M<sup>1,2</sup>, Hays Q<sup>1</sup>, Baron A<sup>3</sup>, Fourmois L<sup>3</sup>, Boulogne I<sup>1</sup>, Vauzeilles B<sup>3</sup>, Mollet J-C<sup>1</sup>, Lerouge P<sup>1</sup>, Lehner A<sup>1</sup>.

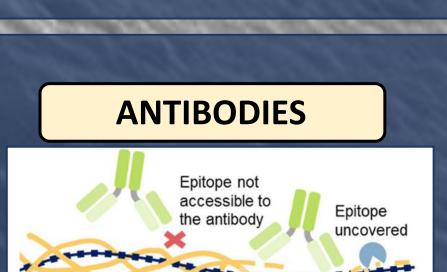
<sup>3</sup>Universite Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France.

<sup>1</sup>Université de Rouen Normandie, Laboratoire GlycoMEV UR 4358, SFR Normandie Végétal FED 4277, Innovation Chimie Carnot, F-76000 Rouen. <sup>2</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>2</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>2</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>2</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>2</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>3</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen. <sup>4</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000 Rouen Rouen

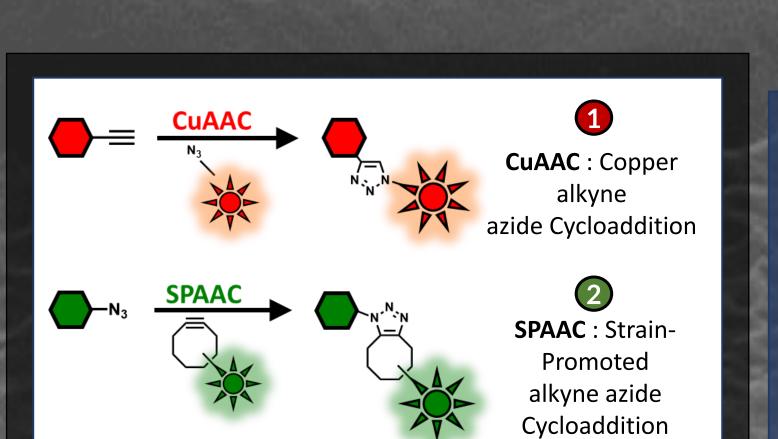
#### **PROTEIN REPORTERS**



Protein tracking in living plant cells has become routine with the emergence of reporter genes encoding fluorescent tags. Unfortunately, this imaging strategy is not applicable to glycans because they are not directly encoded by the genome. Indeed, complex glycans result from sequential additions and/or removals of monosaccharides by glycosyltransferases and glycosidases of the cell's biosynthetic machinery. Currently, the imaging of cell wall polymers mainly relies on the use of antibodies or dyes that exhibit variable specificities. However, as immunolocalization typically requires sample fixation, it does not provide access to the dynamics of living cells.



Rydahl et al, 2018



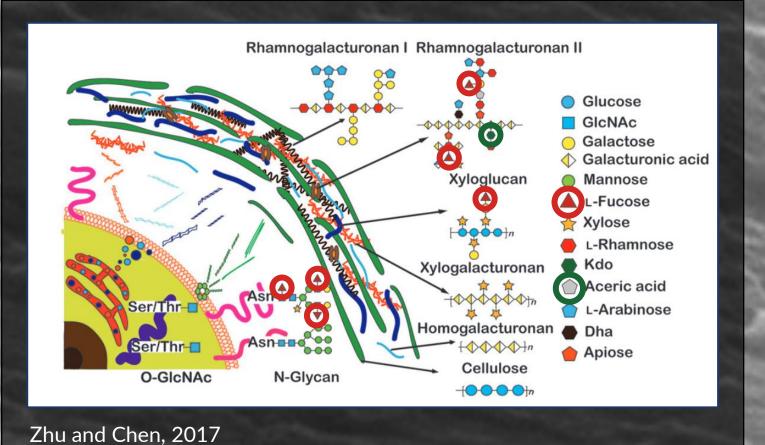
Click labeling catylazed by copper and 2 copper-free click labeling,

### An alternative... The click chemistry

- **ALLOWS DYNAMIC IMAGING**
- **❖** CELL WALL POLYSACCHARIDES LABELING
  - **❖** SPECIFIC LIVE-CELL LABELING

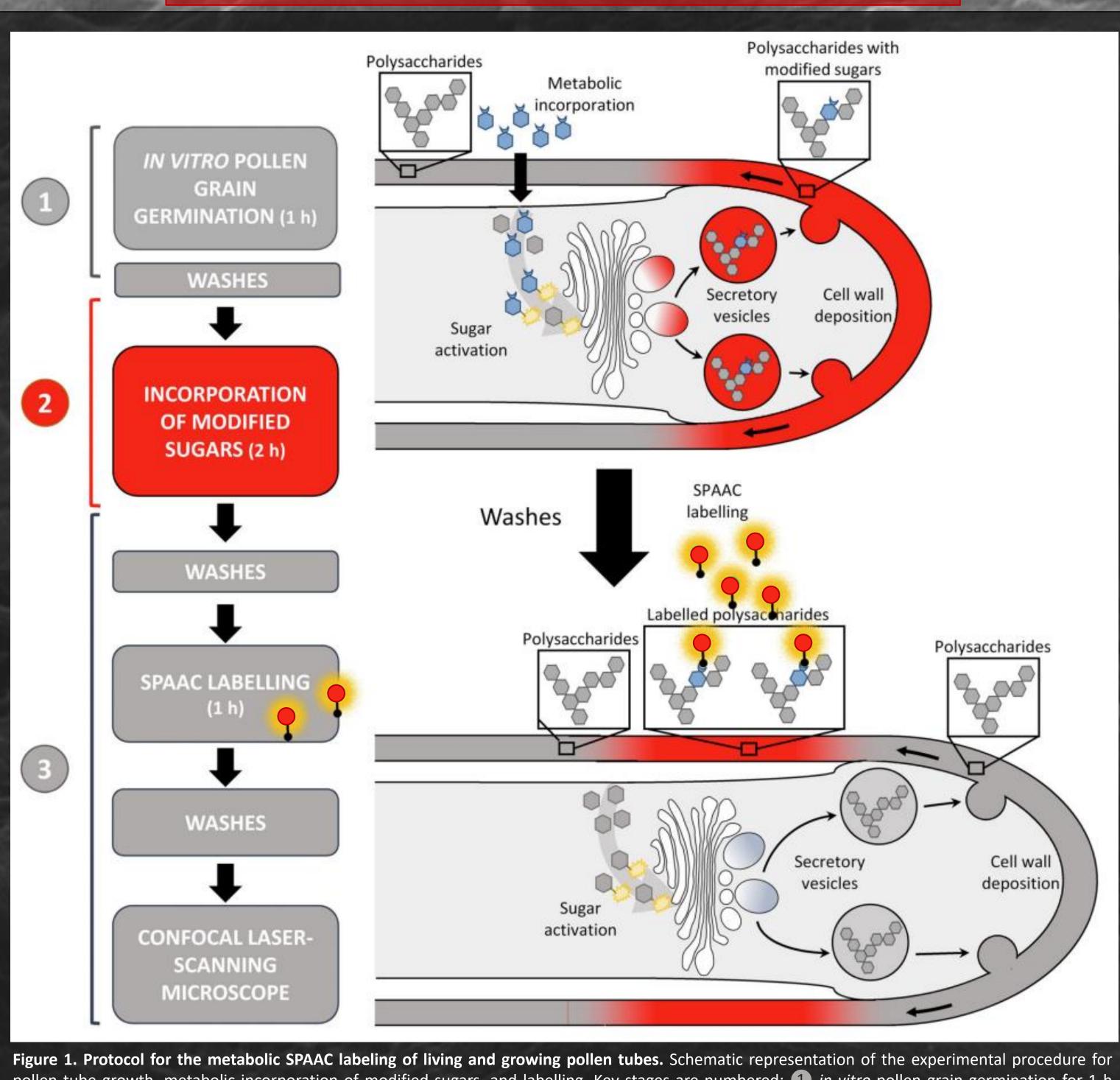
Incorporation of Kdo- $N_3$  (200  $\mu$ M)

Incorporation of Fucose- $N_3$  (50  $\mu$ M)

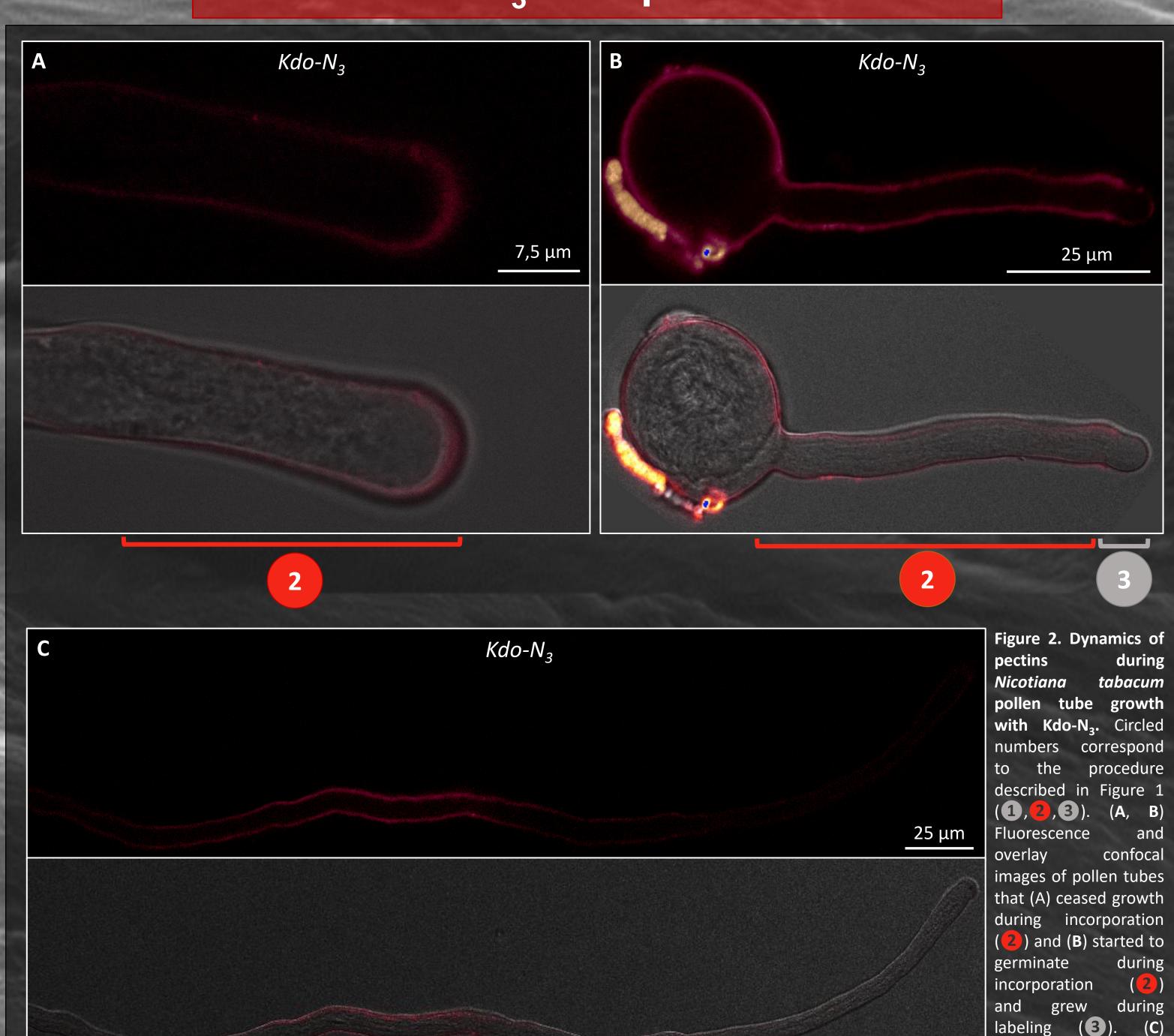


#### How does it work?

## Kdo-N<sub>3</sub> incorporation



pollen tube growth, metabolic incorporation of modified sugars, and labelling. Key stages are numbered: 1 in vitro pollen grain germination for 1 h culture medium at 22°C in darkness; 💋 incorporation of Kdo-N<sub>3</sub> (200 μM) or Fuc-N<sub>3</sub> (50 μM) for 2 h at 22°C in darkness; and 🕄 washes and click labelling with 1 μM alkyne Alexa Fluor 488 sDIBO for 1 h at room temperature (RT) before confocal imaging (Ropitaux et al., 2021).



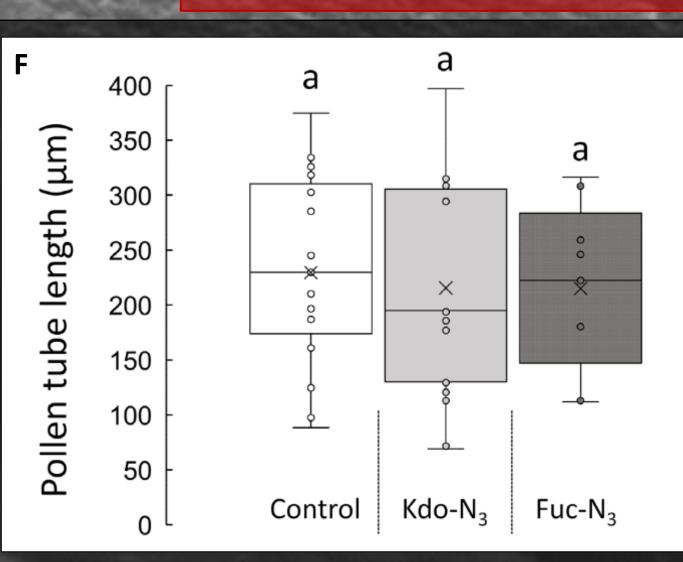
## Fuc-N<sub>3</sub> incorporation

# Fuc-N<sub>3</sub> 25 μm

**Negative Control** 

Figure 3. Dynamics of fucose containing during polymers Nicotiana tabacum pollen tube growth with Fuc-N<sub>3</sub>. Circled numbers correspond to the procedure described in Figure 1 ((1,2,3)). (D) Fluorescence and overlay confocal images of pollen tubes that grew during the three steps after incorporation of Fuc-N3 ). (E) Negative control samples were submitted to steps 📵, 包, and 🔞 but without addition of Kdo-N<sub>3</sub> or Fuc-N<sub>3</sub> during the incorporation step (Ropitaux et al., 2021).

#### Effect of metabolic SPAAC labeling



200 150 Kdo-N<sub>3</sub> Fuc-N<sub>3</sub> Kdo-N<sub>3</sub> Fuc-N<sub>3</sub> Kdo-N<sub>3</sub> Fuc-N<sub>3</sub>

Fluorescence

images of pollen tubes

that expanded during

(Ropitaux et al., 2021).

confocal

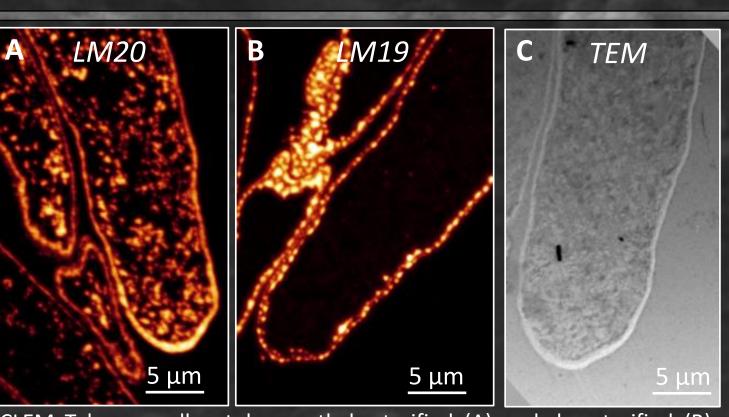
Figure 4. Effect of metabolic SPAAC labeling on pollen tube growth. (F) Distribution of pollen tube length after metabolic incorporation and SPAAC labeling in control conditions (n = 17) or with Kdo-N<sub>3</sub> (200  $\mu$ M, n = 20) or Fuc-N<sub>3</sub> (50  $\mu$ M, n = 13). (G) Distribution of pollen tube length before metabolic incorporation (11), during incorporation (22), and during SPAAC labeling (33) of Kdo-N<sub>3</sub> (n = 15) or Fuc-N<sub>3</sub> (n = 10) (Ropitaux *et al.*, 2021).

# **CLICK CHEMISTRY SPAAC** Strain-Promoted alkyne azide Cycloaddition Rhamnogalacturonan II (RG-II)

Lerouge et al., 2021

#### Conclusion and prospects

- **ALLOW THE STUDY OF POLYSACCHARIDES IN LIVING CELLS** 
  - **CELL WALL POLYSACCHARIDES LABELING**
  - \* NEW TOOL FOR RHAMNOGALACTURONAN II (RG-II) **VISUALIZATION** 
    - CORRELATIVE LIGHT-ELECTRON MICROSCOPY ( )



CLEM Tobacco pollen tube, methyl-esterified (A) and de-esterified (B) (Homogalacturonan immunolabeling Ropitaux et al., unpublished

Acknowledgements: The authors are grateful to the Region Normandie for the financial support of the Normandy Plant Technologies (NPT) project. The authors also acknowledge the Cell Imaging Platform (PRIMACEN-IRIB, University of Rouen

Normandie, France, http://www.primacen.fr) Lerouge et al., Carbohydrate Chemistry 2021, 553–571. Lund et al., 2020, Frontiers in Plant Science, 11: 10.3389/fpls.2020.585774. Ropitaux et al., 2022, The Plant Journal, 110, 916-924. Rydahl et al, 2018, Frontiers in Plant Science 9: 10.3389/fpls.2018.00581. Zhu and Chen, 2017, ChemBioChem, 8: 1286 – 1296.