



**HAL**  
open science

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

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

### ► To cite this version:

Marc Ropitiaux, 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-04153829>**

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.

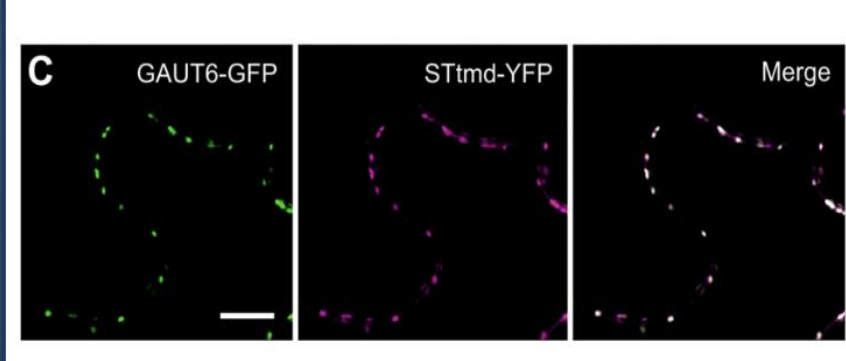


# Studying the dynamics of cell wall polysaccharides by metabolic click-mediated labeling of pectins.

Ropitiaux 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>1</sup>Université de Rouen Normandie, Laboratoire GlycoMEV UR 4358, SFR Normandie Végétal FED 4277, Innovation Chimie Carnot, RMT BESTIM, F-76000 Rouen. <sup>2</sup>Université de Rouen Normandie, INSERM US 51, CNRS UAR 2026, HeRacLeS- PRIMACEN, Innovation Chimie Carnot, F-76000. <sup>3</sup>Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France.

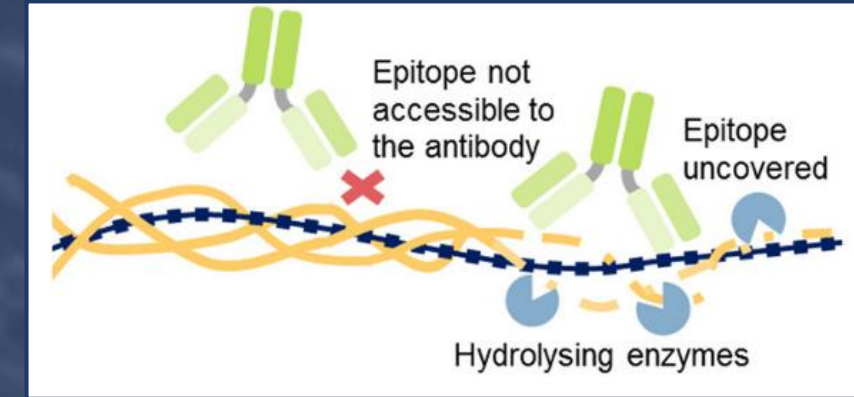
## PROTEIN REPORTERS



Lund et al., 2020

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.

## ANTIBODIES



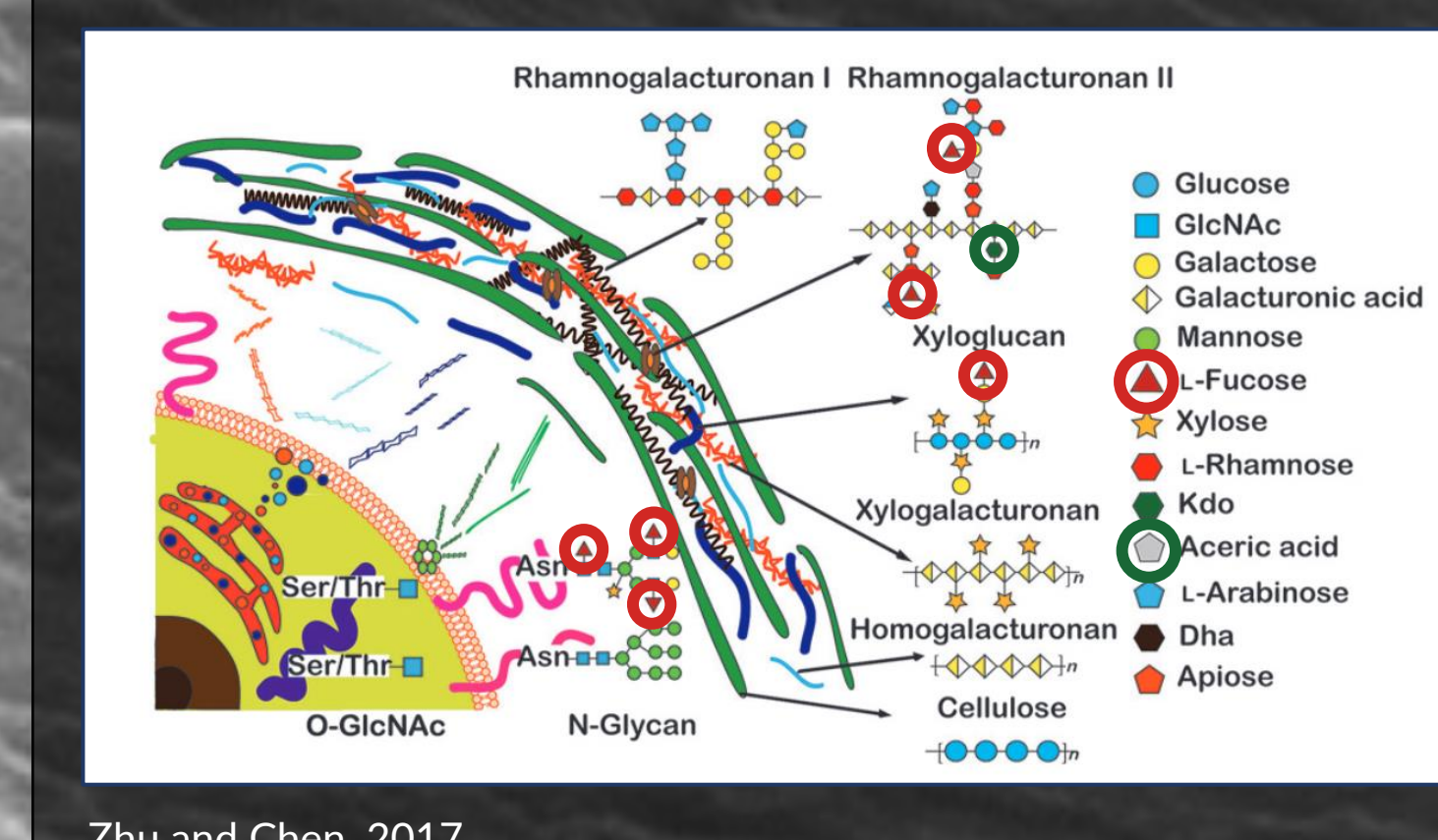
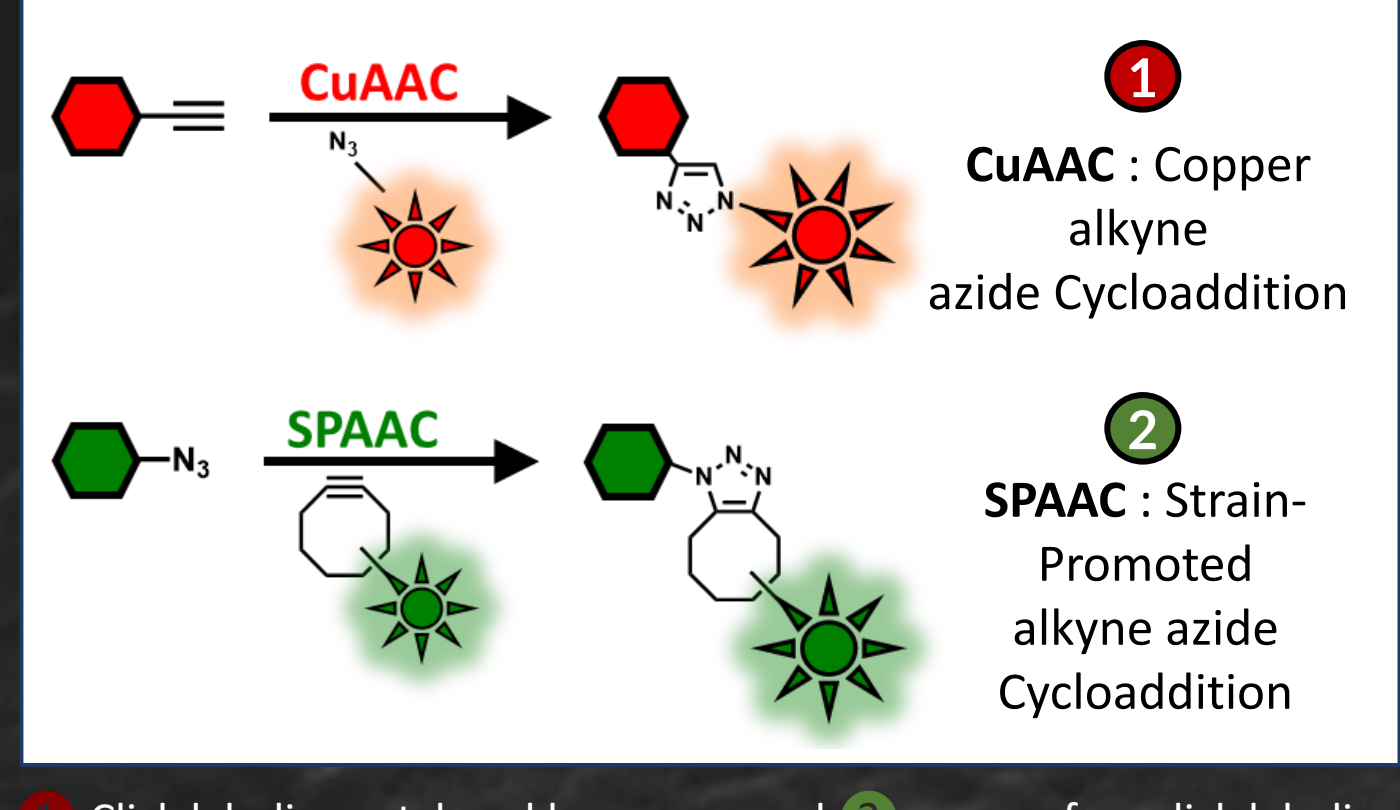
Rydahl et al., 2018

## An alternative... The click chemistry

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

Incorporation of Kdo-N<sub>3</sub> (200 μM)

Incorporation of Fucose-N<sub>3</sub> (50 μM)



## How does it work?

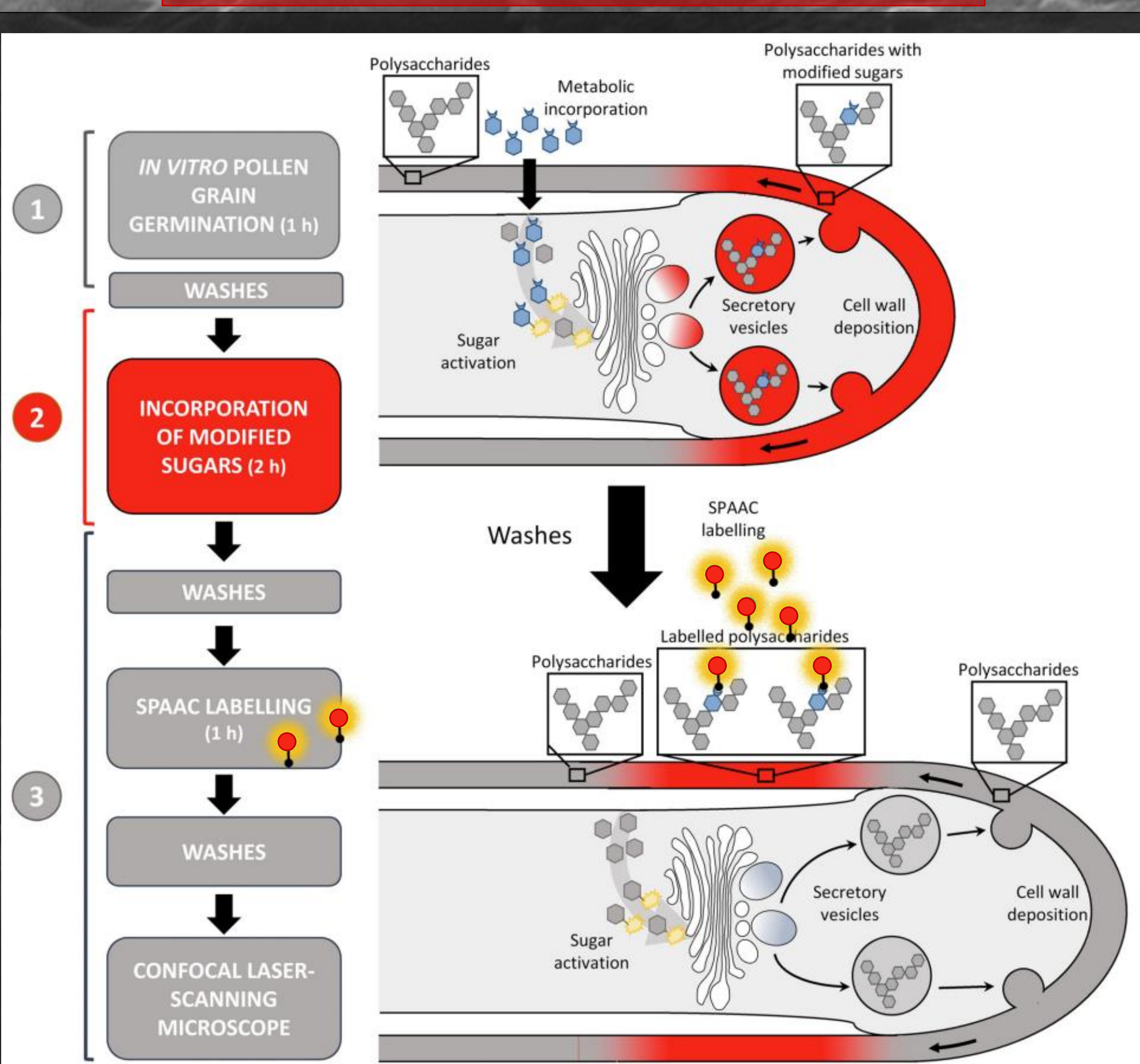
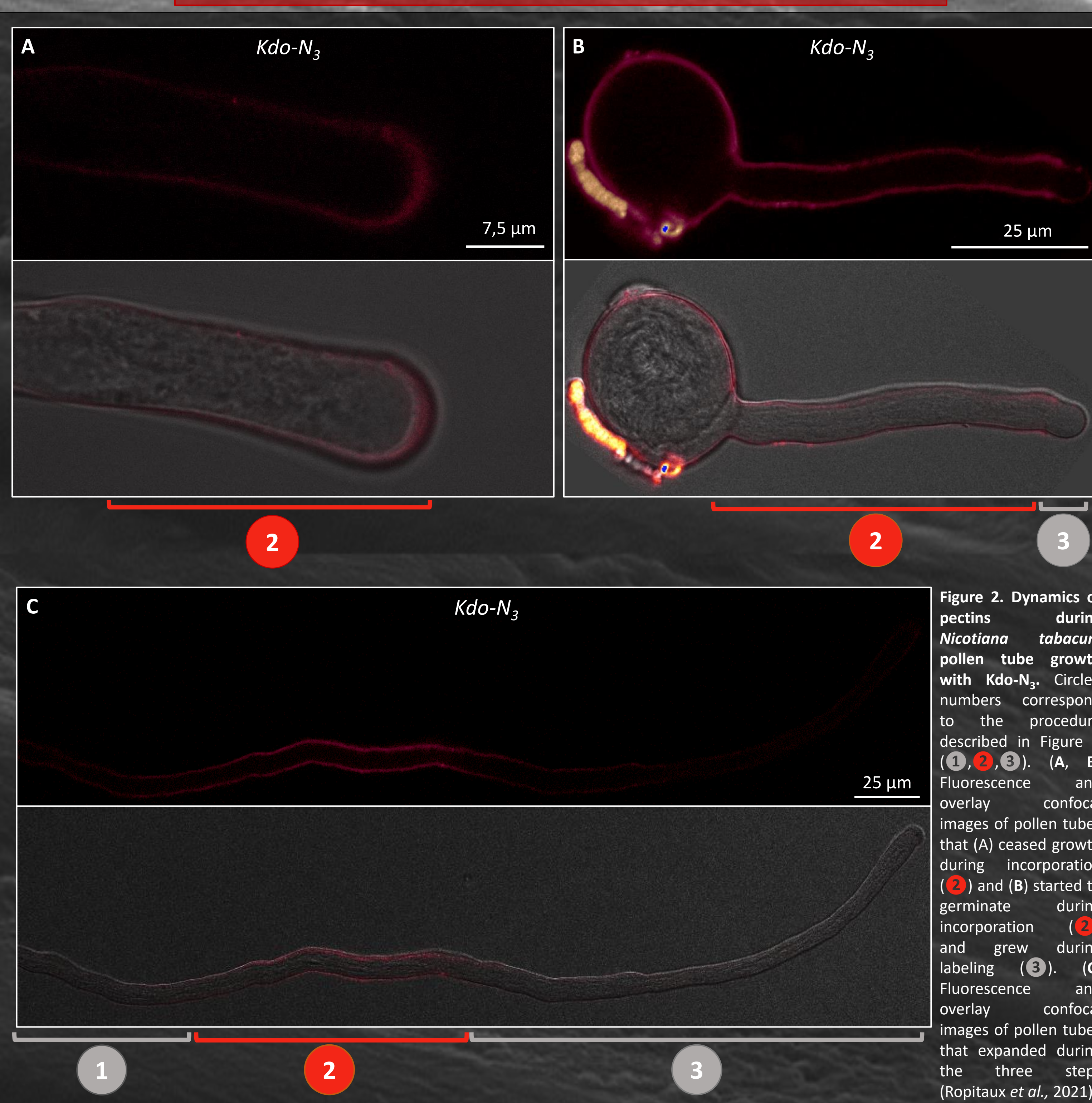
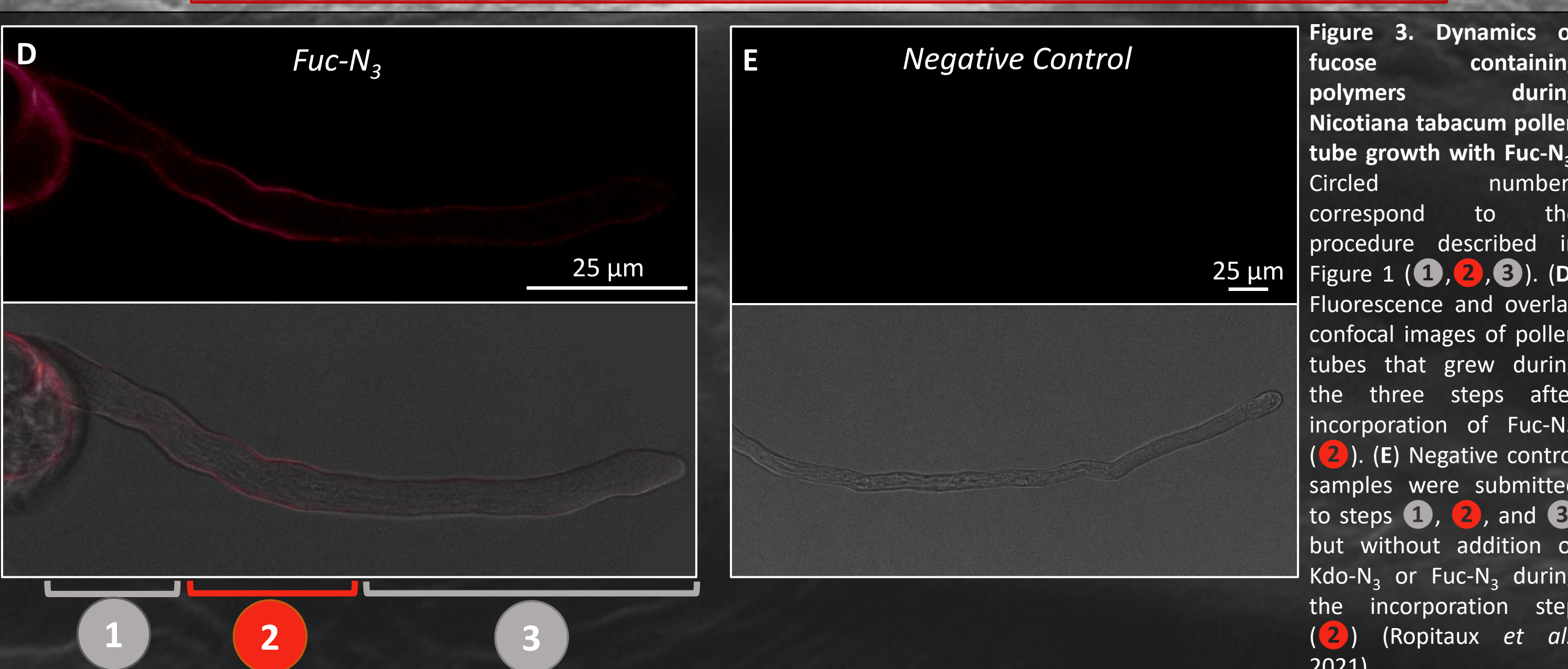


Figure 1. Protocol for the metabolic SPAAC labeling of living and growing pollen tubes. Schematic representation of the experimental procedure for pollen tube growth, metabolic incorporation of modified sugars, and labeling. Key stages are numbered: 1 *in vitro* pollen grain germination for 1 h culture medium at 22°C in darkness; 2 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 3 washes and click-labelling with 1 μM alkyne Alexa Fluor 488 sDIBO for 1 h at room temperature (RT) before confocal imaging (Ropitiaux et al., 2021).

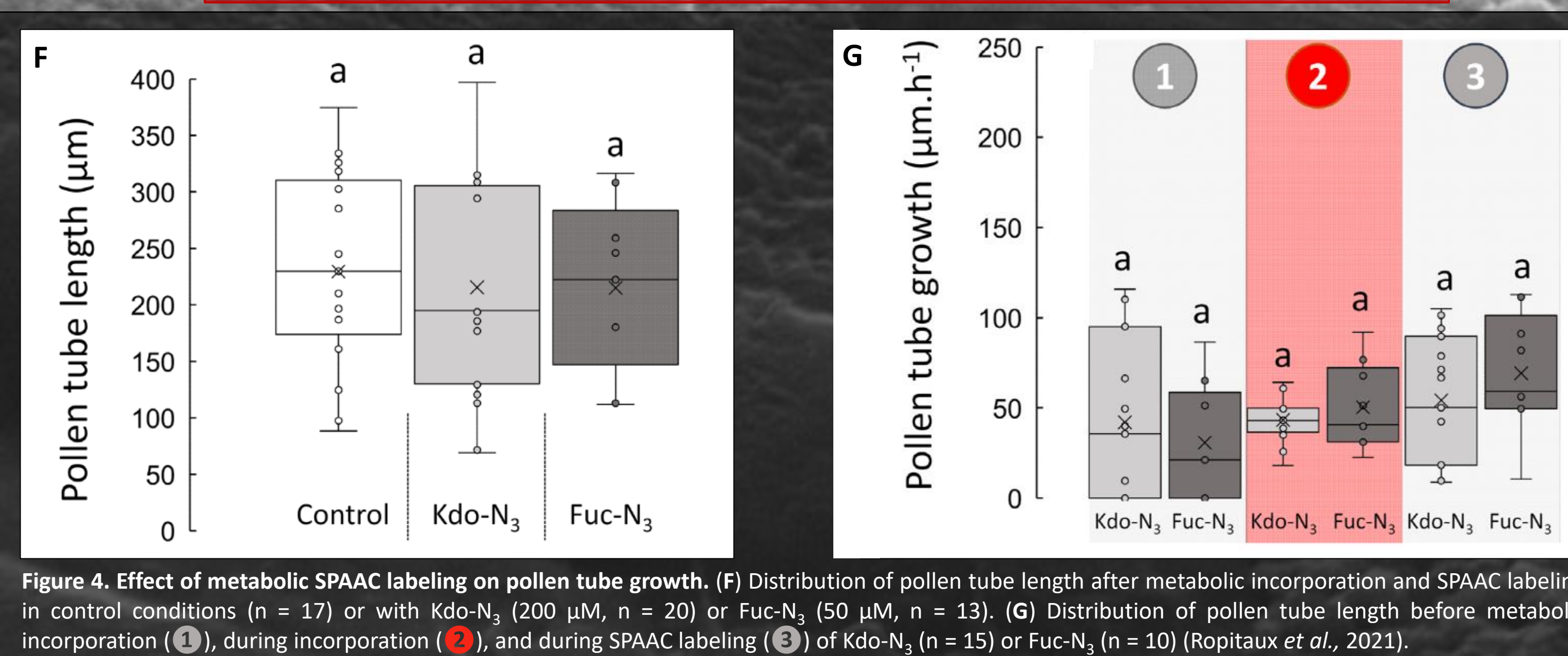
## Kdo-N<sub>3</sub> incorporation



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

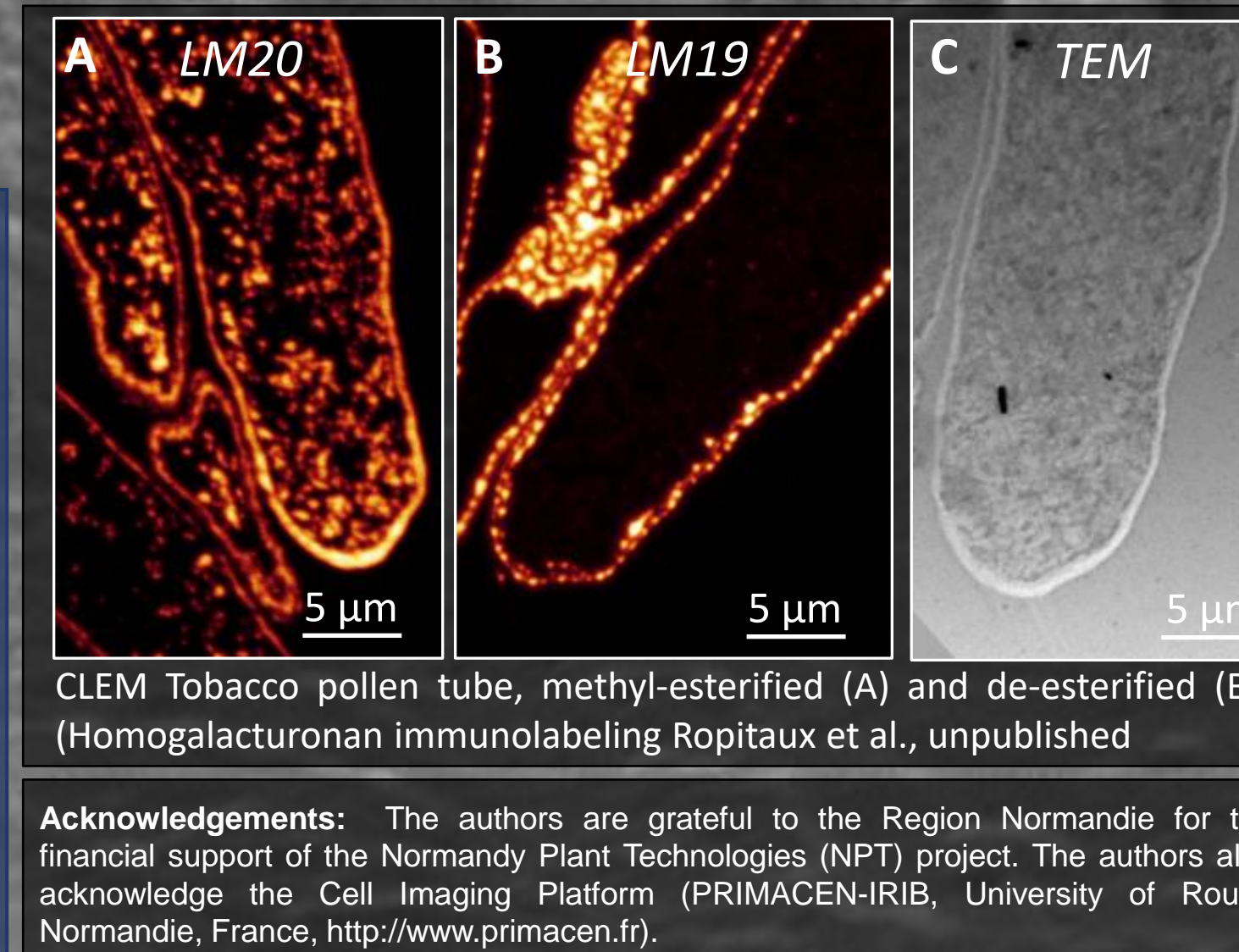
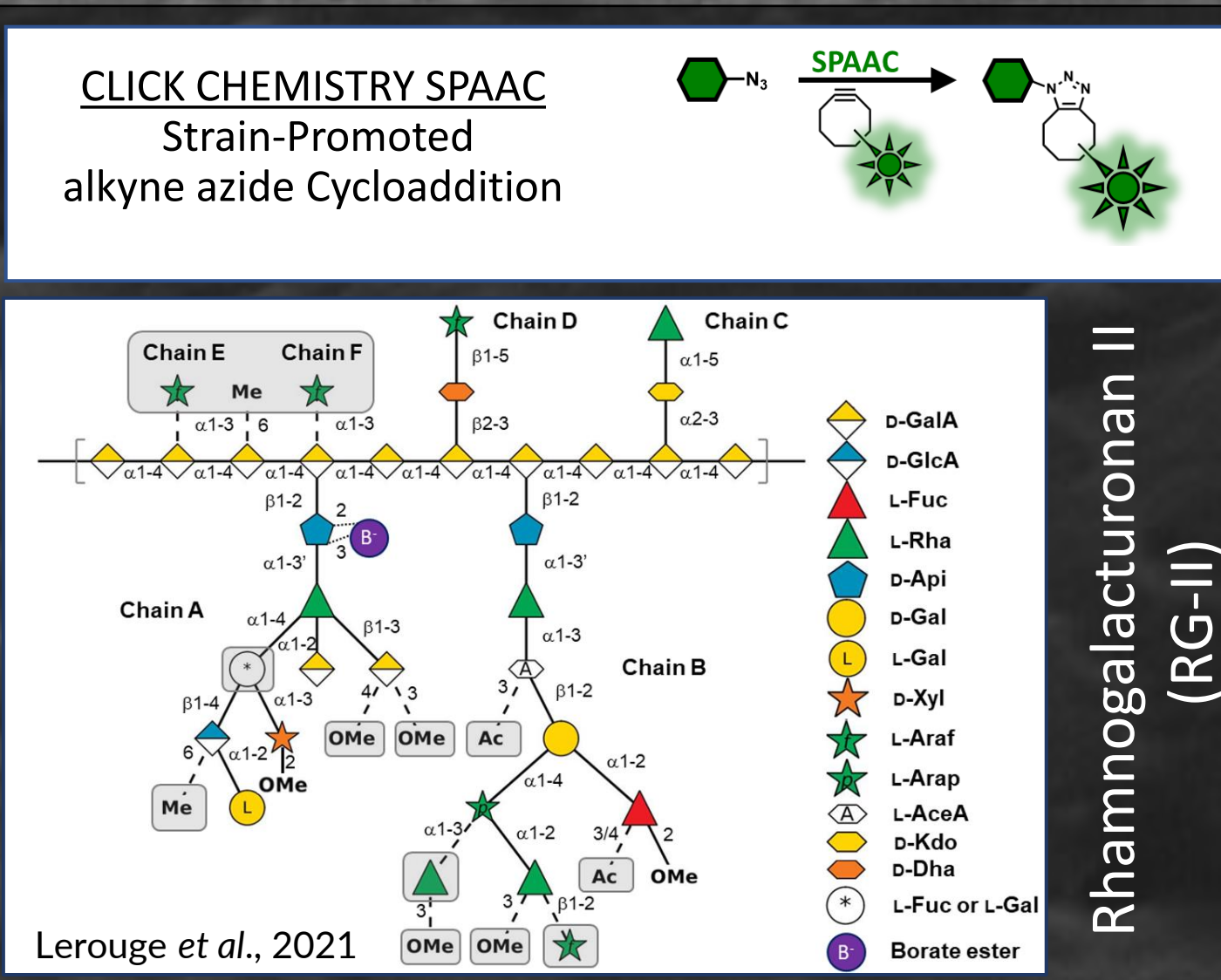


## Effect of metabolic SPAAC labeling



## 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 ←



**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>).