



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

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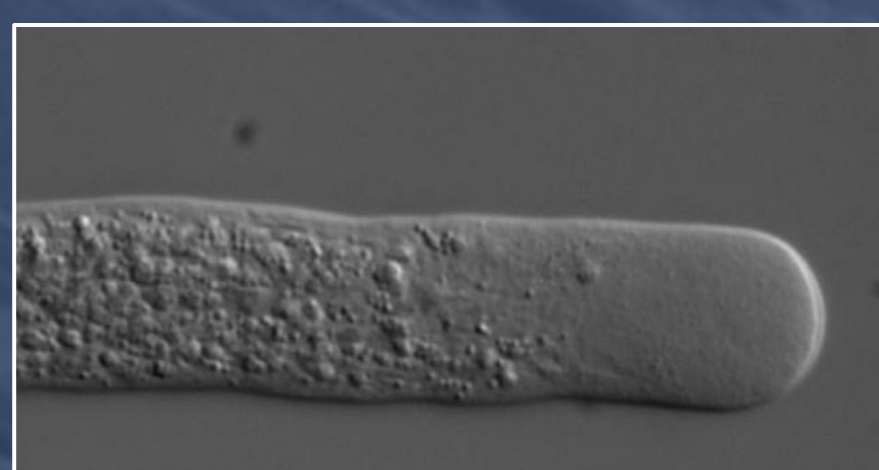
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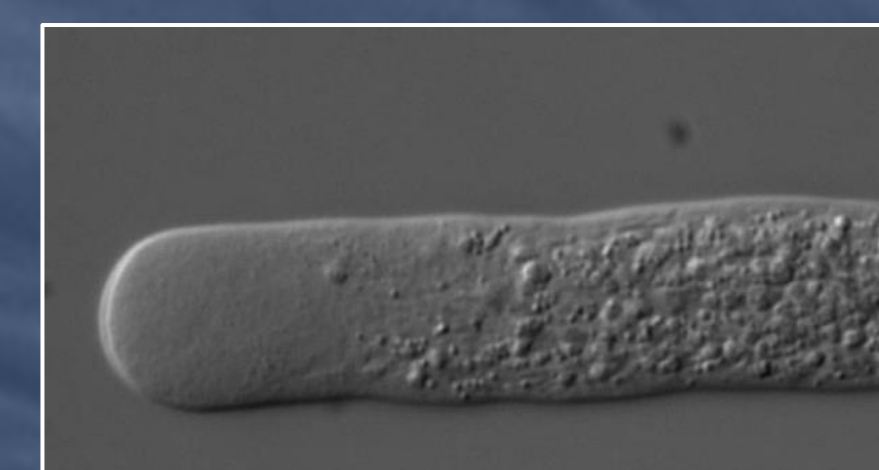
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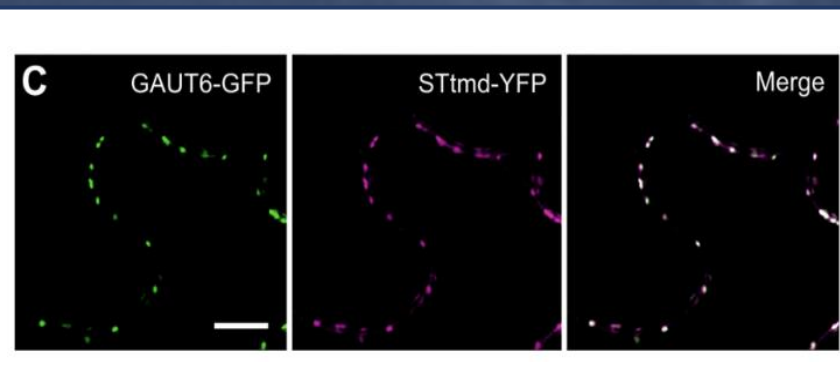
# Studying the dynamics of cell wall polysaccharides by metabolic click-mediated labeling of pectins.



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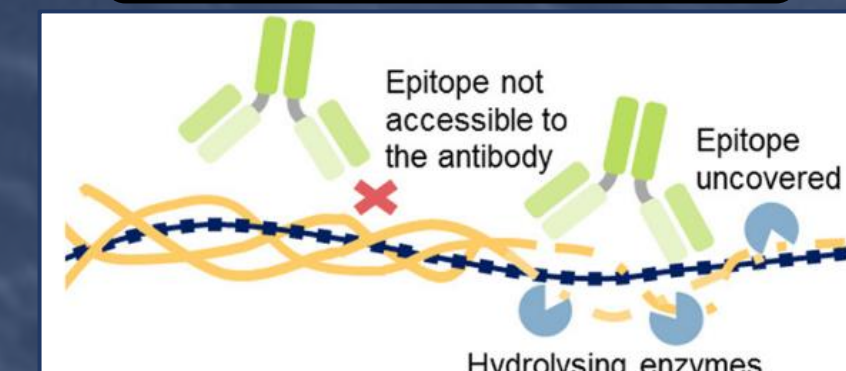
## PROTEIN REPORTERS



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



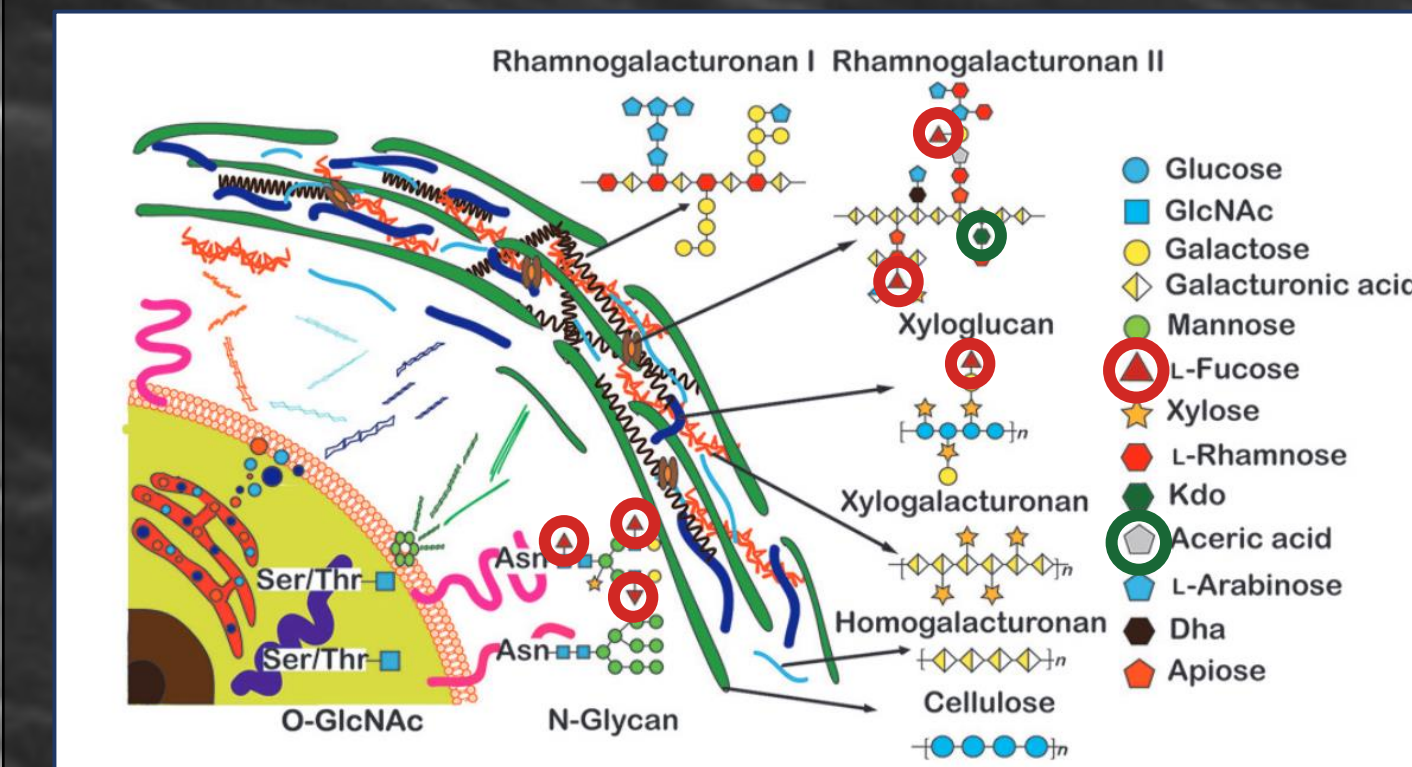
Rydahl et al, 2018

## An alternative... The click chemistry

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

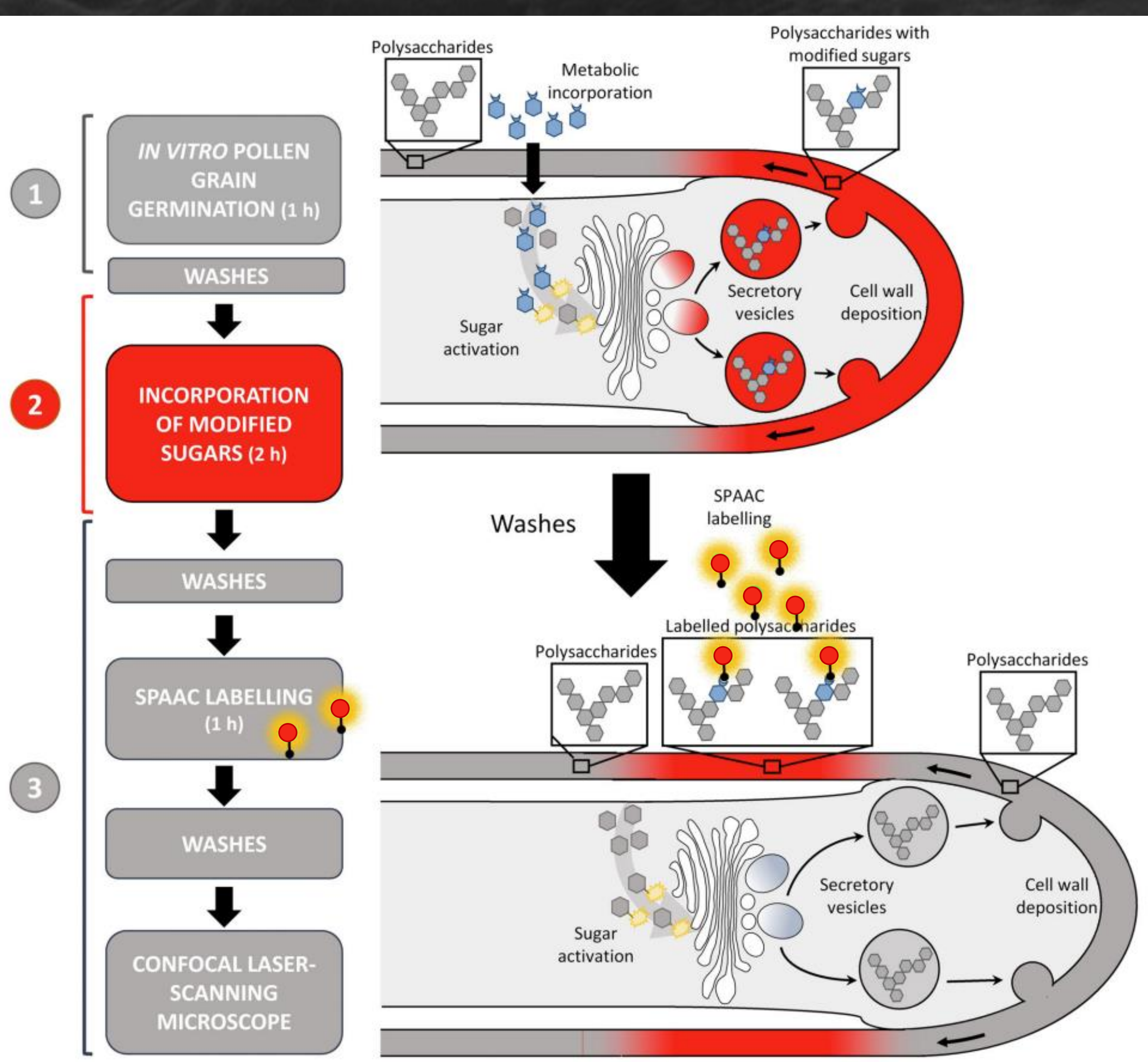
Incorporation of Kdo-N<sub>3</sub> (200 μM)

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



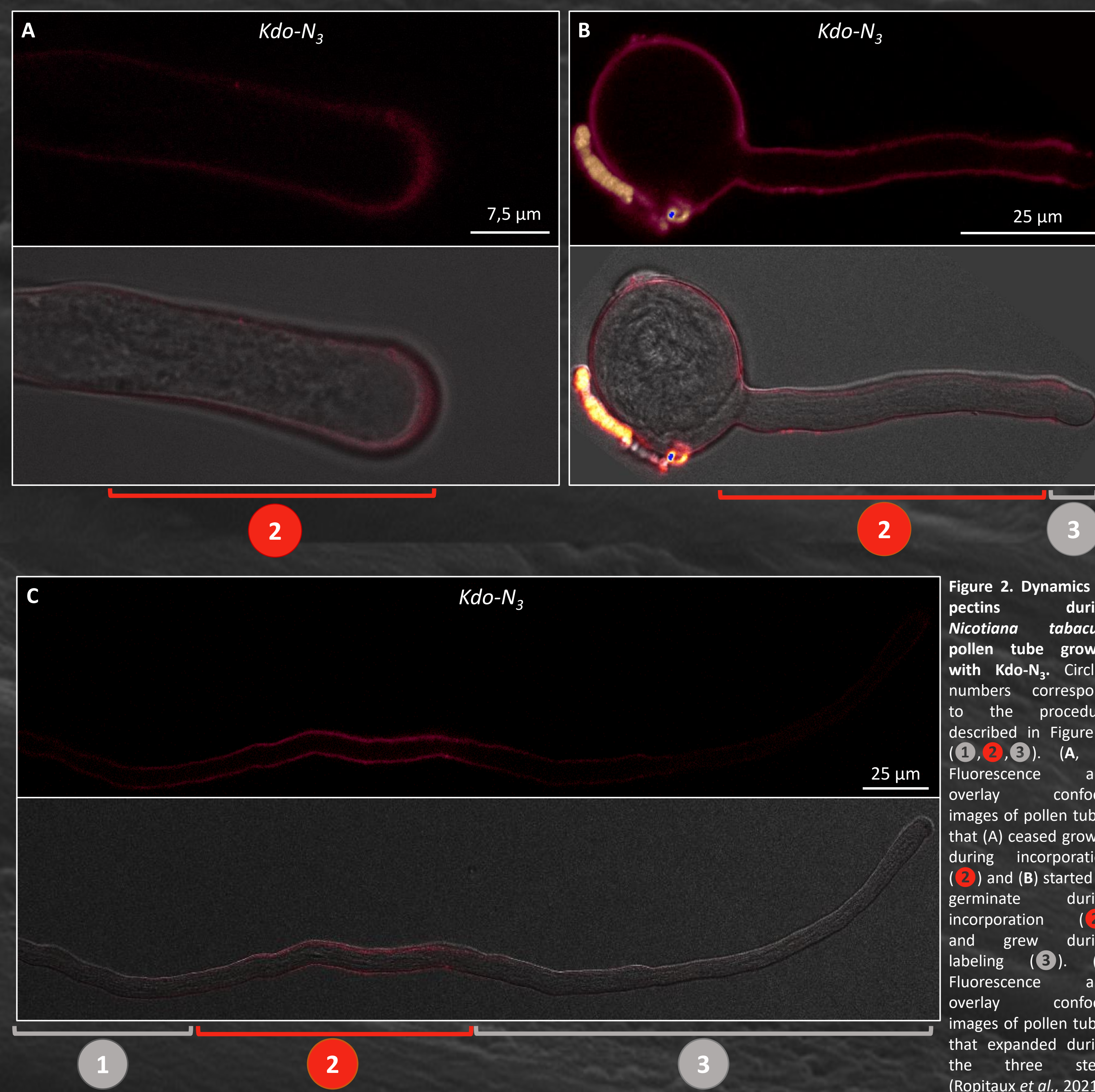
Zhu and Chen, 2017

## How does it work?



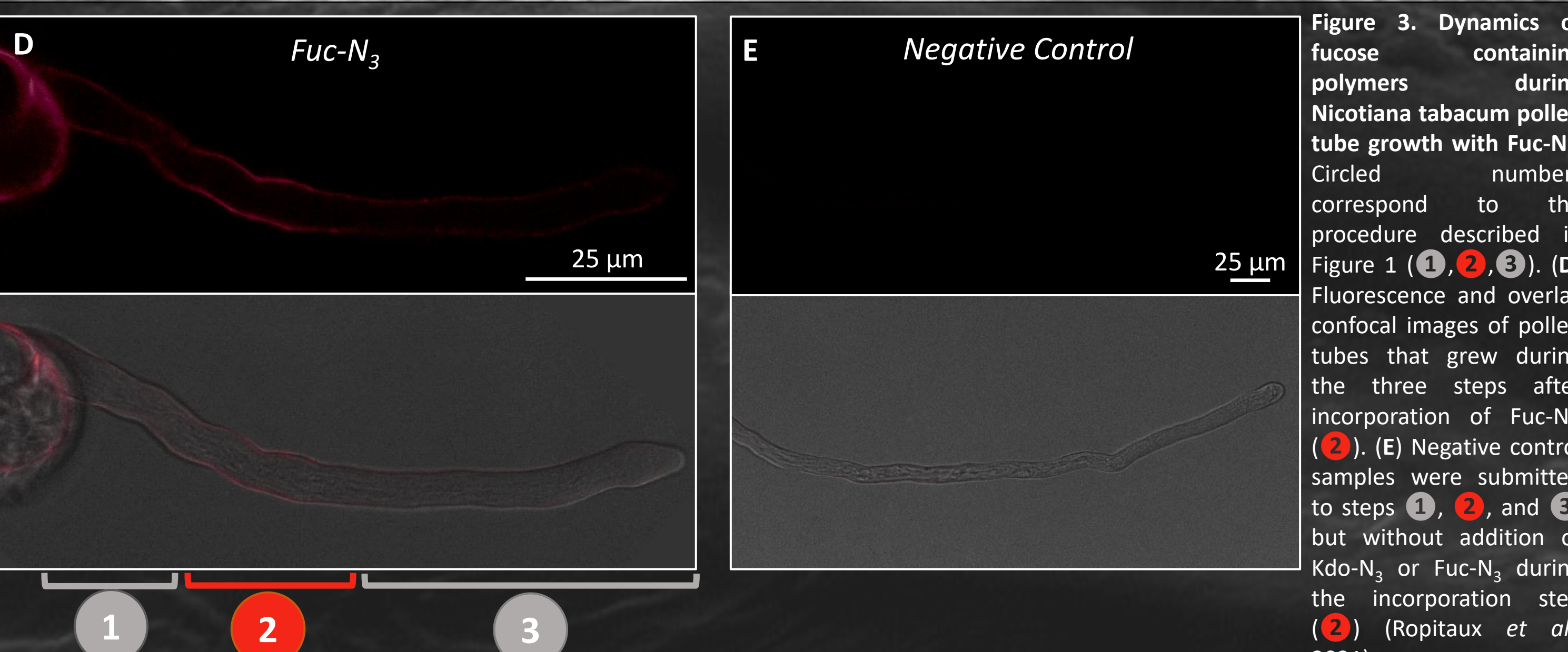
**Figure 1. Protocol for the metabolic SPAAC labeling of living and growing pollen tubes.** Schematic representation of the experimental procedure for pollen tube growth, metabolic incorporation of modified sugars, and labelling. Key stages are numbered: **1** *in vitro* pollen grain germination for 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



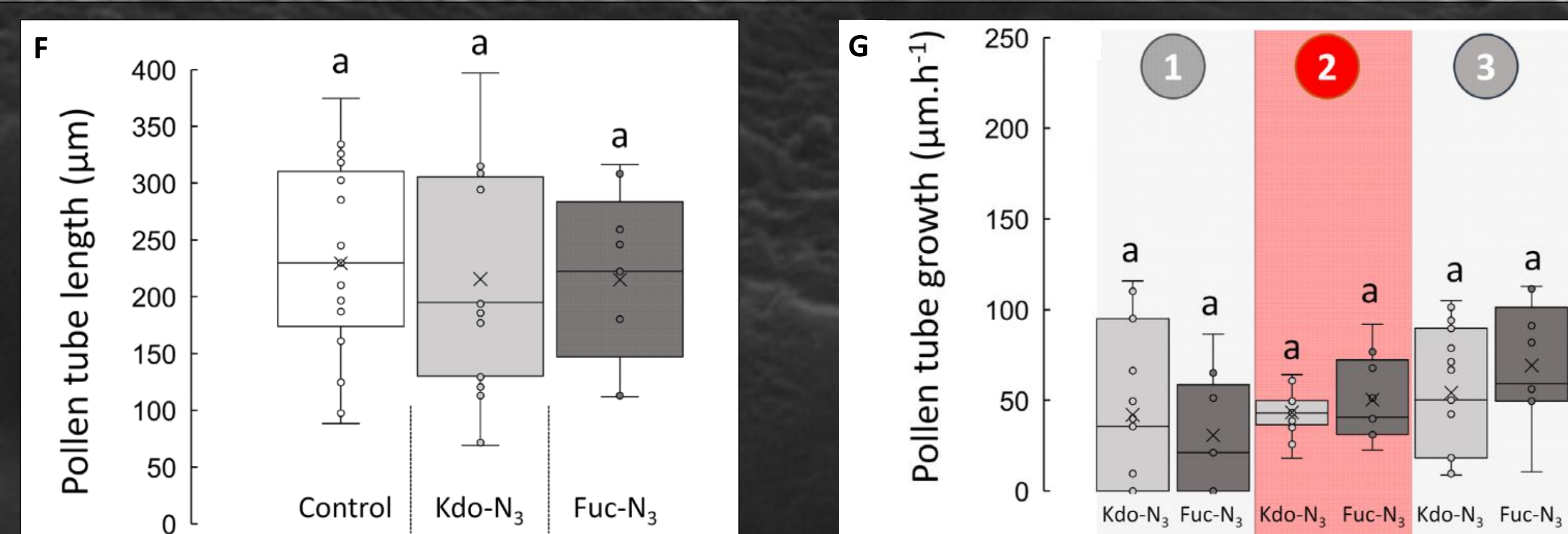
**Figure 2. Dynamics of pectins during *Nicotiana tabacum* pollen tube growth with Kdo-N<sub>3</sub>.** Circled numbers correspond to the procedure described in Figure 1 (**1**, **2**, **3**). (A, B) Fluorescence and overlay confocal images of pollen tubes that (A) ceased growth during incorporation (**2**) and (B) started to germinate during incorporation (**2**) and grew during labeling (**3**). (C) Fluorescence and overlay confocal images of pollen tubes that expanded during the three steps (Ropitiaux et al., 2021).

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



**Figure 3. Dynamics of fucose containing polymers during *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-N<sub>3</sub> (**2**). (E) Negative control samples were submitted to steps **1**, **2**, and **3** but without addition of Kdo-N<sub>3</sub> or Fuc-N<sub>3</sub> during the incorporation step (**2**) (Ropitiaux et al., 2021).

## Effect of metabolic SPAAC labeling

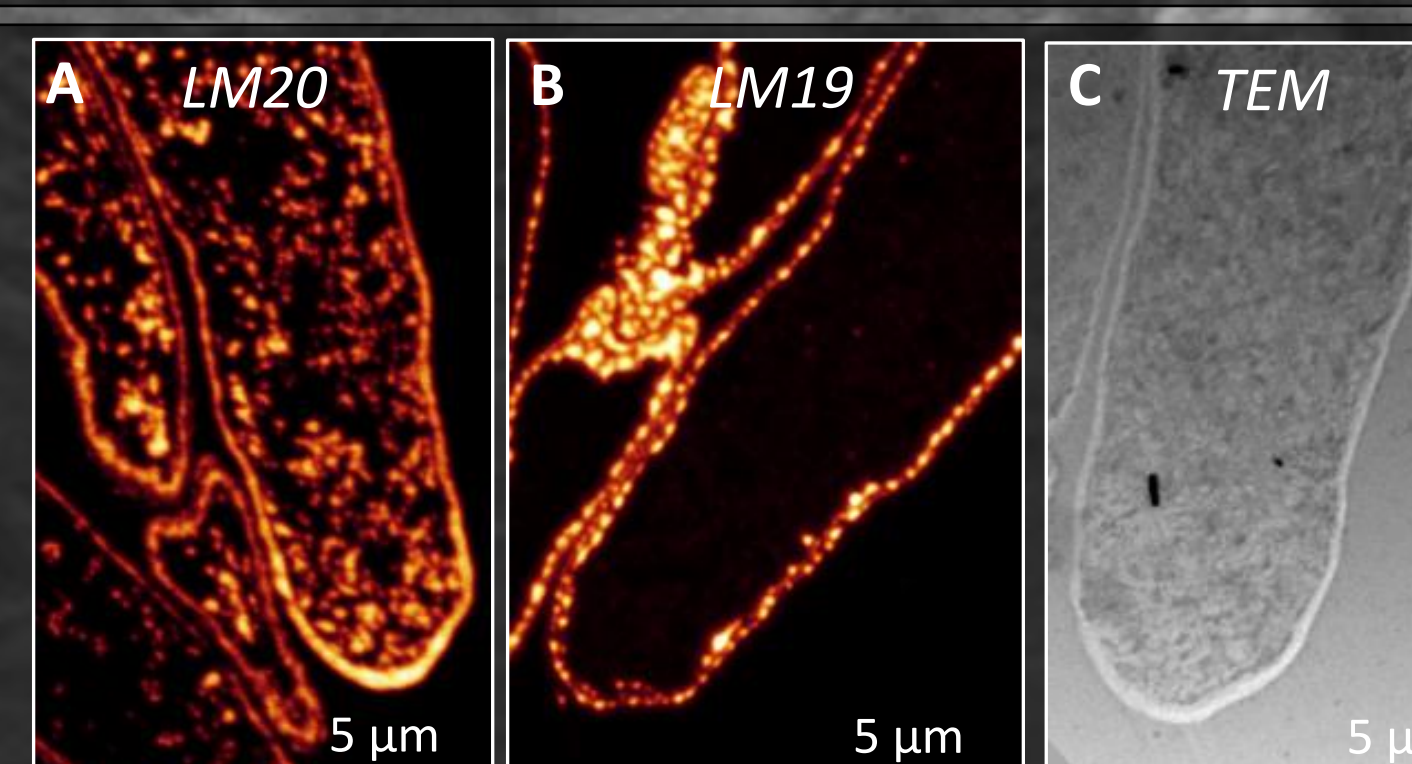


**Figure 4. Effect of metabolic SPAAC labeling on pollen tube growth.** (F) Distribution of pollen tube length after metabolic incorporation and SPAAC labeling in control conditions (n = 17) or with Kdo-N<sub>3</sub> (200 μM, n = 20) or Fuc-N<sub>3</sub> (50 μM, n = 13). (G) Distribution of pollen tube length before metabolic incorporation (**1**), during incorporation (**2**), and during SPAAC labeling (**3**) of Kdo-N<sub>3</sub> (n = 15) or Fuc-N<sub>3</sub> (n = 10) (Ropitiaux 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 Ropitiaux et al., unpublished)

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