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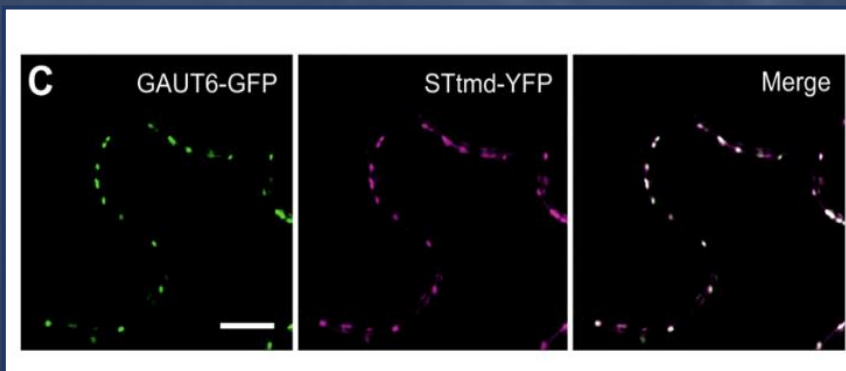
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Studying the dynamics of cell wall polysaccharides by metabolic click-mediated labeling of pectins.

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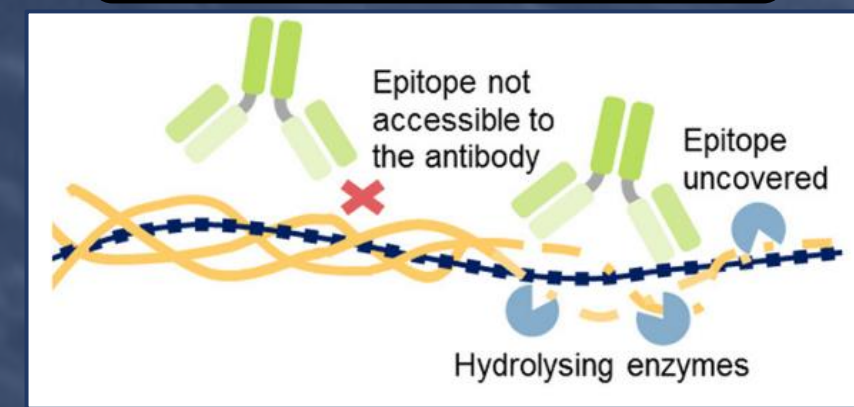
PROTEIN REPORTERS



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



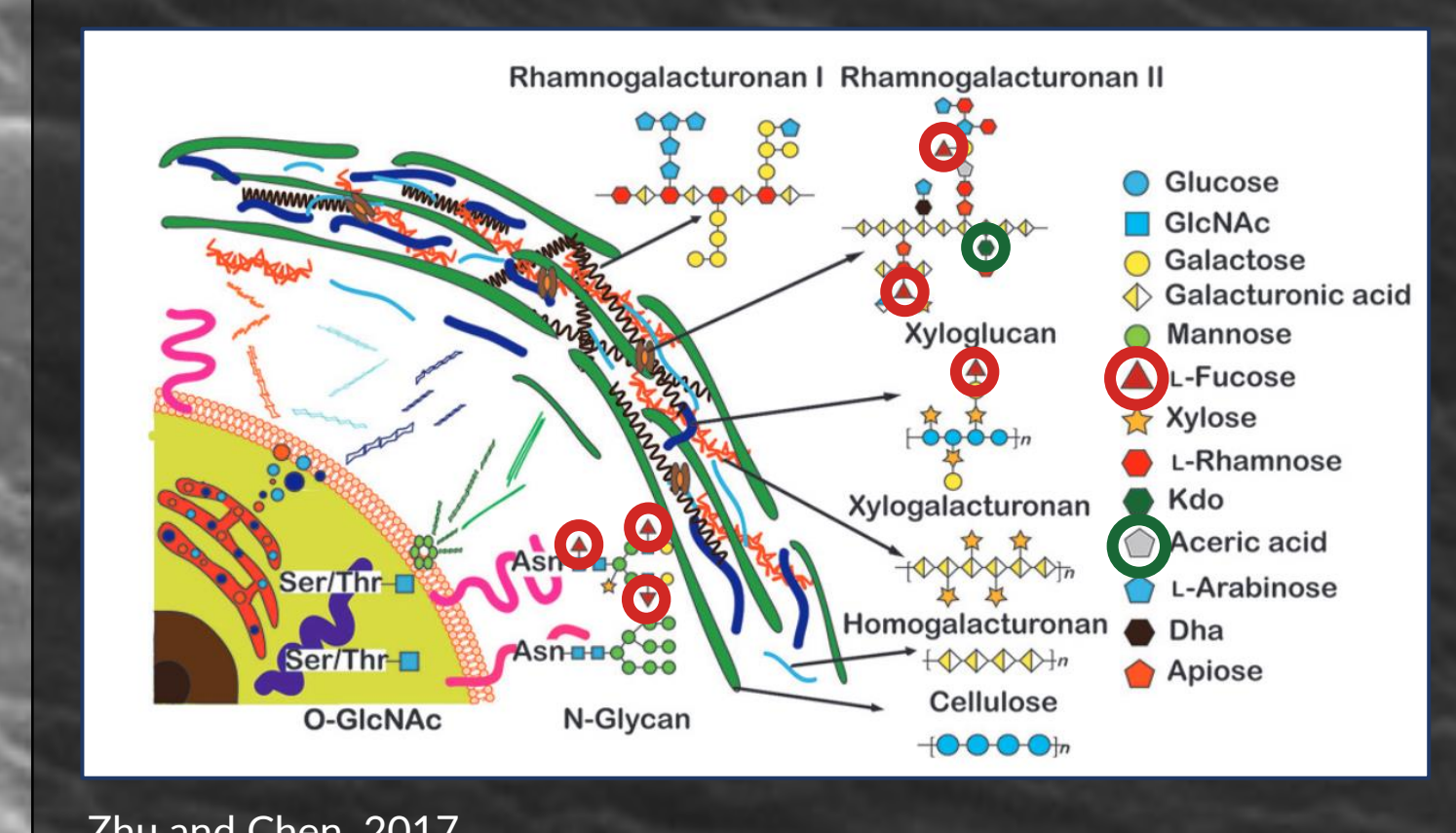
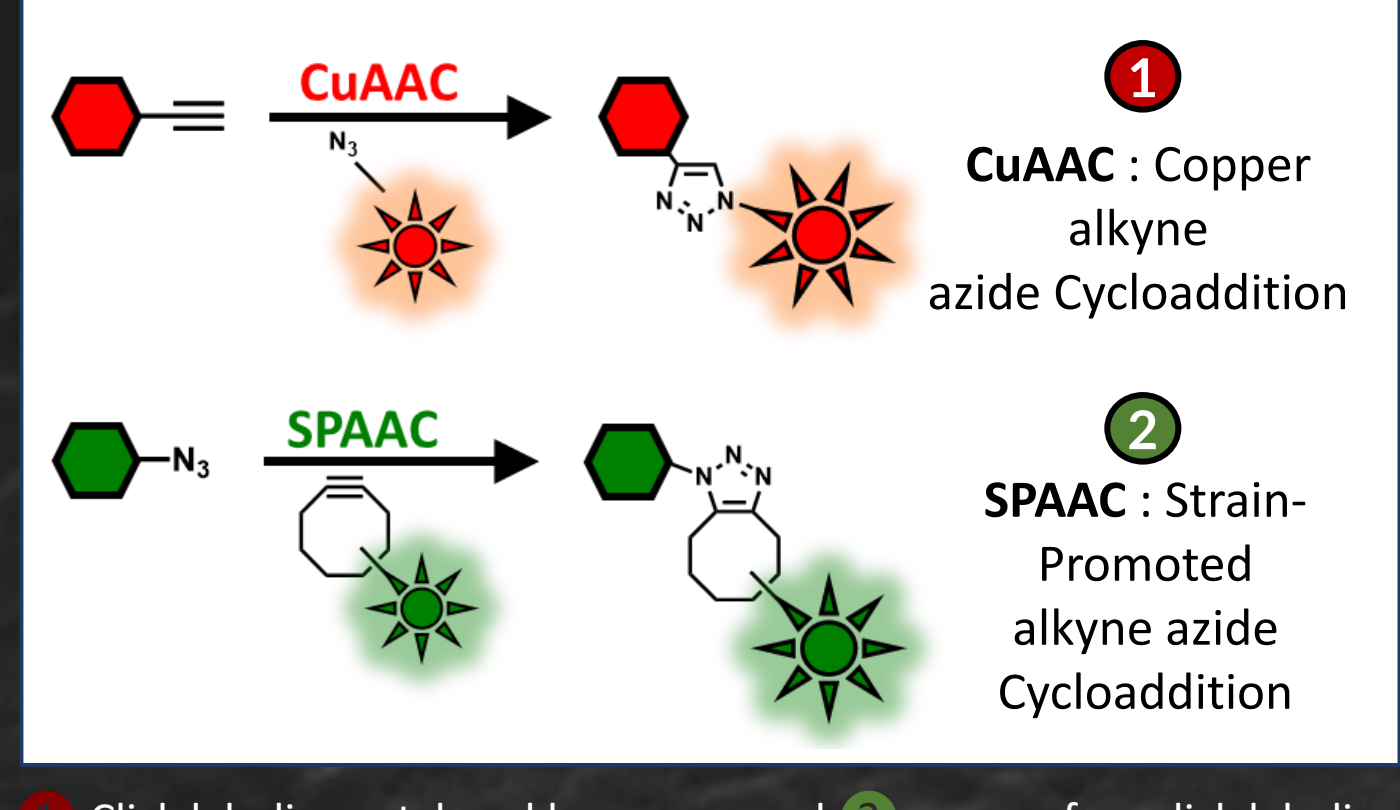
Rydahl et al., 2018

An alternative... The click chemistry

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

Incorporation of Kdo-N₃ (200 μM)

Incorporation of Fucose-N₃ (50 μM)



How does it work?

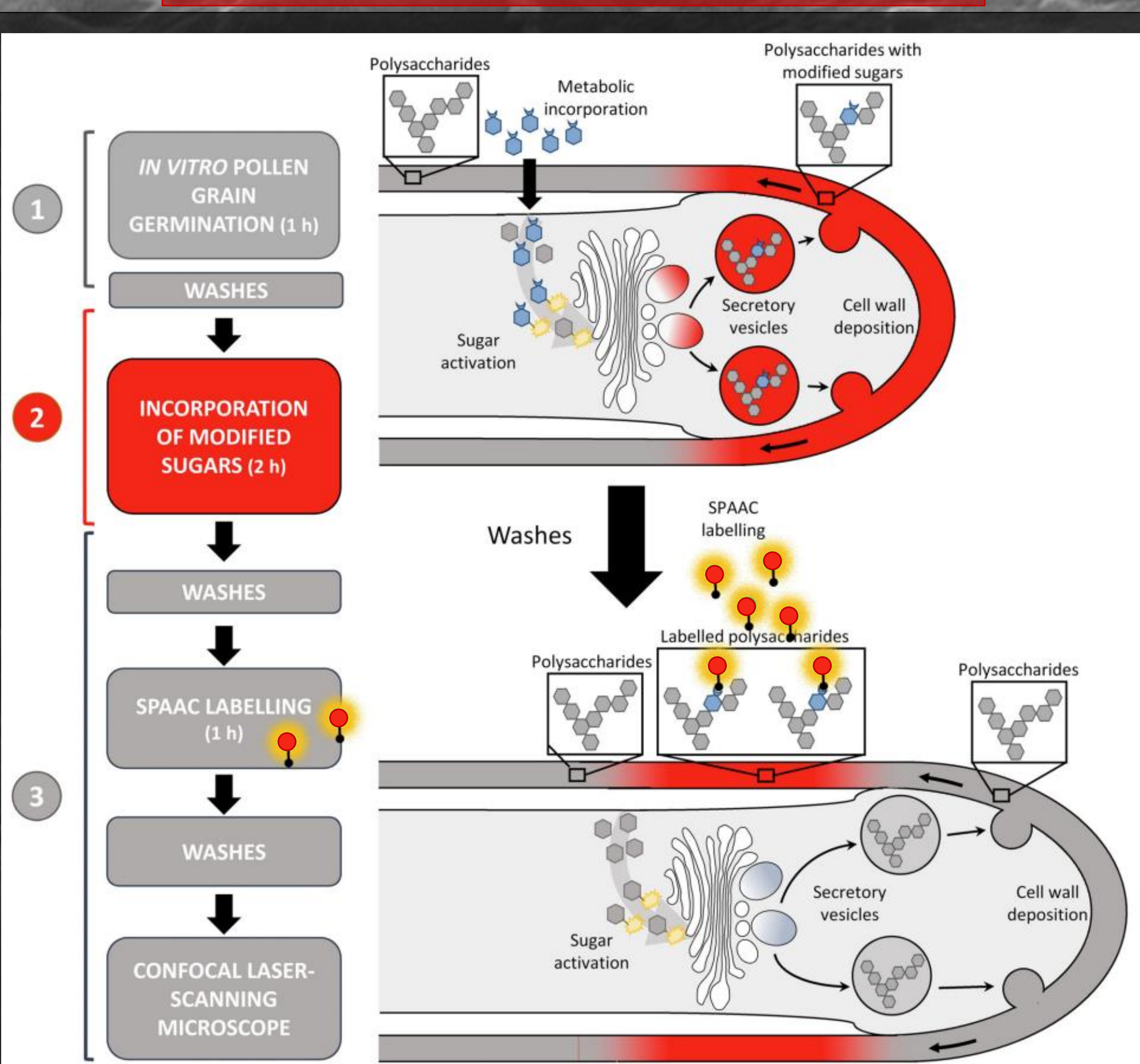
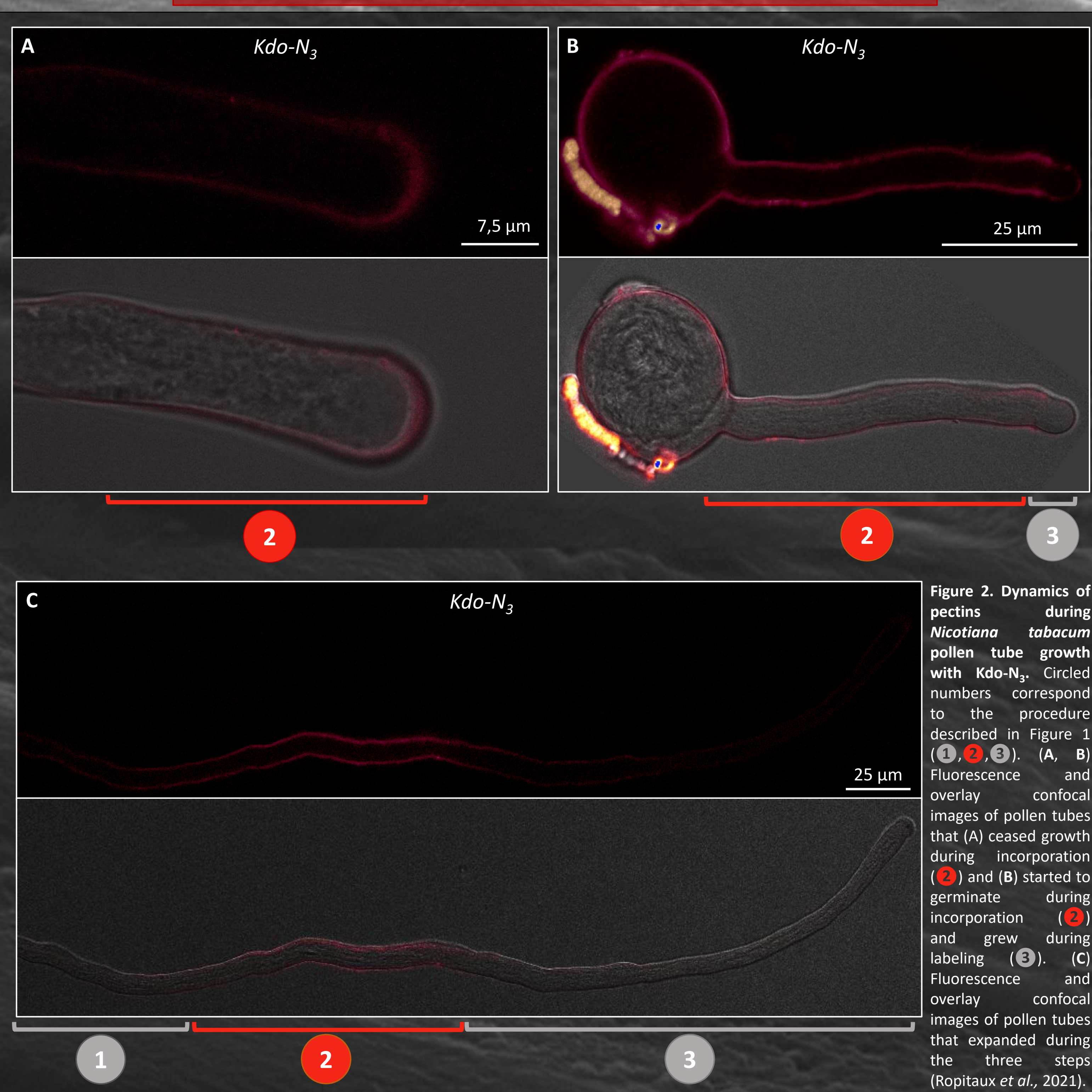
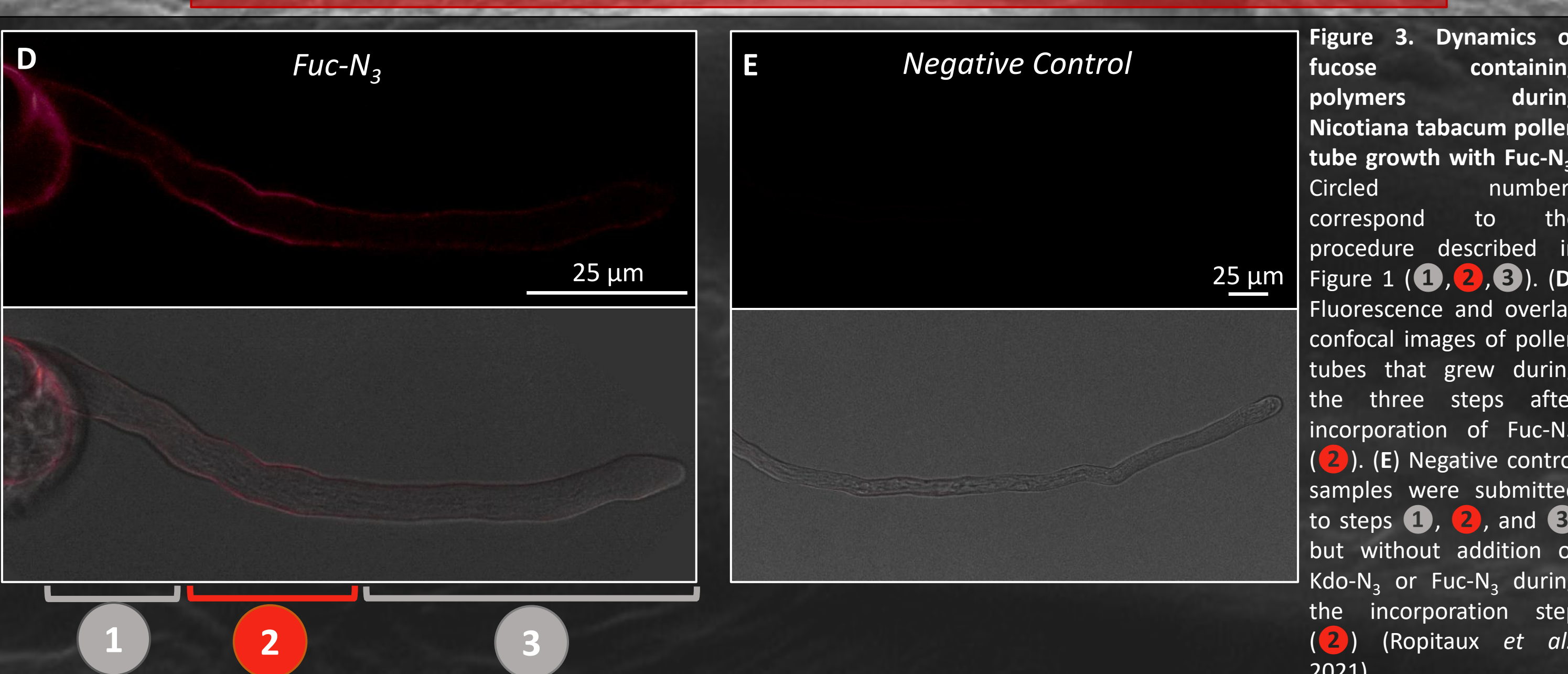


Figure 1. Protocol for the metabolic SPAAC labeling of living and growing pollen tubes. Schematic representation of the experimental procedure for pollen tube growth, metabolic incorporation of modified sugars, and labeling. Key stages are numbered: 1 *in vitro* pollen grain germination for 1 h culture medium at 22°C in darkness; 2 incorporation of Kdo-N₃ (200 μM) or Fuc-N₃ (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).

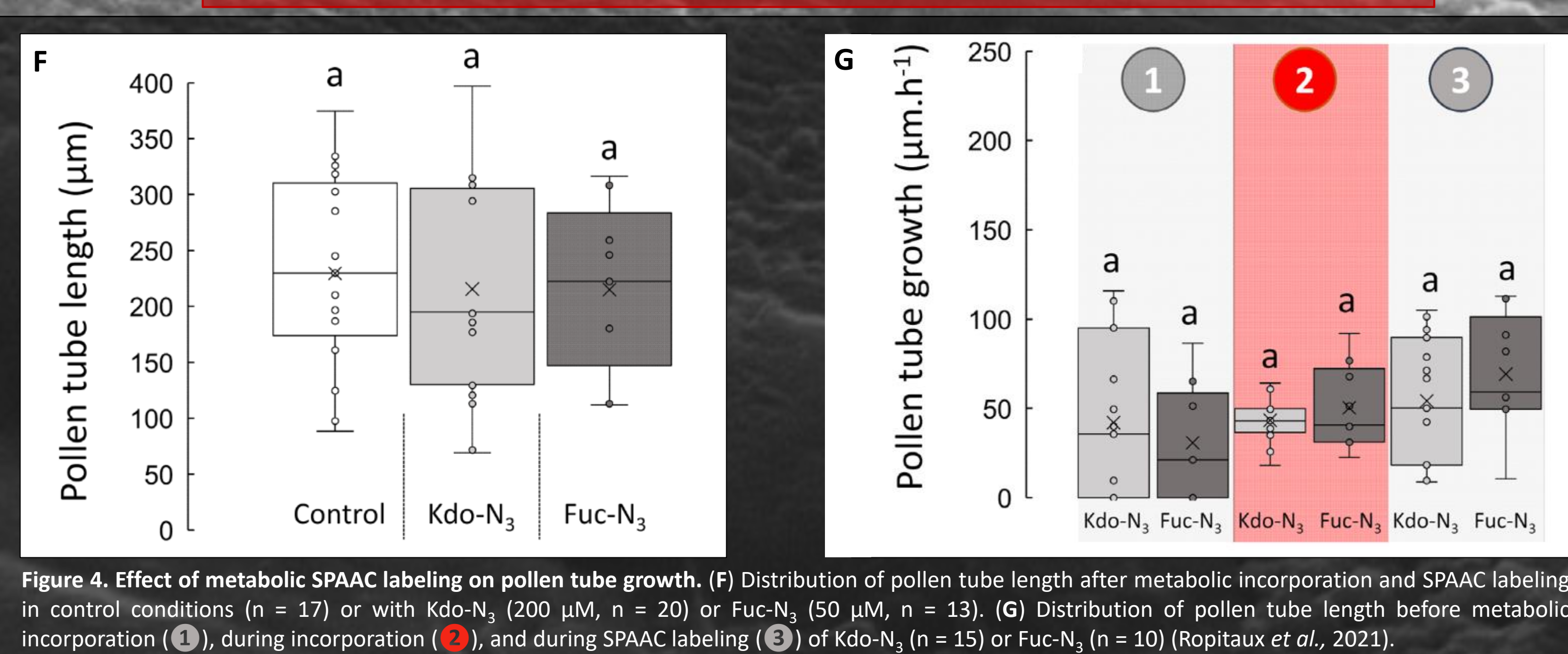
Kdo-N₃ incorporation



Fuc-N₃ 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 ←

