A new vision on plant growth reviled by super resolution microsocpy of plant cell wall

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INRAE
la science pour la vie, l'humain, la terre

How do plants grow and generate their great diversity of shapes?

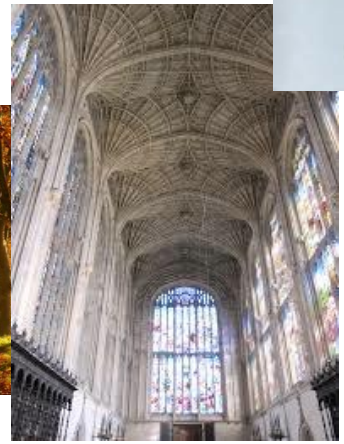
Beauty

Classic

Renaissance

Art Deco

Gothic

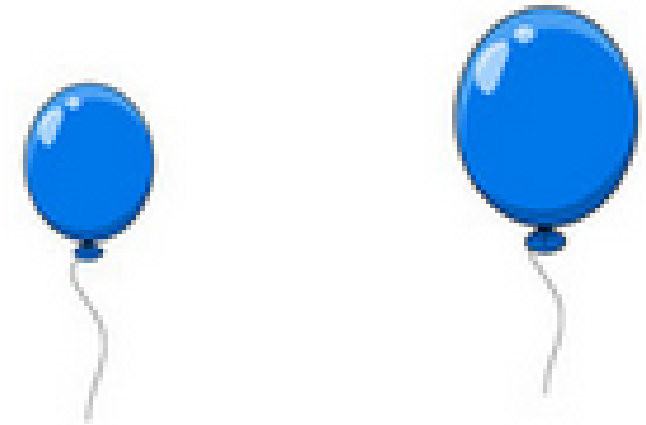


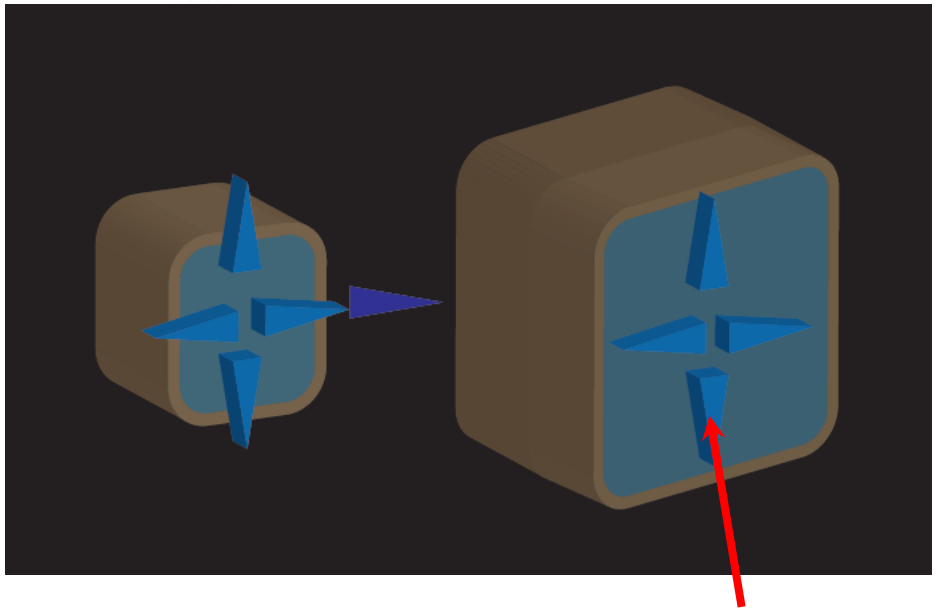
What is the subcellular mechanism of shape formation ?

Beast

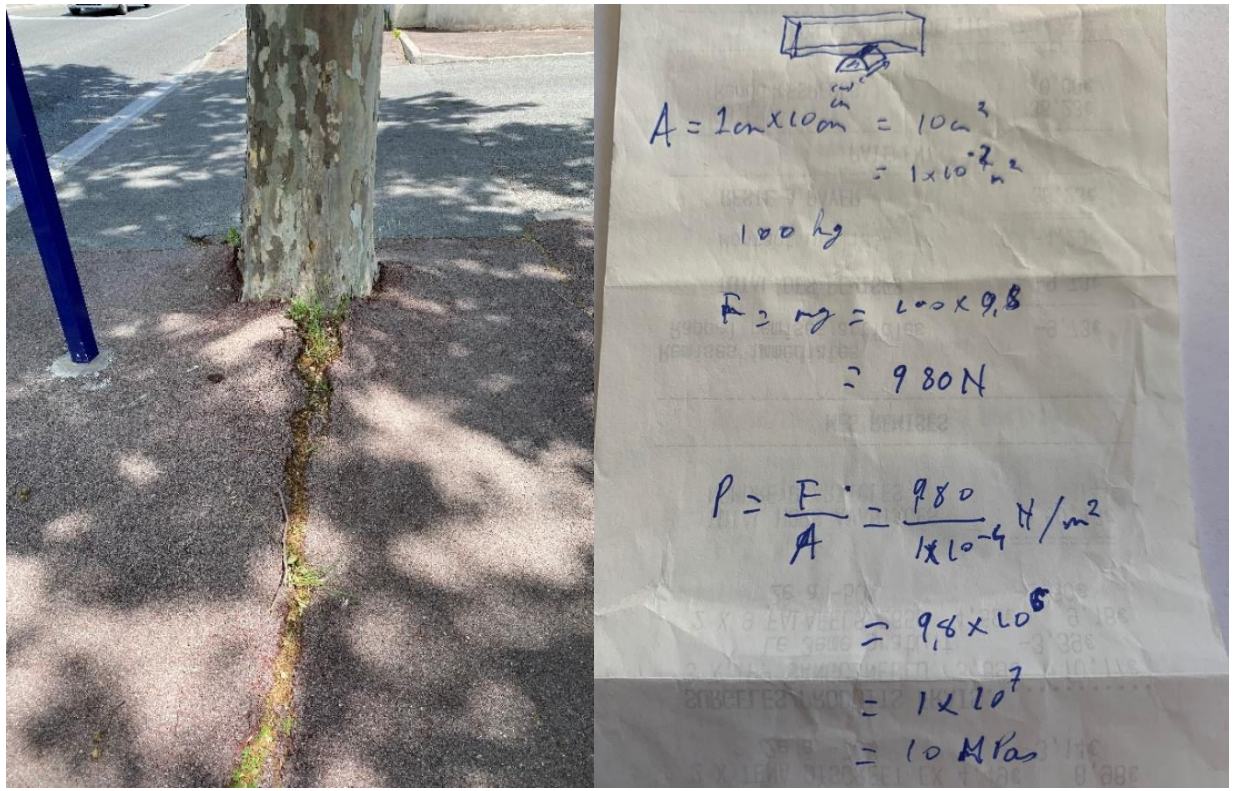
What is the force driving plant growth?

Current paradigm – plant growth is fueled by turgor pressure



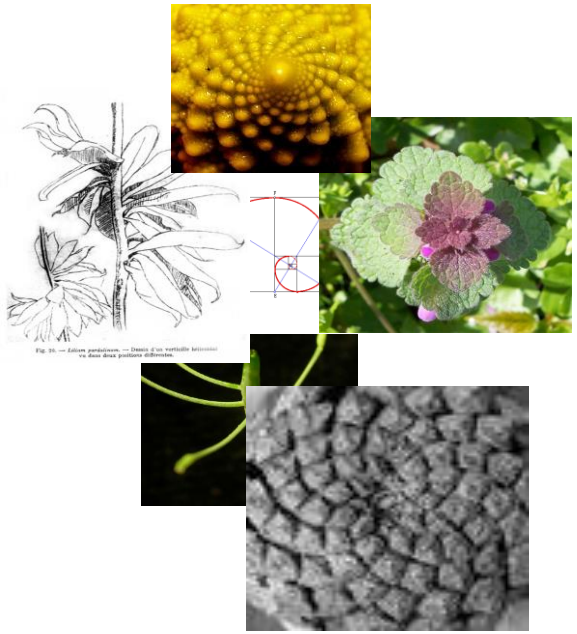


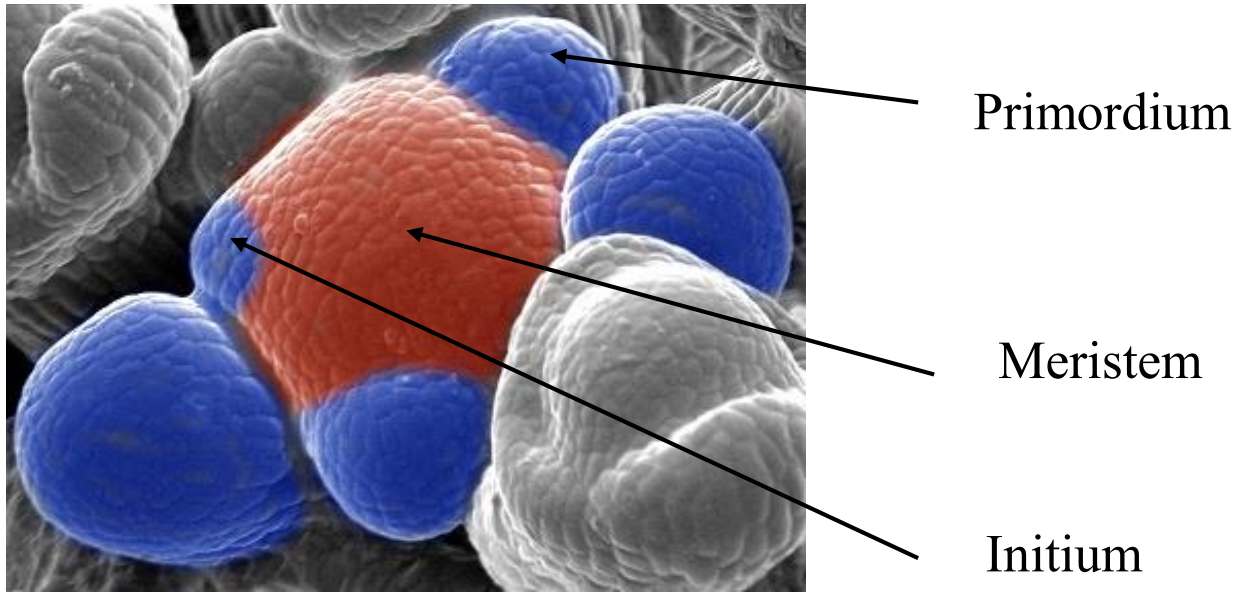
Turgor pressure



Growth rate not proportional to turgor (Zhu, Plant Phys. 1992 Proseus, Plant, Cell and Environment 2008)

Apical meristem generates the lateral organs

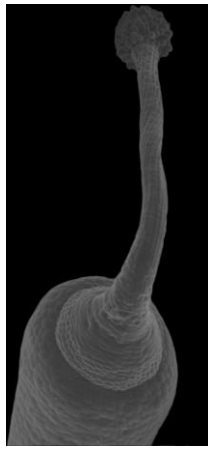


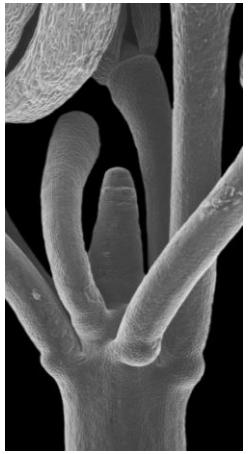


The de-methylesterification of Pectins (Homogalacturonanes) is necessery and sufficient for lateral organe formation

Inhibition PME

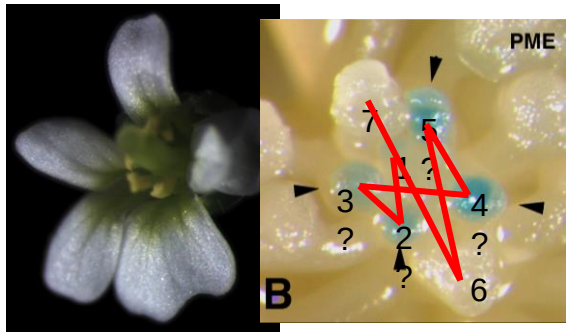
Formation des organes bloquée





WT

Activation PME



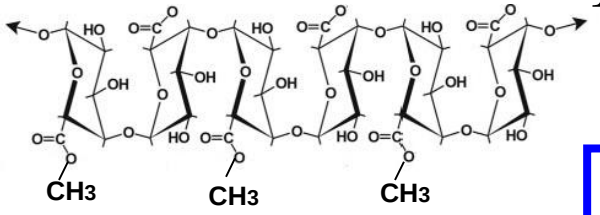
Organes surnuméraires

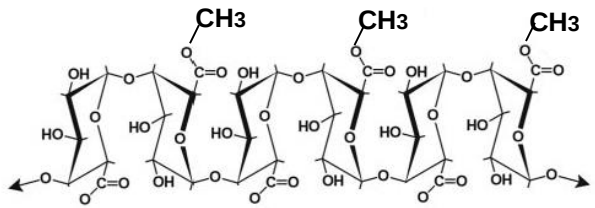
PMEI

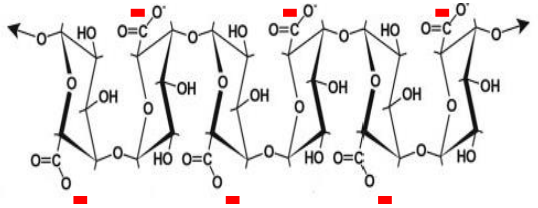
⊥

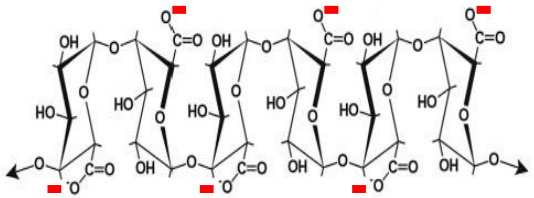
PME

→







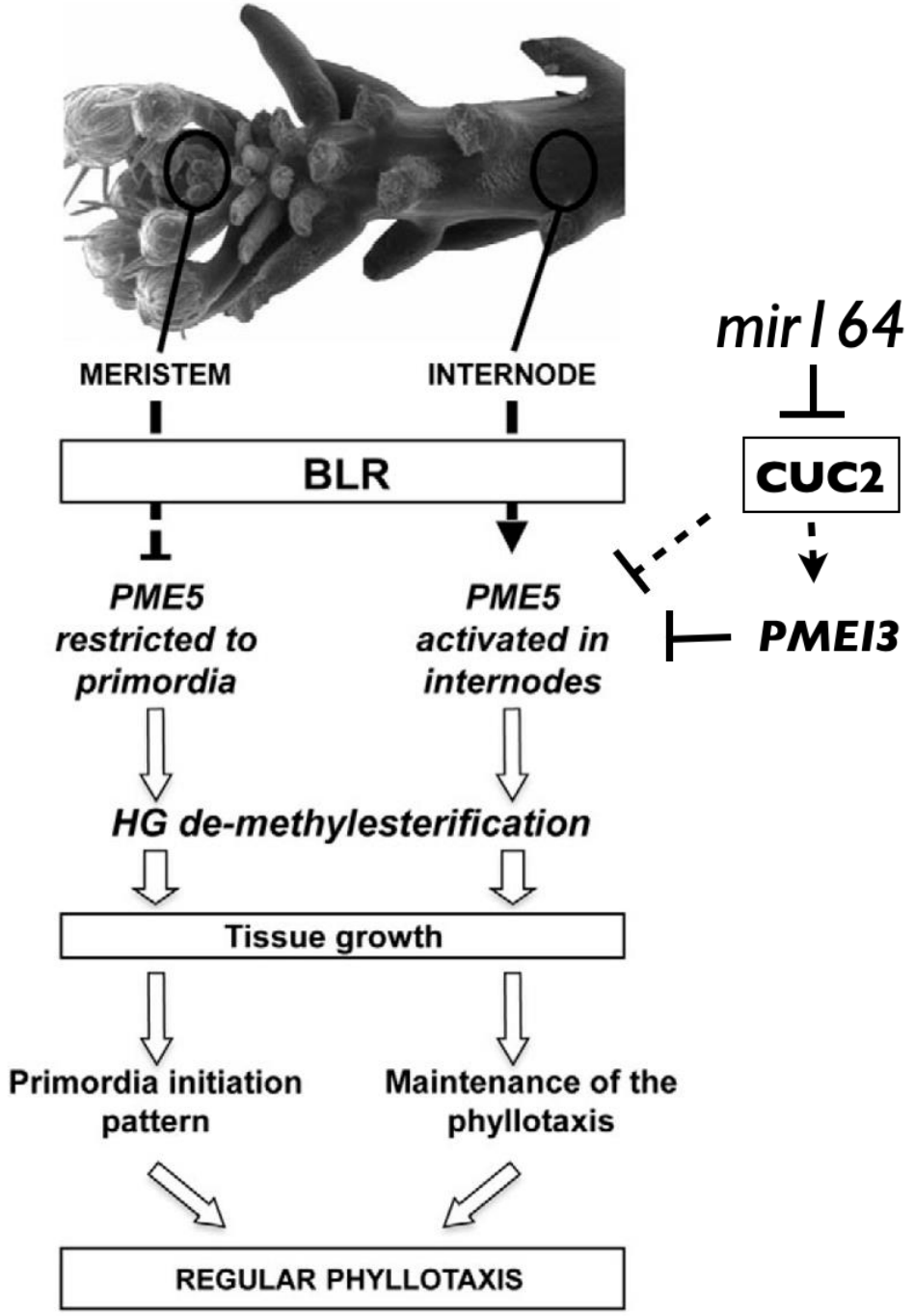


Peaucelle et al, (2008)

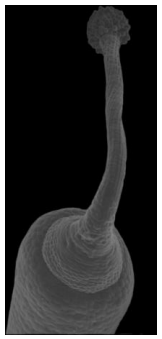
Transcription factor regulation of the PME activity

Peaucelle et all 2008-2011

Similar regulation in maize
Jia Yu, Li Pu and all 2023



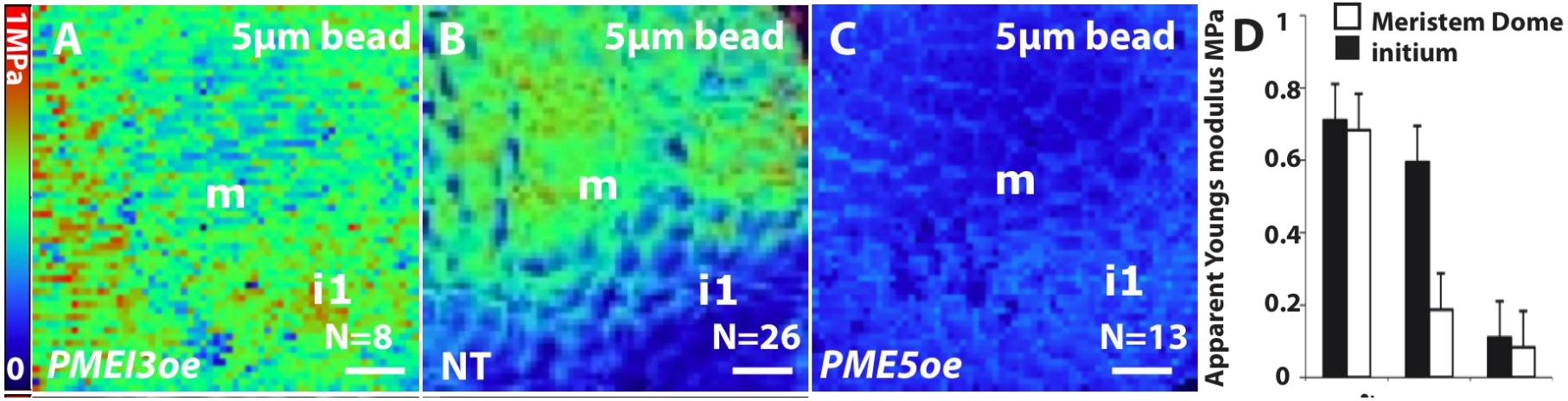
The de-methylesterification of HG is sufficient for laterale organe formation related cell wall losening



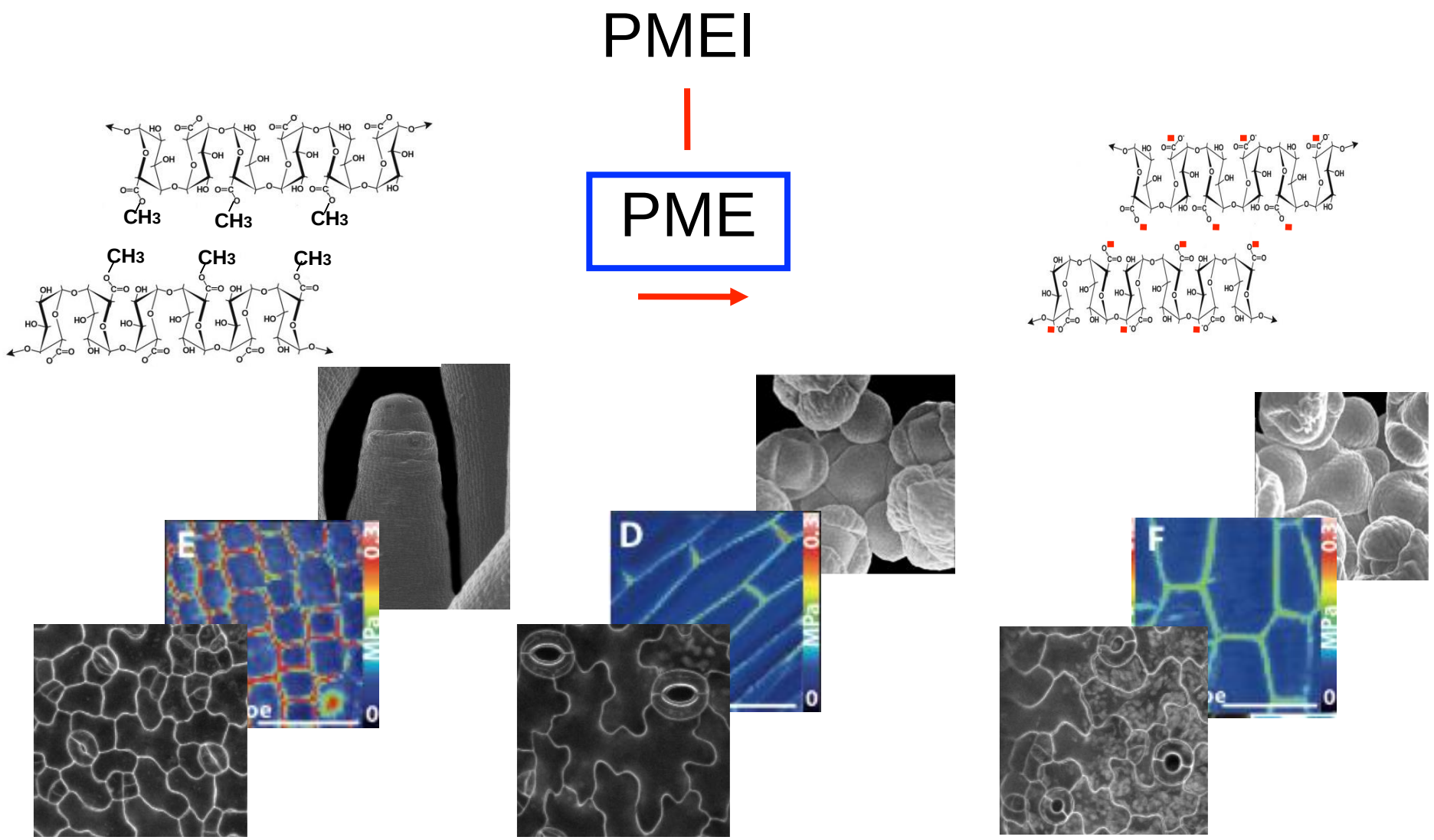
Inhibition des PME

WT

Activation des PME



The de-methylesterification of HG is also implicated in tissue elongation and cell morphogenesis



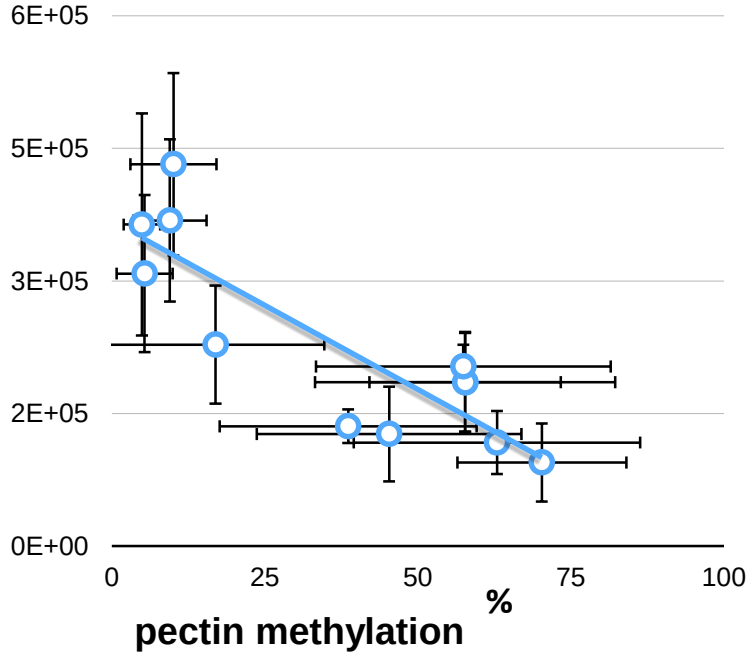
The correlation between pectin methylation, elasticity and growth, but we could not demonstrate any causality (epifenomenon?)

Ea : elasticity

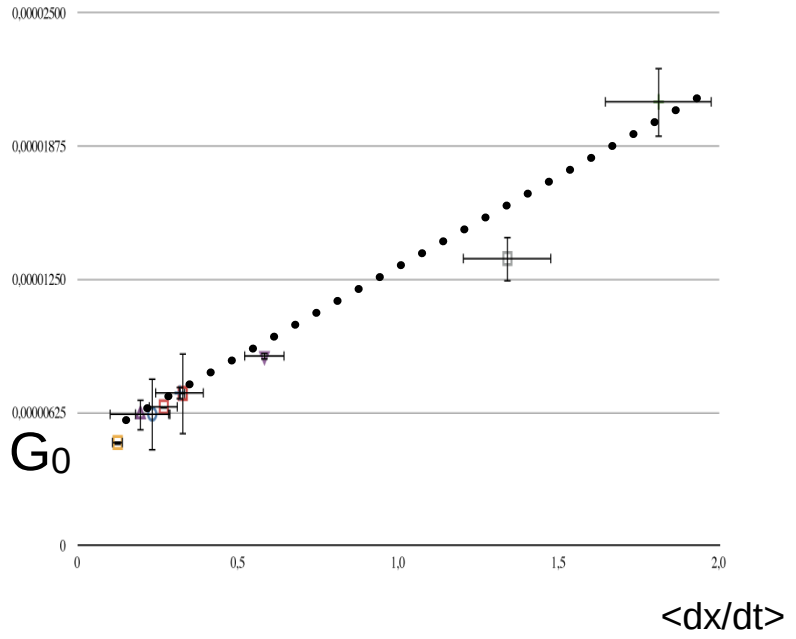
P : pressure

$$Growth = R\frac{P}{Ea} - G_0$$

Ea : elasticity pas

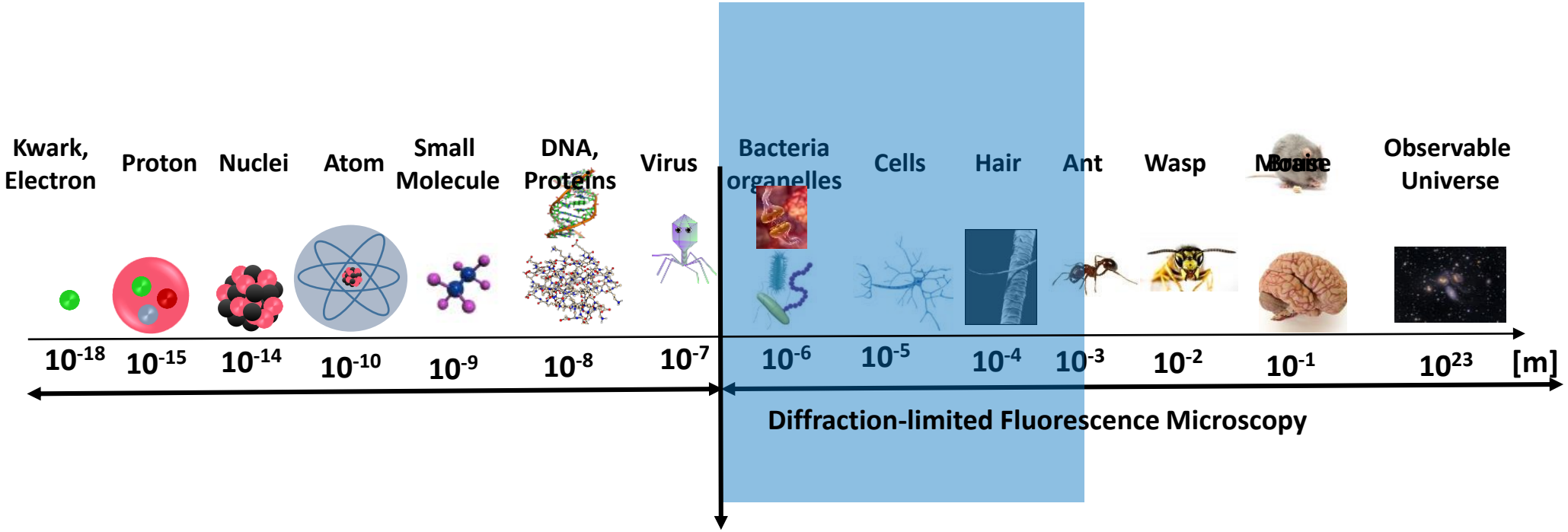


<P/Ea>



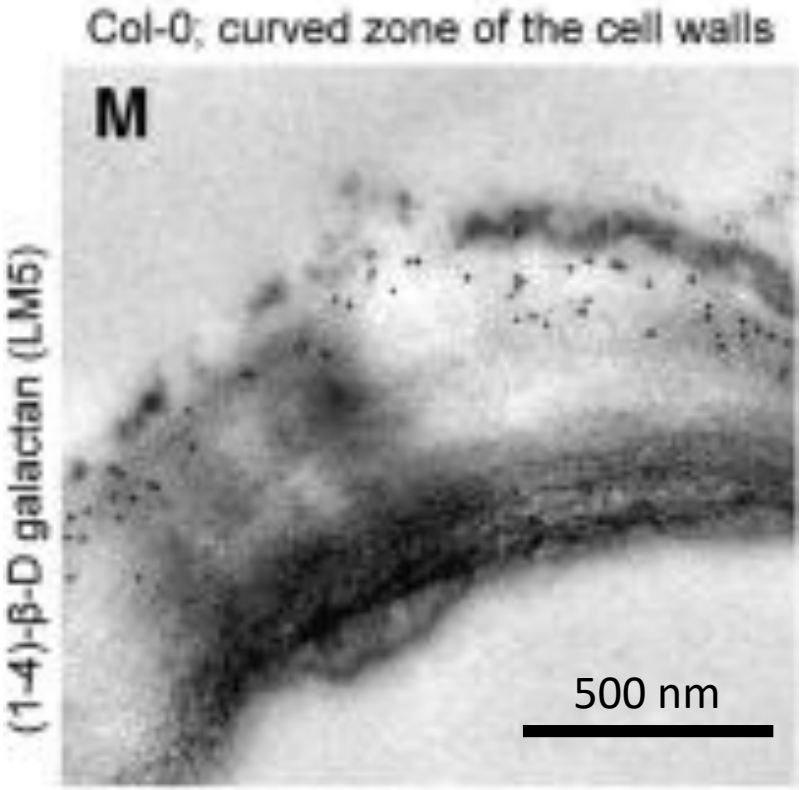
Data gathered on the hypocotyl following different treatment

Length scales and resolution of fluorescence microscope



Diffraction Limit
(Rayleigh Criterion) ~250 nm

Gold-Immuno adds biochemical contrast to Electron Microscopy



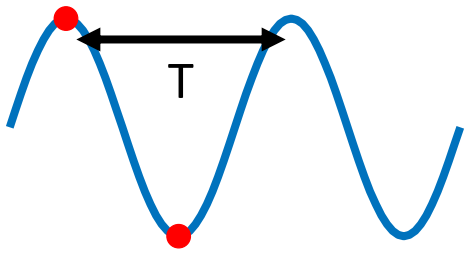
Majda 2017

Here around 100 particles/um^2
A = 200 nm (confocal resolution)

Rayleigh Criterion
$d_{Ray} < 1$ nm

$$d_{Ray} = \frac{\lambda}{2NA}$$

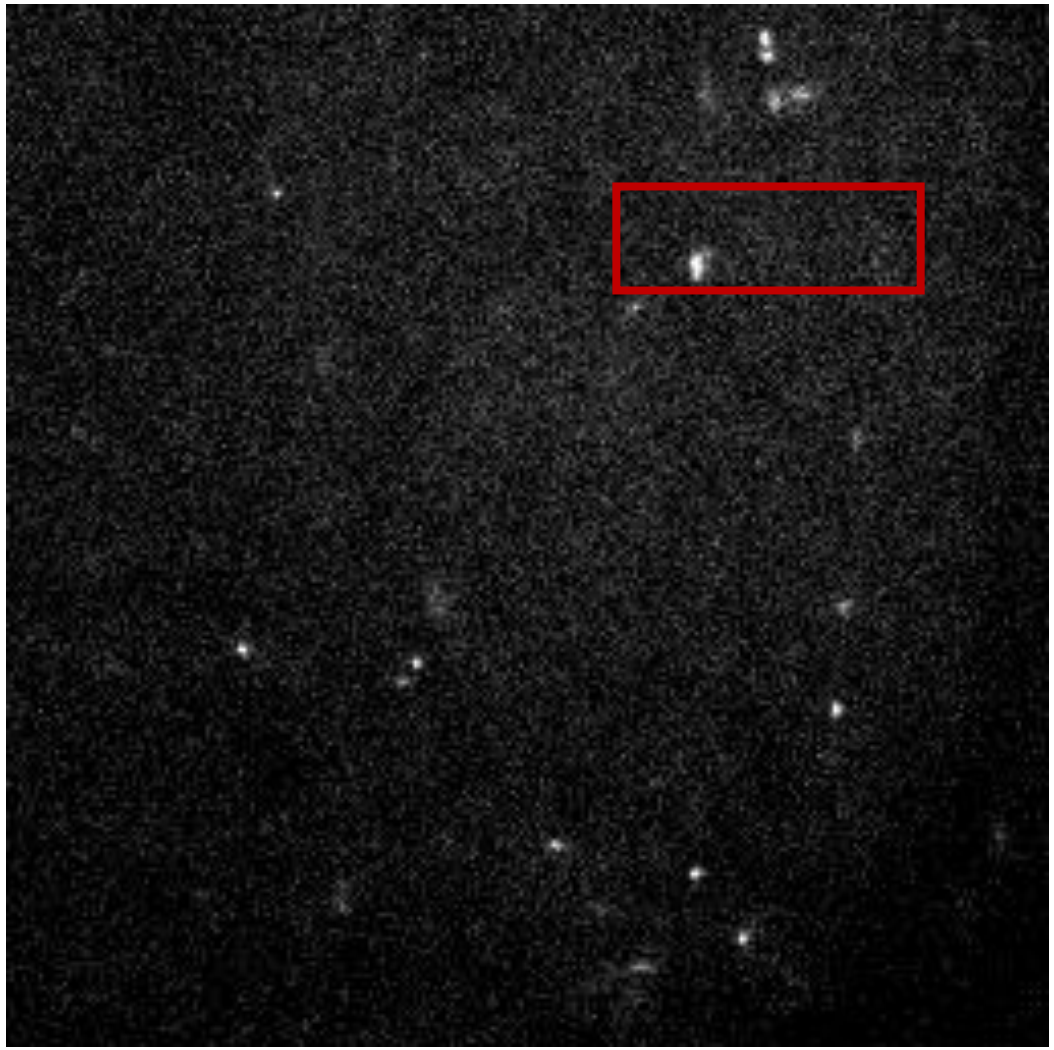
Nyquist–Shannon sampling theorem
two data points per resolution unit



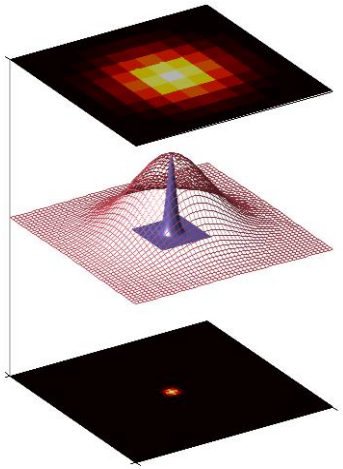
Nyquist Molecular Density (NMD) ≈ $(2/a)^D$
For a = 20 nm:
1 molecule every 10 nm,
NMD = 10,000 mol/um^2 in 2D

dSTORM acquisition and data analysis

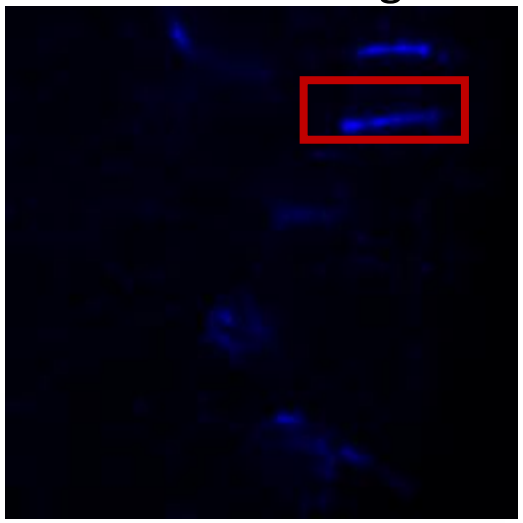
dSTORM single molecule raw image stack



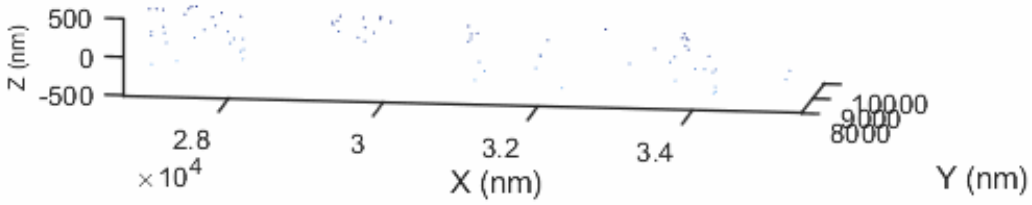
Single molecule localization



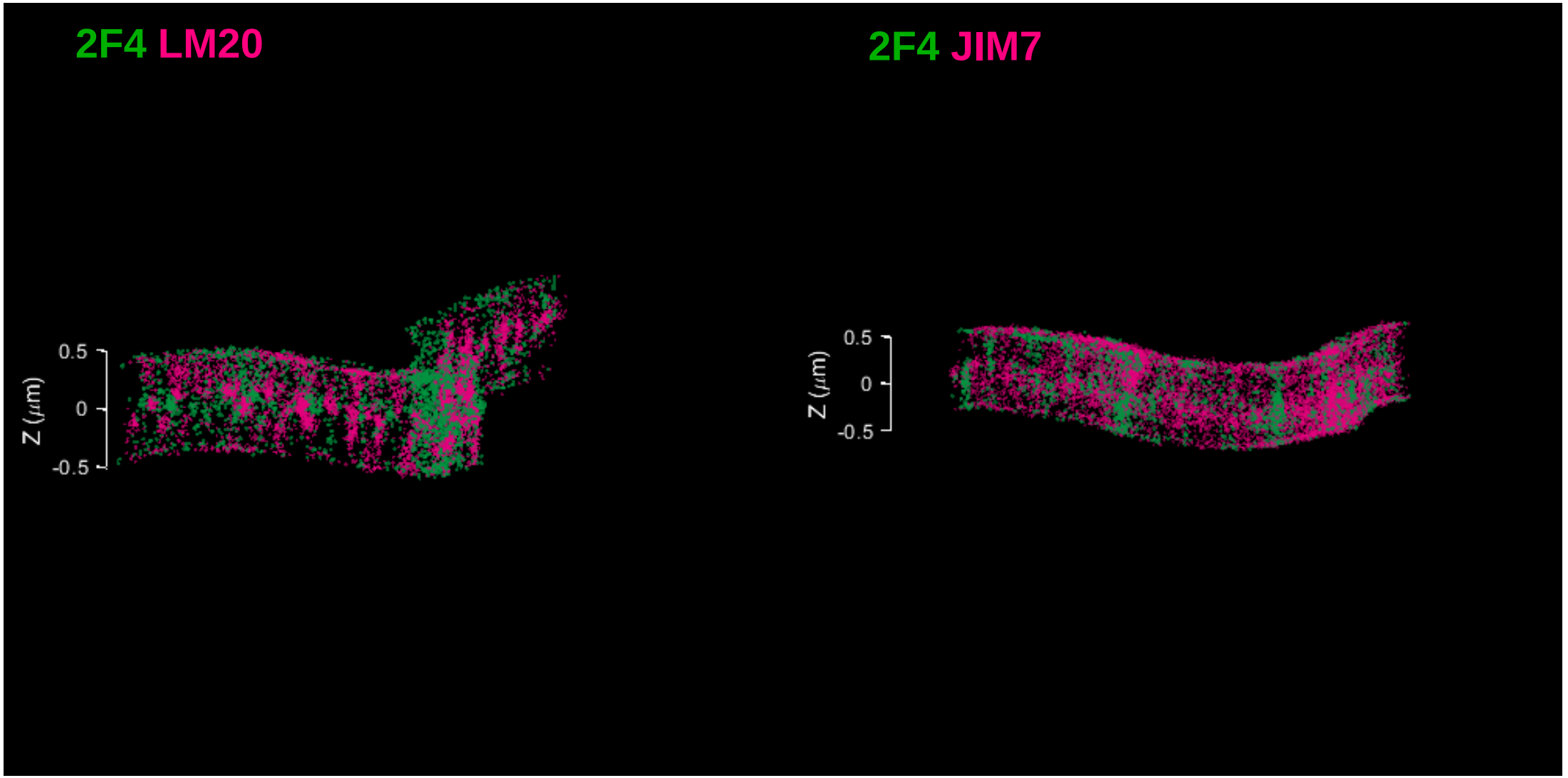
Fluorescence image



3D dSTORM analysed data scatter plot

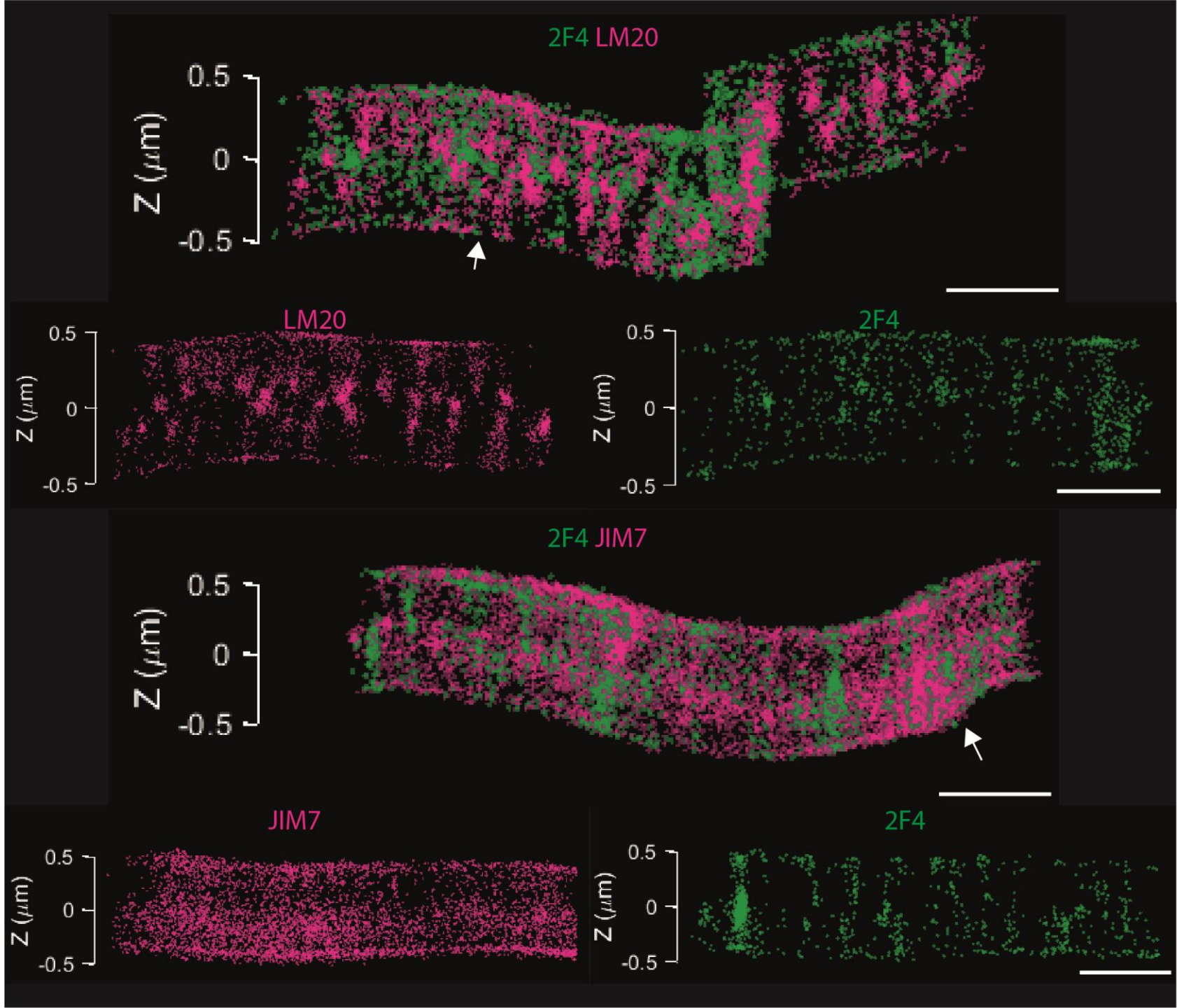


Different HG antibody present different structure in the cell wall



Haas 2020

HG presents at least 3 forms in the primary cell wall



Haas 2021

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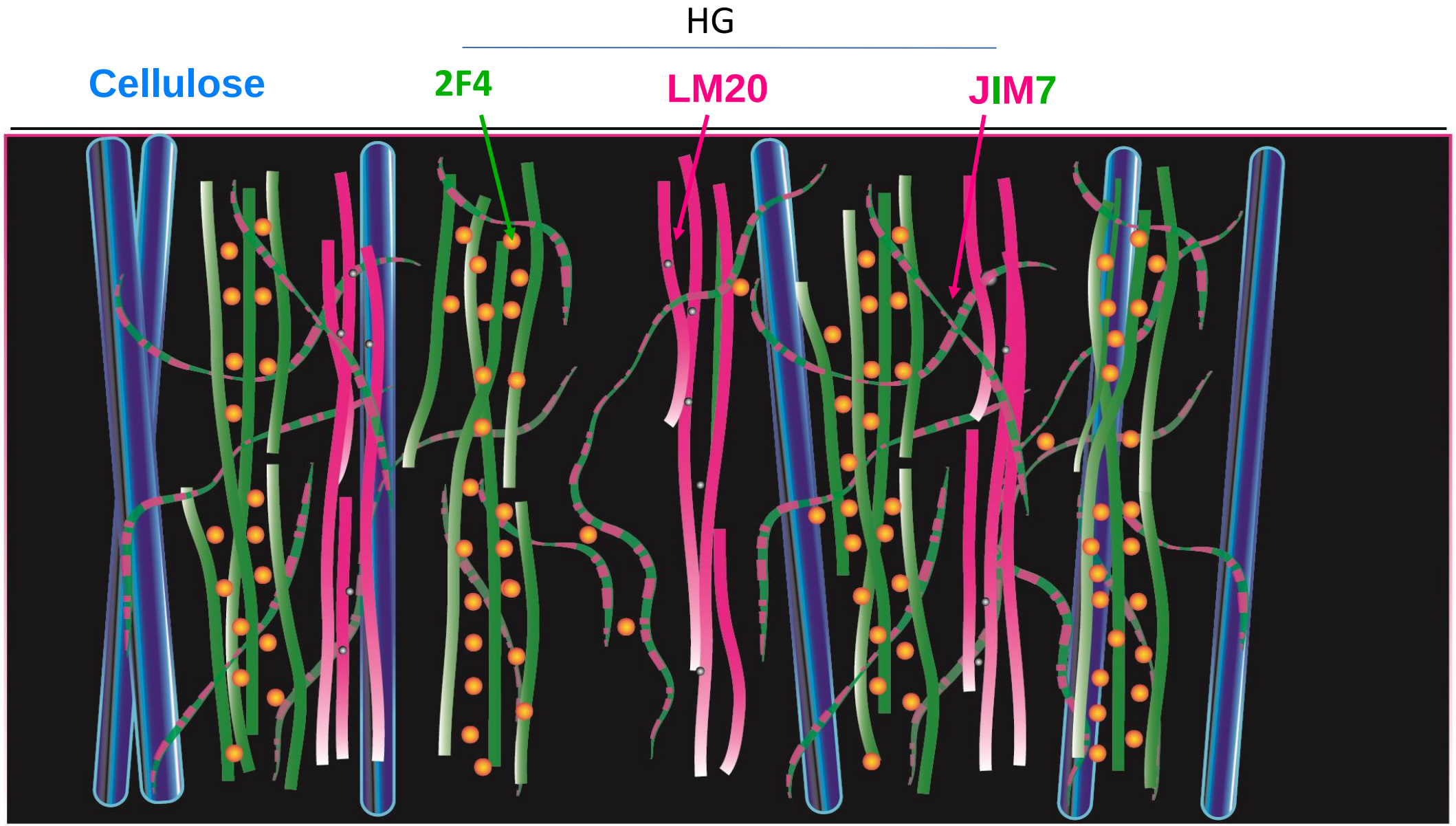
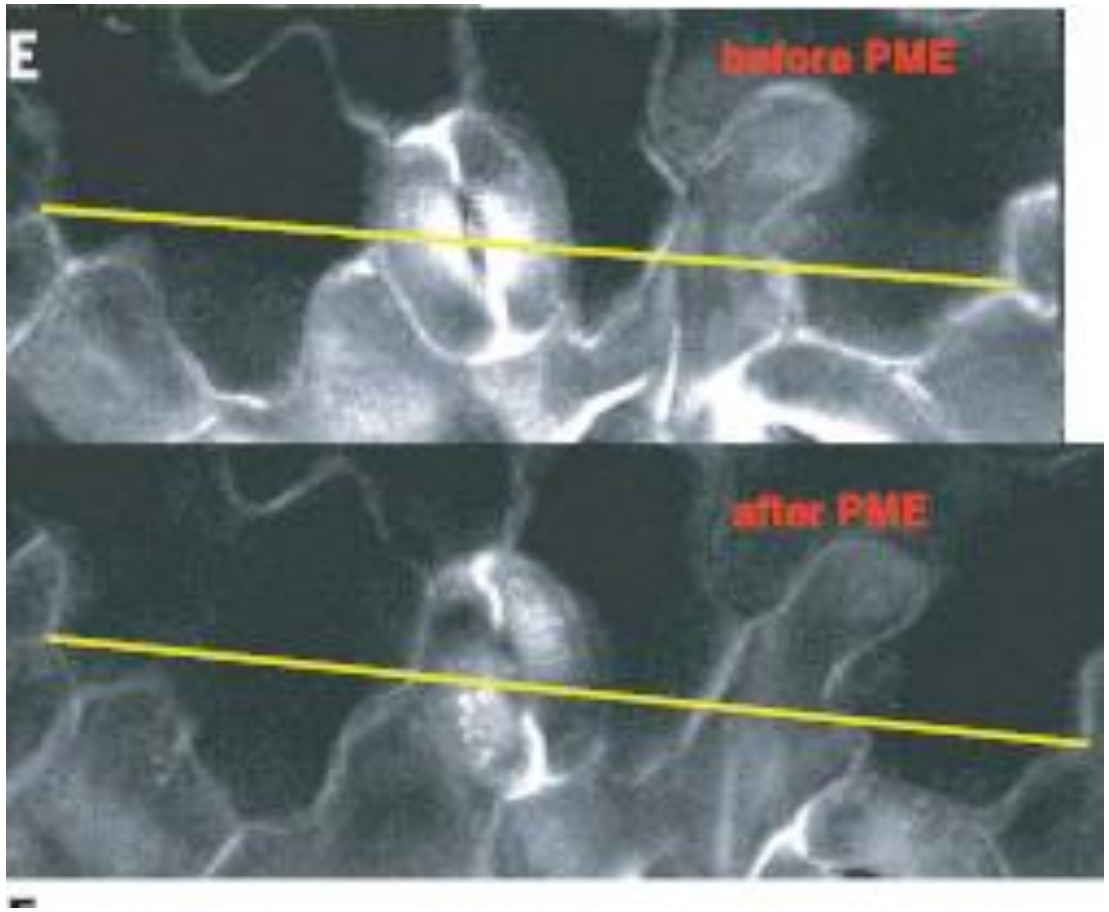
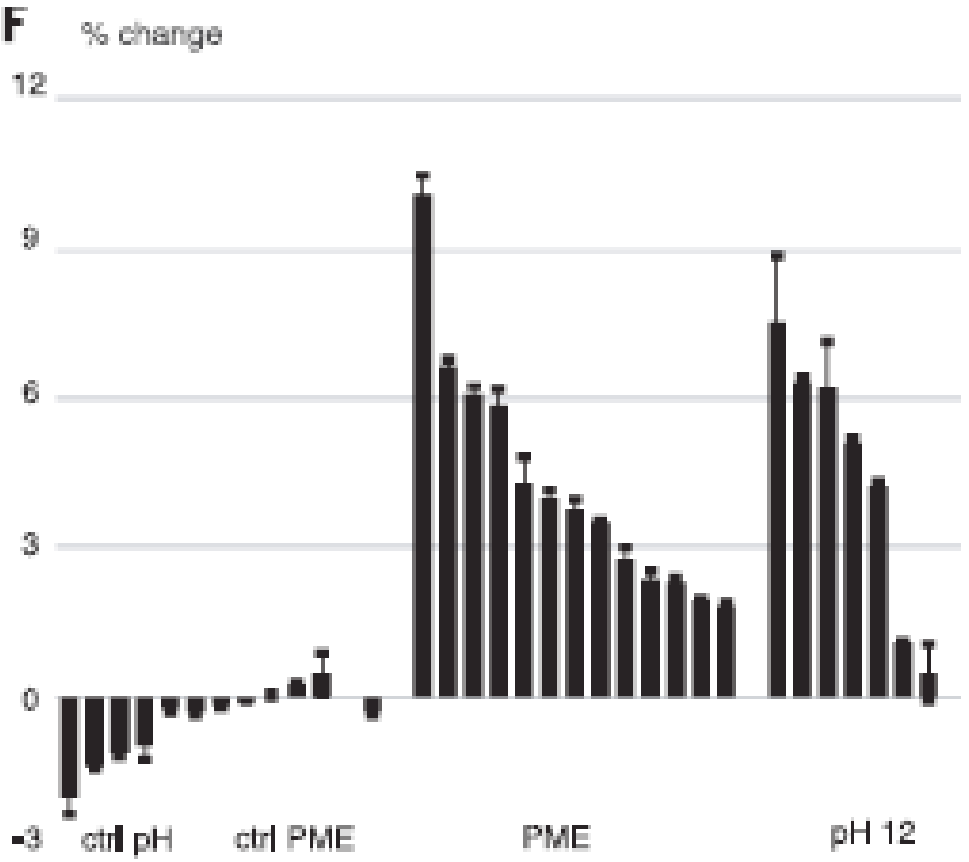


Haas 2021

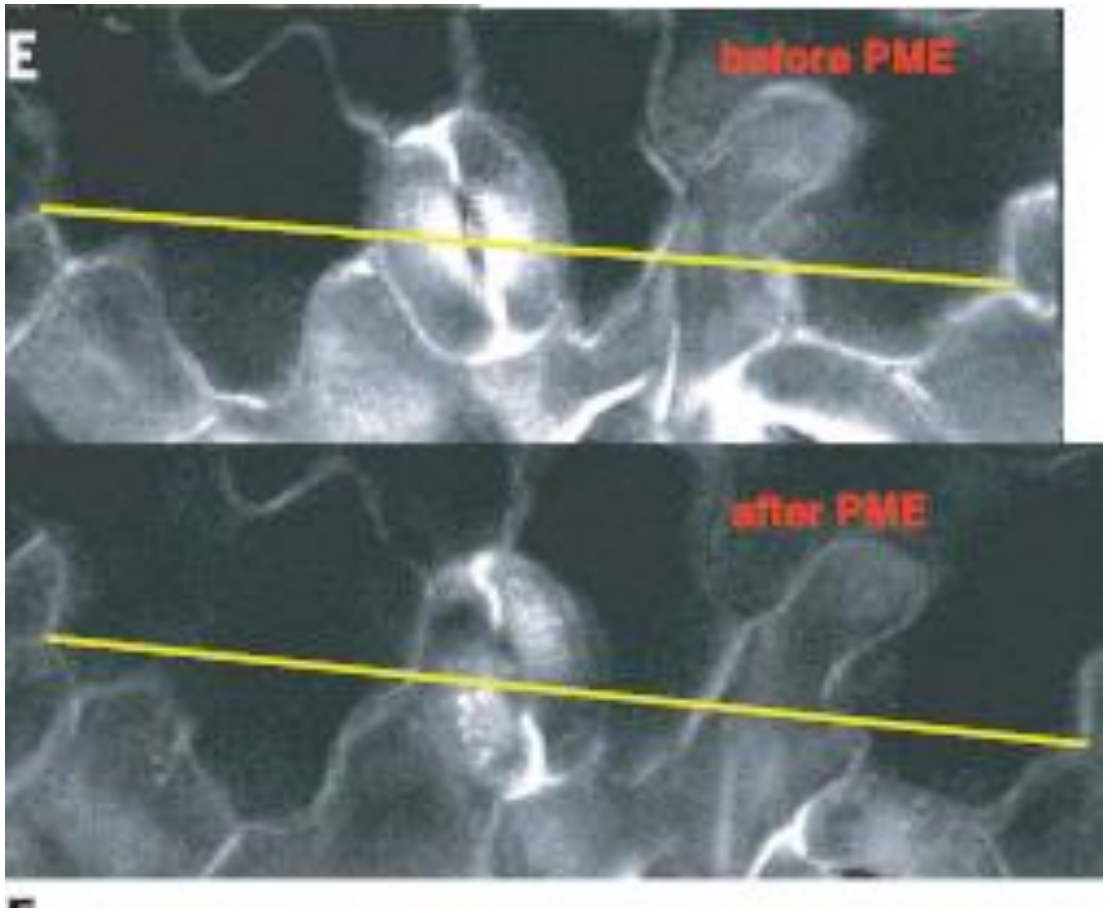
HG demethylation is sufficient for tissue expansion in the absence of turgor



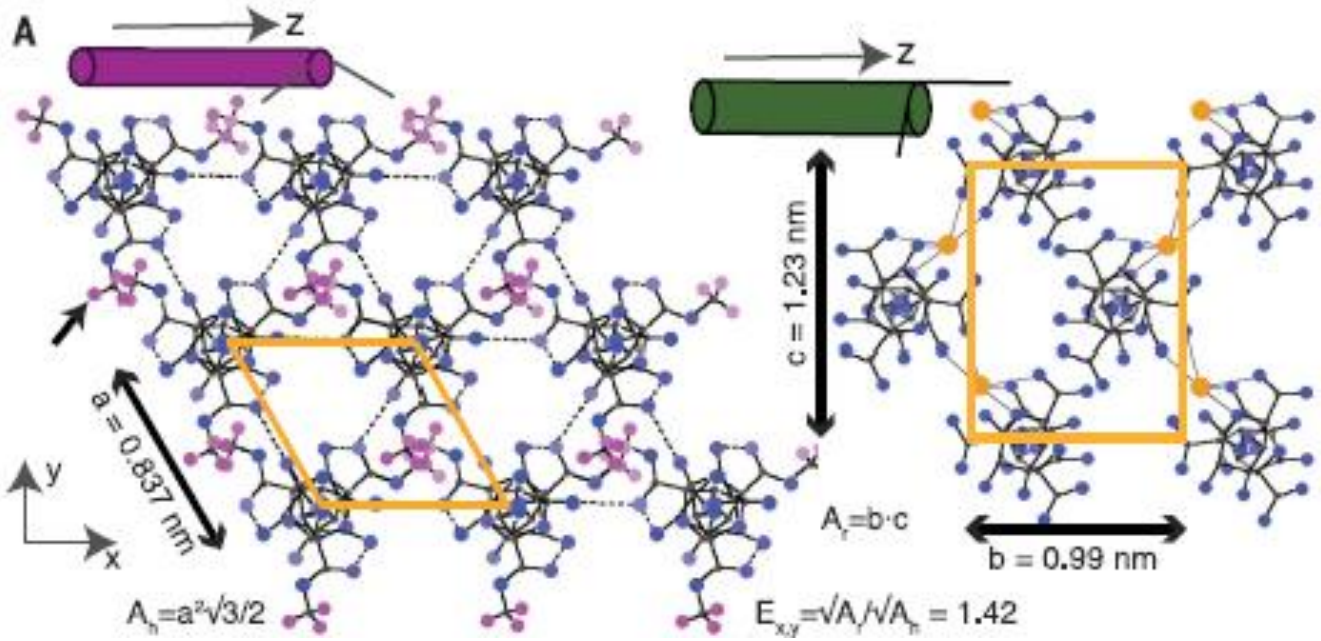


Haas 2020

HG demethylation-mediated growth matches the predicted expansion of the crystalline HG observed *in vitro*



Haas 2020

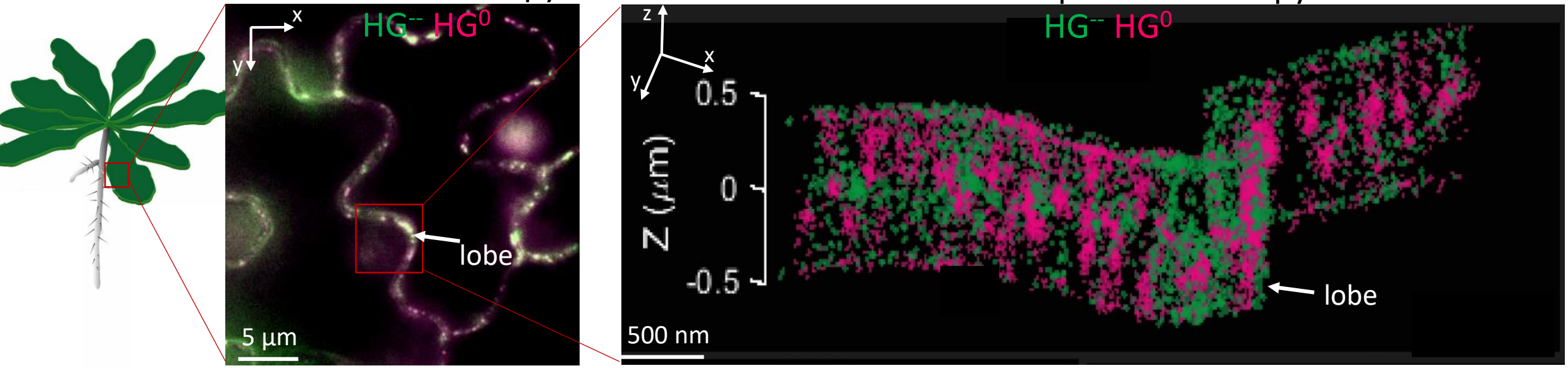


Inspired from Walkinshaw 1981

Morphogenesis through expansion of oriented nanofilaments

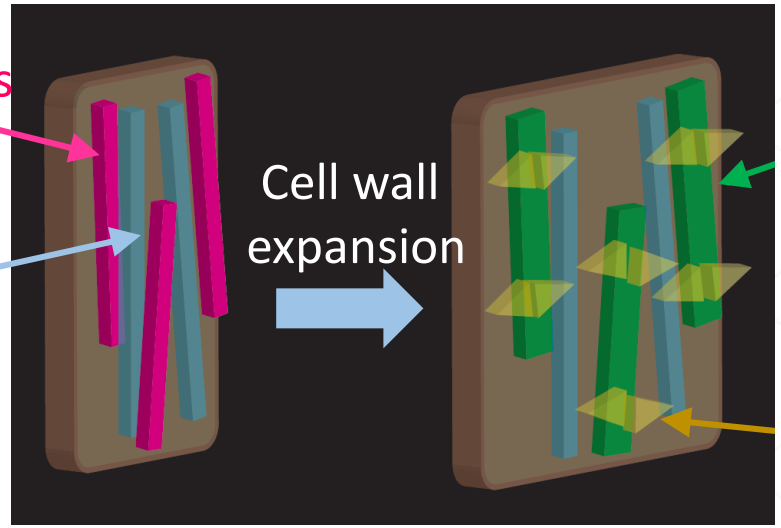
Classical microscopy

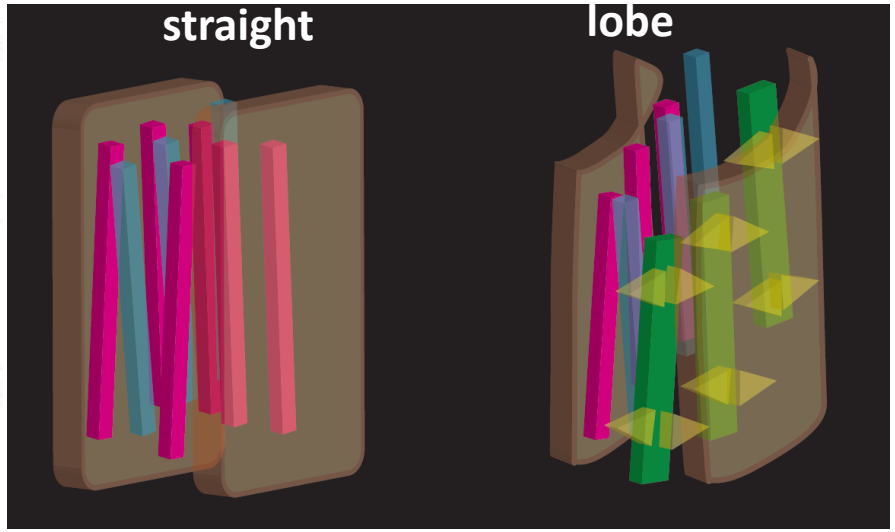
3D dSTORM optical nanoscopy



pectin nanofilaments

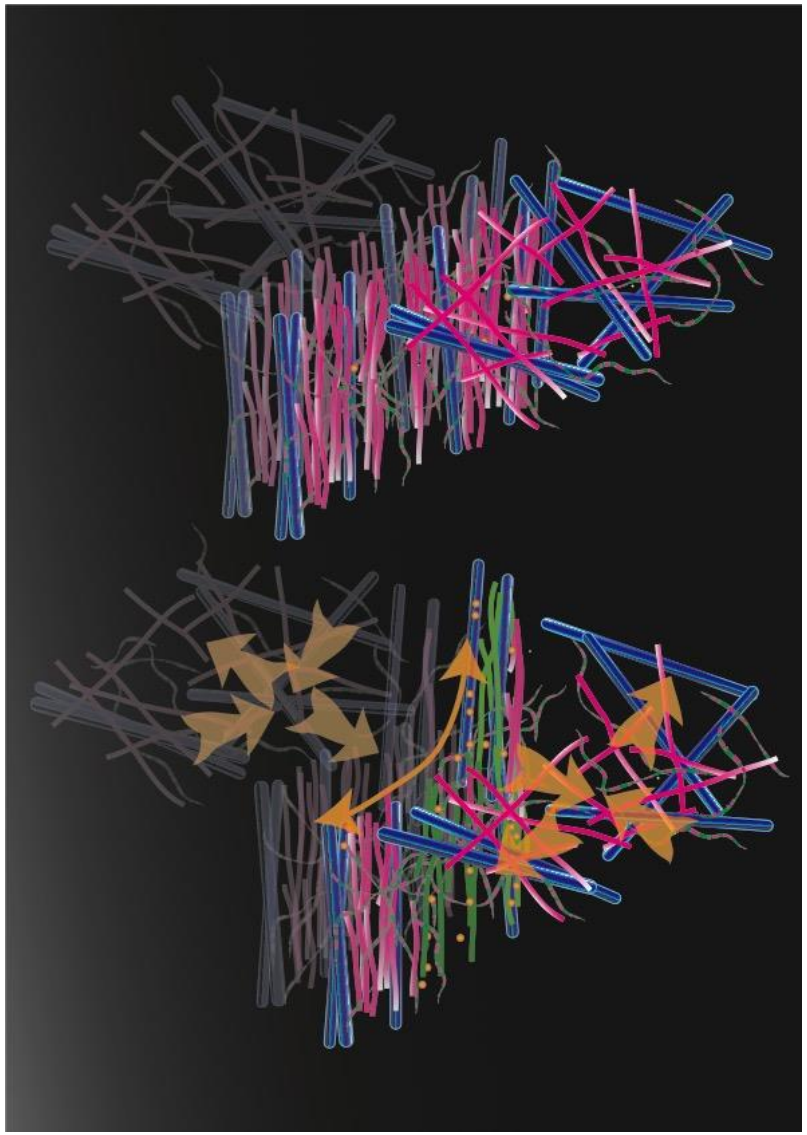
cellulose microfibrils





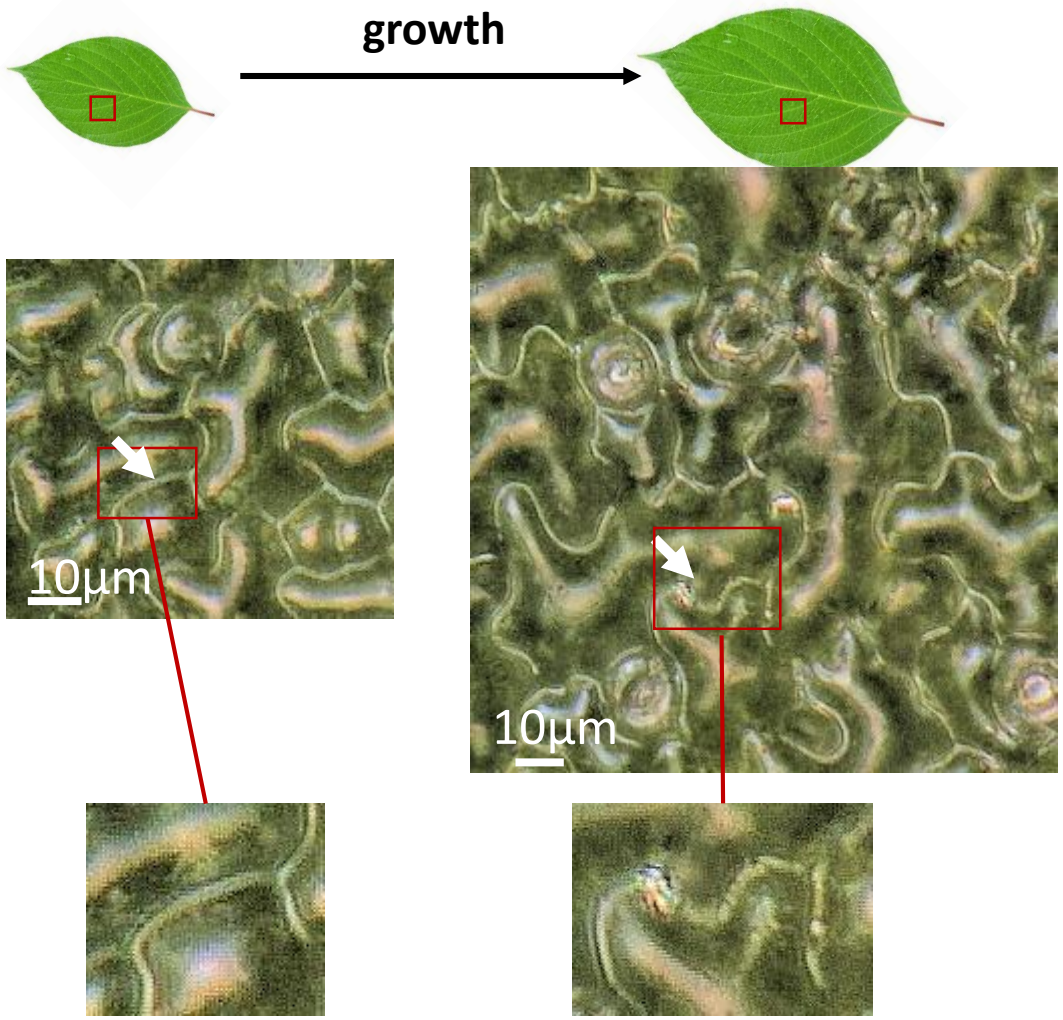
Haas Science 2020, Haas Cell surface 2021

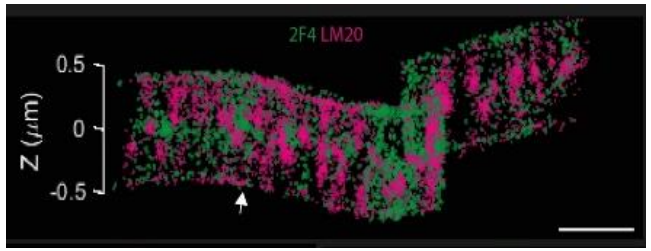
Growth induced tension



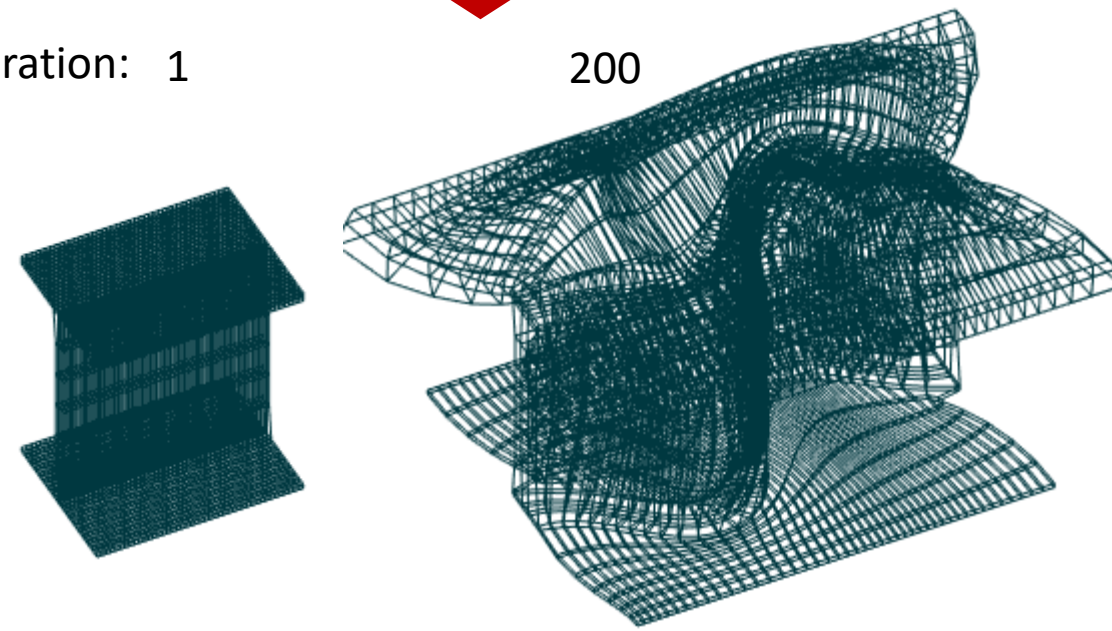
Haas, Cell Surface 2021

Computational model of growth and shape formation by anisotropic nanofilament expansion





Growth iteration: 1 200

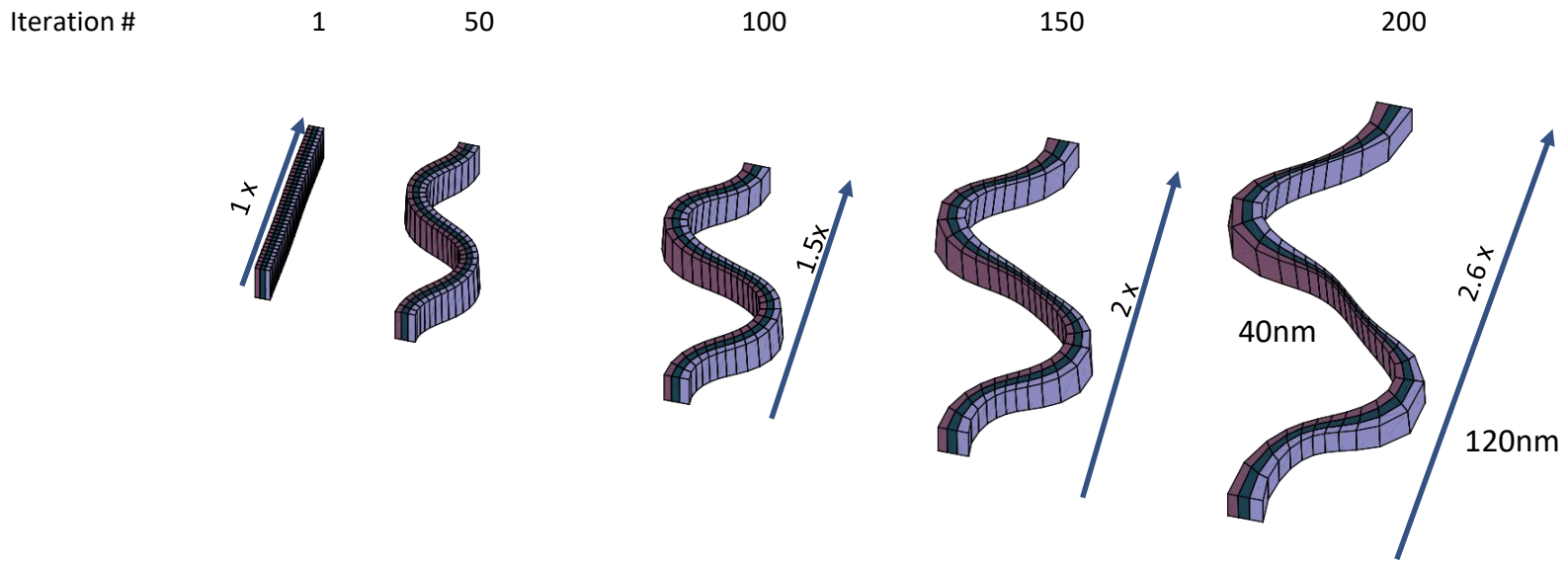


straight lobed

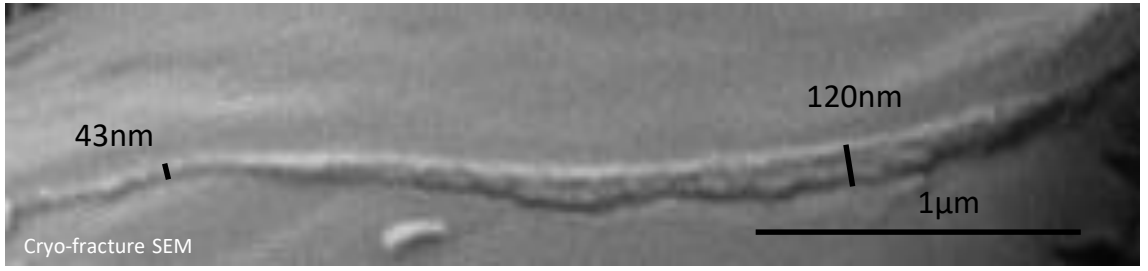
Haas et all, Science 2020

Testing the model prediction throe the indirect prediction of cell wall thickness

Cell wall width model prediction



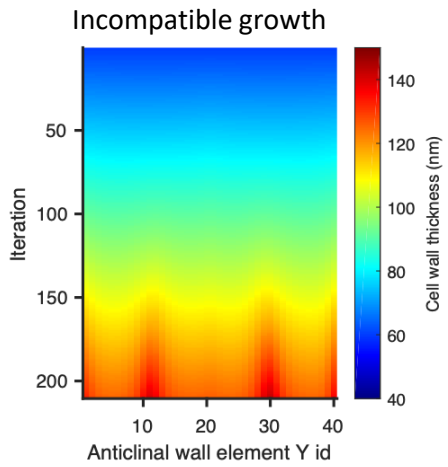
Crio SEM cell wall thiknes observed



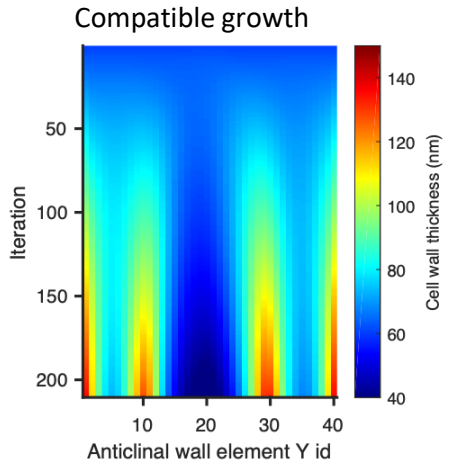
Testing the model prediction through the indirect prediction of cell wall thickness with or without tention

Our model predicts a discrepancy in cell wall thickness observed with TEM and SEM

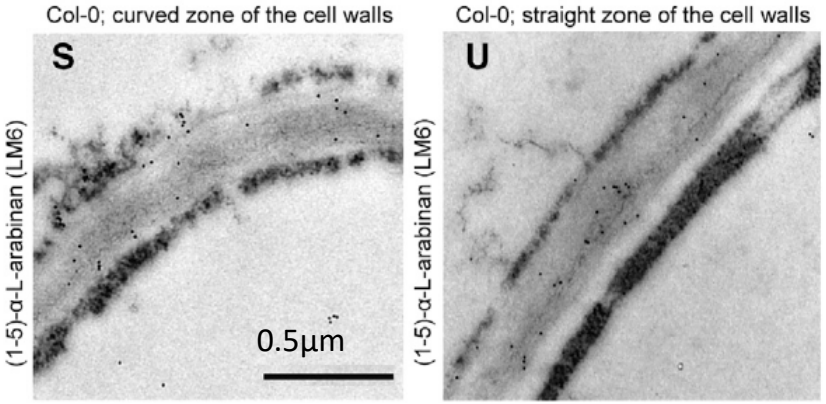
With relaxed tentions



With native tentions

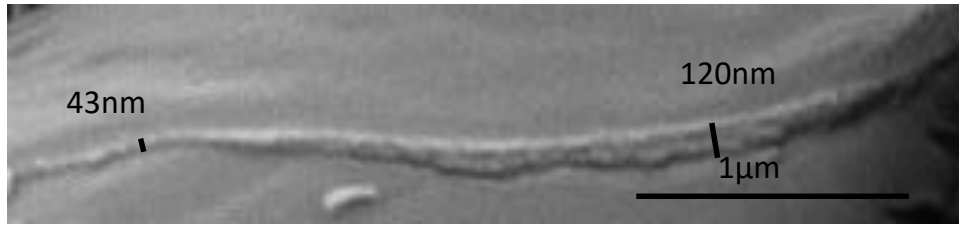


TEM

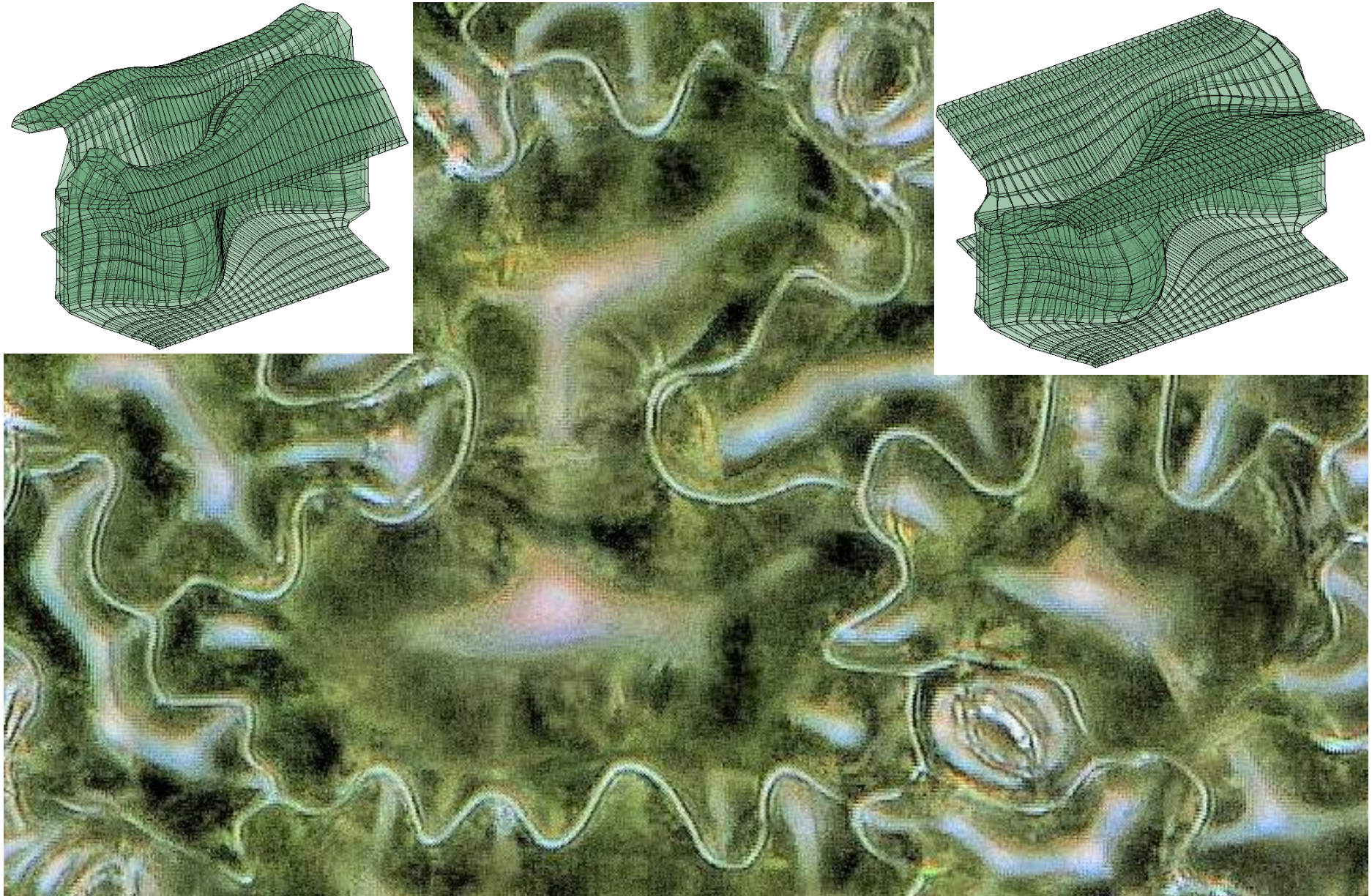


Majda et al., 2017

Cryo-fracture SEM



Testing the model prediction through comparing turgid and non turgid tissue



The cases of cell wall expansion that cannot be driven by turgor pressure

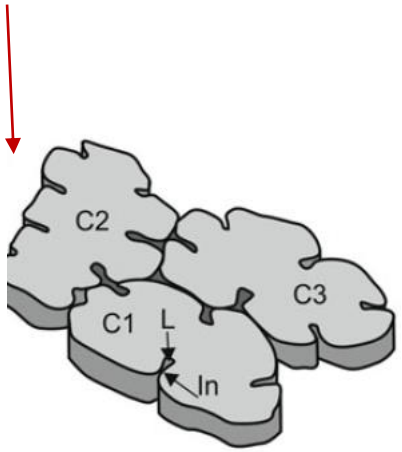
- Expansion of convoluted anticlinal walls in leaf epidermis
- Stomata opening

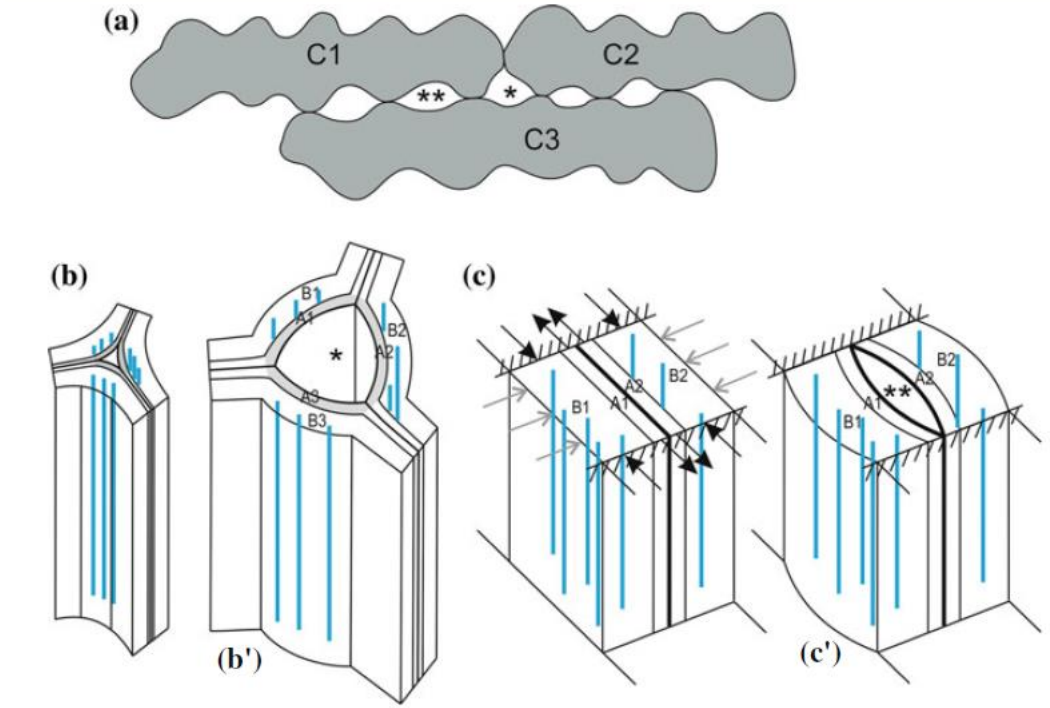
- Formation of gas spaces

**Thre- or four-way junction, ** two cell junction (intercalary).

- expansion of cell wall invaginations in Pinus mesophyll

- Cuticles





Three-way junction

Two cell junction (Stomata opening)

Hejnowicz 2011, Borowska-wykret 2018

STORMtheWALL ERC starting grant

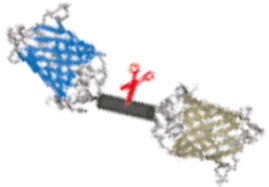
Super-resolution

Novel nanoprobes

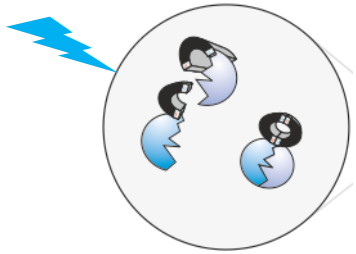
VHH

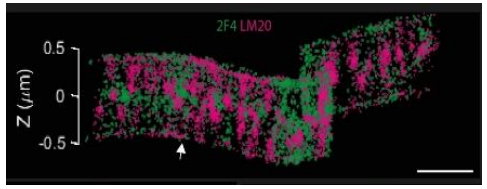
Multiplexed imaging

Biosensors



optogenetics





Subcellular control of Growth orientation

Toolbox

Quantitative growth Modeling

Subcellular dynamics of Growth motor

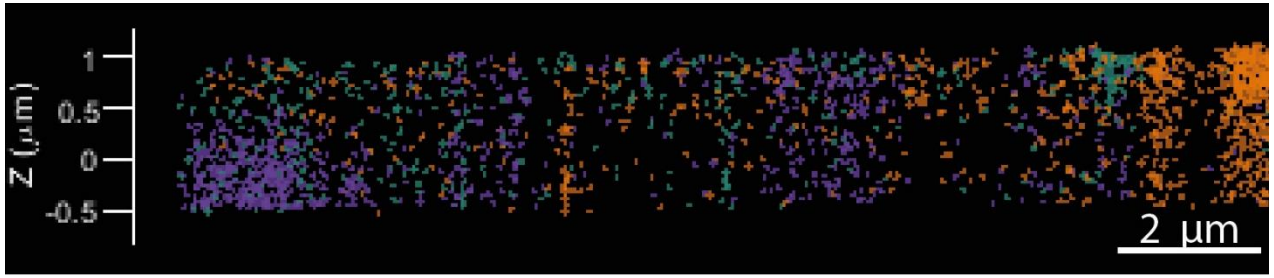
Kalina T. Haas

2 posts open: 1 Engineer, 1 Post-doc

Are other pectins organized into nanofilaments?

Hypocotyl epidermis anticlinal wall HG⁻ HG⁰ RGII

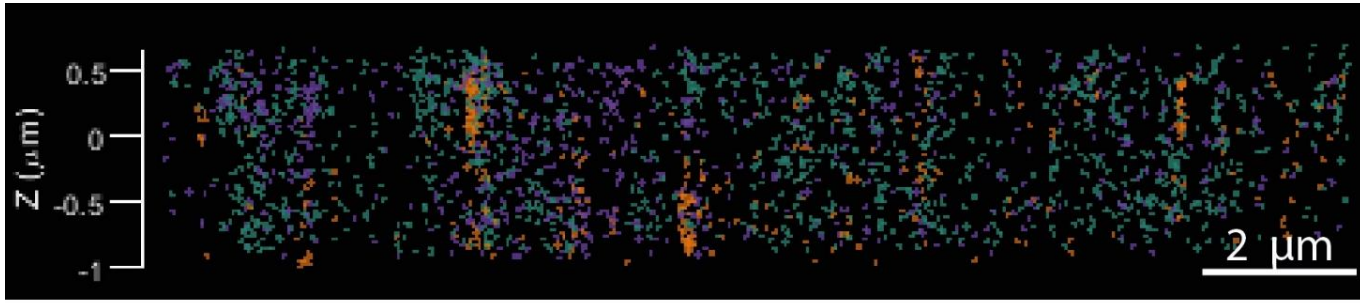
(longitudinal cut in transversal view, AL-2)



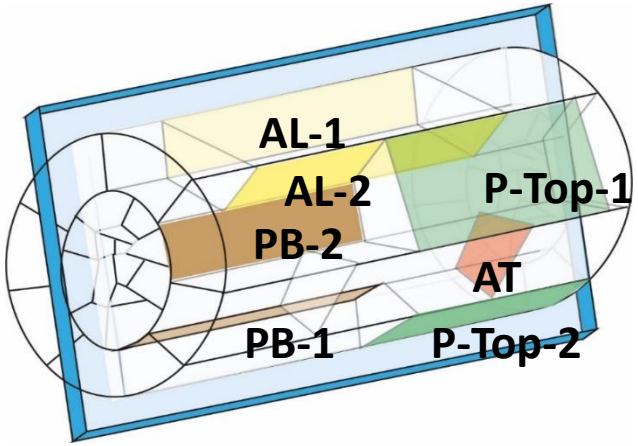
anti-RGII antibodies kindly provided by Prof Masaru Kobayashi, Univ. Kyoto

Root, cortex/endodermis separation HG⁻ HG⁰ cellulose

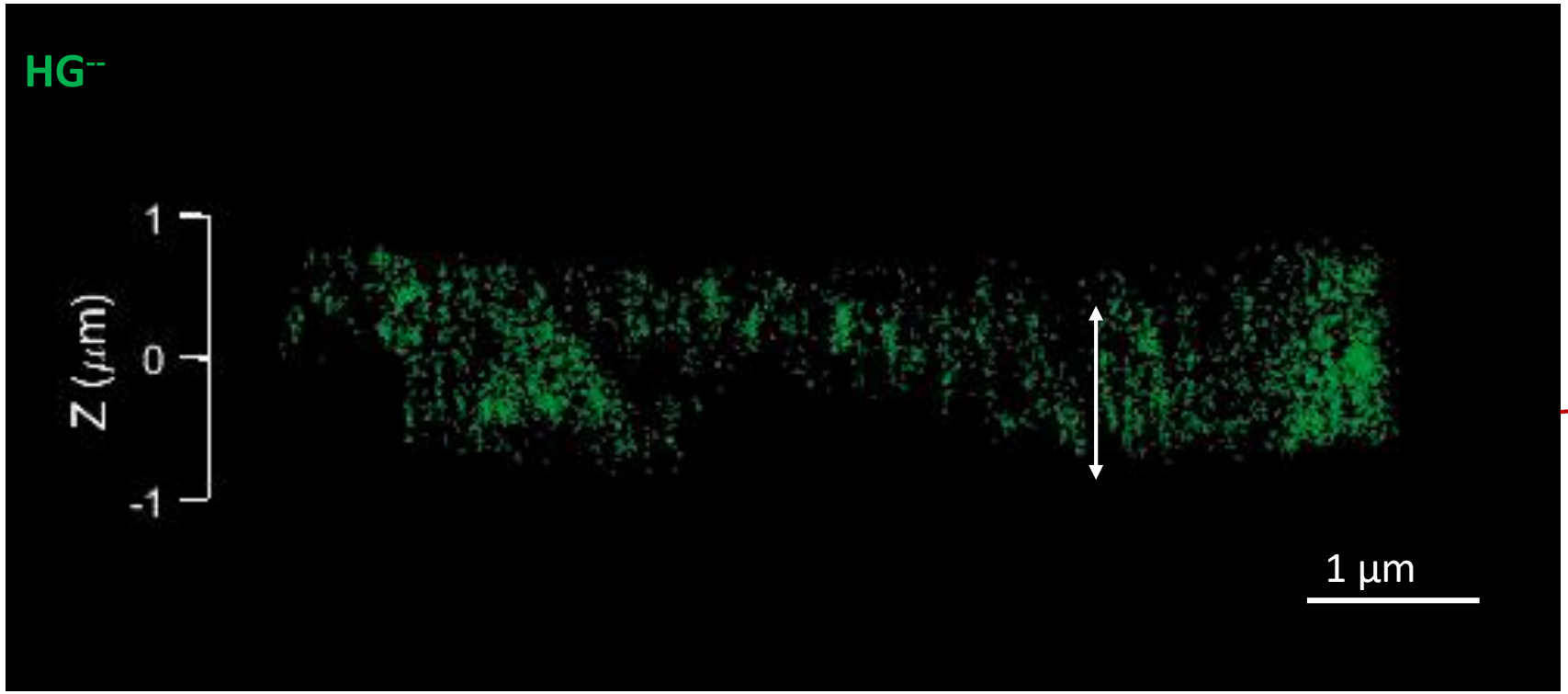
(longitudinal cut in transversal view, AL-2)

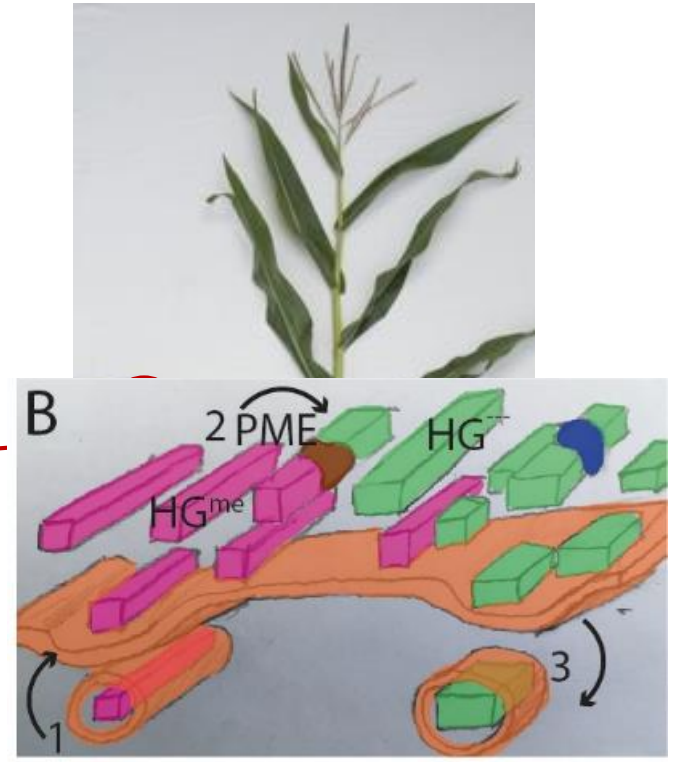


A – Anticlinal wall
P – Periclinal wall
L – Longitudinal
T – Transversal
B - Bottom



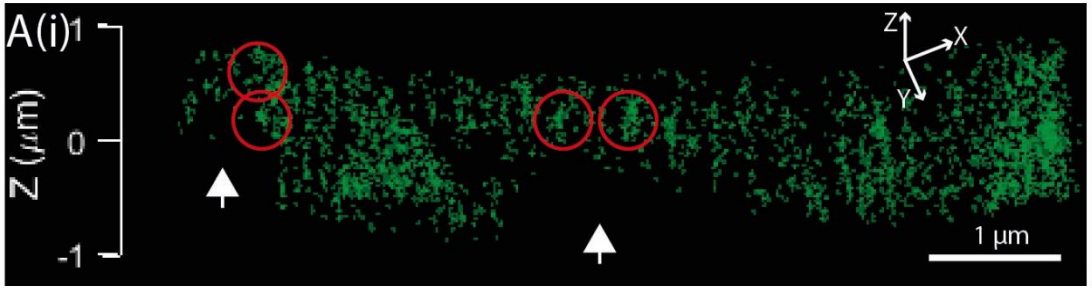
Pectin nanofilaments were also observed in grasses



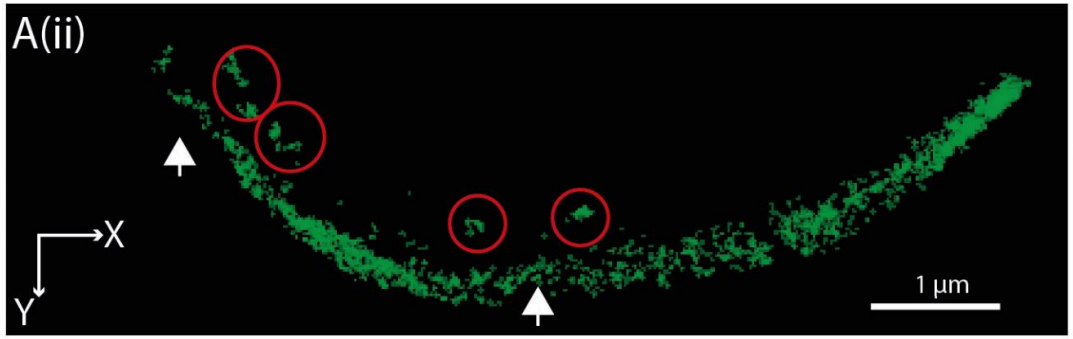


Haas, Bio-Protocol 2020, Haas, J. Exp. Botany 2021

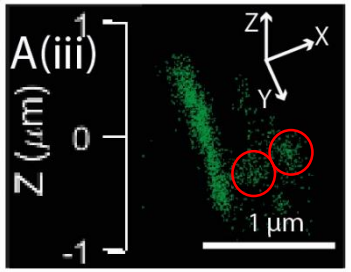
3D XZ view



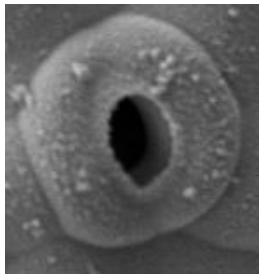
2D XY view



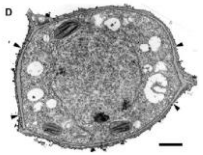
3D YZ view

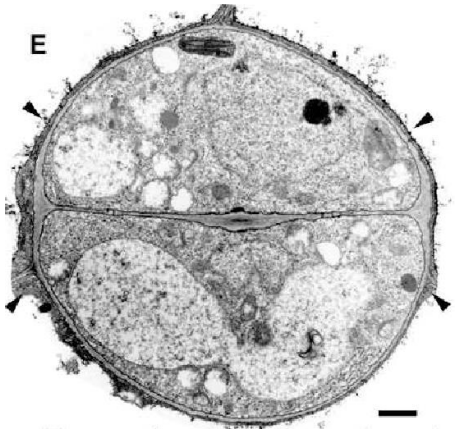


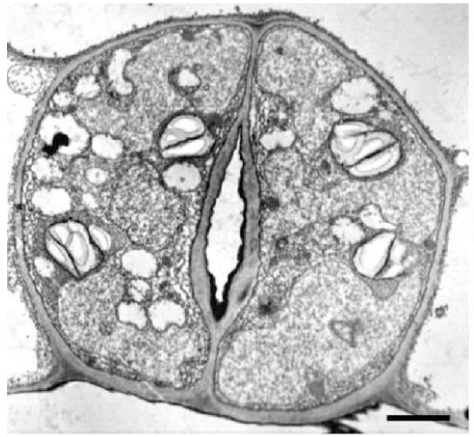
Differentiation is associated with local cell wall thickening



Haas et al 2020







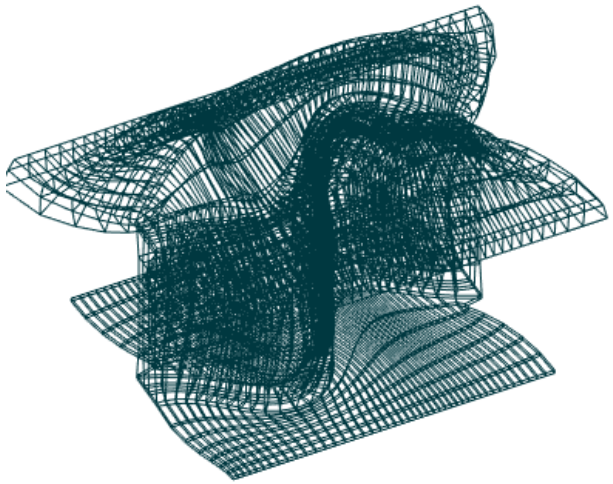
(D) The division site in GMCs is marked by wall thickenings before symmetric division. (E) The GMC division site wall thickenings are still visible in a developing stoma (arrowheads). Adapted from Zhao and Sack (1999). Bars = 1 µm.

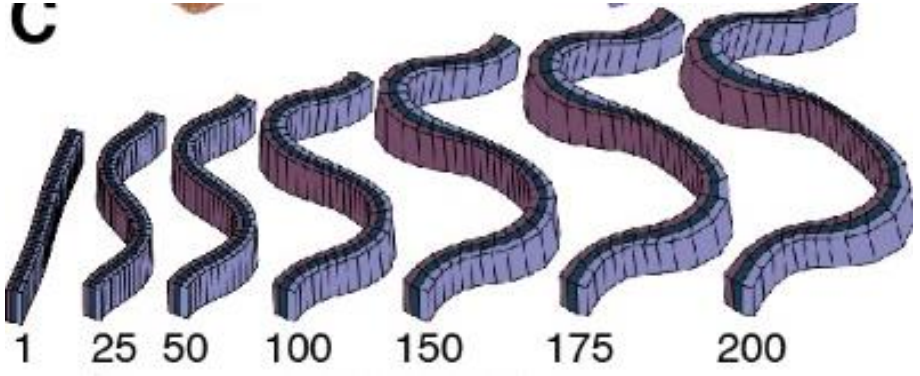
from Zhao and Sack (1999). (Right) Cryo-scanning electron micrograph of maturing epidermis from a cotyledon. Bars = 2 µm.

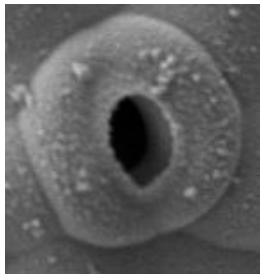
Jeanette A. Nadeau, Fred David Sack

Published 2002 in The arabidopsis book

cell wall thickening observed and confirmed by our model in pavement cells





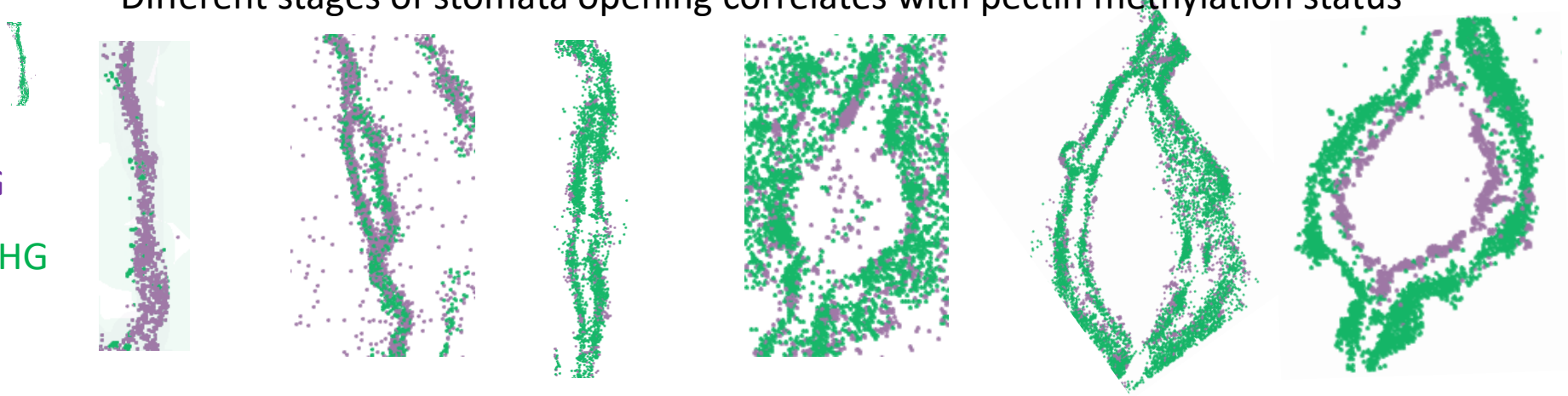


Dynamic changes in pectin methylation during stomata opening seen by super-resolution microscopy

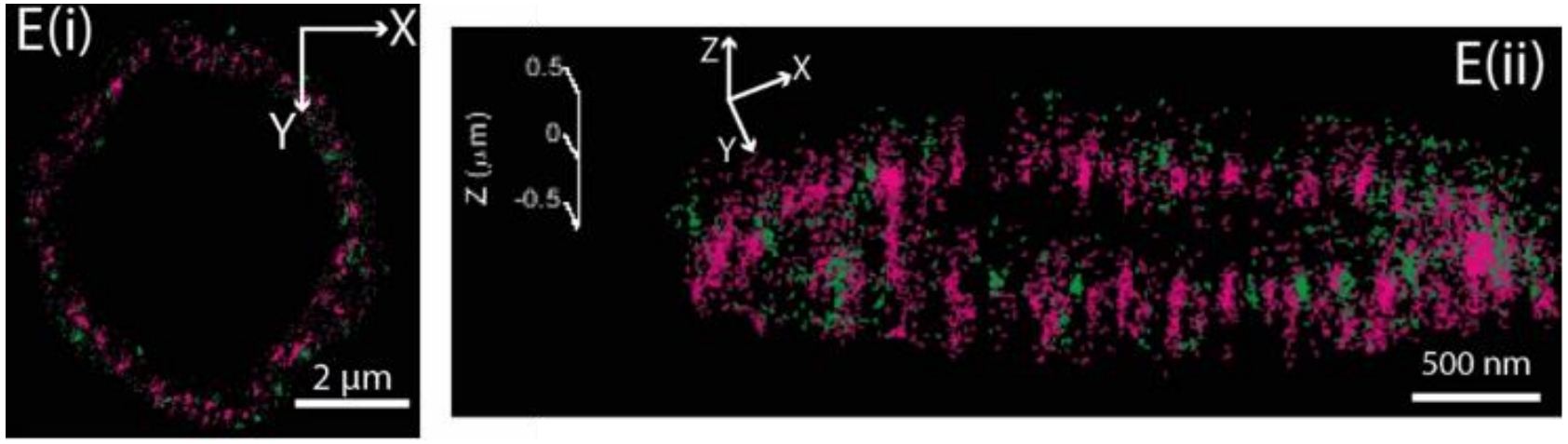
Different stages of stomata opening correlates with pectin methylation status

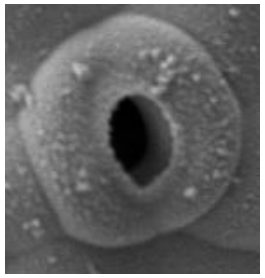
Methylated HG

Demethylated HG



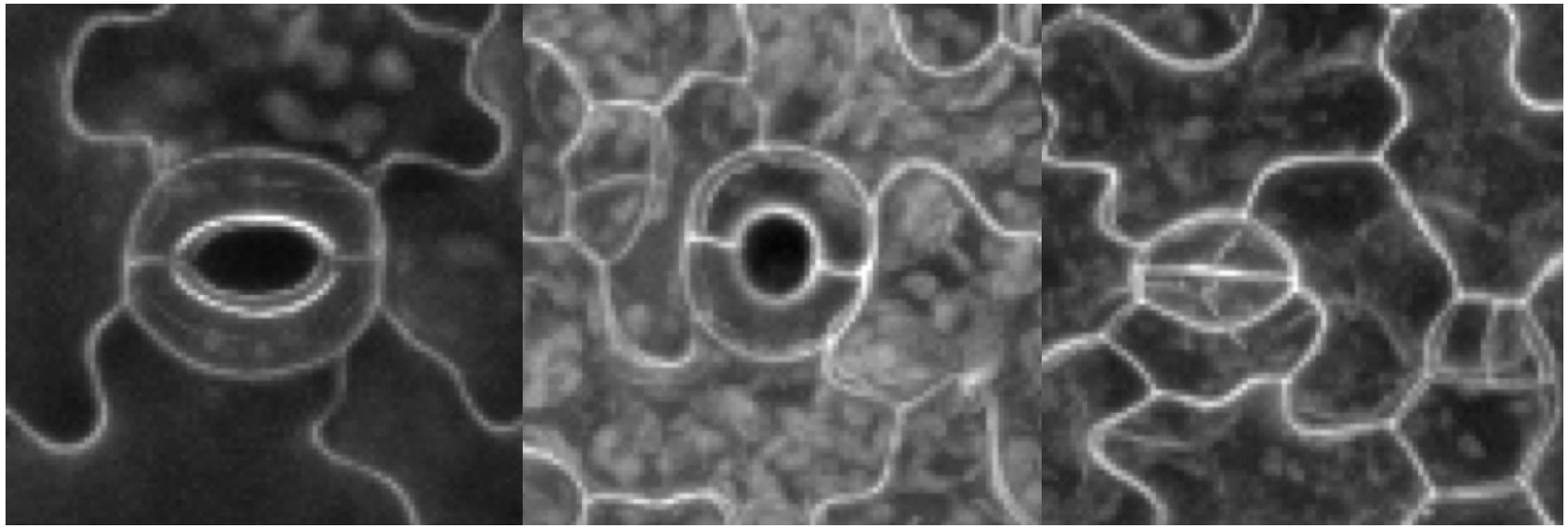
Stomata pore (anticlinal wall)



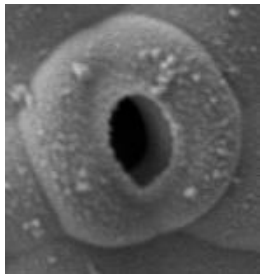


Gard cell differentiation is sensitive to pectin methylation status

Control PMEoe PMEIoe

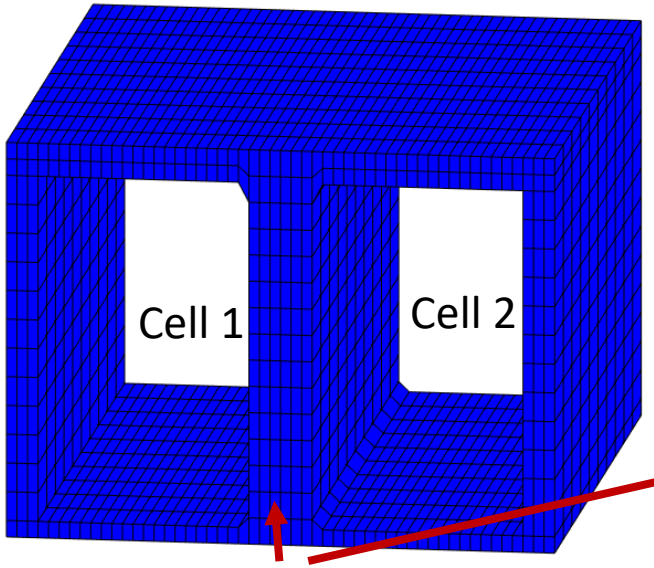


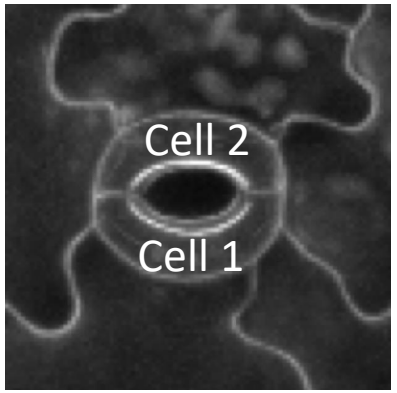
A. Peaucelle, unpublished data



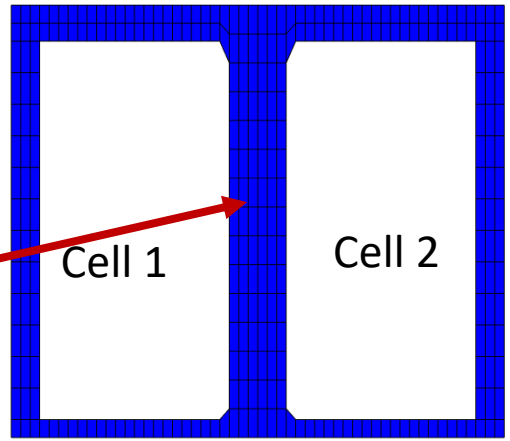
3D FEM model of stomata opening using our expanding beam model

XYZ view





XZ view

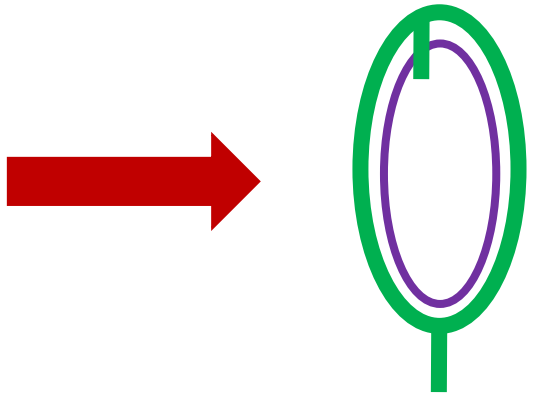


Ventral anticlinal wall with the future stomata pore

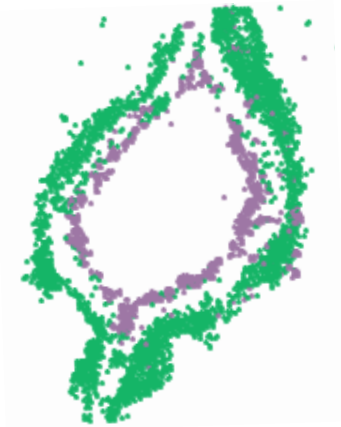
Methylated HG

dSTORM

Closed pore



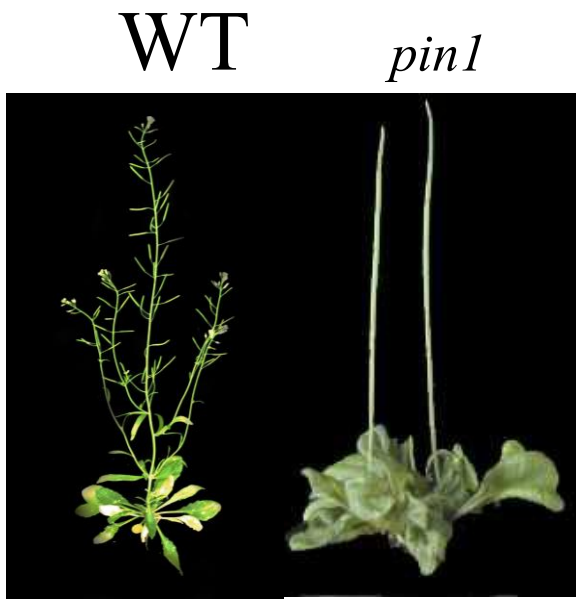
Open pore



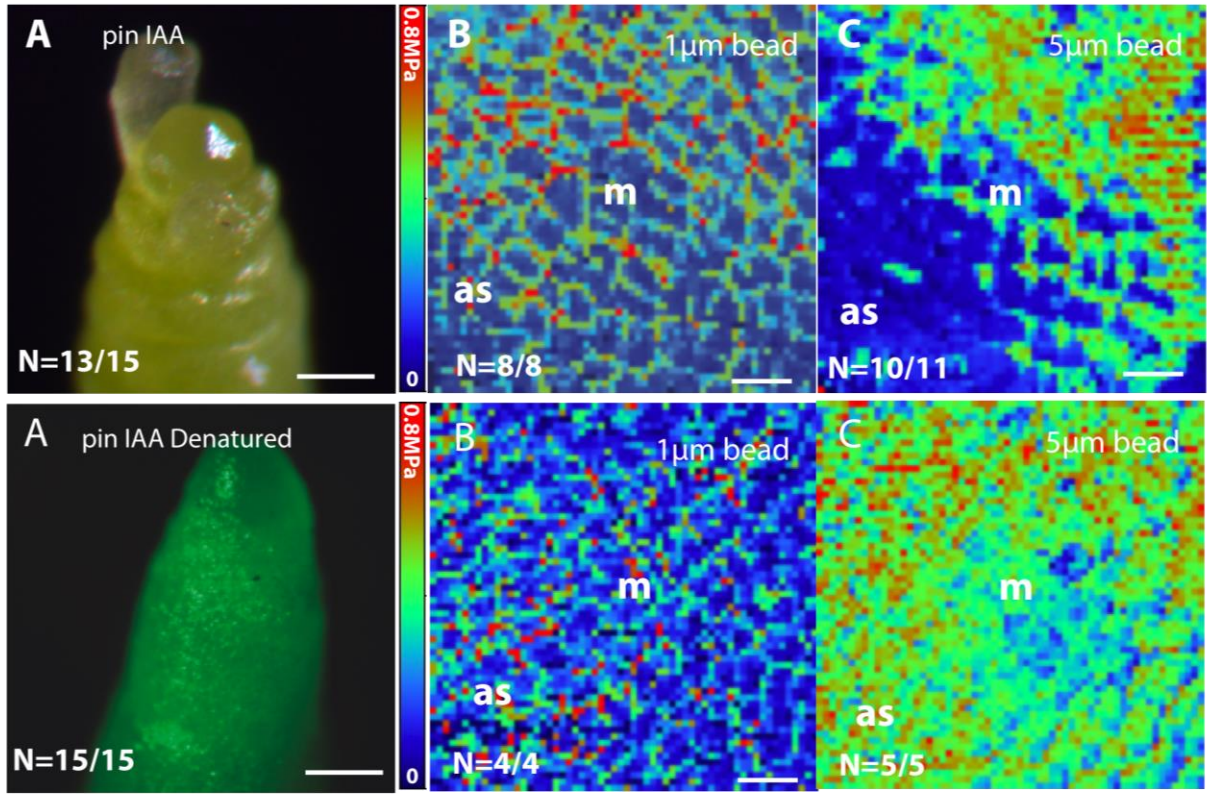
dSTORM

Demethylated HG

L’auxine induit le ramollissement des parois



Reinhardt et al., (2000)



La formation de primordia à la suite d’une application d’auxine chez le mutant *pin1* est précédée par un ramollissement